TEXTBOOK OF

Gastroenterology

FIFTH EDITION

EDITED BY

Tadataka Yamada

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David H. Alpers
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VOLUME 1

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Textbook of Gastroenterology

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Preface

It is over 20 years since the *Textbook of Gastroenterology* was launched. From the very first edition the aim has been to present the scientific basis of gastroenterology in a way that gives insight into common clinical problems, to provide at its core an encyclopedic discussion of virtually all the disease states encountered in practice, and to describe all the major diagnostic and therapeutic technologies of gastroenterology, both the long standing and the very recent, available to clinicians today. As in the past, we have approached this fifth edition of the *Textbook* with the aim of updating every chapter and seeking new authors for at least 30 percent in order to provide a fresh approach to the material.

We have undertaken some major new initiatives in association with the *Textbook* over the years. For example, from the beginning, we recognized that the practice of gastroenterology is a highly visual one and so, on the basis of the old adage that a picture is worth a thousand words, we have published an *Atlas of Gastroenterology* to accompany the *Textbook*. As the *Textbook* itself is of sufficient size and detail to be difficult for students and house staff to carry with them, we published an abridged paperback version, the *Handbook of Gastroenterology*. In the fourth edition of the *Textbook*, we added an extensive hepatology section, recognizing that many practicing gastroenterologists are also hepatologists. In this fifth edition, we have undertaken another substantive change. The reader will note that the section in previous editions that described approaches that a clinician might take to common symptoms and signs encountered in gastroenterology or hepatology has been deleted. The rationale behind this decision was as follows: (1) the *Textbook* was becoming very large and needed to be shortened in a way that would not alter the basic strategy that defined its design; (2) much of what was available in these "Approaches to . . ." chapters is included in the chapters that describe the clinical conditions; and (3) the "Approaches to . . ." section, organized and edited appropriately, could serve as a textbook on its own merits, directed at an audience of students, house staff, and practitioners who are not primarily gastroenterologists. Accordingly, the "Approaches to . . ." section of previous editions has been incorporated into a separate volume, *Principles of Clinical Gastroenterology*.

Much has changed in the science and practice of gastroenterology and hepatology since the publication of the first edition of the *Textbook*. Over the years we have endeavored to keep pace with the many advances that define our clinical discipline in order to maintain the *Textbook*'s modernity, freshness, and up-to-date accuracy. In this spirit, we are delighted to welcome Tony Kalloo to the editorial team. His hands-on experience in the most modern procedural techniques of gastroenterology, in addition to his broad understanding of the discipline, have added immensely to this edition.

We are delighted to have a new publisher, Wiley-Blackwell, for this edition. Their keen insight into the publishing industry and the way in which textbooks are utilized today has been the basis for some of the changes made to the *Textbook*. We are also grateful for their knowledge of the international world of medicine, which will help us to distribute the contents of the *Textbook* to a global audience. The editors would like especially to thank Elisabeth Dodds at Wiley-Blackwell, whose commitment to excellence has contributed materially to the quality of the book. In addition, without the assistance of Alison Brown this fifth edition of the *Textbook* would not have been published.

Our efforts were especially facilitated by the expert assistance of Lori Ennis and Barbara Boughen, who collaborated as a team, complementing editorial talents with interpersonal skills to maintain the high quality of the text and deliver the manuscripts in a timely fashion. The editors are indebted to their administrative and secretarial assistants, Patricia Lai, Terri Astin, Jennifer Mayes, Sue Sparrow, Patty Pool, Gracie Bernal-Muñoz, and Maria L. Vidrio. In addition, the faculty and fellows of the Gastroenterology Divisions at the University of Michigan, Washington University in St Louis, and the University of Texas Medical Branch in Galveston provided valuable assistance in reviewing the chapters in the fourth edition of the *Textbook* in preparation for this, the fifth edition.

Tadataka Yamada, MD

1 Basic mechanisms of normal and abnormal gastrointestinal function

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A COMPONENTS

1 Integrative responses of the gastrointestinal tract and liver to a meal

Stephen J. Pandol, Helen E. Raybould, Hal F. Yee Jr

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This chapter provides an overview of the mechanisms involved in the regulation of food intake and the various responses in the gastrointestinal (GI) tract, the liver, and the pancreas to the ingestion of a meal. The coordination of these processes is essential for the regulation of food intake; the digestion and absorption of nutrients in the food; the distribution, storage, and release of nutrients to meet demand; and the elimination of wastes. Each of the processes described in this introductory chapter is explored in greater depth and with extensive references to the literature elsewhere in the textbook.

The GI tract consists of the alimentary canal from the mouth to the anus and the associated glandular organs (i.e., salivary glands, liver, and pancreas) that empty their contents into the canal. In a general sense, the GI tract adds water, ions, and enzymes to a meal to convert it into an aqueous solution of molecules that can be transported within the body. Importantly, most of the added substances are absorbed for reuse. The assimilation of a meal involves major physiological processes that occur in the GI tract: motility, secretion, digestion, absorption, and elimination. Food is taken into the mouth as large particles containing macromolecules that are not absorbable. The breaking down of food into absorbable material occurs by grinding and mixing the food (motility) with various secretions containing enzymes, ions, and water that enter the GI tract. The enzymes convert the macromolecules into absorbable molecules in a process termed digestion. The products of digestion, as well as the secretions from the upper parts of the GI tract, are then transported across the epithelium to enter the blood or lymph by a process termed absorption. Secretions and luminal contents are moved from the mouth to the anus and eliminated by GI motility. The coordination of GI function is regulated in a synchronized way to maximize digestion and absorption by means of multiple control mechanisms.

The liver and pancreas are anatomically coupled to the GI tract through the biliary and pancreatic ductal systems, respectively. Further, the liver receives blood circulation directly from the GI tract and the pancreas through the portal circulation. The liver has numerous vital functions, including bile formation and secretion; uptake, storage, and release of nutrients; generation of plasma proteins; detoxification; and immune surveillance. The exocrine pancreas provides the major digestive enzymes, whereas the endocrine pancreas plays a central role in the uptake, storage, and release of nutrients in the liver, as well as in other tissues such as skeletal muscle and adipose tissue.

Control systems

Because the macronutrient content of a meal can vary considerably, the GI tract is endowed with mechanisms that can detect the presence of food and mount appropriate physiological responses. In addition, the GI tract communicates with associated organs (e.g., pancreas) such that when events occur in the more proximal GI tract, signals are sent to the more distal parts, and vice versa. Three principal control mechanisms regulate GI function: endocrine, paracrine, and neural (Fig. 1.1).

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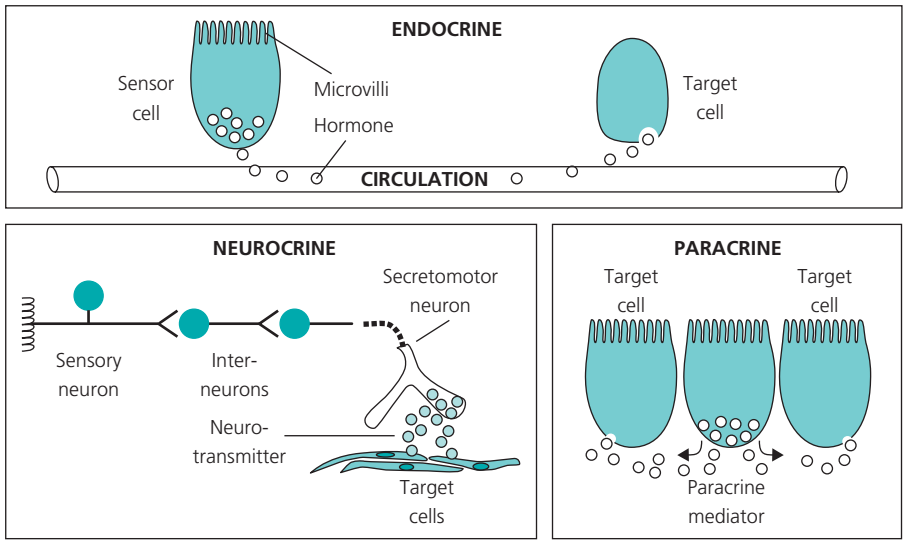

Figure 1.1 Three principal types of control mechanism regulate the function of the gastrointestinal tract: paracrine, endocrine, and neurocrine (neural). Examples of paracrine mechanisms are numerous. In this figure, the target cell is shown as being adjacent to the sensor cell. However, paracrine mechanisms may be mediated over tens or hundreds of micrometers and may involve many different cell types, including epithelial cells, endocrine cells, nerve terminals, and smooth muscle. From Undergraduate Teaching Program of the American Gastroenterological Association, 1996.

Endocrine regulation

Endocrine regulation describes the process whereby the sensing cells in the GI tract, the enteroendocrine cells, respond to a stimulus and secrete regulatory peptides or hormones that travel by way of the bloodstream to target cells. Effector cells respond to the hormones and express specific receptors. Hormones released from the GI tract have effects on cells located in other regions of the GI tract and also on the glandular structures associated with the GI tract, such as the pancreas and the liver.

The secretory products of the enteroendocrine cells are released in response to chemical (nutrients, acid, osmolarity) and mechanical (distention, stretch) stimuli to the wall of the GI tract, although the exact mechanisms by which this occurs are not well understood. Most enteroendocrine cells in the gut wall are of the "open" type; these cells have an apical membrane that is in contact with the lumen of the GI tract (generally regarded as where the sensing occurs) and a basolateral membrane over which secretion occurs.

Examples of hormones secreted by the GI tract abound. Gastrin, one of the better characterized GI hormones, plays a central role in the regulation of gastric acid secretion. Gastrin is released from endocrine cells located in the antral mucosa in response to activation of the parasympathetic outflow to the GI tract. Other hormones include glucoinsulinotropic peptide, which is released by glucose in the intestine and augments insulin secretion from the pancreatic β-cell; cholecystokinin (CCK), which plays a role in the intestinal feedback regulation of proximal GI function, food intake, and the secretion of pancreatic and biliary constituents into the intestine; secretin, which plays a role in neutralizing gastric acid emptied into the intestine; and motilin, which regulates gastrointestinal motility.

Paracrine regulation

Paracrine regulation describes the process whereby a chemical messenger or regulatory peptide is released from enteroendocrine cells and diffuses through the interstitial space to reach target cells. Paracrine agents exert their actions on several different cell types in the wall of the GI tract, including smooth muscle cells, absorptive enterocytes, secretory cells in glands, and other enteroendocrine cells. Histamine is an important paracrine mediator in the gut wall. In the stomach, histamine is stored and released by enterochromaffin-like cells located in the gastric glands, and acts by diffusing through the interstitial space of the lamina propria to neighboring parietal cells to stimulate the production of acid. Somatostatin also is an important paracrine mediator. Released from the antral mucosa in response to acid, somatostatin inhibits the release of gastrin from neighboring gastrin-containing endocrine cells, which results in the inhibition of meal-stimulated gastric acid secretion.

Neural regulation

The functions of the GI tract are controlled by activity in the extrinsic and intrinsic nervous systems. The extrinsic nervous system, defined as those nerves that innervate the gut with cell bodies located outside of the gut wall, is part of the autonomic nervous system. The GI tract is innervated by both the parasympathetic and sympathetic subdivisions of the autonomic nervous system. The parasympathetic innervation to the gut is through the vagus and the pelvic nerves. The vagus nerve (cranial nerve X) innervates the esophagus, stomach, gallbladder, pancreas, small intestine, cecum, and proximal colon. The pelvic nerves innervate the distal colon and anorectal region. The sympathetic innervation is supplied by cell bodies in the spinal cord and by fibers that

terminate in the prevertebral ganglia (i.e., celiac, superior and inferior mesenteric ganglia). The postganglionic neurons innervate all parts of the GI tract.

The intrinsic nervous system, also referred to as the enteric nervous system, has cell bodies that are contained within the wall of the gut in the submucosal plexus and myenteric plexus.

The afferent (sensory) innervation of the GI tract is derived from both parasympathetic and sympathetic innervation. The cell bodies of the vagal afferents are in the nodose ganglion with a central projection terminating in the brainstem and a distal projection to the gut wall. The cell bodies of the afferent neurons that run with the sympathetic pathway are in the dorsal root ganglia. The peripheral terminals of the spinal and vagal afferents are located in all layers of the gut wall, where they detect chemical and mechanical stimuli. The afferent nerves send information to the central nervous system (CNS), which drives reflexes to change secretory and motor function in the GI tract. The afferent innervation is also responsible for transmitting painful stimuli to the CNS.

Enteric nervous system

The enteric nervous system comprises two major plexuses consisting of cell bodies (ganglia) and interconnecting strands that contain nerve fibers. The myenteric plexus lies between the longitudinal and circular muscle layers and the submucosal plexus lies in the submucosa. Neurons in the enteric nervous system are characterized by their function and also by the neurotransmitters they contain. The enteric nervous system contains the components of a reflex pathway (afferent neurons, interneurons, efferent neurons) and thus is capable of functioning in the absence of input from the CNS. However, enteric nervous system function is often modulated by the CNS.

Many GI hormones have been identified in the neurons of the enteric nervous system, where they may act as neurotransmitters, and in regions of the brain, where they may be involved in the regulation of autonomic outflow. These mediators and regulatory peptides are thus referred to as brain–gut peptides and the extrinsic and intrinsic components innervating the gut are sometimes referred to as the brain–gut axis.

Regulation of food intake by the gastrointestinal tract

Multiple complex signals from metabolically active tissues and the CNS contribute to the long-term regulation of body weight (Fig. 1.2). In addition, signals derived from the presence of food in the GI tract regulate food intake, particularly in the short term. Perfusion of the intestine with single macronutrients, or mixtures thereof, results in the inhibition

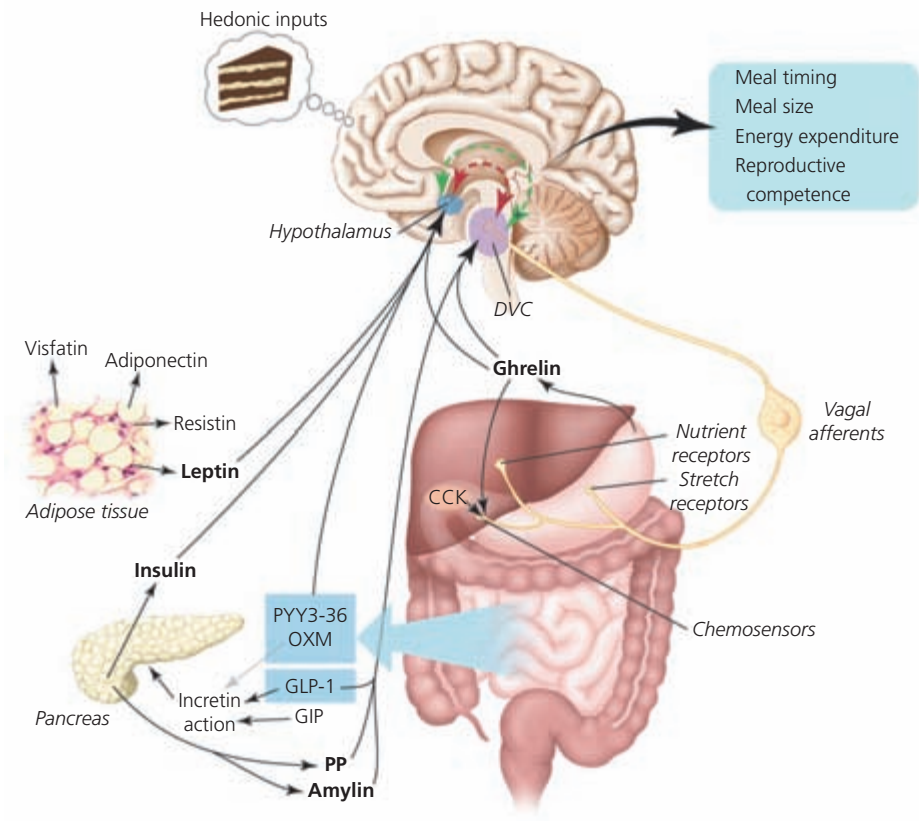

Figure 1.2 Many signaling systems regulate food intake. This graphic presents an overview of inputs from the central nervous system and from peripheral tissues such as adipose tissue (leptin) and metabolic tissue (endocrine pancreas with signals insulin and pancreatic polypeptide [PP]). CCK, cholecystokinin; DVC, dorsal vagal complex; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; OXM, oxyntomodulin. Adapted from Badman MK, Flier JS. The gut and energy balance: visceral allies in the obesity wars. Science 2005;307:1909.

of food intake and intestinal feedback regulation of GI function. These observations have led to the notion of nutrient sensors in the GI tract. Vagal afferents innervating the proximal GI tract are activated by the presence of lumenal nutrients, leading to the activation of CNS pathways involved in the regulation of food intake, including the nuclei of solitary tract (the region in the brainstem where vagal afferents terminate) and the arcuate nucleus of hypothalamus.

Humoral factors are also involved in the regulation of food intake. Many GI hormones are termed satiety signals. These signals are characterized by their release from enteroendocrine cells when nutrients are in the GI tract, and by observations that exogenous injection of these hormones, including CCK, glucagon-like peptide (GLP)-1, peptide YY (PYY), and ghrelin, in experimental animals and humans produces a decrease in food intake.

Possibly the best characterized of these satiety signals is CCK. CCK is released from enteroendocrine cells by protein hydrolysates and free fatty acids in the intestinal lumen. Exogenous administration of CCK to humans and experimental animals has determined that CCK inhibits meal size by activating CCK_1 receptors. These receptors are most likely localized to the peripheral terminals of vagal afferent fibers innervating the mucosal wall of the duodenum. However, although it is clear that CCK can inhibit the size of subsequent meals, its role in the long-term regulation of food intake is uncertain. CCK_1-receptor null mice that receive long-term administration of CCK_1 receptor antagonists maintain normal body weight when fed ad libitum, possibly as a result of the effects of longer-term satiety signals.

GLP-1 is a product of the proglucagon gene that is secreted from the distal small intestine in response to glucose and fatty acids and is proposed to be involved in the regulation of appetite and energy intake. Plasma levels of GLP-1 in humans increase after a meal. Exogenous administration of GLP-1 or agonists of the GLP-1 receptor in humans and experimental animals leads to decreased food intake. The site of action is thought to be directly on the arcuate nucleus of the hypothalamus, an area of the brain involved in the integration of short- and long-term (metabolic) satiety signals.

Despite the controversy surrounding the role of PYY, the weight of evidence now suggests that it does play a physiological role in the regulation of food intake and body weight. Plasma levels of PYY increase after a meal, and the hormone is converted rapidly from PYY1–36 to PYY3–36 by the enzyme dipeptidyl peptidase IV. PYY3–36 acts as specific agonist for the PYY Y2 receptor and is the most potent form of the peptide to inhibit food intake. PYY likely acts by way of the vagal afferent pathway but also by exerting a direct effect on the hypothalamus.

Ghrelin is secreted by endocrine cells in the stomach and is an unusual GI regulatory peptide, because, unlike those already discussed, it increases food intake rapidly and transiently after exogenous administration. Circulating levels of ghrelin decrease with feeding and increase during the fasting period, thus suggesting that it may act as a physiological regulator of appetite. Interestingly, circulating levels of ghrelin are low after gastric bypass surgery, an effect that has been associated with the effectiveness of this procedure in reducing body weight.

The role of water

Water is necessary for the digestion and absorption of nutrients and for the elimination of wastes. Water provides a fluid environment for the movement of lumenal contents, a solution for digestive enzyme action, and a medium to deliver solute to absorptive surfaces.

The daily input of water into the GI tract is about 9.0 L per day, which comprises 2 L oral intake, 1.5 L salivary saliva, 2.5 L gastric juice, 0.5 L bile, 1.5 L pancreatic juice, and 1.0 L small intestinal secretion. The daily intestinal content of water entering the colon is 1.5–2.0 L, whereas fecal output consists of only 0.1–0.2 L water. Thus, the distal small intestine and colon reabsorb almost all the water delivered to them.

Electrolytes play a central role in the mechanisms of secretion and absorption of water. For example, electrolytes are secreted or absorbed across the epithelium of the GI tract by ion transport systems. The actively transported electrolytes then cause water to move so that isosmolality is maintained. Another result of the transport of a particular ion across the epithelium is that other ions with the opposite charge flow passively and paracellularly along with water through the paracellular spaces between epithelial cells.

In each organ, specific combinations of ion transport systems mediate the secretion or absorption of ions and water, thus providing the overall effect of that organ on water flow in the GI tract. Although these systems are described in detail in chapters dedicated to specific organs, the illustrations in Fig. 1.3 show prototypes of a secretory epithelium (Fig. 1.3a) and an absorptive epithelium (Fig. 1.3b). In the secretory epithelium, activation of a Cl^- channel on the lumenal surface along with activation of a K^+ channel on the basolateral surface of the cell drives Cl^- across the epithelium into the lumen. This effect is promoted by the high intracellular K^+ concentration produced by the basolateral Na^+,K^+-ATPase and the increased intracellular Cl^- concentration resulting from the effect of the Na^+,K^+-ATPase on facilitating the movement of Cl^- into the cell using the $Na^+/K^+/Cl^-$ cotransport system. The end result is that Cl^- is transported into the lumen. The electrical and osmotic effect of this transport is the subsequent movement of Na^+ and water through the paracellular space into the lumen. The net result is water and NaCl secretion.

An example of absorptive transport is provided in Fig. 1.3b. The Na^+/glucose cotransport on the lumenal surface results in absorption of the ion Na^+ and the nutrient glucose. This

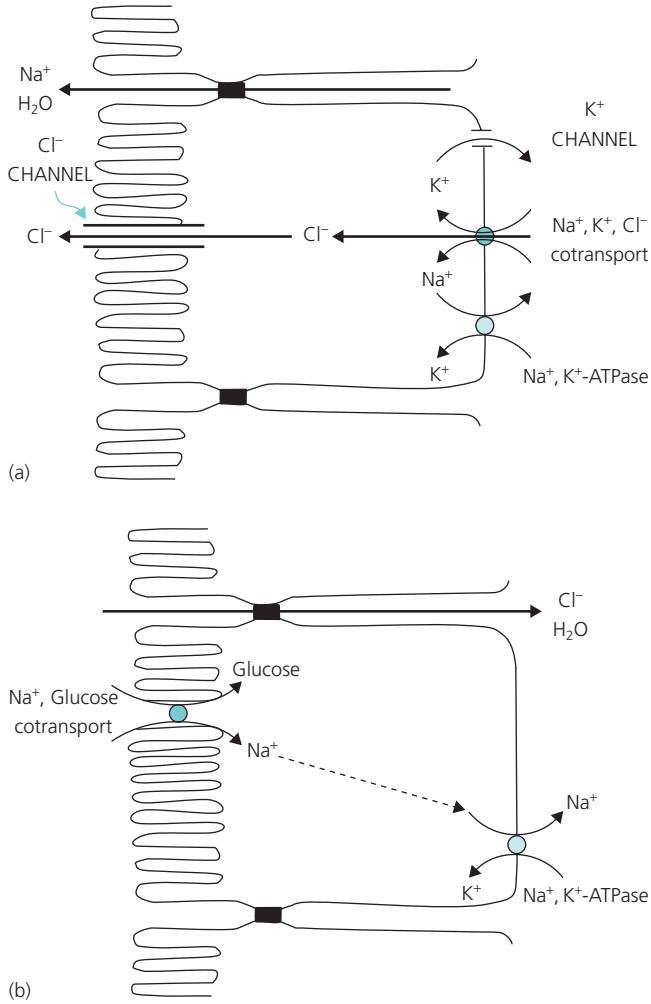

Figure 1.3 Transports of intestinal secretory **(a)** and absorptive **(b)** cells.

transport is also facilitated by the Na^+,K^+-ATPase. The resulting electrical and osmotic effects of the transport of both Na^+ and glucose cause the additional absorption of Cl^- and water across the epithelium. Of note, much of the water-absorptive process in the small intestine is coupled to nutrient transport. That is, sugars, amino acids, and fatty acids are absorbed using Na^+-coupled transport systems, accounting for more rapid absorption of liquids containing combinations of nutrients and NaCl.

As suggested previously, absorption can be regulated by lumenal factors, such as nutrients. Lumenal contents can also regulate secretion. An example is salivary secretion stimulated by food in the mouth or even the thought of food before it enters the mouth. In addition, the meal and nutrients in the meal can activate gastric, biliary, and pancreatic water secretion. The mechanisms that regulate each are specific to the organ and involve both neural and humoral pathways as briefly described in the present chapter and in more detail in other chapters in this textbook.

Cephalic and oral phases of a meal

The main feature of this part of the meal is the activation of the GI tract in readiness for the meal. The cephalic phase consists of responses to auditory, cognitive, visual, and olfactory stimuli induced by the meal. The oral phase includes many of the same stimuli, as well as those initiated in the mouth, which are both chemical and mechanical. The effector responses are mediated through various higher brain centers (many involved in cognition) and ultimately converge on the brainstem to increase parasympathetic outflow to the gut. This outflow activates secretory and motor responses including salivary, gastric, and pancreatic secretion; gallbladder contraction and relaxation of the sphincter of Oddi; and relaxation of smooth muscle activity in the proximal stomach. These responses supply the gut with water, ions, digestive enzymes, and bile necessary to initiate digestion.

During the oral phase, contact of the food with the buccal mucosa presents additional chemical and mechanical stimuli. The responses initiated by these stimuli are, in general, the same as those stimulated during the cephalic phase because they share a common efferent pathway of activation through the vagus nerve. Functions of salivary secretions are listed in Table 1.1. Chewing, an important component of the oral phase of the meal, subdivides the food and mixes it with salivary secretions. Digestion starts in the mouth with the action of salivary amylase and lingual lipase. Mucus in the saliva lubricates the food for both chewing and swallowing.

Neural pathways mediate the components of the oral and cephalic phases. For example, salivary secretion is mediated by parasympathetic effector neurons. In the stomach, vagal efferent activity results in the stimulation of chief cells, enterochromaffin-like cells, parietal cells, and G cells. Thus, there is stimulation of function by way of the enteric neurons, which then activate release of paracrine (histamine) and humoral (gastrin) mediators that will further stimulate parietal and chief cell secretion. These mechanisms ensure the rapid initiation of protein digestion as food enters the stomach.

Table 1.1 Functions of chewing and salivary secretion

Disruption of food resulting in smaller particles
Formation of bolus for swallowing
Initiation of starch and lipid digestion
Facilitation of taste
Cleansing of mouth and provision of antibacterial action
Clearance and neutralization of refluxed gastric material in the esophagus
Regulation of gastric and duodenal phases
Regulation of food intake and eating behavior
Assistance in speech

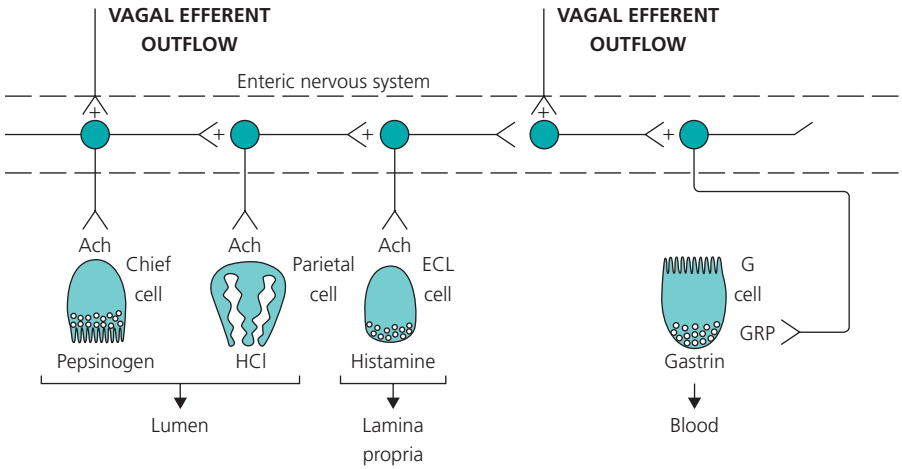

Figure 1.4 Stimulation of gastric acid secretion by increased parasympathetic outflow is effectively amplified by intrinsic neuronal circuitry in the gastric wall. Vagal efferents synapse with intrinsic cholinergic neurons and with neurons containing gastrin-releasing peptide (GRP). Activation of these intrinisic neurons stimulates a number of cell types in the gastric mucosa. Neurocrine (GRP and acetylcholine [Ach]), paracrine (histamine), and endocrine (gastrin) pathways all contribute to this response. ECL, enterochromaffin-like cell; G cell, gastrin cell; HCl, hydrochloric acid. From Undergraduate Teaching Program of the American Gastroenterological Association, 1996.

Gastric phase of a meal

The presence of food in the stomach initiates several responses within the stomach itself and in other parts of the GI tract. The main functions of the stomach, among many, are to act as a temporary reservoir for the meal and to initiate protein digestion through the secretion of acid and the enzyme precursor pepsinogen. As food enters the stomach from the esophagus it presents two principal stimuli: a mechanical stimulation of the gastric wall caused by distention and stretch of the smooth muscle wall and a chemical stimulation caused by oligopeptides and amino acids in the gastric lumen. The regulation of gastric acid secretion during the gastric phase is dependent on the integrated action of endocrine, paracrine, and neural pathways (Fig. 1.4). Both intrinsic and extrinsic neural reflex pathways are important for the regulation of gastric function. Afferent neurons that pass from the GI tract to the CNS by way of the vagus nerve (and to a lesser extent to the spinal cord) respond to mechanical and chemical stimuli and activate parasympathetic outflow that is both excitatory and inhibitory.

The endocrine pathways include the release of gastrin, which stimulates gastric acid secretion. Important paracrine pathways include the release of histamine from enterochromaffin-like cells, which stimulates gastric acid secretion; and the release of somatostatin from cells in the antral mucosa, which inhibits gastric acid secretion. Activation of these pathways elicits both secretory and motor responses. Secretory responses include acid secretion, pepsinogen secretion, and the production of mucus, intrinsic factor, gastrin, lipase, and bicarbonate (Table 1.2). These secretions initiate protein digestion and protect the gastric mucosa. Motor responses, which are described as changes in the activity of smooth muscle, include inhibition of proximal stomach motility (i.e., receptive relaxation) and stimulation of distal stomach motility causing antral peristalsis. The function of these changes in smooth muscle function is to store the meal, mix it with secretions, and regulate the flow of contents out of the stomach.

Table 1.2 Major functions of the gastric phase

Gastric responses
Storage of the meal
Secretion of pepsinogen and lipase to initiate digestion
Secretion of H^+ to kill microorganisms and convert pepsinogen to active form
Secretion of intrinsic factor to bind vitamin B-12 (cobalamin) for absorption
Secretion of mucus and bicarbonate for gastric mucosal barrier and lubrication
Secretion of water for aqueous suspension of nutrients and to make hyposmotic
Mixing secretions with food and reduction of particle size (grinding) of the meal
Regulation of emptying of contents into the duodenum
Distal gastrointestinal tract responses
Stimulation of pancreatic secretion
Contraction of the gallbladder
Increased colonic motor activity
Relaxation of the sphincter of Oddi

The gastric phase also includes the activation of the more distal GI tract, including pancreatic secretion, gallbladder contraction, and relaxation of the sphincter of Oddi. These effects are mediated by the same afferent and efferent pathways that regulate gastric responses and prepare the intestine for the emptying of food from the stomach into the intestine.

Duodenal signals regulating gastric, hepatic, and pancreatic functions

The duodenum and proximal small intestine initiate several regulatory systems that mediate the controlled delivery of

Table 1.3 Duodenal signal-mediated responses

Inhibition of gastric acid secretion
Inhibition of gastric emptying
Stimulation of pancreatic enzyme secretion
Stimulation of pancreatic and biliary ductal ion and water secretion
Stimulation of gallbladder contraction
Relaxation of the sphincter of Oddi
Alteration of intestinal motility from the fasted to the fed pattern

chyme from the stomach and the secretion of pancreatic juice and bile into the duodenum to match the digestive and absorptive capacity of the intestine (Table 1.3). The combination of organs regulated during this phase has been termed the duodenal cluster unit: stomach, duodenum, liver, biliary tract, gallbladder, and pancreas. Lumenal and wall stimuli activate neural and endocrine pathways to mediate the responses of these organs. The different organ systems in the duodenal cluster unit have a common embryological origin and are regulated by similar sensory mechanisms and effector pathways. The gastric phase of the response to a meal provides some of the stimuli for the duodenal phase. For example, the products of gastric lipid and protein digestion are potent stimulants of regulation in the duodenum. The important duodenal stimuli are distention, acid, osmotic load, and different nutrients. The sensory pathways consist of spinal and vagal afferents and intrinsic sensory neurons that initiate reflex pathways. Hormonal pathways, especially those mediated by CCK and secretin, are of central importance (Fig. 1.5). Of note, the effector systems regulated include the inhibition of gastric emptying and secretion, the stimulation of pancreatic secretion, gallbladder contraction, relaxation of the sphincter of Oddi, pancreatic and biliary water and bicarbonate secretion, intestinal secretion, and the conversion of small bowel motility from the fasted to fed pattern.

Gastric emptying depends on the chemical and physical composition of the gastric contents (i.e., chyme) entering the duodenum. Sensory neurons, both vagal and spinal, respond to nutrients, H^+, distention, and the hyperosmolality of chyme. The effector responses that result in the inhibition or slowing of gastric emptying are not completely understood but involve activation of vagal efferent outflow, which produces a decrease in antral contractions, contraction of the pylorus, and a decrease in proximal gastric tone. In addition to these extrinsic neural pathways, there may also be local pathways, such as an intrinsic neural reflex, mediating the pyloric contraction induced by acid in the duodenum.

The availability of CCK antagonists has contributed to the appreciation that CCK is physiologically important in regulating the function of the duodenal cluster unit. The administration of CCK_1 receptor antagonists reverses the inhibition of gastric emptying, gallbladder contraction, relaxation of the sphincter of Oddi, and stimulation of pancreatic enzyme secretion in response to either a meal or the infusion of nutrients into the duodenum. CCK is released from endocrine cells in the proximal intestine in response to lumenal lipid and protein. The action of CCK to inhibit gastric acid secretion and gastric emptying, and to increase pancreatic secretion, is dependent on neural pathways (Fig. 1.6). CCK stimulates

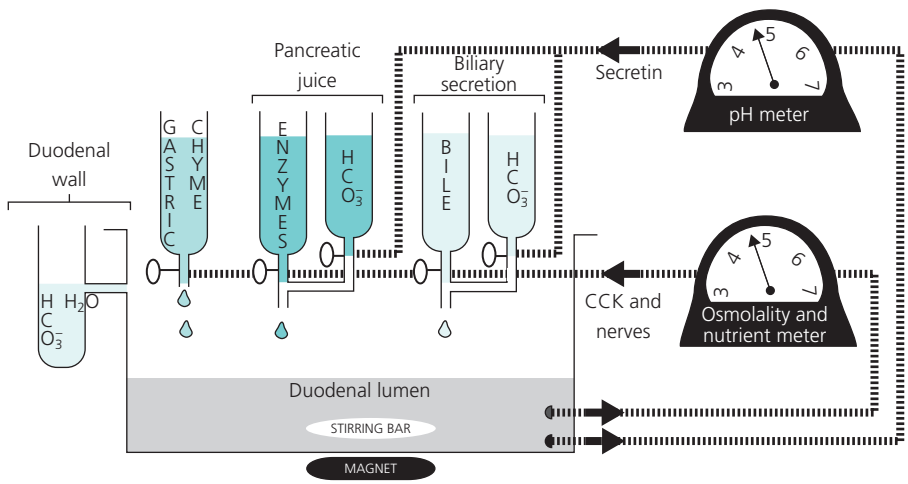

Figure 1.5 Diagrammatic representation of the duodenal cluster unit illustrates the control mechanisms and pathways involved in intestinal feedback regulation during the duodenal phase. The nutrient content and osmolality of the meal are sensed (represented as a meter) and activate predominantly neural pathways involving cholecystokinin (CCK) to open or close various stopcocks to increase pancreatic secretion or delay gastric emptying, for example. The acidity of the meal is sensed and activates the release of secretin and neural pathways to increase bicarbonate secretion from the pancreas, biliary tract, and probably also from the duodenal wall. The contents of the duodenum are mixed with the secretions by the segmenting contractions of the smooth muscle wall, represented as a stirring bar and magnetic stirrer. From Undergraduate Teaching Program of the American Gastroenterological Association, 1996.

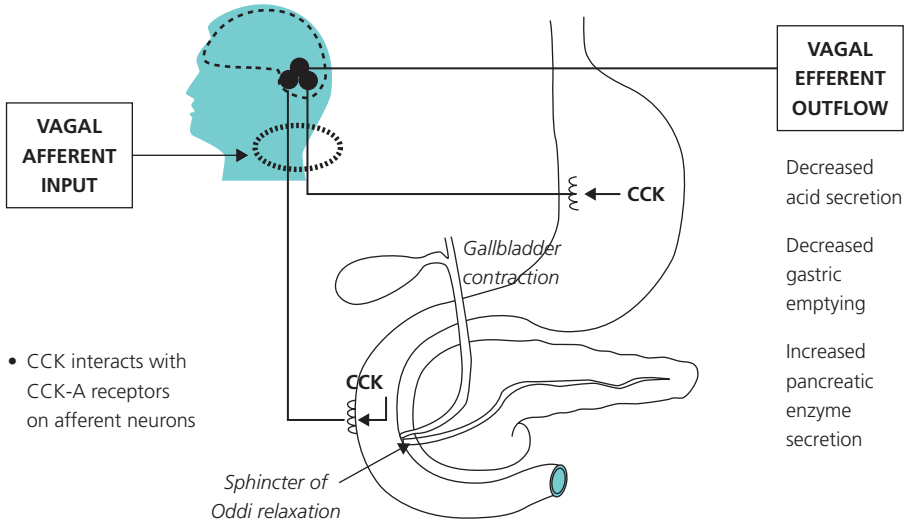

Figure 1.6 Cholecystokinin (CCK) acts predominantly by way of a vagal afferent pathway to integrate intestinal feedback regulation of the duodenal phase of the meal. From Undergraduate Teaching Program of the American Gastroenterological Association, 1996.

specific CCK_1 receptors on vagal afferent nerve terminals in the intestinal mucosa, which results in the activation of vagovagal reflexes to modify gastric, biliary, and pancreatic function. Thus, CCK, long thought of as a classic hormone, actually functions locally as a paracrine effector to stimulate afferent neurons, leading to activation of reflex neural responses.

The physiological release of CCK in response to a meal is modulated by at least two peptides. One is monitor peptide, which is secreted by pancreatic acinar cells. In addition, there is at least one lumenal CCK-releasing factor. The proposed mechanism of action for both of these releasing factors is that they are constitutively secreted into the intestinal lumen. In the absence of protein or protein products in the intestinal lumen, both factors are efficiently broken down by digestive enzymes and thus are not available to stimulate CCK release. On ingestion of a meal, however, dietary proteins compete with the releasing factors as substrates for the digestive enzymes. The net effect is that the releasing factors escape digestion and become available to stimulate CCK secretion from endocrine cells in the mucosa.

The most important function of the pancreas in the integrated response to a meal is the production, storage, and secretion of digestive enzymes along with fluid. The major control of pancreatic secretion is exerted by efferent fibers from the parasympathetic nervous system and by the hormones CCK and secretin. As indicated in the previous sections, parasympathetic activation of pancreatic secretion occurs during the cephalic, oral, gastric, and duodenal phases of the meal. Secretion during the duodenal phase accounts for about 70% of the total response to a meal. In this phase, secretion is activated by the presence of chyme in the duodenum by way of extrinsic neural pathways, enteropancreatic neural pathways (i.e., through intrinsic neurons that terminate in both the duodenum and pancreas), and endocrine pathways (i.e., through CCK and secretin). Of note, research indicates that the effect of CCK on pancreatic secretion is predominantly mediated through neural reflexes similar to those described for the regulation of gastric function.

As the meal enters the intestine, it is acidic and hyperosmotic; however, by the time it leaves the duodenum, it has a neutral pH and is isosmotic. These necessary changes for optimal digestive enzyme activity are achieved by the secretion of large volumes of water and bicarbonate ions by the pancreatic and biliary systems and the intestinal mucosa, as well as by the regulation of gastric motility and secretion as previously described. The alkaline secretions are regulated mainly by the release of secretin from the duodenal mucosa. Acting as an endocrine agent, secretin increases bicarbonate and water secretion from the pancreatic and biliary ductal systems.

Contraction of the gallbladder and relaxation of the sphincter of Oddi result in the addition of biliary secretion to the meal. During the cephalic, oral, and gastric phases, parasympathetic efferent nerves mediate these responses. During the duodenal phase, CCK acting either alone or through a vagal reflex pathway has a pronounced effect on the delivery of bile to the duodenum. Bile is composed of both inorganic and organic constituents. As previously indicated, the inorganic constituents (e.g., water, bicarbonate ions, and other electrolytes) serve to convert gastric chyme into a neutral isosmotic solution. The major organic constituents of bile are conjugated bile acids, phospholipids, cholesterol, and bilirubin; the latter two are excretory products. Both bile acids and phospholipids are essential in maintaining cholesterol in a soluble state and thereby preventing the formation of gallstones. Conjugated bile acids traverse the entire small intestine and are taken up by a receptor-mediated transport system in the terminal ileum. The conjugated bile acids return to the liver by way of the portal circulation and then are secreted back into the biliary system and stored in the gallbladder. This cycling of bile acids is termed the enterohepatic circulation. Bile acids have multiple

important functions in the process of digestion and absorption of lipids and fat-soluble vitamins. They accomplish these functions in part by possessing polar (hydrophilic) and nonpolar (hydrophobic) regions that allow them to interface between aqueous and lipid environments. Thus, bile acids are involved in the formation of emulsions and micelles and in the binding of lipolytic digestive products. All these effects enhance the digestion and the absorption of fat in the meal.

The highly regulated duodenal phase is responsible for the addition of water, ions, enzymes, bile acids, and other secretions to the meal in proportions that result in an optimal environment for the digestion of nutrients. The process of digestion in the intestinal lumen is facilitated further by the induction of patterns of motility in the duodenum that promote the mixing of intestinal contents with digestive enzymes.

During the intervals between meals, motility of the GI tract is characterized by periods of intense contractions and periods of quiescence. In humans, these periods cycle at about 1.5 h. The sequential contractions migrate aborally (i.e., toward the anus) and have been termed the migrating motor complex (MMC). This complex of migrating contractions starts in the stomach and moves through the intestine and into the colon, sweeping undigested material and contents through the GI tract in the interdigestive period. Initiation of the MMC is dependent on the integrity of the vagal innervation, which serves to release the hormone motilin, which in turn activates the MMC. Food in the intestine abolishes the MMC and changes intestinal motility from this fasted pattern to a fed pattern of motility.

Nutrient digestion and absorption systems in the small intestine

The small intestine is divided into three functional regions: duodenum, jejunum, and ileum. The jejunum is the major organ for intralumenal and surface digestion and absorption of nutrients. In addition, a significant amount of water and ions are secreted and absorbed in the jejunum to facilitate digestion and absorption. The ileum provides another site for absorption and has two unique absorptive roles. One is the absorption of conjugated bile acids for reuse after they have traversed the length of the small intestine participating in fat digestion and absorption. The other is the absorption of cobalamin (vitamin B-12) bound to intrinsic factor secreted by the stomach. The ileum also releases hormones that have important functions in the gut. For example, PYY inhibits gastric function and satiety regulation. The ileocecal valve prevents the large reservoir of colonic bacteria from entering the small intestine.

Three characteristics of the small intestine make it especially well adapted for its critical role in nutrient assimilation. First, the small intestine has an enormous surface area as a result of folds in the mucosa, villi on the mucosa, and microvilli on the epithelial cells. Second, the blood flow to the intestine markedly increases with a meal to facilitate the transport of nutrients; it can account for up to 25% of cardiac output during a meal. Third, the enteric nervous system is highly developed in the small intestine, enabling this region of the GI tract to function independently of the extrinsic innervation.

The main responses initiated in the small intestinal phase are alterations in intestinal motility patterns, intestinal secretion, and blood flow, as well as regulation of tight junction permeability. The small intestine exhibits two motor patterns: peristalsis and segmentation. Both of these motility patterns are generated by the enteric nervous system and are independent of the extrinsic nervous system. Peristalsis is characterized by a wave of relaxation followed by a ring of contraction that develops at a point and moves aborally over variable distances. Peristalsis is the primary mechanism by which contents are moved along the intestine in the interdigestive period. Segmentation is characteristic of the fed state and is the process by which rings of contraction develop at uniform intervals, dividing the lumen into segments. Segmentation is the primary mechanism by which the contents of the intestine are mixed with secretions and moved across the mucosa to enhance absorption. Another major effect of segmentation is to slow the transit of the meal in the small intestine to further enhance absorption.

The major changes in the chemical and physical characteristics of food, as well as the absorption of nutrients, trace elements, vitamins, water, and ions, occur in the intestinal phase. Hydrolysis of proteins and carbohydrates and the solubilization and hydrolysis of fats are catalyzed by enzymes in the intestinal lumen (from salivary, gastric, and pancreatic secretions) and on the brush border of the surface of the intestinal mucosa. Examples of lumenal and surface digestive systems are listed in Table 1.4. Absorption is the process by which molecules produced by lumenal surface digestion are transported into epithelial cells and then carried into the blood or lymph. The duodenum and jejunum have the highest absorptive capacities; most absorption occurs in the upper small intestine, although some occurs in the ileum. Molecules

Table 1.4 Digestive enzymes of the lumen and mucosal surface

Intralumenal
Salivary amylase
Gastric pepsin and lipase
Pancreatic amylase, lipase, phospholipase, cholesterol esterase, trypsin, chymotrypsin, elastase, carboxypeptidase A, carboxypeptidase B, DNase, RNase
Mucosal surface
Lactase, sucrase, isomaltase
Amino-oligopeptidase, aminopeptidase A, dipeptidase, dipeptidyl aminopeptidase, carboxypeptidase P, γ-glutamyltransferase, folate conjugase

Table 1.5 Mucosal absorptive transporters

Na^+-glucose/galactose cotransporter (SGLT1), fructose transporter (GLUT5)
Neutral amino acid transporter (NBB system); PHE system for phenylalanine and methionine; IMINO system for imino amino acids, proline and hydroxyproline; basic amino acid transporter, acidic amino acid transporter
Na^+-dependent transporters for folate, thiamine, riboflavin, pantothenic acid, biotin
Na^+-dependent conjugated bile acid transporter in the ileum
Cobalamin–intrinsic factor receptor in the ileum

are absorbed by two principal mechanisms: transcellular (i.e., passing through enterocytes) and paracellular (i.e., passing between enterocytes). Monosaccharides, amino acids, electrolytes, minerals, and water-soluble vitamins enter the portal circulation and pass through the liver into the systemic circulation. Lipids and fat-soluble vitamins and cholesterol esters (together with lipophilic drugs) enter the lymph, which drains into the systemic circulation. Examples of mucosal absorptive systems are listed in Table 1.5.

Colonic phase of a meal

The colonic phase is important for the further reabsorption of water and ions and for the storage and elimination of waste products. The cecum and ascending colon receive about 2 L of ileal effluent daily. Absorptive transport mechanisms reduce the volume to about 200 mL per day. The transverse, descending, and sigmoid portions of the colon store fecal material and transport the material to the rectum. The rectum signals the defecation reflex. In addition, some fermentation of monosaccharides yields free fatty acids that can be taken up by colonic epithelial cells.

The stimuli induced in the colon are both mechanical (e.g., rectal distention) and chemical (e.g., free fatty acids). Extrinsic and intrinsic neural pathways mediate the motility responses of the colon and the defecation response. Hormonal pathways affecting other parts of the GI tract may also be excited in the colon. For example, PYY may be released from the colonic mucosa if the fat content of the meal is high and not all is absorbed by passage through the small intestine. PYY, in turn, slows gastric emptying and transit of the meal through the small intestine, resulting in increased fat digestion and absorption. Further, PYY decreases food intake through its regulatory effect on satiety.

The effectors in the colonic phase are both motor and secretory. The colon exhibits both storage motor patterns (i.e., changes in tone) and propulsive motor patterns (i.e., phasic contractions). Rectal distention initiates the defecation reflex. This response consists of both involuntary and voluntary components. Propulsive motility in the descending colon and rectum is increased to move the feces to the anus, where relaxation of internal and external anal sphincters facilitates elimination.

Regulation of nutrient storage and release: roles of the liver and endocrine pancreas

An essential function of the liver and endocrine pancreas is to coordinate the distribution of vital metabolic substrates to the tissues of the body. It must perform this task during both the fed (i.e., absorptive) state, when nutrients ingested during a meal enter the circulatory system from the gut, and the fasted (i.e., postabsorptive) state, when the gut contains no nutrients (Table 1.6). During the fed state, the body's energy requirements are met by a portion of the ingested nutrients, and any remaining nutrients must be stored for later use. Conversely, during the fasted state, the energy needs of the tissues must be satisfied by metabolic substrates generated from the body's stores, chiefly the liver, the adipose tissue, and the skeletal muscle. The integrated responses of the liver and endocrine pancreas to a meal direct the crucial metabolic

Table 1.6 Major characteristics of the fed and fasted states

Fed state
Nutrients absorbed from the gut
Energy needs met by using ingested nutrients
Insulin released by the pancreas
Net hepatic glucose uptake
Hepatic glycogen synthesis and storage
Hepatic protein and triglyceride synthesis
Muscle glycogen synthesis and storage
Muscle protein synthesis
Adipose triglyceride storage
Glucose is predominant fuel source
Fasted state
No nutrients in the gut
Energy needs met by using stored fuel
Glucagon released by the pancreas
Net hepatic glucose release
Hepatic glycogenolysis and gluconeogenesis
Hepatic protein and fat catabolism
Muscle glycogenolysis
Muscle protein catabolism
Adipocyte fat catabolism
Enhanced fatty acid utilization by most tissues
Long-term fast
Hepatic ketogenesis
Enhanced ketone utilization by the brain and other tissues
Proportional increase in renal gluconeogenesis

transition from the fed to the fasted state and back. These responses are controlled by a complex network of endocrine, paracrine, and neural signals, as well as by the concentration of metabolic substrates in the portal blood that feeds the liver and pancreas. Insulin and glucagon, hormones produced in the islet cells of the pancreas, are the principal regulators of the metabolic state, such that the transition from the fed to the fasted state can be explained by a reduction in the ratio of insulin to glucagon in the blood.

Fed state

Extrinsic nutrients enter the bloodstream from the gut while a meal is digested and absorbed. During this period, termed the fed state, ingested glucose is the body's principal energy source. A portion of this glucose, absorbed directly from the portal system, enters the liver where some of it is used to meet the liver's energy needs, and the remainder is converted to glycogen, which will replete the hepatic stores, and to fatty acids and glycerol for synthesis of and storage as triacylglycerols in the adipose tissue. Thus, a key feature of the fed state is net glucose uptake by the liver. Glucose that reaches the skeletal muscle is catabolized for energy and converted to glycogen to replete muscle energy stores. Adipocytes catabolize glucose, and also use it to form α-glycerol phosphate and fatty acids, which are triacylglycerol precursors. Ingested amino acids are taken up by all of the body's tissues and organs, including the liver, for use in protein synthesis. However, excess amino acids are not stored as protein but are converted by the liver to α-keto acids, which are catabolized or converted to fatty acids for storage as fat. Thus, the net synthesis of protein during the fed state simply replenishes the protein degraded during the fasted state, except in growing children and vigorously exercising adults. The lipid contents of a meal are transported to the circulation by way of the lymphatics in the form of triacylglycerols. Lipoprotein lipases in the endothelium hydrolyze the ingested triacylglycerols into fatty acids and glycerol, which are a source of energy for tissues, and the adipocytes store the excess as fat.

Fasted state

During the fasted state, the absence of nutrient absorption from the gut forces the liver to maintain the concentration of glucose in the blood. This is imperative because, under ordinary circumstances, glucose is the exclusive energy source for the brain and the erythrocytes. The blood glucose concentration is sustained by hepatic glucose production and by a reduction in glucose use by most tissues and organs. An early event in the fasted state is the hydrolysis of glycogen (i.e., glycogenolysis) in the liver into glucose, which is released into the circulation. However, the hepatic stores of glycogen are limited, so that during prolonged fasts the liver must synthesize glucose from a variety of precursors (i.e., gluconeogenesis), including lactate, pyruvate, amino acids, and glycerol. These glucose precursors result from several extrahepatic metabolic processes that are stimulated during a prolonged fast. Glycogen is stored in skeletal muscle, as well as the liver. During a prolonged fast or when a muscle is working, skeletal muscle glycogen is hydrolyzed to form lactate and pyruvate. Lipid hydrolysis (i.e., lipolysis) in adipocytes is stimulated in the fasted state, resulting in the formation of glycerol, as well as fatty acids. As the fasted state proceeds, protein mainly located in skeletal muscle is catabolized to amino acids. These precursors are circulated to the liver where they are converted into glucose to help preserve the concentration of glucose in the blood. Consequently, during the fasted state, the liver is a site of net glucose release.

Gluconeogenesis is capable of supplying less than half of the energy necessary for survival. Therefore, the fasted state is also characterized by a substantial reduction in glucose utilization by most tissues and organs of the body. Glucose generated by the liver is thus spared for use by the CNS. Glucose use is reduced by an increase in fat utilization by other tissues. As previously discussed, the enhanced lipolysis associated with the fasted state produces fatty acids in addition to the glucose precursor, glycerol. These fatty acids are released to the systemic circulation where they are used as an energy source by nearly all tissues except, most notably, the CNS.

During long-term fasts (i.e., longer than 24–48 h), further metabolic changes occur that facilitate survival. The kidneys, which also are capable of performing gluconeogenesis, take on an increasingly prominent role in glucose production. In addition, the liver converts acetyl-CoA, a product of fatty acid catabolism, into ketones. These ketones are used as an energy source by many tissues, including the brain. A distinct advantage of shifting the fuel source from glucose to ketones is that muscle is spared as a source of amino acids for gluconeogenesis.

Regulation of the metabolic state

During the fed state, serum glucose in particular, but also amino acids and other sugars in the blood, absorbed from a meal stimulate the secretion of insulin from the β-cells of the pancreatic islets (Table 1.7). In addition to the metabolic substrates absorbed during a meal, gastrointestinal hormones released from duodenal cells facilitate insulin release. These hormones, called incretins, include GLP-1 and glucoinsulinotropic peptide. In addition, vagal neural pathways and β-adrenergic stimuli regulate insulin release. In contrast, somatostatin and α-adrenergic stimuli inhibit insulin release. Insulin affects mainly the metabolism of the liver, adipose tissue, and muscle, which are the primary sites of fuel storage and release. The hepatic response to insulin is to promote net glucose uptake, glycogen synthesis and storage, and protein and triglyceride synthesis. In adipocytes, insulin stimulates the uptake of glucose, triglyceride synthesis from glucose,

Table 1.7 Regulation and function of key hormonal and paracrine regulators of nutrient storage and release

Peptide	Source	Stimuli and facilitators	Inhibitors	Functions
Insulin	Pancreatic β-cells	Glucose Other sugars (e.g., mannose) Amino acids (e.g., leucine, arginine) GI hormones (e.g., glucagon-like peptides, cholecystokinin, secretin) Vagal input β-Adrenergic signals	α-Adrenergic signals Somatostatin (paracrine)	**Liver** ↑ Glycogen synthesis and storage ↓ Glycogenolysis ↑ Protein and triglyceride synthesis ↑ Very low-density lipoprotein production ↓ Gluconeogenesis ↓ Ketogenesis **Muscle** ↑ Protein synthesis ↑ Glycogen synthesis and storage **Adipose tissue** ↑ Triglyceride storage ↓ Lipolysis
Glucagon	Pancreatic α-cells	↓ Glucose Amino acids (e.g., arginine, alanine)	Glucose Insulin Somatostatin Fatty acids	**Hepatic** ↑ Glycogenolysis ↑ Gluconeogenesis ↑ Ketogenesis
Somatostatin	Pancreatic δ-cells	Glucose Amino acids (e.g., arginine) GI hormones (e.g., glucagon-like peptides, cholecystokinin, secretin)		**Pancreatic** ↓ Glucagon release ↓ Insulin release ↓ Secretion by the exocrine pancreas **Gastrointestinal** ↓ Gastric emptying ↓ Gastric acid and gastrin production ↓ Splanchnic blood flow
Glucagon-like peptide-1	Duodenal L-cells	Glucose Lipids		**Pancreatic** ↑ Insulin release **Gastrointestinal** ↓ Gastric emptying

and net triglyceride storage. The effect of insulin on muscle is to stimulate net glucose and amino acid uptake and use, glycogen synthesis and storage, and protein synthesis.

During the fasted state when there are no nutrients being absorbed from the gut, glucagon secretion is highest. This observation results from the fact that glucagon release is mainly stimulated by hypoglycemia, although certain amino acids, epinephrine (adrenaline), and sympathetic neural activity also stimulate its secretion. Conversely, glucose, insulin, somatostatin, and certain fatty acids inhibit secretion of this peptide. Glucagon, which acts primarily on the liver, counters the effects of insulin by stimulating glycogenolysis, gluconeogenesis, and ketogenesis.

Aside from regulating the secretion of insulin and glucagon, catecholamines and the autonomic nervous system stimulate glycogenolysis by the liver and muscle, gluconeogenesis by the liver, and lipolysis by adipocytes. Hypoglycemia leads to reflex epinephrine secretion and sympathetic neural activity. Although other hormones, such as cortisol and growth hormone, modulate hepatic metabolism, they do not play an important role in the liver's response to a meal.

In addition to the roles that blood glucose and amino acid concentrations play in the hormonal regulation of the metabolic state, the concentration of glucose in the blood of the sinusoids directly modulates hepatic metabolism. As a result of the activity of certain glucose concentration-dependent enzymes in the liver, elevation of the sinusoidal blood glucose concentration stimulates glycogen synthesis and inhibits glycogenolysis. Together, hormones, the autonomic nervous system, and the concentration of metabolic substrates in blood precisely modulate the hepatic response to a meal.

A COMPONENTS

2 The enteric nervous system and its extrinsic connections

John B. Furness, Trung V. Nguyen, Kulmira Nurgali, Yasutake Shimizu

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The proper functioning of the digestive tract is absolutely dependent on the enteric nervous system. If this system of neurons is missing or depleted, then humans or other mammals die or suffer deficiencies in digestive function, depending on the extent of the loss of enteric neurons. An extreme manifestation of enteric neuron loss is Hirschsprung disease, which involves congenital absence of enteric neurons (aganglionosis) in all, or part, of the large intestine. Patients with Hirschsprung disease die if the aganglionic segment is not removed; conversely, removal of the aganglionic segment and the presence of intact enteric nervous system throughout the remaining bowel restores good health [1]. Partial degeneration of the enteric nervous system during life leads to severe dysmotility. Such degeneration occurs, for example, when the enteric nervous system is targeted by the infective organism of Chagas disease, *Trypanosoma cruzi* [2].

The enteric nervous system circuits have essential roles in the control of motility, blood flow, water and electrolyte transport, and acid secretion in the digestive tract, and the enteric nervous system is most commonly the medium through which extrinsic neurons control gastrointestinal function [3]. The enteric nervous system is capable of functioning independently of central control, although the central nervous system normally modifies its activity.

Structural organization of the enteric nervous system

Nerve cell bodies, their processes, and enteric glial cells embedded in the wall of the gut form the enteric nervous system (Fig. 2.1). The number of enteric neurons in a human is estimated to be 200–600 million, which is about the same number of nerve cells as in the spinal cord [3]. The nerve cell bodies are grouped in small aggregates, the enteric ganglia, which are connected by bundles of nerve cell processes to form two major ganglionated plexuses in the tubular digestive tract: the myenteric plexus, also called the *Auerbach plexus*, and the submucosal plexus, often referred to as the *Meissner plexus*.

Locations of the enteric ganglia

The myenteric plexus of ganglia and connecting nerve strands lies between the longitudinal and circular layers of the muscularis externa and forms a continuous network around the circumference of the tubular digestive tract from the upper esophagus to the internal anal sphincter. In the parts of the large intestine where the longitudinal muscle is gathered into taeniae, the myenteric plexus is prominent underneath the taeniae and is sparser over the rest of the colonic surface.

Ganglia are numerous only in the submucosal plexus of the small and large intestines; extensive networks of linked ganglia are absent from the submucosa of the esophagus and stomach, although isolated ganglia may be encountered in these regions.

Ganglia are occasionally found in the mucosa, specifically in the connective tissue close to the muscularis mucosae. Small ganglia are also found along the extrinsic nerves (i.e., vagus, pelvic, and mesenteric nerves) as they enter the gut. Ganglia associated with the extrinsic nerves located on the surface of the gut, particularly in the stomach and rectum, are referred to as subserosal ganglia. Enteric ganglia are also present in the gallbladder, biliary ducts, and pancreas.

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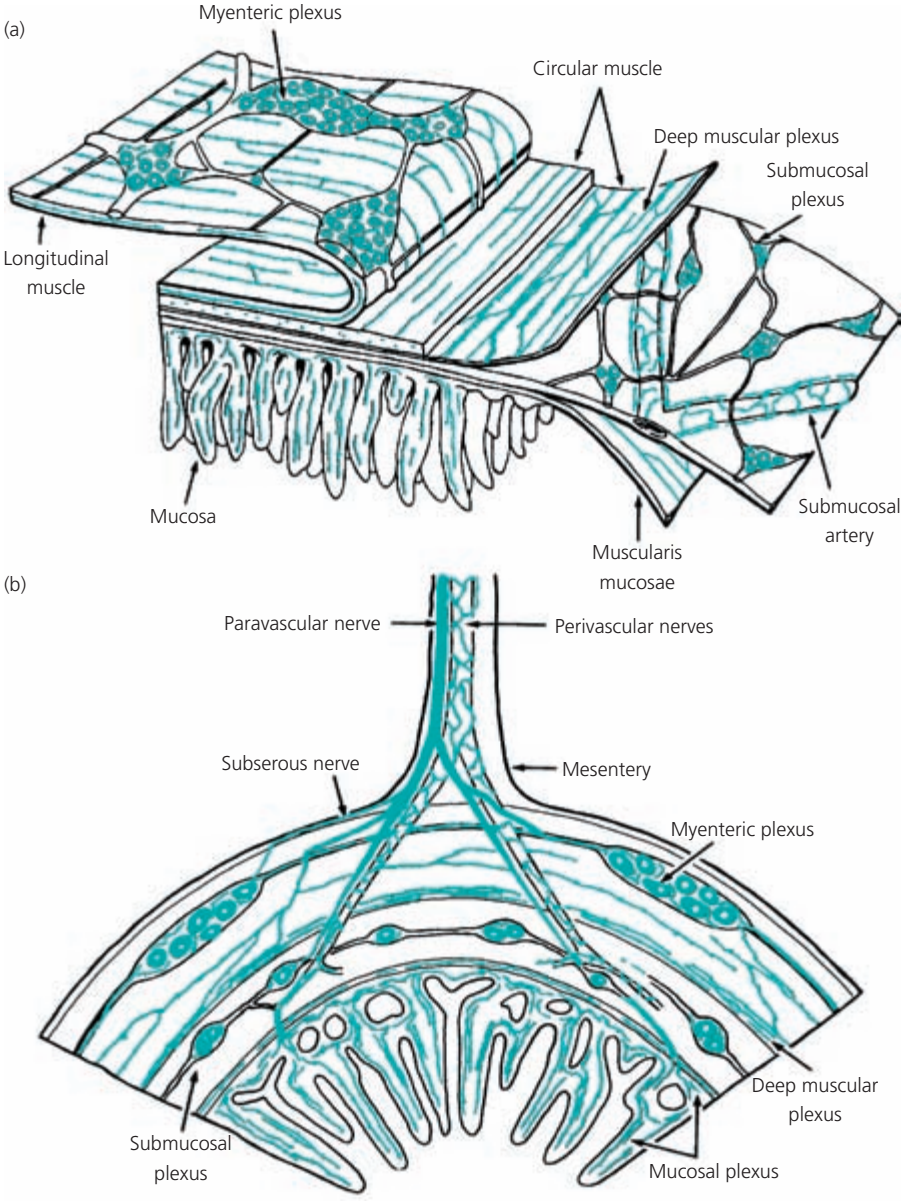

Figure 2.1 The enteric plexuses as seen **(a)** in whole mounts and **(b)** in transverse section of the small intestine. There are two ganglionated plexuses, the myenteric and the submucosal, in addition to plexuses of nerve fibers in the muscle and the mucosa and around the arterioles. Adapted from Furness and Costa [33], with permission.

Nonganglionated plexuses

The enteric neurons provide axons that form a series of nonganglionated plexuses that supply effector tissues of the tubular digestive tract: the longitudinal muscle plexus, the circular muscle plexus (and its subdivisions), the plexus of the muscularis mucosae, the mucosal plexus, and the perivascular plexuses. The exception is the striated component of the external musculature of the esophagus, which is found in the upper third of the human esophagus and in a greater proportion of the esophagus in most other species. The striated muscle cells are innervated at motor endplates, not by a nerve plexus.

The pattern of innervation of the longitudinal muscle differs according to its bulk. In humans, and in other species in which the longitudinal muscle is a thick layer throughout the intestine, the nerve fiber bundles run parallel to the muscle and comprise the longitudinal muscle plexus. In some species, such as the rabbit, guinea pig, and mouse, the thickness of the longitudinal muscle of the small intestine is less than about 10 muscle cells. In these species, the nerve fiber bundles do not form a plexus within the longitudinal muscle, but rather a tertiary plexus, a component of the myenteric plexus, that lies against the inner surface of the longitudinal muscle (Fig. 2.2) [4,5]. The processes of individual tertiary plexus neurons ramify extensively on the inner surface of the longitudinal muscle [6].

The circular muscle plexus is formed by parallel bundles of nerve fibers throughout the thickness of this muscle layer. In

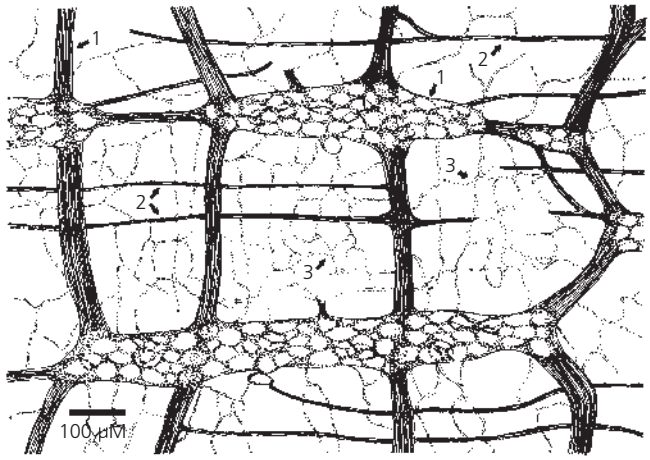

Figure 2.2 The three components of the myenteric plexus in small animals are shown in a drawing of a whole mount of guinea pig small intestine. Common to all species are the primary component of the plexus (1), consisting of the ganglia and internodal strands (interganglionic connectives), and the secondary component (2), consisting of nerve strands lying parallel to the circular muscle. The tertiary plexus (3) is found only where the longitudinal muscle is thin; in such regions, little or no longitudinal muscle plexus is found within the longitudinal layer. Adapted from Furness and Costa [16], with permission.

some regions of the gastrointestinal tract, a dense layer of nerve fiber bundles provides additional innervation of the inner part of the circular muscle. The presence of this dense layer and its position in relation to the circular muscle differs among regions [3]. In the mammalian small intestine, the circular muscle consists of a thick outer layer and a thin inner layer of muscle cells [7,8]. The dense plexus of nerve fibers located between these two muscle layers in the small intestine is called the deep muscular plexus [9]. The colon of most species lacks an inner layer of specialized circular muscle; a dense layer of nerve fibers, the submuscular plexus, similar to the deep muscular plexus, lies close against the inner surface of the circular muscle, adjacent to connective tissue of the submucosa [9–11]. The circular muscle plexus continues into the smooth muscle sphincters of the digestive tract without any apparent change in form. The myenteric plexus also continues into the sphincter regions.

The muscularis mucosae throughout the digestive tube consists of outer longitudinal and inner circular layers of smooth muscle innervated by nerve fibers running parallel to the muscle bundles. Muscle bundles extend into the cores of the small intestinal villi, and similar strands of muscle are found between the gastric glands [12]. In the small intestine of small animals, such as mice and rats, the muscularis mucosae is thin and barely discernible in histological sections.

The mucosal plexus is a network of fine nerve fiber bundles that lies beneath the mucosal epithelium. Sparse in the esophagus, the mucosal plexus is prominent in the stomach, small intestine, colon, and gallbladder. The mucosal plexus in the small intestine is sometimes described as having subglandular, periglandular, and villous components. These components are continuous with one another, despite being innervated by slightly different nerve fiber populations.

Perivascular plexuses are found around the arterioles within the gut wall. The plexuses have mixed origins, from enteric neurons, extrinsic sympathetic neurons, and spinal afferent neurons. Innervation of veins within the gut wall is sparse or nonexistent, and lymphatic vessels in the gut wall appear to lack innervation. The lymphatic tissue within the gut wall, notably the Peyer patches, is innervated from intrinsic and extrinsic sources [13].

Myenteric plexus

The myenteric plexus consists of small aggregations of neurons, the myenteric ganglia, nerve fiber bundles that connect the ganglia, and nerve bundles that connect the plexus with other structures (Fig. 2.3). The ganglia are flattened in the plane of the plexus, and usually have a thickness of one to four nerve cells, depending on the state of contraction and the size of the intestine [14]. In any region of the gastrointestinal tract, ganglion size varies widely. In the guinea pig ileum, ganglia range in size from a single cell to about 200 nerve cell bodies.

The pattern of ganglia, determined by shape and orientation, differs among regions and species but is often readily identifiable as belonging to a particular part of the intestine [15,16]. Small bands of nerve fibers known as internodal strands or interganglionic connectives connect the ganglia (see Figs 2.2 and 2.3). The meshwork formed by the ganglia and the internodal strands is called the primary component of the myenteric plexus. Nerve strands that connect with the primary plexus and run circumferentially constitute the secondary component of the plexus (see Figs 2.2 and 2.3) [4,17]. Branches from these secondary strands innervate the circular muscle [18]. Nerve fiber bundles (called penetrating fiber bundles or vertical connections) also run through the circular muscle to connect the myenteric plexus with the submucosal and mucosal plexuses [19].

The major targets for nerve cells of the myenteric plexus are the muscularis externa (most of its innervation is from this source), the submucosal ganglia, and other myenteric nerve cells. Intrinsic primary afferent neurons (IPANs) with cell bodies in the myenteric plexus, and a small number of secretomotor neurons in the myenteric ganglia, also innervate the mucosa. The intestinofugal neurons comprise a minor population of myenteric neurons that project to sympathetic prevertebral ganglia.

Submucosal plexus

A continuous network of numerous small ganglia is found in the submucosa throughout the small and large intestine. In many species, plexuses of ganglia are located in inner and outer layers, but some regions in certain species of small

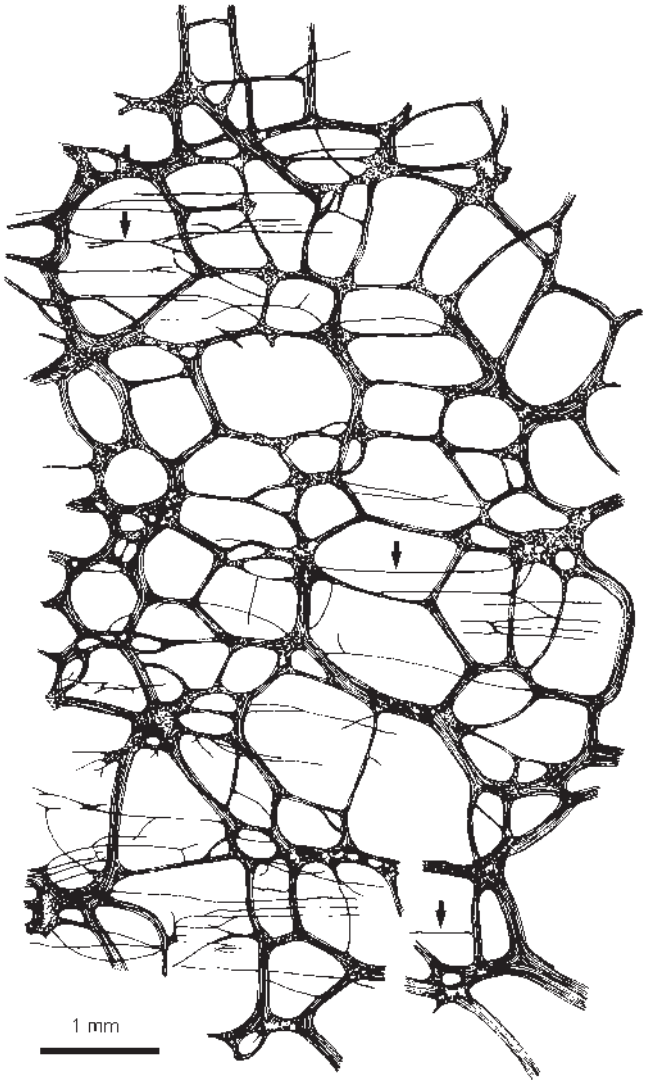

Figure 2.3 Drawing of a whole mount of the myenteric plexus of the human small intestine prepared by Auerbach and published in Henle's Textbook of Histology in 1871. Myenteric ganglia, internodal strands, and small nerve trunks of the secondary component of the myenteric plexus (arrows) can be seen.

animals have only a single layer of submucosal ganglia [3,20]. The inner and outer plexuses contain different populations of neurons, defined by their morphological and histochemical characteristics [21]. The electrophysiological properties of the nerve cell populations in the two plexuses also differ [22]. It is assumed that the inner plexus (closer to the mucosa) is mainly concerned with the control of fluid movement, and the outer plexus contributes to control of motility of the external muscle. Submucosal ganglia are smaller and less regularly arranged than myenteric ganglia.

The submucosal plexus harbors the cell bodies of neurons with dual secretomotor/vasodilator function, as well as the cell bodies of neurons that are solely secretomotor [3]. Some neurons, notably submucosal IPANs, project from the submucosa to the myenteric plexus. Other submucosal neurons innervate the muscularis mucosae. The inner circular muscle in some species, including human, is innervated in part by submucosal neurons [11,23,24]. In the stomach, which almost entirely lacks submucosal ganglia, the intrinsic innervation of the mucosa and muscularis mucosae comes from the myenteric plexus.

Ganglia of the gallbladder, biliary ducts, and pancreas

The biliary system and the pancreas develop from diverticula of the small intestine, and the ganglia in their walls are part of the enteric nervous system. Plexuses of ganglia, similar to the enteric plexuses of the small intestine but differing between species in their relations to tissue layers, are found in the gallbladder, cystic duct, and common bile duct [3]. Numerous nerve fibers occur in the muscle, around blood vessels, and in the mucosa of the biliary tract. Ganglia, connected to each other by small nerve trunks, are scattered through the pancreas, forming a three-dimensional plexus in this solid organ. Nerve fibers are found around the acini and the blood vessels and in the islets. The intrinsic neurons of the gallbladder control motility and the flux of water and electrolytes. In the pancreas, intrinsic neurons appear to be involved in the control of both endocrine and exocrine components, although the most complete data exist for exocrine control [25]. Nerve fibers connect the plexuses of the biliary system and the pancreas with the ganglionated plexuses of the upper small intestine [26–28]. These connections are presumed to contribute to coordination between these organs and the tubular gastrointestinal tract.

Microscopic structure of the enteric nervous system

Shapes of neurons

The nerve cells of the enteric ganglia can be classified into subgroups according to their shapes [3,19]. The first effective classification was by Dogiel [29], who proposed that the shapes of nerve cells are related to their functions. He defined three cell shapes: types I, II, and III. The first two are readily recognized in different species and with various staining techniques; the third type is less well defined, but bears some resemblance to a group later referred to as filamentous neurons. Type I neurons are generally flattened in the plane of the ganglia; they have oval cell bodies, prominent flattened (lamellar) dendrites, and a single long axon often characterized by spiny protuberances close to the cell body. Muscle motor neurons and many interneurons are type I neurons. Type II neurons have a spheroidal shape and give rise to several axons, usually three to ten, although some type II neurons are pseudounipolar [19,30]. A few type II neurons

have tapering dendrites in addition to several long axon-like processes and are referred to as dendritic type II cells [31]. Intrinsic primary afferent neurons have Dogiel type II morphology.

A variety of other shapes have been described, notably in the pig and guinea pig intestines, which have been studied intensively, and in human [19]. Stach [31] extended Dogiel's classification to include types IV, V, and VI and mini-neurons, based primarily on work in the pig [19]. Type IV neurons are secretomotor neurons in the guinea pig and probably in the pig. In the guinea pig small intestine, filamentous and small simple neurons (the latter being similar to the mini-neurons in the pig) have been described [30].

Numbers of neurons

The numbers of nerve cells in the myenteric and submucosal plexuses have been estimated by counting the nerve cell bodies per unit area of gut surface [16,32]. The counts indicate that the number of nerve cells in the enteric nervous system is about the same as the number of nerve cells in the spinal cord [33]. The accuracy of counts of neurons depends on the methods used and many published counts are probably underestimates [34]. Myenteric nerve cells are numerous throughout the digestive tube, varying in density from about 1000 to about 15 000 cell bodies/cm^2. The esophagus and proximal stomach have lower densities, compared with high densities in the distal stomach and large intestine, and intermediate densities in the small intestine. In total, the enteric nervous system of an individual mammal contains 2×10^6–10^9 nerve cells, depending on the size of the mature animal [16,32,34]. The total number of nerve cells in the myenteric plexus of the sheep small intestine has been estimated to be 31.5 million, in the guinea pig 2.75 million, and in the mouse 403 000 [32]. Estimates of total numbers in the submucosa of the small intestine are 50 million in sheep, 950 000 in guinea pig, and 330 000 in mouse. The estimated total number of enteric neurons in the human gastrointestinal tract is 200–600 million [3].

Ultrastructure

The enteric nervous system consists of compact arrangements of nerve cell bodies, nerve fiber terminals, bundles of nerve fibers, and neuroglia. Unlike other autonomic ganglia, the enteric ganglia do not contain blood vessels or connective tissue cells, although septa of connective tissue sometimes separate parts of the ganglia in large species, including human. Nutrients are supplied to the ganglia through the surrounding interstitial fluid. Similarly, hormones and drugs that act on the enteric ganglia diffuse from the interstitial fluid. It may be significant for the actions of hormones that the cell bodies of many enteric neurons present large surface areas, devoid of neuroglia, to the extraganglionic space [3].

Nerve fibers in the enteric plexuses are of two types: fibers of relatively uniform diameter, about 0.2–0.5 μm; and fibers that consist of varicosities, usually about 1–2 μm in diameter, connected by short intervening segments that measure about 0.2–0.5 μm. The nonvaricose axonal segments are regarded as nonterminal regions that do not release transmitter, whereas the varicosities contain numerous transmitter vesicles and are considered to be points of transmitter release. The varicose segments of enteric axons, including those within ganglionated plexuses and those associated with muscle or mucosal epithelium, are often hundreds of micrometers or several millimeters long. This means that a single axon can contact many nerve cell bodies or other effector cells. The close contacts with nerve cells and their dendrites are of two types: those that have synaptic specializations, consisting of presynaptic accumulations of vesicles and postsynaptic densities; and those in which varicosities containing numerous synaptic vesicles come close to the target neuron, with no intervening glial cell and an absence of postsynaptic density. The latter nonspecialized association is the most common. Most enteric neurons receive both specialized and nonspecialized contacts [35–37].

Nerve fibers that innervate muscle and epithelium are arranged in bundles in which the individual fibers are partly surrounded by enteric neuroglia [38]. Although there are no obvious postjunctional specializations in which the varicosities come close to muscle cells, such varicosities may make close contacts with muscle cells that are associated with prejunctional specializations, particularly junctional clumping of vesicles [39].

Histochemical profiles and transmitter multiplicity of enteric neurons

The enteric nervous system contains more than 30 potential neurotransmitters that affect the activity of neurons, muscle, and epithelial cells [3,40]. Moreover, individual neurons contain several possible neurotransmitters in addition to other neuron-specific proteins (Table 2.1). The combinations of chemical attributes, which are related to the functions of the neurons and their places in nerve circuits, provide a chemical code that identifies neurons. In general, more than one substance contributes to the transmission process, which is therefore described as plurichemical [41].

Colocalized substances do not have equal status as transmitters. One or more substances have the major role(s) in transmission; these are the primary transmitters. For example, sympathetic vasoconstrictor neurons in many species contain norepinephrine (noradrenaline) and neuropeptide Y (NPY). The primary transmitter is norepinephrine; NPY has a subsidiary or modifying role. The relative contributions of different transmitters can vary between neurons with common functions, depending on the location and the activity of the neurons; the inhibitory muscle motor neurons of the gut are a good example (see section on motor neurons). Some

Table 2.1 Major types of neurons in the enteric nervous system and some of their defining characteristics. The numbers in parentheses identify the anatomical depictions of the neurons in Fig. 2.6

Functional definition	Primary transmitters	Comments
Excitatory circular muscle motor neurons (6)	ACh, cotransmitter TK ACh receptors: muscarinic TK receptors: NK_1, NK_2	Excitatory neurons supply all smooth muscle regions, including sphincters. Most innervate muscle close to cell bodies; some run a short distance orally to supply muscle
Inhibitory circular muscle motor neurons (7)	NO plus several cotransmitters with varying prominence: ATP, VIP, PACAP	Inhibitory neurons supply all smooth muscle regions, including sphincters. Most innervate muscle close to cell bodies; some run anally to supply muscle
Excitatory longitudinal muscle motor neurons (4)	ACh, cotransmitter TK	Supply muscle adjacent to cell bodies
Inhibitory longitudinal muscle motor neurons (5)	NO plus several cotransmitters with varying prominence: ATP, VIP, PACAP	Supply muscle adjacent to cell bodies
Excitatory neurons to the muscularis mucosae	ACh, cotransmitter TK	Similar properties to other excitatory muscle motor neurons (see above)
Inhibitory neurons to the muscularis mucosae	NO plus several cotransmitters: ATP, VIP, PACAP	Similar properties to other inhibitory muscle motor neurons (see above)
Myenteric ascending interneurons (1)	ACh, nicotinic receptors postsynaptic	In the small intestine, only one type. Involved in local peristaltic reflex
Myenteric descending interneurons (local reflex) (8)	ACh, ATP may be a cotransmitter	These neurons also contain NOS
Myenteric descending interneurons (secretomotor and motility reflexes) (9)	ACh, 5-HT (at 5-HT_3 receptors)	This type of descending interneuron has been documented in several species. In guinea pig, it is in a pathway of descending excitation
Myenteric descending interneurons (MMC) (10)	ACh	Neurons contain somatostatin. These neurons may be involved in conducting the MMC along the intestine
Myenteric intrinsic primary afferent neurons (IPANs) (2)	TK, CGRP, and ACh (see text)	These neurons detect intralumenal chemistry and distortion of their processes
Submucosal IPANs (11)	TK, CGRP, and ACh (see text)	These neurons respond to intralumenal chemistry and mucosal distortion
Intestinofugal neurons (3)	ACh, cotransmitter VIP	These neurons directly or indirectly detect conditions in the gut wall and are in the afferent pathways of enteroenteric reflexes
Motor neurons to gut endocrine cells	Various. Neurons innervating G cells use GRP	The innervation of the different endocrine cell types has not been well described
Noncholinergic secretomotor/vasodilator neurons (12)	VIP. Other peptides of the VIP family may contribute	Neurons project both to the mucosa and to arterioles. Most of these neurons are in submucosal ganglia
Cholinergic secretomotor/vasodilator neurons (13)	ACh	Dual projection, to the mucosa and to arterioles. Innervate bases of glands. Most of these neurons are in submucosal ganglia
Cholinergic secretomotor (nonvasodilator) neurons (14)	ACh	Innervate mucosal epithelium only. Most of these neurons are in submucosal ganglia
Submucosal uniaxonal neurons projecting to the myenteric plexus (15)	ACh (deduced). Contain VIP (NOS?)	Possibly displaced myenteric interneurons

ACh, acetylcholine; ATP, adenosine triphosphate; CGRP, calcitonin gene-related peptide; GRP, gastrin-releasing peptide; 5-HT, 5-hydroxytryptamine; MMC, migrating myoelectric complex; NK, neurokinin (receptor); NO, nitric oxide; NOS, nitric oxide synthase; PACAP, pituitary adenylyl cyclase-activating peptide; TK, tachykinin; VIP, vasoactive intestinal peptide.

neurons contain more than one primary transmitter and several subsidiary transmitters or neuromodulators; some classes of enteric neurons have five or more transmitters or neuromodulators.

A broad range of experiments suggests a constancy of primary transmitters between species; many of the observed species differences in neuronal chemistry are probably differences in subsidiary transmitters or neuromodulators.

Although beyond the scope of this chapter, information about all the substances that may participate in enteric neurotransmission in different species is provided in published reviews [3,40,42,43].

The immunohistochemical localization of specific combinations of compounds in neurons provides a valuable investigative tool that allows the projections of individual neurons to be determined and their functions to be deduced, even if the roles of the detected chemicals are not known. For example, the inhibitory and excitatory muscle motor neurons in the gastrointestinal tract can be localized by immunoreactivity to vasoactive intestinal peptide (VIP) and substance P, respectively.

Physiological characteristics of enteric neurons

Electrophysiological properties of enteric neurons

The electrophysiological classification of enteric neurons is confounded because the properties of the neurons differ between regions of the digestive tract and, even in a specific region, properties can vary depending on the circumstances of the experiment [3,44,45]. Classification is most useful when the electrophysiological type can be correlated with the physiological function of the neuron and with its shape and histochemical profile.

In the small intestine of the guinea pig, most neurons can be separated into two groups, AH and S, according to their electrophysiological properties (Fig. 2.4). These terms were introduced in 1974 by Hirst and colleagues [46] and the classification remains useful, although the reasons for calling them AH and S are no longer well justified.

The S neurons exhibit brief action potentials that are followed by short-duration afterhyperpolarizing potentials (AHPs) lasting 20–100 ms. The action potentials are blocked by tetrodotoxin. S neurons exhibit fast excitatory postsynaptic potentials (EPSPs), and compound fast EPSPs of sufficient amplitude to generate action potentials can be evoked in all S neurons. These EPSPs are referred to as compound because they are the summed effects of transmission from several axons. Slow EPSPs are also recorded from S neurons. S neurons have a variety of shapes, which include the Dogiel type I and filamentous morphologies, and have a single axon.

Intensive study of the currents and ion channels of AH neurons in the guinea pig have has helped to explain how the excitability and firing patterns of these neurons are controlled [47]. Action potentials of the AH neurons in the guinea pig small intestine have greater amplitudes and longer durations than those of the S neurons, and they have an inflection (hump) on the falling phase. They are normally followed by two separate phases of hyperpolarization (see Fig. 2.4). An early AHP lasts 20–100 ms and is followed by a second hyperpolarization, the late AHP, which lasts 4–25 s. The late AHP is the result of the opening of IK_{Ca} channels, which are Ca^{2+}-sensitive K^+ channels [48–51]. A hyperpolarization-activated cation current contributes to the repolarization of the late AHP [47,52]. The action potentials in AH neurons are carried by a combination of Na^+ and Ca^{2+} [46,53]. The Ca^{2+} that enters during the action potential triggers the release of Ca^{2+} from intracellular stores; it is this Ca^{2+} that opens the K^+ channels necessary to generate the slow AHP [54]. Many transmitters and hormones that excite enteric neurons block the AHP [44,47]. All AH neurons in the guinea pig small intestine have Dogiel type II morphology. AH neurons in the guinea pig ileum usually do not exhibit fast EPSPs, and when recorded the EPSPs have small amplitudes [45,46,55]. In contrast, slow EPSPs do occur in AH neurons and can trigger action potentials.

The S and AH nomenclature does not apply, or is less useful, for some other gut regions. For example, in the guinea pig rectum, some neurons that exhibit a prolonged AHP have a single axon and receive fast EPSPs [56]. In the gastric corpus, all myenteric neurons have S characteristics and do not

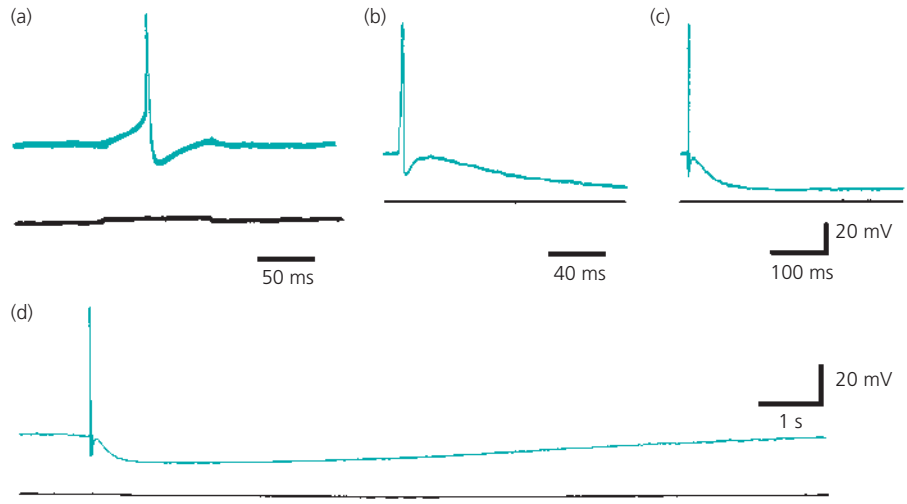

Figure 2.4 Action potentials elicited in an S neuron **(a)** and in AH neurons **(b–d)** by intracellular current pulses. The action potential in the S neuron is followed by a brief period of hyperpolarization, whereas the action potential in an AH neuron is followed by two phases of hyperpolarization (early and late afterhyperpolarizing potentials), one brief **(b)** and the other prolonged **(c, d)**. Note the differences in the time scales. From Hirst et al. [46], with permission.

exhibit a prolonged hyperpolarization after the action potential [57,58].

Synaptic transmission in the enteric nervous system

Enteric neurons receive fast and slow EPSPs and inhibitory postsynaptic potentials (IPSPs). In addition, transmitter release from terminal axons may be reduced by presynaptic inhibition.

Fast EPSPs are graded in amplitude in relation to the strength of electrical stimulation, and when they are evoked reflexly, individual EPSPs of different amplitudes are observed (see below). Thus, enteric neurons receive multiple fast synaptic inputs. The fast EPSPs, which can be elicited by single pulses, last about 15–30 ms. They are associated with an increased membrane conductance, which is attributed primarily to Na^+. Several transmitters contribute to fast EPSPs in S neurons (Fig. 2.5). The use of nicotinic receptor blockers, and other experimental data, show that acetylcholine (ACh) is the most prominent transmitter of fast EPSPs [42]. Some fast EPSPs are in fact completely blocked by nicotinic receptor antagonists, whereas others are reduced in amplitude, some by as little as 20% (see Fig. 2.5) [59–61]. In about 60%–80% of neurons with a nicotinic antagonist-resistant fast EPSP, the residual EPSP is reduced in amplitude or abolished by PPADS, an antagonist of P_{2X} receptors [60,61]. This indicates that purinergic (i.e., ATP-mediated) transmission underlies some fast EPSPs. In about 10% of neurons, a 5-HT_3 receptor antagonist blocks or reduces fast EPSPs that remain in the presence of hexamethonium [61,62]. At some synapses, ATP and 5-HT are probably cotransmitters with ACh. Antagonists of receptors for these three transmitters, in combination, do not fully block fast EPSPs in some S neurons, which implies the existence of one or more other transmitters of fast EPSPs.

In the guinea pig small intestine, slow EPSPs usually last 15–120 s and occur in at least 75% of S neurons and in probably all AH neurons [63,64]. The slow EPSPs are evoked by short trains of stimuli, usually applied at 5–20 Hz for 1–2 s. Slow EPSPs evoked by electrical stimulation are likely to be the result of the superimposed actions of several different transmitters, each producing superficially similar synaptic potentials [63,65]. Simultaneous recording from an AH neuron and from an S or another AH neuron in the same ganglion has shown that AH neurons provide slow excitatory synaptic inputs to both S and AH neurons [66]. Other experiments indicate that AH neurons activated by sensory stimuli cause slow EPSPs in AH and S neurons [64,67]. No direct evidence suggests that S neurons are a source of slow EPSPs, although adequate research to exclude this possibility has not been performed.

When connecting strands are stimulated close to the recorded AH neuron, the main source of input is probably other AH neurons. Although several substances found within enteric nerve terminals mimic slow EPSPs, including 5-HT, substance P, VIP, and somatostatin, most AH neurons have tachykinin immunoreactivity but they never contain 5-HT, somatostatin, or VIP [40]. Slow EPSPs in IPANs are mimicked by the NK_3 tachykinin receptor agonist senktide [68] and are partially blocked by the NK_3 receptor antagonist SR142801, by the NK_1 antagonist SR140333, or by a combination of NK_1 and NK_3 receptor antagonists [69,70]. Stimulation of descending pathways that impinge on IPANs elicits slow EPSPs that can be antagonized by a 5-HT_7 receptor antagonist, although this compound does not reduce the tachykinin-mediated transmission elicited by stimulation of local inputs [65]. Thus, transmission from local connections is through tachykinins, whereas long descending inputs use 5-HT.

Slow EPSPs have been recorded in S neurons in the myenteric and submucosal ganglia. Slow EPSPs in inhibitory motor neurons are blocked by NK_1 receptor antagonists [71]. However, slow transmission to descending interneurons is not affected by NK_1 or NK_3 receptor block.

A long-lasting form of slow excitatory transmission, termed *sustained slow postsynaptic excitation*, has been described in myenteric AH neurons [72]. Activation of presynaptic axons supplying AH neurons for extended periods (1–30 min) at

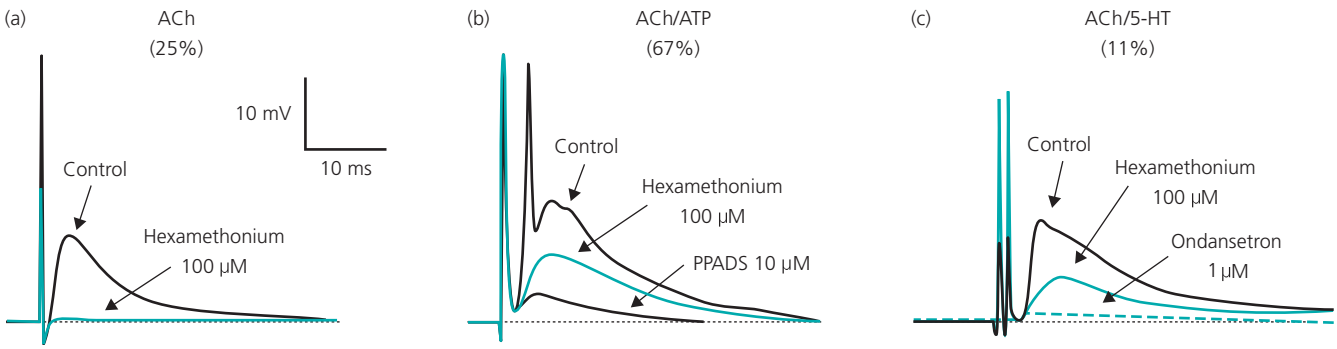

Figure 2.5 Pharmacological dissection of fast excitatory postsynaptic potentials (EPSPs) in S neurons of the myenteric plexus of the guinea pig ileum. **(a)** A fully cholinergic EPSP is blocked by the nicotinic receptor blocker hexamethonium. **(b)** A fast EPSP is partly reduced by hexamethonium. The remaining component is largely blocked by the purinergic P_{2X} receptor antagonist PPADS. **(c)** A fast EPSP in which the response remaining after the administration of hexamethonium is blocked by the 5-hydroxytryptamine $(5\text{-HT})_3$ receptor blocker ondansetron. ACh, acetylcholine. From Galligan et al. [42], with permission.

low frequency (1 Hz) gives rise to a sustained increase in the excitability of the neurons that develops over several minutes and is associated with depolarization, increased input resistance, and suppression of the late AHP. This state of excitation declines more slowly. Successive stimulus trains (duration > 4 min) elicit successively greater increases in excitability. Increased excitability can last up to 3.5 h after a stimulus period of 30 min.

Inhibitory postsynaptic potentials in myenteric neurons have been measured only in a small proportion of neurons in the guinea pig small intestine [73,74]. It is possible that electrical stimulation of internodal strands with one or more pulses affects both excitatory and inhibitory fibers. The excitatory responses may obscure any IPSPs that are evoked simultaneously. Studies in which enteric reflex pathways are activated by physiological stimuli have also failed to evoke IPSPs in myenteric neurons. This suggests that any physiological role for this type of synaptic potential may be confined to a small group of myenteric neurons.

Electrophysiological studies of submucosal neurons have been performed in preparations from the small intestine, cecum, and distal colon of the guinea pig [75–79]. About 90% of all submucosal neurons exhibit fast EPSPs, and a large proportion of these also exhibit slow EPSPs, similar to those observed in myenteric neurons. The fast EPSPs are blocked by hexamethonium and are presumably mediated by ACh acting through nicotinic receptors. Pharmacological data indicate that slow transmission to secretomotor neurons in the submucosa is mediated through purinergic P_{2Y} receptors [80,81].

Although slow IPSPs are rare in myenteric neurons, they are common in submucosal neurons. Inhibitory postsynaptic potentials occur in about 50% of submucosal neurons in the guinea pig small intestine and in as many as 90% of the neurons in the cecum [75–78]. Most submucosal neurons with slow IPSPs also exhibit slow EPSPs, and all have fast EPSPs. In the small intestine, neurons with slow IPSPs contain VIP and are noncholinergic secretomotor/vasodilator neurons [82]. The IPSP is caused by a substantial increase in the K^+ permeability of the membrane, which leads to a hyperpolarization of up to 30 mV. Two separate contributions to IPSPs have been identified: adrenergic transmission from the terminals of sympathetic secretomotor inhibitory neurons (see Sympathetic effects on motility and secretion) and nonadrenergic transmission from intrinsic neurons.

Lesion studies reveal that most submucosal neurons receive fast cholinergic input from neurons in the myenteric plexus and from other neurons in the submucosal plexus [83]. The slow EPSPs in many submucosal neurons appear to come from myenteric neurons, but some may come from submucosal neurons [81–83].

Presynaptic inhibition may also have a role in the enteric nervous system. Sympathetic nerve stimulation reduces the amplitudes of fast EPSPs in both myenteric and submucosal neurons, probably by decreasing the amount of ACh released from the synapses [84,85], although postsynaptic inhibitory effects may also play a role. It appears that the presynaptic inhibitory effect of the sympathetic neurons is the primary mechanism by which they diminish the contractile activity of the gut [3]. This inhibition is mediated through α-adrenergic receptors. ACh released from enteric nerve terminals can act presynaptically to regulate the subsequent release of ACh and possibly to modify the release of the transmitters mediating slow EPSPs [86]. Several other putative transmitters contained within enteric nerve terminals (e.g., dynorphin, enkephalin, γ-aminobutyric acid, 5-HT) have been observed in pharmacological experiments to reduce transmitter release in the gastrointestinal tract. No physiological roles have yet been established for these latter substances in presynaptic inhibition in the enteric nervous system.

Functionally defined enteric neurons

The identification of neurons by function has been derived by combining data from studies of the physiological properties of enteric reflexes, the morphological identification of those neurons that are present (and could thus fulfill the physiological functions), and the correlation of neurochemical (primarily immunohistochemical) and pharmacological properties of enteric neurons (see Table 2.1). The model species has been the guinea pig (Fig. 2.6). These data have contributed to a complete definition of the types of neurons in the small intestine, and a thorough, but not yet complete, definition of the neuron types in other regions [3]. Sufficient data are available from other species, notably human, pig, rat, and mouse, to suggest that the data from guinea pig can be extrapolated. One interspecies difference is the placement of cell bodies of some neurons; specifically, the cell bodies of motor neurons of the external muscle are present in both the myenteric and submucosal plexuses in human and pig, whereas they are almost completely confined to the myenteric plexus in guinea pig, rat, and mouse.

Motor neurons

Muscle motor neurons of the stomach and intestines

Excitatory neurons innervate the longitudinal and circular smooth muscle and the muscularis mucosae throughout the digestive tract. The primary transmitter of these neurons is ACh, which acts on the muscle through muscarinic receptors [3]. Tachykinins contribute to the excitatory transmission but have a lesser role than ACh. The tachykinins in the enteric neurons are substance P, neurokinin A, neuropeptide K, and neuropeptide-γ. The tachykinin component of excitatory transmission appears to be more prominent at high rates of neuron firing. Immunohistochemical studies and quantitative electron microscopy reveal that ACh and

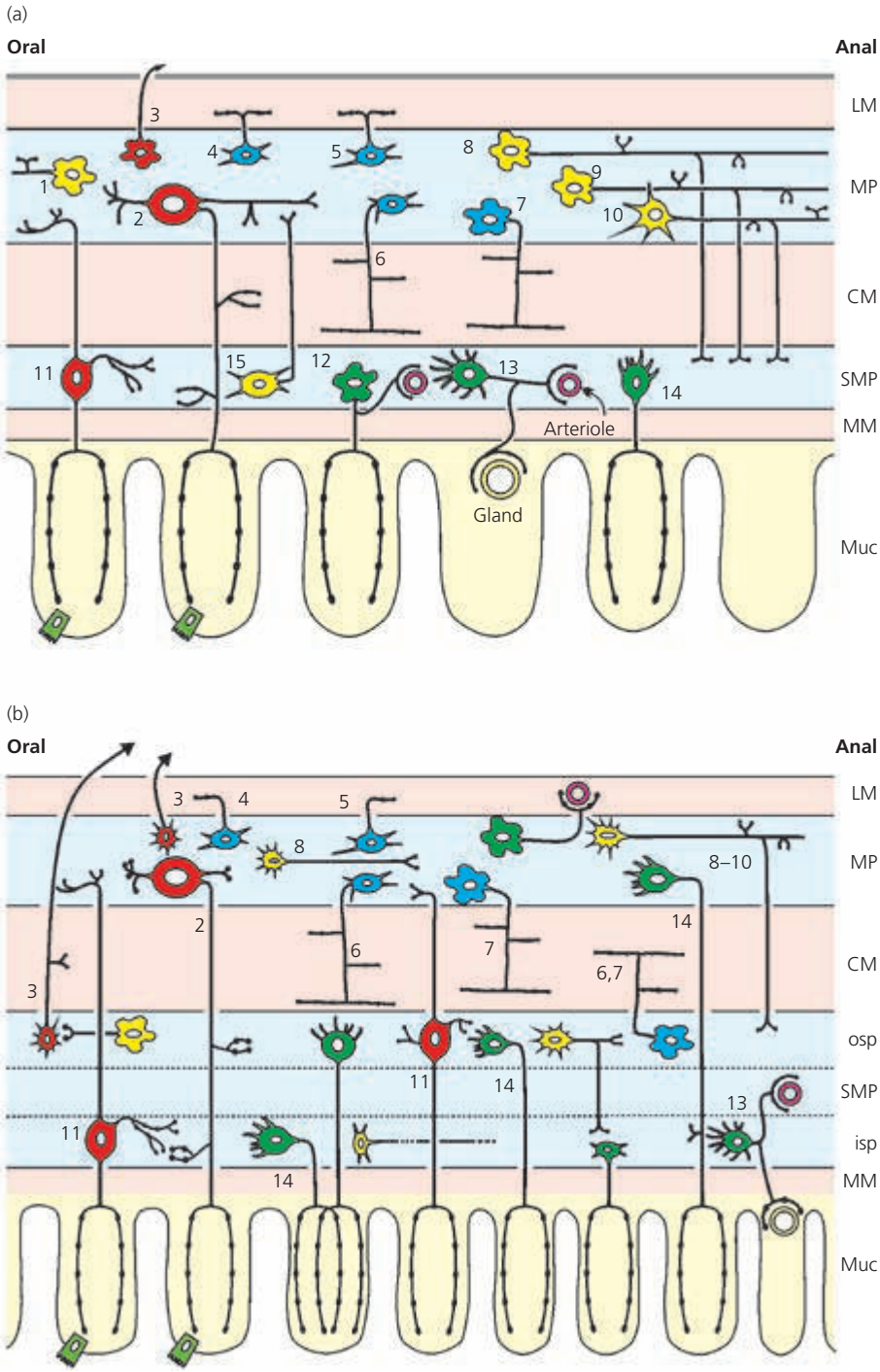

Figure 2.6 The types of neurons in the small intestine of the guinea pig **(a)**, all of which have been defined by their functions, cell body morphologies, chemistries, key transmitters, and projections to targets, and in the small intestine of the pig **(b)**. The numbers adjacent to the neurons correspond to the numbers referred to in Table 2.1. Note that the muscularis mucosae (MM) is very thin in the guinea pig small intestine and the neurons that innervate this muscle have not been identified in this species. In other species they are in the submucosal ganglia. In many cases the equivalent neurons have been identified in the pig and guinea pig, and they have been given the same numbers. LM, longitudinal muscle; MP, myenteric plexus; CM, circular muscle; SMP, submucosal plexus; Muc, mucosa; isp, inner submucosal plexus; osp, outer submucosal plexus. **(a)** Adapted from Furness et al. [47] with permission; **(b)** modified from Timmermans et al. [21], with permission.

tachykinins are contained in the same excitatory muscle motor neurons [87,88]. In the guinea pig small intestine, these neurons, and the inhibitory motor neurons, are S neurons by electrophysiological classification.

Intrinsic motor neurons that relax the muscle are involved in descending reflexes that both facilitate the passage of contents along the bowel and relax regions, such as the stomach, that expand to accommodate and retain their contents. These neurons, called *enteric inhibitory neurons,* release a combination of transmitters that contribute to relaxation in varying degrees, depending on the region and the species [41,89–91]. The neurotransmitters are nitric oxide (NO), ATP (or a related compound), pituitary adenylate cyclase-activating peptide (PACAP), and VIP. VIP and the enzyme nitric oxide synthase (NOS) are found together in the inhibitory motor neurons. NOS inhibitors and scavengers of NO reduce

inhibitory transmission in many species, including human, indicating that NO is an inhibitory transmitter [92,93]. However, electrophysiological and pharmacological evidence indicates that at least one other primary transmitter must be involved in the transmission from these neurons [41].

ATP relaxes the muscle, and when the inhibitory neurons are active, increased levels of ATP and its metabolites are detected in perfusates [94]. Suramin, an antagonist of the action of ATP on P_2 purinoceptors in intestinal muscle, can depress transmission from the inhibitory neurons of the guinea pig taenia coli [95]. VIP contributes to inhibitory transmission in some gastrointestinal muscles, but its involvement is highly variable between regions and in different species [96]. Peptide histidine isoleucine (PHI), or its human equivalent, peptide histidine methioninamide (PHM), is derived from the same gene as VIP and has similar effects. Another member of the VIP family, PACAP, also relaxes intestinal muscle and is found in enteric neurons. Immunoneutralization and pharmacological antagonism of PACAP receptors each indicate that PACAP contributes to enteric inhibitory transmission in some regions of the gut [91,97].

Thus, NO, VIP or PHI, PACAP, and ATP (or a related compound) probably all contribute to inhibitory transmission to muscle. In most instances, NO is the primary transmitter, and the relative contributions of VIP, PACAP, and ATP vary.

Muscle motor neurons of the striated muscle of the esophagus

The striated muscle of the esophagus is innervated by axons that form motor endplates, but unlike motor endplates elsewhere, individual endplates in the esophagus receive dual innervation, one axon being from a vagal motor neuron with its cell body in the medulla oblongata and the other axon originating from a cell body in the myenteric plexus [98]. In the rat, the vagal endings are immunoreactive for calcitonin gene-related peptide (CGRP), and the endings of myenteric origin exhibit NOS immunoreactivity. Double staining for these markers indicates that both fibers make synaptic connections with the muscle, and that the two fiber types are often closely apposed, facilitating presynaptic interaction [99]. The vagal motor neurons are essential to esophageal function, and if their axons are severed, esophageal paralysis results [100]. The enteric innervation of motor endplates is presynaptic to the vagal motor innervation and causes inhibitory modulation of the vagal input [101].

Secretomotor and secretomotor/vasodilator neurons controlling fluid exchange

Secretomotor neurons in the small and large intestines and gallbladder enhance water and electrolyte secretion. There are two types of neuron: secretomotor/vasodilator neurons that send axons to both the mucosal epithelium (causing water and electrolyte secretion) and the submucosal arterioles (causing vasodilation), and secretomotor (only) neurons that supply the epithelium but not the arterioles. Secretomotor transmission to the mucosa has both cholinergic and noncholinergic components [102,103]. ACh released from the cholinergic neurons acts on muscarinic receptors on the mucosal epithelium. The primary transmitter of the noncholinergic secretomotor effect is VIP. Stimulation of single neurons and measurement of the resulting changes in the diameter of submucosal blood vessels provide direct evidence for the presence of both cholinergic and noncholinergic vasodilator neurons [104–106]. Immunohistochemical studies, combined with denervation, confirm that both intrinsic cholinergic and noncholinergic neurons innervate submucosal arterioles [107]. VIP is likely the primary transmitter for noncholinergic vasodilation [3].

Gastric vasodilator neurons

Gastric acid secretion and blood flow are enhanced when the vagus nerve is stimulated; these effects are reduced by muscarinic antagonists. It is usually impossible to determine experimentally whether vasodilation is the result of a direct vascular action of cholinergic neurons, in addition to a functional hyperemia consequent to the increased secretion [108]. However, Thiefin and colleagues showed that centrally administered thyrotropin-releasing hormone stimulated a vagal pathway in the rat that caused gastric vasodilation after acid secretion was blocked by omeprazole [109]. The increased blood flow in the absence of secretory change was antagonized by atropine. There is also evidence of noncholinergic transmission from gastric vasodilator neurons that use VIP [110].

Gastric secretomotor neurons that stimulate acid output

Some secretomotor neurons govern gastric acid secretion. These neurons are cholinergic and act on the parietal cells through muscarinic receptors. For a description of the roles of these neurons and their relation to the hormonal control of gastric acid secretion, see Chapter 13.

Motor neurons that innervate enteric endocrine cells

A variety of endocrine cells reside in the mucosa of the gastrointestinal tract, and because the mucosa is densely innervated, most of these cells have nerve fibers in close proximity. The best-documented motor neurons that provide functional innervation of enteric endocrine cells are those controlling the release of gastrin. Vagal and intrinsic gastric pathways influence the release of gastrin. The final neurons in both pathways are enteric neurons in the stomach wall. Transmission from the final secretomotor neurons is mediated at least partly by gastrin-releasing peptide [3]. Release from other enteroendocrine cells is also likely to be under neural control. For example, the basal release of

motilin is reduced by atropine and by tetrodotoxin and stimulated by muscarinic agonists; these findings suggest that motilin cells receive an excitatory cholinergic input [111].

Innervation of lymphoid tissue (Peyer patches)

Lymphoid aggregations of the gastrointestinal tract (i.e., Peyer patches) are surrounded by nerve fibers, some of which penetrate the capsules of the follicles [13,112]. Receptors for transmitters of enteric neurons occur on lymphocytes within the lymphoid follicles and on lymphocytes that are scattered in the connective tissue of the mucosa [113,114]. Moreover, close axonal approaches to scattered lymphocytes suggest that there may be functional innervation of isolated lymphocytes within the mucosa [112,115]. Close appositions between axons and mast cells also occur in the mucosa [116].

Enteric interneurons

Interneurons have been identified in all gut regions, although their characteristics may vary between regions more than those of other neuron types. For example, the ileum and colon contain the same, or very similar, motor neurons and IPANs, but their complements of interneurons are quite different.

Within the myenteric plexus, the interneurons form chains of like neurons that run both orally and aborally [117,118]. The guinea pig small intestine has at least three classes of descending interneurons and one class of ascending interneurons. Detailed studies show synaptic interconnections between the chains formed by two of the types of descending interneurons [119]. The ascending interneurons appear to be involved in local motility reflexes, as are the descending cholinergic neurons that contain NOS [3]. Another type of descending interneuron, the ACh/somatostatin interneuron, may be linked to the passage of migrating myoelectric complexes (MMCs). These neurons have a distinctive appearance, with numerous branching, tapering, and filamentous dendrites [117]. They have very rare connections from IPANs [120,121]. Migrating myoelectric complexes are waves of excitatory activity that are conducted aborally along the gut. They are mediated through the intrinsic neural pathways of the small intestine [122]. Interruption of the continuity of the enteric nervous system blocks conduction of the MMCs [123], which begin in the duodenum and travel the full length of the small intestine, but not along the colon. It is thus pertinent that the ACh/somatostatin filamentous descending interneurons are found in both the ileum and the duodenum but not in the proximal or distal colon. The third type of descending interneuron, the ACh/5-HT interneuron, is involved in descending excitatory reflexes and possibly in secretomotor reflexes [124]. Interneurons also make connections between the myenteric and submucosal plexuses.

Pharmacological analysis indicates that transmission in the ascending pathway is cholinergic, through nicotinic receptors. Descending reflexes are also blocked by nicotinic antagonists in most species [3], but descending reflexes close to the stimulus, at least in guinea pigs, are resistant to blockade by the nicotinic antagonist hexamethonium [89,125,126]. Pharmacological data reveal that other transmitters of descending pathways are ATP, acting at P_{2X} receptors, and tachykinins, acting at NK_1 and NK_3 receptors [71,127,128]. Nitric oxide, which is probably released from descending interneurons containing NOS, does not appear to be a primary transmitter of enteric interneurons. It may act as a retrograde transmitter, being released from the cell bodies of the interneurons and acting retrogradely on the endings of the terminals of IPANs to reduce transmitter release [129].

Intrinsic primary afferent neurons

About 100 years ago, several investigators showed that enteric motility reflexes could be elicited in segments of intestine that had no neural connections with the central nervous system [130–132]. It was therefore assumed that primary afferent neurons were contained in the gut wall. However, it was discovered at about the same time that reflexes, notably cutaneous vasodilator reflexes, could be initiated by way of axon collaterals even when the axons bearing the collaterals were disconnected from their cell bodies. It is thus necessary to leave sufficient time between lesioning the nerves (so that endings and their collaterals die) and testing the integrity of reflexes. These experiments applied to the small and large intestines of guinea pig, cat, and dog indicate that the motility reflexes are intrinsic [3]. Nevertheless, it is possible to elicit reflexes through activation of axon reflexes [133].

The intrinsic reflex pathways involved in the control of gut movement, blood flow, and secretion are activated through neurons that respond to several stimuli, such as distention, lumenal chemistry, and mechanical stimulation of the mucosa. The IPANs in the guinea pig small intestine have been positively identified as AH neurons with Dogiel type II morphology (Fig. 2.7). Evidence, primarily comparisons of neuron projections and chemistry, suggests that Dogiel type II neurons in other species are also IPANs. However, other neuron types may also have roles as IPANs [134,135].

The AH electrophysiological properties and Dogiel type II morphology of IPANs were confirmed by experiments in which activity-dependent changes were used to reveal the neurons [136,137], and by intracellular recording [67,138] in the small intestine of the guinea pig. These indirect findings agree with deductions made from studies of the morphology and projections of type II neurons [139,140]. Neurons with similar morphology, histochemistry, and projections have been found in the small and large intestines of guinea pig, mouse, rat, human, pig, and sheep. Various experiments indicate that the cell bodies of the mucosal mechanoreceptor primary afferent neurons are in the submucosal ganglia and the cell bodies of chemoreceptor afferent

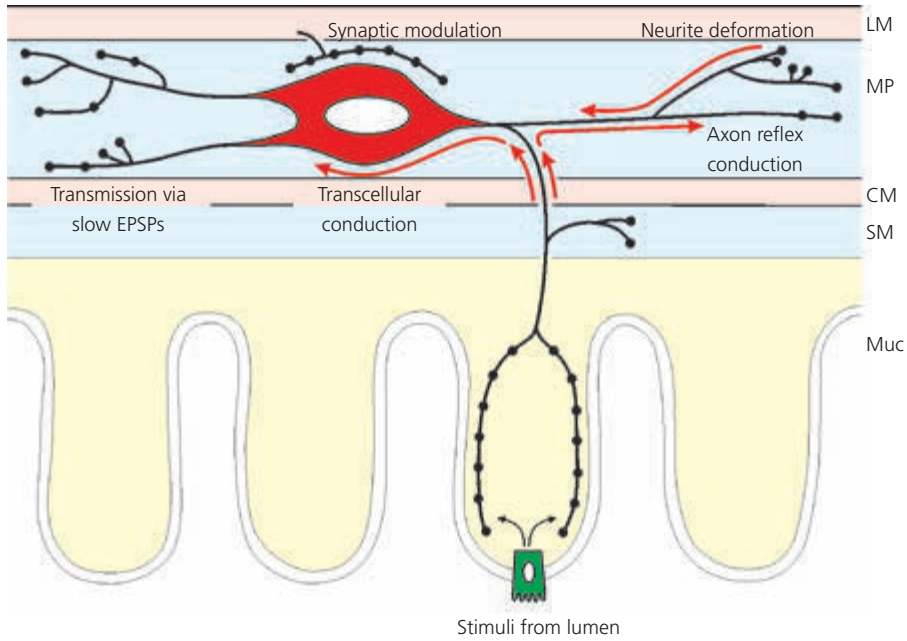

Figure 2.7 A myenteric intrinsic primary afferent neuron (IPAN) (multipolar Dogiel type II) and its conduction pathways are depicted. Action potentials initiated by physiologically appropriate stimuli and by noxious stimuli can traverse the cell bodies (transcellular conduction) or can be conducted to output synapses by way of an axon reflex (axon reflex conduction). Conduction across the cell body is modified by the synaptic inputs that it receives. Myenteric IPANs make synaptic connections with other neurons in the myenteric and submucosal ganglia. EPSPs, excitatory postsynaptic potentials; LM, longitudinal muscle; MP, myenteric plexus; CM, circular muscle; SM, submucosa; Muc, mucosa. Adapted from Furness [3], with permission.

neurons are in the myenteric ganglia, as are the cell bodies of stretch-responsive primary afferent neurons.

Intrinsic primary afferent neurons are one of several types of afferent neuron associated with the gut (Fig. 2.8). Others types are vagal afferent neurons, spinal afferent neurons, and intestinofugal neurons. The intestinofugal neurons are probably second-order neurons in afferent pathways leading from the gut to the prevertebral ganglia [141]. The extrinsic primary afferent neurons are subdivided into two groups: those with cell bodies in the vagal (nodose) ganglia, and those with cell bodies in the dorsal root ganglia. In general, the vagal afferent pathways carry information about the physiological state of the digestive organs. Impulses conveying pain or discomfort are conducted through the dorsal root ganglion (i.e., spinal afferent) pathways.

Extrinsic motor connections

Sympathetic neurons

Norepinephrine is the primary transmitter of the sympathetic postganglionic neurons that supply the gastrointestinal tract. Their cell bodies are in prevertebral and paravertebral ganglia, and their axons run to the gut with the mesenteric nerves. The major roles of these neurons are to regulate blood flow to the gastrointestinal tract, regulate intestinal fluid and electrolyte secretion to keep it in balance with whole-body requirements, and inhibit gastrointestinal motility (see Sympathetic effects on motility and secretion).

Vagal and pelvic motor pathways

The vagus nerves contain the axons of neurons whose cell bodies lie within the brainstem. A variety of effects, primarily on the upper gastrointestinal tract, are mediated through these neurons, including control of esophageal peristalsis, relaxation of the proximal stomach, enhancement of gastric peristalsis, stimulation of gastric acid secretion, promotion of gastrin secretion, stimulation of pancreatic secretion, and contraction of the gallbladder [3]. Except within the striated muscle of the esophagus, the vagal neuron does not act directly; it forms synaptic connections with enteric neurons. Vagal efferent neurons also project to the small intestine and proximal colon. It is interesting that Brunner glands in the small intestine appear to be innervated directly by vagal pathways, but not by local intrinsic neurons [142].

According to the convention used for other cranial autonomic pathways, the vagal neurons of these motor pathways have been called parasympathetic preganglionic or vagal preganglionic neurons. Reference to vagal input neurons as preganglionic wrongly implies that enteric neurons are relay neurons in parasympathetic pathways. Vagal input neurons are neurons in complex circuits in which enteric reflexes of several types are integrated with signals from the central nervous system and from other parts of the gastrointestinal tract.

Transmission from vagal input neurons to enteric neurons is mediated principally by ACh acting on nicotinic receptors; the effects of stimulating vagal motor pathways are blocked or substantially attenuated by drugs that block nicotinic receptors.

The major gastrointestinal effects of the pelvic nerves are on movement, secretion, and blood flow in the distal colon and rectum. The pathways are analogous to those of the vagus; pelvic efferent neurons with cell bodies in the sacral spinal cord form synapses on enteric neurons at which ACh is an excitatory transmitter acting through nicotinic receptors. The pelvic nerves initiate defecatory reflexes.

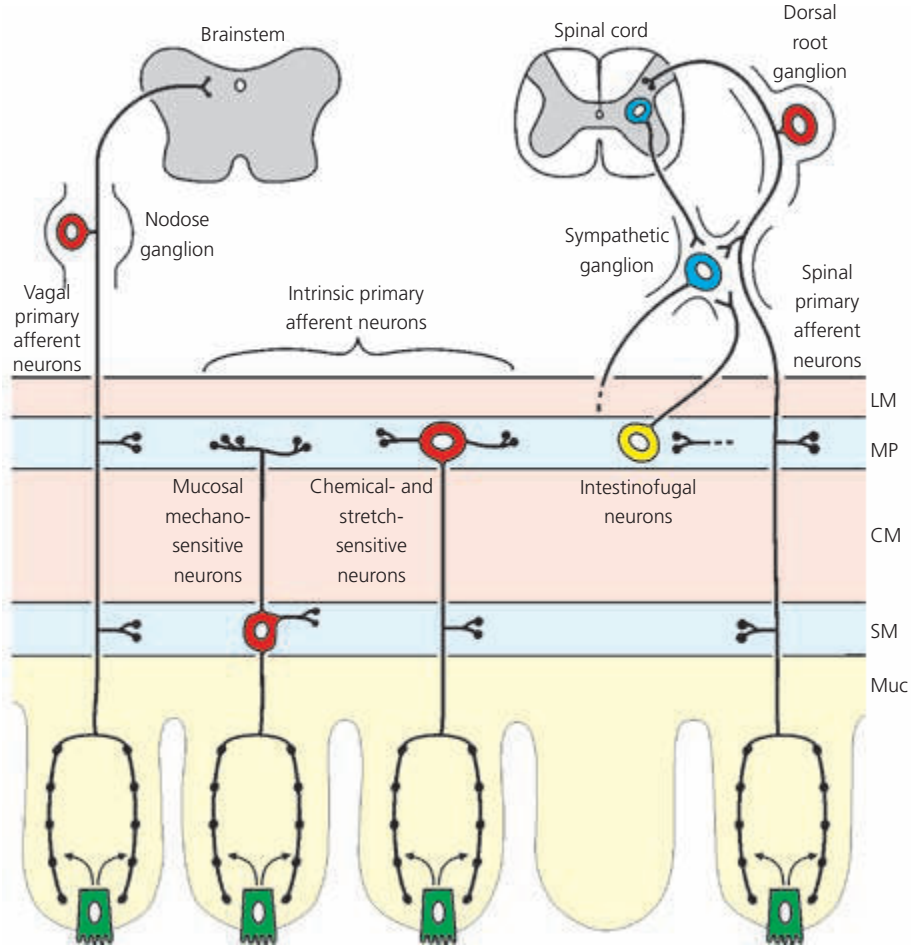

Figure 2.8 The afferent neurons of the digestive tract. Two classes of intrinsic primary afferent neuron (IPAN) have been identified: myenteric IPANs that respond to distortion of their processes in the external muscle layers and, by way of processes in the mucosa, to changes in lumenal chemistry; and submucosal IPANs that detect mechanical distortion of the mucosa and lumenal chemistry. Extrinsic primary afferent neurons have cell bodies in dorsal root ganglia (spinal primary afferent neurons) and vagal (nodose and jugular) ganglia. Spinal primary afferent neurons supply collateral branches in prevertebral (sympathetic) ganglia and in the gut wall. Intestinofugal neurons are parts of the afferent limbs of enteroenteric reflex pathways. Nerve endings in the mucosa are activated by hormones, most prominently 5-hydroxytryptamine, released from enteroendocrine cells (arrows). LM, longitudinal muscle; CM, circular muscle; MP, myenteric plexus; SM, submucosa; Muc, mucosa. Adapted from Furness [3], with permission.

Enteric control of motility

Intrinsic reflexes of the enteric nervous system are essential to the generation of the patterns of motility observed in the small and large intestines. The major patterns of muscle movements in the small intestine are mixing movements, propulsive reflexes that travel short distances, the MMC, peristaltic rushes, and retropulsion associated with vomiting. The same neurons participate in each of these actions, but how the nervous system is programmed to produce such diverse outcomes is not known. In contrast to the intestines, peristalsis in the stomach is a consequence of spontaneous electrical events (i.e., slow waves) in the muscle that begin in the corpus and travel to the pyloric sphincter [3]. The intensity of contraction is determined by the actions of the vagus nerves, which form connections with enteric motor neurons in the gastric wall. The proximal stomach relaxes to accommodate the arrival of food. This relaxation is also mediated through the vagus. Thus, the primary integrative centers for control of gastric motility are in the brainstem, whereas those for control of the small and large intestines are in the enteric nervous system. Reflexes can be generated in the gastric enteric circuits, but their importance, relative to control through the vagus, is not clear. In most mammalian species, the contractile tissue of the external wall of the esophagus is striated muscle; in other species, including human, the proximal half or more is striated muscle and the remainder is smooth muscle. The striated muscle of the esophagus is controlled by an integrative circuitry in the brainstem. Thus, although the myenteric ganglia are prominent in the striated muscle part of the esophagus, they are modifiers, not essential control centers, for esophageal peristalsis.

The smooth muscle sphincters restrict and regulate the passage of the lumenal contents between regions. In general, reflexes that are initiated proximal to the sphincters relax the sphincter muscle and facilitate the passage of the contents, whereas reflexes that are initiated distally restrict retrograde passage of contents into more proximal parts of the digestive tract.

Circuits in the small intestine

The circuits involved in local peristaltic reflexes in the small intestine, and the projections of the component neurons,

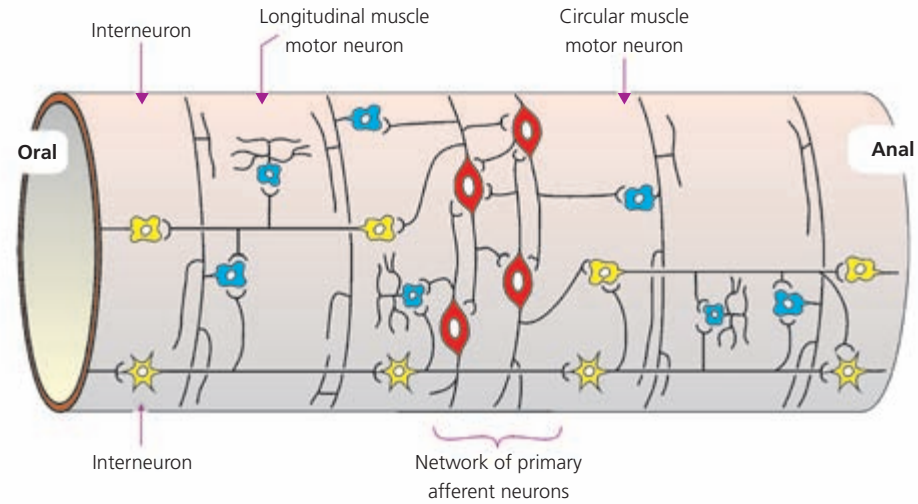

Figure 2.9 The pathways for propulsive reflexes in the intestine. The first parts of the descending inhibitory and the ascending excitatory reflex pathways are depicted in a short segment of intestine. The patterns in the circuitry for the ascending and descending pathways are similar. Intrinsic primary afferent (sensory) neurons (IPANs) are circumferentially oriented and form self-reinforcing networks. They provide outputs to ascending and descending interneurons and monosynaptic connections to motor neurons. The interneurons form descending and ascending chains and also provide outputs to motor neurons. In the descending pathway, some neurons excite the longitudinal muscle, and some neurons inhibit the circular muscle. Ascending reflex pathways supply inputs to excitatory longitudinal muscle motor neurons and excitatory circular muscle motor neurons. A set of descending interneurons **(bottom)** involved in conducting the migrating myoelectric complex receives few inputs from IPANs but connects with motor neurons.

have been deduced from studies of guinea pig small intestine (Fig. 2.9; see also Fig. 2.6). Experiments in other mammals, including cat, dog, and rat, suggest that the pathways are similarly organized in other mammals [3]. In all species, excitatory and inhibitory motor neurons supply the muscle, the general patterns of small intestine motility are similar, and intrinsic reflexes are evoked by the bulk and chemical nature of the lumenal contents.

The movements of the intestine are a result of the contractions and relaxations of the external longitudinal and circular muscles and of the muscularis mucosae. Neural control is superimposed on an underlying rhythm of muscle activity (i.e., slow waves), which occurs at frequencies of about 3–12/min in human. The major role in forming the pattern of mixing and propulsive movements appears to be taken by the circular layer of the external muscle, areas of which contract (or relax) like an annulus to close or increase the lumen during both propulsive and mixing movements.

To study enteric muscle motor reflexes, investigators isolate segments of intestine to eliminate the influence of the central nervous system and circulating hormones. This simplifies recording from the muscle and enteric neurons. In these preparations, lumenal distention, or chemical or mechanical stimulation of the mucosa, elicits reflexes whose effects on the circular muscle can be recorded with intracellular microelectrodes [125,143–148]. The major responses of the muscle are depolarizing potentials (i.e., excitation) in the circular layer oral to the site of the stimulus and aboral hyperpolarizing potentials (i.e., inhibition). This polarization of electrical responses is analogous to that of the mechanical events that occur when the intestine is distended. Longitudinal muscle motor neurons are also activated by reflex stimuli [149]. The longitudinal muscle contracts at the same time as the circular muscle, although there may be a delay between the commencement of contraction in the two layers. The longitudinal muscle appears to restrict the lengthening of the intestine when the circular muscle contracts [3].

If the myenteric plexus is cut, neither the reflex responses to mucosal stimulation nor those to lumenal distention pass along the gut, whereas interruption of the submucosal plexus has no effect [125,150,151]. Therefore, the reflex pathways excited by mucosal stimulation must pass locally from the sensory receptors in the mucosa to the myenteric plexus. Responses evoked by distention persist if the mucosa is removed [125,152,153], a finding consistent with the presence of mechanosensitive processes of IPANs in the external muscle. Mucosal distortion releases 5-HT from enterochromaffin cells in the epithelial lining [154], which in turn activates the endings of the IPANs to initiate or potentiate peristaltic reflexes [155].

In the guinea pig small intestine, IPANs with cell bodies in the myenteric plexus provide extensive varicose networks in the ganglia, primarily close to and surrounding the cell bodies [139,156]. A few neurons have aborally projecting collateral axons [157]. The varicosities of IPAN terminals provide synaptic inputs to most neurons in the myenteric plexus [158], and electrophysiological studies of the effects of activation of IPANs through their nerve endings in the mucosa

confirm that IPANs communicate with other neurons by means of slow EPSPs [64,67], whereas recording at a distance in a circumferential direction implies that some synapses from IPANs cause fast EPSPs [121]. Slow EPSPs are evoked consistently only at rates of electrical stimulation of presynaptic neurons of about 3 Hz or more. In accordance with this finding, the primary afferent neurons fire in response to physiological stimuli in bursts that commonly achieve rates of 10–40 Hz [64,67].

It is notable that physiological as well as ultrastructural studies indicate that IPANs synapse with other IPANs to form a self-reinforcing network. IPANs are numerous, about 500 per millimeter-length of the small intestine. Because the stimuli giving rise to intestinal reflexes (e.g., lumenal chemicals, distention, mucosal distortion) are not spatially confined to submillimeter distances, it can be deduced that reflexes are usually initiated by the more-or-less synchronous activation of a population of several hundred interconnected IPANs. The summation of the synaptic events caused by transmission from the IPANs results in the nearly simultaneous activation of numerous interneurons and motor neurons. The IPANs in the submucosal plexus project to the myenteric plexus and appear to be directly sensitive to mechanical and chemical stimulation of the mucosa. At least some portion of the reflexes evoked by stimulation of the mucosa is mediated by submucosal IPANs.

Enteric reflexes often extend for several centimeters along the intestine from a single point of stimulus, unlike the processes of most IPANs and of motor neurons. This implies that most enteric reflex pathways include interneurons, which, as previously explained, form interconnecting chains that run along the intestine and which may individually run for more than 1 cm [159]. The pathway may be longer in species other than guinea pig. Immunohistochemical data indicate that, in guinea pig small intestine, there is one population of orally directed interneurons, which are cholinergic, and several populations of aborally directed interneurons, many of which are immunoreactive for choline acetyltransferase (see Table 2.1 and Fig. 2.6). Fast EPSPs, which are output events of interneurons, occur in neurons at distances of 0.5–2.5 cm from the stimuli. Physiological and structural studies indicate that interneurons make connections with both motor neurons and other interneurons, confirming the pattern of connectivity shown in Fig. 2.9. The circuits in Fig. 2.9 depict individual neurons, although cohorts of neurons are actually activated, as previously described.

The projections of motor neurons to the circular muscle have been determined by several techniques:

- mapping the responses to transmural nerve stimulation in normal pieces of the intestine and in pieces in which the nerve paths were lesioned and allowed to degenerate [160,161]
- microscopic analysis of the effects of nerve lesions [16]
- observation of retrograde transport of microcrystals of dye placed on the inner surface of the muscle [88]
- filling the neurons by using intracellular microelectrodes [162].

Most excitatory and inhibitory motor neurons supplying the circular muscle extend only 1–2 mm along guinea pig intestine, but they run up to half the distance around its circumference, about 6–8 mm in guinea pig small intestine. Thus, the response to reflex activation is spread around the intestine. This finding is consistent with the common observation that the intestine undergoes annular, not eccentric, contractions or relaxations. The reflex probably involves a summed response to transmission from many motor neurons; each smooth muscle cell is influenced by about 25 inhibitory motor neurons, and a similar convergence of excitatory influence can be expected [160]. Furthermore, the electrical communication between smooth muscle cells ensures summation of the effects of the population of motor neurons. Some excitatory motor neurons project orally as far as 10 mm, and some inhibitory motor neurons project aborally as far as 30 mm.

In addition to the simple reflex whose neural circuits have now been deduced (Fig. 2.9), the intestine exhibits mixing movements and cyclic changes of activity: MMCs, retropulsion, and peristaltic rushes [3]. The MMCs pass along the intestine from the stomach to the terminal ileum. In humans, these cycles last about 90 min and occur between digestive periods. The MMC is generated by the enteric nervous system. During digestion, the contents of the intestine trigger irregular mixing contractions. The neural pathways depicted in Fig. 2.9 presumably provide the building blocks for both the irregular contractile activity and the MMCs, with the irregular contractile activity perhaps representing the superimposition and interaction of simple reflexes. It has been deduced that the descending neuron chains for the MMC are separate from those of local reflexes (see Enteric interneurons).

Enteric control of secretion and vasodilation

About 5–10 L of water, derived from food and drink, salivary secretions, gastric secretion, secretions of the pancreas and biliary system, and from the intestine itself, enters the lumen of the human intestine each day. To maintain the equilibrium of fluids in the body, electrolytes absorbed in the gastrointestinal tract must be matched by losses through perspiration, respiration, urination, and defecation. Secretion and absorption are balanced so that the osmolarity of the fluid in the small intestine is closely matched to that in the interstitial fluid, although there are local variations from isosmolarity (e.g., in the lamina propria of the villi). The critical role played by regulation within the intestine itself is exemplified by the life-threatening effects of bacterial toxins,

such as cholera toxin, which act on enteric neurons to cause copious secretion of fluid across the intestinal epithelium, and the deleterious effect of deficiency of intestinal fluid secretion, as occurs in cystic fibrosis.

Water moves from the lumen in company with nutrient molecules [163,164]. The major process for the absorption of nutrients, such as glucose, is cotransport with Na^+, in this case through the Na^+/glucose cotransporter SGLT1. The entry of these solute molecules draws water across the epithelium, which is one reason to add glucose to oral rehydrating fluids. Direct measurements indicate that the cotransport comprises 1 glucose and 210–260 water molecules [164]. It has been estimated that SGLT1 absorbs 8–9 L of water each day from the small intestine [164]. Another way to consider the relationship between dissolved nutrient molecules and the amount of associated fluid is that 100 g of carbohydrate, if broken down to monosaccharides, would require about 1.8 L of water to be absorbed and be isotonic with blood and tissue fluids. Amino acid transport is also ion-coupled and similarly involves water movement in concert with absorption. The large fluxes of water from the lumen to the fluid compartments of the body are partly balanced by intestinal secretion that is mediated by enteric secretomotor reflexes.

As implied in the previous paragraph, the most efficient way to shift water molecules across the epithelium is to move solute molecules, and this is indeed how most water molecules move. A small proportion may pass through specialized water channels, the aquaporins, and a small proportion uses a paracellular route to cross the epithelium [164]. The secretomotor neurons stimulate the epithelial cells to pump Cl^- into the lumen, which takes with it counter ions, mostly Na^+, and water. A major component of Cl^- secretion is through the cystic fibrosis transmembrane conductance regulator (CFTR) channel. It is also likely that the secretion of K^+ ions, which is stimulated by muscarinic agonists [165], is increased by the activity of cholinergic secretomotor neurons. K^+ secretion is also accompanied by water secretion.

Secretion in the small intestine is primarily from the crypts and the bases of the villi, and in the colon is from the deep parts of the glands; conversely, absorptive processes are dominant in the more superficial parts of the mucosa [166,167]. These functions are not mutually exclusive, and in fact both functions can occur at all levels of the mucosa [168].

Enteric secretomotor and vasomotor reflexes related to fluid exchange

Distention, mechanical stimulation of the mucosa, intraluminal nutrients, and the application of chemicals to the mucosa, including noxious substances, evoke secretomotor and vasodilator reflexes [169–174]. Histochemical studies in the guinea pig small intestine indicate that two types of the motor neurons for secretion are also motor neurons for vasodilation; that is, these secretomotor neurons cause a physiologically appropriate vasodilation, concomitant with secretion, through collaterals to submucosal arterioles [3]. A third type of secretomotor neuron does not project to the vasculature. As previously indicated, the control of secretion in the small intestine contributes to the regulation of whole-body water and electrolyte status. If more fluid is absorbed with nutrients or across the gastric mucosa than is lost elsewhere, some of the excess can be passed back under the control of secretomotor reflexes. Thus, the source of secreted fluid in the small intestine is a mixture of serum electrolytes and locally absorbed electrolytes. We postulate that local computation of the need for vasodilation and transmucosal absorption to supply electrolytes for secretion determines the relative activation of vasodilator and nonvasodilator secretomotor neurons (Fig. 2.10).

Glucose or its uptake stimulates the enteric secretomotor reflex [169]. Enteric reflexes also cause HCO_3^- secretion in response to duodenal acidification, although other acid-sensitive mechanisms, including a neurally independent stimulation of prostaglandin production, also cause secretion of HCO_3^- [175]. It is likely that the same ion channels are permeable to Cl^- and HCO_3^-, the relative amounts of Cl^- and HCO_3^- transported being dependent on the lumenal pH [176]. Secretomotor reflexes can also be initiated pathologically by toxins, such as cholera toxin or enterotoxins, in the lumen (see Enteric nervous system responses to noxious stimuli).

The enteric secretomotor/vasodilator circuits consist of IPANs with their endings in the mucosa and an integrating circuitry that feeds back to motor neurons with cell bodies in the submucosal ganglia [3]. In some cases, the reflex pathways involve the myenteric ganglia (e.g., cholera toxin-induced secretion) [177], whereas reflexes initiated by mechanical stimulation of the mucosa can be mediated entirely through the submucosal plexus [103,171,178]. Pathways from the myenteric plexus also cause vasodilation [179,180]. There are two types of secretomotor neurons, cholinergic and noncholinergic. The noncholinergic neurons appear to mediate most of the local reflex response, using VIP, or a related peptide, as their primary transmitter. In the guinea pig small intestine, there are two types of cholinergic neurons: those that also contain NPY (and other peptides) and those that contain calretinin. The ACh/calretinin neurons preferentially innervate the glands at the base of the mucosa and have collaterals to submucosal arterioles, whereas the ACh/NPY neurons do not appear to innervate the arterioles. The presence of three classes of secretomotor neurons, two of which also have vasodilator collaterals, probably contributes to the balance of secretion and vasodilation appropriate to the digestive state (see above and Fig. 2.10).

In addition to reflexes that have secretomotor neurons as their outputs, IPANs may have direct secretomotor effects.

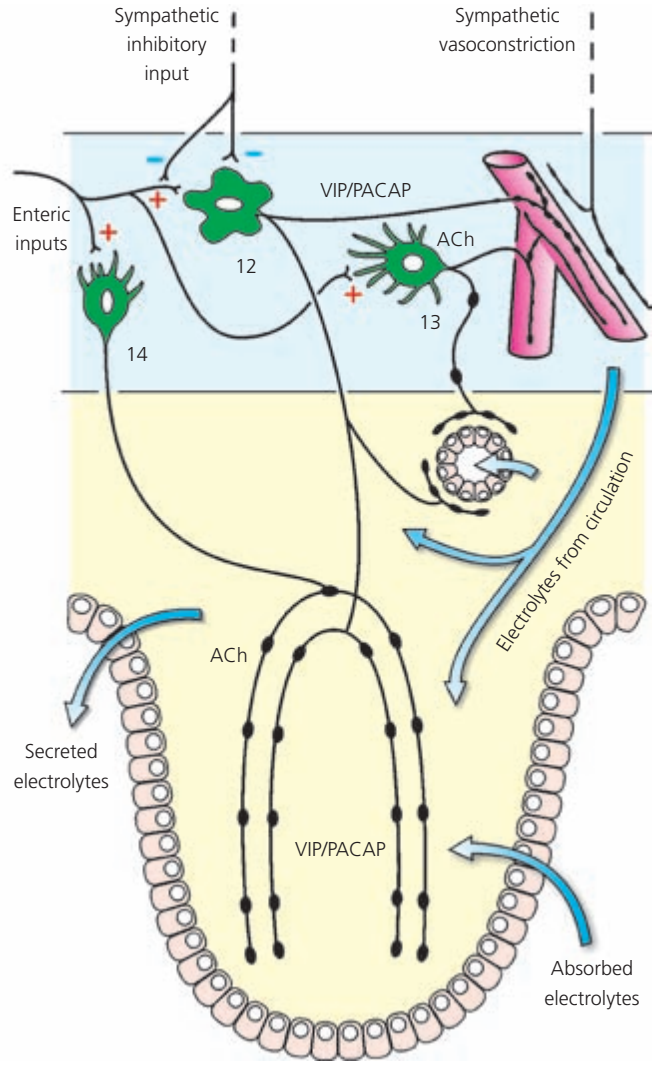

Figure 2.10 Schematic representation of the integration of enteric neuronal control of secretomotor and vasomotor activity in the small intestine. The circuitry provides for the adjustment of fluid and electrolyte secretion into the intestinal lumen, according to the sources of the fluid and the need of the whole body to maintain fluid homeostasis. The secretory flux is supplied by fluid that is absorbed across the epithelium in company with nutrients and ions and by fluid from the circulation. The existence of both secretomotor only (neuron 14) and secretomotor/vasodilator neurons (neurons 12 and 13) facilitates the adjustment of the relative supply from these two sources. The enteric secretomotor reflexes are under tonic inhibitory control from the sympathetic neurons that innervate secretomotor neurons, and the availability of fluid from the circulation is limited by the activity of the sympathetic vasoconstrictor neurons. Decreases in blood pressure or blood volume (e.g., hemorrhage) inhibit both secretion and blood flow to the mucosa. The neuron numbers correspond to the numbering of neurons in Table 2.1 and their depiction in Fig. 2.6. ACh, acetylcholine; VIP/PACAP, vasoactive intestinal peptide/pituitary adenylyl cyclase-activating peptide.

Intrinsic primary afferent neurons are immunoreactive for tachykinins, and their varicose processes are immunoreactive for the vesicular ACh transporter [181]. Thus, their mucosal endings are likely to release ACh and tachykinins, both of which cause secretion. It has been directly shown that action potentials in one process of an IPAN traverse the cell body to invade other processes [182], and the pattern of branching of the neurons indicates that action potentials could be conducted, as an axon reflex, between terminals that branch within the mucosa [3]. Interestingly, the secretory responses to distention and to mucosal stroking in the guinea pig colon are reduced by tetrodotoxin (which blocks nerve conduction) and by atropine (which blocks the ACh receptors on the epithelium), but not by mecamylamine, an antagonist of cholinergic fast neuroneuronal transmission [171,173]. The concentration of mecamylamine that was used was shown to block nicotinic receptors in the colon [173]. Moreover, the responses to stroking were not reduced by extrinsic denervation, a finding that indicates they are dependent on the activation of intrinsic neurons [183]. Thus, there is good evidence that ACh released by axon reflex, or by mononeuronal reflexes crossing the IPAN soma, contributes to secretory responses.

Enteric nervous system responses to noxious stimuli

In the small intestine and colon, protective secretory and motility responses are initiated by irritants that are included in enemas, by noxious products of bacteria, and by parasitic infestations. The intestine exhibits exaggerated secretomotor reflexes, causing diarrhea, when levels of bacteria and bacterial toxins in the gut lumen are excessive [184,185]. Bacteria and parasites in the gut lumen also trigger powerful propulsive reflexes that help to expel these organisms [186–188]. Vomiting, which involves retropulsive reflexes in the small intestine, is also used to eliminate undesirable contents from the gut. Thus, enteric reflexes are initiated by noxious conditions in the gut, as well as being initiated by benign stimuli in normal physiological conditions.

Sympathetic effects on motility and secretion

The movement of the contents in an oral to aboral direction is restricted when sympathetic nerve activity increases. Under resting conditions, the sympathetic pathways exert little influence on motility. They come into action when protective reflexes are activated. Sympathetic pathways involved with fluid secretion are continuously active to regulate the intensity of the enteric reflexes to balance intestinal secretion with whole-body fluid status.

Sympathetic neurons slow transit by constricting the sphincters and by inhibiting the contractile activity of the nonsphincter regions [189]. Inhibition of muscle movement in the nonsphincter parts of the gastrointestinal tract is primarily through actions of the noradrenergic axons on enteric neurons, including the presynaptic inhibition of excitatory transmitter release. Sympathetic nerve fibers also have direct inhibitory actions on the nonsphincter muscle. In the sphincter regions, the sympathetic neurons contract the muscle. Other sympathetic neurons innervate and inhibit submucosal secretomotor neurons. Another group of noradrenergic sympathetic neurons supply the gastrointestinal vasculature, constricting the arterioles and arteries and the major mesenteric veins. Their primary roles are to adjust the proportion of the cardiac output going to digestive organs, according to relative physiological need.

Reflex pathways through which motility is inhibited are of two types. In some cases the reflexes pass by way of the central nervous system and then back to the intestine; reflexes also are conducted from one part of the gastrointestinal tract to another through sympathetic prevertebral ganglia [3,190,191].

The peripheral sympathetic enteroenteric reflex pathways and their associations with other sympathetic pathways are illustrated in Fig. 2.11. Because there is insufficient space in this chapter to survey all the evidence for the circuits shown, only the major points are discussed. Intestinofugal neurons that synapse in the prevertebral ganglia were demonstrated by intracellular recordings from the ganglia in preparations consisting only of a segment of intestine connected to a ganglion that had been completely removed from the body [192]. Activation of intestinal tension receptors evoked fast EPSPs in many nerve cells [192–194]. The EPSPs were blocked by the application of nicotinic antagonists to the ganglia, suggesting that they are cholinergic. This conclusion has been supported by the observation that all intestinofugal neurons are immunoreactive for choline acetyltransferase [195]. There is also a component of slow excitatory transmission

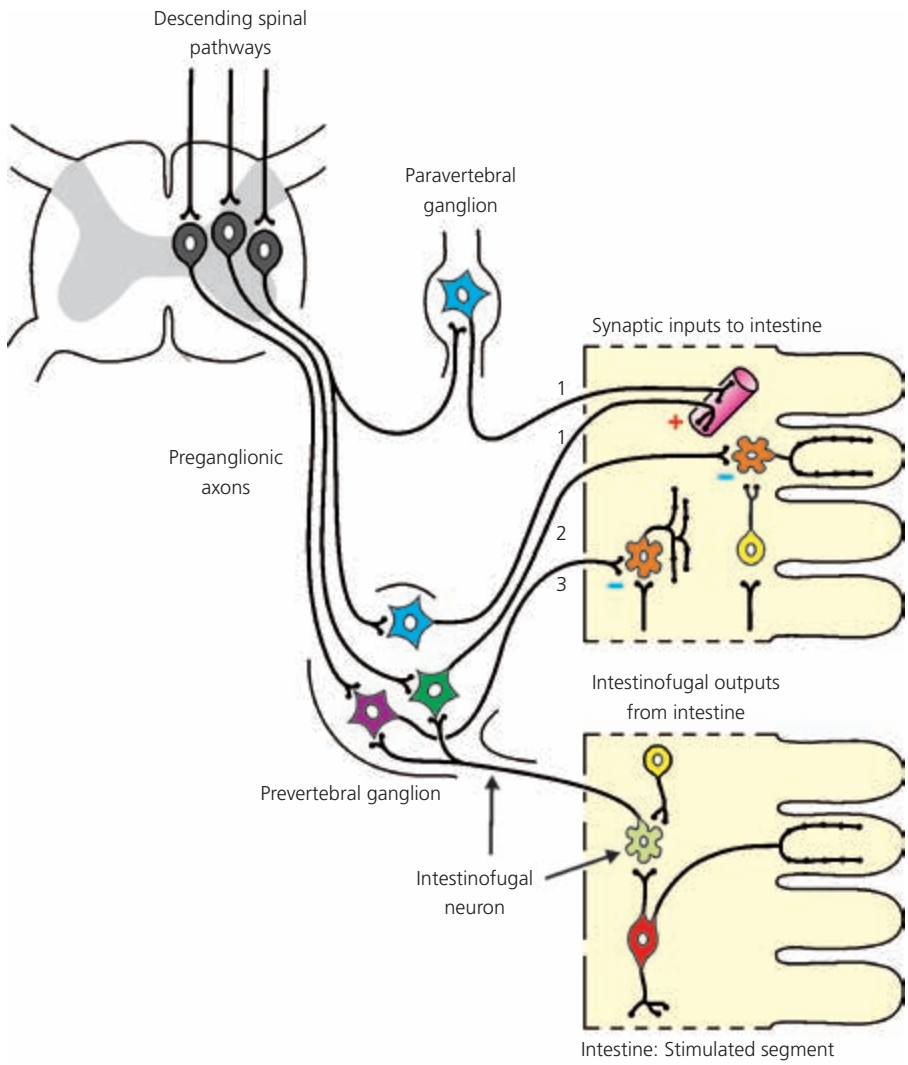

Figure 2.11 Connections of sympathetic pathways that affect intestinal motility, fluid exchange, and blood flow. Three main types of sympathetic neurons supply the nonsphincter parts of the intestine: 1, vasoconstrictor neurons; 2, secretion-inhibiting neurons; 3, motility-inhibiting neurons. These neurons all receive synaptic inputs from the spinal cord. In addition, the secretion-inhibiting and motility-inhibiting neurons receive inputs from intestinofugal neurons. Intestinofugal neurons do not connect with vasoconstrictor neurons. See text for further details.

from the intestinofugal neurons, which is a result of the release of a cotransmitter, probably VIP [191]. Most intestinofugal neurons are second-order neurons that nevertheless may also be directly activated by stretch [141].

Sympathetic motility-inhibiting neurons probably have little or no activity in the resting state in both humans and animals [16]. The enteroenteric inhibitory reflexes appear to have a protective role for the gastrointestinal tract [3]. For example, in the case of enterogastric reflexes initiated by acidity or hypertonicity in the lumen of the upper small intestine, the slowing of gastric emptying protects the duodenal mucosa from acid and osmotic stress. Enteroenteric inhibitory reflexes affecting other parts of the intestine can be initiated by the distention of any region. Most studies have examined reflexes affecting the stomach and intestine, but similar reflex pathways affect the biliary system [196]. The reflexes that pass through the central nervous system are commonly evoked by noxious stimuli or by pain of visceral origin. For example, biliary colic, abdominal injury, and irritation within the abdominal cavity caused by chemicals or infection activate sympathetic reflexes that inhibit gut motility.

The net movement of water and electrolytes between the gut lumen and body fluid compartments is regulated to maintain overall fluid homeostasis. This is accomplished by sympathetic pathways that can override the enteric secretomotor reflexes (see Figs 2.10 and 2.11). Axons arising from the sympathetic secretomotor-inhibiting neurons in the prevertebral ganglia innervate secretomotor neurons, in which they elicit inhibitory synaptic potentials. Persuasive evidence indicates that normal intrinsic secretomotor reflex activity is inhibited by tonic activity of the sympathetic secretomotor inhibitory pathways. Cutting the sympathetic pathways releases the brake on the enteric secretomotor reflex and results in what Bernard, in 1859, called *paralytic secretion* [197]. The level of activity of the sympathetic secretomotor inhibitory neurons responds to changes in whole-body water content and electrolyte status; thus, sympathetic activity is increased and water and electrolyte secretion reduced in response to hemorrhagic hypotension, unloading of the baroreceptors, or reduction in right atrial pressure [198–201]. HCO_3^- secretion is also inhibited by sympathetic nerves [202].

Summary

The enteric nervous system is by far the largest grouping of neurons outside the central nervous system, in the human containing 200–600 million neurons. It contains primary afferent (sensory) neurons, interneurons, and several classes of motor neuron that together form complete reflex circuits that influence many aspects of digestive tract function, prominent among these being motility, secretion, and blood flow. Enteric reflexes are essential to generate the patterns of motility of the small and large intestines, whereas motility in the esophagus and stomach is under dominant vagal control. Intramural secretomotor and vasodilator reflexes control the return to the lumen of fluid that is absorbed with nutrients. Sympathetic pathways inhibit motility in nonsphincter regions and contract the sphincters. These are protective reflexes. Sympathetic pathways regulate the secretomotor reflexes to maintain secretion appropriate to whole-body fluid balance.

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A COMPONENTS

3 Sensory neurons of the gastrointestinal tract

John B. Furness

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Detection of the physiological state and environment of the gastrointestinal tract involves three systems of sensors: primary afferent neurons, enteroendocrine cells, and immune cells (Fig. 3.1). Each of these detecting systems is more extensive than those of other organs [1]. About 20% of the neurons in the enteric nervous system, which contains about 400 million neurons in humans, are sensory, and more than 50 000 axons that reach the gut through the vagus and splanchnic nerves are also sensory. The gastroenteropancreatic endocrine system contains thousands of enteroendocrine cells, many of which react to their local environment, and from which more than 20 identified hormones are released. The gut immune system senses foreign proteins and contains 70%–80% of the body's immune cells.

The neurons that detect the states of tissues are known as primary afferent neurons, *primary* because they are the first neurons in reflex pathways, and *afferent* because they convey information toward reflex control centers. Three broad classes of afferent neurons are associated with the gut: extrinsic primary afferent neurons, which have cell bodies in vagal and dorsal root (spinal) ganglia; intrinsic primary afferent neurons (IPANs), which have cell bodies, processes, and synaptic connections in the gut wall; and intestinofugal neurons, which have cell bodies in the gut but innervate sympathetic ganglia outside the gut wall.

The monitoring and control of the digestive system by neurons is hierarchical. The gut contains an extensive collection of neurons, the enteric nervous system, within its walls (see Chapter 2). This intrinsic nervous system is capable of generating appropriate reflex responses to the contents of the intestinal lumen; for example, local reflexes generate mixing and propulsive movements of the muscle, local changes in blood flow, and secretion of water and electrolytes. These intrinsic reflexes have important roles in the small and large intestines, but they are not prominent in the functions of the stomach, nor in control of the esophagus, pancreas, and gallbladder. At the next level, reflexes occur between parts of the digestive system, for example between the duodenum and stomach to regulate gastric emptying. At the next level, the digestive organs send signals to the central nervous system. These include afferent signals for reflexes that act on the digestive system, signals that mediate coordination with other body systems, and signals that relate to sensations including discomfort, nausea, pain, and satiety.

Extrinsic primary afferent neurons

Extrinsic primary afferent neurons have cell bodies in the nodose and jugular ganglia, from which axons follow vagal pathways to innervate the gut (vagal primary afferent neurons), or in dorsal root ganglia (spinal primary afferent neurons). The fibers of spinal primary afferent neurons in the thoracic and lumbar regions (thoracolumbar spinal afferents, Fig. 3.2) pass through the sympathetic ganglia to reach the gut by way of the splanchnic and mesenteric nerves. Some fibers originating from thoracic dorsal root ganglia join the vagus nerve, either through the stellate ganglion or directly through small connecting nerves in the thorax. Conversely, many axons of vagal afferent neurons supplying the small intestine are in the celiac branch of the vagus nerve and join with spinal afferent fibers in the mesenteric nerves as they run to the gut. The axons of spinal primary afferent neurons with cell bodies in the sacral ganglia follow the pelvic nerves to reach the colon and rectum (pelvic spinal afferents, Fig. 3.2).

The types of information carried to the central nervous system by extrinsic afferent neurons are summarized in Table 3.1. Very broadly, the afferent information can be divided into *that which reaches conscious sensation*, such as

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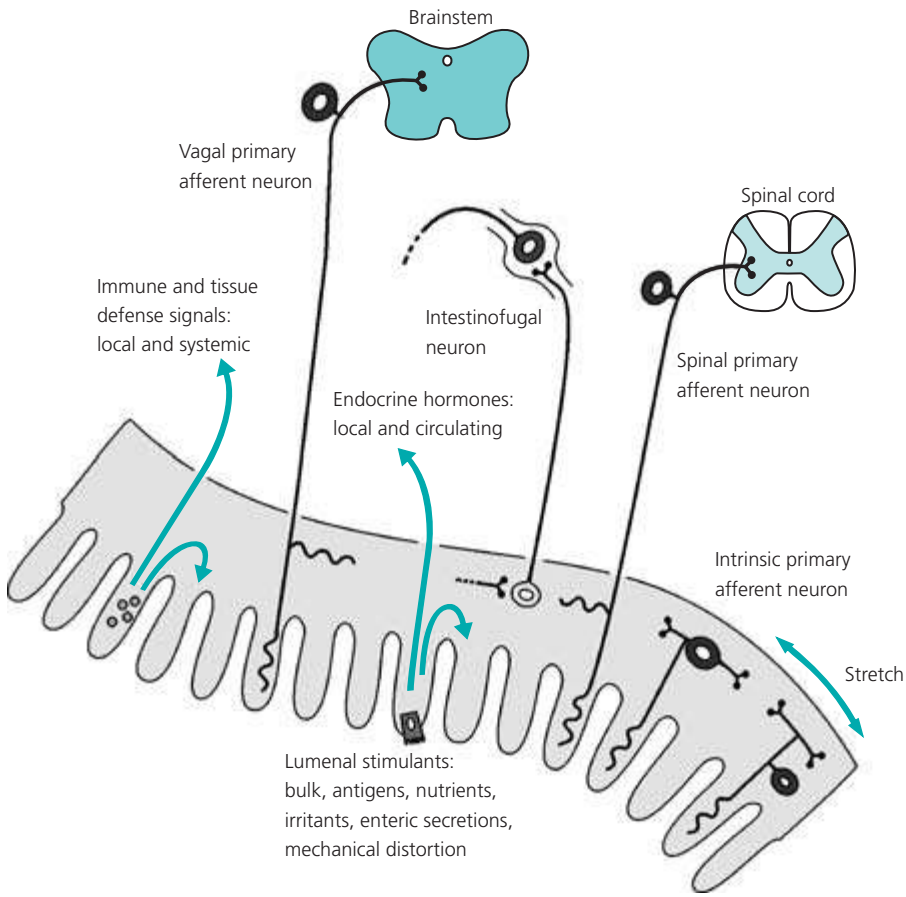

Figure 3.1 Three types of sensory signals originate from the gastrointestinal tract. Cells in the mucosal epithelium (enteroendocrine cells) detect chemicals in the lumen and mechanical stimuli applied to the mucosa. These release endocrine messages in the form of hormones, which enter the circulation and can act at remote sites. Gut hormones can also act locally, on nerve endings, on the epithelium, and on cells of the immune system. Immune messages are conveyed by circulating lymphocytes, which are activated if antigens are presented to them from the lumen or if the mucosal epithelium is breached, and by the local release of cytokines. Neural messages are conveyed by neurons whose sensitive endings are in the lamina propria, beneath the mucosal epithelium, in the muscle, and in enteric ganglia. Some afferent neurons have cell bodies in the gut wall (intrinsic primary afferent neurons and intestinofugal neurons), and others are in extrinsic ganglia (extrinsic primary afferent neurons).

sensations of gastric or intestinal fullness, warmth, various types of pain (cramp, colicky pain, sharp pain), discomfort, nausea, hunger, and satiety, and *that which is not perceived*, such as the chemical nature of the contents of the intestines and the mechanical activity of the gut during normal digestion, which initiate responses such as gastric acid secretion, pancreatic secretion, gallbladder contraction, and gastric and intestinal motor activity.

The level of specificity and detail of the afferent information that reaches the central nervous system is not known. The central nervous system may be informed of the general state of the digestive organs by the summation of afferent signals. This is suggested by the substantial sizes of the receptive fields of the afferent neurons [2]. It is also suggested by the responses of the organs, for example acid or enzyme secretion, gallbladder contraction, or gastric relaxation, none of which appears to be finely graded or highly localized in the sense that, say, the movements of the fingers are finely controlled. Moreover, hormones such as cholecystokinin (CCK) that diffuse locally and in the bloodstream act on many afferent nerve endings. Consciously perceived sensations that are conducted by extrinsic primary afferent neurons are also generalized and usually difficult to locate to any particular abdominal organ. Satiety is indirectly perceived in that one feels sated after a meal but the feeling of satiety is not directed to a particular organ.

Conscious perception of the state of the gastrointestinal tract is subject to psychosensory modulation [3–5]. Perception is enhanced when attention is paid to the gut and is diminished with inattention. Sensation is also diminished when a painless somatic stimulus is simultaneously applied [3]. A classification of extrinsic primary afferent neurons by the stimuli to which they respond, by the sensations to which they give rise, and by the functions they regulate is shown in Table 3.1.

Structural studies

Large numbers of peripheral axons of extrinsic primary afferent neurons enter the gut. In animals such as cat and rabbit, the abdominal vagus contains about 20 000–30 000 primary afferent nerve fibers; the human abdominal vagus contains about 40 000–50 000 [6]. Spinal afferent fibers are also plentiful: in the greater splanchnic nerve of the cat, there are about 3000–4000; in the lumbar splanchnic nerves, about 4000–5000; and in the pelvic nerves, about 3500 [6]. Almost all of the afferent fibers entering the gut are unmyelinated

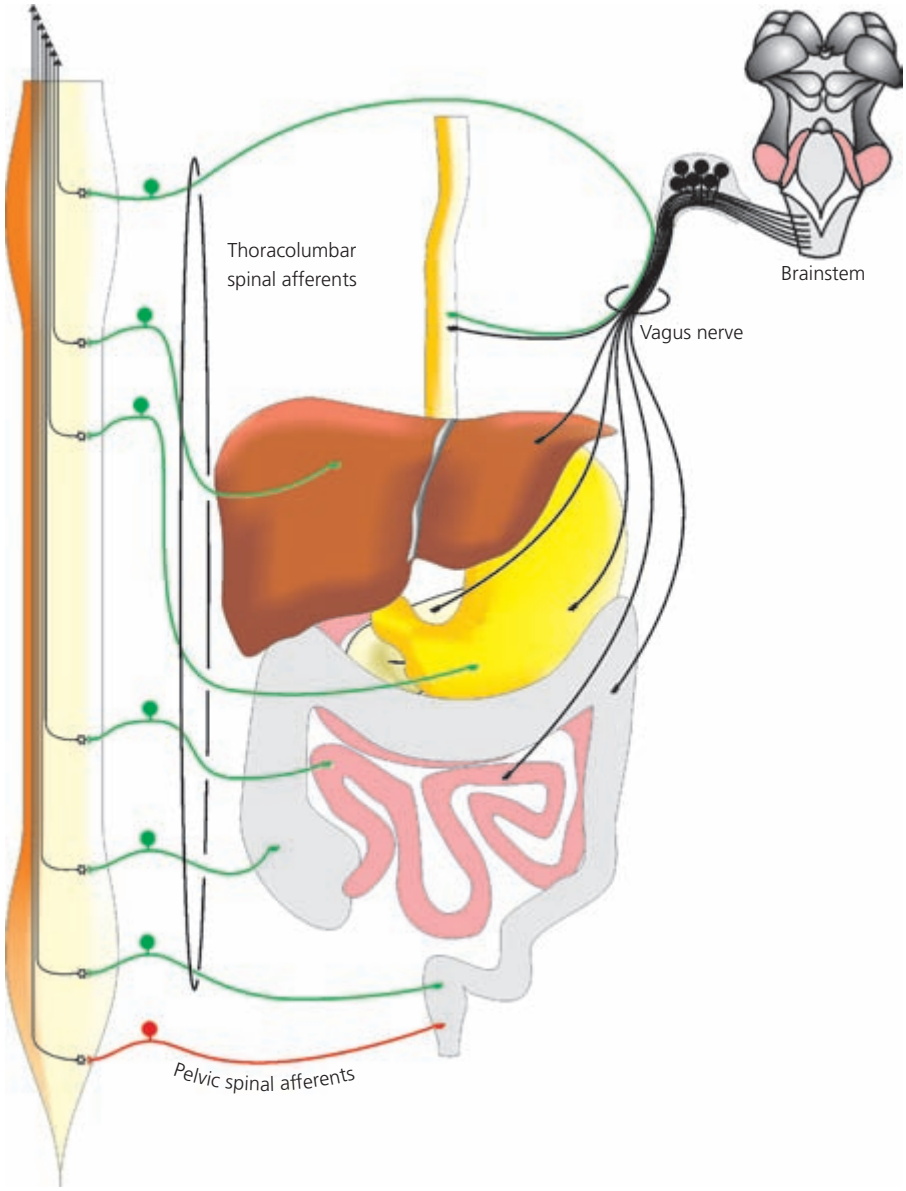

Figure 3.2 The extrinsic primary afferent neurons that supply the digestive system. These can be divided into three broad categories: vagal primary afferent neurons (black), thoracolumbar spinal primary afferent neurons (green), and pelvic spinal primary afferent neurons (red).

and they become mixed with the processes of enteric neurons, from which they are indistinguishable by normal light and electron microscopy, with the exception of the intraganglionic laminar endings (IGLEs).

Three types of vagal afferent endings have been identified, mostly by anterograde tracing studies: intramuscular arrays [7,8], branching varicose axon endings that innervate the mucosa [9,10], and IGLEs.

An intramuscular array consists of an axon that supplies branches, sometimes 10 or more, several millimeters long and running parallel to the longitudinal or circular muscle. They are most numerous in the stomach but are found throughout the gastrointestinal tract, from the smooth muscle part of the esophagus to the distal colon, where they are relatively rare [11]. The morphology of intramuscular arrays suggests that they are in-parallel length receptors, even though most physiological evidence suggests that vagal mechanoreceptors in the stomach are tension receptors [12].

Intraganglionic laminar endings were illustrated in 1929 by Lawrentjew [13], who showed, by their degeneration after vagal nerve section, that they are of vagal origin. Subsequently, their afferent nature was demonstrated when they were found to degenerate after extirpation of nodose ganglion cells but not after section of the vagus nerve central to the nodose ganglion [14]. The IGLE consists of numerous lamellae, generally about 2–5 μm across, that interconnect with each other and form a discontinuous covering of myenteric ganglia. In the rat stomach, about six neurons are

Table 3.1 Extrinsic vagal and spinal gastrointestinal afferent neurons: simplified classification of neurons, sensations, and regulated functions

Region	Afferent neuron	Sensations	Regulated function
Esophagus	V, mechanoreceptor (L)	None	Propulsion
	V, thermoreceptor	Heat	
	S, mechanoreceptor	Discomfort, pain	Regurgitation
	S, mucosal receptor	Unknown	
Stomach	V, mechanoreceptor (tension)	Fullness, satiety	Gastric motility, feeding
	V, mucosal receptor (chemical, acid?)[a]	None	Acid secretion, gastrin release
	V, hormone receptor	Satiety	Feeding
	V, hormone receptor	Nausea	Vomiting
	S, mechanoreceptor[a]	Discomfort, pain	Guarding, autonomic responses, illness behavior
	S, collaterals to arterioles	Unknown	Gastric protection
Duodenum	V, mechanoreceptor (L)	None	Gastric emptying, acid secretion
	V, chemoreceptor	None	Gastric and pancreatic secretion
	V, chemoreceptor	Satiety	Feeding
	S, mechanoreceptor	Discomfort, pain	Guarding, autonomic responses, illness behavior
	S, mucosal receptor[b]	Unknown	Involved in inflammatory response
Jejunum, ileum	V, mechanoreceptor (L)	None	Not known
	S, mechanoreceptor[a]	Discomfort, pain	Guarding, autonomic responses, illness behavior, enteroenteric inhibitory reflexes
Gallbladder	S, mechanoreceptor[a]	Discomfort, pain, biliary colic	Guarding, autonomic responses, illness behavior, enteroenteric inhibitory reflexes
Colon, rectum	S, mechanoreceptor[a] (L)	Fullness, urge to defecate	Defecation
	S, chemoreceptor	Urge to defecate	Defecation
	S, mechanoreceptor	Discomfort, pain	Guarding, autonomic responses, illness behavior, enteroenteric inhibitory reflexes
Anal canal	S, mechanoreceptor, chemoreceptor	Touch, presence of feces	Defecation
	S, thermoreceptor, nociceptor	Discomfort, pain	Guarding

a These and probably other receptors are sensitized and become reactive with inflammation, irritation, and mucosal damage.
b Spinal afferent mucosal receptors are found throughout the small and large intestines.
L, low threshold receptor; S, spinal; V, vagal.

covered by each IGLE [7]. Experiments have confirmed that IGLEs are mechanoreceptors [15]. Common in the esophageal, gastric, and duodenal myenteric ganglia, IGLEs occur at declining densities along the digestive tract until the rectum, where they are again common [11,16,17]. The IGLEs in the rectum arise from pelvic spinal afferent neurons. In rat, IGLEs have been reported to occur at a density of 6.3/mm^2 in the gastric corpus, 3.3/mm^2 in the duodenum, 0.6/mm^2 in the jejunum and ileum, and 0.2/mm^2 in the distal colon [11]. The proportion of ganglia innervated also decreases along the digestive tract until the rectum. Labeling by left vagal ganglion injection shows that IGLEs supply 88% of ganglia in the esophagus and 31% in the corpus; the proportion decreases to 1% in the distal colon [18,19]. The density of supply in the rectum is about 60 times that in the colon [17].

The mucosal vagal afferent endings consist of branching varicose fibers that come close up against the mucosal epithelium, and, in the esophagus, may enter the epithelium [9,10,20,21]. Morphologically, these fibers seem indistinguishable from the motor fibers and intrinsic afferent endings that supply the epithelium.

Less attention has been devoted to determining the identities of spinal afferent neurons with the use of neuronal tracers. In some species, however, a high proportion of spinal afferent neurons contains neuropeptides, particularly tachykinins and calcitonin gene-related peptide (CGRP) [22]. Where spinal nerve endings containing these peptides can be distinguished from nerve fibers of intrinsic origin, the peptides have proved to be useful markers. The axons of spinal afferent neurons provide a sparse network of varicose axons in the myenteric ganglia of the esophagus [23], stomach

[22,24], and small intestine [25]. They are prominent around arterioles in the gut wall [22,23,26,27] and they also branch within the lamina propria of the mucosa throughout the gastrointestinal tract. Rare fibers are found in the muscle layers [22,24,28]. In the cat esophagus, they penetrate deep into the squamous epithelium [23].

Functional subtypes identified by location of afferent endings

Extrinsic primary afferents have been classified as mucosal receptors, muscle receptors, and serosal receptors on the basis of electrophysiological studies of the response patterns to various mechanical and chemical stimuli. The location of sensory endings – superficial (in the mucosa or the serosa) or deep (in the muscle or myenteric ganglia) – can be determined by testing the effect of removing or anesthetizing the mucosa or serosa. Electrophysiological studies, with the use of single-unit recording, imply that most vagal primary afferent fibers innervate the mucosa and muscle layers, whereas the spinal afferent fibers innervate the muscle layers and serosa but not the mucosa [29–31]. However, morphological data show that the axons of spinal primary afferent neurons innervate the mucosa of the esophagus, stomach, and intestines. These spinal afferents may have nonmechanoreceptor functions.

Muscle and myenteric ganglion receptors

Receptors that are sensitive to variations in muscle tension are found throughout the gastrointestinal tract [6]. These are almost always referred to as muscle receptors, although it is now clear that IGLEs are intimately associated with myenteric ganglia, even though they respond to muscle tension [17,32]. Increased discharge when the muscle contracts (i.e., when tension is increased but length is decreased) implies that most muscle receptors are tension receptors [33,34]. Muscle receptors exhibit a dynamic response that adapts slowly. Most vagal mechanoreceptors that innervate the gastric muscle fire at low frequency even when the stomach is empty. Thresholds for the activation of muscle receptors vary within the normal (i.e., nonpathological) range of pressures [35,36]. The same neurons may transmit noxious stimuli to the central nervous system in response to supraphysiological tensions.

Spinal afferent neurons include low- and high-threshold muscle receptors and silent nociceptors [37–39]. Silent nociceptors are neurons whose endings are not activated by mechanical forces, except when the tissue they innervate is injured or inflamed. Persuasive evidence suggests that the term "silent nociceptor" is a misnomer for most fibers so identified because these fibers, although unresponsive to intense mechanical stimuli, may be responsive to other (untested) stimuli; furthermore, not all mechanically insensitive neurons are nociceptors [40]. High-threshold muscle receptors of spinal primary afferent neurons, which are sensitive to ischemia, are likely to be nociceptors [37,38]. Spinal mechanoreceptive afferent neurons are generally polymodal. Afferent neurons that respond to colonic distention also respond to bile salts, inflammatory mediators (a mixture of bradykinin, prostaglandin, 5-hydroxytryptamine [5-HT], and histamine), and temperature [41]. These neurons may in fact be mucosal receptors or have collaterals in the mucosa.

Serosal receptors

Serosal receptors seem to be activated by high-threshold mechanical stimuli, such as bowel movement, distention, and squeezing, but responses to gentle probing, light squeezing or puffs of air have also been reported [6]. Serosal receptors are also sensitive to ischemia. The serosa is continuous with the mesentery and the parietal peritoneum, where receptors with similar properties reside.

Mucosal receptors

Mucosal receptors are found all along the gut. They respond vigorously to mucosal deformation but are likely to be polymodal – that is, able to respond to two or more stimuli, including mechanical deformation (stroking), lumenal chemicals (acid or nutrients), heat or cold, and, in some cases, increased lumenal osmolarity [6,42–46]. In contrast, studies in cats suggest that some mucosal receptors in the esophagus and stomach are selective for temperature [47], and that others in the small intestine are selective for nutrients, such as amino acids [48] and lipids [49]. This apparent selectivity, however, may be a consequence of testing a narrow range of stimuli [6,31]. A study of sheep showed that duodenal mucosal endings that respond to amino acids are also mechanoreceptors [44]. Nerve fiber recording did not reveal mucosal receptors that are sensitive to carbohydrates in rat [50], rabbit [51] or ferret [46] small intestine. Nevertheless, infusions of glucose, maltose, and mannose into the duodenum evoke reflex responses in rat stomach and pancreas by activating vagal afferents [52,53], implying that carbohydrate-sensitive endings are indeed present. Both slow and rapid adaptation to mucosal mechanical stimuli are encountered [6,44]. Responses to lumenal chemicals are generally slowly adapting [48,49]. Although present in the stomach and esophagus, thermal receptors appear to be absent from the duodenum [44].

Indirect activation of mucosal receptors by enteroendocrine cell products

The stimulatory effects of nutrients on mucosal afferent endings in the small intestine are indirect, which is in accord with the separation of the lumenal environment from mucosal nerve endings by the intestinal epithelium. The alternative method of activation, directly after passage of lumenal chemicals across the mucosa, probably also occurs (e.g., diffusion of inorganic acid). CCK and 5-HT, which are released in the mucosa, activate the mucosal receptors but

not the muscle receptors of vagal primary afferent neurons [46,54–57]. A physiological role for CCK released from enteroendocrine cells and acting on vagal afferent endings has been demonstrated; the activation of vagal primary afferent neuron endings by lipid or protein in the duodenum is blocked by CCK_1 receptor antagonists [58,59]. 5-HT appears to be an intermediate in the excitation of vagal primary afferent neurons by intralumenal hyperosmotic or hypoosmotic solution in the duodenum and by high concentrations (> 275 mM) of the monosaccharides glucose and mannose [53]. Increased discharge of vagal afferent neurons caused by these agents was blocked by the intravenous infusion of the $5\text{-}HT_{3/4}$ antagonist tropisetron, and by the $5\text{-}HT_3$ antagonist granisetron. In contrast, activation of afferent fibers by duodenal distention was not affected. The intraduodenal chemical stimuli did not elevate plasma 5-HT, implying that the site of action of 5-HT was in the mucosa near its points of release. The activation of afferents mediating vagovagal pancreatic reflexes depends on the release of both CCK and 5-HT from enteroendocrine cells [60].

Lumenal fat is a stimulus for reflexes that travel from the distal to more proximal ileum, the ileal brake [61]. The release of peptide YY from mucosal enteroendocrine cells of the mid and distal small intestine and colon leads to slowing of small intestinal transit. Although the neural pathway of this effect is not completely determined, it appears to be a neural reflex that passes through sympathetic ganglia [62].

Efferent effects of primary afferent neurons

Ultrastructural studies show that afferent fibers contain numerous synaptic vesicles, similar to the endings of unmyelinated motor neurons or interneurons [20,63]. The axolemma of these fibers is sometimes naked, which suggests that neurotransmitters released by afferent fibers have good access to effector cells. Myenteric neurons are a target because frequent appositions of naked fibers arising from spinal primary afferent neurons occur on the cell bodies [63]. The afferent nerve endings contain bioactive peptides, including CGRP and tachykinins [22,25]. Glutamate, the primary transmitter at the central ends of primary afferent neurons, may also be released at their peripheral ends.

Several pharmacological and physiological studies have revealed muscle contraction in response to antidromic stimulation of spinal afferent nerve fibers [64,65], and one study has implicated axon reflexes in the initiation of the peristaltic reflex (see Chapter 2). Neuropeptides released from primary afferent nerve endings cause vasodilation [66–68] and stimulate myenteric neurons [69] and mast cells [70].

The effects of antidromic release of transmitter from afferent nerve endings on gastric and duodenal motility are most likely of trivial physiological importance [71]. By contrast, vasodilation caused by transmitter released from the peripheral processes of afferent neurons has an important role in mucosal protection (see section on tissue protection).

Roles of extrinsic primary afferent neurons

Extrinsic primary afferent neurons signal to the central nervous system information that is necessary to regulate organs and behaviors beyond the immediate local territories of the afferent endings. The signals that reach the central nervous system convey information about several qualities of the gastrointestinal tract, including the state of distention, the presence of chemicals in the lumen (e.g., nutrients, irritants, and toxins), and the presence of tissue injury and inflammation. The signals can be decoded and interpreted consciously as satiety, pain, or hunger. They can also be used to direct digestive functions automatically: for example, esophageal propulsion, gastric relaxation in response to a meal, and gastric acid secretion can all be controlled through the vagus; defecation is controlled by the pelvic nerves; and water and electrolyte transport and blood flow, in relation to the relative needs of all organs, is regulated by sympathetic motor pathways.

Pain and discomfort

Innervation of the gastrointestinal tract by pain fibers was long considered doubtful, because clearly injurious insults to the stomach, small intestine, and most of the large intestine are not perceived [6,72]. Conversely, there is conscious sensation from the esophagus (e.g., hot food or drink) and from the anal canal. Pain from the abdomen is diffuse, and its origin from a particular organ or site is usually not obvious.

However, it is now clear that the gastrointestinal tract is an origin of pain. Excessive distention (i.e., intralumenal pressure ≥ 30 mmHg) evokes pain and inflammation exacerbates it [73]. In humans, excessive distention of the stomach, gallbladder, small intestine, colon, or rectum causes pain that is not felt after bilateral section of the splanchnic (spinal afferent) nerves, which carry sensations of pain or discomfort from the abdominal viscera [74,75]. However, although pain from the gastrointestinal organs is mostly conducted through spinal afferent neurons, nociceptive information, notably from the esophagus, can also be conducted by vagal afferents [76]. The quality of the sensation from the digestive tract changes with the intensity of distention, i.e., from feelings of fullness, to discomfort, to pain [6].

Pain signals are believed to be mediated through the high-threshold activation of mechanoreceptors and, in pathological conditions, through afferent fibers that are unresponsive to pressures above the pain threshold in nonpathological conditions (e.g., unresponsive to a lumenal pressure of up to 70 mmHg). These unresponsive neurons (silent nociceptors) become mechanosensitive when tissue is inflamed or when they are exposed to inflammatory mediators [39]. At moderate pressures, low-threshold mechanosensitive afferent neurons are activated to cause reflexes that are unnoticed by a subject without inflammation or other gut pathology. Greater distention continues to activate these neurons and also activates higher threshold mechanoreceptors and

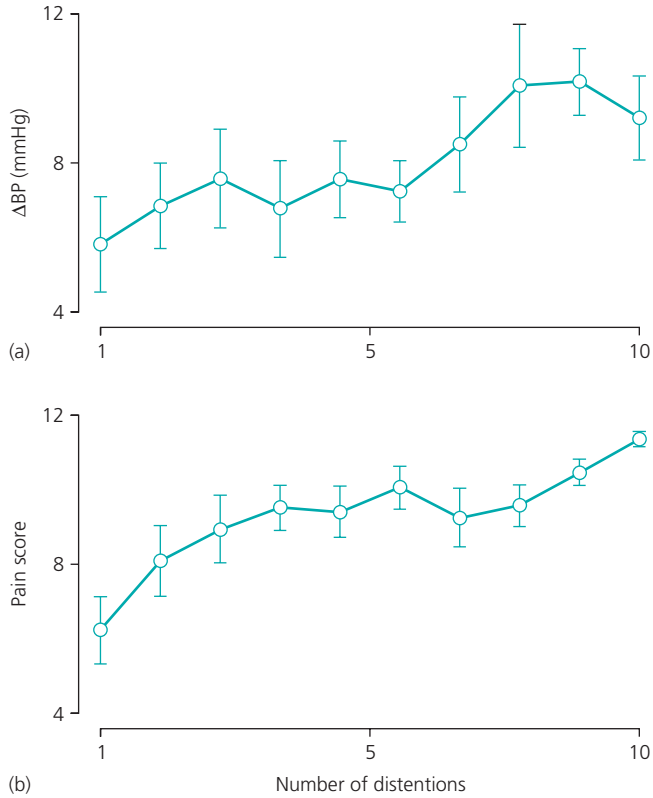

Figure 3.3 Changes in blood pressure **(a)** and psychometric measure of pain perception **(b)** in human volunteers subjected to inflation of an intrarectal balloon. Successive inflations of the balloon cause more pronounced increases in blood pressure (a pseudoaffective response) and increased discomfort or pain. From Ness et al. [189], with permission from the International Association for the Study of Pain.

evokes feelings of fullness. Further distention can lead to discomfort and pain. Painful sensations are more likely, and occur at lower thresholds, if the gut is inflamed or if a functional bowel disorder exists, such as irritable bowel syndrome (IBS). This is because the nerve endings in inflamed tissues and in tissue affected by IBS are sensitized. In general, low-threshold primary afferent endings are vagal in origin, and high-threshold nerve endings are of spinal origin. However, some spinal primary afferent neurons are activated by low-threshold mechanical stimuli [37,77,78].

Visceral pain is accompanied by somatic and general autonomic reactions, including hunching, abdominal contraction, elevated heart rate, and increased blood pressure. In humans, the intensity of these reactions is directly related to the degree of pain (Fig. 3.3). The somatic and autonomic accompaniments to pain persist when pain itself is prevented by anesthesia. Because they are indications of a stimulus that would normally come to conscious attention, the somatic and autonomic responses observed in a subject under general anesthesia have been called pseudoaffective responses [72,79]. Animal studies confirm that pain is conducted from the intestine through spinal afferent pathways. The neurons are rendered unresponsive by pretreatment with capsaicin, which, acting through type 1 transient receptor potential vanilloid (TRPV1) receptors, initially stimulates and then desensitizes the endings of primary afferent neurons. In newborn animals, and at high doses in older animals, capsaicin can cause degeneration of primary afferent neurons.

Chronic changes in the sensitivity of the intestine in IBS manifest as a decreased pain threshold to intestinal distention and exaggerated pseudoaffective responses [80–82]. Patients with IBS can feel motility changes that healthy individuals rarely detect. Moreover, contractions within the normal range of amplitude sometimes cause pain in patients with IBS [83,84]. These and other observations imply a long-lasting increase in the sensitivity of spinal primary afferent mechanisms in IBS.

Nausea and vomiting

The activation of vagal afferent fibers contributes to the nausea and vomiting induced by a number of agents, notably chemotherapy agents such as cisplatin. In animal models, retching or bursts of synchronous activity in respiratory motor neurons are indicators of nausea or vomiting activity. In ferrets, retching and emesis induced by cisplatin or cyclophosphamide were abolished by combined subdiaphragmatic vagotomy and splanchnic nerve section, and were substantially reduced by 5-HT_3 receptor antagonists [85]. However, 5-HT_3 receptor antagonists did not block retching caused by electrical stimulation of the central end of the severed vagus, leading to the hypothesis that the relevant 5-HT receptors are located peripherally, likely on the endings of vagal afferent neurons [86]. Subsequent demonstration of 5-HT release by nauseants led to the hypothesis that toxins that induce nausea and vomiting do so by provoking the release of 5-HT from enterochromaffin cells in the proximal small intestine [87]. The 5-HT then stimulates vagal afferent nerve endings, which take signals to the rather loosely defined medullary vomiting centers, including the area postrema and nucleus tractus solitarius. Consistent with the animal studies, 5-HT_3 receptor antagonists effectively reduce chemotherapy-induced and postoperative nausea and vomiting in humans, their effectiveness as a treatment for vomiting being more profound [88–90]. Vagally mediated nausea and vomiting are also inhibited by type 1 neurokinin (NK_1) receptor antagonists, which act at the central end of the vagal pathway [90].

Satiety

The ingestion of food is dependent on feelings of hunger and satiety, as well as on social and cultural influences. The physiological control center for ingestive behavior is in the hypothalamus and is influenced by circulating hormones (e.g., ghrelin, leptin), olfactory and taste receptors, and

afferent nerve signals conducted from the digestive system to the central nervous system through the vagus nerves. The vagal signals for satiety relate to the volume of food in the stomach and to the chemical nature of the food products in the duodenum. An animal whose food is diverted by an esophageal cannula and never reaches the stomach provides a model for investigating control through vagal afferents. Studies in such sham-fed animals indicate that gastric distention has a satiating effect, and that the inhibition of eating caused by distention is lost after vagus nerve section [91,92]. Studies in which the movement of gastric contents is prevented and the composition of the contents is varied indicate that gastric volume, not the nutrients present, is signaled by the vagus [93,94]. Once the food has entered the duodenum, its nutrient content becomes a factor in influencing satiety. Thus, fat in the duodenum releases CCK from enteroendocrine cells, and the CCK, through CCK_1 receptors, activates vagal endings and feeding is reduced [95]. CCK acts on mechanoreceptive tension receptors, presumably muscle receptors, as well as on mucosal chemosensitive receptors [43,96]. Other peptides of gut origin may have roles in determining satiety, possibly through actions on the vagus. These include bombesin, glucagon-like peptide, and ghrelin [95,97].

Vagovagal reflexes

Stimuli resulting from the ingestion of food, and the presence of food in the stomach and upper intestine, activate vagovagal reflexes that regulate the activity of the stomach itself and the activity of the pancreas and gallbladder. These reflexes use afferent neurons that end in the nucleus tractus solitarius of the medulla oblongata, integrating circuits within the brainstem, and outputs through vagal efferent neurons that form synapses in enteric ganglia, from which neural connections are made with final effectors (smooth muscle or glands). Vagal afferent endings in the esophagus that are stimulated by swallowing cause a reflex relaxation of the proximal stomach, which is called receptive relaxation (i.e., a relaxation in advance of the arrival of the food bolus) [98]. The arrival of food in the stomach triggers another reflex through the vagus, the vagovagal accommodation reflex, which involves relaxation of the proximal stomach as well as acid secretion and increased contractile activity in the antrum [99,100]. A duodenal phase of vagovagal gastric relaxation is triggered by CCK, which is released in response to nutrients, particularly fat, in the lumen of the duodenum [59,101]. CCK acts on the peripheral ends of primary afferent neurons in the mucosa, leading to relaxation of the stomach and thus slowed gastric emptying. The presumed function of this reflex is to allow time for the assimilation of nutrients in the small intestine.

Afferent signals from the proximal stomach trigger transient lower esophageal sphincter relaxations through vagovagal reflexes. These have a role in permitting the escape of gas from the stomach [102]. However, they also contribute to the pathology of gastroesophageal reflux disease, and thus considerable effort has been expended to find ways to inhibit the activity of the receptors on these afferents [103,104].

Pancreatic function is also influenced by vagovagal reflexes. Nutrients in the upper intestine and stroking of the intestinal mucosa increase pancreatic secretion. This effect is reduced by treatment with capsaicin or by blocking efferent transmission in the pancreas with atropine [105,106]. Distention of the body of the stomach, but not the antrum, also increases pancreatic enzyme secretion, again through a vagovagal reflex [107]. Vagovagal reflexes also contribute to the conversion of intestinal motility from the fasted to the fed pattern [108]. The gallbladder is also primarily controlled through the vagus, rather than through enteric reflexes [109].

Reflexes mediated through spinal primary afferent neurons

The nonnociceptive spinal afferents of the terminal bowel that are concerned with visceral reflexes (pelvic spinal afferent neurons, Fig. 3.2) are analogous to the vagal afferents involved in visceral reflexes [110]. Pelvic spinal afferents participate in the control of fecal continence and defecation; thus, injuries to the pelvic nerves, which carry many of the spinal primary afferent fibers from the descending colon and anal canal, cause derangements of defecation [111]. Defecation reflexes are initiated by rectal distention and by chemical or mechanical irritation of the rectoanal mucosa, implying that both muscle tension receptors and mucosal spinal afferent nerve fibers are involved in these reflexes [112].

The section on intestinofugal neurons discusses enteroenteric inhibitory reflexes that pass through prevertebral ganglia without entering the central nervous system. There are also intestinointestinal reflexes, passing through the central nervous system, in which spinal afferent neurons form the afferent arm and sympathetic pathways are the efferent pathway [113]. These pathways can be activated by distention and also by irritation within the abdominal cavity, such as handling of the intestine, or by infection of the abdominal cavity. It has been suggested that the reflex inhibition of motility through these spinal afferent pathways and through the prevertebral ganglia may facilitate each other, because synaptic excitation converges on the noradrenergic neurons in the prevertebral ganglia [114].

Tissue protection

Neurotransmitters released from the peripheral ends of extrinsic primary afferent neurons may effectively restrict the deleterious consequences of tissue damage [115]. In the stomach, pretreatment with capsaicin compromises sensory neuron function and aggravates mucosal damage caused by acid, ethanol, nonsteroidal antiinflammatory agents, and other chemicals [116–119]. Capsaicin alone is not injurious;

Table 3.2 Pharmacological profile of extrinsic afferent endings and their therapeutic targets

Molecular target, compounds	Nature of afferent	Therapeutic purpose	References
5-HT_3 receptors: granisetron, odansetron	Upper gastrointestinal tract	Vomiting, nausea	Sanger and Andrews, 2006 [90]
GABA-B receptor agonists	Gastroesophageal junction	Treatment of gastroesophageal reflux disease	Blackshaw, 2001 [103], Cange et al., 2002 [176]
TRP (transient receptor potential), TRPV1 channels: antagonists, e.g., capsazepine	Pain afferents	Irritable bowel syndrome	Chan et al., 2003 [177], Sugiura et al., 2004 [178]
NMDA receptors: peripherally restricted antagonists	Pain afferents	Irritable bowel syndrome	McRoberts et al., 2001 [179]
Somatostatin receptors: agonists, e.g., octreotide	Pain afferents	Irritable bowel syndrome	Bradette et al., 1994 [180], Schwetz et al., 2004 [181]
Purine P2X receptors	Pain afferents	Inflammatory pain	Wynn and Burnstock, 2006 [182]
Bradykinin receptors	Pain afferents	Irritable bowel syndrome, inflammatory pain	Longhurst et al., 1984 [183], McLean et al., 1998 [184]
Cholecystokinin 1 receptor agonists	Satiety afferents	Obesity (inhibition of food intake)	Barrachina et al., 1997 [185]

Other receptors and ion channels on the endings of extrinsic afferent neurons include cannabinoid CB1 receptors, peptide YY receptors, acid-sensitive ion channels, galanin receptors, leptin receptors, and ghrelin receptors [82,186–188].
GABA, γ-aminobutyric acid; NMDA, *N*-methyl-D-aspartate.

in fact, the short-term effect of capsaicin is protective, primarily through the capsaicin-induced release of the sensory neurotransmitter, CGRP, from spinal afferent nerve endings [115]. Moreover, blocking CGRP receptors, or reducing the effectiveness of CGRP by immunoneutralization, diminishes gastric protection [120,121]. Application of capsaicin to the spinal afferent pathways, but not to the vagus nerves, blocks the CGRP-dependent hyperemic response to the acute application of capsaicin, indicating that gastric protection is dependent on the integrity of spinal afferent neurons [122]. Because the protection is reduced by the nerve conduction blocker tetrodotoxin applied to the stomach, axon reflexes are likely to be involved (i.e., action potential invasion of collateral branches of activated nerve endings) [117]. Tachykinins are often colocalized with CGRP in the gastric spinal afferent neurons, and data indicate that tachykinins, released from the spinal primary afferent endings and acting through NK_2 receptors, contribute to gastric protection [123]. The protective mechanism involves vasodilation and increased mucosal blood flow; CGRP and tachykinins cause vasodilation by releasing nitric oxide from vascular endothelial cells and, thus, inhibition of nitric oxide synthase increases gastric damage [124]. Transmitter release from afferent endings in the colon also reduces the severity of damage caused by inflammation, at least in the acute phase [125].

Pharmacology of receptors and ion channels on extrinsic afferent endings

The receptors and ion channels expressed by the endings of afferent nerves are potential therapeutic targets [82,126]. 5-HT_3 receptors on the endings of vagal afferents in the gut have been targeted in the treatment of nausea and vomiting, particularly when induced by cancer chemotherapy [90]. γ-Aminobutyric acid (GABA)-B receptors have been identified as targets to inhibit vagovagal reflexes, thereby antagonizing vagovagal lower esophageal relaxation, which is the major cause of gastroesophageal reflux disease [103]. Other therapeutic targets are listed in Table 3.2.

Intrinsic primary afferent neurons

Intrinsic primary afferent neurons are the first neurons of motility, secretomotor and vasomotor reflexes in the small intestine and colon [127]. In these regions, the enteric nervous system is capable of controlling such functions in the absence of connections with the central nervous system. By contrast, the nerve circuits for reflexes that control motility, secretion, and blood flow in the stomach pass through the central nervous system. Esophageal functions are controlled through the brainstem, and the neural control of the pancreas and gallbladder is also extrinsic.

Neurons with Dogiel type II morphology have been identified as IPANs in the small intestine, and these neurons are also common in the large intestine [128]. However, they are absent from the esophagus, proximal stomach, and gallbladder, and are rare in the gastric antrum. Studies of neurons in the pancreas have not clarified whether Dogiel type II neurons are present. Although analysis of the circuitry in the small intestine indicates that the first neurons in the reflex pathways are Dogiel type II neurons, it is possible that other neurons in the circuits also respond to their physical and chemical environment. In fact, research suggests that reflexes in the colon can be initiated through other types of neuron [129].

Properties of intrinsic primary afferent neurons

Dogiel type II neurons are multiaxonal, with one or more axons that lead to and branch in the lamina propria of the mucosa, just beneath the absorptive epithelium, and axons that enter the enteric ganglia and supply terminals around several types of nerve cells, including other IPANs, interneurons, and motor neurons. In addition, these neurons have distinct electrophysiological properties. They have broad action potentials that are carried by both Na^+ and Ca^{2+} currents and are followed by early and late afterhyperpolarizing potentials. These neurons, unlike all other enteric neurons (at least in the guinea pig small intestine), do not receive prominent fast excitatory postsynaptic potentials. In other regions and species, fast excitatory postsynaptic potentials are more readily recorded from presumed IPANs. However, they do receive synaptic inputs through which slow excitation is mediated. This is unusual because neither spinal nor vagal afferent neurons receive synapses at their cell bodies [130], although the cell bodies of mesencephalic trigeminal primary afferent neurons do receive synapses [131]. Thus, IPANs differ from extrinsic sensory neurons of the digestive tract in that their excitabilities can be modified by synapses at the cell body.

Chemosensitive intrinsic primary afferent neurons

Intracellular recordings taken from IPANs in the guinea pig small intestine show that IPANs respond to chemicals, such as inorganic acid and short-chain fatty acids at neutral pH, applied to the lumenal surface of the mucosa of the small intestine [132,133]. Application of a solution with a low Ca^{2+} and a high Mg^{2+} concentration did not prevent synaptic transmission. Thus, IPANs are not activated indirectly through other neurons that react to stimulation. Activation of submucosal and myenteric neurons by the application of glucose to the mucosa has also been reported [134].

Intrinsic primary afferent neurons probably detect changes in the chemical content of the gut lumen indirectly, through the release of hormones from enteroendocrine cells. Indirect action is inferred because the mucosal epithelium separates the nerve endings from the lumenal environment. Substantial evidence suggests that 5-HT, a potent IPAN stimulant, acts as an intermediate in enteric reflexes. 5-HT is released when the mucosa is mechanically stimulated to elicit motility reflexes, and the reflex responses are antagonized by drugs that block 5-HT receptors [135–137]. Other hormones in gut endocrine cells, such as CCK and motilin, are released by nutrients and act on neurons; however, their roles as reflex intermediaries are untested. Research suggests that ATP released from mucosal epithelial cells can activate chemosensitive IPANs [138].

Stretch-sensitive intrinsic primary afferent neurons

Intrinsic primary afferent neurons also respond to tension in the muscle and to direct distortion of their processes [139–141]. The neurons respond phasically at the beginning of a stretch or when their processes are directly deformed [140]. During maintained stretch, the muscle contracts more or less rhythmically, and IPANs continue to be activated [139]. The rate of discharge is proportional to the degree of distention. However, the ongoing discharge of action potentials is abolished if the muscle contraction is prevented by muscle relaxants, indicating that active tension in the muscle contributes to the excitation of the tension-sensitive IPANs. The involvement of the muscle is interesting because it has long been known that intestinal muscle cells are directly sensitive to stretch and respond to it by contracting [142]. This reaction of the smooth muscle may be integral to the response of IPANs during sustained stretch. The neurons themselves possess mechanosensitive ion channels, and when the processes of the neurons are distorted, they discharge action potentials [140,141,143].

Interestingly, IPANs are inhibited if their cell bodies are distorted or if a small area of their surface membrane is stretched [140]. This occurs through the opening of BK-type K^+ channels on the cell soma, which appear to be directly stretch-sensitive. Myenteric nerve cell bodies are distorted by muscle movement [144], and it is possible that sufficient pressure in the intestinal wall may inhibit IPANs as part of a protective mechanism that limits the strength of reflex contraction of the intestine.

Mucosal mechanoreceptors

Functional evidence for IPANs with cell bodies in submucosal ganglia comes from experiments that have localized the activity-dependent induction of c-Fos and the activity-dependent uptake of styryl dyes. c-Fos immunoreactivity was detected in submucosal nerve cells after mucosal distortion by puffs of nitrogen gas ejected from a pipette [145]. Styryl dyes, which are taken up by active neuron endings and transported back to the cell bodies, have also been used to identify IPANs that are mucosal mechanoreceptors [134]. The results suggest that cell bodies of mucosal mechanoreceptors are in submucosal ganglia and project to the myenteric plexus. The activation of mucosal mechanoreceptors is

likely to be partly indirect, through the release of 5-HT from enterochromaffin cells in the mucosa [146]. Mechanical stimulation of the mucosa induces c-Fos expression in IPANs in submucosal ganglia, and this induction is blocked by 5-HT receptor antagonists [145].

Distention stimuli can activate both mucosal mechanoreceptors and distention-sensitive neurons (myenteric IPANs), explaining why both myenteric and submucosal neurons are activated by distention [134]. The presence of stretch-sensitivity of neuron cell bodies in the submucosal ganglia, similar to that observed in myenteric neurons, has not yet been determined.

Possible polymodal nature of intrinsic primary afferent neurons

It appears that intrinsic afferent neurons may be modality preferring, rather than modality specific [128]. For example, primary afferent neurons in myenteric ganglia that respond to chemicals applied to the mucosa can also respond to mechanical stimulation of the mucosa [133,147], whereas, under some circumstances, mechanical stimulation excites only neurons with cell bodies in submucosal ganglia [134].

Physiological roles of intrinsic primary afferent neurons

Intrinsic reflexes that affect motility, water and electrolyte secretion, and blood flow all occur in the small intestine (for details of the reflex circuits, see Chapter 2). Each of these reflexes is evoked by similar stimuli, although it is not known whether the same, different, or overlapping populations of IPANs contribute to motility, secretomotor, and vasomotor reflexes.

Muscle motor reflexes have been studied as stereotypical responses of the circular muscle; that is, excitation oral and relaxation aboral can be evoked by distention of the muscle (effected without distorting the mucosa), by the application of chemicals to the mucosal surface, and by distortion of the mucosa [148–150]. These reflexes occur after the degeneration of extrinsic afferent nerve endings and therefore are the consequences of IPAN activation by sensory stimuli (see Chapter 2).

Secretomotor reflexes are initiated physiologically by chemical or mechanical interaction of the lumenal contents with the mucosa, or pathologically by toxins, such as cholera toxin or enterotoxins, in the lumen [151–153]. Enteric reflexes also cause HCO_3^- secretion in response to duodenal acidification [154]. The enteric secretomotor circuits consist of IPANs with their endings in the mucosa and nerve circuits that pass through the myenteric and submucosal plexuses and feed back to secretomotor neurons with cell bodies in the submucosal ganglia [128,155]. The secretomotor neurons stimulate the epithelial cells to pump Cl^- ions, which are accompanied by water, into the lumen.

Local vasodilator reflexes in the small intestine are caused by mechanical or chemical irritation of the mucosa, and substantial evidence indicates that the vasodilator neurons are intrinsic to the intestine and transmission from them is predominantly noncholinergic [156–158]. It is presumed that the first neurons in these reflexes are the IPANs, but this has not been directly shown. In fact, of the reflexes in the intestine, the vasomotor reflexes are the least studied. The same motor neurons have axons that branch to supply both the secretory epithelium and arterioles; thus, some secretomotor and vasodilator reflexes may share the same final neurons (see Chapter 2). This makes physiological sense because a proportion of the secreted water and electrolytes comes from the vasculature [128].

In summary, the roles of IPANs are to signal changes in the state of the intestine that are the consequences of the presence and nature of its contents. The information is conveyed to other neurons of the enteric nervous system that integrate the information and cause appropriate changes in mixing and propulsive activity, in water and electrolyte transport, and in local blood flow.

Intrinsic primary afferent neurons as nociceptors

In the small intestine and colon, protective secretory and motility responses are initiated by irritants that are included in enemas, by toxins, by bacterial products, and by parasitic infestations. The intestine exhibits exaggerated secretomotor reflexes, which are manifested as diarrhea, when excessive levels of bacteria and bacterial toxins overwhelm the gut lumen [153,159]. Powerful propulsive reflexes are triggered by bacteria or parasites in the gut lumen to expel these organisms [160–163]. Thus the IPANs appear to act as detectors of noxious conditions in the gut, in addition to reacting to benign stimuli to elicit enteric reflexes. The plant lectin IB4 appears to bind specifically to nociceptive neurons, but not to nonnociceptive neurons, in dorsal root and nodose ganglia [164,165]. It is thus significant that enteric IPANs also have high-affinity binding sites for IB4 [166].

Intestinofugal neurons

Intestinofugal neurons are an unusual type of neuron. Their cell bodies are in the gut wall, and their processes run toward the central nervous system and form synapses in prevertebral sympathetic ganglia. Their presence was deduced by Kuntz and Saccomanno in the 1930s [167], who found that distention of one region of the gastrointestinal tract inhibits motility in other regions and that these enteroenteric inhibitory reflexes persist after connections with the central nervous system are severed, provided that the integrity of the connections with prevertebral sympathetic ganglia is maintained. Methods to study these reflexes in vitro were developed in the early 1970s [168], and, since that time, the organization of the pathways has been studied in considerable detail [114,128]. The location of the cell bodies of intestinofugal

neurons in the myenteric ganglia of rats and guinea pigs was determined by injecting retrogradely transported dye into prevertebral ganglia [169–172]. In pig, some intestinofugal neurons have cell bodies in submucosal ganglia [173]. Intestinofugal neurons are most numerous in the large intestine; in the small intestine they increase in number distally, and they are rare in the stomach. The axons of intestinofugal neurons form excitatory synapses with the cell bodies of those sympathetic neurons in the prevertebral ganglia that regulate motility and secretion. They do not connect with vasoconstrictor neurons. The axons of the sympathetic neurons project back to the gut. All intestinofugal neurons have the same primary transmitter, acetylcholine [114,174,175].

Roles of intestinofugal neurons

The roles of intestinofugal neurons have been analyzed almost exclusively in relation to motility control, although these neurons also innervate sympathetic neurons that inhibit water and electrolyte secretion in the intestine (see Chapter 2). The intestinofugal neurons that affect motility are in the afferent limbs of enteroenteric inhibitory reflexes. These reflexes appear to act primarily on parts of the gastrointestinal tract that are proximal to the sites from which they are initiated [114]. Thus, the reflexes are one of the mechanisms by which more distal parts of the intestine regulate the proximal regions from which they receive products of digestion. The enteroenteric inhibitory reflexes also appear to have a protective role in the gastrointestinal tract. For example, enterogastric reflexes slow gastric emptying, thereby protecting the duodenal mucosa from acid and osmotic stress.

Overview

The gastrointestinal tract can be regarded as a sensory organ. It has three detecting systems: neurons, endocrine cells, and the gut immune system. The neural detection of sensory information is through both intrinsic sensory (primary afferent) neurons and extrinsic sensory neurons. The neural sensory system is responsible for detecting physiological and pathological states of the digestive system and its environment. The detected information is used to govern: (1) reflexes confined within the gut wall (intrinsic reflexes) that change motility, blood flow, and transepithelial fluid transfer; (2) extrinsic, but peripheral, reflexes between digestive organs; (3) reflexes through the central nervous system that are not consciously perceived; and (4) conscious sensation, such as satiety, hunger, discomfort, pain, and nausea. Although many of the effects mediated through the afferent neurons of the digestive system have been elegantly dissected, the subtleties of their interactions are still being unraveled. The challenge remains to establish a good linkage between what is known of the responses of individual primary afferent neurons and the integration of the information they provide to yield an output, either a conscious experience or a physiological response.

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A COMPONENTS

4 Gastrointestinal hormones and receptors

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The gastrointestinal endocrine system represents the largest and most complex endocrine organ in the body. Whereas traditional endocrine organs are composed of a solid mass of hormone-secreting cells that are homogeneous, or at least organized with a defined spatial orientation relative to one another, the sources of the gastrointestinal regulatory molecules are single endocrine cells and peptidergic neurons that are scattered along the length of the digestive tract. They all share the property of producing and secreting polypeptides. Because these hydrophilic molecules cannot cross a lipid bilayer, they must interact with other molecules that span the plasma membrane of target cells, which can activate the intracellular machinery of those cells. These membrane protein targets of gastrointestinal hormone action are called receptors. They have specialized domains for ligand recognition that are accessible to the extracellular space, as well as effector domains for molecular interactions or enzymatic activity that are located intracellularly. The diversity and design of the gastrointestinal endocrine system also necessitate broadening the basic concept of classical endocrinology, which requires that a hormone be delivered to its targets by way of the bloodstream. Although the bloodstream delivers some gastrointestinal hormones, the gastrointestinal endocrine system may also use more direct and focused means of transmitter delivery.

The endocrine cells are scattered among the epithelial cells of the mucosal lining of the gut and the ductular elements that enter the gut. They have an open or closed morphology. Endocrine cells with an open morphology can sense the composition of chyme and secretions through processes that extend into the lumen. A closed morphology facilitates sensing of the interstitial environment [1,2]. Peptidergic neurons also exist and are organized into syncytia, which extend between and along layers of the mucosa and into the solid digestive organs. These neurons are classified as extrinsic or intrinsic depending on the location of their cell bodies. Extrinsic parasympathetic innervation comes from the vagus and pelvic nerves, whereas sympathetic innervation comes from various sympathetic ganglia. The major source of peptidergic nerves is intrinsic to the gut and is affected little by sympathectomy or vagotomy. Numerous varieties of both endocrine cells and peptidergic neurons exist in the gastrointestinal tract. Each type of cell has its own distinct distribution and is able to sense and respond to a unique set of stimuli, resulting in the synthesis and secretion of a single predominant peptide.

Each hormone or neurotransmitter affects only those target cells possessing the appropriate receptors on their surface. This area of research has become even more complex and interesting in recent years with the realization that receptors can interact with each other and with additional allosteric modulators to change their selectivity and biological responsiveness [3–5]. Cells that can be regulated include the vast spectrum of epithelia, smooth muscle cells, and neurons residing at all levels of the digestive tract. Responses span the temporal spectrum, from rapid and transient to delayed and prolonged. The former includes effects on behavior or sensation, secretory or absorptive phenomena, motor events, and metabolic events. The more prolonged effects typically involve cellular growth, differentiation, or morphogenesis.

The combination of unique stimuli, limited distribution of distinct types of hormone-secreting cells, and exquisite selectivity of target cells and their responses provides almost unlimited opportunities for regulation. This elegant system integrates digestive functions at all levels of the gastrointestinal tract, thereby optimizing the digestion and absorption of

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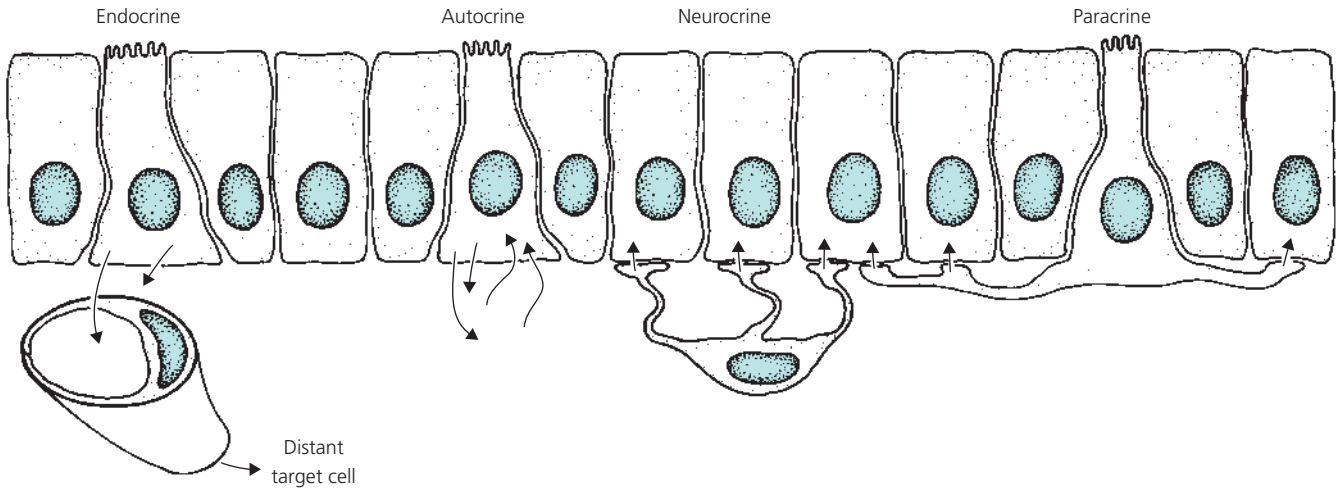

Figure 4.1 Modes of delivery of gastrointestinal regulatory peptides from endocrine cells and nerves, illustrating endocrine, autocrine, neurocrine, and paracrine mechanisms.

nutrients. For all these reasons, the gastrointestinal endocrine system has been referred to as the *brain of the gut*.

Organization of the gut endocrine system

The organization of the gastrointestinal endocrine system is the key to its power and flexibility. Acutely regulated gastrointestinal events are dynamic, complex, and interrelated; the ingestion of a meal initiates changes in secretion, absorption, and transit along the gut and in its associated digestive organs, and the net composition of chyme and its rate of delivery vary markedly over time and space. More delayed and prolonged effects on the differentiation and growth of enteric cells are equally important for the optimal digestive responses to an individual's dietary habits and nutritional requirements. Given the diversity in size and composition of ingested meals, the gastrointestinal endocrine system must be capable of highly varied programs of responses, not only to ensure proper assimilation of the nutrients but also to make eating the pleasurable experience it represents for most people.

The gastrointestinal endocrine system must be responsive both to intralumenal digestive events and to systemic status. The combination of open and closed endocrine cells and nerves provides sensitivity toward both worlds, recognizing that the general health, nutritional status, and metabolic needs of the organism can modify the optimal nutritional impact of a meal. With each hormone-secreting cell sensitive to its local environment, and with each cell strategically located, endocrine cells of the same type in two different locations likely secrete their peptides at different times, reflecting the dynamic changes occurring along the length of the gut after a meal. An additional level of control is made possible by the route of delivery and the mode of action of the secreted regulatory peptide. This can be endocrine, autocrine, neurocrine, or paracrine (Fig. 4.1).

The term endocrine specifically refers to hormonal delivery by way of the bloodstream, providing a mechanism to transport the hormone to distant sites. If this circulation traverses a specialized organ, such as the portal circulation through the liver, the opportunity exists to extract a particular hormone efficiently from the blood, thereby preventing broad systemic distribution. This can differentially affect certain molecular forms of the same hormone; for example, short molecular forms of cholecystokinin (CCK) are extracted in a single pass through the liver, whereas long forms pass through to the systemic circulation [6]. Specialized circulatory delivery systems are also relevant to gastrointestinal peptides, such as the islet–acinar portal circulation that provides high concentrations of peptides to the surrounding pancreatic acinar cells [7].

Autocrine loops are postulated to exist whereby the peptide synthesized by a cell affects that same cell. This may occur after secretion into the interstitial space, such that back-diffusion allows high local concentrations of peptide to interact with receptors on the plasmalemmal surface of the hormone-secreting cell. Such regulatory loops may even exist within cells. Guanine nucleotide-binding proteins (G proteins), which are proximal effectors of hormonally stimulated events at the level of the plasmalemma, may also reside on secretory granule membranes [8].

Neurocrine delivery of peptides usually involves their local release adjacent to a target cell bearing relevant receptors [9]. Neurons are particularly well suited for this mode of delivery because they can have highly specialized long processes that

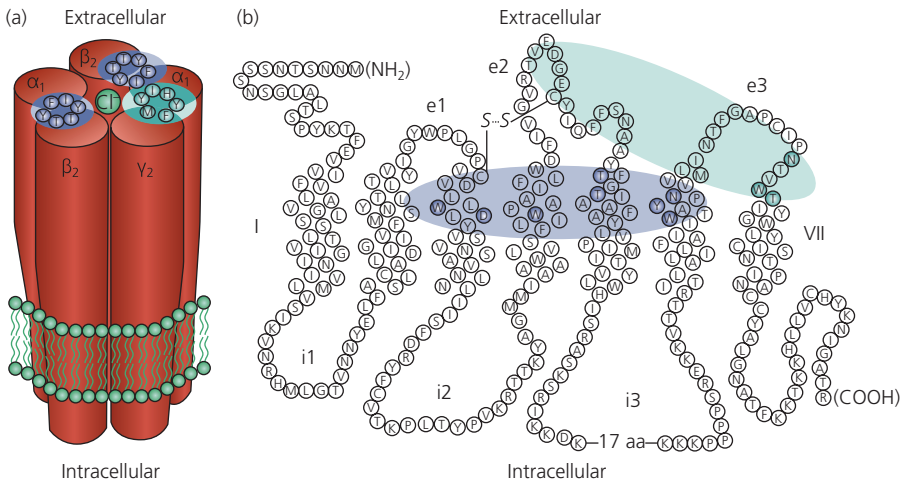

Figure 4.2 Allosteric binding sites on receptors as possible drug targets. Illustrated models show a ligand-gated ion channel **(a)** and the muscarinic acetylcholine G protein-coupled receptor **(b)**. The proposed orthosteric sites for ligands are shown in purple, and the allosteric sites for ligands are shown in turquoise. From Christopoulos [217], with permission from Macmillan Publishers Ltd.

terminate adjacent to potential target cells. This mode of delivery also provides a steep gradient of transmitter concentrations from the point of release, thereby limiting the action to receptor-bearing cells in the region of release.

Paracrine delivery reflects the identical process originating within an endocrine cell rather than a peptidergic neuron. These cells are also capable of developing specialized processes that allow the secretion of a key transmitter in approximation with its target cell. The first identified example of this was the relationship between somatostatin-secreting cells and gastrin-secreting cells in the gastric antrum [10].

Once a regulatory peptide is delivered to its target cell, a high level of regulatory diversity is still provided by the specificity of receptors expressed on each cell and by an array of potential signaling and regulatory molecules. A given endocrine cell can further regulate hormone-stimulated events in several different ways: by altering coupling events between receptor and proximal effector, by modifying the complement of molecules intrinsic to each signaling pathway, and by cross-talk between the molecules involved in signaling pathways. Each cell can also regulate the receptors it expresses, a phenomenon highly relevant to any understanding of the gastrointestinal endocrine system. This regulation can take the form of modification of the number of receptors on the cell surface, modification of the ability of those receptors to bind ligands or to initiate signaling, movement of receptors into different cellular compartments, and modification of the total cellular complement of receptors by effects on receptor synthesis and degradation [11–13].

A given type of receptor may be present on a wide variety of cells of the body, but the secretion of its hormonal agonist may affect only a small subset of those cells. This may reflect the different limited delivery systems previously discussed. It may also reflect a gradient in delivery by diffusion from a point source, with potential target cells also expressing different numbers of receptors on their surfaces. Receptors on distinct cells may be in different states of sensitization or desensitization. A certain type of cell may express a receptor differently at each stage of its life, depending on cell growth, the stage in the cell cycle, and other active metabolic and biochemical processes within the cell.

Receptor molecules can also be allosterically modulated by interactions with other receptors or with nonreceptor membrane or cytoplasmic proteins (Fig. 4.2) [3,5,14]. This type of interaction has been shown to modify specificity or binding affinity, as well as biological responses to agonist ligands. It also can modify the duration of action and regulation of the receptor. The cellular environment of a receptor is thus particularly important.

The gastrointestinal endocrine system represents a system of substantial complexity, which is dynamic and responsive to feedback and cross-regulation at the levels of both the hormones and the receptors. It is precisely because of this complexity that the system is so sensitive and has such extraordinary capabilities to keep us well nourished and pleased with our dining experiences.

History of gastrointestinal endocrinology

Classical endocrinology and gastrointestinal endocrinology were born together in 1902 with the discovery of the first hormone, secretin, by Bayliss and Starling [15] and the recognition that chemical messengers can be carried in the bloodstream. This earliest period in gastrointestinal endocrinology has been termed the juice-physiology era [16]. The discovery of gastrin by Edkins followed quickly in 1905 [17]. This flourish of activity in the gastrointestinal hormone field was followed by many quiescent years, while the field of classical endocrinology achieved steady and substantial growth. The solid and homogeneous endocrine organs could be studied with relative ease, whereas methods had not yet been developed to gain analogous insights into the scattered diverse gastrointestinal endocrine cells.

Gastrointestinal endocrinology finally achieved its status as a distinct discipline in the chemical era, when a long series of candidate hormones was discovered by means of the techniques of purification and peptide sequencing. The first was gastrin, whose structure was determined in 1964 after it had been extracted from a tumor [18]. Subsequently, the number of recognized potential regulators of digestive function expanded logarithmically. The Gastrointestinal Hormone Laboratory, founded by Mutt and Jorpes at the Karolinska Institutet [19], was the site of the initial chemical characterization of secretin, CCK, glucose-dependent insulinotropic peptide (GIP), vasoactive intestinal polypeptide (VIP), motilin, gastrin-releasing peptide (GRP), and many other candidate structures.

The next key advance was also methodological – the development of radioimmunoassay to detect or quantify minute quantities of molecules. Application of this methodology to the quantification of a hormone by Yalow and Berson [20,21] and its application to CCK [22] initiated the immunological era of gastrointestinal endocrinology. The usefulness of antibodies was appreciated quickly; these were applied to the localization of hormone-secreting cells by immunohistochemistry and to the quantification of peptide hormones by radioimmunoassay. The usefulness of radiolabeled peptide hormones was also recognized quickly. The radiolabeling of gastrointestinal hormones to high specific radioactivity made it possible to directly demonstrate the binding of hormones to receptors. The competition-binding technique made it possible to identify receptor subtypes, to define the cellular localization of receptors, and to quantify receptor density on a cell.

Since the 1970s, gastrointestinal endocrinology has become increasingly biochemical and molecular. Gastrin led the way into the new era of the molecular biology of peptides [23]. A more biochemical understanding of hormones excited interest in the biochemistry of receptors. During this era of molecular receptorology [23], receptors increased in status from representing simple sites of hormone binding to being the focus of intense interest. It was critical to understand the structure of receptor molecules before the details of binding, activation, and regulation became meaningful.

The first insights into the structure of receptors came from affinity labeling approaches [24,25], which represented a logical extension of the receptor-binding assay described. These approaches involved the use of a radiolabeled form of the hormone, as rich a source of receptor as possible, and a chemical method to link the two molecules together covalently. The siting of a photolabile group within the pharmacophoric domain of a hormone has been particularly successful in the covalent labeling of hormone receptors and their domains [26].

The most powerful insights have come from cloning the complementary DNA (cDNA) encoding the receptors. Hybridization screening based on partial sequence information quickly moved to expression cloning, bypassing the need for receptor purification and sequencing. Ultimately, the most powerful approach became in silico cloning, which is based on the identification of recognized signatures in sequence databases.

Once identified, a receptor cDNA offers remarkable possibilities. The deduced amino acid sequence provides insights into the receptor class represented. By finding its position in a phylogenetic tree, additional insights can come from analogy with studies of the closest known members of the family [27]. This can take the form of mutagenesis studies that provide insights into structure–function relationships. Chimeric receptors that combine specific regions of two or more receptors can be useful in identifying which domain is important for a given function [28]. Also, truncations and site mutants can help localize such regions [29]. Such receptor constructs can be constructed rapidly and expressed transiently to gain new insights. It is also possible to produce stable cell lines that express such constructs, and to modify the expression of natural or mutagenized hormones and receptors in laboratory animals. A variety of transgenic and null (i.e., knockout) animals are available to explore the impact of such modifications in an intact animal.

The era of molecular conformation and molecular interaction is ongoing [23]. Now that the primary structures of receptors have been collected and categorized, investigators are studying molecular conformations, both resting and active. Also generating substantial interest are the sites and molecular determinants of binding and activation, as well as the use of such insights for the rational design of useful pharmaceuticals. We are witnessing dramatic advances in drug development resulting from novel strategies of synthesizing and screening vast libraries of potential receptor ligands [30]. These have led to the successful development of numerous small-molecule nonpeptide antagonists, and even nonpeptide agonists, for peptide hormone receptors [31].

Hormone secretory cells

Several cellular sources exist for the synthesis and secretion of gut hormones. As previously noted, these are the numerous types of endocrine cells that are isolated and scattered throughout the gut mucosa, as well as the neurons contained within the enteric nervous system. Both cell types share many features: (1) the machinery for peptide biosynthesis and processing; (2) the packaging of products for export into secretory granules and the exocytic secretion of these products; and (3) the specialized processes that may facilitate focused delivery of secretory products. Based on these shared characteristics, a similar embryogenesis has also been postulated. This section focuses on each of these features as they relate to the sources of gastrointestinal hormones and neurotransmitters.

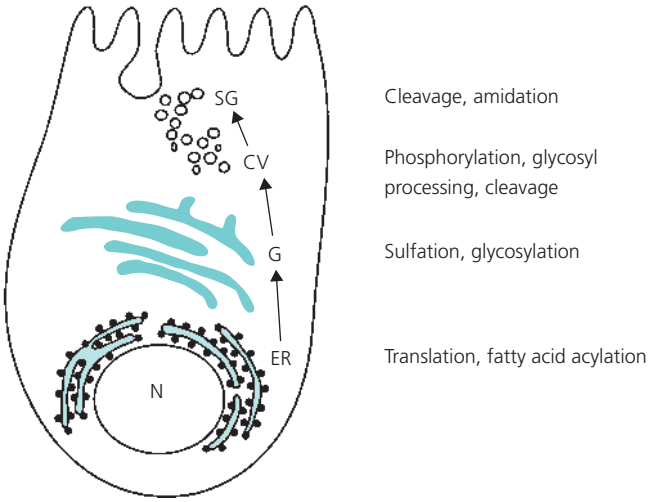

Figure 4.3 The synthesis of proteins or peptides for export (i.e., secretion) from polarized epithelial cells follows a highly conserved biosynthetic route. Synthesis begins at the level of the rough endoplasmic reticulum (ER) and proceeds through the Golgi apparatus (G), condensing vacuoles (CV), and secretory granules (SG) before exocytosis at the apical pole of the cell.

All types of intestinal epithelial cell appear to be derived from a single type of pluripotent stem cell precursor [32,33]. Gastrointestinal endocrine cells continuously differentiate and follow the highly organized vertical paths of migration along the crypt to villous axis. These cells resemble the endocrine cells of the pancreatic islets, with similar embryological associations and evolutionary relationships reflecting common endodermal cell origins.

In contrast, the enteric neurons are derived from the neural crest and the neuroectoderm [34,35]. Of note, the phenotype of cells derived from the neural crest is most dependent on the environmental influences in the regions to which they migrate, rather than on the level of the neural axis from which they originate [34,36]. The complexities of cell types and interactions typical of the enteric nervous system result largely from the mesenchymal substrate that these cells contact. The factors responsible for the development and differentiation of each of the gastrointestinal mucosal endocrine cells are even less well understood.

Despite this apparent difference in their developmental origins, the gastrointestinal endocrine cells and the enteric peptidergic neurons share many biological and biochemical specializations that are necessary for polypeptide synthesis, processing, and secretion. Key in this sequence of events is the packaging of the peptide product for export into a secretory granule. The details of this process were clearly defined by Jamieson and Palade [37], who studied the pancreatic acinar cell (Fig. 4.3). The identical processes appear to exist in the gastrointestinal endocrine cell.

Figure 4.4 Electron micrograph of a gastrointestinal endocrine cell in the human jejunum. This cell extends from the lumen to just above the basal membrane and includes the typical electron-dense secretory granules in its basal pole. From Solcia et al. [218], with permission from Lippincott, Williams & Wilkins.

Endocrine cell localization and characterization

Some gastrointestinal endocrine cells can be recognized by the relatively distinct ultrastructural appearance of their secretory granules [1,2] (Fig. 4.4). Other gastrointestinal endocrine cells can be tentatively identified not only according to the appearance of their secretory granules but also according to their position along the digestive tract. However, more specific techniques are required to identify most endocrine cells and any of the peptidergic nerves. These techniques are directed toward the polypeptides themselves or to the genes and messenger RNA (mRNA) molecules encoding them. Peptide antisera have been most useful in

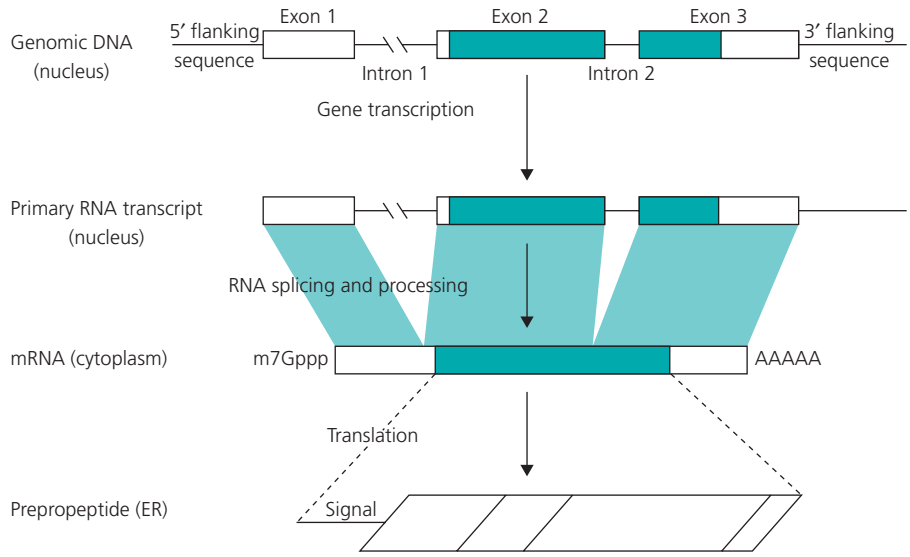

Figure 4.5 The gene structure and the molecular biological events leading to protein synthesis. Posttranscriptional processing events that occur in the nucleus include splicing, capping, and polyadenylation of the RNA to produce mature mRNA. Mature mRNA is transported into the cell cytoplasm, where it directs translation at the surface of the rough endoplasmic reticulum (ER).

the immunohistochemical localization of peptides, while in situ hybridization has been successfully used to localize relevant hormone mRNA.

Immunohistochemistry has become a standard technique to localize any antigen in a tissue section to which specific antisera have been raised. With the current understanding of the primary structure of the molecular forms of a given hormone, and of the unique and shared domains within that structure, it is easy to choose a potentially useful antigenic epitope. By conjugating that peptide to a hapten, a strongly immunogenic antigen can be produced. Most endocrine cells and peptidergic nerves concentrate their peptides in secretory granules in preparation for secretion, providing an ideal target with multiple copies for immunolocalization. A potential problem relates to the ability to fix these soluble compounds without interfering with antibody recognition. Trials of different fixatives and conditions of fixation may be necessary to achieve an optimal signal.

In situ hybridization is an analogous localization technique based on the specific interaction of an oligonucleotide probe with its complementary sequence within the cell [38]. A complementary, single-stranded, antisense riboprobe to an mRNA molecule provides optimal sensitivity and specificity. Here, too, multiple choices exist: fluorescent, colorimetric, or radioactive detection [39]. Limited copy number for a specific mRNA may represent a problem when applying this technique to some hormone genes.

Biosynthesis and processing of gastrointestinal hormones

Most gastrointestinal hormones and neurotransmitters discussed in this chapter are single-chain polypeptides. All are encoded by single-copy genes containing one or more exons that determine the primary amino acid sequence by a series of nucleotide triplets known as codons (Fig. 4.5). For the multiple-exon genes, these coding regions are separated by noncoding introns. While still in the nucleus, the DNA sequences are transcribed by RNA polymerase II to produce pre-mRNA. The noncoding regions of the gene, particularly those upstream of the site of the initiation of transcription (i.e., the 5′ flanking sequence), often contain cis-active regulatory sequences that influence the rate of transcription. Regulation of gene expression typically occurs slowly, over a number of hours, whereas hormone secretion of prepackaged product occurs rapidly, being initiated within seconds of stimulation. The pre-mRNA sequences complementary to the exons are normally spliced together whereas those complementary to the introns are eliminated to form the mature mRNA.

Alternative processing of a single gene may occur and there may be more than one distinct product within a single gene. Examples of this include the proopiomelanocortin gene, which contains sequences for β-endorphin, corticotropin, and melanocyte-stimulating hormone [40], and the proenkephalin gene, which contains five copies of the [Met]enkephalin sequence and one copy of the [Leu]enkephalin sequence [41]. The VIP, neuromedin A, and peptide histidine isoleucine (PHI) sequences all exist within a single gene [42]. Another phenomenon is the production of distinct gene products by alternative splicing of a single gene, such as occurs in the production of calcitonin and calcitonin gene-related peptide [43]. Similarly, alternative splicing of a single gene product may also occur [44]. Alternative splicing of several hormone receptors can yield products of different lengths with short deletions or insertions compared with the predominant species [45,46].

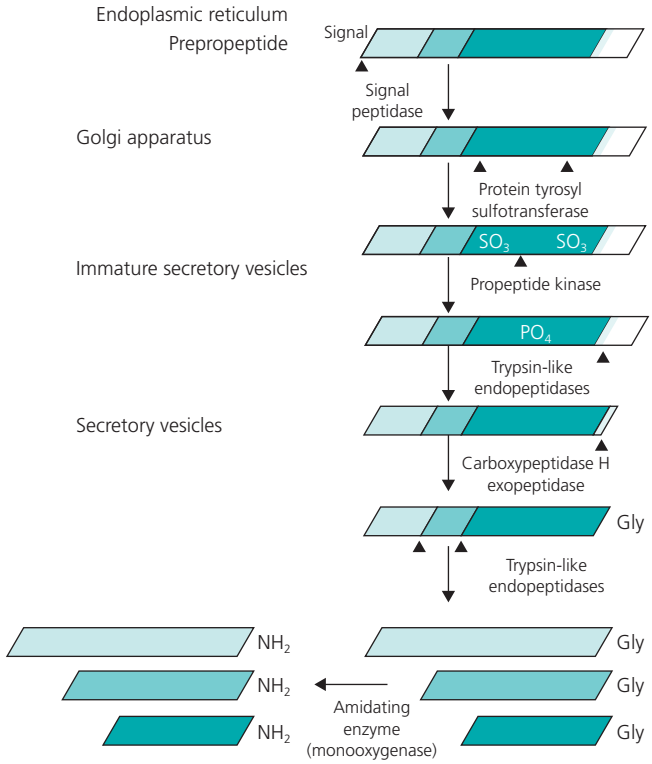

Figure 4.6 Posttranslational processing events that occur in distinct cellular compartments in preparation for secretion of a peptide from the cell. These types of reactions are used selectively for different secretory products.

The fully spliced mRNA is translocated to the cell cytoplasm where translation occurs (Fig. 4.6). Translation is the process of producing the preprohormone polypeptide chain that is encoded by the mRNA. Peptide synthesis begins on the ribosome bound to the rough endoplasmic reticulum. Peptides destined for export have a characteristic amino-terminal signal sequence that leads to their segregation within the lumen of the endoplasmic reticulum [47]. As the nascent chain crosses into the lumen of the endoplasmic reticulum, the signal sequence is bound to a signal-recognition particle and cleaved by a signal peptidase to yield the prohormone. Also, posttranslational modifications may begin cotranslationally. For example, sulfation, phosphorylation, glycosylation, and amidation may continue as the peptide moves through the Golgi apparatus and into secretory granules that are at various stages of maturity. The ultrastructural appearance of these secretory granules can be characteristic of a particular type of gastrointestinal endocrine cell. The large secretory granules typify regulated pathways of exocytosis, with constitutive secretory pathways most commonly using much smaller vesicular structures. Glycosylation is a multistep process that occurs as the peptide moves through each of these compartments. Tyrosine sulfation seems to occur early in biosynthesis, within the Golgi apparatus [48].

A unique posttranslational processing event that is particularly relevant for peptide hormones is the selective cleavage of the prohormone into fragments of different lengths, with each form possessing biological activity [49]. Examples of multiple, biologically active forms of a hormone include gastrin and CCK; the multiple forms of these hormones share the carboxyl-terminal sequence that is critical for activity. This endoproteolytic processing occurs during biosynthesis while the product traverses the late Golgi cisternae and early secretory granule compartments. Dibasic amino acid residues are a common location for such processing. Trypsin-like endopeptidases, such as PC1 and PC2, which are uniquely expressed in endocrine and neuroendocrine cells, have been described to be important in the endoproteolytic processing of gastrointestinal hormones [50,51].

Carboxyl-terminal amidation is a very common posttranslational modification of gastrointestinal hormones [52,53]. It occurs in about half of the recognized hormones and peptide neurotransmitters and was the basis of a discovery strategy used early in the chemical era by Tatemoto and Mutt [54]. A series of enzymatic reactions is necessary to yield an amidated product: (1) cleavage at a site of dibasic residues; (2) trimming of the remaining basic residues by carboxypeptidase H; and (3) conversion of the glycine-extended intermediate to the corresponding carboxyl-terminal amide with an α-amidating monooxygenase. These processes occur within the secretory granules.

The processing of gastrin in the antral G cell is an excellent example of these processes [55]. The biologically active region of this peptide that is key for binding to its classic receptor has been carefully isolated to the carboxyl-terminal tetrapeptide amide of the mature forms of gastrin [56,57]. The gastrin gene is composed of three exons, with exons 2 and 3 possessing the coding sequences [58–60]. The primary mRNA transcript is processed to eliminate about three kilobases of intron sequence between the first two exons and 130 bases of intron sequence between exons 2 and 3. This is translated to yield preprogastrins of 101-amino acid residues in man and 104-amino acid residues in rat and pig. The signal peptide is then cleaved to yield progastrin, which rapidly undergoes further proteolysis at Arg57,58, Lys74,75, and Arg94,95 to yield four fragments. The Arg94,95 cleavage is followed by carboxypeptidase H trimming of carboxyl-terminal basic residues and conversion of the Gly-extended intermediate to the carboxyl-terminal amide. G34 and G17 are the major products of these cleavages. Serine phosphorylation and tyrosine sulfation may also occur [61,62]. One of the sites of serine phosphorylation is Ser96, immediately adjacent to the site of cleavage at the dibasic residues, which ultimately results in carboxyl-terminal amidation. Tyrosine sulfation can occur at Tyr87 and Tyr103. Whether these modifications affect physiological processing remains unclear [63].

Posttranslational modifications of gastrointestinal hormones may affect their activity. Most of the amidated hormones

require this modification for recognizable activity. A critical modification in CCK peptides is the sulfation of the tyrosine that is seven residues from the carboxyl terminus, with desulfated CCK more than 1000-fold less potent than the sulfated form [64]. Although the structurally related gastrin peptides can also exhibit tyrosine sulfation six residues from the carboxyl terminus, this modification has little or no impact on gastrin receptor binding or hormone activity.

Hormone and transmitter molecules

The hydrophilicity and the solubility of hormones and neurotransmitters prevent them from traversing the lipid bilayer in their target cells and necessitate the presence of a receptor molecule in those target cells with which they can interact. By first principles, peptides are also flexible. The peptide bonds linking the amino acid residues are stable and strong, broken only by peptidase enzymes under normal biological conditions. This flexibility enables peptides to sample a large range of potential conformational space, but makes it somewhat difficult to predict the relevant conformation of these molecules as they bind to their targets. Some insights have come from observing the conformationally constrained analogues of some hormones, but such insights are limited [65].

Advances in peptide chemistry have facilitated the synthesis of small peptides. Peptide synthesis can be achieved in solution or by solid phase techniques in which one amino acid residue is added at a time to a growing chain of residues [66]. This technique can be used to produce the natural peptides themselves, or analogues that can provide important primary structure and activity information.

Gastrointestinal hormones can be categorized according to: (1) their predominant localization in endocrine cells or peptidergic neurons; (2) their localization to different levels of the gastrointestinal tract; (3) their rapid effects vs more prolonged effects on growth and differentiation; or (4) the presence of structural similarities between the hormones themselves (Table 4.1). A single peptide hormone can be found in endocrine cells and nerves, it can be present at several levels of the gastrointestinal tract, and it can have both acute and chronic growth-regulatory effects. This is also true for families of structurally related hormones. A single peptide can interact with and activate more than one type of receptor. Only by appreciating the scope of options for these peptides and their receptors can the complexity and the power of the gastrointestinal endocrine system be understood.

Measurement of hormones

Hormone concentrations can be quantified by bioassay or by a type of competition binding assay, such as radioimmunoassay or receptor-binding assay [21,67]. Before the chemical

Table 4.1 Organization schemes for gastrointestinal hormones, neuropeptides, and locally secreted growth factors according to their cell of origin, anatomical location, and structural similarities

Location	Hormone	Cell type
Endocrine cells		
Stomach	Gastrin	G
	Somatostatin	D
Duodenum/jejunum	Secretin	S
	CCK	I
	GIP	K
	Somatostatin	D
	Motilin	M
Ileum/colon	Enteroglucagon	L
	PYY	L
	Neurotensin	N
	Somatostatin	D
Pancreas	Insulin	B
	Glucagon	A
	PP	D_1
	Somatostatin	D
Neuropeptides		
CGRP		
CCK		
Dynorphin		
Enkephalins (Leu and Met)		
Galanin		
GRP		
Motilin		
NPY		
PHM		
PYY		
Somatostatin		
Substance K (neurokinin A)		
Substance P		
VIP		
Locally secreted growth factors (fibroblasts, endothelial cells, epithelial cells, hematopoietic cells)		
EGF		
FGF		
IGF		
PDGF		
TGF-α, TGF-β		
Structurally similar families		
Gastrin–CCK family: gastrin, CCK		
Pancreatic polypeptide family: PP, PYY, NPY		
Tachykinin family: substance P, GRP		
Somatostatin family: somatostatin		
Motilin family: motilin, ghrelin		
Secretin family: secretin, VIP, PACAP, GIP, glucagon, GLP-1		
Tyrosine kinase receptor family: EGF, FGF, IGF, PDGF		

CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; EGF, epidermal growth factor; FGF, fibroblast growth factor; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide-1; GRP, gastrin-releasing peptide; IGF, insulin-like growth factor; NPY, neuropeptide Y; PACAP, pituitary adenylate cyclase-activating peptide; PDGF, platelet-derived growth factor; PHM, peptide HM; PP, pancreatic polypeptide; PYY, peptide YY; TGF, transforming growth factor; VIP, vasoactive intestinal polypeptide.

characterization of hormones, concentrations could be determined only in an activity assay. However, the specificity of an activity assay is unreliable because more than one hormone can produce the same response and because antagonistic agents can influence the net activity. The determination of the chemical identity of many hormones has facilitated the assay of unpurified samples.

Competition binding assays are based on the competitive inhibition of the specific binding of a radiolabeled form of the hormone to an antibody or receptor. The radiolabeled and natural hormones compete for binding to the antibody or receptor. Radioimmunoassay benefits from the availability of specific antibodies that are easy to handle and store. In contrast, radioreceptor assay depends on the preparation of a receptor, which may be difficult to produce and store in an active form.

For all hormone assays, sample collection and handling are critical, and sampling conditions should be reproducible. Samples are usually collected during fasting because hormone concentrations may increase in the postcibal period. Gastrin, CCK, secretin, GIP, and pancreatic polypeptide (PP) all increase transiently after a meal, whereas concentrations of most neurotransmitters (e.g., VIP) change minimally [68]. After sample collection, low temperature and protease inhibitors minimize hormone degradation.

Knowledge of hormone concentrations in the circulation can provide important insights. Elevated basal levels may reflect excess secretion or reduced clearance. Excess secretion may develop if hormone-secreting cells proliferate, as occurs in gastrin cell hyperplasia, or if cells have lost their feedback inhibitory mechanisms and secrete constitutively, as occurs in various types of endocrine cell neoplasia. Because polypeptide hormones are cleared by the kidneys, high levels of hormones may be measured in patients with renal failure [69]. In some settings it may be useful to measure hormone concentrations serially after some type of stimulus, such as the ingestion of a meal or the perfusion of a region of the intestine, to define the timing and characteristics of a normal stimulated response. There are provocative tests for some endocrine tumors, such as secretin stimulation of gastrin in gastrinoma [70].

The confirmation of the role of a hormone in a particular physiological process is not simple. Determination of the physiological activity of a particular gut peptide is based on the following criteria: (1) there is a relationship between the appearance of the hormone in the circulation and the physiological event; (2) the physiological effect can be reproduced by infusion of pure or synthetic hormone to achieve the same serum concentration observed under physiological conditions; and (3) the physiological activity can be inhibited by infusion of a specific antibody or receptor antagonist, when available [71]. The circulating concentrations of the hormone that produce the biological effect should be similar for the natural peptide released physiologically and the synthetic peptide infused into the experimental subject. When multiple molecular forms of a hormone exist, it is important for the infusion to reproduce the forms naturally observed as closely as possible.

Unfortunately, it is more difficult to determine the relevant concentration of gastrointestinal hormones at the level of their receptors. Interstitial concentrations can vary significantly over short distances. There is no good, reproducible method to collect the relevant fluid to measure hormone concentrations at their site of action. This is a particular problem for hormones and neurotransmitters released through paracrine or neurocrine mechanisms.

Receptors

Receptor molecules also have a special place in gastrointestinal endocrinology. A molecule that spans the lipid bilayer in the target cell, such as a receptor, is required for mediating the effects of the hydrophilic hormones and transmitters that cannot cross the plasma membrane to activate intracellular machinery. The receptor molecule has two spatially distinct domains: one domain to bind ligands that approach the molecule from the extracellular side of the membrane, and a second to initiate activity cascades within the cell. The cellular response can be triggered by a ligand-induced conformational change that activates an enzymatic activity intrinsic to the receptor. Alternatively, the ligand-induced change in the conformation of the receptor can expose a receptor domain that interacts with an important intracellular molecule, with the interaction initiating the cascade of signaling events typical of that receptor and ligand pair.

With the primary structures of most receptors now known, insights into the structural basis of cell activation are being advanced rapidly. Gastrointestinal hormone receptors are grouped into three broad classes according to their membrane topology: (1) the single-transmembrane receptors; (2) receptors with subunits that traverse the membrane multiple times; and (3) the G protein-coupled receptors that traverse the membrane seven times (Fig. 4.7).

The largest group of single-transmembrane receptors possess intrinsic ligand-triggered enzyme activity. This group includes ligand-triggered protein tyrosine kinases, such as the epidermal growth factor receptor, insulin-like growth factor receptors, platelet-derived growth factor receptor, fibroblast growth factor receptor, and transforming growth factor receptors [72–74]. These molecules have extracellular ligand-binding domains and intracellular kinase domains. On activation, these molecules associate into dimers and are autophosphorylated. In addition to the phosphorylation of the receptors themselves, various members of this group phosphorylate and activate phospholipase C, GTPase-activator proteins, and intracellular tyrosine and serine/threonine kinase cascades. These events are closely associated with cell

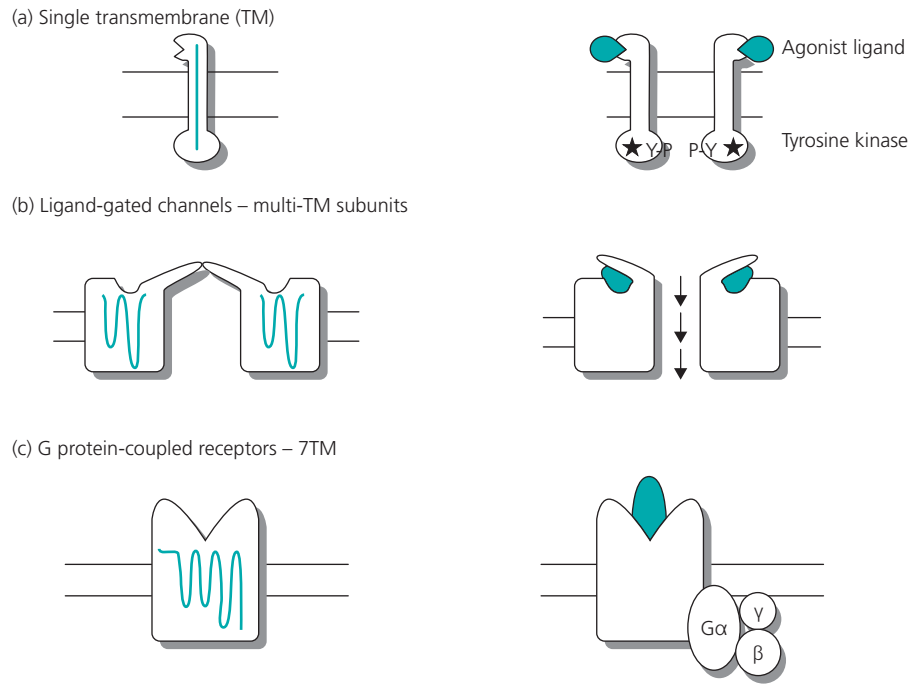

Figure 4.7 Peptide hormone receptors can be divided into three groups according to their membrane topology. The basal conformations are shown on the left and activated states on the right. **(a)** The single-transmembrane (TM) receptors dimerize; some also possess intrinsic tyrosine kinase activity that contributes to the autophosphorylation of the complex. **(b)** The ligand-gated complexes undergo a conformational change that opens the channel. **(c)** The G protein-coupled receptors become associated with their heterotrimeric G proteins, which then initiate the signaling cascade.

growth, differentiation, and oncogenesis. Ligand-triggered membrane guanylate cyclases also exist, such as the receptor for atrial natriuretic peptide [75]. There is also a group of single-transmembrane receptors that does not appear to have any intrinsic enzymatic activity, but rather function by dimerization and initiation of cascades of modular interactions. These include receptors for growth hormone, erythropoietin, prolactin, and several cytokines [76,77].

The second group of receptors (ligand-gated channels) have subunits that span the membrane multiple times and associate to form pores or channels [78]. Proteins with four transmembrane domains are the most common subunit structure, with ligand binding resulting in a conformational change that regulates the size and permeability of the channel. Examples include the nicotinic acetylcholine, GABA-A, 5-HT_3 (serotonin), glycine, and glutamate receptors. The best characterized is the nicotinic acetylcholine receptor. This complex is composed of five membrane-spanning units (α, α, β, γ, and δ), each having four transmembrane domains, which associate to form a central cation-conducting channel.

The last group – the heptahelical G protein-coupled receptors – is by far the largest group of gastrointestinal hormone receptors and the largest group of receptors in the body [79–81]. More than 2000 of these have been identified, if receptors for taste and odorants are included, representing 1%–5% of the genome of different species. They are activated by extraordinarily diverse ligands, ranging from photons to larger peptides and even to very large glycoproteins and viral particles. There is a somewhat parallel organization of groups of these receptors with structurally similar groups of ligands [27]. The heptahelical receptor proteins typically have no intrinsic enzymatic activity, but rather signal by associating on their cytosolic face with heterotrimeric G proteins [82,83]. These can then regulate a variety of effectors, such as phospholipase C and adenylate cyclase. These receptors have seven segments of about 20–25 hydrophobic residues representing the putative transmembrane helices (Fig. 4.8). The most highly conserved residues in each family of G protein-coupled receptors are located in these domains. Typically, there are sites of potential N-linked glycosylation in predicted ectodomains. This posttranslational modification likely plays an important role in helping the receptors fold and maintain their solubilities during biosynthesis. There are often serine- and threonine-rich areas in predicted cytosolic domains of these receptors that may be sites for phosphorylation and, thereby, receptor regulation.

The heterotrimeric G proteins consist of three subunits – α, β, and γ [84,85]. The α-subunit has intrinsic GTPase activity and is the site of guanine nucleotide binding. The β- and γ-subunits remain bound together, binding to the GDP-bound α-subunit as a paired unit. There is a cycle of agonist binding to the receptor, association of the GDP-bound G protein heterotrimer, GTP displacement of GDP, dissociation of the GTP-bound α-subunit from the βγ-dimer, hydrolysis of the GTP, and reassociation of the GDP-bound α-subunit with βγ (Fig. 4.9). Crystal structures have been solved for these G protein complexes, providing insights into the molecular interactions between receptor and G protein and between G protein and effectors. Although early attention focused on the GTP-bound α-subunit as the molecule that interacts with effector

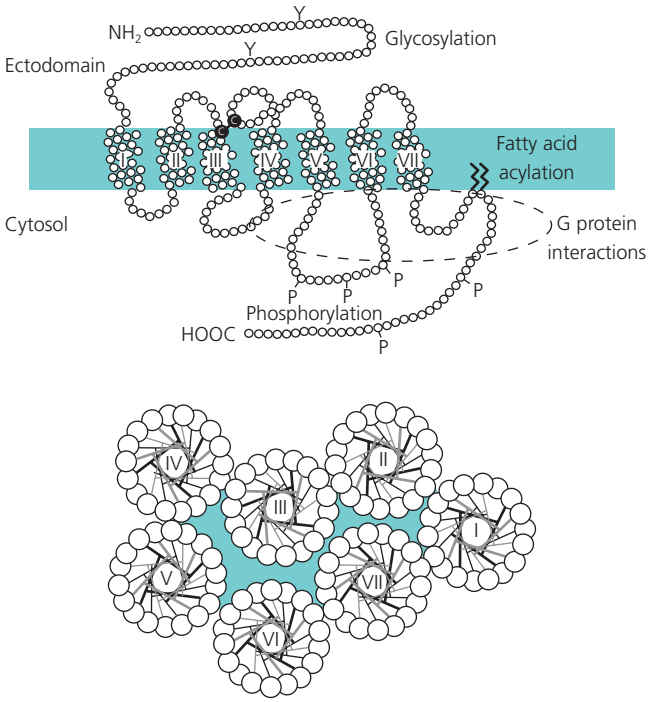

Figure 4.8 A prototypical G protein-coupled receptor depicted from the side (**top**) and looking down on the transmembrane helices (**bottom**). Each receptor is predicted to have seven hydrophobic segments traversing the lipid bilayer, with most having sites of N-linked glycosylation in the ectodomain and sites of phosphorylation in cytosolic domains. Also shown is the position of a highly conserved disulfide bond linking the second and third extracellular loops, and a position of fatty acid acylation that is present in the carboxyl-terminal tail of some of these receptors.

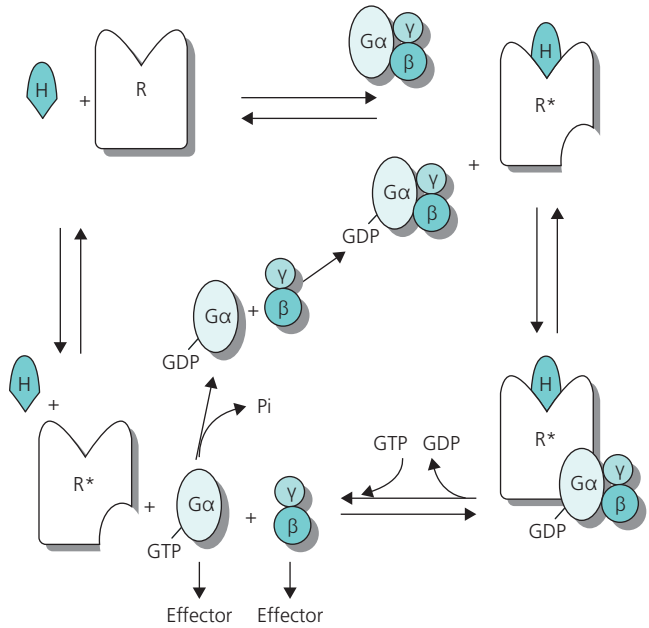

Figure 4.9 Cycles of G protein-coupled receptor activation and proximal signaling events at the level of the plasma membrane, which occur in response to agonist occupation. The earliest step is an induced conformational change in the receptor (asterisk) that facilitates its association with a G protein. At that point, the G protein is in its GDP-bound trimeric state. The receptor association facilitates GTP exchange for the GDP, which in turn results in the dissociation of the ternary complex and dissociation of the GTP-bound α-subunit from the βγ-dimer. Both of these can move on to interact with effectors in other compartments within the cell. The α-subunit of the G protein has intrinsic GTPase activity that results in the elimination of one of the phosphates and reassociation of the trimeric form of the G protein.

proteins to activate them, the βγ-dimer clearly also plays this role for many effector proteins [86,87]. In mammals, genes for 16 α-subunits, 4 β-subunits, and 7 γ-subunits have been isolated. The innumerable combination of these various subunits into heterotrimers contributes to the vast diversity of effects and regulatory properties of peptide hormones.

The G protein-coupled receptors relevant to the gastrointestinal hormones fall into two families. Most of the small and carboxyl terminal-amidated peptides, which have focused domains for receptor binding at the carboxyl terminus, are members of the rhodopsin/β-adrenergic receptor family. The larger peptides with diffuse pharmacophoric domains with amino-terminal determinants of specificity tend to be members of the secretin receptor family [88]. A notable exception is the amino-terminal-specific receptor for motilin, which has been cloned and identified as a member of the rhodopsin/β-adrenergic receptor family [89]. The binding of most agonists acting through this superfamily of receptors is influenced by hydrolysis-resistant analogues of GTP. This property provides an important clue that an action is mediated by this class of receptors.

Like the hormones, the gastrointestinal hormone receptors are all the products of single genes. Most do not have signal sequences and they do not undergo extensive proteolytic processing. Alternative splicing is particularly common in the secretin family of G protein-coupled receptors, in which multiple exons are the rule [90]. The alternatively spliced products can differ substantially in ligand-binding specificity and activation characteristics. The genes encoding the gastrointestinal hormone receptors have been known for less time than those encoding the hormones themselves. As a result, less is known about regulation of the receptor genes. Many receptors undergo substantial posttranslational modifications. Most of the G protein-coupled receptors are glycosylated. This feature helps to establish the appropriate folding and solubilities for biosynthetic transport to the cell surface, and likely helps to protect the receptors from proteolysis [91,92]. Palmitoylation of Cys residues within the carboxyl-terminal tail is common in many G protein-coupled receptors [93]. This modification may bring another loop up to the plasma membrane, and may have a role in regulating access for G protein coupling. Receptor phosphorylation is

another common posttranslational modification, but it occurs predominantly after the mature receptor has been inserted into the plasma membrane [94].

Most of the gastrointestinal hormone receptors are present on the cell surface in small to moderate numbers; there are typically fewer than several thousand molecules per cell. In such low numbers and given the very low concentrations of most of the circulating natural ligands, high-affinity and specific-binding interactions between hormone and receptor are critical. The receptor is an ideal target for regulating the sensitivity of the system. The pharmaceutical industry recognizes this and targets numerous drugs to act at cell surface receptors. Receptors are also a site of substantial endogenous regulation. A shift in the accessibility of the receptor-binding domain to the ligand or a reduction in the number of receptors on the cell surface affects the sensitivity of the signaling system.

Receptors are dynamic molecules that have a life cycle in the cell (Fig. 4.10). Most receptors are synthesized at a slow rate, relying on reversible regulatory processes that conserve receptor molecules. The resting cell has a fixed complement of mature receptors on its surface and a small number of molecules in the biosynthetic pathway within the cell. Agonist occupation often initiates many events that can influence the receptor. By definition, agonist occupation of every receptor initiates signaling processes within the cell. The details of these events vary with the receptor class, family, and even the particular ligand being bound or the cell in which the receptor is expressed. Within many signaling cascades are protein kinases and phosphatases that feed back and act on the receptor itself. For G protein-coupled receptors, this can uncouple the receptor from its proximal G protein effector [13]. For receptor tyrosine kinases, autophosphorylation is critical. Many receptors undergo internalization processes that remove them from the surface, thus removing the potential for further stimulation [11,12]. This mechanism protects the cell from damage caused by overstimulation. Some internalized receptors travel to the lysosome and are degraded, whereas others are recycled to the cell surface. For many ligand-occupied receptors, both molecules internalize together and move through the proximal endocytic cascade, in which the endosome becomes acidified and releases the ligand. The ligand can then move independently from its receptor. To be certain of the details of these processes, both ligand and receptor must be studied.

Receptor characterization and quantification

Receptors can be characterized on the basis of their biological activities or their receptor-binding properties. The availability of analogues of a given hormone that have a spectrum of activities from full agonist to partial agonist to antagonist simplify the characterization of biological activity. If an activity is mediated by a particular receptor, the receptor antagonist should inhibit it in a competitive manner. Such an activity assay can be used to screen for compounds that are

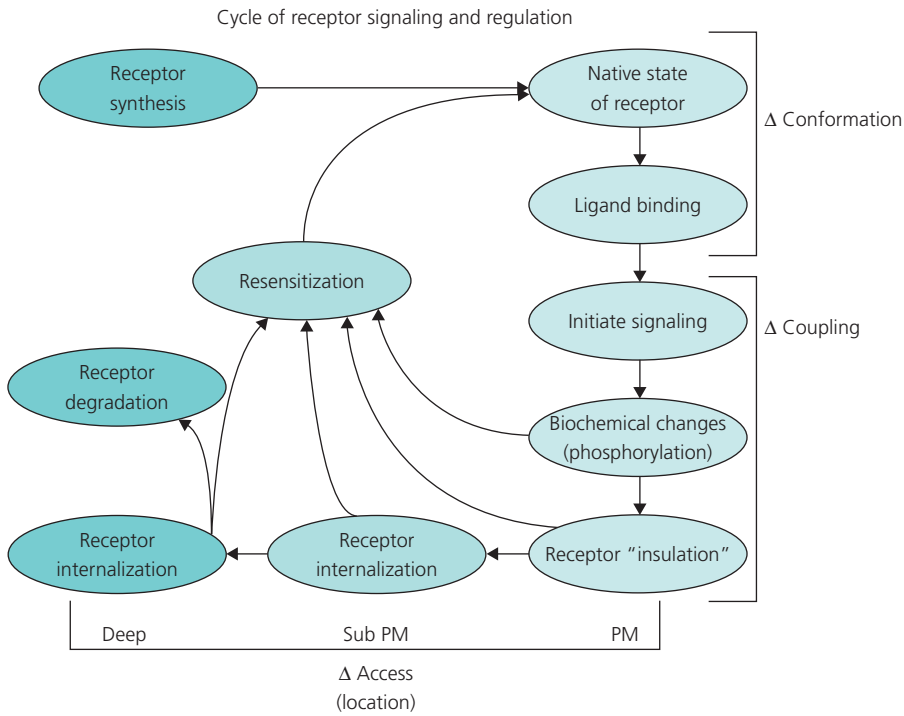

Figure 4.10 Events in the life cycle of a receptor in a cell. In the basal state, most of the receptor is usually at the level of the plasma membrane (PM). Agonist occupation results in changes in receptor conformation, biochemical modifications, and trafficking through the cell, which impact on accessibility for ligand binding, proximal effector coupling, and signaling events. The cycle is completed by reversal of the biochemical changes and the return of the receptor to the original cellular compartment. Because some receptor is lost to degradation, new synthesis can also replenish the cellular complement of receptor. The details of these cycles differ depending on the ligand, receptor, and cell type.

active at a particular receptor. This type of assay is necessary to determine if a new receptor ligand is an agonist or an antagonist.

The binding characteristics of receptors have been the subject of intense investigation [95]. The availability of radiolabeled forms of hormones with high specific radioactivities that bind normally to their receptors provides a tool to analyze binding. The law of mass action should apply to the binding. Numerous software programs are capable of analyzing the binding data generated from this type of experiment. For such data and analysis to be valid, binding must be performed under conditions in which the concentration of radioligand is below the dissociation constant (K_d) and in which binding is permitted to reach steady state or equilibrium. The method of separating bound and free ligand should be rapid and efficient. The saturability of the binding is a critical feature that focuses on biologically relevant binding of appropriate affinity.

Biochemical characterization of receptors can be performed by affinity labeling [26,96]. This represents a variant of a receptor-binding study in which the specifically bound radioligand is covalently bound to the receptor molecule and subsequently purified. This method, which has provided many important insights into the nature of the receptor molecules, is the only way to study posttranslational modifications of the receptor. By far the most valuable insight into biochemical characterization of receptors has come from cDNA cloning and sequence analysis.

Hormone-specific insights

Gastrin–cholecystokinin family

The gastrin–CCK family of peptides is composed of the multiple molecular forms of the gastrin and CCK peptides, all sharing the same carboxyl-terminal pentapeptide amide (Fig. 4.11). Evolutionary studies of gastrin and CCK suggest a common precursor peptide, with these hormones diverging late in evolution [97–99]. Because the biological information in both is highly localized to relatively small pharmacophoric domains at the carboxyl terminus, various molecular forms of each of different lengths share these domains. These forms provide an opportunity for differential regulation, clearance, and activity. Both gastrin and CCK have well-established physiological actions.

Gastrin

The major biologically active forms of gastrin are 17- and 34-amino acid peptides that have a tyrosine residue six residues from the carboxyl terminus, which may (gastrin-17-II, gastrin-34-II) or may not (gastrin-17-I, gastrin-34-I) be sulfated. This posttranslational modification of gastrin appears to have no

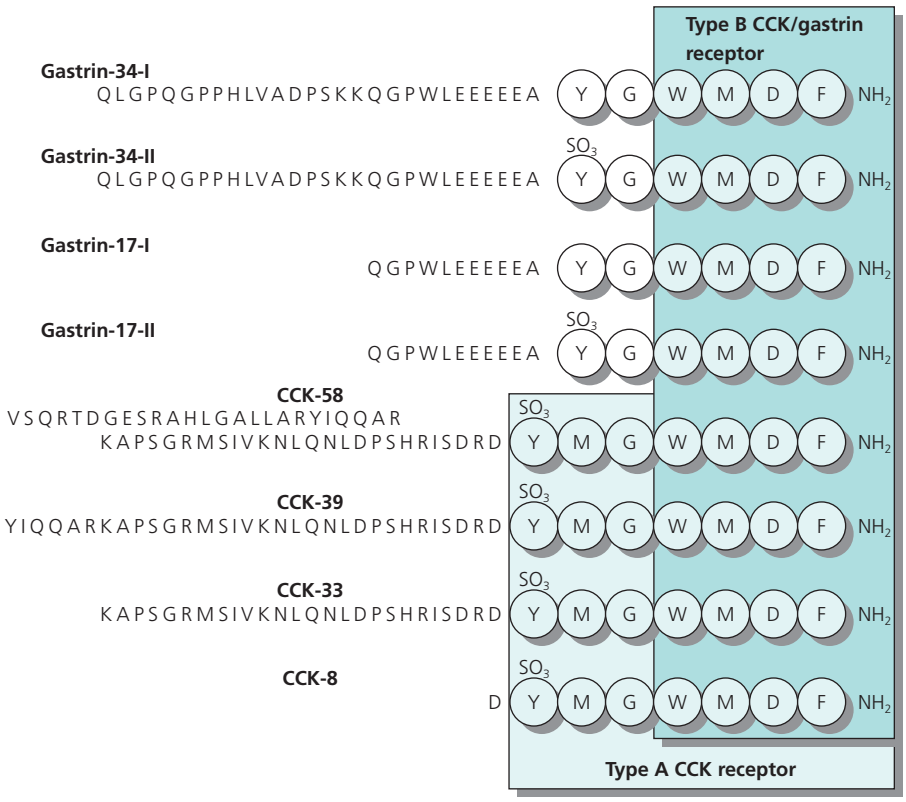

Figure 4.11 The variable molecular forms of gastrin and cholecystokinin (CCK). All share the same carboxyl-terminal pentapeptide amide. Shown are the critical domains for recognition by type A CCK receptors and type B CCK/gastrin receptors.

functional significance, as all forms are equally potent and efficacious at the gastrin (type B CCK) receptor. The critical information in gastrin peptides is in the carboxyl-terminal tetrapeptide amide, with most extensions and modifications of that domain well tolerated. Gastrin-17 is cleared from the circulation faster (half-life of 7 min) than gastrin-34 (half-life of 30 min) [60], meaning that most gastrin in the circulation during fasting is gastrin-34, whereas the major form released after a meal is gastrin-17. The greatest proportion (> 95%) of gastrin in the normal circulation is fully processed and contains a carboxyl-terminal phenylalanine amide [100]. Within some tissues, gastrin can be produced but not fully processed, resulting in the presence of nonamidated and carboxyl-extended forms of this peptide. Also, in gastrin-producing neoplasms, incomplete processing may result in the release of large quantities of nonamidated forms into the peripheral circulation. This has even been proposed as an assay for such neoplasms.

The major site of gastrin expression in the adult is open endocrine cells (G cells) in the gastric antrum. Low levels of gastrin are present in the vagus nerve and in pituitary corticotrophs, and transient expression is observed in fetal pancreatic islets and colon [101,102]. This hormone is found in a number of neoplasms [103,104]. The highest levels of processed and autonomously secreted gastrin come from islet cell tumors (gastrinomas) in the Zollinger–Ellison syndrome (see Chapter 71) [105]. Unprocessed forms of gastrin have been found in some colon, lung, and ovarian cancers. Sensitive radioimmunoassays for mature and biologically active gastrin are widely available, and specialized assays have been developed to detect unprocessed forms of this hormone.

Gastrin secretion from antral G cells is regulated by lumenal, paracrine, endocrine, and neural stimuli [106]. Ingestion of a meal is a strong stimulant of the secretion of this hormone. Small peptides, aromatic amino acids (e.g., phenylalanine and tryptophan), and calcium in a meal are key contributors to its stimulatory effect. Coffee, wine, and beer have also been reported to be stimulants, but the ethanol and caffeine components are not major contributors. Of interest, the carbohydrate and fat components of a meal contribute little to the gastrin response. There is an important feedback loop whereby the major result of gastrin stimulation, acid secretion, results in a lowered intragastric pH and reduced G cell secretion of gastrin (Fig. 4.12). This is mediated by the paracrine effect of somatostatin. The neurocrine mediator GRP stimulates G cell secretion. Sympathetic innervation and parasympathetic innervation have complex effects. Sham feeding and gastric distention both stimulate gastrin release, and atropine can enhance this effect.

The classic target for gastrin is the gastric parietal cell, which secretes hydrochloric acid. This effect is mediated by a G protein-coupled receptor, which is identical to the most abundant CCK receptor present in the central nervous system (i.e., type B CCK receptor). This receptor is in the rhodopsin/β-adrenergic receptor family and closely resembles

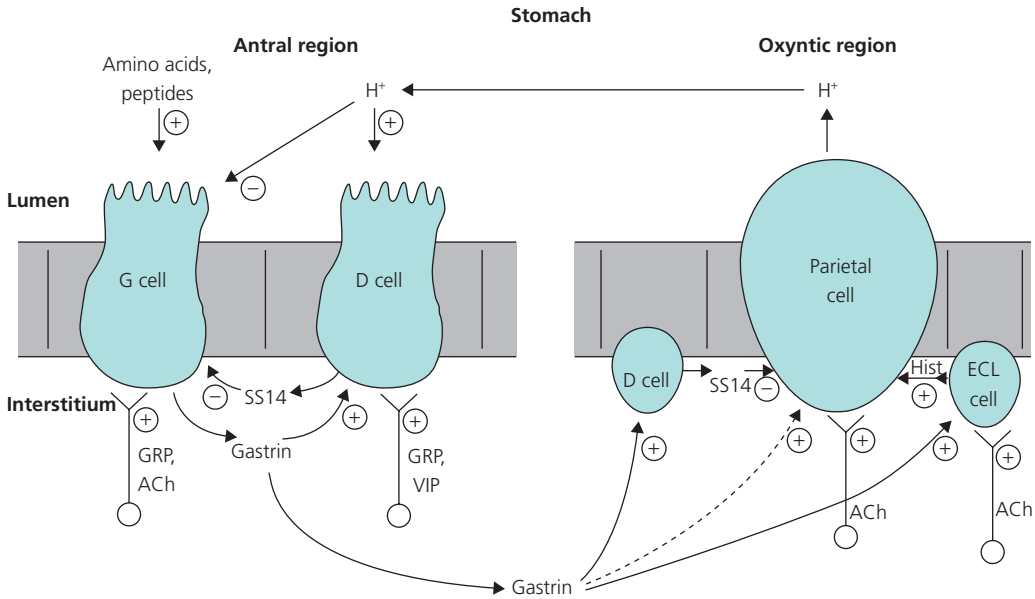

Figure 4.12 The regulation of gastric acid secretion by gastrin represents a complex series of interactions and feedback mechanisms. Some of the key components of this cycle are stimulatory events (+) and inhibitory events (–). Acetylcholine (ACh) stimulates the ECL (enterochromaffin-like) cell, the parietal cell, and the gastrin-producing G cell. Gastrin-releasing peptide (GRP) stimulates both the G cell and the D cell. Gastrin stimulates the D cell, the parietal cell, and also the ECL cell, which then secretes histamine (Hist) to also stimulate the parietal cell. Somatostatin (SS14) secreted by the D cell inhibits both the G cell and the parietal cell. Acid (H^+) secreted by the parietal cell has regulatory effects as well. VIP, vasoactive intestinal polypeptide.

the type A CCK receptor. This is particularly interesting given the likely shared evolutionary relationship between gastrin and CCK. The receptors also probably evolved from a common precursor, with divergence at some point. It is not yet clear whether the hormones or receptors diverged first, or how the relationship between the molecular basis of binding and activation might compare at these two receptors. Both receptors are coupled with Gq/11.

The structural specificity of ligand binding to these two receptors is quite distinct [107,108]. The type B CCK/gastrin receptor requires only the carboxyl-terminal tetrapeptide amide for high-affinity binding and activation. It recognizes all forms of gastrin and CCK almost equally (CCK-8 ≥ gastrin = CCK-8-desulfate = CCK-4). In contrast, the type A CCK receptor has more stringent requirements. At this target, the carboxyl-terminal heptapeptide amide of CCK is required, with the sulfated tyrosine being critical for high-affinity interaction (CCK-8 >> gastrin = CCK-8-desulfate > CCK-4). Gastrin is recognized poorly, being more than 1000-fold less potent and binding with similarly low affinity.

The type B CCK receptor is also expressed on enterochromaffin-like (ECL) cells of the gastric mucosa, on smooth muscle cells at various levels along the gastrointestinal tract, and on diffuse domains of the brain. Antagonists acting at this receptor (such as L-365260) have been reported to inhibit gastric acid secretion [109]. However, this result can also be accomplished by a number of other pharmacological routes, such as the use of histamine H_2 receptor antagonists or hydrogen–potassium ATPase inhibitors, which are already in wide clinical use. The most useful application for gastrin receptor antagonists may be for their neuronal effects, which reduce anxiety and panic attacks [110].

As noted, a major physiological effect of gastrin is the stimulation of gastric acid secretion. Of interest, isolated parietal cells respond weakly to gastrin alone [111,112]. This response is augmented in the presence of histamine or acetylcholine, both of which provide potentiating interactions. Thus, the action of gastrin in vivo to stimulate its receptor on the ECL cell and thereby stimulate histamine release helps provide the optimal background for a brisk acid secretory response. Gastrin has also been shown to stimulate histidine decarboxylase, a key enzyme in histamine synthesis.

In addition to its acute roles in stimulating acid and histamine secretion, gastrin also appears to function as a growth factor to stimulate mucosal proliferation [113,114]. Its role as a growth factor may be unique, with different tissue-specific expression during development and in adult life. During the fetal period, gastrin is expressed in pancreatic islets and in colonic epithelium. Islet expression ends at birth as these cells undergo terminal differentiation. The trophic effect on the gastric oxyntic epithelium has been suggested by the hyperplasia associated with the hypergastrinemia of Zollinger–Ellison syndrome and by the atrophy associated with low levels of gastrin after antrectomy. Chronic high-level gastrin stimulation has also been correlated with enterochromaffin cell hyperplasia. Further, in gastrin receptor-deficient mice generated by gene targeting (i.e., receptor knockout animals), the gastric mucosa was remarkably atrophic, even in the presence of hypergastrinemia [115].

In humans, more than 95% of gastrin peptides normally secreted are fully processed and amidated; the percentage of Gly-extended forms is small. Levels of Gly-extended gastrin may be higher in achlorhydria and in neoplasms such as gastrinomas and colon carcinoma [116]. Although the Gly-extended gastrins are several orders of magnitude less potent than processed gastrin in stimulating acid secretion, they may be equipotent or even more potent than processed gastrin in some growth activities that may be observed in these pathological states [117,118].

Cholecystokinin

Like gastrin, CCK is found as diverse forms sharing a carboxyl-terminal heptapeptide amide. Unlike gastrin, which has sulfated and unsulfated forms, only unsulfated forms of CCK are found in the normal circulation. This is consistent with the structure–activity relationship previously described. Although CCK-8 and possibly CCK-33/39 were thought to be the major forms of this hormone in the circulation, CCK-58 and possibly CCK-83 may be more prominent [119]. It is difficult to measure these very large forms because they are readily cleaved to smaller forms and because little standardized peptide has been available for characterization and validation of assays. No substantial differential biological effects of the molecular forms of CCK have been described. Much less is understood about the proteolytic processing of CCK than of gastrin. Although the processing enzymes are likely to be analogous, the order of cleavages and the mechanisms for tissue-specific processing are not understood. Like gastrin, the various molecular forms are cleared from the circulation at different rates. The small form, CCK-8, has a half-life of only about 1 min and is cleared almost quantitatively in a single pass through the portal circulation [120,121]. Larger forms are not cleared by the liver but seem to be metabolized by various capillary beds and by the kidney.

CCK is predominantly produced in endocrine I cells scattered throughout the proximal two-thirds of the small intestine [122]. It is also found in less abundance in various enteric neurons and in pancreatic islets. CCK-secreting endocrine neoplasms have not been well documented in the literature. The brain contains a large amount of CCK, but the regulation of peptide synthesis and release in the brain is poorly understood.

Both protein and fat components of meals stimulate secretion of CCK from I cells; carbohydrates are ineffective [123]. Long-chain triglycerides and fatty acids, both aromatic and aliphatic amino acids (e.g., phenylalanine, tryptophan, valine, and methionine), and small peptides can all stimulate CCK secretion. Unlike the tests used to measure gastrin

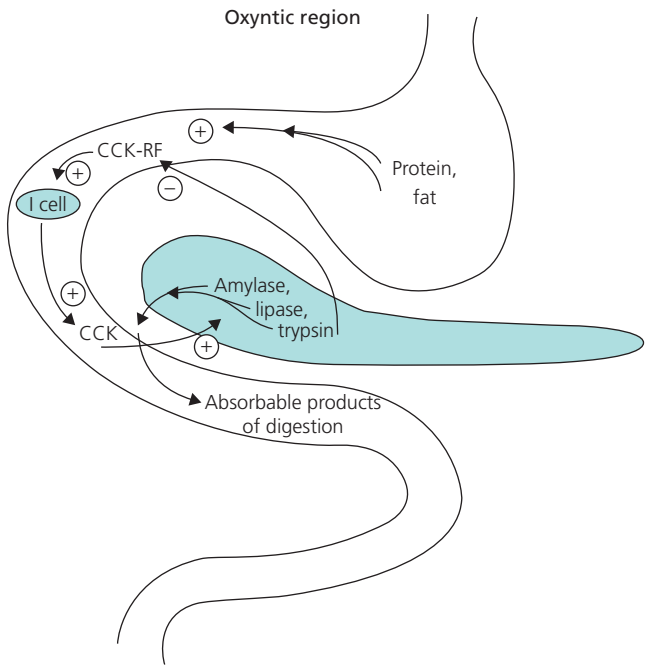

Figure 4.13 One of the regulatory cycles for CCK action on the pancreatic acinar cell. Nutrient ingestion results in the release of CCK-releasing factors (CCK-RF) into the intestinal lumen. These act on the I cells to secrete CCK, which stimulates pancreatic exocrine secretion. The proteolytic enzymes (particularly trypsin) then degrade the CCK-releasing factors, thereby terminating the cycle.

levels, the measurement of CCK is not straightforward. CCK appears to be present in the circulation in much lower concentrations (basal values: < 1 pM CCK vs 100 pM gastrin). Also, because the most common carboxyl terminal-directed antibodies can cross-react with both hormones, a selective radioimmunoassay is difficult to establish. A few radioimmunoassays are used in research that incorporate antisera directed against the amino-terminal region of CCK-8 that includes the tyrosine sulfate [124]. A bioassay that can distinguish CCK from gastrin is commonly used for research applications [125].

Feedback regulation is also involved in CCK secretion (Fig. 4.13) [126–128]. The major targets of this hormone are the rodent pancreatic acinar cells and human pancreatic neurons. When pancreatic exocrine cells are stimulated to release digestive enzymes that make their way into the lumen of the intestine, CCK secretion is reduced. Lumenal administration of a trypsin inhibitor results in increased levels of CCK and increased pancreatic enzyme secretion. Bile acids in the lumen also appear to have a regulatory effect on this feedback phenomenon [129]. Two candidates for endogenous CCK-releasing factors have been described [130,131]. Both have their activities inhibited by trypsin and fulfill the initial expectations of such a mediator, but additional studies are still necessary to define their potential roles in normal physiology.

The type A CCK receptor is structurally similar to the type B CCK/gastrin receptor (50% identical, 66% homologous) [108,132]. It is a member of the rhodopsin/β-adrenergic receptor family. This receptor has substantial structural specificity, recognizing the carboxyl-terminal heptapeptide amide of CCK, with a clear requirement for the tyrosine sulfate, the carboxyl-terminal phenylalanine amide, the nonoxidized state of the methionine residues, and the residues in almost every position of this peptide. Only the amino terminus is freely available for extension or modification without interference with the binding and biological activity of CCK. The understanding of the molecular basis of the binding of the natural agonist peptide to this receptor has improved substantially as direct evidence of contacts in the extracellular loop and tail domains have been defined for almost every residue within the CCK pharmacophore [133]. Nonpeptidyl antagonists (e.g., L-364718, lorglumide, PD140548) and even a nonpeptidyl agonist are available for selective action on this receptor [31,134]. None of these reagents has reached clinical use. The antagonists induce gallbladder stasis; their use, therefore, is complicated by gallstone formation. The agonist theoretically could provide a new mechanism to achieve satiety, but its trophic effect may carry unacceptable risks.

CCK is interesting for the diversity of its established physiological and biological effects [135]. Almost all of its actions relate to nutrient homeostasis. Its major digestive effects are to stimulate pancreatic acinar cell secretion of zymogens and to stimulate gallbladder muscularis smooth muscle cell contraction. Both of these events result in the delivery of key ingredients for digestion to the intestinal lumen. It also causes contraction of the pylorus to slow gastric emptying, providing optimal nutrient delivery for digestion. A key neuronal effect of this hormone is to satiate appetite. Less well-established effects include modification of enteric and colonic transit, reduction in acid secretion by its role as a somatostatin secretagogue, stimulation of secretion of PP, and an insulinotropic effect.

Pancreatic polypeptide family

The pancreatic polypeptide family includes PP, peptide YY (PYY), and neuropeptide Y (NPY). These peptides have extensive homology within their carboxyl-terminal domains, with absolute conservation of an arginine–tyrosine amide. PYY and NPY have extensive overall homology with each other. These three peptides also show cross-recognition of a group of three receptors. Each peptide has a unique distribution and unique functions. Their role in appetite regulation has become a subject of considerable interest [136,137].

Pancreatic polypeptide

PP was originally identified as a contaminant in preparations of insulin extracted from pancreatic islets. It is a 36-amino acid peptide that is predicted to have a globular shape, resulting

from interactions between a polyproline helix and an α-helix [138,139]. Its biological activity resides in its carboxyl-terminal hexapeptide amide, with the carboxyl-terminal tyrosine amide being the critical residue for this activity. It has a short half-life in the circulation (about 7 min), with renal clearance apparently most important.

PP cells are found in the periphery of pancreatic islets and scattered within the parenchyma of the pancreatic head and uncinate lobe, in contrast to the distribution of glucagon in the pancreatic body and tail. There is a clear PP response to the ingestion of a meal. The response is biphasic; the early increase is neurally mediated and inclusive of cephalic and vagal phases. In many ways, secretion of PP correlates with vagal tone. The nutrients that stimulate gastrin and CCK secretion also stimulate PP secretion. Secretion of this hormone also appears to be stimulated by CCK, gastrin, GRP, neuromedins B and C, and secretin.

Although several biological effects of PP have been proposed, debate continues. This hormone has been thought to inhibit pancreatic exocrine secretion, but antibody neutralization studies have failed to show any effect on pancreatic secretion. A metabolic role as an inhibitor of hepatic glucose production has also been proposed.

A series of receptor subtypes that recognize the pancreatic polypeptide family of peptides has been proposed, which are identified as Y1–Y6; only the Y1, Y2, Y4, and Y5 receptors have been found in humans [137]. All are structurally related members of the rhodopsin/β-adrenergic receptor family of the G protein-coupled superfamily of receptors. The PP1-preferring receptor is able to recognize pancreatic polypeptide better than either of the other family members (PP >> NPY = PYY) [140]. Both of the Y1 and Y2 receptors recognize PYY and NPY similarly, and much better than PP (PYY ≥ NPY >> PP). There are selective agonists that distinguish between these receptors, with [Leu31,Pro34]PYY and [Leu31,Pro34]NPY selective for the Y1 receptor, and carboxyl-terminal fragments (PYY[3–36], PYY[13–36], and NPY[13–36]) selective for the Y2 receptor [141].

Perhaps the best current use of PP is as a marker for islet cell tumors. Plasma levels of PP are also elevated in diabetes mellitus and in a high percentage of islet cell carcinomas and multiple endocrine neoplasia. The percentage of positive values varies with the type of endocrine tumor; VIP-secreting tumors have very high levels and gastrin-secreting tumors have substantially lower levels.

Peptide YY

PYY is also a 36-amino acid peptide. It is found in highest concentrations in enteroendocrine cells in the distal small intestine and colon, mainly in the basal crypts of L cells [142–144]. It can act through endocrine mechanisms after release into the bloodstream, or through paracrine mechanisms with specialized basal processes surrounding columnar cells in the vicinity of the endocrine cells. It is released in response to meals. Fat is the major stimulant but other nutrients (e.g., carbohydrate) and bile acids also stimulate secretion. Neuronal release mechanisms have also been postulated, and in some malabsorptive states, high circulating levels have been observed. GRP can also stimulate its release. Like PP, PYY has a short half-life in the circulation.

The actions of PYY are largely inhibitory [145,146]. It inhibits gastrointestinal motility, pancreatic and gastric secretion, and chloride secretion. It has been given the name ileal brake because it helps to establish longer contact times when nutrients reach the distal gut and colon. PYY has also been shown to have trophic effects, but the physiological significance of this action is not yet clear.

Neuropeptide Y

NPY is a 36-amino acid peptide that is structurally homologous to PYY [147,148]. It appears to be the neurotransmitter equivalent of PYY, found in the central and peripheral nervous systems. It is one of the most abundant peptides in the brain, along with CCK. It is found in enteric neurons, alone or colocalized with other neuropeptides [149,150]. It is especially prominent in sympathetic neurons that innervate blood vessels.

Postulated effects of NPY include the stimulation of appetite, alteration of circadian rhythms, vasoconstriction, ethanol consumption, and neuronal development [151–153]. It has many of the same effects as PYY because they both recognize the same receptors, but its physiological role relates mainly to its anatomical distribution.

Tachykinin family

The tachykinins are a group of biologically active peptides sharing the carboxyl-terminal consensus sequence -Phe-X-Gly-Leu-Met-NH_2 [154–157]. Included in this group are substance P, GRP, the neuromedins, and many mollusk and amphibian peptides (bombesin, physalaemin, eledoisin, kassinin, and phyllomedusin). These peptides are neurotransmitters in both the central and peripheral nervous systems. The pharmacophoric domain of the tachykinins is predominantly within the carboxyl terminus and well circumscribed. Consistent with this feature, the receptors for this family are in the rhodopsin/β-adrenergic receptor family of the G protein-coupled superfamily of receptors. Tachykinins are present in a wide variety of cells, including glial, smooth muscle, epithelial, endothelial, glandular, and immune cells.

Substance P (neurokinin A)

Substance P is an 11-amino acid peptide amide that is widely distributed in the brain, spinal cord, and peripheral and enteric nervous systems [157,158]. Its highest concentrations along the digestive tract are in neurons of the esophagus, proximal small intestine, and colon. Substance P stimulates esophageal and intestinal peristalsis and pancreatic

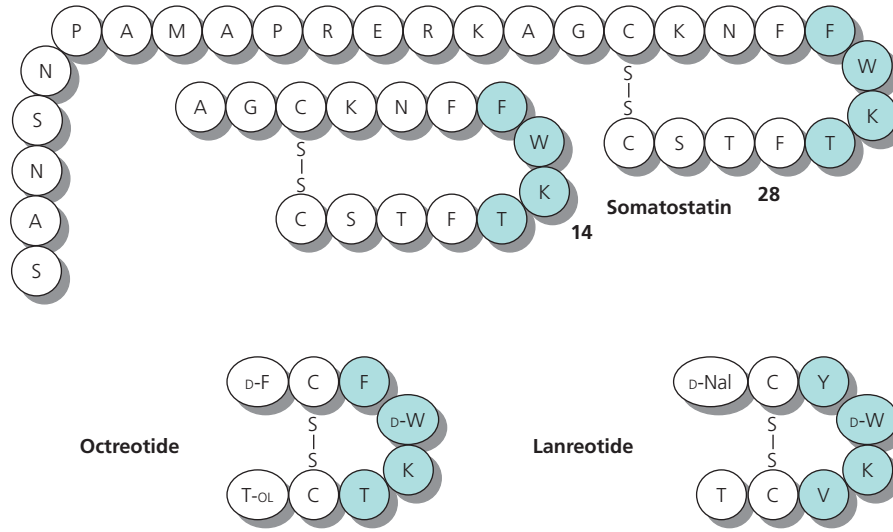

Figure 4.14 Structures of somatostatin-14 and somatostatin-28, as well as two prominent synthetic agonist analogues, octreotide and lanreotide, that incorporate unnatural amino acids and terminal blocking groups, prolonging biological action.

secretion, and it inhibits biliary secretion and somatostatin secretion. It likely plays a sensory role along the digestive tract, participating in the afferent limb of various reflexes and possibly mediating pain impulses. In the central nervous system it probably acts as a neuromodulator.

Gastrin-releasing peptide

GRP was originally isolated during a search for an analogue to the amphibian peptide bombesin [159]. Like bombesin, GRP stimulates gastrin secretion. It is present in neurons of the stomach, intestine, and colon, as well as in the brain and spinal cord. It also stimulates the release of CCK, motilin, neurotensin, enteroglucagon, insulin, somatostatin, and substance P, and it can stimulate pancreatic acinar cell secretion and gut motility. The release of secretin from S cells is not stimulated by GRP. It may also play a role as an excitatory neurotransmitter, acting on enteric interneurons. Like several of the peptides discussed, GRP appears to have trophic activity. Receptors for this peptide have been described on small cell carcinomas of the lung, where a trophic response may have clinical significance [160].

Several of the neuromedins were isolated during a search for mammalian homologs of the amphibian peptides. Neuromedin C shares its carboxyl-terminal decapeptide amide with GRP. Neuromedin B is related to ranatensin.

Four receptor subtypes, BB1, BB2, BB3, and BB4, are recognized for this class of ligands [161]. The BB1 receptor recognizes neuromedin B with highest affinity (neuromedin B ≥ bombesin > GRP). The BB2 receptor recognizes GRP best (GRP ≥ bombesin >> neuromedin B). The BB3 receptor has a high degree of homology with the BB1 and BB2 receptors but binds both GRP and neuromedin B with low affinities. Its natural ligand has not yet been identified. Its presence has been reported in reproductive organs and lung carcinoma; it may not have a relationship with the gastrointestinal tract. The BB4 receptor was isolated from frog brain and has a unique pharmacological profile – it is highly dependent on the penultimate phenylalanine residue for agonist activity, and it has a unique capability for antagonist recognition [162].

Somatostatin family

The two molecular forms of somatostatin are 28 and 14 amino acids in length, with somatostatin-14 representing the carboxyl-terminal domain of somatostatin-28 [163–166]. Both forms of somatostatin contain a critical disulfide bond giving them a cyclic structure (Fig. 4.14). Amino acid residues 7–10 within the short cyclic structure of somatostatin-14 are also critical for activity. Although this hormone was originally identified for its ability to inhibit growth hormone, it is now recognized as having a much broader significance.

Somatostatin is synthesized and secreted from both neurons and enteroendocrine cells, with the latter (D cells) present as either open or closed [167,168]. In the stomach, there are highly specialized D cells with characteristic long processes that extend to G cells, parietal cells, and chief cells. These configurations make somatostatin one of the most flexible regulators of the gastrointestinal tract. Its multiple roles as hormone, paracrine transmitter, neurotransmitter, and neuromodulator reflect this flexibility. Along the digestive tract, 90% of somatostatin is present in the mucosa and 10% in the neuromuscular layers. In the stomach, pancreas, and neuromuscular layers of the intestine, somatostatin-14 is the predominant form, whereas both somatostatin-14 and somatostatin-28 are found in the mucosa of the bowel. Somatostatin-14 has a very short half-life of 1–3 min, whereas somatostatin-28 has a half-life of about 15 min. The degradation sites of this hormone are not well defined, but they likely involve capillary beds.

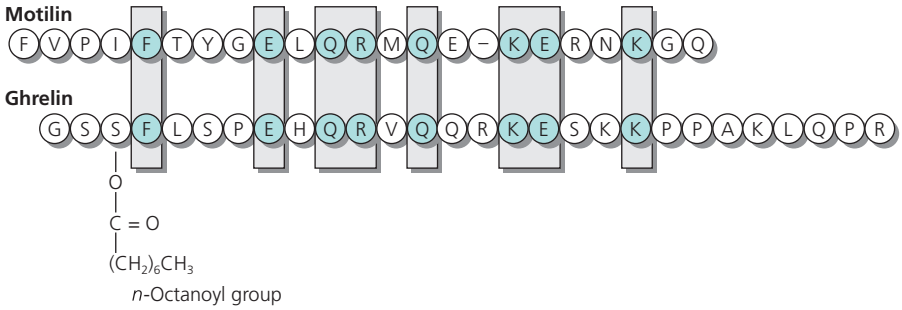

Figure 4.15 Alignment of sequences of motilin and ghrelin, members of the motilin family. Ghrelin has a unique posttranslational modification of the serine residue in position 3, representing O-*n*-octanoylation.

Most of the biological effects of somatostatin are inhibitory. It inhibits gastric, pancreatic, biliary, and even salivary secretion, in addition to inhibiting the release of a broad variety of gastrointestinal hormones. These include gastrin, CCK, secretin, PP, GIP, motilin, glucagon, and insulin. It also inhibits gut motility, but this effect results from its ability to inhibit cholinergic neurons. Somatostatin decreases splanchnic and portal blood flow.

Somatostatin receptors are in the rhodopsin/β-adrenergic receptor family of the G protein-coupled superfamily of receptors. There are five recognized receptor subtypes: SSTR1, SSTR2, SSTR3, SSTR4, and SSTR5 [169–171]. These receptors are structurally homologous but each couples to distinct G proteins and has a distinct tissue distribution. They are most closely related to the opioid receptors, with which they share about 40% homology. Based on structural similarity, there are two groups of somatostatin receptors: SSTR1 is related to SSTR4, and SSTR2, SSTR3, and SSTR5 are related to each other. SSTR5 is unique among the somatostatin receptors because it has a higher affinity for somatostatin-28 than for somatostatin-14. SSTR1 is found in the stomach and intestine. These receptors most prominently couple to adenylate cyclase; some receptors also activate potassium channels and voltage- and potassium-dependent calcium channels. The somatostatin receptor may also activate a protein tyrosine phosphatase, although the precise molecular mechanism for this is unclear.

An analogue of somatostatin that is a long-acting cyclic agonist peptide has become a highly useful clinical reagent. It represents an 8-amino acid cyclic peptide with unnatural residues that make it resistant to proteolysis. It has been used in the imaging and treatment of gastrointestinal hormone-secreting tumors, diarrheal disorders, and carcinoid syndrome [166,172].

Motilin family

Motilin is a 22-amino acid linear peptide that was originally recognized as being secreted into the circulation in a cyclic manner that correlated with increased motor activity along the bowel. It has become one of the most important endogenous prokinetic peptides, and its receptor is recognized as a useful pharmacological target, activated by erythromycin [173,174]. The cDNA encoding the motilin receptor was cloned in 1999. Sequence analysis showed that it was a member of the rhodopsin/β-adrenergic receptor family of the G protein-coupled superfamily of receptors, having closest homology to a group of growth hormone secretagogue receptors. The molecular basis of its binding has received more recent attention [175,176]. One extremely interesting aspect of studying the growth hormone secretagogue receptor was the discovery of ghrelin, an endogenous agonist ligand representing a 28-amino acid peptide that has a high degree of homology with motilin, and a unique posttranslational modification of O-*n*-octanoylation of the Ser residue in position 3 (Fig. 4.15) [177].

Only a single motilin receptor cDNA has been identified, even though there is pharmacological evidence for more than one pattern of response to this hormone [178]. Examination of the molecular basis of these varied responses will likely become an area of active exploration.

Protease-activated receptor family

Proteases, such as trypsin, have traditionally not been thought of as signaling molecules. There is evidence, however, of distinct G protein-coupled receptors in the rhodopsin/β-adrenergic receptor family of G protein-coupled receptors that are activated by proteolytic cleavage of an amino-terminal peptide sequence [179,180]. This makes an endogenous peptide ligand at the new amino-terminal end of the receptor accessible for binding to the remainder of the receptor, thus providing a novel molecular mechanism of action. To date, two such receptors have been identified – the thrombin receptor (or protease-activated receptor 1) and the protease-activated receptor 2 [181,182]. A receptor activation mechanism that requires proteolytic cleavage has substantial implications for receptor regulation, particularly for ensuring the inability to resensitize [183].

These receptors are believed to play roles in the linkage of tissue injury and vascular leakage to cellular responses, such as occurs in hemostasis, inflammation, and angiogenesis. Roles have also been postulated for these receptors in the intestine and pancreas.

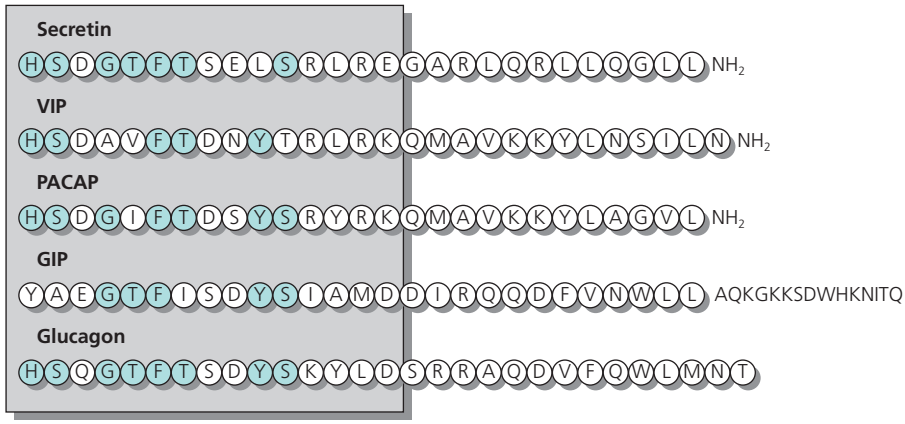

Figure 4.16 Alignment of sequences of members of the secretin family. The amino-terminal domain is most critical for agonist action, whereas the pharmacophore extends throughout the entire peptide sequence.

Chemokine family

The chemokines constitute an extremely large family of ligands and receptors, with biological activities best described for leukocytes [184–186]. Although not specific to the gastrointestinal tract, this system has great relevance to it. More than 40 distinct human chemokines have been described and assigned extremely varied original names based on the tissue or cell of origin, the target cell type, the size of the molecule, or the action. A more coherent nomenclature that correlates with the organization of the receptors for these ligands was proposed in 1999 [187]. This system classifies chemokines by their structure into four groups, based on the number and the spacing of conserved cysteine residues: C, CC, CXC, and CX3C. The CC, CXC, and CX3C cytokines have 4 conserved cysteine residues, with the first two such residues being adjacent to one another or having one or three other residues between them respectively. The C cytokines have only two conserved cysteine residues, representing the second and fourth cysteine residues that are present in the other groups of chemokines.

The chemokine receptors are in the G protein-coupled receptor superfamily. They typically recognize more than one chemokine, but these are usually members of a single chemokine family. The receptor nomenclature is based on the ligand specificity, starting with CC or CXC, and followed by R and a number. Of this group, 18 chemokine receptors have been cloned, and these express 25%–80% sequence identity among themselves.

Another interesting feature of the chemokines and their receptors is the substantial diversity of structure across species, with up to 55% sequence divergence reported. The pressure for the rapidity of evolution of these structures has not been established. Also of note are the nonchemokine ligands for these receptors, which include HIV; the chemokine receptor seems to play an important role as a coreceptor for the virus, permitting its entry into the target cell. On tumor cells, these receptors may determine the metastatic pattern.

Secretin family

The secretin family of hormones is characterized by homology that is most evident in the amino-terminal half of these moderately large polypeptides (Fig. 4.16). Members of this family with known hormone and receptor sequences include secretin [188], VIP [189], pituitary adenylate cyclase-activating peptide (PACAP), GIP [190], glucagon, glucagon-like peptide-1 (GLP-1), calcitonin, calcitonin gene-related peptide (CGRP), parathyroid hormone [191], corticotropin-releasing factor (CRF), and growth hormone-releasing factor (GHRF) [192,193]. Some of these are not relevant to the gastrointestinal tract and are not discussed further. It must be recognized, however, that insights from any members of this family may provide important clues relevant to the entire family. Based on the structural homology of hormones, it is expected that the PHI receptor also belongs to this family, although a distinct receptor for this peptide has not yet been cloned.

The pharmacophoric domains of these hormones are rather diffuse and spread throughout their primary sequences, usually requiring peptide analogues to span essentially the entire length of the natural hormones for maintenance of biological activity. In some of these hormones, the amino-terminal residue and a free amino terminus are particularly important. This observation implies the interesting notion that amino terminal-extended precursor forms may not be biologically active. In fact, few of the hormones in the secretin family have more than one molecular form, as is so common for peptide hormones belonging to the rhodopsin/β-adrenergic receptor family. Some truncated forms of the secretin family hormones have been synthesized and found to continue to bind with high affinity, but these possess markedly reduced biological activity. Another interesting feature of this family is that its members stimulate dual signaling cascades; low concentrations typically stimulate adenylate cyclase, and higher concentrations stimulate intracellular calcium responses. This may also occur in the rhodopsin/β-adrenergic receptor family, but it is less common.

Secretin

Secretin holds a special place in gastrointestinal endocrinology and in endocrinology generally. Its discovery in 1902 by Bayliss and Starling [15] established the concept of hormones as chemical messengers that travel through the bloodstream to control the function of a target organ. As such, the discovery of secretin gave birth to the whole field of endocrinology. It was therefore the first gastrointestinal hormone, although not the first one to be characterized chemically. Secretin clearly has important physiological functions. Because of its relatively large size and the chemical difficulties encountered in working with this peptide and with its receptor, a detailed molecular understanding of this hormone has been delayed substantially. The cloning of cDNAs for members of this receptor family charged this field with new life and excitement, and it continues to be a fertile area for investigation.

Secretin is a 27-amino acid linear peptide with important residues scattered throughout the length of the hormone [194]. It is synthesized in endocrine cells (S cells) that are most abundant in the duodenum and proximal jejunum and present in reduced numbers throughout the distal small intestine. The major stimulus for secretin secretion is unbuffered hydrogen ions as they traverse the duodenum. This stimulus becomes apparent when the duodenal pH decreases to 4 or lower. Secretin secretion can also be stimulated by nutritional acids, such as fatty acids, and by ethanol and components of spicy foods (e.g., 1-phenylpentanol). Bile acids appear to enhance stimulated secretin release. Of interest, the S cell is one of the few enteroendocrine cells that is not responsive to GRP. However, the existence of a secretin-releasing peptide has been postulated. Like most of the peptide hormones, secretin has a short half-life of less than 3 min in the circulation. Renal clearance appears to be the predominant mode of elimination.

The major effect of secretin is the secretion of bicarbonate from biliary and pancreatic ductular epithelium and Brunner glands. This nicely completes the regulatory cycle, with the acid stimulant of secretin release neutralized by the bicarbonate secreted in response to this hormone. It has been estimated that secretin is responsible for 80% of the bicarbonate response to a meal. In nonhuman species, secretin has also been shown to inhibit gastric secretion and gastric motility, to lower esophageal sphincter tone, and to stimulate insulin release and colonic mucus secretion, but these are likely not physiological actions in humans.

Consistent with these observations, the most prominent targets for secretin include ductular epithelial cells in the biliary tree and pancreas, and Brunner gland cells [193]. Receptors are also believed to be expressed on pancreatic acinar cells of some species, vascular smooth muscle in selected organs, some enteric smooth muscle (particularly in the region of the lower esophageal sphincter), and some enteric mucosal cells. These targets were initially characterized as having a potent biological response to secretin, and a lesser response to VIP. However, such studies are difficult to interpret because of the cross-recognition of many peptides in the family by receptors in the family. Now that the structure of the receptors is better understood, the expression of receptors on distinct cells has become easier to identify.

The secretin receptor cDNA was initially cloned in 1991 by Ishihara and colleagues [188]. Its structure appeared to have the seven hydrophobic helical transmembrane domains typical of the G protein-coupled receptor superfamily, but other features of the receptor were unique. In fact, the level of homology with previously cloned receptors in the rhodopsin/β-adrenergic family was only 12%. Subsequent cloning of cDNAs encoding receptors for calcitonin and parathyroid hormone, which are more similar to secretin receptors, defined a new class of G protein-coupled receptors. The most highly conserved areas are in the transmembrane domains, and a particularly long amino-terminal tail contains six highly conserved cysteine residues that are highly sensitive to reducing and cysteine-reactive reagents. The pattern of these disulfide bonds has been experimentally defined, and is conserved throughout the secretin receptor family [195]. Another such bond that is likely to be present is the connection between cysteine residues in the first and second extracellular loops, in positions that are conserved throughout the entire superfamily of G protein-coupled receptors. This bond has been characterized for other receptors in the rhodopsin/β-adrenergic family [196].

Various studies including mutagenesis and photoaffinity labeling have shown the importance of the amino-terminal tail and extracellular loop domains of this receptor for secretin binding and activation [193]. This finding is consistent with the general theme for this superfamily, in which increasingly larger ligands bind to domains moving from the confluence of intramembranous helices to the cellular surface, and ultimately to the extracellular loop and tail domains. An important and novel mechanism for the activation of the secretin family of receptors has been proposed, suggesting that binding of the peptide ligand to the amino-terminal domain results in a conformational change in this domain, exposing an endogenous agonist that interacts with the core of the receptor to activate it (Fig. 4.17) [197].

Despite having new signature sequences unique to the secretin receptor family, the secretin receptor seems to signal and to be regulated much like the receptors in the rhodopsin/β-adrenergic family [198]. The secretin receptor is coupled to both Gs and Gq/11. The most potent response to hormonal stimulation is an increase in adenylate cyclase, and less potent is the stimulation of intracellular calcium. The secretin receptor is phosphorylated in response to agonist stimulation, and this phosphorylation interferes with G protein coupling. Like most G protein-coupled receptors, this receptor is internalized after agonist occupation. The details of the molecular basis of these events are being explored.

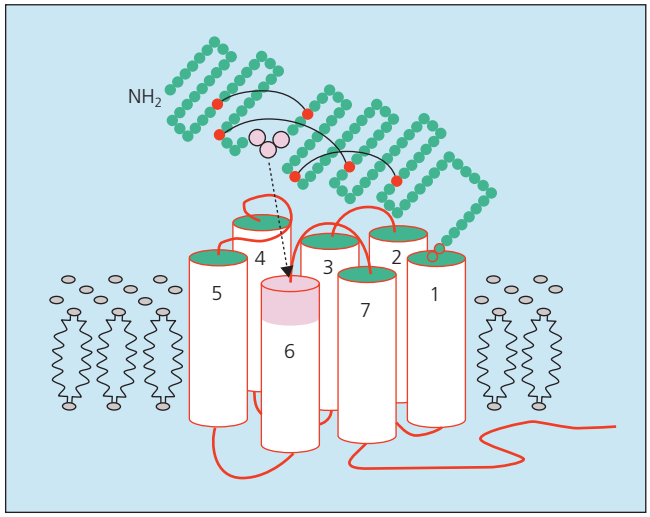

Figure 4.17 Proposed mechanism for the activation of class B G protein-coupled receptors. The peptide ligand-binding domain is within the large and complex amino terminus, exposing an endogenous epitope within this domain which acts as a secondary ligand that interacts with the receptor body. From Beinborn [219], with permission from the American Society for Pharmacology and Experimental Therapeutics.

Vasoactive intestinal polypeptide

VIP is a 28-amino acid linear peptide with close structural homology to secretin [199]. Conformational studies have supported similar structures as well. Unlike the hormone secretin, VIP is exclusively a neurotransmitter. In addition to being present in enteric neurons, VIP is also present in neurons of the brain, spinal cord, lung, urogenital system, and other endocrine organs. VIP has a very short half-life of less than 1 min in the circulation. Accordingly, plasma levels are quite low and unresponsive to the ingestion of a meal. High plasma levels have been reported in the setting of watery diarrhea–hypokalemia–achlorhydria syndrome associated with a VIP-producing endocrine tumor (VIPoma) [200].

The VIPoma syndrome provides insight into potential targets and actions of VIP. The normal physiological role of VIP is difficult to determine because of the local actions and absence of reagents with which to dissect its role from those of other related transmitters. Among the potential actions are stimulation of enteric smooth muscle, stimulation of pancreatic exocrine and intestinal secretion, inhibition of gastric acid secretion, and modification of immune function and gastrointestinal blood flow. The list of tissues and cells containing VIP receptors includes pancreatic acini, pancreatic and biliary ductular epithelial cells, gastric and intestinal epithelial cells, vascular smooth muscle cells, lymphocytes, and a number of epithelial tumors. Direct effects on enteric smooth muscle cells and modulatory effects on interneurons have been demonstrated. VIP is a key candidate for mediating descending relaxation of the peristaltic reflex and relaxation at the lower esophageal sphincter, the internal anal sphincter, and the tenia coli.

Two VIP receptors have been cloned: VIP1 (or VPAC1) and VIP2 (or VPAC2) [201]. Both are typical members of the secretin family of G protein-coupled receptors. VIP is also well recognized by the PACAP (or PAC1) receptor [202]. Both the VIP1 and VIP2 receptors have similar structural specificities for VIP, PACAP, and PHI (VIP = PACAP > PHI). Secretin is recognized weakly by the VIP1 receptor, and not at all by the VIP2 receptor. The selectivity of the PACAP receptor is quite distinct (PACAP > VIP > PHI). Another interesting feature of the PACAP receptor is prominent alternative splicing, yielding variants that differ in both ligand recognition and intracellular signal transduction pathways. The specific tissue and cellular distribution of these receptors is being characterized.

Studies with chimeric constructs incorporating domains of the VIP and secretin receptors have also supported the importance of the amino-terminal domain of these receptors in their selectivity for binding and initiation of active conformations [203]. Such observations indicate the need to understand more about the conformation of the ectodomain of these receptors in an effort to contribute to the rational design of drugs that might act on this important family of receptors.

Pituitary adenylate cyclase-activating peptide

PACAP is a 27-amino acid linear peptide that was initially isolated from ovine brain [204]. Unusual for this family of hormones, there is also a second molecular form, PACAP-38, which consists of PACAP with a carboxyl-terminal extension of 11 amino acids. Like many other brain peptides, PACAP is also present in the gastrointestinal tract, in nerve fibers, and in myenteric and submucous ganglia. PACAP-38 is the predominant peptide in nonenteric locations (e.g., brain, lung, and testes), whereas PACAP-27 is predominant in the intestine. Like VIP, the low circulating levels of PACAP are unresponsive to the ingestion of a meal.

Because of the high degree of homology between PACAP and VIP and the relationship of their receptors, interest has been focused on this neurotransmitter, even though it does not yet have proven physiological functions. Candidate functions include relaxation effects on colonic smooth muscle, stimulation of pancreatic exocrine secretion, and vasodilation.

Glucose-dependent insulinotropic peptide

GIP was originally called *gastric inhibitory peptide*, until it became clear that inhibition of acid secretion is not a physiological action of this hormone. Instead, its most prominent effect is to stimulate insulin production in a glucose-dependent manner, hence the name *glucose-dependent insulinotropic peptide*. A 42-amino acid linear peptide homologous to secretin and glucagons [205,206], GIP is produced in K cells in the proximal intestinal crypts. This peptide hormone is secreted

predominantly in response to the ingestion of a meal, with fat the major nutrient stimulant. Both the secretion of GIP and the response of target organs to GIP are affected by cholinergic tone. GIP has a half-life of 18 min, which is longer than that of most gastrointestinal peptides.

The GIP receptor, like the peptide, is homologous to other members of the secretin receptor family [190]. This is also true for the description of its coupling, signaling, and regulatory events.

The main established biological function for GIP is its role as a stimulant of insulin secretion (incretin) [207]. Incretins provided the explanation for the greater insulin responses to nutrients administered orally as opposed to parenterally. There have been many proposed candidates to mediate this action, but GIP and GLP-1 are considered important. For GIP to exert this effect on the cells of the pancreatic islet, the serum glucose level must be above 110 mg/dL, thus providing protection against hypoglycemia. Other metabolic effects of GIP have been described, such as inhibition of hepatic glucose output, inhibition of fat absorption, and inhibition of lipoprotein lipase. The physiological role of GIP in mediating these effects is not clear.

Tyrosine kinase receptor family

The receptors in this family are quite distinct from the G protein-coupled receptors. All are single-transmembrane receptors and all have prominent effects on cell growth (Fig. 4.18). Some also affect cell survival, differentiation, and movement. Perturbations of several tyrosine kinase receptors have been associated with tumorigenesis. Many G protein-coupled receptors also appear to mediate effects on cell growth, but they do so through a distinct mechanism. Activation of G protein-coupled receptors can also stimulate tyrosine phosphorylation events; however, most of the substrates are distinct from those phosphorylated in response to activation of the tyrosine kinase receptor family [208].

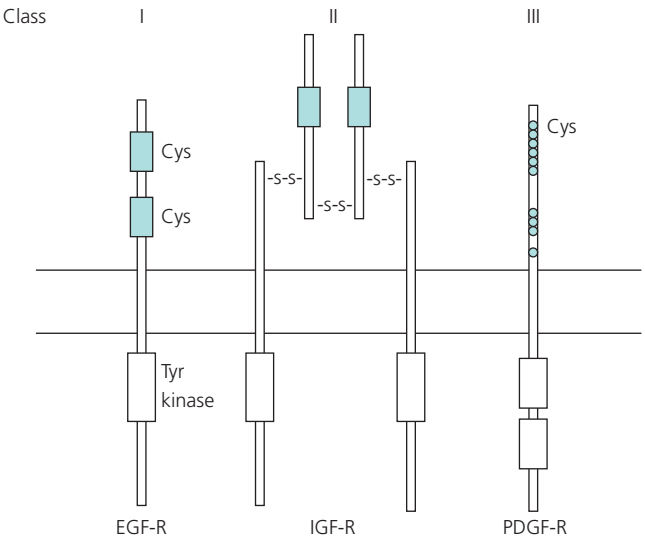

Figure 4.18 The three classes of tyrosine kinase receptor. All possess cysteine-rich regions in the ectodomain and tyrosine kinase catalytic domains in the cytoplasm. The classification of tyrosine kinase receptors into the three classes is dependent on the continuity and spacing of these regions, as well as the subunit structure necessary to achieve a functional unit for the class II receptors.

Epidermal growth factor

Epidermal growth factor (EGF) is a 53-amino acid single-chain polypeptide that contains three intramolecular disulfide bonds. Additional ligands in this family include transforming growth factor-α, amphiregulin, betacellulin, heparin-binding EGF, and the neuregulins. All share the sequence motif CX7 CX2–3 GXCX10–13 CXCX5 GXRC. All are produced as single-transmembrane proteins with an amino-terminal proregion, a mature growth factor region ranging in length from 50 to 87 residues, and a relatively short cytosolic domain. Members of the EGF family are active both while anchored to the membrane and after proteolytic processing to release the mature growth factor domain [72].

There are four members of the EGF receptor subfamily, including the EGF receptor encoded by *erbB* and proteins encoded by *erbB-2*, *erbB-3*, and *erbB-4*. All are single-transmembrane glycoproteins that incorporate two distinct cysteine-rich regions in the ectodomain and a tyrosine kinase catalytic domain and typical sites of potential phosphorylation in cytoplasmic regions. Receptor dimerization or oligomerization is a typical initial step in signaling that is induced by agonist binding. This is followed by cross-phosphorylation on tyrosine residues of the receptor and the recruitment of kinase substrates and other signaling proteins. Many of these substrates have Src-homology-2 (SH2) domains. A large number of potential signaling cascades have been described, including the Ras, Raf, and MAP kinase pathways and the JAK/STAT pathways [209,210]. EGF signaling also includes activation of phosphatidylinositol pathways, with activation of protein kinase C isozymes and increases in intracellular Ca^{2+}.

Many gastrointestinal functions can be affected by this family of hormones and receptors. Included among the most prominent effects are inhibition of gastric acid secretion, protection against injury, and stimulation of intestinal cell growth. There are also putative growth-stimulatory and -inhibitory effects on a number of gastrointestinal tumors. This provides many interesting possibilities for therapeutic uses of agonists and antagonists acting at these receptors [211].

Fibroblast growth factors

Fibroblast growth factors (FGFs) are a group of polypeptides with angiogenic and mitogenic activities that are active in tissue repair and in modulating tissue differentiation. FGFs induce chemotactic activities in fibroblasts and endothelial

cells [74]. Nine members of the FGF family have been identified, each with a conserved core sequence of about 120 residues and with high affinity for heparin and glycosaminoglycans. FGF1, also known as acidic fibroblast growth factor, and FGF2, also known as basic fibroblast growth factor, are not efficiently secreted, whereas all other forms possess signal peptides and are secreted. FGF7 is also known as keratinocyte growth factor (KGF).

Receptors for the FGFs are monomeric and, like other growth factor receptors, they possess a tyrosine kinase domain. Unlike that of most such receptors, the tyrosine kinase domain of these receptors is discontinuous, with a 14-residue interruption. Also, the FGF receptors have two or three immunoglobulin-like domains within an extracellular domain. At least four distinct genes encode FGFR-1 through FGFR-4. FGFR-1 and FGFR-2 also have multiple alternatively spliced forms. All four types of FGFR can be activated by either the acidic or basic forms of FGF (FGF1 or FGF2). FGF7 (KGF) binds only to the KGFR, which is a distinct splice variant of the FGFR-2. Heparin sulfate proteoglycans on the cell surface can also bind these growth factors, but with low affinity. It has been postulated that this is a site for the collection of the ligand in preparation for binding to its high-affinity receptor.

Autophosphorylation of the FGF receptors occurs and is a mechanism for regulation. Tyrosine phosphorylation occurs in response to FGF binding, thereby opening and exposing the kinase domain. Phospholipase C-γ can associate with the tyrosine-phosphorylated receptor. Activation also stimulates Shc association with a kinase domain of the receptor, which leads to activation of Grb2/SOS. This in turn leads to the typical series of events activated by other growth factors, such as activation of Raf-1, MAP kinase kinase, and MAP kinase.

FGF receptors can form homodimers or heterodimers. The composition of the dimer can determine the details of the signaling initiated. Signaling cascades appear to be quite similar to those described for the EGF receptors.

Biological actions for this group of hormones include prominent effects on angiogenesis through effects on endothelial cells. Also described are effects on fibroblasts, smooth muscle cells, and various neuronal cells. Like other growth factors, FGFs also have been implicated in tumorigenesis.

Insulin-like growth factors

Insulin-like growth factors (IGF-1 and IGF-2) are single-chain polypeptides that are homologous to proinsulin [73]. These hormones were first recognized by the demonstration that the addition of insulin antiserum to neutralize insulin activity failed to inhibit all activities attributed to that hormone. The IGFs are structurally related to insulin, but they bind poorly to traditional insulin receptors. Like insulin, the IGFs have both A and B domains. Unlike insulin, they lack the C domain, which is cleaved in the processing of insulin to achieve its mature 2-chain form. IGFs remain a single chain, although they fold to achieve a conformation similar to that of insulin.

Like the insulin receptor, the type 1 IGF receptor consists of a heterotetrameric structure with two α-subunits and two β-subunits, both of which come from a single gene product. Disulfide bonds link the complex, with the α-subunits residing entirely outside the cell and possessing the binding determinants, and the β-subunits spanning the plasma membrane and containing the tyrosine kinase domain intracellularly. As in the insulin receptor, signaling involves tyrosine autophosphorylation, as well as association and phosphorylation of other substrates.

In addition to the receptor, there are soluble circulating and interstitial IGF-binding proteins (IGFBP-1 to -6). They may affect the volumes of distribution and half-lives of IGF and thereby modify its biological activities. IGFs affect the growth and differentiation of a variety of tissues.

Toll-like receptor family

All multicellular organisms have innate immunity as a form of host defense against microbes. Infection or infestation leads to early defenses at the levels of phagocytic leukocytes, endothelial cells, and mucosal epithelial cells, with responses mediated by receptors that recognize pathogen-associated molecular patterns, such as the lipopolysaccharide in bacterial cell walls. Activation of such receptors leads to biological responses that are designed to clear the offending stimulus and to protect the organism. The toll-like receptors are a particularly interesting group of this type of receptor that can recognize and distinguish between various classes of pathogens [212]. They are located on monocytes, macrophages, neutrophils, dendritic cells, intestinal epithelial cells, and endothelial cells. A major signaling target of these receptors is the activation of nuclear factor-kappa B (NF-κB) transcription factors, which can affect the expression of various cytokines, adhesion molecules, acute phase proteins, and inducible enzymes.

The toll-like receptors represent a family of single-transmembrane molecules having multiple copies of leucine-rich repeats in their unique extracellular domain and a cytoplasmic toll/1R (TIR) motif that is related to that found in the interleukin-1 (IL-1) receptor. Because of the latter motif, these receptors signal like the IL-1 receptor. They are thought to dimerize and bind to adapter proteins that initiate signaling. These receptors may represent interesting and possibly productive targets for the treatment of inflammatory and immune-mediated diseases.

Hormones in gastrointestinal disease

As critical as gastrointestinal hormones and their receptors are for the integration and regulation of digestive function in health, their major interest for the clinician is in regard to

hormone-secreting tumor syndromes. These syndromes can be dramatic expressions of unregulated overactivity of one particular hormone, without the advantage of the normally active mechanisms for feedback inhibition. Such tumors are typically of the islet cell type, arising in the pancreas, although the duodenum is another common location for gastrinomas. The two most common and dramatic presentations of such tumors, excluding the insulinomas and glucagonomas typically seen by the endocrinologist, are gastrin-secreting and VIP-secreting islet cell tumors [166,200]. Gastrin is produced in the fetal pancreas but is not normally produced at all in the adult pancreas. VIP is a normal pancreatic neurotransmitter that is present in very low concentrations under normal circumstances. The syndromes of gastrin overproduction in the Zollinger–Ellison syndrome (gastrinoma) and VIP overproduction in the Verner–Morrison syndrome or watery diarrhea–hypokalemia–achlorhydria syndrome (VIPoma) are discussed in detail elsewhere in this book (see Chapter 71). Knowledge of the biological actions of these peptides based on the previous discussion should provide good insight into these clinical syndromes.

The clinical contributions of the gastrointestinal endocrine system to other gastrointestinal diseases is less clear because of the redundancy of the control mechanisms, with more than one hormone and neurotransmitter serving similar functions. Common disturbances of gastrointestinal function, such as irritable bowel syndrome, peptic ulcer disease, gallstone disease, dysmotility states, and even pancreatitis, may be influenced by a diverse menu of hormones and neurotransmitters. Only when the choice of selective pharmacological agonists and antagonists is similarly broad will the roles of these agents be unraveled. The reduced clearance of several of these hormones from the circulation in renal insufficiency can be associated with a broad spectrum of results because of the antagonistic effects of elevated peptides [69]. Because nutrient absorption is needed to stimulate the secretion of many gastrointestinal hormones, circulating levels can be influenced markedly by syndromes of maldigestion, such as occurs in pancreatic insufficiency or celiac disease.

Some gastrointestinal peptides and analogues have reached clinical practice. They have numerous diagnostic applications. Pentagastrin is the agent of choice for gastric acid secretory testing [213]. CCK is used for pancreatic function testing, as well as in radiographic studies of gallbladder emptying and pancreatic duct size [214]. Secretin is administered in a provocative test for gastrinoma [70]. Glucagon is used to relax the gastrointestinal smooth muscle activity to facilitate endoscopic, radiological, and intubation procedures [215].

In addition to having diagnostic applications, potent agonist analogues of somatostatin are now commonly used as therapeutic agents (see structures of octreotide and lanreotide in Fig. 4.14). A long-acting depot form of lanreotide that can be injected intramuscularly is now available. These ligands have highest affinities for receptor subtypes 2 and 5, with moderate affinity for subtype 3 and very low affinity for subtypes 1 and 4. Somatostatin and its analogues suppress hormone release and hormone action of a number of gastrointestinal peptides. They are being used to treat the diarrhea and flushing of metastatic carcinoid syndrome, the secretory diarrhea of the VIPoma syndrome, the skin lesions of the glucagonoma syndrome, and other refractory diarrheal syndromes and fistulae [216]. Although these agents effectively inhibit acid hypersecretion in gastrinoma, there are better methods for controlling acid secretion, such as hydrogen–potassium ATPase inhibitors and histamine H_2 receptor antagonists. Somatostatin agonists have helped to relieve symptoms of islet cell tumors, and in rare cases they have induced islet cell tumor regression. They are also being tested for action against other epithelial carcinomas. More selective somatostatin receptor antagonists are being developed.

Gastrointestinal hormones, such as gastrin-releasing hormone and gastrin, have been implicated as autocrine growth factors in various epithelial malignancies. Antagonists directed toward blocking the action of these peptides may have important clinical applications in the future.

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A COMPONENTS

5 The brain–gut axis

Graham J. Dockray

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The gastrointestinal tract and the central nervous system (CNS) engage each other in two-way communication. The CNS is informed of the state of the gastrointestinal tract by afferent neurons, and is able to control or modulate digestive function through efferent neurons that are part of the autonomic nervous system (Fig. 5.1a). Afferent neurons signal information to the CNS about the chemical environment of the gut lumen, tension in the gut wall, and the condition of tissues (e.g., inflammation) (Fig. 5.1b). Efferent neurons determine gastrointestinal responses during the cephalic phase of digestion. They also mediate gastrointestinal responses after reflex stimulation of afferent pathways, activation of somatosensory inputs, and stimulation by higher centers, including, for example, changes in emotional state.

The major pathways for communication between the brain and gut are the vagal, splanchnic, and sacral nerve trunks; all three contain both afferent and efferent nerve fibers. It has been recognized since the early 20th century that afferent signals associated with the physiological regulation of digestion are typically conveyed by the vagal pathway and painful sensations are conveyed by the splanchnic pathway [1]. Mechanisms that modulate afferent signals have been identified at various levels, including at the peripheral end of afferent fibers and within the CNS, and are a feature in a number of gastrointestinal diseases [2].

Vagal efferent neurons provide the major parasympathetic route regulating gut function during digestion. Splanchnic efferent neurons constitute the sympathetic outflow to the gut and are activated in stress and adaptation to potentially noxious challenges. The neurons of the enteric ganglia are a major target for both types of efferent pathway (Fig. 5.1c). The motor neurons in these ganglia supply smooth muscle, glandular cells, and blood vessels. The enteric ganglia also function as centers for integration; they communicate with each other and they receive inputs from intrinsic afferent neurons and from endocrine cells. Ganglion cells act, then, both to modulate and to mediate efferent signals from the CNS. Not surprisingly, the relative importance of information exchange between the alimentary tract and the CNS varies between different functions and between different regions of the gut.

Developmental biology of the brain–gut axis

The pathway taken by the vagus nerve marks the route by which cells of the neural crest migrate to the gut during development. The molecular mechanisms that determine this migration are of interest because they provide a basis for understanding the developmental biology not only of the brain–gut axis but also of the entire intrinsic nervous system of the gastrointestinal tract. Thus, cells of the vagal neural crest migrate the length of the gut to supply most of the intrinsic innervation, although a small number of cells from the truncal and sacral neural crests also contribute to the colonization of the stomach and distal colon respectively. Neural crest cells give rise to multiple cell lineages, including neurons, neuroglia, and melanocytes, but not to epithelial enteroendocrine cells as previously thought [3]. The consequences of impaired migration or maturation of neural crest cells are illustrated by Hirschsprung disease, in which a failure of neuronal precursors to colonize the distal part of the colon leads to an aganglionic segment associated with tonic smooth muscle contraction and a dilated proximal segment. Mutations in several genes that regulate neural crest cell survival, migration, and maturation have been identified in

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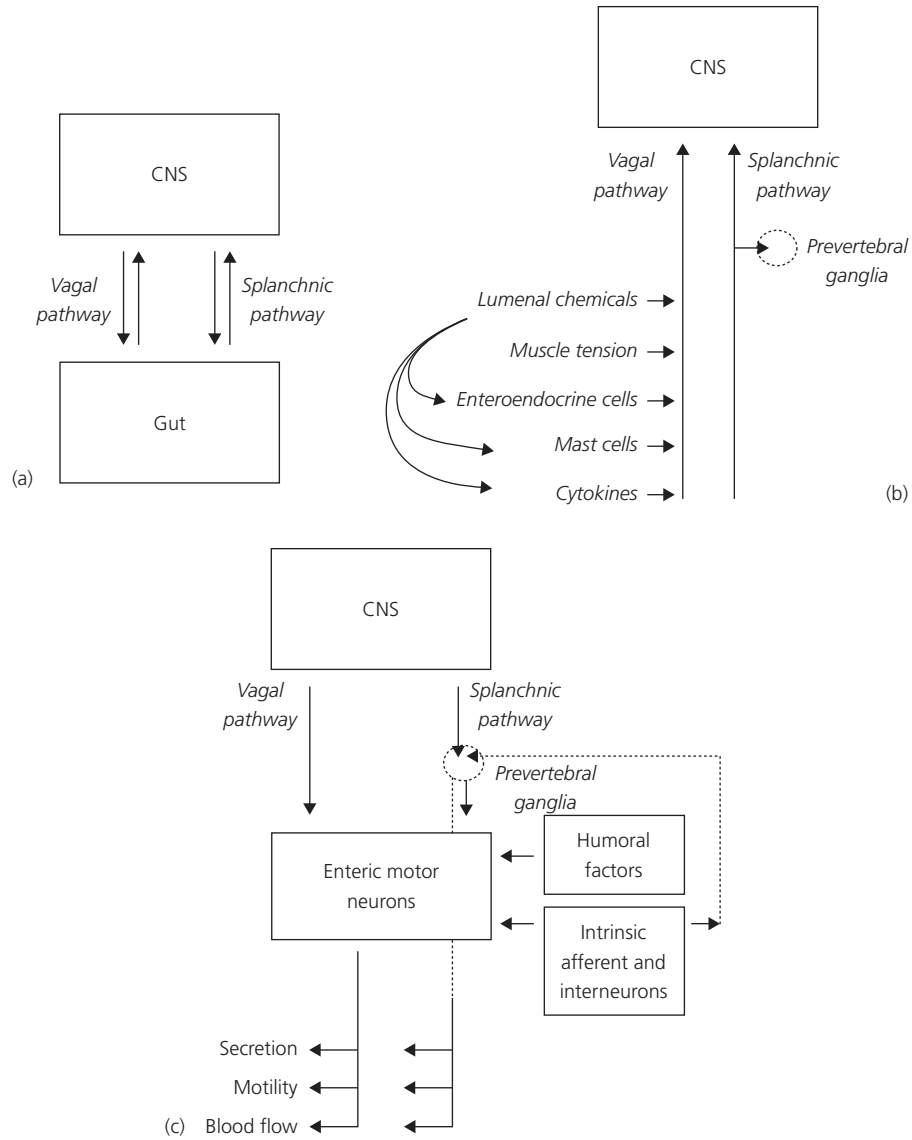

Figure 5.1 Relationships between the brain and the gut. **(a)** The gastrointestinal tract from the mouth to the midcolon is supplied with efferent and afferent nerve fibers running in the vagal and splanchnic trunks. **(b)** Afferent neurons discharge in response to lumenal chemicals, mechanical deformation of their terminal fields, humoral factors, cytokines, and other inflammatory mediators; endocrine cells, mast cells, and cells of the immune system may be influenced by lumenal chemicals. **(c)** Efferent neurons in both splanchnic and vagal nerve trunks terminate on myenteric nerve cell bodies from which intrinsic motor neurons influence secretion and motility; sympathetic efferents may also directly supply blood vessels. The response of the enteric motor neurons is also regulated by humoral factors and by other enteric neurons. CNS, central nervous system.

those with Hirschsprung disease and in mutant mice with an aganglionic colon.

The c-*ret* gene, which encodes a receptor tyrosine kinase, is expressed relatively early by neural crest cells [4] and is thought to be required for the proliferation and survival of enteric neural precursor cells. Loss-of-function mutations of the c-*ret* gene occur in some cases of Hirschsprung disease [5,6]. Interestingly, other mutations of this gene are associated with multiple endocrine neoplasia type II. In mice, deletion of the genes encoding either c-Ret or glial cell line-derived neurotrophic factor (GDNF; which stimulates signaling through c-Ret) leads to a failure in the development of the kidney and of neurons throughout the intestine [7–10]. A second system known to be required for the maturation of neural crest derivatives involves endothelin 3 (ET3) and its G protein-linked receptor, the endothelin B (ETB) receptor. In some patients with Hirschsprung disease, a missense mutation in the gene encoding the ETB receptor is associated with impaired calcium signaling on stimulation. Similarly, targeted disruption of the ETB receptor or mutation of the ET3 gene in mice leads to impaired development of the enteric nervous system and an aganglionic distal colon [11]. It is thought that the ET3/ETB system functions after the c-Ret/GDNF system [12,13] and acts to delay neuronal differentiation so that loss of function leads to a failure in the migration of neuronal precursors as a result of premature differentiation. These are not the only control systems determining the maturation of different populations of intrinsic gut neurons. Others include the homeobox gene *Phox2b*, which is required for expression of c-Ret and of a second

transcription factor, Mash-1, which regulates the expression of a catecholaminergic phenotype in neural crest-derived cells [14,15].

Methods for studying the brain–gut axis

Present knowledge of the organization and function of the brain–gut axis was acquired using a variety of experimental techniques. These methods can be loosely grouped into four categories:

1 *Methods directed at the study of organ function.* Methods that measure changes in secretion or motility in the gut after sham feeding, electrical stimulation of nerve trunks, or loss of function after nerve section (e.g., vagotomy) have often provided the first evidence for functional pathways linking the brain and the gut. Similar experiments can often be performed in humans and animals (e.g., barostat measurements of gastric motility). Taken in isolation, however, the results of such studies seldom allow the precise definition of brain–gut interactions because the relative importance of endocrine mediators and nervous reflexes that are extrinsic or intrinsic to the gut is difficult to evaluate in the intact organism. This type of approach can be coupled with the use of drugs to block or activate particular neurotransmitters or types of neurons, but, again, interpretation of the data may be impeded by the fact that the same neurochemical mechanisms operate at multiple sites in the gut and the CNS.

2 *Morphological methods that define the cellular basis of the brain–gut axis.* The nerve pathways linking the brain and the gut can be defined with precision by using tracers that are taken up by nerve cells and transported along axons, for example, fluorescent compounds such as True Blue, and pseudorabies virus, which is transported across synapses and so reveals chains of neurons in a pathway. In addition, the expression of immediate-early gene proteins (e.g., c-Fos) has been widely used to identify neuronal responses to stimulation, although this technique does not reveal neurons that are inhibited by a particular stimulus. These methods can be coupled with surgical or chemical lesioning of neurons, and with immunocytochemistry for neurochemical characterization of a labeled neuron. Together, such methods provide quantitative information on the organization and neurochemistry of both afferent and efferent pathways, and on CNS pathways. This type of approach is, however, better suited to studies of experimental animals than of humans.

3 *Single-unit electrophysiological recording.* The physiological properties of single afferent and efferent nerve fibers, or of CNS neurons, can be studied by electrophysiological recording of their discharge in response to gastrointestinal stimulation. The data obtained allow rigorous analysis of the patterns of signaling and can provide a formal framework for the description of reflex pathways identified in whole-organism studies or by morphological methods. Again, however, these methods are more readily applied to animal than human studies.

4 *Methods appropriate for the study of the human brain–gut axis.* A number of methods that have proved to be of particular value in human studies are listed. Each has disadvantages, but the combined use of two or more offers obvious advantages.

i *Evoked potentials.* Before the advent of modern imaging techniques, the activation of human CNS neurons after stimulation (e.g., balloon distention, electrical stimulation) was studied by observing the evoked potentials detected by scalp electrodes [16]. This method is useful for measuring cortical neuronal responses (cortical-evoked potentials) that are relatively superficial in the brain, but it is not useful for studies of the activity of deeper neurons. The temporal resolution is good (i.e., milliseconds) but the spatial resolution is only 1–2 cm.

ii *Magnetoencephalography (MEG).* This technique is based on the use of magnetic sensors near the scalp that detect changes in the magnetic field as a consequence of neuronal activity in the underlying part of the brain. The spatial resolution can be 2–5 mm, and images can be obtained in milliseconds. Like cortical-evoked potentials, however, MEG can be difficult to use for recording from subcortical regions.

iii *Positron emission tomography (PET).* This technique provides a view of neuronal activity as reflected by changes in blood flow. However, the time lag between changes in neuronal function and blood flow to the relevant region may mean that it is difficult to detect rapid responses. PET allows the identification of brain regions with a resolution of 2–8 mm, but the temporal resolution is relatively poor (i.e., 40 s). Repeated studies on individuals are difficult because of the risk associated with isotopic exposure, and the cost is relatively high.

iv *Functional magnetic resonance imaging (fMRI).* This imaging technique also detects changes in neuronal activity as reflected by blood flow. The spatial resolution is 2–8 mm. Temporal clustering analysis of fMRI data has shown that it is possible to resolve feeding-related changes in hypothalamic function [17]. fMRI is cheaper than PET, and repeated measurements are harmless.

v *Transcranial magnetic stimulation (TMS).* Unlike the other techniques mentioned, this approach does not depend on the detection of neuronal responses; rather, it facilitates selective activation of specific CNS regions. This is not a new technique but it was only introduced to gastroenterology in the mid 1990s. It can be used repeatedly in humans, and it is useful for the activation of cortical motor regions when coupled with the recording of gut function (e.g., electromyography) [18].

vi *Reporting visceral sensations.* Subjects report sensations after stimulation (mechanical, chemical, electrical) of the gut by using visual analogue scales or by selecting adjectival

descriptors of sensation. This approach is widely used and often valuable when combined with other techniques (e.g., fMRI) [19].

Brain–gut connections

Afferent neurons

The vagus nerve consists predominantly of afferent nerve fibers. In the rat, between 75% and 90% of vagal fibers are thought to be afferent [20]. Almost all of these fibers are unmyelinated or only lightly myelinated and consequently their conduction velocities are relatively low (0.5–5 m/s). The cell bodies of vagal afferent nerve fibers are located in the nodose ganglia, and their central terminals are found in the nucleus of solitary tract (NST). The cell bodies of splanchnic and sacral afferent fibers are found in the dorsal root ganglia, and their central terminals are in the spinal cord [21].

Different populations of afferent fibers respond to stimuli arising in the mucosa, muscle layers, and serosa. The stimulus in the lumen may be mechanical, thermal, or chemical. In addition, afferent neurons express receptors for various hormones, neurotransmitters, and immunomodulators (see Fig. 5.1b). It is convenient to use the term *afferent* to describe the direction in which information is passed relative to the CNS. However, many visceral afferent neurons (particularly splanchnic ones) synthesize neuropeptides that are transported to terminals both in the CNS and in the periphery, where they are released on nerve stimulation, giving rise to motor (or efferent) functions through an axon reflex [22].

Some intrinsic gastrointestinal neurons project to the prevertebral ganglia (see Fig. 5.1c). These neurons are therefore distinct from vagal, splanchnic, and sacral afferents, but they function as the afferent arm in some reflexes operating through the sympathetic ganglia. Electrophysiological recording of the discharge of nerve fibers running in the mesenteric nerve bundles detects both extrinsic afferent and intrinsic afferent nerve fibers, and care is required to distinguish between them.

Organization

The vagal afferent supply of the upper gastrointestinal tract is conveyed by the gastric branches of the vagus nerve. More distal regions are innervated by vagal fibers that pass through the celiac–superior mesenteric ganglia and travel with the mesenteric nerve bundles running along blood vessels. There is a loose viscerotopic representation of vagal afferent terminals within the NST [23]. Tracing experiments suggest that, within the bowel wall, vagal afferent nerve fibers run in the myenteric plexus, where they form characteristic intraganglionic laminar endings that function as slowly adapting tension receptors [24]. The innervation of the mucosa is variable. In the intestine, some villi appear to lack an afferent innervation, whereas others exhibit vagal afferent fibers in the lamina propria; afferent fibers do not appear to penetrate the epithelium [25,26].

Splanchnic afferent nerve fibers enter the spinal cord through the dorsal roots and pass to the gut along blood vessels. They traverse the prevertebral ganglia, where they may supply collateral fibers innervating the ganglion cells, suggesting direct modulatory actions on sympathetic postganglionic discharge (see Fig. 5.1b). In the spinal cord there are fewer terminals of splanchnic afferent neurons than of somatic afferent neurons. Splanchnic afferents terminate mainly in lamina I of the dorsal horn, although some project to lamina V [27]. The spinal cord neurons that respond to visceral afferent stimulation may also have inputs from somatic afferent nerve fibers. The peripheral terminals of splanchnic afferent nerve fibers can be identified within the gastrointestinal tract with the use of peptide markers such as calcitonin gene-related peptide (CGRP), which is found in fibers in the myenteric plexus, in smooth muscle, around submucosal blood vessels, and in the mucosa [28].

Neurochemistry: transmitters

The major transmitter of visceral afferent neurons is thought to be glutamate. In addition, afferent neurons produce many different neuropeptide transmitters that exhibit characteristic distributions in different neuronal populations. About 80% of the spinal afferent neurons serving the rat stomach contain CGRP immunoreactivity, and about 50% contain substance P immunoreactivity [28]. In contrast, fewer than 10% of the vagal afferent neurons projecting to the gut contain these peptides; however, vagal afferent neurons expressing cholecystokinin type 1 (CCK_1) receptors (see Neurochemistry: receptors), which are known to project to the stomach, express the satiety peptide CART (cocaine- and amphetamine-regulated transcript) [29]. The proportion of the spinal afferent neurons associated with substance P or CGRP immunoreactivity is considerably higher in the gastrointestinal tract than in skin or joints. Almost no primary afferent neurons containing somatostatin project to the stomach, although dorsal root ganglia neurons containing somatostatin project to the skin and joints. Low doses of the sensory neurotoxin capsaicin selectively stimulate C-type fibers, whereas high doses selectively destroy C-type fibers, particularly in neonatal animals [30]. Capsaicin-induced lesioning of afferent neurons produces a decrease in the expression of CGRP and substance P and a loss of immunoreactive nerve fibers in the gut [28,31]. Surgical section of afferent nerve trunks also leads to the decreased expression of CGRP and substance P in primary afferent neurons.

Neurochemistry: receptors

Vagal afferent neurons express a variety of receptors for neurotransmitters and hormones [32,33]. One of the best studied is the CCK_1 (also called CCK-A) receptor [34,35]. CCK_1 receptor cDNA has been cloned from rat and human

nodose ganglia, and the mRNA has been localized by in situ hybridization to nodose ganglion cell bodies [35]. After ligation of the vagal nerve trunk, CCK_1 receptors accumulate on the central side of the ligature, indicating transport toward the gut [34,35]. Vagal afferent neurons expressing CCK_1 receptors also express the functional or long form of the leptin receptor, Ob-R [36], cannabinoid CB-1 receptors [37], orexin Ox-R1 receptors [38], galanin receptors [39], melanin-concentrating hormone (MCH)-1 receptors [40], and neuropeptide Y (NPY) Y-2 receptors [41,42]. In addition, vagal afferent neurons express metabotropic glutamate [43], GABA-B [44], and 5-HT_3 receptors [45]. Spinal afferent neurons express a different set of receptors; those involved in sensitization in response to inflammation are likely to be particularly important and include the bradykinin B2 receptor, purinergic P2X receptors, adenosine A1 and A2 receptors, and protease-activated receptors PAR1 and PAR2 [32].

Efferent neurons

Organization

The cell bodies of parasympathetic preganglionic nerve fibers that run in the vagus nerve are found in the dorsal motor nucleus of the vagus. There is a viscerotopic representation of neurons within the dorsal motor nucleus, and columns of cells corresponding approximately to the branches of the vagal nerve can be identified [46]. The peripheral terminals of vagal efferent fibers have been mapped with the use of tracers such as DiI and PHA-L [25,47]. Different tracers elicit quantitatively different results, but several conclusions emerge from the available data. Vagal efferent fibers can be shown to supply the gastrointestinal tract as far as the descending colon, but there is a proximal–distal gradient in the relative abundance of these fibers. In the stomach, where the vagal innervation is most dense, virtually all gastric myenteric ganglia are contacted by vagal efferent nerve fibers, but there is little or no supply to the submucosa or mucosa [25,47] (see Fig. 5.1c). A single vagal efferent fiber may contact many myenteric ganglia. The contacts between preganglionic fibers and ganglion cells exhibit considerable variation; some are clear synaptic-like associations, whereas others are less well defined.

The nerve cell bodies of postganglionic sympathetic neurons to the gut are in the prevertebral ganglia, and the preganglionic cell bodies are in the intermediolateral column of the spinal cord. Most of the upper gut receives its innervation from the celiac–superior mesenteric ganglia, and the lower gut from the inferior mesenteric ganglion. Enteric ganglion cells are a major target, but so too are submucosal blood vessels (see Fig. 5.1c).

Neurochemistry

The predominant phenotype of vagal efferent neurons is cholinergic, although the cell bodies of up to 30% of the neurons of the dorsal motor nucleus projecting to the stomach also appear to express tyrosine hydroxylase and may have a dopaminergic phenotype [48]. A population of the motor neurons of the nucleus ambiguus that project to the esophagus contains CGRP immunoreactivity [49]. It has been recognized for several generations that the main synaptic contact between vagal efferent fibers and cells of the myenteric plexus is cholinergic nicotinic; combined anterograde tracing and immunohistochemistry suggest that gastric myenteric ganglion cells with a vagal input contain 5-HT and vasoactive intestinal polypeptide (VIP) immunoreactivity [47].

The adrenergic phenotype of sympathetic neurons to the gut is well recognized. Many of these neurons also express characteristic neuropeptides depending on their gastrointestinal targets. Thus, neurons of the celiac ganglia that project to submucosal blood vessels in the gut often contain NPY, and those that go to the submucosal plexus contain somatostatin [50].

Central nervous system regions and the digestive tract

Ascending central nervous system pathways stimulated from the gut

Important early studies of the CNS regions responding to gut stimulation were based on recordings of cortical-evoked potentials [51]. Subsequently, many groups have made recordings of the activity of identified CNS neurons responding to gastrointestinal stimuli or humoral mediators [52,53]. Insight into the organization of CNS regions associated with digestive function has been obtained more recently by means of tracing techniques, the induction of immediate-early gene expression (e.g., c-*fos*), and, in the case of the human brain, the application of PET and fMRI [16].

Major CNS projections from the vagal complex identified by anterograde labeling include those to the paraventricular nucleus, the bed nucleus of the stria terminalis, and the central nucleus of the amygdala, as well as those to the parabrachial nuclei and the subthalamus [25]. Many of the same regions are identified by the induction of c-Fos protein in response to nutrients and to the peripheral administration of regulatory peptides [54,55].

Noxious stimulation of spinal afferent neurons increases c-Fos expression in spinal cord neurons [56,57]. Noxious stimulation of the gastric mucosa with HCl and formalin does not increase the spinal cord expression of c-Fos detected by in situ hybridization, but serosal application of formalin produces a significant increase [58]. Because such mucosal stimulation increases blood flow, and this response is recognized to be mediated by splanchnic afferent neurons, it seems possible that noxious stimulation of the gastric mucosa may evoke an afferent fiber response of sufficient strength to trigger a local axon reflex but inadequate to recruit spinal nociceptive circuits.

In healthy human subjects, PET and fMRI studies have identified responses in the anterior cingulate cortex in response to

painful rectal distention. Interestingly, patients with inflammatory bowel disease exhibit differential responses in this region after similar stimulation [59,60]. The anterior cingulate cortex is probably important more generally in visceral pain. Thus, nonnoxious stimulation of the human esophagus activates the central sulcus, the insular cortex, and the frontoparietal operculum [61]; noxious stimulation activates the same regions, and also the anterior cingulate cortex and an expanded region of the insular cortex (Fig. 5.2). The CNS regions that respond to visceral stimuli exhibit plasticity; for example, there are prolonged changes in the cortical responses to short-term stimulation of the esophagus [62]. The cellular mechanisms accounting for plasticity and differential responses in disease are still largely unknown.

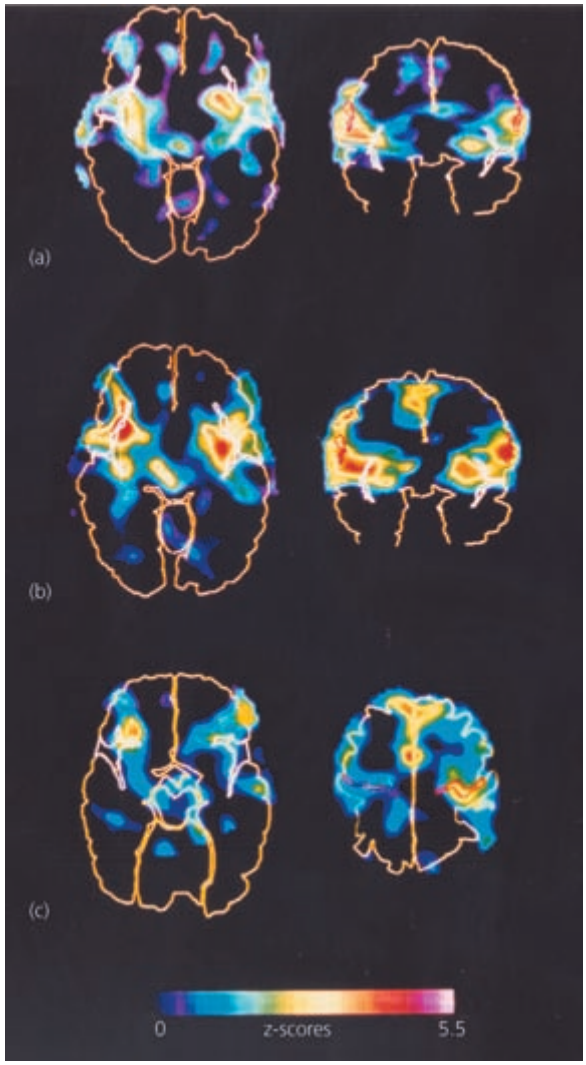

Figure 5.2 Positron emission tomography data after esophageal stimulation in humans. Scans of cortical areas activated during definite sensation–no sensation **(a)**, pain–no sensation **(b)**, and pain–definite sensation **(c)**. **(a)** Left and right scans show bilateral activation of the insular and primary somatosensory cortex. **(b)** There is greater activation of the insular and primary somatosensory cortex, and some activation of the anterior cingulate gyrus. **(c)** The left scan shows activation of the right anterior insular cortex, and the right shows activation of the anterior cingulate cortex. Adapted from Aziz et al. [61], with permission from Elsevier.

Central nervous system projections to the vagal complex

The electrical stimulation of different CNS regions provided convincing early evidence of the role of the CNS in controlling digestive function [63]. Subsequently, retrograde tracing from the vagal complex has revealed the major monosynaptic connections. Thus, the vagal complex receives inputs from the obscure and pallidal raphe nuclei, the paraventricular nucleus, the dorsomedial and lateral areas of the hypothalamus, the central nucleus of the stria terminalis, and the insular and medial prefrontal cortexes. In humans, TMS has been used to examine the cortical control of esophageal function. The relevant area of the cerebral cortex is asymmetric; the greatest esophageal responses were evoked from the right hemisphere in 8 of 10 subjects and from the left hemisphere in the other two subjects [64]. This asymmetry may explain why the dysphagia that develops after stroke is variable in degree and duration [18].

Signaling from gut to brain

Electrophysiological recordings of the discharge of single vagal or splanchnic afferent fibers in anesthetized animals have provided much of the foundation for modern views of visceral afferent nerve function [65,66]. Individual afferent fibers respond to mechanical deformation of their terminal regions, to the presence of chemical stimulants in the gut lumen, and to humoral agents administered into the circulation. Because visceral afferent nerve fibers may be mechanoreceptors, chemoreceptors, or both, classifications of afferent nerve response have been based on the distribution of terminal fields rather than on the modality of the receptor [67,68].

Mucosal, muscle, and serosal afferents

The responses of vagal afferent nerve fibers to mucosal stimulation, typically in the stomach and proximal small intestine, have been reported by many groups. Less is known about splanchnic afferent responses to mucosal stimulation [69]. All of the major macronutrients (i.e., protein, fat, carbohydrate) have been reported to stimulate mucosal afferent nerve fiber discharge. There are also fibers that respond to a variety of nonnutrient chemicals, including acid, alkali, and hypertonic solutions [68,69]. Many mucosal afferent fibers are multimodal and respond to mechanical as well as chemical stimulation. Light mechanical stimulation, such as brushing or stroking of the mucosa, typically evokes a rapidly adapting response from these fibers.

The afferent fibers supplying the muscle layers of the gut typically respond to both passive stretch and active contraction, hence the name *in-series tension receptor*. They are characteristically slowly adapting and, if innervating the stomach and proximal small intestine, they have a single receptive field.

The serosal afferent innervation appears to be predominantly spinal in origin. These afferent fibers respond to deformation of the serosa and mesentery, particularly around the bifurcation of blood vessels, thereby conveying information about visceral tension and movement. The receptive fields of these afferent fibers are often punctate.

Mechanoreceptors: transduction

Sensitivity to mechanical stimulation is a common physiological response and accounts for mechanisms involved in hearing, balance, touch, the regulation of cell volume, and muscle cell responses to stretch. A variety of different cellular mechanisms are now recognized to account for these responses. Many cells have stretch-sensitive ion channels that are gated by changes in membrane tension. The accompanying alterations in membrane potential lead to the propagation of action potentials. Although there is little direct evidence, it is plausible that this type of mechanism is responsible for the sensitivity of muscle and serosal afferent fibers.

Afferent fiber populations differ in sensitivity. For example, pelvic afferent fibers responding to distention in the physiological pressure range (< 5 mmHg) are known as *low-threshold fibers*; discharge of these fibers is proportional to distending pressures up to about 30 mmHg, which is in the range likely to be perceived as noxious. A separate population of fibers (i.e., high threshold), however, discharge only in response to high pressures (> 30 mmHg), suggesting a primary function in noxious signaling [68].

Chemoreceptors

Transduction and the role of enteroendocrine cells

The term *chemoreceptor* is used to describe the capacity of afferent nerve fibers to respond to defined chemical agents. Nonlumenal (neurohumoral) as well as physical or chemical factors within the lumen may influence afferent nerve fiber discharge. In the late 1950s, Bulbring and Lin [70] proposed that enterochromaffin cells released 5-HT in response to mechanical deformation of the lumenal surface, and that 5-HT then stimulated subepithelial nerve fibers to trigger the peristaltic reflex. Many other humoral stimulants of afferent fibers have since been recognized, including bradykinin and hormones such as CCK. The molecular sensing mechanisms involved in taste perception by the tongue provide a model for understanding what may happen within the gastrointestinal tract with respect to the lumenal sensing of chemicals. Thus, compounds associated with the taste of salt or acid act by gating of ion channels [71]. Compounds associated with sweet or bitter tastes are known to act in the tongue through G protein-coupled receptors [71]. α-Gustducin, the GTP-binding subunit of a heterotrimeric G protein, is a putative mediator in these cells. At the cellular and molecular levels, the transduction mechanisms may be similar in the tongue and in the gut, even though taste perception is restricted to the buccal cavity. Consistent with this idea, α-gustducin immunoreactivity and G protein-coupled receptors associated with bitter taste and with the sensing of extracellular calcium and aromatic amino acids (e.g., L-tryptophan and L-phenylalanine) have been shown to be expressed by enteroendocrine cells [72,73].

Some lumenal stimuli (e.g., short-chain fatty acids) may penetrate the mucosa to act directly on afferent nerve fibers [74]. However, many other lumenal stimuli appear to activate enteroendocrine cells as previously described. The secretory products of these cells then act on afferent nerve fibers, thereby signaling from the gut to the brain (Fig. 5.3). Putative messengers that mediate enteroendocrine cell actions in this way include CCK, 5-HT, peptide YY ($PYY_{3–36}$), and glucagon-like peptide-1 (GLP-1). Thus, CCK is thought to mediate the effects of lumenal proteins and long-chain fatty acids in the upper small intestine, and 5-HT may mediate the effects of intestinal carbohydrate stimulation [75]. After the release of putative endocrine cell mediators, there are two general ways that they may signal to the CNS. First, they may penetrate the CNS from the circulation in regions where the blood–brain barrier is absent or leaky. Second, as already noted, primary afferent nerve fibers possess receptors for some of the products of gut endocrine cells (e.g., CCK and 5-HT), which are therefore putative mediators of endocrine–neural signaling pathways. For CCK there may be both stimulation of vagal afferent fibers that also function as gastric in-series tension receptors (i.e., as a hormone after delivery in the systemic circulation) [76] and direct action on intestinal mucosal afferent fibers in the subepithelial regions surrounding the CCK-secreting cells (i.e., in a paracrine manner) [77].

Mast cells and the extrinsic innervation of the gut

There is a close relationship between mast cells and substance P-immunoreactive nerve fibers [78]. Mast cell products (i.e., histamine and 5-HT) stimulate the discharge of intestinal afferent fibers [79], and there is evidence that motor responses to mast cell discharge in vivo are mediated by 5-HT_3 receptors and vagal afferent nerve fibers [80]. In ovalbumin-sensitized rats, antigen challenge increased c-Fos expression in the NST, the lateral parabrachial nucleus, and the paraventricular nucleus. These responses were inhibited by systemic administration of the 5-HT_3 antagonist ondansetron and by perivagal capsaicin, suggesting a mechanism involving peripheral 5-HT_3 receptors and vagal afferent neurons in signaling to the CNS during intestinal anaphylaxis [81]. In addition to the stimulation of afferent fibers by mast cell products, mast cell secretion is stimulated by the

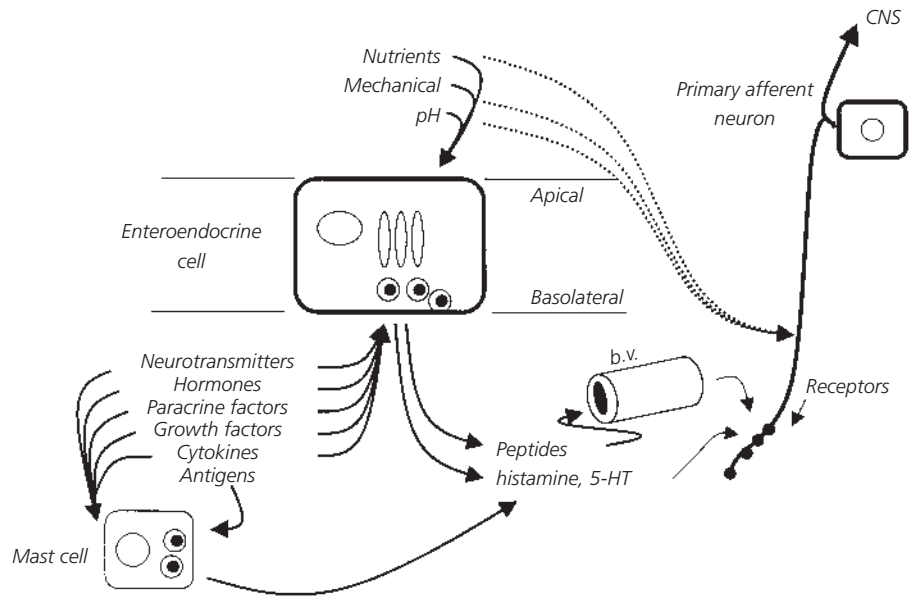

Figure 5.3 Interactions between lumenal factors, mast cells, endocrine cells, and primary afferent nerve fibers. Primary afferent nerve fibers may express receptors for endocrine and mast cell secretory products, and may also respond directly to lumenal chemicals that penetrate the mucosa. The discharge of afferent fibers to the smooth muscle may be secondary to passive stretch (distention caused by a meal) or to the contraction of smooth muscle. Peptides released by endocrine cells are thought to act directly on afferent nerve fibers and after transport in the circulation. The receptors expressed by afferent neurons are synthesized in the cell body and may be transported to peripheral terminals. CNS, central nervous system; b.v., blood vessel.

activation of vagal efferent fibers (see Fig. 5.3). For example, the administration of a thyrotropin-releasing hormone (TRH) analogue that strongly stimulates vagal efferent neurons elicited the release of mast cell markers into the rat intestinal lumen [82].

Inflammatory modulation of afferent function

Inflammation of the gut wall is a strong stimulant of afferent nerve discharge (see Fig. 5.3) [83,84]. Many splanchnic afferent fibers appear normally to be silent; for example, up to 90% of splanchnic afferents to the distal colon are reported to be insensitive to stimulation in the physiological range. Inflammation induced experimentally by the administration of mustard oil, acetic acid, or formaldehyde, however, leads to an increased sensitivity to stimulation. Seemingly silent afferent fibers may play little or no role in the normal physiology of the gastrointestinal tract, but they are important components of the signaling pathway activated by damage or inflammation and may therefore play a role in gastrointestinal disease states. Activation is likely, in part, to be attributable to the release of cytokines such as interleukin-1β, which increase the sensitivity of afferent fibers to stimulation (see Fig. 5.3).

Pain and visceral hypersensitivity

The noxious stimulation of splanchnic afferent nerve fibers is associated with painful sensations. However, the sensation of pain may also be evoked by normal or innocuous stimuli. Note the well-recognized observation that patients with irritable bowel syndrome often report painful sensations in response to distention of the colon at pressures that are not perceived as painful by healthy subjects. The term *allodynia* is used to describe noxious sensations evoked by innocuous stimuli, and the term *hyperalgesia* describes exaggerated responses to noxious stimuli. The mechanisms that account for visceral hypersensitivity have generated considerable interest [19]. These mechanisms are a feature of both inflammatory and noninflammatory conditions (e.g., functional bowel disorders). Changes at any of several different levels in the brain–gut axis may be involved. Thus, in addition to the exaggerated responses of afferent neurons and the recruitment of otherwise "silent" afferent fibers (see previous section), there is also central modulation of afferent stimuli [2]. Perfusion of the lower esophagus with acid increases the sensitivity of the upper esophagus to electrical stimulation, providing direct evidence of central sensitization. Patients with noncardiac chest pain exhibit a marked decrease in their visceral pain threshold [85]. The effects of central sensitization are often relatively long lasting; for example, irritation of the colon in young rats leads to hypersensitivity in later life despite an absence of apparent pathology in the gut [86].

The term *wind-up* is sometimes used to describe the increased synaptic transmission at afferent nerve terminals in the spinal cord; this event is thought to involve enhanced transmission by the neurotransmitter glutamate acting at NMDA receptors. In addition, the inhibitory influences on spinal cord cells from nervous pathways that descend in the spinal cord may be altered. Finally, hyperalgesic states are associated with changes in neuronal activation at supraspinal levels, including, for example, the expansion of cortical regions associated with the response to a defined stimulus [62,84].

Efferent functions of peptidergic visceral afferent neurons

After the stimulation of visceral afferent nerve fibers, the peptides produced by these neurons are released at both central and peripheral terminals. Peptides released peripherally modulate blood flow, motility, secretion, the discharge of enteric ganglion cells, and a number of functions associated with epithelial protection [22,87]. There is now good evidence that noxious agents in the lumen activate splanchnic afferent fibers, which in turn regulate protective or damage-limiting mechanisms within the mucosa, as well as signaling to the CNS (Fig. 5.4). Capsaicin, which is the active ingredient of hot peppers, has been used extensively in studies of primary afferent nerve function [88]. This compound acts on a nonselective cation channel, the vanilloid receptor (VR-1), to increase intracellular calcium concentrations [89]. Because the VR-1 ion channel is located almost exclusively in primary afferent nerve fibers, capsaicin is a valuable selective stimulant of these neurons. At low doses, capsaicin is excitatory, and at high doses it produces long-lasting or permanent loss of function.

Elucidation of the role of peptides released at the peripheral terminals of visceral afferent fibers is complicated for those neuropeptides produced in both afferent and intrinsic neurons (e.g., the tachykinins). However, CGRP-α in the rat stomach has proved to be a valuable model for studies of the relevant mechanisms. CGRP-α is absent from gastric intrinsic neurons and endocrine cells; it is scarce in gastric vagal afferent neurons but abundant in splanchnic afferent nerve fibers. An intact splanchnic afferent innervation mitigates damage of the gastric mucosa inflicted by noxious agents (e.g., ethanol), suggesting that afferent nerve fibers have a protective role [90]. There is good evidence that CGRP-α is a protective mediator in this system. Thus, capsaicin increases the release of CGRP in the perfused rat stomach and, on acute administration, it reduces the lesions produced by ethanol; immunoneutralization of CGRP, or administration of the CGRP antagonist $CGRP_{8–37}$, abolishes capsaicin-induced protection [87]. The release of CGRP is associated with increased mucosal blood flow mediated by nitric oxide; this hyperemic response is a key part of the protective mechanism (see Fig. 5.4). Multiple other actions of CGRP are likely to be protective including stimulation of somatostatin secretion and increased somatostatin synthesis at the level of mRNA abundance [91], which tend to decrease secretagogue-evoked acid secretion.

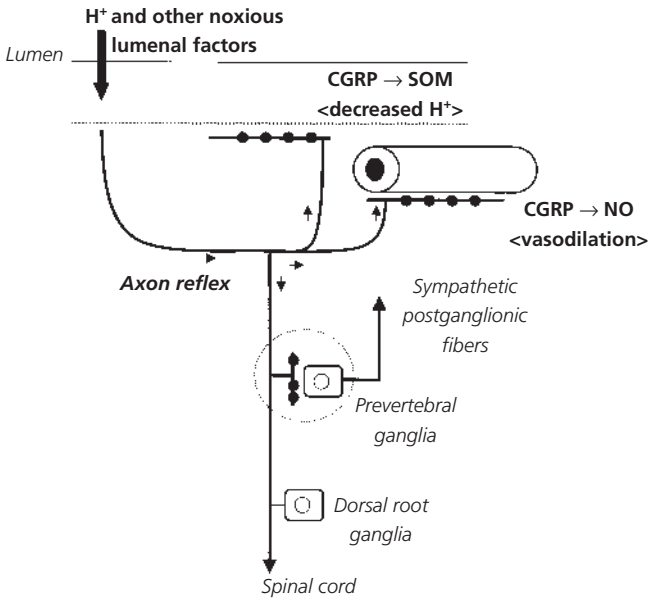

Figure 5.4 Axon reflexes in response to noxious chemical stimuli. These activate primary afferent neurons that also synthesize neuropeptides (e.g., CGRP), which are transported to peripheral terminal fields and released by axon reflexes on stimulation. Responses include increased blood flow, probably secondary to stimulation of nitric oxide synthase, and stimulation of somatostatin-producing D cells, which leads to inhibition of acid secretion. CGRP, calcitonin gene-related peptide; SOM, somatostatin; NO, nitric oxide.

Central nervous system control of the gut

The CNS influences all major gastrointestinal functions, including secretion, motility, blood flow, and defense against noxious challenge. Specific gastrointestinal responses occur after administration to defined CNS regions of a wide range of neurotransmitters, including glutamate, γ-aminobutyric acid, acetylcholine, biogenic amines, corticotropin-releasing factor (CRF), opioids, CGRP, CCK, somatostatin, VIP, and neurotensin. Both stimulation and inhibition of gastrointestinal motor and secretory responses may be evoked by the same substance in different CNS regions. It is unlikely that all of the effects described after the CNS application of neuroactive substances are physiologically relevant. CNS peptide systems regulating gastrointestinal function that have attracted substantial interest include those activated by TRH, bombesin/gastrin-releasing peptide, and CRF.

Thyrotropin-releasing hormone

The stimulation of colonic motility and gastric acid secretion in response to the intracisternal administration of TRH were among the first gastrointestinal effects of a CNS peptide to be reported [92]. It is now clear that TRH is a reliable stimulant of a wide range of gastrointestinal responses, including gastric acid and pepsinogen secretion, gastric histamine secretion, gastric mucosal blood flow, gastric motility, and mast cell secretion [93,94]. In high doses, TRH produces gastric lesions. For the most part, these effects are mediated by the

stimulation of vagal efferent neurons and so are blocked by vagotomy and atropine. They can be mimicked by stable TRH analogues, such as RX77368. There is a rich TRH innervation of the dorsal motor nucleus of the vagus that arises mainly from the obscure and pallidal raphe nuclei [95]. These neurons are also serotonergic. It is likely that the coexisting transmitters, 5-HT and TRH, interact after release. Stimulation of the raphe nuclei, similar to the administration of TRH into the dorsal motor nucleus, increases gastric acid secretion and motility by a vagal cholinergic mechanism [96,97]. Intracerebroventricular administration of TRH antibodies reduces acid secretion in the pylorus-ligated rat, and immunoneutralization also inhibits the response to raphe nuclei stimulation, providing clear evidence that TRH is an endogenous regulator of the vagal efferent outflow to the upper gastrointestinal tract and may participate in vagovagal reflexes [98].

Bombesin/gastrin-releasing peptide

Central administration of peptides of the bombesin/gastrin-releasing peptide group can produce effects opposite to those evoked by peripheral administration. In particular, bombesin and gastrin-releasing peptide administered intracisternally inhibit acid secretion [99], but they generally stimulate it on peripheral administration (reflecting vagal noncholinergic stimulation of the G cell). Retrograde tracing and immunocytochemistry indicate that bombesin-immunoreactive nerve fibers in the dorsal vagal complex originate from cell bodies in the medial parvocellular subdivision of the paraventricular nucleus of the hypothalamus [100], which provides support for the notion that bombesin-related peptides mediate descending inhibitory effects from the paraventricular nucleus to vagal efferent neurons.

Corticotropin-releasing factor

Peptides of the CRF family have emerged as important CNS regulators of gastrointestinal function. The main members of the family are CRF itself and the structurally related urocortins 1, 2, and 3. The CRF-1 receptor is a target for CRF and urocortin 1, and the CRF-2 receptor is a target mainly for urocortins 2 and 3. The identification of a number of selective receptor antagonists supports a role for these peptides in stress-related responses of the gastrointestinal tract, including decreased gastric motility, increased colonic transit, and inhibition of food intake [101,102].

Vagovagal reflex mechanisms

The physiological control of digestion depends on cross-talk between the gastrointestinal tract and the brain; the relevant interactions are well illustrated by vagovagal reflexes. Control of pancreatic and gastric secretion, gallbladder contraction, and the motility of the stomach and intestine may all include a component that involves a vagovagal reflex. The afferent arm of these reflexes was thought for many years to be triggered by direct mechanical or chemical stimulation of the vagal afferent fibers. Thus, distention of the stomach activates vagovagal reflexes that result in relaxation of the gastric corpus and the stimulation of acid secretion [103]. Gastric relaxation is important early in digestion to accommodate the delivery of food, so that gastric volume increases without an increase in pressure (Fig. 5.5). Electrical stimulation of the vagal nerve trunk generally produces gastric contraction, but relaxation is induced by the same stimulus in the presence of the cholinergic muscarinic antagonist atropine. Therefore, a vagal excitatory muscarinic pathway maintains gastric tone and a vagal inhibitory nonmuscarinic pathway leads to relaxation of the body of the stomach (and a decrease in intragastric pressure). Nitric oxide, possibly in combination with VIP, is now recognized as a mediator of this response [104,105].

It is now clear that local paracrine or hormonal factors also stimulate the afferent arm of vagovagal reflexes. One of the best-studied examples involves CCK, which activates vagovagal reflexes regulating gastric motility and pancreatic secretion. These actions can be considered as different aspects of the same role, namely the regulation of protein and fat digestion in the small intestine. On the one hand CCK stimulates the delivery of pancreatic enzymes and bile salts to promote digestion in the intestine [106–108], while at the same time it delays further delivery of nutrients to the small intestine by inhibiting gastric emptying and food intake [109,110]. Taken together, these actions allow matching of the capacity for digestion (enzymes and bile salts) with nutrients already in the small intestine. Neurochemical, electrophysiological, and integrative physiological studies indicate that CCK acts at CCK_1 receptors on vagal afferent fibers which constitute the afferent arm of the vagovagal reflex by which CCK relaxes the gastric corpus, decreasing intragastric pressure and inhibiting the gastric emptying of liquids (see Fig. 5.5) [33]. These responses are reduced after capsaicin is topically administered to the vagal nerve trunk or systemically administered to newborn rats [111,112]. Lumenal nutrients in the intestine induce CCK release, which, like exogenous CCK, results in relaxation of the gastric corpus and inhibition of gastric emptying by a mechanism sensitive to capsaicin and CCK_1 receptor antagonists [113,114]. In parallel with the reflex relaxation of the stomach, CCK also appears to activate vagal reflexes leading to the stimulation of pancreatic secretion. It seems that pancreatic acinar cells are controlled both directly by the effects of CCK delivered in the circulation and indirectly by acetylcholine released from postganglionic parasympathetic neurons as a consequence of CCK-stimulated vagovagal reflexes [106,107,115].

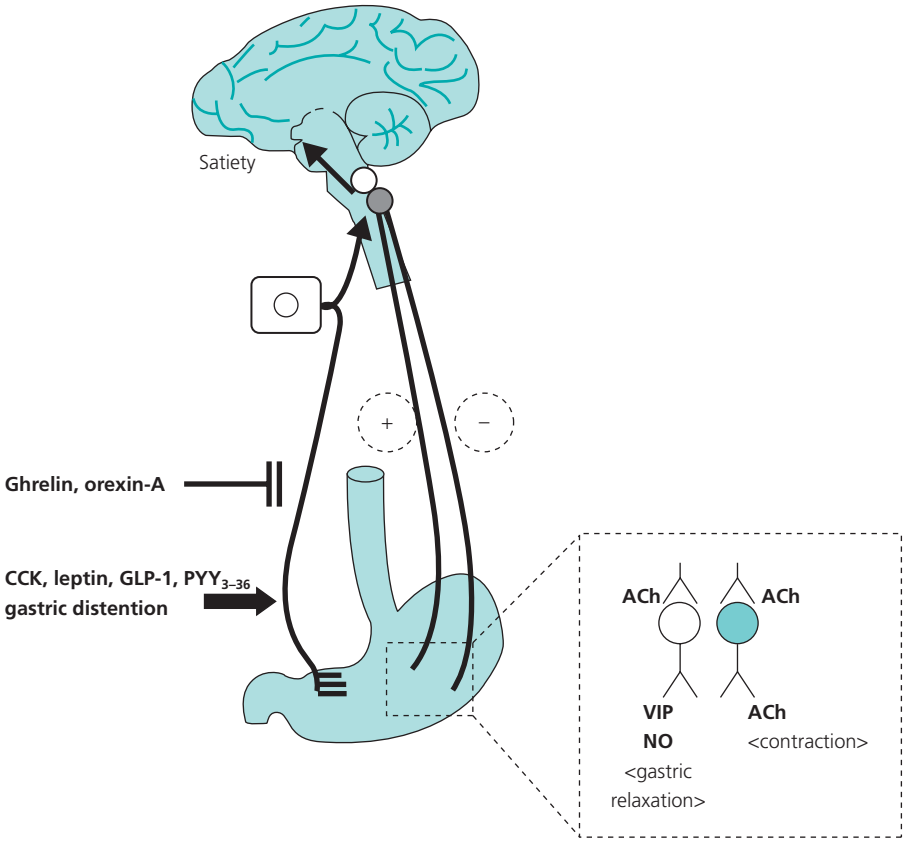

Figure 5.5 Vagovagal reflex control of gastric motility. Gastric mechanoreceptors and cholecystokinin (CCK) act on vagal afferents to trigger a vagovagal reflex that produces inhibition of tone in the gastric corpus. This is likely to be secondary both to inhibition of tonically active vagal cholinergic excitatory pathways (shaded) and to stimulation of vagal inhibitory pathways with vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) as the final mediators. The reflex is important in receptive relaxation of the stomach early in digestion and in inhibition of gastric emptying (by decreasing the pressure difference between corpus and duodenum) later in digestion. In addition, vagal afferent neurons may be stimulated by glucagon-like peptide-1 (GLP-1) and peptide $YY_{3–36}$ ($PYY_{3–36}$), and the effects of CCK are potentiated by leptin; ghrelin and orexin-A inhibit the effects of CCK on vagal afferent nerve discharge. Ach, acetylcholine.

Gastrointestinal signals influencing satiety and appetite

The mechanisms regulating food intake are attracting intense interest [116–120]. An understanding of these systems is central to any approach to the treatment of obesity, which is now widely recognized as a major threat to health. Many different factors are thought to contribute to the obesity epidemic, including genetic factors and changes in lifestyle and diet. Food intake is determined both by mechanisms initiated within the CNS and by a wide range of peripheral factors, including leptin from adipocytes, insulin from the islets of Langerhans, and a variety of gastrointestinal signals [118,119] (Fig. 5.6). Among these signals is the sensing of the absence or presence of ingested nutrients by enteroendocrine cells, and their subsequent release of either appetite-stimulating (orexigenic) or appetite-inhibiting (satiety or anorexic) factors [33,119,121,122]. Signals from the gastrointestinal tract that influence energy intake and expenditure include neurohumoral factors acting either directly on CNS neurons in regions where the blood–brain barrier is leaky or indirectly by stimulating afferent neurons, of which the vagal afferent pathway has been most intensively studied. Together, these mechanisms serve to maintain body weight within narrow limits over long periods in the face of changing metabolic demands. Progress in elucidating the cellular and molecular mechanisms relevant to the control of food intake has been impressive [116] and is in part attributable to studies of genetically modified animals, which have revealed the role of specific transmitters and receptors [123].

The stomach and inhibition of food intake

Distention of the stomach leads to the inhibition of feeding through mechanisms that are not dependent on nutrient content and which are mediated by vagal afferent nerve fibers that function as gastric mechanoreceptors [124,125]. The functional relevance of this system is illustrated by the efficacy of bariatric surgery as a treatment for obesity [126]. A number of different procedures aim to limit the volume of the stomach, including gastric banding (which may be variable) either with or without bypass of the small intestine. In patients with morbid obesity, about 60% of the excess body mass is lost after bariatric surgery and, importantly, the weight loss is maintained for up to 8 years. A metaanalysis by the National Institute for Health and Clinical Excellence in

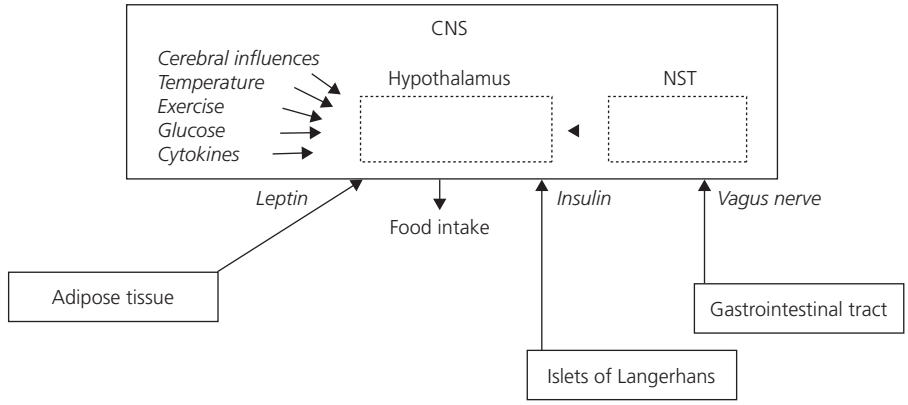

Figure 5.6 Mechanisms controlling food intake. Signals from the gastrointestinal tract that inhibit food intake in the short term (i.e., over the period of a single meal) include gastric distention (which appears not to be nutrient related) and the release of cholecystokinin (CCK) from the small intestine (which is related to the fat and protein content of the meal), and glucagon-like peptide-1 (GLP-1) and peptide $YY_{3–36}$ ($PYY_{3–36}$) from the ileum and colon. These mechanisms stimulate vagal pathways terminating in the nucleus of solitary tract (NST) and projecting to the hypothalamus. Feeding inhibits the release of ghrelin from the stomach; ghrelin acts on the hypothalamus to decrease fat utilization. Over the long term, leptin released from adipocytes regulates food intake. Among other activities, leptin inhibits hypothalamic neuropeptide Y-producing neurons that stimulate food intake.

the United Kingdom concluded that gastric surgery for the treatment of obesity is efficacious and cost-effective [127].

It was thought for many years that fat stores might somehow signal to the CNS to influence food intake (i.e., the lipostatic theory of food intake). The molecular basis for this mechanism has emerged from the characterization of *ob/ob* mice as obese, hyperphagic, less mobile, hypometabolic, diabetic, and infertile. Zhang and colleagues [128] used positional cloning to show that these mice have a mutation in a gene encoding a putative hormone signaling from adipose tissue to the CNS, now known as leptin. Subsequently, mutations in the leptin gene were also reported in two obese human subjects [129]. The receptor for leptin is mutated in a different mouse strain (*db/db*) that also manifests hyperphagia, hypometabolism, and diabetes [130]. In addition to adipocyte secretion, leptin is also produced in gastric chief cells [131] and, moreover, functional leptin receptors are expressed by vagal afferent neurons [36,132]. Therefore, as well as acting as an adipocyte-derived satiety signal, leptin may also act as a gastric satiety peptide through local stimulation of vagal afferent nerve fibers projecting to the stomach.

Postpyloric satiety signals

The intestinal hormone CCK was one of the first hormones to be identified as able to modify food intake [133]. The small intestinal mucosal I cells that secrete CCK function as nutrient sensors responding to ingested fat and protein in the lumen. Fatty acids with a chain of 12 carbon atoms or longer are effective releasers of CCK in humans and in cell lines, whereas fatty acids of 10 or fewer carbon atoms are ineffective [134,135]. A substantial body of evidence indicates that vagal afferent neurons expressing CCK_1 receptors mediate CCK-induced inhibition of gastric emptying and augmentation of sensations of satiety. The action of peripherally administered CCK on food intake is inhibited by abdominal vagotomy, lesioning of the NST, sectioning of vagal afferent rootlets, chemical lesioning of afferent fibers with capsaicin, and CCK_1 receptor antagonists that are excluded from the CNS [136–139]. Other satiety signals modulate the effects of CCK on these neurons; for example, potentiating interactions between CCK and leptin stimulate gastric vagal afferent nerve discharge and inhibit food intake [140,141], as does the interaction between gastric distention and CCK [142,143]. There also may be interactions between CCK and orexigenic factors such as ghrelin and orexin-A at the level of vagal afferent neurons [38,144]. The multiple interactions that occur at the level of vagal afferent neurons to control food intake and autonomic reflexes support the idea of a previously unsuspected degree of integration of signals at this level. In this context, it is therefore worth considering the reports that single nucleotide polymorphisms (SNPs) in the gene encoding the CCK_1 receptor are associated with obesity [145], and that rats in which the CCK_1 receptor locus is deleted (i.e., Otsuka Long Evans Tokushima Fatty strain) are obese [146].

In addition to CCK acting as a satiety signal from the jejunum and duodenum, there are at least two putative satiety hormones from the ileum and colon, notably $PYY_{3–36}$ and GLP-1. The former is a member of the same peptide family as NPY. Cleavage of PYY by dipeptidyl peptidase IV yields the 3–36 C-terminal fragment, which is a Y-2 receptor agonist.

Administration of $PYY_{3–36}$ has been reported to inhibit food intake in rodents and humans [147–149]; there is evidence both for CNS effects of $PYY_{3–36}$ and for an effect mediated by the vagus nerve [42,150]. Some laboratories have been unable to replicate the observation that this peptide administered peripherally inhibits food intake in rodents [151]; the reasons remain unclear but it seems possible that the effects of $PYY_{3–36}$ on food intake are not readily shown in stressed animals.

Cleavage of preproglucagon in intestinal L cells produces the satiety peptide GLP-1 together with a second glucagon-like peptide, GLP-2, and a C-terminally extended form of glucagon, oxyntomodulin [120]. Further cleavage generates $GLP\text{-}1_{7–36amide}$, which is the active form of the peptide that inhibits food intake. A metaanalysis of seven studies in humans concluded that infusion of $GLP\text{-}1_{7–36amide}$ inhibits food intake by 11% with no significant difference between lean and obese subjects [152]. In the circulation, $GLP\text{-}1_{7–36amide}$ is inactivated by dipeptidyl peptidase IV; therefore, inhibition of this enzyme is being considered as a means to increase circulating levels of $GLP\text{-}1_{7–36amide}$. A stable analogue, exendin-4, is also available for the treatment of type II diabetes mellitus. In rats, the inhibitory effect of the peripheral administration of $GLP\text{-}1_{7–36amide}$ on food intake appears to require an intact vagus [150].

Gastrointestinal appetite signals

In addition to signals from the gut that inhibit food intake, it now appears that the gastrointestinal tract produces several stimulants of food intake. The peptide ghrelin was discovered as the ligand of an orphan receptor that mediates the action of synthetic secretagogues on growth hormone release [153]. Ghrelin is mainly produced in the stomach in A-like (or X) cells of the gastric corpus [154]. Plasma concentrations are increased in fasting; they exhibit a rise before each meal, fall after feeding, and correlate inversely with body weight [155–157]. The mechanisms responsible for the rise in plasma concentrations are not well characterized; in contrast, the decrease in plasma concentrations can be produced by glucose and high-protein meals, whereas fat has a lesser effect. The contact of nutrients with the stomach and duodenum is not required for the postprandial fall in plasma ghrelin concentrations. After bariatric surgery by the Roux-en-Y gastric bypass, circulating ghrelin concentrations are suppressed; in contrast, plasma ghrelin concentrations are maintained after variable gastric banding or biliopancreatic diversion, indicating that the functional integrity of the gastric corpus is required to maintain ghrelin secretion [158].

Peripheral administration of ghrelin stimulates food intake and, in experimental animals, this effect requires an intact vagal nerve [159,160]. The vagal afferent neurons that express ghrelin (GHS-1) receptors also express CCK_1 receptors [160], as well as receptors for another orexigenic peptide, orexin-A (i.e., Ox-R1 receptors) [38]. Both ghrelin and orexin-A inhibit vagal afferent nerve discharge, indicating a functional role for these receptors [38,144]. Interestingly, research suggests that a second biologically active peptide, obestatin, also derived from the ghrelin precursor, inhibits food intake and acts through a previously identified G protein-coupled receptor with an unidentified ligand (GPR39) [161].

It is well established that increased appetite (known as *the munchies*) is linked to cannabinoid (CB)-1 receptor activation; for example, by the active ingredient of cannabis, Δ^9-tetrahydrocannabinol. These effects generally are thought to be mediated by receptors on hypothalamic neurons [162]. However, an endogenous ligand of CB-1 receptors, the lipid amide anandamide, is increased in the intestine of fasted rats and, on peripheral administration, stimulates food intake by a mechanism requiring an intact vagal nerve [163]. In both rat and human, CCK_1-expressing vagal neurons also express the CB-1 receptor [37]. Importantly, however, the expression of CB-1 receptors in rat nodose ganglion neurons is critically dependent on the recent nutritional history of the animal. In rats fed ad libitum, expression of CB-1 receptors in vagal neurons projecting to the stomach and duodenum is virtually undetectable; expression is dramatically increased after 12–18 h fasting and this is reversed by refeeding and by administration of exogenous CCK. A CCK_1 receptor antagonist prevented the down-regulation of CB-1 receptor expression indicating that endogenous CCK suppresses CB-1 receptor expression [37]. These observations suggest a novel dimension to signaling through vagal afferent neurons and, in particular, indicate a specific mechanism by which previous nutritional intake, or its absence, may modulate vagal afferent nerve discharge through induction of a receptor associated with stimulation of food intake.

Central nervous system mechanisms

The vagal afferent neurons involved in the control of feeding behavior act on brainstem neurons projecting to the hypothalamus. Many of the central mechanisms that control energy intake also regulate energy expenditure; the hypothalamus is a key CNS region controlling these functions and integrating the peripheral and other central inputs to them. Within the arcuate nucleus of the hypothalamus two broad classes of neuron have been identified. One class expresses genes encoding the peptide transmitters CART and proopiomelancortin (POMC); POMC is cleaved to yield α-melanocyte-stimulating hormone (α-MSH), which, in turn, acts at melanocortin (MC)-4 receptors [119]. Both CART and POMC inhibit food intake; their release is induced by peripheral satiety signals such as leptin and CCK and inhibited by appetite-stimulating factors such as ghrelin. A second class of neuron expresses the genes encoding NPY, which is a powerful stimulant of food intake when administered into the ventromedial and paraventricular hypothalamic nuclei [164], and agouti-related protein (AGRP), which is an

endogenous antagonist of MC-4 receptors and therefore also a stimulant of appetite [165]. These neurons are inhibited by leptin and peripheral satiety signals and excited by ghrelin, orexin-A, and other appetite-stimulating signals. The elucidation of both the neuroendocrine factors influencing these neurons and the neurotransmitter circuits activated by them provide potentially fruitful avenues in the search for novel treatments of obesity.

Overview

The gastrointestinal tract and brain involve each other in two-way communication. These interactions provide a basis for the gastrointestinal control of CNS responses (e.g., control of food intake) and, conversely, the CNS control of gastrointestinal function (e.g., during the cephalic phase of digestion). More importantly, they provide the capacity for integrating information about the state of the gut contents and inputs from other parts of the body. A direct consequence of these integrative functions is modulation of the signaling between brain and gut during normal digestion. However, the modulation of signaling pathways is also likely to be important clinically, and it may account for the hypersensitivity that is a feature of disease states involving inflammation in the gut, as well as those that do not. The results of a variety of experimental approaches have shown that it is possible to explain in molecular and cellular terms the development of the brain–gut axis and its organization, neurochemistry, and physiology. Impressive progress is also being made in the combined application of CNS imaging techniques and functional studies of the human digestive tract. These approaches should make it possible to define how dysfunction of brain–gut signaling contributes to gastrointestinal disease.

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A COMPONENTS

6 Smooth muscle of the gut

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The main function of smooth muscle of the gut is to mix and propel intralumenal contents, enabling efficient digestion of food, progressive absorption of nutrients, and eventual evacuation of residues. This function is regulated by the intrinsic electrical and mechanical properties of smooth muscle, such as the ability to maintain tone or undergo phasic contraction, and by alterations in these properties in response to hormonal and neural signals, particularly signals emanating from the enteric nervous system. A distinctive feature of physiological regulation in the gut is that stimuli of hormonal release and neural activation arise within the lumen from the mechanical and chemical properties of food and digestive secretions.

Structure of smooth muscle

Muscle layers

Smooth muscle of the gut consists of a thin, outer, longitudinal layer and a thick, densely innervated, circular layer; the layers derive their names from the orientation of the long axis of muscle cells in them. The layers are separated by laminar septa into bundles about 1 mm long that probably act as contractile units. The muscle cells are embedded in a connective tissue matrix, a product of their synthetic and secretory activity consisting mainly of elastic and collagen fibrils. The layers include glial cells, fibroblasts, and a distinctive population of muscle-like cells, the interstitial cells of Cajal (ICCs).

Muscle cells: membranes and organelles

Single smooth muscle cells are about 400 μm long and 5 μm wide when fully relaxed. They are spindle-shaped and have a high ratio of surface area–volume (1.5 μm^2:1 μm^3). Their plasma membranes consist of two specialized structures known as *caveolae* and *dense bands* [1–3].

The caveolae, 70 nm wide and 120 nm deep, are basket-shaped invaginations of the membrane that are arranged in clusters (Fig. 6.1). There are about 150 000 caveolae per cell; they occupy about one-third of the outer surface of the cell but constitute a much larger fraction of the surface of the plasma membrane. The bases of caveolae are surrounded by an abundant endoplasmic reticulum, the site of Ca^{2+} storage and release in smooth muscle. The arrangement suggests that caveolae may be functional equivalents of the transverse tubules in striated muscle. Caveolin-1α, caveolin-1β, and caveolin-3 are the main structural proteins in smooth muscle

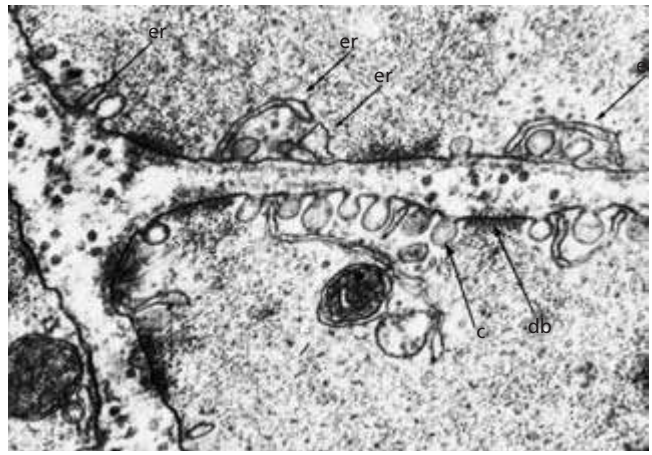

Figure 6.1 Surface organization of three adjacent muscle cells from the circular muscle layer of guinea pig ileum. Clusters of basket-shaped caveolae (c) surrounded by endoplasmic reticulum (er) are separated from each other by dense bands (db) (original magnification × 67 000). From Gabella [1].

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caveolae [4,5]. They bind a variety of signaling molecules and are thought to act as scaffolds to facilitate signaling. In smooth muscle, caveolins also bind transiently to agonist-activated α and βγ subunits of G proteins, prevent their reassociation, and induce desensitization of responses mediated by these G proteins [6].

Clusters of caveolae are separated from each other by electron-dense structures, 1–2 μm long and 0.2–0.4 μm wide, called dense bands (plaques), which occupy about one-half of the surface of the cell (Fig. 6.2). At these sites, thin actin filaments are attached to transmembrane integrins via a series of proteins consisting mainly of α-actinin and various kinases and signaling proteins [7]. Intermediate 10-nm-thick filaments, consisting mainly of desmin in visceral smooth muscle, link dense bands in the membrane to dense bodies in the cytoplasm and transmit the force generated by the contractile apparatus within the cell to the entire surface of the cell [8]. Intermediate junctions consisting of the juxtaposed dense bands of adjacent cells, together with collagen fibrils, transmit force from one cell to the next and couple the contractile apparatus of adjacent cells to the rest of the syncytium (Fig. 6.2).

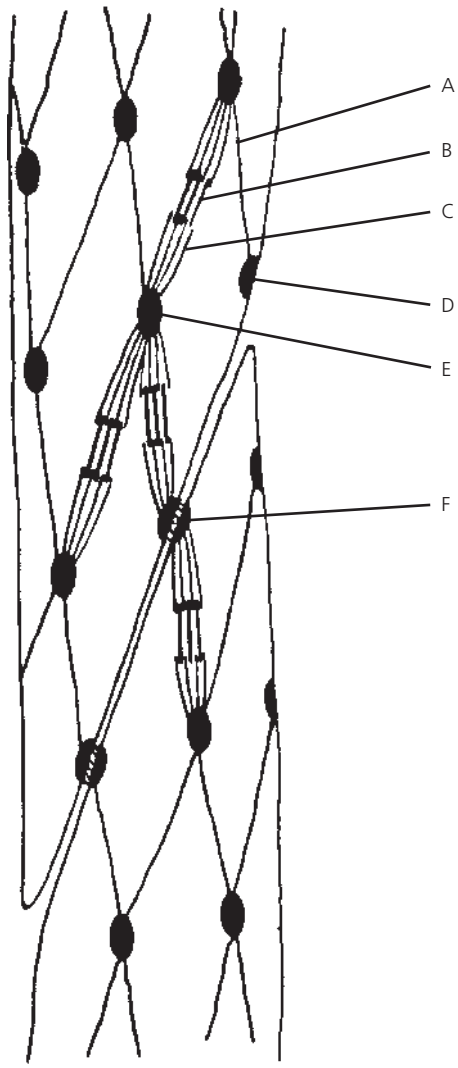

Figure 6.2 Organization of the contractile and cytoskeletal apparatus in smooth muscle cells. Thin actin filaments (C) emerge from the poles of cytoplasmic dense bodies (E) and interdigitate with thick myosin filaments (B). Dense bodies in the plasma membrane (D) are connected to dense bodies in the cytoplasm (E) by intermediate filaments (A). When juxtaposed, dense bodies from adjacent cells can form close intermediate junctions (F). Adapted from Murphy [8].

In some regions, patches of the plasma membrane of adjacent cells are closely apposed and the space between them is bridged by intercellular channels. These patches, known as *gap junctions* or *nexuses*, are the most likely sites of electrical coupling between muscle cells; they permit movement of intracellular regulatory molecules, such as cyclic AMP (cAMP), inositol 1,4,5-triphosphate (IP_3), or Ca^{2+}, and help to propagate the signal from cell to cell [1–3,9].

Dense bodies and contractile filaments occupy about 80% of the interior of the cell; the remainder is occupied by various organelles, including the nucleus, mitochondria, Golgi apparatus, lysosomes, and rough and smooth endoplasmic/sarcoplasmic reticulum. The last, located immediately beneath and parallel to the plasma membrane, occupies 2% of the cell volume and is the site of Ca^{2+} uptake and release [10–13]. It consists of several functional compartments, only one of which is sensitive to IP_3, a membrane-derived messenger responsible for the release of intracellular Ca^{2+} in circular smooth muscle (Fig. 6.1) [14]. The mitochondria, considered a low-affinity, high-capacity storage site, can take up large amounts of Ca^{2+} but only after cell injury, when cytosolic Ca^{2+} increases to more than maximal physiological levels (> 1–5 μM) [15].

Contractile apparatus: thin and thick filaments

Three types of filaments can be differentiated in smooth muscle cells: thin actin filaments (5–7 nm), thick myosin filaments (15 nm), and intermediate desmin filaments (10 nm). Intermediate filaments link dense bodies in the cytoplasm to dense bands on the plasma membrane. Although the arrangement of thin, thick, and intermediate filaments and their attachments to cytoplasmic dense bodies lack the order found in striated muscle, assemblies reminiscent of primitive sarcomeres can be seen.

Thin filaments consist of actin, a ubiquitous 42-kDa globular protein (G actin) that polymerizes to form 2-stranded helical filaments (F actin) of indeterminate length [16]. Inserted into the grooves of the actin helix is another protein, tropomyosin. Thin filaments have a distinct polarity, and they appear to be inserted into or emerge from the poles of dense bodies and are arranged in bundles that run parallel to the long axis of the cells, with their free ends surrounding and interdigitating with thick myosin filaments (Fig. 6.2) [1–3,17]. The insertion of thin filaments in dense bodies is

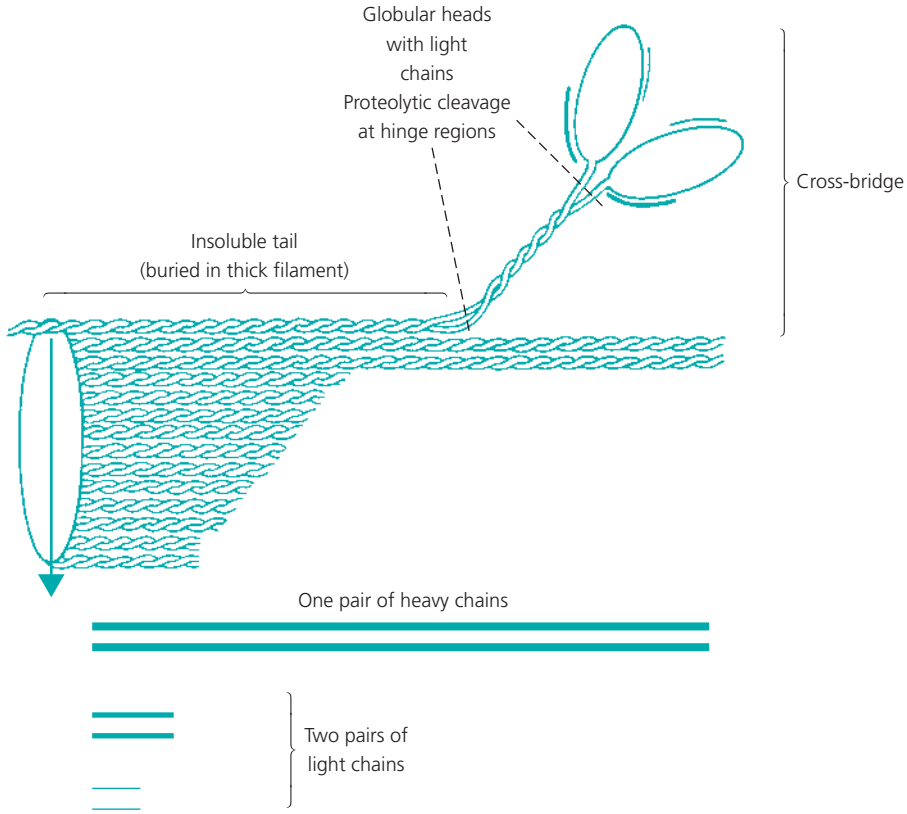

Figure 6.3 Component proteins of the myosin molecule. A cross-bridge projects laterally from the main segment of the myosin core (i.e., tail). Each cross-bridge consists of a pair of myosin heads, each surrounded by a 17-kDa and a 20-kDa myosin light chain and a laterally projecting segment of the myosin core between the two hinge regions. The cores of many myosin molecules form thick filaments with several projecting cross-bridges. Adapted from Murphy [8].

analogous to that found in Z discs of striated muscle cells; in effect, dense bodies may be viewed as dispersed fragments of Z discs held together and anchored to dense bands of the cell membrane by intermediate filaments.

Thick filaments are aggregates of myosin molecules, a complex 480-kDa protein formed by the association of six different proteins [16–18]. These proteins are not covalently linked and can be dissociated from each other into one pair of myosin heavy chains and two pairs of myosin light chains (MLCs) (Fig. 6.3). The heavy chains are coiled around each other to form a rigid, insoluble, helical core or tail. Each strand of the core terminates in a globular head surrounded by two MLCs: a 20-kDa regulatory chain and a 17-kDa essential chain. Each globular head contains a binding site for actin and an actin-activated Mg^{2+}-ATPase.

A hinge located at the junction of the globular head and the core enables the head to rotate about the core. Another hinge in the core enables the globular heads to project laterally. The globular heads and the segments of the core between the two hinges are called *cross-bridges* because they constitute a link or bridge between thick myosin and thin actin filaments.

Only three to five thick filaments are surrounded by and interdigitate with a much larger number of actin filaments. The ratio of thin to thick filaments is reflected in the relative content of actin and myosin. Visceral smooth muscle contains the same amount of actin as striated muscle (22 vs 28 mg/g of cell) but a lower amount of myosin (20 vs 62 mg/g of cell) [16]. Despite the low content of myosin, smooth muscle generates as much force as striated muscle (up to 6 kg/cm^2 of cross-sectional area) [7,19].

Interaction of contractile proteins

The interaction of myosin and actin with hydrolysis of ATP is the fundamental reaction whereby chemical energy is converted into mechanical energy in smooth muscle, generating force and inducing shortening as a result of the sliding of overlapping, interdigitating thin and thick filaments [16,20]. The interaction occurs in a cycle, the cross-bridge cycle, the essential feature of which is a shift in the affinity of myosin for actin (Fig. 6.4). ATP, bound weakly to myosin, is hydrolyzed to ADP and inorganic phosphate (P_i). The products of hydrolysis remain bound to the myosin head, and the energy released is stored in the myosin molecule, which has a high affinity for actin in this state. On release of ADP and P_i, ATP binds again to myosin, which then reverts to a state of low affinity for actin. Cross-bridge cycling ceases after the stimulus is withdrawn; the dephosphorylated myosin

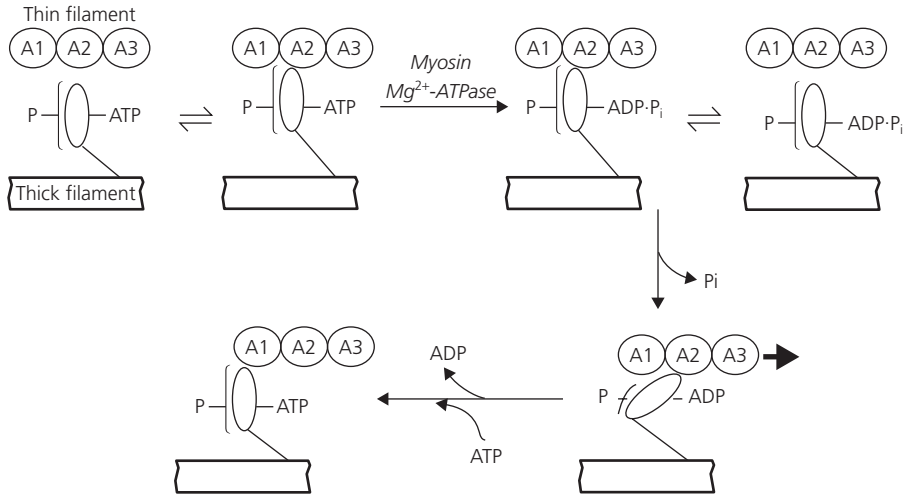

Figure 6.4 Interaction of myosin and actin filaments: the cross-bridge cycle. At the start of the cycle, the myosin head with ATP bound to it (myosin·ATP) is detached or is weakly attached to an actin molecule (A2) in the thin filament. Hydrolysis of ATP by the myosin–Mg^{2+}-ATPase yields an intermediate with the products of hydrolysis still bound to it (myosin·ADP·P_i); the intermediate is detached or weakly bound to the actin molecule. Release of P_i in the next step causes a transition from weak to strong binding of myosin and actin (actin·myosin·ADP) and a change from 90° to 45° in the angle of the myosin head. The strain imposed on the cross-bridge by the change of angle is relieved when the actin molecule slides past the cross-bridge. ADP is released slowly and the myosin head rebinds ATP (myosin·ATP) and reverts to a 90° angle facing the next actin molecule (A1) for the start of another cycle. Dephosphorylation of the myosin light chain surrounding a bound myosin head yields a strongly attached, slowly cycling, latch cross-bridge. The interaction of only one myosin head in the cross-bridge is illustrated. P_i, inorganic phosphate. Adapted from Hartshorne [16].

cross-bridges are arrested in a detached state, which is characteristic of relaxed muscle.

Phosphorylation of myosin light chain

An essential step in smooth muscle contraction is phosphorylation of the 20-kDa regulatory myosin light chain (MLC_{20}) by a Ca^{2+}/calmodulin-dependent or -independent MLC kinase [16,20–22]. When smooth muscle is stimulated, cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) increases as a result of the influx of Ca^{2+} into the cytosol through voltage-gated Ca^{2+} channels and/or the release of Ca^{2+} into the cytosol from intracellular Ca^{2+} stores. Ca^{2+} sequentially binds to the four binding sites on the regulatory protein calmodulin; Ca^{2+}-activated calmodulin binds to MLC kinase to form an active Ca^{2+}/calmodulin–MLC kinase complex [23]. Phosphorylation of MLC_{20} induces a conformational change in the myosin head that greatly enhances the ability of actin to activate myosin–Mg^{2+}-ATPase and stimulate the hydrolysis of ATP bound to the myosin head.

The initial increase in $[Ca^{2+}]_i$ is transient and is rapidly dissipated by reuptake into Ca^{2+} stores or extrusion from the cell. Ca^{2+}/calmodulin-dependent MLC kinase activity decreases coincidentally with the decrease in $[Ca^{2+}]_i$, and its decrease is accelerated via phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II and p21-activated kinase (PAK) (Fig. 6.5) [22,23]. MLC_{20} phosphorylation and contraction, however, are sustained by a Ca^{2+}-independent MLC kinase and a regulated decrease in MLC phosphatase activity [22,24–27]. The signaling pathways that mediate the initial and sustained phases of contraction are discussed in greater detail in the next section.

Unlike the biphasic pattern of agonist-stimulated tonic contraction just described, rhythmic contraction increases and decreases rapidly in phase with the influx and efflux of Ca^{2+} driven by rhythmic changes in membrane potential and the opening and closure of voltage-gated Ca^{2+} channels. A close correlation prevails during phasic activity among Ca^{2+} levels, MLC kinase activity, MLC_{20} phosphorylation, cross-bridge cycling rate, and contraction. Consequently, more energy is required to sustain phasic contraction. Energy is saved, however, because rhythmic contractile activity usually is maintained in abeyance by a dominant inhibitory neural input.

Signal transduction in visceral smooth muscle

Source of activator calcium

The concentration of Ca^{2+} in the cytosol ($[Ca^{2+}]_i$) is the essential determinant of the initial, transient phase of tonic smooth muscle contraction. Two agonist-driven mechanisms lead to an increase in $[Ca^{2+}]_i$. In the first, interaction of a contractile agonist with its receptor on the plasma membrane

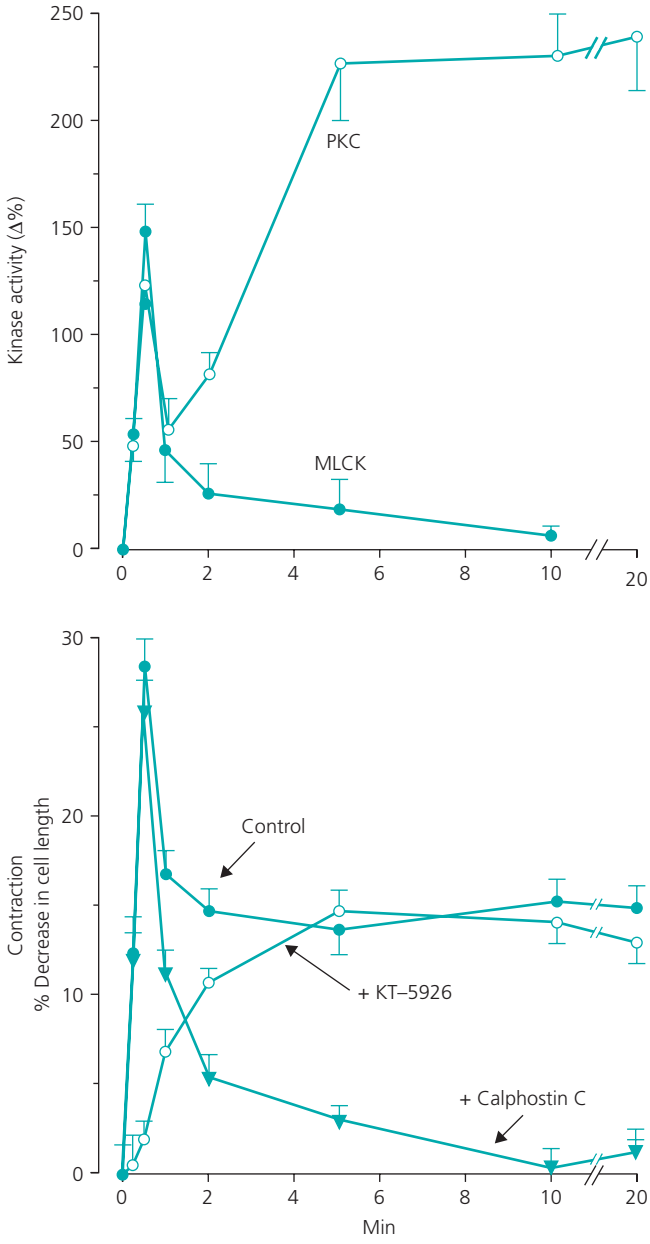

Figure 6.5 **(Upper panel)** Time course of activation of protein kinase C (PKC) and myosin light chain kinase (MLCK). MLCK activity occurs mainly during the initial phase of contraction. PKC activity is derived from phosphoinositide hydrolysis by PLC-β during the initial phase and from phosphatidylcholine hydrolysis by phospholipase D during the sustained phase. **(Lower panel)** Initial contraction is mediated by Ca^{2+}/calmodulin-dependent activation of MLCK and is inhibited by the MLCK inhibitor KT-5926; sustained contraction is mediated by PKC and is inhibited by the PKC inhibitor calphostin C. See details of the pathways in the text and in Figure 6.6.

generates a messenger that causes the release of Ca^{2+} from intracellular stores (Fig. 6.5). In the second, interaction of the contractile agonist with its receptor generates a messenger that induces depolarization of the plasma membrane, which opens voltage-gated Ca^{2+} channels and causes Ca^{2+} influx followed by Ca^{2+}-induced Ca^{2+} release from intracellular stores. The first mechanism occurs in circular muscle, the second in longitudinal muscle.

During rhythmic contraction, spontaneous depolarization of the plasma membrane by slow waves or spike potentials can open voltage-gated Ca^{2+} channels in muscle from both layers. By increasing the frequency or amplitude of these depolarizing events, agonists can cause a further increase in Ca^{2+} influx through voltage-gated Ca^{2+} channels. In addition, agonists stimulate Ca^{2+} release causing a further increase in $[Ca^{2+}]_{i.}$

Components of the transduction pathway

The transduction of an external signal, such as a neurotransmitter or a hormone, into an internal signal involves the sequential activation of three membrane proteins: a receptor and a guanosine triphosphate (GTP)-binding protein (G protein) that couples the receptor to a specific effector enzyme. The effector enzyme acts on membrane-bound or cytoplasmic precursors to generate one or more regulatory signals or second messengers (Figs 6.5, 6.6, 6.7, and 6.8).

Receptors consist of external, membrane-spanning, and cytoplasmic domains with features that determine which specific ligand they bind or agonist they recognize and which G protein or membrane enzyme they activate [28,29].

G proteins are a large family of closely related proteins that act as signal transducers [30]. Among these are G proteins that stimulate (G_s) or inhibit (G_i and G_o) adenylate cyclase activity and G proteins that stimulate phospholipase C (PLC) activity (G_q). G proteins are heterotrimeric with subunits designated α, β, and γ in order of decreasing mass. The α subunit (Gα), which serves to differentiate G proteins, contains a single, high-affinity binding site for GTP and possesses GTPase activity; the latter is crucial for terminating the action of G proteins. In the basal state, guanosine diphosphate (GDP) is tightly bound to the α subunit. The binding of a ligand to its receptor enables the ligand–receptor complex to interact with the G protein and stimulate the dissociation of GDP; this opens up a site that is rapidly filled with abundant cytoplasmic GTP. The binding of GTP to the ligand–receptor–G-protein complex causes the following:

- a decrease in the affinity of the ligand for the receptor and of the receptor for the G protein, thus freeing the receptor for a new cycle of ligand and G-protein binding
- a decrease in the affinity of the α subunit for the βγ subunit, resulting in the dissociation of a Gα–GTP complex and a βγ dimer that activate different effector enzymes.

G proteins act as transducers that conduct and amplify the external signal and as adapters that allow the same receptor to be coupled to different effector enzymes. Both α and βγ subunits activate or inhibit effector enzymes. The involvement of α or βγ subunits depends on the specific receptor activated.

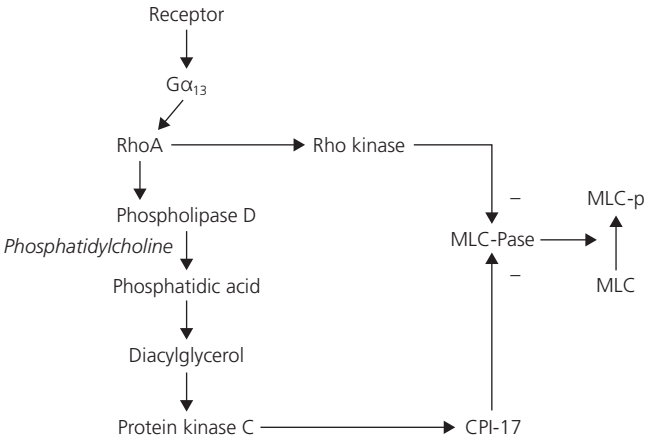

Figure 6.6 Signal transduction pathway mediating sustained contraction of smooth muscle. The pathway involves activation of the α subunit of G_{13}, RhoA and its associated kinase, and phospholipase D. Hydrolysis of phosphatidylcholine by phospholipase D generates phosphatidic acid, which is dephosphorylated to diacylglycerol with resulting activation of protein kinase C. Protein kinase C potently activates CPI-17, an endogenous inhibitor of myosin light chain (MLC) phosphatase activity, thereby maintaining MLC phosphorylation (MLC-p). A parallel pathway links Rho kinase to the inhibition of MLC phosphatase.

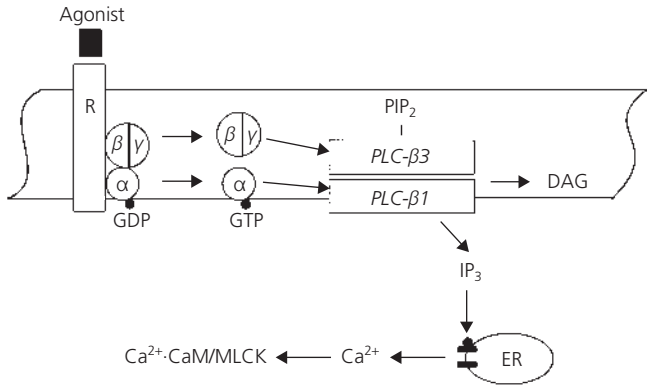

Figure 6.7 Signal transduction pathway for Ca^{2+} mobilization in smooth muscle cells of the circular muscle layer during the initial phase of contraction. The sequence involves binding of an agonist to a specific receptor, activation of a transducing G protein and binding of guanosine triphosphate (GTP) to its mobile α subunit, and dissociation of the βγ subunits. For receptors that couple to G_q (e.g., CCK_1, muscarinic M_3, 5-HT_2), $G\alpha_q$ activates phospholipase C-β1 (PLC-β1); for receptors that couple to G_i (e.g., muscarinic M_2, adenosine A_1, opioid μ, κ, and δ), $G\beta\gamma_i$ activates PLC-β3. In circular muscle cells, the immediate substrate hydrolyzed by PLC-β1 or PLC-β3 is phosphatidylinositol 4,5-bisphosphate (PIP_2), yielding inositol 1,4,5-triphosphate (IP_3) and diacylglycerol (DAG) as second messengers. IP_3 diffuses through the cytosol to interact with IP_3 receptors–Ca^{2+} channels on the membrane of the endoplasmic reticulum (ER), causing release of Ca^{2+} into the cytosol. Ca^{2+} binds to calmodulin (CaM) and the complex activates myosin light chain kinase (MLCK). (See [35] and [48].)

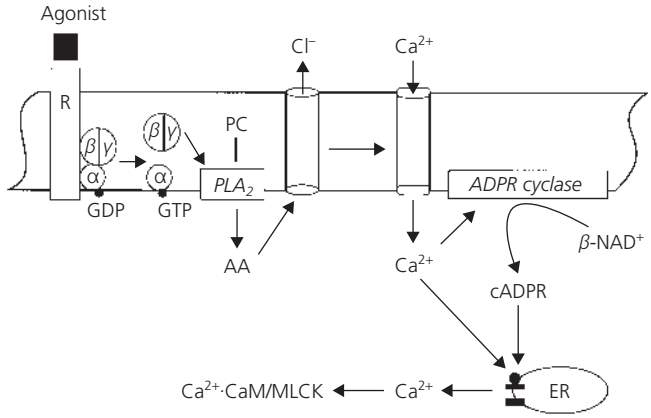

Figure 6.8 Signal transduction pathway for Ca^{2+} mobilization in smooth muscle cells of the longitudinal muscle layer during the initial phase of contraction. The sequence involves activation of phospholipase A_2 (PLA_2) by Gβγ and hydrolysis of membrane-bound phosphatidylcholine (PC) to yield arachidonic acid (AA) and lysophosphatidylcholine. Arachidonic acid activates chloride (Cl^-) channels causing Cl^- efflux from the cell, resulting in depolarization of the plasma membrane, opening of voltage-gated Ca^{2+} channels, and Ca^{2+} influx into the cell. The resultant increase in cytosolic Ca^{2+} stimulates Ca^{2+} release (i.e., Ca^{2+}-induced Ca^{2+} release) from endoplasmic Ca^{2+} stores through ryanodine-sensitive receptor–Ca^{2+} channels. In addition, Ca^{2+} activates a membrane-bound ADP-ribosyl cyclase (ADPR cyclase) that synthesizes cyclic ADP ribose (cADPR) from β-nicotinamide adenine dinucleotide (β-NAD^+). cADPR acts as a Ca^{2+}-mobilizing messenger, stimulating Ca^{2+} release by directly activating ryanodine receptor–Ca^{2+} channels and by potentiating Ca^{2+}-induced Ca^{2+} release. (See [50–53].)

The hydrolysis of GTP by the intrinsic GTPase activity of the Gα–GTP complex terminates the activity of the complex; the inactive Gα–GDP complex binds to and inactivates βγ by fostering the reassociation of the α, β, and γ subunits. The slow hydrolysis of GTP by Gα is accelerated by two classes of GTPase-activating proteins (GAPs): one class includes effector enzymes such as PLC-β; the other consists of a family of Gα GAPs, known as *regulators of G-protein signaling* (RGS) [31]. All members (~ 25) of the RGS family contain a conserved GTPase-activating RGS domain that binds exclusively to Gα–GTP. In some RGS proteins, additional structures outside the RGS domain determine receptor and G-protein selectivity. At least seven RGS proteins are expressed in smooth muscle of the gut, including RGS4 and RGS12, associated with $G\alpha_q$ and $G\alpha_i$ respectively.

Effector enzymes and second messengers

Several effector enzymes capable of yielding different messengers are involved in the regulation of smooth muscle contraction. They include PLC-β isoforms, which are activated by the α subunit of G_q or the βγ subunits of G_i and G_o

(Fig. 6.7); cytosolic phospholipase A_2 ($cPLA_2$), which is activated by G_i (Fig. 6.8); and phospholipase D (PLD), which is activated during the sustained phase of contraction by the α subunit of G_{13} and RhoA (see Fig. 6.6). All can hydrolyze membrane-bound phospholipids. In most cell types, the effector enzyme activated by agonists capable of mobilizing intracellular Ca^{2+} is one of several isoforms of PLC-β (i.e., PLC-β1, PLC-β2, PLC-β3, and PLC-β4); the isozymes hydrolyze inositol phospholipids located on the inner leaflet of the plasma membrane [14,32–36]. These phospholipids are products of the sequential phosphorylation of phosphatidylinositol (PI) to phosphatidylinositol monophosphate (PIP) and phosphatidylinositol 4,5-bisphosphate (PIP_2). The last is the immediate substrate hydrolyzed by PLC-β.

The hydrolysis of PIP_2 generates two messengers: a water-soluble inositol phosphate, IP_3, which diffuses into the cytosol to activate IP_3 receptor–Ca^{2+} channels located in a compartment of the endoplasmic reticulum, and diacylglycerol, which activates various Ca^{2+}-dependent (e.g., α, β, γ) and Ca^{2+}-independent (e.g., ε) isoforms of protein kinase C (PKC) and initiates their translocation from the cytosol to the plasma membrane (Fig. 6.7) [34]. IP_3 can be inactivated by sequential phosphorylation to IP_4, IP_5, or IP_6, or dephosphorylation to IP_2, IP, and inositol. The metabolic products of the two messengers eventually merge to reconstitute PI.

Partial depletion of Ca^{2+} stores by IP_3 triggers Ca^{2+} influx into the cell – a process known as capacitative Ca^{2+} influx – and leads to Ca^{2+} release from adjacent Ca^{2+} stores (i.e., Ca^{2+}-induced Ca^{2+} release). The process is manifested by localized changes in Ca^{2+} concentration (Ca^{2+} oscillations) that sweep through the cell at intervals of 5–60 s, and it is most effective in the presence of IP_3 [14,37]. IP_3 and Ca^{2+} are thought to flow rapidly (about 10 μm/s) through gap junctions to neighboring cells, propagating the intracellular signal and providing a means for a sustained or oscillatory response of the tissue as a whole.

The IP_3 receptor–Ca^{2+} channel is homologous to the ryanodine receptor–Ca^{2+} channel of skeletal and cardiac muscle [38–40]. Both receptor types consist of four subunits surrounding a Ca^{2+} channel; each subunit contains a large N-terminal cytoplasmic domain that includes binding sites for IP_3 and Ca^{2+} and the site for regulatory phosphorylation (i.e., inactivation) by cGMP-dependent protein kinases (PKG). The cytoplasmic domain appears to bridge the space between the endoplasmic reticulum and plasma membranes and is postulated to participate in mediating capacitative Ca^{2+} influx [41].

The involvement of IP_3 in Ca^{2+} mobilization is confined to circular smooth muscle. In longitudinal smooth muscle, Ca^{2+} mobilization involves $cPLA_2$, which hydrolyzes the membrane phospholipid phosphatidylcholine to yield arachidonic acid and lysophosphatidylcholine.

IP_3-dependent calcium mobilization in cells of the circular muscle layer

The transduction pathway initiated by the hydrolysis of PIP_2 is fully expressed in cells from the circular muscle layer of the stomach, intestine, gallbladder, and various sphincters. The various steps in this pathway have been examined in detail in dispersed muscle cells devoid of neural elements. The cellular homogeneity of the suspension makes it possible to characterize receptors and intracellular messengers, such as IP_3, diacylglycerol, cytosolic Ca^{2+}, and cyclic nucleotides, and to determine their coupling to the mechanical response (i.e., contraction or relaxation [12,13,41–48]).

The exposure of cells derived from the circular muscle layer to a contractile agonist induces rapid contraction (i.e., cell shortening) accompanied by a transient increase in IP_3, Ca^{2+} release, and $[Ca^{2+}]_i$ (Fig. 6.9a) [12,13,45,46]. These events are closely correlated, and their magnitudes depend on agonist concentration (Fig. 6.9b). The initial increase in IP_3 and Ca^{2+} is followed by the slow reuptake of Ca^{2+} into the cell (capacitative Ca^{2+} influx). The withdrawal of Ca^{2+} from the medium or the addition of Ca^{2+} channel blockers has no effect on the initial increase in $[Ca^{2+}]_i$ but it blocks Ca^{2+} influx. The pattern of response in muscle cells from the circular muscle layer implies that contractile agonists elicit an initial contraction by means of G-protein-dependent activation of PLC-β, hydrolysis of membrane-bound PIP_2, and generation of IP_3; the last diffuses through the cytosol to interact with sarcoplasmic IP_3 receptor–Ca^{2+} channels to induce Ca^{2+} release and a transient increase in $[Ca^{2+}]_i$. Depletion of Ca^{2+} stores triggers capacitative Ca^{2+} influx.

The coupling of receptors to G proteins and effector enzymes in smooth muscle is summarized in a review by Makhlouf and Murthy [49]. Most receptors mediating contraction in circular muscle are coupled via $G\alpha_q$ to PLC-β1 and PIP_2 hydrolysis; some are coupled via $G\beta\gamma_{i/o}$ to PLC-β3. Acetylcholine acting through muscarinic M_3 receptors, cholecystokinin (CCK) through CCK_1 receptors, the tachykinins (substance P and neurokinin A) through NK_1 and NK_2 receptors, endothelin through ET_A and ET_B receptors, neuropeptide Y (NPY) through Y_2 receptors, and pancreatic polypeptide (PP) through Y_4 receptors are preferentially coupled via $G\alpha_q$ to PLC-β1. Somatostatin acting through SST_3 receptors, opioid peptides through μ, δ, and κ receptors, and adenosine through A_1 receptors are preferentially coupled via the βγ subunits of G_{i1}, G_{i2}, and G_{i3}, respectively, to PLC-β3. Adenosine and uridine triphosphate (ATP and UTP) acting through $P2Y_2$ receptors, and sphingosine-1-phosphate through $S1P_2$ receptors are coupled via $G\alpha_q$ to PLC-β1 as well as via $G\beta\gamma_i$ to PLC-β3. Some G_i-coupled receptors, such as Y_1 receptors, $S1P_1$ receptors, and endocannabinoid CB_1 receptors, do not activate PLC-β: CB_1 and Y_1 receptors are coupled to an aberrant G protein consisting of $G\alpha_{i2}$, $G\beta_5$, and RGS6; the last contains a Gγ-like domain that substitutes for Gγ

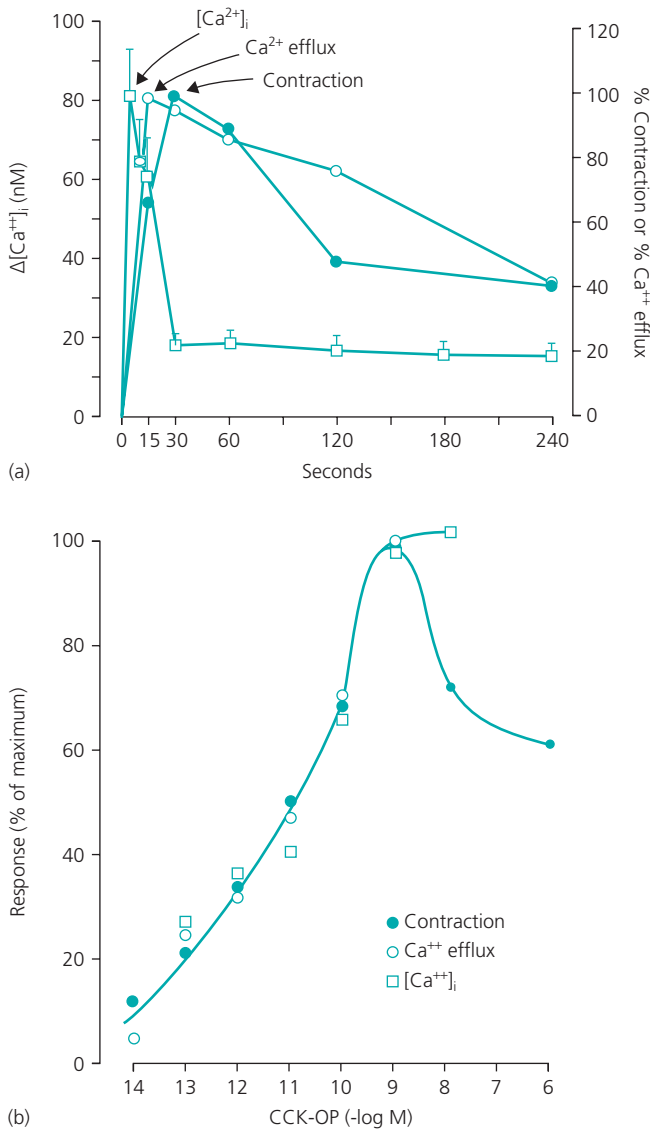

Figure 6.9 Time course **(a)** and stoichiometry **(b)** of contraction, cytosolic Ca^{2+} ($[Ca^{2+}]_i$), and Ca^{2+} release from endoplasmic stores in smooth muscle cells isolated from guinea pig stomach in response to cholecystokinin octapeptide (CCK-OP). Similar results are obtained for muscle cells from human stomach and intestine using other agonists. From Bitar et al. [45], with permission from AAAS.

and renders the $G\beta_5$–RGS6 complex incapable of activating PLC-β. Muscarinic M_2 receptors, activated concurrently with M_3 receptors by acetylcholine, are coupled via $G\beta\gamma_{i3}$ to PLC-β3 and Ca^{2+} mobilization, but are incapable by themselves of inducing contraction; this is because M_2 receptors are coupled to another pathway that leads to inactivation of Ca^{2+}-dependent MLC kinase. The ligands for these receptors are variously derived from excitatory enteric neurons (acetylcholine, tachykinins), from the circulation (PP), from muscle cells (S1P, endocannabinoids), or other cell types (histamine).

IP_3-independent calcium mobilization in cells of the longitudinal muscle layer

All of the receptors listed in the previous section are expressed in longitudinal smooth muscle except for somatostatin, opioid, and Y_1 receptors [49]. The receptors initiate contraction by mobilizing Ca^{2+} via a distinct mechanism that is evident in all mammalian species examined so far, including human, dog, rabbit, guinea pig, rat, and mouse.

The pattern of inositol phospholipid metabolism in longitudinal muscle differs markedly from that in circular muscle. Only a small amount of IP_3 is generated in longitudinal muscle, less than 10% of that generated in circular muscle. The preferred substrate is PIP, which yields the inactive IP_2 and diacylglycerol [35,47–51]. IP_3 does not bind to the endoplasmic and sarcoplasmic membranes of longitudinal muscle cells or cause Ca^{2+} release at maximal concentrations (1 μM); partial Ca^{2+} release is elicited at supramaximal concentrations (10–50 μM).

Contraction and the increase in $[Ca^{2+}]_i$ induced by agonists in muscle cells from the longitudinal muscle layer are IP_3-independent and are abolished in Ca^{2+}-free medium or in the presence of Ca^{2+} channel blockers, implying that an initial step involving Ca^{2+} influx is a prerequisite for Ca^{2+} mobilization in these cells [38,41–43]. The initial influx of Ca^{2+} acts as a trigger for Ca^{2+} release from intracellular stores (i.e., Ca^{2+}-induced Ca^{2+} release) (Fig. 6.8). These stores are highly sensitive to Ca^{2+} alone; increments of $[Ca^{2+}]_i$ in the physiological range (100–500 nM) trigger Ca^{2+} release. In contrast, Ca^{2+} stores in circular muscle develop high sensitivity to Ca^{2+} only in the presence of IP_3. Ryanodine, a specific ligand of ryanodine receptor–Ca^{2+} channels in skeletal and cardiac muscle, binds with high affinity to the sarcoplasmic membranes of longitudinal muscle cells and induces Ca^{2+} release (EC_{50} 2 nM) and contraction (EC_{50} 1 nM) [50]. The characteristics of ryanodine binding and Ca^{2+} release in longitudinal muscle cells are similar to those in cardiac muscle cells [38].

The messenger responsible for initiating Ca^{2+} mobilization in longitudinal muscle cells is arachidonic acid, a product of the hydrolysis of phosphatidylcholine by $cPLA_2$ (Fig. 6.8). An initial agonist-induced, G protein-mediated activation of $cPLA_2$ occurs in longitudinal, but not circular, muscle cells [51]. Suppression of $cPLA_2$ activity by selective inhibitors abolishes contraction and the increase in $[Ca^{2+}]_i$ induced by agonists in longitudinal muscle cells. Arachidonic acid in nanomolar concentrations mimics the effect of contractile agonists and causes an increase in $[Ca^{2+}]_i$ that is abolished by Ca^{2+} channel blockers. The increase in $[Ca^{2+}]_i$ reflects arachidonic acid-dependent Ca^{2+} influx followed by Ca^{2+}-induced Ca^{2+} release from intracellular stores. Depletion of Ca^{2+} stores attenuates the increase in $[Ca^{2+}]_i$ by eliminating the component resulting from Ca^{2+}-induced Ca^{2+} release; the residual increase in $[Ca^{2+}]_i$ reflects the initial step, that is arachidonic acid-dependent Ca^{2+} influx. Ca^{2+} influx results from activation of Cl^- channels by arachidonic acid,

which leads to depolarization of the plasma membrane and opening of voltage-gated Ca^{2+} channels (Fig. 6.8) [52].

The ryanodine receptor–Ca^{2+} channels in longitudinal muscle cells are highly sensitive to cyclic ADP-ribose (cADPR), a product of the hydrolysis of β-nicotinamide adenine dinucleotide (β-NAD^+) by ADP-ribosyl cyclase, a membrane-bound enzyme present in longitudinal, but not circular, muscle cells (Fig. 6.8) [53]. cADPR formation is stimulated in a concentration-dependent fashion by contractile agonists in longitudinal, but not circular, muscle cells. It binds with high affinity to microsomes (IC_{50} 2 nM), stimulates Ca^{2+} release (EC_{50} 4 nM) by itself, and potentiates Ca^{2+}-induced Ca^{2+} release.

In summary, Ca^{2+} mobilization in longitudinal muscle is mediated by an IP_3-independent mechanism that is initiated by G_i-dependent activation of $cPLA_2$ and generation of arachidonic acid; the latter activates Cl^- channels and thus causes depolarization of the plasma membrane and opening of voltage-gated Ca^{2+} channels. Ca^{2+} influx through these channels induces Ca^{2+} release by activating sarcoplasmic reticulum ryanodine receptor–Ca^{2+} channels. Concomitant activation of membrane-bound ADP-ribosyl cyclase generates cADPR, which potentiates Ca^{2+}-induced Ca^{2+} release.

Regulation of cytosolic calcium at rest and during contraction

Resting and agonist-stimulated $[Ca^{2+}]_i$ in cells from the longitudinal or circular muscle layers are remarkably similar in various species (e.g., human, guinea pig, rabbit) [13,50–53]. Resting levels (70–100 nM) increase 2- to 3-fold during half-maximal contraction and 6- to 8-fold during maximal contraction. Exposure of permeabilized muscle cells to these concentrations of Ca^{2+} elicits degrees of contraction similar to those elicited by agonists in intact muscle cells.

Smooth muscle cells, like other cells, possess efficient mechanisms to dispose of the Ca^{2+} transients that occur during contraction. In the resting state, the cells maintain low concentrations of Ca^{2+} in the cytosol despite large chemical (e.g., 2 mM Ca^{2+} outside vs 100 nM Ca^{2+} inside the cell) and electrical (e.g., membrane potential of –40 to –80 mV) gradients favoring the movement of Ca^{2+} into the cell. The gradient for Ca^{2+} is maintained because of low permeability of the plasma membrane to Ca^{2+}, the presence of efficient Ca^{2+} extrusion mechanisms in the plasma membrane, and a Ca^{2+} uptake mechanism in the sarcoplasmic reticulum. The Ca^{2+} extrusion mechanisms in the plasma membrane include a calmodulin-dependent Ca^{2+},Mg^{2+}-ATPase, which acts as a high-affinity Ca^{2+} pump sustained by ATP hydrolysis that responds to Ca^{2+} concentrations, similar to those that occur during contraction, and a low-affinity, high-capacity Na^+/Ca^{2+} exchanger sustained by the Na^+ gradient across the plasma membrane, which responds to more drastic changes in cytosolic Ca^{2+} concentrations.

The Ca^{2+} uptake mechanism is a high-affinity *s*arco–*e*ndoplasmic *r*eticulum *C*a^{2+}-*A*TPase pump (SERCA2) that participates in dissipating the cytosolic Ca^{2+} transients during contraction. Most of the Ca^{2+} required to replenish sarco–endoplasmic Ca^{2+} stores after agonist stimulation of smooth muscle cells in the gut enters from the outside through capacitative Ca^{2+} channels and to a small extent (~ 20%) through voltage-gated Ca^{2+} channels.

Calcium-independent sustained contraction

The initial transient contraction mediated by Ca^{2+} is followed by a sustained contraction. The same receptors that initiate Ca^{2+} mobilization and MLC_{20} phosphorylation and contraction also engage a distinct G protein-dependent pathway that mediates sustained MLC_{20} phosphorylation and contraction. Thus, receptors coupled to G_q are also coupled to G_{13} and they trigger a cascade involving sequential activation of $G\alpha_{13}$ and RhoA guanine nucleotide exchange factor (Rho-GEF), which results in activation of RhoA (Fig. 6.6) [21–27]. Activated RhoA (Rho–GTP) is translocated to the plasma membrane where it activates both Rho kinase and PLD [27,54]. Hydrolysis of phosphatidylcholine by PLD yields phosphatidic acid, which is dephosphorylated to diacylglycerol, resulting in sustained activation of Ca^{2+}-dependent and -independent PKC isozymes (e.g., PKC-ε) (Figs 6.5 and 6.6) [25,55]. Rho kinase and PKC act cooperatively to inhibit MLC phosphatase activity.

The MLC phosphatase holoenzyme consists of three subunits including a 37-kDa catalytic subunit of type 1 phosphatase (PP1cδ) and a 110- to 130-kDa regulatory subunit, known as *myo*sin *p*hosphatase *t*arget subunit *1* or MYPT1; the latter is present in high concentrations in smooth muscle and its binding to the catalytic subunit enhances catalytic activity [21,22,24]. Phosphorylation of MYPT1 by Rho kinase fosters dissociation of the catalytic and regulatory subunits and inhibits catalytic activity. PKC, mainly PKC-ε and PKC-δ, phosphorylates CPI-17, a 17-kDa endogenous inhibitor of MLC phosphatase, greatly increasing its ability to inhibit MLC phosphatase (Fig. 6.6) [22,24,27]. Thus, a dual Rho-dependent mechanism causes sustained inhibition of MLC phosphatase and enables a Ca^{2+}-independent MLC kinase (probably *z*ipper-*i*nteracting *p*rotein kinase; ZIP kinase) to phosphorylate MLC_{20}.

The initial phase of contraction mediated by a Ca^{2+}/calmodulin-dependent MLC kinase and the sustained phase mediated by a Ca^{2+}-independent MLC kinase, whose potency is enhanced by concurrent inhibition of MLC phosphatase, are distinct and can be blocked separately. The initial phase is blocked by inhibitors of Ca^{2+} mobilization (inhibitors of PLC-β activity in circular muscle and $cPLA_2$ activity in longitudinal muscle), by calmodulin antagonists, and by Ca^{2+}/calmodulin-dependent MLC kinase inhibitors. The sustained phase is blocked by RhoA, Rho kinase, and PKC inhibitors.

G_i-coupled receptors activated by somatostatin, adenosine, and opioid peptides are not coupled to G_{13}, RhoA, or downstream effectors yet they can induce an initial Ca^{2+}-dependent and a sustained Ca^{2+}-independent contraction via $G\beta\gamma_i$. This $G\beta\gamma_i$ activates PLC-β3, which elicits a Ca^{2+}-dependent contraction, and cytoskeletal *i*ntegrin-*l*inked *k*inase (ILK) [22], which inhibits MLC phosphatase by phosphorylating CPI-17 and also acts directly as a Ca^{2+}-independent MLC kinase.

Regional differences in smooth muscle signaling

The mechanisms of Ca^{2+}-dependent and Ca^{2+}-independent contraction operate in smooth muscle of the stomach, small intestine, colon, and various sphincters, including the lower esophageal sphincter. Signaling for contraction in smooth muscle of the esophageal body is different and is dependent on Ca^{2+} influx but not on activation of MLC kinase; contraction appears to be mediated by PKC-ε and a Ca^{2+}-independent MLC kinase [56]. Although guinea pig taeniae coli are a condensation of longitudinal muscle, signaling in this tissue resembles that observed in circular muscle [57].

Mechanisms of relaxation by PKA and PKG

Regulation of cAMP and cGMP levels by cyclases, phosphodiesterases, and protein kinases

Inhibition of initial contraction (i.e., relaxation) results from inhibition of the initial increase in $[Ca^{2+}]_i$. Most agents cause relaxation by stimulating the production of cAMP (e.g., β-adrenergic agonists), cGMP (e.g., nitric oxide [NO]), or both cAMP and cGMP (e.g., vasoactive intestinal polypeptide [VIP] and its homologue pituitary adenylate cyclase-activating peptide [PACAP]), leading to the activation of PKA, PKG, or both [58–63]. The levels of cAMP and cGMP in gastrointestinal smooth muscle are regulated by the combined activities of cyclases (i.e., membrane-bound adenylate cyclase types V and VI and soluble guanylate cyclase) and phosphodiesterases (PDEs) [64–67]. Cyclic AMP, which is produced in amounts 10–15 times greater than cGMP, is rapidly degraded by cAMP-specific PDE3A and PDE4D5 [64,65]. Both enzymes are activated by PKA, but only PDE3A is inhibited by cGMP. cGMP is rapidly degraded by cGMP-specific PDE5, which is activated by PKG; when both cAMP and cGMP are present, PDE5 is also activated by PKA [65,66]. Each protein kinase selectively inhibits the activity of the corresponding synthetic enzyme: PKA inhibits adenylate cyclase and PKG inhibits soluble guanylate cyclase [64,66]. Thus, regulatory feedback from the protein kinases inhibits synthesis and accelerates degradation, thereby maintaining the levels of cyclic nucleotides within narrow ranges. The interplay of protein kinases, cyclases, and PDEs in the regulation of cyclic nucleotide levels in smooth muscle is depicted in Fig. 6.10.

Although cAMP preferentially activates PKA, it can, at higher concentrations (e.g., with high concentrations of relaxant agonists), also cross-activate PKG (Fig. 6.10) [62]. An increase in both cAMP and cGMP, such as that brought about by corelease of NO, VIP, and PACAP from the same or adjacent nerve terminals, is the physiological norm during nerve-induced relaxation in the gut. Inhibition of PDE3A by cGMP enhances cAMP levels, whereas activation of PDE5 by PKA and PKG attenuates cGMP levels. Autophosphorylation of PKG by cGMP greatly increases its affinity for the more abundant cAMP. Under these conditions, PKG is activated by both cGMP and cAMP [67,68].

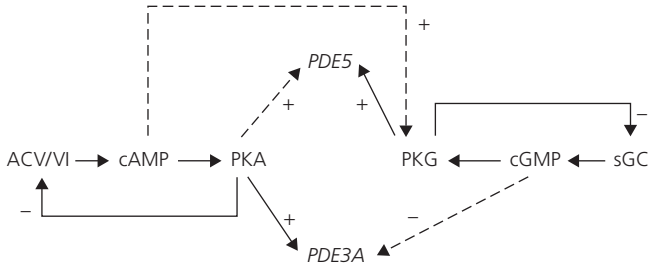

Figure 6.10 Interplay of adenylate and guanylate cyclases with cyclic nucleotide-dependent phosphodiesterases and protein kinases. Concurrent release of nitric oxide, vasoactive intestinal polypeptide, and pituitary adenylate cyclase-activating peptide is the physiological norm in gastrointestinal smooth muscle, leading to activation of adenylate cyclase type V/VI (ACV/VI) and soluble guanylate cyclase (sGC). This leads to concurrent generation of cyclic AMP (cAMP) and cyclic GMP (cGMP) and activation of both cAMP-dependent and cGMP-dependent protein kinases (PKA and PKG). PKA activates cAMP-specific phosphodiesterase 3 and 4 (PDE3 and PDE4) and inhibits adenylate cyclase activity, thus stimulating degradation and inhibiting further synthesis of cAMP. PKG activates cGMP-specific PDE5 and inhibits soluble guanylate cyclase activity, thus stimulating degradation and inhibiting further synthesis of cGMP. PDE5 is further activated by PKA, whereas PDE3 is inhibited by cGMP. Thus, concurrent generation of cAMP and cGMP leads to enhancement of cAMP levels (through inhibition of PDE3 by cGMP) and attenuation of cGMP levels (through activation of PDE5 by PKA). Furthermore, the affinity of cAMP for, and its ability to activate, PKG is greatly enhanced in the presence of cGMP. Because cAMP is present at 10–15 times higher levels than cGMP, it becomes the main activator of PKG. From Murthy et al. [64] (see also ref. 66).

Relaxation of Ca^{2+}-dependent contraction by PKA and PKG

Although both PKA and PKG decrease $[Ca^{2+}]_i$, they do so by different mechanisms. Common and distinctive cellular targets of PKA and PKG in smooth muscle are depicted in Fig. 6.11.

- Both protein kinases can inhibit Ca^{2+} mobilization by inhibiting IP_3 formation in circular muscle and arachidonic acid formation in longitudinal muscle. The mechanism of inhibition of IP_3 formation involves phosphorylation of RGS4 by either kinase, leading to more rapid degradation of $G\alpha_q$–GTP and inhibition of PLC-β1 activity.

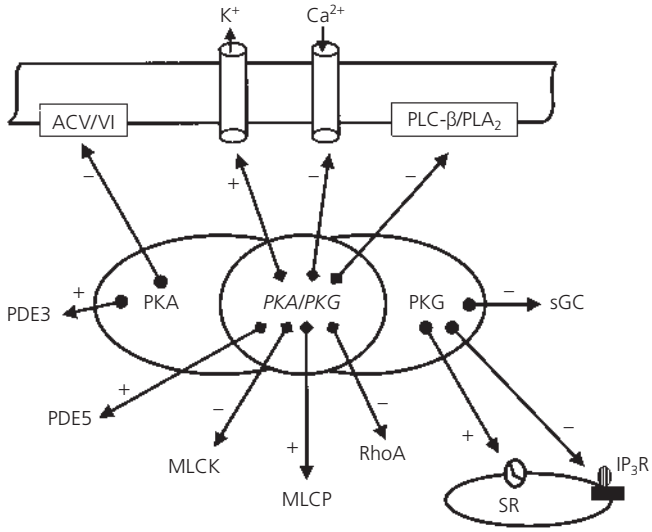

Figure 6.11 Distinct and shared cellular targets of cyclic AMP (cAMP)- and cyclic GMP (cGMP)-dependent protein kinases (PKA and PKG). Only PKA activates cAMP-specific phosphodiesterases 3 and 4 (PDE3 and PDE4) and inhibits adenylate cyclase type V/VI (ACV/VI), thus attenuating cAMP levels. Only PKG inhibits soluble guanylate cyclase (sGC) activity, stimulates Ca^{2+} uptake into the sarcoplasmic reticulum stores, and inhibits sarcoplasmic Ca^{2+} channels. Other targets are shared by both protein kinases. As shown, either one or both kinases decrease cytosolic Ca^{2+} levels by: (1) inhibiting the activity of phospholipase C-β (PLC-β) in circular muscle and PLA_2 in longitudinal muscle, thereby diminishing the synthesis of Ca^{2+}-mobilizing messengers; (2) inhibiting Ca^{2+} release from sarcoplasmic Ca^{2+} stores (SR) by phosphorylating inositol 1,4,5-triphosphate (IP_3) and ryanodine receptors/Ca^{2+} channels; (3) stimulating Ca^{2+} uptake into stores by activating the endoplasmic and sarcoplasmic Ca^{2+}-ATPase pump; (4) inhibiting the activity of plasmalemmal Ca^{2+} channels; and (5) stimulating the activity of plasmalemmal K^+ channels. The resultant hyperpolarization causes further inactivation of plasmalemmal Ca^{2+} channels and suppresses Ca^{2+} influx into the cell. Both PKA and PKG inhibit Ca^{2+}/calmodulin-dependent myosin light chain (MLC) kinase (MLCK) activity and stimulate MLC phosphatase (MLCP) activity, thereby decreasing MLC phosphorylation during the initial and sustained phases of contraction, and both kinases inhibit RhoA activity, thereby interrupting the pathway that mediates sustained contraction. The net effects of PKA and PKG are to inhibit muscle contraction and to induce relaxation.

- Only PKG can phosphorylate SERCA2 and thus accelerate Ca^{2+} reuptake into the stores [66].
- Both PKA and PKG can phosphorylate the IP_3 receptor in vitro or in permeabilized smooth muscle cells, but only PKG can phosphorylate the IP_3 receptor in vivo and thus inhibit IP_3-induced Ca^{2+} release, implying that PKA does not gain access to the IP_3 receptor under physiological conditions.
- Finally, both kinases inhibit the activity of membrane Ca^{2+} channels and stimulate the activity of membrane K^+ channels, leading to hyperpolarization of the plasma membrane and interruption of Ca^{2+} influx into the cell. This mechanism is important in the relaxation of rhythmic contractions.

In summary, both PKA and PKG can inhibit the formation of Ca^{2+}-mobilizing messengers, but only PKG can directly inhibit IP_3-induced Ca^{2+} release in circular muscle by phosphorylating the IP_3 receptor–Ca^{2+} channel. PKG probably inhibits Ca^{2+}-induced Ca^{2+} release in longitudinal muscle by phosphorylating the ryanodine receptor–Ca^{2+} channel.

Relaxation of Ca^{2+}-independent contraction

Inhibition of sustained contraction is chiefly mediated by phosphorylation of activated RhoA at Ser188 causing its inactivation and translocation back to the cytosol [69]. The importance of this mechanism is in its location at the beginning of the signaling pathway that mediates sustained contraction. Further downstream, PKA and PKG can phosphorylate MYPT1 at Ser695, which prevents phosphorylation of MYPT1 at an adjacent site by Rho kinase [70]. In addition, both kinases are able to phospshorylate and enhance the activity of telokin, an endogenous activator of MLC phosphatase [71].

Desensitization of receptor function

Signaling by G protein-coupled receptors is rapidly attenuated or terminated by mechanisms that target receptors or G proteins. Agonist-occupied receptors are phosphorylated by specific G protein-coupled receptor kinases (GRKs, usually GRK2 in smooth muscle), whose effect may be enhanced via feedback phosphorylation of GRK2 by second messenger-activated kinases such as PKA. Binding of the phosphorylated receptor to β-arrestin initiates internalization (endocytosis). β-arrestin targets the phosphorylated receptors to clathrin-coated pits and acts as a scaffold for various MAP kinases. The cytosolic tyrosine kinase, c-Src, which also binds to β-arrestin, phosphorylates a large GTPase, dynamin, and promotes its ability to cleave clathrin-coated vesicles from the membrane. Vesicles fuse with endosomes where receptors are either degraded or recycled to the cell surface.

Two mechanisms that target G proteins participate in desensitization of response; they involve RGS proteins and caveolin-3 [6,31,72]. Spontaneous deactivation of Gα subunits via their intrinsic GTPase activity is accelerated by RGS proteins (see Components of the transduction pathway). The activated G proteins can also bind to caveolin-3, which hinders the reassociation of Gα and Gβγ subunits and impedes the subsequent response mediated by a different receptor that couples to the same G protein.

Electrical properties of smooth muscle

Resting membrane potential

The *resting membrane potential*, defined as the steady state potential at which the net flow of current (i.e., ions) across the plasma membrane is zero, varies from about –40 to –80 mV

in muscle cells of the gut [73–77]. Graded differences in resting membrane potential exist between muscle cells in different regions, such as the fundus, corpus, and antrum of the stomach, and between muscle cells located at different depths in the same region, such as cells near the myenteric border and cells near the submucosal border of circular muscle in the antrum or colon.

The membrane potential is largely determined by the activity of the Na^+,K^+ pump (i.e., Na^+,K^+-ATPase), which sets up diffusion gradients for K^+ (162 mM inside vs 5 mM outside) and Na^+ (136 mM outside vs 14 mM inside) across the membrane. The permeability of the membrane to K^+ is much greater than the permeability to Na^+, and the flow of K^+ ions down their electrochemical gradient creates a diffusion potential that is the major contributor to the resting membrane potential. K^+ ions flow through passive K^+-selective channels that remain open at rest [78].

In addition to setting up ionic gradients, the Na^+,K^+ pump is electrogenic, moving three Na^+ ions out of the cell for every two K^+ ions into the cell; the net outward flow of positive charge can contribute up to 30 mV to the resting membrane potential. Variability in the direct contribution of this pump may account for regional differences in resting membrane potential. A Cl^- pump, which maintains low Cl^- concentrations (55 mM) in the cell, can contribute up to 10 mV to the resting membrane potential; its effect is partly offset by the tendency of Cl^- ions to diffuse out of the cell.

Gated ion-selective channels

In addition to passive ion-selective channels, the plasma membrane contains ion-selective channels that can be regulated by membrane potential (i.e., voltage-gated channels) and by various humoral, hormonal, or neural agents (i.e., agonist- or ligand-gated channels). Ligands can activate channels directly and through G proteins in the membrane. Ligands can also activate, inhibit, or modulate voltage-gated channels through second messengers.

Channels in the plasma membrane usually are selective for one ion, such as K^+ or Ca^{2+}, although some allow the passage of more than one ion. The two main types of ion channels involved in the regulation of rhythmic activity of smooth muscle of the gut are selective for K^+ or Ca^{2+}. The properties of Ca^{2+} and K^+ channels were first characterized in amphibian gastric muscle cells [79–81] and subsequently in muscle cells from various regions of the mammalian gut [82–86]. The flow of ions in single channels can be measured in small patches of plasma membrane [74,75,82,84]. The patches can be electrically isolated by suction into the tip of a micropipette, where they form a tight seal that makes it possible to record current flow in one or only a few channels in the patch. The patches can remain attached to the rest of the plasma membrane or can become fully detached such that the inner (i.e., inside-out patch) or outer (i.e., outside-out patch) surface of the membrane faces the external medium. Each configuration has its advantages: inside-out patches are useful for examining the role of intracellular messengers; outside-out patches are useful for examining the influence of extracellular ions; and patches in the whole cell configuration are useful for examining the effect of ligands and second messengers.

The use of patches has made it possible to characterize ion channels in terms of their ion selectivity, membrane density, activation and inactivation kinetics, voltage or ligand dependence, and dependence on changes in intracellular Ca^{2+}. The channels are electrically defined by their conductance (i.e., reciprocal of resistance), which is expressed in picosiemens (pS) as the amount of current flowing through the channel in response to an electrical gradient (i.e., current/voltage).

Voltage-gated calcium ion channels

Voltage-gated Ca^{2+} channels have been identified in muscle cells from the stomach and intestine of several mammals [84,86]. The channels carry the inward Ca^{2+} current responsible for the upstroke of the fast action potential. The channels are activated rapidly by depolarization of the plasma membrane to about −40 mV but are inactivated more slowly. Inactivation occurs as a result of Ca^{2+} influx and membrane depolarization. The voltage range of activation (−40 to −10 mV) overlaps with that of inactivation (−60 to 0 mV); at −40 to −50 mV, some channels remain open and can carry a steady inward Ca^{2+} current [87,88]. The potential at which such a current may flow is close to resting membrane potential in some muscle cells and usually is attained during the plateau phase of a slow wave.

Voltage-gated potassium ion channels

Several types of K^+ channel have been identified in gastric and intestinal smooth muscle [83–95]. The channels differ in their conductance, ranges of voltage activation, and Ca^{2+} sensitivities. The most widely distributed is a high-conductance (100 pS), Ca^{2+}-activated, voltage-sensitive K^+ channel [83,85,89–91]. During resting conditions, when $[Ca^{2+}]_i$ is low ($< 10^{-7}$ M), relatively few channels are open. On stimulation, the increase in $[Ca^{2+}]_i$ induces activation of large numbers of K^+ channels, which carry an outward current that drives the membrane potential to its resting state (i.e., the K^+ equilibrium potential). A stimulus that acts by inducing membrane depolarization and Ca^{2+} influx is thus terminated. A second voltage-sensitive K^+ channel with lower conductance (50 pS) has been identified; it opens up on prolonged depolarization, as occurs during the plateau phase of slow waves [84]. Other K^+ channels detected in gastrointestinal smooth muscle include delayed-rectifier, apamin-sensitive, and ATP-sensitive K^+ channels. An apamin-sensitive K^+ channel expressed in some human and guinea pig smooth muscles (chiefly taeniae coli) mediates the relaxant effect of PACAP in these tissues [92].

Rhythmic electrical activity of smooth muscle

Control of rhythmic electrical activity by Ca^{2+} and K^+ ion channels

Ca^{2+} channels and Ca^{2+}-activated K^+ channels constitute the electrical apparatus that sustains rhythmicity in smooth muscle. The Ca^{2+} sensitivity of K^+ channels links their activity to that of Ca^{2+} channels and creates the dynamic framework for rhythmic electrical activity. Activation of Ca^{2+} channels induces an inward flow of Ca^{2+} ions that depolarizes the membrane and increases $[Ca^{2+}]_i$. Depolarization and an increase in $[Ca^{2+}]_i$ inactivate the Ca^{2+} channels and activate the K^+ channels by inducing an outward flow of K^+ ions. Suppression of the inward flow of Ca^{2+} ions and enhancement of the outward flow of K^+ ions restore the resting membrane potential. The speed, amplitude, and duration of these cycles of depolarization and repolarization depend on the relative proportions of Ca^{2+} and K^+ channels, modulation by neural and humoral agents, participation of other voltage-gated channels, and coupling of muscle cells to each other and to pacemaker cells.

Fast action potentials

Only *fast action potentials* (i.e., spike potentials) occur in isolated muscle cells, spontaneously or after application of small depolarizing currents; slow waves are not seen [74,79,84,87]. Cell dispersion uncouples muscle cells from pacemaker regions responsible for slow wave activity and from other muscle cells in the syncytium. Small inward currents can cause rapid and complete depolarization of isolated muscle cells, with resulting maximal activation of Ca^{2+} channels and rapid influx of Ca^{2+}. The depolarization and substantial increase in $[Ca^{2+}]_i$ inactivate the Ca^{2+} channels and induce massive activation of the K^+ channels. The rapid depolarization and repolarization are characteristic of fast action potentials.

Fast action potentials lasting 0.1–0.2 s can occur in intact muscle [74–77]. They occur spontaneously in regions of the gut where the resting membrane potential is more positive than a threshold of −30 mV or where it can be raised above that threshold by neural stimulation. In other regions spike potentials occur only after the membrane has been depolarized by slow waves. Whether they occur spontaneously or are superimposed on slow waves, spike potentials are accompanied by muscle contraction. However, they are not essential for contraction, which can also be generated by changes in the amplitude and duration of the plateau potential of slow waves.

Interstitial cells of Cajal: electrical pacemakers and mediators of enteric neurotransmission

Interstitial cells of Cajal (ICC) are a distinctive population of enteric cells that, depending on their location, are either spindle-shaped muscle-like cells or stellate cells [96,97]. Both cell types express c-*kit*, the protooncogene that encodes the receptor tyrosine kinase c-kit; the latter is activated by its natural ligand, stem cell factor or steel factor, and its activation is required for normal development of the cells. The availability of c-kit antibody has enabled immunohistochemical analysis of ICC distribution and morphology and functional analysis using neutralizing concentrations of antibody. Double immunohistochemical labeling for enteric neurotransmitters and c-kit has shown ICC to be closely associated with enteric nerve fibers (~ 25 nm), compared with greater distances between nerve fibers and smooth muscle cells (50–100 nm) [98]. The closeness of nerve fibers to ICC, and ICC to smooth muscle cells led Cajal to propose that ICC were functionally interposed between nerve terminals and smooth muscle cells.

Stellate ICC located near the myenteric plexus at the interface of circular and longitudinal muscle (designated IC-MY) and similar cells located at the interface of circular muscle and submucosa in the colon are pacemaker cells responsible for generating rhythmic activity [99–101]. Intramuscular, spindle-shaped ICC (designated IC-IM) located within the musculature of the esophagus, stomach, colon, and various sphincters, and morphologically similar ICC located in the deep muscular plexus of the small intestine (designated IC-DMP) have been implicated in enteric neurotransmission [97,99]. These distinct classes of ICC, first identified in the mouse, are also present in other species.

Evidence for pacemaker function of ICC was first obtained in dispersed ICC from the pacemaker region of the canine colon [102]. The cells were spontaneously active, generating slow-wave depolarizations similar to those observed in situ (see section on slow waves). In the intestine of the mutant W/W^v mouse, which is devoid of intestinal IC-MY but retains IC-DMP, intestinal rhythmic activity is absent [103,104] whereas enteric neurotransmission in the form of excitatory and inhibitory junction potentials is retained, providing clear evidence that IC-MY in the intestine are responsible for pacemaker activity (generation of slow waves) whereas IC-DMP are responsible for enteric neurotransmission. In the stomach of the W/W^v mouse, which is devoid of IC-IM (functionally similar to intestinal IC-DMP), enteric neurotransmission (identified by junction potentials) is greatly inhibited [97]. Detailed analysis of rhythmic electrical activity suggests that pacemaker waves initiated in IC-MY are conducted passively to IC-IM and from there to smooth muscle; when recorded in smooth muscle, slow wave activity reflects these sequential, combined processes. Viewed in this light, IC-IM facilitate the transmission of rhythmic activity initiated in pacemaker IC-MY and mediate neural activity initiated by enteric nerves. The regulation of muscle tone, however, is not mediated by changes in smooth muscle membrane potential (except in depolarized muscle) but by signaling cascades initiated by enteric neurotransmitters acting directly on smooth muscle receptors.

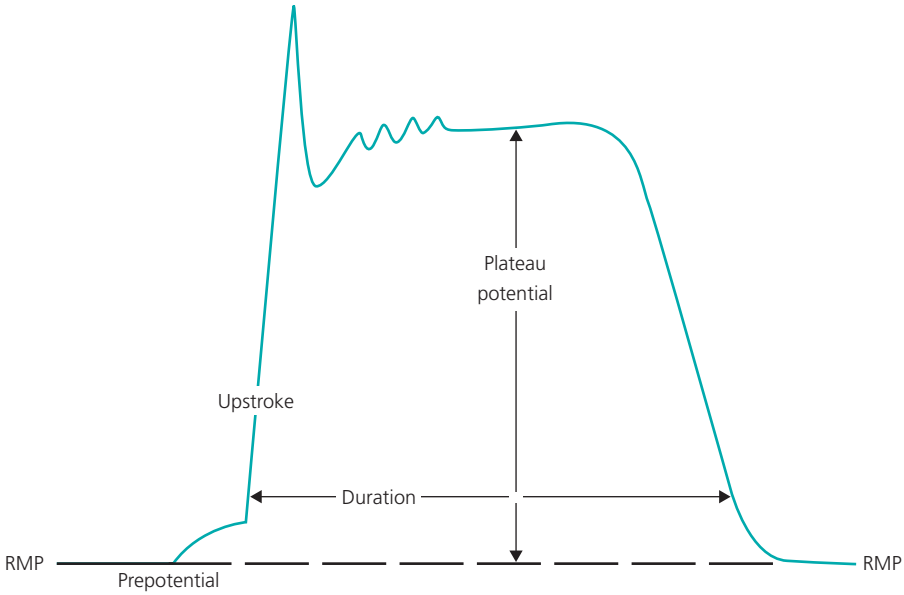

Figure 6.12 Profile of a typical slow wave. A slight depolarization (i.e., prepotential) of the resting membrane potential (RMP) precedes and may trigger slow waves in some regions. A rapid upstroke is followed by partial repolarization, a plateau potential of variable duration on which may be superimposed small oscillations or spike potentials, and complete repolarization.

Slow waves

Profile

A typical *slow wave* consists of the following sequence: rapid depolarization (i.e., upstroke), partial repolarization, a sustained plateau lasting several seconds, and complete repolarization to the resting membrane potential (Fig. 6.12) [76,77].

The amplitudes, durations, and frequencies of slow waves vary with the location of the muscle [81]. Frequency decreases aborally in human and canine stomach (i.e., 3–5 cycles/min in the corpus to 1.5 cycles/min in the antrum) and intestine (i.e., 12 cycles/min in the duodenum, 7 cycles/min in the ileum, and 5–6 cycles/min in the proximal colon). The frequency gradient is continuous and intrinsic to each region; segments of intestine obtained sequentially along the main axis oscillate at progressively decreasing frequencies. In the canine stomach, the decreasing gradient in frequency is accompanied by an increasing gradient both in the resting membrane potential (i.e., –51 mV in the corpus to –71 mV in the antrum) and in the duration of the plateau potential (i.e., 5 s in the corpus to 20 s in the antrum).

Gradients in resting membrane potential and in the amplitude of plateau potential also occur in the transverse direction within the thickness of circular muscle [76]. The decrease in the amplitude of the plateau potential in the transverse direction reflects the decay of slow waves with increasing distance from the main pacemaker regions at the boundaries of circular muscle.

Site of origin

As noted above, slow waves originate in pacemaker regions located at the myenteric and submucosal borders of circular muscle [75–77]. The ICC network in these regions initiates rhythmic electrical activity [96–110].

Propagation

Slow waves originating in pacemaker regions propagate rapidly around circular muscle and throughout its thickness in the transverse and long axes of the gut. Propagation is rapid and is facilitated by the network of intramuscular ICC (IC-IM) and the abundance of gap junctions between muscle cells. Slow waves originating in the myenteric pacemaker region of circular muscle (IC-MY) also spread to the longitudinal muscle [105,106,110–114].

After spreading circumferentially, slow waves propagate in oral and aboral directions as discrete rings of excitation capable of eliciting segmental contractions. Propagation in only one direction (i.e., oral or aboral) can occur if the frequency gradient is steep and if the conduction velocity is fast enough to allow slow waves originating in one segment to set the pace of slow waves in another segment [83] or if inhibitory neural input to one segment limits propagation to that segment [112]. Inhibitory neural input reaching muscle cells directly or via intramuscular ICC (IC-IM) hyperpolarizes the resting membrane potential and prevents the occurrence of a slow wave, or it decreases the plateau potential and prevents the development of a contraction. Inhibitory neural input reflecting the corelease of NO, VIP, and related neuropeptides appears to predominate normally, masking rhythmic electrical and contractile activity [112].

Ionic mechanisms

Pacemaker ICC possess distinct channels responsible for initiation of slow waves and their propagation to electrically coupled smooth muscle cells. The mechanism appears to involve a localized decrease in $[Ca^{2+}]_i$ that causes the opening of nonselective cationic channels in ICC [101]. The decrease in $[Ca^{2+}]_i$ is preceded by release of Ca^{2+} from sarcoplasmic stores

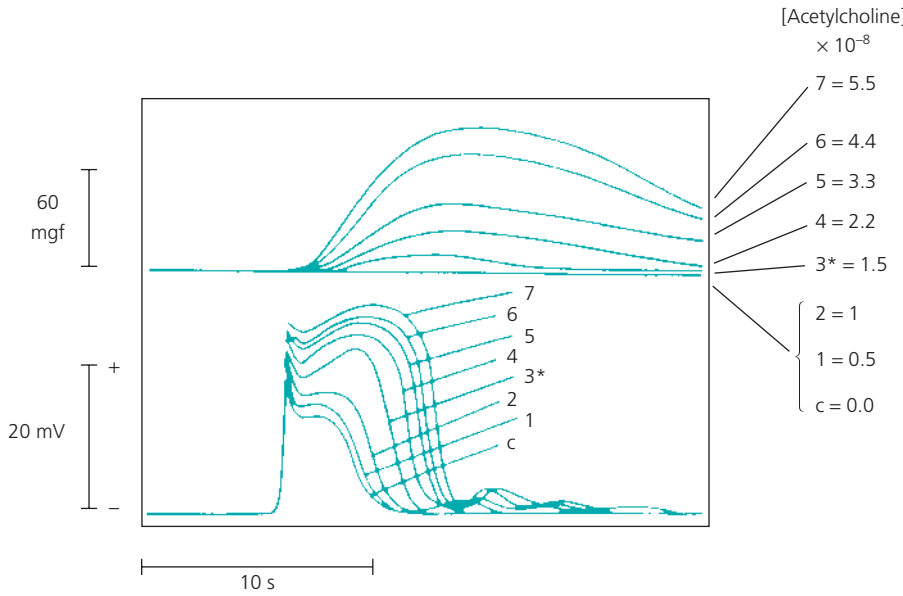

Figure 6.13 Relation between the amplitude and duration of the plateau potential **(lower panel)** and the corresponding contraction **(upper panel)** of longitudinal muscle of canine antrum in response to acetylcholine. From Szurszewski [106].

via IP_3 type I receptors followed by Ca^{2+} uptake into mitochondria to create a zone of low $[Ca^{2+}]_i$ below the plasma membrane. The involvement of IP_3 receptors is based on the fact that mice lacking these receptors do not exhibit rhythmic activity. The precise trigger for Ca^{2+} release via IP_3 receptors in the absence of agonist is unknown. Ca^{2+} influx via the nonselective cationic channels appears to be involved, because the upstroke potential is reduced in the absence of extracellular Ca^{2+}. The plateau potential corresponds to the level at which Ca^{2+} channels are activated, and its amplitude in smooth muscle reflects the combined activity of voltage-gated Ca^{2+} and K^+ channels. Ca^{2+}-activated K^+ channels appear to be the main type of K^+ channel involved in repolarization of the membrane.

Role in phasic contraction

The amplitude and duration of the plateau potential determine the magnitude of Ca^{2+} influx through voltage-gated Ca^{2+} channels and can be modulated by excitatory and inhibitory neurotransmitters acting either directly on muscle cells or via intramuscular ICC (IC-IM) coupled electrically to muscle cells (Fig. 6.13). Excitatory neurotransmitters, such as acetylcholine or tachykinins, increase the amplitude and duration of the plateau potential and cause a concentration-dependent increase in $[Ca^{2+}]_i$ and contraction [106,115,116]. The increase in Ca^{2+} is accentuated by Ca^{2+} release from Ca^{2+} stores. Spike potentials can be superimposed on plateau potentials and can further augment $[Ca^{2+}]_i$ and contraction. In some regions, such as the distal antrum and inner lamella of circular muscle in the small intestine, spike potentials appear to be necessary for contraction [75,77,111,117]. Inhibitory neurotransmitters (e.g., NO, VIP, PACAP) decrease the amplitude and duration of the plateau potential or reduce the frequency of spike potentials and prevent the development of contraction associated with a slow wave. These neurotransmitters act predominantly by activating PKA and PKG, which inhibit Ca^{2+} channel activity and stimulate K^+ channel activity (Fig. 6.11).

Regional patterns of contractile activity

The magnitude of the resting membrane potential, the form, frequency, and site of origin of slow waves, the occurrence and frequency of spike potentials, and the extent of excitatory or inhibitory neural input can be correlated with the neuromuscular function of various regions of the gut. The pattern in humans parallels the pattern observed in dogs.

Stomach

The proximal-to-distal gradient in the resting membrane potential of circular smooth muscle in the stomach is depicted in Fig. 6.14. The membrane potential in the orad segment or fundus is low, about −50 mV, and lies near or above the threshold for the opening of Ca^{2+} channels and contraction [75,118]. The segment normally is tonically contracted and is devoid of rhythmic electrical activity. Small changes in stimulatory (i.e., depolarizing) or inhibitory (i.e., hyperpolarizing) neural input can cause further contraction or can induce relaxation of the fundus, features that render this tonic segment suitable for receiving (i.e., receptive relaxation) and discharging (i.e., tonic contraction) a meal into the middle segment or corpus of the stomach [118].

The corpus is the site of spontaneous pacemaker activity in the stomach and has a higher resting membrane potential.

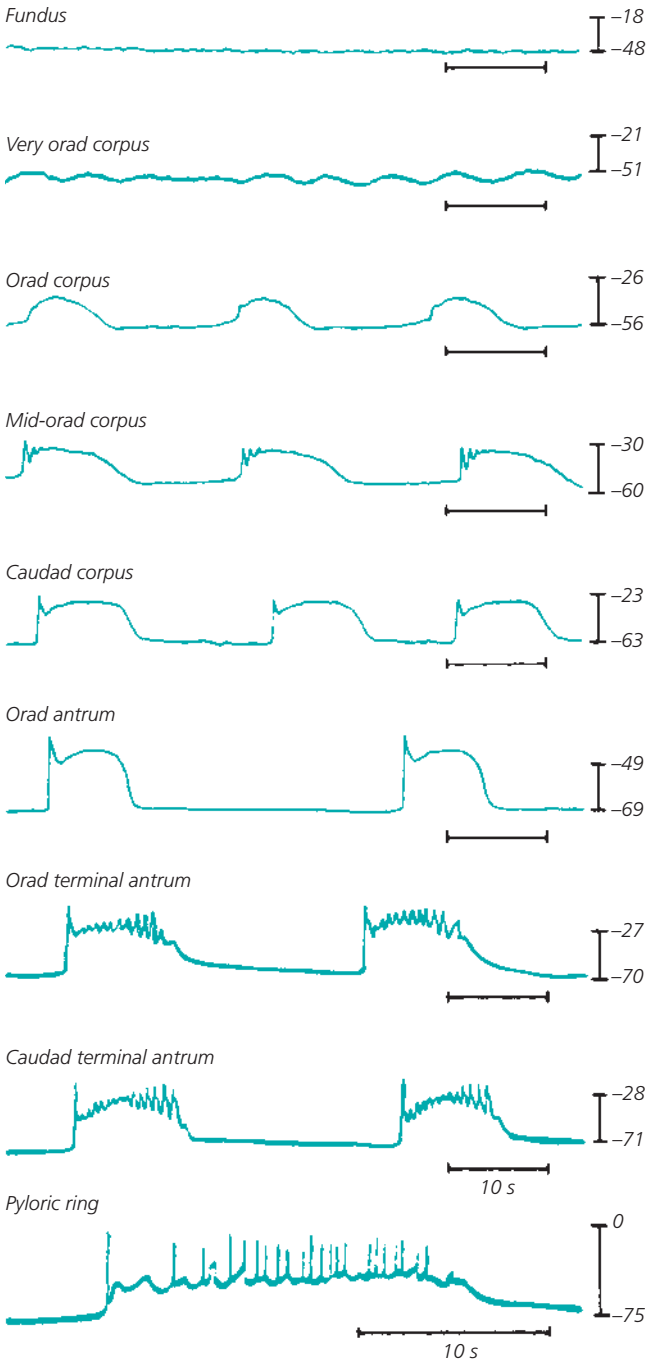

Figure 6.14 Gradients in resting membrane potential and profile of slow waves in various regions of the canine stomach recorded with intracellular electrodes. From the proximal to the distal part of the stomach, the resting membrane potential becomes increasingly negative and the duration of slow waves becomes increasingly longer; potential oscillations and spike potentials are evident in the distal stomach and pylorus. From Szurszewski [75].

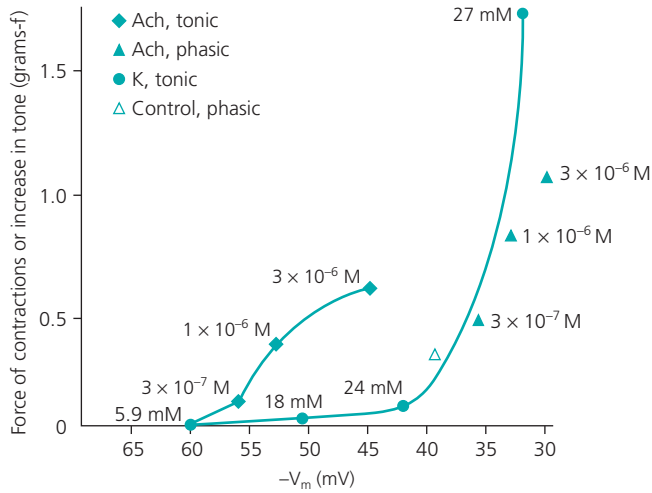

Figure 6.15 Comparative effects of acetylcholine (Ach) and extracellular K^+ on membrane potential and contraction in circular muscle from the corpus of the canine stomach. Acetylcholine caused an increase in tonic contraction; the depolarization induced by acetylcholine does not exceed the threshold for opening of Ca^{2+} channels, implying that the source of Ca^{2+} for agonist-induced tonic contraction is intracellular. During slow wave activity, the magnitude of phasic contraction in response to acetylcholine correlates with the amplitude of the plateau potential. Contraction induced by extracellular K^+ results from depolarization and an influx of Ca^{2+} and coincides with the pattern of phasic contraction induced by acetylcholine. From Szurszewski [75].

The corpus can undergo both tonic and phasic (i.e., rhythmic) contraction. The initial phase of tonic contraction induced by excitatory neurotransmitters is mediated by the release of intracellular Ca^{2+}; the concomitant decrease in the resting membrane potential is not sufficient to activate Ca^{2+} channels and cause Ca^{2+} influx (Fig. 6.15). Phasic contraction of the corpus is determined by the amplitude and duration of the plateau potential. Small changes in amplitude or duration of the plateau potential caused by excitatory neurotransmitters result in substantial changes in the magnitude of phasic contraction.

Intrinsic pacemaker rhythm is highest in the orad corpus (5 cycles/min) and decreases progressively throughout the rest of the corpus, antrum, and pylorus. Slow waves originating in the corpus propagate to and pace antral muscle. Slow waves originating in the distal antrum and pyloric sphincter have prolonged plateau potentials on which spike potentials are usually superimposed.

As a whole, the antrum has little tone and is best suited for propagation of slow waves and contractions originating in the corpus [75,116,117]. Antral slow waves propagate aborally to the pyloric sphincter, where they pace longitudinal muscle and the outer layer of circular muscle. The slow waves decay before reaching muscle cells in the inner layer of circular muscle; muscle cells in this region are electrically quiescent and may be responsible for the intrinsic tone of the sphincter. Opening of the sphincter is mediated by an inhibitory (i.e., relaxant) neural reflex triggered by distention of the distal antrum; distention occurs when the gastric contents are propelled aborally by phasic or peristaltic

contractions originating in the pacemaker region in the corpus of the stomach.

Small intestine

In most mammals, including humans, there is a decreasing gradient in slow wave frequency from the duodenum to the ileum. Slow waves originate in pacemaker ICC (IC-MY) located at the myenteric border of circular muscle from where they propagate to the bulk of circular muscle and to longitudinal muscle [110]. Removal of a thin layer at the myenteric border of circular muscle abolishes slow waves in longitudinal muscle and in the bulk of circular muscle. Propagation around and in the long axis of the intestine occurs preferentially through circular muscle.

The configuration of slow waves in the small intestine is similar to that in the corpus of the stomach and consists of a rapid upstroke followed by a sustained plateau potential. Excitatory and inhibitory neural inputs influence the amplitude of the plateau potential and the frequency of spike potentials, and determines the occurrence and magnitude of phasic contraction in the intestine [111].

Colon

There is a decreasing gradient in resting membrane potential across circular muscle of the canine colon from −80 mV in muscle cells at the submucosal border to −45 mV in muscle cells at the myenteric border (Fig. 6.16). Rhythmic electrical activity is mediated by two pacemaker regions, one at the myenteric border (IC-MY) and the other at the submucosal border (IC-SM). Each pacemaker region generates slow waves with distinctive forms and frequencies that spread passively and summate in the bulk of circular muscle to yield waves of mixed form [76,106,120,121].

Slow waves originating at the submucosal border have a frequency of 5–6 cycles/min, a configuration similar to that found in the small intestine and corpus of the stomach, and a plateau potential lasting 3–15 s (Fig. 6.16) [76]. The amplitude of the plateau potential decreases as the waves spread toward the myenteric border; the decrease is matched and offset by the decrease in resting membrane potential such that the plateau potential is maintained at about −45 mV, which is close to the threshold for mechanical activity (i.e., threshold for Ca^{2+} influx).

Slow waves originating at the myenteric border of circular muscle have a frequency of 17 cycles/min and a sinusoidal configuration [76,106,120,121]. These oscillatory waves spread to and pace muscle cells in the longitudinal muscle layer. They also spread in circular muscle toward the submucosal border, their amplitude decreasing as a function of distance from the myenteric border. The waves summate with waves originating at the submucosal border; the encounter boosts the plateau potential of waves that originate at the submucosal border and elicits contractions at the rate of 6 per min. In longitudinal muscle, myenteric potential oscillations generate fast action potentials. The frequency of these fast transients and of longitudinal muscle contractions is regulated by neural input from the enteric nervous system. Fast action potentials are not generated in circular muscle and only rarely propagate from longitudinal to adjacent circular muscle cells.

Stimulus–contraction coupling in syncytia: tonic and phasic contraction

The signal transduction pathways described in isolated circular and longitudinal muscle cells regulate tonic contraction and relaxation of intact, syncytial muscle. Small depolarizations caused by excitatory neurotransmitters are not sufficient to cause tonic contraction, except in some regions of the gut, such as the fundus of the stomach; there the resting membrane potential is close to the mechanical

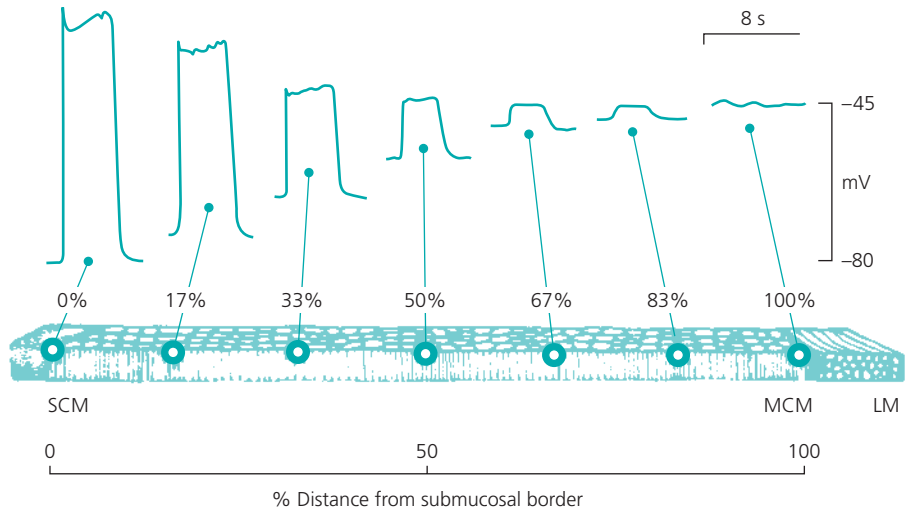

Figure 6.16 Records of slow waves obtained at various depths from the submucosal border of circular muscle in canine colon. Two gradients are evident: a decline in the amplitude of the plateau potential from the submucosal (SCM) to myenteric (MCM) border is matched and offset by a decrease in the resting membrane potential. The plateau potential during slow wave activity attains or exceeds the threshold potential throughout the thickness of circular muscle. Slow waves of a different configuration originate at the myenteric border; as they propagate in circular muscle, they summate with slow waves originating in the submucosal border. From Smith et al. [119].

threshold, which is the membrane potential at which Ca^{2+} channels are presumed to open. In circular muscle of the intestine and corpus of the stomach, tonic contraction induced by excitatory neurotransmitters occurs at membrane potentials more negative than the mechanical threshold, implying that the source of Ca^{2+} responsible for initial tonic contraction in these regions is intracellular (Fig. 6.15) [110]. However, when circular or longitudinal muscle is depolarized by a slow wave, relatively small changes in the plateau potential imposed by the effect of an excitatory neurotransmitter are sufficient to induce Ca^{2+} influx and contraction. The contraction is phasic, coinciding with and determined by the amplitude of the plateau potential; mobilization of intracellular Ca^{2+} by the excitatory neurotransmitter amplifies the phasic contraction.

As previously noted, inhibitory (i.e., relaxant) neurotransmitters act through PKA or PKG to decrease $[Ca^{2+}]_i$ (Fig. 6.11). They do so by inhibiting Ca^{2+} release and enhancing sequestration of Ca^{2+} in intracellular stores [60,62], mechanisms that are well suited to the relaxation of tonic contraction, or by hyperpolarizing the plasma membrane during slow wave activity and reducing Ca^{2+} influx, a mechanism well suited to the relaxation of phasic contraction (Fig. 6.11). The hyperpolarization is mediated by inhibitory phosphorylation of voltage-gated Ca^{2+} channels and stimulatory phosphorylation of voltage-gated, Ca^{2+}-dependent K^+ channels. The hyperpolarization determines the profile or occurrence of slow waves and thus determines the occurrence of phasic contractions. It has traditionally been labeled the inhibitory junction potential because it leads to inhibition of phasic electrical and contractile activity.

Neural regulation of smooth muscle by the myenteric plexus

The intrinsic electrical and mechanical properties of smooth muscle are modulated by neurotransmitters released from neurons of the enteric nervous system, especially neurons of the myenteric plexus. These neurons constitute the final neural pathway regulating smooth muscle activity. Neurons of the submucosal plexus innervate the innermost layers of circular muscle in large species, such as humans and dogs.

Two populations of sensory neurons have been identified. The first, activated by mucosal stimuli, is wholly intrinsic, and the second, activated by muscle stretch and painful stimuli, has neuronal cell bodies in the dorsal root ganglia. Extrinsic neurons of the sympathetic and parasympathetic systems influence smooth muscle indirectly by acting on neurons of the myenteric plexus. Adrenergic and peptidergic neurons in prevertebral and paravertebral ganglia synapse with and inhibit the activity of cholinergic and noncholinergic neurons of the myenteric and submucosal plexuses.

Neuronal topography

The neural organization of the enteric nervous system is well conserved in mammals and has been extensively studied in guinea pigs, rats, dogs, and humans (see Chapter 2) [122–126]. Neurons in the myenteric plexus synapse with neurons in the myenteric and submucosal plexuses and paravertebral ganglia and innervate cells in the circular and longitudinal muscle layers. The fibers that make up the deep plexus close to the submucosal border of circular muscle are derived from neurons of the myenteric and submucosal plexuses in large species.

Advances in immunocytochemical and imaging techniques have made it possible to map these neurons and to correlate their morphological, electrophysiological, and neurochemical properties, specifically their content of neurotransmitters. Neurons of the myenteric plexus fall into two broad categories: about 25% contain VIP or PACAP together with NO synthase (NOS), the enzyme responsible for synthesis of NO in nerve terminals; about 60% contain acetylcholine, usually together with the tachykinins, substance P and neurokinin A (NKA). There is virtually no overlap between these categories of neurons [122–128]. VIP neurons also contain a homologous peptide, designated PHM (i.e., *p*eptide with N-terminal *h*istidine and C-terminal *m*ethionine) in humans and PHI in animals, which is derived from the same precursor, pro-VIP. Substance P neurons contain NKA, also known as substance K, which is derived from the same precursor, β-protachykinin. The two main categories of neurons correspond to major roles for acetylcholine and the tachykinins as excitatory neurotransmitters, and for VIP, its homologues, and NO as inhibitory neurotransmitters.

Excitatory neurotransmitters stimulate Ca^{2+} release and increase muscle tone, and depolarize the plasma membrane (i.e., trigger *excitatory junction potentials or EJPs*). The depolarization can result from a direct action on muscle cells or may be relayed via intramuscular ICC (IC-IM) coupled electrically to muscle cells. EJPs can induce Ca^{2+} influx and contraction if the depolarization attains a level at which voltage-gated Ca^{2+} channels are open; this is likely to happen in tonic smooth muscle when the membrane potential is close to the Ca^{2+} threshold, or in phasic smooth muscle during slow wave activity. Inhibitory neurotransmitters inhibit Ca^{2+} release and decrease (i.e., relax) muscle tone, and hyperpolarize the plasma membrane directly or via IC-IM (i.e., trigger *inhibitory junction potentials or IJPs*). IJPs lower the plateau potential of slow waves and inhibit Ca^{2+} influx and phasic contraction.

Subpopulations of neurons in the two main categories contain one or more of the following: bombesin, also known as gastrin-releasing peptide; neuropeptide Y; the opioid peptides, dynorphin, [Met]enkephalin, and their derivatives; and galanin. A few neurons contain γ-aminobutyric acid (GABA), serotonin (< 3%), or somatostatin (< 5%). Neurons that contain serotonin or somatostatin act mainly as

interneurons and influence smooth muscle cells indirectly by means of other neurons.

Peptide and other neurotransmitters

Pharmacological profile

Peptide and nonpeptide neurotransmitters are released from axonal varicosities near muscle cells. They diffuse across distances ranging from 50 to 100 nm to interact with receptors located on muscle cells and on the same (autoreceptors) or adjacent nerve terminals. The presence of receptors on muscle cells and the possibility of direct action can be determined in isolated muscle cells by measuring the binding of specific radioligands, the release of intracellular messengers (e.g., $[Ca^{2+}]_i$, IP_3, or cyclic nucleotides), or the mechanical response of the cell (i.e., contraction or relaxation) [129,130]. Determining the presence of receptors on neurons or their terminals and the possibility of indirect, neurally mediated action of neurotransmitters on muscle cells requires the use of innervated muscle strips; the neurally mediated component of a mechanical response is identified by its sensitivity to blockade by neurotoxins [129–135].

Direct and neurally mediated response

When given exogenously, peptide and nonpeptide neurotransmitters can cause contraction or relaxation of smooth muscle cells directly or indirectly by stimulating or inhibiting the release of other excitatory or inhibitory neurotransmitters. Bombesin and CCK, for example, cause contraction in isolated muscle cells and innervated muscle strips; in the latter, they also elicit release of acetylcholine and substance P [132–135]. Their effect in strips is in part neurally mediated and can be partially blocked by the axonal blocker tetrodotoxin or by a combination of muscarinic and tachykinin antagonists.

Linkage between the inhibitory neurotransmitters VIP/PACAP and NO

VIP, PACAP, PHI, and PHM cause relaxation of isolated muscle cells and muscle strips [54–58,62,136,137]. The effects of PHI and its human counterpart PHM are mediated by cAMP, whereas the effects of VIP and PACAP are mediated by both cAMP and cGMP [48,49,52]. The increase in cGMP is the result of stimulation of NO production in smooth muscle cells by VIP and PACAP [57,61,62]. A unique interplay exists between VIP or PACAP and NO in smooth muscle of the gut. NO produced in nerve terminals regulates VIP and PACAP release from the same or adjacent nerve terminals (Fig. 6.17). The release of VIP and PACAP from these terminals regenerates NO in target muscle cells (Fig. 6.18; see Fig. 6.17) [58,61,62,138–145].

The interplay between VIP or PACAP and NO is present in various regions of the gut in mammalian species (human, dog, opossum, rabbit, guinea pig, rat, and mouse). In isolated myenteric ganglia devoid of muscle cells, the nicotinic agonist, dimethylphenylpiperazinium, stimulates NO production and VIP release; NOS inhibitors abolish both NO production and VIP release, implying a dependence of VIP release on NO production [138,139]. Exogenous NO causes VIP release from these ganglia, but exogenous VIP has no effect on NO production [138]. Studies in synaptosomal membranes confirm that NO donors stimulate VIP release, whereas VIP does not stimulate NO production [146]. In isolated muscle cells devoid of neural elements, VIP and PACAP, but not the homologous peptides PHI, PHM, or secretin, stimulate NO production [57,61,139,140].

The processes that occur in neurons and muscle cells are evident during nerve stimulation of muscle strips [57,61,139,141]. NOS inhibitors abolish NO production induced by all neural stimuli, and they also abolish VIP

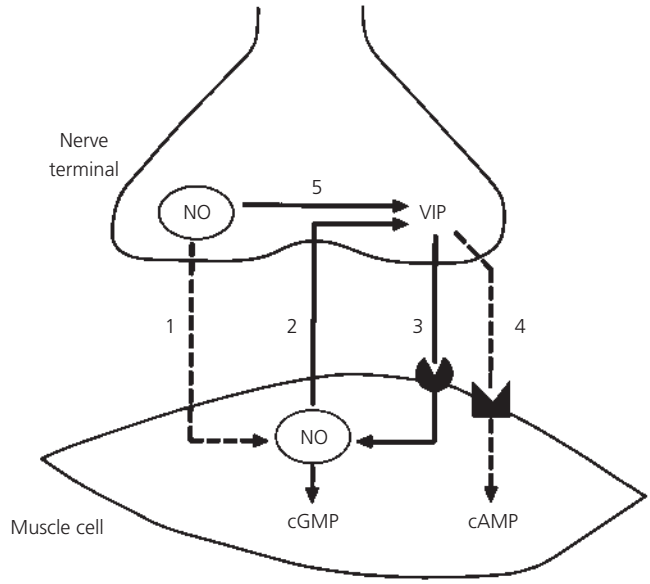

Figure 6.17 Interplay of vasoactive intestinal polypeptide (VIP) and nitric oxide (NO) in the regulation of smooth muscle relaxation. VIP and neuronal NO synthase are often located in the same enteric neurons. NO produced in nerve terminals regulates VIP release (5) and diffuses to muscle cells (1). VIP released from nerve terminals interacts with one class of natriuretic peptide receptors (NPR-C) coupled to an endothelial-type NO synthase in muscle cells, leading to the production of NO and cyclic GMP (cGMP) (3). NO produced in muscle cells diffuses to nerve terminals where it facilitates VIP release (2). VIP also interacts with $VPAC_2$ receptors (previously known as VIP_2 or $PACAP_3$ receptors) coupled to adenylate cyclase to generate cyclic AMP (cAMP) (4). The amount of NO produced in muscle cells (60%–80%) is much larger than that produced in nerve terminals (20%–40%). The linkage between NO production and VIP release and the dual sources of NO explain why NO synthase inhibitors are potent inhibitors of NO production, VIP release, and relaxation, and why the VIP antagonist $VIP_{10–28}$ is a potent inhibitor of NO production and relaxation. Oxyhemoglobin neutralizes extracellular NO and blocks only pathways 1 and 2. Identical pathways link the release of pituitary adenylate cyclase-activating peptide (PACAP) and NO; PACAP interacts with the same two classes of receptors as VIP.

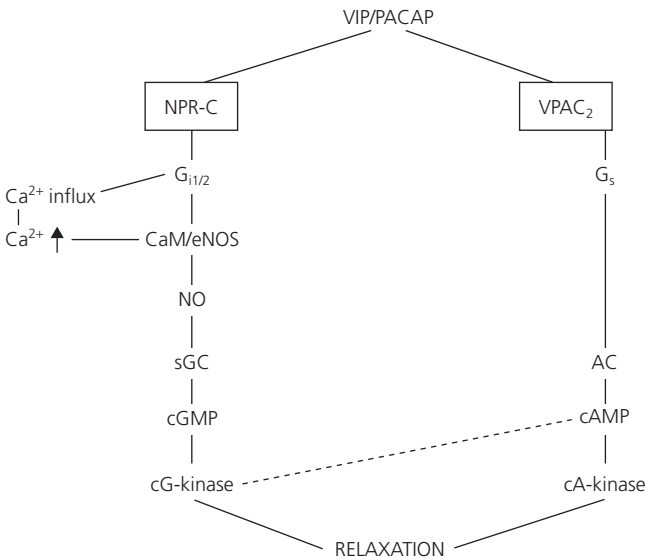

Figure 6.18 Dual signaling cascades initiated by the interaction of vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase-activating peptide (PACAP) with two classes of receptors. VIP and PACAP interact with $VPAC_2$ receptors (previously known as VIP_2 or $PACAP_3$ receptors) coupled by G protein G_s to activation of adenylate cyclase (AC), formation of cyclic AMP (cAMP), and activation of cAMP-dependent protein kinase (PKA); high agonist concentrations generate higher levels of cAMP that can also cross-activate cyclic GMP (cGMP)-dependent protein kinase (PKG; dotted line). VIP and PACAP interact with one class of natriuretic peptide receptors (NPR-C) coupled by G proteins G_{i1} and G_{i2} to stimulation of Ca^{2+} influx into the cell and activation of a constitutive Ca^{2+}/calmodulin (CaM)-dependent isoform of nitric oxide synthase (endothelial type NOS [eNOS]). The resultant increase in nitric oxide (NO) activates soluble guanylate cyclase (sGC), stimulates cGMP formation, and leads to activation of PKG. Both kinases act concurrently to cause relaxation and hyperpolarization of smooth muscle cells. cG-kinase, PKG; cA-kinase, PKA. Adapted from Murthy et al. [61] and Murthy and Makhlouf [147].

release induced by low stimuli and partially inhibit VIP release induced by intense stimuli, indicating that VIP and PACAP release is mediated by NO but can occur independently of it during intense nerve stimulation [139]. The VIP antagonist, $VIP_{10–28}$, which blocks the postjunctional effects of VIP on muscle cells, inhibits NO production by over 60%, indicating that VIP- and PACAP-induced NO production in muscle cells is a major source of NO [139,142]. The effectiveness of NOS inhibitors in blocking muscle relaxation induced by nerve stimulation has led some investigators to conclude incorrectly that relaxation of muscle tone is exclusively mediated by NO released from nerve terminals. NOS inhibitors elicit their potent effects by acting at several locations: (1) they suppress NO production in nerve terminals and muscle cells, and (2) they suppress NO-mediated VIP and PACAP release from nerve terminals, thereby eliminating the postjunctional effects of peptide neurotransmitters.

VIP or PACAP induce relaxation by interacting with two distinct receptor types (Fig. 6.18): $VPAC_2$ receptors, which possess equal affinity for VIP and PACAP (previously known as VIP_2 or $PACAP_3$ receptors) and which are coupled through G_s to the activation of adenylate cyclase; and type C natriuretic peptide receptors (NPR-C), which are coupled through G_{i1} and G_{i2} to activation of NOS in smooth muscle cells. $VPAC_2$ receptors are also recognized by PHI and PHM but with lesser affinity, whereas NPR-C receptors are recognized by VIP and PACAP, as well as by natriuretic peptides [61,143]. The distinctive feature of NPR-C is the presence of a truncated 37-amino acid intracellular domain devoid of kinase or guanylate cyclase activity. Site-directed mutagenesis and studies using peptide fragments derived from the intracellular sequence of NPR-C have identified a 17-amino acid intracellular segment that determines the ability of NPR-C to activate G_{i1} and G_{i2} [147–149]. The interaction of VIP and PACAP with NPR-C initiates a signaling cascade involving Ca^{2+} influx that leads to Ca^{2+}/calmodulin-dependent activation of a membrane-bound constitutive NOS, identified by in situ polymerase chain reaction on single muscle cells as endothelial NOS (eNOS or NOS III) (Fig. 6.18) [144,145]. The resultant increase in intracellular NO activates soluble guanylate cyclase, and this leads to the generation of cGMP and the activation of PKG. Together, PKA and PKG are responsible for the relaxation of muscle tone [57,61,62]. As previously discussed, the two kinases also cause hyperpolarization of muscle cells, which can prevent the development of phasic contractions in innervated muscle strips. Although the amount of eNOS in muscle cells is small and immunocytochemical detection is difficult, eNOS coupling to G proteins greatly amplifies its effect. In smooth muscle cells of the gut, eNOS is susceptible to inactivation by PKC [150]. A common experimental flaw, whereby the tone of muscle strips is increased using contractile agonists that stimulate PI hydrolysis and activate PKC, leads to the suppression of NOS activity in muscle cells and precludes detection of the VIP–NOS linkage in these cells.

VIP differs from PACAP in its action in taeniae coli, and the receptors for each peptide are distinct from those in other regions of the gut. VIP interacts with a specific receptor that has been cloned and which does not recognize PACAP; the VIP-specific receptor differs from the $VPAC_2$ receptor by a pair of adjacent phenylalanine residues in the ligand-binding extracellular domain. PACAP interacts with a specific receptor that does not recognize VIP or couple to adenylate cyclase but which couples to the activation of apamin-sensitive K^+ channels [92,151]. Muscle cells in taeniae coli are the only muscle cells found so far in the gut that are devoid of NOS.

GABA, somatostatin, and opioid peptides

GABA, acting through GABA-A receptors, causes relaxation in muscle strips but not in isolated muscle cells. The relaxation induced by GABA in muscle strips is accompanied by

the release of VIP and NO and can be blocked by VIP antagonists and NOS inhibitors (Figs 6.17 and 6.18).

Opioid peptides cause a transient tonic contraction followed by phasic contractions in previously quiescent muscle strips. The transient tonic contraction is a direct effect of opioid peptides on smooth muscle and is demonstrable in isolated muscle cells [152]. The phasic contractions result from inhibition of the background release of inhibitory neurotransmitters (e.g., VIP, PACAP, and NO) that normally mask phasic contractile activity [137]. Elimination of this background release with VIP antibody, VIP antagonists, or NOS inhibitors induces phasic contractions in quiescent muscle strips.

Somatostatin stimulates VIP (and probably PACAP) release and NO production indirectly through pathways that inhibit opioid peptide release. The inhibition of opioid peptide release eliminates the restraint exerted by opioid neurons on VIP and PACAP neurons, resulting in stimulation of VIP and PACAP release and NO production. The interplay of somatostatin and opioid neurons is evident in the regulation of VIP and PACAP release and NO production during the descending phase of peristalsis (Fig. 6.19).

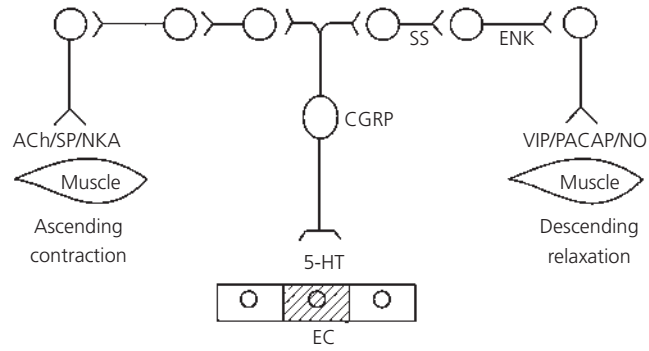

Figure 6.19 Regulation of the peristaltic reflex by neurons of the myenteric plexus. The reflex has ascending and descending phases: the descending relaxation and the ascending contraction in circular muscle shown here are accompanied by reciprocal descending contraction and ascending relaxation of longitudinal muscle (not shown). The reflex can be triggered by mucosal stimuli and circular muscle stretch. Mucosal stimuli (but not muscle stretch) release serotonin (5-hydroxytryptamine; 5-HT) from intestinal enterochromaffin (EC) cells that acts on 5-HT_4 receptors located on the nerve terminals of intrinsic sensory calcitonin gene-related peptide (CGRP)-containing neurons with cell bodies in the wall of the intestine. CGRP acts on modulatory interneurons coupled to (a) inhibitory motor neurons that release the relaxant neurotransmitters vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), and nitric oxide (NO), and (b) excitatory motor neurons that release the contractile neurotransmitters acetylcholine (ACh), substance P (SP), and neurokinin A (NKA). The interneurons consist of somatostatin (SS) and opioid neurons (enkephalin; ENK) connected in series. The increase in somatostatin inhibits the release of opioid peptides, thereby suppressing the inhibitory restraint of opioid neurotransmitters on release of VIP, PACAP, and NO. The reflex triggered by muscle stretch does not involve release of 5-HT; muscle stretch activates extrinsic CGRP-containing sensory neurons with cell bodies in the dorsal root ganglion. These sensory neurons couple to the same modulatory interneurons and motor neurons. The pathways mediating the reflex are identical in rats and humans.

Physiological profile

The pharmacological profile of actions described in the previous sections provides a framework for understanding the regulatory role of neurotransmitters released from neurons of the myenteric plexus. Excitatory motor neurons release one or both types of the contractile neurotransmitters: acetylcholine and the tachykinins, substance P and NKA [153]. Inhibitory motor neurons release the relaxant neurotransmitters, VIP (together with PHI or PHM), PACAP, and NO. Other neurotransmitters modulate the release of excitatory and inhibitory neurotransmitters from motor neurons, as previously noted.

Peptides expressed in neurons of the myenteric plexus may be identified as neurotransmitters if they meet the following criteria [130]:

1 Demonstration of peptide synthesis in neurons and presence of the peptide in nerve terminals close to target cells, such as muscle cells; or ICC

2 Release of the peptide by physiological stimuli, such as chemically or mechanically induced reflexes

3 Coupling of peptide release to a putative function, as evident from blockade of a physiological response by a specific antiserum or antagonist

4 Mimicry of the response by exogenous application of the peptide, provided that the substance does not evoke unrelated pharmacological effects that mask the physiological response.

Several excitatory and inhibitory peptide motor neurotransmitters, such as VIP, PACAP, substance P, and NKA, fulfill these criteria.

The example of VIP demonstrates its role as an inhibitory (relaxant) motor neurotransmitter (see Figs 6.18 and 6.19). VIP and neuronal NOS (nNOS) are colocalized in neurons of the myenteric plexus that innervate circular muscle cells and ICC [122–128]. VIP (together with PACAP) and NO are released concurrently in response to neural stimulation, and their release is accompanied by a proportional increase in relaxation of muscle tone and/or inhibition of phasic contraction [136,137,139]. VIP and its homologues cause direct relaxation in smooth muscle from all regions of the gut, including the stomach, intestine, gallbladder, and sphincters [57,61,139,140,154,155]. The relaxant effect of VIP on muscle tone is partially the result of its ability to stimulate NO production in muscle cells (Fig. 6.18). NO is released from nerve terminals by activation of nNOS and from smooth muscle cells by activation of a VIP/PACAP-dependent eNOS [139]. The effect of VIP/PACAP/NO on phasic contraction probably reflects their ability to generate IJPs that inhibit slow wave activity in ICC coupled electrically to smooth muscle cells.

Neutralization of VIP with specific VIP antiserum, blockade of its effect with selective VIP antagonists, inhibition of VIP release from nerve terminals, and inhibition of its relaxant effect in muscle cells by NOS inhibitors inhibit neurally induced relaxation in all regions of the gut [58,139,142,154–156].

Reflex activation of myenteric neurons by physiological stimuli (e.g., stretch or mucosal stimulation) causes VIP and PACAP release, NO production, relaxation of muscle tone, and inhibition of phasic contraction. VIP or PACAP antiserum and VIP or PACAP antagonists inhibit relaxation and NO production by suppressing the effects of VIP and PACAP on muscle relaxation and NO production. NOS inhibitors block NO production in nerve terminals and muscle cells and inhibit NO-dependent VIP and PACAP release, effectively blocking nerve-induced relaxation (see Figs 6.18 and 6.19) [139,141].

Peristaltic reflex

The peristaltic reflex exemplifies the role of enteric neurons in regulating a physiological motor function (Fig. 6.19) [142,156–170]. The reflex can be evoked by stroking, which stimulates sensory nerve terminals in the mucosa, or by radial stretch, which stimulates sensory nerve terminals in circular muscle. The reflex consists of an orad (or ascending) and a caudad (or descending) component or phase. During the caudad phase, circular muscle relaxes while longitudinal muscle contracts; during the orad phase, circular muscle contracts while longitudinal muscle relaxes. Reciprocal contraction and relaxation of the two muscle layers maintain the dimensions of the segment (Fig. 6.19) [163]. In vitro stimulation of hollow or flat segments of intestine at the orad end to elicit only the caudad (i.e., descending) phase, or at the caudad end to elicit the orad (i.e., ascending) phase, makes it possible to identify the types of neurotransmitter released and their functional coupling to each component of the peristaltic reflex. Compartmented flat-sheet colonic preparations are particularly useful for examining the reflex in humans, which is identical to that observed in rat and guinea pig. Sensory neurotransmitters are released into the central compartment, where stimuli are applied, but not into the peripheral compartments, where orad and caudad mechanical responses are measured [164–166].

Excitatory and inhibitory motor neurons mediating the peristaltic reflex

VIP, PACAP, and NO are released during and are responsible for the descending relaxation of circular muscle; VIP or PACAP antiserum, VIP or PACAP antagonists, and NOS inhibitors inhibit the descending relaxation (Fig. 6.19) [142,156,157]. Acetylcholine, substance P, and NKA are released during and are responsible for the ascending contraction of circular muscle; contraction is partially inhibited by muscarinic antagonists and is abolished by a combination of muscarinic antagonists and tachykinin antagonists or antibodies (Fig. 6.19) [157,158]. The pattern illustrates the involvement of the main excitatory and inhibitory motor neurotransmitters of the myenteric plexus in regulating the motor limb of the peristaltic reflex.

Modulatory interneurons

Interneurons of the myenteric plexus participate in the peristaltic reflex by modulating the release of excitatory and inhibitory neurotransmitters from motor neurons [159–162]. The release of somatostatin increases during the descending phase, whereas the release of opioid peptides decreases [160,161]. In the resting state, opioid interneurons exert a continuous restraint on VIP/PACAP/NOS motor neurons. This restraint is eliminated during the descending phase when opioid peptide release is decreased, leading to an increase in VIP, PACAP, and NO release [159–162]. Opioid inhibitors applied during the descending phase enhance the release of VIP, PACAP, and NO and increase muscle relaxation; the application of opioid peptides has the reverse effect.

The decrease in opioid peptide release, which eliminates opioid restraint on VIP/PACAP/NOS motor neurons, is mediated by the increase in somatostatin during the descending phase. Neutralization of somatostatin with somatostatin antibodies increases opioid peptide release and decreases VIP, PACAP, and NO release and muscle relaxation. Exogenous somatostatin has the opposite effect, enhancing VIP, PACAP, and NO release, as well as circular muscle relaxation [159,161].

The interplay of somatostatin and opioid interneurons that regulates the activity of VIP/PACAP/NOS motor neurons also regulates the activity of VIP/PACAP/NOS interneurons. These interneurons synapse with cholinergic/tachykinin motor neurons that innervate longitudinal muscle [163]. The activation of these interneurons results in contraction of longitudinal muscle during the descending phase concurrently with relaxation of circular muscle. A switch in the activity of somatostatin interneurons during the ascending phase presumably leads to a reverse pattern of circular muscle contraction and longitudinal muscle relaxation, which is characteristic of the ascending phase of the peristaltic reflex.

Sensory neurons mediating the peristaltic reflex

Two distinct populations of sensory neurons that can be differentially activated and desensitized mediate the peristaltic reflex, which is elicited by muscle stretch and mucosal stimulation [164–166]. Muscle stretch activates the intramuscular nerve terminals of extrinsic sensory neurons, which have cell bodies in the dorsal root ganglion and axonal projections to myenteric neurons. Mucosal stimulation activates intrinsic (i.e., enteric) sensory neurons with nerve endings in the mucosa. Activation is initiated by the release of 5-HT from mucosal enterochromaffin cells, which acts on 5-HT_4 receptors

located on sensory nerve terminals and causes the release of the sensory neurotransmitter, calcitonin gene-related peptide (CGRP) [165–170]. The addition of lumenal 5-HT or selective 5-HT_4 agonists induces CGRP release and triggers the peristaltic reflex. Selective 5-HT_4 antagonists block CGRP release and the mechanical components of the reflex induced by mucosal stimulation. CGRP antagonists block the ascending and descending phases of the reflex. The release of 5-HT and CGRP and the effects of 5-HT_4 agonists, 5-HT_4 antagonists, and CGRP antagonists are elicited only in the compartment where stimuli are applied, clearly identifying 5-HT and CGRP as components of the sensory limb of the reflex. Exposure of the intestinal lumen to 5-HT_4 agonists initiates peristaltic activity and stimulates propulsion of intralumenal contents [168–170]. Addition of opioid δ-receptor antagonists suppresses the inhibitory restraint exerted by opioid interneurons on excitatory and inhibitory motor neurons and greatly enhances the propulsive effect of 5-HT_4 agonists [169]. The potent synergism between opioid antagonists and 5-HT_4 agonists at near-threshold concentrations endows the combination with therapeutic potential.

Events corresponding to the two phases of the peristaltic reflex underlie propulsion and the opening and closure of various sphincters, including the lower esophageal, pyloric, choledochal, ileocecal, and internal anal sphincters [154,155,171]. In these regions, VIP (and likely PACAP) acting directly and by means of NO appears to be the main neurotransmitter responsible for sphincter relaxation; acetylcholine and probably tachykinins participate in sphincter contraction.

Hormonal regulation of smooth muscle function

Hormonal influences on smooth muscle activity are evident during and between meals. The example of CCK illustrates the interplay of hormonal and neural influences. After ingestion of a meal, CCK is released into the circulation from the upper small intestine; it causes both direct and cholinergically mediated contraction of muscle cells in the gallbladder and neurally mediated relaxation of muscle cells in the sphincter of Oddi. Relaxation of the sphincter is accompanied by the release of VIP and is blocked by VIP antiserum, VIP antagonists, and NOS inhibitors, implying that CCK causes relaxation by stimulating the release of VIP and NO from intramural neurons [155].

Motilin illustrates the participation of a hormone in the regulation of smooth muscle activity between meals. In humans and other mammals, cycles of electrical and contractile activity, described in greater detail in Chapter 11, recur at 1.5- to 2-h intervals between meals. The cycles consist of four distinct phases, collectively known as the *interdigestive myoelectric complex* or *migrating motor complex (MMC)*. The cycles culminate in phase III, a 5- to 10-min period of intense phasic contractile activity. Cycles typically begin in the stomach and migrate aborally throughout the small intestine; some cycles appear to begin in the small intestine. Motilin, a peptide released from endocrine cells of the upper small intestine, appears to be responsible for initiating cycles that begin in the stomach. Peaks of motilin coincide with the onset of phase III in the stomach. Neutralization of circulating motilin with motilin antiserum disrupts phase III activity, and infusion of motilin in concentrations that mimic circulating levels induces premature phase III activity [172,173]. Cycles that begin in the intestine are not controlled by motilin and may be regulated by input from enteric and extrinsic neurons. The neural and hormonal mechanisms that cause suppression of the cycles on ingestion of a meal are unknown.

Humoral regulation of smooth muscle function

In addition to neurotransmitters and circulating hormones, humoral agents produced by various nonneural cells, including smooth muscle cells, influence smooth muscle activity. These include ATP, histamine, serotonin, adenosine, sphingosine-1-phosphate, lysophosphatidic acid, and eicosanoids, such as prostaglandins, thromboxanes, and leukotrienes. Receptors for most of these agents have been identified on smooth muscle cells of the gut [49].

ATP, whether released as a neurotransmitter or as a metabolic product, interacts with two different P_2 receptors on muscle cells [174]. The term P_2 is preferable to purinergic because it recognizes that purine and pyrimidine nucleotides can act as preferential ligands of receptor subtypes. At low concentrations, ATP interacts with $P2Y_2$ receptors coupled via $G\alpha_q$ to PLC-β1 and via $G\beta\gamma_{i3}$ to PLC-β3 and elicits IP_3-dependent Ca^{2+} release and muscle contraction. At higher concentrations, ATP also interacts with ligand-gated $P2X_1$ receptors, which act as cationic channels and cause depolarization of the plasma membrane and Ca^{2+} influx through voltage-gated Ca^{2+} channels. At high concentrations, ATP also activates K^+ channels; the notion that activation of these channels on release of ATP from inhibitory nerves is responsible for the initial phase of the IJP has some proponents.

ATP is rapidly degraded to adenosine, which also interacts with two types of receptor, A_1 and A_2, on smooth muscle cells of the intestine [175]. These receptors are coupled to three signaling pathways. A_2 receptors are coupled via $G\alpha_s$ to activation of adenylate cyclase, whereas A_1 receptors are coupled via $G\alpha_{i3}$ to inhibition of adenylate cyclase and via $G\beta\gamma_{i3}$ to activation of PLC-β3 and IP_3-dependent Ca^{2+} release in circular muscle and to activation of $cPLA_2$ and Ca^{2+}-induced Ca^{2+} release in longitudinal muscle [175]. The net effect of adenosine is contraction, which is augmented

after blockade of A_2 receptors; blockade of A_1 receptors unmasks relaxation.

Two types of receptors for serotonin (5-HT_2 and 5-HT_4) and histamine (H_1 and H_2) coexist on smooth muscle cells of the stomach and intestine. 5-HT_2 and H_1 receptors mediate initial contraction through an increase in $[Ca^{2+}]_i$, and 5-HT_4 and H_2 receptors mediate relaxation through an increase in cAMP [176,177]. For both 5-HT_2 and H_1 receptors, Ca^{2+} mobilization in circular muscle is mediated by means of PLC-β1 and IP_3-dependent Ca^{2+} release, while in longitudinal muscle it is mediated by means of $cPLA_2$ and Ca^{2+}-induced Ca^{2+} release. The net effect of 5-HT or histamine is contraction, reflecting the dominant influence of 5-HT_2 and H_1 receptors. The blockade of 5-HT_4 or H_2 receptors augments the contraction, whereas blockade of 5-HT_2 or H_1 receptors unmasks relaxation.

Inflammation and smooth muscle

Smooth muscle cells: targets and source of inflammatory mediators

Smooth muscle cells respond to inflammatory mediators by synthesizing and secreting various pro- and antiinflammatory mediators that, in turn, act in an autocrine and paracrine fashion to stimulate a time-dependent expression of other cytokines, chemokines, growth factors, and cell-adhesion molecules [178–180]. Thus, smooth muscle cells act as both source and target of these mediators setting up a dynamic balance that maintains the inflammatory response but eventually facilitates its resolution. Different stimuli elicit different patterns of mediators in smooth muscle cells that either increase or decrease smooth muscle cell contractility. Helminth infections stimulate transient synthesis of the Th2 cytokines, interleukin (IL)-4 and IL-13, which cause a sustained expression of transforming growth factor (TGF)-β1, cyclooxygenase (COX)-2, and protease-activated receptor (PAR)-1 in smooth muscle cells, resulting in persistent hypercontractility [181,182]. Other infections elicit a time-dependent expression of IL-1β, IL-6, IL-8, and tumor necrosis factor (TNF)-α, accompanied by a decrease in muscle contractility [179,183]. A delayed expression of IL-11 fosters resolution of the inflammatory response [179].

Role of NF-κB in inhibition of smooth muscle contractility

The decrease in smooth muscle contractility on exposure of smooth muscle to IL-1β or TNF-α appears to be mediated mainly by nuclear factor-kappa B (NF-κB) [183]. NF-κB is activated via a canonical pathway in which the NF-κB inhibitor, IκBα, is first phosphorylated by IK kinase-β (IKKβ) and then degraded via a proteasomal pathway [184]. Elimination of the inhibitor exposes the nuclear localization signal on the p65/p50 heterodimer, enabling phosphorylation of p65, nuclear translocation of the heterodimer, and binding to a specific DNA sequence resulting in gene transcription. For some constitutively expressed genes (e.g., the α1c subunit of the voltage-gated Ca^{2+} channel), transcription can be repressed on phosphorylation and binding of both p50 and p65 [185]. Various kinases (e.g., ERK1/2, p38 MAP kinase, PI 3-kinase) are also activated by the cytokines and can modulate gene transcription either directly or via transcription factors [186].

The decrease in contractility caused by IL-1β or TNF-α has been attributed to inhibition of Ca^{2+} mobilization resulting from production of H_2O_2 or inhibition of voltage-gated Ca^{2+} channel expression [183,187,188]. Inhibition of Ca^{2+} channel expression suppresses phasic muscle activity, which is dependent on Ca^{2+} influx, but has no effect on muscle tone. Studies have identified various targets in the signaling pathways mediating initial and sustained tonic contraction whose expression or activity are affected by cytokines such as IL-1β [189].

Effect of cytokines on targets in the signaling cascade mediating contraction

Expression of RGS4, the sarcoplasmic Ca^{2+} uptake pump SERCA2, and the NADPH oxidases, NOX-1 and NOX-4, is increased on exposure of cultured muscle cells to IL-1β; the increase in expression of RGS4, NOX-1, and NOX-4 is mediated by NF-κB [190]. The expression of targets upstream of RGS4, such as receptors and G proteins, is not affected. Up-regulation of RGS4 accelerates deactivation of $G\alpha_q$, leading to a decrease in PI hydrolysis and IP_3-dependent Ca^{2+} release, and inhibition of initial contraction and MLC_{20} phosphorylation [189]. The increase in SERCA2 expression, which is not mediated by NF-κB, induces Ca^{2+} sequestration into sarcoplasmic Ca^{2+} stores, an effect that could be enhanced by formation of H_2O_2 [187,188]. The increase in NOX-1 and NOX-4 leads to sustained formation of superoxide, which can be readily dismuted to H_2O_2.

The expression of various targets involved in mediating sustained contraction, including G_{13}, RhoA, Rho kinase, and MYPT1, is not affected by IL-1β. The expression of the endogenous MLC phosphatase inhibitor, CPI-17, however, is decreased, resulting in a decrease in sustained MLC_{20} phosphorylation and contraction [189]. The decrease in CPI-17 expression appears to be mediated by NF-κB. Upregulation of RGS4 and downregulation of CPI-17 expression have been demonstrated in experimental colitis.

Effect of cytokines on targets in the signaling cascade mediating relaxation

Exposure to IL-1β can also affect the expression and activity of signaling targets mediating relaxation. Induction of NOS II (iNOS) causes sustained NO production and activation of soluble guanylate cyclase and PKG. PKG initiates a feedback mechanism that leads to a decrease in soluble guanylate cyclase expression and cGMP production [191].

The increase in iNOS expression can also limit cAMP production. NO generated by iNOS causes *S*-nitrosylation of adenylate cyclase V/VI in smooth muscle cells and inhibits its ability to stimulate cAMP production [192]. An increase in cAMP-specific PDE4 expression enhances cAMP degradation, further limiting cAMP production.

Thus, exposure to cytokines, such as IL-1β and TNF-α, has specific effects on the expression and activity of various targets in the signaling pathways mediating contraction and relaxation. Some, but not all, of these effects are mediated by NF-κB, but in each instance the outcome is to limit both contraction and relaxation.

Summary

Smooth muscle of the gut exhibits variable tone on which are superimposed rhythmic contractions, driven by cycles of depolarization and repolarization (slow waves) that originate in interstitial cells of Cajal (pacemaker type) located mainly at the periphery of circular muscle. Ca^{2+} influx occurs when the depolarization attains a level at which voltage-gated Ca^{2+} channels are activated, triggering a transient contraction superimposed on muscle tone. Release of excitatory neurotransmitters (mainly acetylcholine and tachykinins) from enteric motor neurons accentuates depolarization and Ca^{2+} influx and initiates Ca^{2+} release, causing an increase in both tone and rhythmic contraction. Release of inhibitory/relaxant neurotransmitters (mainly VIP, PACAP, and NO), enhanced by VIP/PACAP-dependent generation of NO from smooth muscle cells, induces hyperpolarization of the plasma membrane and suppresses rhythmic contractile activity (hence the term inhibitory). Evidence suggests that, during phasic activity, the effect of neurotransmitters is preferentially relayed to muscle cells by spindle-shaped ICC (IC-IM) located within the muscle mass. The ICC-mediated neural input generates excitatory and inhibitory junction potentials that regulate the phasic activity of smooth muscle cells. These potentials have little or no effect on muscle tone, except in regions where the muscle cells are depolarized.

The effects of excitatory and inhibitory neurotransmitters on muscle tone (and, to a lesser extent, on rhythmic contractions) are mediated by G protein-coupled receptors. Muscle contraction occurs in two phases. The initial phase consists of a transient contraction mediated by phosphorylation of MLC_{20} via a Ca^{2+}/calmodulin-dependent MLC kinase. Ca^{2+} mobilization during the initial phase involves G protein-dependent activation of PLC-β and stimulation of IP_3-dependent Ca^{2+} release in circular muscle; Ca^{2+} mobilization in longitudinal muscle involves G protein-dependent activation of $cPLA_2$ and arachidonic acid-stimulated Ca^{2+} influx, followed by Ca^{2+}- and cyclic ADP ribose-induced Ca^{2+} release. The second phase consists of a sustained Ca^{2+}-independent contraction during which MLC_{20} phosphorylation is maintained by a Ca^{2+}-independent MLC kinase while MLC phosphatase is inhibited via dual PKC- and Rho kinase-dependent pathways. The pathways are triggered by sequential activation of G_{13} and RhoA.

Relaxation is mediated by NO- and VIP/PACAP-dependent stimulation of cAMP and cGMP and activation of both PKA and PKG. cAMP and cGMP levels are maintained within narrow limits by PKA/PKG-dependent feedback mechanisms that activate PDEs and inhibit cyclases. PKA inhibits adenylate cyclase and activates cAMP-specific PDE3A and PDE4D5, whereas PKG inhibits soluble guanylate cyclase and activates PDE5. The concurrent stimulation of cGMP greatly increases the affinity of cAMP for PKG. Thus, during physiological neural activity when both cyclic nucleotides are present, the more abundant cAMP preferentially activates PKG. Both protein kinases inhibit PLC-β and $cPLA_2$, but only PKG directly inhibits IP_3-induced Ca^{2+} release. These properties are mainly responsible for the ability of PKA and PKG to inhibit initial contraction. The inhibition of sustained contraction is mediated by the ability of both protein kinases to inhibit RhoA activity and to stimulate MLC phosphatase activity.

The interplay of smooth muscle cells, interstitial cells, and enteric neurons (interneurons, sensory neurons, and excitatory and inhibitory motor neurons) is seen to best advantage in the regulation of the peristaltic reflex. The reflex is triggered by mucosal stimuli produced by the passage of digesta through the intestine and only exceptionally by distention, which uses an extrinsic sensory pathway. Mucosal stimulation releases 5-HT from enterochromaffin cells; 5-HT acts on $5\text{-}HT_4$ receptors located on the nerve terminals of intrinsic, sensory CGRP-containing neurons; the information is relayed sequentially through somatostatin and opioid interneurons to ascending and descending excitatory and inhibitory motor neurons. The ascending limb of the reflex involves the release of acetylcholine and tachykinins from excitatory motor neurons and mediates orad contraction of circular muscle; the descending limb, which mediates caudad relaxation of circular muscle, involves the combined release of VIP, PACAP, and NO from inhibitory motor neurons, accentuated by VIP/PACAP-mediated NO production in muscle cells. A distinct set of VIP/PACAP/NOS interneurons that synapse with tonic cholinergic/tachykinin motor neurons innervating longitudinal muscle mediates reciprocal caudad contraction and orad relaxation of longitudinal muscle. The ability of $5\text{-}HT_4$ agonists to initiate peristalsis, particularly when combined with opioid antagonists, underlies their therapeutic potential.

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A COMPONENTS

7

The mucosal immune system and gastrointestinal inflammation

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The mucosal immune system

The intestinal mucosal immune system is one of the largest immunological compartments in the body. This system of epithelial cells, lymphoid cells (i.e., B and T cells), and myeloid cells (i.e., macrophages, neutrophils, dendritic cells, eosinophils, and mast cells) is charged with the daunting task of maintaining a delicate homeostatic balance – defending against the many pathogenic organisms and toxic antigens that can penetrate the epithelial barrier and cause intestinal injury, and ignoring the multitude of commensal organisms and dietary antigens present in the gut that are of no threat to the host. Of all the organ systems, only the gastrointestinal tract and a few other epithelial surfaces are challenged to distinguish between foreign or non-self-antigens and self-antigens in such a dramatic fashion.

To meet this challenge, the intestinal mucosal immune system has evolved several important gut-specific modifications: flattened epithelial microfold (M) cells that transport antigen; specialized epithelial cells (follicle-associated epithelium) overlying lymphoid aggregates and dendritic cells that selectively sample lumenal antigens; the immunoglobulin A (IgA) system that helps to exclude and remove foreign antigens; and unique mechanisms that generate local, specific secretory immunity in the context of systemic tolerance [1–6]. The functional segregation of intestinal from systemic compartments emphasizes the separate role of the intestine as a unique lymphoid organ that is linked to other mucosal surfaces, such as those of the lung, breast, and genitourinary tract, to create a common mucosa-associated lymphoid tissue (MALT) [7]. Functional linkage of the MALT is accomplished by the regulated traffic of lymphocytes between the affiliated tissues [8].

Advances in cellular and molecular biology have greatly facilitated the understanding of the general operation of the intestinal immune system, which protects the organism against the invasion and systemic dissemination of pathogens while limiting the infiltration of commensal bacteria. These commensal bacteria confer benefit to the host by promoting the digestion of nutrients and the development of gut immunity, as well as by preventing colonization by pathogens. In this context, toll-like receptors and NOD-like receptors, which are expressed on intestinal epithelial cells and other mucosal cells, play a critical role in controlling mucosal immunity. Foreign antigens are sampled, processed, presented, and recognized through the major histocompatibility complex (MHC), T-cell receptors, and immunoglobulins. Responsive cellular elements are stimulated after antigen contact through accessory molecules, adhesion molecules, and signal transduction. Activated immune cells then perform their functional role through the production of cytokines and other humoral factors, as well as through cytolysis. The immune response generated by these events is self-limited after the foreign antigen is cleared. However, under pathological conditions, such as inflammatory bowel disease (IBD), the persistence of an aberrant immune response can result in chronic intestinal inflammation and tissue injury.

Components of the immune system

T lymphocytes

T lymphocytes are a critical component of the gut mucosal immune system, which must maintain tight regulation of innate and adaptive immune responses against infectious agents and tumors in an environment that is rich with benign non-self-antigens. As important regulators of effector function, T lymphocytes recognize and eliminate infectious agents and tumors, and orchestrate a coordinated immune

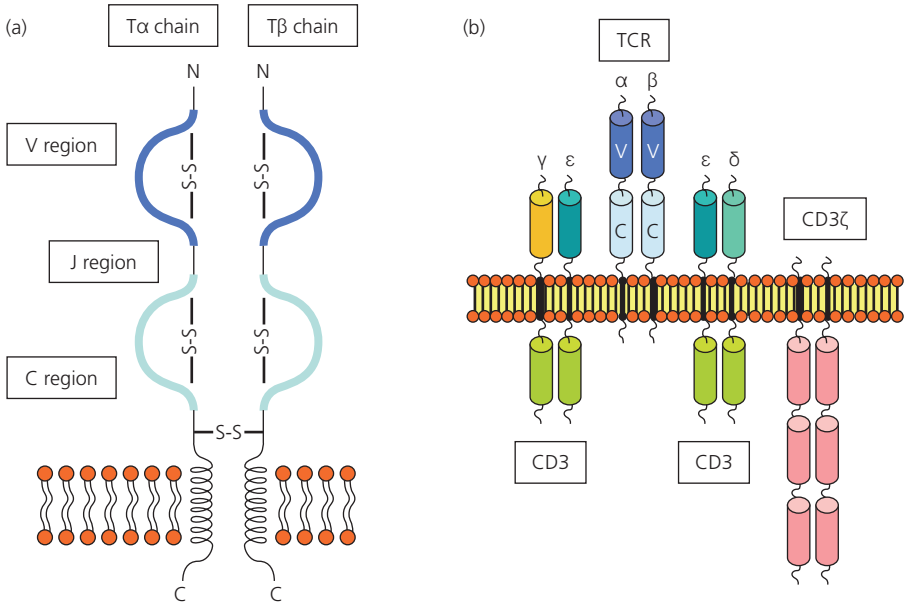

Figure 7.1 Structure of the T-cell receptor (TCR). **(a)** The TCR comprises αβ and γδ heterodimers and is responsible for antigen recognition. Its structure consists of a leader peptide, a long extracellular domain, a transmembrane region, and a short cytoplasmic tail, and is organized into variable (V), joining (J), constant (C), and, for the β and γ chains, diversity (D) regions. **(b)** The cluster of differentiation-3 (CD3) complex, which forms noncovalent associations with the αβ and γδ chains of the TCR, is composed of at least four proteins: γ, δ, ε, and ζ chains. The TCR and associated CD3 complex together are responsible for antigen-specific interactions with antigen-presenting cells.

defense through their regulatory effects on other cell populations.

T cells are derived from bone marrow stem cells and migrate to the thymus, where they mature and are educated about self-antigen. Self-tolerant T cells then migrate to secondary lymphoid tissues throughout the body, including the Peyer patches of the gut. These naïve T cells populate the Peyer patches and await sensitization by lumenal antigens, which are transported to the Peyer patch follicles by specialized follicle-associated epithelial cells (M cells). A specific mucosal immune response is initiated by this interaction and directs the naïve T cell to a functional activated phenotype. The activated T cells migrate out of the intestinal tract to the afferent lymphatics that drain into the mesenteric lymph nodes, enter the efferent lymphatics of the mesenteric lymph nodes, and pass through the thoracic duct into the peripheral blood. Circulating activated T lymphocytes home to the original sites of antigenic stimulation and reenter the gut mucosa through flat endothelial cells on postcapillary venules, where they provide protective immunity within the lamina propria.

During antigen presentation, most T cells recognize processed nominal antigens or peptide fragments from the whole antigen in association with components of the MHC on the surface of an antigen-presenting cell [9]. The antigen-specific or clonotypic T-cell receptor (TCR) on the surface of the lymphocyte is responsible for antigen recognition, and is composed of immunoglobulin-like heterodimeric glycoproteins formed primarily as αβ and γδ *heterodimers*. Most circulating mature T cells express the αβ heterodimer on their receptor; only 5% express the γδ heterodimer and, rarely, the TCR exists on the cell surface as a ββ homodimer. However, among intraepithelial lymphocytes throughout the gastrointestinal mucosa, γδ T cells predominate. Indeed, their proximity to the epithelium means that γδ T cells are ideally situated to contribute to the initial stages of mucosal immunity. Overexpression experiments and x-ray crystallography have proved that the αβ heterodimer recognizes antigen in association with an MHC molecule [10]. The mechanisms by which γδ T cells recognize antigen are not as clearly defined; however, most γδ T cells recognize ligands that are fundamentally different from the short peptides that αβ T cells see in the context of MHC molecules [11]. Subsets of γδ T cells can recognize small bacterial phosphoantigens, alkylamines, synthetic aminobisphosphonates, and stress-inducible MHC-related molecules MICA/B, as well as several other ligands [12].

The TCR is composed of a leader peptide, a long extracellular domain, a transmembrane region, and a short cytoplasmic tail [13]. These protein segments are organized into variable (V), joining (J), constant (C), and, for the β- and γ-chains, diversity (D) regions, which are each encoded by distant gene segments on chromosomes 7 (β- and γ-chains) and 14 (α- and δ-chains) that recombine during T-cell development in the thymus (Fig. 7.1a). Several mechanisms contribute to the antigen-recognizing capacity of the TCR: (1) multiple V-, J-, and D-region genes; (2) recombination of the D region in multiple reading frames; (3) addition of nucleotides not included in the germline configuration at the V–D and D–J junctions (non-germline or N-region additions); and (4) different combinations of either the α- and β- or the γ- and δ-chains [13]. As a result, most of the variability of the TCR is concentrated in the V–D–J region, otherwise known as the *complementarity-determining region 3* (CDR3). This hypervariable region represents the clonotypic deter-

minant of the receptor, whereas sequences closer to the amino-terminal region (CDR1 and CDR2) are more involved in interactions with MHC molecules. Simultaneous crystallization of the TCR αβ heterodimer and the MHC class I molecule has shown that the CDR3 region of the TCR is a major contact site for antigenic peptide [10]. At least four proteins (γ, δ, ε, and ζ) of the *cluster of differentiation-3* (CD3) complex form noncovalent associations with the αβ and γδ chains of the TCRs on the surface of T cells (see Fig. 7.1b). The CD3 complex is invariant and therefore does not contribute to the antigen specificity of the TCR, but the CD3 proteins are involved in the assembly and transport of the TCR to the cell surface and in signal transduction after the TCR binds antigen in the context of the MHC. Together, the TCR and the associated CD3 complex are responsible for *cognate*, or antigen-specific, interactions with the antigen-presenting cell.

Several molecules play important roles in *non-cognate*, or antigen-nonspecific, interactions between a T cell and an antigen-presenting cell. T cells are classified into distinct functional subtypes according to the combinations of these molecules expressed on their cell surfaces. T cells that express the 60-kDa glycoprotein CD4 help coordinate immune responses and recognize antigenic peptides in the context of class II MHC molecules, whereas T cells that express the 32-kDa glycoprotein CD8 recognize antigenic peptides in the context of class I MHC molecules and are either cytotoxic or they secrete cytokines that influence immune response networks [14]. The interactions between CD4 and class II MHC molecules and between CD8 and class I MHC molecules provide a stabilizing environment for the cognate interactions between TCRs and antigenic peptides. CD4 and CD8 also play important roles in signal transduction through intracellular interactions with nonreceptor ligands.

Before activation, T cells must adhere to the local connective tissue matrix and antigen-presenting cells. Two intracellular signals activate the T cell: an antigen-specific signal through the TCR and the CD3 complex (i.e., a cognate signal), and an antigen-independent signal through accessory molecules on the T cell, such as CD4 or CD8, and their ligand counterparts on the antigen-presenting cell (i.e., a non-cognate signal). An intracellular signaling cascade is then initiated that leads to the expression of new cell surface antigens, cell growth, and the functional expression of the T cell.

On activation of the antigen-presenting cells by interleukin (IL)-12 and IL-23, *helper T* (Th) *cells*, which express CD4 on their surface, differentiate into one of several distinct functional phenotypes (Fig. 7.2). *Th1 cells* participate in the early events of T cell and B cell development (IgG1 production) and cell-mediated immunity (delayed hypersensitivity). Differentiation of Th1 cells is regulated by polarizing cytokines such as IL-12, IL-18, IL-23, and IL-27, and transcription factors such as TBET, STAT1, and STAT4 [15]. Th1 cells express a characteristic profile of Th1 cytokines, including interferon-γ (IFN-γ), IL-2, and lymphotoxin, which

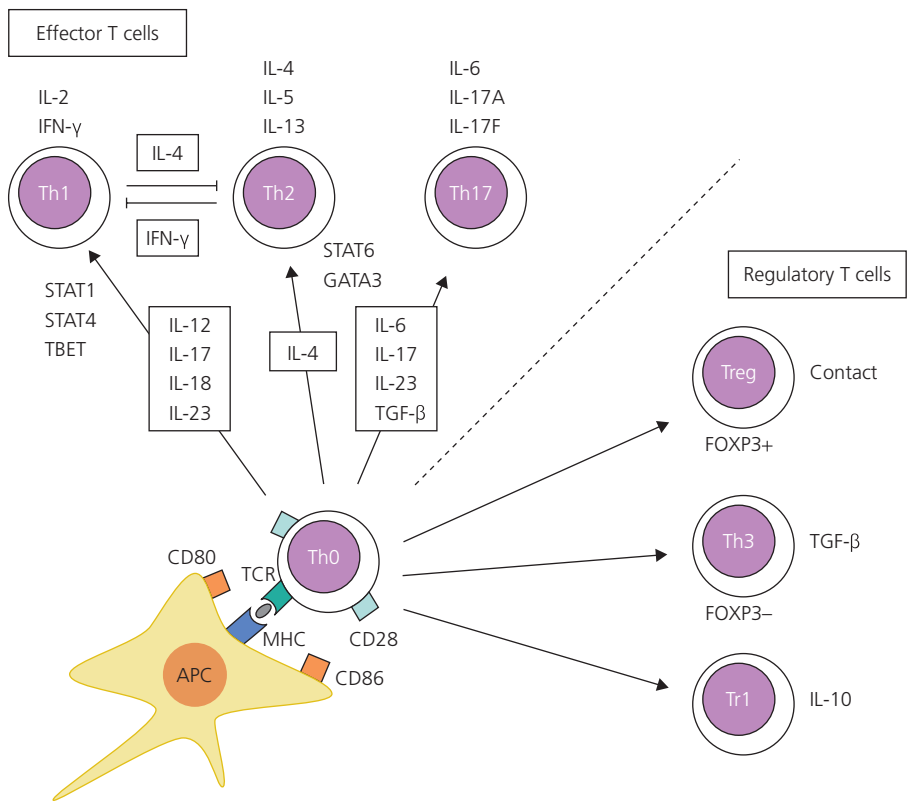

Figure 7.2 Differentiation of T lymphocytes into distinct functional phenotypes. Type 1 helper T (Th1) cells are regulated by polarizing cytokines, such as IL-12, IL-18, IL-23, and IL-27, and transcription factors, including STAT1, STAT4, and TBET. Th1 cells produce specific cytokines, such as IL-2 and IFN-γ. Th2 cells are regulated by IL-4 and the transcription factors STAT6 and GATA3. Cytokines produced by these Th2 cells include IL-4, IL-5, and IL-13. IL-4 and IFN-γ serve as negative regulators of Th1 and Th2 polarization, respectively. Th17 cells are generated by TGF-β and are also dependent on other cytokines, including IL-6 and IL-23. Th17 cells produce a distinct profile of cytokines, including IL-6, IL-17A, and IL-17F. Regulatory T cells (Tregs) include two distinct subsets of CD4+ T cells: natural Tregs, which express the specific transcription factor FOXP3 and suppress effector T cells by contact; and the adaptive Tregs, Tr1 and Th3 cells, which are FOXP3-negative and produce immunosuppressive cytokines such as TGF-β and IL-10, respectively.

mediate inflammatory immune responses within the gastrointestinal mucosa. *Th2 cells* provide help for B cells and are involved in antibody-mediated immunity (humoral immunity). Th2 differentiation is mediated by IL-4 and the transcription factors STAT6 and GATA3. Cytokines secreted by Th2 cells include IL-4, IL-5, and IL-13.

Although the Th1 and Th2 paradigm has been used for nearly two decades, researchers have now identified the Th17 component of the CD4 T-cell effector response. The participation of the Th17 lineage has furthered the understanding of CD4 T-cell biology in many organ systems, including the gastrointestinal tract [16]. This discovery may revolutionize the understanding of immune regulation in health and disease. Interestingly, the development of the Th17 lineage depends on the pleiotropic cytokine, transforming growth factor (TGF)-β, which is also required for the development of regulatory T cells, establishing an important link between Th17 and regulatory T-cell development. Th17 cells are characterized by the production of a distinct profile of cytokines, including IL-6, IL-17A, and IL-17F.

Regulatory T cells (Tregs), also known as suppressor cells, suppress the activation of other T cells at sites of inflammation and minimize collateral tissue damage. Tregs include two distinct subsets of CD4+ T cells: natural Tregs, which mature in the thymus, are self-antigen-specific and express CD25, GITR, CTLA4, and the lineage-specific transcription factor FOXP3 on their cell surface [17,18]; adaptive Tregs (Tr1 and Th3 cells) develop when mature T cells are activated under particular conditions of suboptimal antigen exposure or costimulation. Adaptive Tregs produce immunosuppressive cytokines such as TGF-β and IL-10 [19]. Tregs may be particularly important in the prevention of autoimmune gastritis and IBD [20,21]. Tregs suppress the activation of T cells (primarily Th1 cells) using a contact-dependent mechanism through surface expression of the T-cell inhibitory receptor CTLA4 in the presence of TGF-β. Adoptive transfer experiments have shown that natural Tregs maintain homeostasis in the healthy gastrointestinal tract by acting as primary regulators of the immune response to commensal bacteria. These effects are mediated by IL-10, TGF-β, and CTLA4. Consistent with these phenotypes, T cells are often considered to be either effector cells (i.e., Th1 and Th2) or regulatory cells (i.e., Treg, Th3, and Tr1) [22].

Major histocompatibility complex and antigen presentation

The strict dichotomy between CD4+ and CD8+ T cells in terms of their recognition of antigens in the context of class II and class I MHC, respectively, has important functional implications. An understanding of the molecular basis of antigen processing is essential to appreciate these effects.

The human equivalent of the mouse MHC is known as the *human leukocyte antigen* (HLA) *complex*. The MHC or HLA products and the genes that encode them are subdivided into three classes. The MHC class I and class II glycoproteins are involved in antigen presentation to T cells. The three main MHC class I equivalent genes in humans are designated *HLA-A*, *HLA-B*, and *HLA-C*; and the three main MHC class II equivalent genes are designated *HLA-DP*, *HLA-DQ*, and *HLA-DR*. The class III MHC genes encode for a variety of functionally distinct proteins (e.g., complement components, heat shock proteins, and tumor necrosis factors).

Each MHC class I protein is expressed on the cell surface as a dimer composed of two noncovalently linked polypeptide chains: a 43-kDa integral membrane glycoprotein called the *heavy* or *α-chain*, and an associated non-MHC-encoded, nonglycosylated 12-kDa protein called *β_2-microglobulin* (Fig. 7.3a). The α- or heavy chain consists of three extracellular folded domains, α_1, α_2, and α_3, that are encoded by separate exons and which share structural homology with immunoglobulins. The α_1 and α_2 domains form a groove consisting of a series of β-pleated sheets bound by two α-helices that bind peptide of relatively strict length (nine amino acids) [23,24]. The degree of observed variability within the α_1 and α_2 domains suggests that antigenic peptides reside in the groove and that exposed surfaces of the α-helices interact with the V region of the TCR. Class II molecules are non-disulfide-linked heterodimeric glycoproteins consisting of a 32- to 34-kDa α-chain and a 29- to 32-kDa β-chain (see Fig. 7.3b). The extracellular portion of a class II molecule consists of two domains called α_1-α_2 and β_1-β_2. X-ray crystallography shows that, similar to the class I MHC molecule, the α_1 and β_1 domains of a class II MHC molecule form a peptide-binding pocket with somewhat less stringency in peptide length (13–18 amino acids).

Despite the structural similarities between these two classes of MHC molecules, distinct cellular compartments and trafficking pathways determine the source of antigenic peptides for each class. MHC class I molecules primarily bind cytoplasmic proteins, whereas class II molecules bind peptides derived from exogenous proteins that have been internalized by antigen-presenting cells. At any given time, cells display a summary of their internal antigenic exposure through class I MHC molecules and their external antigenic exposure through class II MHC molecules. Under normal circumstances, self-proteins are displayed, leading to a state of tolerant inactivity. Under abnormal circumstances, deleterious intracellular events lead to the display of MHC class I molecules bound with foreign peptides, the activation of CD8+ T cells, and the generation of cytolytic T cells. Similarly, extracellular exposure to foreign agents results in the cell-surface display of class II molecules bound with foreign peptides, leading to the activation of CD4+ T cells, the induction of B cells through Th cell activity, and the production of antibodies.

The CD8–MHC class I pathway is propelled by internal cellular events. Because virtually all nucleated cells express MHC class I molecules, essentially all cells can act as antigen-presenting cells for CD8+ T cells, providing important

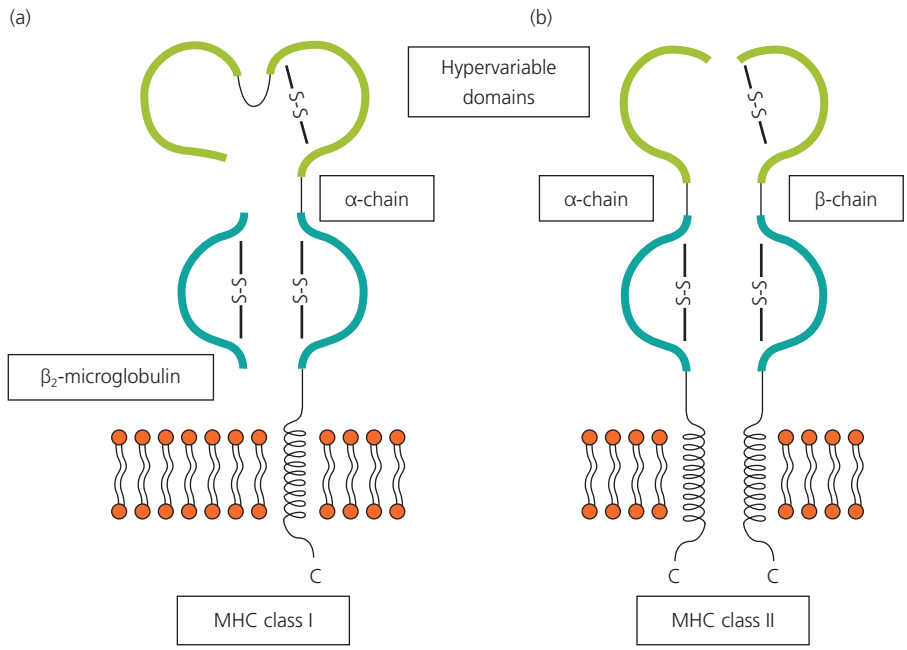

Figure 7.3 Structure of the major histocompatibility complex (MHC). **(a)** Each MHC class I protein consists of a dimer composed of two noncovalently linked polypeptide chains: the heavy (or α-) chain and an associated non-MHC-encoded nonglycosylated protein called β_2-microglobulin. **(b)** Class II molecules are nondisulfide-linked heterodimeric glycoproteins consisting of an α-chain and a β-chain.

protection against harmful intracellular processes such as viral infection or neoplasia. The CD4–MHC class II pathway responds to external cellular events, but the restriction of constitutive MHC class II expression and antigen-presenting capacity to B cells, macrophages, dendritic cells, venular endothelial cells, and probably intestinal epithelial cells during inflammation limits the possibilities and consequences of activating CD4+ T cells. Moreover, the restricted expression of accessory molecules essential to T-cell activation also imposes strict limits on antigen-initiated T-cell activation.

B lymphocytes

B cells mediate *humoral immunity* – the production of antibodies in response to an antigen – which is part of the adaptive immune system. B cells are produced in and, unlike T cells, mature in the bone marrow (Fig. 7.4). Mature B cells express a B-cell antigen receptor (BCR) that is composed of surface immunoglobulin noncovalently associated with a disulfide-linked Ig-α and Ig-β heterodimer. The membrane-bound immunoglobulin forms the antigen-recognition component of the BCR, and the Ig-α/Ig-β heterodimer is the BCR signaling component [25]. Similar to the CD3 complex associated with the TCR, Ig-α and Ig-β link the surface immunoglobulin with cytoplasmic protein kinases, which become functionally active after antigen binding. The functional response depends on the specific signaling cascade that is activated, as well as on signal strength and duration, and modulation of the signal by coreceptor molecules (i.e., the CD19/CD21 complex, CD22, and CD72) [26,27].

Antigenic stimulation of mature B cells causes clonal expansion of the responding B cell and the generation of a secreted form of membrane-bound, antigen-specific immunoglobulin. Some of the clonally expanded B cells differentiate into memory B cells. After further modulation by antigen-specific T cells, B cells switch to the isotype form associated with the antigen-specific V region (usually to isotypes of IgG in the periphery and IgA in the gastrointestinal-associated lymphoid tissues) and differentiate into plasma cells, which secrete extremely high levels of immunoglobulin.

Like T cells, subsets of B cells can be identified on the basis of phenotypic markers other than membrane immunoglobulins. CD5, a T-cell marker, is expressed on 5%–10% of the B-cell lineage. CD5+ cells, or *B1 cells*, are capable of producing autoantibody in autoimmune disease. They are generated most often from fetal and neonatal splenic B-cell populations and transferred fetal precursors, and rarely from adult bone marrow [28,29]. Most B cells do not express CD5 and are known as *B2* or *common B cells*. CD40, which is expressed on all B cells, binds to CD40 ligand on activated T cells and participates in the isotype switch. CD40 is also an important costimulatory molecule.

Immunoglobulins

Immunoglobulins are antibodies expressed on the surface of B cells, serving as their clonotypic antigen receptors, similar to the TCR on T cells [30]. After binding their cognate antigens, the surface immunoglobulins initiate a cascade of signaling events that activate the B cell, prompt clonal proliferation, and generate plasma cells. This results in the production of antibodies that travel throughout tissue fluids to detect and bind to the antigenic molecules that first triggered their production.

Each B cell expresses a unique immunoglobulin molecule that contains variable regions conferring its antigen

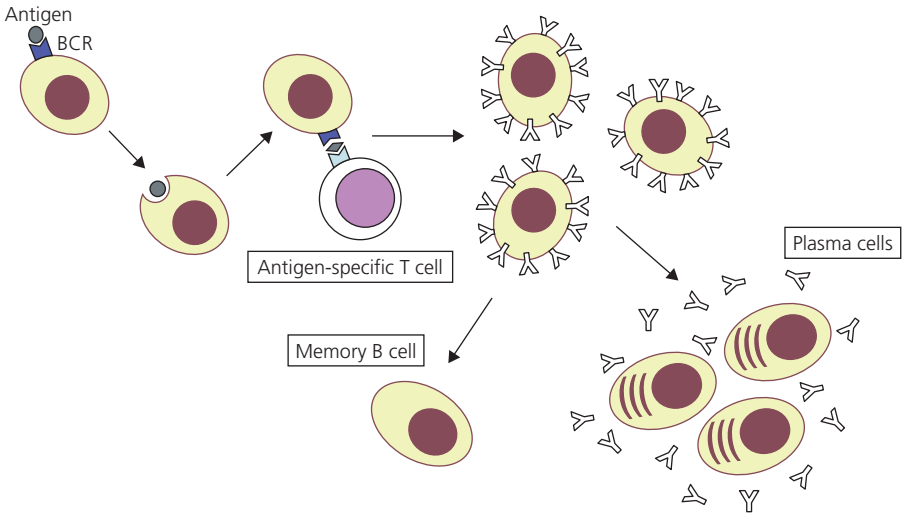

Figure 7.4 Production and differentiation of B cells. B cells mediate humoral immunity and the production of antibodies after interaction between specific antigens and the B-cell antigen receptor (BCR). B cells are produced and mature in the bone marrow. After further modulation by antigen-specific T cells, the responding B cells undergo clonal expansion and differentiate into plasma cells, which are responsible for the secretion of antigen-specific immunoglobulin G (IgG). Some of the clonally expanded B cells differentiate into memory B cells.

specificity. The secreted antibodies consist of two identical light chains linked by disulfide bridges to two identical heavy chains, which are cross-linked by disulfide bridges to each other. Each of the five classes of immunoglobulins are defined by differences in effector function and a unique heavy chain isotype, designated α (IgA), δ (IgD), ε (IgE), γ (IgG), and μ (IgM). These five heavy chain isotypes associate with light chains of two isotypes, κ and λ. In humans, there are four different IgG subclasses (IgG1, IgG2, IgG3, and IgG4) and two different IgA subclasses (IgA1 and IgA2). Although some of the immunoglobulin heterogeneity can be attributed to the different classes and subclasses, most derives from the variable regions located on the amino terminals of the heavy and light chains and encoded by the V-, D-, and J-region gene segments. It is estimated that an individual B cell can theoretically produce more than 10 000 distinct immunoglobulin variable regions.

Natural killer cells

Natural killer (NK) cells, which comprise about 10% of the peripheral blood lymphocytes, play an important role in early innate immune responses. These large, granular lymphocytes lack cognate receptors but express a variety of toll-like receptors (TLRs) on the cell surface that recognize highly conserved pathogen-associated molecular patterns (PAMPs), suggesting that NK cells provide a nonspecific first line of defense through their ability to kill pathogen-infected and transformed cells [31]. Natural killer cells may also have some function in adaptive immunity through interactions with antigen-presenting dendritic cells. Natural killer cells elicit *antibody-dependent cell-mediated cytotoxicity* through the binding of a target cell opsonized with IgG to CD16 on the NK cell.

Natural killer cell function is best characterized by its cytotoxic effects, which are tightly regulated by a balance of class I HLA-specific inhibitory and activating signaling receptors (i.e., killer immunoglobulin-like receptors [KIRs], C94/NKG2, CD2, CD16, lymphocyte function-associated antigen [LAF]-1, and TLRs). During innate immune responses, the NK cell recognizes target cells that lack surface expression of class I MHC molecules. Because many viruses and tumors, including intestinal cancers, down-regulate class I MHC molecules to evade adaptive immune responses, this mechanism of recognition is extremely important. On contact with a target cell, the NK cell releases pore-forming proteins called *perforins*, proteolytic enzymes called *granzymes*, and chemokines into the target cell, which leads to destruction of the cytoskeletal proteins, chromosomal degradation, and apoptosis.

Myelomonocytic cells

Cells of the myelomonocytic lineage include monocytes and their progeny (macrophages and dendritic cells) and polymorphonuclear leukocytes (neutrophils, eosinophils, and basophils) (Fig. 7.5). They evolve from a common myeloid progenitor cell in the bone marrow in response to cytokines, including several interleukins (e.g., IL-1, IL-3, and IL-6), and macrophage and granulocyte colony-stimulating factors (e.g., G-CSF, GM-CSF, and M-CSF).

Monocytes, mononuclear phagocytes that mature into macrophages when residing within tissues, and dendritic cells play central roles in both innate and adaptive immunity. In innate immunity, their phagocytic function and inflammatory cytokine secretion are important for the accumulation of inflammatory cells and the destruction of invading pathogens, whereas in adaptive immunity they mainly exert their effects through their ability to endocytose, process, and present foreign antigens to T cells [32]. Peripheral blood monocytes almost uniformly express the CD14 marker, which they shed on entering tissues during inflammatory responses, such as infiltration into the lamina propria

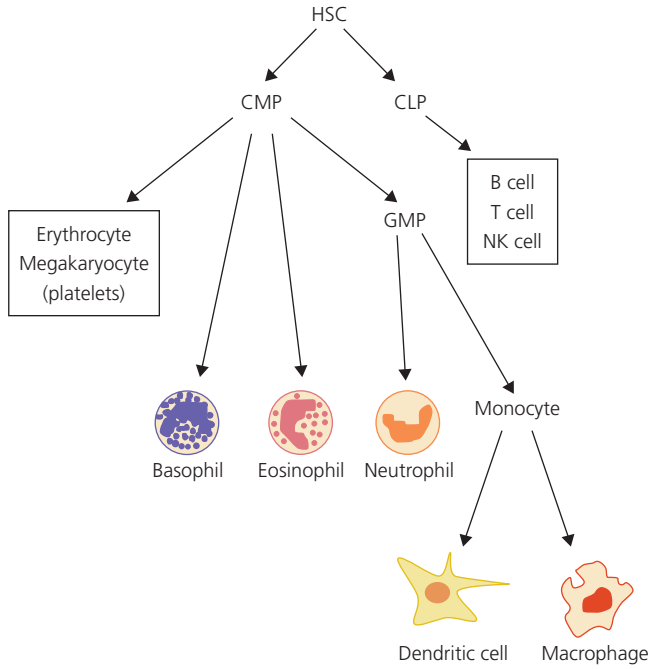

Figure 7.5 Differentiation of myelomonocytic cells. Myelomonocytic cells include monocytes and their progeny (macrophages and dendritic cells), and polymorphonuclear leukocytes (neutrophils, eosinophils, and basophils). Myelomonocytic cells are derived from a common hematopoietic stem cell (HSC), which differentiates into a common myeloid progenitor (CMP) cell in the bone marrow in response to cytokines and colony-stimulating factors. Thus, basophils and eosinophils on the one hand and neutrophils and macrophages on the other are derived from a common granulocyte–macrophage progenitor (GMP) cell. CLP, common lymphoid progenitor cell; NK cell, natural killer cell.

during IBD. These recruited monocytes evolve into tissue macrophages that have active phagocytic functions, which is important not only for the eradication of invading microorganisms but also as the essential first step in initiating antigen processing and presentation for MHC class II pathways, a prerequisite for activating CD4+ T cells that orchestrate further immune responses. Macrophages promote inflammation by secreting IL-12 and IL-23, which drive CD4+ T cells to the Th1 phenotype. This leads to the secretion of IFN-γ and subsequent activation of the phagocytic and antigen-presentation functions of the macrophage.

Dendritic cells are the most potent antigen-presenting cells and are found in the Peyer patches, lamina propria, and epithelium of the gastrointestinal mucosa [33]. Within tertiary lymphoid tissues such as the lamina propria, dendritic cells are considered to be immature, exhibiting active phagocytic functions but poor antigen-presentation functions. When exposed to microbial products – specifically, phylogenetically conserved PAMPs – dendritic cells mature and migrate to secondary lymphoid organs, including the mesenteric lymph nodes. Here they mature further, up-regulate their antigen-presentation functions through the expression of costimulatory molecules, such as CD80 and CD86, and activate naïve T lymphocytes [34]. It is now clear that multiple dendritic cell lineages exist, and that each contributes in a specific way to innate and adaptive immune responses within the gastrointestinal tract. A network of dendritic cells in the lamina propria of the ileum, colon, and the dome region of the Peyer patches express membrane-bound CX3CR1 (fractalkine) and appear to play a unique role in lumenal antigen sampling. These CX3CR1+ cells extend their dendrites through the epithelium to directly sample microbial products, in addition to the more conventional phagocytotic mechanism for recognizing bacteria delivered via M cells and intestinal epithelial cells [6,35]. CX3CR1+CD11c+CD11b+ cells are the predominant dendritic cell subset observed in the terminal ilea of mice, and the expression of the integrin α-chain CD103 (α_E) is characteristic of dendritic cells in the colonic lamina propria [36,37]. The unique physiological roles and functions of these dendritic cell subsets require further investigation.

Neutrophilic polymorphonuclear leukocytes (neutrophils) are an essential component of innate immunity and function as the first responders to infection [38]. They are continuously produced in the bone marrow and circulate through the peripheral blood, where they remain acutely posed for activation and response to invading pathogens. When infection occurs, activated neutrophils rapidly extravasate across the endothelium and into the affected mucosa with the help of adhesion molecules expressed on their surface. They infiltrate infected tissues within hours of the initial insult and phagocytose and kill the invading pathogen. Neutrophil granules contain antimicrobial cationic peptides, proteases, and reactive oxygen species that can be secreted directly into the phagosome or into the extracellular spaces to form neutrophil extracellular traps (NETs) which degrade virulence factors and microbes that have not yet been ingested [39]. Neutrophil secretion of reactive oxygen species can also regulate intracellular signaling mechanisms, allowing neutrophils to mediate the nature of subsequent inflammatory responses through the production of specific cytokines and chemokines that polarize the resulting adaptive immune response toward a unique phenotype. To mediate their proinflammatory effects and contain collateral tissue damage, neutrophils constitutively undergo apoptosis and are phagocytosed by macrophages.

Evidence that the initiating factor in Crohn's disease may be caused by a defect in innate immunity, as opposed to an abnormality in adaptive cell-mediated immunity, has cast new light on the role of neutrophils in the pathogenesis of this disease [40]. In support of this theory, genetic diseases that have a known effect on neutrophil function, such as chronic granulomatous disease, glycogen storage disease type IB, and Turner syndrome, manifest abnormalities similar to those seen in Crohn's disease. Impaired migration,

decreased bactericidal and phagocytic functions, and lower superoxide production characterize neutrophils in patients with Crohn's disease. Moreover, mice that express a cell-specific disrupted *Stat3* gene in their neutrophils and macrophages develop enterocolitis, suggesting that these cell types are indeed involved in Crohn's disease pathogenesis.

Neutrophils also contribute to acute liver damage associated with hepatic ischemia/reperfusion injury and alcoholic hepatitis [41]. In response to signals from proinflammatory cytokines, CXC chemokines, and complement factors, neutrophils are primed and accumulate in the sinusoids, postsinusoidal venules, and portal venules of the hepatic vasculature. When a chemotactic signal is received, the neutrophils extravasate across the endothelium into the parenchyma, where they adhere to hepatocytes and are activated to produce reactive oxygen species and proteases, similar to their function in the intestine. Protease secretion is associated with the promotion of subsequent inflammatory responses through the production of proinflammatory cytokines and chemokines; secretion of reactive oxygen species leads to oxidative stress and neutrophil-mediated cellular necrosis.

Nonimmune parenchymal cells

A variety of parenchymal cells, which are not classical immune cells, exhibit important immunological functions within the gastrointestinal tract. These cell types likely do not initiate specific immune responses through the education of naïve lymphocytes but rather integrate and enhance ongoing immune responses and contribute to immunopathology in disease states. These cell types include intestinal epithelial cells, mesenchymal cells (e.g., fibroblasts), smooth muscle cells, and endothelial cells. Intestinal epithelial cells form a barrier between the intestine and the lumen and perform several immune-related functions, such as the expression of cytokines and chemokines. Fibroblasts, through the expression of cell surface molecules, connective tissue components, and cytokines, regulate local lymphocyte survival and function and contribute to TGF-β-mediated fibrosis associated with chronic inflammation. Smooth muscle cells can present antigens in an MHC class II-restricted fashion in the context of inflammation and respond to inflammatory cytokines such as IL-4. This alters intestinal motility, which may play a role in peristalsis and the elimination of lumenal pathogens. Endothelial cells play a key role in regulating tissue inflammation by both directing the recruitment of leukocytes and myeloid cells and secreting a variety of soluble mediators that enhance inflammation.

The intestinal mucosal immune system

The intestinal mucosal immune system, also known as the gut-associated lymphoid tissue (GALT), can be divided into several functionally and anatomically contained compartments: (1) the loosely organized effector sites (lamina propria and intestinal epithelium), (2) the macroscopic intestinal lymphoid structures (Peyer patches and mesenteric lymph nodes), and (3) the microscopic intestinal lymphoid structures (cryptopatches and isolated lymphoid follicles). Peyer patches, which are rounded lymphoid follicles found in the mucosa and submucosa of the ileum, constitute an afferent limb of the GALT that recognizes antigens through specialized sampling mechanisms involving M cells contained within the follicle-associated epithelium adjacent to the Peyer patches and dendritic cells that extend their dendrites through open tight junctions in the epithelial barrier (Fig. 7.6). Detection of lumenal antigens that cross the epithelial barrier by way of M cells results in the education and dissemination of B and T lymphoblasts to other tissues linked to the mucosal-associated lymphoid tissue (MALT), such as the lungs, breasts, and genitourinary tract, and the loosely affiliated compartments of the lamina propria. The lamina propria represents an efferent or effector limb of the intestinal mucosal immune system; it is populated with lymphoid effector cells, such as B cells, plasma cells, T cells, and NK cells, and with mononuclear and polymorphonuclear phagocytes and mast cells (see Fig. 7.6). Plasma cells in the lamina propria secrete IgA immunoglobulin, which provides protection against bacteria, viruses, and lumenal antigens. The immune compartment within the epithelium consists of a unique resident population of T cells, known as the *intraepithelial lymphocytes*, which, unlike peripheral T cells, predominantly express the γδ TCR. The intestinal epithelial lymphocytes help maintain barrier function through their cytotoxic effects on abnormal epithelial cells.

Organized lymphoid structures and lymphoblast homing

Peyer patches are the most clearly defined of the organized lymphoid structures in the gastrointestinal mucosal immune system (see Fig. 7.6). They are surrounded by the follicle-associated epithelium, which contains specialized M cells. M cells play an important role in mucosal immune surveillance by delivering selected samples of intralumenal antigens to the organized mucosal lymphoid tissues. M cells are derived directly from undifferentiated, immature epithelial stem cells in the crypts. Their differentiation to mature follicle-associated epithelial M cells may be influenced by subjacent B cells within the lamina propria [42]. M cells have fewer, shorter, and wider microvilli than absorptive epithelial cells. Similar to the M cell antigen-sampling mechanism, dendritic cells found adjacent to intestinal epithelial cells can also directly sample lumenal antigens across the epithelium by opening tight junctions and extending their dendrites into the lumen [35]. Although M cell transport is necessary for induction of mucosal immune responses, this portal system also provides a means for pathogenic bacteria and viruses to enter the intestinal mucosa [43]. Among the many infectious agents known to undergo endocytosis and transport by way of M

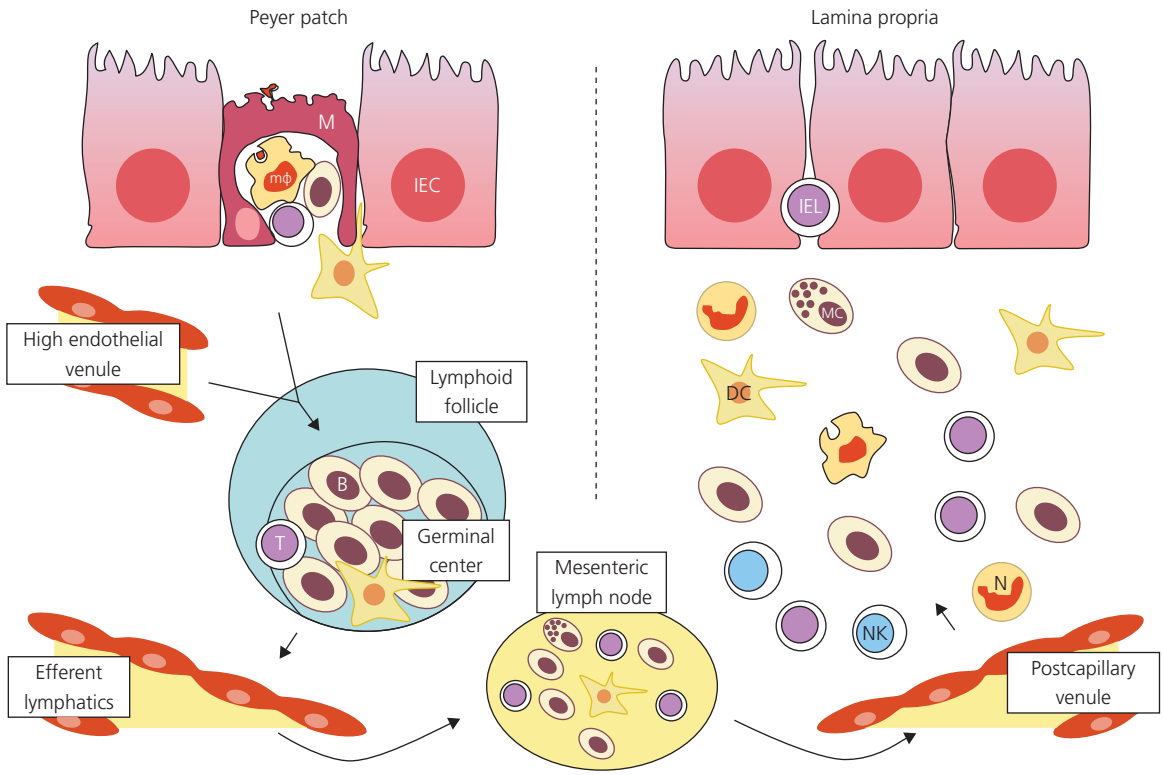

Figure 7.6 The distinct compartments of the intestinal mucosal immune system or gut-associated lymphoid tissue (GALT): (1) Peyer patches and mesenteric lymph nodes. Peyer patches take part in antigen recognition through specialized sampling mechanisms involving microfold (M) cells contained within the follicle-associated epithelium adjacent to the Peyer patches and dendritic cells (DC) that extend their dendrites through open tight junctions in the epithelial barrier. (2) Lamina propria and intestinal epithelium. The lamina propria is populated with lymphoid effector cells, including B cells, plasma cells, T cells, and natural killer cells, and by mononuclear and polymorphonuclear phagocytes and mast cells. Intraepithelial lymphocytes (IELs) are a unique resident population of T cells. mϕ, macrophage; MC, mast cell; NK, natural killer cell; N, neutrophil; B, B cell.

cells are the human immunodeficiency virus, reoviruses, *Vibrio cholerae*, and species of mycobacteria. M cells also transport commensal bacteria into the Peyer patches, thereby initiating IgA antibody immune responses and tolerance.

Intestinal cryptopatches and isolated lymphoid follicles are two other organized lymphoid structures within the mucosal immune system. It is hypothesized that they combine with the Peyer patches to form a primary lymphoid organ. *Cryptopatches* are aggregates of immature T cell precursors found adjacent to the crypts in mouse small intestine, and are a site of extrathymic development of T lymphocytes [44]. The T-cell precursors residing in cryptopatches can differentiate into mature intraepithelial lymphocytes expressing either the αβ TCR or the γδ TCR [45]. *Isolated lymphoid follicles* are an organized lymphoid structure of unknown function also found in the murine small intestine. Similar to Peyer patches, they are covered by a follicle-associated epithelium containing M cells and are composed primarily of mature B2 B cells and CD4+ T cells [46]. Isolated lymphoid follicles can develop de novo in response to lumenal stimulation and in the presence of lymphotoxin-sufficient B cells and a functioning tumor necrosis factor (TNF-α) receptor 1 [47].

Antigens and microorganisms transported out of the lumen by M cells come into contact with lymphocytes, macrophages, and dendritic cells that have migrated into the lymphoid aggregates or Peyer patches below the M cells. Some of these cells enter the intraepithelial pocket, a large invaginated subdomain on the basolateral surface of the M cell that forms a docking site for specific populations of lymphocytes and occasionally dendritic cells and which effectively shortens the distance that antigen-containing transcytotic vesicles must travel to cross the epithelial barrier [48]. Naïve B and T lymphocytes that have never encountered their cognate antigen express a combination of cell surface receptors that directs them to emigrate into lymphoid aggregates such as Peyer patches associated with the follicle-associated epithelium [49]. A vast network of immature dendritic cells exists in the *subepithelial dome* or apical portion of the lymphoid follicle immediately below the follicle-associated epithelium. These immature dendritic cells likely capture most of the antigens from the intestinal lumen and present them to the naïve lymphocytes in the intestinal lymphoid follicles. A specific mucosal immune response is initiated by these interactions, which may preferentially

direct naïve T cells to a Th2, Th3, or Tr1 phenotype over a Th1 phenotype [50]. Such an outcome is likely a consequence of the properties of the dendritic cells within the Peyer patches and plays an important role in generating mucosal tolerance as opposed to an immune response.

Activated lymphocytes from intestinal lymphoid follicles begin a maturational journey during which they leave the intestinal tract and migrate into the afferent lymphatics that drain into the mesenteric lymph nodes [51,52]. During this process, the lymphocytes mature into T and B lymphoblasts enriched in IgA-bearing B cells. The B lymphocytes become surface IgA-bearing lymphoblasts after being promoted to switch their immunoglobulin isotype by regulatory (i.e., "switch") T cells within the Peyer patches [4,5,53]. Lymphocytes then enter the efferent lymphatics of the mesenteric lymph nodes and pass through the thoracic duct into the peripheral blood. These lymphocytes subsequently reenter the loosely affiliated lamina propria through interactions with flat endothelial cells of the postcapillary venules and home to mucosal sites. B lymphoblasts mature into IgA-secreting plasma cells under the control of antigen-activated T lymphocytes that have completed a similar maturational journey. Lymphoblasts that have homed to the gastrointestinal mucosa and matured into effector cells provide protective immunity within the lamina propria.

Lymphoblasts recirculate or home to the sites of the original antigenic stimulation and to other mucosal secretory sites. After antigenic stimulation in the gastrointestinal tract, IgA lymphoblasts circulate to the mucosal secretory sites of the breast, lung, and eye, where antigen-specific antibodies are secreted [52,54]. For example, a breast-feeding mother can passively transfer secretory IgA in her breast milk to her nursing child. The secretory IgA in the transferred breast milk protects the infant against bacteria or viruses in the mother's gastrointestinal tract, supporting the importance of the common mucosal immune system. The intestinal immune system thus has the capacity to selectively sample antigens from the gut and subsequently induce immune responses that provide protection for the gastrointestinal tract and other mucosal surfaces.

Nonorganized lamina propria

The lamina propria lies beneath the intestinal epithelium and serves as a loosely affiliated effector compartment of T and B lymphocytes, plasma cells, NK cells, macrophages, dendritic cells, and mast cells. Virtually all T cells within the lamina propria express the αβ TCR, as well as the CD45 surface marker, which indicates that these are memory cells that have had previous encounters with antigens, presumably in the Peyer patches [55]. The CD4+ cells in the lamina propria exert an important helper–inducer function for immunoglobulin production, and respond to antigen by producing cytokines rather than by proliferating [56]. Most CD8+ lamina propria lymphocytes, in addition to a significant proportion of CD4+ cells, express a unique β_7-integrin associated with a novel α-chain, α_E, which plays a role in intestinal epithelial cell binding [57]. Cytotoxic effector precursor cells in the intestine participate in host mucosal defense mechanisms when necessary, but do not damage the surrounding tissue when they are not needed. Lamina propria lymphocytes can be induced to mediate cell-mediated cytotoxicity by incubation with IL-2, interferons, lectins, and monoclonal antibodies directed against the TCR. This is typical of antigen-primed effector cells and is consistent with the CD45RO memory phenotype [58–64]. Although cytotoxic effector cells are present, the lymphocytes in the intestinal lamina propria are poor mediators of cell-mediated cytotoxicity, antibody-dependent cellular cytotoxicity, and cell-mediated cytolysis [58,59]. Activated cytotoxic CD4+ and CD8+ lymphocytes are more abundant in the lamina propria of patients with IBD [65].

Most mucosal T lymphocytes are also CD95L+ and CD69+ and exhibit elevated levels of cytoplasmic Ca^{2+}, consistent with an activated phenotype [66]. Controlled activation of the intestinal immune system may be important in regulating effector cell function. This includes cytotoxic function, which may be directed at the lymphocytes themselves to down-regulate immune responses and regulate the level of cytotoxic function within the various compartments of the bowel wall [67,68]. For example, administration of anti-TNF-α therapeutics, which are used to treat human IBD, can attenuate murine ileitis through a mechanism that involves down-regulation of intestinal epithelial cell apoptosis and simultaneous up-regulation of apoptosis among lamina propria lymphocytes [68]. In this way, the gut remains in a state of physiological inflammation, poised for intervention when necessary but generally held in abeyance.

About 40% of the lymphoid cells in the lamina propria are B cells derived primarily from precursors in Peyer patches [69]. These B cells and their progeny plasma cells are predominantly focused on the synthesis of IgA, rather than of IgM, IgG, or IgE. Lamina propria B cells are induced to differentiate terminally into IgA-secreting cells cytokines derived from Th1 cells (IFN-γ) and Th2 cells (IL-4, IL-5, and IL-10), indicating the importance of this antibody to mucosal protection [2,4,54,70–76]. In diseases such as IBD, the numbers of lamina propria B cells and plasma cells that produce IgG are markedly increased [77].

Immunoglobulin secretion

The synthesis and secretion of dimeric IgA is a major protective mechanism of the intestinal tract, which contains more than 70% of the immunoglobulin-producing cells in the body [78]. Plasma cells produce dimeric IgA – two IgA monomers joined by a polypeptide J-chain. The J-chain participates in the transport of IgA and IgM molecules across the intestinal epithelium through interaction with the polymeric immunoglobulin receptor (pIgR) on the basal surface of

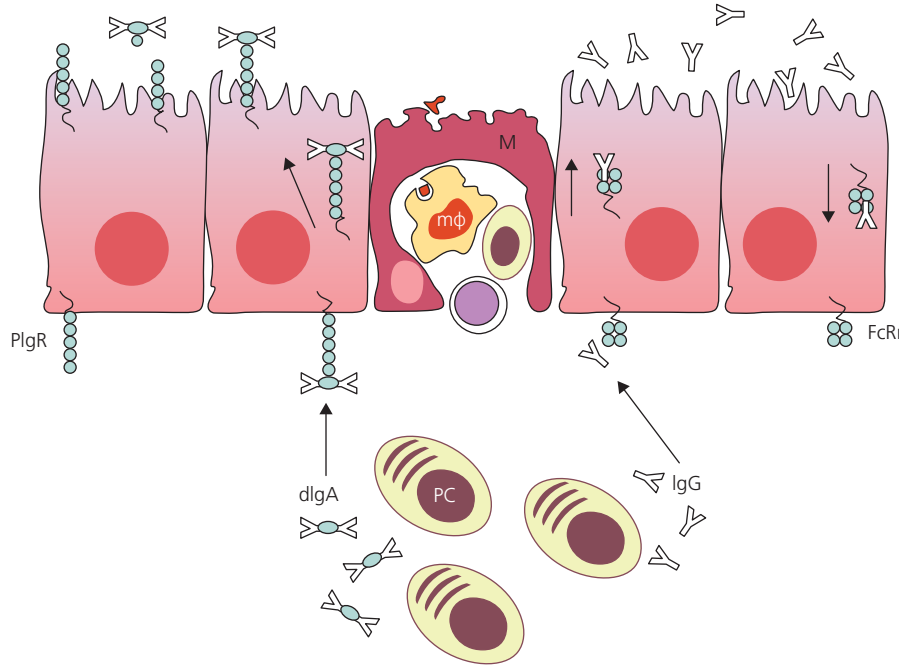

Figure 7.7 Immunoglobulin (Ig) secretion in the gut. The synthesis and secretion of IgA is a major protective mechanism for the intestinal mucosa. Dimeric IgA (dIgA) produced by plasma cells (PC) is transported across the intestinal epithelium through interaction with the polymeric IgR (PIgR). IgA bound to a portion of the PIgR, known as secretory component, is then secreted into the intestinal lumen. IgG transport is mediated by the major histocompatibility complex class I-related molecule FcRn (neonatal Fc receptor for IgG). IgG transport is bidirectional, unlike that for IgA. M, microfold cell; mϕ, macrophage.

epithelial cells (Fig. 7.7) [79–81]. IgA bound to a portion of the pIgR that is known as *secretory component* is then secreted into the intestinal lumen. Secretory component may protect secretory IgA from proteolytic degradation in the hostile lumenal environment and stabilize the structure of the polymeric IgA complex.

IgA is also translocated across hepatocytes or bile duct epithelium into the bile and carried to the duodenum [82,83]. The presence of secretory IgA in bile provides passive immunity and protects the biliary tract and the proximal parts of the small intestine. A second implication of the hepatobiliary secretion of IgA is that complexes of IgA and antigen can be transported into the bile from the circulation. Hepatic removal of IgA–antigen complexes may protect against harmful absorbed substances, including dietary antigens and bacterial products.

The major function of secretory IgA in host defense is protection against bacteria, viruses, and lumenal antigens [2,52,78]. Secretory IgA provides passive immunity by inhibiting the adherence of bacteria to epithelial cells and preventing their effective colonization and proliferation. Secretory IgA neutralizes bacterial toxins and viruses, thereby preventing their action on intestinal epithelial cells. Secretory IgA also blocks the absorption of antigens from the gut and may be particularly important in disease states in which the mucosal barrier is broken. Intracellularly, IgA can neutralize pathogens that possess a transcytotic pathway for IgA [84]. Unlike other immunoglobulins, such as IgG, which can be secreted by intestinal B cells, IgA does not activate complement.

Transcellular pathways for the transport of IgE and IgG also exist in intestinal epithelial cells. IgE transport is mediated by the expression of CD23 (FcεR1 or IgE receptor) on epithelial cells and may be important in intestinal allergic responses and parasitic infestations [85]. IgG transport is mediated by the MHC class I-related molecule FcRn (neonatal Fc receptor for IgG). Unlike the transport of IgA and IgE, IgG transport is bidirectional (apical to basal and basal to apical).

Intraepithelial lymphocytes

The human intestinal epithelium contains a unique population of lymphoid cells, the intraepithelial lymphocytes (IELs), which reside between intestinal epithelial cells along their basolateral surfaces [86]. About 10–20 IELs exist per 100 villi within the small intestine. Given the vast surface area of the intestinal epithelium, IELs represent a significant proportion of all the T cells in the human body [87]. The composition of the IEL subsets differs substantially from that of the circulating T-cell subsets. IEL populations are dominated by CD8+ T cells (> 70% in the small intestine) and largely express the CD8αα homodimer, which is uncommon among circulating T cells [88]. T cells lacking both CD8 and CD4 (CD8–CD4–), or *double-negative* cells, comprise more than 10% of murine small intestinal IELs, as well as the majority of IELs found in other compartments, but they are rarely found among circulating lymphocytes [89–91]. CD4+αβ T cells, which are common in the systemic circulation, are underrepresented among IELs and mostly exist as *double-positive* cells that also express CD8αα. These

double-positive CD8αα + CD4 + αβ T cells are not found among circulating lymphocytes. Lastly, IELs contain an increased proportion of cells expressing the γ™ TCR compared with systemic T cells.

The αβ TCR is expressed on all of the IELs in the large intestine and on about half of the IELs in the small intestine, indicating that these cells respond to MHC class I or class I-like molecules. IELs that express the αβ TCR are mostly CD8αβ+ and are cytolytic in function, and they secrete Th1 cytokines [86]. It has been hypothesized that these cells are primed to antigen in the Peyer patches and enter the circulation through the mesenteric lymph nodes and the thoracic duct [48]. They home to the lamina propria and intestinal epithelium of the small and large intestines through their expression of the $\alpha_4\beta_7$ and $\alpha_E\beta_7$ integrins [57,92].

IELs that express the γδ TCR, as well as those that express the αβ TCR in conjunction with the CD8αα homodimer, comprise the remaining half of the small intestinal IEL population [86]. Unlike the TCRαβ+ CD8αβ+ IELs in the large intestine, these IEL subsets do not circulate through the lymphatics and the blood and do not appear to be memory driven. As they do not recognize antigen presented by MHC class I and class I-like molecules, it has been proposed that these IEL subsets may be primed in situ by the epithelial cells, possibly through an autoantigen that is induced in response to infection or transformation events [93]. However, the identity and nature of this autoantigen remains unclear. A unique feature of TCRγδ+ IELs is that, unlike TCRαβ+ IELs, they can develop independently of the thymus, most likely within the intestinal cryptopatches [94,95].

Despite their contiguity to the gut lumen, potential exposure to a variety of antigens, and the expectation that these cells express a diverse, polyclonal array of αβ and γδ TCRs, IELs within the small and large intestines are oligoclonal and express a small number of αβ and γδ TCRs, based on an analysis of CDR3 regions [96–98]. A limited variety of T-cell clones is widely disseminated throughout the intestinal epithelium [99]. The expression of CD8 by IELs suggests that they function biologically as cytolytic effectors as a consequence of antigen recognition. IELs exhibit a high level of cytolytic activity in a variety of in vitro systems, especially after activation, and it is likely that they also do so in disease states in vivo [100]. A major biological function of IELs in health is the secretion of cytokines (e.g., IFN-γ and keratinocyte growth factor), which regulate epithelial cell function and possibly responses to lumenal antigens [101]. On activation, IELs may acquire cytolytic machinery that can contribute to epithelial cell death through apoptosis. Their cytolytic capabilities, large number, and extremely limited TCR repertoire indicate that IELs are a regionally specific population of cells involved in immunosurveillance against abnormal epithelial cells. IELs may be the first line of defense against deleterious epithelial events. Their numbers are markedly increased in intestinal graft-versus-host disease, gluten-sensitive enteropathy, and protozoal infections of the epithelium, such as those caused by *Cryptosporidium* and *Isospora* species.

Innate and adaptive immunity

Two types of immunity normally function along the mucosal surfaces: innate (natural) immunity and adaptive (acquired) immunity. Natural immunity is nonspecific and rapidly mobilized, whereas acquired immunity is specific for antigens on foreign substances and is mobilized slowly over several days and weeks after an initial or primary exposure. In general, specific immunity amplifies the initial protection provided by innate immunity in a focused, antigen-specific manner, in part through the further mobilization of the natural immune components. In the adaptive immune system, lymphocytes interact with antigens through receptors (the T-cell receptor and the B-cell receptor) that are specific for that antigen. When a specific antigen is presented to a lymphocyte with a receptor capable of recognizing it, that lymphocyte undergoes clonal expression. Unlike innate immunity, adaptive immunity is characterized by the acquisition of memory during the course of a specific immune response, which produces a more rapid mobilization of directed, specific immunity in future or secondary antigen encounters.

Innate immune system

Innate intestinal immunity has two components: nonimmunological and immunological. The mucosal surface of the intestine expresses a variety of nonimmunological physiochemical barriers to exclude, inactivate, or clear pathogenic substances and organisms. These barriers include gastric acid, digestive enzymes, and other potentially antipathogenic factors, such as bile acids, lysozymes, intestinal mucus, normal peristalsis, indigenous microbial flora, and the epithelial cell itself, whose tight junctions are usually impenetrable when intact. In diseases such as AIDS, defects in one or more of these factors may increase susceptibility to invasive pathogens.

A second class of innate immunity is composed of immunological factors [102]. These include cellular and soluble elements. Virtually all classes of intestinal cells participate in innate immunity, including NK cells, a subclass of T cells called NK-T cells, phagocytes, mast cells, and epithelial cells, among others. In response to specific cell surface signals delivered by a variety of receptors, these cells either engulf microorganisms (e.g., phagocytes) or secrete a wide variety of soluble substances. The latter function to remove pathogens (e.g., cryptdins from intestinal epithelial cells), recruit other cell types (e.g., IL-8 from intestinal epithelial cells), arm other cell types against microbial invasion (e.g., IFN-γ from NK-T cells), remove cells altered by infection or malignancy (e.g., granzymes and perforins from NK cells), or modify specific immune responses (i.e., IL-4 from NK-T cells). Many of these responses are initiated by interactions between specific components of microbes and either soluble

or cell surface receptors on host cells that recognize characteristic microbial structures, such as the recognition of bacterial lipopolysaccharide by TLRs.

In contrast to the adaptive immune system, in which there are many thousands of distinct T-cell and B-cell receptors, there are relatively few (several hundred) receptors in the innate immune system. The receptors present on cells of the innate immune system recognize a few highly conserved molecules that exist in large groups of microorganisms and are termed pathogen-associated molecular patterns (PAMPs). These microbial molecules, which include lipopolysaccharides, peptidoglycans, and lipoteichoic acids, share certain attributes: (1) they are not expressed by the host, (2) they are required for microbial survival, and (3) they are shared by a broad group of pathogens. The receptors of the innate immune system are expressed on antigen-presenting cells, which include monocytes, macrophages, dendritic cells, and B cells; some are also expressed on intestinal epithelial cells. The receptors of the innate immune system – the pattern recognition receptors – include TLRs, which recognize lipopolysaccharides, and macrophage mannose receptors, which recognize carbohydrates with large numbers of mannose units, characteristic of microorganisms. Pattern recognition receptors are expressed uniformly on large numbers of cells of a given class. For example, all macrophages express the same mannose receptor, in contrast to the acquired immune system, in which different T cells express thousands of different TCRs. Recognition of bacterial products by pattern recognition receptors results in prompt intracellular signaling events and a biological response. For example, recognition of lipopolysaccharide by TLRs results in the activation of nuclear factor-kappa B (NF-κB) and, as a consequence, the induction of a wide variety of inflammatory- and immune-response genes. This prompt biological response in the innate immune system occurs over a few hours, in contrast to the much slower response in the adaptive immune system, which involves clonal expression of relevant lymphocytes over several days. The innate immune system is therefore much better situated than the adaptive immune system to deal with acute infections caused by pathogens new to the host.

Adaptive immune system

Adaptive immunity also has two components: humoral and cellular immunity. Humoral immunity is mediated by antibodies derived from B lymphocytes, and defends against extracellular events. Cellular immunity is derived from T lymphocytes and provides protection from deleterious intracellular events that are not amenable to the effects of antibodies. Both types of adaptive immunity possess several properties that differentiate them from innate immunity. First, to respond to the wide variety of potential antigens to which humans may be exposed during a lifetime, a large number of clonally distinct lymphocytes specific for particular determinants or epitopes on a molecule are maintained. The molecular basis for this clonotypic specificity is determined by a cell surface receptor (i.e., immunoglobulin for B cells and TCRs for T cells) that is unique to each cell or clone. Second, each cell or clone expressing its unique or clonotypic receptor is selected to differentiate a self-antigen from a non-self- or foreign antigen. This process, primarily the result of positive selection (i.e., selection of receptors that recognize self) and negative selection (i.e., deletion of receptors that recognize non-self) of B cells in the bone marrow and T cells in the thymus during lymphocyte development, results in the production of an army of lymphocytes that are tolerant or unresponsive to self [29,103,104].

In the absence of tolerance, inappropriate recognition of self may occur, resulting in autoimmunity. The intestine, liver, and peritoneum participate in the extrathymic pathways of T-cell and B-cell development, and self-reactive lymphocytes that escape from the normal selection processes may reside within these organs [29,105]. The fact that intestinal autoimmunity is not commonly observed suggests that peripheral mechanisms in the intestine prevent the activation of potentially pathogenic clones.

The adaptive immune system generates memory during the course of the immune response that results in the expansion of long-lived cells, which are the progeny of the original responsive antigen-specific B-cell or T-cell clones. After reexposure to the original offending antigen, the adaptive immune system responds more rapidly with a response that is quantifiably larger and more specific. For example, B-cell immunoglobulins are generated that bind more avidly to their antigens.

Autoimmunity and oral unresponsiveness

Despite the processes of negative and positive selection, healthy persons maintain low numbers of autoreactive T-cell and B-cell clones. Rather than a failure of immunological selection within the central immunological compartments, the existence of these autoreactive clones may indicate the presence of B-cell and T-cell clones that recognize dominant microbe-derived peptides to which a person may be exposed and which also resemble or mimic peptides derived from normal self-antigens. The healthy host must therefore maintain active peripheral mechanisms to prevent potential autoreactive clones from becoming autoimmune clones that participate in immune-mediated diseases. This is likely accomplished through suppressor T-cell networks, the effects of immunoregulatory cytokines (e.g. TGF-β, IL-10), the negative feedback effects of antibodies on B cells, the presentation of antigenic peptides to the TCR in the absence of an appropriate costimulatory signal, and the existence of antiidiotype networks (i.e., antibodies that bind to the antigen-binding site of an antibody and block its function) [106]. The end result of these mechanisms is active suppression, clonal anergy, or clonal deletion through apoptosis.

The gastrointestinal tract must continuously mount immune responses against bacteria, viruses, and other antigens. Individuals with a genetic predisposition for the dysregulation of self-reactive T cells are vulnerable to autoimmune disorders. Autoimmunity appears to play a role in the development of several chronic inflammatory disorders of the gastrointestinal tract, including celiac sprue, autoimmune gastritis, and IBD [107–112].

The gastrointestinal tract exhibits a fascinating example of specific tolerance to orally ingested antigens, called *oral tolerance* [113,114]. The oral administration of antigens can lead to systemic antigen-specific unresponsiveness, which results in the lack of specific T-cell and B-cell responsiveness to those antigens. Concurrently, local specific secretory immunity can develop, resulting in lymphoblasts capable of IgA production [115]. This dichotomy between systemic and mucosal compartments appears to reflect a solution to the need for excluding the specific antigen during future encounters and avoiding inappropriate systemic responsiveness. Not surprisingly, the gastrointestinal tract possesses mechanisms to protect against mounting adverse immunological reactions. If this were not the case, numerous bacterial and viral antigens and food components could lead to frequent cross-reactive immunological stimulatory events and result in a high frequency of intestinal autoimmune disorders, and many foods would give rise to diverse and uncontrollable food-induced allergic reactions.

Gastrointestinal inflammation

Inflammation is a prominent component of several important gastrointestinal diseases, including gastroesophageal reflux disease, *Helicobacter pylori* gastritis, eosinophilic gastritis, peptic ulcer disease, IBD, celiac disease, pancreatitis, primary sclerosing cholangitis, autoimmune hepatitis, and various other enteric infections. The inflammatory response can be viewed as an effector mechanism for the immune response. For example, when a bacterial infection activates the immune system, antibodies that are specific to the invading bacteria are produced and bind to the bacteria, and neutrophils that express antibody receptors on their surface efficiently phagocytose the antibody-coated bacteria. In this example, a portion of the inflammatory response – phagocytosis by neutrophils – acts as an effector mechanism for the humoral component of the immune response. The inflammatory response can also be viewed as a damage control mechanism that is invoked only if the immune response breaks down or is overwhelmed. Lastly, inflammation can be viewed as a component of the larger process of wound healing. After injury, blood clotting and fibrinolysis are followed by the infiltration of inflammatory cells. Repair of the epithelium and the migration of inflammatory cells are regulated by the same cytokines and mediators. Events that affect inflammation also affect epithelial healing, and events that affect epithelial healing also affect inflammation.

The immune processes largely eliminate foreign antigens without the development of clinically apparent inflammation. In fact, a limited state of immune activation is present in the healthy small intestine and colon due to the stimulation of lamina propria immune cells by the enormous antigen load in the intestinal lumen. Indeed, no other organ is faced with foreign antigens in such abundance. This basal inflammatory state is clearly demonstrated by comparing the gastrointestinal histology of rodents raised in germ-free conditions with those raised in conventional conditions. Germ-free rodents have fewer lymphocytes and macrophages, smaller Peyer patches with fewer germinal centers, and no plasma cells. The villi in germ-free rodents are thinner and the crypts are shallower. Epithelial proliferation is diminished, and the time required for epithelial cells to migrate from the crypt to the villus tip is doubled.

The development of clinically apparent inflammation suggests that the immune system has been overwhelmed by an abnormally large or invasive antigen load, by an antigen in a location inaccessible to the immune response (e.g., osteomyelitis), or by an antigen that is resistant to the immune response (e.g., tuberculosis). The inflammatory response likely evolved when our ancestors were confronted with unsanitary environments and infectious agents in contaminated food and water. Ingestion of viral, bacterial, and parasitic agents led to gastrointestinal tract infections, and the inflammatory response evolved to deal with them. Many of the inflammatory diseases of the gastrointestinal tract today reflect the activation of defense mechanisms that evolved in response to infectious agents but which have been adapted (or maladapted) to defend against noninfectious insults. Some chronic inflammatory diseases, such as IBD, may result from dysregulation of the immune response, resulting in an inappropriately prolonged and inappropriately amplified immune activation.

The epithelium is essential to the control and regulation of the inflammatory response in the gastrointestinal tract. The intact epithelium acts as a barrier, preventing the immune cells in the lamina propria from being overwhelmed by lumenal antigens. Breaks in the epithelial monolayer expose lamina propria immune cells to the several hundred species of bacteria that flourish in the human colon, resulting in a clinically apparent inflammatory response. The diversity of antigenic stimuli triggers nonspecific inflammatory responses. In diseases marked by breaks in the epithelial barrier, the immune or inflammatory response to the initiating agent may be difficult to distinguish in the midst of the response to the normal intestinal flora that invade through the epithelial breaks.

Gastrointestinal inflammation is remarkable in that diverse initiating events, such as infections, ischemia, radiation, and chemical toxins, all induce inflammatory responses that are clinically, endoscopically, and histologically similar.

For example, the colonoscopic and histological appearances of ulcerative colitis, radiation proctitis, shigellosis, and ischemic colitis are similar. The commonality of the inflammatory responses is explained partly by the similarity between the proinflammatory cytokines, the patterns of leukocyte migration, and the inflammatory mediators induced by these initiating events. Moreover, in each of these diseases, the inflammatory response is largely directed against normal colonic flora that has activated the immune cells of the lamina propria as a result of the loss of epithelial integrity.

Gastrointestinal inflammation is also distinguished by the organ-level physiological response, primarily increased motility and secretion [116,117]. The gastrointestinal tract responds to enteric infections by attempting to wash out the offending microbes with increased electrolyte and water secretion and increased motility, resulting in cramps and diarrhea. The increases in secretion and motility are mediated by the inflammatory response. Regardless of the initiating event, the gastrointestinal tract mounts a stereotypical inflammatory response. Therefore, the same increases in secretion and motility (and resultant diarrhea and cramping) are the responses to noninfectious inflammatory events (e.g., radiation enterocolitis and IBD) for which there are no pathogenic organisms to wash out. Consequently, both infectious and noninfectious gastrointestinal inflammatory disorders manifest similar clinical symptoms.

Immune cell trafficking and adhesion molecules

Neutrophil and monocyte trafficking

Intestinal inflammation and tissue damage cause circulating neutrophils, macrophages, and lymphocytes to home to the postcapillary venules located near the inflamed regions, and to migrate into the lamina propria through the spaces between adjacent endothelial cells. This process is mediated by the activation of distinct families of adhesion molecules expressed on the surfaces of leukocytes and endothelial cells.

Neutrophils enter the gastrointestinal tissues by binding to adhesion molecules on the endothelium of postcapillary venules. After 1 or 2 days in the gastrointestinal mucosa, they pass between epithelial cells into the intestinal lumen, where they die and are expelled in the stool. Although neutrophils differentiate in the bone marrow, they are primed and activated in the lamina propria [118]. Priming enhances the ability of neutrophils to produce reactive oxygen species. Neutrophil priming and activation are mediated by interaction with particulate stimuli (e.g., bacteria) or by stimulation with soluble factors, such as cytokines, inflammatory mediators, and bacterial products (e.g., endotoxins). Because neutrophils are incapable of proliferation, elevated numbers of neutrophils at sites of inflammation reflects increased trafficking out of the bloodstream and into the gastrointestinal tissues.

Gastrointestinal macrophages are derived from circulating monocytes produced in the bone marrow [119,120]. Monocytes enter the circulation and, like neutrophils, bind to adhesion molecules expressed on endothelial cells in the postcapillary venules of the intestine. Bound monocytes pass between endothelial cells and enter the gastrointestinal tissue, where they begin to differentiate into mature macrophages. As a monocyte differentiates, it can acquire capacities for phagocytosis, proliferation, and bacterial killing. Macrophage differentiation is controlled by cytokines and other soluble factors present in the lamina propria, and different combinations of cytokines and mediators result in macrophages with different phenotypes. As with neutrophils, the large increase in the number of macrophages during clinically apparent inflammation reflects increased migration of monocytes out of the bloodstream, rather than the proliferation of macrophages already in the lamina propria. Receptors on the surface of macrophages determine the stimuli to which the macrophage can respond, as well as the macrophage phenotype.

For the most part, neutrophil and monocyte trafficking in the gastrointestinal tract is similar to that in other organ systems; however, the number of leukocytes passing through the gastrointestinal tract greatly exceeds that in other organs. Because the surface area of the gastrointestinal tract is large, even the modest degree of inflammation seen in the healthy small intestine and colon involves the trafficking of a substantial number of monocytes and neutrophils. In diffuse inflammatory diseases of the gastrointestinal tract, such as ulcerative colitis, the trafficking of leukocytes through the inflamed mucosa expands to the point that most leukocytes produced in the bone marrow travel through the gastrointestinal mucosa into the lumen [121].

Adhesion molecules on leukocytes and on the vascular surface of endothelial cells mediate the migration of neutrophils and monocytes from the peripheral circulation into the lamina propria of the gastrointestinal tract (Fig. 7.8) [122,123]. The adhesion molecules that participate in the binding of inflammatory cells to the vascular endothelium fall into three subgroups: selectins, β_2-integrins, and the immunoglobulin superfamily of adhesion molecules. The initial adherence of leukocytes to the endothelium is mediated by selectins expressed on the surface of both the circulating leukocytes and the endothelial cells. The three members of the selectin family are L-selectin (leukocyte adhesion molecule-1 [LAM-1]), E-selectin (endothelial leukocyte adhesion molecule-1 [ELAM-1]), and P-selectin [124]. The natural ligands for all three selectins are sialylated Lewis X oligosaccharides, which are found on almost all cell types. Among the molecules with sialylated Lewis X moieties are the selectins themselves, so that L-selectin on neutrophils can bind to E-selectin or P-selectin on endothelial cells.

Selectin bonds are responsible for leukocyte rolling. Because only weak selectin bonds form between circulating leukocytes and endothelial cells, leukocytes can roll along the surface of the endothelium by making and breaking

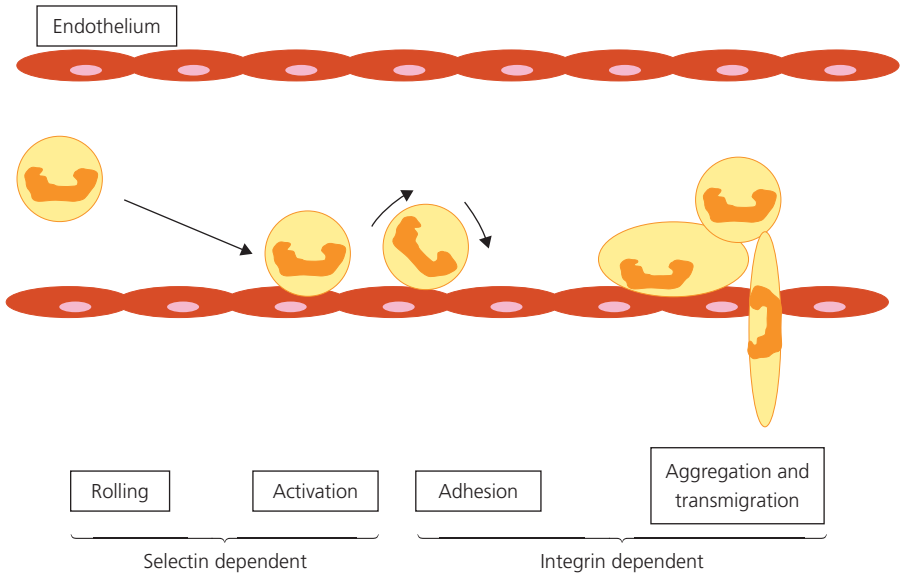

Figure 7.8 Adhesion and transmigration of leukocytes during intestinal inflammation. Leukocytes adhere to the vascular endothelium through cellular adhesion molecules. The processes of rolling and activation are dependent on selectins, whereas adhesion, aggregation, and transmigration are dependent on integrins. In the gut, specific integrins that mediate these processes are expressed on endothelial cells, such as mucosal addressin cell adhesion molecule-1 (MAdCAM-1), with its natural ligand on leukocytes being $\alpha_4\beta_7$.

selectin-mediated bonds. Rolling reduces leukocyte velocity before the formation of stronger adhesion bonds that fully immobilize leukocytes on the surface of the endothelium. These stronger bonds are formed between the remaining two families of adhesion molecules, β_2-integrins, which are expressed on the surface of the leukocytes, and members of the immunoglobulin superfamily of adhesion molecules (e.g., intracellular adhesion molecule-1 [ICAM-1], vascular cell adhesion molecule-1 [VCAM-1], and mucosal addressin cell adhesion molecule-1 [MAdCAM-1]), which are expressed on endothelial cells.

The integrins form a large group of adhesion molecules [125], each of which is a heterodimer that consists of noncovalently associated α and β subunits. Integrins are divided into subfamilies based on common β subunits, with the β_1 and β_2 subunits being the most important for inflammation (e.g., CD18, VLA, $\alpha_4\beta_7$). β_1-integrins are involved in lymphocyte trafficking, whereas β_2-integrins are involved in the adhesion of monocytes and neutrophils to endothelial cells [126]. The β_2-integrins CD11a/CD18 (leukocyte function-associated antigen-1 [LFA-1] or $\alpha_L\beta_2$) and CD11b/CD18 (macrophage-1 antigen [Mac-1] or $\alpha_M\beta_2$) bind to ICAM-1 on endothelial cells. CD11a/CD18 also binds to ICAM-2 and is expressed on neutrophils in their basal state. ICAM-1 and ICAM-2 are the most prominent members of the immunoglobulin superfamily of adhesion molecules and are expressed on endothelial cells in the basal state. They bind to β_2-integrins expressed on the surface of adhering leukocytes [122]. ICAM-2 is expressed at higher levels than ICAM-1. ICAM-2 expression is not increased by cytokine stimulation, whereas ICAM-1 expression is enhanced by IL-1, TNF-α, and IFN-γ. Thus, the relative importance of ICAM-1 increases in conditions of inflammation.

The final step in leukocyte trafficking in intestinal inflammation is the passage of leukocytes between epithelial cells and out into the lumen [127,128]. Leukocytes that have passed into the lumen can be found in the stool, which is a clinical sign of inflammation of the gastrointestinal tract. Adhesion molecules, including ICAM-1, and neutrophil chemotactic factors are expressed on epithelial cells in the presence of inflammation. Chemotactic factors have also been identified in the colonic lumen.

Lymphocyte trafficking

Lymphocyte trafficking shares many similarities with neutrophil and monocyte migration into the gastrointestinal tract. Lymphocyte trafficking is mediated by the expression of receptors and counterreceptors on lymphocytes and endothelial cells. As with leukocyte migration, the interaction of lymphocytes with endothelial cells involves three families of cell surface proteins: integrins, selectins, and immunoglobulin-like adhesion receptors. Although lymphocytes destined for the intestine display some integrins that are commonly expressed on leukocytes (e.g., CD11a/CD18), they also express α_4 integrins, which are predominantly expressed on lymphocytes (e.g., $\alpha_4\beta_7$, an integrin expressed in CD4 and CD8 gut trophic lymphocytes), and $\alpha_E\beta_7$, which is expressed on almost all intraepithelial lymphocytes and 40% of lamina propria lymphocytes. The $\alpha_4\beta_7$ integrin binds to MAdCAM-1, a member of the immunoglobulin superfamily that is selectively expressed on the high endothelial venules of mucosal lymphoid organs. Thus, the migration of selected lymphocyte populations to the intestine is directed by the selective expression of $\alpha_4\beta_7$ on these lymphocytes and the selective expression of MAdCAM-1 on certain endothelial populations. A highly specific receptor, CCR9, is expressed

on T cells that selectively migrate to the small intestine [129]. CCR9+ lymphocytes bind to a single chemokine ligand called CCL25, or TECK, which appears to be specifically expressed in the gastrointestinal tract. Regulated expression of selectins, integrins, and adhesion molecules controls the migration of naïve lymphocytes into Peyer patches through high endothelial venules and the subsequent homing of activated memory lymphocytes into the effector compartments of the loosely affiliated lamina propria.

Antiadhesion molecule therapy in gastrointestinal disease

Adhesion molecules are gatekeepers to circulating immune cells programmed to home to areas of intestinal inflammation. Interactions between integrins and adhesion molecules permit neutrophils, monocytes, and lymphocytes to cross the endothelial barrier and enter the inflamed lamina propria. Therapeutic strategies that block the ability of immune cells to migrate into the inflamed intestine could therefore be very effective for the treatment of acute and chronic inflammatory disorders of the gastrointestinal tract. This has been demonstrated in animal models of IBD in which blockade of ICAM-1 attenuates acute ileitis in the SAMP1/Yit mouse model of human Crohn's disease, as well as colitis induced in rats by administration of dextran sodium sulfate [130,131]. As a result, drug development strategies targeting adhesion molecules in humans first focused on the β_2-integrin/ICAM-1 interaction. Although blockade of ICAM-1 using antisense oligonucleotide therapy (alicaforsen, ISIS-2302) was inconclusive for the treatment of Crohn's disease, it may be effective for ulcerative colitis [132–135].

Clinical investigations of Crohn's disease have focused on monoclonal antibodies against the α_4 integrin, specifically blocking the interaction between $\alpha_4\beta_7$ and MAdCAM-1. MAdCAM-1 expression is specific to the gastrointestinal tract and during intestinal inflammation it is significantly upregulated. A large, randomized, placebo-controlled trial of 905 patients with active Crohn's disease showed that anti-α_4 integrin therapy (natalizumab) did not induce a significant clinical response or remission [136]. However, in patients who did respond, their response could be maintained by ongoing administration of natalizumab. The safety of natalizumab is an area of debate after a patient died of progressive multifocal leukoencephalopathy during an open-label extension of the clinical trial [137]. A second humanized antibody targeting the $\alpha_4\beta_7$ integrin (MLN02) has been extensively studied in patients with ulcerative colitis. A short-term clinical trial of 188 patients with ulcerative colitis showed that 33% of patients administered MLN02 achieved clinical remission by week 6 compared with only 14% of placebo-treated patients [138]. Preclinical studies in mouse models of Crohn's-like ileitis have also shown promising results using antibody neutralization of CCL25/CCR9, the chemokine receptor complex involved in lymphocyte homing to the small intestine [139]. Clinical studies using CCR9 antagonists are in progress. Taken together, these results indicate the potential value of treatment strategies that target adhesion molecules and related mechanisms of leukocyte trafficking for the treatment of IBD.

Leukocyte chemotaxis and activation

The migration of neutrophils across a polarized epithelium and into the lumen is a characteristic event of the active phase of several inflammatory diseases that affect the gastrointestinal and hepatobiliary tracts, including *H. pylori* gastritis, ulcerative colitis, Crohn's disease, bacterial enterocolitis, cholangitis, and acute cholecystitis. After the neutrophil has adhered to the endothelium and passed between the endothelial cells, it must migrate to the site of bacterial invasion or other injury by a process called *chemotaxis*. After entering the affected tissue, the neutrophil must acquire the ability to produce reactive oxygen species in maximal amounts [140]. The process of enhancing the capacity of the neutrophil for producing reactive oxygen species is called priming, and the stimulation of their production is called activation.

Chemoattractants are molecules that stimulate the migration of neutrophils, monocytes, eosinophils, and other cells. Chemotaxis is the migration of a cell in the direction of the higher concentration of chemoattractant. Chemotactic agents act by binding to specific receptors on the leukocyte plasma membrane, which triggers signaling, reorganization of the cytoskeleton, and movement of the leukocyte. Among the agents that induce neutrophil chemotaxis are IL-8 (CXCL8), formylmethionylleucylphenylalanine (fMLP), the complement component C5a, platelet-activating factor, and leukotriene B_4 (LTB_4). Each chemotactic agent has a separate receptor on the neutrophil plasma membrane. The direction of neutrophil migration after the binding of a chemoattractant to its receptor is determined by the location of the receptor on the neutrophil membrane. Receptors are distributed over the surface of the cells, and migration occurs in the direction of the receptor where ligand binding has occurred. Monocytes and eosinophils also are capable of chemotaxis, but they respond to a different range of agents.

The adhesion of neutrophils and monocytes to endothelial cells and their migration to the site of inflammation are two highly integrated components of the inflammatory response. The regulation of these events is coordinated by cytokines and inflammatory mediators. A single biological event, such as the phagocytosis of bacteria by a macrophage, results in the production of agents (e.g., IL-1) that induce the expression of adhesion molecules but not neutrophil chemotaxis, and other agents (e.g., IL-8 and LTB_4) that induce the expression of adhesion molecules and neutrophil chemotaxis. Some soluble mediators, such as eosinophil chemotactic factor, are chemotactic for only one cell type, whereas others act on multiple cell types. Thus, the mediators that induce the

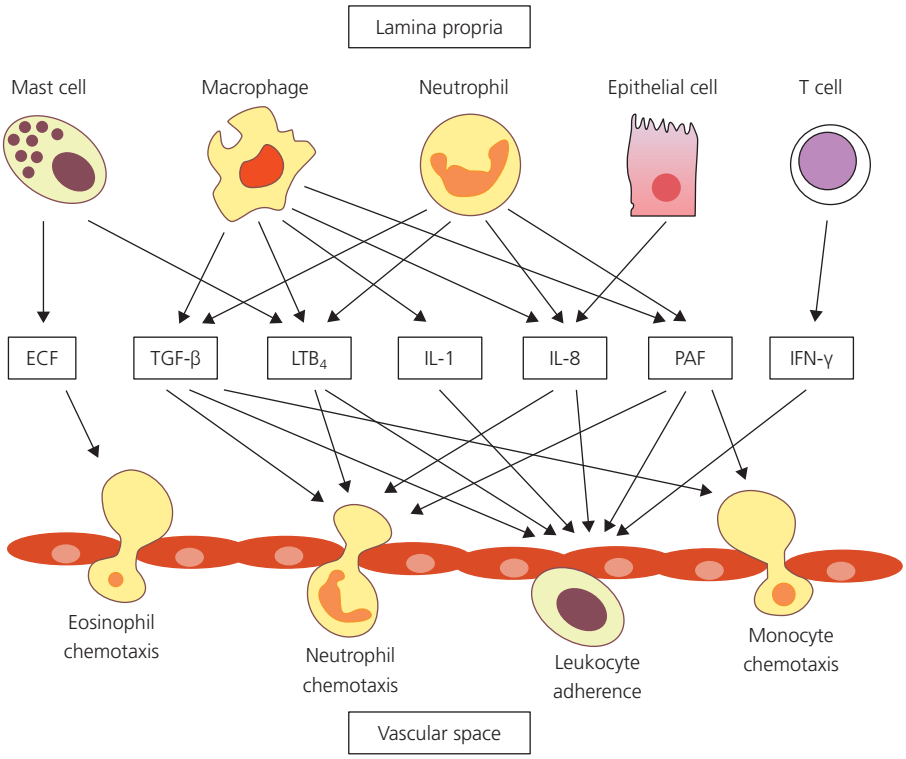

Figure 7.9 Network of chemotactic factors during intestinal inflammation. In the intestine, multiple cell types in the epithelium and lamina propria are capable of producing chemotactic factors including eosinophil chemotactic factor (ECF), transforming growth factor-β (TGF-β), leukotriene B_4 (LTB_4), interleukin (IL)-1, IL-8, platelet-activating factor (PAF), and interferon (IFN)-γ. Some inflammatory cells, especially macrophages and neutrophils, produce several of these factors, which can be specific for one cell type or multiple cell types.

expression of adhesion molecules and leukocyte chemotaxis constitute a complex and highly redundant network (Fig. 7.9). In chronic inflammatory diseases, multiple agents that induce leukocyte adhesion and chemotaxis act in parallel. The redundancy suggests that therapeutic agents aimed at one specific element in this complex network may not successfully block the inflammatory response.

The complexity of the network of chemotactic agents raises the question of whether all of these factors operate in every gastrointestinal inflammatory event, or whether some factors are prominent in certain inflammatory events but not in others. The composition of the inflammatory infiltrate may yield some insight. For example, a large number of eosinophils suggests the presence of eosinophil chemotactic factors. In most inflammatory states, it is likely that more than one chemotactic factor is operative and that different factors are more or less prominent as the inflammatory response develops. In response to infectious agents, neutrophil infiltration typically occurs within a few hours and monocyte infiltration occurs later, suggesting that factors promoting neutrophil chemotaxis appear earlier in the development of inflammation than those promoting monocyte chemotaxis.

Circulating neutrophils do not produce the maximum amount of reactive oxygen species that they are capable of producing. After entering the tissue, neutrophils are primed by IL-1, TNF-α, granulocyte macrophage colony-stimulating factor (GM-CSF), or lipopolysaccharide, which enhances their ability to make reactive oxygen species. Neutrophils can also be primed by exposure to the extracellular matrix. Exposure to C5a, LTB_4, or fMLP can activate the neutrophil respiratory burst with the production of superoxide, singlet oxygen, and hydroxyl radical. More typically, activation of the respiratory burst in neutrophils is the result of the phagocytosis of bacteria, which neutrophils kill with reactive oxygen species generated intracellularly within the phagosome. Although the generation of reactive oxygen species serves the useful purpose of killing bacteria, considerable evidence indicates that reactive oxygen species produced by neutrophils contribute to the tissue destruction seen in acute and chronic inflammatory diseases of the gastrointestinal tract, including IBD [141].

Neutrophil response to bacterial infection

Study of the chemotactic agents that affect neutrophils reveals connections between neutrophil chemotaxis and bacterial infection. The nature of the agents that induce neutrophil chemotaxis suggests that the inflammatory response evolved to deal with bacterial infections. fMLP is a synthetic peptide that mimics the activity of bacterially derived peptides with formylated N-terminal methionine groups. The binding of fMLP to its receptor results in the migration of neutrophils towards the bacteria [142]. The chemokine IL-8 is produced by a variety of cell types, including intestinal epithelial cells and activated monocytes. Intestinal epithelial cells produce IL-8 when infected with invasive bacterial

species such as *Salmonella* [143]. IL-8 serves as an early warning system for host events by triggering the influx of neutrophils to sites of bacterial invasion in the intestine. C5a production is also part of the immune response to bacterial invasion [144]. The binding of immunoglobulins to invading bacteria results in complement activation and the production of C5a, which attracts neutrophils to the site of the antibody-coated bacteria. Platelet-activating factor and LTB_4 are secondary amplification agents for the inflammatory response. Phagocytosis of invading bacteria activates neutrophils and macrophages, resulting in the production of platelet-activating factor and LTB_4, which in turn induce the migration of other neutrophils and macrophages to the site of bacterial invasion [145]. Thus, each of the neutrophil chemoattractant receptors can be related, directly or indirectly, to neutrophil migration to sites of bacterial infection. The multiplicity of mechanisms stimulating neutrophil migration towards sites of bacterial infection suggests the evolutionary importance of this response.

fMLP is a molecule of particular interest in neutrophil chemotaxis and activation in the gut [142]. *Escherichia coli* and other bacteria found in the normal colon produce a series of formulated oligopeptides, of which fMLP is the prototype. These formulated oligopeptides bind to fMLP receptors on neutrophils and macrophages. The intact epithelial monolayer acts as a barrier to the passage of fMLP, preventing the activation of lamina propria neutrophils by fMLP. Diseases marked by defects in the epithelial monolayer result in the exposure of lamina propria neutrophils to fMLP and the recruitment of additional neutrophils to the area of epithelial damage. Neutrophils have two classes of fMLP receptors: high-affinity receptors and low-affinity receptors. Binding of fMLP to the high-affinity receptor results in chemotaxis; binding to the low-affinity receptor activates the respiratory burst and other neutrophil functions involved in bacterial killing, suggesting a mechanism mediated by high-affinity fMLP receptors, by which neutrophil migration is induced by low concentrations of a bacterial product at a considerable distance from the bacteria. As the neutrophils migrate toward the bacteria, the concentration of fMLP increases and low-affinity receptors are activated. Binding of fMLP to low-affinity receptors initiates a series of events, particularly activation of the enzymes required for producing reactive oxygen species that prepare the neutrophils for bacterial killing.

Leukocyte chemotaxis in gastrointestinal disease

The activities of leukocyte chemotactic agents can be disease specific. Infection with the ulcer-causing pathogen *H. pylori* is characterized by the continuous recruitment of neutrophils to the inflamed gastric tissues. The *H. pylori* bacterium contains several chemotactic signaling proteins (cheW, cheA, and cheY) that are essential not only for the establishment of *H. pylori* infection but also for achieving and maintaining a high level of infection, as well as achieving colonization of all regions of the stomach [146]. The neutrophil-activating protein of *H. pylori*, HP-NAP, is a virulence factor that stimulates the transmigration of neutrophils across the endothelium and into the inflamed gastric mucosa. HP-NAP is also capable of inducing activated neutrophils of the innate immune response to express IL-12 and IL-23, which in turn promote an adaptive Th1 polarizing immune response within the *H. pylori*-infected mucosa [147].

Neutrophil migration has been studied more extensively in ulcerative colitis than in other gastrointestinal diseases. Ulcerative colitis is characterized by a massive influx of neutrophils into colonic sites of active inflammation. In contrast, defective neutrophil recruitment to inflamed lesions of the gastrointestinal tract is a primary pathological abnormality associated with Crohn's disease, the other IBD [148]. *Chemokines* are chemotactic cytokines that both promote leukocyte migration to areas of inflammation and initiate leukocyte activation. Mucosa affected by ulcerative colitis has high levels of several important chemokines and neutrophil chemotactic factors, including IL-8 (CXCL8), epithelial cell-derived neutrophil activator (ENA)-78 (CXCL5), growth-related oncogene (GRO)-α (CXCL1), and LTB_4 [149,150]. In ulcerative colitis, production of IL-8 by both macrophages and epithelial cells is induced by IL-1 and TNF-α, proinflammatory cytokines that are produced at increased levels. LTB_4 is produced primarily by activated neutrophils, and the mucosa affected by ulcerative colitis is heavily infiltrated with activated neutrophils. Inhibitors of 5-lipoxygenase, the key enzyme in the synthesis of LTB_4, are effective in the treatment of chemically induced animal models of colitis. Based on these animal studies, clinical trials of an inhibitor of 5-lipoxygenase and an inhibitor of 5-lipoxygenase-activating protein (FLAP) were performed in patients with acute ulcerative colitis. However, no differences in clinical response rates were observed between treated and untreated patients [151]. The failure of leukotriene synthesis inhibitors in human trials, despite success in animal models, may reflect the fact that inflammation had been present for weeks to months in the patients with ulcerative colitis, allowing time for the development of a more complex and redundant network of chemotactic agents.

Cytokines

Cytokines are an ever-expanding group of small protein hormones secreted by a variety of cell types, including immune cells (e.g., lymphocytes and macrophages) and nonimmune cells (e.g., endothelium, epithelium, fibroblasts, and smooth muscle cells). These regulatory proteins have autocrine, paracrine, and endocrine functions, and mediate immunity, inflammation, and hematopoiesis. Cytokines are produced de novo in response to immune stimulation. They generally act in a temporally and spatially limited manner, at very low concentrations. Cytokines have a high level of redundancy in

their effector functions, which include regulating the expression of membrane-bound proteins (including cytokine receptors) and regulating the proliferation, differentiation, and secretion of effector molecules. This redundancy, along with the pleiotropism of cytokine action, creates a complex network of cytokines that are difficult to classify.

Classification schemes for cytokines have focused on their functional roles (e.g., effector molecules vs regulatory molecules) and on the cells that produce them (e.g., Th1, Th2, Treg). For example, Th1 cells predominantly secrete IL-2 and IFN-γ and are associated with cell-mediated immunity; Th2 cells predominantly secrete IL-4, IL-5, IL-10, and IL-13 and are associated with humoral immunity; and Treg cells predominantly secrete IL-10 and TGF-β and are associated with suppression [152]. Despite the appeal of defining cytokines and T-cell clones in this manner, the pleiotropism and redundancy of cytokine action makes such classification difficult. Therefore it has become more common to describe combinations of cytokines as type 1 or type 2 when identifying patterns of cytokine production, so as not to overemphasize the source of the cytokine. These issues are important considering the increased evidence that certain idiopathic diseases, such as IBD, and inappropriate immune responses to certain infectious pathogens may be associated with distinct cytokine profiles.

The redundancy in cytokine effects is likely related to redundancy in their receptors, many of which are composed of common subunits shared between receptors [153]. Cytokine receptors are grouped into families and exert their effects by activating intracellular signaling pathways within a target cell. The hematopoietic cytokine receptor (R) family includes many of the interleukin receptors. A common feature of these receptors is that they function as multimers with each other and other nonhematopoietic cytokine receptor family members, such as IL-2αR or IL-15αR. The interferon receptor family includes the receptors for type I interferons (IFN-α and IFN-β) and type II interferon (IFN-γ); they share extracellular fibronectin-like domains with the hematopoietic cytokine receptors. There are two receptors for TNF-α, p55 (TNFR1) and p75 (TNFR2), which are homologous to CD95 (Fas antigen), CD40, CD27, and nerve growth factor receptor, and which are involved in proliferation and programmed cell death (apoptosis) [154]. Chemokine receptors are seven membrane-spanning G protein-linked receptors that are coupled to cell activation through calcium mobilization.

Cytokine expression in the gastrointestinal tract is tightly regulated to maintain homeostasis between proinflammatory and antiinflammatory cytokines. Some of the mechanisms used to regulate this balance include soluble cytokine receptors, such as the IL-18 binding protein (IL-18BP), which competitively binds secreted IL-18 and neutralizes its activity, and naturally occurring receptor antagonists, such as the IL-1 receptor antagonist (IL-1ra) [154]. IL-1ra specifically inhibits the proinflammatory actions of IL-1 by competitively binding to IL-1 receptors located on target cells with no known agonistic effects.

The relationship between chronic active inflammatory states and the development of malignancies is well established. Hepatitis B and hepatitis C can lead to hepatocellular carcinoma, and infection with certain strains of human papilloma virus can cause cervical cancer. Within the gastrointestinal tract, ulcerative colitis is associated with an elevated risk of developing colorectal cancer, and infection with *H. pylori* is a risk factor for gastric carcinoma. Although the mechanisms by which a chronic inflammatory response leads to carcinoma are still unclear, cytokine production may be an integral part of the process. Genetic polymorphisms for the proinflammatory cytokine IL-1β are associated with an elevated relative risk of developing gastric cancer [155]. In addition to its proinflammatory properties, IL-1 is also a potent inhibitor of acid secretion. Whether or not these polymorphisms are causative for gastric cancer remains unknown. However, the associations emphasize the importance of cytokines as mediators of inflammatory responses and tumorigenicity. Cytokine polymorphism profiles may also prove to be useful screening tools for identifying patients at risk of developing gastric carcinomas.

In chronic inflammatory diseases that result from a dysregulated immune response to pathogens, neutralizing monoclonal antibodies targeting key proinflammatory cytokines can mitigate inflammation and restore gut homeostasis. A classic example of such cytokine-targeted therapies is the use of anti-TNF monoclonal antibodies to treat IBD. In clinical trials, the chimeric anti-TNF monoclonal antibody infliximab induced a clinical response in more than 60% of patients with steroid-refractory and fistulizing Crohn's disease and nearly 70% of patients with steroid-refractory ulcerative colitis [156–158].

Lipid mediators of inflammation

Prostaglandins, leukotrienes, and platelet-activating factor are referred to collectively as lipid mediators of inflammation. They are produced under many circumstances by the same cell types in response to similar stimuli. Phagocytosis in macrophages results in the production of prostaglandin E_2 (PGE_2), LTB_4, and platelet-activating factor. Although the lipid mediators are structurally distinct, they have many overlapping biological effects: PGE_2, LTB_4, and platelet-activating factor enhance vascular permeability; LTB_4 and platelet-activating factor activate neutrophils.

Prostaglandins and leukotrienes are the products of arachidonic acid metabolism and are referred to as eicosanoids [150,159,160]. Prostaglandins are produced through the cyclooxygenase (COX) pathway, whereas leukotrienes are produced through the 5-lipoxygenase pathway. Two distinct cyclooxygenases are involved in prostaglandin synthesis. COX-1 is a constitutive enzyme found in most mammalian

cell types. In the gastrointestinal tract, COX-1 is expressed in lamina propria mononuclear cells, fibroblasts, muscle cells, epithelial cells, and vascular endothelial cells. COX-2 is expressed in response to inducing stimuli, including IL-1, TNF-α, and other proinflammatory cytokines. COX-2 expression can be induced in macrophages, fibroblasts, epithelial cells, and other cell types.

Under most circumstances, the rate-limiting step in the synthesis of prostaglandins is the availability of arachidonic acid. Under basal conditions, cells have almost no free arachidonic acid. The activation of phospholipases (particularly phospholipase A_2) makes arachidonate available by releasing it from membrane phospholipids. A wide variety of stimuli can activate phospholipase A_2, resulting in the release of arachidonate. Phagocytosis in macrophages activates phospholipase A_2, as do receptor-mediated events such as the binding of bradykinin to its receptor. Activation of phospholipase typically results in the rapid release of arachidonate and the production of prostaglandins within a few seconds up to 1 or 2 min. Synthesis of prostaglandins can also be regulated by the infiltration of prostaglandin-producing cells into the gastrointestinal tract. The large increase in prostaglandin levels in IBD may reflect the trafficking of prostaglandin-producing monocytes and macrophages into the lamina propria. A third mechanism for the regulation of prostaglandin production is the promotion of the synthesis of COX-1 and COX-2. Synthesis of COX-2 can be induced by proinflammatory cytokines (e.g., IL-1, TNF-α) and phorbol esters, and occurs over a few hours. Thus, the regulation of prostaglandin production by the induction of COX-2 occurs over a longer time frame than the regulation of prostaglandin production by phospholipase activation.

Among the products of arachidonic acid metabolism through the COX pathway are PGE_2, thromboxane A_2, and prostacyclin. PGE_2 is made by macrophages, epithelial cells, and fibroblasts, and has several biological functions that suggest a role in inflammation and tumor growth. The binding of PGE_2 to its receptor initiates a signaling cascade that controls cell proliferation and migration, apoptosis, and angiogenesis. PGE_2 is also capable of stimulating tumor metastasis and inhibiting tumor surveillance through the down-regulation of cytokine production and natural killer activity. Finally, PGE_2 can cause DNA oxidation, which can lead to carcinogenic mutations. Epidemiological studies of colorectal cancer have demonstrated that the use of nonsteroidal antiinflammatory drugs (NSAIDs), which block cyclooxygenase activity and inhibit PGE_2 production, is associated with a lower incidence of colorectal cancer [161]. These studies further point to the link between chronic inflammation and the subsequent development of malignancy, and have resulted in a renewed interest in NSAIDs as chemopreventive agents for patients who are at high risk for developing colorectal cancer, including patients with ulcerative colitis, familial adenomatous polyposis, or hereditary nonpolyposis colon cancer. Newer NSAIDs (e.g., celecoxib, rofecoxib) selectively block COX-2 production and were hypothesized to have better safety profiles than the original inhibitors, which block both COX-1 and COX-2; however, large clinical trials of both drugs have shown that long-term use is associated with an increased risk of cardiovascular events [162–164].

Leukotrienes are made through the 5-lipoxygenase pathway. The cellular distribution of 5-lipoxygenase is much more limited than that of COX-1 and COX-2. LTB_4, a potent neutrophil chemoattractant, is produced by neutrophils, macrophages, and mast cells. The peptidyl leukotrienes (LTC_4, LTD_4, and LTE_4) are made in macrophages, mast cells, and eosinophils. They increase vascular permeability and induce vasoconstriction and smooth muscle contraction, and they may induce epithelial Cl^- secretion.

Inflamed mucosa from patients with Crohn's disease and ulcerative colitis contains markedly elevated levels of PGE_2 and LTB_4 [165]. Analysis of neutrophil chemotaxis in ulcerative colitis showed that LTB_4 is a major neutrophil chemoattractant; however, randomized controlled trials in which 5-lipoxygenase inhibitors are used as single therapeutic agents have not shown effective results in treating ulcerative colitis. In many inflammatory diseases, NSAIDs, which block COX-1 and COX-2 production, diminish inflammation and relieve clinical symptoms; however, in ulcerative colitis, NSAIDs exacerbate clinical activity. Elevated PGE_2 levels in ulcerative colitis are associated with enhanced COX-2 expression. Immunohistochemical studies demonstrated COX-2 expression in epithelial cells from inflamed tissue affected by ulcerative colitis [166].

Platelet-activating factor is a phosphatidylcholine with an ether-linked alcohol fatty acid on the first carbon and an acyl-linked acetyl group on the second carbon. There are no intracellular stores of platelet-activating factor and its synthesis is initiated by the remodeling of phosphatidylcholine through the activation of phospholipase A_2. Platelet-activating factor is made by neutrophils, macrophages, mast cells, and eosinophils, and it is often produced in parallel with prostaglandins and leukotrienes. The biological effects of platelet-activating factor include enhanced vascular permeability, vasoconstriction, platelet aggregation, neutrophil chemotaxis, smooth muscle contraction, and epithelial Cl^- secretion. Many of these biological effects overlap with those of prostaglandins and leukotrienes. Platelet-activating factor is rapidly degraded to the inactive metabolite lyso-platelet-activating factor by the enzyme acetylhydrolase. Acetylhydrolase has both intracellular and secreted forms. Intestinal epithelial cells secrete acetylhydrolase, which may be an important mechanism for the defense of the gastrointestinal tract against platelet-activating factor [167]. Exposure of neonatal piglets to platelet-activating factor results in intestinal hemorrhagic lesions and intestinal injury consistent with necrotizing enterocolitis [168].

Nitric oxide

Nitric oxide is a small, biologically active compound formed when nitric oxide synthase (NOS) oxidizes the guanidine nitrogen of arginine. The biological effects of nitric oxide include actions as a vasodilator, a neurotransmitter, and an important component of the inflammatory response [169,170].

The three distinct isoforms of NOS differ in their cofactor requirements, tissue distribution, transcriptional regulation, and posttranscriptional modification. Neuronal NOS (nNOS) is constitutively expressed and is found in neuronal tissue. The nitric oxide produced by nNOS is the principal nonadrenergic noncholinergic (NANC) neurotransmitter in the gastrointestinal tract. nNOS is found in enteric nerves in the myenteric plexus and circular muscle and is present in neurons containing vasoactive intestinal polypeptide (VIP) [171]. Nitric oxide produced by nNOS participates in the control of peristalsis and sphincter function in the gut. Impaired nitric oxide production through nNOS may contribute to several disorders of bowel motility, including achalasia, functional dyspepsia, diabetic gastroparesis, infantile hypertrophic pyloric stenosis, intestinal pseudoobstruction, and colitis [172].

Endothelial NOS (eNOS) is also constitutively expressed and was originally found in endothelial cells, although it is also present in epithelial cells, smooth muscle cells, platelets, and T cells. Nitric oxide produced by endothelial cells relaxes vascular smooth muscle cells and dilates the vasculature. Inhibition of eNOS in vivo causes an increase in blood pressure. Hypertension is the major side effect of nonspecific NOS inhibitors. nNOS and eNOS synthesize nitric oxide in relatively small quantities. The physiological effects of nitric oxide as a vasodilator and as a neurotransmitter occur at quite low concentrations and are transient. Calcium-dependent enzymes control the transient expression of both nNOS and eNOS, and both are inhibited by calmodulin.

Unlike nNOS and eNOS, which are constitutively expressed, the third NOS isoform, inducible NOS (iNOS), can be induced by proinflammatory cytokines (e.g., IL-1, TNF-α, IFN-γ) and by bacterial lipopolysaccharide. iNOS is produced in nanomolar concentrations compared with nNOS and eNOS, which are produced in picomolar concentrations. Therefore, iNOS produces nitric oxide in quantities that greatly exceed those required for its physiological function as a vasodilator or neurotransmitter. In addition, nitric oxide production by iNOS is delayed for several hours after stimulation but remains active for up to 5 days. In macrophages, the large quantity of nitric oxide produced by iNOS is used for killing bacteria and tumor cells [173]. Nitric oxide works in conjunction with reactive oxygen species (i.e., hydroxyl radical, superoxide, and hydrogen peroxide) generated within phagosomes to kill phagocytosed bacteria.

iNOS is induced in intestinal epithelial cells in inflammatory states, such as IBD, infectious colitis, and diverticulitis [174,175]. IL-1, TNF-α, IFN-γ, and lipopolysaccharide induce iNOS in epithelial cells in vitro. However, a critical question is whether or not nitric oxide plays a pathogenic or protective role during intestinal inflammation [176]. Many studies have demonstrated that the biological effects of nitric oxide are organ specific and vary depending on the amount, the timing of production, and the particular inflammatory state. Administration of nitric oxide is protective to the intestinal mucosa, and results in maintenance of blood flow, inhibition of platelets and leukocyte adhesion, down-regulation of mast cell reactivity, and reduction of superoxide-induced damage. However, increased production of iNOS-induced nitric oxide by intestinal epithelial cells is associated with prolonged colonic inflammation. Studies using animal models of colitis have generated interesting hypotheses regarding the complex role of nitric oxide in intestinal inflammation. Animal models of chemically induced colitis that used NOS inhibitors to block nitric oxide production produced conflicting results – nitric oxide either improved or exacerbated the colitis [177–182]. Time-dependent studies of endotoxin-induced vascular damage have shown that early administration of NOS inhibitors results in increased tissue damage, whereas late administration results in a dose-dependent reduction in damage [183]. The specific NOS isoform has also been proposed to be a factor influencing the beneficial or pathogenic effects of nitric oxide in intestinal inflammation. However, studies using mice that lack specific NOS isoforms (e.g., nNOS–/–, eNOS–/–, iNOS–/–) have also produced conflicting results [184,185]. A possible explanation may be that iNOS-induced nitric oxide is protective during the early phases of acute inflammation but that continuous overproduction during chronic inflammation contributes to inflammation. Because of this functional complexity, it is likely that the clinical efficacy of any drug therapies that are developed to target nitric oxide or NOS production will be influenced primarily by the specific NOS isoform present and cell types affected, as well as by the timing of therapy.

Intestinal epithelial cells

Intestinal epithelial cells interact with the inflammatory response at several levels. The secretion of electrolytes and water by epithelial cells is an important part of the gastrointestinal response to inflammation. The interaction of inflammation and epithelial cell electrolyte and water secretion has been studied by assessing the effects of individual inflammatory mediators on electrolyte and water secretion, or by identifying the mediators that regulate electrolyte and water secretion in specific inflammatory states. Histamine, PGE_2, serotonin (5-hydroxytryptamine, 5-HT), and LTB_4 are just a few of the inflammatory mediators that induce epithelial cell Cl^- secretion (Fig. 7.10). Neural mechanisms also affect epithelial cell Cl^- secretion directly or indirectly through inflammatory cells. The neurotransmitter acetylcholine induces Cl^- secretion in epithelial cells directly [186].

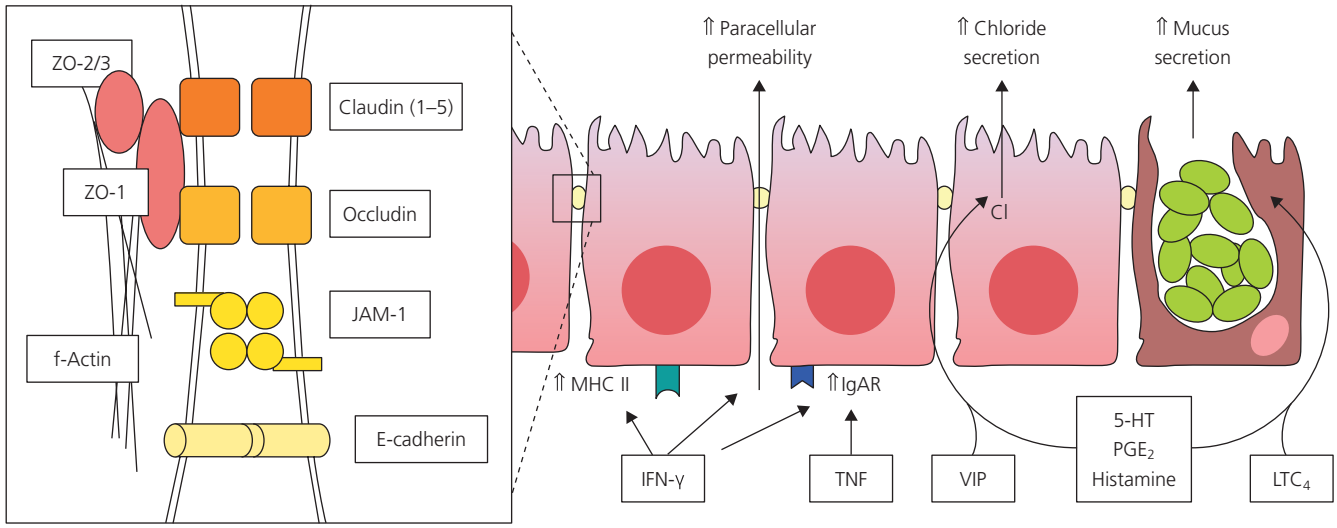

Figure 7.10 Epithelial barrier structure and function. The epithelium serves as a barrier that separates lumenal antigens from the inflammatory cells of the lamina propria. The epithelial barrier is regulated by paracellular pathways that are controlled by tight junctions between adjacent epithelial cells. Tight junctions are composed of several tight junctional proteins, including occludins, members of the claudin family, the junctional adhesion molecule (JAM)-1, ZO-1, ZO-2, ZO-3, f-actin, and E-cadherin. The tight junctional proteins function together as a complex to regulate selective intestinal permeability through the epithelial barrier (inset). The secretion of electrolytes and water by epithelial cells is an important component of the gastrointestinal response to inflammation. Histamine, prostaglandin E_2 (PGE_2), serotonin (5-HT), and leukotriene C_4 (LTC_4) are inflammatory mediators that induce epithelial cell Cl^- secretion within the intestine. Neuropeptides, such as vasoactive intestinal polypeptide (VIP), induce mast cell activation with release of histamine and 5-HT, resulting in epithelial cell Cl^- secretion. Cl^- secretion is accompanied by Na^+ secretion and, consequently, by the passage of water across the epithelium into the intestinal lumen, with resulting diarrhea. IFN-γ, interferon-γ; IgAR, receptor for immunoglobulin A; MHC II, major histocompatibility complex class II molecules; TNF, tumor necrosis factor.

Neuropeptides such as substance P, VIP, and neuropeptide Y induce mast cell activation, resulting in the release of histamine and 5-HT and thus the activation of epithelial cell Cl^- secretion [187]. Cl^- secretion is accompanied by Na^+ secretion and, consequently, by the passage of water across the epithelium into the intestinal lumen. Diarrhea is the clinical manifestation of the enhanced enterocyte Cl^- secretion induced by these mediators. These same inflammatory mediators induce the secretion of mucus by goblet cells in the gastrointestinal tract. Diarrhea protects the host from infectious agents and their toxins by speeding their passage through the gastrointestinal tract and out of the organism. Mucus secretion protects the host from infectious agents in the gastrointestinal tract by preventing the binding of the infectious agents and their toxins to intestinal epithelial cells.

The interaction of inflammation and epithelial electrolyte and water secretion has also been studied by identifying the inflammatory mediators that regulate electrolyte and water secretion in specific inflammatory conditions. One relatively simple animal model of intestinal inflammation is the sensitization of rats to egg albumin followed by antigenic challenge. In this model, antigenic challenge results in increased Cl^- secretion and increased paracellular permeability [188]. The increase in paracellular permeability was demonstrated by the increase in the uptake of chromium 51-EDTA from the intestinal lumen after antigen challenge of previously sensitized rats. The relative contribution of various inflammatory mediators to the increase in Cl^- secretion seen in this model was tested with a series of blocking agents. Ketanserin (a 5-HT antagonist), diphenhydramine (a histamine H_1 antagonist), and piroxicam (a COX inhibitor) inhibited egg albumin-induced Cl^- secretion by 30%, 42%, and 52% respectively. The combination of piroxicam and diphenhydramine inhibited the secretory response by 82%. These data suggest that, even in this simple model, more than one inflammatory mediator contributes to the increase in Cl^- secretion, and the mediators involved indicate that more than one cell type produces mediators. Histamine and 5-HT are products of mast cell activation. PGE_2, the prostaglandin most likely to be involved in Cl^- secretion, is produced by fibroblasts or epithelial cells themselves. In a similar study, challenge of *Trichinella*-immunized rats with *Trichinella* larvae resulted in increased intestinal fluid secretion [189]. Treatment of the rats with the combination of indomethacin (indometacin), a COX inhibitor, and diphenhydramine ablated the increase in the fluid secretion induced by exposure to *Trichinella*. In the *Trichinella* model, increased fluid secretion is caused by a combination of prostaglandins and histamine, whereas in the egg albumin model, increased secretion results from a combination of prostaglandins,

histamine, and 5-HT. Stimulation of intestinal epithelial cells with proinflammatory cytokines such as TNF-α and IFN-γ inhibits Cl^- secretion. Interestingly, pretreatment with probiotics or commensal bacteria, which are essential to the normal development of the mucosal immune system, restores normal levels of Cl^- secretion, suggesting a possible role for these agents in gastrointestinal inflammatory disorders [190].

Epithelial barrier function

The epithelium serves as a barrier that physically separates lumenal antigens from the lamina propria inflammatory cells, thus preventing activation of the inflammatory cells by antigens. However, the epithelial barrier is not impenetrable and it uses sophisticated mechanisms to regulate the trafficking of macromolecules between the environment and the host, primarily through paracellular pathways that are controlled by tight junctions (see Fig. 7.10) [191,192]. Tight junctions were originally believed to be impermeable, but are now recognized to be composed of several tight junctional proteins (e.g., occludin, members of the claudin family, the junctional adhesion molecule [JAM], ZO-1, ZO-2, and ZO-3) that function together as a complex to regulate selective intestinal permeability through the epithelial barrier. Tight junctions are dynamic structures that are capable of rapidly altering permeability in response to extracellular stimuli. Barrier disruption is seen in several gastrointestinal disorders. Enteropathogenic *E. coli* and *Giardia* infections can both induce large increases in tight junction permeability in vitro [193,194]. During inflammation, increased production of proinflammatory cytokines, including TNF-α and IFN-γ, causes reorganization of several tight junctional proteins, including ZO-1, JAM-1, occludin, claudin-1, and claudin-4, and results in increased permeability, or a "leaky gut." Intestinal barrier dysfunction has been proposed to be a major mechanism of disease pathogenesis in IBD, and may possibly even represent the primary defect. Under normal conditions, SAMP-1/Yit mice naturally develop a chronic ileitis that closely resembles human Crohn's disease. Wild-type mice that receive bone marrow chimera from SAMP-1/Yit mice do not develop ileitis. However, SAMP-1/Yit mice that are reconstituted with bone marrow chimera from wild-type mice develop severe ileitis and have increased epithelial permeability, which precedes the onset of ileitis [195]. These data suggest that the primary defect in the SAMP-1/Yit mouse model of human Crohn's disease has a non-hematopoietic source and likely relates to epithelial barrier dysfunction.

One consequence of many forms of intestinal injury is the development of breaks in the epithelial barrier. These breaks are seen in infectious disease, IBD, celiac disease, and injury caused by radiation and chemotherapeutic agents. There is an orchestrated response to these breaks beginning with the rapid migration of epithelial cells shouldering the wound to cover the defect. Epithelial cells elongate and cover the broad areas of denuded mucosal surface (see Chapter 8).

Epithelial cells and antigen presentation

Absorptive intestinal epithelial cells can also function as antigen-presenting cells. Epithelial cells of the small intestine constitutively express MHC class II molecules, possibly as a consequence of the intraepithelial lymphocyte secretion of IFN-γ [196]. Colonic epithelial cells do not normally express measurable levels of MHC class II molecules, except in the setting of inflammation, presumably in response to local cytokine production [197]. In vitro studies of intestinal epithelial cell function show that they take up, process, and present soluble antigens to CD4+ T cells in the context of MHC class II molecules [198]. Although soluble antigens can be taken up apically and basolaterally, MHC class II molecules primarily segregate in the basolateral region, where antigen presentation to antigen-specific, MHC class II-restricted T cells occurs. There is evidence that, under normal conditions, intestinal epithelial cells take up, process, and present soluble antigens from the lumen in vivo. The implications of these studies are that intestinal epithelial cells may augment or modify afferent pathways that normally result from antigenic events within Peyer patches. Despite in vivo evidence for functional class II MHC expression, intestinal epithelial cells seem to preferentially engage and stimulate CD8+ cells that exhibit suppressor activity, an activity that may contribute to the suppressor tone of the intestine [199,200].

Epithelial cell gene expression

In the presence of inflammation, epithelial cells express a series of genes that are not expressed in the absence of inflammation, including genes coding for COX-2 [166,201], iNOS [174,202], and IL-8 [143]. An immunohistochemical study of the human colon revealed expression of iNOS in epithelial cells from areas of inflammation in ulcerative colitis, Crohn's disease, and diverticulitis, but not in epithelial cells from uninflamed areas of the same surgical resections [174]. In parallel studies of the cellular distribution of iNOS and COX-2 in ulcerative colitis resections, both of these proteins were expressed in exactly the same populations of epithelial cells in areas of inflammation [166]. This colocalization suggests a common regulatory mechanism for the expression of these genes. The genes for IL-8, iNOS, and COX-2 each have an NF-κB site in their promoters. NF-κB is an important transcription factor in the regulation of the synthesis of numerous inflammation-related proteins (e.g., TNF-α, IL-1, TLR-2, ICAM-1, E-selectin, IL-8, iNOS, and COX-2) [203].

NF-κB is a heterodimer that consists of p50 and p65 subunits. In unstimulated cells, NF-κB is bound to IκB-α, which is found in the cytoplasm. When cells are stimulated, IκB-α is phosphorylated and degraded, and NF-κB is released. The

release of NF-κB from IκB-α allows NF-κB to enter the nucleus, where it binds to the promoter regions of target genes. NF-κB is activated by proinflammatory cytokines (e.g., TNF-α and IL-1), oxidants, phorbol esters, platelet-activating factor, and lipopolysaccharide. The importance of NF-κB in the response to infection is demonstrated by the finding that mice lacking the p50 subunit of NF-κB are unable to clear *Listeria* species and other organisms effectively [204]. However, given its many functions in regulating the immune response, cell cycle, and cell death, it is unclear whether NF-κB has potential as a therapeutic target for inflammatory disease [205]. Drug-induced inhibition of NF-κB could lead to serious detrimental effects on normal cellular function and response.

Activation of NF-κB results in the parallel stimulation of a number of important genes involved in the innate and adaptive inflammatory responses. It is this parallel stimulation that probably accounts for the coexpression of iNOS and COX-2 in the same population of epithelial cells in ulcerative colitis. The presence of NF-κB response elements in the genes for E-selectin, ICAM-1, IL-8, and TNF-α allows for the coordinated expression of a series of proteins involved in the adhesion of neutrophils to the endothelium, the migration of neutrophils from the vascular space into gastrointestinal tissue, and the activation of those neutrophils. IL-1, TNF-α, and NF-κB are involved in a cycle of activation that results in amplification of the inflammatory response. IL-1 and TNF-α both activate NF-κB; in turn, the synthesis of IL-1 and TNF-α is promoted by the binding of NF-κB to response elements in their promoters. This positive regulatory cycle amplifies and perpetuates the inflammatory response.

Epithelial cells and toll-like receptors

Toll-like receptors (TLRs) are members of the pattern-recognition receptor family and are important components of the innate immune system of the gastrointestinal tract [206]. TLRs recognize pathogen-associated molecular patterns (PAMPs), which are highly conserved and found on a broad spectrum of microorganisms, but not expressed by host cells. On activation, TLRs initiate a signaling cascade that results in the induction of several genes involved in the innate immune response to infection, including cytokines and chemokines, as well as molecules involved in antigen presentation and costimulatory molecules. TLRs are therefore a frontline mechanism of host defense and can result in rapid detection of a wide variety of microorganisms.

TLRs are sometimes expressed on the surface of intestinal epithelial cells. However, as the lumen is full of commensal bacteria, TLRs must possess a mechanism for tolerance to the PAMPs expressed by the nonpathogenic resident bacteria in the lumen, while also maintaining the capacity to mount an effective immune response against any invasive microorganisms. As a result, intestinal epithelial cells down-regulate surface expression of TLR2 and TLR4, which recognize bacterial lipoproteins (e.g., peptidoglycan and lipopolysaccharide). TLR2 and TLR4 are most commonly found intracellularly or on the basolateral surface of the intestinal epithelial cell, thereby effectively limiting their exposure to PAMP-expressing commensal bacteria that reside in the lumen [207–210]. Crypt epithelial cells express TLR2 and TLR4, while mature intestinal epithelial cells express TLR3 [211]. Because crypt epithelial cells are protected by their location from exposure to lumenal bacteria and their PAMPs, surface expression of TLR2 and TLR4 poses a minimal risk to the host defense. Similarly, expression of TLR3 on exposed intestinal epithelial cells does not lead to overstimulation of the innate immune system because TLR3 binds double-stranded RNA from viruses, which are not found in the lumen under normal conditions. A second mechanism for regulating TLR activation is the expression of high levels of TLR antagonists. These *TLR-attenuating factors* (TOLLIP, PPAR-γ, SIGIRR) block TLR signaling by interacting with components of the signaling cascade [212]. Through these regulatory mechanisms, TLRs maintain a careful balance between achieving tolerance for the resident microflora of the gut and rapidly detecting invasive pathogens and mounting immune responses against their presence in the epithelium and lamina propria of the gastrointestinal tract.

Because of their pivotal role in maintaining gut homeostasis, TLRs have been implicated in several gastrointestinal diseases. The cause of IBD is unknown; however, it is believed to result from a dysregulated immune response to lumenal antigens in a genetically susceptible host. Loss of tolerance to the commensal microflora in genetically susceptible hosts, possibly as a result of abnormalities in the TLR/TLR antagonist system, may be the primary defect that leads to the development of Crohn's disease and ulcerative colitis [213]. Patients with Crohn's disease and ulcerative colitis express elevated levels of TLR4 in their colons [214], and polymorphisms in the TLR4 gene have been associated with the development of both diseases [215,216]. In addition, PPAR-γ expression by intestinal epithelial cells is decreased in patients with ulcerative colitis [217]. IL-2-deficient mice, which develop colitis similar to human ulcerative colitis, show increased TLR2 responsiveness to colonic bacteria [218]. These observations suggest that abnormalities in the expression of TLRs or their antagonists may be central to the development of IBD. TLRs may also play a role in *H. pylori*-induced gastritis, as well as gastrointestinal bacterial, viral, and parasitic infections.

Mast cells

Mast cells are inflammatory cells with large granules containing preformed mediators of inflammation (e.g., histamine and 5-HT). In response to stimulation, mast cells release these granules and produce newly formed non-granule-associated mediators (e.g., NO, PGD_2, platelet-activating factor, and leukotrienes). In the healthy gastrointestinal

tract, mast cells are found in the lamina propria, submucosa, and muscle layers, as well as on the serosal surface [219]. More numerous and activated mast cells have been observed in the gastrointestinal mucosa of patients with helminthic infections, ulcerative colitis, Crohn's disease, gastritis, and celiac sprue.

Mast cells can be activated by a variety of factors, but IgE-dependent antigen activation is the most common. Antigen-specific IgE binds to receptors on the surface of the mast cell through its Fc component. Exposure of the mast cell to an appropriate antigen results in cross-linking of the IgE molecules, which in turn results in activation of the mast cell. IgE-mediated mast cell activation is an effective defense mechanism against intestinal worms and other parasites. Mast cell activation results in the release of substances (e.g., histamine, PGD_2, and peptidyl leukotrienes) that promote intestinal motility and increase electrolyte and water secretion. These physiological responses allow the infected host to wash the parasites out of the digestive tract. IgE-mediated mast cell activation is also important in allergic disorders. Pollen cross-links IgE molecules on mast cells in the nasal mucosa, causing allergic rhinitis. Food antigens activate mast cells in the gastrointestinal tract by similar mechanisms. There would appear to be no evolutionary advantage to being able to mount an allergic response to gut parasites that would clear them from the gastrointestinal tract. It may be that food allergies represent the maladaptation of an inflammatory response designed to deal with intestinal parasites.

In addition to IgE-dependent antigen activation, mast cells also can be activated by the calcium ionophore A23187 and by the complement components C3a and C5a. Some subpopulations can be activated by substance P, which is a neurotransmitter that can be released by neural activation in the gastrointestinal tract. Substance P activation is of particular interest because it could provide a mechanism for the induction of intestinal inflammation by neural activation. There are both functional and anatomical interactions between mast cells and the enteric nervous system. In the rat, infection with the intestinal nematode *Nippostrongylus brasiliensis* results in mast cell hyperplasia. Immunohistochemical studies reveal that most of these mast cells are juxtaposed to enteric nerves. There are bidirectional interactions between nerves and mast cells. Neurotransmitters, particularly substance P, cause mast cell degranulation, and mast cells in turn release VIP, which can act as a neurotransmitter.

Mast cells are involved in the mediation of enhanced Cl^- secretion after antigen exposure. Evidence for this functional relationship comes from studies of the mast cell-deficient mouse (W-WV). Antigen challenge in sensitized W-WV mice results in a 70% decrease in Cl^- secretion in comparison with antigen challenge in wild-type littermates [220]. The Cl^- secretion measured in mast cell-deficient mice (30% of that in wild-type mice) could be inhibited with NSAIDs, suggesting that the prostaglandins that induce Cl^- secretion are not of mast cell origin.

Immune-mediated gastrointestinal and liver diseases

A well-functioning immune system is essential to gastrointestinal health. Abnormalities in the mucosal immune system underlie a broad range of gastrointestinal diseases. This section provides a survey of the gastrointestinal, liver, and pancreatic diseases in which immunological abnormalities are known to contribute to disease pathogenesis.

Eosinophilic esophagitis

Eosinophilic esophagitis is characterized by the abnormal presence of eosinophils in the esophagus [221]. The symptoms of this disease mimic those of gastroesophageal reflux diseases (GERD) and include difficulty eating, failure to thrive, vomiting, epigastric or chest pain, dysphagia, and food impaction. Patients with eosinophilic esophagitis have normal pH studies but show an increased number of eosinophils in the mucosa of the esophagus, accompanied by epithelial hyperplasia and an increased incidence of atopic disease. The underlying cause of the disease remains unclear but may involve allergies, in the form of food allergies, aeroallergens, or skin sensitization. The disease appears to involve Th2-mediated immune responses generated from eosinophils or mast cells. Animal models of eosiniphilic esophagitis can be created through exposure to aeroallergens or through overexposure to Th2 cytokines, such as IL-5 and IL-13 [222,223]. These studies point to a connection between eosinophilic inflammation in the respiratory tract and esophagus. In support of this concept, patients with eosinophilic esophagitis report seasonal variations in their symptoms, and preliminary studies have documented an association between seasonal changes and the number of eosinophils in the esophagus [224,225]. Although there is no consensus on standard therapy for eosinophilic esophagitis, elemental diets and glucocorticoids have shown efficacy in clinical trials [226], and anti-IL-5 therapy has shown strong potential in preclinical and early clinical studies [227,228]. A strong familial association suggests a genetic predisposition. Linkage studies have identified an association with the gene encoding eotaxin-3 among patients with eosinophilic esophagitis [229]. Eotaxin-3 is a chemokine for cells that express the eotaxin receptor CCR3+; it is overexpressed by hyperplastic epithelial cells in the esophagus of patients with eosinophilic esophagitis, most likely in response to IL-13 (see Chapter 33).

Autoimmune gastritis

Autoimmune gastritis is a chronic inflammatory disorder of the gastric corpus in which the heterodimeric H^+,K^+-ATPase proton pump found on gastric parietal cells acts as an

autoantigen. Although T cells specific for both the α subunit and the β subunit of the H^+,K^+-ATPase are found within the gastric mucosa during autoimmune gastritis, autoreactivity to the β subunit is essential to the initiation of disease. Mouse models have demonstrated that deletion of the β subunit prevents gastritis, whereas deletion of the α subunit has little effect on the onset of disease. Autoimmune gastritis represents one of the few autoimmune diseases in which the specific autoantigen has been clearly defined.

Autoimmune gastritis is a Th1-mediated disease that involves a massive infiltration of pathogenic CD4+ T cells into the gastric mucosa, with an early increase in IFN-γ production, loss of parietal cells, and a subsequent loss of zymogenic cells due to disruptions in normal parietal cell development. Lymphocyte homing to the corpus mucosa is dependent on the specific interaction between $\alpha_4\beta_7$ and MAdCAM-1. The subset of CD4+ T cells that is responsible for disease pathogenesis appears to be effector cells, as adoptive transfer experiments have shown that CD4+CD25– T cells can cause autoimmune gastritis whereas CD4+CD25+ T cells (a regulatory phenotype) suppress the pathogenic effects, possibly by inhibiting IL-2 production or CTLA-4 expression. During autoimmune gastritis, natural Treg cells (CD4+CD25+ FOXP3+) are activated in a nonspecific manner by MHC class II molecules and by cytokines secreted from other activated effector cells. On activation, these natural Treg cells inhibit the activation of naïve T cells to the Th1 phenotype, thereby suppressing the resulting inflammatory response.

Autoimmune gastritis commonly occurs in association with other autoimmune or infectious diseases. Type I diabetes, autoimmune thyroid disease, and autoimmune gastritis often occur concomitantly in patients and are together known as autoimmune polyendocrine syndrome (APS) type 3. One-third of patients with autoimmune gastritis also have thyroid disease. In genetically susceptible individuals, *H. pylori* infection can either cause or aggravate autoimmune gastritis through a mechanism involving molecular mimicry. Activated CD4+ T cells from patients infected with *H. pylori* may cross-recognize the epitopes of *H. pylori* proteins and the autoantigenic H^+,K^+-ATPase, resulting in the generation of autoantibodies against gastric H^+,K^+-ATPase. This *H. pylori*-associated autoimmune gastritis occurs in 20%–30% of patients infected with the bacteria (see Chapter 42).

Peptic ulcer disease and *Helicobacter pylori*

About 50% of the world's population is infected with the stomach bacteria *H. pylori*. Most of these cases are asymptomatic; however, infection can lead to chronic gastritis, gastric and duodenal ulcers, and an elevated risk of developing gastric cancer. *H. pylori* is a unique bacterium that achieves its pathogenic effects by eliciting an inefficient immune response in the host that fails to eradicate the bacteria from the gut and which causes inflammation and gastric tissue damage [230]. This orchestrated equilibrium of the host immune response is achieved through several mechanisms: inhibition of nitric oxide production and phagocytosis by macrophages; production of the VacA virulence factor that interferes with antigen presentation and inhibits T-cell activation; and activation of Tregs that partially inhibit Th1 immune responses against the bacteria.

Infection with *H. pylori* stimulates both innate and adaptive immune responses within the gastric mucosa, as well as a specific humoral response. Infection is characterized by a Th1-polarized gastric immune response. Contact between the bacterium and the gastric epithelium leads to production of IL-8 through a mechanism involving injection of CagA into the epithelial cytosol [231]. In addition, interactions between the bacterium and TLRs on the surface of epithelial cells initiate signaling pathways that lead to epithelial secretion of a variety of proinflammatory chemokines and cytokines. IL-8 acts as a chemokine for recruitment and activation of polymorphonuclear cells into the gastric mucosa. Antigen-presenting cells produce additional Th1 polarizing cytokines (i.e., IL-6, IL-1β, TNF-α, and IL-12), which leads to recruitment and activation of CD4+ lymphocytes within the gastric mucosa that secrete IFN-γ, but not IL-4 and IL-5, consistent with a Th1 phenotype. Individuals who are positive for *H. pylori* infection develop specific antibodies against the bacterium, primarily in the form of IgG and IgA. Antibodies against membrane proteins, flagellin, urease, lipopolysaccharide, and *H. pylori* adhesion A are increased in infected patients, regardless of whether or not they display symptoms [232,233]. In addition, 20%–30% of patients infected with *H. pylori* also develop autoantibodies against the H^+,K^+-ATPase proton pump on the surface of gastric parietal cells, resulting in chronic autoimmune gastritis (see Chapter 40).

Inflammatory bowel disease

Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis, both of which are chronic relapsing and remitting inflammatory disorders of the gut. Although the exact cause remains unclear, IBD is believed to develop as the result of an overly aggressive adaptive mucosal immune response to intralumenal bacteria in a genetically susceptible host [234]. Chronic inflammation results from the production of proinflammatory cytokines and the recruitment and activation of effector lamina propria lymphocytes. The important role of cytokine mediators in promoting IBD pathogenesis is most clearly demonstrated by the therapeutic success of monoclonal antibodies targeting the proinflammatory cytokine TNF-α. In clinical trials, anti-TNF antibody therapy induced a clinical response in 63% of patients with refractory Crohn's disease and 69% of patients with refractory ulcerative colitis [156,157]. Other mechanisms that may contribute to the chronic inflammation seen in IBD include a failure of regulatory T cells to suppress the initiated immune responses and the resistance of effector T cells to undergo apoptosis.

Traditionally, Crohn's disease has been described as a Th1-mediated immune disease marked by increased production of TNF-α, IL-1β, IL-12, and IL-18, whereas ulcerative colitis displayed cytokine profiles more representative of a Th2-mediated disease (i.e., IL-4 and IL-5 production). However, this Th1/Th2 paradigm has been challenged by evidence that individual cytokines from both pathways may have distinct effects during the acute and chronic phases of Crohn's disease and ulcerative colitis; the Th1/Th2 classification no longer appear to be mutually exclusive in the case of IBD [235].

In contrast to the overly aggressive adaptive immune responses associated with the chronic stages of IBD, the acute phase of disease is marked by defects in innate immunity. A focus on the role of innate immunity in IBD pathogenesis developed in response to the identification of *CARD15* as the first susceptibility gene for Crohn's disease [236,237]. *CARD15* encodes the NOD2 gene product, an intracellular protein expressed by intestinal mucosal cells, including epithelial cells, which recognizes components of the bacterial cell wall and initiates a signaling cascade that activates NF-κB and the innate immune system. The commensal bacteria play an important role in promoting IBD pathogenesis, and loss of tolerance has been proposed as a possible underlying cause for initiation of the disease. In support of this hypothesis, most animal models of IBD do not develop intestinal inflammation when raised under germ-free conditions, and probiotic bacteria taken as dietary supplements are effective therapy for patients with ulcerative colitis [238–243]. The intestinal epithelium plays an active role in maintaining innate immunity. It serves as a protective barrier between intestinal pathogens, the benign commensal flora, and the mucosal compartments. The epithelium also plays a role in antigen presentation and secretes cytokines and chemokines in response to bacterial interaction. Epithelial barrier dysfunction could represent a primary defect that leads to loss of tolerance in patients with IBD.

The prevailing model of the immunopathogenesis of IBD suggests that an initiating factor, most likely an invasive bacteria or a component of the commensal flora, breaches the epithelial barrier and triggers a defective innate immune response in a genetically susceptible host. T lymphocytes are then recruited into the lamina propria and mount a dysregulated adaptive immune response against the antigen, which is marked by overproduction of proinflammatory cytokines, resulting in chronic intestinal inflammation and fibrosis (see Chapter 57).

Celiac disease

Celiac disease is an intestinal inflammatory disorder that occurs in genetically susceptible individuals in response to dietary intake of wheat, rye, and barley [244]. Gluten found in wheat contains two types of disease-activating proteins – gliadins and glutenins. Similarly, hordeins found in barley and secalins found in rye can also activate celiac disease. Gluten-related peptides are insufficiently digested by gastric, pancreatic, and brush border enzymes, resulting in the presence of large gluten-related peptides rich in proline and glutamine within the lumen of the small intestine. Histologically, manifestations of celiac disease are villous atrophy and crypt hyperplasia within the small intestine. The presentation of gluten-related antigens to CD4+ lamina propria lymphocytes initiates a Th1-mediated immune response and the formation of gluten-reactive activated T cells within the mucosa. Notably, the immune response is characterized by the expression of IFN-γ and the transcription factor TBET, which polarizes immune responses towards a Th1 phenotype but no subsequent increase in expression of IL-12 or STAT4, as occurs in other intestinal inflammatory diseases [245]. Patients with celiac disease characteristically display an elevated number of intraepithelial lymphocytes, which function more like natural killer cells than typical antigen-specific T cells. This functional change is mediated through an up-regulation of IL-15 expression by intestinal epithelial cells.

Genetic susceptibility plays an important role in the immunopathogenesis of celiac disease. Celiac disease represents a complex polygenic trait that involves multiple susceptibility genes. A strong association has been described with specific MHC class II alleles that are found at the HLA-DQ locus. In fact, almost all patients with celiac disease have one of two HLA-DQ alleles that encode specific HLA-DQ2 or HLA-DQ8 heterodimers, both of which are common in Caucasians. The HLA-DQ2 heterodimer that confers susceptibility occurs in 90%–95% of patients with celiac disease and is composed of the β-chain of the MHC class II molecule encoded by either HLA-DQB1*0201 or 0202, in association with the α-chain encoded by HLA-DQA1*05. The remaining 5%–10% of patients have the HLA-DQ8 heterodimer, which is composed of the β-chain encoded by HLA-DQB1*0302 in association with the α-chain encoded by HLA-DQA1*03 [246,247]. The HLA-DQ2 and -DQ8 heterodimers of the MHC class II complex have unique properties that augment their ability to bind and process gluten-related proteins. The architecture of their peptide-binding groove contains pockets that have an affinity for binding negatively charged molecules [248]. In celiac patients, neutrally charged gluten is deamidated and converted into glutamic acid by the intestinal mucosal enzyme TGase [249,250]. The negatively charged proline-rich deamidated gluten peptides then bind with high affinity to the HLA-DQ2 and -DQ8 heterodimers within the MHC class II complex, leading to the generation of gluten-reactive CD4+ T cells within the lamina propria (see Chapter 50).

Autoimmune pancreatitis

Autoimmune pancreatitis is an extremely rare autoimmune disorder of unknown incidence and prevalence [251]. The hallmarks of autoimmune pancreatitis are lymphoplasma-

cytic infiltration around the pancreatic ducts and granulocytic epithelial lesions in the duct epithelium. Because of these features the condition is also called duct-destructive chronic pancreatitis. The disease often occurs in association with other autoimmune diseases, such as Sjögren syndrome, variants of primary sclerosing cholangitis, IBD, Hashimoto thyroiditis, and lupus erythematosus, suggesting that this unique form of chronic pancreatitis is also an autoimmune disease. The location of the inflammatory infiltrate suggests that the target antigen is likely contained within the duct epithelium. The association of autoimmune pancreatitis with other autoimmune diseases suggests the potential for a common antigen. Several autoantibodies present in other autoimmune diseases have been detected in patients with autoimmune pancreatitis, including antibodies targeting lactoferrin, carbonic anhydrase type II, rheumatoid factors, and antinuclear antibodies. The inflammatory response is mediated by T cells including both CD4+ and CD8+ lymphocytes; the CD4+ lymphocytes are skewed towards a Th1 phenotype [252,253]. As with other autoimmune diseases, HLA haplotypes have been reported in association with autoimmune pancreatitis, most notably DRB1*0405-DQB1*0401 [254]. Relatively little information is known regarding the environmental and immunological conditions that trigger disease (see Chapter 69).

Autoimmune liver diseases

The autoimmune liver diseases include three separate disease processes: autoimmune hepatitis, primary biliary cholangitis, and primary sclerosing cholangitis. These diseases often occur in association with one another and share common immunopathogenic features related to autoimmunity.

Autoimmune hepatitis

Autoimmune hepatitis is characterized by an accumulation of oligoclonal autoreactive T cells in the liver, and hepatitis in the periportal and periseptal interface [255]. Nearly 90% of patients with type I autoimmune hepatitis have pathogenic autoantibodies targeting the asialoglycoprotein receptor (ASGR), which is expressed exclusively on hepatocytes. Other possible antigens include liver cytosolic antigen and soluble liver antigen. Recognition of these self-antigens leads to the generation of autoreactive CD4+ and CD8+ lymphocytes, and the development of chronic hepatitis in the form of antibody-dependent cell-mediated toxicity and liver damage. In type II autoimmune hepatitis, the self-antigen is believed to be the intracellular protein liver–kidney microsomal antibody type 1 (LKM-1, cytochrome P450 [CYP2D6]), which is expressed on the surface of hepatocytes. Patients with autoimmune hepatitis also present with anti-nuclear autoantibodies (ANA) and anti-smooth muscle autoantibodies (SMA). The dominant subset of T cells in autoimmune hepatitis express the Vβ-chain T-cell receptor, indicating that the autoreactive T cells descend from a common lineage [256]. Most of the T cells are αβ+, but the number of γδ + T cells in the peripheral blood is increased compared with other autoimmune liver diseases [257]. The apoptotic pathway is also altered in autoimmune hepatitis. Activated lymphocytes from patients with autoimmune hepatitis express elevated levels of CD95, which is a marker for deletion, suggesting a defect in the apoptosis pathway [258]. Additional evidence for such a defect is seen in studies showing that activated lymphocytes from patients with autoimmune hepatitis fail to down-regulate expression of the antiapoptotic protein bcl-2 [259]. Finally, molecular mimicry may also be involved through cross-reactivity between specific viral proteins, such as hepatitis C virus core 178–187, and the self-antigens CYP2D6 or CYP2A7, resulting in hepatitis C virus-induced autoreactive CD8+ T cells [260].

The prevailing model of autoimmune hepatitis immunopathogenesis proposes that an initiating event, most likely some viral infection, leads to the activation of CD8+ lymphocytes and cell-mediated toxicity. In an HLA genetically susceptible host, antigenic CYP2D6, CYP2A7, and ASGR are released and phagocytosed by macrophages and other antigen-presenting cells. The antigens are then presented to CD4+ T cells, which undergo clonal expansion. Defects in the apoptotic pathway contribute to the presence of even more T-cell clones in hepatic tissues, which produce proinflammatory cytokines that mediate autoantibody production from B cells. The result of this inflammatory process is hepatocyte cytolysis, the formation of fibrosis, and eventually end-stage liver disease (see Chapter 84).

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease of the intrahepatic and extrahepatic biliary duct [261]. About 75% of patients with PSC also have IBD, primarily in the form of ulcerative colitis. Significant attention has been given to the study of polymorphic HLA genes that may confer genetic susceptibility; however, conflicting results have been reported, probably because of heterogeneity in the study populations. Some associations have been reported for the MHC class I chain-related (MIC) genes [262]. Because of the strong association between PSC and ulcerative colitis, it has been proposed that the self-antigen responsible for PSC may be a component of the gut microflora. Increased permeability of the gut due to ulcerative colitis could allow commensal bacteria to translocate across the intestinal epithelial barrier, enter the portal circulation, and migrate to the biliary ducts. In support of this theory, a study of liver explants from patients with PSC and primary biliary cholangitis found that bacteria could be cultured from 21 of 36 explants from patients with PSC, but 0 of 14 explants from patients with primary biliary cholangitis [263]. The primary autoantibody in PSC is the IgG class of p-antineutrophil cytoplasmic antibodies (ANCA). Potential epitopes for this autoantibody include catalase, which is an

antioxidant enzyme found in hepatocytes and biliary epithelial cells [264], the neutrophil protein bactericidal/permeability-increasing (BPI) protein [261], or biliary epithelial cells themselves [265].

Similar to autoimmune hepatitis, the hepatic T-cell repertoire in PSC is mostly composed of αβ+ cells that express the Vβ3-chain; levels of γδ + T cells are also increased. CD4+ T cells predominate in the portal tract, whereas CD8+ cells are primarily found at sites of necrosis. Macrophages also accumulate in the sinusoidal and perisinusoidal spaces. It has been postulated that the memory T cells found in the liver are originally activated in the gut as part of the immune response generated by IBD [266]. Autoreactive T cells are polarized towards the Th1 phenotype and predominantly produce the proinflammatory cytokine TNF-α.

A model for PSC immunopathogenesis proposes that the disease is triggered by the presence of foreign antigen, possibly bacteria secondary to intestinal inflammation, within the biliary tract. Memory T cells from the gut bind to biliary epithelial cells and proliferate, leading to the increased expression of TNF-α and other proinflammatory cytokines. The cytokines stimulate B cells to secrete immunoglobulins, including autoantibodies against biliary epithelial cells. The resulting inflammation leads to fibrosis formation in concentric rings around the biliary ducts, ischemia, atrophy, cholestasis, and eventually biliary cirrhosis (see Chapter 75).

Primary biliary cirrhosis

Primary biliary cirrhosis is an autoimmune disease characterized by damage to the biliary epithelial cells that line the intrahepatic duct, chronic cholestasis, and fibrosis that leads to cirrhosis [267,268]. Evidence from familial case reports and a study of monozygotic twins suggest a strong genetic component to primary biliary cirrhosis [269,270]; however, only weak associations with particular genes have been identified. It is clear that the environment also plays a role in triggering the onset of disease. More than 95% of patients with primary biliary cirrhosis possess antimitochondrial antibodies (AMA) that target the E2 component of the pyruvate dehydrogenase complex (PDC-E2). Biliary epithelial cells and salivary epithelial cells express PDC, or PDC-like epitopes that cross-react with PDC, on their apical surface and are the main target cells of the disease. PDC-E2-reactive T-cell subsets can be found in the liver and peripheral blood of patients with primary biliary cirrhosis.

Under normal conditions, PDC is located on the inner surface of the inner mitochondrial membrane, and is therefore separated from the extracellular immune system by three separate membranes. It is unclear how the antigen is capable of generating immune responses within this context. One theory suggests that mitochondria undergo apoptosis and release PDC into the cytoplasm, making PDC freely available in the cytoplasm until the cell completes its apoptotic cycle and it is released into the tissue. It is interesting to note that primary biliary cirrhosis is limited to the biliary tissues even though PDC is widely distributed throughout the body. Although several questions regarding the immunopathogenesis of primary biliary cirrhosis still remain, it does appear that the hallmark of the disease is a loss of tolerance to PDC-E2 by T cells, which results in proliferation of reactive T-cell and B-cell clones, production of proinflammatory cytokines, and subsequent secretion of autoantibodies (see Chapter 85).

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A COMPONENTS

8 Epithelia: biological principles of organization

Jerrold R. Turner, James L. Madara

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All cavities within the alimentary tract, from the small ducts and acini of the pancreas to the gastric lumen, are lined by sheets of polarized epithelial cells. Common to all of these epithelia is the ability to create selective barriers that separate lumenal and tissue spaces. Most epithelia are also able to direct vectorial transport of solutes and solvents. These essential functions are based on the structural polarity of individual cells, the complex organization of membrane domains, cell–cell and cell–substrate interactions, and integration with other cell types. This chapter reviews intestinal wall structure and examines how mucosal functions are supported by the organization of the gut and the biological properties of the epithelial barrier and epithelial transport. More detailed discussions of epithelial transport and nutrient processing can be found in Chapters 13–21.

Organization of the gut wall

The relation of the epithelial layer to other components of the gut wall is shown in Fig. 8.1. Four principal layers exist: mucosa, submucosa, muscularis propria, and serosa or adventitia. The mucosa consists of the epithelium, an underlying layer of loose connective tissue carrying nerves and vessels (i.e., lamina propria), and a thin layer of smooth muscle (i.e., muscularis mucosa). The mucosa also contains an array of lymphocytes, mast cells, macrophages, and, in disease states, polymorphonuclear leukocytes, all of which are capable of modulating epithelial function.

An underlying layer of fibroconnective tissue called the submucosa, which contains nerves, vessels, and lymphatics, supports the mucosa. The submucosa rests on the muscularis propria, which is composed of two or three layers of smooth muscle and is home to the myenteric plexus (see Chapters 2 and 6). In most instances, gastrointestinal organs are encased by an outermost delicate layer of fibrofatty tissue, the serosa, encircled by a continuous layer of mesothelial cells. In areas where no serosa exists, as in portions of the esophagus and in the distal colorectum, fibrofatty tissues interface with the external portion of the muscularis propria. These organs are said to have an adventitial, rather than a serosal, encasement.

Forceps biopsies retrieved endoscopically usually go no deeper than the muscularis mucosae, although thin wisps of submucosal tissue may occasionally be present. Suction biopsies more consistently penetrate the submucosa, although only the most superficial portion of the submucosa is obtained. Deeper portions of the wall appear in endoscopic samples by accident, such as in an aggressive snare of a sessile mucosal lesion, or by intention, as in endoscopic mucosal resection (see Chapter 137).

Similar to the gross anatomy, the microscopic anatomy of the gastrointestinal tract varies along its length. A simple columnar epithelium lines the stomach, small intestine, colon, pancreatobiliary ducts, and exocrine pancreas. In contrast, the oral cavity, esophagus, and anus are lined by a nonkeratinizing, stratified squamous epithelium that is capable of withstanding the mechanical stresses of swallowing and defecation but plays no role in transepithelial transport. The three-dimensional structure of epithelia also exhibits significant variation within the gastrointestinal tract, such as the prominent mucosal folds and villi in the small intestine (Fig. 8.1), the lobular organization of the exocrine pancreatic acini (Chapter 67), and the deep epithelial extensions into salivary and Brunner glands in the esophagus and duodenum, respectively. The liver, which lacks a single large lumen, is composed primarily of hepatocytes. These possess only a small apical (canalicular) surface and lack complex, deeper cell layers; capillaries and hepatocytes are separated by a thin basement membrane in the space of Dissé (see Chapter 79).

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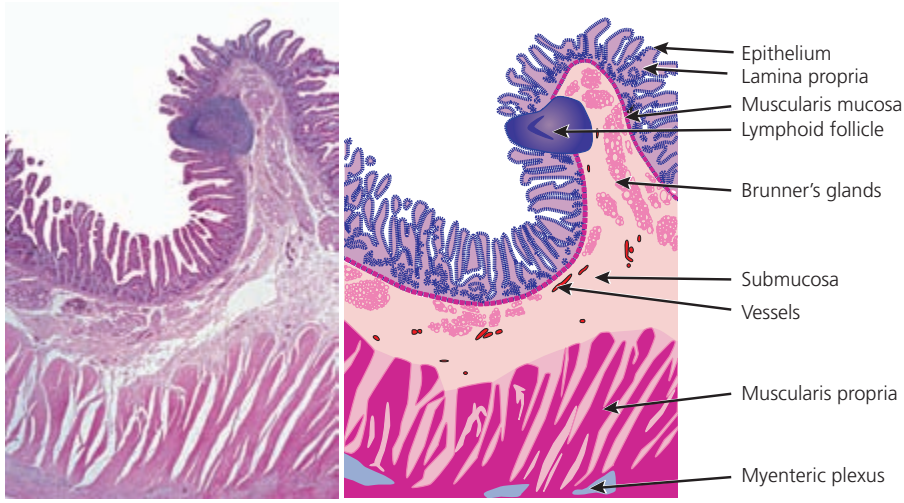

Figure 8.1 Organization of the intestinal wall. This transmural section of normal human duodenum exemplifies many of the structural features that are common throughout the gastrointestinal tract. The corresponding line diagram delineates specific structures.

Despite these complexities of regional specialization, central structural features critical to epithelial function are present throughout the gastrointestinal tract.

Organization of epithelial cells and sheets

To function properly as a barrier, epithelial cells must assemble into a multicellular sheet. This is a complex task that requires individual cells to establish a uniformly oriented polarity; to form intercellular junctions; and to develop stable interactions with the basement membrane (Fig. 8.2).

The need for cell polarity

A central function of many gastrointestinal epithelia is the vectorial transport of solutes and solvents. For example, parietal cells are polarized to effect acid secretion into the lumen (see Chapter 13). Imagine the ensuing havoc if acid were inadvertently secreted into the interstitium. Absorptive villous enterocytes of the small intestine are specialized to accomplish vectorial transport of ions, nutrients, and water from the lumen to the interstitium (Fig. 8.3) by expressing specific transporters within the apical (lumenal), but not basolateral, membrane domain [1–3]. These transporters often rely on a lumenal Na^+ concentration that is much higher than the intracellular Na^+ concentration required for cotransport of sugars, amino acids, ions, bile salt, and xenobiotics. In general, the absorbed solutes exit the cytosol by way of Na^+-independent facilitated transporters present in basolateral, but not apical, membranes, resulting in efficient transcellular transport. The high extracellular and low intracellular Na^+ concentrations that provide energy for this system are maintained by the exclusively basolateral Na^+,K^+-ATPase that pumps Na^+ out of the cell in exchange for K^+. Thus, the net result of apical Na^+-coupled transport is vectorial transport of both the specific solute and Na^+ from the lumen to the interstitium. The polarized distribution of these three classes of transport proteins – apical Na^+-coupled transporters, basolateral Na^+-independent transporters, and the basolateral Na^+,K^+-ATPase – is critical for active transepithelial transport. Note that the deposition of osmotically active molecules in the subepithelial interstitial space provides the driving force for passive water absorption. This integration of active transcellular and passive, primarily paracellular, transport explains the improved efficacy of oral rehydration solutions supplemented with Na^+ and carbohydrates [4,5]. Although details of these transport processes will be addressed in further detail, this outline of vectorial transport should emphasize that polarized distribution of surface membrane components within individual cells is essential to epithelial function. It should also be obvious that were the entire epithelial sheet not polarized uniformly, i.e., with all cells polarized in the same orientation, adjacent cells could

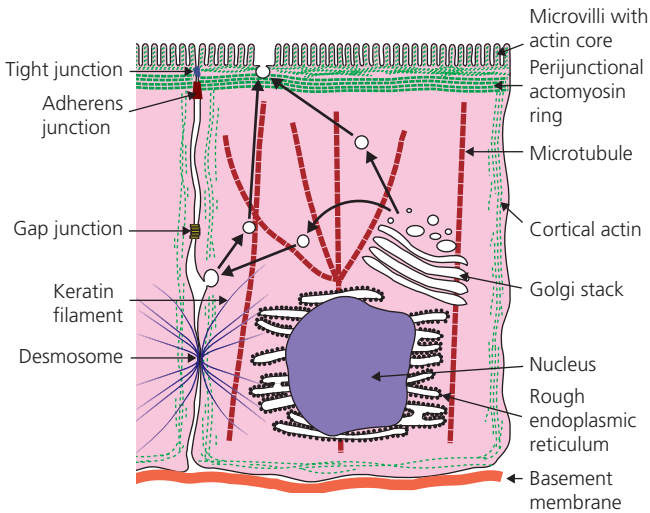

Figure 8.2 Organization of a typical gastrointestinal columnar epithelial cell. The precisely orchestrated architecture of polarized epithelia includes numerous specialized structures, such as cytoskeletal elements, transport vesicles, and intercellular junctions.

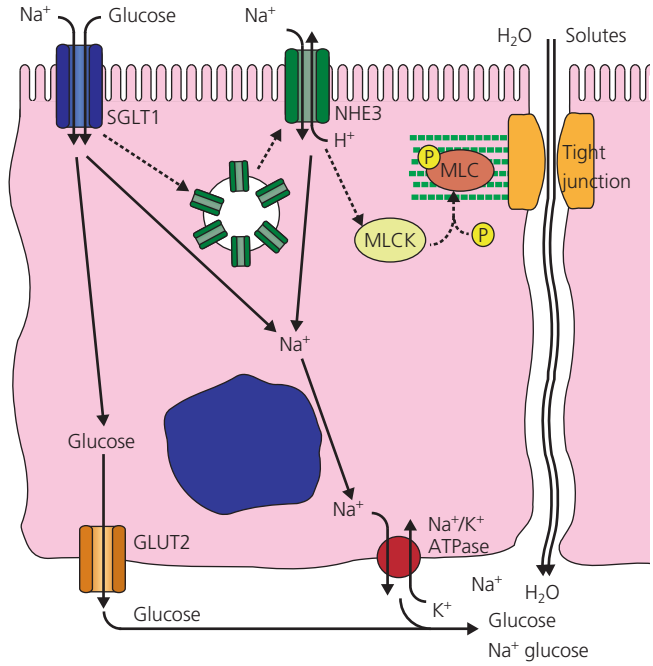

Figure 8.3 Coordination of transcellular and paracellular absorption of water, nutrients, and ions. The polarized delivery of SGLT1, GLUT2, and Na^+,K^+-ATPase to the appropriate apical (SGLT1) and basolateral (GLUT2 and Na^+,K^+-ATPase) membranes is essential for efficient vectorial glucose absorption. Research has shown that SGLT1 activation also stimulates apical NHE3-mediated Na^+ absorption, thereby linking these absorptive processes. Incompletely defined downstream signaling events subsequently activate myosin light chain kinase (MLCK). This, in turn, phosphorylates MLC and increases paracellular permeability, thereby linking transcellular and paracellular absorption.

negate one another's contributions, making net transport impossible. Thus, in addition to polarization of individual cells, it is critical that all cells within an epithelium respond to common cues to generate a unified, polarized epithelial sheet.

Cues to trigger polarization

The spatial cues that induce initial cell polarization and maintain it have generally been thought to require contact with the extracellular matrix and with other cells. For example, the interaction between the epithelial Ca^{2+}-dependent adherens junction protein, E-cadherin, on adjacent cells is a critical trigger for polarization. E-cadherin is concentrated at the adherens junction, the basal-most aspect of the apical junction complex (Fig. 8.2), where, through cytoplasmic linker proteins (e.g., α-actinin and α- and β-catenins), it is coupled to a perijunctional ring of actin and myosin filaments [6,7]. In addition to E-cadherin-mediated intercellular adhesion, interactions with matrix components through integrins, heparan sulfate proteoglycans, and other membrane proteins are also important in inducing polarization and maintaining differentiation [8–10].

The discovery of polarity genes encoding Par proteins in *Caenorhabditis elegans* and *Drosophila* has facilitated the identification of mammalian homologues, complexes of which define epithelial apical and basolateral plasma membrane domains [11,12]. Not surprisingly, Par protein mutations have been linked to disease. For example, many individuals with Peutz–Jeghers syndrome exhibit mutation of LKB1, the mammalian homologue of the *C. elegans* protein Par-4 [13]. LKB1 is a serine/threonine kinase that is activated by the STRAD adapter protein, which may also be mutated in Peutz–Jeghers syndrome [14]. STRAD-mediated LKB1 activation triggers early cell polarization in epithelial cells despite the absence of intercellular junctions [15]. One mediator of LKB1-initiated epithelial polarization may be AMP-activated protein kinase, which is activated by LKB1 and plays an important role in maintaining cellular energy balance [16].

The mutation of β-catenin that is often observed in human colorectal cancers and the increased risk of malignancy associated with Peutz–Jeghers syndrome suggest that disrupted cell polarization may contribute to neoplasia. The association of transcription factor-dependent E-cadherin repression with the epithelial–mesenchymal transition that is typical of invasive cancer [17] and the strong correlation between the loss of E-cadherin expression and the invasive phenotype of many human gastrointestinal neoplasms [18,19] further support this concept. Conversely, loss of E-cadherin allows unbound β-catenin to activate gene transcription. Cytosolic β-catenin activity is normally down-regulated by the adenomatous polyposis coli (APC) protein, explaining why mutations in APC lead to enhanced β-catenin activity and growth of adenomas in patients with familial adenomatous polyposis, as well as in those with spontaneous somatic APC mutations [20,21]. Clearly many functional connections exist between cell adhesion, polarity, growth, and tumor invasion (see detailed discussions in Chapter 24).

Structure of intercellular junctions

All polarized epithelia share a set of distinct intercellular junctions. These include, from the lumenal aspect, the tight and adherens junctions, which form continuous circumferential contacts, and, below these, desmosomes and gap junctions, which form macular or spot contacts. Together these junctions maintain polarity, seal the paracellular space, provide intercellular communication, and stabilize the monolayer.

A critical event in the generation and maintenance of cell polarity is the assembly of the apical junctional complex, which is composed of the adherens junction and the tight junction. The latter defines the boundary between apical and basolateral membrane domains; it prevents the mixing of transmembrane proteins and outer leaflet membrane

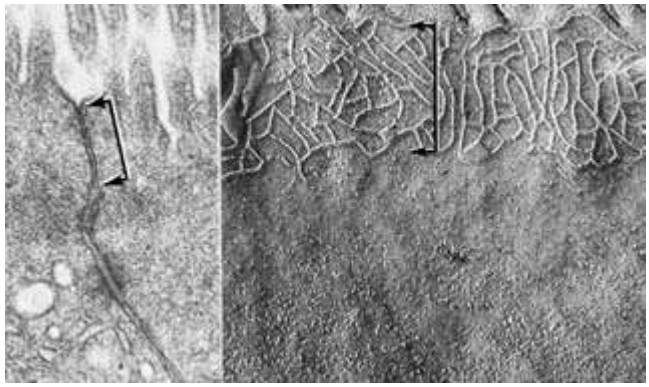

Figure 8.4 Tight junction ultrastructure. Electron microscopic appearance of the tight junction of a small intestinal absorptive epithelial cell. The transmission electron micrograph **(left)** shows that the tight junction is a zone of closely apposed cell membranes. Freeze-fracture electron microscopy **(right)** reveals the dense interconnecting network of strands that define the tight junction.

lipids between these domains. When examined by electron microscopy, tight junctions appear as 100- to 300-nm-deep zones where adjacent cells closely abut (Fig. 8.4). Series of punctate fusions or "kisses" between these plasma membranes form a seal between adjacent cells. These fusion sites are arrayed in a linear anastomosing fashion around the cell and correspond to the net-like series of grooves and strands seen in freeze-fracture replicas of epithelial cells (Fig. 8.4). The strands are formed by members of the claudin family of tetraspanning cell–cell adhesion proteins [22], which make contact in the intercellular space and define the ion selectivity of flow across the tight junction [23–25].

Another tetraspanning membrane protein, occludin, is present in tight junction strands, in intracellular vesicles, and, in some cell types, along the lateral membranes. Although occludin knockout mice are viable and appear to have intact tight junctions [26], abundant in vitro and in vivo data suggest that occludin plays a critical role in the organization and regulation of tight junctions [27–32] and, potentially, in the maintenance of the differentiated epithelial phenotype [33]. Like occludin, several members of the junctional adhesion molecule (JAM) family of proteins localize to tight junctions and along lateral membranes. JAM proteins appear to be necessary for the transepithelial migration of inflammatory cells [34–36]. A variety of other transmembrane proteins, including the coxsackievirus and adenovirus receptor [37,38], are also sequestered within tight junctions.

The cytoplasmic face of tight junctions contains a large number of peripheral membrane proteins that fall into different categories. Various peripheral membrane proteins link transmembrane proteins to the actin cytoskeleton, help to establish cell polarity, define specialized zones for vesicle targeting, or regulate gene transcription. An extensive review of these proteins is beyond the scope of this chapter.

Of special interest are the numerous proteins, such as ZO-1, with multiple protein interaction (PDZ) domains [39]. Although incompletely understood, ZO-1 appears to play a unique role in tight junction assembly and regulation. Initially recruited to nascent adherens junctions through interactions with α-catenin, ZO-1 is present from the earliest stages of tight junction assembly, prompting the suggestion that ZO-1 defines the site of tight junction assembly. A report that tight junction assembly is delayed in ZO-1 knockout epithelial cells gives credence to this idea [40]. ZO-1 may also serve as a scaffold for the assembly of multiprotein complexes. For example, ZO-1 interacts with claudin proteins, occludin, and actin filaments [41–43] and binds to other peripheral membrane proteins, such as the myosin-binding protein cingulin [44]. Most striking, ZO-1 and the related protein ZO-2 can each determine whether and where claudin polymerization occurs [45]. This multitude of protein–protein interactions has led to the hypothesis that the tight junction itself is composed of a stable multiprotein complex. However, measurement of unique rates of protein exchange within the tight junction, as well as between junctional and cytoplasmic pools, contradicts this appealing concept [46].

Directly below the tight junction lies the adherens (or intermediate) junction, in which the lateral membranes of adjacent cells lie parallel to each other and adhere by means of E-cadherin. At this site, the perijunctional ring of actin and myosin interacts with E-cadherin through α-actinin, vinculin, and α- and β-catenin. This perijunctional actomyosin ring is also essential to the maintenance of the tight junction [29,47]. Directly below the adherens junctions are desmosomes (Fig. 8.2). Distant relatives of the cadherin family, desmogelins and desmocollins, form cell contacts at desmosomes. These transmembrane glycoproteins associate with intermediate filaments rather than with actin filaments. Consequently, they anchor the cytokeratin-based cytoskeleton between neighboring cells and provide resistance to mechanical stress. Keratin gene mutations in some patients with inflammatory bowel disease and the spontaneous chronic colitis that develops in keratin-8 knockout mice emphasize the importance of keratin proteins in epithelial function [48,49].

To varying degrees, all epithelial cells of the gastrointestinal tract express gap junctions. At the site of the gap junction the cytoplasm of adjacent cells is in physical continuity through transmembrane channels formed by members of the connexin protein family [50]. Six connexin molecules assemble on each membrane to form a channel and, by adhering across the paracellular space, they create a lumen isolated from the extracellular space. Signaling molecules up to about 1500 Da (e.g., Ca^{2+}, inositol triphosphate) and small nucleotides can diffuse freely between cells and coordinate

physiological responses. There are numerous connexin genes in humans and each shows organ-specific diversity, which allows for organ-specific regulation of communication. Gap junctions coordinate epithelial function by allowing sheets of cells to behave as syncytia, for example coordinating exocytosis of zymogen granules from the pancreas (see Chapter 15). Gap junction communication in the liver, working through intracellular Ca^{2+} waves, modifies bile secretion in response to glucagon and vasopressin. Gap junctions are often mutated or down-regulated in gastrointestinal cancers [51], probably reflecting the aberrant intercellular communication that is characteristic of the neoplastic phenotype.

Polarized protein delivery

Plasma membrane proteins and secreted proteins pass through a series of distinct vesicular compartments as they are sorted to either the apical or basolateral surface. They share a common site of synthesis on ribosomes bound to the rough endoplasmic reticulum and undergo posttranslational modification (e.g., glycosylation) in the Golgi apparatus. Membrane proteins and secreted proteins are then sorted into distinct vesicles in the trans-Golgi network (Fig. 8.2). In most cells, proteins destined for the basolateral surface are delivered directly to that domain; studies using live cell imaging have suggested that this basolateral delivery may even be targeted to specific sites along the lateral membrane [52]. Detailed analysis of basolaterally targeted proteins has shown that specific amino acid sequences located within the cytoplasmic tail are sufficient to direct basolateral delivery [53]. Several of these sequences, including those with conserved tyrosine residues, are sorted by the epithelial adapter protein AP-1B [54]. This protein selects cargo destined for the basolateral membrane and also coordinates the assembly of the exocytic machinery necessary for fusion of transport vesicles with the plasma membrane. The exocytic machinery includes members of the Rab family of small guanosine triphosphate (GTP)-binding proteins and SNARE proteins that target delivery of transport vesicles to specific membrane domains [55,56]. Although not yet identified, other adapter proteins must also exist, because the sorting of E-cadherin, Na^+,K^+-ATPase, and proteins with dileucine-containing basolateral targeting motifs is AP-1B independent.

In contrast to basolateral proteins, apically targeted proteins are transported by both direct and indirect pathways [57,58]. Proteins that traffic directly to the apical membrane include those that associate with glycolipid- and cholesterol-rich membrane domains, such as the brush border hydrolase sucrose-isomaltase, as well as proteins that are targeted independently of these membrane domains, such as lactase–phlorizin hydrolase. Dependence on actin also differentiates these two direct transport pathways, as transport of sucrose-isomaltase occurs along actin tracks and is inhibited by actin depolymerization, whereas lactase–phlorizin hydrolase transport is actin independent. The targeting motifs that direct apical delivery have been more difficult to identify than their basolateral counterparts. Thus far, ectodomain glycosylation sites and transmembrane protein domains, including those that allow association with glycolipid- and cholesterol-rich membranes, have been implicated in apical targeting [59].

Direct trafficking to the apical membrane is not used by all proteins nor in all cell types. In hepatocytes, for example, all membrane proteins are first delivered to the basolateral surface. The apically destined proteins are then transcytosed to the apical, or canalicular, surface. A simple example of this type of sorting in hepatocytes and intestinal epithelia is provided by the polyimmunoglobulin (IgA) receptor, which binds IgA on the basal surface and is then transcytosed and released as secretory component into bile or the intestinal lumen [60]. In addition to specific targeting sequences that direct basolateral and then apical delivery, transcytosis also requires microtubules, which serve as tracks for the movement of transport vesicles from basolateral to apical surfaces. Why apical proteins without specific basolateral duties take this indirect pathway remains unclear. However, this mechanism is useful for the redistribution of apical proteins errantly targeted to the basolateral membrane, as well as for the sorting of membrane proteins during the initial stages of epithelial polarization.

Maintenance of membrane domains

Once delivered to the correct plasma membrane domain, proteins can be retained through interactions with actin-based cytoskeletal proteins. For example, the Na^+,K^+-ATPase is stabilized on the basolateral membrane domain by attachment to the cytoskeleton through the linker proteins ankyrin and spectrin. The complex functions of the apical membrane require intricate structures, such as parietal cell secretory canaliculi (see Chapter 13) and enterocyte microvilli. Assembly and maintenance of these membrane domains depends, in part, on ezrin–radixin–moesin (ERM) proteins, which play a critical role in the organization of apical, or free, membrane domains in species as diverse as *C. elegans*, *Drosophila*, and mammals, and in multiple cell types from epithelia to lymphocytes [61]. These highly conserved cytoskeletal proteins link membrane proteins to the actin cytoskeleton by way of an amino-terminal cargo-binding domain and a carboxy-terminal actin-binding domain. The cargo-binding domain can interact with transmembrane proteins either directly or through accessory proteins, such as NHERF-1, NHERF-2, and PDZK1 [62–67]. These accessory proteins can also anchor protein kinases (e.g., protein kinase A), thus serving as a scaffold for the organization of signaling complexes [66,68,69]. For example, the cystic fibrosis transmembrane regulator (CFTR) is bound to PDZ domains in NHERF-1 or NHERF-2. These interactions stabilize CFTR at the apical membrane and tether CFTR to protein kinase A, thereby enhancing the ability of protein kinase A to activate CFTR [68,70]. Ezrin-dependent mechanisms have also been implicated in

stimulated apical delivery of the apical Na^+/H^+ exchanger NHE3, after the initiation of Na^+/glucose cotransport in enterocytes, and in the acute surface delivery of the H^+,K^+-ATPase in histamine-stimulated parietal cells [71,72]. Studies in knockout mice lacking ezrin, the only ERM protein expressed in enterocytes, confirm the central role of this protein in organizing the apical membrane [73]. Enterocytes in these mice develop only primitive microvilli and fail to correctly target some proteins to the apical membrane. Moreover, the villi are irregularly shaped, with intravillous lumens and fused profiles. Not surprisingly, these mice fail to wean and die in the early postnatal period.

Organization of the cytoskeleton

The cytoskeleton is considered here in the context of the villous absorptive enterocyte, a cell type that has become an important model for studies of cytoskeletal structure and function in nonmuscle cells. The cytoskeletal organization of columnar epithelia throughout the gastrointestinal tract exhibits only relatively minor differences. The stabilization of epithelial cell structure first requires support for the tall columnar shape; in the absence of the cytoskeleton, a sphere would have the most thermodynamically favorable properties. Maintenance of cell shape is primarily a function of the actin microfilaments that form a network beneath the entire plasma membrane (Fig. 8.2). Bundles of 20–30 actin filaments also form the submembranous cores responsible for microvillous architecture. Within these cores, individual microfilaments are cross-linked to each other by actin-bundling proteins and to the microvillous membrane by a member of the myosin family, myosin IA. The microvillous actin bundles jut into the apical pole of the cell and associate with a terminal web composed of actin and type II myosin that interfaces with the apical junctional complex. The tension of this perijunctional actomyosin ring can be adjusted in response to physiological and pathophysiological stimuli, allowing modulation of epithelial barrier function (see Regulation of barrier function by physiological stimuli and Dysregulation of barrier function in intact epithelium).

Cables composed of intermediate filaments course through the cells and function as support cables for structural buttressing. Such tonofilament cables associate with plasma membranes and insert into the desmosomes. This network of intercellular junctions and intermediate filaments is required for the intestinal epithelia to interface with the turbulent environment of the gut lumen.

Microtubules also form a unique array in polarized epithelial cells. In contrast to nonpolarized cells in which microtubules radiate from a single microtubule organizing center adjacent to the nucleus, microtubules in polarized epithelia are aligned apicobasally. With the assistance of microtubule-dependent motor proteins, kinesins and dyneins, which can transport vesicles along microtubule arrays, membrane-bound structures are trafficked throughout the cell. This microtubule network is particularly important in transcytosis; microtubule disruption markedly slows this process.

Basement membrane

In addition to its important structural and supportive roles, the basement membrane serves as a source of signals for inducing epithelial cell polarity. All alimentary epithelia reside on a basement membrane that is 20–40 nm deep, consists of a fibrillar network, and rests on an underlying extracellular matrix (Fig. 8.1). The basement membrane in the alimentary tract, similar to basement membranes in other tissues, is composed primarily of laminin, heparan sulfate proteoglycans, and type IV collagen. Minor constituents that may be functionally important include thrombospondin and entactin/nidogen-1.

Laminin exhibits specific binding sites for type IV collagen, heparan sulfate proteoglycans, cell surface laminin receptors, and entactin. Other matrix components also possess binding sites for additional components, adding to the complexity of interactions between the epithelial cell and its surrounding environment. The major proteoglycans of the basement membrane, heparan sulfate proteoglycans, consist of long chains of glycosaminoglycans linked to a protein core. The structure of these massive molecules is often likened to a test-tube brush, with the bristles representing the glycosaminoglycan extensions. Proteoglycans probably organize water within the basement membrane, hydrating this environment through their capacity to bind water, and possibly imparting solute-sieving characteristics under conditions of bulk water flow. Although a controversial concept, impaired water and electrolyte absorption may cause the watery diarrhea seen in collagenous colitis, a disease that manifests thickening of the basement membrane [74,75].

Type IV collagen originates as a triple-stranded helical molecule, which, unlike other collagens, does not have its propeptides sheared from it after deposition in the extracellular space. Partially as a result of this, collagen IV does not cross-link into dense fibrils; instead it assumes a loose, net-like structure by associating with other collagen IV molecules. This mesh-like structure of collagen IV may provide the basic structure to the basement membrane.

Basement membrane components can exert significant effects on epithelia, including modulation of proliferation, adhesion, migration, differentiation, and even barrier function. In the intestine, type IV collagen is produced primarily by mesenchymal cells, heparan sulfate proteoglycans by epithelial cells, and laminin by both mesenchymal and epithelial cells. Many basement membrane components bind to a family of epithelial cell surface molecules, the integrins, which are connected to the actin cytoskeleton through linker proteins. Through such associations, structural elements within the cell are able to connect with, and potentially be influenced by, events occurring within the basement membrane and more deeply within the extracellular matrix.

Epithelial barriers

Various sites in the alimentary tract are threatened by the presence of acid, bile, undigested potentially antigenic proteins, bacterial proteins, and live bacteria. It is not surprising, therefore, that the epithelial barrier consists of numerous components that prevent injury from these varying insults. Some of these components are site specific and others are universally present. To discuss these barriers, we arbitrarily divide them into two major categories: those that are extrinsic to the epithelium (although in some instances produced by the epithelium) and those provided by the physical presence of the epithelium, which we describe as intrinsic barriers.

Extrinsic barriers

Extrinsic barriers confront the microenvironment overlying the epithelia.

Mucus

All alimentary epithelia are coated with a layer of mucus that protects against bacteria and surface shear forces (Fig. 8.5). Most surfaces, including those of the stomach, the intestine, the pancreatobiliary ducts, and the gallbladder, contain specialized cell types that synthesize, package, and secrete mucin. In the esophagus, mucin is derived from small glands that lie under the epithelium and connect to the lumen by way of delicate ducts. Although the precise chemical nature of mucus varies throughout the alimentary tract, the various mucin molecules share common features. They are viscous, polydispersed glycoproteins (250–20 000 kDa) of which about 80% of the mass is carbohydrate. At least eight human mucin-producing genes (*MUC*) have been identified. *MUC2* is the predominant form in intestinal and colonic surfaces. Esophageal *MUC2* expression can be a marker of Barrett esophagus: In inflammatory bowel disease and cancer, altered *MUC2* expression may occur.

Mucins act as a barrier by behaving as a viscous hydrated gel which undoubtedly attenuates shear forces that the epithelium would otherwise experience from lumenal particulates that are driven down the alimentary tract by peristaltic propulsion. In addition, carbohydrate groups on mucin molecules may bind to bacterial surfaces, thereby inhibiting surface adhesion and colonization. In some instances, mucin carbohydrates replicate epithelial carbohydrate binding sites to which bacteria can attach, presumably preventing colonization by acting as a molecular decoy. Given their extensive glycosylation, mucins can cross-link and aggregate bacteria. Such aggregation presumably aids in bacterial clearance. Exposure of epithelial surfaces to threats such as bacterial toxins and noxious chemicals often results in a reflexive secretory release of mucins, further augmenting their protective effects. Mucin depletion is a nonspecific histological indicator of ongoing injury often noted in biopsy specimens. The expression of mucin genes and the secretion of mucin by goblet cells respond to intestinal microbes and host-derived inflammatory mediators and are altered by infections, such as *Helicobacter pylori* in the stomach. Consistent with the essential protective role ascribed to mucins, mice deficient in Muc2, which is down-regulated in human inflammatory bowel disease, develop spontaneous colitis [76].

It has been observed that the diffusion coefficients of hydrophilic molecules are substantially lower in mucin than in free solution. Some researchers have suggested that this alteration would diminish contact between the epithelial surface and lumenal threats such as acid. Given the depth of the mucin layer and the duration that lumenal contents are in contact with the epithelium, however, small molecules probably have sufficient time to equilibrate within the mucous gel.

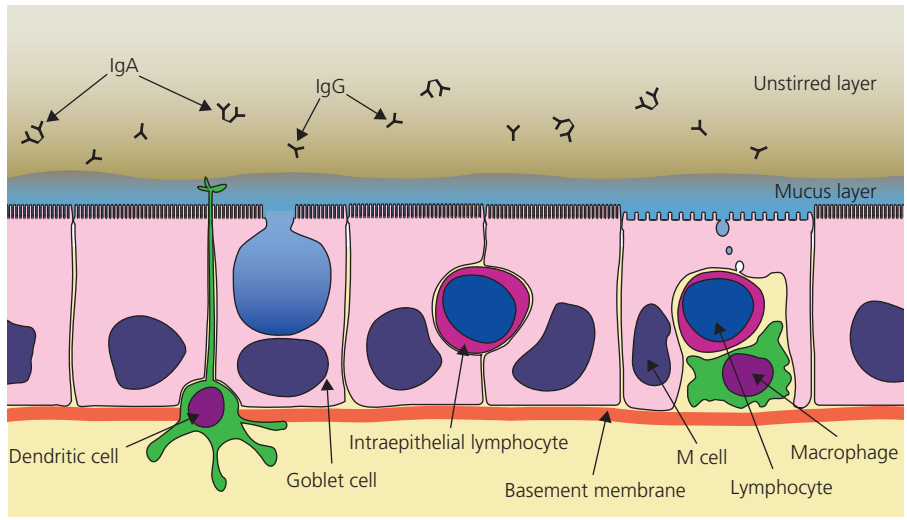

Figure 8.5 Epithelial barriers. The intestinal epithelium is the focal point around which the interaction of lumenal material and subepithelial cells, including those of the immune system, is organized. In addition to conducting vectorial transport and maintaining a cellular barrier, epithelia also contribute to host defense by elaborating mucus and transporting immunoglubulins.

Unstirred layer

Peristalsis creates a rather turbulent environment in the gastrointestinal lumen. This turbulence or convective force does not extend to the epithelial surface. The best estimate is that an aqueous layer with a thickness of 300–800 μm lies above the epithelia. This apical microenvironment is still, that is, it is unstirred. Convective forces then increase rapidly with increasing distance from the mucosal surface. Although not fully determined, the physiological impact of the unstirred layer on the immediate environment to which epithelial cells are exposed must be profound.

The presence and volume of the unstirred layer may significantly affect nutrient absorption. For example, if the epithelial transport system can transport a given nutrient more rapidly than the nutrient can diffuse into the unstirred layer, diffusion becomes the rate-limiting step in absorption. In contrast, polymeric nutrients that are broken down into monomers at the brush border (e.g., carbohydrates) may be formed at very high rates within the unstirred layer. Should local nutrient concentrations exceed transporter capacity, this in itself represents the rate-limiting step in absorption. Because the concentration of a molecule at the epithelial surface is unlikely to be equivalent to the concentration of that molecule in the center of the lumen, the challenge of measuring solute concentrations within the unstirred layer obscures the biophysics of many transport reactions within the intact intestine. Thus, the confounding issue of the unstirred layer must be confronted when analyzing molecular transport kinetics.

Secreted immunoglobulins

The epithelial surfaces in the alimentary tract are for the most part bathed by secretory IgA and IgG (Fig. 8.5). Secretory IgA is produced as a dimer by lamina propria plasma cells, transcytosed by the polyimmunoglublin receptor, and released into the lumen as a consequence of proteolytic clipping of the receptor. IgG is transcytosed by the neonatal Fc receptor [77]. By binding to lumenal threats such as pathogenic bacteria or toxins, secretory IgA and IgG act as barriers to antigenic material [78]. Although of extreme importance in host defense, this barrier is highly specific and dependent on prior antigenic sensitization [79]. Secretory IgA binding to the surfaces of pathogens may not only impede pathogen–epithelial interactions over most of the epithelial surface but also actually enhance pathogen–epithelial interactions at selected sites such as the M cells [80], a cell type responsible for the afferent limb of intestinal immunity.

Secreted bicarbonate

In contrast to the extrinsic barriers discussed previously, some extrinsic barriers have regional variation. One well-described example is the net bicarbonate (HCO_3^-) secretion by epithelia that interface with the acidic lumenal environment of the stomach. The proximal duodenum also must protect itself from gastric acid, as pancreatic bicarbonate secretions enter the gut lumen further downstream. The effects of epithelial bicarbonate secretion on the stomach and the duodenum are of central interest [81]. Clearly the first line of defense is the neutralization of intralumenal acid before it reaches the epithelium. As a result, the unstirred layer overlying the epithelium has a pH much closer to neutral than layers more distant from the mucosal surface [82–85]. In vivo confocal imaging of the gastric juxtamucosal alkaline layer in anesthetized mice shows that the pH set point of this layer is determined by the balance between epithelial H^+ and HCO_3^- secretion and not by the thickness of the unstirred layer [86]. Further, the presence or absence of the gastric mucus layer appears to have no effect on surface pH. Thus, apical HCO_3^- secretion by gastric surface foveolar cells and duodenal villous absorptive cells is an important example of a highly specific and regionally localized extrinsic epithelial barrier. Intracellular HCO_3^- may also play an important role in cytoprotection of the duodenal epithelium [87], providing further evidence of the complex interactions between multiple ion transporters [85].

Antimicrobial peptides

Gut epithelial cells produce and secrete peptides with antimicrobial functions. Several classes of peptides have been isolated from humans, including members of the defensin, cathelicidin, and histatin families. Paneth cells at the base of the crypts in the small intestine and ascending colon release certain enzymes with antimicrobial activity, including lysozyme and type II phospholipase A_2. Paneth cells also produce defensin peptides [88], of which some are released specifically in response to bacteria [89,90]. Some defensins not only exert direct antibacterial activity [91] but also orchestrate a protective host response by signaling to immune cells and by stimulating apical Cl^- and water secretion to flush the lumen [92,93]. The observations that the susceptibility gene *NOD2* for Crohn's disease is required for expression of some defensins [94] and that decreased defensin gene copy number may be related to colonic Crohn's disease [95] suggest that disruption of essential protective defensin functions may contribute to the pathogenesis of Crohn's disease [96].

Intrinsic barriers

The contribution and presence of specific extrinsic barriers vary in different regions of the gastrointestinal tract. In contrast, the intrinsic barrier is formed by the continuous sheet of epithelial cells that lines the entire gastrointestinal tract. This uninterrupted epithelial layer separates lumenal material from the subepithelial space.

Classically, discussions of epithelial barrier function consider two routes by which material may traverse the barrier;

the transcellular and paracellular pathways (Fig. 8.3). The exact physical site where solutes cross the epithelium was a topic of considerable controversy until it was recognized that proteins insert into and form channels across lipid bilayers. Similarly, the paracellular pathway was thought to be impermeable and unregulated, a misconception perpetuated well into the 20th century because of the static appearance of intercellular contacts seen in early electron micrographs. Indeed, even the term "tight" junction is a misnomer, as tight junctions form a transcellular barrier that selectively allows paracellular flux of ions and small molecules. Our present understanding of transcellular and paracellular transport emerged along with the recognition that both are physiologically regulated and vary widely in different tissues. Transepithelial transport of hydrophilic solutes along these two pathways will be discussed below. The movement of water and hydrophobic molecules across the epithelium poses unique challenges. Hydrophobic compounds can cross epithelial cells directly by virtue of their solubility in the lipid bilayer. For example, saturated fatty acids cross jejunal epithelial cell microvillous membranes at rates more than 10^{11}-fold faster than they diffuse in aqueous solution. Fat absorption, the most physiologically important transepithelial movement of hydrophobic compounds, is considered separately (see Chapter 18). Transmucosal water movement, although incompletely understood, is discussed later in this chapter (see Water movement across the epithelial barrier).

Transcellular pathway

The transcellular pathway is highly restrictive to the passive flow of hydrophilic solutes. To traverse an epithelial cell, an ion or other hydrophilic solute must interact with three barriers in series: the apical membrane, the cytosol, and the basolateral membrane (Fig. 8.3). Although the cytosol has the potential to limit transcellular molecular flux, the two plasma membranes are the key barriers that restrict the passive movement of hydrophilic solutes across epithelial cells.

The lipid bilayers of the apical and basolateral membranes prevent massive flux of hydrophilic solutes and preserve transmembrane electrochemical gradients; the high resistance to passive ion flow across model lipid bilayers approaches impermeability. Biological membranes, which are composed of lipid bilayers and membrane proteins, are slightly less impermeable, but still capable of considerable resistances to passive ion flow that are several orders of magnitude greater than those of intact alimentary epithelial cell membranes. Integral membrane proteins such as transporters, pumps, and channels contribute to the relative permeability of alimentary epithelia. These proteins serve a critical need as the transmembrane movement of ions is essential for cellular homeostasis.

As discussed previously, vectorial transport relies on the polarized delivery of transporters, pumps, and channels to the apical and basolateral membranes. The specific example of glucose absorption in the small intestine will be considered here (Fig. 8.3). For detailed discussions of absorption and secretion see Chapters 13–20. Glucose is actively transported across the apical plasma membrane by the Na^+/glucose cotransporter SGLT1 [1]. The absence of this critical transporter results in glucose–galactose malabsorption, an autosomal recessive disease characterized by the failure to absorb these carbohydrates from the diet [97]. The energy source that allows efficient uptake of lumenal glucose by SGLT1 is the high extracellular, and low intracellular, Na^+ concentration; two Na^+ ions are absorbed along with each glucose molecule. The apical positioning of SGLT1 ensures that glucose is never secreted into the lumen, as the Na^+ gradient makes this thermodynamically unfavorable. The necessity for this apical SGLT1 targeting was clearly shown by defects in transepithelial Na^+ and glucose transport when an SGLT1 molecule engineered to include a basolateral targeting sequence was transgenically expressed in intestinal epithelial cells [2]. Once within the cytosol, Na^+ and glucose diffuse to the basolateral membrane. Here, Na^+ ions are pumped out of the cell and into the subepithelial and basolateral interstitium by the Na^+,K^+-ATPase, and glucose molecules diffuse across the membrane in a concentration-dependent manner facilitated by the glucose transporter GLUT2. The basolateral positioning of GLUT2 allows it to operate in the reverse direction, bringing glucose into the epithelial cell from the subepithelial interstitium, in the absence of lumenal nutrients. SGLT1 and GLUT2 are specific transporters for sugars; other transporters with similar properties manage the transport of amino acids and other nutrients. It should be apparent that the subepithelial deposition of Na^+ and glucose results in an osmotic gradient that drives water absorption (Fig. 8.3). This is exemplified by the severe diarrhea and dehydration that are usually fatal in patients with glucose–galactose malabsorption unless these sugars are eliminated from the diet (see Chapter 51).

Paracellular pathway

The paracellular pathway is a major pathway for passive solute permeation. Although plasma membranes tend toward high resistance, alimentary epithelia, with the exception of the esophageal epithelium, have low net resistance, meaning that they are relatively leaky. Detailed molecular, biophysical, and morphological analyses have shown that the paracellular pathway is largely responsible for the leakiness of these epithelia.

The paracellular pathway consists of the apical intercellular tight junction and the underlying paracellular space. Under most conditions the tight junctions are the rate-limiting barrier, restricting passive movement of hydrophilic solutes through the paracellular space. The permeability of tight junctions to ions and solutes varies between tissues and even between sites within tissues (e.g., crypt vs villus) and, in the resting physiological state, tight junctions may leak small

quantities of molecules the size of monosaccharides and disaccharides. The degree of this leakiness is regulated and, as will be described, greatly increased under some conditions [30,98,99].

The selective ionic permeability of the tight junction is largely defined by the expression of specific members of the claudin family of proteins [25,100,101]. Familial hypomagnesemia, a disease of deficient renal tubular Mg^{2+} reabsorption resulting from the loss of a single claudin isoform [23,102], is the best example of this selective mechanism. Although similar genetic losses of claudin family members have not been described in gastrointestinal disease, the unique claudin protein distributions along the length of the gastrointestinal tract, as well as along the crypt–villus axis, explain, at least in part, the variation in paracellular permeability at different sites [103]. Changes in the specific pattern of claudins expressed by intestinal epithelia may also contribute to disease. For example, inflammatory bowel disease is associated with increased claudin-2 expression and decreased claudin-5 and -8 expression [104–107]. In vitro studies suggest that claudin-2 expression increases tight junction permeability [101,108], which is consistent with the reported increased permeability in inflammatory bowel disease.

Water movement across the epithelial barrier

Despite the obvious importance of fluid transport across gastrointestinal epithelia, controversy remains about the relative importance of the transcellular vs paracellular routes. One route for transcellular water movement is through transmembrane channels created by members of the aquaporin protein family [109]. These small integral membrane proteins are well studied in tissues specialized for regulated water transport, such as the collecting duct of the kidney. Although aquaporins are expressed in gastrointestinal epithelia, and expression may be reduced in colitis [110], their contribution to water movement in the gastrointestinal tract remains unknown. Numerous mouse models deficient in specific aquaporins have shown defective water movement in salivary glands, pancreas, and liver, but only minor difficulty in handling water in the intestine [109,111,112]. Other models have suggested that the apical Na^+/glucose cotransporter SGLT1 serves as a molecular water pump, carrying a large number of water molecules with each glucose molecule transported [113]. However, the osmotic gradient discussed previously can completely explain the enhanced water absorption induced by Na^+/glucose cotransport, thereby raising some doubt as to the quantitative contribution of this pathway [114,115]. Transcellular water movement may also occur across lipid membranes; water movement is much less restricted than that of hydrophilic solutes. However, the mechanism by which water permeates biological membranes in the absence of specific channels is uncertain.

Although there is general agreement that water flux requires both transcellular and paracellular routes, the relative contribution of each is controversial. Some data suggest that about 50% of the water absorption stimulated by Na^+/glucose absorption is paracellular. Data from a variety of other epithelia show that interepithelial differences in hydraulic conductivity, a measure of force-induced water flux, correlate reasonably well with transepithelial electrical resistance. Finally, data suggest that a leaky paracellular pathway is necessary to support the massive water secretion that accompanies acute immune-mediated diarrhea [30]. These observations suggest that the paracellular pathway is a major route for water flow across intestinal epithelia whether such flow is driven by hydrostatic or osmotic pressures [116–118].

Transport of xenobiotics

The gastrointestinal tract, particularly the small intestine, is actively involved in the transport and metabolism of foreign chemical compounds, including environmental toxins and therapeutic agents. Many of these so-called xenobiotics are absorbed transcellularly; some are lipophilic and dissolve easily in lipid membranes. Others take advantage of apical uptake pathways that are normally expressed, such as the apical Na^+-dependent bile salt transporter or members of the organic anion transporting polypeptide family [3,119,120]. Basolateral transporters, such as multidrug resistance associated protein 3, ABCC3, may then allow xenobiotics to traverse the basolateral membrane [121]. A significant fraction of these compounds may never reach the basolateral membrane, as members of the cytochrome P450 system expressed in enterocytes may contribute a "first-pass" effect of their own [122]. Analogous to the effects of drugs metabolized by the hepatic cytochrome P450 system, enterocyte nuclear receptors can up-regulate expression of enterocyte drug transporters and cytochrome P450 enzymes [3,119]. These layers of regulation not only challenge the maintenance of steady-state drug levels but also increase the potential for drug interactions [123]. Dysregulation of xenobiotic metabolism and transporter activity may be a pathogenetic mechanism in inflammatory bowel disease [124]. Genetic polymorphisms in these transporter proteins may also lead to significant variation in clinical responses [125,126].

In addition to metabolic clearance, active secretion rapidly clears many xenobiotics from the intestine. This secretory activity, which is primarily mediated by MDR1, an apical multidrug resistance transporter family member [124,127], exhibits significant interindividual variability, similar to absorption and metabolism. Interestingly, *MDR1* mutations have been associated with inflammatory bowel disease in some patient populations [128], and a knockout mouse lacking *mdr1* spontaneously develops colitis [129,130]. *MDR1* polymorphisms may also be related to disease behavior in ulcerative colitis [127,131], suggesting that defective export of an unidentified xenobiotic contributes to the pathophysiology of intestinal disease [132].

Epithelial homeostasis and responses to disease and injury

Commensal bacteria

Bacteria normally colonize the entire human gastrointestinal tract, with the highest concentration and number of species in the colon. Although bacterial cells outnumber the cells of the human body by about 10-fold, most of these organisms belong to only three evolutionary divisions that are, at least in part, modified in a host-specific manner [133–135] (see Chapter 25). These nonpathogenic bacteria are termed commensal, to distinguish them from well-characterized pathogenic species such as *Salmonella*, *Shigella*, and *Clostridium difficile*. It appears that the commensal host–bacteria relationship is beneficial [136], as normal gut function is highly dependent on resident bacteria. Such probiotic effects take several forms, including competing with pathogens for attachment to the epithelial surface, triggering intracellular signal transduction events that limit disease, and inciting the epithelium to release antimicrobial compounds [137–139]. Studies in germ-free mice show that the normal development of immune cell lineages in the bone marrow and the lamina propria and local humoral defense depends on the presence of the commensal bacteria in the gut [140,141]. Inductive effects on epithelial cell gene transcription have also been observed [142]. For example, introducing commensal *Bacteroides* species into germ-free mice extensively alters the bacterial and epithelial transcription profiles toward gene products that enhance nutrient uptake and metabolism [143–145], revealing a possible association with obesity [146,147].

Although the molecular mechanisms remain poorly understood, published reports of the probiotic effects of commensal bacteria are increasing. For example, sterilization of the gut greatly enhances disease severity in some murine models of colitis [138,139,148]. This effect appears to be at least partially mediated by toll-like receptors (TLRs) that specifically recognize bacterial products [138,139,149–152]. Beneficial effects of probiotics have been reported in experimental disease as well as in ulcerative colitis and pouchitis [153–155]. The effects of bacteria on intestinal function are discussed in detail in Chapters 25, 48, 49, and 52.

Physiological epithelial injury

Epithelial injury is most readily apparent when gaps within the epithelium such as erosions or ulcerations are present. However, because the gut has a remarkable ability for repair, many forms of focal acute injury do not result in functionally significant defects. One example is the rapid sealing of wounds that must occur during the physiologically normal turnover of gastrointestinal epithelia. Gut epithelial cells turn over, on average, once each week through coordinated proliferation, migration, apoptosis, and sloughing. For example, small intestinal enterocytes arise from the stem cell compartment, i.e., the crypt, migrate upward through the proliferative zone, and undergo an ordered process of differentiation as their phenotype is modified from undifferentiated secretory cell to fully differentiated villous absorptive cell. They are then sloughed from the villous surface. Membrane proteins, with half-lives considerably shorter than that of the epithelial cell, also turn over. The composition of these proteins changes remarkably during the process of differentiation. Lipid turnover undoubtedly occurs as well but technical challenges hinder its documentation. Perhaps most remarkable in this continual process of renewal is that, at sites of epithelial cell detachment, the barrier remains intact [156,157]. In vivo imaging studies suggest that a presently undefined substance fills the "gap" left as the epithelial cell exits the villus, preventing diffusion of lumenal material into the subepithelial villous core [156,158]. The renewal process must then be completed by some form of wound closure, possibly involving cytoskeletal contraction [158–161].

Regulation of barrier function by physiological stimuli

Intestinal permeability can be regulated by physiological processes. For example, it is well documented that Na^+/nutrient cotransport enhances the permeability of absorptive tight junctions to molecules the size of amino acids and glucose [99,162,163]. Although the physiological significance of these Na^+/nutrient cotransport-induced increases in tight junction permeability remains controversial, the process probably underlies the observations that, at high lumenal glucose concentrations, both glucose and amino acid absorption exceed the capacity of their respective transcellular transport systems [164–166]. The concept of "solvent drag" offers an explanation [167]. For example, as described previously, active transcellular Na^+ and nutrient absorption result in the development of a transepithelial osmotic gradient that drives water absorption. Solvent drag is the mechanism by which water absorption across the tight junctions with increased permeability allows the solvent (i.e., water) to drag nutrient-sized molecules (i.e., free glucose within the unstirred layer) across the tight junction. In this manner, transcellular absorption is amplified by paracellular absorption [167]. The latter depends on generation of a suitable transepithelial osmotic gradient and increased permeability of tight junctions to small molecules. This mechanism can also enhance paracellular absorption of undegradable compounds, such as D-amino acid-substituted short peptides and creatinine [168,169]. Oral pharmaceutical delivery may benefit from use of this pathway.

Physiological tight junction regulation is initially triggered by the apical Na^+/glucose cotransporter SGLT1 (Fig. 8.3). This initiation of Na^+/glucose cotransport activates a signal transduction pathway that induces increased NHE3-mediated apical Na^+/H^+ exchange, resulting in mild cytoplasmic

alkalinization and enhance Na^+ absorption [170,171]. These events are accompanied by the activation of myosin light chain kinase, which phosphorylates the myosin II regulatory light chain and triggers contraction of the perijunctional actomyosin ring [172]. Although the molecular details of the subsequent tight junction remodeling are not yet defined, it is clear that this myosin II regulatory light chain phosphorylation is required for Na^+/glucose cotransport-induced increases in tight junction permeability.

Dysregulation of barrier function in intact epithelium

Despite an intact epithelium, tight junction permeation to inert solutes is enhanced in many inflammatory, infectious, ischemic, and immune-mediated intestinal diseases [173,174]. For example, permeability defects in celiac sprue can be reversed by a gluten-free diet [175]. Similarly, in both graft-versus-host disease and HIV infection, increased serum lipopolysaccharide levels (reflecting leakage of lumenal contents) correlate with disease severity [176,177].

Permeability defects are also seen in Crohn's disease; increased permeability in patients with inactive disease can predict disease reactivation [178,179]. In addition, permeability defects are present in a subset of healthy first-degree relatives of patients with Crohn's disease [180,181]. This has led to speculation that a primary defect in tight junction barrier function may cause Crohn's disease [181–183]. Despite this, it is clear that the inflammation of Crohn's disease can also cause increased permeability, as barrier function can be restored by treatment with antibodies that neutralize tumor necrosis factor [184]. Conversely, tumor necrosis factor acutely reduces barrier function in cultured intestinal epithelial monolayers and jejunal epithelia of intact mice [116,185–187].

In a remarkable demonstration of how pathophysiological events can hijack physiological regulatory mechanisms, investigators have shown that tumor necrosis factor disrupts the intestinal epithelial tight junction by way of myosin II regulatory light chain phosphorylation [188]. In vitro studies have shown that this is due to both transcriptional and enzymatic activation of myosin light chain kinase [185,186,189,190], and that similar increases in myosin light chain kinase expression and enzymatic activity correlate with disease activity in inflammatory bowel disease [191]. Moreover, in vivo work has shown that myosin light chain kinase-driven loss of barrier function is required for the development of acute tumor necrosis factor-mediated diarrhea [30]. These data have led to the proposal of a disease model in which impaired mucosal barrier function leads to increased leakage of lumenal contents and inappropriate immune stimulation, subsequent interferon-γ and tumor necrosis factor release, and further loss of barrier function. The result is a self-amplifying cycle of barrier dysfunction and inappropriate immune activation [173]. Thus, compromised barrier function may be a critical event in the initial pathogenesis and subsequent exacerbation of inflammatory bowel disease and other intestinal diseases.

Healing of epithelial wounds

The destruction of gastrointestinal epithelial cells, as occurs in erosions and ulcers, leads to the loss of epithelial barrier function. Both the magnitude and duration of injury determine the epithelial response. Wounds representing loss of approximately 1–10 epithelial cells close extremely rapidly, within 30 min or less, by a purse-string closure mechanism [159–161,192]. This response depends on some of the same cytoskeletal mechanisms discussed previously, with small GTPases (e.g., Rho) directing the assembly of a ring of actin cables at the edge of the wound (Fig. 8.6) [159,193]. These cables, which are connected across adjacent cells by intercellular junctions, assemble within minutes of wounding and then begin to contract by a mechanism that requires myosin light chain kinase activity [159]. As noted, some data suggest that a similar purse-string mechanism maintains the epithelium after the extrusion of single apoptotic cells, which is a normal physiological process.

Larger epithelial wounds must also be sealed quickly. The initial rapid cell migration, termed restitution, involves a dramatic cytoskeletally directed modification of cell shape (Fig. 8.6) [194]. The columnar cells bordering a wound, normally tall, spread to become flattened, taking on an almost squamoid appearance and maximizing the basement membrane surface area covered by each cell [194]. Often seen in intestinal endoscopic biopsies, this flattened appearance is an easily recognized marker of ongoing epithelial restitution. Persistent injury also stimulates cell growth. The same stimuli often evoke both motogenic (migration-promoting) and mitogenic (proliferation-promoting) effects [195–199]. Thus, inflammatory mediators and growth factors may promote initial reepithelialization of wounds by initiating restitution, and support this process over extended periods by enhancing cell proliferation.

Interactions of epithelia with subepithelial cells

Gut epithelial function may be modulated by a host of local factors derived from nonepithelial sources, such as growth factors, cytokines, and chemokines, which are discussed in detail in Chapters 4 and 7. Direct interactions between epithelial cells and the immune system also occur; an obvious example involves the M, or microfold, cell [200]. This specialized epithelial cell resides in the convex dome epithelium that overlies mucosal lymphoid follicles. Although indistinguishable from adjacent enterocytes by light microscopy, electron microscopic evaluation shows that the basal membrane of M cells is retracted from the basement membrane, forming a cleft into which lymphocytes and macrophages migrate (Fig. 8.5). M cells actively sample lumenal material by bulk endocytosis; the transport vesicles are then released into the cleft, permitting extremely rapid delivery of

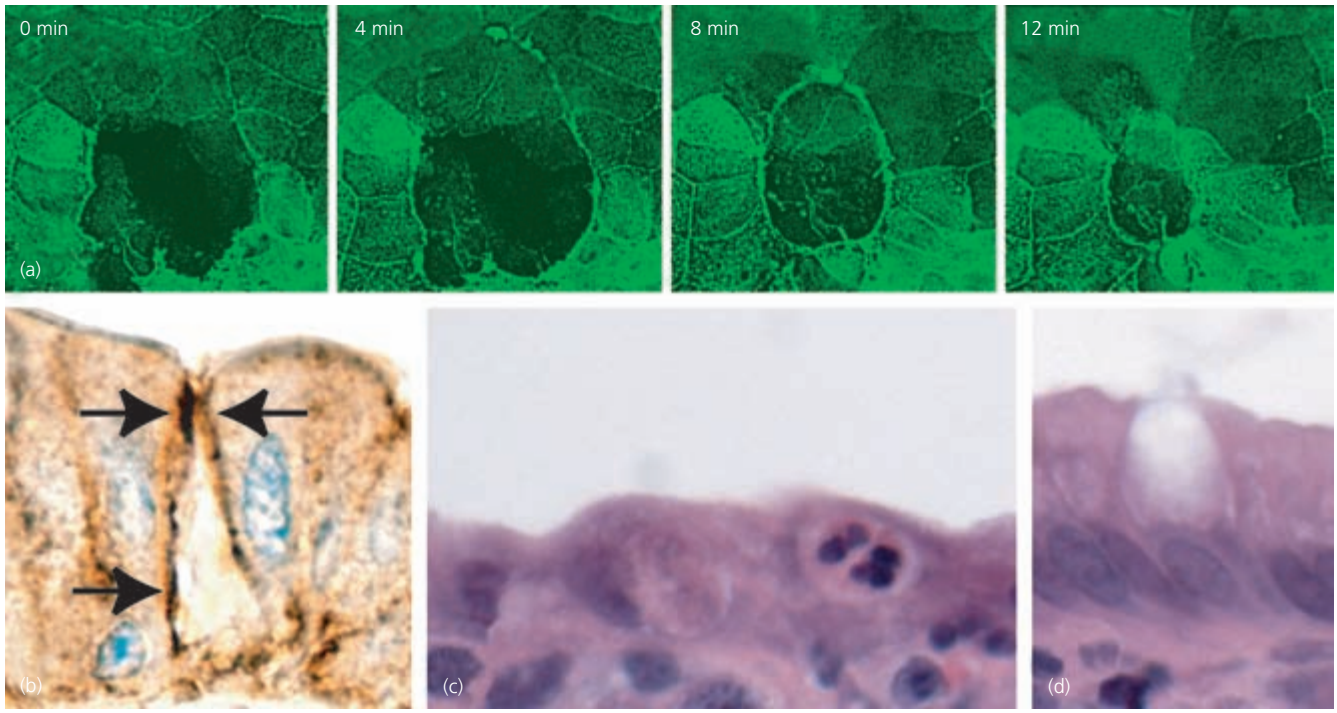

Figure 8.6 Mechanisms of epithelial wound closure. **(a)** A time-lapse series of cultured intestinal epithelial cells expressing fluorescent actin shows that a ring of actin forms to surround a wound within minutes after injury. This ring then contracts in a purse-string manner, rapidly closing the wound and restoring barrier function. **(b)** Immunohistochemical analysis of a small wound present in a patient with Crohn's disease shows that, similar to in vitro experimental wounds, phosphorylated myosin II regulatory light chain is concentrated at the closing edge of the injury site. **(c)** Larger wounds cannot heal by simple purse-string wound closure. This specimen from a patient with ulcerative colitis shows that intestinal epithelia flatten to spread and rapidly reseal the surface, a process called restitution. **(d)** Contrast the flattened shape of epithelia during restitution with the tall columnar shape of epithelial cells in an area of intact mucosa. Panels **(a)** and **(b)** are from Russo et al [159], with permission from Elsevier.

lumenal material to immune cells. Infectious organisms may exploit this pathway as a route of invasion. Other epithelial cells not confined to follicle-associated epithelium may also be capable of transporting antigens to mucosal immune cells [201], and a specialized population of dendritic cells within the ileal lamina propria actually extend slender processes across the tight junction to directly sample lumenal antigens and bacteria (Fig. 8.5) [202–204]. Finally, although not yet well described, additional cell types, beyond epithelial and immune cells, clearly regulate epithelial function [205–207].

Integration of mucosal function

The gastrointestinal mucosa is a complex structure that coordinates a variety of critical functions. These include balancing barrier function with the need for transepithelial movement of ions, nutrients, and antigens; rapid repair of mucosal injuries; and beneficial interactions with the array of mucosal immune cells. The precise integration of these functions centers on the epithelium, which is continuously repaired by rapid wound closure, restitution, and other mechanisms. The gut epithelial barrier, which restricts passive movement of molecules, is complex and dynamic. Maintenance of this barrier depends on the integrity of cellular plasma membranes and intercellular tight junctions. The mucosal barrier also benefits from the contributions of mucus, epithelial secretory products, such as HCO_3^-, and secreted immunoglobulins. Potential threats within the lumen are continuously surveyed and managed by M cells, intraepithelial and lamina propria lymphocytes, dendritic cells, and macrophages. From the examples provided in this chapter, and other chapters in this textbook, it is evident that dysregulation of any of these functions can result in diseases with overlapping clinical presentations. Thus, future studies should aim to better understand the interplay between the intricate systems that comprise the gastrointestinal mucosa.

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B MOTILITY

9 Esophageal motor function

Peter J. Kahrilas, John E. Pandolfino

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The esophagus is the simplest of organs, a hollow muscular tube with a sphincter at each end, designed to keep itself empty in the face of frequent intrusions from above and below. Despite such simplicity of function, the structure and control mechanisms of the esophagus are far from simple. One of the many complexities of the esophagus is that it encompasses the anatomical and physiological transition between two distinct regions, the oropharynx and the gut. The oropharynx is composed of striated muscle, controlled by the cerebral cortex and the medulla, and is capable of precise tactile sensation; the distal esophagus is composed of smooth muscle, controlled by the vagus nerve and the enteric nervous system, and only capable of notoriously imprecise sensation. Although there is a gradual transition between these end points, motor function in the oropharynx and esophageal body are quite distinct. The ensuing discussion exemplifies this distinction, describing selected aspects of pharyngeal, gastric, and diaphragmatic functions that are inextricably entwined with esophageal functions. Similarly, in the interest of presenting swallowing as the complex, highly integrated motor response that it is, the discussion of swallowing encompasses the oropharyngeal swallow and central nervous system control mechanism as well as the strictly esophageal aspects of swallowing.

Pharynx and upper esophageal sphincter

Anatomy and function

Pharynx

The oral cavity, pharynx, and larynx are all involved in the task of transferring food from the palate to the esophagus. Within the oral cavity, the lips, teeth, hard palate, soft palate, mandible, floor of the mouth, and tongue serve functions in masticating, containing, and forming food into a bolus suitable for transfer to the pharynx. The pharynx is a hollow cylinder extending from the base of the skull to the lower border of the cricoid cartilage. Anatomically it is separated into three segments: nasopharynx, oropharynx, and hypopharynx (Fig. 9.1). The nasopharynx extends from the base of the skull to the distal edge of the soft palate. Although the nasopharynx is not part of the alimentary tract, muscles located in the nasopharynx contribute to elevating the soft palate and sealing the nasopharynx during swallowing to prevent bolus entry. The oropharynx extends from the soft palate to the base of the tongue. The inferior margin of the oropharynx is demarcated by the valleculae anteriorly and the mobile tip of the epiglottis posteriorly. The hypopharynx extends from the valleculae to the inferior margin of the cricoid cartilage and includes the upper esophageal sphincter (UES).

Muscle groups participating in deglutition include those of the soft palate, tongue, and pharynx. In addition, muscles that elevate and pull the pharynx forward, sometimes referred to as extrinsic muscles, alter the shape of the pharynx and seal the airway during deglutition. Intrinsic muscles, on the other hand, are responsible for collapsing the lumen of the pharynx and propelling the bolus into the esophagus. Within the nasopharynx, the levator veli palatini, tensor veli palatini, and palatoglossus act to raise and tense the soft palate and uvula, sealing the nasopharynx and preventing the dissipation of pressure through the nose. Posteriorly, the stylohyoid, styloglossus, palatopharyngeus, stylopharyngeus, and posterior digastric elevate the larynx, while the geniohyoid, mylohyoid, anterior digastric, and thyrohyoid pull the larynx forward and contribute to the opening of the UES.

The intrinsic muscles of the pharynx are the superior, middle, and inferior pharyngeal constrictors (see Fig. 9.1). The constrictors overlap and insert into a collagenous sheet, the buccopharyngeal aponeurosis. The superior pharyngeal constrictor arises from the pterygoid hamulus, pterygomandibular raphe, mandible, and tongue, passes posteromedially, and inserts in the posterior raphe. The middle constrictor arises from the hyoid bone and stylohyoid ligament, passes

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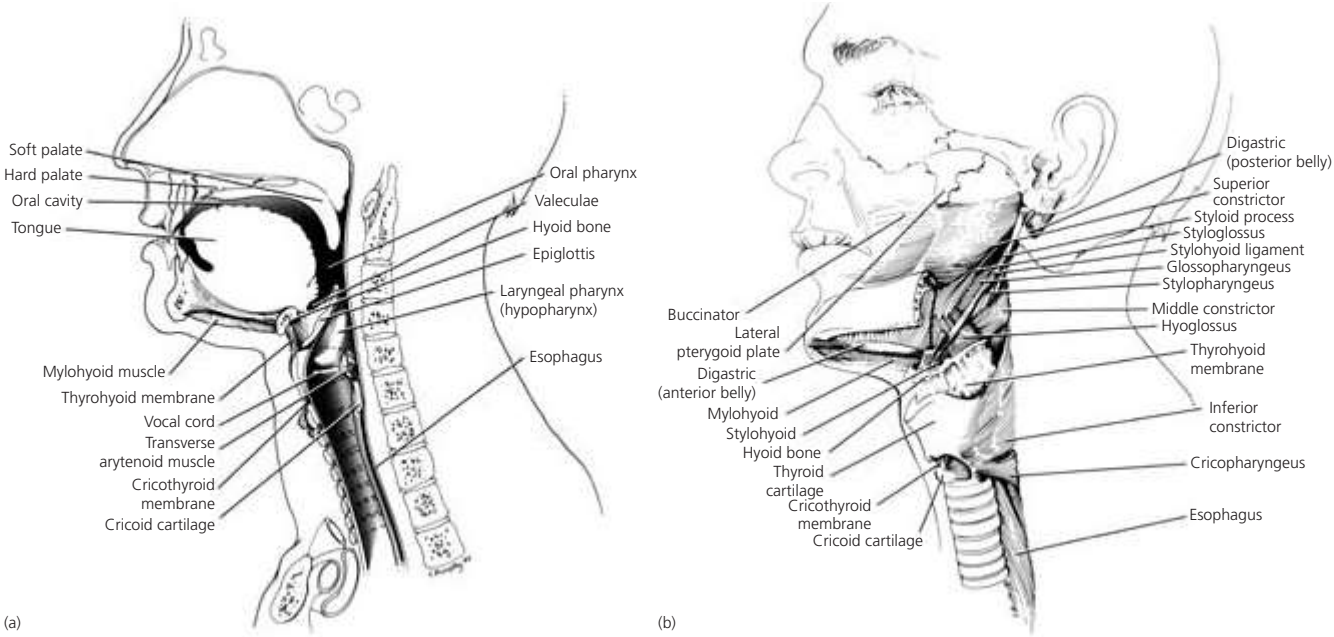

Figure 9.1 Anatomy of the pharynx. **(a)** Sagittal view of the pharynx showing the musculoskeletal structures involved in swallowing. At rest, the esophagus is collapsed and empty. In the course of a swallow, the laryngeal inlet is sealed and the mouth of the esophagus is opened by highly coordinated muscular activity. **(b)** Cutaway view of the musculature of the pharynx. Note that the hyoid bone is positioned as a fulcrum and is instrumental in directing anteriorly and superiorly acting forces toward the larynx, opening the esophageal inlet.

posteromedially, and inserts in the posterior median raphe. The inferior constrictor comprises the thyropharyngeus (superior part) and the cricopharyngeus (inferior part). The thyropharyngeus arises from the thyroid cartilage, passes posteromedially, and inserts in the median raphe. The cricopharyngeus has superior and inferior components, each of which arise bilaterally from the sides of the cricoid lamina; the superior fibers course posteromedially to the median raphe, whereas the inferior fibers loop around the esophageal inlet without a median raphe. The Killian triangle, a triangular area of thin muscular wall, is formed posteriorly between these components of the cricopharyngeus and is the site of origin of Zenker diverticulum.

The pharyngeal walls contain or are supported by five cartilages: epiglottic, arytenoid, cuneiform, corniculate, and cricoid (see Fig. 9.1). The spaces formed between the lateral insertion of the inferior constrictor and the lateral walls of the thyroid cartilage are the pyriform sinuses, which end inferiorly at the cricopharyngeus muscle, separating the pharynx from the esophagus. The larynx and trachea are suspended in the neck between the hyoid bone superiorly and the sternum inferiorly. A number of muscles, categorized as the laryngeal strap muscles, contribute to this suspension and, together with the intrinsic elasticity of the trachea, permit the larynx to be raised and lowered. The tongue rests on the hyoid bone, which also serves as its base. Laryngeal movement is crucial to the successful enactment of the swallow response as the laryngeal inlet is both closed and physically removed from the bolus path in the course of a swallow. Failure to achieve this complex laryngeal movement can result in aspiration.

The motor neurons that innervate the pharyngeal muscles have their cell bodies in the nuclei of the trigeminal, facial, glossopharyngeal, and hypoglossal nerves, as well as in the nucleus ambiguus and spinal segments C1–C3. The pharynx is densely innervated, with a ratio of motor fibers to muscle fibers of between 1:2 and 1:6 [1], which is comparable to ratios in the extraocular muscles and suggests the capacity for exquisite motor control. The innervation of the major pharyngeal muscles is as follows: mylohyoid, tensor veli palatini, and anterior digastric muscles – trigeminal nerve; stylohyoid and posterior part of the digastric – facial nerve; stylopharyngeus – glossopharyngeal nerve; levator veli palatini, palatopharyngeus, salpingopharyngeus, thryroarytenoid, arytenoid, pharyngeal constrictors, and cricopharyngeus – vagus nerve; thyrohyoid, geniohyoid, and tongue – hypoglossal nerve [2]. The nucleus ambiguus is the vagal nucleus responsible for the innervation of the striated muscle of the pharynx, larynx, and esophagus [3]. Within the nucleus ambiguus, all motor neurons seem to participate in swallowing, with those innervating the esophagus situated rostrally and those innervating the larynx situated more caudally [4]. The significance of this internal organization is highlighted by the observation that polio victims with damage to the rostral portion of the nucleus ambiguus experience dysphagia, whereas those with damage to the caudal portion experience primarily dysarthria.

Upper esophageal sphincter

The muscular elements of the UES are the cricopharyngeus, adjacent esophagus, and adjacent inferior constrictor. The cricopharyngeus inserts bilaterally into the inferior–lateral margins of the cricoid lamina and the zone of maximal UES pressure is about 1 cm in length at precisely this location [5]. The closed sphincter has a slit-like configuration with the cricoid lamina anteriorly and the cricopharyngeus making up the lateral and posterior walls. Thus, it is not surprising that the resting UES pressure is markedly asymmetric with the greatest values anteriorly and posteriorly [6]. The asymmetry disappears in patients who have undergone laryngectomy, supporting the concept that the asymmetry is attributable to the cross-sectional shape imposed by the laryngeal cartilage that forms the anterior wall of the sphincter. The maintenance of high resting pressure and the precise coordination of relaxation with swallowing requires neural input. In the opossum, sphincter tone is mediated through neural fibers in the vagal trunks that originate in the nucleus ambiguus [7]. Electrophysiologically, the muscles of the UES exhibit continuous spike activity, indicating that motor neuron activity is responsible for the maintenance of tone. Cessation of firing, or administration of curare, causes relaxation, whereas increased spike activity increases tone. Vagal transection abolishes contractile activity in the cricopharyngeus and inferior pharyngeal constrictor muscles [8].

Manometric evaluation of UES function is difficult because the UES is a short, complex anatomical zone that moves briskly during swallowing. Furthermore, UES pressure measurement is heavily influenced by recording methodology because of both the marked asymmetry of the UES and the fact that the measurement per se stimulates sphincter contraction. The less movement applied to the recording catheter and the smaller the measuring device, the lower the recorded pressures [9]. Thus, considerable variability in reported "normal" UES pressure ranges makes it impossible to define a meaningful normal range [9]. Measurement of UES relaxation during swallowing also poses substantial recording challenges. Relaxation occurs during swallow-associated laryngeal elevation [10]. However, movements of the sphincter and the transnasally positioned catheter are dysynchronous. The UES may move 2–3 cm proximally during swallowing, whereas the sensor may move only 1 cm [10]. Given the short length of the high-pressure zone, this dissociation simulates relaxation when recorded with a point sensor. Although positioning the recording site at the proximal aspect of the UES to anticipate subsequent movement would appear to be a logical solution [11], variable movement of both the UES and the catheter among individuals and certainly among various disease conditions diminishes the reliability of such an approach. High-resolution manometry is capable of a resolution of 1 cm and can accurately measure UES pressure changes during swallowing, the degree of UES relaxation, and intrabolus pressure (Fig. 9.2).

The main function of the UES is to maintain closure of the proximal end of the esophagus unless opening is required for either swallowing or belching [12]. It constitutes an

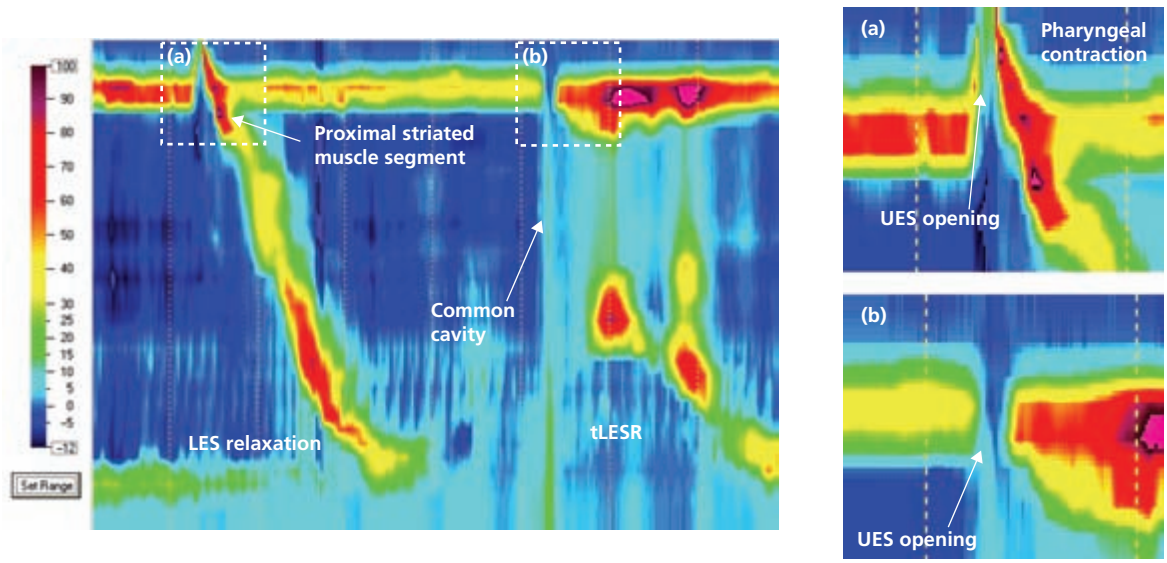

Figure 9.2 High-resolution manometric analysis of upper esophageal sphincter (UES) function. High-resolution manometry can quantify the completeness of lower esophageal sphincter (LES) relaxation, intrabolus pressure, and the duration of UES opening without movement artifact. **(a)** UES opening during swallowing. Typical UES relaxation pressures during swallowing are less than 10 mmHg. UES closure coincides with the arrival of the propagated pharyngeal contraction. The contractile wave front propagates through the striated portion of the proximal esophagus. **(b)** UES opening during belching. During a transient LES relaxation (tLESR) a common cavity develops that leads to UES relaxation and gas venting. Note the lack of pharyngeal activity and the esophageal shortening during the tLESR.

additional barrier to refluxed materials entering the pharynx and also prevents air from entering the esophagus during inspiration. A common observation during intralumenal UES manometry or cricopharyngeal electromyography is that the UES contracts in synchrony with inspiration, a response that probably serves to prevent the inhaling of air into the esophagus. Inspiratory augmentation is most evident during periods of low UES pressure and is often undetectable during periods of higher sphincter pressures, presumably because it is obscured by more vigorous responses. Balloon distention of the esophagus stimulates UES contraction with the effect being more pronounced with proximal balloon positions [13]. However, when the distention pattern of gas reflux is imitated by using a long cylindrical balloon or by rapid air injection into the esophagus, UES relaxation rather than contraction occurs [5]. Belch-induced relaxation is also associated with glottic closure [14]. Figure 9.2 details the unique aspects of belch-associated UES relaxation compared with swallow-associated relaxation. Note especially the absence of a pharyngeal contraction and the more prolonged UES relaxation during belching. The larger the volume of air injected into the esophageal body, the longer the duration of the ensuing UES relaxation [5]. Stress augments UES pressure in humans [15], whereas anesthesia [16] or sleep [17] virtually eliminates it. Esophageal acidification was once thought to induce UES contraction [18,19] but this response has not been confirmed, even in studies involving 30 min of esophageal acid perfusion [20]. Probably the most relevant observation is that spontaneous gastroesophageal acid reflux has no effect on continuously recorded UES pressure in either healthy volunteers [17] or in individuals with peptic esophagitis [20].

Mechanics of oropharyngeal swallowing

Oropharyngeal swallowing begins with an oral phase, which is followed by a pharyngeal phase. The largely voluntary and highly variable oral phase of swallowing is influenced by such factors as taste, environment, hunger, and motivation. Conditions characterized by global neurological dysfunction, such as head trauma, cerebral tumors, or chorea, often manifest disorders of the oral phase of swallowing. Detailed discussions of these conditions may be found in texts on swallow evaluation and therapy [21,22]. The pharyngeal phase of swallowing is the complex oropharyngeal contractile event referred to as the swallow response. A typical individual swallows about 600 times a day (350 times while awake, 200 times while eating and 50 times while asleep) without giving significant thought or effort to this activity [23]. Afferent sensory fibers capable of triggering the pharyngeal swallow travel centrally by way of the internal branch of the superior laryngeal nerve (from the larynx) and by way of the glossopharyngeal nerve (from the pharynx) [24]. These sensory fibers converge before terminating in the swallowing center of the medulla, the location and architecture of which have been extensively investigated [25]. The evolving model is summarized in Figure 9.3 [26]. The clinical significance of this neuronal architecture is that swallowing is relatively resistant to disturbance by diseases affecting

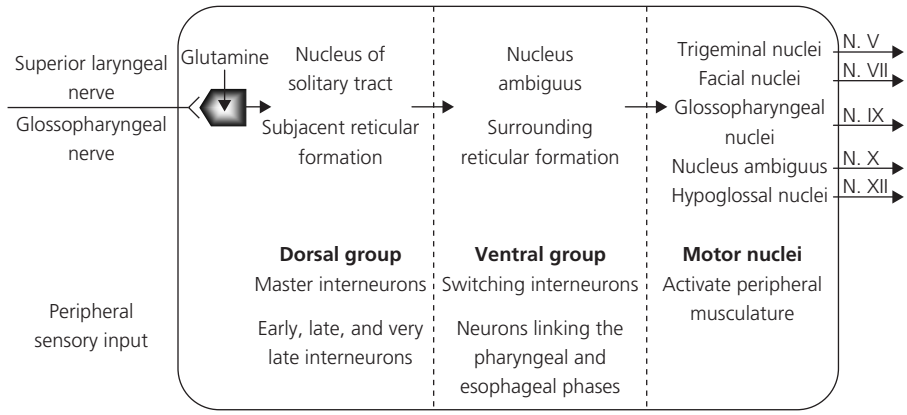

Figure 9.3 Neural organization of the pharyngeal swallow response. Sensory input enters the medulla at and below the nucleus of solitary tract and motor output exits by way of five cranial nerves. The presumed function of each group of neurons is itemized beneath each anatomical grouping. The "master" interneurons establish the timing of sequential excitation of specific cranial motor nuclei. These neurons are believed to be among the dorsal group, located in and around the nucleus of solitary tract. Once activated, the master neurons can establish the entire motor sequence of the swallow without further sensory input. Also among the dorsal group are interneurons that are activated at specific times within the swallow pattern, corresponding to the activity of specific groups of pharyngeal and esophageal muscles. Depending on the temporal relationship of neuronal activity with the onset of deglutition, these medullary neurons are classified as "early," "late," or "very late." The ventral group of interneurons, which are located in and around the nucleus ambiguus, probably function as switching neurons, relaying the swallowing orders from the dorsal pattern generators to the various motor neuron pools involved in enacting the muscular response. From Kahrilas [166].

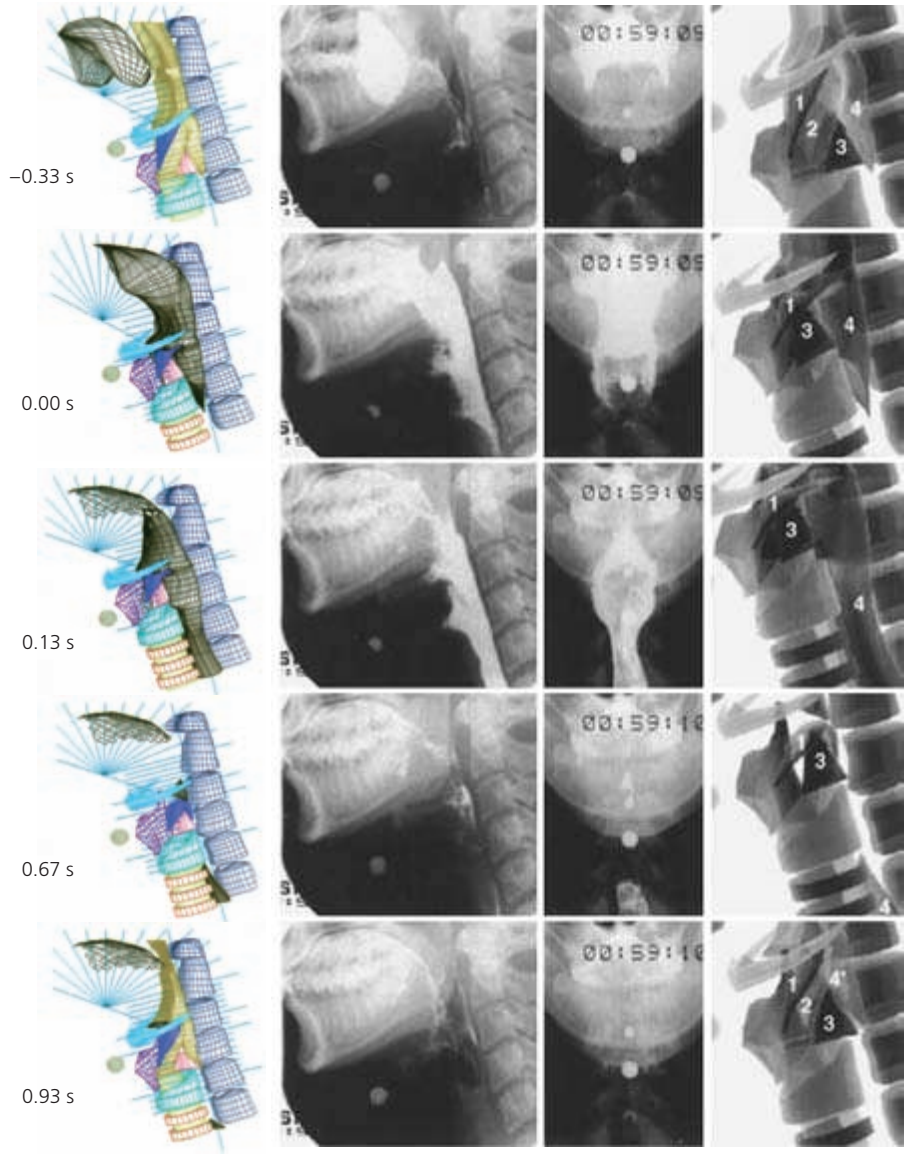

Figure 9.4 The oropharyngeal swallow as imaged by videofluoroscopy and reconstructed in three dimensions with computer graphics. From left to right, each horizontal band of images contains the three-dimensional reconstruction of the pharyngeal cavity and surrounding structures, the lateral radiographic appearance of the pharynx during a 10-mL barium swallow, the corresponding posteroanterior radiographic appearance, and a magnified view of the hypopharynx reconstructed at the instant in time indicated at the far left. Time 0.00 s is the instant of upper esophageal sphincter (UES) opening; the entire sequence transpires within 1.00 s. The metal sphere under the chin is used to compare images. In the magnified hypopharyngeal reconstructions, 1 is the epiglottis, 2 is the laryngeal vestibule, 3 is the arytenoid cartilage, 4 is the esophagus, and 4′ is the pyriform sinus after closure of the UES. Note the laryngeal elevation during the pharyngeal reconfiguration and the synchrony of UES opening with laryngeal vestibule closure.

brain centers higher than the medulla. However, medullary motor neuron diseases such as bulbar polio or amyotrophic lateral sclerosis can lead to severe dysfunction.

Although understood physiologically as the patterned activation of motor neurons and their corresponding motor units, swallowing is clinically evaluated in mechanical terms, specifically, the effect of this motor activity on the configuration of the oropharyngeal cavity. The anatomical complexity of the oropharynx is best evaluated by videofluoroscopic or cineradiographic analysis. The major events comprising the pharyngeal swallow are illustrated by the representative radiographs and three-dimensional reconstructions in Figure 9.4. As the images reveal, swallowing results in the transient geometric rearrangement of pharyngeal structures from a respiratory to an alimentary pathway, which is normally accomplished and reversed within 1 s. The overall swallow response can be subdivided into a series of closely coordinated actions:

- nasopharyngeal closure by elevation and retraction of the soft palate
- UES opening
- laryngeal closure
- tongue loading (ramping)
- tongue pulsion
- pharyngeal clearance.

Early attempts to define this coordination focused on the timing of the individual elements of the swallow response relative to each other. However, the effects of volition and the volume of the swallowed bolus make it difficult to establish a universal temporal reference among elements. A

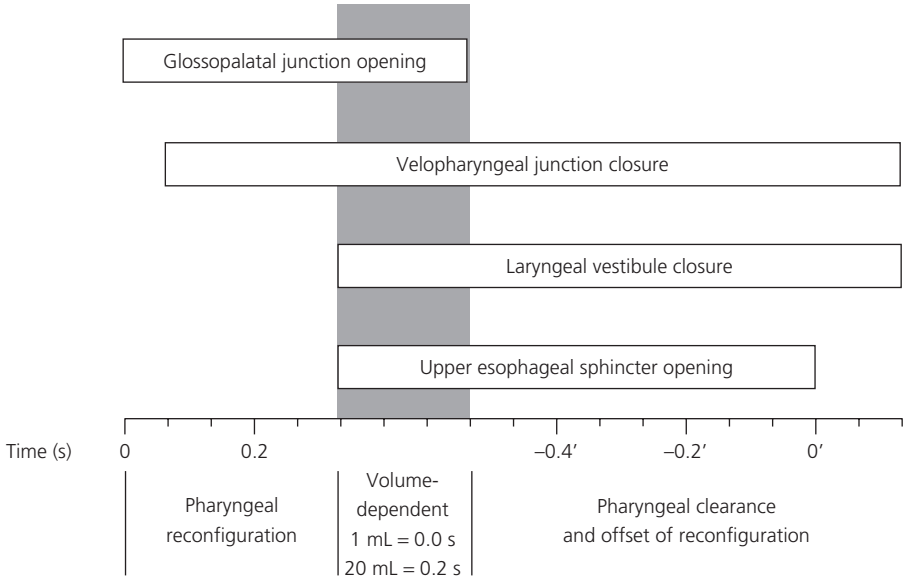

Figure 9.5 Time line showing the coordinated events and volume-induced modifications that comprise the pharyngeal swallow. Each horizontal bar depicts the period during which one of the oropharyngeal valves is in its swallow configuration as opposed to its respiratory configuration. Note that events at the onset and offset of pharyngeal reconfiguration bear a fixed time relationship to each other regardless of swallow bolus volume. The stereotypy of these phases is demonstrated by referencing onset events from time 0 counting forward, and offset events from time 0′ counting forward or backward. This timing scheme defines the volume-dependent, middle portion of the time line (shaded), which has a value of 0.0 s for 1-mL swallows and 0.2 s for 20-mL swallows. Thus, the alteration in the timing of the swallow response with larger volume swallows occurs by prolonging the persistence of pharyngeal reconfiguration without changing the synchrony of events at onset or offset. From Kahrilas et al. [167], with permission from Elsevier.

detailed analysis of the coordination among swallow events has concluded that there is, in fact, constant coordination at the beginning and end of the swallow but variability in how long the alimentary pharyngeal configuration persists before reverting to the respiratory configuration [27]. Thus, to construct a time line of the biomechanical events within the swallow, and still preserve the constancy of the most stereotypical aspects of the swallow, onset events must be timed from the beginning and offset events from the end as illustrated in Figure 9.5.

The most fundamental anatomical reconfiguration required to transform the oropharynx from a respiratory to an alimentary pathway is to open the inlet to the esophagus and seal the inlet to the larynx. The images in Figures 9.4 and 9.5 suggest that these events are synchronized. Examination of Figure 9.4 reveals that the larynx exhibits substantial axial mobility and that the UES is obligated to move with the larynx because the cricopharyngeus attaches to the lateral aspects of the cricoid cartilage and the lamina of the cricoid underlies the anterior wall of the sphincter. The mechanical determinants of UES opening are laryngeal elevation and anterior traction on the hyoid [28,29]. The mechanical determinants of closure of the laryngeal vestibule, which is almost exactly synchronized with UES opening, are laryngeal elevation and anterior tilting of the arytenoid cartilages against the base of the epiglottis [21]. Thus, analysis of the efficacy of either of these events inevitably focuses on laryngeal elevation, which is greater and persists longer for swallows of larger volumes [10]. Upper sphincter relaxation occurs at roughly the same degree of elevation regardless of swallow volume, and precedes sphincter opening by about 0.1 s. What changes with swallow volume is the persistence of laryngeal elevation above this critical value, consistent with the scheme depicted in Figure 9.5. The distinction between UES relaxation and UES opening must be recognized. UES relaxation occurs as a result of cessation of excitatory neural input while the larynx is elevating. Once the larynx is elevated, opening of the UES results from traction on the anterior sphincter wall caused by contraction of the supra- and infrahyoid musculature, which also results in a characteristic pattern of hyoid displacement [28,29]. Both the diameter and the duration of sphincter opening increase with increased bolus volume.

The two main determinants of bolus transport out of the oropharynx are the actions of the tongue and the pharyngeal constrictors. The propagated pharyngeal contraction exhibits similar propagative abilities and vigor regardless of bolus volume [30]. However, the propagated pharyngeal contraction is more involved with bolus clearance than with bolus propulsion; it strips the last residue from the pharyngeal walls. Tongue motion, in contrast, varies substantially with bolus volume suggesting that it has a cardinal role in determining differences in bolus propulsion among swallow volumes [31].

The closure of the UES coincides with the arrival of the propagated pharyngeal contraction, as evidenced by the fixed time relationship between these events [29]. However, the contractile activity of the sphincter has an added dimension in that it exhibits increased electromyographic activity

during laryngeal descent [32]. The magnitude of this deglutitive UES contractile activity is further augmented by either sphincteric or proximal esophageal distention. The net result is a grabbing effect in which reflexive contraction of the sphincter and laryngeal (UES) descent complement each other to clear residue from the hypopharynx [33]. This clearing function probably acts to minimize the risk of postswallow aspiration by preventing residual material from adhering to the laryngeal inlet when respiration resumes.

Intralumenal manometry can quantify the strength of pharyngeal contraction, the completeness of UES relaxation, and the relative timing of these events. When coupled with concurrent videoflouroscopy, manometry may provide useful complementary information regarding UES dysfunction. High intrabolus pressures may distinguish impaired UES opening from impaired UES relaxation and weak pharyngeal contractions as a cause of oropharyngeal dysphagia [34] (see Fig. 9.2).

Esophagus

Anatomy and function

Esophageal body

The esophagus is a 20- to 22-cm-long muscular tube with a wall composed of skeletal muscle and smooth muscle. The proportion of each muscle type is species dependent. In the human esophagus, the proximal 5% including the UES is striated, the middle 35%–40% is mixed with an increasing proportion of smooth muscle moving distally, and the distal 50%–60% is entirely smooth muscle [35]. The bundles of the outer longitudinal muscle arise from the cricoid cartilage, receive slips from the cricopharyngeus, and pass dorsolaterally to fuse posteriorly about 3 cm below the cricoid cartilage. This arrangement results in a posterior triangular area devoid of longitudinal muscle known as the Laimer triangle. Distal to the Laimer triangle, the longitudinal muscles form a continuous sheath of uniform thickness about the esophagus [36]. The adjacent, inner circular muscle layer also forms a sheath of uniform thickness throughout the length of the esophageal body. Unlike the distal gastrointestinal tract, the esophagus lacks a serosal layer.

Both the striated and smooth muscle portions of the esophagus contain a nerve network, known as the myenteric plexus, situated between the longitudinal and circular muscle layers [37]. The inner circular and outer longitudinal muscle layers of the proximal esophagus are composed of striated muscle and are controlled by somatic motor fibers from lower motor neurons in the nucleus retrofacialis and the nucleus ambiguus. Axons of these lower motor neurons course through the vagus nerve by way of the recurrent laryngeal nerve. The thoracic esophagus is composed mainly of smooth muscle that receives innervation from preganglionic neurons in the dorsal motor nucleus of the vagus. Vagal fibers synapse in the myenteric plexus ganglia and are generally considered to be the relay neurons between the vagus and the smooth muscle. A second nerve network, the submucosal or Meissner plexus, is situated between the muscularis mucosae and the circular muscle layer. The ganglia of the myenteric plexus are more numerous in the smooth muscle region than in the striated muscle region of the esophagus but, throughout, they are far less dense and smaller than in other regions of the gut [38,39]. The submucosal plexus of the esophagus is exceedingly sparse; only a few ganglion cells are present in the submucosal plexus of the human esophagus [39].

The vagus nerve provides the extrinsic innervation of the esophagus. Fibers innervating the striated muscle are axons of lower motor neurons with cell bodies situated in the nucleus ambiguous, whereas innervation of the smooth muscle esophagus is provided by the dorsal motor nucleus of the vagus [40,41]. Efferent nerve fibers reach the cervical esophagus by way of the pharyngoesophageal nerve [42], and histological studies show that vagal efferents synapse directly on striated muscle neuromuscular junctions [43,44]. The vagus nerve also provides sensory innervation; in the cervical esophagus this is through the superior laryngeal nerve with cell bodies in the nodose ganglion, whereas in the remainder of the esophagus sensory fibers travel by way of the recurrent laryngeal nerve or, in the most distal esophagus, by way of the esophageal branches of the vagus. Histological studies show many free nerve endings in the mucosa, submucosa, and muscular layers [3,45]. A few encapsulated structures resembling spindles have also been described in humans. These vagal afferents are strongly stimulated by esophageal distention.

Esophageal manometry shows that the normal esophagus does not exhibit spontaneous contractions and its resting pressure is an approximate reflection of pleural pressure, becoming negative during inspiration. Analyses of the vigor and propagation of esophageal peristalsis have concluded that progression along the length of the tubular esophagus is not seamless. Rather, there is a distinct transition zone between the striated and smooth muscle segments that is characterized by low peristaltic amplitude, a slight delay in progression, and an increased likelihood of failed transmission [46]. This transition zone becomes quite evident when peristaltic amplitude and progression are plotted topographically (Fig. 9.6). The topographic analysis also reveals the segmental characteristic of peristaltic progression through the smooth muscle esophagus. Two distinct contractile segments are followed by the lower esophageal sphincter (LES), which contracts with vigor and persistence quite dissimilar to the adjacent smooth muscle esophagus [47].

Mechanics of esophageal peristalsis

Esophageal peristalsis commences as the pharyngeal contraction traverses the UES and progresses along the esophagus at

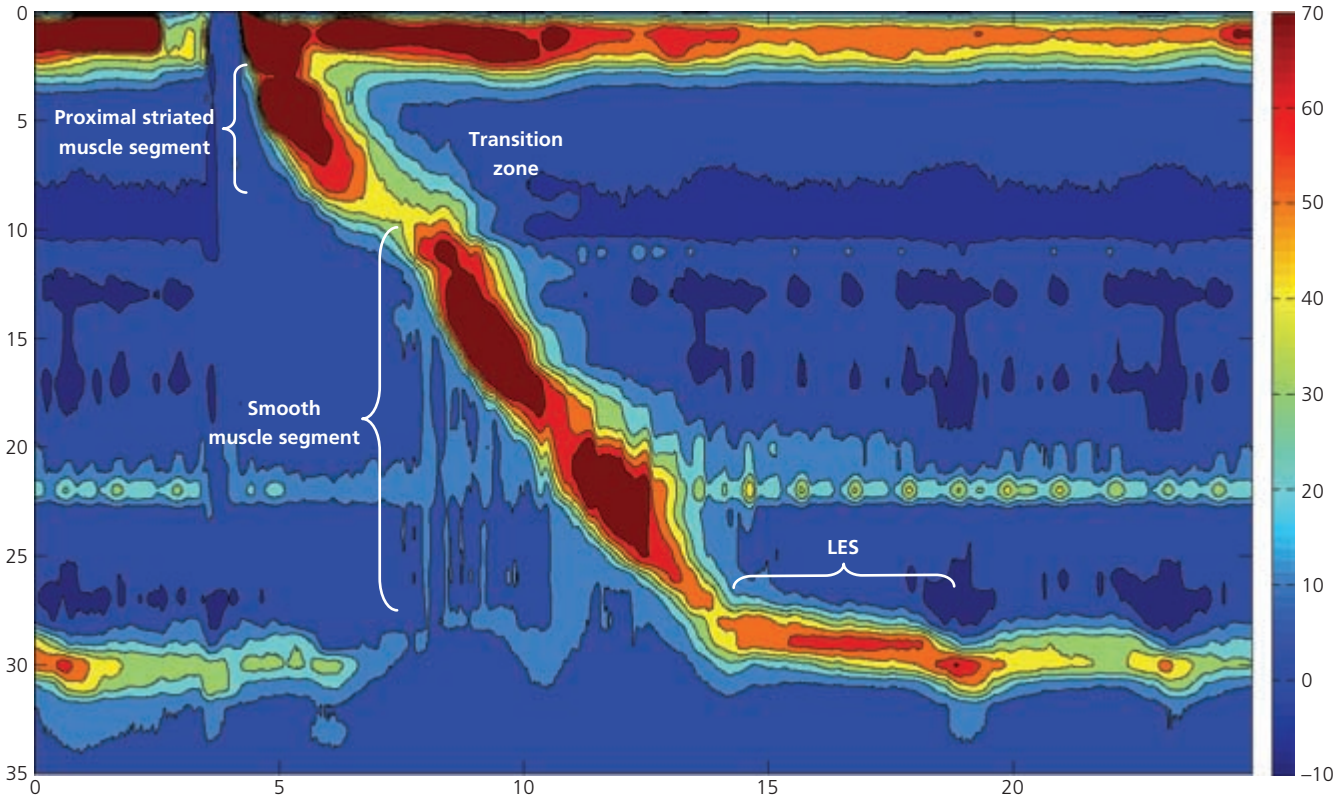

Figure 9.6 Topographic analysis of esophageal peristalsis using high-resolution manometry. The isocontour plot clearly shows that peristaltic progression through the esophagus is not seamless. A transition zone separates the proximal striated and distal smooth muscle esophageal contractile segments. The smooth muscle esophagus is also divided into two distinct contractile segments. The region of the esophagogastric junction is distinguishable as a separate contractile segment that contracts with vigor and persistence dissimilar to the adjacent smooth muscle segment. LES, lower esophageal sphincter.

2–4 cm/s. Whereas primary peristalsis is initiated by a swallow, secondary peristalsis can be elicited in response to focal esophageal distention with air, fluid, or a balloon [48]. The mechanical correlate of peristalsis is of a stripping wave that milks the esophagus clean from its proximal to distal end (Fig. 9.7). The velocity of the stripping wave corresponds closely with that of the manometrically recorded contraction, such that the point of the inverted "V" seen fluoroscopically at each esophageal locus occurs with the upstroke of the pressure wave [49]. The efficacy of distal esophageal emptying is inversely related to peristaltic amplitude, such that emptying becomes progressively impaired with peristaltic amplitudes of 30 mmHg [49] or less. This threshold amplitude was initially determined from simultaneous videofluoroscopic and manometric recordings of a relatively small number of subjects. Multichannel intralumenal impedance uses a much greater number of swallows and subjects to assess the efficacy of esophageal emptying as a function of peristaltic amplitude [50]. Receiver operating characteristic (ROC) analysis of combined manometric and impedance data revealed that a 30-mmHg cutoff for distal esophageal peristaltic amplitude had a sensitivity of 85% and a specificity of 66% for identifying incomplete bolus transit. With diminishing peristaltic amplitudes, the sensitivity progressively decreased and the specificity progressively increased.

Deglutitive inhibition is another crucial feature of the peristaltic mechanism. A second swallow, initiated while an earlier peristaltic contraction is still progressing in the striated muscle esophagus, causes rapid and complete inhibition of the contraction induced by the first swallow [51]. If the first swallow has reached the smooth muscle esophagus, it may proceed distally for a few seconds after the second swallow but its peristaltic amplitude diminishes progressively until it disappears [52]. Deglutitive inhibition is secondary to hyperpolarization of the circular smooth muscle and is mediated via nonadrenergic, noncholinergic neurons in the myenteric plexus. Sifrim and colleagues [53] developed an experimental model to illustrate deglutitive inhibition in the tubular esophagus (Fig. 9.8). They created an artificial high-pressure zone by distending the esophageal lumen with a balloon and recorded intralumenal pressure between the balloon and the esophageal wall. Once a high-pressure zone is established in the normally flaccid tubular esophagus, deglutitive

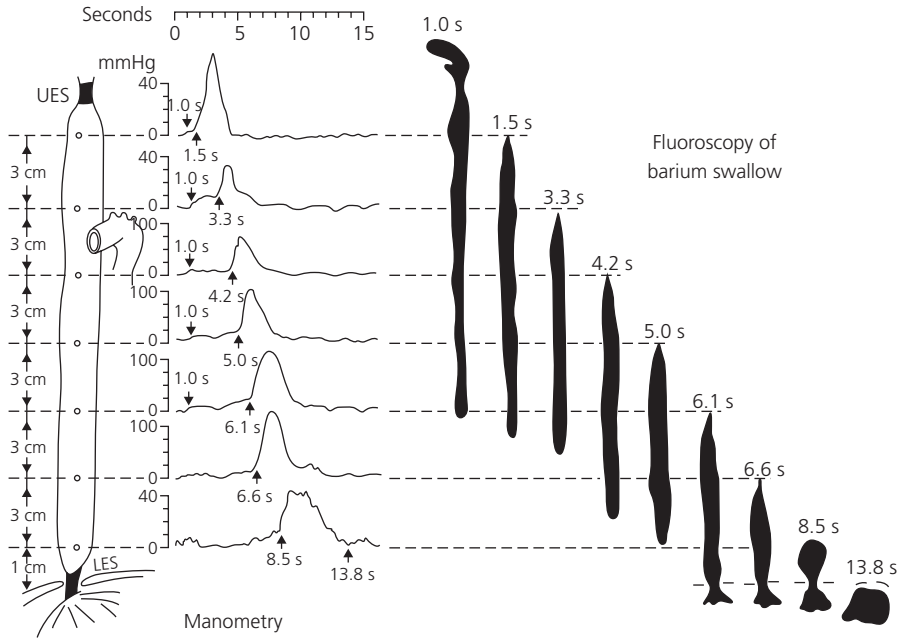

Figure 9.7 The relationship between manometric and fluoroscopic recordings of esophageal peristalsis during concurrent manometric and video recordings of a 5-mL liquid barium swallow. The tracings from the video images of the fluoroscopic sequence (right) show the distribution of the barium column at the time specified above each tracing and also indicated by arrows on the manometric record (middle). In this example, a single peristaltic sequence completely cleared the barium bolus from the esophagus. Pharyngeal injection of barium into the esophagus occurred at the 1.0-s mark. The entry of barium causes distention and a slightly increased intralumenal pressure, indicated by the downward pointing arrows marked "1.0 s." Shortly thereafter, esophageal peristalsis is initiated. During esophageal peristalsis, lumenal closure and hence the passage of the tail of the barium bolus at each recording site is concurrent with the onset of the manometric pressure wave. At 1.5 s, the peristaltic contraction had just reached the proximal recording site and barium had been stripped from the esophagus proximal to that point. Similarly, at 4.2 s, the peristaltic contraction was beginning at the third recording site and, correspondingly, the tail of the barium bolus was passing the third recording site. Finally, after completion of the peristaltic contraction (at 13.8 s), all of the barium was cleared into the stomach. From Kahrilas et al. [168], with permission from Elsevier.

inhibition can be demonstrated throughout the length of the esophagus, commencing concurrently with the pharyngeal swallow [53].

Control of esophageal peristalsis

Esophageal striated muscle

Distinct physiological control mechanisms govern the esophageal striated and smooth musculature. The striated muscle of the esophagus receives exclusively excitatory vagal innervation and its peristaltic contraction results from the sequential activation of motor units in a craniocaudal sequence. These fibers release acetylcholine and stimulate nicotinic cholinergic receptors on the motor endplates of the striated muscle cells. An ingenious series of experiments using the nerve suture technique provided physiological evidence of this arrangement [3]. In these experiments, the vagal branch innervating the esophagus was severed and the central end was sutured to the peripheral end of the severed spinal accessory nerve. Thus, after a period of nerve regeneration, the vagal branch effectively innervates the sternocleidomastoid and trapezius muscles. The occurrence of excitatory vagal discharges can then be surmised from the contractile activity of these readily accessible muscles. Nerve suture experiments allowed for the description of several properties of vagal control of esophageal striated muscle:

- vagal efferent fibers do not discharge spontaneously but fire in spike bursts (15–30 spikes/s) during primary or secondary peristalsis
- vagal fibers innervating different levels of the esophagus fire sequentially during peristaltic contractions demonstrating peristaltic programming by the medullary swallowing center
- vagal motor discharges during primary peristalsis are potentiated by stimulation of afferent fibers from the esophagus (designed to mimic the effect of a bolus being pushed ahead of a peristaltic contraction)
- vagal motor fibers exhibit stronger discharges during primary peristalsis than during secondary peristalsis
- vagal motor fibers are inhibited during the pharyngeal stage of deglutition or after distention of a proximal esophageal segment, supporting the notion that deglutitive inhibition has a central origin.

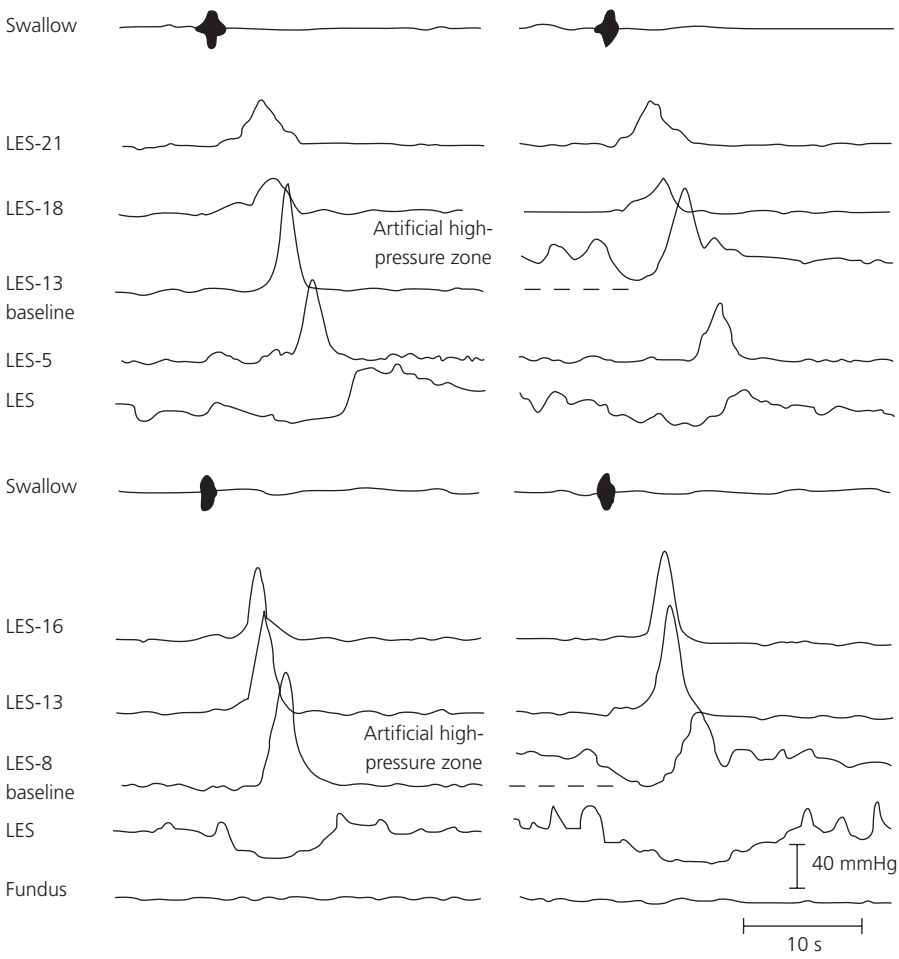

Figure 9.8 Demonstration of deglutitive inhibition in the tubular esophagus by the creation of an artificial high-pressure zone. The top tracing is a swallow marker, and the position from which each of the other tracings was obtained is referenced to the lower esophageal sphincter (LES). The artificial high-pressure zone was created 13 cm above the LES in the upper panel and 8 cm above the LES in the lower panel by inflating a balloon within the esophagus. Pressure was measured by the manometric sensor interposed between the wall of the esophagus and the balloon. The tracings on the left were recorded before balloon inflation and the tracings on the right were recorded when the balloon was inflated and the high-pressure zone had developed. Note that the contraction within the artificial high-pressure zone is inhibited concurrently with the pharyngeal swallow. From Sifrim et al. [169], with permission from Elsevier.

These properties provide convincing evidence that the organization of peristalsis in the striated muscle esophagus is controlled by the swallowing center of the medulla in much the same way as is the swallow response of the oropharyngeal musculature.

The contractile strength of the esophageal striated muscle may be modulated by a variety of sensory inputs [54,55]. An increase in bolus volume will augment the amplitude and duration of the peristaltic contraction. In addition, the velocity of the contractile wavefront will slow to extend the time available for bolus clearance. Temperature has a moderate effect on contractile strength, as warm boluses will increase the amplitude of the peristaltic contraction and cold boluses will decrease the amplitude [56].

Esophageal smooth muscle

Evidence supporting vagal control of primary peristalsis in the smooth muscle esophagus comes from bolus deviation and curarization experiments. Deviation of a swallowed bolus at the level of the cervical esophagus (thereby eliminating the potential for bolus-initiated afferent feedback) does not eliminate the primary peristaltic contraction in the distal esophagus [57]. Furthermore, in baboons, primary peristalsis of the smooth muscle persists even after curarization. As curarization paralyzes the oropharyngeal and cervical esophagus, the persistence of distal esophageal peristalsis in these experiments strongly suggests that primary peristalsis in the smooth muscle segment is triggered by the medullary swallowing center, which can elicit the entire motor sequence of primary peristalsis without receiving afferent feedback.

Vagal control of the esophageal smooth muscle is more complex than that of the striated muscle. Vagal fibers synapse on myenteric plexus neurons rather than directly on muscle cells, and vagal stimulation either excites or inhibits esophageal musculature depending on the stimulation parameters [58,59]. In response to vagal or swallow-induced stimulation in the opossum, longitudinal muscle showed depolarization with superimposed spikes, and circular muscle showed initial hyperpolarization followed by depolarization and spike burst [60]. In studying swallowing initiated by

superior laryngeal nerve stimulation, the response characteristics of 188 single fibers participating in smooth muscle peristalsis could be divided into two groups [61]. Type I fibers exhibited short latencies (< 1 s) that correlated temporally with the onset of deglutitive inhibition along the esophagus, and type II fibers exhibited long latencies that correlated temporally with the onset of contraction at each esophageal segment. Thus, the activity of neurons in the dorsal motor nucleus of the vagus reflects several properties of primary peristalsis in the smooth muscle esophagus, including deglutitive inhibition and both the speed and vigor of peristaltic contraction.

Control of peristalsis may also arise in the myenteric plexus. Stimulation of decentralized vagal efferents evokes peristalsis similar to that seen with swallowing. Transection of the esophageal smooth muscle obliterates this response, which suggests that an intact intramural neural myenteric plexus is necessary for peristaltic propagation within the smooth muscle segment. In contrast, transection of the striated muscle in the proximal esophagus does not inhibit peristaltic progression across the transection site or down the entire length of the esophagus [57]. Further evidence supporting the potential autonomy of peripheral mechanisms is that distention anywhere within the smooth muscle esophagus elicits secondary peristalsis despite extrinsic denervation [62].

Smooth muscle contraction is ultimately elicited by ganglionic cholinergic neurons during peristalsis. Swallow-induced peristalsis is highly sensitive to atropine, and it can be augmented by cholinergic agonists and inhibited by acetylcholinesterase [63–65]. Less clear, however, are the control mechanisms for the direction and velocity of the peristaltic wavefront. Nerve conduction studies indicate that neural stimuli initiated by swallowing propagate with a speed of 5–6 m/s and therefore reach the ganglionic neurons along the length of the esophagus essentially simultaneously [61]. However, the latency between the arrival of the vagal stimulus and muscle contraction progressively increases moving aborally. In humans, the latent period is 2 s in the proximal smooth muscle esophagus and 5–7 s just proximal to the LES. In vitro electrical stimulation evokes longer latencies to contraction in distal compared with proximal esophageal muscle strips [66,67]. The genesis of the latent gradient is controversial, but as it can be changed by varying the parameters of vagal stimulation or by pharmacological manipulation it may be the result of an interaction between the initial inhibition and subsequent excitation of esophageal smooth muscle [68].

Although not completely understood, the directionality of peristalsis is thought to be a manifestation of an intramural neural gradient that progressively delays contraction distally. Originally, Dodds and colleagues [69,70] characterized two intramural neural mechanisms that controlled peristalsis: an "on" contraction that was atropine sensitive and propagated similarly to swallow-induced peristalsis and an "off" contraction that was atropine resistant and rapidly propagated. The "on" contraction was associated with activation of excitatory cholinergic neurons, whereas the "off" contraction was associated with the initial muscle hyperpolarization caused by activation of nonadrenergic, noncholinergic inhibitory neurons, followed by rebound contraction after cessation of the stimulus. It is now known that the primary inhibitory neurotransmitter (formerly referred to as the nonadrenergic, noncholinergic transmitter) is nitric oxide (NO), which is produced from L-arginine by the enzyme NO synthase in myenteric neurons [71,72]. NO synthase can be reversibly inhibited by *N*-nitro-L-arginine methyl ester and *N*-nitro-L-arginine. NO synthase inhibitors decrease the latency and amplitude of "off" contractions in muscle strip experiments [73] and reduce the latency to contraction in vivo in response to swallowing [74,75]. Thus, the current hypothesis is that peristaltic direction results from a neural gradient along the esophagus in which excitatory ganglionic neurons dominate proximally and inhibitory ganglionic neurons dominate distally. In addition to NO-containing neurons, vasoactive intestinal polypeptide (VIP)-containing neurons may also have a role in the initial inhibition [76,77].

Sympathectomy has no apparent effect on esophageal peristalsis [78,79]. In contrast, bilateral vagotomy results in paralysis of the striated muscle esophagus. Severing only the afferent nerve supply to the striated muscle abolishes secondary peristalsis while leaving primary peristalsis intact, highlighting the role of central programming in the latter and the necessity of afferent sensory signals in the former [3]. Recordings from the cervical esophageal vagal afferents show these fibers to be highly sensitive to intralumenal distention, implicating them as the sensory basis for secondary peristalsis. In the smooth muscle segment of the esophagus, vagal cooling or vagotomy reduces the amplitude of primary peristalsis [80,81] but does not affect secondary peristalsis [82].

Esophageal longitudinal muscle

The longitudinal muscle of the esophagus also contracts during peristalsis with the net effect of transiently shortening the structure by 2–2.5 cm [83]. Similar to the pattern of circular muscle contraction, longitudinal muscle contraction is propagated distally as an active segment of contraction at a rate of 2–4 cm/s [33]. The segment of contracting longitudinal muscle precedes but overlaps with the contracting segment of circular muscle. Thus, within a given esophageal segment, the contractions of the longitudinal and circular muscle are slightly out of phase with each other. Propulsive force occurs in the zone of overlap as the delayed circular muscle contraction "catches up" with the distal longitudinal muscle contraction [33].

Central mechanisms also control the contraction of esophageal longitudinal muscle. Swallowing induces peristaltic sequences with gradual orad–caudad activation of

longitudinal muscle. This progression is associated with a progressive increase in latency, which is similar to the increase that occurs in esophageal circular smooth muscle [84]. However, unlike the responses observed in circular muscle, stimulation of decentralized vagal efferent fibers causes simultaneous contractions in the longitudinal muscle layer, suggesting that this muscle is free of inhibitory neuron control [68].

Esophagogastric junction

Anatomy and function

The anatomy of the esophagogastric junction is complex. The distal end of the esophagus is anchored to the diaphragm by the phrenoesophageal membrane, which inserts circumferentially into the esophageal musculature close to the squamocolumnar junction. The esophagus then traverses the diaphragmatic hiatus and joins the stomach in almost a tangential fashion. Thus, there are several potential contributors to the esophagogastric junction high-pressure zone: the intrinsic LES, the influence of the diaphragmatic hiatus, and the muscular architecture of the gastric cardia, which constitutes the distal aspect of this zone.

The LES is a 3- to 4-cm-long segment of tonically contracted smooth muscle at the distal extreme of the tubular esophagus. Surrounding the LES at the level of the squamocolumnar junction is the crural diaphragm – most commonly the right diaphragmatic crus. The right crus of the diaphragm arises as two flattened muscle bundles from the upper lumbar vertebra, inclines forward to arch around the esophagus, diverges in a scissor-like movement approaching the hiatus, surrounds the esophagus, and then merges anteriorly with about 1 cm of muscle separating the anterior rim of the hiatus from the central tendon of the diaphragm [85,86] (Fig. 9.9). Thus, the diaphragmatic hiatus is a teardrop-shaped canal about 2 cm long on its major axis. Elegant anatomical studies suggest that the component of the esophagogastric junction high-pressure zone distal to the squamocolumnar junction is largely a function of the opposing sling and clasp fibers of the middle layer of gastric cardia musculature [85,87]. In this region, the lateral wall of the esophagus meets the medial aspect of the dome of the stomach at an acute angle, defined as the angle of His. Viewed intralumenally, this region extends within the gastric lumen, appearing as a large fold that has been referred to as a flap valve because increased intragastric pressure forces it to close, sealing off the entry to the esophagus [88,89]. Physiologically, both the LES and the surrounding crural diaphragm contribute to the esophagogastric junction high-pressure zone. Concurrent fluoroscopy and manometry, aided by the placement of an endoclip at the squamocolumnar junction, localized the esophagogastric junction high-pressure zone as extending 1–1.5 cm proximal to the squamocolumnar junction and about 2 cm distal to it (Fig. 9.10) [90]. Manometric and intralumenal ultrasound studies suggest that the axial asymmetry of the pressure profile is attributable to the varying thickness of the muscularis propria, whereas the radial pressure asymmetry results from asymmetric compression by the surrounding crural diaphragm [90,91].

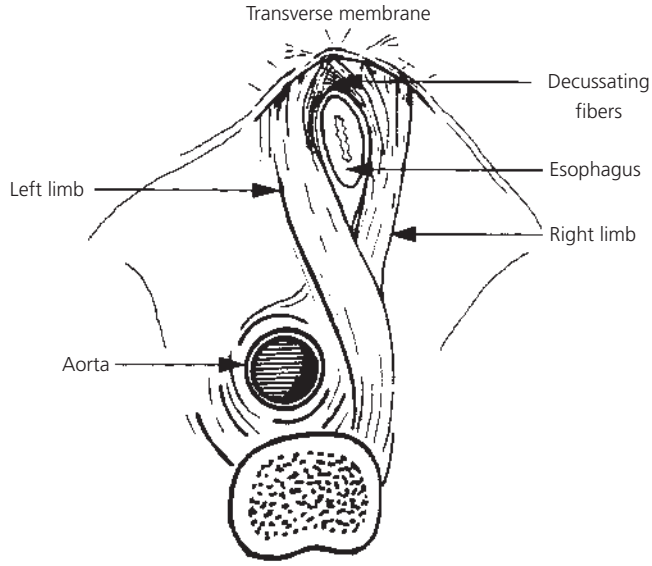

Figure 9.9 The most common anatomy of the diaphragmatic hiatus, in which the muscular elements of the crural diaphragm derive from the right diaphragmatic crus. The right crus arises from the anterior longitudinal ligament overlying the lumbar vertebrae. Once muscular elements emerge from the tendon, two flat muscular bands form that cross each other in a scissor-like fashion, form the walls of the hiatus, and decussate with each other anterior to the esophagus. Modified from Marchand [170].

Resting LES tone normally varies from 10 to 30 mmHg relative to the intragastric pressure, with considerable temporal fluctuation. Although not fully understood, the mechanism of LES tonic contraction seems to display properties of both the muscle itself and the nerves controlling the sphincter. This conclusion is supported by the observation that pressure within the sphincter is minimally affected after the elimination of neural activity by close intraarterial injection of tetrodotoxin [92]. Myogenic LES tone is associated with a depolarized state and electrical spike activity, which leads to an influx of Ca^{2+} during rest. Asoh and Goyal [93] studied this phenomenon in the opposum LES and observed continuous spike activity and a direct relationship between the level of spike activity and muscle tone. Experimental evidence has also shown that LES tone is maintained in the absence of spike activity and that its magnitude varies directly with membrane potential [94]. In addition, direct intracellular recordings demonstrate a direct relationship between membrane potential and basal LES tone. Zelcer and Weisbrodt [95] reported that the resting membrane potential of the

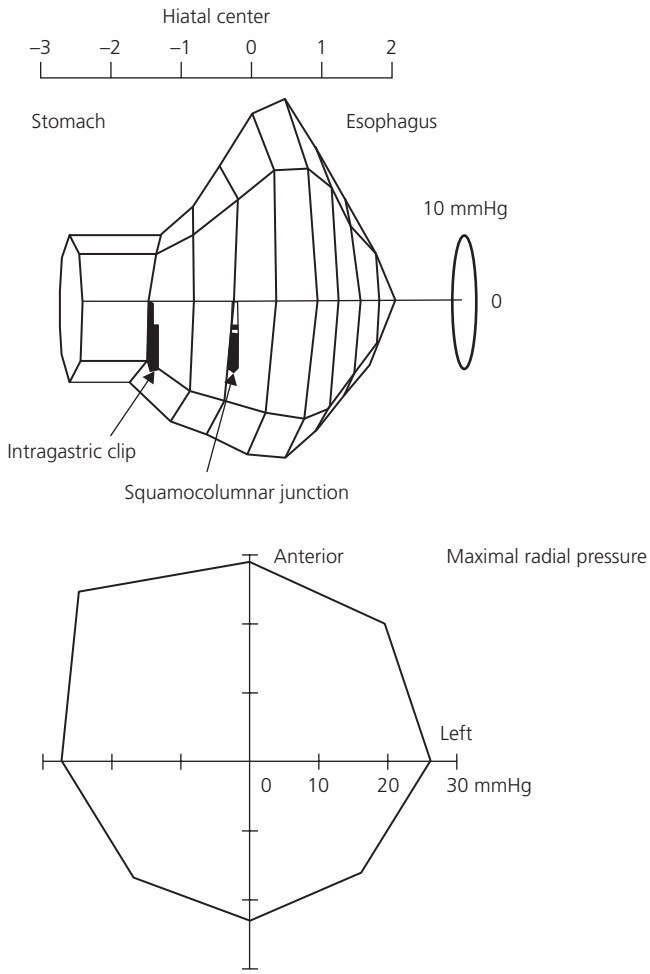

Figure 9.10 Pressure topography of the normal esophagogastric junction illustrating axial and radial asymmetry. Position zero on the axial scale is the midpoint of the diaphragmatic hiatus. The proximal clip indicates the position of the squamocolumnar junction and the distal clip marks the median position of the intragastric aspect to the esophagogastric junction as imaged endoscopically. All values of length and pressure are the medians of seven subjects. The bottom tracings represent maximal radial pressure for normal subjects. From Kahrilas et al. [90].

smooth muscle LES was slightly higher (–40 mV) than the resting membrane potential (–50 mV) of the esophageal body. Modulation of spike activity and membrane potential are not completely understood; however, it is believed that spike activity may be regulated by K^+- and Ca^{2+}-activated Cl^- channels [96]. Sphincter tone may be maintained by inositol phosphate-mediated continuous release of intracellular Ca^{2+} [97,98]. Inositol phosphates are found in higher concentrations in the LES than in the adjacent circular smooth muscle.

Although the cellular mechanism involved in maintaining the tonic LES contraction is unclear, input generated by vagal, adrenergic, hormonal, and mechanical influences can alter basal LES pressure. Vagal influence is similar to that observed in the esophageal body, with vagal stimulation activating both excitatory and inhibitory myenteric neurons [59]. The excitatory intramural neurons are acetylcholine sensitive and they have both nicotinic and muscarinic receptors [99]. Presumably, the excitatory component is selectively activated under resting conditions and the inhibitory component mediates LES relaxation. Experimental data confirming these effects were obtained from a canine study using the nerve suture technique [100]. Dogs provide an interesting model for these studies because the canine esophagus is composed entirely of striated muscle except for a smooth muscle band at the LES. Vagal fibers innervating the LES are of two types: (1) spontaneously active fibers (1.5–4.5 spikes/s) that exhibit a sudden increase in firing with swallowing, abruptly cease firing when the peristaltic contraction arrives, and then resume a spontaneous rate, and (2) spontaneously active fibers (1–3 spikes/s) that cease activity with swallowing and resume normal activity when the bolus reaches the stomach. Thus, the LES pressure at any instant reflects the balance between excitatory and inhibitory neural input. Altering the pattern of vagal discharge can result in swallow-mediated LES relaxation. Data from human studies suggest that, similar to the situation in the dog, basal LES tone is partially generated by cholinergic input [63]. Adrenergic influences on LES pressure are complex and mostly mediated through the myenteric neurons [41,101]. Fibers from the stellate and proximal thoracic ganglia follow the splanchnic nerve, form a recurrent loop through the celiac ganglion, and then synapse on both the excitatory and inhibitory myenteric neurons. These fibers cause excitation of the excitatory neurons and inhibition of the inhibitory neurons via α-adrenergic receptors, resulting in a net augmentation of LES pressure. The adrenergic effect on the esophageal body is the opposite to that on the sphincter; direct inhibition of the muscle and inhibition of the excitatory myenteric neurons.

At any given moment, LES pressure may be affected by myogenic factors, intraabdominal pressure, gastric distention, peptides, hormones, various foods, and many medications (Table 9.1). Large fluctuations of LES pressure occur with the migrating motor complex; during phase III, LES pressure may exceed 80 mmHg. Lesser fluctuations occur throughout the day with pressure decreasing in the postcibal state and increasing during sleep [102].

Another important anatomical consideration of the esophagogastric junction high-pressure zone is its relationship to the crural diaphragm. Even after esophagogastrectomy, with consequent removal of the smooth muscle LES, a high-pressure zone of about 6 mmHg persists at the esophagogastric junction [103]. Furthermore, manometric recordings of esophagogastric junction pressure are often characterized by inspiratory augmentation. Although this has been partly attributed to catheter movement along an asymmetric axial pressure profile, evidence in both cats and humans suggests

Table 9.1 Factors that influence lower esophageal sphincter (LES) pressure and transient lower esophageal sphincter relaxation (tLESR) frequency

	Increase LES pressure	Decrease LES pressure	Increase tLESR frequency	Decrease tLESR frequency
Food(s)	Protein	Fat, chocolate, ethanol peppermint	Fat	
Hormone(s)	Gastrin, motilin, substance P	Secretin, cholecystokinin, glucagon, gastric inhibitory polypeptide, vasoactive intestinal polypeptide, progesterone	Cholecystokinin	
Neural agent(s)	α-Adrenergic agonists, β-adrenergic antagonists, cholinergic agonists	α-Adrenergic antagonists, β-adrenergic agonists, cholinergic antagonists, serotonin	L-Arginine	Baclofen, metabotropic glutamate receptor antagonists, cannaboid receptor agonists, L-NAME, serotonin
Medication(s)	Metoclopramide, domperidone, prostaglandin F2α, cisapride	Nitrates, calcium channel blockers, theophylline, morphine, meperidine, diazepam, barbituates	Sumatriptan	Atropine, morphine, loxiglumide

L-NAME, N(G)-nitro-L-arginine methyl ester.

that the inspiratory increase mainly results from contraction of the crural diaphragm encircling the LES (Fig. 9.11) [104,105]. Experimentally, the inspiratory augmentation of esophagogastric junction pressure persists despite pinning the manometric catheter in place, and the amplitude of respiratory oscillations can be increased with increased respiratory effort or eliminated by manual ventilation. The augmentation of LES pressure observed during sustained inspiration corresponds both temporally and quantitatively with the augmentation of crural electromyographic activity, and this augmented esophagogastric junction pressure can obscure intrinsic LES relaxation induced by esophageal distention [106]. Crural diaphragm contraction is also augmented during abdominal compression, straining, or coughing [107]. In contrast, during esophageal distention, vomiting, and belching, electrical activity in the crural diaphragm is selectively inhibited despite continued respiration, demonstrating a control mechanism independent of the costal diaphragm [108,109]. This reflex inhibition of crural activity is eliminated with vagotomy.

Esophagogastric junction opening

Although the terms are often used synonymously, there is an important distinction between LES relaxation and esophagogastric junction opening. LES relaxation is a permissive event for esophagogastric junction opening, evident in manometric studies by the elimination, or near elimination, of intralumenal pressure within the sphincter. However, LES relaxation is not necessarily synchronous with esophagogastric junction opening and, in fact, not always associated with opening. Whether or not esophagogastric junction opening follows LES relaxation depends on complex mechanical relationships involving peristaltic forces, the gastroesophageal pressure gradient and, most importantly, the transmural pressure gradient between the lumenal pressure within the sphincter and the extrinsic pressure surrounding the structure. Thus, in conceptualizing esophagogastric junction opening, it is necessary to consider three related topics: LES relaxation, esophagogastric junction opening in the setting of swallowing, and esophagogastric junction opening in the setting of reflux (retrograde flow).

Lower esophageal sphincter relaxation

The neural mediation of LES relaxation has been studied extensively [36,99,110]. LES relaxation can be triggered by distention on either side of the esophagogastric junction or by swallowing [111]. Relaxation induced by esophageal distention can be modulated by bolus volume and is unaffected by vagotomy, which shows that it is an intramural process. Relaxation is, however, antagonized by tetrodotoxin (which denervates smooth muscle without affecting its contractile function), proving that it is mediated by postganglionic nerves [112,113]. Electrical stimulation of decentralized efferent vagal fibers or field stimulation of LES muscle strips causes complete relaxation, which is associated with concomitant circular muscle hyperpolarization [93,95]. Deglutition induces an initial inhibition of the entire smooth muscle of the esophagus, and LES relaxation is part of this inhibitory response. Deglutitive LES relaxation is mediated by the vagus nerve, which synapses with inhibitory neurons of the myenteric plexus. Ganglionic transmission is through nicotinic and muscarinic acetylcholine receptors and can be blocked by a

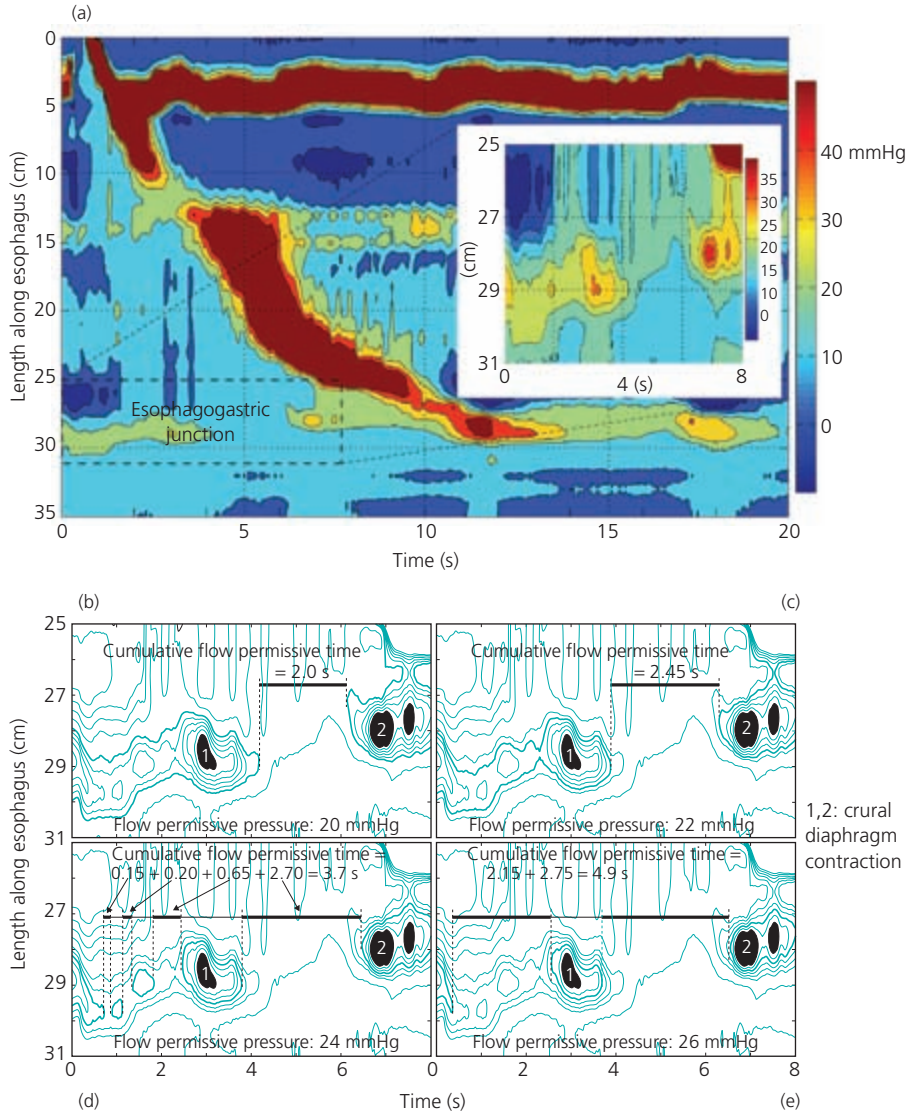

Figure 9.11 Contribution of the intrinsic lower esophageal sphincter and the extrinsic crural diaphragm to the esophagogastric junction high-pressure zone. **(a)** An isobaric contour plot of a swallow highlights the interval of potential esophagogastric junction relaxation extending from upper esophageal sphincter relaxation to the arrival of the peristaltic contraction (black dashed box). The inset magnifies the esophagogastric junction relaxation in time, spatial resolution, and pressure resolution; note the clear definition of the two contractions of the crural diaphragm (1 and 2). **(b–e)** Each focus on the same data set as in the inset in **(a)** but illustrate the calculation of the period of time in which flow is permissive at various pressure settings. In each of these panels, the heavy black isobaric contour line indicates the flow-permissive pressure. Note the dramatic effect of crural contraction (1 and 2) on instantaneous esophagogastric junction relaxation pressure during swallowing. This suggests that flow through the esophagogastric junction would not be permissive during crural contraction.

combination of hexamethonium (i.e., nicotinic blocker) and atropine (i.e., muscarinic blocker).

Evidence implicates NO as the main neurotransmitter in the postganglionic neurons responsible for LES relaxation. NO is produced by neuronal NO synthase, a soluble cytosolic enzyme that has been identified in neurons of the myenteric plexus, colocalizing with VIP, which may be a second inhibitory neurotransmitter in the LES as well as in the esophageal body [114–116]. Neural stimulation in the esophagus, the LES, and the stomach induces NO release [71,117–119]. In the LES, NO has a marked inhibitory effect and multiple in vitro and in vivo studies have shown that NO synthase inhibitors block neurally mediated LES relaxation. Other evidence that neuronal NO synthase plays a role in LES relaxation has been obtained by comparing wild-type and genetically engineered mice that are deficient in neuronal NO synthase. In wild-type mice, electrical field stimulation produces frequency-dependent relaxation, which can be abolished by the NO synthase inhibitor N(ω)-nitro-L-arginine methyl ester. In neuronal NO synthase-deficient mice, electrical field stimulation does not produce relaxation [120].

Although the evidence implicating NO as the main inhibitory transmitter facilitating LES relaxation is convincing, NO may not act alone. VIP-containing neurons have been demonstrated in the submucosal plexus and VIP relaxes the LES by direct muscle action [121–125]. Electrical stimulation of LES muscle strips also causes an LES relaxation-stimulated release of VIP in the muscle bath. Furthermore, VIP antiserum partially reduces LES relaxation evoked by vagal or field stimulation [77,125]. It is thought that VIP has two functions: it acts on NO synthase-containing neural terminals as a prejunctional neurotransmitter, facilitating the release of NO, and it acts on gastric muscle cells to stimulate the production of NO by the muscle [126–130]. In addition to

VIP, there is some evidence that peptide histidine isoleucine (PHI) in the cat and, to a lesser extent, calcitonin gene-related peptide (CGRP) in the opossum may participate as inhibitory neurotransmitters [125,131]. In a similar way to VIP, PHI, and CGRP relax the LES by a direct action on the muscle [125,131,132]. PHI is of interest because it is derived from the same precursor as VIP and coexists with VIP in the same neurons [133].

The notion that multiple neurotransmitters may interact to produce LES relaxation may resolve inconsistencies and discrepancies that follow from the assumption that any one neurotransmitter is uniquely responsible for LES relaxation. Reports of the colocalization of NO synthase, VIP, pituitary adenylate cyclase-activating peptide (PACAP), CGRP, and galanin in myenteric neurons of the distal esophagus support this concept [134–136].

Antegrade opening

The passage of an ingested bolus across the esophagogastric junction is facilitated by LES relaxation and by the force of the peristaltic contraction. LES relaxation occurs within 2 s of deglutition at a time when the peristaltic wave appears in the middle esophagus, at the beginning of the smooth muscle segment. Although accurate measurements are difficult because of motion artifacts associated with esophageal shortening, it is estimated that the LES relaxes to pressures equal to those of the stomach or the esophagus within 1 s of swallowing [137]. When the ingested boluses reach the LES, the LES is relaxed but closed. Subsequently, intrabolus pressure, generated either by its own weight (hydrostatic) or with the aid of the peristaltic contraction, forces the LES open and flow across the esophagogastric junction ensues. After 5–7 s, the LES recovers its initial pressure and then undergoes an aftercontraction, which probably represents the termination of the peristaltic sequence as it reaches the distal end of the esophagus. Circular muscle strips obtained from the LES proper exhibit only relaxation, but more proximal strips exhibit relaxation followed by an aftercontraction, perhaps because of the presence of mixed LES and adjacent esophageal muscle fibers [138]. If esophageal emptying is not completed in the 5- to 7-s window set by the time-limited nature of the peristaltic sequence, residual bolus escapes retrogradely through the contractile front and remains in the esophagus at the termination of the sequence.

Retrograde opening

Physiologically, the esophagogastric junction must perform seemingly contradictory functions. During swallowing it must facilitate the esophagogastric flow of swallowed material. At the same time it prevents reflux of gastric content into the esophagus, which might be an effect of the positive abdomen-to-thoracic pressure gradient. During rest, the esophagogastric junction must, again, contain caustic gastric juice but also be able to transiently relax and permit gas venting of the stomach. These functions are accomplished by the delicate interplay of anatomical elements and physiological responses of the esophagogastric junction. LES relaxation may occur transiently without swallowing or esophageal contraction. These transient LES relaxations (tLESRs) are thought to be an important mechanism in the pathogenesis of gastroesophageal reflux disease (GERD) and a multitude of evidence suggests that tLESRs are the most frequent mechanism for reflux during periods of normal LES pressure (> 10 mmHg). tLESRs occur independently of swallowing, are not accompanied by peristalsis, are accompanied by crural diaphragm inhibition, and persist for longer periods than swallow-induced LES relaxations (> 10 s). [137,139] Prolonged manometric recordings have not consistently demonstrated an increased frequency of tLESRs in patients with GERD compared with healthy controls [140]; however, the proportion of tLESRs associated with acid reflux (as opposed to gas reflux) has consistently been observed to be greater among patients with GERD [140]. tLESRs occur most often in the postprandial state during gastric accommodation, which is attributable to the vagally mediated receptive relaxation of the fundus. In the setting of the completely relaxed esophagogastric junction during tLESRs, even the minimal gastroesophageal pressure gradients observed with gastric distention (3–4 mmHg) are sufficient to facilitate gas venting of the stomach (i.e., belching). Thus, tLESRs are the physiological mechanism of belching [141].

Proximal gastric distention is the major stimulus for tLESRs. Distention stimulates mechanoreceptors (intraganglionic lamellar endings) in the proximal stomach, activating vagal afferent fibers that project to the nucleus of solitary tract [139,142,143]. The afferent and efferent neural pathways responsible for swallow-induced and non-swallow-induced LES relaxations have been compared in the mouse [142,143] The efferent limb of both types of LES relaxation lies in the preganglionic vagal inhibitory pathway to the LES [142,143]. Both relaxations can be blocked by bilateral cervical vagotomy, cervical vagal cooling, or NO synthase inhibitors [139,144]. The afferent arms of these two types of relaxation, however, differ from one another. The afferent arm of the swallow-induced relaxation lies in the pharyngeal and superior laryngeal nerves, with the central neural circuit in the medullary subnuclei [145–149]. Non-swallow-induced relaxations, in contrast, are initiated through gastric afferents in the subdiaphragmatic vagus and activate neurons in the medial, dorsomedial, and commissural solitary subnuclei and motor neurons in the caudal part of the dorsal motor nucleus of the vagus [150]. Vagal outflow from the dorsal motor nucleus completely inhibits both the LES and the crural diaphragm, an important distinction from the swallow-induced LES relaxation, which is not associated with concomitant inhibition of the crural diaphragm.

tLESRs triggered by gastric distention likely use NO and cholecystokinin (CCK) as neurotransmitters, as evidenced by their increased frequency after intravenous administration of CCK and blockade by either NO synthase inhibitors or CCK_1 antagonists [151–154]. The increased frequency of tLESRs during intravenous infusions of CCK or after fatty meals is mediated through CCK_1 receptors, as evidenced by inhibition of CCK_1 [155,156]. Muscarinic receptor involvement in the tLESR pathway is suggested by atropine inhibition [157–160]. Finally, γ-aminobutyric acid (GABA)-B agonists, such as baclofen, inhibit tLESRs, possibly by acting on receptors located in the dorsal motor nucleus of the vagus [161–165].

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B MOTILITY

10 The physiology of gastric motility and gastric emptying

William L. Hasler

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Gastric motor activity serves distinct roles under fasting and fed conditions. Interdigestive patterns clear the stomach of undigested debris and sloughed epithelial cells. After eating, the stomach accommodates and then grinds the ingested food into fine particles that are delivered to the duodenum at a controlled rate. The stomach is divided into three regions: the proximal stomach (cardia, fundus, and proximal body), the distal stomach (distal body and antrum), and the pylorus. Additional feedback control from the small intestine regulates gastric motor function.

Gastric smooth muscle

The gastric smooth muscle layers are oriented to facilitate nutrient dispersal and emptying. The longitudinal muscle layer is prominent distally and is continuous with duodenal longitudinal muscle, although some fibers terminate in the pylorus. The circular muscle layer is more uniformly distributed in the stomach, but it is electrically isolated from its duodenal counterpart by a connective tissue septum at the pylorus. The oblique layer on the lesser curvature, in continuity with the gastroesophageal junction, is innermost. Distal gastric smooth muscle is thicker than that of the proximal stomach, and it is less compliant in response to distention [1]. Specialized pyloric circular smooth muscle comprises two circumferential loops that coalesce over the lesser curvature. The pylorus is reinforced by connective tissue from the mucosa and smooth muscle.

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Innervation of the stomach

The stomach is innervated by extrinsic nerves that relay information to and from the extragastrointestinal ganglia, the spinal cord, and the central nervous system (CNS), and by intrinsic nerves in the gastric wall.

Extrinsic innervation

Efferent supply

Although only a minority of vagal fibers provide efferent input to the gastric myenteric plexus, studies in rats measuring myenteric c-Fos expression indicate extensive functional efferent vagal effects on the enteric nervous system [2]. Efferent fibers within the vagus include preganglionic parasympathetic cholinergic nerves that supply excitatory enteric neurons, preganglionic cholinergic nerves that supply inhibitory enteric neurons, and sympathetic fibers from the superior cervical and stellate ganglia. Cell bodies of most efferent vagal fibers reside in the dorsal motor nucleus of the vagus (DMNV). Different neurons in the DMNV project to the proximal stomach and the pylorus, indicating regional variability in the internal structure of the nucleus [3]. The dorsal vagal complex receives input from the insular cortex of the brain [4]. Insular cortical microstimulation elicits changes in gastric motor activity. Stimulation of low-threshold efferent vagal cholinergic neurons activates nicotinic receptors within enteric ganglia, increasing gastric contractions. Some efferent fibers that exhibit a high threshold to electrical stimulation inhibit motor activity through nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) release [5].

Sympathetic innervation from splanchnic nerves originates from neuronal cell bodies in the prevertebral celiac ganglia. Preganglionic cholinergic neurons project from the inferomedial spinal cord to the prevertebral ganglia, where they synapse by way of nicotinic receptors. Postganglionic

noradrenergic neurons project by way of the splanchnic nerves to the enteric ganglia and inhibit excitatory myenteric transmission. A smaller fraction of sympathetic fibers exert direct inhibitory effects on gastric smooth muscle.

Afferent supply

Afferent fibers greatly outnumber efferents in the vagus and splanchnic nerves. Vagal afferents terminate in the nucleus tractus solitarius (NTS) and the area postrema. Neurons of the NTS project to the DMNV and the nucleus ambiguus, and to higher centers. Sensory information from the stomach is also transmitted to the dorsal horn of the spinal cord through the splanchnic nerves, from where second-order neurons project centrally. The stomach is richly supplied with sensory fibers. Free mucosal nerve endings respond to stroking or chemical stimuli (e.g., hydrochloric acid). Smooth muscle mechanoreceptors respond to passive distention, active contractions, or exposure to extreme temperatures [6]. Two types of mechanoreceptors are evident. Intramuscular arrays serve as stretch receptors to mediate tonic activities, whereas intraganglionic laminar endings integrate tension and neuronal activity into propagative motor activity [7]. Neurons in the NTS and DMNV are activated by gastric distention, indicating vagal mediation of mechanoreceptor-evoked afferent function [8]. Mesenteric and serosal receptors respond to visceral tension and may mediate perception of visceral pain. In many regions of the gut, splanchnic nerves carry nociceptive sensory input.

Intrinsic innervation

The enteric nervous system contains afferent neurons, interneurons, and motor neurons, and can initiate many programmed motor patterns in the absence of extrinsic input. Most intrinsic neurons participating in gastric motor control are located in the myenteric plexus, although submucous plexus neurons may play a minor role in some reflexes. Excitatory neurons containing acetylcholine and tachykinins project to circular muscle. Inhibitory motor neurons containing NO and VIP project aborally [9]. Neurons containing other neurotransmitters and expressing other neurotransmitter receptors are prominent, indicating the complexity of regulation of gastric motor activity.

Interstitial cells of Cajal

Interstitial cells of Cajal (ICC) are histologically distinct from neurons and smooth muscle cells and represent a third major cell type responsible for the control of gastric motor function. Four classes of ICC are identified in the stomach: ICC-MY in the myenteric region are in contact with other ICC-MY and with smooth muscle cells; ICC-IM are present within the smooth muscle layers; ICC-SEP are present along the septa between circular muscle bundles; and ICC-SM are located submucosally [10,11]. In human tissue, fundic ICC are bipolar, whereas corpus and antral ICC are multipolar [12]. The density of ICC is greatest in the fundus; ICC in the corpus and antrum show close contact with inhibitory and excitatory nerves. ICC-IM decrease in density from the greater to the lesser curvature and longitudinally to the antrum [13]. Similarly, ICC-MY predominate along the greater curvature and are less dense along the lesser curvature [14].

Most evidence suggests that ICC-MY serve as the cells of origin of pacemaker activity in the stomach. In living guinea pig antral tissues, ICC form networks in the myenteric region that exhibit an electrical slow wave which oscillates in phase with intracellular calcium fluctuations (Fig. 10.1) [15]. The slow waves have large initial depolarizations along the greater curvature in the corpus that are difficult to demonstrate along the lesser curvature, suggesting that ICC-MY serve as slow wave generators in the region of the physiological gastric pacemaker [14]. Electrical pacemaker activity exhibits an initial fast transient current involving calcium release from inositol triphosphate-sensitive internal stores, followed by a plateau potential that is maintained by activation of a calcium-sensitive chloride channel [16]. Mdx mice, which lack full-length dystrophin, exhibit significant antral ICC structural abnormalities [17]. The slow waves in these mice are highly dysrhythmic with blunting of the plateau potential.

ICC-IM facilitate enteric neurotransmission. ICC in the circular and longitudinal smooth muscle layers form synaptic structures with nerve terminals and muscle cells from both layers [18,19]. ICC also form gap junctions with adjacent smooth muscle cells [10]. Connexins, which are present

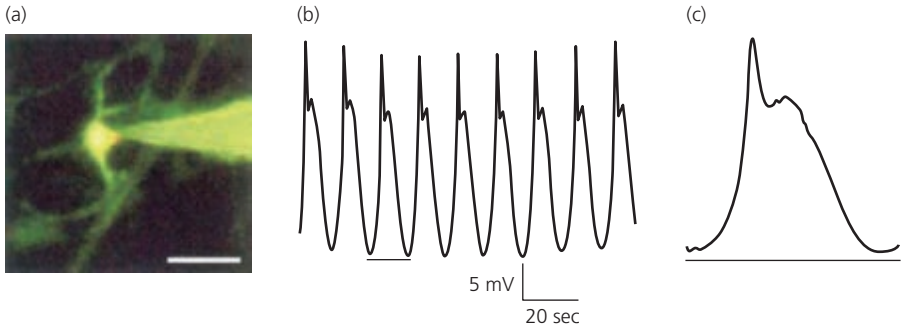

Figure 10.1 **(a)** A single ICC-MY from guinea pig antrum impaled with a recording electrode. **(b)** Rhythmic changes in intracellular membrane potential reflect the generation of slow wave activity by these cells. **(c)** A single slow wave on an expanded time scale. From Rich et al. [15].

in circular smooth muscle near ICC-IM, provide a means of electrical coupling, but their absence in longitudinal muscle indicates a different method of coupling in this region [20]. The close apposition of synaptotagmin-, syntaxin-, and SNAP-25-immunopositive nerve varicosities to ICC-IM suggests specialized synaptic connections between enteric nerves and ICC [21]. Gastric peristalsis depends on a decreasing gradient in slow wave frequency from the corpus to the antrum [22]. Slow wave propagation is mediated by an orderly activation sequence from ICC in the myenteric plexus and smooth muscle and may be followed by a sustained smooth muscle calcium wave that is responsible for contraction [23]. Knockout mice lacking these ICC populations exhibit disruption of the normal proximal-to-distal frequency gradient with the generation of ectopic pacemakers [24]. Slow antegrade propagation of gastric slow waves occurs because of restricted conduction within ICC-MY, whereas rapid circumferential slow wave propagation occurs because of brisk conduction within ICC-IM [25]. Rapid antegrade conduction does not occur within ICC-IM because of the high resistance between circular muscle bundles. ICC-IM appear to mediate mechanosensitive responses of the stomach [26]. Stretching of antral muscles changes slow wave frequencies and leads to uncoupling of slow wave propagation. These effects are not observed in knockout mice lacking ICC-IM [26].

The genes that regulate ICC function have been studied using gene knockout animal models. In the gastric fundus, 11 gene transcripts show higher levels of mRNA expression in mutant mice without ICC-IM, whereas 10 transcripts show higher mRNA expression in wild-type animals [27]. Membrane-bound stem cell factor is necessary for ICC development. Mice that lack stem cell factor exhibit a loss of fundic ICC and show decreased responses to enteric nerve stimulation but not to direct smooth muscle stimulants such as acetylcholine [28].

Regional motor patterns in the stomach and duodenum

Proximal stomach

The proximal stomach accommodates and stores ingested food, regulates intragastric pressure, and propels chyme to the distal stomach in a tonic fashion.

Basic myoelectric and motor patterns

The fundus is in a state of continual partial tonic contraction, as determined by its resting membrane potential. In contrast to more distal regions, the resting membrane potential in the fundus (–48 mV) is higher than the electrical threshold for gastric contraction (–50 mV). Minor depolarizations or hyperpolarizations in membrane potential produce significant increases or decreases in tone respectively. The proximal stomach does not exhibit slow wave activity. This region exhibits two basic contractile patterns. Slow, sustained contractions lasting up to 6 min constitute 80% of the motor activity in the proximal stomach and determine the intragastric pressure. Superimposed on these slow tonic changes are more rapid phasic contractions that last up to 30 s. In dogs, intragastric pressure initially drops after eating and then increases above basal levels 30–90 min later [29]. In humans, intragastric pressure decreases from 12.9 to 9.8 mmHg in the first 30 min after eating; tone does not return to normal until all solids have emptied from the stomach [30]. Maintenance of intragastric pressure is controlled by two reflexes: receptive relaxation and gastric accommodation. Receptive relaxation is the reduction in proximal gastric tone that occurs with the act of swallowing. The reflex may be elicited by a dry swallow or with pharyngeal stimulation, indicating that transfer of the bolus into the stomach is not required for its activation. Gastric accommodation, the relaxation of the proximal stomach in response to gastric distention, is mediated by gastric mechanoreceptor stimulation and may permit up to 80% of a swallowed bolus to be retained in the fundus and proximal corpus. Increases in gastric volume are greater after consuming a liquid meal than after eating an isocaloric mixed meal, indicating differential reflex activation based on meal consistency (Fig. 10.2) [31]. Receptive relaxation and accommodation are mediated by vagovagal reflex arcs through the NTS [32].

Neurohumoral control

Extrinsic nerves exert regulatory influences on proximal gastric tone. Vagal cooling reduces fundic motor activity, whereas truncal or proximal gastric vagotomy produces decreased compliance and increased intragastric pressure after bolus ingestion [33]. Electrical activation of low-threshold vagal fibers increases fundic tone, whereas high-threshold fiber stimulation elicits relaxation [34]. Splanchnicectomy augments the increase in intragastric pressure evoked by vagal activation.

Motor activity of the fundus is modulated by several neurohumoral factors. The nitrergic agent nitroglycerin relaxes the proximal stomach, whereas NO synthase inhibitors increase fundic tone, an effect blocked by atropine or vagal cooling, indicating that NO acts presynaptically on vagal cholinergic efferent nerves [35]. Similarly, the phosphodiesterase-5 inhibitor sildenafil promotes proximal relaxation by enhancing NO action [36]. The accommodation reflex is initiated by activation of NO neural pathways [37]. Accommodation is further modulated by activation of 5-$HT_{1B/D}$ (5-hydroxytryptamine) receptors [38]. In cats, stimulation of 5-HT_{1F} receptors elicits proximal gastric relaxation [39]. Receptive relaxation is postulated to result from VIP release by fundic nerves. Secretin relaxes the proximal stomach by activating vagal afferent pathways originating in the gastroduodenal mucosa [40]. Pentagastrin-evoked

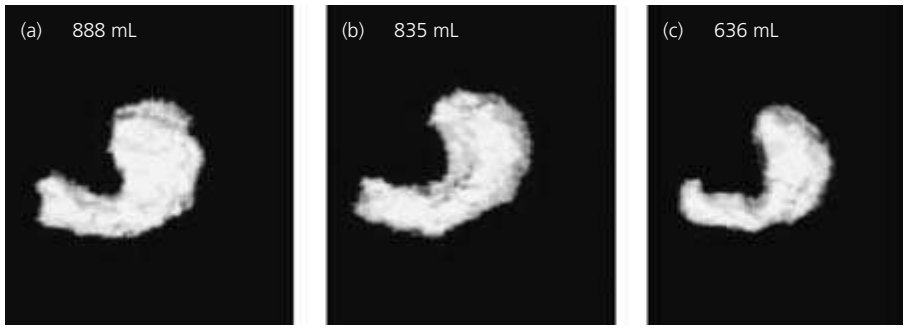

Figure 10.2 Single photon emission computed tomography scans of gastric volume are shown after the consumption of two liquid meals and a mixed liquid–solid meal. Gastric volume increases are greater after liquid ingestion, indicating modulation of the accommodation reflex by meal consistency. From De Schepper et al. [31].

relaxation is mimicked by histamine and is abolished by acid-suppressing agents, suggesting mediation by gastric secretory mechanisms. The sensory neurotoxin capsaicin reduces proximal gastric tone and inhibits phasic contractions by acting on the vanilloid receptor 1 [41]. Other agents that relax the proximal stomach or enhance fundic compliance include cholecystokinin (CCK), somatostatin, dopamine, gastrin-releasing peptide (GRP), glucagon, bombesin, and the α_2-adrenergic receptor agonist clonidine. Motilin and thyrotropin-releasing hormone (TRH), on the other hand, increase fundic pressure.

Neural reflex modulation

Proximal gastric motor function is subject to reflex control by stimulation of other gut regions. Duodenal distention reduces fundic tone by way of vagal and splanchnic nonadrenergic, noncholinergic pathways. Treatment of the vagus nerves with capsaicin attenuates the decrease in fundic pressure after low-volume duodenal distention, whereas capsaicin treatment of the celiac and superior mesenteric ganglia reduces the response to all volumes of distention [42]. Vagal nonadrenergic, noncholinergic pathways variably participate in other enterogastric reflexes, including the inhibition of proximal gastric activity by duodenal protein or lipid perfusion. Nutrient perfusion of the small intestine reduces fundic tone. CCK is postulated to mediate the inhibition of proximal tone during duodenal delivery of lipids or amino acids (especially L-tryptophan) by capsaicin-sensitive pathways, indicating afferent pathway involvement [43]. Fundic relaxation in response to low-level duodenal acid exposure is mediated by the release of secretin, which acts on vagal afferent pathways; increased exposure to acid induces CCK release and directly activates afferent nerves [44]. Duodenal acidification also increases perceptual sensitivity to mechanical fundic distention and blunts gastric accommodation to eating [45]. Esophageal distention elicits proximal gastric relaxation by way of NO release in the NTS, which then inhibits neurons in the DMNV [46]. Rectal distention inhibits proximal gastric tone but also blunts accommodation [47].

Stimulation of the proximal stomach also elicits reflex responses elsewhere in the gut. Transient relaxations of the lower esophageal sphincter are evoked by proximal gastric distention, apparently in response to activation of fundic stretch receptors [48].

Distal stomach

The distal stomach grinds and triturates solid food, and regulates the emptying of solids and, to a lesser extent, liquids.

Basic myoelectric patterns

The distal stomach exhibits a more negative membrane potential than the proximal stomach. Because the membrane potential is less than the threshold for spontaneous contractions or tone, this region is quiescent at rest. Superimposed on the resting membrane potential is the slow wave, generated by ICC-MY. A site along the greater curvature in the proximal body with the highest slow wave frequency [3 cycles per min (cpm) in humans] serves as the dominant pacemaker to entrain the rest of the stomach. Slow waves propagate circumferentially and distally with increasing velocity in the distal antrum (4 cm/s) vs the proximal body (0.5 cm/s). Propagation is slightly faster along the greater curvature, such that slow waves from the greater and lesser curvatures reach the pylorus simultaneously. Slow waves do not propagate proximally into the fundus because of its less negative resting membrane potential and other properties that limit its excitability.

Slow wave rhythm may be modulated by neurochemical input. Acute vagotomy disrupts normal cycling with the generation of ectopic antral pacemakers. Muscarinic agonists and acetylcholinesterase inhibitors increase slow wave frequency by increasing the production of inositol triphosphate [22]. This effect is not seen in mutant mice lacking ICC-IM, indicating the importance of these cells in the regulation of slow wave transmission [49]. NO reduces slow wave amplitude through cyclic GMP-dependent mechanisms [50]. Prostaglandin E_2 elicits positive chronotropic effects by stimulating E-prostanoid-3 receptors [51].

Basic motor patterns

At rest, the gastric slow wave is of insufficient amplitude to evoke significant contractions. Contractile agonists increase the duration and amplitude of the slow wave plateau potential and, in some cases, induce action potentials (< 100 ms) superimposed on the plateau potential that provide adequate membrane depolarization to exceed the contractile threshold [52]. Conversely, most relaxing agents reduce the amplitude or duration of the plateau potential or prevent the stimulatory effects of contractile agonists. VIP inhibits motor activity without altering slow wave morphology, which suggests the involvement of other mechanisms with this mediator. Because the contractile threshold is exceeded only during the plateau potential, the slow wave determines the maximal distal gastric contractile frequency (3 cpm in humans). Phasic antral contractions, sometimes exceeding 100 mmHg, migrate with the propagating slow wave as a ring, increasing in amplitude and velocity as they approach the pylorus.

The distal stomach exhibits distinct motor patterns under fasting and postprandial conditions. The migrating motor complex (MMC) is the stereotypical pattern observed during fasting. Its loss in certain disease states promotes gastric bezoar formation. After eating, the MMC is replaced by a fed contractile pattern of variable intensity and duration that grinds and triturates the ingested meal.

Migrating motor complex

The MMC clears the upper gut of undigested food residue, mucus, and sloughed epithelial cells during fasting and is termed the gastrointestinal housekeeper. The MMC comprises three phases with a combined duration of 84–112 min (Fig. 10.3) [53]. Phase I (40%–60% of the cycle length) is a period of relative motor quiescence. High-amplitude contractions are not observed during phase I, but diminutive pressure waves (< 9 mmHg) occur in phase with the slow wave [54]. Phase II (20%–30% of the cycle) is characterized by increasing but irregular contractions. Phase III is a 5- to 10-min period of intense, lumenally occlusive contractions, which originate in the gastric body and propagate at the frequency of the slow wave to the pylorus. Of the phase III complexes, 71% begin in the stomach, 18% originate in the proximal duodenum, and 11% start more distally [55]. The propulsive characteristics of interdigestive gastric motor activity vary depending on the MMC phase. Phase III contractions are highly propagative, whereas pressure waves during phase II show little coordination between adjacent regions, suggesting that phase II may serve as a period of lumenal mixing. Emptying of inert liquids and endogenous secretions is more rapid during phase III than during phases I and II [56]. MMC cycling exhibits diurnal variability, with fewer phase III complexes occurring during sleep (0.25/h) than while awake (0.64/h) [57]. Phase I is prolonged during sleep, whereas phase II is shortened. MMC cycling is more frequent and phase III complexes are shorter in women than in men.

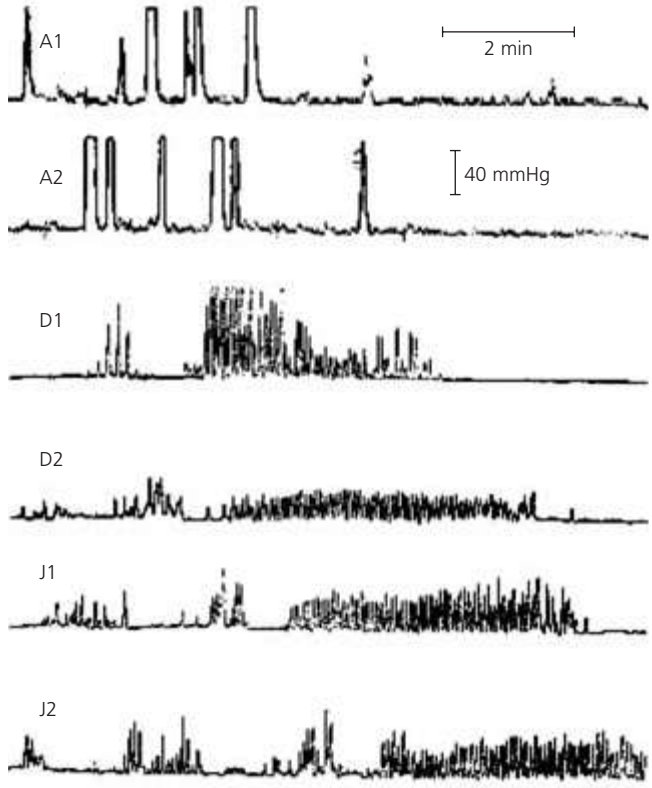

Figure 10.3 A representative migrating motor complex from the antrum (A), duodenum (D), and jejunum (J) is shown. In this tracing, a phase III complex is shown beginning in the distal stomach, which then propagates into the jejunum. From Dooley et al. [55].

Fed motor pattern

Intermittent phasic contractions of variable amplitude similar to those of phase II of the MMC comprise the fed pattern, which is induced 5–10 min after eating and persists for the time that food remains in the stomach. Fluoroscopy shows that fed antral contractions propel the gastric contents distally, only for them to be repelled back into the proximal stomach, thus producing a mixing and grinding action. The interaction of fed contractions with the incisura facilitates the disruption of solid food. Ultrasound shows that fed activity is associated with shortening gastric length and an alteration of the configuration of the distal stomach [58]. In addition to its stimulatory effects on phasic motor activity, meal ingestion elicits antral relaxation similar to proximal gastric accommodation [1].

Fed pattern profiles depend on the consistency and composition of the ingested meal. The duration of the fed period correlates with the number of calories consumed, with fats inducing a more prolonged response than proteins or carbohydrates. The threshold for induction of the fed pattern is as little as 17 g of fat. Antral contractions evoked by eating solids are more intense than those induced by an equivalent homogenized meal, indicating modulation by

meal consistency. Increasing meal viscosity with polycarbophil, a gel-forming compound, reduces antral contractile amplitudes but prolongs the fed pattern for several hours [59]. Sham feeding elicits transient increases in antral contractility and delays recovery of phase III, indicating that nonnutritive factors may participate in the induction of the fed pattern. This response is blocked by the CCK antagonist loxiglumide, suggesting mediation by endogenous CCK [60]. Half of individuals receiving continuous intragastric feeding revert to a fasting pattern, indicating that nutrient inhibition of interdigestive motor activity is self-limited [61]. Intravenous amino acid solutions reduce phase II duration and suppress antral phase III activity [62]. Total parenteral nutrition (TPN) prolongs the MMC cycle length in the antrum but not the jejunoileum of dogs and reduces the intestinal circumference in rats, indicating that systemic factors contribute to the induction of the fed pattern [63,64].

Neurohumoral control

Distal gastric motor activity is regulated by extrinsic nerve pathways. Efferent vagal stimulation of low-threshold fibers evokes atropine-sensitive antral contractions, whereas stimulation of high-threshold fibers decreases antral activity, possibly through VIP release [65]. However, phase III of the MMC persists after sectioning of the vagus and splanchnic nerves, indicating a lack of dependence on extrinsic nerves. Likewise, gastric autotransplantation with extrinsic denervation does not interrupt spontaneous phase III events in the excluded stomach [66]. Nevertheless, vagal firing increases during antral phase III, whereas vagal cooling abolishes these complexes, indicating that distal gastric function during fasting is modulated by vagal input. Bilateral vagotomy blunts phase II activity, suggesting differential regulation of the three MMC phases.

Evidence strongly suggests that antral phase III activity is initiated by cyclic changes in levels of motilin, a hormone produced by specialized duodenal mucosal cells [67]. Motilin infusion evokes premature antral phase III in dogs and humans. Conversely, both motilin antibody administration and duodenal resection of motilin-secreting tissues suppress plasma motilin levels and abolish phase III activity in dogs for several hours [68]. Motilin-evoked antral phase III activity is atropine sensitive, whereas duodenal phase III activity is partly atropine resistant, indicating differential regulation in different regions of the gut [69]. Motilin receptors are present on antral smooth muscle and on myenteric nerves. The existence of two or more motilin receptor subtypes is supported by neurotransmitter binding studies that show different affinities for motilin agonists in membranes from neural and muscular tissues of the stomach [70]. Motilin receptors have also been characterized in the brain. In rats, the injection of motilin into the hippocampus increases gastric contractile amplitude by way of a vagal pathway, suggesting participation of a vagovagal reflex [71].

The physiological stimulus for motilin release is unknown. Although motilin levels increase after vagal stimulation, extrinsic denervation does not interrupt motilin cycling [66]. Intraduodenal capsaicin inhibits motilin release with concomitant loss of the antral MMC, indicating involvement of mucosal afferents [72]. Motilin regulation of antral phase III activity exhibits plasticity. Gastric MMC cycling recovers 1–4 months after resection of the pylorus, duodenum, and upper jejunum in dogs by recruiting vagal cholinergic and adrenergic efferent pathways and without the return of motilin cycling [73].

Other neurohumoral factors participate in motilin-evoked MMC activity. Most motilin-immunoreactive neurons in the myenteric plexus are positive for neuronal NO synthase, whereas few are positive for choline acetyltransferase [74]. In general, NO is an inhibitory transmitter in the distal stomach. In dogs, NO synthase inhibitors increase antral contractions, whereas NO precursors decrease motor function. The phosphodiesterase-5 inhibitor sildenafil inhibits antral motor activity by enhancing the action of endogenous NO [75]. However, motilin also induces the release of NO. NO synthase inhibitors evoke MMC-like contractions in fed rats, induce premature antral phase III in dogs, and increase MMC frequency and trigger premature phase III in humans, all with associated increases in plasma motilin, reflecting regulation of motilin release by endogenous NO (Fig. 10.4) [76,77]. Serotonin depletion with *p*-chlorophenylalanine suppresses spontaneous and motilin-induced antral phase III, suggesting involvement of serotonergic pathways [78]. The μ-opioid receptor antagonist naloxone doubles the MMC cycle length and delays plasma motilin peaking, indicating regulation of motilin release by endogenous opiates. Naloxone does not block motilin-evoked phase III, confirming that motilin does not act by way of opiate-dependent pathways.

Motilin-independent factors modulate gastric MMC activity. The 5-HT_1 agonist sumatriptan prolongs the MMC cycle [79]. The histamine H_2 antagonist famotidine shortens the length of the MMC cycle. The leukotriene inhibitor pranlukast prolongs the MMC period in dogs [80]. Pancreatic polypeptide levels cycle in phase with antral phase III, but exogenous pancreatic polypeptide does not induce a premature MMC. Pancreatic resection, which removes pancreatic polypeptide-secreting cells, does not disrupt the antral MMC. However, motilin stimulates pancreatic polypeptide release, indicating a possible physiological role for this hormone [81]. Serotonin is released cyclically into the duodenal lumen, in phase with the MMC, but as intraduodenal serotonin has no effect on gastroduodenal motor function, the physiological significance of this response is uncertain [82]. CCK, bombesin, substance P, and some opiates increase antral contractions, whereas secretin, somatostatin, glucagon, GRP, TRH, neurotensin, calcitonin, peptide YY, prostaglandin E_2, the α_1-adrenergic receptor agonist phenylephrine, and the

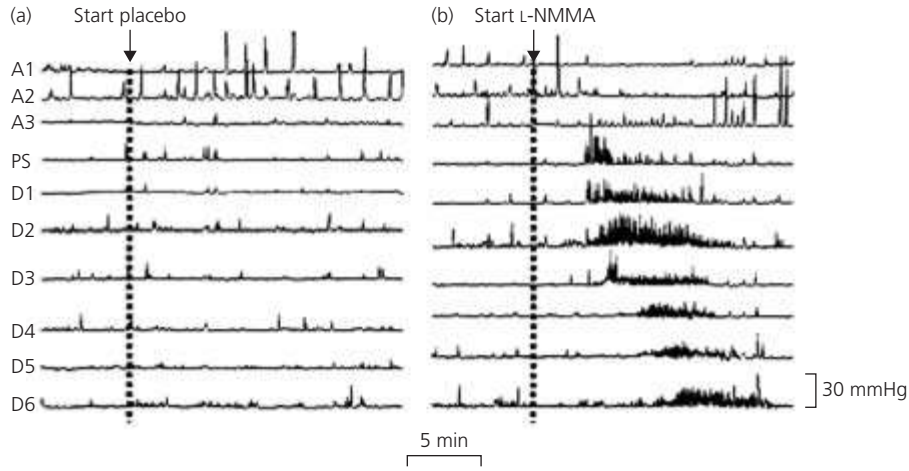

Figure 10.4 Antroduodenal manometry tracings are shown after infusion of a placebo **(a)** or the nitric oxide synthase inhibitor N(G)-monomethyl-L-arginine (L-NMMA) **(b)**. In this individual, inhibition of NO synthase elicited a premature phase III complex, beginning in the proximal duodenum. From Kuiken et al. [77]. A, antrum; D, duodenum; PS, pyloric sphincter.

α_2-adrenoceptor agonist clonidine inhibit distal motor activity. The 5-HT_3 agonist *m*-chlorophenylbiguanide increases antral motility during fasting but reduces postprandial antral contractions.

Secretory activities in the upper gut influence gastric MMC activity. Some, but not all, fasting pancreaticobiliary and secretory immunoglobulin A outputs cycle with phase III activity and motilin peaks [83]. However, duodenal bile acid perfusion suppresses antral contractions suggesting that endogenous bile release is not a stimulus for gastric phase III. Antral phase III complexes do not develop if the duodenal pH is less than 7.0, even during peak motilin levels, and duodenal acid perfusion abolishes phase III activity, indicating that duodenal alkalinization is a prerequisite for MMC cycling [84]. Gastric acidification to pH 1.0 inhibits spontaneous and motilin-induced gastric phase III through a vagovagal reflex. Patients with chronic pancreatic insufficiency exhibit MMC cycles of shorter duration, indicating an interaction between antroduodenal and pancreatic function [85].

Neurohumoral control of the fed motor pattern is less well understood than that of the MMC. Proximal gastric distention evokes irregular phasic contractions that mimic the fed pattern in the proximal intestine, indicating possible participation by gastric mechanoreceptors [86]. Perfusion of a meal into a denervated, autotransplanted, small intestinal loop interrupts antral MMC activity, indicating that external innervation is not needed to induce the fed pattern. However, both vagotomy and total gut autotransplantation increase the threshold number of calories required to induce the fed pattern, reflecting the ability of extrinsic nerves to modulate postprandial activity [87]. Vagal cooling replaces the fed pattern with intermittent phase III complexes, implicating vagal pathways in the recovery of the MMC after a meal. Perfusion of blood from a fed animal into an isolated stomach preparation elicits a motor response similar to the fed pattern, suggesting the presence of a circulating mediator of postprandial motility [88]. Several humoral candidates have been proposed to be the physiological trigger of the fed pattern, but no studies have convincingly implicated any one hormone.

Neural reflex modulation

As with the proximal stomach, distal gastric motor function is modified by reflex stimulation of other regions. An excitatory vagal cholinergic fundoantral reflex is induced by distention of the ferret, human, and canine proximal stomach [89,90]. Circumferential myotomy of the midstomach promotes increased antral motor activity after eating, suggesting an additional tonic inhibitory effect of the fundus on the distal stomach mediated by intrinsic pathways. Liquid stimulation of the larynx and epiglottis inhibits antral contractions by inhibiting neurons in the DMNV [2,91]. Rectal distention reduces antral motor activity and disrupts slow wave cycling [92]. Reflex inhibition of antral motor function by duodenal distention is mediated by vagal and splanchnic innervation. In contrast, this reflex is unaffected by duodenal transection, excluding participation of intrinsic neural pathways. In rats, duodenal glucose or amino acid perfusion increases vagal afferent firing [93]. However, reductions in antral contractions during intraduodenal lipid, protein, or hydrochloric acid perfusion are incompletely blocked by vagotomy. Vagal capsaicin treatment partly reverses the inhibitory effects of protein, glucose, or trypsin inhibitor on distal motor activity, clarifying the importance of the afferent arm of this reflex [94]. Hormonal mediation is suggested by the observation that reflex inhibition of motor activity by duodenal lipids persists after gastric denervation and autotransplantation. Long-chain triglycerides are more potent inhibitors of distal gastric contractions than short- or medium-chain lipids [95]. Protein or amino acid perfusates containing L-tryptophan effectively inhibit antral motility. Participation of CCK_1 receptors in the reflex inhibition of gastric motility by duodenal protein is suggested by studies in rats that use specific antagonists [94].

Pylorus

The pylorus sieves the outflow of gastric contents. The mechanical properties imparted by its thickness and dense connective tissue restrict the passage of large particles. The sphincteric action of the pylorus is facilitated by a redundant, highly folded mucosa that further narrows the lumenal diameter.

Basic myoelectric and motor patterns

Pyloric slow wave activity is entrained to the rate of slow waves in the distal stomach (3 cpm), although most slow waves do not propagate into the duodenum. In rodent models, a slow wave-free gap secondary to a loss of pyloric ICC-MY may contribute to the independence of the antral and duodenal pacemaker systems (Fig. 10.5) [96]. In contrast, some action potentials in cats cross the pyloric region in patches and may form the basis for gastroduodenal coordination [97].

The pylorus exhibits both tonic and phasic motor activity in many species. The resting pressure is 10 mmHg in dogs; however, in humans, a basal pressure cannot be reliably demonstrated. Human pyloric muscle tissue relaxes in response to electrical stimulation, reflecting basal inhibitory neural input, whereas canine pyloric muscle tissue contracts in response to electrical depolarization. The ability of the neural toxin tetrodotoxin to enhance pyloric contractions indicates that phasic motor activity is an inherent property of pyloric smooth muscle.

The pylorus exhibits characteristic motor patterns under fasting and fed conditions. During phase III of the MMC, the pylorus is open, facilitating exit of gastric contents into the duodenum. If the lumen is impeded, retrograde flow is common during phase I, whereas more than 80% of flow is antegrade during phases II and III [98]. After eating, the pylorus exhibits prolonged periods of closure interrupted by brief relaxations that permit gastric evacuation. Large particles are emptied during fasting but not in the fed state, reflecting enhanced sieving after eating. Pyloric resection does not prevent postprandial sieving, suggesting that other gastroduodenal regions also participate in this process; however, removal of both the antrum and the pylorus permits passage of large, untriturated food particles. In general, pyloric contractions are synchronous with fed antral activity, although some foods (e.g., milk) evoke isolated pyloric contractions with initial antral inhibition. On cinefluoroscopy, meal ingestion initiates a minor ring contraction in the gastric body, which is followed 2–3 s later by an intense lumen-occluding contractile ring. The minor ring contraction induces pyloric closure as the larger contraction approaches the midantrum. As the intense contractile ring propagates further, food is trapped by

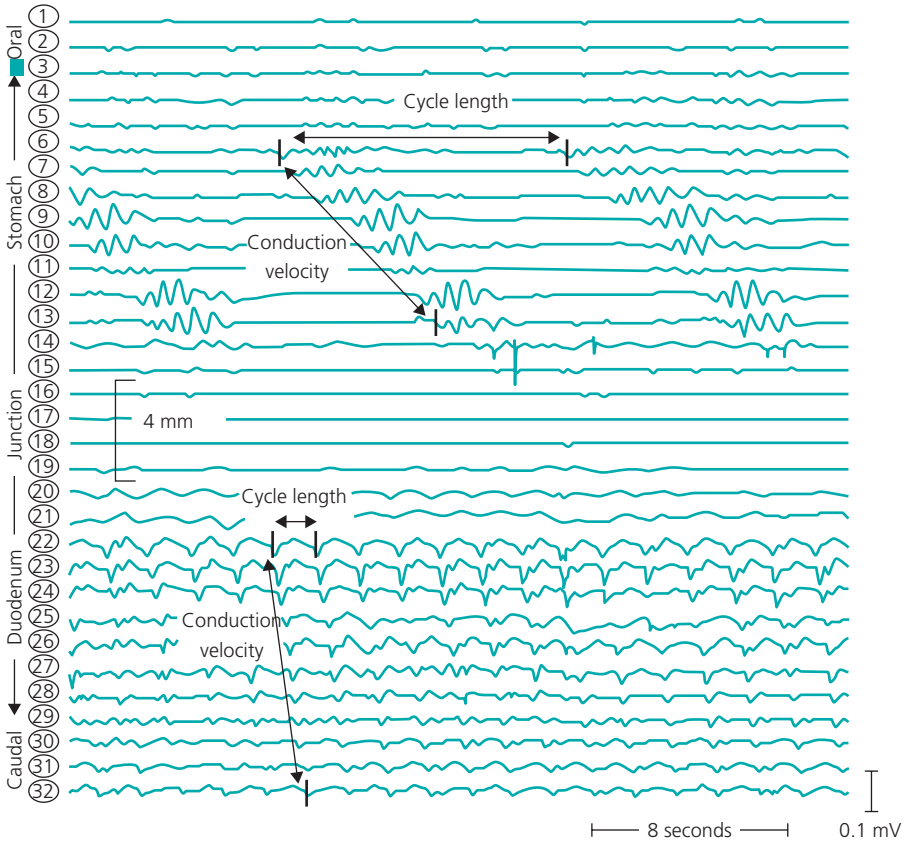

Figure 10.5 Slow wave activity in the stomach, pylorus (junction), and duodenum. Slow waves are more frequent and exhibit faster conduction in the duodenum than in the stomach. There is a slow wave-free zone in the pyloric region that may contribute to the independence of the pacemakers in the different gut regions. From Wang et al. [96].

the occluded pylorus and is ground, mixed, and repelled into the proximal stomach. Because the pylorus is closed by the initial minor ring contraction, all emptying occurs early in the cycle, before the major contraction reaches the midantrum. Ultrasound shows that transpyloric flow occurs with the formation of a common antropyloroduodenal chamber [99]. Bursts of duodenogastric reflux are demonstrable before pyloric closure, suggesting the occurrence of duodenal mixing and retropulsion [99]. The relative timing of the two ring contractions depends on the latency between the initial rapid depolarization of the slow wave, which induces the minor contraction, and the peak of the plateau potential, which elicits the major contraction, and is not affected by neurohumoral or meal-related factors. In dogs, the late postprandial period is characterized by profound pyloric relaxations synchronized with propagating antral contractions [100].

Neurohumoral control

The pylorus exhibits distinct neurohumoral regulation separate from that of adjacent tissues. Nerve densities in pyloric circular muscle are 3- to 5-fold greater than in the antral circular muscle. Likewise, more afferent nerves project from the pylorus to the vagal nuclei of the brainstem than from the duodenum. The pylorus exhibits increased levels of neuronal VIP, substance P, enkephalins, neuropeptide Y, and galanin. Vagal stimulation elicits pyloric contraction at low frequencies and relaxation at high frequencies, suggesting both excitatory and inhibitory efferent supply. Splanchnic stimulation evokes pyloric contraction. Vagally mediated contractions are blocked by naloxone, correlating with observations of enkephalin-containing fibers in pyloric smooth muscle and in pyloric branches of the vagus [101]. NO donors blunt basal pyloric pressure waves and enhance transpyloric flow after eating, whereas NO synthase inhibitors block relaxation of the pylorus evoked by high-frequency vagal stimulation and direct electrical depolarization, and during the late postprandial period, suggesting that NO is a common mediator of pyloric relaxation [100,102]. Combined administration of NO synthase inhibitors and P2X purinoceptor antagonists more potently inhibits electrically induced pyloric relaxations than NO synthase inhibitors alone, suggesting the involvement of purinergic pathways [103]. Motor responses of the antrum and pylorus to intravenous CCK facilitate retropulsion of gastric contents [104]. Inhibition of fed pyloric motility by a CCK receptor antagonist supports a physiological role for CCK in the postprandial state [105]. VIP, peptide histidine isoleucine, galanin, prostaglandin E_1, and serotonin relax the pylorus, whereas secretin and histamine produce contraction.

Neural reflex modulation

Pyloric motor activity is modified by ascending and descending reflexes. Electrical duodenal stimulation induces pyloric contraction, whereas antral depolarization relaxes the pylorus. Duodenal perfusion of lipids, amino acids, glucose, hypertonic saline, or hydrochloric acid produces pyloric closure and decreases transpyloric flow. The effects of fatty acids are dependent on chain length; intraduodenal lauric acid (12 carbons) more potently evokes isolated pyloric contractions, whereas decanoic acid (10 carbons) has little effect [106,107]. Duodenal lipids blunt pyloric contractility by inhibiting lipase activity, which suggests that products of lipolysis mediate this enterogastric reflex [108]. Chronic exposure to a high-fat diet attenuates the excitatory effects of duodenal lipids on pyloric motility, indicating plasticity in this reflex [109]. Ileal triglycerides evoke isolated pyloric contractions, whereas ileal short-chain fatty acids increase pyloric tone and reduce transpyloric fluid pulses [110].

Neurohumoral pathways mediate reflex activation of pyloric motor activity. Pyloric contractions evoked by duodenal hydrochloric acid are antagonized by atropine and hexamethonium but not by vagotomy, indicating control by nonvagal cholinergic pathways [111]. Involvement of serotonin pathways is suggested by the observation that the 5-HT_3 antagonist zacopride reduces pyloric responses to intraduodenal hydrochloric acid. Prevention of acid-induced pyloric contraction by the topical anesthetic xylocaine suggests that mucosal receptors play a role in initiating this reflex [111]. Opioid pathway participation is suggested by the ability of naloxone to block pyloric contractions induced by duodenal amino acids. The inability of naloxone to block lipid-evoked pyloric contractility is evidence of the differential regulation of responses to different stimuli. Antagonists to glucagon-like peptide-1 (GLP-1) stimulate pyloric motor activity and blunt responses to intraduodenal glucose, indicating a physiological role for GLP-1 in this enteropyloric reflex [112]. The role of other hormones such as CCK in enteropyloric reflex activity is undefined.

Proximal small intestine

The small intestine provides feedback regulation of gastric and pyloric motor activity. The dominant pacemaker of the duodenum is distinct from that of the stomach, and cycles at 11–12 cpm. Some gastric myoelectric activity propagates across the pylorus through longitudinal muscle tissue, producing intermittent antroduodenal coordination, with one gastric slow wave in phase with every four duodenal cycles. This permits propagation of some antral pressure waves into the duodenum, most commonly during phase II.

The duodenum exhibits characteristic motor patterns. High-resolution manometry reveals that most duodenal contractions during phase III travel antegradely [113]. Under some conditions, strong retroperistaltic contractions in the duodenum propel intestinal contents orally and induce duodenogastric reflux. Fasting duodenal pH may be regulated by a retroperistaltic pump during late phase III. Retrograde propulsion of bile is limited by phase III-associated closure of

the sphincter of Oddi [114]. After eating, isolated duodenal contractions segment and mix food particles with pancreaticobiliary secretions. In the proximal 5–6 cm of the duodenum, 40%–50% of postprandial pressure waves are retrograde and may participate in the control of gastric emptying [115]. Intraduodenal lipid and bile perfusions increase duodenal diameter, decrease contractile frequency, reduce propagation distances, and slow intestinal flow [116]. In contrast, hydrochloric acid and hyperosmolar solutions retard propagation by inducing tonic duodenal lumenal occlusion.

The small intestine exhibits reflex responsiveness to gastric stimulation. Gastric distention delays intestinal transit. This gastroenteric reflex is unaffected by vagotomy but is reduced by small intestinal denervation and by severing connections from the celiac plexus. L-Arginine applied to celiac ganglion neurons enhances the reflex, whereas NO synthase inhibitors block it, indicating mediation by nitrergic pathways [117]. The gastroileal reflex, defined as the increase in ileal propulsion after eating, is abolished by intestinal transection, indicating mediation by intrinsic nerves. Decreased ileocecal junction pressure is observed after nutrient ingestion.

Gastric emptying

Coordinated actions of the different gastric regions with feedback from the small intestine regulate the emptying of stomach contents. Gastric emptying profiles of liquids, digestible solids, fats, and indigestible solids exhibit distinctive characteristics.

Gastric emptying of liquids

Kinetics

Gastric emptying of inert liquids (e.g., water, isotonic saline) follows a single exponential curve, termed first-order kinetics, with a time to 50% emptying of 8–18 min. The volume emptied into the duodenum in a given time is a constant fraction of the volume that remains in the stomach. Thus, 300 mL of saline empties twice as fast as a 150-mL load. Liquid emptying is slower in women than in men [118]. Nutrient-containing liquids are emptied more slowly in elderly individuals than in young controls [119].

Factors that modify liquid emptying

The nutritional properties of a liquid modify the rate of gastric emptying to ensure that all carbohydrates, proteins, and fats are digested and absorbed in the small intestine. Feedback mechanisms in the small intestine delay liquid emptying by modifying the first-order kinetic pattern. Emptying of nutrient liquids exhibits an initial rapid phase lasting 5–30 min, followed by a slower phase of linear emptying lasting as long as 120 min. During the linear phase, 300 mL of 11% glucose empties at the same rate as 150 mL of the same liquid. Liquids of high caloric density empty more slowly than those with fewer calories per unit volume [120]. Nutrient liquid emptying is regulated such that about 200 kcal/h are delivered to the duodenum, regardless of whether fats, proteins, or carbohydrates are consumed [121]. However, some nutrient liquids that are more concentrated than 1 M empty at rates exceeding 200 kcal/h, indicating that caloric control of liquid emptying operates within a limited range of concentrations [120].

Other nutrient characteristics modulate liquid emptying. Carbohydrates and most amino acids modulate duodenal delivery, in part by acting on intestinal osmoreceptors. Glucose solutions empty more slowly than isocaloric, hypotonic glucose polymer solutions [122]. This discrimination is mediated by vagal and celiac ganglionic pathways. The relative inability of fructose to retard liquid emptying, compared with glucose or xylose, suggests the involvement of additional nonosmotic factors. Stimulation of specific intestinal L-tryptophan receptors may contribute to the delay in emptying elicited by isotonic L-tryptophan solutions. Isocaloric solutions of protein and amino acids delay gastric evacuation to similar degrees, as do isocaloric starch, disaccharide, and monosaccharide solutions, whereas nonhydrolyzable sucrose polymers have no effect [123]. These observations indicate that the digestive products of carbohydrate and protein hydrolysis are the major regulators of liquid emptying. The inhibitory effects of proteins are prevented by VIP antibodies, suggesting mediation by VIP [124]. Liquid peptone emptying is faster in rats fed a high-fat diet for 3 weeks than in those fed a low-protein diet, indicating plasticity in emptying kinetics [125]. Emptying of liquid lipids is not inhibited by pancreatic insufficiency or by biliary diversion, suggesting that fatty acids modulate gastric motor function. Medium-chain fatty acids (i.e., 12–14 carbons) induce more potent inhibitory actions than longer or shorter chain lengths.

Liquid gastric emptying is also modulated by physicochemical factors. Duodenal acid delivery is constant regardless of the pH or lipid solubility of acids within the stomach. Secretin is released by duodenal acid exposure, but the rapidity of the inhibition of gastric emptying with duodenal acidification is more consistent with a neurally mediated reflex. The inhibitory actions of duodenal hydrochloric acid on liquid emptying are observed only with an intact pylorus, suggesting mediation by acid-evoked pyloric contractions [124]. Increased viscosity slows liquid emptying without affecting gastric motor or myoelectric activity [126]. Assuming a supine position slightly delays emptying of saline or nutrient liquids when consumed as part of a mixed meal [127].

Finally, liquid emptying is modified by postabsorptive factors. In healthy humans, TPN slows liquid emptying by 38% [128]. The emptying delay is attenuated when half of the amino acids in TPN are replaced with branched-chain amino

acids, suggesting modulation of motility by different parenteral formulations. Similarly, patients receiving intravenous fat emulsions show delays in gastric emptying.

Roles of different regions

Each region of the proximal gut participates in the regulation of liquid gastric emptying. Extrinsic denervation of the proximal stomach impairs receptive relaxation and accommodation and accelerates liquid emptying. Proximal gastric resection or fundoplication produce similar effects. Liquid meals empty more rapidly when delivered by orogastric perfusion than when swallowed, confirming a modulatory role for receptive relaxation. The importance of a fundus-generated gastroduodenal pressure gradient in the control of liquid emptying has not been resolved. Diabetic patients with normal fundic tone may exhibit delayed liquid emptying [129]. Liquid emptying proceeds normally even if the pressure in the proximal stomach is maintained at a constant 2 cmH_2O below the pressure in the duodenum [130].

The antrum stimulates or inhibits liquid emptying under different conditions. In humans, a substantial degree of low calorie liquid emptying occurs in the absence of peristaltic antroduodenal motor activity [131]. Although not required for liquid emptying, the magnitude of antral contractile activity correlates with liquid expulsion into the duodenum [132]. In pigs, emptying of nonnutrient liquids occurs as a consequence of phasic contractions that originate in the gastric body and propagate to the pylorus, producing discrete flow pulses into the duodenum [133]. Magnetic resonance imaging shows that antral contractions of greater amplitude increase the emptying of liquid nutrients of low caloric density (e.g., 10% dextrose) vs more concentrated solutions (e.g., 25% dextrose), indicating differential nutrient regulation of distal gastric motor activity [134]. If the proximal stomach is excised, coordinated antropyloric pressure waves generate fluid pulses that preserve normal liquid emptying. Antral resection accelerates initial liquid emptying, suggesting inhibition by the distal stomach.

The pylorus and duodenum also participate in liquid emptying. The initial rapid phase of liquid emptying is accelerated with pyloric stenting, while the slower linear phase is unaffected. Likewise, pyloromyotomy and pyloroplasty with or without highly selective vagotomy increase early liquid emptying [130]. The absence of duodenal motor activity is associated with accelerated emptying, whereas the presence of continuous duodenal contractile activity correlates with delayed liquid gastric emptying. Liquid emptying is enhanced by circular myotomy of the duodenal wall [135].

Gastric emptying of digestible solids

Kinetics

Gastric emptying of digestible solids proceeds more slowly than emptying of liquids. Solid-phase emptying exhibits an initial lag phase that lasts for up to 1 h, during which little food residue is expelled into the duodenum [136]. Cinefluoroscopy shows that extensive mixing and retropulsion occur during the lag phase, dispersing the food into fine particles. The linear emptying phase follows the lag phase and is characterized by slow, controlled delivery of the dispersed food residue to the duodenum [136]. The linear phase exhibits zero-order kinetics, during which time lumenal contents exit the stomach at a constant rate independent of the residual gastric volume. Solid-phase emptying terminates in a third phase of very slow transit of the remaining digestible residue [136]. In healthy humans, more than 40% of a low-fat digestible meal empties the stomach in 2 h and more than 90% empties within 4 h (Fig. 10.6) [137]. Solid-phase emptying is slower in premenopausal women than in men, regardless of the phase of the menstrual cycle, because of differences in the linear emptying rate [138]. The rate of digestible solid emptying correlates positively with body mass index [139].

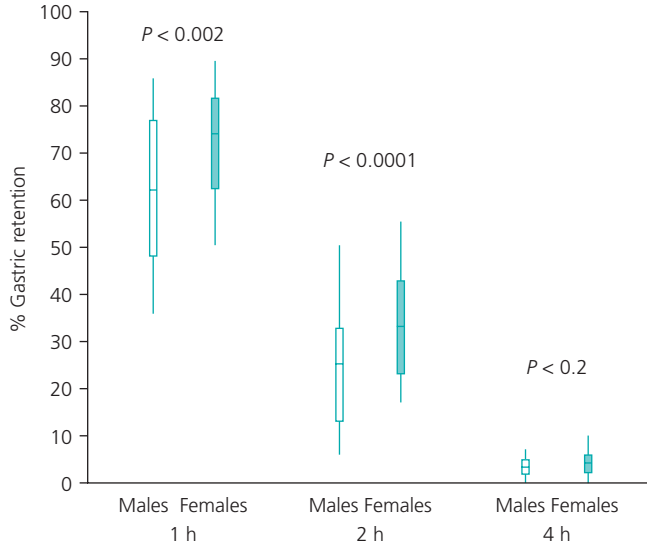

Figure 10.6 Box and whisker plots for gastric retention of a low-fat solid meal in healthy men and women at 1, 2, and 4 h after eating. Normal values from these studies show < 60% retention at 2 h and < 10% retention at 4 h. Emptying rates for women were slightly slower than for men at 1 h and 2 h. From Tougas et al. [137].

Physical factors regulate the differential gastric emptying of solids and liquids. The J shape of the stomach may promote selective retention of solids that settle out along the greater curvature of the gastric body and proximal antrum, while liquids layered on top of the solids are expelled by gastric motor activity [140]. The principles of fluid mechanics dictate that liquids and small dispersed particles will be transported in the center of the fluid stream, which is expelled before pyloric closure. Larger food particles at the periphery of the fluid stream are not propelled into the distal antrum until pyloric closure has occurred. As a consequence, the

residue of larger particles is repulsed proximally, where it is mixed and triturated into finer particles. Dispersal of the digestible solid residue is facilitated by shear forces generated by the sudden changes in fluid direction that occur during this retropulsion.

Factors that modify digestible solid emptying

Physical and nutritional properties of a digestible solid meal determine its rate of emptying from the stomach. In humans and dogs, digestible solids are delivered to the duodenum as finely dispersed particle suspensions of less than 1 mm in diameter. After a chicken meal, the mean particle size recovered in the duodenum is 0.05 mm and 95% of particles are less than 0.5 mm in diameter. Eggs and noodles are expelled into the duodenum faster than an equicaloric meal of 10-mm liver cubes [136]. Similarly, homogenized eggs empty more rapidly than 2.5- or 5-mm cubes [141]. These differences are caused by modulation of the lag phase. Larger particles prolong the lag phase, whereas homogenized food of less than 0.25 mm in diameter is emptied without a significant lag. Emptying is influenced by the amount of liquid consumed with the solid meal. After consumption of a mixed solid–liquid meal, the liquid phase is emptied more rapidly, indicating that the stomach distinguishes between the separate meal phases. Ingestion of liquid can prolong the lag phase of digestible solid emptying. Indeed, liquid consumption 90–180 min after a solid meal blunts antral motor activity and can induce a second lag phase [142]. Increasing the viscosity of a dispersed solid meal suspension impairs the discrimination of large from small particles and thus promotes the expulsion of larger particles into the duodenum. The addition of fats, triglycerides, or carbohydrates (e.g., glucose, fructose, or xylose) to a solid meal prolongs the lag phase of emptying. Conversely, solid emptying is accelerated by adding low-calorie foods (e.g., lettuce) that increase meal volume without enhancing nutritive value. The effects of posture on digestible solid-phase emptying have been debated.

Roles of different regions

Digestible solid emptying results primarily from the combined actions of the distal stomach and pylorus. The rate of solid emptying correlates strongly with the degree of antral motor activity. After pyloroplasty, with or without vagotomy, there is no increase in the particle sizes emptied from the stomach, indicating that the pylorus is not solely responsible for regulating the delivery of fine suspensions to the duodenum [143]. Conversely, after antrectomy with vagotomy, early acceleration of solid emptying occurs with loss of the lag phase. Of the particles expelled into the duodenum after antrectomy, 30% are less than 1 mm in diameter [143]. Thus, antral resection may promote impaired digestion. In dogs that have ingested chicken liver with carbon 14-labeled fat, 85% of the radiolabel is absorbed in the upper intestine [144]; however, after antrectomy, 57% of the radioactive tracer reaches the distal intestine. The role of the proximal stomach in regulating digestible solid emptying is not well defined. That 70% of food material expelled into the duodenum after antrectomy is appropriately dispersed suggests that the proximal stomach has some capacity to mix and grind solid food [143].

Gastric emptying of fats

Foods with high levels of fat are handled differently by the stomach, and their emptying patterns are distinct from those of other liquids and solids. After consumption, high-fat foods are warmed to body temperature and may be converted from a semisolid or solid consistency to a liquid phase, which is emptied more slowly than nonlipid liquids. As with digestible solids, emptying of fats exhibits an initial lag period during which to-and-fro movements in the antrum enhance fat emulsification [145]. Solid and liquid fats empty at similar rates in the first hour after ingestion; thereafter, solid high-fat foods empty more slowly [146]. Prolonged ingestion of a high-fat diet promotes acceleration of the intrinsic gastric emptying rate of fats but not carbohydrates, indicating plasticity in this parameter [147].

Emptying of fats is slow for many reasons. Lipids have specific gravities of less than 1 g/cm^3 and they float on top of aqueous liquids in the stomach, whereas nonlipid solids exhibit densities of greater than 1.2 g/cm^3 and they settle in dependent fashion [148]. Indeed, emptying rates of fats and low-density spheres are similar, emphasizing the importance of relative density as a determinant of gastric emptying. Because of poor aqueous solubility, fats coalesce into large globules and do not easily disperse into fine particles. Liquid lipids adhere to solid food particles and are emptied at the rate of digestible solids.

Gastric emptying of indigestible solids

Indigestible solid residue from a meal is emptied from the stomach in a manner dependent on particle size. Plastic spheres ≤ 3 mm in diameter can pass into the intestine during the fed period, whereas larger spheres pass more slowly, often after a lag period [149]. Spheres greater than 7 mm in diameter do not empty with the digestible solid meal; rather, they are expelled after the fed motor pattern terminates and antral phase III activity resumes. This observation likely relates to the functional differences of the pylorus, which is mostly closed during the fed period but is widely patent during fasting. Indeed, during fasting, indigestible objects of greater than 2 cm may pass from the stomach into the intestine. Emptying of indigestible solid rings is slower in women than in men [150]. From a clinical perspective, particle size is relevant to the design of encapsulated drugs. Drug absorption from 0.7-mm pellets is more rapid than absorption from 3.6-mm pellets, which are emptied more slowly from the stomach [151]. Specific gravity is an independent determinant of indigestible solid emptying, with spheres having a density

greater than 2 g/cm^3 or less than 0.5 g/cm^3 emptying more slowly than spheres with a density of 1.0 g/cm^3 [152]. Increasing intragastric liquid viscosity with guar accelerates emptying of 3.2-mm indigestible spheres. Compressibility of an indigestible compound is important, with soft solids emptying faster than hard ones [153].

Emptying of indigestible solids is regulated by distal gastric motor activity. Antrectomy promotes accelerated indigestible solid emptying, whereas fundic resection has no effect. Interruption of vagal innervation of the distal stomach impairs emptying of indigestible spheres, whereas selective proximal vagotomy does not affect the emptying rate.

Differential intragastric distribution

The sequestration of different meal components in the distinct regions of the stomach contributes to the differential rates of emptying observed for liquids and solids. Fluoroscopy shows that large solid food particles are retained in the fundus, whereas fine particles are mixed in the antrum. After consumption of a radiolabeled solid meal, antral filling to a maximal level of radioactivity is observed during the lag period. This level of radioactivity remains constant during the linear emptying phase, while radioactivity in the fundus progressively declines [141]. Ingested liquids are also retained proximally to limit the volume delivered to the distal stomach. However, nonnutritive liquids are stored to lesser degrees than caloric liquid meals, thus promoting increased antral diameter. The addition of substances with high lipid levels (e.g., margarine) to a liquid meal promotes greater fundic storage with further delays in emptying [154]. When liquids and solids are consumed together, the solid fraction is retained in the proximal stomach while the liquid portion is delivered to the antrum. Large solid meals retard the transfer of liquid from the proximal stomach to the antrum and delay liquid emptying [155]. If solids are ingested with carbonated water, the proximal stomach retains a greater proportion of the solid meal, suggesting that gaseous distention modulates intragastric distribution [156]. Indigestible and digestible solids are distributed separately. After the ingestion of liver with 2.4-mm plastic spheres, the liver is retained in the fundus, whereas the spheres are propelled to the distal stomach where they remain until the late postprandial period.

External regulation of gastric emptying

Gastric emptying is extensively modulated by external influences, including reflex inhibition by intestinal stimuli, input from extrinsic nerve pathways and neurohumoral factors, interaction with the CNS, and disruption by immune activation.

Neural reflex modulation

Gastric emptying is modulated by stimulation of the small intestine. Duodenal acidification slows gastric emptying by acting on pH receptors on the duodenal mucosa. Nutrient perfusion (e.g., amino acids, fats) of the small intestine delays emptying of digestible solids by prolonging the initial lag phase [157]. Duodenal amino acid delivery also enhances pyloric sieving, increasing the percentage of particles of less than 1 mm delivered to the duodenum from 66% to 82%. Duodenal osmoreceptors mediate hypertonic saline-evoked inhibition of emptying, whereas longer segments of small intestine participate in the inhibitory effects of acids, lipids, and glucose [158]. Exposure of as little as 15 cm of the proximal duodenum to hydrochloric acid or oleic acid delays liquid gastric emptying [159]. Maximal inhibition of liquid emptying is seen after contact of the proximal 150 cm of small intestine with acid, glucose, or oleic acid. Solid gastric emptying is inhibited by ileal glucose or oleic acid perfusion, a phenomenon known as the ileal brake. Lipids that activate the ileal brake include corn oil, lecithin, and petroselinic, oleic, myristoleic, erucic, linoleic, linolenic, and deoxycholic acids, but not taurocholic acid [160].

Mechanisms of small intestinal feedback inhibition of gastric emptying have been extensively investigated. Antrectomy does not prevent the inhibition of solid emptying by fats, indicating persistence of enterogastric reflex inhibition. Conversely, duodenal transection prevents the induction of isolated pyloric contractions and retardation of liquid emptying by nutrient perfusion, indicating involvement of intrinsic duodenal innervation in feedback control of gastric motor function [161]. Placement of a duodenal fistula to divert nutrients from mucosal receptors converts liquid nutrient emptying to the first-order kinetic profile seen with nonnutrient liquids. The inhibitory effects of lipids on gastric emptying are mediated by the products of their digestion, as evidenced by the observation that patients with pancreatic exocrine insufficiency exhibit rapid emptying of fats [162]. Similarly, inhibition of lipolysis by orlistat accelerates gastric emptying of lipids [163]. Conversely, intestinal perfusion of nonhydrolyzable fats does not inhibit emptying [123]. Administration of surfactants that prevent chylomicron formation abolish duodenal lipid-evoked inhibition of gastric emptying, indicating that chylomicron generation is required for this enterogastric reflex [164]. Similarly, in rats, lymph with high levels of chylomicrons collected during intestinal lipid infusion significantly inhibits gastric motility when given intraarterially [165]. Data suggests that CCK release evoked by chylomicrons activates CCK_1 receptors on vagal afferent nerves and leads to reflex inhibition of gastric motility (Fig. 10.7) [166]. Apolipoprotein A-IV, a peptide released by enterocytes in response to triglyceride absorption, also delays gastric emptying [167]. Intestinal capsaicin perfusion abolishes inhibition of emptying induced by intestinal hydrochloric acid, but it reduces the inhibition evoked by duodenal glucose or lipid by only 59% and 42%, respectively [168]. Likewise, vagal capsaicin attenuates lipid-induced inhibition of gastric emptying by 57% and prevents the

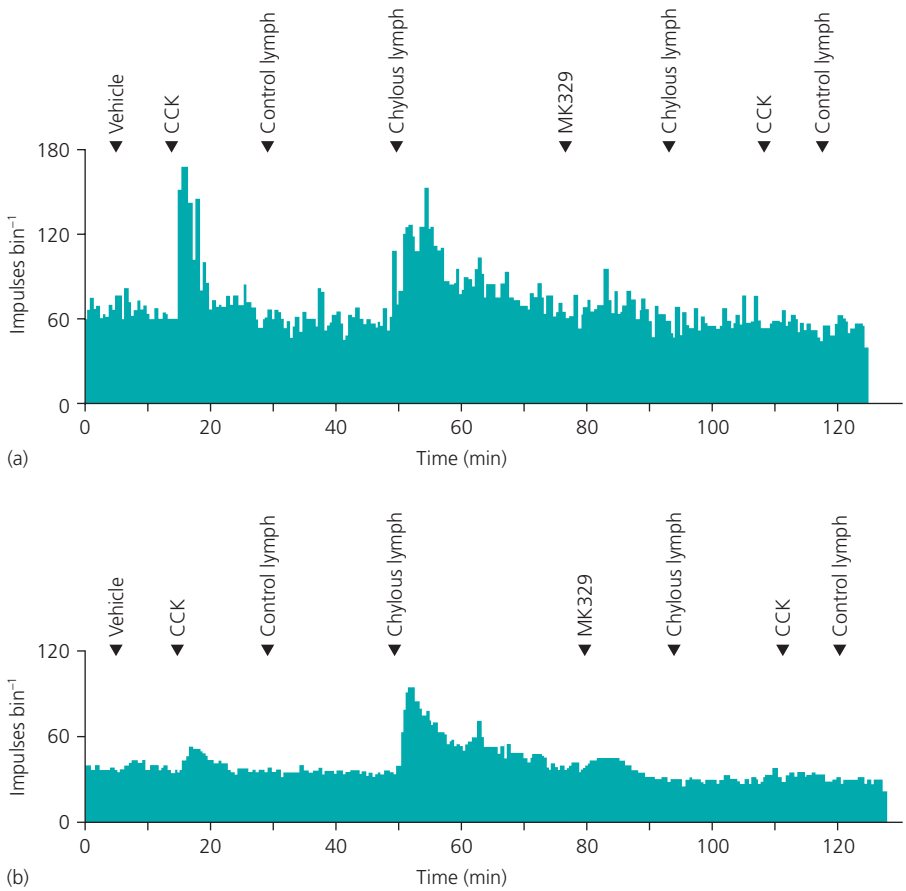

Figure 10.7 The effects of cholecystokinin (CCK), lymph (control), and chylous lymph on discharge from multiunit **(a)** and single-unit **(b)** duodenal vagal afferent fibers. CCK and chylous lymph, but not the control lymph, increased afferent vagal activity. The ability of the CCK antagonist MK329 to block the response to chylous lymph suggests that chylomicrons in chylous lymph activate vagal afferents by way of CCK-dependent pathways. From Glatzle et al. [166].

inhibitory effects of duodenal distention [169]. The enterogastric inhibition of gastric emptying by duodenal maltose is blocked by a selective CCK antagonist, indicating mediation by CCK release [170]. The inhibition of gastric motility by perfusion of the duodenum with the products of protein digestion is mediated by activation of CCK-sensitive duodenal afferent nerves by way of a mechanism involving the oligopeptide transporter PepT1 [171].

Regions other than the small intestine regulate gastric emptying. Nonpainful rectal distention slows the emptying of solids [172]. In rats, proximal colonic distention induces Fos expression in the brain and inhibits gastric emptying by way of capsaicin-sensitive afferent nerves [173]. Soups with high levels of fat delay gastric emptying more when ingested orally than when given by intragastric perfusion, indicating modulation by orosensory stimulation [174].

Neurohumoral control

The extrinsic and intrinsic nerve supplies are essential for normal gastric emptying. Thoracic spinal cord transection acutely delays nutrient liquid emptying [175]. Truncal or proximal vagotomy produces rapid liquid evacuation, which is enhanced by the addition of pyloroplasty [176]. Truncal vagotomy accelerates the earliest phase of solid emptying but retards overall emptying of digestible and indigestible solids and can promote bezoar formation in some patients. Conversely, proximal vagotomy has little effect on solid emptying [176]. Denervation of the myenteric plexus with benzalkonium chloride leads to delayed liquid gastric emptying [177]. Antral transection and reanastomosis reduces the volume of transpyloric flow pulses and retards gastric emptying. However, this operation does not affect the inhibition of antral contractions, the stimulation of pyloric motility, or the inhibition of gastric emptying elicited by duodenal glucose, indicating that intrinsic gastric pathways are not involved in this enterogastric reflex. Conversely, participation of intrinsic duodenal innervation is shown by the observation that duodenal transection impairs nutrient-evoked delays in gastric emptying [161].

Gastric emptying is modified by many neurotransmitters and hormones. In humans, NO donors delay gastric emptying, whereas NO synthase inhibitors have accelerating properties [178]. Conversely, in pigs and rodents, NO synthase inhibitors delay gastric emptying by modulating motor function in the proximal stomach and by increasing pyloric and duodenal contractions (Fig. 10.8) [179]. The phosphodiesterase-5 inhibitor sildenafil alters the intragastric distribution of food and delays gastric emptying by augmenting the

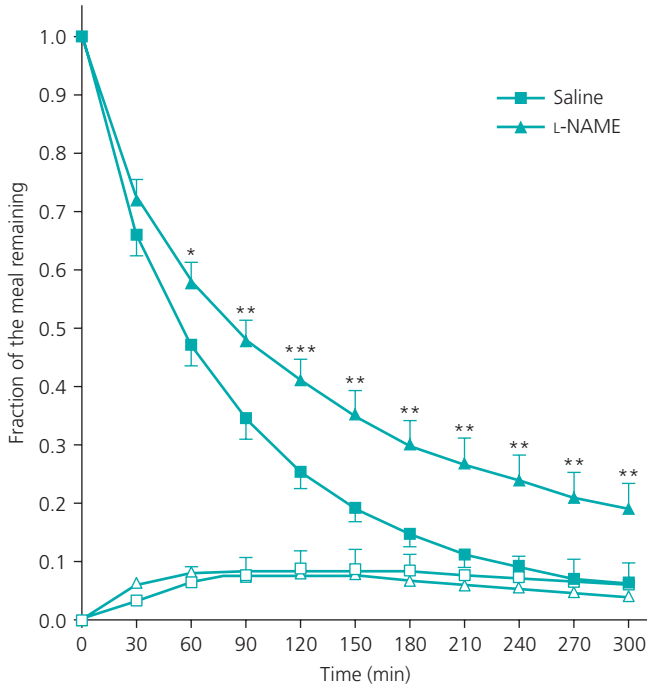

Figure 10.8 The effects of the nitric oxide synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) on retention of a semisolid meal in the proximal (filled symbols) and distal (open symbols) stomach. L-NAME (triangles) delayed gastric emptying compared with the control (squares) by prolonging retention in the proximal stomach. From Lefebvre et al. [179].

actions of NO [180,181]. Opiates of different receptor subclasses exert inhibitory and excitatory effects on emptying through naloxone-sensitive and naloxone-insensitive pathways. Activation of the ileal brake by lipids is antagonized by naloxone, indicating involvement of opiate pathways [182]. Emptying delays induced by meperidine (pethidine) are reversed by the α-adrenergic receptor antagonist phentolamine, suggesting mediation by adrenergic pathways. 5-HT_3 antagonists and 5-HT_1 agonists (e.g., sumatriptan) both delay gastric emptying, indicating the opposing effects of the two serotonin receptor pathways [183]. Inhibition of gastric emptying by duodenal glucose perfusion is mediated by 5-HT_3 receptor activation on extrinsic afferent nerves [184]. The enhancement of emptying by the histamine H_2 antagonist nizatidine suggests involvement of endogenous histamine pathways in the regulation of gastric emptying [185]. Blockade of GRP receptors delays solid emptying, also indicating a physiological role for GRP [186]. Likewise, a role for endogenous secretin is indicated by the observation that secretin antibodies accelerate emptying [187]. Baclofen accelerates solid emptying but delays liquid emptying, reflecting differential actions of γ-aminobutyric acid B receptors on different gastric regions [188]. GLP-1 increases postprandial gastric volume and retards gastric emptying [189]. GLP-2 also inhibits gastric emptying but is less potent [190]. Antibodies to calcitonin gene-related peptide (CGRP) prevent the inhibition of emptying by intestinal hydrochloric acid or hypertonic saline, suggesting the participation of CGRP pathways in these enterogastric reflexes [191]. Other agents that delay gastric emptying include gastrin, somatostatin, neurotensin, pancreatic polypeptide, peptide YY, enteroglucagon, oxyntomodulin, prostaglandins E_1 and E_2, neuromedin B, amylin, adrenomedullin, epidermal growth factor, transforming growth factor-α, interleukin-1β, and cannabinoid agonists.

CCK has been proposed to be an important physiological regulator of gastric emptying. Exogenous CCK inhibits liquid emptying and stimulates antral motor activity, and higher doses increase pyloric contractions. CCK infusions that mimic the postprandial state produce fundic relaxation and increase proximal gastric compliance [192]. In humans, administration of an oral CCK_1 agonist delays gastric emptying of solids [193]. Selective CCK antagonists accelerate the emptying of mixed meals, glucose, lipids, and radiopaque markers, and enhance antral contractility in most but not all studies [194,195]. The ability of the CCK_1 antagonist devazepide to blunt retardation of emptying evoked by a peptone meal decreases as the caloric density increases, indicating recruitment of CCK-independent factors with greater nutrient loads [196]. Gastric emptying of liquids is accelerated in mice lacking CCK_2 receptors [197]. Oleate-induced inhibition of gastric emptying is attenuated in rats fed a high-fat diet [198]. In the same rats, exogenous CCK less potently inhibits gastric emptying, indicating the plasticity of the response to CCK.

The actions of CCK on gastric emptying involve the participation of several regions and neural pathways. Antrectomy in combination with pyloroplasty inhibits the effects of CCK on gastric emptying. Fundic resection prevents CCK-induced inhibition of emptying in some studies but not in others. The application of benzalkonium chloride to the antral serosa does not prevent inhibition of gastric emptying induced by CCK, suggesting that intrinsic antral neurons are not involved [199]. In rats, intraarterial or intraperitoneal CCK increases firing in gastric vagal mechanoreceptor afferents [200]. Perivagal capsaicin abolishes the delay in emptying evoked by CCK, indicating mediation by vagal afferents [42]. The ability of devazepide to block the increase in vagal afferent firing evoked by CCK indicates mediation by CCK_1 receptors [201]. Devazepide attenuates the inhibition of emptying evoked by duodenal lipids, but vagal capsaicin offers no additive effect, indicating that CCK acts solely on vagal afferent CCK_1 receptors [202]. Similarly, the effects of apolipoprotein A-IV on gastric emptying are mediated by CCK release, which activates CCK_1 receptors on duodenal vagal afferents [167]. CCK antagonists that do not cross the blood–brain barrier, such as A-70104, also block nutrient-induced inhibition of gastric emptying, indicating the involvement of peripheral

CCK receptors [203]. In rats, serotonin 5-$HT_{2A/2C}$ receptors are postulated to mediate the inhibitory effects on gastric emptying of endogenous CCK released by intestinal lipid perfusion [204]. An additional modulatory role for splanchnic pathways has been proposed. In cats, CCK-evoked catecholamine release from splanchnic efferent nerves produces excitatory and inhibitory effects on gastric muscle by acting on α_2- and β-adrenergic, receptors, respectively [205].

Central nervous system modulation

The CNS has potent modulatory effects on gastric motor function. Acoustic stress modifies both fasting and fed motor activity [206]. Physical restraint stress in rats disrupts MMC activity, as does mental stress in humans. Cold stress induces isolated pyloric contractions, inhibits antral contractions, stimulates duodenal phase III, alters fed motor activity, and prolongs liquid emptying (Fig. 10.9) [207]. Experimentally induced vertigo, noise stress, restraint stress, and ischemic pain retard gastric emptying. In humans, anger enhances gastric motor activity, in contrast to fear and depression, which evoke decreased levels. Many of these stress responses are associated with norepinephrine (noradrenaline) and endorphin release and they are blocked by vagotomy or by adrenergic or opioid antagonism [208].

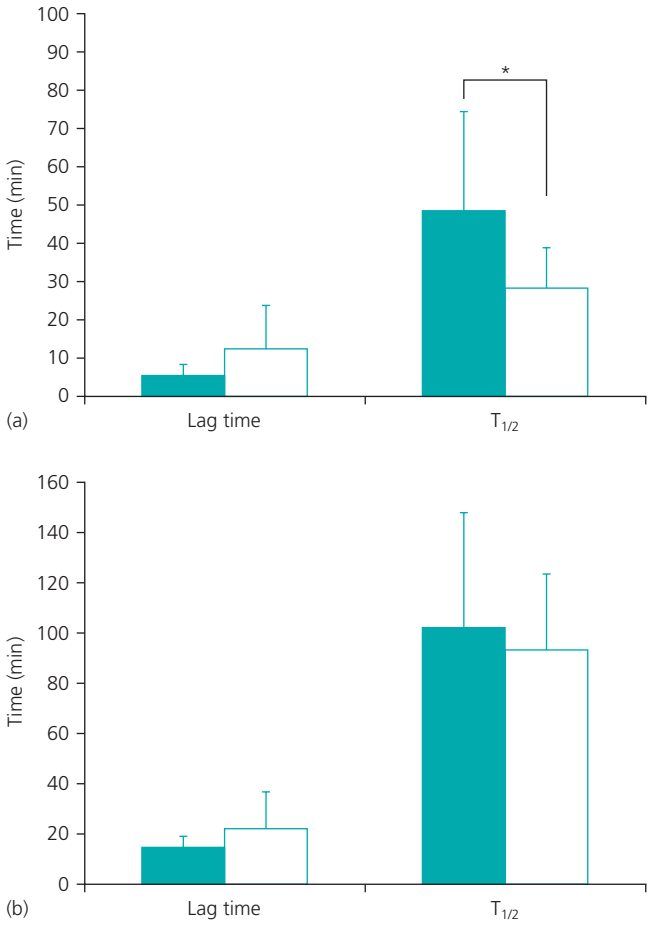

Figure 10.9 The lag times and half-emptying times ($T_{1/2}$) for liquid **(a)** and solid **(b)** meals. Induction of cold pain (solid bars) prolonged the half-time of liquid emptying but had no effect on solid-phase emptying parameters. From Nakae et al. [207].

Corticotropin-releasing factor (CRF) has been proposed to be a major mediator of stress effects on gastric motor function. The peripheral effects of many stressors are inhibited by CRF antagonists, confirming a physiological role for CRF [209]. Studies of the neural pathways and receptor subtypes mediating the actions of CRF have yielded conflicting results. CRF infusion into the brain or spinal cord delays gastric emptying by way of a vagal cholinergic pathway [209]. Similarly, in a study testing its effects on DMNV neurons, CRF excited efferent vagal activity by activating CRF_2 receptors (Fig. 10.10) [210]. The ability of the CRF_2 ligand urocortin to disrupt gastric motility when given intracisternally and the inability of CRF_1 antagonists to block the effects of CRF or urocortin also support a role for CRF_2 receptors in mediating the responses to CRF [211]. However, another group reported that CRF delays gastric emptying by acting on CRF_2 receptors – a response that is blocked by guanethidine, propranolol, and celiac ganglionectomy, indicating the involvement of sympathetic pathways [212]. Similarly, different researchers observed that intracisternal injection of urocortin-2 inhibits gastric emptying by acting through CRF_2 pathways involving sympathetic innervation of the stomach [213]. However, the inhibitory effects of urocortin-1 and CRF on gastric emptying were reported to be mediated by vagal pathways. CRF released by the CNS may interact with other transmitters. TRH-stimulated gastric motility is inhibited by intracisternal urocortin acting on CRF_2 receptors [214]. Central administration of GLP-1 delays gastric emptying by way of central CRF and peripheral sympathetic pathways [215]. Centrally administered CRF antagonists reverse intracisternal interleukin-1β-evoked delays in gastric emptying, indicating an interaction of inflammatory mediators with central CRF pathways [216]. Infusion of a somatostatin analogue into the fourth ventricle blocks the inhibitory effects of CRF on gastric emptying [217]. Inhibition of gastric emptying by nociceptin/orphanin FQ is mediated by CRF pathways [218].

The peripheral actions of CRF differ from its central effects. CRF_1 and CRF_2 receptors are present in rat gastroduodenal nerve fibers and myenteric neurons [219,220]. Specific urocortin-2 receptors are also expressed in antral smooth muscle. Activation of peripheral CRF_2 receptors inhibits phasic antral contractions. In humans, intravenous administration of CRF elicits phasic pyloric and duodenal pressure waves [221]. In mice, peripheral administration of a CRF_1 antagonist prevents gastric ileus induced by surgical visceral manipulation [222]. Likewise, CRF_1 receptor-deficient mice do not exhibit impaired gastric emptying in this model of postoperative ileus.

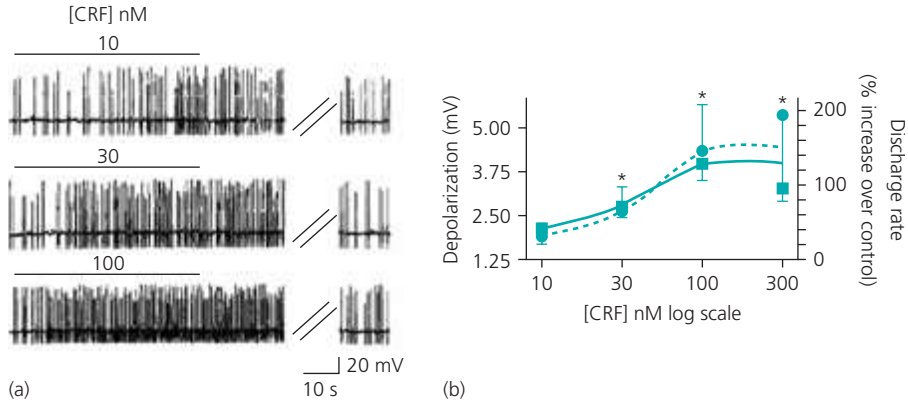

Figure 10.10 **(a)** Representative discharge traces from a gastric-projecting dorsal motor nucleus of the vagus neuron. Corticotropin-releasing factor (CRF) produced dose-dependent increases in neuronal firing. **(b)** Neuronal discharge rate (solid line) and depolarization (dashed line) plotted as a function of CRF dose. Both parameters increased in a dose-dependent fashion. From Lewis et al. [210].

Other CNS neurohumoral factors participate in the regulation of gastric motor function. NO synthase is present in the dorsal vagal complex of the brainstem. Inhibition of central NO synthase suppresses antroduodenal phase III of the MMC and increases pyloric motor activity but has no effect on jejunal cycling or on solid emptying, indicating differential central regulation of proximal and distal motor function and of fasting and fed contractile patterns [223]. Central TRH administration increases gastric motor activity by acting on vagal cholinergic pathways [224]. TRH perfusion into the DMNV or the cisterna magna increases gastric vagal efferent discharge and induces atropine-sensitive stimulation of gastric motor function [225]. TRH antibodies block the gastric contractile response to central glutamate injection, and TRH_1 antisense oligonucleotides prevent the delay in gastric emptying evoked by cold stress, suggesting a stimulatory physiological role for central TRH [226]. Oxytocin infusion into the DMNV reduces gastric motor activity, whereas central administration of an oxytocin antagonist increases fasting gastric activity [227]. Oxytocin antagonists also prevent the inhibition of gastric motor activity evoked by electrical stimulation of the paraventricular nucleus of the hypothalamus. The central action of adrenomedullin inhibits gastric emptying by acting on CRF-independent, β-adrenergic pathways [228]. Central administration of cocaine- and amphetamine-regulated transcript-derived peptides (CARTp) in the dorsal vagal complex inhibits gastric emptying [229]. The central inhibitory effects of μ-opioid receptor agonists on antral motility are mediated by sympathetic pathways [230]. Centrally administered CCK, bombesin, tachykinins, atrial natriuretic factor, γ-aminobutyric acid, calcitonin, CGRP, substance P, and peptide YY inhibit gastric emptying or motility, whereas pituitary adenylate cyclase-activating polypeptide (PACAP)-38, pancreatic polypeptide, serotonin, glutamate, and prolactin-releasing peptide increase gastric motor activity when they are administered into selected brainstem nuclei.

The brain–stomach axis plays an important role in regulating food intake. The effects of centrally acting agents on gastric motor function may contribute to appetite control. CCK acts on gut CCK_1 receptors to suppress food intake by activating vagal pathways that project to the NTS, lateral parabrachial nucleus, amygdala, and higher sites [231]. Rats lacking CCK_1 receptors develop obesity, reflecting the importance of CCK as a satiety hormone. The role of the gastric motor effects of CCK in mediating satiety is unresolved. One group has reported that blockade of $5\text{-}HT_3$ receptors attenuates CCK-induced inhibition of food intake by reversing CCK-induced delays in gastric emptying [232]. However, another group observed that central administration of a CCK antagonist increases food intake without affecting the gastric emptying rate [233]. Leptin is a hormone secreted by adipose tissue and gastric mucosa. Intracisternal injection of leptin inhibits gastric emptying by acting on leptin receptors located on cholinergic neurons in the DMNV [234]. Leptin-deficient obese mice exhibit accelerated gastric emptying and increased jejunal responses to CCK [235]. Leptin applied to the stomach activates vagal afferent neurons that also respond to CCK [236]. When given together, leptin and CCK have an additive effect on vagal discharges. Similarly, leptin stimulation of intestinal electromyographic activity occurs only in the presence of CCK, supporting the hypothesis that leptin may modulate the effects of CCK to regulate food intake [237]. Conversely, ghrelin, a peptide secreted by the stomach, decreases vagal afferent activity and increases gastric motor activity in rats [238]. Ghrelin levels are suppressed by leptin, suggesting that it may act downstream from leptin to control appetite. Ghrelin potently stimulates gastric emptying and small intestinal transit of liquid meals by acting on motilin-independent, cholinergic pathways (Fig. 10.11) [239,240]. Ghrelin colocalizes with choline acetyltransferase in the gastric myenteric plexus [74]. In rats, intravenous and intracisternal administration of ghrelin induce fasting motor activity in the stomach and duodenum [241]. In humans, ghrelin elicits premature antral phase III activity that is not mediated by motilin release [242]. The effects of intravenous injection of ghrelin on gastric motility are inhibited by intracisternal injection of a CRF_2 antagonist [243]. Ghrelin

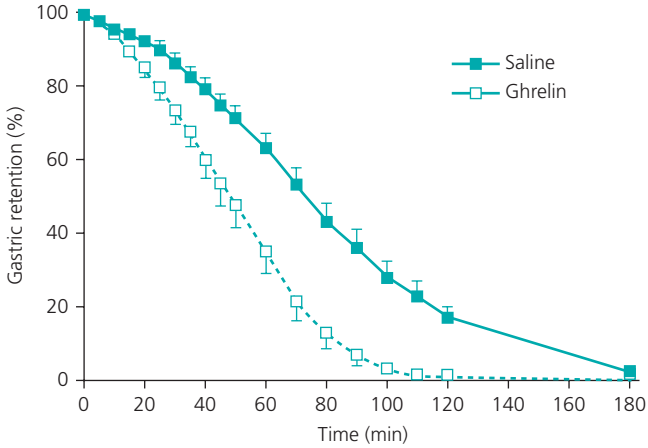

Figure 10.11 Gastric retention is plotted as a function of time after consuming a nutrient meal during intravenous administration of saline or ghrelin. Ghrelin evoked an acceleration of gastric emptying, as reflected by decreased retention throughout the emptying curve. From Levin et al. [240], with permission.

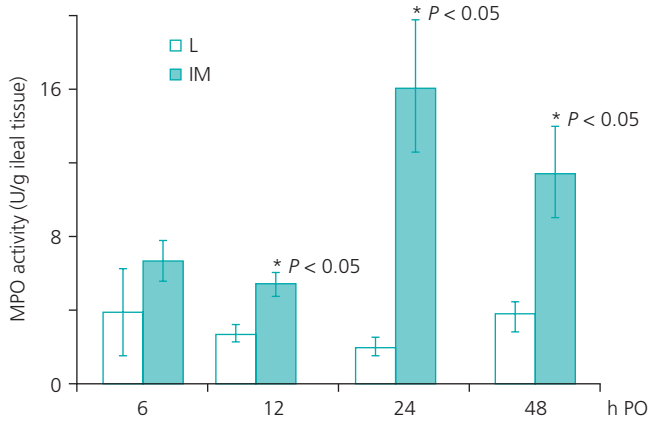

Figure 10.12 Ileal myeloperoxidase activity (MPO), reflecting leukocyte recruitment, is plotted as a function of time after laparotomy (L) and after laparotomy with intestinal manipulation (IM). Increases in MPO activity were seen for at least 48 h postoperatively (PO) after intestinal manipulation, but not after laparotomy alone, in the intestine but not the stomach, reflecting leukocyte infiltration. From De Jonge et al. [257].

may also act through Y_1 receptors for neuropeptide Y in the arcuate hypothalamic nucleus to increase food intake and decrease energy expenditure [238,244]. The neuropeptides orexin-A (OXA) and -B (OXB) also stimulate food intake. OXA-like immunoreactivity is present in duodenal neurons, circular muscle, and mucosal enterochromaffin cells [245]. Both peripheral and intracisternal administration of OXA and OXB induce proximal gastric relaxation and prolong MMC cycle lengths in rats by acting on vagal pathways [245,246]. Peripheral OXA administration slightly delays gastric emptying [247]. Conversely, microinjections of OXA and OXB into the DMNV rostral to the obex increase intragastric pressure and antral contractions [248].

Immune system disruption

Activation of the gut immune system disrupts normal gastric motor function. Immune abnormalities are postulated to underlie several clinical disorders. Gastric dysmotility often occurs with bacterial infection. Lipopolysaccharide treatment of rats elicits initial strong repetitive antral contractions mediated by 5-HT_4 pathways, followed by motor suppression associated with increased inducible NO synthase levels [249]. Rats treated with endotoxin exhibit increases in NO synthesis in fundic soma, axons, and nerve endings of the fundus [250]. Inhibition of inducible NO synthase reverses the inhibition of gastric emptying by lipopolysaccharide [251]. Endotoxin treatment also increases prostaglandin synthesis in the rat antrum [252]. Polyethylene glycol–superoxide dismutase reverses delays in gastric emptying evoked by endotoxin, indicating a role for oxidative stress [253]. Intracisternal perfusion of a tumor necrosis factor-α (TNF-α) construct with a TNF receptor linked to the Fc portion of immunoglobulin G1 to neutralize endogenous TNF-α prevents the inhibition of gastric motility induced by lipopolysaccharide, indicating participation of central TNF-α in the inflammatory response [254,255]. Similarly, the effects of lipopolysaccharide are prevented by intracisternal perfusion of indomethacin (indometacin), suggesting a role for central prostaglandin pathways [255]. Intraperitoneal lipopolysaccharide also reduces fasting levels of ghrelin, an effect blocked by indomethacin and an interleukin-1 antagonist [256]. Exogenous ghrelin significantly improves the impairments in gastric emptying and food intake elicited by lipopolysaccharide. In a model of postoperative gastric ileus, intestinal manipulation delays gastric emptying in mice for as long as 48 h (Fig. 10.12) [257]. This delay is associated with leukocyte infiltrates in the manipulated small intestine but not the stomach, and it is blocked by inhibitors of leukocyte recruitment. Similarly, the delay in gastric emptying elicited by intestinal manipulation is partly reversed by antibodies or antisense nucleotides to intercellular adhesion molecule-1, indicating a mediating role for this substance [258].

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B MOTILITY

11 Motility of the small intestine and colon

William L. Hasler

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Contractile patterns in the small intestine and colon serve the functions of each organ. During fasting, undigested solids and sloughed enterocytes are cleared from the small intestine by mixing and propulsion. After eating, triturated chyme from the stomach is mixed with bile and pancreatic juice for digestion and is propelled aborally over variable distances. Thus, motor function of the small intestine regulates nutrient absorption rates. The small intestine terminates at the ileocolonic junction (ICJ), a sphincteric structure that prevents reflux of cecal contents into the ileum. In the colon, prominent mixing patterns with less uniform aboral propagating waves provide time for the slow process of fecal desiccation. The internal and external anal sphincters in concert with pelvic floor muscles permit volitional control of solid waste evacuation. High-pressure zones have been reported at the duodenojejunal junction, the cecocolonic junction, the colosigmoid junction, and the rectosigmoid junction, suggesting that several regions of tubular bowel serve additional sphincter-like functions [1–3].

Anatomic and functional considerations

The small intestinal wall has two regions of smooth muscle, the muscularis mucosae and the muscularis externa. The muscularis externa, which comprises an outer longitudinal layer and an inner circular layer, is the major effector of mixing and propulsion. The role of the muscularis mucosa is poorly defined. Most mixing and propulsion is mediated by circular muscle, as lumenal occlusion and displacement of gut contents require contraction of this layer. Segmentation is produced by reciprocal inhibition and disinhibition of adjacent circular muscle. Longitudinal contraction shortens the gut and increases lumenal diameter to facilitate bolus transit.

The colonic anatomy is strikingly different from that of the small intestine. The cecum is a sac-like structure that serves a storage function. The ascending, transverse, and descending regions possess a circumferential circular muscle layer with three overlying longitudinal muscle strips, the taeniae coli, 120 degrees apart. Haustra in these regions are produced by circular and longitudinal muscle contractions that narrow the lumen and shorten the colon. The longitudinal muscle spreads to envelop the rectosigmoid colon. Transverse folds in the rectum extending beyond the midline and containing mucosa and smooth muscle provide a shelving function to retard fecal passage and promote storage in the rectosigmoid region.

The anus possesses both smooth and striated muscle. The internal anal sphincter (IAS) is a thickened extension of the rectal circular smooth muscle. Rectal longitudinal fibers fan out at the anal verge, pass through the sphincter muscles, and terminate in subcutaneous perianal tissue. The external anal sphincter (EAS) comprises three striated muscles: the deep external sphincter, the superficial external sphincter, and the subcutaneous external sphincter. The levator ani muscles (puborectalis, pubococcygeus, iliococcygeus) form a sling to surround the rectum and maintain continence when tonically contracted.

Small intestinal and colonic transit

Small intestinal transit ranges from 78 to 264 min in healthy humans and is slower in women, especially those who are postmenopausal or obese [4,5]. Transit time is unaffected by age, although one study observed accelerated transit in older persons [6]. Most flow events, measured by impedance, propagate aborally for only short distances (≤ 10 cm) [7]. In rats, transit is more rapid in the proximal (30 min) than in

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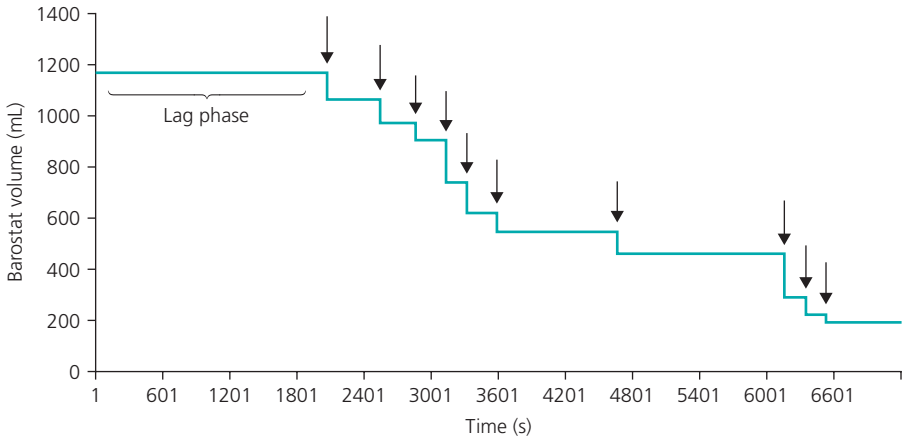

Figure 11.1 A rectal gas evacuation profile from a healthy volunteer during jejunal perfusion of a physiological gas mixture. Before gas perfusion, the barostat cylinder used to collect the expelled gas was emptied (starting volume = 1170 mL). After initiation of gas perfusion, there was a lag of about 1900 s when no gas was evacuated at the level of the rectum. Thereafter, gas evacuation into the barostat cylinder was pulsatile, with passage of discrete boluses (arrows) totaling about 900 mL over 2 h. From Gonlachanvit et al. [13].

the distal intestine (2.5 h) [8]. In contrast to the stomach, in which solids and liquids are handled differently, iodine 131-labeled solids and technetium 99m-DTPA-labeled water are propelled at similar speeds in the small intestine [9]. The ileum selectively retains indigestible solids while permitting the passage of liquids into the colon. Small intestinal nutrient transit depends on caloric density and nutrient class. Transit of protein and lipid solutions is slowed in proportion to the number of calories, which promotes more efficient digestion and absorption.

Lumenal contents in the colon advance slowly from the cecum to the rectum, over 1–2 days in healthy individuals. Transit time is slightly faster in men with consequent increases in fecal weight [5]. Middle-aged women exhibit slower transit than younger women. Transit is slower during the follicular phase of the menstrual cycle than in the earlier luteal phase [10]. Colonic transit is modulated by alterations in lumenal microflora; for example, the administration of *Bifidobacterium animalis* shortens the colonic transit time in women [11]. Ascending colon transit may be retrograde at times; however, higher fecal volumes elicit lumenally occlusive, aborally propagating contractions. Solid residue is initially retained in the ascending colon, while liquids are propelled aborally. In contrast, solids and liquids are propelled similarly in the transverse colon. Efficiency of fecal evacuation with defecation varies by colonic region, with the right colon exhibiting 20% emptying, the left colon showing 32% emptying, and the rectum evacuating 66% of content [12].

Lumenal gas is handled in a distinct manner by the small intestine and colon. In healthy humans, jejunal perfusion of physiological gas mixtures produces steady state flow with little distention and few symptoms. Gas collected from the rectum in such studies is expelled in a pulsatile fashion, indicating that flow is regulated by intrinsic motor patterns in the distal gut (Fig. 11.1) [13]. Gas transit is accelerated by caloric liquid or solid meals, whereas noncaloric liquids have no stimulatory effects, findings that correlate with the observation of increased postprandial gas passage [14]. Conversely, intestinal lipid perfusion retards gas propulsion, demonstrating nutrient-induced reflex modulation of gas transit [15]. Consuming a high-fiber diet retards evacuation of jejunally perfused gas mixtures, reflecting regulation by lumenal contents [13]. Assuming an upright posture expedites gas flow [16]. Likewise, physical activity accelerates gas transit and reduces gas retention in healthy individuals [17].

Specialized small intestinal and colonic cell types

Motor function in the small intestine and colon is controlled by smooth muscle cells, nervous tissues, interstitial cells of Cajal (ICCs), and supporting tissues.

Smooth muscle

Small intestinal and colonic myocytes are uninucleate spindle-shaped cells that are electrically active with resting membrane potentials of −40 to −80 mV maintained by Na^+,K^+-ATPase activity. In the small intestine, ubiquitous membrane potential fluctuations of 3–15 mV oscillating at 11–12 cycles per minute (cpm) are demonstrable in the duodenum and at lower frequencies more distally. Extracellular recordings of these slow waves show sinusoidal morphology or rapid biphasic deflections from zero potential. Intracellular recordings demonstrate rapid depolarizations followed by partial repolarizations to prolonged plateau phases of depolarization and then to full repolarization. Frequencies and propagation directions of phasic contractions are controlled by the slow wave, although tonic contractions lasting from 10 s to 8 min have been recorded from circular muscle. By themselves, slow waves produce insufficient depolarization to initiate contraction. Intestinal stimulants induce motor activity by increasing the duration and amplitude of the slow wave plateau potential or by inducing spike potentials of brief duration (10–100 ms) but high amplitude (50 mV) in

phase with the slow wave. Spike potentials generated in isolated longitudinal and circular muscle propagate only a few millimeters. Intestinal relaxation results from stimuli that inhibit spike potentials, reduce plateau potential amplitude or duration, or induce hyperpolarization.

Slow wave activity in the colon cycles in two frequency ranges, from 2 to 6 cpm and from 9 to 13 cpm, and correlates with short-duration contractions. Additional rapid electrical oscillations at 25–40 cpm, termed myenteric potential oscillations, regulate long-duration colon contractions [18]. Excised human colonic circular muscle strips exhibit spontaneous slow waves with an amplitude of 12 mV, a duration of 9.4 s, and a frequency of 2–4 cpm [19]. However, near the myenteric edge of these strips, rapid membrane potential fluctuations at 18 cpm are observed. In colonic circular muscle, slow wave activity generated along the submucosal border is summed with myenteric potential oscillations generated along the myenteric border [20]. Contraction occurs as a consequence of increased amplitude of the myenteric potential oscillation, enhanced duration and amplitude of the slow wave plateau potential, or the generation of spike potentials [21].

Nervous tissues

Small intestinal and colonic smooth muscle is innervated by intrinsic nerves within the gut wall and by extrinsic nerves connecting to the extraintestinal ganglia, the spinal cord, and the central nervous system (CNS). The myenteric plexus is the major intrinsic neural regulator of motor function in the small intestine and colon, although the submucous plexus may participate in some motor reflexes. The vagus and splanchnic nerves supply the small intestine, ileocecal junction, and proximal colon, whereas the pelvic nerves provide input to the colon and IAS. The EAS and pelvic floor muscles receive sacral spinal input by way of the pudendal nerves. There is bilateral cerebral motor cortex representation in the innervation of the anus and rectum (Fig. 11.2) [22]. Intrinsic neurons greatly exceed vagal or splanchnic fibers in number; thus, most motor activities are directed by intrinsic neural programs, with extrinsic innervation providing a modulatory function.

Intrinsic innervation

The density of myenteric neurons approximates that of the spinal cord. An additional deep muscular plexus innervates the interface of the inner and outer circular muscle layers and receives input from the myenteric plexus. Most submucous plexus neurons project to the mucosa where they regulate secretion and absorption. However, some submucous plexus neurons innervate both the circular and longitudinal muscle layers [23]. Similarly, in the colons of some species, the outer submucous plexus provides input to the circular muscle. The enteric nervous system possesses afferent neurons, interneurons, and motor neurons, and thus can initiate physiological motor activity in the absence of extrinsic input. As in the CNS, myenteric ganglia consist only of neurons and glial cells. Blood vessels and connective tissue are absent, thus neuronal nourishment is received by diffusion in the interstitial fluid. Axonal varicosities release neurotransmitters that diffuse 20–100 nm to specific receptors on muscle cells or neurons. In the small intestine, most motor neurons project 1–2 mm longitudinally although some fibers extend more than 30 mm. Excitatory fibers run cephalad, whereas inhibitory fibers project in a caudad direction. Extensive interneuronal connections mediate extended reflexes, which in some cases project 100 cm or more.

Most myenteric neurons (80%–90%) contain tachykinins (40%–45%) or vasoactive intestinal polypeptide (VIP) (40%–45%), with no overlap between the two groups. Substance P, neurokinin A, and acetylcholine released by tachykinin neurons mediate most excitatory functions of intestinal smooth muscle. Both neurokinin 1 (NK_1) and M_2 muscarinic receptors are prominent on smooth muscle cells [24,25]. Neurokinin 1 receptors are also expressed on myenteric neurons [26]. Atropine-resistant contractions evoked by neural stimulation of the small intestine in porcine ileum are abolished by NK_1 antagonists [27]. Nitric oxide (NO)- and VIP-containing myenteric neurons provide the inhibitory supply to the small intestine and colon. Inhibitors of NO synthesis block ileal relaxations evoked by electrical depolarization, serotonin, adenosine triphosphate (ATP), and γ-aminobutyric acid (GABA), and elicit duodenal contractions, reflecting a basal NO-mediated tone [28]. Conversely, enhanced nitrergic action reduces contractile amplitudes in canine small intestine [29]. Mice with a knockout of the caveolin-1 gene exhibit impaired NO function, indicating participation of membrane caveolae in nitrergic neurotransmission [30]. Carbon monoxide activates L-type calcium channels by activating a pathway that involves NO synthase [31]. Antagonists or antisera to VIP prevent many neurally mediated relaxations [32]. NO synthase colocalizes with VIP in selected neurons [33]. In some models, VIP evokes NO-dependent relaxations by acting through an intermediate transmitter such as GABA [34]. Some VIP neurons contain neuropeptide Y (NPY), calcitonin gene-related peptide (CGRP), gastrin-releasing peptide (GRP), and galanin. A related transmitter, pituitary adenylate cyclase-activating peptide (PACAP), acts as a physiological relaxant in some regions through action on $PACAP_1$/VIP receptors [35]. PACAP immunoreactivity is prominent in myenteric neuronal cell bodies and in nerve fibers in the muscle layers and mucosa [36]. In rat colon, most ATP-containing myenteric neurons synthesize NO [37]. NO pathways also interact with some contractile pathways. In guinea pig small intestine, NK_1 receptor activation inhibits distention-evoked contractions by releasing NO [38]. Inhibitors of NO synthase and guanylate cyclase increase acetylcholine release and cholinergically mediated contractions in mouse ileum, effects not observed in mutant mice lacking NO synthase [39].

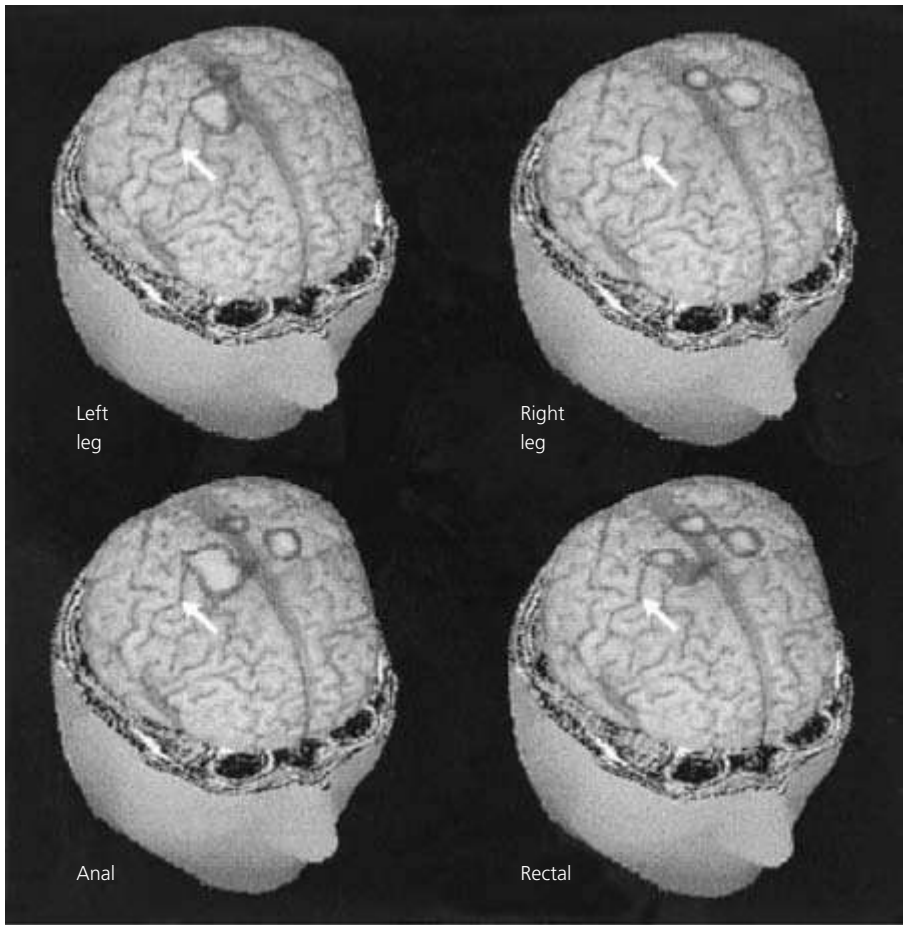

Figure 11.2 Magnetic resonance images from a healthy volunteer after stimulation of the left and right legs, the anus, and the rectum. Responses from all four muscle groups localize to the superomedial motor cortex; however, each stimulus produces responses of differing morphology and extent. In this individual, cortical representations of anal and rectal stimulation are bilateral, whereas leg responses localize to the contralateral hemisphere. From Turnbull et al. [22].

Other intrinsic neurons modulate gut motor activity. Serotonin 5-HT_4 receptors are demonstrable on enteric neurons [40]. 5-HT_4 agonists activate presynaptic receptors to stimulate motor function. Inhibition of serotonin reuptake with citalopram increases phasic colonic contractions in healthy humans [41]. However, in mice, the serotonin reuptake inhibitor paroxetine slows gastrointestinal transit and decreases stool output [42]. Opioid neurons regulate both excitatory and inhibitory transmission. Enkephalinergic neurons project orally to both muscle layers, whereas neurons containing GRP and NPY project aborally [43]. Orphanin FQ immunoreactivity is expressed in excitatory neurons projecting to both muscle layers and to some interneurons [44]. Receptor proteins for dopamine receptor subtypes 1, 2, 3, and 5 are expressed in mouse colon [45]. Colonic smooth muscle from mutant mice lacking the dopamine transporter exhibits impaired contractions to electrical stimulation, whereas mice lacking the D_2 receptor show accelerated colon transit, indicating a physiological role for endogenous dopamine [45]. An inhibitory action of endogenous cannabinoid pathways on small intestinal and colonic motility through action on CB_1 cannabinoid receptors has been proposed [46,47]. Cyclooxygenase 1 (COX-1) and COX-2 isoforms are demonstrable in myenteric ganglia and modulate cholinergic excitation of colonic motor activity [48].

Extrinsic innervation

Efferent extrinsic fibers are carried in parasympathetic and sympathetic tracts. Most efferent fibers terminate in the myenteric plexus and form connections with enteric ganglia, although some sympathetic axons terminate directly on sphincteric smooth muscle. The vagus nerves contain preganglionic parasympathetic excitatory cholinergic nerves projecting to the enteric plexuses, preganglionic inhibitory cholinergic nerves projecting to the myenteric plexus, and sympathetic fibers from the cervical ganglia. Excitatory efferent vagal cholinergic neurons activate nicotinic receptors in enteric ganglia. The cell bodies of these efferent nerves reside in the brainstem dorsal motor nucleus of the vagus. Other fibers exhibiting high thresholds to electrical stimulation are inhibitory to motor activity through NO and VIP release. The efferent vagus supplies the upper gut, although anterograde tracing studies from the rat dorsal motor nucleus of the vagus show vagal innervation to much of the colon as well [49]. Pelvic nerve fibers arise from the sacral spinal cord and enter the colon at the rectosigmoid junction, where they travel in

shunt fascicles to innervate the myenteric plexus. Pelvic nerve stimulation evokes colorectal contractions, accelerates colonic transit, and elicits anal relaxation [50]. Stimulation of the hypogastric nerve increases anal pressure through action on the IAS, whereas pudendal nerve stimulation increases EAS activity [50]. Neuronal cell bodies providing the sympathetic splanchnic innervation reside outside the gut in the prevertebral ganglia (celiac and superior mesenteric ganglia). Preganglionic cholinergic neurons project from the spinal cord to the prevertebral ganglia. Noradrenergic postganglionic neurons then project to the enteric ganglia. This sympathetic innervation inhibits excitatory myenteric cholinergic transmission. Experiments that quantify c-Fos protein expression show that neuronal activity in the distal gut is under tonic inhibitory control by the sympathetic nervous system [51].

Afferent fibers greatly outnumber efferent fibers in the vagus and splanchnic nerves. Vagal afferent fibers terminate in the brainstem nucleus tractus solitarius. Neurons in the nucleus tractus solitarius project to the dorsal motor nucleus of the vagus and the nucleus ambiguus. Sensory information is transmitted to the dorsal horn of the spinal cord by way of the splanchnic nerves. From there, second-order neurons project to the brainstem and cerebral cortex. The anus is served by sensory fibers that project by way of the pudendal nerve. Free mucosal nerve endings respond to stroking or to chemical stimuli such as hydrochloric acid. Small intestinal thermoreceptor activation elicits reflex motor responses in the stomach. Mechanoreceptors are stimulated by passive distention or active contractions. Pelvic nerve afferent fibers respond to distention, heat, and bile salts, reflecting their polymodal nature. Excision of the mucosa, submucosa, and inner circular muscle does not abolish responses to stretch, localizing mechanoreceptors to the outer muscle layers or the myenteric plexus [52]. Mesenteric and serosal receptors respond to tension or to forceful contraction and may mediate visceral pain perception. Intrinsic afferent neurons that mediate local reflexes project within the enteric plexuses, whereas vagal and splanchnic afferents participate in extended reflexes. Mechanosensory afferent nerves project to the superior mesenteric ganglion and monitor intracolonic pressures in mice [53]. Perception of nonnoxious stimulation is mediated by afferent vagal, pelvic, or splanchnic pathways, whereas nociceptive input is carried mainly by the splanchnic nerves. Plasticity of sensory nerve activity is indicated by experiments that show reductions in ileal vagal afferent sensitivity with previous exposure of the distal ileum to short-chain fatty acids [54].

Interstitial cells of Cajal

The ICCs are the third major cell type that participate in the regulation of small intestinal and colonic motor function. ICCs are uninucleate with numerous mitochondria, reflecting high metabolic activity, and abundant surface membrane caveoli with prominent endoplasmic reticula, indicating active ion transport. Immunohistochemical studies measuring immunoreactivitiy to c-Kit, a receptor tyrosine kinase, identify at least six distinct populations of ICCs in the small intestine, including intramuscular ICCs, ICCs within the myenteric plexus (ICC-MY), and ICCs in the deep muscular plexus [55]. ICCs in the submucosal region are prominent in the colon. Specialized ICCs form dense networks at the submucosal and myenteric borders of the rectum and along muscle bundles in the IAS [56]. ICCs are also observed in the EAS, a somatic muscle structure [57].

ICCs are important mediators of neurotransmission. In the small intestine, ICCs located in the deep muscular plexus are closely associated with excitatory muscarinic tachykinin neurons and inhibitory nitrergic neurons [58]. In neonatal mice, excitatory and inhibitory neurotransmission is not demonstrable until ICCs in the smooth muscle layers are developed [59]. In mutant mice lacking intramuscular ICCs, cholinergic neural responses to electrical stimulation are reduced and peristalsis is inhibited [60]. ICCs in the deep muscular plexus exhibit NO synthase activity and substance P-like immunoreactive axonal varicosities [61]. Both cholinergic and nitrergic nerves form synapses with intramuscular ICCs [62]. ICCs express M_2 and M_3 muscarinic receptors, NK_1 and NK_3 neurokinin receptors, VIP_1 receptors, and stem cell factor (steel factor), indicating modulation by neuronal pathways [63].

A second function of ICCs is the generation of electrical pacemaker activity, which is measured as the slow wave in smooth muscle tissues. Selective injury of ICCs with methylene blue plus intense illumination disrupts slow wave cycling [64]. In cat small intestine, rhythmic oscillations are generated only by tissue containing ICC-MY [65]. Intrauterine initiation of electrical rhythmicity occurs only after maturation of ICCs [66]. The membrane-bound form of steel factor, the ligand for c-Kit, increases the expression of c-Kit-immunoreactive ICCs [67]. In animals with steel factor mutations, ICCs are absent in the myenteric plexus of the small intestine and slow wave cycling is not detected [68]. In neonatal mice, administration of neutralizing monoclonal antibodies to c-Kit abolishes small intestinal rhythmicity and reduces the number of ICCs, which then assume the appearance of smooth muscle cells [69]. Electrical recordings from small intestinal ICC-MY exhibit pacesetter potentials characterized by initial upstroke depolarizations followed by plateau phases [70]. In contrast to the small intestine, colonic slow waves are maximal at the submucosal border and become weaker in the myenteric region, reflecting the importance of submucosal ICCs in this organ. Intracellular recordings from submucosal ICCs in canine colon show cyclic oscillations [71]. In mouse colon, high-frequency, low-amplitude contractions cycle in phase with the slow wave and are abolished if the submucous plexus is removed [72,73]. However, exogenous NO elicits electrical oscillations

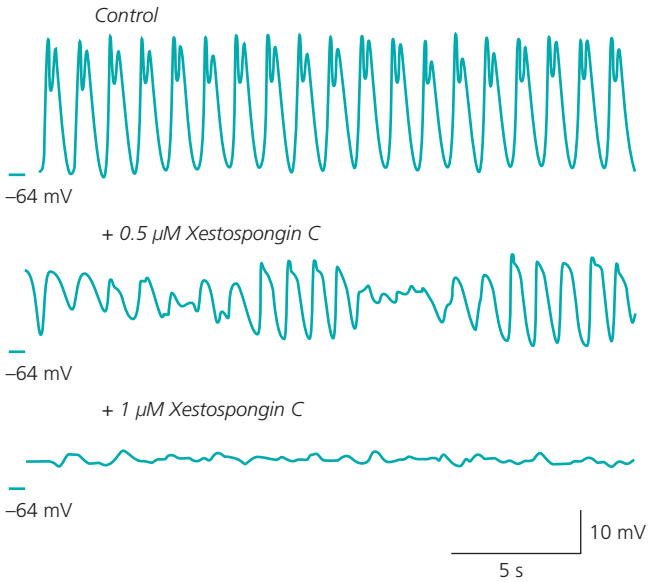

Figure 11.3 Small intestinal slow wave recordings under control conditions and with different concentrations of xestospongin C, a reversible, membrane-permeable inhibitor of inositol triphosphate-sensitive calcium release. Increasing concentrations of xestospongin C disrupt and then abolish intestinal slow wave activity. From Malysz et al. [77].

near the myenteric plexus in colonic circular muscle indicating that this region also has pacemaker capability in the colon [74]. Furthermore, rhythmic depolarizations generated by colonic longitudinal muscle tissue propagate over short distances (< 1 mm) in a manner dependent on L-type calcium channels [75].

Cyclic ion fluctuations are associated with pacemaker activity. ICC-generated pacesetter potentials are reduced by the removal of extracellular calcium and by depolarization with a high potassium solution, but L-type calcium channel antagonists have no effect [70,76]. Rather, electrical rhythm depends on calcium release from inositol triphosphate-sensitive intracellular stores and the rate of sarcoplasmic reticulum calcium refilling (Fig. 11.3) [77]. Caveolin-1 is present in the plasma membranes of all ICCs [78]. Disruption of the caveolae in small intestinal ICC-MY alters pacing frequency, showing the importance of these structures [79]. Dihydropyridine-insensitive calcium oscillations are observed that also depend on calcium influx through nonselective cation channels, which is mediated by the melastatin-type transient receptor potential-like channel 4 (TRPM4) in the caveolae of ICC-MY [80]. Treatment of cultured ICCs with TRPM7-specific small interfering RNA disrupts their pacemaking [81]. The plateau component of the pacesetter potential is maintained by calcium-activated chloride channels [82]. ICCs also express calcium-inhibited nonselective cation channels that activate at the frequency of the pacemaker currents, as well as a mechanosensitive sodium channel current that may modulate slow wave frequency [83]. ICC-MY potassium channels regulate membrane potentials and may control intestinal pacemaking activities [84]. Blockade of ether-a-go-go (ERG) potassium channels increases slow wave duration [85]. High-conductance chloride channels are rhythmically active at the slow wave frequency and likely participate in pacemaking as well [86,87]. The sodium/potassium/chloride cotransporter NKCC1 is differentially expressed in mutant and wild-type mice [88]. Inhibition of NKCC1 with bumetanide alters slow wave shape, amplitude, and frequency, suggesting an important role for this transporter.

Other cell types

Other cell types in the smooth muscle and myenteric plexus have less defined roles in modulating small intestinal and colonic motor function. However, calcium-activated potassium channels are present in the fibroblast-like cells that form gap junctions with small intestinal smooth muscle cells in mice, suggesting a role in the control of motility [89].

Coupling of small intestinal and colonic contractions

Propagation of small intestinal myoelectric and motor activity is regulated by intrinsic coupling in both smooth muscle layers. Slow wave propagation is reduced to distances of 3–6 mm after the excision of a 1-mm band of circular muscle and is abolished by removal of more than 5 mm of longitudinal muscle [90]. In circular muscle, gap junctions or nexuses provide low-resistance pathways for electrical conduction and for the passage of low-molecular-weight, second-messenger compounds. Tight coupling is also observed in the longitudinal layer, which possesses few or no nexuses. In this layer, other structures such as peg-and-socket junctions may mediate intercellular communication. Interconnecting bridges between the circular and longitudinal layers may provide low-resistance coupling across the myenteric region.

Small intestinal ICC-MY are mutually connected to as many as 35 other ICC-MY, most likely by gap junctions, permitting extended propagation of pacing currents [91]. Synchrony of rhythmic calcium transients in ICC-MY and longitudinal muscle indicates coupling of these two structures [92]. The gap junction uncoupler glycyrrhetinic acid disrupts ICC coupling [93]. L-type calcium channel antagonists prevent the propagation of calcium transient wavefronts in longitudinal muscle, but have no effect on propagation in ICC-MY [94]. Mitochondrial uncouplers and inhibitors of the inositol triphosphate receptor abolish cycling and propagation in ICC-MY. Similarly, slow wave propagation depends on inositol triphosphate receptor activation and

mitochondrial calcium uptake in colonic muscle [95]. Mixing movements in the longitudinal layer result from spontaneous calcium waves generated by pacemaker sites that spread in all directions and terminate by colliding with calcium waves from other pacing sites [96]. In mutant mice lacking ICCs, regular contractions are observed in the longitudinal layer but contractions are uncoordinated in the circular muscle, suggesting that ICC control of neurotransmission differs in the two layers [97]. ICCs in the deep muscular plexus and smooth muscle are extensively innervated and form close electrical contact with smooth muscle cells from both layers. In the circular layer, ICCs interface with smooth muscle cells through gap junctions and further interact with one to five ICCs in the deep muscular plexus [98]. Gap junction proteins such as connexin 43 and connexin 40 are prominent in this region [78].

Electrically active regions with the highest slow wave frequency in the small intestine entrain adjacent regions in a phenomenon termed phase lock. In humans, the dominant pacemaker extends from the pylorus to the ligament of Treitz. Circumferential slow wave propagation is more rapid than axial propagation. Conversely, spike potential migration is faster axially than circumferentially. Retrograde slow wave propagation into the stomach is rare because of an electrically insulating septum in the pylorus. In the small intestine, slow wave uncoupling over long distances occurs and is more pronounced in the ileum than in the duodenum [99]. As a consequence, slow wave frequencies decrease from 11–12 cpm in human duodenum to 7–8 cpm in the distal ileum. In most species, this decrease in frequency occurs in a stepwise fashion, with alternating frequency plateaus and regions of variable frequency. Frequency plateaus are more prominent in the proximal intestine than in the ileum [99]. This tighter coupling in the proximal intestine promotes propagation of contractions over long distances in this region, whereas poor ileal coupling limits contractile propagation and slows transit. In addition, slow wave propagation velocities decrease by 50% from the duodenum to the distal ileum. These phenomena promote efficient digestion by distributing nutrients over a large mucosal surface area in the proximal intestine where they can be rapidly digested and absorbed, and by retarding propulsion in the distal intestine to permit the more deliberate assimilation of slowly digested and absorbed substances such as fats and bile.

In the colon, slow wave migration requires continuity of the submucosal aspect of the circular muscle. The colonic slow wave is less well entrained across the different regions than the small intestinal complexes because of a lesser degree of intercellular electrical coupling. Colonic slow wave frequencies and propagation velocities are variable, in part because of the reduction in circular muscle nexus density in the colon compared with the small intestine [100]. Several dominant pacemakers in different colonic regions have been documented in humans, including a rectosigmoid pacemaker that may control motor activities of the distal colon [101–103]. Anesthetization of each pacemaker abolishes slow waves in that segment, but does not affect oscillations in other regions [104]. Circumferential colonic slow wave propagation is more rapid than longitudinal propagation.

Control of small intestinal and colonic peristalsis

The peristaltic reflex in the small intestine and colon produces aboral propulsion of lumenal contents and comprises an excitatory response proximal to stimulation (ascending contraction) and a distal response, which includes an initial relaxation (descending relaxation) followed in some cases by contraction. Radial stretch is the most potent stimulus for inducing peristalsis, although the reflex is also activated by mucosal stimulation. The threshold for peristalsis is lowered by more rapid stretching and by using longer intestinal preparations. Ascending contractions result from simultaneous circular muscle shortening and longitudinal muscle relaxation, whereas descending relaxations are consequences of simultaneous longitudinal muscle contraction and circular muscle relaxation. Lumenal distention with oil or cellulose increases the length of contraction but decreases clearance from an isolated intestinal segment, and cellulose but not oil slows propagation velocity (Fig. 11.4) [105]. Distention with air produces short contraction segments with rapid propagation velocities. The ascending contraction in isolated rat colon is sensitized by repeated radial distention, leading to enhanced responsiveness [106].

Coordinated actions of the major cell types are necessary for generation of the peristaltic reflex. Peristaltic activity is inhibited by smooth muscle relaxants [107]. Jejunal distention evokes initial nerve-mediated pressure elevations through stretch-activated generation of circular muscle contractions, which are followed by pressure decreases secondary to the inherent viscoelastic properties of intestinal smooth muscle [108]. Tetrodotoxin and a solution with a low calcium concentration and a high magnesium concentration abolish peristaltic propagation, indicating neural mediation, whereas the L-type calcium channel antagonist nifedipine attenuates contractile amplitudes but does not prevent peristaltic propagation [109]. Cell bodies of primary sensory neurons initiating the peristaltic reflex are intrinsic to the intestinal wall. Capsaicin-sensitive afferent neurons mediate both ascending and descending components of the reflex. Stripping the mucosa or applying lumenal anesthetics abolishes the reflex to radial stretch, localizing receptors to the mucosa. Nonmucosal receptors are also suggested by the observation that blunted reflexes persist despite chemical destruction of the mucosa by silver nitrate or tannic acid [110]. Mutant mice lacking ICCs exhibit loss of both the ascending and the descending arms of the small intestinal reflex,

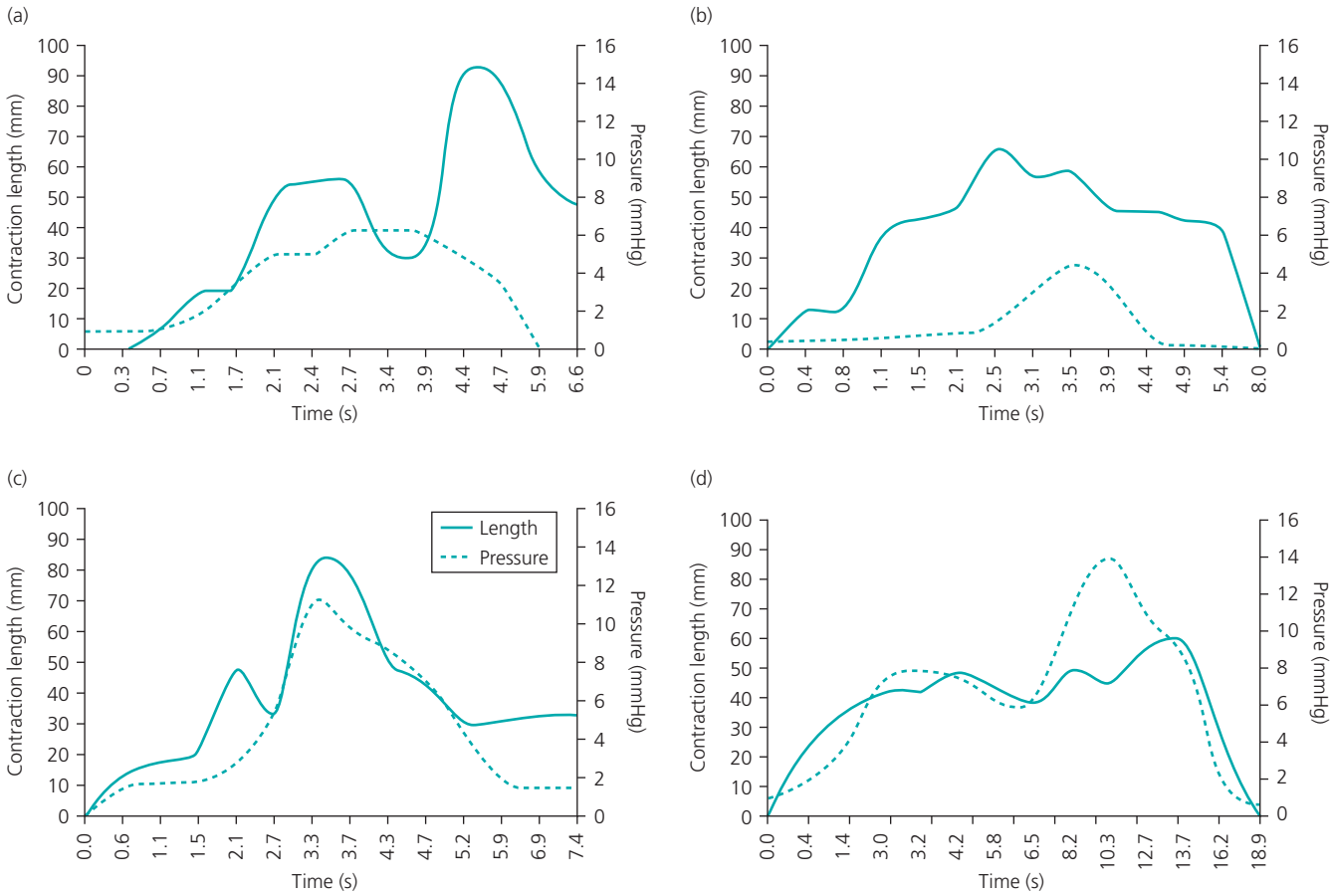

Figure 11.4 Contraction length (solid lines) and pressure (dashed lines) are plotted as a function of time during activation of peristalsis with intralumenal Krebs buffer **(a)**, air **(b)**, oil **(c)**, and cellulose **(d)**. Both contraction length and pressure increase and decrease in parallel over the duration of the peristaltic contraction. From Larson and Schulze [105].

indicating the importance of the ICC network [111,112]. However, the preservation of the peristaltic reflex in the colons of these mice suggests a lesser role for the ICCs in this organ [113].

The peristaltic reflex is mediated by the activation of several neurotransmitter pathways. Acetylcholine, substance P, and neurokinin A are released by radial stretch [114]. Atropine partly inhibits ascending contractions induced by low-level stimulation, whereas tachykinin antagonists or antisera block contractions induced by intense radial stretching. Muscarinic M_1 receptors are involved in neuronal transmission, whereas M_3 receptor activation elicits smooth muscle contraction [115]. NK_1, NK_2, and NK_3 receptors all participate in peristalsis in concert with cholinergic pathways [116]. NK_1 receptor activation increases action potentials in circular muscle, superimposed on the intestinal slow wave frequency [117]. NO and VIP are released during peristalsis and likely mediate descending relaxations [118]. NO synthase inhibitors inhibit descending relaxations elicited by colonic distention.

Serotonin is an important mediator of peristaltic reflex activity. Serotonin is released with gut distention and lowers the threshold for eliciting peristalsis [119]. 5-HT_4 receptors are present on intrinsic primary afferent neurons [120]. Mucosal stimulation initiates peristalsis by activating 5-HT_4/5-HT_{1P} receptors on sensory neurons in human intestine and 5-HT_4/5-HT_{1P} and 5-HT_3 receptors in other species [121]. 5-HT_3 and 5-HT_4 agonists elicit colonic propagation [122–124]; however, in human colon, the 5-HT_3 antagonist granisetron has no effect on the ascending contraction or descending relaxation [125]. In guinea pig colon, 5-HT_3 and 5-HT_4 receptor antagonists are additive in their inhibition of colonic peristalsis, indicating dual mediation in this species [126]. In marmoset small intestine, 5-HT_4 receptors are activated by low concentrations of serotonin, whereas higher concentrations activate 5-HT_3 receptors [127]. In isolated colonic tissue, 5-HT_4 agonists activate CGRP pathways, which then evoke ascending substance P release and descending VIP release [128]. Prolonged exposure of colonic tissues to a 5-HT_4 agonist desensitizes the peristaltic reflex

response to mucosal stroking, which is accompanied by a decrease in CGRP release [129]. 5-HT_7 receptor immunoreactivity is also detected in myenteric neurons [130]. Investigations have characterized an inhibitory effect of serotonin on peristalsis that is blocked by a 5-HT_7 antagonist [131]. Research suggests that serotonin modulates circular muscle accommodation during the preparatory phase of peristalsis by action on 5-HT_7 receptors [130].

Peristalsis is modulated by other neurohumoral substances. Brain-derived neurotrophic factor enhances both limbs of the reflex by increasing serotonin and CGRP release [132]. Opioids acting on μ and κ receptors blunt peristalsis in guinea pig small intestine, whereas somatostatin inhibits peristalsis in rat and mouse jejunum through somatostatin type 2 receptor (SSR_2) activation [133]. Stretch activates aborally projecting somatostatin neurons, which inhibit the activity of opioid neurons and reduce [Met]enkephalin release [134]. This decrease in [Met]enkephalin then elicits relaxation through the release of VIP and NO. Thresholds for eliciting peristalsis are increased by clonidine, indicating inhibitory effects of α_2-adrenergic receptor pathways [135]. Endogenous purinergic pathways suppress peristalsis through the activation of P2 receptors [136]. In rats, ascending responses are augmented by the activation of adenosine A_1 receptors and are inhibited by A_2 receptor stimulation, whereas A_1 receptor activation inhibits descending relaxations [137]. Inhibition of the tonic influence of NPY neurons by graded stretch contributes to the ascending contraction [138]. GRP acts on bombesin type 2 receptors to enhance the descending response [139]. Stimulation of cannabinoid CB_1 receptor pathways inhibits peristalsis in guinea pig ileum and mouse colon [140]. The demonstration that a CB_1 antagonist increases activity in mouse colon suggests that endogenous cannabinoids may physiologically modulate peristalsis [141]. In rat duodenum and ileum, ascending contractions are blocked by cholecystokinin (CCK) by action on CCK_1 and CCK_2 receptors [142]. In mouse jejunum, orexin A enhances both the ascending and descending limbs of the peristaltic reflex [143]. Endothelin ETA receptor activation stimulates peristalsis in guinea pig small intestine, whereas ETB receptor activation inhibits, and ETB antagonists enhance, peristaltic activity [144]. The ability of ETB antagonists to enhance peristalsis suggests a physiological modulatory role for ETB receptors. Estradiol and progesterone increase the threshold for eliciting the peristaltic reflex in guinea pig small intestine [145]. Different prostanoids have receptor-selective effects on peristaltic activity. Interleukin (IL)-1β increases substance P release and circular muscle contraction during the ascending contraction [146]. Bradykinin acting on bradykinin B_2 receptors inhibits peristalsis [147].

Extrinsic neural pathways may also modulate peristaltic activity. In guinea pig colon, sympathetic nerve stimulation inhibits cholinergic components of the reflex. In isolated tissue, extrinsic denervation decreases basal CGRP levels and abolishes peristaltic reflexes activated by muscle stretch but not mucosal stimulation [148]. CGRP antagonists abolish peristalsis with both stimuli, indicating CGRP involvement in reflexes activated by both extrinsic and intrinsic pathways.

Stereotypical motor patterns

Several stereotypical motor patterns have been characterized that control propulsion in the small intestine and colon under physiological and pathophysiological conditions.

Small intestine

Migrating motor complex

Physiological characteristics

The migrating motor complex (MMC) is an organized fasting contractile pattern that propels undigested food residue and sloughed enterocytes from the proximal gut. It is controlled by the migrating myoelectric complex [149]. The development of small intestinal bacterial overgrowth in rats given morphine disrupts MMC cycling, showing the importance of the complex. The MMC lasts for 84–112 min on average and consists of three phases. Phase I (40%–60% of the cycle) is a period of motor quiescence. Phase II (20%–30% of the cycle) is characterized by irregular phasic contractions in phase with about half of the slow wave cycles. Duodenal cross-sectional area is greater during phase II than phase I, possibly to accommodate pancreaticobiliary secretions [150]. Phase III is a 5- to 10-min period of lumenally occlusive contractions, most of which propagate aborally. Some duodenal phase III contractions propagate orally, indicative of a physiological retroperistaltic pump (Fig. 11.5) [151]. This retroperistaltic activity promotes duodenogastric reflux of bicarbonate and immunoglobulin A, which may reconstitute the antral mucosa during fasting. Retrograde duodenal contractions may also increase fasting duodenal pH and nocturnal antral pH, serving to protect both regions [152]. The maximal phase III contractile frequency is determined by the slow wave frequency (11–12 cpm in the duodenum, 7–8 cpm in the ileum). Individual contractions during phase III propagate over longer distances than during phase II. The length of intestine in a given phase III complex is 40–60 cm in the duodenum, compared with 5–10 cm in the ileum. Intestinal transit of inert substances is four times faster in phase III than in phase I [153]. Transit also occurs in phase II, with rapid propulsion occurring during the transition from phases II to III. Cineradiography shows that transit during phase III is characterized by intermittent boluses of 4–5 cm in length separated by 1- to 2-cm ring contractions [154]. In most healthy subjects, at least one phase III develops during 6 h of fasting. In total, 71% of phase III complexes originate in the stomach, with 28% beginning in the duodenum and 1% in the proximal jejunum [155]. The MMC cycle period is

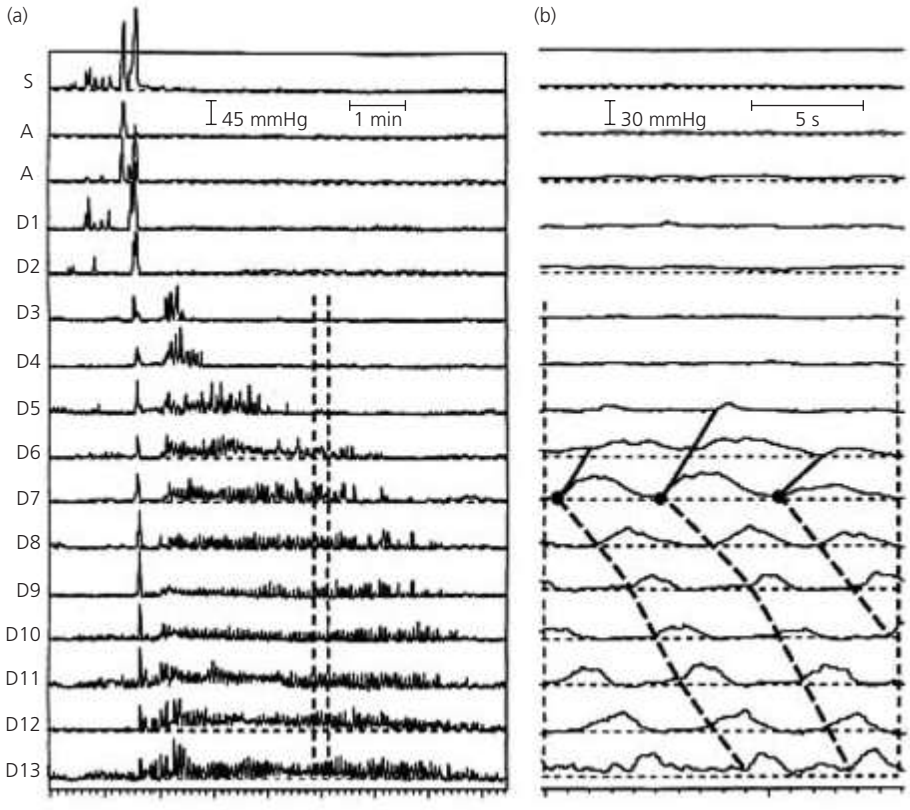

Figure 11.5 **(a)** An antroduodenal phase III complex (A, antrum; D, duodenum). **(b)** High-resolution manometry of phase III contractions shows retrograde propagation in the proximal duodenum and antegrade propagation more distally. From Castedal and Abrahamsson [152].

twice as long if the previous phase III complex originated in the stomach compared with the duodenum [156]. In total, 50% of the MMCs in humans propagate beyond the mid-jejunum and only 10% reach the distal ileum. There are few differences in MMC properties between young and older individuals, although phase III propagation velocity may decrease after age 80 [157]. Phase III propagation velocities are slower and contractile amplitudes are increased during both the follicular and luteal phases of the menstrual cycle [158].

Neural regulation

Enteric neural function coordinates small intestinal MMC propagation. Isolated denervated intestinal segments exhibit spontaneous phase III complexes that propagate aborally; however, cycling in the excluded segment is out of phase with the main segment, showing that continuity of enteric neural connections is needed for synchronized propagation [159]. Enteric nerve connections regenerate 28 days after transection and coordinated MMC cycling resumes after 45 days [159]. Interposition of a colonic segment between two small intestinal segments or construction of end-to-side or side-to-side anastomoses prevents resumption of normal MMC propagation [160]. MMC cycling is disrupted but not abolished by serosal application of benzalkonium hydrochloride, which selectively destroys myenteric ganglia, suggesting that the deep muscular plexus and submucous plexus participate in the maintenance of the MMC [161]. In mutant mice lacking ICC-MY, generation and propagation of the MMC is preserved indicating that the enteric nervous system possesses the requisite program for the complex and that slow waves are not needed [162].

The small intestinal MMC is modulated by extrinsic neural input. Bilateral truncal vagotomy, removal of the superior and inferior mesenteric ganglia, total sympathectomy, and complete extrinsic denervation of the small intestine do not prevent MMC cycling [163]. However, bilateral vagotomy increases cycle periodicity, whereas sectioning extrinsic nerves to the jejunum and ileum decreases MMC cycle length, reduces phase III propagation to the ileum, and disrupts duodenojejunal phase III. Vagal cooling in dogs has no effect on intestinal phase III but it shortens phase II activity, suggesting that vagal dependence varies between the different MMC phases [164].

Selected neurotransmitters modify MMC propagation. In dogs, atropine, the ganglionic blocker hexamethonium, and the neural toxin tetrodotoxin all eliminate MMC cycling. Close intraarterial injection of these agents disrupts MMC propagation distal to the site of infusion [165]. Adrenergic receptor antagonists disrupt but do not abolish MMC cycling. NO synthase inhibitors elicit premature MMC activity in humans, whereas NO donors disrupt MMC activity in rats,

inducing a postprandial-like pattern [166]. Similarly, the phosphodiesterase inhibitor sildenafil, which enhances NO actions by preventing cyclic guanosine monophosphate (GMP) degradation, disrupts MMC cycling [167]. VIP antagonists block the disruptive effects of NO donors, indicating that NO may act by stimulating VIP release [168]. Serotonin shortens the MMC cycle length, accelerates propagation velocities, and converts mixing patterns to propulsive patterns [169]. Ablation of myenteric serotonin neurons in rats with 5,6- and 5,7-dihydroxytryptamine prolongs MMC periodicity and decreases propagation velocities [170]. 5-HT_3 and 5-HT_4 antagonists inhibit phase III or prolong cycle length [171]. The 5-HT_1 agonist sumatriptan prolongs MMC duration by increasing phase II activity [172]. Neurokinin A evokes phase II-like activity in rats, an effect that can be augmented by an NO synthase inhibitor [173]. Morphine induces premature intestinal phase III activity, whereas the μ-opioid antagonist naloxone prolongs MMC duration [174]. δ-Opioid antagonists do not inhibit MMC cycling, but they decrease motility indices during fasting [175]. Orexin A prolongs MMC cycle duration, an effect blocked by NO synthase inhibitors, indicating mediation by nitrergic pathways [176]. Orexin A and NO synthase colocalize in duodenal myenteric neurons. Glucagon-like peptide 1 (GLP-1) and GLP-2 prolong MMC cycle duration and slow transit by way of NO-dependent pathways [177]. Ileal resections reduce phase I duration, shorten MMC cycles, and induce intestinal clusters, suggesting the presence of modulator peptides released by the distal small intestine [178]. After small intestinal transection and reanastomosis in guinea pigs, immunoreactivities for VIP, GRP, and somatostatin are decreased distal to the anastomosis [159]. MMC recovery is associated with normalization of these peptides, suggesting that peptidergic enteric nerves contribute to MMC coordination.

Neurohumoral mediators that cycle with the MMC

Phase III complexes in the antrum and proximal intestine are elicited by motilin release from the duodenal mucosa [179]. Motilin receptors are expressed in human antroduodenal enteric neurons and smooth muscle [180]. Motilin is also localized to the hippocampus, thalamus, hypothalamus, amygdala, cerebellum, and vagus, although its physiological function in the CNS is uncertain [181]. Motilin release is evoked by vagal stimulation, cholinergic agonists, opioid agents, and duodenal pH changes; however, the importance of these stimuli in the physiological regulation of motilin release is undefined [182]. Cyclic motilin fluctuations are blocked by atropine and hexamethonium, suggesting regulation by cholinergic pathways. Antral phase III complexes correlate temporally with plasma motilin elevations in healthy humans, and premature antral phase III in dogs is inducible by motilin infusion [183]. Motilin-evoked phase III is identical in duration, amplitude, and propagation velocity to spontaneous complexes [184]. In dogs, antroduodenal phase III is abolished for several hours after the administration of motilin antisera [185]. Similarly, excision of motilin-secreting duodenal tissue disrupts fasting antroduodenal motility in dogs [186]. Gastroduodenal MMC-like activity recovers 1–4 months after duodenectomy in dogs, an effect that is vagally mediated and dependent on cholinergic and adrenergic efferent pathways, indicating the adaptation and recruitment of new motilin-independent neural pathways to maintain normal upper gut function [187].

Ectopic MMC cycles originating distal to the ligament of Treitz are not usually associated with motilin release [188]. The effects of motilin antisera on distal jejunal and ileal phase III complexes are minimal [185]. Initiation of ectopic phase III activity in the jejunum and ileum are unaffected by duodenectomy [186]. These findings suggest that "programming" of phase III activity in the mid- and distal intestine is a motilin-independent phenomenon that is entrained by the actions of motilin on the antrum and duodenum.

Other peptides that cycle in phase with the MMC are somatostatin, pancreatic polypeptide, and xenin. Exogenous somatostatin evokes intestinal phase III complexes every 20–30 min [189]. However, somatostatin is not a regulator of physiological proximal gut motor activity, as gastric contractions are suppressed, duodenal phase II is abolished, and transit is delayed rather than accelerated. Somatostatin has been proposed to participate in motilin-independent cycling in the distal intestine. Plasma levels of pancreatic polypeptide, a peptide produced by the pancreas, peak just before phase III. Motilin infusion stimulates pancreatic polypeptide release by way of vagal cholinergic pathways involving 5-HT_3 receptors [190]. However, pancreatic polypeptide infusion has no effect on fasting intestinal motor activity and pancreatic autotransplantation has no effect on intestinal phase III, despite disrupting the cyclic secretion of pancreatic polypeptide [191]. Peak levels of xenin in plasma are associated with phase III and xenin infusion evokes premature phase III in healthy humans, suggesting a modulatory role for xenin in regulating fasting motor activity [192].

Other associated synchronous cyclic phenomena

The MMC cycles in phase with motor activity of the gallbladder and sphincter of Oddi, as well as with several secretory functions [193]. Gastric acid and pepsin production, intestinal bicarbonate secretion, bile acid release, pancreatic enzyme release, and lumenal secretion of immunoglobulin A peak just before or during phase III [194]. In healthy humans, intestinal absorptive flux is twice as high as secretory flux during phase I, but four times as high later in the MMC cycle [195]. Duodenectomy also disrupts cyclic sphincter of Oddi motility and increases total sphincteric contractile activity throughout the cycle [193]. However, duodenectomy and diseases such as chronic pancreatitis disrupt the synchrony of MMC and pancreatic exocrine cycling, suggesting differential regulation of the two phenomena [186].

Cyclic release of bile into the duodenum modulates MMC periodicity. Plasma motilin is released after exposing the duodenal mucosa to bile [196]. Furthermore, biliary diversion from the duodenum disrupts motilin cycling with loss of duodenal phase III and induction of ectopic jejunoileal complexes – effects that are reversed with bile flow restoration or exogenous bile acid perfusion [197]. In addition, ileal mixed micelle perfusion evokes premature phase III [198]. However, despite these observations, the role of bile in MMC generation remains in dispute.

Fed motor pattern

Physiological characteristics

After eating, the MMC is replaced by a fed pattern of intermittent phasic contractions of varying amplitude at all levels of the small intestine [199]. Myoelectric recordings show random bursts of spike potentials in phase with the slow wave. The fed pattern both mixes and propels intestinal contents. Postprandial transit is more rapid and fluctuates less than transit during fasting [153]. In total, 44% of fed contractions do not propagate. Of those that propagate, 90% migrate less than 30 cm and 66% migrate less than 9 cm. In the proximal duodenum, 40%–50% of fed contractions are retrograde [200]. Occasionally, a transitional motor pattern occurs immediately after eating, characterized by highly propagative contractile clusters that rapidly distribute nutrients over an extended mucosal surface area [201]. Intense individual migrating contractions may occur postprandially, with amplitudes twice those of normal fed phasic contractions and durations of two slow wave cycles [105]. High-amplitude pressure waves at frequencies of 19–24 cpm develop in the distal ileum 1–4 h after eating, when chyme reaches the colon [202]. Myoelectric recordings show that this activity is associated with spike bursts similar to colonic myenteric potential oscillations. Delayed tonic relaxations of the ileum are induced by local exposure to bile acids and triglycerides that are not absorbed in the proximal intestine [203].

The duration of the fed motor pattern depends on the caloric content and other properties of the meal. In dogs, a 450-kcal meal induces a fed pattern that lasts for longer than 3 h. The threshold for inducing the fed state in humans is not defined, although a 345-kcal meal disrupts MMC cycling for more than 90 min [204]. Duodenal nutrient perfusions at 0.5 kcal/min can disrupt MMC activity in dogs [205]. Peanut oil, consisting of 18-carbon triglycerides, induces fed contractions for longer periods than equicaloric sucrose or milk protein meals [206]. In humans, a 400-kcal meal with 9% fat disrupts MMC activity for 294 ± 21 min, whereas a meal with 50% fat prolongs the fed period to 410 ± 42 min [207]. Long-chain triglycerides elicit a postprandial pattern, whereas equicaloric medium-chain triglycerides have no effect [208]. The addition of guar to a glucose drink prolongs the duration of the fed pattern [209]. Intravenous administration of amino acids shortens the MMC cycle length but does not induce a fed motor pattern, confirming that participation of the gut lumen is requisite [210].

Neurohumoral regulation

Extrinsic and intrinsic small intestinal nerves are important for modulation of the fed pattern. In dogs, the presence of a cephalic phase is suggested by the observation that the sight or smell of food disrupts MMC cycling [211]. Bilateral vagotomy, splanchnicectomy, mesenteric ganglionectomy, and total extrinsic denervation do not prevent induction of the fed state. However, bilateral vagotomy shortens the duration of the fed state and increases the latency from the time of eating to the onset of fed contractions, whereas complete denervation reduces the number of fed contractions, shortens the mean propagation distance, slows intestinal transit, and increases the threshold number of calories needed to initiate the pattern [163]. Vagal cooling converts the fed pattern to one of intermittent phase III activity [164]. If nutrients are perfused into an isolated, but extrinsically innervated, intestinal loop, the MMC is disrupted in the unconnected main portion of the intestine, showing that extrinsic nerves alone are sufficient for suppression of fasting motility [212]. Intestinal transection with reanastomosis decreases the frequency and amplitude of fed contractions in dogs and impairs propagation [213]. Atropine and hexamethonium abolish fed motor and spike potential activity, demonstrating cholinergic dependence of the fed pattern [214]. NO synthase inhibition in healthy humans delays fed duodenocecal transit in association with increases in frequency and amplitude of duodenal pressure waves, indicating modulation by nitrergic pathways [215].

The specific neurohumoral mediators responsible for inducing the fed state are undefined. CCK has an uncertain role in the initiation of the postprandial motor pattern. CCK levels increase five- to tenfold after eating. CCK increases intestinal contractile activity that exhibits characteristics dissimilar to the fed state, such as prominent retrograde propagation and a preferential stimulation of the proximal intestine [216]. CCK inhibits MMC activity but does not prevent motilin cycling. In rats, duodenal perfusion of trypsin inhibitor releases endogenous CCK, which activates vagal afferent CCK_2 receptors to stimulate a central CCK_1 pathway to disrupt MMC cycling [217]. Intravenous CCK_2 blockade or central administration of a CCK_1 antagonist in rats prevents disruption of MMC activity in response to nutrients [218]. However, in dogs, CCK antagonists reduce but do not prevent the fed response or the interruption of MMC cycling after eating [219]. Other compounds inhibit the MMC and induce complexes similar to the fed pattern, including gastrin, insulin, glucagon, neurotensin, neuromedin N, enkephalins, and prostaglandin E_2. Gastrin increases proximal intestinal motility; however, stimuli that produce a fed pattern, such as peanut oil, do not evoke gastrin release

[216]. Glucose promotes insulin release but does not induce a fed pattern. Neurotensin converts fasting motility to a fed pattern along the entire small intestine in rats and humans [220], and, in rats, the fed pattern duration is reduced by neurotensin antagonists [221]. The decrease in plasma motilin levels may contribute to postprandial motility, as suggested by the observation that the administration of motilin antisera generates motor patterns that are similar to the fed state [185].

Reversion to the MMC

Reversion to MMC cycling after completion of the fed pattern is poorly understood. The initiation of duodenal phase III correlates with the completion of gastric emptying of digestible solids [222]. However, the first postprandial MMC begins ectopically distal to the duodenum, implying that factors that initiate more proximal complexes are not yet operational [223]. In dogs and humans, continuous duodenal feedings produce persistence of the fed pattern for only a finite time, after which phase III activity resumes [224]. The ability of NO synthase inhibitors to convert fed duodenal motor patterns to phase III-like activity in healthy humans suggests involvement of nitrergic pathways [225].

Giant migrating contractions

Intense pressure waves that propagate aborally for long distances in the small intestine, termed *giant migrating contractions* (GMCs), are observed experimentally during hypoxia, anemia, gangrene, after laparotomy, and after death. GMCs are two to three times greater in amplitude and four to five times longer in duration than individual phasic contractions and they propagate at 1 cm/s (Fig. 11.6) [226,227]. GMCs typically are more prolonged than a single slow wave cycle, indicating slow wave independence. Myoelectric recordings of GMCs measure intense spike potential bursts that last 4–16 s, obscuring the slow wave. GMCs commonly originate in the jejunum or ileum during fasting, propagate to the ICJ, and are intensely propulsive of ileal contents. Nearly half of ileal GMCs are associated with propagating sequences in the cecum [228]. Consequently, intestinal GMCs are proposed to clear ileal debris and prevent coloileal reflux. Intestinal GMCs, rare (0.03/h) in health, may be induced by intravenous morphine, intragastric vinegar, ileal perfusion of feces or short-chain fatty acids, ionizing radiation, and infection with *Vibrio cholerae*, *Clostridium perfringens*, *Clostridium difficile*, noninvasive *Escherichia coli*, *Shigella*, and *Trichinella spiralis* [227]. The initiation of GMCs is associated with decreased ileal pH, suggesting that coloileal reflux may be a physiological stimulant of this motor pattern [229]. Intestinal GMC propagation is controlled by enteric neural pathways, whereas extrinsic nerves regulate inhibition of motor activity orad to the complex [230]. GMCs elicited by arterial CGRP infusion are blocked by atropine, hexamethonium, and tetrodotoxin [231]. NK_3 receptors on presynaptic neurons may also participate in small intestinal GMCs [232].

Other aborally migrating patterns

Other aborally migrating patterns occur at irregular intervals. Discrete clustered contractions (DCCs) are intensely propulsive contractions that occur during fasting and after eating, comprising 3–10 contractions followed by 1 min of motor quiescence [226,233]. DCCs migrate at 5–10 cm/min over distances of 2–40 cm and are proposed, along with GMCs, to be a physiological means of emptying the ileum. Unlike GMCs, DCCs rarely extend into the proximal colon [228]. An extremely propagative pattern, called the rapidly migrating contraction, migrates for 200 cm at more than 30 cm/s [234]. In contrast to intestinal GMCs, rapidly migrating contractions occur predominantly in the proximal small intestine and are associated with disruption of slow wave activity.

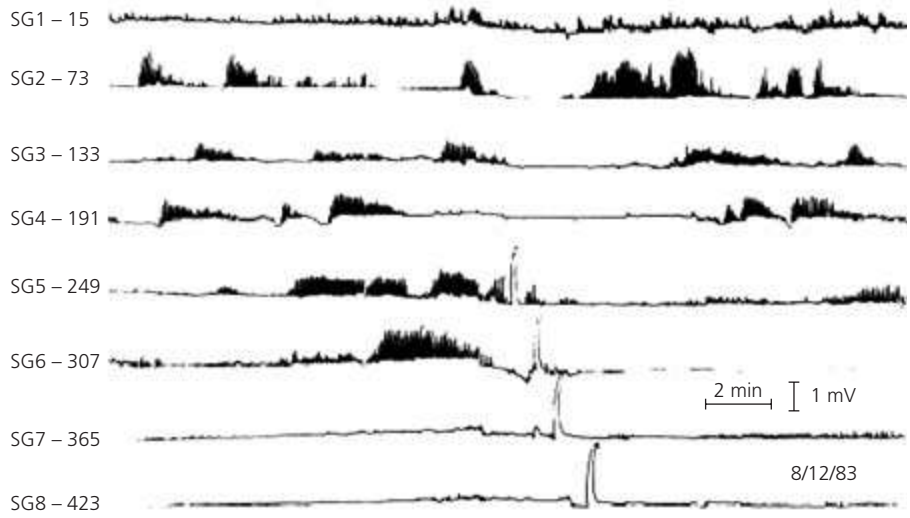

Figure 11.6 A spontaneous giant migrating contraction in canine small intestine. The labels before each tracing reflect the strain gauge number and distance in centimeters from the pylorus. From Sarna [227].

Retrograde peristaltic contractions

After administration of emetic agents, retrograde peristaltic contractions (RPCs) migrate orally from the mid-small intestine, moving lumenal contents into the stomach so that they may be expelled during vomiting. RPCs exhibit motor inhibition immediately before and after the complex and are followed by several phasic contractions and a second inhibitory period [235]. They exhibit contractile amplitudes that are 1.3–1.8 times greater than those of normal intestinal contractions, with durations that are 2–4 times longer, and they migrate rapidly (8–10 cm/s) over distances of more than 100 cm [153]. RPCs are preceded by slow wave obliteration prior to the generation of electrical bursts that migrate orally [236]. The entire phenomenon is abolished by vagotomy, but only the RPC itself is prevented by atropine, hexamethonium, and tetrodotoxin [231,235]. Although commonly associated with retching or vomiting, RPCs occur in their absence. Retching and vomiting also occur without RPCs. Investigations involving autotransplantation of small intestinal segments in dogs indicate that retrograde motor activity is controlled by extrinsic neural pathways [237].

Colon

Local contractile activity

Under basal conditions, the colon exhibits poorly organized local contractions that may be of short or long duration. Stationary short-duration contractions (SDCs) mix fecal material to effect water extraction. The myoelectric pattern underlying SDCs consists of short spike bursts that occur in phase with colonic slow waves. SDCs persist for a mean of 8 s, occur at 4–6 cpm in dogs and 2–13 cpm in humans, and are recorded from in vitro circular muscle preparations [238]. Long-duration contractions (LDCs) are of greater amplitude than SDCs and can be stationary or propagate for short distances in either direction, thus promoting both mixing and local fecal propulsion [239]. LDCs typically propagate orally in the ascending and transverse colon, whereas they migrate aborally in the distal colon [240]. The myoelectrical correlates of LDCs, long spike bursts, are action potentials generated by myenteric potential oscillations that are asynchronous with colonic slow waves. Although long spike bursts oscillate at 25–40 cpm, a single tetanic LDC persists for the duration of the electrical complex because of temporal limitations in relaxation rates of colonic smooth muscle. In contrast to SDCs, LDCs are recorded from in vitro longitudinal muscle preparations [238]. Most LDCs occur during the day and are increased on awakening and after meals [241]. Propagating contractions in the proximal human colon are enhanced by NO synthase inhibitors, suggesting the presence of tonic inhibitory nitrergic control of colonic motor activity [242].

Gastrocolonic response

As in the small intestine, eating elicits increased motor activity throughout the colon. Greater in the sigmoid than in the transverse colon, this increased activity is termed the gastrocolonic response (Fig. 11.7) [243]. In addition to enhanced phasic activity, meal ingestion increases colonic tone. Myoelectric recordings show increases in propagative and stationary long spike bursts peaking 15 min after eating. In some instances, mass fecal movements occur during the gastrocolonic response with subsequent defecation. Differences in meal composition likely influence the magnitude of the gastrocolonic response, although responses to low- and high-fat meals are reportedly similar [244].

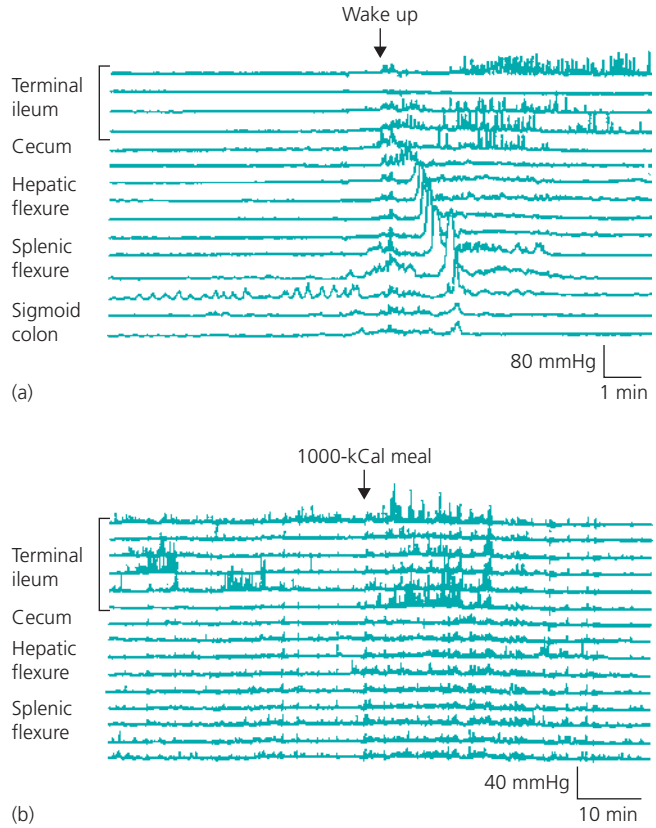

Figure 11.7 Manometric recordings of colonic motor activity in a healthy human. **(a)** On awakening, motor activity increases and a propagating sequence is initiated. **(b)** Ingestion of a 1000-kcal meal elicits a pronounced increase in motor activity at all recording sites. From Bampton et al. [259].

The gastrocolonic response is regulated by several neurohumoral factors. Extrinsic denervation prevents its induction [245]. In dogs, paraaortic and presacral denervation reduces the colonic responses to intragastric lipids, suggesting modulation by the pelvic plexus [246]. Gastrocolonic responses are also blunted by the anticholinergic agent clidinium and the 5-HT_3 antagonist ondansetron, indicating involvement of both cholinergic and serotonergic innervation [247]. The gastrocolonic response in humans consists of gastric mechanoreceptor and small intestinal chemoreceptor components

that are partly mediated by 5-HT_3 pathways [125]. Neurotensin antagonists suppress the early fed response in rat distal colon and block late responses in the proximal colon [221]. CCK increases colonic motor and myoelectrical activity in some models, but does not elicit colonic contractions in humans at doses that stimulate pancreatic enzyme secretion and gallbladder contraction [248]. In dogs, responses to meals and exogenous CCK differ in that CCK reduces rather than increases colonic tone [249]. However, the CCK antagonist loxiglumide blunts meal-evoked increases in colonic motor activity, indicating some CCK-dependent involvement in the response [250]. Similar studies in rats using selective antagonists have shown potential roles for both CCK_1 and CCK_2 receptor mediation of the gastrocolonic response.

High-amplitude propagated contractions

High-amplitude propagated contractions (HAPCs) propagate aborally over extended distances and elicit mass movements of feces that may precede defecation. The myoelectrical correlates of HAPCs are migrating, long spike bursts generated from myenteric potential oscillations. In ambulatory manometric studies of unprepared colons in healthy humans, HAPCs of high amplitude (> 105 mmHg) and prolonged duration (> 14 s) occur 6–10 times daily, often after awakening and after meals, or up to 1 h before defecation (Fig. 11.8) [251]. The time to defecation after the development of a HAPC correlates negatively with the amplitude of the contractile complex. Most HAPCs in humans originate in the cecum, whereas 90% of HAPCs associated with defecation in dogs propagate to the rectum and induce IAS relaxation [252]. HAPCs may be experimentally evoked by colonic distention, stimulant laxatives, abdominal irradiation, intracolonic acetic acid or oleic acid, and orphanin FQ administration [253]. Substance P-evoked HAPCs are enhanced by experimental colonic inflammation [254]. HAPCs elicited by intracolonic glycerol are reduced by lidocaine, hexamethonium, and atropine, indicating mediation by mucosally activated cholinergic pathways [255]. HAPCs are mediated by neural acetylcholine release acting on M_3 receptors and by the activation of NK_1 receptors on smooth muscle, and they are partially inhibited by NO release [256].

Rectal motor complexes

Ambulatory manometric studies demonstrate rectal motor complexes that occur about 16 times per day with mean durations of 5–15 min and which may facilitate the storage function of the rectum [257]. Rectal complexes occur more commonly in the distal than in the proximal rectum and comprise two to three contractions per minute with a mean amplitude of 58 mmHg. Such rectal motor activity occurs during the day and nocturnally and is not related to meal ingestion, whereas the timing of most rectal events relates to contractions more proximally in the colon [258]. Rectal motor complexes usually do not propagate either orally or aborally [259]. Anal relaxations are not observed during rectal motor complexes, consistent with a role in the maintenance of fecal continence.

Migrating motor complexes

Colonic MMCs are observed in some species but not in humans. The myoelectrical correlates, migrating myoelectric

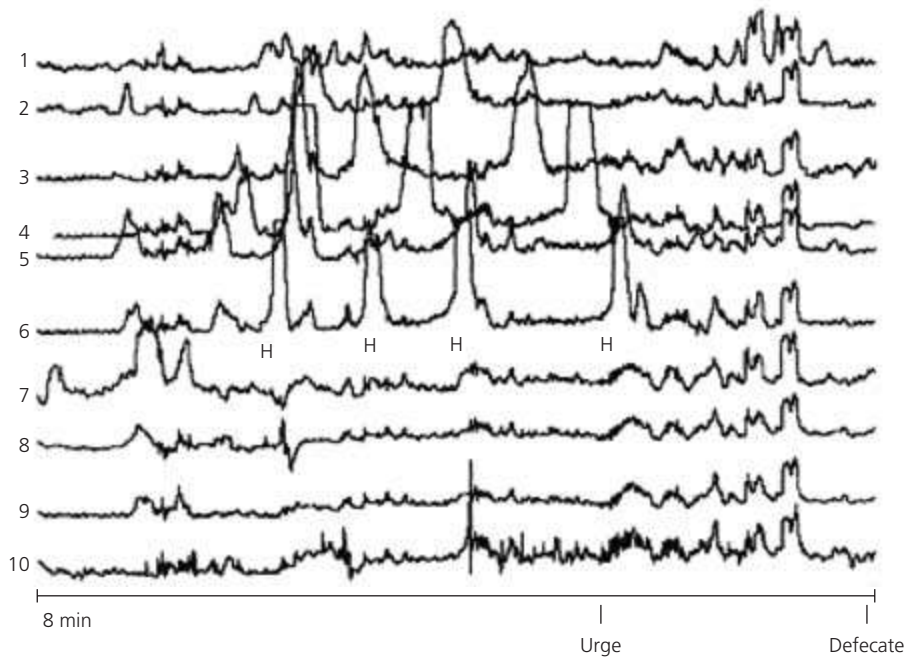

Figure 11.8 A cluster of high-amplitude propagating contractions (H) prior to defecation. The complex is most prominent in recording leads two to six. From Hagger et al. [251].

complexes, cycle every 40–50 min in dogs. Colonic MMCs propagate over more than half of the length of the colon with durations of 30–120 s [260]. Colonic MMCs do not cycle in phase with small intestinal MMCs.

Sphincteric motor function

Ileocolonic junction

The ICJ is a sphincteric region possessing a localized high-pressure zone that is not abolished by neural toxins. In dogs, the ICJ has a basal pressure of 30–40 cmH_2O with phasic contractions of more than 100 cmH_2O [261]. In humans, a high-pressure zone is less reliably observed, although phasic contractions are common. Slow waves and spike potentials migrate across the ICJ into the colon in cats and dogs. In dogs, more than half of the MMCs traverse the ICJ and may elicit colonic contractions; however, in humans, the structure participates in very few MMC cycles [262]. Scintigraphy studies show that fasting flow across the canine ICJ is maximal before phase III, whereas, in humans, changes in flow do not correlate with MMC phases. In dogs, meal consumption increases motor activity and flow across the ICJ, which peaks 4 h after eating [263]. In humans, meals increase ICJ tone and phasic activity [264]. Small intestinal GMCs and DCCs traverse the ICJ and decrease phasic and tonic activity in the structure [262,264]. Both intrinsic and extrinsic nerves control ICJ responses. Colonic distention evokes reflex ICJ contraction, which is not blocked by transection of vagal or pelvic nerves [265]. Conversely, splanchnic nerve transection blocks the reflex, indicating the importance of extrinsic sympathetic pathways. Similarly, total extrinsic denervation increases phasic ICJ pressures as a result of increased muscarinic receptor sensitivity [266].

The ICJ safeguards against coloileal fecal reflux. The acute angulation of the ICJ at its insertion into the cecum likely plays an important role in this response. If the fibrous tissue sustaining this angulation is severed, the ICJ cannot prevent retrograde reflux [267]. Similarly, excision of the ICJ increases ileal fecal bacterial counts [268]. The role of the ICJ as a barrier to aboral flow is less well defined. Some studies observe reflex ICJ relaxations with ileal distention, whereas others note reflex contractions. In a dog study, ICJ relaxations in response to ileal distention were abolished by extrinsic denervation [269]. Colonic filling from the ileum is characterized by bolus movements alternating with periods of stasis, suggesting that the ileum regulates cecal delivery [270]. Studies with Heidelberg capsules show lag times of 0.8–2.5 h for the passage of solids across the ICJ, but other investigations show no differences in solid and liquid transit into the cecum [271]. Patients who have undergone ICJ resection exhibit normal transit of radiolabeled beads, suggesting little role for the structure as a regulator of forward flow [272]. However, with extensive small intestinal resection, excision of the ICJ further accelerates transit, showing that the structure can restrict aboral propagation during conditions of high flow.

Anus and pelvic floor

The anal canal is 2.8 cm in length, with a smaller circumference in women than in men [273]. An additional functional region of elevated pressure extending 6 cm into the rectum helps maintain fecal continence. The IAS provides 85% of resting tone, which in humans is 40–80 mmHg. Three regions of spontaneous electrical activity are observed in the IAS: an upper region 15–20 mm from the anal verge with a frequency of 6.8 cpm, a transitional region with a frequency of 15.9 cpm, and a lower region within 5 mm of the anal verge with a frequency of 24.1 cpm [274]. Tone is generated in the two lower regions, whereas relaxant responses are observed in all regions with electrical depolarization. Voluntary contraction of the EAS increases pressures to more than 150 mmHg. With sudden and constant rectal distention, the IAS contribution to resting tone decreases to 40% while the EAS provides the remaining 60% [275]. Ambulatory manometry reveals 14–19 spontaneous anal relaxations per hour while awake and 4 per hour during sleep, some of which correlate with flatus passage [257]. Tone is similar in men and women, but maximal squeeze pressures and durations are higher in men [276]. Both tone and maximal squeeze pressures decrease with aging. With advanced age, EAS thickness decreases and IAS thickness increases [273].

Fecal dehydration occurs during colonic transit such that the stool which reaches the rectum is semisolid to solid. The compliance of the rectal wall permits the rectum to serve as a reservoir for fecal material until it can be expelled. The tone of the IAS prevents inadvertent leakage, whereas the EAS voluntarily contracts if unwanted fecal expulsion is impending. In contrast to other striated muscle structures, the EAS and puborectalis exhibit spontaneous tone to promote fecal continence. Maximal squeeze pressures are observed where the puborectalis overlaps the EAS. The uppermost loop of the EAS, formed from the deep external sphincter muscle, attaches anteriorly on the pubis. The intermediate loop, formed from the superficial external sphincter, attaches posteriorly to the coccyx. The base loop, formed from the subcutaneous external sphincter, attaches to the perianal skin. Tight contraction of the three loops produces anal occlusion. Electromyographic studies indicate that the pelvic floor musculature behaves as a single unit, with all muscle groups activating in response to local stimulation [277]. An anorectal angle of 90° created by the puborectalis muscle provides a functional obstruction to accidental loss of stool at rest.

Defecation involves coordinated interaction of the different structures in and around the rectum and anus. The transitional zone above the pectinate line, the anal crypt region, and the anal canal possess many free and organized nerve

endings that sense the contents of the anal canal, thereby permitting the differentiation of solids, liquids, and gases. Sitting upright contributes to continence by tightening the anorectal angle [278]. Fecal evacuation is aided by assuming a position in which the hips are flexed to open the anorectal angle. Comparisons of different positions report that assuming a squatting posture promotes less straining effort than sitting upright [279]. With voluntary defecation, puborectalis relaxation further opens the anorectal angle (110°) and elicits IAS relaxation. Rectal contraction increases lumenal pressure to provide a propulsive force for defecation. Contraction of the rectus abdominus muscles, diaphragm, and terminal aspects of the other levator ani muscles increases intra-abdominal pressure to provide additional force. With straining, the pelvic musculature descends about 1 cm. After fecal passage, a rebound contraction of the EAS and pelvic floor muscles occurs. In contrast, flatus passage is not associated with opening of the anorectal angle. Rapid abdominal pressure increases and pelvic floor muscle contractions, coupled with colonic contractions and increased rectal pressure, force gas past the acutely angled anorectum while retaining solid and liquid feces [280].

Extended reflexes involving the small intestine and colon

The small intestine and colon participate in several nerve-mediated reflex responses locally, within the organs themselves, as well as in more distant regions of the gastrointestinal tract.

Intestinointestinal reflex

The inhibitory intestinointestinal reflex is the profound motor inhibition of up to several hundred centimeters of intestine in response to abrupt stretching or dilation of a localized intestinal segment. It is proposed to serve a protective role when there is bowel distention resulting from mechanical obstruction or with adynamic ileus. In humans, the inhibitory effects of distention are more pronounced in the proximal intestine than in the ileum [281]. The reflex produces slow wave disruption and uncoupling on both sides of the distended segment [282]. Removal of the longitudinal muscle prevents activation of the reflex, whereas excision of the mucosa and submucosa has no effect. In dogs, abrupt distention of an excluded but extrinsically innervated intestinal segment abolishes spike activity in the excluded loop and in the intact intestine through the splanchnic pathways [283]. The reflex is abolished by spinal cord transection below T7, indicating a need for intact thoracolumbar spinal pathways.

Nutrient-evoked small intestinal reflexes

The ileal and jejunal brakes are the inhibitors of proximal motor activity in response to nutrient stimulation of the ileum and jejunum that protect the distal intestine from being overwhelmed by massive caloric loads. Ileal glucose or lipid perfusion more potently inhibits gastric and proximal intestinal motor activity than duodenal perfusion [284]. Similarly, ileal short-chain fatty acid perfusion reduces antral contractions and reduces transpyloric flow [285]. Lipids are most effective at activating the ileal brake; ileal oleate is 20-fold more potent than glucose at inhibiting gastric emptying [286]. Lipase inhibition with tetrahydrolipstatin attenuates the inhibitory effects of triglyceride on proximal function, indicating that products of fat hydrolysis mediate lipid-induced intestinal braking (Fig. 11.9) [287]. More proximal intestinal stimulation also has inhibitory effects on gastric emptying. Duodenal peptone administration is more potent than equicaloric glucose perfusion [288].

Several neurohumoral factors participate in nutrient inhibition of proximal gut activity. Extrinsic innervation is necessary for the inhibition of duodenal and jejunal motility

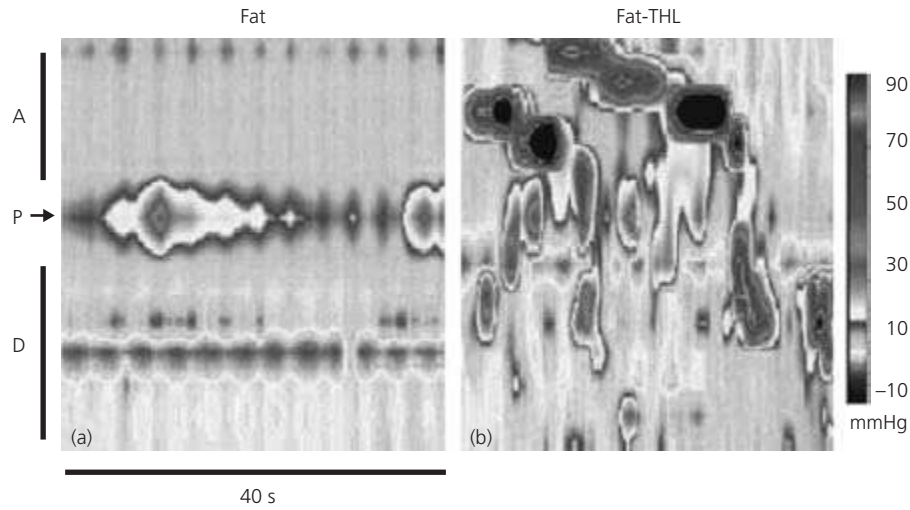

Figure 11.9 High-resolution antro(A)-pyloro(P)-duodenal(D) pressure patterns. **(a)** Duodenal triglyceride perfusion (Fat) elicits isolated pyloric contractions with inhibition of the antrum and duodenum. **(b)** The lipase inhibitor tetrahydrolipstatin (THL) prevents reflex induction of pyloric contractions. Instead, a normal fed pattern of increased contractions occurs in both the antrum and the duodenum. From Feinle et al. [287].

evoked by ileal triglycerides but not oleic acid [289]. Capsaicin abolishes the inhibitory effects of ileal lipids on gastric emptying, indicating participation of extrinsic sensory neurons [290]. Similarly, delays in gastric emptying evoked by duodenal carbohydrate and protein perfusion are associated with increased vagal afferent activity and are inhibited by capsaicin administration through the vagus nerve and the spinal cord [291]. Antagonists of α_1- and β_1-adrenergic, opioid, and 5-HT_3 receptors blunt intestinal nutrient-induced proximal motor inhibition [292]. Inhibition of the ileal brake by the 5-HT_3 antagonist ondansetron or the μ-opioid antagonist naloxone is observed only when the agent is given in the proximal intestine, suggesting that the action occurs on the efferent limb of the reflex [293]. Enterogastric reflexes are blocked by CCK antagonists in some models [291]. In dogs, the lipid-induced jejunal brake is slowed initially by the CCK_1 antagonist devazepide with later acceleration by naloxone, indicating dual mediation by CCK and opioid pathways [294]. Knockout mice without CCK_1 receptors exhibit a blunted ileal brake, suggesting that ileal lipids act through CCK_1 receptor-dependent mechanisms [295]. Apolipoprotein A-IV, released by enterocytes in response to triglyceride absorption, stimulates duodenal vagal afferents through CCK-dependent mechanisms [296]. Similarly, chylous lymph increases discharge of CCK-responsive duodenal fibers, which elicits vagovagal inhibition of gastric motility [297]. Ileal lipids and short-chain fatty acids release peptide YY (PYY), GLP-1, and neurotensin [298]. Conversely, PYY release is not stimulated by ileal triglycerides [289]. PYY_2 receptor mRNA localizes to the ileum and colon in humans [299]. Intravenous PYY prolongs the duodenal MMC cycle length and delays intestinal transit, mimicking the effects of ileal nutrients [300]. PYY immunoneutralization accelerates intestinal transit inhibited by ileal lipids, showing dependence of the ileal brake on endogenous PYY [301]. Slowing of intestinal transit by PYY is blocked by propranolol, indicating mediation of β-adrenoceptor pathways [302]. Similarly, slowing of intestinal transit by PYY is abolished when ondansetron or naloxone is administered to the proximal intestine, mimicking the response to ileal lipids [303]. Inhibition of duodenal motility by ileal protein is reversed by a GLP-1 antagonist [304]. Endogenous melatonin exerts a modulatory influence to decrease the action of the ileal brake [305]. Finally, nutrient-evoked intestinal brakes may be modulated by nonnutrient stimuli. Slowing of intestinal transit by jejunal oleate is reversed by distending the ileum with a perfusion of buffer solution [306].

Reflexes activated by nonnutrient small intestinal stimulation

Nonnutrient small intestinal stimuli also elicit reflex motor effects on other gastrointestinal regions. Intraduodenal application of capsaicin inhibits plasma motilin cycling with abolition of gastric MMCs [307]. Intraileal capsaicin inhibits gastrointestinal contractions by way of NO, 5-HT_3, and opiate pathways [308]. Duodenal distention reduces intragastric pressure, inhibits antral motility, and induces pyloric contractions. Capsaicin-sensitive vagal afferents mediate the inhibitory response to low volumes, whereas spinal afferents mediate responses to high-volume distention of the duodenum [309]. Duodenal transection prevents duodenal distention-evoked pyloric contractile responses, reflecting the importance of intrinsic pathways. Conversely, duodenal transection does not block the inhibition of antral contractions or the delay in gastric emptying induced by duodenal distention, showing the involvement of pathways other than ascending intraduodenal nerves. The exposure of 15 cm of duodenum to hydrochloric acid inhibits gastric emptying, whereas lactic acid has no effect [310].

Colocolonic reflexes

Colonic stimulation evokes reflex responses within the colon. Inflation of a distal colonic balloon inhibits contractions in a proximal colonic loop by α_2-adrenergic receptor-mediated pathways [311]. In humans, rectal distention reduces proximal colonic tone and propagating contractions, whereas rectal perfusion of chenodeoxycholic acid promotes such propagating sequences [312,313]. Distention of the rectosigmoid junction elicits increased rectal pressure and IAS relaxation, with resultant expulsion of a rectal balloon. Conversely, distention of the sigmoid colon increases pressure in the rectosigmoid junction by activating the mucosal receptors, which may promote fecal continence and retention [314]. Both inhibitory rectocolonic and excitatory colorectal reflexes are blunted by neurotensin [315]. Colonic perfusion of short-chain fatty acids accelerates colonic transit by stimulating serotonin release from enterochromaffin cells [316]. Increases in colonic smooth muscle contraction with short-chain fatty acid exposure are mediated by the activation of calcium influx [317].

Rectoanal inhibitory reflex

Rectal distention or electrical stimulation produces a volume-dependent decrease in IAS pressure, a response termed the rectoanal inhibitory reflex. In contrast, EAS pressure increases on rectal distention. The rectoanal inhibitory reflex permits efficient defecation on rectal filling, whereas reflex EAS contraction prevents accidental leakage of rectal contents. The reflex also participates in anal canal sampling to discriminate between solids, liquids, and gas. Intrinsic neural pathways between the rectum and the IAS mediate the rectoanal inhibitory reflex, although a role for spinal pathways has been proposed. The reflex is abolished by rectal transection at the anal verge, but it can regenerate within 2 years of surgery in humans [318]. IAS relaxation in response to rectal stimulation is a consequence of VIP and NO release [319]. In neuronal NO synthase-deficient mice, the reflex is absent, indicating the importance of nitrergic innervation [320]. The

rectoanal inhibitory reflex is diminished in mutant mice lacking ICCs [321].

Other small intestinal and colonic reflexes

Stimulation of the small intestine and colon elicits additional reflex responses in other regions of the gastrointestinal tract. Fluid distention of the duodenum increases sphincter of Oddi motor activity, a reflex that may prevent duodenobiliary and duodenopancreatic reflux [322]. Ileal distention elicits colonic relaxation by way of the splanchnic nerves and prevertebral ganglia [323]. Colonic distention retards intestinal transit, reduces ileal motility, and disrupts intestinal slow wave cycling by way of the extrinsic sympathetic pathways within the splanchnic nerves. Colonic perfusion of mixed nutrients inhibits duodenal fasting activity, whereas colonic distention prolongs MMC cycling by activating nicotinic ganglionic receptors [324]. Topical anesthesia of the rectum prevents the inhibition of small intestinal contractions by rectal distention, indicating the participation of mucosal receptors [325]. Colonic perfusion of lactose and short-chain fatty acids reduces gastric tone, indicative of a colonic brake [326]. Colonic perfusion of short-chain fatty acids releases PYY; however, the role of this peptide in the colonic brake is unproved [327].

Small intestinal and colonic motor functions are reflexively modulated by stimulation of other structures. In dogs, gastric distention abolishes fasting duodenojejunal motor activity and delays intestinal transit – a reflex unaffected by vagotomy but reduced by celiac plexus sectioning or small intestinal denervation [328]. Other investigations report mediation of this reflex by NO release in the celiac plexus [329]. Distention of the vagina promotes rectal relaxation and increased IAS tone, a reflex purported to prevent fecal leakage during coitus [330]. Urine passage through the urethra increases EAS activity, preventing inadvertent defecation while urinating [331].

External influences on motor activity

Motor function during sleep

Small intestinal and colonic motor patterns exhibit subtle differences during wakefulness and sleep. MMCs migrate faster during the day but exhibit higher contractile amplitudes at night. MMC periodicity and phase II duration are shorter during sleep, whereas phase I duration is prolonged [332]. Prolonged manometric recordings show no correlation of the sleep stage with MMC cycling nor any synchrony of rapid eye movement sleep with intestinal phase III [333]. In contrast to fasting activity, fed small intestinal patterns in humans who fall asleep soon after eating are not different from complexes recorded while awake [334]. Propagating contractions in the colon are infrequent during sleep but increase after awakening. Awakening evokes a threefold increase in colonic motility, including high-amplitude events in isolated segments as well as propagated complexes over extended distances in the colon (see Fig. 11.7) [251,335]. Nocturnal suppression of colonic motility correlates with the depth of sleep. Propagated contractions are absent during slow wave sleep, but contraction frequencies increase during rapid eye movement sleep [336].

Central nervous system modulation

CNS modulation of small intestinal and colonic function is evidenced by studies of the effects of stress on motor activity. Phases II and III of the MMC and the DCCs are suppressed by acoustic and mental stress, whereas the fed pattern is prolonged by acoustic stress [337]. Stress-induced effects on small intestinal motility are usually associated with delayed transit. In contrast, abrupt noxious night-time awakening has no effect on MMC cycling, suggesting possible diurnal variability in gut sensitivity to stress. Stressful interviews elicit increased rectal motor activity in healthy humans. Similarly, in rats, conditioned fear increases colonic spike bursts. Different forms of stress elicit distinct effects on colonic motility, with psychological stress inducing propagated contractions and physical stress evoking simultaneous contractions [338]. In rats, tail shock suppresses proximal colonic motility but increases fecal output from the distal colon [339]. The delay in intestinal transit evoked by cold stress is attenuated by β-adrenergic receptor blockade [340]. In rats, the effects of restraint stress on small intestinal motility are reversed by β_3-adrenergic receptor antagonists [341].

Corticotropin-releasing factor (CRF) is the physiological mediator of many stress effects on the small intestine and colon. Stress is associated with intracerebral CRF release. The effects of cold stress on intestinal transit in mice are mimicked by intracerebroventricular (ICV) administration of CRF [342]. In rats, modulation of intestinal transit by ICV CRF and the CRF-related peptide urocortin is prevented by vagotomy and naloxone administration [343]. MMC suppression by ICV CRF in dogs is associated with suppression of motilin cycling [344]. ICV CRF, ICV urocortin, and stress increase colonic activity in rats by acting on the locus caeruleus and the hypothalamic paraventricular nucleus [345]. CRF antagonists reverse the effects of stress, supporting a physiological role for this peptide (Fig. 11.10). Neither urocortin antisera nor CRF antagonists affect small intestinal motility in the absence of stress, indicating that endogenous urocortin and CRF do not modulate basal motor activity [343]. Stimulatory effects of CRF on motor activity are mediated by activation of CRF_1 receptors, whereas inhibition of motor function relies on stimulation of CRF_2 receptor pathways [346,347].

CRF mediation of stress effects on the gut involves the participation of other neuroactive agents. The increase in colonic transit elicited by NPY infusion into the paraventricular nucleus is blocked by CRF antagonist administration [348]. Ghrelin stimulates colonic propulsion through actions

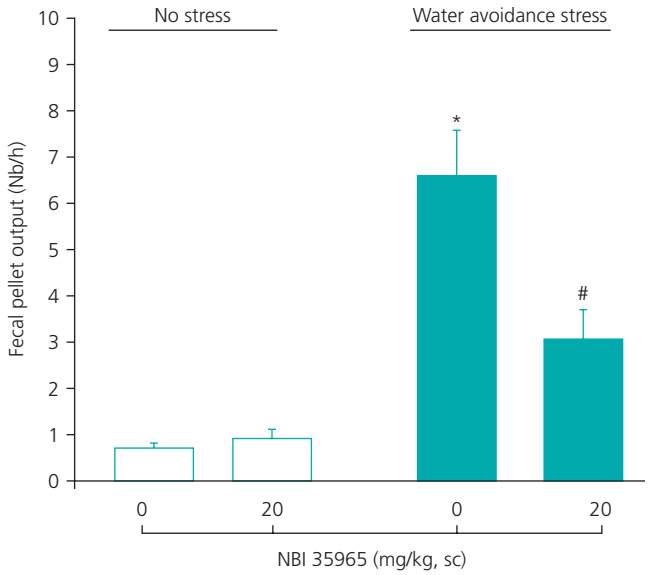

Figure 11.10 Fecal pellet output during periods of no stress (open bars) and water avoidance stress (filled bars). Water avoidance stress significantly increased fecal output. Subcutaneous (sc) administration of the corticotropin-releasing factor antagonist NBI 35965 blunted the increase in output during stressful stimulation. From Million et al. [346].

in the paraventricular nucleus controlled by NPY_1 and CRF_1 receptor-dependent mechanisms [349,350]. Kainate injection into the arcuate nucleus stimulates colonic motility, an effect blocked by the injection of CRF antagonists into the paraventricular nucleus [351]. Vasopressin may be an intermediate transmitter as vasopressin antagonists block the effects of stress and ICV CRF on colonic motility in rats [352]. CRF-stimulated defecation in rats may be mediated by serotonin release, with action on $5\text{-}HT_3$ receptors [353]. The ability of CRF antagonists to block the increase in colonic contractions evoked by IL-1β suggests that central CRF mediates the motor effects of cytokine release in inflammatory states [354].

The effects of peripheral administration of CRF differ from those achieved by central administration. Both CRF_1 and CRF_2 receptors are expressed in rat small intestine and colon, whereas the CRF_2 receptor ligand urocortin-3 is detected in the human colonic myenteric and submucosal plexuses [355,356]. CRF elicits calcium currents in myenteric neurons through the activation of CRF_1 receptors [357]. In rats, peripheral CRF_1 receptor activation elicits duodenal contractions, whereas CRF_2 receptor stimulation inhibits ileal motility [358]. Intraperitoneal CRF acting on CRF_1 receptors elicits spike bursts in rat colon by stimulating myenteric cholinergic neurons [359,360]. However, peripheral effects of CRF may be mediated by the activation of afferent pathways projecting to several brain nuclei (amygdala, area postrema, nucleus tractus solitarius, locus caeruleus, and hypothalamus), which then activate the efferent pathways to the viscera [361]. In rats, subcutaneous administration of CRF elicits colonic contractions by way of vagal cholinergic pathways and activation of CRF receptors in the brain [362].

CNS transmitters other than CRF may function independently to regulate small intestinal and colonic motility. The inhibition of NO synthase in the brain suppresses duodenal phase III in dogs through vagal pathways [363]. NO synthase inhibitors suppress colonic activity when given centrally, but increase colonic contractions when given peripherally. Destruction of hypothalamic and locus caeruleus adrenergic pathways with 6-hydroxydopamine prolongs the MMC cycle [364]. ICV CCK increases whereas ICV somatostatin decreases MMC cycle duration. CCK infusion into the paraventricular nucleus slows colonic transit by acting on CCK_2 receptors [365]. ICV calcitonin, CGRP, neurotensin, and opioids evoke intestinal phase III activity. ICV neurotensin delays MMC disruption by a meal, whereas ICV atropine or substance P shortens the duration of the fed state [366]. ICV galanin restores MMC cycling during the fed state by way of opioid pathways [367]. Injection of a GABA antagonist into the dorsomedial hypothalamic nucleus increases jejunal contractions in rats [368]. ICV GLP-1 increases fecal output, whereas orphanin FQ retards colonic transit [369]. ICV thyrotropin-releasing hormone accelerates intestinal transit and evokes colonic contractions in rats by way of cholinergic and serotonergic pathways [370]. ICV ghrelin induces fasting duodenal motor activity in fed rats, an effect blocked by ICV injection of growth hormone secretagogue receptor and by NPY immunoneutralization in the brain [371]. ICV injections of α_2-adrenoceptor agonists, morphine, and [Leu]enkephalin blunt the increase in EAS activity elicited by pudendal nerve stimulation [372].

Immune and inflammatory modulation

Activation of the immune system elicits profound changes in the motor activity of the small intestine and colon, as evidenced in several animal models. In rats that are antigen sensitized by intraperitoneal egg albumin injection, subsequent oral albumin evokes diarrhea associated with MMC disruption and induction of propagating clustered contractions, whereas colonic antigen challenge increases colonic myoelectric activity [373]. In animal models of postoperative ileus, intestinal manipulation leads to acute disruption of ICC networks, altered slow wave rhythmicity, suppressed jejunal contractions, and delayed transit (Fig. 11.11) [374]. In a rat model of peritonitis and sepsis induced by cecal puncture, intestinal transit is delayed and in vivo jejunal muscle contractility is impaired [375]. Ileal muscle strips from these animals exhibit reduced contractile responses to potassium, substance P, and cholinergic stimulation. Responses to specific organisms have been characterized. In rats, *Yersinia enterocolitica* and *E. coli* alter MMC cycling by free-radical

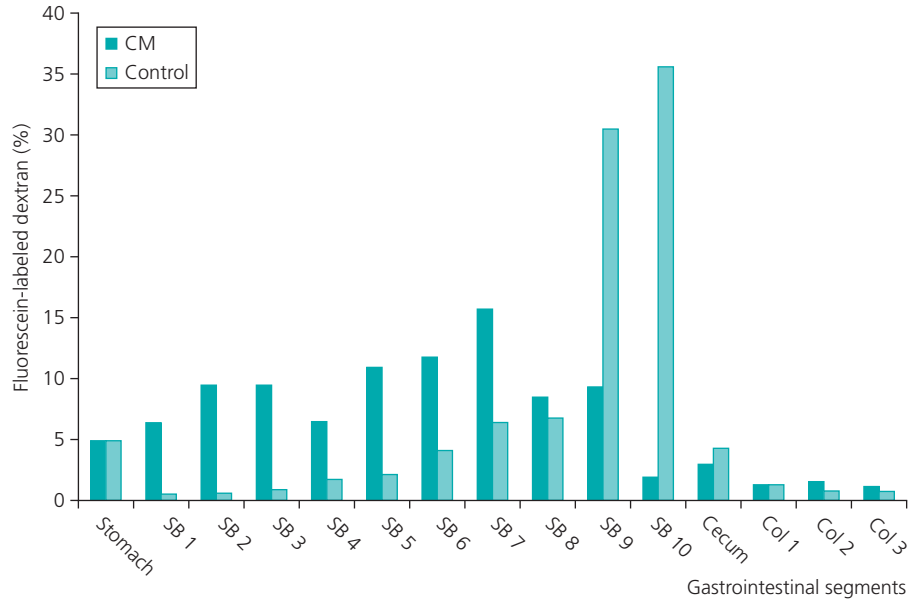

Figure 11.11 Transit histograms for control animals (light bars) and animals undergoing colonic manipulation (CM) to induce postoperative ileus (dark bars). Colonic manipulation led to marker accumulation in the stomach and proximal small intestine, whereas markers passed to the distal small intestine and cecum in the control animals. From Turler et al. [398].

generation [376]. In germ-free rats, *Clostridium tabificum* vp 4 promotes intestinal spike burst activity [377]. *C. tabificum* vp 4, *Lactobacillus acidophilus* A10, and *Bifidobacterium bifidum* B11 reduce MMC periodicity and accelerate small intestinal transit, whereas *Micrococcus luteus* increases MMC duration [377]. The heat-labile enterotoxin of *E. coli* prolongs phase II, increases the MMC period, and accelerates phase III propagation in pigs [378]. In vitro, *E. coli* endotoxin modifies peristalsis and blunts the inhibitory effects of adrenergic receptors on motor function in the guinea pig small intestine [379]. Similarly, lipopolysaccharide reduces spontaneous and acetylcholine-evoked contractions in isolated rabbit small intestine. In pigs, endotoxin accelerates jejunal MMC migration and shortens MMC duration [380]. In rats given *Serratia marcescens* endotoxin, ileal responses are suppressed [381]. Rats infected with *Hymenolepis diminuta* exhibit an increase in fasting intestinal myoelectric activity and the generation of spike potential bursts, responses mediated by the myenteric plexus [382]. Gastrointestinal transit is slowed and ileal smooth muscle is thickened in mice infected with *Schistosoma mansoni* [383]. *Nippostrongylus brasiliensis* enhances jejunal motor responses to carbamylcholine and neurokinin A [384]. Increased responses to CCK after infection with this organism are blocked by vagotomy [385]. With peak inflammation at day 14 of infection with *N. brasiliensis*, there is a loss of NK_1 receptors in myenteric neurons and in substance P-immunoreactive nerve endings [386]. By day 30 of infection there is loss of deep muscular plexus ICCs.

Significant insight into immune modulation of gut function has been provided by study of the nematode *Trichinella spiralis*. This organism induces GMCs in the small intestine, inhibits myenteric nerve activity, decreases neuron membrane potential, and reduces acetylcholine and norepinephrine (noradrenaline) release, with peak effects 6 days after infection [387]. *T. spiralis* reduces intestinal substance P levels by 73% and VIP levels by 59% and inhibits NO synthase gene transcription, protein expression, and enzyme activity [388]. Damage to ICCs with slow wave destabilization, impaired distention-evoked peristalsis, and the loss of contact between ICCs and smooth muscle are observed [389,390]. Jejunal muscle from rats infected with *T. spiralis* exhibits increased responses to muscarinic agonists, CCK, and serotonin [391]. Nerve and smooth muscle effects of *T. spiralis* persist after resolution of acute infection [392]. In mice previously infected, frequencies and propagation velocities of intestinal contractions decrease and retroperistaltic contractions increase more than 40 days after eradication of infection [393].

A hallmark of all of these animal models is the recruitment of inflammatory cell infiltrates that contribute to gut motor impairment. Intestines from egg albumin-sensitized rats exhibit increased numbers of mast cells and increased levels of mast cell protease [394]. Mast cell stabilizers and degranulation inhibitors blunt antigen-induced motor responses and diarrhea, and granulated mast cells are reduced at sites of antigen challenge [395]. In rat colon longitudinal muscle, antigen-induced contraction results from immunoglobulin E-mediated mast cell activation, which then evokes release of contractile stimulants [396]. Similarly, the motor effects caused by infection with *N. brasiliensis* are prevented by mast cell degranulation inhibitors and mast cell stabilizers [384]. In animal models of postoperative ileus, intestinal and colonic manipulation increases intestinal permeability and promotes extravasation of leukocytes and macrophages into

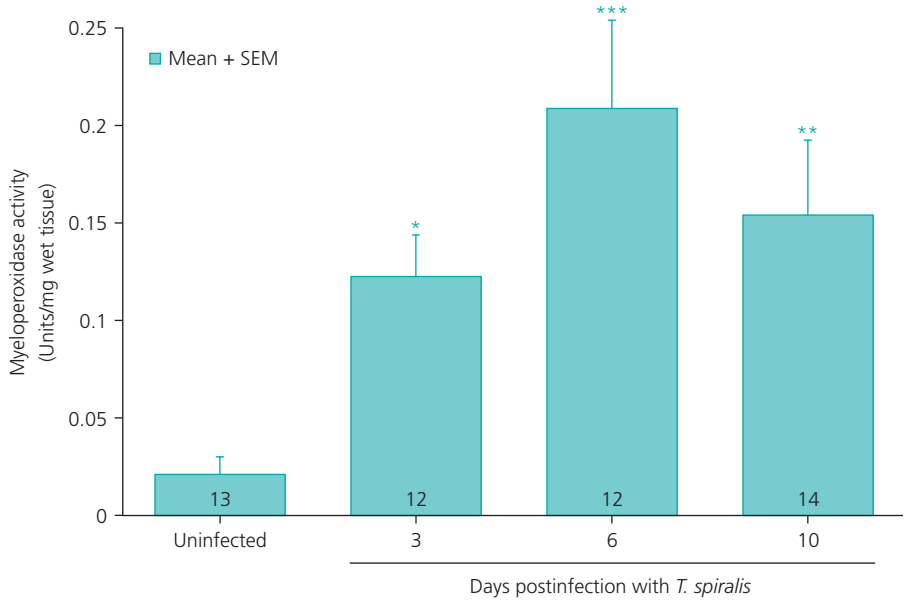

Figure 11.12 Myeloperoxidase activity, a reflection of inflammation intensity, before and after infection with *Trichinella spiralis* in rat. Infection elicited a marked increase in myeloperoxidase activity, which peaked 6 days after infection and persisted for at least 10 days. From Palmer et al. [387].

the intestinal smooth muscle layers [397,398]. Similarly, experimental peritonitis induces prominent mononuclear smooth muscle infiltrates, whereas *T. spiralis* causes transient inflammatory cell infiltration into the longitudinal muscle (Fig. 11.12) [387,389]. The inhibition of myenteric nerve activity that accompanies infection with *T. spiralis* is preserved in athymic rats, suggesting the involvement of T-cell independent pathways [399]. Conversely, athymic animals do not exhibit increased contractions to muscarinic agonists and serotonin, indicating T-cell dependent responses as well. Furthermore, reconstitution of CD4 T cells to mice that are athymic, or deficient in CD4 cells, or major histocompatibility complex II deficient reverses the enhanced muscle contractility [400].

Many of the motor effects of experimental inflammation are postulated to result from release of proinflammatory cytokines and other mediators. Models of postoperative ileus are associated with up-regulation of IL-6 mRNA, tumor necrosis factor-α (TNF-α), COX-2, and NO synthase, as well as activation of signal transducer and activation of transcription (STAT) proteins in the smooth muscle layers, responses that are blunted by dexamethasone or by a protein tyrosine kinase inhibitor [398,401,402]. Human gut smooth muscle tissues harvested at various times after laparotomy demonstrate time-dependent increases in IL-6, IL-1β, TNF-α, NO synthase, and COX-2, which are localized to macrophages [403]. Experimental peritonitis is associated with increases in IL-6, IL-1β, monocyte chemoattractant protein 1, and inducible NO synthase. Mononuclear cells may be recruited by monocyte chemoattractant protein 1, a chemokine secreted by macrophages [404]. In a model of hemorrhagic shock, increases in intercellular adhesion molecule 1 (ICAM-1), IL-6, and granulocyte colony-stimulating factor contribute to neutrophil recruitment with subsequent tissue injury [405]. ICAM-1 antisense oligonucleotides prevent postoperative ileus in mice and reduce manipulation-induced inflammation [406]. In other models, COX-2 predominates over COX-1 in maintaining inhibition of colonic neuromuscular function [407]. The effects of endotoxin on intestinal motility are reversed by polyethylene glycol–superoxide dismutase, indicating a role for oxidative stress in the development of septic ileus [408]. TNF-α plays a crucial role in impairing motor activity in other models. In contrast to wild-type animals, knockout mice lacking TNF-α do not show suppressed colonic contractile responses to motor stimulants in animals with experimental colitis induced by intracolonic administration of 2,4,6-trinitrobenzenesulphonic acid [409]. Cytokine responses evoked by infection with *T. spiralis* are affected by CD40–CD40 ligand interactions with the T helper cell response [410]. Acute *T. spiralis* infection increases levels of IL-4, IL-13, transforming growth factor-β1 (TGF-β1), and COX-2 in muscle tissue [411]. The effects of this organism on nerves are blocked by an IL-1 antagonist, whereas increases in muscle contractility are blunted in IL-5-deficient mice [412]. IL-4 and IL-13 induce smooth muscle hypercontractility through activation of STAT-6 [413]. Many cytokine responses observed on infection with *T. spiralis* (i.e., increased levels of TGF-β1, COX-2, prostaglandin E_2) are reversed by administration of probiotics containing *L. paracasei*, indicating modulation of the immune response by changes in the enteric microbiota [414].

Interactions between cytokines and neurohumoral pathways underlie many of the modulatory effects of the immune system on motor function in the small intestine and colon. Capsaicin prevents egg albumin antigen effects in the small intestine, reflecting the participation of afferent pathways

[415]. Antigen-induced motor responses in the colon are prevented by the serotonin antagonists methysergide and cinanserin and the prostaglandin synthesis inhibitor indomethacin (indometacin) [416]. In sensitized rats, egg albumin MMC disruption is associated with increased c-Fos expression in the nucleus tractus solitarius and lateral parabrachial and paraventricular hypothalamic nuclei by way of vagal afferent pathways [417]. Inflammatory changes in experimental postoperative ileus occur in association with increased Fos expression in the spinal cord, suggesting activation of primary afferent pathways [418]. Neurotransmitter defects in ileus are prominent. α_2-Adrenergic receptor activation aggravates postoperative ileus in rats by enhancing NO synthase induction, thereby increasing NO release [419]. Mice deficient in inducible NO synthase have nearly normal intestinal transit and in vitro smooth muscle contractility after intestinal manipulation, supporting a role for NO pathways in ileus [420]. Similarly, in hemorrhagic shock and with bacterial infection or infection with *T. spiralis*, inhibition of inducible NO synthase reduces cytokine production, limits inflammatory cell infiltration, normalizes intestinal transit, and restores muscle contractility [405,421,422]. In a model of ileus induced by intraperitoneal acetic acid, endocannabinoid CB_1 receptors are overexpressed; acetic acid-induced ileus is reversed by CB_1 antagonists [423]. Carbon monoxide, a purported gaseous transmitter, prevents postoperative ileus by modulating cytokine levels within the inflammatory cascade [424,425]. The motor effects evoked by infection with *S. marcescens* are reversed by lipoxygenase inhibition, indicating mediation by peptidoleukotrienes. In rats, lipopolysaccharide-induced acceleration of intestinal transit is blocked by CB_2 antagonists [426]. Lipopolysaccharide-evoked disruption of small intestinal motor function is also partially blocked by an antagonist to adrenomedullin [427]. In previously infected rats, subsequent exposure to *T. spiralis* larvae evokes muscle contraction, which can be blocked by a serotonin antagonist and the mast cell stabilizer doxantrazole [428]. Motor responses to *T. spiralis* are prevented by treatment with an antibody to nerve growth factor, although the inflammatory infiltrate is unaffected by this therapy [429].

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B MOTILITY

12

Motility of the biliary tract

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Understanding biliary tract function is a clinically significant goal. In the United States alone, over 20 million people have gallstones or have had a cholecystectomy. Furthermore, the total direct costs associated with biliary tract disease, which amount to billions of dollars annually, are greater than the costs for any other gastrointestinal ailment, including colorectal cancer and peptic ulcer disease [1]. Abnormal motility is a hallmark of biliary tract disorders, including both calculous and acalculous etiologies, and it is therefore important to understand how motility of the gallbladder and sphincter of Oddi (SO) are normally regulated, and the changes in the physiology of these structures that lead to dysmotility under pathophysiological conditions.

This chapter provides an overview of biliary tract motor activity and its regulation. As motility primarily involves the activities of two cell types, neurons and smooth muscle, the chapter is organized to provide a summary of the basic physiology of the nerves and smooth muscle in the gallbladder and SO with clinical correlations provided whenever possible. These sections also describe how the nerves and smooth muscle of these organs function during the bile retention and bile flow phases of the feeding cycle. Finally, a section at the end of the chapter describes what is known about the roles of nerves and smooth muscle in the biliary tract under pathophysiological conditions.

The gallbladder

Given that biliary tract dysmotility is so prevalent in gallbladder disease, a comprehensive understanding of the neurons and smooth muscle that are responsible for gallbladder contractile activity is critical. Determination of the cellular mechanisms that are responsible for normal and pathological gallbladder motility is difficult in humans; therefore, much of what is known about the structure and function of gallbladder neurons and smooth muscle is derived from animal studies.

Gallbladder neurobiology

The wall of the gallbladder consists of a mucosal layer with an underlying lamina propria, a muscularis layer, and a serosal layer. Neural networks can be found in each of these layers, and these neural networks are interconnected by nerve bundles. The morphology of nerves of the gallbladder has been investigated in many species including humans, rhesus monkeys, pigs, dogs, cats, marmosets, guinea pigs, North American opossums, Australian brush-tailed possums, and mice [2–8]. The serosal plexus is the most prominent network of nerves in the gallbladder. It contains small, irregularly shaped ganglia that are connected by bundles of unmyelinated axons. The serosal plexus is connected to nerve bundles that follow the extensive vascular distribution in this layer. The neural plexus of the muscularis, which does not contain ganglia, is prominent in humans and other larger species. The mucosal plexus consists of nerve bundles that travel in the lamina propria, with branches that pass through the mucosa and often terminate near the epithelial cells. In some species, including humans, the mucosal plexus contains occasional small ganglia consisting of one to three neurons, and these neurons project to the mucosal epithelium [9].

Neuroactive compounds in gallbladder nerves

Gallbladder neurons

Evaluating the influence of gallbladder neurons on gallbladder function requires knowledge of the neurotransmitters made by these cells. The complexity of the enteric nervous system is reflected by the large number of neuroactive compounds that are found in the neural plexuses of the gut. Studies of the neurotransmitter content of gallbladder neurons have revealed that many putative neurotransmit-

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ters also exist in this system. However, unlike the gut, where distinct populations of cholinergic and noncholinergic neurons exist, all gallbladder neurons are apparently cholinergic as all are immunoreactive for choline acetyltransferase (ChAT), the essential biosynthetic enzyme for acetylcholine [5,9,10].

In the guinea pig, the most extensively studied species, the overall population of cholinergic neurons can be divided into two distinct subpopulations based on chemical coding patterns [11,12]. The larger population, representing over 80% of the neurons, is immunoreactive for substance P, neuropeptide Y (NPY), somatostatin, and orphanin FQ (OFQ, also known as nociceptin), as well as ChAT. The remaining neurons are immunoreactive for ChAT, vasoactive intestinal polypeptide (VIP), pituitary adenylate cyclase-activating polypeptide (PACAP) and nitric oxide synthase (NOS).

Chemical coding of gallbladder neurons has also been described in other species, including humans [5,7–9,13,14]. Most human gallbladder neurons express VIP, NPY, somatostatin, and PACAP, and most of these neurons are also tachykinin immunoreactive [5,7,8,13]. NOS immunoreactivity and nicotinamide adenine dinucleotide phosphate (NADPH) dehydrogenase staining have also been reported in a small subset of human gallbladder neurons; however, unlike the pattern seen in the guinea pig, the NOS-positive neurons are VIP negative [13].

Sympathetic nerve fibers

Catecholamine histofluorescence, and antibodies directed against the biosynthetic enzymes tyrosine hydroxylase and dopamine β-hydroxylase, have been used to identify the sympathetic postganglionic nerves in the wall of the gallbladder [2,4,9]. Injection of axon tracers into the gallbladder wall shows that sympathetic postganglionic projections to the gallbladder arise in the celiac ganglia [4]. Sympathetic postganglionic nerves pass along serosal blood vessels and they are also abundant in the ganglionated plexus of the gallbladder, with numerous axonal varicosities surrounding gallbladder neurons [4]. In the perivascular plexus of the guinea pig and human gallbladders, the sympathetic nerve fibers are also immunoreactive for NPY, and research in the guinea pig has confirmed OFQ immunoreactivity [12].

Sensory nerve fibers

It is not yet possible to distinguish sensory (afferent) fibers that arise from spinal ganglia from those that arise from nodose ganglia; however, the ganglionated plexus of the gallbladder is rich in afferent nerve fibers that are immunoreactive for both substance P and calcitonin gene-related peptide (CGRP) [4,5,9,10,15]. These nerves are likely to be extrinsic primary afferent fibers as gallbladder neurons are not immunoreactive for CGRP [5,8]. The substance P/CGRP-immunoreactive nerve fibers are abundant in ganglia, interganglionic fiber bundles, and the perivascular plexus. Within the ganglia, the substance P/CGRP fibers appear to ramify and give rise to processes that terminate on gallbladder neurons. Substance P/CGRP-positive nerve fibers are also immunoreactive for PACAP [12], suggesting that activated afferent nerves can release tachykinins, CGRP, and PACAP.

Electrical and synaptic properties of gallbladder neurons

The electrical properties of gallbladder neurons have been studied in three species: guinea pig, opossum, and human [6,16,17]. Unlike enteric neurons, gallbladder neurons can be classified into a single group on the basis of their electrical properties. Relatively inexcitable, gallbladder neurons rarely exhibit spontaneous action potentials, and they fire stimulus-induced action potentials only at the onset of a depolarizing current pulse. These features indicate that gallbladder neurons are normally quiescent and must be driven by excitatory inputs to release their neurotransmitters onto their target tissues, such as smooth muscle. Therefore, the neural regulation of the gallbladder is more reminiscent of the dual parasympathetic–sympathetic innervation seen in organs such as the heart and the urinary bladder, as opposed to the intrinsic reflex circuitry that is characteristic of the intestines.

All gallbladder neurons receive fast excitatory synaptic input – the principal driving force in the neuromuscular axis of the gallbladder [6,16–18]. Fast excitatory postsynaptic potentials (EPSPs) in the gallbladder are mediated exclusively by acetylcholine. Unlike the ganglia of the intestines, where most fast synaptic inputs arise from nearby neurons, the main source of fast synaptic input to gallbladder neurons is vagal preganglionic nerve terminals [18]. Stimulation of vagus nerves in vivo elicits gallbladder contraction [19–21]. Furthermore, neurons in the dorsal motor nucleus of the vagus are labeled after retrograde axonal tracers are injected into the gallbladder wall [4]. About 20% of the neurons in the gallbladder appear to receive interganglionic input from other gallbladder neurons [18].

Slow EPSPs are detected in about 25% of gallbladder neurons and are activated by an influx of Na^+ through nonselective cation channels [6,16,22]. They are mediated by the release of peptides from extrinsic afferent fibers, which may be associated with the subjective experience of biliary pain. A subset of the sensory fibers in the wall of the gallbladder is activated only by noxious stimulation of the biliary system, indicating that these are pure visceral nociceptors [23]. Within the gallbladder, the pain afferents are likely to be immunoreactive for both substance P and CGRP [4,5,24]. Capsaicin, which causes peptide release from small-diameter afferent fibers, induces the release of substance P and CGRP when it is applied to the gallbladder in vitro [24]. When applied to gallbladder neurons, tachykinin, CGRP, and capsaicin all cause a membrane depolarization that is mediated by neurokinin (NK) type 3 receptors [22,25]; NK_3 receptor blockade inhibits the slow EPSP and the depolarization

caused by capsaicin [22]. Therefore, sensory fibers that pass through gallbladder ganglia can act locally to modulate ganglionic output by synapsing on gallbladder neurons and increasing their excitability. This local axon reflex circuit may contribute to a cycle that induces the subjective experience of biliary pain in gallbladder disease. Increased ganglionic output caused by slow EPSPs may lead to increased gallbladder contractility. If bile outflow from the gallbladder is inhibited or obstructed, pressure may then increase to higher levels leading to a feed-forward cycle of sensory fiber activation. Activated sensory fibers elicit additional stimulation of gallbladder neurons, leading to further increases in intralumenal pressure and enhanced perception of pain.

Gallbladder smooth muscle cells

Gallbladder smooth muscle cells are arranged in interposed bundles that are oriented in various directions within the muscularis of the gallbladder [26]. Within each bundle these smooth muscle cells appear to be coupled because intact cells have a very low input resistance [27] and rapid Ca^{2+} transients occur simultaneously in all of the smooth muscle cells of a given bundle [28].

Electrical properties of gallbladder smooth muscle

The electrical properties of gallbladder smooth muscle cells have been investigated in intact preparations using intracellular microelectrodes and in isolated myocytes using patch clamp recording techniques [27,29]. Intact gallbladder smooth muscle cells generate spontaneous action potentials that consist of a rapid spike followed by a plateau phase [27]. Spontaneous action potentials typically occur at a frequency of 0.3–0.4 Hz, which is significantly faster than the frequency of slow wave potentials in gastrointestinal smooth muscle. Two of the principal channels involved in the gallbladder smooth muscle action potential are the L-type Ca^{2+} channel and the delayed rectifier K^+ channel. The L-type Ca^{2+} channel blocker, nifedipine, abolishes the gallbladder smooth muscle action potential in intact tissue, as well as the voltage-activated inward current in isolated cells [27,29]. The amplitude and duration of the spike and plateau phases of the action potential are augmented by the voltage-activated K^+ (K_V) channel blocker, 4-aminopyridine, and most of the voltage-activated outward current is mediated by K_V channels [27,30]. Despite the clear involvement of dihydropyridine-sensitive Ca^{2+} channels and K_V channels in the action potential of gallbladder smooth muscle, the mechanisms responsible for pacing of the action potential and the generation of the plateau phase have not been resolved. ERG channels, which are expressed in the gallbladder smooth muscle, likely contribute to the repolarization of the plateau. ERG channel blockers prolong the plateau phase of the action potential, resulting in prolonged action potentials with multiple spikes and a depolarization of the membrane potential [31]. Another current that contributes to the background activity of gallbladder smooth muscle cells is a steady state nonselective cation current. When this current is inhibited by Na^{2+} substitution, the cells become hyperpolarized with an associated elimination of spontaneous action potentials [32].

Other channels that have been identified in gallbladder smooth muscle include ATP-sensitive K^+ (K_{ATP}) channels and large-conductance Ca^{2+}-activated K (BK) channels. BK channels have been identified in single-channel recordings from gallbladder smooth muscle [27] and are responsible for spontaneous transient outward currents in these cells [33]. BK channel currents are activated by local Ca^{2+} release (i.e., sparks) through ryanodine receptor (RyR) channels of the endoplasmic reticulum. Although the functional relevance of sparks and transient outward currents in gallbladder smooth muscle is not completely understood, a role in the regulation of gallbladder smooth muscle excitability seems plausible as they are inhibited by cholecystokinin (CCK) [33].

K_{ATP} channels appear to play an important role in gallbladder smooth muscle hyperpolarization and relaxation [34–36]. Activation of the K_{ATP} channel with the activators lemakalim or pinacidil causes a prolonged hyperpolarization that is associated with an elimination of spontaneous action potentials. K_{ATP} channel activation is responsible for the inhibitory effects of CGRP and histamine H_2 receptor agonists [35,37]. Activation of the K_{ATP} channel involves the activation of the cyclic AMP adenylate cyclase–protein kinase A signal transduction cascade [34,36], whereas activation of protein kinase C inhibits K_{ATP} channel function [36,38].

Calcium handling in gallbladder smooth muscle

Ca^{2+} influx and Ca^{2+} release from intracellular stores is critical for gallbladder smooth muscle function as gallbladder contractility depends mainly on increases in the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$. Whole-mount preparations treated with a Ca^{2+}-sensitive dye have shown the presence of two types of rhythmic spontaneous Ca^{2+} transients in gallbladder smooth muscle cells: Ca^{2+} flashes and Ca^{2+} waves [28,39]. Flashes appear to be Ca^{2+} transients that are directly associated with the spontaneous action potentials in gallbladder smooth muscle [28]. They occur simultaneously in all of the smooth muscle cells within a given bundle, but are not synchronized among nonintersecting bundles. Waves are rhythmic Ca^{2+} transients that spread within a given smooth muscle cell, apparently corresponding to subthreshold depolarization of gallbladder smooth muscle cells [39]. Similar to spontaneous electrical activity, flashes and waves are sensitive to L-type Ca^{2+} channel and inositol triphosphate (IP_3) inhibitors, and are augmented by excitatory agonists in a phospholipase C-dependent manner, which suggests that they play a role in spontaneous excitability and pacemaking in gallbladder smooth muscle. Interestingly, synchronous Ca^{2+} flashes occur among smooth muscle bundles in the presence of CCK or muscarinic agonists. The spatiotemporal pat-

terns of these events support a model in which asynchronous electrical and contractile activity of gallbladder smooth muscle bundles throughout the muscularis layer is responsible for maintenance of net tone in the organ. Furthermore, synchronous global electrical rhythms that likely result from excitatory agonist stimulation may contribute to gallbladder emptying.

Functional studies indicate that Ca^{2+} entry through L-type Ca^{2+} channels is required for gallbladder smooth muscle to respond to excitatory neurohormonal stimulation. However, CCK-induced contraction involves Ca^{2+} release from intracellular stores, which have both IP_3 receptors and RyRs and which are dependent on sarcoplasmic/endoplasmic reticulum calcium ATPase (SERCA) pumps for refilling and maintenance [40]. Ca^{2+} release from intracellular stores not only induces contraction but also relaxation (Figs 12.1 and 12.2). As previously described, release of Ca^{2+} through RyR channels as sparks activates BK channels causing hyperpolarization and relaxation [33]. These divergent functions of Ca^{2+} stored in a single intracellular domain can only be explained by the discrete location of the IP_3 and RyR Ca^{2+} release channels. This cellular model allows for the heterogeneity of the Ca^{2+} signaling, supporting the more versatile and complex role of intracellular Ca^{2+} mobilization. In addition, Ca^{2+} release and Ca^{2+} influx mechanisms are interrelated, as emptying of stores induces the coactivation of capacitative and L-type Ca^{2+} channels [40], underscoring the important role of L-type Ca^{2+} channels in gallbladder function. Evidence that reorganization of the actin cytoskeleton is necessary for Ca^{2+} depletion-induced Ca^{2+} entry supports a conformational coupling model in gallbladder smooth muscle cells [41]. Alterations in Ca^{2+} handling (related to both Ca^{2+} influx and Ca^{2+} release mechanisms) can be responsible for gallbladder stasis in inflammation [42].

Gallbladder interstitial cell of Cajal-like cells

Spontaneous rhythmic electrical activity is a striking feature of gallbladder smooth muscle cells. Evidence of this activity is twofold: (1) action potentials detected in individual cells using intracellular microelectrodes and (2) Ca^{2+} flashes detected in muscle bundles of intact preparations using laser confocal Ca^{2+} imaging. Our laboratory has provided abundant evidence of specialized pacemakers cells capable of generating spontaneous rhythmic activity in the gallbladder muscularis. We refer to these cells as interstitial cell of Cajal (ICC)-like cells because they share a number of features with the intestinal cells described by Ramon y Cajal over a century ago and, further, they have been shown to generate pacemaker activity in the gut and to play a role in mediating neuromuscular signaling [43].

Light microscopy shows fusiform Kit-immunoreactive cells that are oriented parallel to adjacent muscle fibers in the muscularis layer. Electron microscopy shows cells in muscle bundles that have features of ICCs, including an electron-dense cytoplasm and an abundance of mitochondria and calveoli. These cells are in direct contact with each other and with gallbladder smooth muscle cells. Furthermore, Ca^{2+} imaging also reveals cells with a higher intensity of fluo-4 fluorescence than the surrounding muscle cells. Ca^{2+} flashes in the ICC-like cells that are synchronized with the gallbladder smooth muscle cell flashes can be detected but, interestingly, in the presence of gap junction blockers the flashes persist in the ICC-like cells but not in the gallbladder smooth muscle cells. Finally, in isolated gallbladder smooth muscle

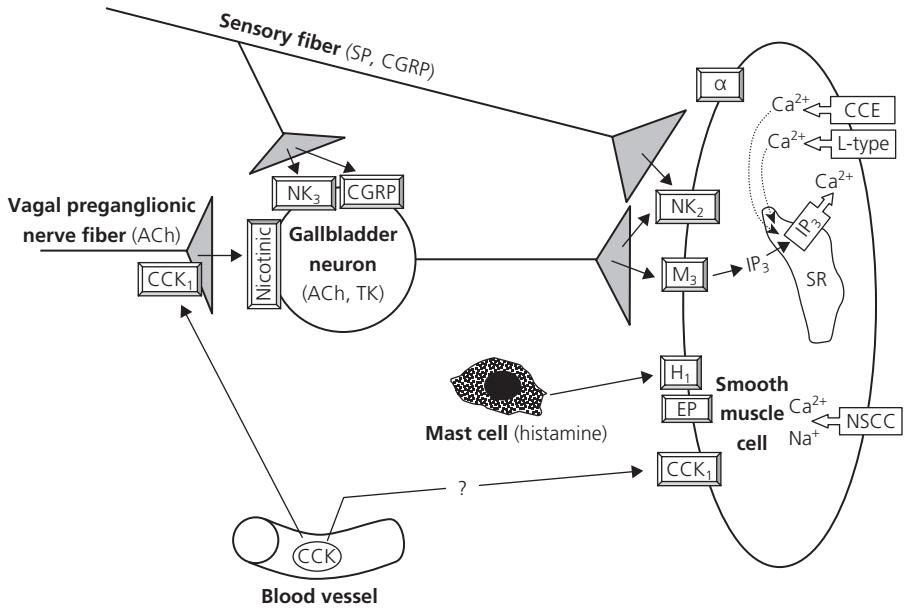

Figure 12.1 Excitatory transmitters, hormones, inflammatory mediators, receptors, and ion channels that have been identified in the neuromuscular axis of the gallbladder. Nerve terminals are represented by triangles, receptors by framed boxes, and ion channels by boxes with arrows indicating the direction of ion flow. α, α-Adrenergic receptor; ACh, acetylcholine; CCE, capacitative calcium entry; CCK, cholecystokinin; CGRP, calcitonin gene-related peptide; EP, prostaglandin E; H, histamine; IP_3, inositol 1,4,5-triphosphate; M, muscarinic; NK, neurokinin; NSCC, nonselective cation channel; SP, substance P; SR, sarcoplasmic reticulum; TK, tachykinins.

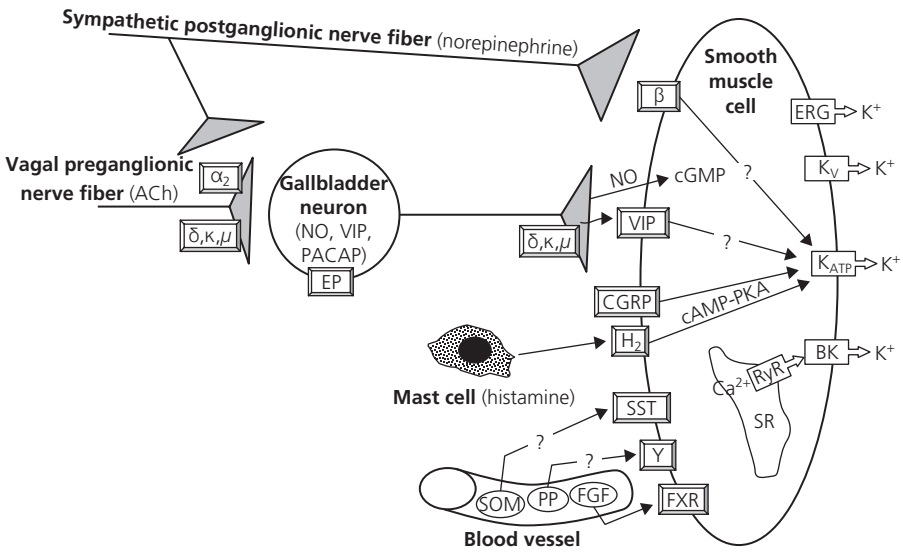

Figure 12.2 Inhibitory transmitters, hormones, inflammatory mediators, receptors, and ion channels that have been identified in the neuromuscular axis of the gallbladder. Nerve terminals are represented by triangles, receptors by framed boxes, and ion channels by boxes with arrows indicating the direction of ion flow. α_2 and β, adrenergic receptors; δ, κ, and μ, opioid receptors; ACh, acetylcholine; BK, large-conductance Ca^{2+}-activated K^+ channel; CGRP, calcitonin gene-related peptide; EP, prostaglandin E; FGF, fibroblast growth factor; FXR, farsenoid X receptor; H, histamine; K_{ATP}, ATP-sensitive K^+ channel; K_V, voltage-activated K^+ channel; NO, nitric oxide; PACAP, pituitary adenylate cyclase-activating peptide; PKA, protein kinase A; PP, pancreatic polypeptide; RyR, ryanodine receptor; SOM, somatostatin; SR, sarcoplasmic reticulum; SST, somatostatin receptor; VIP, vasoactive intestinal polypeptide; Y, neuropeptide Y/PP receptor.

cells, spontaneous rhythmic activity was not detected even when the cells were warmed to 36°C. Collectively, these findings indicate that the spontaneous rhythmic activity that is detected in gallbladder smooth muscle, and which corresponds to smooth muscle bundle contractions, is generated by specialized ICC-like cells and is not an intrinsic property of gallbladder smooth muscle.

Gallbladder emptying

Neuroendocrine control of gallbladder contraction

Numerous neurotransmitters and hormones are capable of causing gallbladder contraction, as demonstrated by functional studies involving measurement of gallbladder pressure or muscle strip tension (see Fig. 12.1). The three compounds that participate physiologically in gallbladder contractile events are CCK, acetylcholine, and tachykinins.

Cholecystokinin

One of the first and most significant hormones to be identified, CCK was named in 1928 by Ivy and Oldberg [44] for its ability to contract the gallbladder. The mechanisms of CCK action in the gallbladder are described in greater detail in the section on postprandial gallbladder contraction.

Acetylcholine

As described previously, all gallbladder neurons are cholinergic, and stimulus-induced release of acetylcholine from these neurons results in the contraction of gallbladder smooth muscle. Although evidence of M_1, M_2, M_3, and M_4 muscarinic receptors has been reported in the gallbladder [45–48], the main muscarinic receptor subtype in the smooth muscle cells is M_3, whose activation induces smooth muscle contraction [45,46]. Activation of M_3 receptors leads to phosphatidylinositol hydrolysis by phospholipase C as well as inhibition of cAMP formation [49,50].

Tachykinins

Tachykinins are prevalent in the nerves of the gallbladder, including extrinsic afferent nerves as well as gallbladder neurons and their axons. Tachykinins produce a direct, concentration-dependent contraction of the isolated gallbladder, with a rank order potency of NKA > NKB > substance P [21,24,51], which is characteristic of NK_2 receptors. In the gallbladder, the binding of tachykinins to NK receptors is linked to protein kinase C activation [51]. The coexpression of tachykinins with acetylcholine in gallbladder neurons indicates that these compounds may act together to promote gallbladder emptying on vagal stimulation.

Postprandial gallbladder contractions

Postprandial gallbladder contraction is triggered by gastric emptying leading to the release of CCK from enterochromaffin cells in the epithelial lining of the duodenum. Although the concept that CCK acts as a hormone to cause gallbladder emptying is well established, it is also likely that CCK acts at several sites to promote functional gallbladder motility (see Fig. 12.1). The most direct means of gallbladder contraction is for hormonal CCK to act on receptors located on gallbladder smooth muscle, which expresses the CCK_1 but not the CCK_2 receptor [52]. It is likely that low-affinity CCK_1 receptors are present on gallbladder smooth muscle, as the EC_{50} for CCK-induced gallbladder contraction is in the 10–50 nM range [53–55]. However, it is unclear whether the CCK receptors on gallbladder smooth muscle are a normal physiological site of action for CCK because postprandial serum concentrations of CCK are in the 10–20 pM range

[56,57], which is far below the threshold necessary for a direct action of CCK on gallbladder muscle strips. Therefore, a neural mechanism must be involved in the prokinetic effects of postprandial CCK release.

Meal-induced gallbladder contractions and contractions induced by physiological concentrations of CCK in vivo are significantly attenuated by neural blockade in several species, including humans [57–66]. Furthermore, hexamethonium, which blocks the vagal preganglionic input to gallbladder neurons, inhibits CCK- and meal-induced gallbladder contractions [57,63,64]. These data indicate that CCK can act on nerves to promote gallbladder motility.

Results of electrophysiological studies suggest that CCK does not have a direct effect on gallbladder neurons, but rather a potent presynaptic excitatory effect on nerve terminals in gallbladder ganglia, thereby increasing the release of acetylcholine onto gallbladder neurons [6,18,67]. CCK enhances vagally mediated fast EPSPs in gallbladder ganglia by way of a presynaptic facilitory action on vagal postganglionic nerve terminals [6,67]. Note that the action of CCK in gallbladder ganglia occurs within the physiological concentration range for CCK (EC_{50} of 33 pM).

Vagal afferent nerve fibers in the duodenum are another likely site of action for CCK released from enterochromaffin cells. Subdiaphragmatic vagal afferent fibers are sensitive to CCK, and postprandial physiological responses, such as increased gastric motility and pancreatic secretion, have been attributed to CCK-mediated increases in vagal afferent activity [68,69]. After a meal, CCK stimulates vagal afferent nerve fibers, which act in the vagal motor complex to increase the rate of firing of vagal preganglionic neurons. Furthermore, CCK acts in gallbladder ganglia, as previously discussed, to increase the amount of acetylcholine released from the vagal motor terminals each time they are activated.

The questions remain of how CCK-induced gallbladder emptying still occurs (albeit diminished) after vagotomy, or after liver transplantation with the donor gallbladder intact, if CCK normally acts through the neural mechanisms described. The answers to these questions probably lie in the fact that gallbladder emptying involves increased gallbladder tone in concert with a decrease in resistance at the SO. As described in the section on interprandial sphincter of Oddi activity, it is likely that the circuitry responsible for the CCK-induced decrease in SO resistance remains intact after vagotomy or liver transplantation. Therefore, changes in SO tone, in combination with the myogenic tone of the gallbladder, may lead to the flow of bile. Another potential mechanism for CCK-induced gallbladder emptying after vagotomy is that gallbladder denervation could lead to a sensitization of gallbladder smooth muscle, resulting in a leftward shift of the concentration–effect curve for CCK. This phenomenon is observed in patients who have had a truncal vagotomy, as the gallbladder contraction in response to CCK is significantly enhanced [70]. Gallbladder emptying after vagotomy could also be mediated through an enterobiliary neural reflex. Studies in the guinea pig and the Australian possum have demonstrated that the gallbladder receives projections from neurons located in the myenteric plexus of the duodenum [4,71]. It is plausible that lumenal stimuli or mucosal CCK release may result in the activation of these neurons, which directly signal to the gallbladder. Although this scheme could not contribute to gallbladder emptying after liver transplantation, the enterobiliary circuitry may contribute to gallbladder emptying after truncal vagotomy.

Interprandial gallbladder contractions

During duodenal phase II of the migrating myoelectric complex (MMC) there is an increase in gallbladder pressure accompanied by a transient flow of bile from the gallbladder to the duodenal lumen. This interprandial gallbladder motor response, which occurs in association with elevated antral and duodenal motor activity, has been demonstrated in several species, including humans, and is thought to help maintain the enterohepatic circulation of bile salts [72,73].

The mechanisms responsible for the phase II gallbladder contraction have not been clearly resolved, but they appear to involve a neural component because spontaneous and motilin-induced gallbladder contractions are reduced or abolished with atropine or hexamethonium [74]. However, it is not clear whether the neural component involves the activation of a vagal reflex or whether it is limited to actions on intrinsic reflex circuits of the bowel. In a canine study, inhibition of vagal reflex activity by transient vagal chilling or by acute vagotomy led to a decrease in MMC-related gallbladder emptying [75]. In contrast, MMC-related contractile activity changes little after chronic vagotomy [76–78]. If the biliary motor response that accompanies the MMC does not involve an extrinsic neural circuit, it may involve direct neural interactions between the gut and the biliary tree, or humoral factors may contribute.

The gastrointestinal hormone motilin is thought to initiate the MMC as plasma motilin levels increase during or just before the onset of MMC activity, and exogenous motilin administration initiates an early burst phase of the MMC in the upper gastrointestinal tract [79,80], including the gallbladder [58]. Exogenous administration of motilin, or motilin analogues such as erythromycin, increases gallbladder motor activity [81], and motilin induces the contraction of isolated gallbladder myocytes [82]. However, it is not yet clear how endogenous motilin would directly activate MMC-related gallbladder activity, which is primarily limited to phase II, as peak levels of motilin in the serum correspond to duodenal phase III of the MMC, when the gallbladder is quiescent.

Gallbladder filling

Between meals, gallbladder filling is related to the rate of hepatic secretion from the liver, as well as SO resistance. During this process, hepatic secretory pressure is relatively

high (≥ 25–30 mmHg), as is SO pressure (~ 11–30 mmHg), whereas pressure within the biliary tree is maintained at a relatively low level (~ 5–15 mmHg) because the gallbladder is able to accommodate newly synthesized bile [72]. The accommodation of the gallbladder during fasting relies on the ability of the epithelium to concentrate bile and on the receptive expansion of the gallbladder. The gallbladder thereby contributes to the modulation of pressure within the biliary system through both passive and active mechanisms.

Passive filling

The fibroelastic properties of the gallbladder wall likely facilitate filling, allowing the organ to expand as bile is routed from the biliary tree to the gallbladder. The sheer size of this organ relative to other structures in the biliary tree could favor the passage of bile into the gallbladder. According to the law of Laplace, tension in the wall of a hollow elastic structure is proportional to the product of pressure multiplied by diameter. Because the diameter of the gallbladder is significantly greater than that of the bile ducts, the path of least resistance would be gallbladder filling and expansion while the SO is resisting bile flow. As the gallbladder fills and its diameter increases further, the propensity for bile to flow into the gallbladder increases. Although this principle has never been applied to the gallbladder, many visceral structures that undergo expansion, including the urinary bladder, alveoli, the heart, and the eyeball, have been shown to behave according to the law of Laplace.

Several stretch-activated channels have been identified. It is possible that gallbladder smooth muscle cells express stretch-activated channels that are opened as gallbladder tone increases slightly as a result of filling. These could be channels that induce a depolarization of the smooth muscle resulting in increased gallbladder basal tension, or channels that elicit hyperpolarization and decreased tension.

Active filling

Several candidates exist as potential mediators of active gallbladder relaxation and resultant gallbladder filling (see Fig. 12.2). Neurotransmitters that have an inhibitory effect on gallbladder smooth muscle include CGRP, norepinephrine (noradrenaline), VIP, PACAP, and nitric oxide (NO). Humoral factors that relax the gallbladder include pancreatic polypeptide, somatostatin, and fibroblast growth factor. However, it is difficult to determine which of these compounds actually contribute to normal physiological gallbladder expansion, as many of their effects on the gallbladder have been observed at supraphysiological concentrations. Several potential schemes that could contribute to this process will be described.

Release of inhibitory neuroactive compounds from gallbladder neurons

In the bowel, distinct subpopulations of enteric neurons (i.e., inhibitory motor neurons) act to decrease muscle tone. Compounds that have been proposed to contribute to the relaxation of intestinal smooth muscle include NO, VIP, PACAP, and ATP. As mentioned, subsets of gallbladder neurons are VIP- or PACAP-immunoreactive [8,11–13], and immunoreactive for NOS [8–10,12,13,83]. The neuroactive peptides VIP and PACAP cause relaxation of resting or precontracted gallbladder muscle strips from several species including humans [84–87], probably through the activation of adenylate cyclase. VIP is released by nerves in the gallbladder in response to electrical stimulation of the vagus nerves, further evidence of its neurotransmitter capability [88,89]. Relaxant responses to exogenous VIP-vagal stimulation can be antagonized by VIP antisera [86].

Evidence indicating that NO relaxes gallbladder smooth muscle includes the finding that inhibition of NOS results in increased gallbladder tone and an enhancement of agonist-induced contractions [90,91]. NOS inhibition also reduces stimulation-induced neurogenic relaxations, and NO donors have an inhibitory effect on gallbladder tone [90–93].

CGRP, which is present in extrinsic sensory fibers, can decrease tension in gallbladder muscle strips and cause a hyperpolarization of gallbladder smooth muscle cells. It is unlikely, however, that CGRP released from gallbladder afferent fibers contributes to relaxation because tachykinins are costored with CGRP in these nerve fibers [4,5,10], and tachykinins have an excitatory effect on gallbladder smooth muscle [15,21,24,51,94].

Carbon monoxide may also play a role in gallbladder function. The enzyme that synthesizes carbon monoxide, heme oxygenase 2, is present in canine gallbladder nerves, and copper protoporphyrin IX, an inhibitor of heme oxygenase, inhibits nonadrenergic noncholinergic stimulus-induced relaxations [95].

In the bowel, ATP is a mediator of nerve-induced relaxations. However, it is unlikely that ATP contributes to gallbladder relaxation as application of exogenous ATP leads to gallbladder contraction [96].

Despite the results described, it is difficult to conceive how gallbladder neurons could provide an unambiguous inhibitory signal to gallbladder smooth muscle as all gallbladder neurons express ChAT and are therefore likely to be cholinergic and excitatory [5]. Activation of these neurons would lead to the release of acetylcholine plus other compounds such as NO and/or VIP, thus providing the gallbladder smooth muscle with a mixed signal.

Increased sympathetic neural activity in the gallbladder

Another possibility is that gallbladder relaxation involves the release of norepinephrine from sympathetic nerves. Stimulation of the splanchnic nerves leads to a decrease in gallbladder tone [97–99], indicating that sympathetic activity contributes to interprandial gallbladder filling. It is likely that activation of sympathetic neural inputs can lead to decreased gallbladder tone through direct effects on gallbladder smooth

muscle [87] and by inhibiting vagal input to gallbladder neurons [18,100]. However, it is still unclear whether such a system is active primarily at times of physiological stress or whether it contributes to the normal accommodation of the gallbladder wall during interprandial filling. Indeed, in models of hemorrhagic shock, gallbladder contractility is significantly decreased [101].

Humoral factors that relax gallbladder smooth muscle

Because pancreatic polypeptide relaxes gallbladder muscle strips [102], this peptide has been implicated as a potential contributor to gallbladder filling. Furthermore, elevated pancreatic polypeptide levels have been implicated in the etiology of clinically significant biliary stasis, as is seen in patients with diabetes mellitus [103]. Increases in pancreatic polypeptide levels have been measured in response to liquid test meals, intraduodenal feeding, and CCK infusion [104,105]. However, there is no correlation between pancreatic polypeptide levels and gallbladder motor activity when observed in healthy individuals [106], indicating that pancreatic polypeptide probably does not contribute to interprandial gallbladder filling. Pancreatic polypeptide may play an indirect role in the control of gallbladder motility by modifying pancreatic secretion or intestinal motility, but this has not been resolved.

Somatostatin is another compound that may contribute to gallbladder filling as several lines of evidence indicate that somatostatin decreases gallbladder contractility. Long-term therapy with the somatostatin analogue octreotide for diseases such as acromegaly [107,108], or the existence of somatostatin-secreting tumors [109], both interfere with gallbladder contractility. Gallbladder hypomotility in celiac disease has been associated with elevated somatostatin levels, increased gallbladder fasting volume, and decreased CCK release [110]. In addition to its well-known inhibitory effect on the release of mucosal CCK, somatostatin can act locally to decrease gallbladder motility. Somatostatin decreases CCK- and carbachol-induced gallbladder contractions, gallbladder pressure, and the release of acetylcholine from gallbladder muscle strips [111,112]. Somatostatin also inhibits CCK-induced gallbladder contractions in isolated cells [113]. In healthy subjects, somatostatin enhances gallbladder relaxation and reduces CCK secretion in the late postprandial phase [114]. Although these results infer an association between somatostatin and gallbladder volume under pharmacological or pathological conditions, a physiological role for somatostatin in gallbladder filling has not been proven.

A study in mice has provided convincing evidence that fibroblast growth factor (FGF) plays a role in gallbladder relaxation and filling [115]. The investigators reported that gallbladders of FGF15 –/– mice were almost devoid of bile, and administration of FGF15 or its human orthologue FGF19 caused gallbladder filling. Furthermore, they showed that FGF induced relaxation of precontracted gallbladder muscle strips, probably through a cAMP-dependent mechanism. Although the neuromuscular mechanisms are not yet understood, these findings support the concept that FGF15/19 released from the ileum in response to bile salts causes gallbladder relaxation and filling.

The sphincter of Oddi

The sphincter of Oddi (SO) oversees the flow of about 3 L of bile and pancreatic secretions per day, and it is the busiest nonvascular intersection in the body. A vast interspecies variation in morphology and function of the SO has led to more discussion and controversy than any other structure in the gastrointestinal tract. The tasks of the SO are to regulate the flow of bile and pancreatic secretions into the duodenum, to facilitate gallbladder filling, and to prevent the reflux of lumenal contents from the duodenum into the pancreatic ducts and biliary tree. Although the precise mechanisms of SO function are not yet fully understood, research has demonstrated that changes in SO motility involve neurohormonal regulation of nontraditional neural circuits that orchestrate coordinated signals from SO ganglia to the surrounding circular smooth muscle.

Sphincter of Oddi neurobiology

Most studies of SO ganglia have involved the guinea pig and the Australian possum. Therefore, most of the following information relates to ganglia in the SO region of these two species, notable considering the aforementioned interspecies variation [116].

Morphology of the sphincter of Oddi ganglia

Ganglia in the guinea pig SO are similar in shape and density to the ganglia of the myenteric plexus of the duodenum [117,118]. In fact, interganglionic nerve bundles can be traced between the myenteric plexus of the duodenum and that of the SO [117]. The processes of SO neurons typically leave the ganglionated plexus to innervate the smooth muscle of the SO, and therefore most SO neurons are thought to be motor neurons.

Neurotransmitters of the sphincter of Oddi

Immunohistochemical studies have revealed the presence of numerous neuroactive compounds in SO nerves [113–130]. For example, among the species investigated there is evidence of acetylcholine, NO, VIP, tachykinins, CGRP, NPY, OFQ, somatostatin, enkephalin (ENK), galanin (GAL), serotonin (5-hydroxytryptamine, 5-HT), peptide histidine isoleucine (PHI), gastrin-releasing peptide (GRP), and bombesin.

The most thorough investigation of neurochemical content and distribution in the SO has been conducted in the guinea pig. Guinea pig SO ganglia consist of two major subpopulations of neurons: those that are immunoreactive for

ChAT and those that are immunoreactive for NOS [10,118]. The cholinergic (ChAT-positive) neurons, which are thought to be excitatory motor neurons, represent about two-thirds of the population and are also immunoreactive for tachykinins and/or ENK. The nitrergic neurons, which are thought to be inhibitory motor neurons, are also immunoreactive for either VIP or NPY, but not both. SO neurons have also been found to be immunoreactive for bombesin [119], OFQ [120], and 5-HT [121]; whether these compounds are expressed by cholinergic and/or nitrergic neurons is not yet known. Nerve fibers, thought to be extrinsic afferent axons, are immunoreactive for tachykinins and CGRP and are abundant in the ganglionated plexus of the guinea pig SO [118]. Sympathetic postganglionic nerves immunoreactive for tyrosine hydroxylase are also abundant in guinea pig SO ganglia and nerve bundles [122].

Less is known about the colocalization of various compounds in other species. Considerable neurotransmitter diversity exists, and a nitrergic innervation of the SO has been reported in all species in which this has been investigated. In the human SO, neural immunostaining has demonstrated NOS, VIP, PHI, NPY, CGRP, GAL, somatostatin, tachykinin, and ENK, and histochemical staining was positive for NADPH dehydrogenase [7,123–126]. Nerve fibers of the rhesus monkey SO are immunoreactive for ENK, NPY, and tyrosine hydroxylase, and NADPH dehydrogenase staining has also been reported [7,127]. In the pig, immunoreactivities for NOS, VIP, NPY, GAL, PHI, CGRP, tachykinin, and bombesin have been reported [14,124,128]. Feline SO nerves have been shown to be VIP and tachykinin immunoreactive [21,86,129,130]. In studies of the Australian possum SO, ChAT, tachykinin, GAL, GRP, and somatostatin immunoreactivities have been demonstrated [131–134], and NADPH dehydrogenase staining suggests a nitrergic innervation [135].

Sphincter of Oddi smooth muscle

Human and animal studies of the SO have established that the sphincter is not a simple and passive smooth muscle portion of the biliary system. Rather, it exhibits basal tonicity, spontaneous phasic contractions, and peristaltic activity that modulates bile flow into the duodenum [136–140]. The human SO is normally characterized by prominent phasic contractions that are superimposed on a modest basal pressure [141]. In a landmark study, pressures were recorded from the human SO to determine the effect of neurohumoral mediators on SO function [142]. The basal SO pressure was 4 mmHg higher than the common bile duct or pancreatic duct pressure. Pronounced phasic contractions superimposed on the basal SO pressure occurred at a frequency of 4.1/min. The phasic SO contractions were about 100 mmHg in amplitude and 4.3 s in duration. These contractions were unique to the SO and did not exist in the duodenum, the common bile duct, or the pancreatic duct. The phasic contractions were altered by intravenous infusions of CCK, glucagon, and secretin, suggesting a neurohumoral component to the modulation of SO motility in humans.

Animal studies have revealed that the proximal and distal segments of the SO can respond differently to a variety of bioactive compounds, further evidence of the complexity of the regulation and function of this neuromuscular structure. Exogenous CCK, secretin, adenosine, and ATP have been used to explore the concept that the SO is not a single-function entity but that, under defined conditions, it can function as one, two, three, and four separate sphincters [143–145]. This complexity may reflect the various inputs that the SO receives from the autonomic nervous system, including those inputs from local reflex circuits, as described in the following text.

In general, the interpretation of the actions of various compounds in the SO has been difficult because most compounds mediate their effects through both direct actions on the smooth muscle and indirect neural responses. For example, exogenously administered CCK has a direct contractile effect on SO smooth muscle as well as contractile and relaxant effects that are mediated by neurotransmitter release. The neurally mediated effects of CCK are species specific; both contraction and relaxation are detected in species whose sphincter acts as a pump [146], whereas simple relaxation is detected in species such as humans and cats in which the sphincter acts primarily as a resistor [147–149].

Electrical field stimulation of the SO evokes a twitch-like contraction followed by relaxation [135,150–152]. Muscarinic blockade in the presence of guanethidine alters this pattern to a simple relaxation [150,151,153], consistent with the existence of inhibitory nonadrenergic noncholinergic innervation. NO is the primary inhibitory neurotransmitter in the SO [135,151,154,155]. Neurally released NO increases cyclic AMP and cyclic GMP levels in SO smooth muscle [154,156] and induces the activation of K_{ATP} channels [157]. This pathway may be important in the physiological regulation of bile duct pressure, as NOS blockers increase SO resistance to flow [153] and NO donors inhibit SO contraction frequency and decrease basal pressure [123].

Whereas the understanding of purinergic regulation of gastrointestinal motility is well advanced, the action of purines in the regulation of SO motility is rudimentary. Exogenous ATP is known to reduce rabbit SO contractility and inhibit spontaneous activity in cat SO [158,159]. In contrast, exogenous ATP produces an excitatory response in the guinea pig SO and the Australian brush-tailed possum SO. [145] Purines have no effect on human SO contractility [160]. Exogenous adenosine exerts an inhibitory effect on the guinea pig SO [161] in contrast to the excitatory response elicited in the human SO and the Australian brush-tailed possum SO [145,162].

In some species, such as guinea pig, rabbit, and North American opossum, CCK release leads to a pumping

response that involves rhythmic fluctuations in SO tension [53,163–165]. In the opossum, bile flow appears to involve peristalsis through the particularly long SO region characteristic of this species. Regardless of the species or the type of response, it appears that the physiological effect of CCK is to decrease resistance and therefore promote bile flow.

The effect of CCK on SO tone involves a neural mechanism, as the CCK-induced increase in bile flow through the SO is inhibited by muscarinic blockade with atropine or by complete neural blockade with tetrodotoxin, [62,63,163–165]. Furthermore, inhibition of VIPergic signals with VIP antiserum, or NO release by NOS inhibitors, suppresses CCK actions [155,166]. These observations led to the theory that CCK alters SO tone through a hormonal effect on SO neurons. Indeed, the application of CCK to guinea pig SO neurons causes a prolonged depolarization and bursts of action potentials [167]. However, as for gallbladder smooth muscle, the concentration-dependent nature of this response is outside of the physiological range [56,57]. These data indicate that the neural mechanism by which CCK decreases SO resistance probably does not involve a direct action of hormonal CCK on SO neurons. One possibility is that CCK, released locally at high concentrations from enterochromaffin cells, has a paracrine effect on SO neurons; another possibility is that CCK acts on duodenal neurons that provide regulatory input to SO ganglia (Fig. 12.4).

Evidence is mounting in support of the concept that CCK released from duodenal enterochromaffin cells activates duodenal enteric neurons, and that these neurons signal SO ganglia, initiating postprandial changes in SO function (see Fig. 12.4) [168]. In the guinea pig [169] and the Australian possum [71], retrograde axonal tracing studies have confirmed the existence of a direct neural projection from the myenteric plexus of the duodenum to the SO. In the guinea pig, the neurons of this duodenum–SO projection are cholinergic [169], and neurons in SO ganglia receive nicotinic excitatory synaptic input from the duodenal myenteric plexus [170]. Experiments involving electrical recordings from duodenal neurons that have been retrogradely labeled from the SO have shown that all of these neurons express CCK receptors and many of these neurons are intrinsic primary afferent neurons [169]. Intrinsic primary afferent neurons in the intestines are known to send projections to the lamina propria, where they may be activated by local release of CCK from enterochromaffin cells [171–173]. These data support the view that CCK released from duodenal enterochromaffin cells could initiate changes in SO resistance by way of a local neural circuit (Fig. 12.4). However, the involvement of this neural circuit in postprandial changes in SO tone is not certain. Other potential functions for this circuit include the mediation of changes in SO tone that accompany interprandial bile flow during phase II of the MMC (see next section). Alternatively, it may provide a means of increasing SO tone when lumenal pressure in the duodenum is elevated, thus inhibiting the flow of duodenal contents into the biliary tract.

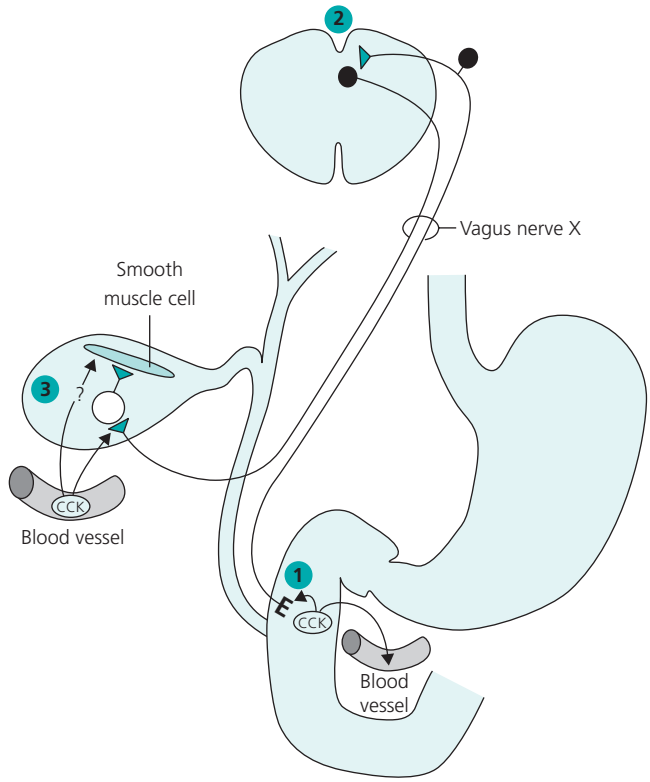

Figure 12.3 Sequence of events leading to postprandial gallbladder contraction. 1. Cholecystokinin (CCK) released from the duodenal mucosa activates vagal afferent fibers and enters the bloodstream. 2. Increased vagal afferent activity leads to increased vagal preganglionic output. 3. Within the gallbladder, hormonal CCK acts at physiological concentrations on receptors located on vagal preganglionic nerve terminals in gallbladder ganglia. Activation of these presynaptic receptors leads to an increase in acetylcholine release and enhanced activation of gallbladder motor neurons. CCK may also act directly on gallbladder smooth muscle, although the receptors at this location appear to be low-affinity CCK_1 receptors, which normally would not be activated by physiological concentrations of CCK.

Interprandial sphincter of Oddi activity

During the fasting state, bile flowing toward the small bowel is diverted into the gallbladder by the resistance encountered at the SO – resistance maintained by phasic contractions of the smooth muscle superimposed upon a basal level of tonic pressure. Thus, intraductal pressures are increased and bile flow is diverted to the region of lowest resistance, the cystic duct and gallbladder. High pressures in the biliary system also trigger relaxation and compliance of the gallbladder. Resistance at the SO and increased compliance of the gallbladder allow for the diversion of most of the bile away from the gut and into the gallbladder. There are, however,

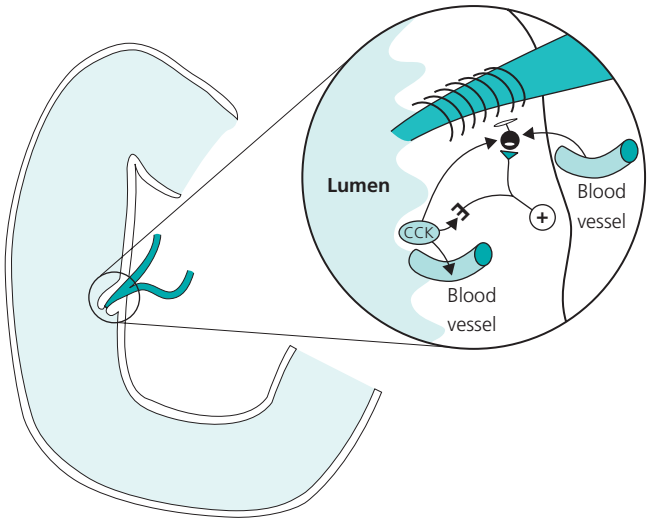

Figure 12.4 Postprandial relaxation of the sphincter of Oddi (SO). Cholecystokinin (CCK) released from the duodenal mucosa activates inhibitory motor neurons in SO ganglia, which in turn decrease the basal tone of SO. CCK probably causes a relaxation of the sphincter by activating duodenal neurons that provide synaptic input to SO ganglia. Hormonal CCK may activate SO neurons, although these neurons are not sensitive to the concentrations of CCK that are found in the serum. Another possibility is that locally released CCK acts directly on SO neurons through a paracrine mechanism.

interprandial changes in SO activity that correspond to phase II of the MMC.

The interprandial SO activity associated with the MMC involves a decrease in SO resistance that accompanies increased gallbladder motor activity and bile flow. However, the MMC-related flow of bile does not depend on increased gallbladder motility, as dogs that have undergone cholecystectomy also exhibit cyclic bile flow that corresponds to the MMC [174]. Changes in SO activity that accompany the MMC have been documented in many species, including humans [74,174–176].

As for the gallbladder, the mechanisms underlying the MMC-related changes in SO activity are not entirely clear; however, it appears likely that the duodenum–SO neural circuitry is involved. After truncal vagotomy, MMC-related SO activity is normal in the opossum. Furthermore, relocation of the duodenal papilla in the opossum, which leaves the blood supply and extrinsic nerves intact but eliminates myoneural continuity between the duodenum and the SO, leads to a marked reduction in electrical activity of the SO during phases II and III of the MMC [177]. Also, surgical relocation of the duodenal papilla reduces the SO responsiveness to motilin [178]. Together, these data support the concept that an intrinsic neural circuit mediates the interprandial SO motor response, although evidence of cyclic activity after translocation of the SO suggests the involvement of vagal reflexes or humoral factors.

Pathophysiology of biliary tract motility

Mechanisms of biliary stasis in cholesterol disease

Patients with cholesterol gallstones often have abnormal gallbladder motility. These individuals have an increased residual gallbladder volume interprandially, as well as decreased gallbladder emptying after a meal [179,180] or in response to exogenous CCK administration [179,181]. Furthermore, gallbladder muscle strips from patients with cholesterol gallstones demonstrate impaired contractile responses to a variety of stimuli [182,183].

Cholesterol and biliary stasis

Decreased gallbladder smooth muscle contractility is a critical link in the chain of events that leads to cholesterol gallstone formation and resultant cholecystitis. Ground squirrels and prairie dogs, as well as CCK_1 receptor knockout mice, that are fed a high-cholesterol diet exhibit a progressive increase in cholesterol saturation in the bile, a decrease in gallbladder contractility, and, ultimately, cholesterol stone formation and cholecystitis [184–187]. If the decrease in gallbladder motility is offset by the administration of the prokinetic agents cisapride or erythromycin, the formation of cholesterol crystals is prevented [188,189]. Conversely, if gallbladder stasis is pharmacologically induced by the administration of a CCK_1 antagonist or by knockout of the CCK_1 receptor, gallstones form in animals fed a high-cholesterol diet [186,187,190].

These clinical and basic scientific data indicate that gallstone formation results from an elevation in cholesterol concentrations within the bile and an associated decrease in gallbladder contractility. The decrease in contractility is directly linked to cholesterol enrichment. Gallbladder smooth muscle that is exposed to cholesterol-rich liposomes exhibits diminished contractility similar to that observed in human gallbladders with cholesterol stones and in animals fed a high-cholesterol diet [191]. These results support the hypothesis that cholesterol itself can have a direct effect on gallbladder smooth muscle.

Gallbladder stasis probably involves changes in the cytosolic membranes of gallbladder smooth muscle cells. An elevated level of cholesterol in the cystolic membranes disrupts spontaneous action potentials and suppresses voltage-activated Ca^{2+} currents [192]. The effect of cholesterol appears to be somewhat selective because K_V and K_{ATP} currents are not affected by cholesterol enrichment. In addition to altering Ca^{2+} channel activity, cholesterol enrichment affects cytosolic membrane receptors. For example, the hyperpolarization elicited by CGRP is suppressed after cholesterol enrichment, even though the K_{ATP} channel that mediates this response is not altered by cholesterol. Furthermore, gallbladders from patients with cholesterol stones have diminished CCK receptor binding and function [193,194]. These data indicate that

cholesterol enrichment results in selective deficits rather than a nondiscriminatory disruption of membrane protein function, and that the biliary stasis associated with cholesterol stone formation is likely to involve altered Ca^{2+} channel and membrane receptor function, possibly caused by changes in membrane fluidity [195]. In vitro studies have shown that cholesterol-induced gallbladder dysmotility can be reversed to normal levels after the defective muscle cells (either from cholesterol-enrichment protocols or patients with cholesterol stones) are incubated with cholesterol-free liposomes [191,194,195].

Bile salts and biliary stasis

Modifications in bile acid metabolism, associated with prolonged intestinal transit, may also contribute to the formation of cholesterol stones [196]. Reduced gallbladder refilling in gallbladder stasis leads to altered enterohepatic circulation [179]. In patients with gallbladder dysmotility, slow bowel transit contributes to the alterations in the metabolism of bile salts, resulting in an enrichment of the bile acid pool with hydrophobic secondary acids such as deoxycholic acid [197]. This enrichment induces hypersecretion of cholesterol into the bile, an increase in the cholesterol saturation index, and acceleration of cholesterol crystallization [198], thus elevating the risk of gallstone formation. In addition, an elevated concentration of hydrophobic acids in bile decreases gallbladder motility and emptying [199–201]. The dysmotility caused by hydrophobic bile acids involves H_2O_2 production through the activation of NADPH and xanthine oxidases [201]. H_2O_2 causes lipid peroxidation and activates cytosolic phospholipase A_2 to increase prostaglandin E_2 (PGE_2) production [201]. In contrast, the hydrophilic bile salt ursodeoxycholic acid does not impair gallbladder contractility and can attenuate the deleterious effects of hydrophobic salts [200,201]. Furthermore, ursodeoxycholic acid treatment can prevent gallbladder smooth muscle dysfunction in acute cholecystitis [202]. These findings may explain why ursodeoxycholic acid therapy for patients with symptomatic gallstones reduces the risk for colicky biliary pain and acute cholecystitis, and improves bile turnover [203,204].

Inflammation and gallbladder dysmotility

Acalculous cholecystitis

Conditions other than elevated biliary cholesterol also lead to decreased gallbladder contractility and cholecystitis, with or without stones. For example, biliary colic in the absence of gallstones is an increasingly prevalent complication among patients in the intensive care unit and in patients without predisposing illness [205–207]. This condition, commonly referred to as acute acalculous cholecystitis, is often associated with trauma or extensive surgery. In a retrospective chart review over 53 months in a tertiary care center, 27 cases of acalculous cholecystitis were observed. Of these cases, 52% occurred in the intensive care unit, 63% occurred in patients recovering from unrelated surgery, and acalculous cholecystitis accounted for 14% of all cases of acute cholecystitis [208]. The mortality rate for all patients with acalculous cholecystitis was 41%, illustrating the potential lethality of this clinical entity.

The development of acalculous biliary colic, like cholesterol-related gallbladder disease, involves decreased gallbladder motility leading to an increase in the resting volume of the gallbladder and cholecystitis [209–211]. The clinical complexity of a critically ill patient makes it difficult to distinguish between interrelated factors and to determine the etiology of a complication such as acute acalculous cholecystitis. Factors implicated in the pathophysiology of acalculous cholecystitis include parenteral feeding, altered bile composition, ischemia, infection, and functional obstruction of gallbladder outflow [209–211]. Decreased gallbladder motility may also be a consequence of the use of therapeutic agents administered to alleviate traumatic or postoperative pain. A summary of the major findings in these areas follows.

Parenteral feeding

During total parenteral nutrition (TPN), biliary stasis and hypomotility have been well documented, contributing to the development of biliary dilation, sludge accumulation, and acute cholecystitis. In most patients, TPN induces gallbladder stasis but does not increase the biliary lithogenic index [212]. Interruption of the enterohepatic circulation and gallbladder stasis are part of the pathogenesis as a consequence of the lack of a physiological stimulus for CCK release. However, during prolonged enteral nutrition, plasma CCK levels are significantly increased and gallbladder contractility is preserved, thus minimizing the risk of acute acalculous cholecystitis [105]. Furthermore, intravascular infusion of CCK in human or animal models ameliorates the hepatobiliary dysfunction caused by TPN [212,213]. Increased circulating levels of CCK can also be induced by intermittent, rapid intravenous infusion of amino acids [214,215]. However, continuous administration of amino acids for longer than 2 h (as occurs in TPN) is accompanied by a decline in CCK release [216]. Taken together, these findings indicate that intravenous administration of amino acids or CCK may circumvent the biliary dysmotility associated with TPN, but therapy of this kind would probably be most beneficial if administered periodically rather than continuously.

Opiate analgesic administration

Another factor that likely contributes to the development of gallbladder stasis in the critically ill patient is opiate analgesic administration. In addition to acting as potent analgesics, opiate compounds are well known for their ability to alter gastrointestinal motility [217]. Opioid agonists do not act directly on gallbladder neurons or gallbladder smooth muscle cells; rather, they cause a decrease in the synaptic activity

between neurons, and between neurons and smooth muscle. Thus, opioid agonists have a potent inhibitory effect on gallbladder motility by decreasing the release of acetylcholine and other excitatory neurotransmitters from vagal terminals in gallbladder ganglia and the neuromuscular terminals of gallbladder neurons. A study showing that the μ-opiate receptor agonist loperamide decreases bethanechol-induced gallbladder contraction even though plasma CCK levels are increased supports this inhibitory mechanism [218]. Because opioid agonists also increase SO resistance, bile flow is likely to be severely inhibited by opioid compounds, and continued administration of these substances may contribute to the onset of acalculous cholecystitis.

Inflammatory mediators

The cause-and-effect relationship between biliary stasis and cholecystitis is not well understood. However, the actions of several proinflammatory agents that are likely to be involved in acute cholecystitis have been investigated. These include prostaglandins, reactive oxygen species, histamine, and endothelins.

Prostaglandins

Prostaglandins, particularly PGE_2, have been shown to be intimately associated with cholecystitis [219]. Early studies of diseased human gallbladders have shown that both the mucosa and the muscularis of the organ produce high levels of PGE_2 [220]. Furthermore, a correlation between severity of inflammation and PGE_2 concentrations has been described [221]. Prostaglandin release may be prolonged or augmented by bradykinin, as gallbladder distention and progressive inflammation stimulate local bradykinin formation, thereby stimulating PGI_2 and PGE_2 release [222] through the activation of cyclooxygenase and prostacyclin synthase [223]. Other stimulants of prostaglandin synthesis that participate in experimental and human gallbladder disease are interleukin-1 and tumor necrosis factor [224]. These cytokines activate prostaglandin synthesis through phospholipase A_2 stimulation [225]. In addition, protease-activated receptors induce gallbladder contraction, an effect that is mediated by prostaglandin synthesis [226] and correlated with the presence of high levels of trypsin in the bile of patients with gallbladder disease [227].

The concept that prostaglandins play a role in cholecystitis is supported by human and animal model studies showing that symptoms of acute cholecystitis are significantly reduced by the cyclooxygenase inhibitor indomethacin (indometacin) [228]. For example, in an experimental cholecystitis model, inflammation and defects in contraction were partially reversed during the first 24 h by indomethacin [229], contrary to the protective role for PGE_2 suggested by other studies. PGE_2 causes a concentration-dependent contraction of the gallbladder [230], as well as a significant reversal in net fluid movement from absorption to secretion, including an increase in mucin secretion [231,232]. Furthermore, PGE_2 stimulates the synthesis of free radical scavengers that attenuate the noxious effects of reactive oxygen species [233]. These actions are consistent with a cytoprotective role for PGE_2 in the gallbladder, at least in acute inflammation, which acts by expelling gallbladder contents and preserving mucosal integrity.

Conversely, PGE_2 also has been shown to decrease gallbladder ganglionic output by hyperpolarizing gallbladder neurons [234], thereby inhibiting neurogenic contractions of the gallbladder. Thus, the direct contractile effects of prostaglandin may contribute to the pain reported by patients suffering acute cholecystitis, as these contractions would not be accompanied by SO relaxation. Furthermore, the neural effects of prostaglandin in the gallbladder would lead to a decrease in postprandial contractions because, as previously described, excitatory neural activity is involved in normal postprandial gallbladder emptying. Therefore, in acute cholecystitis, prostaglandins may contribute to gallbladder dysmotility by causing the gallbladder to contract when it normally would not, and by preventing the gallbladder from contracting when it normally would.

Reactive oxygen species

The generation of reactive oxygen species is a common feature of inflammatory states, including ischemia and reperfusion injury. Under normal conditions, reactive oxygen species produced during oxidative metabolism are cleared by antioxidant mechanisms that include the antioxidant enzymes superoxide dismutase and catalase, and the glutathione antioxidant systems. Superoxide dismutase and catalase are present in the ganglionated plexus of the gallbladder [235]. During inflammation, oxygen-derived free radical production may exceed the scavenger capability, resulting in the accumulation of reactive oxygen species and deleterious effects. Consistent with this, experimental gallbladder inflammation results in an increase in the H_2O_2 content associated with an increase in superoxide dismutase and catalase activities [233]. Exogenous H_2O_2 causes smooth muscle contraction and impairs the gallbladder response to agonists that are membrane dependent [236] and related to membrane desensitization [237]. Similarly, other reactive oxygen species, such as monochloramine or superoxide anions, induce gallbladder contraction [235,238] and smooth muscle impairment [238], indicating that this is a common feature of reactive oxygen species. Reactive oxygen species-induced gallbladder contractions are blocked by indomethacin [236,238] and are associated with an increase in PGE_2 production, which, in turn, induces an increase in superoxide dismutase and catalase activities and a reduction in lipid peroxidation and contraction [233], possibly as part of the protective mechanism against the reactive oxygen species-induced gallbladder stasis. In inflammation, excessive production of NO, often through inducible NOS [239], with

concurrent reactive oxygen species production [233] increases peroxynitrite formation. Although NO causes gallbladder relaxation, peroxynitrite induces contraction [240], consistent with the contractile effects reported for other oxidants, suggesting that peroxynitrite also mediates the dysmotility induced by cholecystitis.

Histamine

Histamine is a well-recognized inflammatory mediator that can be released from mast cells to cause vasodilation, increased vascular permeability, gastric secretion, and contraction of bronchiolar and gastrointestinal smooth muscle. The gallbladder wall is rich in histamine-containing mast cells, which are distributed in the mucosa and muscularis/serosa layers [241]. In gallbladder smooth muscle, both H_1 and H_2 receptors are present, with activation of H_1 receptors causing contraction and H_2 receptors mediating relaxation [241]. Consistent with this, activation of H_1 receptors depolarizes gallbladder smooth muscle, whereas activation of H_2 receptors causes a hyperpolarization that is mediated by K_{ATP} channels [37]. However, the net effect of histamine in gallbladder muscle strips is contraction [241,242]. Although there is no direct evidence for a role of histamine in gallbladder inflammation, it is possible that acute cholecystitis is associated with increased mast cell infiltration and degranulation. Thus, endogenously released histamine may exert an excitatory effect on gallbladder smooth muscle, resulting in a prokinetic protective effect.

Endothelins

Endothelins are another class of inflammatory mediators. Studies in the Australian brush-tailed possum have shown that endothelins increase gallbladder activity [243–245]. In a model of acalculous cholecystitis in this same species, induction of the disease was associated with a significant production of endothelins by gallbladder epithelial cells and an increase in gallbladder tone. Pretreatment with a nonselective endothelin antagonist prevented the onset of inflammation. This study showed that gallbladder endothelin production is an early event in the process of acute cholecystitis, increasing gallbladder tone and playing a crucial role in the inflammatory process [246].

The sphincter of Oddi and postcholecystectomy syndrome

During the fasting state, bile flow into the small bowel is diverted into the gallbladder by the resistance at the SO because of the basal and phasic contractions previously described. After cholecystectomy, the pressure reservoir represented by the gallbladder is lost and the ability of the biliary tree to accommodate hepatic bile production is compromised. In most individuals, bile manages to flow through the SO interprandially, without dramatic increases in pressure within the biliary tree. In some individuals, however, bile does not flow as readily through the SO, leading to increased intrabiliary pressure and postoperative pain [247,248]. In some patients with postoperative biliary pain, biliary manometry has demonstrated abnormal pressure profiles in the SO with changes in amplitude, frequency, and direction of contractions [248–250]. In an attempt to alleviate the pain experienced by these individuals, endoscopic sphincterotomy, stent placement, and surgical sphincteroplasty have all been used with variable results [251].

Overview

Although the neuromuscular reflexes of the gallbladder and SO appear to be less complicated than those of the bowel, bile retention and bile flow require coordinated neuromuscular reflex interactions. Normal gallbladder filling requires relatively high basal pressure in the SO, in concert with an expansion of the gallbladder. This increase in gallbladder diameter requires the passive fibroelastic features of the gallbladder wall as well as myogenic and neurohormonal relaxation of gallbladder smooth muscle. Postprandial bile flow occurs in response to the release of CCK from the duodenal mucosa. To contract the gallbladder CCK acts locally to activate vagal afferent nerves; it also acts within gallbladder ganglia to increase acetylcholine release from vagal preganglionic nerve terminals. CCK release also leads to a decrease in the resistance of the SO by activating inhibitory motor neurons in SO ganglia by way of a duodenum–SO neural circuit.

A common feature of biliary tract disease is abnormal motor activity. In cholesterol gallstone disease, decreased motility involves a disruption of Ca^{2+} channels in gallbladder smooth muscle and a decrease in the ability of agonists to bind to membrane receptors. Acalculous cholecystitis is also associated with biliary stasis, but the contributing factors have not been clearly resolved; likely etiologies include decreased CCK release caused by parenteral feeding, opioid inhibition of excitatory neurotransmission in the gallbladder when opiate analgesics are administered, and the effects of inflammatory mediators. As detailed in this chapter, much has been learned about how gallbladder nerves and smooth muscle operate. However, a clearer understanding of the precise subcellular mechanisms of gallbladder smooth muscle excitability and contractility will aid in the development of clinical strategies for preventing biliary stasis and subsequent biliary disease.

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C SECRETION AND ABSORPTION

13

Gastric secretion

John Del Valle, Andrea Todisco

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The stomach is a complex organ capable of secreting a great variety of products into the gastric lumen, vasculature, and interstitium. Although hydrochloric acid (HCl) is the primary gastric secretion, the stomach also secretes pepsinogen, mucus, bicarbonate (HCO_3^-), intrinsic factor, prostaglandins, regulatory peptides, and other chemical messengers. This chapter discusses each of these, with particular emphasis on gastric acid secretion. Classic human and whole-animal studies are integrated with information gained at the cellular and molecular level, and this integrated model of acid secretion is placed within the fabric of modern clinical practice.

Anatomy of gastric mucosa

A detailed understanding of gastric mucosal structure provides insight into the functional events occurring during gastric secretion. The epithelial lining of the stomach lumen consists of thick vascular folds, called rugae, invaginated with microscopic gastric pits. Each pit opens into four to five gastric glands. The epithelial cells lining the gastric glands are highly specialized and different from the surface epithelial cells. Glands from the cardiac region of the stomach provide the transition from esophageal squamous epithelium to gastric columnar epithelium. They contain mucous and endocrine cells and comprise less than 5% of the gastric gland area. Most gastric glands (75%) occur in the oxyntic mucosa and are responsible for acid secretion (Fig. 13.1). They include parietal, chief, mucous neck, endocrine, and enterochromaffin cells [1]. The pyloric glands cover the gastric antrum and pylorus and contain gastrin cells (G cells), mucous cells, and other endocrine cells. Each of these cell types has evolved into a highly specialized secretory cell that contributes to gastric secretion.

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Cells

The parietal or oxyntic cell is the most distinctive cell of the gastric mucosa. It is usually found in the neck or isthmus of oxyntic glands bulging into the glandular lumen. The

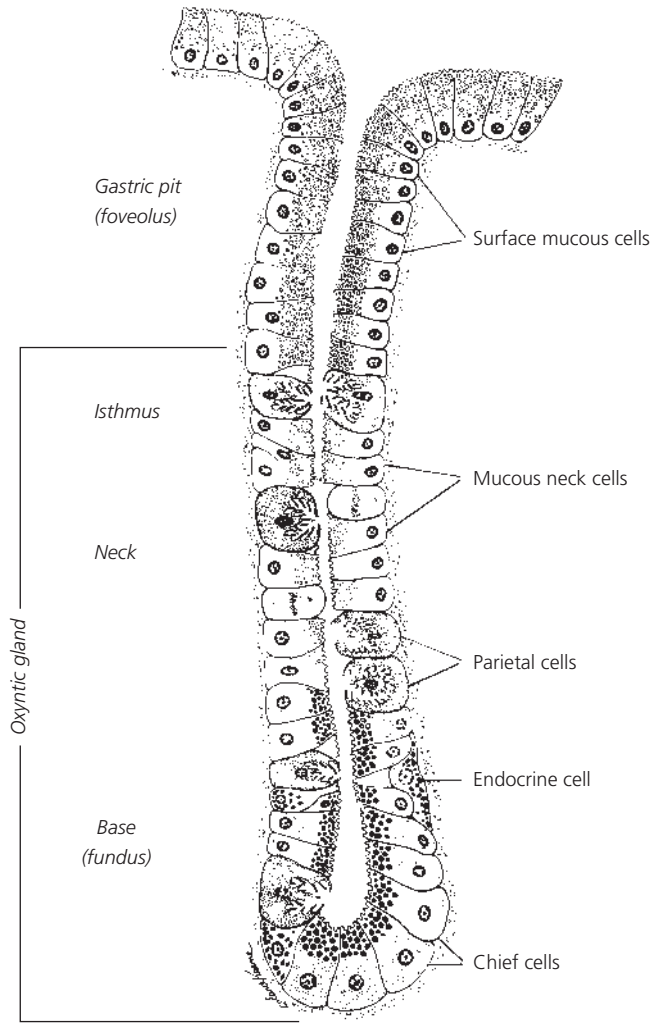

Figure 13.1 Oxyntic gastric gland. Adapted from Ito & Winchester [508].

unstimulated parietal cell has prominent cytoplasmic tubulovesicles and an apical intracellular canaliculus lined with stubby microvilli. On stimulation, a dense meshwork of intracellular canaliculi rapidly forms while tubulovesicles disappear [2,3]. The canaliculi contain a large number of elongated microvilli formed by extensive microfilaments that have a central cytoskeletal core of actin filaments stabilized by other proteins. It is across this apical canalicular surface that HCl is secreted. Acid secretion is an active transport process and requires significant amounts of energy. To provide this energy, parietal cells have numerous mitochondria, which account for 30%–40% of total cellular volume [4].

One prominent feature of parietal cells is their lack of the microvillous glycocalyx that is present on other cells in the gastric glands. Parietal cells are characterized by basolateral membrane folds that increase the surface area for HCO_3^- exchange.

In addition to their well-established role in the production of gastric acid, parietal cells exert important biological actions in the regulation of gastric mucosal homeostasis. Parietal cells are the major site for the production of growth factors and morphogens in the gastric epithelium – growth factors such as transforming growth factor (TGF)-α and morphogens such as sonic hedgehog (Shh), a peptide that contributes to the regulation of complex programs of cellular growth and differentiation in the stomach [5–7]. Interestingly, the loss of mature parietal cells, achieved by genetic, pharmacological, and immunological methods, appears to be associated with an increase in the number of pit cells and a block in the differentiation program of the zymogenic and endocrine lineages, leading to the accumulation of preneck cells and the depletion of their mature zymogenic and endocrine cell descendents [8–12]. Accordingly, these observations suggest that parietal cells, possibly through their ability to produce and secrete TGF-α and Shh, are necessary for the normal differentiation and development of multiple cell lineages in the stomach.

Chief cells are pepsinogen-secreting exocrine cells found in the base or fundus of oxyntic glands. Zymogen granules containing proenzymes are located in the apical cytoplasm and release their contents by exocytosis. The apical membrane has a few short microvilli covered by a thin coating of glycoprotein or glycocalyx. An abundant rough endoplasmic reticulum extends upward from the basal cytoplasm toward the apical granules. Functional characterization of chief cells has been aided by the ability to obtain relatively pure populations of these cells.

Mucous neck cells are located in the isthmus or neck region of oxyntic glands (see Fig. 13.1). A transition zone from mucous neck cells to surface mucous cells appears near the junction of gastric glands and gastric pits. The mucous neck cells are the stem cell precursors for all of the gastric epithelial cells, including the surface mucous, parietal, chief, and endocrine cells [13,14].

Mucous neck cells differ in appearance from surface mucous cells. All mucous cells synthesize large amounts of mucin in prominent Golgi stacks, and these glycoproteins are transported by vesicles to large apical mucous granules. Mucous neck cells contain acidic glycoproteins, indicating sulfated forms, whereas surface mucous cells contain a neutral mucosubstance [15,16]. Mucous granules are larger and often paranuclear in mucous neck cells compared with surface mucous cells. Mucous neck cells have abundant ribosomes and moderate amounts of rough endoplasmic reticulum. Their function as secretory cells and mucosal stem cells is different from the presumed function of surface mucous cells in mucosal defense. Surface mucous cells line the gastric pits and cover the entire lumenal surface of the stomach. They migrate up from the gastric pits and are replaced every 1–3 days [17]. They are thought to protect the stomach from injury by acid, pepsin, ingested materials, and pathogens by secreting mucus and HCO_3^- to form a protective gel. The apical portion of the surface mucous cell is packed with secretory granules. Short microvilli extend from the apical membrane and are covered by a glycocalyx. Secretion of granular mucus appears to occur by exocytosis, apical expulsion, and cell exfoliation [18].

There are many different types of endocrine cells scattered throughout the gastric mucosa. Their secretory products have important endocrine and paracrine effects on acid secretion. Immunohistochemical techniques have enabled characterization of these cells based on their secretory granule contents [19]. Gastric endocrine cells secrete gastrin, somatostatin, and enteroglucagon. Other morphologically distinct gastric endocrine cells may contain additional candidate hormones, but they await further characterization.

Gastric endocrine cells can be classified as open cells, which have apical membranes in contact with the glandular lumen, or closed cells, which are located near the epithelial basement membrane and do not border on the lumen of the gland. The prototypical open endocrine cell is the G cell. The basilar portion of the cell is packed with secretory granules [20] from which gastrin is released by basilar exocytosis [21], consistent with the rapid postprandial appearance of the hormone in the bloodstream. The apical portion of the cell narrows until only a small microvillous border opens on the glandular lumen. The apical membrane may contain lumenal receptors that can detect amino acids or their amine derivatives, which are thought to stimulate G cells during feeding [22,23]. The model of a closed gastric endocrine cell is the fundic somatostatin cell (D cell). Immunohistochemical staining of these cells reveals long, slender processes that terminate on or near parietal and chief cells [24]. These processes presumably mediate the paracrine effect of somatostatin.

The primary cell containing mucosal histamine in the dog stomach is the mast cell [25]. Histamine-containing mast

cells also occur in the human stomach. Mast cells from canine fundic mucosa have been enriched by elutriation and shown to contain characteristic dense granules that stain metachromatically [26]. In some species, including rat and human, histamine is also present in endocrine-like cells that contain large granules and have the characteristic appearance of enterochromaffin-like (ECL) cells [27]. The relative proportion of these two histamine cell types in humans is unknown. Although in situ morphological studies have not been definitive, it appears that these cells exist in the lamina propria in proximity to the glandular cells.

The origin of gastric epithelial cells and the molecular events important in their differentiation are becoming increasingly clear. Electron microscopic examination and the measurement of DNA synthesis in gastric cells indicate that the isthmus of the gastric gland (see Fig. 13.1) constitutes the proliferative zone for the stomach [28–30]. Within this region are three populations of cells that do not contain granules and which are characterized on the basis of their Golgi apparatus. Parietal cells originate from precursor cells (preparietal cells), which mature as they migrate toward the neck and base of the gland.

Karam and colleagues [31] demonstrated that there is functional heterogeneity of parietal cells along the pit–gland axis. Using quantitative morphometry, these investigators observed that parietal cells in the isthmus and neck of the rabbit gastric gland responded appropriately to secretagogues, whereas those in the base showed minimal morphological change in response to stimulation. The factors responsible for decreased function of senescent parietal cells are unclear.

Innervation

As is the case for the entire gastrointestinal tract, the stomach is innervated by the central and enteric nervous systems. This innervation mediates secretion and motor activity through efferent fibers and detects chemical or mechanical stimuli by means of afferent fibers. A full description of the structure and function of the enteric nervous system is provided in Chapter 2.

Central efferents are carried by the parasympathetic vagal branches and the sympathetic greater splanchnic nerve [32]. Afferent sensory neurons are characterized by mucosal chemoreceptors and myenteric mechanoreceptors. These receptors communicate with the central and the enteric nervous systems. Afferent nerve processes account for about 80% of vagal fibers and 20% of fibers of the greater splanchnic nerve [33,34]. The incoming information carried by these nerves is processed in central sensory nuclei and initiates neurally mediated gastric reflexes. Sensory neurons in enteric ganglia also initiate local reflexes such as gastric peristalsis. Cross-talk between central and enteric fibers occurs continually in the enteric plexus as it modulates the effects of motor neurons.

Vasculature

Gastric mucosal blood flow maintains epithelial integrity and is an essential component of mucosal defense. The stomach receives its blood supply from the celiac axis through six major arteries. The right and left gastric arteries supply the lesser curvature and extend over the anterior and posterior surfaces of the stomach. In a similar fashion, the left and right gastroepiploic arteries supply the greater curvature. Short gastric arteries from the splenic artery perfuse the upper stomach, and the gastroduodenal artery serves the pyloroantral region. These arteries provide large arterioles that pierce the gastric muscle wall.

The microvasculature of the stomach is illustrated in Figure 13.2. The entering arterioles provide smaller arterioles that extend to the submucosal plexus and to the muscle layers [35]. A capillary layer in the muscle drains into the venous collecting system. The afferent arterioles supplying the submucosal plexus are innervated by sympathetic fibers coursing with the entering blood vessels. The submucosal arteriolar plexus does not communicate with the venous plexus directly but rather provides mucosal arterioles to the base of the gastric glands. The mucosal arterioles branch into capillaries that ascend perpendicularly between the glands to the epithelial surface. These ascending capillaries interconnect horizontally, forming a lattice around the gastric glands. Ascending capillaries receive HCO_3^- secreted from the basolateral surface of stimulated parietal cells and carry it to the surface epithelium. This phenomenon is called the *alkaline tide*. At the surface epithelium, the HCO_3^- can assist in buffering any H^+ ion back-diffusion. At the mucosal surface the fenestrated capillaries empty into venules that drain into the submucosal venous plexus and efferent veins that follow the course of the primary arterial branches [36]. This anatomic arrangement allows muscle layer blood flow to be in parallel with mucosal flow while submucosal flow is in series with the mucosa. This type of microvascular system allows selective decreases in mucosal blood flow while muscular blood flow is maintained.

Mucosal blood flow accounts for 70%–80% of total gastric blood flow in basal and stimulated states [37,38]. Previous studies using aminopyrine clearance techniques suggest that stimulation of acid secretion increases mucosal blood flow, but interpretation of these results must be tempered by the knowledge that acid secretion affects aminopyrine clearance and may account for the apparent increase in mucosal blood flow [39]. Indicator dilution methods suggest that acid secretagogues have no effect on mucosal blood flow. When gastric perfusion pressures are adequate, gastric acid secretion varies independently of blood flow [40]. These conclusions have

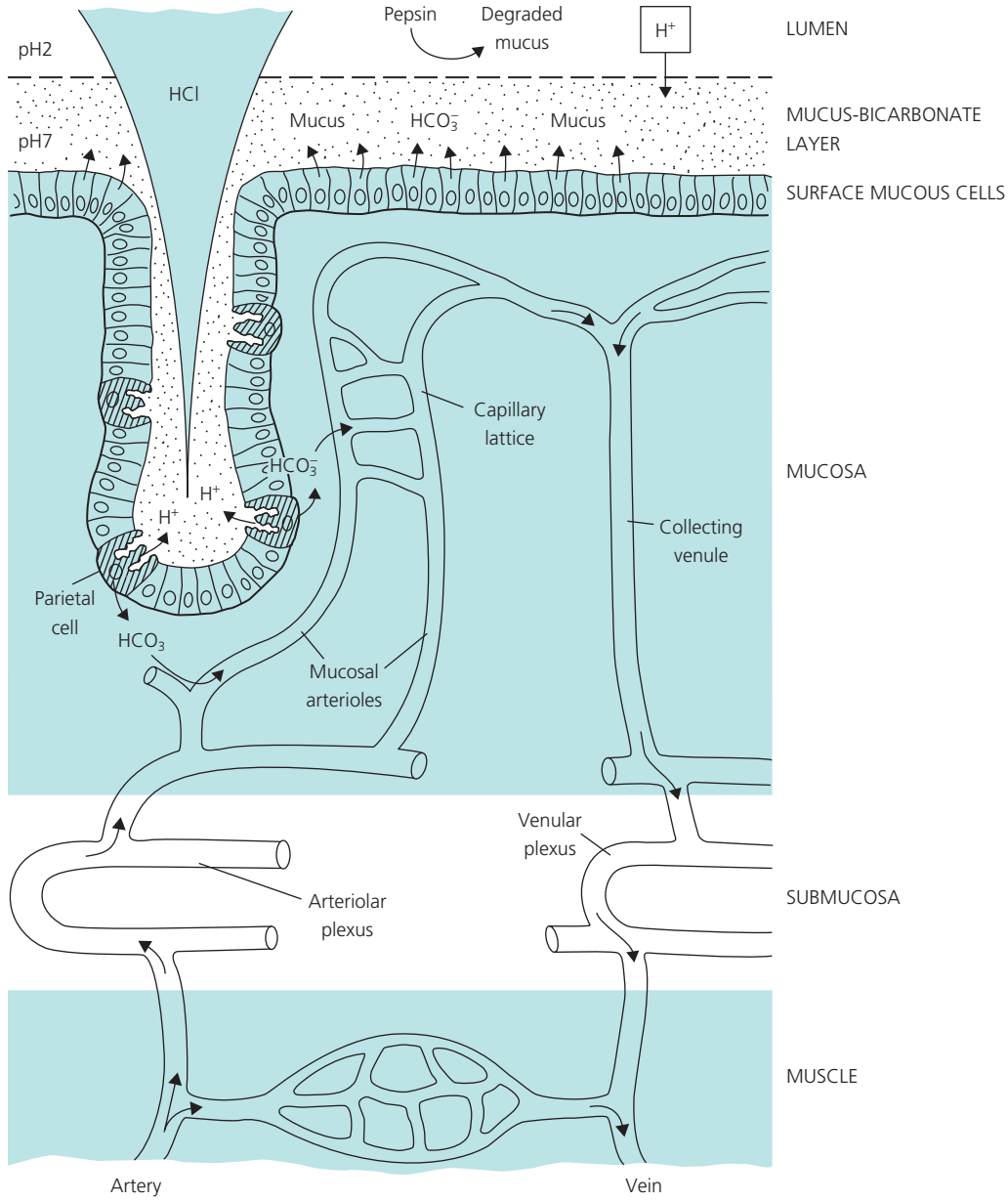

Figure 13.2 Gastric microvasculature. Adapted from Guth & Leung [35] and Koelz et al. [509].

challenged the long-held hypothesis that secretagogue stimulation induces a parallel increase in acid secretion and mucosal blood flow [41].

The innervation of the gastric microvasculature partially reveals the mechanisms regulating mucosal blood flow. The submucosal arterioles appear to be innervated by sympathetic nerve fibers [42]. When stimulated, these fibers constrict the arterioles and temporarily decrease mucosal blood flow [43,44]. After 3–4 min of prolonged sympathetic stimulation, flow increases [45]. This response has been described as autoregulatory escape from adrenergic vasoconstrictor influence [46]. Anatomical details support these physiological findings and suggest a regulatory function at the level of the submucosal arterioles [42].

Regulation of acid secretion

The regulation of acid secretion can be subdivided into the supracellular influences that have been the focus of classic

physiology since Beaumont and the cellular mechanisms that have been elucidated more recently. This section concentrates on supracellular influences and reviews the in vivo studies of acid secretion.

Integrated control

The integrated mechanisms that control acid secretion can be viewed as an arrangement of regulatory strata. These include two forms of neural control: long reflex or cephalovagal arcs and local intragastric reflex arcs. A second tier of control is exerted by humoral substances acting in an endocrine fashion, such as gastrin, or in a paracrine manner, such as histamine. Somatostatin may function as an endocrine and paracrine factor. The role of many such peptides remains uncertain. The direct influence of chemical factors is an additional tier of regulation, typified by the stimulatory action of amino acids and amines on gastrin release and the inhibitory effect of gastric acid on gastrin release.

These strata are not distinct but rather are closely intertwined; when discussing the phases of postprandial acid secretion, Grossman [47] asserted that the individual contributions made by the various phases could not be differentiated. Nevertheless, classic physiology has attempted to isolate and study factors that influence acid secretion. The results indicate that there are three principal stimuli of parietal cell acid secretion: histamine, acetylcholine, and gastrin. The principal inhibitory secretagogue appears to be somatostatin, although other chemical modulators, including peptides, growth factors, and prostaglandins, may also have roles.

When reviewing these in vivo studies, several confounding facts should not be forgotten. First, acute responses should be differentiated from chronic adaptive changes. For example, the effect on gastrin and somatostatin cell number seen during acute achlorhydria differs from the changes seen in chronic achlorhydria [48]. By analogy, data from studies using vagotomy may signify the chronic adaptive response to achlorhydria rather than the effect of withdrawal of vagal innervation alone. Second, acid secretagogues potentiate the response to one another. This central point, which is crucial to the understanding of studies of supracellular and cellular regulation, was first appreciated by Grossman [47]. He defined a potentiated response as one in which the effect of two agents in combination is greater than the sum of their separate effects when administered alone. Gillespie and Grossman [49] demonstrated in dogs with Heidenhain pouches that the acid secretory response to a combination of Urecholine (bethanechol chloride) and gastrin or to Urecholine and histamine greatly exceeded the maximal response to gastrin, histamine, or Urecholine alone (Fig. 13.3). These investigators subsequently demonstrated the corollary effect using antagonists of acid secretion. Metiamide, an H_2-receptor antagonist, inhibited acid secretion stimulated by histamine, pentagastrin, 2-deoxyglucose (which activates central vagal stimulation), and food. Atropine sulfate also inhibited the response to all of these stimulants except histamine [50]. With great perception, Grossman deduced that there were receptors for histamine, gastrin, and acetylcholine on the parietal cell that interacted with one another. Because of these interactions, it is impossible to predict the relative importance of each secretagogue on the basis of individual in vivo stimulation or inhibition studies. The phenomenon of potentiation provides the basis for the efficacy of many of the acid-reducing therapies for peptic ulceration, especially the use of H_2 antagonists. A third fact to consider when reviewing in vivo studies of acid secretion is that stimulatory and inhibitory influences are active simultaneously. This is true in the basal interprandial and the postprandial state.

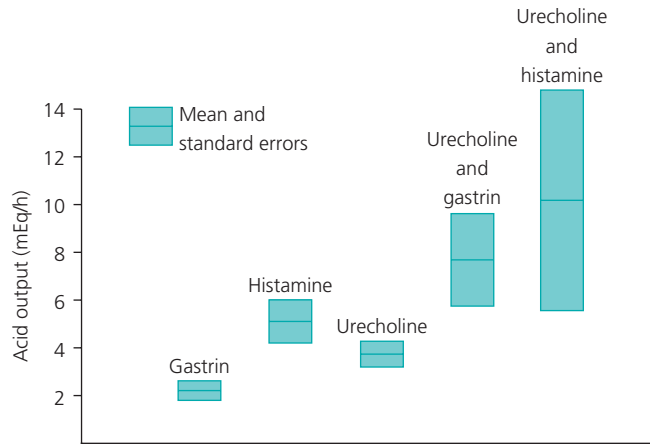

Figure 13.3 Maximal acid responses to gastrin, histamine, and Urecholine, alone and in combination, in dogs with Heidenhain pouches. From Gillespie & Grossman [49], with permission from the BMJ Publishing Group.

Methods for measuring acid secretion

Animal studies have used dogs, rats, rabbits, and mice. There are great species differences in basal secretion and responsiveness to secretagogues [51]. Classic studies of dogs have used vascularly perfused isolated pouches of gastric mucosa. The fundic pouches with intact vagal innervation are called *Pavlov pouches*, and vagotomized fundic pouches are called *Heidenhain pouches*. It is assumed that secretion from a vagally innervated fundic pouch is an accurate index of secretion from the main stomach [47]. These gastric pouches have been used to define many aspects of regulation of gastric acid secretion, including cephalic influences, the role of gastrin, long cephalovagal and local reflex neural arcs, and the inhibitory feedback control of acid secretion by intralumenal acid.

Aspiration of gastric juice is the simplest and most widely used method of estimating acid secretion in humans. To perform this test, a fine-bore nasogastric tube is inserted under fluoroscopic control into the most dependent part of the

stomach of a fasted volunteer, and several measurements are made: basal acid output (BAO), which estimates resting secretion, and maximal acid output (MAO) or peak acid output (PAO), which estimates the acid secretory response to an exogenous secretagogue. The BAO is measured by aspirating the gastric contents for four consecutive 15-min periods. The H^+ ion concentration of the aspirate is estimated by titration with a basic solution of known concentration. The BAO is expressed as mEq H^+/h and is the sum of the measured acid output in four unstimulated test periods. The expected range for BAO in healthy adults is 0–11 mEq H^+/h (Fig. 13.4) [52]. The measurement of BAO is usually combined with the measurement of MAO or PAO. In this test, acid output is stimulated by a supraphysiological dose of an exogenous secretagogue; this is usually pentagastrin, which may be administered by a subcutaneous or intramuscular injection (6 µg/kg) or by continuous intravenous infusion (6 µg/kg/h). Other secretagogues that have been used are histamine or a histamine analogue, betazole [1]. The MAO is the sum of acid output of four consecutive 15-min collection periods. The PAO is calculated by multiplying by two the sum of the two highest outputs recorded in the four test periods. The expected range for PAO in healthy adults is 10–63 mEq H^+/h (see Fig. 13.4) [52].

The MAO and PAO are a reflection of the total number of parietal cells, also called the *parietal cell mass* [53], which is influenced by gender, body weight, lean body mass, and age [54]. The MAO and PAO are lower in women than men because of a smaller parietal cell mass and a lower sensitivity of that parietal cell mass to exogenous secretagogues [55]. Stimulated acid output in children is comparable to that in adults when expressed as a function of body weight, but acid secretion declines in the elderly [56,57].

The BAO, MAO, and PAO under-represent actual basal and stimulated acid secretion because these methods do not account for acid lost through the pylorus, acid neutralization by gastric HCO_3^- and refluxed duodenal juice, and acid losses resulting from back-diffusion of H^+ through the gastric mucosa. Nonetheless, these measurements have proved useful in defining the pathophysiology of peptic ulcer disease [52] and in diagnosing Zollinger–Ellison syndrome.

Another method of estimating acid secretory ability was introduced by Fordtran and Walsh [58] using continuous in vivo intragastric titration. By this means, the acid secretory response to the physiological stimulation of ingested food is estimated. Continuous intragastric titration requires placement of a double-lumen tube into the most dependent part of the stomach, usually under fluoroscopic visualization. One lumen allows frequent sampling of small volumes (2–3 mL) of gastric contents. The pH of the sample is immediately measured and the gastric juice is returned to the stomach. The port of the second tube is positioned 10 cm proximal to the sampling port; throughout the study, the gastric pH is maintained at an arbitrary value, usually pH 5.5, by infusing $NaHCO_3$ through this second tube. A homogenized meal buffered to pH 5.5 is eaten. The amount of $NaHCO_3$ necessary to maintain the pH of the gastric juice at pH 5.5 is a measure of the postprandial acid secretory response. Because this is a cumbersome procedure, it has not gained widespread use outside specific research investigations. However, continuous intragastric titration has proven to be a useful method of measuring postprandial acid secretory responses [59,60].

A different approach to studying continuous acid responses is to measure intragastric pH by using an indwelling probe or radiotelemetric capsule [61,62]. This does not

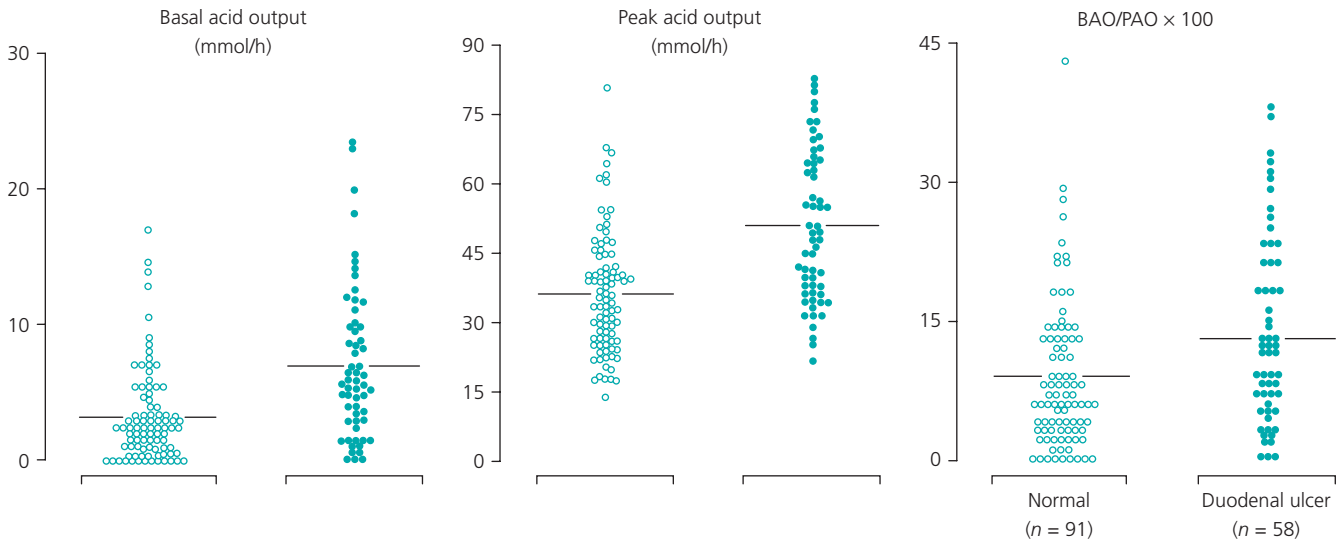

Figure 13.4 Basal acid output (BAO), peak acid output (PAO), and BAO–PAO ratio in 91 healthy adults and in 58 patients with duodenal ulcers. From Blair et al. [52], with permission from the American Society for Clinical Investigation.

estimate total H^+ concentration because it does not account for the volume of gastric juice and buffering capacity. Further studies with these techniques are required before their specific utility in humans can be determined.

Basal or interprandial acid secretion

Basal unstimulated acid secretion exhibits a circadian variation, with the highest secretion at night and the lowest in the early morning [63]. This variation is not matched by changes in circulating serum gastrin levels. In humans, vagotomy greatly reduces or abolishes interprandial acid secretion in patients with duodenal ulcer [53,60,64]. The caveats expressed about the influence of potentiation and chronic adaptive changes on studies of the effect of vagotomy on gastric endocrine and exocrine responses are pertinent when interpreting these data, although vagotomy does not affect parietal cell number [65]. Twice-daily administration of an H_2 receptor antagonist to patients with a duodenal ulcer reduces interprandial acid secretion, although it does not abolish it [60]. This suggests that H_2 receptor activation is also capable of influencing BAO. Basal interprandial acid secretion is likely the result of combined cholinergic and histaminergic stimulation.

The cholinergic regulation of basal gastric acid secretion is complex. In dogs with gastric pouches, denervation of the antral pouch markedly reduces resting output of acid from the main innervated stomach and from the denervated fundic pouch without changing plasma gastrin levels [66]. This suggests that there is a local interneuronal reflex arc innervated by the vagus and independent of plasma gastrin which carries acid stimulatory signals from the antrum to the fundus. These data are mirrored in humans; the small amount of basal acid secretion found in some patients who have undergone vagotomy can be abolished by antrectomy plus vagotomy [64].

Stimulation of acid secretion

The physiological stimulus for acid secretion is food. Traditionally, the food-stimulated acid response has been described in three phases: cephalic, gastric, and intestinal. These phases refer to the sites of origin of the stimuli and do not imply mechanisms by which acid secretion is stimulated or inhibited. These phases occur concurrently, not consecutively. The acid secretory response at any instant represents the sum of all of the stimulatory and inhibitory influences.

Cephalic phase

The influence of the nervous system on gastric acid secretion was first recognized by William Beaumont and Pavlov. In 1928, Farrell [67] showed that the vagus was the sole gastrocephalic neural link involved in gastric secretion, and this observation remains valid. The cephalic phase contributes in some degree to total postprandial acid but was estimated by Richardson and colleagues [68] to be as much as 50%. Feldman and Richardson [69] studied the relative contributions of thought, sight, smell, and taste of food to the cephalic phase of gastric acid secretion in humans (Fig. 13.5). Merely discussing appetizing food for 30 min without sight, smell,

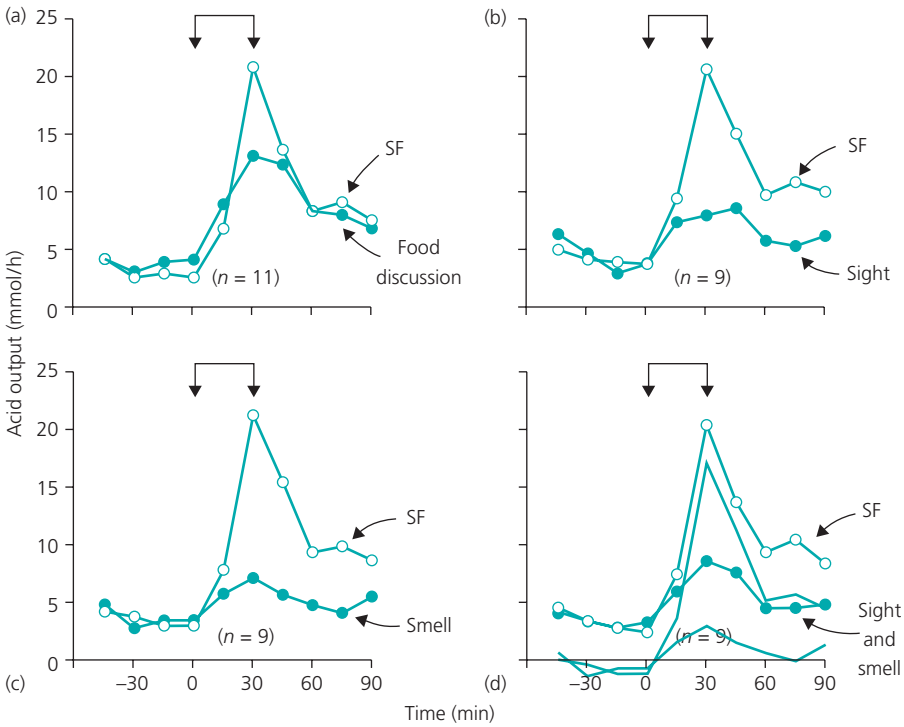

Figure 13.5 Mean gastric output in the same persons in response to sham feeding (SF) compared with **(a)** discussion of favorite foods, **(b)** sight of appetizing food, **(c)** smell of appetizing food, and **(d)** a combination of sight and smell. From Feldman & Richardson [69], with permission from Elsevier.

or taste produced an average of 66% of the total cephalic response estimated by the time-honored method of modified sham feeding. Serum gastrin levels also significantly increased. The sight of food or smell of food alone, although still producing significant acid secretion and gastrin release, was a considerably less potent stimulus than conversation. These data show by subtraction that taste is also an important component of the cephalic phase.

There are many interrelated mechanisms by which cephalovagal input mediates the cephalic phase of stimulated acid secretion. Vagal innervation acts directly on parietal cells. This was deduced from studies of dogs with antral and fundic gastric pouches. Denervation of the antral pouch reduced or abolished the serum gastrin response to sham feeding but did not abolish acid secretion by the vagally innervated fundic pouch [66]. Corroborative evidence for this mechanism is afforded by the demonstration of acetylcholine receptors on isolated canine parietal cells.

A second pathway in the cephalic phase of gastric acid secretion is the release of circulating gastrin from the gastric antrum. The thought, sight, or smell of food and modified sham feeding increase serum gastrin levels [64]. Isolated canine gastrin cells possess acetylcholine receptors. The plasma gastrin response to sham feeding in humans is regulated by intragastric pH and is not observable when gastric pH is maintained at pH 2.5 [70]. This effect is also atropine sensitive. However, vagal control of postprandial gastrin release is complex. Low doses of atropine, a muscarinic antagonist, enhance rather than reduce the gastrin response to sham feeding, insulin-induced hypoglycemia, or feeding [71–75]. Similarly, parietal cell vagotomy, although markedly reducing acid secretion, enhances gastrin release in response to sham feeding [71,76], insulin-induced hypoglycemia, or intragastric nutrient infusion [77]. These effects are independent of changes in intragastric pH [73]. This result suggests that the vagus carries cholinergic fibers that, in the absence of atropine, mediate directly or indirectly an inhibitory control of gastrin release. Studies of dogs with Heidenhain pouches and gastric fistulae demonstrate that truncal vagal denervation reduces gastrin release but elevates acid secretion [78]. These data can be interpreted to suggest that there is cholinergic release of a substance which can inhibit acid secretion independently of its effects on gastrin release. Although the nature of this substance has yet to be elucidated, somatostatin is one candidate [75]. However, Feldman and colleagues [72] were unable to demonstrate a rise in the level of plasma somatostatin in humans during sham feeding, and the role of somatostatin in regulating the cephalovagal control of gastric acid secretion, whether by acting directly on the parietal cell or by inhibition of gastrin release, remains uncertain. To define more precisely the role of gastrin in sham feeding-mediated gastric acid secretion in dogs, Kovacs and colleagues [79] developed a monoclonal antigastrin antibody capable of immunoneutralizing circulating gastrin in vivo. By means of this antibody, these investigators demonstrated that immunoneutralization of gastrin leads to a significant inhibition of sham feeding-stimulated gastric acid output, suggesting that gastrin released by central vagal stimulation is an important mediator of sham feeding-stimulated gastric acid secretion in dogs.

The components of the central nervous system involved in modulating gastric secretion include the dorsal motor nucleus of the vagus (DMNV), the nucleus tractus solitarius (NTS), and the hypothalamus. The DMNV supplies stimulatory efferent fibers to the stomach through the vagus nerve [80,81]. Stimulation of the DMNV results in activation of gastric secretion [82,83], whereas ablation of this nucleus abolishes the secretory process [84]. It appears that the role of the DMNV is not to initiate secretion [85] but instead to integrate central input from the hypothalamus and visceral input from the NTS. Regions of the hypothalamus that are important for regulating gastric secretion include the ventromedial hypothalamus, which appears to provide tonic inhibition [86,87], the lateral hypothalamus, and the adjacent medial forebrain bundle, which together mediate secretion in response to hypoglycemia [88–90]. Visceral afferents and taste fibers deliver input into the NTS demonstrating its potential involvement in stimulating gastric secretion in response to taste. The NTS is also thought to mediate stimulation of acid secretion in response to hypoglycemia [91]. Sympathetic and vagal afferents relay sensory information (mechanical, chemical, and thermal) from the stomach to the central nervous system [92].

Peptidergic factors also play an important role in modulating central nervous system-regulated gastric secretion. Direct injection of peptides into the brain has led to the discovery of factors that either stimulate or inhibit gastric secretion [93]. The peptides observed to either activate or inhibit gastric acid secretion by central injection are summarized in Table 13.1. This represents a partial summary of these peptides, the result of the numerous elegant studies examining this important subject [93–104].

Gastric phase

When food enters the stomach it initiates the gastric phase of acid secretion. This is usually divided into two components: a physical component caused by distention of the stomach and a chemical component in which chemical effectors interact with gastric cells.

The acid secretory response to distention results from presumed stretch receptors in the gastric tissue. This response is the stereotypical long vasovagal reflex arc. Distention of the gastric fundus and corpus in humans stimulates acid secretion. The effect is almost abolished by proximal gastric vagotomy and at least in part is independent of changes in serum gastrin levels [105–107].

Distention-induced acid secretion is a complex phenomenon in which antral and fundic responses can be

Table 13.1 Peptides that affect gastric acid secretion after injection into the central nervous system

Inhibitors	Stimulants
CRF	TRH
β-Endorphin	Somatostatin
Bombesin	Neuropeptide Y
Neurotensin	Galanin
Calcitonin	Peptide YY
CGRP	Ghrelinyama
Interleukin-1	Orexin A

CGRP, calcitonin gene-related peptide; CRF, corticotropin-releasing factor; TRH, thyrotropin-releasing hormone.

viewed separately. Antral distention produces gastrin release in dogs [108] and humans [109] – Debas and associates [108] called this the *pylorooxyntic reflex*. In dogs with antral and fundic pouches, this response was inhibited by antral acidification [108]; however, in humans, albeit those with intact stomachs, it appeared to be independent of lumenal pH [109]. In dogs, the pH sensitivity of the antral distention response required an intact cerebrovagal link to the antrum. In humans, low-dose atropine, which inhibited the acid secretory response to antral distention, nonetheless enhanced the elevation of serum gastrin levels. This suggests that there is an atropine-sensitive inhibitory pathway restraining the gastrin response to antral distention. Fundic distention in dogs with vagally innervated fundic and antral pouches produces gastrin release and acid secretion [110]. Both responses are lost when the antral pouch is maintained at pH 2.5. This vasovagal reflex has been called the *oxyntopyloric reflex*. A similar vagally dependent acid stimulatory response to fundic distention occurs in humans, but it appears not to be mediated through elevated serum gastrin [105].

The roles of histamine and acetylcholine in mediating distention-induced acid secretion are unclear. As may be predicted from our knowledge of the potentiation interrelations of the three principal secretagogues – gastrin, acetylcholine, and histamine – the acid response to gastric distention in humans is abolished by vagotomy or cimetidine [111,112]. However, this may indicate the facilitory effect of background secretion of these secretagogues only. Gastric release of acetylcholine or histamine in response to distention has not been shown.

Food interacts with the gastric mucosa to cause acid secretion in a manner that is independent of stretch by the food bolus. At least four constituents of food produce this stimulatory effect: peptic digests of proteins, ethanol, coffee, and Ca^{2+}. Whole proteins are poor stimuli of gastric acid secretion, but peptic digests of the same proteins are effective [113]. Amino acids, the breakdown products of proteins and amines produce acid secretion principally through the release of gastrin [23,113,114]. The aromatic amino acids phenylalanine and tryptophan are the most potent stimuli of gastrin release and gastric acid secretion in vivo [115]. Their amine derivatives may contribute significantly to this response [23,116]. Circulating gastrin is the principal mediator of postprandial gastric acid secretion. This has been demonstrated in human studies in which the increment in plasma gastrin that occurred after an intragastric infusion of amino acids or ingestion of a protein-rich meal was reproduced with an intravenous infusion of gastrin and a similar acid secretory response to endogenous and exogenous gastrin was observed [58,114]. These studies suggest that, despite the cephalovagal component of gastrin release, it is the chemical response to protein breakdown products that is the principal stimulus for postprandial gastrin release [114].

Although amines and amino acids can cause gastrin release by direct action on the G cell [23,115], this phenomenon must be viewed within the larger orchestrated physiological response to ingested nutrients. Nutrients stimulate the release of many peptides into the circulation, including somatostatin, cholecystokinin (CCK), secretin, gastric inhibitory peptides, enteroglucagon, and peptide YY, which may influence acid secretion directly or by affecting gastrin release.

Integrating these data with data derived from whole animal physiology has proven difficult. Atropine enhances gastrin release in many circumstances, although, according to the in vitro model previously described, inhibition of gastrin release or no change would be expected. Agents that block the gastrin response to bombesin do not affect the gastrin response to food. Studies in humans also suggest that β-adrenergic innervation may play a role in gastric acid secretion. Administration of terbutaline, a β_2-adrenergic receptor agonist, enhances serum gastrin levels but inhibits acid secretion in response to intragastric infusion of a homogenized meal [117]. Although it is likely that numerous neurohumoral mediators, such as somatostatin and gastrin-releasing peptide, and cholinergic and adrenergic innervation are significant factors in postprandial gastrin release, their relative roles in the integrated physiology of gastrin release remain as yet undefined.

Studies in humans have demonstrated a modest acid stimulatory response or no response to direct intragastric infusions of pure alcohol [118–120]. Red wine and beer, however, are potent stimuli of acid secretion and serum gastrin [118–120]. These effects are probably caused by amines or amino acids in the beverage stimulating gastrin release rather than by a direct effect of the alcohol content.

Caffeine stimulates acid release in humans [121]. McArthur and colleagues [122] showed that many beverages are potent stimulants of gastric acid secretion, including Tab, coffee, beer, and milk; each of these caused a pentagastrin-stimulated MAO of greater than 70%. Decaffeinated coffee was also a potent stimulant, showing that it is not just the caffeine in coffee that is an acid stimulant. These studies did not attempt to control for the cephalic phase of acid secretion, nor was gastrin measured; it is therefore impossible to draw conclusions about the mechanisms that underlie these observations.

Oral ingestion of calcium carbonate stimulates gastrin release and acid secretion in humans [123]. This action is independent of acid buffering capacity and is presumed to be an effect of dissociated Ca^{2+}.

Intestinal phase

The entry of chyme into the small intestine initiates the intestinal phase of the acid secretory process. The primary stimulatory factors are distention, proteins, and the products of protein digestion [124,125]. Quantitating the significance of the intestinal phase to the stimulatory limb of acid secretion has proven controversial, perhaps because there are definite potentiation phenomena between the acid secretory response to intestinal nutrients and gastrin or histamine [126]. Serum gastrin levels do not appear to mediate the intestinal phase of acid secretion in dogs or humans [125,127]. The acid stimulatory response to intestinal nutrients is preserved in vagotomized animals, indicating that circulating stimuli are involved to some degree [128]. This role may be filled in part by circulating amino acids, which have been shown to stimulate acid secretion without elevating serum gastrin [129]. There have been numerous attempts to isolate a distinct acid stimulatory peptide hormone (i.e., enterooxyntin) from small bowel mucosa, albeit without convincing success [130–133].

Inhibition of acid secretion

Cephalic influence

Evidence suggesting that there may be a cephalic inhibitory influence acting on gastric acid secretion comes from intracerebral microinjection studies [134]. Several of the peptides that can lead to this inhibitory event after intracerebroventricular injection are outlined in Table 13.1. It appears that vagal fibers carry inhibitory and stimulatory messages to the parietal cells. As with the cerebral microinjection studies in which gastric acid secretion is stimulated, however, there are no data to indicate which, if any, of these observations are relevant to the cephalic phase of gastric acid secretion. In addition, the complex response of stimulated serum gastrin levels to low-dose atropine previously described suggests that there are vagal inhibitory influences on gastrin release.

Gastric mediation

Just as vagal inhibitory fibers have been implicated in the cephalic acid secretory response, there appear to be vagally mediated inhibitory neural arcs involved in the distention-induced acid secretory response. Studies of dogs and humans led Debas and colleagues [108,135,136] to conclude that antral distention, in addition to stimulating serum gastrin release, results in the release of an inhibitor of acid secretion. Whether this effect is humoral or neurocrine is not clear, but it does depend on an intact vagus.

Gastrin release in response to nutrients, sham feeding, and antral distention is inhibited by the presence of acid in the gastric antrum [70,108,137]. An intralumenal pH of 3 appears to be the threshold for initiating this response [138]. The mechanisms of this negative-feedback loop probably include somatostatin release as a paracrine or endocrine gastrin inhibitor [138]. Evidence from in vitro human studies suggests that gastric acid regulates local release of somatostatin in the stomach and postprandial release into the circulation [139,140]. An alternative mechanism for the inhibition of gastrin release at low intralumenal pH is that an acidic milieu causes amines to be protonated and, as charged particles, they are not taken up by G cells [141]. This cannot be the complete answer, however, because at pH 2.5 cephalovagal stimulation of gastrin release is inhibited [70]. It is probable that somatostatin acts directly on the parietal cell to inhibit acid secretion by means of paracrine or endocrine pathways [1,142].

Intestinal inhibition

The observation that nutrient infusion into the small intestine inhibits acid secretion dates to the 1920s. Feng and colleagues [143] proposed that fat infusion into the small bowel inhibited acid secretion by the release from the small bowel mucosa of a circulating inhibitory hormone they called enterogastrone. There have been at least seven candidate peptides for this role: somatostatin, neurotensin, gastric inhibitory peptides, peptide YY, secretin, CCK, and galanin [143–151]. It is probable that enterogastrone is not a single entity but rather a physiological response to more than one circulating acid inhibitor.

Cellular basis of acid secretion

Analysis of the regulatory effects of gastric secretagogues in vivo has provided important insights into the mechanisms of acid secretion in animals and humans. Interactions among neurocrine, paracrine, and endocrine signals and their effects on the parietal cell ultimately must be studied in the intact organism. However, the complexity of the regulatory effects converging on the parietal cell makes integrated in vitro analysis a necessity. In vitro models of acid secretion include

preparations of gastric mucosa, gastric glands, and isolated enriched parietal cells. Gastric endocrine cells effecting acid secretion have also been enriched and analyzed. These models have provided an understanding of the cellular events leading to acid secretion.

In vitro models

Isolated preparations of intact gastric mucosa allow the study of secretory events while maintaining all of the mucosal cell types in their usual cellular environment. Cell polarity, tight junctions, gap junctions, desmosomes, and certain paracrine effects are maintained in such models. Physiological neurocrine and endocrine effects are absent but can be mimicked by bathing one or both surfaces with the desired neurotransmitter or hormone. One such model, the isolated bullfrog mucosa, has been used to study histamine release and acid secretion [152]. Mucosal strips are bluntly dissected and are mounted as a sheet between two sides of a Lucite chamber. A flow-through system can be used to ensure rapid changes of the serosal solution. Acid secretion into the mucosal solution is measured with a pH stat by maintaining the pH at 7.0 with isotonic 15 mM NaOH. Other types of mucosal studies use explants maintained in organ culture or fragments of mucosa suspended in tissue culture solutions [153–155]. However, studies using these types of models have been supplanted by gastric gland and isolated cell studies.

The initial steps in the preparation of glands and cells are similar [156,157]. The gastric mucosa is bluntly dissected away from the submucosa, finely minced, and then dispersed with pronase or crude collagenase. The viability rate of parietal cells obtained with this technique generally exceeds 95% as judged by trypan blue exclusion.

After digestion of the gastric mucosa, the glands can be separated from the cells and debris by several sedimentation washes at unit gravity. The large size of the glands allows them to sediment rapidly, essentially free of nonglandular material. Separation of isolated cells can be achieved by velocity and density separation techniques. Counterflow elutriation is a velocity separation technique that separates cells predominantly on the basis of size [157]. Enriched fractions routinely contain 50%–70% parietal cells, which maintain their viability and biological activity. Antral G cells and fundic D cells have also been enriched by elutriation [158]. A second purification step using a density gradient can be added to enrich parietal or endocrine cells further.

There is a potential limitation in using isolated parietal cells for physiological studies. Once the cells are separated from the microenvironment found within the gastric mucosa, their ability to respond to secretogogues in a tissue-specific fashion diminishes within 8–10 h after isolation (Del Valle and Yamada, unpublished observations). To circumvent this problem, Chew and Ljunstrom [159] developed a system for culturing isolated parietal cells so that they retain their differentiated structure and function for as long as 72 h after isolation. These cultured cells express receptors for the appropriate secretagogues and maintain expression of parietal cell-specific genes such as *ATP4B*, which codes for H^+,K^+-ATPase. This model provides an important tool for characterizing the multiple factors involved in regulating parietal cell physiology.

Gastric secretion in different species has been analyzed with each of these models. Interspecies variation may account for certain discrepant results occasionally observed with different models. Many analyses of the cellular events controlling acid secretion have used canine gastric mucosal cells enriched by counterflow elutriation. The data obtained using canine cells can be correlated with the large body of data obtained from in vivo acid secretory studies in dogs. Moreover, canine parietal cells have several features in common with human parietal cells.

Indirect measures of parietal cell function have been developed to quantitate the biological effects of various secretagogues and inhibitors. The development of these assays has made it possible to correlate the binding of ligands with their functional effects. Oxygen consumption has been shown to correlate with HCl secretion in both in vivo and ex vivo studies [160,161]. Oxygen consumption in isolated enriched parietal cells increases in response to gastrin, carbachol, and histamine, probably reflecting the activity of the proton pump [157]. Glucose oxidation can also be used as a measure of parietal cell metabolic activity [162]. Morphological transformation of parietal cells in response to secretagogues produces dramatic changes in cell appearance. Resting cells are filled with tubulovesicles whereas stimulated cells rapidly develop a dense intracellular canalicular network communicating with the apical surface of the cell. Nomarski optics can visualize this transformation in living cells, and fluorescent microscopy with acridine orange can show the accumulation of fluorescent dye in the newly generated acidic spaces [163].

The accumulation of weak bases in membrane-bound acidic spaces can be measured using the carbon 14-labeled aminopyrine uptake assay [164]. Uncharged aminopyrine is lipid soluble and easily crosses cellular membranes. On entering an acidic compartment, aminopyrine is protonated and loses its lipid solubility. This sequestration of [^{14}C]aminopyrine correlates with intracellular acid formation and is the most commonly used assay for parietal cell stimulation. Other methods involving measurement of proton pump association with canalicular membranes and correlation of morphological changes in parietal cells with functional measurement of acid production using fluorescence microscopy techniques permit the dynamic study of parietal cell physiology [165,166].

Receptors

Separation of gastric cells into highly enriched cell populations has made it possible to identify and characterize specific

receptors on each cell type [167]. Cells maintain intact receptors throughout the separation procedure and purified cells bind radiolabeled ligands specifically. The binding of a ligand should correlate with a functional assay over the same dosage range to confirm that a specific cell type has biologically active receptors. An example is the correlation of carbachol binding to parietal cells and the induction of aminopyrine uptake by carbachol.

After functional receptors have been demonstrated, they can be characterized using binding studies with available receptor agonists and antagonists. Further characterization of receptors has been achieved by solubilizing them from cell membranes and cross-linking them to specific radiolabeled ligands. The solubilized form of the receptor can be examined for size, subunit structure, or ligand-induced autophosphorylation.

Receptors can be purified, their amino acid sequences analyzed, and their genes eventually cloned. The application of molecular biological techniques to the study of cell surface receptors has greatly enhanced our understanding of their structure and function. Several receptors that are critical for gastric secretory function have been cloned. These include the histamine H_2, somatostatin, muscarinic M_3, and gastrin or CCK_2 receptors [168–171]. Analysis of the amino acid sequences of these cloned receptors indicates that they belong to the family of heptahelical or G protein-linked receptors. Structurally, these receptors consist of a single amino acid chain containing seven hydrophobic domains, which presumably traverse the cell surface membrane (i.e., transmembrane regions). Recombinant molecular technology (e.g., site-directed mutagenesis, chimeric receptor studies) enabled the characterization of the structural components of G protein-linked receptors that are important for ligand binding and biological action. For example, the specificity of ligand binding can be determined by the hydrophobic transmembrane regions of the receptor and the extracellular domains. Furthermore, coupling to the G proteins that are responsible for secretagogue-mediated signal transduction involves interaction principally with the third intracellular loop of the receptor. Receptor regulation (e.g., uncoupling, sequestration, down-regulation) appears to involve kinase-mediated phosphorylation events targeted to serine and threonine residues found within the C-terminal portion of these receptors. Detailed structure–function analyses of the receptors that are important in gastric secretion are in progress.

The question of whether acid secretagogues act directly or indirectly on parietal cells could be answered after isolated parietal cell preparations were developed. Initial studies showed that histamine, carbachol, and gastrin increased canine parietal cell oxygen uptake [157,172]. Later studies revealed that [^{14}C]aminopyrine accumulation was also increased by each of these secretagogues. By using isolated rabbit gastric glands, however, somewhat different results were obtained; aminopyrine accumulation could be demonstrated in response to histamine and carbachol but not to gastrin [173]. These differences may reflect species variability. For example, rabbits, which are known to feed continuously, may not have developed the ability to marshal an acid secretory response to intermittent bolus feeding as mediated by gastrin. Alternatively, these differences may reflect differences in the study methods used. The experiments of Del Valle and colleagues [174] have demonstrated marked increases in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in response to the administration of gastrin 17 in single isolated rabbit parietal cells, suggesting that gastrin receptors are present on rabbit cells as well.

The concept that secretagogues such as acetylcholine and gastrin directly activate parietal cells was placed in doubt by a study that demonstrated the expression of histamine H_2, gastrin, and muscarinic receptors on immunocytes found in gastric lamina propria instead of on parietal cells [175]. Diaz and colleagues [176] revisited this controversial issue. Using in situ hybridization histochemistry and autoradiography with a highly selective H_2 receptor radioligand (iodine 125-labeled aminopotentidine), a specific signal for H_2-specific gene transcripts was detected only within parietal cells of the gastric epithelium. The discrepant results obtained by these two investigative teams may be attributed to the less sensitive oligonucleotide probes used by the former group compared with the highly specific H_2 receptor antisense riboprobe used by the latter. The classic secretagogues previously described clearly act directly on gastric parietal cells as initially postulated by multiple investigators, and the possibility that receptors for these ligands are also located on immunocytes in the lamina propria should not be excluded.

Although the critical role of the H_2 receptor in regulating gastric acid secretion had been well established, the structural components of this receptor that determine H_2 selectivity were unknown. The success of Gantz and associates [168] in cloning the gene encoding the histamine H_2 receptor provided the essential tools required for the further study of this question. Key areas of homology in the structures of the histamine H_2 and β_2-adrenergic receptors suggested specific transmembrane amino acids that could be important for histamine binding. On the basis of studies involving the expression of histamine H_2 receptors with site-directed mutations, a model for histamine binding and action on the H_2 receptor was proposed [177]. An aspartic acid residue (Asp98) in the third transmembrane domain is essential for histamine binding and action, and an aspartic acid (Asp186) in the fifth transmembrane domain defines H_2 selectivity. A threonine (Thr190) in the fifth transmembrane domain is important in establishing the kinetics of histamine binding but is not essential for H_2 selectivity.

The presence of a specific muscarinic receptor on canine parietal cells is supported by specific blockade of the biological

effects of carbachol with atropine. The dissociation constant for atropine inhibition of carbachol-induced aminopyrine uptake, 1.3 nM, is consistent with dissociation constants observed with muscarinic receptors in other tissues [173]. In addition to inducing oxygen uptake and aminopyrine accumulation in a dose-dependent manner, carbachol produces a parallel increase in the turnover of membrane inositol phospholipids [178]. Pharmacological studies indicate that the parietal cell muscarinic receptor is of the M_3 subtype, which has been confirmed by the molecular cloning of this receptor [179,180]. Rat parietal cells have also been shown to have M_3 muscarinic receptors that stimulate aminopyrine uptake, increase inositol phospholipid turnover, and bind [*N*-methyl-^{3}H]scopolamine [181].

Gastrin receptors have been localized to isolated rat [182] and canine [183] parietal cells. Binding studies with purified parietal cells reveal specific binding that is rapid and saturable. Proglumide, a CCK or gastrin receptor blocker, inhibits gastrin binding and stimulation of parietal cell function. CCK-8 is equipotent with gastrin 17 in displacing radioligand and in stimulating aminopyrine uptake [183]. This suggests that the parietal cell gastrin receptor binds and responds to either ligand in a similar fashion. The gastrin receptor has been characterized further by cross-linking studies in two species. Using canine parietal cell membranes, cross-linking studies with ^{125}I-gastrin 2–7 revealed a single gastrin receptor with a molecular weight of 74 kDa [184]. Half-maximal inhibition of radiolabeled gastrin binding in these canine parietal cell preparations was achieved at 3×10^{-10} M, in agreement with the potency of gastrin in stimulating aminopyrine uptake [183]. Similar studies with detergent extracts of porcine gastric mucosal membranes using ^{125}I-[Nleu15]gastrin 2–17 as a ligand resulted in the cross-linking of a 78-kDa binding protein [185]. In contrast to the studies using canine parietal cells, however, 50% inhibition of binding required 2×10^{-6} M [Nleu15]gastrin. The apparent reduction in affinity may be an artifact of the extraction technique used. In both species the receptor appears to be a single protein with no disulfide-linked subunits. These studies confirm the presence of a specific gastrin receptor on parietal cells. The gastrin receptor appears to be coupled to membrane inositol phospholipid turnover and protein kinase C (PKC) activation [178].

Kopin and colleagues [171] isolated a cDNA clone encoding the gastrin receptor found on canine parietal cells. They determined that it is a member of the G protein-linked receptor family and, when expressed, it has a molecular weight similar to those reported in the receptor cross-linking studies. Expression of the cloned receptor confirmed that it is coupled to membrane inositol phospholipid turnover and mobilization of $[Ca^{2+}]_i$. Song and colleagues [186] cloned the gene encoding the human gastrin or CCK_2 receptor and localized it to a region of chromosome 11 (11p15.4). It appears that the gene produces two different receptor proteins as a result of alternative RNA splicing. The functional difference between the two receptor isoforms remains to be established.

As with many peptides, gastrin is synthesized as a precursor molecule that undergoes posttranslational processing to become a C-terminally amidated product, which is presumed to be the sole biologically active form of this hormone [187]. Indeed, posttranslational processing intermediates of gastrin, specifically the C-terminally glycine-extended form (G-Gly), serve as substrates for amidation, are stored in gastric tissues, and are secreted into the circulation with amidated peptide reaching plasma concentrations roughly equivalent to those of processed gastrin.

The question of whether posttranslational processing intermediates of gastrin exert a biological effect was addressed by Seva and colleagues [188]. These investigators showed that G-Gly exerts growth-promoting effects by activation of non-CCK_2 receptors [188]. This finding demonstrated that glycine-extended intermediates of prohormone processing have independent and hitherto unrecognized important biological actions. Subsequently, Kaise and associates [189] showed specific binding of ^{125}I-[Leu15]G2–17-Gly to gastric canine parietal cells, which was dose-dependently displaced by G2–17-Gly but not by gastrin or by the specific gastrin/CCK_2 receptor antagonist L365 260. Thus, gastric parietal cells appear to express specific and distinct receptors for both gastrin and G-Gly.

The role of G-Gly in gastric acid secretion has been the focus of ongoing investigative efforts. An early study conducted in isolated canine gastric parietal cells demonstrated that G-Gly was at least four orders of magnitude less potent than gastrin in stimulating gastric acid secretion [190]. Interestingly, inhibition of gastrin amidation by copper chelation with diethyldithiocarbamate led to an unexpected increase in both basal and gastrin-stimulated gastric acid outputs, suggesting that chronically elevated levels of G-Gly could have a stimulatory effect on the gastric secretory process [191]. Higashide and colleagues [192] showed that infusion of G-Gly alone did not stimulate gastric acid secretion in rats. However, administration of G-Gly in combination with gastrin significantly potentiated the response observed in the presence of gastrin alone, further supporting a physiological stimulatory role for G-Gly in gastric acid secretion [192]. Chen and associates [193] observed similar potentiating effects on gastric acid secretion after administering G-Gly in combination with gastrin to gastrin-deficient mice. Interestingly, in these animals G-Gly infusion did not affect the number of ECL or parietal cells, the proliferation rate of the fundic epithelium, or the level of expression of the H^+,K^+-ATPase. Administration of G-Gly, however, appeared to prevent the formation of vacuolar canaliculi and lipofuscin bodies, suggesting that G-Gly could synergize with gastrin to stimulate gastric acid secretion by preventing parietal cell degradation [193].

Another possible mechanism for these observations was presented in a study performed in cultured canine gastric parietal cells [189]. Preincubation of parietal cells with G-Gly enhanced the acid secretory response of the cells to histamine, whereas acutely administered G-Gly had no effect [189]. G-Gly dose-dependently increased both the transcription and expression of the gene encoding the α-subunit of H^+,K^+-ATPase, suggesting that G-Gly may have a functional role in potentiating gastric acid secretion by enhancing the expression of the gene encoding the protein responsible for H^+ generation [189]. Although these observations are in apparent discordance with those reported in the study of the gastrin-deficient mice [193], it is possible that these genetically engineered animals exhibit developmental abnormalities in the mechanisms regulating the expression of the H^+,K^+-ATPase. Alternatively, these findings may reflect species-specific differences in the ability of G-Gly to induce H^+,K^+-ATPase gene expression.

The effects of somatostatin on isolated enriched canine parietal cells have been evaluated. Somatostatin dose-dependently inhibits histamine-induced aminopyrine uptake and cAMP production and pentagastrin-stimulated aminopyrine accumulation [194]. Somatostatin binding sites have been identified using ^{125}I-[Leu8-D-Trp22-Tyr25]somatostatin 28 as a radioligand. Somatostatin 14 and somatostatin 28 are equally potent at displacing bound ligand and at inhibiting aminopyrine accumulation. Scatchard analysis of the binding data revealed two binding sites with dissociation constants of 3.2×10^{-9} M and 2.1×10^{-7} M respectively. Crude membranes prepared from 95%–100% pure parietal cells were incubated with ^{125}I-[Leu8-D-Trp22-Tyr25]somatostatin 28 and cross-linked with disuccinimidyl suberate [195]. After solubilization, a single sharp band with no disulfide linkages was identified corresponding to a membrane receptor with a molecular weight of 99 kDa. These parietal cell somatostatin receptors may be activated by means of a direct paracrine effect mediated by long cytoplasmic processes that extend from fundic mucosal D cells and appear to terminate on parietal cells [24]. Inhibition of acid secretion by postprandial serum somatostatin may also be mediated through these receptors.

Five members of the somatostatin receptor family ($SSTR_1$–$SSTR_5$) have been cloned and characterized; they have distinct pharmacological properties and tissue distribution [196]. All five SSTR subtypes are expressed throughout the rat gastrointestinal tract and, specifically, in all layers of the gastric mucosa [196]. The development of highly specific and selective agonists [197] has led to more precise functional characterization of these different somatostatin receptors. In particular, $SSTR_2$ appears to mediate the inhibition of gastric acid secretion in rats, dogs, and humans [197,198], a finding confirmed by selective ablation of the *SSTR2* gene [199]. Moreover, Prinz and colleagues [200] showed that $SSTR_2$ is the predominant somatostatin receptor subtype expressed on rat ECL cells, where it mediates the inhibition of histamine release. Therefore, although all five SSTRs are expressed in the stomach, $SSTR_2$ appears to be the receptor subtype most involved in regulating the gastric secretory process.

The presence of other receptors on parietal cells has been suggested but awaits confirmation. Prostaglandins E_2 (PGE_2) and I_2 (PGI_2) inhibit histamine-stimulated aminopyrine accumulation and cAMP generation in enriched canine parietal cells [201]. These agents have no effect on aminopyrine uptake induced by gastrin, carbachol, or dibutyryl cAMP. Specific binding sites for PGE_2 exist in subcellular membrane fractions of porcine fundic mucosa, suggesting localization to the plasma membrane [202–204]. Prostaglandins can displace bound [^{3}H]PGE_2 and inhibit histamine-stimulated aminopyrine uptake in isolated rabbit parietal cells [205]. Together, these findings strongly suggest the presence of prostaglandin receptors on parietal cells. Ding and colleagues [206] confirmed this observation by documenting gene expression of the prostaglandin receptor subtypes EP_3 and EP_4 on rat gastric parietal cells.

A calcium-sensing receptor (CaSR) has been identified on rat and human parietal cells. Activation of this receptor by Ca^{2+} and L-amino acids appears to lead to an increase in $[Ca^{2+}]_i$ and to stimulate H^+,K^+-ATPase activity [207,208].

Although it is fairly clear that H_3 receptors are expressed on ECL cells, serving a role in autocrine regulation of histamine release, their presence on parietal cells remains controversial. The H_3 receptor agonist methylhistamine is a potent stimulant of aminopyrine uptake in isolated cultured rabbit parietal cells [209]. This observed stimulatory effect can be blocked by the H_2 receptor antagonist ranitidine, placing in question the selectivity of the H_3 receptor agonist and the significance of the H_3 receptor in parietal cell physiology.

The sympathetic arm of the autonomic nervous system may be expected to counterbalance the stimulatory effect of muscarinic receptors on acid secretion. Although it is possible that β-adrenergic agonists may inhibit acid secretion through stimulation of somatostatin release [210] and inhibition of histamine release by fundic mucosal mast cells [167,211], direct studies with parietal cells support the presence of stimulatory β-adrenergic receptors [212].

Epidermal growth factor (EGF) and TGF-α have been implicated in the inhibitory modulation of gastric acid secretion [213–215]. TGF-α shares structural homology with EGF and is expressed within parietal cells. Parietal cells express the receptor for EGF, which is recognized by both EGF and TGF-α [216,217]. Both these growth factors inhibit parietal cell function in a similar fashion [218–221]. TGF-α is secreted by parietal cells and it may function as an autocrine regulatory factor. The presence of secretin, glucagon, or opioid receptors on parietal cells remains unconfirmed.

Beales and Calam [222] have suggested that parietal cells may express receptors for cytokines. In a series of studies

conducted in isolated rabbit gastric parietal cells, tumor necrosis factor-α and interleukin-1β were shown to inhibit gastric acid secretion, as assessed by the accumulation of the weak base aminopyrine, both in the basal state and after stimulation with histamine, gastrin, and carbachol [222]. In contrast to these observations, Yakabi and colleagues [223] reported that interleukin-8 enhanced in vivo gastrin-stimulated gastric acid secretion in rats. Thus, cytokines appear to have complex regulatory effects on gastric acid secretion, which may be mediated, at least in part, by a direct action of these agents on gastric parietal cells.

An overview of the interactions of ligands and receptors involved in acid secretion is provided in Figure 13.6. The parietal cell has stimulatory receptors for gastrin, acetylcholine, and histamine. Several lines of evidence suggest that parietal cells in humans may be exposed continuously to basal levels of acetylcholine and histamine. Gastrin can account for most of the postprandial increase in gastric acid secretion [114]. Although a portion of the postprandial response is the result of a direct effect of gastrin on parietal cells, a significant component may be caused by the ability of gastrin to stimulate histamine release from ECL cells (see the section on histamine). The parietal cell also has inhibitory somatostatin receptors that counteract the secretagogue effects. Vagal nerve fibers may enhance their acid stimulatory effect through stimulatory muscarinic receptors on G cells and inhibitory muscarinic receptors on D cells [209]. Adrenergic fibers appear to stimulate D-cell secretion and inhibit release by histamine-containing cells, counterbalancing the vagal effects [209]. Gastrin and CCK stimulate parietal cells and D cells [224]. Gastrin-releasing peptide acts as a neurotransmitter that stimulates G cells, and somatostatin may function as a paracrine G-cell inhibitor [225–227]. Further structural and functional analyses of each of these

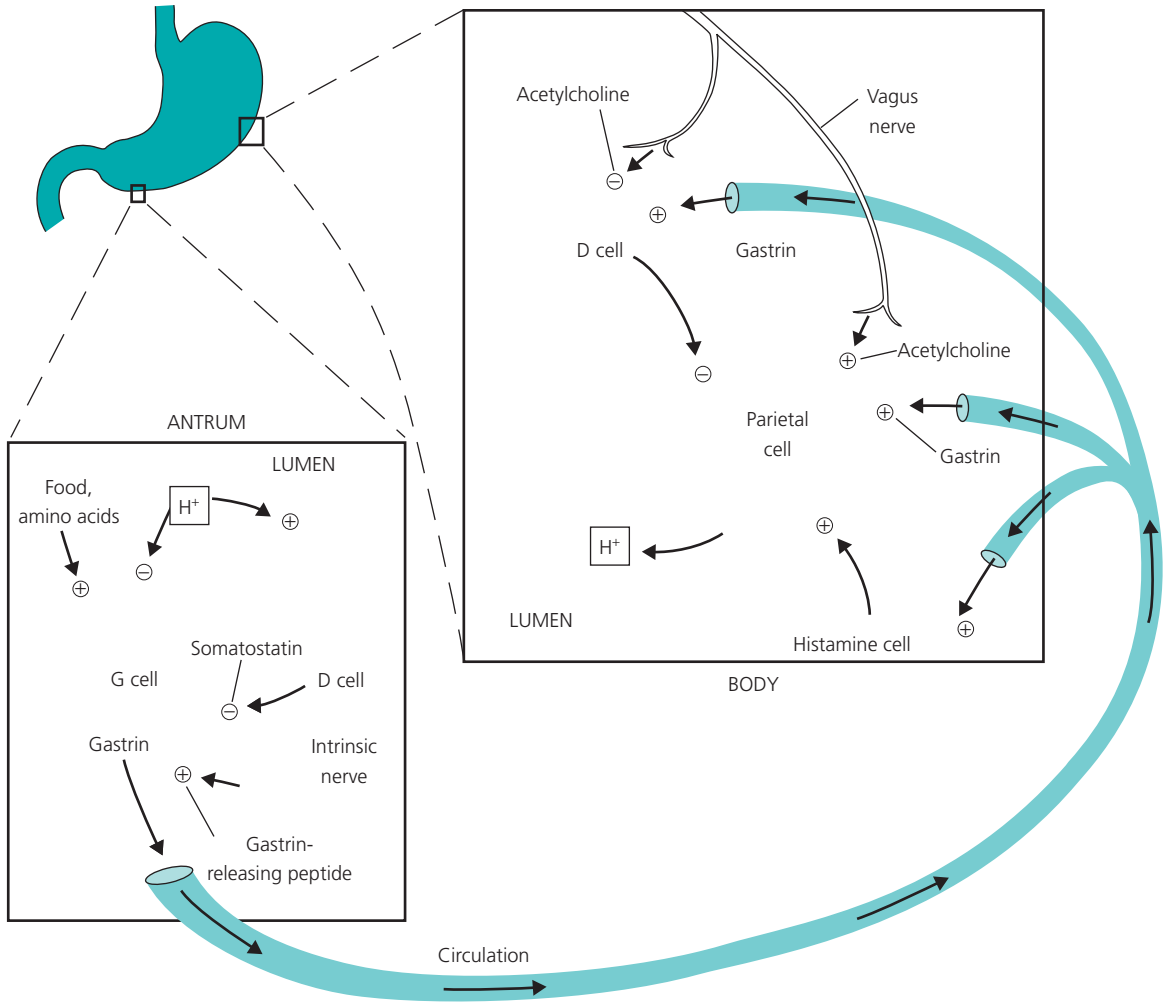

Figure 13.6 Regulation of gastric acid secretion. Major gastric mucosal ligand–receptor interactions regulate parietal cell HCl secretion. D cell, somatostatin cell; G cell, gastrin cell. Adapted from Feldman [510].

receptors will greatly enhance the understanding of gastric secretion.

Intracellular signal transduction

As previously described, the gastric parietal cell is a complex biological structure that is controlled by a broad variety of growth factors, hormones, and neurotransmitters. These agents interact with specific receptors on the cell surface, initiating a flow of information that moves to the cell nucleus along highly organized and complex signal transduction pathways. Once in the nucleus, these signals are known to activate specific programs of transcriptional events that lead to the expression of specialized cellular functions. Numerous signal transduction pathways have been studied and characterized in gastric parietal cells.

Cyclic adenosine monophosphate

Receptors linked to adenylate cyclase influence intracellular levels of cAMP. Such receptors are coupled to inhibitory (G_i) or stimulatory (G_s) GTP-binding proteins (Fig. 13.7) [228]. G_i attenuates adenylate cyclase activity, decreasing cAMP levels. The ability of pertussis toxin to block the actions of G_i can be used experimentally to dissect second messenger pathways. G_s, which increases adenylate cyclase activity and cAMP levels, can be stimulated selectively by cholera toxin. Increases in cytoplasmic cAMP levels result in activation of cAMP-dependent protein kinases and consequent phosphorylation of various intracellular proteins that appear to mediate the effects of the ligand–receptor interaction.

Inositol phospholipids, Ca^{2+}, and protein kinase C

A second major intracellular signal transduction cascade involves the turnover of membrane phospholipids, specifically the inositol phospholipids. Receptors are linked to this pathway through a G protein, followed by phospholipase C-induced hydrolysis of phosphatidylinositol bisphosphate (PIP_2) to diacylglycerol and inositol triphosphate (IP_3) [229]. IP_3 causes the release of Ca^{2+} from intracellular stores, and diacylglycerol promotes the translocation of a Ca^{2+}/phospholipid-dependent protein kinase (i.e., PKC) from the cytoplasm to its active site on the cell membrane [230,231]. Increases in $[Ca^{2+}]_i$ activate various Ca^{2+}-dependent enzyme systems, such as the calmodulin kinases, and promote the translocation and activation of PKC.

Although receptor activation was originally thought to be linked to a single signal transduction cascade, it is evident that interactions between postreceptor signaling pathways can occur at multiple levels [170]. One example of this in gastric secretion is the ability of the histamine H_2 receptor to stimulate increases in cAMP and $[Ca^{2+}]_i$ through separate pathways [232]. Del Valle and colleagues [232] showed that

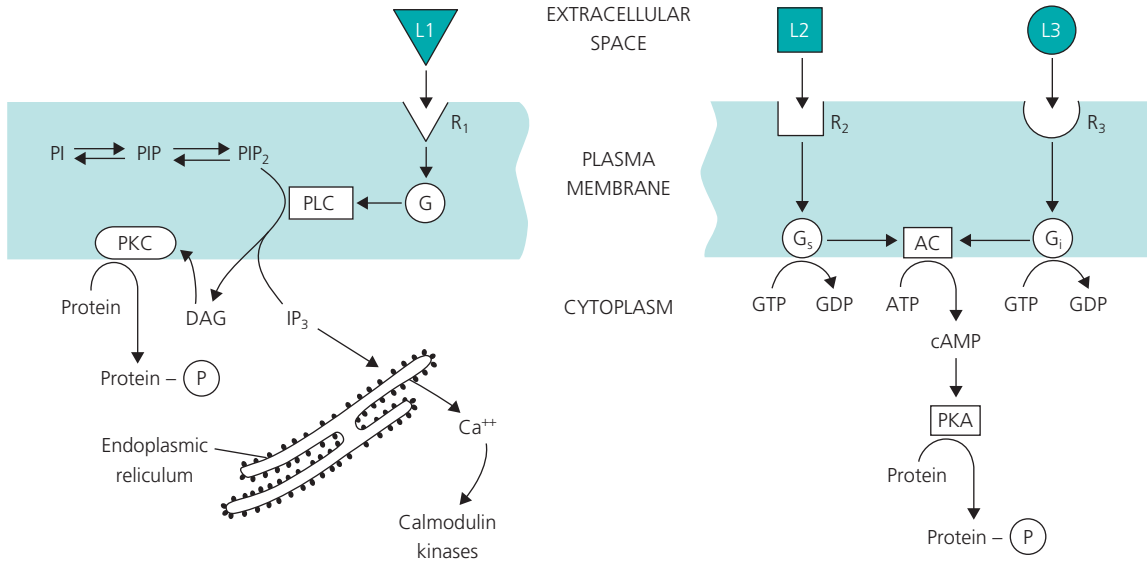

Figure 13.7 Signal transduction pathways in parietal cells. Ligands (L) interact with receptors (R) to initiate the target cell response. The **left panel** shows the membrane inositol phospholipid pathway activated by gastrin and acetylcholine (L1). A guanine nucleotide-binding protein (G) activates phospholipase C (PLC), which hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) to diacylglycerol (DAG) and inositol 1,4,5-triphosphate (IP_3). DAG activates protein kinase C (PKC), which phosphorylates (P) target proteins, whereas IP_3 induces intracellular calcium mobilization, which activates kinases. The **right panel** depicts the cAMP pathway used by histamine (L2) and somatostatin (L3) in parietal cells. The histamine receptor (R_2) acts through a stimulatory G protein (G_s), which activates adenylate cyclase (AC) and generates cAMP. An increased level of cytoplasmic cAMP activates protein kinase A (PKA), which phosphorylates parietal cell effector proteins. The somatostatin receptor (R_3) activates an inhibitory G protein (G_i), which inhibits adenylate cyclase and decreases cAMP levels.

stimulation of the cloned canine H_2 receptor leads to an increase in adenylate cyclase and IP_3 or $[Ca^{2+}]_i$ activity through independent pathways.

The acid stimulatory action of histamine appears to be mediated primarily through its ability to increase cAMP production in parietal cells [233]. This increase parallels the ability of histamine to stimulate aminopyrine uptake and oxygen consumption. Histamine increases cAMP-dependent protein kinase activity in enriched rabbit parietal cells [234]. The substrates for this enzyme activity have not been fully characterized, but they are thought to mediate the effects of histamine stimulation [235].

The acid stimulatory actions of gastrin and carbachol appear to be mediated by Ca^{2+}-dependent pathways. Gastrin and carbachol exert similar effects on membrane inositol phospholipid turnover in parietal cells. Both agents induce a time-dependent decrease in PIP_2 and increase in the formation of IP_3 [178]. These effects parallel increases in [^{14}C]aminopyrine accumulation. The increase in IP_3 may mediate the mobilization of $[Ca^{2+}]_i$ observed in parietal cells stimulated with gastrin or carbachol. In the case of carbachol, increased $[Ca^{2+}]_i$ may occur as a result of enhanced mobilization of Ca^{2+} from intracellular stores or through influx of extracellular Ca^{2+} across the cell membrane [236–238]. It is presumed that the elevated levels of cytoplasmic Ca^{2+} then activate several enzyme cascades, including the calmodulin kinase family and, in concert with diacylglycerol, the second product of PIP_2 breakdown, PKC. Gastrin and carbachol dose-dependently increase membrane-associated PKC activity [185]. Although gastrin and carbachol increase membrane inositol phospholipid turnover and PKC activity in canine gastric parietal cells to a similar degree, carbachol is a more potent stimulant of acid production [185]. Takeda and associates [239] observed that carbachol is more efficacious than gastrin in stimulating the entry of extracellular Ca^{2+} into parietal cells. These two secretagogues appear to regulate the entry of Ca^{2+} into parietal cells by distinct mechanisms. Gastrin stimulates Ca^{2+} entry from extracellular sites by opening Ca^{2+} channels. Carbachol opens similar Ca^{2+} channels and also opens channels that are not regulated by the depletion of $[Ca^{2+}]_i$ pools. These differences in Ca^{2+} regulation may account for the more potent stimulatory action of carbachol on canine gastric parietal cells.

Furthermore, research suggests that the induction of $[Ca^{2+}]_i$ release in response to gastrin but not carbachol is a function of activation of the cAMP pathway [240]. In fact, incubation of rabbit parietal cells with gastrin in the presence of the histamine H_2 receptor blocker cimetidine inhibits gastrin-mediated increases in $[Ca^{2+}]_i$. The addition of dibutyryl adenosine 3′,5′-phosphate in addition to cimetidine completely restores this response, suggesting that, in the rabbit, H_2 receptor activity potentiates the effect of gastrin on parietal cell function. Athmann and colleagues [241] confirmed these observations, demonstrating that, in isolated rabbit gastric glands, ranitidine blocks $[Ca^{2+}]_i$ release induced by gastrin (1 nM). Interestingly, these investigators also reported that a supraphysiological, 10-fold higher dose of gastrin (10 nM) induced a Ca^{2+} signal that was not inhibited by ranitidine [241]. The specific intracellular targets and the biological significance of this ranitidine-insensitive Ca^{2+} signal remain to be elucidated.

Although it appears that the plateau or steady phase of carbachol-mediated increases in $[Ca^{2+}]_i$ is essential for activation of the secretory process [242], this may not be the only signaling event that is important in muscarinic receptor-mediated acid secretion. Similar Ca^{2+} levels can be achieved with the calcium ionophore ionomycin and the Ca^{2+}-ATPase inhibitor thapsigargin [243], but a corresponding increase in acid production similar to that observed with carbachol is not observed. The additional signaling events linking M_3 receptor activation and acid secretion require further investigation, but they may include differential activation of downstream kinase pathways.

Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is one of the best-characterized targets of $[Ca^{2+}]_i$. Tsunoda and colleagues [244] showed that carbachol can lead to the activation of CaMKII in parietal cells. The specific components of the carbachol-activated signaling cascade remain largely unknown. Parente and colleagues [245] purified and cloned a novel Ca^{2+}-sensitive phosphoprotein of 28 kDa (CSPP28) from rabbit parietal cells. CSPP28 was phosphorylated by CaMKII in response to cholinergic stimulation, but not when cells were treated with phorbol esters. CSPP28 may represent an important element in Ca^{2+} signal transduction, but its specific role in parietal cell biology remains unknown. Because CaMKII regulates cytoskeletal function, it may play a role in the dramatic morphological transformation of parietal cells during stimulation.

Carbachol-mediated stimulation of various cell types leads to alterations in cell volume that are linked to the regulation of ion channel conductance. Negulescu and colleagues [246] examined the role of parietal cell volume in regulating carbachol-mediated changes in cytosolic Ca^{2+}. These investigators observed that carbachol-mediated opening of Ca^{2+} channels leads to the loss of ions and water from the parietal cell and subsequent cell shrinkage. The decrease in cell volume may inhibit Ca^{2+} channels, providing a feedback mechanism to reduce further cell shrinkage resulting from ion fluxes.

The action of PKC in parietal cells is complex. Direct activation of PKC with phorbol esters results in enhanced acid secretory activity [247], and inhibition of PKC by the specific inhibitor Ro 31-8220 blocks carbachol-stimulated aminopyrine uptake in isolated rat parietal cells [248]. However, pretreatment of canine parietal cells with phorbol esters decreases the stimulatory effects of subsequent treatment with carbachol and gastrin [247], whereas Ro 31-8220 potentiates [^{14}C]aminopyrine uptake from isolated rabbit gastric glands stimulated by both carbachol and histamine

[249]. Numerous factors may account for these contradictory observations. PKC may induce down-regulation of muscarinic and gastrin receptors on parietal cells [247]. The mechanism for this effect is unclear but may involve receptor phosphorylation. In addition, because PKC comprises a large family of proteins with different biochemical and functional properties [250], species-specific differences in both the function and the cellular localization of PKC isoforms may exist [249]. Chew and colleagues [249] analyzed the different PKC subtypes in rabbit parietal cells and reported abundant levels of both the novel isoforms PKC-ε and PKC-μ and the atypical isoforms PKC-τ, PKC-λ, and PKC-ζ. In contrast to a previous study performed in canine parietal cells [251], low levels of the classic isoforms PKC-α and -β were measured in the rabbit parietal cells. Confocal microscopy revealed that PKC-ε is localized to a parietal cell compartment which bears a resemblance to that containing filamentous actin, suggesting that PKC could negatively regulate gastric acid secretion through phosphorylation and modification of cytoskeletal proteins [249]. In addition, characterization of protein kinase D (PKD) revealed that it is induced by phorbol esters [252,253]. Although it is unknown whether PKD or other related protein kinases are present in gastric cells, this intriguing finding suggests that protein kinases other than PKC may mediate the effects of phorbol esters on acid secretion. Further studies will clarify the function of PKC in the stomach.

Mitogen-activated protein kinases

In addition to these well-established signal transduction pathways, mammalian parietal cells express multiple members of a family of protein kinases known as mitogen-activated protein kinases (MAPKs) [254–256]. In particular, parietal cells appear to express the extracellular signal-regulated protein kinases (ERKs) [254–256], the Jun N-terminal kinases (JNKs) [257], and the p38 kinases [258], molecules known to regulate multiple important cellular functions [259,260] (Fig. 13.8). The ERKs are important elements in a cascade of biochemical reactions that involves the small GTP-binding protein Ras, as well as upstream protein kinases such as Raf and MAPK/ERK [261,262]. ERK activation is known to target numerous cellular proteins, including downstream protein kinases such as the 90-kDa S6 kinase (RSK) [261–264] and transcription factors, such as Elk-1, that regulate the activity of the promoter of the early response gene c-*fos* through the serum response element (SRE) [263,264]. These kinases appear to play a crucial role in the process of amplification, integration, and transmission of extracellular signals from the cell surface to the nucleus, leading to the induction of cellular growth and proliferation and, in some systems, cellular differentiation [260–262].

Several studies have examined the regulation of ERKs by gastric acid secretagogues. Takeuchi and colleagues [256] reported that carbachol was the most potent inducer of ERK2 activity in isolated gastric canine parietal cells. Gastrin and EGF had weaker stimulatory effects, whereas histamine induced no response. The effect of carbachol appeared to be independent of Ca^{2+} signaling. PD98059, a selective inhibitor of the upstream ERK activator MEK, dose-dependently inhibited both carbachol- and EGF-stimulated ERK2 activity [256,265]. Similarly, Nakamura and associates [254] observed that carbachol and EGF induced the ERKs in isolated rabbit parietal cells.

The functional relevance of ERK activation in the stomach has been the focus of continued investigation. Initial studies

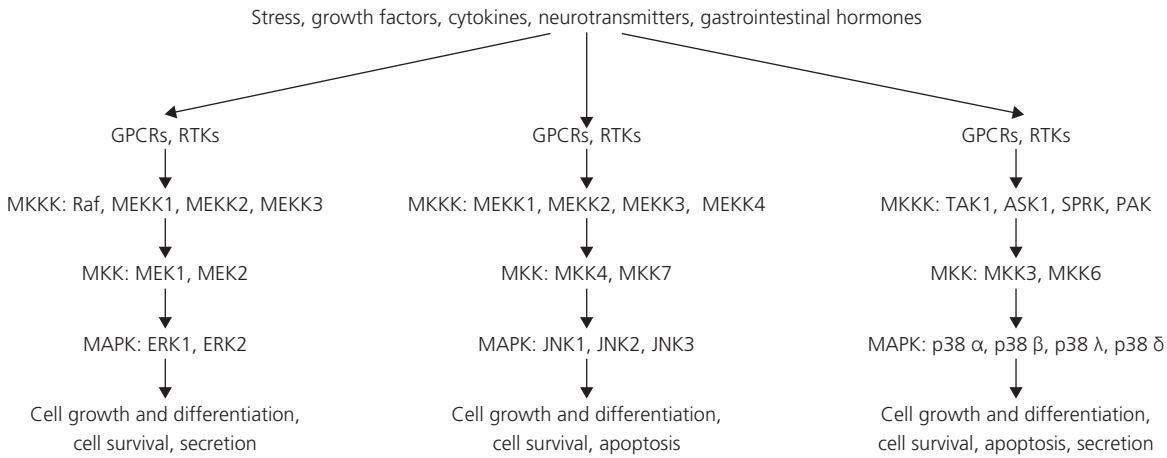

Figure 13.8 Mitogen-activated protein kinase (MAPK) pathways in the gastric parietal cell. Many different extracellular signals activate kinase cascades that lead to the induction of members of the MAPK family of protein kinases. These include the extracellular signal-regulated protein kinases (ERKs), p38 kinases, and the Jun N-terminal kinases (JNKs). These protein kinases appear to regulate several important cellular functions, including growth, apoptosis, survival, differentiation, and secretion. GPCRs, G protein-coupled receptors; RTKs, receptor tyrosine kinases.

reported the divergent effects of EGF on gastric acid secretion. Under acute conditions, EGF had an inhibitory effect on acid secretion, whereas prolonged administration of EGF increased both basal and maximal acid secretion in vivo and acid production in isolated parietal cells in vitro [255,266,267]. Some studies suggested that these effects of EGF could be mediated by the activation of protein tyrosine kinases because they were fully reversed by the addition of protein tyrosine kinase inhibitors [255,266]. In addition, Chew and associates [255] observed that inhibition of the chronic stimulatory effect of EGF by these agents was associated with a decrease in phosphorylation of a 44-kDa protein identified as an ERK isoform. Taken together, these results suggested that both the ERKs and an unidentified protein tyrosine kinase were likely to be important in the regulation of gastric parietal cells.

Understanding of the function and physiological role of the ERK pathway was significantly enhanced by the use of PD98059. To examine further the importance of ERK activation in gastric acid secretion, Takeuchi and colleagues [256] tested the effect of PD98059 on carbachol-stimulated uptake of [^{14}C]aminopyrine. Acute inhibition of the ERKs by PD98059 led to a small increase in [^{14}C]aminopyrine uptake and to complete reversal of the inhibitory effect of EGF on parietal cell activation induced by either carbachol or histamine [256]. In contrast, exposure of the cells to PD98059 for 16 h reversed the chronic stimulatory effect of EGF on [^{14}C]aminopyrine uptake induced by carbachol, leading the investigators to conclude that, whereas the acute effect of the ERKs on gastric acid secretion appears to be inhibitory, the activation of transcription factors and of early gene expression could produce chronic stimulatory effects (Fig. 13.9) [256]. Further research showed that the acute inhibitory effect of EGF on gastric acid secretion could be mediated by a PKC-dependent pathway [251]. Thus, because PKC has been shown to activate c-Raf, a kinase involved in the activation of the ERKs, it is possible that the acute inhibitory effect of EGF on aminopyrine uptake could be mediated by a signaling cascade involving activation of PKC, c-Raf, and MEK, leading finally to induction of the ERKs [268]. Furthermore, Kaise and associates [267] demonstrated that EGF is able to induce transcription of the α-subunit of the H^+,K^+-ATPase gene through a novel EGF response element (ERE) located between bases –162 and –156 (5′-GACATGG-3′) relative to the cap site. This ERE is homologous to the 3′ half-site of the c-*fos* serum response element. Accordingly, the authors suggested that the stimulatory effect of EGF on gastric acid secretion could be mediated by induction of the H^+,K^+-ATPase gene [267]. Although a direct link between induction of the ERKs and c-*fos* and stimulation of the H^+,K^+-ATPase gene has not been demonstrated, it is possible that this gene may contain specific DNA regulatory elements that receive input from signaling pathways involving the ERKs and c-*fos*.

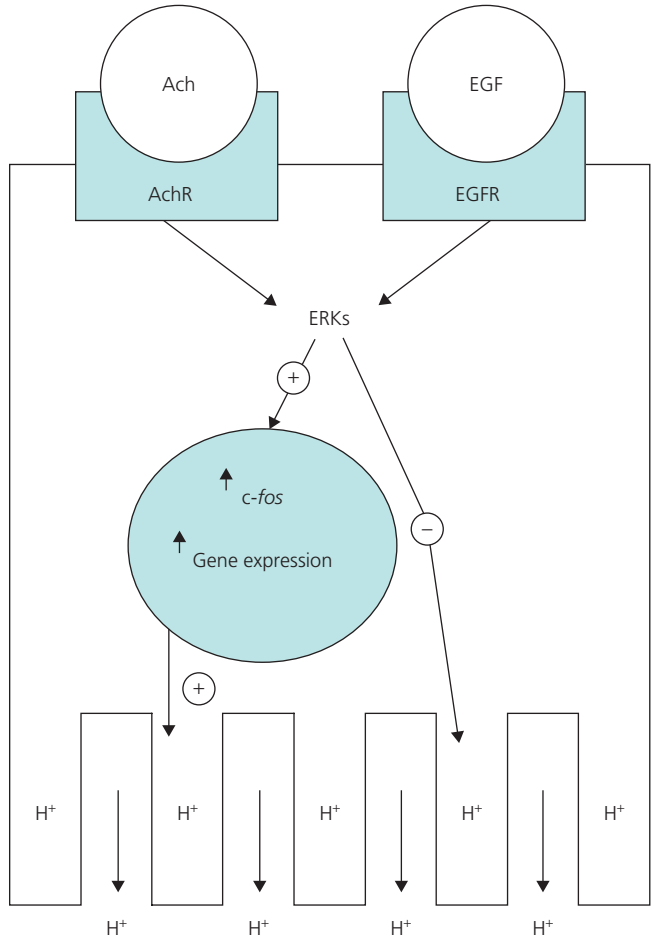

Figure 13.9 Function of the extracellular signal-regulated protein kinases (ERKs) in the gastric parietal cell. Both acetylcholine (Ach) and epidermal growth factor (EGF) induce a cascade of events in parietal cells that results in the activation of ERKs. Whereas the acute effect of the ERKs on gastric acid secretion appears to be inhibitory, the activation of transcription factors and early gene expression may be responsible for their chronic stimulatory effects.

p38 kinase is another member of the MAPK family of protein kinases [259]. Like the MAPKs, p38 kinase is regulated by a multistep cascade of biochemical reactions. One of the first steps in the complex chain of events that leads to the induction of p38 kinase is the activation of Rac and Cdc42, small GTP-binding proteins belonging to the Rho family of GTPases [259]. These molecules are responsible for the induction of downstream protein kinases such as the dual-specificity kinases MKK3 and MKK6, which, in turn, phosphorylate p38 kinase on both tyrosine and threonine residues, leading to its activation [259]. Research indicates that p38 kinase is activated in response to both physical and chemical stress, cytokines, hematopoietic growth factors, neurotransmitters such as carbachol and isoproterenol, and CCK [258,259,269,270]. Activation of p38 kinase has been

linked to regulation of programmed cell death, differentiation of skeletal muscle, organization of the cellular cytoskeleton, and phosphorylation of transcription factors [259,270–272]. Pausawasdi and colleagues [258] used SB-203580, a specific p38 kinase inhibitor [272], to investigate the regulation and the functional relevance of p38 kinase in carbachol-stimulated gastric acid secretion. They observed that SB-203580 dose-dependently potentiates carbachol induction of aminopyrine uptake in isolated and cultured canine gastric parietal cells, suggesting that p38 kinase has a negative regulatory effect on gastric acid secretion [258].

Because p38 kinase is known to play an important role in the organization of the actin cytoskeleton, a possible mechanism of p38 kinase-induced inhibition of gastric acid secretion could involve changes in the actin cytoskeleton of gastric parietal cells [259,270]. The cytoskeleton is an important element in secretagogue-stimulated gastric acid secretion because, during this process, the parietal cell undergoes dramatic morphological modifications that lead to the translocation of H^+,K^+-ATPase from cytoplasmic tubulovesicular structures to the apical plasma membrane [2–4]. Accordingly, p38 induction could result in phosphorylation of cytoskeletal proteins and significant changes in the organization of the actin cytoskeleton. These events could be responsible for alterations in the process of H^+,K^+-ATPase insertion into the parietal cell apical membrane and for the inhibition of gastric acid production.

Considering the lack of specific JNK inhibitors, it is unclear whether these kinases are involved in the process of gastric acid secretion. However, studies conducted in canine parietal cells suggest that JNK activation may be an important step in the parietal cell response to stress and inflammation [257]. The observation that mice deficient in the *JNK1* gene appear to be resistant to the development of cold-/restraint stress-induced gastric lesions further supports this hypothesis [273].

Akt

Another signal transduction pathway that has been shown to play an important role in the mediation of some of the physiological actions of EGF is that involving phosphoinositide 3-kinase (PI3K) and protein kinase B/Akt [274–277]. Activation of Akt is known to induce cellular growth and survival and to promote the expression and maintenance of highly differentiated cellular phenotypes [274,275,278,279]. Furthermore, Akt has been shown to contribute to the regulation of vesicular trafficking and endocytosis [279]. Akt is homologous to the PKA and PKC families of protein kinases, and its activity is regulated by growth factors through the induction of PI3K [275,280]. Phosphorylation of Akt appears to be critical for its activation [275,280,281]. Todisco and associates [282] reported that EGF regulates the expression of the H^+,K^+-ATPase α-subunit gene through a signal transduction pathway that involves the activation of Akt. These findings suggest a novel role for Akt in the regulation of the secretory function of gastric parietal cells.

The hedgehog signal transduction pathway

Sonic hedgehog, indian hedgehog, and desert hedgehog are members of the family of hedgehog (Hh) proteins, which are known to have important regulatory functions in embryonic patterning and growth in a large number of tissues [283,284]. In the mammalian stomach, sonic hedgehog (Shh) is an important factor in the regulation of gastric epithelial cell maturation and differentiation [6,7,283–285]. Indeed, the gastric epithelium of Shh null mice exhibits intestinal transformation [283]. Moreover, Shh appears to be crucial for the induction and maintenance of a normal fundic gland cell phenotype, as the mucosa of patients with gastric atrophy and intestinal metaplasia is characterized by loss of Shh expression [285]. It has also been reported that inhibition of Shh signaling in the gastric mucosa leads to enhanced cellular proliferation. [6]

The intracellular signal transduction pathways that mediate the actions of Shh on target cells have been the focus of several studies. Shh is known to bind to a transmembrane receptor protein known as "patched" (Ptc), which, in the absence of Shh, exerts an inhibitory effect on the seven-transmembrane receptor "smoothened" (Smo). The binding of Shh to Ptc blocks the inhibitory effect of Ptc on Smo (Fig. 13.10). Once activated, Smo induces a complex series of intracellular reactions that target the Gli family of transcription factors [286,287]. At least three members of this family of nuclear proteins have been identified in mammalian tissues [287]; whereas Gli1 and Gli2 are transcriptional activators, Gli3 seems to function primarily as a transcriptional repressor [287]. Gli2 appears to be the principal effector of Shh signaling, as disruption of the Gli2 gene leads to developmental defects involving several Shh target tissues whereas Gli1 null mice are born without detectable abnormalities [287–293]. The Gli transcription factors accumulate in the nucleus where they activate hedgehog-responsive genes, such as Ptc, Gli1, BMP4, HNF3β, Hox genes, and hedgehog-interacting protein (HIP), an extracellular hedgehog antagonist [286]. Of these genes, the up-regulation of Ptc and HIP appears to be involved in a negative feedback response to Shh signaling [286]. The functional significance of the Shh signal transduction pathway in the stomach was initially characterized in studies of isolated canine parietal cells [6]. It was shown that these cells express Shh and Ptc and that incubation of the cells with EGF for 16 h induces both the release and the expression of Shh through mechanisms that involve the activation of Akt. Furthermore, Shh induces H^+,K^+-ATPase α-subunit gene expression and enhances the stimulatory action of histamine on gastric acid production. Considered together, these observations suggest that Shh mediates EGF action in the stomach through both paracrine and autocrine mechanisms.

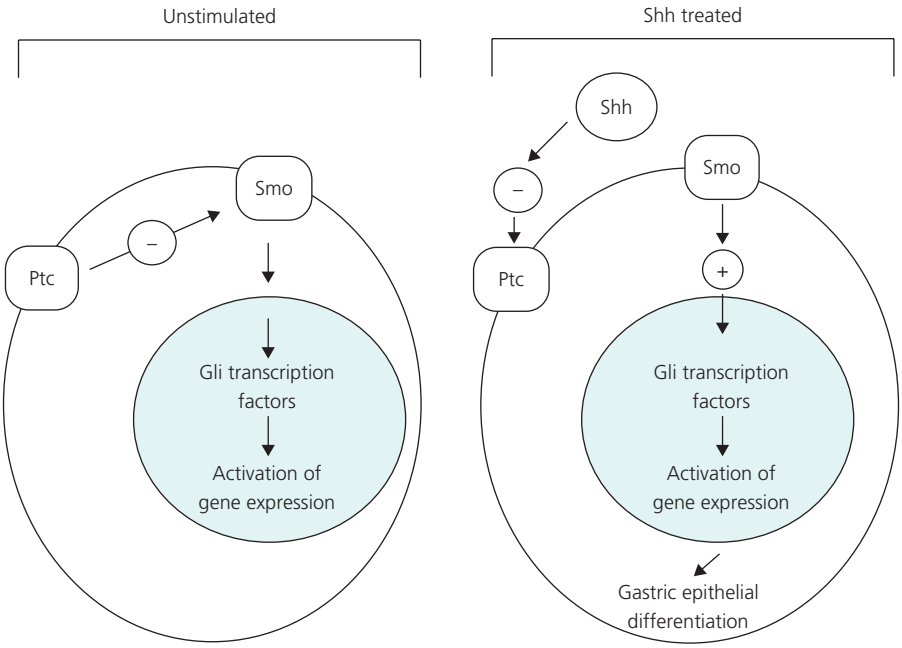

Figure 13.10 Activation of sonic hedgehog (Shh) signaling. Shh binds to the transmembrane receptor protein "Patched" (Ptc) which, in the absence of Shh, exerts an inhibitory effect on the seven-transmembrane receptor "Smoothened" (Smo). Binding of Shh to Ptc blocks the inhibitory effect of Ptc on Smo. Once activated, Smo induces a complex series of intracellular reactions that target the Gli family of transcription factors.

Smad signaling

In the stomach, Shh has been shown to regulate the expression of BMP4, a member of the bone morphogenetic protein (BMP) family of regulatory peptides. BMP4 expression has been detected in myofibroblast-like cells located in the submucosal layers of the stomach but not in gastric epithelial cells, in contrast to Shh, which is expressed in parietal cells [6,7]. The stimulatory effect of Shh on BMP4 expression appears to be highly specific, as Shh fails to affect the expression of BMP2, another member of the BMP family whose expression has been documented in the gastric epithelium [7]. Thus, BMP4, but not BMP2, seems to be a target of the Shh signal transduction pathway in the stomach. The BMPs activate several complex signal transduction pathways to exert their biological actions [294–296]. In particular, the binding of BMPs to the BMP type I receptor (BMPR-I) leads to the dimerization of BMPR-I with the BMP type II receptor (BMPR-II), a molecule that has serine/threonine kinase activity. This event triggers the phosphorylation of BMPR-I and Smad 1, 5, and 8, proteins known to mediate the intracellular actions of BMP. Once phosphorylated, Smad 1, 5, and 8 each associate with Smad 4 to form a heterodimeric complex that translocates to the nucleus, where it activates gene transcription [294–296].

BMP signaling plays a crucial role in the regulation of growth and differentiation of the gastrointestinal epithelium [296]. Studies of isolated parietal cells have shown that BMP4 stimulates the phosphorylation of Smad 1, 5, and 8 and induces H^+,K^+-ATPase α-subunit gene expression [297]. Moreover, BMP4 appears to enhance the stimulatory effect of histamine on gastric acid secretion, suggesting an important role in the regulation of parietal cell maturation and differentiation. Mice with a targeted mutation of the transcription factor Fkh6 exhibit a decreased expression of BMP4 in the gastric mucosa, along with an increased proliferation of the gastric epithelium and a decreased number of parietal cells [298]. Taken together, these observations support the notion that BMP4, and possibly other BMPs, may be important regulators of gastric epithelial cell biology through the activation of intracellular signal transduction pathways that require the phosphorylation and activation of the Smad proteins.

Nitric oxide

Nitric oxide is a physiologically relevant signaling molecule that controls biological processes, such as blood flow, leukocyte and platelet adhesion, and neurotransmission. Nitric oxide may protect gastric cells through its effects on mucosal blood flow and mucus and HCO_3^- secretion [299]. The intracellular actions of nitric oxide are mediated by soluble guanylyl cyclase [299]. Nitric oxide binds to guanylyl cyclase leading to its conformational modification and activation. Active guanylyl cyclase is responsible for the conversion of GTP into cGMP, the main transducer of the biological actions of nitric oxide. The ability of nitric oxide to stimulate cGMP generation appears to mediate nitric oxide inhibition of gastric acid secretion in isolated parietal cells [299].

Somatostatin-activated signaling pathways

The mechanism by which somatostatin inhibits parietal cells has been explored [194]. In the case of histamine-stimulated acid secretion, somatostatin appears to inhibit the generation of cAMP through an inhibitory guanine nucleotide-binding protein that regulates adenylate cyclase activity. However,

somatostatin is also able to inhibit acid secretion induced by dibutyryl cAMP. In addition, the stimulatory effects of gastrin and carbachol are inhibited without altering the turnover of membrane inositol phospholipids or the activation of PKC induced by these agents. Somatostatin appears to act on parietal cells at a site distal to the activation of the intracellular signal transduction cascades. This action may be mediated through the induction of protein dephosphorylation, the inhibition of cellular secretion, or some other yet undetermined mechanism [300–302]. For example, somatostatin inhibits the induction of the early response gene c-*fos* in canine parietal cells stimulated by both carbachol and histamine [303]. As in many other physiological systems, c-*fos* plays an important role in cellular activation. Thus, the inhibitory effect of somatostatin on c-*fos* expression may represent an important regulatory mechanism in gastric parietal cell physiology.

Regulation of parietal cell genes

The stimulation of parietal cells induces several cellular events, including morphological transformation, rapid changes in enzyme location and activity, and opening of ion channels. The resting parietal cell has a collapsed canalicular system and cytoplasmic tubulovesicles containing the gastric proton pump, H^+,K^+-ATPase. The stimulated cell rapidly develops a richly interdigitating intracellular canalicular system bulging with microvilli with a concomitant loss of cytoplasmic tubulovesicles (Fig. 13.11) [3,304]. The microvilli have a central cytoskeletal core of actin filaments stabilized by other proteins [305]. These filaments appear to mediate the fusion of tubulovesicles with the canalicular system. This fusion translocates H^+,K^+-ATPase from vesicular membranes to the canalicular membrane, where it actively pumps H^+ ions in exchange for K^+ (Fig. 13.12) [306,307]. For each proton that is secreted, an intracellular OH^- ion is generated. This alkaline challenge is handled by carbonic anhydrase II (CAII)-mediated conversion of OH^- to HCO_3^-, which is exchanged for Cl^- at the basolateral membrane.

There are many proteins involved in the generation and secretion of gastric acid. Stimulation of parietal cells presumably involves the activation of nascent proteins and the induction of the genes responsible for these effector molecules. The effects of acid secretagogues on the genes coding for these enzymes and structural proteins have been analyzed.

The induction of specific gene transcription has been studied in isolated canine parietal cells [308]. Significant increases in CAII mRNA levels were induced by carbachol, gastrin, and histamine. Maximal stimulation was reached within 20 min for histamine and carbachol and within 60 min for gastrin. To determine whether the observed increases in mRNA levels were the result of increased transcription or decreased degradation, nuclear run-off experiments were performed. They revealed increased transcription within 15 min for each agent. H^+,K^+-ATPase mRNA levels were also induced in the same fashion as for CAII [309]. This coordinated induction of both enzymes could be blocked with the competitive inhibitors of each secretagogue.

Because CAII appears to be the catalyst of the reaction responsible for the elimination of OH^- produced in the generation of H^+, increased expression of CAII mRNA in

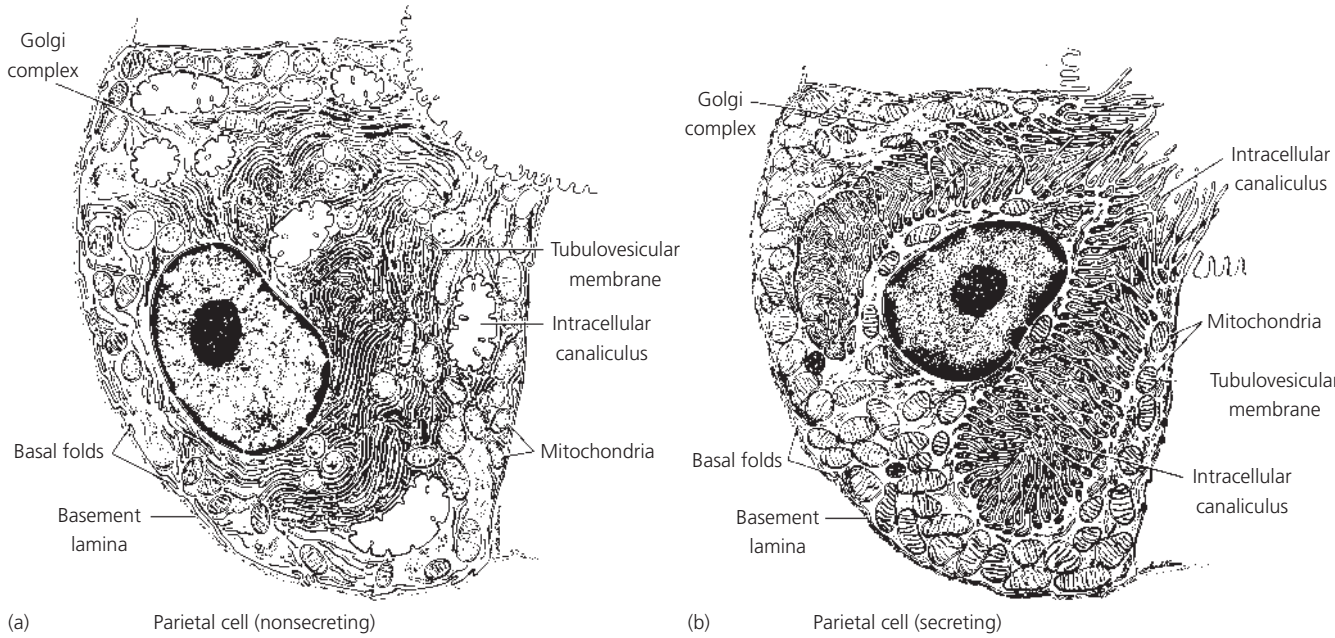

Figure 13.11 **(a)** Resting nonsecretory parietal cell. **(b)** Stimulated acid secretory parietal cell. The stimulated cell develops a richly interdigitating intracellular canalicular system that bulges with microvilli. There is a concomitant loss of cytoplasmic tubulovesicles. From Ito [4].

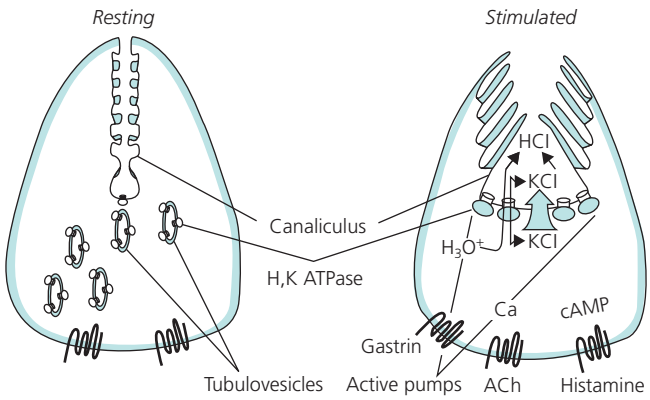

Figure 13.12 Functional transformation of a secreting parietal cell. This model illustrates a resting parietal cell **(left)** with collapsed secretory canaliculi and cytoplasmic tubulovesicles expressing H^+,K^+-ATPase, and a stimulated parietal cell **(right)** with formed secretory canaliculi expressing active H^+,K^+-ATPase pumps. Adapted from Hershey & Sachs [348].

stimulated parietal cells could result as a secondary effect of the induction of H^+,K^+-ATPase. To explore this possibility, parietal cells were pretreated with omeprazole, an agent known to inactivate H^+,K^+-ATPase irreversibly [310]. Under these circumstances, carbachol still induced expression of CAII mRNA, with the same kinetics as in untreated cells. Carbachol appears to stimulate CAII gene expression without dependence on OH^- ion generation by H^+,K^+-ATPase.

Secretagogues stimulate actin expression. Although actin is often used as a control or housekeeper gene for such studies because its expression tends to remain constant, in the case of the parietal cell, actin plays a crucial role in the acid secretory process. The induction of actin gene expression may serve as a particularly useful marker for acid secretion instead of using H^+ generation. This type of analysis can elucidate the effect of acid secretagogues on parietal cell function at the molecular level.

Muraoka and associates [311] undertook an extensive evaluation of the regulatory elements controlling H^+,K^+-ATPase gene expression. They transfected cultured canine gastric parietal cells with reporter gene constructs composed of the first exon and various lengths of the 5′-upstream regulatory region of the canine H^+,K^+-ATPase α-subunit ligated to a luciferase reporter plasmid. Their data indicate that basal transcriptional activity of the H^+,K^+-ATPase gene is mediated through binding of the nuclear transcriptional factor SP1 to the 5′-GCTCCGCCTC-3′ nucleotide sequence residing between bases –47 and –38 relative to the putative cap site [311].

Shigehiko and Maeda and their colleagues [312,313] identified regions within the promoters of both the α- and β-subunits of the H^+,K^+-ATPase gene that are recognized by gastric-specific nuclear proteins. These investigators subsequently cloned two novel nuclear proteins, named GATA-GT1 and GATA-GT2, from pig gastric mucosa. These bind specifically to the sequences (G or C)RR(G or C)NGAT(A or T)RY, in which R and Y are unspecified purine and pyrimidine bases respectively. GATA-GT1 and GATA-GT2 belong to the family of GATA transcription factors that are known to play an important role in tissue-specific gene expression [313]. Northern blot analysis of several different tissues revealed that these nuclear proteins are expressed predominantly in the gastric mucosa and to a lesser degree in the intestine, but not in the brain, heart, liver, kidney, spleen, or lung [312]. Maeda's group speculated that these transcription factors are responsible for the specific expression of the H^+,K^+-ATPase gene in the gastric mucosa.

The regulation of parietal cell gene expression by gastrin has been analyzed using flow cytometry-purified parietal cells isolated from gastrin knockout mice. The expression levels of several genes involved in the regulation of gastric acid secretion – the H^+,K^+-ATPase α- and β-subunits, the KCNQ1 potassium channel, the aquaporin-4 water channel, and creatinine kinase B – were reduced in the parietal cells of the gastrin-deficient mice [314]. The involvement of gastrin in the regulation of these genes was confirmed by the reversal of these observations after a 1-week infusion of gastrin [314].

Acid secretory processes

The gastric epithelium secretes a fluid of almost isotonic HCl through an active transport process. Acid is secreted at a pH of 0.8, but the parietal cell cytosolic pH is about 7.2. The parietal cell alone is responsible for this remarkable H^+ ion concentration gradient of 2.5 million-fold. Significant amounts of mitochondrial energy are required when the parietal cell is signaled to transform from a relatively quiet resting state to an actively secreting state. Dramatic changes in cellular membranes, cytoskeletal architecture, membrane ion conductances, and ATPase activities are only a portion of the events that accompany cell activation. Equally impressive is the compressed time frame over which these changes occur. It has been suggested that the family of isoenzymes, creatine kinase/phosphocreatine (CK/PCr), which catalyze the exchange of high-energy phosphate groups between phosphocreatine and ADP, are involved in parietal cell transformation. The CK/PCr system couples to the H^+,K^+-ATPase [315] and the Ca^{2+}-ATPase found in sarcoplasmic reticulum [316]. The brain-type CCK isoform, BB-CK, has been identified in the parietal cells of different species [317], and this enzyme appears to colocalize with H^+,K^+-ATPase in purified vesicles prepared from porcine parietal cells. These data suggest that H^+,K^+-ATPase may obtain the required ATP from BB-CK colocalized in the same cellular compartment [318].

The stimulation of parietal cells induces the formation of a dense apical meshwork of intracellular canaliculi packed with long microvilli [319]. The apical cell membrane sur-

face area increases five- to tenfold after stimulation. This increase coincides with the disappearance of most of the cytoplasmic tubulovesicles seen in the resting parietal cell. The mechanism of this increase in surface area appears to be the fusion of tubulovesicles and apical membrane. Immunocytochemical studies using a monoclonal antibody to H^+,K^+-ATPase demonstrate the translocation of this enzyme from tubulovesicles in the resting cell to the apical membranes in stimulated cells, supporting the concept of secretagogue-induced membrane fusion and enzyme redistribution [306]. The fusion of tubulovesicles with the apical membrane is directed by cytoskeletal microfilaments composed of actin and other regulatory proteins. Membranes are recycled back to the tubulovesicles as cells return to the resting state, a process that also appears to be mediated by actin-containing microfilaments [305]. These microfilaments anchor at regularly spaced intervals in the apical membrane and do not appear to associate with resting tubulovesicles. Cytochalasins, agents that inhibit actin polymerization, inhibit acid secretion [320]. Colchicine and vinblastine are also capable of inhibiting acid secretion [321]. These studies indicate the essential importance of actin-mediated H^+,K^+-ATPase translocation in the initiation of acid secretion.

Forte and colleagues [322] analyzed the biochemical and functional properties of actin in gastric parietal cells. In a series of elegant studies, these authors showed that most parietal cell actin exists in the filamentous form (F-actin), which is an important component of the apical cell microvilli, whereas only a small fraction appears to be in the monomeric state (G-actin).

In some systems of regulated vesicular transport, F-actin is known to form a meshwork of filaments just beneath the cell surface, creating a barrier for the recruitment of vesicles to the apical plasma membrane. According to this model, F-actin depolymerization to the monomeric G form facilitates vesicle translocation. In contrast to this theory, Forte's group [322] did not detect any significant changes in the steady-state ratio of F-actin to G-actin during the process of parietal cell activation by gastric acid secretagogues. Thus, although these observations do not exclude the possibility that rapid exchange between F-actin and G-actin may occur, it appears that during induction of gastric acid secretion, the parietal cell maintains actin in a highly polymerized state [322].

Forte's laboratory also demonstrated that parietal cells contain pools of actin that display considerable functional differences [323]. Using latrunculin B, a compound known to bind to and inhibit the function of G-actin, they showed that, whereas G-actin is important for lammellipodia formation, F-actin, which is less sensitive to latrunculin B, appears to play a crucial role in the process of secretagogue-stimulated gastric acid secretion. In fact, alteration of F-actin function by either a high dose of latrunculin B or by cytochalasin D, an agent known to fragment F-actin filaments, resulted in inhibition of gastric acid secretion [323].

Hanzel and colleagues [324] identified one of the membrane proteins involved in parietal cell cytoskeletal transformation as ezrin, an 80-kDa membrane protein found in several cell types. Ezrin is associated with the actin filaments in the microvilli of stimulated parietal cells and is phosphorylated during cAMP-mediated stimulation. Actin, an important structural protein for cellular scaffolding, is expressed as several isoforms, including the β- and γ-subtypes found in parietal cells [325]. In a series of elegant experiments using ultrastructural immunocytochemistry and biochemical protein purification, Yao and associates [326] confirmed that β-actin is localized in canalicular microvilli and in the apical portion of parietal cells [326]. The authors also reported the selective association of ezrin with β-actin. These important observations support a potential role for ezrin in parietal cell secretion. Moreover, they support the novel concept that actin isoforms may be distributed in different cellular domains, exerting differential functions by association with isoform-specific actin-binding proteins.

The molecular events regulating the dramatic recycling of H^+,K^+-ATPase-containing membranes have been the focus of intense investigative efforts. Studies of other cell systems have begun to elucidate the important factors involved in the secretion process. One such family of factors is the low-molecular-weight GTP-binding proteins [327]. The members of this superfamily of proteins have certain characteristics: molecular weights of 20–30 kDa, significant homology with the oncogenic peptide Ras, ubiquitous expression, and involvement in a host of cellular functions, including vesicle trafficking. Several members of the Rab category of Ras proteins are expressed in gastric parietal cells [328–332]. Rab 25, Rab 324, and Rab 11 (previously thought to be Rab 2) have been identified in rabbit parietal cells [330,331]. More importantly, these specific proteins cosegregate with α-H^+,K^+-ATPase during parietal cell activation, suggesting a role for these small GTP-binding proteins in membrane recycling [330–332]. The importance of Rab 11a in the process of parietal cell activation has been demonstrated by Duman and colleagues [333]. Transduction of isolated and cultured rabbit parietal cells with an adenoviral vector expressing a dominant negative Rab 11a gene led to a block in the translocation of the H^+,K^+-ATPase to the apical plasma membrane and to the inhibition of gastric acid secretion [333].

Developments in the field of cell biology have enabled the molecular pathways that are important in cellular secretory processes to be elucidated. Studies in neuronal systems and yeast have demonstrated the potential involvement of highly conserved protein complexes in vesicle fusion and docking, such as the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) [334,335]. These can be vesicle-related (v-SNARE) or target membrane-related (t-SNARE), binding to each other to provide a molecular

framework for the attachment of soluble factors [336]. Members of these protein families include VAMP-2 (vesicle-associated membrane protein 2, identified as a v-SNARE in brain) and the syntaxin 1A/1B and SNAP-25 (synaptosome-associated protein), which are categorized as t-SNAREs and are thought to play a role in the recycling of synaptic vesicles [337]. The secretory carrier membrane proteins or SCAMPs found in pancreatic zymogen granules and synaptic vesicles may also play a role in vesicle recycling. Calhoun and Goldenring [332] explored the potential role of these novel trafficking-related proteins in the parietal cell secretory process. The involvement of VAMP-2, SCAMPs, Rab 11, and Rab 25 in membrane trafficking was determined by immunoisolation of highly purified parietal cell tubulovesicles. These investigators reported that VAMP-2, SCAMPs, and the two Rab proteins are present on H^+,K^+-ATPase-containing tubulovesicles and suggested that Rab proteins take part in the assembly of the SNARE complex. These observations support a theory originally proposed by Forte that parietal cell tubulovesicles may be derived from a cell surface recycling system.

Other studies have demonstrated that the cytoskeletal proteins actin, spectrin, and ankyrin copurify with the H^+,K^+-ATPase from parietal cell microsomal membranes and cosegregate with H^+,K^+-ATPase in resting and secreting parietal cells. Ankyrin may mediate the interaction of H^+,K^+-ATPase with the structural membrane proteins actin and spectrin and may thereby function to maintain the polarized distribution of the enzyme to the apical portion of the parietal cell. Festy and colleagues [338] screened a cDNA bank from rabbit gastric fundic mucosa using a two-hybrid assay and demonstrated that the rabbit H^+,K^+-ATPase binds through its N-terminal end to the spectrin-binding domain of ankyrin III. The two proteins bind directly to one another, constituting an important anchoring system for the H^+,K^+-ATPase to the plasma membrane [338].

Studies of H^+,K^+-ATPase activity have been performed using membrane vesicles obtained from resting and stimulated parietal cells [319]. Vesicles from light microsomal membrane fractions, presumably containing the tubulovesicles, exhibit most of the H^+,K^+-ATPase activity in resting parietal cells [339]. Heavier membrane vesicles associated with microfilaments contain most of the H^+,K^+-ATPase activity in stimulated parietal cells. The membranes of these acid-secreting vesicles have a K^+/Cl^- cotransport system that is lacking in membrane preparations from resting cells [340].

Studies have confirmed that the H^+,K^+-ATPase requires extracellular K^+ for electroneutral exchange with H^+ [341,342]. It appears that, concomitant with membrane or enzyme translocation, associated K^+ and Cl^- conductances are activated [340]. Opening of this conductance as it moves from tubulovesicles to the canalicular membrane allows H^+,K^+-ATPase to generate H^+. The K^+/Cl^- conductance is intimately associated with the H^+,K^+-ATPase, and its function is required for acid secretion. Several other ion channels are involved in the maintenance of cellular homeostasis during acid secretion. A schematic representation of parietal cell conductances and pumps is shown in Figure 13.13. Each H^+ ion that is generated results in the formation of an intracellular OH^- ion, which reacts with CO_2 in a reaction catalyzed by CAII to form HCO_3^-. A mechanism located on the basolateral membrane permits the exchange of intracellular HCO_3^- for extracellular Cl^-. This Cl^- provides part of the intracellular pool for transport by the apical K^+/Cl^- conductance system. Several types of Cl^-/HCO_3^- exchanger have been described. The exchanger expressed in parietal cell basolateral membranes has been identified as the anion exchanger 2 [343]. Parietal cells also express the Na^+/H^+ exchanger NHE1 on basolateral membranes, but to a much lesser degree than in mucous neck and chief cells. Moreover, the level of CAII expression appears to correlate with the level of NHE1 expression in these cells. The low level of NHE1 in parietal cells may suggest that another exchanger such as NHE4 [344] may play a more important role in the acid secretory process. Although not shown in Figure 13.13, a Na^+/HCO_3^- cotransporter has also been located on parietal cells [345].

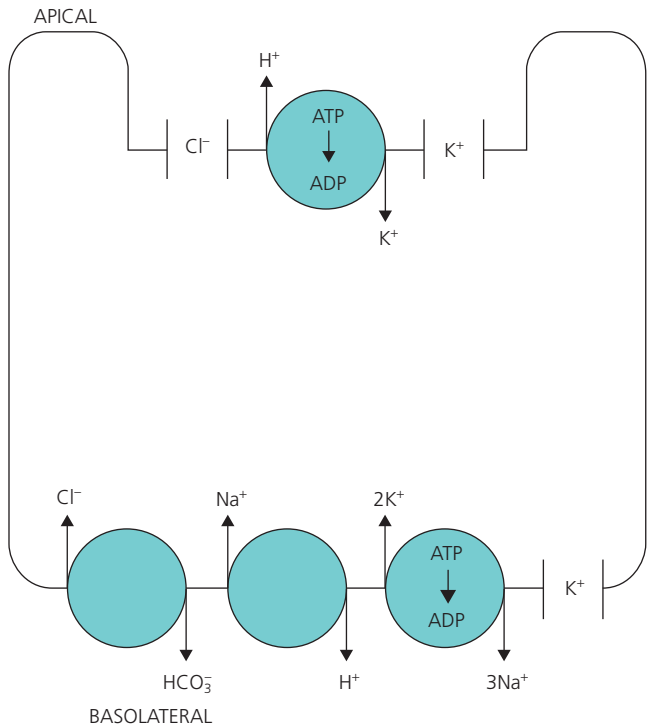

Figure 13.13 Ion transport pathways in parietal cells. The apical membrane contains the H^+,K^+-ATPase pump and K^+ and Cl^- conductances. The basolateral membrane also has a K^+ conductance, Cl^-/HCO_3^- exchange, Na^+/H^+ exchange, and Na^+,K^+-ATPase to maintain cellular homeostasis during secretory and resting states.

The role of this cotransporter in maintaining parietal cell homeostasis has not been determined.

A second mechanism located on the basolateral cell membrane allows the exchange of extracellular Na^+ for intracellular H^+. The Na^+/H^+ and Cl^-/HCO_3^- exchangers are coupled functionally as cellular pH monitors to handle excess acid or base loads respectively. Although physically separate, these exchange mechanisms act as a functional basolateral Na^+ and Cl^- cotransport mechanism. The basolateral membrane also has a K^+ conductance, which allows intracellular K^+ to follow the concentration gradient. K^+ uptake by the parietal cell can occur by means of a basolateral Na^+,K^+-ATPase or a Na^+ pump, which appears to translocate three intracellular Na^+ ions for two extracellular K^+ ions. The net effect of these ion channels is to maintain cellular homeostasis in the face of HCl secretion. The osmotically active HCl generated in the canalicular lumen results in the flow of H_2O across the cell. This flow leads to the formation of gastric fluid associated with acid secretion.

The gastric H^+,K^+-ATPase or H^+ ion pump has been studied in detail [346]. This membrane protein is a member of the P-ATPase family that includes the Na^+- or K^+- and Ca^+-ATPases. Structural homology between these family members suggests that they probably diverged from a common ancestor molecule [347].

The structural characteristics of the gastric H^+,K^+-ATPase are well understood [348]. This critical enzyme in parietal cell biology consists of two subunits, α and β, with the α-subunit being larger. The amino acid sequences of the rat, pig, and rabbit enzymes have been deduced from the corresponding cloned cDNA structures [347,349,350]. Interspecific conservation of the α-subunit sequence is greater than 97%; the protein length ranges from 1033 amino acids in the rat to 1035 residues in the rabbit. The gene corresponding to the human α-subunit has been cloned and sequenced [351]; it contains 22 exons and has a corresponding deduced protein length of 1035 amino acids.

Several experimental procedures have been used to establish the secondary structure of the α-subunit (Fig. 13.14). Initial hydropathy plots of the deduced amino acid sequence suggested that this protein was anchored by seven to nine potential transmembrane domains with a large cytoplasmic

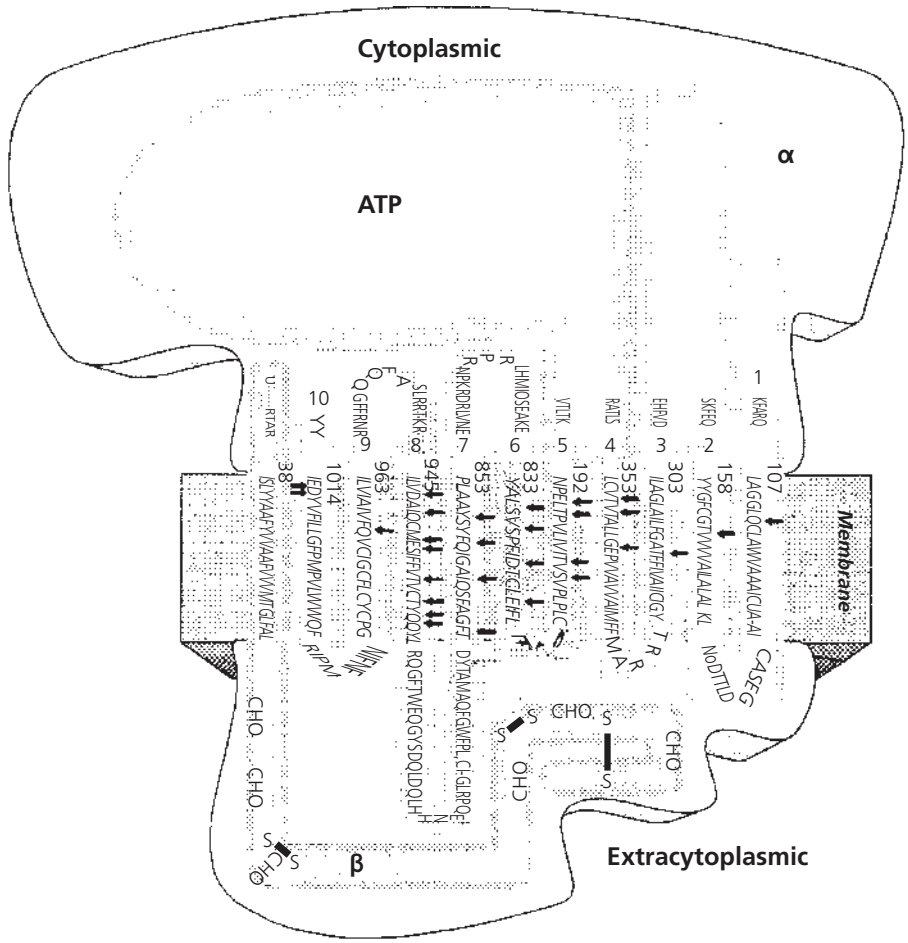

Figure 13.14 Model of the two-dimensional structure of the H^+,K^+-ATPase illustrating the α- and β-subunits. The α-subunit contains 10 putative transmembrane domains. Hydrophilic amino acids within the postulated membrane-anchored domains are shown. Adapted from Hershey & Sachs [348].

site containing an ATP-binding region [347]. Although still controversial, subsequent studies, capitalizing on a combination of proteolytic cleavage of the enzyme and in vitro translation and inhibitor-binding experiments, suggest that the α-subunit may contain ten transmembrane segments with a large cytoplasmic domain [348]. The cytoplasmic domain is presumed to include ATP-binding, phosphorylation, and energy transduction sites. Labeling studies suggest that the proton pump inhibitors omeprazole, lansoprazole, and pantoprazole bind to the cysteine residues located at positions 813 and 822. These residues lie near a predicted extracytoplasmic loop between transmembrane domains five and six, and they are thought to be essential for the inhibition of H^+,K^+-ATPase [352–354].

The cDNA of the H^+,K^+-ATPase β-subunit has been isolated and sequenced from several species, including rabbit [355], rat [356–358], hog [359], mouse [360], and human [361]. The average length of this subunit ranges from 290 to 294 amino acids, and hydropathy plots suggest a single transmembrane domain. Although the role of the β-subunit has not been established, several lines of investigation suggest that it may be important for assembly and stabilization of the functional α-subunit. Insight into the functional role of the H^+,K^+-ATPase β-subunit has been gained by studies conducted in transgenic animals [362]. Courtois-Coutry and colleagues [362] noted that the cytoplasmic tail of the H^+,K^+-ATPase β-subunit contains a four-residue sequence that is homologous to tyrosine-based endocytic signals. Mutation of the tyrosine residue in this motif and expression of the mutated H^+,K^+-ATPase β-subunit in transgenic animals lead to constitutive secretion of acid and the continuous expression of the mutated H^+,K^+-ATPase at the cell surface. Thus, the H^+,K^+-ATPase β-subunit appears to play an important role in the process of H^+,K^+-ATPase internalization and in the termination of gastric acid secretion [362].

The timing of the association between the α- and β-subunits of the H^+,K^+-ATPase and the initial steps in cellular trafficking for this enzyme have been determined. Crothers and associates [363] used in vivo protein metabolic labeling in conjunction with subcellular fractionation to examine these issues in rabbit parietal cells. These investigators observed that the α- and β-subunits associated early in the endoplasmic reticulum. Moreover, the newly synthesized H^+,K^+-ATPase migrated sequentially through at least three distinct membrane pools.

H^+,K^+-ATPase can be inactivated completely by omeprazole, lansoprazole, rabeprazole, and pantoprazole. These compounds become cyclic in the presence of acid and react with available sulfhydryl groups to form a covalent interaction that irreversibly inactivates the enzyme. The blockade of proton transport is virtually complete and has been used clinically as described in Chapter 40.

Other gastric secretory products

Histamine

Histamine plays a critical role in regulating gastric acid secretion through the activation of the parietal cell H_2 receptor. There is evidence that histamine mediates in part the stimulatory effect of other secretagogues (e.g., gastrin, carbachol) on parietal cells. The relative importance of the two histamine-containing cell types found in the stomach (i.e., mast cells and ECL cells) in regulating gastric acid secretion has been the source of some debate. The ratio of the two cell types in gastric mucosa depends on the species examined. Early studies using isolated mast cells obtained from canine gastric mucosa indicated that gastrin and cholinergic agents did not stimulate histamine release, and this observation was used to support the contention that these secretagogues induced acid secretion by direct action on parietal cells rather than by stimulating histamine release. However, these data were at odds with studies demonstrating that pentagastrin stimulated the release of histamine from gastric mucosal preparations. Chuang and colleagues [364] shed light on this controversy by successfully isolating and characterizing a population of non-mast cells from canine oxyntic mucosa that contain histamine and have the morphological characteristics of ECL cells. Gastrin and carbachol stimulate histamine release from ECL cells when they are isolated and cultured. It is possible that the secretagogue actions of gastrin and carbachol can be mediated, at least in part, through the action of histamine. Such studies reinforce the concept that regulation of gastric acid secretion involves a complex interplay between multiple pathways and receptors.

Development of the isolated ECL cell model has facilitated the study of this important source of gastric histamine. Gastrin-stimulated histamine release from ECL cells occurs through the CCK_2/gastrin receptor, which is coupled to a Ca^{2+} signaling pathway [365,366]. Histamine release is also stimulated by factors that increase cAMP, such as agonists of the β-adrenergic receptor, forskolin, and pituitary adenylate cyclase-activating peptide (PACAP) [365,367]. Research indicates that the PACAP type 1 receptor subtype regulates histamine release and $[Ca^{2+}]_i$ mobilization [368]. As one would anticipate, ECL cells are also under inhibitory restraints. Somatostatin binds to an $SSTR_2$ receptor subtype, inhibiting histamine release and Ca^{2+} signaling [369]. Histamine appears to regulate its own release through an H_3 receptor subtype located on ECL cells, thus creating an autocrine feedback loop [365]. Peptide YY has also been observed to inhibit histamine release through a Y_1 receptor subtype [367].

Histamine is generated in ECL cells by decarboxylation of histidine by the enzyme histidine decarboxylase [370,371]. Histidine decarboxylase is transcriptionally activated by both gastrin and acetylcholine. Studies by Hocker and colleagues

[372,373] have demonstrated that gastrin stimulates histidine decarboxylase transcription through a PKC- and MAP kinase-dependent pathway. In view of reports demonstrating the development of ECL hyperplasia and gastric carcinoid tumors in animals receiving large doses of proton pump inhibitors, there has been increased interest in understanding the regulation of ECL cell proliferation [374]. Both in vivo and in vitro studies have demonstrated that gastrin has a direct proliferative effect on these cells, thus supporting the concept that hypergastrinemia resulting from potent acid suppression is responsible for increased ECL proliferation in these animal models [375–381]. The relevance of these observations to human physiology is unclear, considering the studies demonstrating that the human ECL cell is terminally differentiated and is not capable of undergoing proliferation [382].

An attempt to determine the relative importance of histamine-containing mast cells and ECL cells in the regulation of gastric acid secretion has also been made by Stechschulte and colleagues [383]. These investigators observed that mast cell-deficient mice had about 50% of the normal quantity of gastric histamine in addition to diminished basal and stimulated acid secretory activity. They concluded that non-mast cell histamine may account for only part of the basal and stimulated gastric secretory response.

Pepsinogen

The secretion of pepsinogen by the gastric mucosa occurs in response to food ingestion, as described by Langley and Edkins in 1886 [384]. Pepsinogens are inactive proenzymes that are autocatalytically cleaved under acidic conditions to generate their active form, pepsin. The proenzyme is synthesized in exocrine chief cells found at the base of oxyntic glands and in mucous neck and mucous cells in cardiac, oxyntic, and pyloric glands [385]. Chief cells package pepsinogen in apical granules, where it is stored until the cells are stimulated. Stimulation of chief cells by various secretagogues induces exocytosis of granule contents into the glandular lumen. Concomitant stimulation of parietal cells provides the acidic lumenal conditions needed for rapid production of pepsin, the major acid protease activity in the stomach. Pepsin is a member of the group of proteolytic enzymes classified as aspartic proteases [386]. Members of this enzyme family are found in an array of organisms extending from plants and retroviruses to humans. The stomach is the best source of aspartic proteases in mammals, and pepsin is the representative member.

Pepsin initiates protein digestion and is particularly active in the proteolysis of collagen, a major protein component of meat. Peptides generated by the proteolytic activity of pepsin act as signals for the secretion of digestive hormones such as gastrin and CCK. These peptide signals initiate the coordinated digestive response necessary for the absorption of nutrients.

Pepsinogens have been electrophoretically separated into seven isozymogens [387]. Five fractions (i.e., fractions 1–5) that migrate rapidly toward the anode are immunologically similar and have been named group I pepsinogens (PGI or PGA). Group II pepsinogens (i.e., fractions 6 and 7; PGII, PGC, or progastricsin) migrate slightly slower than PGI and are antigenically similar. Both groups are active in acidic conditions (pH 2–3.5) and are inactivated at a pH higher than 5. Despite these similarities, there are many biochemical and immunochemical differences between PGI and PGII, which are the subject of intense investigation.

The distribution of the two pepsinogen groups in gastrointestinal tissues varies. Although both groups are found in the gastric body, PGI being the most abundant, only PGII are found in the gastric antrum, proximal duodenum, and the Brunner glands. The presence of different isozymogens could be the result of multiple gene loci, multiple alleles at a single gene locus, alternate posttranscriptional processing of RNA, or posttranslational modifications of primary gene products. Genetic analysis has revealed that the two groups have different gene loci. A human PGI gene has been isolated, sequenced, and localized to chromosome 11q12–13 [388–390]. Analysis of its locus indicates the presence of multiple PGI genes [391]. This type of multigene protein polymorphism is rare and suggests that the pepsinogen gene complex is undergoing evolutionary gene duplication and selection. Other mechanisms of isozymogen diversity, such as posttranslational modification, may also be active.

Taggart and colleagues [392] isolated and sequenced the cDNA and gene coding human PGII. Analysis of the genomic clones suggests that there is a single gene for PGII located on chromosome 6. Structural comparisons between PGI and PGII demonstrate that they share 60% nucleotide and 50% amino acid identity. No immunological cross-reactivity was detected between the two groups of human pepsinogens. This lack of immunological cross-reactivity may result from divergent evolution of sequences located on the surface of the enzymes, in contrast to the highly conserved sequences at the biologically active sites of the zymogen located within the binding cleft of the enzyme.

The stimulation of pepsinogen secretion has been analyzed in intact animals, gastric mucosal preparations, gastric glands, and isolated chief cells. Despite the use of different species in these models, certain generalizations about the mechanisms of chief cell activation are supported by the data. Acetylcholine and its analogues appear to stimulate chief cells directly through muscarinic receptors [393,394]. Other neuronal mediators, such as gastrin-releasing peptide, may also mediate vagal stimulation of pepsinogen secretion [395]. The adrenergic agonist isoproterenol (isoprenaline) has a stimulatory effect in vitro [396], although the physiological significance of this effect has not been demonstrated in intact animals. The ability of histamine to stimulate pepsinogen

secretion remains controversial, and conflicting data preclude any definitive conclusion [385]. Secretin has been reported to stimulate pepsinogen secretion in vivo and in vitro [397,398].

Although gastrin stimulates chief cells only weakly, CCK-8 is a potent stimulus of pepsinogen secretion in vitro [397]. The finding that peripherally administered CCK in vivo only weakly stimulates pepsinogen secretion may reflect the function of CCK as a neurocrine, rather than endocrine, mediator of chief cell stimulation. Alternatively, it may reflect the mixed effects of a direct stimulatory effect on chief cells and an indirect inhibitory effect such as could be mediated by CCK-induced somatostatin release.

The intracellular signal transduction mechanisms activated by the various chief cell secretagogues have been analyzed. Secretin, isoproterenol, and vasoactive intestinal polypeptide stimulate pepsinogen release through cell surface receptors linked to the activation of adenylate cyclase and cAMP formation [385]. Several peptide hormones including somatostatin, neuropeptide Y, and peptide YY have been found to inhibit pepsinogen release from isolated chief cells through a pertussis toxin-sensitive mechanism. Other secretagogues, such as cholinergic agonists and CCK, stimulate pepsinogen release in chief cells by activating the IP_3 and $[Ca^{2+}]_i$ signaling pathway [399,400]. Activation of the chief cell muscarinic receptor leads to a modest increase (15%) in PKC enzyme activity while stimulating a threefold increase in pepsinogen secretion [401]. Of interest, doses of carbachol capable of inducing pepsinogen release did not alter PKC activity, suggesting that other pathways independent of PKC, such as changes in cytosolic Ca^{2+}, may be sufficient for enzyme release. However, a maximal stimulatory effect of carbachol on pepsinogen release was achieved only at doses that also led to PKC activation. From these data, it appears that PKC, although not essential for enzyme release, may be involved in modulating the maximal response achieved by activation of the muscarinic receptor.

Further insight into the postreceptor events involved in secretagogue-mediated pepsinogen secretion has been gained. The involvement of the Ca^{2+} signaling pathway as an important mechanism for chief cell activation has been reaffirmed [402]. Moreover, it appears that the increase in $[Ca^{2+}]_i$ is important for the ability of ligands such as CCK-8 to potentiate the stimulatory effect of agents such as secretin, which activates cAMP production in chief cells. This potentiation in pepsinogen release occurs independently of the Ca^{2+} source (intracellular or extracellular). As is the case in other cell systems, Ca^{2+}/CaMKII is also involved in Ca^{2+}-mediated pepsinogen secretion [403]. Protein phosphatase 2B (calcineurin) is a Ca^{2+}/calmodulin-dependent serine/threonine protein phosphatase involved in cell signaling in a variety of tissues [404,405]. Raufman and colleagues [406] showed that gastric chief cells express this phosphatase and that it modulates Ca^{2+}-induced potentiation of the cAMP signaling pathways at the level of adenylate cyclase [407]. Moreover, this specific phosphatase appears to be involved in Ca^{2+}-induced pepsinogen secretion by facilitating dephosphorylation of a specific 55-kDa cytoskeletal protein, which, in turn, regulates exocytosis.

As in gastric parietal cells, Rab proteins appear to be involved in chief cell intracellular trafficking [408,409]. Specifically, Rab3 proteins appear to regulate pepsinogen secretion at one of the final steps of exocytosis [408]. Chief cells also express two isoforms of Rab–GDP dissociation inhibitor (Rab-GDI) [409], which are proteins that, in addition to inhibiting the dissociation of GDP from Rab, inhibit the binding of GTP to GDP-bound Rab, thus removing Rab proteins from membranes [410–412].

The mechanism by which PKC modulates (i.e., augments) Ca^{2+}-induced pepsinogen secretion is unclear. A member of the family of proteins known as MARCKS (myristoylated alanine-rich C-kinase substrate) has been suggested to play an important role in mediating the cross-talk between PKC and Ca^{2+}/calmodulin-stimulated pepsinogen secretion [413]. Specifically, a MARCKS protein may serve as a Ca^{2+}/calmodulin buffer, which, on phosphorylation, releases calmodulin from membranes and makes it available to bind and activate other signaling proteins such as Ca^{2+}/CaMKII and calcineurin [414]. In a series of immunological and biochemical studies, Raufman's group presented evidence to suggest that an acidic 72-kDa protein (pp72) serves as a MARCKS protein in chief cells. It appears that PKC-induced phosphorylation and Ca^{2+}/calmodulin binding compete for similar regions on pp72. Therefore, PKC may enhance Ca^{2+}-induced pepsinogen secretion by phosphorylating MARCKS, decreasing its affinity for calmodulin, and facilitating activation of downstream targets that are important for pepsinogen secretion such as Ca^{2+}/CaMKII and calcineurin [414].

An additional signaling pathway involved in pepsinogen secretion is the nitric oxide (NO) system [415,416]. Gastric chief cells express a Ca^{2+}/calmodulin-dependent NO synthase that initially was observed to mediate pepsinogen secretion stimulated by leukotrienes (LTB_4, LTC_4, LTD_4, and LTE_4) [415]. This pathway also appears to be involved in the action of Ca^{2+}-dependent agonists such as carbachol, gastrin, CCK, thapsigargin, and Ca^{2+} ionophore.

Pepsinogens are found in the gastric secretions, serum, urine, and seminal fluid [417,418]. Their physiological role in nongastric fluids is unknown. Measurement of pepsinogens in these fluids is performed with a specific radioimmunoassay [417]. Attempts to correlate serum pepsinogen groups with risk of peptic ulcer disease have proven to be of modest clinical value (see Chapter 40). Azuma and colleagues [419], studying Japanese patients with gastric ulcers, found no genetic polymorphism for the pepsinogen A gene, but observed a 100-base pair insertion–deletion restriction fragment length polymorphism in the pepsinogen C gene.

Genotypes that contained the small fragment were significantly more common in patients with gastric body ulcers than in patients with angular or antral ulcers or in control subjects. The clinical implication of this interesting observation has not been determined. Advances in the understanding of pepsinogen physiology and pathophysiology may lend these measurements new clinical significance.

Mucus

Gastric epithelium is partially protected from acidic autodigestion by a mucous gel that covers the entire surface of the stomach (for further discussion of gastric mucosal defense mechanisms, see Chapter 40). This gel acts as a barrier, protecting the gastric mucosa from acid, pepsin, bile salts, alcohol, and other injurious agents. The barrier consists of an unstirred layer of mucus, HCO_3^-, surface phospholipids, and water. A prominent pH gradient extending from the lumen (pH 2) to the epithelial cell surface (pH 7) is maintained by this gel [420]. Bhaskar and colleagues [421] shed light on the physicochemical basis for the barrier function of gastric mucus. In a series of elegant in vitro experiments, these researchers demonstrated that injection of HCl through solutions of porcine gastric mucin produces viscous fingering patterns that depend on pH, mucin concentration, and acid flow rate. If the pH is greater than 4, discrete fingers are observed; at a pH of less than 4, these fingers are not seen, and HCl does not penetrate the mucin solution. Acid secreted by the gastric gland that exists in an environment of pH 5–7 can penetrate the mucous gel layer through narrow fingers. In contrast, HCl in the gastric lumen (pH 2) is prevented from diffusing back to the epithelium by the high viscosity of the gastric mucous gel on the lumenal side.

Mucin, a high-molecular-weight glycoprotein, is secreted by surface mucous cells, mucous neck cells, and glandular mucous cells. Polymerization of mucin subunits by means of disulfide bonds is essential for the formation of the hydrated gel [422]. The precise structural organization and approximate molecular weight of mucin polymers are unknown. Two significantly different models of gastric mucin polymerization have been proposed and are described in other reviews [423,424]. The peptide backbone of mucin contains many serine, threonine, and proline residues (> 40% molecular weight) that serve as the amino acid anchors for the glycosyl residues that branch from the protein. Partial and complete cDNA clones for mucin have been isolated, and deduced peptide sequences confirm this amino acid preponderance [425–428]. The structural analysis indicates that the mucin backbone contains extended arrays of tandemly repetitive peptides rich in threonine or serine residues which are potential sites for *O*-glycosylation.

Multiple mucin gene products are expressed at various sites. Specific cDNA clones coding for mucins found in submaxillary glands, the tracheobronchial tree, and intestinal mucosa have been isolated and characterized [429–432]. Toribara and colleagues [428] isolated a cDNA clone coding for human gastric mucin, which is characterized by tandem repeat sequences rich in threonine, serine, and proline [428]. This gastric mucin gene, which has been called *MUC6*, is expressed primarily in the stomach and the gallbladder and has been localized to chromosome 11 (11p15.4–11p15.5).

The mucin that is synthesized in the rough endoplasmic reticulum undergoes vesicular transport to the Golgi apparatus for glycosylation. The major portion of mucin is heavily glycosylated, and nonglycosylated or "naked" regions of the peptide are joined to other mucins by disulfide bridges. The initial peptide–carbohydrate linkage involves glycosidic bond formation between *N*-acetylgalactosamine and the hydroxyl groups of serine or threonine residues. This type of *O*-linked glycosylation involves the addition of individual carbohydrate moieties, rather than the transfer of preassembled oligosaccharides, to gradually lengthening chains [433]. Fucose, galactose, *N*-acetylglucosamine, and *N*-acetylgalactosamine make up more than 95% of the sugar moieties in each chain [434]. Each mucin peptide contains up to several hundred linear or branched-chain oligosaccharides. This high carbohydrate content (> 50% by weight) results in a highly viscoelastic substance that expands when hydrated. These properties may be essential for the protective function served by gastric mucus.

The intracellular transport of mucin proceeds through the cis-, medial-, and trans-Golgi cisternae before transfer to the apical mucous granule. Cytoplasmic transport vesicles presumably bud off Golgi membranes and transfer mucus to its target organelles. Fusion of vesicle and granule membranes results in gradually enlarging mucous granules that ultimately pack the apical cytoplasm. Intact microtubules appear to be needed for transport of secretory granules from the Golgi apparatus to the cell surface. Basal secretion of mucus occurs continually throughout the life span of mucous cells [435]. In vitro pulse labeling of human mucous granules reveals that intracellular transit and release occurs in 20–24 h. In contrast to basal secretion, stimulation of mucous cells results in fairly rapid fusion of granular and apical cell membranes or extrusion of mucous granule contents. Fusion of subjacent mucous granules enhances the secretory response. This process is called *compound exocytosis* and results in a dramatically cavitated apical cell surface during stimulated secretion.

Study of the regulation of mucous cell secretion has been impeded by the inherent difficulties in quantitatively retrieving and measuring secreted mucus. Hydration of secreted mucus forms a viscous gel that adheres to mucosal cells. Attempts to quantify mucus secretion by morphological methods [436], release of radiolabeled glycoproteins [437], and radioimmunoassay [438] have met with only limited success. Despite these limitations, several factors that affect mucin secretion have been identified and can be summarized.

Cholinergic agonists stimulate secretion in a portion of gastric mucous cells, but adrenergic neurotransmitters do not appear to influence release of secretory granules. Gastrin and CCK may increase feline gastric lumenal carbohydrate content, but the source of this presumed mucus secretion has not been determined [439]. Similarly, secretin has also been shown to increase the carbohydrate content of secreted gastric mucus in humans, but the cellular origin of this effect is also unknown [440]. PGE and PGF have been reported to stimulate the release of soluble and insoluble mucin from gastric mucosa, with a resultant increase in mucous gel thickness [441,442]. This increase may be produced by prostaglandin-induced HCO_3^- secretion, creating an outward alkaline flow carrying previously secreted mucins from gastric crypts and glands [443]. After gastric mucosal injury has occurred, multiple inflammatory and immune cytokines may be present in the mucosa. It seems plausible that these factors directly or indirectly stimulate mucous cell secretion as part of the reparative process [422]. Candidate secretagogues include leukotrienes, immune complexes, and mast cell histamine.

Boland and colleagues [444] established a system for isolating and culturing canine gastric mucous cells and demonstrated that the cells synthesize and secrete mucin and phospholipids [444]. Mucin glycoprotein and surface-active phospholipids may form a biochemical complex that is analogous to pulmonary surfactant, and it is this complex that may be critical for providing the gastric epithelium with its protective barrier. PGE_2 may enhance mucosal defense through the release of surface-active phospholipids by gastric mucous cells. Other studies have demonstrated that the same secretagogues that stimulate gastric acid secretion (e.g., histamine, gastrin, carbachol) can stimulate mucin synthesis and release of surface-active phospholipids [445,446]. Gastrin, in particular, has been shown to stimulate the biosynthesis of mucin in surface mucous cells of the rat through a NO-dependent mechanism, because NO synthase inhibitors were able to block the stimulatory action of gastrin [447]. These observations suggest that gastric acid secretion (i.e., aggressive factor) and gastric mucosal defense are regulated in a coordinated or parallel fashion.

Mucous cells presumably have various receptors mediating stimulatory effects on the basolateral membrane, but no specific receptors have been demonstrated on these cells. Part of the difficulty in performing radioreceptor binding studies in this cell type is the high level of nonspecific ligand binding to the mucous gel. Functional studies have suggested that carbachol, histamine, and gastrin act directly on gastric mucous cells. Seidler and Pfeiffer [448] demonstrated that acetylcholine stimulates IP_3 formation, $[Ca^{2+}]_i$ mobilization, and mucin release from isolated gastric mucous cells. However, neither dibutyryl AMP nor phosphodiesterase inhibitors have been shown to stimulate mucin release from intestinal mucous cells [449]. Conversely, a Ca^{2+} ionophore has been shown to induce visible loss of mucous granules from guinea pig gastric mucosa and to increase the thickness of the mucous gel [450]. Together, these results suggest that gastric mucous cells may be stimulated by secretagogues that use Ca^{2+}-mediated intracellular pathways.

Research has indicated that activation of protease-activated receptor 2 (PAR-2) leads to the secretion of gastric mucus through the release of calcitonin gene-related peptide and tachykinins from sensory neurons, suggesting a novel mechanism for the regulation of mucus secretion in the stomach [451]. Much remains to be learned about the regulation of gastric mucous cell secretion in the basal and stimulated states.

Bicarbonate

Gastric mucosal defense depends on a mucus–HCO_3^- barrier coating the entire lumen of the stomach. This barrier exists as a gel with a pH gradient that provides a neutral microenvironment at the epithelial surface. The gradient is generated by the secretion of HCO_3^- by gastric surface mucous cells. HCO_3^- uptake at the basolateral membrane and secretion at the apical membrane are metabolically dependent processes [452]. Carbonic anhydrase, the enzyme responsible for HCO_3^- generation, has also been localized to the apical matrix and microvillous cores of surface epithelial cells [453]. HCO_3^- secretion appears to be mediated by a Cl^- and HCO_3^- exchange mechanism on the lumenal surface of gastric epithelial cells [454]. In addition to active secretion, passive efflux of HCO_3^- presumably occurs through a paracellular route. Mucosal delivery of HCO_3^- ions is enhanced by fenestrations of the capillaries supplying the epithelial cells [36]. The relative proportions of passive and active HCO_3^- secretion in the basal and stimulated state have been difficult to determine and depend on the type of model studied.

Several models have been used to measure gastric HCO_3^- secretion. Mucosal membranes can be mounted in chambers and bathed with appropriate solutions [455–457]. Titration of lumenal secretions is used to calculate HCO_3^- release. In vivo measurements have been performed in fundic and antral pouches and are described elsewhere [458,459]. In humans, gastric HCO_3^- secretion can be measured in vivo by back-titration after complete pharmacological inhibition of acid secretion [460]. Another technique involves rapid perfusion of the stomach (30 mL/min) with continuous measurement of P_{CO_2} and pH in the gastric aspirate [461]. HCO_3^- and H^+ secretion can then be calculated using the Henderson–Hasselbalch equation. This technique relies on the assumption that all lumenal CO_2 results from neutralization of H^+ ions by HCO_3^-.

Because of the quantitative differences observed with the various methods used, Odes and colleagues [462] performed detailed in vivo and in vitro studies using the different techniques outlined. They observed that in the acid-suppressed stomach, gastric HCO_3^- is accurately determined by back-titration. However, measurement of HCO_3^- in the

acid-secreting stomach is not accurate with any of the methods tested. Despite these quantitative differences, responses to various stimuli are qualitatively similar with all of the techniques.

Regulation of gastric HCO_3^- secretion is an important component of gastric mucosal defense. Sham feeding and electrical stimulation of the vagus induce gastric HCO_3^- secretion [463–465]. This neural control mechanism is effectively blocked by atropine and benzilonium bromide. Intravenous infusion of cholinergic agents such as bethanechol also stimulates HCO_3^- and H^+ secretion [460,466]. The coordinated stimulation of acid and HCO_3^- secretion by vagal transmitters is not reproduced by gastrin or histamine. Local regulation of HCO_3^- secretion is initiated by the presence of lumenal acid. Studies using canine denervated pouches [467] and isolated frog mucosal strips [468] suggest that a humoral factor that stimulates HCO_3^- secretion is released in response to lumenal acid. Although exogenous prostaglandins have been shown to stimulate gastric and duodenal HCO_3^- secretion, the physiological role of endogenous prostaglandins in HCO_3^- control is less clear [452,469]. The inhibition of gastric HCO_3^- secretion is not well characterized. Vagal stimuli and lumenal acid appear to stimulate surface mucous cells to secrete HCO_3^-. Acid stimulation also results in delivery of HCO_3^- from the parietal cells to the surface epithelium by ascending mucosal capillaries (i.e., the alkaline tide). These regulatory controls ensure simultaneous secretion of gastric acid and HCO_3^-.

Intrinsic factor

Intrinsic factor (IF), a 45-kDa glycoprotein present in gastric secretions, is essential for the absorption of cobalamin (vitamin B-12) in the terminal ileum by receptor-mediated endocytosis. Its existence was first postulated by Castle in 1929 [470]. Human IF is synthesized and secreted by parietal cells [471]. Parietal cells are also the source of IF in cats, rabbits, monkeys, guinea pigs, and oxen [472]. In rodents such as the rat and mouse, chief cells produce IF, whereas its source in pigs is gastric mucous cells [473,474]. The structure of rat IF, as deduced from a cDNA clone, indicates a primary amino acid sequence of 421 amino acids, with a putative signal sequence of 22 amino acids [474]. The cobalamin-binding domain is thought to reside in the N-terminal half of the protein. There is 80% identity between the predicted amino acid sequences of rat IF and human IF [475]. The single human IF gene is located on chromosome 11; human IF appears to have a high degree of conservation with monkey, rat, mouse, and cow IF.

IF is secreted in amounts far exceeding that necessary for cobalamin absorption [476]. Secretion of IF is stimulated by the same pharmacological agents that stimulate acid secretion – pentagastrin, histamine, and cholinergic agonists – but this secretory response is not linked to acid secretion [477]. For example, omeprazole does not alter basal or stimulated IF secretion in humans, nor does it alter the absorption of labeled cobalamin [478,479]. The intracellular responses mediating IF secretion involve the cAMP pathway when stimulated by histamine [477]. Whether pentagastrin and acetylcholine, which appear to act through Ca^{2+}/phospholipid-dependent pathways when stimulating acid secretion, act in a similar fashion when stimulating IF secretion is unknown.

Inhibitory regulation of IF secretion is poorly understood. Somatostatin inhibits histamine- or pentagastrin-stimulated IF secretion in isolated guinea pig gastric glands [480]. EGF is reported to inhibit histamine-stimulated IF secretion in isolated rabbit gastric glands and in humans [481,482]. The physiological significance of these observations is unknown.

IF resists digestion by gastric acid and proteolytic enzymes under normal circumstances. A rare kindred with cobalamin malabsorption caused by an abnormal IF that is susceptible to acid and proteolysis has been reported [483]. An absence of IF secretion occurs rarely in those with normal acid secretion, usually in children [484,485]. The significance of IF in health and disease is discussed further in Chapter 20.

Human tissue studies have demonstrated that IF is expressed not only in parietal cells but also in gastric chief cells and enteroendocrine cells [486]. The expression of IF in these sites appears to be lower than the level of expression in parietal cells. Some investigators have suggested that the principal reason for the differential expression of IF in different cell types lies in the variation between the untranslated regions of the human IF and rat IF genes [487]; however, the observation that IF can also be expressed in human chief cells goes somewhat against this theory [486]. Questions still remain regarding the interspecific variation in IF expression and the cellular pathways that regulate IF synthesis and secretion.

Prostaglandins

The role of prostaglandins in gastric acid secretion and mucosal defense has been an area of active research. Prostaglandins are 20-carbon fatty acid derivatives that have diverse biological activities and which are produced in many different tissues. They are synthesized from arachidonic acid, a product of enzymatic cleavage of cell membrane phospholipids by phospholipases, particularly phospholipase A_2 [488]. Cyclooxygenase rapidly metabolizes free arachidonic acid to cyclic endoperoxides, which are transformed to various prostaglandin subtypes by tissue-specific processing enzymes (Fig. 13.15). PGE_2 and PGI_2 (or prostacyclin) are synthesized and secreted by the gastric mucosa of various species. The presence of these two predominant forms in homogenates of gastric mucosa has been confirmed by bioassay, radioimmunoassay, and gas chromatography with mass spectrometry. Biosynthetic studies using radiolabeled arachidonic acid have confirmed that gastric mucosa can synthesize both of these prostaglandins. The specific mucosal cell types synthesizing the various prostaglandins have not

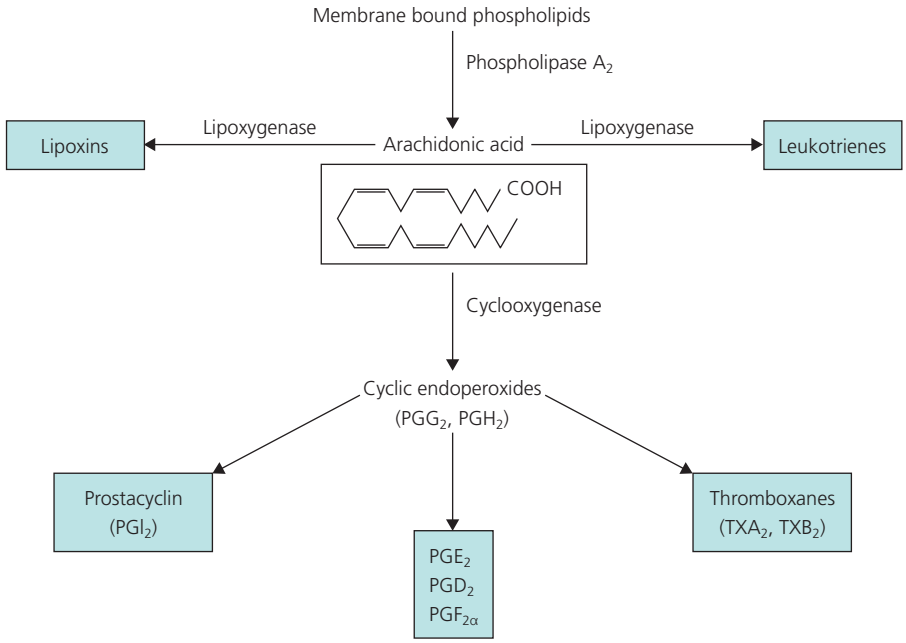

Figure 13.15 Pathways of arachidonic acid metabolism.

been identified. Although canine and rat parietal cell fractions appear to form several prostaglandin subtypes, other cells, such as mucous and chief cells, may also synthesize prostaglandins.

Gastric prostaglandins are likely to have paracrine effects on other gastric mucosal cells. One example is direct inhibition of acid secretion by prostaglandins. Studies of canine parietal cells show that PGE_2 decreases [^{14}C]aminopyrine accumulation and cAMP production [201]. This effect appears to be mediated through prostaglandin receptors activating inhibitory guanine nucleotide-binding proteins [489]. Inhibition of parietal cells could be one effect of a prostaglandin-mediated negative-feedback loop regulating gastric acid secretion.

Prostaglandins may also function as lumenal hormones. PGE_2 has been demonstrated in the gastric juice of several species, including human, cat, dog, and rat [488]. Secretion of PGE_2 into lumenal fluid is maximal during pentagastrin-stimulated acid secretion. The presence of prostaglandins in gastric juice and gastric mucosa may facilitate the diverse cytoprotective functions ascribed to these agents, in addition to their antisecretory effects. These functions include stimulation of mucus, phospholipid, and HCO_3^- secretion; enhancement of mucosal blood flow; reduction of mucosal H^+ ion back-diffusion; and stimulation of mucosal cell turnover. Prostaglandins may function in this way to mediate mucosal defense.

Prostaglandin deficiency may predispose to gastric mucosal injury. Cyclooxygenase inhibitors such as aspirin and nonsteroidal antiinflammatory agents produce a spectrum of mucosal injuries in the stomach. Immunization against prostaglandins has caused ulcers in rabbits [490,491]. Part of the cause of these ulcerations is a decrease in prostaglandin-mediated mucosal defense. These observations have served as the basis for the development of prostaglandins for use in the treatment of acid or peptic diseases of the stomach (see Chapter 40).

Mouse models for the study of gastric secretion

Molecular approaches with various types of transgenic and knockout mouse models have been used to unravel the molecular signals directing the maturation, differentiation, and biological functions of the distinct gastric cell lineages.

Gastric epithelial cell maturation and differentiation

Li and colleagues [8] capitalized on the unique expression of the β-subunit of H^+,K^+-ATPase in parietal cells to target the expression of the simian virus 40 T antigen (SV40TAg) to gastric glands [8]. These investigators successfully amplified the population of preparietal cells in this animal model and noted a block in cell differentiation accompanied by hypochlorhydria and iron deficiency. In addition, SV40TAg-induced preparietal cell proliferation was accompanied by apoptosis, which appeared to occur in a p53-independent manner. The block in the development of mature parietal cells in this model was accompanied by the absence of zymogenic cells. This key observation supports the concept that mature parietal cells generate signals that are important in regulating the differentiation of other cell lineages within the stomach. This hypothesis was further confirmed by analyz-

ing the gastric glands of transgenic mice in which parietal cells were ablated by either diphtheria or cholera toxins [9,492]. In addition to inhibited development of mature parietal cells, these investigators observed that the differentiation of zymogenic cells was blocked, with the accumulation of preneck cells and the amplification of pit cells. These studies corroborate the hypothesis that the interaction between different cell lineages is important in regulating cell differentiation in the stomach. Moreover, these observations emphasize the importance of parietal cells in the regulation of the growth and differentiation of multiple cell lineages in the gastric mucosa.

Some of the mechanisms regulating the process of zymogenic cell differentiation and maturation have been defined in studies conducted in mice lacking the transcription factor Mist1 [493]. The zymogenic cells of Mist1 knockout mice exhibit multiple apical cytoplasmic structural abnormalities. Moreover, the fundic glands of these animals exhibit a significant increase in the number of transitional cells that retain molecular and ultrastructural characteristics of both enzyme-secreting zymogenic cells and mucus-secreting neck cells [493]. Thus, Mist1 may be an important factor in the maturation of mucus-secreting gastric epithelial progenitors into digestive enzyme-secreting zymogenic cells.

Significant alterations in gastric mucosal morphology and gastric acid secretion have also been detected in mice deficient in the trefoil factor 2 (TFF2)/spasmolytic polypeptide (SP) gene [494]. TFF2/SP is a small polypeptide that is secreted by mucous neck cells [494]. Expression of TFF2/SP appears to be increased at sites of gastrointestinal ulceration and inflammation, suggesting that it plays an important role in the stimulation of reparative and cytoprotective mechanisms [494]. TFF2/SP-deficient mice appear to have an increased number of activated parietal cells, increased gastric acid secretion, and undetectable gastrin levels. In addition, these animals exhibit a decrease in gastric mucosal thickness and gastric mucosal proliferation rates, and an increase in susceptibility to indomethacin (indometacin)-induced gastric ulcerations [494]. Taken together, these observations suggest that TFF2/SP promotes mucosal healing through the stimulation of cell proliferation and the inhibition of gastric acid secretion. The precise mechanisms responsible for TFF2/SP-mediated inhibition of gastric acid secretion remain to be elucidated.

Ligands and their receptors

Genetically engineered mice in which the CCK_2 receptor or the peptide gastrin have been ablated develop achlorhydria accompanied by a reduced number of parietal and ECL cells. These findings confirm the importance of both gastrin and the CCK_2 receptor in the regulation of gastric mucosal cell development and differentiation [495,496].

Ablation of the gene encoding the histamine H_2 receptor causes a series of morphological abnormalities of the gastric mucosa [497]. In particular, the histamine H_2 receptor knockout mice have elevated gastrin levels and a markedly hypertrophic gastric mucosa with enlarged folds. Immunohistochemical analysis of the gastric mucosa of these mice reveals an increased number of parietal and ECL cells. Similar, although less severe, abnormalities have also been detected in mice with targeted disruption of the gene for histidine decarboxylase, the key enzyme in the synthesis of histamine [498,499]. Interestingly, the parietal cells of both the histamine H_2 receptor- and the histidine decarboxylase-deficient mice appear to be smaller than in the wild-type mice. These morphological changes cause abnormalities in gastric acid secretion.

Impaired gastric acid secretion also occurs in M_3 muscarinic receptor knockout mice [500]. These animals exhibit a higher intragastric pH, a lower proportion of active parietal cells, higher serum gastrin levels, and a markedly impaired acid secretory response to carbachol, histamine, gastrin 17, and 2-deoxy-D-glucose. Interestingly, although the M_3 muscarinic receptor knockout mice display a significant level of hypergastrinemia, the oxyntic mucosa of these mice does not exhibit any hypertrophic response [500]. These observations suggest that the M_3 receptors play a crucial role in the regulation of both basal and stimulated gastric acid secretion and that they mediate the trophic actions of gastrin in the stomach. Studies conducted with genetically engineered mice in which both the M_5 and the M_1 receptors are ablated indicate that, in addition to the M_3 receptor, the M_5, but not the M_1, receptor mediates cholinergically stimulated gastric acid secretion [501]. Interestingly, pirenzepine, a putative M_1 receptor antagonist, inhibits gastric acid secretion in the M_1 receptor knockout mice, suggesting that inhibition of gastric acid secretion by pirenzepine is unlikely to result from M_1 receptor inhibition [501].

Ion transporters

Selective deletion of either the H^+,K^+-ATPase α- or β-subunit gene causes significant abnormalities in the morphology of parietal cells. The parietal cells of animals with either deletion exhibit enlarged secretory canaliculi and a reduced number of microvilli and tubulovesicles [502,503]. In addition, the H^+,K^+-ATPase β-, but not α-, subunit knockout mice display significant decreases in the numbers of parietal and chief cells and increases in the numbers of pit and neck cells, suggesting that the β-subunit of the H^+,K^+-ATPase might be required for the normal maintenance and distribution of several cell types in the gastric glands [502,503].

Similarly, targeted deletion of the genes for the Na^+/H^+ exchanger isoforms 2 (*NHE2*) and 4 (*NHE4*) leads to hypochlorhydria, a decrease in the number of parietal and zymogenic cells, and a marked increase in parietal cell degeneration, indicating that these molecules play an important role in parietal cell maturation, differentiation, and viability [504,505].

Genetic ablation of the voltage-gated potassium channel gene *KCNQ2* also leads to hypochlorydria and an increase in the number of non-acid secretory cells in the gastric epithelium, underscoring the importance of these transporters in the regulation of gastric epithelial cell homeostasis [506].

Finally, targeted disruption of the *AE2* gene encoding the Cl^-/HCO_3^- exchanger leads to the development of achlorhydria and of morphologically abnormal parietal cells, characterized by immature secretory canaliculi and few tubulovescicles [507].

Considered together, these observations suggest that disruption of the normal mechanisms that regulate ion exchange and membrane gradients can alter the morphology of the gastric mucosa. The exact mechanisms responsible for these observations remain to be characterized.

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C SECRETION AND ABSORPTION

14

Electrolyte secretion and absorption: small intestine and colon

Stephen J. Keely, Marshall H. Montrose, Kim E. Barrett

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The goal of this chapter is to review the cellular, molecular, and regulatory aspects of fluid and electrolyte transport in the mammalian intestinal tract. The intestine can absorb vast quantities of fluid from the intestinal lumen. This is a vital function as about 9 L of fluid are presented to the intestine each day (Fig. 14.1). Greater than 98% of this fluid load is absorbed to preserve health. In addition to its ability to absorb fluid, the intestine is also capable of secretion. In fact, most of the daily fluid load originates from secreted rather than ingested fluids. Fluid secretion is necessary to aid in the digestion and absorption of nutrients, by maintaining a fluid environment for the mixing of food with digestive enzymes. The balance between absorptive and secretory processes is closely regulated. A net absorption of fluid is normal but certain conditions may result in excessive stimulation of secretion, which can overwhelm the combined absorptive capacity of the small and large intestines and result in the pathological condition of secretory diarrhea. The most dramatic example is cholera; patients can experience drastic fluid loss in the stool of up to 20 L per day.

Intestinal fluid movement is predominantly controlled by the active transport of Na^+, K^+, HCO_3^-, and Cl^- ions across the intestinal epithelium. Fluid follows the direction of electrolyte movement to maintain isotonicity between the intestinal lumen and tissue compartments. In addition to this vital role in fluid homeostasis, electrolyte transport has important roles in other physiological processes. For example, mucosal protection in the duodenum is dependent on duodenal secretion of HCO_3^- [1]. Plasma electrolyte homeostasis is affected by electrolyte transport in both the colon and the kidney [2]. The uptake of peptides, amino acids, sugars, and bile acids requires various specialized ion-dependent transport mechanisms in the small intestine [3–5].

Many dramatic advances have been made in understanding intestinal electrolyte transport, from the molecular to the whole-tissue level. Molecular cloning of transport, regulatory, and structural proteins has provided new insight into the regulation of electrolyte transport processes at the molecular level and has defined the underlying basis for genetic disorders of intestinal transport. Advances in cellular biochemistry have shown how different second messenger pathways interact with one another, with transport proteins, and with the cytoskeleton to allow the intestinal epithelium to respond appropriately to changes in the extracellular environment. Enhanced knowledge of epithelial development and the structure of tight junctions has increased the understanding of cellular diversity and of how the epithelium acts as a barrier to lumenal toxins. At the whole-tissue level, our knowledge of intercellular communication between the mucosal immune system, the enteric nervous system, and the intestinal epithelium is growing constantly. This chapter therefore emphasizes molecular mechanisms of intestinal epithelial ion transport processes as well as the intracellular and extracellular factors involved in their regulation.

The intestinal epithelium

Structural properties of epithelial cells

The epithelium is a continuous monolayer of cells that lines the entire lumen of the intestine. The primary functions of the epithelial layer are essentially twofold. First, it must form an effective barrier to prevent the entry of harmful substances, such as pathogens and toxins, from the intestinal contents into the systemic circulation. Second, the epithelium transports ions, nutrients, and fluid to and from the intestinal lumen. Both of these epithelial functions are

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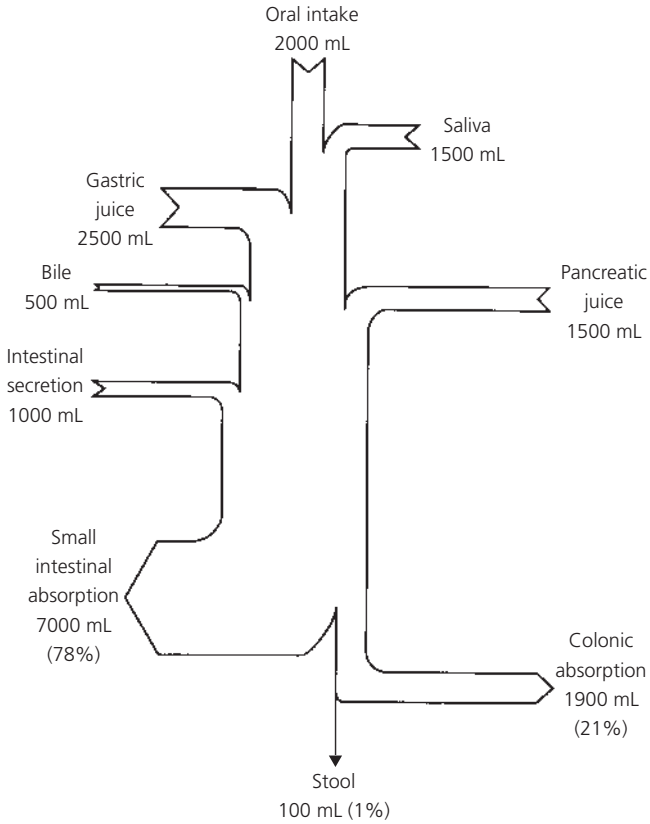

Figure 14.1 Daily water balance in the healthy human gastrointestinal tract. The amount of oral intake varies between individuals depending on the types of meals taken. Note that even in health there is a significant secretory flux of fluid from the intestine (1000 mL). The small intestine is responsible for absorbing almost 80% of the daily fluid load, largely in association with nutrient uptake. The colon absorbs most of the remaining fluid, with an efficiency of about 95%, leaving only about 1% of the daily fluid load to be lost to the stool. Reprinted from Barrett KE, Dharmsathaphorn K. Transport of water and electrolytes in the gastrointestinal tract: physiological mechanisms, regulation and methods for study. In: Narins RG (ed.). Maxwell and Kleeman's Clinical Disorders of Fluid and Electrolyte Metabolism, 5th edn. New York: McGraw-Hill, 1994:493, with permission from McGraw-Hill.

closely integrated in that vectorial transport of fluid and solutes cannot occur when barrier function is compromised. The capacity of the intestine to absorb and secrete fluid and solutes is greatly enhanced by the massive surface area of the epithelial layer, which is amplified by folds, villi, and microvilli.

Two essential features of epithelial cells that enable them to perform their barrier and transport functions are the ability to form intercellular tight junctions with one another and the ability to develop a functionally polarized phenotype.

Tight junctions

Toward the lumenal pole of epithelial cells are found the apical junction complexes, which comprise tight junctions and adherens junctions. These two structures are involved in the generation of barrier function and are therefore critical for the development of the epithelial transport phenotype. Adherens junctions are the more basally located of the two structures and are characterized by a high level of E-cadherin. The adherens junction is believed to be important in the initial development of cell–cell contacts through the formation of homotypic E-cadherin bonds between neighbouring cells [6]. The formation of cell–cell contacts initiates the development of tight junctions, which constitute the pores through which fluid and solutes must pass when following the paracellular route. At the ultrastructural level, freeze-fracture techniques reveal that tight junctions consist of a dense network of protein strands. In general, the number of strands constituting the tight junction is directly related to the permeability of the pore [7–10]. Tight junction permeability is not constant along the length of the intestinal tract and generally decreases aborally. Thus, the epithelium of the proximal small intestine is considered to be leaky, whereas that of the colon is considered to be tight.

Since the mid-1990s there have been great advances in our understanding of the molecular architecture of tight junctions (Fig. 14.2). Transmembrane components of the tight junctions include occludin, claudins, and junctional adhesion molecules (JAMs). Whereas the expression of occludin and JAMs is relatively constant throughout the intestinal tract, claudins are differentially expressed along both the jejunocolonic and crypt–villus axes [11]. It is generally accepted that the claudins constitute the barrier-forming components of tight junctions and are responsible for dictating the size and ionic permeability of the paracellular pores [12]. The claudin family comprises at least 24 proteins, each of which has four transmembrane domains and two extracellular loops. The extracellular loops differ in their charge-carrying amino acids and thus differentially restrict the passage of some ions over others. As several different claudins can be expressed within individual tight junctions, it is their combined permselectivity that ultimately determines the overall ionic permeability of the paracellular pathway [8]. Occludin is structurally similar to the claudins in that it has two extracellular loops that interact with partners on adjacent cells to form homotypic intercellular bonds. The precise function of occludin remains unclear; however, as gene deletion of the protein in mice does not alter tight junction structure or function, it likely plays a more important role in signaling than in tight junction biogenesis [13]. The JAM family is structurally unrelated to claudins and occludin. Three members of the JAM family have been identified and designated as JAM-A, JAM-B, and JAM-C [14–16]. The JAM proteins are single membrane-spanning proteins. Their extracellular N-terminal domains contain two immunoglobulin (Ig)-like repeats that can form homo- or heterotypic bonds with partner JAM proteins on other cells. Although JAMs are clearly important in regulating the

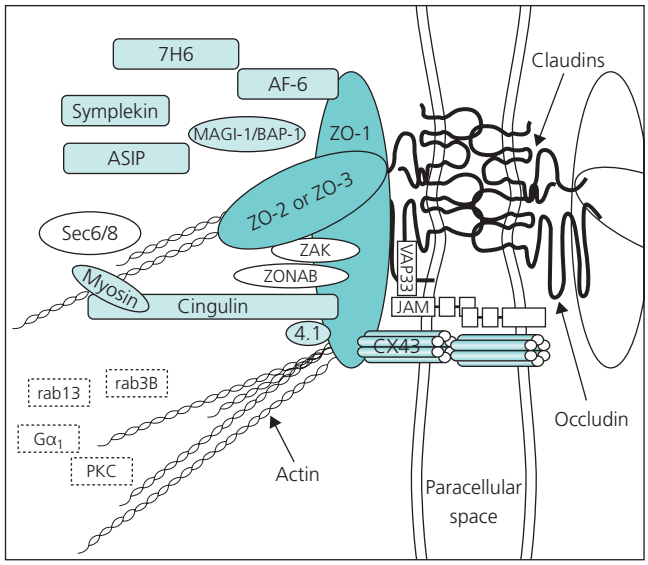

Figure 14.2 Molecular architecture of epithelial tight junctions. The transmembrane proteins occludin, the claudins, and junctional adhesion molecules (JAMs) are the pore-forming constituents of the tight junction. Each of these proteins form extracellular homotypic contacts with partners on neighboring cells. It is thought that the claudins, of which there are multiple isoforms, confer ionic selectivity on the tight junction. On the intracellular face of the tight junction, claudins and occludin associate directly with the zonula occludens (ZO) proteins, ZO-1, ZO-2, and ZO-3. Through several motifs that allow protein–protein interactions, the ZO proteins are, in turn, associated with the cellular actin cytoskeleton and a variety of intracellular signaling proteins that are involved in the modification of tight junction permeability. ASIP, atypical PKC isotype-specific interacting protein; BAP-1, brain-specific angiogenesis inhibitor (BAI)-associated protein; PKC, protein kinase C; VAP, vesicle-associated membrane protein (VAMP)-associated protein; ZONAB, ZO-1-associated nucleic acid-binding protein. Reprinted from Mitic LC, Van Itallie CM, Anderson JM. Tight junction structure and function: lessons from mutant animals and proteins. Am J Physiol Gastrointest Liver Physiol 2000;279:G250, with permission from the American Physiological Society.

sealing and permeability of tight junctions, the mechanisms remain unclear [17]. They may act by recruiting and tethering other regulatory proteins to the tight junction. Interestingly, JAM proteins are not only expressed in epithelial tight junctions but are also found on circulating leukocytes. Thus, JAM proteins are responsible for the cell–cell adhesion processes that are necessary for leukocyte migration through epithelial junctions in conditions of inflammation [18,19]. JAM proteins appear to be members of a larger subfamily of proteins within the Ig superfamily known as the CTX-like proteins [20]. Other members of this family have been identified in tight junctions, including the Coxsackie virus and adenovirus receptor (CAR) [18,21].

Tight junctions are dynamic structures that have the capability to open and close, thereby regulating the extent of fluid flow through the paracellular route. The transduction of regulatory signals to and from tight junctions occurs through a complex network of signaling proteins that are associated with the tight junction structural proteins, namely occludin, claudins, and JAM proteins. Each of these structural proteins contains motifs that facilitate either direct or indirect interactions with cytosolic proteins. Perhaps the most studied of these are the zonula occludens (ZO) proteins, which belong to a class of proteins called the membrane-associated guanylate kinase homologues (MAGUKs). In addition to a guanylate kinase domain (which is inactive), these proteins contain several motifs involved in protein–protein interactions and serve as important scaffolds that link the structural proteins of tight junctions with intracellular signaling molecules and the cytoskeleton [22]. Interestingly, ZO proteins also contain nuclear sorting signals and they colocalize with transcription factors both within tight junctions and the nucleus [23,24]. In cultured epithelial cells, the distribution of ZO1 and ZO2 changes from the nucleus in subconfluent cells to the tight junction in confluent cells, implying an important role for these proteins in coordinating epithelial barrier function with growth and differentiation.

Tight junction permeability is closely coordinated with ion and nutrient transport processes, allowing optimal synchronization of water and solute transport. Thus, increases in Na^+/nutrient-coupled transport leads to an opening of tight junctions and a concomitant increase in fluid absorption [25–27]. Conversely, increases in Cl^- secretion promote epithelial barrier function in a porcine model of intestinal ischemia [28]. This effect is specific for Cl^- secretion through CLC-2 chloride channels, which are colocalized with the tight junctions in injured epithelial cells [29]. Other factors that can induce alterations in tight junction permeability include cytokines, growth factors, neurotransmitters, and hypoxia [30,31].

Bacteria and their toxins also have the capability to interact with and disrupt tight junction function. This can be achieved either through direct alterations in the expression and function of structural proteins within the junctions, through alterations in the cellular cytoskeleton, through the induction of signaling cascades, or through the production of inflammatory cytokines [32–37]. Whatever the mechanism involved, the opening of tight junctions in response to colonization of the epithelium by pathogenic bacteria leads to an increase in paracellular permeability with a concomitant increase in fluid loss and the onset of diarrhea. In addition to bacteria, many other microorganisms, including viruses and protozoans, can disrupt tight junctions leading to the onset of diarrhea [38–40]. Conversely, probiotics, which are commensal bacteria normally found in the intestine and which have therapeutic actions in intestinal disease, have been shown to promote epithelial barrier function and to restore normal transport function [41,42].

In summary, tight junctions are essential for maintaining both the intestinal epithelial barrier function and the osmotic gradients required for active transport processes. Consequently, the opening of tight junctions, whether in response to physiological or pathophysiological stimuli, can have significant consequences for intestinal physiology, as the increase in the permeability of the paracellular pathway compromises the barrier to toxigenic substances, nutrients, electrolytes, and water.

Functional polarity

For epithelial cells to transport ions in a vectorial fashion, they must be able to differentially express specific transport proteins at the apical and basolateral poles of the cells. For example, for Cl^- secretion to occur, an entry pathway for the ion must exist in the basolateral domain of the cell, with an exit pathway in the apical membrane. Conversely, Na^+ absorption requires an entry pathway in the apical side of the cell, with an exit pathway in the basolateral membrane. Functional polarity is important not only for the appropriate distribution of transport proteins but also for the appropriate localization of receptors for hormones, neurotransmitters, and other mediators that regulate epithelial transport processes. Most receptors for such regulatory factors are expressed on the basolateral side of the intestinal epithelium, which is not surprising considering the close association of the basolateral domain with cells of the mucosal immune system and the enteric nervous system. Conversely, receptors for regulatory factors in the lumen (e.g., bacterial toxins, guanylin) are expressed on the apical pole of the cell. Some receptors are expressed bilaterally, such as those for kinins and adenosine. The apical expression of these receptors likely is important when leukocytes migrate through the tight junctions to form crypt abscesses.

Significant advances have been made in the understanding of the mechanisms that regulate the development of cell polarity. [43,44]. The process is initiated by cell–cell adhesion and is reinforced by the formation of homotypic E-cadherin bonds at the point of contact between two cells. It is around this "landmark" in the cell membrane that the structural proteins and machinery for the development of cell polarity are assembled [44]. The clustering of cadherin in the membrane leads to the recruitment of catenins, which in turn leads to changes in the actin cytoskeleton at the site of cell adhesion. The membrane cytoskeleton is formed into which is associated a scaffold of proteins, such as fodrin, ankyrin, and SNAREs (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors), which functions to direct proteins to the appropriate cellular domain. At the same time, the tight junction is assembled and acts as a physical fence, maintaining separation between the forming apical and basolateral domains [43,44].

The signals that direct the sorting of newly synthesized proteins to the apical or basolateral domain of the cell are becoming increasingly well understood. Some proteins, such as the Na^+,K^+-ATPase, have a high affinity for docking proteins within the scaffold that develops at the site of cell–cell contact; thus scaffolds direct the proteins to the appropriate domain [45]. However, most proteins are sorted within the trans-Golgi network into vesicles that are destined for either the apical or the basolateral domains. Signals directing proteins to the basolateral side of the cell are typically located in the cell's cytoplasmic domains and often contain a critical tyrosine residue within highly conserved amino acid sequences. Such signals induce a certain conformation within the protein that targets it to vesicles bound for the basolateral side of the cell [46]. Signals directing proteins to the apical side of the cell are generally found within their transmembrane or extracellular domains. One such signal is the glycosylphosphatidylinositol anchor that serves to cluster proteins into microdomains or rafts enriched in glycolipids and cholesterol [47]. These rafts eventually fuse, partition from the Golgi network, and travel to the cell surface [48]. N- and O-linked glycans have also been identified as important apical sorting signals [49]. Posttranslational modification of proteins by the addition of glycan side chains is determined by the relative expression and specificities of the glycosyltransferases and glycosidases within each cell, which, in turn, is dependent on cell type and differentiation state. Thus, glycosylation offers great potential for the diversification of protein sorting signals in cells from different organs and at different stages of differentiation.

Once proteins have been sorted into vesicles, they are trafficked to the membrane cytoskeleton at the site of cell–cell adhesion. Specific targets, such as SNAREs, exist that direct the vesicles to either the apical or basolateral side of the structure [44]. Transport vesicles express v-SNAREs on their surface that recognize t-SNAREs at the target membrane. Different t-SNAREs, namely syntaxin 3 and syntaxin 4, direct vesicles to the apical and basolateral membranes respectively [50]. Another target involved in directing transport vesicles is the Sec6/8, or exocyst, complex [51]. This protein complex is recruited to sites of cell–cell adhesion during polarization and is involved in targeting vesicles to the basolateral cell domain. Once proteins are resident in the appropriate membrane, their movement between the apical and basolateral domains is restricted by the cytoskeletal anchoring mechanism and by the fence function of tight junctions.

Epithelial organization and diversity

Although all epithelial cells display the common characteristics of functional polarity and the ability to generate tight junctions, cell type and function varies widely throughout the intestine. This diversity is apparent along the length of the intestine (the jejunocolonic axis) and also among the cells that constitute an individual crypt and villus unit (the crypt–villus axis).

Crypt–villus axis

The mucosa of the small intestine is organized into villi, which are finger-like projections that extend into the lumen and which surround deep invaginations into the mucosa, termed the crypts of Lieberkühn. The transporting epithelial cells, or enterocytes, are the most common cells of the epithelium, and it is these cells that perform the absorptive and secretory functions of the intestine. Although the spatial segregation of transport functions is less strict than was previously thought, fluid secretion predominantly occurs in the crypt region, whereas absorption mainly occurs across the villus cells (or surface cells of the colon) [52,53]. Other epithelial cells located along the crypt–villus axis regulate enterocyte function. Goblet cells in the crypt region secrete mucus that lubricates and protects the epithelial surface [54]. Intestinal fluid and electrolyte transport is coordinated with mucus secretion, such that many of the neuroimmune mediators that promote electrolyte and fluid secretion across enterocytes also induce mucus secretion from goblet cells [55]. Paneth cells are located toward the base of the crypts. These cells play an important role in host defense by secreting antibacterial peptides, such as lysozyme and defensins [54,56,57]. Interestingly, cryptdins, which belong to the family of β-defensins, can form anion channels in the apical membrane of enterocytes, thereby eliciting Cl^- secretion. Thus, Paneth cells regulate epithelial fluid transport, and thereby crypt hydration, in a paracrine fashion [58]. Enteroendocrine cells are found rarely, interspersed between enterocytes. At least 14 different populations of enteroendocrine cells have been distinguished by their chemical content, with the 5-HT-containing enterochromaffin cells being the most prominent [59]. The primary function of enteroendocrine cells is to sense or "taste" the lumenal environment to detect changes in intralumenal nutrient content, pH, and distention, and to transduce these signals to the sensory afferents of the enteric nervous system and to the enterocytes, thereby eliciting appropriate changes in transport function [60].

All four epithelial cell types are derived from common stem cells anchored toward the base of the crypts in an area known as the stem cell niche [61]. Stem cells give rise to proliferating progenitor cells whose offspring migrate upward toward the villus, except for Paneth cells, which migrate toward the crypt base. As the cells migrate they proliferate and differentiate into the different epithelial cell populations. The factors that regulate epithelial cell growth, migration, and differentiation are complex and not fully understood. However, of the several partially described signaling mechanisms, the Wnt/β-catenin and EphB signaling pathways appear to be particularly important [62–64]. As enterocytes migrate upward along the crypt to the villus tip they differentiate from a secretory to an absorptive phenotype. After 4–7 days they reach the villus tip and, as they detach and are sloughed off into the intestinal lumen, they undergo a specific form of apoptosis termed anoikis [65].

Jejunocolonic axis

Along its entire length from the duodenum to the colon, the intestinal epithelium absorbs and secretes fluid and electrolytes. However, the contents of the lumenal environment change dramatically along the length of the gut and, as a result, epithelial cells from different regions display marked differences in their structure and function. For example, although anion secretion occurs along the entire intestinal tract, there is an aboral gradient of the ratio between HCO_3^- and Cl^- secretion, with HCO_3^- secretion being highest in the proximal small intestine to neutralize gastric acid entering from the stomach. Similarly, mechanisms of Na^+ absorption differ along the intestine [66]: in the proximal small intestine, Na^+ absorption occurs predominantly through nutrient-coupled pathways; in the proximal colon, Na^+ absorption is mediated by electroneutral transport; and, in the distal colon, electrogenic Na^+ absorption through the epithelial Na^+ channel predominates [67,68]. The epithelium not only displays regional heterogeneity in terms of electrolyte transport but also in the transport of nutrients, vitamins, minerals, bile acids, and drugs [69]. Accordingly, epithelial cells in different intestinal segments express distinct repertoires of transport proteins on their surfaces [70–73]. Regional differences in the expression of transmembrane structural components of the apical junction complex are also known to occur along the jejunocolonic axis and this likely underlies segmental differences in transepithelial permeability along the intestine [11,74].

Principles of epithelial transport

Transepithelial and housekeeping transport

Substances may traverse the intestinal epithelium by multiple routes. Transcellular processes require entry of electrolytes across one membrane barrier, transit through the cell cytosol, and exit across the opposite membrane. The functional polarity of epithelial cells is the basis for this net (vectorial) movement of electrolytes through the asymmetrical distribution of different membrane transport proteins in the opposing apical and basolateral membranes of the cells. Paracellular transport is the passive flux of electrolytes and water across tight junctions. Paracellular flux occurs entirely extracellular to the epithelial cells, and can only produce vectorial transport as a dissipation of the transepithelial chemical and electrical gradients established by active transcellular transport.

Types of transmembrane transport

Charged species such as ions cannot traverse the lipid core of the plasma membrane to any appreciable degree. This is vital to life, because the regulation of cellular biochemistry is dependent on the tight control of intracellular ionic composition. Therefore, specialized proteins are inserted into the plasma membrane to mediate and regulate ion movement.

Ions can cross the membrane by way of these proteins, with either the consumption of cellular energy (*active transport*) or by flowing down existing electrical and/or chemical gradients (*passive transport*). Overall, transepithelial transport involves a combination of both active and passive transport mechanisms. Ultimately, all of the net transepithelial transport mechanisms discussed in this chapter require at least one active transport step to drive vectorial transport. In these cases, net passive transport of a substance through the paracellular or transcellular route occurs only in response to transepithelial electrochemical gradients established by the active transport processes.

In addition to the distinction between active and passive transport across a membrane, all transport reactions can also be grouped into one of two classes: electrogenic or electroneutral. *Electrogenic* transport moves net charge across the membrane by the flux of either single ions (e.g., ion channels) or combinations of transport substrates whose charges are unequal (e.g., Na^+/glucose cotransporter). Because no aqueous solution can have unequal amounts of cations and anions, electrogenic transport requires compensatory flux by another electrogenic transport process to maintain electrical neutrality. Very often, electrical neutrality is maintained by compensatory paracellular transport of an oppositely charged ion through the tight junctions. *Electroneutral* transporters also mediate a net flux of ions, but do so without moving net charge across the membrane during a transport cycle. Electroneutral transporters may mediate an exchange of equal charges across the membrane (e.g., Na^+/H^+ exchanger) or a cotransport of equal and opposite charges (e.g., $Na^+/K^+/2Cl^-$ cotransporter). Importantly, the electrical gradient across the membrane is not a driving force affecting electroneutral carriers, and so the rate and direction of transport by electroneutral carriers is determined solely by the transmembrane chemical gradients of the transported ions. As discussed later (see section on transepithelial electrolyte transport), combinations of different transport proteins can also result in overall transepithelial transport that is either electrogenic or electroneutral.

Fluid transport

Physiology of water flux

Water is crucial for physiological processes, and the amount of water in the intestinal lumen is closely regulated. A large amount of water is secreted by various gastrointestinal organs to facilitate digestive and other intestinal processes (see Fig. 14.1). The daily fluid load varies according to the amount and composition of meals but it approximates 9 L. In healthy humans, 65%–80% of the secreted or ingested water is absorbed in association with nutrient and electrolyte absorption in the small intestine and so the colon receives only 1500–2000 mL. The colon absorbs most of this remaining fluid with high efficiency so that normally only about 100 mL is excreted in the stool.

Molecular mechanisms of water transport

Researchers are still developing an understanding of how water crosses the intestinal epithelium. One general principle, however, is that water always flows across membranes in response to electrolyte and/or osmolyte fluxes. The net movement of electrolytes causes local accumulation of osmolytes, which drives compensatory water diffusion to balance osmolarity. In most cases, water molecules are moved into and out of the lumen by passive processes requiring no further input of cellular energy beyond that required to cause electrolyte transport. There are multiple routes for passive water diffusion across epithelia. If the actively transported electrolytes accumulate in the small extracellular spaces between adjacent epithelial cells (during absorption) or in the gut lumen (during secretion), there is an osmotic driving force for water to equilibrate through the tight junctions. This is believed to be the mechanism for fluid absorption in the proximal small intestine. Conversely, if the electrolytes accumulate intracellularly, there is a driving force for water to equilibrate across the cell membrane, which provides a transcellular route for water transport. The transcellular route is predicted to be more important in tight epithelia (e.g., the distal colon), because ion flow through the tight junctions is restricted in this setting.

Similarly to ions, water has difficulty crossing lipid membranes; therefore, membrane proteins must enhance water transport across these barriers. Attention has been focused on two classes of proteins that increase water permeability across intestinal membranes: aquaporins (AQPs) and Na^+-dependent solute transporters. The AQPs are a family of water-selective channels in the plasma membrane. Since their initial discovery [75], six AQP isoforms have been reported in the intestine, although only three have been suggested to contribute to intestinal water transport [76]. AQP3 is abundant in the surface/villus cells of the colon, jejunum, and ileum, although whether AQP3 is apical [77] or basolateral [78] remains controversial. In contrast, AQP4 is a basolateral protein in the small intestine and colon, but its predominant location – crypt cells or surface/villus cells – is uncertain [78,79]. The AQP8 isoform has also been found in surface/villus cells of the jejunum and colon [78]. The only functional evidence that links any specific isoform to intestinal water transport comes from AQP4 knockout mice, which show a modest (30%–40%) decrease in water absorption by the proximal colon [79]. This highlights the existence of redundant pathways for intestinal water flux under normal circumstances. Nevertheless, evidence suggests that altered aquaporin activity or localization may contribute to some forms of infectious diarrhea [80].

Another potential route for transcellular water flux is through Na^+-dependent solute transporters. Some authors have argued that the Na^+/glucose cotransporter SGLT1 can move water molecules across the membrane each time it transports two Na^+ ions and a single glucose molecule into

the cell, possibly in the form of molecules that hydrate the cotransporter glucose-binding site [81]. Results suggest that other cotransporters also enhance water fluxes [82]. Water may be a transport substrate of SGLT1 even under isotonic conditions [83], although evidence suggests that at least part of the SGLT1-stimulated water flow is due to microscopic osmotic gradients driving conventional passive water diffusion [84]. Independent of the precise model, it has been estimated that the absorption of up to 5 L of water per day may be mediated by SGLT1 alone [81].

In the intestine, water transport is also regulated indirectly by the regulation of electrolyte transport. When electrolytes and osmolytes remain in the intestinal lumen (e.g., lumenal lactose in lactase-deficient subjects, or during active electrolyte secretion), lumenal hyperosmolarity decreases water absorption. The opposite is also true: when molecules are absorbed and leave the lumen, they increase water absorption. Use of this osmotic driving force constitutes the basis for oral rehydration solutions, in which a low osmolarity substrate (e.g., starch) in the lumen is digested (e.g., to glucose) and the products are avidly absorbed (e.g., by SGLT1), thus stimulating water absorption.

Electrolyte transport proteins

This section describes the building blocks of epithelial transport – individual membrane transport proteins. Different combinations of these transport molecules are expressed in individual cells to perform specific electrolyte transport events. Many electrolyte transport proteins have been cloned, providing unprecedented information about their structures and function, including the identification of mutations that underlie inherited diseases of intestinal transport. The electrolyte transport proteins known to exist in gastrointestinal epithelia and their participation in transepithelial electrolyte transport are summarized in Table 14.1. The following sections emphasize cloned transport proteins that are expressed in the intestine. Because each transport protein is a member of a gene family of related protein isoforms, it is sometimes unknown which isoforms participate in particular transepithelial intestinal electrolyte transport mechanisms. Consequently, some proposed mechanisms of transepithelial transport remain more speculative than confirmed.

ATPase pumps

A transport ATPase provides the necessary driving force for net transepithelial ion transport to occur in mammalian cells. Among the known transport ATPases, Na^+,K^+-ATPase and H^+,K^+-ATPase are the best understood and have well-established roles in intestinal electrolyte transport. These proteins have related sequences (60%–70% homology) and are members of the P-type ATPase multigene family, so named because all members of this family become phosphorylated during part of the transport cycle.

Na^+,K^+-ATPase

In the presence of Mg^{2+}, the Na^+,K^+-ATPase catalyzes the efflux of three Na^+ ions from the cell and the uptake of two K^+ ions at the expense of hydrolyzing one ATP molecule per cycle [85]. Because more cation is pumped out than is replaced, the Na^+ pump is electrogenic and generates a negative intracellular electrical potential. Na^+,K^+-ATPase is localized basolaterally and is expressed in all intestinal epithelial cells. It acts to maintain a low intracellular Na^+ concentration and a high intracellular K^+ concentration. The Na^+ electrochemical gradient is then used as a driving force for Na^+ influx through Na^+ channels or through the many secondary active cotransporters and exchangers for which Na^+ is required (see Figs 14.3, 14.4, and 14.5). Inactivation of Na^+,K^+-ATPase with cardiac glycosides (e.g., ouabain) inhibits all these active transport mechanisms in the intestine.

The Na^+ pump exists in the membrane as a heterodimer of α- and β-subunits, and the α1/β1 isoforms of these subunits predominate in the intestine [86]. The α-subunit is an integral membrane protein with at least eight transmembrane segments, and it contains the ouabain binding site and all ion binding and catalytic sites. The β-subunit has no known role in mediating ion transport but is an integral membrane protein essential for chaperoning the heterodimer through correct assembly and membrane targeting [87]. A newly described family of proteins known as FXYD proteins has been shown to interact with the Na^+ pump in a tissue-specific manner, including in the intestine, significantly increasing its activity [88].

H^+,K^+-ATPase

In several species the apical membranes of cells in the distal colon contain H^+,K^+-ATPase activity. Similar to the gastric H^+,K^+-ATPase to which it is related, the colonic form mediates cellular efflux of H^+ into the gut lumen in exchange for K^+ uptake, with hydrolysis of ATP providing energy [89]. The activity of this pump is variably inhibited by the Na^+,K^+-ATPase inhibitor ouabain and the gastric H^+,K^+-ATPase inhibitor SCH28080, suggesting that the colonic enzyme shares common structural features with both of these pumps [90–92]. One reason for the variability in inhibitor responses is that a second, ouabain-insensitive K^+-ATPase probably exists [90]. Moreover, because the only cloned isoform of the colonic H^+,K^+-ATPase is not inhibited by SCH28080, there is some controversy about whether it is the predominant apical isoform [92]. Antibodies detect H^+,K^+-ATPase in both the surface cells and crypt cells, although the cloned isoform may be predominantly expressed in the surface cells [92].

Unlike the Na^+,K^+-ATPase, the colonic H^+,K^+-ATPase does not play a dominant role in regulating intracellular levels of

Table 14.1 Key epithelial transport proteins and their participation in transepithelial electrolyte transport

Transport protein	Polarity	Genes	Transepithelial function	Notes
ATPase pumps				
Na^+,K^+-ATPase	Basolateral	α1-subunit: *ATP1A1*; β1-subunit: *ATP1B1*	All active transport	α1β1 dimer is the only intestinal epithelial isoform
H^+,K^+-ATPase	Apical	α-subunit: *ATP4A* *RATATPASEZ*?	Colonic K^+ and SCFA absorption	β-subunit needed for function
Exchangers and cotransporters				
Na^+/glucose cotransporter	Apical	*SGLT1* (*SLC5A1*)	Solute-coupled Na^+ uptake	Example of Na^+-coupled cotransporter
H^+/peptide cotransporter	Apical	*HPEPT1* (*SLC15A1*)	Proton-coupled osmolyte absorption	Example of proton-coupled cotransporter
H^+/monocarboxylate cotransporter	Apical and basolateral	*MCT1* (*SLC16A1*)	SCFA absorption?	SCFA transporter
$Na^+/K^+/2Cl^-$ cotransporter	Basolateral	*NKCC1* (*SLC12A2*)	Cl^- and K^+ secretion	
Na^+/H^+ exchangers	Apical	*NHE3* (*SLC9A3*)	Small intestinal electroneutral Na^+ absorption	
	Apical	*NHE2* (*SLC9A2*)	Electroneutral Na^+ absorption?; HCO_3^- secretion	Potential role in colonic Na^+ absorption
	Basolateral	*NHE1* (*SLC9A1*)	HCO_3^- secretion	Ubiquitously expressed; cellular pH regulator
Cl^-/HCO_3^- exchangers	Apical	*SLC26A3* (*DRA*), *SLC26A6* (*PAT1*), *AE1*?, *AE4*?	Electroneutral Cl^- and K^+ absorption; HCO_3^- secretion	AE1 controversial; AE4 only involved in HCO_3^- secretion?
	Basolateral	*AE2*, *AE3*?	Electroneutral Cl^- absorption?	AE3 controversial
Na^+/Cl^- cotransporter	Unknown	*NCCT* (*SLC12A3*)	Electroneutral Na^+ and Cl^- absorption?	Putative apical NaCl uptake mechanism
K^+/Cl^- cotransporter	Unproven	KCC1 (*SLC12A4*)	Electroneutral K^+ and Cl^- absorption	Basolateral route for KCl exit
Channels				
Na^+ channel	Apical	α-subunit: *SCNN1A*; β-subunit: *SCNN1B*; γ-subunit: *SCNN1G*	Electrogenic Na^+ absorption	Amiloride-sensitive channel; others may exist
Cl^- channels	Apical	*CFTR*	Cl^- secretion	cAMP-activated; also conducts bicarbonate
	Apical	*CLCA1*	Cl^- secretion?	Calcium-activated
	Apical	*CLC2* (*CLCN2*)	Cl^- secretion?	Outwardly rectifying; volume sensitive
	Endosomal	*CLC5* (*CLCN5*)	Cl^- secretion?	Outwardly rectifying
K^+ channels	Apical	unknown	K^+ secretion	
	Basolateral	*KCNQ1*, *RSK1* (*RPS6KA1*)	Cl^- secretion; electrogenic and solute-coupled Na^+ absorption	
HCO_3^- channel	Apical	unknown	HCO_3^- secretion	Likely CFTR

CFTR, cystic fibrosis transmembrane conductance regulator; SCFA, short-chain fatty acid.

its transported ions. Although cytosolic pH can be affected by the activity of the H^+,K^+-ATPase, other transporters (e.g., Na^+/H^+ exchangers) are likely to have a greater impact on the control of the resting cytosolic pH [93]. In the distal colon of the guinea pig, protons secreted by the colonic H^+,K^+-ATPase are important for stimulating uptake of lumenal short-chain fatty acids (SCFAs), facilitating absorption of SCFAs in their nonionized (i.e., protonated) form [94]. The colonic H^+,K^+-ATPase is also important for K^+ absorption, especially in infant animals who need to retain more K^+ than adults to avoid growth retardation [95].

Both an α- and β-subunit of the colonic H^+,K^+-ATPase have been cloned [96,97]. Coexpression of the α-subunit with either the cloned H^+,K^+-ATPase β-subunit or a heterologous β-subunit (either from gastric H^+,K^+-ATPase or Na^+,K^+-ATPase) is sufficient to reconstitute observable H^+,K^+-ATPase activity in the plasma membrane [98]. Evidence suggests that the β-subunit of the Na^+,K^+-ATPase may serve this role physiologically, as it associates with the H^+,K^+-ATPase α-subunit in the apical colonocyte membrane. [99]. The α-subunit is predicted to have multiple transmembrane-spanning segments [96], but no detailed mapping of functional sites has been reported.

Exchangers and cotransporters

Using this limited number of ATPase pumps, an abundant assortment of electrolytes and solutes are actively transported across the intestinal epithelium. This is possible because a large number of different carrier proteins convert the energy of the Na^+ or proton gradients into net transport of other electrolytes and solutes. In addition to these secondary active exchangers and cotransporters, the passive transporters play a key role in facilitating the movement of ions across the plasma membrane at faster rates than could be expected by simple diffusion.

Na^+/solute cotransporters

Many food-derived products including glucose, amino acids, and vitamins, as well as endogenous lumenal solutes such as bile acids, are taken up by specific Na^+-coupled cotransport proteins in the apical membrane of enterocytes. These cotransporters are the predominant route by which such diverse substances enter the body, and they are also responsible for driving the majority of Na^+ and water absorption after a meal. Most of these cotransporters are found in the small intestine, the main site of organic nutrient absorption.

Among the mammalian Na^+-coupled nutrient transporters, the intestinal Na^+/glucose cotransporter (SGLT1) was the first to be cloned and has been studied most extensively [100,101]. Each transport cycle of SGLT1 results in the uptake of two Na^+ ions and one glucose molecule; its electrogenic nature renders it sensitive to changes in membrane potential. SGLT1 consists of a single polypeptide chain with 14 transmembrane segments. Studies of chimeras between SGLT1 and a related cotransporter (SGLT2) clearly show that the C-terminal portion of SGLT1 contains the sugar-binding site [102]. Further, a naturally occurring missense mutation near the C-terminus (R499H) has been identified in a patient with glucose–galactose malabsorption; the mutant protein has a 10-fold lower sugar affinity than the wild-type protein but no alteration in Na^+ affinity [103].

Similar approaches are being used to evaluate the mechanisms of action of a number of cloned intestinal Na^+/amino acid, Na^+/bile acid, and Na^+/vitamin cotransporters [104]. These Na^+-coupled nutrient cotransporters are predicted to contribute to water and sodium absorption in the intestine; they are discussed in more detail in Chapters 19 and 20.

H^+/solute cotransporters

The intestinal proton-coupled peptide cotransporter (PEPT1) is expressed in the apical membrane of cells in the small intestine and can transport a wide variety of dipeptides and tripeptides. The cloned transporter is electrogenic, and the proton activation kinetics suggest a 1:1 stoichiometry of proton–dipeptide transport for uncharged peptide substrates [105]. From the standpoint of fluid and electrolyte absorption, the major contribution of PEPT1 is to stimulate water transport, in cooperation with the Na^+/H^+ exchanger NHE3, which supplies protons [106]. PEPT1 remains an enigma for transport physiologists because of its capacity to accommodate an extremely broad range of peptide substrates. The molecular basis of this promiscuity is not fully understood.

A cloned proton-coupled monocarboxylate cotransporter (MCT1) has been identified in the epithelial cell membranes of both the small and large intestines [107]. MCT1 functions to reproduce nonionic diffusion through the coupling of fluxes of monocarboxylate anions and protons [108]. The transporter is inhibited by α-cyanohydroxycinnamates, and lactate is a favored substrate [108]. Evidence suggests a physiological role for colonic MCT1 as a route for basolateral SCFA flux from cells as part of transcellular SCFA absorption [109]. In this regard, MCT1 may be most important physiologically in the transport of monocarboxylates that are too hydrophilic for efficient flux by nonionic diffusion through the lipid bilayer.

$Na^+/K^+/2Cl^-$ cotransporters

The $Na^+/K^+/2Cl^-$ cotransporter is present in the basolateral membrane of certain intestinal epithelial cells, and participates in electrolyte secretion. This cotransport is electroneutral because each transport cycle moves equal numbers of cations (one Na^+ plus one K^+) and anions (two Cl^-) into the cell. The $Na^+/K^+/2Cl^-$ cotransporter plays a key role in Cl^- secretion as it provides the predominant route for basolateral Cl^- uptake [110], and it also participates in K^+ secretion by providing a second route for basolateral K^+ uptake in addition to the activity of the Na^+,K^+-ATPase [111].

The $Na^+/K^+/2Cl^-$ cotransporter isoform NKCC1 is expressed in the basolateral membrane of human intestinal

epithelial cells, predominantly in the colon rather than the small intestine [112]. NKCC1 consists of a single polypeptide chain and has 12 putative transmembrane segments. It is inhibited by bumetanide and furosemide, which affect anion binding to the transporter [113].

A distinct Na^+/Cl^- (or $Na^+/K^+/Cl^-$) cotransporter has been proposed to exist in the apical membrane of enterocytes, as part of the electroneutral sodium absorptive mechanism. Similar apical cotransporters have been cloned from the kidney medulla where they participate in Na^+ reabsorption, and a highly homologous isoform is expressed in the human small intestine and colon [114]. Research implicates this transporter in the control of intestinal Ca^{2+} absorption, secondary to an interaction with a voltage-dependent channel for this cation [115].

Na^+/H^+ exchangers

Na^+/H^+ exchange activity is present in all segments of the small intestine and colon. It is defined as the tightly coupled uptake of one Na^+ ion in exchange for the efflux of a proton [116]. This electroneutral exchange reaction is important for intracellular pH regulation as well as transepithelial Na^+ absorption. Na^+/H^+ exchange is observed in both the apical and basolateral membrane [117,118].

Several isoforms of the Na^+/H^+ exchanger (NHE) gene family have been detected in gastrointestinal epithelia; they are characterized by their localization (membrane vs intracellular) and also by the extent to which the intracellular isoforms remain resident or traffic into and out of the plasma membrane [119]. Of the isoforms considered important for epithelial ion transport, NHE1 is present in virtually all cells of the body and is believed to be responsible for cellular pH regulation. NHE1 is found in the basolateral membrane of intestinal epithelial cells [120]. NHE2 and NHE3 are predominantly epithelial isoforms of the exchanger that are expressed in the apical membranes of intestinal cells [121]. NHE4 is also expressed in gastrointestinal tissues (predominantly stomach) but there is no evidence that it contributes to transport responses in the small or large intestines. Each epithelial NHE isoform has a distinct distribution of mRNA abundance in the small and large intestines, which varies among species [122]. There are also gradients of NHE isoforms and Na^+/H^+ exchange function along the crypt-to-villus and crypt-to-surface axes. Immunocytochemical analyses have shown that NHE1 is expressed at all sites along these axes [120] but that NHE3 is expressed preferentially on the villus in the small intestine and on the surface and in the upper one-third of the colonic crypts [123]. In contrast, functional apical Na^+/H^+ exchange activity has been observed along most of the ileal villus–crypt axis using isolated membranes and in colonic crypts using optical approaches to study crypts either in situ or in isolation [124,125].

All NHE isoforms have 10–12 predicted transmembrane segments with cytoplasmic C- and N-terminals [121]. Overall, NHE isoforms are 50%–60% homologous within a species, with the C-terminal cytoplasmic tail having the most sequence divergence. The C-terminal tail is important for second messenger regulation of NHE isoforms, and several portions of this domain have been identified as being crucial for mediating the effects of Ca^{2+}, calmodulin, cAMP, activation by intracellular protons, and growth factors [121,126,127]. Evidence also suggests that NHE regulation requires accessory phosphoproteins, because signaling pathways do not always alter phosphorylation of NHE proteins. As demonstrated by kinetic studies, all isoforms have different affinities for Na^+ and different inhibition constants for amiloride and other inhibitors, reflecting their different primary structures [121].

NHE2 and NHE3 are apical membrane proteins in intestinal cells [121] and therefore may contribute to Na^+ absorption. The relative roles of NHE2 and NHE3 in electroneutral Na^+ absorption in the intestine are still debated [119]. Mineralocorticoids increase the rate of ileal brush border Na^+/H^+ exchange in rabbits, which correlates with an increase in the abundance of NHE3 mRNA without an effect on mRNA for NHE1 or NHE2 [128]. Further, the dose-dependent inhibition of Na^+ and water absorption in dogs by dimethylamiloride is most consistent with NHE3 inhibition [129]. Finally, the patterns of second messenger regulation of Na^+ absorption and NHE3 expression are closely matched (i.e., inhibition by Ca^{2+} and stimulation by phorbol esters), but differ from the responses of NHE2 [121]. Conversely, in rabbit ileum, NHE2 and NHE3 each contribute about equally to basal Na^+ uptake in brush border vesicles [130]. Evidence further suggests that colonic NHE2 may play a role in regulating colonic NaCl absorption [131]. In rat proximal colon, NHE2 is the predominant contributor to basal Na^+ absorption [132], although the role of NHE3 seems more dominant in isolated membrane vesicles from the same tissue [133,134]. Overall, it appears that the role of NHE3 predominates, at least in mice, because NHE3 knockout mice have obvious diarrheal disease, whereas NHE2 knockout mice have no identified intestinal disorder [135,136]. This may result from compensatory up-regulation of another NHE activity in the NHE knockout mice, making the normal role of NHE2 more challenging to identify [137].

Cl^-/HCO_3^- exchangers

Cl^-/HCO_3^- exchange in the apical membrane acts in concert with Na^+/H^+ exchange to mediate electroneutral NaCl uptake in colonocytes and small intestinal cells [138]. Unlike the apical membrane Na^+/H^+ exchange, which is expressed predominantly in villus cells of the small intestine, Cl^-/HCO_3^- exchange appears to be equally prevalent in the apical membranes of both crypt and villus cells, suggesting that it contributes to other transport mechanisms in addition to NaCl absorption. In the colon, apical Cl^-/HCO_3^- exchange is observed predominantly in the surface cells, although Cl^-/OH^-

exchange remains relatively active in crypt membranes [139]. This and other kinetic evidence suggests that apical Cl^-/HCO_3^- and Cl^-/OH^- exchange may not be mediated by the same protein in either the small intestine or colon [139,140].

Molecular biological approaches have identified many candidate proteins that may perform the apical Cl^-/HCO_3^- exchange reaction. Multiple isoforms of the anion exchanger (AE) gene family (also known as solute carrier family 9 [SLC9]) are expressed in the small and large intestines. Among the three known AE2 transcripts (AE2a, AE2b, AE2c1), AE2a and AE2b are abundant in the intestine and, similar to most AE2 epitopes, are localized basolaterally [141,142]. Results have also suggested the presence of an apical AE1 isoform and a basolateral AE3 isoform in the intestines, but the presence of both of these remains highly controversial [142,143]. The AE4 isoform is involved in HCO_3^- secretion in both the stomach and duodenum [144].

More definite roles in intestinal Cl^- absorption and HCO_3^- secretion have been assigned to members of the SLC26 family of anion exchangers. One such transporter, SLC26A3, also known as DRA (down-regulated in adenoma), plays a key role as mutations in this transporter are responsible for congenital chloride diarrhea [145], a disease characterized by a Cl^--rich, acidic stool. The protein is known to be expressed in the apical membrane of colonocytes [146], and is a Cl^-, HCO_3^-, and divalent anion transporter [147,148]. *SLC26A3* mutations identified in patients with congenital chloride diarrhea do, in fact, lead to defective anion transport function [148]. In addition, SLC26A6, also known as PAT1 (putative anion transporter 1), has been characterized as a major contributor to anion exchange in the apical membranes of intestinal epithelial cells on the basis of studies in knockout mice [149]. As for the Na^+/H^+ exchangers, the relative roles of these proteins have yet to be fully determined and may be complicated by compensatory changes that occur when one is absent.

Other exchangers and cotransporters

Other intestinal electrolyte cotransporters have been cloned, including Na^+/PO_4^- and Na^+/SO_4^- cotransporters [150,151]. For phosphate, at least, these likely participate in a homeostatic axis linking bone, kidney, and intestinal transport processes to ensure appropriate levels of the circulating cation [152]. An intestinal K^+/Cl^- cotransporter has also been cloned [153]. This cotransporter provides a logical mechanism for electroneutral basolateral exit of K^+ and Cl^- from intestinal epithelial cells that absorb K^+ and Cl^- in an electroneutral fashion, and its expression increases during dietary potassium depletion [154].

Ion channels

An ion channel acts as a gated pore that is selective for certain ions. Open channels are passive pathways for electrolyte movement. *Gating* controls the amount of time that the pore spends in an open vs a closed configuration. A single open channel conducts a rapid flux of ions across the membrane with a characteristic *unitary conductance* (1 pS ≈ 500 univalent ions/ms). The conductance of a given channel is an intrinsic biophysical signature, whereas gating of epithelial channels is most frequently regulated by second messengers. Compared with ion channels in excitable cells, membrane voltage has only modest effects on gating of epithelial channels, although this should be distinguished from the large effect of membrane voltage on the magnitude of ionic currents across the open channel, a completely separate feature.

Na^+ channels

Apical Na^+ channels contribute to electrogenic Na^+ absorption in many distal colonic epithelia. Epithelial Na^+ channels are selective for Na^+ (> 10:1 vs K^+), have 5 pS unitary conductances, and are *inwardly rectifying* (i.e., they mediate cation influx more efficiently than cation efflux) [155]. Epithelial Na^+ channels are inhibited by nanomolar concentrations of phenamil and amiloride analogues, but the profile of inhibitor sensitivity is distinct from that of Na^+/H^+ exchangers [156]. Studies show that not all electrogenic Na^+ flux is equally sensitive to the same channel inhibitors. It is unclear whether this is due to different regulatory or structural states of the known epithelial Na^+ channel, or whether a second biochemically distinct and amiloride-insensitive Na^+ channel exists in some portions of the colon [157].

The amiloride-sensitive epithelial Na^+ channel (ENaC) is composed of three subunits, α, β, and γ, which share 35% homology [158]. Each subunit is an integral membrane protein predicted to span the membrane twice. Expression of the β- and γ-subunits is required to efficiently target the channel to the plasma membrane [158]. This is consistent with the observation that certain activating mutations in the β- or γ-subunit (see section on defects in electrogenic Na^+ absorption for a description of Liddle syndrome) cause increased expression of Na^+ channels in the membrane as well as increased activity of the expressed channels [159,160]. In contrast, a glycine near the N-terminal part of the α-subunit is involved in channel gating [161]. Although the predicted minimum configuration of the epithelial Na^+ channel is a heterotrimeric protein, it is likely that the native channel is more complex. Biochemical purification suggests that five or six polypeptides of widely different molecular weight are components of the channel. Some of these components may be differentially glycoslyated forms of the known subunits, but some of the peptides have been identified as regulatory proteins and cytoskeletal elements that modify channel function [157].

Cl^- channels

Apical chloride channels are essential components of Cl^- secretion. Cl^- secretion in all parts of the intestine and colon is controlled predominantly by the ability of specific second messengers to regulate channel gating and localization.

However, because there are multiple Cl^- channel types in intestinal epithelial cells, it can be challenging to assign a functional role to individual proteins.

A cAMP-activated channel in the apical membrane is known to participate in hormone-stimulated Cl^- secretion and is encoded by the cystic fibrosis transmembrane conductance regulator gene (*CFTR*), which is responsible for cystic fibrosis (see section on defects in Cl^- secretion) [162]. The normal CFTR channel is selective for $Cl^- > Br^- > I^-$, has an 8–10 pS unitary conductance, and is *nonrectifying* or *linear* (i.e., it conducts ions with equal efficiency in either an inward or an outward direction). The CFTR protein is also able to mediate the conductance of other ions (notably HCO_3^-)[163], it participates in a subset of cAMP-regulated apical membrane endocytosis and exocytosis events [164], and it regulates the activity of other Cl^- and Na^+ channels [165,166]. The CFTR channel is a single polypeptide with 12 membrane-spanning domains, two cytoplasmic nucleotide-binding domains, and a "regulatory" (R) domain that contains most of the phosphorylation sites. CFTR Cl^- channel gating is complex, involving coordinated phosphorylation of multiple R-domain serine residues and ATP hydrolysis at, and interaction between, the two nucleotide-binding domains [167].

In healthy intestine, agonists acting through cAMP or Ca^{2+} stimulate Cl^- secretion. However, in patients with cystic fibrosis, neither cAMP nor Ca^{2+} are able to elicit Cl^- secretion [168]. This is in contrast to the airway epithelia of patients with cystic fibrosis, in whom a Ca^{2+}-activated Cl^- channel functions in the absence of normal CFTR [169]. The reasons underlying the failure to detect such a Ca^{2+}-activated channel in the intestines of patients with cystic fibrosis is not yet understood at a molecular level, but could involve the lack of an appropriate channel protein, a defect in the ability of CFTR to regulate another anion channel, or the existence of negative regulatory mechanisms in the intestinal epithelium (see section on the regulation of electrolyte transport).

Molecular cloning has revealed a number of other candidate Cl^- channels. CLC2 and CLC5 are cloned Cl^- channels that are expressed in the intestine [170–172]. CLC5 is particularly intriguing because it is expressed only in kidney and colon, has a permselectivity of $I^- > Cl^-$, is outwardly rectifying, but is not activated by cAMP [170]. Because CLC5 is located in the endosomes of intestinal epithelial cells [172], it may be inserted into the plasma membrane on receiving the appropriate regulatory signals. CLC2 is expressed in the apical membrane of intestinal epithelial cells and may contribute to Cl^- secretion [173]. As CLC2 is apparently operational in CFTR knockout mice, it is unlikely to be one of the proteins that requires CFTR to function [173]. CLC channels are activated by cell swelling and, at a minimum, probably participate in the ion efflux necessary for cell volume regulation. The CLC2 channel has attracted considerable attention as the presumed target of a channel-opening drug known as lubiprostone, which is marketed for the treatment of constipation [174,175]. This implies that intentional activation of intestinal Cl^- secretion may provide additional hydration of intestinal contents that could be beneficial in patients with altered motility.

K^+ channels

Potassium channels in the apical membrane of epithelial cells are involved in K^+ secretion, and K^+ channels in the basolateral membrane are involved in Cl^- secretion. K^+ channels are also involved in electrogenic Na^+ absorption and Na^+-coupled solute absorption, but the polarity of K^+ channels involved in these processes is less certain.

The characterization of apical K^+ channels has come predominantly from biophysical and physiological studies. The presence of apical K^+ channels has been inferred indirectly by adding lumenal K^+ channel blockers (e.g., barium and tetraethylammonium [TEA]) and monitoring the effects on global electrical properties of the rat colonic mucosa [176]. Treatment with aldosterone increases the apical K^+ conductance, suggesting an increase in K^+ channel activity or number [177]. One report identified an apical K^+ channel with a unitary conductance of 210 pS in rat distal colon as a candidate for the predominant apical K^+ conductance [178].

Basolateral K^+ channels have been studied more extensively. Patch clamping has identified a basolateral channel in human and rat colonic crypts that is selective for K^+ (49:1 vs Na^+) and which has a unitary conductance of 23 pS [179]. The open probability of this channel was increased four- to tenfold by cAMP or micromolar Ca^{2+}, and the channel was blocked by quinidine and diphenylamine carboxylate (DPC) but not by TEA [179,180]. The second messenger sensitivity of the 23-pS channel suggests that it contributes to both Ca^{2+}- and cAMP-stimulated Cl^- secretion. A second K^+ channel with a unitary conductance of 138 pS has also been observed in human colonic crypts. The 138-pS channel has a distinct inhibitor profile; it is inhibited by TEA and quinidine, but not DPC, and is highly K^+ selective (190:1 vs Na^+) [180]. The RSK1 and KCNQ1 channels are basolateral K^+ channels in the colon that respond to Ca^{2+} and cAMP respectively [181].

Transepithelial electrolyte transport

The preceding section reviewed the *transmembrane* transport proteins that serve as basic building blocks for intestinal electrolyte transport of Na^+, K^+, Cl^- and HCO_3^-. This section discusses how these proteins are combined to produce the major *transepithelial* electrolyte transport events observed in the intact intestine.

In general, net transepithelial transport requires the participation of at least two transport proteins to mediate the uptake of ions across one plasma membrane domain and the exit of ions across the plasma membrane domain at the opposite pole of the epithelial cell. By definition, active

transepithelial transport also requires the involvement of a primary active transporter to provide the energy for net absorption or secretion of the ion. When the pump also serves as an uptake or exit step for the ion in question, only two transport proteins are required. Net transepithelial electrolyte transport across the epithelium can be either electrogenic or electroneutral. However, transepithelial transport mechanisms can only be electrogenic when both the apical and the basolateral membranes mediate electrogenic ion fluxes as part of the mechanism, or when transcellular fluxes drive electrogenic paracellular fluxes. For instance, the electrogenic Na^+,K^+-ATPase is involved in both electroneutral and electrogenic sodium absorption depending on the accessory Na^+ transport events.

The voltage clamp and Ussing chamber are used in combination to define the presence of active electrolyte transport between the lumenal and serosal surfaces of a tissue. The *Ussing chamber* permits mounting of intestinal tissue (or a cultured cell monolayer) between two compartments to measure transport between the lumenal and serosal reservoirs. If tissue in an Ussing chamber is *voltage clamped*, eliminating the driving force for transepithelial ion movement (i.e., transepithelial voltage is clamped to zero), the magnitude of the current required to offset the potential difference (termed *short circuit current*, or I_{sc}) will reflect active, net electrogenic ion transport. In the same configuration, the measurement of net transepithelial ion fluxes with isotopes can be used to detect transport of specific ions that is either electrogenic or electroneutral.

Classically, the absorptive and secretory functions of the intestine are considered to be independent, and absorptive function may remain intact during periods when the secretory function is excessively stimulated. This is a clinically important concept because some antidiarrheal strategies (e.g., oral rehydration solutions) rely on stimulating absorption in the presence of abundant secretion. However, evidence suggests that there are functional, and potentially molecular, linkages between the absorptive and secretory mechanisms. As discussed earlier, in the section on the crypt–villus axis, the long-held dogma that villus cells are purely absorptive and crypt cells are purely secretory was challenged when the presence of a subset of cells that mediate both absorption and secretion (at least in the colon) was confirmed [182]. Further, evidence that expression of the CFTR Cl^- channel affects activity of the epithelial Na^+ channel provides an explanation for the observation that patients with cystic fibrosis have increased electrogenic Na^+ absorption [183]. Thus, regulation of Cl^- secretion and Na^+ absorption can be intertwined, and cells that express machinery for both secretory and absorptive functions may have a significant role in Na^+ absorption.

Electrolyte absorptive mechanisms

The small intestine performs electroneutral NaCl absorption and Na^+-coupled nutrient absorption, and is responsible for most of the absorption of nutrients and water by the intestine as a whole. Na^+-absorptive mechanisms in the colon vary considerably as a function of both species and segment; however, in most species, including human, the large intestine avidly absorbs Na^+ through both an electrogenic mechanism involving apical Na^+ channels and an electroneutral NaCl absorptive mechanism similar to that in the small intestine. The colon is also responsible for SCFA absorption and some K^+ absorption, and it is essential for conservation of fluid and electrolytes [184].

Electroneutral NaCl absorption

A significant fraction of the Na^+ and Cl^- absorbed by the intestinal tract is electroneutral and is mutually dependent on the alternative ion (i.e., electroneutral Na^+ absorption requires the presence of Cl^- and vice versa) [185]. Postprandially, after the absorption of nutrients has been completed, or in the fasting state, electroneutral NaCl absorption is the major route for Na^+ absorption in the small intestine [129]. This NaCl-absorptive mechanism is also the principal route for Na^+ absorption in the proximal colon, with less prominence in the distal colon [2]. The relative importance of electroneutral vs electrogenic Na^+ absorption in the colon also varies as a function of mineralocorticoid status [2]. To explain these coupled fluxes, two models of NaCl uptake in the apical membrane have been proposed: an electroneutral Na^+/Cl^- cotransporter [186] or the combined actions of Na^+/H^+ and Cl^-/HCO_3^- exchangers. In both models the consensus is that the Na^+,K^+-ATPase in the basolateral membrane serves as the exit step for Na^+. Figure 14.3 outlines the dual exchanger model, which is the most widely accepted model for human NaCl absorption.

Abundant information supports the existence of an electroneutral NaCl absorption mechanism involving a Na^+/H^+ exchange pathway working in parallel with a Cl^-/HCO_3^- exchange pathway [187]. Both Na^+/H^+ and Cl^-/HCO_3^- exchange are present in vesicles prepared from the apical membranes of rabbit ileal villus cells. In this model (see Fig. 14.3), coupling of the paired exchangers occurs through changes in intracellular pH. For example, the Na^+ gradient drives Na^+ uptake and H^+ efflux by way of Na^+/H^+ exchange, which alkalinizes the cytoplasm and increases the activity of the Cl^-/HCO_3^- exchanger. The action of carbonic anhydrase produces HCO_3^-, which then leaves the cell in exchange for uptake of lumenal Cl^-. The net reaction is Na^+ and Cl^- uptake in exchange for H^+ and HCO_3^- efflux. Once in the cell, Na^+ is pumped out by the Na^+,K^+-ATPase and Cl^- follows by way of an electroneutral transport protein, likely a K^+/Cl^- cotransporter that has yet to be identified [153].

Electrogenic Na^+ absorption

In humans, electrogenic amiloride-sensitive Na^+ absorption accounts for about half of the Na^+ reabsorbed in the distal colon. There is a decreasing gradient of activity through

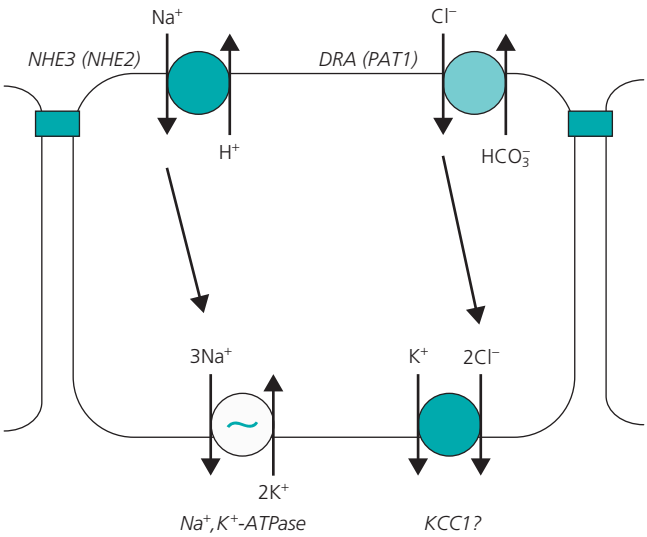

Figure 14.3 Electroneutral NaCl absorption in the small intestine and colon. Apical NaCl entry through Na^+/H^+ and Cl^-/HCO_3^- exchange is the most strongly supported model in mammalian intestine, although directly coupled NaCl cotransport remains a possibility. In both models, Na^+ exits the cell by way of the basolateral Na^+,K^+-ATPase but the route of Cl^- efflux is more speculative. Details of the model are described in the text.

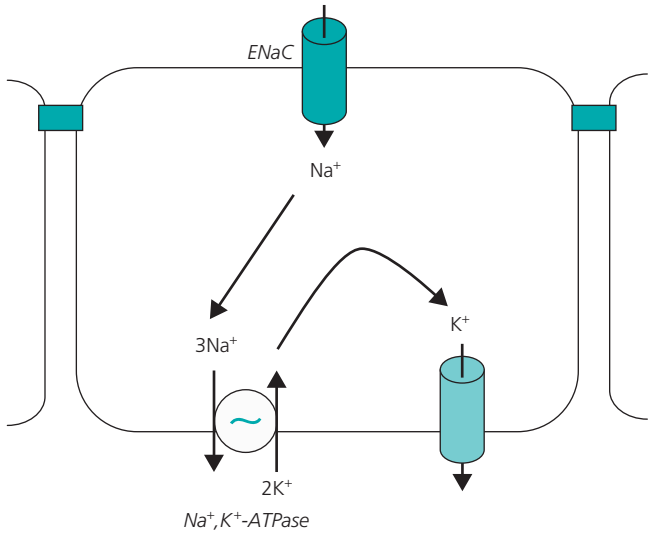

Figure 14.4 Electrogenic Na^+ absorption in the distal colon. Na^+ enters cells at the apical membrane through epithelial Na^+ channels (ENaC) and leaves cells at the basolateral membrane through the Na^+,K^+-ATPase. Details of the model are described in the text.

the transverse colon and only minimal levels in the proximal colon [188,189]. Aldosterone, a mineralocorticoid well known for its Na^+-retaining and K^+-wasting effects on the distal nephron, also exerts profound effects on the distal colon. Induction of secondary hyperaldosteronism by the feeding of a low Na^+ diet or the administration of exogenous aldosterone converts electroneutral NaCl absorption in the rat distal colon to amiloride-sensitive electrogenic Na^+ absorption [189]. Conversely, reduced electrogenic absorption of Na^+ has been described in patients with inflammatory bowel disease and in animal models of colitis [190,191]. This may contribute to diarrheal symptoms, emphasizing the importance of Na^+ and water salvage in the distal colon.

Figure 14.4 shows the proposed mechanism of electrogenic Na^+ absorption by the surface epithelium of the distal colon. Lumenal Na^+ enters colonocytes through ENaC Na^+ channels in the apical membrane and exits by way of the basolateral Na^+,K^+-ATPase. In the process of driving transepithelial Na^+ absorption, the Na^+,K^+-ATPase also catalyzes K^+ uptake and creates a charge imbalance, and both effects must be compensated to sustain Na^+ absorption. The compensatory ion flux is probably achieved by electrogenic efflux of K^+ through K^+ channels. Whether apical or basolateral K^+ channels perform this function remains controversial. It is likely that an apical K^+ conductance is predominantly involved in K^+ secretion, a crypt cell function that is not linked to Na^+ absorption [111]. The simplest model suggests that basolateral K^+ channels provide the compensatory flux and recycle K^+ across the basolateral membrane. Finally, paracellular flux of Cl^- is driven by Na^+ movement to restore electroneutrality between lumenal and serosal compartments.

Solute-coupled Na^+ absorption

The absorption of many nutrients, and also of bile acids (in the terminal ileum), is mediated by Na^+-coupled cotransporters in the small intestine. The combined action of these transporters contributes significantly to Na^+ and water absorption [192]. A common cellular mechanism (Fig. 14.5) is believed to mediate all solute-coupled Na^+ absorption that occurs in villus cells. Lumenal Na^+ enters by way of apical Na^+-coupled cotransport and exits by way of the basolateral Na^+,K^+-ATPase. The Na^+/solute cotransporter uses the energy of the electrochemical gradient for Na^+ to drive intracellular accumulation of the cotransported solute above its equilibrium value. When two Na^+ ions are coupled to the uptake of a single solute molecule (e.g., Na^+/glucose cotransporter), the intracellular accumulation of solute can be nearly 100-fold [193]. Efflux of solute down its steep concentration gradient across the basolateral membrane is then efficiently mediated by facilitated diffusion carriers, which are passive in nature. For example, the GLUT2 passive glucose transporter performs this function for glucose absorption [101]. Electrogenic Na^+ absorption stimulates absorption of a compensatory Cl^- anion through the paracellular route, and recycling of K^+ through basolateral K^+ channels assures cellular electroneutrality.

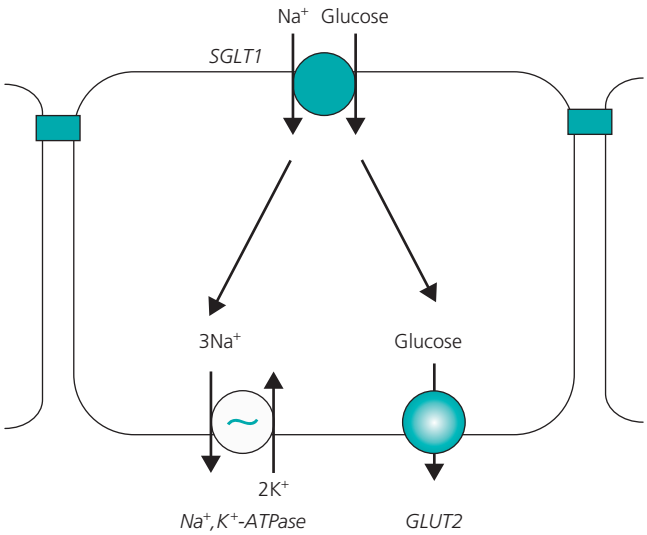

Figure 14.5 Solute-coupled Na^+ absorption in the small intestine. This model shows the mechanism for glucose as a generic solute, but is representative of Na^+-coupled absorption of glucose, many amino acids, certain B vitamins, and bile salts. The details of the model vary between solutes as a result of differences in the charge of the different solutes and the stoichiometry with transported Na^+ ions. Details of the model are discussed in the text.

SCFA absorption and SCFA-stimulated Na^+ absorption

SCFAs are produced in the colon by bacterial catabolism of unabsorbed carbohydrate and protein. The combined concentration of the predominant lumenal SCFAs (acetate, propionate, and butyrate) is 100–150 mM, so they are the major anions and osmolytes in the colonic lumen [194]. Absorbed SCFAs account for 7%–10% of ingested calories, and they serve as an important energy source for colonic epithelial cells [195]. SCFAs also stimulate electroneutral Na^+ absorption, up to fivefold in humans [196].

SCFAs stimulate Na^+ absorption by activation of apical Na^+/H^+ exchange in colonocytes, which leads to the subsequent exit of Na^+ by way of the basolateral Na^+,K^+-ATPase. Evidence suggests that lumenal SCFA uptake causes intracellular acidification and lumenal alkalinization, which are both known activators of Na^+/H^+ exchangers [197,198]. The mechanisms of SCFA uptake that lead to pH change are controversial, but it appears that both nonionic diffusion (uptake of the protonated form of these weak acids without intervention of a transport protein) and carrier-mediated transport by way of anion transporters (e.g., SCFA/HCO_3^- exchange, MCT1) play a role [199–202]. The ability of SCFA-induced acidification to stimulate apical but not basolateral Na^+/H^+ exchangers of colonocytes has been perplexing. The regulation of extracellular pH in microenvironments directly adjacent to colonocyte membranes may provide a partial explanation [203]. Physiological SCFA gradients cause alkalinization of the lumenal surface and acidification of the basolateral surface [197], which preferentially activates apical but not basolateral Na^+/H^+ exchange [198]. There are several candidate mechanisms for SCFA efflux across the basolateral membrane, including nonionic diffusion, SCFA anion transport by SCFA/HCO_3^- exchange, or SCFA/H^+ cotransport by way of MCT1 [108,204].

K^+ absorption

In human metabolic balance studies, about 85% of ingested K^+ is absorbed in the small intestine, with passive absorption driven by prevailing electrochemical gradients being sufficient to explain this uptake [205]. In contrast, active electroneutral K^+ absorption occurs in the distal colon [89] and is believed to mediate absorption of 5%–7% of the K^+ ingested daily [205], leaving 3%–5% to be lost in fecal water. These values underestimate the absolute magnitude of colonic K^+ fluxes because colonic K^+ secretion also occurs.

No single model of colonic K^+ absorption predominates. An apical H^+,K^+-ATPase promotes uptake of K^+ from the lumen [91]. High-conductance lumenal K^+ (BK) channels may also contribute to electrogenic absorption [206]. K^+ likely leaves the cell by way of the K^+/Cl^- cotransporter KCC1, shown to be expressed in the intestine [207]. Because not all K^+ absorption is Cl^- dependent, there is likely to be a second (or alternative) route for basolateral K^+ efflux that is not as tightly coupled to Cl^- anions [2]. The Cl^--dependent portion of K^+ absorption probably uses apical Cl^-/HCO_3^- exchange to neutralize protons extruded by the H^+,K^+-ATPase, providing a source of intracellular Cl^- to drive the basolateral K^+/Cl^- cotransporter. Evidence has also shown that SCFA uptake by the guinea pig distal colon requires activity of the H^+,K^+-ATPase; therefore, an alternative model may exist in which K^+ absorption regulates SCFA fluxes and vice versa [94].

Genetic defects in electrolyte absorptive process

Defects in NaCl absorption

Congenital chloride diarrhea produces profound Cl^--rich, acidic diarrhea commencing at birth, resulting in systemic hypochloremic, hypokalemic acidosis with volume depletion. First described in 1945 [208,209], the disease has been described in more than 60 children worldwide, although this number is certainly an underestimate of prevalence. The histology of the intestinal mucosa is found to be normal on routine examination. Intubation studies have revealed that the transport defect is limited to the ileum and colon [210]. Na^+ absorption against a chemical gradient remains intact and is accompanied by H^+ secretion [211,212].

SLC26A3 (*DRA*), the gene that is responsible for congenital chloride diarrhea, has been cloned; mice lacking *slc26a3* exhibit the symptoms of congenital chloride diarrhea [145,213]. Although the cause of the disease can be attributed to a defective Cl^-/HCO_3^- exchanger, the defect may be more

subtle than a total lack of anion exchange. Indeed, increases in lumenal HCO_3^- concentration increase Cl^- secretion in these patients, suggesting that residual Cl^-/HCO_3^- exchange may still occur [210,211]. It is unclear if this is due to the presence of an alternative anion exchange protein in the apical membrane or residual DRA function.

Congenital sodium diarrhea is an even rarer disorder. This disease produces a Na^+-rich, alkaline stool and results in systemic acidosis. Intubation studies have revealed that the usual inverse relation between Na^+ and H^+ fluxes does not hold, suggesting a defect in Na^+/H^+ exchange [214]. This defect has been confirmed in studies demonstrating a clear lack of proton-driven Na^+ uptake in jejunal vesicles [215]. Field and Semrad [216] reported that the distribution of NHE3 mRNA does not correspond to the site of the transport defect in congenital sodium diarrhea, tentatively suggesting that another Na^+/H^+ exchanger isoform may be affected. However, a genetic study failed to find an association with mutations of NHE1, NHE2, NHE3, or NHE5 [217], demonstrating that the defect is associated with an as yet uncloned NHE isoform or with an essential regulator of a known apical NHE.

Defects in electrogenic Na^+ absorption

Liddle syndrome is an autosomal dominant disorder leading to salt-sensitive hypertension. It is caused by mutations in the amiloride-sensitive Na^+ channel that is present in both distal colonic and renal epithelia. [218]. The disorder leads to increased expression and activity of Na^+ channels because of truncation or frame shift mutations in the cytoplasmic C-terminal tail of the β- or γ-subunit of the channel that may block its negative regulation (see section on the regulation of electrolyte transport) [159,160]. Curiously, no disorders of intestinal electrolyte absorption have been reported in Liddle syndrome despite severe defects in renal function, suggesting that down-regulation of alternative Na^+ absorptive processes in the colon may compensate for any increased electrogenic absorption; however, this hypothesis remains untested.

At least a subset of type 1 pseudohypoaldosteronism (the autosomal recessive version) is also caused by mutations in the epithelial Na^+ channel. In contrast to the gain-of-function mutants seen in Liddle syndrome, the mutations in type 1 pseudohypoaldosteronism diminish channel function and cause salt wasting. Intestinal malfunctions have been noted in this disease, including an increased level of Na^+ in the stool [219] and a lack of colonic responsiveness to mineralocorticoids [220]. The disorder can be caused by frameshift, premature termination, and missense mutations in the α-, β-, or γ-subunit of the channel [221].

Cystic fibrosis leads to an increase in electrogenic Na^+ absorption, although cystic fibrosis is primarily a defect of Cl^- secretory function caused by malfunction of a Cl^- channel (CFTR) (see section on defects in Cl^- secretion). However, the expression of normal CFTR protein is thought to limit Na^+ channel activity, and lack of CFTR (or presence of mutant CFTR) therefore removes an inhibitory influence on the amiloride-sensitive Na^+ channel [166,183]. An activated CFTR Cl^- channel is required to mediate the inhibitory regulation [166].

Defects in nutrient-coupled Na^+ absorption

Glucose–galactose malabsorption is a rare, autosomal recessive disorder in which Na^+-coupled uptake of glucose and galactose is defective [101]. Food ingestion leads to osmotic diarrhea, which can be treated by eliminating glucose and galactose from the diet. Children with this disorder can maintain normal Na^+ balance without glucose or galactose in their diets, illustrating the reserve capacity of the distal small intestine and colon for water and Na^+ absorption. Screening of 33 patients identified multiple mutations in the Na^+/glucose cotransporter gene *SGLT1*. Mutations in *SGLT* cause transport defects, including impaired trafficking of SGLT1 to the plasma membrane and defects in glucose transport kinetics [103].

Electrolyte secretory mechanisms

Secretory mechanisms throughout the gastrointestinal tract center around the Cl^- anion. HCl is the major secretory product of the stomach. In other intestinal segments, the predominant secreted ion is either Cl^- or HCO_3^- [222,223]. However, HCO_3^- secretion may be related to, or require, active Cl^- secretion.

Electrogenic Cl^- secretion

Electrogenic Cl^- secretion is found in all segments of the gastrointestinal tract from the duodenum to the distal colon, presumably reflecting the common need for a mechanism to maintain hydration of the lumenal contents. Figure 14.6 introduces the model of Cl^- secretion. Uptake of Cl^- across the basolateral membrane involves the electroneutral $Na^+/K^+/2Cl^-$ cotransporter [110]. A key function of the $Na^+/K^+/2Cl^-$ cotransporter is to use the energy of the Na^+ gradient to accumulate Cl^- intracellularly above its electrochemical equilibrium. Under these conditions, Cl^- will exit the cell across the apical membrane when Cl^- channels are opened. Na^+,K^+-ATPase provides energy for this overall mechanism and recycles Na^+ across the basolateral membrane. K^+ channels in the basolateral membrane allow for K^+ recycling, maintain cellular electroneutrality by compensating for Cl^- efflux, and keep the cell hyperpolarized (i.e., negative intracellularly compared with extracellular voltage) so that Cl^- efflux across the apical membrane can be sustained [224]. It is believed that Na^+ follows passively by paracellular flux through tight junctions to maintain electroneutrality between lumenal and serosal compartments. The cystic fibrosis gene, *CFTR*, encodes a Cl^- channel that is located in the apical membrane, but other channels may also be important.

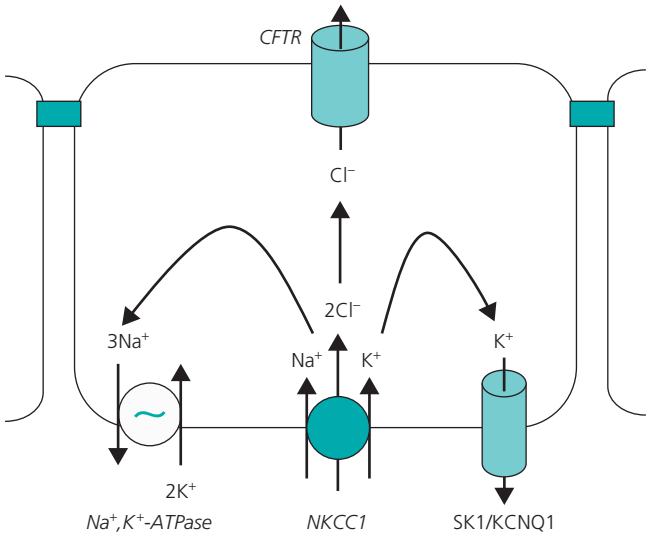

Figure 14.6 Cl^- secretion in the small intestine and colon. Cl^- enters cells at the basolateral membrane through $Na^+/K^+/2Cl^-$ cotransport and leaves cells at the apical membrane through Cl^- channels. Details of the model are discussed in the text.

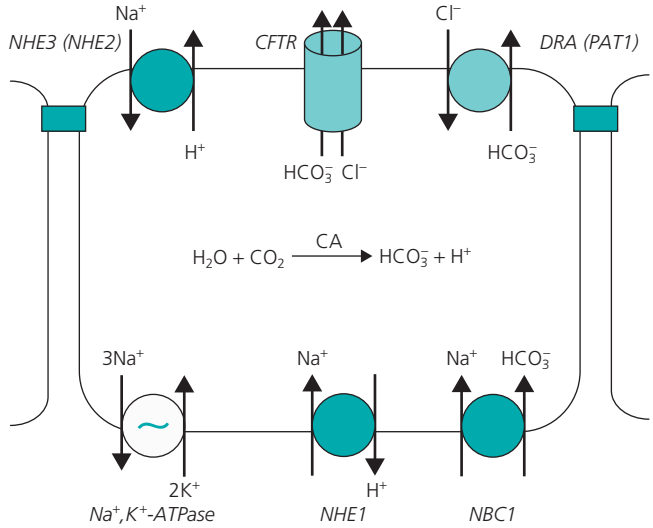

Figure 14.7 HCO_3^- secretion in the duodenum. The cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel provides apical Cl^- to drive the activity of an apical anion exchanger, or it serves as the pathway for HCO_3^- exit at the apical membrane. Both mechanisms may operate in the mammalian duodenum. In both, HCO_3^- is produced by carbonic anhydrase (CA), which requires compensatory efflux of a proton by Na^+/H^+ exchange, or it is taken up across the basolateral membrane by a Na^+-coupled cotransporter, NBC1. Details of the model are described in the text.

HCO_3^- secretion

HCO_3^- secreted by the duodenal epithelium contributes to the mucus–HCO_3^- layer that overlies duodenal epithelial cells and thus may be an important protective factor that defends against duodenal ulceration [223]. Although the duodenum secretes large amounts of HCO_3^-, lumenal HCO_3^- concentrations in the upper small intestine are relatively low because of neutralization by gastric acid. HCO_3^- secretion also occurs in the ileum and colon, but its physiological role in these sites is less clear [222]. One suggestion is that apical Cl^-/HCO_3^- exchange in the colon acts to conserve Cl^- at the expense of HCO_3^- secretion. Conversely, as congenital chloride diarrhea causes systemic alkalosis, the lower gastrointestinal tract may play a significant role in acid–base homeostasis.

Duodenal HCO_3^- secretion likely involves more than one mechanism. In the two mechanisms depicted in Figure 14.7, a common method of production of secreted HCO_3^- anions is provided by carbonic anhydrase, which hydrates CO_2 to produce intracellular HCO_3^- and a proton. The proton is eliminated by basolateral Na^+/H^+ exchange, and Na^+ is recycled across the basolateral membrane by the Na^+,K^+-ATPase or NHE1. HCO_3^- can also be derived from the bloodstream by way of the basolateral Na^+/HCO_3^- cotransporter NBC [225]. The models diverge in describing how HCO_3^- exits across the apical membrane. In an electroneutral mechanism, HCO_3^- is exchanged for intralumenal Cl^- [226]. An electrogenic mechanism has also been proposed, involving HCO_3^- secretion through apical channels, such as CFTR [163]. Evidence from patients with cystic fibrosis and from CFTR knockout mice suggests that CFTR plays an essential role in HCO_3^- secretion, both by conducting HCO_3^- and by regulating Cl^-/HCO_3^- exchange [227,228].

HCO_3^- secretion in the colon is stimulated by SCFAs [194,229] and may occur by a mechanism that differs from those previously described. SCFA uptake is known to cause alkalinization of the colonic lumen, most likely because of nonionic uptake of protonated SCFA by tissues, which removes acid equivalents from the lumen [199]. The resulting transepithelial pH gradient causes a vectorial titration of other weak acids (e.g., CO_2) across the epithelium and results in lumenal accumulation of the basic form (e.g., HCO_3^-). In support of this model, transepithelial CO_2/HCO_3^- gradients have been shown to drive lumenal pH changes consistent with transepithelial nonionic diffusion of CO_2 [199]. The relative importance of this nonionic diffusion model vs one using apical Cl^-/HCO_3^- exchange has not been assessed.

K^+ secretion

All portions of the mammalian colon can perform active K^+ secretion [2]. K^+ secretion is enhanced by a low-sodium diet, which leads to increased plasma levels of aldosterone, and by acute K^+ loading [111,230]. K^+ secretion can also be stimulated by cAMP-dependent secretagogues and seems to occur concurrently with Cl^- secretion [231]. However, despite this evidence for acute regulatory mechanisms, the physiological role of active K^+ secretion remains unknown.

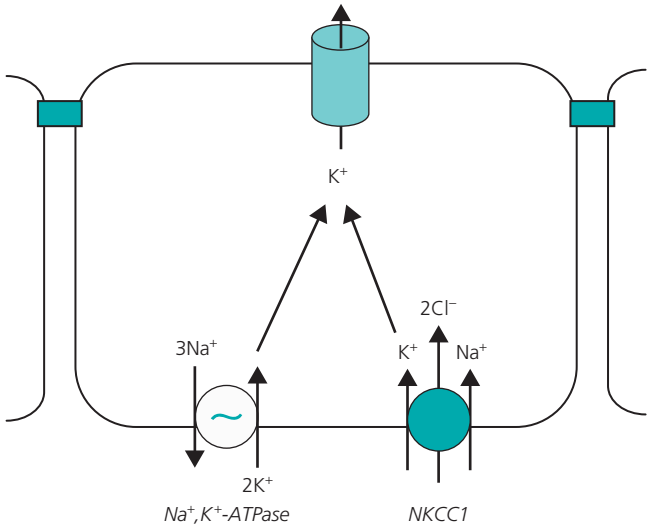

Figure 14.8 K^+ secretion in the colon. K^+ uptake at the basolateral membrane is mediated by either the Na^+,K^+-ATPase or $Na^+/K^+/2Cl^-$ cotransport, depending on the species and intestinal segment. K^+ efflux at the apical membrane is through K^+ channels. Details of the model are discussed in the text.

The model of active K^+ secretion (Fig. 14.8) closely parallels that of Cl^- secretion. As inhibitors of the $Na^+/K^+/2Cl^-$ cotransporter block K^+ secretion, at least in some tissues (e.g., rat and guinea pig colon), the $Na^+/K^+/2Cl^-$ cotransporter may be an important route for basolateral K^+ uptake [232]. K^+ secretion is ultimately dependent on the Na^+,K^+-ATPase pump to establish driving gradients, and in some tissues (e.g., rabbit proximal colon) the ATPase may supply the secreted K^+ instead of the $Na^+/K^+/2Cl^-$ cotransporter. In contrast to Cl^- secretion, active K^+ secretion is sensitive to inhibition by K^+ channel blockers (e.g., barium and TEA) applied lumenally [233]. The entire process is electrogenic, producing a current opposite to that produced by Cl^- secretion or Na^+ absorption. Although K^+ secretion is believed to be a function of colonic crypts, the interrelationship with Cl^- secretory cells at the same site is largely unexplored [231].

Genetic defects in electrolyte secretory mechanisms

Defects in Cl^- secretion

Cystic fibrosis is an autosomal recessive disease that causes defective epithelial transport in numerous organs. It is the most common genetic disease of ion transport, with one of every 20 white persons being a heterozygote carrier and harboring a mutant *CFTR* allele. There are more than 800 naturally occurring missense, frameshift, and truncation mutations in *CFTR*, which cause defective Cl^- secretion by either diminishing the amount of CFTR protein in the membrane (i.e., defective trafficking or synthesis) or decreasing appropriate Cl^- channel opening (i.e., defective ATP gating, cAMP activation, or ion conductance) [234]. Mutations occur in all portions of the CFTR molecule, but the highest frequency occur in the first nucleotide-binding domain, which controls channel gating. The most common mutation, found in 70% of all mutant *CFTR* alleles, is a triplet deletion that removes a phenylalanine (ΔF508) and results mainly in defective trafficking of CFTR to the plasma membrane.

Intestinal obstruction and meconium ileus associated with cystic fibrosis in newborns appears to result from the inability to maintain appropriate viscosity of the lumenal contents. Excised segments of intestine from patients with cystic fibrosis fail to exhibit normal secretory responses in vitro [168,235]. Some investigators have suggested that one reason for the prevalence of the disease may be a heterozygote advantage of being partially protected from the severe, life-threatening consequences of infectious secretory diarrhea, although others have disputed this hypothesis [236,237]. Another finding in this regard is that the ability of *Salmonella typhi* to invade intestinal epithelial cells is significantly impaired in cells carrying one mutant allele of *CFTR*, which might confer some resistance to typhoid fever [238].

Other disorders of water and electrolyte transport

Although genetic diseases associated with specific alterations in transport proteins have provided unique insights into the underlying molecular physiology of electrolyte transport, it should be recognized that the most common diseases of fluid and electrolyte absorption are not related to transporter mutations. This section briefly describes other disorders of intestinal fluid and electrolyte transport, some of which are far more common than the diseases discussed above. The reader is referred to later chapters in this text that provide more detailed descriptions of the pathogenesis of these disorders.

Infectious diarrhea

Diarrhea caused by infectious agents can be only an inconvenience in the developed world, but it is a major killer in non-industrialized nations, responsible for about 5 million deaths per year worldwide. Many infectious diarrheas result from infections with microorganisms that are capable of producing classical enterotoxins. Cholera is the prototype – a multimer toxin binds to receptors on the apical surface of intestinal epithelial cells and thereby simulates a sustained increase in intracellular cAMP, which, in turn, inhibits electroneutral NaCl absorption and evokes profound Cl^- secretion. Other bacterial toxins may subvert different cellular signaling mechanisms, such as the ability of the heat-stable toxin of *Escherichia coli* to evoke large increases in cGMP by binding to a cell surface receptor with intrinsic guanylyl cyclase activity. Both cholera and other enterotoxins may also activate subepithelial cell types to evoke secretion (see section on the regulation of electrolyte transport). Information is emerging regarding the mechanisms by which nontoxigenic

pathogens, such as invasive bacteria, evoke diarrhea. Such organisms may evoke a program of gene expression in intestinal epithelial cells that predisposes them to display increased secretory responses to endogenous agonists, as well as diminished barrier function [239–241]. More complete information on these topics is provided in Chapter 48.

Microvillus inclusion disease

This is a rare hereditary disease that commences in the first few days of life with the appearance of severe diarrhea that can be fatal if left untreated. The histological appearance of the intestinal epithelium is abnormal, characterized by inclusions of brush border membranes as vesicular structures within the cytoplasm of villus cells and a corresponding absence of apical microvilli. It has been suggested that cytoplasmic transport of Golgi-derived vesicles destined to fuse with the apical membrane may be defective in microvillus inclusion disease [242]. Endoscopic biopsies from patients with this disorder show markedly reduced levels of several apical transport proteins, with those transporters instead residing diffusely in the apical cytoplasm. Levels of basolateral transporters, in contrast, are normal. These observations led to the hypothesis that the disease results from a genetic defect specifically affecting a late stage of apical membrane trafficking, although the precise defect remains unknown [243,244]. A related neonatal enteropathy that causes severe diarrhea, known as tufting enteropathy or intestinal epithelial dysplasia, is similarly caused by an as yet unidentified genetic defect [245].

Disaccharide intolerance

This term encompasses genetic variability in the ability to conduct brush border digestion of carbohydrates, such as reduced levels of lactase in adulthood or sucrase–isomaltase deficiency. Such conditions cause osmotic diarrhea because of a reduced rate of brush border hydrolysis of specific disaccharides – only monosaccharides, not disaccharides, can be absorbed by villus enterocytes, and the undigested disaccharides thus remain in the lumen. This is in contrast to specific peptidase deficiencies, which usually do not result in fluid and electrolyte transport abnormalities because the intestinal epithelium can absorb both free amino acids and small oligopeptides.

Celiac disease

This genetic disorder is characterized by sensitivity to gluten, a water-insoluble protein found in certain cereal grains, notably wheat. The disease results in diarrhea and nonspecific nutrient malabsorption, which can be rapidly reversed by the elimination of offending substances from the diet. Celiac disease causes characteristic and striking morphological changes in the small intestine, including loss of villi, damage to remaining epithelial cells, and crypt hyperplasia. The underlying cause of the disease is unknown, but may include defective digestion of gluten or an inappropriate immune response to normal or improperly processed gluten molecules or to peptides from these molecules that have been cross-linked to host proteins through the activity of tissue transglutaminase [246]. The accompanying diarrhea may have both osmotic and secretory components, with the latter mediated, in part, by inflammatory mediators released by activated immune cells in the lamina propria. There is also emerging evidence that the active constituent of gluten in the disease, gliadin, may trigger the inappropriate release of an endogenous factor known as zonulin which impairs epithelial barrier properties [247].

Inflammatory bowel diseases

Patients with inflammatory bowel diseases often present with diarrheal disease, although constipation is also common. It is widely believed that the disorders of fluid and electrolyte absorption seen in patients with inflammatory bowel disease are caused by elevated levels of cytokines and other mediators in the inflamed bowel, although disruption of genes involved in maintaining an epithelial barrier may also play a role. Evidence for altered barrier function as a predisposing factor in inflammatory bowel disease has been derived from studies of mice lacking the multidrug resistance protein 1a [248]. This protein, expressed in the epithelium, may be responsible for effluxing toxic substances inappropriately absorbed from the gut lumen, and it may also regulate the expression and function of other transport proteins, such as CFTR [249]. Similarly, disruption of electrolyte absorption, including Na^+/nutrient cotransport, electrogenic Na^+ absorption, and electroneutral NaCl absorption, occurs in animal models of intestinal inflammation and in humans with inflammatory bowel disease [190,250,251]. Paradoxically, Cl^- secretion may also be inhibited in the setting of colitis, which is the result, in part, of the ability of specific inflammatory mediators to down-regulate the expression of key components of the Cl^- secretory mechanism [190,252]. This suggests that reduced absorption, rather than increased secretion, may be the predominant contributor to inflammatory diarrhea, and perhaps that normal levels of secretion from the crypts are needed to maintain relative crypt sterility and the integrity of the epithelium.

Regulation of electrolyte transport

With molecular information regarding the transport proteins that constitute specific transport mechanisms in the small and large intestines as a foundation, one can study the pathways that regulate these transport mechanisms. Ultimately, regulatory mechanisms are targeted to the specific protein components of the transport mechanisms, by modulating their activity, abundance, or localization within the epithelial cell, or by influencing the permeability of the epithelium

and thus the paracellular pathways for the flow of fluid and electrolytes.

Intestinal water and electrolyte transport are subject to both acute and chronic regulation. Acute regulation accommodates rapid changes in the lumenal or neurohumoral environment of the epithelium, such as occurs after ingestion of a meal. This acute level of regulation usually is provided for by signal transduction events that alter the chemical nature or location of transporter proteins. In general, transport rate is increased either by making individual transport molecules work more rapidly or by inserting more transport molecules into the plasma membrane. The former type of regulation usually occurs on a rapid time scale, in a matter of seconds to minutes. Membrane insertion of transport molecules can also occur on the same time scale if it is mediated by trafficking of transporter-laden vesicles to fuse with the plasma membrane, or it may take several hours if new proteins must be translated. Rapid vesicle trafficking (and interaction with cytoskeletal proteins) is important for the regulation of many electrolyte transport proteins. Second messenger-induced phosphorylation of the transport molecule or associated regulatory molecules, with or without transporter insertion, often mediates the rapid regulatory effects. Transport can also be acutely regulated in a negative sense by the retrieval of transporter proteins from the membrane and, in some cases, their degradation after internalization. Conversely, more protracted changes in whole-body electrolyte status, food intake, or development are balanced by long-term effects on transport regulation that are mediated at a genomic level and which are often, although not invariably, the result of alterations in the expression levels of specific transport proteins or the proteins that regulate them. A schematic depiction of the various levels at which transport can be regulated is provided in Figure 14.9.

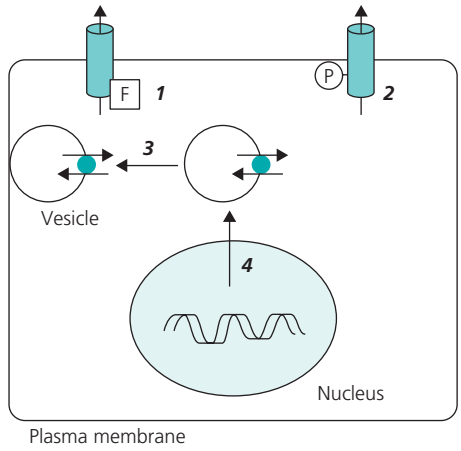

Figure 14.9 Mechanisms for the regulation of transport protein function at the level of the epithelium. Four levels of regulation are recognized. Mechanism 1 involves direct interactions of regulatory factors (F) with transport proteins. An example of this mechanism is the interaction of Ca^{2+} with the Ca^{2+}-activated K^+ channel. Mechanism 2 involves covalent modification of transport proteins, most often mediated by kinase-dependent phosphorylation events. An example of this mechanism is the opening of the cystic fibrosis transmembrane conductance regulator (CFTR) Cl^- channel in response to phosphorylation by protein kinase A. Mechanism 3 involves changes in the insertion of preformed transport proteins from vesicular pools into the plasma membrane or a change in their rate of retrieval from the plasma membrane. This mechanism appears to involve cytoskeletal cooperation. An example is the increase in the number of $Na^+/K^+/2Cl^-$ (NKCC1) cotransporters in the basolateral membrane of secretory epithelial cells after an increase in cAMP levels. Retrieval of transporters from the plasma membrane may also result in a decrease in their overall abundance secondary to degradation, particularly if they are modified by ubiquitination. Mechanism 3 participates in the acute regulation of the epithelial Na^+ channel (ENaC). Mechanism 4 involves changes in the synthesis of specific transport proteins at the level of gene transcription or translation. An example is the increase in ENaC expression in response to mineralocorticoids. Further details are given in the text.

Intracellular regulatory mechanisms

This section focuses on a detailed analysis of the mechanisms intrinsic to the epithelium that ultimately mediate changes in transport function. The intestinal epithelium has evolved a complex network of pathways that regulate its function in a largely autonomous fashion once an initial signal has been received. In addition, signaling cascades intrinsic to the epithelium may modulate its responsiveness to subsequent stimulation.

Receptor binding

The initial step in the activation of the intracellular regulatory mechanisms that control ion transport is the binding of various hormones and neurotransmitters to membrane receptors on epithelial cells. Generally, endogenous regulatory peptides and neurotransmitters bind to and activate receptors on the basolateral aspect of epithelial cells [253], whereas bacterial toxins act on the apical membrane [254]. There are, however, well-recognized exceptions to this polarization. For example, receptors for endogenous purinergic agonists are found on both sides of the epithelium [255]. This may partly reflect the fact that these agonists (or their precursors) are the products of inflammatory cells that are capable of traversing the epithelium and releasing mediators into the lumenal compartment [256]. Similarly, receptors for guanylin, the endogenous ligand for the receptor that binds the heat-stable enterotoxin of *E. coli* (ST_a), are located in the apical membrane, implying that this endogenous peptide is specifically secreted into the lumen to produce its biological effects. Conversely, epidermal growth factor (EGF), normally present in the lumen, has basolaterally localized receptors. EGF may therefore function as a "lumenal surveillance peptide" in that it may only regulate epithelial function in the setting of mucosal damage, when it can gain access to the basolateral aspect of the epithelium [257].

Table 14.2 Endogenous and exogenous regulators of intestinal chloride secretion

Substance	Second messenger	Notes
Endogenous stimuli (hormones, neurotransmitters, and immune/inflammatory mediators)		
Acetylcholine	Ca^{2+}	Acts through M_3 muscarinic receptor on epithelial cells
Histamine	Ca^{2+}	Acts through H_1 histamine receptor on epithelium; indirect effects also possible[a]
5-Hydroxytryptamine	Ca^{2+}, cAMP	Indirect effects also possible[a]
Prostaglandins	cAMP	E-series prostaglandins particularly potent
Bradykinin	Ca^{2+}	Acts through bilateral BK_2 receptors; indirect effects also possible[a]
Reactive oxygen species, H_2O_2	Ca^{2+}, cAMP	Effects likely to be largely indirect[a]
Platelet-activating factor	cAMP	Indirect effects[a]
Vasoactive intestinal polypeptide	cAMP	Direct effect through basolateral receptors
Guanylin, uroguanylin	cGMP	Apical receptor
Adenosine, 5'AMP	cAMP? Other effectors?	Acts through bilateral adenosine A_{2b} receptors on epithelium
Lipoxygenase metabolites of arachidonic acid	Unknown	Effects largely indirect
Exogenous stimuli (bacterial products and lumenally active agents)		
Cholera toxin	cAMP	
Escherichia coli heat-labile toxin	cAMP	
E. coli heat-stable toxin (ST_a)	cGMP	Binds to apical guanylin receptor
Clostridium difficile toxins A and B	Ca^{2+}, other effectors?	Indirect effects and effects on paracellular pathway likely to be important
Bile acids	Ca^{2+}, cAMP?	Indirect effects also likely to contribute
Pharmaceutical agents (e.g., laxatives)	Largely unknown	

a Indicates agonists that may mediate at least a portion of their effects through stimulation of mucosal prostaglandin production or activation of enteric nerves, which, in turn, can alter epithelial cAMP or Ca^{2+} levels.

Many substances have been identified that directly affect the Cl^- secretory function of intestinal epithelial cells and which, accordingly, are thought to bind to epithelial receptors. The effects of such agents, and the second messenger mechanisms that they initiate, are summarized in Table 14.2. The agonists listed in Table 14.2 are limited to those that are thought to be of physiological relevance. Note that many of the agonists listed are capable of simultaneously inhibiting electroneutral NaCl absorption (although not, importantly, Na^+/nutrient absorption). Conversely, other substances, such as peptide growth factors, inhibit Cl^- secretion yet up-regulate NaCl absorption. This reciprocity in the regulation of Cl^- secretion vs NaCl absorption ensures that the transport functions of different epithelial cells normally do not work at cross-purposes. Further, the independence of the nutrient-coupled processes allows ongoing secretion even during the digestion of a meal and provides for the efficacy of oral rehydration. Paradoxically, information extrapolated from studies in renal epithelia, along with more limited work using colonic tissue segments, suggests that at least some cAMP-dependent agonists may stimulate electrogenic Na^+ absorption in the distal colon [190,258–260]. The physiological significance of the likely dual stimulatory effect of cAMP on Cl^- secretion and Na^+ absorption in the distal colon is unknown but it may suggest that it is important to continuously flush the colonic crypts and then reclaim the water lost in doing so by absorbing Na^+ across the surface epithelium. Conversely, ENaC is reportedly inhibited by agonists that elevate levels of cytoplasmic Ca^{2+} [261]. Thus, mediators that increase levels of cytoplasmic Ca^{2+} should evoke colonic

fluid loss without a concomitant reabsorption, albeit only transiently.

Some of the effects of various agonists on intestinal transport may actually be the result of promiscuous coupling to alternative signal transduction pathways. Similarly, the spatial segregation of receptors and effectors in epithelial cells influences the precise mechanisms underlying transport regulation. This can result in signaling asymmetry, whereby the binding of an agonist to the basolateral membrane results in the assembly of signaling components at the apical membrane. Certain protein kinases, including one isoform of the cAMP-dependent protein kinase, protein kinase A, may be selectively anchored in specific subcellular domains. The anchoring of protein kinase A is mediated by a family of proteins known as AKAPs (A-kinase anchoring proteins). These proteins specifically scaffold protein kinase A, as well as other regulatory factors, in the immediate vicinity of ion channels and other transporter targets [262,263].

Epithelial signal transduction pathways

The classical second messengers recognized for their involvement in transport regulation are cAMP, cGMP, and free cytosolic Ca^{2+}. Many novel additional messengers that either amplify, antagonize, or modify the effects of these classical messengers within the epithelium continue to be identified.

Cyclic nucleotides

cAMP and cGMP have long been recognized to play important roles in the control of intestinal epithelial ion transport [264]. An increase in the level of either cAMP or cGMP within the epithelium stimulates Cl^- secretion and inhibits the neutral Na^+/Cl^- absorptive mechanism. cAMP, at least, may also play a role in stimulating the Na^+/glucose absorptive pathway [265], duodenal HCO_3^- secretion [266], and electrogenic Na^+ absorption, as previously described.

Several key endogenous hormonal regulators of ion transport, including prostaglandins and vasoactive intestinal polypeptide (VIP), act through an increase in cAMP production [267]. Other agonists of intestinal ion transport may act not by stimulating increases in cAMP but by relocating protein kinase A that is already active [259]. Exogenous substances may also activate the cAMP pathway. The classical example is cholera toxin, which causes irreversible activation of the G_s protein by ADP-ribosylation, leading to prolonged adenylate cyclase activation and an increase in cAMP levels.

Similarly, the apical receptor for the *E. coli* heat-stable enterotoxin ST_a is a membrane-bound guanylyl cyclase [268]. The binding of toxin to this receptor thereby increases intracellular cGMP levels, stimulating Cl^- secretion and inhibiting Na^+/Cl^- absorption [269]. The existence of a receptor for ST_a implied that there was an endogenous ligand for this system [270]; it was subsequently identified and named guanylin [270]. Guanylin may play an important role in normal intestinal water and electrolyte homeostasis, although the details of this role have yet to be fully elucidated [271]. The guanylin family of peptides may also serve to link the salt homeostatic mechanisms of the intestine and kidney.

Intracellular Ca^{2+}

The other primary second messenger system known to regulate intestinal ion transport involves changes in the levels of free cytosolic Ca^{2+}. Increases in this messenger stimulate both Cl^- and HCO_3^- secretion while inhibiting electroneutral NaCl absorption and electrogenic Na^+ absorption.

The actions of hormones and neurotransmitters that mediate their effects through cytoplasmic Ca^{2+} are more complicated than the direct pathways described for the cyclic nucleotides. There is a relatively poor correlation between the magnitude and duration of increases in cytoplasmic Ca^{2+} and the resulting ion transport responses [272]. In general, whereas ion transport responses mediated by cyclic nucleotides are prolonged, those evoked by Ca^{2+} are transient and terminate even if the Ca^{2+} signal persists [239]. Ca^{2+} alone appears to be sufficient to activate epithelial Cl^- secretion, although the end effects of this messenger can also be modified by other substances within the cell. For example, other products of phospholipid turnover, including diacylglycerol, inositol 3,4,5,6-tetrakisphosphate (IP_4), or 3-phosphorylated inositol phospholipids produced by the enzyme phosphatidylinositol 3-kinase (PI3K), may be involved in modulating Ca^{2+}-dependent ion transport responses [273,274]. These auxiliary messengers have been shown to have reciprocal effects on secretory and absorptive processes, for example inhibiting Ca^{2+}-dependent Cl^- secretion while stimulating electroneutral NaCl absorption [273,275].

Downstream targets of epithelial signaling pathways

The downstream effects of cyclic nucleotides and cytosolic Ca^{2+} within epithelial cells are largely mediated through the actions of specific kinases and phosphatases, as well as by enzymes that conjugate other molecules to ion transporters, such as ubiquitin, which marks proteins for internalization and degradation. In total, therefore, agonist-generated signals can either up-regulate or down-regulate the overall level of a given transport mechanism through the coordinated regulation of a number of membrane transporters.

Electroneutral NaCl absorption, which predominantly involves NHE3 in both the small and large intestines, with a minor contribution from NHE2, is regulated at multiple levels. Various kinases, including protein kinase A, alter the activity of NHE exchanger isoforms [276]. For the predominant isoform involved in small intestinal NaCl absorption, NHE3, the effect of protein kinase A is to inhibit transporter activity. This phosphorylation requires a cofactor – one of two closely related accessory proteins, E3KARP (NH3 kinase regulatory protein) or NHERF (NHE regulatory factor) [277]. Further, complex scaffolding interactions involving ezrin are critical to ensure proper NHE3 regulation by protein kinase A

[278]. The C-terminal part of NHE3 is responsible for binding E3KARP or NHERF, ezrin, and, by way of ezrin, the catalytic subunit of protein kinase A. This multiprotein complex regulates the trafficking of NHE3 into and out of the apical membrane, and also establishes a spatial relationship between protein kinase A and the residues that it phosphorylates on the transporter [119]. NHE3 can similarly be down-regulated by agonists that cause elevations in cytoplasmic Ca^{2+}. In this case, protein kinase C rather than protein kinase A appears to be the kinase effector, with the specific protein kinase C-α isoform identified as mediating the inhibitory effects of neurohumoral effectors such as serotonin or acetylcholine on transport [279,280]. Endocytosis of NHE3 in response to the elevation of cAMP or Ca^{2+} levels, thereby reducing transporter activity, has also been shown to depend on the binding of synaptotagmin 1 and subsequent recruitment of adapter protein 2 (AP2) and clathrin [281]. NHE2 may be regulated similarly when it is assembled into large complexes but, unlike NHE3, it remains resident in the apical membrane and does not appear to undergo agonist-sensitive trafficking [119]. This presumably underlies distinct physiological roles of these two complementary transporters involved in NaCl absorption, although the details of these roles are not yet known.

Electroneutral NaCl absorption may also be up-regulated by signals transmitted coincident with meal intake that enhance fluid reclamation. For example, Turner and colleagues have shown that when Na^+ and glucose are absorbed by way of SGLT1, there is concomitant up-regulation of NHE3 activity that is mediated by translocation of this transporter to the apical membrane. This process involves the sequential activation of p38 mitogen-activated protein kinase (MAPK), its substrate MAPKAPK-2, the kinase Akt2, and ezrin, which ultimately enhances NaCl absorption [282–284]. Similarly, growth factors such as EGF and fibroblast growth factor activate NHE3 activity and membrane insertion through mechanisms that involve the activation of PI3K and subsequent activation of Akt [285].

In Cl^- secretion, multiple targets underlie the regulation of transport in response to changes in the levels of either cyclic nucleotides or cytosolic Ca^{2+}. For example, protein kinase A directly phosphorylates CFTR, resulting in channel opening and trafficking to the apical membrane [286]. The efficiency of this process is enhanced by the clustering of protein kinase A and CFTR in a signaling complex, with the participation of scaffolding proteins, including AKAPs [262], and adaptor proteins, such as E3KARP and NHERF, that assemble the signaling complex through interactions with PDZ domains. Similarly, guanylin and ST_a appear to activate Cl^- secretion through the type II cGMP-dependent protein kinase that is expressed in the intestinal epithelium [287]. Although there are reports that this kinase neither phosphorylates nor activates CFTR in exogenous expression systems, evidence suggests that the stimulatory effect of cGMP on Cl^- secretion, which clearly depends on CFTR, is mediated through insertion of this channel into the apical membrane [288]. Nevertheless, in some segments of the intestine, the effects of cGMP may be mediated in part by cross-reactivity with protein kinase A [287].

Other transport proteins involved in Cl^- secretion may also be modified as a result of kinase cascades. For example, the basolateral NKCC1 cotransporter involved in Cl^- secretion is phosphorylated by protein kinase A and is thereby activated; this activation is balanced, in turn, by phosphatase activity [289]. Similarly, in native colonic crypts, an elevated cAMP level results in prolonged retention of NKCC1 in the basolateral membrane [290]. Furthermore, the intracellular concentration of Cl^- also seems to regulate cotransporter activity by impeding its phosphorylation when Cl^- concentrations are high [289]. Thus, intracellular Cl^- self-regulates the rate of anion entry into the cell. Overall, these mechanisms serve to balance Cl^- exit and entry in the actively secreting epithelial cell. SPAK (Ste20p-related proline alanine-rich kinase) and WNK4 (with no lysine kinase 4) have similarly been shown to phosphorylate and thereby stimulate NKCC1, although the relevance of these kinases for intestinal transport physiology is not yet known. cAMP is also responsible for gating KCNQ1/KCNE3 K^+ channels on the basolateral membrane. The activity of these channels is critical for preventing cell depolarization, which would otherwise mitigate the driving force for apical Cl^- exit during active Cl^- secretion [291].

The protein kinases or other effectors responsible for mediating the effects of Ca^{2+} on Cl^- secretion are less well understood. Some effects of Ca^{2+} are mediated by a direct effect of the cation itself on basolateral, Ca^{2+}-activated K^+ channels [292]. For the Cl^- secretory process, K^+ channel opening increases the driving force for Cl^- exit across the apical membrane [292]. In fact, molecular characterization of hIK1, the Ca^{2+}-sensitive K^+ channel implicated in intestinal Cl^- secretion, has revealed that the channel is activated by either Ca^{2+} or protein kinase A in an ATP-dependent fashion, but not by Ca^{2+}/calmodulin-dependent kinase (CaMK) or protein kinase C [293].

Increases in cytosolic Ca^{2+} can also activate CaMK and various isoforms of protein kinase C. A family of Cl^- channels activated by Ca^{2+} and CaMKII has been cloned. These channels may also contribute to Cl^- secretion in response to Ca^{2+}-mobilizing agonists in the intestine [294]. A channel with similar properties may account for residual Cl^- secretion in the intestine of some patients with cystic fibrosis and in murine models of the disease [295]. Protein kinase C, in comparison, although capable of inducing active Cl^- secretion in some systems, more commonly reduces Cl^- secretory responses induced by both cAMP and Ca^{2+}-mobilizing agonists [296]. This appears to reflect, in part, the ability of specific protein kinase C isoforms and other Ca^{2+}-dependent signals to regulate vesicular trafficking of basolateral transport proteins, including NKCC1 [290,297].

A significant body of information has emerged with respect to the signaling events and targets that regulate electrogenic Na^+ absorption through the ENaC Na^+ channel. Although most findings have come from studies of renal cells, it is reasonable to assume that the regulation of this channel in the distal colon is similar. There is a dynamic association between ENaC and the ubiquitin protein ligase Nedd4-2 in response to increases in the intracellular Na^+ concentration [298]. In turn, the modification of ENaC subunits by covalent association with ubiquitin targets the channel for internalization and degradation in the proteosome [299]. This provides a mechanism whereby Na^+ absorption can appropriately be reduced if cytoplasmic Na^+ levels rise. However, this regulatory machinery also provides for the upregulation of electrogenic Na^+ absorption in response to agonists that increase levels of cAMP. cAMP activates protein kinase A, which, in turn, phosphorylates Nedd4-2, reducing its association with ENaC. Thus, by relieving the inhibitory effect on ENaC membrane abundance, cAMP can increase overall levels of Na^+ absorption [260,300]. Similarly, acute effects of the mineralocorticoid aldosterone on Na^+ absorption may reflect, in part, the insertion of additional Na^+ channels or active Na^+,K^+-ATPase pumps into the appropriate membrane of the epithelial cell [301]. These effects of aldosterone and similar effects of glucocorticoids may be mediated by the acute activation of the serum/glucocorticoid-regulated kinase, SGK1. This kinase, similar to protein kinase A, increases ENaC activity secondary to phosphorylation of Nedd4-2, thereby reducing its ability to associate with ENaC and mediate its degradation [302]. In contrast, cytoplasmic Ca^{2+} inhibits ENaC activity. The mechanism for this effect is unknown; although Ca^{2+} is required to target Nedd4-2 to the plasma membrane, it does not appear to mediate subsequent inhibition of ENaC activity [303].

A final mechanism whereby epithelial signaling cascades can alter transport activity is brought into play for chronic regulation. Certain hormones, particularly steroids, alter transport activity by transcriptional regulation, such that the total pool of transporters within the cell is increased with an accompanying increase in transport capacity. Chronic regulation is an adaptive response to long-term changes in whole-body status, such as occur during fasting and malnutrition or if salt intake is modified substantially [304]. This mechanism is best understood for the regulation of colonic Na^+ transport by aldosterone [305]. This hormone stimulates the expression of both epithelial Na^+ channels (ENaC) and Na^+,K^+-ATPase, thereby increasing the ability of the tissue to perform electrogenic Na^+ absorption [306,307]. Similar mechanisms almost certainly alter the expression of other transport proteins in the intestine, but they remain relatively underexplored. Note, however, that not all effects of steroids or other hormones on transport regulation are mediated at the genomic level. For example, estradiol rapidly downregulates Cl^- secretion in the female rat colon without altering Na^+ absorption [308]. Some of the effects of aldosterone on colonic Na^+ absorption can likewise be dissociated from de novo protein synthesis and likely reflect transporter trafficking [305].

One setting in which transcriptional regulation of transporters has a clearly established role is during development [309]. The fluid and electrolyte transport capabilities of the gut are not fully developed at birth in most mammalian species. Development of the intestine is characterized by the appearance and modification of specific transport proteins and changes in the regulation of these pathways. The intestine of a newborn infant has elevated levels of ion absorption, in particular Na^+-dependent glucose absorption in the small intestine and amiloride-sensitive Na^+ absorption in the colon [310]. In preterm human infants, the appearance of colonic Na^+ absorption precedes that in the kidney, suggesting that the colon may be a major site for Na^+ conservation at this stage [311]. A subsequent decline in amiloride-sensitive Na^+ absorption correlates with a sharp decline in circulating levels of mineralocorticoids [312]. In contrast, the Cl^-/HCO_3^- exchanger is absent in the colon of preterm infants and only slowly develops during the first year of life [313]. Low levels of Cl^-/HCO_3^- exchange may account for the susceptibility of infants to Cl^- depletion.

In the rat colon, Na^+,K^+-ATPase activity is elevated at birth and declines to adult levels during the first few days of life [314]. Conversely, Na^+,K^+-ATPase mRNA expression is low at birth and increases with age [86]. Cyclic nucleotide-dependent Cl^- secretion is also fully functional in newborn and infant intestine. Interestingly, the number of guanylin/ST_a receptors is high in infants and declines rapidly with increasing age [315]. Some investigators have speculated that the ability of guanylin to stimulate Cl^- secretion through CFTR may be involved in the clearance of meconium from the newborn intestine [316]. This may account for the finding of meconium ileus in babies with cystic fibrosis. Conversely, bile acids, which normally elicit a Cl^- secretory response in adult colon, do not stimulate Cl^- secretion in newborns [317]. There is also a developmental delay in the appearance of active ileal reabsorptive mechanisms for bile acids [318]. Thus, the concomitant absence of a Cl^- secretory response to these agonists in the colon would have obvious beneficial consequences. In fact, newborn intestine may have a more general maturational delay in all receptor-mediated, Ca^{2+}-dependent secretion [317], secondary to reduced phospholipase C activity [319].

Interactions among second messengers

Hormones that act via different second messengers may have synergistic effects on intestinal ion transport if supplied in combination. The physiological implication of this observation is that greater effects on ion transport can be achieved for a given level of a hormone. The pathophysiological implication is that the combinations of mediators often

encountered in the setting of intestinal inflammation can readily cause profound secretory diarrhea.

The underlying basis of these synergistic interactions between messengers has been studied extensively. For Cl^- secretion, the synergism appears to depend, in part, on the transport proteins activated by a given messenger. Thus, Cl^- secretion induced by cAMP is regulated primarily by the opening of apical CFTR Cl^- channels, whereas Ca^{2+} appears to act predominantly by opening basolateral K^+ channels. When the levels of both second messengers are elevated simultaneously, the rate-limiting step for each type of secretion is effectively removed. Synergism may also result from interactions at the level of second messenger generation. cAMP has been shown to modify Ca^{2+} mobilization responses within cultured epithelial cells [320].

Some agonists of secretory processes may also stimulate the secondary production of additional messengers that inhibit or antagonize the effects of the initial messenger [275]. Similarly, some of these agents may serve only to induce negative signals within the epithelial cell, without serving as agonists of secretion themselves. An example of such an agonist is EGF, which inhibits epithelial Cl^- secretion evoked by Ca^{2+}-dependent agonists, secondary to an inhibitory effect on basolateral K^+ channels, without itself acting as a secretagogue [321,322]. Conversely, EGF acts to stimulate both NaCl and glucose-coupled Na^+ absorption [323,324]. All of these processes appear to involve common signaling pathways, in which PI3K plays a central role [257]. The EGF receptor also participates in the long-term ability of Ca^{2+}-dependent agonists, such as acetylcholine, to down-regulate intestinal Cl^- and fluid secretion. However, in this case, EGF receptor-mediated signals are redirected to alternative targets such as Cl^- channels or NKCC1 [325]. Evidence gained from the study of native colonic crypts implies that the primary long-term effect of cholinergic stimulation is the EGF receptor-dependent internalization of NKCC1, which can thereby limit fluid loss unless offset by the simultaneous presence of an agonist that elevates cAMP [290]. Other neurohumoral agents may interfere with the initial production of second messengers. Somatostatin, acting through an $SSTR_1$ receptor, inhibits agonist-stimulated accumulation of cAMP in colonic epithelial cells, presumably through the activation of a linked inhibitory G protein (G_i) [326]. This effect may explain, in part, the efficacy of using somatostatin analogues to treat severe secretory diarrhea.

In summary, a plethora of intracellular signals and targets underlies both the acute regulation of ion transport in the intestine, such as is required to respond appropriately to the presence of a meal in the lumen, and the more chronic adaptation of transport capacity that is needed to ensure whole-body fluid and electrolyte homeostasis with changing external conditions. We are beginning to learn that specific mutations in ion transport proteins themselves, or in regulatory proteins, can derange normal regulatory mechanisms, thereby resulting in specific disease states such as inappropriate reabsorption of Na^+ and accompanying hypernatremia, or diarrheal illness. Thus, a detailed understanding of the signaling pathways that regulate ion transport in the intestine may ultimately lead to improved treatments for such conditions.

Intercellular regulatory mechanisms

Epithelial ion transport is regulated by a wide array of extracellular stimuli, which include lumenal factors and blood-borne, neuronal, and immunological mediators (Fig. 14.10). Histological examination of the lamina propria reveals various immune cell types (the mucosal immune system), an extensive neuronal network (the enteric nervous system), and enteroendocrine cells that are interspersed among the cells of the epithelial layer. The epithelium also has an underlying layer of specialized mesenchymal cells called myofibroblasts. Mediators released from all of these cell types contribute to the regulation of epithelial transport function.

Lumenal factors

The intestinal lumen is an ever-changing environment to which epithelial cells must rapidly adapt. For example, the intermittent presence of nutrients in the lumen induces up-regulation of the surface expression of nutrient-coupled cotransporters, thereby coupling fluid to nutrient absorption on ingestion of a meal. As digestion proceeds, the movement of a food bolus through the intestine also exerts physical forces on the epithelium to induce fluid secretion, thereby lubricating the epithelium and protecting it from abrasion. Such responses are mediated by enteroendocrine cells that release mediators which act in a paracrine fashion to activate neuronal secretomotor reflexes [327]. In addition to food and digested nutrients, there are many other factors present in the intestinal lumen that have the capability to dramatically alter transport function. Bile acids are required for the efficient digestion and absorption of fats in the small intestine. Normally, most of these molecules are reabsorbed in the ileum through the Na^+-dependent apical bile salt transporters (ABST). However, in certain pathological conditions, malabsorption in the small intestine can result in increased delivery of bile acids into the colon, where they exert prosecretory effects, leading to the onset of diarrhea [328]. Bile acids can also be metabolized by the colonic flora, which can alter their secretory activities [329]. Colonic bacteria also metabolize dietary fiber to produce SCFAs, such as butyrate, which serve as a primary source of energy for enterocytes. However, SCFAs also induce differentiation of epithelial cells and have been shown to up-regulate both electroneutral and electrogenic Na^+ absorption and to down-regulate epithelial secretory capacity [330,331]. In this manner, SCFAs serve to promote normal colonic absorptive function. The ingestion of pathogens can also have profound effects on epithelial

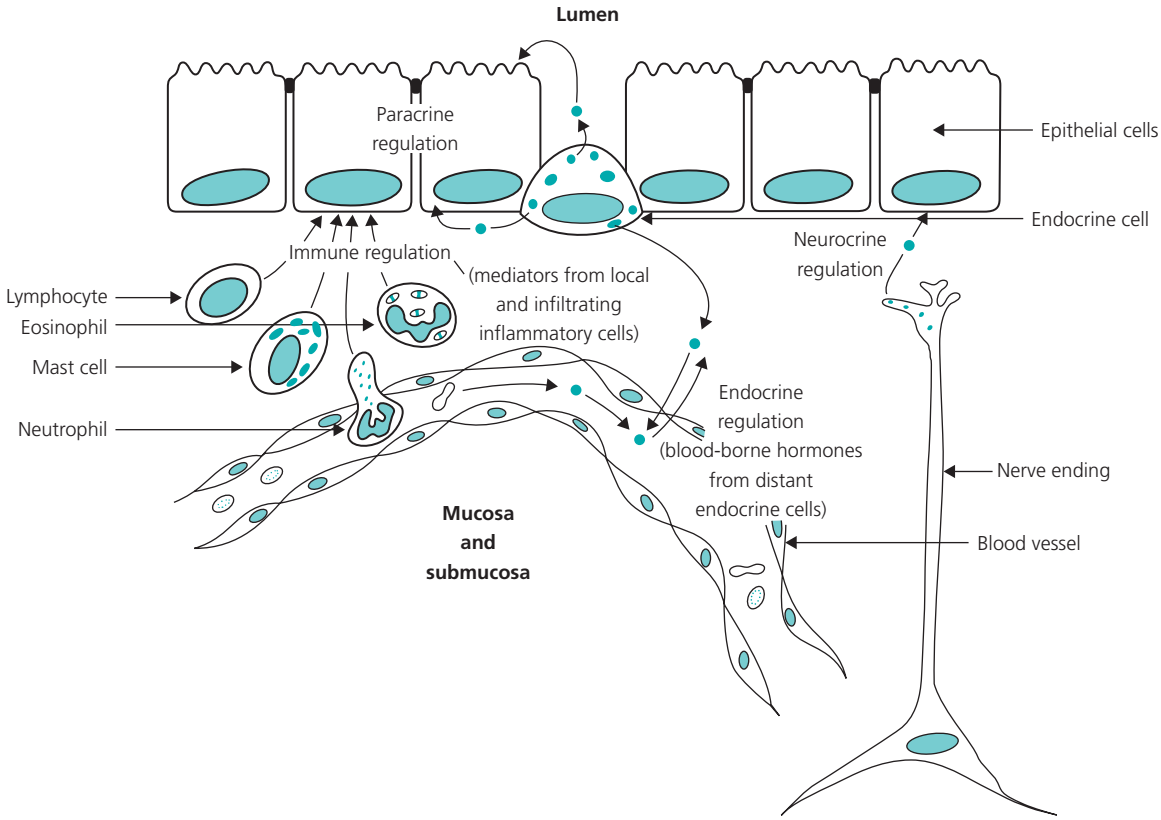

Figure 14.10 Neurohumoral and immune regulation of the intestinal epithelium. Endocrine cells in the crypt region release peptides and other substances across either their basolateral or their apical membranes. Local diffusion of these bioactive substances often regulates nearby ion transporting cells (paracrine regulation). These substances, or others from more distant endocrine organs, can also enter the bloodstream to regulate the function of distant portions of the epithelium (endocrine regulation). Enteric nerve endings release peptides and other neurotransmitters that regulate both the epithelium and muscle layers (neurocrine regulation). Immunological effector cells (e.g., mast cells, eosinophils, neutrophils, and lymphocytes) in the lamina propria can also be stimulated to release substances that regulate the epithelium (immune regulation).

transport. Bacterial toxins, such as anthrax, cholera toxin, and the heat-stable enterotoxin of *E. coli*, ST_a, promote the generation of cyclic nucleotides in epithelial cells, thereby up-regulating secretion and causing diarrhea [288]. Other pathogenic bacteria, for example *Salmonella* organisms, do not produce enterotoxins but can induce gastroenteritis and diarrhea through direct manipulation of host cell signaling pathways. In addition to their direct actions on epithelial cells, bacteria can also exert prosecretory actions in the intestine by indirect means through activation of the enteric nervous system [35]. Many other pathogens, including viruses [332] and protozoans [39], can invade the intestinal lumen and induce diarrhea through alterations in epithelial transport.

Endocrine and paracrine regulation

Both endocrine (i.e., regulation by bloodborne hormones from distant sites) and paracrine (i.e., local) factors are of critical importance in the regulation of epithelial transport. Endocrine cells located within the epithelium sense the nature and composition of lumenal contents and can also mediate responses to extraintestinal signals, such as those from neural inputs. The hormones released by these cells can then act on epithelial receptors to alter ion transport. Table 14.2 describes some of the neurohumoral agents that have been identified as potential physiological regulators of intestinal ion transport. The topic of gastrointestinal hormones is covered in detail in Chapter 4.

Hormonal regulation

The intestine is a highly vascularized organ receiving a rich blood supply through the splanchnic circulation. Disruption of this blood flow to the mucosa can lead to a loss of tissue oxygenation, or hypoxia, and this can have drastic effects on the ability of epithelial cells to secrete fluid and electrolytes [333,334]. In addition to oxygen and nutrients, the intestinal blood supply also carries hormones that can profoundly affect epithelial transport function. Several gastrointestinal peptide hormones, such as gastrin, cholecystokinin, and secretin, are released in response to the ingestion of a meal

and can alter electrolyte and fluid absorption and secretion. These hormones are discussed in detail in Chapters 4, 13, and 15. Many circulating hormones from distant organs are delivered to the intestine. In this way, interorgan signaling enables intestinal electrolyte transport to be integrated and synchronized with the requirements of other organ systems, thereby contributing to long-term control of whole-body fluid homeostasis. As previously discussed (see section on downstream targets of epithelial signaling pathways), aldosterone, a mineralocorticoid produced by the adrenal cortex, is probably the best studied hormone with respect to chronic effects on intestinal transport. Other hormones that can induce chronic alterations in intestinal transport function include glucocorticoids, natriuretic peptides, and thyroid hormone [335–338]. Although hormones have long been known to have the ability to alter intestinal function through the activation of nuclear receptors and the induction of gene transcription and transport protein expression, it is increasingly clear that many hormones can also exert rapid nongenomic effects through the activation of various signaling pathways. For example, in addition to promoting ENaC expression in the colon, aldosterone can rapidly activate Na^+/H^+ exchange leading to cytoplasmic alkalinization [339] and inhibit K^+ channel activity [340]. Sex steroids such as estrogen can also exert rapid antisecretory effects in the colon that may contribute to the retention of body fluid when levels of circulating estrogen are high [308]. Other hormones that exert rapid nongenomic effects on intestinal transport include corticosteroids, prolactin, and growth hormone [341–343].

Immune regulation

The gastrointestinal tract has an abundance of immune effector cells, which is not surprising considering its continuous exposure to bacterial, viral, and dietary antigens. Immune cells of the gut are contained primarily within the lamina propria and consist of lymphocytes, plasma cells, mast cells, and phagocytes. Classically, the mucosal immune system is broadly divided on a functional basis into afferent and efferent components; the afferent component consists of lymphocyte-mediated antigen recognition and presentation and cytokine production, which, in turn, recruits the efferent component consisting of mast cells, phagocytes, and their mediators. However, as our understanding of intestinal mucosal immunology develops, the division between the afferent and efferent components of the mucosal immune system has become less distinct. Practically all cells within the intestinal mucosa are involved in both the initiation and the propagation of mucosal immune responses. Much has been learned of the ways in which cells and mediators of the intestinal immune system affect ion transport [344]. This knowledge will likely enhance our understanding of the dysfunction of intestinal ion transport that accompanies intestinal inflammatory disorders.

Mast cells

Mast cells are the primary effector cells involved in allergic reactions, including those to foods, and they have also been implicated in the pathogenesis of inflammatory bowel disease and irritable bowel syndrome [345,346]. In a variety of animal models, immunological (IgE-dependent) activation of resident mast cells leads to a stimulation of Cl^- secretion [344]. These responses reflect the combined effects of the various mediators that are released from mast cells on activation; the relative contribution of various substances is species dependent. Treatment of human small and large intestine tissue segments with anti-IgE has been reported to produce Cl^- secretory responses that involve histamine, serotonin, and functional enteric neurons. Other mast cell mediators capable of inducing Cl^- secretion include adenosine, platelet-activating factor, cysteinyl leukotrienes, and prostaglandin D_2 [347].

In addition to their role in promoting secretion in conditions of inflammation, mast cells may also play a broader role in the control of intestinal fluid and electrolyte homeostasis in noninflammatory conditions. For example, mice that are genetically deficient in tissue mast cells not only display defective ion transport responses to antigen challenge but also have reduced responsiveness to the Cl^- secretory effects of electrical field stimulation and bile acids [348,349]. In cooperation with the enteric nervous system, mast cells may also mediate Cl^- secretory responses to certain bacterial enterotoxins, most notably the toxins produced by *Clostridium difficile* [350]. The functional association of mast cells and nerves in controlling ion transport is mirrored by a morphological association; mast cells and nerves exist in proximity in the intestinal mucosa. Such intimate contact between these cell types appears to play a role in the manifestation of intestinal disturbances in conditions of acute psychological stress [351,352]. Mast cells may also be important indirect regulators of ion transport by virtue of their ability to influence other inflammatory or mesenchymal cell types. The mast cell produces various cytokines and other chemotactic factors that can attract inflammatory cells. These inflammatory cells can then, in turn, exert regulatory effects on ion transport.

Neutrophils

Neutrophils accumulate at inflammatory foci in response to peptide and lipid chemotactic factors. The bacterial chemotactic peptide f-met-leu-phe has been used experimentally to activate resident neutrophils. In colonic and ileal tissues from rats, rabbits, and humans, f-met-leu-phe induces a Cl^- secretory response that is attenuated by cyclooxygenase inhibitors and which is at least partially dependent on enteric nerves [353,354]. Several mediators released from activated neutrophils may contribute to secretory responses, including neutrophil-derived oxidants [355] as well as 5′AMP. This latter mediator is interesting in that it exhibits only apical

activity [256], reflecting a requirement for an apically localized 5′-ectonucleotidase that cleaves the 5′AMP to its final active mediator, adenosine. Adenosine may be an important stimulus of intestinal Cl^- secretion after neutrophil migration into the intestinal lumen, as occurs in a crypt abscess [356].

Other immune effector cells

Other inflammatory cells have not been examined as extensively as the mast cell or neutrophil for their ability to regulate epithelial ion transport functions. However, it seems likely that such cells, including eosinophils, lymphocytes, and monocyte/macrophages, also participate in the control of electrolyte transport, because all of them can synthesize potent mediators that have either direct or indirect effects on the epithelium [344]. Such cells may also cooperate to influence the transport and barrier properties of the epithelium. For example, the activation of T cells with the consequent synthesis of interferon-γ and tumor necrosis factor (TNF)-α leads to down-regulation of Na^+,K^+-ATPase activity, a loss of epithelial barrier function, and a decrease in intestinal absorptive function [31,357]. Lymphocyte products also induce other mucosal immune cell populations to produce mediators that down-regulate epithelial transport and barrier function [358]. In fact, many studies reveal that the overall effect of ongoing inflammation is to down-regulate the transport function of the intestine, including Na^+ or NaCl absorption [190,359], and to render the epithelium more permeable. These effects, in sum, predipose to diarrhea, a common feature of intestinal inflammatory disorders. An important population of lymphocytes that are present in the intestine are the intraepithelial lymphocytes. These specialized cells reside within the epithelial layer and, although their activation has been demonstrated to elicit production of mediators that increase epithelial permeability, they have also been shown to be important in maintaining barrier function during microbial infection [360]. This protective role of intraepithelial lymphocytes may be the result of their ability to express tight junction proteins and thereby physically contribute to barrier function in times of inflammatory stress [360].

Paneth cells form part of the innate immune system and are localized at the base of intestinal crypts [56,57]. These cells synthesize a variety of antimicrobial products, including a family of cationic peptides known as cryptdins. Because these peptides are secreted into the crypt lumen by activated Paneth cells [361], their electrophysiological effects may also represent another mode of immune–epithelial interactions that modulate transport.

Epithelial cells themselves are widely considered to be an important component of the mucosal immune system. In conditions of inflammation or bacterial infection, enterocytes are induced to express major histocompatiblity complex (MHC) class I and II molecules and can thereby directly participate in antigen presentation to immune cells [362,363]. Intraepithelial lymphocytes appear to play an important role in the induction of this immune accessory function of epithelial cells [364]. Activated epithelial cells also produce cytokines and other chemotactic substances that recruit inflammatory cells to the mucosa, thereby promoting ongoing inflammatory responses [365].

Role of mesenchymal cells

Immune cells may also interact with the structural elements of the mucosa to control the function of the epithelium. The myofibroblast sheath underlying the epithelium is thought to act as a regulatory site whereby signals from elements in the lamina propria, including immune cells, can be amplified, suppressed, translated, or spatially restricted [366]. An example of such indirect regulation by myofibroblasts is demonstrated by the observation that coculture of epithelial cells with myofibroblasts up-regulates the secretory responses of the epithelial cells to products of both mast cells and neutrophils [366]. This effect may be attributable, in part, to the ability of the myofibroblasts to synthesize prostaglandin E_2. In turn, the capacity of the myofibroblast layer to synthesize this mediator may be selectively enhanced in the setting of local inflammation through the induction of cyclooxygenase 2 [367]. Endothelial cells lining the blood vessels within the mucosal layer may act in a similar manner in that they produce prostaglandins in response to inflammatory stimuli, which could stimulate epithelial secretion in a paracrine fashion [368].

Neural regulation

Another key regulatory mechanism for intestinal ion transport is that provided by the enteric nervous system. Various ion transport responses are modified in the absence of neuronal transmission, and electrolyte transport can likewise be stimulated by directly activating resident nerves using electrical stimulation. Neurotransmitters released by enteric nerve endings can bind directly to enterocytes, as described for acetylcholine [369], and can affect their function in much the same way as has been described for immune mediators. Enteric neurotransmitters identified as being able to alter intestinal ion transport are included in Table 14.2. Neurotransmitters may also have indirect effects on ion transport through their ability to release secondary hormones and mediators. In addition, the enteric nervous system appears to be able to respond to mediators released from endocrine or immune cells [370] or to lumenal secretagogues such as bacterial toxins and bile acids [371]. Neural regulation, particularly through cholinergic pathways, appears to be important in maintaining the basal ion transport tone of the intestine. The enteric nervous system probably is also critical for the integration of intestinal electrolyte transport with motility.

Activation of the enteric nervous system has various consequences depending on the segment of the intestine examined. In the duodenum, nerve stimulation evokes HCO_3^-

secretory responses [372]. In the jejunum, ileum, and distal colon, the most prominent effect is a stimulation of electrogenic Cl^- secretion [371]. In contrast, electrical field stimulation of the guinea pig proximal colon inhibits electroneutral Na^+ and Cl^- absorption [373]. Neural regulation of transport function is also likely to be important in coordinating net fluid transport with the passage of contents through the intestine. Thus, mucosal distention during peristalsis evokes the secretion of both fluid and mucus that serve to lubricate the mucosa and protect it from mechanical damage [374,375]. Neural regulation of epithelial function, in part mediated by central input, has also been implicated in pathophysiological settings. Rats subjected to acute systemic stress show significant increases in baseline secretion and permeability in their intestinal tissues [376]. Likewise, the enteric nervous system clearly plays a role in mediating at least a portion of the Cl^- secretory response to specific enteric pathogenic agents such as cholera toxin, *C. difficile*, and rotavirus [332,350,377].

Interactions between intercellular regulatory mechanisms

The epithelium can respond to signals from a variety of regulatory systems, including those supplied by the endocrine, neurocrine, and immune systems, and there is substantial interplay between the mediators produced. As noted previously, mediators that exert their effects on ion transport through different intracellular signaling mechanisms can display synergistic interactions when supplied to the epithelium in combination. Further, because neural input provides a basal tone to the epithelial cells, they can respond more readily to increased levels of other hormones or inflammatory mediators. Evidence of the importance of this neurally defined tone is provided by the observation that the secretory responses of intestinal tissues to a wide variety of substances are reduced by inhibitors of neurotransmission such as tetrodotoxin or atropine [378]. Thus, even for hormones and immune mediators that are known to have direct effects on the epithelium, the degree of neural input appears to be of paramount importance in setting the cellular sensitivity of the system [379].

The topic of intestinal neuroimmunophysiology [380] has aroused interest because of the intimate spatial relationships between the enteric nervous system and the immune elements of the intestine. These spatial relationships appear to have functional correlates. Ion transport responses stimulated by electrical field stimulation can be reduced by antagonists of inflammatory mediators; conversely, the stimulation of immune cells such as mast cells has measurable effects on enteric nerves and consequent indirect effects on ion transport [344]. Another indirect means whereby immune and inflammatory mediators have been shown to influence ion transport function is by the chronic regulation of epithelial responsiveness. Tissues from sensitized animals are more responsive to the Cl^- secretory effects of histamine and substance P [381]. Long-term exposure to mast cell products has similarly been shown to enhance the responsiveness of cultured colonic epithelial cells to the Cl^- secretagogues carbachol, VIP, and ST_a, without altering their basal electrical properties or their responsiveness to prostaglandins or adenosine [382]. Although the underlying mechanisms of these effects are not known, their existence suggests that the epithelium may be primed to display secretory responses in some settings of intestinal inflammation or infection. The idea that communication between nerves and the immune system is important in disease is supported by the observations that interactions between these systems are highly plastic and can undergo extensive remodeling in pathological states [383]. Sensitization primes the epithelium for rapid antigen transfer from the lumen through the expression of the low-affinity IgE receptor CD23 [384]. This would serve to further amplify abnormal transport responses in the setting of gastrointestinal allergies [384].

Other factors may indirectly influence fluid and electrolyte transport by intestinal epithelial cells. These include acid–base homeostasis [385], gastric and intestinal motility [386], lumenal flow rates [387], intestinal permeability [385], oncotic pressure of the blood, arterial pressure, venous pressure, plasma volume, lumenal pressure [388], and physical and psychological stress [389]. Some of these factors likely have important clinical implications. Retardation of the flow of intestinal contents achieved by the coordinated contraction of gastrointestinal smooth muscle allows more contact time for absorption and may thereby decrease stool volume. Antidiarrheal drugs, particularly the synthetic opiates, act mainly through their effects on gut motility, although neuronal, hormonal, and immunological influences are also involved. Intestinal permeability is increased in diseases that cause mucosal injury, such as inflammatory bowel disease and celiac disease [390]. In theory, an increase in intestinal permeability may allow oncotic and hydrostatic pressure gradients to exert greater effects on electrolyte transport. Increased permeability may also allow macromolecules, normally excluded by the epithelium, to diffuse more readily across the intestinal mucosa. This nonspecific increase in macromolecular permeability should be contrasted with the specific CD23-mediated increase in transcytotic antigen transfer previously suggested [384]. Nevertheless, both mechanisms may amplify the immune responses to transferred macromolecules and thereby further enhance immune regulation of epithelial function.

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C SECRETION AND ABSORPTION

15

Pancreatic secretion

Chung Owyang, John A. Williams

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The pancreas is an organ with exocrine and endocrine functions. The secretions of the exocrine pancreas, including digestive enzymes and bicarbonate, affect the digestion and absorption of nutrients. The endocrine pancreas releases hormones that regulate metabolism and the disposition of the breakdown products of food within the body. The combined exocrine and endocrine functions make the pancreas one of the most important and complex organs involved in the assimilation of food.

In humans and other mammals, the exocrine pancreas consists of clusters of acini that form lobules separated by loose connective tissue. Eighty percent or more of the pancreas consists of acini. Each acinus is a sphere composed of 20–50 pyramidal cells arranged with their broad bases around the circumference and their apices pointed toward a central lumen. Each acinus is drained by a ductule; the most proximal cells of the ductules, which extend into the lumen of the acinus, are called centroacinar cells. The ductules drain through a series of ducts of increasing caliber until the main ducts are reached.

Distributed within the pancreas are the islets of Langerhans, containing the cells of the endocrine pancreas. Morphological studies have revealed cell-to-cell contact between the exocrine and endocrine tissue and direct connections between the capillaries of the islets and the acini [1,2]. These morphological arrangements may reflect the regulatory influences of the islet hormones on the function of the exocrine pancreas and vice versa. Of the pancreatic hormones, glucagon, somatostatin, and pancreatic polypeptide inhibit pancreatic exocrine secretion [3–6]. Insulin potentiates the stimulatory effect of cholecystokinin (CCK) on pancreatic exocrine secretion [7]. In addition, exocrine pancreatic secretion can influence pancreatic hormone release.

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The control of pancreatic exocrine function is complex and highly regulated by neural and hormonal factors. The cloning of the CCK receptor with the subsequent recognition that the CCK_1 receptor was virtually absent from the human pancreas [8,9] emphasized the importance of regulation at extrapancreatic sites. The discovery that CCK acts on CCK_1 receptors on vagal afferent fibers to mediate pancreatic secretion [10–12], together with the observations that the most inhibitory mediators of pancreatic exocrine secretion target the dorsal vagal complex [9,13–16], led to a paradigm shift in the understanding of the control of pancreatic secretion and recognition that pancreatic secretion is under neurohormonal control rather than direct hormonal control.

Formation and composition of pancreatic juice

The human pancreas secretes about 1 L of juice daily, consisting mostly of water, electrolytes, and digestive enzymes. The morphological appearance of the different cells of the exocrine pancreas and the results of micropuncture studies suggest that the acinar cells secrete digestive enzymes and that the ductal cells are mainly responsible for an electrolyte secretion that is rich in bicarbonate [17]. This thesis is further supported by animal experiments in which the administration of a toxin or a special diet selectively destroys acinar or ductal cells. Administration of alloxan, which destroys ductal but not acinar cells, results in diminished secretion of fluid and bicarbonate [18]. Ethionine administration damages acinar cells with a concomitant reduction in enzyme secretion, but the secretion of fluid and electrolytes is relatively unaffected [18].

Water and electrolytes

Pancreatic electrolytes are secreted in a clear, alkaline fluid that is isosmotic with extracellular fluid. Evidence suggests that water enters the juice passively along osmotic gradients

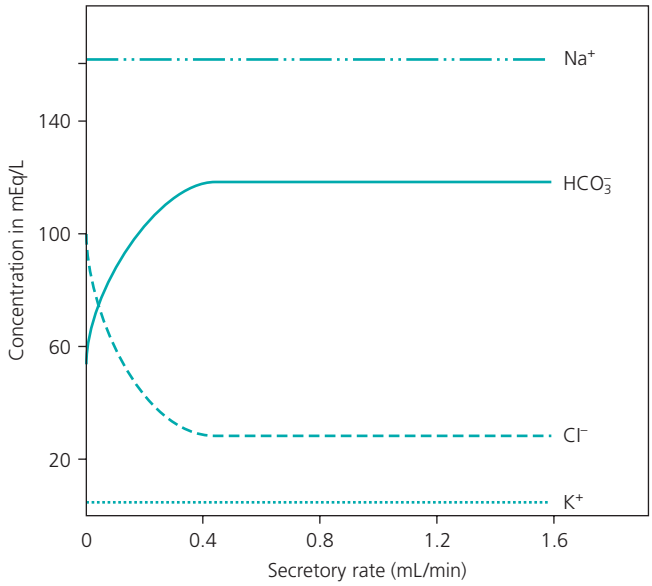

Figure 15.1 Relation of the secretory rate to the electrolyte composition of pancreatic juice.

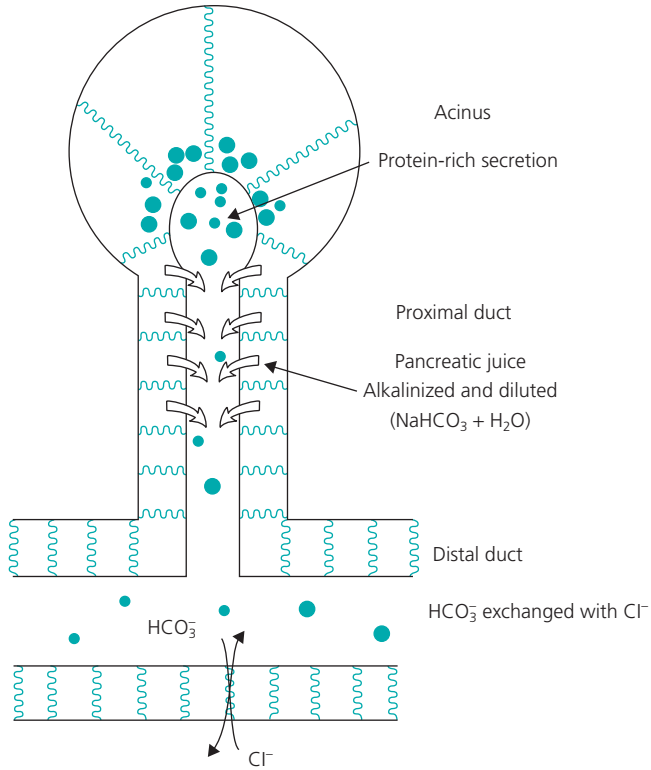

Figure 15.2 Ductal origin of secretin-dependent pancreatic HCO_3^- secretion. Open arrows indicate secretion of $NaHCO_3$ and H_2O from the proximal duct cells. Some of the secreted HCO_3^- is exchanged with Cl^- in the distal ducts (curved, solid arrows). From Raeder [19], with permission from Elsevier.

established by active secretion of electrolytes or other solutes. The major cations in the pancreatic juice are Na^+ and K^+; both are secreted at concentrations similar to their plasma concentrations. The concentrations of both cations are constant and independent of secretory rates (Fig. 15.1). The major anions in the pancreatic juice are HCO_3^- and Cl^-, the concentrations of which depend on flow rates. As the flow rate increases, the HCO_3^- concentration rises asymptotically, approaching a plateau value in humans of ~ 150 mEq/L at 30%–50% of the maximal secretory rate. Because the Cl^- concentration falls reciprocally with the increasing secretory rate, the sum of the two anions remains constant and about equal to the sum of Na^+ and K^+ at all secretory rates. In humans and other animal species, the pancreatic juices also contain Ca^{2+} (1–2 mEq/L) and traces of Mg^{2+}, Zn^{2+}, HPO_4^{2-}, and SO_4^{2-}.

The application of micropuncture and electrophysiological measurements and the use of fluorescent probes and ultrastructural analysis in the study of pancreatic duct cell function have provided important information on the mechanism of ductal electrolyte secretion. Comprehensive reviews of this topic have been published [19,20]. Micropuncture studies in rats, cats, and rabbits show that secreted HCO_3^- originates from the intralobular ducts and small interlobular ducts. In the large ducts, some of the HCO_3^- may be exchanged with Cl^- (Fig. 15.2). Evidence supporting a ductal origin of HCO_3^- in humans comes from studies demonstrating that the biochemical lesion of cystic fibrosis is a defect in cyclic adenosine monophosphate (cAMP) – regulated membrane Cl^- conductance in epithelial cells [21,22]. In the pancreas, this is associated with defective secretion of HCO_3^- [23]. Immunofluorescence studies show that the cystic fibrosis transmembrane conductance regulator (CFTR) is localized to the apical domain of centroacinar cells and intralobular duct cells [24]. CFTR is identical to the small conductance (4 pS) Cl^- channels in the apical plasma membrane of rat pancreatic duct cells, which are regulated by cAMP [25].

Electrophysiological studies have shown that by increasing Cl^- exit into the lumen, the CFTR Cl^- channels facilitate the function of a Cl^-/HCO_3^- exchange protein in the apical membrane. The molecular nature of this exchange has been characterized and the protein is known as SLC26AC. It normally transports two HCO_3^- ions into the lumen for each Cl^- ion absorbed [26]. The net result is recycling of Cl^- and secretion of HCO_3^- [20,27]. CFTR and SLC26AC exhibit intracellular protein–protein interactions, explaining why the exchanger fails to function in the absence of CFTR. The CFTR channel has a limited permeability to HCO_3^- under basal conditions, but when lumenal Cl^- is depleted, the channel switches its selectivity and becomes a major pathway for HCO_3^- exit into the lumen. An additional function of CFTR is to depolarize the duct cell, thereby "affecting" or "increasing" basolateral membrane transport (see discussion at the end of this section).

The mechanism operating at the basolateral membrane of the duct cell is becoming better understood. Originally, HCO_3^- was thought to be derived in duct cells from CO_2 under the influence of carbonic anhydrase, which is present in the duct epithelium [28]. Although H^+ extrusion is often shown to be mediated by a Na^+/H^+ exchanger in the basolateral membrane, this protein is known to play a role in basal cell pH regulation and to account for no more than 1%–2% of stimulated H^+ extrusion (i.e., equivalent to HCO_3^- uptake) [29]. Moreover, experimental evidence and mathematical models indicate that some form of energy input is necessary to obtain the secretion of 150 mM HCO_3^-. Unfortunately, the rat is a poor experimental model because it only secretes HCO_3^- at 70–90 mM. Two competing theories account for higher rates of HCO_3^- secretion in other species. Based on study primarily of pig pancreas, Raeder and colleagues presented evidence for a primary active proton efflux mediated by a vacuolar H^+-ATPase [19]. The ATPase at rest is located in a tubulovesicular compartment and inserted into the basolateral plasma membrane by exocytosis in response to stimulation by secretion [30]. The action of the ATPase to extrude H^+ allows HCO_3^- to accumulate in the cell and to exit by the apical mechanism just described. Colchicine, a microtubule depolymerizing drug, has been shown to block secretion-stimulated insertion of tubulovesicles into the basolateral plasma membranes, causing a 60% inhibition of secretin-invoked HCO_3^- secretion [31].

Details of the other mechanism emerged from work on isolated pancreatic ducts from guinea pigs, which are capable of secreting 150 mM HCO_3^-. Secretion was blocked by omitting Na^+ from the bath but not by inhibitors of Na^+/H^+ exchange or of the vacuolar H^+-ATPase [20]. After analyzing intracellular pH and Na^+ influx, Ishiguro and colleagues concluded that Na^+–HCO_3^- cotransport accounts for 75% of secretion-stimulated HCO_3^- secretion [32,33]. Their model established that the energy of the Na^+ gradient maintained by Na^+,K^+-ATPase on the basolateral plasma membrane [34] serves to drive HCO_3^- into the cell. This model substantiated earlier data demonstrating that fluid secretion from the perfused cat pancreas increases as a function of perfusate HCO_3^- [28].

Some details of the nature of the protein mediating Na^+–HCO_3^- transport have been described. A Na^+–HCO_3^- cotransporter (NBC) was originally cloned from amphibian kidney, and subsequently, at least four NBCs have been characterized molecularly. The NBC1 form has two variants, termed p and k for pancreas and kidney, which differ at the amino terminus as a result of alternate messenger RNA (mRNA) splicing [35]. pNBC1 has been localized to the basolateral membrane of pancreatic duct cells by immunohistochemistry [36]. The human pNBC1 analogue codes for a 1079-amino-acid protein, and when expressed in *Xenopus* oocytes it induces $NaHCO_3$ uptake [35]. The stoichiometry of Na^+ and HCO_3^- varies in different cells; in pancreatic duct cells it appears to be one Na^+ to two HCO_3^- [37]. Because the transporter is electrogenic, it is influenced by the Na^+ gradient, the HCO_3^- gradient, and the membrane potential. In normal duct cells, the transporter mediates HCO_3^- uptake, and this process is enhanced by depolarization induced by Cl^- exit through CFTR [38]. In this model, secretin activates CFTR and thus increases HCO_3^- uptake by way of the pNBC exchanger. The transcellular secretion of anions sets up a lumenal electronegative potential, enabling cations such as Na^+ and K^+ to reach the pancreatic juice by moving passively through the paracellular pathway driven by the electrical gradient that increases in response to stimulation [39]. A cellular model depicting these events in a pancreatic duct cell is shown in Fig. 15.3.

Enzymes

Depending on the species, the enzyme component of pancreatic juice is mixed in various proportions with the aqueous component. Human pancreatic juice contains a concentration of 0.7%–10% protein. Most proteins are enzymes and proenzymes; the remaining proteins are plasma proteins, trypsin inhibitors, and mucoproteins. The four major enzyme groups are amylolytic, lipolytic, proteolytic, and nucleolytic. The proteolytic enzymes, which include trypsinogen, chymotrypsinogen, procarboxypeptidase, and proelastase, account for most enzymes in the juice and are secreted as inactive proenzymes. After entering the intestinal lumen, trypsinogen is converted by enterokinase, an enzyme secreted by the duodenal mucosa, to the biologically active trypsin. Trypsin autocatalytically activates trypsinogen and converts chymotrypsinogen and other proteolytic enzymes into their active forms.

Pancreatic juice also contains a low concentration of trypsin inhibitor, a polypeptide that, at pH of 3–7, combines with and inactivates trypsin in a 1 : 1 ratio. Trypsin inhibitor also partially inhibits chymotrypsin. Trypsin inhibitor in the pancreas is thought to protect the organ against autodigestion by the small amounts of active trypsin within the pancreas. Because trypsin inhibitor is present in minute quantities, the proteolytic activity of fully activated pancreatic juice in the intestinal lumen is not inhibited. Unlike the proteolytic enzymes, amylase, lipase, and ribonuclease are secreted by the acinar cells in their active forms. Pancreatic juice also contains a 10-kDa peptide called colipase, which is essential for optimal lipolysis [40]. It facilitates lipase action by binding with bile salt–lipid surfaces to increase the interaction of lipase with triglyceride [41]. In the presence of bile salts, colipase lowers the optimal pH of lipase from 8.5 to 6.5, the normal pH in the proximal intestine.

Pancreatic enzymes are synthesized within acinar cells and packaged into zymogen granules [17,42]. The entire process, from synthesis to the point at which the enzymes are ready to be secreted into the lumen, takes about 50 min. Total enzyme synthesis is estimated at 20 mg/g dry tissue per hour or 10 million enzyme molecules per acinar cell per minute

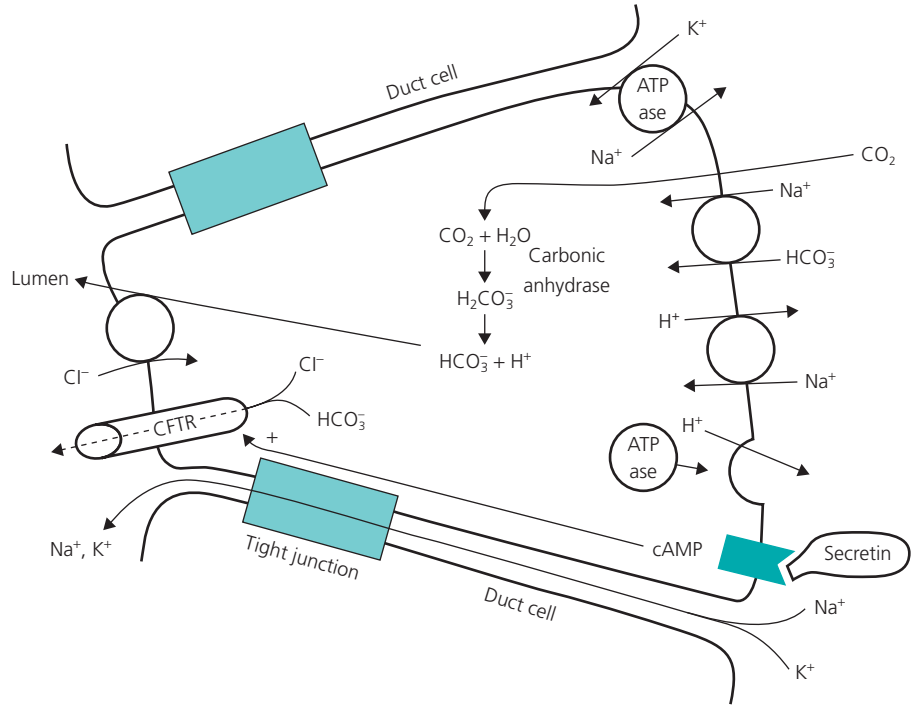

Figure 15.3 Cellular model of ion transport events in the pancreatic duct cells that cause the primary secretion of pancreatic juice. HCO_3^- enters the cell by means of the Na^+–HCO_3^- cotransporter on the basolateral membrane or by the diffusion of CO_2, which becomes hydrated to carbonic acid under the influence of carbonic anhydrase. Transport of H^+ out of the cell by the Na^+/H^+ exchanger or the vascular H^+-ATPase contributes to the provision of intracellular HCO_3^-. Other important components at the basolateral membrane are the secretin receptor, which activates the production of cAMP; a Na^+,K^+-ATPase, which maintains the low level of intracellular Na^+; and a K^+ channel (not shown), which facilitates K^+ exit from the cell. The apical membrane contains the cystic fibrosis transmembrane conductance regulator (CFTR) anion channel and a Cl^-/HCO_3^- exchanger (SLC26A6), which together bring about secretion of HCO_3^- and a net lumenal negative potential. At low lumenal Cl^- concentration, the CFTR channel switches from conducting Cl^- to conducting HCO_3^-. The transcellular electrical gradient between blood and lumenal fluid drives the secretion of Na^+ and K^+. The activation of the CFTR Cl^- channel also depolarizes the duct cells and thereby links the events at the apical membrane to the activation of Na^+–HCO_3^- cotransport at the basolateral membrane.

[43]. Studies in rats showed that CCK plays an important role in the regulation of gene expression of pancreatic enzymes [44]. Intraduodenal infusion of soybean trypsin inhibitor raises plasma CCK and increases trypsinogen I and chymotrypsinogen β mRNA levels fivefold after 48 h. In contrast, soybean trypsin inhibitor infusion has no effect on amylase mRNA levels. Similar effects on pancreatic enzyme mRNA levels are observed after intravenous infusion of CCK to plasma levels comparable to those obtained with soybean trypsin inhibitor. In addition to their effects on gene regulation, CCK and other hormones may exert posttranscriptional control to regulate the synthesis of specific digestive enzymes, because mRNA is translated by ribosomes [45].

Research has begun to reveal the mechanisms by which hormones, especially CCK and insulin, regulate protein synthesis at a translational level and account for the stimulation of pancreatic digestive enzyme synthesis with each meal. CCK and insulin can stimulate acinar protein synthesis in vitro without changes in mRNA [45]. These actions appear primarily at the initiation step in translation and involve the rate-limiting initiation factor eIF4E. Acinar secretagogues and insulin, through a series of steps most likely involving phosphatidylinositol 3-kinase (PI_3K) and the mammalian target of rapamycin (mTOR), lead to the phosphorylation of the binding protein for eIF4E, and thereby the release of eIF4E, which becomes incorporated into a complex that binds the mRNA 5′ cap [46,47]. Through a separate pathway, CCK also activates the phosphorylation of eIF4E, which increases its affinity for the mRNA cap. In addition, mTOR activates ribosomal S6 kinase ($p70^{S6K}$), thereby phosphorylating S6, which increases the translation of messages with terminal polypyrimidine tracts [48]. The importance of the mTOR pathway is shown by the fact that rapamycin can block the stimulation of acinar protein synthesis in vitro [46].

According to the classical model of Palade [49], amino acids are actively transported into the acinar cells, and protein synthesis occurs in the ribosomes. Pancreatic enzymes and a variety of other exportable proteins are synthesized with an amino acid terminal peptide extension called the *signal peptide*, which recognizes a receptor in the rough endoplasmic

membrane and allows the attachment of the ribosome to this membrane [50,51]. Translation is temporarily halted when the signal peptide emerges from the ribosomal subunit and interacts with the signal recognition particle, which is associated with the large ribosomal subunit. The protein–RNA complexes then interact with another protein on the endoplasmic reticulum membrane known as the docking protein [50]. Interaction between the signal recognition particle and the docking protein permits the completion of translocation of the nascent polypeptide into the rough endoplasmic reticulum lumen, after which the ribosomal subunits, signal recognition particle, and RNA dissociate from the endoplasmic reticulum while the protein crosses the endoplasmic reticulum membrane into the cisternae. In fact, most protein synthesis occurs on polysomes that consist of multiple ribosomes simultaneously reading down a single mRNA. When translation is stimulated, the number of ribosomes per polysome increases.

The need for the signal recognition particle and docking protein complex to form on the endoplasmic reticulum before the translation of RNAs containing a signal peptide codon can be completed has important physiological implications. This process ensures that potentially noxious proteins, such as proteinases, cannot gain access to the cytosolic compartment. It also provides a mechanism for sorting the proteins not destined for export from those that must be processed by the endoplasmic reticulum–Golgi pathway and packaged for later secretion [52]. Inside the cisternae of the endoplasmic reticulum, pancreatic secretory proteins interact with chaperones and undergo conformational changes, assuming tertiary and, in some cases, quaternary structures. These structural changes may account for the irreversible segregation of proteins within the rough endoplasmic reticulum. Abnormalities in this process result in the endoplasmic reticulum stress response [53].

The transfer of pancreatic enzyme proteins from the endoplasmic reticulum to the Golgi complex occurs within 20–30 min of synthesis [54]. Their transfer is mediated by vesicles arising from pinched-off transitional elements of the rough endoplasmic reticulum, which act as transport containers for the secretory proteins [54]. Further modification and concentration occur in the Golgi complex and may result partially from the interaction of the predominantly basic secretory proteins with polyanionic substances formed in the Golgi complex [55]. It is also facilitated by the relatively acidic pH within the Golgi complex. After their formation in the Golgi complex, secretory granules move to the apical portions of the acinar cell by a mechanism involving microtubules, where they remain until an appropriate neurohormonal stimulus triggers exocytosis.

Pancreatic enzymes from a single cell probably are secreted in a fixed ratio that is independent of the nature of the stimulus and of the rate of secretion but is determined at the time of synthesis. This phenomenon may be explained by the model proposed by Scheele and Palade [56], which states that secretory proteins are mixed together in the zymogen granule and discharged in parallel. Under certain experimental conditions, nonparallel secretion of pancreatic enzymes may occur. In humans, increasing the doses of CCK infusion results in pancreatic secretion characterized by a greater response of lipase than chymotrypsin concentrations, both of which are greater than the amylase concentration [57]. Similar nonparallel enzyme secretion was reported by Dagorn and colleagues in both rats and humans [58,59].

Several possible explanations for nonparallel secretion have been proposed:

- different rates of enzyme synthesis in response to different degrees of stimulation
- the existence of a soluble cytoplasmic pool of enzyme proteins that are in equilibrium with those contained in zymogen granules
- different enzymatic contents within populations of acinar cells.

Morphological studies show considerable differences in cell sizes in peninsular or teleinsular acinar cells [60]. This, coupled with the observation that the enzyme content and the ratio of amylase to chymotrypsin vary widely among granules taken from the same animal, supports the third hypothesis and suggests that nonparallel secretion is the result of exocytosis from heterogeneous cells within the pancreas [61,62].

Although much controversy exists regarding short-term deviations from parallel secretion, there is little doubt that long-term adaptation of enzymes to diet occurs in animals. Adaptation occurs in rats fed diets containing a preponderance of carbohydrate, protein, or fat, as indicated by increases in the pancreatic content, mRNA levels, and rates of synthesis and secretion of the appropriate class of hydrolytic enzymes by the pancreas [63]. Moreover, the dietary effects are thought to be mediated by specific hormones. Insulin mediates the increased amylase synthesis, and CCK released by protein increases the synthesis of proteases. Although such adaptive changes have not been reported in humans, preferential secretion of lipase occurs in the chronic renal failure associated with hypercholecystokininemia, which is consistent with the adaptive change found in animals receiving chronic administration of CCK [64].

Other biological substances

In addition to producing many digestive enzymes and secretory products that are necessary for nutrient digestion, the acinar cells also secrete a high-density lipoprotein-like substance containing caveolin-1, a membrane protein implicated in cholesterol trafficking and endocytosis [65]. Serine phosphorylation of caveolin-1 directs its entry into the secretory pathway rather than its insertion into the endoplasmic reticulum membrane [65]. Not only does pancreatic juice

contain a significant amount of caveolin-1, but also caveolin-1 is present in zymogen granules from isolated pancreatic acinar cells. Stimulation of the pancreas with CCK also resulted in a large increase in menaquinone-4 secretion, which was accompanied by a marked increase in protein and fluid secretion [66]. These results suggest that menaquinone-4 and caveolin-1 participate in the secretory pathway of acinar cells and have a role in digestive enzyme synthesis or processing.

Stimulation of pancreatic secretion

Mediation of postprandial pancreatic secretion has been ascribed mainly to the hormones secretin and CCK and to vagovagal reflexes that activate cholinergic postganglionic neurons in the pancreas. Considerable knowledge has been gained about these classical regulatory mechanisms, but the picture has become increasingly complicated by evidence suggesting that other regulatory peptide hormones and neurotransmitters are also involved.

Hormonal mechanisms

Secretin

Secretin is synthesized by S-type enteroendocrine cells of the small intestine and is released during a meal. Mutoh and colleagues identified the expression of transcription factor BETA2/NeuroD in the intestinal mucosa cells of mice that also express secretin [67]. This may be the first transcription factor identified that specifically activates cell type-specific expression of an intestinal hormone gene. Secretin is the most potent and efficacious stimulant of pancreatic fluid and HCO_3^- secretion in humans and all other species tested. Duodenal pH is the major regulator of secretin release. A pH of 4.5 is the threshold for secretin release and stimulation of pancreatic HCO_3^- secretion occurs at pH 4.5 [68,69]. Below this pH, pancreatic HCO_3^- output is related to the total amount of titratable acid presented to the duodenum. The increase in postprandial secretin levels in humans amounts to only a few picomolar increments because of the buffering of an appreciable amount of acid produced in the stomach by food and the neutralization of the remaining acid entering the duodenum by pancreaticobiliary secretion [70]. The pH of gastric chyme in the first portion of the duodenum is in the range of 4–5.0 [71]. However, dilute HCl infused into duodenum at a rate of 2–4 mmol/h can increase plasma secretin significantly in humans and dogs [70–73]. H^+ bound to solid food particles may be a potent stimulus of pancreatic HCO_3^- secretion [68]. The slow diffusion of H^+ from the particles stimulates pancreatic HCO_3^- secretion by triggering H^+ receptors located in the more distal small intestine. The mechanism by which HCl stimulates the release of secretin is unclear. Studies of rats indicate that H^+ may release a secretin-releasing factor into the upper intestinal lumen to stimulate the release of secretin [74]. This factor has a molecular weight of less than 5 kDa and is heat stable and trypsin sensitive. The secretin-releasing peptide(s) has not been completely characterized, but investigators have reported that purified porcine pancreatic phospholipase A_2 (PLA_2) stimulates secretin release from rat intestinal secretin-producing cells [75]. Furthermore PLA_2-like immunoreactivity is present in the intestinal mucosa and is released into the lumen during duodenal acidification [76]. Pretreatment with a specific anti-PLA_2 antiserum abolishes the secretin-releasing bioactivity of the intestinal acid perfusate, suggesting that intestinal PLA_2 is a secretin-releasing peptide. Secretin-producing cells appear to have acid-sensing ion channels. Hence, lumenal acid likely stimulates the release of secretin by more than one mechanism.

Nonacid factors may play a role in the postprandial release of secretin. Among the major components of a mixed meal, fatty acids such as oleic acid and other digestive products of fat can increase plasma secretin levels and pancreatic HCO_3^- secretion [77,78]. Bile in the upper small intestine can also stimulate the release of secretin [79]. However, the physiological importance of these nonacid factors in the release of secretin is questionable, because postprandial plasma secretin does not increase in subjects who are achlorhydric or in healthy subjects in whom meal-induced acid secretion is neutralized with $NaHCO_3$.

The pancreas appears to be sensitive to the small amounts of secretin released into the circulation after a meal. Secretin given in a dose that mimics postprandial plasma secretin levels can stimulate pancreatic secretion of water and HCO_3^- [80,81]. Administration of secretin antiserum to conscious dogs greatly reduces the pancreatic HCO_3^- response to a meal [82]. The sites where secretin acts to stimulate pancreatic secretion have not been fully identified. In vitro animal models clearly show that secretin stimulates HCO_3^- secretion by isolated ducts or duct fragments [32,33]. With the use of radiolabeled ligand ^{125}I-labeled secretin and autoradiography, a secretin-binding site was demonstrated on pancreatic acini and duct cells [83]. Binding was not evident on pancreatic islets or vascular structures. This supports, but does not necessarily prove, the idea that secretin acts directly on the pancreas to stimulate pancreatic secretion. Studies have shown that the effect of physiological doses of secretin on exocrine pancreatic secretion is highly sensitive to atropine. In fact, similar to CCK, vagal afferent pathways mediate the effect of secretin on pancreatic exocrine secretion, gastric acid secretion, and motility [84–86]. Receptor autoradiography, immunocytochemical studies, and electrophysiological recordings strongly support the presence of secretin receptors on vagal afferent fibers [87–89]. Close intraarterial injection of secretin (2.5 and 5 pmol) elicits dose-dependent increases in neuronal firing in a subgroup of nodose ganglia neurons. This same group of neurons also contains high-affinity CCK_1 receptors [89]. Injection of a subthreshold dose of CCK-8 (5 pmol) greatly enhances the neural responses to 5 pmol

secretin. This synergistic interaction helps to explain the robust postprandial secretion of pancreatic HCO_3^- and enzyme despite an only modest postprandial increase in levels of CCK and secretin.

Cholecystokinin

CCK is the other gut hormone that plays an important role in pancreatic secretion. It is released by hydrolytic products of digestion such as amino acids and fatty acids. In dogs, proteins do not stimulate pancreatic secretion, but crude enzyme digests of protein that contain peptides and amino acids are effective stimulants of pancreatic enzyme secretion, presumably through the release of CCK [90,91]. Undigested fat is ineffective, but products of lipolysis such as fatty acids are the most potent stimulants of CCK release [78]. Factors that influence the CCK response to fatty acids include their chain length, degree of saturation, concentration, and total load [92].

The mechanism by which nutrients stimulate the release of CCK is not clear. In species such as the rat, in which feedback inhibition of pancreatic enzyme secretion occurs, CCK release may be mediated by a trypsin-sensitive CCK-releasing peptide [93]. Peptone in the duodenum stimulates serotonin (5-hydroxytryptamine, 5-HT) release from the intestinal enterochromaffin cells. The 5-HT released into the submucosa activates the sensory substance P neurons. Signals would then be transmitted to cholinergic interneurons and to epithelial CCK-releasing peptide-containing cells by way of cholinergic secretomotor neurons [93]. In this manner, CCK release may be controlled by the level of active intralumenal proteases [94–96]. Proteins, the major food stimulants of CCK secretion in rats, may bind or inhibit intralumenal endopeptidases, which would otherwise inactivate the CCK-releasing peptide [97]. Research indicates that ethanol-stimulated CCK release is also mediated by a CCK-releasing peptide [98]. In dogs, the mechanism of CCK release may be nutrient dependent [99]. CCK release in response to sodium oleate, but not tryptophan or HCl, is atropine sensitive [99]. It is conceivable that the release of CCK by sodium oleate may be mediated by a CCK-releasing peptide, the release of which is mediated by cholinergic input [97], and further, tryptophan or HCl may stimulate CCK cells directly.

Under fasting conditions, the plasma CCK levels are low, averaging about 1 pmol/L in humans [100–102]. After the ingestion of a meal rich in protein and fat, the CCK concentration increases to 6–8 pmol/L within 10–30 min, followed by a gradual decline to basal levels during the ensuing 3 h [101,102]. Several molecular forms of CCK appear to be released into the circulation postprandially, including CCK-58, CCK-33, CCK-22, CCK-12, and CCK-8 [103]. Their relative contributions to the CCK activity of plasma in basal and stimulated states remain to be determined. In rats, CCK-58 appears to be the only detectable endocrine form of CCK in the circulation [104].

CCK plays an important role in the stimulation of pancreatic enzyme secretion during the postprandial state. The infusion of physiological doses of CCK produces the same levels of pancreatic enzyme secretion as during the postprandial state [105]. Furthermore, the administration of the potent CCK antagonists lorglumide or MK-329 produces a 50%–60% inhibition of meal-stimulated pancreatic secretion in dogs [106]. These antagonists have similar effects on meal-stimulated pancreatic secretion in humans [107].

CCK can stimulate fluid and HCO_3^- secretion to some extent [108]. The effect on HCO_3^- secretion is weak but physiologically relevant because CCK potentiates the action of secretin on the pancreas [109]. In intact dogs and humans, CCK-stimulated pancreatic enzyme secretion is not potentiated by secretin [105,110,111].

The mechanisms by which CCK acts to stimulate pancreatic enzyme secretion remain controversial. In vitro studies using dispersed pancreatic acini demonstrate that CCK-stimulated amylase release is insensitive to atropine or tetrodotoxin, indicating a direct action on pancreatic acini [112]. The effects of cholinergic agonists and CCK on isolated preparations of pancreatic acini are additive, suggesting that CCK may stimulate enzyme secretion by a cholinergically independent mechanism [113]. However, in vivo studies of humans and dogs have shown that pancreatic secretion stimulated by CCK can be blocked by atropine, implying the involvement of cholinergic pathways [114–116]. Furthermore, enzyme output in response to low doses of CCK is reduced in patients after vagotomy compared with normal controls [117]. It appears that CCK can act through atropine-sensitive and atropine-insensitive pathways to stimulate pancreatic exocrine secretion. Human studies have shown that infusions of CCK-8 able to produce plasma CCK levels similar to those observed after ingestion of a standard mixed meal stimulate pancreatic enzyme output predominantly in an atropine-sensitive fashion [114]. Furthermore, studies in rats indicate that physiological doses of CCK act through stimulation of vagal afferent pathways originating from the duodenal mucosa [118] (Fig. 15.4). CCK receptors in the rat vagus nerve have been detected using in vitro receptor autoradiography [119]. As in pancreatic acini, vagal CCK receptors exist in both high-affinity and low-affinity states [11,12,120]. Under physiological conditions, CCK appears to act through high-affinity vagal CCK_1 receptors to mediate pancreatic enzyme secretion [12]. In contrast, the effect of CCK on satiety is mediated by vagal low-affinity CCK_1 receptors [10]. These findings suggest that different affinity states of the vagal CCK receptors mediate different digestive functions. The vagal neurotransmitter used by the CCK_1 receptor to activate the brainstem is likely to be glutamate [121,122], as it has been demonstrated that CCK-8 increases glutamate release from nerve terminals onto neurons of the nucleus tractus solitarius pars centralis (cNTS). With the use of whole cell patch-clamp techniques in thin brainstem slices, it has

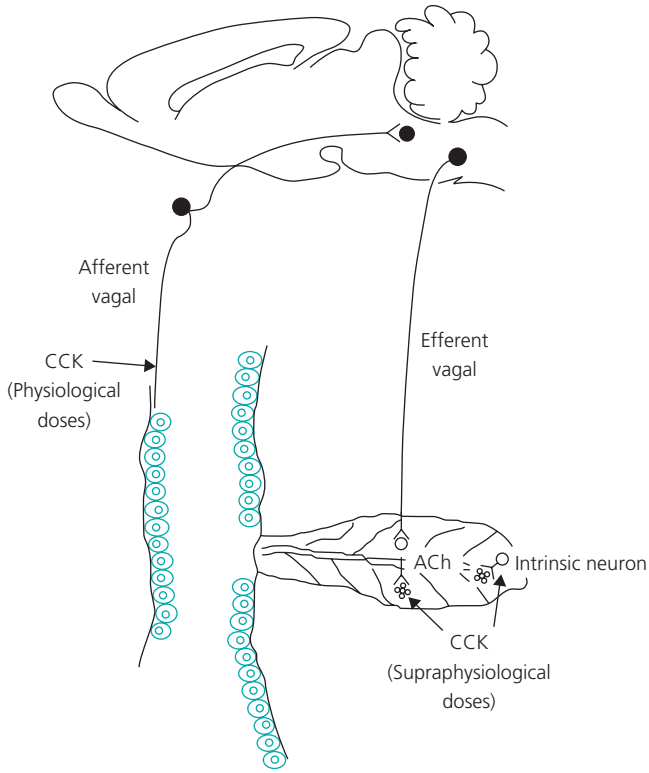

Figure 15.4 Sites and mechanisms of action of cholecystokinin (CCK) to stimulate pancreatic enzyme secretion. Dosages of CCK-8 that produce physiological plasma CCK levels act through stimulation of the vagal afferent pathway, which originates from the gastroduodenal mucosa. In contrast, dosages that produce supraphysiological plasma CCK levels act on intrapancreatic neurons and, to a lesser extent, on pancreatic acini. ACh, acetylcholine. From Li & Owyang [118], with permission from the American Society for Clinical Investigation.

been observed that CCK-8 increases the frequency of spontaneous and miniature excitatory postsynaptic currents in 43% of the cNTS neurons by way of a presynaptic mechanism [123]. In slices from deafferented rats, there was a 50% reduction in the number of cNTS neurons receiving glutamatergic inputs responding to CCK-8 [123]. This suggests that the central terminals of vagal afferent terminals may not be the sole sites for the action of CCK-8 in the brainstem. Although CCK does not cross the blood–brain barrier, the NTS is a circumventricular organ with a leaky blood–brain barrier, fenestrated capillaries, and an enlarged perivascular space that allow the passage of large molecules [124–126]. It is therefore conceivable that CCK may act on other sites of the brainstem, such as the NTS and the dorsal motor nucleus of the vagus (DMV) [123,127,128]. Electrophysiological studies show that CCK-8 induces a concentration-dependent excitation in 60% of pancreas-projecting DMV neurons [127]. This action is mediated by the closure of a K^+ conductance. Under physiological conditions, CCK seems to stimulate postprandial pancreatic enzyme secretion through cholinergic pathways rather than through direct action on the pancreatic acinar cell. Both M_1 and M_3 muscarinic receptors on the pancreatic acini appear to mediate these responses [129,130].

The molecular cloning of the CCK receptor gene and the subsequent recognition that its expression is virtually absent in the human pancreas [8,131] further supported the possibility that CCK acts at an extrapancreatic site. One study indicated that human acini did not respond to CCK agonists, although they responded to a muscarinic agonist [8]. In contrast, the cells responded to CCK agonists after adenovirus-mediated gene transfer of CCK_1 receptors [8]. Quantitative reverse transcriptase–polymerase chain reaction showed that the message levels for CCK_1 receptors were ~ 30-fold lower than those for CCK_2 receptors, which were ~ 10-fold lower than those for M_3 muscarinic receptors. In situ hybridization did not detect CCK_1 receptor mRNAs in adult human pancreas. These observations indicate that human pancreatic acinar cells do not respond to CCK receptor agonists because the level of receptor expression is insufficient.

Serotonin

Intestinal 5-HT appears to play an important role in the mediation of postprandial pancreatic secretion [132–135]. A large amount of 5-HT is present in the mammalian gastrointestinal tract. Serotonergic neurons in the intestinal myenteric plexus may mediate local reflexes. However, the major source of 5-HT in the intestine appears to be gastrointestinal mucosal enterochromaffin cells [136]. Many 5-HT-containing enterochromaffin cells exist in the proximal duodenum and have a morphology consistent with a "sensory" paracrine role [137]. 5-HT is released in response to a wide variety of stimuli [136], including acidification of the duodenum [136], instillation of hypertonic glucose, sucrose, or maltose solutions [132,138], vagal stimulation [139], and mechanical stimulation [140]. Morphological studies have shown that vagal terminal branches come in close contact with the basal lamina, but do not appear to penetrate it [141]. Other studies have shown that 5-HT may increase the discharge of vagal afferent fibers from the stomach and proximal intestine in ferrets [142,143] and may stimulate vagal nodose ganglia activity in rats [133]. This in turn stimulates pancreatic secretion by way of the vagovagal reflex mediated by a cholinergic efferent pathway [132]. 5-HT appears to activate two different groups of afferent fibers innervating the rat jejunum [144]. One group of mucosal nerve fibers is directly activated by stimulation of $5\text{-}HT_3$ receptors and another group responds to contractile activities induced by stimulating $5\text{-}HT_{2A}$ receptors on smooth muscle cells (i.e., mechanosensitive afferents). Direct physiological recordings of the rat nodose ganglia have shown that lumenal factors such as intestinal osmotic stimuli and perfusion of carbohydrates elicit powerful vagal nodose responses that are antagonized by the $5\text{-}HT_{3/4}$ antagonist tropisetron or the $5\text{-}HT_3$

antagonist granisetron [133]. Parallel studies in rats show that lumenal factors such as osmolarity and disaccharides stimulate pancreatic secretion by activating 5-HT_3 receptors whereas mechanical stimulation activates both 5-HT_3 and 5-HT_2 receptors on mucosal afferent fibers in the intestine to induce pancreatic secretion [132]. Pharmacological depletion of 5-HT stores using *p*-chlorophenylalanine, an inhibitor of 5-HT synthesis, abolishes nodose neuronal responses stimulated by lumenal factors [133]. In contrast, pretreatment with 5,7-dihydroxytryptamine, a specific neurotoxin that destroys neurons containing 5-HT without affecting mucosal cells that contain 5-HT, does not affect these responses [133]. These observations suggest that the nodal neuronal responses to lumenal osmolarity and the digestion products of carbohydrates depend on the release of endogenous 5-HT from the mucosal enterochromaffin cells, which acts on 5-HT_3 receptors on vagal afferent fibers. These studies indicate that lumenal stimuli induce 5-HT release, which, in turn, activates 5-HT_3 receptors on mucosal vagal afferent terminals. In this manner, 5-HT acts as a paracrine substance to stimulate pancreatic secretion by way of a vagal cholinergic pathway. It appears that both 5-HT and CCK evoke pancreatic enzyme secretion by stimulating vagal afferent pathways that originate in the duodenal mucosa. These primary vagal afferent neurons contain mainly glutamate and substance P [87], which, on release, stimulate the NTS and subsequently the DMV in the brainstem to mediate postprandial enzyme secretion.

Both 5-HT and CCK are the principal stimulators of postprandial pancreatic secretion. Studies in rats indicate that administration of a CCK_1 receptor antagonist inhibits 54% of postprandial pancreatic protein secretion. The combination of the CCK_1 receptor antagonist and a 5-HT_3 antagonist almost completely abolish exocrine pancreatic secretion [132], suggesting that 5-HT-dependent pancreatic stimulants account for about 50% of postprandial pancreatic secretion.

There is synergistic interaction between vagal CCK and 5-HT receptors in the mediation of pancreatic secretion [135]. Infusion of a subthreshold dose of CCK potentiates vagovagal reflex-mediated pancreatic secretion stimulated by lumenal 5-HT-dependent factors. Discharge recordings of single vagal primary afferent neurons innervating the duodenum reveal distinct groups of nodose ganglia neurons that possess only high- or low-affinity CCK receptors or 5-HT_3 receptors [145]. In one group of nodose neurons, previous exposure to lumenal 5-HT enhanced the subsequent response to a subthreshold dose of CCK. This synergistic interaction provides an explanation of how a small increase in the plasma CCK level is sufficient to produce a robust postprandial pancreatic secretion.

Pancreatic HCO_3^- secretion and secretin release in response to duodenal acidification in rats are inhibited by the 5-HT_3 antagonist ondansetron and the 5-HT_2 antagonist ketanserin[146]. In addition, pancreatic fluid and HCO_3^- secretion stimulated by exogenous secretin are also inhibited by these 5-HT antagonists. Thus, 5-HT appears to regulate acid-stimulated exocrine pancreatic secretion by modulating both the release and action of secretin through two 5-HT receptor subtypes.

Under certain experimental conditions, activation of the 5-HT pathway may inhibit pancreatic exocrine secretion. It has been reported that in isolated guinea pig pancreatic ducts, 5-HT inhibits basal and secretin- or acetylcholine-stimulated fluid secretion. This effect is antagonized by a 5-HT_3 agonist, indicating the involvement of a direct 5-HT_3 inhibitory pathway [147]. In contrast, administration of the 5-HT_3 antagonist azasetron in rats stimulates pancreatic secretion of fluid and protein [148]. Atropine but not vagotomy abolishes this effect, suggesting the involvement of a vagus-independent cholinergic pathway. Researchers have confirmed that guinea pig pancreatic ducts have 5-HT containing cells and identified 5-HT_3 receptors on their basolateral cell membranes [146]. These investigators proposed a distention-stimulated negative feedback on fluid secretion by pancreatic duct cells.

Other hormones and stimulatory factors

Insulin plays a significant role in modulating exocrine pancreatic secretion [7]. Studies have demonstrated that insulin potentiates the secretory response to secretin plus CCK [149], and that ouabain, an inhibitor of Na^+,K^+-ATPase activity, abolishes this stimulatory action of insulin. Insulin potentiates the stimulatory effect of secretin on Na^+,K^+-ATPase activity, without affecting the binding of secretin to pancreatic acini. Physiologically, this action of insulin is important because immunoneutralization experiments in conscious rats show that pancreatic secretion of water, HCO_3^-, and protein stimulated by a meal or by a combined intravenous administration of physiological doses of secretin and CCK-8 is markedly reduced when the circulating insulin is neutralized with a rabbit anti-insulin antibody [150]. Similar observations have been made studying an isolated perfused rat pancreas preparation. These studies indicate that insulin is needed locally for the action of secretin and CCK on the exocrine pancreas. Pancreatic enzyme secretion is often reduced in human diabetics who have no overt pancreatic disease [151]. Similar observations have been made in rats with streptozotocin-induced diabetes [152]. Because physiological doses of CCK act through vagal afferent pathways, it is conceivable that the potentiation between CCK and insulin occurred at the level of the nodose ganglia.

In view of the structural similarity between CCK and gastrin (see Chapter 4), it is not surprising that gastrin stimulates pancreatic enzyme secretion. In the dog, gastrin is about one-third as effective as CCK on a molar basis in stimulating the pancreas. Irrigation of a canine antral pouch with peptone and liver extracts at neutral pH stimulates pancreatic protein secretion, but irrigation with acidified solutions does not

[153]. The stimulatory effects of the protein solution may be caused by the release of gastrin, and the inhibitory effects of acidification may be the result of the suppression of gastrin release. The evidence, however, is indirect; it is unlikely that the postprandial increase in plasma gastrin is sufficient to significantly stimulate pancreatic secretion. In other species, gastrin has much less effect on pancreatic enzyme secretion.

Bombesin (i.e., gastrin-releasing peptide in mammals), a polypeptide isolated from the skin of frogs and the human alimentary tract, stimulates pancreatic secretions that contain small amounts of HCO_3^- and high concentrations of enzymes in humans [154,155]. Bombesin can act directly on the pancreas through specific receptors identified on pancreatic acinar cells [156]. It has been suggested that bombesin exerts its stimulating effect on the exocrine pancreas indirectly by promoting the release of CCK from the mucosa of the small intestine [157]. In other systems, bombesin has been reported to exert its effect by way of a cholinergic pathway [158]. Bombesin stimulates amylase release from rat pancreatic lobules through the release of acetylcholine from intralobular nerves [159]. In contrast, in vivo studies indicate that the action of bombesin in rats is probably direct, because the combined administration of atropine and the CCK antagonist proglumide does not affect pancreatic protein output stimulated by bombesin [160]. Similarly, the stimulatory action of bombesin on pancreatic secretion in humans is not affected by the CCK receptor antagonist loxiglumide [161]. The physiological importance of bombesin in pancreatic secretion was questioned by studies demonstrating that bombesin receptor antagonists do not influence postprandial enzyme secretion in intact rats [162].

The tridecapeptide neurotensin has been shown to stimulate pancreatic secretion in humans and dogs [163,164]. In rats, the mechanism of stimulation appears to be neurally mediated, involving capsaicin-sensitive sensory fibers and cholinergic vagal efferent pathways [165]. Neurotensin is released by intestinal fatty acids, raising the interesting possibility that neurotensin may play a significant role in mediating pancreatic secretion stimulated by fat [164]. However, exogenous infusion of neurotensin in doses that stimulate pancreatic secretion results in a plasma level much higher than that measured after a normal meal [163,164]. Neurotensin stimulates HCO_3^- secretion but decreases enzyme secretion stimulated by secretin and cerulein, a CCK analogue, in humans [163]. These observations do not support a role for neurotensin as a regulator of meal-stimulated pancreatic secretion.

Ghrelin, a novel acylated peptide localized in endocrine cells of the stomach and neurons of the hypothalamic arcuate nucleus [166–168], has been shown to stimulate pancreatic secretion [169]. It acts as an endogenous ligand for the growth hormone secretagogue receptor [166–168], which is found throughout the body, in the pituitary, hypothalamus, stomach, heart, lung, intestine, adrenal cortex, adipose tissue, immune system, human breast carcinoma, and pancreatic islet cells. Growth hormone secretagogue receptor mRNA is expressed in rat pancreas and in the exocrine pancreatic-derived cell line, AR42J [170]. Administration of ghrelin causes a dose-dependent increase in intracellular calcium. However, in vivo rat studies indicate that ghrelin stimulates pancreatic secretion by acting centrally through the vagal cholinergic pathways [169]. Pretreatment with atropine or hexamethonium or acute vagotomy, but not perivagal application of capsaicin, abolishes pancreatic secretion in response to intravenous administration of ghrelin. Furthermore, selective ablation of the area postrema blocks the pancreatic response to ghrelin. Immunocytochemical staining shows a marked increase in the number of cFos-expressing neurons in the area postrema, the NTS, and the DMV after intravenous infusion of ghrelin; selective ablation of the area postrema eliminates this increase [169]. These observations suggest that circulating ghrelin gains access to the brainstem vagal circuitry by way of the area postrema, which represents the primary target on which peripheral ghrelin may act as an endocrine substance to stimulate pancreatic secretion. Further studies are needed to determine whether endogenous ghrelin can reach the central nervous system in sufficient concentrations to interact with the brainstem circuits to regulate pancreatic secretion.

Nitric oxide (NO), a ubiquitous substance that is present in the neurons and vascular endothelium of the pancreas [171], also appears to play a significant role in regulating pancreatic secretion. In rat, inhibition of NO production with N^G-nitro-L-arginine methyl ester (L-NAME) reduces basal amylase secretion by 60% [172]. A NO synthase (NOS) inhibitor also inhibits pancreatic enzyme secretion in response to a meal, duodenal infusion of acid, or intravenous infusion of physiological doses of secretin or CCK in anesthetized and conscious rats [173]. In humans, graded doses of the NOS inhibitor, N^G-monomethyl-L-arginine, dose-dependently reduced pancreatic enzyme secretion stimulated by secretin and cerulein [174]. Because NOS inhibition has no effect on amylase release nor on the changes in intracellular Ca^{2+} concentration in rat pancreatic acini stimulated by carbachol and CCK-8 [175], the effect of NO on exocrine pancreatic secretion is likely to be indirect. NOS is abundant in intrapancreatic nerves [176] and ducts, the enteric nervous system, and the vagus nerve [177]. The source of NO that mediates the action of CCK and secretin on pancreatic secretion is not clear.

Administration of L-NAME reduces CCK-stimulated pancreatic microvascular blood flow and at the same time decreases pancreatic fluid and protein output in cats [178]. This observation may have clinical importance, because inadequate blood flow has been associated with clinical pancreatitis. It has been reported that administration of a NOS inhibitor in rats with edematous pancreatitis causes a decrease in pancreatic blood flow and exacerbates cerulein-induced

pancreatitis [179]. Conversely, treatment with the NO donor L-arginine before and after cerulein injection increases pancreatic blood flow and reduces the severity of cerulein/water-immersion-induced hemorrhagic pancreatitis. These observations suggest that NO may have a protective role against the development of pancreatitis, possibly because it increases pancreatic blood perfusion.

Neural mechanisms

Parasympathetic nervous system

The pancreas is innervated by parasympathetic and sympathetic nerve fibers. The parasympathetic fibers pass to the pancreas directly through the vagus nerves and indirectly through the celiac ganglion, the splanchnic nerves, and perhaps through the intramural plexus of the duodenum.

The functional effect of vagal stimulation of the pancreas varies greatly with the species and with the experimental conditions [180]. In rat, both CCK- and non-CCK-mediated lumenal stimuli evoke pancreatic enzyme secretion through stimulation of a vagal afferent pathway originating from the duodenal mucosa [135,181]. Acute vagotomy or atropine completely abolishes pancreatic secretion stimulated by these agents [135,181]. In dog and rabbit, vagal stimulation has a small stimulatory effect on the output of enzymes, but the increase is smaller in magnitude than with CCK stimulation. This response persists after removal of the stomach and intestine, indicating a direct stimulatory effect on the pancreas. In cat, stimulation of the vagus results in some increase in the secretion rate that does not depend on hormones. Atropine blocks the secretion of enzymes but has no effect on HCO_3^- secretion. In pigs, vagal stimulation results in copious secretion of pancreatic juice with high levels of enzymes and HCO_3^-, even after extirpation of the stomach and intestine.

In humans, the vagus appears to play an important role in the mediation of pancreatic secretion. Insulin-induced hypoglycemia, which is presumed to stimulate the vagus centrally, augments secretin-stimulated pancreatic protein output [182]. Vagotomy reduces the HCO_3^- secretory response to exogenous hormones. Maximal enzyme secretion is not significantly affected, but the sensitivity of the pancreas to submaximal doses of CCK is decreased [117]. Vagotomy reduces pancreatic enzyme responses to intestinal stimulants and food [117,183]. It seems that the cholinergic stimulation primarily modulates the action of gut peptides on pancreatic secretion but has no physiologically relevant effect on the release of CCK or secretin [184].

There are volume receptors and osmoreceptors in the human duodenum. Stimulation of these receptors by distention or administration of a hyperosmolar solution elicits a pancreatic enzyme response mediated by cholinergic neurons [185,186]. Increased firing rates in peripheral afferent vagal neurons and in central sites have been recorded after gastric distention and intestinal perfusion with amino acids and HCl [187–189].

In addition to glutamate, other neurotransmitters in the central nervous system are likely involved in the regulation of pancreatic secretion. Microinjection of the thyrotropin-releasing hormone analogue in the dorsal vagal complex of rats stimulates pancreatic juice flow and enzyme output in a dose-dependent manner [190]. Vagotomy and atropine eliminate this stimulatory effect, indicating that thyrotropin-releasing hormone modulates pancreatic exocrine secretion through the dorsal vagal complex.

Intrapancreatic postganglionic cholinergic neurons regulate enzyme and HCO_3^- secretion. These neurons are activated by central input during the cephalic phase and by vagovagal reflexes initiated by gastric- and intestinal-phase stimulation. Acetylcholine released by the intrapancreatic neurons may act directly on acinar cells or potentiate the action of secretin on HCO_3^- secretion from duct cells in vitro. The interaction of acetylcholine and CCK is additive. The enteropancreatic reflex may also play a role in mediating postprandial enzyme secretion [186]. This is especially important after chronic vagotomy [191].

Sympathetic nervous system

Adrenergic innervation of the pancreas occurs mainly through the splanchnic nerves. In the pancreas, most of the fibers are distributed to the blood vessels, and a few pass to the acini or ducts [109]. One laboratory has shown that rabbit pancreatic ganglia contain high levels of norepinephrine and dopamine [192]. Further, the pancreatic ducts also contain very high amounts of epinephrine and moderate amounts of norepinephrine and dopamine. The physiological significance of these findings remains to be determined.

Activation of the splanchnic nerves usually inhibits exocrine and endocrine pancreatic secretion; stimulation of the nerves decreases the response to pancreatic stimulants, but splanchnicectomy increases it [109,193]. The pancreatic inhibitory effect of splanchnic nerve stimulation appears to be synchronous with and dependent on the intense vasoconstriction that is caused by stimulation of the β-adrenergic receptors on blood vessels. In isolated guinea pig pancreatic acini, norepinephrine alone has no effect on the response to submaximal concentrations of CCK-8 [194]. Epinephrine produces a modest stimulation of enzyme output in mouse and rat pancreas in vitro, and the stimulatory effect is inhibited by β-adrenergic receptor antagonists [195]. No clear pattern emerges from the many studies of regulation of exocrine pancreatic secretion by the sympathetic nervous system. The major role for the adrenergic mechanism appears to be the inhibition of fluid and HCO_3^- secretion, which is mediated partially by vasoconstriction.

Hypothalamic regulation

Although pancreatic secretion is mediated mainly by vagovagal reflexes located within the brainstem, these reflexes are modulated by input from higher centers [196]. Animal

studies have shown that after chronic decerebration, which interrupts the entire descending neural input to the brainstem, there is a 35% reduction in basal pancreatic protein output and a 40% decrease in pancreatic protein secretion stimulated by lumenal perfusion of peptone [197], suggesting that the forebrain plays an important role in modulating the vagovagal reflex in the brainstem. The hypothalamus receives a wide variety of convergent afferent inputs from the viscera and regulates autonomic activities by modulating neuronal input to the autonomic preganglionic neurons. Cholinergic pathways in the hypothalamus play a major role in the stimulation of pancreatic secretion. Microinjection of methscopolamine, a blood–brain barrier-impermeant, cholinergic muscarinic receptor antagonist, into the lateral hypothalamic nucleus or paraventricular nucleus produces inhibitory effects similar to those observed after surgical decerebration [197]. Intracerebroventricular injection of hemicholinium-3 at doses known to deplete the endogenous acetylcholine store produces inhibitory results similar to those produced by methscopolamine. In contrast, microinjection of acetylcholine into the lateral hypothalamic nucleus and the paraventricular nucleus evokes 46% and 40% increases in pancreatic secretion over basal, respectively [197]. Selective lesions of the lateral septal cholinergic neurons or lateral parabrachial nucleus produce significant inhibition of peptone-induced pancreatic secretion [197]. Hence, cholinergic inputs from the lateral septal cholinergic neurons and the parabrachial nucleus to the hypothalamus play a major role in modulating vagal pancreatic efferent nerve activities and pancreatic secretion evoked by the vagovagal reflex.

Enteropancreatic neural reflex

Functional and anatomic enteropancreatic neural connections have been demonstrated by antegrade and retrograde tracer studies; neurons in the ganglia of the myenteric plexuses of the stomach and the duodenum project directly to the pancreas [198]. Activation of the myenteric neurons in the duodenum can influence the exocrine and endocrine pancreatic functions in the rat. These enteropancreatic neural pathways have cholinergic and serotonergic components [198,199]. The cholinergic nerves from the duodenum stimulate intrapancreatic neurons by way of nicotinic synapses. Abundant enteropancreatic serotonergic axons may inhibit pancreatic secretion through presynaptic 5-HT_{1P} receptors on cholinergic nerves [199]. Further studies are needed to define the physiological role of the serotonergic enteropancreatic neural pathways.

Peptidergic nervous system

Immunocytochemical studies have revealed several peptides in the nerve cell bodies and fibers of the pancreas. Among these, nerve fibers and cell bodies containing vasoactive intestinal polypeptide (VIP) are the most abundant [200]. The fibers containing VIP appear to surround the cell bodies of intrapancreatic ganglia and innervate duct cells.

In pigs, VIP is the neurotransmitter that mediates much of the HCO_3^- secretory response to electrical stimulation of the vagus nerve [201]. Vagal stimulation after the administration of atropine increases the pancreatic venous outflow of VIP and pancreatic HCO_3^- secretion; somatostatin blocks both effects [201]. The time courses for increased venous efflux of VIP and for increased HCO_3^- secretion after vagal stimulation are similar. A specific VIP antiserum reduces the HCO_3^- response to vagal stimulation [200]. The importance of intrapancreatic neuronal VIP as a regulator of pancreatic secretion may be species specific. VIP is a weak partial agonist in humans [201]. In some species, VIP may also induce pancreatic vasodilation and increase blood flow in response to the activation of the exocrine pancreas.

The neuropeptide galanin, which has been found in intrapancreatic nerve endings surrounding the endocrine pancreas and within the exocrine pancreas, appears to have a modulatory effect on exocrine pancreatic secretion. Depending on the experimental models used, galanin may stimulate or inhibit pancreatic secretion. With the use of an isolated perfused rat pancreas, it has been shown that porcine galanin in concentrations of 0.001 and 0.01 pmol/L significantly enhances CCK-stimulated amylase secretion and stimulates insulin release [202]. However, higher concentrations of galanin (10 and 100 pmol/L) inhibit insulin secretion and have no effect on CCK-stimulated amylase secretion. Because insulin potentiates CCK-stimulated enzyme secretion, low concentrations of galanin probably act through the release of insulin to stimulate pancreatic secretion. In contrast, galanin appears to be a potent inhibitor of the pancreatic exocrine secretion stimulated by either bombesin, secretin, or CCK in intact animals [203]. Galanin completely inhibits 2-deoxy-D-glucose-stimulated amylase secretion in anesthetized rats and significantly inhibits veratridine-stimulated release of acetylcholine in rat pancreatic lobules [204]. Thus, in intact animals, galanin appears to inhibit pancreatic secretion by inhibiting cholinergic transmission.

Other peptidergic neurotransmitters identified in the pancreas include the carboxyl-terminal tetrapeptide of gastrin/CCK [193,205], gastrin-releasing peptide [206], substance P [193,205], peptide histidine isoleucine [207], neurotensin [208], neuropeptide Y [207,209], enkephalin [193,205], and calcitonin gene-related peptide (CGRP) [210]. Pharmacological studies have found that CCK, gastrin, substance P, gastrin-releasing peptide, peptide histidine isoleucine, neurotensin, and CGRP stimulate, whereas enkephalin and neuropeptide Y inhibit exocrine pancreatic secretion. The physiological relevance of their mediation of pancreatic secretion is unknown.

Intrapancreatic nerves

Pancreatic ganglia share many of the characteristics of the enteric neurons and receive input from the parasympathetic,

sympathetic, and enteric nervous systems. Most pancreatic neurons (86%) are choline acetyltransferase positive [176]. Rabbit pancreatic ganglia contain high levels of norepinephrine and dopamine but 5-HT is absent [192]. All choline acetyltransferase-positive pancreatic neurons possess neuropeptide Y immunoreactivity, and most express NOS [176]. Most pancreatic neurons innervate the acini. Intrapancreatic neurons receive fast and slow excitatory postsynaptic potentials; 5-HT appears to inhibit the slow excitatory postsynaptic potentials through a 5-HT_{1P} receptor in some neurons. 5-HT and 5-HT_{1P} agonists are known to inhibit amylase secretion. Furthermore, intravenous injections of 5-HT_3 receptor antagonists, such as azasetron and granisetron, increase pancreatic protein output in conscious rats [148]. Blockade of this effect by atropine, but not truncal vagotomy [148], is consistent with a site of action on intrapancreatic ganglia and nerves.

Intracellular control of pancreatic secretion

Receptors

Most of the hormones and neurotransmitters that stimulate pancreatic secretion do so by directly regulating acinar and duct cells, but some may regulate indirectly by their actions on nerves or blood vessels. To determine the regulatory pathway, it is important to identify the physiological effects on isolated acinar and duct cells and to localize the high-affinity receptors for each regulator to its target cell. Because of the preponderance of acinar cells in the pancreas, methods to prepare isolated cells or pancreatic acini have been developed. By using amylase secretion as the criterion for functional response, studies of the effects of agonists and antagonists on secretion have identified the presence of specific receptors on acinar cells. The receptors have been confirmed by binding studies with radiolabeled analogues and antagonists [211,212]. Electron microscopic autoradiography and confocal fluorescence microscopy have localized binding to the basolateral membrane domain, although bound ligand may be internalized subsequently by an energy-dependent process [213,214]. Techniques of preparing isolated duct segments or cultured monolayers of duct cells also have been developed [32,215].

Through such studies, acinar cells from a variety of species, including humans, have been shown to bear receptors for CCK, bombesin, acetylcholine, VIP, and secretin. CCK interacts with both CCK_1 receptors, which are highly specific for CCK, and CCK_2 (or gastrin), receptors, which respond to both CCK and gastrin. Much is known about CCK_1 and M_3 muscarinic receptors and their signaling because of their presence on rodent acinar cells; however, the human pancreas contains few CCK_1 or CCK_2 receptors, and little is known about their function on acinar cells. CCK receptors on afferent nerves appear to have properties similar to CCK receptors on acinar cells. Less is known about duct cell receptors because of the relatively small number of these cells in the pancreas and the challenges encountered in studying their physiological functions (e.g., ion transport). Duct cells bear receptors for secretin, ATP, CCK, and acetylcholine and may have receptors for VIP.

Receptors for major pancreatic secretagogues belong to the receptor family characterized structurally by seven hydrophobic transmembrane domains and functionally by their interaction with guanine nucleotide-binding proteins (i.e., G proteins) [216]. M_3 muscarinic receptor, the bombesin receptor, and the CCK_1, and CCK_2 receptors have been cloned and found to possess similar structures. The secretin receptor also possesses seven transmembrane regions but has a somewhat different amino acid sequence [217].

Structure–function studies with site-directed mutagenesis and chimeric receptors have established some general principles of function for the G protein-coupled receptor family [216,218]. The transmembrane segments may form a pocket for the binding of small molecules, such as acetylcholine, and the extracellular amino-terminal end and loops may be important in the interactions with peptide molecules. The third cytoplasmic loop, projecting between the fifth and sixth transmembrane domains, is thought to interact with the appropriate G protein, and the serine and threonine residues in the cytoplasmic carboxyl-terminal end may be involved with regulatory mechanisms, such as desensitization and down-regulation by way of phosphorylation. The function of glycosylation on externally directed sites at the amino-terminal is not yet well established, but these sites may play a role in ligand binding or in the intracellular processing of new receptors and their insertion into the plasma membrane. Numerous techniques are available to elucidate ligand–receptor interactions.

Transmembrane signaling

Although all membrane receptors are integral proteins spanning the lipid bilayer, the pancreatic secretagogue receptors convey information by interacting with G proteins. G proteins are heterotrimeric proteins with unique α subunits and a smaller number of shared $\beta\gamma$ subunits [219]. Acinar cells possess α_s and α_i subunits, which respectively stimulate and inhibit adenylate cyclase. These subunits can be ADP-ribosylated by cholera toxin and pertussis toxin, which respectively activate and inhibit adenylate cyclase, permanently. Acinar cells also possess α_q and α_{11} subunits that activate phospholipase C [220]. The full complement of α and $\beta\gamma$ subunits expressed in acinar and duct cells and their functions require further exploration.

The α subunit possesses the guanine nucleotide-binding site, which, in the resting state, is occupied by GDP. After the receptor binds its ligand, it interacts with the G protein to catalyze the exchange of GDP for GTP. The GTP–α subunit complex dissociates from the $\beta\gamma$ complex and activates its

effector (i.e., phospholipase C, adenylate cyclase). The system is amplified because the lifetime of the GTP–α subunit complex is much longer than that of the hormone–receptor complex. Eventually, GTP is cleaved to GDP by an intrinsic GTPase activity, and the α subunit reassociates with the βγ subunit. It is because of this cycle that secretagogues stimulate GTPase activity in pancreatic membranes and that nonhydrolyzable analogues of GTP, such as GTP γS, activate adenylate cyclase or phospholipase C in pancreatic membranes or permeabilized acinar cells [221].

The final component in transmembrane signaling is the membrane effector that generates the intracellular messenger. There are two major effector enzymes in acinar cell membranes: polyphosphoinositide-specific phospholipase C, which cleaves phosphatidylinositol 4,5-bisphosphate (PIP_2), producing inositol-1,4,5-trisphosphate (IP_3) and 1,2-diacylglycerol (DAG); and adenylate cyclase, which converts ATP to cAMP [222]. These enzymes are presumed to be similar in structure and function to those expressed in a variety of cells. Multiple forms of phospholipase C have been purified, cloned, and shown to be expressed in many tissues [223]. The β_1 and β_3 forms are differentially activated in rat acini by CCK, carbachol, and bombesin [224]. Adenylate cyclase also has been cloned, and its primary structure is consistent with its role as an integral membrane protein with multiple membrane-spanning domains, although its catalytic site is clearly intracellular [225]. Multiple isoforms of adenylate cyclase exist, some of which are also regulated by G protein βγ subunits, Ca^{2+}, and protein kinases. Adenylate cyclase is clearly the major effector enzyme in duct cells, but there is some evidence for the participation of a phospholipase C. Other membrane effectors in the pancreas may include phosphatidylcholine-specific phospholipase C [226] and phospholipase D [227], phospholipase A_2, Na^+/H^+ ion exchanger, and various ion channels. However, these may be regulated by intracellular messengers rather than directly by G proteins.

Intracellular messengers

The major intracellular messengers involved in the regulation of pancreatic secretion are IP_3, Ca^{2+}, DAG, and cAMP [222,228]. The first three are predominant in the acinar cell and increase after the activation of phosphoinositide-specific phospholipase C by CCK and acetylcholine, whereas cAMP is the predominant messenger in duct cells, where it is produced in response to secretin.

Phosphatidylinositol and its polyphosphate derivatives – phosphatidylinositol 4-phosphate (PIP) and PIP_2 – constitute about 10% of membrane phospholipids. As mentioned, PIP_2 serves as a precursor for IP_3. When acinar cells are stimulated with acetylcholine or CCK, prelabeled PIP_2 and PIP levels fall rapidly, DAG increases, and there is a delayed rise in the level of phosphatidic acid. This indicates the primary breakdown of polyphosphoinositides, with production of DAG, which is subsequently converted to phosphatidic acid by DAG kinase. When the production of the water-soluble inositol phosphatases is assessed after labeling with ^{3}H-labeled inositol, the levels of IP_3, IP_2, IP_1, and the inositol products are all increased. Because IP_3 can only be produced by the hydrolysis of PIP_2, this is presumed to be the primary event, with the activation of a phosphatidylinositol kinase leading to further synthesis of PIP and PIP_2 from phosphatidylinositol.

Although IP_3 production can be measured by prelabeling with ^{3}H-labeled inositol, it is possible to measure the actual mass of cellular IP_3 with the use of a competitive binding assay. Such measurements show a rapid increase in IP_3 levels within 5 s in rat acini stimulated with CCK, carbachol, or bombesin. This 10-fold to 30-fold increase declines quickly after 30–60 s to a smaller, sustained plateau. Coincident with this rapid peak of IP_3 is a rapid increase in DAG of similar magnitude, which is thought to arise simultaneously from the hydrolysis of PIP_2. A later, larger increase may result from the hydrolysis of phosphatidylinositol or phosphatidylcholine by distinct phospholipases [226,227]. These results mean that IP_3 and DAG can act as separate signals and are not necessarily in lockstep. Phosphorylation of IP_3 produces inositol 1,3,4,5-tetraphosphate (IP_4), which can be dephosphorylated to yield inositol 1,3,4-triphosphate. Whether a biological role exists for these other inositol phosphates and for additional degradation products, such as IP_2 and IP_1, has not been verified.

The established function of IP_3 is to bind to a receptor on an intracellular Ca^{2+} storage site and trigger the release of Ca^{2+} into the cytoplasm. The IP_3 receptors are large 260-kDa molecules that form tetramers, which function as ligand-gated ion channels. Three forms of IP_3 receptors (type I, II, and III) are known, and all exist in acinar cells [229]. The sensitivity of these receptors to IP_3 can also be regulated by phosphorylation of the IP_3 receptor channel, and by G protein βγ subunits, Ca^{2+}, and thiol-reactive reagents. Modulation of IP_3 sensitivity may explain how certain agonists can release Ca^{2+} without a measurable increase in IP_3 levels. IP_3 receptors undergo down-regulation in response to prolonged stimulation. Another homologous gated intracellular Ca^{2+} channel, the ryanodine receptor RyR, well known as the Ca^{2+} release channel in muscle is also present in acinar cells. Ryanodine receptors open in response to Ca^{2+} and mediate Ca^{2+}-induced Ca^{2+} release. Another intracellular messenger, cyclic ADP-ribose (cADPR), can also activate ryanodine receptors. Although the activation of cADPR production is not fully understood, investigators have identified a membrane enzyme CD38 with ADP-ribosyl cyclase activity that can be stimulated by CCK and acetylcholine [230].

The morphological identity of the Ca^{2+}-sequestering and releasing organelle in the pancreas is not clear. Probably multiple membrane-bound compartments all related to the endoplasmic reticulum have the ability to take up and release Ca^{2+}. Some investigators have suggested the existence of multiple populations of vesicles with different Ca^{2+} transport

or release characteristics [231]. CCK receptors, but not muscarinic receptors, release Ca^{2+} from a lysosome-related (acidic) organelle through the action of nicotinic acid adenine dinucleotide phosphate (NAADP) [232]. The mechanism of NAADP production and the receptor on which NAADP acts remain poorly understood. Immunocytochemistry is used to localize IP_3 receptors in intact cells [233,234]. All three IP_3 receptor types are present primarily in the apical pole of the cell just under the lumenal membrane and partially overlapping the submembranous network of actin filaments. All three types exist in the nuclear membrane but in fewer numbers. Ryanodine receptors have a more diffuse distribution and are found throughout the acinar cells.

Much of the knowledge about secretagogue-induced changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in acinar cells has been obtained by using fluorescent Ca^{2+} probes such as fura-2. These studies have been performed on suspensions of cells or acini and on individual cells with the use of microspectrofluorometry [235] and digital imaging [226,236]. Such studies have shown that high concentrations of CCK, bombesin, and cholinergic analogues cause a rapid 5-fold to 10-fold increase of $[Ca^{2+}]_i$, which declines over 2–5 min to a level slightly above the basal level (Fig. 15.5). This initial large increase is essentially independent of extracellular Ca^{2+}, but the small, sustained plateau increase in $[Ca^{2+}]_i$ absolutely depends on extracellular Ca^{2+}. The initial increase shows a similar time course and dependence on secretagogue concentrations, as does the rapid increase in IP_3, and is presumed to depend on IP_3-stimulated or NAADP-stimulated release of intracellular Ca^{2+}. Much of this released Ca^{2+} is extruded from the cell, as shown by studies documenting an increased efflux of prelabeled $^{45}Ca^{2+}$ and a decline in total acinar Ca^{2+} [228,237]. All three of the major phospholipase C-activating secretagogues (i.e., CCK, acetylcholine, and bombesin) access the same intracellular pool of Ca^{2+}, and after maximal stimulation by one agonist, the addition of another has no added effect. After removal of the agonist, the IP_3-releasable intracellular pool refills over 2–10 min as Ca^{2+} is taken up from the medium and resequestered.

The sustained increase in $[Ca^{2+}]_i$ and the refilling of the intracellular Ca^{2+} pool involve activation of a poorly understood Ca^{2+} entry mechanism. Studies measuring $^{45}Ca^{2+}$ uptake and the influx of Mn^{2+}, which quenches the intracellular fluorescence of fura-2, indicate that the phospholipase C-coupled secretagogues increase Ca^{2+} influx twofold to fourfold [237]. The influx mechanism is blocked by La^{3+} but is not voltage-dependent or sensitive to organic Ca^{2+} channel blockers. Patch-clamp studies have not yet clearly defined a constituent channel. It is better defined as an entry mechanism. The mechanisms controlling Ca^{2+} influx are sensitive

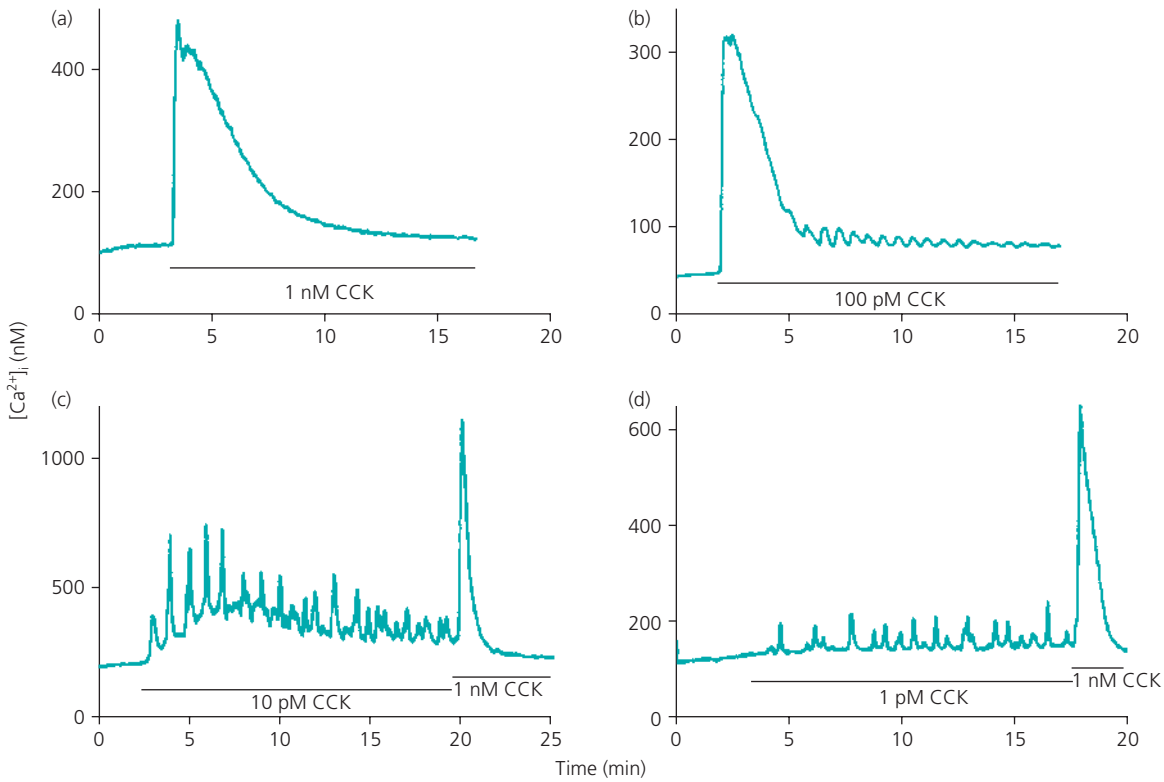

Figure 15.5 Increase in intracellular Ca^{2+} ion concentration ($[Ca^{2+}]_i$) in individual rat acinar cells in response to various concentrations of cholecystokinin (CCK). From Tsunoda et al. [235], with permission from the American Physiological Society.

to the state of the intracellular Ca^{2+} stores, as most directly shown using inhibitors of the microsomal Ca^{2+}-ATPase, such as thapsigargin, which release intracellular Ca^{2+} and activate the Ca^{2+} influx mechanism, bypassing the receptor and the generation of inositol phosphates. Evidence also exists for tyrosine phosphorylation in gating Ca^{2+} influx. That a sustained increase in $[Ca^{2+}]_i$ is important for sustained amylase release is demonstrated by the fact that La^{3+} inhibits the sustained component of amylase release, similar to the removal of Ca^{2+} from the medium [238].

Recordings of the $[Ca^{2+}]_i$ of individual cells of rat and mouse acini have revealed that low physiological concentrations of CCK, acetylcholine, and bombesin induce a different pattern of Ca^{2+} increase characterized by oscillations in $[Ca^{2+}]_i$ increases [235,236,239]. Superimposed on any steady increase are phasic increases in $[Ca^{2+}]$ of up to 250 nmol/L (see Fig. 15.5). These oscillations, which occur between one and four times per minute, are relatively independent of extracellular Ca^{2+} and involve the release and reuptake from the intracellular Ca^{2+} stores. That $[Ca^{2+}]$ oscillations can drive secretion is shown by the fact that the CCK analogue JMV-180 induces Ca^{2+} oscillations and can stimulate maximal amylase release [240].

Confocal digital imaging of Ca^{2+} in acinar cells has shown that the $[Ca^{2+}]_i$ increase is initiated in the apical pole of the cell and then spreads basally [241]. The response to lower agonist concentrations is a series of local increases in Ca^{2+} in the apical pole of the cell [242]. Improved imaging techniques have shown that local Ca^{2+} spikes are the result of pacemaker hot spots of Ca^{2+} release that entrain the surrounding region [243]. Interestingly, in the same cell, different agonists (i.e., carbachol, CCK, and bombesin) initiate $[Ca^{2+}]$ increases in distinct apical areas, indicating compartmentalization of signaling [244,245]. The intracellular application of IP_3, NAADP, or cADPR can all induce Ca^{2+} spiking. Further complicating the process, phosphorylation of IP_3 receptors by protein kinase A can modulate the pattern of Ca^{2+} increase [246]. Besides the apical-to-basal spread of Ca^{2+} in intact acini, Ca^{2+} waves appear to spread from cell to cell around an acinus. Gap junction coupling remains open as these Ca^{2+} waves spread but closes in response to supermaximal stimulation [247]. This cell-to-cell spread [247] increases the cellular sensitivity, allowing acinar activation to be triggered by the most sensitive cell.

Intracellular messenger-induced secretion

The evidence to support the importance of Ca^{2+}, DAG, and cAMP as intracellular mediators of pancreatic secretion is based on the ability of artificial changes in the level of each messenger to influence secretion [211,228]. The discovery that certain antibiotics, such as A23187 and ionomycin, function as Ca^{2+} ionophores and can be used to increase Ca^{2+} influx and trigger secretion provided one of the cornerstones of evidence for the importance of Ca^{2+} in the regulation of pancreatic secretion. The Ca^{2+}-ATPase inhibitor thapsigargin also increases $[Ca^{2+}]_i$ and can be used to define the contribution of Ca^{2+} in stimulating secretion [248]. The discovery that certain phorbol esters can activate protein kinase C in a manner similar to DAG led to extensive studies of the activation of this pathway [249]. Ca^{2+} ionophores and phorbol esters stimulate acinar cell secretion, and their effects are additive or potentiative. In the case of cAMP, derivatives such as dibutyryl cAMP or Br-cAMP, which are lipophilic or phosphodiesterase-resistant, have been used to activate the pathway normally initiated by secretin or VIP. In most species, cAMP derivatives used alone have minimal effects on acinar cell secretion but they potentiate the effects of agents working by way of Ca^{2+} and DAG.

Mechanism of action of intracellular messengers

The intracellular messengers active in pancreatic acinar cells have been identified and characterized but much less is known about the mechanisms by which they act to induce granule exocytosis, fluid secretion, protein synthesis, and gene expression. Although other mechanisms may exist, all of the intracellular messengers activate protein kinases and phosphatases and thereby regulate the state of protein phosphorylation. Considerable data suggest that changes in the phosphorylation of regulatory proteins mediate the action of hormones and neurotransmitters in a variety of tissues.

In support of this postulate, more than 25 phosphoproteins (not all identified) that are regulated by pancreatic secretagogues have been visualized by two-dimensional electrophoresis in pancreatic acini [250,251]. Some are uniquely regulated by Ca^{2+}, phorbol esters, or cAMP, and others are regulated by multiple second messengers. When Ca^{2+} ionophore and phorbol ester are combined, they reproduce all of the phosphorylation changes induced by secretagogues.

Several Ca^{2+}-activated kinases have been identified in pancreatic acinar cells including Ca^{2+}/calmodulin-activated type II and type III kinases and myosin light chain kinase [223,229]. Although some kinases are highly substrate specific, calmodulin-activated type II kinase is a multifunctional kinase that acts on several proteins. Its activation by CCK is supported by its temporary conversion to a Ca^{2+}-independent form [252]. Protein kinase C, originally described as a Ca^{2+}-, phospholipid-, and DAG-dependent kinase [249], is also present in acinar cells. Multiple isoforms of protein kinase C – both classical and atypical forms – are present, including α, δ, ε, and ζ [253]. Research suggests that protein kinase C δ is the isoform involved in mediating amylase release [254]. In addition to Ca^{2+} and cAMP-activated kinases, acinar cells contain the major classes of serine/threonine protein phosphatases (i.e., PP1, PP2A, and PP2B) [255,256]. Whereas some of these are constitutively active and are involved in reversing phosphorylation induced by kinases, PP2B, or calcineurin, is specifically activated by Ca^{2+} through calmodulin. This phosphatase, which is activated

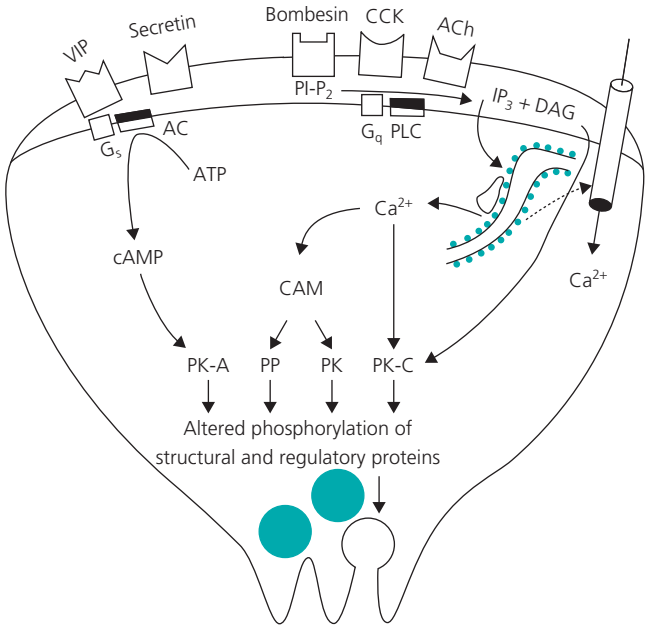

Figure 15.6 Stimulus–secretion coupling of pancreatic acinar cell protein secretion. In one intracellular pathway, receptors for vasoactive intestinal polypeptide (VIP) and secretin couple through a G protein (G_s) to activate adenylate cyclase (AC), and the cAMP produced activates protein kinase A (PK-A). In the other and quantitatively more important pathway, receptors for bombesin, cholecystokinin (CCK), and acetylcholine (ACh) couple through G proteins of the G_q family to activate phospholipase C (PLC). This enzyme hydrolyzes phosphatidylinositol 4,5-bisphophate ($PI\text{-}P_2$) to produce inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). IP_3 releases Ca^{2+} from intracellular stores in the endoplasmic reticulum, activating Ca^{2+} influx across the plasma membrane. Ca^{2+}, by binding to calmodulin (CAM), activates several protein kinases (PK) and one protein phosphatase (PP). DAG with Ca^{2+} activates several species of protein kinase C (PK-C). This battery of protein kinases and phosphatases, by altering the phosphorylation of diverse proteins, brings about the secretion of digestive enzymes and other cellular effects on growth and metabolism.

by pancreatic secretagogues, is blocked by the immunosuppressant cyclosporine, which also partially inhibits secretagogue-activated amylase secretion [256].

The role of intracellular messengers and effectors in pancreatic enzyme secretion is summarized in Fig. 15.6. Stimulation of secretion normally involves synergistic interactions among intracellular messengers. In the case of acetylcholine and CCK, this includes interactions between Ca^{2+}- and DAG-activated pathways. Agents such as VIP and secretin, which increase cAMP, add a further interaction at the postintracellular messenger level. Proteins localized on the granule and lumenal plasma membrane and several soluble and cytoskeletal proteins may be involved in exocytosis. In pancreatic duct cells, the same intracellular messengers and kinases may regulate ion pumps, carriers, and channels involved in fluid and electrolyte secretion.

Zymogen granules and exocytosis

The terminal steps in secretion involve fusion of the zymogen granules with the lumenal membrane of the acinar cell. These granule dynamics were observed by real-time video imaging [257]. A more dramatic view emerged using confocal microscopy to image a lumenal tracer that enters the granule on formation of a connection to the lumen [258]. This fusion event shares basic mechanisms with other membrane fusion events from yeast to neurons. Two types of proteins, SNARE proteins and small G proteins of the Rab family, appear to play prominent roles (Fig. 15.7). The term SNARE was derived from work with the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors. There are more than 35 mammalian SNARE proteins that share a homologous sequence, the SNARE motif. In the SNARE paradigm, transport vesicles destined to fuse with another membrane possess a set of proteins, termed v-SNARE, that interact with

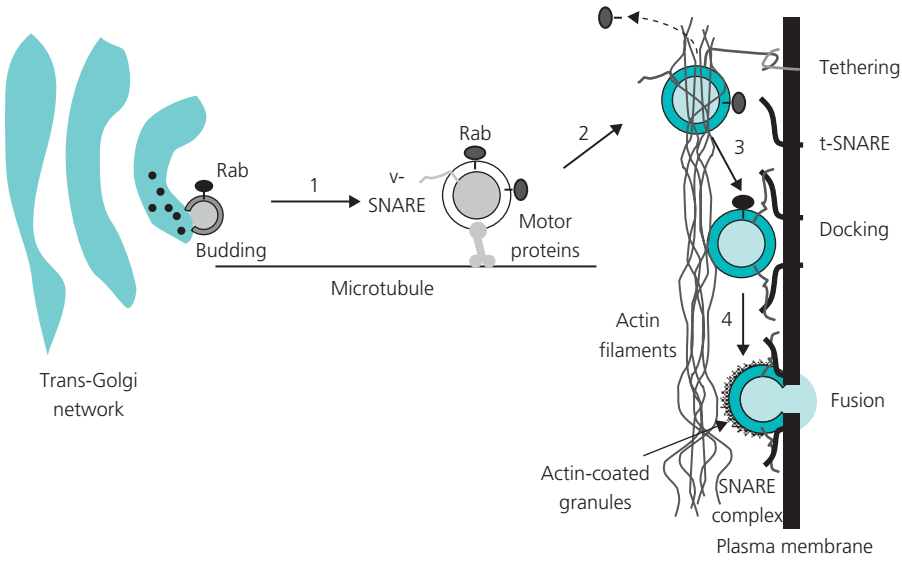

Figure 15.7 Model of the steps in the exocytosis of zymogen granules, including movement to the lumenal membrane, passage through the terminal web of actin filaments, tethering, docking, and fusion. Note, for simplicity, not all of the SNARE complex proteins are shown. From Williams & Yule [273], with permission from Elsevier.

proteins on the target membrane, termed t-SNARE, and with soluble attachment factors [259]. The best-characterized SNARE complex is the synaptic complex made up of synaptobrevin/VAMP on the synaptic vesicle and SNAP25 and syntaxin 1 on the presynaptic plasma membrane. SNARE proteins associate into a complex of four helical bundles with SNAP25 providing two bundles and the other two proteins each providing one. Assembly is by a zipper-like mechanism that may provide the energy for membrane fusion [260]. Modulatory proteins, such as Munc18 and tomosyn, regulate the assembly of SNARE complexes.

Synaptobrevins/VAMPS, which act as v-SNARES on synaptic vesicles, are present on zymogen granules; VAMP2, VAMP3 (cellubrevin), and VAMP8 (endobrevin) have been identified [261,262]. The t-SNAREs on the plasma membrane of neurons include SNAP25 and syntaxin. Multiple isoforms of syntaxin in acini have been identified. Syntaxin 2 is present on the apical plasma membrane [263], and other syntaxins are present on the basolateral membranes and zymogen granules. The interaction of VAMPS and syntaxins in acini remains incompletely understood. VAMP8 and syntaxin 4 have been reported to exist in a complex [261]. SNAP29 has been shown to be associated with VAMP8, and SNAP23 with VAMP2 [264]. Truncated cytosolic peptides of both VAMP2 and VAMP8 inhibited amylase release from permeabilized cells. When syntaxin peptides were tested, only syntaxin 2 inhibited Ca^{2+}-stimulated secretion. These results suggest that a VAMP8, SNAP29, and syntaxin 4 complex may mediate regulated secretion, whereas VAMP2 may mediate constitutive secretion. The role of regulatory proteins, such as Munc18c, which is present on the basolateral membrane, has not been elucidated. Moreover, a Ca^{2+} receptor such as synaptotagmin 1, which is present on synaptic vesicles, has not been identified on zymogen granules.

The additional importance of cytosolic proteins was shown by the observation that permeabilizing acini with α-toxin, which produces small pores that stop protein leakage, prevented the rundown of secretion seen with streptolysin-O permeabilization, which generates large pores [265]. Moreover, cytosol from brain or lacrimal glands restores secretion in streptolysin-O-permeabilized acini. One of the responsible proteins is CRHSP-28, a Ca^{2+}-regulated phosphoprotein, which associates with zymogen granules through a 70-kDa protein [266]. Whether Ca^{2+} in acini regulates directly through a SNARE or cytosolic protein or indirectly by activating a kinase or phosphatase is unknown. An experimental approach that may prove useful in this work is to monitor secretion by measuring the capacitance of patch-clamped acinar cells. Low concentrations of acetylcholine, which are known to induce [Ca^{2+}] oscillations in the apical pole of the cell, also induced an increase in membrane capacitance without an increase in membrane conductance [267].

Work on species of Rab proteins in pancreas has focused on Rab3, because Rab3a is present on synaptic vesicles and an immunoreactive Rab3 species has been identified in pancreas. Studies using polymerase chain reaction amplification and immunocytochemistry with specific antibody have identified conclusively the presence of Rab3D on zymogen granules [268,269] as well as on granules in salivary and lacrimal glands and in chief cells of the stomach. Overexpression of Rab3D in acinar cells of transgenic mice enhances a component of amylase secretion [270], indicating that Rab3D may be rate limiting for secretion. Other Rab proteins have been identified on zymogen granules by immunocytochemistry and mass spectrometry [271]. Of the Rab proteins identified, Rab27B has been related to secretion in other cells. Overexpression in acini of constitutively active Rab27B enhanced amylase secretion, whereas expression of a dominant negative mutant of Rab27B inhibited amylase secretion [272]. Whether Rab3D and Rab27B have distinct effectors or whether they can compensate for each other remains to be determined.

Another cellular component believed to be involved in exocytosis is the actin cytoskeleton [273]. It may serve as a barrier, preventing premature secretion, and it may be involved in the movement of zymogen granules to the cell surface. The former role may be mediated by an actin network under the plasma membrane. Evidence of this role for actin is the observation that introducing the monomeric actin-binding protein β-thymosin into permeabilized acini to shift the monomer–polymer equilibrium induces secretion in a Ca^{2+}-independent manner [274]. Moreover, high concentrations of CCK and β-thymosin, the actin monomer-binding protein, decrease polymerized actin at the apical membrane. In contrast, phalloidin, which binds and stabilizes polymerized actin, inhibits secretion by the permeabilized cells. Filamentous actin has been shown to form a coating on fused granules, and may serve to promote the exit of granule contents after fusion occurs [275]. The small G proteins RhoA and Rac1 have been shown to be activated by secretagogues and to regulate secretion, presumably through effects on actin polymerization [276].

Intracellular pathways leading to growth and gene regulation

Pancreatic secretagogues, particularly CCK, have long been known to induce pancreatic hypertrophy, hyperplasia, and gene expression. Initially, it was assumed that the same intracellular mediators that regulate secretion might mediate these effects. It is clear, however, that nonsecretagogues, such as insulin and epidermal growth factor, can also regulate nonsecretory pancreatic functions and that classical secretagogues, such as CCK, activate novel signaling pathways, many of which involve tyrosine phosphorylation. Furthermore, nonsecretagogue input, such as the extracellular ligation of integrins, can lead to both tyrosyl phosphorylation and an increase in intracellular Ca^{2+} [277]. Increasing evidence that cytokines can affect the pancreas, at least in pancreatitis,

suggests that cytokine receptors also activate intracellular signaling.

G protein-coupled receptors can activate tyrosine kinase signaling pathways in a variety of cells [278]. Most growth factor receptors include an intracellular tyrosine kinase domain, and autophosphorylation of the receptor on tyrosines leads to docking of signaling molecules, such as phosphoinositol 3-kinase, phospholipase C-γ, and adapter molecules, such as Grb2 and Shc. G protein-coupled receptors do not possess tyrosine kinase activity nor are they phosphorylated by tyrosyl. However, CCK is known to increase the tyrosyl phosphorylation of multiple acinar proteins [279] and to activate the mitogen-activated protein kinase (MAPK) cascade involving Ras–Raf–MAP/ERK kinase (MEK)–extracellular signal regulated kinase (ERK) [280,281]. The kinase $p90^{rsk}$, which is downstream of ERKs and acts to phosphorylate nuclear transcription factors and cytoplasmic targets, also is activated by CCK [282].

The early events by which secretagogues may activate Ras and the MAPK cascade leading to ERKs have been studied. In rat acini, CCK, carbachol, and bombesin, as well as epidermal growth factor, increase the tyrosyl phosphorylation of Shc [283], leading to the binding of Grb2 and SOS. Because SOS is a guanine nucleotide exchange factor for Ras, this could mediate an activation of Ras in response to CCK. Shc phosphorylation induced by CCK has been shown to be largely dependent on protein kinase C, whereas that induced by epidermal growth factor was fully protein kinase C independent [283]. In the pancreatic acinar cell line AR42J, which possesses primarily CCK_2 receptors, gastrin has also been shown to increase tyrosyl phosphorylation of Shc [284]. In AR42J cells, gastrin also induces the tyrosyl phosphorylation of the docking protein, insulin receptor substrate 1 (IRS-1) [285]. Because Grb2 also binds to IRS-1, this is a potential alternative means of activating the MAPK cascade. IRS-1 has not been identified in normal acinar cells, although it is overexpressed in some pancreatic cancer cells. The identity of the tyrosine kinase that phosphorylates Shc and IRS-1 and how it is activated remains a major question. Interest has centered on cytoplasmic tyrosine kinases of the Src family, and published data would suggest that somehow protein kinase C activates Src.

Two other MAPK cascades, stress-activated protein kinase (SAPK) and p38 MAPK, lead to activation of Jun kinase. Both of these pathways exist in acinar cells and can be activated by CCK as well as other extracellular signals [286],[287]. Activation of SAPK is an early event in secretagogue-induced pancreatitis, although it is not clear whether it is a cause or an effect [288]. A known effect of p38 MAPK is to activate MAPKAP kinase-2, which phosphorylates the heat-shock protein Hsp27, thereby regulating actin polymerization [287,289]. Both SAPK and p38 MAPK are activated by dual phosphorylation on tyrosine and threonine residues by upstream dual function kinases related to MEKs [290]. These are activated by a serine threonine kinase analogous to Raf that is farther upstream. However, the three MAPK cascades can be activated independently.

Evidence exists for two other signaling pathways in pancreatic acini [222]. The first is the activation of focal adhesion kinase, or $p125^{FAK}$, and its downstream target, paxillin. Both of these molecules are associated with the cytoskeleton and, in other cells, are activated by growth factors and integrins. CCK has been shown to increase the tyrosine phosphorylation of $p125^{FAK}$ and paxillin in rat acini [291]. This effect is probably mediated by the small G protein Rho. The second is the pathway through PI_3K to $p70^{S6K}$, the kinase that phosphorylates ribosomal protein S6. CCK, carbachol, and bombesin activate $p70^{S6K}$ in rat acini by a pathway that can be blocked by PI_3K inhibitors such as wortmannin and, rapamycin, which bind intracellularly to the immunophilin, FKBP-12 [48]. As $p70^{S6K}$ also mediates the initiation of protein translation, this pathway probably underlies, at least in part, the stimulation of protein synthesis in acinar cells by pancreatic secretagogues previously discussed. Identification of intracellular regulation by both conventional and novel pathways is a rapidly developing field, and further progress is expected.

Adaptive growth of the pancreas can occur in response to nutrients and hormones to synthesize more digestive enzymes and prevent malabsorption [45]. Both a high protein diet and hyperphagia induced by pregnancy, lactation, or cold exposure can induce pancreatic growth. Much of this growth is mediated by CCK and can be modeled by feeding trypsin inhibitor to increase endogenous CCK release. Information has emerged on the intracellular signaling pathways responsible for this growth (Fig. 15.8). CCK, in addition to increasing intracellular Ca^{2+}, activates three MAPK pathways and the PI_3K–mTOR pathway, and most of these can be activated in vivo by feeding trypsin inhibitor [222]. One pathway that is key for growth is the Ca^{2+}-activated phosphatase, calcineurin. Two inhibitors of calcineurin, FK506 and cyclosporine, block trypsin inhibitor-stimulated growth [292]. The mTOR pathway has also been shown to be essential for pancreatic growth, as this growth is blocked by rapamycin [293]. These pathways are important regulators of mRNA transcription and translation. Calcineurin-mediated dephosphorylation of the transcription factor NFAT (nuclear factor of activated T cells) is associated with adaptive growth in cardiac and skeletal muscle. CCK leads to NFAT activation in pancreatic acini. The role of the mTOR pathway in translational regulation was discussed earlier (see section on formation and composition of pancreatic juice – enzymes), although whether global or specific protein synthesis affect growth is not fully understood. High levels of protein can also stimulate pancreatic growth, and can do so without CCK [294]. This effect of high protein levels is likely mediated by a direct activation of the mTOR pathway by amino acids, as occurs in a variety of cell types.

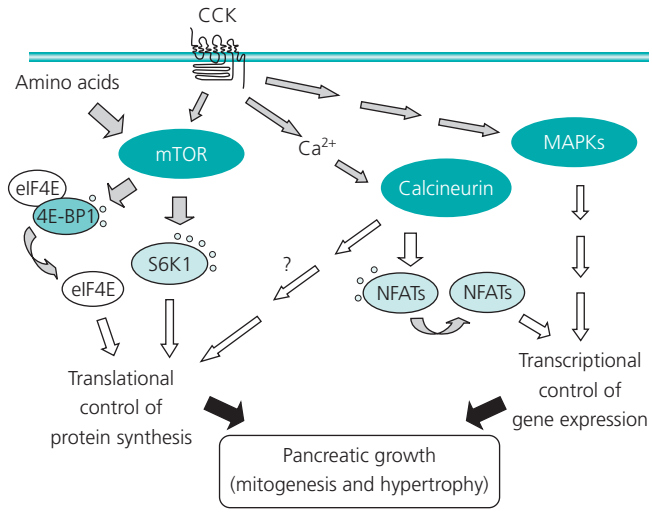

Figure 15.8 Intracellular pathways through which CCK stimulates pancreatic growth. At least three pathways involving calcineurin, mammalian target of rapamycin (mTOR), and mitogen-activated protein (MAP) kinases (extracellular signal-regulated kinases [ERKs] and Jun N-terminal kinases [JNKs]) have been shown to be necessary for the growth of rodent pancreas, either in vivo or in vitro. From Sans et al. [45], with permission from Blackwell Publishing.

Inhibition of pancreatic secretion

The regulation of pancreatic secretion depends on a balance between inhibitory and stimulatory influences on the gland, which are exerted through hormones and the autonomic nervous system. Although much has been written about pancreatic stimulation, less is known about the inhibitory influences on the pancreas.

Inhibitory phase of pancreatic secretion

In humans, hyperglycemia induced by intravenous infusion of glucose inhibits the pancreatic secretory response to a test meal [295]. Similarly, intravenous infusion of amino acids inhibits the human pancreatic enzyme response to intestinal amino acid perfusion [296]. Although the mechanisms responsible for these observations are unknown, the secondary release of inhibitory hormones is postulated. Pancreatic glucagon exhibits characteristics consistent with such an inhibitory hormone. In most of the studies, glucagon inhibits pancreatic secretion stimulated by secretin and CCK, alone or in combination, or by ingestion of a test meal in dogs, cats, rats, and humans [3,297–299]. The inhibitory effect is characterized by reduction of the volume of flow and of HCO_3^- and enzyme secretion.

Pancreatic glucagon is secreted concomitantly with the hyperaminoacidemia observed after the intestinal perfusion of amino acids or after a high-protein meal [228]. This postprandial level of glucagon may be sufficient to inhibit secretin- or CCK-stimulated pancreatic secretion.

Another pancreatic hormone, somatostatin, may also play a role in the inhibition of pancreatic secretion. Somatostatin-containing cells are also present in the upper gastrointestinal tract and the central nervous system. Somatostatin is processed to multiple molecular sizes and interacts with a variety of receptors on acinar cells, dorsal root ganglia, and islets of Langerhans, and on the nerves of the peripheral and central nervous systems. One mechanism to provide a degree of specificity could be the release of different forms of somatostatin. For example, glucagon-like peptide 1, oleic acid, and gastrin-releasing peptide stimulate the secretion of both somatostatin-28 and somatostatin-14, and secretin induces a preferential release of somatostatin-14 [300]. In humans, pharmacological doses of somatostatin cause marked inhibition of CCK-stimulated pancreatic enzyme secretion and modest inhibition of secretin-stimulated HCO_3^- secretion [5,301]. Studies in rats show that somatostatin inhibits 2-deoxy-D-glucose- and CCK-evoked pancreatic enzyme secretion through a vagal pathway [14]. Somatostatin does not act on peripheral vagal afferent or efferent pathways or directly on pancreatic acini; it exerts its inhibitory action at a central vagal site [14]. Somatostatin injected into the dorsal vagal complex significantly inhibits pancreatic secretion evoked by intravenous administration of CCK-8 or 2-deoxy-D-glucose. This suggests that somatostatin is probably acting through the dorsal vagal complex in the brainstem to inhibit pancreatic secretion. Studies in the perfused canine pancreas have demonstrated that somatostatin is released from the pancreas during perfusion with high concentrations of amino acids or glucose [302]. This peptide may exert a paracrine inhibitory effect on the exocrine pancreas.

Six somatostatin receptor (SSTR) subtypes have been cloned from five genes; SSTR2 is expressed in an A and a B form by variant mRNA splicing. SSTR2 receptor agonists (e.g., octreotide) mediate inhibition of pancreatic secretion, whereas SSTR5 inhibits insulin release. Injection of the SSRT2 agonist seglitide into the dorsal vagal complex inhibits CCK-8/2-deoxy-D-glucose-induced pancreatic protein secretion, indicating that the central action of somatostatin is mediated by SSTR2 in the dorsal vagal complex [303]. In addition, SSTR2A has been localized to acinar cells, and to glucagon and pancreatic polypeptide immunoreactive islet cells [304]. Although somatostatin is one of the few peptides to inhibit pancreatic secretion in an isolated pancreas, the role of the acinar cell SSTR may be to modulate the nonparallel secretion of digestive enzymes [305] rather than to inhibit secretion. It is believed that somatostatin inhibits pancreatic exocrine secretion mainly through a central cholinergic mechanism [14].

Intrajejunal perfusion of hypertonic glucose (50%) produces dose-related inhibition of secretin-stimulated pancreatic fluid and HCO_3^- secretion in humans [306,307]. Similar inhibition occurs with intraintestinal hypertonic (9%) NaCl infusion in dogs. At least part of this inhibitory effect has

been attributed to the release of enteric glucagon [296]. Infusion of oxyntomodulin, a 37-amino-acid, glucagon-containing peptide isolated from porcine lower intestine, inhibits basal and cerulein-stimulated pancreatic secretion of HCO_3^- and enzymes [308]. This intestinal glucagon is 10 times more potent than pancreatic glucagon.

Factors in the ileum and colon can inhibit pancreatic secretion. Hage and colleagues showed that infusion of oleic acid into the proximal colon of conscious dogs inhibits secretin-stimulated pancreatic secretion [309]. Similar inhibitory effects have been observed in rats [310]. In the anesthetized cat, infusion of oleic acid or hypertonic solutions of glucose or saline into the colon or terminal ileum inhibits secretin- or CCK-stimulated secretory volume, as well as HCO_3^- and enzyme output from the pancreas [311].

In humans, nutrients (e.g., lipid) in the colon inhibit CCK-stimulated pancreatic enzyme and HCO_3^- output [312]. These late postprandial events may serve as physiological signals to reduce exocrine pancreatic secretion after the digestion and the absorption of nutrients are complete. The inhibitory effect of nutrients in the distal gut on pancreatic secretion appears to be independent of the vagus and splanchnic nerves [311]. Cross-circulation studies in the rat have shown that a humoral factor mediates the inhibition of pancreatic enzyme secretion induced by colonic perfusion of oleic acid.

Harper and colleagues used the term *pancreotone* to describe an inhibitory substance extracted from the colonic mucosa [313]. The function of pancreotone is abolished when the extract is preincubated with trypsin, demonstrating it is a peptide. Peptide YY, a 36-amino-acid peptide named for its amino- and carboxyl-terminal tyrosines, is ubiquitous in the distal small intestine, colon, and rectum [314]. This peptide is released by fat and, to a lesser degree, protein in the distal gut or colon. The infusion of this peptide in dogs significantly inhibits basal and meal-stimulated pancreatic HCO_3^- and enzyme secretion [315]. Physiological experiments demonstrate that intraileal, but not colonic, carbohydrate increases plasma peptide YY levels and decreases amylase secretion in dogs [316]. These observations support the hypothesis that peptide YY is at least a component of pancreotone in dogs. In humans, ileal perfusion of carbohydrate inhibits exocrine pancreatic secretion. Glucagon-like peptide 1 (GLP-1), another ileal hormone, but not peptide YY is elevated in the circulation during ileal infusion of carbohydrate. GLP-1 does not appear to act directly on the pancreas to inhibit exocrine secretion. In anesthetized pigs [317] with cut splanchnic nerves, intravenous infusion of GLP-1 inhibited hypoglycemia-induced pancreatic HCO_3^- and protein secretion. These effects were not observed in vagally stimulated isolated and perfused porcine pancreas [317], suggesting that GLP-1 acts through a central mechanism. Subsequent studies in rats indicated that the inhibitory action of GLP-1 depends on intact vagus nerves [16]. GLP-1 acts on the dorsal vagal complex to inhibit pancreatic enzyme secretion [16]. Similarly, oxyntomodulin, which is released after ileal administration of nutrients, also inhibits pancreatic secretion by a vagal-dependent central mechanism [318]. Because oxyntomodulin does not interact with the receptor for glucagon or truncated GLP-1, its effects appear to involve an oxyntomodulin-specific receptor. Although the mediators of ileal carbohydrate-induced inhibition of exocrine pancreatic secretion have not been firmly identified, it appears that the action of these potential mediators is dependent on central neural pathways.

Pancreatic polypeptide (PP), a peptide closely related to peptide YY, is another hormone that may play an important role in regulating pancreatic exocrine secretion. PP is localized in the islets of Langerhans and between the acinar cells of the exocrine pancreas [319]. Its only apparent physiological actions are to inhibit pancreatic and biliary secretions. The secretion of PP is governed mainly by a cholinergic mechanism [320]. Postprandial release of PP is mediated by a long vagovagal reflex and short local cholinergic pathways [320]. Vagal cholinergic activity is the most powerful stimulant of PP release, and it is also key to most other stimulation of the PP cell [320].

In humans and dogs, infusion of physiological concentrations of PP inhibits basal and stimulated pancreatic secretion [6,321]. In vivo, PP appears to act preferentially by inhibiting vagal stimulation [322]. In vitro, PP inhibits pancreatic enzyme secretion by way of the presynaptic modulation of acetylcholine release [323]. Because its secretion is under cholinergic control and it acts by interfering with cholinergic transmission, PP is an ideal candidate to modulate pancreatic secretion stimulated by the cholinergic enteropancreatic reflex. After ingestion of a meal, the enteropancreatic reflex is activated to stimulate pancreatic enzyme secretion and PP release. PP inhibits cholinergic transmission and reduces pancreatic enzyme secretion. PP may play an important role in the feedback regulation of pancreatic enzyme secretion activated by the enteropancreatic reflex. The primary target of PP appears to be the central nervous system [15]. PP receptors have been identified in discrete locations in the hypothalamus, limbic system, brainstem, and other central locations with the use of receptor autoradiography [324]. In contrast, mRNA for PP is almost undetectable [325], suggesting that peripheral PP is modulating central neural function at sites that have an incomplete blood–brain barrier. Microinjection of PP into the DMV inhibits pancreatic secretion stimulated by CCK, suggesting that the DMV is a site for neural feedback inhibition of pancreatic exocrine secretion [326]. Patch-clamp recordings of pancreas-projecting neurons in the rat DMV show that synapses other than those of the area postrema–DMV circuit may also be involved in mediating the effect of PP on pancreatic exocrine functions. Hence, PP and its structurally related analogues likely act at multiple sites in the brainstem to modulate vagal cholinergic efferent output to the pancreas [327].

Although the list of peptides known to inhibit exocrine pancreatic secretion has expanded, little is known about the mechanisms through which hormones or neurotransmitters inhibit pancreatic enzyme secretion. An important feature shared by these agents is the lack of direct inhibition of the pancreatic acinar cells. Many substances suppress pancreatic enzyme secretion in vivo but do not act directly on the acinar cell to suppress enzyme release. Animal studies suggest that peptides such as PP, somatostatin, CGRP, enkephalin, and pancreastatin inhibit pancreatic enzyme secretion by modulating cholinergic transmission, and most, if not all, act through a central vagal site [303,322,323,328–331]. Intracerebroventricular administration of CGRP inhibitors stimulates (basal) pancreatic secretion in conscious rats and this appears to be mediated by sympathetic noradrenergic efferents through the α-adrenergic receptor [332]. In contrast, CGRP in the central nervous system inhibits pancreatic enzyme secretion stimulated by 2-deoxy-D-glucose and CCK by modulating vagal parasympathetic outflow [333].

Feedback regulation of pancreatic secretion

A series of observations suggest that the intralumenal action of pancreatic proteases plays an important role in regulating pancreatic enzyme secretion [94,334]. The underlying concept of feedback regulation of the pancreas is based primarily on studies of rats that show that diversion of pancreatic juice from the duodenum stimulates CCK release and pancreatic enzyme secretion [95]. However, intraduodenal administration of trypsin or chymotrypsin inhibits the release of CCK and pancreatic enzymes [95]. This phenomenon is specific for activated proteases and is not observed with inactivated trypsin, amylase, lipase, or $NaHCO_3$. Subsequent studies demonstrated that intravenous infusion of proglumide or L364,718, a specific CCK receptor antagonist, abolishes the increase in pancreatic exocrine secretion evoked by diversion of bile–pancreatic juice [95,335]. These observations indicate that feedback inhibition of pancreatic secretion by trypsin is mediated by inhibiting the release or action of CCK.

The elevated plasma CCK levels and the increase in pancreatic secretion after diversion of pancreatic juice appear to be mediated by a trypsin-sensitive substance secreted by the proximal small intestine, originally designated CCK-releasing factor (CCK-RF) [97]. When trypsin is present, this peptide is cleaved and inactivated. CCK-RF may act as a mediator of pancreatic enzyme secretion in response to dietary protein intake in rats. Dietary protein in the intestine competes for the trypsin that would otherwise inactivate CCK-RF [96]. The resulting increase of CCK-RF in the intestinal lumen enhances CCK release, stimulating pancreatic enzyme secretion. Although this appears to be the principal mechanism regulating CCK release in rats, it is not known whether the same mechanism operates in other species.

Many CCK-releasing peptides have been identified, including one from porcine mucosa [336] and another from rat upper intestinal fluid [337]. Herzig and colleagues [336] isolated and purified a CCK-releasing peptide, identical to the porcine diazepam-binding inhibitor, from porcine small bowel mucosal extracts. This trypsin-sensitive peptide, which is secreted intraduodenally, releases CCK and stimulates pancreatic secretion in rats (Fig. 15.9). Diazepam-binding inhibitor-like immunoreactivity has been found in intestinal mucosal cells [338] and this peptide (CCK-releasing peptide) is secreted into the lumen under neural regulation [93]. Peptone in the duodenum stimulates 5-HT release, which activates the sensory substance P neurons in the submucous plexus. Signals are then transmitted to cholinergic interneurons and to epithelial cells containing CCK-releasing peptide by way of cholinergic secretomotor neurons [93]. This enteric neural circuitry, which is responsible for the secretion of CCK-releasing peptide, may in turn play an important role in the postprandial release of CCK. Spannagel and colleagues [337] successfully purified another lumenal CCK-releasing peptide from the jejunal secretion of rats. Partially purified fractions increased the release of CCK and stimulated pancreatic secretion of fluid and protein. Amino acid sequencing and mass spectral analysis of this peptide revealed that it is composed of 70–75 amino acid residues and has a mass of 8136 kDa. The monitor peptide [339] in rat pancreatic juice, which is secreted into the duodenal lumen, is another CCK-releasing peptide. The physiological significance of these releasing peptides is unknown.

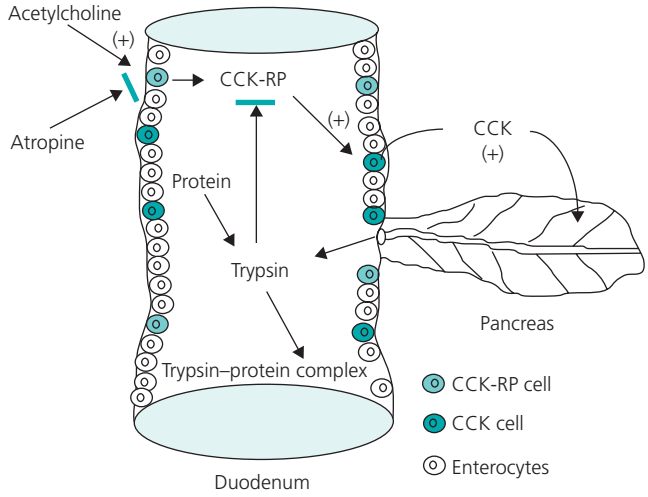

Figure 15.9 The postulated mechanism by which cholecystokinin-releasing peptide (CCK-RP) stimulates the postprandial secretion of CCK. CCK-RP is secreted into the proximal small intestine and inactivated by trypsin. When food enters the duodenum postprandially, protein binds to trypsin and prevents the inactivation of CCK-RP. CCK-RP stimulates CCK cells in the duodenum to release CCK into the bloodstream. CCK, in turn, stimulates pancreatic enzyme secretion. From Herzig et al. [336], with permission. Copyright 1996 National Academy of Sciences, U.S.A.

Despite several attempts to demonstrate a protease-sensitive feedback mechanism in humans, the issue remained controversial because the technical limitations in removing or blocking intralumenal protease activity made human studies challenging. Using a different approach, researchers reported that intestinal administration of trypsin or chymotrypsin in humans suppresses CCK release and partially blocks the pancreatic response to intestinal administration of amino acids or oral ingestion of a test meal [102,340]. These results support the existence of feedback regulation of pancreatic enzyme secretion in humans.

Using an alternative approach, Dlugosz [341] and Hotz [342] and their colleagues observed that duodenal infusion of aprotinin, a trypsin inhibitor, had no effect on basal pancreatic enzyme secretion. Similar findings were reported with the use of the trypsin inhibitor FOY-305 [343]. Neither compound, however, strongly inhibits human chymotrypsin. Liener and colleagues demonstrated that Bowman–Birk soybean trypsin inhibitor, an inhibitor of chymotrypsin and elastase, markedly stimulates pancreatic enzyme secretion in humans [344]. These observations suggest that trypsin and other proteases such as chymotrypsin and elastase should be removed to evoke pancreatic enzyme secretion in humans.

Studies of rats show that biliary secretion may also participate in the feedback regulation of CCK release. Secretion of bile into the proximal intestine inhibits CCK release and pancreatic secretion by protecting pancreatic proteases from autodigestion in the lumen [345].

Postprandial pancreatic enzyme secretion is under hormonal and neural control. Distention of the duodenum and administration of hyperosmolar solutions into the duodenum each elicits pancreatic enzyme secretion without raising plasma CCK levels [186]. This stimulatory effect is inhibited by atropine, suggesting that it is cholinergically mediated. In contrast to amino-acid-stimulated pancreatic enzyme secretion, pancreatic responses to stimulation by volume or osmolality in the duodenum are not suppressed by trypsin [186]. This indicates that feedback regulation of pancreatic secretion by trypsin is stimulus specific and is mediated by inhibiting CCK release. The enteropancreatic reflex is unaffected by intralumenal proteases.

The existence of a feedback regulation of pancreatic enzyme secretion in humans may have important clinical implications. It is conceivable that in patients with chronic pancreatitis, decreased pancreatic enzyme secretion may result in elevated plasma CCK levels, reflecting a failure in the feedback modulation of CCK release. This may cause hyperstimulation of the pancreas and produce pain. Effective enzyme replacement therapy may reduce pancreatic stimulation, decrease intraductal pressure, and diminish pain. Large doses of pancreatic extract have reduced pain in some patients with chronic pancreatitis (see Chapter 69) [346,347]. This exciting observation awaits confirmation.

Patterns of secretion

Basal secretion

Under basal conditions, pancreatic secretion occurs at very low rates, although a small amount of enzymes is always present in the pancreatic juice. Basal secretion rates of enzymes and HCO_3^- are about 10% and 2% of maximal levels, respectively. A pattern of cyclic change in basal pancreatic secretion has been demonstrated in dogs [348] and humans [349], and this is characterized by brief increases in HCO_3^- and enzyme secretion, which recur every 60–120 min during the interdigestive period. These bursts of pancreatic secretory activity are temporarily associated with periods of increased motor activity in the stomach and proximal intestine known as the interdigestive migrating motor complexes (IMMCs) [348,349].

Associated with the bursts of pancreatic secretion are brief increases in gastric acid and biliary secretions [349]. Plasma motilin and PP levels also fluctuate in phase with the IMMC [349,350]. The concentrations of pancreatic enzymes and bile acids during the transient surge of pancreaticobiliary secretion are similar to maximal postprandial outputs, although the concentrations diminish rapidly with the onset of type III duodenal motor activity [349,350]. It has been postulated that the cyclic secretion of pancreatic and biliary juice may be important in the digestion of residual food particles or cellular debris in the gastrointestinal tract during the interdigestive period.

The mechanism of control of the cyclic patterns of pancreatic secretion is unclear. Bursts of increased acid secretion are unlikely to be the principal mediators for the cyclic changes in pancreatic secretion, because removal of gastric acid by aspiration or through a fistula does not affect the pattern of interdigestive pancreatic secretion [349,350]. Infusion of motilin prematurely initiates cyclic pancreatic secretion and shortens the periodicity between peaks [351]. Administration of motilin antiserum abolishes the cyclic pattern of pancreatic secretion [352]. Cholinergic blockade with atropine also markedly decreases trypsin output and abolishes interdigestive motor activity. However, administration of phentolamine, an α-adrenergic blocker, increases basal trypsin output fourfold without disrupting the periodicity of pancreatic secretion [353]. These observations suggest that motilin and the autonomic nervous system are important in the initiation of the cyclic pancreatic secretion that occurs during fasting.

Prandial and postprandial secretion

After the ingestion of a meal, the exocrine pancreas is stimulated to secrete enzymes and HCO_3^-. Total postprandial pancreatic output is about 60%–70% of the output attained in response to maximal stimulation with intravenous infusion of CCK [105]. The stimulatory effect of a meal can be

Table 15.1 Three phases of postprandial pancreatic secretion

Phases	Pancreatic response (%)	Stimulants	Mediators
Cephalic	25	Sight, smell, taste, eating	Vagal innervation
Gastric	10	Distention	Vagal–cholinergic pathways
Intestinal	50–75	Amino acids Fatty acids Ca^{2+}, H^+ Distention	Cholecystokinin, secretin Enteropancreatic reflexes Other hormones (?)

described by separating its components into cephalic, gastric, and intestinal phases (Table 15.1).

Cephalic phase

In humans and experimental animals, pancreatic secretions rich in enzymes are stimulated by the sight, smell, and taste of appetizing food [354]. This cephalic effect in dogs amounts to 25% of the enzyme response to an ordinary meal. In humans, the contribution of the cephalic phase to the postprandial pancreatic enzyme secretion appears to be larger, amounting to 50% of the maximal responses induced by exogenous secretin and CCK [355]. The pancreatic response to sham feeding lasts only for the duration of feeding [355].

The vagus nerve appears to be important in mediating the cephalic phase, because this phase can be completely abolished by vagotomy in rats [356]. Administration of an anticholinergic drug decreases or abolishes the pancreatic response to sham feeding in humans [355]. Efferent cholinergic fibers probably act directly on the pancreas, because vagal stimulation causes pancreatic secretion in dogs even when the pancreas is perfused extracorporeally to eliminate any humoral effects of nerve stimulation [357]. Sham feeding increases gastric acid secretion, but because sham feeding induces a pancreatic response in patients who are achlorhydric, it is unlikely that gastric acid secretion contributes significantly to the cephalic phase of pancreatic enzyme secretion.

Gastric phase

In dogs and humans, gastric distention increases the rate of pancreatic enzyme secretion [358,359]. Gastric distention to a volume of 250–400 mL with a balloon doubles the basal pancreatic protein output [360]. Although the actual contribution of the gastric phase to the total postprandial pancreatic secretion has not been determined in humans, the magnitude of the distention-induced pancreatic response in dogs is about 20% of the maximal CCK response over a range of distention from 300 to 1500 mL [358]. Vagotomy and atropine reduce or abolish the pancreatic response to gastric distention, suggesting that it is mainly mediated by vagal cholinergic pathways [359,360]. The mechanoreceptors appear to be located in the body of the stomach because distention of antral pouches does not stimulate pancreatic secretion, whereas distention of the remaining stomach does [360].

The presence of food in the stomach also releases antral hormones such as gastrin or gastrin-releasing peptide, which can stimulate pancreatic secretion directly or indirectly. However, this possibility is considered unlikely because a transplanted portion of pancreas does not respond to gastric distention in dogs [360]. Moreover, distention of the intact stomach has only a slight effect on gastrin release in humans and dogs [361,362].

The stomach facilitates digestion by fractionating solid food into small particles and by initiating digestion of dietary proteins and lipids by pepsin and gastric lipase, respectively [363,364]. Gastric emptying is important in determining the rate of delivery of acid and nutrients into the duodenum, thereby determining the pattern and magnitude of the intestinal phase of pancreatic secretion. Postprandial pancreatic enzyme secretion is often abnormal in patients after gastric surgery [183].

Intestinal phase

The intestinal phase is the most important phase of postprandial pancreatic secretion. In humans and animals, the delivery of food into the small intestine stimulates pancreatic enzyme secretion to about 70% of the maximal level [183]. The major hormonal mediators of the intestinal phase of pancreatic secretion are secretin and CCK. Intestinal 5-HT also appears to play an important role in the mediation of postprandial pancreatic secretion through the vagal cholinergic pathway [132]. The intestinal mucosa has receptors for important vagal cholinergic reflexes that regulate pancreatic HCO_3^- and enzyme secretion.

The proximal intestine plays an important role in the stimulation of pancreatic HCO_3^- secretion, primarily by the release of secretin. Although duodenal pH is the major regulator for the release of secretin, nonacid factors such as fatty acids and bile may also participate. The physiology of secretin was discussed in a previous section (see section on stimulation of pancreatic secretion – hormonal mechanisms – secretin).

Among the hydrolytic products of digestion, amino acids and fatty acids are potent stimulants of enzyme secretion but have only a weak effect on water and HCO_3^- secretion. Amino acid mixtures are more potent than fatty acids, and among the amino acids, only phenylalanine, valine, and methionine stimulate enzyme secretion in humans, whereas in dogs, phenylalanine, leucine, tryptophan, oligopeptides, and casein are effective [365]. The pancreatic response to intestinal perfusion with amino acids above a concentration of 8 mmol/L depends on the total load administered [366]. This

dependence on load rather than concentration is the result of exposure of longer segments of small intestine to amino acids at a concentration above a threshold value. In humans, the mechanisms responsible for the pancreatic response to amino acids are confined to the duodenum and jejunum; amino acid perfusion into the ileum elicits no response.

Undigested fats are ineffective in stimulating pancreatic secretion, but fatty acids are potent pancreatic stimulants when present in micellar form [78]. Monoglycerols, the other product of lipolysis, also stimulate pancreatic secretion [78,365]. The chain length of fatty acids influences their potency in stimulating pancreatic secretion. In humans, the order of potency is C18 > C12 > C8 [92]. Other factors that influence the pancreatic response to fatty acids include the degree of saturation, the concentration, and the total load, as well as the concentration of bile salts relative to fatty acids [364]. In humans, intestinal perfusion of of monoolein (10 mM) produces a pancreatic enzyme output greater than that stimulated by intestinal amino acids and almost equal to the maximal response to exogenous CCK [367].

The release of CCK and intestinal 5-HT by nutrients and mechanical factors appears to play a major role in mediating the intestinal phase of pancreatic enzyme secretion. Plasma CCK levels increase after oral or intraduodenal administration of fat and protein or amino acids [100–102,368,369]. Administration of proglumide, a CCK receptor antagonist, partially inhibits pancreatic secretory responses to intestinally perfused amino acids and fat emulsions. In contrast, a wide variety of non-CCK-dependent stimuli, such as acid, carbohydrates, and mechanical factors, stimulate pancreatic secretion through intestinal 5-HT [132,135]. The 5-HT, in turn, stimulates submucosal vagal afferent fibers to evoke pancreatic exocrine secretion through a vagal cholinergic pathway. Increased firing rates in peripheral afferent neurons and in central sites have been recorded during intestinal perfusion with amino acids [188,370]. This finding, coupled with the observation that truncal vagotomy or administration of atropine markedly increases the latency of the pancreatic secretory response to intestinal nutrients but not to CCK, indicates the participation of vagovagal cholinergic reflexes [371].

Calcium, which is intimately involved in the action of CCK on pancreatic acinar cells, stimulates the pancreas. In humans, intraduodenal perfusion of Ca^{2+} solutions in concentrations similar to those measured in the duodenum after ingestion of a meal stimulates pancreatic enzyme secretion and gallbladder contraction. Intraduodenal Ca^{2+} concentrations of 12–25 mmol/L induce pancreatic enzyme responses similar to the maximal enzyme output evoked by intravenous infusion of CCK [372]. Intestinal perfusion with $MgSO_4$, $MgCl_2$, and $ZnSO_4$ also stimulates pancreatic enzyme secretion by undetermined mechanisms [373].

The human duodenum contains receptors for volume and osmolality that mediate pancreatic enzyme secretion. Volume distention or hyperosmolar solutions in the duodenum elicit pancreatic enzyme secretion by way of intestinal 5-HT without raising the plasma CCK levels [132,186]. This enzyme secretion is inhibited by atropine, suggesting mediation by cholinergic pathways [186]. The volumes of saline required to induce pancreatic secretion are as low as 1–5 mL/min, within the range observed postprandially in the duodenum. The degree of stimulation by volume receptor or osmoreceptor activation is 15%–20% of the maximal enzyme response to CCK [185]. Therefore, both CCK- and non-CCK-dependent stimuli act in concert to mediate the intestinal phase of pancreatic secretion.

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C SECRETION AND ABSORPTION

16 Bile secretion and cholestasis

Steven A. Weinman, Sajid Jalil

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The formation and secretion of bile is one of the central and earliest recognized functions of the liver [1–4]. In addition to the well-known importance of bile in solubilization and digestion of lipids [5], bile formation is necessary for a number of other functions of the liver. These include the obvious such as elimination of toxins, heavy metals and xenobiotics [6,7] and the less obvious such as regulation of whole-body lipid homeostasis [8] and prevention of liver toxicity from endogenously produced bile acids [9–12]. Cholestasis is a term that was initially defined in terms of morphological criteria [13] but has now come to describe a syndrome of liver toxicity that is associated with abnormalities of the bile secretory apparatus. The hepatotoxicity present in cholestasis is due to intracellular accumulation of toxic bile acids and other substrates, alterations in signaling pathways in hepatocytes and bile duct epithelial cells, and the compensatory responses to these events [14,15].

This chapter describes the cellular and molecular processes responsible for bile formation and the mechanisms that result in cholestasis. Knowledge in this field has increased dramatically and many excellent detailed review articles on this subject are available [9–11,15–17]. In particular there have been major advances in understanding the mechanisms responsible for the control of transport in cholestasis and this knowledge has been critical in the development of new but still experimental approaches to therapy of cholestasis. Specific cholestatic states are discussed as examples of the mechanisms involved but the focus of this chapter is on cellular processes and the reader is referred to other chapters for specific discussions of diagnosis and management of cholestatic liver diseases such as drug-induced cholestasis, primary sclerosing cholangitis, primary biliary cirrhosis, and the approach to the jaundiced patient.

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Overview of bile formation

Bile is the secretory product of the liver and bile ducts. It flows through the biliary tract and empties into the duodenum. Bile is formed as a result of the transport activities of several different epithelia working in concert. It is initially produced by hepatocytes and its volume and composition are subsequently altered as it passes through the bile ducts [4,18]. Bile is a complex suspension that has an electrolyte composition similar to that of plasma except for a higher bicarbonate concentration. In addition, bile contains significant concentrations of bile acids, phospholipids, cholesterol, proteins, amino acids, and peptides [19]. Heavy metals, particularly copper, are also excreted in bile and biliary excretion is the major mechanism for their elimination.

Anatomy and physiology of bile secretion and enterohepatic circulation

Hepatocytes are specialized epithelial cells with distinct basolateral and apical membrane domains. They are depicted schematically in Fig. 16.1. The sinusoidal, or basolateral, membrane surrounds the majority of the cell and the canalicular, or apical, membrane occupies a small area of the cell surface near the points of contact between adjacent cells. The canalicular membranes, from which numerous microvilli protrude, make up only about 15% of the total surface area [20]. Opposing canalicular membrane domains border an enclosed space called the bile canaliculus measuring about 1–2 μm [4]. At the point of contact of the two cell membranes is the tight junction, also known as the zonula occludens. Tight junctions constitute a permeability barrier

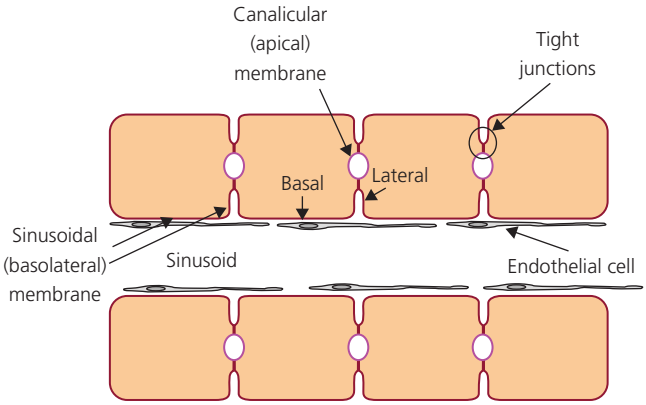

Figure 16.1 Organization of hepatocyte membrane domains. The relationship between the canalicular and basolateral membrane domains is represented for the hepatocyte plates and sinusoidal space. The sinusoidal space and canalicular space are separated from each other by the tight junctional permeability barrier.

that consists of several transmembrane proteins including members of the claudin family and occludin. These proteins form a seal between the membranes of adjacent cells [21]. These tight junctions are cation selective and form a barrier between the bile canaliculus and the sinusoidal space preventing back-diffusion of the secreted organic anions [22,23].

Bile is produced as a result of the osmotic consequences of solute secretion [24]. The hepatocytes directly secrete solutes, primarily bile acids, into the canalicular space and water follows passively driven by osmotic forces across the tight junctions and the cell membranes themselves [19]. The bile canaliculi encircle the hepatocytes in a belt-like fashion and form an interconnecting three-dimensional network. This network communicates with the canals of Hering, which form the transitions to the smallest bile ductules. Flow into the canalicular network thus results in net fluid flux into the biliary tree. Small bile ducts can secrete or absorb fluid and consequently modify the composition and amount of bile [25–27]. They serve as tributaries of the larger ducts. During fasting the sphincter of Oddi contracts and the gallbladder relaxes causing bile to enter the gallbladder for storage, but postprandially the gallbladder remains contracted and the sphincter of Oddi relaxes, resulting in bile bypassing the gallbladder and emptying directly into the duodenum.

Bile acids undergo a process of enterohepatic circulation in which they are absorbed by the intestine, returned to the liver via the portal circulation, taken up into the hepatocytes, and resecreted into the duodenum. In the duodenum and proximal ileum, some bile acids, particularly unconjugated bile acids, are passively absorbed, but conjugated bile acids are primarily absorbed in the terminal ileum. This absorption results from the apical sodium-dependent bile acid transporter (ASBT), a Na^+-dependent transport protein located in the apical membrane of the ileal enterocytes [28]. Within the enterocytes, bile acids interact with a cytosolic bile acid binder, the ileal bile acid binding protein [29,30] and are transported to the basolateral membrane. From here they are secreted into the superior mesenteric vein via a Na^+-independent organic anion exchange protein that is a heterodimeric complex of OSTα and OSTβ [31]. Bile acids then bind to albumin and are transported into the portal venous blood, through the fenestrae between the sinusoidal endothelial cells, and into the space of Disse. They are efficiently extracted by specific transporters present in the basolateral membrane of hepatocytes [32]. The uptake of bile acids occurs predominantly in periportal zone 1 hepatocytes [33]. Although 98% of bile acids are typically extracted from the portal blood during a single pass, the fractional extraction varies considerably according to the particular bile acid molecule. The general scheme for this enterohepatic circulation of bile acids is depicted in Fig. 16.2.

Multiple solutes are responsible for bile flow, but the primary ones are organic anions, and the most important of these are bile acids. Bile acids are amphipathic products of cholesterol metabolism that have several important functions [34]. They are natural detergent molecules that assist in solubilizing the lipid components of bile and are critical for solubilization, digestion, and absorption of lipids in the intestine. In addition they serve as the primary vehicle for cholesterol elimination [8], can specifically trigger apoptosis [35], and have been recognized to be important signaling molecules that bind to transcription factors and regulate gene expression important for lipid homeostasis and hepatic transport [10,11,36,37]. Bile acids are synthesized de novo from cholesterol within the hepatocytes. They are also efficiently recycled through the enterohepatic circulation. Each day 20–40 g of bile acid are secreted into bile but only approximately 3 g are newly synthesized and only 0.5 g are lost through fecal excretion. Under physiological conditions, 70% of the human bile acid pool is composed of cholic acid and cholic acid metabolites while the remaining 30% is chenodeoxycholic acid [38]. Bile acids are conjugated with glycine and taurine after their synthesis, which improves their solubility. These conjugated bile acids are present as anionic salts under physiological pH conditions and are therefore frequently called bile salts. In addition to bile acids, other solutes are also responsible for bile flow. The most important of these is the antioxidant tripeptide, glutathione (GSH), which accounts for most of the bile acid-independent fraction of bile flow [39].

During its transit through the bile ducts, the composition and volume of bile is modified [40]. Some bile acids in the biliary tract are absorbed by the biliary epithelial cells and returned into the portal circulation without entering the intestine. This process is known as cholehepatic shunting and

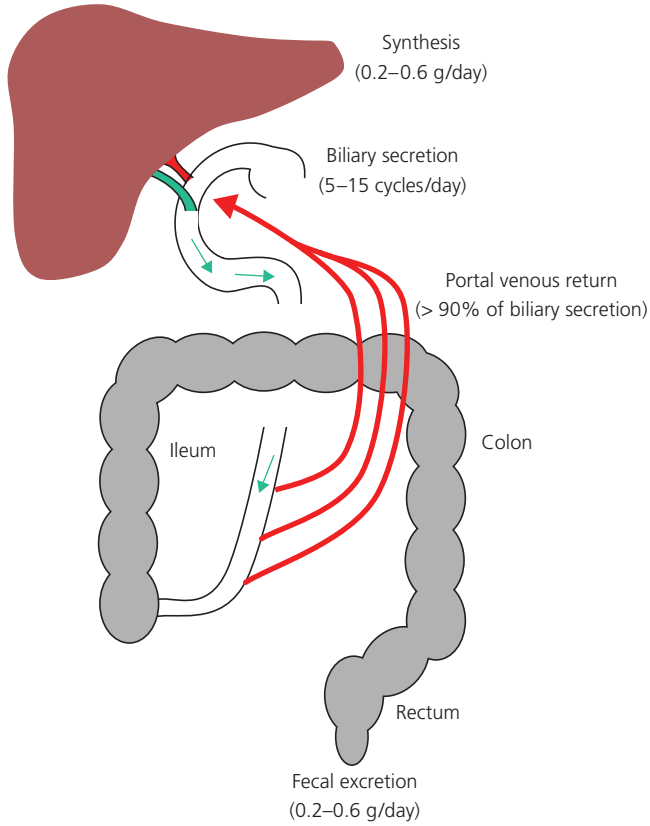

Figure 16.2 Schematic representation of the enterohepatic circulation. The routes of secretion and absorption of bile acids are indicated.

serves to amplify the amount of fluid flow produced by a given net bile acid secretion [41]. Cholangiocytes take up unconjugated bile acids passively after protonation by H^+ equivalents generated from the activity of carbonic anhydrase present in the cholangiocytes [41]. In addition conjugated bile acids can be taken up via the activity of a specific Na^+/bile acid cotransport protein, SLC10A2/ASBT, which is present in the apical membrane of both cholangiocytes and ileal absorptive epithelial cells [28,42]. Intracellular bile acids are then transferred to the basolateral membrane and transported via the periductal capillary plexus into the sinusoids for uptake into the hepatocytes. Cholehepatic shunting plays an important role in bile formation but is also a mechanism of removal of potentially toxic bile acids during pathological states, such as bile duct obstruction where bile acids accumulate proximal to the obstruction in the bile duct. It has also been postulated to play a signaling role where hepatic bile acid secretion can alter the function of the cholangiocytes themselves [43]. These general processes are all mediated by specific transport proteins. The function and regulation of these transporters explain both normal function and pathological manifestations of cholestasis. In order to understand the mechanisms of bile formation and cholestasis, therefore, it is critical to consider the identities and regulation of the specific transport proteins at each of the steps of the process.

Mechanisms of canalicular bile formation

Inorganic ion transport

Hepatocytes possess multiple transport proteins for inorganic ions. These primarily function to generate transmembrane ion gradients, regulate intracellular pH and cell volume, and create the electrical potential difference between the cell interior and the extracellular space. While inorganic ion transport in hepatocytes generates the electrochemical gradients necessary for bile acid transport, it is not directly responsible for bile secretion. This differs from the situation in most fluid-transporting epithelia such as bile duct epithelial cells, where inorganic ion transport is directly responsible for fluid secretion. The inorganic ion transport proteins in hepatocytes are indicated in Fig. 16.3.

The Na^+,K^+-ATPase is present in the basolateral membrane [44] and it utilizes the energy of ATP hydrolysis to exchange Na^+ and K^+, thus generating the low Na^+, high K^+ intracellular environment present in all mammalian cells. A cell-negative membrane potential is generated by the presence of K^+ channels in the plasma membranes [45,46]. The efflux of K^+ through these channels produces a cell-negative diffusion potential. Hepatocytes also have multiple chloride channels in both the sinusoidal and canalicular membranes [47]. This allows chloride to distribute at its electrochemical equilibrium and this process reduces the membrane potential to approximately –30 to –40 mV (cell interior negative) [48].

Several other transporters are primarily involved in pH regulation. These are the Na^+/H^+ exchanger NHE1 [49] and a Na^+/HCO_3^- cotransporter NBC4 [50]. Both of these regulate intracellular pH in response to an acid load. The Na^+/HCO_3^- cotransporter is quantitatively more important in this response [51]. At the canalicular membrane a Cl^- channel [52] and the Cl^-/HCO_3^- exchanger AE2 [53,54] are present. In bile duct epithelial cells this combination of a chloride channel and Cl^-/HCO_3^- exchanger results in electrogenic chloride and bicarbonate secretion (see below). However, chloride is at electrochemical equilibrium in the hepatocyte and there is thus no driving force for net chloride efflux. In hepatocytes, therefore, the AE2 transporter is primarily involved in pH regulation and not fluid secretion.

Cell volume regulation is also extremely important for hepatocytes. Unlike most other cells, hepatocyte function requires cycles of swelling and shrinkage. After a meal the concentrations of amino acids and sugars increase in the portal circulation. Hepatocytes take up large quantities of amino acids and other nutrients and consequently swell [55]. They respond to cell swelling by activation of K^+ and Cl^- channels [56–58]. This produces a net flux of KCl out of the cell and

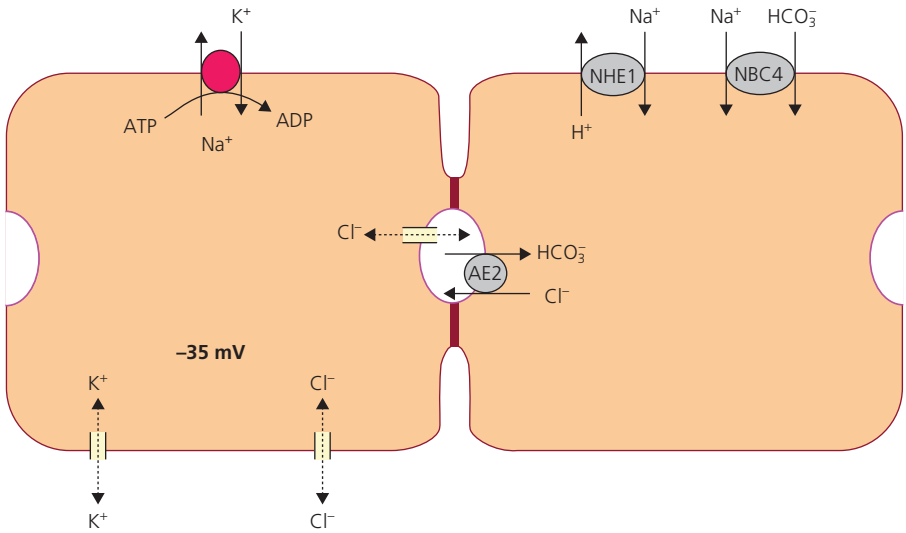

Figure 16.3 Inorganic ion transport pathways of hepatocytes. Ionic gradients are maintained by the Na^+,K^+-ATPase, which in conjunction with K^+ and Cl^- channels maintains the cell interior at an electrical potential of approximately –35 mV with respect to the sinusoid. Chloride is distributed across the membrane passively in accordance with electrochemical equilibrium and there is normally no net driving force for chloride secretion. The Na^+/H^+ exchange protein NHE1, the Na^+/HCO_3^- transporter NBC4, and the anion exchange protein AE2 play important roles in pH regulation.

reduces cell volume. In addition, the chloride channels activated by cell swelling are also permeable to organic substrates such as taurine, and efflux of taurine contributes to volume regulation [59]. Cell swelling activates ion channels by several mechanisms including direct stretch-activated signaling cascades [60,61]. In some cases an autocrine phenomenon of ATP release and binding to purinergic receptors on the cell membrane further activates chloride channels and contributes to cell volume regulation [62].

Organic solute transport

Basolateral (sinusoidal) transport systems

Specific transport proteins at the sinusoidal membrane of hepatocytes account for the ability of the hepatocyte to take up substances from sinusoidal blood and move them into the cells. With few exceptions, the transported molecules do not cross lipid bilayers and their uptake is entirely determined by the properties of the transport proteins present in the sinusoidal membrane. Differences in the identities and quantities of these account for the different uptake properties of hepatocytes in different species, between different individuals, and at different times in the same individual. The most important uptake transporters are SLC10A1/NTCP, the Na^+-dependent bile acid transporter [63], and a family of organic anion conjugate transporters known as the OATP family [64]. Members of the OATP family are responsible for the Na^+-independent uptake of bile acids [10,65] , unconjugated bilirubin, many other organic anions, and even some cationic and amphipathic substances [66,67]. Other important groups of transporters include the OAT family of anion transporters [68] and the OCT family of cation transporters [69]. These transporters are summarized in Table 16.1 and a schematic representation of their localization is shown in Fig. 16.4.

A note on nomenclature

Historically, as the individual transport proteins of the liver were identified they were given names and corresponding acronyms that denoted their function, for example organic anion transport protein (OATP) or bile salt export pump (BSEP). As more of these proteins were discovered and as different species were examined it became clear that most of these proteins were members of families of similar proteins and there was not a one-to-one correspondence of protein functions between species. This led to considerable confusion. Multiple names for the same protein proliferated and in some cases, such as the canalicular membrane phospholipid transport protein, the homologous protein was called mdr2 in mice but MDR3 in humans. Thankfully, the nomenclature of these transport proteins has now been standardized and systematic gene names have been created to classify the proteins by sequence homology. This has been especially helpful for the OATP family in which sequence homology has been used to place the members in evolutionarily related groups. Unfortunately, these systematic names no longer designate function and are thus harder to remember. In the following sections the proteins are introduced using the systematic name in conjunction with the older, more descriptive term. In subsequent sections, for the sake of brevity and in cases where there is no confusion, the descriptive name may be used alone.

Sodium/bile acid cotransport protein (NTCP)

The sodium-taurocholate transport protein SLC10A1/NTCP was the first bile acid transporter to be identified [32,70]. Its expression is highly liver specific, it localizes exclusively to the basolateral membrane of hepatocytes, and it is expressed similarly in periportal and pericentral hepatocytes [71]. Human SLC10A1/NTCP is a 349 amino acid, 50-kDa protein

Table 16.1 Basolateral uptake transporters of the human hepatocyte

Gene symbol	Systematic protein name	Functional protein name	Human gene locus	Predominant substrates	Tissue expression
Primarily liver specific					
SLC10A1	SLC10A1	NTCP	14q24.1	Bile acids, conjugated steroids	Liver, pancreas
SLCO1B1	OATP1B1	OATP-C	12p12	Bile acids, unconjugated bilirubin, organic anions	Liver
SLCO1B3	OATP1B3	OATP-8	12p12	Bile acids, organic anions, digoxin, xenobiotics, peptides	Liver, cancer cell lines
SLCO2B1	OATP2B1	OATP-B	11q13	Sulfated estrogens, DHEAS, BSP	Liver, placenta
Liver and other locations					
SLCO1A2	OATP1A2	OATP-A	12p12	Bile acids, xenobiotics, organic anions, conjugated steroids	Ubiquitous
SLCO3A1	OATP3A1	OATP-D	15q26	E-3-S, prostaglandins, benzylpenicillin	Ubiquitous
SLCO4A1	OATP4A1	OATP-E	20q13.1	Taurocholate, T3, prostaglandins	Ubiquitous
SLCO1C1	OATP1C1	OATP-F	12p12	T4, rT3, BSP	Brain, testes
SLC22A7	SLC22A7	OAT2	6p21.2–p21.1	Organic anions, polyspecific (salicylates, prostaglandin E_2)	Liver, kidney
SLC22A1	SLC22A1	OCT1	6q26	Organic cations, polyspecific (*N*-methylquinine, tetraethylammonium)	Liver, kidney, intestine

BSP, bromosulfophthalein; DHEAS, dehydroepiandrosterone sulfate; E-3-S, estrone-3-sulfate; T3, triiodothyronine; rT3, reverse triiodothyronine; T4, thyroxine.

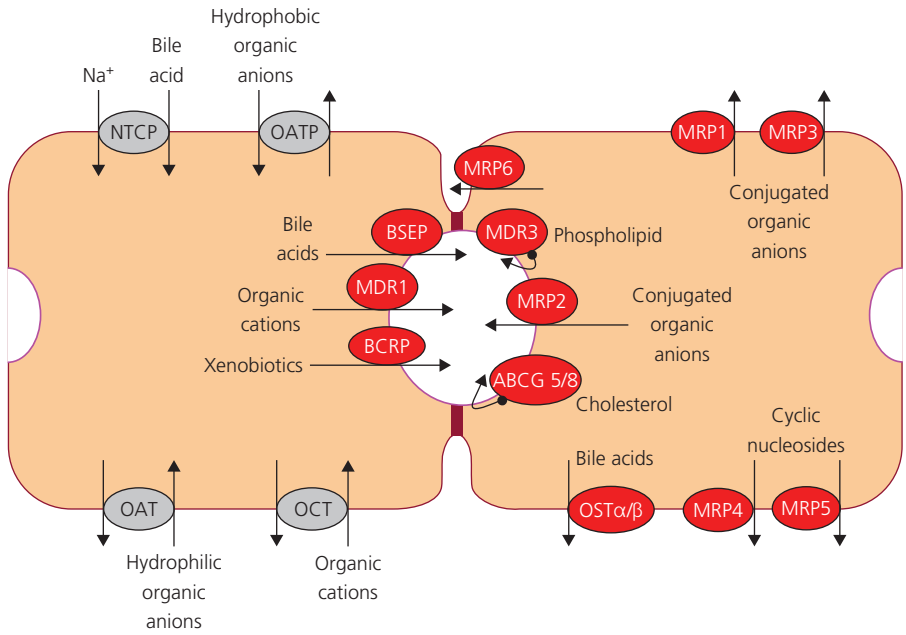

Figure 16.4 Organic ion transporters of hepatocytes. Solute uptake at the basolateral membrane is mediated by a Na^+/bile acid cotransport protein, NTCP, and three families of organic ion exchanger transporters: OATP, the organic anion transporter protein family; OCT, the organic cation transporter family; and OAT, the organic anion transporter family. Export from the hepatocyte into the canalicular space is due to the ATP-dependent transport proteins BSEP, MRP2, MDR1, and ABCB4/MDR3. The ATP-dependent transport proteins MRP1, MRP3, MRP4, MRP5, and MRP6 transport substrates out of the cells into the sinusoidal blood. See text for details.

with a substrate specificity that is primarily limited to bile salts and certain sulfated steroids [11]. Transport is strongly coupled to Na^+, with a stoichiometry of $2Na^+$ transported for one bile acid molecule [72,73]. This coupling strategy results in an electrogenic transport process [74] that greatly increases the driving force for uptake and increases the maximum intracellular bile acid concentrations that can be achieved. It has been estimated that it can elevate intracellular bile acid concentrations at least 10-fold above that in the sinusoidal blood [75]. SLC10A1/NTCP is critical for bile acid transport and is entirely responsible for Na^+-dependent bile acid transport, which accounts for up to 80% of bile acid uptake. SLC10A1/NTCP is strongly conserved among mammalian species.

SLC10A1/NTCP is a member of a small family of sodium/bile salt cotransporters within the superfamily of sodium/solute symporters [76]. In addition to SLC10A1/NTCP, a closely related protein called the apical sodium bile acid transporter SLC10A2/ASBT is expressed at the brush border membrane of ileal epithelial cells and cholangiocytes. It efficiently removes bile salts from the intestinal lumen and allows their return to the liver via the portal circulation [63].

OATP family of transporters

The OATPs are a family of overlapping multispecific transporters with broad tissue distribution and substrate specificity [64]. These transporters are classified within the gene superfamily of solute carriers (SLC) as their own family SLCO. OATP transporters are present in multiple other tissues as well as liver, and they mediate the Na^+-independent transport of bile acids, conjugated estrogens, conjugated bilirubin, cysteinyl leukotrienes, thyroid hormones and many other substrates by a process of anion exchange [77,78]. They are present in many species, but unlike the situation for NTCP, there is no clear correspondence in terms of sequence or substrate specificity between species. The new, species independent, nomenclature divides the OATP family members into six groups based solely on sequence homology [10,64,69]. This system is used in Table 16.1.

The OATPs represent a major mechanism for hepatocellular organic anion uptake and hepatic first-pass clearance of drugs. Numerous drugs are substrates of OATPs, including the antihistamine fexofenadine, opioid peptides, the hydroxymethylglutaryl (HMG)-CoA reductase inhibitor pravastatin, the angiotensin-converting enzyme inhibitor enalapril, and methotrexate [79]. They are anion exchangers and the uptake of substrates into the cell is mediated by the efflux of GSH from hepatocytes into the sinusoid [80]. Since GSH is synthesized in hepatocytes and its intracellular concentrations are much higher than those in sinusoidal blood, efflux of GSH is likely to provide the driving force for electroneutral organic ion uptake [81].

In humans, the OATP family comprises nine members [65], three of which appear to be liver specific, although with marked variation in the level of expression. SLCO1B1/OATP1B1 is highly expressed in hepatocytes and appears to be the primary non-bile acid organic ion uptake transporter [82,83]. It is a 691 amino acid protein that is primarily expressed in the basolateral membrane of hepatocytes and transports bile acids, unconjugated bilirubin, conjugated sterols, eicosanoids, and thyroid hormone. Its significance is demonstrated by its ability to transport albumin-bound unconjugated bilirubin, unlike most of the other members of the OATP family [84]. SLCO1B3/OATP1B3 is another important multispecific transport molecule at the basolateral membrane of human hepatocytes. It is has 80% sequence homology to SLCO1B1/OATP1B1 [85], and also has broad substrate specificity, mediating transport of bile acids, conjugated sterols, bromosulfophthalein, thyroid hormones, and organic cations [66]. In addition, cardiac glycosides including digoxin are transported by SLCO1B3/OATP1B3 [10,79]. SLCO2B1/OATP2B1, expressed predominantly in the basolateral membrane of hepatocytes [66], is the third human liver OATP. It has a more limited substrate specificity than SLCO1B1/OATP1B1 or SLCO1B3/OATP1B3 and does not transport bile acids [66].

The other human OATPs are not primarily hepatic transporters. SLCO1A2/OATP1A2 is a 60-kDa bile salt transporter with ubiquitous expression and broad substrate specificity for xenobiotics [86,87]. It is found primarily in the endothelial cells of the blood–brain barrier where it transports bile salts, organic anions, conjugated steroids, and numerous drugs [88,89]. Expression of SLCO1A2/OATP1A2 is noted to be increased in liver tissues of patients with cholestatic liver disease [90]. SLCO3A1/OATP3A1 and SLCO4A1/OATP4A1 are expressed in many tissues, and SLCO1C1/OATP1C1 expression is limited to brain and testes [64].

OAT/OCT transporters

Several other groups of transporters are present in the basolateral membrane of hepatocytes, although their overall significance is less clear. The OAT family comprises a group of transporters that play a role in anion transport in both liver and kidney [68]. The substrate specificity of OAT family members differs from that of OATP family members in that substrates tend to be more hydrophilic. Nonetheless, there is considerable overlap. SLC22A6/OAT1, the initially identified member of the group, is present only in kidney where it functions as the high-affinity *p*-aminohippuric acid transporter. This is the probenecid-inhibitable transporter that is largely responsible for efficient tubular anion secretion [91]. A similar transporter, SLC22A7/OAT2, is present exclusively in the liver [92], a third family member, SLC22A8/OAT3, is present in both kidney and liver [93], and SLC22A9/OAT4 is present in kidney and placenta [94]. The relative distribution of OATP and OAT family members between kidney and liver plays an important role in the well-known transport differences of these two organs. More bulky hydrophobic anions

Table 16.2 Canalicular export transporters of the human hepatocyte

Gene symbol	Functional protein name	Human gene locus	Predominant substrates	Tissue expression	Disease association
ABCB11	BSEP	2q24	Bile acids	Liver	PFIC II, BRIC 2, ICP
ABCC2	MRP2	10q24	Conjugated bilirubin, organic anions, glutathione conjugates, leukotriene C_4	Liver, kidney, intestine	Dubin–Johnson syndrome
ABCB1	MDR1	7q21	Organic cations, xenobiotics	Liver, kidney, BBB	
ABCB4	MDR3	7q21.1	Phosphatidylcholine	Liver	PFIC III, ICP, gallstone disease
ABCG5, *ABCG8*	Cholesterol half transporters	2p21	Plant sterols, cholesterol	Liver, intestine	Sitosterolemia
ABCG2	BCRP	4q22	Xenobiotics	Liver, placenta	
ATP8B1	FIC1	18q21	Aminophospholipid	Liver, intestine	PFIC I, BRIC 1

BBB, blood–brain barrier; BRIC, benign recurrent intrahepatic cholestasis; ICP intrahepatic cholestasis of pregnancy; PFIC, progressive familial intrahepatic cholestasis.

(OATP substrates) are preferentially taken up by hepatocytes and excreted largely in bile rather than urine. More hydrophilic organic anions (OAT substrates) are preferentially secreted by the renal tubules and excreted largely in urine rather than bile. Substrates of SLC22A7/OAT2 include *p*-aminohippuric acid, salicylates, and prostaglandin $(PG)E_2$ [95].

The members of the OAT family have a 12-transmembrane-domain structure and function largely as electroneutral anion exchangers [68]. In spite of these similarities they do not share sequence homology with the OATP family. However, they do share homology with the organic cation transporters of the OCT family [96]. While the organic cation transporters appear to play little part in bile formation, their substrates include many important therapeutic drugs and xenobiotics.

Canalicular transport

The secretion of bile salts and other molecules from the cytoplasm of the hepatocytes across the canalicular membrane into the bile canaliculi is the rate-limiting step in bile formation and requires direct energy input [4,19]. It is achieved by the action of several different ATP-dependent transport proteins. These transport proteins are part of the superfamily of ATP-binding cassette (ABC) proteins and share similar structural and functional characteristics with each other [97]. The ABC transport proteins of the canalicular membrane can be divided into two functional classes. The first is the solute transporters such as the cation transporter ABCB1/MDR1, the salt export pump ABCB11/BSEP, the xenobiotic transporter ABCG2/BCRP, and the multispecific organic anion transporter ABCC2/MRP2. The second class of ABC proteins in the canalicular membrane are lipid translocators such as the phospholipid *flippase* ABCB4/MDR3, and the ABC half-transporters involved in cholesterol transport ABCG5/ABCG8 [36]. These transporters are summarized in Table 16.2.

Bile salt exporting protein (ABCB11/BSEP)

In humans, ABCB11/BSEP is the predominant bile salt efflux system of hepatocytes [98]. It is localized in the canalicular membrane and in subcanalicular vesicles [99]. Loss of human ABCB11/BSEP results in near total elimination of biliary bile acid excretion [98], whereas a knockout mouse lacking Bsep displayed only 50% reduction in bile acid secretion [100]. This demonstrates that in mice, unlike in humans, other proteins at the canalicular membrane can transport bile salts. ABCB11/BSEP-dependent bile acid secretion is the primary driver of bile flow and conditions that reduce its function or expression generally result in cholestasis. ABCB11/BSEP inhibition occurs in drug-induced cholestasis caused by rifampicin, cyclosporine (ciclosporin), or conjugated estrogens [101,102]. Inherited BSEP dysfunction results in progressive familial intrahepatic cholestasis type 2 and its less aggressive form, benign recurrent intrahepatic cholestasis type 2 [103] (see below).

Multidrug resistance associated protein 2 (MRP2)

ABCC2/MRP2 is an ABC transport protein that is primarily expressed in the canalicular membrane of hepatocytes [104,105] and serves as a primary high-affinity transporter for conjugated organic anions including conjugated bilirubin [106], GSH conjugates, and cysteinyl leukotrienes [107]. In addition it transports other substrates including sulfated bile salts, conjugated estrogens, antibiotics, and a number of other exogenous compounds [108]. ABCC2/MRP2 also transports unconjugated reduced GSH from the hepatocyte

into the bile [81] albeit with a relatively low affinity [109]. However, since intracellular GSH concentrations are high, it is responsible for a significant fraction of canalicular GSH secretion. It is a member of a family of closely related transport proteins that differ more in their tissue and membrane localization than in their transport function. In humans the MRP family consists of six members. ABCC2/MRP2 is the only MRP detected in the canalicular membrane [36,110].

ABCC2/MRP2 is specifically absent from the canalicular membrane in the human disease Dubin–Johnson syndrome. While ABCC2/MRP2 is important in organic anion and bilirubin transport, it plays little role in bile formation. Patients with Dubin–Johnson syndrome have an almost complete absence of bilirubin conjugates and GSH conjugates in bile and develop a black pigment in their liver, but are otherwise normal. They exhibit up-regulation of other MRP family members in the sinusoidal membrane and as a consequence are able to eliminate MRP substrates into the blood for eventual renal rather than biliary excretion [111].

Multidrug resistance protein 1 (MDR1)

ABCB1/MDR1 is a 1280 amino acid, 170-kDa protein that is responsible for the secretion of a variety of large hydrophobic compounds, especially organic cations [112]. This transporter was first identified as a protein that becomes overexpressed in some cancer cells and consequently renders these cells resistant to a diverse array of chemotherapeutic agents. It was subsequently determined that the mechanism of this multidrug resistance is that ABCB1/MDR1 functions as a drug efflux pump with broad specificity for cationic chemotherapeutic agents [113]. The protein is normally expressed at the apical membranes of several epithelia but most notably in the canalicular membrane of hepatocytes [114] where it functions as an important mechanism for the secretion of drugs and organic cations into bile [115]. No specific endogenous substrate has been identified for ABCB1/MDR1.

Phospholipid transporter: multidrug resistance protein 3 (MDR3)

ABCB4/MDR3 is an ABC transport protein that is expressed in high levels in the canalicular membrane of the hepatocytes where it functions as a phospholipid transporter [116]. Several studies have demonstrated that ABCB4/MDR3 and the homologous protein in mice, mdr2, serve as ATP-dependent phospholipid flippases that translocate phosphatidylcholine (PC) from the inner to the outer leaflet of the lipid bilayer of the canalicular membrane [117,118]. This process is a critical step in the mechanism by which phospholipid is secreted into bile (see below).

ABCB4/MDR3 is a 170-kDa protein that represents the major ABC protein of the canalicular membrane [114]. Knockout mice with disruption of the *mdr2* gene develop progressive hepatic fibrosis and bile duct destruction and have complete absence of phospholipid secretion into bile [119]. A similar syndrome, progressive familial intrahepatic cholestasis type 3 (see below), also occurs in humans with mutations of the ABCB4/MDR3 protein [120]. Defects in this gene also contribute to gallstone susceptibility in association with cholestasis [98,103] and intrahepatic cholestasis of pregnancy [121].

ABC half-transporters

While most members of the ABC transporter family contain two highly homologous domains and function as a monomer, several ABC transporters have just a single domain in which the functional unit is a dimer. Several of these so-called *half-transporters* are important canalicular membrane transporters [122]. ABCG2 or breast cancer resistance protein (BCRP) [102], so-called for its overexpression in breast cancer cells, confers resistance against chemotherapeutic agents such as mitoxantrone, bisantrene, etoposide, prazosin, and flavopiridol by extruding these drugs from the cell. The BCRP protein functions as a homodimer and is present at the canalicular membrane of hepatocytes.

ABCG5 and ABCG8 are two other half-transporter members of the ABCG family. Both ABCG5 and ABCG8 are expressed mainly in liver and intestine [123]. These half-transporters function as a heterodimer and are involved in hepatobiliary excretion of plant sterols and cholesterol. Mutations in either the *ABCG5* or *ABCG8* gene cause sitosterolemia, which manifests as elevated plasma levels of plant sterols especially sitosterol [123], leading to hypercholesterolemia and premature atherosclerosis. Overexpression of *ABCG5/ABCG8* in transgenic mice caused increased biliary cholesterol secretion and reduced intestinal absorption of dietary cholesterol, lending further support for ABCG5/ABCG8 involvement in hepatobiliary excretion of cholesterol [124].

ATP8B1/FIC1

The *FIC1* or *ATP8B1* gene was first identified in 1998 as the mutated gene responsible for familial intrahepatic cholestasis (Byler disease) and benign recurrent intrahepatic cholestasis type 1 [103,125]. FIC1 protein is expressed primarily in small intestine and to a lesser extent in the cholangiocyte and canalicular membrane of hepatocytes [126,127]. FIC1 does not appear to be an important plasma membrane transport protein and therefore the reason that its ablation produces cholestasis is not fully understood. However, findings have suggested that it is a phospholipid translocator capable of translocating phosphatidylserine [128]. Its ability to prevent cholestasis may result from its effects on the activation of nuclear receptors such as FXR that control expression of the other transport proteins [129,130] (see below).

Sinusoidal efflux

Secretion of proteins from the hepatocyte, across the sinusoidal membrane into the blood, is a major function of the

Table 16.3 Sinusoidal efflux transporters of the human hepatocyte

Gene symbol	Functional protein name	Human gene locus	Predominant substrates	Localization	Tissue expression	Disease association
ABCC1	MRP1	16p13.1	Organic anions (glutathione, glucuronide and sulfate conjugates)	Basolateral	Absent/low in normal conditions	
ABCC3	MRP3	17q21.3	Bile acids, sulfated bile acids	Basolateral	Absent/low in normal conditions	
ABCC4	MRP4	13q32	Bile acids, nucleoside analogues and cyclic nucleosides, glutathione	Basolateral	Ubiquitous	
ABCC5	MRP5	3q27	Purine analogues and cyclic nucleosides	Basolateral	Ubiquitous	
ABCC6	MRP6	16p13.1	Unknown	Lateral membrane	Ubiquitous	Pseudoxanthoma elasticum
OSTα/OSTβ	OSTα/OSTβ	3q29/15q22.3	Bile acids, sterols, prostaglandin E_2	Basolateral	Small intestine, kidney, liver	

liver, but under normal conditions, the polarity of organic solute transport primarily involves sinusoidal uptake and canalicular efflux. However, in a number of pathological conditions this polarity is reversed and efflux across the sinusoidal membrane becomes quantitatively important. This is partially accomplished by reversal of gradients and passive transport via carriers such as the OATPs, but the primary mechanism of sinusoidal efflux is via insertion of a series of new transport molecules into the sinusoidal membrane. Five members of the ABCC (MRP) family with somewhat varying substrate specificity and another transporter, OSTα/OSTβ [131], are involved in efflux of organic anions across the basolateral membrane during cholestasis [79] (Table 16.3). This explains the widely recognized increase in plasma conjugated bilirubin that occurs in cholestasis.

ABCC1/MRP1 is involved in cellular efflux of various organic anions including GSH, glucuronide and sulfate conjugates of a number of drugs. ABCC3/MRP3 is an organic anion transporter expressed prominently in liver, kidney, pancreas and gut that primarily transports bile salts. The expression of both ABCC1/MRP1 and ABCC3/MRP3 is low under physiological conditions, but both are substantially induced during cholestatic states. Upregulation of ABCC3/MRP3 is an important rescue mechanism allowing for renal excretion of bile salts and organic anion efflux during conditions such as primary biliary cirrhosis and Dubin–Johnson syndrome [132,133]. ABCC4/MRP4 and ABCC5/MRP5 are both organic anion pumps and have the ability to transport cyclic nucleotides and nucleoside analogues [134]. ABCC6/MRP6 is mainly expressed in liver and kidney. The substrate specificity of ABCC6/MRP6 is still unclear [102].

Another transporter, the heterodimer of OSTα and OSTβ, is primarily a bile acid export transporter that has been identified in intestine as well as in liver and is responsible for the efflux of intestinal bile acids from epithelial cells into blood [31]. It is a unique transporter consisting of a heterodimer between OSTα, a seven-transmembrane-domain protein, and OSTβ, a single transmembrane domain protein. Its transport specificity is primarily for bile acids [131].

Intracellular movement

In addition to transport across the sinusoidal and canalicular membranes, movement of solutes through the cytosol is critical for transport. Many of the solutes transported by hepatocytes are hydrophobic organic ions and as a consequence they tend to bind to proteins, partition into various cellular membrane fractions, and become associated with intracellular organelles. It has been estimated that these interactions reduce the effective diffusion constants for intracellular molecules by a factor of 1000 compared with what would be expected for diffusion in free solution [135]. Several high-affinity intracellular binding proteins are present in hepatocytes. These include bile acid-binding proteins, primarily 3α-hydroxysterol dehydrogenases, which bind bile acids in the low micromolar range [136]; fatty acid-binding protein, which binds free fatty acids [137]; and glutathione-*S*-transferases, which, in addition to the enzymatic activities, serve as binding proteins for bilirubin [138]. Intracellular binding proteins serve to prevent bile acids from partitioning into fixed lipid membranes and organelles [139], and serve as an intracellular sink preventing reflux of bile acids through the sinusoidal membrane.

Transcytotic vesicular transport also plays a role in hepatic transport in some situations. A number of proteins, particularly monomeric IgA, bind to specific receptors at the sinusoidal membrane and undergo receptor-mediated endocytosis, transcellular trafficking, and exocytosis at the canalicular membrane [140]. Other molecules including bulky organic solutes such as class II cations, and proteins such as transferrin translocate via this mechanism [141]. Although copper appears to be transported into vesicles and secreted across the canalicular membrane by exocytosis [142], this process does not play a major role in the transport of other small molecules, such as bile acids.

Biliary lipid secretion

Regulation of lipid homeostasis is a key function of the liver and biliary lipid secretion plays a critical role in this process [143]. In addition to its role in maintenance of whole-body lipid homeostasis, biliary secretion of phospholipids and cholesterol is necessary for prevention of intrahepatic bile acid toxicity and cholestatic bile duct injury. The major lipid components of bile are bile salts, phospholipid primarily as PC, and cholesterol. The monomeric forms of phospholipid and cholesterol have limited aqueous solubility and exist in bile in the form of micelles and vesicles. The mechanism of biliary lipid secretion differs from that of soluble small molecules. The general process is one in which PC and cholesterol are inserted into the canalicular membrane and then subsequently solubilized from the outer leaflet of the canalicular membrane into bile by a bile acid-dependent process [8,144].

Phospholipid secretion depends on several steps. First, cellular PC is inserted into the canalicular membrane. The primary process responsible for this is a membrane–membrane exchange process mediated by the phosphatidylcholine-transfer protein (PC-TP) [145,146]. This protein results in the selective addition of PC to the inner leaflet of the canalicular membrane. Flipping of PC from the inner leaflet to the outer leaflet of the membrane is accomplished by the ABCB4/MDR3 protein [116,147,148]. This step is critical and the absence of a functional flippase in the canalicular membrane results in the complete absence of biliary phospholipids in both humans [120] and mice [119]. Phospholipid is ultimately removed from the outer leaflet of the canalicular membrane and appears in bile as small vesicles. This process is dependent on bile salts. Electron microscopic evidence suggests that it involves direct budding of vesicles off the canalicular membrane [144] in addition to transfer from the canalicular membrane to biliary micelles [149].

Biliary cholesterol transport is also dependent on a series of membrane transporters and intracellular transport processes. Cholesterol enters hepatocytes by several mechanisms including endocytic uptake of low-density lipoprotein (LDL) via the LDL receptor and the LDL receptor-related protein. High-density lipoprotein (HDL) is also an important source for hepatic cholesterol uptake and is part of the *reverse cholesterol transport pathway* by which cholesterol is removed from peripheral tissues and returned to the liver for excretion [150]. HDL binds to the hepatic HDL receptor, SR-BI, which results in the uptake of cholesterol destined for biliary secretion [151,152]. With a normal cholesterol diet, this pathway appears to involve direct lateral diffusion of the cholesterol within the plasma membrane and accounts for most of biliary cholesterol. Intracellularly, cholesterol is bound to several binding proteins including the sterol carrier protein SCP-2. In addition it is transported intracellularly by vesicular transport via endosomes and lysosomes. A second pathway for biliary secretion of cholesterol occurs with high dietary cholesterol in which LDL appears to be the source. This involves a trafficking pathway of endocytosed cholesterol from endosomes to lysosomes and then from lysosomes to the canalicular membrane for excretion. The precise mechanisms of this pathway have not been determined but it involves two proteins that have been associated with Niemann–Pick Disease, NPC1 and NPC2 [153].

Secretion of cholesterol across the canalicular membrane into bile has been shown to involve a heterodimeric transport complex consisting of ABCG5 and ABCG8. Absence of either of these proteins leads to hepatic accumulation of plant sterols in patients with sitosterolemia [123,154] and defects in biliary cholesterol secretion. This role of ABCG5/ABCG8 in cholesterol transport has been confirmed in an animal model, showing that coexpression of both proteins permits their transport to the apical surface [155,156]. Humans and mice lacking ABCG5 and ABCG8 have a marked reduction in biliary cholesterol secretion [157], whereas transgenic mice overexpressing the human proteins have increased biliary cholesterol concentration [158]. Another ABC transporter ABCA1 is ubiquitously expressed and is present in hepatocytes. It mediates cholesterol efflux from cells and is responsible for loading of nascent HDL with tissue-derived cholesterol. Although present in hepatocytes, its role in biliary cholesterol secretion has not been established [159].

Hepatic copper transport

Biliary excretion of copper is the exclusive mechanism by which copper is eliminated from the body and a defect in this process, as occurs in Wilson disease, results in massive accumulation of copper in liver, brain and other organs [160]. Copper is taken up by hepatocytes from the sinusoidal circulation by means of a Cu transporter, CTR1 [161]. Due to its ability to catalyze redox reactions and hence its toxicity, intracellular Cu is tightly bound to specific carrier proteins called chaperones. In hepatocytes, copper is primarily destined for incorporation into cytochrome c oxidase via the chaperone COX17, incorporation into the Cu/Zn superoxide dismutase via the chaperone CCS1, and interaction with the Cu pump protein ATP7B via the chaperone ATOX1 [162]. ATP7B is a P-type ATPase that is required both for Cu incorporation

into ceruloplasmin as well as biliary Cu excretion. It is exclusively found in the liver and is localized primarily in an intracellular trans-Golgi site [163].

ATP7B, the protein that is defective in Wilson disease, is exclusively responsible for copper secretion in bile [164,165]. It is primarily an intracellular Cu transport protein that concentrates Cu in vesicular structures where it can be incorporated into ceruloplasmin, such as occurs in the trans-Golgi. When hepatocellular Cu content is low, ATP7B is primarily located in the trans-Golgi. In response to high Cu conditions, it redistributes and translocates to a closer association with the canalicular membrane where its primary role appears to shift to biliary excretion. It is uncertain whether the primary mechanism of biliary secretion is ATP7B-dependent Cu loading and subsequent fusion of subcanalicular vesicles with the canalicular membrane, or whether ATP7B actually resides in the canalicular membrane and pumps Cu directly into bile [166]. The best evidence suggests that ATP7B resides in subcanalicular vesicles and biliary Cu secretion occurs via a process of vesicular fusion [142].

In addition to ATP7B, other proteins are necessary for biliary Cu secretion. Several diseases, such as Indian childhood cirrhosis and endemic Tyrolean infantile cirrhosis, result in massive hepatic Cu accumulation without a defect in ATP7B. A protein called MURR1/COMMD1 has been identified as the gene defect responsible for copper toxicosis in Bedlington terriers [167]. Abnormalities of this protein result in a defect in the biliary excretion of Cu in humans as well as dogs without any defect in Cu incorporation into ceruloplasmin. Although it is ubiquitously expressed and appears to be necessary for a final step in Cu excretion, its precise function is unknown. It does bind directly to ATP7B and one hypothesis is that it is required for the fusion of copper-loaded vesicles with the canalicular membrane [168]. Because the affected dogs show massive copper accumulation in lysosomes of the hepatocyte, a possible role for MURR1 might be to facilitate degranulation of lysosomal contents into the bile [169]. However, MURR1 is not responsible for most non-Wilson disease hepatic copper toxicosis syndromes in humans and thus other elements of the Cu secretion pathway remain to be determined [170].

Mechanisms of bile ductular secretion

Following the process of fluid secretion into the canalicular space, bile flows through the bile ductular system prior to its emptying into the duodenum. Far from a passive conduit, the biliary epithelium serves many functions including secretion and absorption of fluid and electrolytes [171]. In addition, bile ducts are able to specifically absorb bile acids [172]. This serves to both detoxify bile and amplify bile flow through cholehepatic shunting [173]. The biliary ductules comprise only 3%–5% of the total liver cell population but they can secrete up to 40% of total bile flow in humans [174]. Unlike the situation in hepatocytes, fluid secretion by the biliary epithelium is not the product of organic ion secretion. Rather it is a process of chloride-dependent bicarbonate secretion similar to that which occurs in other salt-transporting epithelia such as the airway epithelium [175]. Biliary secretion is under intense hormonal control, particularly by the secretagogue secretin, which increases intracellular cyclic adenosine monophosphate (cAMP), stimulating secretion of bicarbonate-rich fluid. Other hormones and neuropeptides such as acetylcholine, somatostatin, gastrin, bombesin, substance P, vasoactive intestinal polypeptide (VIP), and endothelin also modulate bile flow [171,176]. Figure 16.5 shows the transport proteins responsible for bile ductular secretion.

The cholangiocytes are the epithelial cells that line the biliary ductules. They play an important role in bile secretion and express transporter systems and water channels necessary for secretory and absorptive functions. The basolateral membrane contains a Na^+,K^+-ATPase that establishes the ionic gradients and a K^+ channel that serves to maintain the intracellular negative potential [177]. An electroneutral $Na^+/K^+/2Cl^-$ cotransporter at the basolateral membrane (NKCC) is driven by the Na^+ gradient and results in the accumulation of intracellular chloride concentrations greater than that which would be expected by electrochemical equilibrium [178]. This is important because of the presence of the cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel at the apical membrane facing the ductular lumen [179]. Since Cl^- is above electrochemical equilibrium, a stimulus, such as secretin, that opens the apical membrane chloride channels will result in Cl^- secretion into the lumen [180,181]. A water channel, aquaporin-1, is present in the apical membrane and allows a water flux to be driven by osmotic forces [182].

At the same time, the Na^+ gradient across the basolateral membrane drives two processes that tend to alkalinize the cytosol. These are electrogenic Na^+/HCO_3^- cotransport [183,184] and Na^+/H^+ exchange [185]. The cycle is completed by an apical membrane Cl^-/HCO_3^- exchanger, AE2, which exchanges the secreted Cl^- in the lumen for intracellular HCO_3^- [53]. The net process is one of electrogenic bicarbonate secretion. The entire system is maintained by the Na^+,K^+-ATPase and the energy supplied by this transporter is transduced into net electrogenic bicarbonate secretion from blood to bile, chloride recycling into and out of the cell across the apical membrane, Na^+ and K^+ recycling across the basolateral membrane, and passive Na^+ entry into bile through the tight junctions.

This process of secretin-stimulated fluid secretion occurs primarily in the larger branches of the intrahepatic ducts, those with diameters greater than 15 μm and not primarily in the smallest ducts with diameters less than 8 μm [186]. The CTFR is a critical component of this process. It is a cyclic AMP-dependent apical membrane chloride channel on which this process depends. Similar to the situation in respiratory

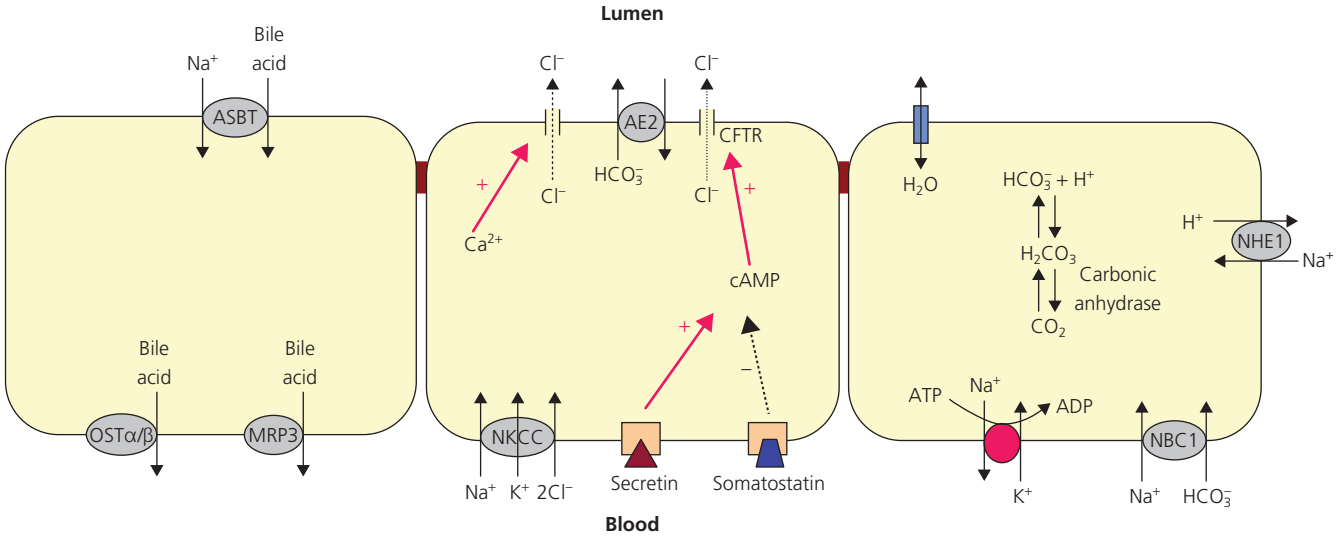

Figure 16.5 Bile duct epithelial cell transport. Electrogenic fluid and bicarbonate transport results from the involvement of multiple transporters. The $Na^+/K^+/2Cl^-$ cotransporter uses the energy of the Na^+ gradient to bring chloride into the cell at concentrations above its electrochemical equilibrium. Chloride is secreted into the lumen primarily via the cAMP-dependent chloride channel CFTR (cystic fibrosis transmembrane conductance regulator). It is exchanged for bicarbonate via AE2 at the apical membrane. The process is primarily regulated by secretin, which increases cAMP levels in the cell. Bile acids are absorbed from the lumen by the ASBT transport protein and transported into the blood by OSTα/OSTβ and MRP3. A water channel, aquaporin-1, is present at the apical membrane. These processes are represented in different cells only for clarity. See text for further details.

epithelia, a defect in this Cl^- channel, as occurs in cystic fibrosis, reduces fluid secretion [187]. Under these circumstances, a calcium-dependent chloride channel can substitute for CFTR and allow some biliary secretion to occur [188].

Hormonal regulation is prominent in ductular secretion. Secretagogues that raise intracellular cAMP, such as secretin, both directly activate the CFTR channel and induce insertion of CFTR, AE2, and aquaporin-1 into the apical membrane [189]. Calcium-mediated signaling induced by secretagogues such as acetylcholine and extracellular ATP also activate K^+ channels and thus stimulate secretion by a CFTR-independent mechanism [190].

In addition to transport of water and electrolytes, cholangiocytes transport bile acids. Bile acids serve both as transported substrates as well as regulators of cholangiocyte function [191]. Uptake of bile salts from the apical membrane of cholangiocytes is mediated by the apical sodium-dependent bile salt transporter SLC10A2/ASBT that is identical to the ASBT found in the brush border of ileocytes [42]. Efflux of bile salts from cholangiocytes across the basolateral membrane into portal circulation is mediated by several different proteins. These include the OSTα/OSTβ transporter [31,131], a truncated form of ASBT [192], the anion exchanger SLCO1A2/OATP1A2 [43], and in part by the ABC transporter ABCC3/MRP3 [65,193].

Mechanisms of regulation of bile formation

A fundamental characteristic of the liver is that it serves multiple different functions and is able to adapt its functional activity to suit physiological and pathophysiological demands. The ability of the liver to produce bile is thus quite variable and under some circumstances can be dramatically reduced. This reduction in the transport activity of hepatocytes and bile ducts manifests itself as cholestasis. Since bile formation is largely a consequence of the activity of organic ion transport proteins, the major mechanisms involved in regulation of bile flow are changes in the function, localization, and expression of these transport proteins. Other changes such as alterations in structure and function of tight junctions [194] and bile duct secretory processes [176,189] also play a role. This allows the liver to adapt to conditions that require changes in bile flow. The liver is able to modify its transport function by several mechanisms. In the short term (minutes to hours) changes in intracellular signaling result in modifications of transport proteins themselves [195], and redistribution of transport proteins between intracellular and plasma membrane pools [196]. In the longer term (hours to days), changes in expression of specific transport proteins play the more significant role.

Alterations in function of hepatocellular transporters

Signaling events within hepatocytes and biliary epithelial cells produce rapid changes in transport [197]. SLC10A1/NTCP is subject to rapid regulation of its function on several levels. First, since it transports two Na^+ ions with each negatively charged bile acid [72], the net transport is electrogenic and its rate is strongly influenced by changes in the intracellular negative membrane potential [74]. Hepatocytes normally have a membrane voltage of –30 to –40 mV but this changes in pathological states [48,198,199] with a consequent increase or decrease in bile acid entry via SLC10A1/NTCP.

In addition, SLC10A1/NTCP is phosphorylated and its phosphorylation state controls its distribution between intracellular and membrane sites [200–202]. The phosphorylation state of SLC10A1/NTCP is controlled by a balance of kinase and phosphatase activity and a cAMP-mediated increase in phosphatase activity reduces the phosphorylation state of SLC10A1/NTCP. The dephosphorylated molecule is preferentially translocated to the sinusoidal membranes from an intracellular pool [200].

Other hepatocyte transport proteins can also be directly phosphorylated but regulation by protein phosphorylation does not appear to be a major mechanism of control. Protein kinase (PK)C phosphorylation sites are present on MRP2 and BSEP [203] and canalicular organic anion transport is modulated by PKC activators [204]. The rat Oatp transporters, SLCO1A1 and SLCO1A4, have also been shown to be phosphorylated [205] and are inhibited by the activity of PKC [206]. There is additional evidence that phosphoinositide (PI)3-kinase plays a role in transporter regulation. It regulates insertion of transporters into the canalicular membrane (see below) and its activation also increases the transport capacity of canalicular membrane vesicles [207]. Thus, although phosphorylation of hepatic transporters does occur, its direct effect on transport activity appears to be minor and its more important effect is related to changes in trafficking of transporters to membrane sites.

Alterations in transporter targeting

Transport proteins exist in at least two separate pools, one at the appropriate plasma membrane, and the other in intracellular vesicles that have the ability to fuse and rapidly insert into the plasma membrane. Translocation between these pools is an important mechanism for rapid changes in the transport capacity of hepatocytes [196,208,209]. Rapid transporter redistribution is induced by bile acid accumulation [210,211], changes in cell volume [212,213], and hormone-induced increases in intracellular cAMP and intracellular calcium signals [209]. Regulated trafficking occurs for the bile acid uptake protein SLC10A1/NTCP, as well as most of the canalicular membrane efflux pumps including ABCB11/BSEP, ABCC/MRP2, ABCB1/MDR1, and ABCB4/MDR3 [208]. It is a more critical mechanism at the canalicular membrane where transport capacity is rate limiting for overall bile flow.

A complex interaction of signaling events controls transporter trafficking and many details are not yet understood. Trafficking of SLC10A1/NTCP is largely controlled by cAMP through it activation of PKA, release of intracellular calcium stores, and subsequent activation of PI3-kinase, calmodulin-dependent kinases and protein phosphatase 2B, which dephosphorylates SLC10A1/NTCP resulting in its translocation to the sinusoidal membrane [214].

The canalicular membrane transport proteins all actively shuttle between the canalicular membrane and an intracellular subapical vesicular pool [196]. Membrane translocation is stimulated by cAMP, cell swelling, or bile acid exposure and is dependent on the generation of lipid signaling molecules through the action of PI3-kinase [215]. Transporter recruitment to the canalicular membrane is entirely blocked by inhibitors of PI3-kinase and the lipid products of PI3-kinase themselves directly stimulate transporter insertion [196]. As discussed above, PI3-kinase therefore plays a dual role in regulation of transport, stimulating transporter insertion and directly modulating transporter activity. The response to cell volume changes also appears to involve PI3-kinase but hepatocyte swelling activates several different members of the mitogen-activated protein kinase family including Erk1/2 and p38 [197] as well as Src kinase.

Intracellular bile acids themselves rapidly increase the cellular capacity for maximal bile acid secretion. This process depends on the function of microtubules [216], and involves transformation of the morphology of a subcanalicular vesicle network from primarily small vesicles to one of elongated tubules [217]. This process results in the insertion of ABCB11/BSEP-containing vesicles into the canalicular membrane [207] and an increase in the capacity of the canalicular membrane to transport bile acids [218]. The mechanisms by which intracellular bile acids promote transport protein insertion into the plasma membrane involve direct activation of PKC isoforms, increases in intracellular calcium, and activation of stress kinases.

Dynamic redistribution of canalicular membrane transporters between intracellular and canalicular membrane sites is important for liver function during physiological conditions. Hepatocytes normally undergo cycles of swelling and shrinkage in response to the solute load imposed by meals and subsequent uptake of amino acids and sugars. Since cell swelling causes insertion of ABCC2/MRP2 and ABCB11/BSEP into the canalicular membrane and cell shrinkage causes their internalization from the canalicular membrane [197], this mechanism coordinates bile secretion rates with meals. Rapid redistribution of transporters appears to be less important for the uptake transporters at the sinusoidal membrane. Although cAMP-dependent translocation occurs for SLC10A1/NTCP, this uptake step is generally not rate

limiting and may not play a major role in regulation of bile formation. Transporter translocation has not been shown to be important for regulation of the OATP family of transport proteins.

Regulation of transporter gene expression

The primary mechanism by which the transport function of the liver is regulated is through changes in the expression levels of individual transport proteins. This mechanism allows the liver to adapt to changing metabolic needs and respond to the presence of drugs and xenobiotics. Coordinated expression changes occur after a number of pathological insults such as inflammation [16,219] and bile duct obstruction [220], and serve to both generate the cholestatic state and protect the liver from accumulation of toxic compounds such as intracellular bile acids. Control of expression of hepatic transport proteins has been actively investigated and a detailed understanding of the processes involved has now emerged [16,221].

Transcription of hepatic transporters, like all other cellular genes, is under the control of 5′ upstream regulatory element sequences and the presence of activator proteins in the nucleus that bind to those elements [222]. Transporter mRNA transcription rates are rapidly altered by changes in the amount or function of these nuclear transcription factors. Regulation of transcription factor activity can occur by activation of the transcription factors themselves, after proteolysis steps that activate or inactivate factors, or most importantly after ligand binding, which creates an active factor complex [222,223].

A major breakthrough in understanding how liver transporter gene expression is regulated came with the discovery that one particular nuclear receptor, the farnesoid X receptor (FXR), is a bile acid sensor [224–226]. FXR is a cytosolic transcription factor that binds bile acids with low micromolar affinity [226,227]. It is a member of a group of type II nuclear receptors that bind an array of hydrophobic ligands. Bile acid binding induces heterodimerization with another member of the family, RXR [228], and the heterodimer serves as the active transcription factor complex [229]. At least 12 different type II nuclear receptors are important in regulating a number of aspects of hepatic function including bile formation, lipid metabolism, drug metabolism, and xenobiotic excretion. In addition to bile acids, other activating ligands include oxysterol cholesterol derivatives, drugs and xenobiotics, fatty acids, and retinoids. The major transcription factors responsible for regulation of hepatic function are listed in Table 16.4.

This system represents a web of interactions whereby changes in the cellular concentration of a ligand, such as bile acid, produces a series of coordinated changes, both positive and negative, in expression of transporters, metabolizing enzymes, biosynthetic pathways, and even the transcription factors themselves. In many cases the activating ligands themselves are substrates for uptake, excretion, degradation or synthesis by the same proteins whose expression they regulate. The system can thus serve important protective functions by limiting accumulation and returning the liver to a state of homeostasis. In some circumstances, such as in cholestasis, these compensatory changes are insufficient to prevent liver injury or can even contribute to injury. The extent to which nuclear receptors control hepatic function, and their proven ability to prevent toxicity in some circumstances makes hepatic nuclear receptors attractive therapeutic targets [17,221,230].

The complexity and coordinated action of the pathway is best illustrated by considering the responses initiated by individual nuclear receptors. FXR is a bile acid-binding protein that preferentially binds the moderately hydrophobic bile acids chenodeoxycholate and cholate, but not hydrophilic bile acids like ursodeoxycholate or extremely hydrophobic ones such as lithocholate. On ligand binding it dimerizes with RXR and activates expression of a number of important genes involved in bile acid and lipid metabolism [231,232]. Key FXR-activated genes are those of the canalicular bile salt export pump ABCB11/BSEP, the canalicular conjugated bilirubin transporter ABCC2/MRP2, the phospholipid flippase ABCB4, the basolateral bile salt export transporter OSTα/OSTβ, and the small heterodimer partner protein (SHP). SHP is important in that it is a member of the nuclear receptor family that lacks DNA-binding properties of its own, but interferes with activation by other nuclear receptors such as RAR/RXR heterodimers, HNF4α, LRH-1 and PPARα. Partially as a result of induction of SHP, FXR activation by bile acids subsequently decreases expression of the bile acid uptake protein SLC10A1/NTCP and 7α-cholesterol monooxygenase (CYP7A1), the first committed step in bile acid synthesis from cholesterol. Elevated cellular bile acids thus decrease their own synthesis and uptake while increasing their excretion. The net effect is to diminish cellular bile acid accumulation and prevent cytotoxicity.

Two other nuclear receptors, PXR and CAR, are primarily xenobiotic receptors and are responsible for the metabolic response of the liver to many drugs as well as the regulation of non-bile acid organic ion transport [233,234]. PXR is activated by many drugs including rifampicin as well as hydrophobic bile salts such as lithocholic acid. On ligand binding and dimerization with RXR it directly activates expression of drug-metabolizing members of the P450 family, CYP3A and CYP2B, as well as the cation transporter ABCB1/MDR1, the anion transporter ABCC2/MRP2, and members of the drug-conjugating family of glutathione-*S*-transferases. CAR, the constitutive androstane receptor, is the only member of the type II nuclear receptor family that has activity in the absence of ligand. It is activated by phenobarbital, and bilirubin is also a ligand. In response to CAR activation there is up-regulation of the drug-metabolizing members of the CYP3A and CYP2B family. In addition, it

Table 16.4 Nuclear receptors/transcription factors regulating hepatic transport protein expression

Nuclear receptor	Alternative name	Ligands	Genes activated	Genes inactivated	Major functions
FXR	NR1H4	Bile acids, especially CDCA, DCA, CA, LCA	BSEP, SHP, MRP2, OATP1B3, ABCB4, PXR, OSTα/β, CYP3A4, UCT2B4, UGT2B7	NTCP, CYP7A1	Major bile acid sensor Increases detoxification and excretion Decreases bile acid synthesis and uptake
SHP	NR0B2			CYP7A1, CYP8B1, NTCP, ASBT, HNF4α	Induced by FXR Inhibits bile acid synthesis and uptake via effects on HNF4α and HNF1α
PXR	SXR, NR1I2	Numerous xenobiotics, rifampicin, statins, LCA, dexamethasone, UDCA	CYP3A4, CYP2B6, MDR1, MRP2, GSTA2, UGT1A1	CYP7A1	Induction of drug metabolism and excretion pathways Considerable overlap with CAR
CAR	NR1I3	Bilirubin, phenobarbital, xenobiotics	CYP3A, CYP2B6, CYP2A6, OATP1B1, MRP2, UGT1A1		Induction of drug metabolism and excretion pathways Considerable overlap with PXR Activated by bilirubin Phenobarbital promotes nuclear translocation
LXRα	NR1H3	Oxysterols, 6α-hydroxy bile acids	ABCG5/8, ABCA1, CYP7A, CYP8B, CYP3A4, SHP		Regulator of cholesterol metabolism and elimination
RXR	NR2B1	9-*cis*-retinoic acid	Required for function of FXR, PXR, LXR, and CAR		Heterodimerization partner for FXR, PXR, CAR , LXR, RAR Activity inhibited by cytokines
RARα	NR1B4	All-*trans*-retinoic acid	NTCP, MRP2, ASBT		
VDR	NR1I1	1α,25-dihydroxyvitamin D3, LCA	CYP3A, SULTs		Induction of bile acid hydroxylation, sulfation, and export
HNF4α	NR2A1		CYP7A1, CYP8B1, NTCP, HNF1α		Activity reduced by bile acids, which reduces HNF1α expression and down-regulates bile acid synthesis
HNF1α	MODY3, TCF1		OATP1B1, NTCP, CYP7A		Major regulator of glucose homeostasis Reduced activity contributes to decreased bile acid uptake and synthesis
PPARα	NR1C1	Fatty acids, fibrates, statins, NSAIDs	ASBT, ABCB4, UGT2B4	CYP7A1	Increases β-oxidation, biliary phospholipid secretion Down-regulates bile acid synthesis

CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; LCA, lithocholic acid; NSAID, nonsteroidal antiinflammatory drug; UDCA, ursodeoxycholic acid.

increases expression of each of the genes involved in bilirubin clearance, including OATP1B1, ABCC2/MRP2, and the conjugating enzyme UDP-glucuronosyl transferase (UGT1A1) [235].

LXR is an oxysterol-binding protein that is critical in the regulation of hepatic lipid metabolism and cholesterol transport [236,237]. Its ligands are primarily oxysterol cholesterol derivatives and fatty acids but it can be activated by bile acids as well. Important proteins that are under the control of LXR are the cholesterol export protein ABCA1, the canalicular cholesterol half-transporters ABCG5/ABCG8, and other genes involved in cholesterol and fatty acid metabolism such as lipoprotein lipase, cholesterol ester transfer protein and apolipoprotein E. PPARα, another hepatic type II nuclear

receptor, binds fatty acids and fibrates and plays a key role in regulation of hepatic fatty acid biosynthesis and oxidation. Its activation up-regulates expression of the canalicular phospholipid transport protein ABCB4, thus promoting biliary phospholipid secretion [238].

Bile acid-induced expression changes

Bile acid transport is regulated by coordinated actions of nuclear receptors, particularly FXR, on specific transport proteins. In cholestatic syndromes, hepatic bile acid concentrations rise, FXR is acitivated, and a bile acid program of transcription changes ensues. The ability of the liver to down-regulate NTCP expression during cholestasis is critical because this transporter is primarily responsible for bile acid entry into the hepatocytes and preventing entry of bile acids while secretion is impaired is necessary to avoid cellular toxicity. Promoter elements that control NTCP expression are complex and vary between species. In humans important elements are HNF1, HNF4α, STAT5 [239], and the RAR/RXR heterodimer [240,241]. Its expression is sharply down-regulated in response to an increase in intracellular bile acids and this is mediated by several mechanisms. First, there is an FXR-mediated increase in SHP expression. This non-DNA binding member of the nuclear receptor family is a negative regulator and inhibits activation by the RXR/RAR heterodimer important for SLC10A1/NTCP expression [242,243]. Second, both NTCP and OATP1B1 expression depend on the nuclear receptor HNF1α, which is itself transactivated by HNF4α. Bile acids inhibit HNF4α-mediated transactivation and may directly inhibit HNF1α as well [241,244].

In contrast to NTCP, which is down-regulated in cholestasis, expression of the canalicular bile salt exporter BSEP is maintained. The promoter elements of the *BSEP* gene include C/EBP-β, HNF3β, and importantly the FXR/RXR heterodimer. BSEP is thus directly up-regulated by bile salt accumulation. This is physiologically appropriate as a mechanism to keep intracellular bile salts low in the face of cholestasis. Other important proteins that must respond to changes in bile acid concentrations are the enzymes involved in bile acid synthesis, CYP7A1 and CYP8B1. Expression of both these proteins is inhibited by an SHP-dependent mechanism [245]. Bile acid conjugation reactions are up-regulated as well via FXR elements in the promoters for the enzymes responsible for conjugation and sulfation. The overall consequences of this bile acid loop are up-regulation of canalicular export and conjugation, and down-regulation of sinusoidal entry and bile acid synthesis in a coordinated fashion that minimizes cellular bile acid toxicity.

Organic anion-induced expression changes

Similar coordinated changes occur for organic anions primarily under the control of PXR and CAR. Rising bilirubin concentrations activate CAR with subsequent effects on expression of transport proteins and drug-metabolizing enzymes. The primary bilirubin uptake protein, OATP1B1, has both PXR and CAR elements in its promoter and the canalicular organic ion transporter, MRP2, has promoter elements for PXR, CAR, FXR, and the RAR/RXR heterodimer [234,246]. These can result in both up-regulation and down-regulation depending on the circumstance. Many drugs stimulate net organic anion transport. Rifampicin and phenobarbital are agonists for PXR and CAR respectively, and thus stimulate expression of both uptake and export transporters. The drug-metabolizing enzymes of the CYP3A and CYP2B families and the bilirubin-conjugating enzyme UGT1A1 all have CAR and/or PXR elements. Consequently elevated intracellular bilirubin and drug concentrations lead to increased expression of the proteins necessary for uptake, metabolism, and excretion. Under normal conditions this serves a detoxification function, although this well-tuned response can be altered by pathological conditions. For example, during inflammation, cytokine-dependent changes in expression levels of the nuclear receptors themselves appear to dominate [240,247] and both OATP1B1 and MRP2 expression are down-regulated [248,249], thus interfering with the detoxification function of the liver. This is discussed in detail below.

Regulation of the cytoskeleton and tight junctions

Alterations in the structure of the cytoskeleton and tight junctions occur in several conditions in which bile flow is reduced. Actin filaments serve as an intracellular scaffolding mechanism and are necessary for the targeted delivery of vesicles and the maintenance of cell polarity. Loss of the normal uniform actin filament distribution and clumping of actin filaments around the bile canalicular membrane have been observed in multiple different models of cholestasis including bile duct obstruction and with cholestatic bile acids and drugs [250,251]. Vesicular transport along microtubules mediated by the molecular motor protein kinesin is also inhibited in cholestasis [252] and these processes together alter transport function by changing the pattern of transport protein insertion and retrieval from the cell membranes.

The paracellular pathway, controlled by the proteins that make up the tight junctional complex, is also altered in cholestasis [194]. Both bile duct obstruction and estrogen-induced cholestasis produce changes in the distribution of the intracellular tight junction protein ZO-1 [253] and a reduction in the number of claudin strands that compose the tight junctional seal [254]. These effects produce an increase in the permeability of the tight junctions to large molecules [254]. Alterations in the organization of tight junction proteins occur in human cholestatic diseases [255] and may contribute to the decrease in bile flow under these conditions.

Regulation of bile duct secretion

Short-term regulation as a consequence of transport protein phosphorylation is the major mechanism of regulation of bile

duct secretion [171,176]. The rate-limiting step for biliary bicarbonate secretion is the activity of the CFTR chloride channel at the apical membrane (see Fig. 16.5). Channel activity of CFTR is controlled by PKA-mediated phosphorylation of its regulatory domain [256,257]. In the biliary epithelium, a secretin-induced increase in intracellular cAMP is the primary mechanism that activates chloride channels and net secretion [180]. In addition secretin also causes a microtubule-dependent insertion of aquaporin-1 water channels into the apical membrane, further enhancing fluid secretion [182].

Other hormones and neuropeptides also play a role in regulation of ductular secretion, particularly in modulating the effects of secretin. Somatostatin interacts with $SSSTR_2$ receptors on the bile duct epithelial cells and decreases cAMP and biliary secretion [258]. Gastrin has no effect on basal secretion but inhibits secretin-induced choleresis [259]. Ductular fluid secretion is stimulated by bombesin [260], VIP [261,262], and acetylcholine [261,262]. It is inhibited by endothelin [263].

An additional mechanism that regulates bile ductular secretion is the presence of micromolar concentrations of ATP in the ductular lumen [264]. In response to cell swelling and other stimuli, hepatocytes are able to release ATP into the canalicular space [265,266]. This ATP interacts with purinergic P_{2U} receptors on the apical membrane of cholangiocytes, which increases intracellular calcium, activates chloride channels, and stimulates secretion [267]. In addition ATP may be released by the cholangiocytes themselves, resulting in a mechanism for both autocrine and paracrine regulation of bile duct function.

Mechanisms of cholestasis

Cholestasis is defined as the cessation or reduction of bile flow in a pathological situation. Cholestasis is a coordinated response of the liver and does not reflect merely nonspecific injury. It may be hereditary, due to genetic defects in transporter proteins or bile duct development, or acquired as a result of exposure to toxins or immune responses. The general principle is that transcellular transport into bile is diminished but at the same time bile acid accumulation in the cell is minimized. Cholestasis is typically associated with the up-regulation of alternative sinusoidal efflux pathways for toxins, conjugated bile acids, conjugated bilirubin, leukotrienes, and GSH. There is frequently preservation of biliary excretion of drugs and an increase in hepatocellular uptake of amino acids, perhaps as a mechanism to support the massive protein synthesis required for the acute-phase reaction.

Other changes in liver function and structure also occur in many cholestatic settings. These include changes in cytoskeletal architecture [251], alterations in tight junctional permeability [254], a decrease in the fluidity of the canalicular membrane [268], alteration in cell polarity, and disruption of cell-to-cell communication via gap junctions [110,220,269]. Generally, cholestasis is associated with disruption of the normal enterohepatic and nephrohepatic circulations. There is increased cholehepatic shunting and an increase in renal elimination of compounds generally excreted in bile as a result of sinusoidal efflux from hepatocytes into blood [10]. This section considers the specific changes that occur in several cholestatic situations. A number of detailed reviews have appeared on this subject [9,10,15,270–273].

Acquired cholestatic syndromes

Inflammatory cholestasis

Cholestasis is frequently associated with inflammation, particularly that caused by Gram-negative bacteremia. Examples include sepsis, alcoholic hepatitis, idiosyncratic drug reactions, total parenteral nutrition, and cholestatic variants of viral hepatitis [274]. Inflammatory cholestasis is one of the most frequent causes of jaundice in hospitalized patients [219]. Inflammatory cholestasis is mediated primarily by cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6, which are released in response to bacterial lipopolysaccharide (LPS) stimulation of activated macrophages and Kupffer cells. The effect of these cytokines has been extensively studied in several experimental models [247,275] and to a lesser extent directly in human disease.

The phenomenon of inflammatory cholestasis is manifested by decreased expression of the transporters responsible for bile acid (NTCP) and organic ion (OATP1B1) uptake, and decreased expression of the canalicular membrane transporters responsible for secretion of bile acids and organic anions (BSEP and MRP2) [249,272,276]. Bile acid synthesis is decreased as well, with reduction in the activity of CYP7A1 [277]. At the same time there is preservation or up-regulation of transport systems responsible for preventing intracellular accumulation of toxic substrates such as the sinusoidal anion efflux pump ABCC1/MRP1 and the canalicular cation efflux pump ABCB1/MDR1.

Although the mechanisms for these changes are multifactorial and only partially understood, they result primarily from cytokine-induced changes in the expression of transcription factors and only secondarily from ligand-induced effects such as the activation of FXR by increased levels of intracellular bile acids [278]. In rodents, LPS causes a decrease in transcriptional activity of the RXRα/RARα heterodimer, and HNF1α. Both of these bind to the *NTCP* promoter and thus the net effect is to decrease NTCP expression. IL-1β plays a major role in this effect as it has been shown to cause export of RXR from the nucleus [279]. Since RXR is the common heterodimer partner for the type II nuclear receptors FXR, PXR, and CAR, its export from the nucleus reduces the expression of multiple genes including those of NTCP, MRP2, BSEP, and OATPs. NTCP expression is further

down-regulated by an additional reduction in transcription factors that bind to specific response elements in the NTCP promoter region, specifically HNF1α [280] and footprint B binding protein [240]. In addition to IL-1β, TNF-α and IL-6 also cause decreases in transporter gene expression [275].

The net result of all these effects is a profound and rather global phenotypic change in the transport function of the liver. There is impaired uptake of bile acids and other organic ions, impaired canalicular secretion of these and many other substrates, and increased sinusoidal export of bile acids, conjugated bilirubin and other molecules that would normally have been secreted into bile. While some of these substances are eliminated by renal excretion, in general this does not keep pace with production and will produce the characteristic cholestatic syndrome of conjugated hyperbilirubinemia and elevated serum bile acids.

Bile duct obstruction

Obstructive cholestasis is a pathological condition resulting from obstruction in the extrahepatic bile duct, commonly caused by choledocholithiasis or neoplasm. Bile duct obstruction rapidly results in an increase in biliary pressure, an increase in biliary and intracellular bile acid concentrations, and a series of adaptive changes in the liver and bile ducts that result in cholestasis [281]. The molecular events occurring after bile duct obstruction have been extensively studied in the model of common bile duct ligation in rats [282,283]. Although there have been fewer studies in humans, the overall picture is similar in both species and several important observations from the rat model help to illustrate the changes in hepatic function that occur in response to obstruction.

Although conceptually simple, bile duct obstruction produces complex effects on the liver. The initial obstruction results in an increase in bile acid concentrations in the bile duct lumen and within the hepatocytes [281] and an increase in the pressure within the biliary tree. This produces a primary effect in which elevated bile acids, primarily by acting as ligands for FXR and other nuclear receptors, induce a series of changes in transporter expression, both positive and negative, in both hepatocytes and cholangiocytes. Secondarily, the chronic obstruction results in inflammation and cytokine production and many of the cytokine effects on transcription factors occur as described for inflammatory cholestasis.

There are several characteristic changes in gene expression [10]. First, there is a decrease in the expression and activity of the sinusoidal uptake transporters for bile acids (NTCP), bilirubin, and other organic ions (OATP family members). Second, there is decreased expression of the enzymes responsible for bile acid synthesis, primarily CYP7A1 and CYP8B1. Third, canalicular secretion of organic anions via MRP2 is strongly down-regulated [249], but canalicular bile acid excretion via BSEP, although decreased, is less severely affected [284]. This results in a decrease in transport with relative preservation of export of bile acids to minimize the toxic accumulation of bile acids in hepatocytes. Finally, transporters involved in detoxification and providing alternative exit pathways for bile acids are up-regulated. These include the canalicular organic cation transporter ABCB1/MDR1, the phospholipid transporter ABCB4/MDR3, and the sinusoidal efflux pumps ABCC3/MRP3 and ABCC4/MRP4 [285,286]. The net effect of these changes is to slow or stop the formation of bile, limit the intracellular accumulation of bile acids, and retain or increase the ability to efflux potentially toxic compounds from the hepatocytes into the blood where they can undergo renal excretion.

Simultaneously, changes in cholangiocytes occur as well [287]. Bile duct cells proliferate, the apical membrane bile acid uptake protein in cholangiocytes, ASBT, is preserved or possibly up-regulated, and the expression of the cholangiocyte basolateral efflux transporter OSTα/OSTβ is increased via the activity of FXR [288]. The net effect of these changes is to allow a greater fraction of biliary bile acids to be reabsorbed by the bile duct.

Gene expression changes after bile duct obstruction are dominated by the effects of bile acid binding to FXR, subsequent increased transcription of the nuclear receptor SHP, and direct inhibitory bile acid effects on two other important transcription factors, HNF4α and HNF1α [16]. As discussed above, the most critical molecular event for prevention of hepatocellular toxicity is the down-regulation of NTCP by mechanisms including an FXR-induced increase in SHP [242], and bile acid-induced inhibition of HNF4α and HNF1α [289]. Finally, SHP also inhibits the ability of the glucocorticoid receptor to activate SLC10A1/NTCP expression and this may play a role as well [290].

Posttranslational mechanisms also play a role in the short-term response to bile duct obstruction [12]. These include relocalization of BSEP and MRP2 from the canalicular membrane to an intracellular site and similar relocalization of NTCP as well. These effects may be partially mediated by an increase in cAMP within the hepatocytes [291].

Estrogen-induced cholestasis

Estrogens and anabolic steroids frequently cause a form of bland hepatocellular cholestasis that manifests clinically as jaundice and pruritis but histologically shows only the morphological characteristics of cholestasis, such as inspissated bile and intrahepatocyte bile plugs, without inflammation or bile duct injury. Sensitivity to estrogen-induced cholestasis varies considerably between individuals and correlates with the risk of development of intrahepatic cholestasis of pregnancy [292,293].

Estrogen-induced cholestasis is a complex process resulting from changes in gene expression, direct inhibition of transport proteins, and possibly other factors. The administration of ethinylestradiol to male rats has frequently been

used as an experimental model to study the pathogenesis of estrogen-induced cholestasis. In this model there is a dramatic decrease in expression of sinusoidal uptake transporters as well as canalicular membrane export transporters. Ntcp expression is reduced and this is associated with a reduction in bile flow [284,294]. In addition, decreased expression of Oatp1A1, the rat OATP responsible for the sodium-independent uptake of bile acids, has also been observed [294]. At the canalicular membrane, Bsep expression is also reduced but as in the other models of cholestasis, this effect is much smaller than that on Ntcp [220]. Repeated administration of ethinylestradiol also reduces Mrp2 protein levels and causes impaired canalicular excretion of Mrp2 substrates such as bromosulfophthalein, bilirubin, and GSH conjugates [249]. In addition to the down-regulation of transporters, there is also evidence that conjugated estrogens can directly inhibit Bsep. The inhibitory site appears to be on the external (bile) side of the canalicular membrane and occurs after the conjugated estrogens have been secreted by ABCC2/Mrp2 [295,296]. The net result is impaired bile acid secretion.

The exact mechanisms of the gene expression changes induced by estrogens are not known and it does not appear that conjugated estrogens themselves are direct ligands for FXR. It is conceivable that once cholestasis is initiated it can be perpetuated by the retained compounds such as hormone metabolites and bilirubin, which act as nuclear receptor ligands and may alter the transcriptional activity of the respective target gene [10]. Recent studies in transgenic and knockout mouse models suggest that estrogen-induced cholestasis and hepatotoxicity requires the estrogen receptor (ER)α and not other nuclear receptors such as ERβ, FXR, PXR, or CAR [297]. Activation of ERα by the estrogen receptor agonist 17α-ethinylestradiol leads to decreased expression of the bile acid transporters (NTCP, OATP1B1 and BSEP), likely through antagonizing HNF4 activity. It also alters the bile acid biosynthesis enzymes CYP7A1, CYP7B1, and CYP8B1. The net result is inhibition of bile acid secretion and alteration of bile acid composition that causes the characteristic hepatocyte degeneration and intrahepatic cholestasis. Although ERα appears to have the primary initiating role, the changes in bile acid concentrations clearly have secondary effects, although these may not be enough to prevent toxicity. It is notable that an FXR agonist, 6-ethylchenodeoxycholic acid, has protective effects in estrogen-induced cholestasis [298], suggesting that FXR-mediated gene expression changes may be compensatory but insufficient to provide full protection.

Intrahepatic cholestasis of pregnancy

Intrahepatic cholestasis of pregnancy (ICP) is a reversible form of cholestasis that may develop in susceptible individuals during the third trimester of pregnancy. This syndrome is characterized by pruritus and cholestasis during the third trimester and accounts for about 20% of pregnancy-associated liver disorders [292,293]. An increased risk for stillbirths and subsequent gallstones in these individuals has been described [121]. Serum bile acids are normal and γ-glutamyltranspeptidase (GGT) may be normal to elevated. Another subtype of ICP with elevated serum bile acids and normal GGT has also been described.

While the causative mechanism of ICP has not been determined, several well-demonstrated gene associations have begun to provide insights into this disorder. First, there is a strong genetic and or environmental component to this phenomenon. For example, up to 14% of pregnancies in the Chilean population may be affected [299], and other regional and familial clusterings have been observed [300,301]. Polymorphisms of the gene for the canalicular membrane phospholipid transporter ABCB4/MDR3 have been identified in high frequency in patients experiencing ICP [302,303]. The elevated bile acid subtype of ICP has also been associated with dysfunction of ABCB11/BSEP [304,305]. However, a group of Finnish women with ICP failed to exhibit genetic mutations of *ABCB11/BSEP*, *ATP8B1/FIC1* or *ABCB4/MDR3*, suggesting the etiology of ICP may be heterogenous [306] and that cholestatic estrogen metabolites may play a critical role.

Primary biliary cirrhosis

Primary biliary cirrhosis (PBC) is an autoimmune disease that develops in genetically susceptible individuals exposed to an environmental trigger. The inflammatory process of PBC primarily affects the small bile ducts and manifests as intense pruritus initially and jaundice in late stages.

Knowledge of adaptive changes in transporter gene expression in PBC is limited due to lack of animal models and is derived mainly from patient liver biopsy specimens. Available data suggest that the alterations in transporter gene expression in PBC are stage specific [133,307]. In early stages of PBC (Ludwig stage I and II), expression of bile salt and organic anion transporters is well preserved [276]. In later stages (III and IV), OATP1B1 and to a lesser extent NTCP expression are down-regulated, while MRP3 and MDR1 expression are up-regulated. MRP2 expression decreases with progressive cholestasis [307]. Unlike ICP and familial cholestasis syndromes, neither *BSEP* nor *ABCB4/MDR3* gene mutations are more frequent in patients with PBC than controls [308]. Eventually, immune-mediated bile duct injury in PBC results in progressive cholestasis. The resulting inflammation with production of cytokines together with regurgitated toxic bile acids leads to the observed changes in transporter expression [10]. Together, these changes represent adaptive rather than causative changes in chronic cholestasis.

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is characterized by inflammation, fibrosis and obstruction of large and medium-sized intrahepatic and extrahepatic bile ducts [309]. The

disease course is variable and prognosis is difficult to predict because of the lack of good models. The majority of patients with PSC have a history of inflammatory bowel disease, particularly ulcerative colitis [310].

Changes in transporter proteins in PSC have been studied less systematically than for PBC. Recently, the ABCB4 knockout mouse has been suggested as a model for PBC [311]. These mice do not secrete phospholipids and this results in high biliary concentrations of toxic free bile salts that weaken tight junctions and damage basement membranes. This allows leakage of bile salts into the portal area inducing inflammation and subsequently concentric rings of fibrosis by activation of myofibroblasts [14].

The steroid and xenobiotic receptor (PXR) mediates protection against bile acid-induced liver injury in cholestatic animal models [312]. Functional polymorphisms of the *PXR* gene influencing disease susceptibility or disease progression in patients with PSC have been evaluated and it has been suggested that *PXR* gene variants may modify disease course in PSC [313]. It is interesting to note that ursodeoxycholic acid and rifampicin are known ligands for PXR, which may explain their therapeutic benefits at least in the early stages of PSC.

Hereditary cholestasis syndromes

Several inherited defects in hepatic transport proteins result in clinically significant cholestatic liver diseases. In addition they provide fundamental insights into the function of the transporters and the consequences of their failure. Progressive familial intrahepatic cholestasis (PFIC), also known as Byler disease, is an autosomal recessive disorder that was first described in an Amish kindred [314]. It is now known that there are several distinct subtypes of this disorder and molecular and genetic studies have identified the genes responsible for three of these.

PFIC I is caused by a defect in a protein produced by the familial intrahepatic cholestasis (*FIC*)*1* gene. This gene maps to chromosome 18q21–22 and at least five mutations have been identified [315]. The *FIC1* gene encodes a P-type ATPase that may be involved in the transport of aminophospholipids. It is expressed in the liver, small intestine and pancreas, and a defect can cause cholestasis, diarrhea, or pancreatitis. How deficiency of FIC1 activity leads to cholestasis is unclear, but a reduction in FIC1 activity appears to cause reduced FXR activity and nuclear translocation resulting in diminished canalicular secretion of bile acids secondary to down-regulation of ABCB11/BSEP [129]. Biochemical findings include normal serum GGT but high serum bile acid concentrations [316]. Liver transplantation is presently the main therapeutic option for this progressive disease, although some success with external partial bile diversion through a jejunal stoma has been described [317–319].

Benign recurrent intrahepatic cholestasis (BRIC) is a clinically milder variant of PFIC I. It is a recurrent cholestatic disorder of adults that was first described by Summerskill and Walshe in 1959 [320]. Houwen and colleagues [321] have mapped the genetic defect in BRIC to the *FIC1* gene. While PFIC I leads to progressive liver disease, BRIC is a milder nonprogressive disease presenting either in childhood or adulthood. DNA sequencing of the *FIC1* gene showed that deletions, frameshifts and nonsense mutations appear to lead to the PFIC I phenotype while missense mutations were found in the patients with BRIC [322,323], suggesting that the *FIC1* gene mutation in BRIC may have residual activity, while the protein is absent or nonfunctional in patients with PFIC I. Another subset of BRIC has been described as BRIC 2 disease, which results from ABCB11/BSEP dysfunction [324]. There has also been a suggestion that temporary endoscopic nasobiliary drainage may induce long-lasting remission of cholestasis in BRIC patients [325].

PFIC II is a subtype of Byler syndrome that presents with permanent jaundice from the onset and progressive liver failure but is caused by a different genetic abnormality. The PFIC II locus has been mapped to the *ABCB11/BSEP* gene on chromosome 2q24 [103]. Immunostaining of the liver of these patients shows an absence of BSEP at the canalicular membrane [98]. These patients have a near total absence of bile acids from the bile and consequently develop bile acid hepatotoxicity due to accumulation within the hepatocytes. The relatively severe phenotype and progressive liver failure resulting from absence of ABCB11/BSEP in PFIC II demonstrates the importance of this molecule in preventing the toxic effects of bile acid accumulation in hepatocytes [326]. It is noteworthy therefore that ABCB11/BSEP expression is relatively preserved in most acquired cholestatic syndromes. Total loss of ABCB11/BSEP, as in PFIC II, results in severe liver toxicity, while ABCB11/BSEP expression is somewhat preserved in BRIC 2 [327].

PFIC III is a subtype that presents as somewhat more aggressive disease and, unlike PFIC I and PFIC II, affected patients have a high GGT and normal serum bile acids [120]. It is caused by a mutation of the *ABCB4/MDR3* gene on chromosome 7q21 [328] and leads to near absence of PC secretion into the bile. Since phospholipids are crucial for forming micelles with bile acids, the absence of biliary phospholipid increases free bile acid concentrations in the biliary tract. The consequences of this are injury and proliferation of bile ductules, resulting in the release of GGT. Heterozygous *ABCB4/MDR3* mutations are associated with milder diseases that manifest in adulthood. Recent studies suggest that defects in *ABCB4/MDR3* may be involved in hereditary susceptibility to cholelithiasis in association with cholestasis as well as cholestasis of pregnancy [329,330].

Dubin–Johnson syndrome

Dubin–Johnson syndrome is a rare autosomal recessive disorder characterized by chronic predominantly conjugated hyperbilirubinemia. Serum bile salt levels are normal and

hepatobiliary transport of bile salts is unaffected. This syndrome results from the absence of ABCC2/MRP2 protein in the canalicular hepatocyte membrane because of mutations of the *ABCC2/MRP2* gene [331,332]. Absent ABCC2/MRP2 function may be compensated by increased expression of ABCC3/MRP3 at the basolateral membrane, as suggested by immunofluorescence studies [132].

Prospects for pharmacotherapy

The goal of therapy for cholestatic disease is to first eliminate the primary cause of the injury and, if that is not possible, to secondarily prevent the hepatic injury that results primarily from the accumulation of bile acids and other toxic metabolites. As discussed above, the response to cholestasis and bile acid accumulation already serves this purpose but in many cases it is inadequate to prevent liver injury and in some cases the response itself may exacerbate injury. With increased understanding of how nuclear receptors regulate liver function there is active research effort to use this information to develop specific and more effective treatments for cholestasis.

Ursodeoxycholic acid (UDCA) and rifampicin have both been used as treatments for cholestasis and appear to act partly by nuclear receptor-mediated changes in gene expression. In a landmark randomized control study [333], 10 otherwise healthy individuals with cholelithiasis undergoing elective cholecystectomy were each assigned to receive UDCA (1 g/day for 3 weeks), rifampicin (600 mg/day for 1 week), or no medication prior to surgery. Subsequently, wedge liver biopsy specimens were analyzed for changes in gene expression. UDCA stimulated the expression of BSEP, ABCB4/MDR3 and MRP4. UDCA also became the predominant bile acid and lowered the biliary cholesterol saturation index, providing functional support for the observed increased expression of the canalicular phospholipid transporter ABCB4/MDR3. Rifampicin enhanced bile acid detoxification as well as bilirubin conjugation and excretion as reflected by increased expression of CYP3A4, UGT1A1, and MRP2. The beneficial effects of UDCA and rifampicin were therefore complementary in the treatment of cholestatic liver diseases [333].

Effects of UDCA on expression of hepatobiliary transporters takes place at multiple transcriptional and posttranscriptional levels and results in increased expression of transporters, promoting elimination and detoxification of potentially harmful compounds [334,335]. UDCA stimulates CYP3A4/CYP3A11 expression in human hepatocytes [336,337], while CYP7A1, the key enzyme system for bile acid synthesis, is down-regulated [338]. UDCA effects on transporter and enzyme expression, except up-regulation of BSEP, are independent of FXR, although other nuclear factors including PXR and CAR may be involved [339].

Activation or inhibition of specific nuclear receptors with synthetic agonists/antagonists offers tremendous therapeutic possibilities but is complicated by the many interactions and potential undesired effects of some target compounds. FXR, as master controller of bile acid-mediated effects, is an obvious target [340]. FXR activation increases expression of BSEP and ABCB4 thus increasing bile salt and phospholipid content of bile. Both of these effects are potentially beneficial for cholesterol gallstone disease. However, reduced expression of CYP7A1 would reduce de novo bile acid synthesis and could have the opposite effect. Perhaps for these reasons, initial experimental results with specific FXR agonists have produced contradictory findings. FXR agonists have shown hepatoprotective effects in models of obstructive [341] and estrogen-induced [298] cholestasis, but FXR inhibition is beneficial in other models suggesting a complex role of FXR in the response to cholestasis [342].

PXR and CAR are both involved in the response to xenobiotics and promote excretion and detoxification. Rifampicin, long known to relieve cholestasis-associated pruritis [343], is a ligand for PXR and activation of this nuclear receptor induces expression of drug export proteins MRP2 and MDR1 as well as the cytochrome P450 isoforms that detoxify hydrophobic bile salts [312,344]. PXR agonists have been shown to prevent lithocholic acid-induced liver toxicity [344] and may have potential for reducing liver toxicity from other cholestatic conditions. It is likely that knowledge of the control of expression of hepatobiliary transporters and detoxification enzymes will lead to the development of a new generation of nuclear receptor agonists/antagonists to enhance cytoprotective responses, prevent toxicity and improve the long-term outcome of patients with cholestatic liver diseases.

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D NUTRITION

17 Carbohydrate assimilation

Eric Sibley

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Carbohydrate assimilation in the gastrointestinal tract requires the breakdown of carbohydrate molecules into their constituent building blocks, monosaccharides, for absorption and transport into the circulatory system. The process of carbohydrate digestion involves the hydrolysis of starch in the lumen of the intestine (*lumenal digestion*) and the hydrolysis of oligosaccharides and disaccharides by enzymes located in the apical membrane of enterocytes (*mucosal digestion*). Following these phases of digestion, the process of carbohydrate assimilation is completed with the transport of monosaccharides across the apical and then basolateral membranes of enterocytes [1,2].

Structure and intake of carbohydrates

Carbohydrates account for 40%–60% of the average caloric energy intake in humans. The primary carbohydrates in the human diet are starches and simple sugars, including disaccharides such as sucrose and lactose, and monosaccharides such as glucose and fructose [3–5]. The basic molecular structure of carbohydrates consists of a series of aldehyde and ketone sugars linked by hydroxyl groups [6]. Monosaccharides consist of a single aldehyde or ketone unit and have the molecular formula $(CH_2O)_n$. Disaccharides consist of two monosaccharide units and oligosaccharides contain three to nine monosaccharide units. Polysaccharides consist of more than nine monosaccharide units joined in long linear or branched chains, as occurs in starches.

The linear and closed-ring forms of the major monosaccharides are shown in Figure 17.1. The five-carbon hexose sugars D-glucose and D-galactose are the major monosaccharides used as fuel sources and are derived from the three-carbon precursor D-glyceraldehyde (Fig. 17.1a,b). The hexose sugars form six-member rings called pyranoses. D-fructose is the major six-carbon ketone sugar and is derived from the three-carbon precursor D-hyroxyacetone (Fig. 17.1c). Fructose forms a five-membered ring called a furanose. The presence of asymmetric carbon atoms allows for stereoisomerization and, thus, a diversity of chemical structures for these sugars.

The various monosaccharide units, which form the building blocks of disaccharides, oligosaccharides, and polysaccharides, are joined by glycosidic linkages. The linkages are covalent chemical bonds, normally formed between the carbon-1 on one sugar and the carbon-4 on the other, and resulting in the removal of a molecule of water. An α-glycosidic bond is formed when the hydroxyl group on carbon-1 is below the plane of the sugar ring and a β-glycosidic bond is formed when it is above the plane. The type of glycosidic bond linking monosaccharides to form oligosaccharides and polysaccharides determines the distinct biological properties of the resultant molecules. For example, the α-(1,4)-glycosidic bond linking two D-glucose molecules to form the disaccharide maltose is easily hydrolyzed by intestinal enzymes (Fig. 17.2). However, the β-(1,4)-glycosidic bond linking two D-glucose molecules to form cellobiose, the structural building block of cellulose fiber, cannot be hydrolyzed by human saccharidases.

The two major starch polysaccharides in the human diet are amylose and amylopectin (Fig. 17.3). Amylose is a linear chain of glucose molecules linked by α-(1,4)-glycosidic bonds. Amylopectin is a branching chain of α-(1,4)-linked glucose molecules with an α-(1,6)-linkage occurring every 15–25 molecules [7]. Starches comprise about 50% of the total amount of carbohydrate consumed in the average adult diet, or about 22% of total calories [3,7].

Sucrose and lactose are the most common disaccharides available as human fuel sources (see chemical structures, Fig. 17.2). Sucrose is a natural sugar of most fruits and vegetables and consists of glucose and fructose molecules linked by an α-(1,4)-glycosidic bond [8]. Sucrose ingestion accounts

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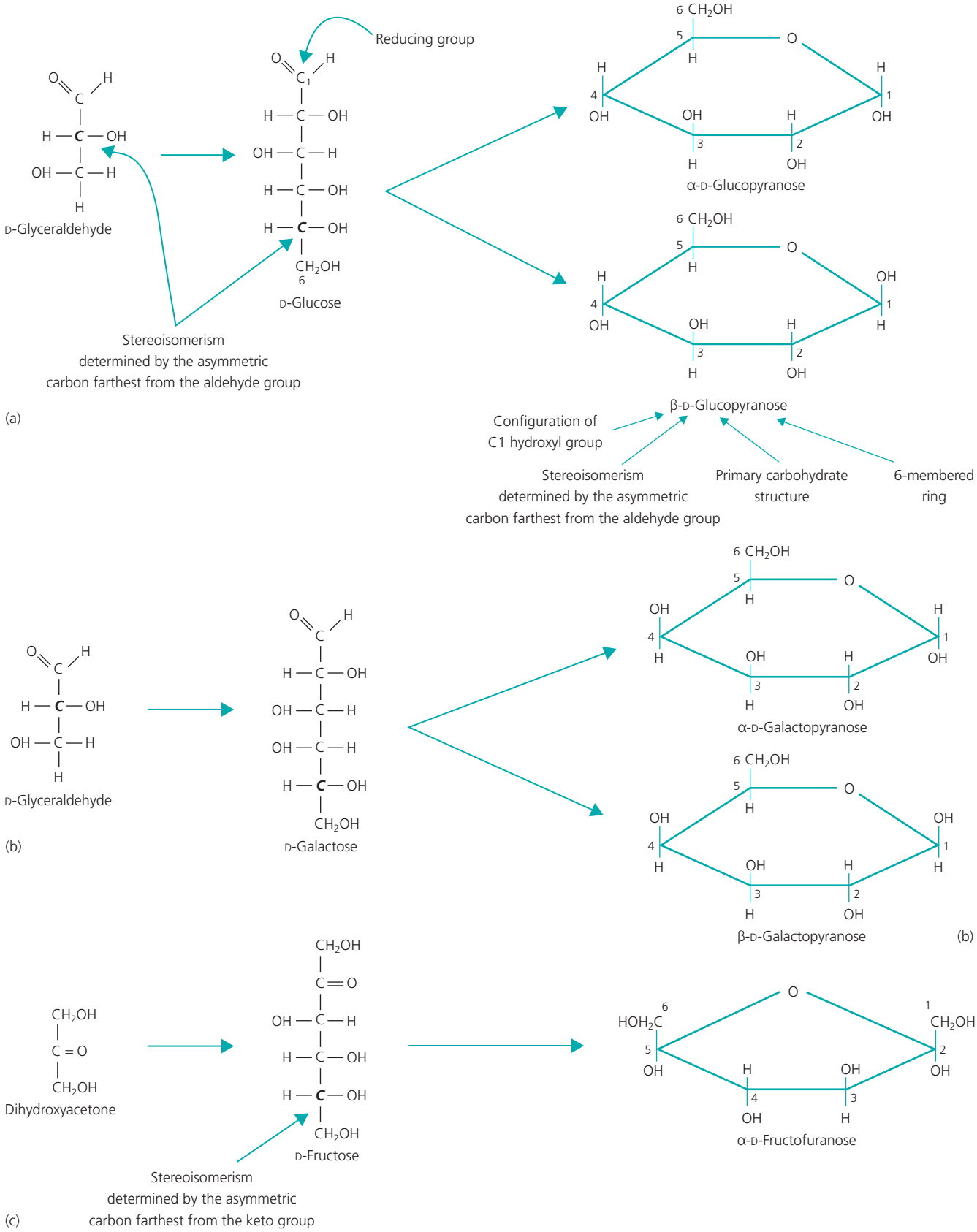

Figure 17.1 Carbohydrate structure. Linear and closed-ring forms of the major nutritional carbohydrates: **(a)** D-glucose; **(b)** D-galactose; and **(c)** D-fructose.

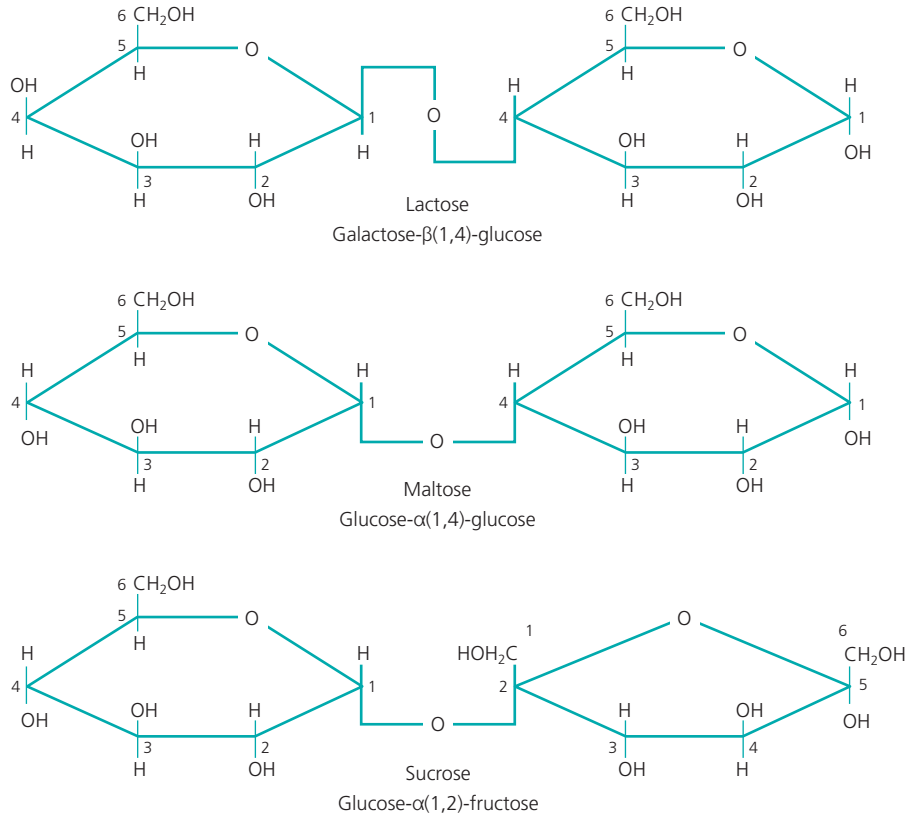

Figure 17.2 Structure of the three major nutritional disaccharides: lactose, maltose, and sucrose.

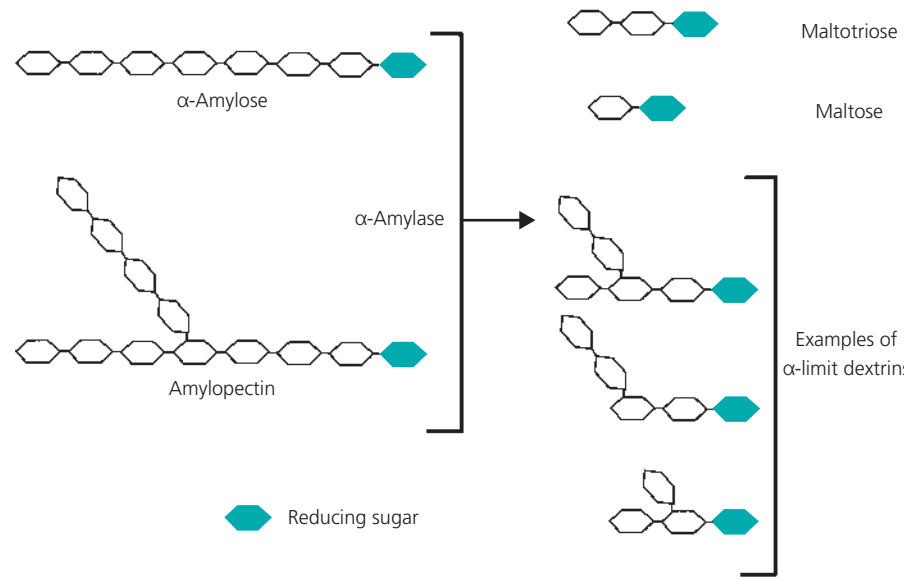

Figure 17.3 Starch digestion products. α-Amylose and amylopectin, the two molecular forms of starch, are digested by lumenal α-amylase into maltotriose, maltose, and a series of α-limit dextrins, a few examples of which are shown.

for about 30% of the carbohydrate intake in the adult diet in the United States [3,9]. Lactose consists of glucose and galactose molecules linked by a β-(1,4)-glycosidic bond. Lactose is the major carbohydrate in milk. Lactose is also used as a food additive in many processed foods. Lactose accounts for about 10% of the total carbohydrate calories consumed by the population in the United States. Individual lactose consumption varies widely, age and lactose tolerance being dependent factors. Maltose is a disaccharide comprising two glucose molecules linked by a α-(1,4)-glycosidic bond and accounts for 1%–2% of carbohydrates consumed (see Fig. 17.2).

Fructose and glucose are the primary monosaccharides

Table 17.1 Food content of fructose, glucose, sucrose, and sorbital

Food	Fructose (g/100 g edible portion)	Glucose (g/100 g edible portion)	Sucrose (g/100 g edible portion)	Sorbitol (g/100 g edible portion)	Excess free fructose (g)
Apple[a]	6–8	1–4	1–5	0.2–1.0	2–7
Cherry	5–7	5–7	0.2	1.4–2.1	0
Banana	2–4	3–6	6–14		0
Grape	5–7	5–7	0.5	0.2	0
Orange	2–3	2–3	4–7		0
Pear[a]	5–9	1–2	1–2	1.2–3.5	3–8
Plum	1–4	2–5	1–5	0.3–2.8	0
Prune	15	30	2	9.4–18.8	0
Strawberry	1–3	1–3	1–2	< 0.1	0
Honey	41	34	2		7
Beans	11–1.5	0.5–1	0.6		0–1
Carrot	1	1	4		0
Melon	2–4	1.2	1.5		0–3

a Juice has similar content.

Adapted from Rumessen [3], with permission from Taylor & Francis Ltd., www.tandf.co.uk/journals, www.informaworld.com.

in foods and are present in varying amounts in fruits and vegetables (Table 17.1). The average daily intake of glucose and fructose in the human diet is estimated to be about 20 g/day and 90 g/day, respectively, in the United States [3,9]. The fructose intake has increased significantly with the use of high-fructose corn syrup as a nutritive sweetener in foods. There is concern that the increasing consumption of refined carbohydrates, such as in corn syrup, is correlated with the rising prevalence of type II diabetes in the United States [10].

Nondigestible carbohydrates

The human diet also contains a significant amount of nondigestible carbohydrates. These carbohydrates consist of nondigestible, nonstarch polysaccharides, largely in the form of plant fibers that comprise the structural components of plant cell walls. As previously mentioned, cellulose fiber is formed from the cellobiose polysaccharide. The multiple types of fiber in different plants can be categorized into soluble and insoluble forms [11]. The different forms of dietary fiber are shown in Table 17.2.

Table 17.2 Structure, properties, and metabolism of dietary fiber

Fiber type	Structure	Properties	Colonic metabolism
Insoluble			
Cellulose	Linear polymer of β-(1,4)-linked glucose	Microfibrils in crystalline form	Moderate
Soluble			
Hemicellulose (xyloglucans, xylans, β-glucans)	Linear polymer of β-(1,4)-linked D-glucan with substituted xylosyl units	Water soluble; binds to cellulose to disperse fibrils	Moderate
Pectin	Galacturonic acid polymers	Acts as cellular glue for cellulose fibrils in cell walls	Extensive
Gums (guar gum, gum karaya, gum arabic)	Complex polysaccharides containing galactose, mannose, glucuronic acid, and other sugars	Used in processed foods; as additives they may contribute < 2% of a food	Extensive
Noncarbohydrate			
Lignins			None
Phenolic esters			None
Cutin			None
Waxy materials			None
Suberin			None

The human diet also consists of nondigestible sugars such as stachyose and raffinose, which are present in legumes, and sugar alcohols such as sorbitol, xylitol, mannitol, lactilol, and maltitol, which are used as sweeteners [1,3,12]. Humans lack digestive enzymes capable of efficiently hydrolyzing these nondigestible carbohydrates. Thus, dietary fibers and nondigestible sugars pass through the gastrointestinal tract undigested. On reaching the colon, the nondigested carbohydrates are metabolized by intestinal bacterial flora to yield short-chain fatty acids, hydrogen, carbon dioxide, and methane [13,14]. The short-chain fatty acids generated by the colonic bacteria can be absorbed by the colonic mucosa and serve as a primary source of energy for colonocytes [15–17]. The nutritional aspects of human dietary intake of digestible and nondigestible carbohydrates are described in Chapter 21.

Carbohydrate digestion

Lumenal digestion

On consumption of dietary food sources, carbohydrates are passaged through the alimentary tract for digestion and absorption. Starch hydrolysis is initiated during a lumenal digestion phase and completed by a mucosal digestion phase. The hydrolytic enzyme α-amylase initiates the digestion of starch in the lumen of the alimentary tract [18–20].

Salivary and pancreatic amylase

Two different isoenzymatic forms of α-amylase – salivary and pancreatic – mediate the lumenal phase of starch digestion. Both amylase isoenzymes have a high level of activity for the cleavage of internal α-(1,4)-glycosidic linkages [18]. Therefore, the terminal products of lumenal digestion of α-amylose are maltose and maltotriose (see Fig. 17.3). α-Amylase, however, has no activity for the hydrolysis of α-(1,6)-linkages, terminal glucose residues, or α-(1,4)-bonds adjacent to α-(1,6)-linkages [18]. Thus, the terminal products of lumenal digestion of amylopectin are maltose, maltotriose, and α-limit dextrins, which are a series of oligosaccharides containing four or more glucose molecules linked by α-(1,6)-glycosidic bonds [7,18,19]. Lumenal starch hydrolysis is an efficient process that is completed in the proximal jejunum [19,21]. A percentage of starch consumed, however, reaches the colon undigested and is metabolized by colonic bacteria.

Salivary α-amylase is synthesized and secreted into the alimentary tract by the parotid gland. The optimal enzymatic activity of salivary α-amylase is limited to the narrow pH range of 6.6–6.8. Therefore, although salivary α-amylase initiates starch digestion, its contribution is minor because it is rapidly inactivated in the acidic environment of the stomach. Pancreatic α-amylase is synthesized in the acinar cells of the pancreas and secreted into the small intestine by way of the pancreatic duct. Starch digestion is mediated largely by pancreatic amylase, which is secreted into the intestinal lumen in amounts exceeding that required for the hydrolysis of starch.

The genes encoding salivary amylase (*AMY1*), and pancreatic amylase (*AMY2*), are present in a clustered gene family located on human chromosome 1 at locus 1p21 [22]. As would be expected, *AMY1* is expressed in the parotid gland and *AMY2* is expressed in the pancreas [23].

Clinical symptoms of starch maldigestion occur only in severe cases of pancreatic insufficiency in which lumenal amylase activity levels are reduced to less than 10% of normal [24] (see Chapter 69). Some salivary amylase activity persists even at an acidic pH because of stabilization by short-chain glucose polymers [25]. Such stabilization of salivary amylase may allow for partial starch digestion in neonates and in patients who have chronic pancreatic insufficiency [4,26]. Human milk contains amylase activity, which facilitates carbohydrate digestion in infants [27]. For additional information on the structure, synthesis, and secretion of amylase, see Chapter 15.

Mucosal digestion

The terminal digestion of dietary carbohydrates is mediated by hydrolytic enzymes located on the apical brush border membrane of epithelial cells lining the small intestinal mucosa. These saccharidases are responsible for the hydrolysis of the products of lumenal starch digestion (i.e., maltose, maltotriose, and α-limit dextrins) and of ingested disaccharidases (i.e., lactose and sucrose). The oligosaccharide products of lumenal starch digestion are hydrolyzed to glucose monomers by the membrane-bound brush border digestive enzymes maltase–glucoamylase, sucrase, and isomaltase (also called α-dextrinase) (Fig. 17.4). The enzymatic action of the brush border saccharidases results in the production of monosaccharides that can be transported across the apical membrane. Table 17.3 outlines the characteristics of each enzyme. The enzyme activity, gene and protein structure, and spatiotemporal expression pattern are described below for the mucosal saccharidases.

Sucrase–isomaltase

Sucrase–isomaltase, also called sucrase–α-limit dextrinase, is a bifunctional brush border membrane enzyme with two active sites. The sucrase active site is capable of hydrolyzing the α-(1,4)-glycosidic bonds of short oligosaccharides including maltose, maltotriose, and sucrose. The isomaltase active site is also capable of hydrolyzing α-(1,4)-glycosidic bonds and is the only saccharidase capable of hydrolyzing the α-(1,6)-glycosidic linkage in the α-limit dextrins [28,29]. The sucrase active site is the only intestinal enzyme capable of hydrolyzing sucrose to glucose and fructose. The enzyme also accounts for 90% of intestinal isomaltase activity and 80% of maltase activity [30].

The human sucrase–isomaltase gene (*SI*) is located on chromosome 3 at locus 3q25-26 [31], and is estimated to be

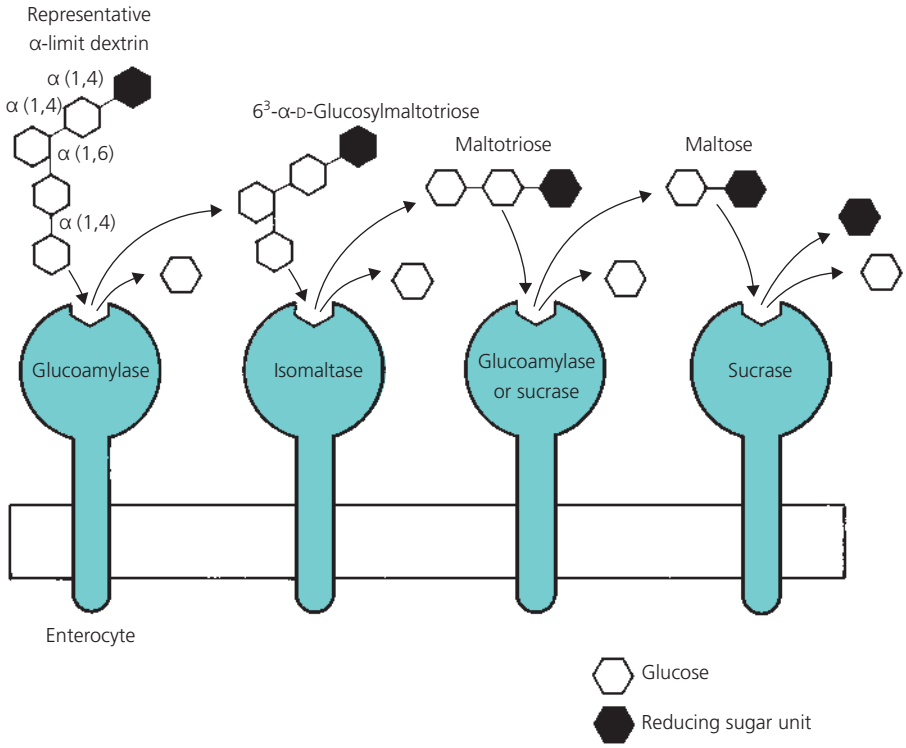

Figure 17.4 Overall process of digestion to monosaccharides. The hydrolysis of α-limit dextrins is a collaborative effort between multiple brush border enzymes.

Table 17.3 Human brush border proteins with carbohydrase activity

Brush border protein	Size of proprotein chains (kDa)	Size of protein chains (kDa)	Site of protein cleavage	Major substrates	Comments
Sucrase–isomaltase	185 (nonglycosylated); 210 (high mannose); 245 (fully glycosylated)		Extracellular (trypsin)		Size estimates from Naim et al. [34]
Sucrase		130		Sucrose	
Isomaltase		145		Maltose and α-limit dextrins	
Lactase–phlorizin hydrolase	210 (nonglycosylated); 215 (high mannose); 225 (fully glycosylated)	160; 145	Intracellular and extracellular		Size estimates from Jacob & Naim [69]
Lactase				Lactose	Single chain contains both active sites
Phlorizin hydrolase				Glycosylceramides	
Maltase–glucoamylase	255 (nonglycosylated); 285 (high mannose); 335 (fully glycosylated)	335	None	α-(1,4)-linked glucose oligomers	
Trehalase	65.5 (calculated from cDNA)	75	None	Trehalose	Anchored to membrane by glycosylphosphatidylinositol; based on cloned rabbit enzyme

about 55 kilobases (kb) in size [32]. The coding sequence of the human *SI* mRNA is 5481 bases long and encodes a 1827-amino-acid polypeptide [32]. The translation and post-translational processing of the sucrase–isomaltase protein has been studied extensively. Sucrase–isomaltase is initially synthesized in the enterocyte as a single glycoprotein chain. The protein is glycosylated as it is transported to the brush border membrane, where it is it is cleaved by lumenal trypsin to yield the sucrase and isomaltase units [28,30,33,34]. The two subunits reassociate noncovalently by forming hydrostatic bonds and exist as a membrane-bound dimer [35] (see Table 17.3).

Spatially, along the longitudinal (anteroposterior) axis of the small intestine, sucrase–isomaltase protein activity is four- to fivefold greater in the jejunum than in the ileum; however, sucrase mRNA expression appears to be similar in the two regions [36,37]. Sucrase–isomaltase is expressed transiently in the colon of humans during fetal development and is absent in the newborn colon [38–40]. Along the crypt–villus axis, there is little sucrase RNA, protein, or enzyme activity in the crypt, maximal activity in the lower and mid-villus cells, and decreased activity in the villus tip cells [33,37,41–44]. Transcriptional regulation of the sucrase–isomaltase gene has been characterized in cell culture and in transgenic animals [37,45,46]. Similar to other enterocyte genes, the sucrase–isomaltase promoter can be activated in cell culture by intestine-specific transcription factors including Cdx2 [47,48], HNF1 [48–52], GATA4 [48], and GATA5 [51].

Temporally, the expression of sucrase–isomaltase is minimal at birth and then is induced dramatically at the time of weaning in most mammalian species. In humans, however, sucrase–isomaltase expression is induced prenatally (10–14 weeks' gestation) and is maximal at birth [53,54]. This precocious expression pattern allows for the digestion and absorption of solid sugars prior to weaning in human infants.

Clinical deficiency of sucrase–isomaltase results in the inability to digest and absorb dietary sucrose [55]. The condition manifests during infancy on the introduction of sucrose in fruits and juices or glucose polymers in infant formula. A late-onset deficiency has also been described in adults. The inability to digest sucrose produces signs and symptoms of malabsorption including diarrhea, increased gas production, and abdominal distention. Sucrase–isomaltase deficiency occurs with a frequency of 0.2% in North American people and a frequency of greater than 10% in the Inuit people of Greenland and Alaska. Genetic studies support autosomal recessive inheritance. Several different mutations in the sucrase–isomaltase gene have been described, which lead to the synthesis of a transport-incompetent or functionally altered enzyme [56,57]. Treatment for congenital sucrase–isomaltase deficiency consists primarily of the avoidance of sucrose in the diet. Sacrosidase, produced from *Saccharomyces cerevisiae*, is a β-D-fructofuranoside fructohydrolase that hydrolyzes sucrose. It is reported to be safe and effective in preventing symptoms of intolerance in patients with sucrase–isomaltase deficiency [58].

Lactase–phlorizin hydrolase

The disaccharide lactose is the major carbohydrate in milk and is produced exclusively in the mammary glands by lactose synthase. As previously described, lactose consists of a glucose molecule and a galactose molecule linked by a β-(1,4)-glycosidic bond. The digestive enzyme lactase–phlorizin hydrolase (also known as lactase) catalyzes the hydrolysis of lactose to yield glucose and galactose, which can then be absorbed across the intestinal mucosal membrane. Lactase is a brush border membrane protein and has phlorizin hydrolase, glycosyl-ceramidase, and β-galactosidase enzymatic activities [59,60]. Lactase is the only intestinal enzyme that cleaves the β-(1,4)-glycosidic bond in lactose to yield glucose and galactose. Lactase activity is therefore essential for the survival of newborn mammals.

The human lactase–phlorizin hydrolase gene (*LPH*) is located on the long arm of chromosome 2 at loci 2q21 and consists of 17 exons spanning about 55 kb [61,62]. The human *LPH* cDNA encodes a single polypeptide chain containing 1972 amino acids [63]. The human lactase enzyme is a membrane-bound glycoprotein that is initially synthesized as a 210- to 220-kDa immature peptide [64,65]. Extensive evidence indicates that the precursor peptide is processed by glycosylation and cleavage and finally inserted into the brush border membrane as a mature 145-kDa subunit homodimer [28,64,66–74] (see Table 17.3).

Lactase gene expression is spatially restricted along both vertical and longitudinal axes in the gut [75,76]. Along the vertical axis, immature enterocytes derived from stem cells in the crypts migrate from the crypt to the villus tip, differentiate, and begin to express the lactase protein. Along the longitudinal axis, the lactase gene is expressed maximally in the distal duodenum and jejunum with significantly lower expression in the proximal duodenal and distal ileal segments of the intestine [76,77]. Transcriptional regulation of the lactase gene has been characterized in transgenic mice [78–80] and in cell culture. The lactase promoter is activated in cell culture by cooperative interaction between intestine-specific transcription factors including Cdx2 [81–85], GATA family members [51,86–89], and HNF1 [51,82,89,90].

During gut development and maturation, lactase enzyme activity is maximal in the small intestine of preweaned mammals and declines markedly during maturation. In most mammalian species, the maturational decline in lactase expression begins at the time of weaning [28,59,91–93]. The age of onset of this maturational decline in humans is variable, ranging from the toddler years to young adulthood [94–96]. After this decline, the level of lactase activity is 5%–10% of childhood levels in most populations worldwide [28,97]. The temporal decline in lactase activity is largely the

result of a decline in the high level of lactase mRNA expression in the enterocytes of the small intestine. Studies in humans and other mammals indicate that regulation of gene transcription is the primary mechanism mediating changes in lactase expression during gut development and maturation [98–101]. In vivo characterization of fragments of the lactase gene promoter in transgenic mice has revealed that 1 kb of the pig lactase promoter and 2 kb of the rat lactase promoter are capable of mediating a decline in reporter transgene expression after weaning [79,80]. Characterization of the specific mechanisms mediating the temporal decline in lactase gene expression is ongoing [102–104].

As described, the temporal decline in human lactase gene expression occurs with maturation in most populations worldwide. In some adults, however, high levels of lactase activity persist in adulthood. This hereditary persistence of lactase is common primarily in people of northern European descent and in select African populations [94,96,105]. It is believed that selection has played a major role in determining the prevalence of lactase persistence in different human populations. Factors that may have contributed to such selection include the nutritional benefit of milk in dairy farming populations and improved calcium absorption in areas of decreased solar irradiation (e.g., northern Europe) [96].

The hereditary persistence of lactase is attributed to inheritance of an autosomal dominant mutation that prevents the normal maturational decline in lactase expression [94,96,105]. Based on analysis of allelic human lactase mRNA transcript levels, it was initially determined that the hereditary lactase persistence/nonpersistence phenotype is controlled by a *cis*-acting DNA element on chromosome 2 in the general region of the lactase gene [105]. Subsequently, linkage disequilibrium and haplotype analysis in humans resulted in identification of genetic variants located –13 910 base pairs (bp) and –22 018 bp upstream of the human lactase gene that are associated with lactase persistence/nonpersistence phenotypes [106]. The –13 910 bp variant ($C/T_{-13\,910}$) is a single nucleotide polymorphism, C to T. Complete correlation between the lactase nonpersistence phenotype and homozygosity for the C nucleotide polymorphism at position –13 910 was described in the initial population studied. The report also described complete correlation between the lactase persistence phenotype and the presence of an allele with a T nucleotide at position –13 910. The –22 018 bp variant ($G/A_{-22\,018}$) was also associated with the lactase persistence trait, although not completely correlated.

Although strongly correlated with the lactase persistence/nonpersistence phenotypes, the $C/T_{-13\,910}$ variant has not yet been shown to be causative of the traits, nor has a functional mechanism for lactase nonpersistence been clearly defined [104]. Some investigators hypothesize that the DNA in the region of the –13 910-bp variant functions to regulate human lactase gene transcription. Transfection experiments have shown that the DNA in the region of the $C/T_{-13\,910}$ lactase persistence/nonpersistence variant can interact with the Oct1 transcription factor and function as a *cis* element capable of enhancing differential transcriptional activation of the lactase promoter in cell culture [107–109]. If determined to be functional, however, the $C/T_{-13\,910}$ variant may not account for all of the variation in lactase expression, as exceptions to the phenotypic correlations have been described [110–112].

As distinguished from the primary decline in intestinal lactase activity that occurs with hypolactasia in adults, secondary lactase deficiency can result from injury of the small intestinal mucosa. Such injury may occur with gastrointestinal infections (e.g., viral enteritis), inflammatory bowel disease (e.g., Crohn's disease), hypoxic ischemia, radiation, or chemotherapy, resulting in villus destruction, loss of total enterocyte number, and relative lactase deficiency. Rare cases of primary congenital lactase deficiency have been described, mainly in Finnish kindreds. The gene defect in congenital lactase deficiency is inherited in an autosomal recessive fashion and is reported to be due to a separate gene in the vicinity of the lactase gene on human chromosome 2 [113].

The clinical manifestations of reduced lactase activity (primary or secondary) can be symptoms associated with lactase intolerance. In individuals with reduced lactase activity, lactose consumed in excess cannot be fully digested in the small intestine. Bacteria in the distal ileum and colon ferment the undigested lactose to yield short-chain fatty acids, hydrogen, carbon dioxide, and methane. The fermentative products and undigested lactose molecules can result in symptoms of flatulence, diarrhea, and abdominal pain characteristic of lactose intolerance [97]. The maturational decline in lactase activity, adult-onset hypolactasia, and lactase nonpersistence render much of the world's adult human population intolerant of excessive consumption of milk and other dairy products. Therefore, the term lactase deficiency is somewhat of a misnomer. In the United States, the prevalence of lactose malabsorption is 80%–95% in African American and Asian American populations and 25% in the white American population [94,97]. Diagnosis of lactose intolerance is often made on clinical grounds and in response to an empirical trail of dietary avoidance. Biochemical methods for assessing lactose malabsorption in the form of the lactose breath hydrogen test and the direct measurement of lactase enzyme activity in small intestinal tissue biopsy samples are also used for diagnosis. Treatment of lactose intolerance consists primarily of avoiding lactose-containing foods or supplementing the diet with exogenous lactase derived from microbial sources [114–116].

Maltase–glucoamylase

Maltase–glucoamylase is present on the brush border membrane of enterocytes and catalyzes the removal of glucose from the nonreducing end of short glucose polymers at α-(1,4)-

glycosidic bonds [117]. The enzyme also has low enzymatic activity for the hydrolysis of α-(1,6)-glycosidic bonds [118].

The human maltase–glucoamylase gene (*MGAM*) is located on chromosome 7 at locus 7q34. The maltase–glucoamylase gene has structural homology with the sucrase–isomaltase gene, suggesting evolution from a common ancestral gene [119]. The maltase–glucoamylase gene spans approximately 82 kb with 48 identified exons. The 6513-bp cDNA contains an open reading frame that encodes a 1857-amino-acid protein. Maltase–glucoamylase is synthesized as a single-chain polypeptide precursor and is heavily glycosylated to produce a 335- to 355-kDa mature protein [120,121]. The maltase–glucoamylase enzyme does not undergo intracellular or extracellular proteolytic cleavage and is expressed in the brush border membrane as a monomeric protein [120] (see Table 17.3).

In humans, maltase–glucoamylase is expressed prenatally, with similar levels after birth and into adulthood. A disorder involving the absorption of short polymers of glucose and starch resulting from primary maltase–glucoamylase deficiency was initially detected with a prevalence of 1.8% in children with chronic diarrhea [122]. A report of a patient with congenital maltase–glucoamylase deficiency with associated lactase and sucrase deficiencies raises the possibility of a rare global carbohydrate digestive disorder caused by a shared regulator [123].

Trehalase

Trehalose is a disaccharide found in mushrooms, yeast, algae, and insect hemolymph. Trehalase is a brush border enzyme that specifically hydrolyzes trehalose to its two constituent glucose molecules [124,125]. The human trehalase gene (*TREH*) is located on chromosome 11 at locus 11q23 [126]. The gene encodes a 583-amino-acid protein with a molecular mass of about 75 kDa [127] (see Table 17.3).

The mouse trehalase gene is expressed in a gradient along the anteroposterior axis of the small intestine, with maximal trehalase mRNA expression in the proximal small intestine and significantly decreased expression in the distal intestine. Levels of primary and mature trehalase RNA transcripts increase dramatically during the third postnatal week in mice [92,128–130]. In humans, trehalase is expressed at comparable levels in the fetal and adult small intestines [92].

Isolated trehalase deficiency is essentially undescribed outside of Greenland, where at least 8% of the population have been reported to have trehalase deficiency. This deficiency can result in severe diarrhea after the ingestion of mushrooms [124,125,131–134].

Absorption of monosaccharides

The products of lumenal and mucosal hydrolysis of starches and sugars are the constituent monosaccharides, primarily glucose, galactose, and fructose. These monosaccharides, present at the enterocyte brush border membrane, must be transported across both the apical and basolateral enterocyte membranes and into the portal circulation to be utilized as nutrient sources. The transport function, gene and protein structure, and spatiotemporal expression pattern of the intestinal sugar transporters mediating monosaccharide absorption are described in the following sections.

Glucose and galactose absorption

The apical membrane absorption of glucose and galactose is mediated by a sodium-dependent active transport mechanism in which each monosaccharide is transported along with two sodium ions into the enterocyte (Fig. 17.5). This classical model of sugar absorption appears to be complemented by another apical membrane transport mechanism under conditions of high glucose concentration. These apical membrane sugar absorption mechanisms involve the SGLT1 and GLUT2 transporters. Glucose and galactose are transported across the enterocyte basolateral membrane into the portal circulation by a facilitative diffusion mechanism involving GLUT2.

SGLT1 and apical sugar transport

SGLT1 has been characterized as the sodium/glucose cotransporter [135]. It is capable of mediating the apical membrane transport of glucose or galactose into the enterocyte. Sodium ions enter the enterocyte by way of the electrochemical gradient, which is maintained by the Na^+,K^+-ATPase, a sodium pump that extrudes sodium ions across the basolateral surface (see Chapter 14). By coupling transport to the sodium gradient, the SGLT1 transporter actively transports glucose and galactose molecules into the cell [136–140]. Transport studies suggest that there is a stoichiometry of two sodium ions for every hexose molecule [138,140]. An alternating access kinetic model has been proposed for SGLT1-mediated sugar transport. The initial binding of the two sodium ions to SGLT1 increases the transporter's affinity for sugar. On binding with sugar, the protein undergoes a conformational change that allows transport of the sodium and sugar molecules across the membrane. The ligand-free protein then cycles back to the original conformation. The gene for the sodium–glucose cotransporter, *SGLT1* (also called *SLC5A1*, solute carrier family 5 [sodium/glucose cotransporter], member 1), is located on human chromosome 22 at locus 22q13.1. *SGLT1* encodes a 664-amino-acid transmembrane protein, estimated to be about 73–75 kDa in size [138,141]. The protein is expressed on the apical surface of the intestinal epithelial cell and exists in the membrane as a homotetramer with 14 membrane-spanning domains [46,138,141–143].

The biological properties of SGLT1 have been characterized by expressing the transporter in oocytes, cultured mammalian and insect cells, and bacteria, and by reconstitution of

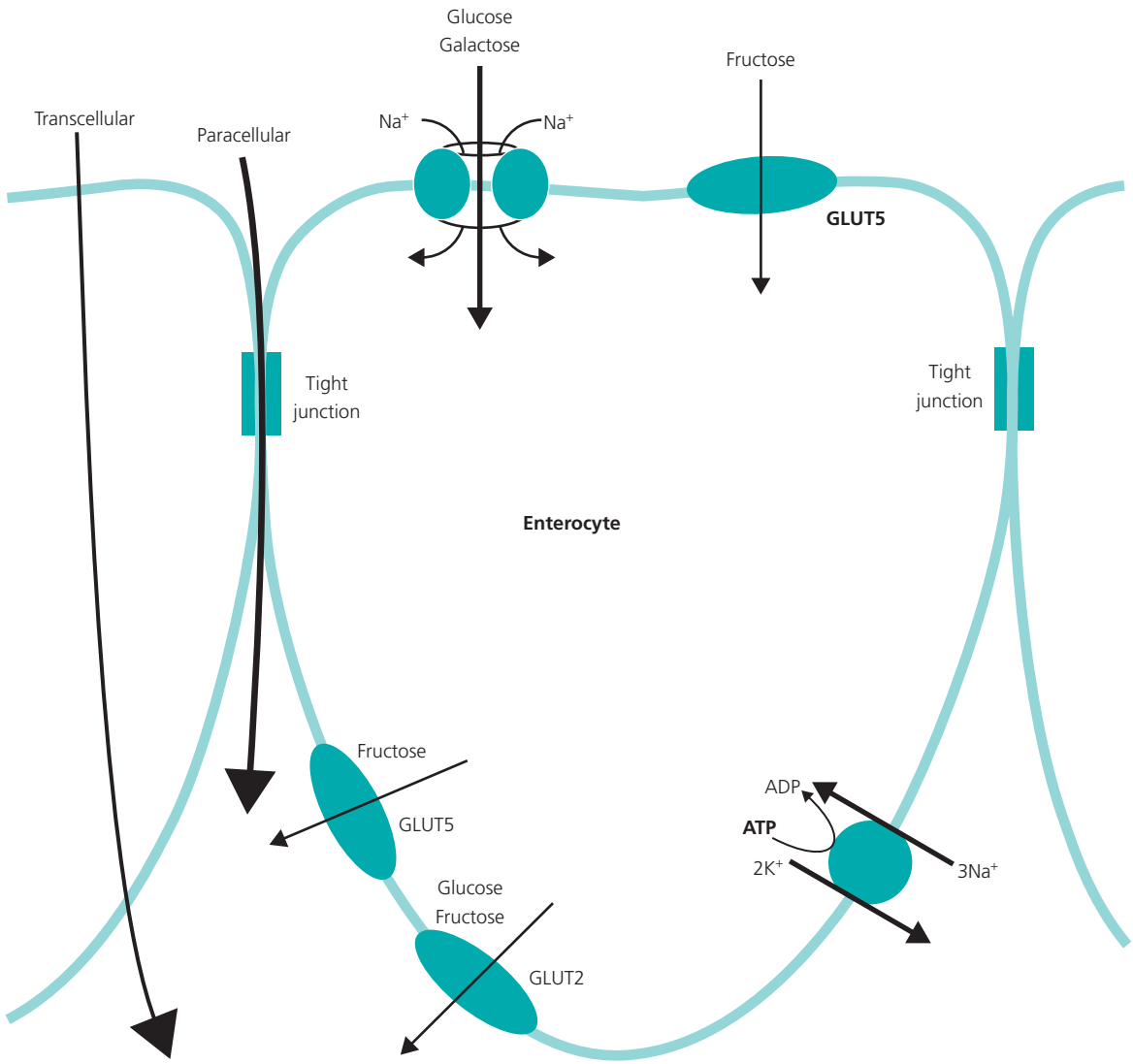

Figure 17.5 Transport processes in the enterocyte responsible for the movement of monosaccharides across the intestinal epithelium. Studies in some animals suggest that GLUT2 may also exist in the apical membrane when lumenal glucose levels are high.

the purified recombinant protein in proteoliposomes [144]. Strong support for the importance of the cloned transporter as a major sodium/glucose cotransporter in the intestine has been provided by the identification of a variety of mutations in the coding region of *SGLT1* in families with hereditary glucose and galactose malabsorption [145]. Expression of the mutant *SGLT1* cDNAs results in proteins that are nonfunctional for D-glucose transport, often as a result of cellular trafficking abnormalities [136,145–150]. Short-term induction of glucose transport results from an increase in the number of SGLT1 transporters at the brush border membrane and is mediated by changes in exocytosis and endocytosis of membrane vesicles that contain the SGLT1 protein [151]. Such changes in membrane vesicle recruitment are regulated by protein kinase A- and protein kinase C-dependent processes [151].

SGLT1 and glucose absorption play a major role in intestinal water transport [152–155]. The human SGLT1 transporter can cotransport approximately 264 water molecules for every molecule of glucose absorbed in enterocytes. It is estimated that ingestion of an average carbohydrate diet results in 5–6 L of water being transported by way of this SGLT1 cotransport mechanism in the human intestine. Increased intestinal water transport coupled to glucose absorption is the rationale for adding glucose to oral rehydration solutions used to treat conditions of clinical dehydration.

Congenital defects of the SGLT1 protein resulting from missense and nonsense mutations have been reported as rare clinical causes of severe glucose–galactose malabsorption in infancy [136,145–150,156]. Inheritance of glucose–galactose malabsorption occurs in an autosomal recessive manner. Affected children present in the neonatal period

with life-threatening acidic diarrhea and dehydration while consuming breast milk or standard infant formulas. Treatment consists of rehydration and initiation of a glucose- and galactose-free diet. As fructose is tolerated, most of the carbohydrate initially can be given as fructose.

GLUT2 and basolateral sugar transport

Glucose and galactose are transported out of the enterocyte into the portal circulation by the GLUT2 transporter located on the basolateral membrane. Transport of these sugars out of the cell occurs by way of a sodium-independent facilitative diffusion mechanism (see Fig. 17.5) [157]. The transport properties of the GLUT2 protein have been studied by expressing it in *Xenopus* oocytes [158–160]. The transporter has a high-level capacity for glucose transport with a high K_m for glucose (> 17 mM) in the intestine [158–160]. Evidence suggests that this protein can also transport fructose [161].

The human glucose transporter 2 gene, *GLUT2* (also called *SLC2A2*, solute carrier family 2 [facilitated glucose transporter], member 2), is located on chromosome 3 at locus 3q26.1-q26.3 [162]. The gene consists of 11 exons and 10 introns spanning approximately 30 kb [163]. The *GLUT2* cDNA encodes a 500-amino-acid protein with 12 membrane-spanning domains (Fig. 17.6). The GLUT2 transporter protein is present predominately on the basolateral membrane of enterocytes and renal tubular cells [160]. Studies show that GLUT2 protein can also be recruited to the apical membrane of enterocytes.

GLUT2 and apical sugar absorption

In addition to the well-characterized role of GLUT2 in mediating the transport of monosaccharides across the basolateral enterocyte membrane and into the circulation, it also appears that GLUT2 is capable of mediating apical membrane transport of sugars into the enterocyte under certain experimental conditions [164,165]. GLUT2 was first located at the apical enterocyte membrane in a diabetic rat model in which diabetes enhanced fructose absorption by approximately 60% [166]. Intestinal apical membrane vesicles revealed significant GLUT2 protein in diabetic rats but not in normal control rats. In addition, immunocytochemical detection revealed GLUT2 protein at the apical membrane [167–169]. GLUT2 located in the basolateral membrane is recruited to the apical brush border membrane in mice after perfusion with, or ingestion of, substances of high glucose [165] or fructose [168] concentration. Based on these data, a modification of the classical model of glucose absorption has been proposed [170]. In this modified model, under conditions of low lumenal glucose concentration (less than that in plasma), SGLT1 mediates high-affinity glucose transport into the enterocyte against a concentration gradient. Under conditions of high lumenal glucose concentration, GLUT2 is recruited to the apical membrane and mediates additional facilitated absorption of glucose. The significance of this modified model in humans is unclear, as patients with defective SGLT1-mediated glucose transport (as occurs in glucose–galactose malabsorption) lack significant glucose absorption. It has been proposed, however, that apical GLUT2 recruitment may be dependent on SGLT1-mediated glucose transport, thereby accounting for the lack of glucose absorption in such patients [164].

Fructose absorption

The fructose monosaccharide is transported across the apical intestinal brush border membrane by a sodium-independent facilitated diffusion mechanism involving the GLUT5 transporter. Although GLUT5 is the predominant fructose transporter at the brush border membrane, GLUT2 may assist

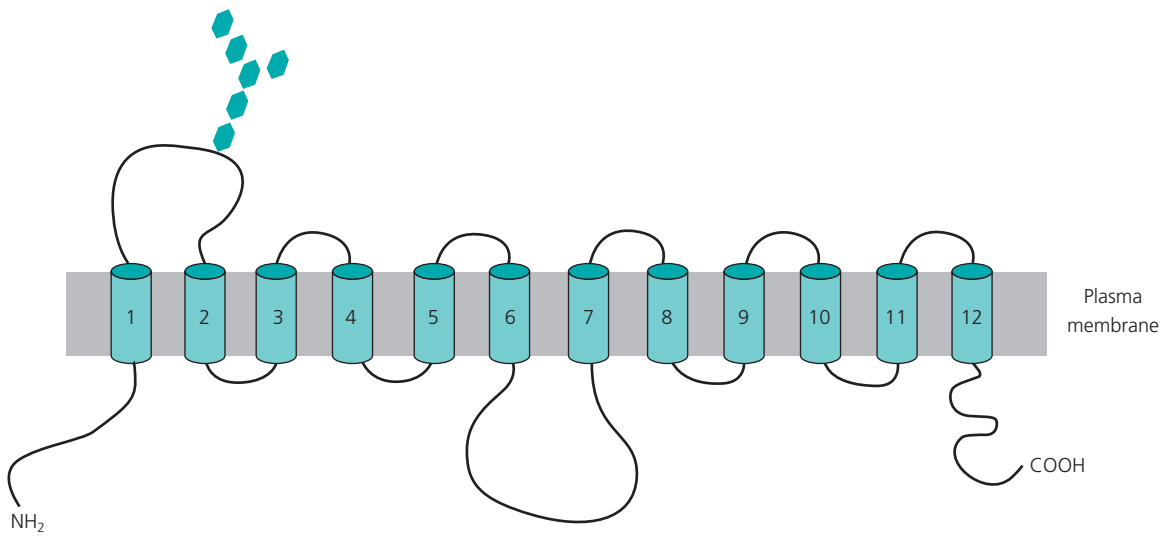

Figure 17.6 GLUT2 glucose transporter. This model of the monomer is suggested by the primary amino acid sequence. From Elsas and Longo [158], with permission from Annual Reviews, www.annualreviews.org.

Table 17.4 Glucose transporters

	Protein size (kDa)	Sites of expression	Function
Facilitative glucose transporters			
GLUT1	55	Many tissues; abundant in erythrocytes, blood–brain barrier, placenta, transformed cells in culture	Basal glucose transport; transport across blood–tissue barriers
GLUT2	58	Liver, pancreatic β-cells, kidney, small intestine	Low-affinity glucose transport; release of absorbed glucose across basolateral membrane of kidney and intestine
GLUT3	54	Many tissues; abundant in brain	Basal glucose transport
GLUT4	55	Skeletal and cardiac muscle, brown and white adipose tissue	Insulin-stimulated glucose uptake
GLUT5	50	Small intestine, kidney, testes, adipose tissue, skeletal muscle	Fructose transport across enterocyte apical membrane
Sodium-dependent glucose transporters			
SGLT1	75	Intestine, kidney	Intestinal and renal absorption of glucose and galactose

in the absorption of excess lumenal fructose [164,165]. Only a small amount of fructose is metabolized in the enterocyte [3]. Most intracellular fructose appears to be transported across the basolateral membrane by GLUT2 for delivery into the circulation [171,172]. GLUT5 expression on the basolateral membrane suggests that it may also transport fructose out of the enterocyte [173]. GLUT5 and GLUT2 are members of a family of genes that encode facilitative sugar transport proteins [158–160,165,171]. The GLUT family of genes, their transport properties, and their tissue distributions are outlined in Table 17.4.

GLUT5 and sugar transport

The GLUT5 transporter is the protein responsible for most apical membrane fructose transport. The gene encoding the GLUT5 transporter, *SLC2A5* (solute carrier family 2 (facilitated glucose/fructose transporter), member 5), is localized on chromosome 1 at locus 1p36.2. GLUT5 is a 501-amino-acid transmembrane protein that transports fructose and glucose molecules [174]. Analysis of this amino acid sequence suggests that there are 12 membrane-spanning domains. Human GLUT5 mRNA and protein are expressed in the small intestine, kidney, testes, adipose tissue, and skeletal muscle [175,176]. This uniporter is fairly exclusive for fructose, with a K_m of 6–14 mM [177].

Expression of the GLUT5 protein is induced by the presence of its substrate fructose [174,178–181]. Precocious expression of GLUT5 in suckling animals is also induced by lumenal fructose [182]. A sugar-response element has been localized to the first 272 bp of the GLUT5 promoter [179]. In addition, fructose stimulation results in increased GLUT5 mRNA stability mediated by cAMP signaling and by binding to PABP (polyadenylated-binding protein)-interacting protein 2 (Paip2) [179]. Although GLUT5 is the predominant fructose transporter at the brush border membrane, GLUT2 may assist in the absorption of excess lumenal fructose. GLUT2, located in the basolateral membrane of mice fed a meal devoid of sugar, is recruited to the apical brush border membrane on ingestion of a meal with a high level of fructose [168].

Fructose is not as well absorbed as glucose. Therefore, ingestion of high levels of fructose in the diet can lead to carbohydrate intolerance. In children, drinking excessive amounts of juices with high levels of fructose may cause nonspecific diarrhea, excessive intestinal gas, and recurrent abdominal pain [183]. In children and adults, fructose malabsorption has been associated with irritable bowel syndrome [184,185]. Previous studies support the hypothesis that isolated fructose malabsorption in children does not result from the expression of mutant GLUT5 protein [186]. Investigators have therefore speculated that children with fructose intolerance may exhibit defective GLUT2 intestinal membrane insertion and recruitment.

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D NUTRITION

18 Intestinal lipid absorption

William Sun, Chun-Min Lo, Patrick Tso

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Types of dietary lipids

Because dietary lipids and many lipophilic vitamins and drugs are carried by chylomicrons and are transported in lymph, it is important to understand the processes involved in the digestion, uptake, intracellular metabolism, and packaging of dietary lipids into chylomicrons. Considering the excellent reviews of intestinal lipid absorption [1–4], this chapter will both update current knowledge on the digestion, uptake, and transport of dietary lipids, and provide a comprehensive look at the current understanding of the role of the transporters in enterocytes in the uptake of lipids.

Dietary fat, simply defined, is the component of a diet that can be extracted by organic solvents [5]. An array of compounds comprises dietary fat, from the highly nonpolar hydrocarbons to the highly polar phospholipids and glycolipids. The classification of these various lipids and their behavior in an aqueous medium has been thoroughly reviewed by Carey and Small [6]. Classification of a lipid as polar or nonpolar depends on its interaction with water. Nonpolar lipids are insoluble in the bulk water phase and therefore do not interact with water (Fig. 18.1). Cholesteryl ester, hydrocarbons, and carotene are examples of nonpolar lipids. Polar lipids can be further classified into one of three groups: insoluble nonswelling amphiphiles, insoluble swelling amphiphiles, and soluble amphiphiles. The insoluble nonswelling amphiphiles include triacylglycerol (TG), diacylglycerol (DG), nonionized long-chain fatty acids (FAs), cholesterol, and fat-soluble vitamins. Nonswelling amphiphiles are so named because they interact little with water in the bulk phase; hence, when added to water, a thin monolayer of lipid molecules is formed. The second group of polar lipids, the insoluble swelling amphiphiles, includes monoacylglycerol (MG), ionized FAs, and polar lipids. Like the insoluble nonswelling amphiphiles, when added to water, the insoluble swelling amphiphiles form a monolayer on the surface of water. Further, this group of lipids has the unique ability to interact with water to form a laminated lipid–water structure that is known as a liquid crystal structure. In the liquid crystal state, nonpolar groups of lipid molecules face one another, which results in the sandwiching of water between the polar groups. This unique behavior is referred to as "swelling", hence the designation swelling amphiphiles. Soluble amphiphiles, the third group of lipid molecules, possess strong polar groups, which render these molecules soluble in water at low concentrations. This group of lipids can be divided further into soluble amphiphiles with lyotropic mesomorphism and those without. Sodium salts of long-chain FAs are examples of soluble amphiphiles with lyotropic mesomorphism. When the lipid concentration in solution reaches the critical micellar concentration, monomers aggregate to form micelles. In the micelle, the polar group of monomers faces the surrounding aqueous medium, whereas the nonpolar group faces inward. In the case of sodium oleate molecules, when the critical micellular concentration is reached but before micelle formation, there is an intermediate liquid crystal phase, a phenomenon known as lyotropic mesomorphism. In contrast, soluble amphiphiles without lyotropic mesomorphism do not form the intermediate liquid crystal phase before micelle aggregation. Bile salts, secreted by the liver, are an example of soluble amphiphiles without mesomorphism.

The major focus of this chapter will be the digestion, absorption, and transport of TG, which is undoubtedly the predominant dietary lipid. Readers also interested in cholesterol absorption should refer to the excellent review by Dawson and Rudel [7], as well as those by other investigators [8,9].

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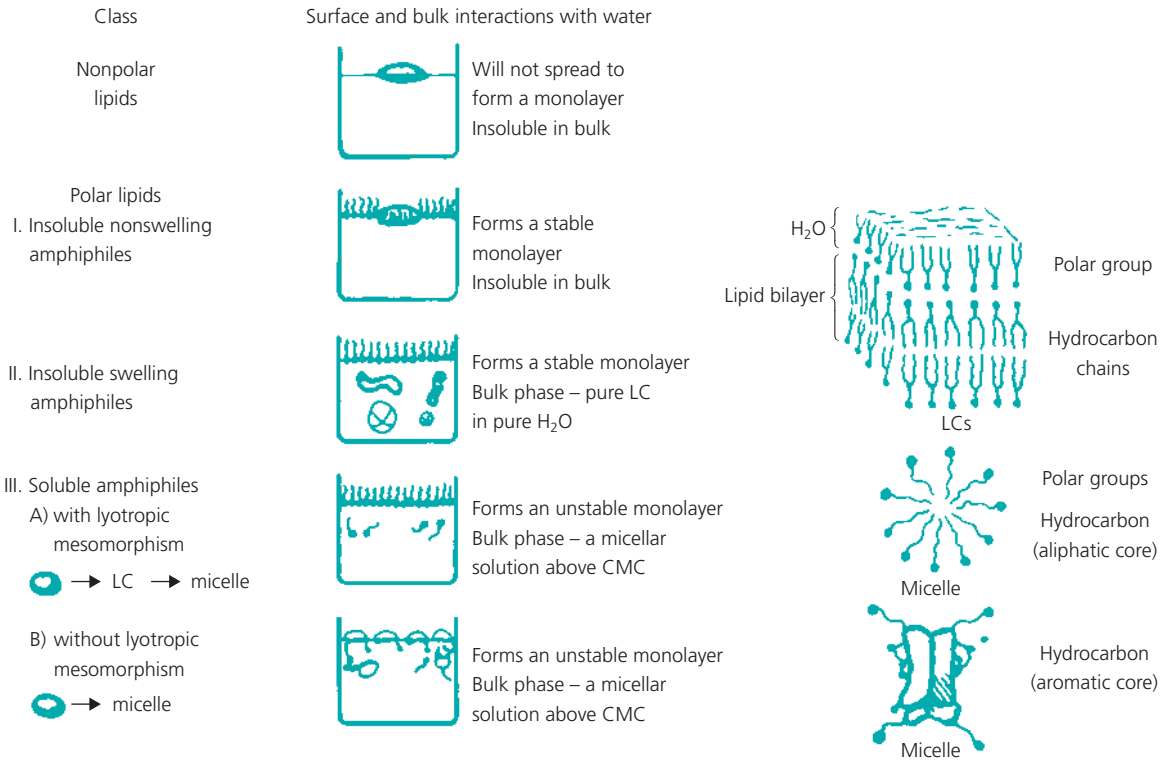

Figure 18.1 Classification of lipids based on their ability to interact with water. Nonpolar lipids: octadecane, carotene, squalene, cholesteryl oleate, cholesteryl linoleate, and paraffin oil. Polar lipids: I – triacylglycerols, diacylglycerols, long-chain protonated fatty acids, and fat-soluble vitamins. II – phospholipids, monoacylglycerols, monoethers, and α-hydroxy fatty acids. IIIA – sodium salts of long-chain fatty acids; many anionic, cationic, and nonionic detergents; and lysophosphatidylcholine. IIIB – bile salts, sulfated bile alcohols, and saponins. CMC, critical micelle concentration; LC, liquid crystal. From Carey and Small [6], with permission from Elsevier.

Dietary fat constitutes a significant source of calories in the Western diet – as much as 30% of the total caloric intake, or 90–100 g/day. Dietary intake of fat has received considerable attention in the last few decades, as diets with high levels of fat have been linked to high levels of serum lipids, especially cholesterol, and increased risk of coronary heart disease [10–12]. It is generally accepted that diets with high levels of saturated FAs are more cholesterolemic (i.e., tending to raise the level of serum cholesterol) than diets with high levels of polyunsaturated FAs [13]. Trans FAs have double bonds, yet they behave more like saturated FAs and are considered just as atherogenic as saturated FAs [14,15]. The Surgeon General of the United States recommends that all individuals should maintain overall fat consumption at or below an average level of 30% of the total caloric intake, and that no more than 300 mg cholesterol should be consumed in one day. This standard has also been adopted by the American Heart Association.

Over the past decade, there has been increased effort to modify, either chemically or biologically, the FA profile of naturally occurring TG to obtain a particular physical property or physiological function. A new approach to optimize the metabolic benefits of specific lipid mixtures has been the development and manufacture of structured triacylglycerols (STGs). STGs are produced by the chemical interesterification of both medium-chain and long-chain FAs incorporated on the same glycerol backbone by hydrolysis and random reesterification [16,17]. These FA molecules are chemically distinct and offer unique advantages depending on their constituent physical mixture of medium-chain FAs and long-chain FAs. For example, STGs that contain medium-chain FAs may provide a useful vehicle for rapid hydrolysis and absorption because of their smaller molecular size and greater water solubility compared to long-chain FAs. The STGs are manufactured both for their potential clinical benefits and for their lower fat-producing value. The clinical applications of STGs have been thoroughly reviewed [18–20]. The benefits of STGs include improved absorption of linoleic acid by patients with cystic fibrosis [21,22], and also a protein-sparing effect in STGs derived from safflower oil [23] and fish oil [24]. Although STGs retain some characteristics of medium-chain and long-chain TG, they may provide an alternative lipid source that could overcome the gastrointestinal intolerance related to the sole use of medium-chain or long-chain TG in patients with malabsorptive diseases. Studies from our laboratory have shown that

STGs promote the lymphatic absorption of both vitamin A and vitamin E better than the constituent physical mixture with a similar FA composition. Note that the enhanced delivery of vitamins A and E into lymph by STGs is limited to healthy animals but also includes animals with impaired gastrointestinal absorption caused by ischemia/reperfusion injury [25,26]. Ascertaining the capability of STGs to enhance the lymphatic absorption of lipid soluble drugs is worthy of investigation.

Lumenal digestion of dietary lipids

Gastric lumen

The digestion of dietary lipids in humans begins in the stomach. The initial step involves the enzyme acid lipase, which is secreted by the gastric mucosa. Acid lipase has its highest activity in the acidic environment of the stomach. The distribution of gastric lipase varies in different parts of the human stomach, with the highest activity detected in the fundus (Fig. 18.2) [27]. Human gastric lipase has a pH optimum ranging from 3.0 to 6.0 [27,28], and it hydrolyzes medium-chain TG more efficiently than long-chain TG [29]. The main hydrolytic products of gastric lipase are DG and FAs [27,28,30]. Gastric lipase does not hydrolyze phospholipids or cholesterol ester. Human gastric lipase has been cloned; it has 379 amino acid residues [31], and interestingly, there is considerable homology (78%) between the amino acid sequences of rat lingual lipase and human gastric lipase. Little homology exists between human gastric lipase and human pancreatic lipase.

The digestion of TG by gastric lipase in the stomach plays an important role in lipid digestion, particularly in neonates. Milk fat, the primary source of nourishment for neonates, largely consists of medium-chain TG, which, as mentioned, are more efficiently hydrolyzed by acid lipases. Furthermore, the pancreatic lipase system is not fully developed in the neonate [32]; gastric lipases therefore play a crucial role in the neonatal digestion and absorption of milk fat.

The integral role played by gastric lipases in lipid digestion in adults is especially evident in patients with cystic fibrosis, who maintain the ability to absorb dietary lipid, despite markedly or completely inhibited pancreatic lipase secretion [33]. Gastric lipase activity in the stomach is not compromised in patients with cystic fibrosis; in fact, gastric lipase activity is high in these patients when they are maintained on diets with moderate and high fat levels [34]. The specific activity of adult human gastric lipase is much lower in vivo than in vitro; interestingly, the relatively low specific activity observed in vivo is sufficient to digest a significant portion of the TG ingested [35].

The stomach is the major site for emulsification of dietary fat, an important prerequisite for efficient hydrolysis by pancreatic lipase. Gastric chyme is propelled forward through the antrum to the pylorus by the peristaltic waves of the corpus (Fig. 18.3). Liquid is squirted into the duodenum along with small solid particles in the chyme. The pylorus closes, and the antrum contracts forcefully, grinding the solid particles. As a result of these contractions, the antral contents are retropelled from the terminal antrum back into the corpus. The squirting of the antral contents into the duodenum, the grinding action of the antrum, and the retropulsion of antral contents back to the corpus provide most of the mechanical action involved in the initial emulsification of dietary TG. The DG and FAs resulting from the action of acid lipases in the stomach and the phospholipids normally present in the diet further aid the emulsification of dietary fat.

Intestinal lumen

The lipid emulsion enters the small intestine as fine lipid droplets less than 0.5 μm in diameter, [36,37]; therefore the emulsification process that occurs in the stomach ensures

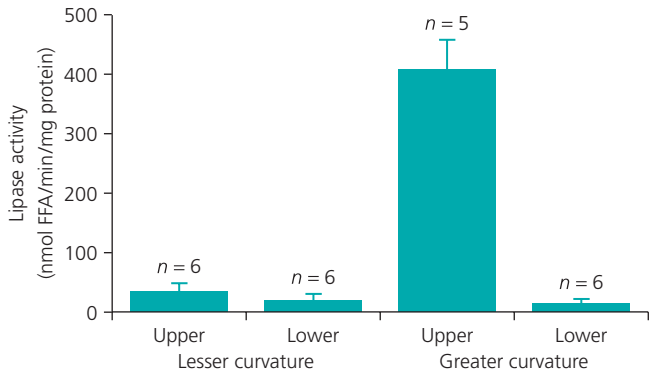

Figure 18.2 Lipolytic activity in the human stomach measured at four sampling sites in the gastric mucosa. FFA, free fatty acid. From Abrams et al. [27], with permission from Elsevier.

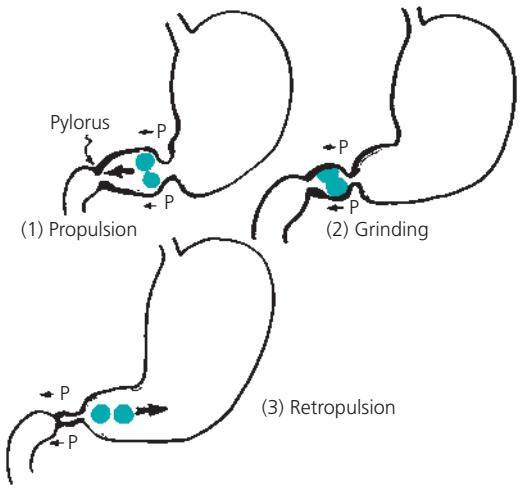

Figure 18.3 The consequences of antral peristalsis. From Kelly [288], with permission.

that lipid is emptied efficiently from the stomach. The combined action of bile and pancreatic juice in the small intestine produces a marked change in the chemical and physical form of the ingested lipid emulsion. Pancreatic lipase is responsible for most of the digestion of TG in the upper part of the intestinal lumen. Pancreatic lipase is active at the interface between the oil and aqueous phases [38–41]. Verger used the monolayer technique to elucidate the effects of the oil–water interface on lipolysis [42]. The application of a monomolecular lipid film at the air–water interface facilitates characterization of the effect of physicochemical properties of the interface, and also the effects of the surface area on the rate of lipolysis. The monolayer technique requires only a small amount of lipid, an advantage when using rare synthetic lipids. A problem with this technique, however, is the denaturation of enzymes that occurs at the lipid–water interface [43,44]. The velocity of lipolysis is dependent on those factors that modify both the physicochemical properties of the interface as well as the surface area [45–47]. The landmark paper of DiMagno and colleagues [48] convinced gastroenterologists that the human pancreas secretes more lipase than is needed to digest the fat ingested. Carriere and colleagues [49] challenged this concept with the measurement of the specific activity of human pancreatic lipase on dietary TG, which is three orders of magnitude lower than the very high specific activity measured under the usual in vitro experimental conditions. They proposed that the lipase activity in the human small intestine is not as high as believed, providing an explanation for why fat absorption takes a long time despite the seemingly large amount of lipase typically secreted by the human pancreas.

Pancreatic lipase acts mainly at the *sn*-1 and *sn*-3 positions of the TG molecule to release 2-monoacylglycerol (2-MG) and free FAs [46,50–52]. Although 1-monoacylglycerol (1-MG) is formed from 2-MG through isomerization in an aqueous medium, 2-MG is probably the predominant form in which MG is absorbed by the small intestine because 1-MG formation is slower than 2-MG uptake [53]. Further hydrolysis of 1-MG or 2-MG by pancreatic lipase results in the formation of glycerol and FAs [54]. Although pancreatic lipase hydrolyzes 1-MG more efficiently than 2-MG, the absorptive rate is so fast that most of the 2-MG is absorbed before degrading or isomerizing to form 1-MG.

Pancreatic lipase is abundant in pancreatic juice, accounting for 2%–3% of the total protein [55]. Its high concentration in pancreatic secretions and its high catalytic efficiency ensure the efficient digestion of dietary fat. Most humans absorb between 90% and 95% of the fat ingested. Consequently, only severe pancreatic deficiencies result in the malabsorption of fat. Pancreatic lipase has been purified from a number of species, including humans [56–59]. Porcine pancreatic lipase is a glycoprotein that contains 449 amino acid residues and a carbohydrate chain of about 2000 Da attached to asparagine at position 166 [60–62]. For pancreatic lipase to exert its enzymatic activity, it must anchor itself at the interface. The serine residue at position 152 plays an important role in this binding site [63].

Pure pancreatic lipase functions extremely inefficiently when acting in a bile salt–lipid mixture, but in pancreatic juice, lipase hydrolyzes TG extremely effectively. This interesting observation led to the discovery of the cofactor known as colipase. In 1969, Morgan and colleagues made the crucial observation that colipase is necessary for efficient lipase activity only if bile salt is present [64]. Studies performed in the laboratories of Professor Borgström in Lund and Professor Desnuelle in Marseille investigated the biochemistry of colipase and its interaction with pancreatic lipase. Colipase has been purified from a number of animal species [65–68]. The mechanism of how colipase works has been discussed in detail by Erlanson-Albertsson [69], who observed that TG lipid droplets covered with bile salts are not accessible to pancreatic lipase. However, the binding of colipase to the TG–aqueous interface allows the lipase molecule to access the lipid–aqueous interface. With the use of site-directed mutagenesis, Freie and colleagues [70] demonstrated that a valine residue at position 407 and a leucine at position 412 are important in the interaction of lipase with colipase and the bile salt micelles. The bile salt micelle, in addition to its role in transporting digested lipids to the enterocytes, also plays a crucial role in the lipolysis of TG by pancreatic lipase. Pancreatic lipase binds with colipase at a 1 : 1 molar ratio [71].

Digestion of phospholipid

The digestion of phospholipid occurs in the small intestine; gastric lipase is incapable of digesting phospholipid. In bile, phospholipid (predominantly phosphatydylcholine [PC]) is found in mixed micelles along with cholesterol and bile salts. In the intestinal lumen, PC distributes itself between the mixed micelles and the TG droplets, with a preference for the micellar phase over the oil phase [72]. PC is then acted on by pancreatic phospholipase A_2 (PLA_2) at the *sn*-2 position to yield a FA and lysophosphatidylcholine (LPC) [73,74]. Some phospholipase A_1 (PLA_1) activity may also occur in pancreatic juice as a result of the presence of pancreatic lipase [75]. PLA_2 was first purified and characterized from porcine pancreas [76]; it has a molecular weight of about 14 000 Da and is secreted as an anionic zymogen that is activated by tryptic cleavage of an amino-terminal heptapeptide [76–78]. PLA_2 requires calcium for activation [79,80] and its activity requires the presence of bile salts [81]. It has multiple isoforms [82,83] and apparently requires a bile salt : PC molar ratio of 2 : 1 for optimal activity [84]. It is heat stable, and acts most efficiently within a pH range of 8–9 [81].

Porcine, canine, and human phospholipases have been sequenced [85–88]. Richmond and Hui [89] reported the genomic organization of the PLA_2 gene of the mouse and showed that the mouse protein was highly homologous to

the rat, dog, and human proteins. Although most intestinal PLA_2 activity is derived from pancreatic juice, the intestinal mucosa likely contributes some where the enzyme is concentrated in the brush border [90].

Digestion of cholesteryl ester

Most dietary cholesterol is present as the free sterol, with only 10%–15% existing as the sterol ester [91]. Cholesteryl ester entering the small intestine must first be hydrolyzed before free cholesterol can be absorbed. The enzyme involved in the hydrolyzation process is cholesterol esterase, also known as carboxylic ester hydrolase or sterol ester hydrolase. It has been purified from porcine [92,93], rat [94], and human [95,96] pancreas. Human cholesterol esterase has a molecular weight of about 100 kDa [95], whereas rat cholesterol esterase has a molecular weight of 70 kDa [94]. Rat, bovine, and human pancreatic cholesterol esterases have been cloned [97–99] and seem to be well conserved among species, sharing 78% homology in rat and human. With the use of site-directed mutagenesis, DiPersio and colleagues [100] showed that substitution of the serine at position 194 with threonine or alanine abolished enzyme activity, thus identifying part of the catalytic site of pancreatic cholesterol esterase. This laboratory further demonstrated that histidine in position 435 is probably also a component of the catalytic triad of this enzyme [101]. The third component of the trio is an aspartic acid residue at position 320 [102]. Human cholesterol esterase likely shares the same catalytic sites as those of the rat esterase.

Human cholesterol esterase has a broad specificity and can hydrolyze TG, cholesterol esterases, and phosphoglycerides [103]. Cholesterol esterase activity is greatly enhanced by the presence of bile salts, particularly trihydroxy bile salts such as sodium cholate. A unique property of cholesterol esterase is its unique ability to self-associate. For example, the presence of trihydroxy bile salts promotes the self-aggregation of the enzyme into a polymeric form [94]. The self-association of cholesterol esterase protects the enzyme from proteolytic inactivation, so retaining its activity. Cholesterol esterase isolated from the pancreas exists mainly as dimers and tetramers [96].

Micellar solubilization and uptake of dietary lipids by enterocytes

Hofmann and Borgström discovered the importance of micellar solubilization in the uptake of lipid digestion products by enterocytes [104,105]. This concept was later challenged by Carey and associates, who discovered the coexistence of unilamellar liposomes and bile salt–lipid mixed micelles in the small intestine [37]. Only recently has the uptake of lipid digestion products by enterocytes been accepted as a passive process. However, several investigators have raised the possibility that some lipids may be taken up by the enterocytes by way of carrier-mediated processes that are energy dependent. This theory will be reviewed in the next section.

Importance of micellar solubilization

Understanding the importance of micellar solubilization of 2-MG and FAs in their uptake by enterocytes requires an understanding of the role of the unstirred water layer – a concept introduced by Dietschy and colleagues [106–108]. The brush border membrane of the enterocytes is separated from the bulk fluid phase in the intestinal lumen by an unstirred water layer (Fig. 18.4). This unstirred water layer mixes poorly with the bulk fluid phase; consequently, solute molecules in the bulk phase gain access to the brush border membrane by diffusion across the unstirred water layer. The solubility of FAs and MG in an aqueous medium is extremely low so very few molecules gain access to the brush border membrane (see Fig. 18.4, arrow 1). In contrast, the micellar solubilization of MG and FAs greatly enhances the number of molecules available for uptake by the enterocytes (see Fig. 18.4, arrow 2). Micellar solubilization increases the aqueous concentration of FAs and MG many fold. Despite the slower rate of diffusion of the micelle relative to the monomolecular FA molecule (because of size differences), micellar solubilization still markedly enhances the diffusion of FA and MG molecules across the unstirred water layer. The same mechanism applies to the transport of lipid-soluble drugs and compounds across the unstirred water layer to the enterocytes.

If micellar solubilization is so important in the absorption of lipid-soluble molecules such as fatty acids and cholesterol, is the absorption of lipid-soluble molecules purely a function of the degree of solubilization by micelles? The answer is "No." Several investigators have demonstrated that trihydroxy bile acids are more effective in promoting cholesterol absorption than dihydroxy bile acids [109–111]; however, the degree of solubilization was not measured in these experiments. Watt and Simmonds [111] showed a linear relationship between the amount of cholesterol taken up by the small intestine and the micellar cholesterol concentration, demonstrating the importance of micellar solubilization in the uptake of cholesterol. With the use of the nonionic surfactant Pluronic F-68 (BASF, Mount Olive, NJ) to promote the micellar solubilization of cholesterol but not its uptake by enterocytes, Watt and Simmonds demonstrated that the uptake of cholesterol by the enterocytes is dependent on the planar structure of the bile acid [111]. The role of bile salt in intestinal cholesterol uptake thus extends beyond its participation in cholesterol solubilization. This is further illustrated by the fact that chenodeoxycholyl taurine (CDC-tau) is a better micellar solubilizer of cholesterol than cholyl taurine (C-tau), even though cholesterol uptake is significantly greater with C-tau than it is with CDC-tau [112–114].

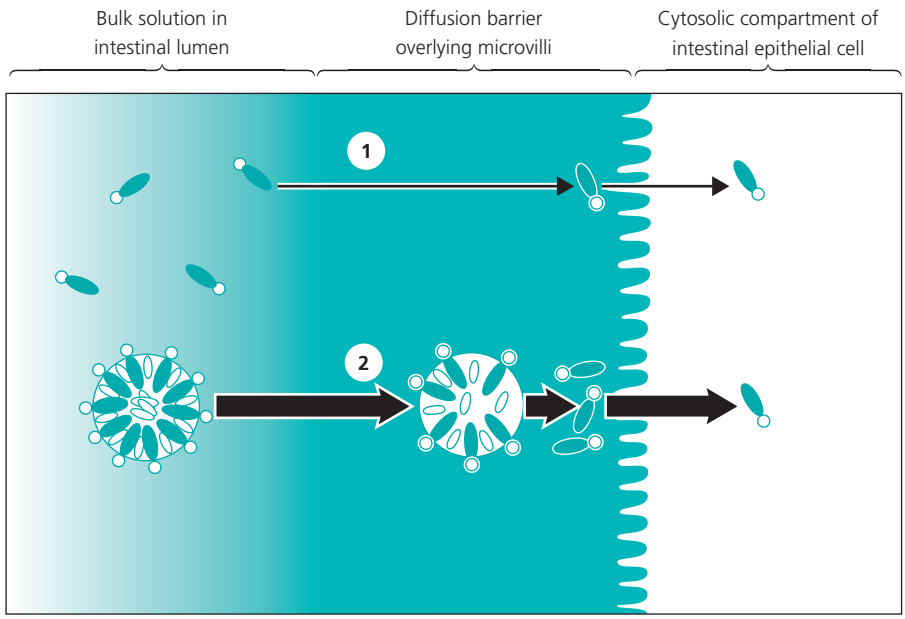

Figure 18.4 The effect of bile salt micelles (or vesicles) in overcoming the diffusion barrier resistance presented by the unstirred water layer. In the absence of bile acids, individual lipid molecules must diffuse across the barriers overlying the microvillus border of the intestinal epithelial cells (arrow 1). Hence, uptake of these molecules is largely diffusion limited. In the presence of bile acids (arrow 2), large amounts of these lipid molecules are delivered directly to the aqueous–membrane interface so that the rate of uptake is greatly enhanced. From Westergaard and Dietschy [108], with permission from the American Society of Clinical Investigation.

A particularly important point about the study of Watt and Simmonds [111] is that the uptake of cholesterol by a hydrophobic acceptor (silicone tubing) was the same for the cholesterol micellar solubilized with either Pluronic F-68 or bile acid. This suggests that the uptake of cholesterol by the enterocytes is an active process possibly mediated by a transporter. This supports an earlier finding by Sylven [115], who found that both cholesterol and β-sitosterol (plant sterol) are present in the human diet, although cholesterol, unlike β-sitosterol, is well absorbed by the small intestine. Sylven observed that the ability of the small intestine to discriminate between cholesterol and β-sitosterol absorption is energy-dependent and compromised by deprivation of blood supply [115]. The possibility that transporters are present at the brush border membrane is witnessed in patients with sitosterolemia, a condition in which the intestine fails to discriminate between cholesterol and β-sitosterol [116–118]. The importance of bile salts in facilitating the uptake of lipid-soluble molecules, including drugs, by the small intestine may therefore extend beyond their detergent properties to potential therapeutic applications. This topic will be discussed in the section on mucosal transporters.

Importance of unilamellar vesicles

When human jejunal contents collected during the digestion of a lipid meal are spun in an ultracentrifuge, there is a solid particulate layer on the bottom of the tube, a clear micellar layer in the middle, and an oily phase on the top [105]. The oily phase consists mostly of TG, partial glycerides, and FAs. The clear micellar phase consists of bile salts, FAs, and MG. However, when Porter and Saunders [119] carefully analyzed the aqueous phase after ultracentrifugation and after the intestinal contents had passed through a series of filters with progressively smaller pores (the smallest being 100 nm in diameter), they found that the resultant fraction did not display a clear micellar phase. Instead, a slightly turbid phase was observed. Furthermore, the micellar phase exhibited a concentration gradient of lipids. The full implication of this finding was not appreciated until later, when Patton and Carey observed the digestion of fat in vitro using light microscopy [120]. These investigators observed the constituent presence of at least three phases during fat digestion:

- the oil phase (mainly TG, partial glycerides, and FAs)
- the calcium soap phase (Ca^{2+} ions and protonated long-chain FAs)
- the viscous isotropic phase (MG and FAs).

Therefore, to divide the intestinal contents into an oil phase and a micellar phase oversimplifies the process of fat digestion.

Carey and colleagues [37] proposed that when the bile salt concentration in the lumen exceeds the critical micellar concentration, the lipid in the intestinal lumen is incorporated into mixed micelles, and that these probably assume the form of mixed disc-like particles, more or less saturated with lipids, with a hydrodynamic radius of about 20 nm. Further increases in the amount of lipid in the aqueous phase lead to the formation of liquid crystalline vesicles (liposomes) with hydrodynamic radii of 40–60 nm [37,121]. This finding may have important clinical implications. Patients with low intralumenal bile salt concentrations [122], or those with biliary fistulae [123], seem to be able to absorb fat well. Carey and colleagues proposed that the liquid crystalline vesicles play an important role in the uptake of FAs and MG by enterocytes [37] in these types of conditions. Woollett and

colleagues [124] addressed this question in a study of human subjects with inborn errors of bile acid synthesis who were being treated with supplemental bile acid therapy. The study commenced 3 weeks after the withdrawal of the supplements, and involved the administration of a lipid meal with or without bile acid added. With the absorption of the more polar phospholipids and fatty acids, it would appear that lipids incorporated in either the micelle or the vesicle are taken up by the enterocytes as well. In contrast, cholesterol uptake by the enterocytes is dependent on the cholesterol being incorporated into mixed micelles and the uptake from vesicular cholesterol is very poor.

The aqueous phase may contain liquid crystalline vesicles in addition to mixed disk-shaped micelles [125]. Both cholesterol and LPC are presumably incorporated into both the disc-shaped micelles and into liquid crystalline vesicles before their uptake by enterocytes. Shoemaker and Nichols [126] observed that bile salts and lysophospholipids form submicellar aggregates that can coexist with artificial phospholipid vesicles in an aqueous medium. Shoemaker and Nichols subsequently provided convincing evidence that these submicellar aggregates serve as an efficient shuttling mechanism for the transfer of lysophospholipids between membranes [127]. The existence of these submicellar aggregates in the intestinal lumen, and their role in delivering lysophospholipids and, potentially, lipid-soluble drugs, to the enterocytes for absorption, remain to be demonstrated.

Mucosal brush border membrane lipid transporters

It was generally believed that FAs and MG were absorbed by the enterocytes through simple diffusion [1]. Strauss demonstrated simply and elegantly that the uptake of FAs is passive and not temperature dependent [128]. The MG and FAs enter the enterocytes as monomers [1]. Studies by Stremmel have indicated the existence of a FA-binding protein associated with the brush border membrane that plays a role in the uptake of FAs by enterocytes [129]. The idea that FAs may be taken up by enterocytes by way of a carrier-mediated process was implied by Chow and Hollander [130] who showed that linoleate uptake by the small intestine reflects a concentration-dependent dual mechanism of transport. At low linoleate concentrations, FAs are taken up by way of a carrier-dependent process, whereas at a higher linoleate concentration, FAs are taken up predominantly by passive diffusion. Stremmel also raised the possibility that some lipids, especially FAs, may be taken up by enterocytes by way of carrier-mediated processes [129,131]. Although Stremmel mainly focused on FA uptake, he observed that this FA-binding protein was also capable of transporting cholesterol, but not cholesterol ester. This finding came after the discovery that cholesterol uptake by a jejunal loop treated with the antibody against this FA-binding protein was significantly reduced. This protein is mainly present in the apical and lateral regions of the villus (where tight junctions are located) and in the crypt [131]. The results of this research suggest that this FA-binding protein is a plausible candidate transporter for an array of lipid molecules, including cholesterol. This conclusion, however, raised questions. First, this transporter was later identified to be the mitochondrial glutamic oxaloacetic aminotransferase, which is an intracellular enzyme not involved in lipid absorption [132]. Second, why do crypt cells express this protein when they are not involved in fat absorption?

Thurnhofer and colleagues first described the presence of a binding protein in the small intestinal brush border membrane that facilitates the uptake of cholesterol by the small intestine [133–136]. The protein has an apparent molecular weight of about 14 kDa as determined by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, or a value of 11.5 kDa if determined by gel filtration on Sephadex G-75 SF (Pharmacia, Döbendorf, Switzerland). With the use of the brush border membrane, Thurnhofer and colleagues [135] demonstrated that cholesterol uptake is most effective from cholate-mixed micelles and is characterized by short half-times of seconds. As expected, proteolytic treatment of the brush border membrane vesicles significantly reduced the capacity of these vesicles to take up cholesterol. When polyclonal antibodies raised against the 14-kDa protein were present in the incubation medium, cholesterol uptake by these vesicles was significantly reduced. This 14-kDa protein was later identified as SCP-2 (synaptonemal complex protein 2), which is an intracellular protein [137]. Thus, the identity of the plasma membrane-bound cholesterol transport protein, if it exists, remains obscure. These searches were fruitless, in part, because of problems associated with the use of hydrophobic ligands as the bait, which often bind nonspecifically to proteins in the brush border preparations. The cross-contamination of the brush border membrane with proteins from other subcellular fractions is another complication.

A number of other proteins have also been shown to bind lipids. These include GP330 (also called megalin), CD36, and caveolin. GP330, a member of the low-density lipoprotein receptor gene family, is an endocytic receptor expressed in many absorptive epithelia, including the kidney proximal tubules, type II pneumocytes, mammary epithelium, and thyroid follicular cells [138]. GP330 is involved in the renal uptake of polybasic drugs [138–140], vitamin B-12 [140], cholesterol-carrying lipoproteins [141], albumin [142], and proteases [139]. The failure of the forebrain of GP330 knockout mice to develop has been attributed to the deficient supply of cholesterol to the brain of the fetus through the maternal–fetal lipoprotein transport system [143]. The expression of GP330, or a related protein, in the intestine remains uncertain [138]. Moreover, whether or not GP330 participates in cholesterol uptake by the small intestine remains unknown. Hauser and colleagues [144] provided evidence that the scavenger receptor BI (SRBI) is involved in

intestinal uptake of cholesterol. Schulthess and colleagues [145] demonstrated that the uptake of cholesterol mediated by scavenger receptors is inhibited by an amphipathic α-helix of 18 amino acids, lending further support to the involvement of SRBI in intestinal cholesterol absorption. Van Bennekum and colleagues [146] have provided additional information that SRBI is involved in intestinal cholesterol uptake and that this uptake can be inhibited by ezetimibe, which is a potent inhibitor of cholesterol uptake by enterocytes.

A membrane transporter for FAs has been identified in adipocytes. This FA transporter is inhibited by sulfo-*N*-succinimidyl derivatives of long-chain FAs [147–151] and by 4,4′-diisothiocyanostilbene-2-2′-sulfonate. It has an apparent molecular weight of 88 kDa, an isolectric point of 6.9, and a strong sequence homology to CD36 and PAS IV. PAS IV is a protein enriched in the apical membranes of lipid-secreting mammary cells during lactation, and cloning of this binding protein revealed that it bears 85% homology to CD36, which is present in human platelets and lactating mammary epithelia [148]. Northern blot analysis revealed that the message is abundant in the heart, intestine, fat, muscle, and testis [149–151]. Fatty-acid transporters have been localized to the brush border membrane, and expression is highest in the jejunum, less abundant in the duodenum, and least in the ileum [151]. Research has shown that a high-fat diet, rich in long-chain FAs, but not medium-chain FAs, results in increased fatty acid transporter expression [152]. With the use of the lymph fistula mouse model, Nauli and colleagues [153] have demonstrated that CD36 is involved in promoting the uptake of cholesterol by the small intestine, and although it does not seem to be involved in the uptake of fatty acids by the small intestine, it plays an important role in the packaging of absorbed fatty acids as TGs into chylomicrons.

Other potential binding proteins have been identified. One binding protein for long-chain FAs was cloned in adipocytes by Schaffer and Lodish [154]. Recently, Stahl and colleagues [155] identified the presence of FATP4, a member of the large family of fatty acid transport proteins, in the small intestine. Moulson and colleagues [156] reported that a wrinkle-free phenotype is associated with the spontaneous autosomal recessive mutation of the gene for FATP4, resulting in a very tight and thick-skinned phenotype. This observation was confirmed in a subsequent report by Herrmann and colleagues [157], which showed that the lack of FATP specifically in the epidermis of the skin results in the same phenotype. Hall and colleagues [158] showed that FATP4, in addition to its role in the uptake of FAs, might also exhibit acyl-coenzyme A (CoA) synthetase activity. As yet, the role of FATP4 in the uptake of FAs in vivo is unclear. Caveolin is another protein that binds cholesterol [159]. Caveolin was the first-reported protein associated with caveolae, the non-clathrin-coated invaginations present on the cell surface that function to bind glycolipids [160], cholesterol [161], glycosyl-phosphatidylinositol-linked proteins [162], and other proteins for potocytosis [163]. It is unknown if caveolin plays a role in the intestinal absorption of cholesterol and FAs.

The investigation of transporters involved in cholesterol absorption has yielded two exciting developments. The first involves the drug ezetimibe, a potent inhibitor of intestinal cholesterol absorption [164–166]. Earlier studies showed that photoaffinity derivatives of ezetimibe specifically labeled the SRBI protein [167]. In vitro studies from two independent laboratories showed that mammalian cells transfected with SRBI cDNA display an increased ability for cholesterol uptake from micellar substrates in comparison to mock-transfected cells with constitutively low SRBI expression [167–169]. Most importantly, the increased capacity for cholesterol uptake by SRBI-transfected cells was indeed subject to ezetimibe inhibition in both of these studies [167,169]. When considered together, these data support a role for SRBI on the membrane of enterocytes as the cholesterol transporter responsible for cholesterol absorption from the intestinal lumen. The distribution of SRBI along the gastrocolic axis (mostly in the duodenum and the jejunum) and on the apical side of the brush border membrane is also consistent with the hypothesis that SRBI participates in cholesterol absorption [169–171]. Another cholesterol transporter that has been implicated in cholesterol absorption is the NPC1L1 (Niemann–Pick C1-like 1) protein. Altmann and colleagues [172] demonstrated that NPC1L1 plays an important role in the absorption of cholesterol by the small intestine. NPC1L1 is expressed mostly in the duodenum and the jejunum, although substantial protein levels may be measured in the ileum. Davies and colleagues observed that the inactivation of NPC1-L1 results in the reduction of intestinal cholesterol absorption, and the prevention of diet-induced hypercholesterolemia [173]. As expected, Davis and colleagues reported that cholesterol/cholate feeding results in the down-regulation of intestinal NPC1L1 messenger RNA (mRNA) expression in wild-type mice [174]. NPC1L1 deficiency results in an up-regulation of intestinal hydroxymethylglutaryl–CoA synthase mRNA expression, an increase in intestinal cholesterol synthesis, down-regulation of ABCA1 mRNA, but no change in ABCG5 and ABCG8 mRNA expression. The studies of Altmann [172], Garcia-Calvo [175], and Iyer [176] and their colleagues seem to imply that NPC1L1 is expressed in the intestinal epithelial cells, probably at the apical membrane.

The second exciting development in the area of cholesterol absorption is the discovery that cholesterol absorption in the small intestine is regulated by ATP-binding cassette (ABC) transporters. Repa and colleagues [177] reported that intestinal absorption of cholesterol is regulated by the expression of the reverse cholesterol transporter ABCA1. In turn, ABCA1 is regulated by the formation of heterodimers of retinoid X

receptors (RXRs) with either oxysterols (LXRs) or with the bile acid receptor (FXR). Berge and colleagues identified seven different mutations in two adjacent genes encoding two ABC transporters ABCG5 and ABCG8 in β-sitosterolemic patients [178]. The same group hypothesized that ABCG5 and ABCG8 normally cooperate to restrict the intestinal uptake and biliary secretion of sterols. Mutation of these genes results in the excess uptake of sterols, including plant sterols such as β-sitosterol. Lee and colleagues [179] reported the mutation of ABCG5 in nine unrelated patients with sitosterolemia. Although these new findings are of integral importance in our mechanistic understanding of how the gut discriminates between the uptake of cholesterol and plant sterols, many questions remain. First, the mechanism of how the various ABC transporters in the gut regulate cholesterol absorption is still not clear. Second, are ABC transporters involved in the reverse transport of other lipid-soluble compounds, including lipid-soluble drugs? Lastly, it would seem that the physiological and therapeutic manipulations of ABC transporters are potential ways to modify intestinal cholesterol absorption in animals and humans. This subject has been reviewed by Wang [180].

Intracellular metabolism of dietary lipids

It is not known how the various absorbed lipids migrate from the site of absorption to the endoplasmic reticulum, where biosynthesis of complex lipids occurs. A FA-binding protein (FABP) in the small intestine has been isolated and characterized by Ockner and Manning [182]. It has been suggested that FABP plays an important role in the intracellular transport of absorbed FAs. This is partly supported by the findings that FABP concentration is greater in villi than in crypts, greater in the jejunum than in the ileum, and greater in the intestinal mucosa of animals fed a high-fat diet than in those fed a low-fat diet [181,182]. There are at least two FABP types in enterocytes – the I-FABP and L-FABP. The preceding letter indicates the organ from which the form was first isolated (i.e., I = intestine and L = liver). These two FABPs differ in their binding specificities. I-FABP binds strongly with FAs, and L-FABP binds with long-chain FAs, LPC, retinoids, bilirubin, carcinogens, and even selenium [183–185]. Considering the results of nuclear magnetic resonance binding studies, Cistola and colleagues [186] speculated that the binding of I-FABP is involved in the intracellular transport of FAs, whereas L-FABP is involved in the intracellular transport of MG and LPC. However, these functions of FABP have not been tested rigorously in vivo. A comprehensive review of the binding proteins of FAs has been published by Storch and Thumser [187].

Demonstrating the in vivo functions of the FABPs is challenging. One strategy has been to knock out the gene of a particular FABP. This, however, has not yielded definitive results because other FABPs are often capable of compensating for the deficit in function that the knocked out FABP assumed. For example, when Shaughnessy and colleagues [188] used the adipocyte FABP knockout mouse model to study its function, the keratinocyte FABP compensated for the absence of the adipocyte FABP. Using the I-FABP knockout mouse model, investigators found that males have higher plasma TG levels and heavier body weight than wild-type mice [189], implying that fat absorption is probably normal in these genetically modified animals. The female knockout mice weighed more than the wild-type mice but less than the male knockout mice. When the investigators who had generated the mice determined if fat absorption was compromised, they found no difference between I-FABP null animals and wild-type animals [190]. As noted earlier, one should not conclude from these data that I-FABP is not physiologically important for fat absorption, as other intracellular FABPs may be compensating for the lack of I-FABP. Newberry and colleagues [191] reported that the L-FABP animals did not exhibit fat malabsorption, but the kinetics of intestinal lipid secretion was slower in the null animals than in the wild-type animals. Investigation of how these FABPs function in a concerted manner to facilitate fat absorption in the intestine is ongoing in a number of laboratories.

Two sterol carrier proteins, SCP1 (47 kDa) and SCP2 (13.5 kDa), have been isolated and characterized [192,193]. SCP1 is important in the microsomal conversion of squalene to lanosterol [194], whereas SCP2 (also known as the nonspecific lipid transfer protein) participates in the microsomal conversion of lanosterol to cholesterol [195]. In addition, SCP2 is involved in the intracellular transport of cholesterol from cytoplasmic lipid droplets to mitochondria and also in the translocation of cholesterol from the outer mitochondrial membrane to the inner mitochondrial membrane [196]. Further, SPC2 appears to be involved in the intracellular trafficking of PC and FAs, and it may play a role in peroxisomal FA–CoA binding [197–200].

Intracellular metabolism of monoacylglycerols and fatty acid

2-MG and FAs are reconstituted to form TG, mainly by way of the MG pathway. As shown in Figure 18.5, 2-MG is reacylated into TG by the consecutive action of MG acyltransferase and DG acyltransferase (DGAT) [201,202]. The enzymes involved in this MG pathway reside in a complex called TG synthetase [201,203], which has been purified by Lehner and Kuksis [204]. It is thought that the synthesis of TG from DG is catalyzed by the enzyme acyl-CoA : diacylglycerol acyltransferase. The gene for this enzyme, *DGAT1*, has been isolated, and a knockout mouse has been generated. Interestingly, this mouse is able to synthesize normal

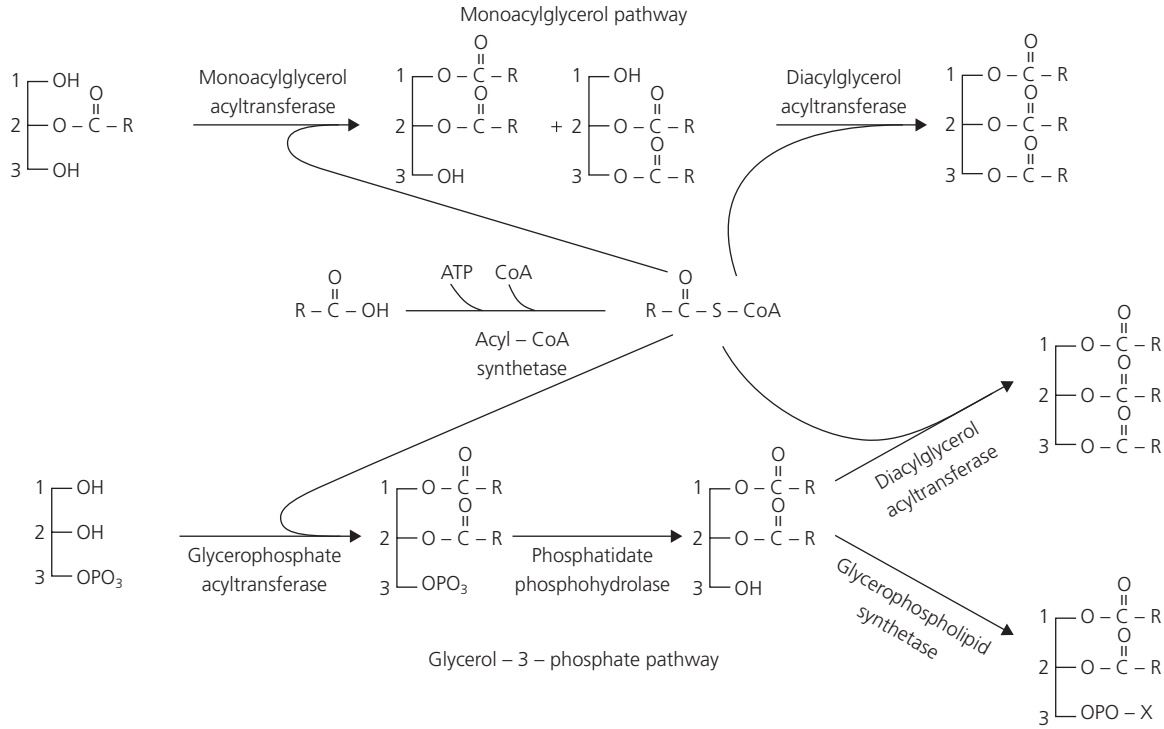

Figure 18.5 Pathways of triacylglycerol biosynthesis in the intestinal mucosa.

amounts of TG in the intestinal mucosa [205], which implies that another enzyme may be involved in the formation of TG from DG. This notion is supported by data from Oelkers and colleagues [206] showing that a lecithin cholesterol acyltransferase-like gene produces an enzyme that mediates DG esterification in yeast. Further, data generated from the DGAT knockout animals [204] suggest that a related protein is also present in intestinal epithelial cells.

Several studies have demonstrated that the enzymes involved in the MG pathway are located on the cytoplasmic surface of the endoplasmic reticulum [207]. This finding has important implications for the understanding of the intracellular packaging of lipoproteins. The data indicate that TG is formed at the cytoplasmic surface of the endoplasmic reticulum, and then enters the cisternae of the endoplasmic reticulum. Because TG has a low solubility in phospholipid bilayers (~ 3 mol%), Atkinson and Small [208] postulated that TG molecules rapidly saturate the membrane. Once the solubility of TG is exceeded, the TG splits the bilayer and forms a small lens. As the lens grows, it bulges into the cytoplasmic or cisternal side of the endoplasmic reticulum. Finally, this protrusion pinches off from the membrane and forms lipid droplets in the cytoplasm or precursors of lipoproteins in the cisternae of the endoplasmic reticulum. Using ultrastructural studies, Wetterau and Zilversmit [209–211] showed that a protein in the liver, small intestine, and several other organs promotes the transfer of TG and cholesterol esterase between membranes. The function of this transfer protein will be discussed in the section of disorders of intestinal lipid transport.

A second pathway in the intestinal mucosa leading to TG formation is the α-glycerophosphate pathway [201,202]. This pathway involves the stepwise acylation of glycerol-3-phosphate to form phosphatidic acid (see Fig. 18.5). In the presence of phosphatidate phosphohydrolase, phosphatidic acid is hydrolyzed to form DG, which is then converted to TG. The relative importance of the MG pathway and the α-glycerophosphate pathway depends on the supply of 2-MG and FA. During normal lipid absorption, when the level of 2-MG is adequate, the 2-MG pathway facilitates the conversion of 2-MG and FA to form TG and aids in inhibiting the α-glycerophosphate pathway [201,202,212]. Conversely, when the supply of 2-MG is lacking or insufficient, the α-glycerophosphate pathway becomes the major pathway for the formation of TG.

Phospholipids

Some absorbed LPC is reacylated to form PC (213–216). The remaining absorbed LPC is hydrolyzed to form glycero-3-phosphorylcholine [217]. The liberated FA is used for TG synthesis, whereas the glycero-3-phosphorylcholine is readily transported by way of the portal circulation for use in the liver [218]. Another reaction that occurs in the intestinal mucosa is the combining of two molecules of LPC to yield one molecule of PC and one molecule of glycero-3-phosphorylcholine [104,219].

Cholesterol

Exogenous cholesterol absorbed by the enterocytes enters a pool of free cholesterol within the enterocytes in which cholesterol from endogenous sources is also stored. Endogenous cholesterol is derived from three sources:

- nondietary cholesterol absorbed from the lumen (biliary cholesterol and cholesterol from cells shed from the intestinal mucosa)
- cholesterol derived from plasma lipoproteins [220]
- cholesterol synthesized de novo [221–223].

The cholesterol from each source is handled uniquely. For example, cholesterol derived from the intestinal lumen does not mix evenly with the free cholesterol pool and is preferentially esterified in the enterocytes for export into lymph as TG-rich lipoproteins [224–226]. Stange and Dietschy observed that very little newly synthesized cholesterol is transported into lymph during fasting [225]. However, during active lipid absorption, significantly more newly synthesized cholesterol is incorporated into the TG-rich lipoproteins that are transported in lymph. Cholesterol synthesis and the uptake of lipoproteins by the small intestine are discussed in articles and reviews by Spady and colleagues [220, 228], Dietschy and colleagues [227], and Stange and Dietschy [225].

Cholesterol is transported mainly as esterified cholesterol and almost exclusively by the lymphatic system. The rate of esterification of cholesterol regulates its lymphatic transport [229]. Two proposed enzymes involved in cholesterol esterification are cholesterol esterase (230,231) and acyl-CoA cholesterol acyltransferase (ACAT) [230,232,233]. The distribution and regulation of ACAT in the small intestinal epithelium has been studied extensively. The rat jejunum and ileum both have high specific activities of ACAT, albeit slightly higher activity in the jejunum than the ileum. Conversely, the specific activity of ACAT in the guinea pig, rabbit, and human is significantly higher in the jejunum than in the ileum [234–236]. This enzyme is stimulated by ingestion of a diet with high levels of cholesterol [234,235]. However, it is not clear whether this increase in ACAT activity is caused by the high levels of cholesterol or by the increase in microsomal cholesterol that is isolated from these animals for the purpose of conducting the ACAT bioassay.

With the use of immunocytochemistry, Gallo and colleagues [237] demonstrated that intracellular cholesterol esterase is derived from pancreatic cholesterol esterase. A later study by Field [238] confirmed the presence of cholesterol esterase in the enterocyte. It remains unclear how pancreatic cholesterol esterase is taken up by the enterocyte. An understanding of this process is of biological and therapeutic interest because it will facilitate the delivery of peptides to the body through the gut. Normally, most peptides are digested by the proteases and peptides located both in the lumen and in the brush border membrane of the enterocytes.

Most investigators believe that ACAT plays a more important role in mucosal cholesterol esterification than does cholesterol esterase. Several substantiated observations support this hypothesis. First, ACAT displays the highest activity in the segment of the small intestine that is most actively involved in cholesterol absorption [234–236]. Second, the ACAT activity that is present in the intestinal epithelium adequately accounts for all of the cholesterol esterase transported by the small intestine [239]. Third, Watt and Simmonds [240] failed to demonstrate an effect of the diversion of pancreatic juice (the origin of mucosal cholesterol esterase) on the absorption and esterification of lumenal dietary cholesterol in rats with lymph fistulae. Finally, using a number of specific ACAT inhibitors, several studies have shown a reduction in lymphatic cholesterol output [230,241–243]. ACAT inhibitors continue to be synthesized, and interested readers are referred to the report by Lee and colleagues [244] for more information.

Chang and associates have played a pivotal role in the purification and identification of ACAT proteins [245–247]. Two ACAT proteins have been identified – ACAT1 and ACAT2. With the use of immunohistochemistry, Chang and colleagues [233] reported that ACAT1 is present in fetal and adult hepatocytes, and ACAT2 is present only in fetal hepatocytes. Furthermore, Chang and colleagues [246] found that the ACAT activity in the small intestine can be immunodepleted by antibodies raised against ACAT2, and that most of the ACAT2 is located at the apices of the villi. The role of ACAT2 in intestinal cholesterol absorption is supported by the findings of Buhman and colleagues [248], who reported that ACAT2 knockout mice are resistant to diet-induced hypercholesterolemia because they have defective cholesterol esterification and poor cholesterol absorption by the small intestinal epithelial cells.

Formation of intestinal chylomicrons and very-low-density lipoproteins

Despite the wealth of information gathered over the last 30 years about intestinal lipid absorption, the mechanism of the intracellular assembly, modification, and secretion of lipoproteins from small intestinal epithelial cells is not fully understood. This is partly because of the complexity of the processes involved, and the lack of good experimental models to study the various steps. Intestinal cell culture systems (e.g., CaCo-2 cells) have been used extensively to study the formation and secretion of lipoproteins [3, 249–252] and the genetic expression and posttranslational modification of apolipoproteins [253]. During fasting, very-low-density lipoproteins (VLDLs) are the only lipoproteins produced by the small intestine [254–256]. After a meal, the small intestine produces predominantly chylomicrons (CMs) [256,257]. The distinction between intestinal CMs and VLDLs is based

on an operational criterion. Lipoproteins that have a Svedberg flotation (S_f) rate exceeding 400 are classified as CMs; those with an S_f rate of 20–400 are defined as VLDLs [258]. Numerous studies support the idea that intestinal CMs and VLDLs represent lipoproteins produced by two separate pathways. Ockner and colleagues [254] showed that the intraduodenal infusion of palmitate causes a marked increase in VLDL transport, but that VLDL output remains unchanged when oleate and linoleate are infused. In contrast, CM output is markedly increased when oleate and linoleate are infused. The FA composition of the VLDL-TG is different from that of the CM-TG, suggesting the presence of two different pathways for VLDL and CM assembly. An ultrastructural and biochemical study conducted by Mahley and colleagues [259] showed that intestinal Golgi vesicles contain either CMs or VLDL particles and that little mixing of particle sizes occurs; conclusive evidence of separate biosynthetic pathways for the assembly of these distinct lipoproteins. Further evidence was provided by Vahouny and colleagues [260], who demonstrated that although puromycin had no significant effect on the incorporation of radioactive leucine into VLDL peptides in male rats, the incorporation of radioactive leucine into CM peptides was markedly inhibited.

Studies conducted by Tso and colleagues [261,262] have shown that rats fed as little as 0.5 mg/h of a hydrophobic surfactant Pluronic L-81 (BASF, Mount Olive, NJ) (L-81) have markedly impaired lymphatic transport of TG and cholesterol. These studies further demonstrated that L-81 inhibited lipid transport by blocking the formation of intestinal CMs, but not the formation of VLDL particles. With the use of this unique tool, Tso and colleagues further showed that intraduodenal infusion of egg PC in rats resulted mostly in the lymphatic transport of VLDLs, and that this transport was not affected by the administration of L-81 [263]. However, when triolein was infused, the lymphatic transport of lipid was inhibited because the feeding of triolein resulted primarily in the formation of CMs [261,262]. These results led Tso and colleagues to propose two pathways for the formation of intestinal lipoproteins – one that forms predominantly CMs and one that forms predominantly VLDLs (Fig. 18.6) [263]. Moreover, these investigators proposed that the pathway forming predominantly CMs is inhibited by L-81, but the pathway forming predominately VLDLs is not, and that preCM and preVLDL particles are packaged in the Golgi apparatus into either preCM or preVLDL-containing vesicles. The Golgi-derived vesicles containing either preCMs or preVLDLs probably correspond to the vesicle types observed by Mahley and colleagues [259]. This hypothesis is supported by the study conducted by Nutting and colleagues [264]. This laboratory measured the appearance time for CMs in control rats and for VLDLs in L-81-treated rats. The appearance time (defined as the time between placement of radioactive FA into the intestinal lumen and the appearance of radioactive lipid in lymph) was 10.8 min in control rats – significantly shorter than the 16.2 min in the L-81-treated rats. This difference in appearance time further supports the hypothesis that CMs and VLDLs are packaged separately in enterocytes. Finally, this in vivo observation can be duplicated in cell culture systems. With the use of CaCo-2 cells, Luchoomun and Hussain [266] also demonstrated that L-81 inhibits CM formation but not VLDL formation by enterocytes.

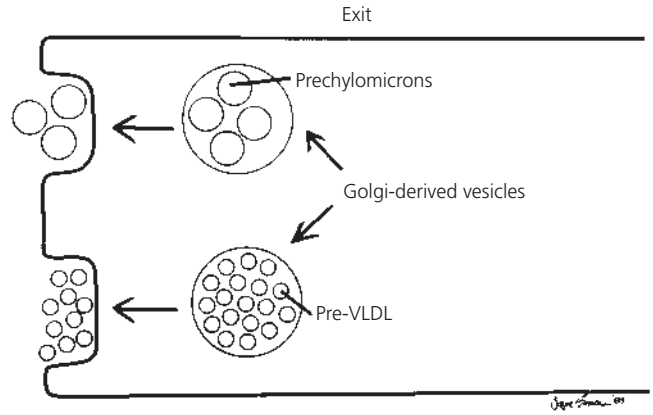

Figure 18.6 Packaging and secretion of intestinal chylomicrons and very-low-density lipoproteins (VLDLs). Golgi-derived vesicles contain either prechylomicron or pre-VLDL particles. Mixing of prechylomicron and pre-VLDL particles in these vesicles seldom occurs.

The fact that the infusion of PC results in the formation of VLDL whereas the infusion of TG results in the formation of CM is certainly of interest with respect to the delivery of lipid-soluble vitamins and drugs. Not only is the amount of drug that can be carried by a VLDL particle (smaller) different from that able to be carried by a CM particle, the difference in the metabolism of VLDL and CM particles may also affect the delivery of fat-soluble vitamins and drugs to different parts of the body. This is an interesting but poorly explored area of investigation.

The CaCo-2 cells have been used extensively to study the uptake as well as the formation and secretion of TG-rich lipoproteins. Based on their CaCo-2 studies, Hussain and colleagues proposed a sequential assembly model to study VLDL and CM formation [265]. This model provides information on the assembly of primordial lipoprotein particles, the synthesis of TG-rich lipid droplets, and the core expansion involving the fusion of primordial lipoproteins with lipid droplets. Readers interested in the details of this model are referred to an excellent review by Hussain [3]. CaCo-2 cells differ from the enterocytes in the small intestine in a number of important ways. First, CaCo-2 cells are derived from human colon carcinoma. Second, the CaCo-2 cells use the glycerol-3-phosphate pathway for the formation TG from fatty acids and glycerol, whereas intestinal epithelial cells use the MG pathway for the formation of TG [267]. Third, the intestinal epithelial cells synthesize only apolipoprotein (apo) B48, whereas CaCo-2 cells synthesize both apo B-48

and apo B-100. Lastly, the intestinal epithelial cells produce mainly CM particles during active fat absorption, whereas CM particles are never the major lipoproteins produced by the CaCo-2 cells. Therefore, although CaCo-2 cells have certainly been used successfully as surrogates of intestinal epithelial cells, cautious interpretation of the data generated by the CaCo-2 cells is warranted.

Clinical disorders of intestinal lipid transport

In humans, the liver secretes only apo B-100, and the small intestine secretes only apo B-48 [268–270]. Both apo B-100 and apo B-48 are encoded by the same gene [271,272]. The biogenesis of apo B-48 involves a unique mechanism by which the CAA codon encoding Gln at 2153 of the apo B-100 mRNA is changed to UAA (a stop codon), thus forming apo B-48. Rat apo B-48 has a molecular weight of about 240 kDa [273] and is an extremely hydrophobic protein [274]. Each CM or VLDL particle has one apo B-48 [275]. The absolute requirement of apo B for the formation of CM is well illustrated by the apo B knockout animals. We do not know, however, whether the production of apo B is physiologically rate limiting in the formation of CM by the enterocytes. Data from several laboratories indicate that the supply of apo B is probably not the rate-limiting step for forming CM. Hayashi and colleagues [276] demonstrated that apo B output in lymph, an indication of the number of CM produced by the small intestine, does not change after intraduodenal infusion of lipid, despite the fact that lymphatic TG output increases sevenfold to eightfold. These data support the finding by Davidson and colleagues [278] that lipid absorption does not lead to an increase in apo B synthesis.

Abetalipoproteinemia is a rare genetic disorder involving the complete failure of the liver and gut to make TG-rich lipoproteins [278,279]. It is believed that patients with abetalipoproteinemia have difficulty synthesizing apo B. Glickman and colleagues [269] reported that apo B synthesis, as determined by ^{3}H-labeled leucine incorporation, is decreased but not abolished in patients with abetalipoproteinemia, suggesting that failure to synthesize apo B by the gut and liver may not be the only reason that these patients do not produce CM and VLDL. This was later confirmed by the finding that abetalipoproteinemia results from the mutation of the microsomal TG transfer protein gene [280–283]. The role of microsomal TG transfer protein in the formation of chylomicrons by the small intestine is well covered in the reviews by Berriot-Varoqueaux and colleagues [284] and Hussain [3].

Anderson disease is another disorder in which there is a deficiency in the ability of the small intestine to form CMs [284–286]. Anderson disease is also known as chylomicron retention disorder because although observable CM particles are present in the enterocytes, there are none in the intercellular spaces, which suggests that the secretion of CMs is defective. Dannoura and colleagues has demonstrated that patients with Anderson disease do not have a defect in those genes that code for known apoproteins or microsomal TG transfer protein, suggesting that this condition is caused by a an unknown factor crucial for the secretion of chylomicrons [287]. There is no animal model available with which to study this perplexing disorder.

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D NUTRITION

19

Protein digestion and assimilation

Vadivel Ganapathy, Malliga E. Ganapathy, Frederick H. Leibach

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The digestion and assimilation of proteins differ from that of carbohydrates and lipids in a number of aspects. Unlike the carbohydrates in the diet, which consist of quantitatively significant amounts of monosaccharides (e.g., glucose and fructose) and disaccharides (e.g., sucrose and lactose) in addition to polysaccharides (e.g., starch and glycogen), dietary amino acids are ingested predominantly in the form of proteins rather than in the form of free amino acids or small peptides. Dietary carbohydrates are absorbed by the enterocytes exclusively as monosaccharides. Therefore, the polysaccharides and disaccharides in the diet must be completely digested in the intestinal lumen to monosaccharides before absorption can occur. In contrast, the digestion of proteins in the intestinal lumen is incomplete, resulting in the generation of a mixture of free amino acids and small peptides in which peptides predominate. A major proportion of the protein digestion products are absorbed by the enterocytes as small peptides, which are subsequently digested to free amino acids inside enterocytes. Thus, whereas specific transport systems exist in the small intestine only for the absorption of monosaccharides, distinct transport systems exist in this organ for the absorption of not only free amino acids but also small peptides. Furthermore, unlike dietary carbohydrates, which consist predominantly of only three structurally different monosaccharides (glucose, galactose, and fructose), dietary proteins comprise a much greater number of structurally and physicochemically diverse amino acids. Consequently, the enzymes and transport systems involved in the digestion and absorption of proteins outnumber those involved in the digestion and absorption of carbohydrates. Moreover, unlike dietary lipids and their digestion products, which are highly hydrophobic, dietary proteins and their digestion products are largely hydrophilic. Therefore, bile salts, which play an obligatory role in the solubilization of dietary lipids to facilitate their digestion and assimilation, do not play a direct role in the digestion and assimilation of dietary proteins. However, bile salts and bile salt–facilitated fat digestion may influence protein digestion indirectly because bile salts and fat digestion products in the intestinal lumen are important modulators of cholecystokinin secretion, a process that has a profound influence on the secretion of pancreatic enzymes involved in protein digestion.

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Nutritional aspects of dietary proteins

The recommended daily allowance for dietary proteins in humans, when expressed per kilogram of body weight, declines in an age-dependent manner. In adults, this value is 0.75 g/kg; it is twice as much in newborns. The recommended daily allowance for dietary proteins increases during illnesses such as sepsis and during trauma; it also increases in women during pregnancy and lactation. The protein content of the average daily American diet is 70–100 g, which is more than adequate to meet the recommended daily allowance. Dietary proteins are required for two purposes: (1) to provide the amino acids that are nutritionally essential under all conditions (i.e., methionine, threonine, tryptophan, valine, isoleucine, leucine, phenylalanine, and lysine) and the amino acids that are conditionally essential under specific physiological and pathological conditions (i.e., cysteine, tyrosine, glutamine, histidine, and arginine); and (2) to provide nitrogen for the synthesis of nutritionally nonessential amino acids and other biologically important nitrogen-containing compounds. The nutritive value of various dietary protein sources depends on their content of the essential amino acids as well as on their digestibility. Proteins from animal sources have a high content of essential amino acids. Egg and milk proteins are routinely used as reference standards for

comparison of protein quality. Proteins from certain specific plant sources are "incomplete" because they lack or contain only limited amounts of some of the essential amino acids. However, by complementation with proteins that contain the missing amino acids, it is possible to overcome this deficit in a complete diet. Just as the essential amino acid content varies, the digestibility of different proteins also varies considerably. Protein digestibility is influenced by the type of protein and by the method of food processing before ingestion. Generally, plant proteins are less digestible than animal proteins. The relative contributions of animal proteins and plant proteins in the daily intake of dietary proteins differ markedly between developed and developing countries. For example, in North America and Western Europe, animal proteins contribute about 60%–70% of the total proteins in the diet. In contrast, this contribution is as low as 20% in Africa, the Middle East, and the Far East.

In addition to the proteins in the diet, substantial amounts of endogenous proteins enter the gastrointestinal tract and are digested and assimilated just like the dietary proteins. These endogenous proteins arise partly from saliva and gastric, biliary, pancreatic, and intestinal secretions, and partly from desquamated epithelial cells of the gastrointestinal tract. The secretions account for about 20–30 g of endogenous proteins per day, and desquamated cells account for an additional 30 g per day.

Digestion of proteins

The digestion of proteins occurs in two phases, defined by the site of digestion along the gastrointestinal tract: a gastric phase and an intestinal phase. The overall scheme of protein digestion in the gastrointestinal tract is depicted in Fig. 19.1.

Gastric phase

Protein digestion begins in the lumen of the stomach with the action of proteases elaborated by the chief cells. These enzymes, called pepsins, belong to the category of acidic proteases that are active at acid pH. The carboxyl group of aspartate, an anionic amino acid, at the catalytic site of these enzymes plays an obligatory role in the enzymatic activity. The enzymes are secreted as inactive precursors into the gastric lumen (i.e., pepsinogens), where they are autocatalytically activated to pepsins under acidic conditions (Fig. 19.2). Pepsinogens possess an amino-terminal region that is positively charged because of the high content of cationic amino acids. At neutral or alkaline pH, this region is folded in such a way that it masks the catalytic site. This inactive state is stabilized by electrostatic interaction between the cationic amino-terminal region and the acidic amino acids present at the catalytic site in pepsinogens, which are ionized at neutral or alkaline pH. In the acidic environment of the gastric lumen, these acidic amino acids are protonated, so that electrostatic interaction is destabilized, uncovering the catalytic site. The unmasked active site removes the amino-terminal region, consisting of about 40 amino acids, from pepsinogens by autocatalytic digestion to generate the active forms of the enzymes, the pepsins. Pepsins are endoproteases that hydrolyze internal peptide bonds in proteins and liberate large peptide fragments. Pepsins act preferentially on peptide bonds formed by the aromatic amino acids phenylalanine and tyrosine, as well as by the branched-chain amino acid leucine (Fig. 19.3). The action of these enzymes on proteins in the

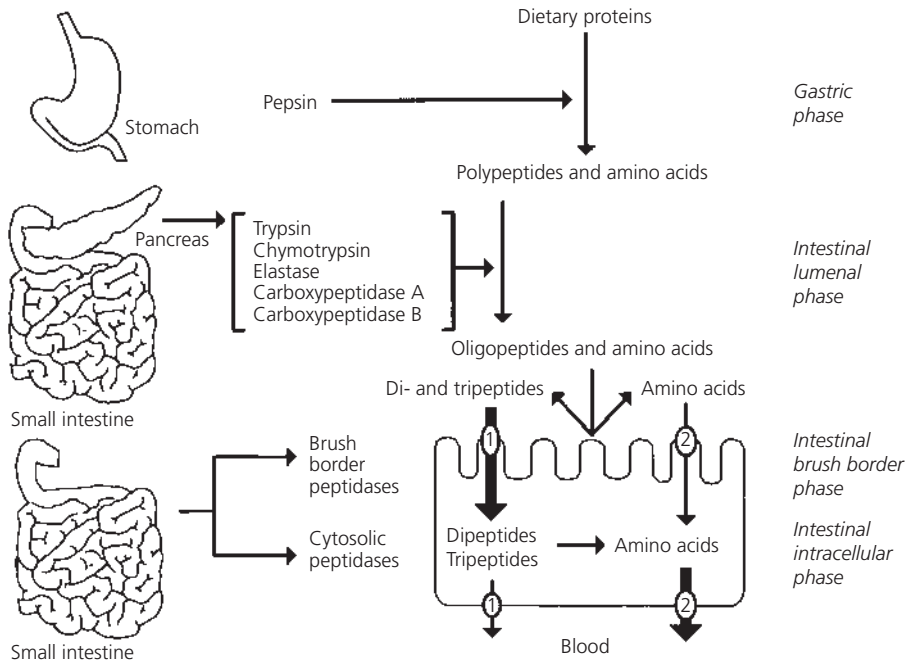

Figure 19.1 Digestion of dietary proteins and absorption of digestion products in the gastrointestinal tract. 1, Transport system for dipeptides and tripeptides; 2, transport systems for free amino acids.

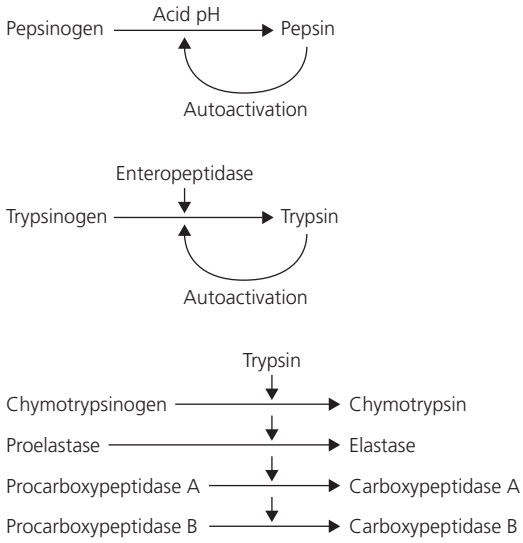

Figure 19.2 Activation of gastric and pancreatic zymogens.

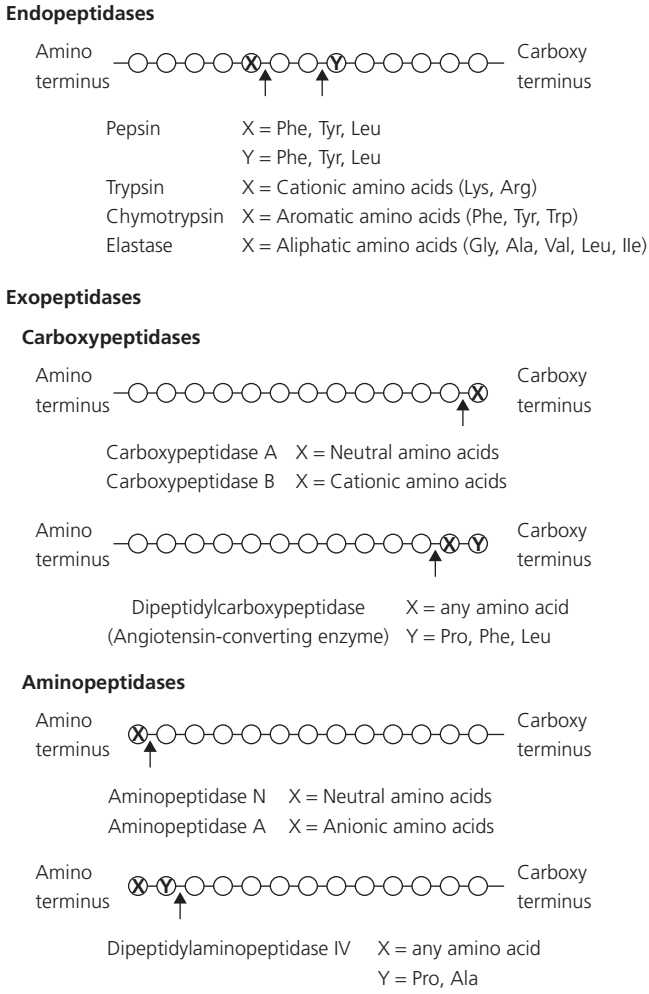

Figure 19.3 Substrate specificity of proteases and peptidases involved in the digestion of proteins in the gastrointestinal tract.

gastric lumen results in a mixture of large polypeptides and smaller oligopeptides.

Gastric juice contains HCl, secreted by the parietal cells, and thus has a pH of less than 2. The acid pH aids the digestive action of pepsins in three ways: (1) proteins are denatured, rendering them more susceptible to proteolytic attack; (2) pepsinogens are converted autocatalytically to active pepsins; and (3) the catalytic mechanism mediated by the carboxyl group of the aspartate residue in the active site of pepsins is effective. Pepsins are not active at a pH greater than 4. Therefore, the digestive action of pepsins on proteins is restricted to the stomach. The acidic contents of the stomach are neutralized by the pancreatic and intestinal secretions in the duodenum, leading to the inactivation of pepsins.

The products of protein digestion by pepsins in the stomach lumen may play an essential role in modulating gastric functions, such as acid and pepsinogen secretion and gastric emptying. These products may also act as stimulants for the release of cholecystokinin in the duodenum, thus influencing pancreatic secretion. Despite the apparent functional consequences of the action of pepsins on proteins, the gastric phase is not essential for overall protein digestion inasmuch as protein digestion and assimilation are not significantly impaired by partial or total gastrectomy.

Intestinal phase

The intestinal phase of protein digestion consists of three stages, defined by the exact location of digestion in the intestine: the lumenal phase, the brush border phase, and the intracellular phase (see Fig. 19.1). The lumenal and brush border phases occur before the protein digestion products are absorbed into enterocytes across the brush border membrane. In the intracellular phase, the absorbed peptides are digested inside enterocytes. Protein digestion comes to an end at this stage. The final products of digestion leave the enterocytes across the basolateral membrane and enter the portal circulation.

Lumenal phase

The lumenal phase of protein digestion in the small intestine is mediated by pancreatic proteases and peptidases. This phase begins in the duodenum, where the pancreatic juice enters the small intestine. The exocrine pancreas produces three endoproteases and two exopeptidases, and each of these enzymes is synthesized and secreted as an inactive precursor (i.e., a zymogen). The inactive forms of the three endoproteases are trypsinogen, chymotrypsinogen, and proelastase. The inactive forms of the two exopeptidases are procarboxypeptidase A and procarboxypeptidase B. Activation of these inactive zymogens is initiated in the duodenum by enteropeptidase (formerly known as enterokinase), an enzyme associated with the brush border membrane in the duodenum. Enteropeptidase is an integral membrane protein and consists of a heterodimer linked with disulfide

bonds. The heavy chain is anchored to the brush border membrane, and the light chain contains the catalytic site. This enzyme is synthesized by enterocytes, initially as a single polypeptide precursor, which is then processed to the heterodimeric form [1]. The only known substrate for enteropeptidase is trypsinogen. The action of enteropeptidase on trypsinogen results in the proteolytic removal of a hexapeptide from the amino terminus of trypsinogen, generating trypsin, the catalytically active form. This is the key step in the activation of pancreatic proteases because, thus generated, trypsin can activate trypsinogen autocatalytically and can also activate the remaining pancreatic zymogens (see Fig. 19.2). The action of trypsin leads to the conversion of chymotrypsinogen to chymotrypsin, proelastase to elastase, procarboxypeptidase A to carboxypeptidase A, and procarboxypeptidase B to carboxypeptidase B.

Trypsin, chymotrypsin, and elastase are endoproteases capable of hydrolyzing the internal peptide bonds in proteins and polypeptides. The substrate specificities of these enzymes are given in Figure 19.3. Trypsin hydrolyzes peptide bonds formed by the carboxyl group of cationic (i.e., basic) amino acids (lysine and arginine); chymotrypsin hydrolyzes peptide bonds formed by the carboxyl group of aromatic amino acids (phenylalanine, tyrosine, and tryptophan); and elastase hydrolyzes peptide bonds formed by the carboxyl group of aliphatic amino acids (glycine, alanine, valine, leucine, and isoleucine). Carboxypeptidase A and carboxypeptidase B are exopeptidases, capable of hydrolyzing the terminal peptide bonds on the carboxyl terminal of polypeptides. Carboxypeptidase A cleaves carboxyl-terminal peptide bonds if the amino acid at the terminus is a neutral amino acid (aromatic or aliphatic), and carboxypeptidase B cleaves carboxyl-terminal peptide bonds if the amino acid at the terminal is a cationic amino acid (lysine or arginine) (see Fig. 19.3). These two enzymes thus release free amino acids from polypeptides. Pancreatic endoproteases and exopeptidases function in a complementary manner to carry out the efficient digestion of proteins. Chymotrypsin and elastase generate oligopeptides that contain neutral amino acids at their carboxyl termini, and these peptides are subsequently acted upon by carboxypeptidase A. Trypsin generates oligopeptides that contain cationic amino acids at their carboxyl termini, and these peptides are subsequently acted upon by carboxypeptidase B. The action of these pancreatic enzymes on proteins in the intestinal lumen produces a mixture of oligopeptides and free amino acids, of which oligopeptides constitute a major fraction (60%–70%) of the total amino nitrogen.

Brush border phase

The amino acids generated by the concerted action of pancreatic enzymes on proteins are absorbed as such into enterocytes across the brush border membrane. In contrast, before absorption, the oligopeptides undergo further hydrolysis by the action of a battery of peptidases associated with the brush border membrane, resulting in a mixture of free amino acids, dipeptides, and tripeptides.

The intestinal brush border membrane exhibits a particularly high level of aminopeptidase activity. Aminopeptidases are exopeptidases that are capable of hydrolyzing the terminal peptide bonds on the amino termini of oligopeptides. This provides functional complementation to the carboxypeptidases present in the pancreatic juice. In addition to several aminopeptidases, the intestinal brush border membrane also contains endopeptidase and dipeptidase activities. The two major aminopeptidases associated with the intestinal brush border membrane are aminopeptidase N and aminopeptidase A. The substrate specificity of these two enzymes is given in Figure 19.3. Aminopeptidase N hydrolyzes the aminoterminal peptide bond of an oligopeptide if the amino acid at the terminus is a neutral amino acid. Aminopeptidase A, in contrast, hydrolyzes the aminoterminal peptide bond of an oligopeptide if the amino acid at the terminus is an anionic amino acid. In addition to these enzymes, two other peptidases are associated with the intestinal brush border membrane. Dipeptidylcarboxypeptidase is an exopeptidase (also called angiotensin-converting enzyme) that hydrolyzes the peptide bond adjacent to the carboxyl-terminal peptide bond. Dipeptidylaminopeptidase IV is also an exopeptidase, but it hydrolyzes the peptide bond adjacent to the amino-terminal peptide bond. Thus, unlike aminopeptidase N and aminopeptidase A, which release free amino acids from oligopeptides, dipeptidylcarboxypeptidase and dipeptidylaminopeptidase IV release dipeptides from oligopeptides. The substrate specificities of these two enzymes are such that the dipeptides released are generally of the X-proline type, where X represents any amino acid (see Fig. 19.3). Animal studies have established unequivocally the essential role of dipeptidylcarboxypeptidase and dipeptidylaminopeptidase IV in the intestinal assimilation of oligopeptides [2–5].

Intracellular phase

The end products of protein digestion by gastric and pancreatic proteases and by peptidases associated with the intestinal brush border membrane comprise a mixture of free amino acids and small peptides. The peptides consist primarily of 2–6 amino acids. Of the total amino acid content in the intestinal lumen, the peptide-bound amino acids represent a predominant fraction. The absorption of free amino acids into enterocytes across the brush border membrane is mediated by a number of distinct amino acid transport systems. Peptides consisting of two or three amino acids are absorbed intact across the brush border membrane by a specific peptide transport system. Once inside the enterocyte, these peptides are hydrolyzed to free amino acids in the cytoplasm by various intracellular peptidases. The peptidases exhibit a preference for dipeptide and tripeptide substrates. The similarity between the substrate specificities of the intracellular peptidases and the peptide transport system is of functional

importance because the transport system makes the peptide substrates available for the intracellular peptidases by importing them across the brush border membrane from the intestinal lumen. The intracellular peptidases that have been characterized in detail include an aminotripeptidase, which releases the amino acid present at the amino terminus of the tripeptide, and several dipeptidases, which show differential substrate specificities toward dipeptides containing specific amino acids. Among the dipeptidases, iminodipeptidase (also called prolidase) is of special interest because of its restricted specificity toward dipeptides of the X-proline or X-hydroxyproline type. Peptide bonds formed by the imino acids proline and hydroxyproline in proteins are generally resistant to hydrolysis by proteases and peptidases involved in the digestion of proteins in the intestinal lumen. Consequently, in the intestinal lumen the imino acids exist to a large extent as iminodipeptides, which are transported intact into the enterocyte by the peptide transport system. These dipeptides are then efficiently cleaved by the intracellular prolidase.

Amino acids, which are either transported as such into the cell in the free form or generated inside the cell by the action of intracellular peptidases, exit the cell across the basolateral membrane by way of specific amino acid transport systems and then enter the portal circulation.

Absorption of protein digestion products

Peptide absorption

The intestinal brush border membrane possesses a specific transport system that accepts dipeptides and tripeptides as substrates. Free amino acids are not recognized by this system. The most interesting feature of this transport process is that it uses a transmembrane electrochemical H^+ gradient rather than a transmembrane electrochemical Na^+ gradient as the driving force [6–8]. The physiological significance of this feature is obvious because there is evidence for the existence of an acid pH microclimate on the lumenal surface of the intestinal brush border membrane that creates a H^+ gradient across the brush border membrane in vivo. This acid pH microclimate is generated and maintained by the combined action of the Na^+/H^+ exchanger in the brush border membrane and the Na^+,K^+-ATPase in the basolateral membrane of the enterocyte (Fig. 19.4). Another interesting and functionally important feature of the intestinal peptide transport system is its broad substrate specificity. This system accepts as substrates dipeptides and tripeptides consisting of neutral amino acids, anionic amino acids, and cationic amino acids. There is only a single peptide transport system in the intestine that is capable of handling the large number of chemically and structurally diverse di- and tripeptides that are expected to be generated from the digestion of dietary proteins.

The peptide transporter from the small intestine of various animal species, including human, has been cloned [7–10].

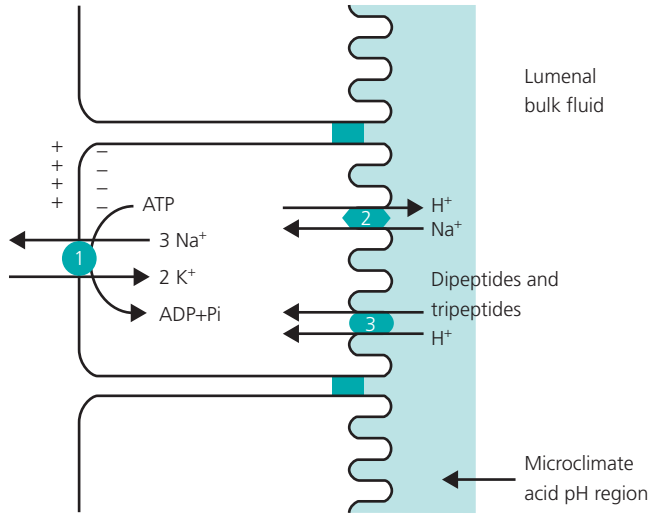

Figure 19.4 Generation of an electrochemical proton gradient across the intestinal brush border membrane. 1, Na^+,K^+-ATPase; 2, Na^+/H^+ exchanger; 3, H^+/peptide cotransporter.

The human protein, known as PEPT1 or PepT1, consists of 708 amino acids, with a predicted core molecular size of 79 kDa. Hydropathy analysis of the primary amino acid sequence shows the presence of 12 transmembrane domains. The gene for this protein is located on human chromosome 13. This transporter is a member of a superfamily of H^+-coupled peptide transporters expressed in animals, yeast, bacteria, and plants. It is expressed in the small intestine (duodenum, jejunum, and ileum), but not in the esophagus and stomach. It is also expressed in the colon, but at much lower levels than are found in the small intestine [11,12]. In the small intestine, the expression is restricted to the absorptive epithelium. Studies of cloned intestinal peptide transporters have established that the transporter recognizes a variety of neutral, anionic, and cationic dipeptides as substrates [13], explaining its broad substrate specificity. The mechanism of the transport process is a simultaneous translocation of H^+ and peptide substrate. The transport process is electrogenic, resulting in a net transfer of a positive charge per transport cycle into the cell, irrespective of the net charge of the transported peptide substrate.

Amino acid absorption

The absorption of free amino acids from the intestinal lumen into enterocytes is mediated by amino acid transport systems expressed in the intestinal brush border membrane [14,15]. Several distinct amino acid transport systems have been functionally characterized in the intestinal brush border membrane, and these transport systems exhibit significant overlap in substrate specificity [16]. It is interesting to note the differences between the processes of peptide absorption and amino acid absorption in terms of the number of physiologically occurring amino acids and peptides and the number

of transport systems that are involved in their absorption. About 20 different amino acids constitute dietary proteins. Absorption of these amino acids in the free form is mediated not by a single transport system but by several distinct transport systems. In contrast, the theoretically possible number of structurally different dipeptides and tripeptides arising from dietary proteins during the digestive process is several times greater than the number of structurally distinct amino acids (400 dipeptides and 8000 tripeptides). Nevertheless, a single transport system (PEPT1) mediates the absorption of all of these diverse peptides. Another notable difference between the peptide and amino acid absorptive processes is in the energetics. The energy source for the peptide transport process is the electrochemical H^+ gradient; a Na^+ gradient is not directly involved. In contrast, several amino acid transport systems are dependent on the Na^+ gradient as the driving force. Some amino acid transport systems, however, are independent of any ion gradient.

The different amino acid transport systems known to be present in the intestinal brush border membrane, their substrate specificities, and their dependence on Na^+ and other ions are given in Table 19.1. The major transport system responsible for the absorption of neutral amino acids across the intestinal brush border membrane is the Na^+-dependent system B^0. The molecular nature of this transport system has been elucidated [17,18]. The protein responsible for this transport activity is called B^0AT1 and the gene encoding this protein is located on human chromosome 5. Immunolocalization studies have established unequivocally that the expression of B^0AT1 is restricted to the brush border membrane of the absorptive epithelial cells in the small intestine [18,19]. This transporter is defective in Hartnup disease, an inheritable amino acid transport defect specific for neutral amino acids.

System $B^{0,+}$ is also present in the brush border membrane. This unique amino acid transporter mediates the Na^+- and Cl^--coupled electrogenic transport of neutral as well as cationic amino acids across the brush border membrane [20]. The protein mediating this transport activity is known as $ATB^{0,+}$ and the gene coding for the protein is located on human chromosome X [21]. Interestingly, this transporter is expressed predominantly in the ileum and colon, not in the proximal part of the small intestine [22–24].

A separate transport system that is specific for anionic amino acids exists in the intestinal brush border membrane. It is known as X^-_{AG} (i.e., the transport system for the negatively charged amino acids aspartate and glutamate) and is Na^+ dependent. The protein responsible for this transport activity has been cloned and functionally characterized [25]. Because this transporter is also expressed in glutamatergic neurons in the brain, where it functions in the neuronal reuptake of glutamate (an excitatory amino acid), system X^-_{AG} is currently classified as a member of the excitatory amino acid transporter (EAAT) family. The glutamate transporter expressed in the intestinal brush border membrane is known as EAAT3. This transporter is defective in the inheritable amino acid transport defect known as dicarboxylic aciduria, which is associated with defective absorption of anionic amino acids in the intestine and kidney.

A specific transport system that mediates the Na^+-coupled transport of alanine, serine, cysteine, glutamine, and asparagine has been cloned from the small intestine [26]. This transporter was originally thought to be responsible for the transport activity known as system B^0 in the intestine; therefore, it was named ATB^0. However, subsequent studies have shown that this transporter functions as an obligatory amino acid exchanger, a characteristic not found with system B^0. Accordingly, this transporter is currently identified as ASCT2 (i.e., the second isoform of the transporter responsible for the transport activity known as ASC, specific for the amino acids alanine, serine, and cysteine) rather than ATB^0. The ASCT2 protein is localized to the brush border

Table 19.1 Amino acid transport systems in the intestinal brush border membrane

Transport system	Molecular identity	HUGO nomenclature	Substrates	Ion dependence	Genetic disease
B^0	B^0AT1	*SLC6A19*	Neutral amino acids	Na^+	Hartnup disease
$B^{0,+}$	$ATB^{0,+}$	*SLC6A14*	Neutral amino acids, cationic amino acids	Na^+, Cl^-	
X^-_{AG}	EAAC1/EAAT3	*SLC1A1*	Anionic amino acids	Na^+, K^+, H^+	Dicarboxylic aciduria
ASC	ASCT2/ATB^0	*SLC1A5*	Neutral amino acids (Ala, Ser, Cys, Gln, Asn)	Na^+	
$b^{0,+}$	$b^{0,+}$AT–rBAT (heterodimer)	*SLC7A9–SLC3A1*	Neutral amino acids, cationic amino acids, cystine	None	Cystinuria
IMINO	SIT1/IMINO	*SLC6A20*	Imino acids	Na^+, Cl^-	
PAT	PAT1	*SLC36A1*	Small neutral amino acids	H^+	
β	TauT	*SLC6A6*	β-Amino acids (taurine, β-alanine)	Na^+, Cl^-	

HUGO, Human Genome Organization; SLC, solute-linked carrier.

membrane in the small intestine and hence may play an essential role in the entry of glutamine into enterocytes from the lumen [27].

Another transport system in the intestinal brush border membrane recognizes neutral and cationic amino acids as high-affinity substrates. This system, $b^{0,+}$, is Na^+ independent and, interestingly, it also recognizes the disulfide amino acid cystine as a substrate. System $b^{0,+}$ is a heterodimer consisting of a light chain (known as $b^{0,+}$AT) and a heavy chain (known as rBAT) [28–30]. The light chain possesses 12 putative transmembrane domains and exhibits a membrane topology typical of most transport proteins. The heavy chain is responsible for directing the recruitment of the light chain specifically to the enterocyte brush border membrane. The genes coding for the heavy chain and the light chain are located on human chromosomes 2 and 19 respectively. This transporter is defective in cystinuria, an inheritable amino acid transport defect specific for cationic amino acids.

Additional amino acid transport systems known to be expressed in the intestinal brush border membrane include IMINO, PAT, and β. System IMINO is a Na^+-dependent transporter with specificity toward the imino acids proline and hydroxyproline. The protein responsible for this transport activity has been cloned recently [31,32]. It is identified as SIT1 (Na^+-coupled imino acid transporter 1) or IMINO. PAT is a transport system for small aliphatic amino acids, and its transport function is coupled to a transmembrane electrochemical H^+ gradient rather than to a transmembrane Na^+ gradient. The protein responsible for this transport activity has been identified at the molecular level. It is known as PAT1 (proton-coupled amino acid transporter 1) [33]. Taurine is a nonprotein amino acid that has its own specific transport system in the small intestine for active absorption, coupled to Na^+ and Cl^- gradients [34]. The protein mediating this transport activity is known as TauT [35].

Fate of absorbed protein digestion products

At the end of the intracellular phase of protein digestion within the enterocyte, after the absorption of small peptides and free amino acids from the intestinal lumen across the brush border membrane and subsequent hydrolysis of the absorbed peptides inside the cells, free amino acids constitute the predominant form of the protein digestion products in the enterocyte cytoplasm. It is probable, however, that a small but significant portion of the protein digestion products may exist in the form of small peptides that are absorbed by way of the brush border membrane peptide transporter but are resistant to hydrolysis by intracellular peptidases. There is some evidence for the presence of a peptide transport system in the basolateral membrane of intestinal epithelial cells [36]. However, it is not PEPT1, and its molecular identity remains unknown. Free amino acids in the cytoplasm of the enterocyte enter into various metabolic pathways, such as degradation, conversion into other amino acids, and incorporation into cellular proteins; they also enter the portal circulation by way of specific amino acid transport systems in the basolateral membrane. Glutamine, glutamate, aspartate, and arginine are preferentially used by the enterocyte as metabolic fuel.

The intestinal basolateral membrane possesses a set of amino acid transport systems that are different to those in the brush border membrane (Table 19.2) [14,15]. The amino acid transport systems in the basolateral membrane function to export amino acids from the enterocyte into the portal circulation during periods of feeding. They also participate in the import of amino acids from the portal circulation into the enterocyte for cellular metabolism when amino acids are not available from the intestinal lumen, such as between meals. Of the amino acid transport systems present in the basolateral membrane, systems y^+L and A are of particular importance. System y^+L is an amino acid exchanger that mediates

Table 19.2 Amino acid transport systems in the intestinal basolateral membrane

Transport system	Molecular identity	HUGO nomenclature	Substrates	Ion dependence	Genetic disease
y^+L	y^+LAT1–4F2hc (heterodimer)	*SLC7A7–SLC3A2*	Neutral amino acids	Na^+	Lysinuric protein intolerance
			Cationic amino acids	None	
	y^+LAT2–4F2hc (heterodimer)	*SLC7A6–SLC3A2*	Neutral amino acids	Na^+	
			Cationic amino acids	None	
A	ATA2/SNAT2	*SLC38A2*	Neutral amino acids	Na^+	
GLY	GLYT1	*SLC6A9*	Glycine	Na^+, Cl^-	
y^+	CAT1	*SLC7A1*	Cationic amino acids	None	
L	LAT2–4F2hc (heterodimer)	*SLC7A8–SLC3A2*	Neutral amino acids	None	

HUGO, Human Genome Organization; SLC, solute-linked carrier.

the Na^+-independent efflux of cationic amino acids from intestinal cells into the blood coupled to the Na^+-dependent influx of neutral amino acids from the blood into intestinal cells [28–30]. There are two isoforms of this transport system and both are heterodimers consisting of a common heavy chain, known as 4F2hc, and an isoform-specific light chain, known as y^+LAT1 and y^+LAT2. Mutations in the gene coding for y^+LAT1 are responsible for the inheritable amino acid transport defect known as lysinuric protein intolerance. System A is a Na^+-coupled transport system for neutral amino acids, including glutamine, that plays a role in the entry of amino acids from the blood into intestinal cells for use in cellular metabolism. System A consists of at least three subtypes, Na^+-coupled neutral amino acid transporter 1 (SNAT1), SNAT2, and SNAT4 [37]. Of these three isoforms, SNAT2 is expressed in the small intestine. Other amino acid transport systems present in the basolateral membrane include GlyT1 (a Na^+- and Cl^--coupled transporter for glycine), CAT1 (a Na^+-independent transporter for cationic amino acids), and system L (a heterodimer consisting of the heavy chain 4F2hc and the light chain LAT2), which functions as a Na^+-independent obligatory amino acid exchanger [14,15].

Physiological and clinical significance

Compelling evidence exists for the absorption of protein digestion products predominantly in the form of small peptides rather than free amino acids. Therefore, the physiological importance of the intestinal peptide transport system in the maintenance of protein nutrition is clearly evident. In addition, the intestinal peptide transporter has been shown to interact with several pharmacologically relevant drugs that possess peptide-like chemical structures. These drugs are efficiently absorbed from the intestinal lumen after oral administration by way of the peptide transporter. Consequently, the peptide transporter in the intestine is an important determinant of the bioavailability of orally active peptidomimetic drugs [38–40]. Examples of peptidomimetic drugs that are absorbed in the intestine by way of the peptide transporter include several β-lactam antibiotics (e.g., cephalexin [cefalexin], cefadroxil, cephradine [cefradine], and ceftibuten), angiotensin-converting enzyme inhibitors (captopril and lisinopril), the antitumor agent bestatin, and inhibitors of renin and thrombin. The transporter is also responsible for the enhanced oral bioavailability of the prodrugs valacyclovir (valaciclovir) and valganciclovir. The parent drugs acyclovir (aciclovir) and ganciclovir are not recognized as substrates by the intestinal peptide transporter; however, when a valine residue is added to these drugs by way of an ester linkage, the resultant derivatives become substrates for the transporter.

The peptide transport process in the intestine also has the potential for therapeutic applications. Peptide-based formula solutions have many advantages over amino acid-based elemental solutions for enteral nutrition [41]. The efficacy of peptide-based enteral solutions is facilitated by the intestinal peptide absorptive process. The osmolality of these solutions can be kept low while amino acid content and composition are maintained. Furthermore, amino acids are absorbed more efficiently and more evenly from peptides than from an equivalent mixture of free amino acids. Another clinical application of the peptide transport system is the practice of including heat-labile and water-insoluble amino acids such as glutamine, cysteine, and tyrosine in the form of peptides in enteral solutions to promote their absorption.

The amino acid transporter $ATB^{0,+}$ also has the potential for drug delivery [42]. It has broad specificity among the amino acids, accepting the neutral as well as cationic amino acids as substrates. In addition, it can transport a variety of amino acid-based drugs and prodrugs.

Regulation of amino acid and peptide absorption

The capacity of the small intestine to absorb protein digestion products changes significantly under various physiological and pathological conditions. The underlying mechanisms of these changes may be nonspecific, such as alterations in the absorptive surface area, or specific, such as alterations in the number and activity of particular transporters. The transport systems responsible for the absorption of amino acids and peptides are present in the small intestine even before birth in several animal species, including humans. The ability of the small intestine to absorb amino acids and peptides, when expressed per wet weight of intestinal tissue, is maximal at birth and during early development, decreasing thereafter with age. Several hormones have been shown to alter the intestinal amino acid and peptide transport systems. Somatostatin and vasoactive intestinal polypeptide inhibit these transport processes, whereas epidermal growth factor, neurotensin, cholecystokinin, and secretin enhance them. Studies of cultured cells indicate that various intracellular second messengers regulate the intestinal peptide transporter. In Caco-2 cells, a human intestinal cell line, the peptide transporter is stimulated by insulin [43], leptin [44], and growth hormone [45]. The transporter is inhibited by thyroid hormones [46], protein kinase C [47], and cyclic adenosine monophosphate (cAMP) [48]. There is evidence of direct coupling between the peptide transporter and the Na^+/H^+ exchanger by way of the transmembrane H^+ gradient in Caco-2 cells [49]. Therefore, the observed effects of some of these regulators (e.g., protein kinase C and cAMP) on peptide transporter activity are likely mediated indirectly by alterations in the activity of the Na^+/H^+ exchanger.

In animal studies, the expression of the intestinal peptide transporter has also been shown to be modulated by dietary protein content and protein digestion products [50,51]. Even

though the peptide transporter is expressed along the entire small intestine, the diet-induced changes in its expression are specific to certain regions. A high-protein diet increases the steady-state levels of the transporter-specific messenger RNA in the middle and distal regions of the small intestine. The expression of the brush border peptidases dipeptidyl-carboxypeptidase and dipeptidylaminopeptidase IV, which release dipeptides from oligopeptides, is also enhanced by a high-protein diet, again only in the middle and distal regions of the small intestine. This coordinated regulation of the peptide transporter and the peptidases that generate the substrates for the transporter is likely to be of physiological importance. Similarly, the protein digestion products, particularly certain specific dipeptides and amino acids, also enhance the expression of the peptide transporter in the intestine. Up-regulation of the peptide transporter by dipeptides has been demonstrated in Caco-2 cells [52,53].

Although the peptide transporter is expressed at very low levels in the large intestine under normal conditions, it is up-regulated in short bowel syndrome [11], Crohn's disease, and ulcerative colitis [54]. Note that the intestinal peptide transporter can transport the bacterial peptide *N*-formyl-Met-Leu-Phe (a chemotactic peptide) [54,55]. The enhanced expression of the peptide transporter in the large intestine in Crohn's disease and ulcerative colitis may have a pathogenic role in these inflammatory diseases by facilitating the entry of this bacterial peptide into intestinal epithelial cells and consequently affecting local immune function [54–56]. Bacterial and parasitic infections have also been shown to influence the expression and activity of the intestinal peptide transporter [57,58].

Protein–energy malnutrition

Protein–energy malnutrition represents the most common type of malnutrition, seen primarily in developing countries. This condition is classified into two subtypes: kwashiorkor and marasmus. In kwashiorkor, intake of protein is inadequate, but energy intake in the form of carbohydrate and fat is adequate. In marasmus, the intake of both protein and energy is inadequate. The crucial clinical features that differentiate these two subtypes are the degree of body wasting and the presence or absence of edema. Kwashiorkor is characterized by edema and a body weight that is 60%–80% of that expected for the given age. This condition is also associated with fatty liver. Inadequate protein intake in the presence of adequate energy in kwashiorkor results in decreased hepatic synthesis of blood proteins and β-lipoprotein. Muscle wasting does not occur in kwashiorkor, but there is growth impairment because of decreased amino acid supply in the form of dietary proteins. Decreased levels of albumin in the blood cause edema, and decreased levels of β-lipoprotein in the liver cause defective handling of lipids, leading to fatty liver. Children with kwashiorkor may appear healthy because of the edema, but there are profound metabolic disturbances associated with the condition. Marasmus is characterized by the absence of edema and a body weight that is less than 60% of that expected for the given age. Insufficient energy intake in marasmus results in decreased levels of glucose in the blood, which leads to elevated levels of glucogenic hormones such as glucocorticoids. Muscle proteins are degraded to provide glucogenic amino acids for hepatic gluconeogenesis in an attempt to maintain the blood levels of glucose. Degradation of muscle proteins helps to maintain the levels of amino acids in the circulation and the hepatic synthesis of blood proteins. Therefore, edema is absent in marasmus. Both kwashiorkor and marasmus manifest a decreased cell-mediated immune response that reduces the ability to fight infection.

Defects in digestion and absorption of proteins

A number of defects, primary as well as secondary, associated with the impairment of protein digestion and assimilation are known to occur in humans (Table 19.3).

Primary defects

Defects in the digestion of proteins can be caused by genetic diseases involving the expression and activity of any of the proteases or peptidases necessary for the digestive process. Cases of a genetic deficiency of pancreatic trypsinogen have been reported [59,60]. Because trypsin is a key enzyme in the activation of other pancreatic zymogens (e.g., chymotrypsinogen), a deficiency of trypsinogen results in a marked impairment of protein digestion, noticeable in early infancy. Infants with trypsinogen deficiency fail to gain weight and present with hypoproteinemia and edema. These infants improve dramatically if fed protein hydrolysates instead of intact proteins. Several cases of a genetic deficiency of enteropeptidase have been reported [61]. Infants with enteropeptidase deficiency present with symptoms similar to those observed in cases of trypsinogen deficiency, which include failure of growth, hypoproteinemia, and edema. Because enteropeptidase deficiency leads to an impairment in the activation of trypsinogen, and consequently impairments in the activation of other pancreatic proteases, the digestion of proteins is markedly affected in these patients. Treatment with activated pancreatic enzymes results in improvement of protein digestion and assimilation. Genetic defects in enteropeptidase and trypsin cause impairment not only in protein digestion but also in the digestion of fat. This is because trypsin is obligatory for the activation of procolipase to colipase, a protein necessary for the hydrolytic action of pancreatic lipase on dietary triglycerides after emulsification with bile acids. Similarly, because pancreatic

Table 19.3 Primary and secondary defects associated with an impairment of protein digestion and assimilation

Disease	Defective protein and metabolic abnormality	Clinical features
Primary defects		
Digestive process		
Trypsin deficiency	Genetic defects in trypsinogen; impairment of activation of pancreatic zymogens involved in protein digestion	Impaired growth and weight gain, hypoproteinemia, edema
Enteropeptidase deficiency	Genetic defects in enteropeptidase; impairment of trypsinogen activation leading to impairment of activation of other pancreatic zymogens involved in protein digestion	Impaired growth and weight gain, hypoproteinemia, edema
Prolidase deficiency	Genetic defects in prolidase; impairment in the hydrolysis of proline-containing peptides	Impaired collagen turnover, skin abnormalities, increased urinary excretion of proline-containing peptides
Absorptive process		
Cystinuria	Genetic defects in $b^{0,+}$, a heterodimeric transporter ($b^{0,+}$AT–rBAT) for cystine and cationic amino acids; disease-causing mutations are found in either of the subunits ($b^{0,+}$AT and rBAT)	Increased urinary excretion of cystine and cationic amino acids, nephropathy secondary to cystine calculi
Hartnup disease	Genetic defects in B^0AT1, a transporter for neutral amino acids; impairment in the endogenous synthesis of niacin	Increased urinary excretion of neutral amino acids, pellagra, cerebellar ataxia, mental retardation
Lysinuric protein intolerance	Genetic defects in a heterodimeric transporter (y^+LAT1–4F2hc) for neutral and cationic amino acids; disease-causing mutations are found only in the y^+LAT1 subunit	Increased urinary excretion of cationic amino acids, protein intolerance, hyperammonemia, diarrhea, vomiting, coma
Secondary defects		
Cystic fibrosis	Genetic defects in a cAMP-activated Cl^- channel in exocrine tissues, including pancreas; impairment of exocrine tissue function	Impaired digestion and assimilation of dietary proteins and fat secondary to decreased exocrine pancreatic function, impaired lung function, and increased lung infections
Celiac sprue	Defect not known; presence of autoantibodies to tissue transglutaminase	Intolerance to dietary gliadin, enteropathy, nutrient malabsorption, and diarrhea on gliadin ingestion
Dermatitis herpetiformis	Defect not known; presence of autoantibodies to epidermal transglutaminase	Intolerance to dietary gliadin, enteropathy, dermatitis
Tropical sprue	Defect not known	Impairment of digestive and absorptive function of intestine, malabsorption of nutrients, diarrhea

proteases play an essential role in the intestinal absorption of vitamin B-12, genetic defects in enteropeptidase and trypsin may also interfere with the absorption of this vitamin.

With the exception of enteropeptidase deficiency, no other documented inheritable defects in peptidases associated with the intestinal brush border membrane are known in humans. However, a mutant rat strain is available that exhibits a genetic deficiency of dipeptidylaminopeptidase IV [5]. These mutant rats are apparently normal with no alteration in growth rate if fed a diet with protein constituents with a modest proline content [5]. However, if the dietary protein source is changed to gliadin, a proline-rich protein, the enzyme-deficient mutant rats experience a significant weight loss, whereas wild-type rats do not. These studies show that, as a result of the unique substrate specificity of dipeptidylaminopeptidase IV, a functional role for this enzyme becomes evident under physiological conditions only if the diet contains proline-rich proteins. These observations also point to the physiological advantage of the presence of multiple peptidases in the intestinal brush border membrane with significant overlapping substrate specificities.

An inheritable deficiency of the intracellular dipeptidase prolidase is known in humans [62]. In healthy persons, this enzyme is expressed not only in the intestine but also in several other tissues, such as the kidney; its functions are multiple, including an important role in collagen turnover. It is therefore not surprising that manifestations of prolidase deficiency are pleiotropic. However, no information is available on the intestinal assimilation of proteins in these patients. It is likely that prolidase deficiency does not lead to detectable

alterations in the intestinal assimilation of proteins under normal dietary conditions, as seen in the mutant rat strain lacking dipeptidylaminopeptidase IV.

Defects in the absorption of protein digestion products can be caused by diseases involving the expression and activity of amino acid and peptide transport systems in the intestine. Several inheritable amino acid transport defects have been identified in humans. Cystinuria is an autosomal recessive disorder characterized by the increased excretion of cationic amino acids (lysine and arginine) and cystine [63]. The disease is associated with a defect in the renal reabsorption of these amino acids. A similar defect also occurs in the small intestine, leading to impaired absorption of cationic amino acids and cystine from the intestinal lumen. This suggests that an identical transport system is involved in the absorption of these amino acids in the kidney and the intestine. The primary clinical problem associated with the disease is nephropathy resulting from renal stones enriched in cystine. This disulfide derivative is poorly soluble in water (300 mg/L). In cystinuria, the concentration of cystine is greater than this solubility level in the renal tubule because of defective reabsorption, and this results in the formation of cystine stones. This disease is caused by mutations in either the light chain ($b^{0,+}$AT) or the heavy chain (rBAT) of the heterodimeric transporter $b^{0,+}$ [64]. Thus, two genes, one on chromosome 2 (heavy chain) and the other on chromosome 19 (light chain), are associated with cystinuria. Because system $b^{0,+}$ is present predominantly in the brush border membrane of the intestinal and renal tubular epithelial cells, the transport defect in cystinuria is restricted to this membrane. Therapeutic strategies are directed toward facilitating cystine excretion in the urine by increasing urine volume, alkalinizing the urine to increase cystine solubility, and using penicillamine, which reacts with cystine to form a more soluble mixed disulfide.

Hartnup disease is also an autosomal recessive disorder of amino acid transport, but this condition is associated with impaired absorption of neutral amino acids (Ala, Ser, Cys, Thr, Gln, Asn, Val, Ile, Leu, Met, Phe, Tyr, and Trp) in the kidney and the intestine [65]. Consequently, this disease is associated with the hyperexcretion of several neutral amino acids in urine. The symptoms of the disease, which include photosensitive skin rash, ataxia, and neurological complications, are similar to those of pellagra (i.e., niacin deficiency). These symptoms occur despite an apparently normal dietary intake of niacin but disappear in response to niacin therapy. In humans, endogenous synthesis of niacin from the neutral amino acid tryptophan contributes significantly to normal niacin requirements. In Hartnup disease, the endogenous synthesis of niacin is most likely impaired because of defective intestinal absorption of tryptophan coupled with defective renal reclamation of tryptophan. Because people in industrialized nations generally consume a nutritious diet rich in protein, the disease is relatively benign, except for symptoms of niacin deficiency, cerebellar ataxia, and occasional psychological problems. However, in developing or underdeveloped nations, where suboptimal protein intake is common, the disease is characterized by more severe clinical symptoms, including mental retardation. The amino acid transport system B^0, expressed in the intestinal and renal brush border membrane, is defective in this disease [66]. The protein responsible for this transport system is B^0AT1, and loss-of-function mutations in the gene encoding this protein, located on human chromosome 5, have been demonstrated in patients with Hartnup disease [17,18].

Interestingly, persons with cystinuria or Hartnup disease usually do not show obvious symptoms of protein malnutrition, despite the clearly established defects in the intestinal absorption of specific amino acids. This was puzzling until it was discovered that intestinal absorption of the affected amino acids (i.e., cationic amino acids in cystinuria and neutral amino acids in Hartnup disease) is normal if the amino acids are given in the form of dipeptides [67–70]. Because protein digestion products are absorbed across the intestinal brush border membrane mainly in the form of small peptides, these individuals obtain significant amounts of the affected amino acids through the peptide transport mechanism despite the amino acid transport defect in this membrane. These observations are of historical importance in peptide transport research because they provided early indisputable evidence that the transport mechanisms available for free amino acids and peptides in the intestine are different [71].

Lysinuric protein intolerance is an autosomal recessive disorder caused by impaired absorption of the cationic amino acids lysine and arginine in the intestine and the kidney [72]. The absorption of ornithine, also a cationic amino acid, is impaired as well. Persons with this disease show decreased plasma levels and increased urinary excretion of these three amino acids. Deficiency of arginine and lysine, which are essential amino acids, causes impairment of protein synthesis, resulting in growth retardation and neurological complications. In addition, because arginine and ornithine are the amino acids involved in the urea cycle and hence in the elimination of ammonia, patients with this disease exhibit decreased urea cycle activity and consequently hyperammonemia. Dietary intake of protein induces diarrhea, vomiting, and, in extreme cases, coma. Treatment strategies, which are related to the prevention of hyperammonemia, include dietary protein restriction and ornithine supplementation. The intestinal and renal handling of cystine is not affected in lysinuric protein intolerance, indicating the difference between the molecular defects associated with this disease and those associated with cystinuria, which also involves the renal and intestinal absorption of cationic amino acids. The transport defect in lysinuric protein intolerance is restricted to the basolateral membrane in intestinal and renal epithelial cells [73]. Transport of cationic amino acids across the brush border membrane of these cells is not affected. Linkage analysis [74] assigned the gene for lysinuric protein intolerance

to the long arm of chromosome 14. The defect in this disease occurs in one of the isoforms of the amino acid transport system y^+L [64]. The transporter defective in this disease is a heterodimer consisting of a heavy chain (4F2hc) and a light chain (y^+LAT1). Loss-of-function mutations in the gene coding for the light chain y^+LAT1 have been demonstrated in patients with this disease [75,76]. Even though the heavy chain is obligatory for the proper function of the transport system under in vivo conditions, no mutations have been detected in the gene coding for this subunit (4F2hc) in any of the patients with lysinuric protein intolerance. This is in contrast to cystinuria, also a disease associated with a defective heterodimeric amino acid transporter, in which mutations in either the light chain or the heavy chain lead to the disease.

There are no documented cases of genetic defects in the gene coding for the intestinal peptide transporter PEPT1 in humans. Studies of genetic polymorphisms in the gene encoding this protein have reported a very low level of genetic variability [77,78]. Most of the polymorphisms in the gene are found either in intronic or noncoding regions or in exonic coding regions that do not cause any change in amino acids. Nonsynonymous polymorphisms in the coding region do occur, but most of them have no significant effect on the transport function of the protein. Two exceptions are the nonsynonymous polymorphisms P586L and F28Y, which exert a significant effect on the function of the transporter and are demonstrable in heterologous expression systems. The P586L mutant exhibits defective plasma membrane expression [77] and the F28Y mutant exhibits reduced substrate affinity [78]. The physiological and pharmacological consequences of these mutations for the intestinal absorption of protein digestion products and peptidomimetic drugs have not been investigated.

Secondary defects

Impairment of protein digestion and absorption occurs as a secondary defect in several diseases, including cystic fibrosis, celiac sprue, dermatitis herpetiformis, and tropical sprue. In cystic fibrosis, the primary defect occurs in the CFTR (cystic fibrosis transmembrane conductance regulator) protein, a cAMP-regulated Cl^- channel. The defective function of this protein leads to complications involving diverse organs, such as the lung, pancreas, gastrointestinal tract, and sweat glands. In the exocrine pancreas, the absence of the Cl^- channel in the apical membrane of pancreatic ductal epithelial cells results in failure to secrete salt and water into the duct. This leads to the retention of digestive enzymes in the pancreas and, ultimately, the destruction of pancreatic tissue. Pancreatic insufficiency associated with this disease causes impairment of the digestion and assimilation of protein and fat.

Celiac sprue, or gluten-induced enteropathy, is characterized by an inability to tolerate gluten, a protein found in wheat, barley, and rye. The dietary intake of gluten induces inflammation in the intestine, leading to the blunting and flattening of the villi. Histological findings include villous atrophy, crypt hyperplasia, and intraepithelial lymphocytes. These structural abnormalities reduce the mucosal surface area and result in a deficiency of enterocyte-associated digestive enzymes and transport proteins. This causes a generalized impairment of the digestive and absorptive function of the intestine, including protein malabsorption. The classical symptoms in patients with celiac sprue during active disease include diarrhea, abdominal bloating, weight loss, and anemia due to iron, folic acid, and vitamin B-12 deficiencies. Celiac sprue is an immunologically mediated disorder with a strong genetic component, and immunoglobulin A antibodies to endomysium, a structure of the smooth muscle connective tissue, are specific indicators of the disease. The endomysial autoantigen has been identified as the tissue transglutaminase [79]. Gliadin, which is the major protein component of gluten and the principal causative agent in celiac disease, is a preferred substrate for this enzyme. The exact genetic component that underlies the sensitivity to gluten-induced enteropathy has not been identified. However, if gluten-containing foods are avoided entirely in the diet, patients with celiac sprue recover completely, with the intestinal morphology and structure returning to normal.

Dermatitis herpetiformis is another malabsorption syndrome associated with blunting and flattening of the mucosal villi and consequently impaired digestion and absorption of dietary nutrients. This disease has much in common with celiac disease because both conditions are associated with intestinal sensitivity to gluten [80]. However, patients with dermatitis herpetiformis exhibit unique dermatological symptoms, manifest as pruritic bullous rashes. Intestinal involvement is much milder compared with celiac disease. Patients with dermatitis herpetiformis have immunoglobulin A antibodies against epidermal transglutaminase. As in celiac disease, strict adherence to a gluten-free diet relieves the symptoms of dermatitis herpetiformis. Thus, celiac disease and dermatitis herpetiformis seem to represent two different manifestations of the same disease.

Tropical sprue is a serious gastrointestinal disease, also characterized by a generalized impairment of the digestive and absorptive function of the intestine. A disease of unknown etiology, it occurs among the residents of tropical areas. The gastrointestinal manifestations of tropical sprue are strikingly similar to those observed in celiac sprue.

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D NUTRITION

20 Vitamins and minerals

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Water-soluble vitamins

Folate

Structure, sources, and requirements

Folate in the form of oxidized folylmonoglutamate, or folic acid, contains a pteridine ring linked to *p*-aminobenzoic acid and glutamic acid. Folates appear in the diet and in endogenous storage as folylpolyglutamates with up to six additional glutamates in γ-peptide linkage to folylmonoglutamate. Whereas the term *folic acid* refers to the oxidized monoglutamyl form of the vitamin, reduction in the 5-, 6-, 7- and 8-positions and methylation at the 5-position of the pteridine ring produce 5-methyltetrahydrofolate (5-MTHF), the circulating form of the vitamin. Dietary sources of folate include green leafy vegetables (i.e., foliage), orange juice, grains (in particular, breakfast cereals that are fortified with folic acid), and organ meats such as liver. The Dietary Reference Intake (DRI) for folate from all sources is quantified in dietary folate equivalents (DFEs). This term accounts for the fact that monoglutamyl folic acid that is marketed as a vitamin supplement or as a fortificant in the diet is 1.7 times more available than the natural food folates that occur as folylpolyglutamates in the diet [1]. In calculating DFEs, the amount of supplemental folic acid, as either a grain fortificant or in tablet form, is multiplied by 1.7 and added to the amount of natural food folate present in the mixed diet. The DRI for folate is set at 400 μg of DFE per day for adults, 600 μg/day during pregnancy, and 500 μg/day during lactation. Since 1998, all grain products in the United States have been fortified with folic acid at 140 μg/g to ensure a daily intake equal to or more than the DRI for folate.

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Folate absorption and homeostasis

Folates are absorbed in the duodenum and upper jejunum. During the process of intestinal absorption, dietary folylpolyglutamates are hydrolyzed at the epithelial cell (enterocyte) brush border membrane, followed by active transport of the folylmonoglutamate derivative into the absorbing enterocyte. Studies that followed the movement of labeled compounds across the human jejunal epithelium during jejunal perfusion or when excreted in the urine after oral administration showed the importance of the hydrolytic step, because the absorption of monoglutamyl folic acid was about 85%, compared to about 50% absorption of complex dietary polyglutamyl folates in adult human volunteers [2]. The 85 : 50, or 1.7 : 1, ratio of the absorption of the two different folates provides the basis for the DFE calculation. A study of orally administered, dual-labeled folates largely confirmed the differences in the absorbability of monoglutamyl and polyglutamyl folates in healthy human subjects [3]

Two intestinal brush border proteins – glutamate carboxypeptidase II (GCP2, or folate hydrolase) and the reduced folate carrier – are required, respectively, for the sequential hydrolysis of dietary folylpolyglutamates and the transport of folylmonoglutamate derivatives across the enterocyte brush border and basolateral membranes [4,5]. GCP2 was characterized in pig and human intestine and is a brush border membrane protein with a molecular size of 120 kDa that contains a single 5′ membrane-spanning domain and an extracellular catalytic domain and shares an identical sequence with two other proteins: brain *N*-acetylated α-linked acidic dipeptidase and prostate-specific membrane antigen [6,7]. In its functionally active enzyme form, purified human intestinal GCP2 has an optimal pH of 6.5 and a K_m value of 0.6 μmol/L and is an exopeptidase that releases intermediate products of folylpolyglutamates in a stepwise fashion ending with the folylmonoglutamate derivative [8]. By contrast, the human reduced folate carrier is a 65-kDa brush border membrane protein with 12 membrane-spanning domains that operates at an optimal pH of 5.0 and has a K_m value of 0.5–1.5 μmol/L [9]. Studies that used isolated intestinal brush border membrane vesicles defined a saturable carrier system

for monoglutamyl folates with a preference for reduced and methylated forms [10,11]. As folate is transported through the absorbing enterocyte, pteroylmonoglutamate is reduced and methylated, and 5-MTHF is transported across the intestinal basolateral membrane by the reduced folate carrier to the portal vein and the liver [12].

The uptake of 5-MTHF by hepatocytes involves binding to the liver plasma membrane and carrier-mediated transport into the cell. The binding step is regulated in part by the high-affinity folate receptor, folate-binding protein, a 37-kDa protein that operates at a pH of 6.5–7.5 and has a K_m value in the nanomolar range. Folate-binding protein is attached to the membrane by a glycosylphosphatidylinositol anchor [13]. However, 5-MTHF transport obeys the kinetics of the reduced folate carrier, which also expresses its activity in the liver plasma membrane [5]. Within hepatocytes, uptake of 5-MTHF is followed by conversion to folylpolyglutamates for storage and metabolism. After reconversion to monoglutamyl forms for membrane transport, about 10% of the liver folate pool undergoes biliary secretion as 5-MTHF to an enterohepatic folate circulation, while the remaining nonhepatic folate pool circulates in the blood to other tissues [14]. The urinary excretion of 5-MTHF is regulated by filtration and then efficient reuptake by folate-binding protein and the reduced folate carrier in the renal tubular epithelial cells [15]. About 1% of the total body folate pool is excreted daily in the urine and 0.1% in the feces of nonhuman primates [16].

Folate functions and methionine metabolism

The metabolism of folate is intrinsically tied to methionine metabolism and to the synthesis, nucleotide balance, and methylation of DNA. Figure 20.1 depicts these interactions in the liver. Folates enter the liver either as 5-MTHF from the diet and from intestinal folate metabolism or in the form of supraphysiological folic acid from fortified cereals [17] or as supplemental vitamin preparations [18]. Folic acid generates 5,10-MTHF by way of dihydrofolate and tetrahydrofolate

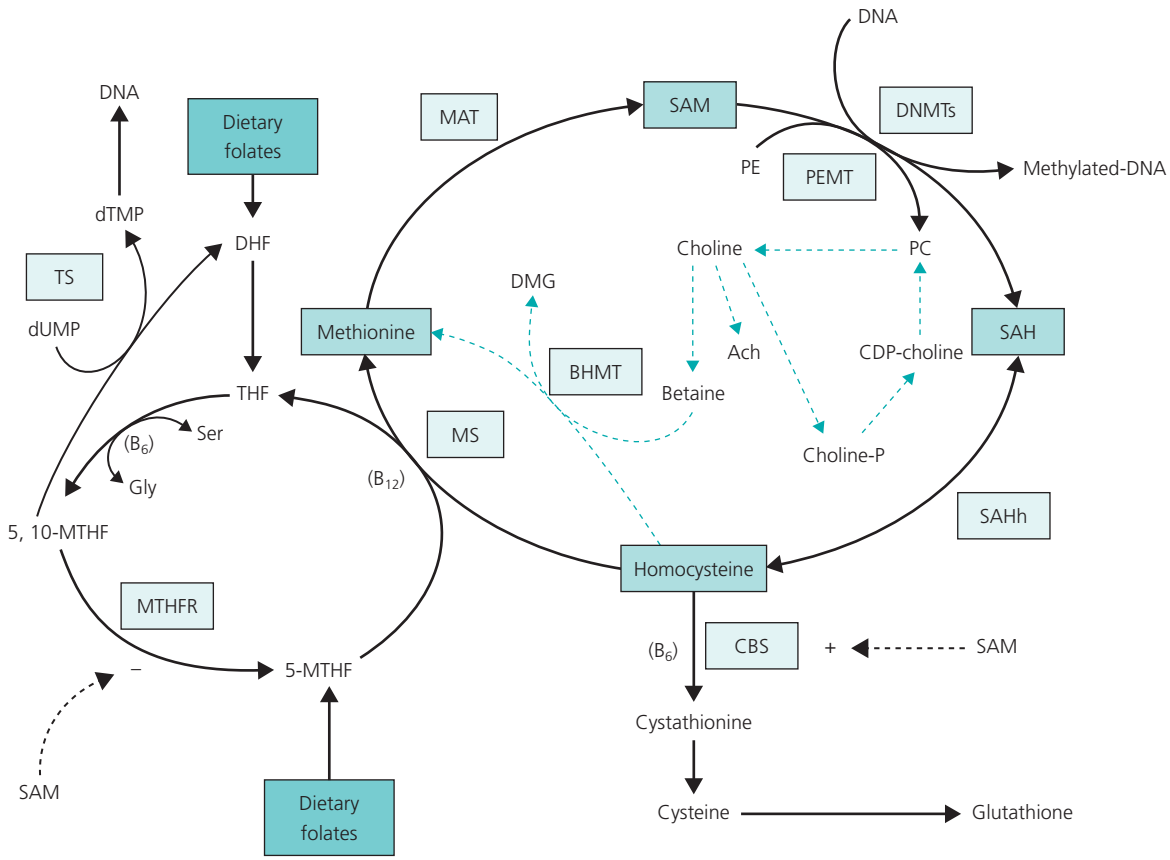

Figure 20.1 Interactions of folate and methionine metabolism. BHMT, betaine homocysteine methyltransferase; CBS, cystathionine β-synthase; CDP-choline, cytidine diphosphorylcholine; choline-P, phosphoryl choline; DHF, dihydrofolate; DNMTs, DNA methyltransferases; dTMP, deoxythymidine monophosphate; dUMP, deoxyuridine monophosphate; GSH, glutathione; MAT, methionine adenosyltransferase; MS, methionine synthase; 5-MTHF, 5-methyltetrahydrofolate; 5,10-MTHF, 5,10-methylenetetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine methyltransferase; SAH, *S*-adenosylhomocysteine; SAM, *S*-adenosylmethionine; SAHh, SAH hydrolase; THF, tetrahydrofolate; TS, thymidylate synthase.

(THF) and their respective reductases. 5,10-MTHF is the substrate for two reactions: those of MTHF reductase (MTHFR) for the production of 5-MTHF, and of thymidylate synthase (TS) for the regeneration of THF and the conversion of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate (dTMP) in the synthesis of DNA. The activity of MTHFR and its regulation by *S*-adenosylmethionine (SAM) are central to DNA synthesis: when MTHFR is less active, as in 677C→T homozygotes or when the level of its inhibitor SAM is increased, more 5,10-MTHF is available as a substrate for TS and the synthesis of dTMP from dUMP for proper DNA nucleotide balance and function. Conversely, when the level of SAM is low as a result of folate deficiency, MTHFR is more active and less 5,10-MTHF is available for TS with resultant nucleotide imbalance and altered DNA synthesis, leading to macrocytosis of nascent red blood cells and increased risk for several cancers. In the form of 5-MTHF, folate plays a central regulatory role in methionine metabolism as the substrate for the methionine synthase (MS) reaction that uses cobalamin as a cofactor and generates endogenous methionine from total homocysteine (tHcy). In the alternate salvage pathway, methionine is generated from tHcy by way of the betaine homocysteine methyltransferase (BHMT) pathway with the substrate betaine, which is a dietary substance and a derivative of choline. Both endogenous and exogenous methionine are converted to SAM by methionine adenosyltransferase (MAT), an enzyme that is produced by two genes: *MAT1A*, which is expressed in the liver, and *MAT2A*, which is expressed in nonhepatic tissues [19]. Through many methylation reactions, including DNA methylation and the synthesis of phosphatidylcholine from phosphatidylethanolamine by way of phosphatidylethanolamine methyltransferase, SAM is metabolized to *S*-adenosylhomocysteine (SAH), which is also generated from tHcy through the reversible SAH hydrolase (SAHH) reaction. Whereas SAM is the universal methyl donor, methylation reactions are inhibited by SAH [20]. SAM also regulates the synthesis of glutathione by stabilizing the cystathionine β-synthase (CBS) reaction and hence the tHcy transsulfuration pathway [21].

Causes of folate deficiency and aberrant folate and methionine metabolism

The principal causes of clinical folate deficiency are inadequate diet, intestinal malabsorption, and altered metabolism as a result of genetic aberrations and toxins such as alcohol.

Dietary deficiency

Since mandatory fortification of the US diet in 1998, the incidence of folate deficiency in the United States has decreased from 22% to 1.7% of the population, while the mean serum folate level of healthy people who do not use vitamin supplements has risen from 4.6 nmol/L to 10.0 nmol/L (from 11 ng/mL to 23 ng/mL) [22]. However, folate deficiency as the result of inadequate diet is common in impoverished populations, in developed countries where the diet does not contain fortified grains, and in susceptible populations including infants, elderly people, and chronic alcoholics whose diets are inadequate. For example, folate deficiency with low serum folate levels was found in more than 80% of chronic alcoholics in the United States in the prefortification era [23], and low levels of red blood cell folates were measured in 35% of chronic alcoholics in Portugal, which does not have a folate fortification policy [24]. The current incidence of folate deficiency in chronic alcoholics living in the United States, who presumably obtain folic acid from fortified diets, is not known. The body stores of folate are sufficient for about 4 months; therefore, before folic acid supplementation of the US food supply, the risk for dietary folate deficiency was high in these susceptible populations.

Malabsorption

Intestinal folate malabsorption and deficiency are associated with diseases and surgical procedures that affect absorbing enterocytes in the duodenum and jejunum, such as celiac disease, severe Crohn's disease, extensive gastric bypass surgery for severe obesity, and extensive small bowel surgery. For example, a Finnish study found that about one-third of adolescents with celiac disease had low serum folate levels, which was double the incidence in control subjects [25]. Sequential intestinal perfusion studies showed malabsorption of both monoglutamyl and polyglutamyl folates in untreated patients with celiac disease, which normalized after treatment with a gluten-free diet [2]. Multivitamin supplementation after gastric bypass surgery has lowered the potential for folate deficiency [26]. Whereas folate deficiency is not considered a risk for patients with inflammatory bowel disease who use mesalamine drugs, sulfasalazine inhibits the absorption of both monoglutamyl and polyglutamyl dietary folates [27]. Studies in the prefortification era identified a high incidence of folate deficiency in chronic alcoholics that was mainly ascribed to poor diet [23], and also demonstrated a significant component of intestinal folate malabsorption in a population of active alcohol consumers [28].

Genetic abnormalities of methionine metabolism

Despite the potential for a variety of functional polymorphisms in the regulatory genes involved in folate and methionine metabolism (see Fig. 20.1), clinically significant polymorphisms affecting folate metabolism are few and focus mainly on those of MTHFR. A thermolabile form of MTHFR occurs in TT homozygotes of the 677C→T variant, who represent 10%–15% of the general population and express moderate hyperhomocysteinemia especially when the polymorphism is combined with low folate levels [29]. A second MTHFR polymorphism at 1298A→C was thought to cause neural tube defects [30], but it does not appear to interact with 677C→T nor to cause elevated homocysteine levels [31]. While functional polymorphisms have been

described in the folate metabolizing genes, *GCP2* and *RFC1* (reduced folate carrier 1), their single or combined effects on folate absorption and status appear to be insignificant [31].

Clinical effects of folate deficiency and aberrant methionine metabolism

Because folate ultimately is required to maintain nucleotide balance during DNA synthesis, its deficiency is expressed by increased apoptosis with compensatory increased proliferation of cells with rapid turnover in the blood-forming bone marrow and in the intestinal mucosa. As indicated in Fig. 20.1, folate deficiency also affects the methionine metabolic cycle, resulting in elevated levels of circulating tHcy. Several common clinical conditions have been linked to folate deficiency with abnormal DNA metabolism and aberrant methionine metabolism.

Anemia and diarrhea

The main clinical expression of overt folate deficiency is megaloblastic anemia, with findings of macrocytic red blood cells with increased mean corpuscular volume and hypersegmented neutrophils in the peripheral blood, which are expressions of reduced levels of DNA and delayed erythropoiesis. Megaloblastic anemia is also a hallmark of cobalamin deficiency, and results from decreased MS activity as the result of folate deficiency with insufficient substrate MTHF or insufficient cofactor cobalamin resulting in diminished production of THF, methionine, and SAM (see Fig. 20.1). Consequently, the level of 5,10-MTHF is reduced by insufficient substrate THF and SAM inhibition. A reduced level of 5,10-MTHF decreases TS production of thymidine from uracil, thereby promoting the DNA nucleotide imbalance and reduced synthesis. Macrocytic enterocytes are evident in small intestinal biopsy specimens from alcoholic patients with folate-deficient megaloblastic anemia [32] and are associated with the diarrhea of chronic alcoholism caused in part by abnormal fluid and electrolyte transport in the intestine [33].

Folate deficiency and neural tube defects

Neural tube defects are errors of fetal spinal cord development that occur within the first 2 weeks of pregnancy and result in anencephaly and spina bifida. Neural tube defects occur at a rate of 1–2 per 1000 live births and probably more frequently in spontaneous abortions. The association of neural tube defects with folate deficiency is based on findings that red blood cell folate levels in early pregnancy correlate with the risk for delivery of a child with a neural tube defect [34] and on the results of trials in which neural tube defects were prevented by periconceptional supplementation with folic acid [35]. About 20% of infants with a neural tube defect and their mothers have the homozygous 677C→T polymorphism of the *MTHFR* gene [36], but it is uncertain whether this variant plays a major role in determining the risk for neural tube defects unless it is associated with folate deficiency. In spite of early high expectations, the public-health policy of folic acid fortification of the US food supply has reduced the incidence of neural tube defects by only about 50% [37].

Cardiovascular disease

Hyperhomocysteinemia clearly increases the risk for stroke and coronary heart disease [38]. A metaanalysis of 15 studies of proven coronary artery disease showed that an increment of 5 μmol/L in the serum level of tHcy above the normal range constitutes a coronary heart disease risk similar to an increase in total cholesterol of 20 mg/dL above normal [39]. The prevention of cardiovascular disease by lowering homocysteine levels with folic acid supplements has been the focus of many clinical trials. A metaanalysis of 12 randomized clinical trials involving 17 000 patients with preexisting vascular disease concluded that folic acid supplementation had no effect on the risk of recurrent stroke or coronary heart disease or on mortality. The authors do not exclude the possibility that lowering homocysteine levels by folic acid supplementation may reduce the incidence of primary cardiovascular events in susceptible populations [40].

Colon cancer

The association of folate deficiency with an increased risk for colon cancer was established by a prospective sigmoidoscopic and dietary survey of more than 20 000 health professionals. The data revealed that a diet containing relatively low folate levels together with the consumption of more than two alcoholic drinks per day increased adenoma risk twofold [41]. A subsequent 6-year prospective study of more than 40 000 male health professionals found a threefold increased risk of colorectal cancer in alcohol consumers ingesting a low-folate diet, whereas a high-folate diet was protective against colorectal cancer despite alcohol consumption [42]. A Norwegian study found a threefold increased risk of large precancerous colorectal adenomas in patients with low red blood cell folate levels, that increased to eightfold in smokers who carried the T allele of the *MTHFR* 677C→T polymorphism [43]. The increased risk of colon cancer can be ascribed to the effects of folate deficiency in promoting DNA nucleotide imbalance, strand breaks, impaired repair, and aberrant DNA methylation [44]. Conversely, the Norwegian study found increased colorectal adenoma risk in smokers with high red blood cell folate levels [43], and a U-shaped curve described the relationship between high plasma folate levels and colorectal cancer risk in a Swedish case–control study [45]. Folate therefore plays a dual role with respect to colon cancer; folate deficiency enhances the likelihood of the initiation of colon cancer through its deleterious effects on DNA, and excessive folate enhances cancer cell proliferation by promoting cell division and the potential methylation of tumor suppressor genes [46,47].

Alcoholic liver disease

The contributory role for folate deficiency and elevated liver homocysteine and SAH in the pathogenesis of alcoholic liver disease has been well established and is summarized in a 2007 symposium report [48]. Studies from European countries where the diet is not fortified with folic acid indicate a continued high incidence of folate deficiency together with elevated serum homocysteine levels in chronic alcoholics [24,49]. In addition to poor diet and intestinal malabsorption, folate deficiency in alcoholism is caused by decreased uptake of folate by the liver and enhanced excretion in the urine [16]. Because liver folate stores are reduced in alcoholic liver disease [50], the combination of these factors accelerates the risk for folate deficiency in these patients. Abnormalities of the methionine metabolic cycle caused by experimental induction of alcoholic liver disease include the inhibition of MS, MAT1A, and SAHH, each of which contribute to increased levels of tHcy and SAH in the liver, and decreased SAM levels (see Fig. 20.1) [19,51]. Reduction in SAM levels was correlated to reduced levels of the principal antioxidant glutathione in the liver of ethanol-fed micropigs [52], while oxidative liver injury was prevented and glutathione levels were preserved by the administration of SAM to ethanol-fed baboons [53]. Elevated levels of homocysteine and SAH appear to activate stress signal pathways in the endoplasmic reticulum leading to increased hepatocellular apoptosis and lipid synthesis [54,55]. While ethanol exposure plays a significant role in altering methionine metabolism, the importance of folate deficiency was underscored by the observation that all these features of alcoholic liver disease were enhanced in micropigs fed a diet that combined both ethanol and the absence of folic acid, compared to either factor alone [52].

Assessment of folate deficiency

Folate deficiency should be suspected in any anemic patient with an elevated mean corpuscular volume of red blood cells, and then confirmed by a measurement of a low level of serum or red blood cell folate. Each of the latter tests can be performed by a radioisotope-binding assay. The serum folate level is more labile and dependent on recent dietary intake, whereas the red blood cell folate level is a more accurate measure of tissue stores. Depending on the laboratory, folate deficiency is defined by a serum folate level less than 7 nmol/L (3 ng/mL), while a level of red blood cell folate level less than 305 nmol/L (140 ng/mL) is predictive of tissue folate deficiency [1]. Measurement of the plasma tHcy level is a more sensitive functional assay for folate deficiency. Since 5-MTHF is the substrate for the MS reaction that converts tHcy to methionine, a low folate level is represented by an increased tHcy level. Normal ranges of tHcy vary among laboratories, and mean normal values for different age groups range from 6.6 to 13.4 μmol/L [1]. In addition to folate deficiency, factors that increase tHcy include male sex, increasing age, smoking, coffee consumption, and decreased renal function with an elevated serum creatinine level [56]. However, because cobalamin is an essential cofactor for the MS reaction, its deficiency is also reflected by an elevated tHcy level, as well as by the same tissue expressions found in folate deficiency (see Fig. 20.1 and Table 20.1). Therefore, folate deficiency can be defined metabolically by high tHcy and normal cobalamin status, as described in the next section.

Folate toxicity

Evidence from many research groups suggests that a considerable portion of supplemental folic acid is absorbed unchanged, and that high circulating levels of folate may alter immune function, increase cancer risk, and promote worsening of cognitive function in elderly subjects [57]. At least 40% of aging Americans take supplemental folic acid at 400 μg/day and consequently their serum folate levels often exceed 40 nmol/L, which is at least double the highest serum folate level in those who do not use supplements [47], and their level of unmetabolized folic acid constitutes more than 15% of serum folate that exceeds 50 nmol/L [58]. A study of postmenopausal women showed that immune function as measured by natural killer cell toxicity was improved by folic acid supplementation at 400 mg/day, but worsened in linear fashion with the increasing levels of unmetabolized serum folic acid in those who took larger doses of supplemental folic acid [18]. Furthermore, the risks for prostate cancer in men and breast cancer in postmenopausal women increased significantly in relationship to increasing plasma folate levels [59,60], especially when associated with the use of folic acid supplements [60]. A study of 1300 elderly people reported an increased risk for megaloblastic anemia and reduced cognitive function in those persons with low vitamin B-12 status who took supplemental folic acid [61]. This interesting relationship of folate and vitamin B-12 is explored in greater detail in the next section.

Vitamin B-12 (cobalamin)

Folate and vitamin B-12 have complex and different mechanisms of absorption, and each has a broad range of clinical effects. Because these vitamins interact metabolically, many of the clinical signs of deficiency are similar, although their structures, food sources, and modes of absorption are dissimilar (Table 20.1).

Structure, nomenclature, and metabolic functions

Vitamin B-12, or cobalamin, contains a central cobalt atom surrounded by a planar corrin ring that is formed from four reduced pyrrole rings linked together and attached at a right angle to a nucleotide containing ribose, phosphate, and 5,6-dimethylbenzimidazole. In its active coenzyme forms, the cobalt atom is bound to specific moieties, resulting in methylcobalamin and adenosylcobalamin, whereas cyanocobalamin is the pharmaceutical and interchangeable form of the

Table 20.1 Folate and cobalamin

	Folate	Cobalamin
Dietary source	Green vegetables, orange juice, fortified grains	Animal protein exclusively
Daily requirement	Adult: 400 µg DFE Pregnancy: 600 µg DFE Lactation: 500 µg DFE	Adult: 2.4 µg Pregnancy: 2.6 µg Lactation: 2.8 µg Older than age 50: supplement
Site of intestinal absorption	Jejunum	Ileum
Regulatory proteins in absorption	Glutamate carboxypeptidase II Reduced folate carrier	R protein (haptocorrin) Intrinsic factor Cubulin Transcobalamin II
Function	Transfer of one-carbon moieties, methylation, DNA synthesis	DNA synthesis, myelin maintenance
Risks for deficiency	Dietary inadequacy Chronic alcoholism Pregnancy Intestinal malabsorption, especially celiac disease Elderly with unbalanced diet	Elderly with achlorhydria Postgastric bypass surgery Chronic use of proton pump inhibitors Pernicious anemia Ileal disease or resection: short bowel syndrome, Crohn's disease, radiation enteritis Intestinal bacterial stasis syndrome
Clinical signs of deficiency	Megaloblastic anemia Hyperhomocysteinemia:cardiovascular risk, neural tube defects	Megaloblastic anemia Cognitive deficit Subacute combined degeneration
Diagnosis	Low serum and red cell folate Elevated serum homocysteine with normal methylmalonic acid	Low serum vitamin B-12 Elevated serum homosysteine and serum methylmalonic acid Low serum holotranscobalamin

DFE, dietary folate equivalent.

vitamin. There are only two known enzymatic reactions that require cobalamin and serve to bind it in tissues. Methylcobalamin is the cofactor for MS and participates in the transfer of the methyl group from 5-MTHF to tHcy, producing methionine and THF (see Fig. 20.1). Cobalamin deficiency therefore reduces activity of MS, elevates tHcy, and "traps" 5-MTHF in a nonfunctioning role. The MS product THF is reduced so less 5,10-MTHF is available for the TS reaction, which results in less generation of thymidine from uracil, nucleotide imbalance, and decreased DNA synthesis with risk of megaloblastic anemia (see Fig. 20.1). Adenosylcobalamin is involved in a separate reaction as a cofactor for methylmalonyl-coenzyme A (CoA) mutase in the conversion of methylmalonyl-CoA to succinyl-CoA. Accordingly, cobalamin deficiency also uniquely results in the elevation of the level of serum methylmalonic acid, which is a side product of methylmalonic CoA [62]. In this section, the term *cobalamin* is used to describe dietary sources, metabolic pathways, and clinical manifestations of deficiency. The term *vitamin B-12* is used to describe the pharmacological and supplemental forms of the vitamin.

Dietary sources and requirements

Cobalamin originates from intestinal bacterial synthesis with subsequent transfer to cells; it therefore appears in the human diet exclusively bound to animal proteins. The usual sources of cobalamin are all animal products, including meat, fish, shellfish, poultry, eggs, milk, and other dairy products. The adult DRI for cobalamin is 2.4 µg/day (2.6 µg during pregnancy and 2.8 µg during lactation), which accounts for an average 50% absorption from all dietary sources. Because cobalamin absorption declines with aging, persons older than 50 years are advised to take supplements containing vitamin B-12 or to ingest food that is fortified with vitamin B-12, such as most breakfast cereals [62].

Intestinal absorption and metabolism of cobalamin

The intestinal absorption of dietary animal-protein-bound cobalamin involves gastric, upper intestinal, and ileal stages, and an enterohepatic circulation (Fig. 20.2). Abnormality in any of these steps may result in cobalamin deficiency. In the presence of gastric acid and pepsin, cobalamin is severed

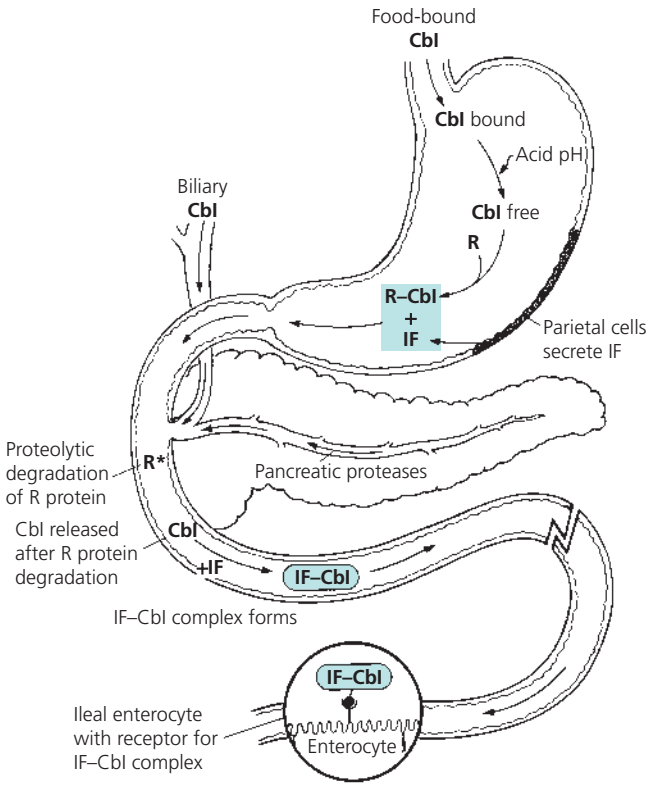

Figure 20.2 Sequential steps in the gastrointestinal absorption of dietary protein-bound cobalamin (Cbl). Gastric acid is required to liberate both methylcobalamin and adenosylcobalamin from dietary protein. In the acidic gastric environment, cobalamin is then bound to salivary R proteins, and gastric parietal cells secrete intrinsic factor (IF). After neutralization of the gastric contents in the upper duodenum and pancreatic protease digestion of the R protein–cobalamin complex, free cobalamin then binds to IF. After transit to the terminal 50 cm of ileum, the IF–cobalamin complex interacts at the microvillus surface with the specific ileal receptors cubulin and amnionless, and is then internalized and binds to megalin and transcobalamin II. From Sitrin [307], with permission from Lippincott, Williams and Wilkins.

from its protein bond and then is transferred to R protein (also called haptocorrin), which is secreted by salivary and parietal cells [63]. The release of cobalamin and its transfer to R protein is optimal at pH 1–1.3, a level that is not achieved by achlorhydric individuals, who mostly include persons older than 65 years who develop gastric atrophy as part of the aging process [64]. The third gastric step involves the secretion of intrinsic factor (IF) from acid-secreting parietal cells. However, IF is ineffective as a cobalamin binder at the acid pH of the healthy stomach, and it follows the R protein–cobalamin complex into the duodenum. Here, the complex is degraded by pancreatic proteases, and at the neutral pH of the duodenum, cobalamin is quickly bound to the protease-resistant IF and in this state traverses the small intestine to the ileum [63,65]. The absorption of cobalamin by ileal enterocytes requires several binding proteins. First, the cobalamin–IF complex is bound to a specific membrane receptor on the ileal enterocyte called cubulin, which collocates with another enterocyte receptor called amnionless (AML). Each of these receptors is active in ileal enterocytes and in renal tubular cells. Subsequent transfer across the ileal enterocyte involves the intracellular cleavage of the cobalamin–IF/cubulin/amnionless complex [66]. Cobalamin then binds to transcobalamin II, a 43-kDa protein with molecular homology to IF that is synthesized in ileal enterocytes and complexes with a protein called megalin, which carries cobalamin out of the ileal mucosa to the portal circulation [67,68]. The transcobalamin II–cobalamin complex, known as holotranscobalamin, accounts for 10%–20% of all circulating cobalamin and is essential for its transport to all cells of the body. Holotranscobalamin is taken up by cells by endocytosis, which is followed by liberation of the two coenzyme forms methylcobalamin and adenosylcobalamin [63]. In an enterohepatic cycle, about 1.4 μg/day of endogenous cobalamin is secreted into the bile as a complex with R protein [69]. The circulating cobalamin is then released from biliary R protein by pancreatic trypsin and then rapidly bound to IF for subsequent passage to the ileal receptor site, where about half, or 0.7 μg/day, is reabsorbed and the other half is excreted in the stool (62).

Clinical expressions of cobalamin deficiency

Folate and cobalamin are required as substrate and cofactor for the MS reaction (see Fig. 20.1), the clinical expression of cobalamin deficiency as megaloblastic anemia and hyperhomocysteinemia is indistinguishable from that of severe folate deficiency. However, because cobalamin also participates in the methylmalonyl-CoA mutase reaction, its deficiency may also be expressed in the nervous system as subacute combined degeneration of the posterior columns of the spinal cord, which is characterized by the loss of position and vibratory sensation and other features of peripheral neuropathy of the lower extremities. Cobalamin deficiency also has more subtle and frequent effects on the central nervous system that include neuropsychiatric disorders such as ataxia, paresthesias, memory loss, cognitive dysfunction, and more severe dementia, even in the absence of anemia [70]. A prospective study of elderly subjects found that the presence of baseline hyperhomocysteinemia predicted the 8-year risk of developing dementia as well as overt Alzheimer disease [71]. A subsequent study showed that the relationship of hyperhomocysteinemia to cognitive defects depends on the adequacy of the serum cobalamin level [72]. Others confirmed this association and also showed that cobalamin-deficient subjects taking folic acid supplements had the most severe cognitive decline [61]. This observation can be explained by the interactions of cobalamin and folate metabolisms (see Fig. 20.1), such that excessive folic acid promotes the use of residual cobalamin by the MS reaction, thereby depleting stores that would otherwise be available for the methylmalonyl-CoA mutase reaction required for brain functions.

Causes of cobalamin deficiency

Cobalamin deficiency has many potential causes owing to the complexity of the mechanisms of its absorption processes.

Dietary inadequacy

The daily requirement of cobalamin of 2.4 μg [62] is very small compared to its body pool size (1–2 mg); therefore, it takes many years to become deficient from dietary inadequacy alone. In populations living in developed countries, only true vegans who do not take vitamin supplements are at risk for dietary cobalamin deficiency, because cobalamin is present in dairy products and in most multivitamins. However, the worldwide incidence of dietary vitamin cobalamin deficiency is substantial, and many populations living in the Indian subcontinent, certain parts of Africa, and in rural Central America obtain less than 1 μg/day and have a higher incidence of megaloblastic anemia, delayed childhood development, and neurological complications [73].

Abnormal gastric events

Gastric acid is required for the release of food-bound cobalamin from dietary protein as the first step in cobalamin absorption so achlorhydric individuals are at risk for cobalamin deficiency. Achlorhydria of aging is the most common cause of cobalamin deficiency in the United States. According to the criteria of elevated levels of methylmalonic acid and tHcy and a serum cobalamin level of less than 222 pmol/L (300 pg/mL), at least 15% of ambulatory individuals older than 65 years are cobalamin-deficient in the United States, with greater prevalence in Hispanic Americans and less in African Americans [73,74]. A similar incidence of age-related cobalamin deficiency has been reported in European countries [63]. The increasing prevalence of age-related cobalamin deficiency, which is 40% in persons older than 80 years [63], could be related to a greater prevalence of *Helicobacter pylori*, which predisposes to more severe gastric atrophy and malabsorption of food-bound cobalamin [75].

Pernicious anemia was first described by Thomas Addison in the early 19th century, and is recognized as an autoimmune disorder characterized by the absence of IF in the gastric juice in association with achlorhydria. Almost all patients have gastric and serum antibodies to either parietal cells or IF, and most have elevated serum gastrin levels [63]. The type A atrophic gastritis pattern of pernicious anemia involves the fundus and upper body of the stomach, in contrast to the more common type B atrophic gastritis of elderly patients, which involves the lower stomach and antrum. Pernicious anemia may be associated with other autoimmune diseases, including Hashimoto thyroiditis, adrenal atrophy, and Sjögren syndrome, and carries increased potential for the development of gastric adenocarcinoma, lymphoma, and carcinoid tumors [63] While the overall incidence of pernicious anemia in the United States is about two cases per 1000 per year [73], it increases with aging, and a survey of over 700 elderly subjects in Los Angeles reported incidences among men and women at 1.4% and 2.7%, respectively, with a somewhat greater incidence in African Americans than in white subjects [76]. Because IF is also required for the absorption of endogenous cobalamin in the enterohepatic circulation, cobalamin deficiency develops more rapidly in patients with untreated pernicious anemia than in strict vegans or elderly persons with achlorhydria. There is an increased risk of gastric neoplasms so persons with proven pernicious anemia should have endoscopic surveillance every 3 years [63]

Many other clinical conditions involving the stomach or small intestine cause cobalamin malabsorption. For example, a case–control study of patients older than 65 years reported that chronic use of proton pump inhibitors is associated with a fourfold greater risk of cobalamin deficiency [77]. Surgical causes of cobalamin deficiency include total gastrectomy and gastric bypass surgery for obesity, each of which is associated with food-bound cobalamin malabsorption secondary to achlorhydria. A survey performed early in the era of gastric bypass surgery found that two-thirds of patients developed cobalamin deficiency within 12 months of surgery [78] However, a more recent survey found a 12% incidence of postoperative cobalamin deficiency among routinely screened patients [26]. Persistent duodenal hyperacidity may inactivate pancreatic trypsin and prevent the transfer of cobalamin from gastric R protein to IF; thus, cobalamin deficiency is a recognized component of Zollinger–Ellison syndrome [79].

Abnormal pancreatic and upper intestinal events

Although cobalamin malabsorption was described in 50% of patients with pancreatic insufficiency, including patients with cystic fibrosis, actual deficiency is uncommon in these patients [80]. During passage through the jejunum, the cobalamin–IF complex is susceptible to bacterial cleavage or uptake; cobalamin deficiency with megaloblastic anemia is an increasingly recognized clinical expression of bacterial overgrowth syndromes in the elderly [81,82]. Intestinal infestation with the fish tapeworm *Diphyllobothrium latum*, which is found in rural Finland, also causes cobalamin deficiency through competition for the cobalamin–IF complex [83].

Abnormal ileal events

According to retrospective studies of patients with ileal resections of various lengths, at least 50–80 cm of the terminal ileum participates in the ileal uptake and transfer of cobalamin, so that patients with inflammatory disease affecting longer lengths of intestine or those who have had surgical resection of the terminal ileum are at increased risk for cobalamin deficiency [84] Diseases that interrupt enterohepatic cycling, such as Crohn's disease affecting the terminal ileum, radiation enteritis [85], and ileal bypass surgery or resection (86), promote increased loss of endogenous cobalamin in the feces. Surgical resection of the ileocecal valve increases the risk for cobalamin deficiency by permitting the migration of

colonic bacteria and subsequent small intestinal bacterial overgrowth. Because the terminal ileum is the site for both dietary cobalamin absorption and the reabsorption of endogenous cobalamin from its enterohepatic circulation, cobalamin deficiency may become evident within several months of surgical resection of more than 50 cm of terminal ileum [86].

Genetic causes of cobalamin malabsorption

Rare congenital deficiency of IF results from the failure of its synthesis by gastric parietal cells. The gastric mucosa and acid secretion are normal and IF antibodies are absent in this condition. Cobalamin deficiency is usually apparent between the ages of 1 and 5 years [87]. The Imerslünd–Grasbeck syndrome, caused by defective cobalamin transport by ileal enterocyctes that results from mutations in cubulin (*CUBN*) or amnionless (*AMN*), has a prevalence of 1 : 200 000 in people of Scandinavian descent, and is usually clinically evident in early childhood but may also be first recognized in adulthood. This autosomal recessive disease represents the failure of synthesis or membrane insertion of cubulin or amnionless and is expressed in both the ileum and the renal tubule by the early onset of cobalamin deficiency and proteinuria [88]. Very rare congenital transcobalamin II deficiency is also an autosomal recessive condition, and is expressed in the first year of life with pancytopenia and megaloblastic anemia [89].

Diagnosis of cobalamin deficiency

Cobalamin deficiency should be suspected in any person who presents with macrocytic anemia with an elevated mean corpuscular red blood cell volume, cognitive decline, glossitis, or classical signs of subacute combined degeneration of the spinal cord that include loss of proprioceptive and position sense. Cobalamin is conventionally measured in the serum by a radioassay with high specificity and sensitivity, with values less than 250 pmol/L considered subnormal [74,90]. The measurement of holotranscobalamin is potentially a more accurate assessment of the amount of cobalamin that is available for cellular uptake, but its utility relative to the cobalamin assay is still uncertain [91]. Measurements of the metabolites tHcy and methylmalonic acid (MMA) are both useful to confirm cobalamin deficiency. An elevated tHcy is nonspecific and does not distinguish between cobalamin deficiency and folate deficiency, whereas the measurement of MMA is costly and often not easily available. MMA measurement has been recommended to confirm cobalamin deficiency when the serum cobalamin level is in the intermediate range between 125 and 250 pmol/L. However, there is also a gray area of uncertain MMA normality; a value greater than 0.75 μmol/L is confirmatory of cobalamin deficiency [90].

The Schilling test of cobalamin deficiency, which was widely available until recently, involved measuring the 24-h urine excretion of labeled cobalamin with and without the inclusion of exogenous IF. This test was used to differentiate dietary causes (normal absorption), pernicious anemia (low absorption corrected with exogenous IF), ileal malabsorption (low in either case), and intestinal bacterial overgrowth (low then corrected with oral antibiotics). However, this "gold standard" test is seldom available as a result of the unavailability of exogenous IF, the need for the use of radioactive dosages, and cumbersome urine collections. The clinician must rely on a careful history to distinguish potential causes before determining the best approach to the treatment of cobalamin deficiency. For practical purposes, dietary cobalamin deficiency occurs in strict vegans or cultural vegetarians, such as Indian immigrants, who allow no source of animal food in their diet and do not use vitamin supplements; it may also occur in patients with long-standing malnutrition secondary to anorexia nervosa [92]. Cobalamin deficiency secondary to the gastric achlorhydria of aging is mainly a diagnosis of exclusion of pernicious anemia and other causes of malabsorption in individuals older than 60 years. Surgical causes including gastric resection or bypass and ileal resection and ileal diseases such as Crohn's disease or radiation enteritis should be self-evident from the history. Intestinal bacterial overgrowth is also diagnosed by symptoms of bloating and history of intestinal diseases such as ileocecal resection, partial bowel obstruction, scleroderma, or autonomic visceral neuropathy of diabetes. The diagnosis of pernicious anemia is problematic with the unavailability of the Schilling test, and is approached by using generally nonspecific testing. While the sensitivities of tests for serum and gastric juice antibodies to IF and parietal cells approach 100%, their specificities are only about 50%–70% [63,90]. Similarly, elevated levels of serum gastrin and pepsinogen are sensitive findings but nonspecific, as is the finding of gastric atrophy by endoscopy. In a series of 120 patients with proven pernicious anemia with antibodies to IF, 91% had low serum pepsinogen values and 87.5% had elevated serum gastrin values [93].

Treatment of cobalamin deficiency

The treatment of cobalamin deficiency is based on both its detection and the identification of its cause. Since cobalamin deficiency secondary to the achlorhydria of aging or gastric surgery is caused by malabsorption of food-bound dietary cobalamin but not of unbound crystalline pharmaceutical vitamin B-12, it can usually be treated by supplemental oral vitamin B-12 at over-the-counter doses of 350 μg/day crystalline vitamin B-12 supplement [94]. Since patients with pernicious anemia lack both IF and gastric acid, they can absorb neither crystalline cobalamin nor dietary food-bound cobalamin and must be treated with parenteral vitamin B-12, starting at 1000 μg/day for a week, then weekly for a month, and then in monthly 1000-μg doses, ideally after training in self-injection. A study of 33 patients with cobalamin deficiency caused by atrophic gastritis with or without pernicious

anemia reported that an oral dose of 2000 μg/day was as effective in correcting the deficiency as a parenteral dose of 1000 μg/month [93]. Patients who develop cobalamin deficiency in the presence of bacterial overgrowth, ileal disease, or surgical resection, each of which limits the absorption of the cobalamin–IF complex, should be similarly treated with regular parenteral cobalamin injections.

Vitamin C

Vitamin C (ascorbate) is a weak acid that acts as a reducing agent that produces dehydroascorbate and subsequently oxalate [95]. Most mammals, except primates and guinea pigs, synthesize ascorbate from glucose [96]. Vitamin C is obtained from fruits and vegetables in the diet, fortified foods and beverages, and vitamin supplements. The DRI for men is 90 mg and for women is 75 mg [97], which, after ingestion as a supplement, produce plasma concentrations of about 50 μM (Table 20.2). However, these same amounts from fruits and vegetables may produce lower plasma concentrations because of the lower bioavailability of ascorbate from food compared to that of pure vitamin C [98]. As a result of increased oxidative stress, smokers require a higher daily intake: 125 mg for men and 110 mg for women [99]. Assuming a variety of five or more servings of fruits and vegetables, the daily dietary intake often exceeds 200 mg. Many vitamin supplements contain ascorbic acid at a variety of doses from 60 to 1000 mg. The dehydroascorbic acid content of foods is estimated to be 10%–20% of the total vitamin content [100].

Ascorbic acid can be measured in plasma by high-performance liquid chromatography (HPLC), whereas there is no sensitive or specific assay for dehydroascorbic acid [101]. Ascorbic acid undergoes carrier-mediated transport by sodium-dependent vitamin C transporters (SVCT) 1 and 2 [102]. SVCT1 is localized to intestine, liver, and kidney, and SVCT2 is distributed in other tissues. Flavonoids in fruits and vegetables may decrease absorption by inhibiting the SVCT1 transporter [98]. Dehydroascorbic acid is transported by facilitated glucose transporters and immediately reduced intracellularly to ascorbic acid [103]. Once absorbed, ascorbic acid, but not dehydroascorbic acid, circulates unbound in plasma [104] and is available for tissue transport by SVCT2

Table 20.2 Other water-soluble vitamins

	Vitamin C	Thiamin	Pyridoxine	Niacin	Riboflavin
Dietary source	Fruits, vegetables, fortified food and beverages	Whole grain, fortified cereals, pork	Meat, fish, poultry, non-citrus fruit, fortified cereals	Tryptophan , fish, legumes, fortified cereals	Milk, eggs, fortified cereals
Daily requirement	Men 90 mg; women 75 mg; smokers 125 mg (men) 110 mg (women); pregnancy 80 mg; lactating 120 mg	Men 1.2 mg; women 1.1 mg; pregnancy and lactation 1.4 mg	Men and women 1.3 mg; pregnancy 1.9 mg, lactation 2.0 mg	Niacin equivalents (NE is 60 mg tryptophan): men 16 NE, women 14 NE; pregnancy 18 NE, lactation 17 NE	Men 1.3 mg; women 1.1 mg; pregnancy 1.4 mg, lactation 1.6 mg
Absorption	Carrier-mediated through SVCT1	Active Na^+-dependent transport, passive diffusion higher dose	Hydrolysis and passive transport	Active tryptophan; passive niacin	Active Na^+-dependent transport
Function	Antioxidant reducing agent; collagen synthesis, neurotransmitter synthesis	Carbohydrate and energy metabolism	Heme synthesis, cofactor for alanine transaminase, CβS	NAD and NADP redox reactions, DNA repair	Redox, especially glutathione reductase; cofactor for MTHFR
Clinical signs of deficiency	Scurvy	High-output heart failure, peripheral neuropathy, Wernicke–Korsakoff syndrome	Elevated homocysteine, altered cognition, glossitis, peripheral neuropathy	Pellagra in malnutrition, AIDS, Hartnup disease, carcinoid syndrome	Glossitis, peripheral neuropathy, elevated homocysteine in TT homozygous MTHFR 677C→T
Toxicity	False-negative hemoccult; hyperoxaluria renal stone potential	None known	Sensory neuropathy in gram amounts	Flushing, exacerbation of peptic ulcer	None known
Diagnosis	HPLC plasma	Erythrocyte transketolase	HPLC	Urine metabolites	Erythrocyte glutathione reductase

HPLC, high-pressure liquid chromatography.

against its concentration gradient into muscle, adrenal, and pituitary tissues, and into circulating neutrophils, monocytes, and lymphocytes [105]. Vitamin C levels are also regulated by glomerular filtration and reabsorption by SVCT1 in the proximal renal tubule [106]. At doses less than 100 mg/day, there is a steep relationship between the ingested dose and the steady-state fasting plasma concentrations of ascorbate, which are saturated at 60–70 μM at oral intakes greater than 100 mg/day. As the ingested dose increases, the amount that is absorbed decreases; for example, 80% of a dose of less than 100 mg is absorbed, compared to less than half of a 1250-mg dose [107]. Even with maximally tolerated pharmacological oral doses of 2–3 g at intervals of several hours, plasma concentrations do not exceed 220 μM [105].

The only certain health benefit of ascorbic acid ingestion is to prevent scurvy, a potentially lethal condition [107]. Scurvy is relatively rare in Western countries, but it may occur under conditions of inadequate diet in substance abusers, alcoholics, and in persons with cancer. Clinical features of scurvy occur at plasma ascorbate concentrations less than 4–5 μM and include bleeding of the gums and around the hair follicles of the lower extremities and lassitude and depression, while more severe deficiency may result in joint effusions, intestinal bleeding with hypotension, and death [108]. Scurvy can usually be corrected with several hundred mg of intravenous or oral ascorbic acid, given daily for several days. Vitamin C deficiency without overt scurvy occurs at plasma concentrations of 5–20 μM, and is reported in persons whose diet consists of fast food [109]. Malabsorption of vitamin C is associated with extensive Crohn's disease, short bowel syndrome, and celiac disease. Vitamin C deficiency also occurs in patients with renal disease undergoing hemodialysis, since each hemodialysis session reduces plasma ascorbate concentrations by as much as 70% [110]. Controversial evidence suggests that eating five or more daily servings of fruits and vegetables that are high in ascorbic acid may prevent some cancers, improve blood pressure, and decrease the risks for cardiovascular disease and stroke [111]. Ascorbate is secreted in gastric juice and enhances iron absorption by reducing Fe^{3+} to Fe^{2+} [112]. Therefore, vitamin C supplements should be avoided by patients with hemochromatosis, thalassemia, or diseases requiring chronic red blood cell transfusions [113]. Diarrhea or bloating occurs in subjects who ingest doses of more than 2–3 g at one time. False-negative results on hemoccult tests can occur with intakes of more than 250 mg vitamin C. Supplemental doses exceeding 1000 mg increase urinary oxalate excretion in patients with known hyperoxaluria. Studies have indicated no increased incidence of renal stones in subjects who ingest at least 1 g of ascorbate daily; however, these studies excluded subjects with a history of renal stone formation who may be at greater risk [113]. Furthermore, patients with end-stage renal disease should avoid consuming more than 200 mg ascorbate daily because compromised renal regulation may increase plasma levels and thus promote hyperoxaluria and stone formation [107,113].

Thiamin

Thiamin, in its active form as thiamin pyrophosphate, is involved in carbohydrate and energy metabolism as a coenzyme for pyruvate dehydrogenase and for the decarboxylation of α-ketoacids. Thiamin is present in fortified or whole grain cereals and breads, and in pork. The DRI for thiamin is 1.2 mg for men and 1.1 mg for women, and 1.4 mg during pregnancy and lactation (Table 20.2) [114]. Thiamin is absorbed by a dual process in the jejunum. At low concentrations (0.2–2.0 mM), thiamin is absorbed by an active Na^+-dependent transport that involves phosphorylation, and at higher concentrations, it is absorbed by passive diffusion [115]. The body pool size of thiamin is about 30 mg, with a half-life of 9–18 days, and plasma and red blood cell levels are sustained by rapid urinary excretion of excess absorbed thiamin. Thiamin deficiency is common in chronic alcoholics because of a combination of poor intake and reduced absorption. Ethanol interferes with Na^+,K^+-ATPase-mediated active transport across the basal enterocyte membrane, which drives thiamin absorption [116]. Other causes of thiamine deficiency include extensive diuretic use and extreme malnutrition of any cause, such as chronic recurrent vomiting, anorexia, and malabsorption syndromes. The clinical signs of thiamin deficiency are related to the heart and the nervous system. Classical wet beriberi, which often accompanies starvation, is characterized by cardiomyopathy with high-output cardiac failure, whereas the principal sign of dry beriberi is progressive stocking–glove polyneuropathy of the lower extremities. The extensive use of diuretics promotes thiamine deficiency in patients with congestive heart failure, whereas the left ventricular ejection fraction improves with thiamine replacement [117]. Additional neurological features of thiamin deficiency occur in chronic alcoholics and are classified under the Wernicke–Korsakoff syndrome as nystagmus and ocular paresis, cerebellar dysfunction with a wide-based gait, and global confusion [118] With the increasing popularity of gastric bypass surgery for obesity, acute Wernicke–Korsakoff syndrome with delirium has been recognized as a severe complication of prolonged postoperative vomiting that may occur with insufficient size of the gastric outlet [119]. Because body stores of thiamin are regulated by carbohydrate metabolism, the administration of intravenous glucose to a malnourished patient may precipitate an acute demand for residual endogenous thiamin with resultant overt thiamin deficiency manifest by cardiac failure and confusion. Patients with Alzheimer disease have decreased levels of brain thiamin pyrophosphate, but do not respond to thiamine therapy [120]. Thiamin status is most accurately assessed by a functional assay of erythrocyte transketolase before and after the addition of thiamin pyrophosphate [121].

Pyridoxine

Pyridoxine, or vitamin B-6, is a coenzyme for many amino acid reactions and exists in six forms: pyridoxine, pyridoxal, pyridoxamine, and the phosphorylated forms of each. Its main food sources include meat, fish, poultry, noncitrus fruits, and fortified cereals. The DRI is 1.3 mg for adult men and women; this rises to 1.5 mg for those older than 50 years, 1.9 mg during pregnancy, and 2.0 mg during lactation (Table 20.2) [122]. The phosphorylated forms of pyridoxine are absorbed after hydrolysis by brush border alkaline phosphates, followed by passive transport of nonphosphorylated forms through the enterocyte. Phosphorylation is restored in the liver, and the principal circulating form of vitamin B-6 is pyridoxal phosphate (PLP) in a complex with albumin. The body pool size of pyridoxine is 60–100 mg. Pyridoxine deficiency is one of the common features of chronic alcoholism and, owing to the role of pyridoxine in heme synthesis, it is characterized by microcytic sideroblastic anemia, as well as by peripheral neuropathy, cheilosis, and glossitis. Pyridoxine deficiency in alcoholism is caused by hepatic degradation of the PLP–protein complex by acetaldehyde followed by rapid urinary excretion of the unbound vitamin [123]. The more than twofold elevation of serum aspartate aminotransferase relative to alanine aminotransferase measured in patients with alcoholic hepatitis may be caused by the greater requirement of the latter enzyme for PLP [124]. Pyridoxine is an essential cofactor for cystathionine β-synthase in the transsulfuration elimination pathway of tHcy (see Fig. 20.1) so its deficiency contributes to hyperhomocysteinemia in elderly patients and other susceptible individuals, and may play a role in the enhanced risk of diseases associated with decreased cognitive function [125]. Pyridoxine toxicity was described in women in whom severe sensory neuropathy of the hands and fingers developed after ingestion of 2–6 g/day for 2 months for premenstrual stress [126]. Plasma PLP can be measured by HPLC [121].

Niacin

Niacin is expressed biologically by its two coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP). Both forms are involved in a vast number of redox reactions involved in the generation of energy from carbohydrates, fats, proteins, and ethanol. NADP is also involved in fatty acid and cholesterol biosynthesis, and NAD is the substrate for poly(ADP-ribose)polymerase, which is involved in DNA replication and repair [127]. Dietary niacin is derived from animal protein as tryptophan, and as nicotinic acid and nicotinamide from enriched or whole grain cereals, leafy vegetables, and legumes. Since 60 mg tryptophan is metabolized to 1 mg niacin, one niacin equivalent is the same as 1 mg niacin or 60 mg tryptophan. The DRI of niacin is 16 mg of niacin equivalents for men and 14 mg for women, with 18 mg during pregnancy and 17 mg during lactation (Table 20.2) [128]. Both nicotinic acid and nicotinamide are absorbed passively and completely from the stomach and the small intestine, while low concentrations are absorbed by sodium-dependent facilitated diffusion. Pellagra is the classical disease of niacin deficiency and occurs in populations whose diets contain insufficient animal protein as a source of tryptophan, in particular if corn is used as a grain staple. Once prevalent as a cause of mental illness among the very poor in the southern United States, pellagra was eliminated after Goldberger's classic finding that the missing vitamin occurred in grains other than corn [129]. Still occurring in persons living in areas of Africa and other less-developed countries where there is a scarcity of animal protein, in malnourished chronic alcoholics [130], and sometimes in patients with acquired immunodeficiency syndrome [131], pellagra is clinically manifest by the "three Ds" of diarrhea, dementia, and dermatitis. The dermatological manifestation is a scaly red rash of sun-exposed areas. Characteristic signs of pellagra may also occur in Hartnup disease of congenital impaired tryptophan absorption and in the carcinoid syndrome, in which endogenous trytophan is diverted into serotonin synthesis [132]. Niacin in doses exceeding 1–3 g/day is often used in the treatment of combined hyperlipidemia. The common toxic side effects of flushing and exacerbation of peptic ulcer disease are greatly minimized by the use of lower doses of niacin together with a statin [133]. Niacin status is assessed by measurement of the urinary ratio of its metabolites, *N*-methyl-2-pyridone-5-carboxamide and *N*-methylnicotinamide [121].

Riboflavin

Riboflavin, or vitamin B-2, exists in coenzyme forms as flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), each of which is involved in redox reactions. The term flavoprotein refers to enzymes that use flavins as cofactors. The food sources of riboflavin include milk, eggs, and fortified cereals. The DRIs for riboflavin are 1.3 mg for men, 1.1 mg for women, 1.4 mg during pregnancy, and 1.6 mg during lactation (Table 20.2) [134]. During the process of assimilation, dietary FAD and FMN are released from food protein by gastric acid and then hydrolyzed by intestinal phosphatases. Riboflavin is absorbed in the proximal small intestine by a Na^+-independent, carrier-mediated system [135] that is maximal at an oral dose of about 25 mg and includes an enterohepatic cycle. Riboflavin circulates in the plasma bound to albumin and enters cells by carrier-mediated transport; excretion in the urine accounts for most of its turnover [136]. Riboflavin metabolism appears to be unaffected by liver disease; similar pharmacokinetics were measured in healthy volunteers and in patients with cirrhosis [137]. A major function of flavin coenzymes is the regulation of redox reactions. For example, FAD is essential as a cofactor for glutathione reductase, which regenerates glutathione, the principal antioxidant substrate for the protection of

cellular functions [138,139]. In addition, FMN is required for the generation and activation of pyridoxal phosphate from nonphosphorylated forms of pyridoxine [140]. FAD plays a role as a cofactor for MTHFR in reducing homocysteine (see Fig. 20.1), particularly in individuals who are homozygous for the 677C→T polymorphism [141]. Riboflavin deficiency is often associated with deficiencies of other water-soluble vitamins in the setting of malnutrition associated with chronic alcoholism, celiac disease, and anorexia of any cause. The clinical manifestations include intense glossitis (magenta tongue), cheilosis, angular stomatitis, seborrheic dermatitis, and peripheral neuropathy [136]. Riboflavin status is assessed by determining the level in the urine using HPLC or by colorimetric measurement of erythrocyte glutathione reductase before and after the addition of FAD [121].

Fat-soluble vitamins

Vitamins A, D, E, and K are unlike the water-soluble vitamins by virtue of their solubility in lipid solvents, their predominant roles as cell-signaling molecules, and their sequestration or storage in cellular organelles. By virtue of their nonpolar properties, the fat-soluble vitamins are usually found in the lipophilic core or domain of proteins, cellular membranes, or micellar structures. As a consequence, the essential features of digestion and assimilation of the fat-soluble vitamins involve the interaction of receptors and transporters for fat-soluble vitamins with intestinal mixed micelles and lipoprotein particles [142]. Table 20.3 summarizes sources, requirements, selected functions, mechanisms

Table 20.3 Fat-soluble vitamins

	Vitamin A	Vitamin D	Vitamin E	Vitamin K
Dietary source	Carotenoids: green, yellow vegetables; vitamin A: liver, milk, butter, eggs	Fish oils, liver, fortified milk and cereals, egg yolk	Soy, corn, sunflower, safflower oils, almonds, avocado	Dark green leafy vegetables, intestinal bacteria
Daily requirement	900 µg RE men 700 µg RE women 750 µg pregnancy 1200 µg lactation	200 IU, to age 50 400 IU, ages 50–70 600 IU, older than age 70 (1300 IU, older than age 50)[a]	15 mg adults 19 mg lactation	120 µg men, 90 µg women, 90 µg pregnancy and lactation
Absorption	Pancreatic esterase, micelle incorporation, facilitated transport, carotenoids cleaved by intestinal monooxygenase	Micelle incorporation, facilitated diffusion	Micelle incorporation, passive and facilitated diffusion	Phylloquinone: micelle incorporation, passive and facilitated diffusion Menaquinone: uncertain
Function	Vision, cell differentiation, immune (particularly T- and B-cell) function	Calcium absorption and homeostasis	Antioxidant	Blood clotting: γ-carboxylation of clotting factors II, VII, IX, X; bone mineralization through osteocalcin
Clinical signs of deficiency	Night blindness, xerophthalmia, corneal ulceration, hyperkeratosis, infection risk	Rickets (children), osteoporosis, potential for immune compromise	Abnormal peripheral vision, peripheral neuropathy, hemolysis (newborns)	Decreased blood clotting, osteoporosis
Risks deficiency	Protein malnourishment in infants, chronic alcoholism, intestinal malabsorption (e.g., short bowel syndrome, celiac disease)	Lack of sunshine, intestinal malabsorption (e.g., short bowel syndrome, celiac disease)	Intestinal malabsorption, especially short bowel syndrome, cholestasis	Intestinal malabsorption, prolonged use of antibiotics
Over consumption	Nausea and irritability, blurred vision, growth retardation, enlargement of liver and spleen, hair loss, bone pain, increased spinal fluid pressure, skin changes	Nausea and weight loss, irritability, soft tissue calcification, kidney damage	Possible increase in coagulation/prothrombin time	Few signs
Diagnosis	HPLC	Competitive protein binding of 25(OH)D	HPLC	Prothrombin response to vitamin K

a New consensus. From Heaney [161].
HPLC, high-pressure liquid chromatography; RE, retinol equivalent: 1 µg retinol or 24 µg mixed carotenes.

of absorption, and clinical features of the fat-soluble vitamins. Symptoms of mild deficiencies of the fat-soluble vitamins may develop as a consequence of decreased absorption of dietary lipid or abnormal systemic metabolism of lipids [143].

Vitamin A

Vitamin A includes dietary retinol in its esterified form and the provitamin carotenoid precursors of retinal. The relationships among the most important of the vitamin A precursors, β-carotene, and the essential retinoids that include retinal, retinol (vitamin A), and retinoic acid are shown in the following equations:

(1) β-carotene → 2 retinal (R-CHO) ↔ 2 retinol (R-CH_2OH)
(2) Retinol (R-CH_2OH) + fatty acid (often palmitic acid, R′-COOH) ↔ retinyl ester (R-O-CO-R′)
(3) Retinal (R-CHO) → retinoic acid (R-COOH)

Carotenoids comprise a group of more than 700 compounds that most often contain red, yellow, and orange pigments in their isolated states and are found in many fruits and vegetables [144]. In plants and many single-cell organisms, carotenoids serve as mediators of photoenergy-related processes. For a carotenoid to be essential in animals, a β-bond and three methyl groups in the terminal ring structure must be connected to an unsaturated chain of 10 or more carbons. β-Carotene is abundant and therefore is an excellent biomarker of the intake of fruits and vegetables. Lycopene is another carotenoid found in fruits and vegetables, especially tomatoes. Lutein and zeaxanthin are carotenoids found in green leafy vegetables that are associated with a decrease in the risk of cataracts. Other sources of vitamin A in animal products include retinol in its retinyl ester form, which is found in liver, eggs, and milk products [142,144]. Carotenoids and retinoids that are released from food during digestion are first partitioned into micelles within the intestinal lumen. The retinoids in the micelles are hydrolyzed by specific pancreatic esterases to retinol, whereas the carotenoids are cleaved to form retinal. The movement of retinyl esters and carotenoids into the enterocytes involves both active and facilitated transport mechanisms. Within the enterocyte, retinol is transported to the smooth endoplasmic reticulum for reesterification and eventual incorporation into chylomicrons in preparation for subsequent transport as retinyl esters by the lymphatics to the liver. In contrast, retinoic acid, which constitutes less than 5% of the total vitamin-related products (see equation 3), can be transported by way of the portal circulation by mechanisms similar to those for free fatty acids [142]. Within the enterocytes, β-carotene is cleaved by β-carotene-15,15′-monooxygenase [144] to two molecules of retinal (see equation 1), which is the aldehyde form of retinol. Retinal is converted to retinol by NADH-requiring retinol dehydrogenase, which is identical to alcohol dehydrogenase. Since the availability of purified β-carotene in oil is only half that of retinol (see equation 1), the retinol activity equivalent (RE) of supplemental β-carotene is considered to be 2 : 1, while the average availability of dietary β-carotene in mixed vegetable and fruit diets is only 8% (1/12) that of retinol. Therefore, after absorption and bioconversion, as much as 24 mg or more of mixed dietary β-carotenes is required to yield the same amount of retinol as 1 mg of dietary retinol [145]. The intestinal absorptions of vitamins E and K are decreased in the presence of high doses of vitamin A, whereas vitamin A absorption may be enhanced in the presence of high doses of vitamin E. At high doses, vitamins A and E can be absorbed directly from water-miscible emulsions that are commercially available [146]. The DRIs for vitamin A as REs are 300–900 μg for children and adolescents, 900 μg for adult men, and 700 μg for adult women (Table 20.3) [147]. Retinyl esters are stored in the liver as fat droplets in the hepatic stellate cells. Carotenoids that reach the liver intact are transported together to the circulation in association with very-low-density lipoprotein and are eventually converted to retinoids by tissue oxygenases. In the liver, active exchange of retinyl esters and other retinoids occurs between hepatic stellate cells and parenchymal hepatocytes [142]. Excess vitamin A is rapidly converted to retinyl ester and is sequestered in lipid vacuoles within the hepatic stellate cells. As vitamin A is needed, retinyl ester is hydrolyzed and released as retinol bound to retinol-binding protein (RBP). When released into the circulation, RBP exists as a complex not only with vitamin A, but also with another protein, transthyretin, which binds thyroxine. The primary targets for the RBP–transthyretin complex are the epithelial cells of the fetus, gastrointestinal mucosa, reproductive tract, pulmonary secretory cells, and salivary gland. Within targeted cells, vitamin A interacts with cellular-binding proteins that control its subsequent cellular translocation and metabolism; e.g., oxidation to retinal or to retinoic acid [142].

Vitamin A is essential for normal vision, for dark adaptation, and for epithelial cell differentiation [147,148]. Retinal is a necessary structural component of rhodopsin or *visual purple*, the light-sensitive pigment within the rod and cone cells of the retina. Retinal is covalently associated with rhodopsin and facilitates conformational changes in rhodopsin when excited by photons of light. This interaction is the first step in a cell-signaling cascade that causes excitation of the optic nerve. If inadequate quantities of vitamin A are present, vision is impaired. The identification of two families of nuclear receptors, retinoic acid receptors (RAR) and retinoid receptors (RAX), has helped to explain the role of vitamin A in cell differentiation. RAX and RAR are essential for the regulation of over 600 genes, particularly those involved in epithelial and immune cell differentiation; skin lesions and increased susceptibility to infection are common signs of vitamin A deficiency [142,145]. Further, the epithelial layers of cells become hyperkeratotic and may undergo metaplasia. Defects in the corneal epithelium can result in xerophthalmia, which may progress with the appearance of conjunctival white deposits known as Bitot spots, eventual corneal ulceration,

scarring, and permanent blindness. Moreover, vitamin A deficiency also results in decreased fluid secretion and phagocytic activity of natural killer cells. Fortunately, international vitamin A supplementation programs have resulted in improvements in innate immune function and have significantly reduced life-threatening infections in children worldwide [145]. Nevertheless, much of the world's preventable blindness continues to be the result of vitamin A deficiency [149].

Vitamin A status may also be influenced and augmented by other dietary factors. Protein deficiency contributes to decreased RBP production [142]. Vitamin A deficiency is also common in patients with alcoholic liver disease [143]. Alcohol metabolism increases the microsomal CYP2E1 ethanol-oxidizing enzyme, which in turn influences the rate of catabolism and biliary excretion of hepatic retinoids. The process in which the function of hepatic stellate cells is transformed from vitamin A storage to active collagen production with the initiation of fibrogenesis is increased as stores of retinoid are depleted in response to alcohol and other toxic agents [150]. In contrast, the signs of supplemental vitamin A hepatotoxicity are enhanced by chronic alcoholism [151]. Excessive alcohol consumption also can interfere with the metabolism of supplemental carotenoids and result in the production of carcinogens, particularly in alcoholics who smoke [142]. In the setting of chronic alcoholism, vitamin A deficiency may also induce the development of esophageal squamous cell cancer [152]. In spite of evidence that retinoids may prevent carcinogenesis in vitro, the use of vitamin A and carotenoids in cancer prevention is unproven. For example, the incidence of lung cancer was increased by 28% among smokers participating in the Carotene and Retinol Efficacy Trial (CARET) [153]. While the incidence of breast cancer was found to be inversely proportional to total daily vitamin A intake in one study of premenstrual women [154], a Swedish study showed no relationship between serum retinol levels and breast cancer risk [155]. The increased consumption of dietary lycopene from tomatoes may be associated with a reduced risk of prostate cancer [156]. Vitamin A toxicity can occur with long-term daily ingestion of 25 000–50 000 IU, amounts that are easily obtained from over-the-counter supplements. Signs of vitamin A toxicity include liver failure, headache, vomiting, and desquamating rash [147]. Doses of vitamin A in this range can lead to alopecia, ataxia, and bone and muscle pain. Carotenoids, in contrast, are generally nontoxic, and may be ingested in gram amounts on a daily basis with no deleterious effects [142]

Vitamin A is measured by HPLC of plasma or serum. Radial immunodiffusion assays are also available. The relative dose–response assay is used in field studies and consists of measuring RBP before and after a standard oral dose of vitamin A. Because apo-RBP accumulates in the liver during vitamin A deficiency, an increase of circulating RBP after the administration of a bolus of vitamin A is indicative of vitamin A deficiency [157]. However, because levels of plasma RBP are decreased in severe protein–calorie malnutrition, acute and chronic infection, and trauma, a liver biopsy is the gold standard for the assessment of vitamin A status. The dichotomy of normal serum levels with low hepatic levels of vitamin A was demonstrated in patients with liver disease of different etiologies, including progressive alcoholic liver disease [158].

Vitamin D

Vitamin D is the generic term for a group of sterols that regulate calcium absorption and homeostasis. Vitamin D receptors are also present in the pancreas and on various cells involved in immune function [142]. The principal forms of vitamin D are vitamin D-3 (cholecalciferol), which includes the endogenous form of the vitamin, and vitamin D-2 (ergocalciferol), which is derived from plant sources. Vitamin D-3 is synthesized from 7-dehydrocholesterol in skin on exposure to sunlight and is found in the diet in fish oils, egg yolks, and fortified milk [159]. Persons living in temperate and tropical climates receive most of their vitamin D-3 by synthesis in the skin. However, synthesis of vitamin D in the skin is compromised in the far northern or southern hemispheres, where sunlight is less intense and more variable in amount, and it is less in dark-skinned than in light-skinned peoples. In 1997, the recommended DRI of vitamin D for healthy adults aged 50 years and younger was 200 IU (equivalent to 5 μg/day), 400 IU for persons aged 50–70 years, and 600 IU for those older than 70 years [160]. However, evidence indicates that these amounts are insufficient for the maintenance of normal bone density [159], and a growing consensus indicates that a minimal amount of 1300 IU is required to increase serum vitamin D to a level sufficient to prevent osteoporosis and fracture risk [161]. A study of serum vitamin D levels suggests that the relative potency for vitamin D-2 in humans is about one-third that of vitamin D-3 [162], and recommended preventive supplemental doses should be based on the use of vitamin D-3.

Like other fat-soluble vitamins, dietary vitamin D is absorbed after micellar solubilization in the upper intestine and subsequent facilitated diffusion across the enterocyte membrane. Vitamin D is then incorporated into chylomicrons for entry into the circulation by way of the lymphatics. Two hydroxylation steps are required for the activation of vitamin D. Both vitamins D-3 and D-2 are converted in the liver to 25-hydroxycholecalciferol [25(OH)D3], which is the principal circulating form of the vitamin. Within proximal renal tubular cells, 25(OH)D3 is the substrate for 1α-hydroxylase in the synthesis of 1α,25-dihydroxycholecalciferol ($1,25(OH)_2D3$), which is the functional form of the vitamin. The activity of renal 1-hydroxylase and the production of $1,25(OH)_2D3$ is tightly up-regulated by circulating levels of parathyroid hormone, which is increased in response to low serum calcium, and also increases renal tubular calcium reabsorption while promoting phosphate excretion. The main action of $1,25(OH)_2D3$ is to promote

intestinal calcium absorption and to act with parathyroid hormone to enhance bone remodeling [163]. The $1,25(OH)_2D3$ also regulates gene transcription by interacting with vitamin D membrane receptors and nuclear receptors in many different tissues, and carries the potential to inhibit cell proliferation in breast, colon, lung, and prostate tumor cells [163]. A specific vitamin D-binding protein (DBP), also known as group-specific component (Gc) or vitamin D-binding α-globulin, delivers $1,25(OH)_2D3$ and other active forms of vitamin D to targeted cells. DBP is a member of the albumin, α-fetoprotein, and albumin/afamin gene family [164].

Vitamin D and calcium are poorly absorbed in malabsorption diseases that are characterized by decreased biliary secretion and micelle formation, such as primary biliary cirrhosis or short bowel syndrome, and in diseases involving mucosal abnormality such as celiac disease. A study of Swedish subjects showed that the positive serology indicative of celiac disease was 10-fold more likely among osteoporotic than nonosteoporotic adults [165]. The amount of vitamin D required to treat deficiency in intestinal malabsorption syndromes is not established, and doses up to 50 000 IU of vitamin D-2 (ergocalciferol) three times a week may be required to sustain normal plasma levels of 25(OH)D3 in patients with short bowel syndromes [159].

Osteoporosis with increased risk of bone fracture is the major disease associated with vitamin D deficiency due to insufficient sun exposure or inadequate diet, which reduces calcium absorption and secondarily increases parathyroid hormone. The latter in turn increases the resorption of calcium from bone, which facilitates its eventual loss. If such loss is significant, it may cause osteomalacia in adults or rickets in children, and, most significantly, it increases the risk of osteoporosis in postmenopausal women and elderly men [161]. Several studies have found that bone density can be improved and osteoporosis can be prevented by supplemental vitamin D-3 (cholecalciferol) up to 800 IU daily in combination with calcium up to 1200 mg/day [166, 167]. More recent recommendations include a minimal dose of 1300 IU vitamin D to sustain an adequate serum level of 25(OH)D3 at more than 80 nmol/L to reduce fracture risk [161]. Because vitamin D receptors have been found in numerous types of cells, treatment with $1,25(OH)_2D3$ (calcitriol) has also been reported to be efficacious for conditions ranging from diabetes to psoriasis [168]. In view of its regulatory effects on cell differentiation, several studies suggest that vitamin D supplementation may be beneficial in the prevention of certain cancers. These included a small study on preventive recurrence of prostate cancer [169], and a case–control study that showed an inverse association of plasma levels of 25(OH)D3 with the risk of precancerous colonic adenomas [170]. Vitamin D toxicity occurs after prolonged use of vitamin D-3 at doses greater than 10 000 IU/day by otherwise healthy humans, and is associated with hypercalcemia accompanied by lethargy, confusion, constipation, polyuria, and abnormal elevation of the serum level of 25(OH)D3 (> 800 nmol/L) [168]. Vitamin D status is determined by HPLC measurement of 25(OH)D3 in the plasma. Whereas most laboratories report a level of 50 nmol/L (20 ng/mL) as the lower limit of normal, emerging evidence indicates that calcium absorption and the prevention of osteoporosis fractures is optimal at 25(OH)D3 plasma levels higher than 80 nmol/L (32 ng/mL) [161].

Vitamin E

Vitamin E comprises eight tocopherols, of which two, α-tocopherol and γ-tocopherol, are the most important to human nutrition. The predominant natural form of vitamin E is the RRR isomer of α-tocopherol [171]. The major food sources of vitamin E include polyunsaturated vegetable and seed oils, whole grains, nuts, and green leafy vegetables. The DRIs of vitamin E are 6–11 mg for children, 15 mg for adults of all ages, 15 mg during pregnancy, and 19 mg during lactation [172]. The intestinal absorption of dietary vitamin E includes deesterification by pancreatic esterases, followed by bile-dependent incorporation into intralumenal micelles, diffusion, or facilitative transport into enterocytes, and incorporation into chylomicrons for transfer by way of the lymphatics. After peripheral hydrolysis of chylomicrons, vitamin E as *RRR*-α-tocopherol returns to the liver with chylomicron remnants and is then transferred by hepatic α-tocopherol transfer protein to high-density or low-density lipoprotein. The subsequent transfer to tissues occurs by receptor-mediated endocytosis of low-density lipoprotein. As a consequence, plasma levels of α-tocopherol vary according to the total plasma lipid concentration and often correlate with values for total cholesterol. Once within cells, vitamin E is incorporated into lipid membranes, about 40% into nuclear membranes and the remaining 60% into lysosomal, mitochondrial, and outer cell wall membranes. The efflux of vitamin E from cells is less well understood, but it appears to be dependent on the ATP-requiring transporters associated with the cholesterol transporter family, [173].

Tocopherols are unique because they act at a chemical level as antioxidants, although possible roles in cell signaling have also been described [173,174]. Vitamin E protects unsaturated fatty acids found in the phospholipids of cell membranes. Cell membranes contain vitamin E at a concentration of about 1 mg per 5–10 g of membrane lipid; a concentration sufficient to retard membrane lipid oxidation. Membrane lipids are constantly engaged in the process of turnover and repair, and vitamin E in membranes is consumed while inhibiting the formation of lipid-derived oxidation products. By prolonging the initiation time before a free-radical chain reaction occurs, vitamin E gives cells time to replace damaged membrane lipids through the process of normal cell turnover [174].

Vitamin E deficiency occurs mainly in patients with malabsorptive disorders involving the biliary circulation, pancreas, and intestinal mucosa. Some infants can also be at risk because

their body stores of vitamin E are lower. Children with biliary atresia or other causes of cholestasis become depleted more rapidly than older children and adults with malabsorption diseases [175]. Vitamin E deficiency is manifested by neurological damage involving the posterior columns, cranial nerves, brainstem, and peripheral nerves with loss of balance and peripheral neuropathy, and by retinal damage with visual field defects [173]. A water-soluble form of vitamin E, RRR-α-tocopherol glycol (Aquasol E), is better absorbed than the dietary, fat-soluble natural vitamin and hence is more effective in the treatment or prevention of vitamin E deficiency in malabsorption diseases such as short bowel syndrome [175].

Vitamin E supplementation in the form of RRR-α-tocopherol at daily doses up to 800 IU has been used in several trials for the prevention of cardiovascular disease with conflicting and nonconclusive results; furthermore vitamin E carries the risk of increasing bleeding when combined with aspirin [173]. Other diseases associated with an inconclusive preventive effect of vitamin E supplementation include Alzheimer disease, several cancers, and cataracts [173]. One study suggested a positive effect of vitamin E in combination with ursodeoxycholic acid in the reduction of liver fat in patients with nonalcoholic steatohepatitis [176]. Although many food sources of vitamin E contain both α and γ forms of tocopherol, the functional potency of supplemental γ-tocopherol is only about 10% that of α-tocopherol; hence only the latter form should be used in preventive trials [173]. Vitamin E status is assessed by HPLC measurement of plasma levels. Normal values of α-tocopherol are greater than 12 μmol/L (5 μg/mL). However, since the plasma level of vitamin E is regulated in part by plasma lipid levels, more correct values include α-tocopherol at greater than 8 mg per the sum of triglycerides and total cholesterol levels, or 2.8 mg per g total cholesterol [173].

Vitamin K

Vitamin K activity is associated with phylloquinone (K1) and menaquinone (K2-5) derivatives of 1,4-naphthoquinone [177]. The main dietary sources of phylloquinone are dark green leafy vegetables and some vegetable oils. Recent comparisons of the forms of vitamin K in the liver suggest that menaquinones produced by enteric bacteria provide substantially less vitamin K than dietary phylloquinone [142]. The major function of vitamin K is to activate blood clotting by serving as a cofactor for microsomal synthesis of γ-carboxyglutamic acid (Gla) residues, which permit the formation of specific calcium-binding sites in prothrombin (factor II) and factors VII, IX, and X. Gla residues are also found in proteins C and S, which are anticoagulants by virtue of their ability to inhibit factor V [177]. Vitamin K is also essential for the γ-carboxylation of osteocalcin, an osteoblast protein that is also up-regulated by $1\alpha,25(OH)_2D3$. Therefore, deficiency of vitamin K may contribute to osteoporosis, whereas vitamin K supplementation has been shown to prevent bone fractures [178].

There is no precise DRI for vitamin K, and the recommended adequate intake for dietary phylloquinone is 120 μg/day in men and 90 μg/day in women, while uncertain amounts are derived from intestinal bacterial synthesis of menaquinone (Table 20.3) [179]. As a fat-soluble vitamin, phylloquinone requires micellar solubilization before diffusion into enterocytes and uptake with chylomicrons. Phylloquinone circulates with chylomicrons with a high triglyceride level and very-low-density lipoproteins, and blood levels are dependent on plasma lipid concentrations. In contrast, menaquinones are synthesized by anaerobic intestinal bacteria and their route of absorption remains unclear [177].

Vitamin K deficiency may occur in neonates as a result of immature metabolic pathways, insufficient intestinal bacterial synthesis, and low levels in breast milk. Hemorrhagic disease of the newborn is prevented by a single injection of phylloquinone to all neonates, as recommended by the American Academy of Pediatrics [180]. Phylloquinone deficiency in adults occurs in biliary diseases, malabsorption disorders such as the short bowel syndrome, and celiac disease. Ineffective use of vitamin K occurs in individuals adhering to long-term regimens of the antagonist coumadin and in patients with severe liver disease that leads to impaired synthesis of coagulation factors irrespective of vitamin K adequacy. Also, long-term oral regimens of antibiotics may suppress menoquinone-synthesizing anaerobic bacteria and lead to vitamin K deficiency [177]. A large study of elderly people found that those in the highest quartile of dietary vitamin K intake had a reduced risk of hip fracture compared to those in the lowest quartile of vitamin K intake, although there was no relationship of these findings to bone mineral density measurements [181]. Vitamin K deficiency can be assessed by a prolonged prothrombin time that responds to parenteral vitamin K administration [177] and by direct measurement of plasma levels of phylloquinone by HPLC [121].

Minerals

This section describes the sources, requirements, absorptive mechanisms, functions, and clinical causes and effects of deficiencies of the macrominerals calcium and magnesium and of the trace minerals iron, zinc, and copper (Table 20.4).

Calcium

Sources and bioavailability

Milk and other dairy products provide up to 75% of dietary calcium; the remainder comes from vegetables such as spinach, cabbage, kale, and various beans. The bioavailability of calcium from milk, dairy products, and meat is high, perhaps because the smaller peptides formed during digestion keep calcium in an absorbable, soluble form [182]. Calcium is

Table 20.4 Minerals and trace elements

	Calcium	Magnesium	Iron	Zinc	Copper
Dietary source	Milk, dairy products, fortified juices, green vegetables, beans	Vegetables, fruits, dairy, animal products	Meat, fish, poultry, fortified cereals	Animal protein, seafood, especially oysters	Meat, grain, nuts
Daily requirement	1000 mg, men and women 19–50 years; 1200 mg, men and women older than 50	420 mg men 320 mg women	8 mg men 18 mg women, 8 mg postmenopause	11 mg men 11 mg women	0.9 mg men and women
Absorption	Active transport by way of vitamin D; passive diffusion	Active transport, passive diffusion	Active transport, passive diffusion	Active transport, passive diffusion	Active transport, passive diffusion
Function	Bone formation; muscle and nerve function	Bone metabolism; nerve, muscle, cardiac functions	Oxygen transport as heme, energy metabolism, neurotransmitter synthesis	Cofactor for many enzymes involved in nucleic acid, protein, lipid, carbohydrate metabolism	Connective tissue synthesis, bone growth, ferroxidase activity
Clinical signs of deficiency	Bone loss, tetany	Cardiac arrhythmia, osteoporosis, tetany	Microcytic anemia, fatigue, pallor	Eczema, alopecia, delayed growth	Bone abnormalities, hypopigmentation, impaired growth
Diagnosis	Bone density scan	Serum magnesium	Hemoglobin, iron-binding capacity, ferritin, transferrin receptor	Serum zinc	Serum copper, red cell superoxide dysmutase

less available from meat products and plants [183]. Plant fiber that contains phytate has a negative effect on calcium absorption. Although not directly affecting calcium absorption, high intakes of caffeine and protein have a negative effect on calcium balance by increasing urinary losses [183]. Adequate intakes for calcium include 1000 mg/day for adults under 50 years, 1200 mg/day for adults over 50 years, and 1300 mg/day for teenagers and young pregnant and lactating women [184]. These levels can most reliably be achieved from supplements and fortified beverages [182].

Absorption and homeostasis

Calcium is ionized at low gastric pH and is absorbed actively in the duodenum and by passive diffusion from the ileum [185]. In addition, about 7% of total calcium absorption occurs by active and passive processes in the colon [186]. Active absorption by way of calcium-binding proteins dominates at low levels of calcium intake, whereas passive diffusion makes a significant contribution to net calcium uptake at high levels of intake [187]. Transepithelial calcium transport in the upper part of the duodenum is dependent on vitamin D ($1,25(OH)_2$D-3) and consists of three mechanisms. First, brush border uptake occurs down a steep electrochemical gradient from the lumen to the cytoplasm through calcium channels that are not voltage gated [185]. Second, rate-limiting transport of calcium ions through the enterocytes occurs by carrier-mediated facilitated diffusion from the brush border membrane to the basolateral membrane [188]. Diffusion of calcium is facilitated by the cytosolic calcium-binding protein known as calbindin 3 (CALB3, or D9K). Transcellular calcium transport increases in a linear fashion according to the cellular content of calbindin [189]. Another calcium-binding protein, calbindin 1 (CALB1, or D28K), is induced by vitamin D and binds four calcium ions, compared to two for calbindin 3 [190]. Third, the extrusion of calcium through the basolateral membrane is mediated by calcium ATPase and the Na^+/Ca^{2+} exchanger, and occurs against an electrochemical gradient [191]. Vitamin D has some effect on both the uptake and the extrusion phases of calcium by increasing the gene expression of membrane-bound calcium ATPase [192]. Passive absorption of calcium occurs by paracellular diffusion down a chemical gradient in all sections of the small intestine [185] and accounts for the major part of calcium absorption when intake is adequate or high. During pregnancy, calcium absorption increases significantly, which may be a response to the high fetal requirement. Lactation, however, does not result in a similar increase in calcium absorption.

Disorders of calcium homeostasis

Intestinal calcium absorption decreases with age, possibly because of lower circulating concentrations or decreased responsiveness of the intestine to vitamin D [193]. Achlorhydria reduces the absorption of calcium carbonate [194], and long-term regimens of proton pump inhibitors increase the risk for osteoporosis and hip fractures [195]. Gastric

surgery often disrupts calcium absorption because of the lack of gastric acid, resulting in osteomalacia as a late complication [196]. However, patients with achlorhydria can absorb calcium normally from milk because of the solubilizing effect of milk peptides, which are not as pH-dependent. Diseases of the small intestine such as celiac disease, Crohn's disease, and short bowel syndrome often result in osteomalacia, usually as a consequence of vitamin D deficiency [196]. Also, increased fat excretion in malabsorption disorders also causes losses of calcium bound to fatty acids. Renal disease can result in lower production of $1,25(OH)_2D3$ and thus decreased calcium absorption. Therefore, $1,25(OH)_2D3$ is used in the management of chronic renal disease to preserve calcium absorption. Whereas some drugs, like glucocorticoids and phenytoin, directly inhibit calcium absorption in the intestine, thiazides increase the renal reabsorption of calcium and thereby decrease parathyroid hormone secretion and its effect on $1,25(OH)_2D3$ synthesis, indirectly reducing calcium absorption. Vitamin D intoxication also increases calcium absorption [197]. Osteoporosis, which is the principal result of calcium deficiency, is caused by a combination of inadequate levels of vitamin D and calcium. Calcium supplementation is often required to prevent osteoporosis and hip fracture – the latter being a leading cause of nursing home admission, and carrying up to 20% mortality [198]. In addition to other measures such as exercise and adequate vitamin D supplementation, calcium should be provided at 1200 mg/day for those at risk of osteoporosis. A case–control study demonstrated that high calcium intake was associated with a decreased risk of colorectal cancer in men with elevated levels of insulin-like growth factor [199]. Doses of calcium higher than 2500 mg/day may be toxic and can induce hypercalciuria and the formation of kidney stones [184].

Magnesium

Sources and bioavailability

Magnesium is abundant in whole grains, nuts, seeds, broccoli, spinach, beans, dairy products, meat, and mineral waters that are high in magnesium. The DRI for magnesium is 420 mg for adult men and 320 mg for women, with 400 mg during pregnancy and 360 mg during lactation [200].

Absorption and homeostasis

Magnesium absorption occurs primarily in the ileum and the colon and much less is absorbed in the duodenum [201]. Human studies over a wide range of magnesium intakes show a saturable absorption curve that is indicative of an active transport mechanism, as well as a linear function that is compatible with passive diffusion [202]. The efficiency of absorption with a normal dietary intake of magnesium is 21%–27% in adult men and women [203]. Magnesium homeostasis is largely regulated by renal excretion and the efficiency of magnesium absorption increases during deficiency.

Deficiency

Magnesium deficiency secondary to low dietary intake is uncommon and difficult to diagnose because of a lack of reliable markers [204]. The serum or plasma magnesium level is relatively tightly regulated and, although used for clinical decision-making, may not reflect actual tissue levels. Congenital primary hypomagnesemia is an uncommon disorder that is correlated to a specific defect in the intestinal absorption of magnesium [205]. Hypomagnesuria, hypocalcemia, hypokalemia, and tetany often occur in association with convulsions [206], which are corrected by the administration of magnesium. The hypocalcemia of magnesium deficiency is caused by the decreased responsiveness of osteoclast parathyroid hormone receptors and the inhibition of parathyroid hormone secretion, which then decreases the renal production of $1,25(OH)_2D$-3, thereby decreasing the intestinal absorption of calcium [207]. Vitamin D does not correct the hypocalcemia, whereas correction of magnesium levels restores calcium levels to normal. Experimentally induced magnesium deficiency in human volunteers resulted in hypocalcemia, hypokalemia, nausea, vomiting, arrhythmia, and personality changes that were resolved by magnesium replacement [204]. Clinical conditions that contribute to magnesium depletion include malabsorption syndromes [208] such as celiac disease and the short bowel syndrome, renal dysfunction, and treatment with diuretics, which may induce excessive magnesium losses [209]. Endocrine disorders that cause magnesium depletion include diabetes, hyperparathyroidism, and hyperthyroidism [210]. Chronic alcoholism and protein–calorie malnutrition are often associated with poor magnesium status [211,212]. Potassium depletion, protein catabolism, and chronic acidosis all reduce cellular magnesium levels and, consequently, levels of magnesium in muscle and bone [204].

Iron

Sources, forms, and bioavailability

The human diet contains both heme iron and nonheme iron, which are absorbed and metabolized differently. The efficiency of iron absorption from heme is 20%–25%, whereas that of nonheme iron ranges from 3% to 10% [213]. Heme iron is present in myoglobin and hemoglobin in meat, poultry, and fish, and it is the presence of globin that increases its absorption. While heme iron absorption is relatively unaffected by dietary factors, food components may have widely different effects on the absorption of nonheme iron. Ascorbic acid has a strong enhancing effect on nonheme iron absorption [214], whereas phytate, tannins, and other polyphenols are inhibitory [215]. Long-term studies of children and lactating and premenopausal women given high daily doses of calcium (500–1000 mg) for 6–12 months showed no change in iron status as assessed by hemoglobin or serum ferritin levels [216–218]. The DRI for iron is 8 mg for men and women older than 50 years; 15 mg

for young women aged 15–18 years, and 18 mg for older premenstrual women (Table 20.4) [219].

Absorption and homeostasis

Heme and nonheme iron are absorbed by way of different pathways and their levels are affected to different degrees by dietary components, physiological conditions, and iron status. Heme-carrier protein 1 (HCP1) was isolated from mouse duodenal plasma membrane. A highly hydrophobic transporter with 12 transmembrane regions, HCP1 has a high expression level in the duodenum and is present on the apical membrane during iron deficiency and in the cytoplasm of iron-loaded mice [220]. Intracellular heme oxygenase breaks down the heme group, releasing iron into the nonheme pool for incorporation into intracellular ferritin and for export out of the cell [221]. Several mechanisms involved in the mucosal regulation of nonheme iron absorption have been discovered (Fig. 20.3). Duodenal cytochrome b (Dcytb) reduces ferric iron (Fe^{3+}) to the more absorbable ferrous (Fe^{2+}) form [222]. Divalent metal transporter (DMT1, also called DCT1 or NRAMP2) is located in the brush border membrane and is involved in the regulation of nonheme iron uptake by the enterocyte. DMT1 is primarily located in villus tip cells and is up-regulated during iron deficiency and down-regulated in situations of iron excess [223]. In addition to Fe^{2+}, many other cations are taken up by brush border DMT1, including Zn^{2+}, Cu^{2+}, Cd^{2+}, Co^{2+}, Mn^{2+}, Ni^{2+}, and Pb^{2+} [224]. Within the mucosal cell, the expression of the iron storage protein ferritin is regulated by the intracellular concentration of iron, such that ferritin synthesis is low when iron status is low and increases when iron is present in excess [225]. The transferrin receptor (TFR) contains five iron-responsive elements that regulate the stability and turnover of iron stores. TFR is located both in endosomal compartments and in the basolateral membrane of the enterocyte [226]. Iron transfer from the enterocyte into the portal circulation requires the oxidation of Fe^{2+} to Fe^{3+}, a process that is facilitated by hephaestin (Hp), a copper-containing ferroxidase that is associated with the basolateral membrane [227]. The oxidation process allows Fe^{3+} to be incorporated into apotransferrin (apoTF) in the circulation. In combination with the hemochromatosis protein (HFE), TFR permits the successful binding of apoTF-bound iron and its reuptake back into the intestinal cell [228]. The export of iron out of the enterocyte is also mediated by ferroportin 1 (FPN1, also called IREG1 or MTP1) [229]. FPN1 is a membrane-bound protein of 62 kDa, with 10 transmembrane regions. FPN1 contains iron-responsive elements [230] and is up-regulated during iron deficiency and down-regulated during iron excess [231]. Hepcidin, an antimicrobial peptide produced by the liver, modulates enterocyte iron transport and is believed to be the communication link between liver iron stores and intestinal iron uptake, facilitating increased iron absorption during iron deficiency and decreased iron absorption during iron repletion [232]. Hepcidin levels parallel liver iron stores in adults and evidence indicates that hepcidin binds to FPN1, stimulating FPN1 internalization and degradation, and so reducing iron absorption when liver iron stores are high [233] (see Fig. 20.3).

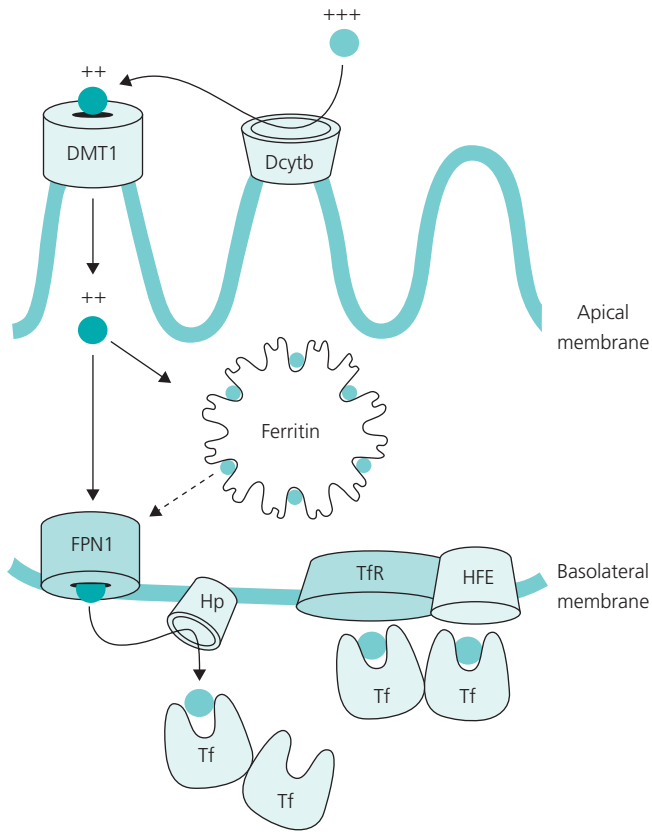

Figure 20.3 Interaction of potential mechanisms for the regulation of iron absorption by the mucosal cell. Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1; FPN1, ferroportin 1; HFE, hemochromatosis gene product; Hp, hephaestin; Tf, transferrin; TfR, transferrin receptor.

Disorders of iron homeostasis

Hereditary hemochromatosis is an autosomal recessive genetic disorder of defective regulation of iron absorption that leads to severe iron overload in various tissues of the body, particularly the liver [234]. The gene frequency of hereditary hemochromatosis is about 1 in 300; it varies with ethnicity and is highest among Northern Europeans. To prevent cirrhosis of the liver secondary to iron loading, patients are either phlebotomized regularly or given chelation therapy with desferrioxamine. The most common form of hereditary hemochromatosis involves mutations in the hemochromatosis gene *HFE*, which leads to inappropriately low levels of hepcidin [235], an inappropriately high degree of iron absorption, and hepatic iron overload. More than 95% of patients with hereditary hemochromatosis have a missense mutation in the *HFE* gene at 282C→Y, which affects a highly

conserved cysteine residue and is expressed at low levels in most tissues including the small intestine. *HFE* is highly expressed in liver and appears to be involved in the down-regulation of iron absorption that normally occurs when iron stores are high, possibly by way of binding to the transferrin receptor. The homozygous mutation at 282C→Y causes a defect in HFE protein trafficking and cell surface expression as a consequence of the inability to bind β_1-microglobulin [236]. An additional *HFE* mutation at 63H→D does not cause iron overload [237], but compound heterozygotes 63H→D/282C→Y develop iron overload with less severe clinical features of hereditary hemochromatosis than patients who are homozygotes for 282C→Y [238]. Iron overload develops in β_1-microglobulin-deficient mice, indicating that HFE must interact with β_1-microglobulin to regulate iron absorption [239]. There are also patients with hereditary hemochromatosis who have mutations in other proteins involved in iron homeostasis, such as hepcidin and transferrin receptor 2 (TFR2). Mutations in TFR2 cause iron loading disease with symptoms similar to those manifest in hemochromatosis as a result of defective HFE [240]. Hemojuvelin, a third member of this regulatory network to be identified [241], is expressed in hepatocytes and is mutated in most cases of juvenile hemochromatosis. Sickle cell disease and β-thalassemia are also genetic disorders that lead to abnormal iron metabolism. Whereas hereditary hemochromatosis is characterized by the preferential loading of iron into parenchymal cells, iron first accumulates in the reticuloendothelial cells in sickle cell disease or β-thalassemia [242]. Clinically, the primary cause of mortality in hereditary hemochromatosis is cirrhosis and hepatocellular carcinoma, whereas it is cardiomyopathy in β-thalassemia, [243]. The toxicity of iron overload is minor in sickle cell disease compared to β-thalassemia.

Deficiency and excess

Iron deficiency as the result of a dietary lack of iron is the most common single nutrient deficiency worldwide, afflicting up to 1 billion people in both less developed and industrialized countries. It is estimated that 500–600 million persons have iron deficiency anemia. In infants and young children, iron deficiency anemia causes delays in cognitive and motor development [244], and adult iron deficiency anemia is typically associated with impaired immune function and energy metabolism [245]. Iron deficiency is diagnosed by the serum ferritin concentration, which is directly proportional to body iron stores and decreases when the body becomes depleted of iron. A serum ferritin value less than 12 μg/L is used as the cutoff for iron deficiency and correlates with lack of stainable iron in the bone marrow. Progressive iron deficiency impairs hematopoiesis, resulting in microcytic anemia. Since the level of serum ferritin, but not of TFR, increases during infection and inflammation, a rising circulating serum TFR level is considered to be a more accurate additional test for iron deficiency [246]. The TFR–log ferritin ratio was proposed as an accurate method to detect iron deficiency in patients with chronic renal disease [247]. The second most common cause of iron deficiency is occult or overt chronic gastrointestinal blood loss from a wide variety of lesions. Excessive menstrual bleeding may contribute to iron deficiency in premenopausal women. Iron deficiency secondary to intestinal malabsorption is often the only clinical feature of celiac disease [25]. Bariatric surgical procedures that exclude the duodenum such as the Roux-en-Y–gastric bypass are significant causes of occult iron deficiency [248]. Iron overload can also occur in a significant proportion of individuals with hereditary hemochromatosis, homozygous thalassemia, or sideroblastic anemia secondary to pyridoxine deficiency [249]. Therefore, an overly zealous approach to providing iron to large population groups may harm individuals who have large iron stores. Concern has also been raised that high iron stores, although not yet characterized as iron overload, can lead to increased free radical formation and chronic disease. For example, Finnish men with serum ferritin levels greater than 200 μg/L have a significantly higher risk for coronary heart disease and non-insulin-dependent diabetes (diabetes type II) than men with normal serum ferritin levels [250]. Adverse effects of iron supplements on infant growth have been reported [251], and a study on children in Zanzibar had to be interrupted because iron supplementation caused increased morbidity and mortality [252].

Zinc

Sources, forms, and bioavailability

Zinc is a cofactor for a diversity of enzymes and transcription factors. Severe zinc deficiency affects many metabolic pathways and tissues, impairing growth, development, reproduction, and immune function [253]. The daily dietary intake of zinc is about 10–15 mg, and generally meets the requirement of men but is often marginal for women, particularly during pregnancy and lactation. The DRI for men is 11 mg; for non-pregnant women it is 8 mg; for young women aged 18 years and younger it is 13 mg; and for pregnant and lactating women it is 14 mg. For women ages 19–50 years the DRI is 11 mg and for women older than 50 years it is 12 mg [253]. The best dietary sources of zinc are meat, meat products, and seafood. Whole grains and cereals are relatively high in zinc, but bioavailability is often low because of the presence of chelating phytates in the diet. Multivitamin and mineral tablets often contain zinc, and some cereal products are fortified with zinc. Phytates in cereals and legumes have a strong inhibitory effect on zinc absorption as a consequence of their chelating properties [254]. Attempts to increase the bioavailability of zinc from human diets include alternative food processing, phytase treatment, and the development of genetically modified cereals that are lower in phytate [255]. The protein content of the diet is positively correlated to zinc absorption, most likely because of the formation of amino acids and small peptides that facilitate zinc uptake by the

enterocyte [256]. Iron in high doses can reduce zinc absorption from oral supplements [257], but this does not occur when both are given with a meal [258].

Absorption and homeostasis

The efficiency of zinc absorption from the diet is about 15%–35% in adults [259]. Active transport dominates at low or normal intake, whereas passive diffusion contributes more significantly at high intake [260]. Metallothionein is a low-molecular-weight (~ 6 kDa) zinc-binding protein, present in the cytosol of the enterocyte. Metallothionein also binds copper and cadmium but induces zinc only at very high intake levels [261]. Members of the ZIP (Zrt-, Irt-like protein) family transport zinc from the extracellular space or organelles into the cytoplasm [262]. The ZIP4 transporter was discovered when mutations in its gene were linked to the human recessive genetic disorder acrodermatitis enteropathica [263]. Since zinc supplements alleviate zinc deficiency in patients with acrodermatitis enteropathica, it is obvious that other zinc transporters exist on the apical membrane of the enterocyte. The ZIP5 protein is expressed on the basolateral membrane of the enterocyte, and may be responsible for zinc transport from the systemic circulation into the enterocyte when dietary zinc is low [262]. ZIP14 is involved in the uptake of zinc by the liver in response to acute inflammation and infection [264]. ZIP14 expression in the liver rises in response to IL-6 during the acute-phase response [262,264], suggesting that the induction of ZIP14 is responsible for the hypozincemia associated with infection. The ZNT family of zinc transporters has nine members in the human genome. ZNT1 production is regulated by dietary zinc [265] and has been implicated in the regulation of zinc homeostasis by controlling zinc efflux, whereas ZNT2 and ZNT4 are involved in the flux of zinc in the endosomes, possibly regulating the intracellular trafficking of zinc. These membrane transporters all have six transmembrane-spanning domains and a conserved histidine-rich region predicted to have a cytoplasmic loop that likely functions to bind zinc [266,267]. Experiments showing zinc sequestration by endosomal vesicles during overexpression of ZNT2 suggest that this transporter may be important for controlling intracellular transport of zinc by the enterocyte [268]. All three transporters occur mainly in villus cells, and the ileum is the major site for ZNT1. ZNT4 is involved in the secretion of zinc by the mammary gland, and mutations of the gene cause the "lethal milk" defect in mice [269]. ZNT3 is localized to synaptic vesicles in some types of neurons [270], and ZNT8 is associated with the secretory granules of pancreatic beta cells [271].

Deficiency and excess

While severe zinc deficiency is rare and is usually seen only in patients with acrodermatitis enteropathica, marginal zinc deficiency is common [272]. The diagnosis of milder forms of zinc deficiency is difficult, partly because the plasma zinc level is affected by many factors other than zinc. Zinc supplementation trials show positive effects on the growth of infants and children [273], pregnancy outcome [274], and infantile diarrhea [275]. Zinc deficiency is a leading cause of preventable deaths in children worldwide [276]. A meta-analysis of zinc supplementation in children reported significant increases in height and weight, with the greatest effects observed in children with stunted growth [277]. Pooled analyses of trials evaluating the effect of daily zinc supplementation on infectious disease reported a robust decrease (~ 40%) in death related to diarrhea and pneumonia [278]. The consistent positive effects on the treatment of infant diarrhea prompted the inclusion of zinc into oral rehydration solution [279], the administration of which reduces stool output and duration of diarrhea [280].

Disorders of zinc homeostasis

Acrodermatitis enteropathica is an inborn autosomal recessive disorder of zinc metabolism with a gene frequency of about 1 in 10 000, and is manifested by eczema, dermatitis, anorexia, compromised immune function, and poor growth when infants are weaned [281]. Patients require daily doses of zinc, usually 30–50 mg, and higher doses during periods of rapid growth, such as at puberty. If doses are too high, however, copper deficiency can result, causing other clinical problems [282]; therefore, both the zinc status and the copper status of these patients must be monitored. More generalized malabsorption may be a secondary problem resulting from mucosal damage caused by local zinc deficiency. Patients treated with zinc appear to absorb zinc normally [282]. Rarely, the features of acrodermatitis enteropathica occur in patients being treated long-term with total parenteral nutrition formulas that do not contain zinc.

Copper

Sources and bioavailability

Copper is relatively abundant in meat, grains, and nuts, but is scarce in milk and dairy products. Copper intakes are relatively close to the DRI of 0.9 mg for men and women [283]. Environmental factors, such as soil, water source, fertilizer use, processing, and cooking, affect the copper content of the diet [284]. Copper absorption from the diet is efficient, and few dietary components strongly affect its bioavailability [285]. High intakes of iron or zinc, however, may lower the copper status if the ratio between these elements and copper remains high for a long period [286]. Also, ascorbic acid may lower copper absorption in animals by reducing well-absorbed divalent cupric to monovalent cuprous copper [287], although young adult volunteers given high levels of ascorbic acid daily (600 mg) showed no change in copper status [206].

Absorption and homeostasis

Copper absorption is mediated by both active transport and passive diffusion [287]. Animal studies suggest that the

duodenum is the major site of absorption, but some absorption also occurs in the stomach and ileum [287]. Copper is also excreted in bile and only 15% is reabsorbed through the enterohepatic circulation [287]. Cellular copper homeostasis is maintained by a delicate balance of copper uptake and efflux, which are mediated by highly conserved membrane proteins [288] that include copper-transporting P-type ATPases [289]. Cytoplasmic low-molecular-weight copper-binding proteins steer copper ions to their incorporation into proper apoproteins [288]. The expression of human copper transporter 1 (CTR1) increases the uptake of copper in yeast cells and transfected human fibroblasts [290], whereas the *Ctr1* knockout mouse is embryolethal [291]. Ceruloplasmin and hephaestin are copper-dependent enzymes involved in iron homeostasis (see previous section on iron absorption and homeostasis), and the heterozygous *Ctr1* knockout mice has decreased tissue levels of iron, most likely a consequence of impaired delivery of copper to its apoproteins. Studies of CaCo-2 cells exposed to excess levels of copper show that Ctr1 is internalized by endocytosis [292]. Ctr1 also localizes to the hepatocyte membrane in adult mouse liver and may participate in copper uptake by the liver [293]. The copper-transporting ATPase ATP7A is responsible for copper export from the enterocyte [294], while ATP7B, which is predominantly expressed in the liver, transports copper into the secretory pathway for incorporation into apoceruloplasmin [295].

Disorders of copper homeostasis

Menkes disease is an X-linked recessive disorder of copper absorption and results in copper deficiency at a very young age [296]. The incidence of Menkes disease is between 1 in 50 000 to 1 in 100 000 live births. Symptoms include pallor, lax skin, bone fractures, osteoporosis, altered pigmentation of skin and hair (secondary to low levels of melanin formation), growth failure, mental retardation, and microcytic anemia (secondary to low levels of ceruloplasmin and hephaestin). Oral copper treatment is ineffective and patients often die at a young age. The gene was identified in mouse models, with altered ATP7A Menkes disease (MNK) expressions [289,297]. Thus, copper is blocked in the enterocyte, and little copper is transported into the systemic circulation, resulting in severe copper deficiency. In Wilson disease, excessive amounts of copper accumulate in the body, particularly in the liver and the brain, and clinical symptoms include cirrhosis, eye lesions (specifically Kayser–Fleisher rings), kidney malfunction, and neurological problems [298]. Despite very high levels of copper in the liver, serum levels of copper and ceruloplasmin are low. Treatment includes chelation therapy with drugs such as penicillamine or trientine, but high oral doses of zinc (40–50 mg/day) are also effective because they inhibit copper absorption [299]. Wilson disease is an autosomal recessive disorder, occurring in 1 in 30 000 live births. This disorder of copper metabolism has also been shown to be caused by a defective transporter, in this case ATP7B or the WD protein [300]. Copper absorption per se does not appear to be dysregulated in these patients; rather, tissue copper metabolism, particularly in the liver, is affected, causing an excessive cellular accumulation of copper. The outcome for these patients, if treated, is usually good, although continuous monitoring of copper, zinc, and iron status is necessary.

Deficiency and excess

Risks for copper deficiency include low iron stores in the liver of premature infants, a rapid growth rate, malabsorption syndromes, and increased copper losses [301]. Low serum levels of copper are associated with anemia that is refractory to iron supplementation because of low ferroxidase and hephaestin activities and are accompanied by neutropenia, bone abnormalities, hypopigmentation of hair and skin, impaired growth, and an increased incidence of infections [302]. Decreased phagocytic capacity of neutrophils and impaired cell immunity affect the immune system [303]. Acute copper toxicity is rare and is usually caused by the consumption of contaminated foods or beverages, or by the accidental or deliberate ingestion of large quantities of copper salts [304]. Symptoms include nausea, vomiting, and diarrhea. Indian childhood cirrhosis has been reported in families consuming milk boiled or stored in brass or copper containers [305]. Children consuming such milk may ingest up to 1 mg of copper per kilogram of body weight per day, which is enough to explain the observed liver damage. Infants and children in the Austrian Tyrol were reported to have died of liver cirrhosis secondary to chronically high levels of copper intake [306]. In these cases, inheritance followed the typical pattern of a mendelian recessive trait in individuals who were particularly sensitive to copper exposure. Whether a genetic disorder of copper metabolism is present in patients with liver cirrhosis is not known, but the possibility should be explored in light of the new findings of copper transporters in humans.

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D NUTRITION

21 General nutritional principles

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Basic nutritional principles

Body composition

The human body consists of 35 components that are organized into five levels of increasing complexity: atomic (e.g., nitrogen, potassium), molecular (e.g., water, protein), cellular (e.g., body cell mass, intra- and extracellular fluid), tissue (e.g., skeletal muscle, adipose tissue), and whole body (e.g., weight, height). A healthy, lean man is composed of 55%–60% water, 15%–20% fat, 15%–20% protein (one-half in skeletal muscle), 1% glycogen (four-fifths in muscle, one-fifth in liver), and 4% minerals [1]. Although sophisticated techniques are available to measure each body component, the definitions of some commonly used terms can be confusing. *Fat mass* represents all body triglycerides, which are present in adipose tissue, muscle, and liver. *Adipose tissue* is about 83% fat (e.g., triglyceride), 15% water, and 2% protein. *Fat-free mass* refers to total body mass minus total fat mass. *Lean body mass* is defined as total body mass minus adipose tissue. The body also can be divided into cellular and extracellular mass. *Body cell mass* is defined as the cellular components of all tissues (35%–45% of the body weight in healthy men, 30%–40% in women) and can be measured by total exchangeable potassium [2]. *Extracellular mass* is defined as the heterogeneous group of tissues and fluids supporting the body cell mass.

Diet for healthy people

Many guidelines have been developed over the years for general use by the US population for health maintenance and disease prevention. Although these have been published at different times and represent the input of a large number of experts with diverse interests, all expert panels have reported remarkably simple and consistent recommendations for healthy adults. Two of these sets of guidelines are widely disseminated.

The 2005 *Dietary Guidelines for Americans,* developed by the US Department of Health and Human Services and the US Department of Agriculture, provides guidelines for a healthy diet and body weight [3] (Table 21.1). These guidelines are similar to those recommended by the American Heart Association [4]. Both reports recommend the following:

- consume a variety of fruits, vegetables, and whole-grain products
- balance energy intake with energy needs and prevent gradual weight gain over time by making small decreases in energy intake while increasing physical activity
- limit intake of foods that contain high levels of saturated fatty acids, and keep consumption of *trans* fatty acid as low as possible
- limit dietary salt intake and alcohol consumption
- engage in regular physical activity.

Although the recommendations listed in Table 21.1 are generalized for all Americans, special considerations may be needed for African Americans and other minority groups.

In the last 25 years, the prevalence of obesity has increased markedly throughout the world. The World Health Organization [5] and the National Institutes of Health (NIH) [6,7] have proposed guidelines for classifying weight status by body mass index (BMI) (Table 21.2).

It is often helpful to estimate a patient's energy requirement when making recommendations of calorie intake. The Harris–Benedict equation is a useful tool for estimating resting energy requirements (Table 21.3). For estimates of overall energy use, the *recommended daily allowance* (RDA) figures are simple and useful (Table 21.4).

Most guidelines suggest an *adequate intake* (AI) of calcium (1300 mg/day for adolescents, 1000 mg/day for adult men and nonpregnant women aged 19–50, and 1200 mg/day for adults older than 50 years) [8]. Milk contains the highest concentration of calcium – 280–300 mg/225-g (8-oz) cup. There is as yet no convincing evidence that additional protection against osteopenia is achieved by ingesting calcium over the recommended AI. Salt (NaCl) should be limited to

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Table 21.1 Dietary guidelines for adult Americans, 2005

Theme	Guideline	Practical recommendations
Aim for fitness	Do not become obese	Maintain body mass index between 18.5 and 24.9 kg/m^2
	Be physically active on a daily basis	To reduce risks of chronic disease, engage in 30 min of moderate exercise (e.g., brisk walking) daily. To help manage body weight, engage in 60 min of moderate exercise daily
Build a healthy base	Enjoy a wide variety of foods	Increase intake of low-energy, nutrient-dense foods (e.g., fruits, vegetables)
	Consume a variety of fruits, vegetables, and grains daily. Eat more dark-green and orange vegetables. Eat at least 85 g (3 oz) of whole-grain cereals, breads, crackers, rice, or pasta	Consume 2 cups of fruit, 2½ cups of vegetables, 170 g (6 oz) of grains daily, 28 g (1 oz) ≈ 1 slice bread, 1 cup breakfast cereal, or ½ cup cooked rice, cereal, or pasta
	Maintain calcium intake	Consume lactose-free products or other calcium sources, such as fortified foods and beverages, if you are unable to ingest milk or milk products
	Maintain moderate protein intake	Consume 150 g (5½ oz) daily of low-fat lean meats and poultry
		Vary your protein sources by choosing fish, beans, peas, nuts, and seeds
Choose sensibly	Choose a diet low in saturated fats and cholesterol	Keep total fat intake between 20% and 35%, and combined saturated and *trans* fatty acids to < 10% of total calories. Limit cholesterol intake to < 300 mg/day
	Moderation in salt intake	Consume < 2300 mg (~ 1 tsp salt) sodium per day. Persons with hypertension, African Americans, and middle-aged and older adults should consume ≤ 1500 mg/day
	Choose and prepare foods and beverages with little added sugars or intense sweetners	
	Do not smoke	
	Drink alcoholic beverages in moderation, if at all	Limit alcoholic drinks to one or two per day
Food safety	Avoid microbial foodborne illness	Clean hands and surfaces prior to contact with food. Cook foods to a safe temperature. Chill perishable food promptly. Avoid unpasteurized milk or milk products, raw or partially cooked eggs, and raw or undercooked meat and poultry

Amounts are based on a daily intake of 8350 kJ (2000 kcal).
Adapted from US Department of Health and Human Services, US Department of Agriculture [3] and Krauss et al. [4].

Table 21.2 Disease risk associated with body mass index

	Obesity class	BMI (kg/m^2)	Disease risk
Underweight		< 18.5	Increased
Normal		18.5–24.9	Normal
Overweight		25.0–29.9	Increased
Obesity	I	30.0–34.9	High
	II	35.0–39.9	Very high
Extreme obesity	III	≥ 40.0	Extremely high

Additional risks: (i) waist circumference > 100 cm (40 in) in men and > 90 cm (35 in) in women; and (ii) poor aerobic fitness.
BMI, body mass index.
Adapted from National Institutes of Health, National Heart, Lung, and Blood Institute [7].

6 g/day or less, and alcohol to 28 g (1 oz) of pure alcohol per day (28 g is the equivalent of two cans of beer, two small glasses of wine, or two average cocktails).

The implementation of the published recommendations depends on two other federally supported guidelines. The RDA [9–13] is the level judged to be high enough to ensure an adequate intake for the majority of the normal population, not the minimal intake necessary to avoid negative balance. The values for the RDA vary with age and gender. These values should not be confused with the US RDAs, standards set by the US Food and Drug Administration in 1973 for the purposes of food labeling for the entire population. The term "US RDA" has been replaced by *reference daily intake* (RDI) and *daily reference value* (DRV). The latter provides for total and saturated fat, cholesterol, total carbohydrates, dietary fiber, sodium, potassium, and protein – nutrients for

Table 21.3 Formulas for estimating resting energy expenditure

Harris–Benedict equation [41]
Men = 66 + (13.7 × W) + (5 × H) – (6.8 × A)
Women = 665 + (9.6 × W) + (1.8 × H) – (4.7 × A)

Owen et al. [43]
Men = 879 + (10.2 × W)
Women = 795 + (7.18 × W)

World Health Organization [56]

Age (years)	Male	Female
0–3	(60.9 × W) – 54	(61.0 × W) – 51
3–10	(22.7 × W) – 495	(22.5 × W) + 499
10–18	(17.5 × W) + 651	(12.2 × W) + 746
18–30	(15.3 × W) + 679	(14.7 × W) + 996
30–60	(11.2 × W) + 879	(8.7 × W) + 829
> 60	(13.5 × W) + 987	(10.5 × W) + 596

A, age (years); H, height (cm); W, weight (kg).

Table 21.4 Calculation of energy requirement

Level of activity	Activity factor (× REE)	Average energy expenditure[a] (kcal/kg per day)
Very light		
Men	1.3	31
Women	1.3	30
Light		
Men	1.6	38
Women	1.5	35
Moderate		
Men	1.7	41
Women	1.6	37
Heavy		
Men	2.1	50
Women	1.9	44

a Estimated from World Health Organization equations for median weights of persons aged 19–74 years; activity factor 1.0 = 100.4 kJ/kg (24.0 kcal/kg) for males, 97 kJ/kg (23.2 kcal/kg) for females.
REE, resting energy expenditure.
Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine [13].

which no US RDA was available [12,13]. The RDI and DRV are the basis for the daily values that appear on all food labels in the United States, and reflect DRVs and RDIs for a 2000-calorie reference diet. The new recommended dietary intake levels are called *dietary reference intakes* (DRIs), a collective term that includes the *estimated average requirement* (EAR), the RDA, the AI, and the *tolerable upper intake level* (UL) (Table 21.5) [8–13]. These terms were developed by the Institute of Medicine in the United States along with Health Canada. The EAR is the daily nutrient intake value estimated to meet the requirements of half of an age- and gender-specific group. It often is derived from balance studies and is used in setting the RDA, the intake level sufficient to meet the nutrient needs of most people within a given life-stage and gender group. The RDA also includes a factor to account for variation within the group, usually estimated at 10% (i.e., coefficient of variation, or CV). If insufficent data are available to calculate an EAR, AI is used, based on observed intake consistent with good health. The UL is the maximum amount of a nutrient that can be ingested without posing a health threat and was included because so many nutrients are ingested at levels far exceeding those possible from the diet.

These guidelines account for group, and in some cases individual, variability, but they are not meant for use in patients with either acute or chronic diseases. Rather, they should be used as baseline estimates for planning individual nutrient intake. Such adjustments are particularly important in diseases characterized by malabsorption or catabolism, in contrast to simple lack of intake. Confirmation of sufficient nutrient provision can then be monitored by tests specific for each macro- or micronutrient (see Chapter 107).

MyPyramid is a food guide that incorporates recommendations from the 2005 *Dietary Guidelines for Americans* and is available on the US Department of Agriculture (USDA) website (www.mypyramid.gov). MyPyramid is part of an overall food guidance system designed to meet the need for a more individualized approach to improving diet and lifestyle. Five basic food groups are emphasized: grains, vegetables, fruits, low-fat milk and milk products, and meat and beans. The concept assumes that meals are built on a basis of grains, fruits, and vegetables, and supplemented with low-fat milk products and other protein sources in the meat group. MyPyramid comes with a warning to use fats and sweets sparingly and advocates a sensible balance between food and physical activity.

Glycemic index

The glycemic index of a food represents the relative increase in blood glucose that occurs over 2 h after consuming that food compared with either glucose or white bread [14]. Glycemic load is defined as the product of the glycemic index of a food and the amount of carbohydrate in a serving. Diets of foods with a low glycemic index and low glycemic load have been proposed to improve glycemic control in diabetes and to enhance weight loss in obesity. Glycemic control is better in subjects who have type 2 diabetes if a portion of carbohydrate intake is replaced by fat [15]. A study of overweight adolescents showed that altering dietary glycemic load by reducing both total carbohydrate content (45%–50%

of energy intake) and consuming foods with a low glycemic index resulted in greater weight loss compared with a conventional low-fat (25%–30%) diet [16]. However, additional research is needed to clarify the long-term efficacy of diets with a low glycemic load for patients with diabetes or obesity.

Chemoprevention of gastrointestinal cancers

One of the special applications of dietary recommendations for healthy people is to prevent gastrointestinal cancers. The general recommendations for such diets are similar to those that support health in the entire population. However, it has been estimated that about one-third of all cancers are related to diet, and that most colorectal cancer in the United States might be prevented by dietary alterations [17]. Although epidemiological data have suggested associations between overall diets or environment and the risk for cancer incidence or mortality, it has proved difficult to identify the dietary components that might influence such risks, and to demonstrate their benefit in a prospective fashion [18–20]. Table 21.6 summarizes many of the data associating risks for colorectal cancer with dietary components. Few of the reported associations have been convincing. When dietary components have been identified and tested prospectively, the data, in general, are negative, even when premalignant end points are examined, such as colorectal polyps [21].

Epidemiological evidence (case–control and cohort studies) has suggested that people with cancer have a lower intake of raw, fresh, leafy green, or cruciferous vegetables, as well as raw or fresh fruits, especially citrus fruits, but a prospective analysis of 285 526 women showed no association between total or specific vegetable consumption and the risk of breast cancer [22]. Similar findings were noted in a prospective study of risk for colorectal cancer [23]. Although the ingestion of such foods has increased in the United States, the average intake is less than 0.7 servings of vegetables or fruits per day. Moreover, a large cohort study (Iowa Women's Health Study of more than 40 000 women) showed no protective effect of vegetables or fruits on colorectal carcinoma incidence, except for garlic [21]. The antioxidant vitamins A, C, and E are among those compounds thought to be responsible for the possible effects of vegetables and fruits on carcinogenesis. A meta-analysis of 14 randomized trials of antioxidant supplements found no evidence that these could prevent gastrointestinal cancer [24]. In fact, the supplements seemed to increase overall mortality, an effect that may be related to high-dose vitamin E supplementation [25]. Other studies have tested the addition of individual or combinations of vitamins (or β-carotene) on the incidence of colorectal adenoma formation, but have found little prophylactic efficacy [17,26]. Epidemiological studies have provided positive associations between current vitamin use and decreased cancer risk. However, the past use of supplements (including multivitamins) may modestly reduce the risk of colorectal cancer, and blunt the effects of added supplements in controlled trials [27].

The other dietary component that has been most extensively studied is fiber. Two extensive reviews have examined the descriptive and case control studies of the association of dietary fiber with colorectal cancer [28,29]. Most studies showed some inverse correlation, suggesting a protective effect of fiber. In the Health Professionals Follow-up Study involving men, a 64% reduction in cancer was noted in those with the highest quintile of fiber intake (> 28.3 g/day) compared with those on the lowest intake (< 16.6 g/day) [30]. Results from two large studies of women, the Nurses Health Study [31] and the Iowa Women's Study [32], showed no effect. However, two large prospective epidemiological studies reported protection against colorectal cancer [33] and colonic adenomas [34] in subjects with high fiber intake. Prospective studies suggest that an effect of fiber may occur only in men. Interventional studies using fiber supplements often yield inconclusive results [17,28,29].

The data regarding the effect of other dietary components on the incidence of colorectal cancer or adenomas are too fragmentary or incomplete to permit strong recommendations [17]. Some evidence suggests an effect of folate, and its role is being tested in interventional studies. Alcohol (> 30 g/day) may increase the incidence of adenomas in the distal colon and rectum. Although many case control and cohort studies suggested that calcium protected against the development of colorectal cancer, other studies with larger cohorts showed no effect. The role of vitamin C has not been confirmed [35]. The results of interventional studies are conflicting, and the end point used has been colonic cell proliferation, not adenoma or carcinoma incidence.

Although numerous studies examining the role of nutrition in prevention of gastrointestinal cancer exist, the interventional studies do not support specific dietary recommendations, as most of the data only demonstrate associations between diet and cancer prevention. However, intake levels of fruits, vegetables, fiber, and calcium as outlined in the Dietary Guidelines for Americans should be given specific dietary recommendations, as most of the data only demonstrate associations between diet and cancer prevention. Intake levels of fruits, vegetables, fiber, and calcium, as outlined in the Dietary Guidelines for Americans, should be recommended.

Energy metabolism

The human body continuously consumes energy for the maintenance of ionic and osmotic gradients, cell transport, nerve conduction, intermediary metabolism, biosynthesis, heat generation, and the performance of involuntary and voluntary mechanical work. Energy is provided largely by the mitochondrial production of high-energy phosphate bonds generated by the oxidation of fat, carbohydrate, and protein. After the hydrolysis of carbohydrates to simple sugars, fats to fatty acids and glycerol, and proteins to amino

Table 21.5 Dietary reference intakes: recommended intakes for individuals

Life-stage group	Calcium (mg/day)	Phosphorus (mg/day)	Magnesium (mg/day)	Vitamin D (μg/day)[a,b]	Fluoride (mg/day)	Thiamin (mg/day)	Riboflavin (mg/day)	Niacin (mg/day)[c]	Vitamin B-6 (mg/day)	Folate (μg/day)[d]	Vitamin B-12 (μg/day)	Pantothenic acid (mg/day)
Infants												
0–6 months	210*	100*	30*	5*	0.01*	0.2*	0.3*	2*	0.1*	65*	0.4*	1.7*
7–12 months	270*	275*	75*	5*	0.5*	0.3*	0.4*	4*	0.3*	80*	0.5*	1.8*
Children												
1–3 years	500*	**460**	**80**	5*	0.7*	**0.5**	**0.5**	**6**	**0.5**	**150**	0.9	2*
4–8 years	800*	**500**	**130**	5*	1*	**0.6**	**0.6**	**8**	**0.6**	**200**	1.2	3*
Males												
9–13 years	1300*	**1250**	**240**	5*	2*	0.9	0.9	12	1.0	**300**	1.8	4*
14–18 years	1300*	**1250**	**410**	5*	3*	1.2	1.3	16	1.3	**400**	2.4	5*
19–30 years	1000*	**700**	**400**	5*	4*	1.2	1.3	16	1.3	**400**	2.4	5*
31–50 years	1000*	**700**	**420**	5*	4*	1.2	1.3	16	1.3	**400**	2.4	5*
51–70 years	1200*	**700**	**420**	10*	4*	1.2	1.3	16	1.7	**400**	2.4[g]	5*
> 70 years	1200*	**700**	**420**	15*	4*	1.2	1.3	16	1.7	**400**	2.4[g]	5*
Females												
9–13 years	1300*	**1250**	**240**	5*	2*	0.9	0.9	12	1.0	**300**	1.8	4*
14–18 years	1300*	**1250**	**360**	5*	3*	1.0	1.0	14	1.2	**400**[h]	2.4	5*
19–30 years	1000*	**700**	**310**	5*	3*	1.1	1.1	14	1.3	**400**[h]	2.4	5*
31–50 years	1000*	**700**	**320**	5*	3*	1.1	1.1	14	1.3	**400**[h]	2.4	5*
51–70 years	1200*	**700**	**320**	10*	3*	1.1	1.1	14	1.5	**400**	2.4[g]	5*
> 70 years	1200*	**700**	**320**	15*	3*	1.1	1.1	14	1.5	**400**	2.4[g]	5*
Pregnancy												
≤ 18 years	1300*	**1250**	**400**	5*	3*	1.4	1.4	18	1.9	**600**[i]	2.6	6*
19–30 years	1000*	**700**	**350**	5*	3*	1.4	1.4	18	1.9	**600**[i]	2.6	6*
31–50 years	1000*	**700**	**360**	5*	3*	1.4	1.4	18	1.9	**600**[i]	2.6	6*
Lactation												
≤ 18 years	1300*	**1250**	**360**	5*	3*	1.4	1.6	17	2.0	**500**	2.8	7*
19–30 years	1000*	**700**	**310**	5*	3*	1.4	1.6	17	2.0	**500**	2.8	7*
31–50 years	1000*	**700**	**320**	5*	3*	1.4	1.6	17	2.0	**500**	2.8	7*

This table presents Recommended Dietary Allowances (RDAs) in **bold type** and Adequate Intakes (AIs) in ordinary type followed by an asterisk (*). RDAs and AIs may both be used as goals for individual intake. RDAs are set to meet the needs of almost all (97%–98%) individuals in a group. For healthy breast-fed infants, the AI is the mean intake. The AI for other life-stage and gender groups is believed to cover the needs of all individuals in the group, but lack of data or uncertainty in the data prevent specification with confidence of either percentage of individuals covered by this intake.

a As cholecalciferol. 1 μg cholecalciferol = 40 IU vitamin D.

b In the absence of adequate exposure to sunlight.

c As niacin equivalents (NE). 1 mg of niacin = 60 mg tryptophan; 0–6 months = preformed niacin (not NE).

d As dietary folate equivalents (DFE). 1 DFE = 1 μg food folate = 0.6 μg folic acid from fortified food or as a supplement consumed with food = 0.5 μg of a supplement taken on an empty stomach.

e Although AIs have been set for choline, there are few data to assess whether a dietary supply of choline is needed at all stages of the life cycle, and it may be that the choline requirement can be met by endogenous synthesis at some of these stages.

acids, most of these small molecules are converted to the acetyl unit of acetyl coenzyme A (CoA), generating a small amount of ATP in the process. Acetyl-CoA is a common breakdown product of the three macronutrients. Acetyl-CoA, carrying most of the chemical energy of the original macronutrients, enters the citric acid cycle and undergoes oxidative phosphorylation, the final common pathways in the oxidation of food molecules (Fig. 21.1). Many amino acids enter the citric acid cycle as α-ketoglutarate or oxaloacetate rather than as acetyl-CoA.

A portion of the energy released during substrate oxidation is not used to perform work and is dissipated as heat. Therefore, energy production is traditionally measured in terms of heat production. One kilocalorie (kcal), equal to

Table 21.5 *continued*

Biotin (µg/day)	Choline[e] (mg/day)	Vitamin C (mg/day)	Vitamin E[f] (mg/day)	Selenium (µg/day)	Vitamin A[j] (µg)	Vitamin K (µg/day)	Chromium (µg/day)	Copper (µg/day)	Iodine (µg/day)	Iron (mg/day)	Manganese (mg/day)	Molybdenum (µg/day)	Zinc (mg/day)
5*	125*	40*	4*	15*	400*	2.0*	0.2*	200*	110*	0.27*	0.003*	2*	2*
6*	150*	50*	5*	20*	500*	2.5*	5.5*	200*	130*	**11**	0.6*	3*	3
8*	200*	**15**	6	**20**	**300**	30*	11*	**340**	**90**	**7**	1.2*	**17**	**3**
12*	250*	**25**	7	**30**	**400**	55*	15*	**440**	**90**	**10**	1.5*	**22**	**5**
20*	375*	**45**	11	**40**	**600**	60*	25*	**700**	**120**	**8**	1.9*	**34**	**8**
25*	550*	**75**	15	**55**	**900**	75*	35*	**890**	**150**	**11**	2.2*	**43**	**11**
30*	550*	**90**	15	**55**	**900**	120*	35*	**900**	**150**	**8**	2.3*	**45**	**11**
30*	550*	**90**	15	**55**	**900**	120*	35*	**900**	**150**	**8**	2.3*	**45**	**11**
30*	550*	**90**	15	**55**	**900**	120*	30*	**900**	**150**	**8**	2.3*	**45**	**11**
30*	550*	**90**	15	**55**	**900**	120*	30*	**900**	**150**	**8**	2.3*	**45**	**11**
20*	375*	**45**	11	**40**	**600**	60*	21*	**700**	**120**	**8**	1.6*	**34**	**8**
25*	400*	**65**	15	**55**	**700**	75*	24*	**890**	**150**	**15**	1.6*	**43**	**9**
30*	425*	**75**	15	**55**	**700**	90*	25*	**900**	**150**	**18**	1.8*	**45**	**8**
30*	425*	**75**	15	**55**	**700**	90*	25*	**900**	**150**	**18**	1.8*	**45**	**8**
30*	425*	**75**	15	**55**	**700**	90*	20*	**900**	**150**	**8**	1.8*	**45**	**8**
30*	425*	**75**	15	**55**	**700**	90*	20*	**900**	**150**	**8**	1.8*	**45**	**8**
30*	450*	**80**	15	**60**	**750**	75*	29*	**1000**	**220**	**27**	2.0*	**50**	**12**
30*	450*	**85**	15	**60**	**770**	90*	30*	**1000**	**220**	**27**	2.0*	**50**	**11**
30*	450*	**85**	15	**60**	**770**	90*	30*	**1000**	**220**	**27**	2.0*	**50**	**11**
35*	550*	**115**	19	**70**	**1200**	75*	44*	**1300**	**290**	**10**	2.6*	**50**	**13**
35*	550*	**120**	19	**70**	**1300**	90*	45*	**1300**	**290**	**9**	2.6*	**50**	**12**
35*	550*	**120**	19	**70**	**1300**	90*	45*	**1300**	**290**	**9**	2.6	**50**	**12**

f As α-tocopherol. α-Tocopherol includes *RRR*-α-tocopherol, the only form of α-tocopherol that occurs naturally in foods, and the 2*R*-stereoisomeric forms of α-tocopherol (*RRR*-, *RSR*-, *RRS*-, and *RSS*-α-tocopherol) that occur in fortified foods and supplements. It does not include the 2*S*-stereoisomeric forms of α-tocopherol (*SRR*-, *SSR*-, *SRS*-, and *SSS*-α-tocopherol), also found in fortified foods and supplements.

g Because 10%–30% of older people may malabsorb foodbound vitamin B-12, it is advisable for those older than 50 years to meet their RDA mainly by consuming foods fortified with vitamin B-12 or a supplement containing vitamin B-12.

h In view of evidence linking folate intake with neural tube defects in the fetus, it is recommended that all women capable of becoming pregnant consume 400 µg from supplements or fortified foods in addition to food folate from a varied diet.

i It is assumed that women will continue consuming 400 µg from supplements or fortified food until their pregnancy is confirmed and they enter prenatal care, which ordinarily occurs after the end of the periconceptional period – the critical time for formation of the neural tube.

j As retinol activity equivalents (RE). 1 RE = 1 µg all-*trans*-retinol, 12 µg β-carotene, 24 µg α-carotene, or 24 µg β-cryptoxanthin.

Data are compiled from the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine [9–11].

4.184 kilojoules (kJ), is the amount of heat required to raise the temperature of 1 kg of water by 1°C. Normally, body temperature is carefully maintained within narrow limits so that heat production equals heat loss. Energy production can be determined directly (direct calorimetry) by measuring the transfer of heat from the body to water circulating in specially designed chambers or suits. Energy production also can be measured indirectly (indirect calorimetry) by measuring carbon dioxide (CO_2) production and oxygen (O_2) consumption, because the amount of heat produced during substrate oxidation is proportional to the amount of CO_2 produced and O_2 consumed [36]. The relationship between CO_2 production and O_2 consumption can be used to estimate the relative oxidation of different substrates [37].

Table 21.6 Epidemiological evidence on dietary factors and colorectal cancer risk

Factor	Correlational studies	Case control studies	Prospective studies
Dietary fiber	1	1	2
Vegetables	3	3	2
Fruits	3	2	1
Cholesterol	1	1	0
Red meat	2	2	1
Antioxidant vitamins	1	1	1
Folate	1	3	3
Alcohol	2	2	2
Calcium	2	2	1
Vitamin D	1	1	1
Selenium	1	1	1

3 = convincing, 2 = probable, 1 = possible, 0 = insufficient.
Adapted from Forman et al. [26].

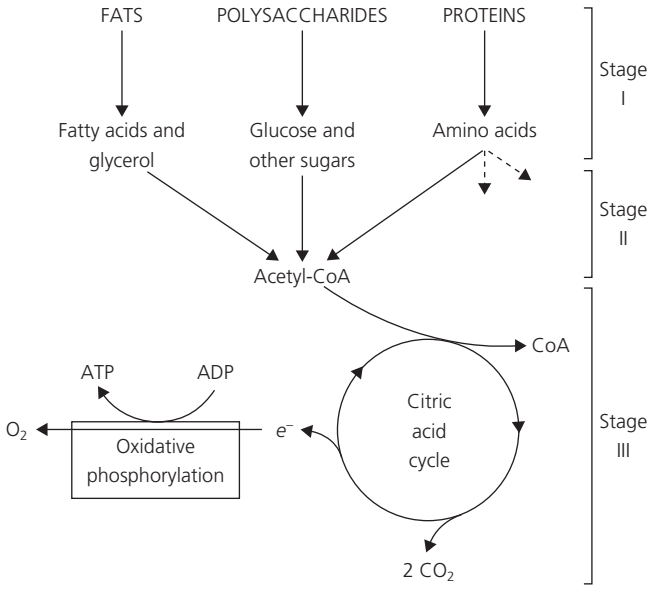

Figure 21.1 Stages in the extraction of energy from foodstuffs. CoA, coenzyme A. From Stryer L. Biochemistry, 3rd edn. New York: WH Freeman, 1988:325.

Dietary carbohydrates, fats, and proteins can be used as fuel soon after their ingestion, or they can be stored by the body for subsequent oxidation. Endogenous energy stores (Table 21.7), which are continuously being mobilized and oxidized, become a critical source of fuel during postabsorptive conditions and when energy intake is inadequate to meet energy demands. The largest source of endogenous energy is triglyceride in adipose tissue, which is uniquely designed to store fuel. Triglycerides have a high energy density and release 39.3 kJ/g (9.4 kcal/g) when oxidized. Adipose tissue is composed almost entirely of triglycerides in an oil form, which constitute 85% of adipocyte weight. In comparison, glycogen, the other major source of endogenous fuel, generates only 17.2 kJ/g (4.1 kcal/g) on oxidation. Glycogen takes up a considerable amount of space because it is stored in liver and muscle tissue as a gel containing 2–4 g of water for every gram of glycogen [38]. The mobilization of adipose tissue yields 25–33 kJ/g (6–8 kcal/g), whereas the mobilization of glycogen yields only 4–8 kJ/g (1–2 kcal/g). The energy stored in the adipose tissue of a lean man can provide enough fuel for him to survive 2 months of total energy restriction [39], whereas the energy present as liver glycogen is consumed within 24 h of fasting. Certain cells and tissues, such as the brain, prefer glucose as a fuel, and others, such as bone marrow, erythrocytes, leukocytes, renal medulla, eye tissues, and peripheral nerve tissue, require glucose because they cannot oxidize fatty acids. None of the macronutrients is completely absorbed; some are excreted in the feces. Based on the average digestibility of fat (95%) and carbohydrate (97%), the digestible energy derived from fat is 37.6 kJ/g (9.0 kcal/g), and that from carbohydrate is 16.7 kJ/g (4.0 kcal/g).

Table 21.7 Body energy stores

Tissue	Fuel	Energy
Adipose tissue	Triglyceride	585 000 kJ (140 000 kcal)
Muscle	Glycogen	8400 kJ (2000 kcal)
	Triglyceride	12 500 kJ (3000 kcal)
Liver	Glycogen	1250 kJ (300 kcal)
	Triglyceride	2100 kJ (500 kcal)

Components of energy expenditure

Total energy requirements include the sum of *resting energy expenditure* (REE), the thermic effect of physical activity (TEPA), the thermic effect of feeding (TEF), and adaptive thermogenesis (AT). The resting energy expenditure is the energy consumed while lying quietly awake in the postabsorptive state. Normally, REE accounts for about 70% of total daily energy expenditure. Energy requirements of different tissues and organs are heterogeneous, however. Energy consumption by the body's most metabolically active organs – the brain, liver, kidney, and heart – accounts for 60% of REE; these constitute only 5% of total body mass (Table 21.8). By contrast, adipose tissue, which accounts for about 20% and 30% of body weight in lean men and women, respectively, consumes less than 5% of REE.

Across mammalian species, REE is related to body weight; REE is proportional to the three-fourths power of body weight ($\text{weight}^{0.75}$) [40]. Several equations have been used to estimate resting energy requirements in humans based on

Table 21.8 Postabsorptive energy requirements

	Mass		Energy expenditure	
Tissue	**g**	**% Total**	**kJ/day (kcal/day)**	**% Total**
Gut	2000	3	1250 (300)	13
Brain	1400	2	1675 (400)	18
Liver	1600	2.2	1840 (440)	19
Heart	300	0.4	985 (235)	10
Kidneys	300	0.5	835 (200)	10
Adipose tissue	14 000	20	290 (70)	4
Skeletal muscle	28 000	40	1675 (400)	18

measurements of REE in healthy subjects (see Table 21.3) [41–44]. These equations generate values that are usually within 10% of measured values in healthy volunteers but are less accurate in persons who are at the extremes of weight (i.e., extremely lean or obese) or who are ill. Starvation and severe hypocaloric feeding decrease the resting metabolic rate to values some 15%–20% below that expected for actual body size, whereas illness and injury can increase energy requirements.

Physical activity usually accounts for 15%–20% of total energy expenditure. The precise contribution of the TEPA to total energy expenditure depends on the intensity and duration of activities. At rest, skeletal muscle accounts for 20% of total energy requirements. However, during moderate- to high-intensity aerobic exercise, energy consumed by working muscles can increase more than 50-fold, causing a 15-fold increase in total energy expenditure.

The TEF represents the energy costs of digestion, absorption, transport, metabolism, and storage of nutrients, and it also may involve AT. Eating or infusing nutrients increases the metabolic rate by about 5%–10% of the ingested or infused calories and depends on the specific foods consumed. Normally, 12%–20% of the energy in ingested protein, 6%–12% of carbohydrate energy, and 2%–3% of fat energy is expended.

Adaptive thermogenesis is a proposed mechanism for wasting excess energy to maintain a constant body weight despite fluctuating amounts of energy intake, or for maintaining body heat during exposure to different environmental temperatures [45]. The concept of a "dual control" system for AT has been introduced [46]. In addition to a "rapid reaction" control system function as the attenuator of energy balance, under conditions of positive energy balance AT may be regarded as a "slow reaction" control system, functioning specifically as an accelerator for fat deposition [45]. It has been proposed that energy expenditure and heat production for AT and TEF involve brown adipose tissue, a specialized, highly vascularized, thermogenic tissue innervated by sympathetic nerves [47]. Brown adipose tissue is packed with large mitochondria possessing an uncoupling protein that uncouples ATP synthesis from respiration [48,49]. In this situation, the rate of substrate oxidization does not depend on the availability of ADP precursor, and the reaction can continue at high rates, permitting even small quantities of brown adipose tissue to increase heat production markedly. Radioimmunoassays for the uncoupling protein have demonstrated the presence of brown adipose tissue in humans of all ages [49]; other studies suggest that uncoupling protein expression also can be induced in white adipose tissue [50]. The physiological importance of AT in energy metabolism in humans is not clear. In societies where food is plentiful and the level of daily physical activity is low, the variations in AT among individuals may be important in determining their trend toward weight maintenance or weight gain.

Proteins

Proteins are composed of amino acids joined together by peptide bonds. Twenty different amino acids are commonly found in human proteins. Differences in the sequences of amino acids in proteins permit diverse structures and functions; proteins serve as enzymes, carriers, receptors, hormones, and structural elements. The amino acid sequence determines the location of sites for covalent attachment of carbohydrate and ultimately determines the protein's three-dimensional configuration and specific function. Nitrogen also is present in the body in the form of free amino acids. Free amino acids are in a dynamic state, whether they are being incorporated into tissue proteins, undergoing catabolic reactions, or being used for the synthesis of other nitrogen-containing compounds [51].

Protein quality

Protein quality is related to the ability of the protein to support metabolic homeostasis and growth, which is determined by amino acid content and bioavailability. Some amino acids (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and possibly arginine) are considered essential because their carbon skeletons cannot be synthesized by the body. These amino acids must be consumed in the diet for normal function and survival. Other amino acids (glycine, alanine, serine, cysteine, cystine, tyrosine, glutamine, glutamic acid, asparagine, and aspartic acid) are nonessential because their carbon skeletons can be produced endogenously. In general, the greater the ratio of essential to nonessential amino acids, the better the quality of protein.

The ability to digest protein and absorb its component amino acids also affects protein quality. True absorption ranges from 97%–99% for proteins in meat, milk, and eggs, to 75% for proteins in potatoes and navy beans [52,53]. Protein bioavailability also can be affected by food preparation. For example, some lysine is lost by heating in the presence of reducing sugars. By contrast, heating increases the

bioavailability of soy protein by inactivating the trypsin inhibitor present in soybeans [54]. In general, the proteins of eggs, milk, fish, red meat, and poultry are high in biological value, and the protein in wheat gluten is low [52,53,55].

Nitrogen balance

Nitrogen balance is the difference between intake and output. Nitrogen is excreted primarily in the urine as urea, creatinine, porphyrins, ammonia, and uric acid. The relative proportions of these compounds can vary, but urea usually accounts for about 80% of urinary nitrogen. During fasting conditions, urinary nitrogen reaches a low level of about 2 mg/kcal of REE, or about 40 mg/kg of body weight. About 1–3 g of nitrogen is normally lost per day from fecal and other sources [13]. Fecal nitrogen losses reflect unabsorbed protein in the diet and in intestinal secretions and sloughed epithelial cells. The amount of endogenous protein that normally enters the intestinal lumen is about 50 g/day. Absorption of exogenous and endogenous protein is so efficient that fecal nitrogen is normally only 1–2 g/day. Minor amounts of nitrogen are lost through intact skin, nasal secretions, semen, menstrual fluid, and hair cuttings.

Nitrogen balance can be used to estimate protein balance because about 16% of protein consists of nitrogen, and it is assumed that almost all body nitrogen is incorporated into protein or amino acids. A positive balance (i.e., intake greater than losses) represents a net increase in total body protein, whereas a negative balance (i.e., losses greater than intake) demonstrates net protein catabolism. One gram of nitrogen represents about 6.25 g of protein, which is equivalent to 30 g of hydrated lean body mass.

Nitrogen balance is affected by protein intake and quality, energy intake, and nutritional status. Inadequate energy intake increases protein requirements. When protein intake is suboptimal, nitrogen balance can be improved by increasing energy intake. Therefore, nitrogen balance reflects both protein intake and energy balance. Most normal adults can maintain nitrogen equilibrium by ingesting 0.5 g of high-quality protein per kilogram of body weight per day. The range of recommended intake for adults is 0.5–0.8 g/kg/day [13,51,56,57], which provides a margin of safety to allow for decreased biological availability and increased requirements in subsets of the population. The average protein in a Western diet has only 75% of the biological value of egg protein [51,58]. Intravenously administered amino acids are as effective in promoting nitrogen balance as oral protein [59].

Infancy is a time of intense growth, and the protein requirements per unit of body weight for infants are higher than those for adults. The healthy infant also requires a higher proportion of essential to nonessential amino acids [60]. The growth spurt of adolescence, the only extrauterine period during which growth velocity increases, occurs between 10 and 13 years of age for US girls and between 12 and 15 years of age for US boys, and contributes about 15% of final adult height and 50% of adult weight [61]. The guidelines for protein and calorie needs in infancy, childhood, and adolescence are summarized in Table 21.9. The nutritional demands of a normal pregnancy average about 335 000 kJ (80 000 kcal) – that is, roughly 1250 kJ/day (300 kcal/day) – and 950 g of protein (i.e., 3.5 g/day). The Food and Nutrition Board of the Institute of Medicine has set the RDA for protein during pregnancy at 1.1 g/kg/day, an increase of 25 g/day over the RDA for nonpregnant women aged 25 years or older [13]. For lactating women over 25 years of age whose average daily output of milk is 850 mL, a protein intake of 1.3 g/kg/day, or 25 g/day of additional protein, is recommended by the Food and Nutrition Board [13].

Protein metabolism

Body proteins are in a state of constant flux, with protein synthesis and breakdown occurring simultaneously. Normal daily protein turnover is 1%–2% of total body protein and results largely from the degradation of muscle and hepatic proteins. Protein degradation involves the enzymatic hydrolysis of protein to its constituent amino acids. More than 75% of the amino acids released by protein breakdown are reused for the synthesis of new proteins; the remaining amino acids are oxidized. Proteases within cell lysosomes are responsible for most protein degradation [62–66]. However, proteases are also found in plasma membranes and in the cytosol. The carbon skeletons of amino acids can be oxidized for energy or used for the synthesis of glucose, ketone bodies, and fatty acids [2]. Nitrogen can be released as ammonia into the bloodstream and delivered to the liver, where it is converted to urea.

The metabolism of amino acids involves the transfer of nitrogen between organs from the periphery to the liver (Fig. 21.2). The liver is a workhorse for amino acid metabolism and is the site of synthesis for urea and plasma proteins. It is the main site of catabolism for the essential amino acids, with the exception of the branched-chain amino acids leucine, isoleucine, and valine, which are degraded in muscle and kidney.

Skeletal muscle preferentially takes up the branched-chain amino acids after each meal and is the primary site of metabolism for these amino acids. Although leucine, isoleucine, and valine constitute only 8% of dietary amino acids, they make up 60% of the amino acids in the systemic circulation [67,68]. When muscle proteins are catabolized, the branched-chain amino acids undergo transamination, yielding alanine, glutamine, and branched-chain keto acids. The keto acids are used by the muscle as fuel, and alanine and glutamine are exported and taken up predominantly by the liver and intestine, respectively [67]. These two amino acids account for more than 50% of the total amino acid nitrogen released from muscle [68–70]. The kidneys also take up glutamine, which is the major substrate for renal ammonia production [71].

Table 21.9 Dietary reference intake values for energy and protein in individuals by life-stage group

			Protein[b]					
	Energy EER[a], kJ/day (kcal/day)		AI or RDA (g/day)		EAR (g/kg/day)		RDA (g/kg/day)	
Life-stage group	M	F	M	F	M	F	M	F
0–6 months	2385 (570)[c]	2218 (530)	9.1[k]	9.1				
7–12 months	3109 (743)[d]	2828 (676)	11	11	1.0	1.0	1.2	1.2
1–2 years	4376 (1046)[e]	4150 (992)						
1–3 years			13	13	0.87	0.87	1.05	1.05
3–8 years	7289 (1742)[f]	6870 (1642)						
4–8 years			19	19	0.76	0.76	0.95	0.95
9–13 years	9535 (2279)[g]	8665 (2071)	34	34	0.76	0.76	0.95	0.95
14–18 years	13 188 (3152)[h]	9908 (2368)	52	46	0.73	0.71	0.85	0.85
> 18 years	12 832 (3067)[i]	10 054 (2403)[j]	56	46	0.66	0.66	0.80	0.80

a Based on energy expenditure plus energy deposition for moderately active Americans and Canadians up to age 18, but only for energy expenditure < 18 years.
b Based on nitrogen equilibrium plus protein deposition, except for 0–6 years (average consumption of protein from human milk) and < 18 years (nitrogen equilibrium only).
c Total for a 3-month-old.
d Total for a 9-month-old.
e Total for a 24-month-old.
f Total for a 6-year-old.
g Total for an 11-year-old.
h Total for a 16-year-old.
i Total for a 19-year-old.
j For subjects older than 19 years subtract 42 kJ/day (10 kcal/day) for males and 29 kJ/day (7 kcal/day) for females.
k Based on AI only.
EER, estimated energy requirement; AI, adequate intake, the observed average or experimentally determined intake by a defined population or subgroup that appears to sustain a defined nutritional status. The AI is not equivalent to an RDA; EAR, estimated average requirement, the intake that meets the estimated nutrient need of half of the individuals in a group; RDA, recommended dietary allowance, the intake that meets the nutrient needs of almost all (97%–98%) individuals in a group. The EAR and RDA for protein for the first half of pregnancy are the same as those of a nonpregnant woman.
Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine [13].

Lipids

Lipids are a heterogeneous group of compounds that are soluble in organic solvents. Lipids include triglycerides (fat), sterols, glycolipids, phospholipids, and fat-soluble vitamins. These compounds serve as a source of energy, structural components of cell membranes, carriers of essential nutrients, and precursors for the synthesis of steroid hormones, prostaglandins, thromboxanes, and leukotrienes. Dietary lipids are composed mainly of triglycerides, which contain mostly saturated and unsaturated long-chain fatty acids with a 16- to 18-carbon chain length.

Lipid metabolism

The use of fat as a fuel requires the hydrolysis of triglyceride to free fatty acid and glycerol and the tissue uptake of free fatty acids for subsequent oxidation. Hormone-sensitive lipase within adipocytes hydrolyzes adipose tissue triglycerides and releases free fatty acids into the bloodstream, where they are bound to plasma proteins and delivered to other tissues. Lipoprotein lipase at the lumenal surface of the capillary endothelium hydrolyzes plasma triglycerides and releases free fatty acids for local tissue uptake. Fatty acids are transported across the cell membrane by passive diffusion, facilitated diffusion, and active transport. Membrane and cytosolic fatty acid-binding proteins are important in transporting fatty acids across the cell membrane and in directing fatty acids from the cell membrane to different metabolic sites. This intracellular fatty acid transport system enhances fatty acid uptake by maintaining a fatty acid concentration gradient and prevents potentially toxic interactions between fatty acids and intracellular organelles. Long-chain fatty acids are delivered across the outer and inner mitochondrial membranes by a carnitine-dependent transport system. Inside the mitochondria, fatty acids are degraded by β-oxidation to acetyl-CoA, which enters the tricarboxylic acid cycle (see Fig. 21.2).

Ketone bodies are produced solely by the liver and are generated by the partial oxidation of fatty acids. Ketone body production increases when the rate of fatty acid production is

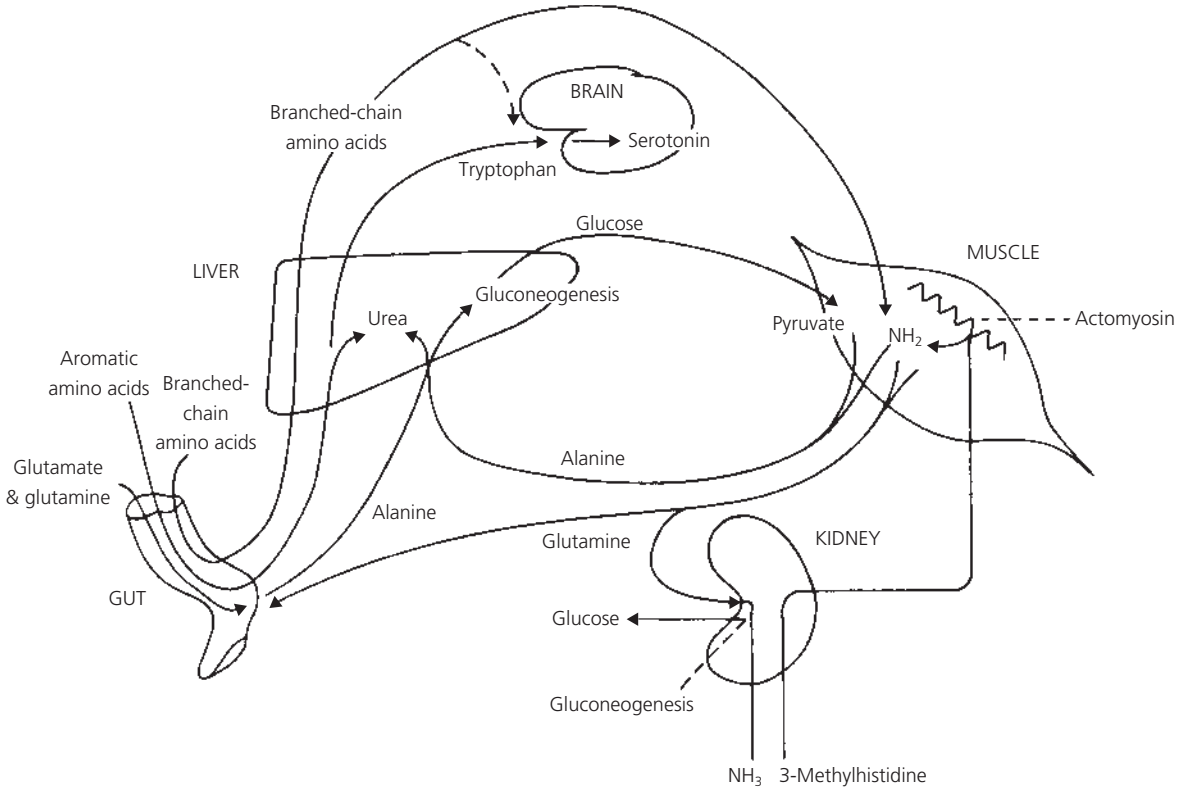

Figure 21.2 Interactions of organs in the metabolism of some major amino acids. Adapted from Munro HN. Interactions of the liver and muscle in the regulation of metabolism in response to nutritional and other factors. In: Arias IM, Popper H, Schachter D, et al. (eds). The Liver: Biology and Pathobiology. New York: Raven Press, 1982:681.

much greater than the rate of fatty acid oxidation, such as during starvation or uncontrolled diabetes mellitus. In these conditions, ketone bodies become an important fuel and are released into the bloodstream for delivery to extrahepatic tissues. Ketone bodies represent a water-soluble fuel derived from water-insoluble fatty acids. Ketone bodies can cross the blood–brain barrier to replace glucose as the major fuel for the brain, sparing plasma glucose for consumption by other tissues [72].

The biosynthesis of fatty acids is mediated by fatty acid synthase, a multienzyme complex embodied in a single polypeptide chain. It elongates the molecule by sequential addition of two-carbon units and stops with the formation of palmitic acid, a 16-carbon fatty acid. The formation of malonyl-CoA from acetyl-CoA is the committed step in fatty acid biosynthesis and the most important step of regulation. The enzyme that catalyzes this step, acetyl-CoA carboxylase, is stimulated by citrate. Citrate is abundant when ATP and acetyl-CoA are abundant, a condition appropriate for fat synthesis. Palmitoyl-CoA, the end product of fatty acid synthesis, antagonizes the activation of acetyl-CoA carboxylase by citrate.

Essential fatty acids

Most fatty acids can be synthesized by the liver, but humans lack the desaturase enzyme needed to produce the *n*-3 double bond (between carbons 3 and 4, counted from the methyl end) and the *n*-6 double bond (between carbons 6 and 7) in the fatty acid series. Essential fatty acids are important constituents of cell membranes and precursors of the eicosanoids [73]. Arachidonic acid (C20:4, *n*-6), a precursor of eicosanoids, prostaglandins, leukotrienes, prostacyclins, and thromboxanes, is synthesized from linoleic acid [73,74]. Vegetable oils, such as corn, soybean, sunflower, peanut, and cottonseed oils, are rich sources of linoleic acid [73,74]. Linoleic acid (C18:2, *n*-6) should constitute at least 2%, and linolenic acid (C18:3, *n*-6, -9, -12) at least 0.5%, of the daily energy intake to prevent the occurrence of essential fatty acid deficiency, usually manifested as a specific alteration in the plasma fatty acid profile and a skin rash. An elevated ratio of triene to tetraene (> 0.4) is characteristic of essential fatty acid deficiency as a result of increased production of eicosatrienoic acid, a fatty acid containing three double bonds (i.e., triene) derived from oleic acid (C18:1), and of decreased arachidonic acid production, a tetraene derived from linoleic acid elongation [75,76]. Essential fatty acid deficiency is rare in adult humans because of sufficient essential fatty acids stored in adipose tissue. However, continuous infusion of lipid-free total parenteral nutrition (TPN) can cause abnormalities of the triene-to-tetraene ratio within

10 days because of increased plasma insulin concentrations, which inhibit lipolysis and the release of essential fatty acids [76].

Fish oils

Fish oils are ω-3 polyunsaturated fatty acids (PUFAs) found in marine animals, particularly fatty fish, such as herring, salmon, bluefish, and tuna [77,78]. Epidemiological studies suggest a possible protective effect of fish oils, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), against cardiovascular disease and breast cancer [79–82]. In sufficient doses, fish oil prolongs the bleeding time and decreases the production of the proaggregating substance thromboxane A_2 [83–86]. Animal models suggest that fish oils have an inhibitory effect on coronary atherosclerosis and intimal hyperplasia [77,83,84]. The significant hypotriglyceridemic effects of fish oils have been confirmed repeatedly in healthy persons and in persons with various degrees of hyperlipidemia [85–92]. In a study involving 11 323 patients with a recent myocardial infarction, a 45% reduction in sudden death was noted at 42 months. Interestingly, the reduction of sudden cardiac death started to be significant at 4 months. This suggests that the benefit of PUFAs may not be mediated only by antiatherosclerotic and antithrombotic effects, but may also involve antiarrhythmic effects [77,81,83,84]. The effects on serum cholesterol and low-density lipoprotein (LDL) levels have varied [77,92,93]. The ω-3 fatty acids generally suppress cellular inflammatory responses by changing the end products of eicosanoid synthesis [77,90,94–96]. Dietary supplementation with fish oil suppresses the production by monocytes of the polypeptide cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF), suggesting an additional mechanism by which fish oils may exert an antiinflammatory effect [94,97]. Successful outcomes of numerous randomized trials led the American Heart Association to release the following guidelines in 2002:

- all adults should eat fish, especially fatty fish, at least two times per week
- patients with documented coronary heart disease should consume about 1 g of EPA and DHA (combined) per day
- an EPA and DHA supplement may be beneficial for patients with hypertriglyceridemia [98].

Structured triglycerides

Structured triglycerides are lipids that have been chemically or enzymatically altered. They are used in nutritional admixtures and generally are prepared with reesterification of a long-chain fatty acid in the *sn*-2 position and medium-chain fatty acids in the *sn*-1 or -3 position of the glycerol molecule. Studies conducted in different animal models of burn injury, endotoxic shock, trauma, and ischemia/reperfusion injury have shown that the use of structured triglycerides improves protein and energy metabolism and increases intestinal absorption, compared with physical mixtures of long-chain and medium-chain fatty acids [99–105]. A study of patients with cancer of the upper gastrointestinal tract found that structured triglycerides reduce gastrointestinal complications and improve hepatic and renal function [106].

Carbohydrates

Carbohydrates, which constitute most of the earth's organic matter, are important sources of metabolic fuel. In the United States, carbohydrates normally account for about 50% of ingested calories; about 60% is complex carbohydrate, primarily starch, and most of the remainder is sucrose and lactose [107]. About 10–20 g of indigestible carbohydrate (i.e., soluble and insoluble fibers) are consumed daily. They all undergo hydrolysis in the colon to yield glucose, other simple sugars, and short-chain fatty acids. Some cells and tissues, such as erythrocytes, leukocytes, renal medulla, eye tissues, and peripheral nerve tissue, do not have the capacity for citric acid cycle activity and require glucose as a fuel for anaerobic glycolysis. The brain prefers glucose as a fuel. Daily glucose requirements include 40 g/day for anaerobic tissues and 140 g/day for the brain [108].

Absorbed glucose that is not directly oxidized can be stored as energy in the form of glycogen or fat, which requires about 5% and 25%, respectively, of the original substrate oxidative energy potential. Glycogen is a branching, long-chain polymer of glucose molecules that has water and electrolytes between the chains. It is found in most tissues but significant amounts are stored only in the liver and skeletal muscle. The primary function of hepatic glycogen, amounting to about 100 g in a healthy adult, is to maintain blood glucose levels. Plasma glucose is an essential fuel for glucose-dependent tissues. Glycogen in skeletal muscle serves to supply glucose to the muscle itself during physical activity.

Glycolysis

The conversion of glucose to pyruvate in the cytosol of cells is known as glycolysis, a process that results in the generation of ATP but does not require oxygen. Pyruvate represents a major metabolic junction; it can be reduced to lactate, transaminated to form alanine, or enter the mitochondria and undergo carboxylation to oxaloacetate or oxidative decarboxylation to acetyl-CoA (Fig. 21.3).

Citric acid cycle and oxidative phosphorylation

The citric acid cycle (i.e., tricarboxylic acid cycle, or Krebs cycle) represents a series of reactions that occur in mitochondria. Carbohydrates, lipids, and amino acids enter the cycle after being metabolized to acetyl-CoA, and are completely oxidized to CO_2 and water. Vital biosynthetic intermediates are produced by the cycle, which plays a major role in gluconeogenesis, lipogenesis, and amino acid transamination and deamination. As acetyl-CoA is oxidized, reduced nicotinamide adenine dinucleotide (NADH) and reduced flavin adenine dinucleotide ($FADH_2$) are formed, which transfer

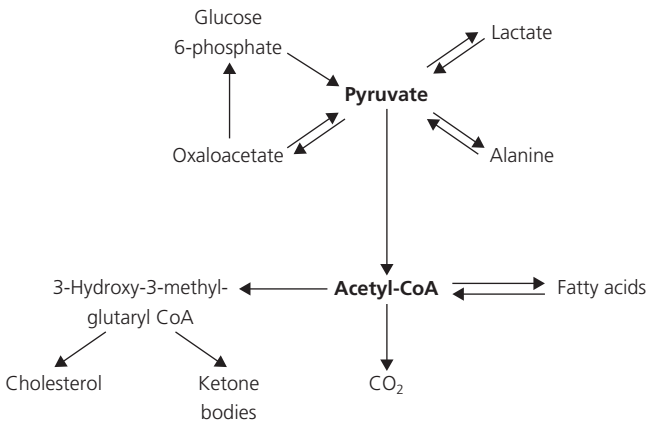

Figure 21.3 Major metabolic end products of pyruvate and acetyl-coenzyme A in mammals. From Stryer L. Biochemistry, 3rd edn. New York: WH Freeman, 1988:633.

the electrons to the respiratory chain in the inner mitochondrial membrane. In the mitochondria, the transfer of high-energy electrons from NADH or $FADH_2$ down the electron transport chain is coupled to the generation of ATP, a process known as *oxidative phosphorylation*. Glycolysis (i.e., anaerobic respiration) yields a net of only 2 ATPs per molecule of glucose, whereas aerobic metabolism (i.e., citric acid cycle and oxidative phosphorylation) yields 36 ATPs for each molecule of glucose oxidized.

Glucose production

Hepatic glycogenolysis is responsible for most of the glucose produced endogenously in the fed and postabsorptive states. Other mechanisms for glucose production are active and become critically important when hepatic glycogen is depleted, such as during prolonged starvation and endurance exercise. Gluconeogenesis is the process by which glucose is synthesized from noncarbohydrate precursors, lactate, glycerol, and most amino acids (principally alanine). Gluconeogenesis occurs primarily in the liver, but the kidneys also produce glucose, especially during prolonged fasting.

The Cori and glucose–alanine cycles provide mechanisms for generating plasma glucose for glucose-dependent tissues from 3-carbon intermediates released from peripheral tissues [109]. The *Cori cycle* (or lactic acid cycle) resynthesizes glucose that has been partially metabolized to lactate by peripheral tissues. Lactate produced principally by muscle, erythrocytes, and adipose tissue is transported to the liver and kidneys, where it is converted to glucose (gluconeogenesis) and released into the bloodstream. The *glucose–alanine cycle* shuttles glucose from the liver to muscle and alanine from muscle to liver. In this cycle, pyruvate is transaminated to alanine, which is transported to the liver and converted to glucose, which is then returned to muscle through the bloodstream.

Fiber

The accepted definition of dietary fiber is plant cell-wall components, both polysaccharides and noncarbodydrate components, that resist digestion by enzymes of the small intestine. The polysaccharide compounds in dietary fiber, which are the structural and matrix components of plant cell walls, consist primarily of cellulose, hemicelluloses, and pectins [110], as well as fructooligosaccharides and resistant starches. Examples of foods with high cellulose content include wheat bran, apple and pear skin, and strawberries; foods with notable hemicellulose content include whole grains; and foods with high levels of pectins include bananas, apples, and oranges. Cellulose is a β1-4-linked polymer of glucose and the main structural component of plant cell walls. Hemicellulose consists of branched polymers of pentose and hexose sugars. Other noncellulose polysaccharides include pectins, which are complex mixtures of colloidal polysaccharides, and several polysaccharides not associated with the cell wall, including mucilages and gums. These compounds are branched polymers containing many uronic acids that hold water and form gels. They are highly branched in growing plants and become less branched as the support structure develops. They act as adhesives and are insoluble in the unripe fruit, becoming soluble only as the fruit matures. Undigested oligosaccharides, such as those associated with flatus (e.g., stachyose and raffinose), are soluble and not included in the definition of fiber.

Fructooligosaccharides are mixtures of β2-1-linked β-D-fructose monomers. These molecules include inulin-type fructans (linear polymers) and levans (branched fructans) and are present in many edible plants, such as wheat grains and members of the onion family. The daily consumption of oligofructoses by North American populations is estimated at 1–12 g, slightly more in Western Europe [111]. The β-C2 linkage makes these polymers resistant to hydrolysis by human digestive enzymes, and they are fermented and metabolized in the colon to short-chain fatty acids. This colonic fermentation produces a change in the microflora, enhancing bifidobacteria and decreasing *Bacteroides* organisms, clostridia, and other anaerobes. Fructooligosaccharides are the best studied of the prebiotics, defined as "a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth or the activity of one or a limited number of bacteria in the colon and thus improves host health" [112].

Resistant starches (RSs) are defined as starches that enter the colon: RS1 is physically inaccessible starch because of the particle size or entrapment in food; RS2 and RS3 are resistant to amylase action because of their compact (unbranched) structure, by being either unbranched (RS2) or made retrograde (RS3) – that is, altered during food processing [113]. Most resistant starches are produced during food preparation. Intake of such starches with a typical Western diet is estimated to be 5–10 g/day.

Other components of dietary fiber include polyphenols (especially flavonoids) and other cell wall-associated nonpolysaccharide substances. Polyphenols are products of plant metabolism and range from single-ring phenols to highly polymerized compounds, such as tannins and lignins. Only lignins are included in the determination of dietary fiber, although they constitute about 12% of plant organic compounds. Lignins include a group of phenylpropane polymers of varying sizes, because they are continually polymerized as the plant ages. They reinforce the cellulose support structure and inhibit microbial cell wall digestion. Lignins are thus resistant to all anaerobic digestion systems and are not partially metabolized in the colon, as are the cell wall polysaccharides. They represent only a small part of the human diet (~ 0.2%).

Phenolic acids and aldehydes, such as vanillin, are common, but the most common of the plant phenolics are flavonoids, consisting of two aromatic rings linked through three carbons that form an oxygenated heterocyclic ring [114]. Flavonoids and other polyphenols are ubiquitous in plants and beverages. They contribute to the bitterness of tea and other beverages [9]. Polyphenols usually account for less than 1% of the dry matter of plants, but they can reach concentrations of 4000–7000 mg/mL in red wines and fruit juices [114]. The typical adult dietary intake of polyphenols in the United States is 1–1.1 g/day, with flavonoids accounting for about 4% of the total. Like other fiber components, polyphenols are degraded and their metabolites are absorbed in the colon, but the effect of these compounds on short-chain fatty acid production and microflora depends on the type of compound and the microorganisms present. Polyphenols bind proteins and precipitate them in the intestinal lumen, and they can decrease the absorption of nitrogen, fat, and some minerals, including iron.

Interest in polyphenols generally has focused on their antioxidant properties, particularly in relation to carcinogens and LDL oxidation. Some evidence suggests that moderate consumption of tea, a rich source of flavonoids, may protect against several forms of cancer, cardiovascular diseases, and kidney stone formation [115]. The blacker the tea, the more the polyphenols have been oxidized, lowering the possible effective role of these compounds. Herbal teas are not true teas (which derive from the shrub *Camellia sinensis*) and have a much lower flavonoid content. Tea contributes more than 60% of dietary flavonoids, onions about 13%, and grapes, apples, red wine, and dairy products most of the rest. The consumption of 1–2 cups of tea a day has been associated with health benefits in epidemiological studies [116], including 50% decreased mortality from stroke in men and 20%–40% decreased from cancer of the mouth, pancreas, colon, esophagus, skin, lung, prostate, and bladder. These data show only associations, not causation, and the results must be confirmed by prospective intervention studies. However, phenolics can, under some conditions, act as prooxidants [116], so the consumption of large amounts of phenolics as foods or supplements cannot be recommended until more data are available.

The heterogeneity of dietary fiber has inspired numerous classification schemes, including those based on source, chemistry, structure, water solubility, detergent solubility, physicochemical properties, and physiological actions. *Crude fiber* was a term commonly used until the early 1970s. It refers to the residue of plant material that remains when food is extracted by dilute acids and alkalis. Although crude fiber is the measurement still referred to in most food tables, it underestimates by 80%–90% the amount of material in foods that is undigestible by human digestive enzymes [13,117].

The physiological effects of dietary fiber on gastrointestinal function are complex because of their heterogeneity and the changes in the lumenal environment along the gastrointestinal tract. Combinations of fibers may have effects that differ from those of individual purified preparations, and the same purified fiber can have different effects depending on how finely or coarsely it is ground. The quantitative measurements of the fiber content of foods alone do not always allow prediction of their biological action.

Many physical properties of dietary fiber are physiologically important, including hydratability, viscosity, ion-exchange properties, and adsorptive capacity. Hydratability relates to the ability of a fiber to form viscous gels. It is a function of the physical and chemical composition of the fiber, including particle size, the age of the plant, and the chemical properties of the surrounding solvent. Some dietary fibers, such as lignins and pectins, have a significant capacity to bind and exchange ions, particularly calcium, iron, magnesium, zinc, and phosphorus, and to adsorb materials such as bile salts, proteins, and bacterial cells [13].

Fermentation of fiber by colonic bacteria generates volatile short-chain fatty acids, acetate, propionate, and butyrate, which serve as a systemic fuel and as the preferred energy substrate of colonocytes [118]. In general, water-insoluble fibers (e.g., wheat bran, bagasse) are less subject to fermentation and hold more water than do the water-soluble fibers (e.g., vegetable fiber, pectins, gums). Therefore, water-insoluble fibers have a greater effect on stool mass than watersoluble fibers. However, the ingestion of degradable fiber stimulates bacterial growth and generates a fecal mass composed largely of bacteria. Although the total number of bacteria can be affected by diet, there is no convincing evidence that dietary changes produce major changes in the composition of colonic microflora [119].

The rate of gastric emptying and the rate of digestion and absorption are influenced by fiber components. Guar gum and pectins increase the viscosity of the chyme and slow gastric emptying, but particulate fibers (e.g., wheat bran) appear to promote more rapid gastric emptying [117]. Fiber can decrease or increase mouth-to-anus transit time depending on fiber type, particle size, and bulk-forming capacity [120].

Intestinal transit time and stool bulk are inversely related. A large particle size (e.g., coarse wheat bran) produces a greater increase in stool bulk and a greater decrease in transit time than does a small particle size (e.g., finely ground bran). The mechanism by which fiber decreases colonic transit time is unknown but may be related to an increase in colonic peristalsis secondary to increased fecal mass [120].

If dietary fiber prevents colon carcinoma (see "Chemoprevention of gastrointestinal cancers" above), it might do so by several mechanisms: a decrease in colonic transit time, so that the time that colonic mucosa is exposed to carcinogens is shortened; adsorption of carcinogenic sterols or other carcinogens; dilution of potential carcinogens by increasing stool volume; and alteration of the relative number of anaerobic and aerobic bacteria in the colon [121]. The treatment of irritable bowel syndrome with a high-fiber diet (especially a diet that includes wheat bran and commercial fiber supplements) has produced conflicting results, but overall no benefit has yet been demonstrated [122]. Several studies have shown that bran and other fiber supplements are effective in preventing constipation, but the side effects include flatulence, distention, and bloating, perhaps related to a long colonic residence time and bacterial fermentation [123].

Fiber may prevent and treat hemorrhoids by decreasing straining during defecation. Straining causes engorgement of the vascular cushion lining the distal rectum and anal canal, making it more vulnerable to shearing stress. The passage of hard fecal masses through the anal canal exacerbates these shearing forces and displaces the vascular cushion caudally, where it may be trapped temporarily by contraction of the anal sphincter [124]. A trial of fiber is a reasonable initial therapeutic approach for many patients who have hemorrhoids.

Micronutrients: minerals and vitamins

The RDAs and AIs are based on the amount of a nutrient needed for an individual to avoid deficiency, or on the average daily amount that populations must consume to prevent deficiency (see Table 21.5). Statistically, the RDA is set as two standard deviations above the mean requirement, so that 97% of healthy persons are covered. The RDAs thus exceed the needs of many healthy persons. They are established only for healthy persons and were never intended as guidelines for therapy. For some nutrients (e.g., sodium, chloride, potassium), the evidence suggests that daily requirements are much lower than the content of the average diet in the United States. The Food and Nutrition Board has provided an estimated minimal requirement for these nutrients (Table 21.10). The RDIs and DRVs were established in 1993 for the purposes of food labeling and are derived from the 1989 RDAs for micronutrients [125]. What appears on food labels are neither DRVs nor RDIs but daily values, reflecting the recommendations for a 2000-calorie reference diet.

Table 21.10 Adequate intakes for healthy persons for sodium, chloride, and potassium

Age	Sodium (mg/day)	Chloride (mg/day)	Potassium (mg/day)
0–6 months	0.12	0.18	0.4
7–12 months	0.37	0.57	0.7
1–3 years	1.0	1.5	3.0
4–8 years	1.2	1.9	3.8
9–12 years	1.5	2.3	4.5
19–50 years	1.5	2.3	4.7
50–70 years	1.3	2.0	4.7
> 70 years	1.2	1.8	4.7

The adequate intake (AI) does not allow for prolonged losses by vomiting, diarrhea, or excessive dieting. The AI is not equivalent to the recommended dietary allowance (RDA).
Adapted from Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, Food and Nutrition Board, Institute of Medicine [12].

Minerals

Sodium [12]

Sodium, the principal cation in extracellular fluid, is necessary for maintenance of intravascular fluid volume and membrane potentials [125–127]. Total body sodium ranges from 48 to 60 mEq/g and is dependent on body size. The range for intake is 0.2–10.3 g/day. A 70-kg man has about 83–97 g of sodium in his body, about one-fourth of which is in the skeleton and cannot be exchanged. The kidney regulates sodium excretion by aldosterone action in the distal tubule in response to intravascular volume. Obligatory sodium losses are small compared with body stores. The minimum requirement to replace sweat, fecal, and urinary losses per day is about 0.18 g (8 mmol). Because almost all sodium is absorbed in the intestine, renal regulation of excessive sodium absorption is crucial.

Sodium is abundant in foods, not only as the chloride salt but as the bicarbonate, glutamate, phosphate, caseinate, benzoate, nitrate, propionate, sulfate, and citrate salts, among others. It is also present in many condiments, such as catsup, meat tenderizer, prepared mustards, olives, pickles, sauces, butter, margarine, and salad dressings. Water softeners can increase the content of sodium in water. Medications may contain sodium, although only a few contain enough to cause a problem.

Deficiency from inadequate intake alone is rarely encountered. Increased losses from the gut (e.g., vomiting, diarrhea, or drainage) or kidney (e.g., diuresis, salt-wasting renal disease, adrenal insufficiency) or excessive perspiration are the usual causes. Approximate mean concentrations of sodium in various fluids (in mmol/L) are sweat, 30–70; saliva, 10–20;

gastric juice, 70; bile, 145; pancreatic juice, 130; jejunal secretion, 115; ileal secretion, 100; and normal stool, 5.

Potassium [12]

Potassium is the primary cation in intracellular fluid, in which its concentration is 140–160 mEq/L [12,126–128]. The 2% of total body potassium present in the extracellular fluid is important in influencing resting membrane function, particularly in the cardiac muscle. The kidney is the major site of potassium excretion, which is normally regulated not by filtered load but by the action of aldosterone and systemic pH in the distal tubule. It is absorbed efficiently in the upper intestine but secreted in the colon, another aldosterone-sensitive tissue. Colonic secretion is aided by the electronegativity of the lumen. As with sodium, the amount lost in the stool is volume dependent. Most adults consume between 2 and 3.5 g daily. Abundant food sources (i.e., > 200 mg per portion) include meats and fish, vegetables (especially potatoes), nuts, fruits, and milk. In unprocessed foods, the usual anions are organic (e.g., citrate), whereas in processed foods with added potassium, the anion usually is chloride. Most salt substitutes use potassium chloride to replace sodium chloride, and contain about 2000 mg potassium per teaspoon. Moderate potassium deficiency occurs usually without hypokalemia, and is characterized by increased blood pressure in response to salt intake, increased risk of kidney stones, and increases in bone turnover. Hyperkalemia causes cardiac arrhythmias, muscle weakness, and glucose intolerance.

Calcium [8]

Calcium is the most abundant cation in the body. About 99% resides in bone; the other 1% is a crucial mediator for neural transmission, myocardial function, excitation and contraction of muscle, coagulation, cell division, maintenance of intercellular tight junctions, and enzyme function [8,129–132]. Many factors affect the intestinal absorption of calcium: lumenal pH, lumenal binders, transit time, the presence in the diet of the few foods that are rich in calcium, and vitamin D status [8,131]. The process is inefficient in that only about 33% of the daily calcium requirement is absorbed by the intestine, in part due to an obligatory loss of calcium each day from the intestine and kidney. During periods of growth or new bone formation, the calcium requirement increases. The risk for a negative calcium balance is great, especially during childhood, adolescence, pregnancy, or lactation. The RDA for adults is 1000 mg/day (see Table 21.5), but typical diets in the United States provide only about 750 mg/day [8]. Unlike many of the other RDAs, these recommendations are meant not as nutrient requirements for individuals, but as an average requirement for nearly all healthy persons in a particular life-stage or gender group. The debate continues regarding the need for calcium guidelines based on the relationship between calcium intake and bone health [133]. The RDA was raised for adolescents to 1300 mg/day, based on increased bone growth during this life stage. The increased recommendation in older persons to 1200 mg/day is based on decreased calcium absorption in that group. Milk and dairy products are the richest source of calcium (providing about 60% of dietary calcium), and it is most bioavailable in those sources in the form of calcium citrate, but, there is little evidence for its superiority in promoting bone mineralization [134]. Each 225-g (8-oz) cup of milk contains about 280–300 mg of calcium. Green leafy vegetables are a good source, but the bioavailability is more irregular than that in milk products because the calcium is present as the phytate, oxalate, or other organic anion salt. These salts are poorly ionized and absorbed. The single universal requirement for calcium intake at each life-stage without reference to intake of protein, sodium, or phytate has been challenged, based on the observation that calcium intakes are low in parts of the world where fracture rates are low, but protein intake also is low, whereas phytate intake is high [135]. Other rich dietary sources are fish with edible bones, such as sardines and salmon.

Lumenal calcium is most actively transported in the duodenum, but in humans most calcium is absorbed in the ileum [129]. About 150–300 mg is secreted into the lumen each day. After glomerular filtration, about 98% of calcium is reabsorbed by the renal tubules [132]. Normal levels of serum calcium are tightly regulated by the action of parathyroid hormone and vitamin D and are not related to total body stores, except when deficiency is severe. Bone density is not a sensitive indicator of calcium deficiency, but it is a measure of body stores, and its determination can detect decreases in bone mass exceeding 1%–2% [136]. Ionized serum calcium is a measure of the hormonally regulated calcium concentration. Half of serum calcium is protein bound, 10% is complexed with anions, and 40% is ionized and physiologically active. Under most circumstances, ionized serum calcium reflects body stores, but when acutely altered, its concentration may be low in the presence of normal total body calcium [137].

Magnesium [8]

Magnesium is the second most abundant intracellular cation. About 70% of the total is in bone, and the rest is in soft tissues. Less than 1% is in extracellular fluids, where 20%–30% is bound to protein. Serum magnesium levels do not closely mimic body stores. The magnesium concentration in cells is high, like that of potassium, but magnesium leaves the cells less readily. Magnesium is important for neuromuscular transmission, and it is an essential cofactor in many enzyme reactions, including oxidative phosphorylation and nucleic acid synthesis. It also plays a role in wound healing, myocardial contractility, membrane stability, and coagulation [138–141].

The RDA averages about 350 mg (29 mEq) for adults: 400–420 mg for men and 310–320 mg for women. The typical

adult diet in the United States provides 20–40 mEq/day. Food sources are well distributed, but nuts, cereals, seafood, meats, legumes, and green vegetables are rich food sources [138,139]. Like that of calcium, the intestinal absorption of magnesium is relatively inefficient (i.e., 30%–40%), and most absorption occurs in the ileum. However, magnesium differs from calcium in that there is little obligatory intestinal loss, so magnesium balance is maintained even with a low dietary intake if no abnormal losses occur. Loss from the body occurs in regulated function, mostly through the kidney (2%–3% of the filtered load) [138–141]. Serum magnesium is the standard for assessing body stores, but it is falsely elevated by hemolysis and does not always reflect either intracellular stores or active extracellular ionized magnesium [125,137].

Phosphorus [8]

Phosphorus is the major intracellular anion (100 mmol/L) [141–143]. It is essential for normal membrane function, regulation of enzyme systems, and generation and storage of energy. It affects the delivery of oxygen to tissue by regulating the concentration of 2,3-diphosphoglycerate in red blood cells. However, 80%–85% of total body phosphorus resides in bone.

The requirements for phosphorus parallel those of calcium. The new RDA is only 700 mg/day for adults, somewhat below the DRI for calcium, although daily intakes in the United States range from 1000 to 1500 mg. Like magnesium, urinary phosphorus excretion does not reflect a low dietary intake, so balance studies are misleading. Moreover, the efficiency of phosphorus absorption varies with the food source and the dietary calcium-to-phosphorus ratio. In general, if protein intake is adequate, so is phosphorus intake [8]. Phosphorus is a constituent of all cells and is abundant in most foods, especially meats, dairy products, and carbonated beverages. The amount of phosphorus in food additives is increasing, a change that can lead to decreased bone mass [144]. Phosphorus is a component of some gut lavage preparations (e.g., Fleet, Accu-Prep, Visicol, Fleet Phospho-soda). The absorption of phosphorus requires hydrolysis of the organic phosphates in food by intestinal alkaline phosphatase and is promoted by the action of 1,25-dihydroxyvitamin D-3. Five sodium-dependent phosphate cotransporters (NPT) have been identified [143]. It is the NPT2b form, expressed in the small intestine, that mediates phosphate absorption. Net absorption is 60%–80% of ingested phosphorus. The kidney adjusts phosphate excretion over a wide range, mostly using NPT2a but also NPT2c and NPT3, so deficiency from low intake or malabsorption is rare. In fact, reabsorption can increase to 99.8% if the dietary intake of phosphorus is low [141].

Iron [11]

The body stores of iron are not regulated by increased or decreased excretion, but rather by control of the rate of intestinal absorption, which is increased during deficiency. Daily losses normally occur from the gastrointestinal tract, skin, and urine. Fecal losses predominate, ranging from 6 to 16 mg/day, most of which is unabsorbed dietary iron. About 1 mg of endogenous iron is lost in the stool per day. Additional losses occur from the uterus in women, amounting to 0.5–1.0 mg/day averaged over a whole month. The requirements for iron per kilogram of body weight are highest during infancy (because of low body stores), periods of rapid growth (adolescence and pregnancy), and periods of excessive loss (menstruation).

The RDA assigned by the 2001 DRI Committee for all age groups of men and postmenopausal women is 8 mg/day; the RDA for premenopausal women is 18 mg/day [11]. The recommended intake during pregnancy is 27 mg/day. At birth, even the child of an iron-deficient mother has normal stores because the fetus has priority for available iron. Milk is a poor dietary source of iron, but the AI for infants aged 0–6 months has been set at 0.27 mg/day, based on the daily amount of iron in ingested milk (~ 0.35 mg/L). It is assumed that milk intake and requirements correlate with body size. Therefore, this AI may not be sufficient for infants with a lower milk intake. Three months after birth, the requirement for iron increases because of growth, and the RDA of 11 mg/day for infants aged 7–12 months assumes that feedings complementary to milk are in place. It is during this period that the infant is most at risk for iron deficiency. During adolescence, the hemoglobin level rises 0.5–1.0 mg/dL per year, requiring 50–100 mg of iron per year, and 300 mg during adolescence.

Food iron is available in a variety of red meats, nuts, seeds, and egg yolks. Milk products, potatoes, and fresh fruit are poor sources. Iron availability can vary by as much as 10-fold between meals with similar iron content [145]. Iron in vegetables varies according to growth conditions of the plant. Heme iron in vegetables is not so readily available for absorption as that in meat. Heme iron requires reduction to the ferrous state (by ascorbic acid) for maximal absorption. Heme iron accounts for less than 40% of dietary iron in the United States. The iron content of some foods (e.g., bread flour) is increased by fortification. Iron supplements can decrease absorption efficiency of dietary iron. Vegetarians are at risk for dietary iron deficiency because of the more limited absorption of nonheme iron. Iron is involved in many reactions as a cofactor for enzymes (heme or otherwise) and as a major constituent of heme as an oxygen-carrying cofactor.

Iron is absorbed primarily in the duodenum and upper jejunum. The absorption of inorganic iron is enhanced by gastric acid, ascorbic acid, and other organic acids, including the amino acids histidine, lysine, and cysteine, which form iron chelates [11,146]. Absorption is decreased when iron forms an insoluble complex in the lumen with dietary phytates or phosphates, or with antacids or other medications. In the duodenal lumen, dietary iron is reduced to the ferrous state by ferric reductase (Dcytb, duodenal cytochrome b),

transported into the cell by the divalent metal transporter, DMT1, and released by way of ferroportin into the circulation [147]. Hephaestin is another protein that facilitates iron release from the enterocyte. Hepatocytes take up iron, either free or bound to transferrin (Tf), by Tf receptor 1 or 2. The TfR2 receptor is the major sensor of circulating Tf–iron complexes and influences the expression of hepcidin, the protein that downregulates ferroportin-mediated iron release from enterocytes, macrophages, and hepatocytes [148]. Hepcidin levels are inappropriately low in hereditary hemochromatosis, and are increased in patients with inflammatory conditions. Hepcidin appears to be the major gatekeeper for iron absorption. Normally about 10% of iron is absorbed, but in iron deficiency states this can increase to 30%. After absorption, iron is stored as ferritin in the liver, spleen, and bone marrow. The functional compartment of iron (hemoglobin, myoglobin) accounts for most of the total body iron, ranging from about 2180 to 2750 mg for the average woman and man, respectively.

Zinc [11]

The body contains 1.5–2.5 g of zinc, so that it is the second most abundant trace mineral after iron. Although the turnover of isotopic zinc in adults is 6 mg/day, balance studies show that 12.5 mg of dietary zinc is needed to maintain positive balance [11]. The daily loss of 2.5 mg/day is mostly in feces, and absorption ranges from 20% to 40% of dietary zinc, depending on the fiber content of the diet (inversely related). Zinc bioavailability from a vegetarian diet is moderate (30%–35% is absorbed) with phytate:zinc ratios of 5:15. More zinc may be needed in diets that are high in legumes, whole grains, seeds, and nuts, because in those diets the phytate:zinc ratio may approach 15. The 2001 DRI Committee report set the RDA at 9 and 8 mg/day for girls and boys aged 9 to 13 years, respectively, and at 8 and 11 mg/day for adult women and men, respectively [11]. The RDA for pregnancy is 13 mg for adolescents and 11 mg for women older than 18 years. During lactation, the RDA is increased by 1 mg/day.

Like other divalent cations, zinc is absorbed inefficiently along the entire length of the small intestine. A series of zinc transporters have been identified in humans. Zinc transporters have a binding domain that is rich in histidine residues. These transporters are thought to be important in absorption, and include: ZnT1, which is expressed in duodenal and jejunal villi; DCT1, which directs the influx of iron, zinc, copper, cadmium, and manganese; and ZnT1 and ZnT2, which mediate the efflux of the same minerals [149]. Zinc absorption is decreased by lumenal binders (phosphate and others), as is the absorption of calcium and magnesium [150]. Inorganic iron, especially when ingested as a supplement, impairs inorganic zinc absorption [151]. Absorption can be enhanced, however, by animal proteins and sulfur-containing amino acids, and by hydroxy acids [152]. Zinc absorption may be increased during states that increase demand for the mineral, including infancy, pregnancy, and lactation.

Zinc plays a critical role in the growth and function of cells. It is a cofactor for many enzymes that participate in the metabolism of carbohydrate, fat, and protein [11]. It is necessary for cell growth and proliferation, sexual maturation, reproduction, and dark adaptation and night vision, and it may play a role in wound healing and immune defenses. Finally, it may activate or inhibit enzymes, modify membrane functions, or bind to DNA transcription factors.

Copper [11]

Copper is an essential trace mineral for humans. Estimates of copper requirements are based on balance studies, fecal and other losses, and absorption at each life stage [11]. Obligatory losses for adults are about 580 μg/day, and absorption averages about 25% of dietary copper intake. Most absorbed copper is excreted in bile, although some biliary copper is reabsorbed by way of an enterohepatic circulation [153]. When intestinal and biliary losses occur, the copper requirement increases. The AI for infants is based on intake of milk (120 μg/L) and is set at 200 μg/day for infants aged 0–6 months and 220 μg/day for infants aged 7–12 months. The RDA is 900 μg/day for men and women aged 19 years or older [11].

The body contains about 100 mg of copper, 30 mg of which is bound to proteins and enzymes. The liver contains about one-third of the body stores of copper, mostly in enzymes, including ceruloplasmin, cytochrome oxidase, superoxide dismutase, tyrosinase, lysyl oxidase, and histaminase [154]. Copper is important for normal skeletal and nervous system development, erythropoiesis, leukopoiesis, and iron absorption, and as an antioxidant.

Copper absorption is efficient (35%–70%) compared with the absorption of other trace elements, but the efficiency is reduced in the elderly. Copper absorption and intracellular metabolism are complex processes [155]. A high-affinity copper transport protein, hCtr1, may transport copper across the apical enterocyte membrane by endocytosis. There is no free copper in the cell because it is bound to chaperones (e.g., to CCS, the chaperone for copper and zinc, and to metallothioneins 1 and 2) [155]. The Menkes disease protein, ATP7A, is a membrane-associated P-type ATPase that is required for copper secretion into the portal vein. Hephaestin is a multicopper oxidase that is a membrane-bound analogue of ceruloplasmin required for iron (but not copper) secretion from the intestine [156]. Absorbed copper is transferred in plasma to ceruloplasmin and albumin, and it is taken up by the liver by hCtr1-mediated transport. It is distributed to various cellular compartments by a series of small cytoplasmic copper chaperones [155]. In all tissues apart from the liver, ATP7A mediates copper efflux in the Golgi apparatus and subsequently in the plasma membrane, as the protein moves to the plasma membrane. In the liver, copper is guided to ATP7B, a copper-transporting ATPase, by the Wilson disease protein,

ATOX1, a metal chaperone [157]. Copper is then bound to glutathione and metallothionein. The multicanalicular organic anion transporter, cMOAT, transports the copper–glutathione complexes into bile. When intracellular levels of copper increase, ATP7B is redistributed to exocytic vesicles for delivery to bile, similar to the process for the Menkes gene protein ATP7A in nonhepatic cells.

Other trace minerals

Selenium is essential to the antioxidant system, being a component of more than 20 selenoproteins, including six glutathione peroxidases [10,158]. Thus, its function is linked with that of vitamin E. Chromium potentiates the action of insulin at the cell receptor level and plays a role as a cofactor for insulin [159]. Manganese is a cofactor for many enzymes with widely varying functions, from superoxide dismutase to hydrolases and kinases, but a deficiency state in humans has not been identified [11]. Iodine is an essential component of the thyroid hormones and is thus integral to their function [11,125]. Iodine is transported by the sodium/glucose cotransport family solute carrier SLC5 [160]. Fluoride is concentrated in bones and teeth and is required for normal growth. The major source of fluoride is fluoridated water. Although deficiency of most of these minerals occurs in humans, the occurrence of such deficiency is unusual in developed countries. For this reason, they are used in clinical practice primarily as TPN supplements.

Vanadium, nickel, cobalt, tin, and silicon are considered essential in mammals because deficiencies have been produced experimentally, but human deficiencies have not been reported [11]. Other elements, including cadmium, lead, boron, aluminum, arsenic, mercury, strontium, and lithium, have not yet been proved essential. Most of these elements, with the exception of boron, are probably present in sufficient quantities as contaminants in TPN solutions [161].

Vitamins

Thiamin (vitamin B-1) [9]

Thiamin is essential for the function of many enzyme systems and plays a major role in energy production. Thiamin pyrophosphate is a coenzyme in the oxidative decarboxylation of α-ketoacids to aldehydes, and it catalyzes transketolase activity in the pentose phosphate cycle. It is important for nucleotide synthesis and provides cofactors for fatty acid synthesis. Blood pyruvate levels increase with thiamin deficiency. The requirement usually is related to the intake of energy, especially carbohydrate. The RDA for adults is 0.5 mg thiamin for every 4184 kJ (1000 kcal) in the diet [9]. Allowances are based on the effects of varying dietary thiamin and the relationship with signs of clinical deficiency or with urinary excretion of thiamin or serum transketolase activity. Thiamin is synthesized by many plants. It is abundant in all foods and is added to many commercial baked products and cereals. Because thiamin is lost easily in the cooking and processing of food, its content in food varies according to preparation. Thiamin is transported into cells by the SLC19 folate/thiamin transporter family; specifically, the A2 and A3 types [162]. In polarized cells, such as enterocytes, thiamin is found at the apical and basolateral membranes (Table 21.11).

Riboflavin (vitamin B-2) [9]

Riboflavin is a major component of two essential coenzymes: flavin adenine dinucleotide (FAD) and flavin mononucleotide (FMN). It forms the active portion of these coenzymes, which are involved in biological oxidations. Riboflavin requirements are linked with metabolic function, like those for thiamin. However, unlike the requirements for thiamin, those for riboflavin are unrelated to food energy intake. The RDA is set at 1.1 mg/day and 1.3 mg/day for adult women and men, respectively [9]. Requirements have been assessed by measuring urinary excretion and by observing signs of deficiency. Riboflavin is available in all leafy vegetables, in meats and fish, and in milk and eggs. The average Western diet contains about 2.7 mg/day. The vitamin can be lost during food processing and by the action of ultraviolet light. Absorption is efficient and occurs by an active carrier-mediated process in the small intestine. Riboflavin enters the plasma as free flavin mononucleotide (FMN) or riboflavin, and after dephosphorylation of flavin adenine dinucleotide (FAD) and FMN, is reformed into FAD and FMN inside the enterocyte. The vitamin is rephosphorylated to permit transport across the basolateral membrane, and is excreted from the kidney unmetabolized [163].

Niacin (vitamin B-3) [9]

The term "niacin" encompasses nicotinic acid and its amide form, nicotinamide. It is a component of two coenzymes, nicotinamide adenine dinucleotide (NAD) and nicotinamide adenine dinucleotide phosphate (NADP), which participate in more than 50 metabolic reactions. Humans can synthesize about 1 mg of niacin from 60 mg of the amino acid tryptophan. Deficiency depends on the limited availability of niacin and tryptophan [164]. The RDA is reported in niacin equivalents (NEs) (1 NE = 1 mg of niacin = 60 mg of tryptophan). The RDA has been estimated at 14 mg and 16 mg NE daily for women and men aged 14 years or older, respectively [9]. Average Western diets provide 16–24 NE/day. Nicotinic acid is present in most foods, except fats and oils. Meat, fish, and grain products are good dietary sources. Much is lost during grain processing, but it is added as fortification to the finished products. Niacin is well absorbed in the small intestine by a sodium-independent, acid-sensitive process.

Pyridoxine (vitamin B-6) [9]

The term "vitamin B-6" refers to three naturally occurring pyridines: pyridoxine, pyridoxal, and pyridoxamine. All forms function similarly. Vitamin B-6 is essential for the function of

Table 21.11 Mechanisms of absorption of water-soluble vitamins

Vitamin	Source of ingested vitamin	Transporter	Extent documented	Intracellular regulation
Thiamin (B-1)	Diet, bacteria	Human thiamin transporters 1 and 2 SLC19A2 and SLC19A3 genes	Small bowel? colon, apical < basolateral	Ca^{2+}/calmodulin
Riboflavin (B-2)	Diet	Na-independent carrier-mediated	Small bowel, colonocytes	PKA, Ca^{2+}/calmodulin
Niacin (B-3)	Diet, metabolism (from tryptophan)	Na-independent, acid-dependent	Small bowel	Tyrosine kinase
Pyridoxine (B-6)	Diet	Na-independent		PKA-mediated
Biotin	Diet, bacteria	Na-dependent multivitamin transporter (SMVT)	Small bowel	PKC, Ca^{2+}/calmodulin, biotin (extracellular)
Pantothenic acid	Diet, bacteria	SMVT	Small bowel, colonocytes	
Folate	Diet, bacteria	Reduced folate carrier	Small bowel, pancreas, apical > basolateral	Tyrosine kinase, cAMP, pH-dependent
Cobalamin (B-12)	Diet	Cubilin–amnionless complex	Small bowel, proximal renal tubule, choroid plexus, apical	Development
Ascorbate (C)	Diet	Na-dependent vitamin C transporter SVCT1 (SLC23A1 gene), facilitated glucose transporters GLUT1, GLUT3, and GLUT4 for dehydro-L-ascorbate	Small bowel, apical SVCT2 basolateral	PKC-mediated Ascorbic acid (extracellular)

cAMP, cyclic adenosine monophosphate; PKA, protein kinase A; PKC, protein kinase C.

many aminotransferases and amino acid decarboxylases. It is involved in the metabolism of all amino acids and in the synthesis of acetylcholine, porphyrin, arachidonic acid, dopamine, serotonin, and bile acids. Requirements are increased with a higher protein intake. The RDA for adults aged 19–50 years is 1.3 mg/day for average protein intake. Low levels of all three forms are present in all foods, with meats, fish, and grains being good sources. The vitamin B-6 allowance has been estimated by using a ratio of 0.016 mg of vitamin per gram of protein ingested. Because energy and protein intakes are lower in the older population, RDAs are increased for persons older than 50 years, and the estimates for women (i.e., 1.5 mg/day) are lower than those for men (i.e., 1.7 mg/day) [9]. Vitamin B-6 is synthesized by microorganisms in the intestine, primarily in the colon, where the vitamin is not absorbed. Vitamin B-6 is rapidly absorbed in the small intestine by a sodium-independent transporter, and is excreted in the urine as the metabolized product, 4-pyridoxic acid. Certain drugs are pyridoxine antagonists (e.g., isoniazid, hydralazine, penicillamine). A deficiency of vitamin B-6 and other B vitamins can occur in chronic alcoholics.

Folate (folacin, folic acid) [9]

"Folacin" is a generic term for compounds that have a structure and function similar to that of folic acid, that is, pteroylglutamic acid (PGA). The many forms differ in the degree of reduction of the double bonds in the ring structure (e.g., tetrahydrofolate), the presence of 1-carbon groups (e.g., methyltetrahydrofolate), and the number of glutamyl residues in the peptide chain (e.g., folate pentaglutamate). Folate functions as a carrier of 1-carbon groups from donor to recipient molecules and is necessary for the synthesis of nucleic acids, the initiation of protein synthesis, and the synthesis of acetylcholine and methionine. Three other vitamins – cobalamin, ascorbic acid, and niacin – are involved in converting folate to its active coenzyme forms [9,165]. Pteroylglutamic acid is the form of folate used commercially, and it is a relatively poor substrate for dihydrofolate reductase. As a result, tissue utilization is poorer for PGA than for natural methylated or reduced folates found in food. However, folate requirements have been based on replacement with PGA.

The RDA for folate has been modified dramatically from the 1989 estimate of 200 μg/day for adults to 400 μg [9]. This change was the result of recognition of the role of folate in reducing the incidence of neural defects [166], and the role of elevated serum homocysteine concentrations as a cardiovascular risk factor [167]. Dietary folate is reported in folate equivalents, in recognition of the greater bioavailability of synthetic folic acid compared with natural folate. The RDA

for women aged 14–50 years is 400 μg/day, and should comprise synthetic folic acid plus dietary folate. During pregnancy, the amount of synthetic folic acid is specified at 400 μg/day [9].

The vitamin is abundant in citrus juices, enriched cereals and breads, legumes, liver, nuts, and green leafy vegetables. To prevent neural defects, the US Food and Drug Administration (FDA) has, since 1998, required that all cereal grains are fortified with 140 μg of folate/100 g of grain [168]. It is estimated that this supplements the average daily dietary intake (50–500 μg) by 215–240 μg, and the incidence of neural defects has decreased by about 25%. Folate occurs in food largely in the polyglutamate form, and its bioavailability generally is high. However, boiling, steaming, or frying can lead to significant losses. Folate is absorbed in the proximal small intestine through active transport mediated by the reduced folate carrier, the apical SLC19A1 sodium/folate cotransporter [162]. It is converted in the liver and other tissues to the 5-methyltetrahydrofolate form and is stored as polyglutamate [169]. Total body stores are relatively small and can be depleted in a few months if dietary intake is negligible. Folate can enter the serum or the bile and be reabsorbed, undergoing an enterohepatic circulation amounting to about 100 μg/day. In diseases that cause malabsorption, body stores of folate are lost more rapidly than in simple dietary deficiency.

Cobalamin (vitamin B-12) [9]

Cobalamin contains a cobalt atom to which are bound active groups, including hydroxy-, methyl, and nitro- moieties. The vitamin functions as a carrier for methyl and hydrogen groups. It is required for reactions catalyzed by two enzymes, methionine synthase and methylmalonic acid mutase, and thus participates in methionine and succinyl-CoA synthesis. The total body content of cobalamin is 2–2.5 mg, most of which is in the liver, and the half-life is 1.5–3.5 years. Thus, daily losses average about 1.3 μg/day. The RDA was set at 2 μg/day for adults, based on 70% absorption efficiency [9]. However, because 10%–30% of adults older than 51 years may have protein-bound cobalamin malabsorption, the new RDA has been set at 2.4 μg/day [9]. Although this malabsorption probably is caused by reduced pepsin and gastric acid secretion, most elderly persons have some intrinsic factor. Thus, it is recommended by some experts that most intake in older persons be in the form of a dietary supplement, to ensure its adequacy [170]. The vitamin is synthesized only by bacteria and enters animal tissues after the ingestion of contaminated foods or production in the gut lumen. It is found only in animal products, including meat, fish, eggs, and milk. The average Western diet contains 5–15 μg/day. Cobalamin is relatively stable during cooking and processing.

The absorption of cobalamin is complex [171]. In food, it is bound to enzymes from which it is released by gastric proteases. It is bound to haptocorrin in the stomach, released from haptocorrin by the action of pancreatic enzymes, and then bound to intrinsic factor, with which it is absorbed in the ileum by the process of receptor-mediated endocytosis. The receptor for the intrinsic factor–cobalamin complex, cubilin, is a large, multifunctional protein that also binds apolipoproteins [172]. Studies of the mutations in Imerslund–Grasbeck syndrome (congenital vitamin B-12 malabsorption) have uncovered a 45–50-kDa transmembrane protein that colocalizes with cubilin, called amnionless (AMN) [173]. The amino-terminal portion of AMN directs endocytosis, and is essential for cobalamin absorption, whereas the carboxy terminal is necessary for embryonic development. In the enterocyte, the vitamin is released from intrinsic factor, bound to transcobalamin II, and delivered to the tissues complexed to that protein. It is released into the bile bound again to haptocorrin, and 5–10 μg/day undergoes an enterohepatic circulation. As with folate, body stores are lost more rapidly if malabsorption is present because endogenous as well as dietary cobalamin is lost.

Ascorbic acid (vitamin C) [10]

Ascorbic acid is an essential cofactor for several hydroxylation reactions and plays a key role in the synthesis of collagen. It may also function as an antioxidant for vitamins A and E, and is involved in the formation of norepinephrine (noradrenaline) and serotonin. This role in neurotransmitter synthesis may explain the fatigue and weakness seen in scurvy [174]. A daily intake of 10 mg of ascorbic acid cures clinical signs of scurvy but does not maintain body stores. The previous RDA of 60 mg/day produced wide fluctuations in plasma levels. The ascorbic acid requirement is also compounded by its possible chemoprotective value in some disorders (e.g., colon cancer, heart diseases, cataracts), at doses far in excess of those needed to prevent scurvy. Intake of 200 mg of ascorbic acid per day is needed to begin to saturate tissues, an intake that approximates the vitamin C content of diets (~ 225 mg/day) that allow maximal protective effects of the vitamin [175]. The new DRI values represent a compromise between the old RDA value and those needed for chemoprevention, and are set at 75 mg/day and 90 mg/day for adult women and men, respectively [10]. During pregnancy and lactation, the RDA should be increased by 10 mg/day and 45 mg/day, respectively. The vitamin is especially concentrated in green vegetables and citrus fruits, although it is widespread in foods. The vitamin C content of food depends on the state of ripeness and on the method of preparation because ascorbic acid is sensitive to heating and oxidation. It is well absorbed in the small intestine by two sodium-dependent cotransporters (SVCT1 and SVCT2) at intakes of up to 180 mg/day [176]. Over that amount, the proportion appearing in the stool increases, and at high doses (> 3–4 g/day), diarrhea can result.

Biotin [9]
Biotin is a coenzyme for various carboxylases and is important in the metabolism of carbohydrate, protein, and fat [177]. It is produced by colonic bacteria and absorbed in the small bowel and colon by facilitated diffusion. Because of this endogenous production, an RDA has not been determined, but the AI for adults has been estimated at a DRI of 30 μg/day [9]. Absorption of the vitamin is mediated by a sodium-dependent multivitamin transporter (SMVT) [178].

Pantothenic acid [9]
Pantothenic acid is a precursor of CoA, which is essential for the metabolism of fats, carbohydrates, and proteins and for the synthesis of steroids and porphyrins [9,179]. It is widely distributed in foods, especially animal tissues, whole-grain cereals, and legumes. Microflora may produce some pantothenic acid, but the data in humans are not clear. Although an RDA has not been established, the estimated AI for older adolescents and for adults is 5 mg/day. Pantothenic acid is well absorbed in the intestine by means of SMVT, and is excreted unchanged in the urine.

Vitamin A [11]
Vitamin A is the collective term for vitamin A alcohol (retinol) and its related biologically active forms. It is essential for growth and development, the maintenance of epithelial cells, the stability of cell membranes, reproduction, and vision in dim light [11,180]. The β-carotenes are precursors of vitamin A and appear to have additional beneficial functions, probably as antioxidants [181]. The DRI allowance for vitamin A is based on many nutritional studies and amounts to 900 μg of retinol per day for men and 700 μg/day for women [11]. The recommendations for β-carotene are even more complex because no reproducible biological activities are available to establish adequate intake. Some epidemiological studies show correlations between low serum levels (still in the normal range) and protection from cancer, but intervention studies have not been positive [182]. Moreover, some carotenoids (e.g., lutein) are accumulated in the retina preferentially, whereas others (e.g., lycopene) lack provitamin A function but exhibit other biological activity. The data were judged by the DRI Committee to be insufficient to make a recommendation on the required percentage of dietary vitamin A that must be derived from provitamin A carotenoids [11].

Vitamin A synthesis is limited to plants and microorganisms. Its activity in foods usually is expressed in international units (IUs) and an equivalent value established with retinol activity, accounting for both vitamin A and provitamin compounds in the diet. The previously accepted 6:1 equivalence of β-carotene to vitamin A has been questioned because of the inefficient bioconversion of plant carotenoids. Thus, the new conversions are 1 retinol equivalent (RE) = 1 μg of all-*trans*-retinal, or 12 μg of β-carotene, or 24 μg of mixed carotenes. One RE is also equivalent to 3.3 IU of retinol activity or 10 IU of β-carotene activity. This means that older food tables have overestimated the vitamin A activity in foods. This change may be of special importance in less-developed countries that rely mostly on vegetable products for their vitamin A. Animal products are rich sources, including liver, kidney, dairy products, and eggs. Carotenoids, especially β-carotene, are found in green and yellow vegetables. There are more than 400 carotenoids in foods, only about 60 of which have provitamin A activity. In the United States, dairy products and margarines are supplemented with retinyl esters, and these products are the major dietary source of the vitamin.

Vitamin A, ingested in the form of long-chain retinyl esters, is hydrolyzed to retinol by lipases and esterases in bile and pancreatic secretions or in milk. More than 80% of the vitamin is absorbed passively by an as yet unknown mechanism, reincorporated into retinyl esters, packaged into chylomicrons, transported in the lymphatic circulation, and stored in the liver, which controls release of the vitamin [11,183]. Carotene is absorbed passively, but less well than vitamin A (40%–60%). Most is hydrolyzed to retinol inside the enterocyte, and a small amount is absorbed intact. After vitamin A is released from the liver, it is transported in the plasma as a trimolecular complex with retinol-binding protein and transthyretin [183]. If stores are adequate, any excess vitamin A is excreted in bile. A small amount is excreted in urine along with other metabolites. About 10% of hepatic retinol is converted to retinoic acid, which in turn is conjugated with glucuronide and undergoes enterohepatic circulation. As in the case of folate, cobalamin, and 1,25-dihydroxyvitamin D, this enterohepatic circulation leads to the loss of endogenous as well as dietary vitamin A if malabsorption occurs.

Vitamin D [8]
Vitamin D is the designation for a group of sterols and their metabolites that have antirachitic activity [8]. Cholecalciferol (vitamin D-3) is formed in the skin from 7-dehydrocholesterol by the action of ultraviolet light, and about 100 IU/day (10 μg = 400 IU) is produced in persons living in the temperate zone [184]. Ergocalciferol (vitamin D-2) is present in plants. Vitamin D is a provitamin, with the active metabolite being 1,25-dihydroxyvitamin D. The active vitamin promotes the intestinal absorption of calcium and phosphorus, in conjunction with parathyroid hormone. The vitamin is necessary for normal bone formation and regulates calcium and phosphorus metabolism in bone and kidney [185]. Vitamin D may play an important role in the prevention of chronic diseases other than osteoporosis, such as cancer and diabetes [184], but more studies will be needed before it is known if providing additional vitamin D will alter the outcomes in these diseases.

Table 21.12 Potential candidates for functions/indicators of vitamin D sufficiency

Indicator	Vitamin D effect on function	Indicator of insufficiency
Serum (s) Ca^{2+}, P	Normal serum levels maintained	↓ [Ca]s, ↓ [P]s
Calcium absorption	Optimizes absorption	↓ % absorption of Ca^{2+}
Parathyroid hormone (PTH)	Active transport of Ca^{2+}, restores [PTH]s	↑ [PTH]s
Fracture risk	↓ PTH levels to normal, ↓ bone turnover	↑ Risk related to D status
Muscle strength	Active transport of PO_4, ↓ PTH, restores intracellular [PO_4]	↓ Muscle strength
Bone turnover	Normalizes turnover	↓ Turnover, ↑ resorption
Immunomodulation	Maturation of antigen-presenting cells	↑ T-cell proliferation, ↓ killer cells
Cell proliferation	↓ Proliferation	More cells in G1 vs G2 phase

A major problem in determining an estimated average requirement (EAR) for vitamin D is that the indicator for adequacy is not clear (Table 21.12) [186]. The potential indicators to identify insufficiency reflect the multiple actions of vitamin D. Because more than 90% of circulating 25-hydroxyvitamin D-3 is derived from skin and is endogenously produced, daily requirements have not been established [186]. The DRIs are based on AIs. Because vitamin D deficiency is more prevalent in older adults, the recommended AI for adults older than 50 years is twice that of younger adults (10 vs 5 μg/day, or 400 vs 200 IU), and for adults older than 70 years it is tripled (15 μg/day) [8]. However, some experts feel that the AI should be as high as 800 IU/day, based on the amount needed to achieve levels of $25(OH)_2$-vitamin D sufficient to suppress parathormone levels [184].

Vitamin D occurs naturally in foods of animal origin, such as fish-liver oils, eggs, liver, and dairy products. Fortified foods supply the major dietary sources, although most vitamin D is made endogenously. Vitamin D is absorbed in the small intestine along with other lipids, but it requires bile salt micelle formation for efficient absorption. It is transported in the lymph with chylomicrons and other lipoproteins. The serum content of vitamin D is not limited by the content of the specific vitamin D-binding protein, and the ingestion of excessive doses can produce toxic levels of vitamin D or its metabolites in the serum. Vitamin D is stored in fat depots (e.g., liver, adipose tissue) and muscle, and it is released slowly. The 25-hydroxyl group is added in the liver, and the 1-hydroxyl group in the kidney. 1,25-Dihydroxyvitamin D and other polar metabolites are excreted in the bile and undergo an enterohepatic circulation. Malabsorption disorders produce earlier deficiency because endogenous as well as exogenous vitamin is lost. Small amounts of the vitamin are excreted in the urine.

Vitamin E [10]

The term "vitamin E" refers to two groups of lipid-soluble compounds, tocopherols and tocotrienols, that are found in plants. α-Tocopherol is the most active and abundant of these compounds. These compounds function as fat-soluble antioxidants and free-radical scavengers, in conjunction with the selenium–glutathione peroxidase system. The natural form is complex, covering eight different structures produced by plants (tocopherols α, β, γ, and δ, plus corresponding tocotrienols) [187]. Tocopherols contain a phytyl tail with three places that could be in either L- or R-configuration. Commercial vitamin E is a mixture of tocopherols and tocotrienols. α-Tocopherol is all *RRR*-α-tocopherol. The bioavailability of these forms varies, but the capsule form of vitamin E may be relatively less readily available [188]. The RDA previously was based on the assumptions that the diet had no more than 0.1 ppm of selenium, average amounts of sulfur amino acids, a ratio of vitamin E to PUFAs of 0.4, and less than 1.5% linoleic acid in a diet containing 7500–12 500 kJ (1800–3000 kcal). Later recommendations also consider the possible role of vitamin E as an antioxidant in preventing disease, the increased intake of PUFAs in the United States, and the serum vitamin E values from NHANES III (Third National Health and Nutrition Examination Survey, 1998–1994) [10]. Thus, the new DRIs are set about 50% higher than the 1989 levels (15 mg/day for adults). The vitamin is abundant in the lipids of green leafy plants, in vegetable oils, and in seeds. Foods high in PUFAs also are excellent sources of vitamin E [10,187].

Vitamin E is absorbed passively from the small intestine with other lipids, and its absorption, like that of other fat-soluble vitamins, requires bile salt micelles. Only about 40% of an oral dose is absorbed. The natural form is an acetate ester; bile salt-dependent pancreatic esterase also is required for absorption. There is no specific serum carrier for vitamin E. Because it is bound to LDL and other lipoproteins, serum levels are proportional to total lipids. The vitamin is delivered to the liver after the action of lipoprotein lipase, and from the liver it is delivered in lipoproteins to adipose tissue, where it is stored. Tocopherol-associated proteins (TAPs) may be important in lipoprotein transfer. α-Tocopherol transfer protein (TTP) activity is mediated by the ABCA1 transporter. Both TTP and TAP belong to a family of hydrophobic ligand-binding proteins with a *cis*-retinal-binding motif sequence (CPAR-TRIO) shared with cellular retinol-binding protein,

but the mechanism of hepatic secretion of vitamin E is unclear [189]. Excess vitamin E is excreted in bile or metabolized by β- or ω-oxidation by cytochrome P450-dependent hydroxylases [190]. Vitamin E destroys tissue peroxides that promote the oxidation of LDL [189] and prevents platelet adhesion by an antioxidant-independent mechanism [191]. These effects are the basis for the potential effect of the vitamin in preventing ischemic damage.

Vitamin K [11]

The term "vitamin K" designates naphthoquinone compounds with antihemorrhagic activity. They are crucial for the production of plasma clotting factors II (prothrombin), VII, IX, and X. Two forms occur naturally: vitamin K-1 (phylloquinones) in green plants and vitamin K-2 (menaquinones) in bacteria. Colonic bacterial synthesis provides an estimated 2 μg/kg of body weight, and absorption of that source presumably occurs by backwash into the terminal ileum. The RDAs of 1989 were based on the function of the vitamin for clotting proteins, but the requirement may be greater for the nonhepatic vitamin K-dependent proteins, especially those in bone [192]. Because of the lack of data on which to estimate an average requirement, the DRIs are based on AIs, set at 90 μg/day and 120 μg/day for adult women and men, respectively [11]. Therapeutic sources of vitamin K are synthetic compounds. Vitamin K (150 μg/day) is included in the new FDA recommended guidelines for adult parenteral multivitamins [193]. The best food sources are green leafy vegetables, and they are considered to be the major source of the vitamin despite some endogenous production.

Vitamin K is passively absorbed in the small intestine, a process that requires bile salt micelles and pancreatic enzymes. Unlike the other fat-soluble vitamins, vitamin K is not stored in large amounts in adipose tissue. The plasma form is carried on lipoproteins, but the storage form is primarily long-chain menaquinones. The vitamin is concentrated in the liver and is excreted in bile, stool, and urine [11,194]. Vitamin K acts by carboxylating selected glutamic acid residues of proteins to form α-carboxyglutamic acid (Gla), which binds calcium [194]. The coagulation function of the vitamin K-dependent hepatic proteins (e.g., prothrombin and factors VII, IX, and X) and the function of the bone proteins (e.g., osteocalcin, matrix Gla protein) are proportional to the degree of carboxylation. Dietary intake of 200–500 μg/day of vitamin K has been suggested as necessary for optimal γ-carboxylation of osteocalcin [195]. Other vitamin K-dependent proteins of unknown function are present in other tissues.

Conditionally essential nutrients

Choline

Choline can be synthesized in sufficient amounts to support normal metabolism of healthy animals and humans, and so was not considered an essential nutrient. However, in some pathological conditions this nutrient is conditionally essential; it is also essential for normal neonatal metabolism [196]. Choline is a precursor for acetylcholine, phospholipids, and the methyl donor betaine. It is absorbed from the intestine by transporter proteins, but it is not clear whether this process is mediated by the same high-affinity choline transporter, CHT1, that mediates endocytosis in neural tissue. Foods with high choline content include liver, eggs, peanut butter, and milk, and lower amounts occur in all raw foods. Human intake is estimated to be about 0.6–1.0 g/day. Gut flora degrade choline to many products, including betaine and methylamines. A choline deficiency syndrome has never been described in humans, but the Food and Drug Nutrition Board of the Institute of Medicine classifies choline as an essential nutrient [9], based on a study that showed lower plasma choline and phosphatidylcholine levels in subjects fed a choline-free diet for 3 weeks. In addition, some data suggest a partial reversal in hepatic abnormalities following choline supplementation [197]. The AI for infants is set at 17–18 mg/kg, and for adults at 550 mg/day for men, and 425 mg/day for women. A 20% lipid emulsion for TPN provides 11.6–132 mmol/L of phosphatidylcholine. The tolerable upper intake level (UL) for choline is 3.5 g/day, as higher amounts may cause hypotension and a fishy body odor from a metabolite, trimethylamine.

Carnitine

Carnitine is not an essential nutrient for adults, although it may be conditionally essential for neonates [196]. It functions to transfer long-chain fatty acids into mitochondria, improves glucose disposal, and may reduce insulin resistance [198]. Carnitine is made in the liver and kidney, and ingested mostly in milk and meats. Various disease states can alter carnitine status, including renal tubular dysfunction (Fanconi syndrome), chronic renal failure and hemodialysis, and certain medications including zidovudine and valproic acid [198]. Levocarnitine (Carnitor) is approved for such deficiencies, and produces modest effects at 2 g/day when given to patients with anemia caused by renal failure, pain in chronic diabetic neuropathy, and cardiac ischemia.

Arginine

Arginine is not considered an essential amino acid by the Food and Drug Nutrition Board of the Institute of Medicine [13]. However, sepsis appears to produce a conditional deficiency [199]. Arginine is a precursor for nitric oxide, and its potential benefits are related to replacing nitric oxide (NO) in severe illness. It can be given safely, and although laboratory end points can improve, its clinical efficacy is still uncertain [200]. Most studies use arginine in therapy as a component of commercial products that also contain *n*-3 fatty acids, branched-chain fatty acids, and nucleotides. There is no reason to recommend arginine or other immunoactive substances to critically ill patients [201].

Glutamine

Glutamine, along with aspartate, is an important energy source for the small intestine, but it has not been classified as an essential amino acid [13]. A special role for glutamine has been suggested for the treatment of critically ill patients, because it is the most abundant extracellular amino acid – it is used at high rates by many tissues, including the central nervous system and immune cells, and its levels fall during critical illness [202]. Supplemental glutamine has been reported to improve some immunological markers and reduce rates of infectious complications in some studies [203]. Glutamine has also been used in the treatment of mucositis caused by chemotherapy or radiotherapy [204]. The problems with assessing the considerable literature on glutamine are many, and include the greatly different patient populations, the variety of end points tested, the relatively short follow-up, the lack of adequately powered studies, and the lack of reproducible improvement of significant clinical end points [205]. Further studies are needed before glutamine supplementation can be recommended for use in critically ill patients.

Altered nutritional states

Starvation

The metabolic response to starvation enhances survival by increasing the use of adipose tissue triglycerides as a source of fuel, preventing severe hypoglycemia, conserving lean tissue, and decreasing the metabolic rate. The duration of survival during starvation depends on the amount of body fat and lean body mass. In lean men, death occurs after about 60 days of starvation [39], whereas obese persons can survive complete energy deprivation for more than 1 year without obvious adverse consequences [206].

Marked metabolic adaptations occur within the first 24 h of fasting. The mobilization of adipose tissue triglycerides, ketone body production, and the oxidation of plasma fatty acids increase, whereas hepatic glucose production and plasma glucose oxidation decrease [207].

The rate of lipolysis of adipose tissue triglycerides increases because of a decrease in circulating insulin, an increase in plasma epinephrine (adrenaline) concentration, and an increase in the lipolytic response to catecholamines [208–210]. After 3 days of fasting, lipolysis of adipose tissue triglycerides increases to more than double the values observed after an overnight (i.e., 12-h) fast. The increases in lipolysis and plasma fatty acid concentrations promote rates of fatty acid oxidation. The increased delivery of free fatty acids to the liver in conjunction with a decrease in the plasma ratio of insulin to glucagon stimulates hepatic ketone body production [211]. The rate of ketogenesis is maximal by 3 days of starvation; plasma ketone body concentration increases 75-fold by 7 days [212]. Ketone bodies are water soluble and able to cross the blood–brain barrier. As plasma ketone body concentrations increase, ketone body oxidation by the brain increases; by 7 days of starvation, ketone bodies provide 70% of the brain's energy needs [213]. The shift in fuel use by the brain helps spare the limited supply of plasma glucose for glucose-requiring tissues.

Whole-body glucose production decreases by more than half during the first few days of fasting because of a marked reduction in hepatic glucose output [214]. Only 15% of hepatic glycogen stores remain after 24 h of fasting [215]. Therefore, the contribution of gluconeogenesis from plasma precursors to total hepatic glucose output increases as the rate of hepatic glycogenolysis declines. As fasting continues, the conversion of glutamine to glucose in the kidney represents almost 50% of total glucose production.

Normally, about 70 g of amino acids is mobilized from protein stores and about 10 g of nitrogen is excreted in the urine [216]. During starvation, it is critical to slow down the rate of protein breakdown to prevent clinically significant protein losses. If protein breakdown proceeded at a normal rate throughout starvation, a potentially lethal amount of muscle protein would be catabolized in less than 3 weeks. The increase in ketone bodies [217] and starvation-induced inactivation of thyroid hormone – conversion of triiodothyronine (T_3) to reverse T_3 [218] – directly inhibit muscle protein breakdown.

Energy expenditure is conserved during fasting because of a decrease in physical activity caused by fatigue and a decrease in the resting metabolic rate, which decreases by 10%–15% at 7 days because of the diminished size and function of metabolically active tissues [219], increased conversion of active thyroid hormone to its inactive form [220], and suppressed sympathetic nervous system activity [221].

Maximum adaptation occurs as starvation continues. After 14 days of fasting, the rates of lipid, carbohydrate, and protein metabolism reach a plateau. Adipose tissue provides more than 90% of daily energy requirements. Muscle protein breakdown decreases to less than 30 g/day, causing a marked decrease in urea nitrogen production and excretion. Fluid requirements decrease because the diminished osmotic load from urea causes a decline in urine volume to 200 mL/day. Total glucose production decreases to about 75 g/day, providing fuel for glycolytic tissues (40 g/day) and the brain (35 g/day) while maintaining a constant plasma glucose concentration. Energy expenditure decreases by 20%–25% after 30 days of fasting [222] and remains relatively constant thereafter despite continued starvation.

During the terminal phase of starvation, body fat mass, muscle protein, and the size of most organs are markedly decreased. The weight and protein content of the brain remain relatively stable throughout starvation. In rodent models, when fat stores are depleted, the energy derived from body fat decreases and muscle protein catabolism increases. Death occurs when 30% of muscle protein is lost [223].

Metabolic response to illness and injury

The metabolic response to illness and injury is characterized by hypermetabolism, negative nitrogen balance, insulin resistance and hyperglycemia, and increased mobilization and oxidation of adipose tissue triglycerides. These events are produced by a complex cascade of endogenous mediators that cause a predictable physiological response. Increased production and secretion of the counterregulatory hormones (e.g., catecholamines, glucagon, and glucocorticoids) and cytokines are probably responsible for most of the observed responses to illness and injury.

In the 1930s, the classic work of Cutherbertson [224] on long-bone fractures provided the basis of our understanding of the metabolic response to injury. Cuthbertson demonstrated that the response to injury could be divided into two phases: the early ebb phase (12–24 h after trauma) and the subsequent flow phase. The ebb phase is characterized by decreases in blood pressure, oxygen consumption, cardiac output, and body temperature. The flow phase is characterized by hypermetabolism and increases in oxygen consumption, cardiac output, body temperature, and the urinary excretion of nitrogen, potassium, and phosphorus. Subsequently, Moore [225] divided the flow phase into the catabolic and anabolic phases. The restoration of tissue perfusion marks the beginning of the catabolic phase and lasts for days to weeks, depending on the severity of injury, medical intervention, and the premorbid health of the patient. This phase is characterized by catabolism, heat production, negative nitrogen balance, and hyperglycemia [224,225]. It ends after volume deficits are corrected, infection is controlled, pain is eliminated, and oxygenation is restored. At this point, net anabolism may occur, resulting in a slow reaccumulation of protein and body fat.

Hormone and cytokine mediators

During the flow phase, the secretion of several hormones is increased, and they act synergistically to generate alterations in intermediary metabolism. Catecholamines increase lipolysis and hepatic glucose production. Glucagon increases hepatic gluconeogenesis and glycogenolysis. Cortisol enhances protein breakdown and increases hepatic gluconeogenesis.

Cytokines, produced by macrophages, lymphocytes, Kupffer cells, and endothelial cells, are also critical mediators of the metabolic response. Tumor necrosis factor is believed to be the primary cytokine mediating many of the responses to infection and trauma, including fever, increased acute-phase protein synthesis, protein catabolism, hypotension, decreased lipoprotein lipase activity, and metabolic acidosis [226–230]. Interleukin-1 and TNF act synergistically in promoting tissue injury and stimulating the release of counterregulatory hormones [231]; IL-1 causes fever, hypotension, and increased insulin and glucagon secretion, and it affects the concentrations of plasma divalent cations. The liver is stimulated by IL-1 to synthesize metallothioneins, which sequester zinc, and IL-1 mediates the sequestration of iron in hemosiderin and ferritin, depriving invading organisms of these trace elements [228–235]. Other aspects of the acute-phase response stimulated by IL-1 include fibrinogen and C-reactive protein production by hepatocytes and the release of lactoferrin by neutrophils [232,233]. Interleukin-1 enhances immunoglobulin production by B lymphocytes and is a potent stimulus for the synthesis and release of IL-2 by T lymphocytes [232,233,236]. Interleukin-6 is also inducible by TNF and functions primarily as a stimulator of the hepatic acute-phase protein response and lymphocyte proliferation [231,236]. Interleukin-8 is inducible by TNF and IL-1 and is a potent neutrophil chemoattractant [236].

Energy metabolism

Increases in metabolic rate correlate directly with the severity of illness and injury [237]. For example, the REE increases after uncomplicated surgery by about 10%, after long-bone fractures by 20%, and after multiple trauma by 50%. The REE rarely exceeds twice the normal rate regardless of the extent of injury [223]. In fact, even patients with severe burns usually do not experience an increase in REE by more than 50% for any prolonged period [238]. In certain types of injury, a temporary resetting of the hypothalamic thermoregulatory set point is responsible for a 1–2°C elevation of body temperature known as *posttraumatic fever* [226,239]. The REE increases about 12% for each 1°C increase in core body temperature. Because the central temperature set point is higher, the comfort temperature for an injured patient is elevated, and elevating the ambient temperature decreases the energy requirement [223].

Protein metabolism

Illness or injury increases protein synthesis and protein breakdown. The increase in protein breakdown is greater than the increase in protein synthesis, resulting in negative nitrogen balance. Skeletal muscle is the major site of protein catabolism, but increased catabolism of extracellular proteins, including acute-phase reactants, coagulation system proteins, and complement system proteins, also occurs [232,240–242]. Considerable protein synthesis is directed toward host defense, including phagocyte activity, hormones, cytokines, intracellular proteins, immunoglobulins, complement, coagulation system proteins, and acute-phase reactant glycoproteins [232].

The composition of amino acids released by muscle does not reflect the composition of muscle proteins. The branched-chain amino acids of skeletal muscle are metabolized within the muscle cell for energy, and transamination generates glutamine and alanine. As a result, alanine and glutamine, which constitute only 12% of muscle protein, make up 50%–60% of the amino acids released into the plasma by muscle. Conversely, branched-chain amino acids make up 15% of the muscle protein but only 6% of the

amino acids released [232,237]. Glutamine is taken up and metabolized by the kidneys at an accelerated rate, providing additional ammonium for excretion to help maintain acid–base balance in the face of the acidosis that frequently accompanies critical illness [237]. In addition to carrying amino groups from the periphery to the liver and kidney, glutamine serves as a major energy source for the lymphocytes, fibroblasts, and the gastrointestinal tract [243]. Alanine is captured by the liver for gluconeogenesis, and its nitrogen contributes to the increase in ureagenesis [244].

During severe illness, nitrogen losses may reach 20–40 g/day. This represents catabolism of 600–1200 g of lean body mass per day. Providing exogenous nitrogen and energy may [245] or may not [246] decrease the rate of catabolism, but it enhances protein synthesis and thereby decreases negative nitrogen balance [243,247].

Carbohydrate metabolism

The stress response is marked by hyperglycemia, in large part related to hepatic gluconeogenesis fueled by lactate, pyruvate, glycerol, alanine, and other glucogenic amino acids. Hepatic glucose production may exceed 500 g/day and is resistant to suppression by insulin [248]. Peripheral insulin resistance decreases skeletal muscle glucose uptake, which also contributes to hyperglycemia [226]. Glucose consumption by wounds and injured extremities is increased to provide fuel for inflammatory cells, such as fibroblasts, macrophages, and leukocytes. These cells metabolize glucose anaerobically and can release large quantities of lactate into the bloodstream, which is subsequently recycled to glucose by the liver.

Lipid metabolism

Fat is a major oxidative fuel in critically ill patients, even when exogenous carbohydrates are administered. The rate of lipolysis correlates directly with the severity of illness [249]. The increased delivery of fatty acids to the liver stimulates very-low-density lipoprotein (VLDL) production. However, the rate of VLDL secretion is not adequate to remove the excessive supply of fatty acids and thus contributes to hepatic fat accumulation. Hypertriglyceridemia can occur because of both increased VLDL production and decreased peripheral lipoprotein lipase activity and VLDL clearance.

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E MISCELLANEOUS

22 Gastrointestinal blood flow

Philip T. Nowicki, D. Neil Granger

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The ultimate function of the gastrointestinal tract is to assimilate nutrients and water from the external environment and make them available to cells throughout the body; in this context, the blood and lymph circulations provide the conduits for transferring absorbed nutrients and water to the entire body. The vascular supply to the gastrointestinal mucosa is particularly well suited for the absorptive and secretory functions of this tissue in that it can accommodate a high rate of blood flow, has a large exchange surface area, and permits easy permeation of nutrients and water, yet largely retains proteins within the plasma compartment. This chapter summarizes current concepts regarding circulatory control and function in the gastrointestinal tract during normal physiological conditions. The chapter also reviews mechanisms by which the gastrointestinal and hepatic circulations interact, discusses unique features of the newborn intestinal circulation, and summarizes information relevant to the pathophysiology of intestinal ischemia.

Anatomy of the gastrointestinal circulation

Extramural vessels

Arteries

In humans, the major arteries supplying the stomach and intestines are the celiac, superior mesenteric, and inferior mesenteric arteries. The celiac artery supplies the stomach, the first portion of the duodenum, a portion of the pancreas, and the liver. The superior mesenteric artery supplies the remainder of the pancreas and duodenum, the jejunum, the ileum, and the colon through two-thirds of the transverse segment. The inferior mesenteric artery supplies the remainder of the colon and rectum, except the distal rectum, which is supplied by rectal arteries arising from the internal iliac arteries. Along the mesenteric border of the intestine, arterial and venous branches form multiple arcades, anastomose with one another, and provide a pathway for collateral blood flow. The arcades give rise to vasa recta, which branch to encircle the intestine and ultimately pierce the circular muscle [1–3].

Veins

Blood from the stomach, pancreas, and intestines drains into the portal vein; the exception is the blood from the distal rectum, which drains into the internal iliac veins. The vessels that drain the intestines course within the mesentery, except those vessels supplying retroperitoneal portions [1–3].

Intramural vessels and microcirculation

Stomach

In the human stomach, submucosal arterioles branch into capillaries at the base of the gastric glands, pass perpendicularly through the mucosa, form a lumenal capillary network, and drain into mucosal venules only at the most lumenal level of the lamina propria (Fig. 22.1). These venular branches converge on infrequent mucosal collecting venules, which then pass directly to the submucous venous plexus without receiving any direct capillary tributaries within the mucosa [4].

Small intestine

Small arteries pierce the muscularis layers and give rise to 1A submucosal arterioles that in turn generate an extensive, anastomosed series of 2A arterioles. These arterioles function as a pressure manifold for 3A arterioles, which descend into the mucosa to form the villus microcirculation; in addition, 4A arterioles branch from the 3A vessels and ascend into the muscularis layers. A tufted pattern of capillaries, derived from the tubular capillary plexus surrounding the epithelium of the crypts, supplies the basal 70%–80% of the villus and also drains into the venule high in the villus (Fig. 22.2) [2,5].

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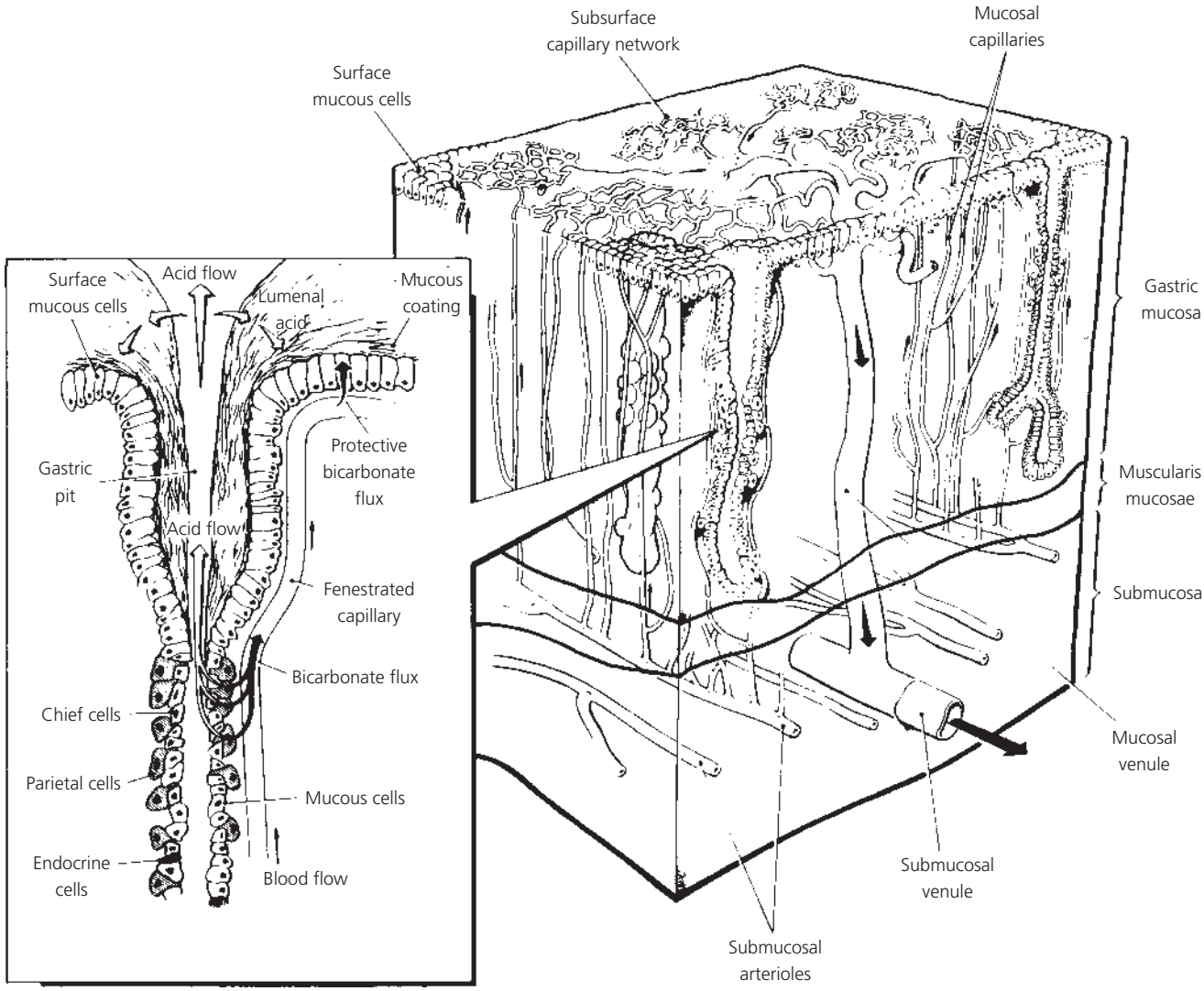


Figure 22.1 The vascular organization in gastric mucosa **(right)**. The proposed mechanism for the vascular transport of bicarbonate is toward the surface mucous cells from deeper within the mucosa (inset left). From Gannon et al. [4], with permission from Elsevier.

Colon

The colonic mucosa is devoid of villi; therefore, the arterioles and their capillary branches pass to the epithelial surface between the crypts to form a network of capillary plexuses around the crypts. The colonic capillaries are much closer o the epithelial cells than are the villus capillaries in the small intestine [6].

Mechanisms of blood flow regulation

Intrinsic systems

It is well recognized that blood flow in the gastrointestinal tract is maintained within narrow limits. This circumstance reflects, in part, the existence of intrinsic vascular regulatory systems; i.e., mechanisms that originate and remain within the intestinal parenchyma and its attendant circulation and that can be identified under isolated or in vitro conditions. Three well-identified systems merit review: the myogenic and metabolic mechanisms, and the system involving nitric oxide (NO) derived from the endothelial isoform of nitric oxide synthase (eNOS). Integration of these systems occurs at a molecular level and their relative activities are most certainly modified by other vasoactive stimuli (peptides, hormones, neural input) that arise from intrinsic or extrinsic sites.

Myogenic vascular response

Vascular smooth muscle cells contract when stretched, a phenomenon termed the myogenic response. The molecular basis for the myogenic response is incompletely understood, although an excellent review is available [7]. The myogenic response has been repeatedly demonstrated within the

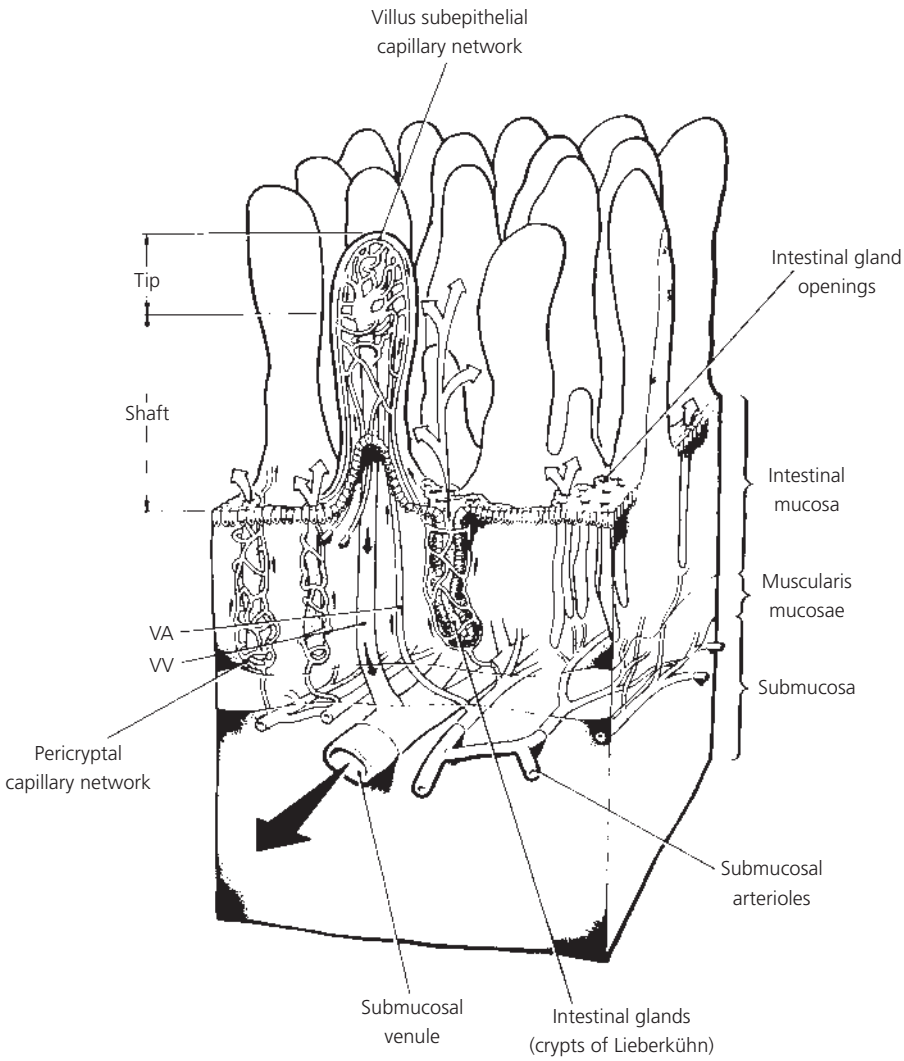


Figure 22.2 Mucosal microcirculatory patterns typical of human and rabbit small intestine. VA, villus arteriole; VV, villus venule. Adapted from Frasher and Wayland [5], with permission from Elsevier.

intestinal circulation, most commonly by noting vasoconstriction in response to increased intravascular pressure [8–13]. Intrinsic myogenicity of the intestinal vasculature has at least two physiological roles. First, it contributes to the basal vascular tone and so participates in setting vascular resistance across the intestine; in this context, the myogenic mechanism generates vascular tone that is fully independent of extrinsic neural stimuli [7,14]. Second, it serves to regulate vascular wall tension and in this capacity participates in pressure-flow autoregulation [8,11,15,16] and helps preserve homeostasis of capillary filtration [9,17–20].

Metabolic vascular regulation

A consistent experimental observation is that the intensity of vasoregulation within the intestine is contingent on its momentary rate of O_2 utilization (O_2 demand), convincing evidence that a factor produced by the gut parenchyma is indexed to tissue metabolic activity, and that this factor regulates the gut circulation. This concept is the basis of the metabolic theory of local blood flow regulation, which states that tissue oxygenation, not blood flow, is the principal variable regulated by intrinsic vascular control mechanisms. The theory partitions microvascular control into two sites: resistance vessels and precapillary sphincters. The former regulate the flow of blood, and hence O_2 delivery to the capillary level, wherein O_2 delivery is defined as the product of blood flow rate and O_2 concentration. Precapillary sphincters govern perfusion of individual capillaries, and hence the surface area available for O_2 diffusion from capillary-to-cell (Fig. 22.3). The metabolic feedback signal serves to vasodilate resistance vessels and open precapillary sphincters. Anything that reduces the O_2 delivery-to-demand ratio (e.g., hypotension, hypoxemia, or the increased tissue O_2 demand during digestion) increases production of the metabolic feedback signal, an action that increases net O_2 transport to the gut parenchyma and restores O_2 homeostasis [21].

An important aspect of the metabolic theory is that precapillary sphincters are more sensitive to the metabolic feedback

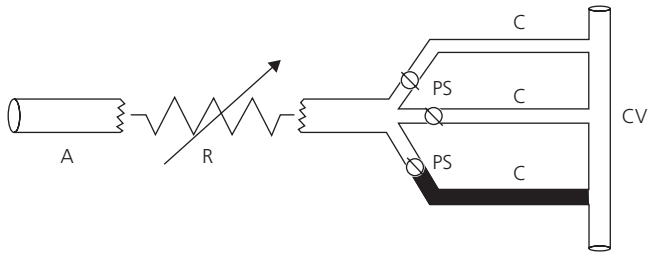


Figure 22.3 Schematic representation of the physiological control sites within the intestinal circulation based on the metabolic theory of local blood flow regulation. Arterioles (A) act as variable resistors, i.e., as resistance vessels (R) that determine the rate of flow into the capillaries (C). Precapillary sphincters (PS) govern perfusion of individual capillaries and exist in an "open" or "closed" state. In the figure, the lowermost capillary is perfused (PS "open"), while the upper capillaries are not perfused (PS "closed"). In response to a reduction in the O_2 delivery-to-demand ratio, the first response would be an opening of the previously closed PS; thereafter, as necessary to maintain capillary Po_2, arteriolar resistance would decrease. CV, collecting venule.

signal than resistance vessels [21]. Hence, the first response to a reduction in the O_2 delivery-to-demand ratio is the opening of closed precapillary sphincters, an effect that has been experimentally confirmed by noting increases in the capillary filtration coefficient ($K_{f,c}$) and the arteriovenous O_2 content difference across the intestine in response to a reduction in the O_2 delivery-to-demand ratio [12,22–24]. Dilation of resistance vessels, and hence augmentation of flow, only occurs when capillary Po_2 is no longer sufficient to drive the capillary-to-cell O_2 diffusion process. This phenomenon has been used to explain why the intrinsic regulation of intestinal blood flow is enhanced when the intestinal metabolic rate, and hence tissue O_2 demand, is increased [10,25,26].

The identity of the metabolic theory is unknown, although several reports support adenosine as a viable candidate for this role. Adenosine is a powerful intestinal vasodilator [27] that is generated during metabolic utilization of adenosine triphosphate (ATP), and its accumulation in venous blood has been demonstrated under conditions where tissue oxygenation might be reduced, such as after arterial hypoxemia or occlusion, or during the active transport of nutrients. The role of adenosine in local vasoregulation has been assessed with the use of substances such as theophylline, a competitive antagonist; by the enzyme adenosine deaminase, which converts adenosine to inosine; and by dipyridamole, an inhibitor of adenosine reuptake. These agents have been shown to attenuate or completely abolish the vasodilation associated with reductions in arterial pressure, release of an arterial occlusion, hypoxemia, and absorption of nutrients [28–32].

Other studies suggest that molecular O_2 itself dictates vascular tone, either directly or through the generation of endothelium-derived autocoids. Microvascular studies demonstrate an inverse correlation between villus Po_2, measured directly with O_2-sensitive electrodes, and the rate of blood flow within the submucosal and villus microvasculature [33,34]. Reduction of Po_2 within vascular smooth muscle generally causes its relaxation, possibly because of the effects of Po_2 on ion channels, such as the ATP-dependent K^+ channels [35,36]. Alternatively, a considerable body of evidence supports a role for the vascular endothelium as an O_2-sensing organ; release of vasorelaxing autocoids such as prostacyclin and NO can occur in response to endothelial hypoxia, this ultimately leads to a reduction in vascular resistance that, in turn, increases the rate of blood flow and hence of O_2 delivery [37,38]. In this context, tissue Po_2 functions as the controlled variable (in accordance with the metabolic theory), whereas secondary agents such as NO and prostacyclin are the effecter mechanisms (the feedback signal).

Nitric oxide

The eNOS-derived NO has emerged as a pivotal 'final common pathway' in vasodilation, and its role in intestinal vascular regulation has been established by anatomical [39,40] and physiological [41–44] studies. The eNOS generates NO during the reduction of L-arginine to L-citrulline, in the presence of Ca^{2+}, calmodulin, O_2, tetrahydrobiopterin, and reduced nicotinamide dinucleotide phosphate. The principal mechanism of NO-based vasodilation is the activation of soluble guanylate cyclase in adjacent vascular smooth muscle, an action that initiates the following cascade: increased cyclic guanosine monophosphate production, reduced Ca^{2+} concentration, cell relaxation, and a net increase in vessel diameter (Fig. 22.4). Excellent reviews of eNOS biochemistry and molecular biology are available [45,46].

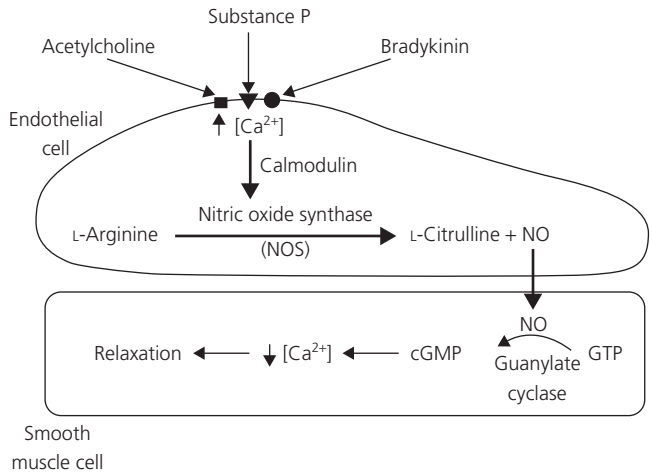


Figure 22.4 Mechanism of nitric oxide (NO)-mediated relaxation of vascular smooth muscle. NO generation by the endothelial cell enzyme NO synthase is induced by certain stimuli, such as acetylcholine, substance P, and shear stress. The endothelial cell-derived NO produced by these stimuli readily diffuses into the underlying smooth muscle cells, where it binds to and consequently activates soluble guanylate cyclase (sGC). The cyclic guanosine monophosphate (cGMP) produced by sGC promotes smooth muscle relaxation by lowering intracellular calcium.

The most convincing evidence for NO-based intestinal vasoregulation comes from microvascular studies using NO-sensitive microelectrodes placed immediately adjacent (~ 10 μm) to submucosal vessels. Periarteriolar NO concentration exceeds the dissociation constant for soluble guanylate cyclase; in addition, this variable is directly correlated to arteriolar diameter and increases in response to shear stress, hypoxemia, and nutrient absorption, perturbations that lead to arteriolar dilation [43,47,48]. Moreover, attenuation of eNOS activity with L-arginine analogues causes relative vasoconstriction of the intestine, indicating that constitutive eNOS activity (and hence NO production) participates in gut vasoregulation [44]. The eNOS-derived NO also participates in flow-induced dilation within the intestine; i.e., the vascular phenomenon by which an increase in flow rate increases vessel diameter [41].

Extrinsic systems

Central nervous system

The gastrointestinal tract is innervated by postganglionic (sympathetic) fibers emanating from celiac, superior mesenteric, and inferior mesenteric ganglia [49]. Both anatomical and physiological studies demonstrate innervation of this type in virtually all portions of the gut circulation, from conduit vessels (e.g., the superior mesenteric artery) to microvessels (e.g., submucosal arterioles) to the mesenteric venous vasculature (e.g., the splanchnic capacitance vessels). Identified neurotransmitters include norepinephrine, ATP, and neuropeptide Y [50,51]. These fibers provide the sole neurogenic vasoconstrictor stimuli to submucosal arterioles [50].

Direct stimulation of the postganglionic mesenteric nerve fibers causes brisk vasoconstriction; however, this effect is not sustained, even in the face of continued nerve stimulation. This process is termed autoregulatory escape (Fig. 22.5) [52–54]. A similar phenomenon occurs during intraarterial infusion of the neurotransmitter norepinephrine [55]. Autoregulatory escape occurs only in arteriolar smooth muscle, not in venous smooth muscle [52]. It is unaltered by β-receptor blockade [53] or by administration of atropine [54]. Three mechanisms commonly invoked to explain autoregulatory escape are redistribution of blood flow from the mucosa to the submucosa, adaptation of adrenergic receptors to continued nerve stimulation, and accumulation of vasodilator metabolites during tissue hypoxia generated during nerve-induced ischemia. The lattermost explanation is the most widely accepted [56–59]. Consistent with this theory is experimental evidence that the propensity for blood flow to escape from sympathetic vasoconstriction is significantly greater in the metabolically active mucosa than in the muscularis [60]. Adenosine appears to play at least a partial role in autoregulatory escape [55], whereas a role for histamine [54,55] and prostaglandins [54] has not been substantiated. Evidence also implicates a role for vasodilator peptidergic neurons in effecting autoregulatory escape after sympathetic nerve stimulation but not during infusion of norepinephrine [61,62]. One neurotransmitter sub-stance produced by these fibers is vasoactive intestinal polypeptide [63].

Extrinsic preganglionic afferent (sensory) fibers that arise from dorsal root ganglia also innervate the gastrointestinal vasculature [50]. Substance P and calcitonin gene-related peptide serve as neurotransmitters for these fibers. Stimulation of extrinsic afferents with capsaicin causes vasodilation of submucosal arterioles [64–66].

The gastrointestinal tract receives extensive cholinergic, parasympathetic innervation by way of the vagus nerve (stomach, small intestine) and pelvic nerve (colon), although the role of these fibers in the regulation of gut perfusion is unclear. Early studies failed to demonstrate significant change in gut vascular tone after direct stimulation of the vagus or after vagotomy [67,68]; subsequent work, however, has revealed vasodilation of intestinal submucosal arterioles in response to vagal stimulation [69].

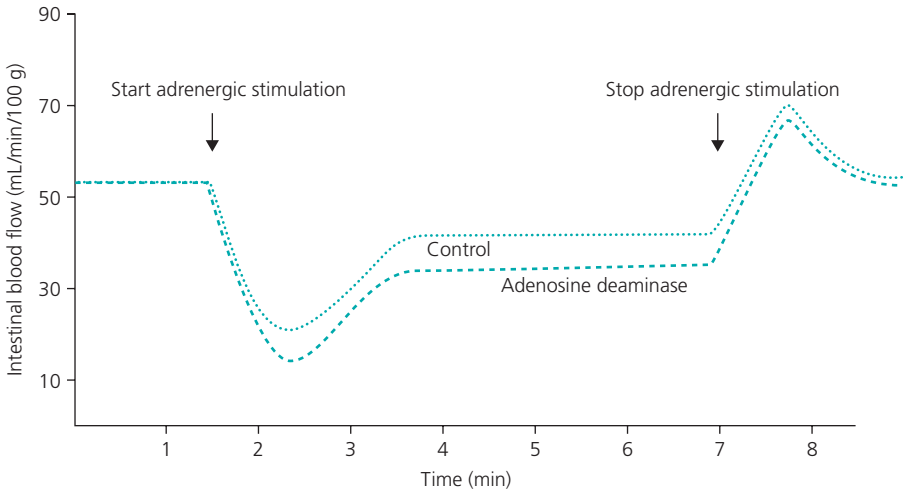


Figure 22.5 Autoregulatory escape from adrenergic stimulation in autoperfused piglet small intestine. Adenosine deaminase pretreatment reduced the steady-state escape response to norepinephrine infusion, whereas pretreatment with chlorpheniramine, a histamine H_1 blocker, had no effect. Data from Crissinger et al. [55].

Enteric nervous system

The gastrointestinal tract is endowed with a complex nervous system that contains sensory and motor neurons capable of function in the absence of extrinsic neural input [70]. It might be cogently argued that this regulatory system is actually *intrinsic* to the gut; however, the myenteric and submucosal neurons that constitute the enteric nervous system receive extensive input from extrinsic fibers arising from the celiac ganglia [50], and so the enteric nervous system is closely integrated at the central nervous system. Submucosal arterioles are innervated by afferent and efferent fibers that emanate from both myenteric and submucosal neurons. Myenteric long fibers are believed to synapse with submucosal neurons that in turn innervate submucosal arterioles [50]. Direct stimulation of the myenteric ganglia induces vasodilation of the submucosal arterioles [71] that is mediated by various neurotransmitters, including acetylcholine [72], substance P [72], calcitonin gene-related peptide [73], and neuropeptide Y [74]. In addition, a reflex arc connecting afferent fibers arising from the villi to the submucosal and myenteric ganglia, and thence to submucosal arterioles, has been identified. Chemical and mechanical stimulation of the mucosal activates this reflex arc and induces submucosal arteriolar vasodilation [73,75]. This phenomenon suggests that the enteric nervous system may participate in generating vasodilation in response to mucosal events, such as nutrient absorption, as well as the mucosal inflammation associated with ulcerative colitis [50]. Reviews of the role of the enteric nervous system in gut vascular regulation are available [50,73].

Circulating vasoactive substances

Circulating vasoactive substances that affect gastrointestinal blood flow include catecholamines, vasopressin, and angiotensin. Norepinephrine, a predominantly α-adrenergic receptor stimulant, causes intestinal vasoconstriction, a decrease in capillary density, and a reduction in O_2 uptake [59,76,77]. With continuous intraarterial infusion, the intense initial vasoconstriction is followed by the return of blood flow towards control levels despite continued norepinephrine infusion. Epinephrine can cause either α-receptor-mediated vasoconstriction at high doses or β-receptor-mediated vasodilation at low doses, as well as a variable response in O_2 uptake [59,78,79].

Both vasopressin and angiotensin II are potent physiological vasoconstrictors that reduce blood flow and increase vascular resistance in all gastrointestinal organs. These agents cause generalized vasoconstriction, with a disproportionate selective reduction in mesenteric blood flow at doses that have been measured in pathophysiological states of hypotension [80]. Vasopressin causes a decrease in capillary density and a reduction in intestinal O_2 uptake, whereas angiotensin II reduces or does not affect splanchnic O_2 uptake [81]. In normal rat intestine, α-adrenergic and vasopressin activity account for most extrinsic vasoconstrictor tone, whereas vasopressin and angiotensin II account for most extrinsic vasoconstrictor tone in portal hypertensive rat intestine [82]. Renin–angiotensin and vasopressin systems are also involved in the intestinal vasoconstrictor response to hemorrhage and hypovolemia, and significant attenuation of this increase in vascular resistance occurs only when both systems are blocked simultaneously [83,84], even in the presence of an intact sympathetic system.

Response of the intestinal circulation to systemic circulatory perturbations

Pressure–flow autoregulation

Pressure–flow autoregulation, or more simply, autoregulation, is a physiological response designed to maintain blood flow at a relatively constant level in the face of fluctuation in the pressure gradient across an organ or tissue. The relationship among flow, pressure, and resistance is given by the equation flow = (Pa – Pv)/R, where Pa and Pv are arterial and venous pressures, respectively, and R is resistance. Assuming that Pv remains constant, evidence of autoregulation would include an increase in R (vasoconstriction) in the face of increased Pa (hypertension), or a reduced R (vasodilation) in response to a lowered Pa (hypotension). Autoregulation can be elicited in the stomach [85,86], small intestine [25,87–95], and colon [11,12,96,97], although it is not the intense phenomenon observed in the renal and cerebral circulations (Fig. 22.6).

Autoregulation is generally ascribed to intrinsic control mechanisms, and both myogenic [91,92] and metabolic [10,25,86,98] mechanisms have been evoked to explain the phenomenon. The myogenic response, i.e., vasoconstriction in response to an increase in pressure and vice versa, would reflect the inherent tendency of the circumferential arterial vascular smooth muscle to contract or relax as a function of

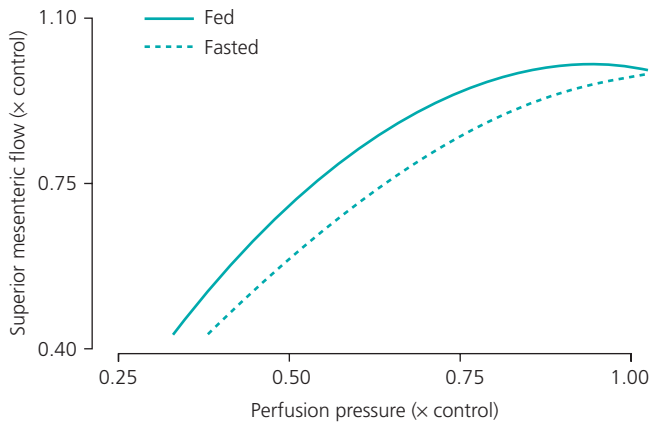


Figure 22.6 Responses of intestinal blood flow to step reductions in perfusion pressure in fed (solid line) vs fasted (dashed line) dogs demonstrate an increased intensity of autoregulation during enhanced functional activity. From Granger & Norris [26], with permission from the American Physiological Society.

the prevailing intravascular pressure, insofar as that pressure affects the stretch stimulus applied to the muscle cells [7]. The metabolic response is less straightforward. For example, hypotension would initially compromise gut perfusion and reduce O_2 delivery. This circumstance reduces the O_2 delivery-to-demand ratio and elicits production of the vasodilator metabolic feedback signal previously described. However, active regulation of precapillary sphincters designed to enhance capillary surface area and hence the diffusive flux of O_2 from capillary-to-cell is the first response to hypotension. This response is indicated by a rise in the capillary filtration coefficient $K_{f,c}$, a marker of the perfused capillary density, as pressure falls [22,24,26]. Evidence of autoregulation (vasodilation) only occurs when capillary Po_2 falls below the level needed to drive O_2 diffusion to maintain tissue oxygenation, the variable controlled by the metabolic mechanism [99]. This circumstance explains why the efficacy of autoregulation is significantly enhanced when the oxidative requirements of the intestine are increased by feeding [10,25,26].

Alterations in arterial blood gas tensions (hypoxemia) and hematocrit

Alterations in arterial blood gases and hematocrit also affect gastrointestinal blood flow. Arterial hypoxemia increases the perfused capillary density and causes vasodilation, and hence increased perfusion in denervated intestinal preparations [99,100]. The vasodilation and increased perfused capillary density tend to minimize the reduction in O_2 uptake induced by the limited O_2 delivery. When blood flow is held constant, the intestine maintains O_2 consumption within 48% of control during arterial hypoxemia. When both blood flow and capillary density are free to increase, however, O_2 uptake remains within 26% of control despite the hypoxia. Similar to hypoxia, hypercapnia induces a marked relaxation of resistance vessels [100]. In contrast to hypoxia, hypercapnia causes the precapillary sphincters to constrict and the capillary density to decrease.

Alterations in arterial hematocrit also influence gastrointestinal blood flow and oxygenation [101–104]. An inverse linear correlation exists between the intestinal blood flow and hematocrit, and a direct linear correlation between the arteriovenous O_2 difference and hematocrit in both the intestine and the stomach. The relation between intestinal O_2 uptake and hematocrit is parabolic (Fig. 22.7) [101], showing a maximal uptake at a hematocrit of 48.7% – i.e., the optimal hematocrit. Intralumenal placement of nutrients increases the optimal hematocrit to 57.1%. In the stomach, the optimal hematocrit is 38.2% during resting conditions, and it increases to 45.7% during pentagastrin stimulation.

Venous pressure elevation

Studies of the stomach, small intestine, and colon in adult animals indicate that vascular resistance rises in response to

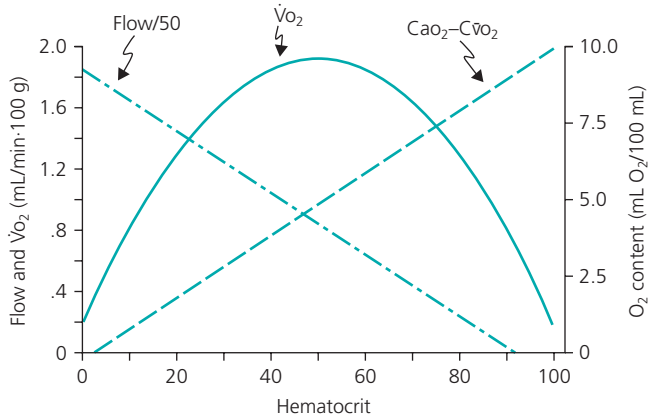


Figure 22.7 Effect of hematocrit on canine intestinal blood flow, O_2 consumption ($\dot{V}o_2$), arterial O_2 content, and arteriovenous O_2 difference ($Cao_2 – C\bar{v}o_2$). From Shepherd & Riedel [101].

venous pressure elevation, findings consistent with a myogenic mechanism (Fig. 22.8) [8–13,15,17–19,96,97,105]. Acute venous hypertension in the adult intestine elicits vasoconstriction as a result of rising precapillary (arteriolar) resistance whereas postcapillary resistance falls [106]. Capillary exchange capacity increases in the stomach and the colon but decreases in the small intestine during venous hypertension [107]. Despite the intense capillary derecruitment initiated by venous hypertension in the small intestine, O_2 extraction increases disproportionately to the reduced blood flow, and

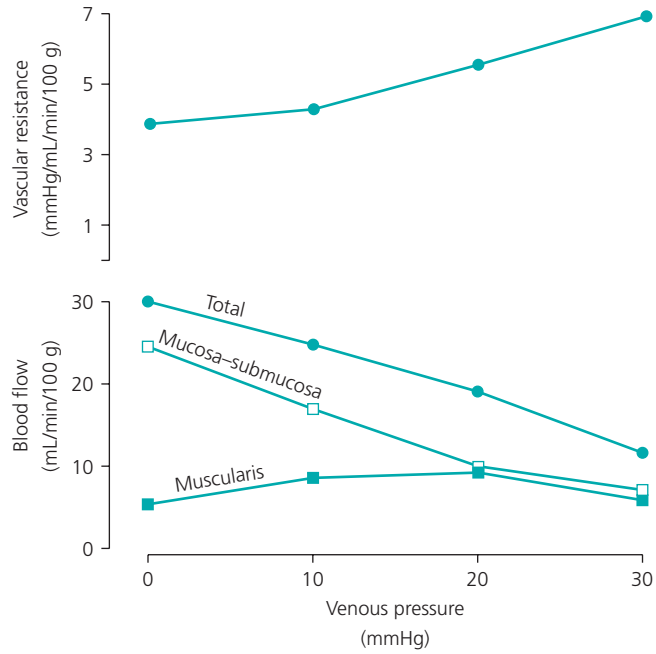


Figure 22.8 Intestinal vascular responses to acute venous hypertension in cats. The increase in vascular resistance and the decrease in blood flow during acute venous pressure elevation are consistent with the myogenic theory of intrinsic vasoregulation. Data from Granger et al. [17].

consequently, O_2 consumption rises [19]. The elevated intestinal O_2 utilization during venous hypertension has been attributed to increased villus motility [108]. There are conflicting reports regarding the influence of enhanced oxidative metabolism on the vascular responses to elevations in venous pressure. Some investigators have observed that increased O_2 demand significantly reduces or abolishes the rise in vascular resistance [26,97], whereas others have noted an exaggerated resistance response to venous pressure elevation [109].

Acute venous hypertension also alters the distribution of blood flow within the bowel wall [17,110]. As venous pressure is elevated, the percentage of total blood flow directed to the mucosa and submucosa is reduced, whereas the muscularis receives a larger fraction of the total blood flow. These observations indicate that the constriction of arteriolar and precapillary sphincter smooth muscles elicited by venous hypertension takes place in the mucosal and submucosal layers, and that the vasculature of the muscularis dilates in response to venous hypertension.

O_2 uptake–blood flow relation: functional implications

Considerable attention has been devoted to the interaction between gastrointestinal blood flow and O_2 uptake, and the relevance of this interaction to mucosal function and integrity. Figure 22.9 depicts the observed relation between intestinal blood flow and O_2 uptake when blood flow is altered with a pump or by graded reductions in perfusion pressure [22]. O_2 uptake remains virtually constant over a wide range of blood flow levels (i.e., it is independent of blood flow), and it is compromised only when blood flow reaches a critically low level. Below this level, O_2 uptake is dependent on blood flow. Resting blood flow in the small intestine, stomach, and colon is usually greater than the critical blood flow at which O_2 uptake is dependent on blood flow [13,111–113].

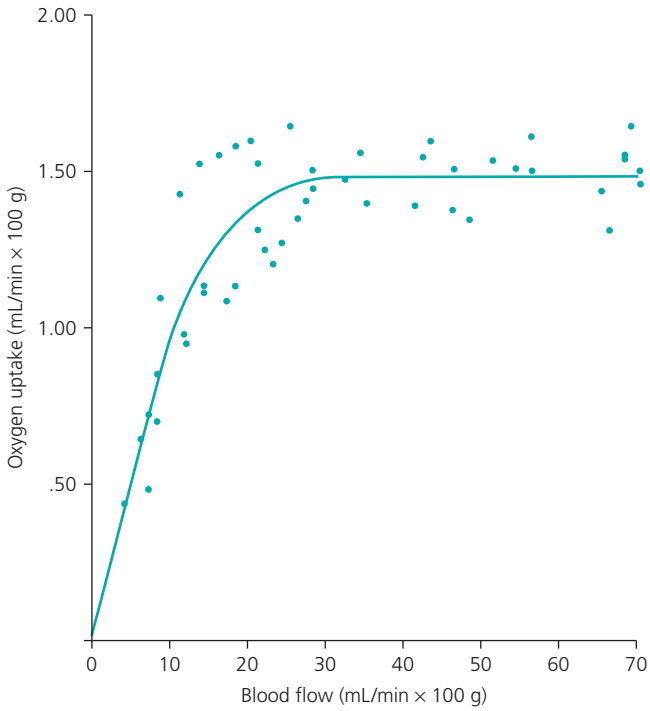


Figure 22.9 Relation between intestinal blood flow and O_2 uptake in feline jejunoileum when blood flow is altered with a pump or by graded reductions in perfusion pressure. From Granger et al. [22], with permission from the American Physiological Society.

The reduction in O_2 uptake that occurs when blood flow falls below a critical level can be explained in terms of the normal relation between mitochondrial O_2 consumption and cell Po_2. This relation predicts that O_2 uptake remains constant over a wide range of cell Po_2 levels, and uptake is reduced only when cell Po_2 falls to a low level, the critical Po_2. The resting cell Po_2 is normally well above the critical Po_2. Evidence indicates that graded reductions in blood flow produce concomitant reductions in cell Po_2 without altering O_2 uptake in the stomach [81]. At very low rates of blood flow, however, the rate of O_2 diffusion to the cells is so low that the intracellular Po_2 falls below the level required to maintain normal oxidative metabolism. The reduction in O_2 uptake observed at low blood flow may simply reflect a depression in oxidative metabolism caused by the limited O_2 availability.

Many conditions alter the relation between O_2 uptake and blood flow such that O_2 uptake is dependent on blood flow even when it is increased above normal. These include lumenal distention [114], alterations in hematocrit [115], and devascularization [96]. All these conditions are thought to reduce tissue oxygenation severely in discrete or generalized regions of the stomach or gut. Lumenal distention and devascularization reduce tissue oxygenation by compromising blood flow, whereas a low hematocrit limits O_2 delivery, even at high blood flows. Normal physiological conditions can also influence the relation between O_2 uptake and blood flow. Stimulation or inhibition of oxidative metabolism will shift the plateau of the blood flow–O_2 uptake curve upward or downward, respectively (Fig. 22.10) [81]. Stimulation of intestinal motility or enhancement of active transport raises the plateau of the blood flow–O_2 uptake curve [24]. Conversely, decreasing the temperature of isolated bowel segments lowers the plateau. Another important aspect of the blood flow–O_2 uptake relation in the small bowel is that it will predict the influence of blood flow reductions on O_2-requiring processes, such as absorption and secretion. For example, it has been shown that the reductions in glucose absorption produced by graded decrements in blood flow parallel the decline in O_2 uptake [116], suggesting that O_2 availability limits solute transport when cell Po_2 falls.

The relation between blood flow and O_2 uptake has also been useful in explaining some of the reported observations regarding the influence of blood flow on gastric acid

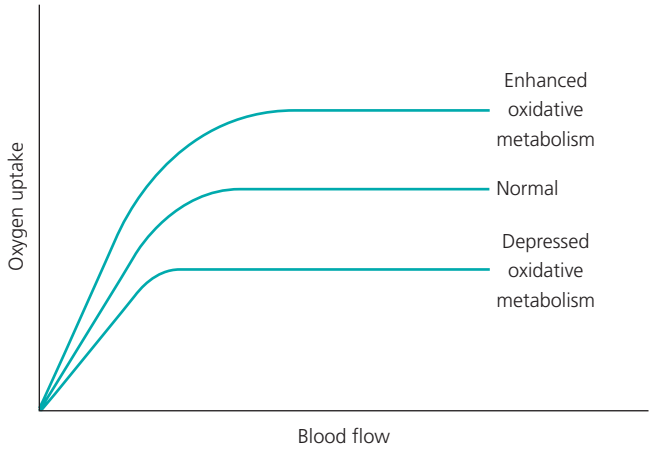


Figure 22.10 Relation between blood flow and O_2 uptake during stimulation or inhibition of oxidative metabolism. From Kvietys & Granger [81], with permission from the American Physiological Society.

secretion [117]. The production of gastric acid is an energy-consuming process that results in an increase in gastric O_2 uptake. The consistent finding that acid secretion and O_2 consumption are highly correlated raises the question of whether acid secretion in the stomach is dependent on blood flow. The answer to this question largely depends on the type of surgical preparation. In some preparations, many vessels supplying the stomach are occluded, and the end result is flow-dependent O_2 uptake. In this situation, an increase or decrease in blood flow is associated with a corresponding change in acid secretion. In normally perfused preparations, however, acid secretion and blood flow exhibit a relation similar to that observed between O_2 consumption and blood flow; acid secretion is dependent on blood flow at low blood flow rates and is independent at higher flow rates. This explains why vasoconstrictors tend to reduce gastric acid output and why some vasodilators (i.e., acid secretagogues) increase acid output whereas others (i.e., nonsecretagogues) do not.

Postprandial hyperemia

The term *postprandial hyperemia* is used to describe the increase in blood flow that occurs in response to a meal. The anticipatory–ingestion phase of digestion is characterized by transient increases in heart rate, cardiac output, and aortic pressure; however, gastrointestinal blood flow is either unchanged or slightly increased [118–122]. These transient hemodynamic responses appear to be mediated by activation of the sympathetic nervous system, because they can be attenuated by adrenergic blocking agents [120].

In conscious animals, blood flow to the stomach and proximal bowel increases 30–90 min after ingestion of a meal [118–121]. Blood flow to the ileum increases 45–120 min postprandially, whereas colonic blood flow generally does not increase [123–125]. Transient decreases in distal colon blood flow have been observed 30 min after a meal, a response attributed to tonic contractions produced by the gastrocolic reflex [125]. Blood flow in the superior mesenteric artery of conscious animals typically increases by 25%–130% after ingestion of a meal [118–120,126]. The splanchnic vasodilation may last for 4–7 h, depending on the nature and quantity of the meal [127,128]. A smaller increase (10%–60%) in blood flow is observed in isolated bowel segments in adult animals after intralumenal placement of digested food or nutrient solutions. This is consistent with findings in human subjects wherein intraduodenal perfusion with a test meal elicits a 35%–45% increase in superior mesenteric artery blood flow, with no gender differences in postprandial splanchnic flow [129].

Constituents of chyme responsible for postprandial hyperemia

Considerable effort has been devoted to defining the lumenal stimuli responsible for postprandial hyperemia. Lumenal placement of undigested food does not elicit hyperemia, whereas digested food significantly increases blood flow [130]. The latter observation indicates that hydrolytic products of food digestion initiate the hyperemia.

Bile appears to play an important role in postprandial intestinal hyperemia. Gallbladder bile at 10% concentration, the steady-state concentration in the proximal bowel in the early postprandial period, does not increase jejunal blood flow, yet it appears to render glucose and long-chain fatty acids vasoactive [131]. At a concentration of 33%, gallbladder bile renders both short-chain fatty acids (e.g., caproic acid) and amino acids vasoactive and further enhances glucose-induced hyperemia. Although intralumenal placement of endogenous or synthetic bile does not have a direct vasoactive effect in the jejunum, bile more than doubles blood flow in the ileum [130,132]. Bile acids are largely responsible for bile-induced hyperemia, an assertion supported by the observation that cholestyramine abolishes the vasodilator effects of endogenous bile on ileal blood flow [132].

Ingestion of meals with high levels of protein in humans and gastric placement of protein in conscious rats produces marked increases in splanchnic blood flow. In isolated loops of proximal small bowel, a high-protein diet (64%) increases blood flow by the same extent as a high-carbohydrate diet (68%) [133]. Although hydrolyzed proteins are known to induce postprandial hyperemia, the specific hydrolytic products of protein digestion that mediate the response remain unknown.

Solubilized long-chain fatty acids appear to be the most potent lumenal stimulus of postprandial intestinal hyperemia. Oleic acid (10–20 mM) solubilized in 10% gallbladder bile produces a 20%–60% increase in intestinal blood flow [131,134,135]. Evidence suggests that this increase in blood flow may be a response to epithelial cell injury [136].

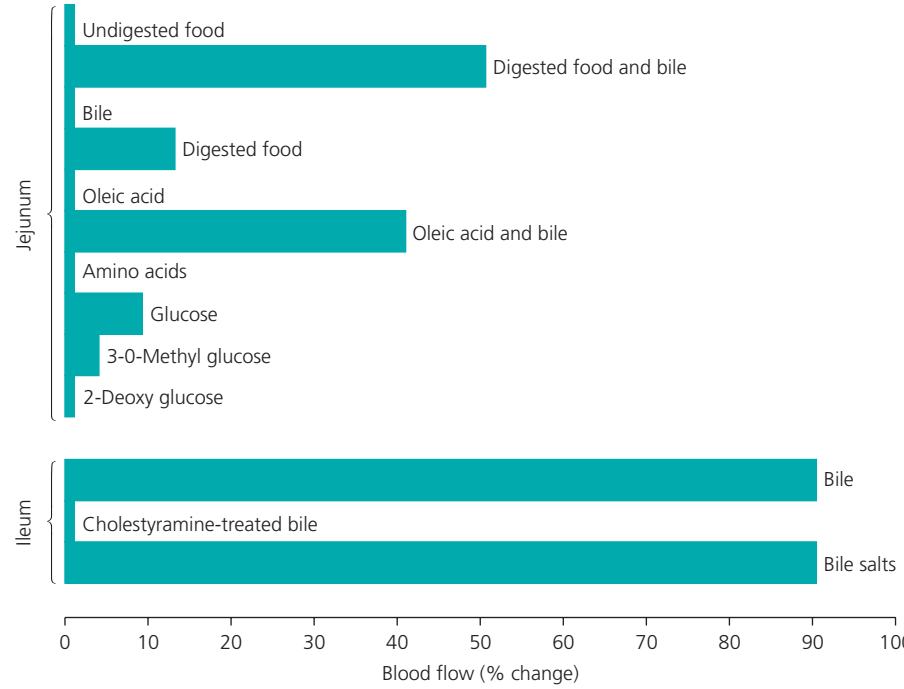


Figure 22.11 Effects of intralumenal placement of various constituents of chyme on intestinal blood flow. From Granger et al. [137], with permission from S. Karger AG, Basel.

Relatively little is known about the vascular response of the gut to the lumenal placement or ingestion of other dietary lipids. Short-chain fatty acids (e.g., caproic acid) do not alter blood flow, even in the presence of 10% gallbladder bile. Although lipids produce the largest intestinal hyperemia, the vascular responses elicited by protein and carbohydrate are not insignificant in that the three major dietary components of food – that is, fats, proteins, and carbohydrates – appear to act synergistically on blood flow when placed in the bowel lumen (Fig. 22.11) [137].

Mechanisms of postprandial hyperemia

Numerous mechanisms relevant to the gut vasodilation that occur during the digestive/absorptive phase have been investigated. The current consensus is that the final vascular effect reflects the integration of multiple inputs and that this orchestration is relatively region specific; i.e., that the inputs relevant to the stomach, jejunum, and ileum are unique.

Tissue metabolic rate

Nutrient absorption is an energy-dependent process and increases intestinal metabolic rate; hence, tissue O_2 demand increases after feeding under in vivo conditions, as well as in gut loops in vitro. This effect reduces the O_2 delivery-to-demand ratio and activates the metabolic mechanism previously described. Mathematical modeling of the intestinal circulation predicts expansion of capillary surface area and intestinal vasodilation in response to this perturbation [138] and these predications have been experimentally confirmed [26]. However, a metabolic mechanism alone does not account for all facets of postprandial hyperemia. For example, intralumenal placement of solubilized oleic acid induces a brisk hyperemia in the absence of any change in tissue O_2 uptake, and hence demand [139].

The putative role of tissue Po_2 as a stimulus for postprandial hyperemia has also been assessed on a microvascular level. Mucosal suffusion with isotonic glucose reduces villus Po_2 from ~ 17 to ~ 8 mmHg and simultaneously increases flow through the villus arteriole [99]. If the Po_2 of the mucosal suffusate is increased so that the glucose suffusion does not reduce villus Po_2, the villus arteriolar vasodilation is attenuated [33]. Once again, however, a metabolic mechanism cannot entirely account for intramural microvascular regulation during glucose suffusion; thus, vasodilation of upstream submucosal arterioles also occurs in response to glucose, despite the fact that periarteriolar Po_2 is unchanged at the submucosal level.

Adenosine

As previously discussed, adenosine has been proposed as an integral component of the metabolic mechanism, possibly functioning as the putative metabolic feedback signal. Adenosine is released into the venous effluent of isolated jejunal gut loops in response to intralumenal placement of a predigested meal [140]. Moreover, theophylline (an adenosine receptor antagonist), dipyridamole (blocks adenosine reuptake), and adenosine deaminase attenuate the magnitude of food-induced vasodilation [31,140]. Other work, however, failed to demonstrate an effect of adenosine antagonism on the magnitude of postprandial hyperemia [29].

Na^+-induced hyperosmolarity/nitric oxide

Na^+ is cotransported during the active uptake of glucose or amino acids across the villus membrane [141]. Ultimately, this process can lead to the activation of eNOS and resultant NO-induced vasodilation insofar as intracellular Na^+ homeostasis is maintained by the exchange of Na^+ for Ca^{2+}, and intracellular Ca^{2+} is a potent stimulus for eNOS [142]. The perivascular concentration of Na^+ increases within both the villus and the submucosal arterioles during glucose absorption [143]. Furthermore, the temporal sequence of increased perivascular Na^+ noted at the villus arterioles and thereafter at the submucosal arterioles is consistent with the timing of the vasodilation of these vessels during glucose absorption [34]. Movement of cotransported Na^+ to the submucosal space during villus glucose absorption may occur by way of the lymphatic system, as indicated by an increase in lymph osmolarity after mucosal glucose suffusion [144]. The anatomical juxtaposition of the extensive submucosal lymphatic plexus and submucosal arterioles is ideally suited for the flux of lymph-borne Na^+ to adjacent arterioles [145]. It also appears that Na^+-induced eNOS activation occurs within the lymphatic system with subsequent diffusion of NO to the immediately adjacent submucosal arteriole, leading to vasodilation [146,147].

The eNOS-derived NO may also participate in dilation of the small arteries that lie upstream from the submucosal arteriolar plexus [48]. These vessels, the vasa recta, represent the terminal portion of the mesenteric arterial arcade and are a vital portion of the intestinal resistance vasculature [148]. The perivascular concentration of NO increases at this site during glucose infusion; moreover, downstream blockade of the immediately adjacent venule reduces both the postglucose infusion rise in periarteriolar NO concentration and the subsequent vasodilation. The subsequent rise in flow rate through the arteriole would generate an additional mechanostimulus for eNOS activation; i.e., shear stress [41].

Gastrointestinal hormones

Feeding induces the production of vasoactive peptides and hormones by the gut. This effect was initially believed to contribute to postprandial hyperemia; however, subsequent work determined that postprandial concentration of these agents is below that necessary to induce a vascular effect [149]. The effects of cholecystokinin illustrate this circumstance. Intraarterial infusion of cholecystokinin induces vasodilation, but only if it is given at concentrations well above those noted after feeding [150,151]. In contrast, the peptide neurotensin, produced by gut neuroendocrine cells in response to feeding [153,154], does induce vasodilation at physiologically relevant concentrations [152]. Interestingly, the vascular effect of neurotensin appears to be region-specific: neurotensin-induced vasodilation occurs in the ileum [152], but not in the jejunum [151].

Enteric nervous system

While it is well accepted that extrinsic nerves play no role in the digestive/absorptive phase of postprandial hyperemia, some evidence supports a role for the enteric nervous system in this process [149]. Afferent fibers from neurons of the submucosal ganglia innervate the villi and synapse with efferent fibers that reach submucosal arterioles [75]. Vasoactive intestinal peptide, an effective intestinal vasodilator, is one of several neurotransmitters localized to these neurons [153]. VIP release and jejunal vasodilation occur in response to lumenal instillation of a bile–oleic acid mixture [154], and to mechanical stimulation of the mucosal surface [73].

Interactions between gastrointestinal and liver circulations

General considerations

Blood draining the gastrointestinal tract, pancreas, and spleen accounts for about 80% of the resting blood supply to the liver, with the remaining 20% of liver blood flow derived from the hepatic artery. This dual supply of blood to the liver reflects a combination of well-oxygenated blood from the high-pressure hepatic artery that mixes with poorly oxygenated blood from the low-pressure portal vein. Because of the higher O_2 content of arterial blood, the hepatic artery and portal vein contribute roughly equal amounts of O_2 to the liver in the fasting state. Within the liver, these vessels give rise to numerous smaller vessels, called the hepatic arterioles and terminal portal venules, which empty into sinusoids (the capillary network of the liver). Blood flow in the liver may be modulated at each of these anatomical sites. The sinusoidal capillaries radiate toward the periphery of the acinus, where they connect with the terminal hepatic venules and ultimately into progressively larger branches of hepatic veins and inferior vena cava [129,155].

A unique feature of the liver circulation is the role of capillary (sinusoidal) constriction in the regulation of intrahepatic vascular resistance and blood flow. This ability of sinusoids to constrict has been attributed to stellate cells that reside in the space of Disse in close contact with sinusoidal endothelial cells. Stellate cells exhibit anatomical features that are remarkably similar to smooth muscle-like pericytes, which have been implicated in the sprouting and remodeling of capillaries and may represent the anatomical equivalent of the precapillary sphincter that controls the perfused capillary density in other vascular beds. A variety of vasoconstrictors, including endothelin-1, angiotensin II, and vasopressin, are known to elicit stellate cell contraction that colocalizes with sinusoidal constriction. The gaseous monoxides, NO and carbon monoxide, also appear to play an important role in modulating stellate cell contraction, possibly through the activation of soluble guanylyl cyclase. The interplay of NO with vasoconstrictors like endothelin-1 may be a major

determinant of liver blood flow, especially after liver injury, which is often associated with stellate cell activation and an enhanced contractility. It has been proposed that exaggerated stellate cell contractility may contribute to the increased intrahepatic resistance and portal hypertension that can accompany liver injury. Through this effect on portal vein pressure, the stellate cell-mediated sinusoidal constriction may exert a significant influence on blood flow in those tissues, including the gastrointestinal tract, that are drained by the portal vein [129,156–158].

Inadequate blood perfusion of the gastrointestinal tract can result in impairment of organ function, tissue necrosis, and mortality. Although the liver receives most of its resting blood flow from venous drainage of the gastrointestinal tract, it appears to be relatively well protected against the deleterious consequences of gastrointestinal hypoperfusion. This protection largely results from the unique hydrodynamic interaction between portal venous and hepatic arterial blood flows that has been termed the hepatic arterial buffer response. With the hepatic arterial buffer response, an increased blood flow in the portal vein leads to an increased hepatic arterial resistance, whereas a reduction in portal vein flow produces hepatic arterial dilation. Adenosine washout from the space of Mall has been proposed as a major determinant of the alteration in hepatic arteriolar tone that accompanies changes in portal venous blood flow. The adenosine washout hypothesis predicts that adenosine levels are inversely related to portal venous blood flow and that adenosine exerts a potent dilator effect on hepatic arterioles. Although the reciprocal relationship between hepatic arterial and portal vein blood flows tends to maintain a constant total blood flow through the liver, hepatic arterial flow can only compensate for about 30% of the change in portal venous inflow. However, compensation relative to O_2 delivery is much greater because of the higher O_2 content of hepatic arterial blood compared to the portal vein blood. For example, it has been shown that liver O_2 supply remains relatively normal during hemorrhage until the blood loss exceeds 30%. The liver is more effective in maintaining constant O_2 consumption than tissues in the gastrointestinal tract because the extraction of O_2 from hepatic blood is highly efficient. This highly efficient O_2 extraction results from the small diffusion distances for O_2 transport between blood and hepatocytes. The combined effects of a large capacity for efficient O_2 extraction and the hepatic arterial buffer response ensures that the liver can tolerate substantial reductions in gastrointestinal blood flow without impairment of hepatocellular function [155,159].

Portal venous hypertension

Whereas exposure of the splanchnic circulation to an acute elevation in portal venous pressure is likely to elicit a myogenically mediated constriction of splanchnic arterioles, chronic portal hypertension tends to dilate the splanchnic vasculature. Furthermore, chronic portal hypertension has a significant impact on other regional vascular beds and on systemic hemodynamics. Blood flow to the gastrointestinal tract, kidneys, and skeletal muscle is significantly elevated. This presumably results from an increase in circulating vasodilators (e.g., glucagon) and a decrease in vascular sensitivity to vasoconstrictors (e.g., norepinephrine). The widespread dilation of arterioles results in a reduction of peripheral vascular resistance and a corresponding reduction of arterial blood pressure. In addition, cardiac output is elevated as a consequence of the increased venous return associated with the splanchnic and peripheral vasodilation. The elevated portal pressure results in the opening of portosystemic shunts to divert portal blood from the liver and reduce portal pressure. These shunts generally run along the esophagus (i.e., esophageal varices). The increase in portal pressure impairs venous drainage from the spleen into the portal vein, resulting in the accumulation of blood within, and distention of, the spleen (i.e., splenomegaly) [160–162].

Organ blood flow is determined by the arterial–venous pressure gradient and vascular resistance. It follows then that portal pressure is determined by portal venous inflow and portal venous resistance. The relationship between portal venous flow and portal pressure at a normal portal vascular resistance is depicted in Fig. 22.12 as a solid line. In this instance, an increase in portal venous flow will produce a proportional increase in portal pressure (point A to point B; flow-induced portal hypertension). When portal vascular resistance is increased, the relationship between portal pressure and portal venous flow is shifted upward and to the left, as depicted by the dashed line. At any given portal venous inflow, an increased portal vascular resistance will result in an increase in portal pressure (point A to point C;

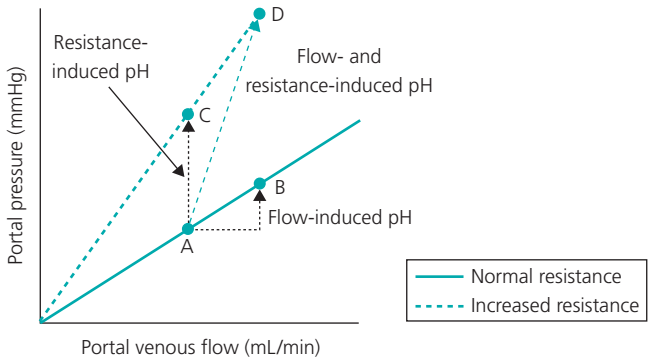


Figure 22.12 Hypothetical relationship between portal pressure and portal venous flow in the presence of a normal (solid line) or elevated (broken line) portal vascular resistance. Even when portal vascular resistance is normal, an increased portal blood flow (from point A to point B) can result in an increased portal pressure – that is, portal hypertension. An elevation in portal vascular resistance in the absence of increased portal blood flow (point A to point C) can also lead to portal hypertension. When both portal blood flow and vascular resistance are increased (point A to point D), the increase in portal pressure is more substantial. From Benoit et al. [163], with permission from Elsevier.

resistance-induced portal hypertension). Portal pressure can be further increased by a concomitant increase in portal venous flow and portal vascular resistance (point A to point D; flow- and resistance-induced portal hypertension). Indeed, the latter situation appears to reflect the vascular changes that account for the elevated portal pressure observed in some experimental models of chronic portal hypertension, and it is likely to account for the portal hypertension associated with some forms of liver disease. With a portal vascular resistance that is 40% higher in the portal hypertensive state than in the control state, it is predicted that increased portal inflow and increased portal vascular resistance account for 40% and 60% of the increase in portal pressure, respectively [163].

The portal hypertensive state leads to the development of collaterals (mostly along the esophagus; esophageal varices) to shunt blood from the congested portal vein, around the liver, to the systemic circulation (i.e., portosystemic shunting). Because a large proportion of portal venous blood bypasses the liver as a result portosystemic shunting, the hepatic degradation of different compounds, including circulating vasodilators, such as glucagon, is reduced. The diminished catabolism of circulating vasodilators increases their concentration in the plasma, allowing these agents to relax arteriolar vascular smooth muscle and reduce splanchnic vascular resistance. Another important action of some of the vasodilators that accumulate in chronic portal hypertension (e.g., glucagon) is to reduce the sensitivity of the splanchnic arterioles to vasoconstrictors such as norepinephrine, vasopressin, and angiotensin. The consequences of the direct and indirect actions of the accumulated circulating vasodilators include increased splanchnic blood flow and higher shear rates in splanchnic arterioles. Mechanical and humoral factors also exert an influence on NO generation by eNOS. The increased NO production that accompanies portal hypertension appears to result from endothelial cell responses that include increased intracellular Ca^{2+}, serine threonine protein kinase (AKT) activation, and the production and mobilization of molecular chaperones. However, some studies have revealed that eNOS-deficient mice exhibit a hyperdynamic state during portal hypertension, suggesting that eNOS-derived NO is not required for the induction of this vascular response. The net result of the vasodilation induced by all of these mechanisms is perpetuation of the portal hypertensive state [160–162,164–167].

Physiology and biochemistry of ischemia

Ischemic damage to the intestine occurs when splanchnic blood flow falls to a level at which the delivery of O_2 and other nutrients is insufficient to maintain oxidative metabolism and hence cell integrity. Blood flow to the gastrointestinal tract may be reduced during generalized nonocclusive ischemia (e.g., circulatory shock and congestive heart failure, especially in patients treated with cardiac glycosides) and in occlusive disorders (e.g., embolism, atherosclerosis, thrombosis) that primarily involve the mesenteric circulation. The mortality of acute mesenteric ischemia in adults has been reported at 70%–90% [168], primarily because of the difficulty of making an early diagnosis before bowel infarction occurs. Surgical intervention (e.g., embolectomy, intestinal resection) [169] and local intraarterial infusion of vasodilators (e.g., papaverine) [170] are used to treat acute mesenteric ischemia, but the mortality of this disease continues to be significant. Experimental nonocclusive mesenteric ischemia in dogs has been treated successfully with intravenously administered selective mesenteric vasodilators (e.g., urotensin I, sauvagine, and corticotropin-releasing factor) [171], thereby potentially obviating the risk of an indwelling angiographic catheter, but the use of these drugs in humans remains to be investigated.

Alterations of intestinal morphology with ischemia

The response of the intestine to decreased blood flow can range from no damage to transmural necrosis [172], and a gradient of sensitivity to ischemic injury has been demonstrated from the villus tips to the muscularis [172–174]. Mesenteric ischemia is associated with characteristic mucosal lesions that progress from subepithelial edema within 30 min after total vascular occlusion, to loss of epithelial cells along the villus after 1 h of total occlusion, to total loss of villi after 2 h of occlusion [172,173]. Within 30–60 min after total mesenteric artery occlusion, changes indicative of cellular failure appear, such as mitochondrial vacuolization and decreased O_2 uptake, loss of ATP, and release of lysosomal enzymes [174].

Changes in vascular and mucosal permeability with ischemia

Increases in the capillary filtration coefficient and microvascular permeability in the small intestine have been observed after ischemia [175]. The osmotic reflection coefficient of ileal capillaries to plasma proteins decreases after 1 h of ischemia and reperfusion; this is indicative of increased vascular permeability. Furthermore, the increase in permeability is derived from an increase in the number of large (20 nm) pores; the small-pore (5 nm) population is unaffected. In the intestine, the increase in the capillary filtration coefficient observed after ischemia and reperfusion is not solely a result of increased capillary surface area.

Increases in mucosal permeability induced by ischemia and reperfusion have been estimated based on the clearance of solutes ranging from 700 to 70 000 d [176,177]. The ischemia/reperfusion-induced increases in mucosal permeability are dependent on both the duration and severity of the ischemic insult (Fig. 22.13) [178]. Mucosal permeability increases significantly after 1–2 h of mesenteric artery occlusion in adult animals.

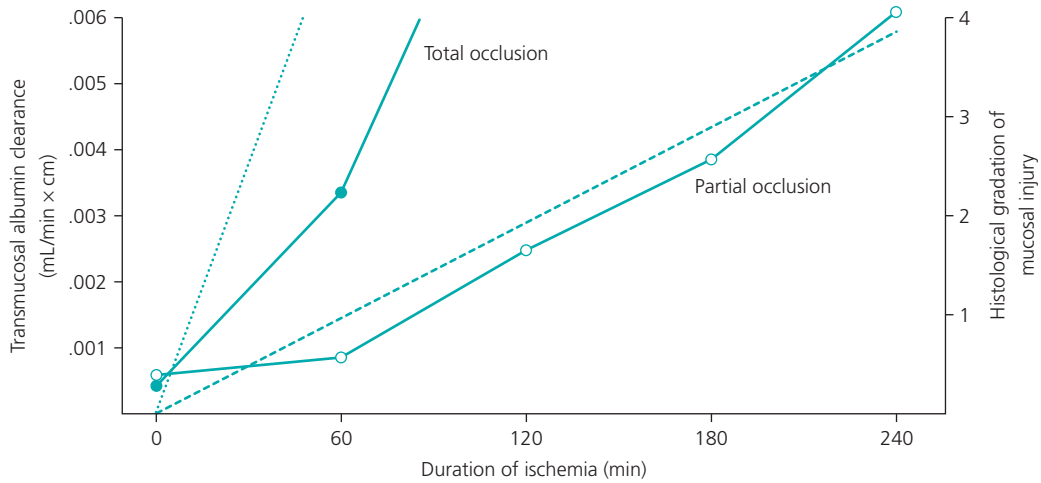
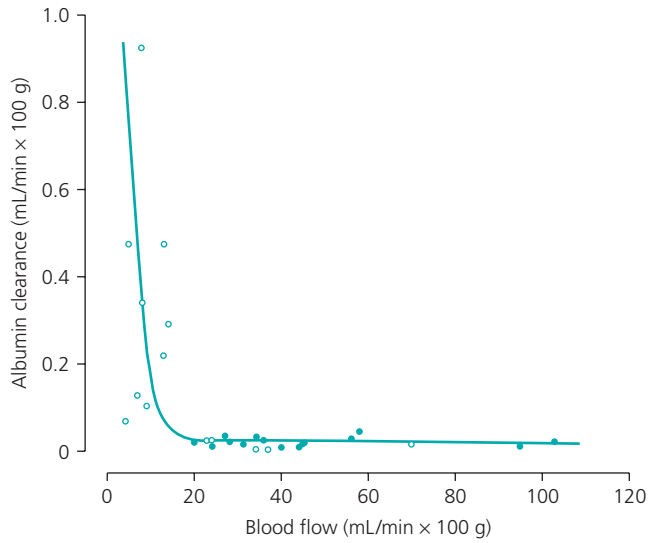


Figure 22.13 Comparison of quantitative morphological data (dotted and broken lines) and mucosal albumin clearance results (solid lines). From Parks et al. [178], with permission from Elsevier.

Blood flow, oxygenation, and ischemic injury

Ischemic injury to the intestine occurs when blood flow is reduced to a level that compromises delivery of O_2 and other nutrients to the tissue. Although the correlation of tissue Po_2, mucosal blood flow, and mucosal injury has not been investigated, reduction of blood flow to levels that do not affect O_2 uptake is not associated with any evidence of mucosal damage in adult animals [33]. Furthermore, substantial increases in mucosal albumin clearance are not seen until the blood flow falls to levels at which O_2 consumption is reduced by about 50% (Fig. 22.14) [33].

The importance of collateral blood flow in the prevention of intestinal ischemia is well recognized in humans [179–181] and in adult animals [182–184]. Intestinal collateral blood flow may occur through anastomotic connections at several levels of vessel branching, including the main arterial trunks (i.e., celiac, superior, and inferior mesenteric arteries) [179,182], extramural vessels (i.e., arterial arcades, marginal arteries) [184,185], and intramural vascular plexuses located within the intestinal wall itself [179,185]. Quantitative studies in adult animals have shown that collateral channels among the major arterial trunks and between adjacent bowel segments both play a role in the prevention of intestinal ischemia. In the adult cat, perfusion through collateral vessels after occlusion of the superior mesenteric artery maintained flow to the small intestine and proximal colon to



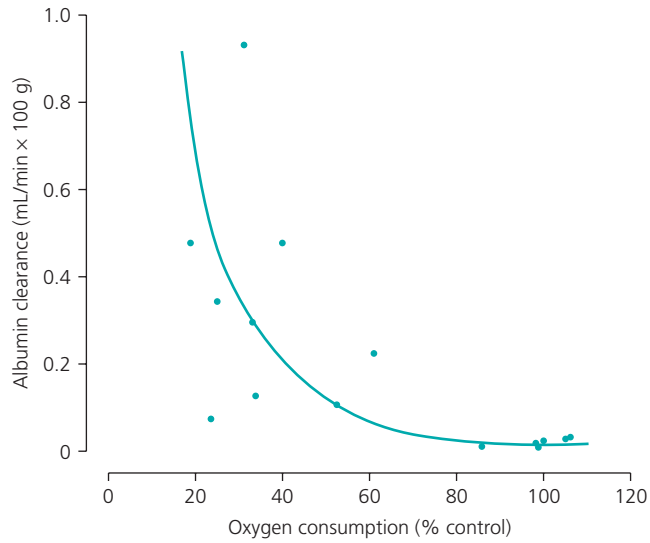


Figure 22.14 Relation between blood flow in control (closed circles) and during ischemia (open circles) and mucosal albumin clearance during reperfusion of canine jejunum (left). Relation between intestinal O_2 consumption in control and during ischemia and mucosal albumin clearance during reperfusion of canine jejunum (right). Substantial increases in albumin clearance were not seen unless blood flow was reduced to below 20 mL/min × 100 g or O_2 consumption was reduced to about 50% of the control values. From Bulkley et al. [294], with permission from Elsevier.

within 30%–65% of preocclusion flow [182]. However, the efficiency of collateral perfusion by way of the celiac and inferior mesenteric arteries is substantially lower in dogs after superior mesenteric artery occlusion [183]. In adjacent segments of canine small bowel, collateral vessels maintain blood flow in one segment at about 55% of its control level when the artery to that segment is totally occluded. The percentage of collateral flow attributed to extramural vessels is 67%, whereas that attributed to intramural vessels is 33% [184].

Possible mechanisms of injury or villous necrosis

Ischemic injury in the intestine appears to be related, either primarily or secondarily, to the effects of tissue hypoxia. In some species, the hypoxic stress induced by ischemia is exacerbated by the presence of a countercurrent exchange mechanism [141]. A role for hypoxia is supported by the observation that intralumenal perfusion with oxygenated saline solution markedly attenuates mucosal injury during ischemia induced by hypotension [186], whereas perfusion with nitrogenated saline solution does not attenuate injury. Possible mechanisms of mucosal injury induced by tissue hypoxia include depletion of high-energy phosphates necessary to produce protective substances, such as mucus, leading to increased susceptibility to the action of intralumenal proteases [187]; accumulation of histamine, leading to increased microvascular permeability [188]; production of metabolic acidosis, leading to release of lysosomal enzymes and cellular digestion [189]; conversion of xanthine dehydrogenase to xanthine oxidase, an enzyme that can produce cytotoxic O_2-derived free radicals during reoxygenation [190]; and attraction of circulating granulocytes into the mucosa, or activation of resident leukocytes within the mucosa, with release of neutrophilic proteases and oxidants to initiate or propagate mucosal injury [191].

It has been shown that changes in the intestinal mucosa induced by circulatory shock lead to an increased vulnerability to the digestive action of trypsin and chymotrypsin. Inhibition of pancreatic proteases by aprotinin [192] or previous ligation of the pancreatic ducts [193] significantly attenuates ischemic mucosal injury. In addition, an intralumenal injection of trypsin exacerbates mucosal injury [192]. The digestive action is caused by enzymes already present along the intestinal wall before shock, because removal of the pancreas has no effect if the animal is subjected to shock immediately after pancreatectomy [194]. Inhibition of pancreatic elastase and bile salts, both of which contribute to the loss of protective brush border glycoproteins [195,196], decreases mucosal injury. It has been proposed that if impairment of mesenteric blood flow prevents the steady regeneration of these brush border glycoproteins, then the mucosa becomes accessible to the digestive action of the pancreatic endopeptidases present along the intestinal wall and lumen [187]. Research has provided strong support for self-digestion by pancreatic enzymes as a major mechanism for the gut injury and multiorgan failure associated with hemorrhagic shock [197].

A role for reactive oxygen metabolites in the pathogenesis of injury associated with reperfusion of the ischemic bowel has received considerable attention during the past 20 years. The digestive system is particularly well endowed with the enzymatic machinery capable of generating significant quantities of reactive oxygen metabolites. For example, the intestine and liver are the richest sources of xanthine oxidase [198], an enzyme that catalyzes the production of both superoxide and hydrogen peroxide. Xanthine oxidase activity in the small intestine is located primarily within the mucosa, with a gradient of activity from villus tip to base [199]. In addition, the intestine contains a large, resident population of phagocytic cells (i.e., neutrophils, eosinophils, macrophages), which, when activated, produce considerable quantities of superoxide, hydrogen peroxide, and hypochlorous acid [200]. Oxidants generated by either xanthine oxidase or activated phagocytes can injure cells by a variety of mechanisms, including lipid peroxidation, degradation of the extracellular matrix, protein and carbohydrate decomposition, and DNA strand breakage [201]. Cellular enzymatic defense mechanisms against these oxidants include superoxide dismutase, which converts the superoxide anion to hydrogen peroxide and O_2, and catalase and glutathione peroxidase, which detoxify hydrogen peroxide [202]. Another important oxidant defense is reduced glutathione, which serves both as a cosubstrate for the glutathione peroxidase-catalyzed decomposition of hydrogen peroxide and as a free radical scavenger [201].

A large body of experimental data supports the hypothesis that reactive oxygen metabolites mediate the microvascular and mucosal permeability changes after reperfusion of the ischemic intestine and stomach in adult animals [191,203–207]. Superoxide dismutase (which scavenges superoxide anions), catalase (which detoxifies hydrogen peroxide), and dimethylsulfoxide (which scavenges hydroxyl radicals and decomposes hypochlorous acid) attenuate the vascular permeability changes observed after reperfusion of the ischemic intestine [203].

A role for xanthine oxidase in the increased vascular permeability and morphological changes induced by reperfusion has been proposed based on the following observations: attenuation of injury by pretreatment with allopurinol or pterin aldehyde, which are inhibitors of xanthine oxidase [204,208,209]; increased vascular and mucosal permeability during intraarterial infusion of hypoxanthine–xanthine oxidase, a superoxide anion-generating system [210]; attenuation of injury by soybean trypsin inhibitor, a substance that prevents the conversion of xanthine dehydrogenase to xanthine oxidase [205]; and attenuation of reperfusion injury by administration of a tungsten-supplemented, molybdenum-deficient diet, which inactivates xanthine oxidase [211].

Evidence to support a role for granulocyte-mediated injury after ischemia and reperfusion is also accumulating. Ischemia may lead to neutrophil activation, release, or production of neutrophilic oxidants (e.g., superoxide, hydrogen peroxide, hypochlorous acid, *N*-chloramines) and proteases, and subsequent tissue injury [200,212–214]. A fivefold to sevenfold increase in myeloperoxidase activity, which is an index of granulocyte number, occurs during ischemia, whereas reperfusion induces an 18-fold increase in myeloperoxidase activity in feline intestine [206]. Both neutrophil depletion and prevention of neutrophil adherence significantly attenuate the increased intestinal microvascular permeability induced by ischemia and reperfusion in cat intestine [191], suggesting that neutrophils, which migrate into the mucosa, mediate the injury produced by reperfusion of the ischemic bowel.

It has also been proposed that xanthine oxidase-derived oxidants may serve as chemoattractants for granulocytes in postischemic adult intestine. Allopurinol (an inhibitor of xanthine oxidase) [200], superoxide dismutase (a superoxide scavenger and inhibitor of neutrophil adherence) [200], catalase (a scavenger of hydrogen peroxide) [215], deferoxamine (an iron chelator) [215], dimethylthiourea (a hydroxyl radical scavenger) [215], and IB4 (a CD18-specific monoclonal antibody inhibitor of neutrophil adherence) [216] all inhibit reperfusion-induced granulocyte accumulation in the small intestine.

The view that leukocyte–endothelial cell adhesion plays an important role in the pathogenesis of ischemia and reperfusion injury, as well as in other inflammatory conditions of the gastrointestinal tract [217,218], has led to an increased interest in defining the factors that modulate leukocyte adherence and emigration in postcapillary venules. Intravital microscopic techniques have been used to monitor and quantify leukocyte–endothelial cell adhesion in venules exposed to ischemia and reperfusion [219–222]. During the final 10 min of a 60-min 80% reduction in mesenteric blood flow, the numbers of adherent and emigrated leukocytes increase fourfold and threefold, respectively. At 60 min after reperfusion, sevenfold and eightfold increases in adherence and emigration are noted. Electron microscopic analyses of postischemic mesenteric venules reveal that more than 85% of the leukocytes that emigrate into the adjacent interstitial compartment are neutrophils [223]. The recruitment of adherent leukocytes after reperfusion is associated with a corresponding accumulation of adherent platelets, with each blood cell exerting an influence on the recruitment of the other. Evidence suggests that platelet attachment to leukocytes and the products of platelet activation greatly enhance the inflammatory response to ischemia/reperfusion [220–222].

Several chemical mediators produced by endothelial or parenchymal cells have been implicated in the leukocyte–endothelial cell adhesion elicited by mesenteric ischemia and reperfusion. A growing body of evidence implicates xanthine oxidase-derived superoxide as a major mediator in this process. A role for superoxide is supported by reports that superoxide dismutase, whether administered before ischemia or after reperfusion, effectively reduces the number of adherent and emigrated leukocytes in mesenteric venules exposed to ischemia and reperfusion [219,224]. Superoxide dismutase is also effective in attenuating the adherence of neutrophils to endothelial cell monolayers exposed to anoxia and reoxygenation [224]. A similar antiadhesive action has been reported to occur with allopurinol [219,225], suggesting that xanthine oxidase is a likely source of the superoxide produced after reperfusion. Xanthine oxidase, rather than neutrophils, also appears to be responsible for generating the oxidants that mediate reperfusion-induced lipid peroxidation in intestinal mucosa. This contention is based on reports showing that although both allopurinol and CD18-specific monoclonal antibodies prevent the reperfusion-induced increase in mucosal myeloperoxidase activity, only allopurinol prevents the rise in tissue-conjugated dienes, an index of membrane lipid peroxidation [226,227].

A likely explanation for the proadhesive action of superoxide is that it inactivates an antiadhesion molecule that is normally produced by endothelial cells. NO, a product of L-arginine metabolism in endothelium that is rapidly inactivated by superoxide, may be such an endogenous antiadhesion molecule. NO donors are very effective in preventing the recruitment of leukocytes, platelet–leukocyte aggregation, and tissue injury associated with ischemia/reperfusion [228]. Inhibitors of NO production lead to a dramatic increase in the number of leukocytes adhering to and emigrating from mesenteric venules [229]. This adhesion response can be prevented or reversed by simultaneous exposure of venules to an NO synthase inhibitor and either L-arginine (but not D-arginine) or nitroprusside, which spontaneously generates NO. The observations that NO synthase inhibitors promote leukocyte adherence whereas superoxide dismutase reduces reperfusion-induced adherence are consistent with the view that the enhanced formation of superoxide by postischemic endothelial cells leads to NO inactivation and consequently results in enhanced leukocyte adhesion. Such a mechanism would explain why superoxide dismutase exerts an antiadhesive effect in postischemic tissues, because the enzyme would prevent inactivation of NO. NO also stabilizes mast cells through a mechanism that involves superoxide [230].

Catalase also has been shown to attenuate reperfusion-induced leukocyte adherence in mesenteric venules [225]. This observation is consistent with reports that demonstrate that hydrogen peroxide is produced by endothelial cell monolayers exposed to anoxia and reoxygenation [231], and hydrogen peroxide promotes neutrophil adherence to cultured endothelial cells and in mesenteric venules [232,233]. The levels of hydrogen peroxide required to promote adherence are well within the range of the hydrogen peroxide concentration produced by activated neutrophils. Both in vivo and in vitro studies indicate that hydrogen peroxide-induced

neutrophil adherence is mediated by platelet-activating factor (PAF) [232,233]. PAF receptor antagonists effectively attenuate the neutrophil adherence induced by hydrogen peroxide in feline mesenteric venules [216] and isolated canine carotid arteries [234]. The hydrogen peroxide-induced, PAF-mediated leukocyte adherence is prevented mostly by monoclonal antibodies directed against the common β-subunit of CD11/CD18.

The proposed role of reactive oxygen (hydrogen peroxide, superoxide) and nitrogen (NO) species in mediating the endothelium-dependent inflammatory responses observed in the gastrointestinal microcirculation after ischemia/reperfusion is summarized in Fig. 22.15. Ischemia/reperfusion leads to an increased production of superoxide and hydrogen peroxide by the enzyme xanthine oxidase, with a corresponding reduced production of NO by eNOS. The enhanced generation of oxidants results in the activation and deposition of complement, and phospholipase A_2-mediated production of leukotriene B_4 and PAF. Oxidants also mediate the initial expression of P-selectin by mobilizing the leukocyte rolling receptor from its preformed pool (Weibel–Palade bodies) in endothelial cells. Firm adhesion of leukocytes, which is mediated by β_2-integrins (CD11/CD18), is induced by the engagement of activated complement, leukotriene B_4, and PAF with their receptors on rolling leukocytes. Sustained rolling and adhesion of leukocytes on endothelial cells is ensured by an oxidant-dependent synthesis of endothelial cell adhesion molecules, such as E-selectin and intracellular adhesion molecule-1. Oxidants, derived from either endothelial cells or leukocytes, elicit this biosynthetic response by activating specific nuclear transcription factors (e.g., nuclear factor-κB) that bind to the genes for these adhesion molecules. The inflammatory responses to ischemia/reperfusion are amplified by mediators released from mast cells and macrophages that normally reside near postcapillary venules [235].

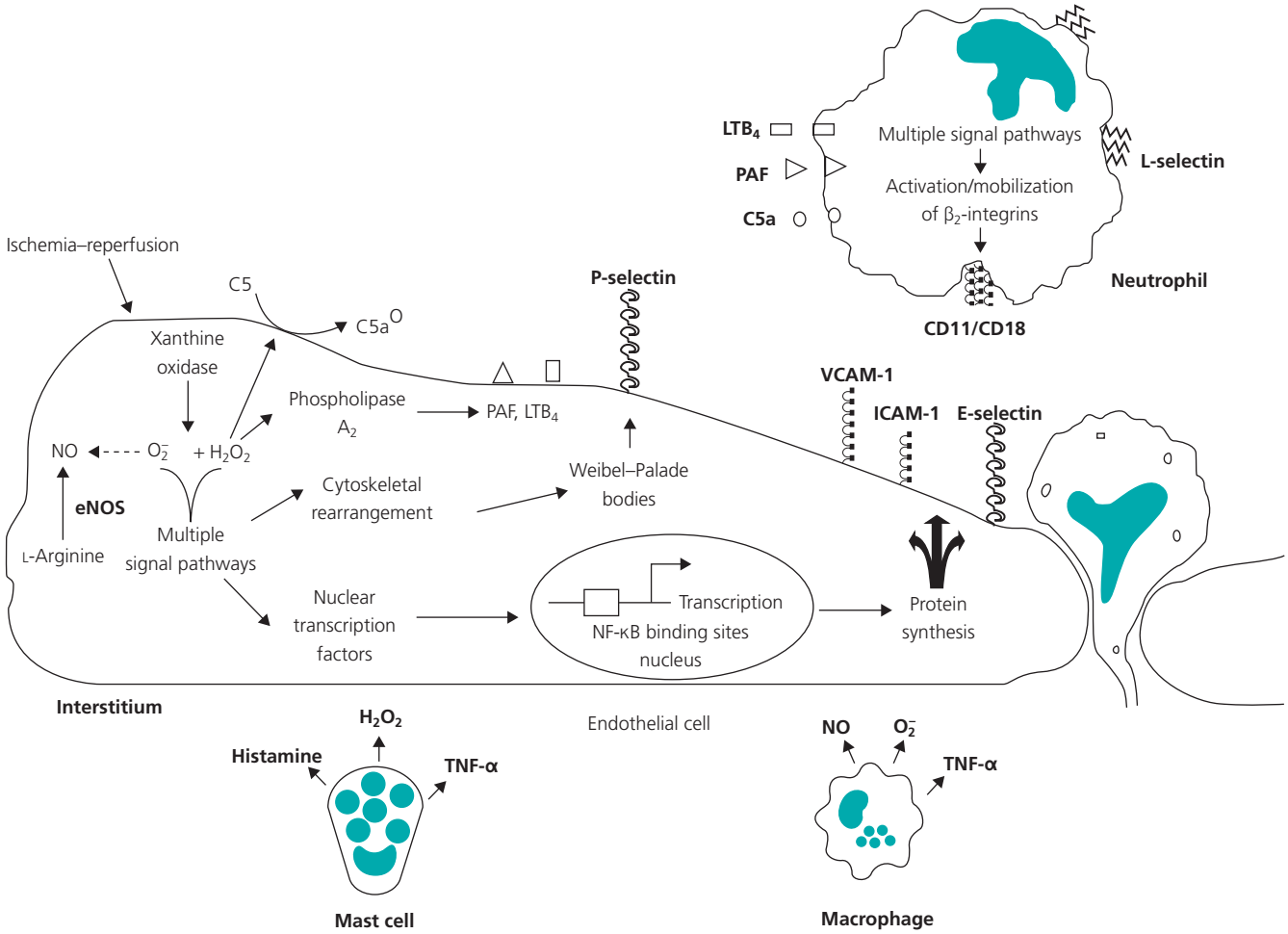


Figure 22.15 Mechanism proposed to explain the endothelium-dependent inflammatory responses observed in the postischemic microvasculature. C5a, complement 5a; CD11/CD18, leukocyte adhesion glycoprotein; eNOS, endothelial nitric oxide synthase; H_2O_2, hydrogen peroxide; ICAM, intracellular adhesion molecule; LTB_4, leukotriene B_4; NF-κB, nuclear factor-κB; NO, nitric oxide; O_2^-, superoxide; PAF, platelet-activating factor; TNF-α, tumor necrosis factor-α; VCAM, vascular cell adhesion molecule. From Carden and Granger [235].

The newborn intestinal circulation

At birth, the intestine assumes sole responsibility for the assimilation of water and nutrients; in addition, it begins a period of rapid growth. During subsequent postnatal life, dietary composition changes from one exclusively of milk to one of varied composition. It thus might be expected that the intestinal hemodynamics in the newborn would be unique when compared to the adult circumstance, a predication that has been experimentally confirmed.

Basal hemodynamic conditions

The basal, or preprandial, hemodynamic conditions within the piglet intestine change significantly during the first postnatal month, during which time the diet transitions from milk to cereal (Table 22.1). Highlights of these changes include:

- a progressive reduction of intestinal vascular resistance and concomitant fall in blood flow
- a progressive reduction of intestinal O_2 consumption (Vo_{2i}) [236].

A similar change in Vo_{2i} and gut vascular resistance and blood flow has been reported in lambs [237–239].

Factors established to induce intestinal vasoconstriction during early postnatal life

Extrinsic neural stimuli

Although sympathetic nerves are present in the intestine at birth, functional maturation of these nerves occurs during early postnatal life [240]. Intestinal denervation causes only a modest reduction of basal vascular resistance in newborn piglets, suggesting that the role of tonic adrenergic stimuli in setting resistance is minimal at birth [241]. Direct stimulation of the mesenteric nerve trunk generates vasoconstriction to a greater extent in weanling than newborn piglets; as well, Vo_{2i} is compromised only in older subjects [242]. "Escape" from adrenergic-induced vasoconstriction [53] occurs in both newborn and weanling subjects, and in the latter group, it is mediated in part by adenosine [55].

Table 22.1 Baseline hemodynamic conditions in swine perinatal intestine.

	3-day-old (newborn)	30-day-old (weanling)
Arterial pressure (mmHg)	75 ± 1	89 ± 1*
Capillary pressure (mmHg)	6 ± 1	6 ± 1
Mesenteric vein pressure (mmHg)	1 ± 1	1 ± 1
Total vascular resistance (mmHg/mL/min/100 g)	0.74 ± 0.06	1.82 ± 0.08*
Capillary filtration coefficient (mL/min/mmHg/100 g)	0.361 ± 0.040	0.203 ± 0.020*
(a–v)O_2 (mL O_2/dL)	2.9 ± 0.2	4.3 ± 0.3*
O_2 consumption (mL O_2/min/100 g)	3.01 ± 0.24	2.00 ± 0.12*

Capillary filtration coefficient reflects the perfused capillary density; (a–v)O_2 is the arteriovenous O_2 content difference across the intestine. O_2 consumption is calculated by the Fick equation as the product of flow and (a–v)O_2. Mean ± SE, $n = 12$.
* $P < 0.01$ vs 3-day old intestine.
Data from Warwick and Williams [1].

Intrinsic myogenic tone

The myogenic response – the intrinsic vasoconstriction noted in response to augmentation of intravascular pressure [7] – is present to a greater extent in the newborn than the weanling intestinal circulation [243–245]. The intensity of this response is reduced when tissue O_2 availability is compromised by ischemia, but only in weanling intestine [245]. Protein kinase C participates in the mediation of the myogenic response in small arteries from newborn, but not from weanling piglets [243].

Endothelin-1

The vasoconstrictor peptide endothelin-1 imparts basal vasoconstrictor tone in an age-dependent manner. This effect is more substantial in newborn intestine, as evidenced by two observations:

- blockade of endothelin receptors leads to vasodilation in newborn intestine
- the concentration of endothelin-1 in the newborn mesenteric venous effluent exceeds the arterial concentration.

These observations indicate a net production of the peptide within the newborn intestinal vasculature [14,246]. This developmental pattern reflects, in part, the postnatal ontogeny of endothelin receptors, whose expression is substantially greater in the newborn than weanling mesenteric artery [247,248].

Angiotensin II

Mesenteric artery rings from newborn piglets contract in response to the vasoconstrictor peptide angiotensin II, indicating the presence of the angiotensin II receptor AT1 within the newborn intestinal circulation. Blockade of AT1 receptors in vivo under resting conditions does not affect vascular resistance; however, intestinal vasoconstriction in response to cardiac tamponade is largely mediated by angiotensin II and the magnitude and duration of this effect is significantly greater in newborn than weanling piglets. While the angiotensin system plays a minimal role in setting basal vascular resistance in newborn intestine, it participates in the response to specific circulatory perturbations [249].

Platelet-activating factor

Platelet-activating factor, a proinflammatory phosphoglyceride [250], is well established as a cause of intestinal

vasoconstriction in adult animals [251]. It also reduces intestinal perfusion in the newborn piglet [252]. Indeed, intraarterial infusion of PAF causes hemorrhagic necrosis of the intestine in a manner indistinguishable from that induced by infusion of lipopolysaccharide.

Factors established to induce intestinal vasodilation during early postnatal life

Nitric oxide

The eNOS-derived NO is the principal vasodilator stimulus in the postnatal intestine and its effect is age-dependent, being more potent in newborn than weanling intestine [14,44]. The age specificity of this action is attributable, in part, to the postnatal change in eNOS expression, which increases on postnatal day 1, but then wanes between postnatal days 10 and 30; interestingly, this reflects posttranscriptional regulation insofar as eNOS messenger RNA expression remains stable during the first postnatal month [253].

Although direct measurement of eNOS activity has not been performed within the postnatal intestinal circulation, one factor known to activate eNOS, the mechanostimulus of flow [254], plays a prominent role in vascular regulation during early postnatal life. The application of flow induces vasodilation to a greater extent in newborn than weanling intestine; moreover, the release of NO by small mesenteric arteries in response to a flow stimulus is greater in newborns [255,256]. Further, flow-induced dilation overrides myogenic vasoconstriction in small mesenteric arteries from newborn piglets but not weanling piglets [14].

Substance P, adenosine

Substance P, a peptide neurotransmitter produced within the perinatal intestine [257], relaxes mesenteric artery rings in vitro and vasodilates the newborn intestinal circulation in vivo in a NO-dependent manner; this effect, although present, is less robust in weanling intestine [258]. Adenosine, an established intestinal vasodilator [81], contributes to the vasodilation that attenuates adrenergic-induced vasoconstriction during sustained mesenteric nerve stimulation (i.e., "escape") [55].

Response of the newborn intestinal circulation to specific perturbations

Hypotension (pressure–flow autoregulation)

Autoregulation in response to manipulation of arterial pressure is weak in the weanling intestine, and absent in the newborn (Fig. 22.16); indeed, prior induction of hypoxia, ischemia, or a postprandial state, factors that generally enhance autoregulation [10,259,260], have no effect on the efficacy of autoregulation in the newborn intestine under in vitro conditions [261,262]. Also, the newborn intestine cannot expand the perfused capillary density in response to reduced perfusion pressure, whereas weanling intestine has this capacity [236]. This circumstance, coupled with a lack of autoregulation, renders the newborn intestine profoundly susceptible to tissue hypoxia. Systemic hypotension generated under in vivo conditions also compromises intestinal perfusion in an age-specific manner, having a greater impact in the newborn [263,264]. The effect of venous pressure fluctuation is also age-dependent. Acute venous hypertension induces intestinal vasoconstriction that is more pronounced in the newborn [243,244].

Hypoxemia

Arterial hypoxemia, a circulatory perturbation common in sick human infants [265], induces intestinal vasoconstriction and tissue hypoxia to a greater extent in newborn than weanling intestine [266–268]. This effect is mediated locally, insofar as it occurs to an equal extent under both in vitro [266] and in vivo [269] conditions.

Sustained ischemia

Mechanically induced, sustained reduction of the intestinal flow rate to about 50% of baseline leads to progressive vasoconstriction, and hence to worsening intestinal ischemia in newborn animals, but not in weanling animals [270]. This age-specific phenomenon is mediated, in part, by at least two factors:

- a reduction of eNOS-derived NO production
- angiotensin II.

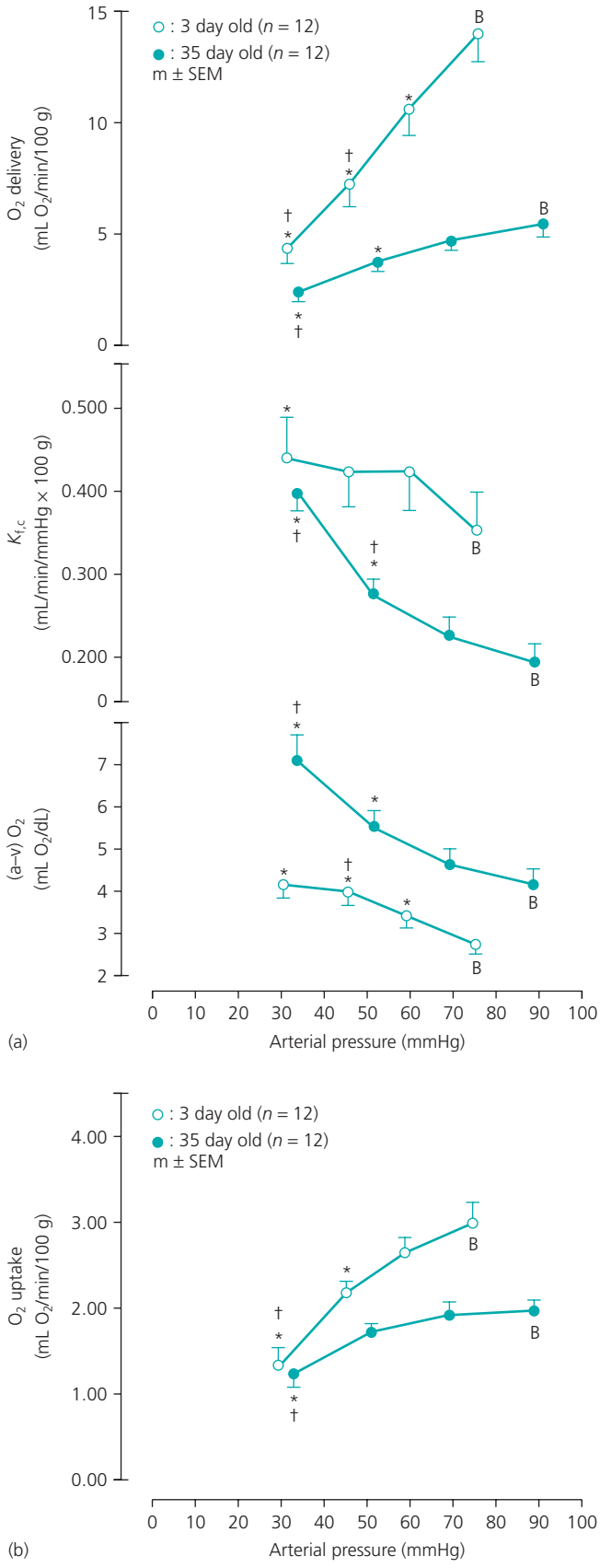
Moreover, exposure of the intestinal circulation of newborns, but not of weanlings, to sustained low flow conditions enhances its response to the vasoconstrictor effect of norepinephrine, angiotensin II, and vasopressin; the mechanisms of this effect are not known [271].

Postprandial hyperemia

Postprandial hyperemia occurs to a similar extent in newborn and weanling intestines [238,272,273]. Interestingly, Crissinger and Burney [272] observed that instillation of artificial formula directly into an in vivo gut loop in 1-day-old, never-nursed piglets decreased total gut perfusion. However, flow rate to the mucosal and submucosal layers of the gut increased in response to formula instillation in this age group; therefore, increased O_2 delivery to the site of nutrient absorption was achieved by redistribution of flow within the gut wall; i.e., mucosal hyperemia occurred at the expense of the muscularis layers [272]. This group also determined that postprandial hyperemia occurred in response to protein (5% casec), bile-acid solubilized fat (5% corn oil), and carbohydrate (5% glucose) to a similar extent in 3-day-old to 30-day-old piglets, showing that postprandial hyperemia occurred irrespective of the nutrient composition of the meal [274].

Putative clinical relevance of postnatal intestinal hemodynamics

Interest in the physiology of postnatal intestinal blood was sparked by its potential relevance to the pathogenesis of



neonatal necrotizing enterocolitis, the most common acquired gastrointestinal disease of infancy [265]. The histopathology of necrotizing enterocolitis clearly implicates preceding ischemia [275,276] and existing thought proposes that the triad of ischemia, inflammation, and exposure to nutrients (i.e., feeding) are the principal factors in disease pathogenesis [277,278].

Intestinal perfusion in the human fetus and infant has been measured by Doppler ultrasonography. This approach has limitations: the technique measures flow velocity or pulsatility, rather than flow per se, and is limited to observations of the superior mesenteric artery. The latter circumstance is especially troubling insofar as the vasculopathy relevant to necrotizing enterocolitis likely occurs within the intramural microvasculature [279]. With these caveats stated, however, several observations merit discussion. Blood flow velocity in the superior mesenteric artery increases within 30 min of feeding in full-term infants; interestingly, milk elicits a greater and more sustained increase [280], although human milk and cows' milk-based formula generated a similar response [281]. Premature infants deemed "at risk" for the development of necrotizing enterocolitis demonstrated decreased superior mesenteric artery flow velocity, although this finding was not ultimately predictive of the occurrence of necrotizing enterocolitis in these infants [282]. Superior mesenteric artery flow velocity in preterm infants with established necrotizing enterocolitis is reduced [282] or increased [283,284] with respect to control. This disparity likely reflects the relative irrelevance of superior mesenteric artery flow in necrotizing enterocolitis pathogenesis.

An alternative means to study the human intestinal circulation in necrotizing enterocolitis is by the in vitro assessment of arterioles removed from the submucosa and mesentery of intestine resected for this disease [285]. When compared to arterioles from control intestine, those removed from necrotizing enterocolitis-afflicted gut showed a greater basal vascular resistance that was attenuated by blockade of endothelin-1 receptors; concomitantly, this tissue demonstrated a greater concentration of endothelin-1. Interestingly, Weir and colleagues [286] demonstrated Doppler evidence of reduced

Figure 22.16 Relationships between perfusion pressure and various hemodynamic parameters in 3-day-old (newborn; milk-fed) and 35-day-old (weanling; cereal-fed) swine. Data were collected using blood-perfused in vitro gut loops. **(a)** O_2 delivery (product of blood flow and arterial O_2 concentration; here, the latter term is fixed, so that the data directly reflect blood flow); $K_{f,c}$ (a marker of the perfused capillary density; under these experimental conditions, an increase in $K_{f,c}$ indicates an expansion of the perfused capillary density, and vice versa); the arteriovenous O_2 content difference across the gut loop. **(b)** Tissue O_2 uptake, or consumption. Data from Nowicki & Miller [236], with permission from the American Physiological Society.

superior mesenteric artery flow velocity in infants as a function of the plasma endothelin-1 concentration.

Finally, a substantial body of work indicates an important role for PAF in the vascular pathogenesis of necrotizing enterocolitis [287]. PAF induces intestinal damage in newborn piglets similar to the damage caused by necrotizing enterocolitis in humans, whereas administration of PAF receptor antagonists attenuates this damage [252,288,289]. Plasma levels of PAF are elevated in infants with necrotizing enterocolitis [290,291]. Interestingly, PAF acetylhydrolase, the enzyme responsible for PAF inactivation, is present in human milk, and exclusive use of human milk in feeding preterm infants reduces the incidence of necrotizing enterocolitis [292,293].

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E MISCELLANEOUS

23 Development of the gastrointestinal system

Ben Z. Stanger, Daniel K. Podolsky

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Developmental biology seeks to understand how organisms are formed. Central to the field are questions about differentiation, morphogenesis, and growth – the processes that give rise to our physical appearance, physiology, and (when perturbed) diseases. Despite many years of intensive research, our understanding of the molecular mechanisms that guide normal vertebrate development remains primitive. Perturbations in these processes, resulting in congenital malformations or functional diseases, are all the more difficult to study because developmental insults may occur weeks or months before a defect is detectable.

While an understanding of how the body is formed is interesting and important in its own right, studies of development can also have a clinical impact. Exploiting developmental processes offers the promise of creating "cell therapies" – growing tissues ex vivo for use in tissue transplantation and augmentation, or coaxing cells in vivo to acquire characteristics that restore function. Fulfilling this promise will undoubtedly require a more complete delineation of developmental mechanisms.

The chapter has been divided into several sections to facilitate an appreciation for the complexity of the development of the gastrointestinal system. *Early development* outlines the basic mechanisms by which the embryo achieves a spatial "pattern," setting the stage for further developmental steps. *Organogenesis* focuses on the known molecular mechanisms that guide development of the liver, the pancreas, and the lumenal gastrointestinal tract. *Developmental physiology* samples important events during the functional maturation of the gastrointestinal tract. *Disorders of development*, the fourth and final section, focuses on specific diseases that highlight the relationship between molecular events and clinical consequences.

The embryology of the human gastrointestinal tract involves many temporally and spatially regulated tissue interactions and the creation of many varied structures. Atlases are excellent resources for detailed consideration of key aspects of embryology [1,2]. The ensuing discussion focuses on the mechanisms of gastrointestinal development. What hurdles must be surmounted to create a gastrointestinal tract with normal form and function and how can these processes be controlled for therapeutic benefit?

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Early development

The complex anatomy of adult mammals has its origins in a single fertilized egg. The transformation from egg to newborn occurs in many steps marked by discrete events (Fig. 23.1). The fertilized egg initially grows in cell number through cleavage divisions into a blastocyst, an asymmetrical collection of cells containing the precursors of both embryo and placenta, which implants in the uterine wall. After implantation, the *endoderm*, from which the epithelium of all gastrointestinal organs is derived, is formed through the process of *gastrulation*. Subsequently, the endoderm is segmented (*patterned*) into domains, which become *committed* to give rise to specific organs. Finally, solid organ buds grow into the gut tube, and organogenesis proceeds with the processes of *differentiation* and *morphogenesis*.

Many studies of gastrointestinal development have been performed in model organisms, including fruit flies (*Drosophila*), frogs (*Xenopus*), zebrafish, and mice. Despite differences in anatomy and timing of development (see Fig. 23.1), most studies suggest that developmental mechanisms in the mouse are comparable to those in the human. Information gained from model organisms can therefore reasonably be extrapolated to humans because of the remarkable conservation of mechanisms throughout evolution. The terminology used in developmental biology is listed and defined in Box 23.1.

Gastrulation and tube formation

To understand gastrointestinal form and function, it is necessary to recognize the steps that precede organogenesis. The

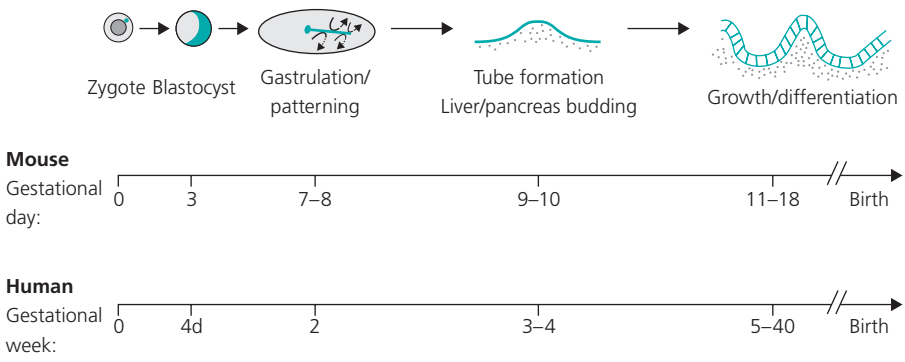


Figure 23.1 Overview of gastrointestinal development. Timelines for milestones of mouse (18-day gestation) and human (40-week gestation) embryogenesis. See text for details of individual steps.

Box 23.1 Terms in developmental biology

Gastrulation – the process by which cells migrate through the primitive streak of the epiblast to form the three germ layers of the embryo: ectoderm, mesoderm, and endoderm. The *endoderm* gives rise to the epithelium of the lumenal gastrointestinal tract, pancreas, liver, and lung, as well as parts of several pharyngeal organs (e.g., thyroid).

Fate/Specification – the assignment of predictive identifying information to a precursor cell or group of cells. Under normal circumstances, cells will adopt their specified *fate*, although cells may retain the *potential* to adopt other fates until the final fate *commitment* occurs. The set of fates that will be adopted in the absence of any external influence is determined by marking cells at one point in time and compiling a map of all the different types of cells derived from the marked cells.

Patterning – the regulated assignment of fates over a large area in a manner that ensures the proper positional relationship between tissues.

Differentiation – the acquisition by a cell of specialized features. Differentiation is generally associated with commitment or loss of potential.

Inductive signals – signals that impart fate-determining information. Such signals may be *permissive* (permit the expression of a previously assigned fate) or *instructive* (cause the cell to divert from a previously assigned fate).

Homeotic transformation – an alteration in the fate of a large tissue segment, such that it adopts the fate of a different tissue segment.

Morphogenesis – the assembly of a higher order structure in development (i.e., the creation of a two-dimensional structure from one dimension, or a three-dimensional structure from two dimensions).

Stem cells – "undifferentiated" cells exhibiting two properties: the capacity to give rise to multiple differentiated cell types, and the capacity for "self-renewal" (i.e., dividing to give rise to additional stem cells).

Embryonic stem cells – cell lines derived from the in vitro culture of dissociated blastocysts. These cells most closely resemble cells from the *inner cell mass* – the portion of the blastocyst that gives rise to the embryo proper. Embryonic stem cells themselves retain the capacity to give rise to all tissues in the animal, including gametes.

Transcription factors – proteins with the ability to bind DNA and activate (or repress) mRNA transcription. These factors are categorized into families according to structure; structural domains include homeobox, forkhead, zinc-finger, high mobility group (HMG) -box elements. In addition, components of some signaling pathways can themselves act as transcription factors (e.g., SMAD proteins).

Imprinting – the differential marking of maternally inherited and paternally inherited alleles of a gene, with resultant effects on gene expression and phenotype. Imprinting is most commonly achieved by differential DNA methylation, which occurs early in embryonic development.

most important of these is *gastrulation*, the process by which three distinct germ layers – ecotoderm, mesoderm, and endoderm – are formed. After implantation in the uterus, the embryo exists as a disc of cells called the epiblast. Two structures – the node and the primitive streak – appear in the posterior half of the epiblast layer, and cells migrate posteriorly toward and down through the primitive streak, giving rise to new layers of cells – the embryonic mesoderm and embryonic endoderm (Fig. 23.2). As a consequence of gastrulation, the three axes of the embryo are also established: the antero-posterior or rostral–caudal axis is defined by the location of the primitive streak (posterior); the dorsal–ventral axis is defined by the ectoderm (dorsal) and endoderm (ventral); and the left–right axis is defined by the other two axes.

How the cells that migrate through the primitive streak are instructed to become mesoderm or endoderm is incompletely understood. Phylogenetic analyses of organisms including fish, frogs, and mice point to a conserved pathway for endoderm

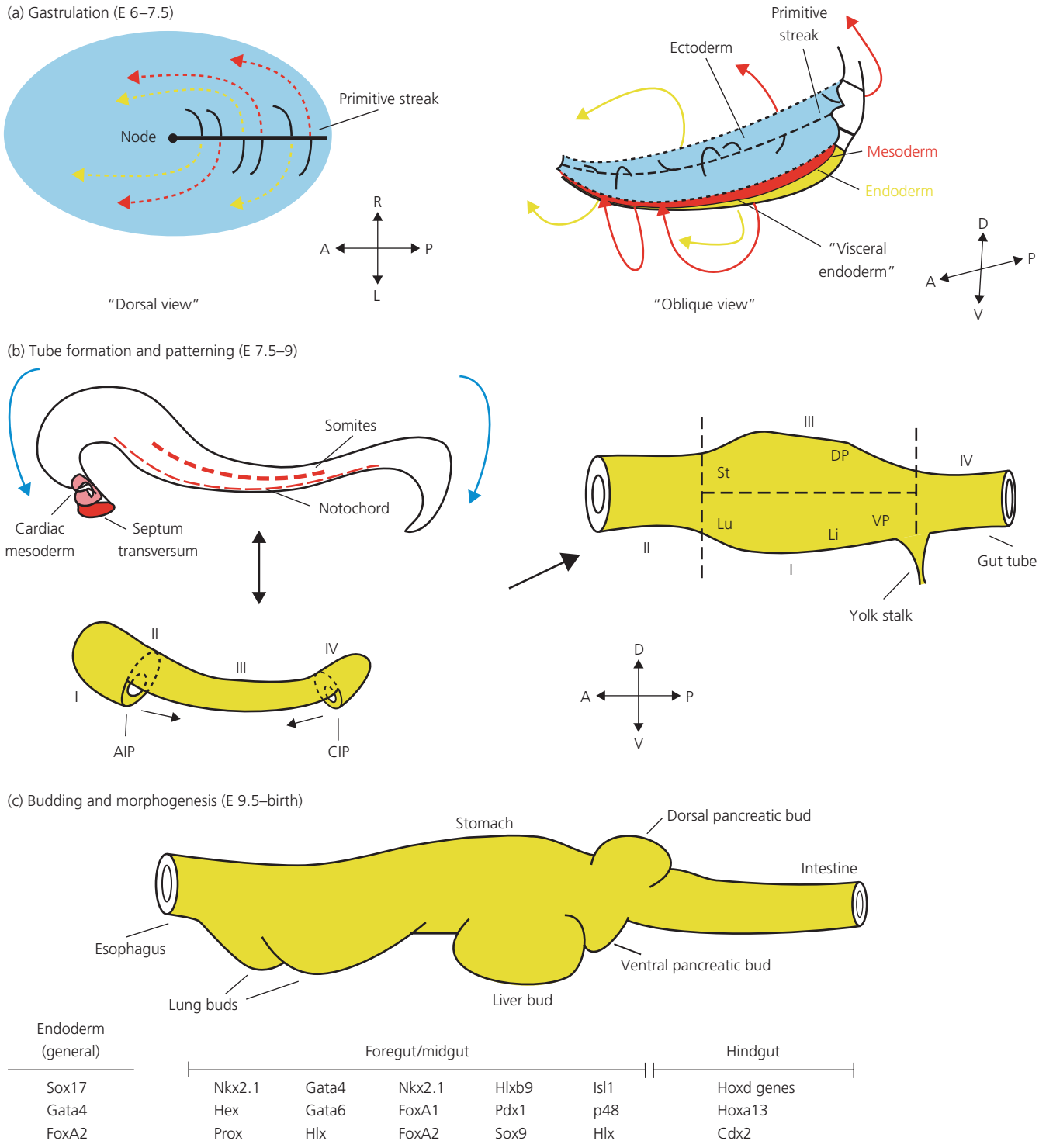


Figure 23.2 Major events in early mammalian endoderm development. **(a)** Gastrulation. (*left*) The embryonic epiblast (blue) viewed from above. Epiblast cells (solid black) migrate down through the primitive streak, becoming mesoderm (dashed red) or endoderm (dashed yellow) cells. (*right*) Oblique view of migrating epiblast cells, in which formation of the new mesoderm and endoderm is visible. **(b)** Tube formation and patterning. (*left*) The mesoderm and ectoderm have been pulled back to reveal the endoderm below. At this stage, the anterior endoderm is adjacent to the cardiac mesoderm and septum transversum (which mediate liver induction) whereas more dorsal portions of the endoderm are in contact with the notochord (which mediates pancreas induction). The folds of the anterior intestinal portal (AIP) and caudal intestinal portal (CIP) form the gut tube as they migrate towards each other at the midline. Blue arrows indicate the process of turning, by which the embryo switches from a convex to a concave shape, with the endoderm on the "inside". The roman numeral designations are derived from fate-mapping studies and indicate the prospective regions of endoderm that will give rise to later endoderm derivatives. (*right*) The relative position of endoderm domains changes with the completion of gut tube folding; the region that previously constituted the most anterior portion of endoderm (I) shifts to the ventral midline and gives rise to lung (Lu), liver (Li), and ventral pancreas (VP). **(c)** Budding and morphogenesis. Budding of endoderm derivatives begins shortly after the gut tube is formed and involves many transcription factors. DP, dorsal pancreas; E, embryonic day; St, stomach. Adapted from Stanger & Melton [240].

development that involves the transforming growth factor-β (TGF-β)-related nodal pathway and several classes of DNA-binding transcription factors that belong to the homeobox, forkhead (winged helix), zinc finger, and high mobility group (HMG) families [3].

The tubular structure of the gut arises from two ventral invaginations that form at the anterior (proximal) and posterior (distal) ends of the embryo after gastrulation (see Fig. 23.2). These will eventually form the structures of the foregut and hindgut, respectively. The anterior fold, or anterior intestinal portal, and caudal intestinal portal, move towards each other and meet in the midline of the embryo at the level of the yolk sac. As a result, ventral structures close to the midline (e.g., lung, liver, and ventral pancreas) derive from endoderm that is distinct and distant from the endoderm that gives rise to dorsal structures (e.g., dorsal pancreas). This arrangement means that the dorsal and ventral portions of the pancreas are independently induced, although these tissues eventually combine to form one functioning organ.

Several genes have been identified that are required for tube formation of the gut (Table 23.1). One of these genes encodes GATA4, a zinc finger-containing, DNA-binding protein. Although endoderm is able to develop in *Gata4* mutant mice, formation of the anterior intestinal portal is faulty and results in failure to form a foregut [4–6]. Other genes that are required for tube formation or closure include those for the forkhead-winged helix DNA-binding transcription factor FOXA2 (previously HNF3B), which has additional roles in foregut and midgut development, and the protease *furin*, which may be necessary to process TGF-β signals [7–9]. A critical and conserved role for two other families – the HMG domain-containing SOX factors and the homeodomain-containing MIX factors – has been demonstrated [10,11]. GATA4-like and FOXA-like factors are involved in gut development in organisms as distantly related to mammals as the fruit fly *Drosophila* and the nematode *Caenorhabditis elegans* [12], whereas the involvement of SOX and MIX factors appears to become important only in higher organisms including zebrafish, *Xenopus*, and mammals.

Pattern formation

Patterning refers to the stereotypical commitment of cells to certain *fates*, constituting in its grandest form the establishment of the "body plan" – the spatial arrangement of all tissue types in three-dimensional space. In the endoderm, patterning establishes the correct relationship, along the anteroposterior axis, of domains that will give rise to the respiratory tract and the gastrointestinal organs. Our understanding of how embryos are patterned comes largely from classical studies in *Drosophila*, which identified homeobox-containing transcription factors (*Hox* genes, in particular) as the major determinants of the body pattern [13]. A testament to the remarkable conservation of biological mechanisms across evolution, these studies have confirmed the critical role that *HOX* genes (and related homeobox-containing genes) also play in establishing the body plan of all higher organisms, including humans.

HOX genes, of which there are 39 in humans, have a distinct organization in the genome. Specifically, *HOX* genes are arranged sequentially within each of four distinct "clusters" (A, B, C, and D). This chromosomal organization of the *HOX* genes mirrors their expression in the embryo, so-called "co-linearity" of expression (e.g., the mouse *Hoxa* cluster consists of 12 genes; *Hoxa1* is expressed more anteriorly in the embryo than *Hoxa2*, which is expressed more anteriorly than *Hoxa3*, and so forth). Loss-of-function or gene knockout analyses in flies and mice have shown that these carefully regulated expression boundaries dictate the *pattern* of the ectoderm and mesoderm. Malformations in *Hoxa2* mutants are therefore more anterior (head) than those in *Hoxa3* mutants (neck and chest); this property holds true for all *Hox* clusters and genes. Conversely, enforced expression of a *Hox* gene in an ectopic segment causes a shift in the identity of that segment to an anterior or posterior fate. This respecification of fate is referred to as *homeotic transformation*.

On the basis of the key role that *Hox* genes play in establishing the anteroposterior pattern of the ectoderm and mesoderm, it would be logical to assume that these genes function similarly in the endoderm. Indeed, there are rare cases of homeotic transformations resulting from the misexpression of homeobox-containing proteins; for example, *Cdx2* is expressed in the early preimplantation embryo and its expression is maintained in the endoderm throughout development [14]. Although *Cdx2*-deficient embryos die before implantation, animals heterozygous for *Cdx2* develop colonic lesions that exhibit an anterior histology [15,16]. Conversely, misexpression of *Cdx2* in the stomach causes intestinal metaplasia [17,18], a more posterior phenotype. Thus, *Cdx2* seems to pattern the endoderm by directing cells to adopt a more posterior fate.

With the exception of *Cdx2*, the rarity of homeotic transformations in the endoderm suggests that homeobox-containing genes are only indirectly responsible for regulating the anteroposterior pattern of the endoderm. Although many *Hox* mutations result in intestinal malformations [19–22], these phenotypes are not specific to the endoderm. Nevertheless, several *Hox* genes are expressed in the endoderm, and the boundaries of *Hox* expression in the endoderm correlate with organ boundaries [23–25].

After gastrulation, the developing gut tube is surrounded by mesoderm from the so-called lateral plate. It has long been appreciated that patterning is normally influenced by interactions between mesoderm derivatives (mesenchyme) and endoderm derivatives (epithelia). Epithelial–mesenchymal interactions can be demonstrated by transplantation experiments in which pieces of endoderm and mesoderm from

Table 23.1 Transcription factors in gastrointestinal development

Gene class	Region	Function
HMG-box genes		
SOX17	Endoderm	Formation of definitive endoderm
SOX10	Enteric nervous system	Development of neural crest derivatives
SOX9	Intestine	Formation of pyloric sphincter
GATA genes		
GATA4	Endoderm	Anterior intestinal portal and foregut development
GATA6	Liver	Liver bud outgrowth, regulates HNF4
FOXA genes		
FOXA1 (*HNF3A*)	Endoderm, liver	FOXA1/A2 cooperate to specify the liver
FOXA2 (*HNF3B*)	Endoderm, liver	FOXA2 required for foregut and midgut development
FOXA3 (*HNF3G*)	Liver	Liver gene transcription
Onecut factors		
HNF6 (*OC1*)	Liver, pancreas	Bile duct, pancreatic duct, and islet development
OC2	Liver	Bile duct development
bHLH genes		
HES1	Liver, pancreas, intestine	Notch signaling; numerous roles in differentiation
NGN3, *NEUROD*	Pancreas, intestine	Pancreatic, gut endocrine cell specification
PTF1/p48	Pancreas	Early development of pancreas; exocrine transcription
MATH1	Intestine	Secretory vs enterocyte cell fate specification
Homeobox genes		
HEX	Liver	Growth of early liver bud
PROX1	Liver, pancreas	Growth of early liver bud, endocrine differentiation
HNF1B	Liver	Cholangiocyte formation
PDX1	Pancreas	Growth of pancreatic progenitor cells
HLXB9	Pancreas	Budding of dorsal pancreas, β-cell development
ISL1	Pancreas	Budding of dorsal pancreas, islet development
NKX2.2	Pancreas	β-cell development
NKX6.1	Pancreas	β-cell development
PAX4	Pancreas	β-cell development
PAX6	Pancreas	Islet development (α cells > β cells)
ARX	Pancreas	α-cell development
HLX	Intestine, liver	Early growth of liver and intestine
NKX2.5	Intestine	Formation of pyloric sphincter
CDX2	Intestine	Anterior–posterior patterning of intestine
HOXA/*HOXD* clusters	Intestine	Anterior–posterior patterning
Other		
HNF4	Liver	Terminal differentiation of hepatocyte
SMAD2	Endoderm	Endoderm development

HMG, high mobility group; bHLH, basic helix-loop-helix

different regions are recombined [26–29]. When tissues from postgastrulation embryos are recombined in this way, the fate of the endoderm is largely dependent on the type of mesoderm with which it is cultured; thus, anterior endoderm becomes "posteriorized" when recombined with posterior mesectoderm, and posterior endoderm becomes "anteriorized" when recombined with anterior mesoderm/ectoderm [30]. The mesoderm may be capable of providing the endoderm with a pattern because the mesoderm itself has already been patterned by *Hox* gene activity. Such a mechanism

regulates endoderm development in *Drosophila*, in which the nonoverlapping mesodermal expression of four homeobox genes (*Sex combs reduced*, *Antennapedia*, *Ultrabithorax*, and *abdominal-A*) guides the pattern of the adjacent endoderm [12].

Finally, other molecules may participate in endoderm patterning. Among these is the vitamin A-derivative, retinoic acid. Embryos exposed to excess doses of retinoic acid exhibit congenital malformations resulting from the transformation of anterior embryonic structures to more posterior fates, a "posteriorization" phenotype that also involves the endoderm [31,32]. The mechanism by which retinoic acid influences patterning in such a global fashion remains unclear.

Fate and potential

Although it is clear that after gastrulation, the fate of epithelial cells is strongly influenced by adjacent mesenchyme, cells in the epiblast contain information about their future identity and position even before gastrulation has occurred. This has been shown through the construction of *fate maps*, in which individual cells are marked at one time-point and their progress is traced during development. Fate maps of the epiblast illustrate a stereotyped pattern of development, in which the endoderm is largely derived from cells that surround the anterior primitive streak before gastrulation [33,34]. However, assignment of cell *fate* may not be irreversible, and cells may remain capable of adopting identities other than their assigned fates. This capacity to change fate in response to environmental cues is referred to as *potential.* Fate and potential represent important and distinct properties of a cell during development. Plasticity of this type, which is common throughout development, probably provides the embryo with a way of correcting errors that may occur during embryogenesis.

It is generally accepted that a loss in the potential (or *competence*) of cells to form different tissue types accompanies gastrulation. The ability of cells within the very early embryo to become any cell type (*totipotency*) is therefore reduced within each germ layer to a more limited set of possibilities after gastrulation. This progressive *commitment* means that the parenchymal cells of the gastrointestinal organs are derived exclusively from endoderm, and at later stages, different organs are derived only from specific portions of the endoderm. This classical notion of progressive commitment has been challenged by studies in which cells appear to be capable of traversing germ layer boundaries. As will be discussed in the section on transdifferentiation, such cellular behavior certainly occurs but whether it contributes significantly to normal tissue homeostasis is not clear.

How is the balance between competence and commitment achieved during development? Attention has focused on the importance of *chromatin* in the regulation of tissue competence. Chromatin defines the structural state of DNA–protein complexes that determine the "availability" of DNA for transcription factor binding. A model in which competence and commitment are achieved through sequential changes in chromatin has been suggested by studies of the regulatory region of the liver-specific albumin gene [35]. Such studies have used in vivo footprint analysis to characterize the binding of GATA and FOXA, endodermally expressed transcription factors previously discussed, to the albumin enhancer in several different cell types. In neural tube cells, which lack GATA and FOXA, the albumin enhancer is empty. In dorsal endoderm, the albumin enhancer is bound by GATA and FOXA (even although albumin is not transcribed in these cells), whereas in cells of the budding liver, the albumin promoter is bound by these and other factors and is transcriptionally active. Consistent with this model, FOXA factors are capable of modifying chromatin [36], and hepatic competence is specifically blocked in *Foxa1*/*Foxa2* mutant murine embryos [37].

Signaling in development

The assignment of cell fate in the endoderm is achieved through cell–cell signaling between neighboring cells or between adjacent cell layers. Such signals can be divided into two classes: *permissive* signals, which allow a tissue to progress to a fate that has already been assigned, and *instructive* or *inductive* signals, which divert a tissue to a new fate that would not otherwise have been followed. Instructive signals play an important role in regulating patterning by committing multipotent cells to specific lineages.

Developmental signals have traditionally been identified through transplantation studies, in which different embryonic structures (e.g., epithelium and mesenchyme) are cocultured. The resulting fate (or absence thereof) indicates whether signals are present or absent, and if present, whether the signals are permissive or instructive. Several features of development complicate the study of the specific ligands that mediate this intercellular communication. As development is a highly dynamic process, cells and cell layers are in constant motion relative to each other. Cell or tissue interactions may exist only transiently – long enough for a signal to be received, but not long enough to be easily characterized experimentally. Furthermore, signaling often occurs in a *reciprocal* manner. For example, the epithelium may respond to signal "A" from the mesenchyme by supplying signal "B", which in turn prompts the mesenchyme to secrete signal "C", and so forth. The number of secreted factors encoded in the genome is vast, leading to the potential for a level of signaling complexity that may preclude straightforward analysis.

While additional layers of complexity will undoubtedly be discovered, it appears that a limited repertoire of signals controls development. At least four signaling *modules*, each consisting of a family of ligands, receptors, and signal-modifying factors, are used iteratively during development: the fibroblast growth factor (FGF), hedgehog (Hh), bone morphogenetic

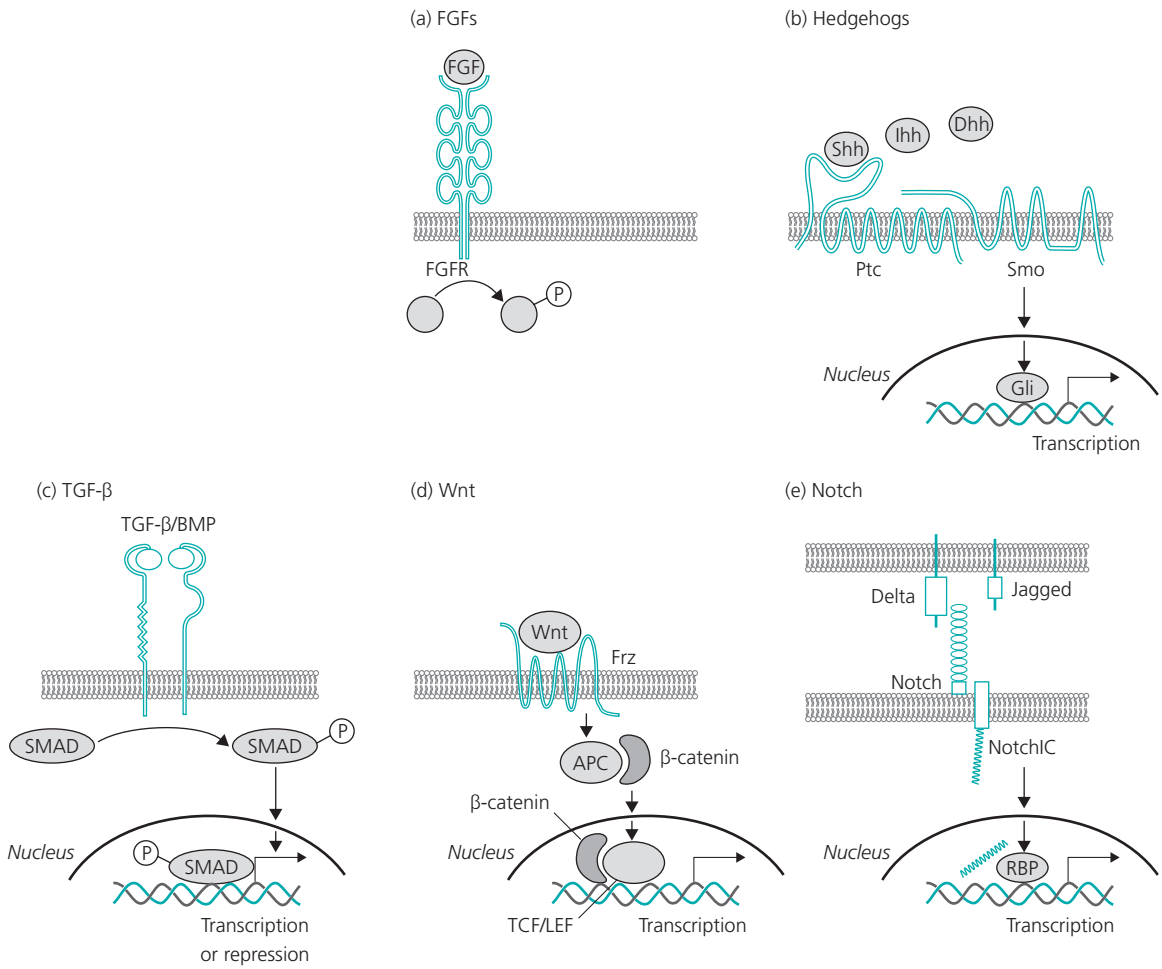


Figure 23.3 Ligand–receptor pairs in gastrointestinal development. **(a)** Fibroblast growth factor (FGF) signaling. Binding of an FGF ligand to one of four FGF receptors (FGFRs) leads to receptor dimerization and activation of FGFR tyrosine kinase activity. Phosphorylation of target proteins leads to the activation of multiple pathways, including Ras, phosphatidylinositol 3-kinase, phospholipase C, and STAT pathways. **(b)** Hedgehog signaling. All three hedgehog ligands – Sonic hedgehog (Shh), Indian hedgehog (Ihh), and Desert hedgehog (Dhh) – are capable of binding to the Patched receptor (Ptc). Ligand binding causes the membrane-bound signaling mediator Smoothened (Smo) to activate downstream transcription factors in the Gli family. These factors migrate to the nucleus and activate transcription. **(c)** Transforming growth factor (TGF)-β/bone morphogenetic protein (BMP) signaling. TGF-β family members bind to a heterodimeric membrane receptor complex consisting of a type I receptor and a type II receptor. The activated receptor complex phosphorylates SMAD transcription factors, which migrate to the nucleus where they mediate or repress transcriptional activation. **(d)** Wnt signaling. Binding of a soluble Wnt ligand to one of the seven transmembrane Frizzled (Frz) receptors results in the activation of the canonical Wnt pathway, in which adenomatous polyposis coli (APC) dissociates from β-catenin, allowing the latter to migrate to the nucleus where it becomes part of a transcriptionally active complex that includes T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors. **(e)** Notch signaling. Cell–cell signaling is mediated by an interaction between one of the membrane-associated Notch ligands, Delta or Jagged, and one of the four Notch receptors on an adjacent cell. Ligand binding causes the intracellular portion of the Notch receptor (NotchIC) to migrate to the nucleus where it activates the retinol-binding protein (RBP) transcription factor.

protein (BMP), and BMP-related tumor growth factor (TGF) families (Fig. 23.3, Table 23.2). In addition, two other classes of signaling modules, Wnt and Notch, act predominantly in regulating differentiation within established organs. Cross-talk between signaling modules active in specific tissue layers (in particular epithelial HH and mesenchymal FGF and BMP) exemplifies the reciprocal nature of epithelial–mesenchymal signaling.

Fibroblast growth factors

The FGFs comprise a large family of ligands that are capable of variably binding to four FGF receptors. As both ligands and receptors are subject to a significant degree of regulation of splicing, the combinatorial ligand–receptor repertoire is vast and subject to complex variability in binding specificity and tissue-specific expression. FGF receptor signaling is largely mediated by the tyrosine kinase activity of the receptor, acting

Table 23.2 Soluble signals in gastrointestinal development

Tissue	Signal	Source	Function
Liver	FGF	Cardiac mesoderm	FGF 1, 2, and/or 8 specify prehepatic endoderm, promote liver bud outgrowth
	BMP	Septum transversum mesenchyme	BMP4 (and other BMPs?) cooperates with FGFs in specification, outgrowth
	HGF	Septum transversum mesenchyme	Mediate hepatoblast growth and suppress apoptosis
	???	Blood vessels	Expansion of liver bud into septum transversum mesenchyme
	Jagged 1	Portal mesenchyme	Specification or survival of cholangiocyte precursors (ductal plate)
Pancreas	Shh	[Endoderm]	Shh repression signals pancreatic specification
	FGF/activin	Notochord	Candidate mediators of Shh repression
	FGF10	Pancreatic mesenchyme	Outgrowth of pancreatic bud, pancreatic epithelium
	Delta/Jagged	???	Notch-mediated inhibition of pancreatic progenitor cell differentiation
	TGF-β family	???	Regulation of endocrine vs exocrine fate decisions
Intestine	Shh	Epithelium	Epithelial–mesenchymal cross-talk (Shh–BMP) regulates intestinal pattern
	BMP	Mesenchyme	Shh mediates radial pattern of gut. BMP regulates intestinal stem cell niche
	GDNF	Mesenchyme	Migration and/or survival of enteric neurons
	Endothelins	Mesenchyme	Migration and/or survival of enteric neurons
	Frizzled	???	Ligands for Wnt regulation of intestinal stem/progenitor cells
	Delta/Jagged	???	Ligands for Notch regulation of intestinal stem/progenitor cells
General	TGF-β family/Nodal	???	Regulation of endoderm specification

through Ras and phospholipase C pathways [38]. FGFs are expressed in the primitive streak, mesodermal structures of the postgastrulation embryo, and in developing organs, and they have important roles in endoderm patterning (possibly through a concentration gradient) and in organogenesis of the liver, pancreas, and intestine (see the section on organogenesis) [30,39,40].

Hedgehogs

The hedgehog genes were first identified through studies of *Drosophila*, and their names, like those of other developmental genes (e.g., *Notch*), arise from the characterization of mutants in that experimental model. There are three mammalian hedgehogs – Indian, Sonic, and Desert – all of which bind to the membrane receptor Patched (Ptc). In the absence of ligand, Ptc acts as a repressor of the signaling mediator Smoothened (Smo); on ligand binding to Ptc, Smo is derepressed and activates Gli transcription factors. Importantly, cells are able to distinguish different concentrations of hedgehog ligand. In traveling long distances, hedgehogs are able to create patterns through a gradient effect, in which cell fate depends on whether a high, intermediate, or low concentration of ligand is sensed. Sonic hedgehog (Shh) is particularly important in gastrointestinal development. Shh is expressed in the endoderm at the time of formation of the gut tube (in the anterior and caudal intestinal portals) and participates in the specification of the pancreas and regionalization/morphogenesis of the gut.

Bone morphogenetic proteins and the TGF-β superfamily

BMPs are members of the TGF-β superfamily of secreted proteins, a family that also includes the activins. Receptors for TGF-β family members are serine–threonine kinases that modulate the activity of TGF-β–responsive transcription factors (termed SMADs) through phosphorylation. The relevance of BMPs to gut development was also first suggested by studies in *Drosophila*, which showed that the BMP orthologue *decapentaplegic* responds to hedgehog signaling and is necessary for midgut development. Such reciprocal signaling between TGF-β and hedgehog family members is conserved in mammals, and represents one example of epithelial–mesenchymal signaling. Specifically, Shh is expressed in the epithelium of the developing gut, and induces expression of particular BMPs in the adjacent mesenchyme.

Wnts

Wnt ligands play a critical role in the formation of differentiated cell types in the embryo, a process called *cell fate determination*. Wnts are a family of secreted factors (there are at least 19 known mammalian Wnts) that bind "frizzled" receptors on the membrane. A complex series of events follow receptor binding. In the best characterized, or canonical, pathway, Wnt signaling leads to the release of β-catenin from the adenomatous polyposis coli (APC) protein, and the former then moves to the nucleus where it activates T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors.

Box 23.2 Cancer and its relationship to development: cancer stem cell hypothesis

The observation that developmental signaling pathways are often activated in adult tumors has forged a bridge between the fields of developmental biology and cancer biology. The notion that cancer recapitulates development dates to the 19th century (reviewed by Sell [244]) and is embodied in the hypothesis that tumors arise from stem cells in adult tissues that retain an embryonic phenotype. Strong evidence for such a model exists in hemopoiesis but it remains to be determined whether stem cells represent a target for malignant transformation in solid organs.

Further evidence for a link between development and cancer comes from the "reemergence" of signals normally prominent in development during the course of tumor initiation and progression. Wnt signaling, normally important during embryogenesis, is activated in many hepatoblastomas and pancreatoblastomas in children. Mutations in the type 1A BMP receptor (BMPR1A) or the downstream signaling element SMAD4 are common in juvenile polyposis syndrome.

Links between developmental signals and tumorigenesis are not limited to cancers that occur in children or inherited cancers. Like their inherited counterparts (see Box 23.4), most sporadic colorectal cancers exhibit activated Wnt signaling. Many adult pancreatic adenocarcinomas exhibit a reactivation of PDX1, Sonic hedgehog, and Notch signaling, which are either completely absent or only present in a subset of cells in the adult pancreas. Furthermore, several gastrointestinal malignancies (esophageal and gastric, in particular) are preceded by metaplasia. This replacement of one tissue type with another may reflect the emergence of more primitive cells with a greater capacity for growth.

Similarly, the concept of "cancer stem cells" – special cells within a tumor that provide the tumor with an inexhaustible supply of new cancer cells – is based on this apparent link between development and cancer. The *cancer stem cell hypothesis* posits that most cells within a tumor have a limited capacity for division and are themselves generated from cells with an unlimited capacity for division. In several tumors (breast and brain, in particular), a small subset of tumor cells have been identified and shown to be uniquely capable of reconstituting the tumor [245,246].

This cancer stem cell hypothesis has enormous implications for cancer therapy. Most cancer therapies are assessed by their effect on tumor mass, the easiest assay for antitumor activity. However, if the cancer stem cell hypothesis is true, these agents would primarily target a cell population with a limited self-renewal capacity – analogous to a "transient amplifying population" – and may inefficiently kill the cancer stem cells. Stem cells that normally reside in adult tissues seem to be more resistant to chemotherapy than other cells [247], giving additional plausibility to this model. Therapies that target stem cells, if specific therapies can be found, may provide more durable cures and simultaneously generate less toxicity.

Despite the involvement of Wnt signaling in multiple developmental systems, including the intestine, developmental disorders with prominent gastrointestinal tract manifestations have not yet been associated with perturbed Wnt signaling. Rather, alterations in Wnt signaling are predominantly associated with carcinogenesis, particularly in the colon (colon adenocarcinoma), liver (hepatoblastoma), and pancreas (pancreatoblastoma) (see Box 23.2).

Notch

Like Wnt, Notch signals regulate the differentiation of cells within established tissues (Fig. 23.4). Of note, a role for Notch in the formation of endoderm itself has also been postulated [41,42]. There are four mammalian Notch receptors, which are activated by two classes of ligands, Delta and Serrate/Jagged. In contrast to ligands from the other important signaling modules, including Wnts, FGFs, BMPs, and hedgehogs, Delta and Serrate/Jagged are transmembrane ligands and so Notch mediates signals that cells receive only from their nearest neighbors. Ligand engagement leads to the detachment of the intracellular portion of the Notch receptor from the membrane, where it travels to the nucleus and alters the transcriptional program of the cell. Like Wnt signals, Notch signals are subject to complex regulatory inputs at all stages of the signal transduction pathway, from ligand binding to cytoplasmic and nuclear activation of downstream mediators.

The role of these signaling modules in adult homeostasis remains to be fully defined. However, it is known that some signals are necessary for function throughout life. For example, Notch and Wnt signals maintain the proper balance of cell types in both the embryonic and the adult intestine. It is not clear how developmental specificity is achieved when signals from a single family are used repeatedly. It is likely that signals are interpreted in the context of cellular identity, thereby causing the same signal to have different effects on different tissues (i.e., pancreas vs liver vs intestine).

Transdifferentiation

Several publications have challenged the notion that commitment imposes a nearly absolute boundary between different lineages. Several investigators have reported that certain somatic cells, particularly the cells derived from bone marrow, have the capacity to give rise to many different tissues in vitro and in vivo, including the cells of skin, lung, kidney, muscle, and all of the gastrointestinal organs [43]. A significant fraction of this apparent plasticity may actually reflect the effect of cell fusion between the bone marrow-derived cells and other differentiated cells, giving rise to tetraploid cells with the characteristics of hepatocytes, cardiomyocytes, and neurons [44–46]. Although it is possible that bone marrow-derived cells can transdifferentiate, albeit with low efficiency, into other somatic cells, the physiological significance of such a rare event is unclear [47], and the paradigms of lineage commitment established by developmental biologists during

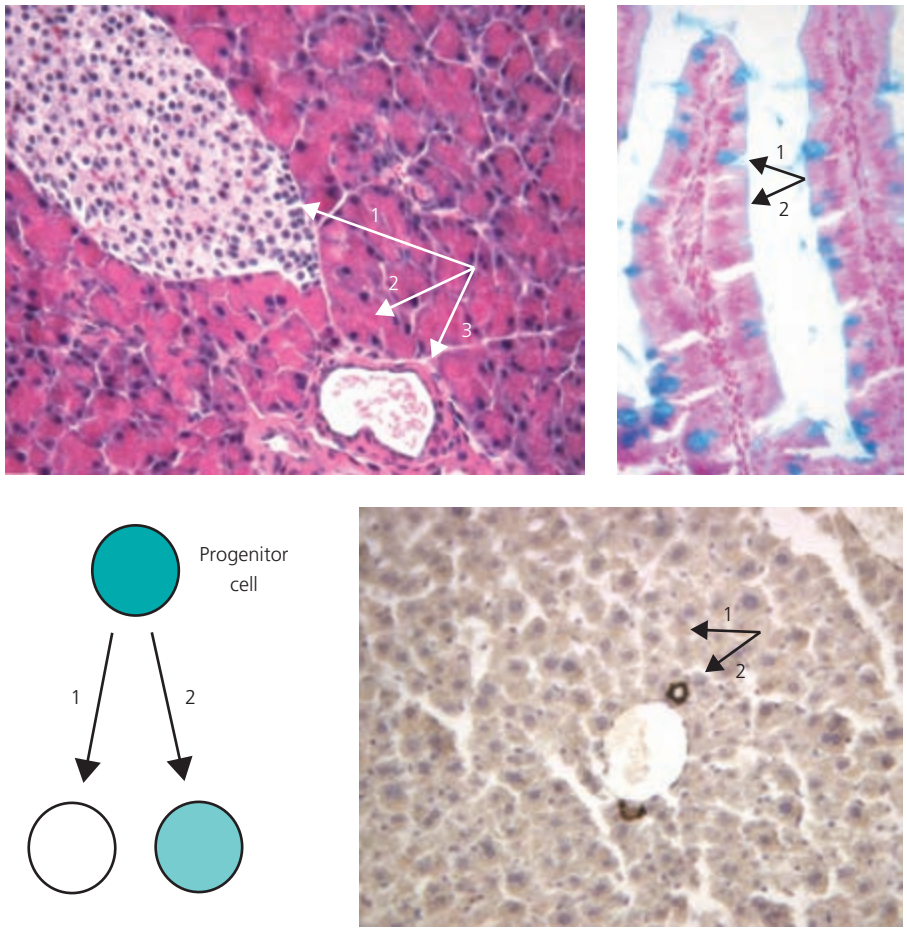


Figure 23.4 Control of gastrointestinal development by Notch. (*Lower left panel*) The fate of a multipotent progenitor cell (dark green) is influenced by whether it receives a signal from a Notch ligand (Delta or Jagged). In this example, active Notch signaling causes the cell to adopt fate 1 (white), whereas absence of signaling results in fate 2 (light green). Evidence supports distinct roles for Notch in various gastrointestinal tissues. In the pancreas (*upper left panel*), a Notch signal prevents the differentiation of the progenitor cell into any of the mature pancreatic cell types – islet (1), acinar (2), or ductal (3). In intestinal progenitor cells (*upper right panel*), activation of Notch signaling promotes the formation of enterocytes (2). The formation of secretory cells (1), such as the goblet cells visualized here with Alcian blue staining, is inhibited by Notch signaling. In the liver (*lower right panel*), Notch signaling is required for the proper formation of bile ducts (2), visualized by staining for cytokeratin 19. It is not known whether Notch acts in the liver by repressing the hepatocyte fate or through some other mechanism.

the 20th century remain largely intact. Nevertheless, the capacity of adult somatic cells to contribute to multiple lineages, whether by fusion or through developmental plasticity, points to the potential feasibility of cell therapy.

Conclusions

Early development of the gastrointestinal tract is characterized by gastrulation and endoderm formation, followed by midline migration of anterior and posterior invaginations (i.e., the anterior and caudal intestinal portals), resulting in a gut tube. The endoderm is patterned into organ domains along its anteroposterior axis through the activity of homeobox-containing transcription factors and epithelial–mesenchymal signaling. Cell fate remains plastic during the initial stages of development, with tissue identity depending on reciprocal signals that are refined until the commitment to a particular fate is made. Unanswered questions still remain concerning gastrointestinal development.

Organogenesis

After gastrulation, the endoderm undergoes more easily recognizable changes of organogenesis. Studies delineating the development of the gastrointestinal tract were performed over the 20th century and the timing of most key biochemical, physiological, and morphological events during human development was established at least 30 years ago [48]. Accordingly, this section will focus primarily on the mechanisms that underlie these remarkably complex and integrated events.

The same signaling pathways described in the previous section on early development are used in the specification of organ domains and the growth and differentiation of tissues. Sometimes, a single signal is involved in the development of two different organs, or one signal may mediate two different effects in the same tissue. In these instances, it is cellular *context*, the identity of the cell on which a given signal acts, that determines the signaling outcome.

Organogenesis can be divided into several overlapping phases:

- *specification* – a direct consequence of the patterning processes previously described, results in the commitment of cells to restricted tissue fates
- *budding* – of liver and pancreas
- *morphogenesis* – the formation of a three-dimensional structure that facilitates the physiological function of the tissue (e.g., hepatic sinusoids and intestinal villi

• *cell fate determination* – the restriction of specific lineages within the tissue (e.g., hepatocytes and cholangiocytes).

Importantly, these components of organogenesis do not occur sequentially or independently, but rather, they occur in parallel, in a coordinated fashion. Finally, differentiation programs are implemented within those lineages, allowing the expression of physiological function (discussed further in the section on developmental physiology).

Liver

Specification

The liver provides a good example of how a prepatterned mesenchyme can influence epithelial fate. The developing cardiac mesoderm, which gives rise to the heart, lies adjacent to the anterior endoderm fated to give rise to the liver (see Fig. 23.2b). Experiments performed decades ago showed that cardiac mesoderm plays a critical role in the formation of the liver. These studies consisted of transplantation assays in which pieces of endoderm and mesenchyme were independently assembled. Such experiments demonstrated that an interaction between endoderm and cardiac mesoderm, during a critical time window (four to six somites), is necessary for the endoderm to activate a liver program [26]. As the cardiac mesoderm moves anteriorly, the space adjacent to the prehepatic endoderm is replaced by the septum transversum, a mesoderm derivative that later gives rise to part of the diaphragm. Other signals mediate the outgrowth of the expanding liver bud into the septum transversum mesenchyme (see also Chapter 79)

Tissue transplantation studies using molecular markers have confirmed an important role for embryonic mesenchyme in liver development. For example, ventral endoderm expresses albumin (a marker of liver specification) when it is cocultured with cardiac mesoderm. However, other studies suggest that a more complex regulatory circuit underlies the process. For example, *dorsal* endoderm expresses albumin when it is simply removed from its adjacent endoderm. This surprising result implies that the normal function of cardiac mesenchyme is permissive rather than inductive, in that it may allow the expression of a "default" liver program [49]. Such a default mechanism may also apply to the ventral pancreas, which forms from a lip of anterior endoderm that constitutes the "leading edge" of the anterior intestinal portal. This piece of endoderm exhibits "bipotential" pancreatic/hepatic properties; i.e., it expresses pancreatic genes if cultured on its own, but represses the pancreatic program and expresses albumin if cocultured with signals from the cardiac mesoderm [39]. Although it is enticing to interpret these experiments as an indication that intrinsic endoderm fates are reprogrammed by specific mesenchymal elements, further confirmation of this model is required. In particular, the assay for reprogramming in these studies (e.g., detection of albumin transcription) may not be a reliable marker of liver specification, and it remains unclear what mechanism would initially program the default fates.

What are the specific signals that mediate these inductive effects? It is likely that a combination of FGF and BMP signals are involved in hepatic specification. Several FGFs are made by the cardiac mesoderm. FGFs are both sufficient and necessary for isolated anterior endoderm to activate albumin expression [50], and BMPs expressed by the septum transversum mesenchyme appear to act in concert with these FGFs [51]. The transcription factors Foxa1 and Foxa2 are critical mediators of these signals within the adjacent endoderm, as liver specification fails to occur in mice with a targeted inactivation of both of these genes [37].

Budding

After hepatic specification by the cardiac mesoderm, the formation of a bud that will become the liver begins. The first morphological evidence of budding is a thickening of the adjacent endoderm into a "hepatic diverticulum", which is followed by the outgrowth of liver cells into the septum transversum mesenchyme. FGFs are also necessary for this outgrowth, although their role in budding appears to be permissive and their actions alone are not sufficient for liver bud outgrowth [50]. BMPs (specifically BMP4) are independently required for liver budding into the septum transversum, as demonstrated with the use of *Bmp4* mutant mice and the BMP antagonist noggin [51]. Endothelial cells within the septum transversum mesenchyme may be the specific source for growth-promoting signals, as *Flk1* mutant embryos (which are incapable of forming mature endothelial cells or blood vessels) exhibit liver specification but fail to undergo liver budding [52].

Many genes are required after endoderm specification for outgrowth into the septum transversum. These include three homeobox-containing transcription factors, Hex, Prox1, and Hlx, and zinc-finger transcription factor GATA6. *Hex* is expressed during gastrulation in the first endoderm cells to pass through the primitive streak that ultimately give rise to the liver (these are the cells that constitute the anterior-most cells of the endoderm before it folds back on itself during formation of the anterior intestinal portal). Mice lacking *Hex* form a small hepatic diverticulum, but subsequent outgrowth and budding fails to occur [53,54]. *Hlx* and *Prox1* mutant mice also exhibit growth arrest at the bud stage, although the livers of *Prox1* mutant mice ultimately reach nearly a third of the size of a normal liver [55,56]. *Hex* and *Prox1* are expressed in the hepatic epithelium, whereas *Hlx* is normally expressed in the septum transversum mesenchyme.

As previously described, the GATA4 zinc finger transcription factor binds to the albumin promoter before albumin expression, suggesting a role in liver specification [35]. Another GATA family member, GATA6, also plays an important role in liver development. GATA6 regulates HNF4, an important transcriptional regulator of hepatocyte genes (described in

the section on morphogenesis and cytodifferentiation), and liver bud outgrowth is retarded in mouse embryos lacking *GATA6* [57]. Further studies are needed to determine whether a regulatory relationship exists between *Hex*, *Hlx*, *Prox1*, and *GATA6*, given the similar phenotypes that mutations of these genes exhibit. Further studies are also required to determine the signaling hierarchy between soluble FGFs and BMPs and the activity of these transcription factors; for example, *Hex* expression can be induced by BMP signaling [58].

Morphogenesis and cytodifferentiation

After this migration into the septum transversum, epithelial cells intercalate with mesenchymal cells, eventually leading to the formation of the hepatic sinusoids, which support embryonic hemopoiesis. These morphogenetic changes are accompanied by dramatic growth of the liver, which seems to require mesenchymal factors. The most important of these is hepatocyte growth factor (HGF), which signals through the c-met receptor. Mutation of either *Hgf* or c-*met* leads to marked liver cell apoptosis in some but not all analyses [59,60]. This signaling pathway also seems to modulate the response to injury in adult liver [61,62]. Mutations in several other genes, including components of the tumor necrosis factor (TNF)–nuclear factor-κB signaling pathway, lead to similar developmental apoptosis phenotypes [63–65]. Hepatocyte apoptosis in many adult liver diseases is mediated by a TNF-like "death receptor" pathway [66], suggesting that these cell death signaling mechanisms remain active throughout life.

The two major parenchymal cell types of the liver – hepatocytes and bile ducts – are believed to arise from multipotent embryonic "hepatoblasts." Intrahepatic bile ducts are derived from "ductal plates", precursor structures that form around branches of the portal vein. Inductive signals from the portal vein induce surrounding hepatoblasts to form the ductal plate, which can be recognized by the expression of distinctive cytokeratin (CK) molecules, such as CK19 (Fig. 23.5a). Mature intrahepatic bile ducts emerge after remodeling of the ductal plate, which involves selective apoptosis of duct precursors. Little is known about the development of the extrahepatic bile ducts and the gallbladder, which are thought to be directly descended from the gut tube.

Several signaling pathways are involved in biliary specification and morphogenesis. Among those first identified were liver-enriched "hepatocyte nuclear factors" (HNFs). HNFs belong to several different transcription factor families and contribute to the expression of liver-specific genes. Inactivation of either HNF6 (a member of the onecut transcription factor family) or HNF1B (a homeodomain factor) perturbs biliary development [67,68]. The reduced HNF1B expression in the livers of *Hnf6* mutant mice suggests that HNF6 likely acts through HNF1B [67]. Alternatively, HNF6 and the onecut transcription factor OC2 act through activin/TGF-β family member(s) to regulate biliary fate decisions. The liver normally exhibits a gradient of TGF-β signaling activity, with high activity near the ductal plates and low activity in the remaining parenchyma. In livers lacking both HNF6 and OC2, this gradient is disrupted, resulting in high levels of activin/TGF-β signaling throughout the liver and the appearance of cells exhibiting features of both hepatocytes and cholangiocytes. Thus, onecut transcription factors may shape a gradient of activin/TGF-β signaling to allow localized induction of the bile ducts.

In addition, there is convincing evidence that Notch signaling is important for biliary development. Mutations in the Notch ligand Jagged 1 (JAG1) result in Alagille disease, a clinical syndrome that includes a paucity of intrahepatic bile ducts [69,70]. The molecular pathogenesis of Alagille syndrome is discussed further in the section on disorders of remodeling.

A cellular differentiation program is executed after the assignment of biliary or hepatocyte fate. Evidence that this program is distinct from the assignment of hepatocyte cell fate comes from the targeted inactivation of HNF4. Remarkably, this transcription factor binds to nearly half of the actively expressed genes in the liver [71]. Among the genes whose expression "defines" the hepatocyte are albumin, apoplipoproteins A and B, and transferrin. Despite exhibiting normal morphogenesis, HNF4-deficient embryos exhibit reduced expression of all of these genes, demonstrating a role for this transcription factor in hepatocyte differentiation, but not liver specification and morphogenesis [72]. Although other transcription factors, including gene products whose importance in liver development has just been described (e.g., HNF1B, HNF6, FoxA), bind to liver-specific promoters, the function of these transcription factors is redundant, as their replacement by other factors preserves liver development [73].

Summary

FGF and BMP signals from the cardiac mesoderm and septum transversum mesenchyme induce a portion of the ventral foregut endoderm to become liver. Budding and parenchymal growth involves homeobox-containing transcription factors and mesenchymal factors, such as HGF, that mediate proliferation and suppress apoptosis. Poorly understood epithelial–mesenchymal interactions mediate the morphogenesis of hepatic sinusoids, which support hemopoiesis during fetal life. Biliary development involves the formation of periportal ductal plates and subsequent remodeling, a process that requires Notch and TGF-β signals and the activity of several onecut transcription factors. In hepatocytes, other transcription factors like HNF4A are required for the expression of the hepatocyte program.

Pancreas

With some important exceptions, development of the pancreas follows a pattern that is similar to that of the liver.

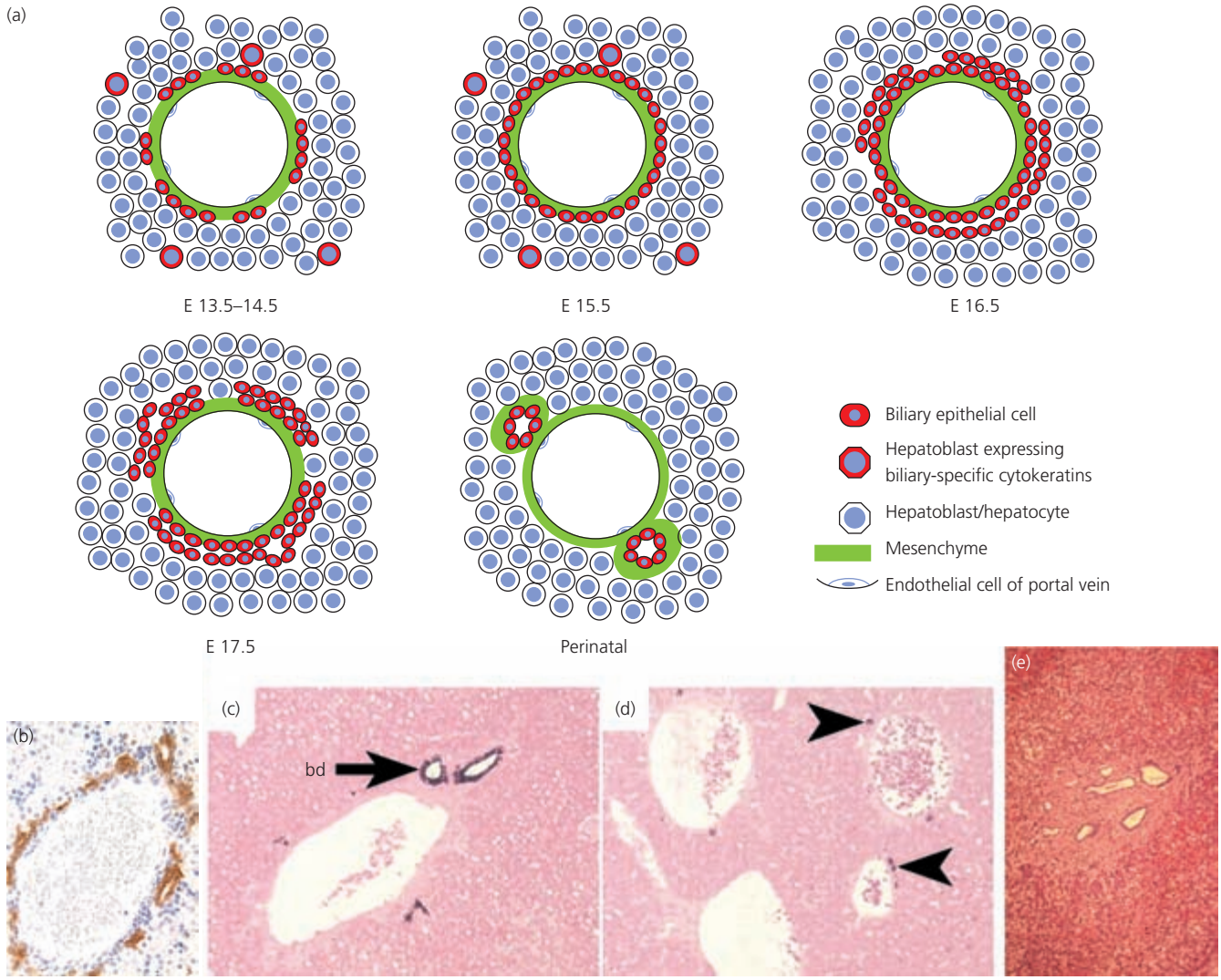


Figure 23.5 Biliary tract development. **(a,b)** Normal biliary development. **(a)** Branches of the portal vein induce embryonic hepatoblasts to form the ductal plate, a bilayered ring of biliary epithelial cells. Late in embryogenesis or early in postnatal life, cells within the ductal plate undergo apoptosis and remodeling to yield mature bile ducts. This process involves Notch and TGF-β signaling. Adapted from Lemaigre [241], with permission from Elsevier. **(b)** The ductal plate expresses cytokeratin 19, a marker of mature bile ducts. **(c,d)** Disorders of biliary development. **(c)** Normal mouse portal tract with bile duct (bd, visualized by DBA lectin staining), hepatic artery, and portal vein branches. **(d)** Portal tract from a mouse lacking one copy of both *Jagged1* and *Notch2*, a model of human Alagille syndrome. Reproduced from McCright et al. [226], with permission from the Company of Biologists, Ltd. **(e)** Periportal expansion of bile ducts in a patient with the ductal plate abnormality characterisitic of congenital hepatic fibrosis.

Specifically, signals from adjacent mesoderm specify the pancreatic endoderm, FGFs mediate pancreatic growth, and a variety of signaling components (including Notch and homeobox-containing transcription factors) regulate the differentiation of the parenchymal cell types – exocrine, endocrine, and duct cells.

Specification

Unlike the liver, the pancreas forms from two distinct pieces of foregut endoderm – a dorsal pancreatic domain and a ventral pancreatic domain (see Fig. 23.2c) – that later fuse into a single integrated gland. Patches of endoderm on opposite sides of the gut tube must therefore somehow be instructed to become pancreas. Transplantation studies similar to those previously described have shown that the dorsal pancreatic region of the endoderm is specified before the 13-somite stage, a period when this endoderm is in contact with the notochord [74]. At a slightly later stage, the prepancreatic endoderm is in contact with the aorta (dorsally) and the vitelline veins (ventrally). Thus, the notochord or blood vessels could be mesenchymal sources for inductive pancreatic signals, akin to the cardiac mesoderm for the liver.

Indeed, evidence suggests that both the notochord and the blood vessels are important for pancreatic specification and growth. Isolated dorsal endoderm fails to express pancreatic markers when cultured on its own, but a pancreatic program

is induced on reassociation with the notochord [75]. Similarly, coculture of dorsal endoderm with aortic cells (or other endothelial cells) induces a pancreatic program, whereas removal of aortic precursor cells from the embryo causes a failure in pancreatic development [76].

The most important consequence of mesenchymal signaling appears to be the repression of Shh expression. Shh is expressed throughout the entire gut tube, with the notable exception of the prepancreatic regions (both dorsal and ventral), where it is excluded. The notochord is directly responsible for repressing Shh in the dorsal prepancreatic endoderm, possibly through the activity of FGF2 or activin β-B [77]. Repression of Shh alone is able to reproduce the pancreatic inductive activity of notochord [77], and blocking Shh signaling with the inhibitor cyclopamine causes ectopic pancreas formation [78]. Furthermore, Shh repression must be maintained throughout pancreatic development, as ectopic expression of Shh after pancreatic budding causes conversion of the mesenchyme to an "intestinal type" and inhibits further development [79]. Shh repression is therefore both necessary and sufficient for pancreas specification. It is unclear what structure serves the function analogous to that of the notochord to repress Shh expression in the ventral prepancreatic endoderm.

Budding

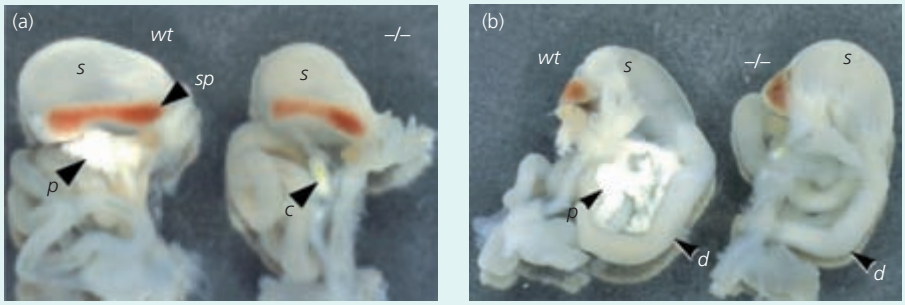
The pancreatic buds form at about 3–4 weeks of embryonic development (E9.5 in the mouse), with formation of the ventral bud lagging behind that of the dorsal bud. One of the earliest and most important genes to be expressed in the nascent buds is the homeobox transcription factor PDX1. All mature pancreatic cell types are derived from cells that expressed PDX1 [80] and ectopic PDX1 expression in the intestine is sufficient to promote the early steps of pancreas formation [81]. Although pancreatic buds form in *Pdx1*-deficient embryos, further pancreas development is arrested at this stage [82,83], a phenotype that has also been observed in humans (see Box 23.3) [84]. In adults, PDX1 is a major transcription factor for insulin, and deletion of *Pdx1* in adult mice causes diabetes [85]. Several other transcription factors are expressed in the early progenitor cells of the pancreas, including p48/PTF, Hes1, and Nkx6.1. In contrast to *Pdx1*, the genes for these transcription factors are not required for the formation of progenitor cells but instead are associated with specific phenotypes in later pancreatic lineages (see section on morphogenesis and cytodifferentiation).

As noted, the means of specification differ for the dorsal and ventral pancreata (notochord for dorsal, unknown for ventral). This differential regulation recurs later, as several genes exhibit distinct activities in dorsal vs ventral pancreatic development. One of these is *Hlxb9*, which encodes a homeobox transcription factor that is required for dorsal, but not ventral, pancreatic budding in mice [86,87]. Similarly, mouse embryos lacking the LIM homeobox gene *Isl1* do not develop a dorsal pancreas, most likely because Isl1 function is required in the dorsal mesenchyme [88]. Mesenchymal *Isl1* expression is maintained in *Hlxb9* mutants, suggesting that *Isl1* is not downstream of *Hlxb9* [86,87]. As there are no profound functional or histological differences between the postnatal derivatives of the ventral (head and uncinate process) and dorsal (body and tail) pancreas, it is unclear why *Isl1* and *Hlxb9* mutations cause such selective phenotypes.

Once formed, the ventral pancreas rotates across the midline to meet the dorsal pancreas. The two pancreatic derivatives undergo complete functional and anatomic integration,

Box 23.3 Pancreatic agenesis

PDX1 homeodomain-containing transcription factor (also known as IDX1, STF1, and IPF1) is absolutely required for development of the pancreas [248], and mice lacking the gene develop a small pancreatic bud, but further pancreatic development is arrested [82]. Pancreatic agenesis is a rare condition in humans [249]. In one patient with pancreatic agenesis [250], sequencing of the *PDX1* gene (*IPF1*) revealed a homozygous frameshift mutation that resulted in a truncated protein [251]. Thus, PDX1 seems to function similarly in mice and humans. Heterozygous mutations in PDX1 caused maturity-onset diabetes of youth (MODY) in a subset of patients, reflecting the protein's later role as the major transcriptional regulator of insulin gene expression [252].



(a,b) Modeling pancreatic agenesis in the mouse. Images of dissected mouse stomach (*s*), spleen (*sp*), duodenum (*d*) and pancreas (*p*) from wild-type (*wt*) and mutant mice lacking the Pdx1/IPF1 gene (*–/–*). In the absence of Pdx1/IPF1, the pancreas does not develop and is instead replaced with a cystic structure (*c*). Image from Offield et al. [82].

and the ventral ductal system (duct of Wirsung) serves as the major conduit for pancreatic secretion through the major papilla. Failure of integration results in the common anatomic variant *pancreatic divisum*, which is marked by persistence of the dorsal duct of Santorini and drainage through the minor papilla.

Morphogenesis and cytodifferentiation

These mutant phenotypes demonstrate that early pancreas organogenesis occurs in two steps: an early phase of pancreatic budding (which requires ISL1), and a later phase of outgrowth and branching (which requires PDX1). Wessells and Cohen [74] suggested a two-step process after observing that the substitution of heterologous mesenchyme for pancreatic mesenchyme supported later stages of development but not early budding. For many years, investigators looked for "mesenchymal factors" that control pancreatic growth, branching, and differentiation [89].

FGF10 was discovered to be such a pancreatic mesenchymal factor. In the lung, FGF10 expression causes budding and branching of the pulmonary epithelium [90]. This growth is "stereotyped" – primary, secondary, and tertiary branch formation is spatially and temporally regulated to ensure a consistent branching pattern. Although pancreatic branching does not appear to be stereotyped, FGF10 has a strikingly similar function in the development of the pancreas. FGF10 is expressed in the mesenchyme and drives the proliferation of progenitor cells expressing PDX1 during branching by binding to the FGFR2b receptor on epithelial cells [91]. Consistent with this, *Fgf10* mutant mice exhibit arrested pancreas development at the bud stage [92]. An additional activity of FGF10 during pancreas growth is to keep the expanding pancreatic epithelium in an undifferentiated state. This is achieved through the activation of Notch signaling, a potent regulator of pancreatic differentiation [93–97].

The different compartments of the mature pancreas include exocrine cells that make digestive enzymes, ducts that carry these enzymes to the gut, and hormone-producing endocrine cells. The exocrine pancreas is the largest compartment, comprising over 80% of the pancreatic mass. The transcription of exocrine-specific genes is dependent on the PTF1 transcriptional complex, which contains a pancreas-specific factor called p48. Like PDX1, p48 is expressed in the early stages in multipotent pancreatic progenitor cells [98], and it is the major transcription factor for the expression of exocrine-specific genes [99]. The transcription factor p48 is required for exocrine differentiation, as null mutant mice develop an endocrine pancreas but lack exocrine cells [100]. Another transcription factor, MIST1, is required for the assembly of the exocrine secretory machinery [101].

During the growth of the pancreatic epithelium within the bud, endocrine cells arise in waves of differentiation (glucagon-producing α cells preceding insulin-producing β cells); these cells delaminate from the epithelium and reaggregate postnatally into the islets of Langerhans (which also include somatostatin-producing δ cells and pancreatic polypeptide-producing cells). The development of these different endocrine lineages is complex and regulated by multiple factors. The bHLH transcription factor Neurogenin 3 (NGN3) is both necessary and sufficient for endocrine differentiation in the pancreas [79,81,102,103]. *NGN3*, and its target gene *BETA2/NEUROD*, are regulated by Notch signals [104] and the one-cut transcription factor HNF6 [105]. Additional transcription factors involved in the delineation of different endocrine lineages in appropriate numbers include NKX6.1, NKX2.2, PAX4, and PAX6 [106].

Of note, islets are not derived from the monoclonal expansion of endocrine precursor cells, but rather from the coalescence of distinct endocrine cells or endocrine precursors [107]. The derivation of endocrine β cells from embryonic progenitors has led many investigators to hypothesize that adult β cells are generated by a similar mechanism. The aggressive search for putative "adult stem cells" in the pancreas has yielded ambiguous results. One laboratory that used a genetic labeling method failed to show that adult stem/progenitor cells give rise to β cells, suggesting that the adult β cell mass is maintained principally by replication [108].

Summary

The repression of Shh signaling induces the formation of dorsal and ventral pancreatic buds from the endoderm. Signals provided by blood vessels, as well as mesenchymal FGF10, promote the outgrowth of multipotent pancreatic progenitor cells into a branched epithelium. Complex signals, including members of the Notch and TGF-β families, as well as numerous bHLH and homeodomain proteins, regulate the subsequent differentiation of pancreatic endocrine, exocrine, and ductal lineages.

Gastrointestinal tract

Specification

Although the gastrointestinal tract is composed of a single continuous tube, it is partitioned into discrete domains from anterior to posterior (esophagus, stomach, small intestine, and colon) that are separated by sphincters (lower esophageal sphincter, pylorus, and ileocecal valve). Each domain has a distinct function and a unique architecture. Similar to the liver and the pancreas, the different functional domains of the intestine are patterned after gastrulation through a repertoire of homeobox genes and epithelial–mesenchymal cross-talk.

Homeobox-containing genes are expressed in a regionalized manner in the gut epithelium and mesenchyme [15,24], and several examples of "homeotic transformations" have been observed after the dysregulated expression of homeobox genes. *Hoxa13* and *Hoxd13* are expressed in the hindgut, and ectopic expression of either of these *Hox* genes in the midgut leads to acquisition of hindgut characteristics [109,110]. The *Hox* gene *Hoxa5* is expressed in stomach mesenchyme, and

is necessary for gastric fate specification [111]. Sphincters constitute a special case of endoderm patterning, as they reside at boundaries between intestinal segments. Again, *Hox* genes are important for the process of sphincter formation. *Hoxa13/Hoxd13* mutant mice have defects in anal sphincter formation, and mice with a large deletion in the *Hoxd* cluster (*Hoxd4–d13*) lack an ileocecal valve [21,22].

In addition to the expression of homeobox genes, epithelial–mesenchymal signaling is also essential for the establishment of an intestinal pattern. In some cases, such as the murine cecum, a clear hierarchy of epithelial–mesenchymal signaling (through FGFs in cecal development) mediates organ growth [112]. In other cases, it is less clear whether transcription factors (such as homeobox-containing proteins) establish an initial pattern that is refined by further epithelial–mesenchymal signaling, or whether epithelial–mesenchymal signaling establishes the pattern of transcription factor gene expression. An alternative possibility is that the basement membrane, an aggregate of extracellular matrix strategically placed between epithelial and mesenchymal cells, regulates cross-talk between the two tissue layers [113].

Pyloric sphincter development is a particularly instructive example of this complex specification process. In the chicken, the pyloric sphincter forms at the junction of the gizzard (caudal stomach) and the small intestine. Two transcription factors – the homeobox factor Nkx2.5 and the HMG-box factor Sox9 – are both markers of the mesenchyme of the pyloric sphincter, and ectopic expression of either gene is sufficient to convert the gizzard into pyloric sphincter-like epithelium [114–116]. Moreover, mesenchymal BMP4 is both necessary and sufficient to induce the expression of these transcription factors [114,116,117]. This result is surprising, because BMP4 is expressed widely throughout the gut mesenchyme. The specificity of BMP4 activity to induce sphincter development likely reflects specific spatial regulation of its own expression and spatial regulation of its receptor [117,118]. Moreover, the ability of the downstream factor Nkx2.5 to induce pyloric sphincter development is spatially regulated; Nkx2.5 can induce pyloric sphincter development when it is expressed anteriorly (in the gizzard), but not posteriorly (in the duodenum) [115]. These studies provide insight into the final steps regulating the development of the pyloric sphincter but a deeper question remains. What regulates the regulators?

Complex signals that are both intrinsic and extrinsic to the developing epithelium regulate tissue identity. The extent to which these or similar inductive events contribute to common congenital anomalies, including intestinal stenoses and atresias, duplications, and anorectal malformations, is unknown. However, congenital anomalies of the gastrointestinal tract are commonly associated with malformations in other organ systems or chromosomal abnormalities, including trisomy 21 (Down syndrome), suggesting that the regulatory signals involved in patterning are disrupted widely.

The clinical relevance of these regulatory networks may extend beyond putative relationships to congenital errors. Intestinal metaplasia, an adult premalignant lesion in which portions of the esophagus or stomach are replaced with intestinal mucosa, may represent reactivation of developmental programs. Studies of BARX1, a homeobox-containing transcription factor that is expressed transiently in the gastric mesenchyme, provide support for this concept. BARX1 mediates gastric specification by inhibiting Wnt signaling, and mouse embryos with a targeted disruption of the gene exhibit a homeotic transformation of stomach to intestine [119]. One interpretation of this result is that intestinal differentiation represents a "default" state for gut endoderm that must be overcome (through inhibition of Wnt signaling) to allow stomach specification. Although unproved, this model may explain why intestinal metaplasia of the stomach and esophagus is common, whereas the converse, gastric metaplasia of the midgut or hindgut, is uncommon. Furthermore, the model gives rise to the testable hypothesis that intestinal metaplasia in later life may result from the unopposed reactivation of Wnt signaling after injury.

Morphogenesis

The lumenal gastrointestinal tract acquires it shape through rotational changes at a gross level, and through tissue remodeling at a microscopic level. Left–right (L–R) asymmetry of the intestine is generated through the same mechanisms that regulate the L–R axis of the body plan. This process involves the clockwise movement of cilia, which promotes the asymmetric distribution of inductive signals [120]. Dysregulation of cilium function leads to randomization of L–R asymmetry and clinical phenotypes including situs inversus.

The intestine undergoes tremendous growth during the initial embryonic period, and elongates about 1000-fold between the 5th and 40th weeks of human development [121]. To accommodate a large embryonic liver, the intestine exists outside the abdominal cavity for much of its early embryonic life ("physiological herniation"). Early in development, the growing midgut and hindgut undergo a two-step rotation (Fig. 23.6) totaling 270° (counterclockwise orientation viewing the embryo en face). Both growth and looping of the intestine require the action of HLX, a homeobox transcription factor that is expressed in the midgut and hindgut mesenchyme and that is also required for liver development (see section on budding of the liver). *Hlx* mutant mouse embryos have a shortened and single-looped gut that undergoes normal differentiation [55]. Many congenital anomalies are related to errors in these gross movements of the intestine, most notably midgut malrotation with risk of ensuing volvulus.

Although our understanding of this dramatic intestinal growth and rotation remains mainly descriptive and phenomenological, the mechanisms controlling the cross-sectional makeup of the intestine are better understood. The

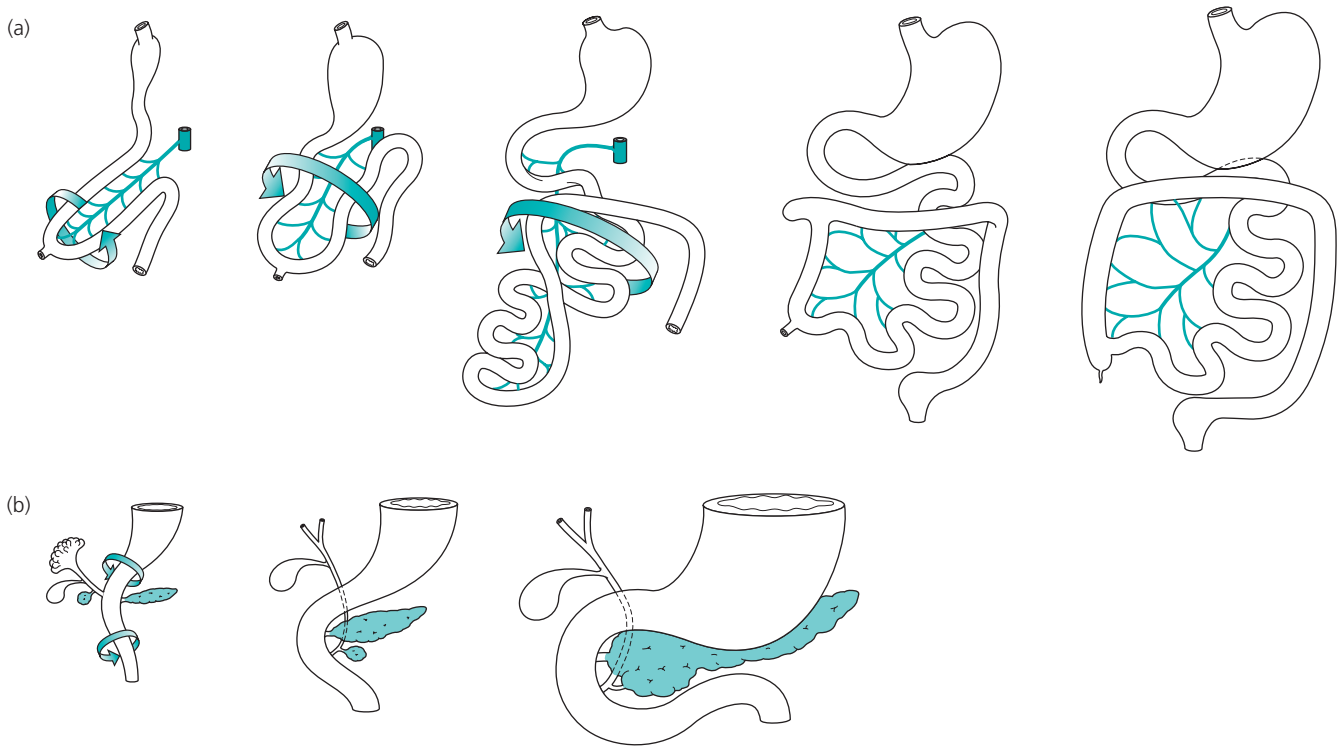


Figure 23.6 Rotation during development of the gastrointestinal tract. **(a)** Rotation of the gut. **(b)** Rotation of the pancreas.

stereotyped circumferential arrangement of cells according to each intestinal segment has been referred to as the *radial axis* of the gastrointestinal tract: innermost epithelium, lamina propria, muscularis mucosae, submucosa, outer muscular layers, and serosa. Each intestinal segment has a unique epithelial and mesenchymal composition; e.g., the stratified squamous epithelium and thin submucosa and muscular layers of the esophagus vs the columnar epithelium and thickly muscled mesenchyme of the stomach.

Shh–BMP cross-talk appears to be important for determining the composition of the radial axis in each intestinal segment. This conclusion is based on several lines of evidence. First, Shh is expressed throughout the gut epithelium (except for the pancreas, as discussed in the section describing the specification of the pancreas) and is a potent activator of mesenchymal BMP expression so that the two signaling pathways regulate each other. Second, ectopic BMP expression affects the degree of muscularity of the mesenchyme along the anteroposterior axis [110], suggesting that it regulates mesenchymal morphology. Third, Shh signaling is necessary for normal crypt–villus structure [122], and Shh regulates mesenchymal fate according to the distance from the epithelium [123]. These results are consistent with a model in which a concentration gradient of Shh (expressed by the epithelium) organizes the mesenchymal rings of the gut, possibly through the activity of BMPs. According to this model, mesenchymal cells closest to the epithelium are induced to adopt a lamina propria or submucosal fate, whereas only those cells furthest from the epithelium adopt a muscle fate [123].

The intestinal lumen forms after 7–8 weeks of gestation (human) and consists of two processes, designated canalization and morphogenesis. The failure of canalization is thought to account for some cases of duodenal atresia, a partial or complete obstruction of the duodenum that occurs with a frequency of 1 in 5000 to 1 in 10 000 births. Morphogenesis involves polarization of the epithelium and transformation of a stratified epithelium to a columnar epithelium. As discrete villi emerge from the stratified epithelium, a distinct crypt–villus architecture is acquired. Research has revealed the importance of cytoskeletal regulation in villus morphogenesis. Mice lacking the "bridge" protein ezrin, which links membrane proteins to the actin cytoskeleton, exhibit abnormal villus morphogenesis, with nascent villus structures that are unable to break away from each other [124]. $Ez^{-/-}$ mice have normal intestinal differentiation and polarity, showing that Ezrin acts specifically to regulate the formation of new villi, presumably by binding to unidentified membrane proteins.

Cell proliferation and kinetics

The adult small intestinal epithelium has a rapid and regular turnover, with the average lifespan of intestinal enterocytes measured in days [125]. To support this constant need for new cells, the intestine recapitulates the embryonic processes of differentiation from stem cells throughout life (Fig. 23.7).

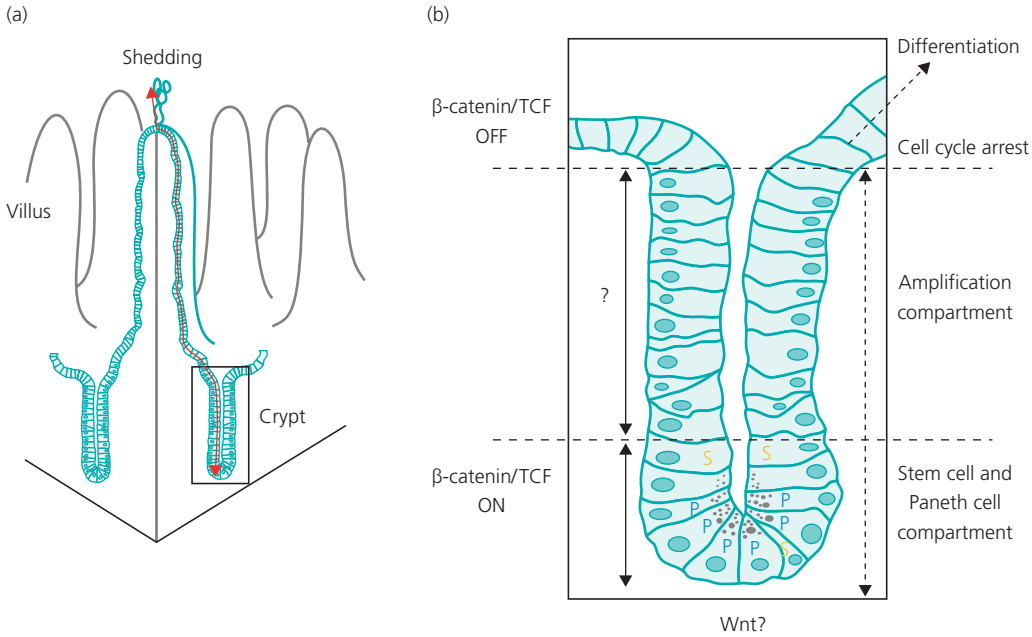


Figure 23.7 Small intestinal maturation. **(a)** Maturation of the crypt–villus axis. Stem cells near the base of the crypt give rise to a transient amplifying multipotent population of cells that reside in the middle and upper portions of the crypt. These cells give rise to mature enterocytes, enteroendocrine cells, and goblet cells, which migrate up the villus and are eventually shed into the intestinal lumen. Paneth cells are also derived from the transient amplifying population, but these cells migrate down to the crypt base where they intermingle with stem cells. **(b)** Small intestinal crypt. Schematic showing the crypt is divided into compartments. At the base are stem cells (S), which are thought to reside slightly above the crypt base, and Paneth cells (P). Canonical Wnt signaling is active in these cells. Above this compartment is the transient amplifying population, containing actively dividing cells. Cells withdraw from the cell cycle as they reach the crypt–villus interface and adopt an absorptive or secretory fate. Adapted from Sancho et al. [242].

Stem cells are specialized cells that can generate multiple differentiated cell types ("multipotentiality") and also produce more stem cells ("self-renewal"). Intestinal stem cells reside near or at the bottom of the crypts and are characterized by their relatively low rate of cell division and long life [126].

Progenitor cells with a more limited potential and shorter half-life coexist with stem cells in the crypts [127]. A subset of stem cell-derived progenitor cells, known as the *transient amplifying population*, undergoes rapid cell division within a region of the crypt–villus axis known as the *proliferative zone* (see Fig. 23.7). Mesenchymal factors, including the winged helix transcription factor Fkh6 [128], and several intercellular signaling pathways regulate cell division in this zone.

Wnt signaling is an important regulator of stem cell maintenance and differentiation in many developmental systems [129]. Wnt signals act during villus maturation by regulating the pool of undifferentiated progenitor cells. Inactivation of the Wnt signaling mediator Tcf4, or inhibition of Wnt signaling with the soluble inhibitor Dickkopf1 (Dkk1), results in the loss of the proliferative zone [130,131]. Conversely, activation of Wnt signaling leads to an expanded proliferative zone [132,133]. Because Wnt ligands are expressed in both the epithelial and the mesenchymal compartments of the intestine [134], their precise source is unclear.

Like Wnt, Notch signals seem to play an important role in the regulation of intestinal progenitor cells [135,136]. This regulatory mechanism appears to be conserved in organisms as distantly related as zebrafish and *Drosophila* [137–139]. Finally, hedgehog signals also appear to regulate intestinal progenitor cells [140,141].

Despite these advances, relatively little is known about the factors that regulate the behavior of intestinal stem cells themselves: when and whether to self-renew or differentiate. Studies of intestinal stem cell biology have been hampered by an absence of stem cell markers and in vitro culture systems. The unique properties of stem cells are conferred by attributes of the cellular microenvironment, which has been termed the *stem cell niche*. In other stem cell systems, notably *Drosophila* germ cells and hemopoietic stem cells, characterization of the niche has revealed several factors that promote maintenance of the stem cell state. Although no equivalent description exists for the intestinal stem cell niche, one group of investigators has proposed that BMP signals (provided by mesenchymal cells at the crypt base) regulate Wnt signals to maintain the stem cell compartment [142].

Cytodifferentiation

The differentiated cells of the intestine can be divided into *absorptive* and *secretory* cells on the basis of the function of various cells. The precise identity and relative abundance of absorptive and secretory cells varies along the anteroposterior axis. The major secretory cells of the stomach (and their

secretory products) are parietal cells (acid), chief cells (digestive enzymes), and endocrine G cells (gastrin). By contrast, the major secretory cells of the small intestine are goblet cells (mucous), Paneth cells (antimicrobial peptides), and enteroendocrine cells (myriad hormones). Nevertheless, the genetic mechanism that regulates the development of these different cells is shared between different segments of the gastrointestinal tract.

As in the pancreas, Notch signaling plays a critical role in the regulation of intestinal cell fate by mediating the expression of several downstream bHLH proteins – NGN3, BETA2/NEUROD, HES1, and MATH1. The general conclusion from studies of mice with mutations of these proteins is that differentiation of the secretory cell lineage is triggered by repression of Notch signaling and HES1, with the resulting derepression of MATH1 [143,144]. Additional signals control the selection of different intestinal secretory cell lineages (endocrine, goblet, and Paneth). One of these signals, NGN3, is absolutely required for endocrine cells to form in the intestine, but not in the stomach; by contrast, the formation of goblet and Paneth cells is normal in *Ngn3*-deficient mice [145,146]. Other complex signals and lineage relationships underlie the development of the 10 or so different types of enteroendocrine cells [147].

There is additional evidence that Notch signals are coordinated with Wnt signals to regulate the balance between progenitor cell proliferation and cytodifferentiation. Either embryonic activation of Notch or inhibition of Wnt results in the loss of secretory cell lineages [131,134,137]. Furthermore, Wnts have a special role in ensuring proper compartmentalization of the crypt–villus axis by regulating another family of cell–cell signaling molecules known as ephrins [148].

The intestine is maintained throughout life by stem cells so the question arises whether the same mechanisms that regulate stem cell differentiation in the embryo also do so in the adult. Indeed, several studies suggest that Notch signals function in both embryonic and adult intestinal homeostasis. In particular, the inhibition of Notch signals using pharmacological γ-secretase inhibitors (which block Notch signaling by preventing proteolytic maturation of the receptor) results in the conversion of crypt proliferative cells into goblet cells [136,149,150]. Conversely, the activation of Notch signaling through the misexpression of a constitutively active mutant form of Notch prevents the development of secretory cell lineages [151].

Wnt signaling also plays a role in adult intestinal homeostasis. Activation of Wnt signaling in the adult intestine causes a marked expansion of the proliferative zone, but has minimal effect on the differentiated cells of the villus [132,152]. Activation of Wnt signaling through the loss of the *APC* gene and the subsequent activation of β-catenin is known to be a key step in colorectal carcinogenesis (see Box 23.4 and Chapters 24, 63, and 65), suggesting that intestinal stem cells or transient amplifying cells are most sensitive to the inactivation of *APC* and represent a likely target for malignant transformation [153].

Despite the observation that Notch and Wnt signals mediate similar activities in the intestine, the precise relationship between these two pathways is unknown, as is the mechanism by which they regulate a balance between proliferation and

Box 23.4 Wnt signaling and colorectal cancer

One of the clearest links between developmental signals and carcinogenesis is exemplified by the role of Wnt signaling in intestinal differentiation and colorectal cancer. Complex cellular events occur after the Wnt ligand binds to a Frizzled receptor, and most colon cancers harbor mutations that lead to activated Wnt signaling. The best-characterized signals, referred to as the canonical arm of the Wnt signaling pathway, involve the stabilization of β-catenin in the nucleus and the formation of β-catenin/TCF-4 transcriptional complexes (see section on signaling in development).

Most Wnt signaling pathway mutations found in colorectal cancer occur in the genes encoding β-catenin or its regulator, adenomatous polyposis coli (APC). These mutations result in pathway activation; they occur as an early event in most sporadic colorectal cancers and cause the familial adenomatous polyposis colorectal cancer syndrome. It is postulated that persistent Wnt signaling in villi (an epithelial population in which Wnt signaling is normally absent) imposes a crypt stem/progenitor cell phenotype [253,254]; this self-renewal phenotype might allow villus cells to accumulate the additional mutations needed for carcinogenesis according to the prevailing model [255].

The first *Wnt* gene to be identified was *Int1*, a candidate oncogene in mammary tumorigenesis [256]. Subsequent studies showed *Int1* to be orthologous to the *Drosophila* segment polarity gene *Wingless*. Furthermore, *Wnt* genes were discovered to play a near-ubiquitous role in organ development. As mentioned, Wnt signaling plays a critical role in the biology of the intestine. Regulation of Wnt signaling appears to be important in the normal patterning of the stomach and intestine [257]. Abrogation of Wnt signaling in the embryonic or adult intestine leads to a depletion of intestinal progenitor cells [258–260]. Moreover, defective Wnt signaling also perturbs the proliferative compartment of the intestine and the ability of cells to differentiate into secretory cell types [258,259].

Among the remaining challenges is the question of how cells with active Wnt signaling are normally kept in check. One possible mechanism involves the β-catenin/TCF-4 target Ephrin (*Eph*) genes, which function to regulate compartmental boundaries within the crypt–villus unit [261]. Mice that harbor mutations in both *APC* and *EphB* have a marked acceleration in tumor formation compared to mice with a mutation in APC alone [262]. These results suggest that EphB normally constrains the growth of cells with active Wnt signaling and that loss of Eph function may serve as a "second hit" by relieving this constraint.

differentiation. The studies described above are intellectually satisfying because they establish links between embryonic development, adult stem cells, and cancer. Nevertheless, they merely hint at the complexity underlying the regulation of intestinal differentiation. Hurdles facing the field include a lack of markers that uniquely identify the various precursor populations (unipotent and multipotent progenitor cells and stem cells), and an understanding of how proliferation and differentiation are coupled and the mechanism by which the appropriate ratio of absorptive and different secretory lineages is achieved.

Summary

The formation of sphincters during midgestation divides the gut tube into segments – esophagus, stomach, small intestine, and large intestine – that prefigure their distinct morphologies and functions. The mechanism of sphincter formation is incompletely understood but involves significant epithelial–mesenchymal cross-talk. Similar cross-talk is involved in the morphogenesis of the different intestinal segments. Subsequently, intestinal development proceeds by differentiation from stem cells, a process that depends on Wnt and Notch signaling, among other pathways. Such signals control proliferation and differentiation in the adult intestine and are dysregulated during carcinogenesis.

Conclusions

After gastrulation, the gut tube is exposed to regional signals. In prespecified organ domains, the epithelium responds by growing into adjacent mesenchyme, resulting in the budding of lung, liver, and pancreas. In the remaining gut epithelium, reciprocal signaling with mesenchyme results in the formation of sphincters or regionally distinct submucosae. Complex morphogenetic changes and cytodifferentiation occur in each of these developing organs, giving rise to organized tissues. Tissue-specific gene expression begins, setting the stage for further refinement of regulated expression and function. In the adult intestine, differentiated cell types are generated throughout life from stem cells that reside within the crypts, an ongoing process that recapitulates many developmental events. In the liver and the pancreas, by contrast, the replication of existing cell types appears to be a major mechanism for tissue maintenance.

Developmental physiology

The development of complex anatomical structures with distinct differentiated cell lineages would be purposeless if it did not facilitate function. The functions of the gastrointestinal tract include assimilation of nutrients, detoxification and elimination of waste, maintenance of blood glucose, and synthesis of plasma proteins. In addition, the gastrointestinal tract subserves secondary roles in water and electrolyte balance and immunological defense. The physiology of the intestine, pancreas, and liver is the subjects of entire textbooks [154] and is beyond the scope of this chapter. Again, the emphasis within this chapter is on mechanism.

The connection between form and function is reflected in an economy of mechanism. Many genes exhibit dual function and are involved in both development and physiological regulation. As previously described, FOXA2 and GATA4 bind to the albumin promoter as part of a program of endoderm commitment, and are thus involved in both patterning as well as the functional expression of a liver-specific gene. There are several other similar instances of developmental "parsimony"; e.g., the *Pdx1* and *p48* genes play important roles in pancreatic development and are also the major transcriptional regulators of insulin and of several exocrine genes, respectively [83,99]. Similarly, the *CDX2* homeobox gene plays an important role in endoderm patterning [15,17,18,155] and also functions as a major transcription factor for the expression of brush border enzymes and intestinal carbonic anhydrase [156–158]. Such economy is not surprising because the use of a limited set of genetic tools reduces the need for cellular reprogramming during specification and differentiation. Fortuitously, this arrangement facilitates mechanistic understanding, as identification of the genes that regulate development provides a list of candidate regulators of function, and vice versa.

Maturation of the gastrointestinal tract

After parturition, the gastrointestinal tract faces two environmental challenges. Immediately after birth, the individual must adjust from a diet that is predominantly parenteral (provided by the maternal circulation), to one that is completely enteral (consisting of colostrum and breast milk). Later, at weaning, the gastrointestinal tract must be able to assimilate nutrients from a vast array of solid foods. These adjustments occur in a hormonal milieu that is increasingly under the control of the infant. Therefore, unlike structural development, which follows a set of preprogrammed genetic events, functional development is likely to be considerably more dependent on environmental forces [159]. Furthermore, a comprehensive understanding of functional development must incorporate studies of both humans and model organisms, which may differ in important ways.

Gastrointestinal "maturation" refers to the progressive attainment of features of adult gastrointestinal physiology during development. Given the imprecise nature of such a definition, several surrogate markers are used to understand how the physiology of the gastrointestinal tract changes over time. These include biochemical measurements of intestinal enzyme and hormone activities, morphological grading, mutant analysis, and measurements of permeability, motility, and immune performance. It has been suggested that the human gastrointestinal tract is structurally and functionally mature at the time of parturition, whereas the rodent gas-

trointestinal tract is altricial, or immature, at birth. However, given the comprehensive nature of maturation, and the fact that the neonatal diet of all mammals is similar (i.e., milk), the implications of such a distinction are unclear. The following sections provide snapshots of some well-studied events during intestinal maturation.

Carbohydrate digestion and absorption

A focal point in the study of intestinal maturation has been the characterization of the major brush border enzymes that digest carbohydrates. One enzyme is lactase–phlorizin hydrolase (LPH), which cleaves lactose, the major dietary carbohydrate of breast milk. In rats, LPH is expressed at high levels early in embryogenesis, whereas peak expression in human embryos occurs during the third trimester. LPH expression declines with age in both species. The observation that LPH expression across species is highest after birth and subsequently lower likely reflects the critical requirement for lactase during nursing [160].

Another well-studied brush border enzyme is sucrase–isomaltase (SI), a bifunctional disaccharidase. In contrast to LPH, the expression of SI is discordant between humans and other mammals. In rats and pigs, SI expression is undetectable before a dramatic burst of expression in the postnatal period, corresponding to the time just before weaning when the major carbohydrate source shifts from milk to starch. By contrast, SI expression in humans begins in the first trimester and reaches its peak level just before birth [160]. The earlier expression of SI during human ontogeny is not understood, and it is unclear whether differences in enzyme expression levels reflect differences in overall functional maturation between species.

Protein digestion and absorption

The embryo has only a limited capacity to digest proteins, a result of the late expression of digestive zymogens, the low-level expression of the activating enzyme enterokinase, and the insensitivity of embryonic pancreatic exocrine cells to the action of secretagogues (see section on hormonal control of gastrointestinal development). Furthermore, the gastric pH is neutral until birth, dropping rapidly from 6.0 to 2.2 in the first day of life [161]. Instead, other systems handle the limited protein load delivered to the intestine pre- and perinatally. Brush border and microvillar peptidases and dipeptidases, which complete peptide digestion, are present in the fetal small intestine at levels of activity comparable to levels in the adult small intestine. High levels of amino acid transporters in the newborn permit the uptake of free amino acids.

Macromolecular transport also plays an important role in the digestion of proteins and lipids in the fetus and the neonate [162–164]. In experimental animals, the small intestinal epithelium is more permeable to amino acids and peptides in the immediate postnatal period than when the intestine is mature. Macromolecular tracers infused into the amniotic fluid or the intestinal lumen late in gestation are absorbed into the enterocytes of humans, monkeys, guinea pigs, and rats, reflecting a high rate of pinocytosis [164]. This process is extremely active in the first 2 weeks postnatally and decreases at the time of weaning. This mechanism accounts for the absorption of intact maternal immunoglobulins and other proteins from milk.

In parallel with pinocytosis, enterocytes exhibit high levels of lysosomal proteases, such as cathepsins and other peptidases, during the first 2 weeks postnatally. These intracellular enzymes provide a mechanism for protein digestion before the appearance of the pancreatic proteolytic enzymes. Intact proteins also are absorbed in premature and term human infants during the first few months of life. Macromolecules may continue to cross the healthy adult small intestine, but the quantity is low compared to those in the newborn. The relative permeability of the intestine during the first months of life may play an important role in conferring tolerance or sensitivity to dietary proteins during the development of immune function.

Lipid digestion and absorption

Fats and unhydrolyzed triglycerides are present in the stools of human neonates at a rate that is higher than that of adults, a phenomenon that correlates with the low activity of pancreatic lipase and the low intralumenal concentrations of bile acids. Although pancreatic lipase levels rise significantly during the third trimester, lipase activity at week 32 of gestation is only 50% of term levels, which are themselves only 10% of adult levels. Fat digestion in human neonates is aided by "preduodenal" lipases (lingual and gastric lipases) and maternal milk lipase. Lingual lipase rises to adult levels by 2 years of age [165]. Gastric lipase appears as early as 10–13 weeks into gestation and reaches adult levels by 16 weeks [166]. Gastric lipase appears to be a major determinant of lipolytic activity in gastric aspirates of premature infants. As with peptides, the newborn intestine exhibits increased permeability to both triglycerides and cholesterol [167].

The synthesis of bile acids from cholesterol and their conjugation with taurine and glycine can be demonstrated in organ culture in vitro with human liver tissue obtained from fetuses after 15 weeks of gestation [168]. Biliary secretion is observed as early as the 22nd week of gestation. Bile acid reabsorption occurs in the neonate by passive diffusion throughout the small intestine, but active sodium-dependent ileal transport of bile acids does not occur until weaning in rats, rabbits, and humans [169]. As a result, the bile acid pool is reduced in neonates; this is of particular concern in premature infants, in whom 10%–20% of ingested fat may not be absorbed.

Dietary control of gastrointestinal development

The expression of SI, LPH, and other brush border enzymes seems to be mostly under autonomous control, because their

normal expression pattern does not change significantly with delayed or early weaning or early introduction of dietary sucrose [170]. Indeed, transplanted human fetal intestine is able to undergo normal cytodifferentiation in an immunodeficient "nude" mouse host [171]. However, other aspects of gastrointestinal development, particularly growth, are regulated by diet. Exposure of the gut lumen to nutrients begins in utero with the swallowing of amniotic fluid, which contains amino acids and carbohydrates, and which the embryo uses to meet some of its nutritional requirements.

The importance of the lumenal environment is supported by studies in which the timing, the composition, or the route of delivery of nutrition is varied. Ligation of the embryonic sheep esophagus causes reversible and specific inhibition of growth of the gastrointestinal tract [172]. Although normally absent in human amniotic fluid, galactose can nevertheless be absorbed by the embryonic jejunum. Intraamniotic infusion (and therefore increased enteral delivery) of galactose induces an increase in the mucosal transport of galactose by the rabbit intestine, as well as an overall increase in mucosal weight, suggesting that the fetal intestine is competent to respond to small changes in enteral carbohydrate composition [173]. Consistent with this, intestinal growth in the first day of life depends on the composition of milk [174]. Importantly, it is not simply the metabolic consequences of feeding that provide a signal; the intestinal mucosa itself must be exposed to these nutritional components. For example, infant rats given enteral nutrition exhibit greater intestinal growth than rats given the same nutritional support intravenously [175]. A requirement for lumenal stimulation has long been appreciated in the "adaptation" observed after massive intestinal resection – a compensatory increase in intestinal surface area that depends on enteral feeding [176]. It is possible that this adaptation reflects a reemergence of an early developmental program that regulates intestinal size and surface area. Indeed, microarray analysis of gene transcription during development and adaptation supports this hypothesis [177].

Hormonal control of gastrointestinal development

A possible regulatory role for corticosteroids and thyroid hormone in intestinal development has been extensively explored because of the dramatic increase in the level of both hormones observed in rats immediately before the spike in SI activity and coinciding with a reduction in LPH activity. Direct effects on the activity of several disaccharidases have been documented after the administration of exogenous hormones. Notably, prenatal administration of cortisone reduces the incidence of necrotizing enterocolitis in a rat model, presumably by accelerating the maturation of the mucosal barrier [178]. Conversely, intestinal maturation is slowed by treatments that reduce levels of circulating corticosteroids. Similar effects are seen with enhancement or inhibition of thyroid hormone expression, although some of these effects may be mediated through corticosteroids [160]. Importantly, mice lacking corticotropin-releasing hormone or thyrotropin-releasing hormone do not exhibit an overt gastrointestinal phenotype [179,180]. The regulation of gastrointestinal maturation by other hormones and circulating growth factors has also been investigated through similar approaches. In particular, cholecystokinin, gastrin, insulin, and members of the insulin-like growth factor (IGF), epidermal growth factor (EGF), and TGF families have been the focus of numerous studies.

These analyses have yielded much evidence for the involvement of hormones and systemic growth factors in gastrointestinal development, the description of which is beyond the scope of this chapter. However, differentiation between primary and secondary effects is challenging, and for the most part, the precise functions of these molecules in development remain to be delineated. It is worth noting the phenotype resulting from a deficiency of gastrin, which is abundantly expressed in the pancreas and proximal intestine during embryonic development. Mice with a targeted inactivation of the gastrin gene exhibit a deficiency of acid-producing parietal cells [181,182], suggesting a role in cellular differentiation rather than maturation per se.

Despite the lingering controversy over the precise role of hormones in intestinal maturation, it is clear that the responsiveness of some gastrointestinal tissues to hormones changes over the course of fetal and postnatal life. The responsiveness of the exocrine pancreas is an example of such regulation. Pancreatic digestive and lipolytic enzymes are detected in the early bud stage, and high levels of protein are detected in the acinar cells before term. Despite the abundance of these proteins, embryonic acini are insensitive to secretagogues until after birth [183]. Similarly, sensitivity to the acid-secretory action of gastrin develops during the first week of life; poor expression of the gastrin receptor in the immediate postnatal period renders newborns relatively insensitive to gastrin [184].

Finally, the intestine and the pancreas are major endocrine organs in their own right. As the source of insulin, the pancreas is the major regulator of glucose homeostasis. The intestine contains numerous peptides that regulate motility, ion transport, feeding, and satiety, and has been dubbed "the largest endocrine organ" in the body [185]. Previous sections have outlined the mechanisms by which endocrine cells arise in the pancreas and the gut. For a more detailed description of the ontogeny and regulation of the gastrointestinal endocrine system, we refer the reader to Williams Textbook of Endocrinology [186].

Development of the enteric nervous system

The enteric nervous system (ENS) regulates multiple aspects of gastrointestinal physiology, including peristalsis and smooth muscle activity, sphincter tone, glandular output,

microcirculation, and possibly, inflammation [187]. Through these activities, the ENS controls the response to feeding by coordinating intestinal transit, secretion, and continence. The cells that give rise to the ENS migrate from the neural crest during the first trimester, induced by complex and poorly understood signals. Similar to other neural crest derivatives, the ENS is part of the peripheral nervous system, constituting its largest subdivision. Although the ENS receives input from the vagus nerve, it possesses marked independence from the central nervous system, exhibiting function even after complete dissociation from all brain and spinal inputs. On the basis of its size and autonomy, the ENS has been referred to as the "second brain" [188]. Although many adult disorders may be related to ENS dysfunction, only Hirschsprung disease is clearly attributable to developmental errors in ENS formation (see section on disorders of specification and formation).

Fate mapping in the chick has shown that enteric neurons are largely derived from rostral (vagal) and caudal (sacral) precursors that migrate from the neural tube and intermingle to populate the entire gut tube [184]. Although some details remain controversial, studies in mice have confirmed the general picture of neural crest migration mapped out by studies in the chick. The ENS is composed of two types of ganglionated plexuses: the Auerbach (myenteric) plexus, which is located in the outer muscular layer and regulates gastrointestinal tract motility and function of extralumenal organs, and the Meissner (submucosal) plexus, which regulates enteral secretory activity [190]. Enteric neurons can be further subclassified according to the neurotransmitters (e.g., vasoactive intestinal polypeptide and serotonin) and enzymes (e.g., tyrosine hydroxylase and choline acetyltransferase) they express. Although the mechanisms that mediate the migration and terminal differentiation of neural crest precursors are unknown, neuronal subtypes appear to arise in overlapping developmental waves [191]. The functional roles of these neuronal subtypes and specific neuropeptides in gastrointestinal physiology are described elsewhere in this textbook (see Chapters 2 and 47).

In contrast to the neural crest-derived cells of the enteric plexuses, interstitial cells of Cajal (ICCs), which serve as the "pacemakers" of the intestine, arise from intestinal mesenchyme [192,193]. The development of these cells requires the function of another receptor tyrosine kinase, c-Kit. Mice with reduced or absent c-Kit function exhibit abnormal slow-wave activity in the small intestine and develop paralytic ileus [194,195]. It has been discovered that a category of mesenchymal tumors known as gastrointestinal stromal tumors (GISTs) have activating mutations in *KIT* that confer constitutive kinase activity in the absence of ligand [196,197]. Ultrastructural similarities between ICCs and GISTS, and other shared features, has led researchers to propose that GISTs arise from ICCs [198] or from a common ICC–smooth muscle precursor cell [199].

The ability to inactivate specific genes in the mouse has provided additional mechanistic details regarding ENS development. In particular, a central role for the c-Ret tyrosine kinase pathway in the development of most enteric neurons has been demonstrated through targeted inactivation of pathway components (see the discussion of Hirschsprung disease). Furthermore, important neural crest subpopulations have been recognized from the more limited phenotypes that result from the targeted mutation of other genes. For example, the basic helix-loop-helix protein MASH1 is required for the development of a subset of enteric neuronal precursors with noradrenergic features, and endothelin B signaling is required to prevent the differentiation of neuronal precursors that will enervate the distal colon [200]. The significance of these different subtypes is unclear, and the mechanisms by which they achieve regulatory integration require further study.

The ENS begins to function early in embryonic development, but its maturation continues well into postnatal life. Fetal swallowing is first detectable during the first trimester [201], and by term, the fetus swallows about 450 mL amniotic fluid (half of the total amniotic volume) per day [202]. A spectrum of neuropeptides is detectable between weeks 11 and 18 of human development [203]. Peak numbers of both neurons and ganglion cells are achieved during the second trimester and decrease during the third trimester [204]. Although the structural elements of the esophagus and stomach are largely developed by midgestation, gastroesophageal motility does not fully mature until after birth. Lower esophageal sphincter pressure increases dramatically during the last trimester and again postnatally [205], achieving adult levels by 3–6 weeks of age. Despite this, free gastroesophageal reflux is common postnatally and persists in up to 10% of infants for the first year [206].

Mucosal immune system

The gastrointestinal tract, particularly the small intestine, contains a highly complex mixture of immune cell populations. The gut-associated lymphoid tissue (GALT) encompasses organized aggregates dominated by lymphocytes (Peyer patches) and a diffuse heterogeneous population of lymphocytes, monocytes, or macrophages, and other cells, such as eosinophils and mast cells in the lamina propria. Intraepithelial lymphocytes are also scattered throughout the surface epithelium. Structures resembling Peyer patches are evident as early as 11 weeks into human gestation; by 14 weeks, $CD4^+$ and $CD8^+$ lymphocytes can be detected. By the end of the second trimester, Peyer patches histologically resemble the adult structure indicating that antigen exposure or bacterial colonization are not necessary for their development; however, germinal centers do not form until after birth. Mice carrying a null mutation for TNF-α do not develop Peyer patches or lymph nodes, and splenic organization is markedly abnormal; if the 55-kDa receptor for TNF-α

is disrupted, lymph nodes and splenic tissue develop normally, but Peyer patches are still absent, suggesting that the 55-kDa receptor provides specificity for Peyer patch development. Other targeted mutations that result in the absence of Peyer patch development in mice include knockout of the inhibitory helix-loop-helix transcription factor Id2, lymphotoxins, and the lymphotoxin-β receptor. Mice lacking Peyer patches do not develop oral tolerance. Targeted disruption of the homeodomain-containing the transcription factor gene *Nkx2.3* in mice results in significant defects in intestinal development and also smaller Peyer patches and loss of expression of the mucosal addressin cell adhesion molecule 1 (MadCam1), which is normally responsible for B-cell and T-cell homing to peripheral lymphoid organs. Research has shown that full maturation of the immune system and specifically Peyer patch formation is dependent on postnatal bacterial colonization.

Lamina propria lymphocytes are first detected after 11 weeks of gestation. Macrophages are present at 12 weeks, but increase greatly in number postnatally. Recruitment and maturation of mucosal lymphocytes depend on retinoic acid, presumably produced by intestinal epithelial populations. During fetal life, lymphocytes consist of increasing numbers of scattered T cells and B cells. In contrast to αβ T cells, γδ T cells, which make up 5%–15% of small intestinal and 40% of colonic intraepithelial lymphocytes, can develop extrathymically as well as in the thymus. The γδ T cells undergo clonal expansion soon after birth but with further maturation they become clonally restricted and unique in each individual. Targeted deletion of γδ T cells in mice results in a lack of mucosal B cells that produce immunoglobulin A (IgA) but has no effect on αβ T-cell development, which is thought to occur intrathymically. IgA- and IgM-producing plasma cells are not found in the lamina propria until after birth and antigenic exposure. Intraepithelial lymphocytes appear at 11 and 12 weeks of gestation. Fetal lamina propria lymphocytes are mostly $CD4^+$ as in the adult lamina propria, and fetal intraepithelial lymphocytes are often $CD4^-$ $CD8^-$; $CD8^+$ cells become more predominant after birth.

As mentioned, exposure to the lumenal flora is necessary for maturation of the mucosal immune compartment. In rats, suckling and germ-free animals have fewer intestinal lymphocytes than adults, and weaning – associated with intestinal maturation and increasing bacterial colonization – is also characterized by marked development of the mucosal immune system. Cyclosporine (cyclosporin), an inhibitor of T-lymphocyte activation, retards normal lymphocyte development in the small intestine. Natural killer activity of intraepithelial and lamina propria lymphocytes is absent before birth, rising dramatically after weaning.

Conclusions

The genes and signals that give rise to the primitive structures of the gastrointestinal tract become progressively invested with functionality during embryogenesis and postnatal life. Some features (e.g., synthesis of pancreatic hormones, neuropeptides, and certain digestive enzymes) are largely under autonomous control, whereas other features (e.g., intestinal growth and development of mucosal immunity) are highly dependent on interactions with the environment.

Disorders of development

The previous sections have described the basic events and mechanisms that allow the normal development of the gastrointestinal tract, the pancreas, and the liver. While dysgenesis may result from disturbances of any one of these steps, errors in gastrulation or endoderm formation do not present clinically because the global importance of these early steps for further development render them lethal during embryonic development. The range of observable clinical phenotypes is therefore confined to those that are compatible with advanced embryonic development. It should be emphasized that developmental disorders involving the gastrointestinal tract are most commonly observed as part of multigenic disorders. Of these, the most common is Down syndrome (trisomy 21 syndrome), which is associated with duodenal atresia, tracheoesophageal fistula, Hirshsprung disease, and imperforate anus. Detailed molecular genetic analysis of karyotypic abnormalities and correlation with clinical phenotypes may provide further insight into developmental mechanisms. In the following sections, several disorders have been selected to illustrate key events in organ formation and organogenesis along with their (known) molecular underpinnings.

Disorders of specification and formation

Congenital gastrointestinal malformations may occur in the setting of Down syndrome or other syndromes, or they may occur as isolated findings. For example, anorectal malformations are common birth defects that may be found in isolation or as part of a syndrome, such as the VACTERL syndrome (vertebral, anal, cardiac, tracheal, esophageal, renal, and limb abnormalities). Clinical features of anorectal malformations are discussed in Chapter 66.

The etiology for most congenital malformations is unknown. Certain anomalies result from lesions in a single gene (e.g., see Box 23.3: Pancreatic agenesis), whereas others may be associated with a disruption of a signaling pathway (e.g., see Box 23.7: Meckel syndrome and the discussion of heterotopias). Another class of congenital syndromes may reflect a common final pathophysiological pathway that can be disrupted by any of a number of events. Hirschsprung disease is an instructive example of this last class.

Hirschsprung disease

As previously described, neural crest cells migrate from the neural tube during midgestation to give rise to the ganglion

cells of the ENS. Absence of these cells (aganglionosis) in the colon results in Hirschsprung disease, a male-predominant disorder that most commonly presents in the perinatal period. Absent peristalsis in the affected segment of colon causes constipation (or failure to pass meconium), distal obstruction, and megacolon. Hirschsprung disease always affects the rectum; more proximal segments are affected in a few patients, and rarely, the small bowel (see Chapter 47 for a detailed clinical discussion). Although Hirschsprung disease can be inherited in an autosomal or recessive fashion, most cases exhibit nonMendelian inheritance with a genetic component. Hirschsprung disease is commonly associated with Down syndrome.

Receptor tyrosine kinase RET

Heterozygous mutations in RET, a transmembrane tyrosine kinase (chromosome 10q11.2), represent the most common genetic alteration in persons with Hirschsprung disease. The gene for RET is expressed in ENS precursors, whereas those for its ligands (which include GDNF and neurturin [NRTN]), are expressed in the mesenchyme of the developing gut. On binding to one of its cognate ligands, RET normally activates a membrane complex that includes a glycosylphosphatidylinositol-anchored signaling component (GFRA1–4) [200]. Mutations in the RET gene cause disease by reducing kinase function, which interferes with the proper differentiation, survival, or migration of these cells. Such mutations are present in up to 50% of patients with familial disease. A small percentage of patients with sporadic disease have inactivating RET mutations, and polymorphisms in the gene may also play a role [200,207,208]. Mutations in the RET ligand GDNF have also been found in patients with Hirschsprung disease [209,210], and mutations in the NRTN ligand may contribute to disease severity [211]. Furthermore, polymorphisms in the homeobox transcription factor PHOX2B, a putative regulator of RET [212], are associated with Hirschsprung disease [213]. Consistent with a specific role for RET in neural crest cell biology, a high frequency of activating mutations occur in patients with multiple endocrine neoplasia type 2, who develop a spectrum of neural crest-derived tumors [214].

Sox10

Mutations in genes with no apparent link to RET signaling are also associated with Hirschsprung disease. One of the first models of Hirschsprung disease was Dominant megacolon (Dom), a naturally occurring mouse mutant that exhibited pigmentary defects and aganglionosis [215]. Mutations in the SRY-related transcription factor SOX10 are responsible for the Dom phenotype [216,217]. In contrast to most mouse models of Hirschsprung disease, haploinsufficiency of SOX10 is sufficient to cause colonic aganglionosis in *Sox10*$^{+/-}$ mice. *SOX10* mutations are also found in patients with Waardenburg–Shah syndrome, who exhibit Hirschsprung disease, pigmentary defects, and deafness. Thus, like RET, SOX10 also likely has a general role in the development of neural crest derivatives.

Endothelins

Mutations in endothelin 3 (EDN3) and its receptor (EDNRB) have been found in patients with isolated Hirschsprung disease or the Waardenburg–Shah syndrome. Similar to RET, EDNRB is expressed in neural crest cells before and during migration, whereas its ligand is expressed by the gut mesenchyme; mutations in these genes account for about 10% of Hirschsprung disease cases [218]. In addition, a mutation in an endothelin-processing enzyme (ECE1) has been found in a patient with Hirschsprung disease [219].

The final common pathology in Hirschsprung disease is aganglionosis so the disorder may reflect defects in the specification, migration, or survival of enteric neurons. Much work remains to be done to understand precisely how the identified genes function in normal ENS development and how mutations in these genes result in a Hirschprung disease phenotype. Given that most patients with Hirschsprung disease lack identifiable mutations, polygenic contributions are likely to be important. Alternatively, "errors" in migration, without a genetic contribution, may play a role in some cases.

Disorders of differentiation and patterning

Clinical phenotypes caused by the developmental failure to form a particular cell type are rarely observed. It is likely that many mutations affecting critical regulatory pathways (e.g., Notch signaling) are incompatible with life. Alternatively, redundancy or plasticity lead to adaptive compensatory changes that permit normal or nearly normal differentiation in a mutant background.

Instead, disorders that affect patterning leading to *misplacement* of differentiated tissues occur with some frequency. These conditions may be the result of an acquired (metaplasia) or congenital (heterotopia) tissue placement. Metaplasia is often the harbinger of malignant transformation, as mentioned in the section on the specification of the gastroeintestinal tract and discussed in greater detail in Chapter 42. While the possible mechanism of heterotopia is discussed in the following section, note that the mechanism of metaplasia is entirely unknown. In particular, it is not clear whether the premalignant intestinal epithelium that replaces the normal squamous mucosa of the esophagus is a consequence of *transdifferentiation* between the two cell types or the growth and replacement of squamous cells by a quiescent stem/progenitor cell that exists within the esophgagus.

Heterotopias

The presence of ectopic cell types (heterotopia) is observed in several tissue types, although in some cases the displacement is the result of faulty migration (e.g., subependymal heterotopia). Heterotopias involving gastrointestinal derivatives

also occur, and ectopic placement of gastric, pancreatic, and liver tissues have all been described, and may occur in the setting of congenital gastrointestinal duplications. Of the simple heterotopias, two types occur with relative frequency: inlet patches and pancreatic heterotopias.

Inlet patches consist of a segment of gastric mucosa within the cervical esophagus and occur with a frequency of up to 4.5% in autopsy studies. Inlet patches contain true gastric mucosa and most exhibit oxyntic histology. Most cases are asymptomatic, although some may be complicated by infection with *Helicobacter pylori*, inflammation, bleeding, and malignant transformation [220]. Inlet patches are sometimes associated with intestinal metaplasia and pancreatic heterotopia.

Pancreatic heterotopias, also known as pancreatic rests, consist of ectopic pancreatic tissue, most often located within the proximal gastrointestinal tract. Autopsy studies estimate their frequency to range from 0.5% to 14%, although the true prevalence is probably on the lower end of the scale [221]. As with inlet patches, most pancreatic heterotopias are asymptomatic.

Both of these conditions are believed to be congenital, but the causes are unknown. One study shed light on a possible mechanism: the segmental absence of a developmental signal. As discussed in the section on pancreatic organogenesis, a key signal during the specification of the pancreas is the *repression* of Shh expression in the endoderm. Consistent with hedgehog repression being sufficient to specify pancreatic development, the exposure of mouse embryos to the drug cyclopamine, an inhibitor of Shh signaling, results in ectopic pancreas formation with an anatomic distribution that mimics that of human pancreatic heterotopia (stomach > duodenum > small intestine; Fig. 23.8a; Box 23.5. Thus, the failure of a patch of endoderm to receive a hedgehog

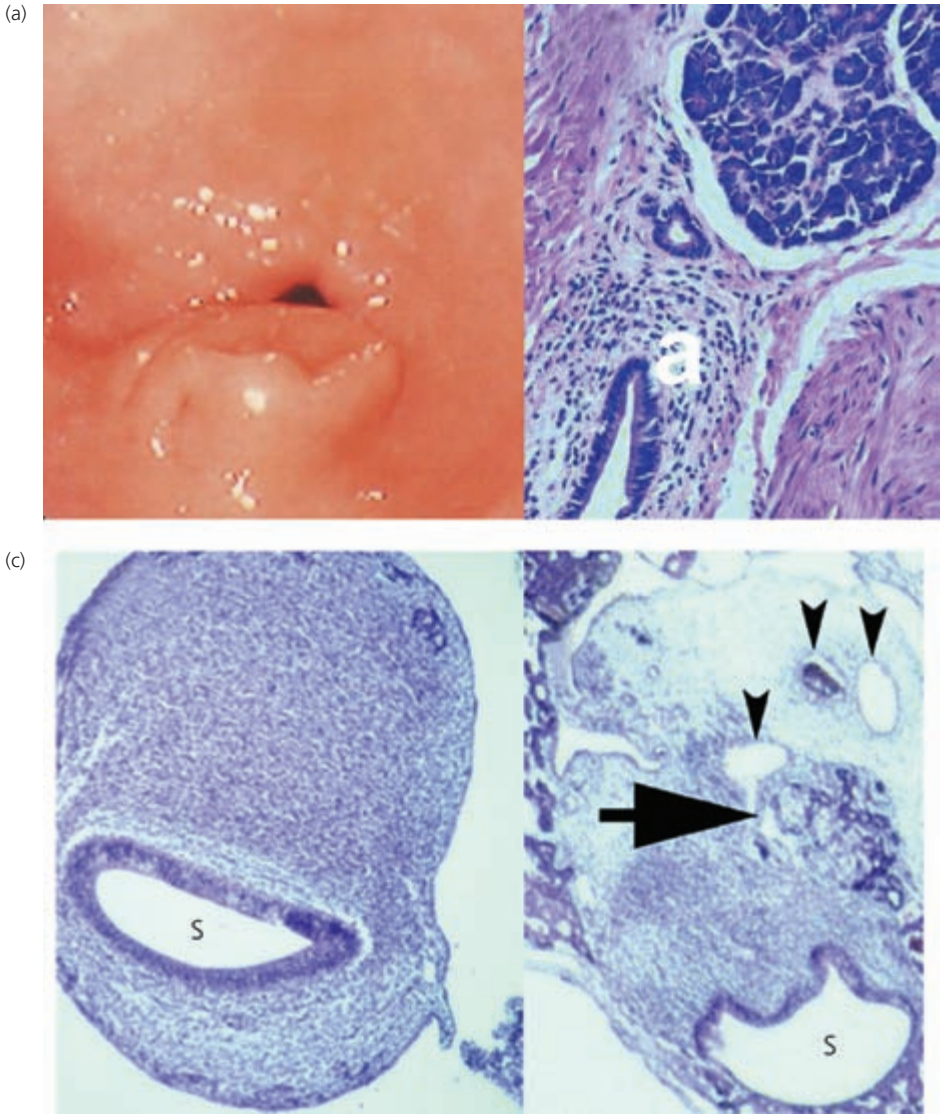
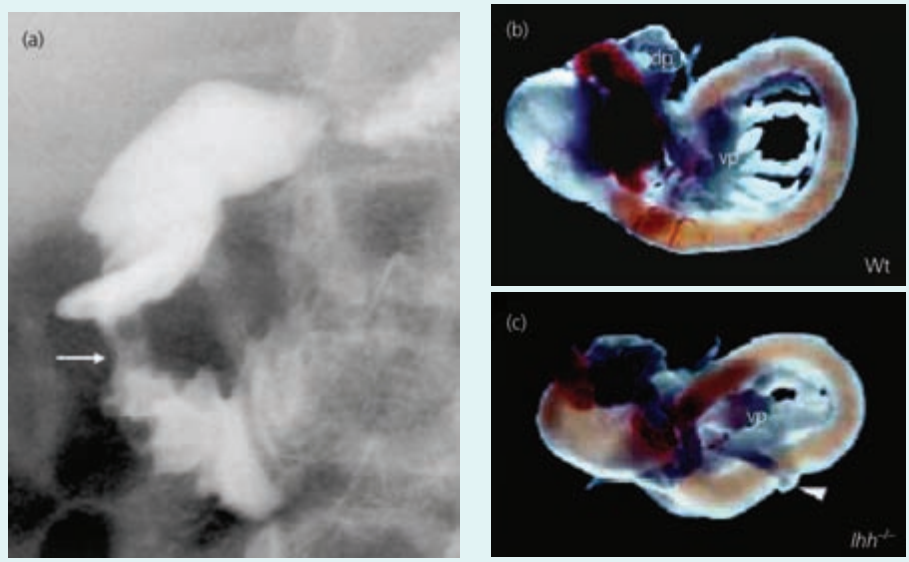


Figure 23.8 Pancreatic heterotopias (rests) in human and mouse. **(a,b)** Pancreatic heterotopia in human **(a)** Endoscopy reveals dimpling of gastric epithelium. **(b)** Histology reveals pancreatic acini (a) adjacent to gastric mucosa. **(c,d)** Mouse model of pancreatic heterotopia. Images in **(c,d)** from Kim and Melton [243], with permission. **(c)** Normal mouse stomach (S). **(d)** Mouse treated with cyclopamine exhibits pancreatic differentiation within the stomach (arrow). Arrowheads show vascular structures. **(a)** Courtesy of William R. Brugge, MD, Massachusetts General Hospital.

Box 23.5 Annular pancreas

The pancreas forms from two buds – a ventral bud and a dorsal bud – that only later fuse into a single integrated gland during the rotation of the abdominal viscera (at which time the ventral portion rotates *behind* the duodenum to meet the dorsal portion). Dysregulation of this process is thought to result in annular pancreas, a condition in which the duodenum is encircled by pancreatic tissue. Annular pancreas was first described in 1818 by Tiedemann [263] and is the most common congenital anomaly of the pancreas to present in childhood, although nearly half of cases are first recognized in adults [264], in whom it presents with early satiety, nausea, and vomiting [265,266]. In pediatric patients, the disorder is associated with other congenital anomalies, and it is more common in patients with Down syndrome.

The etiology of annular pancreas is not understood, although several theories have been proposed, including hypertrophy or failure of atrophy of the left ventral pancreatic bud, fusion of heterotopic pancreatic rests, and malrotation [264]. Others have suggested that annular pancreas is not a cause of duodenal obstruction at all, but is instead secondary to duodenal obstruction from other causes. A mouse model of annular pancreas was serendipitously discovered while looking at the role of hedgehog signaling in pancreas development. Inactivation of Indian hedgehog (Ihh) and rarely Sonic hedgehog (Shh) results in a high frequency of an annular pancreas that encircles the duodenum [267]. This observation provides an experimental framework for determining whether rare cases of familial annular pancreas [268], or the more common annular pancreas associated with Down syndrome, are caused by disruptions in hedgehog signaling.



(a) Upper GI radiograph showing narrowing of the duodenum in the area of a pancreatic annulus. **(b)** Foregut structures from a wild-type (Wt) mouse have been dissected out, revealing strands of ventral pancreatic tissue (vp) within the duodenal loop. **(c)** In mutant animals lacking Indian hedgehog ($Ihh^{-/-}$), a piece of pancreatic tissue encircles the proximal duodenum (arrowhead). Radiographic image and images from Hebrok et al. [267].

signal could result in the specification of an ectopic patch of pancreatic tissue. Similarly, the cervical esophagus could be particularly susceptible to the absence of a normally inhibitory signal during development, giving rise to an inlet patch. This presumptive mechanism could account for the development of ectopic tissues in other organs as well (see also the discussion of Meckel syndrome in Box 23.6.

Disorders of remodeling

Much is known about remodeling – the molding of patterned tissue through growth and development – in certain tissues, especially the developing central nervous system. By contrast, little is known about remodeling during gastrointestinal development. How are the vascular supplies of the intestine, pancreas, and liver tailored to physiological need? What mediates the integration of the ventral and dorsal pancreatic ductal systems (the failure of which causes pancreatic divisum)? How are the different endocrine cells in the pancreas guided to coalesce into the islets of Langerhans? Since the pathophysiology of some developmental disorders (e.g., Hirschsprung disease) may have a component of defective remodeling, the following discussion focuses on biliary tract remodeling as an example.

Abnormal biliary development

As discussed in the section on morphogenesis and cytodifferentiation in the liver, the ductal plate – two adjacent rings of specialized cells surrounding branches of the portal vein

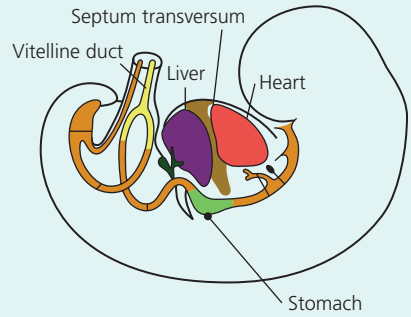
Box 23.6 Meckel syndrome

Meckel diverticulum is the most common congenital malformation of the gastrointestinal tract, occurring with a frequency of 2% of births [269]. The disorder reflects a persistence of the vitelline duct – the embryonic structure connecting the gut to the yolk sac. Meckel diverticula are generally located near the terminal ileum, and in about 50% of patients the diverticulum contains ectopic tissue, most commonly gastric or pancreatic, but occasionally also colonic, duodenal, jejunal, hepatic, and endometrial [270]. Secretion of gastric acid (and in some cases pancreatic bicarbonate) causes ulceration of adjacent small intestinal mucosa; the disorder commonly presents as unexplained gastrointestinal hemorrhage in a child or young adult (see Chapter 46; see also Fig. 23.8a). Note that heterotopia is distinct from metaplasia, which represents an acquired replacement of one tissue type with another over time.

What mechanism might account for the defective patterning leading to heterotopia?

Bossard and Zaret [271] observed that 3% of mouse embryos exhibit an albumin-expressing ectopic bud at the site of the vitelline duct, near the terminal ileum, which led them to propose that Meckel diverticula result from the loss of normal mesenchymal inhibitory signals at the site of the vitelline duct. According to this attractive model, heterotopic tissue forms not as a result of ectopic cells "left behind" by the nonregressed vitelline structure, but because a signal required for patterning and specification was disrupted by the error in regression. (See section on heterotopias in Disorders of development.)

(a)



(b)

(a) Gross specimen showing a Meckel diverticulum in the embryo. Courtesy of Beth Furth, University of Pennsylvania School of Medicine. **(b)** Embryonic vitelline duct.

– gives rise to the intrahepatic bile ducts. Ductal plate remodeling appears to occur in two steps:

- formation of discrete tubules within the two-cell-thick layer of the ductal plate
- elimination of remaining cells through apoptosis or attrition.

The intrahepatic bile ducts anastomose with the extrahepatic biliary system (the origin of the extrahepatic system is unclear but it may derive directly from the gut).

Developmental or neonatal biliary disorders fall into two categories: ductal plate malformations and bile duct paucity. Ductal plate malformations refer to a collection of overlapping disorders that are characterized by faulty remodeling of the intrahepatic bile ducts, resulting in the persistence of the embryonic ductal plate configuration (see Fig. 23.5; [222]). Congenital hepatic fibrosis is an autosomal recessive disease with variable histological and clinical features in which the portal tracts and bile ducts exhibit fibrosis and a ductal plate configuration. The histopathology of congenital hepatic fibrosis is seen in association with both autosomal recessive and autosomal dominant polycystic kidney disease. A related disorder, Caroli syndrome, is characterized by the ductal dilation of Caroli disease (type IVA/type V choledochal cysts, see Chapter 76) with the superimposed fibrosis of congenital hepatic fibrosis, suggesting an overlapping pathophysiology [222]. While the shared histopathological characteristics observed in these and similar abnormalities (e.g., von Meyenburg complexes) are intriguing, the etiology of these disorders remains completely unknown.

Insight into one potential mechanism for biliary malformation comes from studies of patients with a paucity of

intrahepatic bile ducts, also known as Alagille syndrome. Although bile duct paucity is the sine qua non of Alagille syndrome, patients may also have several extrahepatic manifestations, including abnormalities of the great vessels, skeletal and ocular malformations, as well as characteristic facies (see Chapter 108). Two studies in 1997 showed that mutations in the Notch ligand JAG1 are responsible for Alagille syndrome and strengthened the link between this developmental signaling pathway and the disease [69,70].

Consistent with the hypothesis that the formation of the ductal plate is induced by Notch signaling, JAG1 is expressed by portal veins and hepatic arteries [223–225], and mice with compound heterozygous *Jagged*/*Notch* mutations exhibit a paucity of intrahepatic bile ducts [226]. These studies suggest that Alagille syndrome may result from the faulty specification of bile ducts in the absence of Notch signaling. However, an analysis of ductal plate development in mice with defective Notch signaling suggests that Notch has a more direct role in remodeling. Embryos deficient in the Notch target Hes1 develop ductal plates with normal appearance at the appropriate developmental time, but these ductal plates fail to form the tubular structures that precede normal duct development [227].

Notch may therefore mediate biliary development in at least one of two ways. Notch signals may serve as the inductive signal emanating from portal veins or they may be involved in the remodeling of the biliary system. Either mechanism is compatible with the hepatic phenotype observed in Alagille syndrome. As previously mentioned, the onecut transcription factors HNF6 and OC2 play an important role in biliary specification. As 30% of patients with Alagille syndrome lack *JAG1* mutations [228], it is possible that disease-causing mutations in these onecut transcription factors or in other elements of the Notch pathway remain to be discovered.

Biliary atresia, a more common syndrome of bile duct paucity, is characterized by the loss of the extrahepatic bile ducts (see Chapter 73). Biliary atresia is a heterogeneous disorder that presents with two major clinical patterns – a prenatal form that presents almost immediately after birth and is associated with other congenital anomalies, and a perinatal form that presents in the first few weeks of life. Although the etiology of both forms is poorly understood, defective morphogenesis of the bile ducts may play a role in the prenatal form of the disease [229]. Congenital anomalies affecting body symmetry, such as cardiac anomalies, intestinal malrotation, and abdominal situs inversus, often accompany the prenatal form of biliary atresia [230]. Mice with a mutation of the *inversin* gene exhibit abdominal situs inversus and a defective extrahepatic biliary tree [231,232]. Furthermore, missense mutations in *JAG1* have been observed in patients with severe refractory biliary atresia, suggesting that this Notch ligand contributes to disease progression [233]. Consistent with a connection between intra- and extrahepatic ductal pathology, some patients with biliary atresia exhibit the histological characteristics of ductal plate malformation observed with congenital hepatic fibrosis [222].

Disorders of growth control

Several rare disorders that affect the growth of specific parts of the body highlight another developmental phenomenon: genomic *imprinting*. In mammals, which contain sets of paired chromosomes, the maternally inherited chromosome differs from the paternally inherited chromosome both in terms of primary sequence (polymorphisms) and in additional epigenetic (noninherited) differences. Epigenetic differences are conferred by DNA methylation, a process that occurs early in embryonic development and results in the differential expression of genes from maternal and paternal alleles. Imprinting is enormously important in normal development, and improper allele-specific methylation is a major cause of defective embryos and newborns after nuclear transplantation (cloning). Several human disorders that exhibit growth abnormalities and an increased cancer susceptibility are linked to abnormalities in genomic imprinting, and we will focus on one of these: Beckwith–Wiedemann syndrome.

Beckwith–Wiedemann syndrome

This disorder is characterized by variable growth defects, including generalized overgrowth (pre- and postnatal) as well as macroglossia, visceromegaly, and hemihypertrophy (enlargement of one half of the body). Patients with Beckwith–Wiedemann syndrome have an increased frequency of several tumors, including Wilm tumor, hepatoblastoma, and pancreatoblastoma. In the last decade, it has become clear that Beckwith–Wiedemann syndrome is linked to chromosome 11p15, a region containing several imprinted genes.

Two genes in this imprinted region are thought to play a causative role in Beckwith–Wiedemann syndrome: *CDKN1C* (a negative regulator of cell proliferation that acts by inhibiting cyclin-dependent kinase) and *IGF2* (a major regulator of fetal growth). Classical mutations of either of these genes affect growth. For example, mutations in CDKN1C have been described in patients with Beckwith–Wiedemann syndrome [234], and the overexpression of *IGF2* in mice is sufficient to cause an overgrowth syndrome [235]. However, the more common mechanism of gene activation (*IGF2*) or inactivation (*CDKN1C*) is related to abnormalities in methylation-dependent imprinting.

Under conditions of normal imprinting, *CDKN1C* is expressed from the maternal allele and *IGF2* is expressed from the paternal allele. Two different patterns of abnormal imprinting are associated with the development of Beckwith–Wiedemann syndrome. In most cases, abnormal methylation results in the loss of *CDKN1C* expression from both alleles, whereas in a few cases, abnormal methylation results in *IGF2* expression from both alleles [236]. Notably, the converse pattern of dysregulated methylation (resulting in loss of *IGF2* expression from both alleles) is associated with Silver–Russell

Box 23.7 Unanswered questions in gastrointestinal development

What makes the endoderm endoderm? After gastrulation, the cells of the endoderm are uniquely competent to give rise to the gastrointestinal organs. What intrinsic properties of the cell confer this restricted competence? A favored view is epigenetic modification, including chromatin condensation.

What patterns the endoderm? How are the various organ domains partitioned and coordinated with the remaining "body plan" (e.g., skeleton)? Epithelial–mesenchymal signaling clearly plays a key role in coordinating patterning. Although some of the ingredients involved in such signaling have been identified, the recipe is far from complete.

How are three-dimensional structures generated (i.e., morphogenesis)? Complex organized three-dimensional structures arise from simple epithelial buds. In the liver, for example, the three circulatory systems (arterial, portal venous, central venous) must be correctly organized in space with the canalicular and ductal biliary systems. How is this spatial organization achieved with such precision?

What are the differences between embryonic and adult intestinal stem/progenitor cells? Embryonic and adult stem/progenitor cells exhibit different features. Adult intestinal stem cells can give rise to all epithelial cell types. Further, they can mediate villus elongation but cannot mediate gut tube elongation in the manner of embryonic cells. It is unclear whether embryonic gut epithelial cells are true stem cells or committed progenitors with a limited capacity for self-renewal.

Are there adult stem/progenitor cells in the pancreas and liver? Is all tissue maintenance in the pancreas and the liver mediated by cell replication? What is the role, if any, of "facultative stem cells" (e.g., oval cells) in the response to certain types of pancreatic or hepatic injuries?

What are the signals that stimulate functional maturation? The intestine undergoes dramatic physiological changes both before and after birth. Some of these changes appear to be responsive to changes in the environment (e.g., intestinal growth and adaptation), whereas others appear to be "intrinsic" (e.g., the timing of brush-border enzyme expression). To what extent are these functional parameters responsive to environmental changes (and therefore amenable to therapy) and are there underlying principles that dictate which aspects of gastrointestinal physiology are intrinsic or regulated?

What is the mechanism of metaplasia? Does metaplasia arise from the transdifferentiation of one cell type into another (e.g., squamous esophageal epithelium to columnar intestinal epithelium) or does a metaplastic tissue arise from rare progenitor cells (e.g., a rare intestinal stem cell that resides in the esophageal mucosa)?

What is the cellular basis of gastrointestinal cancers? Do cancers arise from normal adult stem cells in adult tissues or from differentiated cells that "dedifferentiate" to acquire the characteristics of stem cells? Are such cancers maintained by "cancer stem cells" – rare cells within the tumor that have an enhanced or unlimited capacity for self-renewal?

syndrome, a congenital disorder characterized by growth retardation and asymmetry [237–239]. Although the mechanism by which dysregulation of *CDKN1C* or *IGF2* results in isolated growth phenotypes is not known, it is likely that alterations in cell proliferation underlie both the abnormal growth and the tumor propensity in patients with Beckwith–Wiedemann syndrome.

Conclusions

Despite a detailed conceptual framework for understanding the events that govern normal patterning, organogenesis, and physiological adaptation of the gastrointestinal tract, the pathogenesis of congenital disorders of the gastrointestinal tract is poorly understood, reflecting the numerous questions about gastrointestinal development that remain unanswered (Box 23.7). To date, most insights have come from human (reverse) genetics and serendipitous similarities between animal and human phenotypes. Specific challenges to further advances include the association of many developmental disorders with complex genetic syndromes and the separation in time between a developmental lesion and its phenotypic manifestations.

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E MISCELLANEOUS

24 Neoplasia of the gastrointestinal tract

Barbara H. Jung, C. Richard Boland, John M. Carethers

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The gastrointestinal tract, including the hollow organs of the gut, pancreas, liver, and biliary tree, is the site of more cancers and the source of more cancer mortality than any other organ system in the body. However, no simple etiological explanation can unify all gut tumors.

The most notable feature of international cancer epidemiology is the wide variability of tumor incidence from country to country by organ site. For example, an esophageal cancer belt extends from northeastern China through central Asia into northern Iran [1]. In parts of these regions, the incidence of squamous cell carcinoma of the esophagus is more than 100-fold higher than that in adjacent low-incidence regions. In the United States, three- to fourfold differences in the incidence of esophageal cancer occur between the sexes and among races [2]. In Japan, the incidence of gastric carcinoma is about 10 times higher than it is in the United States. Colorectal cancer was historically less common in Japan, compared to being the most common gastrointestinal malignancy in North America, Western Europe, and much of the industrialized world. However, over the past decade, the incidence of colorectal cancer in Japan has risen to the same level as, or perhaps higher than, in the West [3]. These marked differences in cancer risk are not based on racial or genetic factors. When people migrate from a high-incidence region to a low-incidence region, the organ-specific rates of some cancers change to match that of the new region, usually within two generations. Collectively, the epidemiological observations strongly indicate the importance of environmental factors in gastrointestinal carcinogenesis; however, individual genetic differences may influence the effects of these factors.

Worldwide, people are exposed to different types of foods, the gastrointestinal flora differs as a result of dietary and host factors, and certain mucosal infections occur with different frequencies. Gastrointestinal carcinogenesis, therefore, is a complex problem to understand and to modify. For example, tobacco smoke causes cancers of the oral and respiratory tracts, and by eliminating smoke from the environment, the risks of tumor development can be controlled. However, everyone consumes some kind of diet, and the elimination of specific items results in the introduction of replacement nutrients. Moreover, the diet consists of a complex combination of macronutrients (i.e., fat, carbohydrate, protein, fiber) and micronutrients (i.e., vitamins, minerals, other agents present in tiny concentrations) that affect cancer risk. Directly acting carcinogens or indirectly acting procarcinogens also are part of each person's diet. Mucosal infections with human papillomavirus and *Helicobacter pylori* probably contribute to the genesis of cancers of specific gastrointestinal organs.

Ultimately, changes at the genetic level can alter the growth characteristics of healthy gastrointestinal cells. Although many deleterious genetic events must occur for a normal cell to become malignant, inactivation of a gatekeeper gene or a pathway specific to each tissue may be required to initiate the neoplastic process [4]. This concept is evident in familial syndromes such as familial adenomatous polyposis, in which thousands of benign colonic adenomas develop at a young age; it can also be applied to the development of sporadic colorectal cancer. After the initial gatekeeper alteration, successive genetic events propel the cell toward the malignant phenotype. The loss of gatekeeper function and the successive genetic events lead to an uncoupling of cellular growth control mechanisms that typically regulate a normal gastrointestinal cell. These include loss of cell cycle control checkpoints, abrogation of programmed cell death, and dysregulation of intracellular signaling pathways. Some genetic perturbations have been identified as markers for the presence of neoplasia as well as prognostic factors for malignancy, but their clinical use has not been fully evaluated.

Our understanding of carcinogenesis in the gastrointestinal tract is far from complete so it is necessary to understand the principles of regulation of normal, nonneoplastic gastrointestinal cell growth before considering the abnormal events. The mechanisms of tumor development appear to be distinct among the gastrointestinal organs, but general pathogenetic principles apply at the cellular and molecular level for most tumors.

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Normal cellular control mechanisms

Cell cycle checkpoints

The cell replication cycle consists of five phases [5]. Resting diploid cells not involved in active replication may be in the G0 or G1 phase. G1 represents the postmitotic period before the initiation of new DNA synthesis as the cell prepares to divide. The cell may spend a variable period of time in G1. Cells not involved in replication may be described arbitrarily as resting in the G0 phase. After receiving the signal to divide, cells enter a phase of active DNA synthesis, the S phase. After duplication of the entire genome, the tetraploid cell pauses briefly in the G2 phase before entering mitosis (the M phase). After cell division into two identical daughter cells, the cell reenters the G1 phase. As the genome becomes more complex with tumor progression, the S and M phases may be prolonged, altering the apparent fraction of cells involved in replication. This complicates a direct comparison of the proliferative activity of tumors with that of normal tissue.

Each transition from one phase of the cell cycle to the next is regulated by a family of cyclin-dependent kinases (CDKs) and their activating partners, the cyclins (Fig. 24.1). The CDK–cyclin complexes are positively and negatively regulated by phosphorylation. The formation and activation of a CDK–cyclin complex requires proper completion of the previous cell cycle phase before the subsequent phase is entered [6,7]. If DNA is damaged by ultraviolet light in the G1 phase of the cell cycle, passage into the S phase will not occur until the damage is repaired. Likewise, if DNA is not replicated with complete fidelity during the S phase, passage into G2 and mitosis are prohibited until an exact copy of the DNA is duplicated. The CDK–cyclin complexes receive input from other cellular proteins that relay information regarding the integrity of each checkpoint. The gene products of the tumor suppressor genes retinoblastoma (*RB1*) and *TP53*, Rb protein and p53, relay information to G1 phase CDK–cyclins to regulate the initiation of DNA synthesis (S phase) [7]. CDK inhibitors, such as $p21^{WAF1/CIP1}$, regulate specific CDKs to prevent entry into the next phase of the cell cycle and to allow repair of damaged DNA during cell cycle arrest [8]. These cell cycle checkpoints are lost in oncogenesis. The CDK–cyclin complexes themselves may be altered in some tumors and, in the gastrointestinal tract, changes occur in the regulators that relay information to CDK–cyclin complexes. These genetic perturbations allow the neoplastic cell to cycle with disregard for mutations and chromosomal rearrangements, essentially establishing progeny cells that differ from the parent. This generation of genetic diversity is a form of genomic instability and a hallmark of oncogenesis.

Programmed cell death

Deregulated proliferation is the sine qua non of the neoplastic process. An increase in the proliferation rate of cells can occur either by increased mitosis or by a decreased cell death rate. Programmed cell death is a physiological process that plays an essential role in normal tissue turnover and embryonic development [9]. This process has been shown to require energy, macromolecular synthesis, and de novo gene transcription [10]. The morphological changes of programmed cell death, first described in 1972 by Kerr and colleagues [11] and termed *apoptosis*, affect single scattered cells

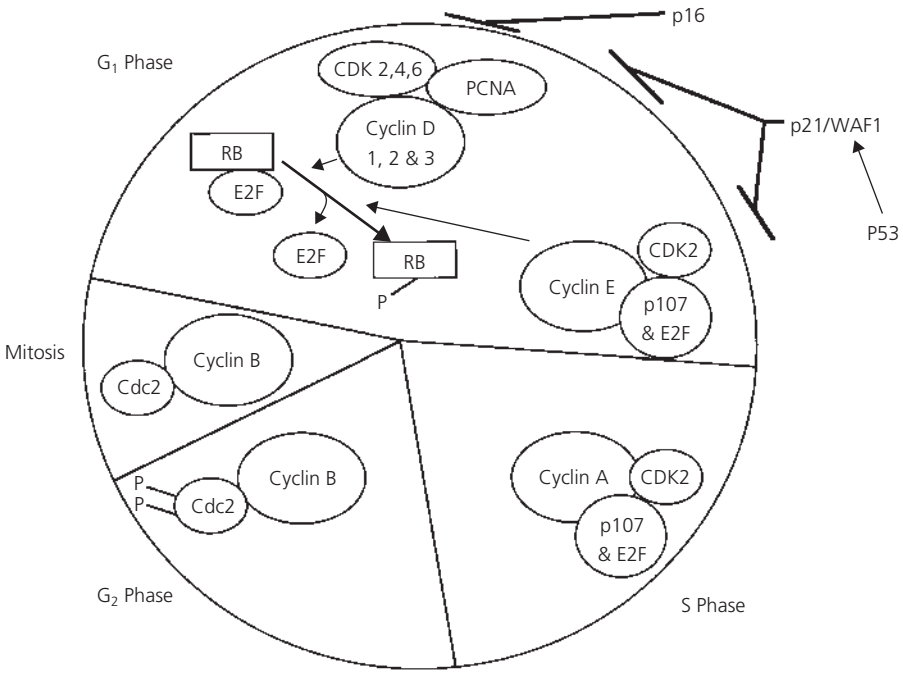

Figure 24.1 Cell replication cycle. Transition through each phase of the cell cycle is regulated by cyclins and cyclin-dependent kinases (CDKs) to ensure completion of one phase before initiation of the subsequent phase. Each cyclin–CDK complex is positively and negatively regulated by phosphorylation. Inhibitors of CDKs include p21 and p16, both of which prevent transition to the S phase of the cell cycle. The transcriptional regulator E2F transactivates genes that are important for S phase entry. E2F is bound by the retinoblastoma protein (RB) until RB is phosphorylated, thus releasing E2F. RB phosphorylation is initiated by cyclin D-dependent kinases and accelerated by cyclin E–CDK2 complexes. P, phosphorylated; PCNA, proliferating cell nuclear antigen.

with characteristic changes in the nucleus (i.e., karyorrhexis), preservation of cytoplasmic organelles, cellular shrinkage, and the formation of apoptotic bodies. The initiation of programmed cell death may involve a sustained increase in intracellular calcium, which activates several calcium-dependent enzymes. These enzymes, in turn, activate stored or newly synthesized transglutaminases (involved in the cross-linking of cytoplasmic proteins), proteases (e.g., caspases) that aid in disruption of the cytoskeleton and cell shrinkage, and endonucleases that cleave DNA into uniformly sized fragments. Once activated, programmed cell death is irreversible.

Activation of a cell death program is best illustrated by the normal turnover of the colonic epithelium. In healthy colonocytes, the surface epithelium is constantly renewed about every 6 days by cellular proliferation and differentiation of crypt cells. The proliferating compartment is in the lower third of the crypt, characterized by mitoses and upward migration from anchored stem cells. As the cells move up the crypt, differentiation and maturation occur, and the capacity to divide is lost. Eventually, cells die and are shed. In this way, programmed cell death of colonocytes maintains a uniform number of cells in the mucosa. The expression of *APC*, the adenomatous polyposis colon gene, controls, in part, the programmed cell death of colonocytes [12], functioning to maintain a normal rate of cell turnover. Other cellular proteins, such as those of the *BCL2* gene family, may also play significant roles in programmed cell death in the gastrointestinal tract.

Activation of programmed cell death is the target of many therapeutic interventions in the treatment of gastrointestinal tumors. Both radiation and chemotherapy can induce apoptosis in tumors of the gastrointestinal tract, in addition to direct toxic tissue injury (i.e., necrosis) [9]. Apoptosis has been proposed to be the mechanism by which the nonsteroidal antiinflammatory agent sulindac and the cyclooxygenase-2 inhibitor celecoxib lead to the involution of colonic adenomas in patients with familial adenomatous polyposis [13,14]. Interference with programmed cell death may be as important as deregulated cell proliferation in some gastrointestinal tumors.

Intercellular and intracellular growth control pathways

Cells that make up the tissues of the gastrointestinal tract are in contact with neighboring cells to form a tight epithelial barrier. In addition to functioning as a barrier, the epithelial junctions that interconnect neighboring cells distribute information about the cell's local environment, such as the presence of a contiguous cell (e.g., cell–cell adhesion and recognition at the zona adherens), and they may allow the direct passage of ions and small molecules through specialized pores called *gap junctions* [15]. Passage of ions and small molecules through gap junctions allows cells to be coupled and permits groups of cells to behave as a syncytium in regard to ion transfer. Cell–cell adhesion is primarily mediated through the intracellular adhesion molecule E (epithelial)-cadherin, which forms homeotypic interactions (i.e., with other E-cadherin molecules) and anchors the zona adherens to the actin cytoskeleton inside the cell [16]. Dysregulation of cell–cell adhesion disrupts normal intercellular communication and allows transformed cells to separate from each other and to spread locally, invading regional tissue. Proteins from the catenin family (α, β, γ) bind to E-cadherin and to the actin cytoskeleton, but any signal relayed by the catenins for cellular adhesion is not known. Importantly, β-catenin, a protooncogene that can transactivate genes associated with cellular proliferation, has its cytoplasmic levels regulated by the adenomatous polyposis coli (APC) protein when its intercellular concentration rises (Fig. 24.2) [17–19]. APC targets β-catenin for degradation; and if β-catenin escapes this homeostatic regulation, it can move to the nucleus, where it associates with T-cell factor-4 (TCF-4) to transactivate genes that promote cell growth and prevent programmed cell death.

Another mode of intercellular communication exists between epithelial cells and other cells that line the gastrointestinal tract. Growth factors are proteins that bind as a ligand to a receptor on the same (autocrine) cell or neighboring (paracrine) cell. Growth factors modulate growth characteristics of the cell. Once the growth factor is bound to its specific receptor, an intracellular signal is amplified and transmitted to the nucleus of the cell to induce or inhibit gene activation; this in turn modifies growth characteristics, metabolism, and ion transport. Although many growth factors use tyrosine phosphorylation at the start of their intracellular signaling pathway, other ligand–receptor complexes often use other pathways, such as serine/threonine phosphorylation, calcium and phosphoinositol formation, and the generation of cyclic nucleotides. Two important examples highlight the signal transduction process in normal and neoplastic growth in the gastrointestinal tract: the KRAS and transforming growth factor-β1 (TGF-β1) signaling pathways.

The protooncogene Kirsten-RAS (*KRAS*) encodes an intracellular protein that binds the cyclic nucleotide guanosine tri- or diphosphate (GTP/GDP). On cell surface ligand–receptor activation (e.g., epithelial growth factor binding to its receptor), the KRAS protein is activated and exchanges the bound GDP for GTP (Fig. 24.3). Active KRAS molecules then interact with downstream intracellular molecules, transmitting a growth response to the nucleus. To turn off this activity, KRAS is deactivated by the hydrolysis of GTP to GDP, which returns KRAS to the inactive GDP-bound state [20]. This signal transduction process is perturbed with a mutant KRAS protein. Stabilization of mutant KRAS in its active state permits continuous signal transduction, allowing the cell to undergo unregulated proliferation. This process may be caused by mutations that inhibit the intrinsic GTPase

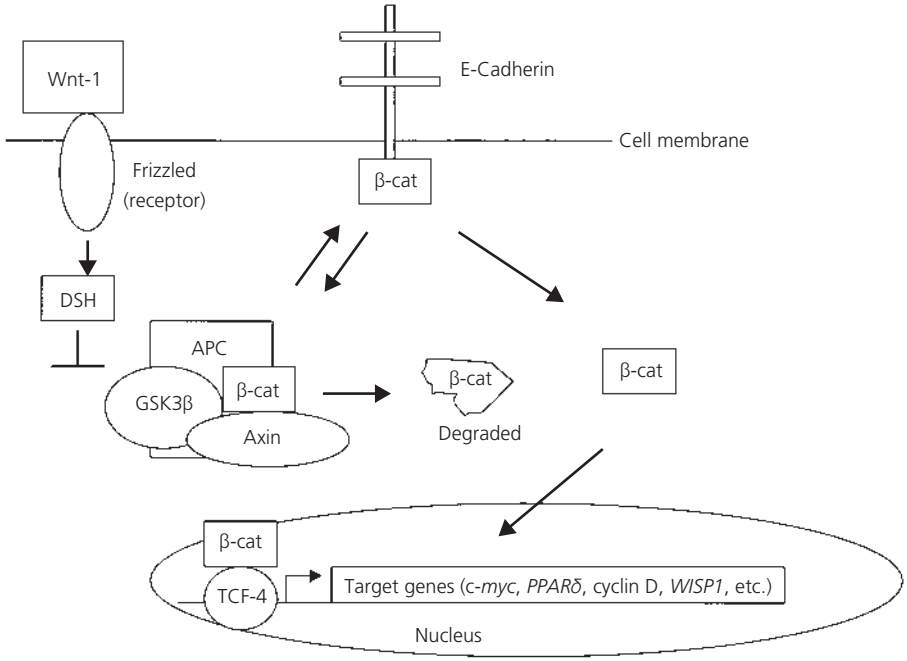

Figure 24.2 Adenomatous polyposis coli gene (*APC*) regulation of β-catenin and its function as a tumor suppressor gene. Normal APC protein, in association with axin and glycogen synthase kinase-3β (GSK3β), binds, phosphorylates, and targets cytoplasmic β-catenin (β-cat) for degradation. Degradation of β-catenin prevents its translocation into the nucleus, where it can initiate the transcription of genes by converting the transcriptional repressor T-cell factor-4 (TCF-4) to a transcriptional activator to promote cellular proliferation and prevent cell death. Cytoplasmic β-catenin can bind to E-cadherin, usually in association with α-catenin. During embryogenesis, blockage of APC-mediated degradation can occur through the Wnt-1 signaling pathway (through the frizzled receptor and disheveled [DSH] signaling protein). Presumably, inactivation of APC would have the same consequences as Wnt-1 signaling.

activity of KRAS, allowing KRAS to stay in its active conformation, or by inducing an active conformation that does not require the binding of the guanine nucleotides [20]. Indeed, the most common mutations of *KRAS* in pancreatic and colon cancers involve codons 12, 13, and 61, corresponding to areas in the GTP/GDP binding domains of the KRAS protein [20]. In pancreatic cancers, mutant *KRAS* has been reported in 95% of tumors [21,22], whereas it occurs in about half of colorectal cancers [23–25] and is uncommon in gastric cancers [26]. *BRAF*, one of three known *Raf* genes, acts as a downstream RAS activator and is somatically mutated in a wide range of cancers [27]. In colon cancer, *BRAF* mutations occur at a high rate in microsatellite unstable colorectal tumors [28] that contain an epigenetic alteration of *hMLH1* [29].

TGF-β1 is a ligand that inhibits the growth of gastrointestinal epithelial cells [30]. To exert its growth-suppressive effect, TGF-β1 in its latent form is activated through an interaction with the insulin-like growth factor II receptor (IGF2R) present on the cell surface (Fig. 24.4) [31]. Activated TGF-β1 then binds to the TGF-β1 type II receptor (TGFBR2), which subsequently phosphorylates (as a serine/threonine kinase) the TGF-β1 type I receptor (TGFBR1), forming a heterotrimeric complex of TGF-β1, TGFBR2, and TGFBR1 [32,33]. The conjoined receptor complex then phosphorylates (as a serine threonine kinase) intracellular proteins that cascade to ultimately affect the transcription of growth-suppressing genes in the nucleus. In particular, SMAD2 (SMAD, small mothers against decapentaplegic) and SMAD3 are phosphorylated by activated TGFBR1, which allows them to bind individually to SMAD4 and subsequently translocate to the nucleus. Similarly, activin, a TGF-β family member, binds to its primary receptor, the activin type II receptor (ACVR2), which in turn binds to and phosphorylates the activin type I receptor (ACVR1). This leads to downstream SMAD phosphorylation, association with SMAD4, and translocation to the nucleus, stimulating the transcription of genes involved in growth suppression (Fig. 24.4) [34]. Ligands from another TGF-β family member, bone morphogenetic proteins (BMPs), bind to another serine/threonine kinase type I receptor (BMPRIA) and type II receptor (BMPRII) (35), which then induce phosphorylation of SMAD1, SMAD5, or SMAD8, association with SMAD4, nuclear translocation, and the ensuing transcriptional effects [361,37]. Inactivation of these pathways occurs in some gastrointestinal tumors. For example, many colonic and gastric tumors that exhibit microsatellite instability – a laboratory marker for the inactivation of the DNA mismatch repair (MMR) system – develop mutations in *TGFBR2* [38–40] or *ACVR2* [41] or in both [42]. Other tumors with microsatellite instability but with normal *TGFBR2* have mutations in *IGF2R* [43]. These mutations prevent expression of the receptor on the cell surface. The absence of either TGFBR2 or IGF2R prevents activation and binding of the TGF-β1 ligand and abrogates its growth-suppressive action. Some colon tumors without microsatellite instability have mutations in the kinase domain of *TGFBR2* [44]. Germ-line mutations in *BMPRIA* cause juvenile polyposis, an autosomal dominantly transmitted hamartomatous polyposis syndrome associated with an increased risk of colon cancer [45]. Inactivation of the potential downstream signaling proteins SMAD4 and SMAD2 also has been found in some gastrointestinal cancers [46,47]. Likewise, germ-line mutations in the downstream effector of BMP signaling,

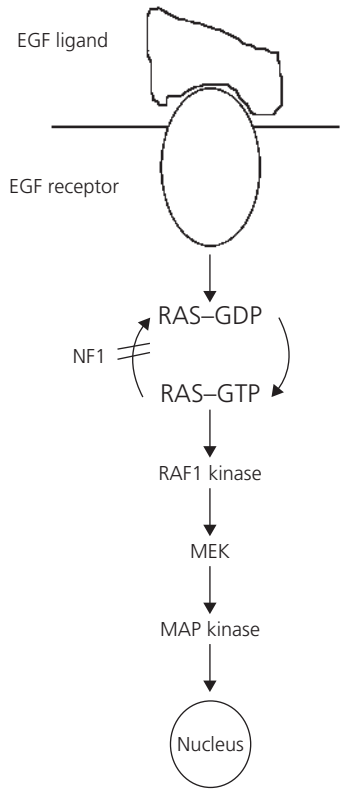

Figure 24.3 RAS intracellular signaling. Cell surface ligand binding to a receptor, such as epithelial growth factor (EGF) to the EGF receptor, triggers the activation of GDP-bound RAS and the exchange of GDP for GTP. RAS–GTP can activate subsequent steps in the intracellular signaling cascade (RAF1 kinase → mitogen-activating protein [MAP] kinase kinase [MEK] → MAP kinase), which leads to the expression of genes that promote cellular proliferation. The tumor suppressor protein neurofibromatosis 1 (NF1) is an activating protein for RAS, keeping RAS in the GTP-bound state. The activation of RAS by mutation allows continued signaling of this pathway.

SMAD4, may lead to juvenile polyposis [48]. The key point is that essential growth-regulatory pathways may be deactivated at any one of several points in the serial cascade.

TGF-β1 may also induce proliferation in colon cancer cells [49], and somatic mutations of *TGFBR2* microsatellite unstable tumors have been reported to predict improved survival [50]. These observations indicate that TGF-β1 may be influencing proliferative as well as suppressive pathways, and when the suppressive pathway is inactivated by somatic mutation, proliferation may become the dominant phenotype.

RNA and protein degradation

Cells can regulate gene products at the posttranscriptional level. Specific inhibitory RNA molecules can bind and target transcripts for degradation [51] as a way to control transcript output; a process that can become dysregulated in neoplastic transformation. Likewise, targeted protein degradation, which can occur much faster than protein synthesis, can control cellular protein levels. Ubiquitination, which involves the addition of multiple 76-amino-acid ubiquitin monomers to proteins, is a major pathway of protein degradation. Proteins with polyubiquinated tails are recognized and processed by the 26S proteasome, which recycles the ubiquitin [52]. Alterations in ubiquitination have been postulated to be associated with loss of growth regulation, as evident in a subset of colon cancers with ubiquitination-resistant β-catenin mutations [53].

Telomerase

The shortening of telomeres at the end of chromosomes during aging causes a decrease in cellular replication. Tumor cells may acquire immortality by continuous telomere maintenance that is predominantly the result of the transcriptional up-regulation of the limiting component of telomerase, hTERT (human telomerase reverse transcriptase). Oncogenic activity is known to stimulate the hTERT promoter [54]. Furthermore, there is evidence that telomerase plays a role in other aspects of carcinogenesis, as silencing of hTERT increases apoptosis [55]. Inhibition of telomerase has become an attractive target for cancer therapeutics [56].

Many of these regulatory pathways are active simultaneously. They are often redundant and sometimes interconnected, but they typically provide a homeostatic balance for normal cellular growth. Alteration of one or more of these pathways may lead toward cellular proliferation and away from controlled growth and regulated cell turnover by programmed cell death, initiating and exacerbating transformation into neoplastic cells. The complexity of cellular growth mechanisms affords the neoplastic cell many opportunities for a pathway to become deregulated. In gastrointestinal carcinogenesis it appears that more than one pathway is affected, which may be necessary for transformation. Specific pathway inactivations are more common in certain types or subtypes of tumors, creating opportunities for therapeutic intervention of gastrointestinal tumors as biotechnology advances.

Molecular carcinogenesis

Mechanisms by which DNA is damaged

The current concept of carcinogenesis in the gastrointestinal tract begins with damage to normal cellular genes. This can occur through a variety of mechanisms. Mutations occur spontaneously because of predictable instability of the purine and pyrimidine bases themselves. The most common spontaneous mutation is depurination, which occurs after disruption of the *N*-glycosyl linkages between the purine base and the ribose chain. In another spontaneous event, deamination, cytosine is converted to uracil, or 5-methylcytosine to thymine. These single base mutations, if not repaired, lead to nucleotide complementary mispairing during the next

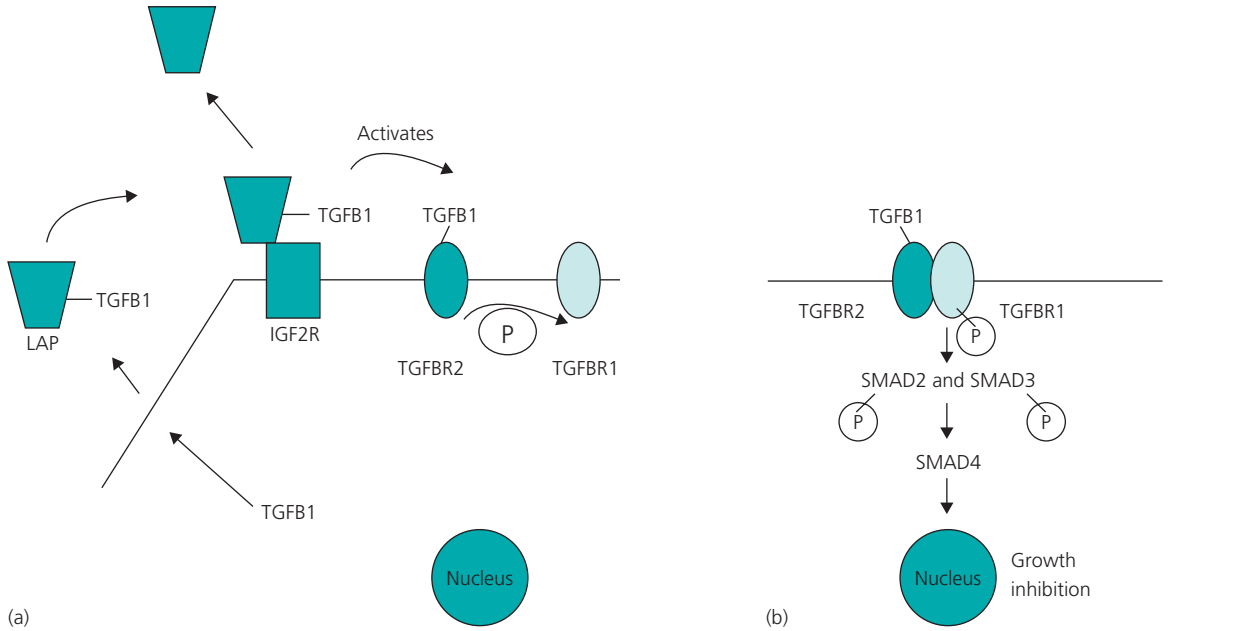

Figure 24.4 Transforming growth factor β-1 signaling. **(a)** TGF-β1 can be produced in an autocrine fashion by epithelial cells, and it is bound by a latency-associated peptide (LAP) once outside the cell. To activate TGF-β-1, LAP is cleaved by the insulin-like growth factor II receptor (IGF2R), leaving TGF-β1 free to bind to the TGF-β1 receptor type II (TGFBR2). This action, in turn, phosphorylates (P) the TGF-β1 receptor type I, which then forms a heteromeric complex with TGFBR2. **(b)** Subsequent intracellular signaling of TGF-β1 (and the TGF-β family member activin) to the nucleus occurs through the SMAD family of proteins, ultimately causing growth suppression. Mutations in *IGF2R*, *TGFBR2*, *ACVR2*, *SMAD2*, and *SMAD4* have been described that uncouple the growth-inhibitory effects of TGF-β1 on the colonocyte.

round of replication. Both events are common and subject to revision by families of DNA repair enzymes.

Several environmental factors predictably damage DNA, including viral infections, chemical carcinogens, and radiation in the form of ultraviolet radiation, ionizing electromagnetic radiation such as x-rays and γ-rays, and particle radiation such as electrons, α particles, and heavy ions (Table 24.1). Radiation injury damages DNA by producing strand breaks. As an internal organ, the gastrointestinal tract is usually not exposed to sufficient radiation for it to be a major factor in gastrointestinal carcinogenesis. However, very high doses of x-rays administered under unusual circumstances have been implicated as a pathogenetic factor in some intestinal cancers. Viral oncogenesis has been documented in several non-human tumor models and in squamous cell carcinoma of the esophagus and anus in humans [57,58]. JC virus, a DNA virus encoding a T antigen, is present in the gut and has been implicated in colorectal carcinogenesis [59,60]. There is evidence to suggest a role for viruses in the genesis of adenocarcinomas of the gastrointestinal tract, such as the role of hepatitis B and C viruses in causing hepatomas. Chemical carcinogens are thought to be most important in initiating adenocarcinoma in the gastrointestinal tract because of their access to the gut mucosa. This form of injury tends to produce mutations in single nucleotide bases.

Table 24.1 Carcinogenic agents

Radiation injury
Ultraviolet radiation
Especially important for skin
Most important wavelengths, 290–320 nm
Ionizing radiation
Electromagnetic radiation (e.g., x-rays, γ-rays)
Particulate radiation
Electrons, protons, neutrons, α particles, heavy ions
Viral oncogenesis
Human papillomavirus
Squamous cell carcinoma of the esophagus
Anal carcinoma
Genital cancers
Epstein–Barr virus
Burkitt lymphoma
Nasopharyngeal carcinoma
Hepatitis B and C viruses
Hepatoma
Human T-cell leukemia viruses
Human polyomavirus
JC virus

Single base-pair mutations are characterized as *transitions* when they change one purine to another or change one pyrimidine to another. They are characterized as *transversions* when they produce a change between nucleotide classes. Losses of base pairs in the DNA sequence are called *deletions*, and gains are called *insertions*. Some single base-pair changes are silent and do not result in amino acid changes. Mutations that change the amino acid in the protein product are called *missense mutations*, and they are characterized as conservative if the amino acid change occurs within the same class (i.e., from one neutral amino acid to another). Missense mutations can be serious if they result in the appearance or removal of charge, cysteines, phosphorylation or glycosylation sites, or other amino acids critical to the folding and function of the protein. Changes that create a stop codon within an open reading frame are called *nonsense mutations*. Nucleotide insertions and deletions (which do not occur in groups of three) create a frameshift, which commonly produces a series of missense and nonsense changes downstream on the DNA strand.

Chemical carcinogenesis

Chemical carcinogens are ubiquitous in the human diet but may not reach all the digestive organs in their active forms [61,62]. Typically, proximate carcinogens are highly reactive, short-lived chemical compounds that bind nucleic acids, proteins, and other macromolecules near the site of their generation. Chemical carcinogens usually have a narrow range of host and tissue specificity. The microbial flora of the gastrointestinal tract and mucosal enzymes are important factors in the activation and inactivation of many carcinogens. For example, cycasin, an extract made from cycad nuts, produces intestinal tumors when administered to most rodents [63]. This compound is a glycoside that must be hydrolyzed by intestinal bacteria to produce methylazoxymethanol, which is unstable and spontaneously decomposes, giving rise to a reactive carbonium ion capable of methylating nucleic acids. Cycasin does not give rise to tumors in germ-free rodents because they lack the necessary glycosidases produced by the intestinal flora.

Mechanisms by which genes are damaged

Point mutation

The significance of point mutations in gene damage was first appreciated when it was determined that animal tumor viruses carry mutated forms of cellular protooncogenes. Transfection of these mutated genes into NIH3T3 cells transformed the fibroblasts into cells that assumed a malignant morphology in vitro, no longer exhibited contact inhibition, and could form tumors when injected into nude mice. A transforming oncogene was first found in the Rous sarcoma virus, and later the *HRAS* gene was found to be oncogenic in this laboratory setting [64,65]. *RAS* gene mutations have been demonstrated in about half of colorectal cancers and in most pancreatic cancers [22,24,25]. Point mutations may be produced in DNA by the administration of alkylating agents; for example, treatment of rats with the carcinogen 1,2-dimethylhydrazine (DMH) results in the alkylation of DNA throughout the genome [66]. After exposure to such an agent, the host attempts to repair damaged DNA, or in some instances, the cell responds to the damage by initiating programmed cell death [9,10]. The action of a carcinogen triggers a dynamic response; the DNA is altered first, and the host then attempts to limit the impact of mutational damage, possibly sacrificing some damaged cells to prevent the replication of a damaged genome.

Carcinogens may produce characteristic damage based on the chemistry of the interaction between the reactive compound and certain susceptible sites in purines and pyrimidines [67]. A single genetic locus may be more likely to undergo a specific mutation, depending on the mutagen involved [68]. Some mutations are silent, but the appearance of others results in a growth advantage for the cell, and the error is amplified through clonal expansion of the mutant cell.

In rats treated with DMH, G-to-A mutations in *KRAS* develop in 66% of the colon cancers, indicating a strong selection for this mutation [69]. Other mutations may occur in locations that result in the inactivation of a gene essential for cell survival, and the cell is immediately eliminated. *KRAS* mutations may occur anywhere in the gene. Mutational inactivation of the guanine nucleotide regulatory region of the *RAS* gene (at codons 12, 13, and 61) needs to occur in only one cell in the colon after carcinogen exposure because this cell will expand clonally, outgrow those around it, and create large numbers of progeny cells.

Chronic inflammation is a common setting in which cancer develops in the gut, as in chronic esophagitis, chronic atrophic gastritis, and inflammatory bowel disease. Active forms of oxygen, such as hydroxyl and superoxide radicals, hydrogen peroxide, and singlet oxygen, arise as by-products of chronic inflammation and oxygen stress. These reactive species also mediate damage induced by ionizing and ultraviolet radiation. Reactive oxygen species create several forms of DNA damage, including strand breaks and single base changes. The two most common mutations induced by reactive oxygen molecules are the creation of 8-hydroxypurines, which result in misreading of those residues and adjacent pyrimidines. Because mispairing occurs in all four bases with almost equal frequency, it is not possible to predict what mutation this mechanism will induce. Evidence suggests that inflammation induces relaxation of DNA mismatch repair, permitting new mutations to be replicated, exacerbating the situation [70]. Furthermore, increased activity of certain nucleotide excision repair enzymes in response to oxidative stress can create additional mutations in microsatellite sequences [71]. Microsatellite instability in chronically inflamed, but not neoplastic, colonic tissues occurs as a result of these processes [72].

DNA rearrangement

Gene expression may be modified by the gross rearrangement of DNA sequences. For example, in chronic myelogenous leukemia, a translocation occurs in which sequences from chromosome 22 are accidentally spliced in frame with the cellular oncogene *ABL* (i.e., the oncogene originally isolated from the Abelson murine leukemia virus and formerly designated v-*abl* or c-*abl*). The translocation creates a chimeric messenger RNA that translates into an abnormally large mutant ABL protein [73]. A similar rearrangement appears in Burkitt lymphoma, in which a reciprocal translation occurring between chromosomes 8 and 14 juxtaposes a portion of the immunoglobulin gene with the oncogene *MYC*. In this instance, *MYC* is not mutated, but the rearrangement causes an amplification of gene expression [74]. The protooncogene *BCL2* was first discovered in human B-cell follicular lymphoma as a t(14;18) chromosomal translocation, which placed *BCL2* under the control of the heavy immunoglobulin chain promoter [75]. Elevated levels of BCL2, a 26-kDa protein, have since been measured in adenocarcinomas of the colon and other tumors without this translocation [76]. BCL2 functions to inhibit programmed cell death and is often highly expressed in tumors resistant to chemotherapy [77].

DNA amplification

DNA rearrangements can increase gene expression without the creation of additional copies of the gene. A normal cellular gene may also become a transforming gene through DNA amplification. This mechanism has attracted attention because resistance to methotrexate may be caused by amplification of the gene for dihydrofolate reductase [78]. Amplification of members of the *MYC* oncogene family is predictive of disease progression in neuroblastomas [79]. Some tumor cell lines have a large number of minichromosomes that exist independently as "double minutes" or are inserted into chromosomes (as homogeneously staining regions). Both represent examples of gene amplification. Amplification of *ERBB2* has been reported by several groups studying stomach cancers [80,81]. Other examples of amplified cellular oncogenes found in tumor cell lines include mutated *KRAS*, *MYB*, and *MYC* [82–84].

Altered methylation of DNA

Only a small percentage of the total genome is expressed in each tissue. One mechanism to control gene expression is to silence the genes through the stable, covalent methylation of the base cytosine at C–G sequences commonly found in promotors. DNA methylation is maintained by specific methyltransferases, and the pattern of methylation is inherited in a stable way through successive generations of cells in a given tissue. A wide variety of genes are substantially hypomethylated in primary colorectal cancers in comparison with the adjacent normal mucosa [85]. These genes include the γ-globulin gene, which is normally hypomethylated only in blood precursor cells, and the γ-crystallin gene, which is normally hypomethylated only in embryonic lens tissue [85,86]. Hypomethylation occurs in even the smallest adenomas. The implications of hypomethylation are not clear [87]. For example, an assessment of tumor formation in $Apc^{(Min/+)}$ mice using DNA methyltransferase (DNMT1) hypomorphic alleles to reduce genomic methylation resulted in a decrease of macroscopic intestinal tumors, in conjunction with the development of multifocal liver tumors [88]. Accordingly, although increases in DNA hypomethylation and hypermethylation in a sample of gastric and colon cancer specimens correlated with the patients' ages, only hypomethylation correlated with genomic damage, possibly because it precedes loss of diploidy [89].

Epigenetic alterations of DNA by methylation of promotor sequences in critical growth regulatory genes such as *p16*, *IGF2*, and *PTEN* have been described in microsatellite unstable tumors [90–92]. The mechanism for promoter methylation is unknown, but there is evidence that the MMR system is not directly responsible for this activity [91]. The promotor of the MMR gene *hMLH1* has been shown to be hypermethylated in about 10% of sporadic colorectal tumors (and is hypomethylated in the surrounding normal tissue) with absence of *hMLH1* expression [93]. This indicates that DNA methylation is a mode of MMR gene inactivation in some sporadic tumors. The correlation between microsatellite instability and promoter methylation suggests that methylation abnormalities precede microsatellite instability in sporadic colorectal cancers, and that other genes with methylated promoters in microsatellite unstable tumors are additional targets of this poorly understood process. The primary mammalian DNA methyltransferase, DNMT1, does not appear to play a key role in regulating this process [94].

Acetylation and deacetylation

Histone acetylation and deacetylation have been shown to be mechanisms for controlling gene expression. Acetylation and deacetylation of DNA-bound histones change DNA conformation to permit or prevent RNA polymerase access to the DNA to initiate transcription. In addition, individuals who are rapid acetylators of potentially carcinogenic compounds display an increased risk of developing colon cancer [95,96].

DNA deletion

Cell proliferation is regulated by a family of genes known as *tumor suppressor genes*. These genes prevent inappropriate cell proliferation and regulate the number of cells in a tissue. Unlike oncogenes, these genes play a role in carcinogenesis through their inactivation.

Even before the first tumor suppressor gene was identified, the presence of such genes was predicted by Knudson [97], who proposed that some human cancers arise through a recessive mechanism in which both alleles of a tumor suppressor gene are independently inactivated. This concept was par-

ticularly important because it provided a framework for the inheritance of enhanced susceptibility to specific cancers. Loss of the first allele could occur as a dominantly inherited trait that is transmitted vertically in the germ line. Inactivation of the first allele can be phenotypically silent because the activity of the remaining second allele is sufficient to maintain the nonmalignant phenotype. However, such individuals are at extremely high risk for the development of cancer because only a single further inactivation mutation in a single cell may be required to trigger tumor development, usually at younger ages than occurs in the general population. In contrast, the formation of sporadic tumors requires that two individual events occur independently, which decreases the likelihood of their occurrence in the same cell and increases the likelihood that they will occur later in life. Mutation of one allele followed by loss of DNA at the second allele (through chromosomal instability) is the "two-hit" mechanism for the complete inactivation of tumor suppressor genes. Loss of heterozygosity in gastrointestinal carcinogenesis is discussed in detail in the section on genomic instability and multistep carcinogenesis.

Inactivation of DNA repair

Nucleotide excision repair

DNA damaged by ultraviolet radiation, chemicals, and toxins is repaired by a complex of proteins that effectively excise the damaged DNA and replicate the interrupted strand using the complementary DNA strand as a template. Collectively known as the nucleotide excision repair complex, the component proteins are designated XP (xeroderma pigmentosum) or ERCC (excision repair cross-complementing) in reference to the discovery of defective nucleotide excision repair in patients with xeroderma pigmentosum [98]. The XP and ERCC proteins are capable of recognizing and binding to damaged DNA, unwinding the DNA double helix by virtue of helicase activity, and excising 27–29 nucleotides containing the damaged DNA [99–101]. The excision gap is then filled in by DNA polymerases. Some of the nucleotide excision proteins form an important transcription factor, TFIIH, which is involved in regulating the activity of all protein-encoding genes [102,103]. Consequences of nucleotide excision repair defects include xeroderma pigmentosum, a disease characterized by sunlight-induced photodermatoses that result in skin cancers and neurological abnormalities [99,100]. Cockayne syndrome and trichothiodystrophy, diseases characterized by neurological abnormalities and mental retardation without a major increase in skin cancers, are caused by mutations that impair the transcription function of nucleotide excision proteins [99,100]. Malfunctioning nucleotide excision repair in cancers of the gastrointestinal tract has not been reported.

Base excision repair

The MutY homologue (*MYH*) gene encodes a member of the nucleotide excision repair pathway that is involved in repairing oxidative damage to DNA. Biallelic germ-line *MYH* mutations predispose to the development of colorectal adenomas in the absence of an *APC* mutation and with a family history compatible with recessive inheritance [104]. Similar to the clinical situation that occurs with xeroderma pigmentosum caused by mutations in nucleotide excision repair genes, *MYH* mutations cause a rare, recessive disease. The affected persons appear to have sporadic (i.e., nonfamilial), attenutated adenomatous polyposis. Carriage of a single mutated *MYH* allele may confer a slight increase in the risk of colorectal cancer, but this remains controversial [105].

DNA mismatch repair

Unlike nucleotide excision repair, inactivation of the DNA MMR system is common in tumors of the gastrointestinal tract. Point mutations can occur in critical growth-regulatory genes and can accumulate in cells with a defective DNA MMR system (Fig. 24.5). The MMR system is responsible for maintaining the replicative fidelity of DNA. Germ-line mutations in *hMSH2*, *hMLH1*, *hPMS2*, and *hMSH6* have been identified and clearly linked to hereditary nonpolyposis colorectal cancer, and somatic inactivation of *hMLH1* by promoter hypermethylation has been identified in sporadic tumors [93,106–109]. No germ-line mutation in *hMSH3* has been identified in families with this syndrome [110]. The proteins that comprise the MMR system bind to nucleotide base–base mismatches of double-stranded DNA or loops of inaccurately replicated repetitive sequences (termed microsatellite DNA) and target the DNA area for excision, resynthesis, and ligation [111]. The DNA sequences repaired by the MMR system have apparently escaped the normal editing function of DNA polymerase. Base mispairing, if not corrected by the MMR system, may cause nucleotide transitions or transversions, allowing a novel base to alter the authentic genetic sequence [112]. Such point mutations in genes that regulate cell growth may promote neoplastic growth.

Inherited premutations

Premutations may elevate familial cancer risk [113]. For example, a T-to-A mutation causing a substitution of lysine for isoleucine at codon 1307 in the *APC* gene is present in 6.1% of Ashkenazi Jews [113]. This missense mutation in itself is not thought to alter the function of the APC protein severely because most *APC* mutations are nonsense mutations, yielding a truncated protein. However, the T-to-A mutation converts the sequence AAATAAAA to $(A)_8$, a repetitive nucleotide tract that is relatively unstable during transcription and replication. In fact, the T-to-A premutation is associated with the acquisition of other nonsense mutations within and surrounding the $(A)_8$ tract, causing truncation of the APC protein [113]. This type of point mutation, which creates an intrinsically unstable repetitive DNA sequence, may lead to further mutations as a mechanism to inactivate gene function.

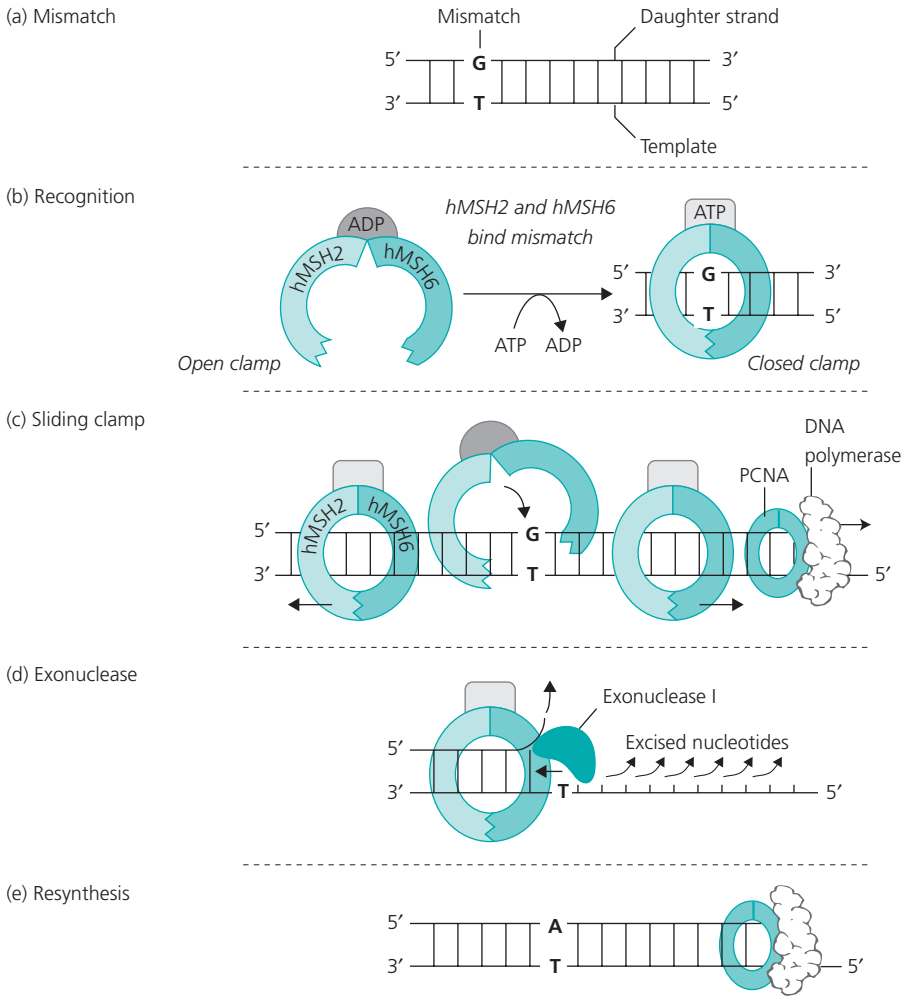

Figure 24.5 DNA mismatch repair (MMR). After DNA replication, mistakes that apparently have escaped the editing function of DNA polymerase are bound by the MMR proteins and targeted for repair. Single base mispairs and insertion or deletion loops of one base pair at microsatellite sequences are recognized by hMutSα (heterodimer of hMSH2 and hMSH6), and insertion or deletion loops of more than one base pair are recognized by hMutSβ (heterodimer of hMSH2 and hMSH3). Subsequent recruitment of hMutLα (heterodimer of hMLH1 and hPMS2) to the altered DNA targets the area for excision, resynthesis, and ligation. Inactivation of the MMR proteins would permit these types of mutations to be passed to progeny cells.

Genetic mechanisms of tumor development

Gatekeeper, caretaker, and landscaper genes

Gatekeeper genes are cellular genes that directly regulate tumor growth by suppressing cell growth or promoting cell death. By definition, gatekeepers are tumor suppressor genes. It has been suggested that each human cell has only one or a few gatekeeper genes that are specific to that cell type. The esophagus, stomach, small intestine, colon, liver, and pancreas are different histologically, and the cells of these tissues express a different array of genes to perform their unique functions. The gastrointestinal organs probably do not share gatekeeper genes. Only the gatekeeper gene of the colon, *APC*, has been clearly identified [114]. Inactivation of a gatekeeper gene is the rate-limiting step for the initiation of tumor growth, and demonstrates a specific tissue distribution of that tumor [4]. As a tumor suppressor gene, inactivation requires alteration of the maternal and paternal alleles, fulfilling the Knudson two-hit hypothesis [97]. Restoration of the gatekeeper function in vitro leads to suppression of the neoplastic growth.

Caretaker genes contribute to genomic stability, typically as part of a DNA repair process (e.g., *hMLH1* and *hMSH2*). Inactivation of a caretaker gene leads to genomic instability by increasing the rate of accumulation of mutations in a cell, which ultimately results in a constellation of altered genes that are associated with neoplastic behavior. This phenomenon might be likened to a constant and excessive exposure to mutagens. Therefore, caretaker genes normally serve to inhibit the development of neoplasia, and conceptually can be thought of as tumor suppressor genes. Restoration of caretaker function to a cancer cell will not affect its growth because these indirectly acting genes are not required for neoplasia.

Defects in landscaper genes create an abnormal stromal environment for the epithelium, which contributes to neoplastic transformation [115]. For instance, chronic inflammation in ulcerative colitis affects the colonic epithelium, initially with the inflammation, but ultimately it may cause neoplastic changes and progression to cancer. The regeneration that occurs to replace damaged epithelium may

increase the probability of somatic mutations in an abnormal microenvironment.

There is increased evidence for the role of stromal cells on tumor initiation and progression [116]. The loss of TGF-β responsiveness in mouse fibroblasts results in increased tumorigenesis in skin, foregut, and prostate [117]. Epithelial-mesenchymal interactions may also explain the link between cancer and inflammation. Deletion of the I-κB kinase (IKK) complex, an activator of the transcription nuclear factor-κB (a key mediator of signaling in inflammation), in myeloid cells but not epithelial cells leads to a decrease in tumor size in a colitis-associated mouse model of cancer [118]. Selective deletion of SMAD4, a key downstream molecule in growth suppressive signaling, in T cells, leads to spontaneous gastrointestinal cancers in mice, likely through the abundant production of T helper type 2 cytokines [119].

Genetically, tumor initiation is caused by inactivation of both alleles of a gatekeeper gene. This may occur as the result of an inherited mutated allele (i.e., a germ-line mutation), coupled with somatic loss within a specific cell, or as the result of two somatic hits within a specific cell. For a caretaker gene to initiate neoplasia, both alleles must be inactivated; in addition, the resulting genomic instability must inactivate both alleles of the gatekeeper. It is believed that inherited susceptibility syndromes involving gatekeeper genes are associated with extremely high rates of cancer development, whereas inherited syndromes involving caretaker genes are associated with cancer rates lower than those of gatekeeper genes because some minimal number of additional mutations must occur [4,115]. Inherited caretaker mutations would still be associated with cancer rates much higher than those in the general population.

Oncogenes

The traditional concept of tumor initiation and promotion is inadequate to explain the full complexities of carcinogenesis. No single mutation or altered gene is sufficient to cause cancer in most cells. Multiple mutations, genetic deletions, and chromosomal rearrangements have been found in cancers that have been studied carefully. However, some single genes, when activated, can cause the phenotype of a cultured fibroblast cell line to become recognizably malignant. These initial cancer-causing genes were called *oncogenes*, and they were discovered by studying viruses that could transform chicken cells [120]. A number of oncogenes have been identified, and each can be activated by a point mutation that alters the activity of the gene product or by genetic rearrangement that results in an increase in gene expression (Table 24.2) [121].

Some oncogenes produce growth factors (e.g., *SIS*), and when production is not regulated, the overproduced peptide ligand binds repeatedly and continuously to the receptor to cause unremitting stimulation. Other protooncogenes produce growth factor receptors (e.g., *ERBB2*, *CSF1R*, *KIT*). The

Table 24.2 Classification of oncogenes and gene products according to function

Class 1 – Growth factors	
sis	PDGF β-chain growth factor
int-2	FGF-related growth factor
hst (*KS3*)	FGF-related growth factor
FGF-5	FGF-related growth factor
int-1	Growth factor?
Class 2 – Receptor and nonreceptor protein tyrosine kinases	
src	Membrane-associated nonreceptor protein-tyrosine kinase
yes	Membrane-associated nonreceptor protein-tyrosine kinase
fgr	Membrane-associated nonreceptor protein-tyrosine kinase
lck	Membrane-associated nonreceptor protein-tyrosine kinase
fps/fes	Nonreceptor protein-tyrosine kinase
abl/bcr-abl	Nonreceptor protein-tyrosine kinase
ros	Membrane-associated receptor-like protein-tyrosine kinase
egfr	Membrane-associated receptor-like protein-tyrosine kinase
erbB	Truncated EGF receptor protein-tyrosine kinase
neu	Receptor-like protein-tyrosine kinase
fms	Mutant CSF-1 receptor protein-tyrosine kinase
met	Soluble truncated receptor-like protein-tyrosine kinase
trk	Soluble truncated receptor-like protein-tyrosine kinase
kit (W locus)	Truncated stem cell receptor protein-tyrosine kinase
sea	Membrane-associated truncated receptor-like protein-tyrosine kinase
ret	Truncated receptor-like protein-tyrosine kinase
jak1/3	Protein tyrosine kinase
fak	Nonreceptor protein-tyrosine kinase
Class 3 – Receptors lacking protein kinase activity	
mas	Angiotensin receptor
Class 4 – Membrane-associated G proteins	
H-*ras*	Membrane-associated GTP-binding/GTPase
K-*ras*	Membrane-associated GTP-binding/GTPase
N-*ras*	Membrane-associated GTP-binding/GTPase
gsp	Mutant activated form of $G_s\alpha$
gip	Mutant activated form of $G_i\alpha$
Class 5 – Cytoplasmic protein-serine kinases	
raf/mil	Cytoplasmic protein-serine kinase
pim-1	Cytoplasmic protein-serine kinase
mos	Cytoplasmic protein-serine kinase (cytostatic factor)
cot	Cytoplasmic protein-serine kinase?
Class 6 – Cytoplasmic regulators	
crk	SH-2/3 protein that binds to (and regulates?) phosphotyrosine-containing proteins
Class 7 – Nuclear transcription factors	
myc	Sequence-specific DNA-binding protein
N-*myc*	Sequence-specific DNA-binding protein?
L-*myc*	Sequence-specific DNA-binding protein?
myb	Sequence-specific DNA-binding protein

Table 24.2 (*cont'd*)

lyl-1	Sequence-specific DNA-binding protein?
p53	Mutant form may sequester wild-type p53 growth suppressor
fos	Combines with c-*jun* product to form AP-1 transcription factor
jun	Sequence-specific DNA-binding protein; part of AP-1
erbA	Dominant negative mutant thyroxine (T_3) receptor
rel	Dominant negative mutant nuclear factor-κB-related protein
vav	Transcription factor?
ets	Sequence-specific DNA-binding protein
ski	Transcription factor?
evi-1	Transcription factor?
gli-1	Transcription factor?
maf	Transcription factor?
pbx	Chimeric E2A-homeobox transcription factor
Hox2,4	Transcription factor?
Unclassified	
dbl	Cytoplasmic truncated cytoskeletal protein?
bcl-2	Plasma membrane signal transducer/apoptosis
Cox-2	Prostaglandin synthase
Survivin	Inhibitor of apoptosis protein (IAP)
Thx3	Transcription repressor
Evi1	Viral integration site

EGF, epithelial growth factor; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor.
Adapted and updated from Hunter [121].

most intensively studied protooncogenes are those involved in cellular signal transduction, including *SRC*, *RAS*, and *RAF* gene families. These oncogenes are activated by point mutations that maintain the protein in a configuration such that signal transduction events are activated, regardless of whether the appropriate ligand or receptor is in place. Some of the protooncogenes, including *MYC*, *JUN*, and *FOS*, encode transcription factors, often nuclear phosphoproteins, that interact with DNA and regulate gene transcription. Many of these genes were found because they had become incorporated into viral genomes, and because their expression had been deregulated by mutation or rearrangement, they created oncogenic viruses. In each case, these genes were capable of transforming cultured fibroblasts after infecting the cells. These oncogenes initially were designated v-oncogenes (e.g., v-*src*) because of their viral origin. Soon it was found that cellular protooncogenes could undergo activation in situ by point mutation or other genetic rearrangement and become oncogenic in the absence of a viral vector.

Tumor suppressor genes

Boveri hypothesized as early as 1914 that the relentless growth of a malignant tumor was as likely to result from the inactivation or deletion of genes that inhibit proliferation as from enhanced activity of genes that promote growth. Tumors often have grossly abnormal karyotypes, and losses of chromosomal arms occur frequently [122]. Advances in molecular biology, specifically the use of restriction fragment length polymorphism and microsatellite analysis, have permitted the examination of specific chromosomal locations for genetic losses.

Using such techniques, Vogelstein and colleagues [123] reported the widespread losses of genetic material in colorectal cancers and the deletion of specific genetic loci in a large proportion of tumors. These investigators reasoned that identifiable hot spots for genetic loss would be the location of tumor suppressor genes that were relevant in the development of colorectal cancer. This hypothesis proved to be correct, and the concept has led to identification of numerous tumor suppressor genes in human cancer (Table 24.3) [124–126]. The mechanism of their activation may differ from gene to gene, but in all cases, it is the inactivation of the protein function that plays a role in carcinogenesis.

Perhaps the most important of these is the *TP53* gene, which normally acts to prevent the cell from initiating DNA replication or cell division. It serves as a critical regulatory gene to prevent inappropriate proliferation [127]. When DNA is damaged, the p53 protein becomes activated, which switches on the transcription of a variety of genes. These include activation of *WAF1/CIP1*, a gene encoding a 21-kDa protein that inhibits CDK2, thus arresting the cell in the G1 phase of the cell cycle and preventing the replication of damaged DNA [8,128,129]. The p53 protein also stimulates the transcription of *GADD45*, and the gene product in turn complexes with proliferating cell nuclear antigen, a necessary component for the synthesis of DNA [130]. By stimulating the synthesis of WAF1/CIP1 and GADD45, p53 effectively halts DNA replication and stimulates DNA repair. It also plays a critical role in programmed cell death, or apoptosis, when DNA is damaged, perhaps beyond repair [8,131–133]. Mutation of *TP53*, therefore, would prevent cell death in the wake of DNA damage.

Inactivation of *TP53* in cancer involves two steps. Often, one *TP53* allele undergoes a missense point mutation, typically in a portion of the molecule involved in the binding to DNA. It is controversial how the presence of one wild-type allele together with one mutated copy of the p53 protein alters the growth characteristics of a cell. However, most colon cancers have lost the wild-type *TP53* gene, leaving a single mutated copy of the gene in the nucleus [134]. The peanut fungus *Aspergillus fumigatus* produces aflatoxin β1, which may cause hepatocellular carcinoma by mutating *TP53* at a critical codon [135]. Other mechanisms have developed in certain cancers to inactivate p53 by interrupting its evolutionarily conserved regions (Fig. 24.6). These include the binding of viral proteins to p53 (i.e., JC virus or simian virus 40 T antigen, human papillomavirus E6 protein, and adenovirus E1b protein), and binding of amplified cellular

Table 24.3 Tumor suppressor genes involved in human cancers

Tumor suppressor gene	Chromosome location	Proposed function	Somatically mutated neoplasms	Inherited syndrome	Typical inherited neoplasms or traits
TP53/p53	17p13.1	Transcription factor, apoptosis, response to DNA damage	Most types of human cancers	Li–Fraumeni cancer syndrome	Sarcomas, carcinomas of breast and adrenal cortex, leukemia, brain tumors
RB1	13q14.1–q14.2	Cell cycle and transcriptional regulator, E2F binding	Retinoblastoma, osteosarcoma, carcinomas of the breast, prostate, bladder, and lung	Familial retinoblastoma	Retinoblastoma, osteosarcoma
APC	5q21–q22	Regulation of β-catenin, microtubule binding	Colorectal adenomas and cancer, carcinomas of the stomach and pancreas	Familial adenomatous polyposis, Gardner syndrome, Turcot syndrome	Colorectal cancer, duodenal and gastric cancers, medulloblastoma
hMSH2 *hMLH1* *hPMS1* *hPMS2* *hMSH6/GTBP*	2p22–p22 3p21.3 2q31–q33 7p22 2p16	DNA mismatch repair	Colorectal cancer; carcinomas of the ovary, endometrium, urinary tract, head and neck; small cell lung cancer	HNPCC, Muir–Torre syndrome, Turcot syndrome	Colorectal cancer, carcinomas of the endometrium, stomach, kidney, ureter, glioblastomas
NF1	17q11.2, 2p22–p21	GTP-activating protein for RAS, microtubule binding	Schwannomas	Neurofibromatosis type 1	Neurofibromas, neurofibrosarcomas, AML, brain tumors
NF2	22q12.2	Links membrane proteins to cytoskeleton	Schwannomas and meningiomas	Neurofibromatosis type 2	Acoustic neuromas, meningiomas, gliomas, ependymomas
WT1	11p13	Transcriptional repressor	Wilms tumor	Wilms tumor	Wilms tumor
BRCA1	17q21	Interacts with RAD51, repair of double-stranded DNA breaks	Ovarian cancer	Familial breast cancer 1	Breast cancer, ovarian cancer
BRCA2	13q12.3	Interacts with RAD51, repair of double-stranded DNA breaks	Ovarian cancer	Familial breast cancer 2	Breast cancer, male breast cancer, pancreatic cancer
VHL	3p26–p25	Regulates transcriptional elongation by RNA polymerase II	Renal clear cell carcinoma, pheochromocytomas, hemangioblastomas	Von Hippel–Lindau syndrome	Renal clear cell carcinoma, pheochromocytomas, retinal angiomas, hemangioblastomas, pancreatic cancer
CDKN2A/p16	9p21	Inhibition of CDK4 and CDK6	Melanoma	Familial melanoma	Melanoma, pancreatic cancer, dysplastic nevi
MEN1	11q13	Unknown	Parathyroid tumors	Multiple endocrine neoplasia type I	Pancreatic islet cell gastrinomas, parathyroid hyperplasia, pituitary adenomas
PTEN/MMAC1	10q23.31	Tyrosine phosphatase	Glioblastomas, carcinomas of the kidney, prostate, breast	Cowden disease, Lhermitte–Duclos disease, Bannayan–Riley–Ruvalcaba syndrome, juvenile polyposis syndrome	Carcinomas of the breast and thyroid, intestinal and skin hamartomas
CDKN1C/p57(KIP2)	11p15.5	Cell cycle regulator	Unknown	Beckwith–Wiedemann syndrome	Wilms tumor, organomegaly, hemi-hypertrophy, hepatoblastoma, adrenocortical cancer

(*cont'd*)

Table 24.3 (*cont'd*)

Tumor suppressor gene	Chromosome location	Proposed function	Somatically mutated neoplasms	Inherited syndrome	Typical inherited neoplasms or traits
PTCH1	9q22.3	Transmembrane receptor for hedgehog signaling	Primitive neuroendocrine tumors, basal cell carcinoma, medulloblastoma	Nevoid basal cell carcinoma	Basal cell skin cancer, jaw cysts, palmar and plantar pits, medulloblastomas, ovarian fibromas
EXT1 *EXT2* *EXT3*	8q24.11–q24.13 11p12–p11 19p	Unknown	Chondrosarcoma	Multiple exostoses	Exostoses
DCKN1A/p21/CIP1/WAF1	6p21.2	CDK inhibitor	Bladder cancer	None known	–
TP73/p73	1p36	Transcription factor, apoptosis (p53-like protein)	Neuroblastoma	None known	–
SMAD4/DPC4	18q21.1	TGF-β signal transduction	Pancreatic cancer, colorectal cancer	Juvenile polyposis syndrome	Juvenile polyps, colorectal and gastric cancers
SMAD2/MADR2 (JV18-1)	18q21	TGF-β signal transduction	Colorectal cancer	None known	–
DCC	18q21.3	Netrin-1 receptor	Colorectal cancer, neuroblastoma	None known	–
ATM	11q22.3	DNA repair, induction of p53	Unknown	Ataxia telangiectasia	Lymphoma, cerebellar ataxia, immunodeficiency
BLM	15q26.1	?DNA helicase	Unknown	Bloom syndrome	Solid tumors, immunodeficiency, small stature
FANCC/FACC	9q22.3	?DNA repair	Unknown	Fanconi anemia	AML, pancytopenia, skeletal abnormalities
FANCA/FACA *XPB, XPD, XPA*	16q24.3 Multiple complementation on groups	DNA repair helicases, nucleotide excision repair	Unknown	Xeroderma pigmentosum	Skin cancer, pigmentation abnormalities, hypogonadism
MUTYH/MYH	1p34.3–p32.1	Base excision repair	Unknown	MYH-associated polyposis syndrome	Autosomal recessive colon cancer
ENG	9q34.1	Accessory-receptor for TGF-β	Unknown	Juvenile polyposis syndrome, hereditary hemorrhagic telangiectasia	Colon cancer
TGFBR2	3p22	Growth suppression	Common mutation in microsatellite unstable colon cancers	Unknown	Colon cancer
ACVR2	2q22.2–q23.3	Growth suppression	Common mutation in microsatellite unstable colon cancers	Unknown	Colon cancer
BMPR1A	10q22.3	Growth suppression	Unknown	Juvenile polyposis syndrome	Hamartomas and cancers of the intestine
STK11/LKB1	19p13.3	Growth suppression, angiogenesis, cell polarization	Unknown	Peutz–Jeghers syndrome	Cancers of the small intestine, stomach, pancreas, colorectum,esophagus, ovaries, lung, uterus, breast, testes

AML, acute myelogenous leukemia; CDK, cyclin-dependent kinases; HNPCC, hereditary nonpolyposis colorectal cancer; TGF, transforming growth factor.
Adapted and updated from Fearon [126].

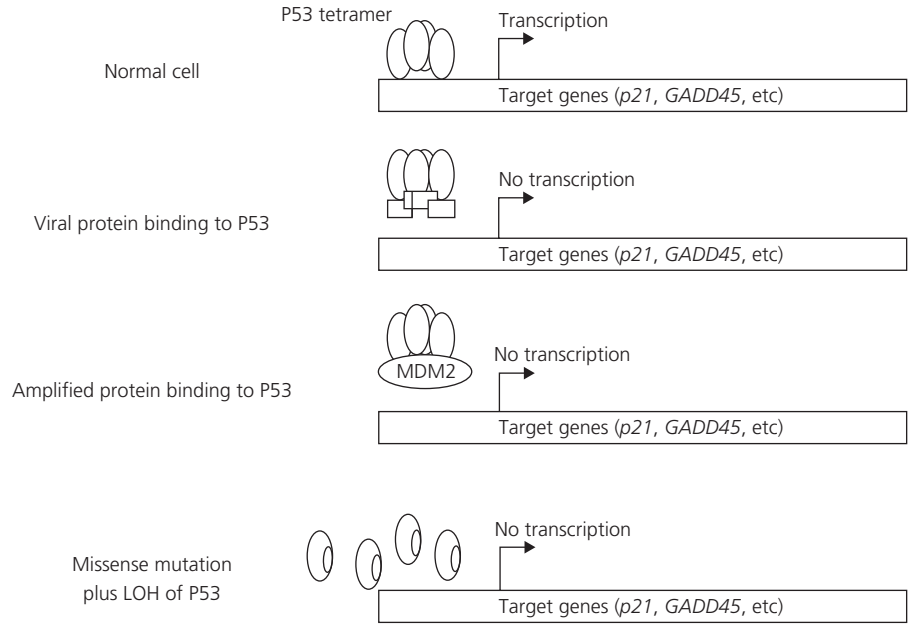

Figure 24.6 Some mechanisms for inactivating p53. The p53 protein binds as a tetramer to a p53-binding site in the promotor region of a target gene to activate genes involved in growth suppression and DNA repair. Viral proteins such as the simian virus 40 T antigen, human papillomavirus E6, and adenovirus E1b can bind p53 protein to prevent its transactivating function. Similarly, amplified cellular proteins such as MDM2 can bind p53 protein to prevent promotor binding. In colon cancer, the typical p53 inactivation involves a missense mutation of one allele and loss of heterozygosity (LOH) of the second allele, leaving only mutated protein available. The mutated p53 protein may have limited or no binding to promotor sites to initiate transcription at target genes.

proteins to p53 (e.g., MDM2 oncoprotein in sarcomas) [59,136]. Mutant *TP53* (with concomitant lack of normal *TP53*) appears to be overexpressed in tumors, including colorectal tumors, and can be detected by immunohistochemical methods [137]. This overexpression often is the result of enhanced stability of the mutant p53 protein, allowing its accumulation within the cell [136,137].

The tumor suppressor gene concept accommodates the notion of familial predisposition to cancer. The first example of this was found in familial retinoblastoma, in which there are germ-line mutations at the *RB1* locus [124]. The *RB1* gene has a tumor suppressor function in many tissues, including the gastrointestinal tract, but its inactivation is the limiting step (gatekeeper) only for the development of retinoblastoma [138,139]. In familial retinoblastoma, every retinal epithelial cell contains one copy of a mutated *RB1* gene and one wild-type gene. The chance occurrence of a mutation in the wild-type *RB1* allele in any cell is sufficient to inactivate completely the tumor suppressive activity of the *RB1* gene. Because this event needs to occur in only a single cell to produce a tumor, the incidence of retinoblastoma is high in families with a mutated *RB1* allele. Essentially every child who inherits the mutated *RB1* allele is at risk for the development of bilateral tumors at an early age. In contrast, unaffected individuals require two inactivational hits in a single cell, one on each allele, for retinoblastoma to occur; accordingly, sporadic tumors are rare. Retinoblasts disappear from the eye after early infancy, so this disease is not seen later in life.

Germ-line mutations at tumor suppressor gene loci may be associated with a normal phenotype at birth, but they are associated with very high risks for cancer. This is the outcome in Li–Fraumeni syndrome, in which there is a germ-line mutation in the *TP53* gene; affected individuals are phenotypically normal at birth but develop breast cancers, sarcomas, and brain tumors at an early age [124]. An animal model for this disorder exists in mice made deficient for the *p53* gene by homologous recombination; these mice can survive with no copies of the *p53* gene, which underscores the redundancy and adaptability of the control of cell growth [140]. Two more genes, *TP73L* (*p63*) and *TP73*, produce proteins that have some functions similar to those of p53 protein [141–144]. Similar to p53, both p63 and p73 can form homooligomers, bind DNA, activate transcription from p53-responsive genes, and induce apoptosis [143–145]. However, in contrast to p53, both p63 and p73 give rise to multiple functionally distinct protein isoforms, some of which can function as "dominant negative" proteins, blocking the function of the corresponding full-length protein [146]. In addition, *TP73L* and *TP73* are only rarely mutated in tumors and so do not function as classical tumor suppressor genes. Furthermore, unlike $p53^{-/-}$ mice or patients with Li–Fraumeni syndrome, $p63^{-/-}$ and $p73^{-/-}$ mice are not tumor prone but instead manifest multiple developmental abnormalities [147–149]. Germ-line *TP73L* mutations in humans cause ectrodactyly–ectodermal dysplasia–clefting (EEC) syndrome, an autosomal dominant disorder characterized by ectrodactyly, ectodermal dysplasia, and cleft lip with or without cleft palate [150]. Although *TP73L* and *TP73* have some overlapping functions and interactions with *TP53*, they are not tumor suppressor genes like *TP53*, and mutations do not pose the same high risk for cancer as *TP53* mutations.

E-cadherin is a calcium-dependent cell–cell adhesion molecule expressed in adherent junctions and coded by the *CDH1* gene. Dysfunctional E-cadherin may render cells less matrix-bound and more prone to metastasis. Germ-line mutations in the *CDH1* gene have been identified in

hereditary diffuse gastric cancer, and somatic mutations of *CDH1* may be associated with sporadic gastric cancer [151].

Clonal expansion

A variety of genetic techniques have demonstrated that neoplasms are derived from a single cell, but most human cancers have multiple mutations, and it is unlikely that a single cell undergoes all these genetic changes at once [152].

The concept of multistage carcinogenesis assumes that the gastrointestinal epithelium undergoes a constant barrage by factors that threaten to damage the DNA, but the mutations occur one by one at critical locations in genes related to the control of cell growth or survival. Some of these mutations may inhibit the function of a gene essential for the life of the cell, which is lethal to that cell. Other mutations provide the cell with a slight growth or survival advantage over the rest of the cells in the tissue. In these instances, the mutated cells proliferate faster than the surrounding cells and gradually represent a larger proportion of the tissue. In other instances, a mutated cell may be less capable of repairing DNA damage, which facilitates the accumulation of mutations in succeeding generations. This expanded pool of cells continues to be susceptible to additional mutations.

The redundancy in the cellular mechanisms regulating proliferation means that loss of any individual function is insufficient for the transformation of a normal cell. The accumulation of cooperating lesions is required to overcome the regulated cell growth. Periodically, an individual cell within an expanding clone experiences another mutation that adds to its growth or survival advantage, and the progeny of this cell then overgrows the population from which it originated. Successive waves of clonal expansion occur by the chance accumulation of new mutational events, which add to the survival advantage of the cell in an evolutionary manner. In early-stage neoplasms, there is evidence for only a small number of mutational events; later in neoplastic progression, however, the cell has a larger number of genetic lesions [22,153]. Figure 24.7 demonstrates tumor progression as successive expansions of cellular clones with new growth characteristics.

Genomic instability and multistep carcinogenesis

Through the elegant work performed in several laboratories, a conceptual interpretation of the genetic basis of colorectal cancer and other cancers has been developed [153]. This framework accommodates the growing evidence that most cancers develop slowly and that multiple genes are involved in the process. Certain types of genetic damage tend to occur early, and different types occur later. It is not yet clear how strictly the sequence of events is followed in most neoplasms, but not all cancers appear to accumulate every mutation or chromosomal deletion during their development.

Some type of genomic instability is required to account for the mutations that occur in multistep carcinogenesis. In most

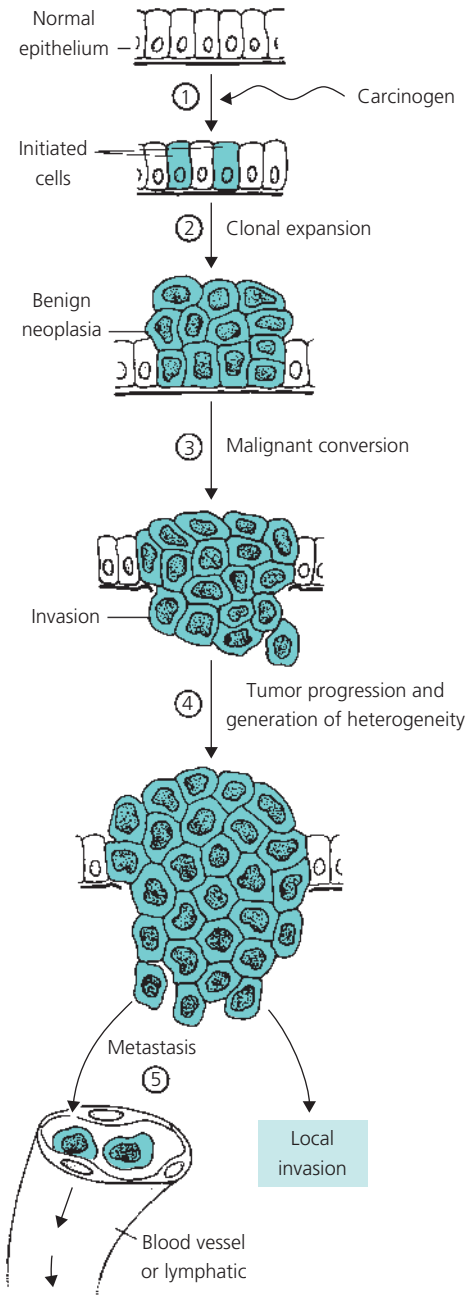

Figure 24.7 Tumor progression. This scheme illustrates the natural history of carcinogenesis. The first step represents a genotoxic event caused by a carcinogen. When a critical number of genes are mutated (the specific site and number remain speculative), a cell becomes neoplastic (step 1). Clonal expansion gives rise to a benign neoplasm (step 2). Unless additional events occur, a benign neoplasm may persist or grow indefinitely without becoming malignant. However, if additional genetic events occur, cells appear within a benign neoplasm that are capable of malignant behavior, such as invasion and metastasis (step 3). A malignant neoplasm may invade locally and damage the host and surrounding organs. However, the genome of the malignant neoplasm is intrinsically unstable, and despite its monoclonal origin, the cancer becomes more heterogeneous with time (steps 4 and 5). Clinically, the generation of tumor cell heterogeneity increases the likelihood of metastasis to one or more distant sites.

colorectal cancers, the process could be called chromosomal instability. Chromosomal segments or entire chromosomes are deleted, duplicated, or rearranged, so that the normal diploid nucleus is changed into an aneuploid one. A common finding in this pathogenic pathway is the allelic mutation of a tumor suppressor gene (e.g., *APC* or *TP53*), followed by the loss of the remaining normal allele (termed *loss of heterozygosity*, or LOH) [123,154]. As an increasing fraction of allelic arms is lost, the prognosis for colorectal cancer worsens. Patients with tumors that have lost more than 20% of the tested alleles are significantly more likely to die from their disease than those with tumors that have not, and this characteristic is a more powerful prognostic factor than the Dukes stage or the histological grade of the tumor [154].

It is not known what specific cellular function becomes disturbed, resulting in multiple LOH episodes throughout the genome. Chromosomal instability occurs in the smallest adenomas, suggesting that it occurs very early during colorectal neoplasia [155]. It has been hypothesized that structural changes to the chromosomes and abnormal mitoses are operative in the development of chromosomal instability. Mitotic checkpoint gene mutations, such as those found in *BUB1*, have been found, but only rarely in colorectal cancers [156]. A mutant APC protein, which is inactivated in the earliest stages of colonic neoplasia, is associated with defective chromosome segregation [157,158]. Wild-type APC localizes to the kinetochore during mitosis and forms complexes with BUB1 and BUB3 mitotic checkpoint proteins [158]. Although mutant APC may contribute to ongoing chromosomal instability in a colonic cell, it is not clear how this causes the initial chromosomal instability responsible for APC inactivation. Other proteins, such as human securin, a protein necessary for completion of the anaphase portion of mitosis, may help maintain euploidy [159]. Telomerase dysfunction also has been implicated as a mechanism for chromosomal instability in mouse models [160]. Foreign proteins such as T antigen from the polyomavirus JC Mad-1 strain have been found in colorectal cancers [59,161]. T antigens are capable of inducing aneuploidy in cells and are known to inactivate important cell cycle proteins, such as p53 and R protein [59,161]. The checkpoint gene *MAD2*, when inactivated, may contribute to chromosomal instability and cancer development in RB-mutated tumors [162] through the formation of abnormal centrosomes, which are organelles involved in cytoskeletal and mitotic organization in genomic homeostasis [163]. However, many questions remain, especially because aneuploidy appears to possess both tumor-promoting and tumor-suppressing properties [162].

The early stages of carcinogenesis in the colon appear to occur slowly, with the accumulation of mutations and LOH at a variety of chromosomal loci. However, transition to the malignant stage is associated with widespread genomic instability and LOH, which accelerates the likelihood that tumor suppressor loci will be lost, including genes that may suppress metastatic behavior. Studies have shown that mutation of one *TP53* allele coupled with LOH of the second allele coincides with the appearance of carcinoma within an adenomatous polyp, making *TP53* the gatekeeper for malignant transformation in the colon [35,164,165]. This model of multistep carcinogenesis reflects the consequences of gene dysfunction. A malignancy occurs when a sufficient combination of these mutated genes accumulates. In some instances, a sequential pattern for the occurrence of their metastasis may be required.

The following sequence of events appears to be relevant to the *chromosomal instability* pathway of tumorigenesis in the human colon (Fig. 24.8) [35,153]. Hypomethylation of DNA occurs in the smallest adenomas and may be one of the early events in carcinogenesis, but the mechanism by which this occurs, the specific genes involved in the process, and its role in carcinogenesis are unknown [85,86]. Mutations (and LOH) at the *APC* locus are also found in small adenomas, and these events have been linked to dysregulated proliferation on the basis of their role in familial adenomatous polyposis [166]. Mutations at the *APC* locus are typically nonsense mutations or insertions and deletions that produce frameshifts resulting in downstream stop codons. Mutations in the *KRAS* and *TP53* genes are found in larger adenomatous polyps and are thought to play a mechanistic role in supporting neoplastic growth. Indeed, activation of *KRAS* mutations seems to facilitate the exophytic growth of adenomatous polyps whereas *KRAS* mutations are more rare in flat adenomas and cancers [167].

Point mutations play a prominent role in the early stages of neoplastic progression, typically by inactivating the function of tumor suppressor genes. It has been hypothesized that mutant APC proteins may bind to the wild-type proteins and interfere with their function through a dominant negative mechanism [168]. In the case of *KRAS*, mutations inactivate the portion of the protein involved in switching off signal transduction [20,169]. In the case of *TP53*, the mutations, which occur in a broad region in the middle of the p53 protein, interfere with the ability of the protein to activate gene expression in the nucleus [136]. It is uncertain whether one mutant p53 protein can bind to and inactivate the wild-type protein and act through a dominant negative mechanism.

A second form of genomic instability that does not result in LOH events is involved in the genesis of about 12%–15% of colon cancers. These tumors demonstrate multiple errors in DNA sequences throughout their genome, which result from a failure to edit errors made during DNA replication [170–172]. This was first observed in microsatellite sequences, which are repetitive sequences that require the DNA mismatch repair system for proper replication because of an intrinsic tendency to be copied inaccurately. In certain tumors, deletion mutations occur in the DNA microsatellite sequences; this is termed microsatellite instability. Microsatellite sequences are ubiquitous throughout the genome, and half

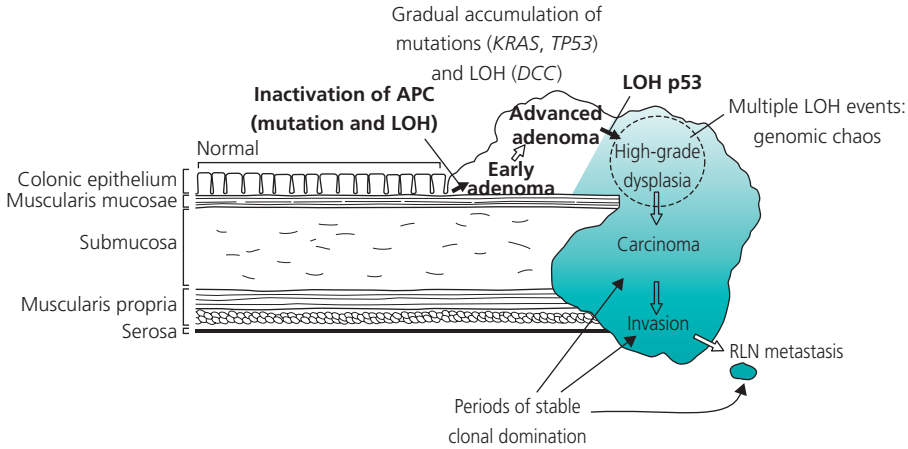

Figure 24.8 Chromosomal instability model of genetic events mediating neoplastic progression during colorectal tumorigenesis. This type of genomic instability is present in familial adenomatous polyposis and in about 85% of sporadic colorectal cancers in which segments or whole chromosomes are deleted, duplicated, or rearranged, forming aneuploid tumors. *APC* is inactivated by mutation and loss of heterozygosity (LOH) at the transition between normal colonic epithelium and adenomatous epithelium. Thereafter, successive rounds of clonal expansion occur, with accumulation of mutations (i.e., *KRAS, TP53*, others) and LOH (i.e., chromosome 18q) that allow acceleration of growth and dominance over less-advantaged clones. *TP53* inactivation by LOH of 17p13.1 first occurs in high-grade dysplasia, and defines the transition between benign and malignant neoplasia. Successive genetic events as well as prior genetic changes enable the colon cancer to invade and metastasize to regional lymph nodes (RLNs), and ultimately to distant organs such as the liver. From Boland et al. [35].

or more may be mutated in tumors that have microsatellite instability.

Tumors with microsatellite instability demonstrate significantly less LOH than usually occurs in colorectal cancers with chromosomal instability. Thus, either chromosomal instability or microsatellite instability develops as the type of instability within a tumor genome [173]. Microsatellite instability is more common in cancers of the proximal colon and is associated with diploid tumors and increased patient survival [170,174]. This hypermutable phenotype appears to be the mechanism for rapid neoplastic progression in Lynch syndrome and in sporadic tumors that exhibit microsatellite instability [172,175].

Lynch syndrome and about 10%–12% of sporadic colorectal tumors exhibit microsatellite instability. An analysis of repetitive sequences in tumor tissue led to the identification of other inactivated genes in these tumors, suggesting a second pathway for developing neoplasia. Each of these targeted genes contains a microsatellite within its (exonic) coding sequence, making it susceptible to mutation in the absence of DNA MMR activity. For example, a repeat of 10 adenines in the *TGFBR2* gene has undergone frameshift mutation in 85% of colorectal tumors with microsatellite instability, inactivating this receptor [30,39], and the cell escapes growth suppression from the ligand TGF-β1. In the remaining 15% of tumors with microsatellite instability, it is estimated that frameshift mutations of *IGF2R* at its polyguanine sequence uncouple TGF-β1 growth suppression [43]. Another important target of microsatellite instability is *BAX*, a member of the *BCL2* gene family. BAX heterodimerizes with BCL2 within the cell, and the ratio of the two heterodimers determines the cell's commitment to programmed cell death. *BAX* contains a polyguanine $(G)_8$ tract that is mutated in 50% of colorectal cancers with microsatellite instability, and in 64% of gastric adenocarcinomas with microsatellite instability [176,177]. Mutation of *BAX* (by diminishing the ratio of BAX to BCL2) prevents programmed cell death. Other genetic targets include the MMR genes *hMSH3* and *hMSH6*, which may broaden and accelerate the accumulation of mutations [178]. A comprehensive search using a genetic database for coding microsatellites and subsequent colorectal tumor analysis revealed nine genes that were mutated in more than 20% of the tumors [179]. Aside from *TGFBR2*, *BAX*, and *hMSH3*, these included the *ACVR2* (activin type II receptor), *SEC63* (human homologue of a yeast DnaJ-like endoplasmic reticulum translocon component protein gene), *AIM2* (an interferon-inducible gene), *NDUFC2* (NADH-ubiquinone oxidoreductase B14.5B subunit gene), *COBLL1*/*KIAA0977* (probable human homologue of the mouse embryonal protein *cordon-bleu*), and *PA2G4*/*EBP1* (homologue of the mouse cell cycle protein p38-2G4) [179]. Subsequently, *ACVR2* has been shown to be mutated in the polyadenine tract of exon 10 in most microsatellite unstable colon cancers, causing the loss of ACVR2 protein expression [41] and an associated increase in local tumor growth [42]. Furthermore, reconsitution of mutated *ACVR2* leads to restitution of growth inhibitory signaling in colon cancer cells [180]. The role of the other often-mutated genes in the pathogenesis of tumors with microsatellite instability is not known.

Several lines of evidence indicate that the Wnt signaling pathway, including the gatekeeper APC or other proteins in

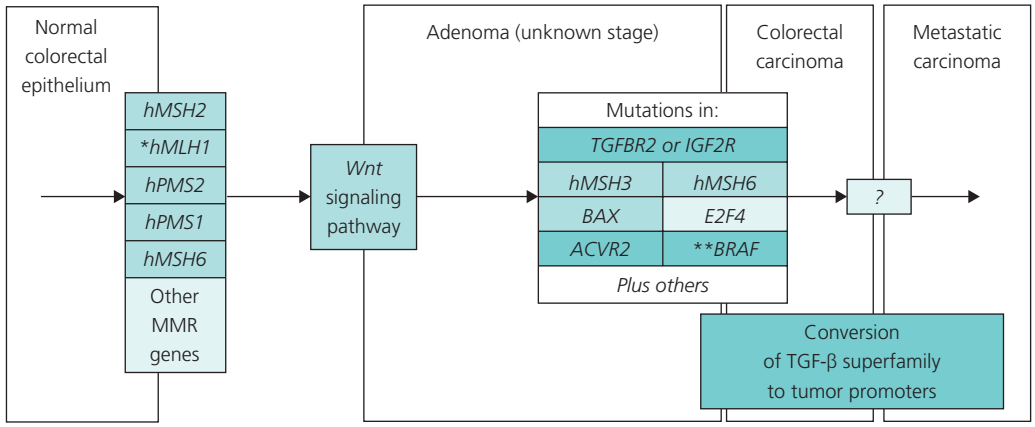

Figure 24.9 Colorectal tumor progression in microsatellite unstable tumors. This type of genomic instability is present in hereditary nonpolyposis colorectal cancer (germ-line mutation of a DNA mismatch repair [MMR] gene) and in about 15% of sporadic colorectal tumors (hypermethylation of the MMR gene promotor *hMLH1*). These tumors tend to be diploid because chromosomal arrangements typically do not occur. Biallelic inactivation of an MMR protein occurs to disrupt the DNA MMR system completely. The Wnt signaling pathway, including the gatekeeper protein APC and other proteins in this pathway, are deregulated. Many genes with encoded microsatellites are targeted for mutation in microsatellite unstable tumors, including *hMSH3*, *TGFBR2*, and *BAX*. Mutations in *TGFBR2*, *ACVR2*, *BRAF*, and *BAX* appear at the transition point during malignant conversion. Other mutated genes are likely to be involved in forming the final tumor and in mediating metastasis. **hMLH1* is hypermethylated in sporadic cancers only. ***BRAF* mutations occur in sporadic microsatellite unstable tumors and are not seen in Lynch syndrome tumors.

this signaling pathway, are also affected in microsatellite unstable tumors (see Fig. 24.2). Some *APC* mutations have been found in colorectal tumors with microsatellite instability [181]. In addition, β-catenin, a protein that binds to and is normally degraded by the APC protein, undergoes a stabilizing mutation in microsatellite unstable primary tumors and cell lines [182]. Activating mutations in β-catenin signal the cell to proliferate incessantly [183,184], and the mutations prevent interaction with the APC protein and its subsequent targeting for degradation. TCF-4, to which β-catenin binds as a partner in the nucleus for transcriptional activation, is mutated at its polyadenine site in 39% of primary colorectal cancers with microsatellite instability [185]. Simultaneous analysis of various components of the Wnt pathway in microsatellite unstable colorectal cancer indicates that there is often a perturbation in the Wnt signaling pathway, including mutations in APC, β-catenin, TCF-4, or axin I (involved as a scaffold protein and negative regulator of Wnt signaling) [186,187]. Taken together, colorectal tumors exhibiting microsatellite instability appear to have initiated their growth through deregulation of Wnt signaling, which has the identical consequence as inactivating the gatekeeper APC.

As described for the chromosomal instability pathway of tumorigenesis, inactivation of several genes likely promotes the progression of the neoplastic process in tumors with microsatellite instability, but a common sequence of genetic alterations has not been elucidated. Mutations of *TGFBR2*, *BAX*, and *ACVR2* appear to be late in the adenoma–carcinoma progression because these mutations tend to occur in high-grade dysplasia at the interface between adenoma and carcinoma [42,188,189], although both *BAX* and *TGFBR2*, but not *ACVR2*, are often mutated in adenomas from relatives of patients with Lynch syndrome [174]. A proposed scheme for the progression of microsatellite unstable colorectal cancer is presented in Figure 24.9.

Much has been learned about multistep carcinogenesis through studies of colon cancer, and the principles may be applicable to cancers of many types. Multiple genetic events are required to overcome the redundancy in regulatory controls in the cell. These events accumulate during successive waves of clonal expansion and cooperate to produce tumor progression. The exact impact on growth regulation is not entirely known for any single genetic lesion. A small proportion of cancers lack the genetic events seen in most others so it is reasonable to speculate that there are additional mechanisms of tumor progression, and there is evidence for alternative pathways [91,167].

CpG island methylator phenotype

A third pathway in colon carcinogenesis involves widespread hypermethylation of gene promoters as the mechanism for epigenetic inactivation and gene silencing [91]; this pathway has been termed the CpG island methylator phenotype (CIMP) [190]. In fact, sporadic colorectal cancers with microsatellite instability lose DNA MMR activity as a consequence of hypermethylation of *hMLH1* [191,192], so those tumors are actually a subset of the CIMP tumors. Other genes often inactivated by hypermethylation at promoter CpG islands include the cell cycle regulator *CDNK2A/p16*, the angiogenesis inhibitor *THBS1*, the estrogen receptor growth suppressor *ESR1*, the metastasis suppressor *TIMP3*, and the O^6-methylguanine DNA methyltransferase DNA repair gene *MGMT* [193], and even *APC* [194]. CpG islands are regions of DNA 0.5–2 kilobase pairs long, rich in cytosine-guanine

dinucleotides, that are found in the 5′ region of about half of all human genes. Methylation of cytosines within the CpG islands is associated with loss of gene expression and can be observed not only physiologically (as with X chromosome inactivation and aging), but also in neoplasia. In a study of colorectal cancers, the CIMP phenotype segregated with tumors that demonstrated microsatellite instability [190]. However, in a study of colonic adenomas, the CIMP and microsatellite instability pathways appeared to be distinct [193], and CIMP cancers were associated with distinctive epidemiological, histological, and molecular features [195]. The CIMP phenotype seems to occur early in colorectal tumorigenesis, but the mechanism for its occurrence is not known.

Loss of genetic imprinting and cancer

Alteration in genetic imprinting is another type of epigenetic aberration seen in cancer. Imprinting refers to the silencing of one parental allele in cells by selective promoter methylation. Loss of imprinting (LOI) is a process that occurs in some neoplastic tissues in which the imprinted allele is inappropriately reexpressed as the result of a loss of promoter methylation. LOI of the insulin like growth factor II gene (*Igf2*) doubles the number of inherited tumors in *Min* mice [196]. LOI of the *IGF2* gene may also occur in humans with colorectal cancer, and the inappropriately expressed allele can be assayed with a DNA-based blood test [197].

While carcinogenesis of the colon has been studied most intensely as a result of availability and accessibility of a common precursor lesion, it appears that other gastrointestinal cancers may have distinct patterns of genomic instability. In gastric cancer for example, microsatellite stable, unstable, and CIMP subsets have been recognized [198]. In hepatocellular carcinoma, chromosomal instability occurs in association with *TP53* mutations, hepatitis B viral infection, and poorly differentiated cancers, while chromosomally stable cancers often harbor activated β-catenin [199,200].

Tumor formation and behavior

Molecular histology

Much of the understanding of the genetics of gastrointestinal tract carcinogenesis has come from analyzing various histological stages of tumors. Neoplasia originates from a single cell that has developed a genetic alteration, and then grows at variable rates to larger sizes with differing histological morphology. In the colonic adenoma there is continued mitosis and lack of cellular differentiation, so that the principal proliferative compartment moves from the base of the crypt to involve the entire crypt. Persistent replication of cells near the crypt surface, in concert with retarded cell maturation and extrusion, results in an increased number of replicating surface cells. Continued proliferation coupled with a lack of cell death leads to a downward infolding of epithelial cells, which branch and interpose themselves between normal crypt elements. This process results in the characteristic branching glandular pattern of tubular adenomas. As the polyp continues to grow, the growth pattern of the underlying mesenchyme may be enhanced [201,202]. If mesenchymal proliferation matches the rate of epithelial growth, resistance to continued epithelial proliferation is unimpeded, resulting in long, finger-like projections of the glandular elements. This histological picture is characteristic of villous adenomas. Perhaps more commonly, an adenoma demonstrates a combination of these two histological types, a tubulovillous adenoma. Villous adenomas tend to be larger than tubular adenomas, consistent with the concept that the villous histology is associated with enhanced growth characteristics. Subsequently, some adenomas develop pathological and biological characteristics of carcinoma, giving rise to the malignant polyp. Malignant cells at some point develop the capacity to invade locally and the potential to spread to distant organs.

Each histological stage of neoplasia has been characterized genetically by analyzing the cells in the specimen. Immunohistochemistry, RNA and DNA extraction, sequencing, and in situ polymerase chain reaction are some of the techniques used to determine the genetic abnormalities of the cells. The sequential biological process of tumor formation has been correlated with specific morphological characteristics of tumors in their various stages [35,42,167].

As mentioned, the general features of the colorectal neoplastic model may be applicable to other gastrointestinal neoplasms. In esophageal, pancreatic, and hepatic neoplasms, however, it is difficult to obtain tissue samples at each stage of a multistep process. These tumors do not have a readily identifiable precursor lesion to obtain and study. Tumors of these organs, particularly of the esophagus and pancreas, are often diagnosed in advanced stages, precluding an opportunity to obtain specimens at early stages of the disease. However, it has been recognized that most esophageal adenocarcinomas arise from the esophageal mucosa with intestinal metaplasia (see Chapter 35, Barrett esophagus and esophageal adenocarcinoma), and that a subset of pancreatic cancers arises from pancreatic intraepithelial neoplasia (see Chapter 70 Nonendocrine tumors of the pancreas). The natural history of these lesions is still not fully appreciated, nor are they readily accessible for scientific study.

Loss of proliferative control and clonal expansion

Proliferation is normally tightly regulated and confined to one compartment of the epithelial unit within the gastrointestinal tract. During inflammation and epithelial repair, cell death and turnover increase, regulation is relaxed, and the rate of proliferation increases to compensate for the losses. When a sufficient number of cells have been produced, the rate of proliferation slows to retain homeostasis.

A chance genetic event may occur in one cell that has not

yet been committed to programmed cell death, enabling it to undergo more cycles of replication than its neighboring cells. When this event affects a gatekeeper gene or pathway function, neoplasia may be initiated. In the colon, the proliferative pool of cells migrates from its normally restricted site near the base of the crypt toward the apex of the crypt. Abnormal clusters of cells may be seen in magnified histological preparations of grossly normal-appearing colonic mucosa stained with methylene blue, and these have been termed *aberrant crypt foci* [203]. Aberrant crypt foci are collections of thickened epithelium with asteroid-shaped or oval glandular lumens [204]. The number of aberrant crypt foci appears to be higher in patients with a previous history of colon cancer, and even higher in patients with familial adenomatous polyposis [203]. Not all aberrant crypt foci are associated with the same types of mutations, and they likely represent more than one distinctive lesion. Most aberrant crypt foci have a nonneoplastic histological appearance, and these contain *KRAS* mutations [204–208]. However, histologically dysplastic aberrant crypt foci (which are much less common) may contain *APC* mutations [205,207]. The abnormalities in APC in these latter lesions suggest that dysplastic aberrant crypt foci may be the earliest neoplastic lesions identifiable in the colon. Dysregulation of the Wnt signaling pathway, of which APC is a part, appears to be critical for the initiation of colonic neoplasia [114,166]. Loss of the Wnt signaling pathway uncouples the cell from programmed cell death, and the resulting increase in β-catenin enhances homotypic intercellular adhesion [12,209]. Because these cells continue to proliferate and adhere to one another, but are incapable of invasion, they tend to expand locally and develop into adenomas. Additional growth without invasion permits the formation of a polypoid lesion. This pattern of development of colonic neoplasia is not necessarily typical of neoplasia of the esophagus, stomach, and pancreas.

The liberation from typical growth restraints allows the cell to grow at a faster rate than any surrounding cell. Subsequently, if additional genetic alterations occur in one of these cells, further acceleration of growth may occur. Each advantage gained in growth, beginning with the adenoma and subsequently progressing to carcinoma, occurs by successive waves of clonal expansion. Each clone gains its advantage by a genetic alteration within the cell that allows more successful proliferation than its predecessors. In the colon, it appears that mutations in the *KRAS* gene, and possibly the *TP53* gene, participate in this process. Alterations in the KRAS signaling pathway may permit enlargement of the adenoma to an exophytic mass [167]. Flat cancers that have mostly been observed in Japan tend to lack *KRAS* mutations [167,210,211]. In microsatellite unstable tumors, clonal expansion may be mediated in part by alterations in the Wnt signaling pathway or the TGF-β1/activin signaling pathway, by escape from BAX-mediated programmed cell death, and by *BRAF* mutations [28–30,39,41,42,176,177,180,186,187].

The development of adenomas is a reversible biological event in the colon, at least by gross appearance. Small, sporadic adenomatous polyps may spontaneously disappear, and small rectal adenomas often spontaneously disappear from patients with familial adenomatous polyposis after subtotal colectomy [212]. Patients who have hundreds of colonic adenomas may experience regression of these lesions after the administration of the nonsteroidal antiinflammatory drug sulindac or the cyclooxygenase-2 inhibitor celecoxib [14,213–215]. The mechanism underlying adenoma involution in response to sulindac or celecoxib has been attributed to the activation of programmed cell death by way of unknown mechanisms [13,14]. The incidence of gastrointestinal cancer is significantly reduced in patients taking aspirin and other nonsteroidal antiinflammatory drugs (see Chapter 65 Malignant tumors of the colon). Pharmacological therapies that may block neoplastic progression at this early stage are being evaluated, but the search is complicated by need for an agent with excellent safety profiles.

Malignant conversion

At least two genetic mechanisms permit destabilization of the cellular genome and the appearance of cancer. Both mechanisms involve the breakdown of a homeostatic mechanism by which the genome is faithfully reproduced.

Chromosome instability reflects a failure in the symmetric separation of sister chromosomes and results in LOH events throughout the genome. When LOH occurs at a tumor suppressor gene locus, additional clonal expansion may be greatly favored. The inability to divide the tetraploid nucleus into two perfect diploid pairs at mitosis results in the random generation of aneuploid cells. Cells that accumulate extra chromosomal pieces become hyperploid, and those that lose chromosomal arms become hypoploid. Randomly occurring events that provide a growth advantage result in clonal expansion of the favored cell. Flow cytometry has been used to detect expanded clones with abnormal DNA contents [216,217]. Highly aneuploid tumors exhibit increased virulence [216,218]. The abnormal clones occur focally rather than diffusely throughout the tumor, and evidence suggests that metastatic lesions are derived from these aneuploid clones [219]. In the colon, the histological appearance of high-grade dysplasia coincides with the complete genetic inactivation of *TP53* in chromosome-unstable tumors [35].

A second type of genetic lesion capable of increasing the rate of mutation is inactivation of the DNA MMR system. This form of genomic instability leads to *microsatellite instability*, and allows an accumulation of mutations throughout the genome. Microsatellite instability leads to an increase in point mutations, as well as insertion/deletion mutations at simple repetitive DNA sequences (microsatellites). Cancer cells with microsatellite instability are typically diploid [171]. The polymerase chain reaction can detect the presence of the

inaccurately copied microsatellite DNA. Because of the high mutation rate, it is probable that tumors with MMR defects may progress to carcinoma at a more rapid pace than other tumors [172,175]. Malignant conversion in colorectal tumors with microsatellite instability involves mutation of *TGFBR2*, *ACVR2*, and *BAX*, all of which appear in high-grade dysplasia at the adenoma–carcinoma transition [42,188,189,220].

Malignant cells are capable of continued growth, invasion, and formation of distant metastases. The malignant phenotype is intrinsically unstable and gives rise to additional phenotypic diversity. Malignant tumors are dynamic by virtue of ongoing genomic instability. Some of the newly created cells lose proteins that are essential for survival and die. Other genetic events result in the generation of more virulent clones that overgrow and replace the parent tumor.

Metastasis

The metastasis of tumor cells is not a random or accidental process. In fact, most circulating tumor cells will not give rise to a metastasis. For a tumor to metastasize, it must degrade the basement membrane and associated matrix components, migrate through the subtending connective tissue, enter into a lymphatic or blood vessel, migrate away from the parent tumor, avoid a gauntlet of naturally occurring defense mechanisms, emigrate from the efferent vessel, lodge at a site favorable for growth, and recruit a sufficient blood supply (Fig. 24.10) [221,222]. Metastasis occurs as a late event in the natural history of a tumor, because time is required for the gradual evolution of cells capable of all these behaviors.

The characteristics required for metastasis are specific. A malignant neoplasm, for example, may acquire the ability

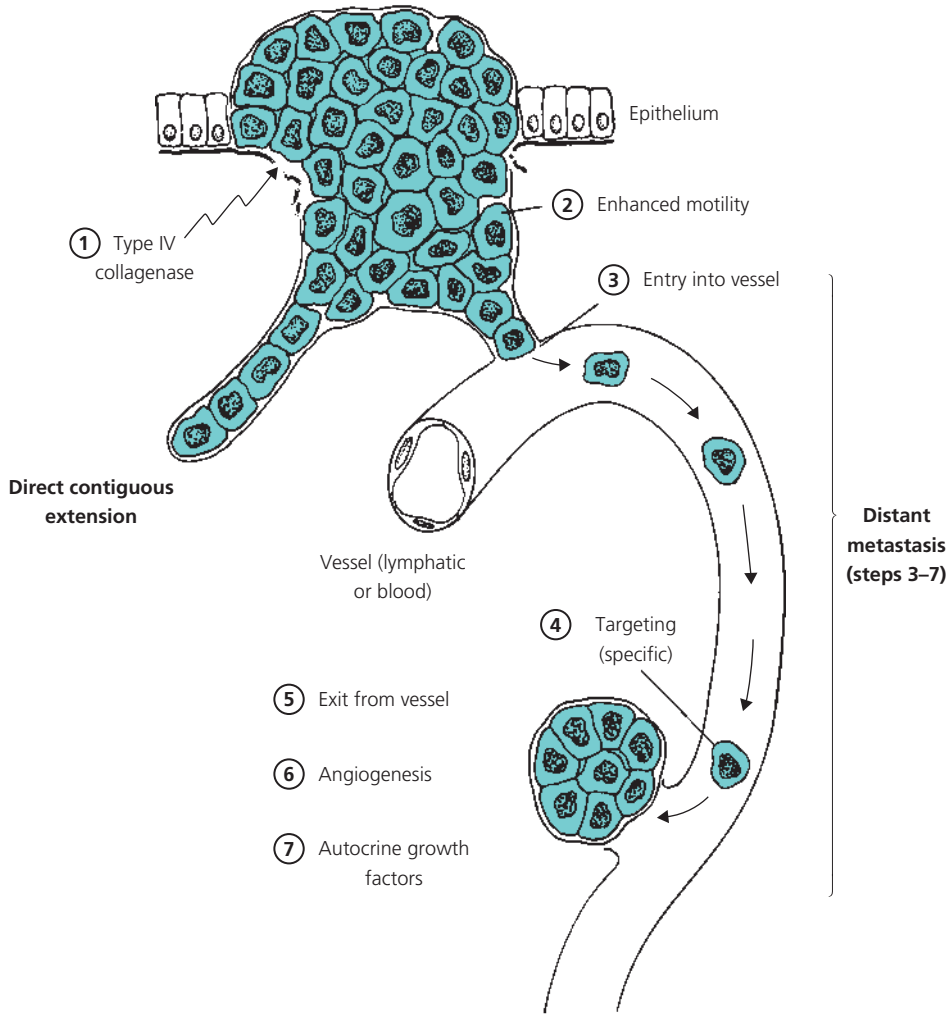

Figure 24.10 Tumor metastasis. Successful tumor metastasis requires several coordinated events. Specific collagenases are required to transgress the basement membrane (step 1). The tumor may grow by direct contiguous extension into surrounding organs. Alternatively, tumor cells may enter a vascular structure for distant metastasis to occur. The spread of malignant cells is enhanced by the development of cellular motility (step 2). Access to a vascular structure is permitted by the expansion of the growing tumor, the motile cells, and the ingrowth of blood vessels, which is stimulated by angiogenesis factors (steps 3 and 6). Entry into the circulation is not sufficient to ensure distant metastasis. Specific targeting mechanisms and adaptations that would enhance survival at a distant site are required for distant metastatic spread.

to digest the basement membrane and slowly expand into the submucosa; however, it may not acquire other properties required for entry into the circulation or for survival at an ectopic site. Such a tumor is pathologically malignant and may become a bulky mass, but there may be a relatively long period during which it can be detected and successfully removed. Alternatively, a highly metastatic clone of cells emerging from a malignant tumor may give rise rapidly to a large number of metastatic units, from which the host may suffer a brisk and relentless downhill clinical course. However, this is likely a dynamic process that may be modeled by selective forces defined by the severity of the microenvironment, allowing a variety of clones to coexist in a heterogeneous tumor mass [223].

Anoikis, Greek for "loss of home", describes the phenomenon whereby normal epithelia undergo apoptosis when detached from the extracellular matrix [224]. Consequently, tumor cells need to be anoikis-resistant to metastasize [225]. The underlying mechanisms rendering tumor cells resistant to anoikis are not fully understood, but may require the stimulation of survival signals that are not dependent on contact with the extracellular matrix. Molecules that change in expression, distribution, and function during the epithelial-to-mesenchymal transition and are causally involved in the process include growth factors (such as TGF-β and IGF1) [226], transcription factors (SMADs and SNAIL) [227], cell adhesion molecules (integrins and extracellular matrix proteins) [226], and cell–cell adhesion molecules (E-cadherin) [228], as well as extracellular proteases (matrix metallproteinases and caveolin) [229]. Molecular candidates in the development of anoikis-inducing agents to block cancer progression to metastasis include galectins, caveolin, and tyrosine kinase receptor B [227,229–231] and are at the center of the development of anoikis-inducing agents to target cancer progression to metastasis [230].

Current insight into the metastatic process is insufficient to predict the clinical course of a malignancy by studying tumor cells in vitro. Tumor cells are often present in the portal and peripheral circulation at the time of colectomy for a colon cancer [232,233]. However, the presence of cytologically identifiable tumor cells in the circulation has no prognostic significance because most of these cells are incapable of growing at a distant site and are susceptible to cytolysis by natural killer lymphocytes and other immunological mechanisms. Clumps of cells appear to be more likely to survive as a metastasis, perhaps because individual cells in the clump have only some of the functions required for successful metastasis, but the entire "social unit" is capable of growing communally.

Tumor cells and patient survival can be affected by local immune cells. The presence of cytotoxic T cells may be a better predictor of patient survival than TNM (tumor/node/metastasis) staging [234]. The type, density, and location of these T cells infiltrating the tumor may protect or slow the metastatic capacity of the primary tumor.

Tumor cells require a blood supply to survive. Oxygen can normally diffuse 100–200 μm from a blood vessel, which is roughly 4–10 cell diameters, depending on the tumor. To facilitate their growth at metastatic sites, tumors stimulate the growth of capillaries. Tumor-associated angiogenesis appears to be mediated by a variety of different tumor growth factors, including angiogenesis factors. The presence of such growth factors can be demonstrated by the tumor's ability to recruit endothelial cells to form capillaries in vitro [235]. Inhibition of vascular endothelial growth factor (VEGF) in mouse models of colon cancer blocks tumor growth and induces apoptosis by inhibiting angiogenesis and endothelial cell survival [235]. Evidence suggests that the expression of transcripts in the endothelium of tumor blood vessels differs from that in the endothelium of nontumor vessels [236]. VEGF is a key mediator in this process. Bevacizumab, a humanized monoclonal antibody that recognizes VEGF receptors, neutralizes the biologically active forms of VEGF that interact with these receptors. In addition, bevacizumab has shown antiangiogenic and antitumor activity in several cancer types, and therefore is used in combination with fluorouracil-based chemotherapy as a first-line treatment for metastatic cancer of the colon or rectum [237].

Not all cells in a primary tumor have identical metastatic potential. From a primary tumor, it is possible to clone cells with an enhanced capacity for metastasis and a propensity to metastasize to specific organs. For example, certain experimental tumor cells form lung metastases in rodents, but others do not. When these metastatic colonies are removed from the rodent's lungs and their numbers are expanded in culture and injected into another animal, each successive cycle produces a larger number of pulmonary metastases. The propensity of the highly metastatic cell line to grow in the lung appears to result from a specific interaction between the injected cells and the pulmonary capillary bed. Lung lesions are seen even after left ventricular injection of the cells, in which the lung is not the first capillary bed encountered, and after transplantation of lung tissue to a subcutaneous location. By means of the same technique, other cell lines can be developed with an enhanced propensity to metastasize to the liver or to specific sites within the brain [238,239]. Site-specific metastasis appears to be mediated by cell surface membrane glycoproteins. Removal of the glycoproteins from the tumor cell surface membranes interferes with the specificity of metastasis. By fusing membrane vesicles from metastatic cells to those of nonmetastatic cells, it is possible to confer homing specificity to the recipient cells.

Therefore, the sites to which malignant cells metastasize in patients with cancer may appear to be random, or based purely on the blood supply of an organ, but the process is more complicated. For example, although tumor cells may be present in the portal blood or peripheral circulation at the time of surgical resection of a colon cancer, hepatic metastases occur only in some instances. Metastases may be

present in the lung, bone, and other distant sites, such as the brain, even in the absence of hepatic metastases. Tumor cells can migrate through the hepatic sinusoids without establishing a malignant deposit in the liver. The phenomenon of cells specifically seeding at the metastatic site is called "homing". Homing may be mediated by factors secreted by the primary tumor to prepare the metastatic sites, including the upregulation of fibronectin and matrix metalloproteinases. This change in local microenvironment in preparation of the cancer cell's arrival is termed the *premetastatic niche*, which may explain the different patterns of metastasis of various tumors [240].

Primary colon cancer differs from metastatic colon cancer in part because metastatic cancer cells express sialylated mucin-associated carbohydrate structures that may play a role in adhesion to the target endothelial glycoproteins and the target cell basement membrane [37]. Other carbohydrate structures found on colon cancer cells appear to bind to molecules such as laminin, a normal component of the basement membrane. Metastases from several gastrointestinal cancers rely to some degree on mutations in growth factor mediators. This observation has been exploited by the development of growth inhibitors to reduce the tumor burden of patients. Gefitinib inhibits epidermal growth factor receptor and has been shown to reduce tumor growth [241]. Imatinib mesylate, which binds and inhibits tyrosine kinase, reduces the tumor burden in chroni myeloid leukemia and many gastrointestinal stromal tumors [242]. The activities of VEGF are mediated by two receptor tyrosine kinases. Bevacizumab has both a direct antiangiogenic effect as well as a local effect, allowing more efficient delivery of chemotherapy by altering tumor vasculature, and has shown promise in treating metastatic colon cancers [243,244].

Metastasis genes

The genetic basis of metastasis is complex and not well understood. One gene has been identified that is associated with distant metastasis in colorectal carcinoma. The *NM23* gene (also designated *NME*) seems to function as a metastasis suppressor gene, inasmuch as it is deleted in distant metastases from some colorectal cancers [245]. Patients without allelic deletions of this gene have a significantly better prognostic outlook. The cellular mechanism by which the *NM23* locus confers metastatic capability is uncertain, but transfection of the gene into certain tumor cell lines inhibits their ability to migrate in response to chemoattractants [246]. *NM23* gene mutation has been reported from human colorectal cancer metastases, suggesting that it may undergo a two-step inactivation, like other tumor suppressor genes [247]. NM23 was down-regulated in colon cancer specimens from patients with poor prognoses, congruent with metastasis suppressor function [248].

Other potential metastasis suppressor genes may be located on the long arm of chromosome 18. Chromosome 18q is lost in 80% of patients with stage IV colorectal cancer [249]. The deleted in colorectal cancer (*DCC*) gene, located on chromosome 18q21, is one putative metastasis suppressor gene, and was identified during the study of commonly deleted regions on chromosome 18q in colorectal cancers [250]. *DCC* encodes a unique protein with homology to neural cell adhesion molecules and other related cell surface glycoproteins. This similarity has led to the hypothesis that the product of the *DCC* gene is involved in cell–cell adhesion and cell matrix interactions, which may be important in preventing tumor growth, invasion, and metastases. Expression of *DCC* was reportedly absent in most colorectal cancers metastatic to the liver, but it was lost in only a minority of nonmetastatic cancers [251]. However, germ-line inactivation of the murine homologue of *DCC* failed to support a tumor-suppressive function for its protein [252]. Other studies have indicated that DCC is a netrin-1 receptor, which, when engaged by a ligand, mediates signals for proliferation. In the absence of ligand, DCC can induce apoptosis [253]. The dependence of DCC on netrin-1 binding suggests a potential role for DCC in colon cancer metastasis.

Additional tumor suppressor genes on chromosome 18q include the TGF-β1 signaling molecule genes *SMAD2* and *SMAD4* [254]. One possibility is that the loss of several of the genes located on 18q cooperates to enhance tumor virulence and metastatic potential.

Other putative metastasis suppressor genes include *S100A4*, which is a direct β-catenin/TCF target that induces colon cancer cell migration and invasion in vitro and metastasis in vivo [255]. Matrix metalloproteinase 9, produced by human colon cancer epithelia, appears to play a role in processes underlying metastasis; it regulates metastatic behavior in vitro, including degradation of extracellular matrix components and formation of organelles involved in migration and hematogenous seeding of mouse lungs by human colon cancer cells in vivo [256].

Clinical markers of neoplasia

Biological tumor markers and oncofetal proteins

Some gastrointestinal cancers acquire and elaborate cell surface determinants that recapitulate structures transiently expressed during normal fetal development. These oncofetal proteins are often glycoproteins and can usually be detected in the plasma. Carcinoembryonic antigen (CEA) was first detected with antibodies developed after rabbits were immunized with colorectal cancer cells; these antibodies are largely directed to the carbohydrate moieties of the glycoprotein [257,258]. Subsequently, elevated levels of CEA were demonstrated in patients with colorectal cancer and other cancers. However, the limitations of CEA measurements in the population as a whole have precluded the use of CEA as a screening or diagnostic marker. Poor sensitivity and

specificity in asymptomatic populations render it less than useful for detecting early, potentially curable cancers [259]. Other conditions besides colorectal cancer are associated with elevated plasma levels of CEA, including cancers of the stomach, pancreas, and liver, nonmalignant diseases of the liver such as alcoholic hepatitis, inflammatory bowel disease, and tobacco use [260]. CEA levels measured at the time of diagnosis may be correlated to the prognosis of colorectal and other cancers (i.e., the higher the CEA elevation, the more advanced is the cancer stage at diagnosis) [261,262]. Nonetheless, this information cannot be used to predict outcome. CEA is recommended as an adjunct in the staging of colon cancers, as well as in surveillance and monitoring the response to systemic therapy, if the levels are elevated at the time of diagnosis and fall after successful resection [263].

Other oncofetal proteins have been used to screen for the presence of gastrointestinal cancer. As with CEA, poor sensitivity and specificity and elevated levels in nonmalignant conditions have limited their usefulness in screening. The determinants for many other markers reflect modifications of blood group antigens (e.g., Lewis blood group) and mucin glycoproteins. Table 24.4 shows some tumor markers and their associated cancers.

Genetic tumor markers

Cancer cells develop as a consequence of multiple genetic insults that affect normal growth regulatory mechanisms. Detection of mutations in tissue samples would therefore suggest the presence of cancer or precursor neoplasia. Techniques in molecular biology, largely through use of the polymerase chain reaction, allow the detection of minute amounts of tumor cells or free nucleic acid in biological samples. For example, *KRAS* mutations have been detected in DNA purified from feces, but benign and malignant colorectal tumors could not be differentiated [264]. In addition, *KRAS* mutations have been detected in the stool of patients with pancreatic adenocarcinoma as well as chronic pancreatitis [265]. The detection of *KRAS* mutations in feces as a means of differentiating neoplasia from inflammation is difficult, and differentiating between colonic and pancreatic neoplasia likewise is problematic. *KRAS* mutations also have been detected in the plasma of patients with colorectal cancer [266]. The detection of *KRAS* mutations in pancreatic juice may eliminate the colon as a source of the mutation, but still cannot distinguish benign from malignant disease [267].

Mutation in *TP53* has been associated with poor prognosis in several malignancies, including colon cancer [268], and the loss of normal *TP53* signals the transition from benign to malignant colorectal neoplasia [35]. Tumors with mutations in *TP53* tend to respond poorly to therapy, whereas malignancies that rarely have *TP53* mutations (e.g., Wilms tumor, testicular cancer, acute lymphoblastic leukemia) often respond to cytotoxic chemotherapy [269]. The finding of *TP53* mutations in the urine of patients with bladder cancer and in the stool of patients with colorectal cancer suggests that detection may eventually be useful for screening [270,271]. The presence of mutated *TP53* suggests the presence of a neoplastic process; however, it may not indicate loss of the second, normal *TP53* allele that is seen in malignant transformation. Distinguishing a benign adenoma from colorectal cancer, as with *KRAS*, may be impossible. The costs and utility of advanced molecular diagnostics in colon cancer in comparison with those of conventional screening methods have not been addressed.

Detection of naked DNA by microsatellite analysis of the serum [272] and plasma [273] of patients with head and neck cancer and of patients with small cell lung cancer, respectively, indicates that these tumors release large amounts of DNA into the blood. For the most part, microsatellite alterations in the circulating tumor DNA were similar to those in the primary tumor. Enrichment of the tumor DNA in the blood may occur. Aberrantly methylated *hMLH1* promoter DNA has also been detected in the serum of patients with microsatellite unstable tumors [274]. LOH can be detected in the serum, which requires higher concentrations of DNA than that needed for the detection of microsatellite instability

Table 24.4 Examples of oncofetal antigen markers in the gastrointestinal tract

Marker	Epitope	Site of primary tumor with serum elevation	Site of other tumors with serum elevation
CEA	Multiple glycosylation sites on an immunoglobin-like protein	Colorectum	Stomach, pancreas, biliary tract, liver
CA 19-9	Sialylated Lewis A	Pancreas	Stomach, biliary tract, liver, colorectum
CAM 17.1	Sialyl blood group antigen	Pancreas	Biliary tract
CA 50	Sialylated Lewis A	Pancreas	Stomach, biliary tract, liver, colorectum
CA 242	Mucin-like antigen	Colorectum	Pancreas
CA 72-4	Mucin-like glycoprotein (sialosyl-2-6α-*N*-acetylgalactosaminyl)	Pancreas, ovary	Colorectum
CA 125	Mucin-like molecule	Ovary	Colorectum

CA, carbohydrate antigen; CEA, carcinoembryonic antigen.

[275]. However, plasma or serum microsatellite alterations in benign or nonneoplastic tumors have not been identified.

The status of chromosome 18q in a tumor may have prognostic significance for patients with colorectal cancer. Rates of survival associated with stage II (Dukes B2) colorectal cancer and LOH at chromosome 18q were similar to those associated with stage III cancer [249]. The survival rates of patients with stage II cancer and no LOH at chromosome 18q were similar to those of patients with stage I cancer. The status of chromosome 18q was determined from the resected tumor and suggested that patients with stage II disease and LOH at chromosome 18q should be offered chemotherapy because patients with stage III disease have been shown to derive survival benefit from chemotherapy [276]. It is not known what genes on chromosome 18q may be metastasis suppressor genes that, if lost from the tumor, may allow spread to the regional lymph nodes (stage III) or distant organs [254].

Data are insufficient to endorse the routine use of p53, RAS, 18q loss of heterozygosity, DCC, thymidine phosphorylase or synthase, and dihydropyrimidine dehydrogenase in the treatment of patients with colon cancer [263].

One promising approach has been the detection of *APC* mutations in fecal DNA [277]. Mutations in *APC* generally initiate colorectal neoplasia, and detection of such mutations would be expected to segregate with neoplasia. In a feasibility study, *APC* mutations were detected in 57% of patients with polyps or cancer, but in none of the control patients without colorectal neoplasia [277]. However, it is technically difficult to identify every alteration in *APC*, and some neoplasms retain wild-type copies of *APC*. Future improvements in proteomics may increase the sensitivity of these assays.

Assaying multiple genetic targets as part of one complete assay is another approach to detect neoplasia. There is some genetic heterogeneity in colorectal tumorigenesis that may extend to other cancers of the gastrointestinal tract. One group assayed mutations of *KRAS*, *TP53*, and *APC* genes, *BAT-26* (a microsatellite instability marker), and highly amplifiable long-strand DNA from human fecal material [278]. *Long-strand DNA* refers to DNA that has not been degraded during apoptosis; it may be present in cells that have escaped programmed cell death. With the use of the panel of multicomponent markers, the sensitivity for detecting neoplasia was 82% for adenomas and 91% for cancers [278]. When *KRAS* was excluded from the analysis, the specificity for detecting neoplasia increased from 93% to 100%, with a positive predictive value for colorectal neoplasia of 100% and a negative predictive value of 85% [278]. A multitarget assay detecting 21 different mutations in stool proved somewhat better than Hemoccult testing, but still failed to detect most neoplastic lesions identified by colonoscopy [279].

The use of genetic tumor markers for screening and determining survival is therefore limited. As our understanding of tumor biology progresses and the sophistication of biotechnology improves, genetic tumor markers will likely play a role in the treatment of patients with cancer.

Genetic profiles affecting patient outcome

Research to understand the molecular mechanisms leading to cancer is ultimately driven toward the goal of being able to apply this knowledge to individual patient treatment. It is known that patients with microsatellite unstable colon cancer have a better clinical outcome than those with chromosomally unstable colon cancers [50]. On the other hand, microsatellite unstable colon cancers do not respond to adjuvant chemotherapy with 5-fluorouracil [280,281]. Although *BRAF* mutations typically occur in microsatellite unstable cancers with no influence on patient outcome, *BRAF* mutations are associated with a poor outcome in patients with microsatellite stable colon cancers [282]. In Barrett esophagus, the size of a clone with *TP53* LOH or ploidy abnormality predicts progression to cancer [283]. While these studies are encouraging, prospective studies are needed to validate individual genetic markers in patient treatment. High-throughput technologies allow the assessment of gene expression profiles of individual tumors to predict their response to chemotherapy. For example, in vitro drug sensitivity data may be validated with response data from cell line studies and coupled with gene array patterns. In the future, such approaches may greatly to develop chemotherapeutic regimens that are customized for individuals [284].

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E MISCELLANEOUS

25 The human intestinal microbiota and microbiome

Ruth E. Ley, Jeffrey I. Gordon

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Our adult bodies are colonized by a multitude of microbes whose collective genome, the *microbiome*, complements and enhances our own genome with genes we have not had to evolve on our own. In the decade before the sequencing of the human genome was completed, scientists anticipated that the number of genes it encoded would be close to 100 000. It came as a surprise that the number of genes encoded in the human genome is in the range of 20 000. Yet, if we consider the genes of our microbial partners, in addition to those encoded in our primate genome, 100 000 is likely an underestimate of our gene content. From this perspective, we are "supraorganisms" coated with a number of microbial cells that is 10 times greater than the sum of all our human somatic and germ cells.

The intestine contains the largest collection of microbes among all of our body "habitats" (locations for microbial colonization). In the colon, for example, anaerobic bacteria reach densities of 10^{11} per gram of lumenal contents. Together, gut microbes form a community, or *microbiota*, that has a major impact on health through interactions with host cells (including components of the innate and adaptive immune systems), through extraction of nutrients and energy from the diet, and through complex biotransformations of a variety of ingested compounds, including potential carcinogens. Long-standing questions abound concerning this ecosystem. How is the gut microbiota assembled and maintained over a lifetime? How personal is each individual's microbiota? How does its composition contribute to well-being or disease predisposition and pathogenesis? The answers to these and other questions are starting to emerge with the application of new experimental technologies to define gut microbial community composition and gene content, such as high-throughput, massively parallel DNA sequencers. Equally important, advances in the computational methods used to analyze the vast amount of data emanating from studies of the DNA, messenger RNA, protein, or metabolite content of the gut microbiota, coupled with the application of principles gleaned from the fields of ecology, evolutionary biology, and population genetics, are illuminating the properties and operations of this remarkable microbial "organ."

Describing microbes: species and phylotypes

Our knowledge of the composition of microbial communities stems from over a century of culture-based work and from almost three decades of culture-independent, nucleic-acid-based analyses. Traditionally, cultured microbes have been identified (typed) on the basis of their phenotypic and physiological properties – a rapid and efficient method for detecting specific microbes with previously described properties. However, culture-based approaches have severe limitations; the most problematic is that at least 60%–80% of the microbial cells in the human gut have not been isolated and grown in pure culture in the laboratory [1]. Because of the difficulties involved in isolating microbes in culture, surveys of diversity (who is there and in what abundance) using a culture step are particularly susceptible to bias.

Molecular phylogenetics is an alternative method for microbial identification that does not rely on a culture step; the central tenet of this approach is that an organism is identified by its evolutionary relationship to others. All living cells have a common ancestor so they contain genes whose history can be traced back to the same gene in their last common ancestor. The modern descendants (homologues) of these ancient genes may perform the same or similar functions in each descendant living cell and can, depending on the degree of conservation/evolution, share a large

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proportion of identical sequence. To measure evolutionary (phylogenetic) distances, homologous genes from different microbes are sequenced, their nucleotide or protein sequences are aligned, and the differences between pairs of sequences are tallied. Comparison of the pairs of sequences of multiple organisms reveals an evolutionary map (phylogeny) of the organisms. This allows classification of previously unknown organisms because each organism is given a coordinate on the evolutionary map; each organism is identified by its evolutionary distance from others.

The gene most widely used to build the tree of microbial life encodes an RNA molecule that is a component of the small-subunit of ribosomes (SSU rRNA: this is 16S rRNA in Bacteria and Archaea and 18S rRNA in Eukarya). This gene is sometimes referred to as a barcode for life. Roughly 1500 base pairs in length, its high level of nucleotide sequence conservation across all three domains of life, Eukarya, Bacteria, and Archaea (~ 50% identity, or % ID), allows a confident alignment, yet there is enough variation to infer evolutionary relationships. Like other components of central information-processing pathways in the cell, rRNA genes are thought to be transmitted primarily from mother cell to daughter cell and so the rRNA gene phylogeny of a microbe is its *vertical lineage*. In contrast, genes encoding proteins can also be exchanged between microbes from different lineages (particularly among Bacteria), a process termed *lateral gene transfer* (i.e., lateral with respect to the rRNA phylogeny). Phylogenies of these laterally transferred genes can be incongruent with the rRNA phylogeny [2]. As a consequence, distantly related microbes may have similar phenotypes. Lateral gene transfer partly accounts for the failure of traditional microbiology to build a coherent and universal classification scheme for microbes based on their physiological or phenotypic characteristics [3].

Although physiological and phenotypic characteristics are used to define genera and species in clinical microbiology, genus/species names often do not map well on the rRNA gene phylogenies derived from reference "type" strains and their associated clinical isolates [4]. The bacterial genus *Clostridium* is a particularly egregious example of the disparity between classifications based on phenotype and those based on 16S rRNA phylogeny [4]. The *Clostridium* genus was once described as "free-living, unicellular, nonbranching, nonphotosynthetic, rod-shaped, anaerobic, heterotrophic, and forming endospores." However, these descriptors apply to many genera named according to their 16S rRNA phylogeny, such as *Eubacterium*, *Syntrophospora*, *Paenibacillus*, *Caloramator*, *Oxalophagus*, *Oxobater*, *Moorella*, *Thermoanaerobacter*, and *Filifactor* [4].

Phylotypes (phylogenetic types) are branches of the phylogenetic tree, and *species-level phylotypes* are closely related clusters of branches. The % ID between any two SSU rRNA gene sequences in a species-level phylotype is typically greater than or equal to 97%. Note that the use of the 97% ID cutoff to delimit a species is based on attempts to match the SSU rRNA gene sequence % ID to previously described species that have been classified according to their phenotype; so rather than being a rule, it is a rule of thumb. Indeed, the species-level phylotype may not include the set of strains described for the species by phenotypic matching. Furthermore, inclusion in a species-level phylotype is not necessarily a good predictor of the gene content of the entire genome. For example, *Escherichia coli* isolates with identical 16S rRNA gene sequences can share only 30% of their genes [5], but are still considered *E. coli* – the genes they do have in common confer the essence of the species. Studies of the differing genome content of strains of the same species (≥ 99% rRNA gene identity) have led to the concept of the *pan-genome*, which is defined as the sum of all genes present in all isolates [6,7]. One of the only generalizations that we can make at present is that bacteria whose 16S rRNA gene sequences are below the threshold of 97% ID have genomes whose gene content is less than 40% similar [8].

Terms

Microbiota refers to the microbial community, and *microbiome* refers to the sum of all the genes encoded in all of the genomes of the microbiota. We use *phylotype* to describe clusters of related 16S rRNA gene sequences characterized by levels of pairwise sequence identity (i.e., phylotypes may describe deep or shallow levels of diversity that may loosely correspond to groups [operational taxonomic units], such as divisions [also known as phyla], genera, species, or strains). We deliberately omit the use of the word "prokaryote" because the prokaryote/eukaryote dichotomy is inconsistent with the three known *domains* of life on Earth (Bacteria, Archaea, Eukarya), and so represents an outdated concept [9].

The microbiota

Methods to determine community composition

To characterize the microbial diversity (i.e., presence and abundance of phylotypes) in a mucosal biopsy or stool sample using molecular phylogenetics, bulk DNA is extracted, and SSU rRNA genes are amplified by polymerase chain reaction (PCR). When the goal is to encompass the greatest possible breadth of diversity, the PCR primer pair used most often is the "universal" primer pair 515F and 1391R, which recognizes SSU RNA genes from members of all three domains of life (the numbers associated with these primers correspond to nucleotide positions [from 5′ to 3′] in the *E. coli* 16S rRNA gene sequence). When members of the domain Bacteria alone are targeted, the Bacteria-specific forward primer 8F is paired with the universal reverse primer 1391R, or with the Bacteria-specific reverse primer 1492R. After PCR amplification and subsequent cloning and sequencing of the 16S rRNA gene amplicons, the sequences are deposited in public databases so that they can be shared with other

researchers. These sequences provide the context for interpreting any new sequence, so allowing immediate comparisons with data from other samples and studies.

Data sets

To date, the two largest data sets of 16S rRNA gene sequences obtained from the human gut consist of 13 335 16S rRNA sequences from three unrelated healthy adults [10], and 18 348 sequences from 14 unrelated adults [11]. Eckburg and colleagues specifically addressed the composition of the normal gut microbiota [10]: each of the three individuals contributed biopsy samples from six regions of their colons, plus one stool sample. Ley and colleagues addressed the effect of obesity and weight loss on gut microbial ecology [11]: stool was collected from 12 obese individuals at four time-points over the course of 1 year, before and while they lost weight on either one of two types of low-calorie diet. Bacteria were surveyed in both studies. Eckburg and colleagues [10] also targeted members of Archaea (~ 1500 sequences), but neither Eckburg nor Ley and their colleagues targeted Eukarya. From these and earlier studies [1,12], broad patterns emerged.

How many species of bacteria are in the human gut?

This a challenging question. Even setting aside the fact that the term "species" has no generally accepted definition in microbiology [13], the technical difficulties faced in searching for an answer are enormous. Obtaining an answer involves attempting to "count the uncountable" in a densely packed microbial society of 10–100 trillion organisms that differs from person to person in an exponentially growing human population whose microbes may be undergoing evolution faster than they can be counted. However, because most reviews and discussions of the human gut microbiota begin with a statement about the "number of species in the human gut", it is important to know the source of these figures, if only to highlight the limits of our present capabilities, what we can know from current methods, and the likely accuracy of statistical extrapolations of "species" data.

The oft-quoted number of 400 species [14] stems from a statistical extrapolation based on 113 different species cultured from the feces of 20 Japanese–Hawaiian subjects (13–30 species per specimen [15]). The more recent estimates of 800–1000 species [16,17] are derived from statistical analyses of 16S rRNA gene sequence data sets. Figure 25.1 shows such an analysis of the combined data sets generated by Eckburg [10] and Ley [11] and their colleagues. At the 97% ID level, the total number of species-level phylotypes is 4000–5000. In this analysis, the number of phylotypes is tallied as sequences are added. Because of the high degree of interpersonal differences, each new person studied adds a substantial number of new species (see Fig. 25.1).

How many people must be sampled before few or no new species-level phylotypes are encountered? This question can only be answered once interpersonal variability is well constrained for humans of both sexes with a wide variety of characteristics, spanning the human life cycle, as well as different demographic factors (e.g., rural vs urban), socioeconomic groups, cultures, diets, and health states. Clearly, more information is needed before generalizations can be made with confidence about the fine-scale (species-level) characteristics of "the" human gut.

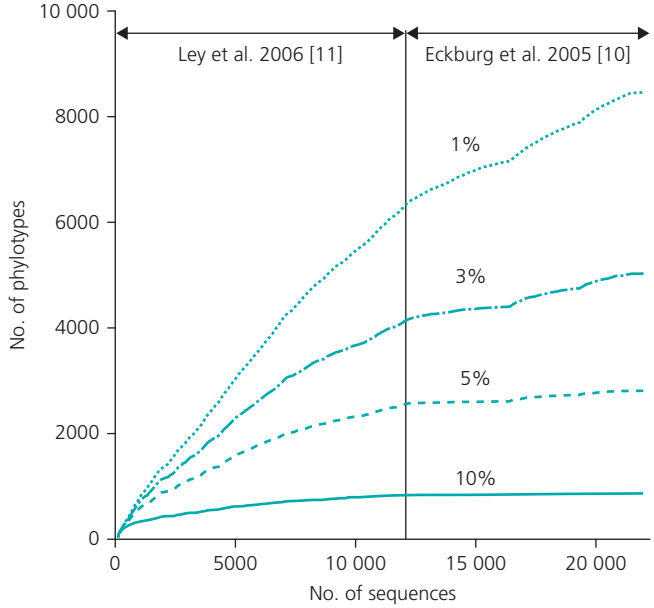

Figure 25.1 Abundance of 16S rRNA gene sequence phylotypes in the human gut. Phylotypes are tallied at different levels of sequence divergence (% identity [% ID]): 1% divergence in 16S rRNA gene sequence identity corresponds grossly to strain level, 3% to species level, 5% to genus level, and 10% to family level. Sequence data for each subject from the studies of Ley et al. [11] and Eckburg et al. [10] were added sequentially. Note that the three inflections in the 1%, 3%, and 5% lines from the data generated by Eckburg and colleagues correspond to the high number of sequences in each of the three subjects in the study. Sequences from both data sets were aligned using the NAST algorithm [67] and phylotypes were generated using the computer program DOTUR [68] (for details, see ref. 11).

Within a *single* distal gut, mucosal communities sampled at different points along the colon are more similar to one another than to communities sampled from the same regions of another individual [10]. The community composition might be expected to change consistently from the proximal to the distal end of the colon, but no consistent order for the colon biopsy samples was observed for the three subjects in the study by Eckburg and colleagues [10] (Fig. 25.2).

These types of conclusions, obtained from large SSU rRNA sequence data sets, could only be reached because of the development of new computational tools. One such tool, the UniFrac metric [18], which was used to display the relationships between all the mucosal biopsy and stool samples in Fig. 25.2, is a measure of the evolutionary distance between the sets of 16S rRNA gene sequences from different samples.

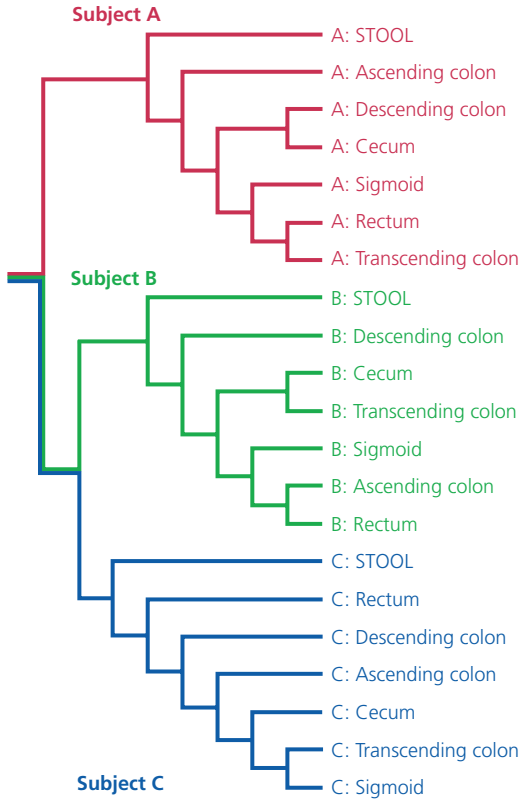

Figure 25.2 Dendrogram illustrating the similarities in bacterial diversity between mucosal and stool samples of three healthy individuals (Subjects A, B, C) from the study of Eckburg et al. [10]. The more similar the communities, the closer together they are on the phylogenetic tree: each subject's samples cluster to the exclusion of the others. There is no consistent rank order to the similarity of communities residing at the different mucosal biopsy sites. However, the stool sample for each subject is the furthest removed from the set, indicating that stool is a mix of mucosal and lumenal communities, but each stool can clearly be attributed to its host. The dendrogram shown was generated with UniFrac, which measures the fraction of total branch length of the 11,831-sequence neighbor-joining tree occupied by each community (for details, see ref. 11).

All sequences from all mucosal and stool communities were assembled into one large phylogenetic tree. For each sample, the fraction of tree branch length that is either unique to its set of sequences, or shared with other sets, is computed using the UniFrac metric: this allows quantification of community overlap on the tree. Communities with the most overlap share the most lineages, and so, are the most similar.

Using UniFrac and other tools to compare communities, the emerging view is that a highly diverse and personalized microbiota, consisting of hundreds, and perhaps thousands, of species-level phylotypes *per person*, resides in our distal gut. Stool is a mix of mucosal and lumenal communities; Eckburg and colleagues [10] determined that the colonic mucosal communities for each person were more closely related to the corresponding stool sample than to any samples from the other individuals (see Fig. 25.2). This underscores the usefulness of stool for providing a general view of an individual's gut microbial community – stool is easy to obtain and representative of the colonic mucosal community.

A detailed spatial analysis of community structure in the intestine is needed but awaits the development of reliable sampling methods that can be applied to individual human hosts. Issues to be addressed about the biogeography of the gut microbiota include the nature of variation along the length of the small intestine and colon, along the crypt-to-villus axis in the small intestine, along the crypt-to-surface epithelial cuff axis in the colon, and from the lumen through the mucus layer to the surface of epithelial cells, as well as variations surrounding or within pathogenic lesions.

Who is there? Bacteria, Archaea, viruses, and eukaryotes

Most cells that comprise the human gut microbiota are bacterial. The roles of the rarer, nonbacterial members of the community are not well known, but the consistent presence of these organisms in the human gut microbiota hints at their importance in the functioning of the system.

Of the numerically dominant Bacteria, two divisions (deep evolutionary lineages, or phyla) predominate: Bacteroidetes and Firmicutes. A few other divisions are represented at low levels. In the large-scale surveys previously described, the Firmicutes and the Bacteroidetes together accounted for most of all the 16S rRNA sequences in each human host studied. Within the Firmicutes, the majority of phylotypes belonged to the clostridial clusters XIVa (*Clostridium coccoides* group) and IV (*Clostridium leptum* group), although most of these were novel and unrelated to species present in publicly accessible culture collections. The remaining sequences belonged to seven other divisions of Bacteria, which, together with divisions described in previous studies [17], brings the total number of divisions detected either in biopsy samples or feces to 10 (Firmicutes, Bacteroidetes, Actinobacteria, Fusobacteria, Verrucomicrobia, Lentispharae, Cyanobacteria, Synergistes, Spirochaetes, Proteobacteria). Some members of the Actinobacteria (i.e., *Bifidobacterium* and *Atopobium*, which are gram-positive organisms with a high guanine and cytosine content), and some members of the Firmicutes (e.g., clostridial cluster IX) may be underrepresented in molecular surveys; for example, fluorescent in situ hybridization studies have revealed higher than expected cell counts for this cluster [19]. The ~ 100 other divisions of Bacteria known from environmental studies [20] either do not find the human gastrointestinal tract a hospitable habitat, or are so rare that their detection requires larger or more focused surveys.

Although only two divisions, Firmicutes and Bacteroidetes, dominate in the gut, the diversity of lineages within those divisions is great. In the data set from the three adults in the study by Eckburg and colleagues [10], over half (7555) of the 13 335 sequences were sampled only once, and each unique sequence can be considered as a strain. The high number of

strains sort into a dramatically lower number of species-level phylotypes: 395 when the threshold for defining membership in a given species is set at ≥ 99% 16S rRNA sequence identity [10]. The number of phylotypes decreases dramatically as their defining phylogenetic resolution becomes coarser (in other words, as the % ID is lowered). This pattern is repeated in other studies of the microbiotas of humans [11,17], and mice [21], and is much more extreme than that which is observed in environmental habitats, such as soil and ocean water, which contain a higher level of intermediate lineages [22]. The evolutionary relationships of the human gut bacteria can be visualized as a grove of 10 palm trees, with a few deep lineages in each division represented by a fan of branches at the top of a given palm. This contrasts with other environments, where more intermediate branches give rise to a pattern more akin to an oak tree. One challenge in the study of gut microbiology is to discern the biological implications of the extreme fan-like phylogenetic architecture [22].

Archaeons are present in most (50%–80%) humans surveyed [23]. Methanogenic archaeal phylotypes can comprise more than 10% of all anaerobe cells in feces [24]. Despite their abundance, the diversity of methanogens appears to be remarkably low. In the single time-point survey of three adults, *Methanobrevibacter smithii* was the only archaeal phylotype encountered [10]. *Methanosphaera stadtmanae* and other members of the Crenarchaeote and Euryarchaeote archaeal divisions are rarer [25]. Archaea are the only known producers of methane. The niche (profession) occupied by mesophilic methanogenic Archaea in the gut is to improve the efficiency of polysaccharide fermentation by preventing build-up of H_2 and other reaction end products. The roles of non-methane-producing Archaea are completely unknown.

A culture-independent study of DNA viral diversity in human feces revealed 1200 different genotypes [26]. Most (81%) were siphophages and prophages within bacterial genomes [26]. A similar survey of the RNA viral diversity in feces revealed that most of the viruses were plant pathogens – the most abundant member, pepper mottle virus, was detected in ~ 70% of 18 fecal samples from healthy donors on two continents [27]. This plant virus likely has a dietary origin. Its capacity to infect plants after passing through the human gastrointestinal tract suggests that we may act as reservoirs for disseminating this and other plant viruses [27].

The fungal components of the normal human intestinal microbiota have not been surveyed with culture-independent methods, but studies in mice and culture-based studies in humans suggest a dynamic and complex community. An 18S rRNA gene sequence diversity study in mice revealed commensals from all four fungal divisions [28]. A culture-based study of feces obtained from Apollo 14 and 15 astronauts, before and after their space flights, revealed a variety of yeast-like fungi (*Candida* species, *Cladosporium* species), at least one filamentous fungal morphotype (*Cladosporium elatum*), a high degree of interpersonal variation, and changes over time [29].

Understanding patterns of diversity: insights from ecology

The patterns of diversity in the gut can be understood using ecology [22]. Conceptual frameworks and hypotheses developed to explain patterns of diversity in plants and animals (macroecology) can be applied to microbial ecology because the data sets are large enough for rigorous statistical comparisons. In macroecology, community composition can depend strongly on the order in which species initially enter a community, a phenomenon known as multiple stable states [30]. In microecology, the influence of the inoculating community on the composition of the later community is evident in mice [21], in fish [31], and in artificial systems [32]. These observations also fit with the predictions of the neutral theory of community assembly, which states that most species will share the same general niche (profession), or the widest niche, and so are likely to be functionally redundant [33]. In the gut, neutral community assembly would result in a high level of microbial lineage variability between guts, but the broad functions encoded in the microbiomes would be the same. Indeed, the very broad functions are the same regardless of the high interpersonal variability: all gut microbiotas help digest the diet [34].

Provenance of the lineages

Cell densities increase from 10^4 cells/mL near the stomach to 10^{11} in the colon. The community in the distal intestine likely is influenced by those further upstream in the mouth, esophagus, stomach, or small intestine. Upstream community members have the potential to translocate to the distal regions of the intestine, either as entrenched residents or as tourists, and indeed, upstream communities may act as reservoirs for recolonization after disturbances (i.e., diarrheal diseases). The microbiota of the stomach shows considerable overlap in composition with the microbiota of the mouth; the mouth has high taxonomic richness, with ~ 700 species-level phylotypes [35]. A single study of the stomach revealed 128 species-level phylotypes, 40% of which had been previously identified in the mouth [36]. Despite the overlap in phylotype composition, their abundance is habitat-specific [35]. Among Firmicutes for example, the genus *Streptococcus* is abundant in the mouth [35], where attachment to surfaces is a selection criterion, but not in the colon, where *Clostridium*, *Eubacterium*, and *Faecalibacterium* species (i.e., members of clostridial cluster XIVa) dominate. If colonization of the lower intestine by upstream residents does occur, therefore, it does not result in the dominance in the colon by the translocated microorganisms. Instead, lineages typical of the distal gut and those typical of the mouth [35] and stomach [36] are likely adapted to their body habitats.

How is our gut microbiota acquired? Pathogens and commensals alike can be said to use the oral–fecal route for host-to-host transmission. In contrast to gut pathogens, which proliferate in environmental reservoirs outside their hosts (e.g., members of the Proteobacteria such as *Vibrio cholera*), gut commensals do not appear to grow in the environment [17]. As a consequence, commensals are most likely to rely on the direct contact of parents and offspring for their transmission. One testable prediction of a parent-to-offspring transmission hypothesis is that microbial communities will be more similar if hosts are related rather than unrelated. In laboratory experiments using families of C57BL/6J inbred mice in which littermates were raised in separate cages after weaning, the family tree could be recapitulated on the basis of gut microbial community structure. Mothers that were sisters had more similar microbiotas than did an "unrelated" but genetically identical C57BL/6J mother [21]. These kinship effects were seen across multiple generations; i.e., in the mothers' offspring and in the mothers' mother so the microbiota and microbiome can be viewed as a heritable genetic factor [22].

Mice are coprophagous. The extent to which human families share microbial diversity remains to be seen, but multiple lines of evidence suggest that mother-to-baby transmission of microbes is a fundamental feature of human biology. Human babies are born germ-free and acquire their gut microbiota from their early environmental exposures: the vagina, feces, skin, and breast milk of their mothers figure prominently as sources of inoculum [37–39]. Birth is a critical period for inoculation: babies delivered by cesarean section show altered patterns of colonization compared to babies delivered vaginally [40]. Direct mother-to-baby transmission has been shown for certain groups of bacteria. For example, the probiotic strain *Lactobacillus rhamnosus* GG, when taken during pregnancy, has been detected in the infant gut up to 24 months after delivery and cessation of maternal consumption of the probiotic [41]. Strains of *Lactobacillus* species and *Enterococcus faecium* isolated from the breast skin and milk of mothers have been matched, by randomly amplified polymorphic DNA analysis, to strains isolated from their babies' feces [38]. Such direct matching of a few hundred isolates picked randomly from bacterial communities suggests that the same bacterial strains are not only present in both mother and child but also colonize to dominance. It is tempting to speculate that the dominance of *Lactobacilli* in the vagina, and their presence in breast milk, are evolutionarily advantageous strategies – when propelling one's newly born, germ-free offspring into the microbial world, immediate dosing with friendly and useful microbes enhances survival.

Another factor that could account for similarities between mother and infant microbiotas is host genotype selection; i.e, related individuals share genetic traits that could select particular lineages of microbes. To tease apart the effect of a shared human genome compared with the effects of the mother and early environment on community composition, two studies have surveyed the microbiota of monozygotic and dizygotic twins and their families [42,43]. Both studies used DNA fingerprinting methods (denaturing and temperature gel gradient electrophoretic techniques, in which mixed PCR amplicons in a sample are separated on gels according to base compositional differences) that yield qualitative estimates of community similarities but no sequence data. Both studies reported the highest similarities between the microbiotas of monozygotic twins.

The twin studies revealed a remarkable pattern: whether the similarities between siblings are the result of a shared early environment or a shared genotype, they persist into adulthood despite the erosional potential of time, cohabitation with marital partners, and the different environmental exposures brought about by separate lives. In an ongoing study of the microbiotas of 12 unrelated adults over the course of 1 year, Ley and colleagues [11] showed that the lineages present were generally stable over time. Methanogen population levels are also stable over time within individuals [44]. Lineage composition appears resilient to antibiotic treatment, but induces a transient shift in their relative abundances [45,46]. Changes in diet [47] and weight loss [11] also affect the relative abundance of lineages. One manifestation of the resiliency of the intestinal microbiota is that its lineages can be maintained, although their relative abundance may change under certain conditions.

The community and its properties

Gut microbes are thought to be highly functionally redundant, such that many different combinations of microbes will still perform basic services. The genes involved in basic services, which can be thought of as host-imposed essential requirements, can be ascertained by comparing the gene complement in the gut microbiome to that in nongut microbes. With the use of a metagenomics approach, in which bulk DNA is isolated from an intact microbial community and then randomly sequenced, Gill and colleagues [34] identified some of these basic services. They compared the genes present in the microbiomes of two unrelated healthy adults to the genes found in all other sequenced bacteria deposited in public databases and in the human genome. The gut microbiomes were enriched in genes involved in energy production and conversion, carbohydrate transport and metabolism, amino acid transport and metabolism, coenzyme transport and metabolism, secondary metabolite biosynthesis, transport and catabolism, and methanogenesis [34]. As might be expected, the gut microbiome has been selected in part for its role in nutrient and energy extraction from the diet.

An example of the impact of differences in microbial community composition impacting energy metabolism comes from studies of obesity. 16S rRNA-based comparisons of the

distal gut microbiota in genetically obese (*ob/ob*) mice and their lean (+/+ or *ob/+*) littermates disclosed that increased adiposity is associated with a division-wide decrease in the proportion of Bacteroidetes and a corresponding division-wide increase in the proportion of Firmicutes [21]. Using metagenomic methods, Turnbaugh and colleagues [48] observed enrichment in the representation of genes involved in polysaccharide metabolism in the microbiomes of genetically obese (*ob/ob*) mice relative to the microbiomes of their lean wild-type (+/+) littermates. The investigators' prediction that the *ob/ob* microbiome was more efficient at energy extraction from a diet was borne out by biochemical assays; compared to lean mice, the *ob/ob* mice had higher levels of short-chain fatty acids in their ceca and less energy remaining in fecal pellets. A direct demonstration of the *ob/ob* microbiome's increased capacity for energy harvest from the diet came from an experiment in which the gut microbiota from *ob/ob* and lean littermates were transplanted to lean germ-free, wild-type mice, and their weights were monitored for 2 weeks. Although food consumption and initial body weight were the same for both groups of mice, the recipients of an *ob/ob* gut microbiota exhibited twice the increase in percentage body fat compared to recipients of a "lean" gut microbiota.

A similar division-wide shift in the ratio of Bacteroidetes and Firmicutes has been observed in the gut (fecal) microbiota of unrelated adult obese humans compared to that of lean humans [11]. Furthermore, the change in relative abundance of the Bacteroidetes was correlated with weight loss on two types of diets (fat-restricted, carbohydrate-restricted), indicating that there is a dynamic linkage between adiposity and gut microbial ecology. Together, these studies suggest that the nutrient and energy value of food should be considered in the context of the consumer's gut microbial ecology.

Metabolic food webs

The relationship between host and microbiota, particularly the dynamics of food webs, including their thermodynamic interactions, are beneficial in most humans. However, the microbial ecology of obesity shows how entire communities may contribute to host pathology. The "behavior" of the community as a whole is an emergent property of the community that is shaped by a multitude of microbe–microbe and microbe–host interactions.

Microbes in the gut are at the intersection of two forms of selective pressure: bottom-up, where intermicrobial competition would drive microbes into distinct functional roles, and top-down, where the host would select for functional redundancy to insure against "reactor failure" [22]. Indeed, gut bacterial species display both overlap and specialization in their fermentative abilities. They generally are capable of a wide range of fermentative activities [19], but divide up the available substrates in a number of ways: by differing in substrate use (see review by Louis and colleagues [49]), by varying the order in which common substrates are used [50], and differing the selection of substrates for attachment [51].

The genome sequences and in vivo behavior of Bacteroidetes species illustrate the plasticity necessary to thrive in a changeable gut environment. Full genome sequences of *Bacteroides thetaiotaomicron*, *B. vulgatus*, and *B. distasonis* show that these species can deploy a large arsenal of genes involved in the acquisition and metabolism of polysaccharides, and that the suite of genes involved in these processes varies between species and can be laterally transferred [52]. *Bacteroides thetaiotaomicron* has the greatest arsenal. Its genome includes 208 paralogues of two outer membrane proteins, SusC and SusD; which are involved in the binding and importing of starch and maltooligosaccharides; 246 predicted glycoside hydrolases and polysaccharide lyases; and a large array of environmental sensors (50 extracytoplasmic function-type σ factors, 25 anti-σ factors, and 32 novel hybrid two-component systems) [52]. The carbohydrate foraging behavior of *B. thetaiotaomicron*, when introduced into the ceca of germ-free mice maintained on standard chow diets with high levels of polysaccharides, includes attachment to small nutrient platforms, consisting of partially digested food particles, and to host mucus, and sequential deployment of its arsenal in response to substrate depletion [50]. For a bacterium like *B. thetaiotaomicron*, which lacks adhesive organelles, attachment to substrates (seating at the "dining table") is determined in part by the suite of glycan-specific SusC/SusD outer membrane-binding proteins it produces, the composition of which is dictated by the menu of available glycans. Attachment presumably increases the efficiency of oligo- and monosaccharide harvest by adaptively expressed bacterial glycoside hydrolases, and their subsequent distribution to other members of the microbiota. This activity, together with the ability of *B. thetaiotaomicron* to endure during episodes of low nutrient availability when it turns to host polysaccharides as a nutrient source [50], contribute to the maintenance of microbial diversity within the system.

The combined activities of diverse members of the microbiota result in interactions that can be very complex, and involve multiple pathways for the processing of given substrates, including the reuse of fermentation products. Thus, there are multiple options for the fate of any given metabolite, and for the types of bacteria that will be involved. Bifidobacteria can ferment carbohydrates to produce lactate, which is then converted to either butyrate or propionate depending on the mix of species and the types of carbohydrate [53,54]. *Roseburia* and *Faecalibacterium* species (clostridial cluster XIVa) are key butyrate producers and are consumers of acetate [19]. They also produce H_2 that can be

consumed by acetogenic bacteria, so that in coculture with an acetogen, H_2 is consumed to produce acetate, which is then used as a substrate by the butyrate producer [19,55]. Methanogenic Archaea and sulfate-reducing bacteria compete with acetogens for H_2 – a competition that may be influenced by the pH of the gut, such that acetogens thrive in acidic conditions [56].

Changes in the chemical environment of the gut can not only alter the competitive balance between functionally redundant microbes, but can also impact the host. The activity of methanogens removes carbon from the system in the form of gas (CH_4) but also depletes the level of H_2, thereby enhancing the thermodynamic yield of the whole system (buildup of H_2 from microbial fermentation of polysaccharides slows down the process). Moreover, because short-chain fatty acids generated from polysaccharide fermentation are absorbed by the host, changes in the proportions of acetate, propionate, and butyrate produced in the gut can affect the overall energy balance of the host [57–59].

Linking phylogeny to function

Most phylotypes encountered in culture-independent surveys of the human gut microbiota lack cultured representatives so their role in the community is not known. New approaches for cultivation are yielding such organisms [19,60,61]. These methods include *gel microdroplet technology*, which allows high-throughput culture of microorganisms in a simulated natural environment, where cells are separated by emulsion but metabolites and signaling molecules can flow freely [62].

Researchers are also beginning to explore culture-independent approaches for linking phylotype identity and function [63]. These include tracking labeled substrates into cells in situ, a technique known as stable isotope probing [64]. Although stable isotope probing has yet to be used in vivo, it has been applied to a model intestine reactor inoculated with a human gut microbiota. The ^{13}C-labeled glucose was added and "heavy" (^{13}C-enriched) RNA identified the bacteria that fermented the glucose (the labeled RNA was recovered by ultracentrifugation through cesium chloride gradients; reverse transcription–PCR, cloning, and sequencing of the transcripts was used to ascertain which organism was using the labeled substrate. Further, the metabolic products have been identified by nuclear magnetic resonance spectroscopy [65]. Fluorescence in situ hybridization, flow cytometry, whole-genome amplification, and shotgun-sequencing have been used to obtain a partial genome sequence for a member of the TM7 phylum found in the mouth, providing a first look at a group of organisms with no available cultured representatives [66].

Prospectus

A better understanding of the intestinal microbial ecosystem is required to define health and characterize disease susceptibility, and to design, as well as predict, the outcome of therapeutic interventions, whether these interventions use prebiotics, probiotics, antibiotics, or man-made drugs. This will require greater understanding in several areas:

- interpersonal variability in gut microbiomes, in terms of both the organisms and gene lineages present and the metabolic functions
- variables controlling community membership, including age and aging, plus genetic, demographic, environmental, and lifestyle factors
- genetic and metabolic networks that are modulated by the microbiota.

To this end, the International Human Microbiome Project aims to sequence the genomes of multiple cultured representatives of the human (gut) microbiota, and to compare SSU rRNA and metagenomic data sets of the microbiomes of communities harvested from a range of locations along the cephalocaudal axis of the gastrointestinal tract of healthy subjects, including twin pairs and their mothers, as well as those with various diseases, such as inflammatory bowel disease. One key challenge will be to define "distances"; i.e., how similar or far apart are the microbiomes (and transcriptomes, proteomes, and metabolomes) of gut microbial communities from a single human, his or her relatives, and unrelated individuals, and how do differences manifest themselves on different timescales? With distances defined (e.g., using methods analogous to UniFrac), statistical tools will have to be developed to integrate the data sets into a unified framework that will facilitate the identification of the components of the microbiome that impact digestive health and disease. With a concerted, integrated interdisciplinary research effort directed to this complex and dynamic microbial organ, characterizing and deliberately manipulating its elements should one day be a part of the gastroenterologist's diagnostic armamentarium and therapeutic arsenal.

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E MISCELLANEOUS

26 Drug metabolism and transport in the liver and intestine

Paul B. Watkins

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General principles

In order for drugs to be absorbed when taken orally, they must generally be fat soluble (lipophilicity). Lipophilicity can also be an undesirable property for a drug. Once inside the body, lipophilic drugs tend to sequester into fat and bind in blood to plasma proteins and are therefore not readily eliminated in urine. If eliminated in bile, highly lipophilic drugs tend to be reabsorbed from the digestive tract and thus undergo enterohepatic cycling. For these reasons, most drugs that gain entry into the body would remain there for a long time if it were not for the body's ability to convert (metabolize) drugs into more water-soluble and hence more readily excreted metabolites. An additional critical process in elimination is active transport of drugs and their metabolites, which involves a growing list of membrane proteins. The liver is the major organ involved in drug metabolism and transport. For some xenobiotics, the small intestine also plays a major role.

Discovery of drug-metabolizing enzymes

A major breakthrough promoting study of drug metabolism was the development of techniques to isolate the endoplasmic reticulum from whole pieces of tissue. During this process, endoplasmic reticulum breaks up into small spheres called microsomes. It was discovered that under certain experimental conditions, liver microsomes could produce most of the metabolites generated from drugs (and other xenobiotics) by the liver in vivo. The reactions characterized generally involved insertion of an oxygen atom into the drug (usually a hydroxylation) or covalent binding (conjugation) of the drug to polar ligands such as glucuronic acid, sulfate, or glutathione. Many drugs had to first undergo hydroxylation in order to produce a reactive site on the molecule suitable for conjugation reactions. This typical sequence of events led to the division of xenobiotic metabolism into two categories: phase 1 (often hydroxylation reactions) and phase 2 (conjugation).

It is now appreciated that most (but not all) of what was described as phase I microsomal metabolism is the result of a large family of enzymes termed *cytochromes P450*, now referred to simply as P450s or CYPs (pronounced "sips").

Liver P450s

The vast majority of drugs in clinical use today are metabolized by liver P450s. Although 57 P450 genes have been identified [1], relatively few of these are important for drug metabolism [2]. The P450s involved in drug metabolism fall predominantly into three gene families, termed CYP1, CYP2, and CYP3 [3]. Within each P450 family, there are subfamilies designated by capital letters. Each subfamily generally contains multiple members, designated by Arabic numbers usually reflecting the order in which they were discovered.

A list of the major P450s involved in human drug metabolism is shown in Table 26.1. The most abundant single P450 in human liver is CYP3A4, and it has been estimated that this specific P450 may be involved in the metabolism of over 50% of drugs used clinically [4]. Only a few of the many substrates for CYP3A4 are listed in Table 26.1.

Some of the principles of P450 metabolism are shown in Fig. 26.1. Drugs either passively diffuse, or are actively transported, into the liver during passage through sinusoidal

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Table 26.1 Characteristics of major human liver P450s

P450	Substrates	Inhibitors	Inducers
CYP1A2	Tacrine Theophylline Tolcapone[a] Dihydralazine[a]	Fluvoxamine Cimetidine Ciprofloxacin	Cigarette smoke Charcoal-broiled foods Omeprazole
CYP2A6	Acetaminophen[a] [74] Halothane[a] Nicotine	8-Methoxy-psoralen	None known
CYP2B6	Efavirenz Bupropion Carbamazepine[a]	None known	Rifampin, phenytoin, carbamazepine, phenobarbital, St John's wort
CYP2C8	Paclitaxel Rosiglitazone	None known	Rifampin, phenytoin, carbamazepine, phenobarbital, St John's wort
CYP2C9	Diclofenac[a] Warfarin Tienilic acid[a] Phenytoin	Fluvoxamine	Rifampin, phenytoin, carbamazepine, phenobarbital, St John's wort
CYP2C19	Omeprazole Mephenytoin Diazepam Phenytoin[a]	Sulfinpyrazone Ticlopidine Fluvoxamine	None identified
CYP2D6	Debrisoquine Dextromethorphan Metoprolol and other β-blockers Perhexiline Amitriptyline and other neuroleptics Encainide Codeine	Fluoxetine and other SSRIs Quinidine	None identified
CYP2E1	Acetaminophen[a] Ethanol Tolcapone[a] Isoniazid[a] Halothane[a]	Disulfiram Ethanol	Ethanol Isoniazid
CYP3A4	Erythromycin Cyclosporine Docetaxel Carbamazepine[a] Midazolam/triazolam Lovastatin and other statins Saquinavir and other protease inhibitors Trazodone[a] Nefazodone[a] Troglitazone[a]	Ketoconazole (other azoles) Troleandomycin Rotinavir	Rifampin, phenytoin, carbamazepine, phenobarbital, St John's wort
CYP3A5	Similar to CYP3A4	Similar to CYP3A4	None identified

a Drugs metabolized to reactive metabolites implicated in hepatotoxicity.
SSRI, selective serotonin reuptake inhibitor.

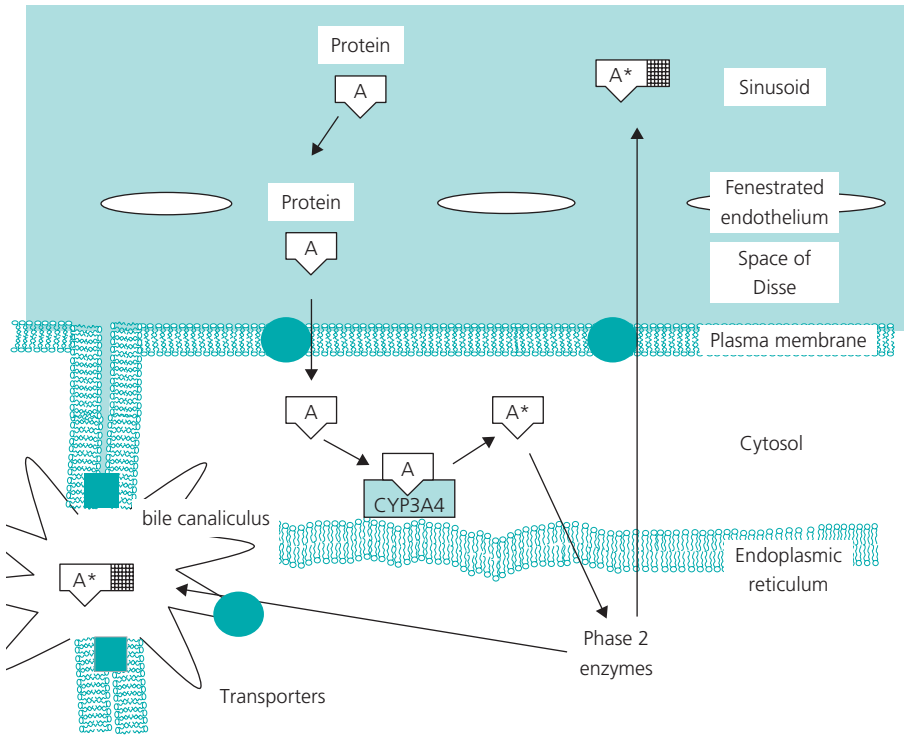

Figure 26.1 Elimination of drugs by the hepatocyte. Lipophilic drugs, such as drug A, exist in sinusoidal blood largely bound to proteins. Drugs can diffuse through the endothelial cell fenestrations into the space of Disse while still protein bound. Drugs then enter the hepatocyte by active transport, or passive diffusion. Once inside the cell, a drug will typically encounter a specific P450 (CYP3A4 in this example) capable of binding to the drug, and converting it to more water-soluble and chemically reactive metabolites (A*). This is referred to as Phase 1 metabolism. Typically, the resultant metabolite undergoes conjugation to a polar ligand in a reaction catalyzed by Phase 2 enzymes. The resultant conjugated metabolite is then secreted by efflux transporters into the biliary canaliculus for elimination in bile, or it will be secreted back into sinusoidal blood (also by efflux transporters) for ultimate excretion in urine. Efflux of metabolites from the hepatocyte is commonly referred to as Phase 3; the dark circles indicate efflux transporters.

blood (illustrated by drug A in Fig. 26.1). Once inside the liver, drugs diffuse to the particular P450s capable of metabolizing them. With some drugs, a single P450 is involved in the majority of the metabolism. The P450 inserts an oxygen atom into the drug, usually in the form of a hydroxyl group. In some cases, however, the immediate product of P450 catalysis is unstable, and rearrangement of the molecule (such as *N*-dealkylation) can rapidly occur.

Intestine P450s

P450 enzymes are detectable throughout the human digestive tract, including the esophagus, stomach, small bowel, and colon [5]. The concentration of total P450 enzymes is highest in the jejunum, and gradually declines to lower levels in the ileum and colon. The major P450 in small bowel enterocytes is CYP3A4, which accounts for at least 70% of the total P450 [6,7]. Other P450s detected in the digestive tract include CYP3A5, CYP2C9, CYP2C19, and CYP1A1 [8,9]. Notably, CYP2C8, CYP2B6, CYP1A2, and CYP2E1 have not generally been detected in the intestine. In most cases studied, P450s are not found in the intestinal crypt cells but appear, probably as a result of transcriptional activation of the corresponding genes, as the epithelial cells migrate out of the crypt toward the villus tip. This location is appropriate for metabolism of xenobiotics in the gut, because absorption is thought to occur chiefly in the upper part of the villus.

The only intestinal P450 shown to catalyze significant drug metabolism is CYP3A4. This was originally demonstrated by intrajejunal administration of the CYP3A4 substrates cyclosporine (ciclosporin) [10] and midazolam [11] during the anhepatic phase of the liver transplant operation. In each case, up to 50% of detectable drug absorbed into portal blood had been converted into metabolites characteristically produced by CYP3A4. Other studies quantitated intestinal metabolism by administering unlabeled and stable-labeled CYP3A4 substrates by intravenous and oral routes, respectively. Such studies have shown that the intestine is a major site for metabolism of verapamil [12] and midazolam [13].

The importance of intestinal CYP3A4 has also been highlighted by drug interactions produced by grapefruit juice [14,15]. A growing number of drugs have enhanced oral availability when consumed with grapefruit juice (compared with water or orange juice) (Table 26.2). This interaction has been shown to result from both competitive and mechanism-based inhibition of CYP3A4. In addition, the enterocyte content of CYP3A4 actually falls within hours of drinking a single glass of grapefruit juice [16,17], presumably because the inactivated enzyme undergoes rapid intracellular degradation [17]. The ingredients in grapefruit juice responsible for CYP3A4 inhibition appear to be furanocoumarins (also called psoralens) [17–19]. Furanocoumarins are not present in most other types of fruit juice. Grapefruit juice does not appear to inhibit liver CYP3A4, at least when consumed in usual quantities [15,16]. This presumably reflects insufficient

Table 26.2 Some drugs affected by grapefruit juice

Drug	Mean fold increase in AUC[a]	Reference
Simvastatin	13.5	Lilja et al., 2000 [141]
Lovastatin	15.0	Kantola et al., 1998 [142]
Atorvastatin acid	1.8	Ando et al., 2005 [143]
Midazolam	1.5	Kupferschmidt et al., 1995 [144]
Triazolam	1.5	Hukkinen et al., 1995 [145]
Saquinavir	2.0	Kupferschmidt et al., 1998 [146]
Halofantrine	2.8	Charbit et al., 2002 [147]
Nifedipine	3.0	Bailey et al., 1991 [148]
Carbamazepine	1.5	Garg et al., 1998 [149]
Buspirone	9.2	Lilja et al., 1998 [150]
Cyclosporine	1.6	Yee et al., 1995 [151]

a Area under the blood concentration vs time curve divided by that observed when drug was taken with water or orange juice.

concentrations of furanocoumarins reaching the liver after absorption. Grapefruit juice can therefore be used experimentally to knock out intestinal CYP3A4 (although it may affect some drug transporters as well) [20,21]. If grapefruit juice does not affect the oral availability of a drug, then intestinal CYP3A4 cannot be contributing much to first-pass metabolism.

The aggregate CYP3A4 in the intestine is probably less than 10% of that in the liver [22]. For significant metabolism to occur in the intestine, it seems likely that a drug must be very rapidly metabolized by CYP3A4, be administered in relatively small amounts, or have a prolonged absorption phase (i.e., prolonged contact with the enzyme). It has been proposed that the physiological role of intestinal CYP3A4 may primarily be to limit entry into the body of potentially harmful dietary compounds. One study [23] suggested that CYP3A4 may convert aflatoxin B1 to reactive metabolites that bind to macromolecules within enterocytes. This, in effect, traps the metabolites within the cells. As the enterocytes are normally sloughed into the digestive tract, the aflatoxin metabolites are eliminated in stool.

Interindividual differences in P450 activity

Although elimination of drugs generally requires multiple processes (Fig. 26.1), the rate-limiting step is often conversion to metabolites by P450s. Hence, interpatient differences in the activity of a certain P450 can account for variation in the pharmacokinetics of drugs metabolized by that P450 [24,25]. In some cases, variation in P450 activity is due to genetic polymorphisms in the P450 genes. For example, approximately 5% of whites completely lack CYP2D6 activity and therefore are CYP2D6 *poor metabolizers* [26]. Patients who are CYP2D6 poor metabolizers have increased sensitivity to the effects of several drugs, including some medications commonly used to treat cardiac arrhythmias, psychosis, and depression. The incidence of CYP2D6 poor metabolizers varies substantially across different ethnic populations [27]. For example, poor metabolizers are present in less than 1% of Japanese. It should also be noted that 5% of whites have gene duplication of CYP2D6 resulting in an *ultrarapid metabolizer* phenotype [26]. These individuals exhibit unusually rapid clearance of at least some CYP2D6 substrates, and this can account for therapeutic failure of some medications.

CYP2C19 activity is absent in approximately 5% of whites, but absent in up to 20% of Asians [27]. CYP2C19 poor metabolizers have been shown to have higher blood levels of omeprazole than others when treated with usual therapeutic doses [28]. This is expected since omeprazole relies on CYP2C19 for its elimination (Table 26.1). This appears to explain why CYP2C19 poor metabolizers have a higher cure rate for *Helicobacter* infection when treated with omeprazole-containing regimens [28].

Allelic variation can result in diminished catalytic function of other P450s, including CYP2C9, resulting in exaggerated effects of warfarin and phenytoin [29,30]; CYP2A6, which may influence cigarette smoking behavior [31]; and CYP2B6, which appears to influence response to efavirenz. [32]. Polymorphisms that appear to affect CYP3A4 activity have been identified [33] and, although not yet linked to drug pharmacokinetics, may be associated with risk of certain cancers [34]. In addition, approximately 20% of whites and 50% of African Americans express CYP3A5 in liver and intestine, and this enzyme appears to metabolize most of the same substrates as CYP3A4. Patients who express CYP3A5 generally have increased dosing requirements for tacrolimus and several other drugs [21,35].

Nongenetic factors such as concomitant disease, diet variation and malnutrition can also underlie large interindividual differences in the activities of liver P450s [36,37]. Diet also likely largely explains why, for a given individual, the relative activity of CYP3A4 in liver does not correlate well with the relative activity of CYP3A4 in the intestine [38,39]. In rats, ethanol treatment and removal of iron or selenium from the diet results in marked reduction in intestinal P450 activity while having little effect on P450 activity in the liver [40–42], a phenomenon similar to the effect of grapefruit juice [15].

Drugs represent an additional cause for nongenetic variation in activity of P450s. This is the basis for many drug interactions.

P450s and drug interactions

Many drug interactions are explained by the ability of one drug to inhibit a P450 involved in the metabolism of another

drug. Inhibition of P450 activity can occur by a variety of mechanisms [43] but the most common form of inhibition reflects simple competition between two drugs for metabolism by the same P450 (i.e., one drug interferes with the ability of the other drug to bind to the substrate-binding site of the P450, reducing its rate of metabolism by the enzyme). Whether two drugs vying for metabolism by the same P450 will result in a clinically important interaction depends on a number of factors. These include the intracellular concentrations of each drug, the relative affinities of each drug for binding to the enzyme active site, and the importance of the P450 to the overall elimination of the drug, Additional important variables are the relative safety (therapeutic index) of the drugs involved and whether the metabolites generated by the P450 are pharmacologically active.

Some clinically important interactions involving inhibition can be inferred from Table 26.1. For example, when patients treated with tricyclic antidepressants are also started on certain selective serotonin reuptake inhibitors (SSRIs), blood levels of the tricyclics will tend to rise due to CYP2D6 inhibition [44]. Of course, patients who are CYP2D6 poor metabolizers will not experience this drug interaction; the inhibition of CYP2D6 by another drug in effect turns a patient into a CYP2D6 poor metabolizer, a process termed *phenocopying*. In addition, cyclosporine blood levels can rise to toxic levels in organ transplant recipients who receive concomitant treatment with erythromycin or ketoconazole and this appears to largely result from inhibition of CYP3A4 [45]. Many interactions between drugs used in HIV patients involve P450 inhibition [46]. Ritonavir is a potent inhibitor of CYP3A4 and is used to boost blood levels of certain protease inhibitors [47]. Some drug interactions involving inhibition of CYP3A4 can largely occur in the intestine, presumably due to higher concentration of the inhibitor at that location after oral delivery [13].

Some medications are such potent and selective inhibitors of specific P450s that they can be used in experimental settings to pharmacologically knock out the enzyme in a human subject. The drugs most frequently used to intentionally inhibit specific P450s are noted in Table 26.1. If elimination of a drug is not affected by the inhibitor, then it can be assumed that the inhibited P450 does not represent a major pathway for elimination of that drug. Some of the most useful P450 inhibitors are not competitive inhibitors but mechanism-based inhibitors [43,48]. Mechanism-based inhibition is irreversible, and occurs when the inhibitor is converted by the target P450 to a metabolite that damages the enzyme, often by covalently binding to the enzyme protein. This appears to be the mechanism by which a single therapeutic dose of disulfiram (Antabuse) results in greater than 90% inhibition of CYP2E1 activity which lasts for hours [49]. Another example of a potent mechanism-based inhibitor is the antibiotic troleandomycin which, when administered in the usual therapeutic dose, produces greater than 90% reduction in liver CYP3A4 activity that also persists for hours [50]. Examples of other mechanism-based inhibitors include the CYP1A2 inhibitor furafylline [51] and the CYP2A6 inhibitor methoxsalen [52].

In addition to inhibition, a medication can sometimes result in an increase in the activity of a particular P450. In most cases, this induction results from increased hepatocyte concentrations of a specific P450. Some medications that can induce P450s are listed in Table 26.1. Induction of P450 activity can occur by several mechanisms, but most commonly reflects an increase in the rate of transcription of the corresponding gene [53]. In some instances, the cellular receptor involved in transcriptional activation of P450s has been identified [54]. For example, induction of CYP3A4 by rifampin, some antiseizure medications, and St John's wort appears to involve a nuclear receptor termed the human pregnane X receptor (PXR) [55], also termed the steroid xenobiotic receptor (SXR) [56] or the pregnane-activated receptor (PAR) [57]. The inducing drug binds PXR and then forms a heterodimer with another receptor (RXR). The complex then binds to regulatory elements in the *CYP3A4* gene. This results in increased transcription of the *CYP3A4* gene, which in turn results in increases in the hepatocyte concentration of CYP3A4 [54]. It appears that PXR also mediates induction of CYP2B6, CYP2C9, and CYP2C19 by rifampin and certain antiseizure drugs in both liver [58–60] and intestine [61,62]. Some drug interactions due to induction of CYP3A4 can chiefly result from increased intestinal metabolism [63].

Induction of CYP1A2 involves a different receptor termed the aryl hydrocarbon (Ah) receptor [64]. Aryl hydrocarbons in cigarette smoke and the drug omeprazole (in high doses) bind to the Ah receptor, mediating transcription of the *CYP1A2* gene and increased production of the enzyme. This accounts for why cigarette smokers tend to require higher doses of theophylline, tacrine, and clozapine [65]. Some P450s, such as CYP2D6, CYP2A6 and CYP3A5, do not appear to be inducible.

Other examples of clinically significant drug interactions resulting from induction can also be deduced from Table 26.1. When transplant recipients are treated with rifampin or certain antiseizure medications, blood levels of cyclosporine can fall to subtherapeutic levels due largely to induction of CYP3A4 [45]. These patients risk organ rejection unless their daily dose of cyclosporine is increased. Likewise, individuals treated with rifampin or antiseizure drugs risk therapeutic failure of oral contraceptives due to induction of CYP3A4 and probably other enzymes and may require increased doses of warfarin (due to CYP2C9 induction) [66–68]. As with inhibition, induction can also be used to assess the contribution of an inducible P450 to the disposition of a drug. For example, if treatment of subjects with rifampin does not increase clearance of a drug, it is unlikely that CYP3A4 is a major pathway for metabolism.

CYP1A1 is not detectable in liver and is generally at low levels in the intestine. However, consumption of chargrilled meats causes marked induction of the enzyme in the intestine [69]. Some evidence suggests that induction of CYP1A1 in small intestine may be protective by reducing absorption of potentially harmful substances present in chargrilled meats, including polyaromatic hydrocarbons [69].

P450s and drug-induced liver injury

It is generally believed that liver injury results from reactive metabolites generated in the liver from otherwise harmless drugs [70]. The identical P450s involved in the safe metabolism of drugs are those that have been most implicated in the production of hepatotoxic metabolites [71] (see Table 26.1 for examples). In general, P450s are expressed in highest concentration in zone 3 hepatocytes, and this partly accounts for the predominance of pericentral (zone 3) necrosis in some forms of drug-induced liver injury (such as that due to acetaminophen) [72]. Interspecies differences in P450 catalytic activities and regulation probably contribute to the imperfect ability of preclinical animal studies to identify human hepatotoxins [71].

Several methods have been used to determine which P450 is responsible for production of toxic metabolites. For example, if an antibody or chemical that specifically inhibits a certain P450 causes significant reduction in the production of the reactive metabolite in liver microsomes, it is assumed that this P450 is involved [73]. In some instances, it is possible to use selective P450 inhibitors to identify the responsible P450s in clinical studies. This strategy is suggested by the listing of specific inhibitors in Table 26.1. For example, acetaminophen is believed to cause toxicity in the liver due to production of the *N*-acetyl-*p*-benzoquinone imine (NAPQI) metabolite. The total production of NAPQI from an oral dose of acetaminophen can be estimated from the production of thiol metabolites eliminated in urine. When healthy volunteers were given a dose of acetaminophen together with a CYP2E1 selective inhibitor (disulfiram), urinary production of NAPQI derivatives fell an average of 69% [74], indicating a major role for CYP2E1 in the production of this metabolite. In contrast, treatment with rifampin, which would induce multiple other P450s (Table 26.1), did not increase urinary elimination of the thiol metabolites [74].

Similar clinical research has been done with halothane. Severe halothane liver toxicity is thought to result from metabolism to a trifluoroacetyl (TFA) intermediate which becomes trifluoroacetic acid that can be readily measured in plasma and urine [75]. Plasma and urine levels of trifluoroacetic acid fell 70% when patients receiving halothane anesthesia were pretreated with the CYP2E1 inhibitor disulfiram [76]. A small reduction in TFA production was also observed when these patients were pretreated with the CYP2A6 inhibitor 8-methoxsalen, but no change was observed after treatment with troleandomycin, a potent inhibitor of CYP3A4 [77]. These studies indicate that the major enzyme involved in the production of the TFA intermediate from halothane in vivo is CYP2E1, with a minor contribution from CYP2A6 and no contribution from CYP3A4.

Anti-P450 antibodies are sometimes also present in patients recovering from liver injury caused by some drugs [78]. In at least some instances, it appears that the antibodies are generated to P450s that are covalently bound to reactive metabolites produced from these drugs. The presence of these antibodies may therefore indicate which P450 is involved in producing reactive metabolites, and may also be helpful in causality assessment. Unfortunately, anti-P450 antibodies have been associated with only a few types of drug-induced liver injury and work in this area remains experimental.

Phase 2 enzymes

In general, phase 2 enzymes have not been as well characterized as the P450s. The best-studied phase 2 enzymes are the uridine 5′-diphosphate (UDP)-glucuronosyltransferases, the glutathione *S*-transferases, and the sulfotransferases, which catalyze conjugation to glucuronic acid, glutathione, and sulfate, respectively. *N*-Acetyltransferases and thiopurine methyltransferase are also discussed.

UDP-glucuronosyltransferases

Conjugation to glucuronic acid results in enhanced water solubility; for this reason, glucuronide conjugates are usually readily excreted into urine. Glucuronide conjugates are also excreted into the bile and, because of their water solubility, generally pass in the stool unless deconjugated by colonic bacteria. In addition, glucuronidation generally results in reduced pharmacological activity. However, glucuronides can occasionally retain pharmacological activity. An example of this is morphine glucuronide, which has roughly 50-fold greater opioid activity than morphine itself [79].

As with the P450s, the UDP-glucuronosyltransferases (UGTs) arise from a multigene family of microsomal enzymes, some of which are inducible [80]. UGT genes comprise two families termed UGT1 and UGT2. The UGT1 family contains eight enzymes (designated by Arabic numerals) and the UGT2 family contains seven enzymes. There is considerable catalytic specificity of UGTs toward drugs, although such characterization is progressing relatively slowly. One problem has been that unlike P450s, which exist on the outside of microsomes, the UGTs are present on the inside of the microsomes. It is therefore often necessary to permeablilize microsomes to assess UGT activity, and this may introduce problems in data interpretation [81].

The complement of UGTs expressed in liver and intestine is not identicial [82]; there are liver UGTs that do not appear

to be expressed in the digestive tract (such as UGT2B4) and other UGTs expressed in intestine but not liver (such as UGT1A8). It has been proposed that UGTs present in the colon may provide a protective function by glucuronidating compounds that are deconjugated by colonic bacteria [83].

Multiple polymorphisms of the UGT1 family have been identified [80]. The best-studied polymorphisms involve UGT1A1, which is the enzyme primarily responsible for bilirubin conjugation. Variation in the *UGT1A1* gene have been shown to account for Gilbert and Crigler–Najjar (types 1 and 2) hyperbilirubinemia and have been associated with toxicity from irinotecan [84]. Drug interactions due to alterations in UGT activity have been described. For example, anticonvulsants have been shown to induce some UGTs and may account for some drug interactions, particularly in patients treated for HIV [85]. Induction of UGTs can involve several receptors, including PXR, CAR, and the Ah receptor [54]. Inhibition of UGTs has been proposed to account in part for the pharmacokinetic interaction between gemfibrozil and cerivastatin [86] and for a few other drug–drug interactions [87]. Nonetheless, it appears that drug interactions due to induction or inhibition of UGTs are generally of low magnitude and rarely clinically significant [88].

Some glucuronides (particularly acyl glucuronides) have been shown to be reactive molecules, and may be involved in drug-induced liver injury [89]. For example, a glucuronide metabolite of diclofenac has been shown to covalently bind multiple proteins in the hepatocyte, and this covalent binding may contribute to hepatotoxicity rarely associated with this drug [90].

Glutathione *S*-transferases

Glutathione *S*-transferases (GSTs) involved in liver or intestinal drug metabolism are cytosolic enzymes belonging to eight separate gene families designated α, μ, θ, π, ζ, σ, κ, and ω [91]. Each family has several members designated by arabic numbers. For example, GSTM1 is the first of five members of the μ gene family. GSTs are expressed in many organs in addition to liver and intestine. The complement of GSTs present in liver and intestine is not identical [92]. GSTs typically function to detoxify electrophilic metabolites of drugs, such as the reactive metabolite produced from acetaminophen (NAPQI). In general, the reactions catalyzed by GSTs are not rate limiting in drug elimination. An exception may be busulfan, which requires glutathione conjugation catalyzed by GSTA1 for elimination. Studies have suggested that busulfan oral availability is limited by GST1A1 activity in the intestine [93].

Polymorphisms in GSTs are common [94]. GSTM1 and GSTT1 are absent in 40% and 15% of whites, respectively. Polymorphisms in these and other GSTs have been linked to susceptibility to a variety of diseases, including asthma, cancer, diabetes, heart disease, and drug-induced liver injury. An example of the latter is hepatotoxicity due to tacrine; patients who lack both GSTM1 and GSTT1 appear to have increased incidence of treatment-associated ALT elevations [95].

Sulfotransferases

Human liver and intestine are capable of sulfating a variety of xenobiotics using sulfotransferases (SULTs). This usually enhances solubility and decreased biological activity. In humans, there are three SULT families, SULT 1, SULT 2, and SULT 4, which contain at least 13 members [96]. The catalytic activity of the sulfotransferases varies considerably among individuals, and polymorphisms in specific sulfotransferases have been identified. Some sulfotransferases are inducible, at least in liver, and this appears to involve the same nuclear receptors involved in P450 and UGT induction [97]. For example, one study suggested that the loss of efficacy of birth control pills during treatment with antiseizure medications or rifampin may be due to induction of sulfation, and not just induction of CYP3A4 (Table 26.1) [98].

N-Acetyltransferases

There are two *N*-acetyltransferase enzymes termed NAT1 and NAT2. NAT2 is polymorphic; up to 50% of whites lack NAT2 activity and are therefore slow acetylators [99]. Slow acetylators have reduced clearance of isoniazid and sulfamethoxazole and may therefore be at higher risk of dose-dependent toxicities from these drugs. The data suggest that slow metabolizers are probably more susceptible to idiosyncratic hepatotoxicity from isoniazid, especially when risk is adjusted for a CYP2E1 polymorphism [100]. NAT2 polyumorphism has also been linked to susceptibility to certain cancers. Slow acetylators appear to be at higher risk for bladder cancer but at lower risk for colon cancer [101].

Thiopurine methyltransferase

Thiopurine methyltransferase (TPMT) catalyzes the *S*-methylation of the thiopurine drugs mercaptopurine, azathioprine, and thioguanine. The methylated products are pharmacologically inactive. There exists a genetic polymorphism in TPMT such that 0.3% of whites completely lack the enzyme (10% have reduced activity) [102]. Children with certain childhood leukemias and who are deficient in TPMT activity exhibit life-threatening myelosuppression when receiving standard doses of thiopurine drugs [103]; screening for TPMT deficiency is therefore recommended. The role for screening patients treated with lower doses of thiopurines, such as patients with autoimmune hepatitis and inflammatory bowel disease, is controversial [104,105].

Phase 3: transporters

The ability of many and perhaps most drugs to cross cell membranes relies on specific transport proteins that can be

Table 26.3 Characteristics of some major human drug transporters

Protein	Substrates	Inhibitors	Inducers
Uptake			
OATP1B1 (SLC1B1)	Cerivastatin, pravastatin, rifampin, hormone conjugates	Gemfibrozil	Unknown
OATP1A2	Fexofenadine	Citrus juices	Unknown
OAT4 (SLC22A1)	Tetracycline, ketoprofen, zidovudine	Unknown	Unknown
Efflux			
MDR1 (ABCB1)	Digoxin, cyclosporine, fexofenadine, paclitaxel, erythromycin, HIV protease inhibitors, loperamide, simvastatin acid	Quinidine, itraconazole, clarithromycin, verapamil	PXR activators[a]
MRP2 (ABCC2)	Irinotecan, methotrexate, vinblastine, mycophenolate, many phase 2 metabolites	Cyclosporine	Unknown
MRP3 (ABCC3)	Methotrexate, doxorubicin, vincristine, acetaminophen glucuronide	8-Methoxy-psoralen	PXR activators[a]
BCRP (ABCG2)	Methotrexate, doxorubicin, topotecan, irinotecan active metabolite (SN-38)	Omeprazole, pantoprazole	PXR activators[a]

a Presumed inducers would include rifampin, phenytoin, carbamazepine, phenobarbital and St John's wort.

divided into two functional categories: efflux transporters and uptake transporters.

Efflux transporters

It has long been appreciated that elimination of drugs and metabolites into bile must involve active transport mechanisms, and the specific proteins involved have now been identified. Some properties of major efflux transporters are listed in Table 26.3. The major canalicular drug transporters that have been identified are the multidrug resistance protein (MDR)1, multidrug resistance associated protein (MRP)2, and the breast cancer resistance protein (BCRP) (Fig. 26.2). As their names suggest, each of these transporters was first discovered in cancer cells as a basis for resistance to multiple chemotherapies [106–108]. Each of these transport proteins utilize ATP and are members of the ATP-binding cassette (ABC) superfamily, which accounts for the gene nomenclature (Table 26.3). MDR3 transports phospholipids and bile salt excretory protein (BSEP) transports bile acids. Polymorphisms in MDR3 have been linked to intrahepatic cholestasis of pregnancy [109] and polymorphisms in BSEP have been linked to inherited forms of cholestasis [110].

MDR1, MRP2, and BCRP are also expressed in the apical membrane of enterocytes, where they function to reduce or prolong absorption by pumping drugs or their metabolites back into the intestinal lumen. It has been proposed that the transporters and metabolizing enzymes function synergistically in the intestine to limit entry of potentially harmful xenobiotics in the diet [111,112]. It also seems likely that inhibitors of these transporters could be added to certain drug formulations to improve oral availability [113].

MRP3, MRP4, MRP5, and MRP6 are also efflux transporters and function to pump drugs and metabolites from within the hepatocyte into the space of Disse. For example, MRP3 largely accounts for efflux of acetaminophen glucuronide into sinusoidal blood after it is formed in the hepatocyte [114].

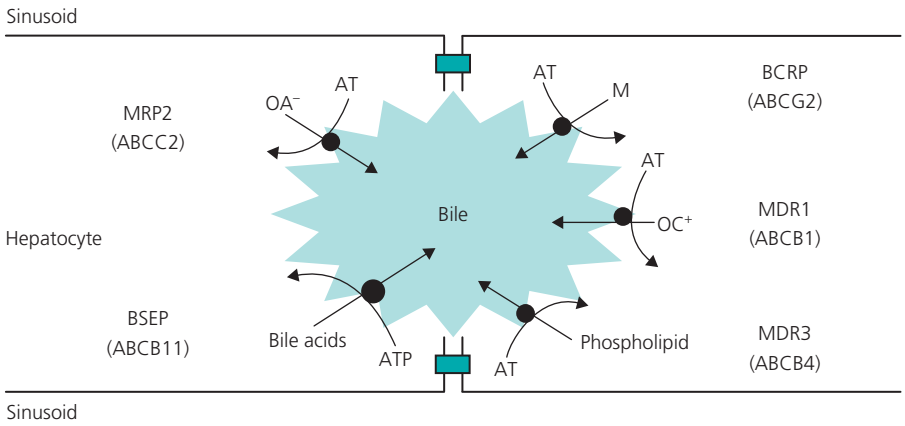

Figure 26.2 Basolateral membrane transport proteins in the hepatocyte. The basolateral membrane contains both efflux and uptake transporters that largely control the entrance and exit of drugs and their metabolites. The major transporters involved in uptake of drugs belong to the OAT and OATP families. The major drug efflux transporters are members of the MRP family and MDR1. Adapted from Chandra & Brouwer [152].

Some of these transporters also appear to be present in enterocytes, where they aid absorption by pumping drugs into the body across the basolateral membrane [115]. Characterization of the substrates for these transporters is an active area of investigation.

Uptake transporters

Uptake of drugs into hepatocytes and enterocytes also involves transporters. Major liver uptake transporters are shown in Fig. 26.3. Uptake transporters do not utilize ATP but sodium independent cotransport (the gene designation SLC stands for *solute carrier*). The best-characterized uptake transporters in liver are the organic anion transporting polypeptides (OATP) and the organic anion transporters (OAT). The sodium taurocholate cotransporting polypeptide (NTCP) transports bile acids into the hepatocyte and the organic cation transporters (OCT) in liver are not well characterized. Only some OATP and OAT transporters are present in the intestine. In addition, there are intestine-specific transporters such as PEPT1, which is involved in the absorption of various peptide drugs, including some cephalosporin antibiotics [116]. One strategy is to improve absorption of some drugs by linking them to peptide substrates for PEPT1 [115].

Drug transporters as a basis for interpatient variation in pharmacokinetics and drug interactions

As previously discussed, it is believed that metabolism catalyzed by cytochrome P450s is generally rate limiting in the elimination of drugs. However, it is becoming clear that transporters may be rate limiting in the elimination of some drugs and their metabolites. Genetic polymorphisms have been reported for most of the efflux and uptake transporters discussed above, and emerging data suggest that polymporphisms can partly account for interpatient variation in efficacy and/or safety of some drugs [117], including simvastatin [118], pravastatin [119], cyclosporine [120], and nelfinavir [121]. Some of these polymorphisms have also been linked to susceptibility to certain diseases, including inflammatory bowel disease [122]. Genetic deficiency in MRP2 activity appears to account for Dubin–Johnson syndrome [123]. Given the broad substrate specificity of this transporter, it is somewhat surprising that patients with Dubin–Johnson syndrome have not yet been reported to be sensitive to any drugs.

It is also clear that some drug interactions can occur because of inhibition or induction of transporters [124], and these can be inferred from Table 26.3. For example, gemfibrozil inhibits OATP1B1 [125] and this may partly account for the increased incidence of muscle injury observed in patients concomitantly receiving gemfibrozil and cerivastatin [126]. Some interactions thought to be due to inhibition or induction of CYP3A4 may result in part by inhibition or induction of MDR1. For example, terfenadine is metabolized by CYP3A4 to the pharmacologically active antihistamine fexofenadine, which is largely eliminated from the body unchanged. Terfenadine blood levels rose to sometimes dangerous levels in patients treated with CYP3A4 inhibitors (including macrolide antibiotics such as clarithromycin and itraconazole) [127] and it was assumed that this predisposition would be removed by replacing terfenadine with fexofenadine. However, most of the drug interactions described with terfenadine were still observed with fexofenadine. Fexofenadine is a substrate for MDR1, and the similarity in drug interactions between fexofenadine and terfenadine likely results because potent inhibitors of CYP3A4 also inhibit MDR1 [128]. In addition, lower blood levels of fexofenadine were observed during treatment with rifampin [129] and other PXR activators [130] and this appears to be

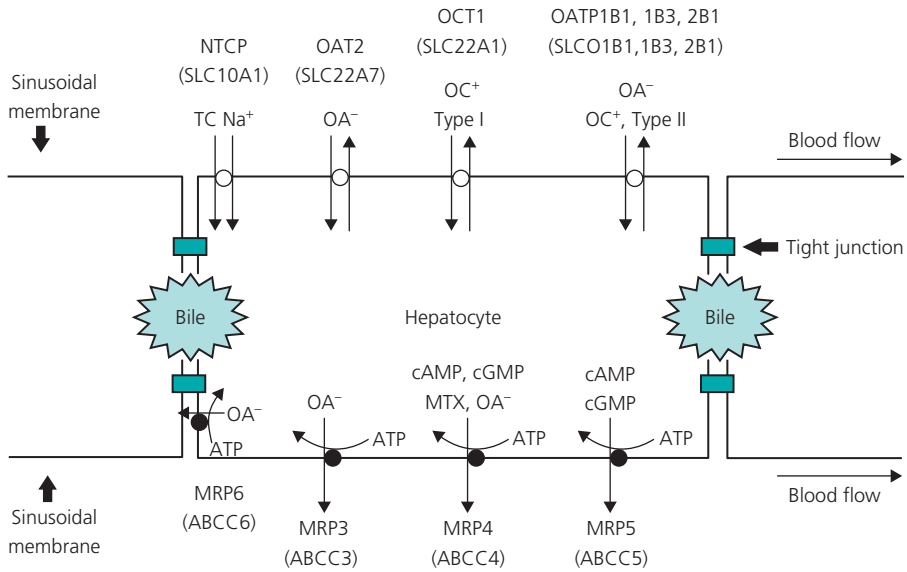

Figure 26.3 Canalicular efflux transporters. The major canalicular efflux transporters that pump drugs and metabolites into bile are MDR1, MRP2, and BCRP. Adapted from Chandra & Brouwer [152].

explained by PXR-mediated induction of MDR1. MDR1 induction in the intestine also appears to significantly contribute to reduction in erythromycin blood levels during treatment with rifampin [131] and may well contribute to interactions between PXR activators and other dual CYP3A4/MDR1 substrates (such as cyclosporine) [132]. Evidence suggests that MRP3 and BRCP are also part of the cassette of genes that are transcriptionally activated by PXR, although the clinical relevance of this remains unclear [133].

Some drug interactions involving uptake transporters appear to largely occur in the intestine. For example, several citrus juices enhance the oral availability of fexofenadine and this appears to result from inhibition of OATP1A2 by as yet unidentified substances [134].

Role of transporters in drug-induced liver injury

It seems reasonable to assume that transporters will be found to play important roles in mechanisms underlying drug-induced liver injury, although work is just beginning in this area. For example, it has been shown that certain drugs inhibit BSEP and thereby lead to accumulation of potentially toxic bile acids within the hepatocyte [135]. This has been proposed as a mechanism underlying hepatotoxicity of bosentan [136] and troglitazone [137]. One study demonstrated that a major metabolite of troglitazone (M-1) potently inhibited some OATPs and proposed that this may contribute to the hepatotoxicity observed with this compound [138].

Transporters may also be involved in helping the hepatocyte recover from drug-induced liver injury. For example, during recovery from hepatotoxicity due to acetaminophen or carbon tetrachloride, there is downregulation of several hepatic OATPs and upregulation of MRP3 and MRP4 in rodents [139]. This presumably protects the injured cell from further drug injury by decreasing uptake and increasing efflux of the drugs and their metabolites. One study has suggested similar changes in transporter expression during liver injury in humans [140].

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E MISCELLANEOUS

27

Pathogenesis of hepatic fibrosis

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Overview of liver fibrosis

Liver fibrosis is the accumulation of extracellular matrix (ECM) proteins, mainly collagen, that occurs in most types of chronic liver disease [1]. The main causes of liver fibrosis in industrialized countries include chronic hepatitis C virus (HCV) infection, alcohol abuse, and nonalcoholic steatohepatitis (NASH). The accumulation of ECM proteins distorts the hepatic architecture by forming a fibrous scar and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis [2]. Cirrhosis produces hepatocellular dysfunction and increased intrahepatic resistance to blood flow, resulting in hepatic insufficiency and portal hypertension, respectively.

Hepatic fibrosis was historically thought to be a passive and irreversible process, due to the collapse of the hepatic parenchyma and its substitution by a collagen-rich tissue [3]. Currently, it is considered a model of the wound healing response to chronic liver injury [4]. Liver fibrosis received little attention until the 1980s, when the hepatic stellate cell (HSC), formerly known as the lipocyte, Ito cell or perisinusoidal cell, was identified as the main collagen-producing cell in the liver [5]. This cell type, first described by von Kupffer in 1876, undergoes a dramatic phenotypic activation in chronic liver diseases with the acquisition of fibrogenic properties [6]. The molecular mechanisms leading to HSC activation and increased collagen synthesis in liver fibrosis have been identified using cultured HSCs and experimental models of chronic liver injury in rodents [7,8]. Besides HSCs, portal myofibroblasts and cells of bone marrow origin have also been shown to have fibrogenic potential [9]. The demonstration that even advanced liver fibrosis is reversible has greatly stimulated researchers to identify antifibrotic therapies [10]. However, the most effective therapy to treat hepatic fibrosis is still to remove the causative agent [11]. A number of drugs are able to reduce the accumulation of scar tissue in experimental models of chronic liver injury. Renin–angiotensin system blockers and antioxidants are promising drugs, yet evidence-based therapies are not yet available [12].

The onset of liver fibrosis is usually insidious and most of the related morbidity and mortality occur after the development of cirrhosis [13]. In the majority of patients, progression to cirrhosis occurs after an interval of 15–20 years. The natural history of liver fibrosis is influenced by both genetic and environmental factors. Epidemiological studies have identified polymorphisms in a number of candidate genes that may influence the progression of liver fibrosis in humans [14]. These genetic factors may explain the broad spectrum of responses to the same etiological agent found in patients with chronic liver diseases. Conceptually, the process of hepatic fibrosis must, at some point, consist of the stimulation of excessive ECM molecule synthesis by one or more populations(s) of cells (or an increase in the population of ECM-producing cells), or a decrease in the rate of ECM degradation or turnover.

This chapter reviews our current understanding of the pathogenesis of hepatic fibrosis and highlights those elements of the process for which intervention has been attempted. The etiology and cellular and molecular mechanisms of liver fibrosis will be detailed. The mechanisms of transition of advanced fibrosis to cirrhosis are also discussed. Finally, the basis for pathophysiologically based therapies will be detailed.

Etiology of liver fibrosis

Etiological factors of liver fibrosis are depicted in Table 27.1. The main causes of fibrosis in industrialized countries are HCV infection and alcoholic liver disease, which account for more

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Table 27.1 Main causes of liver fibrosis

Chronic viral diseases
Hepatitis B
Hepatitis C
Hepatitis D
Alcohol abuse
Autoimmune diseases
Autoimmune hepatitis
Primary biliary cirrhosis
Primary sclerosing cholangitis
Graft-versus-host disease
Hepatotoxic agents
Drugs: methotrexate, α-methyldopa, amiodarone, others
Vitamin A intoxication
Metabolic diseases
Nonalcoholic steatohepatitis
Wilson disease
Hemochromatosis
α_1-Antitrypsin deficiency
Type IV glycogen storage disease
Tyrosinemia
Vascular diseases
Chronic right-sided heart failure
Budd–Chiari syndrome
Venoocclusive disease
Inferior vena cava thrombosis
Miscellaneous
Secondary biliary cirrhosis
Cryptogenic

than half of cases [2]. Other major causes are hepatitis B virus (HBV) infection; autoimmune hepatitis; chronic cholestasis, namely primary biliary cirrhosis and primary sclerosing cholangitis; and genetic metabolic diseases (hemochromatosis and Wilson disease). Due to the current epidemics of obesity, NASH is increasingly recognized as a major cause of fibrosis, yet its actual prevalence is still unknown.

Chronic viral infections

Chronic viral infections are a common cause of liver fibrosis. More than 170 million people worldwide are chronically infected with HCV [13]. Most infected patients develop chronic viral infection. The natural history of patients with chronic HCV is characterized by a slow progression of liver fibrosis. Following infection, cirrhosis may develop after an average of 20–30 years. In some patients, the rate of fibrosis progression is much faster, and cirrhosis develops after 10–15 years, whereas in others the rate of progression is negligible. Several factors are associated with fibrosis progression rate: duration of infection, older age, male sex, alcohol consumption, and HIV coinfection. Metabolic disorders such as obesity and diabetes are emerging as independent cofactors of fibrogenesis [15]. Moreover, genetic factors may also play a role. Current antiviral therapies cure chronic HCV infection in half of infected patients [16]. Viral clearance is associated with improvement of the degree of fibrosis in many patients. Globally, HBV infection is the single most common cause of fibrosis, although the prevalence rate is influenced by geographic region [17]. HBV is endemic in parts of Asia where millions of individuals are infected. About 5%–10% of acutely infected adults become chronically infected. The natural history of liver fibrosis due to chronic HBV infection can vary dramatically between individuals [18]. Some individuals with chronic HBV will have clinically insignificant or minimal liver disease and never develop complications. Others will have clinically apparent chronic hepatitis that can progress to fibrosis and cirrhosis. Patients with chronic HBV and *replicative* infection defined by the presence of detectable HBeAg have a generally worse prognosis and a greater chance of developing advanced fibrosis or cirrhosis than those without HBeAg. The response to therapy in patients with chronic HBV infection is excellent and liver fibrosis can reverse in patients in whom viral infection has been cured [19]. Hepatitis delta virus (HDV) is a defective transmissible pathogen dependent on HBV for replication. Liver fibrosis progresses rapidly to cirrhosis in patients with dual infection with HBV and HDV [20]. Coinfection of HIV with hepatitis viruses is a common cause of liver fibrosis [21]. Importantly, advanced liver fibrosis and cirrhosis are the main causes of mortality among HIV-infected individuals in industrialized countries, in which highly active antiretroviral therapy is widely available. Both HBV and HCV are more common in HIV-infected individuals than in the general population as a result of shared risk factors for viral acquisition. Populations of injecting drug users are at particularly high risk for HIV/HCV coinfection. Coinfection with HIV results in greater likelihood of chronicity and enhanced viral replication in the setting of both HBV and HCV infections. Current evidence suggests that HIV infection may have a negative impact on HBV-related fibrosis progression, although the mechanisms for this are unclear [22]. HIV coinfection hastens HCV-related liver disease, with faster fibrosis progression to cirrhosis, end-stage liver disease, and occurrence of hepatocellular carcinoma.

Alcoholic liver disease

Alcoholic liver disease (ALD) is one of the commonest causes of liver fibrosis worldwide [23]. Although cumulative alcohol dose undoubtedly plays a role in determining fibrosis risk, only a small proportion of heavy drinkers go on to develop the more advanced forms of ALD–hepatitis, fibrosis and cirrhosis. Both genetic and environmental factors are associated with fibrosis progression in these patients [24]. Patients developing alcoholic hepatitis are at high risk of progressing to advanced fibrosis and cirrhosis [25]. This condition is characterized by evidence of hepatocyte necrosis and apoptosis, leukocyte infiltration, and perisinusoidal–pericellular fibrosis. The only effective maneuver leading to

fibrosis regression in these patients is alcohol abstinence. Until now, the development of specific antifibrotic treatments has been hampered by the lack of detailed knowledge of disease mechanisms [26]. This situation might now be changing with improved insight into the pathogenesis obtained from studies that focus on the roles of inflammation, endotoxin, and immunity.

Metabolic causes

Among causes of metabolic origin, nonalcoholic fatty liver disease (NAFLD) has emerged as a major cause of liver fibrosis [27]. NAFLD is a frequent syndrome encompassing fatty liver alone and a subset of patients develop hepatocellular injury, inflammatory changes, and slowly progressive fibrosis [28]. In the general adult population, NAFLD prevalence ranges between 20% and 30%, while the prevalence of NASH is 1%–5%. The presence of necroinflammation has been associated with a significant risk of progression to advanced fibrosis and eventually liver cirrhosis and hepatocellular carcinoma [29]. Age over 45 years, obesity, and diabetes have also been associated with an increased risk of liver fibrosis and progression to cirrhosis. Hereditary hemochromatosis is the most common genetic cause of liver fibrosis [30]. Liver biopsy remains important in management to determine the presence or absence of fibrosis, particularly among patients with serum ferritin levels greater than 1000 ng/mL or elevated liver enzymes. Factors such as moderate alcohol consumption can accelerate the progression of fibrosis [31]. Decompensated cirrhosis due to hemochromatosis carries a bad prognosis unless liver transplantation is performed. Moreover, patients with hemochromatosis are at high risk of developing hepatocellular carcinoma, which is a major cause of mortality among these patients. Wilson disease is an autosomal recessive disorder of copper metabolism that may result in liver fibrosis [32]. Thus far, more than 200 mutations have been detected, causing impairment of ATP7B function and, ultimately, copper accumulation. Resultant liver damage leads to steatosis, inflammation and progressive fibrosis. Wilson disease should be suspected in a patient with liver fibrosis of unknown origin and neurosychiatric symptoms. α_1-Antitrypsin deficiency is the most common genetic cause of liver fibrosis in children [33]. α_1-Antitrypsin is the main protease inhibitor in human plasma. Polymers accumulate within the endoplasmic reticulum of the hepatocyte forming inclusion bodies that are associated with neonatal hepatitis, progressive fibrosis, and juvenile fibrosis. In adults, α_1-antitrypsin deficiency may cause severe liver injury and is a common finding in patients with hepatic fibrosis of unknown origin. Heterozygosity for the Pi*Z allele may also predispose to liver fibrosis in patients with ongoing liver injury (HCV infection or NASH). Other metabolic causes of fibrosis include glycogen storage diseases, galactosemia, and congenital tyrosinosis. Even diabetes mellitus itself has been associated with the development of fibrosis.

Autoimmune liver diseases

Several autoimmune liver diseases may result in liver fibrosis. Primary biliary cirrhosis (PBC) is a chronic cholestatic liver disease characterized histologically by chronic nonsuppurative destruction of interlobular bile ducts leading to advanced fibrosis, cirrhosis, and liver failure [34]. Affected patients are typically middle-aged women with abnormal serum concentrations of alkaline phosphatase. Identification of PBC is important, because effective treatment with ursodeoxycholic acid has been shown to halt fibrosis progression and improve survival without need for liver transplantation [35]. Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by strictures of the biliary tree. It is closely associated with inflammatory bowel disease, particularly ulcerative colitis, although the precise etiology remains unknown [36]. Small-duct PSC is a distinct clinical entity associated with a benign course. Patients eventually show progressive fibrosis and cirrhosis. Autoimmune hepatitis is characterized histologically by interface hepatitis and serologically by the presence of non-organ- and liver-specific autoantibodies and increased levels of IgG [37]. Its onset is often ill-defined, frequently mimicking acute hepatitis. Liver damage is likely to derive from an immune reaction to liver cell antigens. Fibrosis often develops and its progression is attenuated and even reversed if effective immunosuppressant therapy is initiated.

Other causes

Cardiac fibrosis includes a spectrum of hepatic derangements that occur in the setting of right-sided heart failure. It should be suspectd in patients with hepatic fibrosis of unknown origin with clinical signs of right-sided heart failure. Prolonged biliary obstruction (secondary biliary fibrosis) can result in liver fibrosis. It can be caused by gallstones, bile duct or head of pancreas carcinoma, biliary tract infections or strictures and, in children, biliary atresia. This latter condition is the main cause of advanced liver fibrosis requiring liver transplantation among children. When fibrosis is in its earlier stages and the cause of biliary obstruction is resolved, histological improvement has been documented. Granulomatous diseases can cause liver fibrosis. These diseases include sarcoidosis and infections (mycobacterial infections and schistosomiasis). Hepatotoxic drugs can also cause liver cirrhosis and fibrosis. Drug-induced liver disease account for between 10% and 40% of adult patients with elevated enzymes. Liver injury can be cytotoxic, cholestatic, or mixed. Most cases resolve after withdrawal of the drug, but in a few undiagnosed cases the liver disease can progress to cirrhosis. Drug-induced liver disease can mimic autoimmune hepatitis or venoocclusive disorders. The plethora of herbal and traditional agents ingested by many people should always be considered in any patient with liver fibrosis of unknown origin. Venous outflow obstruction (e.g., Budd–Chiari syndrome, venoocclusive disease) can result in severe liver fibrosis and

cirrhosis [38]. Budd–Chiari syndrome is a rare disorder caused by hepatic venous obstruction mainly due to thrombosis. Most cases are due to an underlying hypercoagulable disorder due to myeloproliferative disorders or abnormalities in the coagulation cascade. Fibrosis progresses rapidly, leading to severe portal hypertension and liver failure in some patients. In these acute or subacute cases, pharmacological or mechanical thrombolysis can be effective. Transjugular portosystemic shunt is also used for restoring a normal venous outflow. Hepatic venoocclusive disease is a common complication of bone marrow transplantation. Perivenular fibrosis develops and can result in jaundice and portal hypertension. Hepatic fibrosis of unknown etiology, termed *cryptogenic*, is diagnosed less frequently as modern techniques are available to identify the causative disease. The diagnosis of cryptogenic fibrosis is made when all known causes of liver disease are excluded. Therefore, medical history, consumption of alcohol or drugs, virological tests, tests for metabolic diseases, tests for autoimmune diseases, and careful imaging of the liver should be performed. Importantly, studies suggest that many patients with cryptogenic fibrosis and cirrhosis show features of the metabolic syndrome, including obesity, diabetes, and dyslipemia. Therefore, a role for NASH in the pathogenesis of cryptogenic fibrosis has been suggested [39].

Cellular mechanisms of liver fibrosis

Hepatic fibrosis is the result of the wound healing response of the liver to repeated injury [2]. After an acute liver injury (e.g., viral hepatitis), parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is associated with an inflammatory response and limited deposition of ECM. If the hepatic injury persists, then eventually liver regeneration fails and hepatocytes are substituted by abundant ECM including fibrillar collagen. The distribution of this fibrous material depends on the origin of the liver injury.

The cellular mechanisms of liver fibrosis have now been uncovered. The most relevant finding, which has greatly stimulated research in this field, was the identification of activated HSCs as the major source of ECM in the fibrotic liver [40]. This assumption is based on the following data.

- In vitro studies performed in the early 1980s clearly demonstrated that the collagen found in cultures of primary hepatocytes was due to contaminating HSCs within the cell monolayers [41]. Further studies confirmed that cultured HSCs synthesize and secrete large amounts of collagen and other ECM proteins, while other cells types (i.e., hepatocytes, sinusoidal endothelial cells) only secrete modest amounts of these proteins [5].
- Studies using immunocytochemistry, in situ hybridization, and transgenic mice identified collagen expression in damaged livers in activated HSCs rather than in parenchymal cells [42] (Fig. 27.1).
- Studies assessing gene expression in rat and human HSCs freshly isolated from normal livers showed that quiescent HSCs express low levels of mRNA encoding ECM proteins, while HSCs activated in culture or in vivo show a marked upregulation of these genes and secrete large amounts of collagen [43].
- HSCs isolated from rat and human livers with advanced fibrosis markedly overexpress genes encoding ECM proteins, which is not observed in other hepatic cell types [44].

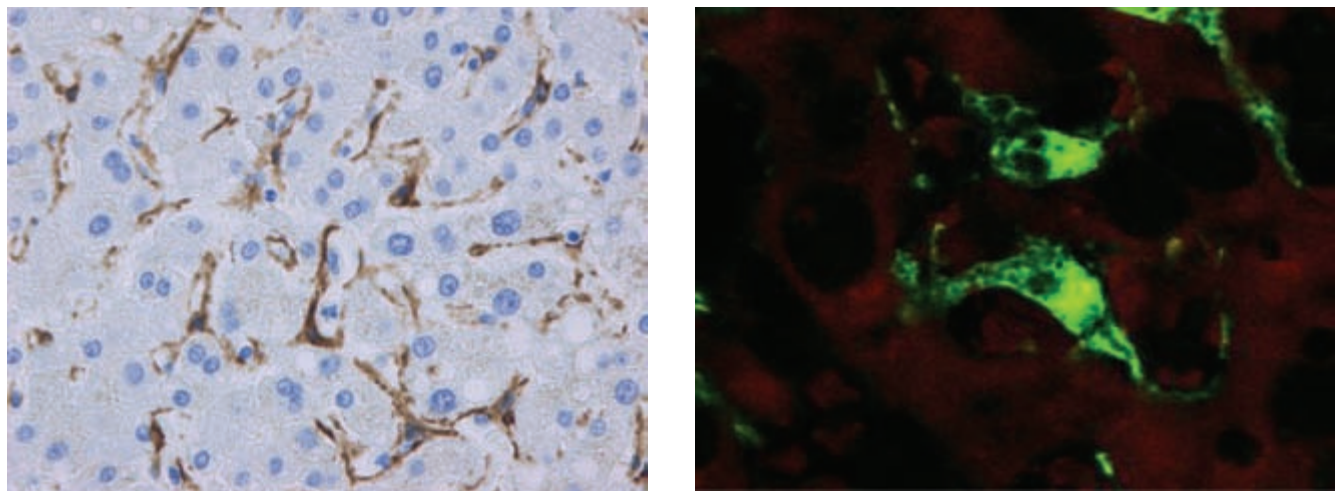

Figure 27.1 Identification of activated hepatic stellate cells in fibrotic livers. **(a)** Demonstration of α-smooth muscle actin (SMA) immunoreactivity in a liver biopsy of a patient with hepatitis C virus-induced liver fibrosis (magnification × 400). **(b)** Identification of activated hepatic stellate cells expressing green fluorescence protein-procollagen α1(I) in transgenic mice with bile duct ligation (magnification × 600).

• Studies assessing both experimental models of liver fibrosis and human fibrogenesis have demonstrated a positive correlation between the degree of fibrosis and the accumulation of activated HSCs in the damaged liver [45].
• Substances that inhibit the activation or proliferation of HSCs also attenuate the progression of hepatic fibrosis in experimental models of chronic liver injury [46–48].

Activation of HSCs is a key step in liver fibrogenesis. HSCs from normal liver represent about 15% of the total liver population and show a *quiescent* phenotype (rich in vitamin A fat droplets). When freshly isolated and cultured, quiescent HSCs show a low proliferative rate and very modest fibrogenic potential, little cytokine secretion, and lack of contractile properties [43,49]. Therefore, the main function of these quiescent HSCs is considered to be the storage and metabolism of vitamin A [6]. However, following liver injury of any etiology, HSCs undergo a process termed *activation*, which represents a transition into proliferative, fibrogenic, proinflammatory, and contractile myofibroblasts [50] (Fig. 27.2). Therefore, substances that inhibit activation of HSCs are of major importance in attenuating their fibrogenic response. In the 1990s, a number of studies characterized the cellular and molecular mechanisms underlying HSC activation. The initiation phase of this process is probably due to paracrine stimuli from injured neighboring cells, which include hepatocytes, Kupffer cells, sinusoidal endothelial cells, platelets, and infiltrating inflammatory cells [51,52]. Some of these cells (i.e., hepatocytes, Kupffer cells) promote HSC activation by producing lipid peroxides leading to oxidative stress [53–55]. Moreover, antioxidants can attenuate liver fibrogenesis by inhibiting HSC activation [56]. A number of cytokines released by damaged neighboring cells can also activate HSCs. These include transforming growth factor (TGF)-β1, platelet-derived growth factor (PDGF), and endothelin-1, which stimulate transcription factors such as Sp1, c-myb, NF-κB, c-jun/AP1, STAT-1 and SMAD proteins that regulate gene expression [8,57,58]. Activated HSCs perpetuate their own activation by several autocrine loops, including the secretion of TGF-β1 and upregulation of its receptors [51]. Based on its importance, several therapeutic options under evaluation are aimed at inhibiting HSC activation (i.e., antioxidants, substances blocking TGF-β1 activity).

One important feature of activated HSCs is their proliferative phenotype. Following liver injury there is a marked accumulation of α-smooth muscle actin-positive cells at the sites of active liver fibrogenesis [7,59]. Growth factors for HSCs and their intracellular signaling pathways have been largely characterized in culture [60]. The most powerful growth factor for HSCs is PDGF, but stimulation of other tyrosine kinase receptors by epidermal growth factor (EGF), fibroblast growth factor (FGF), or insulin-like growth factor (IGF) also results in increased HSC proliferation [8].

Resistance to apoptosis of activated HSCs is considered a key event in liver fibrogenesis [61]. Soluble survival factors are likely to be important to HSC survival. During liver injury, hepatocytes and HSCs express IGF type 1, a potent prosurvival factor. In addition, overexpresion of TGF-β1 in the injured liver may promote stellate cell survival. In contrast to these factors, PDGF, which is potently mitogenic for HSCs, has relatively little antiapoptotic activity. The separate regulation of proliferation and survival in HSCs provides a further control on HSC numbers. Moreover, adipokines such as leptin and factors regulating collagen degradation, such as tissue inhibitor of metalloproteinases (TIMP)-1, may also act

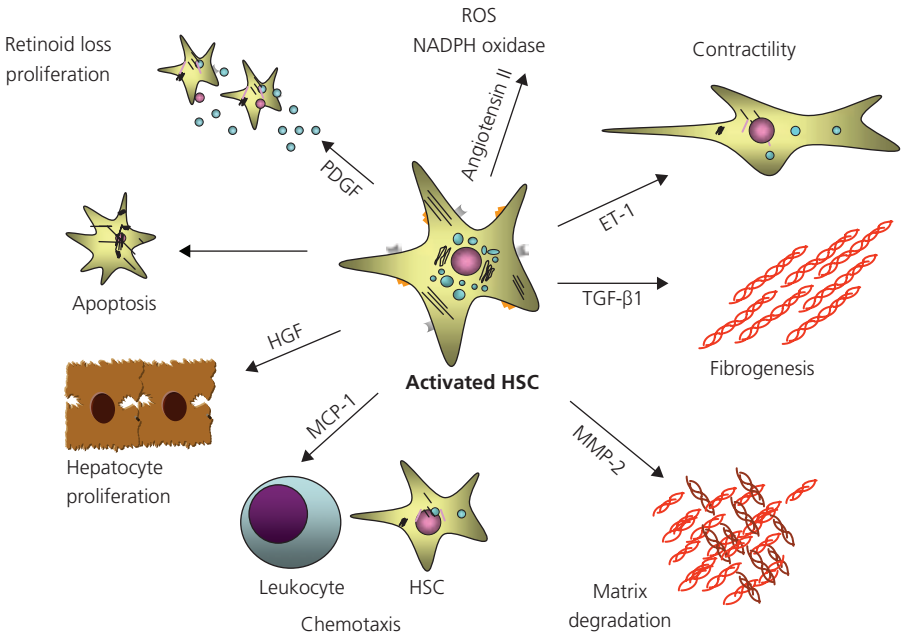

Figure 27.2 Biological properties of activated hepatic stellate cells (HSC) that participate in liver fibrogenesis. ET-1, endothelin-1; HGF, hepatocyte growth factor; MCP-1, monocyte chemotactic protein type 1; MMP, metalloproteinase; PDGF, platelet-derived growth factor; ROS, reactive oxygen species; TGF, transforming growth factor.

as survival factors for activated HSCs [62,63]. The ECM may also provide key survival signals [64]. There is a striking correlation between the degradation of the collagen I-rich matrix during recovery from liver fibrosis and apoptosis of activated HSCs. Preliminary studies indicate that the phenotype of activated HSCs is greatly influenced by the composition of the adjacent subcellular matrix. For example, activated HSCs cultured on a collagen I matrix maintain an activated phenotype with an increased propensity to apoptosis, as compared with cells grown on basement membrane-like matrix [65]. When activated cells are transferred to this latter surface, they revert to a quiescent state with increased resistance to apoptosis, associated with expression of the antiapoptotic protein Bcl-2. These data clearly support the notion that activated HSCs are more susceptible to apoptosis than quiescent cells. A decreasing concentration of soluble survival factors, the degradation of specific matrix components, or separation of cells from matrix contact, either separately or in combination, may then be critical for precipitating an apoptotic response in HSCs. Removal of activated HSCs from the injured liver by promoting their apoptosis is another potential target for treatment. Apoptosis of activated HSCs plays a critical role in the spontaneous recovery from experimental fibrosis in different experimental models (carbon tetrachlolide administration, bile duct ligation) [66]. Conversely, resistance to apoptosis and the consequent prolonged survival of activated HSCs may contribute to the progression of hepatic fibrosis [67]. Further studies are required to delineate the factors and mechanisms regulating apoptosis in HSCs and to design therapeutic strategies aimed at promoting apoptosis of activated HSCs in experimental liver fibrosis.

Migration of HSCs could contribute to their accumulation at sites of liver injury. Following activation, cultured HSCs migrate in response to several stimuli, including growth factors (PDGF), vasoactive substances (angiotensin II, endothelin-1), and chemokines (monocyte chemotactic protein-1) [68,69]. Chemotaxis of HSCs requires Ca^{2+} influx and involves the phosphatidylinositol 3-kinase/Akt pathway. Although the importance of migration of HSCs in vivo has not been determined, substances that inhibit migration of HSCs could theoretically reduce their accumulation in injured liver.

The interaction of HSCs with the ECM is also of major importance in the profibrogenic behavior of activated HSCs. One of the earlier changes observed after chronic liver injury is the change in the composition of the space of Disse. Proteins including type IV collagen, fibronectin, proteoglycans, and urokinase-type plasminogen activator are released by sinusoidal endothelial cells and accumulate around the hepatic sinusoids [70]. These factors contribute to the activation of resident HSCs by the stimulation of latent cytokines such as TGF-β [71]. Of particular interest is a *fetal* isoform of fibronectin, [EIIIA]Fn, which is synthesized de novo by the damaged liver and contributes to the activation of resident HSCs [72]. Once HSCs are activated, they secrete fibrillar collagens and other ECM proteins, which also accumulate in the space of Disse. This altered microenvironment can amplify the fibrogenic activity of HSCs by different mechanisms. First, fibrillar collagens can bind to discoidin domain receptors and activate intracellular signaling pathways [55]. Second, the altered ECM can serve as a reservoir for a number of growth factors (PDGF, TGF-β, FGF) and matrix metalloproteinases (MMPs), which are released and reach neighboring cells including HSCs [73]. This implies that proteolysis of the ECM during liver inflammation can initiate reparative processes by the released growth factors and MMPs. Third, HSCs express a number of integrins, heterodimeric transmembrane proteins whose ligands are matrix molecules rather than cytokines, which transduce the extracellular signals from the ECM into the cells [74]. Several activities of HSCs can be regulated by integrins, including cell proliferation, contraction, migration, and collagen synthesis [75]. Moreover, integrins can also activate latent TGF-β, thus amplifying the fibrogenenic action of this key cytokine. Therefore, pharmacologically modulating the interaction between HSCs and the surrounding ECM could limit liver fibrosis. In this regard, the common presence of Arg-Gly-Asp (RGD) within many integrin ligands has prompted the use of competitive RGD antagonists to block integrin-mediated pathways in experimental liver fibrogenesis [76]. Finally, HSCs express CD44, the hyaluronic acid receptor [77]. Activation of CD44 is involved in cell migration and is required for liver fibrogenesis.

HSCs also play an active role in hepatic inflammation [78]. Activated HSCs migrate in response to cytokines released by monocytes and also secrete a number of proinflammatory cytokines and chemokines that could participate in the activation of lymphocytes and the recruitment of white blood cells, thus amplifying the inflammatory response [79]. The mechanisms involved in cytokine secretion by HSCs include activation of the transcription factor NF-κB. In fact, inhibition of NF-κB by proteosome inhibitiors or by an adenovirus expressing the IκB super-repressor markedly blunt cytokine secretion by activated HSCs [80]. Moreover, activated human HSCs express receptors for a number of chemokines that activate several intracellular pathways stimulating the inflammatory action of these cells [81]. In addition, HSCs can take up and process antigens and, under stimulation with cytokines, express the cell machinery required for antigen presentation and thereby modulate the growth of lymphocytes [82]. In fact, the development of liver fibrosis in most human liver diseases is preceded by chronic inflammation of the hepatic parenchyma, and treatments inhibiting liver inflammation may also attenuate the progression of liver fibrosis [83]. Therefore, the use of substances with combined antifibrotic and antiinflammatory effect should be considered in antifibrotic therapy.

Hepatic cell types other than HSCs may also have fibrogenic potential (Fig. 27.3). Studies in transgenic mice have

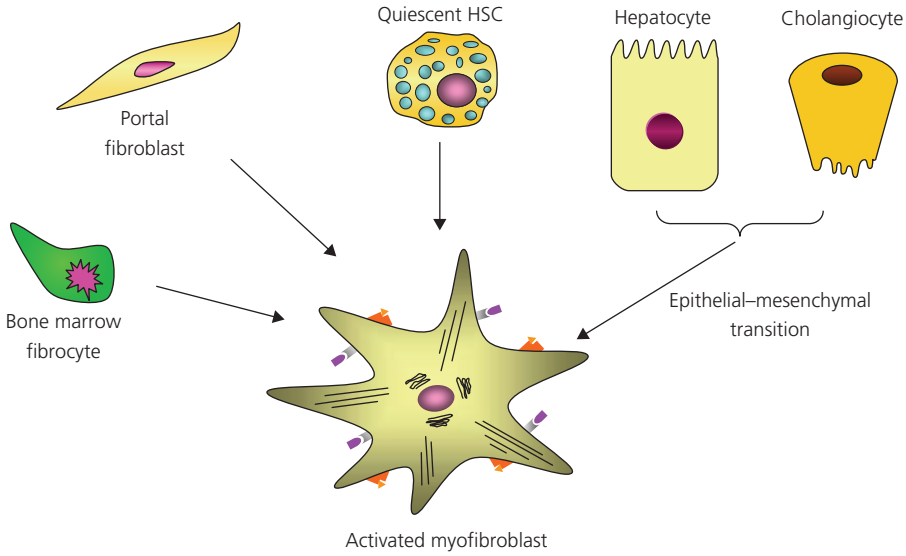

Figure 27.3 Different sources of myofibroblastic cells with fibrogenic potential in livers with chronic damage. HSC, hepatic stellate cell.

shown that myofibroblasts derived from small portal vessels proliferate around biliary tracts in cholestasis-induced liver fibrosis to initiate collagen deposition [84]. HSCs and portal myofibroblasts differ in specific cell markers and response to apoptotic stimuli [9]. Culture of $CD34^{+}CD38^{-}$ hematopoietic stem cells with various growth factors has been shown to generate HSCs and myofibroblasts of bone marrow origin that infiltrate human livers undergoing tissue remodeling [85]. These data suggest that cells of bone marrow origin can be a source of fibrogenic cells in the injured liver. Other potential sources of fibrogenic cells are circulating fibrocytes [86]. In mice with bile duct ligation, the majority of bone marrow-derived cells coexpress collagen and CD45, suggesting that these cells represent a unique population of fibrocytes. Interestingly, fibrocytes cultured in the presence of TGF-β1 differentiated collagen-producing myofibroblasts, potentially contributing to liver fibrosis. Finally, epithelial-to-mesenchymal transition, a TGF-β1-driven mechanism that participates in renal fibrosis, may also occur during chronic liver injury [87]. The relative importance of each cell type in liver fibrogenesis may depend on the origin of the liver injury. While HSCs are the main fibrogenic cell type in pericentral areas, portal myofibroblasts may predominate when liver injury occurs around portal tracts.

A complex interplay among different hepatic cell types takes place during hepatic fibrogenesis [2,88]. Hepatocytes are targets for most hepatotoxic agents, including hepatitis viruses, alcohol metabolites, and bile acids. Damaged hepatocytes release reactive oxygen species (ROS) and fibrogenic mediators and induce infiltration by inflammatory cells [89]. Apoptosis of damaged hepatocytes stimulates the fibrogenic actions of liver myofibroblasts [61]. Inflammatory cells, either lymphocytes or polymorphonuclear cells, activate HSCs to secrete collagen. Activated HSCs secrete inflammatory chemokines, express cell adhesion molecules, and modulate the activation of lymphocytes [78]. Therefore, a vicious circle in which inflammatory and fibrogenic cells stimulate each other is likely to occur. Fibrosis is influenced by different T-helper subsets, the Th2 response being associated with more active fibrogenesis [90]. Kupffer cells are resident macrophages that play a major role in liver inflammation by releasing ROS and cytokines [91,92]. A role for macrophages in hepatic wound healing has been proposed [93]. Macrophage depletion when liver fibrosis was advanced resulted in reduced scarring and fewer myofibroblasts. In contrast, macrophage depletion during recovery led to a failure of matrix degradation. These findings suggest that macrophages regulate both ECM formation and degradation in liver fibrogenesis. In chronic cholestatic disorders (i.e., primary biliary cirrhosis and primary sclerosing cholangitis) epithelial cells stimulate the accumulated portal myofibroblasts to initiate collagen deposition around damaged bile ducts. Finally, changes in the composition of the ECM can directly stimulate fibrogenesis. Type IV collagen, fibrinogen, and urokinase-type plasminogen activator stimulate resident HSCs by activating latent cytokines such as TGF-β1 [94]. Fibrillar collagens can bind and stimulate HSCs via discoidin domain receptor DDR2 and integrins. Moreover, the altered ECM can serve as a reservoir for growth factors and MMPs [73].

Molecular mechanisms of liver fibrosis

Liver fibrosis is associated with major alterations in both the quantity and composition of ECM proteins [95]. In advanced stages, the liver contains approximately six times more ECM, including collagens (I, III and IV), fibronectin, undulin, elastin, laminin, hyaluronan, and proteoglycans. Accumulation of ECM results from both increased synthesis and decreased degradation [24]. Decreased activity of ECM-

removing MMPs is mainly due to overexpression of their specific inhibitors (i.e., TIMPs).

Type 1 collagen is a major ECM component in the fibrotic liver. The mechanisms of increased collagen synthesis in liver fibrosis have been described in HSCs. Type 1 collagen synthesis in HSCs is regulated at the transciptional and posttranscriptional levels [96]. In particular, increased collagen mRNA stability plays a key role in the increased collagen synthesis found in activated HSCs. In these cells, posttranscriptional regulation of collagen is governed by sequences in the 3′ untranslated region via the RNA-binding protein αCP2 as well as a stem–loop structure in the 5′ end of collagen mRNA [97]. Mutations that alter the structure of this stem–loop region lead to rapid degradation of collagen mRNA. Interestingly, this region not only influences mRNA stability, but also controls export of α1(I) collagen mRNA from the nucleus to the cytoplasm and the translation of the different fibrillar chains of collagen. These data suggest that blocking the activity of this stem–loop region decreases collagen synthesis by HSCs. In this regard, an RNA-binding protein binds this 5′ stem–loop region in activated but not quiescent HSCs, suggesting that it could play an important role in collagen synthesis by activated HSCs.

Mechanisms of liver fibrosis include oxidative stress, hypoxia, and angiogenesis. Oxidative stress is believed to play a major role in the pathogenesis of liver fibrogenesis [55]. It results from increased ROS production and decreased removal in the fibrotic liver. Several differentially localized and expressed enzymatic systems contribute to ROS formation in the liver, including endothelial nitric oxide synthases, cytochrome P450 monooxygenases, and NAPDH oxidase [98]. In the healthy liver, hepatocytes produce low amounts of ROS and Kupffer cells, the resident macrophages of the liver, are well equipped to release ROS in response to bacterial stimuli. Antioxidant systems such as superoxide dismutase and catalase efficiently remove excess ROS to maintain the normal cell homeostasis. In chronic liver diseases, there is increased ROS production as well as decreased activity of antioxidant systems, resulting in oxidative stress [91]. This feature is commonly detected in patients with alcohol abuse, HCV infection, iron overload, or chronic cholestasis, as well as in most types of experimental liver fibrogenesis [99–101]. In these conditions, oxidative stress is not only a consequence of chronic liver injury but it contributes to excessive tissue remodeling and fibrogenesis. The cellular and molecular mechanisms leading to oxidative stress in chronic liver diseases are under extensive investigation. Evidence has accumulated showing that HSCs contain a nonphagoctyic form of NADPH oxidase. Activation of this enzyme could therefore modulate the fibrogenic actions of these cells. The finding that cytokines produced at the sites of tissue repair (i.e., angiotensin II or PDGF) activate NADPH oxidase in HSCs, enhancing their inflammatory and fibrogenic actions, supports this hypothesis [69,102]. Thus, the NADPH complex has emerged as a novel regulator and potential target in the pathogenesis of hepatic fibrosis.

Hypoxia is a common environmental stress factor associated with various pathological conditions including fibrogenesis. Several lines of evidence indicate that liver parenchyma in chronically injured liver suffers from hypoxia. Hypoxia seems particularly relevant in ALD, where decreased oxygen delivery is commonly found in perivenular areas. Low oxygen tension in cultured HSCs results in increased proliferation and collagen gene expression [103]. Hypoxia can result in angiogenesis, which is also associated with fibrogenesis [104]. Hepatic neovascularization and the expression of vascular endothelial growth factor (VEGF), a potent angiogenic factor, increase in parallel with fibrogenesis. The CD31-immunopositive neovessels are mainly localized along the fibrotic septa. Cytokines promoting angiogenesis include angiotensin II and leptin [105,106].

Several cytokines regulate ECM synthesis and mediate liver fibrogenesis. Among them, TGF-β is considered the most powerful fibrogenic cytokine in the liver [94]. In HSCs, TGF-β favors the transition to myofibroblast-like cells, stimulates the synthesis of ECM proteins, and inhibits its degradation [107,108]. TGF-β is synthesized in a latent form linked to a glycoprotein (latent TGF-β binding protein, LTBP), which serves as an anchor in the ECM. Proteolytic cleavage of LTBP is a prerequisite for the release and generation of bioactive (mature) TGF-β. Binding of active TGF-β to cell surface receptors results in the phosphorylation and activation of the Smad family of intracellular signaling proteins. Phosphorylated Smad2 and Smad3 recruit the common mediator Sma4 and translocate into the nucleus, regulating gene transcription. Several nuclear oncoproteins, such as Ski and SnoN, as well as Smad7, repress the activity of Smad proteins and limit TGF-β's biological actions [109]. Therapies aimed at disrupting TGF-β synthesis or signaling pathways have decreased fibrosis in experimental models [110,111].

Growth factors such as PDGF, EGF, IGF, and FGF amplify the fibrogenic response by inducing growth and migration of resident HSCs [112]. Among them, PDGF is the most potent proliferative cytokine known for HSCs by binding to a specific tyrosine kinase cell surface receptor [8]. Binding of PDGF to its receptor initiates phosphorylation signaling cascades that lead to increased DNA synthesis and cell proliferation [113]. Following HSC activation there is upregulation of PDGF receptors in HSCs, which in turn secrete this potent mitogen. PDGF activates the focal adhesion kinase (FAK)/phosphatidylinositol 3-kinase (PI3K)/Akt (protein kinase B) signaling pathway that transduces mitogenic signals for the cell and which is critical for cellular chemotaxis [114]. PDGF also activates the Ras/Raf/MEK/extracellular signal-regulated kinase (ERK) signaling pathway and the mitogen-activated protein kinase (MAPK)/Jun N-terminal kinase (JNK) signaling pathway, transducing mitogenic signals for the cell [115]. PDGF, as well as other cytokines that

stimulate tyrosine-kinase receptors such as EGF, FGF, or IGF, regulate HSC proliferation. The expression of growth factors, mainly PDGF, is markedly increased in hepatic tissue after both acute and chronic liver injury [116]. Transgenic mice overexpressing PDGF develop liver fibrosis and genetic or pharmacological abrogation of PDGF results in attenuated liver fibrosis in rodents with chronic injury [117].

Angiotensin II is another cytokine that plays an important role in liver fibrogenesis [12]. Angiotensin II is the main effector of the renin–angiotensin system, which is a key mediator of renal or cardiac fibrosis. Following phenotypic activation, HSCs express AT_1 receptors, the activation of which mediates cell contraction, migration, and proliferation [118] (Fig. 27.4). Moreover, angiotensin II stimulates collagen synthesis and TGF-β expression [69]. These effects are blocked by AT_1 receptor antagonists and are blunted in mouse HSCs lacking AT_{1a} receptors [119]. Importantly, activated HSCs are capable of generating mature angiotensin II, suggesting that it can act in an autocrine or paracrine manner [120]. The molecular mechanisms mediating the inflammatory and fibrogenic effects of angiotensin II in HSCs have been partially delineated [69]. Angiotensin II increases intracellular calcium concentration and induces NADPH oxidase-derived ROS, stimulating intracellular pathways such as PI3K/Akt and MAPKs. It increases AP-1 DNA binding, while NF-κB activity is stimulated in rat, but not human, HSCs [121]. Angiotensin II also stimulates the Smad signaling pathway through upregulation of TGF-β1 [122]. The most convincing evidence supporting a role for the renin–angiotensin system in experimental liver fibrosis is the finding that blockade of the generation of angiotensin II or its binding to AT_1 receptors markedly attenuates experimental liver fibrosis. Remarkably, a high number of studies using four different experimental models of liver fibrosis have yielded similar results (Table 27.2) [47,48,123–129]. A role for AT_1 receptors in liver fibrosis is indicated by data showing that mice lacking AT_{1a} receptors are protected against liver fibrosis after prolonged bile duct ligation [119]. Besides angiotensin II, other vasoactive peptides play a role in liver fibrogenesis. Vasoconstrictors such as endothelin-1 and norepinephrine usually exert fibrogenic effects on activated HSCs and participate in experimental liver fibrogenesis [130–133]. In contrast, vasodilator substances such as adrenomedullin, prostaglandin $(PG)E_2$ and nitric oxide inhibit proliferation and collagen synthesis in HSCs and attenuate fibrogenesis [134,135]. In advanced liver fibrosis, there is a predominance of vasoconstrictors over vasodilators. Whether this unbalanced expression contributes to the progression of liver fibrosis remains unclear.

Adipokines have been implicated in the pathogenesis of liver fibrosis [136]. The adipose tissue, previously considered a passive storage site for excess energy, is now recognized as a hormonally active system, producing numerous molecules, known as adipokines, that exert local, central and peripheral

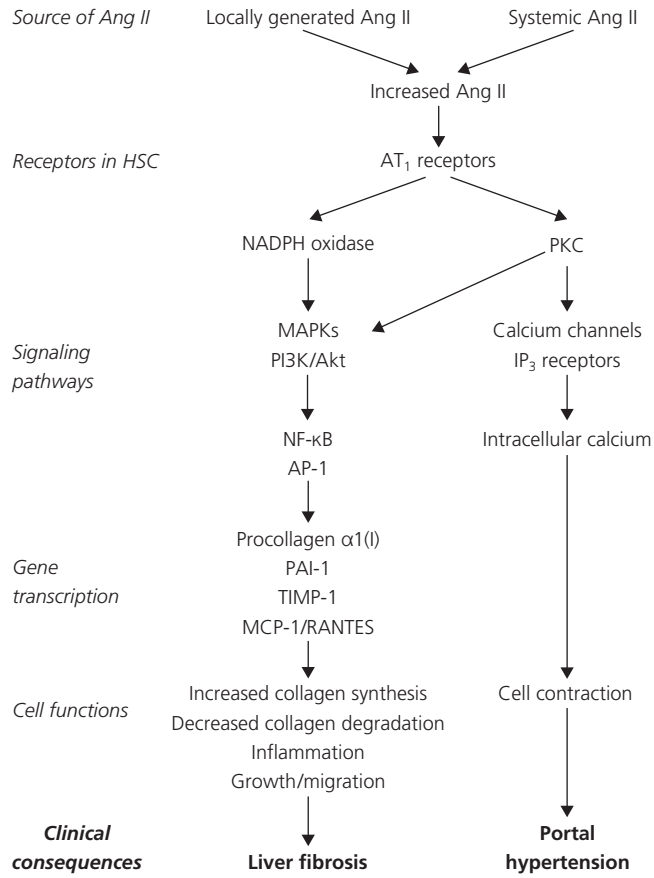

Figure 27.4 Mechanisms of the pathogenic effect of the renin–angiotensin system in the liver. Increased angiotensin II (Ang II) binds to type 1 angiotensin receptors (AT_1) located in activated hepatic stellate cells (HSCs). AT_1 receptors activate a nonphagocytic NADPH oxidase to generate reactive oxygen species that stimulate redox-sensitive intracellular pathways. Increased gene transcription leads to mitogenic, fibrogenic, and inflammatory properties, promoting fibrogenesis. Ang II increases intracellular calcium and induces cell contraction, increasing intrahepatic vascular resistance and participating in the pathogenesis of portal hypertension. AP-1, activating protein-1; IP_3, inositol 1,4,5-trisphosphate; MAPK, mitogen-activated protein kinase; MCP-1, monocyte chemotactic protein type 1; NF-κB, nuclear factor κB; PAI-1, plasminogen activator inhibitor type 1; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; RANTES, regulated on activation normal T-cell expressed and secreted; TIMP-1, tissue inhibitor of metalloproteinases type 1.

actions [137]. Among adipokines, leptin has received special attention. Leptin is produced by HSCs and acts on HSCs and endothelial cells inducing inflammatory and fibrogenic events [106,138,139]. This effect includes increased collagen synthesis and decreased expression of matrix-degrading enzymes such as MMP-1 and protection from apoptotic cell death by increased expression of TIMP-1 [140]. Rodents lacking either leptin or leptin receptor do not develop liver fibrosis after repeated injury [139,141]. These results clearly indicate that leptin mediates liver fibrogenesis. Adiponectin is an adipokine with opposite results. It is produced locally in

Table 27.2 Studies assessing the role of the renin–angiotensin system (RAS) in liver fibrosis in rats

Reference	RAS inhibitor	Experimental model	Proposed mechanism
Ramos et al. [231]	Captopril	Pig serum	Decreased mast cell accumulation
Ohishi et al. [232]	Lisinopril	CCl_4	Decreased stimulation of HSCs Decreased TGF-β expression
Jonsson et al. [233]	Captopril	BDL	Decreased TGF-β expression Regulation MMPs/TIMPs
Yoshiji et al. [125]	Candesartan Perindopril	Pig serum	Reduced αSMA-positive cells Decreased TGF-β expression
Paizis et al. [47]	Irbesartan	BDL	Decreased TGF-β expression AT_1 down-regulation
Wei et al. [128]	Losartan	CCl_4	Reduced αSMA-positive cells Decreased TGF-β expression
Yoshiji et al. [105]	Perindopril	Dimethylnitrosamine	Reduced αSMA-positive cells
Ramalho et al. [124]	Losartan	BDL	Reduced αSMA-positive cells
Croquet et al. [123]	Losartan	CCl_4	
Yoshiji et al. [126]	Perindopril Candesartan	CCl_4 Pig serum	Decreased TIMP-1 expression
Li et al. [234]	Perindopril	CCl_4	Decreased MMP-2, MMP-9 expression Decreased TGF-β, NF-κB
Ueki et al. [235]	Candesartan	BDL	Decreased CTGF expression Decreased TGF-β expression
Yoshiji et al. [126]	Perindopril Candesartan	CCl_4 Pig serum	Decreased TIMP-1 expression
Li et al. [234]	Perindopril Candesartan	CCl_4	Decreased MCP-1 expression Decreased TGF-β expression
Xu et al. [236]	Perindopril Valsartan	CCl_4	Decreased Smad3 expression
Yoshiji et al. [62]	Candesartan	CDD	Decreased VEGF
Kurikawa et al. [237]	Olmesartan	BDL	Reduced αSMA-positive cells Decreased TGF-β expression Decreased CTGF expression Effect in HSCs
Tuncer et al. [127]	Candesartan Captopril	CCl_4	Reduced αSMA-positive cells

BDL, bile duct ligation; CCl_4, carbon tetrachloride; CDD, choline-deficient diet; CTGF, connective tissue growth factor; HSC, hepatic stellate cell; MCP-1, monocyte chemotactic protein type 1; MMP, matrix metalloproteinase; αSMA, α-smooth muscle actin; TGF, transforming growth factor; TIMP-1, tissue inhibitor of metalloproteinases type 1; VEGF, vascular endothelial growth factor.

the liver and inhibits liver fibrosis both in vitro and in vivo [142]. In patients with NASH, the leptin/adiponectin ratio increases, suggesting that these cytokines may play a role in the development of liver fibrosis (Fig. 27.5). Finally, a recent report indicates that resistin is overexpressed in human fibrotic livers and exerts inflammatory actions on HSCs [143].

Data on intracellular pathways regulating liver fibrogenesis is mainly derived from studies using cultured HSCs, while their role in vivo is progressing through experimental fibrogenesis studies using knockout mice [60]. Several MAPKs modulate major fibrogenic actions of HSCs. ERK, which is stimulated in experimentally induced liver injury, mediates proliferation and migration of HSCs [115]. In contrast, JNK regulates apoptosis of hepatocytes as well as the secretion of inflammatory cytokines by cultured HSCs [144]. The FAK/PI3K/Akt signaling pathway mediates agonist-induced fibrogenic actions in HSCs [115]. TGF-β1-induced Smad signaling pathway stimulates experimental hepatic fibrosis and is a potential target for therapy [109,111]. The peroxisome proliferator activated receptor (PPAR) pathway regulates HSC activation and experimental liver fibrosis. PPAR-γ ligands inhibit the fibrogenic actions in HSCs and attenuate liver fibrosis in vivo [145]. NF-κB may have both a profibrogenic and inhibitory action on liver fibrosis [146]. Other transcription factors are involved in HSC activation and may participate in liver fibrogenesis [147]. Other studies have suggested a role for intracellular pathways signaled by Toll-like receptors and β-cathepsin [148].

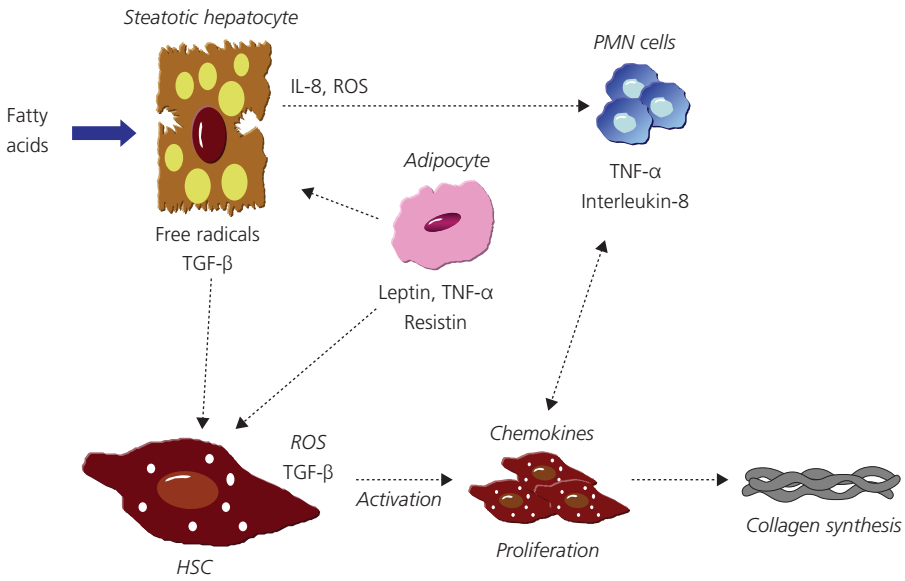

Figure 27.5 Pathogenesis of liver fibrosis in patients with nonalcoholic steatohepatitis. Steatotic hepatocytes take up circulating fatty acids and release interleukin (IL)-8 and reactive oxygen species (ROS) that contribute to recruitment of inflammatory cells. Adipocytes release adipokines and induce fibrogenic actions in hepatic stellate cells (HSC), which in turn interact with inflammatory cells. Accumulation of HSCs results in collagen deposition. PMN, polymorphonuclear; TGF, transforming growth factor; TNF, tumor necrosis factor.

Fibrosis remodeling and resolution

In contrast with the traditional view that cirrhosis is an irreversible disease, recent evidence indicates that even advanced fibrosis is reversible [149]. In experimentally induced fibrosis, cessation of liver injury results in fibrosis regression [150]. In humans, spontaneous resolution of liver fibrosis can occur after successful treatment of the underlying disease. This observation has been described in patients with iron and copper overload, alcohol-induced liver injury, chronic HCV, HBV and HDV infection, hemochromatosis, secondary biliary cirrhosis, NASH, and autoimmune hepatitis [2]. The time to achieve significant regression may take years and varies depending on the underlying cause and the severity of the liver disease. Chronic HCV infection is the most extensively studied condition; successful therapy with interferon (IFN)-α plus ribavirin leads to viral clearance and results in fibrosis improvement. Importantly, nearly half of patients with cirrhosis reverse to a significant degree [151]. Whether this beneficial effect is associated with improvements in long-term clinical outcome, including decreased portal hypertension, is unknown.

Increased collagenolytic activity is a major mechanism of fibrosis resolution [149]. Fibrillar collagens (I and III) are degraded by interstitial MMPs (MMP-1, MMP-8 and MMP-13 in humans, MMP-13 in rodents). During fibrosis resolution, MMP activity increases due to a rapid decrease in the expression of TIMP-1. Partial degradation of fibrillar collagen occurs, and the altered interaction between activated HSCs and ECM favors apoptosis [150]. Removal of activated HSCs by apoptosis precedes fibrosis resolution. Stimulation of death receptors in activated HSCs and a decrease in survival factors, including TIMP-1, can precipitate HSC apoptosis [66].

Activated HSCs are the main hepatic cell type implicated in collagen degradation in fibrotic liver [152]. Following cell activation, HSCs express virtually all the key components required for matrix degradation. In particular, they are a key source of MMP-2, as well as stromelysin/MMP-3. Importantly, through the activation of TIMP-1 and TIMP-2, activated HSCs can also inhibit the activity of interstitial collagenases, which degrade fibrillar collagen, thus favoring the accumulation of ECM [153]. This hypothesis is supported by studies of experimental liver fibrosis and in human liver disease, in which TIMP-1 expression is markedly upregulated in cirrhotic compared with normal liver, whereas expression of collagenases remains unchanged [154]. Moreover, transgenic mice overexpressing TIMP-1 rapidly develop liver fibrosis following injury [117]. Administration of TIMP-1 antibodies to rats with established fibrosis partially removes collagen from the liver, suggesting that this approach may be useful in patients with chronic liver diseases [155].

Genetic determinants of liver fibrosis

Hepatic fibrosis is a condition in which multiple genes interact with environmental factors [2] (Fig. 27.6). Well-characterized causative agents of liver fibrosis include chronic HCV infection and alcohol abuse. However, not all patients exposed to a similar causative agent develop the same degree of liver fibrosis. For example, while viral factors (i.e., genotype, viral load) do not influence fibrosis progression in patients with HCV, host factors (i.e., age, gender) seem to play an important role [13]. Large-scale studies have allowed the identification of patients with rapid fibrosis progression per unit time (*rapid fibrosers*) and those with slow fibrosis progression (*slow fibrosers*) [156]. The genetic deter-

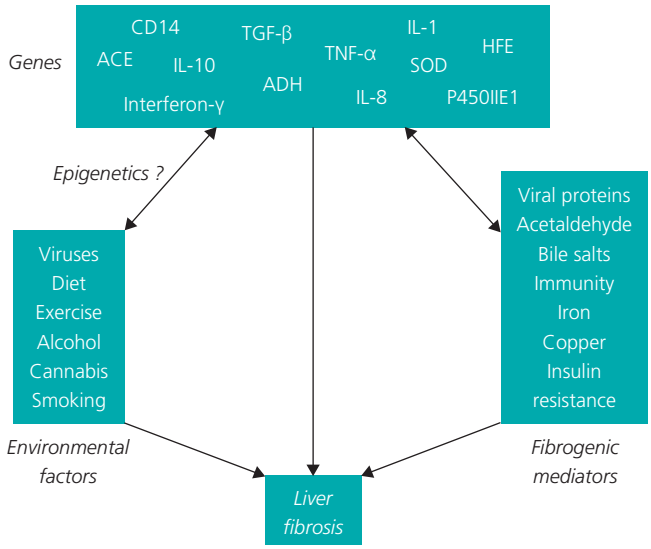

Figure 27.6 Liver fibrosis is the consequence of the interaction between multiple genes, fibrogenic mediators, and environmental factors. ACE, angiotensin-converting enzyme; ADH, alcohol dehydrogenase; IL, interleukin; SOD, superoxide dismutase; TGF, transforming growth factor; TNF, tumor necrosis factor.

minants involved in these different individual behaviors are largely unknown, but candidate genes are currently under evaluation. Although association studies have yielded promising results, many of the candidate genes identified in animal models have not yet been tested in humans. A rational approach is to identify candidate genes in experimental studies and then to carry out association studies to investigate whether functional polymorphisms of these genes influence fibrosis progression in patients.

During the last few years, numerous association studies have investigated the role of gene polymorphisms in the progression of liver fibrosis or development of cirrhosis in patients with different types of chronic liver disease. Gene variations influencing the course of prevalent liver diseases have been identified (Table 27.3). However, studies examining the role of mutations in genes encoding alcohol-metabolizing enzymes and of *HFE* C282Y heterozygosity in the hemochromatosis gene have yielded divergent results. Common methodological limitations of genetic epidemiological studies include low sample size, leading to lack of statistical power, and no investigation of gene–environment interactions.

The role of genetic variations in patients with ALD has been widely studied. ALD results from a complex interaction between behavioral, environmental, and genetic factors [157]. Although a positive correlation between cumulative alcohol intake and degree of liver injury has been reported, extensive individual variability exists. For similar ethanol consumption, some subjects only develop macrovesiculovacuolar steatosis, while others develop cirrhosis. Genes encoding alcohol-metabolizing enzymes and proteins involved in liver toxicity such as antioxidants and proinflammatory

Table 27.3 Main genes with polymorphisms that influence the progression of liver fibrosis in patients with chronic liver diseases

Gene	Liver disease
Interleukin-10	ALD/HCV
Transforming growth factor-β1	HCV/NASH
Interleukin-1β	ALD/PBC/HBV
Interleukin-1 receptor	ALD/PBC
Angiotensinogen	HCV/NASH/HBV
Mannose-binding lectin	HCV
Haptoglobin	HCV
Mannan-binding lectin	HCV
MxA	HCV
Transporter associated with antigen processing (TAP)2	HCV/ALD
Complement C5a	HCV
DEAD box polypeptide 5	HCV
Palmitoyltransferase 1A	HCV
Alcohol dehydrogenase	ALD
Aldehyde dehydrogenase	ALD
CYP2E1	ALD
HLA-II haplotypes	HCV/PBC/AIH
Tumor necrosis factor-α	HCV/PBC/ALD/HBV
Microsomal epoxide hydrolase	HCV
HFE	HCV/NASH
CD14	ALD
Cytotoxic T-lymphocyte antigen-4	ALD
Manganese superoxide dismutase	ALD
Apolipoprotein E	HCV

AIH, autoimmune hepatitis; ALD, alcoholic liver disease; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, nonalcoholic steatohepatitis; PBC, primary biliary cirrhosis.

cytokines have been the main subjects of investigation in genetic studies. The main enzymes involved in alcohol metabolism in humans are alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), and cytochrome P450IIE1 (CYP2E1). It has been recognized for many years that genetic factors influence the activity of these enzymes and the rate of alcohol metabolism. Because of its fibrogenic potential, variations in the generation of acetaldehyde may explain individual differences after abusive alcohol consumption. The majority of studies have been performed in Asia, where the prevalence of polymorphisms of these enzymes is very high. Although it is well accepted that genetic polymorphisms in *ADH2*, *ADH3*, *ALDH2* and in the 5′-flanking region of *CYP2E1* are involved in individual susceptibility to alcoholism [158], their role in the progression of ALD remains controversial. Some studies indicate that the c2/c2, c2/c1 and c3/c2 alleles of the *ADH2* gene and the c2/c2 allele of *CYP2E1* gene, which result in increased enzymatic activity and acetaldehyde production, predispose to more advanced ALD [159]. However, these results have not been confirmed in other investigations [160]. Polymorphisms in genes encoding proinflammatory

cytokines known to participate in the pathogenesis of ALD have also been investigated. A single nucleotide polymorphism (SNP) located in the promoter region of the tumor necrosis factor (TNF)-α gene, which results in increased cytokine production, is associated with more severe steatohepatitis [161]. Polymorphisms in interleukin (IL)-1β and IL-1 receptor antagonist genes have been related with inherited susceptibility to ALD [162]. Similarly, patients with the *A* allele of the IL-10 gene and cytotoxic T-lymphocyte antigen (CTLA)-4 are more prone to develop severe fibrosis, suggesting an important role for the immune system in progression of ALD [163]. Another gene implicated in the pathogenesis of ALD is CD14, which is a cell receptor for lipopolysaccharide. Both clinical and experimental data indicate that activation of Kupffer cells and HSCs by gut-derived endotoxin is an important mediator in liver injury. An SNP in the promoter region of the CD14 gene that confers increased protein synthesis is a risk factor for developing alcoholic cirrhosis [164]. Finally, genetic variations of superoxide dismutase, a powerful antioxidant, are a risk factor for developing severe ALD [165]. This is consistent with a major role for oxidative stress in the hepatic toxicity of ethanol and may support the use of antioxidants in these patients.

PBC is a heterogeneous disease. Some patients have persistent minor histological changes, while others progress to cirrhosis. The current hypothesis of the pathogenesis of PBC is that an initial environmental factor (i.e., infection by *Chlamydia pneumoniae*) triggers an immunological reaction against bile duct epithelial cells [166]. T lymphocytes and cytokine secretion mediate persistent bile duct damage. Accumulation of bile salts and inflammatory cells induce activation of HSCs and progressive fibrosis. Genes encoding human leukocyte antigen class II (HLA-II) haplotypes and proinflammatory cytokines can influence individual susceptibility to develop chronic biliary damage. Twin and family studies strongly suggest a genetic predisposition to PBC [167]. Variations in immunoregulatory genes such as HLA-II alleles, proinflammatory cytokines (TNF-α, IL-1β), CTLA-4, and vitamin D receptor influence disease susceptibility [168]. Moreover, genetic polymorphisms may influence disease progression. HLA-II alleles (DRB1*0801, DQA1*0401, DQB1*0402) and polymorphisms in IL-1β, interleukin receptor antagonist, and TNF-α genes are associated with faster disease progression, although these results have not been confirmed by other studies [169]. These data indicate that a complex interplay between genes encoding immunnoregulatory proteins regulates disease progression. Interestingly, some alleles of the apolipoprotein E gene influence response to the standard therapy, ursodeoxycholic acid [170], suggesting that genetic polymorphisms may predict therapeutic response. Less is known about genetic variants influencing disease progression in PSC, although HLA-II alleles and polymorphisms in TNF-α and stromelysin genes can affect disease susceptibility [171]. Finally, other results have suggested that gender, HLA-II alleles, and polymorphisms in genes encoding proinflammatory cytokines can influence both susceptibility and disease progression in type 1 autoimmune hepatitis [172].

Many studies have analyzed the factors influencing fibrosis progression in patients with chronic HCV infection. The natural history of patients with chronic HCV is characterized by a slow progression of liver fibrosis [13]. Following infection, cirrhosis may develop after an average of 20–30 years. In some patients, the rate of fibrosis progression is much faster, and cirrhosis develops after 10–15 years, whereas in others the rate of progression is negligible. The pathogenesis of HCV-induced liver fibrosis is poorly known, partly due to the lack of a rodent model of persistent HCV infection. HCV escapes surveillance of the HLA-II-directed immune response and infects hepatocytes, causing oxidative stress and inducing the recruitment of inflammatory cells [173]. Both factors lead to HSC activation and collagen deposition. Moreover, HCV proteins (i.e., core protein) stimulate secretion of profibrogenic cytokines by hepatocytes and can directly induce fibrogenic actions in HSCs [174]. Although factors such as duration of infection, gender and alcohol consumption influence fibrosis progression, genetic factors may also play a role. Genetic variations are involved in susceptibility to persistent HCV infection, response to antiviral therapy, and progression of liver disease. Different HLA-II alleles and polymorphisms of genes involved in the immune response (manose-binding lectin, haptoglobin, mannan-binding lectin, solute carrier family 11 member 1) influence susceptibility and resistance to persistent HCV infection [175,176]. Similarly, variations in IL-10 and MxA, an interferon-induced protein, can determine individual response to interferon therapy [177]. Variations in genes involved in the immune response to HCV infection seem to influence disease progression. The TAP2 (transporter associated with antigen processing 2) *0103 allele, which predisposes to stronger antiviral activity and low viral load, is associated with slower disease progression in HCV-infected patients [178]. Variations in mannose-binding lectin and specific HLA-II alleles (DRB1*0405, DQB1*0401), which are involved in host defense against viral infections, and complement C5a also influence cirrhosis development [179]. The role of polymorphisms in proinflammatory cytokines is unclear. While one report indicates that the *TNF2* allele is more frequently found in patients with cirrhosis than in those with less severe liver disease [180], other studies have not confirmed these results. Variations in genes encoding key cytokines involved in human fibrogenesis may regulate fibrosis progression. These cytokines include angiotensin II and TGF-β, which play an important role in experimental liver fibrosis. Polymorphisms in the angiotensinogen gene, the angiotensin II precursor, as well as the TGF-β1 gene are major determinants in fibrosis progression in a series of patients with chronic HCV [181]. Interestingly, patients having mutations in both genes pro-

gress more rapidly than those having only one polymorphism. Similarly, variations of metalloproteinases also influence fibrosis progression [182]. The role of genetic variations of the *HFE* gene is controversial. The available studies investigating the role of C282Y heterozygosity have yielded contradictory results [14]. A few studies have attempted to generate prognostic genetic chips including several polymorphisms. Richardson and colleagues described a six-gene polymorphism panel able to predict fibrosis progression in these patients [183]. Another study that included a large number of patients in whom whole gene analysis was performed identified DEAD box polypeptide 5 and carnitine palmitoyltransferase 1A as major genetic determinants of fibrosis progression [184]. In patients with chronic HBV infection, polymorphisms in the IL-1, TNF-α and angiotensinogen genes influence fibrosis progression [185–187].

Little is known about factors influencing fibrosis progression in patients with NASH, a common cause of chronic liver disease [39]. Obesity, type 2 diabetes mellitus, and dyslipidemia are the most common conditions associated with NASH. At the time of diagnosis, almost one-third of the patients show established fibrosis. However, only a small porportion of these patients progress to liver cirrhosis. Potential fibrogenic factors include oxidative stress, iron accumulation, and hyperglycemia. Genetic variations can explain susceptibility to develop NASH in patients with obesity or diabetes [188]. Genetic factors influencing fibrosis progression in these patients are under investigation. While one study has shown that *HFE* C282Y heterozygosity is associated with faster fibrosis progression [189], other studies have not confirmed these results [190]. In morbidly obese patients, genetic variations in ther angiotensinogen and TGF-β1 genes influence fibrosis development [191].

In patients with hereditary hemochromatosis, additional gene variations such as a polymorphism in the myeloperoxidase promoter can accelerate the progression of fibrosis [192].

Pathogenesis of liver fibrosis/cirrhosis

Advanced fibrosis distorts the hepatic architecture by forming a fibrous scar and the subsequent development of nodules of regenerating hepatocytes defines cirrhosis (Fig. 27.7). While hepatic fibrosis is potentially reversible, established cirrhosis is usually an irreversible process [193]. Established cirrhosis results when bridging fibrosis is eventually accompanied by regenerative nodules. In early phases, incomplete septal cirrhosis can be found. It is characterized by the presence of very slender septa radiating from enlarged fields toward the center of the lobule. There are distended efferent vessels around the septum. This type of cirrhosis produces only portal hypertension and no liver failure is usually observed. The prognosis of these patients is acceptable if the portal hypertension is controlled. If the cause of liver injury (e.g., HCV infection, alcohol abuse) is not removed, the hepatic wound healing response to injury progresses and early cirrhosis develops. At this stage of the liver disease, thin fibrous septa with dissecting nodules are present. As liver damage progresses, wide scars containing clusters of regenerative hepatocytes are common and advanced cirrhosis develops. This stage of the disease is characterized by the accumulation of abundant fibrillar collagen, which is resistant to the collagenolytic actions of MMPs. Moreover, advanced cirrhosis comprises major changes in hepatic microcirculation, endothelial integrity and function, and abnormal hepatocyte organization. In this phase of the disease, cirrhosis is basically irreversible regardless of cessation of the causative agent.

Formation of nodules is the hallmark of advanced cirrhosis. Nodules are divided into dissection and regenerative nodules. Dissection nodules contain remnants of portal tracts and central veins. They contain thin fibrous septa as well as dilated sinusoids especially at their periphery that look like multiple central veins produced by the inflow of arterial blood coming from the surrounding wide scars. Regenerative nodules arise in the midst of scars favored by the rich arterial blood of scar tissue. They are round nodules with a fibrous pseudocapsule with bile ductules due to obstruction of bile flow. Because of their size, they compress the vessels of the capsule, contributing to the perpetuation of the cirrhosis. Importantly, regenerative nodules may undergo dysplastic and malignant changes.

According to the degree of fibrosis and the type of nodule, cirrhosis can be classified into different progressive stages: incomplete septal cirrhosis (incomplete bridging fibrosis, no nodules), early cirrhosis (thin bridging fibrosis with dissecting nodules), moderately advanced cirrhosis (thick bridging fibrosis with dissecting nodules), and advanced cirrhosis (wide septa with regenerative hyperplastic nodules). Histopathologically, advanced cirrhosis can be divided into micronodular and macronodular varieties. Micronodular cirrhosis is characterized by uniformly small nodules (< 3 mm in diameter) and regular bands of connective tissue. Typically, nodules lack portal organization; terminal (central) hepatic venules or portal tracts are difficult to identify. Macronodular cirrhosis is characterized by nodules that vary in size (3 mm to 5 cm in diameter) and which contain some normal lobular structure (portal tracts, terminal hepatic venules). Broad fibrous bands of varying thickness surround the large nodules. Collapse of the normal liver architecture is suggested by the concentration of portal tracts within the fibrous scars. Mixed cirrhosis (incomplete septal cirrhosis) combines elements of micronodular and macronodular cirrhosis. Regeneration in micronodular cirrhosis can result in macronodular or mixed cirrhosis. Conversion from micronodular to macronodular cirrhosis takes more than 2 years.

Vascular changes play a major role in the pathogenesis of advanced cirrhosis [194]. Complete septa may link central veins to central veins, creating anastomoses between

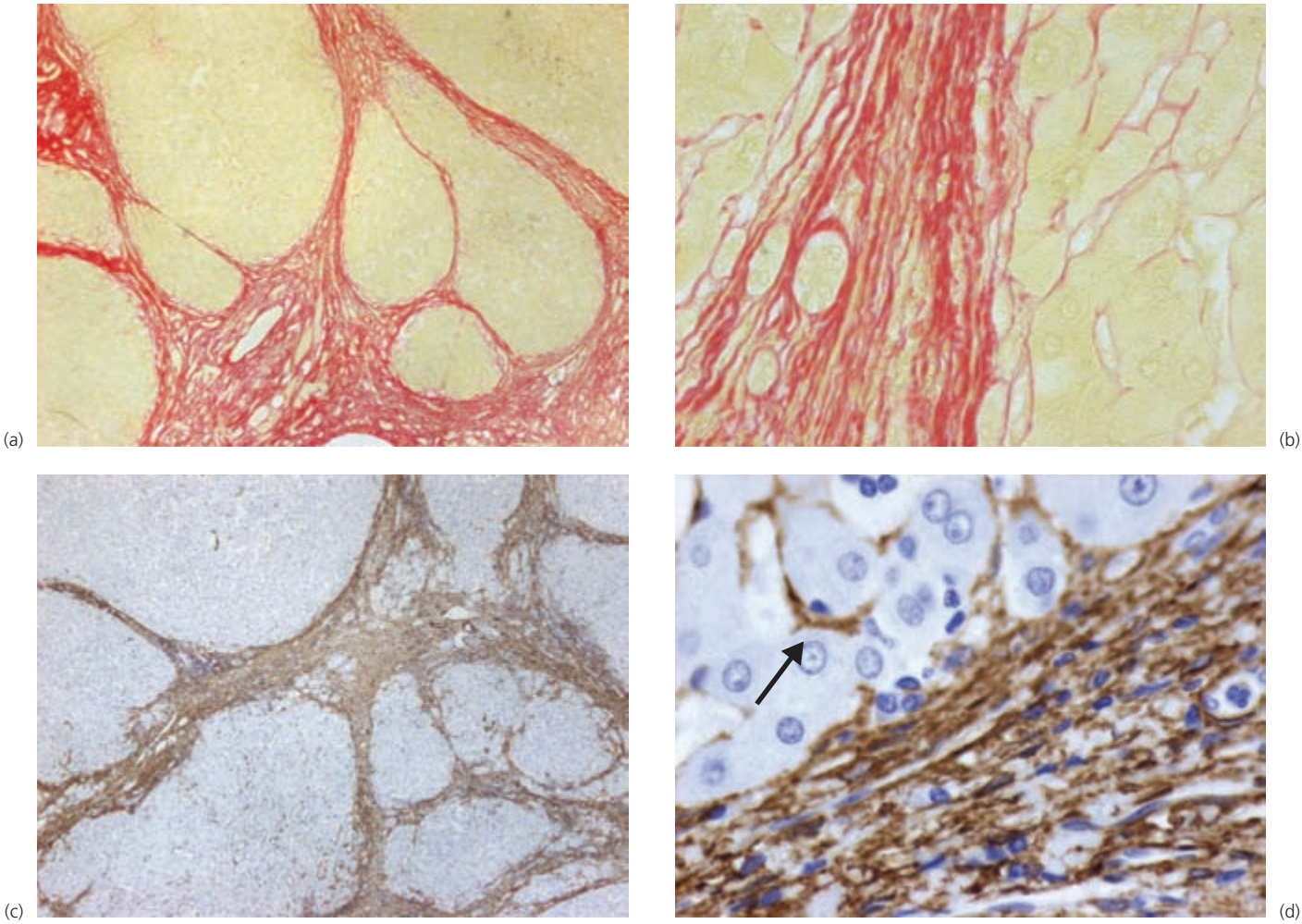

Figure 27.7 Histological appearance of liver cirrhosis in a patient with chronic hepatitis C virus infection. **(a,b)** Sirius red staining reveals bridging fibrosis with the development of regenerative nodules (magnification × 200). **(c,d)** Accumulation of myofibroblastic fibrogenic cells in the fibrous septa, as assessed by positive staining for smooth muscle α-actin cells. Most fibrogenic cells derive from perisinusoidal stellate cells (arrow) (magnification × 400).

draining vessels. Septa linking adjacent portal tracts create vascular anastomoses between afferent vessels of the portal tracts involved. Vascular structures in central–central and portal–portal septa are not the major determinants of a detrimental change in intrahepatic circulation. The key phenomenon in the emergence of a truly cirrhotic state is the development of fibrous vascularized septa linking portal tracts and central veins. Therefore portal–central bridging fibrosis creates direct anastomoses between the afferent (hepatic artery, portal vein) and efferent (centrolobular veins) vessels of the liver, allowing a fraction of the blood to bypass the lobular parenchyma, without functionally contacting a metabolically active parenchyma. In advanced cirrhosis, most of the hepatic blood supply appears to pass through the liver via these channels. Further vascular changes in developing and established liver cirrhosis are due to vascular thrombosis. Thrombosis of medium and large portal veins and hepatic veins is a common occurrence in cirrhosis, and these lesions are important in causing progression of cirrhosis. Investigations on neoangiogenesis in cirrhosis have focused attention on hypoxia of liver tissue. Hypoxia may result from several mechanisms: impairment in sinusoidal permeability and perfusion, intrahepatic shunts, vasoconstriction and thrombosis and capillarization of sinusoids. Liver tissue hypoxia aggravates fibrosis progression, so that fibrosis and hypoxia may aggravate each other in the presence of persistent parenchymal injury, leading to a vicious circle, which disrupts normal tissue repair and thereby promotes the development and progression of cirrhosis.

Pathophysiologically oriented antifibrotic therapies

There is no gold-standard treatment for liver fibrosis. Experimental studies have revealed targets to prevent fibrosis pro-

Table 27.4 Main antifibrotic drugs to treat liver fibrosis

Agent	Antifibrotic effects in hepatic stellate cells	Antifibrotic effect in experimental fibrosis	Antifibrotic effect in humans
Angiotensin inhibitors	Consistent positive data	Consistent positive data	Retrospective study
Colchicine	Not tested	Limited data	Discrepant results
Corticosteroids	Limited data	Limited data	Effective in autoimmune hepatitis
Endothelin inhibitors	Not tested	Limited data	Not tested
Interferon-α	Consistent positive data	Consistent positive data	Effective in chronic hepatitis C
Interleukin-10	Limited data	Consistent positive data	Isolated reports in chronic hepatitis C
Pentoxifylline	Consistent positive data	Consistent positive data	Not tested
Phosphatidylcholine	Limited data	Consistent positive data	Not proven in alcohol-induced fibrosis
PPAR antagonists	Consistent data	Consistent positive data	Isolated reports in NASH
S-Adenosyl-L-methionine	Not tested	Not tested	Effective in alcohol-induced fibrosis
Sho-saiko-to (Asiatic herb)	Consistent positive data	Consistent positive data	Isolated reports in chronic hepatitis C
TGF-β inhibitors	Consistent positive data	Consistent positive data	Not proven
Tocopherol	Consistent positive data	Limited data	Isolated reports in NASH

NASH, nonalcoholic steatohepatitis; PPAR, peroxisome proliferator activated receptor; TGF, transforming growth factor.

gression in rodents [11] (Table 27.4). However, the efficacy of most treatments has not been tested in humans. This is because of the need to perform serial liver biopsies to accurately assess changes in liver fibrosis and the requirement of long follow-up studies. The development of reliable noninvasive markers of liver fibrosis will positively impact the design of clinical trials. The ideal antifibrotic therapy would be the one that is liver-specific, well tolerated when administered for prolonged periods of time, and effective in attenuating excessive collagen deposition without affecting normal ECM synthesis.

Removal of the causative agent is the most effective maneuver in the treatment of liver fibrosis. This strategy has been shown effective in most etiologies of chronic liver diseases [10,19,151,195,196]. For patients with cirrhosis and clinical complications, liver transplantation is currently the only curative approach [197,198]. In randomized clinical trials using IFN-α plus ribavirin in patients with chronic HCV infection, persistent clearance of viral infection is associated not only with resolution of hepatic inflammation but also with improvement in liver fibrosis [151]. Moreover, long-term treatment with IFN-α is commonly associated with improvement of liver fibrosis, including in patients with cirrhosis [199]. These findings raise the possibility that IFN-α has an intrinsic antifibrotic effect independently of its antiviral effect. This hypothesis is supported by experimental models in which IFN-α inhibits the fibrogenic action of HSCs and prevents the development of liver fibrosis [46].

Because inflammation precedes and promotes the progression of liver fibrosis, antiinflammatory drugs have been proposed. Corticosteroids are only indicated for the treatment of hepatic fibrosis in patients with autoimmune hepatitis and acute alcoholic hepatitis [195]. Inhibition of the accumulation of activated HSCs by either inhibiting their activation/proliferation or promoting their apoptosis is another strategy. Antioxidants such as vitamin E, silymarin, phosphatidylcholine, and *S*-adenosyl-L-methionine inhibit HSC activation, protect hepatocytes from undergoing apoptosis, and attenuate experimental liver fibrosis [56,200,201]. Antioxidants exert beneficial effects in patients with alcohol-induced liver disease and NASH [202].

Disrupting TGF-β1 synthesis or signaling pathways prevents scar formation in experimental liver fibrosis [94]. This strategy includes inhibition of key members of the TGF-β superfamily such as bone morphogenetic protein-7 [203]. Moreover, administration of growth factors (e.g. IGF, hepatocyte growth factor and cardiotrophin) or their delivery by gene therapy attenuates experimental liver fibrosis [204,205]. Inhibition of growth factors involved in liver fibrogenesis (i.e., PDGF-BB) is also useful to attenuate liver fibrosis in rats [206]. However, these approaches have not been tested in humans and may favor cancer development.

Substances that inhibit key signal transduction pathways involved in liver fibrogenesis have also potential to treat liver fibrosis [11]. They include pentoxifylline (phosphodiesterase inhibitor), amiloride (Na^+/H^+ pump inhibitor), and *S*-farnesylthiosalicylic acid (Ras antagonist). Stimulation of pathways with antifibrogenic actions (e.g., STAT1) can also reduce collagen deposition [207]. Ligands of PPAR-α or PPAR-γ such as thiazolidinediones exert beneficial effects in experimental liver fibrosis and in patients with NASH [208,209]. Drugs that interfere with PI3K/Akt pathways, such as rapamycin, have been shown to inhibit fibrosis in rats [210]. The usefulness of rapamycin in humans deserves further investigation. Pharmacological manipulation of nuclear factors can also modulate liver fibrosis in vivo. For example, stimulation

of the nuclear pregnane receptor inhibits liver fibrosis in rats [211,212].

Promoting apoptosis of activated HSCs is an alternative approach to the treatment of liver fibrosis. Substances such as gliotoxin induce apoptosis of cultured cells and remove activated HSCs from fibrotic livers [213–215]. These effects can also be achieved by inhibiting antiapoptotic factors in HSCs such as κB kinases or by using proteasome inhibitors [216,217]. Another substance that causes death of HSCs is anandamide [218]. Anandamide is an endogenous cannabinoid and belongs to a family that has been shown to modulate hepatic fibrogenesis both in vitro and in vivo. Endocannabinoids bind to CB1 and CB2 receptors, which exert powerful profibrogenic and antifibrogenic effects, respectively [219,220].

Inhibition of the renin–angiotensin system is probably the most promising strategy for treating liver fibrosis [12]. There is overwhelming experimental data indicating that pharmacological or genetic inhibition of this system attenuates liver fibrogenesis in rodents. Renin–angiotensin inhibitors are widely used as antifibrotic agents in patients with chronic renal and cardiac diseases and appear to be safe when administered for prolonged periods of time [221]. Little information is available on liver fibrosis. Transplanted patients receiving renin–angiotensin inhibitors as antihypertensive therapy show less fibrosis progression than patients receiving other types of drugs [222]. Moreover, AT_1 receptor blockers reduce liver fibrosis in patients with NASH [223]. Large prospective clinical trials should be performed to confirm these promising data. Other vasoactive substances are potential targets to treat liver fibrosis. Endothelin-1 type A receptor blockers and vasodilators (PGE_2 and nitric oxide donors) exert antifibrotic activity in rodents, yet the effects in humans are unknown [131]. Cyclooxygenase (COX)-2, a key enzyme in the generation of vasodilatory prostaglandins, is a novel target to treat liver fibrosis. However, COX-2 inhibition has yielded contradictory results, probably due to differences in specificity between the inhibitors used. In particular, COX-2 inhibitors with intrinsic PPAR-γ activity have net antifibrotic effect [224]. Finally, the mineralocorticoid receptor antagonist spironolactone also suppresses liver fibrogenesis in rodents [225].

Different herbal compounds, many of them traditionally used in Asian countries to treat liver diseases, have been demonstrated to have antifibrotic effects [11]. They include sho-saiko-to, glycyrrhizin, and savia miltiorhiza. An alternative approach is to inhibit collagen production or promote its degradation [226]. Inhibitors of prolyl-4 hydoxylase and halofuginone prevent the development of experimental liver cirrhosis by inhibiting collagen synthesis. MMP-8 and urokinase-type plasminogen activator stimulate collagen degradation in vivo [227]. Antibodies that block tissue inhibitors of metalloproteinases such as TIMP-1 reverse established liver fibrosis, suggesting that they may be useful for patients with advanced liver diseases [155]. The efficacy of these drugs in humans is unknown and may result in undesirable side effects.

A limitation of the current antifibrotic approaches is that antifibrotic drugs are not efficiently taken up by activated HSCs or may produce unwanted side effects. Cell-specific delivery to HSCs could provide a solution to these problems. Promising preliminary results have been obtained using different carriers (e.g., cyclic peptides coupled to HAS recognizing collagen type VI receptor or PDGF receptor) [228]. Drugs such as pentoxifylline or mycophenolic acid are efficiently released into activated HSCs and reduce liver fibrogenesis [229,230]. Interestingly, this approach could also be useful for treating portal hypertension, a condition that involves chronic contraction of activated HSCs.

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Why does the liver need to regenerate?

The liver receives all absorbed products digested by the gastrointestinal tract through the portal circulation. While many of the absorbed compounds are in the broad category of nutrients, an equal number do not belong to the chemistry of normal metabolic pathways and as such are classified as xenobiotics. Many of the xenobiotic compounds are water soluble and can be excreted by the liver through the bile or released into the bloodstream and excreted by the kidneys. A subclass of xenobiotics comprises medicinal drugs and plant alkaloids and these are lipophilic (not water soluble) and processed through a complex set of enzymes (drug-metabolizing enzymes). These enzymes include multiple members of the family of proteins known as cytochrome P450 (CYP), as well as several enzymes involved in conjugation of lipophilic xenobiotics with smaller moieties (e.g., sulfate, acetyl, glucuronide, and other hydrophilic moieties). During the course of these complex reactions, intermediary metabolites generated by the pathways of xenobiotic detoxification acquire electrophilic properties and thus can react directly with cellular nucleophiles (proteins, RNA and DNA). These electrophiles, often generically described as *free radicals*, bind to cellular nucleophiles through covalent bonding and may either inactivate them (e.g., proteins) or permanently change them (e.g., formation of DNA adducts leading to mutations) [1]. Massive covalent bonding of xenobiotics activated to free radicals is toxic to hepatocytes and leads to cell death via necrosis or apoptosis. The potency of the toxic xenobiotic or the amount ingested may be such that most of the hepatocytes die, leading to fulminant hepatitis. In most situations, although a large number of hepatocytes die, the surviving hepatocytes restore the liver architecture and the liver can function; however, permanent residual scars can be visualized internally or on the surface of the liver. Such events are not uncommon in animals living in the wild. Scars on the surface of the liver are so common that they were used in ancient times during animal sacrifices to divine the future. There were clay libraries in ancient Babylon with hundreds of liver samples, instructing ancient soothsayers on all possible scars seen on the surface of the liver and their meaning in terms of predicting the future. The existence of such liver scars in wild animals suggests that massive toxic liver injury in natural settings is very common. Liver regeneration emerged through evolution as a survival adaptation to allow longevity of the organism in a natural, but often nutritionally hostile, environment.

Experimental models and clinical settings

Liver regeneration aims to restore hepatic mass. Hepatocyte proliferation occurs in any situation associated with chronic liver disease or massive injury to the liver, leading to loss of hepatic parenchyma. Chronic hepatic inflammation due to viruses or autoimmune mechanisms is associated with variable rates of hepatocyte death, easily seen as apoptotic bodies. Immunohistochemical staining with nuclear cell cycle

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markers such as proliferating cell nuclear antigen (PCNA) in these cases often shows that the vast majority of surviving hepatocytes are engaged in the cell cycle (G1 to M), in contrast to normal healthy livers in which more than 99% of hepatocytes are not in the cell cycle (G0 stage) [2]. This can be demonstrated even in the mildest forms of liver disease and is also true in the most severe cases of fulminant hepatitis. Acute liver damage (e.g., as a result of acetaminophen overdose) is also associated with hepatocyte death (centrilobular area) and compensatory proliferation of the surviving hepatocytes. Studies with patients suffering from Dubin–Johnson syndrome have been very instructive. In these patients, deposition of a melanin-like substance makes hepatocytes black; newly regenerated hepatocytes do not yet have melanin accumulation and appear white. When these individuals acquire acute viral hepatitis, it can be easily determined from the percentage of hepatocytes of different color that, in a standard clinically overt episode of acute viral hepatitis, more than 90% of the original hepatocytes die [3]. However, compensatory proliferation of the surviving hepatocytes maintains liver histology and function until the virus is cleared and health is restored. Easier to demonstrate is hepatic regeneration following hepatic resections (e.g., to remove solitary tumors, cysts, repair trauma). Up to 70% of the liver may be removed, but liver mass is restored within 8–20 days, depending on the magnitude of the resection, the nutritional status of the patient, age, and so on [4].

Experimental models to study liver regeneration typically employ rodents (rats or mice). Administration of a chemical toxin such as carbon tetrachloride (CCl_4) or acetaminophen causes centrilobular necrosis, since most of the CYP proteins reside in the centrilobular hepatocytes, and CCl_4 (and acetaminophen) require interaction with CYP proteins to be converted into the active electrophilic metabolites that cause hepatocyte death. CYP protein is much less expressed in periportal hepatocytes compared with centrilobular hepatocytes; thus, the periportal hepatocytes are unable to activate CCl_4 and acetaminophen and are spared from damage [5]. Injury caused by most of the hepatotoxic chemicals follows this pattern of histology. Very few chemicals, such as allyl alcohol, produce hepatic injury that is predominantly periportal, and the mechanisms leading to this type of injury are not fully understood [6]. Induction of acute centrilobular necrosis is easily achieved with proper dosing of chemicals and remains a simple way to cause acute liver injury followed by hepatic regeneration. On the other hand, experimental models using this approach are difficult to study because massive hepatocyte necrosis within a localized lobular zone leaves an area of destruction in which dead cells need to be first removed by macrophages and other inflammatory cells prior to initiation of regeneration. Thus, chemical injury models suffer from an inherent difficulty in distinguishing between events related to acute inflammation and early events associated with liver regeneration.

A less complicated model for studying liver regeneration is two-thirds partial hepatectomy (PHx). The liver of rats and mice has five separate lobes. Each lobe has its own pedicle containing vascular structures, bile duct branches, and nerve branches. Higgins and Anderson first described partial hepatectomy in 1931 [7]. Following a small longitudinal mid-abdominal incision, mild abdominal pressure leads to externalization of three of the five liver lobes. These lobes can be easily removed after ligation of their fibrovascular pedicles. The remaining two lobes stay within the abdominal cavity. They start growing in size and restore the mass (but not the five-lobe shape) of the liver within 5–6 days after PHx. Since PHx is not associated with formation of necrotic tissue within the remaining liver lobes, one can study the earliest events after PHx and thus use this model to dissect the earliest pathways associated with the regenerative process without the interference of the complications associated with the inflammatory response seen with CCl_4 treatment.

Unless otherwise stated, our presentation of hepatic regeneration will focus on events leading to liver regeneration after PHx.

Cellular kinetics during liver regeneration after partial hepatectomy

In some tissues such as the skin and intestine, tissue restoration proceeds only from a small fraction of the overall cell population (i.e., *stem cells*). This is not the case in the liver. The entire hepatic cell population remaining after PHx participates in proliferative events that restore liver mass. In rats, PHx is followed by entry of hepatocytes into DNA synthesis within 12 h. The fist peak of hepatocyte mitoses is seen at 24 h. (Patterns of feeding and lighting of the experimental animals can moderately alter the timing of these events. Also, in mice, these phenomena occur about 12 h later than they do in rats [8].) Most of the hepatocytes (> 98%) can be labeled by tagged DNA precursors (tritiated thymidine or bromodeoxyuridine) in the first 24 h after PHx. A similar 24-h labeling pulse given 24–48 h after PHx labels only 50%–60% of the hepatocytes. Thus, the original 30% of hepatocytes doubles following PHx, becoming 60% at 24 h, and slightly more than half of these proliferate to add another 35%–45% of the number of hepatocytes in the original liver (prior to PHx). Thus, by the end of 48 h, the total number of hepatocytes is 100% of the original (or slightly more than 100%: a small wave of apoptosis often follows the end of proliferation after PHx [9]). The first wave of proliferation starts from the periportal hepatocytes [10], with matrix metalloproteinase (MMP)-9 expression seen at 3 h after PHx in this region [11]. The second wave of proliferation occurs predominantly in the pericentral areas. Hepatocyte proliferation, as evidenced by mitoses, nuclear labeling, etc., proceeds as a wave from the periportal to the pericentral region of the lobule [10]. It is

not clear whether the other liver cells types, such as biliary epithelial cells or stellate cells, proliferate following the same zonation pattern. Hepatocytes are the first cell types to enter into proliferation. Biliary epithelial cells and stellate cells follow after a 12–24 h delay, whereas endothelial cells go through a broad proliferative stage 2–6 days after PHx [12].

As discussed later, following PHx, hepatocytes receive several proliferation-related signals from their immediate environment and from the peripheral blood [13–16]. Hepatocytes undergo rapid changes in gene expression and activation of receptors and transcription factors. During proliferation, hepatocytes generate paracrine growth signals consisting of proteins known to be mitogenic to the other populations of liver cells. Proliferation-related events typically cease for most cell types by day 5–6 after PHx. Hepatocytes are typically smaller at the end of their proliferation at 48 h. The size of the now quiescent hepatocytes becomes gradually larger as liver weight slowly increases from days 3–6 after PHx [8].

Functional and structural changes in liver histology during regeneration

Perhaps the most fascinating aspect of liver regeneration is that hepatocytes exhibit minimal alterations in structure and function while regeneration is proceeding. Liver continues to provide all essential functions, from production of plasma proteins to bile acid conjugation, drug metabolism and synthesis of bile, with only minimal changes. Albumin concentrations drop by about 5%–10%; glucose tends to be a little lower but within normal limits. This is remarkable for the following reasons. Firstly, more than 95% of the hepatocytes undergo at least one full DNA synthesis cycle and mitotic division during this process. Secondly, hepatocytes tend to be polyploid. Most human hepatocytes are binucleate, with $2n$ ploidy per nucleus. In the rat and mouse, most hepatocytes are polyploid, with ploidies ranging from $4n$ to $32n$ per nucleus [17,18]. At the end of regeneration, hepatocyte ploidy tends to be less but returns to normal (polyploid) levels within 21 days after PHx [19]. Other changes are also detected. A mild degree of accumulation of fatty droplets appears in hepatocytes in the first 48 h after PHx. Recent studies have determined that changes in fatty acid metabolism and lipid biosynthesis occur during the regenerative process [20]. As hepatocytes enter into mitosis, transient alterations in the structure of bile canaliculi, including transient restructuring of tight and gap junctions, can be observed. However, the bile canaliculi and their function are maintained [21,22]. As hepatocytes proliferate prior to initiation of angiogenesis, newly formed small hepatocytes become arranged in hypovascularized clusters [23]. Angiogenesis is led in large part by growth factors produced by newly proliferated hepatocytes, which stimulate formation of fenestrated endothelium that becomes organized into regular sinusoids. This process eliminates the hypovascularized hepatocytic clumps and restores the characteristic hepatocyte plates [24]. Hepatocyte plates of a liver that has undergone regeneration are, on average, thicker (two cells thick) than the plates of a normal liver (one cell thick) [25]. However, it should also be emphasized that regeneration after PHx does not result in an increased number of lobules. There is no evidence for formation of new portal triads. The existing lobules increase in size.

General functional aspects of cell signaling during liver regeneration

There are common aspects of the experimental approaches used to investigate signals associated with liver regeneration. One approach is to study changes related to specific molecules during the process and examine linkage of these changes to signaling mechanisms. Another approach is to eliminate specific potential signals (by generating genetically engineered mice, using silencing RNA or specific inhibitors) and study the effect of blockade on liver regeneration. There are literally hundreds of studies following these approaches. However, some general conclusions have been drawn from these studies that are applicable to all signaling associated with regeneration.

- PHx triggers multiple and concurrent signaling pathways within minutes. These pathways involve the receptors of, for example, hepatocyte growth factor (HGF), epidermal growth factor (EGF), interleukin (IL)-6, tumor necrosis factor (TNF), transforming growth factor (TGF)-β1, norepinephrine, and Notch.
- Blocking several signals causes different degrees of delay in the regenerative process. However, no block completely arrests liver regeneration. Regenerative signals are highly redundant: the regenerative process can occur even when signaling receptors are eliminated, because the required pathways for activation and completion of the cell cycle can be approached through alternative routes. (Perhaps the only exception to this is the documented inability of the liver to fully regenerate after targeted inactivation of the HGF receptor [cMet], but this is observed only after a full two-thirds PHx.)
- In addition to signals operating in the liver, changes involving multiple growth factors and cytokines (HGF, EGF, IL-6, TNF, norepinephrine, TGF-β1) also occur in the peripheral blood. Liver regeneration is also associated with DNA synthesis and mitoses in hepatocytes or hepatic tissue fragments transplanted in extrahepatic sites.

Extracellular signals leading liver into regeneration

Such signals include growth factors and cytokines and their specific receptors. In addition, other ligand–receptor signals

are also involved, such as Notch/Jagged, catecholamines and α_1-adrenergic receptors, and angiopoietins. The evidence implicating these signals and their potential roles during regeneration is described in this section.

Hepatocyte growth factor and its receptor cMet

HGF was discovered in 1989 on the basis of its mitogenic properties on hepatocytes in culture and the fact that it rises dramatically in the peripheral blood within 1 h after PHx [26–28]. There is an extensive literature on HGF, which has effects on many tissues besides liver [29,30]. Another protein called scatter factor (SF) was subsequently found to be identical to HGF and the name HGF/SF is often used to refer to the protein [31]. This section focuses on HGF in the context of its role in liver regeneration and biology. The HGF receptor cMet recognizes HGF as its only ligand and mediates all the biological effects of HGF [32,33]. General aspects of the biology of HGF and cMet pertinent to this review include the folowing.

- HGF is produced by mesenchymal connective tissue cells, including hepatic stellate cells [34] and fenestrated sinusoidal endothelial cells [35]. cMet is a receptor tyrosine kinase [36–39]. In addition to its direct role as a signaling receptor, cMet also binds the cell death-associated receptor Fas on the plasma membrane of epithelial cells, thus raising the threshold concentration that Fas ligand has to achieve in order to induce apoptosis [40]. cMet is generally present on epithelial cells, but also on endothelial cells. In liver, cMet is expressed by hepatocytes, endothelial cells, biliary epithelial cells, and stellate cells [41]. In the brain, there are select groups of neurons that express either HGF or its receptor [42].
- The full-size HGF molecule is composed of an amino-terminal hairpin loop and four kringles (comprising the HGF heavy chain). This protein is attached to a light chain by S–S bonds. The light chain has an inactive pseudoprotease motif. The whole molecule has high homology to other proteins related to coagulation and fibrinolysis, which also bear kringles but have an active protease site. These proteins include plasminogen, thrombin, and plasminogen activators [28,36].
- HGF is produced as a single-chain molecule and becomes activated to the heterodimeric form by proteolytic cleavage. This is mostly mediated by the urokinase plasminogen activator (uPA), free or attached to the urokinase receptor (uPAR) [43]. Other proteases have also been shown to cleave HGF in vitro, including plasminogen and bradykinin. Another protease, known as HGF activator, is highly homologous to coagulation factor XII and appears to act as a soluble protein [44].
- The single-chain inactive HGF precursor is a heparin-binding protein and as such is present in high concentrations in the extracellular matrix (ECM) of several tissues. It is present in high concentration in the sparse ECM of the liver, concentrated primarily in the periportal region [45].
- Homozygous deletion of HGF or its receptor is associated with embryonic lethality. There are abnormalities in many embryonic tissues, most prominently related to abnormal structure of the placenta, small liver [46,47], and abnormal erythropoiesis (Michalopoulos G, unpublished observations).
- Sustained short-term infusion of HGF causes proliferation of hepatocytes in normal rats [48] and doubling of liver weight in mice within 5 days [49].

While HGF plays a role in the biology of most tissues, it appears to have specific functions related to liver regeneration and liver biology in general. The model emerging as to the role of HGF and cMet in liver regeneration is as follows.

- There is a rapid (within 5 min) increase in uPA activity following PHx [50]. This generates a cascade of proteolytic events involving activation of plasminogen to plasmin [51] and subsequent activation of MMP-9 [11].
- As the hepatic ECM undergoes rapid remodeling, the bound HGF single-chain precursor is rapidly converted within the liver to the active two-chain form. This reaction is catalyzed by uPA [50]. Mice deficient in urokinase have defective liver regeneration due to insufficient activation of HGF [52,53]. Activated HGF is available to act locally and also escapes to the peripheral blood, where it quickly rises within 30–60 min after PHx [54]. The form of HGF appearing in the plasma is the active heterodimeric form [55]. (Other ECM-bound molecules also rise in the plasma after PHx, including TGF-β1 and hyaluronic acid; see relevant sections.)
- The HGF receptor is activated within 15–30 min after PHx [56].
- The above events result in a decrease in HGF in hepatic ECM. However, the synthesis of new HGF begins at 3 h after PHx [57], and both single-chain and heterodimeric forms rise rapidly in the liver, with a peak at 48 h after PHx [55]. The source of the new HGF is primarily hepatic stellate cells [34] and, to a lesser extent, fenestrated sinusoidal endothelial cells [35]. The latter respond to production of vascular endothelial growth factor (VEGF) by proliferating hepatocytes and synthesize HGF by signaling of the VEGF receptor 1.
- In addition to HGF generated in the liver, HGF mRNA also rises in the lungs [58] by mechanisms that are not clearly understood but which may involve IL-1, IL-6 [59], and norepinephrine [60].

Despite all the evidence that HGF is associated with mitogenic signaling very early during regeneration, targeted inactivation of cMet on hepatocytes does not block the early signaling events associated with liver regeneration. However, these mice fail to complete regeneration, and hepatocytes become arrested at some point in the cycle at late S or G2 [61]. In a similar study, it was shown that targeted inactivation of cMet in the liver interferes with activation of extracellular signal-regulated kinase (ERK)1/2, and hepatocytes had decreased rate of entry into S phase [62]. The nature of the block is not clear, but it appears that some of the

signals expected to be delivered by cMet early in the regenerative process are not redundant and cannot be substituted by other receptor tyrosine kinases involved in the process, such as the EGF receptor.

Epidermal growth factor receptor and its associated ligands

The EGF/EGF receptor (EGFR) signaling system involves a very complex array of receptors, coreceptors and multiple ligands and operates on most epithelial cells [63,64]. EGFR is a member of the Erb family of receptor tyrosine kinases, a family that consists of four members. EGFR is also known as ErbB or HER. The other Erb members are ErbB-2 (HER-2, NEU), ErbB-3 (HER-3), and ErbB-4 (HER-4). HER-4 is not expressed in adult or embryonic liver. More than 10 ligands exist for EGFR, including EGF, TGF-α, amphiregulin, heparin-binding EGF (HB-EGF), Cripto, Epiregulin, and Betacellulin. The receptor system and many of the ligands are ancient (in a phylogenetic sense) and have been described in species as primitive as nematodes [65]. EGFR forms homodimers, or it can form heterodimers with ErbB-2. The latter also possesses a kinase domain. Binding of ligands to either the homocomplexes or heterocomplexes triggers phosphorylation of tyrosine sites and activation of the receptors. Activation of EGFR is evident at 60 min after PHx [56,66]. Although all ligands of EGFR bind to the same receptors, their effects are nonetheless significantly different in terms of intensity of elicited response. Also, while many ligands are expressed during regeneration, some deliver more essential signals than others. The basis for such differences in the effects induced by EGFR ligands has been discussed in many reviews [67]. Basically, binding of ligands causes activation of the receptor, which is followed by endocytosis through clathrin pits and processing through the endosome system of membranes and vesicles. EGFR–ErbB-2 complexes are stable through endosomal processing and tend to be recycled back to the plasma membrane, whereas EGFR–EGFR complexes are typically processed for degradation. The complexes between EGFR and ErbB-2 also tend to stay bound to ligand and thus signal for longer periods of time. Thus, much of the observed differences in signaling between EGFR ligands can be explained by their differential affinity for EGFR–EGFR or EGFR–ErbB-2 complexes on the plasma membrane, and the different signaling capabilities of the two complexes [68–71]. All EGFR ligands are directly mitogenic on hepatocytes in primary culture, without requiring the presence of any other cytokines to exert this effect. The EGFR ligands implicated in regeneration are discussed in the following sections.

Epidermal growth factor

EGF is synthesized in multiple exocrine glands, and is found in their exocrine fluids. It is also secreted as an endocrine product from the same sites. Brunner glands of the duodenum are very active in secretion of EGF and the endocrine component of this function sends EGF directly and continually to the liver [72]. EGF injected into the liver through the portal vein is sequestered in large amounts in one pass and accumulates in periportal sites [73]. Mouse salivary glands also produce much EGF, and removal of salivary glands in the male mouse delays and decreases the regenerative response [74]. Sustained injection of EGF leads to hepatic enlargement [75]. However, there is no evidence of a rise in EGF in plasma after PHx. This is not surprising, given the high affinity and capacity of the liver to sequester EGF present in the portal circulation. Even though the role of EGF per se is enigmatic and often disputed, it should be noted that EGF is a direct mitogen for hepatocytes [76]. In view of the fact that (i) EGFR is activated within 30–60 min after PHx, (ii) EGF is always present in the portal circulation, and (iii) the EGF per hepatocyte ratio should increase threefold after two-thirds PHx, EGF is a good candidate for being the original trigger of the whole set of subsequent events following PHx. However, this is highly speculative because studies in mice with homozygous deletion of EGF have not been carried out.

Transforming growth factor-α

This EGFR ligand is produced as a large precursor of 28 kDa, which is inserted into the plasma membrane. The inserted precursor can have effects on adjacent cells expressing EGFR, without ever leaving the originating cell [77,78]. The extracellular portion is cleaved off by specific proteases, such as TACE/ADAM17, generating the active free ligand [79]. TGF-α is the stronger ligand for EGFR in terms of its mitogenic effects on hepatocytes [80]. TGF-α is also produced by hepatocytes as they undergo proliferation. TGF-α mRNA increases dramatically after PHx, but the overall increase in protein is rather small [81,82]. EGFR is expressed in hepatocytes as well as endothelial cells and biliary epithelium. It is not clear whether TGF-α has an autocrine function on hepatocytes that synthesize it or whether it has only a paracrine function on cells adjacent to hepatocytes expressing EGFR. Injection of TGF-α through the portal vein induces hepatocyte DNA synthesis, clearly demonstrating that TGF-α can be a mitogen for hepatocytes [83]. On the other hand, liver regeneration proceeds normally in mice with homozygous deletion of TGF-α [84]. This is not surprising given the availability of multiple EGFR ligands that can complement the function of TGF-α in its absence. Deletion of EGFR itself is not associated with any hepatic abnormalities during development [85], but the response of $EGFR^{-/-}$ mice to PHx has not been studied.

Heparin-binding epidermal growth factor and amphiregulin

HB-EGF and amphiregulin are two additional members of the EGFR ligand family. Injection of HB-EGF to mice subjected to only one-third PHx accelerated DNA synthesis to

levels seen with mice subjected to two-thirds PHx. Mice deficient in HB-EGF have delayed liver regeneration [86]. Similarly, mice deficient in amphiregulin also have decreased hepatic regenerative activity [87]. The results observed in mice deficient in either of these EGFR ligands contrast with those obtained with mice lacking TGF-α and are surprising because TGF-α is overall a stronger mitogen for hepatocytes than these two ligands. Details of the differences in interaction between amphiregulin, HB-EGF and TGF-α with EGFR in the liver need to be better understood.

Tumor necrosis factor-α

TNF has numerous functions on many cell types, depending on cell status and function. In many situations, as in tumor cells, TNF triggers apoptosis. In other cell types, TNF triggers activation of NF-κB and promotes cell survival. The outcome of interaction between TNF and target cells depends on the redox status [88], concentrations of free radicals, and TRAF proteins [89,90]. This is discussed further in the section on NF-κB. TNF is produced by hepatic macrophages (Kupffer cells) [91]. It rises in the peripheral blood after PHx [92]. TNF is not a direct mitogen for hepatocytes. In culture, it enhances the effect of EGF or HGF. Similarly, TNF enhances the mitogenic effect of subthreshold levels of HGF or TGF-α on liver when these growth factors are injected in vivo [83]. Antibodies neutralizing TNF injected at PHx block activation of Jun N-terminal kinase (JNK) and delay the regenerative response [93]. More significantly, there is delay and decrease in liver regeneration in mice with homozygous deletion of TNF receptor 1 (TNFR1) [92,94,95]. The mode by which TNF functions is not entirely clear. It is associated with activation of NF-κB and there is substantially decreased NF-κB activation in TNFR1$^{-/-}$ mice [96]. Even though NF-κB does not play an essential role during liver regeneration [97], the multiplicity of functions associated with NF-κB activation in most cell types suggests that the observed rapid activation of NF-κB after PHx (see below) must play an important role in the process. TNF has also been linked with increased production of the plasma membrane metalloproteinase TACE/ADAM17 associated with proteolytic activation of many entities, including TNF itself and TGF-α [98]. The latter is intercalated in the plasma membrane through a defined transmembrane motif. TACE/ADAM17 cleaves the extracellular portion of TGF-α and generates the smaller active form. This event has been observed in hepatocyte cell lines overexpressing TGF-α, and if it occurs in the context of liver regeneration, it must be a later event because TGF-α mRNA does not rise until about 3 h after PHx. TNF also induces production of IL-6 in hepatic macrophages [99]. IL-6 appears to have some effects on liver regeneration on its own, and administration of IL-6 corrects some of the deficiencies in liver regeneration seen in TNFR1-deficient mice [92]. The overall effects of TNF are multiple and it is thought that TNF provides a priming effect on hepatocytes, making them susceptible to the effects of growth factors (EGF and HGF). The concept is more useful for highlighting cooperative effects between TNF and the growth factors and should not be interpreted as denoting a hierarchy of events in which the effects of TNF precede those of the growth factors. Events triggered by TNF as well as those triggered by HGF, EGF, and Notch are concurrent and interdependent.

Interleukin-6

IL-6 is an intriguing cytokine. It is one of the earliest cytokines expressed during embryonic development (by the fertilized ovum). It attaches to a soluble receptor and forms a complex, which in turn binds to gp130, a receptor that IL-6 shares with other cytokines such as oncostatin M, leukemia inhibitory factor, and ciliary neurotrophic factor. Each one of the latter cytokines also has a unique soluble receptor, forms a complex, and binds to gp130 [100]. As with the EGFR ligand system, many of the functions of the different cytokines that bind to the shared gp130 receptor differ in intensity from one another, leading to a spectrum of responses. IL-6 is produced by macrophages and has been well known for years as the inducer of the hepatic acute-phase response. This response appears in acute body-wide infections and diffuse inflammatory conditions. A large repertoire of proteins is synthesized by hepatocytes during the acute-phase response, including serum amyloid proteins, complement components, and proteins of the opsonin group [101–105]. Mice with homozygous deletion of IL-6 have an overall normal phenotype but appear to be sensitive to parasitic and fungal infections [106]. The same mice were reported as suffering from deficient hepatic regeneration and increased mortality after PHx [107]. However, subsequent studies with IL-6$^{-/-}$ mice found very few deficiencies in the regenerative response [9]. In addition, mice with homozygous deletion of the shared gp130 receptor also have a normal regenerative response [108]. As already mentioned, a common feature associated with all extracellular signals associated with liver regeneration is the redundancy of the associated pathways. Most evidence suggests that the role of IL-6 in hepatic regeneration is not as essential as originally thought. However, this should not be meant to imply that IL-6 does not play a role. All studies agree that activation of the transcription factor STAT3 is deficient in IL6$^{-/-}$ and gp130$^{-/-}$ mice, and STAT3 is well known to be activated at the early stages of entry into the cell cycle in most proliferating cells [109]. IL-6 also rises in the plasma after PHx and replaces the regenerative defects seen in TNFR1-deficient mice [92]. In addition to its other properties in relation to liver, IL-6 is a direct mitogen for biliary epithelial cells in culture [110]. It is also highly expressed in spontaneously growing hepatic organoid cultures and its expression is suppressed by dexamethasone [111]. Double transgenic mice with overexpression of both IL-6 and its soluble receptor targeted to the liver have liver enlargement and focal areas of hepatocyte hyperplasia [112].

Norepinephrine

Several studies have associated small signaling molecules acting through G protein-associated receptors with comitogenic signals. Norepinephrine (NE) and epinephrine are in this category of molecules and act through α- and β-adrenergic receptors [113]. NE enhances the effects of HGF and EGF in hepatocyte cultures and the effect can be blocked by the α_1-specific blocker prazosin [114,115]. In relation to EGF, this is also associated with a heterologous downregulation of EGFR [116]. However, NE is more intriguing because it has a multiplicity of other effects related to liver regeneration. It partially inhibits the mitoinhibitory effects of TGF-β1 on hepatocytes [117]. NE increases production of EGF from salivary glands and Brunner glands of the duodenum [72,118]. Since NE quickly rises in the plasma after PHx, an NE-dependent increased output of EGF would enhance the EGFR-mediated signals for hepatocytes (in addition to the direct effects of NE on EGFR itself through the α_1-adrenergic receptor). In addition, NE induces expression of HGF in fibroblasts [60], suggesting that the rise in plasma NE levels after PHx may be a contributory signal related to increased expression of HGF in liver itself and extrahepatic tissues (lungs, spleen) during hepatic regeneration. From a different perspective, it has also been shown that, in addition to its traditional sources of production (peripheral sympathetic neurons and adrenal medulla), NE is also synthesized by hepatic stellate cells and increases their proliferation in cell culture [119]. In view of all this, it is not surprising that blockade of the α_1-adrenergic receptor or resection of the sympathetic nerves of the liver blocks the first wave of DNA synthesis after PHx and delays the overall process by 24 h [120,121].

Transforming growth factor-β1

TGF-β1 is a member of a family comprising three proteins (TGF-β1, TGF-β2, TGF-β3). Other polypeptides such as activin, inhibin and bone morphogenetic proteins are also members of the broader group of related proteins [122–124]. The active form of TGF-β1 is produced after removal of another protein attached to it by disulfide bonds, a reaction catalyzed by several proteases including plasmin [125]. The TGF-β1 receptor is assembled by interaction between three plasma membrane proteins (TGF-βRI, TGF-βRII, TGF-βRIII). Binding of TGF-βRI to TGF-βRII induces serine phosphorylation of TGF-βRI [126]. This activates a serine-threonine kinase function that phosphorylates downstream targets including that of the Smad family of proteins [127].

TGF-β1 is produced by most mesenchymal-derived cells including fibroblasts, myofibroblasts, and hepatic stellate cells. It is also produced by many epithelial cells and most carcinomas, including hepatocellular carcinomas [128]. It stimulates synthesis of most ECM proteins produced by connective tissue cells, including collagen types I, IV and VI. TGF-β1 is a mitoinhibitor for hepatocytes in primary culture and in a dose-dependent manner blocks the mitogenic effects of HGF and EGF [129,130]. Its inhibitory effects are partially reversed by NE [117]. In view of this, it is surprising that TGF-β1 mRNA rises soon (within 3 h) after PHx and remains elevated until 72 h after PHx [131,132]. TGF-β1 levels in plasma also increase rapidly after PHx and prior to the rise of TGF-β1 mRNA in the liver, in a manner similar to that of HGF [8,133]. This is not surprising because TGF-β1 is present in the ECM immediately adjacent to hepatocytes and bound to the protein decorin [134]. It is probably released in the peripheral blood during remodeling of the hepatic ECM following PHx along with HGF, hyaluronic acid, and other matrix components [8,133]. It is unlikely that TGF-β1 released in the plasma exercises any effects in liver because circulating TGF-β1 is inactivated by binding to α_2-macroglobulin, and the complex is processed and destroyed by intracellular proteolytic pathways involving endosomes in hepatocytes and most epithelial cells [135,136]. TGF-β1 also binds to the insulin-like growth factor (IGF)II/mannose-6-phosphate receptor, and the complex is endocytosed and processed for proteolytic degradation [137–139]. Expression of TGF-β1 receptors I and II in regenerating hepatocytes decreases dramatically after PHx, and it is likely that by this mechanism regenerating hepatocytes become resistant to the mitoinhibitory effects of the rising concentrations of TGF-β1 during regeneration [140]. In addition, signal transduction pathways initiated from TGF-β1 and proceeding through Smad proteins are inhibited during liver regeneration by upregulation of the transcription factors SnoN and Ski [141]. Immunohistochemical studies have shown that TGF-β1 is eliminated in a wave-like way from the periportal to the pericentral areas of the lobules as regeneration proceeds in the same direction. Immediately behind the residual TGF-β1-positive front, there is a front of hepatocytes in mitosis [139]. The multiple mechanisms by which the potential mitoinhibitory effect of TGF-β1 on hepatocytes is eliminated during regeneration suggest that the role of TGF-β1 is not that of an ineffective mitoinhibitor but rather that of a necessary effector for cells other than hepatocytes. As mentioned above, TGF-β1 stimulates production of most proteins of the ECM which, following the early remodeling events, is reconstituted 3–5 days after PHx. TGF-β1 also stimulates formation of capillary tubes in endothelial cells [142], and may be involved in the late angiogenic events that reconstitute the hepatic sinusoidal network at the end of regeneration. TGF-β1 is also a *motogen* for normal and neoplastic hepatocytes [143–145], and its presence may be important for the mediation of events requiring cell migration during tissue remodeling, especially at the late stages of regeneration.

In view of its mitoinhibitory effects on hepatocytes, it has been often hypothesized that TGF-β1 is involved in the termination of liver regeneration. Infusion of TGF-β1 prior to and immediately after PHx suppresses regeneration for 72 h in mice [146]. Liver regeneration in transgenic mice

overexpressing TGF-β1 in hepatocytes under the albumin promoter is slightly suppressed but not by any means eliminated [147]. The overall role of TGF-β1 in the early and late stages of regeneration is further in the section Extracellular matrix changes and angiogenesis.

Notch and Jagged

Notch and Jagged are members of two families of proteins whose members are intercalated in plasma membranes. The names derive from specific phenotypic alterations resulting from mutations of these proteins in *Drosophila*. Jagged proteins, as well as Delta proteins, bind to Notch. This causes an allosteric reconfiguration of Notch and makes it susceptible to specific proteases, including presenilin. Following binding of Jagged or Delta, the extracellular portion of the Notch–ligand complex is cleaved by TACE/ADAM17 protease (an enzyme that also cleaves TNF and TGF-α precursor). The intracellular portion of Notch is cleaved by presenilin and the Notch intracellular domain (NICD) is transferred to the nucleus. There it functions as a coactivator of expression of specific genes associated with the cell cycle, including c-Myc and cyclin D_1 [148,149]. The Notch/Jagged/Delta system is involved in fate determination of cell lineages and has effects on multiple tissues, including nervous system and bone marrow. Mutations in Jagged-1 are associated with Alagille syndrome affecting multiple tissues including liver, where it causes a paucity of intrahepatic bile ductules [150,151]. Activation of Notch and migration of NICD to the nucleus occur prior to entry into the cell cycle in most quiescent and fully differentiated cells. NICD appears in hepatic nuclei within 15 min after PHx and is associated with increased expression of the dependent genes HES1 and HES5 [152]. Both Notch and Jagged increase in hepatocytes, endothelial cells, and biliary cells 24–72 h after PHx. Recombinant Jagged-1 protein is mitogenic for hepatocytes in primary culture, although it is not as potent as HGF or EGF. However, Jagged-1 enhances the mitogenic effects of HGF or EGF [152]. Knockdown of Notch-1 or Jagged-1 proteins by silencing RNA decreases the regenerative response [152]. Silencing RNA would have effects on all cell types of the liver expressing Notch or Jagged. In another study, however, targeted deletion of Notch-1 selectively to hepatocytes was carried out. In these animals, there was a gradually increasing proliferation of all cells of the liver and emergence of hepatic nodular hyperplasia and periportal fibrosis. However, liver regeneration was also decreased in this study [153]. The combined results suggest that Notch and Jagged proteins interact between multiple cell types and that the effects seen depend on which cell type is affected by the experimental approaches taken. Based on paradigms from other tissues and results from the liver literature, it is highly likely that Notch and Jagged proteins are involved in the detailed morphogenetic events of the liver from entry into regeneration to final quiescence.

Other factors implicated in liver regeneration

Members of the fibroblast growth factor (FGF) family of proteins are mildly mitogenic to hepatocytes in culture. FGF-1, FGF-2, and keratinocyte growth factor are expressed by hepatocytes during liver regeneration. It is not clear whether this generates an autocrine mitogenic loop or whether this constitutes a paracrine loop by which hepatocytes stimulate proliferation of adjacent cell types responding to FGF (endothelial cells, stellate cells) [154,155]. Stem cell factor (SCF) may also play a role. Hepatocyte proliferation after PHx is decreased in mice with homozygous deletion of SCF. Administration of SCF to IL-6-deficient mice enhances hepatocyte proliferation after PHx and ameliorates some of the deficiencies in regeneration seen in $IL6^{-/-}$ mice [156].

Intracellular events occurring during regeneration

PHx rapidly induces a series of alterations in gene transcription and activation of transcription factors. Most of these studies have been conducted using extracts from whole liver. Since most of the RNA and cytoplasm in the liver derives from hepatocytes, it is fair to assume that most of the findings described in the literature involve primarily hepatocytes. This is especially true at the earliest stages after PHx, because hepatocytes are the first cells in the liver to enter into DNA synthesis. The end point of the intracellular events occurring in hepatocytes after PHx is a delicate balance between hepatocytes continuing to provide vital functions to maintain body homeostasis (especially glucose regulation) while performing all functions associated with cell proliferation. In addition, given the smaller mass of the remnant liver, some functions (e.g., gluconeogenesis) need to be enhanced so that the output from the smaller remnant liver is sufficient to cover the functions normally provided by the intact organ [157].

Many of the intracellular changes described during liver regeneration, such as posttranslational modifications in cell cycle proteins such as cyclin D_1, histone synthesis, and upregulation of enzymes related to DNA synthesis, are seen in most eukaryotic proliferating cells. Other events appear to be more (though not entirely) liver specific. A group of genes, collectively called *immediate early genes*, are rapidly upregulated within 30 min after PHx and include many gluconeogenic enzymes and IGF-binding protein (IGFBP)-1 [109,158,159]. Later, as with other proliferating cells, hepatocytes show enhanced transcription of several proto-oncogenes including c-Myc, Ras proteins, p53, and others [160,161]. Changes in gene expression are generally the result of altered status of transcription factors, the proteins which regulate gene expression. Several studies have focused on changes in specific transcription factors early during liver regeneration and these are probably responsible for

mediating most of these events. It is likely that others will emerge as more studies focus on liver regeneration.

NF-κB

This transcription factor is a complex of two subunits (p65 and p50) with another protein (inhibitor of κB, IκB-α). Activation of NF-κB results from action of the IκB-β kinase (IKK) which leads to the phosphorylation of IκB-α and its detachment from the p65/p50 complex. The latter migrates into the nucleus and acts on target sites on gene promoter sequences to enhance transcription of target genes. Prominent among these are several genes that enhance cell survival and block apoptosis, such as BCL-X_L, BFL1, and GADD45β [162,163]. Activation of NF-κB occurs within 30 min after PHx [164]. Mice with liver-targeted expression of a form of IκB resistant to the action of IKK-β (super-IκB) are unable to fully activate NF-κB. In these mice, PHx results in massive hepatocyte apoptosis [165]. In another experimental approach, however, there was an inducer-dependent and hepatocyte-targeted expression of super-IκB that resulted in blocking activation of NF-κB. In this system, PHx did not lead to hepatocyte apoptosis, and liver regeneration proceeded normally [97]. The above data suggest that, as with all other molecules involved in liver regeneration, NF-κB is not critically required for regeneration. In its absence, vital functions performed by NF-κB are assumed by other complementing molecules. However, this should not imply that NF-κB does not play a very important role under normal circumstances. Activation of antiapoptotic genes is an important aspect of cell physiology and is likely required for hepatocytes prior to fully engaging in the proliferative process. It has been assumed that the rapid activation of NF-κB seen after PHx is the result of TNF and that activation of NF-κB is a pivotal event in determining hepatocyte apoptosis vs proliferation. This view is reinforced by the finding that activation of NF-κB is dramatically decreased in mice with homozygous deletion of TNFR1. However, numerous studies with other cell types have shown that receptor tyrosine kinase ligands including EGF and HGF can also cause rapid activation of NF-κB in multiple cell types and that this activation is associated with protective effects of these growth factors on target cells [166]. Thus, in view of the fact that multiple and concurrent signaling through growth factors and cytokines occurs early in regeneration, the activation of NF-κB should be viewed as an important event, but it is likely that many contributing signals trigger the pathways leading to its activation. These alternative pathways may be the ones responsible for normal completion of the regenerative process in TNFR1 deficient mice.

STAT3

Signal transducer and activator of transcription (STAT)3 is one of seven members of a family of proteins that are phosphorylated by Janus kinases (Jak) following activation of a variety of plasma membrane receptors. There are more than five members in the family. STAT3 activation occurs within 1 h after PHx and lasts up to 8 h [167,168]. STAT3 migrates to the nucleus and triggers expression of many genes associated with cell cycle alterations including the components of AP-1 transcription factor (jun and fos complex). STAT3 activation is deficient in mice with homozygous deletion of IL-6 [107,169]. As with NF-κB, multiple cytokines and growth factors activate STAT3 in different cell types, and it is likely that several pathways are responsible for activation of STAT3 after PHx. HGF and EGF also activate STAT1 and STAT5 in hepatocyte cultures, and STAT3 and STAT5 can dimerize [170,171]. In mice with targeted elimination of STAT3 from hepatocytes, the regenerative response after PHx was substantially reduced. Activation of STAT1, not normally seen after PHx, occurred in these mice and probably accounts for completion of the regenerative response [172].

C/EBPα and C/EBPβ

C/EBPα and C/EBPβ (CCAAT/enhancer-binding protein α and β) are transcription factors with a leucine zipper motif that are involved in regulation of many genes associated with carbohydrate and lipid metabolism. C/EBPα is the dominant form expressed in hepatocytes. Its expression is relatively restricted to liver and adipose tissue; on the other hand, C/EBPβ is relatively ubiquitous. Mice with homozygous deletion of C/EBPα have alterations in carbohydrate and urea cycle enzymes and die from hypoglycemia soon after birth [173]. Levels of C/EBPα increase whereas those of C/EBPβ decrease after PHx. This affects both whole cell and nuclear concentrations of the two factors [174,175]. Hepatocytes from C/EBP$\alpha^{-/-}$ mice have increased response to growth factors in cell culture [176]. Because of these findings, it is considered that C/EBPα per se has antiproliferative effects but this has not been critically tested. It is not clear whether mice deficient in C/EBPβ have enhanced regeneration, but studies with these mice have shown that the effect of HGF requires the presence of C/EBPβ [177].

β-Catenin

In quiescent liver, β-catenin protein is bound to E-cadherin under the plasma membrane. In hepatocytes and many other cell types, β-catenin becomes part of a signaling system whereby Wnt family members generate signals activating specific gene transcription patterns. Any β-catenin circulating free in the cytoplasm is phosphorylated on serine or threonine residues by a complex composed of glycogen synthase kinase 3β (GSK3β), axin and adenomatous polyposis coli protein (APC). This labels β-catenin for ubiquitination and further destruction by proteasomes. When Wnt proteins bind to their receptor proteins (members of the Frizzled family), the intracellular protein Disheveled becomes activated and inhibits GSK3β, allowing accumulation of β-catenin in the cytoplasm. Accumulating β-catenin enters the nucleus, where it forms complexes with members of the Tcf family

of transcription factors and regulates expression of many cell cycle-related proteins, including cyclin D_1 and cMyc [178–180]. A unique relationship of β-catenin with hepatic proliferative events has emerged from studies of liver cancer. Mutations rendering β-catenin resistant to tagging and ubiquitination are commonly seen in hepatocellular carcinomas and hepatoblastomas [181–183]. β-Catenin levels rise within 15 min after PHx and the protein appears in nuclei in the same period of time, remaining elevated for at least 6 h [184]. The protein APC rises sharply after β-catenin becomes elevated and results in rapid decrease in β-catenin levels in the cytoplasm, after the pulse of β-catenin has entered the nucleus. Thus, the mitogenic events of β-catenin after PHx are limited to a very early pulse of nuclear entry followed by a sustained presence of β-catenin in the nucleus [184]. However, the pathway leading to this process is probably not the canonical Wnt-driven pathway. In hepatocytes and other cell types, β-catenin is also bound to the HGF receptor cMet, as well as EGFR and platelet-derived growth factor receptor (PDGFR) in other cell types. HGF stimulation causes rapid translocation of β-catenin to the nucleus in hepatocyte cultures [185]. Thus, it appears that β-catenin is a very important component of the signaling pathway controlled by cMet and (presumably) EGFR, and it acts within minutes after PHx. This finding places into perspective the association of hepatic neoplasia with mutations rendering β-catenin resistant to proteolytic degradation.

Notch and Jagged

The Notch/Jagged signaling pathway is also rapidly activated after PHx, with NICD appearing in hepatocyte nuclei within 15 min after PHx.

Extracellular matrix changes and angiogenesis

Matrix remodeling

Matrix remodeling is an integral part of cell and tissue growth. It occurs in normal tissues as well as in neoplasms. Much of our knowledge related to matrix remodeling derives from studies of cancer invasion and metastasis [186]. A major pathway of matrix remodeling proceeds from uPA. Active uPA, free or bound to its receptor, activates plasminogen into plasmin. The latter activates MMP-9 which in turn acts as a protease for many ECM proteins. A complex network of positive and negative feedback mediated by tissue inhibitors of metalloproteinases (TIMPs) and plasminogen activator inhibitors (PAIs) provides fine control of the above series of events [187]. Notable is the fact that many of the components of this system are also associated with parallel events of activation or deactivation of growth factors and cytokines. uPA itself is a major activator of single-chain HGF to the active two-chain form [43]. Plasmin activates TGF-β1 by removing an inactivating bound protein [188]. This pathway becomes active very shortly after PHx. uPA activity increases dramatically within 5 min after PHx. This is followed by activation of plasminogen to plasmin within 15–30 min after PHx and subsequent activation of MMP-9 within 1 h after PHx [11,50,51]. These events are associated with release of both HGF and TGF-β1 from their binding sites immediately adjacent to the cell. Released HGF is then cleaved, appearing in the plasma in the active form [55] with subsequent activation of the HGF receptor within 30 min after PHx [56]. Other ECM proteins are also affected by this process [51]. Gene expression of proteins associated with ECM turnover also increases, with significant inductions in mRNA of TIMP-1, TIMP-3, TIMP-4, MMP-2, MMP-9, MMP-13, MMP-14, and MMP-24 [189]. Increased expression of MMP-9 is seen first in periportal hepatocytes within 3 h after PHx [11]. Another prominent pathway associated with matrix remodeling proceeds through a family of membrane-bound metalloproteinases. These proteins act in synergy with TIMP-2 to activate MMP-2. The latter is observed within 6–8 h after PHx [11]. Mice with homozygous deletion of uPA have impaired regeneration, manifesting with delays in DNA synthesis and decreased activation of HGF [52,53]. Conversely, mice with deficiency in the uPA inhibitor PAI-1 have enhanced HGF activation and accelerated regeneration [53]. Also, in mice in which the inhibitory TIMP-1 gene has been deleted, there is more active regeneration, with higher levels of activated HGF and early peak of DNA synthesis [189]. Deficiency of TIMP-3 on the other hand is also associated with deficiency in liver regeneration due to defective activation of TNF [190]. While many studies have focused on the initial remodeling of ECM after PHx, not much is known about the pathways leading to restoration of ECM at the end of regeneration.

Angiogenesis

Angiogenesis is also important to the regenerative process, and typically vessels are laid down in conjunction with new ECM. Proliferation of hepatocytes precedes that of endothelial cells, and clumps of small undervascularized hepatocytes arise at days 2–3 after PHx [191,192]. However, the latter produce a range of angiogenic factors including VEGF and angiopoietins 1 and 2 [193,194]. Increases in VEGF receptors Flt-1 and Flk-1/KDR, angiopoietin receptors Tie-1, Tie-2, and PDGFR-β are seen in isolated membranes from hepatic endothelial cells at days 2–5 after PHx. All these receptors are tyrosine phosphorylated and therefore activated during this process [195]. The period of proliferation of the sinusoidal endothelial cells is broad and stretches from day 2 to 5 after PHx. The undervascularized hepatocyte clumps are penetrated by the endothelial cells stimulated by the paracrine effects of growth factors produced by hepatocytes. This penetration is associated with rearrangement of the hepatocytes into plates and deposition of new ECM [8].

Paracrine signaling interactions between different cell types during liver regeneration

The multiplicity of signals generated during liver regeneration allow for a plethora of cell–cell interactions between the various hepatic cell types. As discussed, hepatocytes produce most ligands of EGFR, including TGF-α, amphiregulin, and HB-EGF. These ligands are likely to have effects on most adjacent cells expressing EGFR, including endothelial cells. Stellate cells express both HGF and TGF-β1 and respond to FGF-1, FGF-2 and PDGF produced by hepatocytes. HGF and TGF-β1 have effects on hepatocytes, endothelial cells, and biliary epithelium. VEGF and angiopoietins are produced by hepatocytes and act on endothelial cells, which in turn (via activation of VEGF receptor 1) produce HGF that then stimulates hepatocytes. Macrophages (Kupffer cells) produce both TNF and IL-6. This complex network of interactive cell–cell stimuli (Fig. 28.1) illustrates that liver regeneration is a highly integrated process in which all cells participate and depend on each other, so that the net result is not just replenishment of hepatocytes but formation of a complete tissue structure.

What starts and ends liver regeneration: a semispeculative view

(For further details and references on this section, see discussion of specific points in the preceding sections.) Studies of liver regeneration in many ways resemble those of astrophysicists trying to understand the origin of the Big Bang that led to the creation of the universe. While the earliest events are well tabulated and understood, the primary trigger (if such a single trigger exists) is not clear.

Initiation

With regard to liver regeneration, the earliest documented events after PHx are rising uPA activity (within 5 min), β-catenin in the nucleus (5 min), NICD in the nucleus (15 min), tyrosine phosphorylation of EGFR and cMet (30 min), activation of NF-κB (30 min), and changes in expression of many genes (within the first 30 min). While the rapid emergence of all these events is clear, it is not apparent which, if any, is the single (or multiple) trigger(s) leading to these events. PHx removes two-thirds of the liver and this is mathematically associated with a threefold increase in blood flow through the remaining hepatic lobules. It is doubtful that a mere

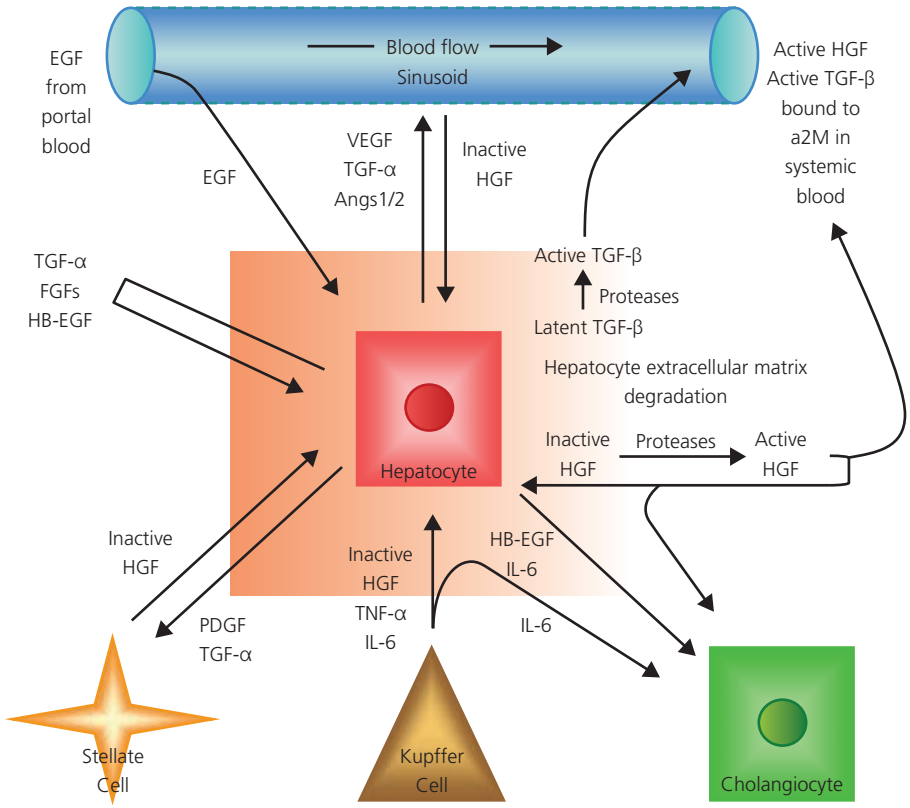

Figure 28.1 Signaling molecules contributing to liver regeneration, deriving or impinging on hepatocytes and the other hepatic cells. Hepatocytes play a key role in the process as being the first to enter into DNA synthesis after partial hepatectomy. a2M, α_2-macroglobulin; Angs, angiopoietins; EGF, epidermal growth factor; FGF, fibroblast growth factor; HB-EGF, heparin-binding epidermal growth factor; HGF, hepatocyte growth factor; IL, interleukin; PDGF, platelet-derived growth factor; TGF, transforming growth factor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

increase in flow through the liver would trigger all these events, because flow fluctuations through organs (though not as extreme as threefold) are a routine part of daily physiology. On the other hand, increased flow of blood has the consequence of increased (threefold) ratios of blood-borne signaling molecules delivered to hepatocytes. Of all the signals described above, EGF is the only one constantly present in the portal circulation in high levels, derived from Brunner glands of the duodenum. This would make EGF a good candidate for the trigger of the earliest events. A serious caveat, however, is the fact that EGFR phosphorylation is not seen before 30 min after PHx. Insulin is another well-known signaling molecule delivered to liver constantly via the portal circulation. However, insulin is not a mitogen for hepatocytes [76,115]. Insulin deprivation (after portacaval shunt) leads to hepatic atrophy, which is subsequently restored (with hepatocyte DNA synthesis and mitoses) following administration of insulin to the animals bearing the portacaval shunts [196,197]. However, insulin administration to normal livers does not cause DNA synthesis. It has been speculated that the increased flow of nutrients to the residual hepatocytes may trigger intracellular events independent of growth factors, which may in turn lead to the subsequent series of events at the level of the whole organ. While this is an attractive hypothesis, there is no known link that would connect such nutrient-driven triggers with all other known events (e.g., changes in levels of growth factors and cytokines). It is highly likely that the threefold increase in circulating growth factors and nutrients triggers the whole series of events, but the molecular signaling pathways that would connect these events to those occurring in the first 60 s of the programme that leads to liver regeneration are not yet understood. Perhaps the best perspective for understanding the dynamic process leading to the early triggers of regeneration is to investigate why hepatocytes in normal liver do not continually proliferate. Normal hepatocytes in a quiescent liver are surrounded by ECM containing both growth factors (HGF and EGF in periportal sites) and mitoinhibitors (TGF-β1 bound to decorin). The process of matrix remodeling triggered by uPA should release single-chain inactive HGF. Activation of single-chain HGF immediately after PHx is mediated by uPA. Antibodies against urokinase block activation of HGF in whole tissue regenerating liver homogenates [50]. Active HGF appears in the plasma within 30 min after PHx. Matrix remodeling also releases TGF-β1, and causes a parallel rise in plasma TGF-β1, which is inactivated by binding to α_2-macroglobulin. Thus, early matrix remodeling results in an acute change of balance between mitogenic growth factors (e.g., HGF, HB-EGF) and mitoinhibitors (TGF-β1). Alterations of this balance in resting hepatocytes in a normal quiescent liver can trigger hepatocyte DNA synthesis (Fig. 28.2). Injection of DNA constructs expressing dominant-negative TGF-β receptor 1 or dominant-negative activin receptor causes hepatocyte DNA synthesis in normal rat liver without PHx [198]. This can only be understood if it is hypothesized that normal hepatocytes are in a quiescent state because of a continued *tonic* effect between mitogens and mitoinhibitors. Inhibition of the tonic effect of the

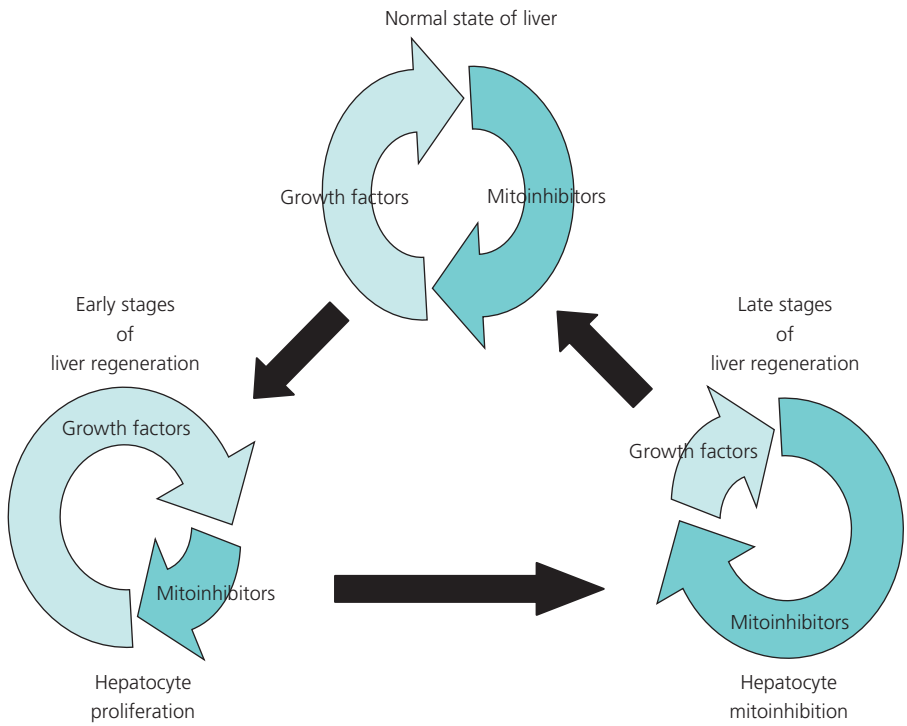

Figure 28.2 In normal (quiescent) livers, hepatocytes exist in a state of tonic balance between mitogens, such as hepatocyte growth factor (HGF) and epidermal growth factor, and mitoinhibitors such as transforming growth factor (TGF)-β1. Most of these molecules are bound to the matrix surrounding hepatocytes, especially in periportal areas of the hepatic lobule. Matrix remodeling after partial hepatectomy alters the balance between mitogens and mitoinhibitors. HGF becomes activated by urokinase and acts locally and through the plasma, whereas TGF-β1 released into the plasma is eliminated by binding to α_2-macroglobulin. Resynthesis of the hepatic matrix at the end of regeneration restores the balance between mitogens and mitoinhibitors and hepatocytes enter into a state of quiescence.

mitoinhibitor TGF-β1 (by the dominant-negative TGF-β1 receptor 1) would only cause hepatocyte DNA synthesis if the mitogens surrounding the hepatocytes were also causing a continual tonic event. Similar findings occur when follistatin, a protein that binds activin, is injected into animals [199]. The role of activin and inhibin, members of the same broad family to which TGF-β1 also belongs, has not been discussed so far. Activin is made by hepatocytes, but its role in either initiation or termination of hepatic regeneration has not been fully explored. Mice overexpressing activin in the liver have normal or accelerated liver regeneration [200].

Termination

Equally (or more) speculative are the events occurring at the termination of the regenerative process. The controversies regarding the role of TGF-β1 as the termination trigger have been discussed in the previous section. An additional study also indicated that deletion of TGF-β1 receptor II was associated with normal termination of liver regeneration [201]. However, no other single molecule has emerged as a good candidate for termination of the regenerative process. Nevertheless, many studies have shown that hepatocytes in primary culture, and stimulated to grow by HGF and EGF, stop growing when exposed to complex preparations of ECM such as collagen type I gels [202,203] or the high laminin and collagen type IV extract of EHS sarcoma known by its commercial name of Matrigel [204,205]. The effect is powerful and reversible. Disruption of the hepatic matrix by collagenase in live animals enhances the effect of HGF on hepatocytes [48], whereas perfusion of liver of anesthetized animals with collagenase (for the purpose of hepatocyte isolation) causes entry of the isolated hepatocytes into the cell cycle [204]. It is quite likely that termination of the regenerative process occurs when (and because) the hepatic ECM is restored. This would bring about the binding of, and increased concentrations of, mitoinhibitors such as TGF-β1 immediately next to hepatocytes. It would also bring about sequestration (and thus inactivation) of growth factors such as HGF and HB-EGF. The combined tonic effect of the two would counteract each other and result in a state of quiescence causing the exit of hepatocytes from the cell cycle.

Proportionality

Another intriguing aspect of liver regeneration is the proportionality of the response. Whereas two-thirds PHx triggers the grand scale of events, smaller resections result in regeneration on a smaller scale. Also, livers from small animals transplanted into large animals grow to a larger size proportionate to the size of the new body that they occupy [206]. Conversely, livers from large animals transplanted in toto into smaller animals decrease in size (by hepatocyte apoptosis) until the size becomes correct for the new animal [8,133]. Is there a *hepatostat* that determines the correct size of the liver in relation to the rest of the body and what relation would there be between such a homeostatic mechanism and the signaling pathways? The studies associated with demonstrations of proportionality involve complex surgical procedures and thus have not been easily adapted to frequently employed experimental models, even though the phenomena studied are very intriguing. Most of the signals associated with liver regeneration (e.g., HGF, EGF, TNF, IL-6, norepinephrine) normally have low but measurable levels in peripheral blood. In addition, liver is the primary site of sequestration and removal from the peripheral blood of HGF [207], EGF [73], and norepinephrine [120]. It is reasonable to hypothesize that the proportionality of the regenerative response and the adjustment of hepatic size based on body size are regulated by the same signals in peripheral blood that rise dramatically during the earliest stages of regeneration. In this scenario, even though circulating levels of these signaling molecules are normally very low, they do provide critical signaling for hepatocyte survival. A liver larger than normal would sequester and deplete these signaling molecules, thus eliminating their antiapoptotic signals and triggering hepatocyte apoptosis. A liver smaller than normal would allow plasma levels of these molecules to rise, triggering hepatic enlargement by a process akin to regeneration. In support of this hypothesis is the fact that sustained elevation of plasma HGF by injection of the protein [49,208] or forced expression via specific HGF-expressing plasmids [209] leads to hepatic enlargement and doubling of liver weight in normal mice. This has not been shown to the same extent for the other signaling molecules, but injection of EGF and TGF-α is associated with DNA synthesis in normal livers, and injection of TNF enhances the effect of coinjected HGF and causes DNA synthesis in hepatocytes [210]. Thus, HGF, EGF, TNF, IL-6, norepinephrine, and perhaps other currently unknown signaling molecules in the peripheral blood do collectively function as a hepatostat that regulates liver mass in proportion to body size.

Alternative pathways to liver regeneration: oval cells and stem cells

Hepatocytes are at the center of the regenerative process following PHx, and they produce and receive multiple paracrine and endocrine stimuli that interact to restore whole hepatic tissue. There are circumstances, however, both in experimental and clinical settings, during which hepatocytes are unable to participate and drive the regenerative process. Experimentally, hepatocyte proliferation can be blocked using genotoxic agents that generate bulky DNA adducts, such as acetylaminofluorene (AAF), or agents that cross-link DNA, such as retrorsine [211–213]. Treatment with these reagents prior to PHx prevents hepatocytes from entering into the cell cycle. Under these circumstances, a large population of small cells with oval-shaped nuclei emerges in the

periportal areas. These cells, named *oval cells* after the shape of their nucleus, proliferate actively and dominate the immediate periportal areas from days 3–10 after PHx. Immediately after their appearance, they express genes and proteins of both biliary and hepatocytic specificity [6,214–220]. For example, they express cytokeratins 7 and 19 (biliary markers) as well as α-fetoprotein (a marker of embryonic hepatocytes) and albumin, a marker of adult hepatocytes. They tend to be arranged in infiltrating sheets or form small ducts. They infiltrate deeply into the lobule, reaching into the centrilobular areas. They eventually transform into small and then normal-size hepatocytes and repopulate the entire lobule [221–223]. Infiltration of oval cells into the lobule is accompanied by concurrent proliferation of stellate cells synthesizing matrix and expressing HGF and SCF. Oval cells themselves express cMet as well the SCF receptor (c-Kit) [224,225]. In addition, oval cells also express certain hematopoietic markers (e.g., Thy-1) [214], not seen normally in either bile duct cells or hepatocytes.

The origin of these cells has been much debated. The preponderance of evidence strongly suggests that these cells are derived from the biliary compartment, most likely from the small extraportal ductules linking hepatocytes with the intraportal bile ducts. These ductules are known as canals of Hering. The evidence for the biliary origin of oval cells is as follows.

- Following PHx and administration of AAF, hepatocyte-associated transcription factors are expressed in the portal biliary ductules within days [226]. This suggests that the biliary compartment is undergoing gene reprogramming toward the hepatocytic lineage.
- Administration of the biliary toxin methylene diamiline (DAPM) prior to AAF and PHx eliminates the appearance of oval cells [227].

These findings demonstrate that under circumstances in which hepatocytes cannot proliferate, biliary epithelial cells can function as facultative stem cells for hepatocytes. Recent evidence shows that the opposite phenomenon is also true. When proliferation of biliary epithelium is blocked (e.g., by DAPM) and biliary epithelium needs to repair by proliferation (e.g., following bile duct ligation), hepatocytes of the immediate periportal area transform into biliary epithelium and become biliary ductules. Up to 50% of the biliary epithelium can be replaced by cells derived from hepatocytes under these circumstances [228,229]. These findings demonstrate that under some specific circumstances, hepatocytes can function as facultative stem cells for biliary epithelium. The term *facultative* implies that the cells that are the source of the apparent stem cells do not perform a stem cell function as their full-time mission. These cells (hepatocytes and biliary epithelium) are fully differentiated and perform specific functions. However, they can differentiate from one cell to another through a gradual process of transient amplification in cell numbers (Fig. 28.3). These findings raise an obvious question: are there true hepatic stem cells? Few issues have

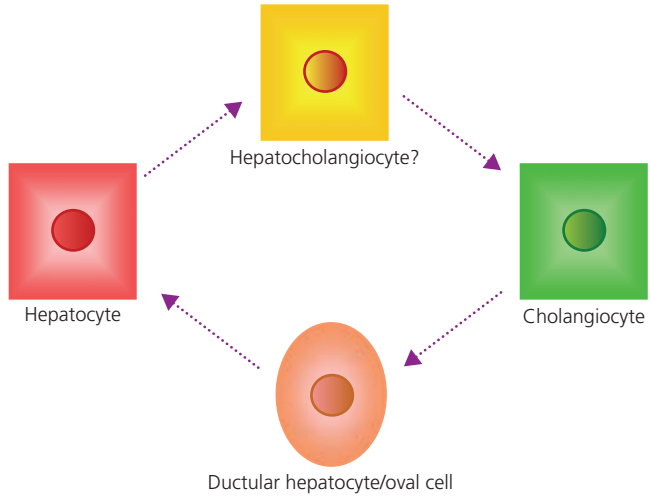

Figure 28.3 Hepatocytes and biliary epithelium (cholangiocytes) function as facultative stem cells for each other by undergoing transient redifferentiation and amplification of the cell population. These pathways allow restoration of cellular compartments whose proliferative capacity is impaired, as in hepatocytes during fulminant hepatitis or cholangiocytes during chronic immune attack (e.g., primary biliary cirrhosis or chronic rejection posttransplant).

divided liver biologists as this one. Of the two alternate views, one suggests that oval cells are derived from true hepatic stem cells, which perform no other function and whose mission is to rescue hepatic tissue under circumstances of blocked hepatocyte proliferation [230,231]. The alternative view is that true hepatic stem cells do not exist and that both hepatocytes and biliary epithelium can function as facultative stem cells for each other [228]. The majority of the evidence supports the second alternative. Also in support of the absence of true, full-time hepatic stem cells is the obvious comparison between the histology of liver and other tissues. Examination of the histology of the liver does not reveal a group of cells that correspond to full-time hepatic stem cells. Such cells are readily seen in the intestine, skin, ependyma of the brain, and bone marrow. This is not the case for the liver.

The capacity of one hepatic epithelial cell to undergo transdifferentiation into the other is very important in a clinical setting. Proliferation of cells with properties akin to oval cells is often seen in human histopathology. In the human, these cells are called *ductular hepatocytes* [232,233]. They appear in situations of massive hepatic injury, such as after administration of large amounts of toxins like acetaminophen, and overwhelming viral infections (hepatitis B or C virus). These cells are seen most obviously during the course of fulminant hepatitis [234]. Most clinical situations that induce liver damage and which therefore require regeneration of hepatic parenchyma do not result from clean hepatic resections. Viruses and toxins are likely to interfere with the capacity of hepatocytes to proliferate. The activation of transient transdifferentiation and amplification of biliary epithelial cells so

that they transform into hepatocytes may be the only pathway to rescue the liver until the proliferative capacity of the hepatocytes is restored. Biliary epithelium is also chronically damaged, most often from immune attack (e.g., primary biliary cirrhosis or biliary epithelium loss in transplanted livers during chronic rejection). Under these circumstances, hepatocytes in the immediate periportal area often express biliary markers, suggesting that an attempt is being made to rescue the biliary epithelium by transdifferentiation of hepatocytes [235].

Often forgotten in this debate is the vast clonogenic potential of the mature adult hepatocyte. This is most aptly demonstrated in rodent models. An extreme example is that of mice with fumarylacetoacetate hydrolase (FAH) deficiency. These mice cannot properly degrade tyrosine and die soon after birth. Administration of 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) prevents accumulation of the toxic metabolites resulting from lack of function of FAH, and mice can be kept alive for a normal lifespan. When NTBC is withdrawn, hepatocytes undergo massive cell death (from necrosis and apoptosis) inducing fulminant liver failure and death of the animal. Injection of normal (FAH-positive) hepatocytes during fulminant hepatic failure results in massive recolonization of the liver by FAH-positive hepatocytes. (Typically the donor of the normal FAH-positive hepatocytes also bears a transgene such as β-galactosidase, which allows FAH-positive hepatocytes to be stained blue by a chemical reaction specific to β-galactosidase.) Livers of the first generation of rescued mice were used as the source of FAH-positive hepatocytes to rescue a second generation of FAH-deficient mice subjected to liver failure. These rescued mice were again used as the source of FAH-positive hepatocytes to rescue a third generation of FAH-deficient mice from liver failure, and so on. This was done for 10 serial rescues and FAH-positive hepatocytes derived from a single original donor mouse were able to rescue liver failure indefinitely. It was estimated from these studies that, at least for the mouse, one hepatocyte could generate enough progeny for 50 mouse livers [236,237]. The vast clonogenic potential of hepatocytes is rather unusual for a fully differentiated cell, and provides a future opportunity for transplanting hepatocytes to recolonize and rescue human livers under conditions in which endogenous hepatocytes are permanently damaged [238].

Another issue that has received much attention is the possibility that hepatocyte precursors or oval cells are generated from cells derived from the bone marrow [239,240]. Injection of bone marrow cells from FAH-positive mice into FAH-deficient mice subjected to liver failure rescued the mice by the emergence of FAH-positive hepatocytes derived from bone marrow precursors, probably from the myelomonocytic series. Subsequent studies demonstrated that FAH-positive hepatocytes that emerged from the injected bone marrow cells did not derive from transdifferentiation of bone marrow cells but from fusion of FAH-positive bone marrow cells with FAH-deficient hepatocytes [241]. Fusion between the two cell types restored the missing functional FAH gene and converted specific FAH-deficient hepatocytes into FAH-positive fusion hybrids. The fusion products rapidly populated and restored the entire liver, as though there had been an injection of true hepatocytes. Currently, there is convincing evidence that most if not all cases in which bone marrow cells apparently transdifferentiate into other cell products (e.g., muscle, neurons) are due to fusion between bone marrow cells and targets in cells of the peripheral tissues. Claims that hepatocytes in transplanted livers are replaced to a large extent by bone marrow cells of the recipient [242] have not been verified by other groups [243].

Clinical aspects of liver regeneration: cirrhosis and fulminant hepatitis

Hepatocytes proliferate to restore loss of hepatocytes. Such loss occurs to some extent in most aspects of liver disease. The proportion of hepatocytes leaving the quiescent state and entering into the cell cycle can be easily monitored by performing immunohistochemistry for a cell cycle marker such as PCNA or Ki67. No more than 1%–5% of hepatocytes are labeled in a normal resting liver. The proportion of PCNA-positive hepatocytes can easily rise to 20%–50% even with a mild increase in the number of dying hepatocytes [2]. In clinically obvious cases of viral hepatitis, in which it is estimated that eventually 90% of hepatocytes die [3], PCNA-positive cells can reach 95% of the population. The same high percentages are seen in cirrhotic nodules of cirrhosis due to chronic toxins, such as alcohol. In cirrhosis due to metabolic etiologies, e.g., hemochromatosis or α_1-antitrypsin deficiency, there are typically two populations of hepatocytes. One population bears the brunt of the metabolic defect (iron deposition, accumulation of α_1-antitrypsin globules); typically, in this subpopulation the proportion of cells in the cell cycle is low. However, a second subpopulation of hepatocytes is always seen which represents cells that are much less affected. In this subpopulation of hepatocytes, the proportion of cells in the cell cycle is very high [244,245]. It is notable that hepatocellular carcinomas emerging in the context of these types of cirrhosis typically comprise neoplastic cells that do not carry the metabolic defect [246]. Neoplasia is often associated with allelic loss. Elimination of some specific gene alleles may confer some protection to the preneoplastic cell (e.g., deficiency in iron uptake in the context of hemochromatosis, loss of α_1-antitrypsin genes) such that it becomes protected from the offending metabolic agent. This setting of *differential resistance* [247,248] tends to selectively promote growth of specific preneoplastic cell populations and eventually leads to neoplasms that frequently do not carry the metabolic defect.

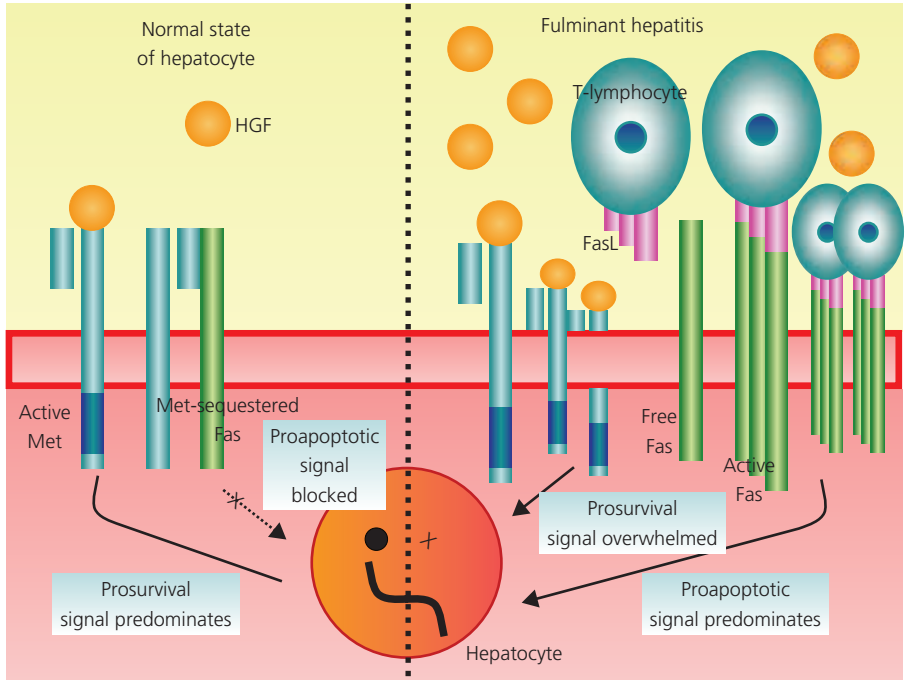

Figure 28.4 Effect of high hepatocyte growth factor (HGF) concentrations during fulminant hepatitis. HGF receptors (cMet) are normally in excess of available HGF and the surplus of cMet molecules dimerize and sequester the apoptosis-inducing receptor Fas. When HGF levels rise to very high levels during fulminant hepatitis, cMet receptors become fully occupied by HGF, and Fas is released and becomes available to form trimers, which can bind Fas ligand and induce apoptosis in hepatocytes.

Fulminant hepatitis remains a puzzling situation from the point of view of regeneration. It is often assumed that fulminant hepatitis is caused by failure of the hepatocytes to regenerate. However, this is untrue. The PCNA labeling index in fulminant hepatitis reaches almost 100% [2]. It is conceivable that the offending agent leading to fulminant hepatitis is so robust that cells die before they proliferate. Supporting evidence for this inability of hepatocytes to effectively proliferate is the increase in numbers of transiently amplifying and transdifferentiating ductules (ductular hepatocytes), which express hepatocytic markers such as albumin, α-fetoprotein and hepatocyte-associated transcription markers such as HNF4 (Michalopoulos G, unpublished observations) [234]. Another way to assess the signaling dynamics of fulminant hepatitis is to examine the role of HGF. The latter reaches extremely high levels during fulminant hepatitis [249–252]. There is an inverse correlation between HGF levels and prognosis of the patient. The mechanism for this paradox is not entirely clear. However, it has been shown that under normal circumstances, the HGF receptor (cMet) dimerizes with the apoptotic receptor Fas on hepatocyte plasma membranes [40]. This prevents Fas from forming trimers and becoming activated by Fas ligand. Typically, receptor occupancy of 10%–20% is sufficient to exert the maximal effect. This would leave the residual receptors unbound to HGF and bound to Fas. HGF displaces Fas from binding to Met. When HGF concentrations reach extremely high levels, it is likely that most of the Fas bound to Met is released and available for activation by Fas ligand and induction of apoptosis. In this scenario, extremely high HGF concentrations may have a deleterious effect on hepatocytes and further accentuate hepatocyte death instead of promoting regeneration (Fig. 28.4).

Augmentative hepatomegaly

This phenomenon is seen in experimental and clinical settings with rodents or humans receiving a variety of lipophilic drugs such as barbiturates, dilantin, diazepam, and peroxisome proliferators. Treatment with these agents induces a dose-dependent enlargement of the liver, which may reach up to 80% above normal size [253–255]. The same phenomenon is seen in some physiological conditions, such as pregnancy or hyperthyroidism [256]. The mechanisms mediating this response are not clear. Most of the molecules inducing this phenomenon (including estrogens) are ligands for nuclear hormone receptors. Several studies have shown that activation of this pathway does not proceed through the typical cell cycle-related events seen during liver regeneration [257–259]. The hepatomegaly is achieved through a mixture of hepatocyte proliferation and hepatocyte enlargement [260]. The proportion of each process that is utilized varies from one agent to another, suggesting a multiplicity of pathways. There is more hepatocyte hypertrophy than hepatocyte proliferation in the response induced by barbiturates. Barbiturates activate the constitutive androstane receptor (CAR) and the pregnane X receptor (PXR). CAR appears to be the chief mediator of signaling leading toward hepatocyte proliferation, but the pathways are not clear [261,262].

Withdrawal of the agents in experimental settings is associated with a gradual decrease of the liver to normal size through a wave of apoptosis. While the gene expression patterns used during this phenomenon do not mimic those of classic regeneration, some of the proteins involved in regeneration may still play a role. For example, estrogens act very similarly to norepinephrine in hepatocyte cultures by enhancing the mitogenic effect of HGF and EGF and by antagonizing the mitoinhibitory effect of TGF-β [263]. It is not clear how this effect relates to activation of the estrogen receptor by estrogens. Augmentative hepatomegaly is also induced (in rodents, but not clearly in humans) by compounds that are members of the class of peroxisome proliferators [264]. These compounds activate the peroxisome proliferation associated receptor (PPAR)-α, -β, -γ, and -δ to various degrees. Hepatocyte proliferation is very prominent in this response. It is interesting that the enlarged livers induced by all xenobiotics respond poorly to two-thirds PHx. Several growth factor receptors (EGFR) are downregulated by treatment with phenobarbital, whereas there is an increase in expression of TGF-β1 receptors [265–267].

Augmentative hepatomegaly has serious clinical implications and should be not overlooked. Hepatic enlargement changes the ability of the liver to metabolize drugs. The larger size of the liver implies that some of the doses need to be adjusted upward to compensate for the increased degradation of drugs by the enlarged liver. However, this cannot be done across the board, because augmentative hepatomegaly is also typically associated with increase in many members of the CYP family of enzymes, which metabolize lipophilic drugs and often generate free radicals. This renders individuals with augmentative hepatomegaly very sensitive to drugs that can be otherwise tolerated by normal individuals [268]. Typical is the hypersensitivity of humans receiving phenobarbital to small doses of acetaminophen and the occurrence of fulminant hepatic failure at high frequency in patients receiving both drugs.

Conclusions

Liver regeneration is a signature hepatic response to all situations associated with loss of hepatic parenchyma. As with all vital functions perfected through millions of years of evolution, the pathways leading to the phenomenon have sufficient redundancy to guarantee the response regardless of any signaling defects. Key players in the phenomenon are the receptors of the growth factors HGF and EGF. However, multiple cytokines, including TNF, IL-6 and norepinephrine, as well as other receptors such as Notch, are also involved in optimizing the early and later stages of the response. The net result is proper maintenance of liver mass in relation to body weight and adjustment of liver mass to sufficient size to guarantee responses to changing homeostatic needs.

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Molecular biological approaches to the diagnosis and treatment of gastrointestinal diseases

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The development and application of the science and techniques of molecular biology has had a profound impact on the diagnosis of gastrointestinal diseases. Furthermore, molecular biology has revolutionized the conceptual approaches to these diseases. An understanding of the regulation of gene expression encompasses key concepts: deoxyribonucleic acid (DNA) transcription, ribonucleic acid (RNA) translation, posttranslational control, and the effects of DNA mutations on gene expression.

DNA

The genetic material of all known organisms and many viruses is DNA, which consists of chemically linked subunits. Each subunit, called a nucleotide, contains a nitrogenous base (a purine [adenosine and guanine] or pyrimidine [cytosine or thymine]), a pentose sugar, and a phosphate group (Fig. 29.1). Nucleotides are linked by phosphodiester bonds forming single-stranded nucleic acids.

The DNA double helix consists of two chains that are complementary and antiparallel. They are complementary because adjacent nucleotides always pair in a specific way: adenine to thymine and guanine to cytosine, so that the base sequence of one strand determines that of the other (see Fig. 29.1). They are antiparallel because the 3′ to 5′ orientation of the sugar–phosphate backbone of one chain is opposite the 5′ to 3′ orientation of the other chain.

Transcription

Transcription is the synthesis of RNA from its DNA template and is catalyzed by DNA-dependent RNA polymerases. A gene includes all DNA sequences required for the synthesis of the RNA, including transcribed and regulatory sequences. Promoters are the regulatory sequence immediately 5′ to the transcription unit and are required for proper transcription initiation. Enhancers are orientation- and position-independent regulatory sequences that increase the transcription rate of a given transcription unit.

Translation

Translation is the synthesis of proteins from the information encoded in its messenger RNA (mRNA). The genetic code consists of groups of three nucleotides called codons that specify the amino acid sequence or define sites for the initiation or termination of translation.

Techniques in molecular biology

Enzymes used in molecular biology techniques

Most of the methodology used in molecular biology involves the application of specific enzymes. These enzymes can be classified into four groups: restriction endonucleases, polymerases, ligases, and modifying enzymes. Many of these enzymes have been cloned and changed by mutation to enhance performance and reduce cost.

The restriction endonucleases comprise a large group of enzymes that recognize specific nucleotide sequences and cleave double-stranded DNA at precisely these locations. Restriction endonucleases are found naturally in bacteria and are important for degrading foreign DNA. The cleavage sites recognized by the restriction endonucleases are typically four to eight nucleotides in length. Digestion products may have either 5′- or 3′-overhanging nucleotides, also called cohesive termini or sticky ends, or the digestion may result in blunt-ended molecules. Joining ends of DNA is accomplished with DNA ligases. DNA ligases catalyze the formation of a

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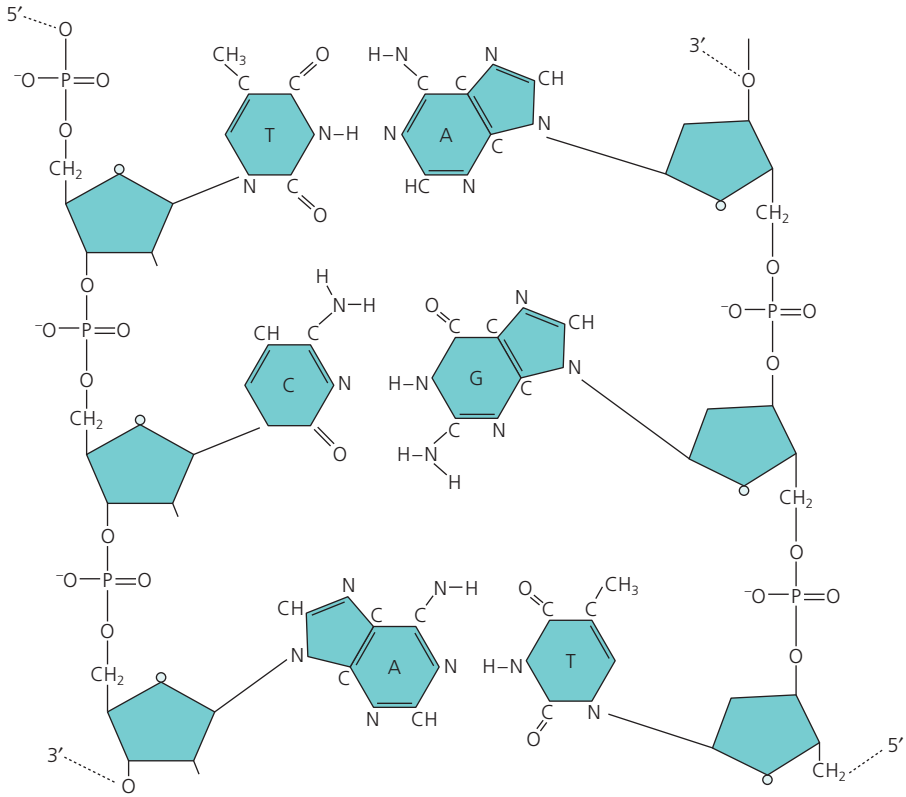

Figure 29.1 DNA double helix. A nucleotide consists of the deoxyribose sugar and phosphate backbone with a nitrogenous base (A, C, G, or T). Adenine (A) always pairs with thymidine (T), and guanine (G) always pairs with cytosine (C). The two nucleotide chains run in antiparallel directions, one strand runs in the 5′ to 3′ direction, and the other strand runs in the 3′ to 5′ direction. From Rippe et al. [78].

phosphodiester bond between the 5′ phosphate and the 3′ hydroxyl ends in duplex DNA. Recombinant DNA molecules can be generated efficiently by digesting double-stranded DNA with appropriate restriction endonucleases, isolating the pieces of interest, and ligating the pieces to create recombinant molecules.

Another major group of enzymes is composed of the polymerases, which catalyze the synthesis of nucleic acids from nucleic acid templates. These enzymes are classified by the type of nucleic acid that is synthesized and the type of nucleic acid template that is replicated. All polymerases synthesize DNA in a 5′ to 3′ direction and complementary to the template strand. The DNA-dependent DNA polymerases synthesize DNA from DNA templates. In addition to synthesis activity, many of the polymerases possess exonuclease activity, an enzymatic property that removes nucleotides one at a time from the ends of the polynucleotide chain, thereby functioning as a biological "proofreader". The exonuclease activity has been used extensively in the laboratory to label double-stranded DNA uniformly.

Another group of DNA-dependent DNA polymerases is used extensively in the polymerase chain reaction (PCR) – the heat-stable DNA polymerases, such as *Taq I*. Originally isolated from *Thermus aquaticus*, *Taq I* has been cloned and engineered genetically to improve its performance. This enzyme is active over a broad temperature range and is stable with repeated temperature cycling.

The RNA-dependent DNA polymerases synthesize a complementary DNA (cDNA) copy of an RNA template. These enzymes are called reverse transcriptases and typically are derived from either retroviruses or the Moloney murine leukemia virus. Reverse transcriptase creates cDNA copies of a population of mRNA molecules. Single-stranded cDNA molecules are converted into double-stranded DNA and cloned into appropriate vectors to generate cDNA libraries. These cDNA libraries are used to isolate genes of interest.

Blotting techniques

Nucleic acids and proteins are readily detected by fixing these molecules to a nitrocellulose membrane and identifying them by hybridization with specific probes. The detection of specific DNA sequences was described originally by E.M. Southern in 1975 [1], and subsequently termed Southern blotting. This technique was modified for the detection of RNA sequences and named Northern blotting. Specific proteins are detected by Western blotting.

Southern blotting

Southern blotting involves digesting high-molecular weight DNA (e.g., genomic DNA) with restriction endonucleases and separating the generated DNA fragments according to size by agarose gel electrophoresis. The DNA fragments are denatured in the gel into single-strand DNA and subsequently transferred (blotted) from the gel to a solid support,

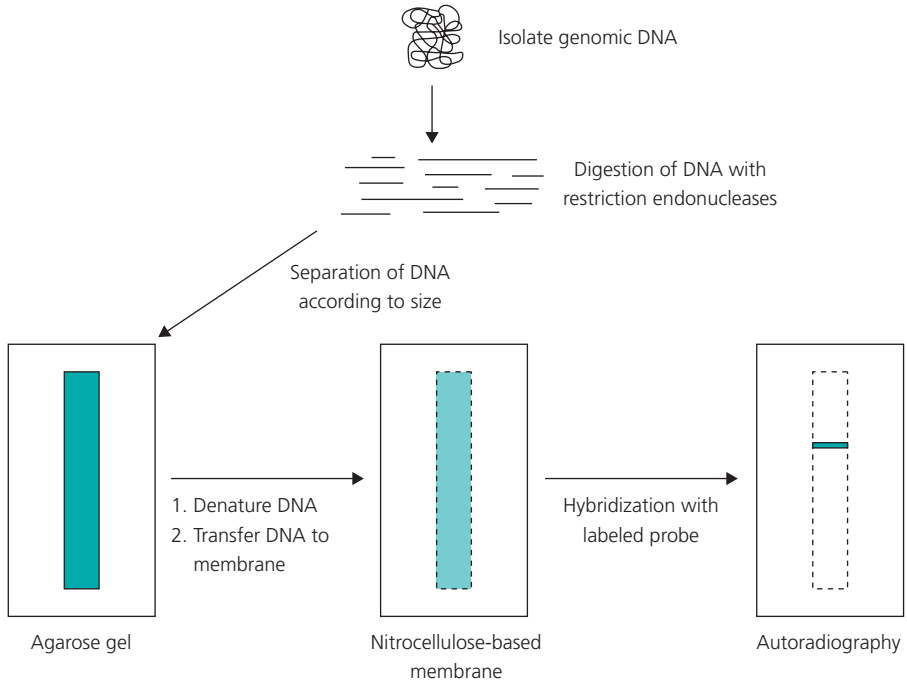

Figure 29.2 Southern blotting. Detection of specific DNA sequence in a complex mixture of DNAs (such as genomic DNA) by hybridization with a labeled probe. The hybridization depends on the normal base-pairing of the probe nucleic acid with the complementary genomic DNA sequence. From Rippe et al. [78].

typically a nitrocellulose membrane. The transferred DNA and a radiolabeled DNA or RNA bind to each other in complementary base-pair fashion to form a hybrid, a process called hybridization. The radiolabeled signals are detected by autoradiography, through exposure to x-rays (Fig. 29.2).

Northern blotting

Northern blotting is an adaptation of the Southern blotting method and is used for the detection of RNA. RNA is isolated, separated by electrophoresis in formaldehyde–agarose gels, transferred to nitrocellulose membranes, and hybridized to specific RNA or DNA probes. Standards of known size allow estimation of the size of RNA of interest. This technique measures the steady-state levels of a particular mRNA under set experimental conditions. Steady-state mRNA levels do not, however, indicate the stage at which gene expression may be regulated. Some conditions may affect the rate at which the gene is transcribed, whereas other conditions may affect the turnover rate, or stability of the mRNA molecule.

Western blotting

Western blotting (immunoblotting) is a method used to detect and quantify specific proteins using antibodies. Moreover, Western blotting can be applied to determine posttranslational modifications, such as phosphorylation, acetylation, or nitrosylation, which often reflect changes in protein activity or function. The procedure involves extracting proteins from a given source, separating the proteins based on their size by electrophoresis in a polyacrylamide gel, and transferring them from the gel to a nitrocellulose membrane. Once transferred, the proteins are spotted for binding to a specific antibody. A primary antibody binds to the protein of interest and is subsequently detected using a secondary antibody complexed to an enzymatic detection system. A substrate that emits light or fluoresces when activated by the enzymatic detection system allows visualization of the antigen–antibody complexes (Fig. 29.3). The radioimmunoassay and enzyme-linked immunosorbent assay techniques were derived in principle from the Western blotting method.

In situ hybridization and immunohistochemical detection

The study of gene expression often requires the detection of proteins or mRNAs within single cells or tissues (in situ analysis). In situ hybridization examines the expression of mRNA whereas immunohistochemical detection indicates the presence of specific proteins. In situ hybridization provides a sensitive method to locate specific cells that express a gene of interest. The technique involves fixing tissue sections to slides, treating the sections with reagents to increase cellular permeability, and then hybridizing the tissue with either labeled RNA or DNA probes. Probes labeled with enzymes or fluorescent tags (fluorescent in situ hybridization) afford excellent resolution and rapid detection, and their use avoids the safety problems associated with radioactive isotopes. In situ hybridization has been used successfully to localize genes on chromosomes, to identify tissues and specific cells in which viruses are replicating, and to locate cells that express messages in low abundance.

Immunohistochemical analysis allows for the localization of proteins to individual cells within tissue samples or to subcellular compartments. Similar to other protein detection

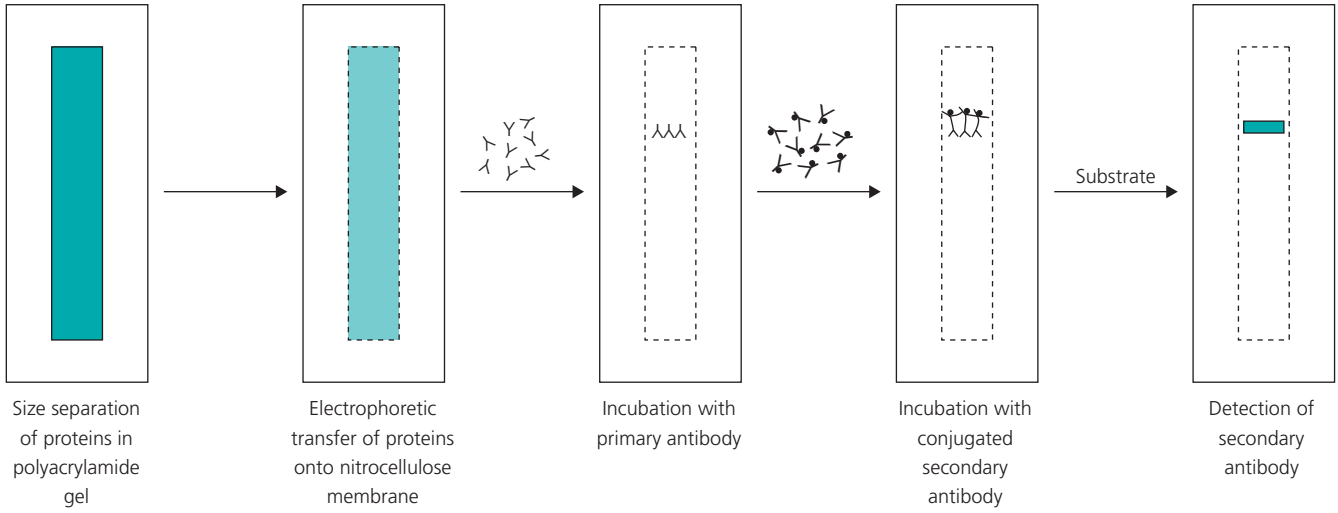

Figure 29.3 Western blotting. Detection and quantification of specific proteins using antibodies. Proteins are separated by size on a polyacrylamide gel and transferred electrophoretically to a nitrocellulose membrane. The primary antibody binds to the protein of interest, and the secondary antibody binds to the primary antibody. The secondary antibody contains a detection system that allows identification of the proteins. From Rippe et al. [78].

methodologies, immunohistochemical analysis uses specific antibodies to bind to the protein of interest, followed by antigen–antibody detection using secondary antibodies directed against the primary antibody.

DNA sequencing techniques

Many applications of molecular biology require determination of the DNA sequence, such as a gene or cDNA. The more popular method of sequencing DNA is called dideoxy sequencing or chain termination sequencing, as originally developed by Sanger and Coulsen [2]. This method is based on the ability of DNA polymerase to synthesize a complementary strand of DNA from a template. Specific complementary primers are hybridized to the DNA template and then DNA polymerase is added to allow elongation of the synthesized strand. Four reactions, each containing four deoxynucleotide triphosphates (dNTPs), one of which is radiolabeled and one of which is a dideoxynucleotide triphosphate (ddNTP), are performed. When the polymerase incorporates the ddNTP in the newly synthesized DNA strand, chain elongation terminates. When one of the four possible ddNTPs (ddATP, ddCTP, ddGTP, ddTTP) is included in each of four reactions, and the reaction products are separated in electrophoretic sequencing gels, a typical sequencing ladder is obtained from which the nucleotide sequence can be read easily. Dideoxy sequencing is automated. The success of the human genome project depended on high-throughput automated DNA sequencing.

Polymerase chain reaction

PCR allows for the rapid amplification of small amounts of nucleic acids [3]. The numerous clinical applications include diagnostic detection of infectious agents, tissue typing, prenatal diagnosis of genetic diseases, and analysis of allelic sequence variations. In the laboratory, PCR methodology is used for DNA sequencing, cloning, gene isolation, and analysis of gene expression.

The basis of PCR is simple but elegant (Fig. 29.4). Two primers are designed that are complementary to the flanking regions of the sequence to be amplified. The primers are added to the template DNA along with a DNA-dependent DNA polymerase that allows new chain synthesis. The reaction mixture is heated to denature (separate the strands) the template DNA, followed by cooling to allow for hybridization of the primers to the template. The DNA polymerase is then allowed to synthesize complementary strands from each primer. Adding the primers in vast excess and repeating the cycles of denaturation, annealing (binding of the primer to the template), and synthesis result in an exponential rate of amplification of the target sequence. The efficiency of PCR has been improved with the discovery of heat-stable DNA polymerases, like *Taq* polymerase, and newer genetically altered enzymes. These heat-stable polymerases retain their enzymatic functions despite thermal cycling. The detection of PCR products has classically been achieved by the addition of ethidium bromide, which intercalates DNA and fluoresces in this hydrophobic environment when exposed to ultraviolet radiation. In quantitative real-time applications, PCR products are detected either by specific probes that fluoresce after binding or nonspecifically by the intercalating fluorescent stain SYBR green, and are monitored after each cycle. Comparison to a known standard, e.g., defined amounts of plasmid or a virtual standard using cDNA samples, allows absolute or relative quantification of PCR products in the linear phase of the PCR. The PCR technology has been used extensively for the detection of genetic mutations in patients.

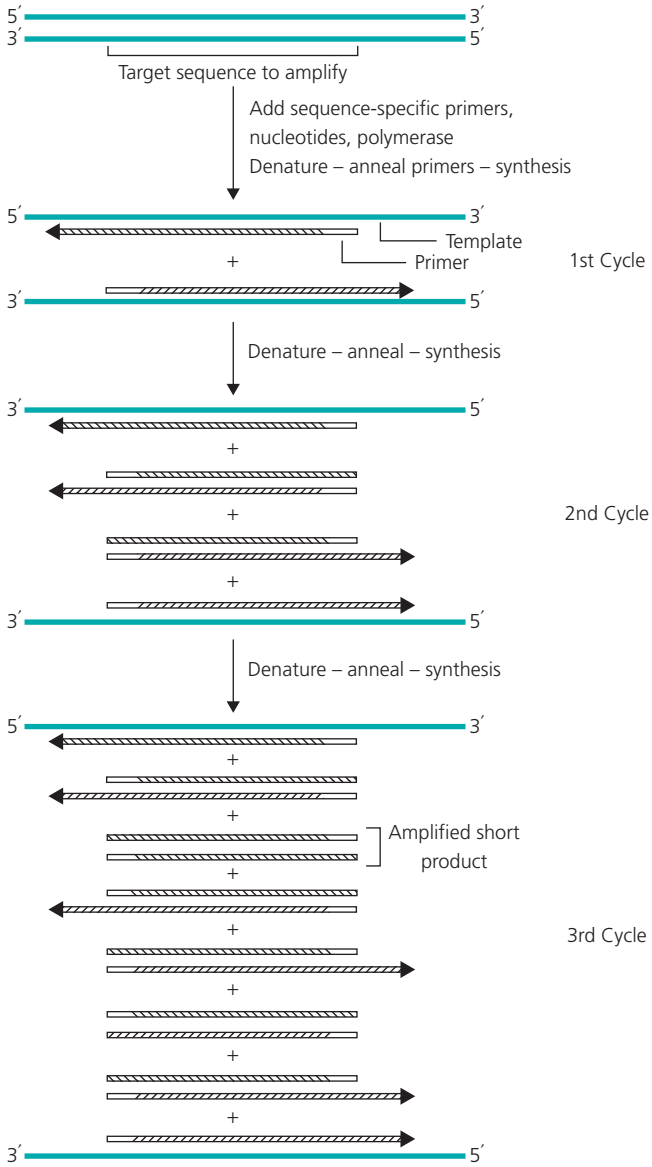

Figure 29.4 Amplification of DNA by the polymerase chain reaction (PCR). The template DNA contains the target sequence of interest. Specific DNA primers hybridize to the 5′ ends of the target sequence. The reaction also requires a heat-stable DNA-dependent DNA polymerase (such as *Taq I*) and four deoxynucleotides. Repeated cycles of denaturation (usually at 94°C), annealing of primers to template DNA, and DNA synthesis (usually at 72°C) results in the exponential amplification of the target DNA. From Rippe et al. [78].

Microarrays

Microarray technology allows the quantification of an extremely large number of transcripts in a specific sample to detect differences in gene expression. Previously, the analysis of gene expression was conducted one gene at a time (e.g., by Northern blotting or PCR as previously discussed). Microarray technology is based on the ability to create chips that contain thousands of probes to detect and quantify the presence of specific targets in a sample. The probes may be cDNAs that are amplified from plasmids or oligonucleotides synthesized onto the array matrix. Microarray analysis of gene expression consists of four steps:

- generating labeled cRNA from a specific tissue or cell
- hybridization of cRNA to the array
- scanning of the chip
- data analysis.

Microarray-based technology is not restricted to the detection of RNA expression, but can also be used to detect single nucleotide polymorphisms (SNPs) (see section on SNPs), protein binding to promoters (ChIP-chip technology, i.e., chromatin immunoprecipitation combined with gene microarray technology), and genomic changes (comparative genomic hybridization) in DNA samples.

For microarray-based gene expression analysis, labeled cRNA is generated after reverse transcription of RNA into a cDNA and a second step in which cDNA is transcribed into cRNA labeled either with radioactivity or a fluorescent label. If different fluorescent labels are used for the experimental and control RNA, then both targets may be incubated on the same microarray. The labeled targets are then hybridized to the probes, which enable the complementary target and probe sequences to bind specifically. The array is then washed to remove nonhybridized probes, reduce nonspecific hybridization, and minimize background. The specific hybridization is then detected by quantifying the amount of label that has annealed to the probe (i.e., either radioisotopic or fluorescence detection) (Fig. 29.5). Biological and technical variation can mask the effects on gene expression so quality checks are required at different steps; e.g., before the chip analysis by assessing RNA quality and during data analysis to identify bad hybridization, corrupt chips, and RNA degradation, which all create typical patterns [4]. Microarray array analysis generates a vast amount of data. To detect those genes that show reproducible differences is the most basic and important step of microarray analysis. A large number of different methods are applied and their success largely relies on a sufficient number of replicates. Microarray analyses generate unusually large sets of data so standard tests (e.g., *t* tests) are not appropriate because they will identify a large number of genes just by chance (e.g., 500 genes in a 25 000-probe microarray using a *P* value of 0.05). The commonly applied statistical algorithm in microarray analysis is the false discovery rate described by Benjamini and Hochberg [5]. Differentially expressed genes are usually displayed as a color-coded heat map with one color showing up-regulated genes and another color showing down-regulated genes. To achieve easier interpretation, differentially expressed genes are often placed clusters of similarly expressed genes or functionally related genes. Using the gene ontology database and specific software tools, further information regarding biological functions and activation of specific pathways can be extracted from the data.

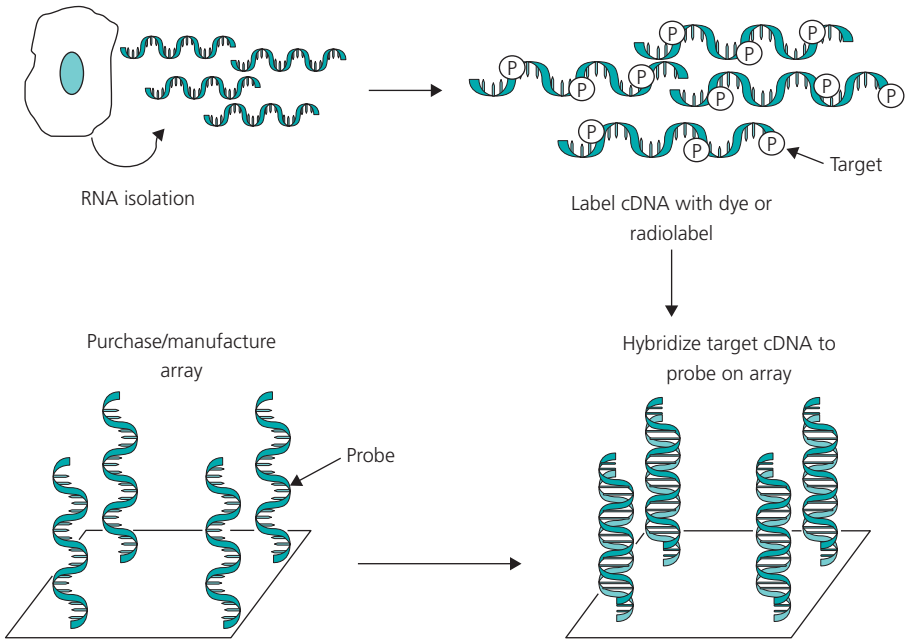

Figure 29.5 DNA microarrays for studying gene expression. RNA from a tissue of interest is converted into a labeled cDNA probe and hybridized to thousands of genes. The relative expression of genes representing the entire human genome can be detected on a single chip.

Microarray analysis is an extremely powerful tool to expand our understanding of the biology of liver disease. Micoarray-based gene expression profiling of diseases, such as infection with hepatitis C virus, nonalcoholic steatohepatitis, and hepatocellular carcinoma, has led to the identification of genes that are abnormally expressed in these diseases and may thus provide the basis for novel therapeutic approaches [6]. Profiling of the response to interferon in patients infected with hepatitis B virus or hepatitis C virus has provided insight into the therapeutic action of interferons [7]. Gene expression profiling has facilitated the classification of hepatocellular carcinoma into different groups, and consequently, enabled the prediction of survival [8]. Researchers have identified a novel subtype of hepatocellular carcinoma that is derived from hepatic progenitors and carries a poor prognosis [9]. It is likely that gene expression profiling and genomic characterization of hepatocellular carcinoma will enter clinical medicine to further classify patients to allow tailored therapies.

Applications of molecular biology to the clinical laboratory

Clinical laboratories are rapidly developing approaches to detect a variety of mutations. The different technical approaches available may be categorized as either targeted or screening mutation detection strategies.

Targeted mutation detection entails straightforward and largely routine procedures by which DNA samples may be analyzed for previously identified mutations using an assay designed for maximum specificity. This approach targets known mutations in potentially large cohorts of clinically indicated patients as well as in disorders marked by one or a few common alleles, and may confirm or establish clinical diagnoses. Furthermore, in families at risk for a particular genetic disease, targeted mutation detection allows for rapid screening of an entire family for the mutation identified in the proband, thereby permitting accurate carrier determinations. Rapid testing of large numbers of patients also permits an assessment of the mutation's frequency among disease-causing alleles, thereby determining which mutations are most prevalent in different patient populations and guiding the creation of effective clinical mutation testing panels.

By contrast, mutation screening analyzes genes for all the sequence variants present. By definition, these strategies are not predicated on allele specificity but rather are designed for highly sensitive detection for all possible variants. In principle, all sequence variants present will be detected without regard to advance knowledge of their pathogenic consequences. If evidence for a sequence variant is found, the sample must be sequenced to identify it fully. Only when combined with appropriate genetic data and in vitro functional studies can investigators distinguish between disease-causing mutations and polymorphisms without clinical consequence. From a basic research perspective, mutation screening is a critical and obligatory final step toward identifying genes that underlie genetic disease. Screening may also be applied toward the detection of mutations in diseases marked by many different mutations (allelic heterogeneity), and may also aid in the establishment of correlations between genotype and phenotype.

Use of restriction endonucleases to detect mutations

Mutations represent a fundamental change in the local DNA sequence, and may involve single nucleotide substitutions, small deletions or insertions, or more complex rearrangements. Beyond their potential for clinical consequences, gene mutations may also, by virtue of their changes in nucleotide sequence, create novel recognition sites for restriction endonucleases or destroy preexisting ones. For example, a DNA fragment harboring a mutation might *not* be cleaved with a particular restriction enzyme that normally cleaves wild-type DNA. Conversely, a different mutation might create a novel restriction site not present in the wild-type DNA. In either case, mutated DNA would produce a restriction digestion pattern distinct from that seen with nonmutated DNA when the digestion products are compared after electrophoresis on either agarose or polyacrylamide gels. An example of this commonplace approach applied to mutation detection in cystic fibrosis [10] is shown in Fig. 29.6. Other examples include mutation detection in Wilson disease [11], intrahepatic cholestasis of pregnancy [12], progressive familial intrahepatic cholestasis [13], and α_1-antichymotrypsin deficiency [14].

Using restriction enzymes to distinguish between alleles is a commonplace technique. This method also has advantages over approaches associated with radioisotope usage and their collateral costs and concerns. The large variety of restriction endonucleases that are commercially available represents a substantial resource to molecular laboratories, and the wide variety of recognition sequences associated with these enzymes affords the investigator many choices for designing straightforward mutation detection strategies.

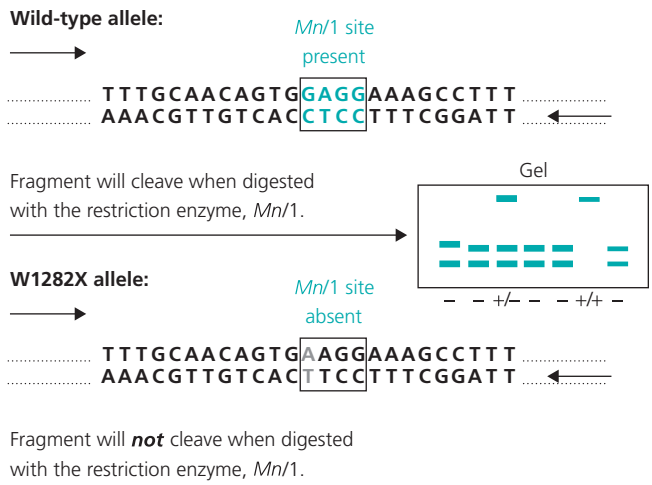

Figure 29.6 Restriction endonuclease-mediated detection of the W1282X mutation that causes cystic fibrosis. A restriction recognition site (CCTC/GAGG) present for the endonuclease *Mnl*1 in the normal CFTR exon 20 is ablated by the W1282X mutation. Arrows: PCR primers. Gel at center right shows W1282X-negative (–), W1282X heterozygote (+/–), and W1282X homozygote (+/+) patterns.

Despite this large supply of different restriction endonucleases, less than half of the known DNA sequence variants independently alter restriction digestion patterns for a commercially available enzyme. In addition, some enzymes are either unreliable or prohibitively expensive for use in routine, high-throughput analyses.

Single nucleotide polymorphisms

A SNP is a specific position in the genome in which the sequence differs only at a single base pair. SNPs account for more than 90% of all human genetic variation and are evenly distributed throughout the human genome at a frequency of 1 every 100–300 bases [15]. To date, nearly 3 million SNPs have been described and more than 10 million are estimated to exist. SNPs should clearly be differentiated from point mutations because they represent normal variations of sequence and do not necessarily cause disease. Point mutations, in contrast, are directly capable of causing disease, such as the H1069Q mutation of the *ATP7b* gene that causes Wilson disease. So, not every SNP is a point mutation, but every point mutation is a SNP. The functional consequence of a SNP is determined by its location within the genome. SNPs within the coding region of a gene can lead to an altered base triplet that potentially codes for a different amino acid, affecting protein structure and function [16].

SNPs in the coding region of genes are the cause of most recessively or dominantly inherited monogenic disorders (e.g., cystic fibrosis); however, they can also influence the course of polygenic diseases (e.g., liver fibrosis). Monogenetic disorders are caused by mutations within one gene, but it is believed that complex traits such as colon cancer, inflammatory bowel disease, or liver fibrosis are caused by a complex interplay of many genes, each of which contributes to the disease trait or phenotype.

Genotyping of SNPs is possible through electrophoretic techniques, such as restriction fragment length polymorphism analysis, in which a restriction enzyme cuts a PCR product at the site of the SNP, thereby generating DNA fragments of different sizes, which are then made visible by agarose gel electrophoresis. This approach is reasonable to screen or test for one particular SNP. However, the explosion of information about new SNPs underscores the need for more robust and scaleable genotyping techniques.

An international project, known as the Human Haplotype Map (Hap-Map) Project, aims to define SNP-based haplotype blocks in a number of populations. This work takes advantage of the fact that the probability of inheritance or independent transmission of two segments of DNA is proportional to their interlocus chromosomal distance. Hence, loci that are located close to each other segregate together and are generally inherited together as so-called haplotype blocks.

The underlying biological principle is that genetic markers in close proximity to the responsible mutant genes are in linkage disequilibrium and can therefore be evaluated as

surrogates for the presence of alleles at other loci. It is estimated that the average size of haplotype blocks ranges from 11 to 44 kilobases [17]. The use of tag-SNPs that cover the entire genome should help identify common haplotypes predisposing to common complex diseases.

Another approach to study a large number of SNPs involves the use of mass spectroscopy. SNPs are genotyped using a primer extension assay. In this assay, DNA fragments of differing size are produced depending on the allele of the assayed SNP and discriminated on the basis of mass.

The methods to perform large-scale SNP analysis are rapidly evolving with the use of microarray chip technology, which allows screening for 3000, 5000, 10 000 or even 100 000 SNPs on a patient in one experiment [18]. For this purpose, genomic DNA is first digested with restriction endonucleases and subsequently ligated to adapters, which then serve as templates for a generic single primer. PCR products are size fragmented and then hybridized with a chip.

Other approaches use molecular inversion probe (MIP) genotyping. This technique uses MIPs to produce inverted sequences, which undergo a unimolecular rearrangement and then are amplified by PCR using common primers and analyzed using universal sequence tag DNA microarrays [19].

Whole genome genotyping is made possible by bead arrays. The whole genomic DNA is amplified, fragmented, denatured, and hybridized to an array of allele-specific probes that are attached to the array by beads. After primer extension with biotin-conjugated oligonucleotides, the SNPs are then stained and evaluated directly on the array by immunohistochemical detection methods [20,21].

Proteomics is an emerging discipline that aims to study the various constituents of the entire human proteome (sum of human proteins) and how they interact with themselves and the environment. This is accomplished by two-dimensional gel electrophoresis, which separates proteins by charge (the first dimension) and mass (the second dimension). After staining, a two-dimensional matrix of dots represents different proteins that can be isolated and further characterized by mass spectroscopy. This approach identifies differentially expressed proteins between samples on a larger scale than simple Western blotting. With the use of labelled methionine, this technique also identifies differences in protein translation between different samples [22].

Polymerase chain reaction-mediated, site-directed mutagenesis

When confounding factors impede the design of facile restriction-based assays, laboratories may use a modification of this approach. PCR-mediated, site-directed mutagenesis is a technique by which a novel restriction digestion pattern can be purposefully generated in association with virtually any mutation. This strategy is known by a variety of names other than PCR-mediated, site-directed mutagenesis [23,24], including restriction site generating PCR [25] and amplification-created restriction site [26]. These techniques, identical in approach, involve the design of one PCR primer that abuts the mutation locus and includes typically one or two nucleotides mismatched relative to the template DNA. If properly designed, this mutagenic primer will bind specifically to its target DNA and support multiple rounds of PCR amplification. All PCR products generated will include not only the mutation locus under study but will also incorporate the base change inherent to the mutagenic primer. The combination of the mutation and the novel base change of this primer creates a novel restriction pattern specific to the different alleles at this locus where none was present previously. This methodology has become commonplace, including applications for mutations in the *ras* oncogene [27], *BRCA1* [28], β-thalassemia [29], medium-chain acyl-coenzyme A dehydrogenase deficiency [30], hereditary hemochromatosis [31], and α_1-antitrypsin deficiency

Amplification refractory mutation system

In the protocol for the amplification refractory mutation system, also known as allele-specific PCR, primers are designed that will amplify only one of the alleles present at the locus of interest [32]. This is accomplished with primers that are substantially mismatched relative to one allele, but that have sufficient complementarity to anneal to, and amplify, the other allele. Typically, the amplification refractory mutation system is designed to amplify one allele, whereas a separate reaction will be specific for the other allele at that locus. This technique has also been applied to cystic fibrosis [33], primary biliary cirrhosis [34], and the drug-metabolizing liver enzyme, cytochrome P450 CYP2D6 (debrisoquine 4-hydroxylase) [35].

In the absence of any advance knowledge of the likelihood of mutations being present in a DNA fragment, screening strategies are applied. Various technologies are available for the detection of any and all sequence variants that may occur. Many of these approaches require subsequent DNA sequencing to elucidate the nature of the mutations detected. These approaches, nonetheless, complement targeted mutation detection strategies because all sequence variants will be detected, not just those that have been previously reported and have highly specific assays available.

Single-stranded conformational polymorphism analysis

Single- and double-stranded DNA fragments may assume distinct secondary structures after they are denatured. These structures are highly dependent on their nucleotide sequences, so that a DNA fragment with a mutation will have an altered structure relative to the normal fragment. The screening strategy of single-stranded conformational polymorphism (SSCP or SSCA) takes advantage of the different

rates at which these fragments will migrate through polyacrylamide gels [36]. After amplification of a genomic fragment, the PCR product is typically digested into smaller fragments. It is then partially denatured to single strands and transferred immediately to ice. The sample is then electrophoresed on a non-denaturing polyacrylamide gel. Both double-stranded and single-stranded fragments will be apparent on the gel as distinctly migrating bands. The presence of a mutation or polymorphism within the PCR product will be apparent through band mobility shifts in either the double- or single-stranded fragments or both, when compared to DNA fragments without sequence variants (Fig. 29.7). The wide range of applications for SSCP includes mutation screening in Wilson disease [37], Alagille syndrome [38], progressive familial intrahepatic cholestasis [39], and cystic fibrosis [40]. The use of permutations of SSCP, such as conformation-sensitive gel electrophoresis (CSGE), has also been reported [41].

Many mutation-screening strategies are predicated on heteroduplex formation. Two single-stranded fragments of DNA, such as synthesized PCR products, will form fully matched, stable homoduplex structures at appropriate chemical and temperature conditions if they have 100% sequence complementarity. DNA fragments that differ at a small number of nucleotides will also form duplexes, but these fragments will fail to pair when the nucleotides are not matched. These heteroduplexes may have subtly altered conformational properties and become substrates for specific chemical as well as enzymatic reactions. When amplifying a DNA fragment from an individual who is heterozygous for a nucleotide substitution, the final PCR product will include both homoduplex structures for each allele, as well as heteroduplexes derived from the imperfect annealing of dissimilar fragments. Whenever heterozygosity is established, either through gel electrophoresis or chemical analysis, direct sequence analysis is used to identify the underlying mutation or polymorphism. A heteroduplex detected in a large gene allows DNA sequencing efforts to be targeted toward the fragment that gave rise to the heteroduplex, making mutation detection more efficient.

Chemical and enzymatic cleavage

Heteroduplex DNA may be cleaved in a sequence-specific manner using either chemical or enzymatic agents. Chemical cleavage of DNA mismatches (CCM) involves agents that modify specific bases when present in the single-stranded form. In imperfectly annealed, heteroduplex fragments, the nucleotide mismatch manifests as a localized single-stranded region with bases vulnerable to chemical modification. Hydroxylamine modifies unpaired cytosines, whereas osmium tetroxide modifies unpaired thymines. Piperidine is then added to cleave the mismatched products, which are then electrophoresed to resolve the digested fragments [42]. Every possible single-base mismatch, as well as small insertions and deletions, is subject to chemical cleavage. Phenylketonuria [43], acute intermittent porphyria [44], and hemophilia A [45] are among the disorders for which CCM has been used. Not only will the presence of a mutation be established, but its approximate location within the fragment will also be apparent from the size of the digestion products.

An enzymatic protocol for mismatch-directed cleavage using T4 endonuclease VII avoids the chemical toxicity of the CCM reagents [46,47]. In vivo, T4 endonuclease VII resolves the complicated structures of genetic recombination by recognizing kinks and loops produced in recombining DNA. These transient structures are similar in nature to the

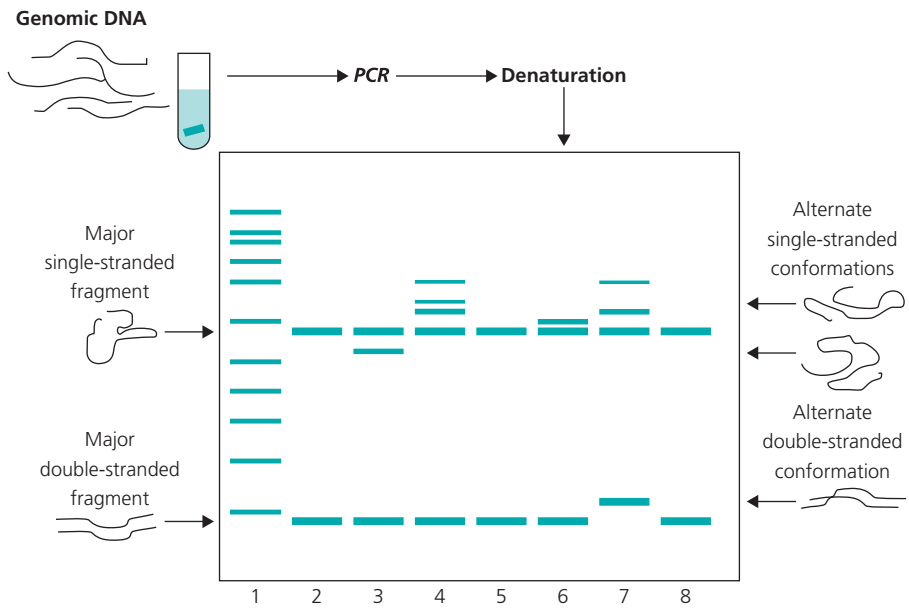

Figure 29.7 Single-stranded conformational polymorphism analysis (SSCP or SSCA). The polymerase chain reaction (PCR) product is denatured, placed on ice, and electrophoresed on nondenaturing polyacrylamide gels. Lane 1, size marker; lanes 2, 5, and 8, no bands of altered mobility are seen in samples with the wild-type sequence; lanes 3, 4, 6, and 7, bands of altered mobility establish presence of sequence variants. Different variants may yield different electrophoretic patterns. Multiple bands reflect distinct fragment conformations.

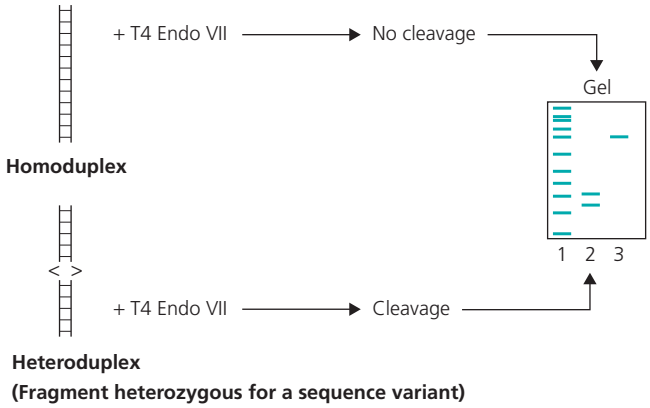

Figure 29.8 T4 endonuclease VII-mediated cleavage. Homoduplex polymerase chain reaction (PCR) fragments have no localized single-stranded domains and are not cleaved by T4 endonuclease VII. Electrophoresis produces a single uncut band (lane 3). Heteroduplex PCR fragments are cleaved by T4 endonuclease VII, giving cleaved fragments (lane 2). Lane 1, size marker.

mismatch-bearing fragments that typify heteroduplexes. Enzyme mismatch cleavage (EMC) cleaves heteroduplex fragments, allowing the digestion products to be characterized by electrophoresis (Fig. 29.8). Mutation detection using EMC has been described for hereditary nonpolyposis colorectal cancer (HNPCC) [48] and for hereditary pancreatitis [49]. As with CCM, some positional information is also be generated.

Heteroduplex analysis

The conformational changes associated with heteroduplex structures often lead to fragments with altered electrophoretic mobility. Heteroduplex fragments may be separable from homoduplex forms, thereby establishing heterozygosity for a sequence variant in that sample. A method that exploits the differential migration of homoduplexes from heteroduplexes uses mutation detection enhancement (MDE) gels (BioWhittaker Molecular Applications, Rockland, ME). This proprietary gel matrix is formulated to enhance the electrophoretic separation between homoduplex and heteroduplex fragments in a straightforward manner [50]. After PCR, DNA products are renatured slowly to enhance heteroduplex formation and are subsequently electrophoresed on MDE gels. Heteroduplex and homoduplex fragments migrate on a gel with different mobilities, an effect that is directly related to the subtle structural differences between them. This technique has been used for mutation detection in Wilson disease [51], acute intermittent porphyria [52], Duchenne muscular dystrophy [53], and hereditary breast cancer (*BRCA1*) [54].

Denaturing gradient gel electrophoresis

The differential conformational properties of heteroduplex fragments may be exploited by other methodologies. As a result of their mismatched bases, heteroduplexes will denature into single strands more readily than their homoduplex counterparts. Denaturing gradient gel electrophoresis (DGGE) uses a chemical gradient within the electrophoretic gel to take advantage of this difference [55,56]. The chemicals involved, typically urea or formamide, denature PCR fragments from double- to single-stranded forms. PCR of heterozygous samples yields heteroduplex fragments with altered denaturation properties. Once denaturation of a fragment occurs, its migration through the gel will effectively cease. The appearance of these slowly migrating bands on DGGE gels is indicative of heterozygosity for a DNA sequence variant in the underlying sample. This "melting process" is sensitive not only to the nature of the mismatch and the underlying mutation but also to the sequence of the fragment as a whole. DGGE is another widely used technique, having found utility for detecting mutations in cystic fibrosis [57], erythropoietic protoporphyria [58], tuberous sclerosis [60], and hereditary breast cancer [61], and *TP53* mutations in hepatocellular carcinoma [59].

Protein truncation test

The current method involves reverse transcription PCR (RT-PCR), in which total RNA is used to produce single-stranded cDNA. PCR amplification, using a forward primer that includes the gene's regulatory sequences necessary for transcription and translation (Fig. 29.9), results in a functional protein-coding segment. Protein synthesis is completed by adding this segment to an in vitro translation/transcription system that includes radiolabeled amino acids and results in a labeled protein. A novel, lower molecular-weight band indicates the presence of a truncated polypeptide, representing a truncating mutation in that sample. The position of the band indicates the relative size of the product, from which the position of the genomic alteration can be extrapolated (see Fig. 29.9). Confirmation of the mutation is achieved by genomic sequencing.

Missense mutations do not usually result in size alterations of proteins; therefore, other procedures must be used for missense detection, so maximum analytical sensitivity is often combined with scanning techniques for nontruncating mutations. Using this approach, the sensitivity for *BRCA1* and *BRCA2* mutation detection is nearly 90%.

Real-time polymerase chain reaction

Allele-specific mutation detection is also amenable to automation. The Perkin-Elmer 7700 TaqMan allelic discrimination protocol enables high-throughput genotyping using real-time PCR. This strategy entails the use of a short DNA probe with a fluorescent dye attached to one end and a quenching agent linked to the other end. This probe is designed to match perfectly one allele at a particular locus but to be mismatched relative to other alleles. As the synthesis of the PCR product advances, the DNA polymerase

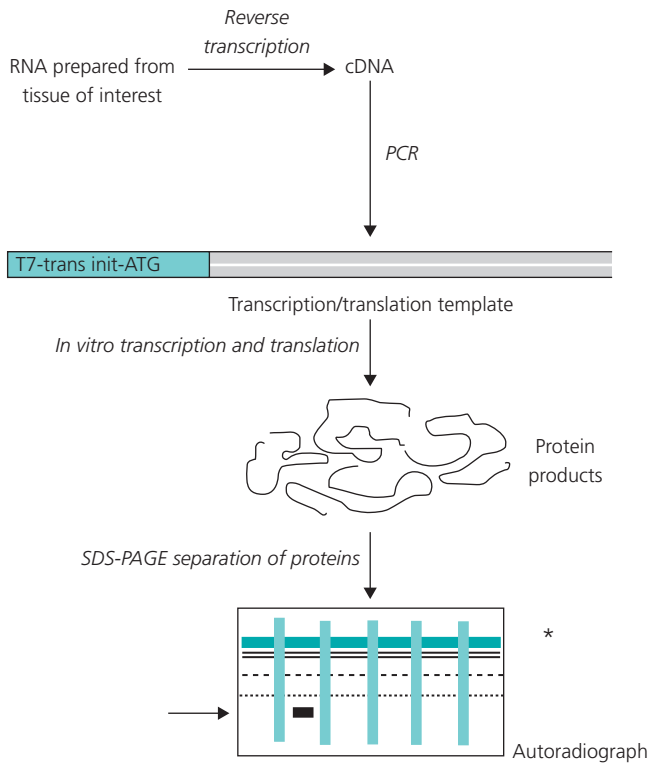

Figure 29.9 The protein truncation test. RNA is usually isolated from peripheral blood lymphocytes from whole blood collected in the presence of anticoagulant citrate dextrose solution or EDTA. (The quality of RNA isolated from tissue is dependent on rapid freezing of tissue to – 70°C or below.) Coupled in vitro transcription/translation kits, using a rabbit reticulocyte extract, are commercially available. A labeled amino acid, usually ^{35}S-labeled lysine, is added to the in vitro assay to label the synthesized proteins for autoradiography. The arrow adjacent to the autoradiograph indicates the presence of a band of lower-than-expected molecular weight, representing a truncated protein. The asterisk identifies the full-length, nontruncated protein.

5′-exonuclease activity digests any probe in its path, releasing the fluorescent dye from the quencher and generating a fluorescent signal. If the probe is mismatched relative to the template, its affinity for the template will be greatly reduced under appropriately stringent conditions. Probe not bound to the template will neither be digested nor generate signal. A similar probe may be designed to detect the other alleles, and these assays may be pooled as long as each probe carries a different, separately detectable, fluorescent dye. Real-time PCR approaches to mutation detection have been reported for α_1-antitrypsin deficiency [62], hemochromatosis [63], Tay–Sachs disease [64], and the study of mephenytoin metabolism by CYP2C19 (mephenytoin 4-prime-hydroxylase) variants [65,66].

Genetic testing for inherited colon cancer

Familial adenomatous polyposis (FAP), which also includes attenuated FAP, Gardner syndrome, and most cases of Turcot syndrome, is an autosomal dominant syndrome. More than 90% of patients with FAP have a mutation in *APC*, which encodes a tumor suppressor gene.

Genetic testing for FAP often starts with the protein truncation test, which can help localize the mutation in the FAP gene. Cell-free expression systems can be used as a bridge between genomics and proteomics by converting nucleic acid sequence (usually in the form of RNA) into protein sequence. The protein truncation test detects mutations that alter the reading frame of the expressed protein, so-called truncating mutations, which lead to a shortened protein product. This method is particularly useful in detecting mutations in large genes that are frequently altered by nonsense and frameshift mutations (the result of small insertions or deletions) in addition to large deletions. Examples of the underlying disorders include FAP [66], cystic fibrosis [67], Duchenne and Becker muscular dystrophies [68], neurofibromatosis type 1 [69], and hereditary breast and ovarian cancer defined by the tumor suppressor genes such as *BRCA1* and *BRCA2* [70,71].

Genetic testing is performed by sequencing the *APC* gene to identify the mutations, which may be preceded by SSCP or DGGE to localize the mutation. DNA for genetic testing is extracted from the white blood cells of a peripheral blood sample. Direct DNA sequencing correctly identifies up to 95% of mutations. Southern blotting identifies additional large deletions or rearrangement mutations [72,73].

Hereditary nonpolyposis colorectal cancer

HNPCC is characterized by early-onset colorectal, endometrial, gastric, and genitourinary cancers in patients with a strong family history of cancer. HNPCC is inherited as an autosomal dominant trait. It results in mutations in one of the DNA mismatch repair genes, most frequently in *MLH1* or *MSH2* and rarely in *MSH6* or *PMS2*. Mismatch repair proteins are responsible for correcting errors that occurred during DNA replication, typically the addition or deletion of one or more nucleotides. Defects in mismatch repair are readily detected in microsatellite DNA, which are sequences found throughout the human genome that contain mononucleotide, dinucleotide, or trinucleotide repeats. These short stretches of repetitive DNA are prone to mutations, which, in patients with HNPCC, are not repaired. Colon cancer that is caused by defects in mismatch repair genes often demonstrates errors in microsatellite DNA, and these cancers are said to exhibit high microsatellite instability (MSI) [74,75].

Several strategies are used for the genetic testing of families with suspected HNPCC. Testing for MSI is often the first step. MSI testing requires the availability of both tumor tissue and normal tissue. As a rule, five specific DNA microsatellites are evaluated for mutations, and if at least two of the five are abnormal, the tumor is considered MSI-high (Fig. 29.10). A corresponding approach to MSI testing is immunohistochemical analysis of the colon cancer. Specific antibodies are

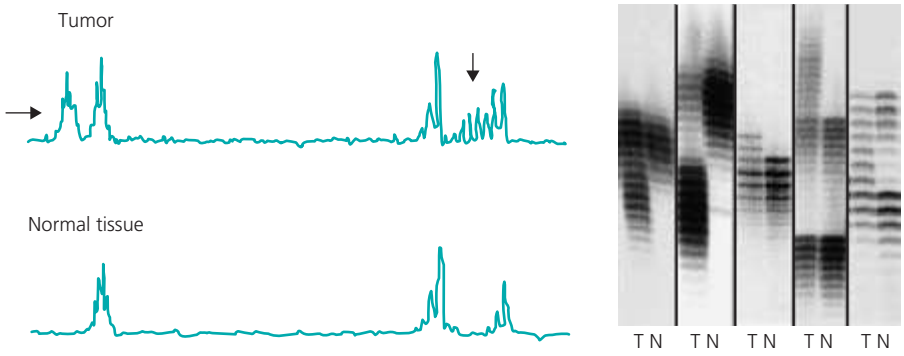

Figure 29.10 Microsatellite instability assay. DNA is extracted from both normal tissue (N) and tumor tissue (T). Microsatellite regions that are unstable because of a mismatch repair defect are amplified by polymerase chain reaction (PCR). The PCR products are detected by either capillary electrophoresis of fluorescently labeled DNA (*left*) or gel electrophoresis (*right*).

used to immunostain for the DNA mismatch repair proteins MLH1, MSH2, and MSH6. Failure to stain for the protein indicates that the corresponding gene is not being appropriately expressed and perhaps results from a germ-line mutation [76,77].

Patients with MSI-high tumors or absence of the DNA mismatch repair proteins after immunohistochemical analysis are tested for mutations in *MLH1* and *MSH2*, which account for at least 90% of the HNPCC mutations that are detected.

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2 Gastrointestinal diseases

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A ESOPHAGUS

30 Esophagus: anatomy and developmental and structural anomalies

Ikuo Hirano

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Adult anatomy, 719
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Developmental anomalies, 725
Structural anomalies, 729

The majority of developmental and structural anomalies covered in this chapter are important but infrequently encountered. Certain entities, including esophageal heterotopic gastric mucosa (inlet patch) and Schatzki rings, are prevalent but only occasionally produce clinical manifestations. With the widespread use of upper gastrointestinal endoscopy, recognition and understanding of both common and uncommon esophageal pathology is of relevance to clinical care. The embryology and normal gross anatomy and histology of the esophagus are reviewed as background pertinent to all esophageal disorders.

Embryology

During the first 2 weeks of gestation, the human embryo forms a bilaminar disc with adjacent layers comprising ectoderm and endoderm [1,2]. In the fourth week, the tracheobronchial diverticulum forms on the ventral surface of the endodermally derived foregut adjacent to the pharyngeal gut. This diverticulum gradually separates from the dorsal foregut with formation of the esophagotracheal septum that separates the trachea and esophagus. During the sixth week of gestation, the muscularis layers form, derived from the splanchnic mesoderm. The striated muscle of the upper esophagus is derived from branchial arches 4, 5 and 6 with innervation by corresponding branches of the vagus nerve. Vagal neural crest cells derived from the ectoderm populate the foregut, forming the enteric nervous system. Unlike the midgut and hindgut, axial migration of the neural crest cells is not an important factor in the development of the foregut enteric nervous system [4]. Complex interactions between the gut microenvironment regulated by control regulatory genes coordinate the development of the myenteric and submucosal plexuses. Growth factors have a direct and indirect effect on neural crest cell migration [5].

The esophageal epithelium rapidly proliferates and almost completely occludes the lumen in the seventh and eighth weeks, leaving residual channels. A single esophageal lumen returns in the tenth week lined by a superficial layer of ciliated epithelial cells. In the fourth month, the ciliated cells are replaced by stratified squamous epithelium, a process that continues until birth. Residual islands of ciliated epithelium at the proximal and distal ends of the esophagus remain and give rise to esophageal glands.

Adult anatomy

Gross anatomy

The proximal origin of the esophagus is at the pharyngoesophageal junction that anatomically corresponds with the anterior thyroid cartilage and physiologically with the upper esophageal sphincter (UES). The UES is identified manometrically as a 2–3 cm focus of elevated resting pressure between the hypopharynx and the esophagus. The UES primarily comprises the transversely oriented cricopharyngeus with contributions from the inferior pharyngeal constrictor and thyropharyngeus muscles [6–9]. The cricopharyngeus is a C-shaped muscle that has attachments to the lateral aspects of the cricoid cartilage at the level of the C5–6 vertebral interspace. The inferior fibers of the cricopharyngeus merge with the circular muscle of the esophageal body, which courses through the posterior mediastinum to end at the esophagogastric junction corresponding with the T10 vertebral level.

The esophageal lumen can distend to approximately 2 cm in anteroposterior diameter and up to 3 cm in lateral diameter. The resting length of the adult esophagus is variable

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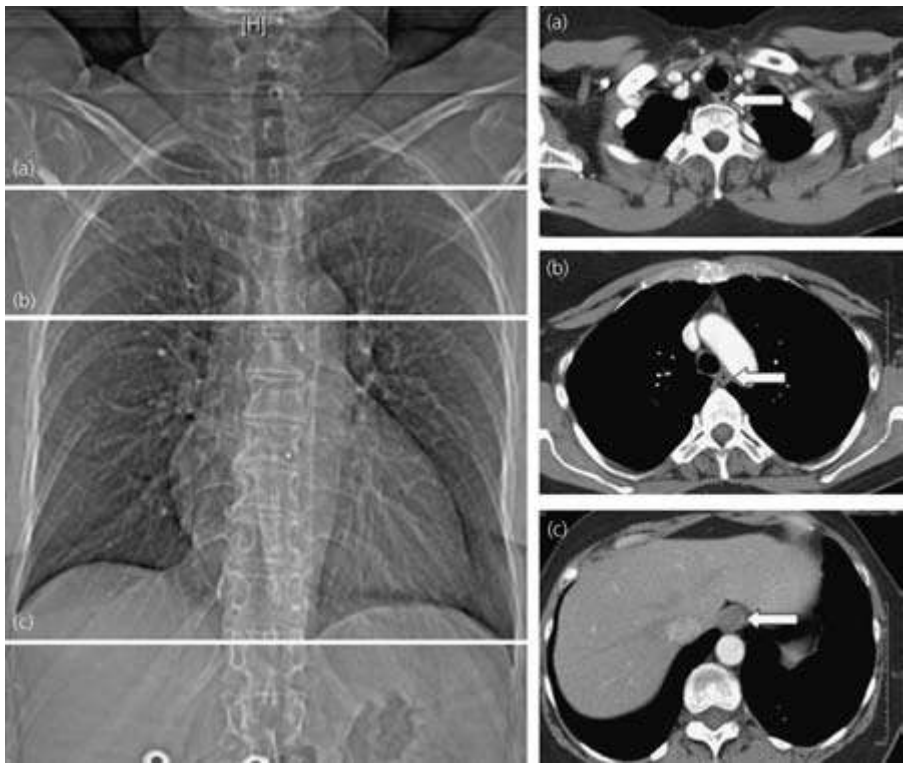


Figure 30.1 Cross-sectional computed tomography of the thorax demonstrating the relationships between the esophagus and adjacent structures. **(a)** Image at the level of the cervical esophagus with the trachea anteriorly and thoracic spine posteriorly. The carotid arteries and jugular veins are situated laterally. **(b)** Image at the level of the thoracic esophagus with the aortic arch visible anteriorly and left laterally and trachea right anterior. **(c)** Image at the level of the abdominal segment of the esophagus with the liver anteriorly and aorta posteriorly.

but ranges from 18 to 26 cm [10]. Significant shortening of the esophagus occurs during both deglutition and transient lower esophageal sphincter (LES) relaxation that can axially displace the esophagogastric junction by several centimeters. The cervical esophagus extends from the pharyngoesophageal junction to the suprasternal notch and is about 4–5 cm long. At this level, the esophagus is surrounded by the trachea anteriorly, the vertebral column posteriorly, and the carotid sheaths and thyroid laterally (Fig. 30.1a).

The thoracic esophagus passes just posterior to the tracheal wall and courses to the right and posterior to the aortic arch that corresponds with the T4 vertebral level and posterior to the tracheal bifurcation and left main stem bronchus (Fig. 30.1b). At the T8 vertebral level, the esophagus turns left and crosses anterior to the aorta at the level of the diaphragmatic hiatus. At the T10 vertebral level, the esophagus passes through the diaphragmatic hiatus and enters into the cardia of the stomach at an oblique angle.

The abdominal portion of the esophagus varies in length from 0.5 to 2.5 cm [11]. At this level, the left lobe of the liver lies anteriorly, the caudate lobe of the liver lies to the right, the fundus of the stomach is to the left, and the right crus of the diaphragm and aorta lie posteriorly (Fig. 30.1c). The intraabdominal segment of the esophagus is compressed by physiological events that increase intraabdominal pressure and therefore provides resistance to esophageal reflux under such circumstances. The borders of the esophageal hiatus are formed by the diaphragmatic crura and median arcuate ligament, if present. The crura arise from the first four lumbar vertebrae, intervertebral discs, and anterior longitudinal ligament. The fibers of the left and right crura pass upward and anteriorly to form the muscle borders of the hiatal ring and then insert into the transverse ligament of the central tendon of the diaphragm [12–14]. At the level of the diaphragm, the phrenoesophageal membrane extends from the hiatal margin to insert into the circumference of the esophagus both above and below the diaphragm [15,16]. With age, the esophagus is less firmly fixed to the hiatus and adipose tissue is incorporated into the membrane [17]. The membrane is absent in patients with hiatal hernia [18].

The esophagogastric junction is represented intralumenally by the cephalic margin of the longitudinal mucosal folds in the gastric cardia [19,20]. These folds are best appreciated when a hiatal hernia is present and used as an anatomical landmark for the esophagogastric junction in patients with Barrett esophagus. In patients without Barrett esophagus, the esophagogastric junction corresponds with the squamocolumnar mucosal junction or Z line that is demarcated by the abrupt disappearance of the visible vascular pattern and a color change from white-tan to the reddish orange, slightly granular appearance of gastric mucosa [19]. Manometrically, the junction is defined by the LES, which has both intrinsic smooth muscle and extrinsic skeletal muscle components [21]. The smooth muscle of the LES generates myogenic tone that is modulated by neurotransmitters from the enteric and autonomic nervous system. The extrinsic

component of the LES represents contractions of the crural diaphragm.

Blood supply

The arterial blood supply to the esophagus is segmental, with limited vascular overlap (Fig. 30.2). The cervical esophagus is supplied mainly by branches of the inferior thyroid artery. Branches of other arteries, such as the common carotid, subclavian, vertebral, and ascending pharyngeal, may provide additional blood supply. The thoracic esophagus is supplied by branches of the aorta and the right intercostal and bronchial arteries. The abdominal esophagus is supplied by branches of the left gastric, short gastric, and left inferior phrenic arteries.

The venous anatomy of the esophagus is depicted in Fig. 30.3. Fine intraepithelial channels drain into a subepithelial superficial venous plexus that connect with intrinsic veins in the submucosa. At the level of the gastroesophageal junction, the superficial venous plexus and deep intrinsic veins communicate with their gastric counterparts [22]. Perforating veins connect the deep intrinsic veins to adventitial veins. At the level of the cervical esophagus, the adventitial veins drain into the inferior thyroid vein, deep cervical vein, vertebral vein, and peritracheal venous plexus.

At the thoracic level, adventitial veins drain into the azygous vein on the right, the hemizygous vein on the left, and the intercostal veins when the hemizygous vein is absent. At the gastroesophageal junction, the portal systemic circulation involves venous drainage of the esophagus, stomach, pancreas, spleen, diaphragm, and retroperitoneum. Anatomical studies have suggested a high-pressure watershed region between the portal and azygous systems in the region of the gastroesophageal junction that is prone to venous dilation in portal hypertension [23]. Esophageal varices may extend up to the level of the aortic arch, the upper limit of the venous drainage of the lower esophagus by way of the azygous vein system into the superior vena cava. Varices in a location cephalic to this level have been termed *downhill varices*. This

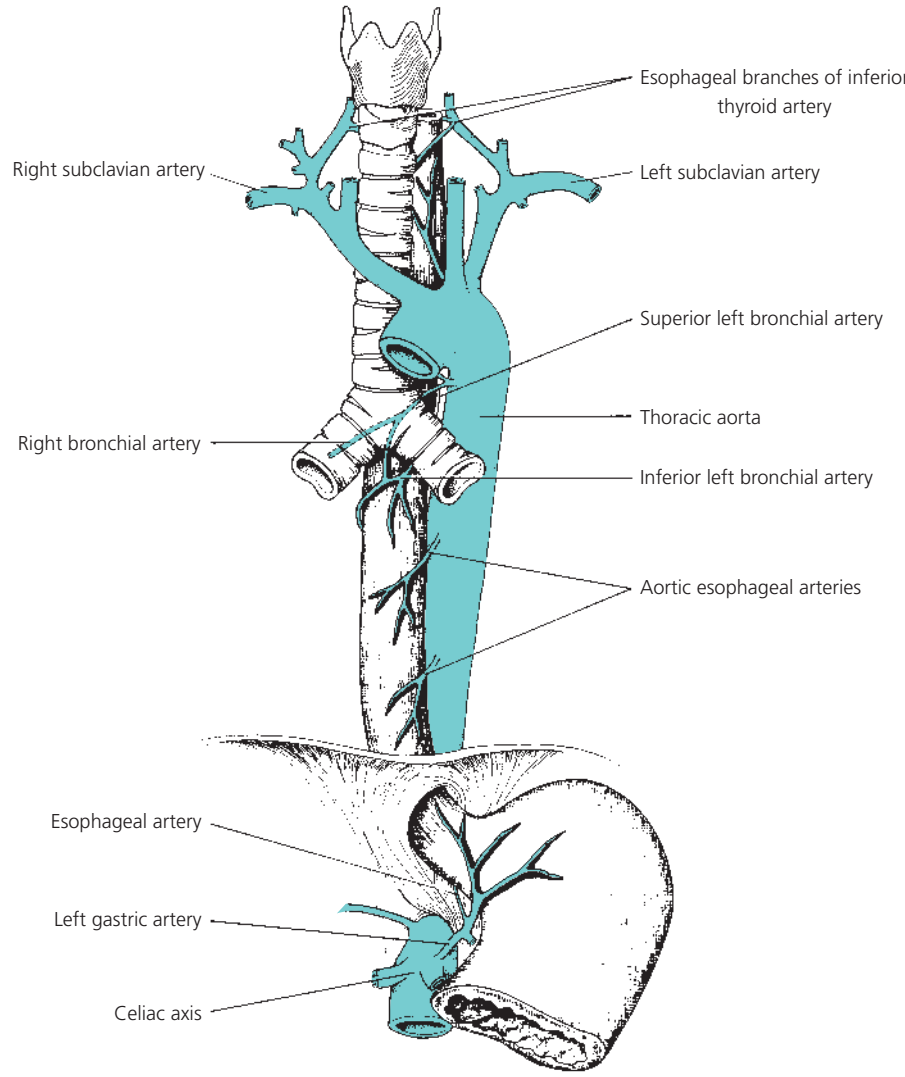


Figure 30.2 Arterial system of the esophagus.

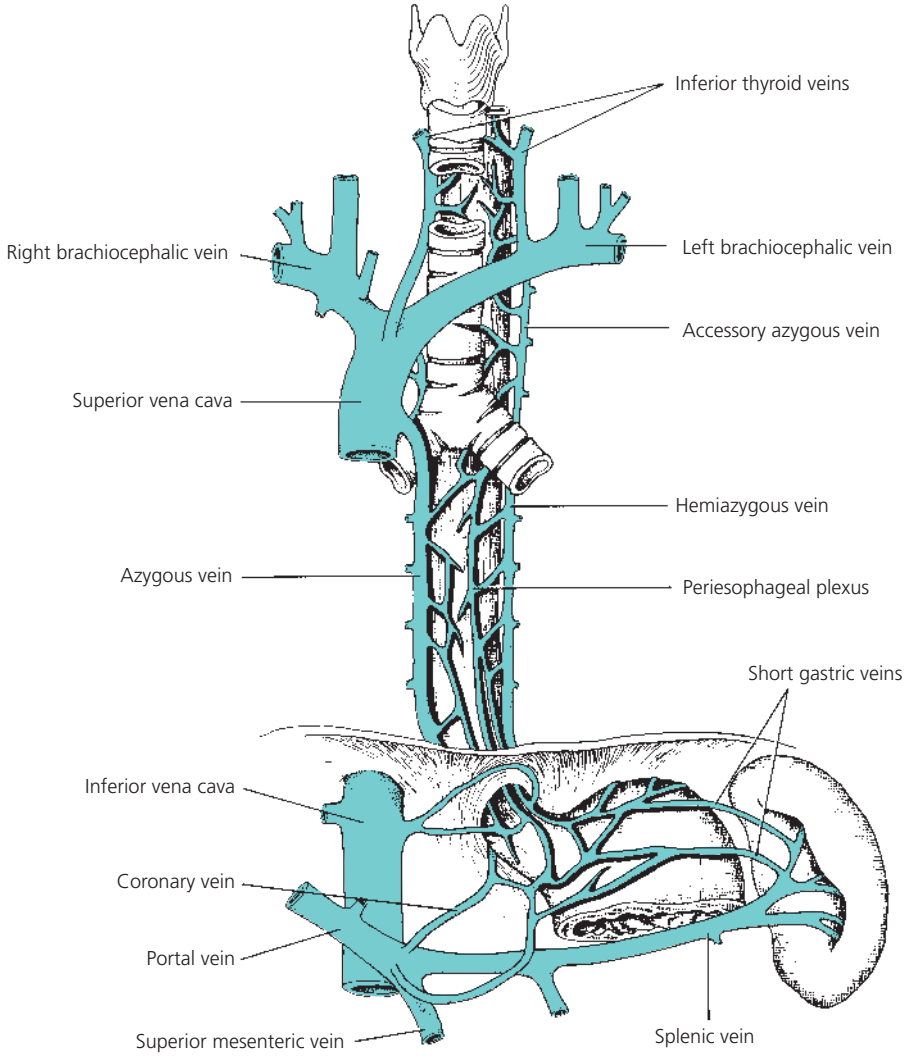


Figure 30.3 Venous drainage of the esophagus.

description refers to the anatomical site of venous obstruction above the level of the varices, in either the cervical venous system or the superior vena cava. The varices then develop below the site of venous obstruction, whereas the more commonly encountered distal esophageal varices develop in a location cephalic to or above the portal venous flow.

Innervation

Motor innervation of the esophagus is dominated by the vagus nerve, which supplies parasympathetic innervation to the esophagus (Fig. 30.4). Vagal fibers responsible for motor innervation of the upper esophageal sphincter and striated muscle esophagus originate from cell bodies in the nucleus ambiguus. The distal esophagus and LES derive vagal projections from the dorsal motor nucleus. Vagal afferent fibers responsive to chemical, thermal and mechanical stimuli have cell bodies in the nodose ganglia with projections to the nucleus solitarius. The cervical esophagus is innervated by the recurrent laryngeal nerves, which arise from the vagus. Branches of the vagus nerves and the left recurrent laryngeal nerve innervate the upper thoracic esophagus. The left and right vagus nerves intertwine with sympathetic fibers to form the esophageal plexus [24,25]. Out of the esophageal plexus, the anterior and posterior vagus trunks form at a variable distance above the diaphragm [24]. Below the diaphragm, the anterior (i.e., left) vagus trunk splits into anterior gastric branches and hepatic branch. The posterior (i.e., right) vagus trunk splits into posterior gastric branches and a branch to the celiac plexus.

Sympathetic innervation is supplied by the superior cervical ganglion, sympathetic chain, major splanchnic nerve, thoracic aortic plexus, and celiac ganglion. Cell bodies for spinal afferent nerves originate in the dorsal root ganglia and project to the spinal column and brainstem nucleus gracilis and nucleus cuneatus [26]. Spinal afferents mediate nociception from both mechanical sensation and chemosensation [27]. The enteric nervous system is responsible for direct neural innervation of the smooth muscle of the esophagus

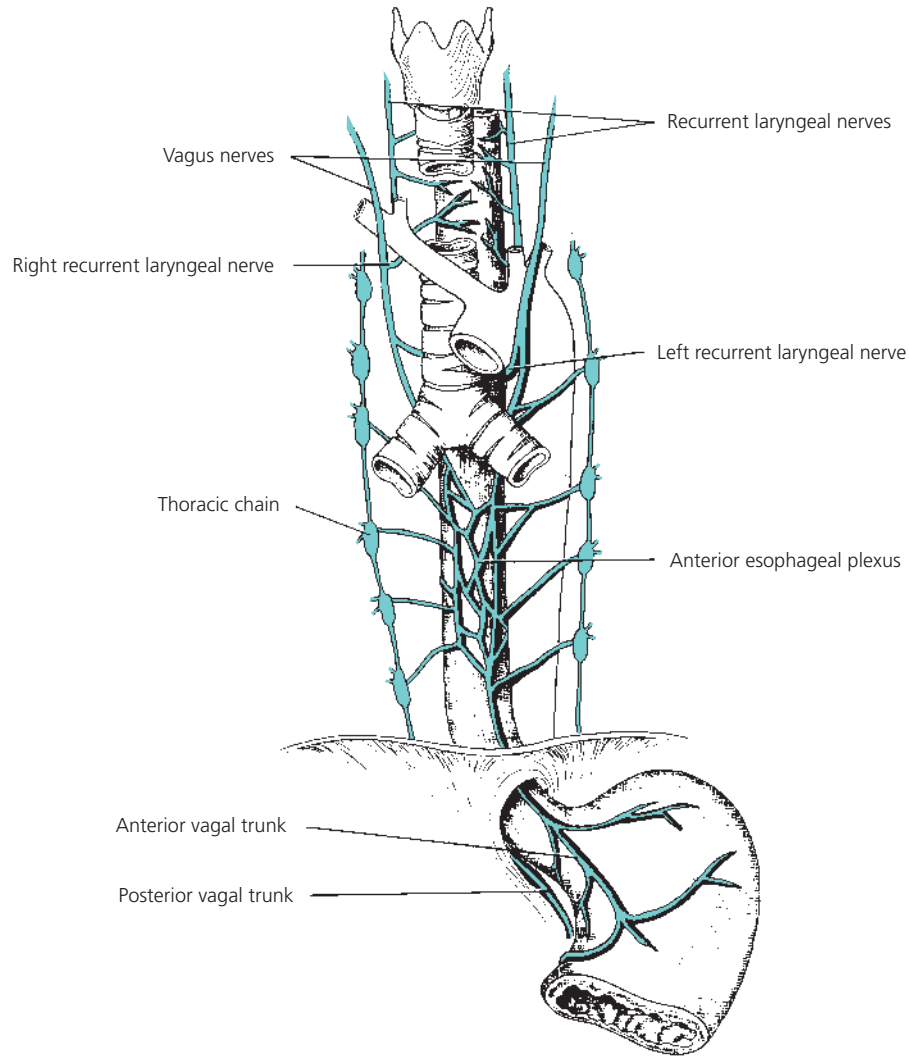


Figure 30.4 Innervation of the esophagus.

and LES. Interestingly, the enteric nervous system is present within the striated portion of the esophagus but its function is unclear.

Lymphatics

Lymphatic vessels in the mucosa and submucosa of the esophageal wall extend through the muscularis propria before emptying into adventitial lymph nodes (Fig. 30.5). Cervical lymph nodes drain the proximal esophagus, while more inferiorly the lymphatics drain into the paraesophageal lymph node chain. Internal jugular, tracheal, tracheobronchial, posterior mediastinal, and pericardial nodes drain adjacent esophageal segments. In contrast to the arterial supply, the lymphatic drainage of the esophagus is not segmental. Multiple interconnections exist between nodal chains. This arrangement accounts for the frequent wide intramural and mediastinal lymphatic spread of esophageal carcinoma.

Histology

Light microscopy

Similar to other regions of the digestive tract, the esophageal wall comprises a mucosa, submucosa, and muscularis propria (Fig. 30.6) [28]. The esophagus does not have a serosa but only a loose connective tissue that comprises the adventitia. The absence of a serosal layer allows esophageal perforations and malignancies to disseminate more readily and makes esophageal anastamosis and surgical repair more difficult.

The esophageal mucosa is composed of nonkeratinized stratified squamous epithelium, connective tissue of the lamina propria, and the muscularis mucosa. The squamous epithelium resembles that of the skin and oral cavity and is composed of a basal cell layer known as the stratum basale or germinativum, the stratum intermedium or spinosum, and a superficial layer known as the stratum superficiale or corneum.

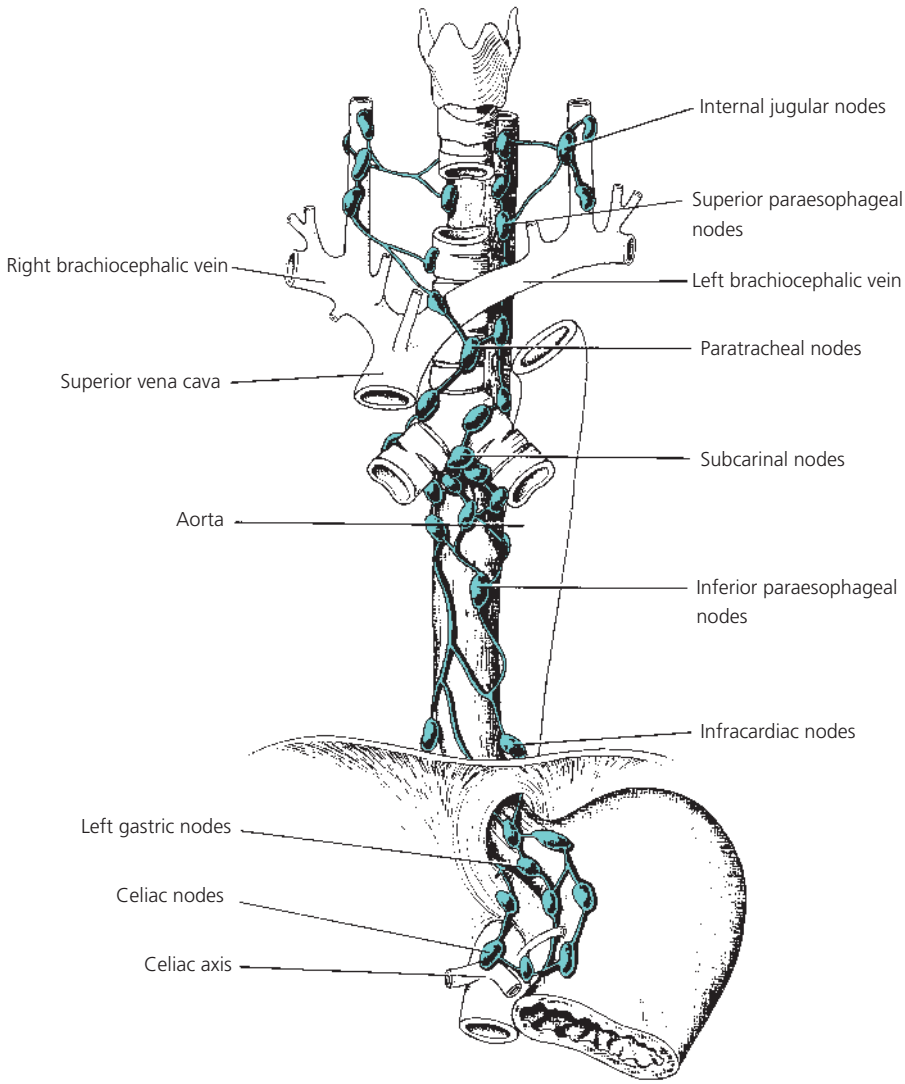
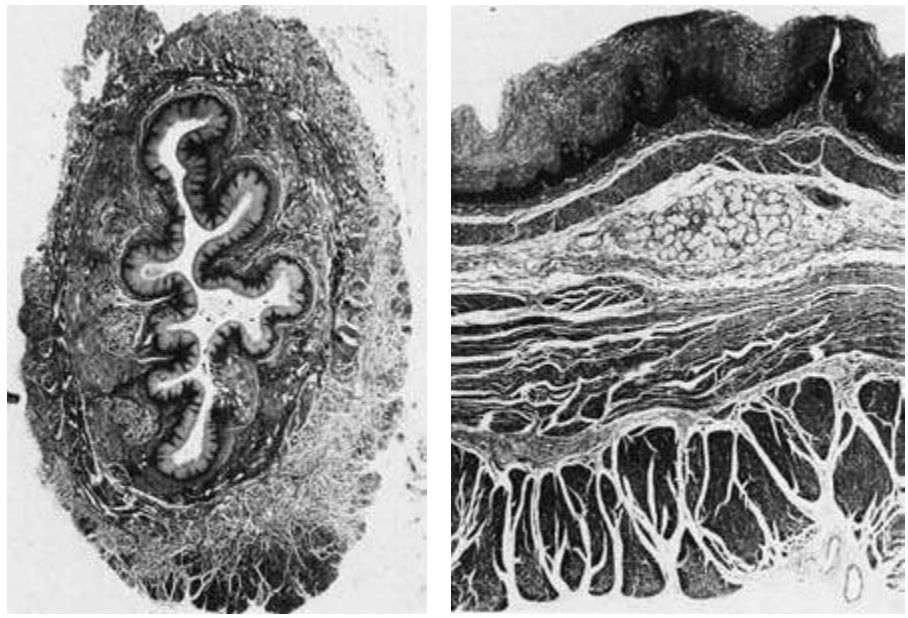


Figure 30.5 Lymphatic system of the esophagus.

The inner aspect of the epithelium undulates owing to protrusions of the lamina propria called dermal papillae or rete pegs. The papillae contain vasculature and normally extend less than two-thirds of the overall thickness of the mucosal layer. The basal cell layer is composed of basophilic cylindrical cells that have the capacity to divide and repopulate the superficial layers [28]. The cell turnover rate for the squamous epithelium is 5–8 days. Cells of the stratum spinosum contain glycogen and membrane-coating granules believed to play a role in cell cohesion [29,30]. The presence of glycogen in the superficial mucosal cells accounts for their brownish black staining by Lugol iodine applied for chromoendoscopy. In the stratum superficiale, the squamous epithelial cells are more flattened and oriented parallel to the surface. Tight junctions and intercellular mucin secretions form a protective seal between cells. Dilation of the intercellular spaces has been demonstrated to be significantly increased in patients with both erosive and nonerosive reflux disease [31,32]. This observation may explain the sensitivity of nonerosive reflux patients to acid perfusion and may serve as a histological marker for reflux disease. Acid and neutral mucosubstances are found on all layers of epithelial cells and may play a protective role [33].

The esophagus contains cells that are a part of the gut-associated lymphoid tissue. Cytotoxic T cells (i.e., intraepithelial lymphocytes) and Langerhans cells are found in the squamous epithelium. The presence of mucosal neutrophils or eosinophils is indicative of inflammatory conditions such as reflux esophagitis or eosinophilic esophagitis. T helper cells and B lymphocytes are seen primarily in the lamina propria [28,34,35].

The muscular components of the esophageal wall include the muscularis mucosa and muscularis propria. The muscularis mucosa is composed of longitudinally oriented smooth muscle cells that separate the lamina propria from the submucosa (Fig. 30.6). The submucosa consists primarily of



(a) (b)

Figure 30.6 **(a)** Cross-section from the middle third of the esophagus shows a mixture of skeletal and predominantly smooth muscle in the muscularis propria. The submucosal glands are clearly shown. At one point, there is an esophageal cardiac gland in which a small focus of glandular epithelium interrupts the squamous mucosa. This is a normal finding, seen in at least 1% of all esophagi (magnification × 2.5). **(b)** Longitudinal section of esophageal wall (magnification × 10). Courtesy of Rodger C. Haggitt MD, Seattle, WA, USA.

loose connective tissue. The vascular network known as the Heller plexus, the submucosal or Meissner plexus of the enteric nervous system, mucin-secreting glands, lymph follicles, and lymphocytes are located at the level of the submucosa. Esophageal submucosal glands are found in the proximal esophagus and near the esophagogastric junction. The muscularis propria is composed of an inner circular and an outer longitudinal layer, the inner circular layer being the thicker of the two (Fig. 30.6). The first centimeter of the proximal esophagus is striated muscle alone, whereas the muscle of the next 6–8 cm consists of interdigitated striated and smooth muscle. The remaining length is smooth muscle alone. In situ, longitudinal muscle fibers run in an elongated spiral. Circular muscle fibers run in an elliptical course, with some fibers leaving their bundle to join higher or lower bundles [3]. Below the diaphragm and proximal to the angle of His (i.e., the abdominal or submerged segment), an area has been described in fixed gastroesophageal specimens in which the inner circular muscle layer thickens and the fibers become semicircular and interlaced. Oblique fibers of gastric type from the greater curve are also present at this level [36].

The myenteric, or Auerbach, plexus is interspersed between the inner circular and outer longitudinal muscle coats. The ganglion cells can be identified as lavender-colored larger cells clustered in small groupings on hematoxylin and eosin staining. The adventitial layer consists of connective tissue with networks of nerve plexus, vascular structures, and elastic fibers. Other specialized elements can be seen in the esophageal wall. Islands of gastric mucosa, sebaceous glands, taste buds, and foci of hyperplastic epithelial cells with intranuclear glycogen (i.e., glycogenic acanthosis) have been described [37–39]. The latter condition is commonly seen during esophagoscopy as scattered, focal, white, sessile lesions, a few to several millimeters in diameter. Glycogenic acanthosis is of no clinical consequence. It stains more intensely with Lugol iodine than the surrounding typical mucosa.

Developmental anomalies

Congenital tracheoesophageal fistula and esophageal atresia

Because of a shared embryologic origin, congenital disorders affecting the esophagus often involve abnormalities of the respiratory tract. During embryogenesis, the process of elongation and separation of the trachea and esophagus can be disrupted. If fusion of the tracheoesophageal septum is incomplete, the result is a tracheoesophageal fistula (TEF). Five basic types of TEF and atresia have been described (Fig. 30.7). Esophageal atresia with lower-pouch fistula is by far the most common [40,41]. Esophageal atresia and congenital TEF occur in 1 in 2400 to 1 in 4500 individuals [42]. Hydramnios and prematurity are common in infants with atresia or TEF [43–45]. Up to 50% of infants may have other associated congenital anomalies that include cardiac, genitourinary, gastrointestinal, skeletal, and central nervous system anomalies [42]. VACTERL is an acronym used to describe the combined occurrence of vertebral, anal, cardiac, tracheoesophageal, renal and limb anomalies in the same patient.

Symptoms vary with the type of tracheoesophageal anomaly. In infants with atresia alone, the diagnosis is often made after birth, with signs of retention and regurgitation of

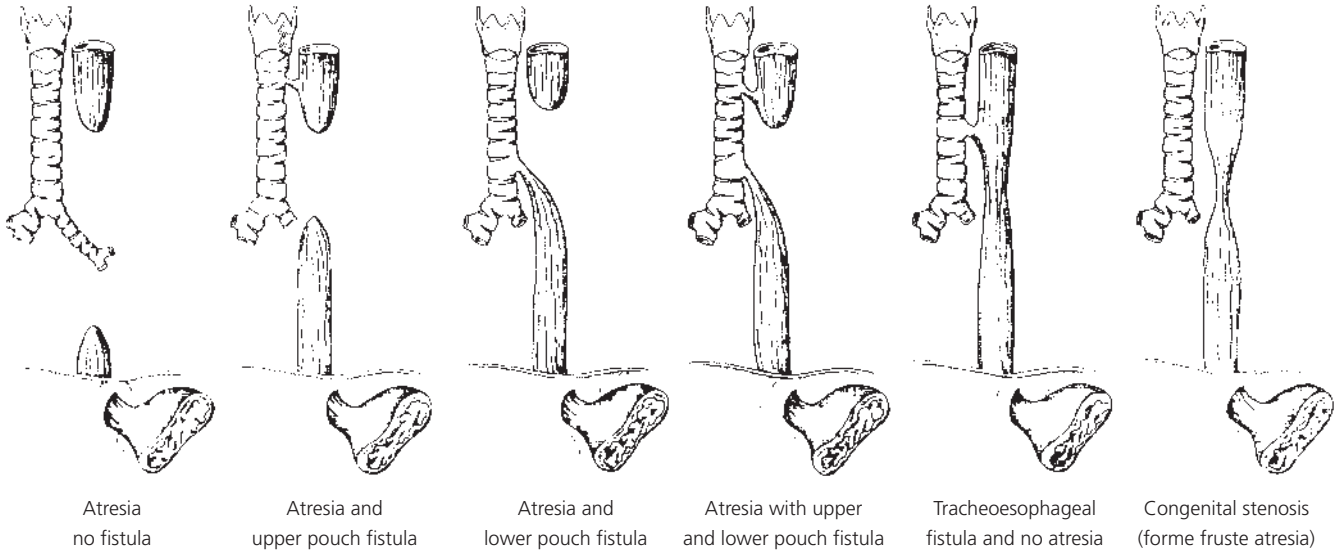


Figure 30.7 The spectrum of esophageal atresia, tracheoesophageal fistula, and congenital stenosis. Atresia with lower pouch fistula (*third from left*) is the most common anomaly.

saliva and feedings. Infants with proximal fistula exhibit respiratory distress during feedings. In infants with atresia and distal fistula, excessive salivation and regurgitation occur along with cyanosis and pneumonia secondary to reflux of gastric contents. The fifth type, isolated TEF or H-type fistula, leads to cough and choking with feedings, recurrent pneumonia, and intermittent abdominal distention. In rare cases, this anomaly may present in adulthood given the absence of associated esophageal atresia. In such cases, secondary causes of TEF such as infection, chronic inflammation and cancer need to be excluded. Adult patients present with a history of recurrent aspiration pneumonia and bronchiectasis [46–49].

Most infants with esophageal atresia and TEF undergo surgical repair with division of the fistula and primary esophageal anastamosis. In cases of atresia with wide separation of the proximal and distal esophageal remnants, esophageal lengthening procedures or interposition using small or large intestine is necessary. Gastrointestinal complications result from both the underlying congenital defect as well as surgical repair. Dysphagia is common and can result from anastamotic stricture formation as well as esophageal dysmotility and rarely from concomitant congenital esophageal stenosis. Abnormalities of the myenteric plexus have been described and may account for the dysmotility [50]. Gastroesophageal reflux and delayed gastric emptying have also been reported in a high proportion of patients.

Congenital esophageal stenosis

Congenital esophageal stenosis is rare, estimated to occur in 1 in 25 000 live births, and is thought to result from failure of the normal embryonic separation of trachea and esophagus that is frequently associated with esophageal atresia and TEF [51]. Stenoses caused by tracheobronchial remnants, and fibromuscular wall hypertrophy and membranous web formation have been described (Fig. 30.8) [52–55]. Unlike atresia and TEF, congenital stenosis is often not diagnosed until later in childhood, and several cases have been reported in adults [56,57,216]. Symptoms include regurgitation, prolonged eating time, and dysphagia with recurrent solid bolus impaction. An esophagram usually demonstrates a focal stenosis in the middle third of the esophagus. Endoscopic ultrasono-

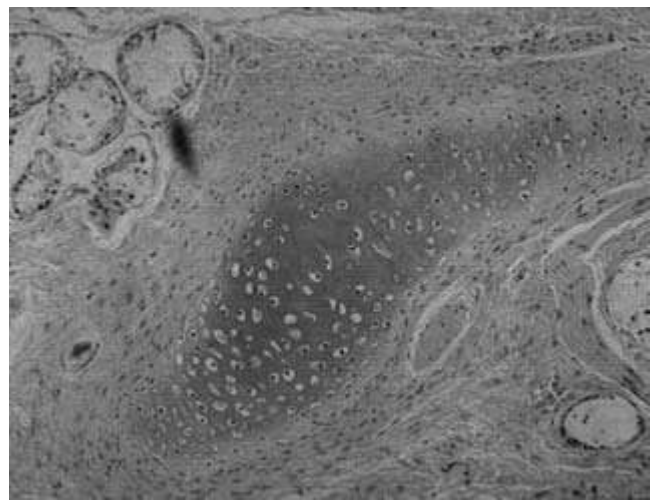


Figure 30.8 Histological section from the esophageal wall of a resected segment of congenital esophageal stenosis with cartilaginous tracheobronchial remnant. From Maeda et al. [58], with permission from Elsevier.

graphy can visualize the presence of tracheobronchial remnants. Surgical myotomy or segmental resection has been advocated for most symptomatic patients [58]. Esophageal dilation has been used in children and adults but perforation does occur [51,59]. Eosinophilic esophagitis can present with proximal esophageal focal strictures as well as concentric mucosal rings in both children and adults and is an important consideration in the evaluation of a patient with possible congenital esophageal stenosis. While the ringed appearance of eosinophilic esophagitis is sometimes referred to as *trachealization* of the esophagus, these are mucosal and not cartilaginous rings. Several reported cases of congenital esophageal stenosis were likely unrecognized cases of eosinophilic esophagitis.

Congenital esophageal duplication

Congenital esophageal duplications, tubular or cystic, represent about 15% of digestive duplications [60]. They develop in the third week of embryonic development as the result of failure of vacuoles to coalesce properly, interfering with normal recanalization of the esophageal lumen. As a result, a cyst or parallel tubular channel forms within the esophageal wall [61]. Duplications of the gastrointestinal tract have three common characteristics: they are contiguous with some segment of the gastrointestinal tract; they are lined by alimentary epithelium; and smooth muscle is present in their walls. Most patients present before 1 year of age, but as many as 25%–30% of cases are first diagnosed in adults [62]. The most common presenting symptom is dysphagia, but the diagnosis is often made as an incidental finding in patients studied for other indications [63].

Congenital duplication cysts constitute less than 3% of mediastinal masses. They are the second most common benign esophageal submucosal lesion, with gastrointestinal stromal tumors being the most common [64,65]. They are lined by squamous columnar, cuboid, pseudostratified, or ciliated epithelium [66,67]; 60% arise in the distal third of the esophagus, 17% in the middle third, and 23% in the upper third [60,68]. Cysts located posterior to the heart have been associated with cardiac arrhythmias [69]. Intraspinal cystic extensions can cause neurological deficits that may be the initial sign of these disorders [70]. In one report, intraspinal anomalies coexisted with mediastinal masses in almost 25% of patients [71]. Gastric cysts, inclusion cysts without a smooth muscle wall, bronchogenic cysts, and neuroenteric cysts are also rarely found in the esophageal wall [60].

Duplication cysts can be seen on chest radiographs as posterior or middle mediastinal masses. On barium esophagram, a smooth curved displacement of the esophagus is seen without the sharp step-like proximal and distal margin seen with a leiomyoma. Computed tomography (CT) can be helpful in determining the location, size, and anatomical relation to other organs [72,73]. At endoscopy, a soft compressible submucosal indentation into the esophageal lumen is apparent [74]. Endoscopic ultrasonography can define the structure of a duplication cyst [75]. Preoperative esophageal biopsies have been discouraged by surgeons because of potential for adhesions between the esophageal mucosa and cyst wall that may make surgical resection more difficult.

Tubular duplications are rare and may be associated with other congenital cardiac, spinal, pulmonary, and extremity abnormalities [63]. Tubular duplications may communicate at both ends with the esophageal lumen or be closed at one end, but more than 80% do not communicate with the lumen [76–78,217]. Spontaneous submucosal dissection of the esophagus has been reported and can radiographically present with a double-barrel esophagus that mimics a tubular duplication [79]. Surgical resection is usually recommended for definitive treatment and pathological diagnosis [80–82]. Both laparoscopic and thoracoscopic resection have been reported [83,84]. Marsupialization has been used for the treatment of large cysts, and needle aspiration has been used to relieve tracheal compression from a duplication cyst [85,86]. Although rare, both adenocarcinoma and squamous cell carcinoma within tubular or cystic duplications have been reported [87–89].

Bronchopulmonary foregut malformation

The term *bronchopulmonary foregut malformation* was proposed by Gerle in 1968 to include pulmonary developmental abnormalities both with and without communication to the gastrointestinal tract. Since then, confusion in the terminology has occurred because of the inclusion and exclusion of a number of gastrointestinal, pulmonary and vascular anomalies as well as attempts to histologically, pathogenetically and anatomically classify the varying presentations described largely in case reports and small series. Bronchopulmonary foregut malformations include a focus of pulmonary parenchyma or sequestration with a patent congenital communication to the upper gastrointestinal tract [90–92,218,219]. They are considered distinct from TEF, which result from initial foregut septation. Bronchopulmonary foregut malformations develop when cell rests with respiratory potential arise from the foregut caudal to the lung bud or when a portion of the lung bud arises from the dorsal esophagus rather than the ventral trachea [91]. The tract within the sequestered pulmonary lobe typically involutes because of outgrowth of its blood supply; incomplete involution of the tract leads to a gastrointestinal tract communication [92]. Bronchopulmonary foregut malformations are most commonly seen in the lower lobes [218]. Up to 40% of children with communicating bronchopulmonary foregut malformations have associated congenital anomalies. The clinical presentation in infants is respiratory distress that is exacerbated with feedings. In older children and adults, recurrent pneumonia, bronchiectasis, hemoptysis, gastrointestinal bleeding, and dysphagia may develop. Contrast esophagram, CT, and angiography are used for diagnosis and surgical planning.

Aortic arch vessel abnormalities

It has been estimated that 3% of the population have a congenital abnormality of the aortic arch vessels, but only rarely does this result in symptomatic compression of the esophagus [93]. In the embryo, the foregut is surrounded by vascular structures of the branchial arches. Normally, portions of the branchial arches obliterate to form the great vessels and aortic arch. Abnormalities in developmental obliteration of the branchial arches may lead to vascular compression of the trachea and esophagus [94].

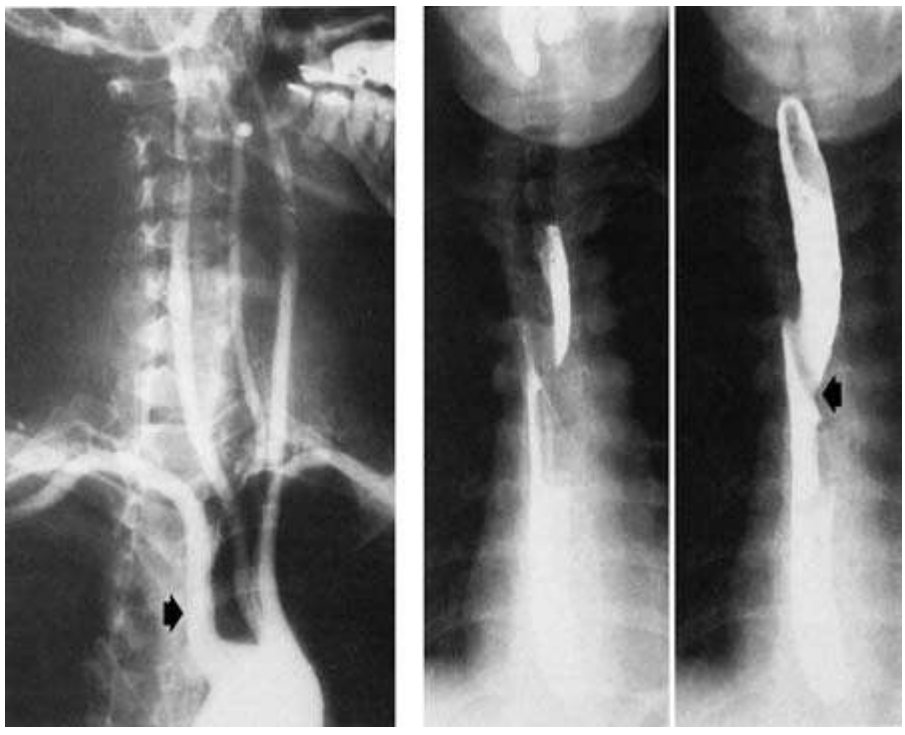
The term *dysphagia lusoria*, literally translated from the Latin *lusus naturae* (meaning trick or freak of nature), is used to describe symptomatic esophageal compression resulting from any vascular anomaly of the aortic arch. Most commonly, it results from an aberrant right subclavian artery [95]. The root of the aberrant artery often has a broad base referred to as Kommerell diverticulum. Symptoms of this anomaly may occur at the onset of semisolid feedings, later in childhood, or in adult life. With this anomaly, the right subclavian artery arises from the left side of the aortic arch and compresses the esophagus from the posterior aspect in an oblique manner (Fig. 30.9a). Based on autopsy studies, an aberrant right subclavian artery occurs in 0.7% of the general population, with only 10% of these affected individuals having symptoms related to compression [96]. During esophageal endosonography performed in 3334 patients, an aberrant right subclavian artery was identified in 12 (0.36%). None of the patients had symptoms from this entity [97]. While esophageal motility abnormalities have been observed, the patterns have been variable and nonspecific [98]. This vascular anomaly most typically causes symptoms later in life [99]. The reason for this is unclear but may be attributed to age-related changes to the vasculature.

Barium esophagram demonstrates an oblique filling defect just above the level of the aortic arch (Fig. 30.9b). Computed tomography and magnetic resonance imaging have largely replaced the need for angiography in the diagnosis. Endoscopy may demonstrate a posterolateral pulsatile compression of the lumen of the proximal esophagus. Correction is performed in children by division and ligation of the aberrant artery. In adults, reanastomosis to the ascending aorta is performed to avoid the development of a subclavian steal syndrome [100]. Surgery is not always needed because many adult patients can easily tolerate minor degrees of dysphagia by simply modifying their diet.

Esophageal compression by an anomalous vertebral artery and right aortic arch with constricting left ligamentum arteriosum has been reported in adults and can be successfully repaired surgically [101,102]. Other vascular anomalies are causes of tracheoesophageal compression in infants, and these require early surgical intervention [103,104].

Heterotopic gastric mucosa (inlet patch)

Heterotopic gastric mucosa, also know as an inlet patch, is a focus of gastric epithelium most commonly located in the cervical esophagus. An autopsy series of 1000 children



(a) (b)

Figure 30.9 **(a)** Angiography reveals an aberrant right subclavian artery (arrow) arising from the aortic arch. **(b)** Barium esophagram in the same patient reveals oblique esophageal compression (arrow) by the aberrant right subclavian artery posterior to the esophagus.

demonstrated the prevalence at 4.5% [105]. Endoscopic studies reported a prevalence of 0.1%–10% [106–109]. Most commonly, the inlet patch is situated immediately distal to the UES, making endoscopic detection difficult. Substantially higher prevalence frequencies are reported if microscopic foci of gastric tissue are included in the definition. Inlet patches have been reported to occur in 5.6% of patients with Barrett esophagus but in none with achalasia [110].

At endoscopy, these areas of gastric mucosa typically are well-circumscribed patches of reddish orange or salmon-colored mucosa that range in size from 2 mm to 4 cm [111]. The lesions can be unifocal, multifocal, or circumferential. Inlet patches can be dramatically demonstrated by injecting topical dilute iodine solution to stain the squamous mucosa around their margin. Histologically, the majority contain fundic-type gastric mucosa that includes parietal cells [106]. The inlet patch is generally considered to be a congenital abnormality that results from incomplete replacement of the early embryonic columnar epithelium with squamous epithelium. On the other hand, some investigators have reported that inlet patches share features with Barrett esophagus, suggesting that the former is an acquired condition associated with acid reflux [112–114]. *Helicobacter pylori* has been detected in mucosal biopsies of inlet patches [115,116].

Complications of heterotopic gastric mucosa include those secondary to acid production and neoplastic transformation. Acid secretion has been demonstrated in several reports [106,117–119]. While the majority of inlet patches are asymptomatic, symptoms of odynophagia, dysphagia and globus have been reported [120]. Cervical esophageal strictures or webs and even esophagotracheal fistula have been demonstrated [121–123]. Plummer–Vinson (or Paterson–Kelly) syndrome is characterized by dysphagia due to a cervical esophageal web with concomitant iron deficiency anemia. The anemia and strictures may be secondary to esophageal erosions produced by acid secretion. In terms of cancer risk, 30 cases of progression of heterotopic gastric mucosa to adenocarcinoma have been reported [124,125]. Given the high prevalence of inlet patches and low prevalence of cervical esophageal adenocarcinoma, both the absolute and relative risk of malignant transformation is quite low. In light of this, biopsies of endoscopically detected inlet patches are generally not performed.

Symptomatic inlet patches should respond to proton pump inhibition through the same mechanism by which gastric acid suppression is achieved. Complications of strictures and webs are amenable to standard esophageal dilation techniques, with the caveat that detection of such strictures can be more difficult given the location adjacent to the UES. Transendoscopic thermal ablation combined with high-dose omeprazole therapy and argon plasma ablation have been shown to allow replacement of the inlet patch by normal squamous mucosa, with resolution of related symptoms [126,127]. As most patients are asymptomatic and symptomatic patients should respond to acid inhibition or dilation, the role for ablation techniques is yet undefined.

Structural anomalies

Esophageal rings and webs

Lower esophageal mucosal ring (Schatzki ring)

The lower esophageal mucosal ring, or B-ring, was initially described by Templeton in 1944 [220]. In 1953, Ingelfinger and Kramer and Schatzki and Gary independently described the association of lower esophageal mucosal rings with dysphagia [128,129]. Schatzki and Gary attributed the symptoms to a fixed mucosal stricture at the squamocolumnar junction. Ingelfinger and Kramer, on the other hand, postulated that a contractile muscular ring at the gastroesophageal junction produced the symptoms. Ring-like narrowings at the esophagogastric junction are now differentiated into two types: the lower esophageal muscular ring, or A-ring, and lower esophageal mucosal ring, or B-ring.

Lower esophageal mucosal rings are located at the level of the squamocolumnar junction. These rings consist of mucosa and submucosa and are covered by squamous mucosa on the proximal aspect and either columnar mucosa or several millimeters of squamous mucosa on the distal or gastric aspect [130,131]. The lower esophageal mucosal ring is circumferential and 3 mm or less in thickness (Fig. 30.10). Based on an autopsy study of 100 subjects mucosal rings have a prevalence of 9%, and are detected in 4%–15% of radiographic studies [132]. The rings are likely acquired and may be a manifestation of reflux disease, although morphologically they are distinct from peptic strictures. The majority of patients with Schatzki rings have abnormal distal esophageal acid exposure on pH monitoring, although this does not prove causation [133]. A congenital origin and association with esophageal shortening have also been proposed [132].

Most patients with lower esophageal mucosal rings are asymptomatic; however, they are one of the most common causes of intermittent dysphagia and food impactions, typically presenting in patients older than 40 years of age [134,135]. The severity of symptoms depends on the inner diameter of the ring. Rings larger than 20 mm in diameter are usually asymptomatic, rings 13–20 mm in diameter cause variable degrees of dysphagia depending on type and size of bolus, and rings less than 13 mm in diameter frequently cause solid food dysphagia [136,137]. Serial esophagrams have demonstrated the development and progressive narrowing of lower esophageal mucosal rings, providing evidence that these are acquired and not congenital lesions [138–140]. Misunderstanding in the literature and in practice has resulted from confusing Schatzki rings with short annular peptic strictures and, more recently, eosinophilic esophagitis.

On esophagram, a lower esophageal mucosal ring appears as a thin (< 3.0 mm) transverse circumferential ridge above

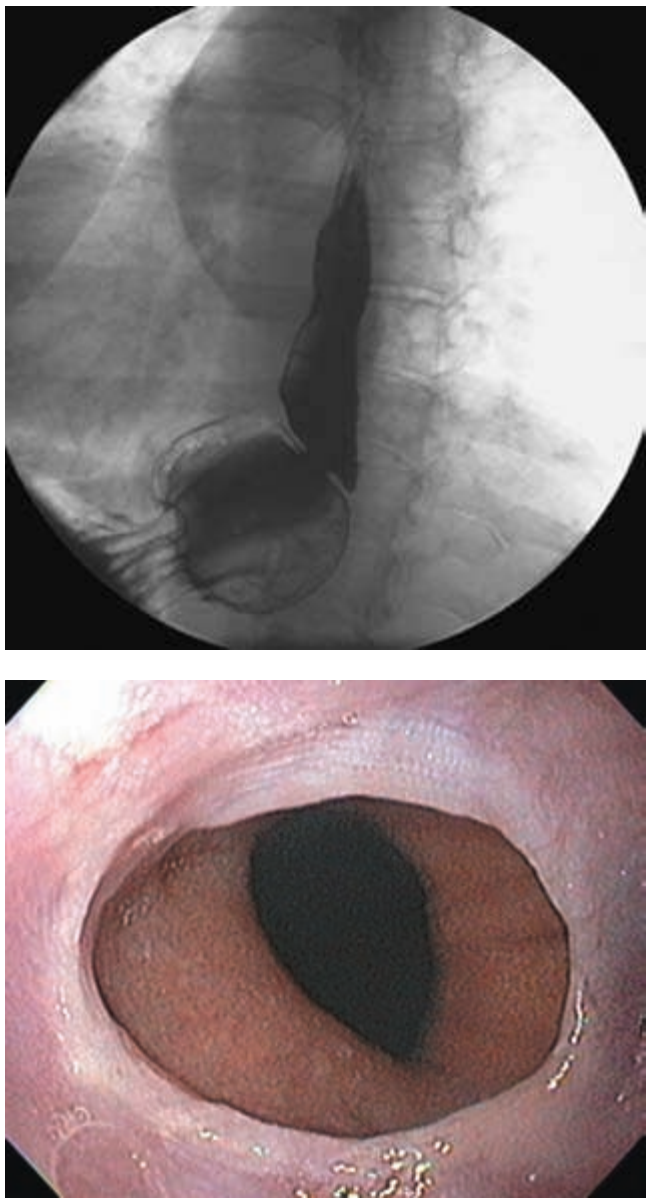


Figure 30.10 **(a)** Barium esophagram demonstrates a lower esophageal mucosal ring, also known as Schatzki or B-ring, at the proximal end of a hiatal hernia pouch. Constriction of the distal end of the hernia is secondary to the diaphragmatic hiatus. The ring is appreciated with both a distended (*left*) and undistended (*right*) hiatal hernia. **(b)** Endoscopic view of a Schatzki ring with hiatal hernia immediately below. The ring margin is smooth, less than 3 mm thick, and without inflammation or evidence of fibrosis.

the hiatus of the diaphragm. To visualize the ring either endoscopically or radiographically, the esophagogastric junction must be adequately distended (Fig. 30.10). By definition, a hiatal hernia is invariably present, although this may be related to a limited ability to visualize the ring when it is collapsed by constriction of the diaphragmatic hiatus. Lower esophageal mucosal rings are absent in patients with long segment Barrett esophagus. Barium esophagram with a prone full-column technique is more sensitive than double-contrast radiography or endoscopy in detecting lower esophageal mucosal rings [141]. Performing a Valsalva maneuver during the esophagram is helpful in demonstrating a ring [142]. The use of a barium tablet or marshmallow bolus may further improve the sensitivity of the barium esophagram to correlate dysphagia with the ring. The standard barium tablet has a diameter of 12.8 mm and will therefore ignore rings in the 13–20 mm category that may be clinically significant.

The endoscopic characteristics of lower esophageal rings and the associated findings are readily recognized (Fig. 30.10b) [143]. Symptomatic lower esophageal rings can be effectively treated with single passage of a 17–20 mm (51F–60F) Maloney or Savary dilator [144]. For very tight rings, some clinicians use the standard gradual sequential dilation technique rather than passage of a single large-diameter bougie. Hydrostatic balloon dilation is also effective. Disruption of the ring using four-quadrant mucosal biopsies and needle knife incision has been described [145,146]. While most patients achieve immediate relief of dysphagia following dilation, a follow-up study noted that 32% of patients had recurrent dysphagia at 1 year and 65% at 2 years [147]. Proton pump inhibitor therapy may reduce the occurrence of recurrent dysphagia, again pointing to a possible link with reflux disease. In a trial of patients with symptomatic Schatzki ring initially treated with dilation, recurrent dysphagia that required repeat dilation occurred in 7% of patients randomized to omeprazole compared with 47% of patients on placebo [148]. It should be kept in mind that Schatzki rings are common and may be an incidental finding when detected in a patient with dysphagia. For patients demonstrating a poor or short-duration response to esophageal dilation of a presumed symptomatic lower esophageal mucosal ring, other etiologies should be sought including motility disorders and eosinophilic esophagitis.

Lower esophageal muscular ring

In an autopsy series, lower esophageal muscular rings were found in 5% of an asymptomatic cohort of 100 patients [132]. The location proximal to the squamocolumnar junction makes it likely that the rings represent an exaggeration of the proximal aspect of the LES. Symptomatic muscular rings are unusual and typically present with dysphagia. Unlike the dysphagia that occurs with Schatzki rings, muscular rings present with intermittent dysphagia for both liquids and solids in the absence of food impaction. On barium esophagram, muscular rings are smooth symmetric narrowings that are several millimeters in axial extent with a lumenal aperture that varies during the course of fluoroscopic examination (Fig. 30.11). Endoscopically, the appearance is that of a constriction with intact overlying squamous mucosa situated 2–3 cm above the squamocolumnar junction. Unlike achalasia, the constriction is very focal and does not encompass the distal aspect of the esophagogastric junction. Lower esophageal muscular rings have been associated

Figure 30.11 Lower esophageal muscular ring (A-ring). Two views from the same patient show marked, 3-cm long constrictions in the distal esophagus proximal to a hiatal hernia (*left*). (*Right*) Partial relaxation of the A-ring with distention of the phrenic ampulla proximal to a concomitant Schatzki ring. The distended hiatal hernia is visible distal to the Schatzki ring.

with esophageal manometric abnormalities that include high-amplitude long-duration esophageal body contractions and esophageal spasm [149]. Esophageal dilation is often attempted but provides incomplete and short-lasting relief. Several case reports have noted significant improvement in dysphagia following injection of botulinum toxin [149–151]. Surgical myotomy has been described but is necessary in only rare instances [128,152–154].

Cervical esophageal and midesophageal webs

Esophageal webs are thin (1–2 mm) transverse membranes of squamous epithelium. They most commonly occur in the cervical esophagus and usually originate on the anterior wall. They are rarely circumferential but may be multiple. Because of their proximity to the UES, cervical webs are easily missed on upper endoscopy. Radiographic studies detected esophageal webs in 6%–12% of patients, with the higher prevalence reported in patients with symptoms of dysphagia [155,156]. Like Schatzki rings, most webs are asymptomatic. Intermittent solid food dysphagia is the usual complaint in symptomatic patients.

Cervical esophageal webs have been reported to occur in association with heterotopic gastric mucosa and in some instances may represent a form of peptic stricture related to local acid secretion [157]. Cervical webs should be distinguished from proximal, mucosal, web-like strictures secondary to a number of other conditions producing esophageal injury, including epidermolysis bullosa, cicatricial pemphigoid, pill esophagitis, chemotherapy-induced mucositis, graft-versus-host disease, radiation and eosinophilic esophagitis.

The association of postcricoid webs with iron deficiency anemia (i.e., Plummer–Vinson or Paterson–Kelly syndrome) is both infrequent and controversial [158,159]. The existence of the syndrome is debated, although some indirect evidence points to the possibility that iron deficiency could predispose to web formation. On the other hand, as discussed in a previous section of this chapter, acid secretion from heterotopic gastric mucosa could be a cause of blood loss from esophageal erosions as well as cervical webs.

Midesophageal webs are rare and typically present with dysphagia. They may be single or multiple and are believed to be of congenital origin [159–163]. In addition to midesophageal mucosal lesions resulting from the conditions causing esophageal injury noted above, they may also be confused with congenital esophageal stenosis. If symptomatic, they are best treated with bougienage. Treatment with transendoscopic incision or surgical resection has been reported but is rarely necessary [160–162,164,165].

Cricopharyngeal bar

Cricopharyngeal (CP) bar is a common radiographic finding, reported in anywhere from 5% to 19% of patients undergoing dynamic pharyngeal radiography [166,167]. It appears as a prominent and persistent posterior indentation at the level of the lower third of the cricoid cartilage (Fig. 30.12). The majority of patients with CP bar do not experience significant dysphagia. In one report, dysphagia was not statistically more prevalent in CP bar patients than in controls (13% vs. 8%) [168]. Symptomatic CP bars most commonly present in the elderly with dysphagia for solids and occasionally liquids as well. Studies have also shown histological changes similar to those found in patients with Zenker diverticulum that include muscle degeneration and fibrosis [169]. It is important to exclude other more common etiologies for

Figure 30.12 Barium swallow depicting a prominent cricopharyngeal bar in the cervical esophagus in a patient presenting with dysphagia.

dysphagia since a CP bar may be an incidental finding. Furthermore, CP bars may be secondary to neuromuscular causes, including central and peripheral nervous system abnormalities (multiple sclerosis, amyotrophic lateral sclerosis, syringomyelia, cerebral vascular disease), inflammatory myopathies, and myoneural junction disorders (myasthenia gravis, diphtheria, tetanus).

Early studies attributed the pathophysiology of CP bars to failed UES relaxation, UES spasm, and basal hypertension. It has subsequently been recognized that the radiographic appearance of a CP bar is neither associated with high basal UES pressure nor with incomplete sphincter relaxation. Moreover, one study found that neither peak pharyngeal pressure nor incoordination were predictors of outcome following cricopharyngeal disruption in a heterogeneous group of patients with oropharyngeal dysphagia [170]. Using simultaneous pharyngeal videoradiography and manometry, these investigators demonstrated that increased hypopharyngeal intrabolus pressure was a significant predictor of positive outcome following CP disruption. Similar to studies in patients with Zenker diverticulum, these authors suggested that increased intrabolus pressure implies impaired sphincter opening and may be an indirect measure of sphincter compliance, all of which predict response to CP disruption. An earlier study by Dantas and colleagues demonstrated significant impairment of cricopharyngeal opening in patients with a CP bar when compared with control subjects without a CP bar, which further supports the concept of sphincter dysfunction in the pathogenesis of CP bars [167].

Treatment options for symptomatic CP bar include esophageal dilation and surgical myotomy. Several small case series have described significant relief of dysphagia with a 1–2 year follow-up after dilation to 17–20 mm [170–173]. Esophageal dilation is an attractive alternative to surgery, especially in older patients with comorbidities.

Pharyngoesophageal and esophageal diverticula

Zenker diverticulum

Pharyngoesophageal diverticulum was first described by Ludlow in 1769 and critically reviewed a century later by the pathologists Zenker and Ziemssen [221]. Pharyngoesophageal or Zenker diverticulum represents an outpouching of the posterior hypopharyngeal mucosa proximal to the esophageal inlet. The site of origin of the diverticulum between the oblique fibers of the inferior pharyngeal constrictor and the transverse fibers of the cricopharyngeus was described by Killian in 1907 and is referred to as Killian dehiscence or the triangle of Killian.

A number of hypotheses have been proposed to explain the pathogenesis of Zenker diverticulum. The observation that both symptomatic and asymptomatic Zenker diverticula occur in the elderly population supports an acquired defect that is a consequence of aging. Early studies suggested that the UES failed to relax in this condition leading to the term *cricopharyngeal achalasia*. Subsequent combined manometric/fluoroscopic studies demonstrated complete UES relaxation but diminished opening [174,175]. As a result, hypopharyngeal pressures are increased and thought to lead to progressive protrusion through an area of relative mural weakness in the triangle of Killian. The limitation in UES opening is most likely a myogenic rather than neurogenic phenomenon. Histological studies of muscle biopsies taken during surgical treatment have demonstrated connective tissue replacement of skeletal muscle fibers and muscle fiber degeneration [175–177]. The cause of the muscle changes is unclear. Evidence linking gastroesophageal reflux disease with Zenker diverticulum is limited. Initial reports of UES hypertension and contraction in response to distal esophageal acid exposure have not been confirmed in subsequent studies [178,179]. Others have theorized that esophageal shortening induces mural weakness, allowing for mucosal herniation in patients with Zenker diverticulum [180].

The prevalence of Zenker diverticulum ranges from 0.01% to 0.11%, with the majority of patients being asymptomatic [181]. The median age is approximately 70 years with a male predominance, and presentation in a patient younger than 30 is unusual. The predominant symptoms are dysphagia and regurgitation. Throat pain, cough, aspiration, halitosis, and neck gurgling or mass are also reported. The diagnosis is apparent on esophagram but the fluoroscopic field needs to visualize the hypopharynx, which is not routinely included by all radiologists (Fig. 30.13). For reasons that are unclear, the majority of diverticula deviate to the left. Complications

Figure 30.13 Anterior view of barium-filled Zenker diverticulum deviating to the left of the esophageal axis.

include aspiration pneumonia, pill retention, ulceration, fistula, and bleeding. Squamous cell cancer was reported in 0.4% of 1249 patients with Zenker diverticulum over a 53-year period [182]. Spindle cell carcinoma and benign tumors have also been reported to arise in pharyngoesophageal diverticula [183,184]. Endoscopy is not essential to the management of symptomatic Zenker diverticulum and carries a risk of perforation. With direct visualization and awareness of the posterior location of the diverticular orifice, endoscopy is not contraindicated and may demonstrate concomitant esophageal pathology or secondary complications. Blind esophageal intubation with nasogastric tubes, manometric catheters, and transesophageal echocardiographic devices can lead to inadvertent perforation. A fluoroscopically placed guidewire and use of smaller, pediatric or transnasal endoscopes can facilitate esophageal intubation in cases with significant stenosis of the UES.

Patients with minimal or no symptoms can be followed clinically for potential progression. Symptomatic pharyngoesophageal diverticulum is managed by endoscopic or surgical approaches [185]. Early surgical reports of diverticulectomy alone were associated with a very high recurrence rate leading to the current recommendation for UES myotomy in most cases. For the open surgical approach, myotomy alone may suffice for small diverticula while a myotomy with either diverticulectomy or diverticulopexy is performed for larger diverticula. Notwithstanding the elderly population that is afflicted with this condition, operative mortality is less than 2% [186]. Reported morbidity is highly variable but approximates 5%–10% and includes esophageal leak, bleeding, mediastinal emphysema, fistula, nerve palsy, and mediastinitis.

The endoscopic approach to Zenker diverticulum dates back to 1917 but has become increasingly popular with advances in the use of electrical or laser cautery or endoscopic stapling devices. The technique involves dividing the common septum between the diverticulum and esophagus that is largely composed of the UES. As of 2006, well over 700 patients have been treated by this approach in the literature with outcomes, morbidity, and recurrence rates that are similar to the open surgical approach [187]. The procedure is often performed with monitored anesthesia care, although some surgeons still use general anesthesia. Advantages include shorter recovery and hospital stays. The technique is primarily being performed by thoracic surgeons and otorhinolaryngologists rather than gastroenterologists. An open surgical approach is generally favored for younger patients who are good surgical candidates and for large diverticula.

Killian–Jamieson diverticula

Killian–Jamieson diverticula are outpouchings arising from the proximal cervical esophagus immediately below the UES [188]. They are less common and less likely to present with dysphagia than Zenker diverticulum [189]. While more commonly unilateral with a left-sided predilection, bilateral diverticula occur in 25% and may coexist with Zenker diverticulum [189]. It is important to recognize these anatomical lesions since they can be confused with Zenker diverticulum. It is interesting to speculate on their pathogenesis given their location below the UES. An underlying esophageal motility disorder of the striated muscle of the cervical esophagus has neither been described nor looked for in these patients.

Midesophageal and epiphrenic diverticula

Diverticula may also occur in the middle or distal esophagus (Fig. 30.14). Prevalence estimates of less than 1% are based on retrospective reviews of radiographic studies but are affected by the indications for the radiographic examination. A century ago, midesophageal diverticula were described secondary to pulmonary tuberculosis. They were termed traction diverticula and felt to result from tethering on the outer aspect of the esophagus created by inflammation of adjacent mediastinal lymph nodes. Midesophageal diverticula have also been reported in cases of anthracosis, sarcoidosis, histoplasmosis, and lymphoma, all with a presumed similar pathogenesis [190]. Over the past 50 years, it has become recognized that the majority of both midesophageal and epiphrenic diverticula are seen in conjunction with esophageal dysmotility [191–195]. They are typically referred to as pulsion-type diverticula, caused by increased intralumenal pressure induced by high-amplitude or simultaneous esophageal body contractions or failed LES relaxation. The predominant associated manometric disorders are

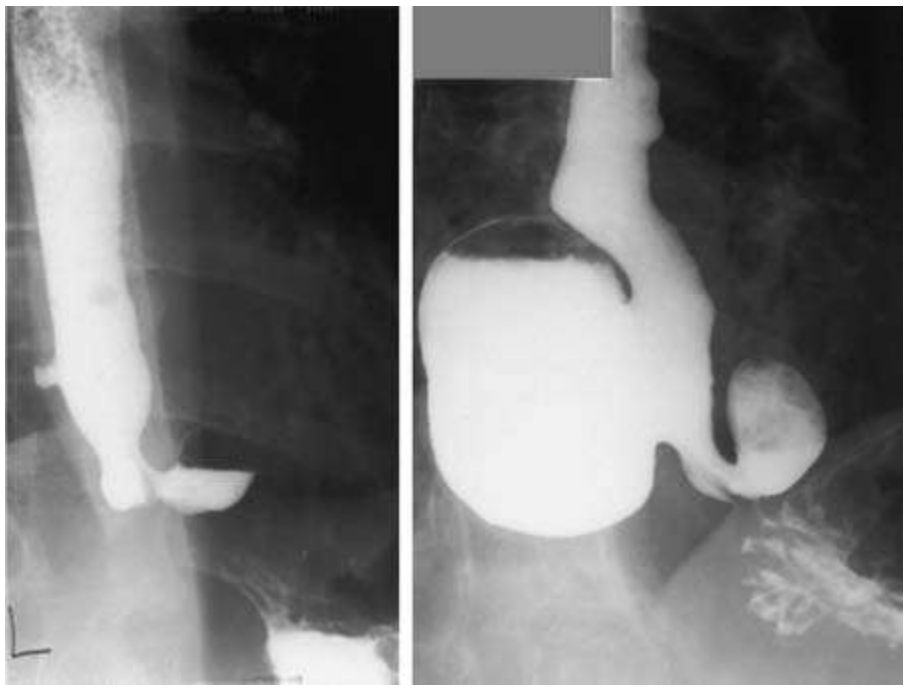


Figure 30.14 Barium radiograph depicting significant enlargement of epiphrenic diverticula in a patient on initial presentation (*left*) and 10 years later (*right*).

achalasia and diffuse esophageal spasm. However, diverticula are uncommon sequelae of either disorder. Cases of hypertensive LES, nutcracker esophagus, and nonspecific motility disorders have also been reported, although the causative nature of these diagnoses in the pathogenesis of diverticula is uncertain. They are manometrically defined conditions that even in the absence of diverticula have shown poor correlation with symptoms or esophageal functional abnormalities. A recent study utilizing high-frequency intralumenal ultrasonography reported temporal incoordination between circular and longitudinal muscle contractions in patients with nutcracker esophagus [196]. The investigators postulated that the asynchrony resulted in increased esophageal wall stress that could be a mechanism for diverticulum formation. Esophageal diverticula may also be secondary to esophageal wall anatomical weakening as in cases following Heller myotomy for achalasia or scleroderma [197].

Symptom presentation for esophageal diverticula closely mirrors that of the underlying esophageal motility disorders, making it difficult to discern whether a particular symptom is a direct result of the diverticulum. Dysphagia and regurgitation are the dominant presenting complaints with additional symptoms including chest pain, heartburn, weight loss, and respiratory symptoms [190]. In general, symptoms tend to correlate more closely with the motility disorder rather than the size of the diverticulum [190,194]. An exception to this are giant diverticula, which increase the retention of food and likely increase the risk of regurgitation and aspiration. Epiphrenic diverticula more commonly protrude from the right. Uncommon complications of esophageal diverticula include ulceration, hemorrhage, perforation, and fistulization. Rare cases of carcinoma arising from esophageal diverticula have been reported [198,199]. Many patients with esophageal diverticula are asymptomatic at the time of presentation with the finding incidentally noted on a radiographic or endoscopic procedure for another indication [190]. Natural history studies of untreated esophageal diverticula are few. In one radiologic series, enlargement of the diverticulum was noted in 16% over a 12-year period [200]. Clinical case series have noted the development or progression of symptoms in less than 10% with a follow-up of several years [190].

Surgery remains the primary means of therapy for symptomatic esophageal diverticula. Esophageal dilation over a guidewire under fluoroscopy provides partial and temporary symptom relief in some patients with diverticula. Botulinum toxin A injection of the LES has been effective in providing relief of dysphagia in a small series of patients with diverticula, although the moderate efficacy and durability of this approach limits its utility [201–203]. Recognizing the importance of underlying esophageal dysmotility, most surgical approaches combine a diverticulectomy with esophageal myotomy [190]. Several retrospective reports have noted a higher rate of postoperative esophageal leak and recurrent diverticula for diverticulectomy compared with diverticulectomy with myotomy. Even when combined with myotomy, esophageal leak is a major source of surgical morbidity and significantly more common than reported with myotomy alone in the treatment of achalasia without diverticula. A thorough presurgical evaluation, including barium esophagram, esophagoscopy and esophageal manometry, should be performed. The proximal extension of the myotomy into the esophageal body is considered in cases of esophageal spasm

and vigorous achalasia and can be guided by the proximal extent of the diverticula or hypercontractile manometric abnormalities. Divertulectomy, accompanied by LES myotomy plus some degree of fundoplication, has been successfully accomplished via the laparoscopic transhiatal approach [190].

Esophageal intramural pseudodiverticulosis

Esophageal intramural pseudodiverticulosis is a rare condition, detected in less than 1% of radiologic studies of the esophagus [204]. First described by Mendl and colleagues in 1960, multiple small diverticula form in the wall of the esophagus by dilation of the excretory ducts of the submucosal esophageal glands [205–207]. The diagnosis is best appreciated on barium studies where intramural tracking of contrast can also be appreciated in 50% of studies [208]. Thickening of the esophageal wall due to chronic submucosal inflammation and fibrosis can be appreciated on CT as well as endoscopic ultrasonography [209,210]. Most cases present after the sixth decade with chronic dysphagia. Esophageal strictures, typically proximal, are seen in 70%–90% of patients, and esophageal manometric abnormalities have been found in two-thirds of those studied [206,211,212]. Unlike the association between achalasia and esophageal spasm with esophageal diverticula, the reported abnormalities are most commonly nonspecific and may not be involved in the pathogenesis of intramural pseudodiverticulosis. Only one case of pseudodiverticulosis in achalasia has been reported [213]. The relation of corrosive injury of the esophagus to intramural pseudodiverticulosis has been reported [214]. A report of 14 cases of this entity in 59 patients with corrosive esophageal injury noted that an esophageal stricture was a constant association. No correlation was found between the length of the stricture and the number of diverticula, and the diverticula regressed in number or disappeared altogether after the stricture was dilated.

As opposed to the surgical management of other esophageal diverticula, esophageal intramural pseudodiverticulosis is managed medically. Dilation of strictures, antireflux therapy, antifungal therapy, and calcium channel blockers have been reported to relieve symptoms [209,214]. Esophageal candidiasis has been described in up to 50% of reported cases; however, its role in the development of esophageal intramural pseudodiverticulosis is unknown and is likely secondary to stasis. Rare complications include perforation, mediastinitis, fistula formation, and bleeding. An association between intramural pseudodiverticulosis and esophageal cancer has been reported but the cause and effect of this observation is unclear [215].

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A ESOPHAGUS

31 Motility disorders of the esophagus

Peter J. Kahrilas, John E. Pandolfino

Oropharyngeal swallowing disorders, 740
Esophageal motility disorders, 747
Esophageal involvement in systemic disease, 766

Esophageal motility disorders can be categorized based on anatomical localization and muscle type involved. The oropharynx and proximal esophagus are composed of striated muscle and are under central nervous system control. In contrast, the distal esophagus is predominantly composed of smooth muscle controlled by the enteric nervous system and vagus nerve. Recognizing these morphological and functional differences, this chapter on motility disorders of the esophagus first considers oropharyngeal dysphagia, which includes most physiological aberrations of the upper esophageal sphincter and proximal esophagus, and then focuses on purely esophageal motor disorders, including considerations mainly relevant to the distal esophagus.

Oropharyngeal swallowing disorders

Oropharyngeal dysphagia is associated with high morbidity, mortality and cost. Estimates of the prevalence of dysphagia among individuals older than 50 years of age range from 16% to 22% [1,2]. Within health-care institutions it is estimated that up to 13% of hospitalized patients [3] and 60% of nursing home residents [4] have feeding problems, of which most are attributed to oropharyngeal dysfunction as opposed to esophageal dysfunction. The consequences of oropharyngeal dysphagia are severe: dehydration, malnutrition, aspiration, choking, pneumonia, and death. In fact, the mortality rate of nursing home residents with dysphagia and aspiration can be as high as 45% over 1 year [5]. As our population continues to age, oropharyngeal dysphagia will become an increasing problem associated with complex medical and ethical issues.

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Evaluation and classification of oropharyngeal dysphagia

The evaluation of patients with presumed oropharyngeal dysphagia should focus on five fundamental questions:

1 Does the patient describe dysphagia as opposed to globus sensation or xerostomia?

2 Is dysphagia oropharyngeal or esophageal in origin?

3 Is the dysphagia secondary to a structural or functional disorder?

4 Is there an underlying related or causative disorder?

5 Should therapy be directed toward the underlying etiology or the dysphagia itself?

These questions can usually be answered with a careful history and physical exam. However, further diagnostic tests may be needed to determine both the cause of oropharyngeal dysphagia and the proper treatment.

The patient history is crucial in the evaluation of oropharyngeal dysphagia. Major objectives of the history are to differentiate oropharyngeal dysphagia from esophageal dysphagia, xerostomia, or globus sensation. Whereas patients almost invariably accurately recognize the locus and consequence of oropharyngeal dysphagia, they mistakenly identify the neck as the locus of bolus hang-up with esophageal dysphagia about 30% of the time. Therefore, elicitation of symptoms such as aspiration, coughing, nasopharyngeal regurgitation, or drooling is of great value in distinguishing oropharyngeal dysphagia from proximally referred esophageal dysphagia.

Distinguishing oropharyngeal dysphagia from globus sensation can be particularly vexing. Unlike dysphagia, which occurs only during swallowing, globus sensation is prominent between swallows. Patients relate the nearly constant sensation of having a lump in their throat or feeling a foreign object caught in their throat. In some instances globus is associated with reflux symptoms and in others with substantial anxiety; that anxiety is sometimes thought to be etiological (globus hystericus). Unfortunately, studies have failed to define an objective anatomical or physiological cause for globus and we are left with the crucial data being in

the history: globus sensation persists regardless of the act of swallowing.

Physical examination may help identify features of the underlying systemic or metabolic disorder or localize the anatomical level and severity of a causative neurological lesion. The patients general condition should be assessed to determine the degree that dysphagia is affecting fluid and nutritional status. Patients may initially require hydration and correction of electrolyte abnormalities. Examination of the oral cavity, head, and neck for masses, lymph nodes, goiter, and evidence of previous surgery or radiation therapy will help define structural abnormalities associated with dysphagia. Neurological examination may indicate cranial nerve dysfunction, neuromuscular disease, cerebellar dysfunction, or an underlying movement disorder. Of note, contrary to popular belief, the gag reflex is not predictive of pharyngeal swallowing efficiency or aspiration risk. The gag reflex is absent in 20%–40% of normal adults [6].

If the etiology of oropharyngeal dysphagia is not readily apparent after initial evaluation, further diagnostic studies are indicated. Because the management implications are so different, the first task in the evaluation of suspected oropharyngeal dysphagia is to distinguish between structural and functional etiologies. Structural abnormalities that may result from trauma, surgery, tumors, caustic injury, congenital anomalies, or acquired deformities are identified by endoscopic or radiographic examination. Functional abnormalities can be attributable to dysfunction of either intrinsic musculature, peripheral nerves, or central nervous system control mechanisms. Endoscopy may be performed either transorally or transanally to identify tumors, webs or hypopharyngeal diverticula. Barium studies may define areas of obstruction and hypopharyngeal diverticula, but add little structural information to endoscopic exams.

After structural defects have been excluded, videofluoroscopy is used for a functional evaluation of swallowing. Frequently referred to as a modified barium swallow, Logemann has described a protocol comprised of a series of swallow tasks [7]. Images are obtained in a lateral projection, framed to include the oropharynx, palate, proximal esophagus, and proximal airway. These images are then evaluated with respect to four major categories of oropharyngeal dysfunction:

1 inability or excessive delay in initiation of pharyngeal swallowing
2 aspiration
3 nasopharyngeal regurgitation
4 residue of the ingestate within the pharyngeal cavity after swallowing.

Furthermore, the procedure allows for evaluation of the efficacy of various compensatory dietary modifications, postures, and swallowing maneuvers in compensating for observed swallowing dysfunction.

Intralumenal manometry can quantify the strength of pharyngeal contraction, the completeness of upper esophageal sphincter (UES) relaxation, and the relative timing of these events. When coupled with concurrent videofluoroscopy it may provide useful complementary information regarding UES dysfunction. High intrabolus pressures may distinguish impaired UES opening from impaired UES relaxation as a cause of oropharyngeal dysphagia. Historically, a pitfall of UES manometry has been artifacts introduced by sphincter movement during swallowing. However, this limitation has recently been overcome with the introduction of high-resolution manometry that allows one to completely define the intralumenal pressure environment of the pharynx and esophagus without spatial gaps between recording sites and, consequently, with minimal movement-related recording artifacts. This can be very useful in discriminating between mechanical obstruction and weak musculature as potential causes of oropharyngeal dysphagia (Fig. 31.1).

Structural etiologies of oropharyngeal dysphagia

Implicit in the mechanical description of swallowing summarized above is that normal swallowing is associated with minimal outflow resistance from the oropharynx. Identification of obstructing lesions that cause dysphagia will lead to specific management in most cases. For example, cervical webs and pharyngeal or cricopharyngeal strictures or tumors are indications for surgery, dilation, antineoplastic therapy or some combination thereof. In the setting of benign strictures or postcricoid webs, simple dilation is safe and effective. Prominent cervical osteophytes can also cause obstructive dysphagia. As the posterior wall of the pharynx is so closely opposed to the anterior aspect of the cervical vertebrae, cervical osteophytes cause an anterior bulging into the hypopharynx that can impede passage of a normal-sized bolus [8]. The most common structural abnormalities of the hypopharynx associated with dysphagia are hypopharyngeal diverticula and cricopharyngeal bars. Given the interplay between structural and functional defects in these disorders, more detailed consideration is merited.

Hypopharyngeal diverticula and cricopharyngeal bars

Acquired hypopharyngeal diverticula occur most commonly in men after the age of 60. The most common type, Zenker diverticulum, originates in the midline posteriorly at Killian's dehiscence, a point of pharyngeal wall weakness between the oblique fibers of the inferior pharyngeal constrictor and the transverse cricopharyngeus muscle (Fig. 31.2) [9]. Other locations of acquired pharyngeal diverticula include the lateral slit separating the cricopharyngeus muscle from the fibers of the proximal end of the esophagus through which the recurrent laryngeal nerve and its accompanying vessels run to supply the larynx; at the penetration of the inferior thyroid artery into the hypopharynx; and at the junction of the middle and inferior constrictor muscles. The unifying theme of these locations is that they are sites of potential weakness of the muscular lining of the hypopharynx.

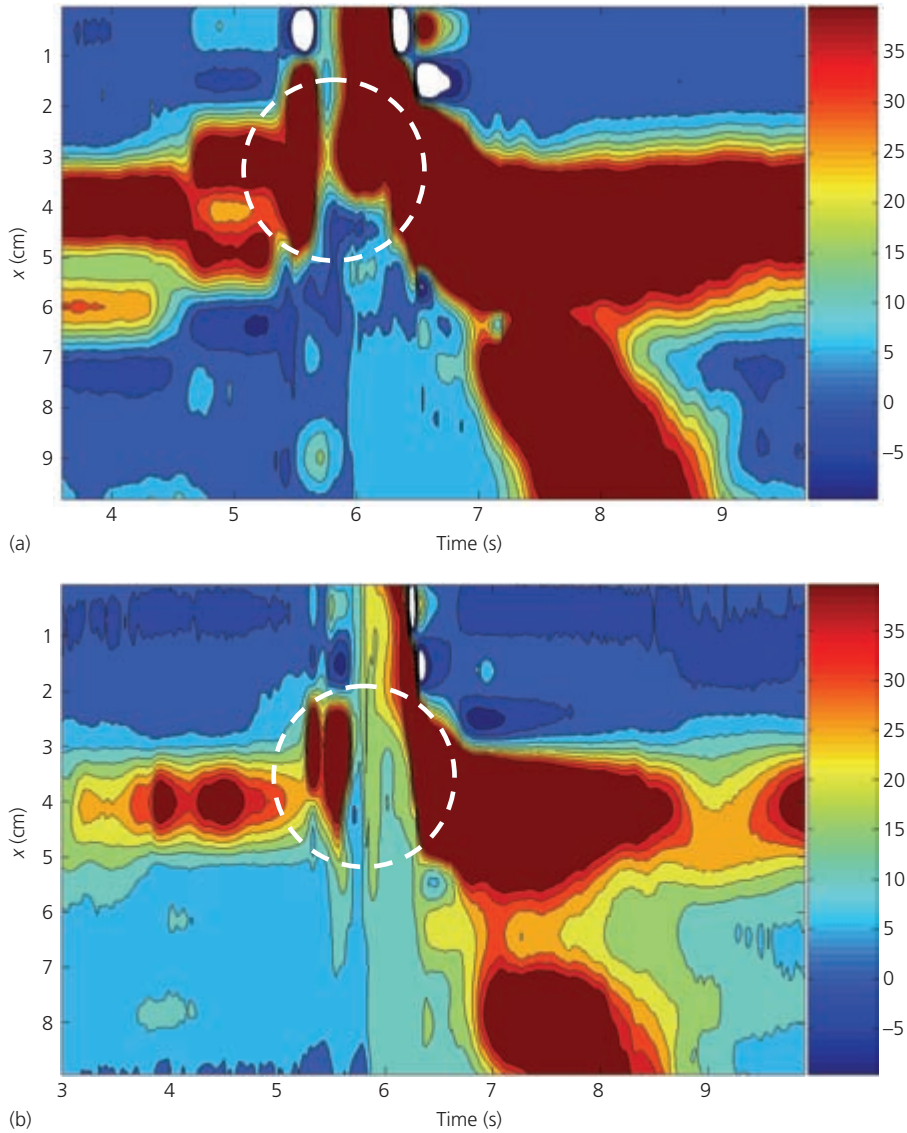

Figure 31.1 High-resolution manometry focused at the upper esophageal sphincter (UES) before and after dilation in a subject with a symptomatic cricopharyngeal bar. Isocontour plots before **(a)** and after dilation **(b)** reveal a decrease in intrabolus pressure and an increase in UES relaxation duration. Typical UES relaxation pressures are below 10 mmHg; however, in **(a)** the mean UES relaxation pressure is greater than 35 mmHg and the relaxation duration is extremely short. After dilation with an over-the-guidewire polyvinyl dilator **(b)** , the intrabolus pressure is substantially decreased and relaxation duration is adequate to allow for normal bolus transit through the UES.

Hypopharyngeal diverticula are often asymptomatic until they enlarge sufficiently to store a significant amount of food or liquid. In most instances, symptoms are of dysphagia, halitosis, post-swallow regurgitation, or even aspiration of material from the pharyngeal pouch.

Hypopharyngeal diverticula have been hypothesized to result from either delayed UES relaxation, failure of relaxation, or premature contraction [10]. However, little data and considerable contradiction accompany each of these hypotheses. A more plausible explanation for the development of diverticula is that they form as a result of a restrictive myopathy associated with diminished compliance of the cricopharyngeus muscle. Surgical specimens of cricopharyngeus muscle strips from 14 patients with hypopharyngeal diverticula demonstrated structural changes that would decrease UES compliance and opening [11]. The cricopharyngeus samples from these patients had "fibro-adipose tissue replacement and (muscle) fiber degeneration." Thus, although the muscle relaxes normally during a swallow, it cannot distend normally, resulting in the appearance of a cricopharyngeal indentation, or bar, during a barium swallow (Fig. 31.3). Diminished sphincter compliance necessitates increased hypopharyngeal intrabolus pressure to maintain trans-sphincteric flow through the smaller UES opening. The increased stress on the hypopharynx from the increased intrabolus pressure may ultimately result in diverticulum formation.

Management of structural oropharyngeal dysphagia

The treatment of hypopharyngeal diverticula is cricopharyngeal myotomy with or without a diverticulectomy. Cricopharyngeal myotomy reduces both the resting sphincter tone and resistance to flow across the UES. Resting UES tone is reduced to approximately 50% of baseline, suggesting that

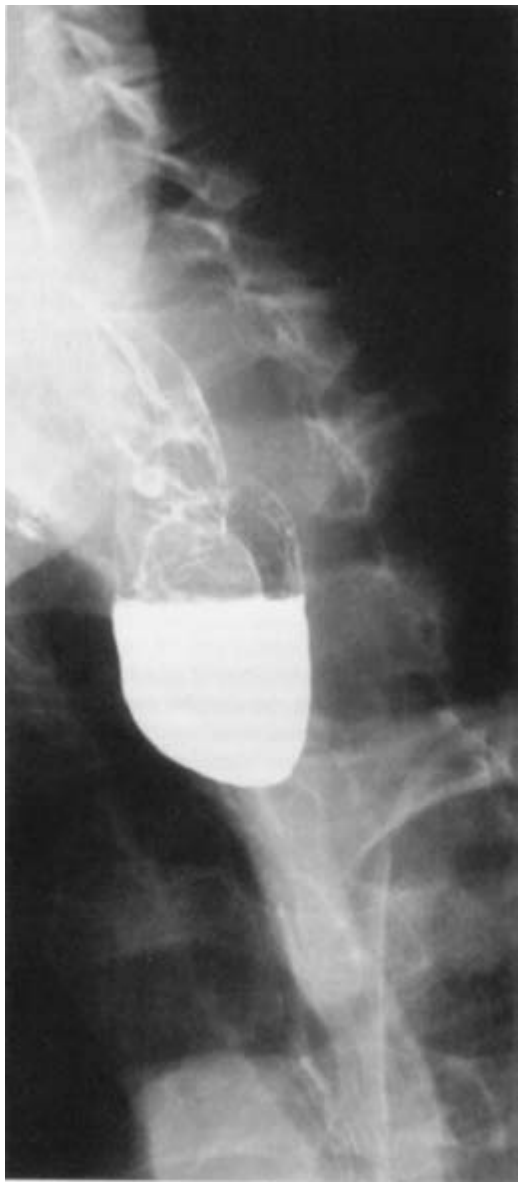

Figure 31.2 Radiograph of a large Zenker diverticulum partially filled with barium. Although the point of herniation is midline posterior, the diverticulum necessarily migrates laterally in the neck because there is no potential space between the posterior pharyngeal wall and the vertebral column.

the derived benefit is from increased compliance at the UES. A recent study found that the compliance of the sphincter following diverticulectomy with myotomy was restored to normal in five patients following surgery, as indicated by normal hypopharyngeal intrabolus pressure during swallowing [12]. Good or excellent results can be expected in 80%–100% of Zenker patients treated by transcervical myotomy combined with diverticulectomy or diverticulopexy [13]. There are instances in which a limited procedure would be adequate, but a definitive approach to the problem of

Figure 31.3 Cricopharyngeal bar in a patient with oropharyngeal dysphagia. The posterior indentation of the barium column is caused by a noncompliant cricopharyngeus muscle. Courtesy of Dr Richard Gore.

pulsion diverticula should involve both diverticulectomy and myotomy. Diverticulectomy alone risks recurrence because the underlying stenosis at the level of the cricopharyngeus is not remedied. Similarly, myotomy alone risks not solving the problem of food accumulation within the diverticula with attendant regurgitation and aspiration. Small diverticula may, however, disappear spontaneously following myotomy.

Whether or not a cricopharyngeal bar in the absence of a diverticula requires treatment is less clear. Certainly, if dysphagia exists and combined fluoroscopic/manometric analysis demonstrates reduced sphincter opening in conjunction with elevated upstream intrabolus pressure, there is good rationale for treatment. One recent uncontrolled series suggests that in this scenario dilation with a large caliber bougie may be efficacious in relieving dysphagia and this is certainly a reasonable treatment option prior to myotomy [14] (Fig. 31.1).

Functional etiologies of oropharyngeal dysphagia

Primary neurological or muscular diseases involving the oropharynx can be associated with dysphagia. Thus, whereas esophageal dysphagia usually results from esophageal diseases, oropharyngeal dysphagia is frequently the result of neurological or muscular diseases with oropharyngeal dysfunction being just one pathological manifestation. Although the specifics of the diseases vary, the net effect on swallowing

Table 31.1 Patterns and manifestations of oropharyngeal dysphagia

Mechanical element	Biomechanical mechanism	Evidence of dysfunction	Typical diseases
Nasopharyngeal closure	Soft palate elevation	Nasopharyngeal regurgitation, nasal voice	Myasthenia gravis
Laryngeal closure	Laryngeal elevation, arytenoid tilt, vocal fold closure	Aspiration during bolus transit	CVA, head trauma
UES opening	UES relaxation, laryngeal elevation, anterior hyoid traction, sphincter distension	Dysphagia, post-swallow residue/aspiration, diverticulum formation	Cricopharyngeal bar, CVA, Parkinson disease
Tongue loading and bolus propulsion	Lingual sensation and control	Sluggish, misdirected bolus	Parkinson disease, surgical defects, cerebral palsy
Pharyngeal clearance	Pharyngeal shortening, pharyngeal contraction, epiglottic flip	Post-swallow residue/aspiration	Polio, postpolio syndrome, oculopharyngeal dystrophy, CVA

CVA, cerebrovascular accident; UES, upper esophageal sphincter.

can be analyzed according to the mechanical description of the swallow outlined above. Table 31.1 summarizes the mechanical elements of the swallow along with the manifestations and consequences of dysfunction and representative pathological conditions in which they may be encountered. Some of the more distinct pathological entities are discussed below.

Neurological causes of oropharyngeal dysphagia

Neurological diseases can damage the neural structures necessary for either the afferent or efferent limbs of the oropharyngeal swallow. As there is nothing unique to neurons controlling swallowing, their involvement in disease processes is usually random. Furthermore, in most instances, functions mediated by adjacent neuronal structures are concurrently involved. Virtually any disease of the central nervous system can potentially cause dysphagia. The following discussion will focus on neuropathological processes with the best characterized patterns of dysphagia.

Cerebrovascular accidents

Aspiration pneumonia has been estimated to inflict a 20% death rate in the first year after a stroke, and 10%–15% each year thereafter [15]. It is usually not the first episode of aspiration pneumonia but the subsequent recurrences over a several-year period that eventually cause death [16]. The ultimate cause of aspiration pneumonia is dysphagia leading to aspiration, which can occur by a number of mechanisms: absence or severe delay in triggering the swallowing reflex (swallow apraxia), reduced lingual control, and unilateral or bilateral weakened laryngopharyngeal musculature [7]. Conceptually, these etiologies can be divided into motor or sensory impairments. Although sensory and motor impairment frequently co-occur, each will be addressed separately.

Cortical strokes are less likely to result in severe dysphagia than are brainstem strokes [17]. In a report of 100 consecutive patients, 37 of 86 (43%) who sustained an acute cerebral infarct experienced dysphagia when evaluated within 4 days of the event. However, 86% of these patients were able to swallow normally 2 weeks later [17]. Two-dimensional scalp topographical maps of the pharyngeal muscles obtained by transcranial magnetic stimulation suggest that recovery occurs as a result of contralateral areas taking over the lost function [18]. Thus, dysphagia is more prevalent among patients incurring larger strokes or patients who have had previous infarcts.

The sensory cues required for eliciting the pharyngeal swallow are unclear. In pathological circumstances in which one or the other neural substrate of the afferent signal has been damaged, patients may experience a relative inability to initiate a swallow. A study of sensory acuity in the supraglottic and pharyngeal regions [19] revealed either unilateral ($n = 9$) or bilateral ($n = 6$) sensory impairment of moderate to severe degree in all 15 stroke patients studied. Impaired sensation in these critical areas likely results in swallow apraxia. An interesting compensatory strategy for this sensory defect is to accentuate oropharyngeal stimulation during eating to facilitate achieving the threshold for triggering the medullary swallowing center [7]. Preliminary data suggest that the sour bolus is particularly effective in some patients with swallow apraxia providing some hope for the treatment of this disabling condition [7].

Poliomyelitis

Most cases of poliomyelitis involve only the spinal cord; however, the fatality rate from bulbar disease far exceeds that of spinal disease with the most common fatal complication being respiratory depression. In addition to its dreaded effect of respiratory depression, bulbar poliomyelitis is also associated with dysphagia. In one analysis of the persistent sequelae of bulbar poliomyelitis, 28 of 47 patients (60%) had recurrent or continued involvement of the pharynx 17 or more months after their acute illness [20]. Speech and

swallowing dysfunction are the result of weakness of the levator veli palatini muscle, pharyngeal constrictors, and hyolaryngeal elevators [20].

In recent years, neurologists have observed an increasing number of patients with new paretic symptoms traceable to their remote polio infection 30–40 years earlier. The new, slowly progressive postpolio muscular atrophy may occur in muscles that were clinically unaffected by the acute illness [21]. One investigation studied 13 patients with postpolio dysphagia and demonstrated palatal, pharyngeal, and laryngeal weakness [22]. Over half of the patients evaluated demonstrated silent aspiration suggesting that the clinician should maintain a low threshold for evaluating such patients with videofluoroscopy. Accumulating evidence suggests that at least some of these patients with postpolio syndrome respond to pyridostigmine bromide (Mestinon), a cholinomimetic agent.

Amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurological disease characterized by degeneration of motoneurons in the brain, brainstem, and spinal cord. Specific symptoms are dependent upon the locations of affected motoneurons and the relative severity of involvement. When the degenerative process involves the cranial nerve nuclei, swallowing difficulties ensue. Oropharyngeal dysfunction characteristically begins with the tongue and progresses to involve the pharyngeal and laryngeal musculature. Patients experience choking attacks, become dehydrated or malnourished, and incur aspiration pneumonia. The decline in swallowing function is progressive and predictable, invariably leading to gastrostomy feeding. A number of patients die as a consequence of their swallowing dysfunction in conjunction with respiratory depression [23].

Parkinson disease

Although only 15%–20% of patients with Parkinson disease complain of swallowing problems, more than 95% have demonstrable defects videofluoroscopically [24]. The disparity between these numbers suggests that patients compensate effectively during the early stages of the disease and complain of dysphagia only when it becomes severe. Abnormalities of the oral phase of swallowing include repetitive lingual pumping before initiation of a pharyngeal swallow, piecemeal swallowing, and oral residue after the swallow [7,25]. Patients may also exhibit a delayed swallow response and a weak pharyngeal contraction, resulting in vallecular and pyriform sinus residue after each swallow [24]. Recent combined manometric and fluoroscopic data suggest that this is related to the combination of incomplete UES relaxation and a weakened pharyngeal contraction [24]. Although no controlled trials are available, dysphagic symptoms have improved following therapy with levodopa and carbidopa [25,26].

Tumors

Medullary or vagal tumors are potentially debilitating with respect to swallowing. Astrocytomas are the most common histological subtype affecting adults whereas medulloblastomas are the most common type encountered in children [27]. The relative inaccessibility of the medulla to surgery usually means that substantial morbidity is incurred during attempted resection or palliation.

Unilateral lesions of the vagus can result in hemiparesis of the soft palate and pharyngeal constrictors, as well as of the laryngeal musculature. Surgical manipulation of this region can even result in complete loss of the pharyngeal swallow response [28]. The recurrent laryngeal nerves can be injured as a result of thyroid surgery, polio, aortic aneurysms, pneumonectomy, primary malignancies of the mediastinum, or metastatic lesions to the mediastinum. Because of its more extensive loop into the chest, the left recurrent laryngeal nerve is more vulnerable to involvement with mediastinal node malignancy. Unilateral recurrent laryngeal nerve injury results in unilateral adductor paralysis of the vocal cords. This defect can result in aspiration during swallowing because of impaired laryngeal closure. Although some reports state the contrary [29], it is probably rare to have any primary pharyngeal dysfunction associated with recurrent laryngeal nerve injury.

Muscular diseases causing oropharyngeal dysphagia

Primary muscular diseases involving the oropharynx are associated with dysphagia, reflective of the pattern of involvement. Nasal voice and nasopharyngeal regurgitation indicate either weakness or paresis of the soft palate elevators, and tongue weakness can cause poor control of the bolus within the mouth. Post-swallow residue in the valleculae or hypopharynx reflects an ineffective, presumably weakened pharyngeal contraction. Aspiration suggests either weakened laryngeal elevators or post-swallow residue that is then aspirated after the swallow sequence is completed. As with neurological disorders, virtually any disorder affecting skeletal muscle can result in dysphagia. The following discussion focuses on a few of the better characterized entities.

Oculopharyngeal dystrophy

Oculopharyngeal muscular dystrophy, first described by Taylor in 1915 [30], is a syndrome characterized by progressive dysphagia and palpebral ptosis. Taylor noted that all of his afflicted patients reaching the age of 50 died of starvation resulting from pharyngeal paralysis. The disease is now known to be a form of muscular dystrophy inherited in an autosomal dominant fashion with occurrences clustered in families of French Canadian descent. Genetic studies of an afflicted family indicate linkage to chromosome 14, perhaps involving the region coding for cardiac α- or β-myosin heavy chains [31].

Oculopharyngeal dystrophy affects the striated pharyngeal

muscles and the levator palpebrae. Other forms of muscular dystrophy occasionally affect the pharyngeal constrictors but rarely, if ever, is this a dominant manifestation. The first symptom of oculopharyngeal dystrophy is usually ptosis, which develops slowly and eventually dominates the patient's appearance. Dysphagia may begin before the ptosis, but more often manifests simultaneously with it or a few years later. The dominant functional abnormalities are weak or absent pharyngeal contraction with hypopharyngeal stasis [32]. Dysphagia progresses slowly but may ultimately lead to starvation, aspiration pneumonia, or asphyxia.

Myotonic dystrophy

Myotonic dystrophy is a rare disorder characterized by prolonged contraction and difficulty in relaxation of involved skeletal musculature. Dysphagia is a common complaint among affected individuals. Early investigations suggested that the dysphagia was the result of myotonia of the cricopharyngeus with resultant overflow into the airway during attempted swallowing, but subsequent manometric and radiographic investigations have not confirmed this [33]. The experience in more recent investigations was that, even though only half of the patients studied complained of dysphagia, motor abnormalities could be demonstrated in every patient. The pattern of abnormality was of a weakened pharyngeal contraction, absent peristalsis in the striated muscle esophagus, and diminished or absent peristalsis in the smooth muscle portion of the esophagus. No study has demonstrated myotonia in any part of the esophagus. Thus, the consequences of the disease are identical to other forms of muscular dystrophy: poor pharyngeal clearance with risk of aspiration during the swallow if there is concurrent weakness of the laryngeal elevators or after the swallow when the substantial pharyngeal residue might fall into the reopened airway.

Myasthenia gravis

Myasthenia gravis is a progressive autoimmune disease characterized by high circulating levels of acetylcholine receptor antibody and destruction of acetylcholine receptors at neuromuscular junctions. Musculature controlled by the cranial nerves is almost always involved, particularly the ocular muscles. Dysphagia is prominent in more than one-third of myasthenia gravis cases and in unusual instances can be the initial manifestation of the disease [7]. In mild cases, dysphagia may not be evident until after 15–20 min of eating. Classically, manometric studies reveal a progressive deterioration in the amplitude of pharyngeal contractions with repeated swallows. Peristaltic amplitude recovers with rest or following the administration of 10 mg of edrophonium chloride. In more advanced cases the dysphagia can be profound and associated with nasopharyngeal regurgitation and nasality of the voice, even to the extent of being confused with bulbar ALS or brainstem stroke [34].

Management of oropharyngeal dysphagia

Management of oropharyngeal dysphagia begins with definition of the aberrant physiology along the lines summarized in Table 31.1. This is most easily accomplished with a videofluoroscopic swallowing study. Following definition of the patient's swallowing dysfunction, four specific issues pertaining to management of oropharyngeal dysphagia can be addressed:

1 identification of an underlying systemic disease
2 characterization of a disorder amenable to surgery or dilation
3 identification of a specific pattern of dysphagia amenable to swallowing therapy
4 assessment of aspiration risk.

Identifying underlying disease

A potential outcome of the swallowing evaluation is the identification of an underlying neuromuscular, neoplastic, or metabolic disorder that will dictate specific management. For example, dysphagia can be the presenting symptom in patients with myopathy, myasthenia, thyrotoxicosis, motor neuron disease, or Parkinson disease. In each instance, identification of the underlying disease will result in a specific treatment. Whether or not identification and treatment of the underlying disorder improves swallowing function depends on both the natural history of the disease and whether or not an effective treatment exists.

Disorders amenable to surgery

In marked contrast to the high efficacy observed with structural cricopharyngeal disorders, the efficacy of myotomy in neurogenic dysphagia is variable. Furthermore, most series evaluating the efficacy of myotomy in neurogenic dysphagia are uncontrolled without specific outcome measures. Thus, although an overall favorable response rate in excess of 60% is reported, there are currently no validated criteria for preoperative selection. Theoretically, the functional limitation in these circumstances is of pharyngeal propulsion and the potential benefit of myotomy is less obvious [35].

Specific patterns of dysphagia amenable to swallowing therapy

Following characterization of a patient's swallow dysfunction, the radiographic study should proceed to test selected compensatory or therapeutic treatment strategies. Compensatory treatments include postural changes, modifying food delivery or consistency, or the use of prosthetics. For instance, head turning can eliminate aspiration or pharyngeal residue by favoring the more functional side in patients with hemiparesis [7]. Similarly, diet modifications can reduce the "difficulty" of the swallow [7]. Therapeutic strategies are designed to alter the physiology of the swallow, usually by improving the range of motion of oral or pharyngeal structures using voluntary control of oropharyngeal movement during swallow. Depending on the severity of the

impairment, level of motivation, and global neurological intactness, defective elements of the swallow can be selectively rehabilitated. For a detailed description of the techniques and limitations of swallow therapy, the reader is referred to treatises on the topic [7,13].

Detection of severe aspiration

Oropharyngeal dysphagia associated with aspiration is responsible for an estimated 40 000 deaths a year as a result of aspiration pneumonia [19]. Videoflouroscopy is believed to be the most sensitive test for detecting aspiration, detecting instances not evident by bedside evaluation in 42%–60% of patients. However, despite the logical association between deglutitive aspiration and the subsequent development of pneumonia, this sequence is not inevitable. In fact, available data suggest that radiographic aspiration has a positive predictive value of only 19%–68% and a negative predictive value of 55%–97% for pneumonia [13]. Nonetheless, the balance of evidence suggests that the detection of aspiration is a predictor of pneumonia risk, and that its detection dictates that compensatory swallowing strategies, nonoral feeding or corrective surgery be instituted. Whether nonoral feeding eliminates the risk of aspiration is controversial. A provocative finding by Croghan et al. [5] was that, out of 22 patients with radiographic aspiration, pneumonia and death were more frequent among patients who received feeding tubes. This finding suggests that aspiration of oral secretions may be important in determining pneumonia risk and has led some to consider procedures such as tracheostomy to protect the airway.

Xerostomia

Although not an esophageal motility disorder, xerostomia (the subjective feeling of oral dryness) will be discussed briefly given its association with dysphagia. Xerostomia is usually associated with reduced salivary secretion. The normal, unstimulated salivary flow rate should be 0.3–0.5 mL/min, whereas flow rates of less than 0.1 mL/min are consistent with xerostomia. The most common etiologies of xerostomia are:

- drugs
- autoimmune diseases including Sjogren syndrome and rheumatoid arthritis
- radiation therapy.

Symptoms associated with xerostomia are:

- pain, burning, and soreness of the oral mucosa, especially the tongue
- difficulty in mastication, swallowing, and speech
- impairment of taste
- painful oral ulcers
- difficulty wearing dentures
- increased dental caries
- increased frequency or volume of fluid intake [36].

The dysphagia associated with xerostomia is primarily attributable to the absence of the lubricating properties of saliva. Therapy consists of attempts to increase secretion with sugarless gum or sour lozenges or with pharmacological agents such as pilocarpine and cholinergic agents. Individuals without a salivary response regardless of stimulation can try commercially available saliva substitutes.

Esophageal motility disorders

Neuromuscular function of the esophagus (motility) has the consistent objective of emptying the esophagus: primary esophageal peristalsis empties swallowed material from the esophagus; secondary peristalsis eliminates air or fluid refluxed from the stomach; the upper esophageal sphincter contracts during inspiration to exclude inspired air from the digestive tract; and elements of the esophagogastric junction (EGJ) contract during transient increases of intra-abdominal pressure, preventing gastroesophageal reflux. A basic characteristic of esophageal motility disorders is the failure to preserve esophageal emptiness. Retained material within the esophagus, or the excessive entry of material into the esophagus, is abnormal. Such dysfunction can be categorized as disorders of peristalsis or of sphincter competence. The main dysfunction of sphincter competence occurs with reflux disease, an entity covered elsewhere in this text. This discussion will focus on less common etiologies of sphincter dysfunction and on disorders of peristalsis.

Evaluation and classification of esophageal dysphagia

Dysphagia is a fundamental symptom of esophageal disorders. Esophageal as opposed to oropharyngeal dysphagia is suggested by the absence of associated aspiration, cough, nasopharyngeal regurgitation, dry mouth, pharyngeal residue following swallow, or co-occurring neuromuscular dysfunction. On the other hand, the associated conditions of heartburn, esophagopharyngeal regurgitation, chest pain, odynophagia, or intermittent esophageal obstruction suggest esophageal dysphagia. However, an important limitation of the patient history in esophageal dysphagia is that patient identification of the location of obstruction is of limited accuracy. Specifically, a distal esophageal obstruction caused by an esophageal ring or achalasia will often be sensed as cervical dysphagia such that patients correctly locate distal dysfunction only 60% of the time [37]. Because of this subjective difficulty in distinguishing proximal from distal lesions within the esophagus, an evaluation for cervical dysphagia should encompass the entire esophagus.

Another important consideration in patient management is that esophageal motility disorders are much less common than mechanical or inflammatory etiologies of dysphagia: tumors, strictures, rings, and peptic, pill, or infectious esophagitis. Historical points suggestive of a motor disorder are difficulty with both solids and liquids as opposed to only

solids, which is more suggestive of mechanical obstruction. However, as will become evident in the ensuing discussion, the functional consequences of mechanical or inflammatory disorders can exactly mimic those of primary motility disorders. Thus, as with oropharyngeal dysphagia, an esophageal motility disorder should be considered only after exclusion of these more common diagnoses by endoscopic or radiographic examination.

As outlined above, the physiological elements of esophageal peristalsis are the coordinated inhibition and excitation of the circular and longitudinal muscle layers of the esophagus. It follows that pathophysiological processes can involve dysfunction of deglutitive inhibition (including sphincter relaxation) or of the propagated contraction. Furthermore, because esophageal motor disorders are rarely diagnosed on histopathological grounds, diagnosis depends upon defining these functional aberrations. Table 31.2 illustrates an attempt at categorizing esophageal motility disorders on the basis of regional function of deglutitive inhibition and sequenced excitation. Examining Table 31.2, there are few well-defined esophageal motility disorders and, as one might predict, in some circumstances it can be very difficult to definitively choose between the diagnostic possibilities. However, such is the state of the art. Furthermore, in the instance of spastic disorders, there is considerable debate over the clinical significance of some manometric findings [38]. With these considerations in mind, the following discussion emphasizes disease entities with clear diagnostic or therapeutic implications rather than manometric phenomena of unproved significance. The exception is gastroesophageal reflux disease (GERD), which, although highly significant, is deferred to Chapter 32.

Diagnostic testing in esophageal disease

Endoscopy

Although upper endoscopy is an excellent tool for evaluating dysphagia, it has significant limitations in assessing extralumenal structures and abnormal esophageal motility. Upper endoscopy should be the first test ordered for evaluating new-onset dysphagia when structural causes requiring either dilation or biopsy are being considered. It has excellent specificity for strictures and tumors; however, it has the potential to miss subtle obstructing lesions, such as webs and rings. In addition, it will also provide very limited information regarding peristaltic function.

Contrast studies

Contrast studies of the oropharynx and esophagus are useful in assessing dysphagia if stricture or tumor is not suspected or upper endoscopy is not readily available. It has the added benefit of providing information regarding UES function, peristalsis, and bolus clearance through the EGJ. With good technique, normal peristalsis can be verified with 91%–95% specificity [39,40]. Peristalsis is best evaluated in the prone position so that clearance does not occur by gravity. In the prone position, the primary peristaltic wave manifests as an inverted "V" that represents the tail of the bolus leading the peristaltic wavefront (Fig. 31.4). Abnormalities of peristalsis are inferred by retrograde escape of the bolus through the peristaltic wavefront resulting in incomplete esophageal emptying. Normally the EGJ will become widely patent when the bolus reaches this area and impaired relaxation can be inferred when either a smooth tapering is noted at the EGJ or bolus transit across the EGJ is impeded.

Table 31.2 Patterns of esophageal dysmotility

	Esophageal region		
Functional impairment	**UES**	**Body**	**LES**
Inadequate deglutitive inhibition (or relaxation)	Parkinson disease, *cricopharyngeal bar*	Achalasia, diffuse esophageal spasm	Achalasia, *pseudoachalasia*
Impaired retrograde inhibition	Belch dysfunction, complicated achalasia	–	Gas bloat (postfundoplication)
Excess retrograde inhibition	–	–	GERD (tLESR)
Inadequate peristaltic excitation (or resting tone)	Oculopharyngeal dystrophy, mixed connective tissue disease	Scleroderma esophagus, advanced achalasia, GERD, nonspecific esophageal motor disorder, *obstruction*	Scleroderma esophagus, GERD
Excess peristaltic excitation (or resting tone)	–	DES, vigorous achalasia, nutcracker esophagus	Hypertensive LES, achalasia

Italics indicate structural abnormalities mimicking the findings of primary motor disorders.
DES, diffuse esophageal spasm; GERD, gastroesophageal reflux disease; LES, lower esophageal sphincter; tLESR, transient LES relaxation; UES, upper esophageal sphincter.

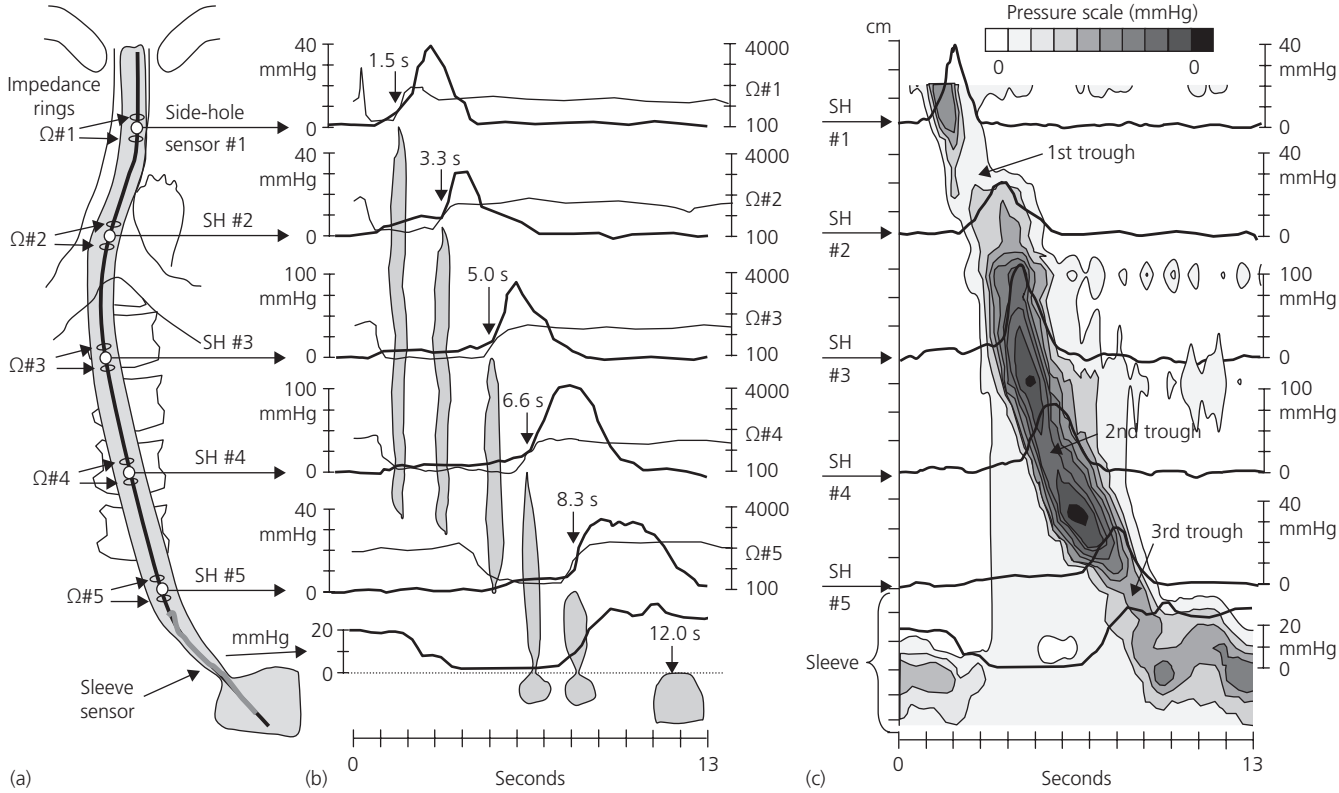

Figure 31.4 Representative physiological data, modified to illustrate the relationship between videofluoroscopic, manometric, impedance, and topographic representations of esophageal peristalsis. **(a)** Schematic drawing of placement of a combined manometry/intralumenal impedance monitoring system with five manometric side holes (SH) spaced 4 cm apart and a 6-cm sleeve sensor placed just distal to the last manometric port. The impedance rings (Ω) are also spaced 4 cm apart with the rings straddling the manometric ports. The arrows to part **(b)** point to the corresponding data tracings obtained from each combined manometry/impedance or sleeve recording site. **(b)** Concurrent videofluoroscopic, manometric, and multichannel intralumenal impedance recordings of a 5-mL renografin swallow that was completely cleared by one peristaltic sequence. Representative tracings from the videofluoroscopic sequence overlayed on the combined manometric/impedance tracing show the distribution of the bolus at the times indicated by the vertical arrows. At each recording site, the thick line intersecting the pressure scale (mmHg) on the left represents the manometric tracing and the fine line intersecting the impedance scale (Ω) on the right represents the impedance recording tracing. Bolus entry at each combined manometry/impedance recording site is signaled by a subtle increase in pressure (intrabolus pressure) and a greater than 50% decrease in impedance. In this example, the bolus propagates past Ω#4 rapidly, indicated by an abrupt reduction in impedance in Ω#2, Ω#3, and Ω#4 at time 1.5 s. Lumenal closure and hence the tail of the barium bolus is evident at each recording site by the upstroke of the peristaltic contraction and a 50% increase in recorded impedance. Hence, at 5.0 s, the peristaltic contraction was beginning at SH#3, corresponding to a 50% increase in impedance and the tail of the barium bolus at the same esophageal locus. Finally, after completion of the peristaltic contraction (time 12.0 s), all renograffin was in the stomach. **(c)** Comparison of conventional manometry obtained with a sleeve assembly as depicted in **(a)** and high-fidelity manometry with recording sites at 1-cm intervals displayed topographically as an isocontour plot. The standard manometric recordings are superimposed on the isocontour plot at axial locations corresponding to the equivalent portion of the high-fidelity manometry it represents. In the isocontour plot, deepening shades of gray indicate higher pressures, revealing four distinct pressure segments separated by three pressure troughs. Physiologically the first trough is at the junction between striated and smooth muscle, the second is within the smooth muscle segment and the third separates the peristaltic segment from the lower esophageal sphincter (LES). Note that LES relaxation is reliably recorded using either methodology, albeit somewhat differently. From the illustration one can see that the end of LES relaxation measured by the sleeve coincides with the peristaltic contraction contacting the proximal portion of the sleeve. In addition to measuring mean residual pressure, topographic analysis allows for more precise measurement of the transphincteric pressure gradient. Isocontour tracing courtesy of Ray Clouse. From Pandolfino & Kahrilas [204], with permission.

Manometry

Manometry is the only technique that can define the contractile characteristics of the esophagus in an attempt to identify pathological conditions. Manometric evaluation of the tubular esophagus assesses the integrity, rate of progression, and morphology of the contractile complex (amplitude, duration, repetitive contractions). Failed peristalsis is defined as the absence of contraction after a swallow, or a contraction that proceeds only part way down the esophagus and either disappears or ends with a simultaneous contraction. Each of

these outcomes results in incomplete esophageal emptying [41]. The progression rate of peristaltic contraction is measured by identifying the timing of the initial upstroke of contraction at adjacent recording sites and knowing the distance between these sites. Progression rates greater than 6.25 cm/s are associated with poor esophageal emptying and are considered simultaneous [42]. Contractile amplitude is considered hypotensive if it has an amplitude of less than 35 mmHg because this too can be associated with impaired esophageal emptying [41]. A typical convention for scoring hypertensive peristalsis is that the mean amplitude of contractile complexes exceeds 180 mmHg; however, this upper limit has varied among investigators. Repetitive contractions are multipeaked complexes. By convention, a contraction is scored as multipeaked if the valley between the peaks is at least 10 mmHg less than and at least 1 s after the preceding peak.

Both the magnitude of EGJ pressure and EGJ relaxation characteristics are measured with manometry. The three most widely used techniques are: (1) a stationary sleeve sensor; (2) a rapid pull-through of side-hole sensors across the EGJ during suspended respiration; or (3) a station pull-through of a side-hole sensor recording pressure activity for 30–60 s at 1 cm increments as the catheter is withdrawn. EGJ pressure varies by as much as 25 mmHg with the method of measurement and depending on whether mid-respiratory or end-expiratory EGJ pressure is reported. In addition to methodology, the minute-to-minute variations of EGJ pressure are attributable to normal physiological events, such as the migrating motor complex. Thus, normal EGJ pressure varies widely with normal values ranging from 15 ± 11 mmHg at end expiration to 40 ± 13 mmHg at end inspiration, even when measured from the same manometric tracings [43]. The most meaningful statement that can be made regarding isolated EGJ pressure measurements is that it is abnormal to find an extremely low value (≤ 5 mmHg).

The manometric evaluation of EGJ relaxation is arguably the most important measurement made during clinical esophageal manometry. Relaxation of the lower esophageal sphincter (LES) occurs with swallowing, esophageal distention, and transient LES relaxation (tLESR). Relaxation characteristics can be evaluated using a sleeve sensor, during a station pull-through, or after repositioning a side hole at the optimal position. Until recently, there was a paucity of quantitative data regarding EGJ relaxation. The deficiency was mostly attributable to the lack of standardized recording methodology and data interpretation. Recognizing this void, recent studies have quantified normal deglutitive EGJ relaxation with techniques suited to the study of a mobile anatomical zone: either a water-perfused sleeve sensor that spans the EGJ or high-resolution manometry with topographic data analysis. Shi and colleagues [44] used a standardized methodology of sleeve-sensor recording and computer-assisted data analysis and concluded that the best single assessment of EGJ relaxation was mean relaxation pressure (Table 31.3). Using the 95th percentile value of control subjects (12 mmHg) as the upper limit of normal, this parameter had a sensitivity and positive predictive value of 92% and 88%, respectively, for the diagnosis of achalasia. In another study aimed at defining the optimal criteria for incomplete EGJ relaxation, Staiano and Clouse [45] utilized high-resolution manometry with and without topographic analysis (Fig. 31.4) [45]. With topographic analysis, a transphincteric pressure gradient exceeding 5 mmHg had 94% sensitivity and 89% specificity for achalasia regardless of the presence or absence of peristalsis. Without topographic analysis, the 3-s mean residual EGJ pressure was most discriminative.

Impedance monitoring

Multichannel intralumenal impedance is a technique that was described more than a decade ago as a method to assess intralumenal bolus transit without fluoroscopy. The technique utilizes a catheter with multiple, closely spaced pairs of metal rings. An alternating current is applied across each pair of adjacent rings and the resultant current flow is dependent on the impedance between the rings. Impedance decreases when the electrodes are bridged by liquid and increases when they are surrounded by air, thereby providing data on the direction, content, and completeness of bolus transit. As illustrated in Figure 31.4, liquid bolus entry is indicated by a 50% drop in impedance, and return of the impedance tracing to 50% of baseline correlates with the passage of the tail of the bolus on fluoroscopy, also indicated by the contractile upstroke noted on manometry. Validation studies of multichannel intralumenal impedance against videofluoroscopy have shown excellent concordance in ascertaining bolus transit, reporting agreement in 97% (83/86) of swallows analyzed [46].

Multichannel intralumenal impedance has also recently been combined with manometry to assess the efficacy of esophageal emptying as a function of distal peristaltic amplitude. In a receiver operating characteristic (ROC) analysis of a large number of swallows, a 30 mmHg cutoff had 85% sensitivity and 66% specificity for identifying incomplete bolus transit [47]. With diminishing peristaltic amplitudes, the sensitivity progressively decreased and the specificity progressively increased (Fig. 31.5). This study illustrates the complementary nature of manometry and impedance testing in assessing esophageal function, which may potentially develop into a valuable clinical tool for the assessment of dysphagia.

Achalasia

Achalasia is the most easily recognized and best defined motor disorder of the esophagus. First recognized more than 300 years ago, the disorder was initially labeled cardiospasm reflecting the observation that it was caused by a functional obstruction of the esophagus at the cardiac sphincter with no

Table 31.3 Esophagogastric junction (EGJ) relaxation parameters and the detection of achalasia. Sensitivity and specificity values are in distinguishing achalasics from control subjects [85] or mixed subject groups [45]

Sleeve sensor **(Shi et al., 1998 [85])**

EGJ relaxation parameter	Threshold value	Sensitivity	Specificity
Basal pressure	> 41 mmHg	38%	93%
Mean relaxation pressure	> 12 mmHg	92%	93%
Percent relaxation	< 57%	88%	93%
Relaxation duration	< 2.2 s	31%	93%

High-resolution manometry with concurrent baseline (subjects with peristalsis) **(Staiano and Clouse, 2001 [45])**

EGJ relaxation parameter	Threshold value	Sensitivity	Specificity
Lowest residual pressure	≥ 4 mmHg	77%	92%
Lowest residual pressure over entire deglutitive period	≥ 4 mmHg	68%	98%
Lowest mean LES relaxation pressure in a 3-s postdeglutitive interval	> 10 mmHg	85%	92%

High-resolution manometry with concurrent baseline (subjects without peristalsis) **(Staiano and Clouse, 2001 [45])**

EGJ relaxation parameter	Threshold value	Sensitivity	Specificity
Lowest residual pressure	> 2 mmHg	81%	89%
Lowest residual pressure over entire deglutitive period	> 2 mmHg	77%	89%
Lowest mean LES relaxation pressure in a 3-s postdeglutitive interval	> 8 mmHg	87%	95%

High-resolution manometry with concurrent baseline (subjects with peristalsis or without peristalsis (in parentheses) **(Staiano and Clouse, 2001 [45])**

EGJ relaxation parameter	Threshold value	Sensitivity	Specificity
Transphincteric gradient	> 2 mmHg	100% (100%)	85% (100%)
	> 5 mmHg	94% (94%)	98% (100%)

LES, lower esophageal sphincter.

obstructing lesion evident in autopsy specimens. The first reported case was treated by passing a piece of carved whalebone with a sponge affixed to the distal end through the esophagus to facilitate esophageal emptying after meals [48]. That patient apparently sustained himself in this fashion for 15 years. During the next two centuries there were sporadic reports of cases similarly treated with crude ramrods or dilators. In 1937, Lendrum [49] proposed that the functional esophageal obstruction in this syndrome resulted from incomplete relaxation of the LES and renamed the disease achalasia ("failure to relax"), ushering in our current concept of the disease [49].

Epidemiology

Achalasia is a rare disease with an estimated incidence of 1/100 000 population per year in the United States and Europe [50,51]. The incidence in black Zimbabweans may be much lower, raising the issue of racial or environmental predisposition [52]. The disease affects both sexes equally and usually presents in adult life, being most common between the ages of 25 and 60 [53]. Because achalasia is a chronic condition, its prevalence greatly exceeds its incidence. Estimates of the prevalence of achalasia in Europe range from 7.1/100 000 in Wales to 13.4/100 000 in Ireland [54].

Reports of familial clustering of achalasia raise the possibility of genetic predisposition; however, the data on this are inconclusive. Achalasia has been reported in monozygotic twins [55], siblings [56], and children of affected parents [57]. However, other reports of the occurrence of achalasia in only one of a pair of monozygotic twins speaks against a strong genetic determinant [58]. Emphasizing this point, a survey of 1012 first-degree relatives of 159 achalasics

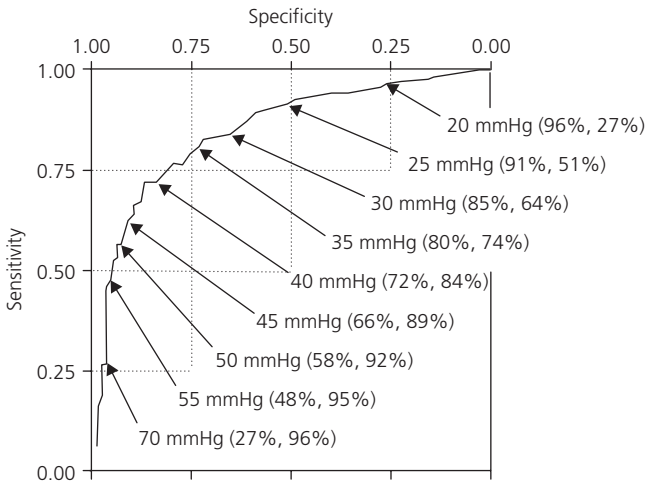

Figure 31.5 Receiver operating characteristic (ROC) curves describing the effectiveness of peristaltic contractions in achieving complete esophageal emptying for liquid swallows in subjects with hypotensive peristalsis. These data were generated from combined impedance and manometry studies of 700 swallows in 70 patients with the impedance/manometry segments under analysis positioned 5 and 10 cm above the lower esophageal sphincter (LES). The curves plot sensitivity against specificity of peristaltic amplitude as a predictor of complete bolus transit. The arrows indicate the position along the ROC curve of several discrete cutoff values of peristaltic amplitude with the corresponding sensitivity and specificity values (respectively) in parentheses. Evident from the curve, the peristaltic amplitude that achieves the optimal sensitivity and specificity is between 35 and 40 mmHg. Modified from Tutuian & Castell [137].

identified no affected relatives [59]. A rare genetic achalasia syndrome has also been described. Familial adrenal insufficiency with alacrima is inherited as an autosomal recessive disease that manifests itself with the childhood onset of autonomic nervous system dysfunction including achalasia, alacrima, sinoatrial dysfunction, abnormal pupillary responses to light, and delayed gastric emptying [60]. It is caused by mutations in the *AAAS* gene, which encodes a protein known as ALADIN.

Neuropathology

Achalasia is characterized by: (1) failure of the LES to relax completely with swallowing; and (2) aperistalsis in the smooth muscle esophagus. The resting LES pressure is elevated in about 60% of achalasia cases. If there are nonperistaltic, spasm-like contractions in the esophageal body, the disease is referred to as vigorous achalasia. These physiological alterations are thought to result from damage to the innervation of the smooth muscle esophagus (including the LES). Proposed neuroanatomical changes responsible for achalasia include loss of ganglion cells within the myenteric (Auerbach's) plexus, degeneration of the vagus nerve, and degeneration of the dorsal motor nucleus of the vagus. Of these possibilities, the loss of ganglion cells is best substantiated. Several observers report fewer ganglion cells and ganglion cells surrounded by mononuclear inflammatory cells in the smooth muscle esophagus of achalasics [61]. One report additionally noted ganglion cell degeneration extending into the proximal stomach in half of 34 specimens analyzed [62]. The degree of ganglion cell loss parallels the duration of disease such that ganglion cells are almost absent in patients afflicted for ≥ 10 years [63]. A morphological study of 42 esophagi resected from patients with advanced achalasia revealed diminished myenteric ganglion cells and inflammation within the myenteric plexus in all cases [64].

The ultimate cause of ganglion cell degeneration in achalasia is unknown; however, there is increasing evidence consistent with an immune-mediated process. Immunohistochemical analysis of the myenteric infiltrate in achalasia patients revealed that the majority of inflammatory cells are CD3/CD8-positive lymphocytes that express T-cell intracellular antigen-1 (TIA-1), indicating that these cells are either resting or activated cytotoxic T cells [65]. In addition to this histological support, there is also an association with the class II human leucocyte antigens (HLA) DQw1 [66] and DQb1 [67]. Achalasia may also be associated with degenerative neurological disorders such as Parkinson disease. Patients with both achalasia and Parkinson disease were noted to have Lewy bodies (intracytoplasmic hyaline or spherical eosinophilic inclusions) in the degenerating ganglion cells of the myenteric plexus [68].

Physiological studies also provide evidence of at least partial postganglionic denervation of esophageal smooth muscle in achalasia. Such damage can potentially affect excitatory (cholinergic) ganglionic neurons, inhibitory (nitric oxide ± vasoactive intestinal polypeptide [VIP]) ganglionic neurons, or both. Consider first the excitatory ganglionic neurons. Muscle strips from the circular layer of the esophageal body of achalasics contract when directly stimulated by acetylcholine but fail to respond to ganglionic stimulation by nicotine, indicating a postganglionic excitatory defect [69]. An in vivo demonstration of smooth muscle sensitivity to acetylcholine is the Mecholyl test in which the injection of this acetylcholine analogue evokes profound contraction of the otherwise flaccid achalasic esophagus [70]. This exaggerated response has been interpreted as a demonstration of Cannon's law of denervation supersensitivity. However, it is likely that loss of excitatory innervation is variable among achalasics. Partial preservation of the postganglionic cholinergic pathway is suggested by the observations that an achalasic's LES pressure increases after administration of the acetycholinesterase inhibitor edrophonium, and decreases after administration of the muscarinic antagonist atropine [71]. These observations are crucial to understanding why botulinum toxin may have therapeutic benefit in achalasia (see Botulinum toxin injection).

Regardless of excitatory ganglionic neuron impairment, it is clear that the inhibitory ganglionic neurons are necessarily impaired as an early manifestation of achalasia. Functionally,

these neurons are responsible for deglutitive inhibition (including LES relaxation) and for the timing of propagation of esophageal peristalsis; their absence offers a unifying hypothesis for the key physiological abnormalities of achalasia: impaired LES relaxation and aperistalsis. It is increasingly evident that the inhibitory ganglionic neurons utilize nitric oxide as a neurotransmitter [72]. In support of this conclusion, patients with achalasia have been shown to lack nitric oxide synthase in the EGJ [72]. Furthermore, VIP may be a cotransmitter in these neurons and immunohistochemical studies have demonstrated a marked reduction of VIP-staining neurons in achalasics [73].

A multitude of evidence supports impaired physiological function of postganglionic inhibitory innervation in the smooth muscle esophagus of achalasics. Muscle strips from the LES of achalasics do not relax in response to ganglionic stimulation as they do in normal control subjects [69]. Cholecystokinin octapeptide, which normally stimulates the inhibitory ganglionic neurons, thereby reducing LES pressure, paradoxically increases the LES pressure in achalasics [74]. Impaired inhibitory innervation of the tubular esophagus above the LES is more difficult to demonstrate than within the sphincter itself because of the absence of resting tone in this region. However, in a clever experiment, Sifrim and colleagues [75] utilized an intraesophageal balloon to create a high-pressure zone in the tubular esophagus that then relaxed with the onset of deglutitive inhibition. This deglutitive relaxation in the esophageal body was absent in early, nondilated cases of achalasia.

Clinical presentation

Clinical manifestations of achalasia may include dysphagia, regurgitation, chest pain, weight loss, and aspiration pneumonia. All patients have solid food dysphagia; the majority of patients also have variable degrees of liquid dysphagia. The onset of dysphagia is usually gradual with the duration of symptoms averaging 2 years at presentation [53]. Dysphagia severity fluctuates but eventually plateaus. Predictably, regurgitation occurs when large amounts of food are retained in the dilated esophagus. The regurgitant is often recognized as food that has been eaten hours, or even days, previously. It tends to be nonbilious, non-acid, and mixed with copious amounts of saliva. Chest pain is a frequent complaint early in the course of achalasia, occurring in approximately two-thirds of patients [76]. Its etiology is unknown but is speculated to be related to the occurrence of esophageal spasms or to the process of esophageal dilation associated with disease progression. Treatment of achalasia is less effective in relieving chest pain than it is in relieving dysphagia or regurgitation. However, unlike dysphagia or regurgitation, chest pain may improve or disappear over time [76].

An estimated 10% of achalasics have bronchopulmonary complications as a result of regurgitation; in some instances, it is these complications rather than dysphagia that prompts them to seek medical care [77]. Another interesting, but fortunately rare, symptom of achalasia is airway compromise and stridor as a result of the dilated esophagus compressing the trachea [78]. This is related to dysfunction of the belch reflex either because of neural degeneration or because esophageal dilation prevents activation of stretch receptors within the esophageal wall [79].

It is paradoxical that many achalasics complain of heartburn, even after the onset of dysphagia [80]. Although reflux may be a common sequela of the treatments for achalasia, it seems physiologically inconsistent to simultaneously have dysphagia from impaired LES relaxation and reflux from excessive LES relaxation. In support of this skepticism, ambulatory 24-h esophageal pH studies of achalasics have only shown periods of esophageal acidification caused by the bacterial fermentation of retained food in the esophagus rather than discrete gastroesophageal reflux events [81]. Furthermore, prolonged LES recordings have shown a nearly complete absence of transient LES relaxations in achalasics [82]. However, there are occasional exceptions to this, evident from a well-documented case of an achalasic patient with intact transient LES relaxation despite the absence of deglutitive LES relaxation [83].

Radiography

A barium swallow radiograph (Fig. 31.6) can demonstrate the physiological abnormalities of achalasia. The characteristic radiograph is of a dilated intrathoracic esophagus with impaired emptying, an air–fluid level, absence of a gastric air bubble, and an LES that tapers to a point giving the distal esophagus a beak-like appearance. Occasionally, an epiphrenic diverticulum, immediately proximal to the LES, is observed with achalasia [84]. With long-standing achalasia the esophagus may assume a sigmoid configuration, and in some instances an air–fluid level, mediastinal widening, and an outline of the dilated esophageal wall are even evident on a plain chest film. Note that the characteristic radiographic findings depend upon esophageal dilation; as dilation is not always present in achalasia, the sensitivity of the radiographic examination is limited [51].

Manometry

The defining manometric features of achalasia are aperistalsis and incomplete LES relaxation. In cases of achalasia that are established by all available clinical criteria, these features are present more than 90% of the time. Given that esophageal aperistalsis also occurs with disorders other than achalasia, including GERD, collagen vascular diseases, and diabetes, the diagnosis of achalasia is highly dependent on accurately detecting impaired LES relaxation. Assuming that the LES can be intubated, an inaccurate diagnosis of impaired relaxation may result from artifactual relaxation associated with inappropriate catheter design or from employing suboptimal diagnostic thresholds. Two studies utilizing assemblies that

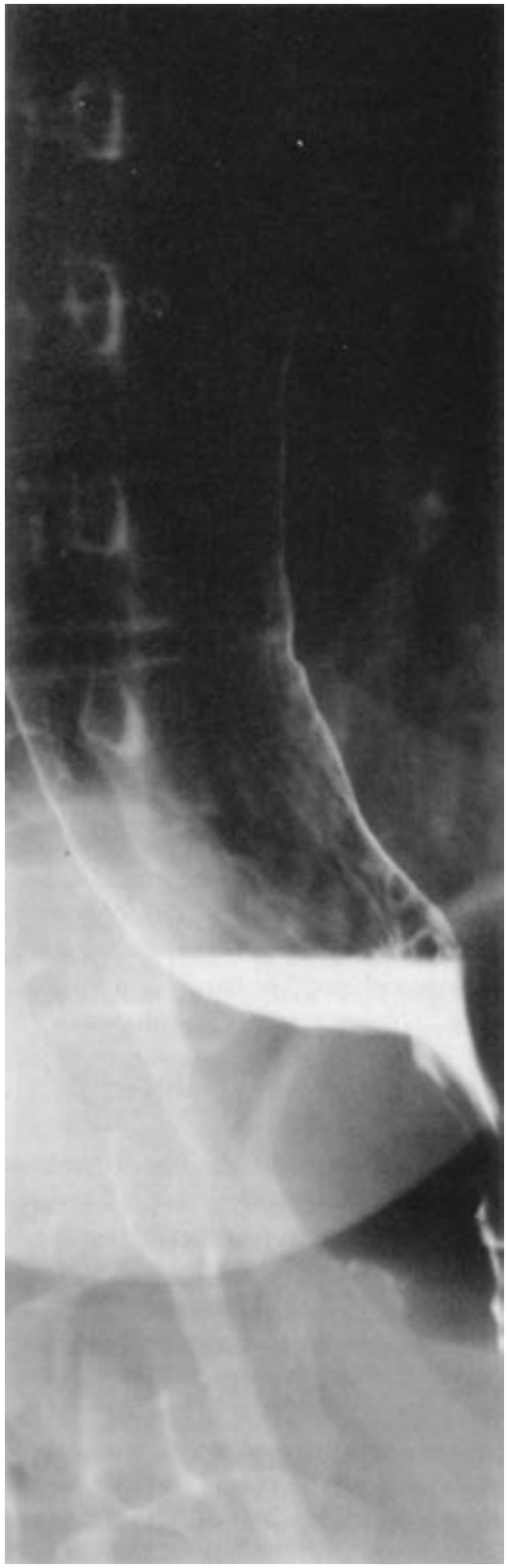

Figure 31.6 Characteristic barium swallow in idiopathic achalasia. Note esophageal dilation with an air–fluid level and the smooth tapering at the gastroesophageal junction. Radiographic findings can be much more subtle in the early phases of the disease.

control for the effect of axial movement (a sleeve sensor and high-resolution manometry with topographic analysis) were recently conducted to minimize such errors. The highlights of these studies are summarized in Table 31.3. With a sleeve sensor, the best single assessment of EGJ relaxation for a diagnosis of achalasia was mean relaxation pressure using the 95th percentile value of control subjects (12 mmHg) as the upper limit of normal [85]. Utilizing high-resolution manometry with topographic analysis, similar accuracy was achieved using a threshold value of 8–10 mmHg for the lowest mean residual pressure in a 3-s postdeglutitive interval [45]. However, the greatest accuracy was achieved utilizing a novel concept for assessing LES relaxation: the transphincteric pressure gradient during the 2- to 6-s post-swallow interval. Pressure gradients exceeding 5 mmHg had a sensitivity of 94% and specificity of 98% for detecting achalasia.

Although utilizing the methods and thresholds defined in Table 31.3 will maximize diagnostic accuracy, manometry remains an imperfect tool for detecting achalasia. Manometric variants exist in achalasia that can still confound the diagnosis [86–88]. Illustrative of this is a report describing four achalasia variants in a retrospective study of 58 patients with idiopathic achalasia [83]. Achalasia variants were characterized by:

1 high amplitude esophageal body contractions ("vigorous achalasia")

2 retained peristalsis in most of the esophagus with only a short segment of aperistalsis

3 retained deglutitive LES relaxation

4 impaired deglutitive relaxation but intact tLESRs (Fig. 31.7).

Despite utilizing state-of-the-art techniques, each of these achalasia variants had atypical features of either peristalsis or LES relaxation, confounding their diagnosis. Nonetheless, in each case the patient was symptomatically improved following Heller myotomy, and histopathological analysis subsequently confirmed inflammation and destruction of the myenteric plexus. In addition to the possibility of misdiagnosis, manometric criteria for achalasia are not 100% specific for either idiopathic achalasia or Chagas disease. Pseudoachalasia accounts for up to 5% of cases with manometric criteria typifying achalasia, being more common with progressive age [53].

Manometry is also useful in evaluating symptomatic patients after Heller myotomy or pneumatic dilation. Data suggest that relief of dysphagia is related to postprocedure EGJ pressure, with a value of less than 10 mmHg being optimal [89]. Identification of residual EGJ high pressure of more than 10 mmHg argues for further therapy targeting LES disruption. On the other hand, symptomatic subjects with basal LES pressures of less than 10 mmHg may not benefit from repeat dilation or surgery and should be evaluated with a timed barium swallow to assess emptying [90]. In instances in which a timed barium swallow demonstrates poor esophageal emptying despite an optimal reduction of EGJ pressure, esophageal resection with gastric pull-up or colon interposition may be indicated.

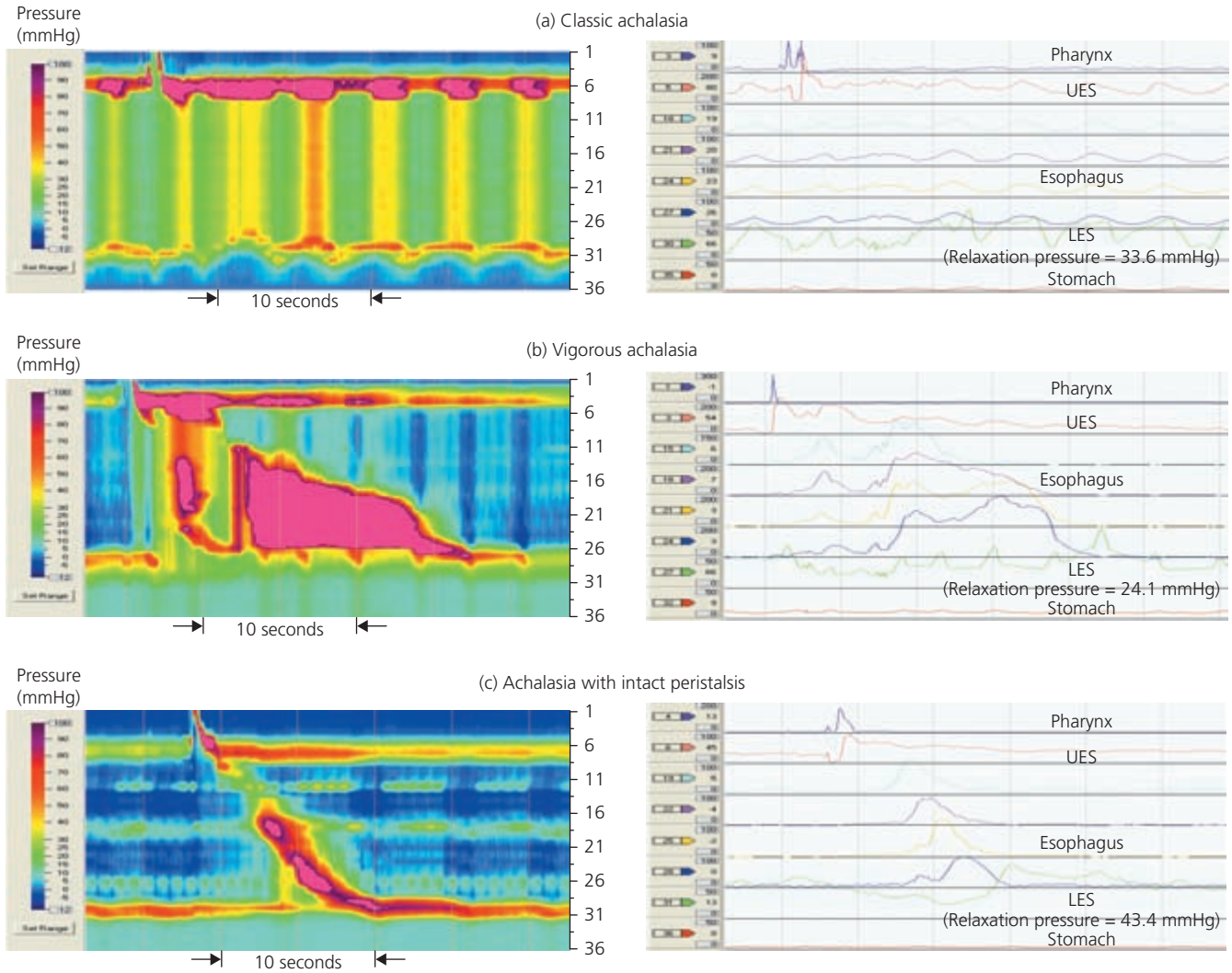

Figure 31.7 High-resolution manometric isocontour plots and conventional manometric recording in three patients with achalasia with varying peristaltic function. **(a)** Classic achalasia with aperistalsis and abnormal lower esophageal sphincter (LES) relaxation. Note the absence of the peristaltic activity and the common cavities with an abnormal increase in the esophagogastric pressure gradient. **(b)** A case of vigorous achalasia and a nondilated esophagus. Note the prolonged simultaneous contraction in the entire smooth muscle esophagus. LES relaxation is poor and the duration of relaxation is shortened as a result of the velocity of the simultaneous contraction reaching the distal esophagus. **(c)** A rare case of achalasia with intact peristalsis. Note that abnormal relaxation is associated with a normally propagating peristaltic wavefront with normal contractile pressures. Each of these patients responded to laparoscopic Heller myotomy.

Endoscopic findings

Endoscopy is relatively insensitive in the detection of achalasia with endoscopists prospectively suggesting the correct diagnosis in less than one-third of patients [51]. Typical endoscopic findings include retained food or saliva, dilation, and atony of the esophageal body. With progressive dilation and stasis, erythema, friability, and superficial ulcerations may be seen. Whitish plaques covering the epithelial surface may be seen as the result of *Candida* overgrowth; these are usually asymptomatic. The achalasic LES has a pinpoint appearance and does not open with air insufflation. Nonetheless, the instrument should pass easily through the sphincter into the stomach with minimal pressure. Resistance, or a feeling of stiffness as the endoscope crosses the EGJ, should immediately raise the suspicion of malignancy (see Pseudoachalasia). Equivocal mucosal abnormalities of the EGJ, evident on forward viewing or retroflexed inspection, should always be biopsied because of the possibility of pseudoachalasia.

Differential diagnosis

The differential diagnosis of achalasia includes both other esophageal motility disorders with functional attributes overlapping those of achalasia and diseases of distinct pathophysiology that duplicate the functional consequences of achalasia. With respect to other motility disorders, inspection of Table 31.2 suggests many similarities between esophageal

spasm and achalasia, especially vigorous achalasia. In fact, the only distinction between these entities is the demonstration of incomplete LES relaxation in vigorous achalasia. Thus, it has been speculated that in some instances esophageal spasm (and vigorous achalasia) may represent an early stage of achalasia and may evolve into full-fledged achalasia. Vantrappen and colleagues [86] first speculated on this relationship and reported several cases demonstrating just such an evolution. Recently, Khatami and colleagues [91] reported on a prospective cohort of patients diagnosed with esophageal spasm between 1992 and 2003. Review of their follow-up manometry by two independent interpreters revealed that achalasia was subsequently diagnosed in one of the twelve patients, suggesting this to be a rare occurrence. However, as will become evident later in this chapter, spastic disorders of the esophagus are a heterogeneous lot and only a small minority, at best, are part of the continuum with achalasia.

With respect to diseases of distinct pathophysiology that duplicate the functional consequences of achalasia, the main considerations are Chagas disease and pseudoachalasia. These disorders will be considered in some detail because they may resemble achalasia so closely that conventional diagnostic tests are misleading.

Chagas disease

Although achalasia is almost universally idiopathic in North America, the disease can be closely mimicked by esophageal involvement in Chagas disease, which is endemic in areas of central Brazil, Venezuela, and northern Argentina. Recently, cases have also been reported in south Texas. Chagas disease is spread by the bite of the reduviid (kissing) bug, which transmits the culprit protozoan, *Trypanosoma cruzi*. After infection, an acute septicemic phase of the illness develops, which varies in severity from being so mild as to go unnoticed to being so severe as to be fatal [92]. The chronic phase of the disease develops many years after infection and results from destruction of autonomic ganglion cells throughout the body, including the heart, gut, urinary tract, and respiratory tract. Chronic cardiomyopathy with conduction system disturbances and arrhythmias is the most common cause of death. The digestive tract organs most often affected are the esophagus, duodenum, and colon. The severity of esophageal dysfunction is directly proportional to the degree of intramural ganglion cell loss; abnormal peristalsis is first detectable after 50% of ganglion cells are destroyed and esophageal dilation only after 90% are destroyed [92]. Paralleling this, the initial dysfunction is confined to the esophageal body, with LES dysfunction occurring late in the course of the disease [92]. The most useful clinical distinction between idiopathic achalasia and esophageal involvement in Chagas disease is evidence of other tubular organ involvement (megaureter, cardiomyopathy, megaduodenum, megacolon, megarectum) in the latter. Otherwise, Chagas disease patients have the same clinical, radiographic, and manometric characteristics as those with idiopathic achalasia. The diagnosis of Chagas disease is confirmed by a serological test utilizing complement fixation or the polymerase chain reaction. The treatment of the achalasia syndrome in Chagas disease is similar to that for idiopathic achalasia. Treatment of the infection itself is of limited efficacy in the acute phase of the disease and of no proven efficacy with chronic disease.

Pseudoachalasia

It is important to emphasize that neither the radiographic nor the manometric features of achalasia are specific for idiopathic achalasia or achalasia associated with Chagas disease; tumor-related pseudoachalasia accounts for up to 5% of cases with manometrically defined achalasia. Pseudoachalasia is more likely with progressive age (> 50 years), abrupt onset of symptoms (< 1 year), and early weight loss in excess of 7 kg [53]. However, even though these criteria make pseudoachalasia more likely, they still have poor predictive value in individual cases [93]. Tumor infiltration (especially carcinoma in the gastric fundus) can completely mimic the functional impairment seen with idiopathic achalasia [94] (see Table 31.2). It is because of this potential pitfall that a thorough anatomical examination, including endoscopy, should be carried out as part of the diagnostic evaluation of every new case of achalasia. A clue to the presence of pseudoachalasia on endoscopic examination is of more than the slightest resistance of passage of the endoscope across the EGJ. In idiopathic achalasia, the endoscope should pop through with only gentle pressure required. If suspicion of pseudoachalasia persists, endoscopic biopsy, computed tomography, magnetic resonance imaging, or endoscopic ultrasound should be considered for further evaluation, depending upon the special circumstances.

Adenocarcinoma of the EGJ accounts for more than half of pseudoachalasia cases, with a myriad of tumors and miscellaneous conditions accounting for the remainder. Within the spectrum of malignancies, pancreatic, oat cell, hepatoma, bronchogenic, esophageal squamous cell, prostate, and lymphoma cases have been reported [53]. These tumors produce an achalasia syndrome by infiltrating the wall of the esophagus at the EGJ, in essence causing a malignant obstruction at the LES with proximal esophageal dilation [94]. Similarly, pseudoachalasia has also been reported to result from esophageal infiltration by amyloid [95], eosinophilic gastroenteritis [96], and sarcoidosis [97]. Although often speculated in the literature, it is less certain, and certainly much less common, that an achalasic syndrome occurs as a paraneoplastic syndrome without direct tumor stenosis of the EGJ [53].

Postfundoplication

Postfundoplication dysphagia is common in the early postoperative period and patients are often advised to consume soft diets for the first 2–4 weeks. Dysphagia that persists for longer than 2–4 weeks should be evaluated with upper

endoscopy or a barium esophagram to assess the integrity of the wrap and evaluate for possible paraesophageal hernia. Subjects without an overt mechanical disruption should be evaluated with manometry to assess peristaltic function, LES pressure, and LES relaxation to determine whether the wrap is too tight or an underlying motility disorder such as achalasia exists. Diagnosing achalasia in the context of fundoplication can be difficult as the manometric findings can be quite similar with both entities: aperistalsis and impaired LES relaxation. To distinguish mechanical obstruction due to a tight wrap or tight crural repair from a functional obstruction related to achalasia one can administer amyl nitrite during manometry and observe the effect on the EGJ high-pressure zone. The mechanical effect of a tight fundoplication should be less affected by the smooth-muscle relaxing effects of the amyl nitrite than the hypertensive sphincter of an achalasic [98].

Management

Because the underlying neuropathology cannot be corrected, treatment of achalasia is directed at compensating for the functional abnormalities and preventing complications. The main functional abnormality in achalasia is poor esophageal emptying, and this can be treated by reducing the LES pressure; with a reduced LES pressure, gravity promotes esophageal emptying. Peristalsis rarely returns with therapy. LES pressure can be reduced by pharmacological therapy, forceful dilation, or surgical myotomy. On the whole, pharmacological treatments are not very effective and are perhaps more indicated as temporizing maneuvers than definitive therapies. The definitive treatment of achalasia is still disruption of the LES either surgically (Heller myotomy) or with a pneumatic dilator. The optimal approach is an issue of debate given the lack of randomized controlled trials with accepted criteria for assessing efficacy. Thus, there is no alternative but to compare therapies based on the numerous uncontrolled series that have been published using a variety of qualitative endpoints as indications of efficacy. Table 31.4 summarizes the pooled estimate of response rate of medical and surgical treatment data in achalasia. Data in the table are uncontrolled and are consecutive series collected both prospectively and retrospectively. As there was no uniformity in assessment of efficacy among trials, the proportion with a good to excellent response was extracted from each study, regardless of criteria. Only studies including more than 10 patients and with a follow-up of 1 year were tabulated.

Pharmacological therapy

Smooth muscle relaxants such as nitrates or calcium channel blockers, administered sublingually immediately before eating, may offer partial relief of the dysphagia accompanying achalasia by reducing the resting sphincter pressure. Amyl nitrite [99], sublingual nitroglycerin [100], theophylline [100], and β2-agonists [100] have also been tried with variable results. The greatest reported experience has been with isosorbide dinitrate and nifedipine [101]. Isosorbide dinitrate (Isordil), 5–10 mg sublingually before meals, reduces the resting LES pressure by 66% for 90 min, with clinical efficacy paralleling the magnitude of LES response [102]. A 19-month trial of this therapy has been reported to provide marked or complete relief of dysphagia [102]. Side effects, particularly headache, are common. Placebo-controlled trials have not been reported.

Calcium channel blockers (diltiazem, nifedipine, verapamil) reduce the LES pressure by 30%–40% for more than an hour, with potential benefit in achalasia [102,103]. The greatest clinical experience has been with nifedipine (Procardia), which can be administered in a dose of 10–30 mg sublingually (capsules are crushed in the mouth) 30–45 min before meals. The benefit of nifedipine, 30–40 mg sublingually per day, was assessed in a group of 29 patients with early achalasia (before esophageal dilation) in a placebo-controlled trial. Nifedipine was significantly better than placebo (which had no significant benefit), yielding good to

Table 31.4 Pooled estimate of response rate of achalasia treatments across referenced studies. In calculating the weighted mean response for each treatment modality, studies were characterized by the number of subjects included (*n*) and the response rate for those subjects (*p*). See text for inclusion criteria

	Without further therapy			Repeat treatment	
Therapy [reference]	**Total (*n*)**	**Weighted mean response (*p*) ± SE (%)[a]**	**Weighted mean follow-up (years)**	**Total (*n*)**	**Weighted mean response (*p*) ± SE (%)**
Botulinum toxin [130–133]	221	35 ± 17	1.3	63	49 ± 26
Pneumatic dilation [130]	1276	72 ± 26	4.9	269	80 ± 42
Heller myotomy:					
Thoracotomy [130]	1221	84 ± 20	5	Scant data	
Laparotomy [130]	732	85 ± 18	7.6	Scant data	
Laparoscopy [130,145–150]	365	91 ± 13	1.4	4.1% converted to open procedure	

excellent results in 70% of achalasics followed for 6–18 months [101]. However, subsequent placebo-controlled crossover trials have found only minimal clinical improvement with nifedipine [104]. Limiting side effects of nifedipine use are flushing, dizziness, headache, peripheral edema, and orthostasis.

Sildenafil is another smooth muscle relaxant that has been shown to decrease the LES pressure and deglutitive relaxation pressure in patients with achalasia by blocking phosphodiesterase type 5, the enzyme that destroys cyclic guanosine monophosphate induced by nitric oxide. A double-blind placebo-controlled trial revealed that 50 mg of sildenafil caused a significant decrease in LES pressure and relaxation pressure when compared with placebo [105]. The effect peaked at 15–20 min after administration and persisted for less than 1 h.

Botulinum toxin injection

Pasricha and colleagues [106] first reported the effects of botulinum toxin on the LES in achalasia patients in 1994. They found that intrasphincteric injection of botulinum toxin decreased LES pressure by 33% and improved dysphagia in 66% of patients for a 6-month period. Botulinum toxin works by irreversibly inhibiting the release of acetylcholine from presynaptic terminals, effectively eliminating the neurogenic component of LES pressure. However, as this effect is eventually reversed by the growth of new axons, it is not surprising that the effect of botulinum toxin is not long lasting. Although most patients initially experience a good response, there is minimal continued efficacy at 1 year (Table 31.4) [107–110]. The technique for administering botulinum toxin involves injecting a total of 80 units into four quadrants of the LES with a sclerotherapy catheter. Doses higher than 100 units have not been shown to be more effective [111]. Studies comparing botulinum toxin with pneumatic dilation suggest that the added expense of repeated injection outweighs the potential economic benefits of the added safety, unless life expectancy is 2 years or less [112]. Thus, this therapeutic option should be mainly reserved for elderly or frail individuals who are poor risks for more definitive treatments.

Pneumatic dilation

Therapeutic dilation for achalasia requires the forceful distension of the LES to a diameter of approximately 3 cm to partially disrupt the circular muscle of the sphincter and effect lasting reduction of LES pressure. Dilation with an endoscope or with standard bougies (up to 60F) provides very temporary benefit at best. Only balloon dilators specifically designed to treat achalasia achieve an adequate diameter for lasting effectiveness. The basic element of an achalasia dilator is a long, noncompliant, cylindrical balloon that can be positioned fluoroscopically (Rigiflex dilator) or endoscopically (Witzel dilator) across the LES and then inflated to a characteristic diameter in a controlled fashion using a hand-held manometer.

The technique of pneumatic dilation is variable among practitioners in terms of patient preparation, parameters of balloon inflation, and postdilation monitoring. There is general agreement that pneumatic dilation can be carried out in an outpatient setting with the patient under conscious sedation. In patients with substantial esophageal retention, it is useful to have them on a liquid diet for one or more days before the procedure. Reported balloon inflation pressures range from 360 mmHg (7 psi) to 775 mmHg (15 psi) with periods of inflation ranging from several seconds to 5 min [113]. Although there is minimal methodological consistency among authors, a cautious approach beginning with relatively low inflation pressures and/or a smaller diameter dilator (3.0–3.5 cm) is fairly universal.

The major complication of pneumatic dilation is esophageal perforation; mortality is very rare [114]. Most series report an incidence of esophageal perforation during pneumatic dilatation of between 1% and 5% [77,113]. Because most perforations are readily evident within a few hours of the procedure, patients should be observed closely for signs of an esophageal leak for 3–6 h after pneumatic dilation. Alternatively, some practitioners routinely obtain a fluoroscopic examination of the esophagus following pneumatic dilation to make sure that perforation has not occurred. Usually, water-soluble contrast is given first, followed by barium. If the perforation appears small and confined, or intramural, conservative management consisting of close observation while maintaining the patient nil per os (NPO) and administering intravenous antibiotics is appropriate [77]. If any substantial perforation has occurred, or if worsening pain and fever occur during observation of what was thought to be a small perforation, surgical repair should be pursued quickly. Patients with perforation from pneumatic dilation that is recognized and promptly treated surgically (within 6–8 h) have outcomes that are comparable to those of patients undergoing elective Heller myotomy [115].

The best predictor of efficacy following a pneumatic dilation is the postdilation LES pressure; neither sphincter relaxation nor peristaltic function is significantly changed. LES pressure values of less than 10 mmHg are associated with prolonged remission whereas values of more than 20 mmHg derive little benefit from the procedure [89]. It is neither unusual nor dangerous to repeat a pneumatic dilation 2–3 times in instances of an unsatisfactory result. The clinical efficacy of dilation has been reported to range from 32% to 98% (Table 31.4) [107]. Patients having a poor initial result or rapid recurrence of symptoms have a diminished likelihood of responding to additional dilations [107]. Subsequent response to surgical myotomy is not influenced by the history of previous dilations [116].

Heller myotomy

The surgical objectives in the treatment of achalasia are to reduce the LES pressure enough to eliminate dysphagia but

not so much as to allow gastroesophageal reflux. Current procedures are variations on the esophageal myotomy described by Heller in 1913 [107]. In Heller's operation, an anterior and posterior myotomy were performed through either a laparotomy or a thoracotomy. Although clearly efficacious, open Heller myotomy is associated with considerable morbidity related to thoracotomy, which led most patients to pursue pneumatic dilation as the initial intervention. However, adoption of the laparoscopic approach for achalasia surgery has led many practitioners to use surgery as an initial intervention because this technique offers similar efficacy to the open procedure and substantially reduced morbidity.

Surgical series of achalasics treated with myotomy report good to excellent results in 62%–100% of patients, with persistent dysphagia troubling less than 10% of patients overall (Table 31.4) [107]. The appeal of myotomy is that it offers a more predictable method of reducing LES pressure than pneumatic dilation [117]. Studies have suggested that laparoscopic and thoracoscopic approaches are associated with similar efficacy and reduced morbidity when compared with myotomy via thoracotomy or laparotomy [107,118–123]. Patti and colleagues [120] reported the clinical outcomes of 168 achalasic patients who underwent thoracoscopic myotomy (35 patients) or laparoscopic Heller myotomy accompanied by a partial fundoplication (133 patients). There were no deaths in this series and only eight patients required reoperation. Relief of dysphagia was obtained in 93% of the patients who underwent the laparoscopic Heller myotomy and 85% of those treated with thoracoscopic myotomy. Not only did the laparoscopic approach more effectively improve dysphagia, it was also associated with a shorter hospital stay and less postoperative gastroesophageal reflux. Historically, postmyotomy reflux in achalasics could be particularly severe [124]. However, with the availability of proton pump inhibitors, reflux is usually easily controlled, making these complications very unlikely. Thus, laparoscopic Heller myotomy combined with a partial fundoplication has become the preferred surgical procedure for achalasia.

Occasionally, patients fail to respond to dilation and myotomy and require more extensive surgical procedures. In extremely advanced or refractory cases of achalasia, esophageal resection with gastric pull-up or interposition of a segment of transverse colon or small bowel may be the only surgical option [125,126]. Indications for this intervention include unresolvable obstructive symptoms, cancer, and perforation during dilation. Although excellent long-term functional results can be achieved, the reported mortality rate of this surgery is about 4%, consistent with the mortality rate of esophagectomy carried out for other indications.

Medical versus surgical treatment

Although pharmacological therapy is simple and safe, it has become increasingly clear that this should be reserved for use as a temporizing measure while more definitive therapy is being considered. Thus, practically speaking, the choice is between pneumatic dilation and laparoscopic Heller myotomy as the primary therapy for achalasia. However, there are no prospective controlled trials comparing these treatments. One controlled trial exists comparing pneumatic dilation with myotomy via thoracotomy [127]. This study reported 95% symptom resolution with myotomy and 51% symptom resolution in the dilation group, but the study was criticized for the methodology of pneumatic dilation utilized. Most studies report symptom resolution in approximately 70% of patients after pneumatic dilation, substantially higher than the 51% reported in the controlled trial but still substantially lower than that reported in uncontrolled series of laparoscopic Heller myotomy (Table 31.4). Furthermore, although laparoscopic Heller myotomy is invasive, its morbidity rate is low. On the other hand, pneumatic dilation has a perforation rate that averages 3%. Even though these patients do well if the perforation is recognized and operated on promptly, they require a thoracotomy. Thus, it appears that, in expert hands, laparoscopic Heller myotomy is likely the optimal initial therapy when one considers both the safety and efficacy of the procedure.

Risk of squamous cell cancer

Numerous series report cases of squamous cell carcinoma developing in the achalasic esophagus [128,129], and the relative risk of developing squamous cell cancer has been estimated to be 33-fold higher than in the nonachalasic population [130]. The pathogenesis of the carcinoma is obscure, but stasis and mucosal irritation may be precipitating factors. The tumors develop many years after the diagnosis of achalasia. Because the tumors often arise in a greatly dilated esophagus, symptoms can be delayed and the neoplasms are large and advanced at the time of detection. These considerations raise the issue of surveillance endoscopy in achalasics to detect early squamous cell cancer. However, an elegant analysis by Sandler and colleagues [131] of a database encompassing the entire Swedish population of 1062 achalasics suggests that, after discounting incident carcinomas, the overall squamous cell cancer risk for achalasics is 17-fold higher than for age-matched control subjects, resulting in a 0.15% incidence of cancer among the achalasics [131]. The authors calculated that if surveillance endoscopy was carried out annually, 406 exams would need to be carried out in men and 2220 in women before one, potentially treatable tumor was found.

Spastic disorders

The concept of "esophageal spasm" is credited to Hamilton Osgood based on his 1889 description of six patients with episodic chest pain and dysphagia: "sudden and often intense constriction in the epigastrium . . . with an arrest of food at the cardiac orifice" [132]. From the time of that report,

further insights into the entity of esophageal spasm have paralleled the development of imaging or measurement technologies that might detect it. "Diffuse spasm of the lower part of the esophagus" was first described radiographically in 1934, and the first manometric descriptions of spasm came in the late 1950s. Introduction of the term "diffuse esophageal spasm" and our present concept of this entity followed Fleshler's 1967 description of a "clinical syndrome characterized by symptoms of substernal distress or dysphagia or both, the roentgenographic appearance of localized, nonprogressive waves (tertiary contractions), and an increased incidence of nonperistaltic contractions recorded by intralumenal manometry" [133].

In broad terms, diffuse esophageal spasm (DES) as described by Fleshler represents a disorder of peristalsis. However, the esophagus of the patient with DES usually retains its ability to propagate normal primary peristaltic waves for the majority of the time, suggesting that the responsible neuromuscular pathology must be subtler than with achalasia. Partly because of this fact, the criteria for diagnosing DES (which is the best accepted of the proposed disorders of peristalsis) remain variable and confusing [134]. Furthermore, along with the evolution of manometric techniques capable of accurately recording intralumenal esophageal contractile activity came the description of other minor aberrations of peristalsis (nutcracker esophagus, hypertensive LES, and nonspecific esophageal motor disorder) to which symptomatology, especially chest pain, might be attributed [135]. Despite this proliferation of literature, it remains controversial whether or not these entities have any clinical significance. The heterogeneity among patients, the absence of specific pathology, and the absence of well-defined clinical implications caution against considering them akin to achalasia. Strict criteria for defining a disease would stipulate that the peristaltic abnormality cause a major alteration of esophageal physiology demonstrable by both manometric and nonmanometric measures, and that it be temporally associated with symptoms, with correction resulting in symptomatic improvement [136]. If such criteria are applied to these aberrations of peristalsis, only severe DES would be judged significant [136]. Indicative of this, esophageal transit was recently assessed in a prospective cohort of 350 patients using multichannel intralumenal impedance [137]. Entities such as nutcracker esophagus and hypertensive LES exhibited normal bolus transit and only 50% of patients with esophageal spasm had abnormal bolus transit. Thus, even esophageal spasm is a heterogeneous group with varying degrees of functional compromise. With this caution in mind, consider what has been learned of spastic disorders of peristalsis.

Neuromuscular pathology

The esophageal muscularis propria and myenteric plexus are not readily accessible for biopsy, resulting in little material being available for histopathological examination. Furthermore, patients with spastic disorders of the esophagus rarely undergo esophageal surgery and the diseases are not fatal, making for a paucity of pathological specimens; the limited information that does exist pertains only to DES as opposed to the minor aberrations of peristalsis. The most striking reported pathological change is diffuse muscular hypertrophy or hyperplasia, mainly of the distal two-thirds of the esophagus. Muscular thickening of up to 2 cm has been reported in patients with clinical and manometric evidence of DES [138]. However, there are other well-documented cases of spasm in which esophageal muscular thickening was not found at thoracotomy [139], and still other instances of patients with muscular thickening not associated with DES symptoms [140]. Similarly, little evidence of neuropathology has been reported; diffuse fragmentation of vagal filaments, increased endoneural collagen, and mitochondrial fragmentation have been described but the significance of these findings is unclear [141].

Pathophysiology of spastic disorders of peristalsis

Esophageal spasm

Despite the absence of defined histopathology, physiological evidence implicates myenteric plexus neuronal dysfunction in spastic disorders of the esophagus. During peristalsis, vagal impulses reach the entire smooth muscle segment of the esophagus simultaneously and activate myenteric plexus neurons between the longitudinal and circular muscle layers [142]. Ganglionic neurons then intervene between the efferent vagal fibers and the smooth muscle, belonging to either an inhibitory population (using nitric oxide as a neurotransmitter) that hyperpolarizes the muscle cell membrane and inhibits contraction or an excitatory (cholinergic) population that depolarizes the membrane thereby prompting contraction. Thus, the instantaneous activity of the musculature at each esophageal locus is determined by the balance between these controlling influences from the myenteric plexus. Experimental evidence suggests heterogeneity among patients with spastic disorders such that some primarily exhibit a defect of inhibitory interneuron function, whereas in others the defect is of excess excitation.

Two in vivo experiments implicate a defect of myenteric plexus inhibitory interneuron function in the genesis of simultaneous contractions in the distal esophagus. Behar and Biancani [143] timed the propagation of a swallow-induced contraction in normal subjects and in a group of patients whose dominant manometric abnormality was of a simultaneous contraction in the distal 10 cm of the esophagus. Figure 31.8 illustrates the key finding of that work. Note that within the proximal (striated muscle) esophagus the two groups exhibited a similar contraction propagation, consistent with this timing being the result of the sequenced activation of motor units by vagal efferents programmed within the medullary swallowing center. However, once entering

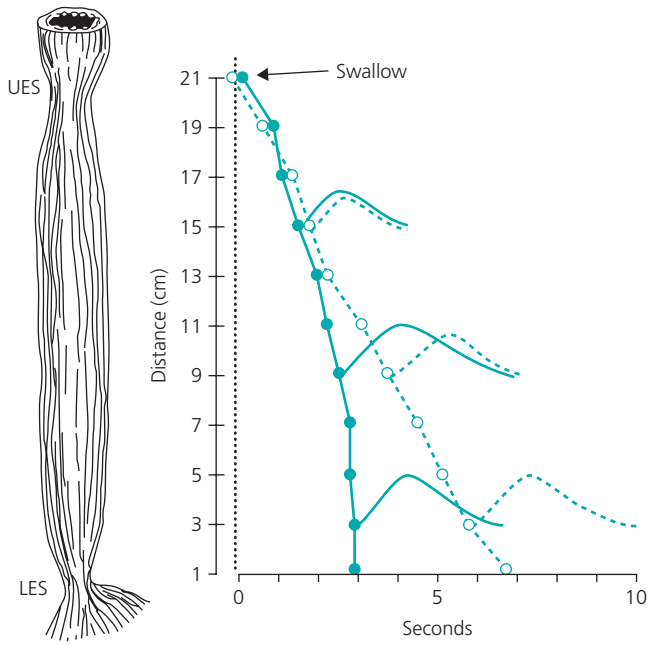

Figure 31.8 Rate of propagation of an esophageal contraction in individuals with normal peristalsis (open circles) and a group of patients with a motor disorder characterized by dysphagia and simultaneous contractions in the distal esophagus (filled circles). Note that the propagation is similar between the two groups in the proximal (striated muscle) esophagus but diverges in the distal (smooth muscle) esophagus. As the prolonged latency in the distal esophagus is believed to be a function of postganglionic inhibitory interneurons within the myenteric plexus, this suggests selective dysfunction of these cells. From Behar and Biancani [143], with permission.

the smooth muscle segment, the patients' contractions diverged from those of the normal subjects, resulting in a simultaneous contraction in the distal esophagus. The duration and amplitude of contraction at each esophageal locus was normal in patients, but the progressive delay of initiation of that contraction, a function attributable to the increasing influence of inhibitory interneurons in the distal esophagus, was absent. Furthermore, if these individuals swallowed twice within a 5-s interval, there was no deglutitive inhibition of the first peristaltic contraction within the smooth muscle esophagus as is observed in normal subjects. Another experiment demonstrating impaired deglutitive inhibition in DES comes from work using an artificial high-pressure zone within the distal esophagus. Patients with motor disorders characterized by rapidly propagating or simultaneous contractions exhibited only partial relaxation of the artificial high-pressure zone, proportional to the impairment of propagation velocity [75]. Taken together, these findings strongly suggest that one potential neuropathological process in DES is a selective, intermittent dysfunction of myenteric plexus inhibitory interneurons.

The above discussion suggests that the physiological abnormalities of patients with spastic disorders are heterogeneous, but all are characterized by an imbalance between the excitatory and inhibitory influences on the esophageal smooth muscle. The suggestion of an impairment of the pathway of deglutitive inhibition is particularly interesting in that it places DES in a pathophysiological continuum with achalasia, consistent with documented case reports of patients undergoing this evolution [86]. Furthermore there are marked similarities between vigorous achalasia and DES, both being characterized by simultaneous contractions of the esophageal body; the only differences are involvement of the LES and constancy of the disorder in vigorous achalasia. Similar to achalasia, the simultaneous contractions typifying diffuse esophageal spasm impair bolus transit through the esophagus, potentially explaining the associated dysphagia [144].

Nutcracker esophagus

Considerable attention has been focused on the clinical significance of the disorders of peristalsis other than DES described in Table 31.5, particularly "nutcracker esophagus." Esophageal bolus transit is normal in this condition [39,137] and no associated neuromuscular pathology is recognized. As outlined earlier, dysfunction of the excitatory population of interneurons within the myenteric plexus, or abnormal excitability of the muscle itself, can also cause an aberration of peristalsis. Several lines of evidence suggest instances of excess excitation in response to hormonal or cholinergic stimulation. Representative of such an abnormality is a second group of patients in the analysis of Behar and Biancani whose main manometric abnormality was of frequent spontaneous distal esophageal contractions. These patients had a normal propagation latency as defined by the parameters of Figure 31.8 but a significantly longer and higher amplitude contraction at each locus within the smooth muscle [143]. Examining the categorization of disorders of peristalsis outlined in Table 31.5, these individuals would be labeled as having nutcracker esophagus. Patients with peristaltic disorders characterized by excess excitation demonstrate heightened sensitivity to stimulation with cholinergic agents [70,145], the cholinesterase inhibitor edrophonium [146], pentagastrin [147], and ergonovine (ergometrine) [148]. An electromyographic correlate of this excitability is found from bipolar ring electrode recordings from the distal esophagus [149]. Whereas normal individuals uniformly exhibited spiking activity before each esophageal contraction, DES patients exhibited spike-independent spontaneous esophageal contractions.

A few instances in which nutcracker esophagus patients are subsequently diagnosed with achalasia have been reported [150,151], but considering the rarity of this observation and the high prevalence of nutcracker esophagus, the association is most likely explained by chance. No correlation has been shown between the severity of nonspecific peristaltic abnormalities and the severity of symptoms [152,153]. Long-term observation has shown neither a parallel

Table 31.5 Manometric criteria for disorders of peristalsis

Peristaltic disorder	Required finding	Associated finding
Diffuse esophageal spasm	Simultaneous contractions with > 30% of water swallows	Repetitive contractions (> 2 peaks); prolonged contractions (> 6 s); high-amplitude contractions (> 180 mmHg); spontaneous contractions, incomplete LES relaxation; increased LES pressure (> 40 mmHg)
Nutcracker esophagus	Mean distal esophageal peristaltic amplitude > 180 mmHg	Repetitive contractions (> 2 peaks); prolonged contractions (> 6 s); increased resting LES pressure (> 40 mmHg)
Hypertensive LES	Increased resting LES pressure (> 40 mmHg); normal LES relaxation	
Nonspecific esophageal motor disorder	Peristaltic abnormalities insufficient to establish any other diagnosis	Frequent nontransmitted contractions (> 20% water swallows); retrograde contractions; repetitive contractions (> 2 peaks); low-amplitude contractions (< 30 mmHg); prolonged contractions (> 6 s); high-amplitude contractions (> 180 mmHg); spontaneous contractions; incomplete LES relaxation

LES, lower esophageal sphincter.

in clinical course with manometric findings nor consistency in manometric diagnosis over time [154]. A therapeutic trial with nifedipine showed that a significant reduction of wave amplitude in nutcracker esophagus patients was not accompanied by a significant reduction in chest pain [155]. On the other hand, a controlled trial of trazodone produced significant symptomatic improvement but no change in manometric findings [156]. One controlled trial demonstrated an association of symptomatic and manometric improvement with diltiazem, but the correlation for individual subjects was not reported [157]. Finally, provocation of nonspecific motility abnormalities with cholinergic stimulation has not proven useful in detecting patients who are subsequently responsive to pharmacological treatment [158]. In summary, it has been difficult to determine a direct relevance of nonspecific motility disturbances to either symptoms or function, making their detection of no generalizable clinical value.

Epidemiology

No population-based studies exist on the incidence or prevalence of esophageal motility disorders other than achalasia. Thus, the only way to estimate the incidence or prevalence of spastic disorders is to examine the data on populations at risk and reference the observed frequency of spastic disorders to the incidence of achalasia, which, as detailed earlier, is about 1/100 000 population per year. Populations at risk for motility disorders are patients with chest pain and/or dysphagia, and so it is among these patients that extensive manometric data have been collected. As detailed in Table 31.6, summarizing representative studies of at least 30 subjects, manometric abnormalities are prevalent among these groups [128,135,159–168]. Asymptomatic volunteers (usually younger than the patients) were evaluated to define normal values in five of these studies [128,160,167–169]. The manometric pattern of achalasia was not detected in any of the volunteers. Similarly, although diffuse esophageal spasm is more variably defined than achalasia [134], simultaneous contractions following 30% or more of swallows were not found in any of the volunteers [167].

The majority of the manometric findings among the patients in Table 31.6 fall under the categories other than DES described in Table 31.5. Of this group, increased wave amplitude (nutcracker esophagus) is the most commonly detected pattern, accounting for a large proportion of the patients in the "nonspecific disorders" category. Variability in the prevalence values of the nonspecific disorders in Table 31.5 is partly attributable to a lack of uniform definitions of the motility disorders listed as well as to the inhomogeneity of patient populations studied.

Clinical presentation

The major symptoms of spastic disorders are dysphagia and chest pain; regurgitation is infrequent compared with achalasia. The prevalence of chest pain is constant among the spastic disorders listed in Table 31.5, but the prevalence and severity of dysphagia is greater with DES [153]. Among the individuals with chest pain as a dominant symptom, there is a high prevalence of psychiatric diagnosis, particularly anxiety and depression [170]. Evidence also exists suggesting a lower visceral pain threshold in this group; balloon distention and esophageal compliance studies reproduce esophageal pain at low distending volumes without corresponding motor changes [171,172]. Finally symptoms compatible with the irritable bowel syndrome may be seen in more than 50% of these patients [173].

Dysphagia for both liquids and solids is reported by 30%–60% of patients with spastic disorders [153,161]. Dysphagia

Table 31.6 Manometric abnormalities in chest pain/dysphagia patients

Study population	Total (*n*) [reference]	Achalasia (%)	DES (%)	Nonspecific disorders	
				Total (%)	Nutcracker esophagus (%)
Unselected manometry patients	363 [191]	8.5	2	17	–
	681 [188]	1	3	23	11
	202 [196]	2	2	–	–
	123 [197]	–	–	–	23
	1013 [155]	6.4	5	> 50	31
	429 [189]	13	4.6	42.8	10
Mean		6.2	3.5	33	18.8
Chest-pain patients with negative cardiological evaluation	72 [197]	–	–	–	–
	112 [155]	12	10	–	12
	34 [189]	0	0	> 50	29
	910 [190]	0.05	3	25	13
	100 [194]	0	4	28	6
	100 [195]	0	2	44	21
	44 [192]	0	0	32	14
Mean		2.1	3.2	35.8	15.8
Chest-pain patients with cardiovascular disease and suspected esophageal pain	220 [194]	0	2–7	20–29	11–16
	31 [193]	0	3	42	13
Mean		0	3.8	35.1	13.3
Patients with dysphagia as the principal symptom	251 [190]	19	7	27	5
Mean		19	7	27	5

–, data not provided; DES, diffuse esophageal spasm.

is usually intermittent in occurrence, sometimes related to swallowing specific substances or liquids at extreme temperature. Dysphagia is usually not progressive and weight loss is rare. In some instances, spasm patients experience episodes of esophageal obstruction while eating that persist until relieved by emesis; these instances are probably related to simultaneous prolonged DES-type contractions. More commonly, the dysphagia reported by patients with spastic disorders does not prevent or prolong eating.

Intermittent substernal chest pain is reported by 80%–90% of patients with spastic disorders [153,161]. Esophageal chest pain is very similar to angina, usually described as crushing or squeezing in character, often radiating to the neck, jaw, arms, or midline of the back. Pain episodes may last from minutes to hours, but swallowing is usually not impaired during these episodes. Severe pain episodes may require narcotics or nitroglycerin for relief, further confusing the distinction between esophageal and cardiac pain. The mechanism producing pain is poorly understood; transient ischemia, lumenal distension, and altered visceral sensitivity have all been hypothesized [171,174,175]. However, it is more likely that the mechanism responsible for esophageal pain is variable among individuals.

Radiographic, impedance, and manometric findings

Spastic motor disorders of the esophagus have no pathognomonic endoscopic features. However, endoscopy is useful in the evaluation of patients with dysphagia or suspected esophageal pain to identify either structural lesions or reflux esophagitis. Typically, spastic disorders are defined by manometry or barium radiography, the abnormal motor events are usually intermittent, and there are no uniform radiograpic or manometric definitions. Radiographically, a "corkscrew esophagus," "rosary bead esophagus," pseudodiverticula, or curling are indicative of DES (Fig. 31.9). It must be emphasized, however, that neither tertiary contractions (nonperistaltic, simultaneous esophageal contractions) on radiography nor an occasional simultaneous contraction on manometry are pathognomonic of esophageal spasm as both may be seen in asymptomatic individuals [135].

The manometric feature that is universal among proposed classification schemes for DES is the occurrence of simultaneous contractions [134]. However, beyond that there is little agreement as to how frequently such contractions need to occur or whether they must exceed a threshold amplitude to qualify [176]. With respect to frequency, the observation

Figure 31.9 Corkscrew esophagus on barium radiography in a patient with symptomatic diffuse esophageal spasm.

that simultaneous contractions with 30% or more of swallows have not been found in normal volunteers argues in favor of accepting that threshold. As for an appropriate threshold amplitude for what constitutes a "spastic" contraction, simultaneous low-amplitude contractions occur in scleroderma, amyloidosis, pseudo-obstruction, alcoholic neuropathies, and GERD, all entities from which DES should be distinguished. Recognizing that the functional significance of these low-amplitude pressure waves is different from that of high-amplitude contractions, Spechler and Castell [177] recently proposed adding a second necessary manometric criterion for DES that would incorporate a minimum amplitude of 30 mmHg for simultaneous contractions, based on functional studies assessing bolus transit. Although this new manometric criterion should improve the specificity of manometry in detecting clinically meaningful DES, it may still be too inclusive of insignificant contractile events and it remains to be seen how much of a difference it will make.

In the unequivocal case, nonperistaltic, high-amplitude, prolonged contractions are seen during esophageal manometry and these are associated with the patient experiencing pain (Fig. 31.10). These unequivocal cases are probably related to a defect in the neuronal architecture of the myenteric plexus, placing them along the continuum of vigorous achalasia and achalasia (Table 31.2) [75]. The LES typically functions normally. One hypothesized explanation for the limited sensitivity of esophageal manometry in detecting spasm is that abnormal motor events escape detection because of sampling error. As both chest pain and spasm are sporadic events, the likelihood of documenting both in a brief bedside study seems slim. This perception fostered the development of ambulatory esophageal manometry systems capable of longer recording intervals with the hope of correlating spontaneously occurring symptoms with abnormal esophageal motility. Unfortunately, despite multiple examinations of this technology, no consensus exists among investigators regarding scoring techniques or the utility of this technology in establishing abnormal esophageal motility as the cause of "noncardiac" chest pain. One report of combined ambulatory manometry and pH studies in a highly selected group of patients acutely hospitalized for attacks of chest pain showed that up to 43% of patients had pain associated with episodes of acid reflux, but there was little additional yield from the manometric data [178]. Thus, at present, ambulatory manometry remains an investigational technique, with scoring criteria and symptom-association criteria remaining to be established. The variability in results evident in published trials can easily be explained by differences in patient populations studied and analysis schemes utilized. Furthermore, the evolving picture suggests that motor abnormalities play a less significant role than previously suggested in chest pain and that reflux disease plays a much more significant role [179]. Bancewicz and colleagues [162] found that intensive antireflux therapy was the most useful therapy for a large group of symptomatic patients without a specific motor disorder. Similarly, Achem and colleagues [180] found that antireflux therapy benefited patients with unexplained chest pain, regardless of the presence or absence of nonspecific motor abnormalities.

Differential diagnosis

Although abnormal peristalsis can potentially cause dysphagia and chest pain, significant abnormalities are rare, as suggested by the data in Table 31.6. Furthermore, unlike angina pectoris, the pain of which it can closely mimic, esophageal dysmotility is not life threatening. Features suggesting an esophageal as opposed to a cardiac etiology include:

- pain that is nonexertional and prolonged
- pain that interrupts sleep
- pain that is meal-related
- pain relieved with antacids
- the presence of additional esophageal symptoms of heartburn, dysphagia, or regurgitation.

However, each of these characteristics still exhibits overlap with cardiac pain in some instances. Thus, an esophageal

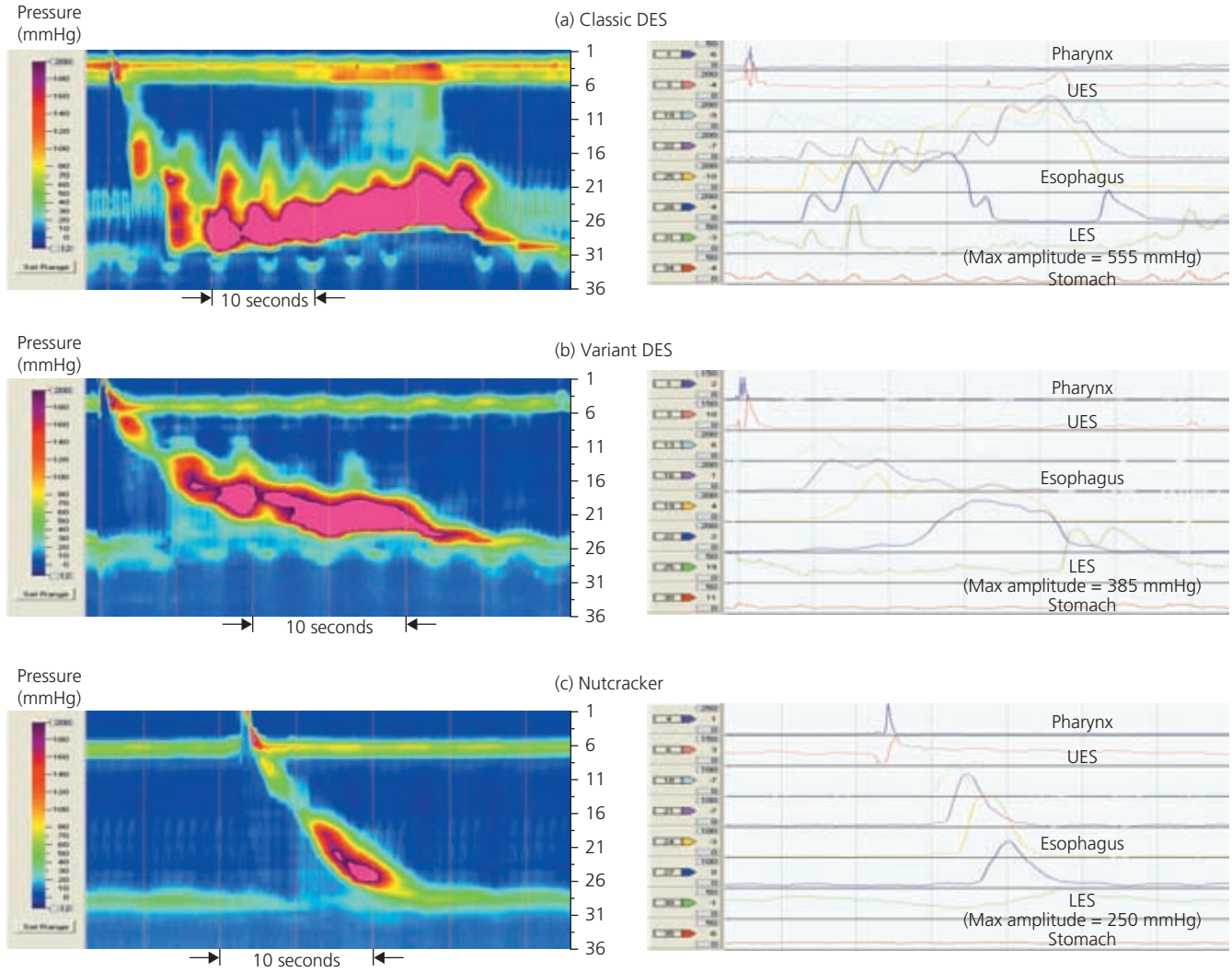

Figure 31.10 High-resolution manometric isocontour plots and conventional manometric recordings of three patients with varying spastic disorders of peristalsis. **(a)** Example of classic diffuse esophageal spasm (DES). This disorder is characterized by simultaneous contractile activity along the smooth muscle esophagus. The peristaltic amplitudes are elevated and the spastic event is prolonged. **(b)** Variant of esophageal spasm that has a normally propagating peristaltic wavefront; however, the peristaltic amplitudes are elevated and prolonged. This is different from **(c)**, representing classic "nutcracker esophagus," which has normal propagation of the peristaltic wavefront with elevated contractile amplitudes and normal contractile duration.

etiology of chest pain should be considered only after careful consideration and evaluation of potential cardiopulmonary etiologies. Furthermore, even within the spectrum of esophageal diseases, neither chest pain nor dysphagia is specific for a spastic disorder as both symptoms are also characteristic of common esophageal disorders, including peptic or infectious esophagitis. After these more common diagnostic possibilities have been excluded by appropriate radiographic evaluation, endoscopic evaluation, and, in some instances, a therapeutic trial of antisecretory medications, spastic disorders should be considered as the etiology of the still unexplained symptoms.

Treatment

Ironically, the medical therapies of esophageal spasm are similar to those of coronary artery disease, a disease with which it can be confused. However, despite the dogma of treatment with smooth muscle relaxants, there is a paucity of data on the medical treatment of esophageal spasm. Long-term outcome studies of the medical treatment of DES with smooth muscle relaxants are not available. Nitrates [181], calcium channel blockers [182], and hydralazine [183] have all been shown to be beneficial in small trials. In addition, botulinum toxin injected at the EGJ has also been used with some success in patients with nonachalasia esophageal

spasm [184]. The only double-blind placebo-controlled trial showing efficacy with medical therapy is in the case of the anxiolytic trazodone, suggesting that reassurance and control of anxiety are important therapeutic goals [156]. Consistent with this conclusion, successful management of symptoms associated with spastic motility disorders have also been reported using behavioral modification programs and biofeedback [185].

Although the rationale is unclear, esophageal dilation with standard mercury-filled dilators has also been suggested as a therapy for dysphagia or chest pain in patients with spastic disorders. However, in the only controlled trial of this therapy, dilation with an 8-mm ("placebo") dilator was as effective as an 18-mm dilator in producing transient symptom relief [186]. Alternatively, pneumatic dilation has been used in DES patients with severe dysphagia [77,187]. In one practitioner's experience, 45% of DES patients noted relief compared with 80% of achalasics [77]. In another series of nine patients with DES and LES dysfunction treated with pneumatic dilation, dysphagia but not chest pain improved during 37 months of observation [187]. However, it is not clear what distinguishes this group of patients from patients with vigorous achalasia, implying that patients likely to derive benefit from pneumatic dilation are those with mixed features of achalasia and DES as described by Vantrappen and colleagues [86].

If dysphagia becomes so severe that weight loss is observed or if pain becomes unbearable, surgical therapy consisting of a Heller myotomy across the LES with proximal extension of the incision to include the involved area of spasm should be considered [188,189]. However, there are no controlled studies of this treatment in well-defined DES patients and the indication for this procedure is extremely rare.

In summary, the therapy of esophageal spastic disorders is as poorly defined as are the spastic disorders themselves. Clearly, most esophageal pain is attributable to reflux, irrespective of the presence or absence of minor motility abnormalities, and antireflux therapy should be extensively pursued before attempting therapy of a spastic disorder. Within the range of motility disorders, achalasia stands alone as a well-defined disease. Partially overlapping achalasia is DES, a disorder characterized by a severe peristaltic defect with functional and symptomatic consequences in some cases. Interestingly, DES most likely responds to specific therapy when it exhibits characteristics indicative of vigorous achalasia, implying that it is part of a pathophysiological continuum in these cases (Fig. 31.11). Alternatively, episodic esophageal spasm may be a manifestation of GERD, a disorder for which very effective therapy exists.

With respect to other esophageal motility disorders (nutcracker esophagus, hypertensive LES, and nonspecific disorders), in extreme circumstances they may mimic DES in symptomatology and can exhibit profound esophageal hypercontractility; however, there are no significant controlled data suggesting that these patients benefit from antispasmotic therapy. In view of the unproven value of detecting these manometric aberrations, practice guidelines do not support pursuing them in the evaluation of chest pain patients. Rather, until the meaning of these aberrations of esophageal motility are clarified, we should not overlook therapy aimed at the most common esophageal disorder, GERD, or more global conditions such as depression or somatization neurosis that are coexistent in these patients (Fig. 31.11).

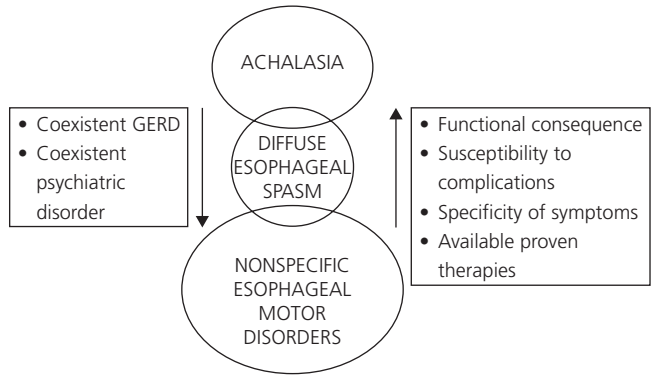

Figure 31.11 The range of esophageal motility disorders. Some overlap exists between adjacent entities as suggested by the Venn diagram. The attributes indicated on the left are increasingly relevant moving down the diagram whereas those on the right are increasingly applicable moving up the diagram. From Kahrilas PJ. Esophageal motility disorders: current concepts of pathogenesis and treatment. Can J Gastroenterol 2000;14:221, with permission from Pulsus Group Inc.

Esophageal involvement in systemic disease

Esophageal dysmotility occurs as a manifestation of several disease processes with the potential to affect smooth muscle or the autonomic nervous system. Included are collagen vascular diseases, infiltrative diseases such as amyloidosis or malignancies, metabolic disorders such as thyrotoxicosis and diabetes, and caustic injury as occurs with gastroesophageal reflux. However, esophageal involvement is well characterized only with collagen vascular diseases, diabetes, and reflux disease. Dysmotility associated with reflux disease is discussed in Chapter 32; the following discussion focuses on esophageal involvement in the collagen vascular diseases and diabetes.

Scleroderma

Scleroderma causes diffuse fibrosis and degenerative changes in the skin and synovium. The disease also has the potential to involve the heart, kidneys, lungs, intestines, and esophagus. Caucasian women between 30 and 50 years of age are the most commonly afflicted. Two forms of scleroderma are recognized: progressive systemic sclerosis with diffuse

scleroderma (the more fulminant form with early involvement of internal organs) and the CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dysfunction, sclerodactyly, telangiectasia). The basic disease process is of smooth muscle atrophy with subsequent fibrosis. With either form of scleroderma, the esophagus is involved in 75%–85% of cases [190,191]. Pathological changes are confined to the smooth muscle portion of the esophagus resulting in aperistalsis and atony of the LES [192].

The main clinical manifestations of scleroderma esophagus are dysphagia and heartburn. Symptomatic patients usually have Raynaud phenomenon, but the severity of the esophageal disease does not covary with the disease severity in other organs [193]. The prevalence of erosive esophagitis may be as high as 60%, with reported cases of Barrett metaplasia and Barrett adenocarcinoma [194,195]. Dysphagia may be attributable to poor peristalsis or to a stricture complicating the peptic esophagitis (seen in as many as one-third of patients) [195]. Radiographic findings typically consist of a slightly dilated aperistaltic esophagus and free reflux. Wide-mouth diverticula of the esophagus, similar to those described in intestines, have also been seen [191]. Manometric abnormalities consist of a hypotensive or absent LES pressure, hypotensive to absent distal esophageal peristalsis, and normal proximal esophageal peristalsis [190]. There is no specific treatment for the esophageal involvement in scleroderma. Gastroesophageal reflux should be identified and treated commensurate with its severity. Strictures may require frequent dilation and, in rare cases of intractable esophagitis, antireflux surgery may be required [196].

Other collagen vascular and connective tissue diseases

Esophageal symptoms are uncommon in systemic lupus erythematosus, although 25%–35% of unselected patients have the manometric findings of hypotensive peristalsis and a hypotensive LES [197,198]. Mixed connective tissue disease exhibits a mixture of the clinical features found in scleroderma, polymyositis, and systemic lupus erythematosus, and is characterized by high titers of a circulating antibody for a nuclear ribonucleoprotein antigen. More than 60% of patients with mixed connective tissue disease have esophageal involvement defined by cineradiography [199] and up to 82% of patients have manometric findings [197]. Abnormalities of both smooth and skeletal muscle are found. In the largest report to date [197], 5 of 17 patients had a manometric pattern consistent with scleroderma, and 10 patients had aperistalsis of the entire esophageal body along with low pressures in both the upper and lower sphincters.

Diabetes mellitus

More than 60% of diabetic patients with peripheral or autonomic neuropathy and an occasional patient without neuropathy have esophageal manometric abnormalities [200]. Reported manometric abnormalities associated with diabetes include hypotensive peristalsis, frequent failed peristalsis, hypotensive LES with impaired deglutitive relaxation, simultaneous contractions, and repetitive contractions [200,201]. The significance of these abnormalities is uncertain because most of these patients are asymptomatic [200]. Histological and pharmacological studies suggest that the esophageal abnormalities are secondary to the degenerative effects of diabetes mellitus on the autonomic nervous system rather than smooth muscle dysfunction.

Presbyesophagus

Manometric studies from the 1960s on men over the age of 80 suggested that aging was associated with failed peristalsis, decreased LES relaxation, and an increase in spontaneous contractions [202]. Symptoms correlated poorly with these findings leading to the introduction of the term "presbyesophagus" to describe incidental asymptomatic peristaltic abnormalities in the elderly. More recently, a study of patients over the age of 70, carefully screened to rule out diabetes or neurological disease, found no increased incidence of peristaltic abnormalities in the elderly compared with young control subjects [203]. The only difference found was decreased peristaltic amplitude in the elderly group suggesting that age in itself does not lead to peristaltic dysfunction. Thus, cases of "presbyesophagus" are likely related to comorbid conditions commonly encountered in the elderly.

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A ESOPHAGUS

32 Gastroesophageal reflux disease

Joel E. Richter

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Gastroesophageal reflux disease (GERD) results from the failure of the normal antireflux mechanism to protect against frequent and abnormal amounts of gastroesophageal reflux (GER), that is, the effortless movement of gastric contents from the stomach to the esophagus. GER is not itself a disease, but a normal physiological process. It occurs in virtually everyone, multiple times a day, especially after large meals, without producing either symptoms or signs of mucosal damage. In contrast, GERD is a spectrum of disease usually producing symptoms of heartburn and acid regurgitation. Most patients have no visible mucosal injury at the time of endoscopic examination (nonerosive GERD), whereas some have esophagitis, peptic strictures, Barrett esophagus, or evidence of extraesophageal diseases such as chest pain, pulmonary symptoms, or ear, nose, and throat symptoms. GERD is a multifactorial process, one of the most common human diseases, and of economic importance, contributing to the expenditure in the United States of US$4–5 billion per year for antacid medications.

Epidemiology

The prevalence of GERD differs depending on whether the analysis is based on symptoms (primarily heartburn) or signs (i.e., esophagitis) of disease. When based on symptoms, GERD is common in Western countries. For example, in a nationwide population-based study by the Gallup Organization in the United States [1], 44% of the population reported having heartburn at least once a month. More convincing data were obtained by Locke and colleagues [2], who mailed out 2200 validated self-report questionnaires to a predominantly white population residing in Olmsted County, Minnesota. The prevalence of heartburn and acid regurgitation in the previous 12 months were noted to be 42% and 45% respectively. Frequent symptoms (at least weekly) were reported by 20% of respondents, with an equal gender distribution across all ages. The majority reported that heartburn was of moderate severity and had a duration of 5 years or more, and only 5.4% reported a physician visit for reflux complaints within the previous year. More variable prevalence rates for symptomatic GERD have been reported from Europe, ranging from 5% in Switzerland to 27% in Finland [3].

Conversely, the true prevalence of esophagitis is very difficult to define because healthy persons rarely undergo endoscopic procedures. Studies suggest that 7% of persons in the United States have erosive esophagitis, whereas European studies identify prevalence rates ranging from 2% to 10% [4]. Analyses of the gender ratio of GERD show nearly equal proportions of affected men and women, but a male predominance occurs in esophagitis and Barrett esophagus [4]. Increasing age is an important factor in the prevalence of GERD complications, probably the result of cumulative acid injury to the esophagus over time [5].

In contrast, the prevalence of GERD and its complications is relatively low among residents of Africa and Asia. For example, a cross-sectional study in Singapore reported prevalence rates for reflux symptoms of 7.5% in Indian people, 0.8% in Chinese people, and 3.0% in Malay people [6]. Possible reasons for the lower prevalence of GERD include low dietary fat, lower body mass index, and lower maximal acid output related to infection with *Helicobacter pylori* [7,8].

The prevalence of GERD is increasing in Western countries. El-Serag and Sonnenberg [9] observed opposing time trends in the prevalence of peptic ulcer disease and GERD in the United States: rates of peptic ulcer disease and gastric cancer fell between 1970 and 1995, whereas the prevalence of GERD and esophageal adenocarcinoma rose significantly.

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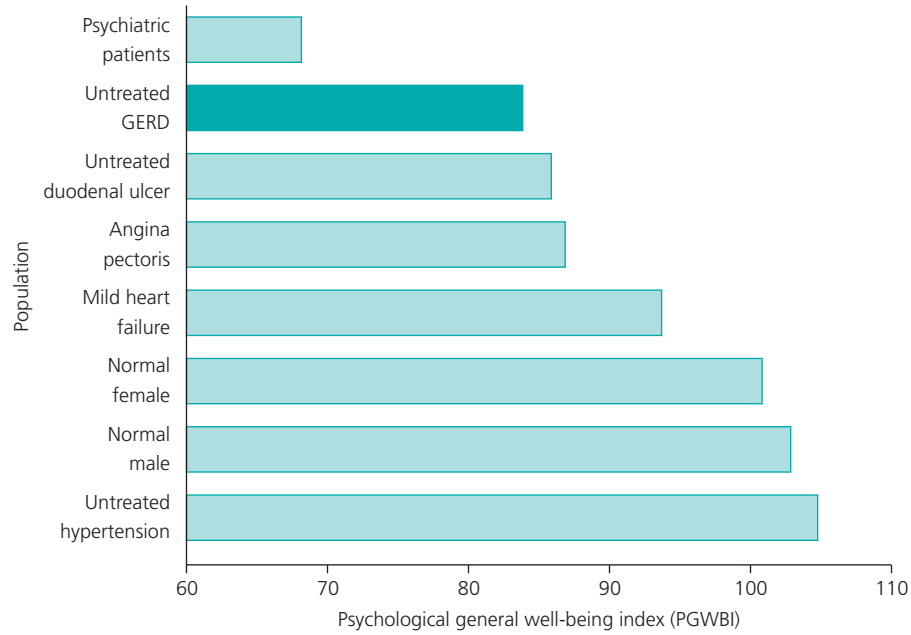


Figure 32.1 Gastroesophageal reflux disease (GERD) and quality of life. GERD has a greater negative impact on the sense of well-being than many common chronic diseases including hypertension and angina. The only illness that has a greater effect than GERD on quality of life is psychiatric disease. Adapted from Enck et al. [11].

The authors speculated that the decreasing prevalence of *H. pylori* may be playing a contributory role in the increasing prevalence of GERD. Data suggest that *H. pylori*-induced gastritis involves both the antrum and corpus, affecting the parietal cells and thus reducing acid secretion and elevating gastric pH [8]. This may have a protective influence on the esophageal mucosa in patients susceptible to GERD. Long-term epidemiological studies will be required to address this question more appropriately.

Other noteworthy epidemiological observations about GERD are that it is more common in white populations than in African American or Native American populations [4], family clustering has been reported [10], and it is rarely the cause of death [4]. GERD, however, is associated with considerable morbidity and with complications such as esophageal ulcerations (5%), peptic stricture (4%–20%), and Barrett esophagus (8%–20%). Furthermore, GERD as a chronic disease significantly impairs quality of life. Compared with other chronic medical conditions, the impairment of quality of life resulting from GERD is similar to, or even greater than, that resulting from arthritis, myocardial infarction, heart failure, or hypertension (Fig. 32.1) [11].

Pathophysiology

The pathophysiology of GERD is complex and results from an imbalance between defensive factors protecting the esophagus (antireflux barriers, esophageal acid clearance, tissue resistance) and aggressive factors from the stomach contents (gastric acidity and volume and duodenal contents). The intermittent nature of symptoms and esophagitis in many patients suggests that the aggressive and defensive forces are part of a delicately balanced system.

Antireflux barriers

The first tier of the three-tiered esophageal defense against acid damage consists of the *antireflux barriers.* This is an anatomically complex region that includes the intrinsic lower esophageal sphincter (LES), the diaphragmatic crura, the intraabdominal location of the LES, the phrenoesophageal ligaments, and the acute angle of His.

The LES is a tonically contracted segment of distal esophagus about 3.0–4.0 cm in length [12]. It is the major component of the antireflux barrier and is capable of preventing reflux even when it is completely displaced from the diaphragmatic crura because of a hiatus hernia [12]. The proximal margin of the LES is normally about 1.5–2.0 cm above the squamocolumnar junction, whereas the distal segment, about 2.0 cm in length, lies within the abdominal cavity [13]. This location of the distal LES contributes to the maintenance of gastroesophageal competence during intraabdominal pressure events. Resting LES pressure ranges between 10 and 30 mmHg and includes a generous reserve capacity, because a minimal basal LES pressure in the range of 5–10 mmHg usually prevents GER [14]. The LES maintains a high-pressure zone by the intrinsic tone of its muscle and by the action of cholinergic excitatory neurons [15]. There is considerable diurnal variation in basal LES pressure; it is lowest after meals and highest at night. It is also influenced by certain circulating peptides and hormones, foods (particularly fat), and numerous drugs, the most clinically relevant of which include theophylline, calcium channel blockers, estrogen/progesterone, and narcotics. During

swallowing, LES relaxation (LESR) occurs for 5–10 s, thus permitting esophageal peristalsis to sweep the swallowed bolus into the stomach.

Anatomically, the LES lies within the hiatus created by the right crus of the diaphragm, and it is anchored by the phrenoesophageal ligaments, which insert at about the level of the squamocolumnar junction [16]. Developmentally, the crural diaphragm arises from the dorsal mesentery of the esophagus and is innervated separately from the costal part of the diaphragm. It is inhibited by esophageal distention, during vomiting, and in association with transient LESRs, but not during swallowing. The crural diaphragm provides extrinsic squeeze to the intrinsic LES, contributing to resting pressure during inspiration and also augmenting LES pressure during periods of increased abdominal pressure such as coughing, sneezing, or bending [17]. Crural contractions impose rhythmic pressure increases of about 5–10 mmHg on the LES pressure recording [18]. During deep inspirations and some periods of increased abdominal straining, these changes may reach 50–150 mmHg [19].

The oblique entrance of the esophagus into the stomach creates a sharp angle on the greater curve aspect of the gastroesophageal junction, the angle of His. This angle has been shown in cadavers to create a flap valve effect that contributes to gastroesophageal junction competency [20].

Mechanisms of reflux

Transient lower esophageal sphincter relaxations

This is the most common mechanism underlying GER and also accounts for the reflux of gases during belching. Figure 32.2 illustrates an example of a transient LESR and highlights differences from swallow-induced LESRs: transient LESRs are not associated with antecedent pharyngeal contractions, are unaccompanied by esophageal peristalsis, persist for longer periods than swallow-induced LESRs (> 10 s), and are always accompanied by inhibition of the crural diaphragm [21]. Transient LESRs account for nearly all reflux episodes in healthy persons and for 50%–80% of episodes in patients with GERD, depending on the severity of associated esophagitis (Fig. 32.3) [22]. However, one study suggested that low basal LES pressure, rather than transient LESRs, may be the primary mechanism of GER in patients with nonreducible hernias [23]. Transient LESRs are not always associated with GER. In healthy persons, about 40%–60% of transient LESRs are accompanied by reflux episodes, compared with 60%–70% in patients with GERD [15,22,24,25]. The rate of transient LESRs is increased by gastric distention, whether by gas or a meal, by stress, and, to a lesser extent, by subthreshold (for swallowing) stimulation of the pharynx [26]. Under normal circumstances, meals are the major stimuli for transient LESRs, but the importance of specific foods is unknown [27]. Transient LESRs are inhibited by the supine position, sleep, general anesthesia, and vagal cooling [26]. Various drugs also impair transient

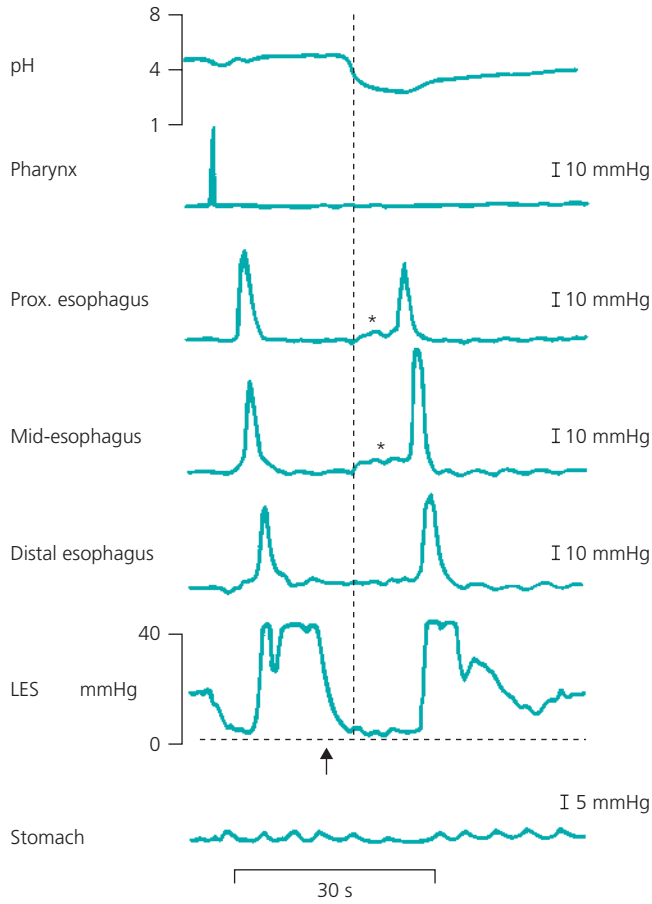


Figure 32.2 Transient lower esophageal sphincter (LES) relaxation (arrow) occurring immediately after the completion of a swallow-induced LES relaxation. Note the substantially longer duration of the transient LES relaxation (TLESR) compared with the preceding swallow-induced LES relaxation. A reflux episode (dotted line) associated with a common cavity (asterisk) occurs after complete LES relaxation has been achieved and is cleared by secondary peristalsis. From Mittal et al. [26], with permission from the American Gastroenterological Association.

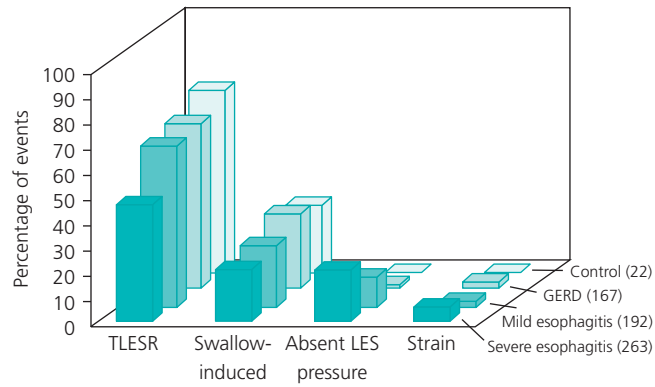


Figure 32.3 Proportion of reflux episodes in the spectrum of gastroesophageal reflux disease (GERD) occurring by the mechanisms of transient lower esophageal sphincter relaxation (TLESR), swallow-induced LES relaxation, absent basal LES pressure, and straining in the presence of low LES pressure. Adapted from Dent et al. [22].

LESRs including cholecystokinin A antagonists, anticholinergic drugs, morphine, somatostatin, nitric oxide inhibitors, 5-hydroxytryptamine 3 (5-HT_3) antagonists, and δ-aminobutyric acid B (GABA-B) agonists [28]. Current evidence indicates that transient LESRs are mediated through vagal pathways [26]. Gastric distention activates mechanoreceptors in the proximal stomach adjacent to the gastric cardia, which send signals to the brainstem center through vagal afferent pathways. The structured sequence of motor events including LESR, inhibition of the crural diaphragm, and contractions of the esophageal body suggest that this process occurs in a programmed manner, probably controlled by a pattern generator within the vagal nuclei. The motor arm is in the vagus nerve and shares common elements with swallow-induced LESRs [29].

Swallow-induced lower esophageal sphincter relaxations

About 5%–10% of reflux episodes occur during swallow-induced LESRs. Most of these episodes are associated with defective or incomplete peristalsis [22,30]. During a normal swallow-induced LESR associated with normal peristalsis, reflux is uncommon because of the absence of concomitant crural diaphragm relaxation, the relatively short duration of LES relaxation (5–10 s), and the prevention of reflux by the oncoming peristaltic wave (see Fig. 32.2). Reflux during a swallow-induced LESR is more common in the presence of a hiatus hernia because of pooling of gastric liquids in the hernia sac and the absence of any residual diaphragmatic support during the LESR [31].

Hypotensive lower esophageal sphincter pressure

Stress reflux and free reflux are two mechanisms by which GER can be associated with diminished LES pressure [14,22]. *Stress reflux* results when a relatively hypotensive LES is overcome and is "blown open" by an abrupt increase in intraabdominal pressure from coughing, straining, or bending. Stress reflux is unlikely when the LES pressure is greater than 10 mmHg. *Free reflux* is characterized by a fall in intraesophageal pH without an identifiable change in intragastric pressure, and it usually occurs when the LES pressure is 0–4 mmHg. Reflux as the result of low or absent LES pressure is uncommon. It is found mostly in patients with severe esophagitis, in whom it may account for up to 23% of reflux episodes (see Fig. 32.3), and rarely in patients without endoscopic evidence of esophagitis [14,22,24,25]. The mechanisms resulting in a low LES pressure are poorly understood. The presence of a hiatus hernia reduces LES pressure because the intrinsic support of the crural diaphragm is lost [12]. Some LES weakness may be secondary to impairment of the excitatory cholinergic pathways to the LES as a result of esophagitis. Induction of experimental esophagitis in cats affects the release of acetylcholine and lowers LES pressure, changes that are reversible on healing of the esophagitis [32]. However, healing of esophagitis in humans is rarely accompanied by an increase in LES pressure [33].

Hiatus hernia

The relationship between *hiatus hernia* and GERD remains controversial. Mainstream opinion has shifted widely from one that virtually equated hiatus hernia with GERD to one that denied it a causal role. Currently, both epidemiological and physiological data confirm the importance of the hiatus hernia in patients with more severe esophagitis, peptic stricture, or Barrett esophagus. Hiatus hernias, identified endoscopically or radiologically, are reported in 54%–94% of patients with reflux esophagitis, a rate that is strikingly higher than that in the healthy population [34].

The functional impact of the hiatus hernia has been clarified by elegant combined manometry and videofluoroscopic studies, which show that hiatus hernia impairs LES function through several mechanisms as well as impairing esophageal clearance (Fig. 32.4) [31,35,36]. Reflux is worse in patients who have a nonreducible as opposed to a reducible hiatus hernia. Nonreducing hernias are those in which the gastric rugal folds remain above the diaphragm between swallows [36]. Furthermore, statistical modeling has revealed a significant interaction between hiatus hernia and LES pressure, such that the likelihood of GER is increased as basal LES pressure decreases, an effect that is substantially amplified by the presence of a hiatus hernia and as hernia size increases [12].

Displacement of the LES from the crural diaphragm into the chest reduces basal LES pressure and shortens the length of the high-pressure zone primarily because of the loss of the intraabdominal LES segment [35]. Both of these effects are caused by the loss of the extrinsic support of the diaphragmatic crura resulting in increased GER [23]. Hiatus hernia virtually eliminates the increase in LES pressure that occurs during straining and may increase the triggering of transient LESRs during gastric insufflation with gas [35,37]. Large, nonreducible hernias also impair esophageal acid clearance because of an increased tendency for reflux to occur from the hernia sac during swallow-induced LESRs [31,36].

The origin of hiatus hernia remains unclear. Familial clustering of GERD [10] suggests the possibility of inherited muscle weakness in this area. Animal studies propose that reflux itself causes esophageal shortening, promoting the development of a hiatus hernia [38]. Other studies find an association with obesity [39] and lifting of heavy weights [40], raising the possibility that, over time, chronic intraabdominal stressors may weaken the esophageal hiatus and may cause the development of a hiatus hernia. This theory is attractive because it helps to reconcile the increased prevalence of hiatus hernias as the population ages [34].

Esophageal acid clearance

The second tier of the esophageal defense against reflux damage is *esophageal acid clearance.* Reflux events determine

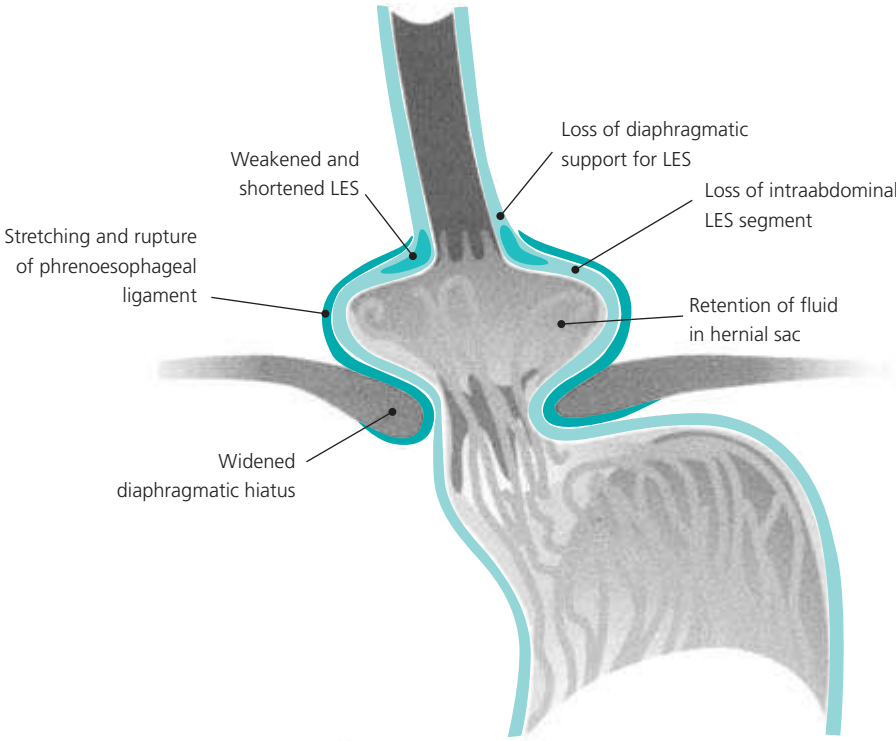


Figure 32.4 The impact of a hiatus hernia on the antireflux barrier. LES, lower esophageal sphincter. From Holloway [28], with permission from Elsevier.

the frequency with which and extent to which gastric contents enter the esophagus, but esophageal acid clearance time determines the duration that the mucosa is exposed to acid and probably the severity of acid damage. Esophageal acid clearance involves two related but separate processes: *volume clearance,* which is the actual removal of the reflux material from the esophagus, and *acid clearance,* which is the restoration of normal pH in the esophagus after acid exposure through titration with base, rather than true removal of the refluxed material.

Volume clearance

Esophageal peristalsis operates to clear the acid volume in both the upright and supine positions, but it is inoperative during deep rapid eye movement sleep [41]. Helm and colleagues [42] showed that one or two primary peristaltic contractions will completely clear a 15-mL fluid bolus from the esophagus. Primary peristalsis is elicited by swallowing, which occurs at a frequency of once per minute in awake subjects, regardless of whether reflux occurs. Secondary peristalsis, initiated by esophageal distention from acid reflux, is much less effective in promoting clearance of refluxate, thus offering only an ancillary protective role.

Peristaltic dysfunction, that is, failed peristaltic contractions and hypotensive (< 30 mmHg) peristaltic contractions that incompletely empty the esophagus, increases in frequency with the severity of esophagitis. Kahrilas and colleagues [43] found that the prevalence of peristaltic dysfunction rose from 25% in patients with mild esophagitis to more than 50% in patients with severe esophagitis (Fig. 32.5). Failed peristalsis results in very poor volume clearance because the foci of feeble contractions clear most, but not all, of the refluxate from the esophagus. Whether esophagitis leads to peristaltic dysfunction or whether an underlying motility disorder predisposes to the development of reflux disease is not clear. Animal studies find that esophageal dysmotility associated with active esophagitis is reversible, but that associated with stricture or extensive fibrosis is not [32]. Clinical observations suggest that impaired motor function does not revert to normal after either effective medical or effective surgical therapy [33].

Gravity contributes to bolus clearance when reflux occurs in the upright position. At night, when patients are supine, this mechanism is not operative unless the head of the bed is elevated. This time-honored treatment of GERD markedly improves acid clearance time and is most beneficial in those patients with aperistalsis (i.e., scleroderma) [44].

Salivary and esophageal gland secretions

Saliva is the second essential factor required for normal esophageal clearance of acid. Saliva has a pH of 6.4–7.8 and therefore is a weak base compared with the acidic gastric contents [45]. The high rate of spontaneous swallowing results in saliva production at a rate of approximately 0.5 mL/min. Although saliva is ineffective in neutralizing large volumes of acid (5–10 mL), it can neutralize small residual amounts of acid remaining in the esophagus after the volume of refluxed material has been cleared by several

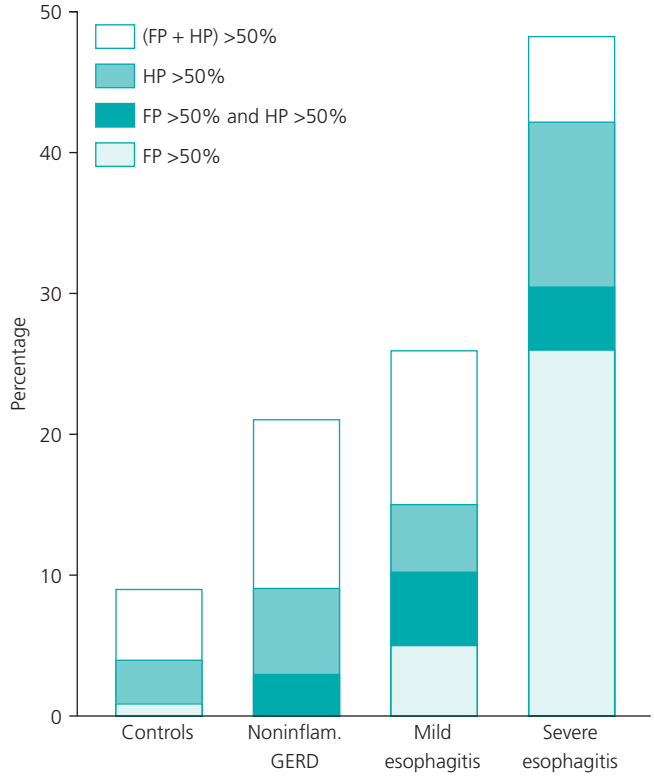


Figure 32.5 The prevalence of peristaltic dysfunction in control subjects and in patients with increasing severity of gastroesophageal reflux disease (GERD). Motility abnormality was defined as the occurrence of either failed, nontransmitted primary peristalsis (FP) or hypotensive peristalsis (< 30 mmHg) in the distal esophagus (HP) with more than half of the swallows. Note the increased prevalence of peristaltic dysfunction as the severity of GERD increases. From Kahrilas et al. [43], with permission from the American Gastroenterological Association.

peristaltic contractions [42]. The importance of swallowed saliva is supported by findings that increased salivation induced by oral lozenges or bethanechol is associated with a significant decrease in acid clearance time. In contrast, suction aspiration of saliva is accompanied by a marked prolongation of esophageal clearance, despite the presence of normal peristaltic contractions [45].

Physiological or pathological compromise of salivation may contribute to GERD. Diminished salivation during sleep explains why nocturnal reflux episodes are associated with markedly prolonged acid clearance times [41]. Similarly, chronic xerostomia is associated with prolonged esophageal acid exposure and esophagitis [46]. Cigarette smoking may promote GER. This was originally attributed to the effects of nicotine on lowering LES pressure, but more recent studies suggest that cigarette smokers have hyposalivation, which may also prolong esophageal acid clearance [47]. Finally, the esophagosalivary reflex may be impaired in patients with reflux esophagitis. This is a vasovagal reflex demonstrated by perfusing acid into the esophagus, thereby stimulating increased salivation [48]. This reflex may explain the symptoms of water brash (copious salivation) observed in some patients with reflux disease. The esophagosalivary reflex is very active in healthy persons, with a doubling or tripling of the salivary flow rate on exposure to acid. However, this reflex is diminished in patients with esophagitis and in those with strictures [48].

In addition to the role of saliva, dilution and neutralization of residual acid are achieved by the aqueous bicarbonate (HCO_3^-)-rich secretions of the esophageal submucosal glands. These glands have been identified in the opossum as well as in the human esophagus [49]. Reflux of acid into the esophageal lumen stimulates these glands and helps to neutralize the acid, even if swallowing does not occur [50].

Tissue resistance

Although clearance mechanisms minimize acid contact time with the epithelium, even healthy persons may have their esophagus exposed to acid for 1–2 h during the day and sometimes at night. Nevertheless, only a few persons experience symptomatic GER, and even fewer suffer GERD. This is the result of the third tier of esophageal defense, known as *tissue resistance*. Tissue resistance is not a single factor but a group of dynamic mucosal structures and functions that interact to minimize mucosal damage from the noxious gastric refluxate [51]. Conceptually, tissue resistance can be subdivided into preepithelial, epithelial, and postepithelial factors.

The preepithelial defense in the esophagus, in contrast to that in the stomach and duodenum, is poorly developed. There is neither a well-defined mucous layer nor a buffering capacity of the surface cells to secrete HCO_3^- into the unstirred water layer. This results in a lumen-to-surface pH gradient in the esophagus of 1:10, in contrast to the stomach and duodenum where the gradient can range from 1:1000 to 1:10 000 [52].

The epithelial defenses in the esophagus consist of both structural and functional components. Structural components include the cell membranes and intercellular junctional complexes of the esophageal mucosa. This structure is a 25- to 30-cell-thick, nonkeratinized squamous epithelium functionally divided into a proliferating basal cell layer (stratum basalis), a midzone layer of metabolically active squamous cells (stratum spinosum), and a 5- to 10-cell-thick layer of dead cells (stratum corneum). The esophageal mucosa is a relatively "tight" epithelium with resistance to ionic movement at the intercellular as well as the cellular level as a result of both tight junctions and the matrix of lipid-rich glucoconjugates in the intercellular space [53].

The functional components of epithelial tissue resistance include the ability of the esophageal epithelium to buffer and extrude H^+ ions. Intracellular buffering is accomplished by negatively charged phosphates and proteins, as well as HCO_3^-. When the buffering capacity of the cell is exceeded

and intracellular pH falls, the cell has the capacity to actively remove H^+ ions. This occurs possibly by the action of two transmembrane proteins, a Na^+/H^+ exchanger and a Na^+-dependent Cl^-/HCO_3^- exchanger [54,55]. After reflux-induced cell acidification, these transporters restore the intracellular pH to neutrality by exchanging H^+ for extracellular Na^+ or by exchanging Cl^- for extracellular HCO_3^- respectively. Additionally, esophageal cells contain a transmembrane Na^+-independent Cl^-/HCO_3^- exchanger that extrudes HCO_3^- from the cytoplasm when the intracellular pH is too high [54]. When the epithelial cells are no longer able to maintain intracellular pH, they lose their ability to regulate volume, edema occurs, and balloon cells develop.

The postepithelial defense is provided by the esophageal blood supply. Blood flow delivers oxygen, nutrients, and HCO_3^- and removes H^+ and carbon dioxide, thereby maintaining a normal tissue acid–base balance. Blood flow to the esophageal mucosa increases in response to the stress of lumenal acid [56]. Cellular injury also stimulates cell proliferation [57], which results in thickening of the basal cell layer of the epithelium. Unlike the stomach, in which superficial mucosal injury is repaired in hours, the esophagus repairs itself more slowly over days to weeks.

Gastric factors

Gastric factors (particularly gastric volume and certain aggressive factors found in the refluxate) are potentially important in the production of reflux esophagitis. Gastric volume is determined by the basal acid secretion rate, concomitant *H. pylori* infection, duodenogastric reflux, and the rate of gastric emptying. Increased gastric volume not only results in more gastric contents being available for reflux but also increases the rate of transient LESRs.

Gastric acid secretion

The primary importance of gastric acid in the production of reflux esophagitis is indisputable, but its mechanism of action may involve activation of pepsin rather than direct damage from acid alone. In animal studies, acid causes minimal injury at a pH of less than 3.0, primarily by protein denaturation. However, the combination of acid and even small amounts of pepsin disrupts the mucosal barrier resulting in increased H^+ ion permeability, histological changes, and gross hemorrhage [58]. Complementing the animal studies, a series of clinical reports showed that patients with various grades of esophagitis, including Barrett esophagus, have an increased frequency and duration of esophageal exposure to gastric refluxate of pH lower than 4.0 [59,60]. Conversely, perfusing the esophagus of animals with a pepsin solution of pH 7.5 produces minimal mucosal disruption or changes in permeability [58]. These observations are the cornerstone of acid suppressive therapy in the treatment of GERD.

Some studies have suggested that patients with reflux, especially those responding poorly to conventional antisecretory therapy, may have higher rates of acid secretion than control subjects [61]. However, most evidence finds no abnormality of gastric acid secretion in patients with GERD. For example, Hirschowitz [62] compared gastric secretion in 115 patients with esophagitis with that in 508 age-, gender-, and disease-matched control patients without esophagitis. Fasting basal or maximal secretion of either acid or pepsin was the same in both groups, and the severity of esophagitis was not related to any of these factors.

Factors that reduce gastric acid secretion naturally, for example concomitant infection with *H. pylori*, especially if it is the $cagA^+$ virulent strain, may protect from the development of severe esophagitis and Barrett esophagus [63,64]. *H. pylori*, particularly this virulent strain, is a biological *antisecretory agent* that lowers gastric acidity. It produces severe corpus gastritis and accelerates the progression to multifocal atrophic gastritis and intestinal metaplasia, with concomitant lower acid output (Fig. 32.6). In addition, the bacteria produce ammonia, which acts as a powerful neutralizing agent under elevated pH conditions [8,65]. The corpus mucosa returns to normal when the *H. pylori* infection is cured, increasing acid secretion and possibly contributing to the reports of esophagitis after successful treatment of *H. pylori* infection [66,67]. The consequences of long-term normalization of parietal cell function and return to higher intragastric acidity are unknown, but they could promote the development of more severe GERD, Barrett esophagus, and adenocarcinoma in Western populations [8].

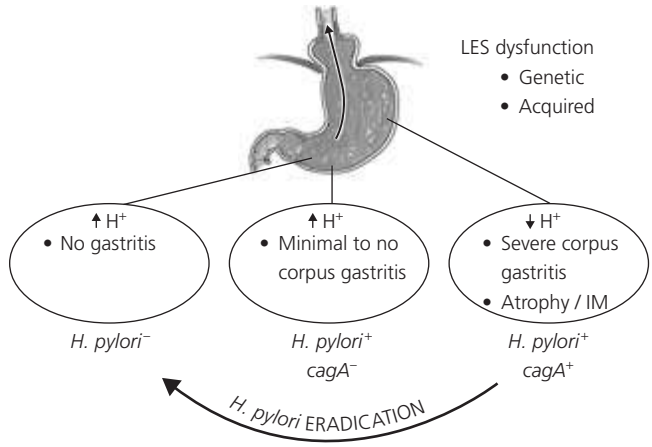


Figure 32.6 The acidity and volume of the gastric refluxate are modulated by the presence or absence of *Helicobacter pylori* and the $cagA^+$ virulence strain through the development of chronic corpus gastritis and gastric atrophy or intestinal metaplasia. Among persons susceptible to gastroesophageal reflux disease (GERD), the more acidic refluxate may increase the probability of esophagitis, Barrett esophagus, and adenocarcinoma, whereas the less acidic refluxate may protect against these complications of GERD. Conceivably, *H. pylori* eradication could shift this delicate balance and could increase the potential for the development of more severe forms of GERD. LES, lower esophageal sphincter. From Richter et al. [8], with permission from Blackwell Publishing.

Duodenogastric reflux

Bile acids have been implicated in the development of esophagitis, especially in the presence of increased *duodenogastric reflux*. Studies in animals demonstrate that conjugated bile acids produce their greatest injury in the presence of acid and pepsin, whereas trypsin, deconjugated bile salts, and the conjugated bile salt taurodeoxycholate are more damaging in the absence of acid [68]. Several surgical reports have suggested that duodenogastric reflux into the esophagus is frequent and may predispose to complications of GERD [59,69]. However, accurate measurement of duodenogastric reflux is difficult. Duodenogastric reflux may be indirectly estimated by ambulatory pH studies using an esophageal pH of less than 7.0 to indicate *alkaline reflux* [70]. However, the reliability of this indirect marker is now questioned by newer techniques, which either spectrophotometrically measure bilirubin, the most common pigment in bile [71], or measure esophageal impedance to the flow of liquids and gases independently of pH [72]. These studies show that acid reflux and bile reflux increase together across the spectrum of GERD, making it nearly impossible to incriminate one agent over the other in the development of esophagitis [73]. In addition, aggressive acid suppression with proton pump inhibitors (PPIs) decreases both acid and duodenogastric reflux, probably by decreasing the volume of gastric contents available to reflux into the esophagus [74]. Finally, the absence of membrane microvesiculation and intracellular bile deposits in human esophageal biopsies, two distinctive morphological features of experimental acid–bile salt injury, also argue against an important role for bile salts in GERD [75].

Delayed gastric emptying

The importance of *delayed gastric emptying* in the pathogenesis of GERD is controversial. Early studies observed a delay in the gastric emptying of solids in up to 50% of patients with reflux [76]. However, methodological problems with these studies including conducting them with the patients supine, scanning only in the anterior position, and the use of unstable radioisotopes may have influenced the results. More recent studies of patients in the upright position, in whom scanning was done both anteriorly and posteriorly, found only a 6%–38% rate of delayed gastric emptying, regardless of the severity of the esophagitis [77,78]. Nevertheless, delayed gastric emptying may be a major factor contributing to GERD in some groups, such as diabetic patients with autonomic peripheral neuropathy.

Associated conditions

Certain medical and surgical conditions can predispose a person to GERD. The most common is *pregnancy*: 30%–50% of pregnant women complain of heartburn, especially in the first trimester. Pregnancy increases the risk of reflux because of the relaxing effects of circulating estrogens and progesterones on LES pressure [79]. Although symptoms may be severe, esophagitis is uncommon, and this type of "situational" GERD is cured with childbirth.

Up to 90% of patients with *scleroderma* have GERD as the result of smooth muscle fibrosis causing low LES pressure and weak or absent peristalsis. Severe disease is common: up to 70% of patients have esophagitis, many have peptic strictures, and Barrett esophagus and carcinoma of the esophagus have been reported [80].

Unlike the previous two conditions, which are characterized by LES dysfunction, hypersecretion of acid and increased gastric volume are the major factors causing GERD in patients with the *Zollinger–Ellison syndrome*. In these patients, the esophagitis and complications may be more difficult to treat than the ulcer disease [81]. After Heller myotomy, 10%–20% of patients may develop GERD [82]. Finally, prolonged *nasogastric tube intubation* may contribute to the development of reflux esophagitis, in part because acid tracks orad along the tube and because the tube mechanically interferes with LES barrier function [83].

Clinical manifestations

Classical reflux symptoms

Heartburn is the classical symptom of GERD, with patients generally reporting a burning feeling, rising from the stomach or lower chest and radiating toward the neck, throat, and occasionally the back [84]. Usually it occurs postprandially, particularly after large meals or the consumption of spicy foods, citrus products, fats, chocolates, and alcohol. Recumbency and bending over may exacerbate heartburn. Nighttime heartburn interferes with restful sleeping and may impair next-day work performance. When heartburn dominates the patient's complaints, it has very high specificity (89%), but low sensitivity (38%), for GERD as diagnosed by abnormal 24-h esophageal pH testing [85]. The diagnosis of GERD is usually based on the occurrence of heartburn on 2 or more days a week, although less frequent symptoms do not preclude the disease [86]. Although this symptom is an aid to diagnosis, the frequency and severity of heartburn do not predict the degree of esophageal damage. Heartburn is caused by acid stimulation of sensory nerve endings in the deeper layers of the esophageal epithelium. These nerve endings are normally protected by a relatively impermeable epithelium but, with epithelial changes caused by reflux, they may be stimulated by H^+ ions or spicy foods [87].

Other common symptoms of GERD are acid regurgitation and dysphagia. The effortless regurgitation of acidic fluid, especially after meals and exacerbated by stooping or recumbency, is highly suggestive of GERD [85]. Among patients with daily regurgitation, the LES pressure usually is low, many have associated gastroparesis, and esophagitis is

common. For these reasons, acid regurgitation may be more difficult to control medically then classical heartburn complaints. Dysphagia is reported by more than 30% of patients with GERD [88]. It usually occurs in the setting of long-standing heartburn, with slowly progressive dysphagia primarily for solids. Weight loss is uncommon because patients have good appetites. The most common causes are a peptic stricture or Schatzki ring, but other causes include severe esophageal inflammation alone, peristaltic dysfunction, and esophageal cancer arising from Barrett esophagus.

Less common reflux-associated symptoms include water brash, odynophagia, burping, hiccups, nausea, and vomiting [89]. *Water brash* is the sudden appearance in the mouth of a slightly sour or salty fluid. It is not regurgitated fluid but rather secretions from the salivary glands in response to acid reflux [45]. *Odynophagia*, pain on swallowing, can occasionally be seen with severe ulcerative esophagitis. However, its presence should raise the suspicion of an alternative cause of esophagitis, especially infections (candidiasis, herpes) or pills (tetracycline, potassium chloride, quinine, vitamin C, alendronate).

In contrast to the previously described symptomatic presentations, some patients with GERD are asymptomatic. This is particularly true in elderly patients because of decreased acidity of the reflux material or decreased pain perception [5]. Many elderly patients present first with complications of GERD because of long-standing disease with minimal symptoms. For example, up to one-third of patients with Barrett esophagus are insensitive to acid at the time of presentation [90].

Extraesophageal manifestations

It has been suggested that GER may be the cause of a wide spectrum of conditions including noncardiac chest pain, asthma, posterior laryngitis, chronic cough, recurrent pneumonitis, and even dental erosion [91]. Some of these patients have classical reflux symptoms, but many are "silent refluxers," contributing to problems in making the diagnosis. Furthermore, it may be difficult to establish a causal relationship even if GER can be documented by testing (e.g., pH studies), because patients may simply have two common diseases without a cause-and-effect relationship.

Chest pain

GER-related *chest pain* may mimic angina pectoris. The chest pain is usually described as squeezing or burning, substernal in location, and radiating to the back, neck, jaw, or arm. It often is worse after meals, awakens the patient from sleep, and may worsen during periods of emotional stress. Heavy exercise, even treadmill testing, may provoke GER [92]. Reflux-related chest pain may last minutes to hours, often resolves spontaneously, and may be eased with antacids. Most patients with GERD-induced chest pain have heartburn symptoms [93].

Early studies suggested that spastic motility disorders were the most common esophageal cause of chest pain. However, studies using ambulatory esophageal pH and pressure monitoring suggest that about 25%–50% of patients with noncardiac chest pain have GERD [94]. Overall, these series of reports found that 41% of patients had abnormal 24-h pH test results, whereas 32% had chest pain that was clearly associated with acid reflux. Patients with coronary artery disease commonly have coexisting esophageal diseases, but the evidence that GER causes ischemic pain is controversial [95]. The mechanism for GERD-related chest pain is not clearly understood and probably is multifactorial, related to H^+ ion concentration, volume and duration of acid reflux, and secondary esophageal spasm.

Asthma and other pulmonary diseases

The association of GERD and *pulmonary diseases* was recognized by Sir William Osler [96], who recommended that asthmatic patients should "learn to take their large daily meal at noon to avoid nighttime asthma which occurred if they ate a full supper." More recent studies suggest the coexistence of the two diseases in up to 80% of asthmatic patients, irrespective of the use of bronchodilators [97,98]. GERD should be considered in asthmatic patients who present in adulthood, those without an intrinsic component, and those not responding to bronchodilators or steroids [99]. Up to 30% of patients with GERD-related asthma have no other esophageal complaints. Other pulmonary diseases associated with GERD include aspiration pneumonia, interstitial pulmonary fibrosis, chronic bronchitis, bronchiectasis, and possibly cystic fibrosis, neonatal bronchopulmonary dysplasia, and sudden infant death syndrome.

Proposed mechanisms of reflux-induced asthma are either aspiration of gastric contents into the lungs with secondary bronchospasm or activation of a vagal reflex from the esophagus to the lungs causing bronchoconstriction. Animal [100] and human [101] studies report bronchoconstriction after esophageal acidification, but the response tends to be mild and unpredictable. In contrast, intratracheal infusion of even small amounts of acid induces profound and reproducible bronchospasm in cats [100]. The reflux of acid into the trachea compared with the esophagus alone predictably caused marked changes in peak expiratory flow rates in asthmatic patients [102]. Although either mechanism may be responsible for reflux-induced asthma, most patients probably suffer from intermittent microaspiration.

Ear, nose, and throat diseases

GERD may be associated with a variety of laryngeal conditions and symptoms, of which *reflux laryngitis* is perhaps the most common [103,104]. These patients present with hoarseness, globus sensation, frequent throat clearing, recurrent sore throat, and prolonged voice warm-up. Ear, nose, and throat signs attributed to GERD include posterior laryn-

gitis with edema and redness, vocal cord ulcers and granulomas, leukoplakia, and even carcinoma. These changes usually are limited to the posterior third of the vocal cords and interarytenoid areas, both in close proximity to the upper esophageal sphincter. GERD is the third leading cause of chronic cough (after sinus problems and asthma), accounting for 20% of cases [105]. *Dental erosion,* defined as the loss of tooth structure by chemical processes not involving bacteria, can be caused by GER in healthy persons and in patients with bulimia [106].

Despite the association between ear, nose, and throat diseases and GERD, overt esophagitis usually is absent, and most patients have only mild reflux symptoms if any [103]. Microaspiration of gastric contents is the most likely cause of these complaints. Animal studies find that the combination of acid and pepsin is very injurious to the larynx [103]. Human studies report that proximal esophageal acid exposure, especially at night while sleeping, is significantly increased in patients with laryngeal symptoms and signs [107].

Table 32.1 Commonly used tests for assessing the presence, mechanism, and consequences of gastroesophageal reflux disease[a]

Tests for reflux
Intraesophageal pH/impedance monitoring
Ambulatory bilirubin monitoring
Radionuclide technetium 99m scintiscanning
Barium esophagram
Tests to assess symptoms
Empiric trial of acid suppression
Intraesophageal pH/impedance monitoring
Acid perfusion (Bernstein) test
Tests to assess esophageal damage
Endoscopy
Esophageal biopsy
Barium esophagram
Tests to assess pathogenesis
Esophageal manometry
Gastric analysis
Radionuclide technetium 99m scintiscanning

a Order of presentation represents the order of diagnostic usefulness.

Diagnostic evaluation

Many tests are available for evaluating patients with suspected GERD. These tests are often unnecessary because the classical symptoms of heartburn and acid regurgitation are sufficiently specific to identify reflux disease and to begin medical treatment. However, this may not always be the case, and the clinician must choose which test to use to arrive at a diagnosis in a reliable, timely, and cost-effective manner, depending on the information desired (Table 32.1).

Empiric trial of acid suppression

The simplest and most definitive method for diagnosing GERD and assessing its relationship to symptoms (either classical or atypical) is the empiric trial of acid suppression. Unlike other tests that only suggest an association (e.g., esophagitis at endoscopy or positive symptom index on pH testing), the response to antireflux therapy ensures a cause-and-effect relationship between GERD and symptoms. Therefore, it has become the first test used in patients with classical or atypical reflux symptoms without "alarm" complaints. The popularity of this approach was aided by the introduction of the PPIs, which, unlike the histamine type 2 receptor antagonists (H_2RAs), can drastically reduce the amount of acid reflux into the esophagus. Symptoms usually respond to a PPI trial within 7–14 days. If symptoms disappear with therapy and then return when the medication is stopped, GERD may be assumed.

In the reported empiric trials with heartburn, the initial dose of PPI was high (e.g., omeprazole 40–80 mg/day) and was given for not less than 14 days. A positive response was defined as at least 50% improvement in heartburn. Using this approach, the PPI empiric trial had a sensitivity of 68%–83% for determining the presence of GERD [108,109]. In noncardiac chest pain, Fass and colleagues [110] found that a 7-day trial of omeprazole, 40 mg in the morning and 20 mg at night, had a sensitivity of 78% and specificity of 86% for predicting GERD when compared with traditional tests. Likewise, Ours and colleagues [111] found omeprazole, 40 mg twice a day for 2 weeks, to be a very reliable method for identifying acid-related cough. Empiric trials using a 2- to 4-month regimen of PPIs taken twice a day are commonly used in patients with suspected GERD-associated asthma and GERD complaints related to the ear, nose and throat.

An empiric trial of PPIs for diagnosing GERD has many advantages. The test is office based, is easily performed, is relatively inexpensive, is available to all physicians, and avoids many needless procedures. For example, Fass and associates [110] showed a saving of more than US$570 per average patient because of a 59% reduction in the number of diagnostic tests performed for noncardiac chest pain. Disadvantages are few, including a placebo response and an uncertain symptomatic end point if symptoms do not resolve totally with extended treatment.

Endoscopy

Upper *endoscopy* is the current standard for documenting the presence of esophagitis and its extent and excluding other etiologies. However, only 40%–60% of patients with abnormal esophageal reflux by pH testing have endoscopic evidence of esophagitis. Thus, the sensitivity of endoscopy for

GERD is 60% at best, although it has excellent specificity, at 90%–95% [112].

The earliest endoscopic signs of acid reflux include edema and erythema. Neither finding is specific for GERD, and both are very dependent on the quality of the endoscopic visual images [112]. More reliable are the findings of friability, granularity, and red streaks. Friability (easy bleeding), occurring with gentle pressure on the mucosa, results from the development of enlarged capillaries near the mucosal surface in response to acid. Red streaks may extend upward from the esophagogastric junction along the ridges of the esophageal folds. In studies evaluating these stigmata, nearly all patients had GERD [113]. With progressive acid injury, erosions develop (Fig. 32.7a), characterized by shallow thinning of the mucosa associated with a white or yellow exudate surrounded by erythema. Commonly located just above the esophagogastric junction, erosions may be either single lesions or coalesced regions and occur along the tops of mucosal folds, areas most prone to acid exposure. Erosions may also be caused by nonsteroidal antiinflammatory drugs (NSAIDs), heavy smoking, and infectious esophagitis [113]. Ulcers reflect more severe esophageal damage. They penetrate the mucosa, tend to have either a white or yellow discolored base, and may be seen either isolated along a fold or surrounding the esophagogastric junction.

Multiple classification systems for esophagitis have been proposed; some are confusing and none has worldwide acceptance [114,115] (Table 32.2). In Europe, the most popular scheme is the Savary–Miller classification [114]. In the United States, the Hetzel [116] and Los Angeles [115] systems are most popular, with the latter gaining the most acceptance.

Most patients with GERD are treated initially without endoscopy. The important exception is the patient experiencing alarm symptoms: dysphagia, odynophagia, weight loss, and gastrointestinal bleeding. With such symptoms, endoscopy should be performed early to rule out other entities such as infections, ulcers, cancer, or varices. The role of endoscopy in GERD without alarm symptoms has changed dramatically in the PPI era. Previously, the degree of esophagitis helped direct management but this is less of an issue with PPIs, which effectively heal all grades of esophagitis. Currently, the most important reason for performing endoscopy in patients with GERD is to identify peptic strictures or Barrett esophagus. Using this rationale, most patients with chronic GERD need only one endoscopic examination while they are receiving therapy.

Esophageal biopsy

Similar to endoscopy, the role of esophageal biopsies in evaluating GERD has evolved over the years. Microscopic changes indicative of reflux may occur even when the mucosa appears normal endoscopically [117]. These classical findings are reactive epithelial changes characterized by an increase in the basal cell layer of greater than 15% of the epithelium thickness or papilla elongation into the upper third of the epithelium (see Fig. 32.7b), both changes representing increased epithelial turnover of the squamous mucosa. Unfortunately, these changes are also noted in up to 50% of healthy persons when biopsies are taken from the distal 2–3 cm of the esophagus [118]. Hence, the changes are sensitive markers for GERD but have poor specificity.

Acute inflammation characterized by the presence of neutrophils and eosinophils is very specific for esophagitis [118]; however, the sensitivity is low, in the range of 15%–40% [119]. Eosinophils are found more often on biopsy

(a)

(b)

Figure 32.7 Endoscopic and histological signs of esophagitis. **(a)** Two linear erosions extending proximally from the squamocolumnar junction at the proximal border of a hiatal hernia. This would be classified as grade II esophagitis by the Savary–Miller and Hetzel systems and as grade B on the Los Angeles scale. **(b)** Reparative changes secondary to reflux disease characterized by basal cell hyperplasia (dark cells crowding the papillae) and marked elongation of the rete pegs (pale papillae with finger-like projections).

Table 32.2 Endoscopic grading systems for esophagitis

	Savary–Miller classification	Hetzel classification
Grade 0	Not applicable	Normal-appearing mucosa
Grade I	Single erosive or exudative lesion on one longitudinal fold	Mucosal edema, hyperemia, or friability of mucosa
Grade II	Multiple erosions on more than one longitudinal fold	Superficial erosions involving < 10% of the mucosal surface of the last 5 cm of distal esophagus
Grade III	Circumferential erosions	Superficial erosions involving 10%–50% of distal esophagus
Grade IV	Ulcer, stricture, or short esophagus isolated or associated with grade I–III lesion	Deep ulceration or confluent erosions involving > 50% of distal esophagus
Grade V	Barrett esophagus ± grade I–III lesion	Not applicable

Los Angeles classification

Grade A	One or more mucosal breaks confined to folds, no longer than 5 mm
Grade B	One or more mucosal breaks of > 5 mm, confined to folds but not continuous between tops of mucosal folds
Grade C	Mucosal breaks continuous between tops of two or more mucosal folds but not circumferential
Grade D	Circumferential mucosal break

(19%–63% of subjects) but are less specific, present in up to 33% of healthy adults [120]. Interestingly, the sensitivity and specificity of eosinophils in children are much stronger, reflecting the lack of eosinophils in the juvenile inflammatory response [118,121].

In clinical practice, the primary indication for esophageal biopsy is to determine the presence of Barrett epithelium and possible eosinophilic esophagitis. When the former diagnosis is suspected, a biopsy is essential and should be carried out when all visual evidence of esophagitis is healed.

Esophageal pH monitoring

Ambulatory intraesophageal pH monitoring is the standard for establishing pathological reflux (see Chapter 137) [122,123]. The test is performed with a pH probe passed nasally, positioned 5 cm above the manometrically determined LES and connected to a battery-powered data logger capable of collecting pH values every 4–6 s. An event marker is activated by the subject in response to symptoms, meals, and body position changes. Patients are encouraged to eat normally and to pursue regular daily activities. Monitoring is carried out usually for 18–24 h. Reflux episodes are detected by a pH drop below 4.0. Commonly measured parameters include the percentage of total time that the pH is below 4.0, the percentage of time upright and time supine that the pH is below 4.0, the total number of reflux episodes, the duration of the longest reflux episode, and the number of episodes that last for longer than 5 min [122]. The total percentage of time that the pH is below 4.0 is the most reproducible measurement for GERD, with reported upper limits of normal ranging from 4% to 5.5% [123]. Ambulatory pH testing can discern positional variations in GER, meal- and sleep-related episodes, and help to relate symptoms to reflux events (Fig. 32.8). As the result of its reliability for measuring GER across normal activities, ambulatory pH testing has replaced other older studies such as the standard acid reflux (Tuttle) test and radionuclide scintigraphy.

One important problem with esophageal pH monitoring is that there exists no absolute threshold value that reliably identifies pathological GER. Validation studies comparing the presence of esophagitis with an abnormal pH test report sensitivities ranging from 77% to 100% with specificities of 85%–100% [123]. However, these patients rarely need pH testing; rather, patients with normal endoscopic findings and suspected reflux symptoms should benefit most from ambulatory pH monitoring. Unfortunately, the data are much less conclusive in this group, with considerable overlap between control subjects and patients with nonerosive reflux [123]. Other drawbacks of pH testing include possible equipment failure, the pH probe missing a reflux event because it is buried in a mucosal fold, and false-negative studies resulting from dietary or activity limitations because of poor tolerability of the nasal probe.

An important advantage of ambulatory esophageal pH monitoring is its ability to record and correlate symptoms with reflux episodes over extended periods. For this indication it has essentially replaced the shorter acid perfusion (Bernstein) test. Because only about 10%–20% of reflux episodes are associated with reported symptoms, different statistical analyses have evolved in an attempt to define a significant association between these two variables, including the symptom index, symptom sensitivity index, and symptom association probability [124]. However, one study found none of these statistical relationships to be superior to a high-dose trial of a PPI in establishing the causal relationship between symptoms and GER [124].

Definite clinical indications for ambulatory pH monitoring have been established [123]. Before fundoplication, pH

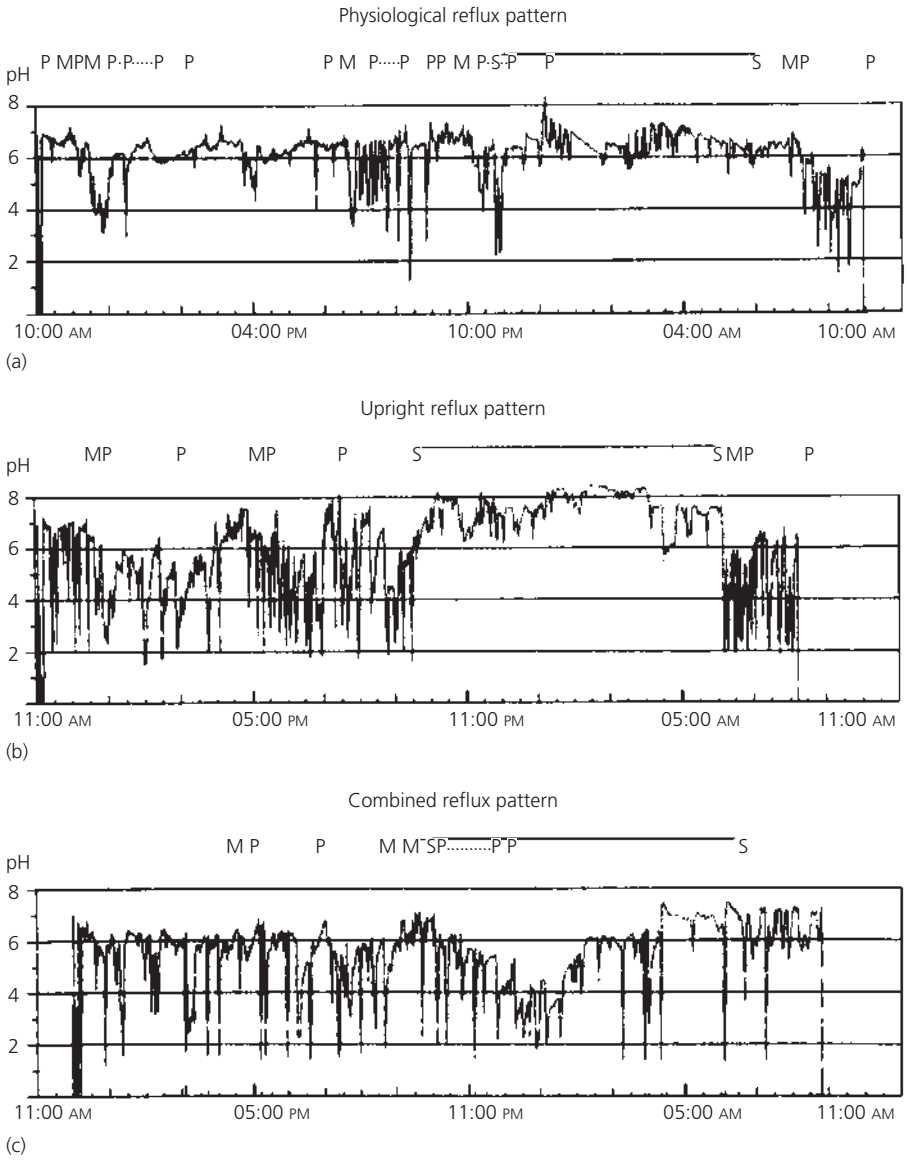


Figure 32.8 Common patterns of 24-h esophageal pH monitoring. MP, meal; P, postprandial; S, supine. **(a)** The physiological pattern of gastroesophageal reflux (GER) in healthy persons. Reflux is noted after meals (M) but not while asleep (S). A reflux episode is defined as a pH drop to less than 4.0. **(b)** Upright reflux pattern with extensive GER during the day but not at night. These patients have frequent symptoms but esophagitis is uncommon. **(c)** Combined pattern with GER during the day and at night (one episode lasted for 3 h). Most of these patients have esophagitis.

testing should be performed in patients with normal endoscopic findings to identify the presence of pathological reflux. If esophagitis is present, pH testing is not necessary because the disease has been established. After antireflux surgery, persistent or recurrent symptoms warrant repeat pH testing. In these situations, pH monitoring is performed with the patient discontinuing all antireflux medications (PPIs for 1 week, H_2RAs for 2 days). Esophageal pH testing is particularly helpful in the evaluation of patients with reflux symptoms resistant to treatment and with normal or equivocal endoscopic findings. For this indication, pH testing is usually also carried out in patients receiving therapy to define two populations: those with and those without continued abnormal esophageal acid exposure times. The group with persistent GER needs intensification of the medical regimen, whereas those patients with symptoms and adequate acid control have another cause of their complaints. Finally, ambulatory pH testing may help in defining patients with extraesophageal manifestations of GERD. In this situation, pH testing is usually carried out with additional pH probes placed in the proximal esophagus or pharynx [125]. Initially, most of these studies were carried out when patients were not taking antireflux medications, to confirm the coexistence of GERD; however, this does not guarantee symptom causality. Therefore, the current approach is to treat the patients aggressively with PPIs first and to reserve pH testing only for those patients not responding after 4–12 weeks of therapy [126].

Two advancements have made pH testing more user-friendly and capable of measuring nonacid reflux. The Bravo pH capsule is a tubeless method of acid monitoring that uses a radiotelemetry capsule attached directly to the esophageal

mucosa. This is more tolerable to the patient, allows 48 h of monitoring, and may improve test accuracy by allowing patients to more comfortably carry out their usual activities [127]. The second improvement combines impedance and pH testing, allowing the measurement of both acid and nonacid reflux. This test may be helpful in patients with regurgitation or those with persistent symptoms despite an adequate medical trial of a PPI [128].

Barium esophagram

The *barium esophagram* is an inexpensive, readily available, and noninvasive esophageal test (see Chapter 127). It is most useful in demonstrating structural narrowing of the esophagus and in assessing the presence and reducibility of a hiatal hernia. Schatzki rings, webs, or minimally narrowed peptic strictures are often seen only with an esophagram, being missed by endoscopy, which may not adequately distend the esophagus. Giving a 13-mm radiopaque pill or marshmallow along with the barium liquid can help to identify these subtle narrowings [129]. By giving the patient in the prone oblique position swallows of barium, the barium esophagram also allows good assessment of peristalsis and is helpful preoperatively in identifying a weak esophageal pump [130]. The ability of a barium esophagram to detect esophagitis varies considerably, with sensitivities of 79%–100% for moderate to severe esophagitis, with mild esophagitis usually missed [130]. Barium testing also falls short when addressing the presence of Barrett esophagus. The spontaneous reflux of barium into the proximal esophagus usually suggests reflux but is infrequent [129]. Provocative maneuvers such as leg lifting, coughing, the Valsalva maneuver, or the water-siphon test can be used to elicit stress reflux. Although these tests can improve the sensitivity of the barium esophagram, some argue that they also decrease its specificity [131,132].

Esophageal manometry

Esophageal manometry allows accurate assessment of LES pressure and relaxation, as well as peristaltic activity, including contraction amplitude, duration, and velocity (see Chapter 137). However, esophageal manometry is generally not indicated in the evaluation of patients with uncomplicated GERD because most of these patients have a normal resting LES pressure [43]. It is an integral component of pH testing to define the LES location accurately, a task poorly performed by endoscopy, fluoroscopy, or the pH pull-through technique. Esophageal manometry is traditionally recommended before antireflux surgery to document adequate esophageal peristalsis [133]. If ineffective peristalsis (low amplitude or frequent failed peristalsis) is identified [134], then a complete fundoplication may be contraindicated. However, several studies have challenged this physiological premise, finding that reflux control was better and dysphagia no more common in patients with weak peristalsis after a complete, compared with a partial, fundoplication [135]. Using impedance combined with traditional manometry, one study found that fewer than 50% of patients with manometric "ineffective" peristalsis have poor esophageal bolus clearance [136]. Thus, this new technology helps to better define a weak esophageal pump and limits the use of the less effective partial fundoplication.

Differential diagnosis

Symptoms associated with GERD may be mimicked by other esophageal and extraesophageal diseases including achalasia, Zenker diverticulum, gastroparesis, gallstones, peptic ulcer disease, functional dyspepsia, and angina pectoris. These disorders can usually be identified by their failure to respond to aggressive antisecretory therapy and by diagnostic tests such as endoscopy, barium esophagram, esophageal manometry, ultrasound, nuclear emptying studies, and various cardiac tests. Although GERD is the most common cause of esophagitis, other causes (pill injury, infections, or radiation esophagitis) need to be considered in cases that are difficult to manage and in older or immunocompromised patients.

Clinical course

The clinical course of reflux esophagitis depends to a great extent on whether the patient has *erosive* or *nonerosive GERD* on initial presentation. Furthermore, patients tend not to cross over from one group to another unless they are treated medically or surgically: in follow-up ranging from 6 months to more than 5 years, only 15% of patients with nonerosive disease evolved over time to having esophagitis or complications of GERD [137,138].

Nonerosive reflux disease

Although early studies from tertiary referral centers suggested that nearly half of patients with GERD had esophagitis [139], studies carried out in community practices reveal that up to 70% of the patients with GERD had a normal endoscopic examination [140,141]. Furthermore, another community-based study of antacid users found that 53% of patients with GERD had nonerosive disease, and two-thirds of the remaining had only minimal erosive changes at endoscopy [142]. Endoscopy-negative patients with GERD are more likely to be female, younger, thin, and without hiatal hernia. Despite their mild mucosal damage, these patients demonstrate a chronic pattern of symptoms with periods of exacerbation and remission [143]. Studies suggest that dilation of intercellular spaces is a histological marker of this disease, irrespective of esophageal acid exposure [144].

Nonerosive GERD is suspected by the presence of typical reflux symptoms with a normal endoscopic examination and is confirmed by the patient's response to antisecretory

therapy. When performed, 24-h esophageal pH monitoring identifies three distinct subsets of patients with nonerosive disease. First, there are the patients with abnormal acid exposure times who are usually responsive to antisecretory therapy. Second are the patients with normal reflux parameters but a good relationship between acid reflux episodes and symptoms. This group represents 30%–50% of patients with nonerosive GERD [145] and has "functional heartburn" [143]. These patients probably have heightened esophageal sensitivity to acid and are less likely to respond to antireflux therapy [146]. The third group is characterized by normal acid exposure times and poor symptom correlation. Despite sometimes having classical reflux symptoms, other diseases such as achalasia, gastroparesis, bile reflux, or functional dyspepsia are the cause of their symptoms. Overall, patients with nonerosive GERD do not respond to antireflux treatments as well as patients with erosive GERD, probably because these three subsets are not carefully defined before treatment [143].

Erosive reflux disease

The clinical course of patients with erosive esophagitis is more predictable and is associated with complications of GERD. Controlled studies have shown that, in the absence of ongoing maintenance therapy, up to 85% of patients with erosive GERD will have a relapse within 6 months, and the relapse rate is highest in those with the more severe grades of esophagitis [147,148]. This observation, however, should not prevent at least one attempt to withdraw medication, because 20% of patients remain in remission for up to 1 year, especially those with milder esophagitis grades. Although the natural history of untreated erosive GERD is well studied, two European studies suggest that these patients are more prone to reflux complications. In a Finnish study, 20 patients with erosive GERD treated with lifestyle changes, antacids, and prokinetic drugs were followed up for a median of 19 years. In total, 14 patients continued to have erosions, and six new cases of Barrett esophagus were detected [149]. Similarly, a large retrospective European study with 6.5 years of follow-up found a high rate of complications (21%) including 13 patients with esophageal ulcers, 15 with strictures, and 45 with Barrett epithelium [150]. However, these data must be contrasted with other studies, for example a 2-year trial in the United States [151] in which no patients with erosive esophagitis developed Barrett esophagus, and a study over a 12-year period of 3800 French patients [152] in whom the development of stricture was reported in only 0.26%.

Complications

Hemorrhage and perforation

Hemorrhage and esophageal perforation are rare complications of reflux esophagitis and are usually associated with deep esophageal ulcers or severe diffuse esophagitis. Clinically important hemorrhage has been reported in 7%–18% of patients with GERD [153]. Esophageal perforations are very rare in the PPI era, but they can result in mediastinitis and can be fatal if they are not rapidly recognized and treated.

Peptic esophageal strictures

Strictures occur in 7%–23% of patients with untreated reflux esophagitis, especially in older men [154]. They usually evolve over many years and may be linked to the long-term use of NSAIDs [155]. The mechanism of stricture formation is complex, starting as a reversible inflammatory process with edema, cellular infiltration, and vascular congestion, progressing to deposition of connective tissue and collagen, and ending in irreversible fibrosis. With the onset of dysphagia there is often less heartburn, reflecting the fact that the stricture is acting as a barrier to reflux. Dysphagia is usually limited to solids but it may progress to liquids. Unlike malignant strictures, patients with peptic strictures have a good appetite, alter their diet, and lose little weight.

Radiographically, peptic strictures are smooth-walled, tapered, circumferential narrowings in the lower esophagus; they are usually less than 1 cm long but can occasionally extend to 8 cm in length (Fig. 32.9). In these unusual cases the clinician should suspect a predisposing condition, such as the Zollinger–Ellison syndrome, superimposed pill esophagitis, or prolonged nasogastric intubation [154]. A stricture in the middle to upper esophagus should raise the suspicion of Barrett esophagus or malignant disease. Although once controversial, most data suggest that a Schatzki ring is a forme fruste of an early peptic stricture [156]. In all cases, the nature of a peptic stricture needs to be confirmed by endoscopy with biopsies because some patients may have Barrett esophagus or unsuspected cancer.

Barrett esophagus

In some patients with GERD, the squamous epithelium of the distal esophagus is replaced by specialized columnar epithelium resembling that of the intestine and containing goblet cells. Although Dr. Norman Barrett thought that this lesion was a congenitally shortened esophagus [157], studies consistently show that these patients have severe GERD with low LES pressures, poor esophageal motility, large hiatal hernias, and extensive acid and bile reflux [158]. Furthermore, most patients have had chronic reflux symptoms for at least 10 years [159]. Animal experiments show that, if the mucosal lining of the distal esophagus is excised in the setting of free acid reflux, columnar epithelium will regenerate in the area previously occupied by squamous epithelium [160]. If reflux is controlled, the mucosal lining will regenerate with squamous epithelium. Pluripotential stem cells derived from the stratified squamous epithelium are the origin of the specialized columnar epithelium [161].

Barrett esophagus was once considered an uncommon condition, but estimates of its frequency at autopsy (1 in 57 to

(a) (b)

Figure 32.9 Barium esophagram of several forms of esophageal stricture. **(a)** Double-contrast study showing distal linear erosive esophagitis, an ulcer with a short stricture, and a large hiatus hernia. **(b)** A long, irregular distal stricture in a patient with Zollinger–Ellison syndrome. The stricture occurred after prolonged nasogastric intubation following head and neck surgery.

1 in 105 cases), on general endoscopy survey (1 in 100 cases), and on endoscopic surveys of patients with GERD (10 in 100 to 15 in 100 cases) indicate that it is not uncommon and that it affects nearly 700 000 adults in the United States [157]. An autopsy series from Olmsted County, Minnesota found that most cases of Barrett esophagus go undetected during life and thus are not accessible for cancer surveillance programs [162]. Barrett esophagus is principally a disorder of white men; it is three times more frequent in men than in women and is rare in African American and Asian populations [163]. It is found predominantly in middle-aged and older adults; the mean age at diagnosis is approximately 55 years, but it has been reported in children older than 5 years of age [164]. The prevalence of Barrett esophagus increases with age, paralleling that of reflux esophagitis, but the length of the columnar-lined segment remains remarkably stable, even over years of endoscopic follow-up [163]. This finding suggests that it arises rapidly in the susceptible reflux-damaged esophagus and early in the course of disease. Families have been reported with multiple members having Barrett esophagus, some with cancer affecting more than one generation [10,165]. Although the columnar-lined esophagus in itself does not cause symptoms, most patients complain of heartburn and regurgitations. Approximately 25% of patients with Barrett esophagus discovered at endoscopy have no esophageal symptoms [90].

Barrett esophagus is suspected at endoscopy and is confirmed by biopsy and histological examination. The columnar epithelium of the stomach is reddish pink, and the junction between the glossy white squamous mucosa and the columnar mucosa (Z-line) is normally found at the lower end of the tubular esophagus, just above the proximal folds of a hiatal hernia if present. In Barrett esophagus, the distal esophagus is lined with columnar epithelium, extending upward for a variable distance, often 3–10 cm, but occasionally involving most of the esophagus (Fig. 32.10a). The

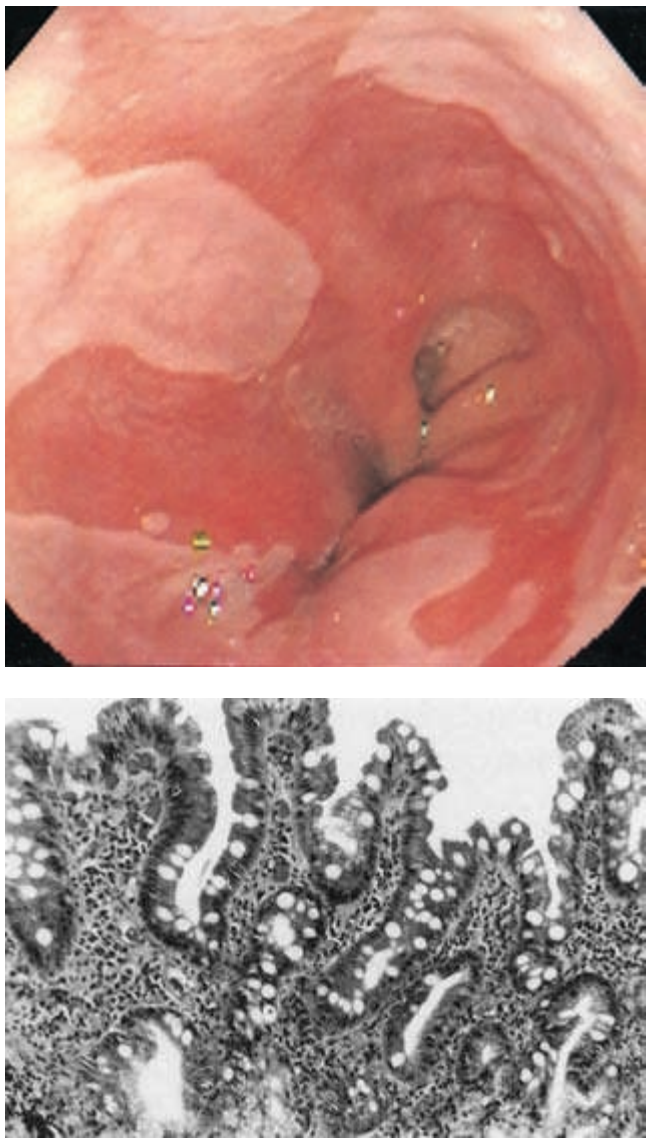


Figure 32.10 Barrett esophagus. **(a)** Reddish pink columnar mucosa with tongue-like projections 3–4 cm into the tubular esophagus. **(b)** Specialized intestinal metaplasia with glandular epithelium and characteristic goblet cells.

proximal margin may be horizontal or there may be irregular, often tongue-shaped upward extensions of columnar mucosa. Some patients have pale islands of regenerative or residual squamous epithelium, whereas others have punched-out benign ulcers in the columnar area. Strictures and esophagitis may be seen at the new squamocolumnar junction. The endoscopist should especially look for evidence of adenocarcinoma, such as nodularity or masses [166].

The characteristic histological finding in Barrett esophagus is a distinctive specialized intestinal epithelium. This is a glandular epithelium with mucin-type cells and the distinguishing presence of goblet cells (see Fig. 32.10b). These are easily seen on hematoxylin and eosin-stained sections and can be demonstrated more prominently in sections stained with Alcian blue. It occupies most or all of the columnar-lined area and is the type of epithelium in which adenocarcinoma arises [167]. Other types of epithelia seen with Barrett esophagus include gastric fundic and cardia-type epithelia, but these alone do not make the diagnosis of Barrett esophagus nor are they associated with adenocarcinoma.

There is some controversy over the classification of Barrett esophagus [157,166]. The classical or *long-segment Barrett esophagus* requires at least 3 cm of esophagus to be lined with columnar epithelium. This is the best-studied subset of Barrett esophagus, with traditional demographic features and a definite increased risk of becoming adenocarcinoma. *Short-segment Barrett esophagus* refers to shorter lengths or tongues of columnar epithelium, less than 3 cm, in the distal esophagus, with intestinal metaplasia on biopsy. This entity is three to five times more common than the long-segment variant, but, based on anecdotal reports, the risk of cancer appears to be lower [168]. *Intestinal metaplasia at the esophagogastric junction* refers to microscopic findings on biopsy but no visible columnar epithelium in the esophagus at endoscopy [157]. This finding has been reported in 10%–32% of biopsies from unselected patients, many of whom have no reflux symptoms [168]. The percentage of woman and African American people with this lesion is also higher than the percentage with either long- or short-segment Barrett esophagus. The cause is controversial; some investigators suggest that this is the earliest form of GERD [169], whereas others believe that these changes are secondary to *H. pylori* infection [170]. Cancer risk is minimal, if it exists at all.

Patients with long-segment Barrett esophagus have an estimated 30–125 times increased risk of developing esophageal cancer compared with the general population [171]. Early studies suggested that the median cancer incidence was 1 per 100 patient-years of follow-up [157], but subsequent studies with longer follow-up suggest a lower cancer incidence of 1 per 200–250 patient-years [172,173]. This represents an annual incidence of approximately 0.5%, with about 500 cases of adenocarcinoma diagnosed annually. However, since the early 1980s, the incidence of squamous cell carcinoma has stayed constant, whereas the incidence of adenocarcinoma of the esophagus and esophagogastric junction has risen fivefold – a growth rate exceeding that of any other cancer [174]. Adenocarcinoma accounts for more than half of all esophageal cancers in the United States. Despite this cancer risk, most patients with Barrett esophagus die of unrelated causes [173]. More than 95% of patients who develop cancer present with symptoms caused by the tumor itself and are unaware of their antecedent Barrett esophagus [175]. Epidemiological data suggest that the mean interval from developing Barrett esophagus to evolution of cancer may be 20–30 years [163].

Medical and surgical therapy

The rationale for GERD therapy depends on a careful definition of specific aims. In patients without esophagitis, the therapeutic goals are simply to relieve the acid-related symptoms and to prevent frequent symptomatic relapses. In patients with esophagitis, the goals are to relieve symptoms and to heal the esophagitis while attempting to prevent further relapses and the development of complications. These goals are set against a complex background: GERD is a chronic disease that may wax and wane in intensity, and relapses are common.

Nonprescription therapy

Although GERD is common in the United States, very few persons seek medical care for their complaints, instead choosing to change their lifestyles and self-medicate with over-the-counter (OTC) antacids and low doses of H_2RAs. These observations have led to the "iceberg" model of the GERD population. Most heartburn sufferers are invisible because they self-medicate and do not seek professional help; only those at the tip of the iceberg, typically patients with severe symptoms or reflux complications, are seen by physicians [176].

Lifestyle modifications

Sensible *changes in lifestyle*, especially if their rationale is explained to the patient, should be part of the initial management of all subjects. These include head of the bed elevation, avoidance of tight-fitting clothes, weight loss, restriction of alcohol consumption, elimination of smoking, dietary therapy, refraining from lying down after meals, and avoidance of evening snacks before bedtime. Physiological studies show that these maneuvers enhance esophageal acid clearance, minimize acid reflux-related events, or ease heartburn symptoms, but their therapeutic efficacy in controlled trials usually has not been evaluated [176,177]. The head of the bed can be elevated either by putting 6- to 8-inch blocks under the legs of the bed or by using a Styrofoam wedge under the mattress to elevate the upper torso. Eating several hours before retiring and avoiding evening snacks keep the stomach empty at bedtime, thereby decreasing the number of nocturnal reflux episodes. These three lifestyle changes are recommended for patients with nocturnal GERD symptoms or laryngeal complaints. One study found that head of the bed elevation was nearly as effective as ranitidine therapy in healing esophagitis [178]. Avoidance of tight-fitting clothes and weight loss are interventions aimed at reducing the incidence of reflux by the abdominal stress mechanism. Although obesity is now well established as a risk factor for GERD, esophagitis and esophageal adenocarcinoma, the efficacy of weight reduction is controversial [179]. Targeted weight loss may be helpful when discrete periods of weight gain can be associated with exacerbation of reflux symptoms. Cessation of smoking and elimination of alcohol are valuable because both agents lower LES pressure, reduce acid clearance, and impair intrinsic squamous epithelial protective functions [47,180,181]. Dietary changes include reducing the sizes of meals and the intake of fats, carminatives, and chocolate, to reduce the frequency of reflux by decreasing gastric distention and by reducing the episodes of transient LESRs, and avoiding foods that lower basal LES pressure [176,179]. Additionally, some patients complain of heartburn after consuming citrus drinks, spicy foods, tomato-based products, coffee, tea, or cola drinks. Stimulation of gastric acid secretion or esophageal sensitivity to low pH or hyperosmolar liquid solutions may account for these symptoms [145]. However, the indiscriminate prohibition of food products should be avoided; rather, to promote dietary compliance, prohibition should be tailored to those foods that bring on individual symptoms. Finally, if possible, patients should avoid drugs that lower LES pressure or which can promote localized esophagitis.

Over-the-counter medications

Over-the-counter antacids, Gaviscon, and H_2RAs are useful in treating mild and infrequent heartburn symptoms, especially when symptoms are brought on by lifestyle indiscretions. Antacids increase LES pressure but work primarily by buffering gastric acid in the esophagus and stomach, albeit for relatively short periods. Heartburn symptoms are rapidly relieved, but patients need to take antacids frequently, usually 1–3 h after meals and at bedtime, depending on symptom severity. Gaviscon, containing alginic acid and antacids, mixes with saliva to form a highly viscous solution that floats on the surface of the gastric pool and acts as a mechanical barrier. Both antacids [182] and Gaviscon [183] are more effective than placebo in relieving symptoms induced by a heartburn-promoting meal. However, these agents do not heal esophagitis, and long-term trials suggest effective symptom relief in only 20% of patients using antacids [184]. H_2RAs are available in an OTC form at doses that are usually one-half of the standard prescription dose. Although their onset of relief is not as rapid as that of antacids, the OTC H_2RAs have a longer duration of action, up to 6–10 h. Therefore, they are particularly useful when taken before a potentially refluxogenic activity, such as a heavy meal or exercise. Similar to antacids, the OTC H_2RAs are ineffective in healing esophagitis [185]. In the summer of 2003, the US Food and Drug Administration (FDA) approved omeprazole (20 mg) as the first OTC PPI. Drug labeling suggested daily use for only 2 weeks and recommended physician follow-up for persistent symptoms. Despite initial fears that patients might abuse this drug and not see a physician, early consumer data suggest that individuals accurately self-select if OTC omeprazole is appropriate to use, comply with a 2-week regimen, and seek physician care for long-term management of frequent heartburn [186].

Prescription medication therapy

Patients with frequent heartburn, esophagitis, or complications of GERD usually see a physician and receive prescription medications for their disease. Although prokinetic drugs attempt to correct the motility disorder associated with GERD, the most clinically effective medications for short- and long-term reflux treatment are the acid-suppressive drugs.

Prokinetic drugs

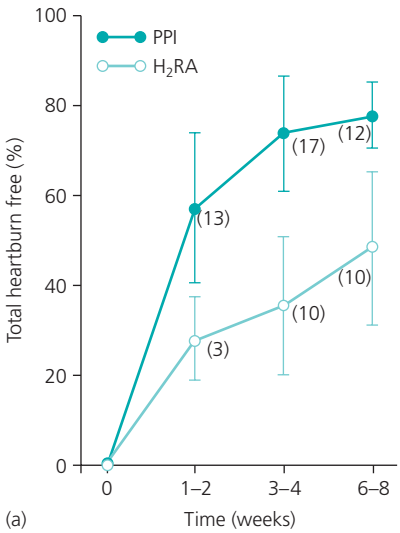
Until recently, three prokinetic drugs were available for the treatment of GERD: bethanechol, a cholinergic agonist; metoclopramide, a dopamine antagonist; and cisapride, a serotonin (5-HT_4) receptor agonist, which increases acetylcholine release in the myenteric plexus. These drugs improve reflux symptoms by increasing LES pressure, acid clearance, or gastric emptying [187]. However, none alters the frequency of transient LESRs, and their physiological activity decreases as the disease severity worsens [188]. Therefore, all of the current prokinetics provide modest benefit in controlling heartburn but they have little efficacy in healing esophagitis unless they are combined with an acid-inhibiting drug [187].

The use of prokinetic drugs is limited by their side-effect profiles. Bethanechol commonly causes flushing, blurred vision, headaches, abdominal cramps, and urinary frequency. Metoclopramide, which crosses the blood–brain barrier, has a 20%–50% incidence of fatigue, lethargy, anxiety, and restlessness, and rarely causes tremor, parkinsonism, or tardive dyskinesia. It is possible to decrease the frequency of these side effects by dose reduction, by increasing the dosing regimen to twice a day, by taking a larger single dose before dinner or at bedtime, or by using a sustained-release tablet. Although cisapride was the best prokinetic drug for treating GERD, with an excellent safety profile, it was withdrawn from the US market because of increased reports of serious cardiac arrhythmias (ventricular tachycardia, ventricular fibrillation, torsades de pointes, and QT prolongation), with associated cardiac arrest and deaths related to possible drug interactions [189].

Histamine type 2 receptor antagonists

Cimetidine, ranitidine, famotidine, and nizatidine reduce acid secretion by competing for histamine receptors on parietal cells. They are more effective in controlling nocturnal than meal-related acid secretion because parietal cells may also be stimulated postprandially by acetylcholine and gastrin [190]. All of the H_2RAs are equally effective when used in proper doses, usually twice a day before meals. Clinical GERD trials show that heartburn, both day and night, can be significantly decreased by H_2RAs compared with placebo, although symptoms are rarely abolished (Fig. 32.11a) [191]. Trials and a metaanalysis found that the overall esophagitis healing rates with H_2RAs rarely exceeded 60% after up to 12 weeks of treatment, even when higher than standard doses were used (see Fig. 32.11b) [191,192]. Healing rates differ in individual trials, depending primarily on the degree of esophagitis being treated: grade I and II esophagitis heals in 60%–90% of patients, whereas grade III and IV heals in 30%–50% of patients despite high-dose regimens [192].

Reflux symptoms associated with nocturnal gastric acid breakthrough during PPI therapy have been recognized [193]. At bedtime, H_2RAs successfully eliminated this problem, suggesting a new indication for H_2RAs in the PPI era [194]. However, this study used only a single evening dose and did not account for the tolerance that frequently develops to H_2RAs over weeks to months [195]. This may impair the ability of chronic long-term nocturnal dosing of H_2RAs to eliminate acid breakthrough symptoms [196], but it suggests an important clinical role as medications to be used on an



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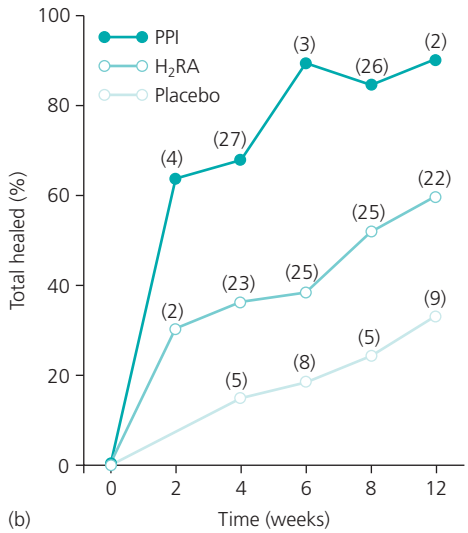

(b)

Figure 32.11 **(a)** Symptom relief–time curve expressed as the mean total heartburn relief for proton pump inhibitors (PPIs) or histamine type 2 receptor antagonists (H_2RA) corrected for patients free of heartburn at baseline and over 8 weeks. By week 2, more patients treated with PPIs are asymptomatic than patients treated with H_2RAs; this superior efficacy is maintained over the 6- to 8-week duration of these studies. **(b)** Esophagitis healing–time curve expressed as the mean total healing for PPIs, H_2RAs, and placebo over 12 weeks. By 4 weeks, PPIs healed esophagitis in more patients than the other two drug classes over 12 weeks, implying a substantial therapeutic gain. The number of studies is shown in parentheses. From Chiba et al. [192], with permission from the American Gastroenterological Association.

as-needed basis when lifestyle indiscretions may promote nocturnal symptoms.

The H_2RAs are very safe, with a side-effect rate (most of which are minor and reversible) of about 4% [190]. Serum concentrations of phenytoin, procainamide, theophylline, and warfarin are altered after the administration of cimetidine and, to a lesser degree, ranitidine, whereas this interaction is not reported with the other two H_2RAs. The concern that these agents could alter blood ethanol levels has been discounted [190].

Proton pump inhibitors

This class of drugs markedly diminishes gastric acid secretion by inhibiting the final common pathway of acid secretion, the H^+,K^+-ATPase pump. PPIs inhibit daytime, nocturnal, and meal-stimulated acid secretion to a significantly greater degree than H_2RAs [197], but they rarely make patients achlorhydric. Unlike H_2RAs, the degree of acid inhibition by PPIs does not correlate with plasma concentration, but is related to the concentration and duration (area under the curve). After oral ingestion, acid inhibition is delayed because PPIs need to accumulate in the secretory canaliculus of the parietal cell to bind irreversibly to actively secreting proton pumps [198]. Therefore, the slower a PPI is cleared from the plasma, the more of it is available for delivery to the proton pumps. PPIs are best taken before the first meal of the day, when most proton pumps are active. Because not all pumps are active at any given time, a single PPI dose does not inhibit all pumps. A second dose, if needed, can be taken before the evening meal.

The five available PPIs are omeprazole, lansoprazole, rabeprazole, pantoprazole, and esomeprazole, the S-isomer of the racemic omeprazole. Their superior efficacy compared with H_2RAs is based on their ability to maintain an intragastric pH higher than 4.0 between 15 and 21 h daily compared with approximately 8 h daily for the H_2RAs [199]. PPIs are superior to H_2RAs in completely relieving heartburn symptoms in most patients with severe GERD, usually within 1–2 weeks (see Fig. 32.11a) [192]. Symptom relief is better in patients with erosive than nonerosive disease [200]. Controlled studies and a large metaanalysis report complete healing of even severe ulcerative esophagitis after 8 weeks in more than 80% of patients taking PPIs compared with 51% of patients taking H_2RAs and 28% receiving placebo (see Fig. 32.11b) [201–205]. In those patients not healing initially, prolonged therapy with the same dose or an increased dose usually resulted in 100% healing [206]. Therapeutic efficacy between PPIs was similar; however, large studies have found that the newest PPI, esomeprazole (40 mg), is superior to omeprazole (20 mg) and lansoprazole (30 mg) in healing esophagitis [207]. The therapeutic advantage is minimal with mild esophagitis (number to treat, 20–40 patients) and greatest with severe esophagitis (number to treat, 7–10 patients). This superiority is related to higher systemic bioavailability and less interpatient variability with esomeprazole. Several PPIs are now available in the United States for intravenous use.

All of the PPIs are well tolerated with headaches and diarrhea described as the most common side effects in clinical trials. Although increased gastrin levels are reported with all of the PPIs, the elevations generally do not exceed the normal range for gastrin and levels return to normal values within 1 week of stopping the drug. Omeprazole may decrease the clearance of diazepam and warfarin because of competition for the cytochrome P450 isoenzyme P2C19 [208]. The four newer PPIs have minimal or no important drug–drug interactions.

Maintenance therapy

GERD tends to be a chronic relapsing disease, especially in patients with low LES pressure, severe grades of esophagitis, and difficult to manage symptoms [185]. Although almost all patients with severe esophagitis can be healed with PPI treatment, recurrence can be anticipated in more than 80% of patients within 6 months of drug discontinuation [147]. The chronicity of less severe forms of GERD is less certain, but relapses probably occur in 15%–30% of patients over 6 months [209]. Therefore, maintenance therapy is needed for many patients.

One-year maintenance studies always find that the PPIs are superior to the H_2RAs or prokinetics, with remission rates higher than 75% [148,210,211]. H_2RAs and prokinetic drugs have lower overall remission rates (20%–50%) and are most useful in patients with mild or no esophagitis [148,212]. The FDA has approved all of the PPIs, sometimes at half the short-term dose, for maintenance therapy, but only ranitidine, 150 mg twice daily, has maintenance indications for mild esophagitis. Conversely, clinicians are now placing their patients with severe erosive esophagitis on long-term PPI therapy indefinitely. The efficacy of this approach is supported by open, compassionate use data, primarily from the Netherlands and Australia [213,214]. In a study of 230 patients with severe esophagitis healed initially with 40 mg of omeprazole, all subjects were kept in remission for up to 11 years. More than 60% were maintained on omeprazole, 20 mg a day, whereas higher doses of 60 mg or more were needed in only 12% of patients, suggesting a lack of tolerance to PPIs. Relapses were rare (1 per 9.4 years of follow-up), strictures did not occur, and Barrett esophagus did not progress [214].

There was concern about the long-term safety of PPIs because of their profound acid suppression. Evidence suggests that this fear is unjustified, as sufficient gastric acid is produced, allowing for normal protein and carbohydrate digestion, iron and calcium absorption, and the prevention of bacterial overgrowth. The clinical effect of omeprazole on vitamin B-12 absorption is controversial [215]. Therefore, it may be prudent to monitor vitamin B-12 levels in patients

receiving long-term PPI therapy, especially elderly patients or those with poor or unusual diets. The main concern over the long-term safety of PPIs stemmed from reports that omeprazole causes hypergastrinemia and gastric carcinoid tumors in rats, changes also subsequently demonstrated with long-term ranitidine therapy and subtotal resection of the gastric fundus [216]. However, the rat has a high density of enterochromaffin-like cells and an exaggerated response to achlorhydria; long-term omeprazole therapy in species with lower densities of enterochromaffin-like cells (mice, dogs, humans) has not caused carcinoid tumors. Furthermore, other groups with massive hypergastrinemia (five to ten times the gastrin values seen with omeprazole), such as patients with pernicious anemia or Zollinger–Ellison syndrome, rarely develop carcinoid tumors [216]. Finally, one study suggested that patients taking long-term omeprazole who are infected with *H. pylori* develop atrophic gastritis, a precursor to gastric adenocarcinoma, at a more rapid rate than noninfected patients [217]. Nevertheless, a subsequent FDA panel determined that the available data were insufficient for recommending screening and treatment of *H. pylori* infection in patients receiving long-term PPI therapy [218].

Treatment in elderly or pregnant patients

Older patients often complain of less severe reflux symptoms than their younger cohorts; however, because of prolonged acid exposure over many years, the elderly may have more complicated disease [5]. Treatment of older patients with GERD follows the same principles as treatment of other adults, although they may require more aggressive acid-suppression therapy [219]. Pill-induced esophagitis may complicate treatment. Metoclopramide must be used with caution because of frequent side effects in the elderly. H_2RAs can be associated with mental changes in older patients, and doses need to be decreased in patients with renal insufficiency. Fewer drug interactions are seen with famotidine and nizatidine. Alternative methods of administering PPIs may be necessary in debilitated older patients who cannot swallow intact omeprazole or lansoprazole capsules. Both capsules can be opened and the granules taken with water, a HCO_3^--based suspension, or apple or orange juice; alternatively, the granules can be sprinkled onto apple sauce or yogurt [220].

Teratogenicity or fetal harm from absorption of medications across the placenta is the foremost consideration in the treatment of GERD during pregnancy [221]. Lifestyle modifications and the use of antacids or Gaviscon remain the cornerstones of treatment, providing adequate relief to the majority of women with mild symptoms. Although rarely used in adults, sucralfate is a nonabsorbable mucosal binder that has been found to be superior to lifestyle changes in a controlled study in pregnant women [222]. Metoclopramide, H_2RAs, and most PPIs (except omeprazole) have a category B FDA safety profile for use during pregnancy, which is based on animal studies showing no risk, as well as on small case series and anecdotal human reports. Ranitidine is the only one of these drugs that has been shown to be effective during pregnancy [223]. PPIs may be safe for aspiration prophylaxis before anesthesia for elective cesarean sections. Antacids, sucralfate, and most H_2RAs (except nizatidine) are safe to use during lactation, even though H_2RAs are excreted in breast milk. PPIs are not recommended for use during breast-feeding, based on safety concerns in animal studies [221].

Treatment of complications

Extraesophageal presentations

Acid reflux-related chest pain is easily treated by H_2RAs or PPIs, with efficacy substantiated by placebo-controlled studies [224]. The efficacy of acid-suppression therapy in asthma, cough, and other pulmonary complications of GERD is more mixed [98]. Medical antireflux therapy improves asthma symptoms and reduces the need for asthma medications in more than 60% of patients, but objective improvement of peak expiratory flow rates is observed in only 25% of patients. Best results are found with higher doses of PPIs (usually twice-daily administration) given for 2–3 months. Potential positive predictors of PPI response include asthma that is difficult to control, associated acid regurgitation, proximal reflux on pH testing, and healing of esophagitis with antireflux therapy. Case studies report that 60%–96% of patients with suspected acid-related ear, nose, and throat symptoms and signs improve with acid suppression, but the results of placebo-controlled studies are inconsistent and much less encouraging [126]. Here again, PPIs are more effective than H_2RAs, and extended therapy for up to 3 months may be required. Predictors of response have not been identified, although patients with milder laryngeal signs show better symptom improvement. In all of these possible extraesophageal presentations of GERD, failure to respond to aggressive PPI therapy, confirmed by adequate acid control on pH testing, suggests a cause of these complaints other than acid.

Esophageal strictures

Dysphagia in patients with esophageal strictures is related to stricture diameter and severity of esophagitis [225]. When the esophageal lumen diameter is less than 13 mm, dysphagia is a major complaint and esophageal dilation is required. Simple short strictures can be dilated by blind peroral passage of rubber Hurst (round ends) or Maloney (tapered ends) mercury-filled dilators of increasing sizes (16–60 French, 3 French = 1 mm) to disrupt the fibrous bands producing the obstruction. Complicated longer, tighter, or more irregular strictures will require bougienage over a guidewire using hollow-centered Savary plastic-covered polyvinyl dilators or balloon (Gruentzig) dilators. The techniques of esophageal dilation are discussed in detail in Chapter 121 [226]. Before and after dilation, medical therapy with PPIs is indicated;

PPIs have been shown to be superior to H_2RAs in relieving symptoms and in reducing the need for repeat dilations [227]. Maintenance PPI therapy for patients with strictures has dramatically reduced the incidence of repeat esophageal dilations and the cost of treating these patients [154]. Recalcitrant strictures requiring surgery are now very uncommon and suggest another aggravating factor such as chronic pill injury.

Barrett esophagus

Esophagitis in the presence of Barrett esophagus can be easily healed with PPI therapy; however, regression of Barrett epithelium, except for small squamous islands, is rarely reported, even with high-dose PPI therapy [228]. Recent ex vivo studies [229] have suggested that intermittent "pulses" of acid enhance Barrett epithelial cell proliferation, possibly increasing the risk of dysplasia and cancer. The clinical relevance of this data is supported by a 20-year follow-up study of 236 veteran patients by a single endoscopist. In multivariate analysis, the use of PPIs after the diagnosis of Barrett esophagus was independently associated with a reduced risk of dysplasia (hazard ratio 0.25, 95% confidence interval 0.13–0.47) [230]. Therefore, all Barrett patients should be on chronic PPI therapy.

Esophageal resection of Barrett esophagus can prevent the progression to cancer; however, this requires a total esophagectomy, which has a high mortality rate except in selected surgical centers. Therefore, ablation of Barrett epithelium in the setting of strict PPI anacidity has been proposed. Photodynamic therapy, laser, multipolar electrocoagulation, argon plasma coagulation, and endoscopic mucosal resection have been used for this purpose [231]. In these studies, Barrett mucosa can be reversed completely in 70%–80% of patients, but intestinal metaplasia underlying the new squamous mucosa is reported in almost all series, with the occasional residual foci of metaplasia developing adenocarcinoma [232]. Adverse effects of ablation therapy have ranged from mild chest pain, sore throat, or odynophagia to esophageal perforation and death. The incidence of adenocarcinoma in patients with Barrett esophagus without dysplasia is probably so low that endoscopic ablation cannot be advocated outside of study protocols. Endoscopic therapy for patients with high-grade dysplasia or early cancer holds more promise, especially in the older patients with comorbid illnesses [233].

Because there is no way of eliminating the malignant risk of Barrett esophagus, regular endoscopic surveillance is recommended. Biopsies should be taken from each quadrant every 2 cm axially within the metaplastic tissue. The rationale is that dysplasia within Barrett epithelium is often multifocal, and obtaining fewer tissue samples increases the risk of missing dysplastic areas [234]. Brush cytology can complement endoscopic biopsies [235]. Biomarkers [236], such as p53, and flow cytometry [237] may augment the yield of histological examination of biopsy specimens. Although prospective studies are not available, case series confirm that esophageal adenocarcinomas detected by endoscopic surveillance are at an earlier stage and have a more favorable survival outcome than cancers detected at the time of diagnosis of Barrett esophagus, typically when patients present with dysphagia [238].

The appropriate surveillance interval for patients with Barrett esophagus has not been studied prospectively. However, current programs, such as proposed by the American College of Gastroenterology, are based on the grade of dysplasia (Table 32.3) [239]. In these recommendations, the management of high-grade dysplasia remains most controversial. Some groups suggest that high-grade dysplasia may regress to lesser grades, and that an intensive biopsy protocol every 3 months will differentiate high-grade dysplasia from cancer [240,241]. The surgical literature contrasts with this experience. Of 126 cases with high-grade dysplasia alone on endoscopic biopsy, 41% had cancer, although usually an early stage, at the time of esophagectomy [239]. Nevertheless, most patients with Barrett esophagus never progress to important degrees of dysplasia. Predictors of cancer progression at the initial diagnosis of Barrett esophagus are needed to define individual surveillance programs more appropriately. One study suggested that patients whose baseline biopsies are negative or show only low-grade dysplasia without increased 4N or aneuploidy on flow cytometry may have surveillance deferred for up to 5 years [241]. Another

Table 32.3 Barrett esophagus: grade of dysplasia and proposed follow-up

	Published data			
Dysplasia	**No. cancer cases/total no. patients**	**Percentage**	**Follow-up (years)**	**Proposed endoscopic follow-up**
None	5/150	3	3.4–10	After two negative biopsies, every 2–3 years
Low-grade	8/45	18	1.5–4.3	Every 6 months for 1 year, then every year
High-grade	21/61	34	0.2–4.5	Expert confirmation; esophageal resection, ablation therapy or endoscopic surveillance every 3 months

From Sampliner [239], with permission from Blackwell Publishing.

prospective multivariate analysis revealed that progression to high-grade dysplasia or cancer was significantly and independently associated with dysplasia at diagnosis or at any time during follow-up, hiatal hernia size, and Barrett esophagus length [242]. These patients may warrant more frequent surveillance programs.

Other unresolved issues in Barrett esophagus include the role of screening endoscopy and chemoprevention in dysplasia [243]. As the majority of Barrett patients remain undiagnosed, screening endoscopy has been recommended for older men with chronic long-standing heartburn [157]. A recent mathematical model [244] found that screening a 50-year-old man with GERD was cost-effective (US$10 000 per quality-adjusted life-year of survival gained) if follow-up endoscopies were carried out only in the subset of Barrett patients with dysplasia at initial biopsy. Two studies [244,245] of patients referred for colon cancer screening who agreed to upper endoscopy demonstrated Barrett esophagus (usually short segment) in 10%–25%, regardless of the presence of reflux symptoms, suggesting a practical method to address this issue. Chemoprevention is a rapidly emerging option that can be used to reduce the risk of developing adenocarcinoma in Barrett esophagus. A metaanalysis of previous cohort studies showed that low-dose aspirin and NSAIDs led to a significant risk reduction in esophageal cancer [246]. These drugs block the up-regulation of cyclooxygenase 2 (COX-2) enzyme activity, which is serially increased along the metaplasia–dysplasia–cancer cascade [247].

Surgical treatment

Antireflux surgery reduces GER by increasing basal LES pressure, decreasing episodes of transient LESR, and inhibiting complete LESR [248]. This is accomplished by reducing the hiatal hernia back into the abdomen and thereby restoring an adequate length of intraabdominal sphincter, reconstructing the diaphragmatic hiatus, and reinforcing the LES [249]. Since the advent of minimally invasive surgery, the two most popular operations are the Nissen fundoplication and the Toupet partial fundoplication (Fig. 32.12). The former is a superior operation with more long-term durability, but it has a higher frequency of postoperative dysphagia and gas bloat symptoms [250,251]. Both are now routinely performed laparoscopically through the abdomen. The hospital stay is 1–2 days, and many patients return to normal activity within 7–10 days. Patients with more severe disease and a short esophagus manifested by a large nonreducible hernia, a tight stricture, or a long-segment Barrett esophagus require a Collis lengthening procedure creating a 3- to 5-cm neoesophagus so that the fundoplication can be placed in the abdomen under minimal tension [252].

In the PPI era, the resolution of symptoms on treatment helps to predicate the success of antireflux surgery for both classical and atypical symptoms [253]. Antireflux surgery is a reasonable option in the following situations: (1) healthy patients with GERD well controlled on PPIs who want alternative therapy because of drug expense, poor medication compliance, or a fear of unknown long-term side effects; (2) patients with atypical GERD symptoms responding to PPIs; and (3) patients with volume regurgitation and aspiration symptoms not controlled on PPIs. Patients recalcitrant to PPI therapy need to be approached cautiously with surgery because there may be another cause of their disorder (i.e., pill esophagitis, gastroparesis, functional heartburn).

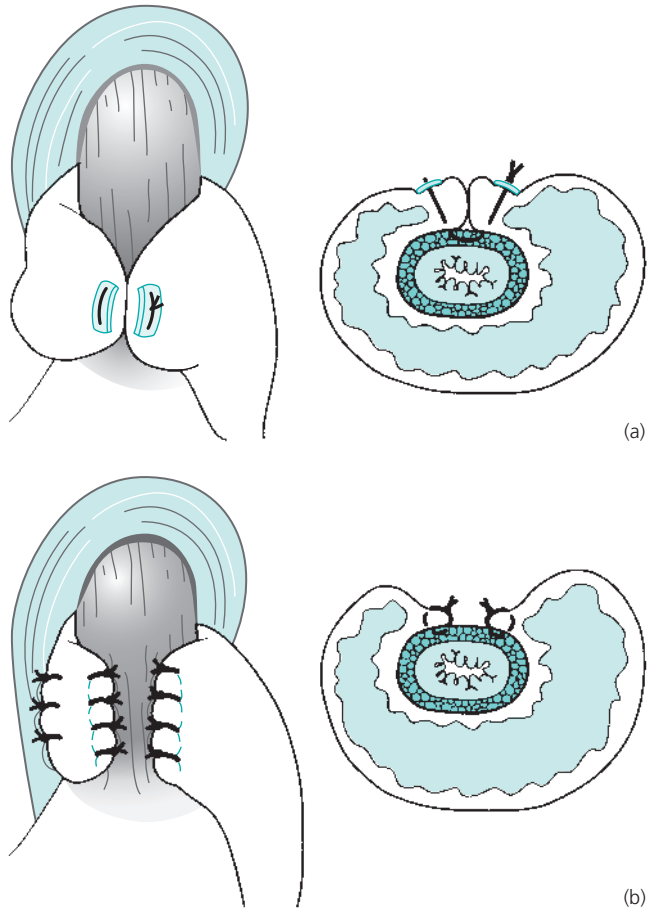

Figure 32.12 Appearance and cross-sectional views of the Nissen fundoplication, a full 360° wrap **(a)**, and the Toupet partial fundoplication **(b)**. These are the two most popular fundoplications used in laparoscopic antireflux surgery.

Extensive physiological testing should be carried out before antireflux surgery is performed. All patients need to undergo endoscopy to exclude strictures, Barrett esophagus, and dysplasia. A barium esophagram can help define a nonreducible hernia, shortened esophagus, and poor esophageal motility. Esophageal manometry combined with impedance testing will identify ineffective esophageal peristalsis and previously misdiagnosed achalasia or scleroderma. In addition, 24-h pH testing is needed in all patients with nonerosive GERD or in those with esophagitis not responding to PPI therapy. Gastric analysis and gastric emptying studies may be indicated in

selected patients. Careful testing will result in modification of the original operation or an alternative diagnosis in approximately 25% of patients [131].

Antireflux surgery relieves reflux symptoms and reduces the need for stricture dilation in more than 90% of patients [251], but Barrett esophagus rarely regresses and there is insufficient evidence that surgery reduces the risk of esophageal cancer [254]. Comparison studies have found that antireflux surgery is superior to lifestyle changes, the use of antacids, and H_2RA and prokinetic therapy [151,184], but not to PPI therapy, especially when dose titration is permitted [255]. Mortality is rare (< 1%) after antireflux surgery, but new postoperative complaints can occur in up to 25% of patients, including dysphagia, gas bloat, diarrhea, and increased flatus [256]. Most symptoms improve over a year, but persistent complaints suggest too tight a wrap, a displaced fundoplication, or inadvertent damage to the vagus nerve. Successful antireflux surgery, however, does not guarantee a permanent cure. The best surgical results are obtained by experienced surgeons in high-volume centers who report long-term symptom recurrence in only 10%–15% of patients [250]. However, most operations are performed by community or Veterans Affairs hospital surgeons. Here the results are not as good, with studies finding relapse of symptoms after 2 years in 32% of patients, with 7% requiring repeat surgery [257], and a return to regular use of antireflux medications in 62% of patients (one-half on PPIs) 10–15 years after fundoplication [258]. Potential factors contributing to these high relapse rates include inexperienced surgeons, low numbers of operations performed yearly per surgeon, and persistence of abdominal stressors (i.e., obesity, heavy isometric exercise or work) that gradually weaken the fundoplication. A suboptimal operation or severe symptom relapse may necessitate a second operation, which has less likelihood of a successful outcome [249]. Because optimal medical therapy is available to all patients with GERD, the risk and benefits of both long-term medical treatment and antireflux surgery must be carefully discussed with patients so that they can take part in this important decision.

New treatments

The future medical treatment of GERD involves drugs that interfere with transient LESRs but which do not cause dysphagia. Baclofen, a GABA-B agonist, has been shown to decrease reflux symptoms and to improve pH studies in healthy persons and in patients with GERD [259]. Newer endoscopic treatments of GERD have been approved by the FDA. These techniques include an endoscopic suturing system, radiofrequency energy delivery to the gastroesophageal junction, and the injection of nonabsorbable polymers into the submucosa surrounding the LES [260–262]. These procedures decrease the frequency of transient LESRs, but LES pressure is unchanged and less than 40% of patients have normal pH tests. To date, these techniques have been applied only to patients with small (< 2 cm) or no hernias and mild esophagitis. The results are encouraging, with improvement of symptoms and decreased medication requirements, but the cost-effectiveness and durability of the procedures is suspect (usually > 50% failure by 1–2 years) and reported complications include perforation, hemorrhage, pain, and death. Controlled studies using the radiofrequency energy delivery and injectable polymer techniques show consistent symptom improvement over the sham procedure, inconsistent reduction in the use of PPIs, and no significant improvement in LES pressure or pH profiles [263,264]. Serious adverse effects after using the injectable techniques, including several deaths, led to the voluntary withdrawal of Enteryx by the manufacturer in September 2005, and suspension of the Gatekeeper clinical program in 2005. An American Gastroenterological Association (AGA) Institute medical position statement recommended that "current data suggests that there are no definite indications for endoscopy therapy for GERD at this time" [265].

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A ESOPHAGUS

33 Eosinophilic esophagitis

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Since 2000 an increasing body of evidence has suggested that eosinophils are likely to play a key role in the pathogenesis of gastrointestinal diseases. In this time frame, eosinophilic esophagitis (EoE) has emerged as a distinct clinicopathological disease affecting both children and adults. Symptoms span a broad range in children but affected adults typically complain of dysphagia and often present with food impaction. These complaints are thought to be secondary to acute esophageal spasm resulting from chronic allergic inflammation with fibrotic stricture formation. Although the natural history of this disease remains uncertain, removal of dietary allergens and medical management with glucocorticoids have been shown to have clear benefit. This chapter will focus on pathogenetic mechanisms of eosinophilic inflammation and the clinicopathological features of EoE.

Eosinophil biology

Life cycle and regulation

Eosinophils are produced in the bone marrow from pluripotential stem cells under the regulation of the transcription factor GATA-1 [1] and the cytokines interleukin (IL)-3, IL-5, and GM-CSF [2]. IL-5 is the most specific to the eosinophil lineage and is responsible for the selective differentiation of eosinophils [3], their release from the bone marrow into the peripheral circulation [4], and their survival [5]. The critical role of IL-5 in the production of eosinophils is best demonstrated by genetic manipulation of mice. Overproduction of IL-5 by a variety of approaches, including transgenic overexpression in enterocytes, results in profound blood eosinophilia, whereas deletion of the IL-5 gene causes a marked reduction of eosinophils in the blood, lungs, and gastrointestinal tract after allergen challenge [6–9]. Based on these results, a humanized anti-IL-5 drug has been developed and tested in asthmatic individuals (as well as in patients with EoE [see section on pathogenesis]). Although this drug lowers the level of circulating eosinophils by 80%, it only reduces lung tissue levels by approximately 55% [10]. Early results with anti-IL-5 have not shown an improvement in airway function (e.g., forced expiratory volume in 1 s [FEV1]) [11], but it appears to induce a significant improvement in markers of remodeling (associated with decreases in transforming growth factor [TGF]-β1 levels) [12]. This suggests the importance of other molecules involved in eosinophil tissue accumulation as well as an important role for eosinophils in the inflammatory changes associated with chronic remodeling, a process that is likely to be important in EoE pathogenesis. Indeed, other products of T helper 2 (Th2) cells (e.g., IL-4 and IL-13) can affect eosinophils [13]. IL-4 and IL-13 induce eosinophil recruitment and survival. They mediate eosinophil function by inducing expression of critical adhesion molecules on the endothelium that bind to the β1- and β2-integrins on eosinophils (such as intercellular adhesion molecule 1 [ICAM-1] and vascular cell adhesion molecule 1 [VCAM-1]), as well as expression of eosinophil-active chemokines, such as the eotaxins, and other molecules that influence eosinophils (e.g., chitinases) [13,14]. IL-4 and IL-13 signal through a common receptor subunit, IL-4Rα, that utilizes the JAK-STAT6 pathway [15]. Mice with targeted deletion of STAT6 have impaired development of Th2-associated responses in the gastrointestinal tract [16,17]. Activated STAT6 dimers bind to specific promoter motifs located in a series of inflammatory genes, such as the eotaxin-1, eotaxin-2, and eotaxin-3 promoters [18–22]. IL-4 and IL-13 receptors are expressed by structural cells such as lung and intestinal epithelium [15,22,23]. In addition to promoting eotaxin production via STAT6-induced transcription, these cytokines promote increased eotaxin-1 mRNA stability by way of

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stabilization motifs in the 3′ untranslated region of eotaxin-1 mRNA [24]. Although not yet studied in the esophagus, analysis of lung tissue from asthmatic patients has revealed expression of the eotaxin chemokines by epithelial cells [13,25]. Whether this also applies to esophageal epithelial cells has not been determined, but it is interesting to note that squamous epithelial cells in the skin produce the eotaxins in response to IL-13 treatment [26].

Chemotaxis – eotaxins and CCR3

Eotaxin was initially discovered using a biological assay in guinea pigs designed to identify the molecules responsible for allergen-induced eosinophil accumulation in the lungs [27]. Using an in vivo chemotaxis assay in guinea pig skin, the partial amino acid sequence of the protein responsible for eosinophil chemoattraction in the bronchoalveolar lavage fluid (BALF) in allergen-challenged guinea pigs was determined [27]. This sequence facilitated the genetic cloning of the genes and cDNA for guinea pig, murine, and human eotaxin, which led to the identification of eotaxin as a member of the CC chemokine family, being most homologous to the macrophage chemoattractant protein (MCP) subfamily [28–31]. Utilizing genomic analyses rather than biological assays, two additional genes have been identified in the human genome that encode CC chemokines with eosinophil-selective chemoattractant activity; these have been designated eotaxin-2 and eotaxin-3 [32–35]. It was subsequently determined that the mouse genome only contains eotaxin-1 and eotaxin-2 [19]; a putative mouse eotaxin-3 gene exists but it does not appear to be expressed [36]. Eotaxin-2 and eotaxin-3 are only distantly related to eotaxin-1 because they are only about 30% identical in sequence and are located in a different chromosomal position (human 7q11.23) [19,35,37]; however, they have a conserved structure [38,39] that allows them to specifically bind to the eotaxin receptor, CCR3, a seven transmembrane-spanning G protein-coupled receptor (GPCR), primarily expressed on eosinophils [31,40,41]. CCR3 is a genetically polymorphic receptor that undergoes ligand-induced internalization, a process that is required for eotaxin-induced cellular activation [42–44]. CCR3 is a promiscuous receptor. It interacts with multiple ligands including MCP-2, -3, -4, RANTES, and HCC-2 (MIP-5, leukotactin); however, the only ligands that signal exclusively through this receptor are the eotaxin chemokines, accounting for the cellular selectivity of the eotaxins [45]. Although all three eotaxin chemokines have been shown to selectively trigger CCR3 with potent binding affinities in the nanomolar range, their relative affinities are different. Eotaxin-1 appears to have a unique ability to weakly bind CCR2b and possibly CCR5 [46], whereas eotaxin-3 has been shown to antagonize CCR1, CCR2, and CCR5 [47,48]; this provides a potential mechanism to explain the nonredundant activity of the eotaxins. Indeed, gene targeting and antibody neutralization experiments have shown distinct and cooperative roles for each eotaxin in regulating baseline and allergen-induced eosinophil responses [36,49–51]. CCR3 appears to be the dominant eosinophil chemokine receptor as suggested by its relatively high level of expression and the ability of an anti-CCR3 reagent to block the activity of RANTES, a chemokine that can signal through CCR1 or CCR3 on eosinophils [52]. Interestingly, other cells involved in allergic responses (e.g., Th2 cells, basophils, mast cells, dendritic cells, and airway epithelial cells) also express CCR3 [53–55]; however, the significance of CCR3 expression on these cells has been less clearly demonstrated than on eosinophils. It is interesting to note that keratinocytes have been shown to express CCR3, raising the possibility that the esophageal epithelium may also have the capacity to express CCR3 [56].

There is now substantial preclinical evidence supporting a role for the eotaxins in human disease. Markedly increased levels of eotaxin-1 mRNA in the intestines of patients with inflammatory bowel disease (IBD) have been demonstrated [30]. Additionally, experimental induction of cutaneous and pulmonary late-phase responses in humans has revealed that the eotaxin chemokines are produced by tissue-resident cells (e.g., respiratory epithelial cells and skin fibroblasts) and allergen-induced infiltrative cells (e.g., macrophages and eosinophils) [13]. Following allergen challenge in the lung, eotaxin-1 is induced early (6 h) and correlates with early eosinophil recruitment; in contrast, eotaxin-2 and eotaxin-3 correlate with eosinophil accumulation at 24 h [57–59]. Collectively, these studies have provided the impetus for the development of therapeutic agents aimed at blocking the actions of eotaxins and CCR3. Indeed, small-molecule inhibitors of CCR3 and a humanized anti-human eotaxin-1 antibody have been developed [13,60,61]. The results of a phase I trial of humanized anti-eotaxin-1 antibody in patients with allergic rhinitis have shown no serious adverse responses when this drug is administered by intravenous or intranasal routes [60,62]. Notably, anti-eotaxin-1 antibody lowers levels of eosinophils in nasal washes and nasal biopsies, and improves nasal patency [60,62].

Gastrointestinal eosinophils

Eosinophils have been noted to be present at low levels in numerous tissues. When a large series of biopsy and autopsy specimens were analyzed, the only organs that demonstrated tissue eosinophils (at substantial levels) were the gastrointestinal tract, spleen, lymph nodes, and thymus [63]. Interestingly, eosinophil infiltrations were only associated with eosinophil degranulation in the gastrointestinal tract. Examination of eosinophils throughout the gastrointestinal tract of conventional healthy mice (untreated mice maintained under pathogen-free conditions) has revealed that eosinophils are normally present in the lamina propria of the stomach, small intestine, cecum, and colon [64]. Notably,

unlike intestinal lymphocytes and mast cells, eosinophils are not normally present in Peyer patches or intraepithelial locations, although they commonly infiltrate these regions in eosinophilic disorders [65]. Interestingly, prenatal mice have eosinophils located in similar regions and concentrations as observed in adult mice [64], providing evidence that eosinophil homing into the gastrointestinal tract occurs independently of endogenous flora. Indeed, germ-free mice and mice deficient in innate signaling responses (Myd88 deficient) have normal levels of gastrointestinal eosinophils (ref. 64 and unpublished findings). These data suggest that eosinophils respond to distinct stimuli compared with other intestinal leukocytes; indeed, constitutive expression of eotaxin-1 has been demonstrated to provide the unique signal that promotes localization of eosinophils into the gastrointestinal tract at baseline. The importance of eotaxin-1 in regulating the baseline level of eosinophils is reinforced by the observation that mice with the targeted deletion of eotaxin-1 or its receptor CCR3 (but not eotaxin-2-deficient mice) have a deficiency in gastrointestinal eosinophils [36,66].

Eosinophil-associated gastrointestinal disorders

Eosinophil accumulation in the gastrointestinal tract is a common feature of numerous gastrointestinal disorders including classic IgE-mediated food allergy [67–69], eosinophilic gastroenteritis [70,71], allergic colitis [72–74], EoE [75–77], IBD [78–80], and gastroesophageal reflux disease (GERD) [81–83] (Table 33.1). In IBD, eosinophils usually represent only a small percentage of the infiltrating leukocytes [80,84], but their level has been proposed to be a negative prognostic indicator [84,85]. Similarly, in GERD there are only low levels of esophageal eosinophils [81,86]. Primary eosinophil-associated gastrointestinal disorders (EGID; e.g., EoE, eosinophilic gastritis, eosinophilic gastroenteritis, eosinophilic enteritis, and eosinophilic colitis) are defined as disorders that primarily affect the gastrointestinal tract with eosinophil-rich inflammation in the absence of known causes of eosinophilia (e.g., drug reactions, parasitic infections, malignancy) [87]. Patients with EGID suffer from a variety of problems including failure to thrive, abdominal pain, irritability, gastric dysmotility, vomiting, diarrhea, and dysphagia [88–90]. Evidence is accumulating to support the concept that EGID arises secondary to the interplay of genetic and environmental factors. Notably, a large percentage (~10%) of patients suffering from EGID, especially EoE, have an immediate family member with EGID [91,92]. Additionally, several lines of evidence support an allergic etiology, including the finding that approximately 75% of patients with EGID are atopic [93–101], the common finding of mast cell degranulation in tissue specimens [102,103], and the finding that the severity of disease can sometimes be reversed by institution of an allergen-free diet [100,101,104]. Importantly, recent murine models of EGID support a potential allergic etiology for these disorders [105,106]. Interestingly, despite the common finding of food-specific IgE in patients with EGID, food-induced anaphylactic responses only occur in a minority of patients [87]. Thus, EGID have properties that fall between pure IgE-mediated food allergies and cellular-mediated hypersensitivity disorders (e.g., celiac disease) [69,87,107,108].

Table 33.1 Differential diagnosis for gastrointestinal eosinophilia

Gastroesophageal reflux disease
Eosinophil-associated gastrointestinal disorders (EGID):
Eosinophilic esophagitis
Eosinophilic gastritis
Eosinophilic enteritis
Eosinophilic colitis
Eosinophilic gastroenteritis
Infections:
Schistosomiasis
Anisakiasis
Gastrointestinal basidiobolomycosis
Toxocara canis
Ancylostoma duodenale
Helicobacter pylori
Celiac disease
Hypereosinophilic syndrome
Food allergies/drug hypersensitivity response
Inflammatory bowel diseases
Transplant-associated eosinophilic enteritis
Churg–Strauss syndrome
Toxic injury

Clinical features

EoE is a clinicopathological disease, predominantly affecting young males, that is often confused with GERD as it presents with symptoms of vomiting, dysphagia, heartburn, and food impaction. Features that help to distinguish it from peptic disease include the finding of large numbers of esophageal eosinophils, a history of other atopic diseases, peripheral eosinophilia, and the lack of response of these features to maximal acid suppression (Table 33.2). It is important to remember that the mere presence of esophageal eosinophils is not pathognomonic for a particular disorder as eosinophil infiltration in the esophagus occurs in a variety of states including EoE, eosinophilic gastroenteritis, GERD, parasitic and fungal infections, IBD, hypereosinophilic syndrome (HES), scleroderma, and drug injury [86,109,110]. Estimates suggest that peptic esophagitis is at least 10 times more common than EoE, emphasizing the fact that GERD must be ruled out before assigning a diagnosis of EoE.

All age groups are affected by EoE but the presentation in adults and children is different (Table 33.3). Commonly

Table 33.2 Differences between gastroesophageal reflux disease (GERD) and eosinophilic esophagitis (EoE)

	GERD	EoE
Prevalence	Common (~ 1:10)	Uncommon (~ 1:2000)
Sex preference	Males = females	Males > females
Food impaction	Uncommon	Common
Symptom response to PPI	Yes	No
Endoscopic appearance	Exudates, friability, ulceration	White exudates, furrowing, longitudinal shearing
Histology	Basal zone hyperplasia, papillary lengthening, distal eosinophilia	Increased basal zone hyperplasia, typically more eosinophils/hpf and eosinophils in proximal and distal region
pH probe analysis	Increased acid reflux	Normal
Treatment	Acid blockade, prokinetics	Elimination diet, elemental diet, corticosteroids (topical or systemic), dilation
Pathogenesis	Transient relaxation of the lower esophageal sphincter	IL-5-, IL-13-, and eotaxin-driven response
Complications	Distal stricture, Barrett esophagus	Proximal stricture, esophageal fragility

hpf, high-power field; PPI, proton pump inhibitors.

Table 33.3 Age-related differences in eosinophilic esophagitis

	Pediatric	Adult
Symptoms	Feeding refusal, vomiting, abdominal pain, GERD unresponsive to acid blockade	Dysphagia, food impaction, GERD unresponsive to acid blockade
Endoscopic findings	Whitish exudates, longitudinal furrows, fixed or transient rings, proximal strictures	Same as pediatric findings, small-caliber esophagus
Treatment	Dietary (elemental and food elimination), corticosteroids (systemic and topical)	Same as pediatric treatments plus esophageal dilation

GERD, gastroesophageal reflux disease.

reported symptoms include difficulty feeding, failure to thrive, vomiting, epigastric or chest pain, dysphagia, and food impaction [77,100,111,112]; in fact, these symptoms appear to occur in progressive order as they are the presenting symptoms from infancy into adulthood [92]. Indeed, the most common symptoms observed in adult patients are dysphagia and food impaction [113–115] whereas affected children typically seek evaluation for non-specific symptoms of vomiting, feeding refusal, failure to thrive, and epigastric and periumbilical abdominal pain. As longitudinal studies from childhood into adulthood are not yet available, it is unclear if childhood EoE evolves into adult EoE and whether adult EoE involves the same pathogenic mechanisms as pediatric EoE. Peripheral eosinophilia does not help as it is not a consistent finding.

Early studies described children who were thought to have recalcitrant GERD who underwent Nissen fundoplication. Because symptoms persisted postoperatively, endoscopy was repeated and showed large numbers of mucosal eosinophils in the esophagus. Esophageal narrowing is more commonly seen in teenage and adult patients than in the young child. Consistent with this finding is the fact that adult patients more often undergo treatment with esophageal dilation than children. Typically, children will respond to medical treatments that include dietary and corticosteroid approaches.

Unlike all other segments of the gastrointestinal tract, the esophagus is normally devoid of eosinophils and so the finding of esophageal eosinophils denotes pathology [65]. Presently, the exact number of eosinophils that define EoE is uncertain. Traditionally, fewer than six eosinophils/high-power field (hpf; 400 ×) has been thought to be most indicative of GERD, whereas more than 20–24 eosinophils/hpf appears to be most indicative of EoE [110,112,116]. The anatomical distribution of eosinophils in both the proximal

and distal esophagus typically denotes EoE whereas the accumulation of eosinophils mainly in the distal esophagus is likely to be more characteristic of GERD [76]. In one study [116], the level of eosinophils in the esophagus negatively correlated with the response to conventional anti-GERD therapy. For instance, esophageal eosinophil levels of more than 23/hpf correlated with lack of responsiveness to anti-GERD therapy. However, recent evidence suggests that large numbers of eosinophils (> 25/hpf) can also be seen in GERD in children (unpublished data). Patients with intermediate levels of eosinophils (7–20/hpf) often present a diagnostic dilemma for several reasons. First, the cutoff value of eosinophil concentration has not been vigorously determined. Second, EoE is often a patchy disease and diagnosis is dependent on the number of biopsies obtained; there may be great variability in the maximum level of eosinophils [77]. Other associated pathological findings suggestive of EoE include superficial layering of eosinophils and eosinophilic abscesses. Both gastric and duodenal biopsies are normal in this disease. Clearly, the detection of a molecular marker(s) for this disease would aid in disease diagnosis, as has occurred with celiac disease [117]. Endoscopic esophageal biopsy from a patient with EoE typically shows a high concentration of eosinophils, readily detectable eosinophil granule proteins in the extracellular regions, and the marked expansion of the basal layer (basal zone hyperplasia) (Fig. 33.1).

Radiographic studies from patients with EoE reveal proximal esophageal stricture, small-caliber esophagus, and Schatzki rings. Endoscopic analysis has shown a number of features including whitish papules or exudates consistent with eosinophilic desquamation. This finding can be confused with *Candida albicans* infection or use of topical anesthetic spray. Additional findings include longitudinal furrows, proximal strictures, and a fragile esophageal mucosa, which can occur when merely passing the endoscope across the esophageal epithelium and which can be quite painful [76,83,111,118,119]. Nearly 20% of patients with EoE have a normal endoscopic appearance [120], emphasizing the need for endoscopic biopsies.

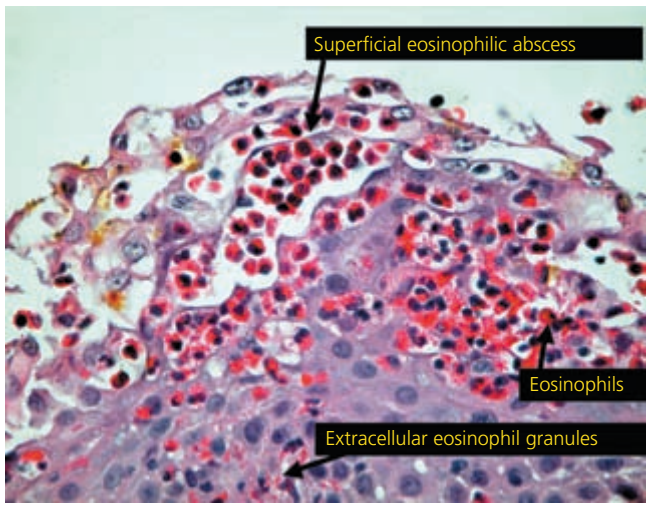

Figure 33.1 A representative microscopic image of eosinophilic esophagitis (EoE). This photomicrograph shows the classic findings of EoE following endoscopic biopsy and histological staining with hematoxylin and eosin. Note the characteristic abundant eosinophils (arrowhead), the readily detectable extracellular eosinophil granule constituents (arrow), and the eosinophilic microabscess near the surface (asterisk). Original magnification × 400.

Preliminary evidence suggests that symptoms associated with EoE result from esophageal dysmotility. Assessment of the esophageal wall with endoscopic ultrasound demonstrates significant submucosal and muscular inflammation in children with EoE compared with non-inflamed esophageal tissue. Although stationary esophageal manometric testing has revealed normal esophageal function, one preliminary study examining prolonged esophageal motility with 24-h ambulatory monitoring demonstrated increased isolated contractions compared with tracings derived from normal non-inflamed esophagi. Importantly, these contractions correlated with observed symptoms. Because food impaction is a primary complaint in EoE, one speculates that the esophagus can undergo intermittent non-fixed contractions leading to dysphagia with or without obstruction. The exact pathogenesis of the motor disturbances is unclear, but eosinophil activation and degranulation has been postulated as a possible etiology [121–123]. This is supported by the finding of increased deposition of the eosinophil granule protein, major basic protein (MBP), in the epithelium of patients affected by EoE with food impaction.

The assessment of EoE includes an extended allergy evaluation looking for food and aeroallergen sensitization, either by skin-prick or RAST (radioallergosorbent) testing, and the exclusion of GERD (typically by using anti-GERD therapy) as well as other causes of eosinophils in the esophagus [76,100,101]. A recent study has suggested that the evaluation of food protein sensitization by delayed skin-patch testing increases the identification of food allergy compared with skin-prick testing alone [101]. Of note, the presence of GERD does not exclude the diagnosis of EoE or food allergy, demonstrating the importance of a food allergy evaluation in these patients [75,86,124]. An essential question in understanding EoE is its relationship with other forms of esophagitis. In particular, it remains possible that the pathological level of eosinophils in the esophagus in patients with GERD and EoE represents a continuum. As such, the pathological processes in the esophagus of patients with EoE and GERD may be shared in part.

Epidemiology

EoE is a global health disease that has been reported in Australia [125], Brazil [124], England [123], Italy [126],

Japan [127], Spain [128,129], and Switzerland [130]. Although the incidence of primary EoE has not been rigorously calculated, a mini-epidemic has been noted over the last decade. Liacouras and his group [131] at the Children's Hospital of Philadelphia have found that 8%–10% of their pediatric patients with GERD-like symptoms unresponsive to acid blockade have EoE. Furuta and colleagues [76] at the Boston Children's Hospital have reported that 6% of their patients with esophagitis have EoE. Over a 16-year observation period, Straumann and Simon [132] have documented a prevalence of approximately 1:4000 adults in Switzerland. Croese and colleagues [125] have reported EoE to be present in 1:70 000 adults in an Australian provincial city. Finally, Rothenberg and colleagues have noted that EoE occurred in 1:2000 children in the Cincinnati metropolitan area over a 5-year time period (ref. 92 and unpublished findings).

Therapy

There is no uniformly agreed upon therapy for EoE, probably because of the lack of controlled clinical trials and studies on the natural history of the disorder [133,134]. At present, therapy for EoE is based on antigen elimination trials, antiinflammatory approaches, and physical dilation when strictures are present. The last approach, which has been the strategy reported for adults, temporarily reduces symptoms of dysphagia; however, it includes the significant risk of rupture and hemorrhage, needs to be repeated regularly, and does not improve the underlying inflammation [135]. An antigen elimination approach in sensitized individuals (e.g., aeroallergen avoidance and food elimination diets) is typically unsatisfactory or practically difficult (when patients are sensitized to many allergens), probably because the current allergen sensitization tests (skin-prick and patch tests, as well as antigen-specific plasma IgE levels) are not optimal for detecting sensitization. A diet consisting exclusively of an elemental (amino acid-based) formula frequently improves symptoms and normalizes esophageal pathology [104,111]. However, this approach is often not tolerated (especially in older individuals), frequently requires a surgically placed feeding tube, and can be financially costly (e.g., thousands of dollars per month). Glucocorticoids (systemic [83] or topical [136,137]) have been used with apparently satisfactory results. Systemic steroids are used for acute exacerbations, whereas topical glucocorticoids are used to provide long-term control. In particular, several studies have shown the usefulness of swallowed fluticasone (patients simply swallow fluticasone administered from a standard asthma inhaler). Fluticasone therapy is associated with normalization of esophageal eosinophilia, levels of CD3 and CD8 T cells, and epithelial hyperplasia, as assessed by staining for the proliferation nuclear antigen Ki-67 [138,139]. It is now appreciated that a significant fraction of patients are glucocorticoid resistant [139]. Swallowed fluticasone likely acts directly upon the esophageal tissue, but this has not yet been proven. Other antiinflammatory agents such as leukotriene receptor antagonists have been advocated, but they have not been shown to reverse esophageal pathology [140,141]. The mechanism by which any therapeutic intervention improves EoE has not been established and is under active investigation.

Pathogenesis

The etiology of EoE is poorly understood, but allergy has been implicated. In fact, the majority of patients have evidence of food and aeroallergen hypersensitivity as defined by skin-prick or RAST tests; however, only a minority have a history of food anaphylaxis [76], indicating distinct mechanisms compared with simple IgE-mediated mast cell/basophil activation. Substantial evidence is accumulating that EoE is associated with a Th2-type immune response. In particular, elevated levels of eosinophil-active Th2 cytokines (e.g., IL-4, IL-5, and IL-13) as well as mast cells are present in the esophagus of EoE patients [100,142]. In addition, experimental models of EoE can be induced in mice by allergen exposure, as well as by overexpression of Th2 cytokines [143,144]. In particular, repeated intranasal exposure to the aeroallergen *Aspergillus fumigatus* induces simultaneous eosinophilic airway and esophageal inflammation (without inducing gastrointestinal eosinophilia) [143]. Furthermore, intratracheal delivery of human or mouse IL-13 also induces experimental EoE [145], and this process can be blocked with a therapeutic anti-human IL-13 antibody [146].

More recently, epicutaneous allergen sensitization has been shown to potently prime for respiratory allergen-induced experimental EoE [147]. This finding may be particularly important for understanding EoE as a large fraction of EoE patients suffer from preceding allergic skin disease (atopic dermatitis) [100]. Collectively, these experimental systems demonstrate an intimate connection between the development of eosinophilic inflammation in the respiratory tract and esophagus and not only external allergic triggers but also intrinsic Th2 cytokines.

It is interesting to note that patients with EoE sometimes report seasonal variations in their symptoms; preliminary studies have recently documented seasonal changes in esophageal eosinophil levels [148]. Based on these results, as well as clinical observations, some investigators have referred to EoE as "asthma" of the esophagus [149].

Experimental modeling in mice has established that Th2 signaling is required for induction of experimental EoE. In particular, mice with the targeted deletion of STAT6 are protected from allergen- and IL-13-induced experimental EoE [145,147]. Furthermore, IL-13-deficient mice have impaired allergen-induced EoE [147]. As IL-13 is a potent inducer of

the eotaxins by a STAT6-dependent mechanism (based on studies primarily in the context of the lung) [13], it is interesting to speculate that IL-13 (and STAT6) may induce EoE by up-regulating the production of eotaxins in the esophagus. Indeed, the promoters of all three eotaxin chemokines contain STAT6 binding sites that have been shown to be operational in vitro [18–22]. However, the mechanism has remained elusive as the eotaxins have not yet been demonstrated to be overproduced in EoE, and eotaxin-1-deficient mice develop only a modest attenuation of experimental EoE [127,142,143].

A central role for IL-5 in EoE pathogenesis is emerging. Overexpression of IL-5 (by pharmacological or transgenic delivery) induces EoE, and neutralization of IL-5 (with antibodies or gene targeting) completely blocks allergen- or IL-13-induced EoE in mice [143–145]. Notably, in an early phase I/II trial designed to test humanized anti-IL-5 antibody therapy in hypereosinophilic patients, a remarkable response in the first treated EoE patient has been observed, with improved clinical symptoms and markedly reduced esophageal eosinophil levels [150].

Potential role of eosinophils in EoE

In vitro studies have shown that eosinophil granule constituents are toxic to a variety of tissues including intestinal epithelium [151]. Eosinophil granules contain a crystalloid core composed of MBP-1 (and MBP-2), and a matrix composed of eosinophil cationic protein (ECP), eosinophil-derived neurotoxin (EDN), and eosinophil peroxidase (EPO) [152]. These cationic proteins share certain proinflammatory properties but differ in other ways. For example, MBP, EPO, and ECP have cytotoxic effects on epithelium in concentrations similar to those found in biological fluids from patients with eosinophilia. Additionally, ECP and EDN belong to the ribonuclease A superfamily and possess antiviral and ribonuclease activity [153,154]. ECP can insert voltage-insensitive, ion-nonselective toxic pores into the membranes of target cells and these pores may facilitate the entry of other toxic molecules [155]. MBP directly increases smooth muscle reactivity by causing dysfunction of vagal muscarinic M_2 receptors [156]. MBP also triggers degranulation of mast cells and basophils [157]. Indeed, evidence is emerging that mast cells and eosinophils are both involved in EoE [142]. Triggering of eosinophils by engagement of receptors for cytokines, immunoglobulins, and complement can lead to the generation of a wide range of inflammatory cytokines including IL-1, -3, -4, -5, -13, GM-CSF, TGF-α/β, tissue necrosis factor (TNF)-α, RANTES, MIP-1α, and eotaxin-1, indicating that they have the potential to modulate multiple aspects of the immune response [158,159]. In fact, eosinophil-derived TGF-β is linked with epithelial growth, fibrosis, and tissue remodeling [160,161]; this may be particularly important in promoting the chronic inflammation and structural changes associated with EoE (e.g., fibrosis and strictures) [113]. Indeed, depletion of murine eosinophils with anti-IL-5 therapy abates allergen-induced epithelial hyperplasia in the esophagus [143], and the level of human esophageal eosinophils correlates with the degree of epithelial cell Ki-67 antigen staining [139]. Additionally, eosinophils can directly activate T cells by antigen presentation, which has been demonstrated in vitro and in vivo [162,163]. Further eosinophil-mediated damage is caused by toxic hydrogen peroxide and halide acids generated by EPO and by superoxide generated by the respiratory burst oxidase enzyme pathway in eosinophils. Eosinophils also generate large amounts of the cysteinyl leukotriene C4 (LTC4), which is metabolized to LTD4 and LTE4. These three lipid mediators increase vascular permeability and mucus secretion, and are potent stimulators of smooth muscle contraction [164]. Indeed, leukotriene modifiers appear to improve clinical symptoms in EoE patients [141].

Clinical investigations have demonstrated extracellular deposition of MBP in the esophagus of patients with EoE and other forms of EGID [71,165–169] and have shown a correlation between the level of eosinophils and disease severity [84]. Electron microscopy studies have revealed ultrastructural changes in the secondary granules (indicative of eosinophil degranulation and mediator release) in duodenal samples from patients with EGID [79,122]. Furthermore, Charcot–Leyden crystals, remnants of eosinophil degranulation, are found on microscopic examination of stools obtained from patients with eosinophilic gastroenteritis [95,170].

Summary and future directions

Eosinophilic esophagitis is a chronic allergic disease that can easily be confused with GERD. Although the exact pathophysiology of EoE has not been defined, it is likely that intermittent esophageal contraction or fibrotic narrowing leads to dysphagia and food impaction. The pathogenesis of EoE appears to be related to overexpression of IL-5, IL-13, and possibly eotaxins, which leads to chronic eosinophilic inflammation. Present studies have focused on clinicopathological features of EoE whereas future studies will likely define the natural history (e.g., the relationship between pediatric and adult EoE), the genetic basis of EoE including the role of particular single nucleotide polymorphisms, and non-invasive markers of disease activity. Whereas current therapy is primarily focused on anti-allergy therapy (including glucocorticoids), it is hopeful that molecular dissection of disease pathogenesis will lead to targeted therapy (such as anti-IL-5 and/or anti-CCR3 antibodies). This will lead to improved treatment guidelines and better care for the affected patients.

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A ESOPHAGUS

34 Esophageal infections and disorders associated with acquired immunodeficiency syndrome

C. Mel Wilcox

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Because of the remarkable effectiveness of highly active antiretroviral therapy (HAART) and continued advancements in prophylaxis for opportunistic infections such as cytomegalovirus (CMV) in high-risk transplant patients, the prevalence of esophageal infections continues to fall in developed countries [1]. In contrast, the human immunodeficiency virus (HIV) epidemic continues unabated in developing countries where gastrointestinal disorders parallel what were seen in developed countries more than two decades ago [2]. Despite these advancements, there still remains a number of patients at risk for these diseases, particularly as our older population grows, immunosuppressive therapy becomes more widespread for a variety of indications, and the use of organ transplantation expands. Timely and accurate diagnosis of esophageal infections is critical because treatment is highly effective, results in rapid symptomatic improvement, and often yields a clinical cure. This chapter focuses on the specific causes of esophageal infections, in particular epidemiology, pathology, presentation, diagnosis, and therapy. Esophageal disorders associated with HIV infection and AIDS are also reviewed here.

Epidemiology and predisposing factors

Primary esophageal infection is rare in an otherwise healthy person in whom no permissive factor is present. In this setting, the most common pathogen is *Candida*, with herpes simplex virus (HSV) less commonly involved [3,4]. In general, immunocompetent patients who develop esophageal infection have conditions, either local or systemic, that weaken esophageal defense mechanisms, such as alteration of normal oroesophageal flora (e.g., antibiotics) or hampering of esophageal emptying (e.g., achalasia). Infection in adjacent structures may spread into and involve the esophagus secondarily.

More commonly, some form of humoral or cellular immunodeficiency, either iatrogenic, inherent, or a combination, leads to esophageal infection. Underlying conditions that predispose to infections include diabetes mellitus, alcoholism, malnutrition, malignant diseases, and advanced age. Transplantation predisposes to infections through variable mechanisms, most of which affect both B-cell and T-cell number and function, including the use of immunosuppressive agents, chemotherapy, and neutropenia. Episodes of rejection in recipients of solid organ transplants are also commonly complicated by infection because these patients are given more immunosuppression, including powerful agents such as antithymocyte globulin.

Without antimicrobial prophylaxis, infection after transplantation has a predictable time course. Bacterial and fungal infections are most common during the initial months after transplantation, because it is during this period that granulocyte number or function is most severely compromised. HSV infection also tends to occur early after transplantation, related to reactivation of disease, whereas CMV typically presents 2–6 months after transplantation at a time when neutropenia is common and T-cell function is most severely impaired. The development of opportunistic infections in HIV-infected patients reflects severe immunodeficiency; the risk for esophageal infections increases markedly when the CD4 lymphocyte count falls below 200/mm^3, with most infections occurring when the count is less than 100/mm^3 [5].

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Fungal infections

Candida species

Epidemiology

Candida species are the most common esophageal pathogens, primarily *C. albicans* but occasionally *C. tropicalis, C. parapsilosis, C. glabrata*, and *C. dublinensis*. These organisms are normal components of the oral flora and their growth is kept in check by bacterial commensals. Conditions predisposing to *Candida* esophagitis in the "normal" host include antibiotic use, inhaled or ingested corticosteroids, antisecretory therapy (histamine H_2 receptor blockers, proton pump inhibitors), or hypochlorhydric states, diabetes mellitus, alcoholism, malnutrition, old age, head and neck radiotherapy, and esophageal motility disturbances. Alterations in cellular immunity lead to candidal colonization and superficial infection, whereas granulocytes function to prevent invasive disease and dissemination. The congenital immunodeficiency disorder chronic mucocutaneous candidiasis may also be complicated by *Candida* esophagitis. Improvements in immunosuppressive regimens, targeted prophylactic antifungal therapy, and empirical use of fluconazole in symptomatic patients have dramatically reduced the incidence of candidal infections in recipients of solid organ and bone marrow transplants [6]. Candidiasis remains the most common esophageal infection in patients with AIDS; it represents approximately 50% of esophageal infections and it frequently coexists with other esophageal diseases [7].

Pathology

The gross pathological appearance of esophageal candidiasis can range from a few white or yellow plaques on the mucosal surface to a dense, thick plaque that coats the esophageal mucosa (Fig. 34.1a). This plaque material is composed of desquamated squamous epithelial cells, admixed with fungal organisms, inflammatory cells, and bacteria [8] (Fig. 34.1b,c). In the absence of granulocytopenia or coinfection, true ulceration is infrequent (Table 34.1).

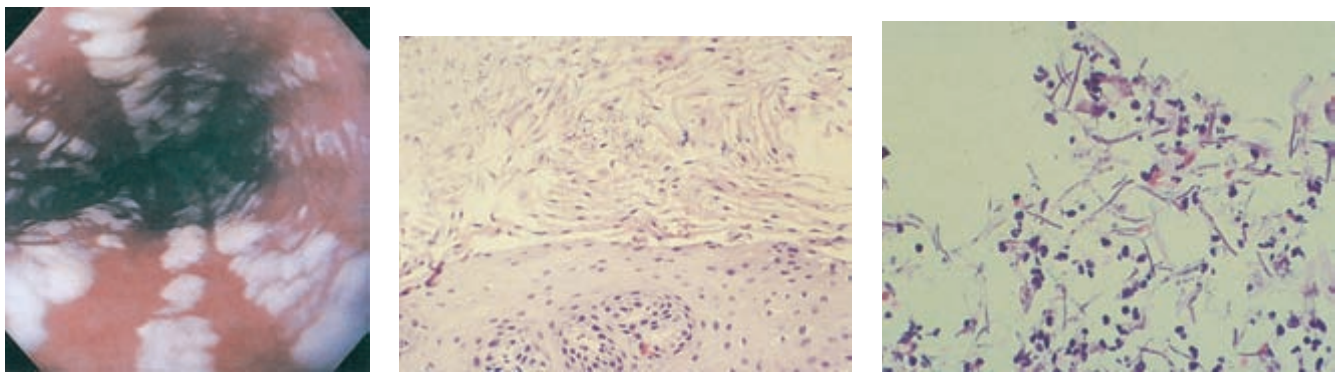
Clinical manifestations and complications

Although on occasion this infection is detected incidentally in an asymptomatic patient [3], the usual clinical presentation of esophageal candidiasis is dysphagia, with odynophagia less prominent (Table 34.2). Esophageal symptoms range from mild difficulty with swallowing to severe pain resulting in an inability to eat and secondary dehydration. When odynophagia is very severe, one must consider other causes or coinfections, particularly in patients with AIDS.

Physical examination may be helpful. Approximately two-thirds of patients with AIDS and esophageal candidiasis have oral candidiasis (thrush) [9]. Patients with chronic mucocutaneous candidiasis may have fungal involvement of various mucous membranes, hair, nails, and skin with a history of adrenal or parathyroid dysfunction. As the infection is generally superficial, complications of esophageal candidiasis are very rare.

Diagnosis

Esophageal candidiasis should be suspected in any patient at risk of esophageal infection who complains of dysphagia or odynophagia. The presence of thrush further supports this diagnosis, but the absence of oral involvement does not exclude esophageal disease. On a barium esophagram, *Candida* esophagitis is characteristically manifested by multiple plaque-like lesions, often in a linear configuration; when severe, these lesions become confluent to cause a "shaggy" appearance of the esophagus resembling ulcerations (see



(a) (b) (c)

Figure 34.1 *Candida* esophagitis. **(a)** Endoscopic photograph shows multiple raised plaques involving the esophagus with normal intervening mucosa. **(b)** Desquamated squamous epithelial cells admixed with fungi and inflammatory cells adherent to the mucosa. The underlying squamous epithelium appears normal. **(c)** Close-up view of the plaque material demonstrates mycelia and spores typical of *Candida*.

Table 34.1 Pathological findings of esophageal infections

	Candida	Other fungi	Viruses	IEU	Bacteria	Mycobacteria	Parasites
Plaque	++++	+	+	–	++	+	++
Ulcer	+	++	++++	++++	+++	+++	+
Stricture	–	+	++	+	–	++	–
Fistula	–	+	+	+	–	+++	–
Mass	+	+	+	+	–	++	–

–, absent; +, rare finding; ++, occasional finding; +++, common finding; ++++, uniformly present.
IEU, idiopathic esophageal ulcer.

Table 34.2 Symptoms and signs of esophageal infections

	Candida	Viruses	IEU	Bacteria	Mycobacteria	Parasites
Dysphagia	++++	+	+	+	++	+
Odynophagia	+++	++++	++++	+++	++	++
Heartburn	+	+	+	+	+	+
Chest pain	+	+++	+++	++	++	+
Fever	–	++	–	+++	++++	–
Bleeding	+	++	++	+	+	+

–, absent; +, rare finding; ++, occasional finding; +++, common finding; ++++, uniformly present.
IEU, idiopathic esophageal ulcer.

Fig. 34.1) [10]. A large, well-circumscribed ulceration should not be attributed to *Candida*. Importantly, a normal barium esophagram does not exclude esophageal candidiasis. The presence of severe odynophagia may limit the ability of the patient to drink barium and thus hampers the utility of barium studies.

Endoscopic examination of the esophagus is the most accurate method of diagnosing esophageal candidiasis. The gross endoscopic appearance of *Candida* esophagitis is usually diagnostic and may be graded according to published criteria [8]. During endoscopy, mucosal lesions can be brushed and submitted for cytological evaluation or examined by biopsy for histological diagnosis. Multiple biopsies are essential to exclude coexisting disorders when ulceration is identified endoscopically. The use of periodic acid–Schiff or Gomori methenamine silver stain helps to highlight the organisms. Cytological examination of esophageal brushings is more sensitive than histological examination of biopsy specimens because organisms may be washed off tissue surfaces in mild superficial candidiasis (i.e., grades 1 and 2) during processing of biopsy specimens. Rarely, positive cytology but negative histology indicates colonization rather than infection. Skin testing and serological tests for candidiasis play no role in the diagnosis of *Candida* esophagitis.

In patients with AIDS and thrush, the presence of dysphagia or odynophagia usually indicates *Candida* esophagitis [7]. Given the prevalence of *Candida* esophagitis in AIDS, in the symptomatic patient with associated thrush, an empirical trial of antifungal therapy may be instituted, reserving endoscopy for those patients who fail to respond. However, this symptom complex does not exclude coinfection with other pathogens, and one-third of patients with esophageal candidiasis do not have thrush. Following an empirical trial, further evaluation should not be delayed for longer than 1 week if severe symptoms persist, because the response to antifungal therapy is rapid, with substantial clinical improvement occurring in most patients within days [7]. When patients fail to improve with empirical antifungal therapy, endoscopy should be performed, given that disorders other than candidiasis are identified in most patients [11]. The role of empirical antifungal therapy in symptomatic immunosuppressed patients who do not have AIDS has not been well studied, although this approach is commonly practiced.

Treatment

Both oral and intravenous medications are available that are highly effective for the treatment of *Candida* esophagitis (Table 34.3). In general, oral therapies should be first initiated,

Table 34.3 Treatment regimens for common esophageal diseases in AIDS

Pathogen	Drug	Dosage	Route	Duration	Efficacy (%)
Candida	Ketoconazole	200–400 mg/day	p.o.	7–14 days	< 80
	Fluconazole	100 mg/day	p.o./i.v.	7–14 days	≈ 80
	Itraconazole	200 mg/day	p.o.	7–14 days	≈ 80
	Amphotericin B	0.5 mg/kg/day	p.o./i.v.	7 days	> 95
	Caspofungin	50 mg/day	i.v.	7 days	> 90
Cytomegalovirus	Ganciclovir	5 mg/kg b.i.d.	i.v.	2–4 weeks	≈ 75
	Foscarnet	90 mg/kg b.i.d.	i.v.	2–4 weeks	≈ 75
	Cidofovir	5 mg/kg weekly	i.v.	2–4 weeks	≈ 75
Herpes simplex virus	Acyclovir (aciclovir)	400 mg 5×/day	p.o./i.v.	14 days	> 90
	Valacyclovir (valaciclovir)	1 g t.i.d.	p.o.	14 days	> 90
	Famciclovir	500 mg t.i.d.	p.o.	14 days	> 90
	Foscarnet	90 mg/kg b.i.d.	i.v.	14 days	> 95
	Ganciclovir	5 mg/kg b.i.d.	i.v.	14 days	> 95
Idiopathic ulcer	Prednisone	40 mg/day taper	p.o.	4 weeks	> 90
	Thalidomide	200–300 mg/day	p.o.	4 weeks	> 90

reserving intravenous treatment for refractory disease or for patients who have contraindications to orally administered medication. Although candidal species other than *C. albicans* may cause esophagitis, speciation is not widely employed because reliable culturing and sensitivity testing are lacking at most centers and the treatment is generally the same. For those patients with mild disease, minimal immunocompromise, or readily reversible immunodeficiency, an abbreviated course of therapy with an oral azole should be given. Immunocompromised transplant recipients and patients with AIDS with *Candida* esophagitis are best treated with systemically absorbed agents (azoles). In addition, patients with granulocytopenia are at significant risk of disseminated candidal infection, thus warranting the use of systemically acting agents.

Orally administered systemic agents, all of which have efficacy for *Candida* esophagitis, include ketoconazole (Nizoral), fluconazole (Diflucan), and itraconazole (Sporanox). These agents, like other azoles, alter fungal cell membrane permeability by cytochrome P450-dependent interference with ergosterol biosynthesis resulting in fungal cell injury and death. The newer triazoles (itraconazole and fluconazole) have a greater affinity than the imidazoles (miconazole and ketoconazole) for fungal cytochrome P450 enzymes [12]. Although other agents such as clotrimazole and nystatin may be effective for oral candidiasis and for prophylaxis against esophageal involvement, these agents are much less effective as primary therapy for esophageal candidiasis.

Although ketoconazole therapy (200–400 mg/day) is effective for the treatment of esophageal candidiasis, its potent inducement of the P450 system has led to its replacement by newer azoles. Like ketoconazole, itraconazole absorption is reduced by increasing gastric pH [13]. Its half-life is 24–42 h and further dose increases lengthen the drug's half-life and improve efficacy [13].

Large randomized studies suggest that fluconazole (100 mg/day), the absorption of which does not depend on pH, has significantly greater efficacy for the treatment of *Candida* esophagitis in AIDS than ketoconazole (200 mg/day) [12] and itraconazole [14]. Both itraconazole and fluconazole are available in oral forms (both pill and solution) and fluconazole is also available in an intravenous preparation [15]. Fluconazole is minimally metabolized and is excreted unchanged in the urine. Unlike ketoconazole and itraconazole, fluconazole is highly water soluble, is minimally protein bound, and has a half-life of approximately 30 h if renal function is normal. The adverse effects of ketoconazole, fluconazole, and itraconazole are primarily dose dependent and include nausea, hepatotoxicity, and inhibition of steroid production. Minor increases in aminotransferases are common with these three agents but do not warrant drug discontinuation [16]. Finally, because of the effects on hepatic microsomal enzymes, all three azoles inhibit the metabolism of cyclosporine (ciclosporin) and result in an increase in cyclosporine blood levels, and this effect is most pronounced with ketoconazole.

Another major family of antifungal agents consists of the polyene antibiotics, represented by amphotericin and nystatin (see Table 34.3). These agents bind irreversibly to sterols in fungal cell membranes, thereby altering the permeability characteristics of the membrane and causing cell death. Nystatin is effective for treating thrush but much less so for esophageal disease. Although amphotericin B (Fungizone) is a highly effective treatment for systemic mycoses, its

severe side effects, coupled with the availability of effective alternative agents, have limited its use for the treatment of esophageal candidiasis.

The newest class of antifungal agents is the echinocandins. These agents have a novel mechanism of action as glucan synthase inhibitors, which inhibits fungal cell wall synthesis [17]. The first agent in this class, caspofungin, has activity against both *Aspergillus* and *Candida* species including non-*albicans* species. Prospective studies have shown it to be as efficacious and as well tolerated as fluconazole for AIDS patients with *Candida* esophagitis and as effective and better tolerated than amphotericin B for azole-resistant esophageal candidiasis [18,19].

Prophylaxis

The use of nystatin or azoles for the prevention of esophageal candidiasis in patients with cancer and in transplant recipients has yielded mixed results, and it can be problematic, especially for those receiving cyclosporine [20]. Primary prophylaxis has not been recommended in patients with AIDS, although the long-term use of fluconazole in AIDS has been associated with a reduction in cryptococcal disease [21].

Drug resistance

Because of widespread use, azole resistance has become a well-recognized problem in HIV-infected patients. This represents a less common clinical problem today with the widespread use of HAART. Both the cumulative dose of azole and severe immunodeficiency have been shown to be highly associated with the development of resistance [22]. In patients with AIDS, clinical resistance correlates with in vitro resistance. When resistance occurs, increasing the dose of azole, switching to another azole, or use of an oral solution of itraconazole may be tried, in higher doses because cross-resistance is often present. Intravenous amphotericin B, in doses of 10–20 mg/day, may be effective when high-dose (> 400 mg/day fluconazole) therapy fails; however, caspofungin is equally as efficacious as amphotericin B in these patients, and is better tolerated, making it the drug of choice in this setting [17,19]. Improvement in immune function with HAART is also an important component of the treatment of resistant candidiasis.

Other fungi

Epidemiology

Esophageal involvement with other fungi is rare [23]. Most instances of histoplasmosis and blastomycosis esophagitis represent secondary esophageal involvement from mediastinal lymph nodes rather than primary esophageal infection [24,25]. Although no particular geographical distribution within the United States has been reported for blastomycosis, histoplasmosis is endemic in the Midwestern states and the Mississippi Valley. Mucormycosis and aspergillosis esophagitis have also been described [26,27].

Pathology, clinical manifestations, and complications

Other than the development of fistula, there are no unique pathological features of these fungal infections (see Table 34.1).

Diagnosis

Histoplasmosis should be considered in endemic areas and if extraesophageal manifestations such as hilar adenopathy, calcification or atelectasis of adjacent pulmonary tissue, or splenic calcification are present. Esophageal blastomycosis should be considered in patients with skin involvement and dysphagia. Barium esophagraphy or endoscopy may show a focal area of extrinsic compression of the esophagus, usually in the region of the carina, ulcer, or fistula. As with other esophageal infections, the appearance of the abnormality at barium esophagram, the endoscopic appearance at endoscopy, and the use of endoscopic brushings and biopsies with appropriate culture and staining will establish the diagnosis. Serological tests are not useful because of the high prevalence of positive results in endemic areas. A urine antigen test is highly specific for the diagnosis of disseminated histoplasmosis [24].

Treatment

Although histoplasmosis may resolve without antifungal therapy in the normal host, therapy should be administered with an induction course of amphotericin B followed by maintenance itraconazole. Blastomycosis responds to itraconazole and amphotericin B. Systemic aspergillosis should be treated with a new azole agent voriconazole [28]. Surgery may be required for drainage of abscesses or excision of fistulae.

Viral infections

As with all esophageal infections, the prevalence of viral esophagitis has fallen in recent years. In the transplant setting, this reduction has resulted from targeted antiviral prophylaxis for herpesviruses in high-risk transplant recipients, the use of CMV-seronegative organs and blood products for seronegative recipients, and the use of leukocyte-depleted platelets for patients after bone marrow transplantation. A overall reduction in viral esophagitis attributable to the implementation of HAART has been also observed in HIV-infected patients [1]. Nevertheless, in patients who fail such therapy or for undiagnosed patients, viral esophagitis continues to be clinically relevant [5].

Herpes simplex virus

Epidemiology

HSV type 1 (HSV-1) is one of three herpesviruses that affect the esophagus, the others being CMV and varicella-zoster virus (see sections on cytomegalovirus and other viruses).

HSV-2 rarely involves the esophagus. After *Candida* species, HSV-1 is the next most frequent agent causing infectious esophagitis. Although well recognized as an esophageal pathogen in otherwise healthy people, HSV-1 esophagitis has been reported most often in patients with immunosuppression or other predisposing factors. After transplantation, HSV occurs as frequently as CMV as a cause of esophageal disease [23], whereas in patients with AIDS, HSV esophagitis is relatively uncommon and is much less frequent than CMV infection [29].

Pathology

HSV infection is generally limited to squamous mucosa, where the earliest manifestation is a vesicle. As these vesicles enlarge and ulcerate, they may coalesce to form larger lesions. Usually, the intervening mucosa between these lesions is normal. Microscopic examination of the squamous epithelial cells at the edge of the ulcers reveals multinucleation, ground-glass nuclei, and eosinophilic Cowdry type A inclusion bodies that may occupy half of the nuclear volume. These inclusion bodies may be surrounded by haloes and with time they become more basophilic, filling, enlarging, and deforming the nucleus.

Clinical manifestations and complications

HSV-1 esophageal infection commonly presents with the sudden onset of severe odynophagia, often resulting in an inability to swallow liquids or solids. Herpes labialis (i.e., cold sores) and oropharyngeal ulcers may frequently coexist, antedate, or develop during the esophageal infection, whereas skin infection is rare [9,29]. In untreated immunocompetent persons, spontaneous resolution of HSV-1 esophageal infection occurs 1–2 weeks after the onset of symptoms. Complications are rare and include bleeding, tracheoesophageal fistula, or dissemination [23].

Diagnosis

Esophageal disease caused by HSV-1 usually appears in radiographic studies as focal ulceration on a background of normal mucosa; vesicles are infrequently present (Fig. 34.2). These ulcers have been described as stellate or volcanic in appearance, with fewer propensities to form the longitudinal or linear lesions that are commonly seen in CMV infection [30]. Severe, diffuse herpetic esophagitis may result in a cobblestone or "shaggy" mucosal appearance resembling *Candida* esophagitis [30]. Although the radiographic appearance may be suggestive, definitive diagnosis of herpetic esophagitis requires endoscopic mucosal biopsies. The endoscopic appearance of herpetic esophagitis reflects the pathological changes (see Table 34.1), with discrete, usually small (< 2 cm), well-circumscribed ulcers [31], diffuse erosive esophagitis, or, rarely, vesicles. Small scattered lesions covered with exudates can mimic esophageal candidiasis. Given the pathophysiological mechanism of disease, deep ulcers, as seen with CMV, are very rare. Cytological or histological brushings or biopsies should be taken from the edge of an ulcer because the viral cytopathic effect is best identified here rather than in the granulation tissue in the ulcer bed (Fig. 34.3). Immunohistochemistry on biopsy samples using specific monoclonal antibodies to HSV will help to confirm the diagnosis when the viral cytopathic effect is infrequent. Viral culture may also help to establish a definitive diagnosis. As with other causes of infectious esophagitis, serological tests are unhelpful in establishing the diagnosis.

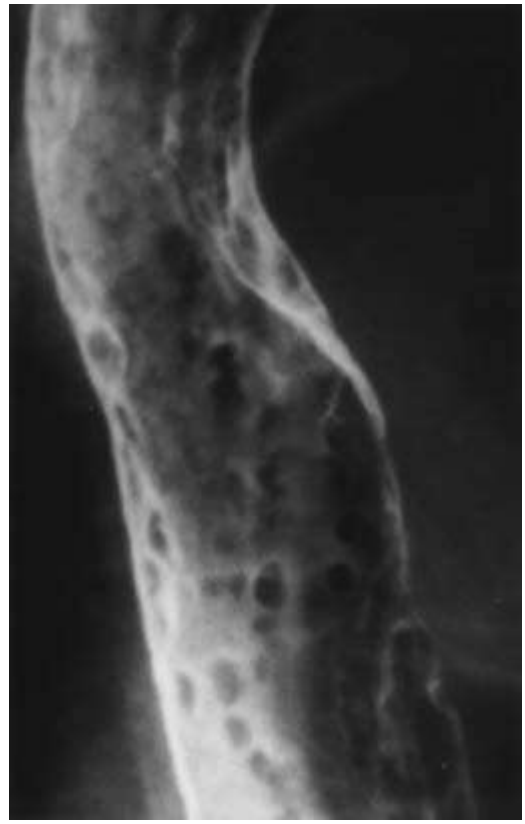


Figure 34.2 Herpes simplex virus esophagitis. Multiple vesicles and ulcers are present throughout the esophagus. Courtesy of Robert Koehler, MD.

Treatment

Uncontrolled trials and vast clinical experience in both immunocompetent and immunodeficient patients, including those with AIDS [32], suggest efficacy of acyclovir (aciclovir), a nucleoside analogue, for the therapy of esophageal disease [33]. Valacyclovir (valaciclovir), a prodrug of acyclovir, and famciclovir have largely replaced acyclovir as oral therapy given their longer half-lives. Although not studied for esophagitis, they have shown equivalency for genital HSV disease and, as with acyclovir, minimal toxicity.

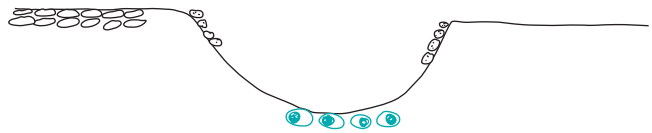


Figure 34.3 Location of viral cytopathic effect in esophageal ulceration.

Although spontaneous resolution of HSV-1 esophagitis is common in the immunologically normal host, because of its safety and efficacy, therapy is commonly instituted in all patients regardless of immune status. When oral intake is hampered by severe odynophagia or when there is a question of drug absorption, intravenous administration is required. Side effects of intravenous acyclovir therapy are few and appear limited to irritation of veins used for drug infusion and rash. Although rare, drug resistance should be suspected when there is clinical failure of acyclovir; in this setting, foscarnet (see Table 34.3) is the drug of choice and will lead to clinical cure in most patients [34]; cidofovir may also be used. Acyclovir is effective prophylaxis for HSV antibody-positive patients undergoing transplantation. Long-term secondary prophylaxis may be required when immunodeficiency persists because the relapse rate is high.

Cytomegalovirus

Epidemiology

CMV continues to dominate as one of the most common opportunistic infections across all risk groups. This frequency relates to the high background seropositivity rates in the general population with even higher rates in developing countries and homosexual men [35]. In transplant recipients who receive no antiviral prophylaxis, CMV and HSV are equally common esophageal pathogens [23]. In contrast, CMV is the most frequent cause of esophageal ulcer in patients with AIDS [29].

Pathology

The most prominent histological feature of CMV esophagitis is mucosal ulceration (see Table 34.1). Although there is variability, deep ulcers are very characteristic of disease in AIDS, whereas in immunosuppressed transplant recipients, lesions tend to remain more superficial. In contrast to HSV, the viral cytopathic effect of CMV is present in endothelial and mesenchymal cells in the granulation tissue of the ulcer base and not in the squamous epithelium. Inclusions are large (*cytomegalo*) and often have an eosinophilic appearance that may be present in the nucleus and cytoplasm (Fig. 34.4a). Because these inclusions may be atypical in appearance in patients with AIDS [36], immunohistochemical stains are useful in confirming the presence of CMV and often highlight more infected cells than can be appreciated on routine hematoxylin and eosin staining. As with other esophageal infections, CMV may coexist with HSV or *Candida*, especially in patients with AIDS. Whereas HSV leads to disease by a direct cytopathic effect on squamous epithelium, the pathogenesis of disease caused by CMV is not well understood. It has been speculated that CMV mucosal disease is the result of ischemia caused by endothelial cell involvement. Inflammatory cytokines, such as tumor necrosis factor-α, have also been suggested to play an etiological role [37].

Clinical manifestations and complications

In contrast to HSV esophagitis, CMV esophagitis has very rarely been documented in immunocompetent persons [38]. Odynophagia is almost uniformly present and is typically severe (see Table 34.2). A previous or coexistent diagnosis of CMV infection in other organs (e.g., retinitis or colitis) is not infrequent. Although rare in transplant recipients, retinitis may be observed in approximately 15% of patients with AIDS at the time of diagnosis of gastrointestinal disease [39]. Complications include gastrointestinal bleeding (~ 5% of patients) and, rarely, strictures [40] or fistulae to the tracheobronchial tree [41].

Diagnosis

Like HSV-1, the radiological appearance of CMV esophagitis is that of either focal or extensive ulceration and is dependent in large part on the epidemiological setting. Barium esophagraphy of CMV esophagitis may reveal thickening of mucosal folds or more typically ulcers; these ulcers may be vertical, linear with central umbilication, solitary, and deep, or occasionally they may manifest as diffuse superficial ulceration (see Fig. 34.4b) [30]. In patients with AIDS, these ulcers are often large and deep and may exceed 2 cm in size. Rarely, the exuberant inflammatory response mimics a malignant disease. The endoscopic appearance of CMV esophagitis is variable, ranging from multiple shallow ulcers, to solitary giant ulcers, to diffuse superficial esophagitis (see Fig. 34.4c) [42]. Given the high rate of previous exposure to CMV, serological testing is not helpful. In addition, some immunosuppressed transplant recipients fail to develop a brisk antibody response. Blood antigenemia is often present at the time of diagnosis and its presence may predict the subsequent development of end-organ disease [43].

Identification of viral cytopathic effect in mucosal biopsies is the best diagnostic method. Multiple biopsies (up to 10) may be required to establish the diagnosis in patients with AIDS and should be taken from the ulcer base because the viral cytopathic effect is found here [44] (Fig. 34.3). Viral culture of mucosal biopsies is less sensitive and specific than histology and cytological brushings are not helpful [45] The identification of an isolated inclusion found by immunohistochemical staining should not be considered CMV disease but rather infection, and other causes of mucosal disease should be sought. Because retinitis may coexist with gastrointestinal disease, and, when present, alters the duration of antiviral therapy, a diagnosis of CMV gastrointestinal disease in any patient with AIDS warrants ophthalmological examination.

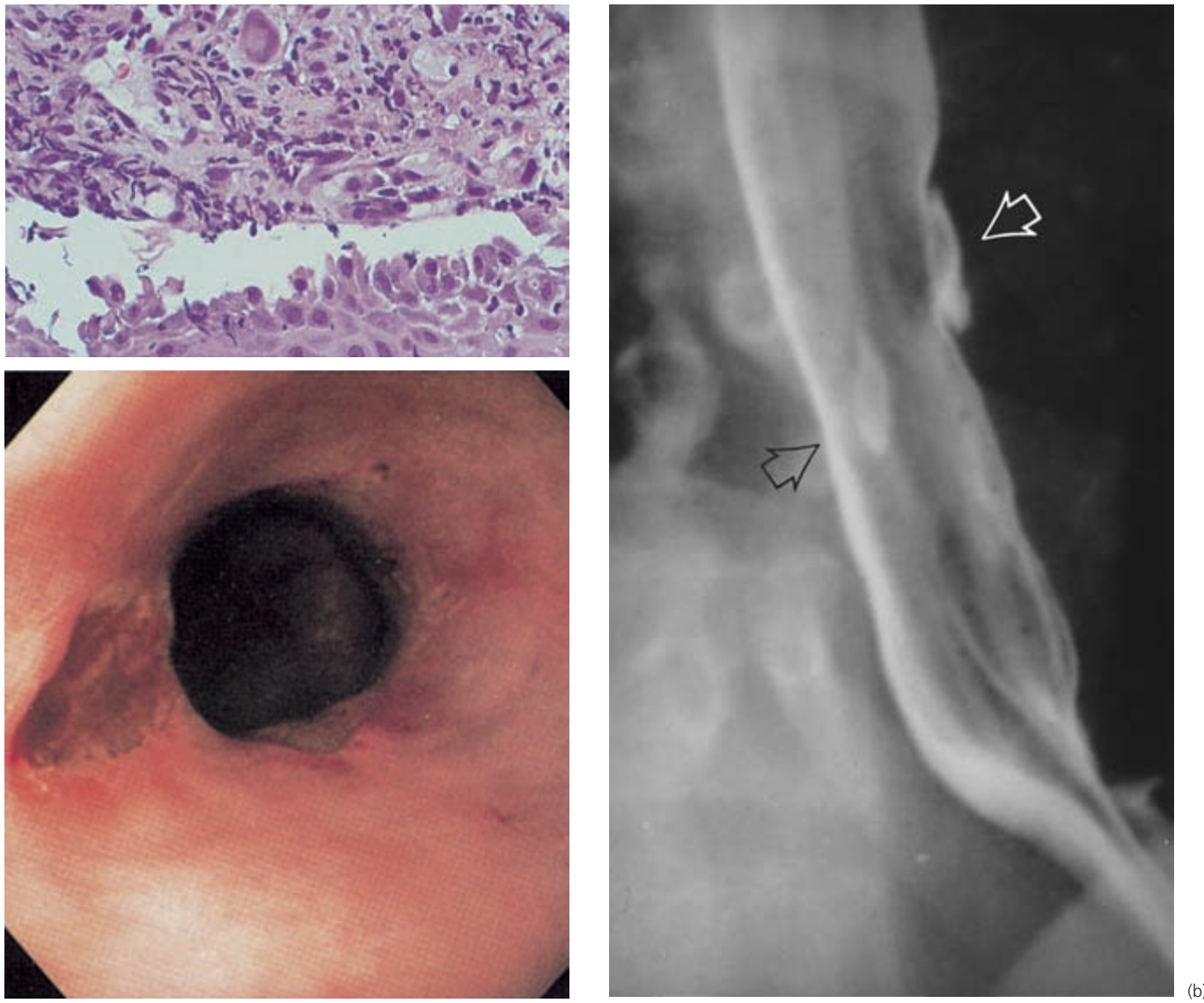


Figure 34.4 Cytomegalovirus esophagitis. **(a)** Biopsy from an ulcer base demonstrates granulation tissue with a large cell with intranuclear and intracytoplasmic inclusions diagnostic for cytomegalovirus. **(b)** Barium swallow demonstrates two well-circumscribed ulcers in the distal esophagus (arrows). **(c)** Endoscopic photograph shows the two ulcers, which are well circumscribed and have some depth; the surrounding mucosa is normal.

Treatment

The therapies available for the treatment of CMV disease generally require intravenous administration and include ganciclovir, foscarnet, and cidofovir. The most widely used therapy, ganciclovir, is an acyclovir derivative. A large prospective open-label trial of ganciclovir in 35 patients with AIDS documented clinical and endoscopic improvement in 77% [39]. The time course of the clinical response to ganciclovir is variable; a week of therapy may be required before there is symptomatic improvement. The total treatment duration should be based on the clinical and endoscopic response, and a 2- to 4-week treatment course is usually adequate. If retinitis is absent and there has been a complete response, the patient may be followed closely without maintenance therapy, especially if immune function can be optimized with HAART. Because of low bioavailability (< 10%), oral ganciclovir is not effective for the treatment of active infections including those of the gastrointestinal tract. After acute CMV disease in transplant recipients, treatment with ganciclovir should be given for approximately 1–2 months until the immunosuppressive regimen is either discontinued (bone marrow transplant) or significantly reduced (solid organ transplant). Ganciclovir is well tolerated with its major side effect being myelosuppression, which may be severe when

other bone marrow suppressive drugs are coadministered. Clinical and virological resistance has been documented, usually in patients receiving prolonged therapy. In this setting, foscarnet is often effective [46].

Valganciclovir, an orally administered prodrug that is rapidly hydrolyzed to ganciclovir, achieves high blood levels that are equivalent to those achieved with intravenous administration of ganciclovir [47]. Given its favorable pharmacokinetics, it has been used successfully for induction therapy for CMV retinitis in AIDS patients, as well as for prophylaxis in high-risk solid organ transplant recipients, such as those who are donor positive/recipient negative (D^+/R^-), and preemptively for those who develop CMV antigenemia [48,49]. Although potentially effective for treatment of end-organ disease such as esophagitis, trials have not been performed and intravenous ganciclovir remains the treatment of choice. Long-term prophylaxis with this agent following successful therapy is a reasonable option for those in whom severe immunodeficiency persists, and long-term use has rarely been associated with the development of resistance [50].

Foscarnet is a pyrophosphate analog that inhibits viral DNA polymerase and reverse transcriptase. A randomized trial comparing ganciclovir with foscarnet in patients with AIDS and gastrointestinal disease found clinical improvement in more than 80%; there was no significant difference in efficacy between the two agents [51]. There are fewer data on its use in immunosuppressed patients who do not have AIDS. Because of its recognized toxicity (reversible renal insufficiency and electrolyte disturbances), foscarnet has been most frequently used when there is clinical resistance or intolerance to ganciclovir. All drugs used for the therapy of herpesviruses only inhibit viral replication; thus, relapse is frequent when therapy is discontinued. The relapse rate for transplant recipients also remains high until immunosuppressive therapy is reduced.

Cidofovir, the newest systemic agent available for the therapy of CMV, has undergone evaluation for the treatment of retinitis in AIDS [52] and less so in other immunocompromised patients. Its long half-life provides the opportunity for once-weekly administration. Like foscarnet, however, this drug is associated with renal insufficiency; probenecid pretreatment is used to decrease this complication. Because of its significant expense, it has been used most often for CMV resistance (ganciclovir, foscarnet) as well as for other DNA viruses in patients with AIDS.

The relapse rate of CMV esophagitis is approximately 50% and is similar for HSV [32,39,51]. Initiation of HAART, when associated with significant improvement in CD4 count, may abrogate the need for long-term maintenance therapy [21].

Prophylaxis

Antiviral prophylaxis will reduce CMV disease and associated mortality in transplant patients [53]. Oral ganciclovir is recommended to reduce the incidence of CMV disease in solid organ transplant patients. Oral ganciclovir is also used preemptively in patients who develop CMV viremia. However, in high-risk patients, such as bone marrow transplant patients and solid organ transplant recipients, valganciclovir is the drug of choice [48,49]. Intravenous ganciclovir prophylaxis may be appropriate in very high-risk patients such as D^+/R^+ transplant recipients. Oral ganciclovir has shown efficacy for prophylaxis for CMV retinitis in AIDS, but HAART represents the ideal choice.

Other viruses

The frequency of esophageal involvement caused by varicella-zoster during the course of chickenpox or herpes zoster infections is unknown but clinically uncommon [23]. Papillomavirus may infect the esophagus and may cause small polypoid lesions. Esophageal ulcers have reportedly been caused by papovavirus and Epstein–Barr virus in patients with AIDS [23].

Mycobacterial infections

Epidemiology

Mycobacterial involvement of the esophagus is very rare, even in immunosuppressed patients. In developing countries the rate of tuberculosis (TB) is much higher, and extrapulmonary manifestations, including esophageal disease, are more common. The combination of the AIDS epidemic and the upsurge in reported cases of systemic TB have increased the incidence of esophageal infection in developed countries. *Mycobacterium avium* complex (MAC) remains primarily a small bowel pathogen with only rare cases of esophageal involvement reported [23].

Pathology

Most commonly, TB affects the middle third of the esophagus at the level of the carina [54]. Esophageal disease is caused by spread of infection from tuberculous-infected mediastinal lymph nodes by way of a draining fistula or obstructed lymphatics, often resulting in tracheoesophageal fistula. TB can also involve the upper third of the esophagus by direct extension from tuberculous pharyngitis or laryngitis. Primary esophageal TB in the absence of extraesophageal disease is exceedingly rare and found in patients from endemic areas.

Clinical manifestations and complications

The symptoms of esophageal TB depend on the degree and type of involvement [23]. Pulmonary complaints often predominate because of the common occurrence of fistula to the trachea, bronchus, or pleural space. Rarely, formation of long strictures or traction diverticula resulting from the fibrotic response cause dysphagia. Upper gastrointestinal

hemorrhage from tuberculous esophageal ulcers and tuberculous arterioesophageal fistulae has been reported [55].

Diagnosis

Esophageal TB should be suspected in patients with pulmonary or systemic TB who develop esophageal symptoms. Chest radiography is generally abnormal and CT scan may demonstrate mediastinal adenopathy [54]. Barium esophagram findings, including ulceration and stricture, are nonspecific. A sinus tract or fistulous connection to the bronchial tree or mediastinum is highly suggestive of TB but can also be seen with malignant disease or other infections; in this setting, the diagnosis may often be made by sputum staining and culture, or CT-guided or surgical biopsy [54]. Endoscopic findings include ulcers, which may be solitary and resemble a malignancy, fistulae or strictures [56,57]. Biopsies from the edges of the lesions may demonstrate granulomata or acid-fast bacilli, and biopsy material may be cultured for further confirmation of the diagnosis and determination of sensitivities to antimycobacterial agents.

Treatment

Regardless of the presence of immunodeficiency, a 9-month course of multidrug therapy (in the absence of drug resistance) will cure esophageal TB and will often close fistulae. If fistulae do not close with medical therapy, surgical intervention will be required. The prevalence of multidrug-resistant TB is an increasingly complex problem; thus, drug sensitivities to antituberculous therapy are essential to guide therapy. The most effective agents for the treatment of MAC are clarithromycin and ethambutol [58]. Although a clinical and bacteriological response is common, long-term therapy for MAC is required in AIDS unless HAART is effective [21].

Bacterial infections

Epidemiology

Bacterial esophagitis is a rare cause of esophageal disease in immunocompromised patients. It has been described almost exclusively in patients with hematological malignant disease complicated by severe granulocytopenia, occasionally after bone marrow transplantation and diabetic ketoacidosis or in those patients receiving radiation and chemotherapy [23,59,60]. For the most part the infecting pathogens are oral flora, particularly gram-positive organisms, including viridans streptococci, staphylococci, and other bacilli. It is likely that these pathogens colonize and then invade mucosa damaged by reflux disease, radiation, or chemotherapy, leading to local infection. Reports in patients with AIDS have broadened the etiological spectrum to include *Bartonella henselae*, the cause of catscratch disease [61], and *Nocardia* [23]. Actinomycosis causes ulcers, sinus tracts, and fistulas [60]. Although esophageal involvement by *Treponema pallidum* was well recognized many years ago, this disease is now only of historical interest [23].

Pathology

The gross pathological appearance of the esophagus in bacterial infection ranges from normal mucosa (colonization) to ulcers associated with erythema, plaques, pseudomembranes, sinus cavities or hemorrhage [59]. Microscopic examination reveals pseudomembranes and bacterial invasion that may be superficial and invade only the squamous epithelium or may be invasive and transmural with infiltration of blood vessels (i.e., phlegmonous esophagitis). *B. henselae* esophagitis causes multiple nodules of the esophagus resulting from a lobulated proliferation of capillary vessels lined by plump endothelial cells [61].

Clinical manifestations and complications

Bacterial esophagitis is usually found in a neutropenic patient with esophageal complaints who has undergone chemotherapy for a hematogenous malignant disease. Esophageal infection may serve as a focus for bacteremia and seeding of other organs, particularly in patients with granulocytopenia [59]. No unique complications have been reported.

Diagnosis

The diagnosis of bacterial esophagitis should be considered in the clinical setting described earlier. Radiographic findings are nonspecific, and endoscopic biopsy and culture are required to establish this diagnosis. Additional stains including Gram stain are required to identify the etiological bacteria. Positive blood cultures also pinpoint the bacterial pathogens and direct antimicrobial therapy.

Treatment

Broad-spectrum antibiotics that effectively treat both gram-positive and gram-negative oropharyngeal flora are required for treatment. Treatment of other bacterial infection found in these patients is similar to treatment of disease in other locations.

Protozoal infections

In developed countries, protozoal infections of the esophagus are very rare, occurring almost exclusively in patients with AIDS. In these patients, pathogens include *Pneumocystis carinii*, *Cryptosporidium parvum*, *Leishmania donovani*, and *Trichomonas* [23,62]. In immunologically normal hosts from endemic areas in South America, *Trypanosoma cruzi* may involve the myenteric plexus of the esophagus resulting in Chagas disease. This disease is indistinguishable clinically, radiographically, endoscopically, and manometrically from idiopathic achalasia. The diagnosis may be established by antibody testing [23].

Specific HIV-related esophageal disorders

In addition to the infections described earlier, certain unique disorders cause esophageal disease in HIV-positive patients.

Disorders associated with primary HIV infection

Although primary HIV infection is largely asymptomatic, in some patients a mononucleosis-like illness occurs around the time of infection consisting of fever, sore throat, and myalgias associated with a maculopapular rash [63]. Spontaneously resolving oropharyngeal and esophageal ulceration or candidal infection may also be observed during this seroconversion illness. Endoscopically, these esophageal ulcerations are multiple, small, and shallow. The diagnosis can be established at the time of presentation by the detection of HIV RNA in serum [63]. Antibody positivity to HIV occurs within 3–18 months after the illness.

Idiopathic esophageal ulcer

Epidemiology

Early in the AIDS epidemic, large esophageal ulcerations were recognized in which no specific cause could be identified despite extensive histopathological examination of ulcer tissue. These ulcers, termed idiopathic esophageal ulcers (IEU) or aphthous ulcers, are very common and were found in 41% of HIV-infected patients with esophageal ulcer in a large prospective study [29]. They are seen in the later stages of immunodeficiency when the CD4 lymphocyte count is less than 100/mm^3 [5,29]. Like all opportunistic disorders, their frequency has also fallen in the era of HAART [1].

Pathology

These ulcers are variable in size, may be large, and are uniformly well circumscribed; diffuse superficial esophagitis has not been described [64]. Ulcer tissue resembles that seen in CMV and HSV infection except that viral cytopathic effect is absent. The presence of a superficial candidal infection overlying a large well-circumscribed lesion with histopathological findings of granulation tissue without viral cytopathic effect should still lead to the diagnosis of IEU [65]. Although HIV has been suggested as the direct cause of these lesions, there is little histopathological evidence to support a direct cytopathic role for HIV [66].

Clinical manifestations and complications

IEU presents in a fashion indistinguishable from CMV esophagitis. Coexistent oropharyngeal aphthous ulcers are infrequent [9], whereas thrush is common, especially if the patient has not been given empirical antifungal therapy. Complications include bleeding and fistulae to the stomach but not to the tracheobronchial tree, and strictures [40].

Diagnosis

The findings of IEU on barium esophagram are typically large, well-circumscribed, and often deep ulcers [67]. Because of the similarity to CMV infection, a definitive diagnosis cannot be based on the radiographic appearance alone. Because IEU is a diagnosis of exclusion, endoscopy and biopsy are the only definitive diagnostic tests. These ulcers are variable in size and appearance, and larger ulcers are endoscopically indistinguishable from CMV infection [42,64]. Distal esophageal ulcer may suggest gastroesophageal reflux disease; the histopathological features alone also cannot distinguish IEU from gastroesophageal reflux disease. Pill-induced esophagitis must be excluded by history because the pathological findings of esophageal biopsies are similar. These ulcers respond rapidly to either prednisone or thalidomide, with clinical and endoscopic cure seen in more than 90% [29,68,69].

Neoplasms associated with AIDS

Kaposi sarcoma

With the advent of HAART, Kaposi sarcoma (KS), caused by human herpesvirus 8, has shown a sharp fall in frequency [70]. Gastrointestinal KS is common in those with cutaneous disease; clinical experience suggests that gastric or duodenal involvement is more common than esophageal disease, with esophageal lesions, like other areas of the gastrointestinal tract, being most frequently an incidental finding. Findings of KS on barium esophagram have not been well studied and small lesions can be easily missed. The endoscopic features of esophageal KS are characteristic and resemble their cutaneous appearance: violaceous macular or plaque-like lesions. These tumors typically involve the submucosa; therefore, mucosal biopsy must sample deeper tissue. Bleeding may occur when the tumor becomes large and ulcerated. Effective relatively nontoxic chemotherapy includes Doxil, a liposomal form of doxorubicin (Adriamycin). HAART therapy alone has been associated with lesion regression and apparent cure [71]. Radiation therapy is effective to treat local lesions of the head and neck including the oropharynx.

Non-Hodgkin lymphoma

Despite HAART, lymphoma remains an important opportunistic neoplasm. Although gastrointestinal involvement is common, esophageal disease remains rare [72]. Extraesophageal disease is common at the time of diagnosis [72]. The lesions appear radiographically as large ulcers or mass lesions typical of any carcinoma. The endoscopic appearance of these lesions has been described as an ulcerated polypoid mass, often with a central ulceration, a submucosal lesion or extensive disease resulting in lumenal narrowing resembling an adenocarcinoma, or a solitary ulcer [73]. Complications are rare and include bleeding. Multidrug chemotherapeutic regimens are usually given for non-Hodgkin lymphoma in AIDS, and complete remission may be realized in approximately 50% or more of patients. Radiation therapy may be a

potential option when the disease is localized. Complications are frequent because of chemotherapy-induced neutropenia. The median survival time for AIDS-associated non-Hodgkin lymphoma has been enhanced by HAART [74].

Miscellaneous neoplasms

Despite the profound immunodeficiency seen in these patients, there does not appear to be an increase in the incidence of other esophageal neoplasms including squamous cell cancer and adenocarcinoma [75].

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A ESOPHAGUS

35

Barrett esophagus and esophageal adenocarcinoma

Stuart Jon Spechler, Rhonda F. Souza

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Barrett esophagus is the condition in which the stratified squamous epithelium that normally lines the distal esophagus is replaced by an abnormal, columnar epithelium (Fig. 35.1) [1]. The condition is judged to be a consequence of gastroesophageal reflux disease (GERD), which both injures the esophageal squamous epithelium and provides the abnormal intraluminal milieu that leads to healing of the injury through the process of columnar metaplasia rather than through the regeneration of more squamous cells. For reasons that are poorly understood, the metaplastic columnar epithelium predisposes to esophageal adenocarcinoma. Indeed, GERD and Barrett esophagus are the major risk factors for esophageal adenocarcinoma, a tumor whose frequency has increased more than sixfold over the past few decades [2,3]. The history of Barrett esophagus has been one of intense controversy, and authorities continue to debate the pathogenesis, proper management, and the very definition of this common disorder.

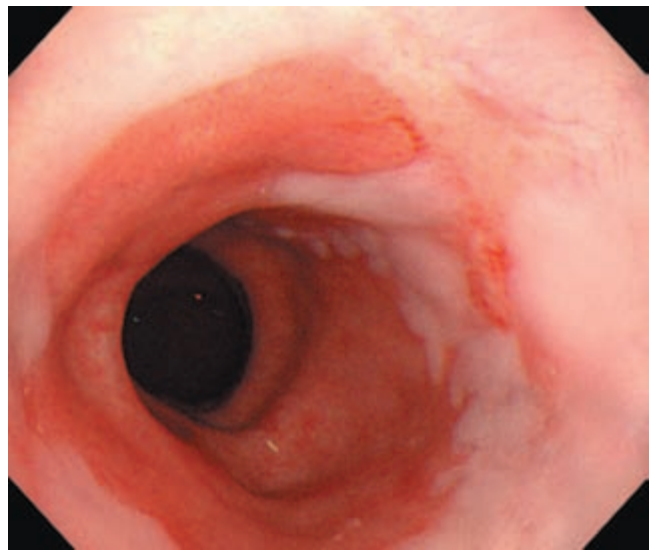

Figure 35.1 Endoscopic photograph of Barrett esophagus. Note the contrast between the pale, glossy squamous epithelium of the proximal esophagus and the red Barrett epithelium of the distal esophagus.

The history of Barrett esophagus – roots of the controversies

Barrett esophagus is named for Norman Rupert Barrett, an Australian surgeon who, in 1950, described a number of patients who had ulcerations in an organ that appeared to be the esophagus, but whose distal portion was lined by a "gastric type" of columnar epithelium [4]. Barrett originally contended that this condition was congenital in origin. In 1959, Moersch and colleagues [5] argued that the esophageal columnar lining was acquired as a result of reflux esophagitis and, since then, most investigators have agreed that Barrett esophagus is an acquired condition [6].

The histology of Barrett esophagus was a highly controversial issue until 1976, when Paull and colleagues [7] reported the results of a landmark study on 11 patients with Barrett's esophagus who had biopsy specimens obtained using manometric guidance to ensure that the specimens came from the esophagus. These patients were found to have one, or a combination, of three types of columnar epithelia lining the distal esophagus: (1) junctional-type epithelium (also known as

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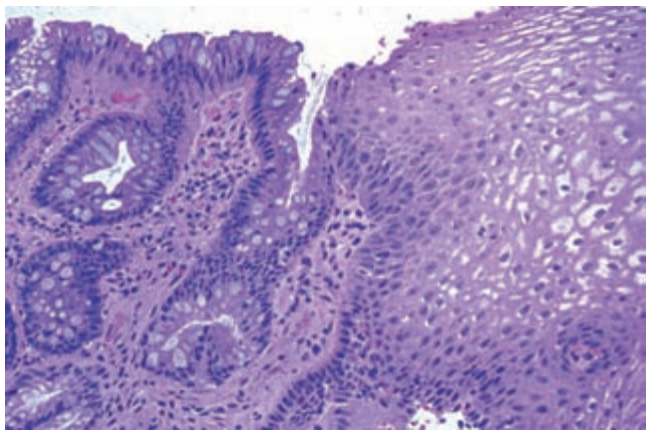

Figure 35.2 Photomicrograph showing the junction between stratified squamous epithelium (right) and specialized intestinal metaplasia (left). Note the numerous goblet cells characteristic of the intestinal-type epithelium.

cardiac epithelium) comprised almost exclusively of mucus-secreting cells; (2) gastric fundic-type epithelium (also called oxyntic epithelium) containing acid-secreting parietal cells; and (3) an incomplete form of intestinal metaplasia that the investigators called specialized columnar epithelium (now called specialized intestinal metaplasia). The three epithelial types were noted to occupy different zones in the esophagus, with specialized intestinal metaplasia found adjacent to squamous epithelium in the most proximal segment of the columnar lining (Fig. 35.2), followed by junctional-type epithelium and, most distally, by gastric fundic-type epithelium.

To recognize Barrett esophagus, endoscopists must identify the gastroesophageal junction (GEJ) and then ascertain that columnar epithelium extends proximal to it. Unfortunately, there are no universally accepted and clear-cut landmarks for the GEJ, a problem that can result in both false-positive and false-negative diagnoses of Barrett esophagus. Large hiatal hernias and severe reflux esophagitis can make it especially difficult to identify the GEJ. In the early 1980s, investigators intent on avoiding false-positive diagnoses established arbitrary criteria for the extent of esophageal columnar lining (ranging from 2 to 5 cm) needed to include patients in studies on Barrett esophagus [8]. Those arbitrary investigative criteria were embraced by clinicians and, as a result, endoscopists in the 1980s often dismissed columnar epithelium limited to the distal few centimeters of the esophagus as normal. In their efforts to avoid false-positive diagnoses, however, these investigators and clinicians had created a potentially larger problem of false-negative diagnoses.

In 1994, Spechler and colleagues [9] reported the results of a study in which patients undergoing elective endoscopic examinations in a general endoscopy unit had biopsy specimens taken at the squamocolumnar junction in the distal esophagus, irrespective of its appearance and location. Among 142 patients who had columnar epithelium involving 3 cm of the distal esophagus, 26 (18%) were found to have specialized intestinal metaplasia typical of Barrett esophagus. That metaplastic epithelium would have gone unrecognized if the study protocol had not mandated the procurement of biopsy specimens from a normal-appearing distal esophagus. A number of subsequent studies have confirmed this observation that intestinal metaplasia is present frequently in short segments of columnar epithelium in the distal esophagus, even in patients with no symptoms or endoscopic signs of GERD [10]. Since the description by Spechler, clinicians have called this condition "short-segment Barrett esophagus" [11].

The association between adenocarcinoma and Barrett esophagus had been well established by the 1970s [12]. By the late 1980s, it was recognized that intestinal metaplasia was the epithelial type that was especially associated with this cancer [13]. Because specialized intestinal metaplasia was the most frequent and distinctive of the epithelial types, the type that was clearly abnormal and the one most often associated with cancer, a number of investigators chose to define Barrett esophagus by the presence of intestinal metaplasia [14,15]. These investigators rejected the diagnosis of Barrett for patients with only junctional- and gastric fundic-type epithelia in the esophagus, arguing that it was too difficult to exclude the possibility that the endoscopist had inadvertently taken biopsy specimens from the stomach and that, without specialized intestinal metaplasia, the condition had no clinical importance anyway. This requirement for intestinal metaplasia to define Barrett esophagus, which was based more on practical than on scientific concerns, was also embraced by clinicians and adopted widely into clinical practice.

The definition of Barrett esophagus has undergone substantial evolution since the original report by Barrett in 1950. Some have argued that the very term "Barrett esophagus" should be discarded, because the condition has been defined so variably by different investigators who have imposed arbitrary criteria to fit their personal perspectives [6]. Nevertheless, the term is so firmly entrenched that it appears unlikely to be abandoned. For the past two decades, most studies on Barrett esophagus have included primarily or exclusively patients who had specialized intestinal metaplasia documented in their esophageal biopsy specimens. However, there remain substantial conceptual and practical problems with this practice.

Chandrasoma has suggested that cardiac (junctional) epithelium might not be a normal epithelial type at all, but rather a metaplastic epithelium acquired as a consequence of reflux esophagitis [16]. According to this hypothesis, reflux-damaged esophageal squamous epithelium is replaced first by cardiac epithelium, which later evolves into intestinal metaplasia. The British Society of Gastroenterology has defined Barrett as "an oesophagus in which any portion of the normal squamous lining has been replaced by a metaplastic columnar epithelium which is visible macroscopically" [17]. This definition, which does not require the presence of intestinal-type epithelium, is fine conceptually but problematic

practically because it is difficult to establish whether junctional- and gastric fundic-type epithelia found at the GEJ are metaplastic or normal. Today, more than half a century after the publication of Norman Barrett's seminal treatise, authorities continue to dispute the definition and nature of Barrett esophagus.

Diagnosis of Barrett esophagus

Endoscopic examination is required to diagnose Barrett esophagus, and most authorities insist on fulfillment of both of the following two diagnostic criteria: (1) the endoscopist must document that columnar epithelium extends above the GEJ to line the distal esophagus; and (2) histological examination of biopsy specimens from the esophageal columnar epithelium must reveal intestinal metaplasia. To document that columnar epithelium lines the esophagus, the endoscopist must identify both the squamocolumnar junction and the GEJ (Fig. 35.3). Columnar epithelium has a reddish color and coarse texture on endoscopic examination, whereas squamous epithelium has a pale, glossy appearance. The juxtaposition of these epithelia at the squamocolumnar junction forms a visible, typically jagged line called the Z-line. The GEJ is recognized endoscopically as the level of the most proximal extent of the gastric folds [8]. When the squamocolumnar junction is located proximal to the GEJ, then there is a columnar-lined segment of esophagus. When the squamocolumnar junction and the GEJ coincide (Fig. 35.4), then the entire esophagus is lined by squamous epithelium. If biopsy specimens from the columnar-lined esophagus show specialized intestinal metaplasia, then the patient has Barrett esophagus. Such a patient has long-segment Barrett esophagus if the distance between the Z-line and the GEJ is 3 cm or more, and short-segment Barrett esophagus if that distance is less than 3 cm (Fig. 35.5).

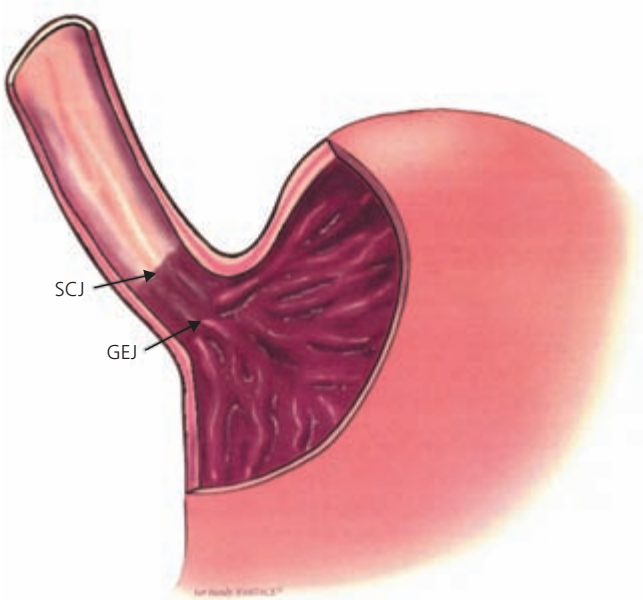

Figure 35.3 Landmarks at the gastroesophageal junction (GEJ) region. The squamocolumnar junction (SCJ, also called the Z-line) is the visible line formed by the juxtaposition of squamous and columnar epithelia. The GEJ is the imaginary line at which the esophagus ends and the stomach begins. The GEJ corresponds to the most proximal extent of the gastric folds. When the SCJ is located proximal to the GEJ, as it is in this figure, then the region between the two landmarks is a columnar-lined segment of esophagus.

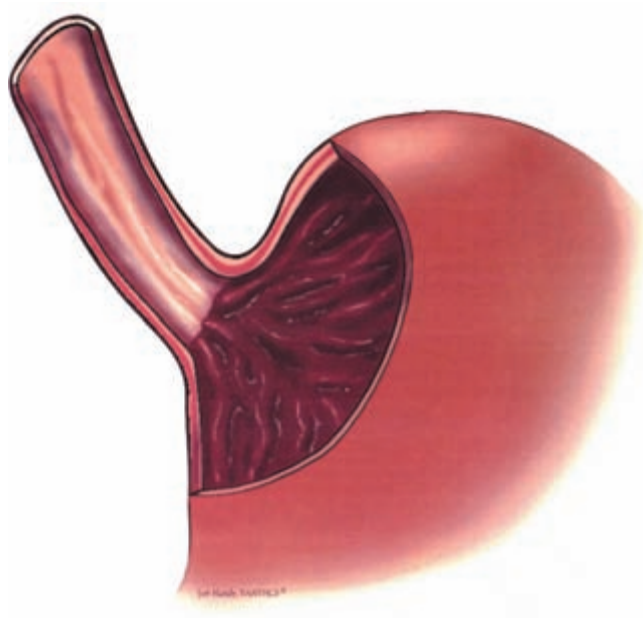

Figure 35.4 The squamocolumnar junction and gastroesophageal junction coincide. In this situation there is no columnar-lined esophagus (i.e., the entire esophagus is lined by squamous epithelium).

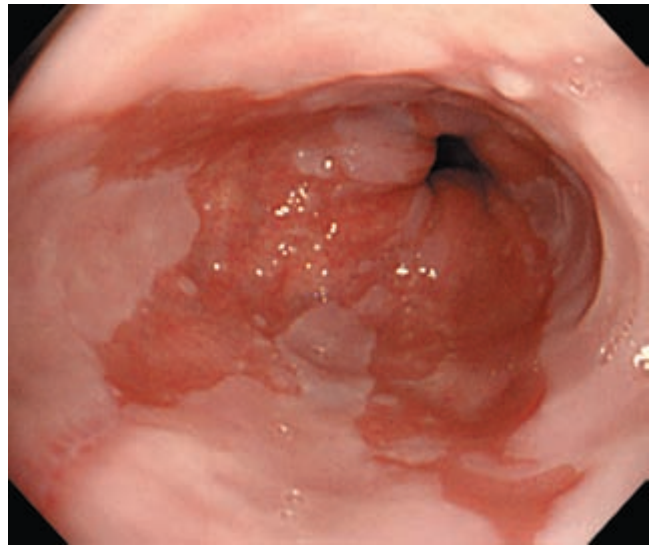

Figure 35.5 Endoscopic photograph of short-segment Barrett esophagus. Columnar epithelium extends for less than 3 cm above the gastroesophageal junction.

Recently, a new system called the "Prague C and M criteria" has been proposed for the endoscopic diagnosis and grading of Barrett esophagus [18]. This system evaluates both the circumferential (C) and the maximum (M) extent of Barrett metaplasia above the GEJ. The Prague C and M system has been found to have excellent reproducibility among endoscopists for a number of features of Barrett esophagus, with the notable exception of short-segment Barrett with metaplasia that extends for less than 1 cm above the GEJ, for which interobserver agreement is poor. However, it is not yet clear whether there is any clinical value to the Prague C and M criteria.

Intestinal metaplasia at the gastroesophageal junction

Chronic reflux esophagitis leads to the development of intestinal metaplasia in the esophagus. Intestinal metaplasia also occurs commonly in the stomach as a consequence of chronic gastritis caused by infection with *Helicobacter pylori* [19]. On histological examination, intestinal metaplasia in the stomach cannot be distinguished reliably from intestinal metaplasia in the esophagus. Because the GEJ cannot be identified with great precision, it can be difficult to determine whether short segments of intestinal metaplasia found in the GEJ region are lining the esophagus (i.e., short-segment Barrett esophagus) or the proximal stomach (i.e., intestinal metaplasia of the gastric cardia). The term "intestinal metaplasia at the GEJ" has been used to describe the condition in which intestinal metaplasia is found at a Z-line that appears to coincide precisely with the GEJ [20]. Rather than constituting an independent condition, however, intestinal metaplasia at the GEJ almost certainly represents either short-segment Barrett esophagus or intestinal metaplasia of the cardia. For technical reasons, it is difficult to distinguish these two conditions.

For clinical purposes, however, it may be important to distinguish short-segment Barrett esophagus from intestinal metaplasia of the gastric cardia because the former condition appears to have a substantially higher risk of malignancy. For example, one study found dysplasia (the precursor of malignancy) in 20 of 177 patients (11.3%) with short-segment Barrett esophagus, but in only 1 of 76 patients (1.3%) with intestinal metaplasia of the gastric cardia [21]. Authorities recommend endoscopic cancer surveillance routinely for patients with Barrett esophagus, but not for patients with intestinal metaplasia in the stomach [22,23]. Therefore, the distinction between the two conditions has implications for patient management. A number of histochemical and immunological biomarkers have been proposed to differentiate intestinal metaplasia of the cardia from short-segment Barrett esophagus (e.g., cytokeratin staining patterns, immunoreactivity for mAb Das-1, expression of colonic-type sulfomucins [24,25]). However, the utility of biomarkers in distinguishing short-segment Barrett esophagus from intestinal metaplasia of the gastric cardia has not been established [26]. For patients found to have intestinal metaplasia at the GEJ, one recommended approach is to assume that the condition is short-segment Barrett esophagus and to manage patients according to the guidelines for Barrett esophagus discussed below [20].

Epidemiology of Barrett esophagus and esophageal adenocarcinoma

Barrett esophagus is typically discovered in middle-aged and older adults, usually during endoscopic examinations performed for the evaluation of GERD symptoms [27]. The average age at the time of endoscopic diagnosis is approximately 55 years, but usually it is not possible to determine when the condition first developed. White men predominate in most series, and Barrett esophagus is decidedly uncommon in black and Asian populations [28]. Among patients who have had endoscopic examinations for the evaluation of GERD symptoms, long-segment Barrett esophagus has been found in 3%–5%, whereas 10%–20% have short-segment Barrett esophagus [1].

The prevalence of Barrett esophagus in the general adult population is not clear. In one study of 961 patients who were scheduled for elective colonoscopy and who agreed to have an upper gastrointestinal endoscopy performed for research purposes, Barrett esophagus (predominantly short-segment) was found in 6.8% [29]. In another study in which individuals in the general population of Sweden were invited to have endoscopic examinations, the prevalence of Barrett esophagus was found to be 1.6% [30].

The prevalence of Barrett esophagus in children is disputed. The condition appears to be very rare in children younger than 10 years of age and virtually non-existent in children younger than 5 years [31]. In a retrospective study of 6731 children who had upper gastrointestinal endoscopy in 12 pediatric facilities, only 17 (0.25%) had suspected Barrett esophagus, and only 9 of those had intestinal metaplasia confirmed by esophageal biopsy [32]. However, it has been proposed that this prevalence was spuriously low because pediatric gastroenterologists may not have recognized Barrett esophagus as readily as their adult counterparts. Another study from a pediatric group with a long-standing, special interest in Barrett esophagus found the condition (with histologically confirmed intestinal metaplasia) in 4.8% of 166 children who were receiving chronic proton pump inhibitor (PPI) therapy [33].

The epidemiology of esophageal adenocarcinoma is similar to that of Barrett esophagus. For example, GERD is a strong risk factor for adenocarcinoma of the esophagus [2], and this tumor affects white men predominantly [27]. In the

1960s, squamous cell cancers comprised well over 90% of all esophageal tumors in the United States, whereas adenocarcinoma of the esophagus was considered so uncommon that some authorities questioned its very existence. Since then, however, the frequency of adenocarcinoma of the esophagus has increased dramatically, to the point that adenocarcinoma surpassed squamous cell carcinoma in frequency in the 1990s [3,34,35]. Squamous cell carcinoma affects black people six times more frequently than white people, whereas adenocarcinoma is at least four times as common in white people [27].

Obesity has also been established as a strong risk factor for esophageal adenocarcinoma [36]. In the United States, the prevalence of obesity has increased substantially over the past three decades, paralleling the increase in the frequency of esophageal adenocarcinoma. The mechanism underlying the association between cancer of the esophagus and obesity is not known, but may be related to the fact that obesity predisposes to the development of GERD [37,38]. In addition, obesity is associated with increased serum levels of insulin-like growth factor 1 and leptin, hormones that can stimulate proliferation [39,40], and with decreased levels of adiponectin, a hormone that has antiproliferative effects [41]. Two studies have suggested that Barrett esophagus is associated with the presence of abdominal fat, not with body mass index (BMI) per se [42,43].

The frequency of infection with *H. pylori* has declined dramatically over the last century in the United States [44]. *H. pylori* is a well-established carcinogen for adenocarcinoma of the stomach, but some studies suggest that this infection may protect against the development and neoplastic progression of Barrett esophagus [45,46]. The mechanism of this alleged protective effect is not clear, but it has been proposed that *H. pylori* infections that cause pangastritis can decrease gastric acid secretion, thereby protecting against the development of GERD [47]. Thus, the declining frequency of *H. pylori* infection may be contributing to the rising frequency of esophageal adenocarcinoma.

In addition to *H. pylori* infection, factors that appear to protect against the development of esophageal adenocarcinoma include the use of aspirin and other nonsteroidal antiinflammatory drugs (NSAIDs) [48], and the consumption of a diet high in fruits and vegetables [49]. Although cigarette smoking and excessive alcohol consumption are very strong risk factors for squamous cell carcinoma of the esophagus, cigarette smoking only modestly increases the risk for esophageal adenocarcinoma and alcohol does not appear to affect that risk at all [50,51].

Cancer risk

Published estimates on the annual incidence of cancer in patients with long-segment Barrett esophagus have ranged from 0.2% to 2.9% [52]. In 2000, Shaheen and colleagues [52] presented compelling evidence that the cancer risk had been overestimated because of publication bias, the selective reporting of studies with positive or extreme results. A number of modern, larger studies, which may be less susceptible to sampling error, have suggested that the risk of cancer in Barrett esophagus is approximately 0.5% per year [53,54]. This estimate has become widely accepted among authorities.

It seems logical to assume that the risk of cancer in Barrett esophagus should vary with the extent of metaplastic epithelium, and data from a number of observational studies support this hypothesis [55,56]. However, there is yet no proof that the risk of cancer varies with the extent of the metaplastic lining. Furthermore, in one report of 235 patients with Barrett esophagus, the cancer risk was not found to vary significantly with the extent of metaplasia [57].

Pathogenesis of metaplasia in the esophagus

Metaplasia, the process in which one adult cell type replaces another, is a response to chronic inflammation in a number of organs [20,58]. Because the metaplastic cells may be more resistant to the factors inducing the inflammation than the native cells, metaplasia can be viewed teleologically as a protective mechanism. Unfortunately, metaplasia also may predispose to malignancy. In the esophagus, chronic inflammation due to GERD results in columnar metaplasia in which the acid-sensitive squamous epithelium is replaced by a columnar epithelium that may be more resistant to peptic injury.

A number of physiological abnormalities that predispose to severe GERD have been described in patients with long-segment Barrett esophagus. For example, gastric acid hypersecretion and duodenogastric reflux have been found in some patients [59,60]. Extreme hypotension of the lower esophageal sphincter, which predisposes to gastroesophageal reflux, is also common in patients with long-segment Barrett esophagus [61]. Manometric studies often reveal ineffective esophageal motility, an abnormality that may delay the clearance of refluxed material from the esophagus [62]. Some patients have diminished esophageal pain sensitivity [63]. If the reflux of caustic material does not induce the warning symptom of heartburn, then GERD may go untreated until a complication develops. Some patients have decreased salivary secretion of epidermal growth factor, a peptide that enhances the healing of peptic ulceration [64]. Individual patients may exhibit any, all, or none of these abnormalities, and their frequency in Barrett esophagus is disputed. For example, some investigators have found normal gastric acid secretion in their patients with long-segment Barrett esophagus [65]. Furthermore, many patients with short-segment Barrett esophagus have no GERD symptoms and no endoscopic signs of esophagitis [10].

Noxious factors that affect the most distal esophagus may play a role in the development of short-segment Barrett esophagus. Even in normal individuals, the environment of the GEJ appears to be especially hostile. After meals, there is a pocket of acid at the GEJ that escapes the buffering effects of ingested food [66]. This postprandial acid pocket has a mean length of approximately 2 cm, and may extend above the squamocolumnar junction to affect the distal esophagus. Studies have shown that the very distal esophagus (5–10 mm above the squamocolumnar junction) of healthy volunteers can be exposed to acid for more than 10% of the day [67,68]. This can result in acid peptic injury to the distal esophagus, and also in exposure to high concentrations of nitric oxide (NO), which is generated from dietary nitrate (NO_3^-) in green leafy vegetables. The ingested nitrate is absorbed by the small intestine, concentrated by the salivary glands, and secreted into the mouth where bacteria on the tongue reduce the nitrate to nitrite (NO_2^-). When swallowed nitrite encounters acidic gastric juice at the GEJ, the nitrite is converted rapidly to NO. After nitrate ingestion, high levels of NO can be found at the GEJ [69]. NO in these concentrations can be genotoxic and potentially carcinogenic. Thus, the GEJ is exposed repeatedly to acid, pepsin, NO, and other noxious agents in gastric juice. Chronic exposure to these agents may induce the injury and inflammation that results in the intestinal metaplasia of short-segment Barrett esophagus [70].

It is not known which cells give rise to the specialized intestinal metaplasia of Barrett esophagus. The prevailing hypothesis is that Barrett esophagus forms when GERD damages the superficial layers of the esophageal squamous epithelium, thereby exposing multipotential stem cells in the basal layers to gastric juice, which stimulates their abnormal differentiation [71]. Others have proposed that specialized intestinal metaplasia develops from progenitor cells located in the ducts of the esophageal mucosal glands [72]. In either case, the progenitor cells for Barrett esophagus have been assumed to reside within the esophagus itself.

One provocative study has suggested that bone marrow stem cells might contribute to the development of Barrett esophagus. In this study [73], female rats were given a lethal dose of radiation to destroy their native bone marrow cells, followed by a bone marrow transplant from male rat donors. The female rats then underwent esophagojejunostomy, a procedure that resulted in severe esophagitis and intestinal metaplasia histologically similar to human Barrett esophagus. Eight weeks after esophagojejunostomy, nuclear staining for Y chromosome was found in both squamous cells and metaplastic columnar cells in the esophagi of the female rats. These observations suggest that multipotential adult progenitor cells of bone marrow origin contribute to esophageal regeneration and metaplasia in this rat model of reflux esophagitis and Barrett esophagus, and raise the fascinating possibility that the progenitor cell for Barrett esophagus might be a circulating stem cell.

Although the identity of the progenitor cell for Barrett esophagus remains unclear, metaplasia must arise from changes in the gene expression of that cell. The protein products of key developmental genes are often transcription factors that regulate other genes and ultimately determine a cell's phenotype. The homeobox family of transcription factors includes those encoded by the *CDX* genes, which are known to mediate the differentiation of intestinal epithelial cells. Intestinal metaplasia in mouse stomach can be induced by forcing gastric epithelial cells to express either Cdx1 or Cdx2 [74,75]. Chronic acid exposure has been shown to activate the *Cdx2* gene promoter in cultures of mouse esophageal keratinocytes [76] and, in a rat model of Barrett esophagus, Cdx2 expression has been found in squamous cells of the basal layer prior to the development of specialized intestinal metaplasia [77]. In humans, CDX2 expression has been found in esophageal squamous epithelium from patients with Barrett esophagus [78], and biopsy specimens of specialized intestinal metaplasia have been shown to express both CDX1 and CDX2 [79,80]. These and other data suggest that gastroesophageal reflux may induce CDX expression by esophageal cells, which mediates their differentiation into the intestinal types characteristic of Barrett esophagus.

Bone morphogenetic protein (BMP)-4 also may be involved in the squamous-to-columnar metaplasia of Barrett esophagus. Early in embryonic development, the entire esophagus is lined by a columnar epithelium. In mice, a drop in levels of BMP-4 has been associated with the change from columnar epithelium to squamous epithelium in the embryonic esophagus [81]. In a rat model and in patients with GERD and Barrett esophagus, BMP-4 has been found by immunostaining in inflamed esophageal squamous epithelium and in specialized intestinal metaplasia, but not in normal esophageal squamous epithelium [82]. When cultures of human esophageal squamous cells are exposed to BMP-4, they express cytokeratins typical of columnar cells, and their gene expression patterns change to resemble those seen in Barrett epithelial cells [82]. These data suggest that reflux esophagitis may induce the esophageal squamous cells to express BMP-4, which mediates the change to the columnar cell types of Barrett esophagus.

Molecular biology of dysplasia and adenocarcinoma in Barrett esophagus

In 2000, Hanahan and Weinberg [83] proposed that, to become cancer cells, normal cells must acquire six essential physiological hallmarks which enable the cells to: (1) proliferate without exogenous stimulation; (2) resist growth-inhibitory signals; (3) avoid apoptosis; (4) resist cell senescence; (5) develop new vascular supplies (angiogenesis); and (6) invade and metastasize. This section will review what is known about how benign Barrett metaplastic cells

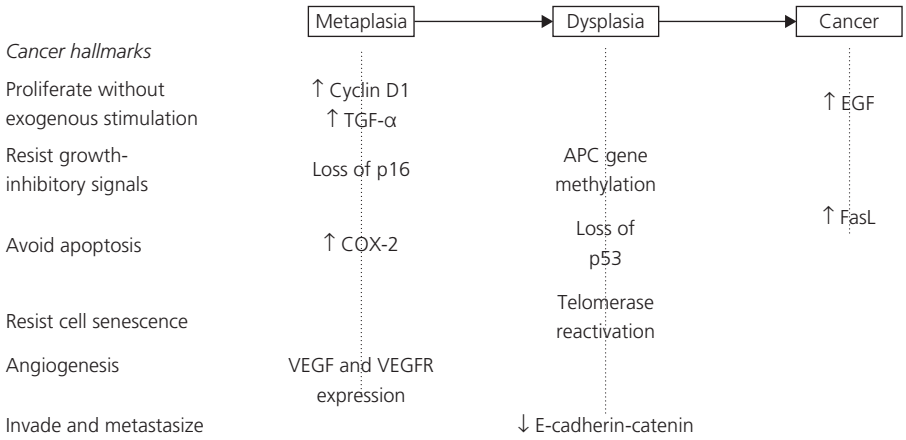

Figure 35.6 Molecular biology of dysplasia and cancer in Barrett esophagus. Some of the major genetic alterations recognized in Barrett esophagus that might allow the cells to acquire the six physiological hallmarks of cancer are shown. The histological stage at which each genetic change has been recognized is also depicted. APC, adenomatous polyposis coli; COX-2, cyclooxygenase 2; EGF, epidermal growth factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

acquire these abilities during the process of carcinogenesis [84,85]. The exact sequence and precise molecular changes necessary to produce esophageal adenocarcinoma remain incompletely understood. Some of the major genetic alterations that have been recognized in Barrett esophagus and which might allow the cells to acquire the six physiological hallmarks of cancer are shown in Figure 35.6.

Proliferation without exogenous stimulation

Oncogene expression is one mechanism whereby cells can proliferate without exogenous stimulation. Examples of oncogenes implicated in cancer progression of Barrett esophagus include cyclins D1, E, and B and c-*myc*. Increased nuclear expression of cyclin D1 protein has been found in 34% of biopsy specimens of Barrett specialized intestinal metaplasia [86]. Amplification of the chromosomal region of cyclin E or overexpression of cyclin E has been detected in Barrett esophagus with low- and high-grade dysplasia and in adenocarcinomas, but not in benign Barrett metaplasia [87,88]. Expression of cyclin B1 protein has been found to increase as the degree of dysplasia increases in Barrett epithelium [89]. Finally, up-regulation of c-Myc protein has been found in 50% of Barrett metaplasias and in 90% of adenocarcinomas [90,91].

Alterations in growth factors, growth factor receptors, and proproliferative signal transduction pathways are other ways in which Barrett cells may proliferate without exogenous stimulation. The Ras/Raf/mitogen-activated protein kinase (MAPK) pathway, which is activated by the binding of certain growth factors to their receptors, is one of the key proproliferative signaling cascades [92]. Data suggest that *KRAS* mutations occur as end-stage events in the process of carcinogenesis in Barrett esophagus [93]. Other studies have revealed genomic amplification or overexpression of the epidermal growth factor receptor (EGFR) and its ligand, transforming growth factor alpha (TGF-α), in biopsy samples of Barrett metaplasia [94]. Gastrin, a hormone that binds the CCK_2 receptor, has been shown to activate the MAPK pathways to induce proliferation in Barrett metaplasia and to prevent apoptosis in esophageal adenocarcinoma cells [95,96].

Acid exposure has been shown to activate the p38 MAPK pathway in metaplastic Barrett mucosa in vivo, and acid-mediated activation of the MAPK pathways has been associated with increased proliferation and decreased apoptosis in a human Barrett-associated adenocarcinoma cell line [97]. Exposure to glycochenodeoxycholic acid, a conjugated bile acid, also activates the MAPK pathways to increase proliferation in both nonneoplastic Barrett cells and in a Barrett-associated adenocarcinoma cell line [98]. Other growth factors and receptors that have been implicated in Barrett carcinogenesis include insulin-like growth factor 2, the insulin-like growth factor 1 receptor, and Met, a hepatocyte growth factor [99–101]. The role of *erb*B-2 (also called HER2 or Neu), an oncogenic form of EGFR, remains controversial [102,103].

Resistance to growth-inhibitory signals

Growth-inhibitory signals can exert their antiproliferative effects through the activation of tumor suppressor genes, and inactivation of these genes can result in hyperproliferation. There are at least three mechanisms by which tumor suppressor genes can be inactivated including mutation, loss of heterozygosity (LOH), and gene promoter methylation. Inactivation of the tumor suppressor genes p53, p16, p15, p27, and adenomatous polyposis coli (APC) has been implicated in the development of Barrett-associated adenocarcinomas.

The role of p53 in the neoplastic progression of Barrett esophagus has been studied extensively. LOH of 17p, the p53 locus, and p53 mutations have been found in nondysplastic cells of specialized intestinal metaplasia [104,105]. In fact, 17p LOH was found in diploid cells of specialized intestinal metaplasia prior to the development of aneuploidy, suggesting that p53 inactivation is an early step in the process of Barrett carcinogenesis [104]. Expression of mutant p53 has been found in nondysplastic Barrett metaplasia, and its levels increase as dysplasia progresses to cancer [106–108]. LOH of

17p and p53 mutations have been found in up to 90% of esophageal adenocarcinomas in Barrett esophagus [107–109].

LOH of 9p21, the chromosomal locus for p16 and p15, has been observed frequently in nondysplastic specialized intestinal metaplasia whose cells are aneuploid, and in Barrett-associated adenocarcinomas [110,111]. Mutations of the p16 and p15 genes are found far less frequently, however [110,112]. Recently, biopsy specimens of metaplastic Barrett esophagus demonstrated alterations of p16 via methylation, LOH, or mutation in 73%–87% of patients, suggesting that p16 alterations are a common and early event in the neoplastic progression of Barrett esophagus [111,113].

Loss of expression of p27, an inhibitor of cyclin E activity, has been found in 83% of esophageal adenocarcinomas, and has been correlated with aggressive tumor behavior and poor patient outcome [114]. LOH of 5q21, the APC locus, has been found in 20%–64% of esophageal adenocarcinomas, but only rarely have mutations been identified [115,116]. More common is APC inactivation by methylation, which has been found in 83%–92% of high-grade dysplasias and adenocarcinomas and in 40%–50% of Barrett metaplasias [117,118]. In one study, APC methylation was identified in the specialized intestinal metaplasia of 100% of patients who progressed to esophageal adenocarcinoma, compared with only 36% in patients who did not progress, suggesting that APC inactivation may be a predictive marker for neoplastic progression [118]. Furthermore, once adenocarcinoma develops, the finding of methylated APC DNA in the plasma may herald a poor survival [119].

Avoidance of apoptosis

Apoptosis is a tightly regulated, intrinsic form of programmed cell death that can occur in all normal cells. Normally, apoptosis is a beneficial process because it eliminates cells with DNA damage that, if perpetuated, might harm the organism [120]. Cancer cells must find ways to overcome apoptosis if they are to survive, albeit to the detriment of the organism. The apoptotic machinery comprises several death-commitment signaling pathways. These can be activated either by external stimuli that activate surface membrane death receptors or by intracellular stimuli including DNA damage and metabolic abnormalities that eventually converge on the caspase cascade, which ultimately leads to cell destruction [121].

The expression of death receptors that are members of the tumor necrosis factor (TNF) receptor superfamily has been investigated in the formation of Barrett-associated adenocarcinomas. Using immunohistochemistry and immunoblotting, one group of investigators has found enhanced expression of TNFR1 and its ligand, TNF-α, in the progression from Barrett metaplasia to adenocarcinoma [122]. Although this may seem counterintuitive, TNF-α binding to TNFR1 does not usually result in apoptosis, but rather in the up-regulation of antiapoptotic genes that promote cell survival [123,124]. Another death receptor–ligand pair implicated in Barrett carcinogenesis is Fas and Fas ligand (FasL). When FasL binds to Fas, apoptosis is induced in the Fas-expressing cells [125]. Therefore, one way for tumor cells to avoid apoptosis is to down-regulate Fas expression. Data on Fas expression in benign Barrett metaplasia as well as in esophageal adenocarcinoma are contradictory, with some studies detecting Fas expression and others finding undetectable or weak Fas staining [126–128]. Another way in which tumor cells can avoid apoptosis is by expressing FasL and mounting a "counterattack" against antitumor lymphocytes [129]. For example, in a study of esophageal adenocarcinoma, Younes et al. [130] found FasL expression in all 13 of the esophageal adenocarcinomas and their associated metastases, but none was detected in samples of normal squamous or metaplastic Barrett esophagus [130].

Intracellular stimuli that activate death-commitment pathways can also trigger apoptosis. Although the precise mechanisms are not known, the Bcl-2 family of proteins appears to be involved. The Bcl-2 protein family includes both proapoptotic members (e.g., Bax) and antiapoptotic members (e.g., Bcl-2 and Bcl-XL). Expression of Bcl-2 has been found in 72% of specimens with specialized intestinal metaplasia, 84%–100% with low-grade dysplasia, 0%–25% with high-grade dysplasia, and 0%–40% with adenocarcinoma [108,131]. Survival in patients whose tumors stained positive for Bcl-2 was significantly better than survival in those whose tumors were Bcl-2 negative [132]. Expression of the antiapoptotic protein Bcl-XL and the proapoptotic protein Bax has been found in nondysplastic specialized intestinal metaplasia, low- and high-grade dysplasia, and adenocarcinoma [100,132].

Other mechanisms for avoiding apoptosis have also been found in Barrett esophagus. For example, down-regulation of 15-lipoxygenase-1 (15-LOX-1), an enzyme involved in the formation of the apoptosis-inducing fatty acid 13-*S*-hydroxyoctadecadienoic acid (13-*S*-HODE), has been described in 75% of Barrett-associated esophageal adenocarcinomas [133]. Cyclooxygenase 2 (COX-2), an enzyme involved in the generation of prostaglandins from arachadonic acid, has been shown to reduce the rate of apoptosis in vitro [134]. Increased expression of COX-2 has been detected both in esophageal adenocarcinomas and in the metaplastic epithelium of Barrett esophagus compared with normal control subjects [135,136]. Souza et al. [137] found that, by inhibiting COX-2 with the selective inhibitor NS-398, apoptosis could be induced in Barrett adenocarcinoma cells, suggesting a potential role for COX-2 inhibition in patients with Barrett-associated cancers [137]. Expression of a transcription factor, nuclear factor-kappa B (NF-κB), that regulates the expression of a number of antiapoptotic genes has been detected in 40%–60% of Barrett specialized intestinal metaplasia and in 61%–80% of esophageal adenocarcinomas [138,139]. Finally, as discussed earlier, p53 inactivation

allows Barrett cancer cells to avoid apoptosis in response to DNA damage.

Resistance to cell senescence

Cellular senescence, a state of permanent growth arrest, is an autonomous, intrinsic mechanism that limits the proliferative capacity of normal cells. Cancer cells must overcome this limitation to maintain unlimited proliferative capacity and hence become immortal. Telomeres are long stretches of noncoding, DNA repeat sequences located on the ends of chromosomes [140]. With each successive round of cell replication, telomeric DNA repeats are lost. When telomere shortening reaches a critical level, the chromosomes becomes unstable, which triggers exit from the cell cycle into senescence. To maintain an unlimited proliferative capacity, therefore, cells must maintain their telomere lengths. Telomerase is the enzyme responsible for maintaining telomere length above the critical level [141]. Most normal human somatic cells do not produce this enzyme, but the large majority of cancer cells express active telomerase [142].

Weak levels of telomerase expression have been detected in benign metaplastic Barrett esophagus, and these levels increase progressively as dysplasia worsens, with the highest levels found in Barrett-associated adenocarcinomas [143,144]. With advances in our understanding of cellular senescence and telomerase, it has now become possible to use the expression of telomerase to immortalize normal, nontumorigenic cells in vitro, including the benign metaplastic cells of Barrett esophagus [145,146]. Shammas et al. [147] found that, by inhibiting telomerase with 2,6-bis[3-(N-Piperidino)propionamido]anthracene-9,10-dione (PPA), growth arrest and apoptosis could be induced in Barrett adenocarcinoma cells, suggesting a potential role for telomerase inhibitors in patients with Barrett-associated cancers.

Development of new vascular supplies (angiogenesis)

To continue to increase in size, tumors must form new blood vessels (angiogenesis) to enable essential nutrients and oxygen to be provided [148]. To do so, tumor cells secrete potent angiogenic factors such as vascular endothelial growth factor (VEGF). VEGF binds to its receptors (VEGFRs), which are located on the surface of endothelial cells, to stimulate the proliferation and migration of endothelial cells into the tumor [148,149].

Several groups have investigated neoangiogenesis and the expression of VEGFs and VEGFRs in Barrett esophagus. Compared with normal esophageal squamous mucosa, specialized intestinal Barrett metaplasia demonstrates an increase in the density of new, immature blood vessels, which increases further as dysplasia progresses to adenocarcinoma [150–153]. The Barrett epithelial cells have been found to express VEGF-A and -C, whereas the blood vessels supplying the metaplastic mucosa express the corresponding receptors VEGFR2 and -3 [150,154]. Investigators have speculated that this neovascularization is responsible for the salmon color characteristic of Barrett metaplasia on endoscopic examination [150]. VEGF-A and VEGF-C mRNA have been found in 100% and 74% of esophageal adenocarcinomas respectively [154]. Moreover, expression levels of VEGF mRNA and protein are higher in adenocarcinomas than in dysplastic and nonneoplastic Barrett metaplasia [150,155].

Invasion and metastasis

With loss of cell–cell anchoring mechanisms, tumor cells can break away from the primary lesion to metastasize and invade surrounding tissues [156]. Although the mechanisms of invasion and metastasis are poorly understood, abnormalities in cell adhesion molecules (cadherins) and their associated proteins (catenins) appear to play a central role. For tumor cells to invade they must disrupt cell–cell adhesion by altering the cadherins or the catenins. Membranous staining for E-cadherin and β-catenin has been found in normal esophageal squamous epithelium and in the nondysplastic, specialized intestinal metaplasia of Barrett esophagus and the intensity of this staining has been found to decreases as the metaplastic mucosa becomes increasingly dysplastic [157–160].

Disruption of the cadherin/catenin-based cell–cell interaction allows cancer cells to break free of the primary tumor. To invade the surrounding tissues and to metastasize through vascular structures, the free tumor cells must penetrate extracellular matrix barriers [161]. The matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases that can digest components of the extracellular matrix and other extracellular proteins including cell adhesion molecules [161,162]. MMP-9 expression is higher in the metaplastic mucosa than in normal esophageal squamous mucosa [150]. Using in situ hybridization, Salmela et al. [163] found MMP-7 to be the principal MMP in Barrett-associated adenocarcinomas and lymph node metastases, and up-regulation of MMP-7 was even found in the epithelial cells of nondysplastic specialized intestinal metaplasia. Moreover, this same group found that aggressiveness of the tumor, as determined by histological criteria, correlated with the expression of MMP-7 [163].

Dysplasia in Barrett esophagus

Cancers in Barrett esophagus develop through a series of genetic alterations that endow the affected cells with growth advantages. As the advantaged cells proliferate, they acquire more genetic damage until, eventually, an autonomous clone of cells emerges that no longer responds to normal growth controls. This autonomous condition is called neoplasia. Some of the same DNA changes that give the cells their growth advantages and autonomy also cause morphological changes that the pathologist recognizes as dysplasia.

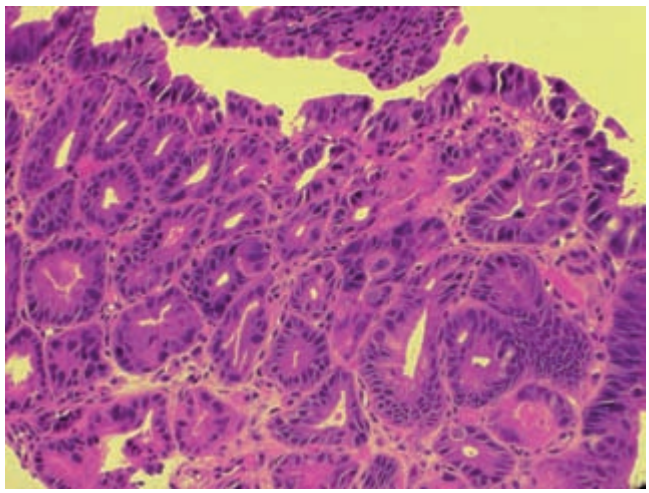

Figure 35.7 Photomicrograph showing high-grade dysplasia. Note that the nuclei are enlarged, pleomorphic, hyperchromatic, and stratified, and there are atypical mitoses.

Thus, dysplasia can be viewed as the histological expression of genetic alterations that favor cell growth and neoplasia [164]. Some prefer to use the term "intraepithelial neoplasia" instead of "dysplasia" to emphasize that the pathologist has judged the tissue to be neoplastic [165].

Pathologists diagnose dysplasia when they recognize a constellation of characteristic cytological and architectural abnormalities in tissue biopsy specimens including: (1) nuclear changes such as enlargement, pleomorphism, hyperchromatism, stratification, and atypical mitoses; (2) loss of cytoplasmic maturation; and (3) crowding of tubules and villiform surfaces (Fig. 35.7) [166]. Dysplasia is categorized as low-grade or high-grade depending on the degree of histological abnormalities, with more pronounced abnormalities assumed to reflect more severe genetic damage and greater potential for carcinogenesis.

Histological changes similar to those of low-grade dysplasia can be seen in nonneoplastic tissue that is regenerating in response to injury, and interobserver agreement among experienced pathologists for the diagnosis of low-grade dysplasia may be less than 50% [167–169]. Interobserver agreement is better (approximately 85%) for high-grade dysplasia, but far from perfect. There is also substantial interobserver disagreement when distinguishing high-grade dysplasia from intramucosal carcinoma [170].

Dysplasia in Barrett esophagus is often flat, inconspicuous, and patchy, both in extent and severity, factors contributing to the substantial problem of biopsy sampling error in identifying dysplasia [171,172]. Endoscopists use a random biopsy sampling system to find dysplasia in Barrett esophagus, and it is clear that this system can miss dysplastic areas. Furthermore, for patients who are found to have dysplasia, foci of invasive cancer can be missed because of sampling error. In a series of patients who had esophagectomies because endoscopic examination revealed high-grade dysplasia with no apparent tumor mass, invasive cancer has been found in 30%–40% of the resected esophagi [173]. Conversely, there are reports of patients whose preoperative biopsy specimens showed intramucosal carcinoma, but whose resected esophagi had no evidence of cancer [174].

Researchers have tried to develop endoscopic techniques for recognizing dysplasia in Barrett esophagus, and have tried to find alternative markers for cancer risk [175]. Techniques that have been proposed to identify areas of dysplasia for biopsy sampling during endoscopy include mucosal staining with vital dyes (chromoendoscopy), endosonography [176], optical coherence tomography [177], and Raman detection methods [178,179]. Some studies have shown that abnormalities in p53 and cyclin D1 expression [180,181], and abnormal cellular DNA content (aneuploidy) demonstrable by flow cytometry are associated with carcinogenesis in Barrett esophagus [182]. Presently, however, none of these tests and techniques has been shown to provide sufficient clinical information to justify its routine application for surveillance purposes. Endoscopy with random biopsy sampling for dysplasia, despite the many shortcomings, remains the clinical standard for managing patients with Barrett esophagus.

Although cancers in Barrett esophagus are thought to evolve from nonneoplastic metaplasia to low-grade dysplasia, to high-grade dysplasia, and to adenocarcinoma, this orderly sequence of progression is documented only infrequently. For patients with verified dysplasia, subsequent endoscopies often reveal no dysplastic epithelium, or patchy areas of dysplasia with varying severities [182,183]. Furthermore, adenocarcinomas have been found in patients whose previous surveillance biopsy specimens revealed only low-grade or even no dysplasia [184]. One recent study suggests that patients who have Barrett esophagus develop low-grade dysplasia at a rate of 4.3% per year, and high-grade dysplasia at a rate of 0.9% per year [54].

Few data are available on the natural history of low-grade dysplasia in Barrett esophagus. In a series of 48 patients with low-grade dysplasia followed for a mean duration of 41 months, five (10%) progressed to high-grade dysplasia (four patients) or adenocarcinoma (one patient) [185]. Another group described a 12% cumulative incidence of adenocarcinoma at 5 years for 43 patients with low-grade dysplasia on baseline endoscopy [182]. Skacel et al. [168] followed 25 patients with low-grade dysplasia for a mean duration of 26 months, during which seven (28%) progressed to high-grade dysplasia (five patients) or adenocarcinoma (two patients). In this series, agreement among pathologists in the diagnosis of low-grade dysplasia was associated with neoplastic progression. In total, seven of the 17 patients (41%) in whom at least two of the three study pathologists agreed on the diagnosis of low-grade dysplasia exhibited progression, whereas

progression was seen in four of the five patients (80%) for whom there was unanimous agreement among the study pathologists. In another study that included 156 patients with low-grade dysplasia, the incidence of cancer in these patients was 0.6% per year [54].

Relatively few studies provide meaningful data on the rate at which high-grade dysplasia in Barrett esophagus progresses to cancer, and reported estimates vary considerably. Hameeteman et al. [186] described eight patients with high-grade dysplasia in Barrett esophagus, five (63%) of whom were found to have adenocarcinoma on repeat endoscopic examination performed within 1 year [186]. Reid et al. [182] reported a 59% 5-year cumulative cancer incidence among 76 patients with high-grade dysplasia in Barrett esophagus [182]. However, Schnell et al. [183] observed a much lower cancer incidence in his 75 patients with high-grade dysplasia in Barrett esophagus, only 12 of whom (16%) developed adenocarcinoma during a mean follow-up period of 7.3 years. The reasons underlying the large disparities in the results of these three studies are not clear. In the series of Schnell et al. [183], it is possible that "overreading" of dysplastic changes contributed to the low incidence of adenocarcinoma [187]. In addition, the analysis of Schnell et al. [183] excluded patients who were found to have cancer within 1 year of the diagnosis of high-grade dysplasia, because the investigators considered these cancers to be prevalent (present at the first endoscopy but missed because of sampling error) rather than incident malignancies (developing during the period of follow-up). This elimination criterion would have excluded all of the five patients with cancer described by Hameeteman et al. [186], and would have reduced the 5-year cumulative cancer incidence in the series of Reid et al. [182] from 59% to 31% [22].

Buttar and colleagues [188] found that the extent of high-grade dysplasia in Barrett esophagus correlated with the risk for adenocarcinoma. In their report, high-grade dysplasia was defined as "focal" if the histological abnormalities were confined to a single focus involving up to five crypts, and as "diffuse" when these features were seen in more than five crypts or in multiple biopsy specimens. The cumulative cancer incidence at 3 years was 56% for the 67 patients with diffuse high-grade dysplasia, compared with only 14% for the 33 patients initially found to have focal high-grade dysplasia. However, the histological criteria for focal and diffuse dysplasia used in this study were arbitrary, and there is no clear consensus on this classification among gastrointestinal pathologists [189]. Furthermore, other investigators have not confirmed the observation that focal high-grade dysplasia is associated with such a low cancer risk [190].

In summary, it appears that patients with Barrett esophagus develop low-grade dysplasia at a rate of 4.3% per year, and high-grade dysplasia at a rate of 0.9% per year. The overall rate of cancer development in patients with Barrett esophagus is approximately 0.5%. Patients with low-grade dysplasia develop cancer at a rate of approximately 0.6% per year, but this estimate must be suspect because of the substantial problem of disagreement among pathologists in the identification of low-grade dysplasia. For patients with high-grade dysplasia, one review estimates that the rate of demonstrable cancer development is 4%–6% per year [164].

Management of patients with Barrett esophagus

The management of patients with Barrett esophagus involves three major components: (1) treatment of the associated GERD; (2) endoscopic surveillance to detect dysplasia; and (3) treatment of dysplasia. All three components are highly controversial.

Treatment of gastroesophageal reflux disease in Barrett esophagus

The general approach to the treatment of GERD in patients with Barrett esophagus is very similar to that recommended for patients who have GERD without Barrett esophagus [191]. One important difference, however, is that many modern authorities advocate initial and maintenance therapy with a PPI, rather than an incremental ("step up") therapeutic approach that is based upon symptom response. This practice is based on indirect evidence suggesting that acid reflux promotes carcinogenesis in specialized intestinal metaplasia, and that aggressive control of acid reflux may interfere with carcinogenesis.

The indirect evidence for acid promoting carcinogenesis in Barrett esophagus includes the observation that biopsy specimens of specialized intestinal metaplasia maintained in organ culture exhibit hyperproliferation and increased expression of COX-2 when exposed to acid [192,193]. In Barrett-associated adenocarcinoma cell lines, acid exposure has been shown to activate MAPK and protein kinase C pathways, to increase proliferation, and to decrease apoptosis [192,194]. Brief esophageal acid exposure in vivo also activates the MAPK pathways in Barrett esophagus [192]. In a clinical study in which esophageal biopsy specimens were taken from Barrett patients at baseline and after 6 months of antireflux therapy, the expression of PCNA (a proliferation marker) was decreased significantly in biopsy specimens from 24 patients in whom treatment had normalized esophageal acid exposure, but not in the 15 with persistently abnormal acid reflux [195]. Another group found no significant change in the proliferative activity of Barrett esophagus in 22 patients treated with a PPI for 2 years, whereas proliferative activity increased significantly in 23 patients treated for the same time with a histamine H_2 receptor antagonist [196]. During PPI therapy, most patients with Barrett esophagus develop partial regression of Barrett epithelium in the form of islands of squamous epithelium

[197]; however, it is not clear whether this partial regression reduces the cancer risk [198]. In a study of long-term follow-up of 236 patients with Barrett esophagus, the cumulative incidence of dysplasia at 10 years was 21% for the patients who received PPI therapy, compared with 58% for the patients not treated with PPIs [199]. All of these studies suggest that aggressive acid suppression might prevent the development of cancer in Barrett esophagus.

One study has challenged the notion that acid exposure exerts only damaging effects in Barrett esophagus [200]. The in vitro studies on this issue mentioned above involved cancer cell lines, which have extensive genetic abnormalities that might alter their responses to acid exposure, and tissue explants, which comprise numerous nonepithelial cell types that might mask the direct effects of acid on epithelial cells. Furthermore, in the clinical studies that suggest potential benefits for aggressive acid suppression, it is not possible to determine whether acid control decreased proliferation in Barrett epithelium directly, by removing an acid-triggered proliferation signal, or indirectly through the antiinflammatory effects of healing reflux esophagitis. Inflammation is well known to be a potent stimulus for proliferation and carcinogenesis in a number of organs. In cultures of a novel, nonneoplastic Barrett epithelial cell line that is immortalized but not transformed, Feagins et al. [200] found that acid exerts direct antiproliferative effects. If acid has beneficial direct effects on Barrett cells, then it is conceivable that the prescription of antisecretory medications in doses beyond those required to heal reflux esophagitis could have some detrimental effects.

For patients with Barrett esophagus, the elimination of heartburn by antisecretory therapy is no guarantee that acid reflux has normalized. A number of studies have shown that Barrett patients may be rendered asymptomatic by PPIs given in dosages that fail to normalize esophageal acid exposure [201–203]. In one study of 48 patients with Barrett esophagus, for example, 50% had persistently abnormal acid exposure documented by esophageal pH monitoring while they were receiving dosages of PPIs that abolished symptoms [203].

In patients with GERD who are treated with PPIs, lower healing rates of reflux esophagitis have been reported for those with Barrett esophagus than for those who have esophagitis of similar severity but without columnar metaplasia [204]. Thus, it has been suggested that patients with Barrett esophagus are unusually resistant to PPI treatment [203]. One study has suggested that this problem is not due to gastric resistance to the antisecretory effects of PPIs, because Barrett patients treated with high doses of esomeprazole exhibited gastric acid suppression to levels similar to those reported for normal individuals [205]. Despite the significant decrease in gastric acidity with the high dose of PPI, however, 16%–23% of the Barrett patients still had abnormal esophageal acid exposure. This suggests that the "PPI resistance" in patients with Barrett esophagus is a consequence of their profound propensity for reflux diathesis.

Some surgeons have proposed that fundoplication might be more effective than antisecretory therapy for preventing cancer in Barrett esophagus [206]; however, this proposal is based far more on speculation than on data. Some authorities have even proposed that antisecretory therapy might predispose to malignancy [207,208], and that the increasing use of antisecretory medications might underlie the rising frequency of esophageal adenocarcinoma in Western countries [209]. However, the limited studies that have addressed this issue directly have not found a significant association between esophageal adenocarcinoma and the use of antisecretory agents per se [210,211].

Although some observational studies have found fewer cases of dysplasia and cancer among patients with Barrett esophagus who had antireflux surgery than among those who had received medical treatment [212], a number of higher quality studies have refuted this observation. For example, during a 10- to 13-year follow-up of patients who had participated in a randomized trial of medical and surgical therapies for complicated GERD, four of 165 patients (2.4%) in the medical group and one of 82 (1.2%) in the surgical group developed an esophageal adenocarcinoma [213]. The difference between the treatment groups in the incidence of this tumor was not statistically significant but, with such a low observed rate of cancer development, the study did not have sufficient statistical power to detect small differences in the incidence of esophageal cancer. In addition, any potential cancer-preventive benefit of surgery was offset by an unexplained, but significant, decrease in survival for the surgical patients as a result of excess deaths from heart disease. Two studies using large patient databases [214,215] and two metaanalyses [216,217] also have found no significant cancer-preventive effect for antireflux surgery. These data suggest that antireflux surgery should not be advised with the expectation that the procedure will prevent esophageal cancer.

Endoscopic surveillance for dysplasia

Although a number of medical societies recommend endoscopic surveillance for patients with Barrett esophagus, there is no proof (in the form of a randomized control trial) that surveillance is beneficial. The unproved assumptions underlying endoscopic surveillance for patients with Barrett esophagus are outlined in Table 35.1.

A number of small studies have suggested that Barrett esophagus does not affect survival [218]. In these small series, survival for patients with Barrett esophagus did not appear to differ significantly from that for control subjects in the general population. However, small studies might not have the statistical power to demonstrate a small decrement in survival. In a large, population-based study comprising 7413 person-years of follow-up in 2373 patients with Barrett esophagus, Anderson et al. [219] found that overall mortality

Table 35.1 Unproved assumptions underlying endoscopic surveillance for patients with Barrett esophagus

1. Barrett esophagus decreases survival as the result of death from esophageal adenocarcinoma
2. Endoscopic surveillance reliably detects dysplasia in Barrett esophagus
3. Treatment of dysplasia found by surveillance prolongs survival by preventing deaths from esophageal cancer

in the Barrett patients was not increased above that of the general population of Northern Ireland. In contrast, Solaymani-Dodaran et al. [220] found that 1677 patients with Barrett esophagus had a 37% increase in mortality compared with a standard reference cohort of 13 416 individuals representing the general population of the United Kingdom. Approximately 45% of the excess mortality in the Barrett patients was due to esophageal cancer, with the remainder due to other causes, mostly cardiovascular disease.

On the basis of available data, one can conclude that the incidence of esophageal adenocarcinoma and the frequency of deaths due to esophageal cancer for patients with Barrett esophagus are increased significantly above those of the general population. Nevertheless, esophageal cancer is an uncommon cause of death in these patients. Older patients with Barrett esophagus succumb far more often to common illnesses such as coronary artery disease than to esophageal adenocarcinoma. Thus, overall mortality in patients with Barrett esophagus appears to be affected only slightly by their higher frequency of esophageal cancer.

Observational studies have shown that some patients with Barrett esophagus have had curable neoplasms detected by endoscopic surveillance, and that asymptomatic cancers discovered in surveillance programs are less advanced than those found when patients present to the hospital with cancer symptoms such as dysphagia and weight loss. For example, Corley et al. [221] reviewed the records of 589 patients who had adenocarcinomas at the GEJ, only 23 of whom were known to have had Barrett esophagus before the cancer was discovered. In total, 15 of those 23 patients had the cancer discovered during endoscopic surveillance for Barrett esophagus and 11 of those (73%) survived, whereas there were no survivors among eight patients who were not in surveillance and whose cancers were discovered when they presented with symptoms of dysphagia and weight loss. However, such observational studies are subject to a number of biases (e.g., healthy volunteer bias, lead-time bias, length-time bias) that might inflate the benefits of surveillance programs [222]. In addition, reports have documented the development of incurable esophageal malignancies in some patients despite their participation in endoscopic surveillance programs [223].

Surveillance programs seek to detect early neoplasia but, to prolong survival, there must be an effective treatment that prevents the progression to incurable cancer. Until recently, the only treatment available for dysplasia and early cancers was esophagectomy, a procedure associated with considerable morbidity and with mortality. For older patients with comorbidities, the risks of esophagectomy might exceed the benefits [224]. When the only treatment for dysplasia was esophagectomy, it made little sense to recommend endoscopic surveillance for any patient who was not fit enough to undergo that major operation. Now, however, there are less invasive treatments for dysplasia such as endoscopic ablative therapies (e.g., photodynamic therapy) and endoscopic mucosal resection (EMR), which are discussed below (see section on treatment of dysplasia in Barrett esophagus). Although studies have not yet proved that these less invasive therapies prolong life, the presence of these therapies has substantially expanded the population of patients who might benefit from surveillance. In other words, patients may no longer need to be fit enough to undergo esophagectomy to justify endoscopic surveillance.

A number of computer models have supported the practice of endoscopic screening and surveillance for Barrett esophagus [225–232]. Cost-efficacy studies often use a benchmark value of up to US$50 000 per life-year saved as something that society deems to be worthwhile. Provenzale et al. [225] estimated that surveillance performed at 5-year intervals would cost US$98 000 per quality-adjusted life-year. Although this exceeds the US$50 000 benchmark value, it remains within a range that might be considered reasonable. All other studies estimate that endoscopic screening and surveillance for patients with Barrett esophagus would cost less than US$50 000 per life-year saved. Unfortunately, the computer models are not definitive because they rely on numerous layers of soft data and questionable assumptions. Furthermore, the internal programs of the models are largely "black boxes," with no external means to validate their accuracy. Despite the problems and the apparent discrepancies in cost estimates among the various models, it is worthwhile to note that all of them have found that screening and surveillance for Barrett esophagus can be beneficial.

The major points of the arguments for and against endoscopic surveillance for Barrett esophagus are summarized in Table 35.2. The opponents argue that, in an era of shrinking financial resources, society can ill afford for physicians to practice the unproved, expensive, and potentially hazardous policy of performing endoscopic surveillance for Barrett esophagus. The proponents contend that it is ethically wrong for physicians to forgo the generally safe and potentially life-saving practice of endoscopic surveillance because of financial concerns or because they are awaiting the results of a definitive study that may never materialize. There have been numerous debates at medical meetings and in medical journals regarding the wisdom of surveillance for Barrett esophagus, with no clear winners.

Table 35.2 Arguments for and against endoscopic surveillance for patients with Barrett esophagus

Against surveillance
There is no proof (no randomized controlled trials) of efficacy
Endoscopy is expensive
Endoscopy has physical risks and potentially adverse, long-term emotional and financial consequences
Most patients derive no demonstrable benefit from surveillance
For surveillance
The rationale is reasonable
No proof of efficacy is likely in the near future
Most observational studies suggest benefit
All available computer models suggest benefit
No study has shown an overall survival disadvantage for patients in surveillance programs

Treatment of dysplasia in Barrett esophagus

When evaluating reports on treatments for dysplasia in Barrett esophagus, it is important to consider the issue of what constitutes adequate follow-up. Specifically, when can the patient treated for dysplasia be considered "cured?" Patients treated for carcinomas traditionally are deemed cured if there is no evidence of recurrence at 5 years, because it is assumed that any cancer cells that survived the treatment would have become clinically manifest within that time period. As discussed, however, it often takes considerably longer than 5 years for dysplasia to progress to invasive cancer. Consequently, it is not appropriate to conclude that the cancer risk has been eliminated in a patient who has survived for 5 years after treatment of dysplasia. Unfortunately, the follow-up duration in most reported studies on treatments for dysplasia in Barrett esophagus is considerably less than 5 years, and this factor severely limits the conclusions that can be drawn regarding the efficacy of therapy.

For patients with verified high-grade dysplasia in Barrett esophagus, there are generally four proposed management options: (1) esophagectomy; (2) endoscopic therapies that ablate the neoplastic tissue; (3) EMR (sometimes used in combination with ablative therapies); and (4) intensive endoscopic surveillance in which invasive therapies are withheld until biopsy specimens reveal adenocarcinoma. All four choices are associated with substantial risks and uncertain benefits.

Esophagectomy

Esophagectomy, which removes all of the abnormal esophageal epithelium in toto, is both the most definitive and the most risky (in terms of procedure-related morbidity and mortality) of the treatments for dysplasia in Barrett esophagus. Histological examination of the resected esophagus has revealed foci of cancer in 30%–40% of patients whose preoperative biopsies revealed only high-grade dysplasia in Barrett esophagus [173]. Studies on esophagectomy for high-grade dysplasia in Barrett esophagus typically have involved small numbers of patients from a single institution, and such reports are of limited value for estimating the morbidity and mortality of esophagectomy in this setting. Much larger series are available on the results of esophagectomy for esophageal cancer, but it may not be appropriate to extrapolate the results of surgery performed on patients with esophageal cancer, who often have substantial comorbidities and nutritional deficiencies, to otherwise healthy patients with precancerous lesions.

Mortality rates for esophagectomy among institutions are inversely related to the frequency with which the operation is performed. In one study of 340 esophagectomies performed at 25 different hospitals, the mortality rate was 3.0% for patients who had the operation at institutions that did five or more esophagectomies per year, compared with 12.2% for patients treated at institutions where the operation was performed less frequently [233]. In a study of data from the Dutch National Medical Registry, the mortality rates for esophagectomy were 12.1%, 7.5%, and 4.9% at centers performing 1–10, 11–20, and more than 50 esophagectomies per year respectively [234].

Esophagectomy has substantial operative morbidity. The average hospital stay for open esophagectomy is approximately 2 weeks, and 30%–50% of patients develop at least one serious postoperative complication such as pneumonia, arrhythmia, myocardial infarction, heart failure, wound infection, and anastomotic leaks [233–236]. Available data on minimally invasive techniques for esophagectomy are promising but limited, and it is not yet clear whether these approaches are preferable to the open procedure [237,238].

Esophagectomy can have substantial long-term morbidity including dysphagia, weight loss, gastroesophageal reflux, and dumping. However, the limited data available suggest that the impact of these symptoms on quality of life is surprisingly small. One group performed a utility assessment of patients who had esophagectomies at least 1 year earlier, and found that they rated their median quality of life at 0.97 on a scale of 0–1 (where 0 = death and 1 = perfect health) [225]. In another study of 53 patients who had esophagectomies for high-grade dysplasia in Barrett esophagus and who completed the MOS SF-36 Health Status Questionnaire, there appeared to be few important differences between the patients and a normal control population [239]. In another study in which quality of life questionnaires were administered to 199 patients before and after esophagectomy, quality of life was found to return to baseline values within 2 years [240].

The perception that the morbidity and mortality of esophagectomy is unacceptably high has prompted physicians to explore less invasive modalities to treat dysplasia in Barrett esophagus. However, it should be pointed out that,

unlike the alternative therapies discussed below, esophagectomy clearly eliminates the dysplasia and, if performed in a high-volume center on fit patients, should have a mortality rate substantially less than 5% with reasonable long-term morbidity. Thus, the esophagectomy option still warrants serious consideration, especially for young and fit patients.

Endoscopic ablative therapies

Endoscopic ablative therapies (e.g. KTP, argon, and Nd:YAG laser; multipolar electrocoagulation; argon plasma coagulation; photodynamic therapy; the HALO system) use thermal, photochemical or radiofrequency energy to ablate the abnormal epithelium in Barrett esophagus [241–244]. After the epithelium is ablated, patients are given potent antireflux therapy (usually PPIs) so that the injured mucosa heals with the growth of new squamous epithelium. The relative merits of the various endoscopic ablative therapies are disputed.

A major concern regarding endoscopic ablative therapies for dysplasia is that the procedures will leave dysplastic cells behind in the esophagus. Indeed, visible foci of metaplastic mucosa are found commonly after endoscopic ablation with all of the techniques available [241–244]. Partially ablated metaplastic mucosa can heal with an overlying layer of squamous epithelium that "buries" the metaplastic tissue and hides it from the endoscopist. There are reports of adenocarcinoma developing from buried metaplastic tissue [245]. Furthermore, partially ablated metaplastic epithelium can develop new abnormalities in the expression of proliferation markers and p53, raising the possibility that incomplete ablation of Barrett esophagus might even increase the risk of carcinogenesis [246]. Techniques to ablate the residual metaplasia are being studied, but limited data are available on their efficacy [247].

The conclusions that can be drawn from reports on ablative therapies for dysplasia in Barrett esophagus are limited because most studies are not randomized or controlled, involve small numbers of patients, and have short durations of follow-up. Photodynamic therapy (PDT) has been the most extensively studied of the ablative techniques. For PDT, patients are given a systemic dose of a light-activated chemical (usually a porphyrin or porphyrin precursor), which is taken up by the esophageal cells. The esophagus is then irradiated using a low-power laser that activates the chemical, which transfers the energy acquired from laser light to oxygen. This results in the formation of singlet oxygen, a toxic molecule that destroys the abnormal cells and their vasculature.

In a multicenter, randomized trial of PDT using porfimer sodium for ablation of high-grade dysplasia in Barrett esophagus, 138 patients were treated with PDT plus 20 mg of omeprazole b.i.d., and 70 received 20 mg of omeprazole b.i.d. alone (without PDT) [248,249]. No dysplasia was seen on repeat endoscopy in 77% of the patients treated with PDT and omeprazole, and in 39% of the patients who received omeprazole alone ($P < 0.0001$). During up to 5 years of follow-up, 15% of the PDT patients developed cancer, compared with 29% of those treated with omeprazole alone ($P = 0.027$). There was no procedure-related mortality, but 69% of the patients who received PDT developed photosensitivity reactions and 36% developed esophageal strictures that required one or more sessions of dilation therapy. Although these results show that PDT is clearly superior to omeprazole alone for eradicating dysplasia and preventing cancer in Barrett esophagus, the frequency of serious complications is disconcerting, as is the fact that 15% of the patients who received PDT developed cancer.

Although a number of reports have documented the feasibility of ablating neoplastic epithelium with endoscopic ablation, available studies have not established the overall benefits of the technique. Endoscopic ablation is expensive, and the treatments entail risks and inconvenience. Preliminary results of radiofrequency ablation for dysplasia in Barrett esophagus are promising, and this technique appears to have substantially fewer adverse effects than PDT [250]. Without histological examination of the resected esophagus or durations of follow-up well beyond 5 years, however, it is not possible to verify claims that dysplasia and cancer indeed are "eliminated" by endoscopic ablation. Residual foci of metaplasia remain in many patients after endoscopic ablation, and some of these foci may be buried under a superficial layer of squamous epithelium where they are invisible to the endoscopist.

Endoscopic mucosal resection

In EMR, a diathermy snare or endoscopic knife is used to remove a large segment of esophageal mucosa down to the submucosa [251,252]. Most centers have used a "suck and cut" method for EMR in which the endoscopist elevates the dysplastic area by injecting fluid into the submucosa, after which the elevated mucosa is suctioned into a cap that fits over the tip of the endoscope. A polypectomy snare is then deployed around the suctioned area to remove it. A recent variation on this technique involves the use of a ligating device, similar to that used for endoscopic variceal ligation, which deploys elastic bands around the suctioned mucosal segment [253]. Unlike the endoscopic ablative techniques, EMR provides large tissue specimens that can be examined by the pathologist to determine the character and extent of the lesion, and the adequacy of resection.

Although there is the theoretical potential for lymphatic dissemination of neoplastic cells that enter the esophageal lamina propria, limited data from surgical series suggest that fewer than 5% of patients with intramucosal adenocarcinoma in Barrett esophagus have lymph node metastases [254,255]. In contrast, for esophageal squamous cell carcinomas with a similar early T stage, lymphatic spread appears to be more common [256]. It has been proposed that the reflux esophagitis that often accompanies Barrett esophagus may

occlude mucosal lymphatic channels and thereby prevent lymphatic spread of early esophageal adenocarcinoma [255]. Once the tumor cells breach the muscularis mucosae to enter the submucosa, however, the frequency of lymph node metastases exceeds 20%, even for patients without bulky tumors [254,255]. The tissue specimens obtained by EMR allow histological examination to determine submucosal invasion. Therefore, EMR can be both diagnostic (revealing submucosal invasion that might not be apparent by less invasive techniques, which would require esophagectomy or other modalities for cure) and therapeutic (if there is no submucosal extension) [257].

Considering the depth and size of the mucosal specimens obtained by EMR, there have been surprisingly few reports of serious complications (bleeding, perforation, stricture) and virtually no procedure-related mortality [258–261]. The mean durations of follow-up in most reports are too short for meaningful conclusions to be made regarding the efficacy of EMR in decreasing the risk of cancer development. Furthermore, it appears that cap-assisted EMR leaves neoplastic cells behind in the large majority of cases. In one report, histological examination of EMR specimens obtained by the cap-assisted technique from 88 patients with high-grade dysplasia revealed dysplastic tissue at the margins of the specimens in 72 cases (82%) [262]. This suggests that EMR should be combined with an ablative procedure to eliminate the residual dysplastic epithelium.

The limited data that are available on long-term survival for patients with dysplasia and early cancers in Barrett esophagus who have been treated by EMR are impressive. For example, Ell et al. [260] performed EMR on 100 patients with early adenocarcinoma (i.e., tumor diameter < 20 mm, well-differentiated histology, no invasion of lymphatics or blood vessels, no metastases, no submucosal invasion) in Barrett esophagus. EMR resulted in no serious complications, and the calculated 5-year survival rate was an extraordinary 98%. However, recurrent or metachronous cancers were found in 11% of the patients during a mean follow-up period of 37 months. The recurrent tumors were treated successfully with more endoscopic therapy, but this high rate of recurrence shows that EMR often leaves behind cells with neoplastic potential. A retrospective study from the Mayo Clinic confirms this observation [261]. The investigators compared long-term survival in patients with high-grade dysplasia who were treated either with esophagectomy or with a combination of EMR and PDT. There was no statistically significant difference in overall long-term survival for patients treated with either of the therapies, even though 6.2% of the patients treated with PDT and EMR developed esophageal cancer during the follow-up period.

EMR for dysplasia and early cancers in Barrett esophagus appears to be safe in experienced hands, and limited data suggest that 5-year survival rates are excellent. However, recurrent neoplasms develop frequently, especially in patients with long-segment Barrett esophagus. Although the results so far are impressive, the clinician should appreciate that the data are still limited in duration and in scope. Most of the studies on EMR have come from only a handful of highly specialized centers, and it is not clear whether these results can be duplicated in a community hospital setting.

Intensive endoscopic surveillance

Some authorities recommend a program of expectant management with intensive endoscopic surveillance (i.e., endoscopic examinations every 3–6 months) for patients with high-grade dysplasia in Barrett esophagus, withholding invasive treatments until biopsy specimens reveal adenocarcinoma [174,183]. Although this practice has been endorsed as a management option by the American College of Gastroenterology [22], few published data directly support the safety and efficacy of intensive surveillance for high-grade dysplasia.

Schnell et al. [183] described 12 patients who developed adenocarcinoma during intensive endoscopic surveillance for high-grade dysplasia in Barrett esophagus. The cancers were deemed potentially curable at the time of detection in all 11 patients who were compliant with the surveillance program, but one patient who was lost to follow-up returned 10 years later with an unresectable tumor. In the series of Reid et al. [263] of 32 patients with high-grade dysplasia who developed adenocarcinoma during intensive endoscopic surveillance, only one patient (3%) had incurable disease (metastases) when the cancer was first detected on surveillance endoscopy. Weston et al. [190] performed intensive endoscopic surveillance in 15 patients with high-grade dysplasia for a mean duration of 36.8 months, during which four developed adenocarcinoma [190]. One of those four had metastatic disease. These studies show that intensive endoscopic surveillance generally is safe, but even patients who are compliant with intensive surveillance programs can develop incurable cancers.

Authorities have recommended that the treatment for patients with high-grade dysplasia in Barrett esophagus should be individualized based on considerations of age and comorbidities [264]. Is the patient a healthy 50 year old or a 90 year old with heart and kidney failure and an inordinate operative risk? Is the life expectancy 30 years or 30 days? What is the extent of the Barrett metaplasia? Is there short-segment Barrett with a single focus of dysplasia that can easily be removed endoscopically, or is there long-segment Barrett that involves the entire esophagus with multiple neoplastic areas that make endoscopic treatments untenable? And importantly, what is the patient's preference when the options are presented fairly?

Nonsteroidal antiinflammatory drugs

Epidemiological studies have shown that the use of aspirin and other NSAIDs, which inhibit COX, are associated with a

decreased risk for developing esophageal cancer; a metaanalysis of these studies suggests that the risk is decreased by approximately 40% [265]. The specialized intestinal metaplasia of Barrett esophagus exhibits increased expression of COX-2 [266], and inhibition of COX-2 has antiproliferative and proapoptotic effects in Barrett-associated esophageal adenocarcinoma cell lines [137]. Furthermore, COX-2 inhibitors have been found to prevent the development of esophageal adenocarcinoma in an animal model of Barrett esophagus [267]. Nevertheless, prospective clinical studies are needed before NSAIDs can be recommended routinely for chemoprevention for patients with Barrett esophagus. Even if efficacy in cancer prevention can be demonstrated, it is not at all clear that the cost and cardiovascular risks of NSAIDs will be justified for routine clinical use. Aspirin, an inexpensive, nonselective NSAID that can prevent cardiovascular as well as neoplastic complications, might be a very useful drug if its protective effects can be shown to outweigh its risk of gastrointestinal complications.

Management recommendations

Clinicians should appreciate that no management strategy for patients with Barrett esophagus has been verified by studies demonstrating that the strategy prolongs life. The bulk of the indirect evidence available suggests that acid suppression with PPIs may reduce the cancer risk in Barrett esophagus. We recommend that Barrett patients should be treated with a PPI using whatever dose is needed to control GERD symptoms and to maintain the healing of esophagitis. We do not routinely recommend esophageal pH monitoring to verify that esophageal acid exposure has been normalized by PPI therapy. We also feel that antireflux surgery should not be prescribed solely as a cancer preventive strategy. There may be other valid reasons to recommend this operation, but cancer prevention is not one of them. We also feel that it is appropriate to consider the prescription of low-dose aspirin, both for its cancer preventive and cardioprotective effects, especially for patients who are not at high risk for complications of NSAID treatment. Because patients will already be taking a PPI, the risks of aspirin causing serious gastrointestinal toxicity in average risk individuals should be minimal.

We endorse the management policy that has been recommended by the Practice Parameters Committee of the American College of Gastroenterology with some modifications as outlined in Table 35.3 [22]. We feel that esophagectomy may be the preferred management strategy for young patients with high-grade dysplasia. For patients with localized dysplasia, it may be reasonable to perform EMR, at least for the purposes of staging. If the EMR specimen shows no submucosal invasion and no dysplasia in the margins, the need for esophagectomy is highly controversial.

Table 35.3 Management of patients with Barrett esophagus recommended by the Practice Parameters Committee of the American College of Gastroenterology

- Patients found to have Barrett esophagus should have regular surveillance endoscopy to obtain esophageal biopsy specimens
  - o GERD should be treated aggressively before surveillance to minimize confusion caused by inflammation in the interpretation of dysplasia
- For patients who have had two consecutive endoscopies that show no dysplasia, surveillance endoscopy is recommended at an interval of every 3 years
- If dysplasia is noted, the finding should be verified by consultation with another expert pathologist:
  - o For patients with verified low-grade dysplasia after extensive biopsy sampling, yearly surveillance endoscopy is recommended
  - o For patients with verified high-grade dysplasia, the risks of esophagectomy must be weighed against those of intensive endoscopic surveillance, endoscopic ablative therapies, and endoscopic mucosal resection

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A ESOPHAGUS

36 Esophageal neoplasms

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Squamous cell carcinoma

Epidemiology and etiology

Esophageal *squamous cell carcinoma* (SCC) is the most common form of esophageal malignancy worldwide and is one of the leading causes of cancer mortality in men, especially African American men. The incidence of esophageal SCC varies according to geographical location, with a global range from 2.5 to 5.0 for men and from 1.5 to 2.5 for women per 100 000 population. High-incidence regions, where rates may exceed 100 per 100 000 population, include northern China, India, northern Iran, areas north and east of the Caspian Sea, and the Transkei area of South Africa [1–5]. Indeed, nearly 50% of all cancers are esophageal in southeastern South Africa [1]. In the United States, African Americans have a 4- to 5-fold increased risk compared with white people, especially African American men, in whom the risk is 15.1 per 100 000 compared with 2.9 per 100 000 in white men [6,7].

The geographical variation in esophageal SCC strongly hints at a contribution from environmental factors. An inherited predisposition to esophageal SCC is rare. The incidence of esophageal SCC is very low in those under 40 years of age, but it increases with each succeeding decade of life [7]. It is more common in men than in women, with a 2- to 3-fold higher risk, regardless of ethnicity and age [7]. However, there are some exceptions to this general finding. In northern Iran, the rate is 263 per 100 000 for women and 206 per 100 000 for men [8], and upper esophageal cancers in association with the Plummer–Vinson syndrome are found in women [9].

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Tobacco and alcohol

Together, tobacco and alcohol constitute the largest risk factors for the development of esophageal SCC in North America and Western Europe [10–13]. Because tobacco and alcohol are commonly consumed together, their individual contribution may be difficult to ascertain. However, a comprehensive study of alcohol consumption in nonsmokers and tobacco use in nondrinkers found that each acts independently as a risk factor [14]. The relative risk was 2.0 for those who smoked fewer than 15 cigarettes per day, and 6.2 for those who smoked more than 25 cigarettes per day [14]. Not surprisingly, there is a documented substantial increase in risk in patients who consume both tobacco and alcohol, with the amount consumed and duration of use being pivotal factors [14–17,18]. Thus, smokers who have a large intake of beer and whiskey harbor a 10- to 25-fold increased risk of esophageal SCC compared with smokers who do not drink alcohol [18,19]. Additionally, the risk may be greater for pipe and cigar smokers than for cigarette smokers [18].

In a manner reminiscent of lung cancer, cessation of smoking does lead to a decreased risk of esophageal SCC after 10 years [18,20,21]. Although cigarette smoking is a key risk factor in North America and Western Europe, chewing tobacco, betel, or combinations of these with lime accounts for the high incidence of esophageal SCC in southern and Southeast Asia [22–24]. Tobacco tars and cigarette smoke contain various chemical carcinogens, such as aromatic amines, lactones, peroxy compounds, halo ethers, *N*-nitroso compounds, and polycyclic aromatic hydrocarbons [25–28], that individually or in combination likely lead to esophageal epithelial hyperproliferation and eventual malignant transformation.

The type of alcohol (beer, whiskey) and the fashion in which it is distilled, but not the duration of ingestion, are risk factors for esophageal cancer. Groups of people who do not consume alcohol have a substantially decreased risk. There are several factors underlying the contribution of alcohol to the pathogenesis of esophageal cancer, as well as other

cancers [29]. Alcoholic beverages may contain congeners or other ingredients, such as *N*-nitroso compounds, urethane, mycotoxins, tannins, pesticide residues, and asbestos products, that are directly or indirectly carcinogenic. Ethanol itself, because it is a solvent for fat-soluble compounds, may facilitate the absorption of other carcinogens. Carcinogens that are oxidants may increase the risk of DNA damage. Acetaldehyde, a metabolite of ethanol, can inhibit DNA methyltransferase activity [30]. Consequently, a state of methyl deficiency may ensue, which may potentiate the carcinogenic effect of a methyl-deficient diet.

Diet and nutrition

Vitamins and deficiencies, especially of vitamins A and C, folic acid, vitamins E and B-12, and riboflavin, are crucial risk factors [31–35]. Diets containing high amounts of green and yellow vegetables that are rich in β-carotene and citrus fruits that are high in vitamin C decrease the incidence of esophageal SCC [35]. Lower plasma levels of folic acid have been documented in patients with esophageal SCC [33]. Riboflavin deficiency manifested by cheilosis and glossitis is common in the Linxian province of China, where esophageal cancer is endemic [36]. It is likely that vitamins A, C, and E exert antioxidant effects; vitamins C and E are known to influence the formation of nitrosamines.

The levels of certain trace elements, such as selenium, molybdenum, and zinc, show an inverse association with mortality from esophageal cancer in high-incidence areas of the world [37–39]. Furthermore, in animal models, a significant increase in esophageal tumors is induced by *N*-nitrosomethylbenzylamine (NMBA) in rats with zinc-deficient diets. Mechanistically, zinc deficiency enhances the microsomal activation of NMBA to a methylating agent that yields *O*-6-methylguanine adducts in esophageal DNA [40]. As a component of superoxide dismutase, zinc also protects against oxygen-derived radical damage and free-radical formation [33]. Similarly, selenium has protective effects, in part mediated through the inhibition of lipid peroxidation of cell membranes through the actions of selenium-dependent glutathione peroxidase [32]. The Linxian and adjoining provinces in China have foodstuffs low in molybdenum and documented reduced levels of this trace element in patients' serum and urine. It is conceivable that molybdenum deficiency leads to the accumulation of nitrates that are precursors of nitrosamines because this trace element is a key constituent of nitrate reductase [24].

Achalasia

Patients with long-standing achalasia have a prevalence of esophageal SCC of 5% [41–46]. The interval from dysphagia, weight loss, and chest pain ascribable to achalasia to the development of cancer is approximately 17–20 years [46,47]. In a study of 195 consecutive patients with achalasia, the incidence of esophageal SCC was 3.4 per 1000 patients per year, representing a 33-fold increase when compared with age- and sex-matched controls [48]. A follow-up study of 146 of 147 patients with achalasia treated with myotomy revealed that 10 patients progressed to esophageal cancer, with a mean interval of 16.7 years; the predicted frequency was less than 1 [49]. Thus, the condition of achalasia eventually may lead to cancer; however, the exact risk and mechanism remain to be determined. Although endoscopic surveillance of patients with achalasia has been advocated, the frequency of examinations remains the subject of debate.

Head and neck squamous cell carcinoma

Reflecting the use of both alcohol and tobacco, patients with head and neck SCC are at an increased risk of developing esophageal cancer [50,51]. Either coexisting or sequential esophageal cancers may develop annually at a rate of 3%–7% [52–55]. As a result, endoscopic surveillance of the esophagus has been advocated in this setting. Toluidine blue staining has been demonstrated to enhance the detection of indolent esophageal cancers in patients with head and neck cancers [56]. Not surprisingly, survival of patients with head and neck cancers is adversely affected by the coexistence of esophageal cancer [57]. A reduction in the incidence of second primary tumors from 24% to 4% in patients with head and neck cancers has been observed with the use of isotretinoin, a chemopreventive agent that induces epithelial differentiation [58].

Tylosis

Although some studies from China have suggested familial aggregation of esophageal cancers [59], it is difficult to discriminate between a common environmental exposure and an actual genetic basis for these observations. However, a genetic predisposition to esophageal cancer is clear in *tylosis palmaris*, an autosomal dominant disorder manifested by hyperkeratosis of the palms and soles [60–66]. Approximately 50% of patients with tylosis palmaris will develop esophageal cancer by the age of 45 years, and perhaps 95% by the age of 65 years [61,62]. There is linkage between esophageal SCC and oropharyngeal leukoplakia. In endoscopic surveillance studies over a 5-year period, 4 of 29 patients were found to have dysplasia, and one patient had carcinoma in situ [67]. The gene responsible for tylosis has yet to be identified although chromosomal 17 linkage has been suggested; once identified, it may also provide insights into sporadic esophageal cancer.

Other factors

Lye ingestion, which results in stricture formation, has been postulated as a risk factor for esophageal cancer, mostly appearing at the level of the bronchial bifurcation [68–70]. Lye strictures constitute only a small proportion of esophageal cancer cases, and the progression to cancer after initial exposure requires 4–5 decades.

Other factors implicated in the pathogenesis of esophageal cancer include ionizing radiation [71,72], celiac sprue [72–75], human papillomavirus (HPV) [76–78], Plummer–Vinson syndrome [79], esophageal diverticula [80], and maté drinking [81,82]. Experimentally, induction of radiation injury is associated with cancer formation [83]. In patients who have undergone high-dose radiation treatments, esophageal cancer is the most frequently observed malignant disease [84–90]. DNA tumor viruses that have a tropism for squamous epithelial cells, such as HPV and Epstein–Barr virus, have been associated with esophageal SCC. Polymerase chain reaction techniques have demonstrated an association of the oncogenic variants of HPV, namely HPV-16 and HPV-18, as well as a novel HPV genotype with esophageal SCC [77,78]. The same has been observed with Epstein–Barr virus [91]. Both viruses infect squamous epithelial cells, replicate, and produce oncogenic proteins that contribute to hyperproliferation and malignant transformation.

Hot maté drinking is common in Uruguay and southern Brazil [81,82], and hot liquid ingestion is prevalent in China [92]. These findings suggest that thermal injury of the mucosa may increase the risk of esophageal cancer.

Biology and genetics

Based on the colon cancer paradigm, it is thought that the development of cancer results from the accumulation of alterations in oncogenes, tumor suppressor genes, and DNA mismatch repair genes. This is probably true for esophageal cancer as well. However, a genetic basis for the pathogenesis of esophageal cancer by no means precludes the importance of environmental factors; rather, it is likely that there is cooperativity among these factors. For example, some of the environmental factors injure the esophageal epithelium, which in turn may act to signal changes in key genes through point mutations or augmented gene expression.

Perhaps the most critical oncogene in esophageal SCC pathogenesis is cyclin D_1. Mammalian cyclins represent a large family of cell cycle regulatory proteins that associate, in turn, with their catalytic subunit partners, designated cyclin-dependent kinases or CDK. (The cell cycle is reviewed in the literature [93].) The activity of cyclin D_1 is restricted to the G1 phase, and it preferentially associates with cyclin-dependent kinase 4 or 6 (CDK4 or CDK6). The complex of cyclin D_1 and CDK4 or CDK6 then phosphorylates the retinoblastoma tumor suppressor gene product (pRb). This relieves the negative regulation of pRb in the G1 phase through the release of key transcriptional factors, such as E_2F. It has been shown that cyclin D_1 is overexpressed in more than 50% of esophageal SCCs and is associated with a poor prognosis [94–96]. A subset of esophageal cancers also show c-*myc* and epidermal growth factor receptor overexpression as a result of gene amplification [97]. Recent research suggests that retinoid receptor-induced gene-1 (RRIG1) plays a role in suppressing tumorigenesis [98].

Knowledge of the association of cyclin D_1 and esophageal cancer has been used to develop a transgenic mouse model in which one of the Epstein–Barr virus promoters has been used to target cyclin D_1 specifically to the oral–esophageal squamous epithelia, resulting in dysplasia and cell cycle abnormalities [99,100].

The key tumor suppressor gene *p53* has many functions, including cell cycle regulation, induction of apoptosis in response to environmental insults, transcriptional regulation of genes, and DNA replication. *p53* is mutated in up to 70% of esophageal cancers [101–104]. One of the functional consequences of *p53* mutation is the transcriptional downregulation of *p21,* a general CDK inhibitor. In addition, *p16,* also referred to as *INK4a* (designated $p16^{INK4a}$), is an inhibitor of CDK4. Located on chromosome 9p22–23, *p16* can be deleted, mutated, or hypermethylated, resulting in loss of its inhibitory function in esophageal cancer [105,106]. A paradigm that emerges in esophageal SCC is that either cyclin D_1 is overexpressed or *p16* is altered to accelerate the cell through the G1 phase. Loss of heterozygosity in other tumor suppressor gene loci, such as *pRb* (chromosome 11) and *APC* (adenomatous polyposis coli) (chromosome 5q), does not appear to occur in esophageal cancer [107], suggesting that other genes in proximity to *pRb* and *APC* are the key targets. Loss of function of E-cadherin and transforming growth factor-β receptor 2 have important implications for epithelial carcinogenesis in esophageal SCC [108].

Clinical manifestations

Early esophageal SCC may be asymptomatic or may be associated with only mild specific symptoms [109–113]. As the cancer grows, dysphagia occurs in nearly 90% and odynophagia in 50% of patients [114–116]. This is usually a reflection of partial or total lumenal obstruction. Anorexia and weight loss from malnourishment may be observed in about 75% of patients [116,117]. Retrosternal pain or radiation of pain to the back suggests mediastinal involvement by the cancer. Nausea, vomiting, and hematemesis may occur. Cough may reflect aspiration pneumonia or, rarely, tracheoesophageal fistula. Recurrent laryngeal nerve involvement may result in hoarseness. Skeletal metastases may cause bone pain. Physical examination may reveal cachexia, lymphadenopathy, hepatomegaly (if distant metastatic disease is present), and the presence of fecal occult blood.

Evaluation of dysphagia is imperative and is discussed in Chapter 31. The differential diagnosis may include peptic acid esophagitis with stricture, caustic ingestion resulting in stricture, malignant disease (either squamous or adenocarcinoma), and motility disorders such as achalasia or scleroderma.

Natural history and complications

Esophageal cancer in Western countries usually presents in an advanced stage and, consequently, nearly 75% of untreated patients succumb to the disease within 1 year [118].

Biologically, esophageal SCC is not a rapidly growing tumor, and there is a prolonged period of time between dysplasia and cancer [119,120]. The early stages of esophageal cancer have been shown to persist for more than two decades in China and Iran [121,122]. Additionally, balloon cytological examinations, used to monitor the natural history of esophageal squamous dysplasia in China, indicate that dysplasia may progress to cancer (26.6% of cases), but it may regress to mild dysplasia or a normal state (40.5% of cases). Furthermore, of the mild dysplastic cases, nearly 15% progressed to severe dysplasia and about 45% regressed [123]. Other studies in China indicate that early superficial cancers remain in this state in 58% of cases for a follow-up period of 19–78 months [120]. Patients with untreated early esophageal cancer in China have a median survival time of 75 months.

There are probably biological differences superimposed on environmental factors in the high-incidence areas compared with Western countries that may account for the apparently lengthy period from dysplasia to cancer in China and the good prognosis of resected early esophageal cancer with a 5-year survival rate approaching 90% [112]. Reminiscent of the high prevalence of early gastric cancer in Japan relative to Western countries, it is not entirely clear whether early esophageal cancer is prevalent in North America and Western Europe. Several nonbiological explanations can be evoked: the use and accuracy of balloon cytology in China, the definition of early esophageal cancer, and proper staging with aggressive therapy [124,125].

The average survival of untreated patients with advanced, symptomatic cancer is approximately 9.5 months [118,122]. Patients may develop aspiration pneumonia complications of esophageal cancer from extension into the tracheobronchial tree or other mediastinal structures [126]. Fistulae have a 4.5% prevalence and most often communicate with the trachea, the main stem bronchi, and the lung parenchyma. Fistulae carry a poor prognosis [127,128]. They may cause presenting symptoms, such as cough, chest pain, dyspnea, hoarseness, and hemoptysis, or may occur within 2 months of the initial diagnosis in untreated patients [127,129]. As a result of fistula formation, patients may develop pneumonia and sepsis. Direct extension of the esophageal cancer, as opposed to fistula formation, may involve the lung and tracheobronchial tree, aorta, pericardium, and upper abdomen. Hoarseness suggests recurrent laryngeal nerve involvement. Apart from direct extension or fistula formation, esophageal cancer may metastasize to lymph nodes, lung, and liver as frequent sites [130–134].

Diagnostic evaluation

Implicit in a diagnostic evaluation is a complete history and physical examination. A laboratory profile may reveal anemia, hypoalbuminemia, elevated prothrombin time reflecting vitamin K deficiency, and possibly elevated liver function tests if metastatic disease is present. Serological markers of cancer, such as carbohydrate antigen 72.4 in gastric cancer, carbohydrate antigen 19-9 in pancreatic cancer, carcinoembryonic antigen in colorectal cancer, and alpha-fetoprotein in hepatocellular carcinoma, have not proven to be of high sensitivity and specificity in esophageal SCC. Evaluation is driven by the establishment of tissue diagnosis through biopsy and cytology, followed by staging for prognosis and therapeutic planning. Initial diagnostic tests usually entail barium swallow supplemented with a tablet and flexible fiberoptic endoscopy. Staging requires computed tomography (CT), endoscopic ultrasound (EUS), and radionuclide bone scan. Bronchoscopy should be considered if tracheal invasion is suspected.

Endoscopy and barium esophagography

The diagnosis of esophageal cancer requires endoscopy with biopsy and cytology. Multiple biopsies increase the diagnostic yield to up to 96%; cytology identifies the remaining cases. For example, in a retrospective study of 155 patients with endoscopic biopsy and cytology of upper gastrointestinal lesions, 2 of 28 patients with malignant disease were diagnosed through cytology alone [135].

Because endoscopy may prove too costly in some regions of the world, barium esophagrams are advocated by some experts, especially in patients with dysphagia. Intrinsic advantages include reduction in cost, noninvasiveness, and reduced complication rate. Additionally, a barium study allows information to be obtained about motility as well as challenge with a solid bolus. Ultimately, endoscopy is a better diagnostic test for patients in whom one suspects esophageal cancer because of the ability to obtain tissue in one test and the greater positive predictive value of upper endoscopy: 100% for endoscopy and 33% for barium swallow [136].

The endoscopic appearance of early esophageal cancer includes superficial erosive ulcer, a raised plaque, a "congestive" lesion marked by red spots on the mucosa, and a small polypoid lesion. Vital staining dyes such as toluidine blue, aniline blue, or Lugol iodine may be used to enhance detection and simultaneously to discriminate early cancers from normal adjacent mucosa. In a study of 178 patients who underwent endoscopy and vital staining, nine esophageal cancers were found, of which seven were early cancers [137]. In contrast, endoscopy without vital staining only detected four of nine cancers. Limiting the usefulness of vital staining is its inability or failure to distinguish inflammation from dysplasia [138].

Advanced esophageal SCCs are typically exophytic, ulcerated, and even circumferential. Submucosal invasion may produce friable nodules or strictures. Whenever possible, the endoscope should be advanced through the stricture to determine the proximal–distal extent of tumor and any distal abnormalities. The majority of SCCs are found in the proximal esophagus to the midesophagus.

Radiologically, early esophageal cancer may have a granular mucosal appearance with single or multiple small ulcerations with tiny filling defects. Polypoid lesions are characterized by small intralumenal filling defects affecting one wall of the esophagus with ulcerations [139]. An infiltrative or an ulcerative appearance can be one of the defining features of advanced esophageal cancer, but the most common presentation of advanced esophageal carcinoma is that of a polypoid mass within the lumen produced by an exophytic cancer [140]. The mass may have the characteristic appearance of an "apple-core" lesion with circumferential involvement by the cancer (Fig. 36.1). Infiltrating carcinomas may result in diminished esophageal motility with or without stricture. Such a stricture may be symmetric or asymmetric and, in fact, can sometimes be difficult to distinguish from a benign stricture. The primary ulcerative type of esophageal cancer is rare, although ulceration can be a component of either polypoid or infiltrating cancer. Ulcerative cancers are characterized by a well-demarcated meniscoid ulcer with a thin rim of lucency representing neoplastic tissue [141].

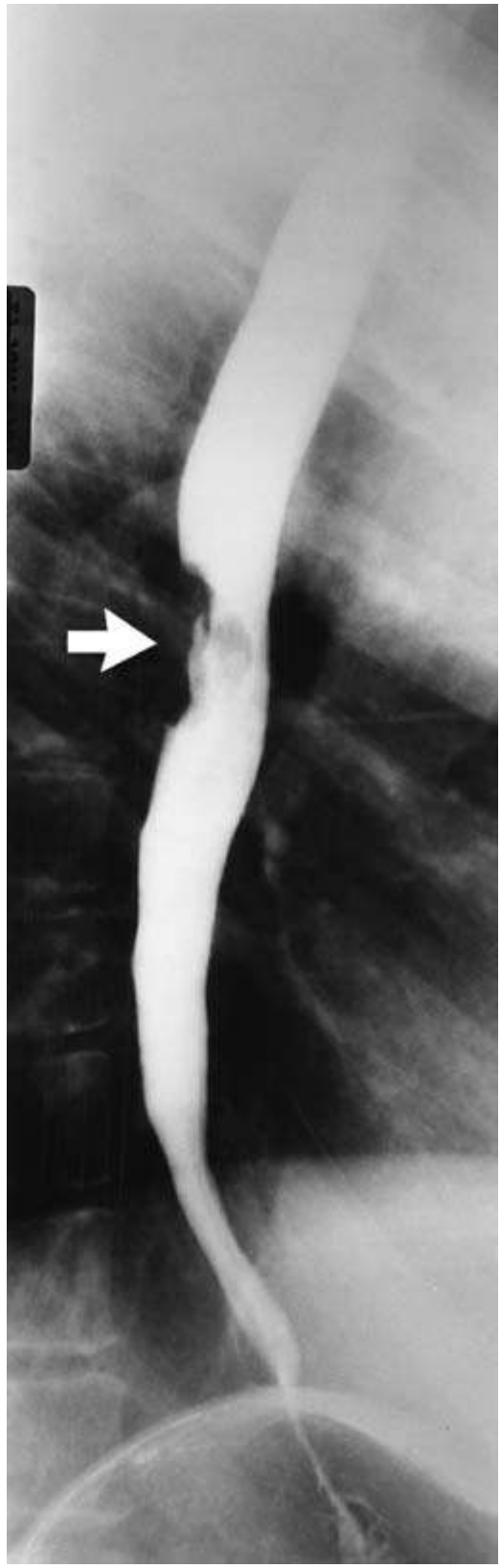


Figure 36.1 Advanced squamous cell carcinoma represented by an ulcerated mass (arrow) in the midesophagus. Courtesy of Deborah Hall, MD.

Screening

Endoscopic screening of the general population may be merited in high-incidence areas, such as in northern China. In China, 5-year survival was nearly 90% after operative resection for cancer cases detected through screening [142,143]. Surprisingly, nearly 80% of patients did not undergo therapy because of noncompliance, unavailability of medical resources, and coexisting medical illnesses [144]. Although balloon cytology has achieved success in China, its applications in US veterans did not prove very successful [145]. The reasons were the low prevalence of esophageal cancer and the low specificity of dysplasia because of a confounding factor such as inflammation resulting from reflux esophagitis. In this US veterans study, follow-up measures included biannual endoscopy with vital staining and biopsy, balloon cytology, laryngoscopy, and chest radiography [146]. However, false-positive results were obtained with cytology in more than 95% of screened patients. Thus, mass screening in the United States is probably not feasible for esophageal SCC, given the lack of cost-effectiveness. However, institution of screening and surveillance endoscopic programs may be applied to certain clinical situations, such as in patients with tylosis, achalasia, and head and neck cancer.

Ingestible esophageal capsule endoscopy (PillCam ESO)

An office-based noninvasive diagnostic approach (PillCam ESO) has been developed as an alternative to conventional endoscopy or other screening techniques to evaluate patients with esophageal abnormalities, especially those with Barrett esophagus and gastroesophageal reflux disease (GERD). A small plastic capsule with a tiny video camera glides down

the esophageal tract taking video images and transmitting these to a recording device. The disposable capsule is passed, usually within 24 h. A pilot study showed that the positive predictive value for any esophageal pathology was 92% and the negative predictive value was 100% [147]. However, large-scale studies are necessary to fully evaluate this novel diagnostic technology before widespread implementation.

Staging and staging systems

After establishment of the diagnosis of cancer, staging is necessary to optimize treatment options and management. This should ideally commence with CT scanning of the chest and abdomen and should be supplemented with EUS for esophageal wall and local lymph node involvement.

In a retrospective study [148], the critical determinants of survival in nearly 4000 surgical specimens from Japanese patients were depth of invasion, the presence or absence of lymph node metastases, and the presence or absence of distant metastases. Five-year survival was 46% with submucosal invasion, 29.5% with invasion of the muscularis propria, 21.7% with invasion into the muscularis propria, and 7% with involvement of adjacent tissues and organs [148]. Worsening survival rates (3.0%) are also correlated with involvement of distant lymph nodes and with metastasis to distant organs. Although several staging systems have been recommended for esophageal cancer, an overriding consideration is that surgical pathological staging is more accurate for predicting survival than pretreatment clinical staging.

Thus, staging involves clinical evaluation before therapy and then pathological staging of the surgically resected esophagus and dissected lymph nodes [149]. A staging system that is widely used because of its accuracy is depicted in Table 36.1.

Table 36.1 TNM staging system for cancer of the esophagus (American Joint Committee on Cancer criteria)

Primary tumor (T)			
TX	Primary tumor cannot be assessed		
T0	No evidence of primary tumor		
Tis	Carcinoma in situ		
T1	Tumor invades lamina propria or submucosa		
T2	Tumor invades mucularis propria		
T3	Tumor invades adventitia		
T4	Tumor invades adjacent structures		
Regional lymph nodes (N)			
NX	Regional lymph nodes cannot be assessed		
N0	No regional lymph node metastasis		
N1	Regional lymph node metastasis		
Distant metastasis (M)			
MX	Distant metastasis cannot be assessed		
M0	No distant metastasis		
M1	Distant metastasis		
Stage grouping			
Stage 00	Tis	N0	M0
Stage I	T1	N0	M0
Stage IIA	T2	N0	M0
	T3	N0	M0
Stage IIB	T1	N1	M0
	T2	N1	M0
Stage III	T3	N1	M0
	T4	N1	M0
Stage IV	Any T	Any N	M1

From American Joint Committee on Cancer (AJCC). Esophagus. In: AJCC Cancer Staging Manual, 6th edn. New York: Springer, 2002: 91–8, with permission of the AJCC, Chicago, IL.

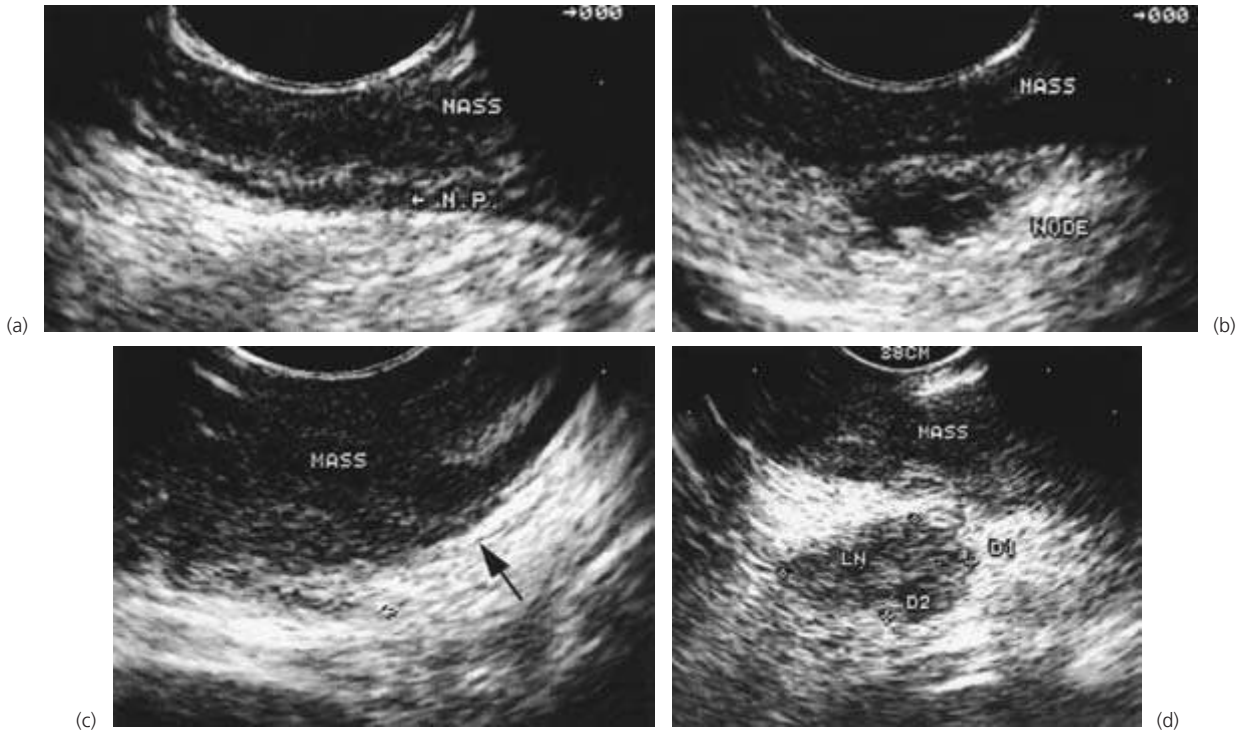
Imaging studies: computed tomography and magnetic resonance imaging

CT scanning has proven useful in the preoperative staging of esophageal cancer [150–156], especially in defining lymph node, pulmonary, and hepatic involvement. The accuracy of CT staging has a broad range, from 39% to 100% [151,152,155,157]. Even documentation of aortic invasion, which precludes surgical resection, has a sensitivity of 80%–100% and a specificity of 50%–100% on CT scanning [151,152,155]. Tracheobronchial involvement is also discernible with CT scanning [149–151,153].

Comparisons between CT and magnetic resonance imaging (MRI) have shown little difference between the two modalities [158–161]. For example, sensitivity, specificity, and accuracy rates between CT scanning (100%, 80%, and 84%, respectively) and MRI (100%, 84%, and 87%, respectively) are comparable [158]. Early spread beyond the esophageal wall, regional lymph node involvement, and aortic invasion have led to problems with both false positivity and false negativity with both imaging modalities [151,152,154,155,157,159–161]. Thus, CT scanning remains the primary staging modality.

Endoscopic ultrasound

EUS has emerged as a key component of staging. It is particularly useful in gauging the depth of esophageal invasion and local lymph node involvement (Fig. 36.2). EUS is superior to CT scanning in the determination of the primary tumor (T) and regional lymph node (N) status of the staging classification scheme and has been compared with surgical–pathological and CT staging in several studies [162–165]. EUS accuracy in staging the depth of tumor invasion varies from 87% to 92%, making it more accurate than CT. The overall accuracy of EUS in detecting N status ranges from 80% to 88%. In these studies, EUS sensitivity varied from 92% to 95% and specificity varied from 50% to 54%, whereas CT accuracy ranged from 51% to 74%, with a sensitivity of 34% and specificity of



(a) (b) (c) (d)

Figure 36.2 Endoscopic ultrasound images of different stages of esophageal cancer. **(a)** T2N0 stage. Note the prominent muscularis propria (m.p.) **(b)** T2N1 stage. The mass is directly adjacent to the muscularis propria. **(c)** T3N0 stage. Note invasion into the muscularis propria (arrow). **(d)** T3N1 stage. A mass with a large lymph node (LN) in mediastinal fat. Courtesy of William Brugge, MD.

88%. EUS-fine needle aspiration (EUS-FNA) has improved the accuracy of EUS for N staging with confirmatory cytological material [166]. EUS-FNA has more than 90% accuracy in confirming celiac axis lymph node metastases, but only a limited role for staging distant metastases [167,168]. Therefore, it is generally recommended that CT or MRI be performed before EUS for detection of distant metastases. CT and EUS are usually considered to be complementary modalities for the staging of esophageal cancer.

EUS is useful in the assessment of resectability. It was able to predict resectability in 147 of 167 patients (89%), more accurately for adenocarcinoma (82%) than for SCC (64%) [169]. Lumenal stenosis, which prevents passage of the scope, portends a higher stage, either III or IV (older staging system), in nearly 90% of cases; conversely, 58% of cancers without stenosis were not stage III or IV [170]. Overstaging (T1 to T3) or understaging (T1 or T2) may be a problem with EUS; 73% of T2 lesions are overstaged, and 71% of T3 lesions are understaged [171]. Although EUS is superior to CT scanning for staging after preoperative chemotherapy and radiation, it is inaccurate, with a sensitivity of only 27%–52% for T2/T3 restaging and 48%–52% for N0/N1 disease. This is because of the inability to distinguish inflammation and fibrosis from residual cancer [172]. However, it has been found that more than a 50% decrease in tumor cross-sectional area or diameter correlates with treatment response [173]. Cost-effectiveness studies of EUS staging are needed.

Positron emission tomography

Positron emission tomography (PET) is a whole-body, metabolic-based imaging modality using fluorine 18-labeled deoxyglucose (FDG). There is an increased accumulation of the radiotracer in malignant tissues, which is believed to be the result of an increased expression of glucose transport enzymes in the tumor cell membranes together with an increased activity of the enzymes in the first steps of the glycolytic pathway. The degree of FDG accumulation in a lesion can be assessed semiquantitatively and expressed as a standard uptake value [174]. The results of a prospective study comparing the staging accuracy of FDG-PET with the standard combined use of CT and EUS showed that FDP-PET significantly improves the detection of stage IV disease [175]. Thus, FDG-PET has been documented as a highly accurate modality for the primary preoperative staging of patients with esophageal cancer. Other studies [176,177] also show that FDP-PET is a promising noninvasive tool for the assessment of preoperative (neoadjuvant) therapy. There is a good correlation between the clinical response evidenced via serial PET scans and the pathological response and survival. Recent studies suggest that PET with the nucleoside analogue

3′-deoxy-3′-(18)F-fluorothymidine (FLT) may be more accurate than FDG-PET in visualizing early changes in tumor proliferation. Using an experimental model of esophageal cancer (human xenographs), FLT-PET was more accurate than FDG-PET in detecting early changes in proliferation following docetaxel and radiation therapy, with a much stronger correlation with histological findings. Clinical studies are needed to determine whether FLT-PET can distinguish between degrees of response to neoadjuvant chemoradiation in patients with esophageal cancer [178].

Laparoscopy

Abdominal metastases are more common from distal esophageal tumors, and these subdiaphragmatic metastases may be confirmed by laparoscopy [179]. Nonetheless, laparoscopy is not common practice for the staging of esophageal cancer.

Therapy for esophageal cancer

The presentation of esophageal cancer at advanced stages mitigates against curative surgical resection on a consistent basis. In fact, although nearly 60% of patients may undergo surgical exploration, only two-thirds of such patients will undergo resection. Thus, more than 40% of patients will require palliative therapy. However, with better understanding of the disease and the development of new anticancer agents, the overall survival of patients with esophageal cancer has been improving. Based on Surveillance, Epidemiology, and End Results (SEER) information, the 5-year overall survival rate in the United States increased from 4.7% in 1976 to 14.9% in 2002 [180]. The following discussion is divided into categories of potential curative modalities as well as palliative measures.

Surgery

Surgery remains the mainstay of treatment of esophageal cancer with curative intent for respectable, local, and locoregional disease. Advances in surgical therapy, staging techniques, patient selection, and supportive care in recent years have resulted in a marked improvement in surgery-related mortality and morbidity. Even if cure is not accomplished, palliative surgical bypass procedures may also have some impact on patients with advanced unresectable disease, relieving symptoms and improving the quality of life.

There are various surgical approaches for esophageal cancer, including transthoracic, transhiatal, and radical en bloc resections. Despite the different advantages and disadvantages of each, and the controversies surrounding them, there is no evidence to suggest improved survival with any particular one [181]. Factors that may influence the decision of which particular technique to select include tumor location, depth of tumor invasion, status of lymph node involvement, overall performance status and body habitus of the patient, previous treatment (e.g., radiation or chemoradiation therapy), and the preferences and biases of the surgeon and institution.

In the 1940s, midesophageal tumors were approached through the left side of the chest. The benefits of a right-sided approach (Ivor Lewis) have been advocated since then and include enhanced accessibility to the upper two-thirds of the esophagus, location of the aorta as a barrier to the left pleural space, and visualization of the entire esophagus after division of the azygous vein. This approach has been applied as well for distal esophageal cancers, thus allowing for a longer segment of esophageal resection with a higher anastomosis. A cervical anastomosis has some inherent advantages, namely decreased morbidity and mortality, reduced reflux, and a better opportunity for postoperative radiation if pursued. The left thoracoabdominal approach may be used for distal esophageal and gastroesophageal cancers.

The early complications of transthoracic esophagectomy include anastomotic leak (0%–13%), pulmonary problems such as pneumonia, retained secretions, and pulmonary emboli (6%–50%), recurrent laryngeal nerve injury (1%–13%), and cardiac problems such as myocardial infarction, arrhythmia, and congestive heart failure (2%–27%). The mortality ranges from 1% to 13%, with a 5-year survival rate of 1%–35% [182–185].

Because of the high rates of morbidity and mortality linked to the thoracotomy associated with transthoracic esophagectomy, the transhiatal approach has also been advocated. The mortality rate ranges between 2% and 13%, and the 5-year survival rate ranges from 12% to 27% [186–192]. Although there are still some arguments about transhiatal esophagectomy (THE) without an en bloc mediastinal lymphadenectomy, THE has been used increasingly in the resection of benign and malignant disease with potential advantages over transthoracic esophagectomy, including significantly decreased respiratory complications and mediastinitis because of the avoidance of thoracotomy and intrathoracic anastomosis [193]. The en bloc resection of cancers of the distal esophagus and the cardia involves removal of 10 cm of the esophagus on either side of the tumor along with contiguous tissues, vascular supply, and lymph nodes. Various series report a 1.4%–11% operative mortality rate, with a 5-year survival rate of 18%–35% [194,195]. Histology cell type (squamous cell carcinoma vs adenocarcinoma) is considered important for the overall outcome after esophagectomy. Patients with squamous cell carcinoma have a poorer perioperative outcome than those with adenocarcinoma; however, in the longer term, squamous cell carcinoma appears to confer a significant survival advantage [196]. Other evidence has demonstrated that the most important determinants of survival appear to be the biological behavior of the tumor and

the stage at the time of resection rather than the operative approach, and esophageal carcinoma will likely require systemic therapy with pre- or postoperative chemotherapy and chemoradiation therapy for a cure.

An important consideration during surgery is the choice of esophageal replacement [197–202]. The stomach, by virtue of its rich blood supply, length, and muscular wall, is preferred, whereas the left colon, because of its similarity to the esophagus in caliber, can be used to replace the esophagus when the stomach cannot be used. Jejunal interposition may be used for reconstitution of the hypopharynx and the cervical esophagus. Overall, complications of esophageal replacement include anastomotic leaks (2%–5%), anastomotic stricture (9%–29%), gastric dysfunction, and chylothorax.

Analysis has demonstrated that, with modern surgical management, leak-associated mortality has markedly reduced and has no impact on long-term survival. Clinical decisions regarding the use of intrathoracic anastomoses should not be affected by concerns of increased mortality from leak [203].

Minimally invasive laparoscopic esophagectomy has been investigated in highly selected esophageal cancer patients with early disease. Limited information suggests that it is a feasible approach and can be safely performed by surgeons with extensive experience in that field. Advantages include less intraoperative blood loss, a smaller incision, and a potentially faster postoperative recovery [204]. Large prospective trials with longer follow up will be required to confirm any advantages of this approach over conventional approaches.

Unresectable esophageal cancer may warrant a bypass procedure using a portion of the stomach, jejunum, or colon as a conduit. However, bypass surgery is fraught with high morbidity and mortality rates, and it should be reserved for patients with a tracheoesophageal fistula or those in whom conservative medical measures have failed. Effective palliative resection of the cervical esophagus usually requires total laryngectomy and esophagectomy (transhiatal approach).

Radiation therapy

Radiation (external beam) alone

Radiation as a single modality is seldom used as either definitive or adjuvant therapy. It is limited to patients who are not candidates for surgical resection because of unresectable disease or patients who are medically unable to tolerate a surgical procedure and multimodality treatment [205–207]. Limited experience with patients treated with radiation therapy alone indicates a 1-year survival rate of 18% and a 5-year survival rate of 6%, reminiscent of survival data after surgical resection [208–210]. Favorable 5-year survival rates may be observed in women and in patients with cancers of less than 5 cm in length (22.4% and 17.7%, respectively) [205–210]. Radiation alone may lead to amelioration of dysphagia, with 50% of patients receiving relief for 2 months or more and 15% of patients for more than 1 year. In the acute setting, radiation-induced esophagitis may lead to dysphagia, and fibrotic strictures may form. Unusual complications of radiation therapy include pulmonary fibrosis, myelitis, and cutaneous burns.

Brachytherapy

Intralumenal radiation (brachytherapy) can ameliorate symptoms resulting from obstructing cancers. Higher doses of radiation can be delivered with this technique without inducing injury to pulmonary, cardiac, and mediastinal structures [211]. A cobalt 60 source is commonly used, either alone or after external beam radiation therapy, and treatment is typically administered over 2 weeks. Most patients experience a prompt subjective response to brachytherapy. Complications of brachytherapy include ulcers, strictures, and tracheoesophageal fistulae in up to 25% of patients [212,213]. Brachytherapy is commonly used for palliation of esophageal obstruction due to inoperable cancer. Studies show that brachytherapy delivers better long-term relief of dysphagia than metal stent placement and is also associated with fewer complications than stent placement [214].

Preoperative (neoadjuvant) and postoperative (adjuvant) radiation therapy

The potential advantages of neoadjuvant radiation therapy include increased resectability, decreased tumor seeding at the time of surgery, and increased radiosensitivity as a result of more oxygenated cells. Neoadjuvant radiation therapy has not been shown to improve the overall survival of patients with potentially resectable disease [215–219]. A metaanalysis [219] of 1147 patients from five randomized neoadjuvant radiation therapy trials (median follow up of 9 years) showed no statistically significant benefit in either the 2-year survival rate (34% vs 30%) or the 5-year survival rate (18% vs 15%). There was benefit demonstrated from radiation treatment, with an overall reduction in the risk of death of 11%.

Adjuvant radiation therapy provides the advantages of accurate pathological staging and the direction of treatment to areas with a high risk of disease recurrence. Postoperative radiation therapy is often given when the surgical margins are positive or the resection is incomplete. A slightly higher total dose range (4500–5500 cGy) than that administered in the neoadjuvant randomized trials has not yielded a survival advantage [220].

Chemotherapy

Because approximately 30% of patients present initially with metastatic disease and at least 50% of others present with stage II and III disease, chemotherapy holds promise for the local control of disease, increased survival, and the prevention of distant metastasis. Cisplatin and 5-fluorouracil (5FU) have been used as a standard regimen, and they have produced response rates of 35%–40% in advanced disease with

a median survival of 33 weeks and a 1-year survival of 38%. Other active agents are mitomycin and bleomycin, as well as many newer agents such as the taxanes (paclitaxel and docetaxel), irinotecan, gemcitabine, and oxaliplatin [221,222].

Neoadjuvant (preoperative) chemotherapy

The rationale of neoadjuvant chemotherapy is to eradicate occult metastases before surgery. No advantages of neoadjuvant therapy were demonstrated in the 1980s or 1990s [223–227]. The initial US intergroup trial 113 [227] did not show survival benefits in patients undergoing preoperative chemotherapy. However, data from a larger (802 patients), randomized, and well-controlled study of neoadjuvant chemotherapy in esophageal cancer conducted by the UK Medical Research Council (MRC) upper gastrointestinal tract cancer group [228] demonstrated that preoperative chemotherapy with 5FU and cisplatin can improve relapse rates, overall survival, and cure rate. Patients with esophageal cancer, 67% of whom had adenocarcinoma and 33% of whom had squamous cell carcinoma, were randomized to neoadjuvant chemotherapy before surgery or to surgery alone. Resectability was significantly higher in the treatment arm (78% vs 70%; $P < 0.001$), as was overall survival ($P = 0.003$). Two-year survival rates were 43% in the neoadjuvant chemotherapy arm vs 34% in the surgery-alone arm. Overall survival was superior in patients who received preoperative chemotherapy (hazard ratio [HR] 0.79; $P = 0.0004$). There was no increase in postoperative complications in patients receiving preoperative chemotherapy (40%–41%). Preoperative chemotherapy has become a standard in the UK and in much of Europe.

Adjuvant (postoperative) chemotherapy

A trial to evaluate the efficacy of postoperative chemotherapy in resectable lesions showed an advantage in disease-free survival in the treatment arm ($P = 0.05$), but there was no overall survival benefit [229]. Several clinical trials are ongoing to evaluate this approach further, using newer chemotherapy agents and regimens.

The MRC Adjuvant Gastric Infusional Chemotherapy (MAGIC) trial was designed to determine whether perioperative chemotherapy could improve outcomes in patients with resectable gastric and lower esophageal adenocarcinomas. In total, 503 patients with operable disease were randomized to receive either three cycles of epirubicin, cisplatin, and 5FU (ECF) before and after surgery or surgery alone [230]. The resected tumors were significantly smaller and less advanced in patients who received perioperative chemotherapy. The perioperative chemotherapy patients also had higher overall survival, with a 5-year survival rate of 36.3% vs 23.0% ($P = 0.009$), and a statistically significant improved progression-free survival ($P < 0.001$). Postoperative complications and mortality were similar between the two groups.

Primary chemoradiation therapy

Cisplatin and 5FU combined with radiation therapy can substantially improve the 5-year survival in patients with esophageal cancer compared with radiation alone. Cisplatin and 5FU are radiosensitizers and improve the response to radiation therapy not only in the cancer cells but also unfortunately in normal tissues.

Definitive primary chemoradiation therapy for locoregional esophageal carcinoma is considered an alternative to surgical resection, although there are no large prospective randomized trials comparing it directly with surgery. The largest single-institution trial of primary chemoradiation was conducted by Coia [231] on 57 patients with stage T1 and T2 esophageal cancer. These patients were treated with 6000 cGy radiation and two cycles of 5FU delivered as a continuous 4-day infusion during the first and fifth week of radiation therapy, and a single bolus of mitomycin C on day 2. The median survival was 18 months, and 3-year and 5-year survival rates were 29% and 18% respectively.

A phase III multiple-institution randomized trial (RTOG 85-01) compared combination therapy with radiation therapy alone [232]. Patients randomized to combination therapy received a total dose of 5000 cGy radiation and 5FU for four consecutive days and cisplatin on day 1 in weeks 1, 5, 8, and 11. Patients in the radiation-alone group were given a total dose of 6400 cGy. This trial was stopped early after an interim analysis demonstrated a survival advantage for patients in the combination therapy group. The 5-year survival rate for patients who had chemoradiation was 26% vs 0% for those receiving radiation only ($P < 0.001$), with a median survival of 14.1 months vs 9.3 months respectively. There was no statistical difference in survival related to histology of the cancer (adenocarcinoma vs SCC). No further benefit but more treatment-related death was shown in a later study (RTOG 94-05) using a higher dose of radiation (6480 cGy) and the same chemoregimen [233].

Neoadjuvant chemoradiation therapy

Data from several nonrandomized studies suggest that neoadjuvant therapy with chemotherapy (5FU and cisplatin) plus radiation before surgery yielded complete response rates of 24%–42%, with a median survival of 12–23 months [220–222]. In a University of Michigan study [223], 100 patients (25 with SCC and 75 with adenocarcinoma) were randomized to either concurrent continuous infusion of 5FU, cisplatin, and vinblastine with 45 Gy radiation followed by surgery or surgery alone. In total, 28% of patients in the neoadjuvant chemoradiation arm achieved a pathological complete response. The 3-year survival rates were 30% in the combination therapy arm vs 16% in the surgery alone arm, but this difference was not statistically significant ($P = 0.15$). Median survivals were similar in both arms of the trial. A small-sized phase III trial in 113 patients with esophageal adenocarcinoma randomized to either 5FU infu-

sion and cisplatin with radiation (40 Gy) plus surgery or surgery alone suggested a survival benefit of combined therapy [224]. However, a trial of 297 patients with SCC randomized to either surgery alone or cisplatin with radiation followed by surgery was inconclusive [225]. Large randomized trials are needed to study this approach further. PET allows the early identification of patients not responding to chemoradiation and is probably helpful when making the decision as to whether a individual patient should continue with chemoradiation or undergo surgery [176,177].

Adjuvant chemoradiation therapy

Medical and radiation oncologists have also administered combined modality chemoradiation therapy postoperatively to patients with esophageal cancer, based on the proven benefit observed in other gastrointestinal malignancies, such as rectal cancer [234–239]. Several small or retrospective studies suggested a survival benefit of adjuvant chemoradiation for patients with esophageal and gastroesophageal junction cancer [240–243]. A large intergroup study from the United States demonstrated a survival benefit of adjuvant chemoradiation in such patients [244]. In total, 603 patients with complete resections, negative surgical margins, and no evidence of residual disease were randomized to receive surgery alone or surgery plus postoperative chemotherapy with 5FU and leucovorin and concurrent radiation therapy. The median survival was 36 months for patients in the adjuvant chemoradiation arm vs 26 months for patients in the surgery-only arm ($P = 0.005$). The 3-year overall survival rates were 50% vs 40% and disease-free survival rates were 48% vs 31% for the treatment group vs the observation group. The median disease-free survival rates were 30 months for chemoradiation-treated patients and 19 months for surgery-only patients ($P < 0.001$). The toxicities of the chemoradiation were acceptable, with hematological and gastrointestinal toxicities comprising the predominant adverse events. In total, three patients (1%) died as a result of toxicities. This study is the first large well-controlled study demonstrating an advantage of adjuvant therapy with respect to both disease-free survival and overall survival. Unfortunately, the study was not designed specifically for determining the role of adjuvant therapy in esophageal cancer. Although this study has been criticized for lack of standardized surgery, a subsequent analysis showed that the survival benefits of adjuvant chemoradiation therapy were retained in patients who had either a D0 (less than complete dissection of the N1 lymph nodes) or D1 dissection [245].

Summary

For patients with stage T1 and T2 lesions without any evidence of nodal involvement, surgery alone may be curative in more than 60%. For patients with locally advanced disease, the emerging recommendation entails perioperative chemotherapy and/or radiation therapy plus surgical resection. Perioperative chemotherapy or chemoradiation can be performed safely with an acceptable operative morbidity and a low operative mortality rate in patients with gastroesophageal cancer, with careful consideration of associated risks such as age and obesity [246]. Patients should be encouraged to enter large randomized clinical trials whenever possible, because definitive survival benefits of either neoadjuvant or adjuvant multimodality therapy with surgery need further study.

Palliation treatment

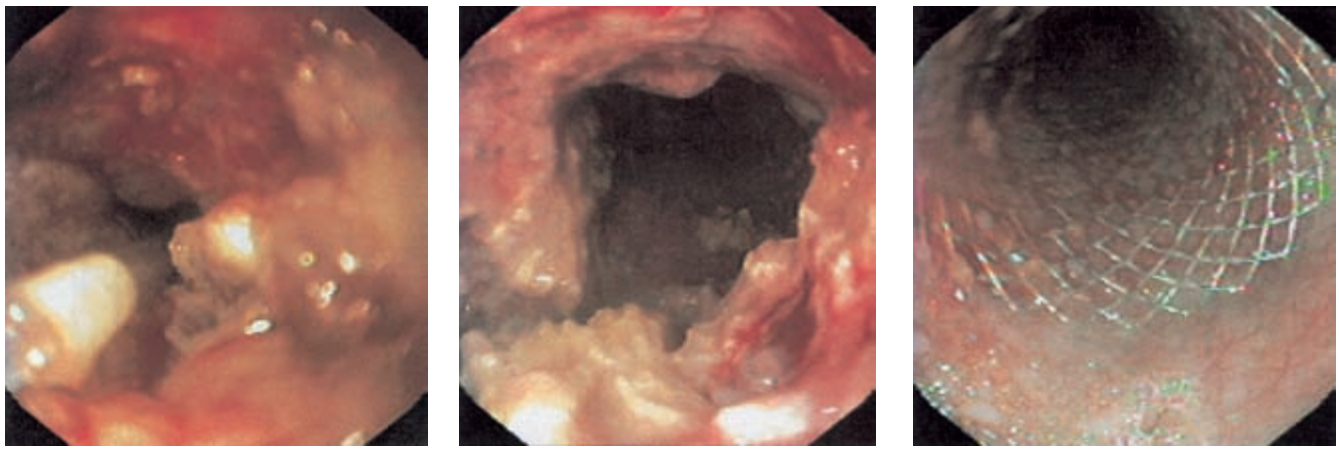
Local disease

Because fewer than 50% of patients are candidates for a curative approach after careful staging and preoperative assessment of comorbid disease, palliative approaches are essential. Because total esophagectomy is fraught with high mortality rates in palliative situations, bypass should be considered for those patients with an expected survival of more than 6 months. External beam radiation therapy (with or without chemotherapy) may be suitable for annular or nearly obstructive lesions, but radiation-induced esophagitis may be problematic. Lower doses may be an option. Brachytherapy should be considered for relief of symptoms (see Brachytherapy). Endoscopic esophageal interventions with various techniques including endoscopic dilation, photodynamic therapy, endoscopic lumen restoration, and self-expanding metal stents can be employed to relieve strictures and are associated with low complication rates [247,248]. Expandable metal stents are a good option to maintain patency; additionally, laser and bipolar electrocoagulation therapy may be helpful (see Chapter 121).

It is worth emphasizing the exciting potential of photodynamic therapy. An intravenously administered photosensitizer, porfimer sodium (approved by the Food and Drug Administration for use in the palliation of esophageal cancer), is administered, and then light of a specific wavelength (630 nm) is shone on the tumor. Cell toxicity is believed to occur through the release of singlet oxygen resulting in ischemia and eventually necrosis. The depth of destruction in photodynamic therapy is 2–5 mm, and it is preferred to laser treatment in tumors of longer than 8 cm (Fig. 36.3).

Metastatic disease

For distant metastatic disease, systemic chemotherapy with 5FU and cisplatin is the main modality for palliative care, although survival rates are not impressive. With newer agents such as paclitaxel, docetaxel, gemcitabine, irinotecan, and oxaliplatin, response rates have improved to 50%–60% and median survival has increased to 10–14 months, with gains in quality of life [249–254]. The combination of epirubicin, cisplatin, and 5FU is considered a standard treatment for esophageal and gastric adenocarcinoma in Europe. A large randomized phase III trial with a 2×2 factorial design



(a) (b) (c)

Figure 36.3 Photodynamic therapy of esophageal cancer with laser after administration of porfimer sodium, a photosensitizer. Light of 630 nm from a laser acts on cells that accumulate in the photosensitizer **(a)**. After 6 days, there is some decrease in the mass size **(b)**. After 12 days, the mass is diminished in size significantly, and a metallic endoprosthesis is endoscopically inserted **(c)**. Courtesy of Norman Nishioka, MD.

is testing whether oxaliplatin and capecitabine may replace cisplatin and 5FU. The preliminary results suggest that there are advantages to the combination of epirubicin, oxaliplatin, and capecitabine [255].

An emerging understanding of the molecular characteristics of cell growth, the cell cycle, apoptosis, angiogenesis, and signaling pathways has provided novel targets in cancer therapy including for esophageal cancer. Success has been demonstrated in the treatment of colorectal, breast, lung, and renal cell carcinomas. Preliminary data from esophageal cancer, while from small studies, are very encouraging [256–258].

Prognostic factors

It would be helpful to be able to identify patients a priori who would benefit from certain types of therapeutic modalities. Performance status, weight loss of more than 10% of body weight, the tumor stage, and lymph node involvement from the primary tumor are also very important [259,260].

The chances of curative surgical resection are optimal for lesions of less than 5 cm, confined to the mucosa, and without lymph node involvement. Poor prognosis is correlated with aneuploidy and histology. For those patients who achieve a pathological complete response to neoadjuvant chemotherapy, long-term survival is significantly improved [261]. Increased cyclin D_1 expression and reduced E-cadherin expression portend a less favorable outcome [262]. A high serum vascular endothelial growth factor (VEGF) level has been associated with tumor progression, poor treatment response, and poor survival in patients with SCC [263].

Patients with higher serum levels of C-reactive protein (CRP) may have a poorer response to chemoradiotherapy and worse overall disease-free survival rates [264]. It has also been suggested that high expression of BAX and $p16^{INK4a}$ are associated with a favorable prognosis [265]. Importantly, patients need to abstain from tobacco and alcohol consumption during treatment.

The extent of disease is an important prognostic factor for esophageal adenocarcinoma. After resection, negative surgical margins are important for improving prognosis; conversely, the development of distant metastasis during treatment portends a poor prognosis. The type of operation is not an independent prognostic factor, nor is ultrasound assessment after neoadjuvant therapy. Identification of micrometastatic disease in lymph nodes of patients with esophageal cancer may permit stratification to better or worse survival [266].

Benign epithelial tumors (squamous cell papilloma)

Squamous cell papillomas are usually discovered incidentally at endoscopy or radiography. Generally, they are small, sessile, and polypoid growths of normal or hyperplastic squamous epithelium covering a core of connective tissue [267,268]. On removal, they typically do not recur [269,270]. The average age of patients is 51.5 years, with a male-to-female ratio of 1.8:1. These tumors occur more frequently in Japan. Most are solitary and located in the distal one-third of the esophagus. Papillomas may result from chronic irritation from reflux esophagitis. Morphological features suggest that papillomas result from HPV infection, and HPV DNA has been found in a subset of papillomas. The actual development of cancer has not been documented in these tumors.

This may reflect the fact that the nononcogenic genotypes of HPV may be associated with esophageal papillomas, as in the skin. NMBA administered subcutaneously in rats leads to the development of esophageal squamous dysplasia and papillomas.

Malignant epithelial tumors

Squamous cell carcinoma with a spindle cell component

SCC with a spindle cell component is also referred to as carcinosarcoma, pseudosarcoma, spindle cell carcinoma, and polypoid carcinoma [271–277]. Originally, carcinosarcoma was believed to be malignant, whereas pseudosarcoma was designated benign without metastatic potential. Local and distant metastasis of spindle cells, however, has been reported.

Spindle cell carcinomas are either solitary or multiple and are large and polypoid. Histologically, the squamous component at the surface is associated with dysplasia or carcinoma in situ. One may also see adenocarcinoma or undifferentiated epithelial components. The spindle cell component may range from mild proliferation to marked proliferation with pleomorphism, giant cells, and mitoses. Differentiation into smooth or skeletal muscle, bone, or cartilage may be rarely observed. Immunohistochemical and electron microscopic analyses indicate that the spindle cells stem from mesenchymal metaplasia of malignant squamous cells. Although there are reports that spindle cells contain epithelial cell markers such as cytokeratins, suggesting multiclonality, most pathologists believe that spindle cell carcinomas arise from SCCs that produce a spindle cell component.

Clinically, men are affected more often than women, and most patients are middle aged or elderly. Located in the middle to distal esophagus, these cancers can produce obstruction and dysphagia by virtue of their large size. Most patients present with esophageal wall invasion and lymph node involvement.

Verrucous squamous cell carcinoma

Classically, *verrucous SCC* of the esophagus is a slow-growing malignant tumor that may be limited to local invasion but without distant metastasis [278,279]. Some, however, do metastasize to other organs, with resulting poor prognosis.

Adenocanthoma and adenosquamous carcinoma

These rare tumors feature adenocarcinoma with squamous metaplasia (*adenocanthoma*) or with squamous carcinoma (*adenosquamous carcinoma*) [280,281]. These lesions can arise in the setting of Barrett esophagus or de novo. It is likely that these tumors arise in the squamous epithelium, which has the ability to achieve squamous and glandular differentiation. Alternatively, squamous metaplasia or transformation within an adenocarcinoma may have occurred. Clinically, these tumors behave like adenocarcinomas.

Adenoid cystic carcinoma

This is a rare tumor that resembles salivary adenoid cystic carcinoma. Carrying a male predilection at an advanced age, these tumors are multilobulated or ulcerated and located in the midesophagus [282–287]. Histologically, *adenoid cystic carcinomas* contain many cystic spaces and are primarily submucosal.

Mucoepidermoid carcinoma

This cancer consists of glandular and squamous elements; however, the mixture is more pronounced than in adenosquamous carcinoma. Found in the middle to distal esophagus, *mucoepidermoid carcinomas* probably arise from submucosal glands or their ducts and carry a dismal prognosis.

Melanoma

Melanoma is a rare esophageal cancer that can be primary or metastatic, although the esophagus is less commonly a metastatic target than the stomach, small intestine, or colon [288–294]. The mean age of patients is 60 years, and there are more cases in Japan than in other countries. Melanomas are polypoid and may be multiple, with a propensity for lateral spread. Primary esophageal melanomas arise from melanocytes in the basal layer. There must not be evidence of melanoma in the skin or internal organs to call an esophageal melanoma a primary tumor. Additionally, in situ or junctional melanoma must be evident in the mucosa adjacent to the tumor. Some cases are amelanotic and may require confirmation with immunohistochemical demonstration of S100 protein or homatropine methylbromide 45; alternatively, electron microscopy may reveal premelanosomes. Overall, the prognosis of malignant melanoma is dismal; most patients succumb to the disease within 2 years.

Neuroendocrine tumors

Small cell carcinoma of the esophagus may include a small cell variant of SCC as well as oat cell carcinoma [295–298]. Oat cell carcinoma of the esophagus can be primary or secondary, but it is rare in either situation. A disease of the elderly, who present with dysphagia, primary small cell carcinoma of the esophagus (typically distal and sometimes multiple) carries a dismal prognosis, with an average survival of 4.7 months. These tumors may occasionally secrete ectopic antidiuretic hormone or calcium. Microscopically, this tumor is an anaplastic small cell carcinoma resembling what is observed in the lung, with solid sheets of small anaplastic cells with mitoses. There can be evidence of squamous or glandular differentiation. Neuroendocrine markers employed are chromogranin and synaptophysin, as well as electron microscopic evidence of neurosecretory granules.

Esophageal carcinoid tumors are rare [299–302], with most occurring at the gastroesophageal junction. Carcinoid syndrome has not been reported. There is little or no malignant potential and, consequently, prognosis is very good.

Choriocarcinoma of the esophagus is an exceedingly rare tumor that may be primary or an adenocarcinoma with trophoblastic differentiation [303,304]. Secretion of human chorionic gonadotropin has been reported.

Benign nonepithelial tumors

Leiomyoma

Leiomyomas are the most common benign esophageal tumors [305–310]. Men are affected more frequently than women, with a ratio of 2:1. These tumors typically are single, and most occur in the distal esophagus and in the inner circular layer of the muscularis propria. Typically, most leiomyomas are not associated with symptoms and are found incidentally. Symptoms, if present, include dysphagia and chest pain; gastrointestinal bleeding is rare.

The diagnosis is usually suggested by barium esophagography, which shows a smooth, round defect with sharp margins (Fig. 36.4), or endoscopy, in which there is a well-circumscribed, rounded mass with normal overlying mucosa. Biopsy is usually not necessary because of its nondiagnostic nature and also because of the potential for complications.

Granular cell tumor

Granular cell tumors are derived from neural or Schwann cell elements, as evidenced by positive staining for S100 protein and neuron-specific enolase. Endoscopically, granular cell tumor is characterized as a small, smooth, sessile polyp with overlying normal mucosa and intramural nodules or plaques [311]. Most tumors occur in the distal esophagus and may be single or sometimes multiple. Patients are typically asymptomatic. Malignant transformation is rare. Microscopically, there are sheets of monomorphic histiocyte-like cells.

Fibrovascular polyp

Intralumenal *fibrovascular polyps* of the esophagus are rare tumors that may grow and may prolapse into the larynx. Other symptoms include dysphagia, nausea, vomiting, and, occasionally, gastrointestinal bleeding from ulceration. The polyps are covered by a smooth mucosa and are composed of fibrous and vascular tissue, although adipose tissue may occasionally predominate, prompting the designation of a fibrolipoma or pedunculated lipoma. Fibrovascular polyps do not have any malignant potential.

Hemangioma

Hemangiomas are small, asymptomatic lesions found incidentally [312].

Figure 36.4 Leiomyoma of the esophagus represented by a smooth intramural mass (arrow) outlined with air-contrast barium. Courtesy of Deborah Hall, MD.

Lymphangioma

Lymphangiomas are rare, with the appearance of a translucent, easily compressed mass on endoscopy [313]. Histologically, they contain dilated endothelial spaces.

Lipoma and fibroma

Sessile submucosal nodules composed of adipose or fibrous tissue are called *lipomas* and *fibromas* respectively. Together, they constitute fewer than 5% of all benign esophageal tumors.

Malignant nonepithelial tumors

Leiomyosarcoma and other sarcomas

Leiomyosarcoma is an uncommon tumor of the esophagus [314–317]. These tumors may be polypoid or infiltrative, and they can occur in all segments of the esophagus. Other esophageal sarcomas include soft tissue sarcoma, rhabdomyosarcoma, neurogenic sarcoma, and Kaposi sarcoma.

Metastatic carcinoma

Metastatic carcinoma of the esophagus is unusual, representing 3% of esophageal malignancies in one autopsy series [318]. The most common metastatic tumor to the esophagus is melanoma, followed by breast cancer and, less commonly, gastric, renal, liver, prostate, testicular, bone, skin, lung, and head and neck cancer. Primary esophageal and head and neck squamous cancers may coexist or may develop sequentially. Metastatic carcinomas to the esophagus cause extrinsic compression. In this context, the esophageal stricture is extrinsic, without mucosal involvement.

Lymphoma

Primary esophageal *lymphoma* may be Hodgkin or non-Hodgkin lymphoma [319–322], although, clearly, primary gastrointestinal lymphomas more commonly affect the stomach and small intestine. Symptomatic esophageal lymphoma may be present in immunocompromised patients, as in those with acquired immunodeficiency syndrome. Alternatively, lymphoma of the esophagus may be attributable to disseminated disease or infiltration from adjacent lymph nodes.

Summary and future directions

Advances have been made in the pathobiology, diagnosis, prognosis, and therapy of both major subtypes of esophageal neoplasms, namely esophageal squamous cell carcinoma and esophageal adenocarcinoma. The development and characterization of in vitro and in vivo model systems will help to facilitate the discovery and implementation of novel approaches in diagnostics and therapeutics in the future. As the last 15 years have witnessed much progress, it is hoped that the next 15 years will mirror that growth and advance the field to new heights to allow more beneficial outcomes for these once deadly diseases.

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A ESOPHAGUS

37 Miscellaneous diseases of the esophagus: foreign bodies, physical injury, and systemic and dermatological diseases

Evan S. Dellon, Nicholas J. Shaheen

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As the portal to the gastrointestinal tract, the esophagus is susceptible to insults from a variety of environmental exposures and to the effects of systemic and dermatological diseases. In this chapter we discuss myriad causes of esophageal injury not addressed elsewhere in this book. Although some of these diseases are rare it is important for the clinician to recognize them, because in many cases a delay in diagnosis of these diseases is the primary predictor of a poor outcome.

Esophageal foreign bodies

Epidemiology

A wide variety of objects can become lodged in the esophagus, the most common site of *foreign body impaction* in the gastrointestinal (GI) tract [1]. The type of object varies by age group. In the general population of adults, the annual incidence of food impaction is approximately 13 episodes per 100 000 people, with meat and bone impactions occurring commonly [2]. Special populations of adults at high risk include denture wearers, prisoners, the mentally challenged, substance abusers, and psychiatric patients, who swallow a panoply of implements [1,3–8]. In children, most cases occur between the ages of 6 months and 6 years, and small objects such as coins, toys, crayons, buttons, and batteries are commonly ingested [1,3–5,7].

Pathogenesis

Foreign body impaction can occur in areas of anatomical esophageal narrowing, such as the cervical esophagus, aortic arch, and gastroesophageal junction [7]. However, adults with food impaction often have an underlying structural esophageal disorder such as a peptic stricture or Schatzki ring (Fig. 37.1) [4]. Data suggest that a sizeable proportion may have eosinophilic esophagitis (see Chapter 33) [9].

Presentation

The clinical presentation is often straightforward. Many older children and adults are able to recognize the impaction when it occurs, and a history of prior impaction is common [1]. Patients may point to the level of the impaction, although this is not always reliable [10]. Dysphagia is the most common symptom and, if the esophagus is obstructed, odynophagia, choking, or drooling can be present [6]. These last few symptoms may be the only clue in young children or adults who cannot communicate. In children with foreign objects lodged proximally, 5%–15% may present with respiratory symptoms [3].

Complications usually depend on the characteristics of the impacted object, and may occur with a larger sized, irregularly shaped, or sharply pointed object [7]. If a foreign body perforates the esophagus, mediastinitis can result in abscess formation requiring drainage [11,12]. Fistulization to surrounding vasculature such as the aorta or innominate artery may lead to life-threatening hemorrhage [13,14]. Equally serious consequences can also occur with fistulization to the airway or pericardium [15,16]. Disk batteries pose a unique threat; leakage of alkali causes direct tissue damage, potentially leading to perforation or fistulization [17,18].

Diagnosis

An accurate history is crucial for diagnosis. Although little extra information is gained on the physical exam, signs of some complications may be detected. For example, proximal esophageal perforation is suggested with neck swelling,

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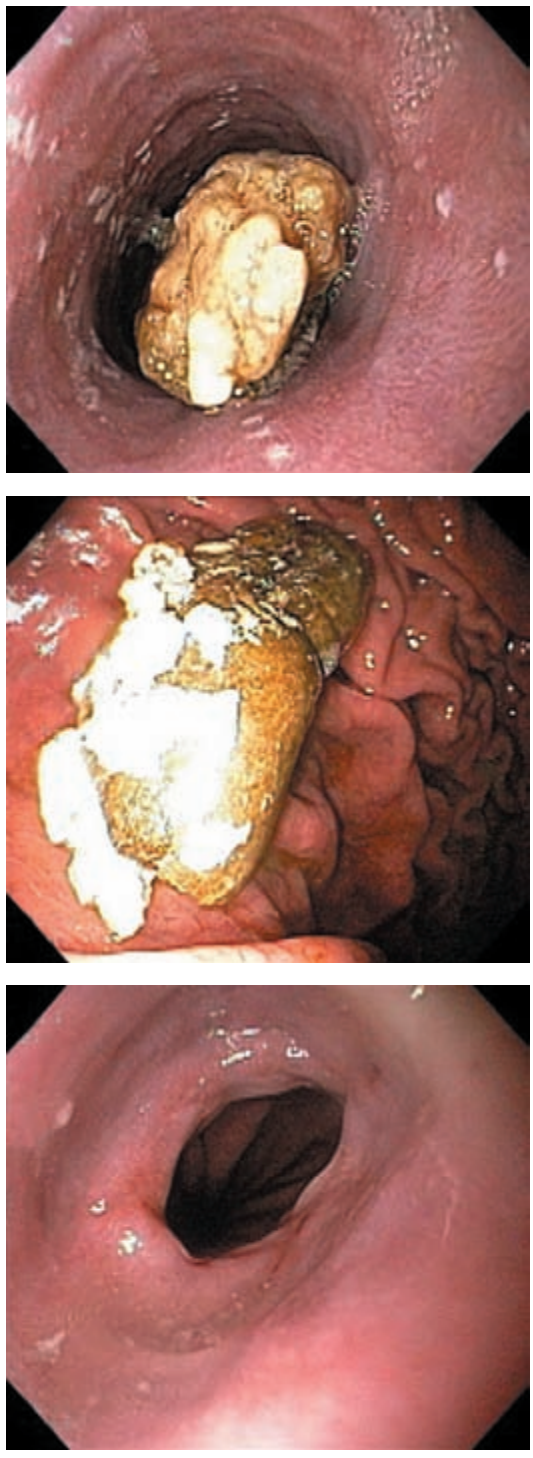


Figure 37.1 Food impaction in the distal esophagus **(a)**, which is cleared by gently advancing the bolus under direct endoscopic vision into the stomach **(b)**. A reexamination of the esophagus reveals a Schatzki ring as the underlying cause **(c)**.

erythema, tenderness, or crepitus. Imaging can confirm the diagnosis prior to an invasive procedure. Neck and chest radiographs show a radiopaque foreign object and, in the case of esophageal perforation, subcutaneous air [6,19,20]. Contrast esophagram should be avoided when obstruction is suspected because of the risk of aspiration. Barium is an opaque contrast agent that may coat both the foreign body and esophagus, hampering endoscopy and removal. Gastrografin is a transparent contrast agent but may cause severe pneumonitis if aspirated [7]. In children, metal detectors can be used to detect metallic foreign bodies [21].

Treatment

The management of an esophageal foreign body is determined by its physical characteristics, location, and length of time in place. In general, flexible endoscopy performed by an experienced endoscopist is the mainstay of therapy with a success rate of up to 98% and very low morbidity [1,7,22–25]. An exception is made for objects in the high cervical esophagus or pharynx, which may require rigid laryngoscopy for removal [4]. Blind bougienage carries a significant perforation risk and is not recommended [6]. As with all procedures, the risks of aspiration and airway obstruction should be minimized, and elective intubation may be necessary to manage an airway prior to endoscopic intervention.

The time frame for management varies. For disk batteries, sharp objects, and objects lodged in the proximal esophagus, immediate intervention is required [7]. Cases of complete esophageal obstruction in which patients have difficulty handling secretions should also be dealt with swiftly [1,6]. In contrast, it is safe to observe coins in the distal esophagus for up to 24 h to determine whether they will pass into the stomach [26]. If they do, they usually will pass through the remainder of the GI tract without incident [27]. Because of the increased risk of progressive mucosal damage and aspiration, frankly impacted objects should not be allowed to remain in the esophagus for more than 24 h, even if they are nonobstructing [6].

Nonendoscopic medical treatments are generally of limited value in esophageal foreign body impaction. Glucagon, given in a 1 mg intravenous bolus, can relax the esophagus to allow spontaneous passage of a food bolus or foreign body, but may only be successful in patients without a fixed esophageal obstruction, and is often not successful at all [28,29]. Effervescent agents are also of limited efficacy, carry a perforation risk, and interfere with subsequent endoscopy [30]. The use of enzymatic digestion of a meat bolus (with papain or another meat tenderizer) is not recommended; both perforation and death have been reported [31]. Even if a patient experiences medication-assisted passage of an object, subsequent esophageal examination is recommended to determine whether there is an underlying cause of mechanical obstruction.

To remove impacted esophageal foreign objects, a number of endoscopic instruments, including snares, nets, and multiple varieties of grasping forceps, are available (Fig. 37.2) [32,33]. Before the procedure, a practice session with a similar foreign body can identify the appropriate endoscopic

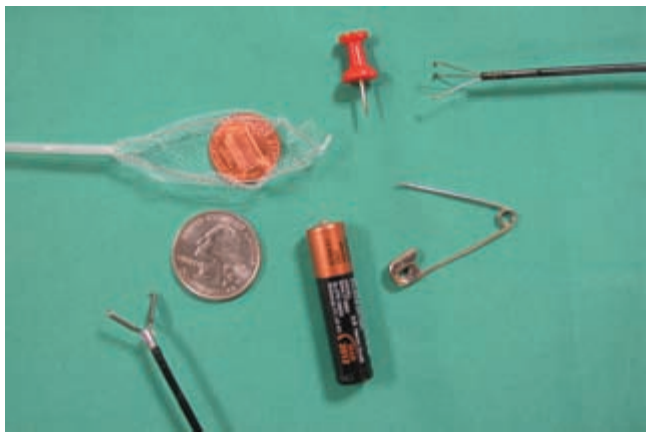


Figure 37.2 An example of the array of devices available for foreign body removal. From top left and proceeding clockwise, a Roth net (with a captured penny), a three-pronged grasping forceps, and a rat-tooth forceps are pictured. Objects that can potentially be impacted in the esophagus are shown for size comparison.

accessories for the actual removal and increase dexterity. An esophageal overtube can be used to protect the airway, but because of the risk of perforation should be used only by experienced personnel [34].

Coins can be removed with either a grasping forceps, net, or retrieval basket, depending on the size and whether the coin has a ridge [26,35]. Of note, special care should be taken when traversing the upper esophageal sphincter with a coin to prevent loss into the trachea. Disc batteries or other round objects are similarly captured in a net [32]. If a small (< 2.0 cm), smooth object can not be securely grasped in the esophagus, it is possible under direct visualization to gently advance it into the stomach where there is more room to maneuver. Sharp objects, however, are best removed where they lie with retrieval forceps or a polypectomy snare [32]. Orienting a sharp object with its point trailing during extraction can minimize mucosal injury, as does fitting a protector hood to the end of the endoscope [6,36]. Methods have been described for the closing of an open safety pin before extraction to minimize the risk of perforation [37].

Techniques for clearing a food impaction are similar. A bolus can often be gently advanced into the stomach under direct visualization with the tip of the endoscope [2,38]. Alternatively, the food bolus can be removed with snares, baskets, or forceps, although an esophageal overtube is usually required to prevent stray food particles from dropping into the airway [6]. A friction-fit adaptor, such as a variceal band ligator, placed on the end of the endoscope can be used as a suction device provided enough food is present to fill the cup and form an airtight seal [39].

After clearing an esophageal foreign body, the endoscopist must still assess for underlying structural abnormalities. If an esophageal stricture is found, it can be biopsied or dilated, provided that the foreign body removal was not delayed and severe esophagitis is not present [1,2,9,38]. Surgery is rarely needed to remove an impacted object or food bolus, and should be reserved for when endoscopic management has failed, an object is embedded in the esophageal wall or extraction carries a high risk of perforation, if a perforation has already occurred, or if endoscopic removal of the object poses an additional risk of systemic effects, such as in the case of ingested packets of illicit drugs [1,6,14].

Esophageal trauma

Mallory–Weiss syndrome

In 1929 Mallory and Weiss described a syndrome in alcoholics of retching and emesis followed by massive, and sometimes fatal, upper GI hemorrhage caused by linear lacerations at the gastroesophageal junction [40]. In subsequent retrospective series, these lacerations, termed *Mallory–Weiss tears*, account for 5%–15% of cases of upper GI bleeding [41,42]. These mucosal lacerations are generally 3–20 mm long, 2–3 mm wide, and parallel to the long axis of the esophagus (Fig. 37.3) [43].

The sudden rise in intraabdominal pressure during vomiting, retching, or coughing is thought to be the major pathogenic mechanism of a Mallory–Weiss tear. Accordingly, tears have been seen after ingestion of ipecac syrup or administration of chemotherapeutic agents, during diabetic ketoacidosis, seizures, hiccups, blunt trauma, straining at stool, asthma exacerbations, heavy lifting, cardiopulmonary resuscitation, or primal scream therapy, after abrupt changes in air pressure, and as a complication of carbon monoxide poisoning [41,44–47]. They can also occur during upper endoscopy, but this is rare (0.5% of all endoscopies) and only seen in patients with excessive retching during the procedure [48]. Hiatal hernias may also play a role in pathogenesis. When the gastroesophageal junction is located above the diaphragm, a large pressure gradient between the intragastric and intrathoracic compartments may result in sudden dilation and laceration of the mucosa at the gastroesophageal junction [43].

The "classic" presentation of a Mallory–Weiss tear is of repeated, violent retching, vomiting, or coughing followed by hematemesis. However, the presentation is often atypical: 40% of patients have no antecedent explanation for the tear, and 50% have hematemesis with the first episode of emesis [41,43]. The mean age at presentation is in the fifth decade, but the syndrome can occur at any age [43,49]. Male patients are affected more frequently (4:1) than female patients [41,43]. In total, 27% of patients have multiple lacerations and up to 77% have other associated upper GI lesions [41,42]. Although bleeding stops spontaneously in up to 90% of patients, blood transfusion may be needed and mortality may be as high as 12% [41,42]. Patients with portal hypertension and coagulation defects are at increased risk of bleeding from Mallory–Weiss tears [42,50]. Presentation

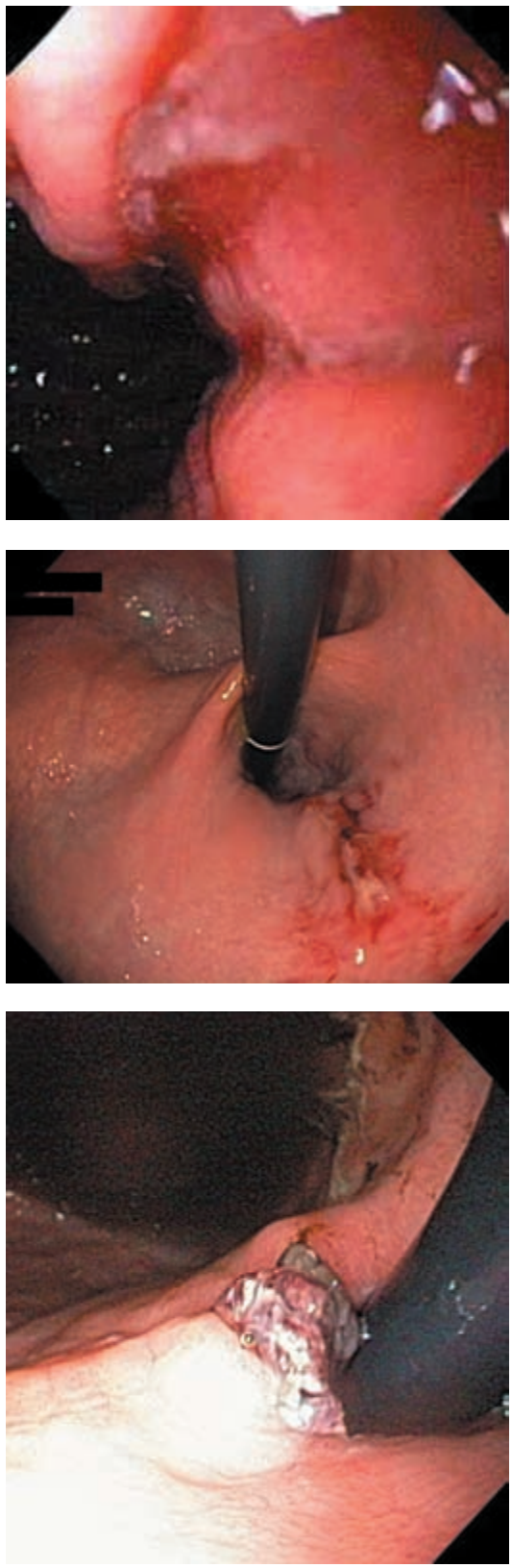


Figure 37.3 Examples of different morphologies of Mallory–Weiss tears in three different patients, seen in antegrade **(a)** and retroflexed **(b,c)** views in the proximity of the gastroesophageal junction.

with shock or ongoing active bleeding predicts recurrent bleeding episodes [51].

Endoscopy is commonly used to diagnose and treat Mallory–Weiss tears, but treatment is recommended only for lesions with active bleeding or stigmata of bleeding [52]. Endoscopic hemostasis has been achieved by the injection of anhydrous ethanol, polidocanol, or 1:10 000 epinephrine (adrenaline), cautery with a multipolar coagulation or heater probe, endoscopic band ligation, and application of hemoclips [41,53–60]. Endoscopic injection with epinephrine and polidocanol has been shown to reduce recurrent bleeding and decrease length of hospital stay [61]. Angiographic embolization has also been used with success, but typically is reserved for situations when endoscopic therapy fails [62]. Surgery may be required in rare instances when bleeding cannot be controlled by these methods [41].

Esophageal perforation and rupture

Injury to the esophagus resulting in perforation or rupture is life-threatening, and successful management depends on early diagnosis and prompt surgical intervention [63]. The most common cause of esophageal perforation, accounting for about 50% of cases, is medical instrumentation, including rigid and flexible endoscopy [64–66]. Therapeutic endoscopic stricture dilation by bougie or balloon caries a perforation risk, and in treatment of achalasia the perforation rate can be as high as 3%–4% [67–73]. Perforation has also been reported following nasogastric tube placement, inflation of a gastric variceal tamponade balloon in the esophagus, sclerotherapy, and insertion of an intragastric balloon for weight loss [66,74–76]. In addition to medical instrumentation, penetrating injury from knife and gunshot wounds or sword-swallowing, as well as blunt trauma to the neck, chest, and upper abdomen, may cause esophageal perforation [77–79]. The degree of blunt trauma may vary from grave, as in blast and crush injuries, to less severe, as in assault or the Heimlich maneuver [80–85].

In 1724 Herman Boerhaave described spontaneous transmural rupture of the esophagus in a patient, Baron von Wassenauer, who died suddenly after severe retching [86]. In total, 90% of cases of spontaneous rupture, or *Boerhaave syndrome*, occur in the distal esophagus, with more than two-thirds on the left side. This is likely due to an anatomical weakness of the left posterolateral aspect of the esophagus just above the diaphragm [87,88]. Most patients have antecedent retching and vomiting, although any maneuver that suddenly increases the intraabdominal pressure may result in rupture [87,89]. Interestingly, some patients with Boerhaave syndrome have been found to lack a muscularis mucosae, which may make them anatomically predisposed to rupture [90].

The etiology and severity of an esophageal rupture dictate the presentation. Chest pain, dysphagia, odynophagia, nausea, vomiting, hoarseness, or aphonia can be seen in an upper

esophageal perforation, whereas lower esophageal perforation manifests with abdominal pain, pneumothorax or hydropneumothorax, or pneumomediastinum. Hematemesis is rarely severe, but occurs in 55% of patients [66,87,89–92]. Of note, iatrogenic perforations often may remain asymptomatic for up to 8 h [66].

Physical examination reveals tachypnea, tachycardia, and sometimes fever. Subcutaneous emphysema is present in at least 30% of patients [93]. If present, Hamman sign (a mediastinal "crunch" appreciated with each heartbeat with patients in the left lateral decubitus position) suggests pneumomediastinum. With pleural involvement, there may be reduced or absent breath sounds on the side of the perforation. Signs of tension pneumothorax, hypotension, or shock are indicative of sudden massive esophageal rupture [94–96].

Early diagnosis and treatment are essential to avoid the high mortality rate (25%–75%) associated with presentation beyond 24 h from the rupture [92,95,97,98]. A high level of suspicion is also required, as misdiagnosis or treatment delay occur in more than 50% of patients [96,99]. After obtaining a history and performing the physical examination, radiographs of the chest and abdomen should be acquired. There are a range of findings, including subcutaneous emphysema, pneumomediastinum, mediastinal widening, mediastinal air–fluid levels, pleural effusion, pneumothorax, hydropneumothorax, or pneumoperitoneum [87,89]. A completely normal chest radiograph is present in fewer than 5% of patients [97]. The next step in evaluation is to perform an esophagram with water-soluble contrast; multidirectional views are diagnostic in most cases [66,87,89]. If this cannot be obtained, a noncontrast computed tomography (CT) scan may identify smalls quantities of air or oral contrast in the pleural space, and provides views of the mediastinum, pleura, and aorta [89,100]. Unfortunately, it does not always allow for localization of the perforation [101]. Flexible upper endoscopy is highly accurate and has been advocated for suspected blunt or penetrating esophageal trauma, but it does present the risk of extending the perforation [102].

Although infection is a common complication of esophageal perforation, until the patient develops fever, mediastinitis, pleural effusion, empyema, or sepsis, the diagnosis may be obscure. If examined, the pleural fluid is exudative, with a pH as low as 6.0 [103]. Because the pleural fluid is salivary in origin, the pleural fluid amylase may be elevated [104]. A wide range of community and nosocomial organisms cause empyema [105,106].

Surgical management is generally required for both spontaneous rupture and traumatic perforation [98,107]. Initially, all patients should take nothing by mouth, and broad-spectrum antibiotics should be administered. Specific therapy depends on the site and etiology of the rupture, the length of time between the perforation and the diagnosis, and the overall health status of the patient. Small perforations of the cervical esophagus can be treated conservatively with antibiotics and without surgery, or with localized drainage of the neck without esophageal repair [108,109]. Intrathoracic perforations, however, must be treated aggressively with debridement and irrigation of the mediastinum, followed by primary closure of the defect [110]. With early intervention, the mortality rate is less than 10%, but this rate triples if surgery is delayed for more than 24 h [64,98]. However, even in patients with a delayed diagnosis, primary esophageal repair remains the treatment of choice [64,110–113]. Esophagectomy is typically performed only when the diagnosis has been significantly delayed such that severe infection has developed [72,114]. Patients with perforation caused by endoscopy with dilation tend to have a good prognosis because of prompt diagnosis and treatment [115]. In addition, minimally invasive interventions are being used more commonly. Thorascopic repair of esophageal rupture and endoscopic repair of small dilation-induced perforations using hemoclips are emerging techniques [116–120]. Covered expandable metal stents have also been successfully deployed to seal immediately recognized esophageal perforations [121–124]. Because this type of stent is generally not removable, it should be reserved for inoperable patients with significant comorbidity (e.g., cancer). Recently, a removable expandable plastic stent has been developed that facilitates temporary stent placement in the case of esophageal perforation [125].

Esophageal intramural hematomas

An *esophageal intramural hematoma* is usually produced by local trauma [126–128]. It typically occurs in the setting of vomiting or retching, although spontaneous hematomas have been seen in patients with coagulopathies or those treated with anticoagulants or aspirin [126–133]. Iatrogenic hematomas have occurred after endoscopic sclerotherapy, cardioversion, thrombolysis, passage of a nasogastric tube, and endoscopic biopsy [134–139].

The pathogenesis is likely due to a mechanical injury of the esophageal wall. Potential causes include sudden or dramatic changes in internal esophageal pressure from blunt external trauma or from retching, coughing, or sneezing [127,128].

Patients typically present with an abrupt onset of substernal or epigastric pain followed by hematemesis. Hemorrhage is usually of small volume, although up to 10% of patients require blood transfusion [126,140]. Dysphagia and odynophagia are common, and in some cases the esophagus can be completely obstructed [132,134,140–142].

Patients presenting with hematemesis and substernal chest pain should immediately have a chest radiograph to rule out perforation. If negative, an esophagram with a water-soluble contrast agent should then be performed [141]. The most common abnormality seen on this test is a filling defect [143]. On endoscopy, a bluish or violet esophageal mass protruding into the lumen is seen, sometimes associated with a mucosal tear [127,144,145]. CT with contrast

or magnetic resonance imaging (MRI) can reveal a non-enhancing esophageal mass that has the density of blood [129,131,145,146]. Endoscopic ultrasound shows an intramural submucosal hypoechoic mass [145].

Most patients respond to conservative management with complete resolution within 2–3 weeks without long-term sequelae [133,138,140]. However, the development of fever or pleural effusions indicates that an esophageal perforation was not detected during the initial evaluation [138,143]. Endoscopic incision and drainage of the hematoma has been described [147,148]. Surgery is needed in 19% of cases to control bleeding or repair perforations [140].

Corrosive esophagitis

Epidemiology

Ingestion of corrosive substances occurs in more than 26 000 patients per year in the United States, approximately 17 000 of whom are children (half of whom are less than 4 years of age) [149]. Nearly half of caustic ingestions injure the esophagus severely enough to cause long-term sequelae, and ingestion of corrosive substances remains a leading cause of pediatric death [149,150]. Although corrosive esophageal injury usually represents accidental ingestion in children, in adolescents and adults most ingestions represent suicide attempts.

Caustic esophageal injury is also a risk factor for squamous cell cancer of the esophagus. One study found that this malignancy may be 1000 times more common in patients after caustic injury than in the general population, even though the mean time between ingestion and cancer is 50 years [149,151]. This association has prompted the recommendation for yearly endoscopic surveillance beginning 20 years after caustic esophageal injury [152].

Pathogenesis

The pathogenesis of corrosive esophagitis is due to direct tissue injury. Alkaline substances produce a deeper depth of injury than acidic substances because liquefaction necrosis occurs in seconds [149,153]. The pH required for esophageal damage is 12.5, but the severity is dependent on both the concentration of the alkaline agent and the mucosal contact time [149]. For example, one study found complications only in those ingesting strong alkaline agents such as lye, whereas detergent ingestion (a weak alkali) caused a complication in only a single case [154].

In contrast to alkaline agents, acidic corrosives cause coagulative necrosis and eschar formation, a mechanism felt to protect deeper esophageal layers from further injury [149]. As with alkalis, the concentration of the acidic agent determines the degree of injury, but the rapid clearance of acid by esophageal peristalsis and partial neutralization by swallowed salivary bicarbonate can limit esophageal injury [149].

The formulation of the corrosive agent is also important. Granular corrosives tend to cause less esophageal injury than liquids, because the granules adhere to the mucous membranes of the mouth rather than being carried into the esophagus [155].

Presentation

Some patients will present immediately after a volitional caustic esophageal injury, but others may not. Symptoms can last days to weeks and include abdominal pain, retrosternal chest pain, dysphagia, nausea, vomiting, and odynophagia. In children unable to describe their symptoms, refusal to drink and increased salivation might be noted.

The most common long-term consequences of severe or circumferential caustic ingestion injuries are esophageal strictures. These strictures are long and narrow, adversely affect distal esophageal peristalsis, and cause dysphagia or food impaction [156]. Strictures and subsequent dysmotility may also lead to decreased clearance of refluxate, esophagitis, and even peptic stricture.

Diagnosis

Most often, the diagnosis is suggested by an accurate history, but upper endoscopy is usually needed to confirm the diagnosis and grade the severity of the injury (Fig. 37.4) [149,153,154]. Injury can be classified as first degree (superficial injury manifest by erythema), second degree (deeper injury manifest by ulceration), or third degree (transmural injury manifest by necrosis). Although not yet widely used for this application, endoscopic ultrasound may provide a more accurate determination of depth of injury [157,158]. Other imaging modalities may also be helpful in the diagnostic evaluation. Plain abdominal or chest radiographs can show evidence of pleural fluid, mediastinitis, or frank perforation. If an esophageal perforation is found, upper endoscopy is relatively contraindicated. Barium swallow may demonstrate esophagitis, gastric abnormalities, or poor distention of the esophagus [159].

Treatment

Once a corrosive injury has occurred, there is no role for inducing emesis, lavaging the esophagus, or using a "neutralizing" agent because these modalities have the potential to cause further mucosal damage [149].

Although frequently recommended for palliation, the use of corticosteroids is somewhat controversial as there have been no placebo-controlled trials [160,161]. However, in a review of 13 publications of 361 patients [160], strictures were seen in 19% of those treated with steroids compared with 41% of those not treated with steroids. This effect was limited to patients who had either second- or third-degree burns; strictures were not seen in either of the treatment groups with first-degree esophageal burns. The timing of resuming oral feeding is not well established, but some

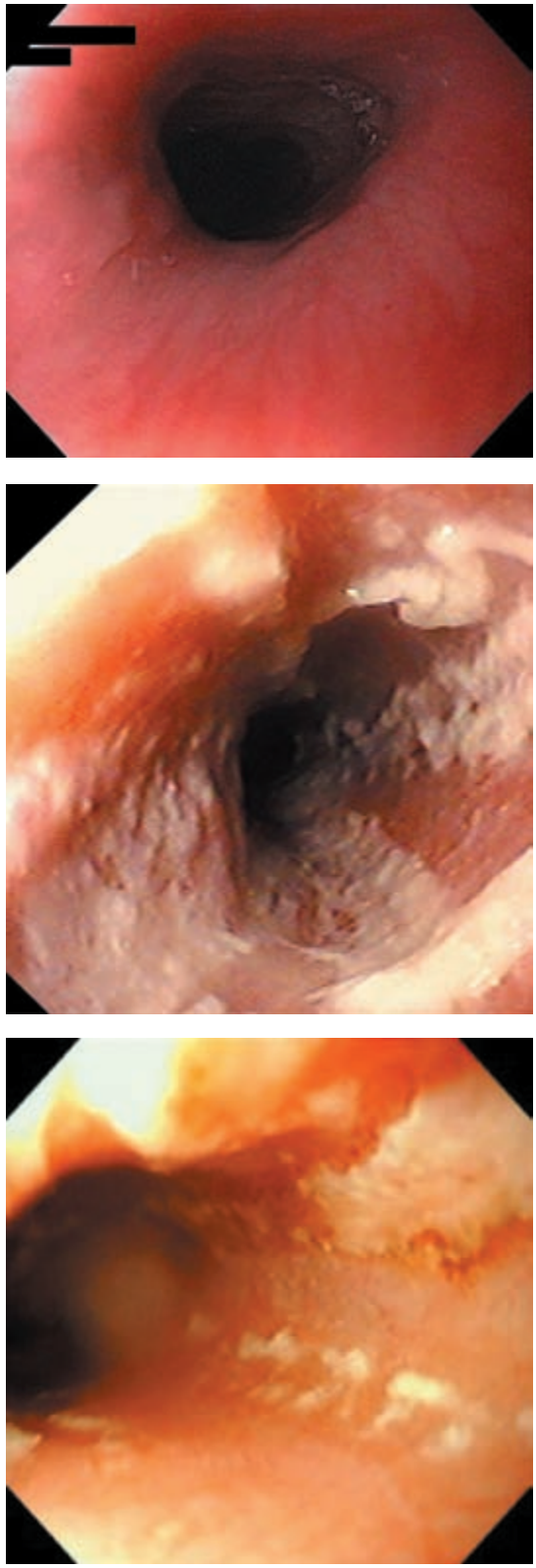


Figure 37.4 Examples of caustic esophageal injury in three pediatric patients after ingestion of a household alkali cleaning agent. First-degree injury is manifest by mucosal erythema **(a)**, whereas second-degree injury results in sloughing of the mucosa with ulceration **(b,c)**.

recommend giving patients nothing by mouth for at least 1 week in cases of severe esophageal burns [162]. Finally, given that concomitant reflux may also contribute to stricture formation, it is reasonable to prescribe antisecretory agents such as proton pump inhibitors [163].

When strictures secondary to caustic esophagitis do occur, endoscopic dilation is effective more than 50% of the time [164]. In recalcitrant cases, a gastrostomy has been placed to facilitate so-called endless-loop bougienage, in which a continuous string loop with plummets of progressively larger size is passed through the mouth and esophagus to the gastrostomy [165]. Patients with severe or dilation-refractory strictures require surgical resection, most commonly with a colonic interposition [166–168].

Pill esophagitis

Epidemiology

Pill esophagitis is widely reported in the literature. Although almost 100 different substances have been implicated in more than 700 cases (Table 37.1), the majority of cases are caused by tetracyclines, potassium chloride, nonsteroidal antiinflammatory drugs (NSAIDs), and quinidine [169–171]. The overall incidence of pill esophagitis has been estimated at 0.004% per year, but ranges from 0.04% for alendronate to as high as 20% for NSAIDs [171–174].

Because of the frequent use of high-risk medications, decreased salivation, and a higher prevalence of esophageal dysmotility, elderly patients are at increased risk for pill esophagitis [170,171]. Similarly, women are more likely to be treated with high-risk medications and are twice as likely than men to have pill esophagitis [170,171].

Pathogenesis

The development of pill esophagitis depends on a combination of patient, esophageal, and drug factors. Patients who lie down or swallow pills without adequate liquid intake may increase their risk of pill esophagitis. This risk is enhanced further by esophageal factors such as dysmotility or mechanical narrowing of the esophagus. Even without these conditions, esophageal transit of standard-sized medication tablets can take as long as 5 min [175,176]. The transit of capsules that adhere to the esophageal wall may also be slowed [169].

Most simply, however, pill esophagitis is caused by prolonged, direct contact of a caustic medication with the esophageal mucosa [171,177]. This is particularly typical of quinidine, potassium, and the bisphosphonates [173]. Tetracycline and ferrous sulfate, in contrast, injure the esophageal epithelium as a result of local pH changes or hyperosmolarity [178]. NSAIDs appear to be absorbed by the esophageal mucosa and cause injury from within, potentially as a result of prostaglandin inhibition [174].

Table 37.1 Selected medications implicated in pill esophagitis [169,171]

Antibiotics
Tetracycline
Tetracycline derivatives (doxycycline, erythromycin)
Penicillin
Penicillin derivatives (amoxicillin, ampicillin)
Trimethoprim
Clindamycin
Rifampin (rifampicin)
Zidovudine
Zalcitabine
Telithromycin

Nonsteroidal antiinflammatory drugs (NSAIDs)
Aspirin
Ibuprofen
Naproxen
Many others in class (e.g., indomethacin [indometacin], diclofenac)

Other drugs
Bisphosphonates (alendronate, pamidronate, etidronate)
Potassium chloride and potassium citrate
Quinidine
Emepronium
Ascorbic acid
Papain
Isotretinoin
Theophyllines
Oxybutynin
Iron preparations (ferrous sulfate)
Captopril
Nifedipine
Diltiazem
Warfarin
Valproic acid
Phenytoin
Phenobarbital
Cromolyn sodium
Chloral hydrate
Alprenolol
Corticosteroids

The mid- and distal esophagus are at highest risk for injury. Extrinsic compression by the left atrium or aortic arch may slow transit and increase contact time with the medication in the middle one-third of the esophagus. In the acid-exposed region of the distal esophagus, pill esophagitis may be worsened by concomitant acid reflux [179].

Presentation

Pain and odynophagia are the hallmarks of pill esophagitis, and these symptoms may be severe enough to inhibit adequate oral intake [178]. Retrosternal chest pain is also a frequent complaint. Dysphagia is less common and usually related to edema and ulceration rather than stricture. Rarely, patients may present with hematemesis or even perforation [170].

Diagnosis

Pill esophagitis should be suspected based on medication ingestion, risk factors, and symptoms elicited in a thorough history. Upper endoscopy typically demonstrates erosions or ulcers, usually in the middle or distal one-third of the esophagus, which are often circular or oval in nature. Rarely, the culprit medication is still in place (Fig. 37.5). Focal epithelial

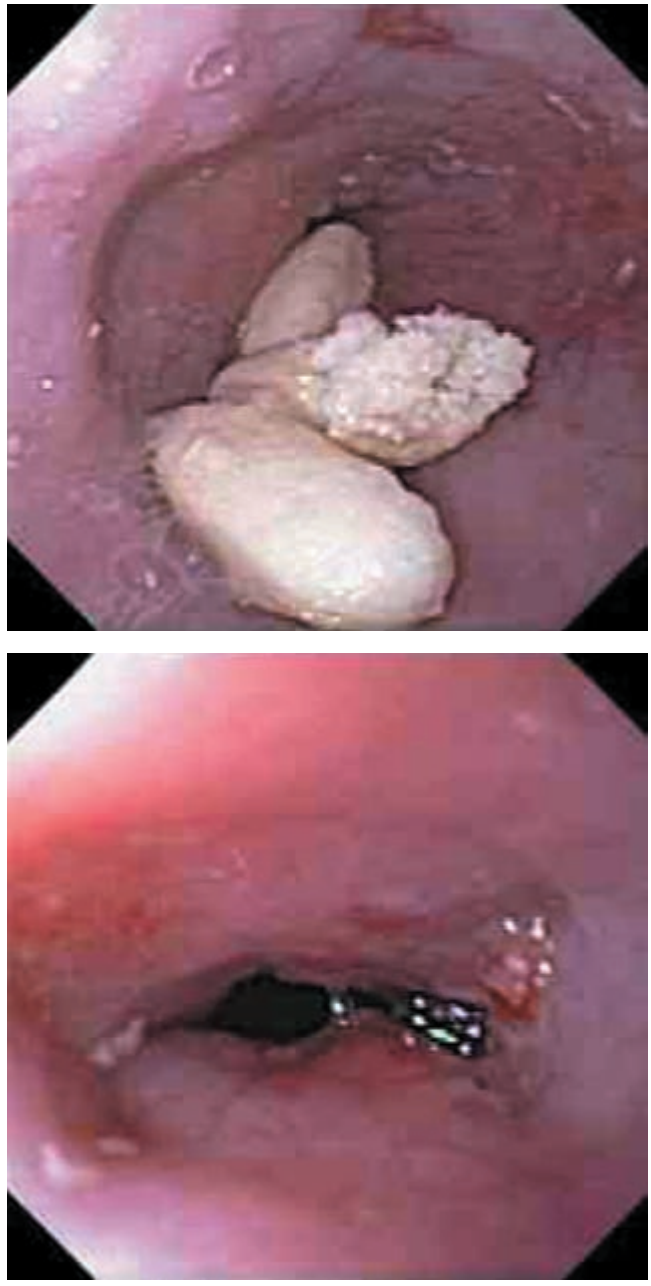

(a) (b)

Figure 37.5 Three pill tablets (one partially dissolved) lodged in the midesophagus with nearby erosions **(a)**. After removal of the tablets, pill esophagitis and an underlying esophageal stricture are evident **(b)**.

damage that spares the majority of the distal esophagus is nearly pathognomonic of pill esophagitis, and effectively excludes reflux esophagitis. "Kissing" ulcers, or ulcers at the same level of the esophagus but on adjacent walls, can also be seen [180]. If a stricture is found, it more likely is the cause, rather than a result, of the esophagitis, and may be responsible for delayed transit of a pill. Finally, although nonspecific, barium esophagram can show discrete ulcers in the mid- or distal esophagus [181,182].

Treatment

Pill esophagitis can largely be prevented with appropriate patient education. Patients should be instructed to ingest medications in an upright position with at least 100 mL of fluid, and to remain upright for 30 min after ingestion. If possible, patients with known esophageal compression, stricture, or dysmotility, and those patients confined to a bed, should avoid medications most frequently associated with esophageal injury [170].

If pill esophagitis does occur, most mucosal lesions heal within days to weeks of withdrawal of the offending agent [169,171]. Occasionally, patients may have such severe symptoms that intravenous fluids or dietary modifications are needed to avoid dehydration. Antisecretory therapy with either an H_2-receptor antagonist or a proton pump inhibitor is usually prescribed to protect the esophageal mucosa from further injury related to reflux of gastric contents [171]. Similarly, agents that provide a protective coating over the esophagus, such as sucralfate, may be of benefit.

Systemic diseases

Sarcoidosis

In total, 10% of patients with known *sarcoidosis*, a systemic granulomatous disease, may have granulomata in the submucosa of their GI tracts, but associated symptoms are rare [183,184]. When symptoms do occur, dysphagia is the most common clinical manifestation of esophageal sarcoidosis. Causes of dysphagia include extrinsic compression from enlarged lymph nodes, an achalasia-like dysmotility syndrome, and granulomatous stricture formation [185–188]. Because granulomata are present in both diseases, esophageal sarcoidosis may be difficult to differentiate from Crohn's disease [188].

Medical therapy primarily targets the underlying systemic disease. Dysphagia may respond to steroid treatment, although endoscopic dilation of strictures may be required [186,187]. Esophageal surgery is very rarely needed [187].

Crohn's disease

Esophageal involvement in *Crohn's disease* is uncommon. The prevalence of esophageal disease in several series ranges from 0.3% to 2% [189,190]. This may be an underestimate because a large number of patients with esophageal involvement are asymptomatic: of 75 case reports of patients with esophageal Crohn's disease, only 55% presented with esophageal symptoms [191]. The prevalence may also be higher in children, although children undergo upper endoscopy more frequently during the evaluation of Crohn's disease than adults [190,192,193].

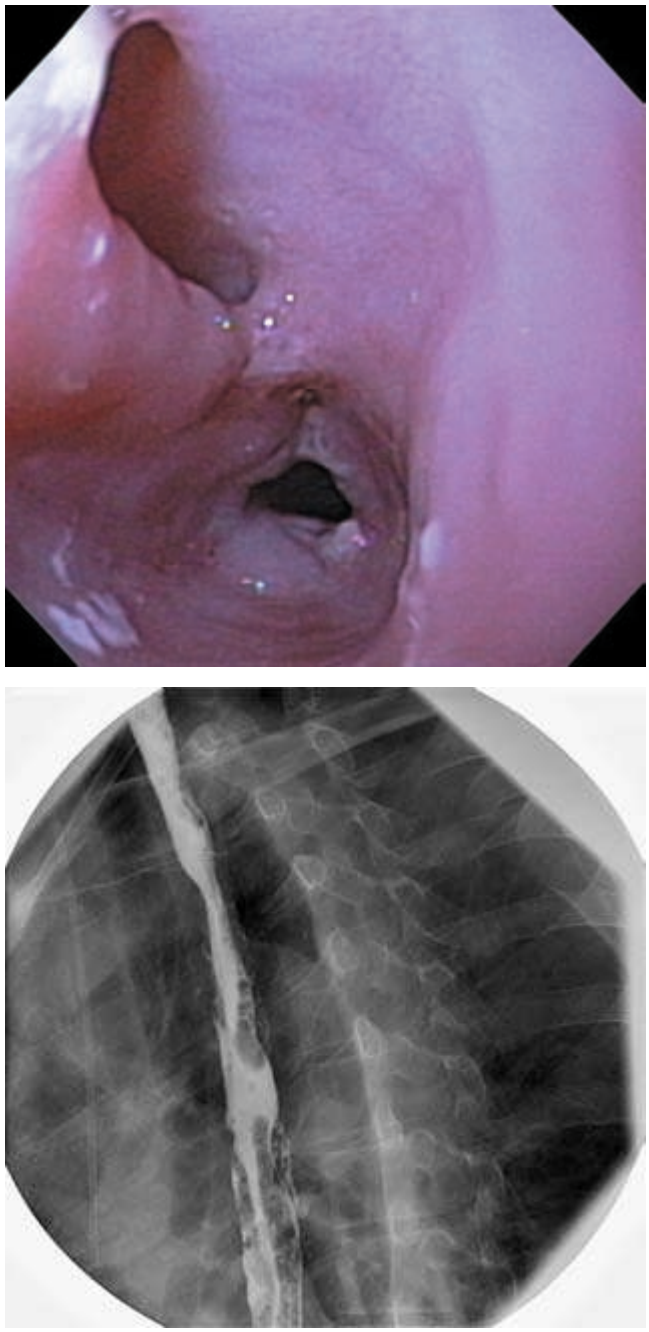
Patients with Crohn's esophagitis usually have active disease elsewhere in the GI tract, and the degree of inflammation typically parallels the activity in the other involved segments [194,195]. Rarely, dysphagia alone can be the presenting symptom of Crohn's disease, and isolated esophageal involvement has been reported [194,196–198]. Other symptoms include odynophagia, pyrosis, and substernal chest pain [189,194].

On upper endoscopy, aphthous ulceration can be seen early in the course of the disease, whereas cobblestoning and deep linear ulcerations may be present later, leading to decreased motility and distensibility (Fig. 37.6). Esophageal stricturing and the formation of sinus tracts, fistulae, or mucosal bridges have been reported [194,195,199]. In severe cases there is perforation and fistulization to the bronchi, mediastinum, pleura or stomach [190,198,200,201]. For diagnostic purposes, endoscopic mucosal biopsies can evaluate the degree of inflammation, but cannot demonstrate transmural inflammation; granulomata are rarely found [189,194,195]. The transmural nature of the inflammatory process may be better illustrated with endoscopic ultrasound, although this is not routine as of yet [202,203].

The tenets of treating Crohn's disease of the esophagus are the same as for the small or large intestine (see Chapter 57), with a focus on controlling systemic inflammation and local complications [190]. Of patients who respond to corticosteroids or other immunosuppressants, more than half will remain free of both esophageal lesions and upper GI symptoms, whereas the remainder will have either refractory lesions or recurrence [189,194,195]. For patients with fistulizing Crohn's disease of the esophagus, infliximab may have value [204]. Strictures may respond to both medical management and bougie dilation; extensive mucosal bridges may be endoscopically excised with a papillotomy needle knife [199]. In some patients, placement of a percutaneous endoscopic gastrostomy may be needed because of malnutrition [205]. Surgical resection is reserved for medically refractory fistulae, endoscopically refractory strictures, or when malignancy cannot be excluded [197].

Graft-versus-host disease

After bone marrow transplantation, the primary signs of *graft-versus-host disease* (GVHD) are a cutaneous rash, abnormal liver function tests, and diarrhea [206]. GVHD involving the esophagus is uncommon and, although isolated upper GI involvement may rarely occur, esophageal GVDH is usually seen in patients with severe multisystem chronic disease with both the upper and lower GI tracts affected [207–210].



(a)

(b)

Figure 37.6 An endoscopic view of Crohn's disease of the esophagus with diffuse esophageal narrowing, a prominent sinus tract, and exudative plaques **(a)**. A barium swallow from the same patient showing esophageal narrowing, mucosal irregularity, ulceration, and nodularity, and sinus tracts parallel to the esophagus **(b)**.

Symptoms of GVHD esophagitis include odynophagia, dysphagia, pyrosis, and retrosternal chest pain.

For diagnostic purposes CT is often normal, but a thickened distal esophagus may represent high-grade disease [211]. Because patients are immunosuppressed, upper endoscopy with biopsy and culture is the most sensitive way to differentiate GVHD esophagitis from opportunistic infections such as herpes simplex virus, cytomegalovirus, or *Candida* [212]. On visualization, the esophageal mucosa may appear erythematous and friable, and strictures, webs, bullae, or esophageal casts have been noted (Fig. 37.7) [207,208,213]. Recently, video capsule endoscopy has been used to image GVHD in the small intestine, but not the esophagus; however, this technique is limited because biopsies can not yet be obtained [214].

Therapy guided by hematologists specializing in bone marrow transplantation utilizes immunosuppressants such as prednisone, azathioprine, cyclosporine (ciclosporin), mycophenolate mofetil, and even infliximab [206,215,216]. Esophageal symptoms can respond to antireflux medications and to dilation of webs and strictures, although the risk of iatrogenic perforation may be increased in these patients [209].

Behçet syndrome

The constellation of oral and genital aphthous ulceration and ocular inflammation characterizes *Behçet syndrome*, which may rarely involve the esophagus [217]. It is most common in the eastern Mediterranean and in Middle Eastern and East Asian populations, primarily affecting young adults [218].

As with other esophageal processes, symptoms can include dysphagia, odynophagia, chest pain, and hematemesis. Upper endoscopy can reveal a range of findings including erosions, esophagitis, and esophageal strictures, most of which have been reported in the middle and distal esophagus [219–222]. Biopsies demonstrate ulceration with nonspecific inflammation and neutrophilic infiltration [217].

The clinical course is one of unpredictable remissions and exacerbations. Although dysphagia caused by esophageal ulcers may resolve spontaneously, corticosteroids have been reported to improve symptoms, and intestinal and esophageal ulcers have also responded to sulfasalazine and low-dose cyclosporine [217–220,223,224].

Miscellaneous autoimmune diseases

Rarely, the esophagus is involved in other systemic autoimmune diseases. Although more than half of patients with *Sjögren syndrome* complain of difficulty swallowing, dysphagia does not appear to correlate with the degree of xerostomia [225]. In up to 10% of affected patients, esophageal webs are responsible for dysphagia [226]. Data are mixed on whether dysphagia correlates with abnormalities on esophageal manometry, although heartburn is associated with reflux severity as measured by pH probe [227–229].

Primary or secondary *amyloidosis* may cause dysphagia because of amyloid deposition in the esophageal muscle or nerves. The resulting motility abnormalities can mimic achalasia [230–232]. In rare cases, amyloid deposition may predispose to spontaneous esophageal rupture [233].

The *anticardiolipin antibody syndrome* may present with esophageal vascular thrombosis and ischemic esophageal perforation or esophageal varices secondary to portal vein thrombosis [234–236].

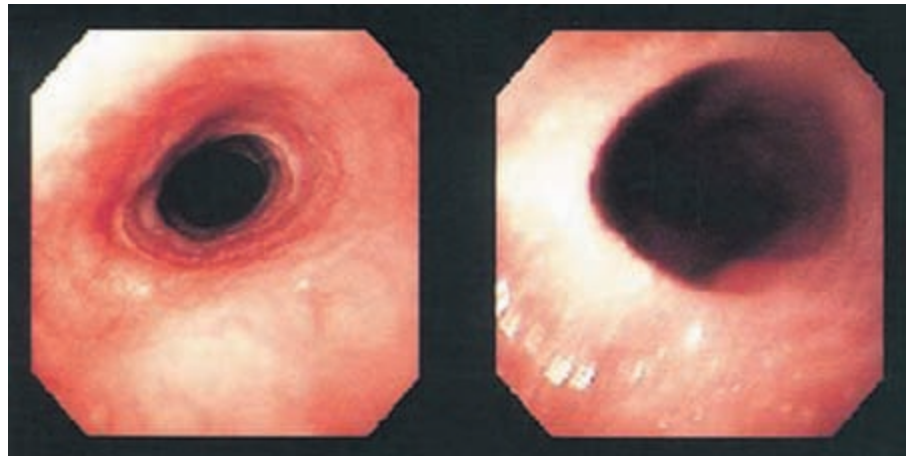


Figure 37.7 An endoscopic view of graft-versus-host disease showing multiple fine mucosal webs in the esophagus.

Dermatological diseases

Pemphigus vulgaris

The esophageal mucosa can be affected by diseases of the squamous epithelium of the skin. One example is *pemphigus vulgaris*, a chronic autoimmune blistering disease of older adults in which flaccid intraepidermal blisters and oral erosions are caused by IgG autoantibodies against desmoglein-3 in keratinocytes [237–239]. Patients may be asymptomatic, but the 50% who have esophageal involvement usually present with dysphagia or food impaction [240–242]. Isolated esophageal involvement without significant skin manifestations has rarely been reported [243,244]. On taking a history, the use of certain medications associated with pemphigus, such as thiol compounds (e.g., penicillamine), angiotensin-converting enzyme inhibitors, and antibiotics (e.g., penicillins, rifampin [rifampicin], cephalosporins), should be elicited [244].

Diagnosis requires upper endoscopy with biopsy [245]. On histopathological examination, immunohistochemistry cannot distinguish idiopathic pemphigus vulgaris from drug-induced or paraneoplastic pemphigus [239,246,247]. Therefore, serological testing for pemphigus antibodies and antidesmoglein-3 antibodies is also needed [248]. Although corticosteroids are the primary treatment modality in pemphigus vulgaris, other immunosuppressive drugs such as azathioprine, cyclophosphamide, cyclosporine, or methotrexate may be needed to induce a complete remission or to spare steroid use [239]. In severe cases, plasmapheresis has been successful [239].

Bullous pemphigoid

In contrast to pemphigus vulgaris, *bullous pemphigoid* is characterized by the presence of tense bullae on the skin of the flexor surfaces [239]. The disease is caused by IgG autoantibodies directed against the basement membrane of the squamous epithelium [239,249]. Although 20% of patients will have involvement of the oropharynx, the prevalence of esophageal bullous pemphigoid is unknown and may be underestimated because symptoms are infrequent [250]. Rarely, patients complain of dysphagia and odynophagia. Upper GI hemorrhage, scarring, mucosal sloughing, and hemorrhage are also rare [251]. Treatment with prednisone is highly effective, but azathioprine, cyclophosphamide, cyclosporine, or methotrexate have also been used [239].

Benign mucous membrane pemphigoid

Benign mucous membrane pemphigoid (BMMP), or cicatricial pemphigoid, is a chronic blistering disease of the conjunctivae, oral and genital mucosa, and adjacent areas of skin. In this disease, IgG or IgA autoantibodies are deposited in the basement membrane zone [239,252,253]. BMMP usually occurs after the sixth decade of life, producing tense blisters with scarring and strictures of the esophagus, trachea, anus, or vagina [254]. In total, 2–13% of affected patients have esophageal involvement, which can present up to 10 years into the course of disease as dysphagia, odynophagia, or chronic cough due to aspiration [255]. Rarely, patients have isolated esophageal BMMP with no other disease manifestations [256]. On endoscopy, esophageal webs or strictures may be seen (Fig. 37.8) [257]. In contrast, bullae are rarely seen endoscopically, although in one case endoscopy induced their formation [256]. Persistent dysphagia can be treated with dilation, although these patients may be at increased risk of esophageal injury [257,258]. In severe cases, BMMP requires prednisone, whereas milder cases often respond to dapsone [239,259]. Surgical treatment of strictures is rarely required [260].

Epidermolysis bullosa dystrophica

A final, and also rare, esophageal manifestation of dermatological disease is *epidermolysis bullosa dystrophica* (EBD), a blistering disease of the squamous epithelium caused by mutations in the collagen type VII gene (*COL7A1*) [261,262].

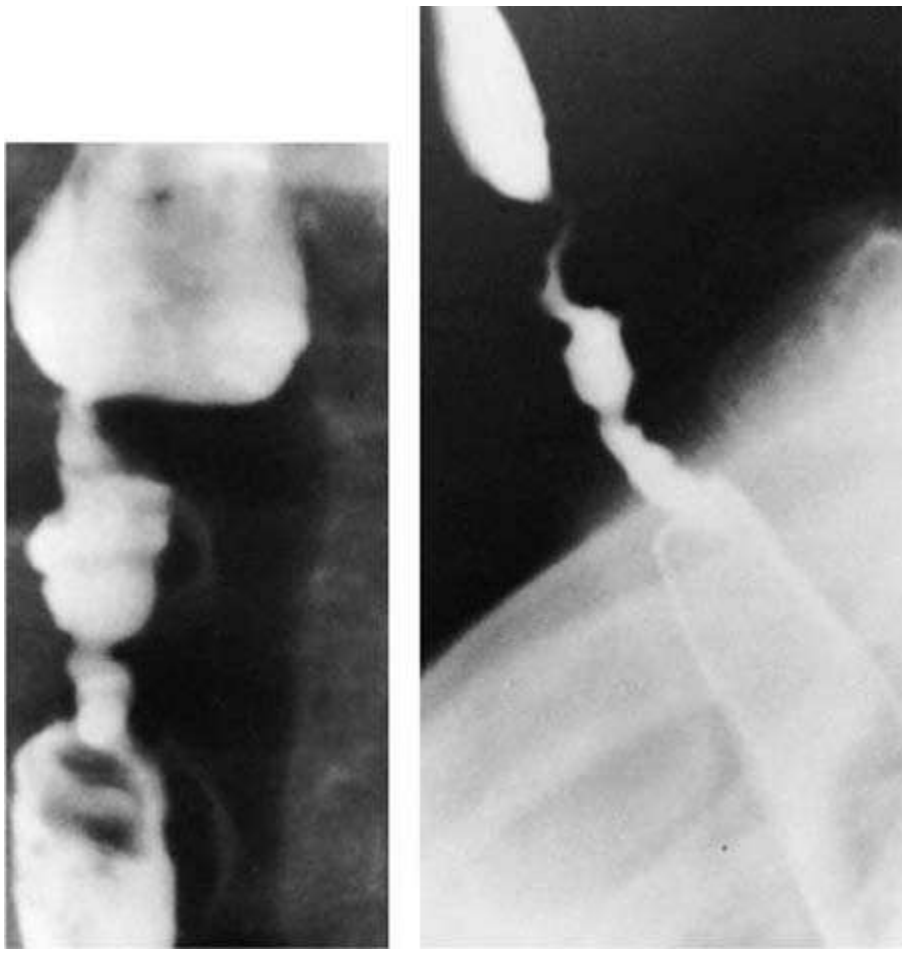


Figure 37.8 Benign mucous membrane pemphigoid. Postinflammatory scarring caused a long, irregular area of narrowing suggestive of a malignant process. From Eisenberg RL. Gastrointestinal Radiology: A Pattern Approach, 4th edn. Philadelphia, PA: Lippincott Williams & Wilkins, 2003.

Histopathologically, the basal lamina separates from the dermis because of a reduction or absence of the fibrils normally attaching these two layers [263]. Cutaneous bullae are evident beginning in early childhood and form at sites of minor trauma, especially in the hands and extremities. Over time, the cycle of chronic scarring and healing leads to contractures and syndactyly, a process termed *mummification*. Both dominant and recessive forms of this inherited disease exist, but esophageal involvement is only clinically important in the recessive form [264]. There is also an acquired form of the disease (*epidermolysis bullosa acquisita*), which causes nonscarring bullous lesions of the esophagus [265,266].

In the esophagus of patients with EBD, trauma from routine swallowing begins a cycle of bullae formation, healing, and scarring, leading to development of strictures, esophageal shortening, and dysmotility (Fig. 37.9) [267,268]. Clinically, these cause symptoms of dysphagia, odynophagia, and food impaction [267,268]. In younger patients, malnourishment is common and parenteral nutrition may be required. The constellation of esophageal symptoms and the prominent cutaneous manifestations suggest the diagnosis of epidermolysis bullosa dystrophica. In uncomplicated cases, the risk of traumatic bullae formation due to endoscopy outweighs the incremental diagnostic yield. If a diagnostic test is needed, barium esophagram can reveal strictures or webs [267,268]. Complications include esophageal perforation and occlusion due to scarring [267,268].

Treatments for EBD are limited, and when the esophagus is involved, the prognosis tends to be poor [268]. From a dietary standpoint, patients should avoid coarse or hot foods and use nutritional supplements to avoid malnutrition. Medical therapies lack efficacy, although corticosteroids or phenytoin have been used [267–269]. For endoscopic therapy of strictures, balloon dilators may be preferable to bougienage to avoid damage to friable mucosa, and a preprocedural barium swallow may help plan the procedure by assessing angulation within the stricture [270]. Because dilation carries an increased risk of esophageal trauma and perforation, fluoroscopy may be a valuable adjunct [268,271]. In patients with esophageal perforation or malnutrition from refractory esophageal stenosis, esophagectomy and conduit reconstruction may be necessary [268,269,272].

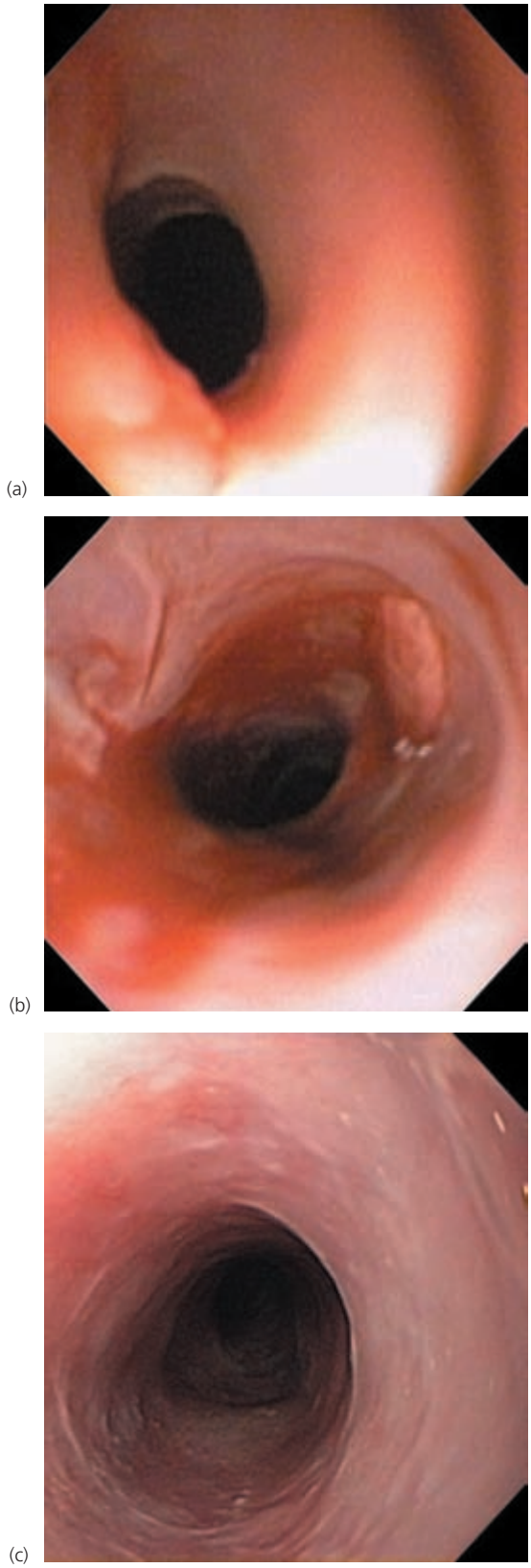


Figure 37.9 An endoscopic view of a tight esophageal stricture due to epidermolysis bullosa dystrophica before **(a)** and after **(b)** initial dilation. After multiple sessions of serial dilation, the stricture zone is much improved **(c)**.

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B STOMACH

38 Stomach and duodenum: anatomy and structural anomalies

Jean-Pierre Raufman, Eric Goldberg

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Anatomy of the stomach and duodenum

The gross anatomy and microscopic anatomy of the stomach and duodenum are intrinsically linked to their functions of digestion and nutrition. The structure and organization of these organs facilitate sequential physiological events that promote the fragmentation, enzymatic digestion, and absorption of nutrients. Moreover, appropriately timed autocrine, paracrine, and hormonal secretions of gastric and duodenal endocrine cells recruit other cells and organs to aid in the digestive process. Despite advancements in our understanding of these complex integrated physiological events, which include secretory, peristaltic, and absorptive actions, much remains to be learned. In this chapter, the anatomy and structural anomalies of the stomach and duodenum are considered together because these organs are structurally and functionally related.

Gross anatomy

Anatomic relationships and divisions

The stomach, a large, distensible sac with the largest diameter of any part of the gastrointestinal tract, is located in the epigastrium, just inferior to the diaphragm (Fig. 38.1). The size and shape of the stomach vary greatly from person to person, depending on age, body habitus, posture, and interval since eating. The ability of the stomach to enlarge to accommodate meals is facilitated by its free mesentery, distensibility, and its location. Although fixed proximally to the esophagus and distally to the duodenum, and by the gastrocolic and gastrophrenic ligaments, the stomach has great latitude in distention and motion. When the stomach is nearly empty, gastric volume approximates a few hundred milliliters; when distended with food, the stomach can accommodate up to 2 L. With full distention, the stomach may extend from the diaphragm to the pelvic brim.

As a consequence of its size and central location in the abdomen, the stomach abuts many organs. These include the diaphragm superiorly, hepatobiliary organs to the right, the spleen to the left, and the pancreas and transverse colon posteriorly (Fig. 38.1). The anterior surface of the stomach abuts the abdominal wall. The stomach is separated from other abdominal organs by visceral peritoneal lining. The close proximity of the stomach to other abdominal organs such as the pancreas allows endolumenal imaging techniques such as endoscopic ultrasonography to evaluate intraabdominal viscera. Furthermore, because the anterior surface of the stomach is adjacent to the abdominal wall, percutaneous feeding tubes can be placed directly into the lumen of the stomach.

By convention, the somewhat J-shaped stomach is divided into the cardia, a 1- to 2-cm segment adjacent to the esophagogastric junction; the fundus, the superior portion of the stomach lying above and slightly posterior to the rest of the stomach; the body or corpus, the voluminous portion of the stomach below the fundus; the antrum, the distal region of the stomach; and, finally, the pylorus or pyloric channel, a narrow (1–2 cm) channel that connects the stomach to the duodenum (Fig. 38.2). The shorter right side of the stomach is the lesser curvature; the opposite, longer left side is the greater curvature. An intrusion, about two-thirds of the way along the distal lesser curvature, near the junction of the body and antrum, is designated the angular notch or incisura angularis.

The tubular, C-shaped duodenum, nestling the head of the pancreas, starts at the pylorus and extends to the ligament of Treitz, a landmark demarcating the fourth portion of the duodenum from the jejunum (Fig. 38.3). The word *duodenum* derives from its length, approximately the same as the breadth of 12 fingers (about 25–30 cm). The duodenum has no mesentery and, in contrast to the stomach, it is largely retroperitoneal and fixed in position.

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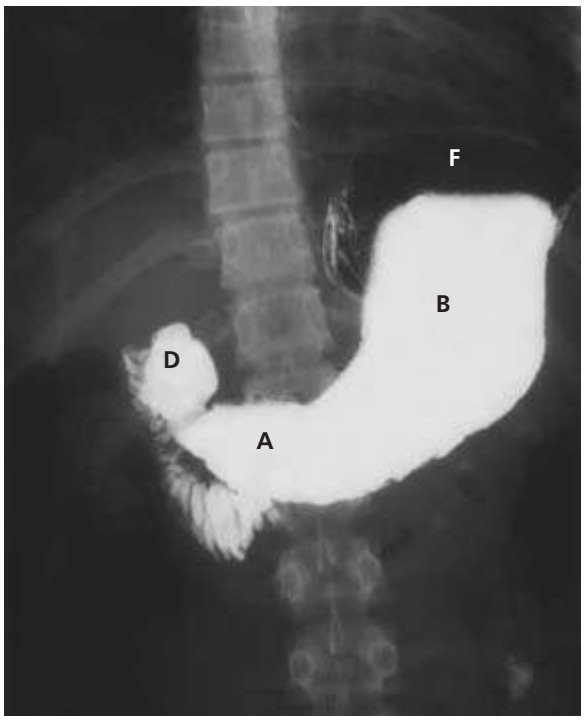
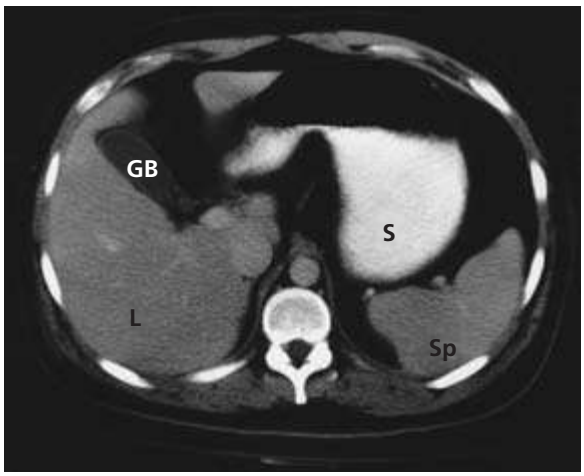


(a)



(b)

Figure 38.1 **(a)** Barium contrast radiograph of the stomach and duodenum. The pyloric channel can be seen connecting the antrum to the duodenal bulb. The second and third parts of the duodenum can be seen as well as the plicae circulares. F, fundus; B, body; A, antrum; D, duodenal bulb. **(b)** A computed tomogram of the abdomen shows the stomach in relation to adjacent organs. S, stomach; GB, gallbladder; L, liver; Sp, spleen. Courtesy of Drs Timothy Carter and Hemendra R. Shah, University of Arkansas for Medical Sciences, Little Rock, AR.

The first, or superior, part of the duodenum is about 5 cm long, starts at the pylorus, and passes posteriorly, upward, and to the right. The initial 2–3 cm of the first part is the duodenal bulb. In contrast to the rest of the duodenum, which is lined by circular folds (plicae circulares), the lining of the bulb is relatively flat. The second, or descending, part of the duodenum takes a sharp curve and descends along the head of the pancreas for 7–10 cm. The close proximity of the second portion of the duodenum to the head of the pancreas allows endoscopic ultrasonography and transduodenal biopsy of lesions in the head of the pancreas. The common bile and pancreatic ducts enter the second portion of the duodenum via the posteromedially located ampulla of Vater. The accessory pancreatic duct (Santorini) enters the duodenum approximately 2 cm proximal to the ampulla of Vater. The third, or horizontal, part of the duodenum passes from right to left across the spine, inclining upward about 5–8 cm. The fourth, or ascending part of the duodenum starts left of the spine, ascends leftward, and terminates at the ligament of Treitz, where the intestine angles anteriorly and downward as the jejunum.

Circulation

The gastric and duodenal blood supply are derived from the celiac artery and the superior mesenteric artery (SMA). Both of these vessels arise from the abdominal aorta. The short celiac artery branches to form the splenic, left gastric, and common hepatic arteries. A dense anastomotic network that encircles the stomach is formed by vessels that branch from these major arteries. These vessels include the left gastric artery, which feeds the anterior and superior portions of the stomach; the right gastric, gastroduodenal, and right gastroepiploic arteries, which arise from the hepatic artery and feed the lower right portion of the stomach and the lower greater curvature; and the short gastric and left gastroepiploic arteries, which arise from the splenic artery to feed the fundus and the upper greater curvature (Fig. 38.4). Because of the extensive anastomotic network surrounding the stomach, gastric ischemia is uncommon and generally occurs only when there are systemic aberrations in blood flow or there is gastric volvulus or previous gastric surgery.

The distal stomach, pylorus, and duodenum are supplied by the inferior pancreaticoduodenal branch of the SMA. The duodenum is also nourished by the right gastric and superior pancreaticoduodenal arteries that arise from the hepatic artery.

Venous drainage from the stomach and duodenum leads directly or indirectly to the portal vein. From the stomach, the left and right gastric veins drain the lesser curvature, and the right and left gastroepiploic and short gastric veins drain the greater curvature. From the duodenum, the anterior inferior and superior pancreaticoduodenal veins drain into the superior mesenteric vein. The posterior superior pancreaticoduodenal vein drains directly into the portal vein.

Lymphatics

The pattern of lymphatic drainage of the stomach is similar to that for its vasculature, with most lymph draining ultimately into celiac nodes. Submucosal, muscular, and serosal lymphatics join to drain into four major groups from the stomach and two from the duodenum. The first group of gastric

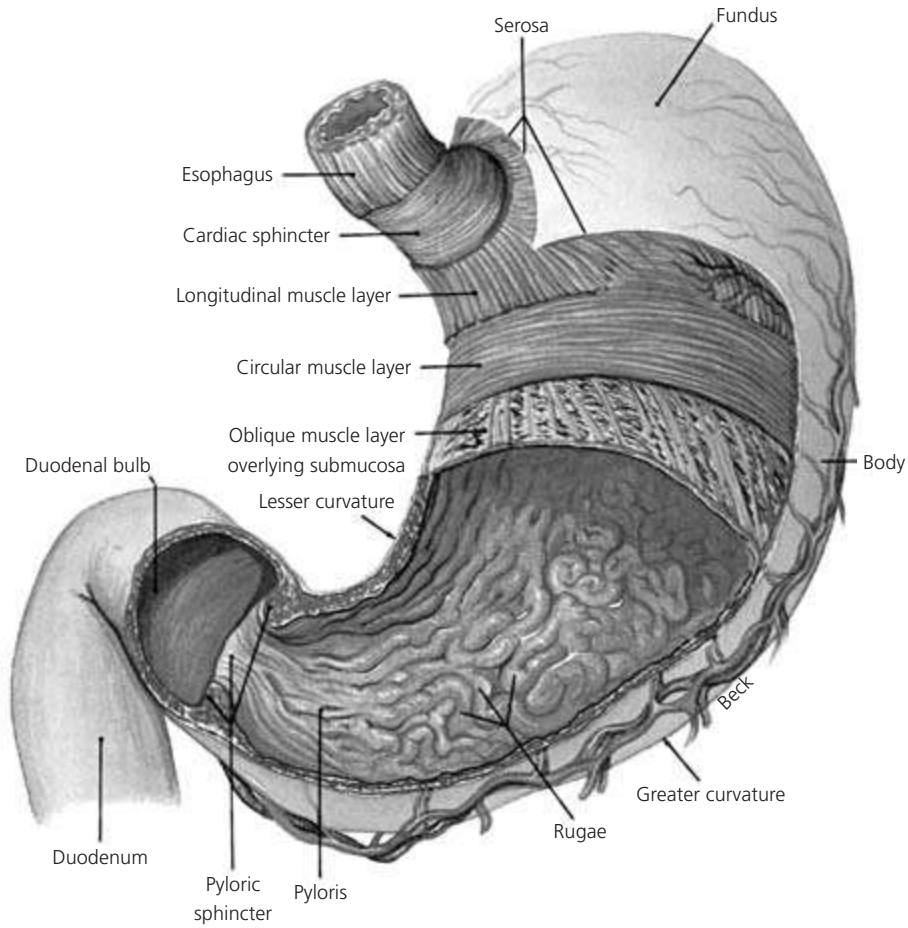


Figure 38.2 Muscle layers and rugal folds of the stomach. From Thibodeau GA, Patton KT. Anthony's Textbook of Anatomy and Physiology. St Louis, MO: Mosby-Year Book, 1996, with permission from Elsevier.

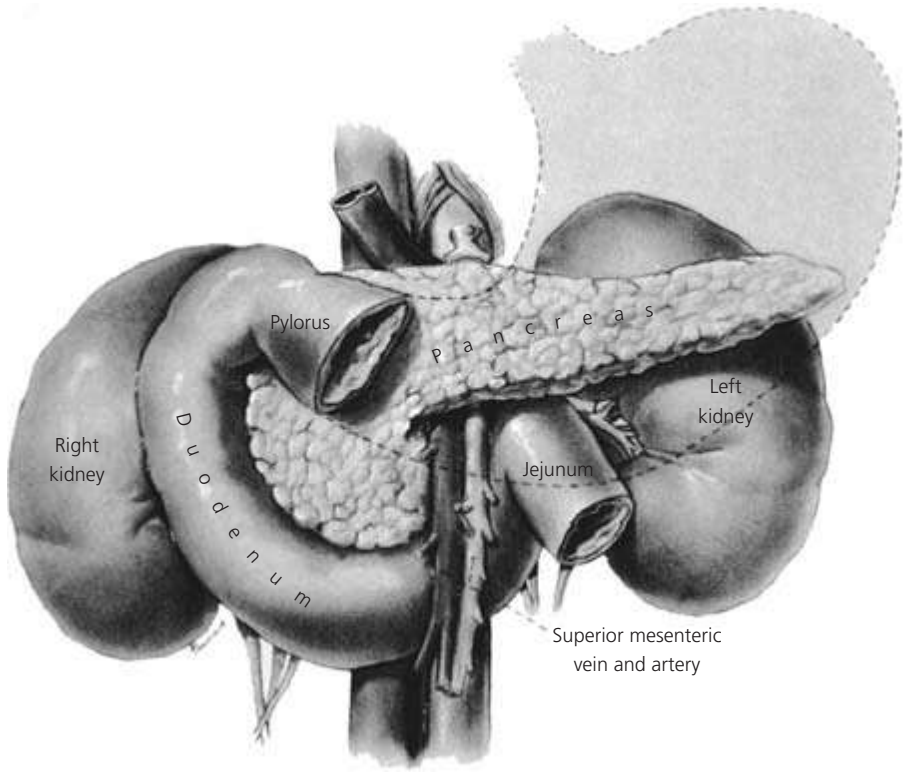


Figure 38.3 Anatomic relationships of the four parts of the duodenum to adjacent organs. The stomach is shown in outline. From Rosse C, Gaddum-Rosse P. Hollinshead's Textbook of Anatomy. Philadelphia, PA: Lippincott-Raven, 1997, with permission from Lippincott, Williams & Wilkins.

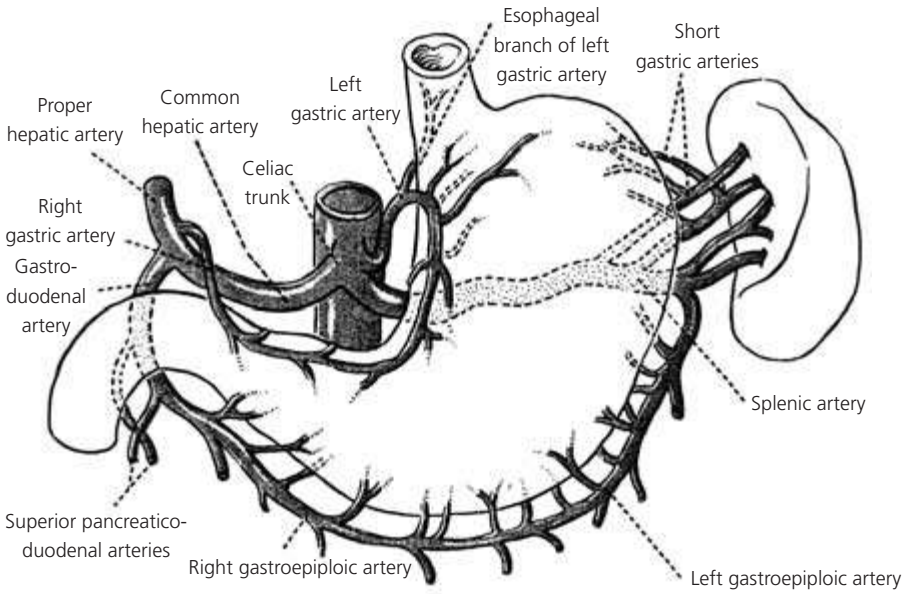


Figure 38.4 Illustration of the arterial supply of the stomach. From Rosse C, Gaddum-Rosse P. Hollinshead's Textbook of Anatomy. Philadelphia, PA: Lippincott-Raven, 1997, with permission from Lippincott, Williams & Wilkins.

lymphatics follows the left gastric artery, receives branches from the upper stomach, and ends in the superior gastric nodes surrounding the gastroesophageal junction. The second group drains the fundus and proximal stomach, follows the short gastric and left gastroepiploic arteries, and ends in the pancreaticolienal and splenic nodes, which drain into celiac nodes. The third group drains the distal greater curvature into inferior gastric nodes, which are connected to subpyloric nodes. The final group of gastric lymphatics drains the pyloric area into superior gastric, hepatic, and subpyloric nodes.

In the duodenum, anterior and posterior lymphatics drain into a series of small pancreaticoduodenal nodes near the boundary between the pancreas and duodenum. Efferents from these lymph nodes run superiorly and inferiorly to hepatic and pancreatic nodes, respectively, and to preaortic (superior mesenteric) nodes near the origin of the SMA.

Innervation

The stomach and duodenum are innervated by sympathetic and parasympathetic neurons of the autonomic nervous system. Sympathetic neuron cell bodies are located in the gray matter of the anterior columns of spinal thoracic segments T6–T12. Neural axons exit the spinal cord by the ventral roots and unite to form the greater and lesser splanchnic nerves that synapse in the celiac ganglia. Postganglionic fibers follow the hepatic, splenic, and left gastric arteries to the stomach, where they form perivascular intramural autonomic plexuses. Gastric afferent fibers follow the same course as efferent fibers; however, they pass through the celiac ganglia without synapsing, reaching cell bodies in dorsal root ganglia of the spinal cord. Afferent fibers transmit visceral pain sensation from the stomach and duodenum.

The vagus nerve, originating in the dorsal motor nucleus in the medulla, provides parasympathetic innervation to the stomach and duodenum. Vagal fibers course along the esophagus and enter the abdomen as the posterior and anterior vagal trunks. These neural trunks contain preganglionic efferent and visceral afferent fibers. Efferent fibers synapse with gastric cholinergic and peptidergic neurons in the wall of the stomach and duodenum, which innervate various cells directly. Although afferent vagal fibers predominate, little is known about their function.

After entering the abdomen at the esophageal hiatus of the diaphragm, the anterior vagal trunk divides almost immediately into anterior gastric and hepatic branches. The anterior branch innervates the cardia and provides a branch running to the right of the lesser curvature as the anterior nerve of Latarjet. The hepatic branch innervates the liver, gallbladder, pylorus, and proximal duodenum. The posterior vagal trunk divides into the celiac and posterior gastric branches. The former innervates the pancreas and other abdominal viscera. The posterior gastric branch innervates both surfaces of the stomach and forms the posterior nerve of Latarjet. The anterior and posterior nerves of Latarjet course along the lesser curvature, give off branches to the fundus and body, and terminate in a crow's foot neural distribution to the antrum and pylorus.

Preganglionic vagal fibers synapse with ganglia in the intrinsic plexuses of the gastric wall: the myenteric (Auerbach) plexus and the submucosal (Meissner) plexus. Postganglionic fibers from these plexuses innervate secretory and muscle cells.

Microscopic anatomy

The stomach and duodenum have four tissue layers: mucosa, submucosa, muscularis propria, and serosa. The submucosa represents a connective tissue layer beneath the mucosa composed of a loose framework containing vasculature,

lymphatics, and nerves. The muscularis propria of the stomach consists of a series of three muscle layers between the submucosa and the serosa (Fig. 38.2). The oblique muscle layer overlies the submucosa. The more substantial circular muscle layer, which thickens at the pylorus to form the pyloric sphincter, is covered by an outer longitudinal muscle layer that is a feature of the muscularis propria of the entire gastrointestinal tract. The thin serosa, the outer layer of the stomach that serves as the visceral peritoneum, is composed of areolar tissue covered by a monolayer of squamous mesothelial cells.

Stomach

The gastric mucosa is divided into epithelium, lamina propria, and muscularis mucosae. At the gastroesophageal junction, the mucosal boundary between the stratified squamous epithelium of the esophagus and the glandular columnar epithelium of the stomach can be seen as an irregular line (Z-line) encircling the lumen. When the stomach is empty, the mucosa and submucosa contract into thick folds called *rugae* (see Fig. 38.2). As the stomach distends, the rugae flatten.

Gastric pits (foveolae gastricae), which serve as exit channels for underlying gastric glands, stud the gastric mucosal surface. This surface epithelium is lined by a single layer of columnar epithelial cells that extends into the gastric pits and glands. Differences in the cellular makeup of glands permits histological division of the gastric mucosa into three types, cardiac (junctional), fundic, and antral (pyloric), which differ in structure and function (Fig. 38.5). The lamina propria is an area of loose connective tissue containing strands of collagen and smooth muscle, lymphatics, blood vessels, nerves, and a variety of cells, including lymphocytes, plasma cells, mast cells, fibroblasts, macrophages, and eosinophils. The muscularis mucosae, a smooth muscle layer, forms the inferior margin of the mucosa and separates it from the submucosa, which contains dense connective tissue, arterioles, venules, lymphatics, and neural plexuses.

Proceeding from the surface epithelium into the pits, the following mucosal cells are considered in detail in the following subsections: mucous cells and mucous neck cells, which line the surface and extend into the pits, respectively; parietal cells, which secrete hydrochloric acid and intrinsic factor;

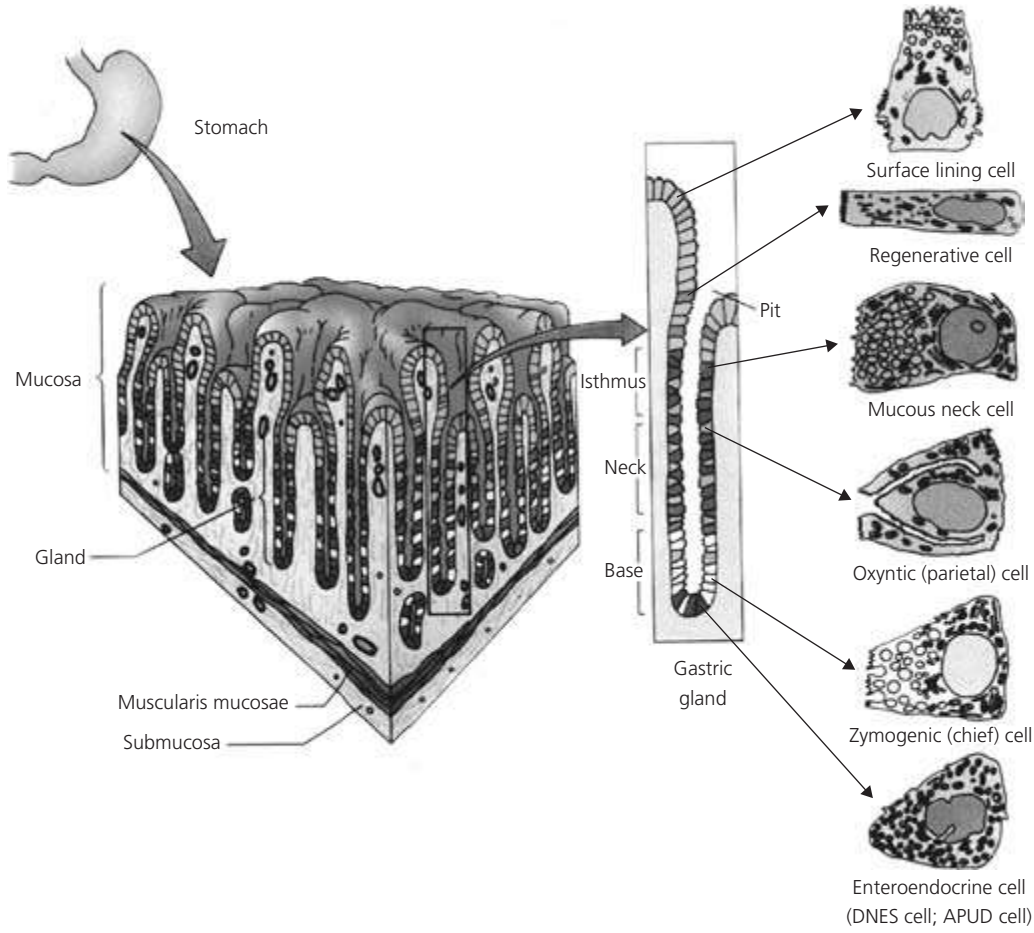
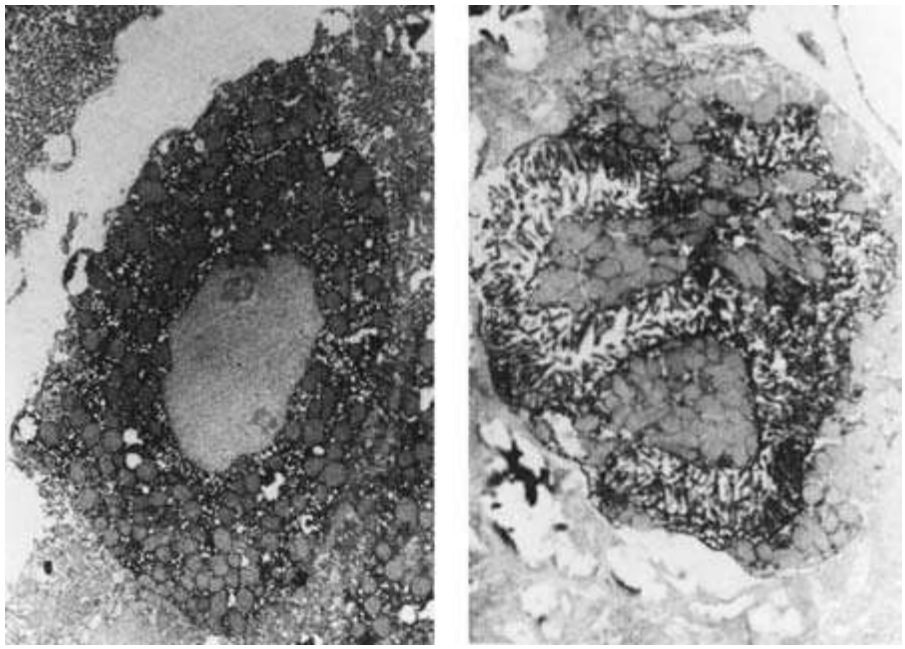


Figure 38.5 Drawing of gastric glands from the fundus/body region of the stomach showing approximate location within a representative gastric gland and typical histology of surface epithelial, mucous neck, parietal, chief, and enterochromaffin-like cells. APUD, amine precursor uptake and decarboxylation; DNES, diffuse neuroendocrine system. From Gartner LP, Hiatt JL. Color Textbook of Anatomy. Philadelphia, PA: WB Saunders, 1997, with permission from Elsevier.



(a) (b)

Figure 38.6 Electron micrographs of parietal cells. **(a)** Resting parietal cell showing concentric nucleus and abundant mitochondria and tubulovesicles. **(b)** Activated parietal cell with expanded canaliculus (approximate magnification × 4000). From Prinz C, Kajimura M, Scott D, et al. Acid secretion and H,K-ATPase of stomach. In: Modlin IM (ed.). Current Clinical and Scientific Perspectives in Gastroenterology. Yale J Biol Med 1992;65:170, with permission from the Yale Journal of Biology and Medicine.

endocrine cells, which secrete a variety of hormonal mediators; and, toward the base of the pits, chief cells, which secrete pepsinogen (see Fig. 38.6) [1–3].

Mucous cells

The rectangular *mucous cells* are polarized (Fig. 38.7). Apically located mucous granules contain mucin, a neutral glycoprotein that can be stained with mucicarmine and periodic acid–Schiff. The mucous cell nucleus, Golgi complexes, and endoplasmic reticulum are located at the base of the cell. Like other columnar epithelial cells, mucous cells are attached at their apical margins by tight junction complexes and are connected by gap junctions and desmosomes at other cellular sites. Mucous neck cells are similar to the mucous cells just described, but they contain more rough endoplasmic reticulum and are found predominantly in the neck of glands and scattered deeper within glands. Because mucous neck cells contain acidic mucus (glycosaminoglycan), their granules are generally less dense than granules in surface mucous cells. Mucus is released by stimulated exocytosis; hence, the degree of granule filling depends on the stage of digestion.

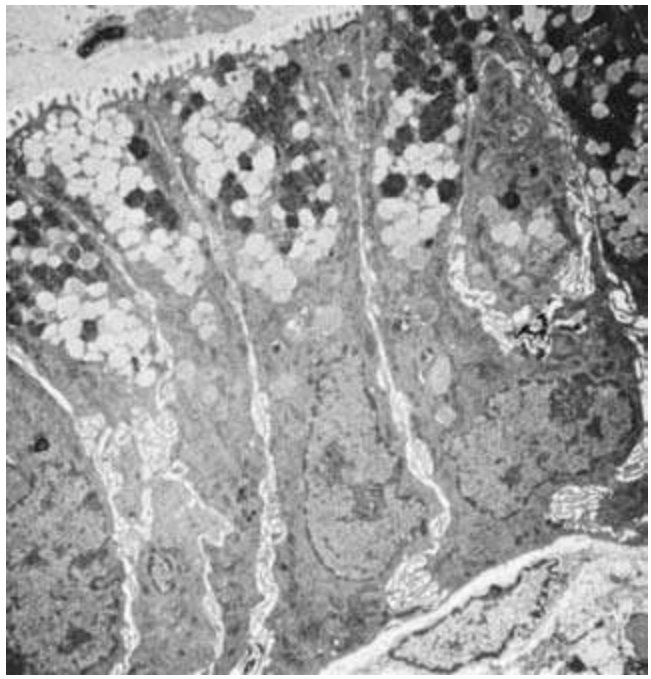


Figure 38.7 Electron micrograph (original magnification × 3200) of gastric mucous cells. Microvilli protrude from the apical surface, mucous granules fill the apical cytoplasm, and the nucleus lies near the base of the cell. From Misiewicz JJ, Forbes A, Price A, et al. Atlas of Clinical Gastroenterology. London: Wolfe, 1994, with permission from Elsevier.

Parietal (oxyntic) cells

Parietal cells, situated in the lower two-thirds of fundic-type glands, secrete hydrochloric acid and intrinsic factor (see Fig. 38.5). In glands of the fundus and body, these cells have a concentric nucleus and appear pyramidal, with the tubular apical end abutting the glandular lumen. Numerous mitochondria and tubulovesicles occupy much of the cytoplasm (Fig. 38.6a). When parietal cells are stimulated, tubulovesicles expand into microvillus-lined canaliculi that course through the cytoplasm and fuse with the apical membrane to connect with the lumen of the gastric gland (Fig. 38.6b) [4]. In unstimulated cells, H^+,K^+-ATPase, which mediates the electroneutral exchange of cytoplasmic hydronium ions for

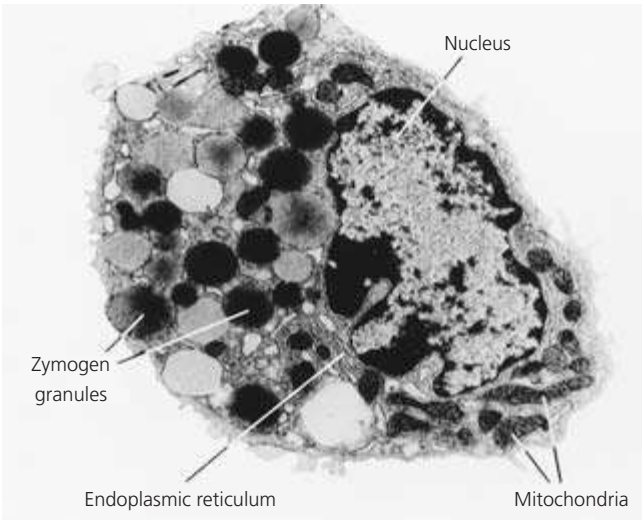


Figure 38.8 Electron micrograph of a dispersed chief cell from a guinea pig stomach (original magnification × 21 000). From Raufman JP. Gastric chief cells: receptors and signal transduction mechanisms. Gastroenterology 1992;102:699, with permission from Elsevier.

potassium in the canalicular lumen, is found in the tubulovesicle membranes, whereas in stimulated cells this enzyme is relocated to canalicular membranes. Translocation of the proton pump and expansion of the canaliculi are necessary for the acid-secreting function of parietal cells.

Chief cells

Chief cells, found predominantly in the lower one-third of gastric glands (see Fig. 38.5), secrete the proenzyme pepsinogen, which is hydrolyzed in an acid environment to the acid protease pepsin. Chief cells are polar, with apically located dense pepsinogen-containing secretory granules. The basal pole contains granular endoplasmic reticulum, the nucleus, and prominent Golgi complexes (Fig. 38.8). When chief cells are stimulated, the zymogen granules migrate to the apical pole, fuse with the cell membrane, and release their contents into the lumen of the gastric gland by exocytosis.

Endocrine cells

Enterochromaffin-like (ECL) cells are small, irregularly shaped cells scattered near parietal cells in fundic-type gastric glands. ECL cells, which do not connect with the lumen of the glands, are packed with histamine-containing granules. Histamine release from ECL cells regulates parietal cell function [5].

G cells, located in the midportions of antral glands and in duodenal crypts, are characterized by a broad base resting against the basement membrane that runs beneath the epithelium [6]. Dense secretory granules containing gastrin cluster near the base. A narrow apex extends to the lumen of the gland. In response to stimuli associated with eating, such as vagal stimulation, antral distention, and the lumenal presence of aromatic amino acids, G cells secrete the hormone gastrin.

D cells, located in fundic and antral glands, contain the inhibitory peptide somatostatin. These cells have neuron-like extensions that terminate near other gastric mucosal cells, particularly G cells, ECL cells, parietal cells, and chief cells. Inhibitory actions of D cells are mediated by paracrine actions of somatostatin released from these extensions [7].

Duodenum

In the pylorus, the mucosal pattern changes from pits and glands (typical of gastric mucosa) to villi and crypts (typical of intestinal mucosa) [8]. From the proximal to distal duodenum, the villi become progressively taller and thinner until a ratio of villus to crypt of 4:1 or 5:1, characteristic of jejunal mucosa, is achieved. A monolayer of epithelial cells, consisting of absorptive, mucous, Paneth, and endocrine cells, covers the villi and lines the crypts. The lamina propria, composed of loose connective tissue, contains lymphatics (lacteals), blood vessels, nerves, and smooth muscle fibers, as well as varying numbers of plasma and mast cells, lymphocytes, fibroblasts, macrophages, and eosinophils. The muscularis mucosae lies below and perpendicular to the crypts.

The submucosa of the duodenum provides a connective tissue framework for blood vessels, lymphatics, and nerves, and contains Brunner glands that secrete alkaline mucus. Brunner glands extend through the muscularis mucosae, occupy part of the mucosa, and empty into the crypts. The muscularis propria consists of a layer of inner circular fibers surrounded by a layer of fibers arranged longitudinally. The myenteric plexus is located between these muscle layers.

Cellular interactions

The juxtaposition of secretory cells and neurons in the stomach and duodenum is crucial for their physiological interactions that regulate digestion. For example, in the fundus and body, ECL cells, D cells, and cholinergic neurons must be sufficiently close to parietal cells to allow paracrine secretory products, such as histamine, somatostatin, and acetylcholine, to reach receptors on the acid-secreting cells [5]. Likewise, in the antrum, the interplay among neurons containing gastrin-releasing peptide and vasoactive intestinal polypeptide, histamine-containing mast cells, somatostatin-containing D cells, and gastrin-containing G cells requires physical proximity [7].

Cellular renewal

Gastric and duodenal mucosal epithelial cells undergo continuous, rapid renewal, a process involving proliferation, migration, differentiation, senescence, and loss of epithelial cells [9]. To replace surface mucous cells, nascent cells migrate upward from the base of the gastric pits or duodenal crypts to the mucosal surface or villous tip. In humans, migration of basal cells to the surface takes 2–6 days [10,11]. In contrast, parietal and chief cells are replaced by cells in the upper proliferative zone of gastric glands, possibly mucous

neck cells, a process that takes weeks to months. Senescent gastric or duodenal mucosal cells are sloughed into the lumen.

Aspirin, indomethacin (indometacin), and ethanol stimulate epithelial proliferation, perhaps by causing mild injury [12–17]. Epithelial proliferation is also increased by chronic inflammation, as in chronic superficial gastritis caused by *Helicobacter pylori* infection, gastric atrophy associated with pernicious anemia, and other hypergastrinemic states, such as Zollinger–Ellison syndrome [18]. In these conditions, the zone of proliferating cells is expanded. In contrast, corticosteroids and physiological stress, such as water immersion and restraint in rats, suppress epithelial proliferation [15]. Prostaglandins appear to delay senescence and loss of epithelial cells [19]. The resulting mucosal thickening contributes to the cytoprotective effects of prostaglandins.

Embryology of the stomach and duodenum

The primitive stomach and proximal duodenum derive from the foregut caudal to the developing lung, whereas the duodenum distal to the ampulla of Vater derives from the cephalic end of the midgut [20,21]. After the fourth week of intrauterine development, the primitive stomach rotates 90° clockwise around its longitudinal axis, ending with the left side facing anteriorly and the right side posteriorly [22]. This accounts for the course of the left vagus along the anterior wall and the right vagus along the posterior wall. The left wall of the stomach grows faster than the right, resulting in the size difference between the curvatures. The dorsal mesentery, which attaches the stomach to the posterior body wall, forms the omental bursa, or lesser peritoneal sac. The ventral mesentery attaches the stomach and duodenum to the liver.

As the stomach grows and rotates, the cephalic end moves leftward and downward, forming the fundus and cardia. The caudal end moves upward and to the right, forming the antrum and pylorus. Hence, the long axis of the stomach runs from above left to below right.

The duodenum also grows rapidly, forms a C-loop projecting ventrally, rotates to the right, and becomes retroperitoneal. Because of rapid epithelial proliferation during the fifth and sixth gestational weeks, the duodenal lumen is temporarily obliterated. The duodenal lumen recanalizes over the ensuing weeks as some cells degenerate.

The epithelium and glands of the stomach and duodenum derive from the embryonic endoderm. The connective tissue, muscle, and serosa derive from the mesoderm.

Congenital anomalies of the stomach

Atresia

Gastric atresia results in a blind end in the antrum or pylorus. Atresia may involve only the mucosa and submucosa, with normal muscle and serosa, or it may involve the entire gastroduodenal wall. Atresia probably results from failure of the antrum and pylorus to recanalize after transient occlusion by epithelium during embryogenesis. Complete atresia is familial, with autosomal recessive transmission [23]. Complete pyloric atresia may be associated with junctional epidermolysis bullosa.

Symptoms of gastric atresia in the newborn include persistent, nonbilious vomiting, upper abdominal distention, dehydration, and hypochloremic, hypokalemic metabolic alkalosis. Gastric rupture may occur. Diagnostic abdominal radiographs demonstrate gaseous gastric distention without intestinal gas.

Initial therapy includes nasogastric suction and fluid resuscitation. Surgical treatment of a short atretic segment involving only the mucosa requires resection of the membrane and pyloroplasty. Extensive atretic segments require resection with gastroduodenostomy. Gastrojejunostomy should be avoided because, in the absence of vagotomy, peptic stomal ulceration may occur. Careful exploration must exclude unsuspected distal atresias.

Mucosal membranes

Congenital mucosal membranes, which are probably related to atresias, occur in the antrum or pylorus and generally encircle, but do not occlude, the lumen. The membrane may contain either squamous or columnar epithelium. Peptic ulceration can result in acquired membranes. Vomiting may occur in infancy, but symptoms usually develop in late childhood or adulthood.

Plain abdominal radiographs usually appear normal, but barium studies reveal a band-like defect in the prepyloric antrum. Gastric emptying may be slowed, and the antrum between the mucosal membrane and the pylorus may simulate a second duodenal bulb. Endoscopic lysis of membranes may be effective, thereby obviating surgical repair [24].

Gastric duplication

Gastric duplications, containing mucosa, submucosa, and muscle, usually involve the greater curvature and share a common wall, but they are separate from the main lumen of the stomach. They sometimes connect with the gastrointestinal tract, usually the stomach or pancreas. Gastric duplications vary tremendously in size, occur more commonly in girls, and may coexist with other duplications of the digestive tract.

Patients usually present in the first year of life. Symptoms depend on size, location, and the presence or absence of communication with the gastrointestinal tract. Small children may present with vomiting, failure to thrive, and weight loss. Projectile vomiting may mimic pyloric stenosis. In older children, epigastric pain, abdominal fullness or mass, gastrointestinal bleeding, or symptoms of gastric obstruction may occur. Rarely, peritonitis results from cyst perforation, hemoptysis results from fistulation to the lung, or cancer

develops in the duplication [25]. Barium radiography, ultrasonography, computed tomography (CT) or magnetic resonance imaging may demonstrate the anomaly. Complete surgical excision may not be feasible if a large part of the gastric wall is involved. Partial excision, partial gastrectomy, and cyst gastrostomy are options.

Microgastria

Microgastria represents a rare failure of the stomach to enlarge after its development from the foregut. The structure becomes tubular, with a small capacity. Esophageal dilation may provide some storage area, but patients with microgastria generally present soon after birth with vomiting, diarrhea, aspiration pneumonia, malnutrition, and anemia. Microgastria is associated with developmental cardiac abnormalities, upper limb and spinal deformities, micrognathia, and asplenia [26,27]. Many patients die within weeks to months. Supportive treatment requires frequent, small, high-caloric feedings and parenteral alimentation. If the child survives, surgical formation of a jejunal reservoir should be attempted, but anastomotic ulcers may occur [28].

Gastric teratoma

These congenital tumors contain all three primary embryonic germ layers. They can occur anywhere, but are rare in the stomach. *Gastric teratomas* may cause gastrointestinal bleeding, obstruction, or an upper abdominal mass, and occur almost exclusively in male patients. Although usually diagnosed in children, gastric teratomas may not be discovered until adulthood.

Plain abdominal radiographs may show calcified teeth or bone in the tumor, and barium contrast or CT studies confirm a gastric mass. The large size of these teratomas may require total gastrectomy and formation of a jejunal pouch [29]. Gastric teratomas are not associated with other congenital anomalies, and the prognosis is good [30].

Gastric diverticula

Congenital *gastric diverticula* are rare, comprise all layers of the gastric wall, and arise primarily from the posterior gastric wall near the gastroesophageal junction. Gastric diverticula may also be found in the antrum and pylorus. They may be associated with ectopic pancreatic tissue, hiatus hernia, and diverticula in another part of the gastrointestinal tract. Ectopic pancreatic tissue, or pancreatic rests, may occur in the mucosa or submucosa of the duodenum or stomach and may mimic tumors, or they may ulcerate and bleed. Acquired diverticula may result from the scarring and dilation that accompany peptic ulceration, intestinal obstruction, cancer, or gastric surgery.

Gastric diverticula are commonly asymptomatic and are discovered as incidental findings on endoscopy or imaging studies [31]. These must be distinguished from acquired false diverticula, peptic ulcers, ulcerated neoplasms, and prominent folds [32]. Occasionally, epigastric or chest pain, heartburn, and vomiting may be reported. Severe symptoms or, rarely, bleeding and perforation require surgical excision and closure of the defect.

Ectopic gastric mucosa

Rests of *ectopic gastric mucosa* can be found in any part of the gastrointestinal tract and may cause ulceration, obstruction, or bleeding. In the upper esophagus, this condition has been reported in as many as 5% of people [33]. In the small intestine, colon, and rectum, ectopic gastric mucosa may form bleeding masses resembling polyps [34,35]. Meckel diverticula in the distal ileum may contain gastric mucosa with acid-secreting parietal cells that cause ulceration and bleeding in children and young adults [36].

Hypertrophic pyloric stenosis

Neonates

In *neonatal hypertrophic pyloric stenosis*, a congenital condition, muscular hypertrophy and mucosal edema of the pylorus result in gastric outlet obstruction. This disorder, the most common indication for surgery during the first 6 months of life, is more frequent in boys (1 case per 150 births) than in girls (1 case per 750 births) [37]. It has a genetic basis, clusters in families, and is more common in white populations than in other races. Although the cause is unknown, lack of nitric oxide synthase in pyloric tissue may contribute to pylorospasm [38,39]. Cases cluster in families and can be associated with other disorders, including maternal myasthenia gravis, fetal rubella, phenylketonuria, Hirschsprung disease, Turner syndrome, Smith–Lemli–Opitz syndrome, Cornelia de Lange Amsterdam dwarf syndrome, and esophageal atresia [37].

Although the symptoms of pyloric stenosis, particularly regurgitation and nonbilious projectile vomiting, typically do not occur until 3–4 weeks after birth, up to 20% of infants may become symptomatic earlier [37]. Vomiting may occur immediately after eating or may be delayed until the stomach is full. The vomitus is classically nonbilious, but may contain blood. Despite vomiting, the infant remains hungry until malnutrition and weakness cause interest in feeding to wane. Decreased delivery of food and fluid to the intestines results in constipation, oliguria, and failure to thrive.

The baby generally appears thin, weak, and dehydrated. Abdominal examination is likely to reveal gastric dilation, visible gastric peristalsis, and a palpable pyloric mass. Whereas gastric dilation and visible peristalsis are more evident during feeding, the olive-like pyloric mass is more likely to be palpable immediately after vomiting, when the abdominal wall is more flaccid.

Typically, upright radiographs demonstrate a large gastric air bubble with little or no intestinal air. In most cases, the detection of an "olive" on physical examination in babies with typical symptoms is sufficient for diagnosis [40]. If

Figure 38.9 Barium contrast upper gastrointestinal series shows the long, narrow, double channel of the pylorus (arrows) in a patient with hypertrophic pyloric stenosis.

further testing is needed, barium radiography appears to be the most cost-effective [41]. It may show a long, narrow pyloric channel, giving the appearance of a double channel, and a mass effect that indents the prepyloric antrum and duodenal bulb (Fig. 38.9).

Initial therapy, before surgery is considered, requires replacement of fluid and electrolytes, as well as correction of the alkalosis resulting from repeated vomiting. Definitive therapy is surgical. The preferred operation is Ramstedt pyloromyotomy: longitudinal division of the anterior pyloric muscle from the serosa to the submucosa. Mild vomiting may persist, but symptoms usually disappear within several days. After surgical correction of the defect, growth and development are normal, and long-term results are excellent.

Adults

Rarely, congenital hypertrophic pyloric stenosis may not present until adulthood. In adults, however, most cases of pyloric stenosis are acquired and result from chronic peptic ulcer disease, severe gastritis, or cancer involving the pyloric area.

In adults with congenital pyloric stenosis, common symptoms, usually dating from infancy, include early satiety, nausea, vomiting, epigastric pain, weight loss, and anorexia. The diagnosis is generally delayed, in part because physical findings observed in infants, such as a pyloric mass, are not present. Imaging may reveal gastric dilation, delayed emptying, and a long, narrow pyloric channel. Endoscopic dilation or surgical pyloromyotomy may be therapeutic, but, to exclude a small focus of cancer, pyloric resection with gastroduodenostomy may be preferable [42].

Congenital anomalies of the duodenum

Atresias, stenosis, and membranes

The duodenal lumen is obliterated by epithelium during the fifth to sixth weeks of development. *Duodenal atresia, stenosis and membranes* likely result from subsequent failure of the lumen to recanalize. This rare cause of duodenal obstruction occurs distal to the ampulla in 80% of cases, has no sexual or racial predilection, and is frequently associated with other congenital anomalies, including Down syndrome, intestinal malrotation, tracheoesophageal fistula, annular pancreas, cardiac and renal anomalies, and anorectal malformations.

In newborns with compete obstruction caused by a duodenal membrane or atresia, vomiting develops within hours to days after birth. The vomitus is nonbilious if obstruction is proximal to the ampulla and generally indistinguishable from that associated with pyloric stenosis. With partial obstruction, symptoms may be delayed and less severe. Rarely, duodenal membranes that persist or develop in adulthood cause upper abdominal distention, vomiting, and weight loss.

When obstruction is distal to the duodenal bulb, plain abdominal radiography or ultrasonography shows a double bubble, caused by a distended stomach and duodenal bulb (Fig. 38.10) [43]. The absence of distal intestinal gas suggests complete obstruction.

Initial treatment consists of nasogastric suction, with fluid and electrolyte repletion. Definitive treatment is surgical, with gastrojejunostomy or duodenojejunostomy, depending on the level of obstruction [44]. At surgery, other correctable anomalies should be sought, as the prognosis usually depends on the outcome of associated anomalies. In adults, endoscopic lysis of duodenal membranes may be successful [45,46].

Annular pancreas

Annular pancreas is a rare condition that affects the sexes equally and may result in complete or partial obstruction of the second part of the duodenum [47]. The cause is unknown but probably involves incomplete migration of the ventral pancreatic bud during embryogenesis. This results in a pancreatic remnant within the duodenal wall or pancreatic encirclement of the duodenum. Annular pancreas is associated with intestinal malrotation, Down syndrome, facial cleft, imperforate anus, and cardiac and tracheoesophageal malformations [48].

Neonates present with intractable vomiting. Children or young adults have abdominal pain and distention with less

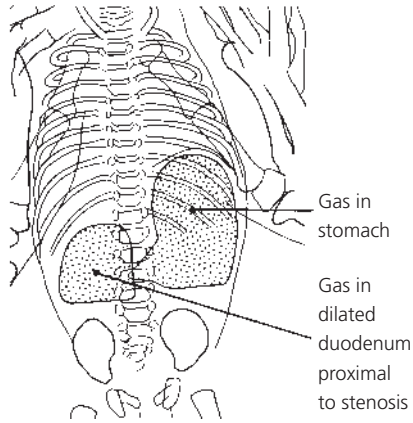


Figure 38.10 Plain abdominal radiograph in an infant with duodenal atresia shows the double bubble sign. The stomach and the proximal duodenum are distended with air. From Misiewicz JJ, Forbes A, Price A, et al. Atlas of Clinical Gastroenterology. London: Wolfe, 1994, with permission from Elsevier.

dramatic vomiting. The clinical presentation and plain abdominal radiographs are similar to those for duodenal obstruction caused by duodenal atresia, including a double bubble sign. Imaging and endoscopy confirm the level of duodenal obstruction and may establish the diagnosis (Fig. 38.11). In adults, endoscopic retrograde cholangiopancreatography can establish the diagnosis if the duct from the annular pancreas joins the main pancreatic duct. Endoscopic ultrasonography can also diagnose annular pancreas by identifying bands of pancreatic tissue encircling the duodenum [49]. Impaired pancreatic ductal flow may result in acute or chronic pancreatitis.

Treatment includes nasogastric decompression and correction of fluid and electrolyte disturbances. Resection or division of the annular pancreatic segment may cause pancreatitis or pancreatic fistula and should not be performed. Definitive therapy requires duodenostomy or duodenojejunostomy. Laparoscopic gastrojejunostomy has also been reported to treat annular pancreas in adults [50]. Surgical results are good, and long-term prognosis depends on the outcomes of associated anomalies.

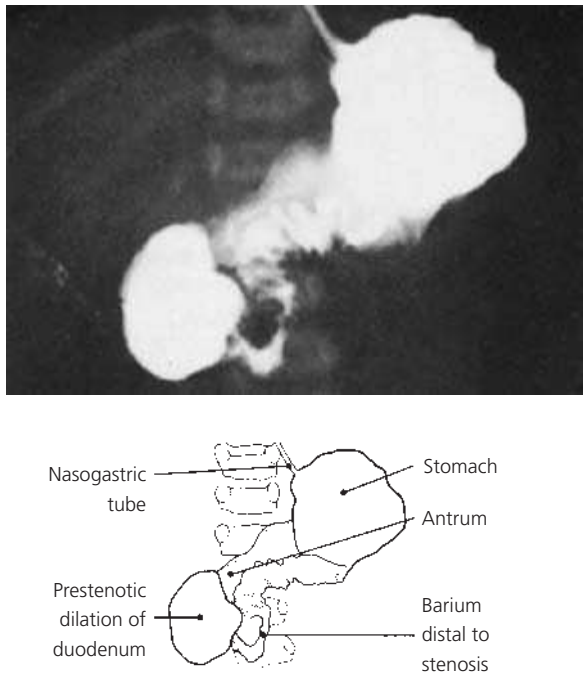


Figure 38.11 Barium contrast upper gastrointestinal series shows partial occlusion of the second part of the duodenum, consistent with annular pancreas, duodenal atresia, or other causes of duodenal obstruction. From Misiewicz JJ, Forbes A, Price A, et al. Atlas of Clinical Gastroenterology. London: Wolfe, 1994, with permission from Elsevier.

Duplication

Duplications are cystic or tubular malformations that contain all layers of the normal duodenum and which may communicate with the main lumen. Intestinal obstruction may occur secondary to compression by a mass effect with resulting symptoms of upper abdominal distention, early satiety, and vomiting. Duodenal duplications may ulcerate, bleed and, rarely, perforate into the head of the pancreas causing pancreatitis [51,52]. Diagnosis can be established by ultrasonography, contrast radiography, or CT. Treatment is surgical, and the approach depends on the relationship of the cyst to the biliary and pancreatic ducts, as well as the vasculature. If total resection is not possible, cystenterostomy may suffice.

Malrotation

In children, *intestinal malrotation* is the most common cause of duodenal obstruction [53]. This may occur in utero if the

duodenojejunal loop does not rotate normally around the SMA and the duodenum fails to complete its rotation. Consequently, the ligament of Treitz may be situated to the right, rather than to the left, of midline, thereby kinking the duodenum. If the cecum fails to rotate completely into the right lower abdomen or when the hepatic flexure lies medial to the duodenum, mesenteric bands may compress the second or third parts of the duodenum. Other developmental anomalies, such as duodenal atresia, annular pancreas, or Hirschsprung disease, are present in 30%–60% of patients with intestinal malrotation.

Patients with malrotation present within the first few weeks of life with bilious vomiting and abdominal distention. Intestinal peristalsis may be visible on the abdominal wall. Occasionally, lax mesentery results in intestinal or cecal volvulus. If the vasculature is twisted, intestinal ischemia can result in bleeding, perforation, peritonitis, and sepsis. A few patients with malrotation present later in life with weight loss, hypoproteinemia, chylous ascites, increased susceptibility to infection, intermittent postprandial vomiting, and abdominal distention.

Plain abdominal radiographs demonstrate a large gastric air bubble and distended duodenum proximal to the obstruction. An upper gastrointestinal series can confirm the obstruction and, if barium is able to pass this area, may identify the type and extent of malrotation. Ultrasonography may reveal obstruction or volvulus caused by malrotation in utero.

Treatment is surgical: division of obstructing bands, resection of infarcted bowel, and fixation of the cecum to prevent further episodes of volvulus. When extensive bowel resection is required, mortality approaches 20%, and survivors may develop short bowel syndrome with profound malabsorption requiring long-term parenteral alimentation.

Superior mesenteric artery syndrome

Rarely, the SMA obstructs the third portion of the duodenum by compressing it against fixed retroperitoneal structures, particularly the spine. Although *SMA syndrome* has been attributed to an acute angle between this vessel and the aorta, thereby trapping the duodenum, the exact cause is unknown. This syndrome has also been associated with rapid childhood growth, major weight loss in adults, immobilization in a cast that increases lordosis and accentuates the angle of the SMA with the aorta, abdominal surgery, and inflammatory diseases of the abdomen [54,55].

Symptoms are nonspecific, and documenting SMA compression of the duodenum as the cause is difficult. Symptoms, including episodic epigastric pain associated with vomiting, may be acute or chronic, sometimes lasting many years.

In adults, plain abdominal radiographs are usually unremarkable, but in children a double bubble sign may be seen (see Fig. 38.10). Upper gastrointestinal contrast studies may show dilation behind an abrupt cutoff in the third portion of the duodenum (see Fig. 38.11). On abdominal angiography, lateral views may show the narrowed angle of the SMA with the aorta. It is important to exclude other causes of abdominal pain before making this diagnosis.

Medical therapy includes small feedings or a liquid diet. Patients may benefit from lying prone or on their left side postprandially. In some cases, weight gain or removal of a body cast improves symptoms. Laparoscopic lysis of the ligament of Treitz has been reported to be beneficial in a few cases [56]. Duodenojejunostomy is required in refractory cases.

Preduodenal portal vein

A *preduodenal portal vein*, sometimes associated with a preduodenal common bile duct, can obstruct the duodenum. Only about 50 cases have been reported. In early embryonic life, both retroduodenal and preduodenal veins drain the primitive gut. The preduodenal vein usually atrophies, leaving a normal retroduodenal portal vein. When the retroduodenal branch atrophies, the preduodenal vein remains patent and may compress the duodenum. A preduodenal vein is

Figure 38.12 Barium contrast upper gastrointestinal series shows two large diverticula arising from the medial border of the descending duodenum. From Misiewicz JJ, Forbes A, Price A, et al. Atlas of Clinical Gastroenterology. London: Wolfe, 1994, with permission from Elsevier.

associated with other congenital malformations, such as duodenal stenosis or atresia, annular pancreas, intestinal malrotation, and biliary tract abnormalities [57].

Duodenal diverticula

Congenital *duodenal diverticula* occur within a few centimeters of the ampulla of Vater (Fig. 38.12) [58]. The ampulla may empty directly into the diverticulum. These diverticula are usually asymptomatic, incidental findings on imaging or endoscopy. Rarely, duodenal diverticula obstruct the common bile duct by external compression on the duct or distortion of its entry into the duodenum. Most cases of biliary obstruction associated with a duodenal diverticulum result from another cause, such as an obstructing common bile duct stone or neoplasm. A symptomatic diverticulum is best treated by choledochoduodenostomy or jejunostomy with Roux-en-Y anastomosis, rather than excision of the diverticulum.

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B STOMACH

39 Disorders of gastric emptying

Henry P. Parkman, Frank K. Friedenberg, Robert S. Fisher

Delayed gastric emptying (gastroparesis), 903
Disorders with rapid gastric emptying, 923
Functional dyspepsia, 925
Summary, 928

Gastric motility disorders include delayed gastric emptying (gastroparesis), rapid gastric emptying (as seen in dumping syndrome), and disorders with motor and sensory abnormalities (e.g., functional dyspepsia). Each disorder can present with a spectrum of symptoms that may be mild to severe and incapacitating. Evaluation of patients with possible gastric motility disorders may include gastric emptying scintigraphy, antroduodenal manometry, electrogastrography (EGG), and possibly tests of gastric accommodation or compliance (satiety testing). Evaluation may help to understand the underlying pathophysiology and guide treatment. Management of these patients requires an understanding of the pathophysiology, clinical tests, and treatment options.

Delayed gastric emptying (gastroparesis)

Symptoms and clinical presentation

Gastroparesis is a chronic motility disorder of the stomach characterized by symptoms of delayed gastric emptying in the absence of mechanical obstruction. The clinical presentation of gastroparesis may vary. Symptoms include nausea, vomiting, early satiety, bloating, and upper abdominal discomfort. Late postprandial vomiting of undigested food is typical. In 146 patients with gastroparesis, nausea was present in 92%, vomiting in 84%, abdominal bloating in 75%, early satiety in 60%, and abdominal pain in 46% [1]. These symptoms, however, are nonspecific and may mimic structural disorders such as ulcer disease, partial gastric or small bowel obstruction, gastric cancer, and gallbladder or pancreatic disorders [2]. At times, vomiting can be difficult to distinguish from the regurgitation of gastroesophageal reflux disease (GERD) or rumination syndrome.

There is an overlap of symptoms of gastroparesis and functional dyspepsia. Abdominal pain or discomfort is present to varying degrees in patients with gastroparesis, but it is not usually the predominant symptom. Abdominal pain is generally described as burning, vague, or crampy [3]. In diabetic gastroparesis, abdominal pain may represent a visceral equivalent of autonomic neuropathy. Pain in gastroparesis responds poorly to prokinetic agents and gastric electrical stimulation [3]. Visceral hypersensitivity to gastric distention has been suggested as a cause of pain in gastroparesis, analogous to that described in functional dyspepsia [4,5].

Symptoms that suggest delayed gastric emptying are primarily postprandial fullness, nausea, and vomiting [6,7]. In patients with diabetes, symptoms that have been associated with delayed gastric emptying are abdominal bloating/fullness and upper abdominal pain [8,9]. Some studies, however, have shown a poor correlation between gastric emptying and the severity of gastric symptoms [8–10].

Symptoms of gastroparesis may be quantified by a validated symptom questionnaire, the Gastroparesis Cardinal Symptom Index (GCSI) [11,12]. The GCSI is based on three subscales (postprandial fullness/early satiety, nausea/vomiting, and bloating) and represents a subset of the longer Patient Assessment of Upper Gastrointestinal Disorders–Symptoms (PAGI-SYM), which quantifies symptoms of gastroparesis, dyspepsia, and GERD [13].

Most gastroparetic patients are women. In the series of Soykan et al. [1], 82% of patients were female. Female gender and the luteal phase of the menstrual cycle have been associated with slower gastric emptying [14,15], thought to result from reduced gastric muscle contractility caused by female reproductive hormones, primarily progesterone. In the study by Stanghellini et al. [6] of functional dyspepsia, female gender was an independent risk factor for delayed gastric emptying.

Evaluation of patients with suspected gastroparesis

Gastroparesis is diagnosed by demonstrating delayed gastric emptying in a symptomatic patient after exclusion of mechanical obstruction by endoscopy or upper gastrointestinal (GI) series (Table 39.1).

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Table 39.1 Evaluation of patients suspected to have gastroparesis

1 Initial investigation
a History and physical examination
b Blood tests
  i Complete blood count
  ii Complete metabolic profile including glucose, potassium, creatinine, total protein, albumin, calcium
  iii Amylase if abdominal pain is a significant symptom
  iv Pregnancy test if appropriate
c Abdominal obstruction series if vomiting or pain is acute or severe
2 Evaluate for organic disorders
a Upper endoscopy to evaluate for mechanical obstruction or mucosal lesions (alternative: barium upper gastrointestinal series, often with small bowel follow-through)
b Biliary ultrasound if abdominal pain is a significant symptom
3 Evaluate for delayed gastric emptying
a Solid phase gastric emptying test
b Screen for secondary causes of gastroparesis
  i Thyroid function tests
  ii Antinuclear antibody
  iii Glycosylated hemoglobin
4 Treatment trial with prokinetic agent or antiemetic agent
5 If no clinical response, consider further investigation
a Electrogastrography
b Antroduodenal manometry
c Small bowel evaluation with enteroclysis

The first step in the evaluation is taking a careful history. Understanding the patient's symptoms should lead to a clinical suspicion of gastroparesis. Typical symptoms in a young female patient may suggest idiopathic gastroparesis, just as typical symptoms in a patient with long-standing insulin-dependent diabetes should suggest diabetic gastroparesis. After abdominal surgery, patients can develop delayed gastric emptying, especially if the vagus nerve has been cut or damaged. On physical examination, a succussion splash, detected by auscultation over the epigastrium while moving the patient from side to side, indicates excessive fluid in the stomach from gastroparesis or mechanical gastric outlet obstruction [16]. Dehydration and weight loss suggest chronic and severe symptoms.

The second step in evaluating gastroparesis is to exclude mechanical obstruction or ulcers by performing an upper endoscopy or upper GI series. Endoscopy is more sensitive for detection of mucosal lesions [16]. Mechanical gastric outlet obstruction can be caused by pyloric stenosis, an active duodenal, pyloric channel, or prepyloric ulcer, scarring from previous ulcers, or neoplasia of the stomach. Gastroparesis is also suggested on endoscopy by the presence of food particles in the stomach after an overnight fast. In severe gastroparesis, bezoars may occur.

Double-contrast techniques have increased the sensitivity of radiological studies. When an upper GI series is ordered, a small bowel follow-through (SBFT) can look for small bowel lesions. The SBFT is accurate for detection of high-grade small bowel obstruction and usually provides an adequate assessment of the terminal ileum. However, it may fail to detect low-grade obstruction and smaller mucosal lesions. A SBFT may occasionally suggest superior mesenteric artery syndrome as a possible cause. Enteroclysis (small bowel enema) can be obtained after placement of a nasoduodenal or oroduodenal tube. Compared with SBFT, enteroclysis is more accurate in detecting small intestinal mucosal lesions, mild to intermediate grades of obstruction, and small bowel cancers [17]. Computed tomographic scanning with oral and intravenous contrast may also be useful for detection and localization of intestinal obstruction.

The third step is to obtain a gastric emptying scintigraphy using a radiolabeled solid meal. Patients should discontinue medications that may affect gastric emptying for 48 h before this test (Table 39.2). In general, the main medications that delay gastric emptying are narcotic analgesic agents and anticholinergic agents. The main medications that accelerate gastric emptying are the prokinetic agents used to treat

Table 39.2 Medications that affect gastric emptying

Medications that delay gastric emptying
Opiate analgesics
Anticholinergic agents
Tricyclic antidepressants
Calcium channel blockers
Progesterone
Octreotide
Pramlitide
Proton pump inhibitors
Histamine H_2 receptor antagonists
Interferon-α
L-Dopa
Fiber
Sucralfate
Aluminum hydroxide antacids
β-Adrenergic receptor agonists
Glucagon
Calcitonin
Dexfenfluramine
Diphenhydramine
Alcohol
Tobacco/nicotine
Tetrahydrocannabinol

Medications that accelerate gastric emptying
Prokinetic agents
  Metoclopramide
  Erythromycin and related antibiotics
  Cisapride
  Domperidone
β-Adrenergic receptor antagonists

gastroparesis. A delayed gastric emptying test suggests, but does not prove, that the symptoms are caused by gastroparesis. If gastric emptying is normal, other causes for symptoms should be sought. However, a gastric motility disorder should not be totally dismissed despite a normal gastric emptying test because localized motor dysfunction, such as impaired fundic relaxation or gastric dysrhythmias, may be associated with symptoms and normal gastric emptying [18].

Evaluation of a patient should not stop once delayed gastric emptying is demonstrated; an underlying cause should be sought. Other causes of gastroparesis besides diabetes and previous gastric surgery include smooth muscle, neurological, metabolic or endocrine disorders. Thyroid function tests should be obtained to evaluate for hypothyroidism. A glycosylated hemoglobin level evaluates long-term glycemic control; poor glucose control can worsen gastric emptying. Idiopathic gastroparesis is diagnosed after all other causes are excluded.

Evaluation of gastric emptying, motor function, myoelectric activity, and radiographic contrast techniques

The upper GI series is primarily helpful in detecting mucosal lesions and mechanical outlet obstruction. It is an insensitive method for measuring gastric emptying because it is difficult to quantitate, and barium is not a "physiological" meal (Table 39.3) [19]. Gastric retention may be suggested by poor emptying of barium from the stomach, gastric dilation, and the presence of retained food or a gastric bezoar. Little or no emptying of barium at 30 min and minimal barium remaining in the stomach at 6 h are suggestive of delayed gastric emptying [20].

Assessment of gastric emptying

Radionuclide gastric emptying scintigraphy

Gastric emptying scintigraphy remains the best current test for measuring gastric emptying because it is sensitive, quantitative, and physiological. It is used to confirm the presence of gastric stasis after excluding structural or mucosal disorders. However, the usefulness of emptying tests in directing therapy and predicting response is debated [21,22].

Measurement of gastric emptying of solids is more sensitive than measurement of gastric emptying of liquids for detection of symptomatic gastroparesis because emptying of liquids is often preserved until the disorder is advanced. In patients who have undergone gastric surgery, a dual solid and liquid emptying test may be indicated because symptoms may result from slow solid emptying or rapid liquid emptying.

The technetium 99m sulfur colloid radiolabeled meal consisting of the equivalent of two large eggs (Eggbeaters) plus two slices of bread with jam and water is suggested as the standardized test meal. Imaging is performed in the anterior and posterior projections at four time points (0, 1, 2, and 4 h)

Table 39.3 Tests to assess gastric motor and myoelectric function

Tests assessing gastric emptying	Advantages	Disadvantages
Upper gastrointestinal barium radiographic study	Assesses for mucosal lesions	Nonphysiological
Scintigraphy	Gold standard, noninvasive, solid and liquid phase	Radiation exposure minimum to moderate
Breath tests using ^{13}C	Noninvasive	Needs normal small intestinal absorption, liver metabolism, pulmonary excretion
Ultrasonography for serial changes in antral area	Noninvasive	Requires expertise for imaging and interpretation, primarily measures liquid emptying
Magnetic resonance imaging	Noninvasive	Expensive, time consuming, needs specialized centers and software
Tests assessing gastric contractile activity	**Advantages**	**Disadvantages**
Antroduodenal manometry	Assesses contractility in fasting and postprandial periods	Invasive, requires expertise to perform and interpret
Gastric barostat	Measures proximal stomach relaxation and contraction	Invasive, research technique
Tests assessing gastric myoelectrical activity	**Advantages**	**Disadvantages**
Electrogastrography	Noninvasive	Movement artifact may make recording difficult to interpret

Adapted from Hasler WL et al. [16], with permission from Elsevier.

[23]. The radiolabel needs to be cooked into the egg white so that the radioisotope binds to the solid phase, thus preventing elution of the radiotracer into the liquid phase with an erroneous measurement of the faster liquid phase of gastric emptying [24].

Imaging for gastric emptying up to 4 h increases the detection of delayed gastric emptying and is now suggested as the standard in all tests to obtain reliable results for the detection of gastroparesis [25,26]. When gastric scintigraphy is performed for shorter durations, the test is less reliable because of large variations in normal gastric emptying.

The simplest approach for interpreting gastric emptying studies is to report the percentage of retention at defined times after meal ingestion (usually 2 and 4 h) (Fig. 39.1).

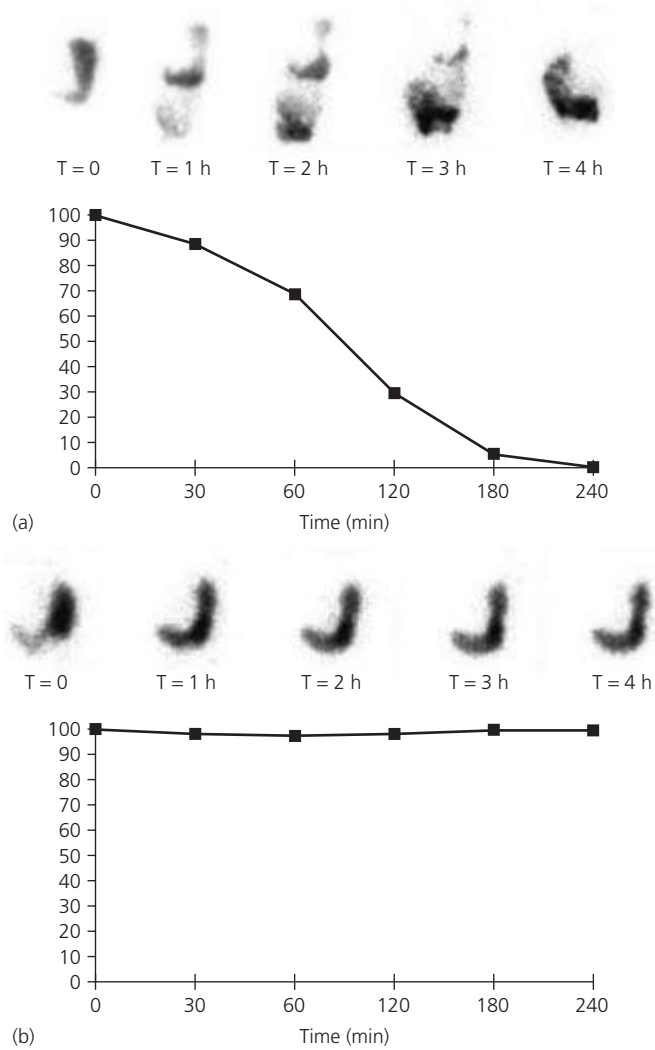

Figure 39.1 Gastric emptying scintigraphy using a technetium 99m-labeled egg sandwich. The percentages of gastric retention are shown at 0, 30, 60, 120, 180, and 240 min after meal ingestion. **(a)** Normal gastric emptying with only 30% retention at 2 h after meal ingestion (normal < 50%) and complete emptying at 4 h (normal < 10%). **(b)** Markedly delayed gastric emptying with little emptying at 4 h.

Curve-fitting techniques can calculate the half-emptying time, the time for half of the stomach contents to have emptied from the stomach. Extrapolation of the emptying curve to predict the half-emptying time may be unreliable if the emptying has not reached 50% during the actual imaging [27]. Other parameters of potential use are the lag phase for solids, which represents the time required for trituration of solid food into 1- to 2-mm particles that can then empty through the pylorus [28].

Advances in scintigraphy may provide information on fundic and antral abnormalities. Regional gastric emptying can assess intragastric meal distribution and transit from the proximal to distal portions of the stomach. Proximal retention may be seen in GERD, distal retention in functional dyspepsia, and global retention in gastroparesis (Fig. 39.2). Dynamic antral scintigraphy with frequent 1-s imaging can evaluate antral wall contractility [19].

Blood glucose, smoking, and gender are factors that affect gastric emptying. Premenopausal women empty the stomach more slowly than men [14,15]. Some investigators have advocated separate reference values for premenopausal women [29]. Marked hyperglycemia with serum glucose levels of 288 mg/dL or more significantly delays gastric emptying in diabetic patients when compared with euglycemia [30]. Blood glucose should be under reasonable control on the day of an emptying test to obtain a reliable measurement of gastric emptying. It is recommended that serum glucose be measured prior to the study, noted in the report, and if the blood glucose is greater than 275 mg/dL on the morning of the test, the glucose should be lowered with insulin to less than 275 mg/dL or the study should be rescheduled for another day when the blood glucose is under better control. Smoking during the test can delay gastric emptying.

SmartPill pH and pressure capsule to assess gastric emptying

The SmartPill is an ingestible capsule that measures pH, pressure, and temperature using miniaturized wireless sensor technology. The SmartPill capsule is swallowed by the patient and acidity and pressures are recorded as it travels through the gastrointestinal tract. From these measurements, gastric emptying and total gastrointestinal tract transit time can be obtained. In addition, the SmartPill capsule will characterize pressure patterns and provide motility indices for the stomach, small intestine, and colon. The gastric residence time of the SmartPill has a high correlation (85%) with the T-90% of gastric emptying scintigraphy suggesting that the gastric residence time of the SmartPill represents a time near the end of the emptying of a solid meal. The gastric residence time of the SmartPill is able to differentiate normal gastric emptying from delayed gastric emptying similarly to scintigraphy [31]. A SmartPill gastric residence cutoff time of 5 h was best in distinguishing between subjects with delayed and those with normal gastric emptying based on scintigraphy

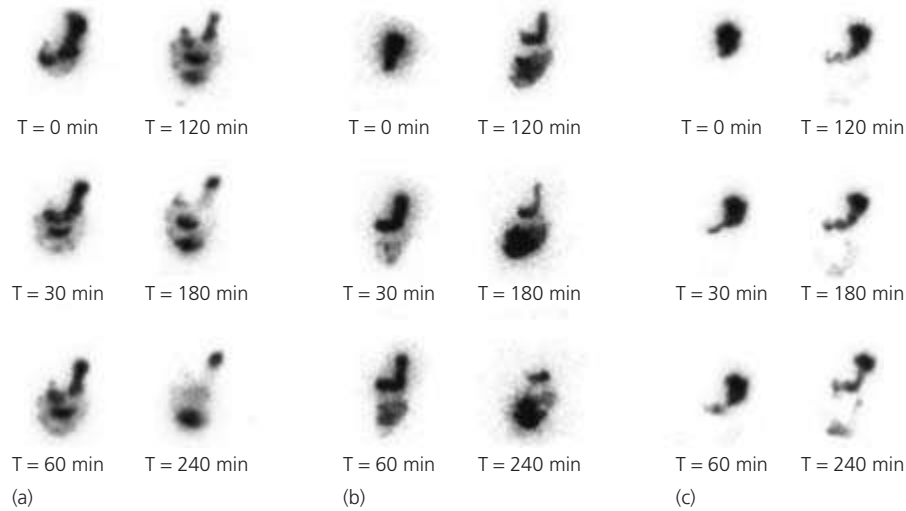

Figure 39.2 Regional gastric emptying abnormalities in gastroesophageal reflux disease, functional dyspepsia, and gastroparesis. **(a)** In patients with gastroesophageal reflux disease, there is retention of the radioactivity in the proximal portion of the stomach. **(b)** In patients with functional dyspepsia, there is retention of radioactivity in the distal portion of the stomach. **(c)** In patients with gastroparesis, there is a global retention throughout the whole stomach.

on the day of the test (83% sensitivity, 83% specificity) [32]. However, the utility of the SmartPill capsule in clinical settings remains to be determined.

There have been several reports of capsule endoscopy used to measure gastric emptying; this technique is able to visualize the capsule emptying from the stomach and thus measure gastric emptying of the capsule from the stomach. These studies have been limited by the need to perform this technique in the fasting stomach. How this relates to a physiological meal has not been determined.

Breath tests for gastric emptying

Breath testing can be used to measure gastric emptying. The nonradioactive isotope carbon 13 is used to label octanoate, a medium-chain triglyceride, which can be bound into a solid meal [33–35]. Studies have also been carried out using ^{13}C-labeled proteinaceous algae (*Spirulina*) [24]. After ingestion and emptying from the stomach, octanoate is absorbed by the small intestine. The octanoate is metabolized to carbon dioxide in the liver and the ^{13}C is excreted from the lungs during respiration. The rate-limiting step for excretion of ^{13}C is gastric emptying. By measuring ^{13}C in breath samples, gastric emptying can be indirectly determined. The octanoate breath test is reproducible, correlates with gastric emptying scintigraphy, and offers promise as a clinical test for gastric emptying. It does not use ionizing radiation, it can be used to study patients without access to gamma camera facilities in community doctors' offices or at the bedside, and breath samples can be shipped to a laboratory for analysis. The octanoate breath test has been used in clinical research and pharmaceutical studies. Validation of this test in patients with emphysema, cirrhosis, celiac sprue, and pancreatic insufficiency is needed, because it is not clear whether substrate metabolism in these disorders may also be a rate limiting step for $^{13}CO_2$ excretion.

Ultrasonography

Transabdominal ultrasonography can measure several parameters of gastric motility. Serial changes in antral cross-sectional area are measured as an index of gastric emptying; emptying is considered complete when the antral area returns to the fasting baseline [36]. Duplex sonography may be used to evaluate transpyloric flow of liquid gastric contents. Ultrasonography has also been employed to measure accommodation in the proximal and distal stomach [24]. Unfortunately, ultrasonography for gastric emptying is operator dependent and generally measures liquid emptying only. The test is suboptimal in obese people.

Assessment of gastric myoelectric activity with electrogastrography

EGG is the recording of gastric myoelectric activity using cutaneous electrodes on the abdomen overlying the stomach [37]. The dominant frequency of the EGG corresponds to the gastric electrical rhythm or frequency of the gastric slow wave. The normal gastric slow wave frequency is approximately 3 cycles/min (Fig. 39.3). Meal ingestion increases the amplitude of the EGG signal by increasing gastric electrical activity and contractility or by distention of the stomach. EGG measures the frequency and regularity of gastric myoelectric activity, detects abnormal rhythms of gastric myoelectric activity, and assesses the amplitude or power increase after a meal [27].

Gastric dysrhythmias (tachygastria, bradygastria) and decreased postprandial amplitude (or power) of the EGG have been described in idiopathic and diabetic gastroparesis [38] (Fig. 39.3). Gastric myoelectric abnormalities have also been described in patients with unexplained nausea and vomiting, motion sickness, and nausea and vomiting of pregnancy [27]. Studies have suggested a good correlation between delayed gastric emptying measured by scintigraphy

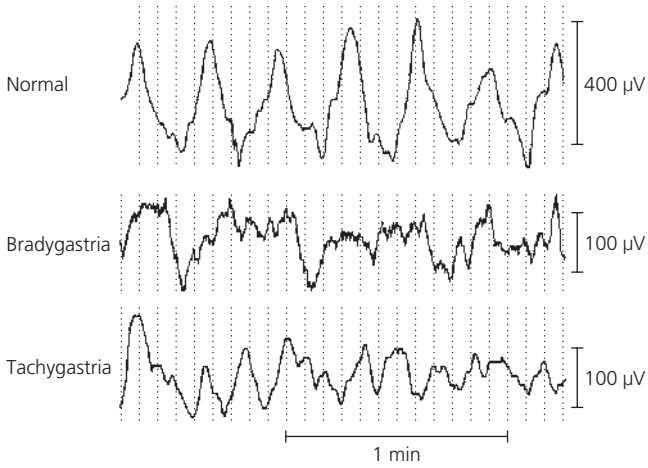

Figure 39.3 Gastric dysrhythmias recorded with electrogastrography (EGG). Representative raw EGG tracings showing normal 3 cycles/min rhythm, bradygastria, and tachygastria. From Parkman HP et al. [42], with permission from Blackwell Publishing.

and an abnormal EGG, particularly postprandial rhythm and amplitude abnormalities [38]. An abnormal EGG is present in 75% of patients with gastroparesis compared with only 25% of symptomatic patients with normal gastric emptying. Gastric dysrhythmias have been suggested to be better predictors of symptoms than delayed gastric emptying [39]. In diabetic patients, hyperglycemia may itself provoke dysrhythmias, primarily tachygastrias [40].

EGG is used to demonstrate gastric myoelectric abnormalities in patients with unexplained nausea and vomiting with abdominal discomfort or functional dyspepsia. The EGG is generally used as an adjunct to gastric emptying scintigraphy, as part of a comprehensive evaluation of patients with refractory symptoms suggestive of an upper GI motility disorder [27,41,42].

Antroduodenal manometry

Antroduodenal manometry provides information about the coordination of gastric and duodenal motor function in both fasting and postprandial periods [27]. The three main indications for antroduodenal manometry are to evaluate: (1) unexplained nausea and vomiting; (2) the cause of gastric or small bowel stasis (e.g., neuropathic or myopathic disorders); and (3) suspected chronic intestinal pseudoobstruction when the diagnosis is unclear [27].

Decreased antral contractility and phase III migrating motor complexes (MMCs) originating in the small intestine rather than in the stomach can be seen in gastroparesis. Occasionally, pylorospasm or irregular bursts of small intestinal contractions, which increase outflow resistance, can be observed. With an accurate stationary recording, a reduced postprandial distal antral motility index is correlated with impaired gastric emptying of solids [28]. An average of less than one antral contraction per minute postprandially has been suggested as a simple estimate of significant hypomotility [43]. Ambulatory studies, performed over 24 h using solid-state transducers, allow correlation of symptoms with abnormal motility but catheter migration in the stomach prevents quantitation of antral contractility.

Antroduodenal manometry may differentiate between neuropathic and myopathic motility disorders, and it may suggest unexpected small bowel obstruction or rumination syndrome [27,44]. Myopathic disorders, such as scleroderma or amyloidosis, have low-amplitude contractions with normal propagation. Neuropathic disorders have normal amplitude but abnormal propagative contractions, seen readily in the phase III MMC, such as bursts and sustained uncoordinated pressure activity, and a failure of a meal to induce the fed-type pattern. Occult mechanical obstruction of the small intestine is suggested by two types of pattern: postprandial clustered contractions for more than 30 min separated by quiescence, and simultaneous prolonged (> 8 s) or summated contractions suggesting a common cavity phenomenon from a dilated segment of intestine [45]. Antroduodenal manometry may demonstrate a characteristic pattern of rumination, with an increase in intraabdominal pressure at all levels of the upper gut (R waves), especially postprandially, indicating the act of rumination [46].

In pediatric studies, the absence of MMCs is an indicator of a poor response to prokinetic agents [47]. Some investigators perform the study with infusions of erythromycin or octreotide to predict the patient's response to treatment with these agents [48].

Gastroparesis and disorders with delayed gastric emptying

Gastroparesis occurs in many clinical settings (Table 39.4); the most common are diabetes mellitus, sequelae of gastric surgery, and idiopathic. In a series of 146 patients, gastroparesis was classified as being idiopathic in 36%, diabetic in 29%, and postsurgical in 13% [1].

Diabetic gastroparesis

Gastroparesis is a well-known complication of *diabetes,* most commonly associated with long-standing, insulin-dependent diabetes mellitus with other complications of retinopathy, nephropathy, and peripheral neuropathy. Frequently, it occurs with autonomic neuropathy as identified by orthostatic hypotension. Longitudinal studies suggest that delayed gastric emptying is present in 30%–40% of patients with long-standing type I diabetes and in 10%–20% of type II diabetics [43]. The prevalence of GI symptoms in insulin-dependent diabetes mellitus is greater in patients with poor glycemic control [49].

Diabetic gastroparesis is clinically important because it is associated with GI symptoms, alterations in glycemic control, and changes in oral drug absorption [50]. Symptoms of

Table 39.4 Etiology of gastroparesis (nonobstructive delayed gastric emptying)

Idiopathic
Endocrine or metabolic:
Diabetes mellitus
Thyroid disease
Renal insufficiency
After gastric surgery:
After resection
After vagotomy
Central nervous system disorders:
Brainstem lesions
Parkinson disease
Peripheral neuromuscular disorders:
Myotonia dystrophica
Duchenne muscular dystrophy
Connective tissue disorders:
Scleroderma
Polymyositis/dermatomyositis
Infiltrative disorders:
Lymphoma
Amyloidosis
Diffuse gastrointestinal motility disorder:
Chronic intestinal pseudoobstruction
Medication-induced (see Table 39.2)
Electrolyte imbalance: potassium, calcium, magnesium
Miscellaneous conditions:
Infections (especially viral)
Paraneoplastic syndrome
Ischemic conditions
Gastric ulcer

Adapted from Parkman HP et al. [19] from Blackwell Publishing.

diabetic gastroparesis primarily include nausea and vomiting, but also early satiety, fullness, and abdominal discomfort. The presence of abdominal bloating or fullness in particular appears to be associated with diabetic gastroparesis [51]. Symptom severity, however, does not necessarily correlate well with the degree of gastric stasis [52,53]. Patients with severe symptoms may have only a mild delay or even normal gastric emptying. One study examining the effect of the prokinetic agent domperidone in diabetic gastroparesis suggested that symptom improvement did not correlate with improvement in gastric emptying, but rather with resolution of dysrhythmias [50]. Diabetic gastroparesis has been considered to be associated with a poor prognosis; however, more recent studies suggest that this may be incorrect and that most patients with diabetic gastroparesis do not deteriorate over time [50].

The motor dysfunction is typically confined to the stomach (gastroparesis), but sometimes a diffuse GI motility disorder may be present. Gastric motor abnormalities include impaired fundic relaxation with antral dilation, gastric dysrhythmias, antroduodenal incoordination, antral hypomotility, and visceral hypersensitivity [4]; gastroparesis may be the end stage of gastric dysfunction. The term *gastropathy* has been used because symptoms do not predict delayed gastric emptying, and the symptomatic response to prokinetic treatment is not necessarily related to improvement in emptying.

Diabetic gastroparesis likely results from impaired neural control of gastric motor function, possibly resulting from a vagal neuropathy. Morphological abnormalities of the gastric myenteric plexus in diabetic patients have not been identified by conventional histology [54]. Abnormal neural innervation and neurotransmission of both inhibitory and excitatory neurotransmitters and underlying muscle dysfunction have been described in animal models of diabetes. Impairment of the inhibitory nitric oxide-containing nerves [55] and damage of the interstitial cells of Cajal [56], the electrical pacemaker cells, have been described in patients with diabetic gastroparesis [57].

Gastroparesis has also been described in type II diabetes mellitus [53]. However, it is also not uncommon for gastric emptying to be accelerated in type II diabetes [58]. Usually, this change occurs early in the disease. It may be related to a lack of a fundic receptive relaxation, a vagally mediated response [59].

Hyperglycemia itself may reversibly affect gastric motility. Hyperglycemia decreases antral contractility, decreases antral phase III MMC activity, increases pyloric contractions, causes gastric dysrhythmias (primarily tachygastria), and delays gastric emptying [40,60]. Normalization of serum glucose in hyperglycemic patients has been shown to improve gastric myoelectric activity, to improve gastric emptying, and to restore antral phase III MMC activity [61]. It is not clear how hyperglycemia adversely affects gastric motility. Hyperglycemia appears to cause a reversible impairment of vagal efferent function [62]. Glucose-responsive neurons have been identified in the central nervous system that may modify vagal efferent activity [63]. Prostaglandins may also be involved; indomethacin (indometacin) may reverse abnormal gastric electrical rhythms that occur during hyperglycemia [64].

Changes of gastric motility may also affect postprandial blood glucose concentrations. Delayed gastric emptying may contribute to poor glucose control because of unpredictable delivery of food into the duodenum [60]. Impaired gastric emptying with administration of exogenous insulin may produce hypoglycemia. Conversely, acceleration of emptying has been reported to cause hyperglycemia [58,65]. Problems with glucose control may be the first indication that a diabetic patient is developing gastroparesis [60]. Interestingly, gastroparetic symptoms may be mild or absent. Long-term oral administration of the prokinetic agents that accelerate gastric emptying have been shown to decrease plasma glucose and glycosylated hemoglobin (HbA_{1c}) levels in diabetic patients in some studies [66,67]. However, several other studies have not confirmed this.

Patients with insulin-dependent diabetes mellitus have an increased perception of gastric distention, with more nausea, fullness, and upper abdominal pain [4]. Hyperglycemia increases nausea and fullness during proximal gastric distention [60,68]. Increased sensitivity of the proximal stomach may be responsible for dyspeptic symptoms postprandially when the stomach is distended by a meal [4,68].

Postsurgical gastroparesis

Postsurgical gastroparesis has been classically described in the past occurring after peptic ulcer surgery [69], most commonly when a truncal vagotomy was performed. Vagal nerve stimulation causes both fundic relaxation and antral contractions; the effect of a total vagotomy is to delay emptying of solids while accelerating gastric emptying of liquids. A gastric drainage procedure such as a pyloroplasty or gastroenterostomy is usually performed in an attempt to offset the gastroparetic effects of vagotomy. In most patients, the net result is that vagotomy combined with a drainage procedure produces little alteration in gastric emptying. The results of different gastric surgical procedures on gastric emptying are shown in Table 39.5.

About 5% of patients undergoing vagotomy with antral resection and gastrojejunostomy develop severe postsurgical gastroparesis; the antrum is not present to triturate solids, and the proximal stomach is unable to generate sufficient pressure to empty solids [69]. Patients with gastric outlet obstruction undergoing surgery may also develop postsurgical gastric stasis; the chronic gastric dilation may have led to irreversible gastric muscle dysfunction.

The Roux-en-Y stasis syndrome is characterized by postprandial abdominal pain, bloating, nausea, and vomiting [69]. The combination of vagotomy, distal gastric resection, and Roux-en-Y gastrojejunostomy predisposes to gastric stasis, which results from both slow emptying from the gastric remnant and delayed small bowel transit in the Roux efferent limb [69]. Ectopic jejunal pacemakers may develop in the transected and reattached jejunal segment. In addition, myoelectric and motor activity in the Roux limb is slower than normal and may be retrograde, causing reverse peristalsis toward the stomach and allowing the Roux limb to cause a functional obstruction [70].

Gastroparesis is unusual in patients with a highly selective parietal cell vagotomy. Transient myoelectric abnormalities may occur, but they usually disappear with time [71]. Development of delayed gastric emptying after highly selective vagotomy may suggest inadvertent complete vagotomy [72].

Post-Nissen fundoplication gastric motor dysfunction

Mild acceleration of gastric emptying has been reported to occur after Nissen fundoplication [73,74]. Proximal gastric emptying after fundoplication is increased because of an increase in intragastric pressure resulting from the fundic wrap preventing receptive relaxation. This may lead to symptoms of early satiety [74]. In patients with GERD and delayed gastric emptying, there can be normalization of emptying after fundoplication [74]. However, this is not universally seen and, often, if Nissen fundoplication is performed in a patient with delayed gastric emptying, a pyloroplasty is sometimes performed.

Table 39.5 Gastric emptying alterations after different types of gastric surgery

Surgical procedure	Anatomic changes	Motility changes	Comments
Proximal gastric vagotomy or parietal cell vagotomy	Selective denervation of acid-producing parietal cells	Impaired receptive relaxation accommodation; accelerated liquid emptying, normal solid emptying	Early satiety is common
Truncal vagotomy with drainage procedure (pyloroplasty or gastroenterostomy)	Vagal denervation of entire stomach; ablation of pyloric sphincter	Same effects as proximal gastric vagotomy plus a significant decrease in antral contractility with delayed emptying of solids	Obligatory drainage procedure is needed to prevent stasis; as a result, larger solids, less suitable for intestinal digestion and absorption, pass directly into the small intestine
Partial gastrectomy	Proximal stomach resected	Impaired accommodation; accelerated liquid emptying	Decreased total reservoir capacity
Subtotal gastrectomy	Distal two-thirds of stomach resected	Decreased accommodation with decreased outlet resistance; rapid emptying into intestine	Usually performed with vagotomy, may account for dumping
Billroth I gastroduodenostomy or Billroth II gastroenterostomy	Antrectomy (distal one-third)		

Adapted from Parkman HP et al. [19], with permission from Blackwell Publishing.

Gas bloat syndrome refers to postoperative symptoms including an inability to belch, pain, and bloating. It is more common in patients with preoperative delayed gastric emptying [75].

Early satiety, postprandial fullness, nausea, and vomiting may develop after fundoplication [76]. In some of these patients, postprandial antral hypomotility or a delay in gastric emptying can be seen [77,78]. Delayed gastric emptying may have been present before surgery but not recognized because of the GERD symptoms of heartburn and regurgitation. The development of postoperative gastroparesis after an open or laparoscopic Nissen fundoplication may result in significant morbidity [78]. Vagal nerve injury has been reported in from 4% to as many as 40% of patients undergoing laparoscopic fundoplication [79,80].

Postbariatric surgery

Surgical therapy for morbid obesity has increased since the late 1990s. Several procedures are designed to restrict stomach size [81]. The most commonly performed surgical procedure is the Roux-en-Y gastric bypass. The stomach is partitioned into a small proximal fundic pouch and a bypassed distal stomach; a loop gastrojejunostomy through a small gastroenterostomy drains the proximal pouch [81]. Ingestion of large meals is prevented by early satiety resulting from the small gastric pouch. The gastrojejunostomy configuration produces a relatively malabsorptive state and can result in dumping if a high carbohydrate liquid meal is ingested. Solid emptying is slower and liquid emptying is faster after gastric bypass surgery [82]. Less commonly performed are gastric restrictive procedures including vertical banding gastroplasty and adjustable gastric banding [80]. Vomiting can occur in 21% of patients after vertical banding gastroplasty [83]; another study found that emptying from the proximal pouch was normal and could not explain the early satiety [84].

Idiopathic gastroparesis

Idiopathic gastroparesis refers to symptomatic gastroparesis in patients with no apparent primary underlying abnormality. In most series, idiopathic gastroparesis is the most common category for gastroparesis [1]. Symptoms may fluctuate, with episodes of pronounced symptoms interspersed with relatively symptom-free intervals.

Patients with idiopathic gastroparesis are typically young or middle-aged women. Even after adjusting for gender differences in gastric emptying, gastroparesis occurs more commonly in women [6]. A potential cause in some patients includes viral injury to the nerves or muscles of the stomach. Postviral gastroparesis was suspected in 23% of patients with idiopathic gastroparesis [85]. This clinical diagnosis is suggested in previously healthy persons with an acute onset of viral illness with nausea, vomiting, diarrhea, fever, and cramps who have persistence of symptoms (nausea, vomiting, early satiety) for more than 3 months with a delay in gastric emptying. Viruses suspected as potential causes are cytomegalovirus (CMV), Epstein–Barr virus, and herpes varicella-zoster. Symptoms of idiopathic gastroparesis after a presumed viral illness tend to be less severe than in gastroparesis from other causes. Overall, these patients appear to have a good prognosis, with many patients having a slow resolution of their symptoms [85].

Other disorders associated with delayed gastric emptying

Gastrointestinal disorders

Gastroesophageal reflux disease

Delayed gastric emptying can be seen in patients with GERD. Gastric stasis with proximal fundic distention may promote transient lower esophageal sphincter relaxation with subsequent gastroesophageal reflux [86]. Some investigators have found delayed emptying in 40% of patients with GERD [87]; others have not shown this high prevalence [88]. Studies suggest that slow emptying from the proximal stomach, but not from the overall stomach, may correlate with esophageal acid exposure [89].

Achalasia

Gastric emptying is difficult to study in achalasia because the ingested meal is often retained in the esophagus. Gastric emptying studies in these patients have given variable results; several reported no abnormalities in gastric emptying [90]. Studies delivering liquid meals into the stomach through a nasogastric tube have shown rapid emptying of liquids [91]. After successful pneumatic dilation, gastric emptying of solid meals was delayed in 50% of patients with achalasia [92]. These emptying abnormalities may be related to abnormalities of the vagus nerve and dorsal motor nuclei of the vagus, which have been reported in achalasia.

Gastric ulcer

Delayed gastric emptying has been reported in some patients with gastric ulcers. In some patients, the gastroparesis has resolved after ulcer healing. It may be difficult to determine whether the ulcer caused the gastric emptying delay or the gastric stasis caused the ulcer.

Atrophic gastritis

Atrophic gastritis is characterized by a decrease in gastric acid secretion, usually leading to hypergastrinemia through acid–gastrin feedback regulation. Atrophic gastritis may result from pernicious anemia or may occur on a nonautoimmune basis, such as in *H. pylori* infection. Delayed gastric emptying is present in two-thirds of patients with achlorhydric atrophic gastritis and in one-third of those with preserved acid secretion. Delayed gastric emptying in atrophic gastritis is associated with reduction of gastric acid output and hypergastrinemia, rather than with severity of mucosal atrophy [93].

Functional dyspepsia

See Functional dyspepsia later in the chapter.

Hypertrophic pyloric stenosis

Infantile hypertrophic pyloric stenosis (IHPS), occurring in up to 0.3% of births, is the most common disorder requiring abdominal surgery during the first 6 months of life. A typical infant begins to vomit within the first 4 weeks of life. Hypertrophy of the inner circular muscle of the pylorus produces pylorospasm or functional gastric outlet obstruction resulting in nonbilious vomiting, dehydration, and metabolic alkalosis. An enlarged pylorus (pyloric olive) can be palpated or detected radiologically. Pylorospasm occurs from a defect in pyloric sphincter relaxation and not from a true obstruction. A lack of inhibitory nerves of the pylorus, primarily nitric oxide-containing nerves, and interstitial cells of Cajal has been shown in IHPS [94,95]. Treatment with surgical pyloromyotomy is effective. Patients usually remain symptom free after surgical treatment; the muscle hypertrophy resolves. The abnormalities of nitric oxide-containing nerves and interstitial cells of Cajal partially reverse [96], although on manometry the pyloric tone is higher and the phasic pyloric pressure waves are less frequent than in physiologically normal infants [97]. Medical treatment with atropine has been suggested by European groups. IHPS has been associated with the use of erythromycin for pertussis prophylaxis in neonates [98].

IHPS in adults is rare [99]. Diagnostic criteria for adults have been variable. Early reported cases were related to local inflammation and fibrosis from previous gastric or duodenal ulcer. In the uncommon idiopathic form, there is hypertrophy of the smooth muscle without any underlying disease. A certain proportion of these primary cases may represent persistence of the juvenile form, presenting later in life. Endoscopic dilation and laparoscopic pyloroplasty have been used for treatment [100].

Celiac disease

Delayed gastric emptying is seen in 50% of patients with celiac disease, which normalizes with a gluten-free diet and recovery of small bowel mucosa. Delayed gastric emptying has been suggested to explain some of the dyspeptic symptoms in celiac disease [101,102].

Gallbladder disease

Delayed gastric emptying, in addition to delayed gallbladder emptying, may be present in cholelithiasis [103]. Some studies have reported delayed solid phase gastric emptying in 20%–42% of patients with gallstones [104], whereas others have not found alterations in liquid phase emptying [103]. Interestingly, cholecystectomy has been shown to normalize gastric emptying in some patients [104]. Many patients with gastroparesis have had a previous cholecystectomy, possibly with the thought that gallbladder dysfunction may be causing the nonspecific symptoms.

Chronic pancreatitis

Some patients with chronic pancreatitis have delayed gastric emptying [105]. This may explain some of the abdominal pain, nausea, and vomiting seen in this group of patients. This phenomenon may be related to the increased serum level of endogenous cholecystokinin (CCK) [105]. One study showed impaired gastric myoelectric activity in chronic pancreatitis [106]; this abnormality was restored toward normal with pancreatic enzyme replacement. Gastric emptying is variable in cystic fibrosis: early in the disease it may be rapid, whereas later in the disease it is delayed [107].

Liver disease

Abnormal gastric emptying has been reported in up to 78% of patients with cirrhosis of the liver [108]. The delay in gastric emptying is correlated with the severity of liver disease, as indicated by Child–Pugh status, bilirubin level, prothrombin time, and presence of esophageal varices [108]. Secretin and endothelin have been suggested to play a role in the gastric dysmotility seen in cirrhosis and portal hypertension [109,110]. Other studies have not shown significant changes in gastric emptying [111] and have even reported acceleration of liquid phase gastric emptying [112].

Interferon-α treatment may delay gastric emptying and may cause dyspeptic symptoms. In one study, interferon treatment delayed gastric emptying in 12 of 14 patients and was associated with development of anorexia and abdominal discomfort [113]. Prokinetic therapy improved gastric emptying and GI symptoms during interferon-α therapy.

Constipation

Delayed gastric emptying is not uncommonly observed in patients with constipation. The finding of delayed gastric emptying suggests the possibility of a diffuse GI disorder [114]. This has implications for treatment as patients with chronic severe constipation and proximal GI tract dysmotility often have poor responses to colectomy. Volitional suppression of defecation can delay gastric emptying, suggesting a "cologastric brake" or the effects of stress [115].

Diffuse gastrointestinal motor disorders

Gastroparesis can occur as a part of a diffuse GI motility disorder. *Chronic intestinal pseudoobstruction* is a syndrome with recurrent symptoms suggestive of intestinal obstruction in the absence of mechanical blockage. Symptoms are caused by ineffective peristalsis and include nausea, vomiting, and abdominal pain with abdominal distention. Radiological findings consist of air–fluid levels within the small intestine. The term *diffuse GI motility disorder* rather than chronic intestinal pseudoobstruction is often used if several portions of the GI tract have delayed transit but no air–fluid levels are seen on abdominal radiographs [19].

Chronic intestinal pseudoobstruction can be caused by several systemic diseases including scleroderma, amyloidosis,

myxedema, and long-standing diabetes mellitus. It is often idiopathic; the two main forms of idiopathic pseudoobstruction are myopathic and neuropathic. Small bowel manometry may help to differentiate between these two forms.

Nongastrointestinal disorders

Eating disorders: anorexia, bulimia, and rumination

Anorexia nervosa is a psychiatric disorder occurring primarily in adolescent and young adult women, characterized by distorted body image and fear of obesity with compulsive dieting and self-imposed starvation to maintain a profoundly low body weight. GI symptoms are common and include lack of appetite, early satiety, epigastric fullness, abdominal bloating, nausea, and vomiting [116,117]. Patients with anorexia nervosa often have delayed gastric emptying primarily for solids [117]. Interestingly, realimentation and maintenance of normal body weight improve gastric emptying and GI symptoms [116,118]. Prokinetic therapy had been suggested to improve gastric emptying and to facilitate refeeding [117]; other studies have not shown an effect on weight gain [119]. The pathogenesis of gastric dysfunction in anorexia is probably multifactorial; starvation and malnutrition with marked endocrine disturbances may play a role; others have suggested a central nervous system disturbance related to the psychiatric abnormality.

Bulimia nervosa is characterized by recurrent episodes of binge eating with a feeling of lack of control over the eating behavior during the binges, often followed by self-induced vomiting, the use of laxatives or diuretics, strict dieting or fasting, or vigorous exercise to prevent weight gain [120]. Self-induced vomiting allows bulimic patients to continue eating or to terminate the binge. In contrast to anorexia nervosa, concern about weight and body size does not lead to a fall in weight below normal. Symptoms of postprandial fullness, early satiety, bloating, nausea, and epigastric pain may occur [121]. Depression may be an important determinant of GI symptoms [121]. Gastric emptying studies in bulimia have yielded conflicting results; some bulimic patients (37%) appear to have delayed emptying [120].

Rumination syndrome is the effortless regurgitation of recently ingested food into the mouth with subsequent remastication and reswallowing or expectorating of food. Initially described in children and mentally retarded institutionalized persons, there is an increasing awareness of this disorder in adults of normal intelligence. Rumination can become a habit, often initiated by a belch, a swallow, or by stimulation of the palate with the tongue. Abdominal muscle contraction with lower esophageal sphincter relaxation in the early postprandial period is responsible for regurgitation [122]. Typically, the effortless repetitive regurgitation occurs within 15 min of starting a meal, in contrast to vomiting from gastroparesis, which occurs later in the postprandial period [46]. Symptoms often cease when regurgitated food becomes acidic to taste. Difficulty in diagnosis results from lack of awareness and the difficulty in differentiating rumination from vomiting caused by gastroparesis or regurgitation from GERD. Gastric emptying and esophageal pH monitoring are usually normal. Gastroduodenal manometry may show characteristic brief simultaneous increases in gastric and small bowel pressure (R waves) seen in all abdominal recording ports during the postprandial period. These represent abdominal wall contractions, and, if performed with esophageal pH monitoring, they are associated with a decrease in distal esophageal pH. These characteristic findings are seen in only one-third of patients with clinical symptoms of rumination [46]. Making the patient aware of this habit may be beneficial. Treatment involves behavioral modification and biofeedback therapy administered in a formal eating regulation program. Concentration on diaphragmatic breathing after eating may help. Biofeedback techniques help to teach patients to relax abdominal muscles during and after eating [123]. Chewing gum postprandially has been suggested to be helpful.

Cyclic vomiting syndrome

Cyclic vomiting syndrome refers to recurrent episodes of intense nausea and vomiting lasting hours to days separated by symptom-free periods of variable lengths. Typically, each episode is similar. Vomiting often starts abruptly, although a prodrome of nausea and abdominal pain can occur. The vomiting reaches its highest intensity during the first hours, diminishes thereafter, and ends rapidly. The duration of vomiting averages 3–4 days and can lead to marked dehydration. This disorder is well characterized in children, but it is now being described in adults [124,125]. In adults, the vomiting episodes are longer (3–5 days), less frequent (every 3–4 months), and triggering events are less evident [125]; there is usually a long delay in diagnosis. Abnormal upper GI motility can be demonstrated by gastric emptying scintigraphy, antroduodenal manometry, and EGG, even in the asymptomatic state; however, these tests are often normal [126]. Recently, gastric emptying has been reported to be rapid in the symptom-free period [127]. Precipitating events, identified in a few patients, include onset of menstrual periods and stress. Cyclic vomiting syndrome, also referred to as *abdominal migraine* or *abdominal epilepsy*, is associated with a history of migraine headaches in patients and other family members. The cause of this disorder is unknown; a mechanistic relationship to migraine, underlying hypothalamic–pituitary axis derangement, or an inappropriate activation of vomiting reflex has been suggested [124]. Mitochondrial disorders and dysfunction of corticotropin releasing factor (CRF) have been identified in some patients [128,129]. There is no standard management for this disorder. A quiet environment, sleep, intravenous fluids (generally dextrose 10% in normal saline [$D_{10}NS$]), and the use of antiemetics, such as ondansetron or metoclopramide, and benzodiazepines, such as lorazepam, may be effective in the acute episodes of vomiting [125,127]. Tricyclic antidepressants

(such as amitriptyline) in low doses [125,127] and β-blockers such as propranolol may prevent attacks. Antimigraine therapies such as the 5-HT_{1D} receptor agonist sumatriptan may reduce the severity of attacks. The use of nonsteroidal antiinflammatory drugs, such as indomethacin or ketorolac, given intramuscularly, has been reported to prevent or reduce the intensity of attacks if these drugs are given during the prodromal period [124]. These agents inhibit prostaglandin formation and may correct gastric dysrhythmias or act centrally.

Neurological disorders

Central nervous system causes of gastroparesis are rare. Any condition associated with increased intracranial pressure may produce emesis, with or without nausea, by activation of brainstem structures mediating vomiting [16]. Focal lesions including tumors, especially those involving the brainstem and posterior fossa, can cause nausea and vomiting and can even present with gastroparesis. Demyelinating diseases such as multiple sclerosis and other demyelinating disorders have been reported to cause gastroparesis [130]. An adult patient with vomiting from a central mass lesion usually has central nervous system symptoms or signs, such as headache, cranial nerve findings, long tract signs, or papilledema [131]. Rarely, objective neurological findings may be absent [132]. Magnetic resonance imaging is better than computed tomography for visualization of the posterior fossa.

Gastroparesis is common in Parkinson disease and has been reported in 70% of patients [133]. The stomach can be affected by the underlying Parkinsonian neurodegenerative pathology; Lewy bodies have been reported in the myenteric plexus of the stomach [134]. Medications used to treat Parkinson disease such as L-dopa can delay gastric emptying. Impaired gastric emptying with delayed levodopa absorption may be a reason for a poor response to medical therapy for Parkinson disease [133]. Domperidone may be a useful prokinetic agent in Parkinson disease; domperidone does not cross the blood-brain barrier and should not interfere with centrally acting antiparkinsonism medications [135].

Collagen vascular disorders

Scleroderma (systemic sclerosis) is a systemic inflammatory disorder affecting the skin and other organs. GI manifestations usually follow cutaneous and other systemic manifestations [136]. The esophagus is involved in about 75% of patients, especially when Raynaud phenomenon is present. Gastric involvement is less frequent than that of the esophagus, small intestine, or colon. Delayed gastric emptying has been reported in 10%–75% of patients [137], usually in those with esophageal involvement [138]. Watermelon stomach is also reported in scleroderma [137]. Antroduodenal manometry classically reveals very low amplitude antral and duodenal contractions with normal MMC propagation [136].

Polymyositis and dermatomyositis classically affect only skeletal muscle, as recognized on esophageal manometry with dysfunction of the upper esophageal sphincter and upper esophagus. However, polymyositis and dermatomyositis may be associated with other GI motor dysfunction. Gastric emptying was found to be delayed in 8 of 13 patients and correlated with the severity of the peripheral (skeletal) muscle weakness [139].

Systemic lupus erythematosus rarely affects gastric motility. Delayed gastric emptying has been reported in a patient with systemic lupus erythematosus; in some patients, functional gastric outlet obstruction with antral narrowing from thickened folds resolved with prednisone treatment [140].

Amyloid-associated GI dysmotility can manifest as intestinal pseudoobstruction, diarrhea, or achalasia [141]. GI symptoms typically include anorexia, difficulty with eating from macroglossia, and altered bowel habits. Nausea may occur in up to 40% of patients with amyloidosis [141]. Clinical gastroparesis occurs rarely in patients with amyloidosis; one study reported only three cases in 769 patients [142].

Endocrine and metabolic disorders: thyroid, parathyroid, and renal failure

Hypothyroidism should be excluded in all patients with gastroparesis. Although it represents only a small percentage of patients with gastroparesis, it is easily treated with thyroid supplementation. There is a poor correlation between the gastric emptying and the elevated thyroid-stimulating hormone level [143]. Intestinal pseudoobstruction has also been described in hypothyroidism. *Hyperthyroid* patients may be expected to have rapid gastric emptying; however, studies have reported normal, rapid, and delayed gastric emptying [138,139,144]. Delayed gastric emptying with gastric dysrhythmias has been reported; these resolve with treatment of the hyperthyroidism [145]. The delayed gastric emptying has been suggested to result from associated autonomic dysfunction [145].

In both hyperthyroidism and *hypoparathyroidism*, small intestine pseudoobstruction has been reported. Parathyroid hormone has been shown to relax gastric and other GI smooth muscle directly [146]. Whether it influences gastric emptying is not known.

GI symptoms, especially anorexia, nausea, and vomiting, are often present in *renal failure*. Symptoms may be from uremia, medications, gastric mucosal ulceration, or gastroparesis. Gastric emptying studies have yielded conflicting results. Delayed emptying has been seen in patients with symptomatic uremia [147]. Normal emptying is most commonly seen in patients undergoing hemodialysis. Delayed emptying has been reported to be associated with changes in nutritional status and autonomic neuropathy [148,149]. Using the octanoate breath test, delayed gastric emptying was present in 62% of patients undergoing long-term hemodialysis; delayed emptying was present in 91% of patients with

dyspeptic symptoms and in 40% of those without symptoms [150]. Chronic ambulatory peritoneal dialysis delays emptying, primarily when the abdomen contains the dialysate; emptying normalizes after the dialysate is emptied [151].

Gastric infections

Delayed gastric emptying can be seen during a variety of acute *viral infections* including herpes zoster and infections with Epstein–Barr virus, CMV, rotavirus, and parvoviruses [152]. Nausea and vomiting have been associated with altered gastric motor function [153]; this has been shown during acute viral gastroenteritis with parvovirus-like agents (Norwalk and Hawaii viruses). Delay in gastric emptying is usually transient and returns to normal with recovery from the viral infection. In rare patients, chronic symptoms and delayed gastric emptying may result [154,155]; this may be one cause of idiopathic gastroparesis.

Gastric CMV occurs often in immunosuppressed persons, particularly organ transplant recipients [156]. Upper GI CMV infection has been reported to occur in one-third of patients after liver transplantation, often associated with typical symptoms of gastroparesis including nausea and abdominal fullness. Endoscopy can show large antral folds, gastric inflammation including acute superficial gastritis, and duodenal erosions and ulcerations. Viral cultures of gastric biopsies and histological presence of CMV inclusions in the gastric mucosa are used for diagnosis [156]. The CMV infection can be treated with ganciclovir. GI infection with CMV is unusual in immunologically normal individuals. A case report documents acute CMV infection causing GI dysmotility in an immunocompetent patient; the disturbance in GI motor function persisted for years after viral infection had been eradicated [154].

Gastric emptying may be delayed in one-third of persons seropositive for human immunodeficiency virus (HIV) [157], particularly those with advanced disease evidenced by low CD4 counts, marked weight loss, and enteric infections [158]. In immunocompromised patients, the acute stage of gastritis and gastroparesis from viruses and other infectious agents, especially CMV and *Mycobacterium avium-intracellulare,* may persist for prolonged times. Some investigators have found delayed gastric emptying of solids with diminished postprandial antral motility but rapid emptying of liquids, suggesting an autonomic neuropathy that is common in HIV infection [159].

It had been speculated that *H. pylori* causes GI motor abnormalities. Although a few studies have shown an association [160], most conclude that *H. pylori* does not cause delayed gastric emptying or functional dyspepsia [161]. One study reported that chronic antral *H. pylori* gastritis was associated with a lower incidence of the phase III MMC starting in the antrum in dyspeptic patients [162]. Another study suggests that dyspeptic patients with *H. pylori* infection have a high prevalence of gastric dysrhythmias; eradication of *H. pylori* normalized gastric myoelectric activity and improved symptoms [163].

Chronic mesenteric ischemia (ischemic gastroparesis)

Gastric ischemia may occur in chronic *atherosclerotic disease*; the gastric ischemia is insidious and often is not recognized. Ischemic gastropathy secondary to atherosclerosis may present as gastritis, ulceration, or gastroparesis [164]. Angiography demonstrates arterial occlusion. Cases of chronic *mesenteric ischemia* with chronic gastric ischemia causing gastroparesis and gastric dysrhythmias have been reported [165]. Surgical revascularization may improve gastric emptying and corrects gastric dysrhythmias in selected patients.

Gastroparesis after lung and heart–lung transplantations

Gastroparesis has been reported after heart and lung transplantations [166,167]. After lung transplantation, gastroparesis may predispose to gastroesophageal reflux with microaspiration and subsequent pulmonary infection, which can have deleterious effects on the transplanted lungs. Symptomatic delayed gastric emptying was present in 25% of patients after single lung transplantation and in 50%–80% of patients after combined heart and lung transplantation [166,167]. Several causes including vagal nerve dysfunction, viral infection, and immunosuppressive medications may explain gastroparesis after heart and lung transplantation [166–168].

Gastroparesis secondary to malignant disease (tumor-associated gastroparesis)

Tumor-associated gastroparesis has been described with esophageal, gastric, pancreatic, breast, and lung carcinoma. The pathophysiology is unknown, but frequently it is attributed to paraneoplastic effects, neural invasion of the tumor, or the side effects of chemotherapy.

In *gastric cancers* that are nonobstructing, tumor infiltration into the stomach wall may disrupt smooth muscle or neural innervation. The symptoms and delay in gastric emptying can be responsive to prokinetic agents, including metoclopramide [169].

Small bowel tumors are rare and can be difficult to diagnose. Infrequently, patients initially diagnosed with gastroparesis are found on further evaluation to have a small bowel adenocarcinoma causing symptoms from partial obstruction [170]. These tumors can be missed on an SBFT examination. Enteroclysis provides a more detailed evaluation.

In *pancreatic cancer,* delayed gastric emptying has been reported in 40%–60% of patients without gastroduodenal invasion or obstruction. Usually, the gastroparesis is asymptomatic, but it may be responsible for the nausea, vomiting, and abdominal or back pain that occur [171]. Retroperitoneal nerve invasion of the tumor has been postulated as a cause of the gastroparesis, although gastroparesis can also occur with resectable cancers.

Pseudoobstruction with delayed gastric emptying may represent a paraneoplastic syndrome that occurs primarily with small cell lung carcinoma [172]. Intestinal dysmotility is suspected to have an autoimmune basis affecting the myenteric plexus. Pathological studies have shown degeneration of the myenteric plexus with infiltration by plasma cells and lymphocytes and markedly reduced numbers of neurons [173]. The onset of GI dysmotility not infrequently precedes the diagnosis of cancer [172]; patients may have a normal chest radiograph at presentation with subsequent diagnosis of cancer by computed tomography, magnetic resonance imaging, or bronchoscopy. Antroduodenal manometry may show neuropathic abnormalities with incoordination of contractions. Type 1 antineuronal nuclear antibody (ANNA-1 or anti-Hu) can often be detected in paraneoplastic pseudoobstruction from small cell lung carcinoma [172].

Delayed gastric emptying with nausea and vomiting can occur during treatment of malignant disease. Gastroparesis can occur after radiation therapy for treatment of tumors [174], during treatment with chemotherapeutic agents [175], after bone marrow transplantation [176], and after celiac plexus block for chronic pain from pancreatic cancer [177].

Medication-induced gastroparesis

Nausea and vomiting can be side effects of medications. Nausea and vomiting in response to a drug are likely to present early in its use; medications usually cause acute rather than chronic nausea and vomiting [16]. Many drugs including anticholinergics, narcotic analgesics, tricyclic antidepressants, and calcium channel blockers are known to delay gastric emptying (see Table 39.2). Several of the newer diabetic medications, including pramlintide, delay gastric emptying. Drug-induced gastroparesis may be asymptomatic, but it needs to be considered when delayed gastric emptying is found in a symptomatic patient. Gastric acid-suppressing agents, such as proton pump inhibitors and H_2 receptor antagonists, have been shown to delay gastric emptying [178]; however, these agents are unlikely to produce clinically significant gastroparesis. Some studies suggest that nizatidine, an H_2 receptor antagonist with cholinergic properties, may induce antral contractility and may promote gastric emptying [179].

Gastric emptying of solids is delayed in patients receiving total parenteral nutrition (TPN) [180]. It is suggested that if assessment of gastric emptying and motility is performed in a patient who is receiving TPN, the TPN should be converted to saline for the test [27]. Slowing of gastric emptying during TPN is correlated with an increase in blood glucose from the intravenous nutrients; hyperglycemia inhibits gastric emptying of solids. The inhibitory effect of hyperglycemia on gastric emptying may be mediated by the vagal–cholinergic system [180]. Oral ingestion of fat is well known to delay gastric emptying through intestinal feedback and release of CCK. Intravenous infusion of fat emulsions also delays gastric emptying [180,181]. The mechanism by which intravenous fat influences gastric motility is poorly understood [180,181].

Treatment of symptomatic gastroparesis

The general principles for treatment of symptomatic gastroparesis are as follows: first, to correct fluid, electrolyte, and nutritional deficiencies; second, to identify and treat the underlying cause of gastroparesis; and third, to reduce symptoms [16,182,183]. The specific treatment depends on the severity of symptoms and the underlying disorder. For mild gastroparetic symptoms, dietary modifications may be used. Medicines are reviewed to ensure that the patient is not taking an anticholinergic agent or narcotic analgesic that could delay gastric emptying or prevent the action of a prokinetic agent. For diabetic patients, blood glucose should be regulated carefully to prevent hyperglycemia. In severe cases of gastroparesis with vomiting, signs of dehydration, and poor glucose control, treatment may include hospitalization, intravenous hydration, nasogastric suction to decompress the stomach, regulation of blood glucose, and intravenous administration of antiemetic and prokinetic agents.

Dietary treatment

In gastroparesis, dietary modification is the initial treatment [184]. The diet may be modified to promote faster gastric emptying. Liquid nutrients in the diet should be increased, because emptying of liquids is usually less affected than solids. Because fat intake releases CCK, which can delay gastric emptying, low-fat meals with complex carbohydrates are suggested. Meals should be small to allow time for emptying to occur; gastric emptying of meals is regulated for a set number of calories to be emptied into the duodenum [185]. To compensate for small meals, patients may need to eat four or five times daily. A diet low in indigestible fiber and roughage will reduce the chance of bezoar formation.

Alcohol and carbonated beverages should be avoided. Even low doses of alcohol can decrease antral contractility and impair gastric emptying. Carbonated beverages release carbon dioxide gas, which can aggravate gastric distention.

Commercial or self-prepared liquid homogenized meals may be tolerated in small quantities; larger volumes needed to cover daily caloric needs are often not. Enteral alimentation may be needed in patients with refractory symptoms unable to maintain adequate nutrition and hydration. In some severe cases, TPN may be needed. This is not a long-term solution because of catheter-induced bacteremia, clot formation, and cost.

Glucose control

Diabetic patients with gastroparesis may have abnormally high glucose concentrations both after fasting and postprandially. Hyperglycemia itself has been shown to delay gastric emptying [60,61]. This slowing is associated with reduced antral contractility and increased pyloric pressure.

Hyperglycemia can inhibit the accelerating effects of prokinetic agents [186]. Improvement of glucose control can increase antral contractility, correct gastric dysrhythmias, and accelerate gastric emptying. Measures should include more aggressive glucose monitoring with frequent dosing of short-acting insulin preparations to prevent profound postprandial hyperglycemia. Prevention of wide fluctuations in serum glucose levels may be more important than maintenance of a given steady state blood sugar value from a gastric emptying perspective. For many type I diabetic patients, a long-acting preparation such as insulin glargine may be administered twice daily with preprandial injections of regular insulin formulas. However in patients with gastroparesis, postprandial hypoglycemic episodes can occur when the glucose-lowering effects of preprandial short-acting insulin precede delivery of nutrients into the small intestine for digestion and absorption. To this end, monitoring 2-h postprandial blood glucose levels may be useful.

Antiemetic agents

Nausea and vomiting are treated with antiemetic agents [16]. These include phenothiazines, antihistamines, anticholinergics, dopamine receptor antagonists, and serotonin receptor antagonists.

The actions of phenothiazine compounds are mediated primarily through a central antidopaminergic mechanism at the chemoreceptor trigger zone in the area postrema of the medulla oblongata [16]. Commonly used agents include prochlorperazine (Compazine), trimethobenzamide (Tigan), and promethazine (Phenergan). These agents are commonly used for severe episodes of nausea and vomiting and are available as tablets, capsules, liquid suspensions, and suppositories, and for injectable use. Sedation and extrapyramidal side effects may develop with these agents [187].

Antihistamines with H_1 receptor antagonistic properties have central antiemetic effects [16]. These include diphenhydramine (Benadryl), dimenhydrinate (Dramamine), and meclizine (Antivert). These agents exert antinausea effects by blocking H_1 receptors in the vestibular apparatus and the chemoreceptor trigger zone within the area postrema. In vection-induced motion sickness, dimenhydrinate reduced tachygastria, decreased symptoms, and caused drowsiness, suggesting that symptom improvement is by improvement of gastric myoelectric activity or depression of central nervous system activity [188].

Serotonin ($5\text{-}HT_3$) receptor antagonists, such as ondansetron (Zofran) and granisetron (Kytril), are useful in chemotherapy-induced nausea and vomiting. The primary site of action is likely the chemoreceptor trigger zone because of the high density of $5\text{-}HT_3$ receptors in the area postrema [16]. They may also act peripherally because $5\text{-}HT_3$ receptors are present in the GI tract [16]. They do not appear to affect gastric emptying [189]. If used, they are best given on an as needed basis because of the lack of evidence of prolonged efficacy and their expense. Benzodiazepines such as lorazepam and diazepam have been shown to be effective as adjunctive agents in the treatment of chemotherapy-related nausea and vomiting [16]. The most recently approved antiemetic agents for chemotherapy-induced nausea and vomiting are the neurokinin-3 receptor antagonists such as aprepitant [190].

Prokinetic agents

Prokinetic agents enhance contractility of the GI tract and accelerate the aboral movement of lumenal contents (Table 39.6). In general, prokinetic agents increase gastric antral contractility, correct gastric dysrhythmias, and improve antroduodenal coordination. Some of the prokinetic agents, metoclopramide and domperidone, also have antiemetic properties.

Table 39.6 Prokinetic agents for gastroparesis

Agent	Major mechanism of action	Comments
Metoclopramide (Reglan)	Dopamine receptor antagonist, central/peripheral	Only FDA-approved agent for gastroparesis Central nervous system side effects in 20%–30% Prokinetic and antiemetic properties
Erythromycin	Motilin receptor agonist	Gastrointestinal side effects in many: nausea, vomiting, abdominal pain Tachyphylaxis with long-term oral administration
Cisapride (Propulsid)	$5\text{-}HT_4$ receptor agonist, facilitates acetylcholine release	Taken off the market in March 2000 for prolonging QT interval Was approved only for nocturnal heartburn Currently not available in the United States
Domperidone (Motilium)	Dopamine receptor antagonist, peripheral	Prokinetic and antiemetic properties Available in Europe, Canada, and Mexico, but not in the United States
Bethanechol (Urecholine)	Muscarinic receptor agonist	Increases amplitude of contractions, not peristalsis Not a true prokinetic agent

Usually, prokinetic agents are administered 30 min before meals to maximize blood levels and gastric prokinetic effects at meal ingestion. In addition, a bedtime dose is often used so that indigestible solids can be emptied from the stomach. Medications with anticholinergic activity may block the effects of prokinetics that work by facilitating acetylcholine release.

In gastroparesis, pharmacological therapy with prokinetic agents is often needed on a prolonged basis. Because GI symptoms correlate poorly with gastric emptying, the response to treatment is usually judged clinically; repeating gastric emptying tests is not necessary.

Prokinetic therapeutic options are limited now that cisapride and tegaserod are not routinely available (see Table 39.6). Prokinetics available for clinical use in the United States are metoclopramide and erythromycin.

Metoclopramide

Metoclopramide (Reglan), a substituted benzamide structurally related to procainamide, has been used since the 1970s to treat gastroparesis [191]. Metoclopramide has both prokinetic and antiemetic effects. It releases acetylcholine from intrinsic cholinergic neurons and blocks peripheral dopamine receptors, where dopamine acts as an inhibitory agent of smooth muscle contraction. The prokinetic properties of metoclopramide are limited to the proximal GI tract. Metoclopramide increases amplitudes of esophageal contractions, resting lower esophageal sphincter pressure, and gastric fundic and antral contractions, and improves antropyloroduodenal coordination. It has antiemetic effects resulting both from dopamine antagonism in the chemoreceptor zone and from a central action at the vomiting center.

Metoclopramide is approved for use in diabetic gastroparesis and for prevention of postoperative and chemotherapy-induced nausea and vomiting. Controlled trials have shown that metoclopramide may provide symptomatic relief while accelerating gastric emptying of solids and liquids in patients with idiopathic, diabetic, and postvagotomy gastroparesis and in patients with GERD [192,193]. Metoclopramide has been reported to be effective in the short-term treatment of gastroparesis for up to several weeks [192,193]. Long-term efficacy for metoclopramide has not been clearly demonstrated; its effect on gastric emptying may diminish during long-term treatment [194]. In diabetic gastroparesis, acute administration of metoclopramide accelerates emptying, but not after 1 month of treatment [194]. However, metoclopramide may continue to relieve symptoms because of its antiemetic effects.

The usual starting dose of metoclopramide in adults is 10 mg 30 min before meals and at bedtime. In patients not responding, the dose can be increased to 20 mg. In severe gastroparesis, oral metoclopramide may not be adequately absorbed because of vomiting or delayed gastric emptying; metoclopramide administered intravenously may improve gastric emptying. Metoclopramide may also be administered subcutaneously, by suppository, or even intraperitoneally in patients undergoing peritoneal dialysis [195].

Side effects of metoclopramide, resulting from its antidopaminergic properties, may occur in up to 30% of patients and are the major factor restricting its use. Acute dystonic reactions including facial spasm, oculogyric crisis, trismus, and torticollis occur in 0.2%–6% of patients [196]. These effects are usually observed during the first 48 h of treatment and are more frequent with higher doses. Drowsiness, fatigue, and lassitude occur in 10% of patients. Mental depression may occur and may range from mild to severe. Metoclopramide can aggravate underlying depression. Other side effects may include restlessness, agitation, irritability, and akathisia. Increased prolactin release may result in breast engorgement, lactation, and menstrual irregularity.

Side effects of long-term use of metoclopramide are of concern. Prolonged treatment with metoclopramide may result in Parkinsonian-like symptoms, more commonly within the first 6 months after beginning treatment, but occasionally after longer periods [196]. These symptoms usually subside within 2–3 months after discontinuation of the drug. Patients with Parkinson disease should be given metoclopramide cautiously, if at all. Tardive dyskinesia, consisting of involuntary movements of the face, tongue, or extremities, may occur with prolonged use and may not reverse after stopping the medication. The prevalence of tardive dyskinesia may range from 0.02% to 2% when patients take metoclopramide for at least 3 months [196]. This study also reported a prevalence as high as 15% [196]. This effect is more common in elderly women. Possible side effects of metoclopramide should be discussed with the patient before starting this medication, and notation of this discussion should be documented in the patient's medical chart.

Erythromycin and motilides

Erythromycin has prokinetic effects because it is a motilin receptor agonist in addition to being an antibiotic [197]. Motilin, an endogenous GI peptide, plays a role in initiating the gastric phase III of the MMC and is a potent stimulator of antral contractility. Erythromycin binds to motilin receptors located on smooth muscle and on cholinergic neurons; the latter appear to be important for actions in vivo [197,198].

Erythromycin stimulates gastric emptying in diabetic, idiopathic, and postvagotomy gastroparesis. It is the most potent prokinetic agent in accelerating gastric emptying, which it achieves by increasing the amplitude and frequency of antral contractions. Interestingly, erythromycin has been reported to accelerate stomach emptying in postsurgical gastroparesis in which the antrum, the primary site of its motor effect, has been resected [199]. The prokinetic effect in this situation may result from a stimulatory effect on the fundus.

Erythromycin is reported to be most effective when it is used intravenously during acute exacerbations of gastroparesis

[200]. Intravenous infusion of erythromycin lactobionate (200 mg) accelerated emptying of solids in patients with diabetic gastroparesis [201]. In these same patients, after 4 weeks of oral erythromycin ethylsuccinate (250 mg orally three times daily, 30 min before meals), the magnitude of the acceleration was less than that observed in response to the single intravenous dose. Studies carried out over longer observation periods have reported a reduction of benefit over time with oral administration. Hyperglycemia attenuates the stimulation of antral contractility and gastric emptying by erythromycin [186]. The motilin receptor agonist ABT-229 was not efficacious in relief of postprandial dyspeptic symptoms in diabetic gastroparesis or functional dyspepsia [202]. This lack of efficacy has been attributed to motilin's propensity to increase fundic tone, impairing accommodation.

Oral administration of erythromycin should be initiated at low doses, such as 125–250 mg three times daily. Many physicians prefer using erythromycin suspension as it takes less time to reach peak concentrations and is easier to adjust dosages [203]. One center reports the use of intravenous erythromycin (100 mg every 8 h) for severe refractory gastroparesis [204]. Side effects of erythromycin include nausea, vomiting, and abdominal pain.

Domperidone

Domperidone (Motilium) is a benzimidazole derivative and is a specific dopamine type 2 receptor antagonist. The effects of domperidone in the upper GI tract are similar to those of metoclopramide. Domperidone stimulates upper GI motility by enhancing gastroduodenal contractions and coordination. It does not readily cross the blood–brain barrier; therefore, it is much less likely to cause extrapyramidal side effects than metoclopramide [205]. However, domperidone has antiemetic properties from its actions at the chemoreceptor trigger zone on the blood side of the blood–brain barrier.

Domperidone has been studied primarily in diabetic gastroparesis. It increases both solid and liquid emptying, especially in those patients with a severe delay in gastric emptying [206]. Symptomatic improvement may not correlate with improvement in emptying; symptom improvement may result from antiemetic effects. A symptomatic benefit of domperidone can occur in diabetic patients with normal gastric emptying [207].

The effect of long-term treatment with domperidone has been variable. In one study, at 6 weeks the effect of domperidone on solid phase gastric emptying was lost, whereas that on liquid gastric emptying was maintained [206]. Another study found that nausea and vomiting were improved compared with baseline values after 6 weeks of treatment, but there was no change in solid phase gastric emptying [207]. Other studies have suggested that improvements in gastric emptying and symptoms were still present after 1 year of treatment [135]. Double-blind studies examining the effects of long-term treatment with domperidone on chronic nausea and vomiting have revealed symptomatic benefit; these observations, however, have not been uniform [207].

Domperidone has been suggested for therapy of nausea and vomiting in Parkinson disease, symptoms that may be related to the use of dopaminergic agents (e.g., L-dopa), which may cause or worsen gastroparesis associated with the disorder [135]. Domperidone would work peripherally for GI dysmotility and symptoms, whereas L-dopa would still work centrally for the parkinsonian symptoms.

The side effects of domperidone are usually secondary to increased prolactin levels, and include breast engorgement and galactorrhea. Central nervous system side effects are minimal.

Domperidone has not been approved by the US Food and Drug Administration (FDA) and is not available by routine prescription in the United States. Physicans who would like to use domperidone for their patients can submit an Investigative New Drug (IND) application to the FDA and their Institutional Review Board (IRB); patients need to purchase the medication (US$50 per month for 10 mg orally four times a day).

Cisapride

Cisapride (Propulsid) is a serotonin (5-HT_4) agonist that facilitates the release of acetylcholine from cholinergic nerves in the myenteric plexus throughout the GI tract. Cisapride stimulates antral and duodenal contractions, improves antroduodenal coordination, and accelerates gastric emptying. Cisapride accelerates gastric emptying and decreases symptoms in patients with gastroparesis, an effect that may last for a year. It also increases esophageal contractions and resting lower esophageal sphincter pressure.

Cisapride was approved by the FDA for the treatment of nocturnal heartburn in patients with GERD. There have been rare instances of cardiac arrhythmias and sudden death after use of this drug [16,187]. These were not due to the 5-HT_4 agonist properties of cisapride, but rather were an effect of its molecular structure on cardiac potassium channels, prolonging the QT interval and predisposing to ventricular arrhythmias, particularly torsades de pointes. Cisapride is metabolized by the cytochrome P450 3A4 system; use of medications that interact with this enzyme system leads to increased cisapride blood levels and increased cardiac toxicity. Besides cardiac risks, side effects include diarrhea, lightheadedness, and abdominal cramps. In patients with a seizure focus, cisapride may rarely increase seizure activity. Cisapride is not available for routine clinical use in the United States; it can be obtained only through limited-access investigational study protocols.

Bethanechol

Bethanechol (Urecholine) is a nonspecific cholinergic muscarinic receptor agonist. It enhances the amplitude of contractions throughout the GI tract. Unfortunately, bethanechol does not coordinate contractions; gastric emptying and small

bowel transit are not necessarily accelerated. Thus, bethanechol is not a true prokinetic agent [187]. Occasionally, it may be helpful as adjuvant treatment in combination with other prokinetic agents. The typical dose is 25 mg orally four times daily. The direct cholinergic action of bethanechol may also stimulate secretion of gastric acid and saliva. Potential side effects of bethanechol include increased salivation, blurred vision, abdominal cramps, and bladder spasm.

Octreotide

Somatostatin has variable effects on GI tract motor activity. Somatostatin inhibits antral motor contractions. In the intestine, somatostatin initiates ectopic myoelectric and motor fronts under basal conditions but inhibits fed motility. Octreotide is a cyclic analog of somatostatin that is administered by subcutaneous injection and which induces small intestinal phase III-like contractile activity. Nocturnal dosing may improve small intestinal motor function in patients with scleroderma with small bowel involvement [208]. In patients with gastroparesis, octreotide initiates phase III activity, which originates in the small intestine and inhibits postprandial antral contractility [209]. Thus, octreotide delays gastric emptying and may worsen symptoms in patients with gastroparesis. Clinically, it may be used for small bowel dysmotility. In this case, prokinetic agents are administered before meals and octreotide is given at bedtime. Prolonged usage may result in gallstones from inhibition of gallbladder emptying.

Refractory gastroparesis

There is no uniform way to handle patients with refractory gastroparesis – patients not responding to standard antiemetic and prokinetic agents or with side effects preventing their use. In one study of 110 patients with "refractory" gastroparesis [1], 74% responded to use of another prokinetic agent and only 26% were refractory to all prokinetic agents. These truly refractory patients underwent enteral or parenteral feedings and gastric electrical stimulation, and some underwent gastrectomy. Poor responders to prokinetic agents include postgastrectomy patients, those with myopathic connective tissue disorders, type I diabetic patients with severely delayed gastric emptying, and patients with idiopathic gastroparesis with abdominal pain [1].

An initial approach to refractory gastroparesis includes ensuring that delayed gastric emptying is present, optimizing the patient's current therapy, and changing prokinetic agents [187]. Why some patients respond to one prokinetic agent and not another is uncertain. Different prokinetic agents have different mechanisms of action (see Table 39.6) and the efficacy of most prokinetic agents diminishes with prolonged use. A "drug holiday," as used for L-dopa in Parkinson disease, can be used for prokinetic agents.

In patients who remain refractory to treatment, one may consider placement of a venting gastrostomy or a feeding jejunostomy. Newer therapies might include gastric electrical stimulation and injection of botulinum toxin into the pylorus. TPN, if used, should be temporary because of the risk of infection and deep venous thrombosis. Surgical subtotal gastric resection, usually accompanied by a Roux-en-Y diversion, should generally be discouraged. Completion gastrectomy can be considered for selected patients with postsurgical gastroparesis.

Combination prokinetic therapy

Prokinetic agents use different mechanisms to enhance gastric emptying (see Table 39.6). Addition of a second prokinetic agent may augment the response of a first agent. Combination prokinetic therapy with two different agents may be useful to accelerate emptying and reduce symptoms in some refractory gastroparetic patients. The combination of the available prokinetic agents, metoclopramide with erythromycin, or domperidone with erythromycin have not been studied.

Newer prokinetic agents

Newer prokinetic agents being tested for gastroparesis include 5-HT_4 receptor agonists, dopamine receptor antagonists, CCK receptor antagonists, motilin receptor agonists, and, more recently, ghrelin receptor agonists.

Tegaserod, a partial 5-HT_4 receptor agonist, has been shown to accelerate gastric emptying in normal subjects and in patients with gastroparesis [210]. However, its effect on symptoms was marginal. Tegaserod was removed from the market in March 2007 because of cardiovascular side effects. Other 5-HT_4 receptor agonsts are being studied.

Clonidine, an α_2-adrenergic agonist, has been reported to decrease symptoms and to accelerate gastric emptying in patients with diabetic gastroparesis [211]. Other studies have shown that clonidine reduces dyspeptic symptoms by improving gastric accommodation [212]. Some studies report that clonidine delays gastric emptying [213]. A side effect is hypotension; clonidine should be used cautiously, especially in diabetic patients with autonomic dysfunction.

Acetylcholinesterase inhibitors, such as physostigmine and neostigmine, may stimulate GI contractility by increasing acetylcholine levels with subsequent muscarinic receptor activation. Anticholinesterase agents may increase gastric contractility, but they may not increase antroduodenal coordination; therefore, they may not increase emptying [214]. In animal studies, GI transit is affected in an inverted U-shaped dose–response curve with acceleration at lower doses but inhibition at higher doses [215]. This dual effect may be related to activation of different muscarinic receptor subtypes. Some H_2 receptor antagonists, such as ranitidine and nizatidine, have been reported to have anticholinesterase activity [178,179].

Psychotropic medications

Tricyclic antidepressants appear to be of some benefit in patients with functional vomiting and gastroparesis [215,216];

they may be helpful for the nausea, vomiting, or abdominal pain. They are used at lower doses (median 50 mg/day) than in the treatment of depression. Low doses may reduce afferent sensory transmission. Some of these agents, especially the tertiary amines such as amitriptyline (Elavil), have anticholinergic properties and may delay gastric emptying. In general, desipramine and nortriptyline are used in patients with gastroparesis because of their reduced anticholinergic effects. Future prospective controlled trials will define the role of this group of medications in the management of gastroparesis.

Botulinum toxin injection into the pyloric sphincter

Gastric emptying is a highly regulated process reflecting the integration of propulsive forces of proximal fundic tone and distal antral contractions with inhibitory forces of pyloric sphincter resistance. Few studies have been performed to see whether inhibition of the pyloric sphincter muscle accelerates gastric emptying. Early studies with surgical pyloromyotomy did not show benefit; more recent studies have shown a beneficial response in diabetic gastroparesis [217]. Pyloromyotomy may be helpful in preventing gastric stasis after truncal vagotomy.

Botulinum toxin is a potent inhibitor of neuromuscular transmission and has been used to treat spastic disorders of muscles by local injection into affected muscles. Injection of botulinum toxin directly into the lower esophageal sphincter reduces pressure and improves symptoms in patients with achalasia [218]. Initial case series included small numbers of patients with idiopathic and diabetic gastroparesis in whom botulinum toxin was injected into the pylorus in an attempt to reduce gastric outlet resistance and accelerate gastric emptying [219–221]. These series showed a mild improvement in gastric emptying and a modest improvement in symptoms after injection of botulinum toxin. A subsequent, much larger case series included 63 patients and demonstrated that approximately 40% of patients experienced clinical benefit from pyloric injection, which lasted a median of 5 months [222]. Results from placebo-controlled trials have shown that, although botulinum toxin injection into the pylorus may mildly accelerate gastric emptying, there is little difference in symptom improvement with botulinum toxin compared with placebo [223,224].

Alternative and unconventional medical therapy primarily for nausea and vomiting

Ginger, a traditional Chinese herbal remedy, is used to treat nausea and vomiting postoperatively [225] and nausea and vomiting during pregnancy [226]. Ginger reduces nausea and associated tachygastria caused by circular vection, an experimental model of motion sickness [227]. Ginger has also been shown to reduce hyperglycemia-induced gastric dysrhythmias and nausea [228].

Stimulation of the PC6 acupuncture point on the wrist with conventional acupuncture, electroacupuncture, or acupressure with a wrist band (e.g., Sea-band) may help to control postoperative nausea, chemotherapy-induced nausea, and nausea associated with morning sickness [229–231]. These studies also suggest that acupressure has similar efficacy to standard antiemetic therapy for postoperative nausea [229]. Another acupuncture point that has been used for influencing gastric motility is the Zusanli point (ST36) located on the leg below the patella [230]. Electroacupuncture reduces the severity of symptoms of vection-induced motion sickness while decreasing gastric tachyarrhythmias [231]. Animal studies suggest that electroacupuncture enhances gastric emptying by vagal stimulation. Electroacupuncture has been postulated to modulate the release of serotonin, substance P, and endogenous opiates in central nervous system pathways [232].

Venting gastrostomy and feeding jejunostomy tubes

In the treatment-refractory patient with severe nausea and vomiting, placement of a gastrostomy tube for intermittent decompression by venting or suctioning may provide relief to the patient who has prominent abdominal distention. The gastrostomy tube can be opened for drainage or aspirated to decompress the stomach; this can be employed when symptoms are distressing to alleviate nausea, pain, and bloating. Venting gastrostomy tubes may be placed by the endoscopist, surgeon, or interventional radiologist. In one series, six of eight patients were able to return to full-time work or school [232]. The percutaneous endoscopic gastrostomy tube can be converted to a button. Gastrostomy tubes are used much less now that refractory patients can be treated with gastric electrical stimulation; the gastrostomy tube may interfere with placement of the gastric stimulating electrodes.

Use of a gastrostomy tube for feeding may aggravate gastroparetic symptoms. A feeding jejunostomy is usually preferred. Although a J-tube extension can be placed through the gastrostomy, these often prolapse into the stomach. To prevent this, enteral feedings through a direct jejunostomy tube can be helpful. Jejunostomy tubes are effective for providing nutrition, fluids, and medications, provided that the small intestine is functioning [233,234]. Carefully regulated nutrient enteral infusion may allow better glucose control in diabetic patients whose glycemic control is otherwise poor because of vomiting and gastroparesis [234]. Jejunostomy tubes are usually inserted laparoscopically or at laparotomy [235]; in some centers they are placed endoscopically.

Gastric electrical stimulation

Gastric electrical stimulation is an emerging treatment for refractory gastroparesis. There are several techniques for stimulating the stomach. First, in gastric electrical pacing the goal is to entrain and pace the gastric slow waves with

low-frequency, high-energy, long-duration pulses. Pacing at 10% higher than the basal rate has been shown to accelerate gastric emptying and to improve dyspeptic symptoms [236]. Second, stimulation with high-frequency, low-energy, short-duration pulses has been shown to decrease symptoms with little effect on gastric emptying [237,238]; it may affect proximal stomach function and activate sensory afferent nerves to reduce symptoms. Third, sequential muscle stimulation using bursts of suprahigh-frequency stimulation to induce direct gastric muscle contractions in a peristaltic sequence has accelerated emptying in animal studies [239]. More recently, sequential gastric neurostimulation of the stomach has been used to entrain gastric slow waves.

High-frequency gastric electrical stimulation at 12 cycles/min has received FDA humanitarian approval for treatment of chronic, refractory nausea and vomiting secondary to diabetic or idiopathic gastroparesis. Stimulating wires are placed into the gastric muscle at the greater curvature during laparoscopy or laparotomy. These leads are attached to an electric stimulator ("pacemaker"), which is placed in a subcutaneous abdominal pouch. An initial study demonstrated effectiveness in 20 of 26 patients with a decrease in nausea and vomiting at 3 and 6 months; gastric emptying of liquids, but not solids, was improved [237]. In long-term follow-up, 3 of 24 patients underwent total gastrectomy because of unsatisfactory results, and three had the stimulator removed because of erosion or infection. A subsequent study reported on 33 patients with chronic gastroparesis [238]. After implantation, the electrical stimulator was turned on or off in a randomized, double-blind, crossover design. Patients felt better when the stimulator was on, although the decrease in vomiting that occurred was not statistically significant. Long-term follow-up over 1 year showed a decrease in the mean vomiting frequency from 25 to 6 times per week. In addition, there was an improvement in the overall quality-of-life score and a mild improvement in gastric emptying. Complications were infection in three patients and electrode penetration of the stomach wall in one patient. An open-label study in 25 patients reported an overall improvement from "severe" to "moderate" in symptoms of nausea and vomiting of 40% [240]. There was an infection rate of 15%, requiring removal of the stimulator. Carefully designed studies are needed to determine the overall effectiveness of gastric electrical stimulation, which type of patient will likely respond, and the optimal stimulation parameters. Preliminary studies suggest that the patients most likely to respond are those with diabetic gastroparesis, main symptoms of nausea and vomiting, and those not taking regular narcotic analgesic medications [241].

Surgical treatment

Surgical treatment of idiopathic and diabetic gastroparesis has generally been disappointing [16]. Surgery is performed only as a last resort. The procedure usually employed is a partial gastrectomy and Roux-en-Y gastrojejunostomy.

For selected patients with postsurgical gastroparesis in whom medical therapy has failed, further resection of the stomach may occasionally be considered. Usually, these patients have had a previous vagotomy. Postsurgical gastroparesis patients require radical surgery to eliminate hold-up in the atonic stomach and to enable the creation or revision of a Roux limb to prevent enterogastric reflux. The resection is performed as a completion of the gastrectomy or a near-total gastrectomy, rather than as a less extensive resection [242,243]. In near-total gastrectomy, only a small rim of proximal stomach (< 1 cm) is retained. Reconstruction is achieved by a Roux loop of at least 45 cm to the rim of the stomach. The extensive gastrectomy is to eliminate the gastroparetic stomach and to allow ingested nutrients to pass directly from the esophagus into the small intestine. The Roux-en-Y jejunal segment helps to prevent reflux of small intestinal contents into the remaining stomach and esophagus. This extensive surgery may lead to improvement in two-thirds of patients in specialized centers [242].

In a series of 62 patients who underwent completion gastrectomy for severe postvagotomy gastric stasis [244], good symptomatic improvement in nausea, vomiting, and postprandial abdominal pain was obtained in only 43%. There was no improvement in chronic pain. The combination of nausea, need for TPN, and retained food at endoscopy predicted a poor outcome.

In another series of 81 patients with severe postsurgical gastroparesis [245], near-complete gastrectomy with a 55-cm Roux-en-Y reconstruction was performed. Follow-up averaged 56 months for 52 patients; 78% reported improvement of their GI symptoms, 7% believed that there was no change, and 15% stated that their condition had worsened.

A subtotal (70%) gastrectomy with resection of the antrum and pylorus, closure of the duodenum, and restoration of GI continuity with a 60-cm Roux-en-Y jejunal loop was reported in four patients with insulin-dependent diabetes with intractable vomiting from gastroparesis [246]. In total, three of the four patients did well, eliminating frequent hospital admissions.

Pancreatic transplantation for diabetic gastroparesis

Pancreatic transplantation and simultaneous kidney and pancreatic transplantation are being performed more often for insulin-dependent diabetes mellitus. With successful pancreatic transplantation, postprandial hyperglycemia resolves, and insulin therapy can be discontinued [241]. Simultaneous pancreatic and kidney transplantation helps to correct both uremia and hyperglycemia. Pancreatic transplantation has been shown to halt progression and slightly improve diabetic polyneuropathy [240]. Improvement during the first year

after pancreatic transplantation may be primarily the result of correction of hyperglycemia, which affects nerve function. A relatively small degree of improvement in peripheral nerve function has been reported over time [247]. Gastric emptying may be improved in some patients after pancreatic transplantation, although the literature is sparse. One study reported an improvement in liquid emptying in six of eight patients with previously delayed emptying, but no improvement in solid phase emptying at 6 months after pancreatic transplantation [241]. Another study found that emptying was improved 1 year after pancreatic and kidney transplantation in 8 of 23 patients [248]. Despite limited improvement in gastric emptying, symptoms and gastric dysrhythmias improved in diabetic patients after organ transplantation [241,248].

Disorders with rapid gastric emptying

Postsurgical dumping syndrome

Description and clinical features

Rapid gastric emptying and its consequences are responsible for *dumping syndrome*, symptoms of which include postprandial sweating, weakness, orthostasis, tachycardia, and diarrhea. Dumping syndrome, especially of liquids, occurs almost exclusively after gastric surgery. It affects up to 20% of patients after gastrectomy (usually antrectomy) or truncal vagotomy with a drainage procedure (see Table 39.5). Postsurgical pathophysiological abnormalities causing the dumping syndrome include the following: (1) loss of proximal stomach receptive relaxation and accommodation secondary to vagotomy; (2) decreased gastric capacity after gastrectomy; (3) loss of controlled emptying from bypass or ablation of pyloric sphincter; (4) loss of duodenal feedback inhibition resulting from a bypassed duodenum from gastrojejunostomy [249,250].

Dumping symptoms are often characterized as early or late relative to their time course after meal ingestion. Early dumping symptoms occur in the first 30 min and result from accelerated early gastric emptying of liquids with rapid filling of the intestine with hypertonic fluid, leading to bloating, crampy abdominal pain, and explosive diarrhea. Rapid filling of the intestine is associated with osmotic fluid shifts into the gut lumen and reduction of plasma volume resulting in secondary release of vasoactive substances that cause vasomotor symptoms such as lightheadedness, sweating, flushing, and palpitations with tachycardia. Late dumping symptoms occur 2–3 h after a meal and are associated with reactive hypoglycemia (weakness, palpitations, and diaphoresis); these result from the hyperinsulinemic response to an overwhelming carbohydrate load [251]. Because of its relatively long half-life, increased serum concentrations of insulin and associated hypoglycemia persist for relatively long periods.

Rapid gastric emptying of solids has been demonstrated in some patients with unexplained nausea, vomiting, bloating, and fullness [252]. Enhanced emptying of gastric contents has also been reported in some patients with functional dyspepsia [253]. Rapid gastric emptying also occurs in some diabetic patients, particularly in the early stages of type II diabetes [254]. Many of these patients have symptoms that are indistinguishable from those of gastroparesis. Rapid movement of food from the stomach into the small bowel with small bowel distention may explain the pain and nausea, symptoms similar to those described in patients with delayed gastric emptying. Rapid emptying has been recently observed as an accompanying factor in adult patients with cyclic vomiting syndrome [255,256]. In one study, rapid gastric emptying was more common than delayed gastric emptying in symptomatic patients with autonomic dysfunction [257].

Diagnosis

Dumping syndrome is usually diagnosed clinically, based on the characteristic symptoms occurring in patients after gastric surgery. Another postsurgical disorder is postvagotomy diarrhea; in this disorder the diarrhea is not related to meals, and postprandial hypotension and hypoglycemia are not seen. Scintigraphic studies of both the solid and liquid phase can document rapid gastric emptying. The rapid emptying is most notable immediately after the meal, occurring in the first 15 min [257]. In patients with suspected dumping syndrome, gastric emptying of liquids may be the preferable measurement. Orocecal transit time may also be helpful.

Treatment

Postsurgical dumping symptoms may resolve over time with conservative management. Specific treatment of the dumping syndrome is aimed at slowing delivery of nutrients into the small intestine and minimizing release of endogenous vasoactive substances. Initial dietary recommendations are to eat small, dry, frequent meals, to increase protein and fat content in the diet, to reduce the intake of carbohydrates by avoiding simple sugars, and to minimize liquid intake [249]. Lying down in a supine position after drinking liquids may delay liquid emptying. Increasing the viscosity of liquid meals by adding gel-forming carbohydrates (pectin or guar gum) may delay transit time and may slow carbohydrate absorption. Late dumping symptoms from hypoglycemia are usually relieved by ingestion of simple carbohydrates.

Medical therapy has centered on delaying gastric emptying. Anticholinergic agents have been employed, but they are usually not beneficial. The somatostatin analog, octreotide, is the primary pharmaceutical agent used for treatment. Octreotide has a much longer half-life than somatostatin and is administered subcutaneously to prevent breakdown by gastric acid. Octreotide has effects on both GI transit and

GI peptide release. Octreotide delays gastric emptying and prevents the accelerated emptying seen in dumping syndrome [249]. Octreotide also inhibits the release of enteric hormones and insulin secretion; this may actually be the primary mechanism of its symptomatic benefit [251]. Octreotide, 50 μg subcutaneously 30 min before meals, reduces the symptoms (diarrhea, lightheadedness, and palpitations) and signs (orthostatic hypotension, tachygastria) and metabolic alterations (late hypoglycemia) seen with dumping syndrome [251]. The dose may be increased to 100 μg three times a day if needed. The side effects of octreotide when administered subcutaneously include injection site pain, iatrogenic diabetes, malabsorption with a worsening of diarrhea, and cholelithiasis. Two studies have reported the long-term utility of octreotide and use of the newer long-acting depot form of octreotide [258,259].

The fatty acid oleic acid, administered before a meal, may slow GI transit and may decrease diarrhea in patients with rapid GI transit including dumping syndrome. Oleic acid activates nutrient-triggered intestinal inhibitory feedback mechanisms (jejunal–ileal brake) [260].

Revisional gastric surgery is performed in a few patients with severe dumping symptoms, with the aim of delaying stomach emptying to alleviate symptoms [261]. The results are variable and often disappointing [249]. Because of the risk of causing severe gastric stasis, surgery is recommended only when other measures have failed. Surgical procedures include takedown of a previous gastrojejunostomy with conversion to Billroth I gastroduodenostomy. A commonly used surgical procedure is creating a Roux-en-Y diversion, which has been reported to slow gastric emptying [260]. Insertion of an antiperistaltic jejunal segment between the stomach and the small bowel has been performed to produce a functional delay of nutrient outflow from the stomach [260]. Pyloric reconstruction has also been attempted.

Other disorders with rapid gastric emptying

Type II diabetes mellitus

Accelerated gastric emptying has been reported in early *type II diabetes* and rarely in early type I diabetes [58,59]. Although this condition primarily applies to liquids, rapid emptying of a high-carbohydrate solid meal may occur. This may result from impaired fundic accommodation from vagal neuropathy with reduction of inhibitory nitric oxide nerves [56]. Accelerated gastric emptying may result in enhanced intestinal absorption of glucose and increased postprandial glucose levels, in part because of a mismatch between absorbed food and insulin release. Excessive hepatic glucose release also contributes to postprandial hyperglycemia [59].

Slowing gastric emptying has been suggested to improve control of postprandial glucose in type II diabetes [262]. This may be the means by which the addition of dietary soluble fiber has been shown to improve glucose control [263,264]. Agents that slow gastric emptying are being used to improve glucose tolerance in diabetic patients; these include amylin and its analog pramlintide, and glucagon-like peptide-1 [262].

Pancreatic exocrine insufficiency

In *pancreatic insufficiency*, accelerated gastric emptying of fatty meals has been reported [265]. Maldigestion and malabsorption of lipids may lead to the inability of unhydrolyzed fats to release CCK, which serves as a feedback inhibitor of gastric emptying. Pancreatic enzyme replacement slows the rapid gastric emptying [266]. Other studies report normal gastric emptying in pancreatic insufficiency [266]. Chronic pancreatitis has also been reported in association with delayed gastric emptying (see Chronic pancreatitis) [105].

Duodenal ulcer

Some patients with *duodenal ulcer* have rapid gastric emptying of liquids with increased acid secretion [267]. Rapid gastric emptying of acid may exceed the duodenal buffering capacity leading to mucosal injury. One study reported that enhanced gastric emptying was still present in patients with healed duodenal ulcers. Many of these studies were performed before the recognition that *H. pylori* is an important factor in ulcer disease and acid secretion.

Zollinger–Ellison syndrome

Patients with *Zollinger–Ellison syndrome*, characterized by hypergastrinemia and increased gastric acid secretion, can have duodenal ulcers, diarrhea, or marked esophagitis. The diarrhea is usually attributed to massive gastric acid secretion leading to an increased amount of liquids propelled into the small intestine. These patients may have accelerated gastric emptying of liquids. However, gastric acid suppression may not normalize the rapid gastric emptying, suggesting that the rapid emptying may not be related to gastric acid hypersecretion [268]. Pentagastrin administration also does not accelerate gastric emptying in physiologically normal subjects; instead, it seems to delay stomach emptying of solids [268].

Hyperthyroidism

Orocecal transit time is rapid in *hyperthyroidism* and may be a factor in hyperthyroid-associated diarrhea [269]. It returns to normal after reestablishment of the euthyroid state. Rapid gastric emptying and intestinal transit have been reported in hyperthyroidism [144]; however, most patients with hyperthyroidism appear to have normal gastric emptying [270] and some have delayed emptying [145].

Obesity

Morbidly obese patients may have accelerated gastric emptying [271]. Dieting with weight loss has been shown to decrease this accelerated gastric emptying to a near normal rate [271]. Gastric volumes are also larger than in normal-weight persons; this may result in satiety signals not being triggered in

response to meal ingestion and gastric distention. In addition, intestinal absorption of nutrients may be more efficient in obesity [272].

Functional dyspepsia

Definition

Dyspepsia refers to symptoms originating in the upper GI tract; the term is used to describe upper abdominal pain or discomfort often exacerbated with eating, early satiety, postprandial abdominal bloating or distention, and nausea [273]. Some examples of structural or biochemical disorders causing dyspepsia include gastroduodenal ulcer, gastritis, GERD, medication side effects, and gastric cancer. In many patients the cause of dyspepsia is idiopathic and it is otherwise termed functional or nonulcer dyspepsia. Recently, the Rome III criteria for functional dyspepsia were developed [274]. The committee developed a general definition of functional dyspepsia for the purposes of clinical management, which includes the following requirements:

1 The presence of one or more of the following: bothersome postprandial fullness, early satiation, epigastric pain, epigastric burning.

2 No evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms.

To satisfy the criteria for functional dyspepsia it is required that these features be fulfilled for the previous 3 months with symptom onset at least 6 months before diagnosis [274].

It should be emphasized that there may be a substantial overlap between patients categorized as having functional dyspepsia and the presence of gastroesophageal reflux disease (GERD). The use of validated reflux questionnaires as well as a trial of adequate acid suppression therapy may be useful to distinguish these two disorders [275]. In some patients, functional dyspepsia may coexist with GERD. This often becomes evident after relief of GERD symptoms with acid suppressive therapy. The following section will address the roles of gastric motility and sensory perception in functional dyspepsia.

Pathogenesis with emphasis on gastric dysmotility

Numerous mechanisms have been proposed for the pathogenesis of functional dyspepsia [276]. The *gastric acid* or *inflammation hypothesis* suggests that gastric acid or inflammation from acid, bile, or *H. pylori* infection is responsible for symptoms. This acid hypothesis also includes occult GERD. The *motor disorder hypothesis* suggests that gastric motility disorders such as gastroparesis, impaired fundic accommodation, antral distention, or gastric dysrhythmias are important. The *visceral hypersensitivity hypothesis* proposes exaggerated symptoms in response to physicochemical stimuli such as distention, contraction, acid, and bile. The *psychological* or *psychiatric hypothesis* proposes that symptoms are related to depression, anxiety, or a somatization disorder.

Gastrointestinal dysmotility

There are several pathophysiological alterations of gastric motility and sensation in functional dyspepsia. Delayed gastric emptying, impaired gastric accommodation to a meal, gastric dysrhythmias, and visceral hypersensitivity are important pathophysiological factors in functional dyspepsia. One or more of these factors may be detected in one-half to two-thirds of patients with functional dyspepsia [277].

Distinct pathophysiological abnormalities may be present in subgroups of patients, perhaps causing different symptoms, and may predict different responses to treatment. One study found that 23% of patients with functional dyspepsia had delayed gastric emptying with associated symptoms of nausea, vomiting, and postprandial fullness, 35% of patients had hypersensitivity to gastric distention associated with symptoms of pain, belching, and weight loss, and 40% had impaired accommodation associated with symptoms of early satiety and weight loss [277]. In total, 23% of patients had two or three of these abnormalities, whereas 26% had none, suggesting still another as yet unidentified mechanism of functional dyspepsia. Unfortunately, individual symptoms do not strongly predict underlying pathophysiology [277]. These disorders may be interrelated: reduced fundic accommodation may lead to increased wall tone in response to distention or meal ingestion and may lead to stimulation of visceral afferents and cerebral perception of gastric events/symptoms [278].

Delayed gastric emptying and antral hypomotility

Delayed gastric emptying is present in approximately 35% of patients with functional dyspepsia, with a range of 25%–80% [6,277]. Specific dyspeptic symptoms and their severity correlate poorly with the degree of gastric stasis [279–281]. However, in a series of 343 patients with functional dyspepsia, the severity of postprandial fullness and vomiting correlated with delayed gastric emptying, which was present in 34% of the dyspeptic patients [6].

Detection of delayed gastric emptying in functional dyspepsia may have prognostic importance because these patients may respond better to prokinetic agents than patients with normal emptying [21]. However, the role of prokinetic agents in improving gastric emptying and symptoms is inconsistent; in some cases both delayed emptying and symptoms may be improved by prokinetic therapy, whereas at other times either emptying or symptoms have improved but not both [21].

Antroduodenal manometry has been used to study patients with functional dyspepsia. The most common abnormality, present in about 50% of patients, is postprandial antral hypomotility [282]. Antral hypomotility correlates

with delayed gastric emptying. Several studies have suggested that small bowel motor dysfunction occurs in a large proportion of patients with nonulcer dyspepsia [282]; other investigators have reported small intestinal dysmotility in only a small percentage [283]. Small intestinal abnormalities may include retrograde, simultaneous, or reduced frequency of fasting MMC activity, as well as a reduced percentage of MMCs that start in the antrum. In the fed state, decreased or low-amplitude duodenal contractions may occur. In a study of 41 consecutive patients with severe chronic dyspepsia, a neuropathic disorder (contractions of normal amplitude but abnormal propagation) was present in 39%, myopathy (low-amplitude contractions with normal coordination) in 2%, and normal small bowel motility in 59% [284].

Regional gastric dysfunction: impaired fundic accommodation and antral distention

Regional gastric function abnormalities may be present in many dyspeptic patients and appear to correlate with dyspeptic symptoms. Normally, the stomach accommodates a meal by the relaxation of the gastric fundus and corpus, providing the meal with a reservoir and enabling a volume increase without a rise in intragastric pressure. Reduced postprandial fundic relaxation and accommodation were found in 40% of patients with functional dyspepsia [285]. Impaired proximal gastric accommodation was associated with early satiety and subsequent weight loss and may be related to a vagal defect. Other studies have not found a clear relationship between postprandial symptoms and proximal stomach function [286].

Scintigraphy and ultrasound studies have shown that, in functional dyspepsia, the intragastric distribution of the meal is characterized by rapid transit of ingested food from the proximal stomach to the distal stomach (Fig. 39.3) [287]. Rapid transit from the proximal stomach may result from poor receptive relaxation or accommodation of the proximal stomach. In a study of 25 patients with functional dyspepsia, rapid proximal gastric transit after ingestion caused abnormally large quantities of food to immediately enter the antrum and remain for a prolonged period [288]. Other studies have confirmed that early antral distention occurs in many dyspeptic patients [287,289]. The degree of postprandial abdominal discomfort has been related to the degree of impaired accommodation of the proximal stomach and antral distention [290].

Radionuclide imaging of the gastric wall with single photon emission computed tomography (SPECT) to calculate gastric volumes has been used as a noninvasive measure of gastric accommodation [278]. SPECT imaging in 32 patients with functional dyspepsia suggested that impaired gastric accommodation (41%) may occur more commonly than delayed emptying (9%) in dyspeptic patients referred to tertiary centers.

Satiety testing with water has been used as a noninvasive technique to evaluate impaired accommodation and sensation. For the water load test, subjects drink water until full (maximum tolerated volume) [291]. Functional dyspeptic patients consume a reduced volume even after adjustment for body mass index [292,293]. Another option for satiety testing is the use of a caloric drink (Nutridrink; NV Nutricia, Zoetermeer, The Netherlands) ingested at 15 mL/min [294]. The maximum tolerated volume consumed during the satiety test has been correlated with gastric accommodation to a meal, as measured by the barostat. Satiety tests therefore offer the potential to evaluate gastric accommodation in a noninvasive way. However, reduced maximum tolerated volume may reflect either impaired accommodation or hypersensitivity of the stomach [24] as there is a poor correlation between maximum tolerated volume and total gastric volume during fasting as measured by SPECT scanning [288,295].

Gastric myoelectric abnormalities

Gastric myoelectric abnormalities measured by EGG, such as tachygastria, bradygastria, and a decreased ratio of postprandial to fasting power, are found in about 40% of patients with functional dyspepsia [41,291,295,296]. The prevalence of EGG abnormalities is similar to that of delayed gastric emptying in functional dyspepsia. EGG and gastric emptying tests appear to complement each other in correlating symptoms to gastric dysmotility [41]. Multichannel EGG recording using electrodes placed at several positions overlying the stomach has been suggested to assess slow wave propagation and coupling. Indeed, patients with functional dyspepsia were frequently found to have inconsistencies in frequency and regularity of gastric slow waves suggesting impaired propagation and coupling [297]. At present, despite these advances, EGG studies are not performed at most centers, and have been a disappointing tool in the management of patients with dyspepsia.

Visceral hypersensitivity

Augmentation of visceral afferent sensation (nociception) may be a major cause of symptoms in functional dyspepsia. In total, 35%–50% of patients with functional dyspepsia have increased sensation to gastric and small intestinal distention [5,298]. When the stomach is stimulated by increasing volumes or increasing intragastric pressures, patients with functional dyspepsia experience symptoms at volumes that physiologically normal subjects do not (allodynia), and they have greater amounts of pain at levels when the physiologically normal subjects begin to have pain (hyperalgesia). The symptoms in functional dyspepsia may result from altered gastric perception with an exaggerated visceral sensory perception of normal physiological events. In addition to hypersensitivity to distention, some dyspeptic patients may be hypersensitive to intragastric or intraduodenal acid, bile salts, or even nutrients [299].

Brain activation by visceral stimulation with gastric distention is being studied in functional dyspepsia using positron emission tomography and functional magnetic resonance imaging [300]. In normal subjects, distal gastric distention produces symptoms of abdominal pain, nausea, and bloating, and it activates some of the same cortical and subcortical central nervous system regions implicated in processing somatic pain [300]. It is conceivable that patients with functional dyspepsia may have abnormal activation of these cortical and subcortical sites in response to gastric distention.

Treatment of functional dyspepsia

There is much literature available on the treatment of functional dyspepsia, although it is inconsistent. Methodological shortcomings are present in many studies including suboptimal study design, unclear inclusion criteria, unvalidated outcome measures, and a short duration of follow-up [301]. In addition, the placebo response in functional dyspepsia can be substantial, approximating 45% [302]. It has been hypothesized that the variability in the efficacy and safety of agents used for the treatment of functional dyspepsia may be due to genetic polymorphisms or differences in the activity of cytochrome P450 isoenzymes [303]. Treatment modalities that have been tested extensively include acid-suppressive agents, promotility compounds, nociceptive modulating compounds, and *H. pylori* eradication. The following sections discuss the therapeutic approach focusing on gastric dysmotility and nociception.

Treatment focusing on gastric dysmotility and nociception

Promotility agents

The relative lack of available promotility compounds limits this treatment modality. Promotility agents including metoclopramide, cisapride, and domperidone have improved dyspeptic symptoms more effectively than placebo in the majority of studies [304]; improvement in symptoms with promotility agents has been 40%–45% greater than with placebo [304]. The few studies that compare the effects of acid-suppressing and promotility agents on dyspeptic symptoms have favored the promotility agents [305].

Several metaanalyses of studies of prokinetic agents in functional dyspepsia have been published [304,306,307]. In a metaanalysis of 17 cisapride studies and four domperidone studies, both cisapride and domperidone seemed to be efficacious [307]. For cisapride, there were overall reductions in epigastric pain, early satiety, abdominal distention, and nausea [308].

Erythromycin and other motilin receptor agonists are potent stimulants of gastric motor function. Intravenous erythromycin significantly enhances solid and liquid gastric emptying; however, it does not reduce meal-related dyspepsia symptoms [309]. Similarly, other motilides have not demonstrated efficacy in functional dyspepsia. The motilin receptor agonist, ABT-229, was ineffective in dyspeptic patients with delayed gastric emptying [310]. It is speculated that, because motilin receptor agonists increase proximal gastric tone, they may aggravate the impaired fundic accommodation in functional dyspepsia, and this could explain the worsening of postprandial symptoms with ABT-229 [310].

Psychotropic medications, sensory modulators, and antinociceptive agents

Strategies to inhibit pain or symptom perception (sensory modulators) are receiving attention because of the augmented visceral hypersensitivity in dyspepsia.

Low-dose tricyclic antidepressants appear to have analgesic effects that are independent of psychological effects. The mechanism of action of these agents in functional bowel disorders is not known, but it may include a reduction in visceral sensitivity [311]. Tricyclic antidepressants have been beneficial in treating abdominal pain syndromes, noncardiac chest pain, and irritable bowel syndrome [312]. Several studies using low-dose tricyclic antidepressants for functional dyspepsia [313,314] have reported a decrease in dyspeptic symptoms, primarily abdominal pain.

In clinical practice, psychotropic medications have been suggested for patients with severe, intractable, refractory dyspeptic symptoms that interfere with daily activities. Dosages required for symptomatic improvement are usually lower than those used to treat psychiatric disorders [311]; the general approach is to start with a low dose, with gradual dose escalations in small increments if required. Typically, for amitriptyline, a starting dose is 10–25 mg at bedtime. If benefit is not observed after several weeks, doses are increased by 25-mg increments up to 75–100 mg. The positive effects are rapidly noticed by many patients, in contrast to the prolonged treatment needed to improve depression. Significant side effects of antidepressant agents are common and can interfere with management, leading to a change in medication in 25% of patients [311]. The sedative effects of many tricyclic compounds may be helpful for patients with difficulty in sleeping. Agents with strong anticholinergic properties, such as amitriptyline, may adversely affect gastric motility. The secondary amines, nortriptyline (Pamelor) and desipramine (Norpramin), have fewer side effects than amitriptyline (Elavil).

There are no published studies demonstrating the utility of the selective serotonin reuptake inhibitors for functional dyspepsia, although some studies have reported their use in irritable bowel syndrome [315]. Interestingly, paroxetine (Paxil) appears to enhance gastric accommodation rather than improve the visceral hypersensitivity [316].

Strategies to inhibit pain or symptom perception also include the use of κ-opioid agonists and 5-HT_3 receptor antagonists. Fedotozine, a κ-opioid agonist, was tested in patients with functional dyspepsia and irritable bowel syndrome.

Concern was expressed on the fairly modest symptom improvement and the exact mechanism of action. Other κ-opioid agonists are now being studied.

5-HT_3 receptors are located on vagal afferents and dorsal root ganglion neurons. In normal subjects, the 5-HT_3 receptor antagonist, alosetron (Lotronex), reduced postprandial nausea and vomiting in response to a dyspepsia-inducing meal, without affecting gastric accommodation or the amount of ingested food [317]. Preliminary studies suggest that alosetron may be mildly effective in decreasing symptoms of functional dyspepsia [318]. Alosetron was FDA approved for treating irritable bowel syndrome. It was withdrawn because of reports of cases of ischemic colitis and is currently available for clinical use in the United States under controlled protocols for patients with diarrhea-predominant irritable bowel syndrome.

Fundic relaxing agents

Agents that relax the fundus and improve the accommodation response may be helpful in some patients with functional dyspepsia, perhaps those with early satiety. 5-HT_1 agonists (sumatriptan, buspirone), α-adrenergic receptor agonists (clonidine), and nitric oxide donors (glyceryl trinitrate) have been tried in several small series [212].

The 5-HT_1 agonists, such as sumatriptan, induce relaxation of the gastric fundus through a nitric oxide-mediated pathway. Sumatriptan allows larger volumes to be accommodated before perception or discomfort is reached, and it improves meal-induced satiety in patients with functional dyspepsia [319]. It is administered by injection, and recently became available orally. Buspirone (Buspar), an oral 5-HT_1 agonist, may have effects similar to those of sumatriptan. It also has anxiolytic properties.

The α-adrenergic agonist clonidine reduces proximal gastric tone and pain perception during gastric distention in healthy subjects [212]. Thus, clonidine has the potential to reduce gastric sensation or increase gastric compliance without altering other physiological motor functions. Some studies have suggested that clonidine may delay gastric emptying [213].

Sildenafil (Viagra) has been shown to increase gastric accommodation [320]. In animal models, it also relaxes the pylorus and improves gastric emptying [321]. In humans, however, it slows gastric emptying [319].

Summary

In this chapter, gastric motility disorders have been reviewed, focusing primarily on the pathophysiology, diagnosis, and treatment of gastroparesis, dumping syndrome, and the gastric dysmotility seen in functional dyspepsia. Refractory symptoms may lead to a comprehensive evaluation including gastric emptying scintigraphy, antroduodenal manometry, EGG, and possibly tests of gastric accommodation or compliance. This evaluation may guide treatments that target the underlying pathophysiological mechanisms.

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B STOMACH

40 Peptic ulcer disease

Andrew H. Soll, David Y. Graham

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Introduction

Peptic ulcers are defects in the gastrointestinal mucosa extending through the muscularis mucosae, which persist and are generally dependent on the acid–peptic activity of gastric juice. The major causes are infection with *Helicobacter pylori* (Hp) and consumption of nonsteroidal antiinflammatory drugs (NSAIDs), including aspirin (Table 40.1). Although the incidence of Hp ulcers has rapidly declined in developed countries and treatment has been greatly facilitated by potent antisecretory agents, peptic ulcer remains a major challenge to patients, clinicians, investigators, and payors.

Etiology and pathophysiology of peptic ulcer

Normal mucosal defense and healing

The regulation of acid–pepsin secretion reflects an intricate balance between inputs from neural, endocrine, paracrine, and autocrine pathways and involves both stimulatory and inhibitory mechanisms. The normal gastroduodenal mucosa has a remarkable ability to defend itself against injury and to rapidly repair injury when it does occur (Fig. 40.1). Chronic ulcer disease only occurs when these repair and healing mechanisms fail.

Ulcer pathogenesis

Considering the aggressive nature of the acid–peptic environment and the noxious agents we ingest, ulcer disease is surprisingly uncommon. Even among individuals at high risk, such as those with Hp infection, the yearly incidence of new clinical ulcer disease is only about 1%. No pathogenic mechanisms have been identified distinguishing the small subset who develop ulcers from those who do not, and there are not good explanations for the focal distribution of ulcers nor for their intermittent and recurring course.

Pathogenesis is multifactorial. Duodenal, but not gastric, ulcers are associated with a high duodenal acid load, and acid secretion is also typically high-normal or high. Duodenal ulcer (DU) is also associated with decreased duodenal defenses, manifest by the presence of duodenal inflammation and the related decreased duodenal bicarbonate secretion [1] (Fig. 40.2). Although environmental factors such as smoking and stress affect the duodenal acid load (e.g., smoking inhibits duodenal bicarbonate secretion), they also have other effects such that their impact on pathogenesis remains incompletely understood.

Pathology of peptic ulcers

Erosions are superficial mucosal defects. In contrast, the defect in ulcers extends through the muscularis mucosa into the submucosa or muscularis propria. Acute lesions, such as those occurring with aspirin injury, are generally multiple and shallow, with minimal surrounding inflammation or fibrosis, and generally heal rapidly. In contrast, chronic ulcers are usually single and surrounded by inflammation and fibrosis. They heal slowly and tend to recur in the same location. Gastric ulcers (GU) typically occur on the lesser curvature near the angularis at the transition of oxyntic and antral mucosa [2,3]. More proximal gastric ulcers are typically located at the junction of the atrophic border as Hp gastritis encroaches on the acid-secreting oxyntic mucosa [4]. Ulcers in the gastric cardia or in a hiatal hernia pouch have been hypothesized to be a distinct entity [5], and in some series they account for up to 10% of GU. Cameron ulcers, initially described in association with anemia, occur on the tips of folds in the region of a hiatal hernia [6].

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Table 40.1 Etiologies and disease associations for peptic ulcer

Ulcers due to defined mechanisms

Infection:
- *Helicobacter pylori*
- HSV
- CMV
- *H. heilmannii*

Other rare infectious causes: e.g., tuberculosis, syphilis

Drug exposure:
- NSAIDs and aspirin
- Corticosteroids (when combined with NSAIDs)
- Bisphosphonates
- Clopidogrel
- Mycophenolate mofetil
- Potassium chloride
- Chemotherapy (e.g., hepatic infusion with 5-fluorouracil)

Hormonal or mediator-induced, including acid hypersecretory states:
- Gastrinoma (Zollinger–Ellison syndrome)
- Systemic mastocytosis
- Basophilia in myeloproliferative disease
- Antral G-cell hyperfunction (existence independent of *H. pylori* is controversial)

Vascular insufficiency including crack cocaine use

Mechanical: duodenal obstruction (e.g., annular pancreas)

Radiation therapy

Infiltrating disease: sarcoidosis, Crohn's disease

Idiopathic peptic ulcer

Idiopathic hypersecretory (*H. pylori*-negative) duodenal ulcer

Non-*H. pylori*, non-NSAID familial peptic ulcer

Non-*H. pylori*, non-NSAID peptic ulcer

Comorbid ulcers associated with decompensated chronic disease or acute multisystem failure

Stress (ICU) ulcers

Chronic obstructive pulmonary disease

Cirrhosis

Renal failure

Organ transplantation

Other comorbidity, such as cardiovascular disease

CMV, cytomegalovirus; HSV, herpes simplex virus; ICU, intensive care unit; NSAIDs, nonsteroidal antiinflammatory drugs.

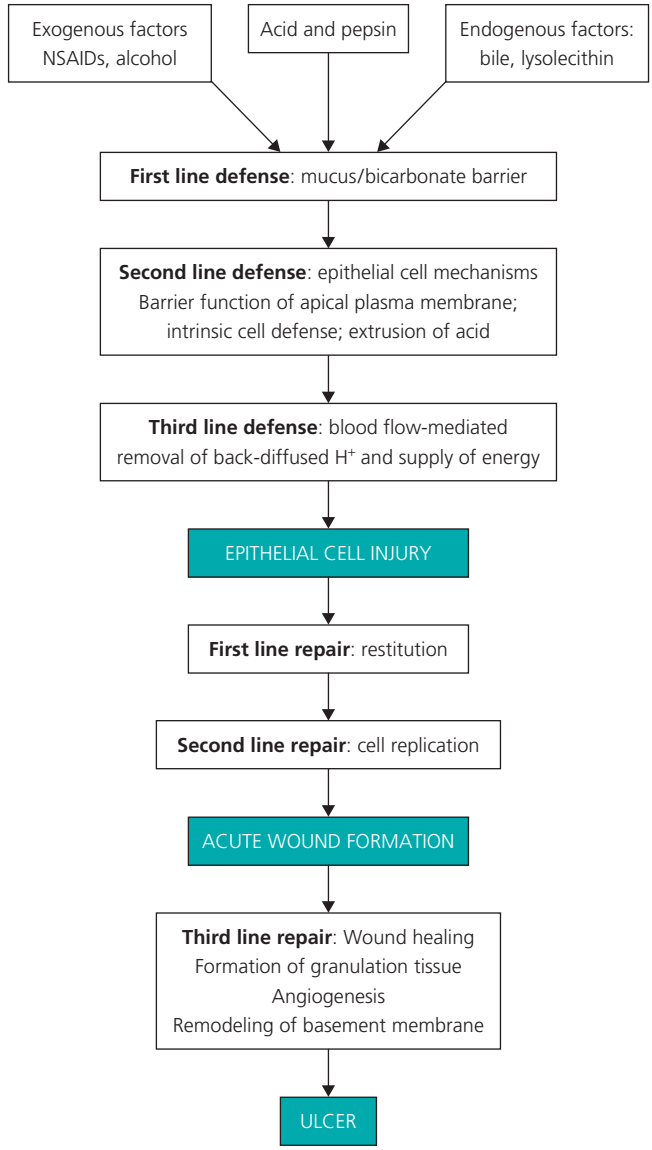


Figure 40.1 Cascade of mucosal defense and repair mechanisms. Damaging effects on epithelial cells of exogenous and endogenous factors are amplified by acid peptic activity. If the three sets of lines of defense mechanisms fail, epithelial cell injury occurs, which will be repaired by restitution and cell replication. If these repair mechanisms fail, an acute wound forms. Ulcers form when these normal defense and repair mechanisms fail or are overwhelmed. NSAIDs, nonsteroidal antiinflammatory drugs.

Helicobacter pylori and peptic ulcer

It is well accepted that, worldwide, Hp and NSAIDs account for the large majority of cases of peptic ulcer disease (PUD). However, the proportions associated with these specific causes vary greatly with demographics and are changing rapidly over time, especially in developed countries. Several studies in the United States have demonstrated rates of Hp infection of less than 75% in those with DU not associated with the use of NSAIDs. Studies from Rochester, New York, for example, found that, after excluding NSAID use, only 61% of DU and 63% of GU were Hp positive [7]. Only 52% of white people with DU were Hp positive, compared to 85% of nonwhite people, underscoring the differences in Hp prevalence in different populations.

Helicobacter pylori, gastritis, and peptic ulcer

Hp is a gram-negative spiral bacteria whose niche is the human stomach (see Chapter 42). The infection causes progressive gastric inflammation and has been etiologically related to gastritis, PUD, gastric adenocarcinoma, and primary

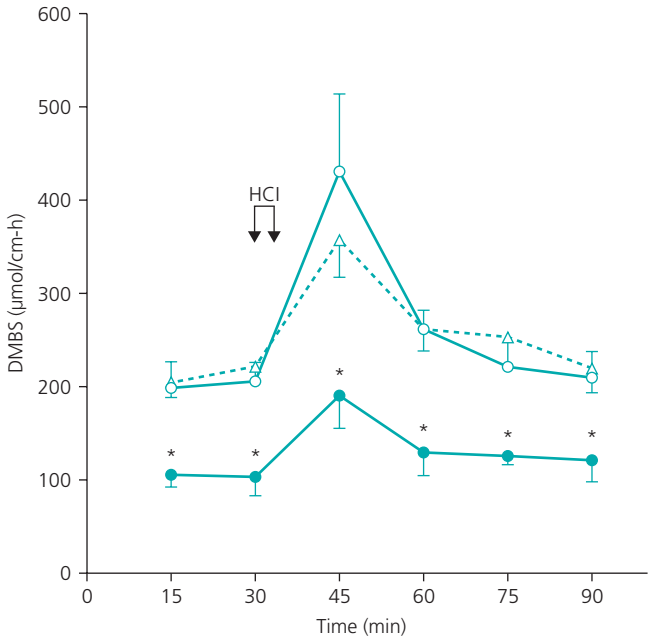


Figure 40.2 Duodenal bicarbonate output in response to cure of *Helicobacter pylori* (Hp) infection. Proximal duodenal bicarbonate secretion (DMBS) is shown for normal subjects (open circles), Hp-positive duodenal ulcer (DU) subjects before antibiotic treatment (solid circles), and DU subjects after successful Hp cure (open triangles). Data are mean (± SE) for basal bicarbonate output (15 and 30 min) and in response to acid stimulation. Redrawn from Hogan et al. [1], with permission from Elsevier.

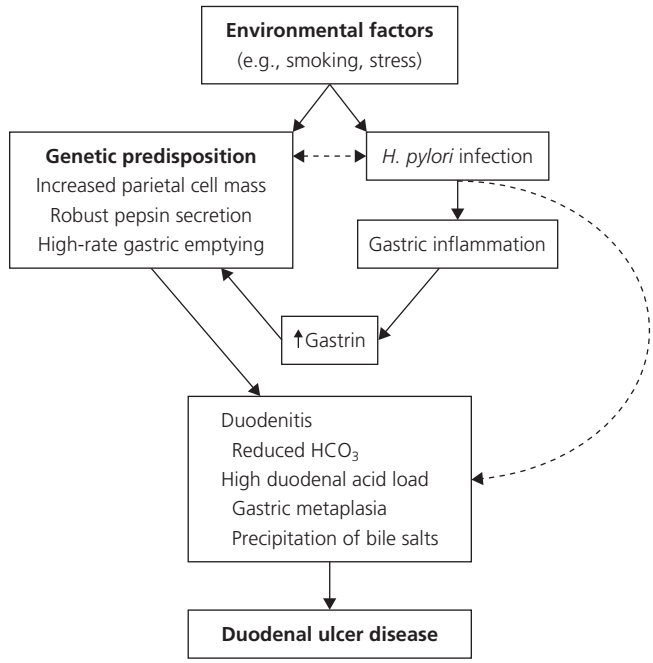


Figure 40.3 Hypothesis regarding the pathogenesis of duodenal ulcer disease showing the interaction of environmental, host, and *Helicobacter pylori* factors leading to an increased duodenal acid load, increased gastric metaplasia in the duodenum, and infection of that metaplastic epithelium, resulting in a duodenal ulcer. Redrawn from Graham [8], with permission from Elsevier.

gastric B-cell lymphoma. Other forms of peptic ulcer (e.g., NSAID, gastrinoma, or stress ulcer) occur without gastritis in the surrounding mucosa. Hp-associated DU is associated with gastric inflammation largely restricted to the antrum, thereby sparing the acid-secreting body of the stomach and allowing robust acid secretion. In contrast, GU are associated with antral and corpus gastritis and, as noted above, typically occur just proximal to the line of demarcation between the advancing atrophic border and the oxyntic mucosa. The natural history of Hp gastritis is to progress into the oxyntic mucosa leading to hyposecretion of acid and pepsin and eventually to atrophy. In contrast, oxyntic mucosal inflammation usually progresses slowly, or not at all, in DU subjects. However, inhibition of acid secretion with either vagotomy or antisecretory therapy releases this block and promotes progression of oxyntic gastritis, which may lead to gastric atrophy [8–11].

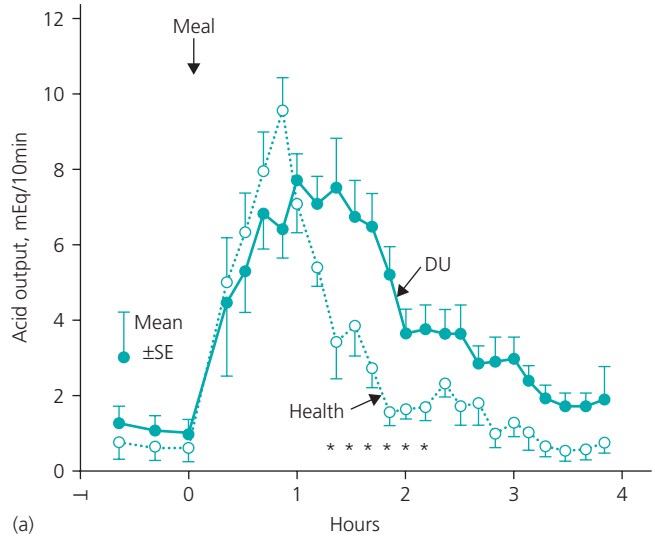
Helicobacter pylori, duodenitis, acid secretion, and gastric metaplasia in duodenal ulcer

Acid causes duodenal damage in DU and gastrinoma resulting in an increase in gastric metaplasia. Hp is trophic for gastric epithelium and, when Hp is found in the duodenum, it is exclusively localized over gastric-type epithelial cells [12–14]. Therefore, Hp-related DU is actually a "gastric mucosal" ulcer occurring in the duodenum. The complex relationship (Fig. 40.3) has additional elements. For example, bile salts inhibit Hp but are precipitated at low pH. Thus, Hp may thrive in the duodenum in individuals where robust acid secretion, usually coupled with low duodenal bicarbonate secretion, lowers duodenal pH, thereby precipitating bile salts and removing their Hp-inhibitory effects [15,16].

Acid secretion

"Peptic" ulcers are by definition dependent upon the acid and peptic activity in gastric juice. An adequate level of acid secretion (12–15 mEq/h) is necessary for DU [17,18], and inhibition of acid secretion promotes healing of both duodenal and gastric ulcers. When ulcers occur in the absence of acid, other factors deserve consideration, including NSAID use, neoplasia, and infection other than Hp [19–21]. However, GU may occur in the face of very low acid concentrations.

The average maximal acid secretory response to stimulation, which reflects an increased parietal cells mass, is greater in DU patients than in normal subjects [22–27]. Gastrin is an important trophic factor for oxyntic mucosa, stimulating oxyntic mucosal growth and clearly accounting for increased secretory mass in gastrinoma patients (see Chapter 41). However, eradication of Hp reverses Hp-associated hypergastrinemia without a significant reduction in parietal cell mass [28].



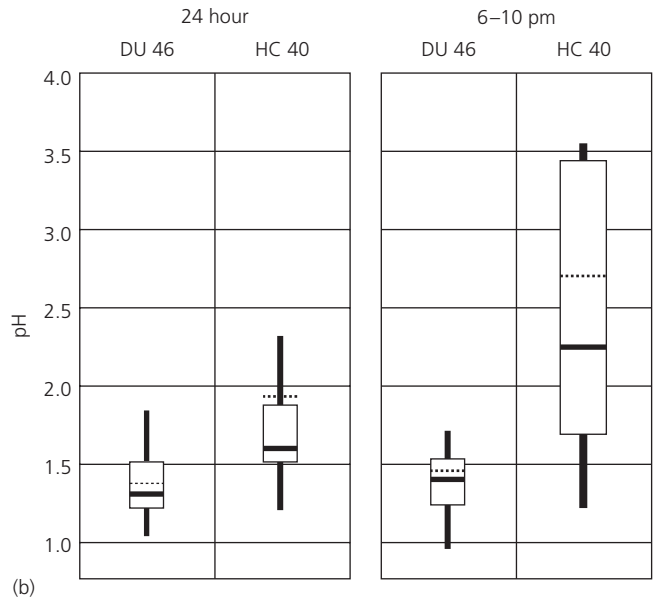


Figure 40.4 **(a)** Acid secretion in response to a meal, comparing duodenal ulcer (DU) and normal subjects. The consistent difference is not the peak acid secretory response, but a more sustained response in DU subjects. This difference is more pronounced in the evening hours, presumably due to circadian rhythm. (From Malagelada et al. [385], with permission from Elsevier.) **(b)** pH in duodenal ulcer: 24-h (left panel) and nocturnal (right panel). Box plots illustrate the median intragastric pH for DU patients and healthy controls (HC). On the left are data for the 24-h period and on the right are data for the evening hours from 6 to 10 PM. Dotted lines show the mean and the solid line the median of the individual values. The box contains the interquartile range and the whiskers stretch to the furthest value within one interquartile distance from the first and third quartiles. The data illustrate the considerably increased difference in gastric pH in DU that is most evident in the evening postprandial period. From Merki et al. [32], with permission.

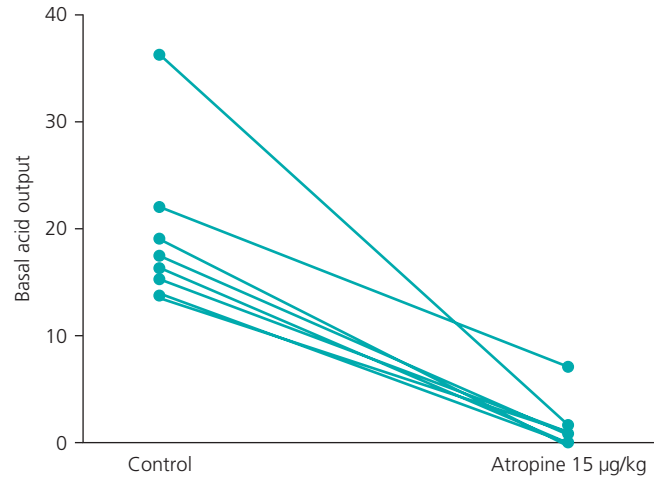


Figure 40.5 Inhibition of basal acid hypersecretion in a subset of duodenal ulcer (DU) patients by intravenous atropine. DU subjects with basal acid hypersecretion were evaluated before and after administration of atropine (15 μg/kg). Marked inhibition was noted in all of these subjects. Data redrawn from Kirkpatrick and Hirschowitz [40].

A number of perturbations of gastric physiology have been hypothesized to underlie acid hypersecretion in DU, including enhanced and prolonged meal-stimulated gastric acid secretion (Fig. 40.4a) and impaired inhibitory mechanisms [24,29–31]. The result of these abnormalities is that the acid secretory response persists long after the food buffer has emptied. The difference in acid secretion or gastric pH is most evident in the evening or night-time hours [32] (Fig. 40.4b). Although the pathogenesis of these abnormalities is incompletely understood, most have all proved to be reversible following eradication of Hp infection [33,34]. The major exception is the acid hypersecretion that persists in a small subset of subjects.

Hypersecretion in Hp-positive DU occurs in at least two subsets: those with hypergastrinemia that is Hp-dependent (reversed with antibiotics, i.e., Hp-positive antral G-cell hyperfunction) and those with acid hypersecretion that appears to be independent of Hp [35–37]. Sometimes the Hp-independent acid hypersecretion is modest, but it can on occasion mimic gastrinoma [39]. Vagal hyperactivity has been hypothesized as an underlying mechanism. In a group of DU subjects with basal acid hypersecretion, atropine was found to inhibit basal acid secretion [40] (Fig. 40.5), implicating the importance of cholinergic pathways. A rare patient may have hypergastrinemia in the absence of gastrinoma and Hp.

Proximal GU, in contrast to GU in the distal antrum, are usually associated with low acid secretion, reflecting a low or low-normal parietal cell mass and the presence of corpus inflammation [3,17,22]. These findings correspond to the encroachment of oxyntic mucosa by advancing antritis and

oxyntic gland atrophy [4,41]. In contrast to proximal GU, patients with distal antral GU or GU associated with DU have normal or even increased levels of acid secretion.

Pepsin and peptic ulcer

Despite the fact that ulcers of the stomach and duodenum are referred to as "peptic," pepsin and peptic activity have received little attention. Acid plus pepsin is much more ulcerogenic than acid alone [42], leaving little question that the "peptic" in peptic ulcer appropriately reflects the critical pathogenic role of proteolytic activity in gastric juice. Peptic activity is closely linked to gastric pH for two reasons. First, the precursor pepsinogen is converted into the active protease at low pH. Second, pepsin is inactivated when the pH is elevated above 4. This pH dependence probably accounts for the healing of refractory ulcers when the pH is elevated above 4. Hp infection is largely responsible for the hyperpepsinogenemia that occurs in peptic ulcer. The degree of hyperpepsinogenemia reflects acid secretion and has been used to help predict the risk for peptic ulcer in Hp-infected subjects [43].

Abnormalities of mucosal defense and healing in peptic ulcer

Impaired healing of mucosal breaks and ulcers appears to be the factor that differentiates patients with chronic ulcer disease from those exposed to the same risk factors (Hp, NSAIDs, or elevated acid secretion) who do not develop ulcers. However, there is no evidence implicating any primary derangement in epithelial repair, ulcer healing, or key mechanisms of mucosal defense in ulcer pathophysiology (Fig. 40.1).

Defense mechanisms are compromised by the presence of Hp and Hp-induced inflammation, and by the NSAID-induced reduction in mucosal prostaglandin synthesis. However, the abnormalities associated with Hp or NSAIDs do not in themselves explain ulcer pathogenesis because they are present throughout the mucosa and in the many subjects who do not develop focal peptic ulcers.

Ulcers tend to recur locally. Wyatt and others have hypothesized that the gastric metaplasia in the duodenum explains the localization of both Hp-induced inflammation and ulcer formation [8,13]. However, there is no good explanation for the localization of ulcers in the stomach. A hypothesis that we consider attractive is that dense fibrosis occurs at the site of ulceration in predisposed subjects and this fibrosis prevents complete healing and mucosal remodeling and predisposes to local recurrence.

Impaired mucosal blood flow is clearly an important factor in ulcer formation, at least in a subset of subjects. Blood flow through normal mucosa removes acid that has diffused across an overwhelmed or injured epithelium and delivers the metabolic energy necessary to support transport and other functions. Maintaining mucosal blood flow at adequate levels for these demands is critical for mucosal integrity. Using reflectance spectrophotometry, mucosal blood volume was found to be decreased in patients with active gastric ulceration compared with those with healing or healed ulcers [44]. However, as with other potential mechanisms, changes were generalized in the antrum and body and not limited to the region or margin of the ulcer. Of interest, when healing had commenced, blood volume at the margins of healing ulcers increased compared with measurements at a distance from the crater.

There is little doubt that the initiation of ulcer healing is marked by an ingrowth of new blood vessels (angiogenesis) into the granulation tissue in the ulcer bed. Pathologically, chronic ulcers are surrounded by fibrinoid necrosis and fibrosis; this is the zone accounting for the decrease in blood flow around nonhealing ulcers [45]. The increase in blood flow at the edge of a healing ulcer probably reflects angiogenesis, and the failure of blood flow to increase probably reflects the failure of normal angiogenesis. Ulcer persistence is thus marked by failure of blood vessel ingrowth into this granulation tissue and, conversely, ulcer healing commences with angiogenesis in the granulation tissue and an increase in blood flow at the ulcer margin [45].

Ulcers from NSAIDs and platelet-active agents

Superficial damage, endoscopic ulcers, and clinical ulcers

NSAID-induced mucosal damage is categorized as either superficial damage consisting of petechiae (intramucosal hemorrhages appearing like "blood under plastic wrap" [46]), erosions, and small, shallow ulcers, or "clinical" ulcers, which can be defined either by presentation in a clinical setting with or without complications or by having a size and depth to clearly be of clinical relevance. Cross-sectional studies of individuals chronically consuming full-dose NSAIDs reveal normal endoscopic appearances in about 35%, erosions or petechiae in about 50%, and endoscopic ulcers in 5%–30% (depending upon defining criteria) [47,48]. In chronic cases, ulcers frequently are present in the absence of superficial injury.

Many studies of the natural history of NSAID-induced mucosal damage and of preventive strategies have focused only on acute superficial injury and erosions or small acute ulcers. Such lesions are especially amenable to "prevention" with acid-suppressive therapies and are not good surrogates for clinical ulcers with respect to natural history or response to therapy. It behooves clinicians to remain skeptical of "marketing" claims based on such surrogate markers (see Prevention of NSAID ulcers).

Mechanisms of NSAID injury

Petechiae and superficial erosions reflect topical mucosal injury in which, in the acidic environment of gastric juice, weak acids are not ionized and freely penetrate the gastric barrier to become concentrated in mucosal cells, thus

amplifying potential damaging effects. Superficial lesions may cause occult bleeding and rarely can cause chronic iron-deficient anemia. Superficial lesions rarely, if ever, cause clinically significant acute GI bleeding.

Clinical NSAID ulcers are related to the systemic effects of NSAID administration. Such damage occurs irrespective of the route of administration of the NSAID (e.g., oral, rectal, or parenteral) and can occur even in patients with achlorhydria. The leading hypothesis is that the systemic action of NSAIDs that produces ulcers is the reduction of mucosal prostaglandin production, which limits the ability of the mucosa to defend itself against injury from superimposed factors.

Helicobacter pylori, NSAIDs, and the peptic ulcer diathesis

The interaction between NSAIDs and Hp is controversial and complex. The confusion is not surprising because Hp causes a spectrum of gastric consequences ranging from antral-predominant gastritis with robust acid secretion, which predisposes to duodenal ulcer formation, to pangastritis with achlorhydria, in which the Hp peptic ulcer risk is nil. Patients with Hp and low acid secretion may even be partially protected from NSAID ulceration, whereas the subset with active or subclinical ulcer disease would be at high risk from NSAID complications. As noted below, 1%–4% of asymptomatic Hp-positive subjects without an ulcer history have silent peptic ulcers evident at surveillance endoscopy; presumably this is the group that would be at the highest risk with exposure to NSAIDs.

The most convincing evidence for the Hp exacerbation of gastrointestinal complications from NSAIDs comes from two controlled trials in which patients who were Hp positive and NSAID-naïve were randomized to Hp eradication treatment or placebo [49,50]. Hp cure lowered the incidence of endoscopic ulcer formation, clinical ulcer development, and complications. The second trial entered a higher risk group with a history of dyspepsia or previous ulcer and followed them for 6 months on NSAIDs; complicated ulcers were reduced from 27% in the control group to 4% in the eradication group.

In patient populations other than those naïve to NSAIDs, the relation to Hp and the benefits of eradication are less clear. A metaanalysis examining observational studies found synergism between Hp and NSAIDs in producing both peptic ulcer and ulcer bleeding [51]. However, there are negative studies that point against a causal relation between Hp and NSAID ulcer risk. Some of these studies were carried out with low-risk patients who had previously been on NSAIDs or examined only endoscopy end points [52], so these conclusions are without much weight [53].

One point is critical. In patients with a history of previous NSAID-induced complications, even if Hp is found and eradicated, the risk of complications on subsequent NSAID therapy is very high. The one exception may be low-dose aspirin treatment. In one controlled trial [54], Hp-positive patients resuming conventional NSAIDs plus placebo after Hp eradication had a 6-month ulcer recurrence rate of 24% compared with 4% in patients treated with NSAIDs plus a proton pump inhibitor (PPI). It should not be surprising that, if an ulcer is caused by Hp, failure to eradicate Hp is associated with a high rate of recurrence. Similarly, if one has an NSAID-induced ulcer and restarts NSAIDs, one should not be surprised that the ulcer recurs. In fact, one would be amazed if eradication of Hp entirely prevented recurrent NSAID ulcers. Aspirin use appears to be different from the use of other NSAIDs; patients who resumed low-dose aspirin after Hp eradication had a recurrence rate at 6 months of only 1.9% compared with 0.9% for those who resumed aspirin after being treated with a PPI without antibiotics [54].

We advise testing and treating Hp infection before starting NSAIDs in naïve users, in patients with a history of a previous ulcer or ulcer complications, and in those who will receive PPI cotherapy. As there are other potential benefits to curing Hp infection, we favor at least offering patients Hp testing and treatment before initiating NSAIDs.

Aspirin vs NSAIDs: platelet vs gastric and intestinal mucosal effects

Aspirin, even at low doses, increases the risks of ulcer complications and GI bleeding [55]. Although it is clear that aspirin can cause peptic ulcers, many bleeding complications and even perforation from aspirin occur at sites other than the stomach and duodenum [56]. Bleeding from these sites most likely reflects the effects of aspirin on platelet function and bleeding time. It is of interest that a subset of patients with a history of bleeding induced by aspirin have markedly prolonged bleeding times on aspirin [57], suggesting a predisposing factor that has received little attention from clinicians or investigators. Other NSAIDs vary in their effects on platelet function and bleeding time. It is important to keep in mind that other NSAIDs also cause small intestinal and colonic damage, which can result in clinical complications.

COX-1 vs COX-2 inhibition

Factors that may determine NSAID propensity for mucosal damage and ulcer formation include relative potency for inhibiting gastric cyclooxygenase 1 (COX-1) compared with COX-2, duration of action, solubility of the NSAID in gastric juice, the gastric absorption of NSAIDs that are soluble in gastric juice, and the concentration by pH partition of weak acid NSAIDs in gastric mucosa. Potency, duration of action, and relative COX inhibition appear to be the most important factors.

Controlled trials with COX-2-selective inhibitors (coxibs) have demonstrated a reduction in risk of clinical peptic ulcers and their complications [58]. Valdecoxib and rofecoxib were removed from the market by the US Food and Drug Administration (FDA) because of data indicating increased risk of thrombotic cardiovascular disease. Although there are

concerns about the cardiovascular risks of celecoxib, especially at higher doses, it remains on the market at present. However, the benefit of celecoxib in reducing the risk of ulcer complications compared with nonselective NSAIDs is less clear than with valdecoxib and rofecoxib. It is also suspected that traditional NSAIDS, with the possible exception of naproxen, also share the propensity for myocardial infarction [59,60].

Although there is evidence that coxibs have a lower complication rate for average-risk patients taking NSAIDs, coxibs may impair ulcer healing [61]. There is no evidence that coxibs have advantages over other NSAIDs for high-risk patients with unhealed clinical ulcers or with a history of previous complications, although there is some evidence that the combination of a coxib and a PPI has a lower rate of recurrent ulceration and/or complications than a PPI plus a traditional NSAID [62].

Platelet-active agents

The antiplatelet agent clopidogrel appears to have a very high risk of complications when used in patients with previous GI complications. Clopidogrel was associated with a 12-month rebleeding rate of 8.6% compared with 0.7% with aspirin plus a PPI [63]. Thus, clopidigrel may be contraindicated in high-risk patients when used alone or in combination with NSAIDs. Any antiplatelet agent should be approached with caution, especially in patients at increased risk for ulcer complications. The mechanism of action has not been established but may be related to the role that platelets play in ulcer healing and in angiogenesis in particular [64].

Risk factors for NSAID ulcers

A large number of individuals consume NSAIDs but only a very few go on to develop clinical ulcers or complications. Several risk factors have been identified that have a major impact on patient outcomes. Risk stratification is essential to make the use of NSAIDs as rational and safe as possible.

Age

Many early studies have demonstrated an increased risk of ulcer complications (hospitalization, operation, and death) from NSAIDs in elderly individuals [65–68], which has been confirmed in more recent controlled trials. Age greater than about 70 years is a factor because older individuals frequently use NSAIDs and they have comorbid diseases that compromise tolerance to complications. However, controlled studies [69,70] provide evidence that age is also an independent risk factor.

Previous history of ulcers or complications

A previous history of ulcers, especially if complicated, has the greatest impact on NSAID ulcer risk. Presumably this group is at risk because of permissive levels of acid secretion, but defects in mucosal defense and healing are also possible. Although there are no firm data available, it is likely that a high risk would be imparted by either a previous history of Hp ulcers or of "virgin" NSAID ulcers.

Duration of therapy

The risk of ulcer complications begins to emerge within the first week of therapy and the absolute risk is greatest within the first month of therapy and then remains constant [71]. Adaptation is the phenomenon by which acute injury resolves despite continued therapy [72]. However, adaptation fails to occur in the 10%–20% who develop endoscopic ulcers and in the few who develop clinically significant ulcers. The mechanisms accounting for adaptation or its failure remain unclear.

Dose and duration of action and use of multiple NSAIDs

In clinical studies, ulcer complications with NSAIDs appear to be dose dependent. However, the threshold for conventional NSAIDs, as well as for aspirin as noted above, appears to be quite low for continuous use [71]. Risk appears to be related to the potency and duration of action of suppression of prostaglandin synthesis; NSAIDs with high COX-1 antiinflammatory activity and duration of action are associated with greater risk than those whose significant antiinflammatory activity requires multiple tablets of the drug (e.g., piroxicam compared with ibuprofen). Simultaneous use of multiple NSAIDs also appears to increase the risk of complications.

Dyspepsia

Previously, it appeared that little relation existed between dyspepsia induced by NSAIDs and clinical ulcer formation. This conclusion is probably valid in unselected subjects in whom there is no predictive value of dyspepsia for either acute mucosal damage or clinical ulcer risk. However, in a controlled study, the development of new dyspepsia did predict ulcer recurrence [73,74]. Therefore, it behooves the clinician to ask patients taking NSAIDs about symptoms and to pay particular attention to changes in symptoms.

NSAIDs plus low-dose aspirin

Aspirin was observed to impair the beneficial effects of celecoxib on reducing the rate of ulcer complications [75]. Subsequent studies have further indicated that the addition of aspirin to coxibs increases the risks of complications and obviates or reduces any ulcer-sparing benefit. Presumably, the addition of aspirin will also exacerbate the risks with nonselective NSAIDs. There is also evidence that the coadministration of NSAIDs (e.g., ibuprofen) may also reduce the antiplatelet effects of aspirin [76].

Cotherapy with corticosteroids

In early studies, the impact of corticosteroids on the risk of peptic ulcer was controversial. However, the relative risk for upper gastrointestinal hemorrhage of adding steroids to

NSAIDs is 10.6-fold above that found with NSAIDs alone [77]. Steroids alone impart insignificant ulcer risk, but these agents potentiate the ulcer risk from NSAIDs. These data argue for avoiding the combination of steroids with NSAIDs whenever possible, particularly in elderly patients. There are no controlled studies indicating that cotherapy with antisecretory agents prevents complications in the face of NSAIDs plus corticosteroids, and so extreme caution is necessary.

Cotherapy with other drugs

In addition to steroids, aspirin, and platelet-active agents, interactions of NSAIDs with other drugs also increase the complication risk. Anticoagulants [78] and alendronate both dramatically increase the complication risk. Any agent that interferes with platelet function or bleeding time or with mucosa integrity or repair deserves caution until proved safe.

Comorbidity

Comorbidity also increases the risk of ulcer complications from NSAIDs [70]. It is unclear if this is a specific effect for NSAIDs or part of the general effect of comorbidity on ulcer formation (see Comorbid ulcers – Disease associations).

Other forms of peptic ulcer (non-Hp, non-NSAID)

Other specific causes of peptic ulcer have been elucidated in addition to Hp and NSAIDs, such as gastrinoma or viral infections (Table 40.1). It remains unclear whether non-Hp, non-NSAID ulcers are becoming more prevalent or are becoming more apparent as the prevalence of Hp and Hp ulcers has fallen. Although one could argue that the defined secondary forms should be listed in the differential diagnosis section, we have listed them here if they present and require treatment as peptic ulcers. However, it is important to keep in mind that several secondary forms of PUD also require specific forms of treatment.

Infections other than *Helicobacter pylori*

Herpes simplex virus type 1

Antibodies to herpes simplex virus type 1 (HSV-1) have been reported to be increased in DU patients. As herpesvirus can reside in vagal ganglia it is possible that some ulcers may represent reactivation of latent HSV infections [79]. HSV-1 DNA and viral proteins have been detected in the mucosa of the margins of a low percentage of ulcers by immunocytochemistry, in situ hybridization, and polymerase chain reaction with sequence confirmation [79]. In one report, four patients with HSV-1 markers had antral or prepyloric ulcers and none had systemic disease or were immunocompromised. A second study reported HSV-1 at the margins of 10% of GU [80]. A recent study using PCR to identify HSV-1 and a rapid urease test and histology to identify Hp found HSV-1 in 30% and 32% of DU and GU respectively [81]. HSV-1 was found more frequently in the absence of Hp. In all three studies, HSV-1 was only present in the margins of ulcers and was absent in gastric mucosa distant from the ulcer and in controls, consistent with a role for HSV-1 in ulcer pathogenesis.

Cytomegalovirus

Although the number of reports is limited, cytomegalovirus (CMV) is a well-documented cause of gastroduodenal ulcers in immunocompromised subjects and it is a common cause of ulceration in HIV patients with low CD4 counts [82]. CMV ulcers occasionally complicate, causing bleeding, perforation, or pyloric stenosis [83]. Although much more common in immunocompromised hosts, CMV can also cause ulceration in immunocompetent subjects [83]. The ulcers are frequently large and multiple.

Drug exposures

A number of drugs besides NSAIDs are probably also capable of causing or complicating peptic ulceration. Platelet-active agents, steroids, and anticoagulants were discussed above. Sirolimus has been associated with aggressive ulcer disease in patients undergoing transplantation [84]. Sirolimus is known to inhibit wound healing and has been associated with small bowel ulceration [85], so this connection is convincing and warrants cautious use of this agent in patients with a history of PUD as well as aggressive management of patients on the drug who develop symptoms or complications suggestive of PUD.

The relation of alendronate to clinically significant GU is more controversial. Damage and acute ulceration have been observed [86], but the proportion that go onto clinically significant events appears limited [87]. An impressive interaction between NSAIDs and alendronate [88] prompts caution in coadministration of these agents.

Hormonal- or mediator-induced ulcers associated with acid hypersecretion

Gastrinoma

See Chapter 41.

Systemic mastocytosis

Systemic mastocytosis is characterized by mast cell infiltration of many tissues. Symptoms include flushing, maculopapular rash, pruritus, abdominal pain, and diarrhea [89]. Dyspepsia, DU, and severe duodenitis occur in 30%–50% of these patients, typically in association with basal acid hypersecretion and elevated serum histamine levels [89,90], suggesting that circulating histamine contributes to the basal acid hypersecretion. Dyspeptic symptoms and the duodenal disease are readily treated by antisecretory drug therapy. Histamine H_2 receptor antagonists (H2RAs) and PPIs have been used with success; no comparative studies are available to guide management.

Basophilia associated with myeloproliferative disorders

Basophilic leukemia and chronic myelogenous leukemia with marked basophilia have been associated with increased acid secretion, increased serum or urine histamine concentrations, and DU [91]. Acid hypersecretion may require a threshold level of basophilia [91]. Polycythemia vera has also been associated with peptic disease [92]. It has been hypothesized that ulcers result from alterations in mucosal blood flow due to increased blood viscosity.

Antral G-cell hyperfunction

Antral G-cell hyperfunction most often occurs as an exaggerated response to Hp infection and responds rapidly to Hp eradication. However, an Hp-independent form has also been reported.

Idiopathic hypersecretory (Hp-negative) duodenal ulcer

As noted (see Laboratory evaluation – Acid secretion), gastric acid hypersecretion occurs in non-Hp, non-NSAID DU. Some cases have acid secretory rates that approach those found in gastrinoma [38]. Mechanisms of acid hypersecretion have not been established; vagal cholinergic mechanisms are a possibility that deserve further study in Hp-negative hypersecretory DU patients (Fig. 40.5). After Hp, NSAIDs, and gastrinoma have been confidently excluded, management is conservative using a PPI [93]. Occasionally it is necessary to titrate PPI dosage, as in gastrinoma.

Vascular insufficiency including crack cocaine use

Mucosal blood flow is critical to mucosal integrity and vascular insufficiency syndromes involving the arterial supply can present with gastric and also duodenal ulcers [94–96]. These ulcers often fail PPI therapy but will respond to revascularization. For example, gastric ulceration associated with stenosis of the inferior mesenteric artery responded to stenting of the inferior mesenteric artery [96]. Large ulcers and perforation of gastroduodenal ulcers has also been described with crack cocaine use, presumably secondary to mucosal vasoconstriction [97,98].

Enterocolic lymphocytic phlebitis is a rare cause of gastrointestinal ischemia apparently due to inflammation and has been reported with gastric and duodenal involvement associated with chronic nonhealing antral ulcer [99].

Mechanical: duodenal obstruction

Unusual cases of DU have been linked to a variety of duodenal abnormalities, including congenital duodenal webs, hypertrophic pyloric stenosis, annular pancreas, and preduodenal portal vein. Although DU in this setting may present in infancy or childhood, presentation in adolescence or adulthood also occurs [100]. Although annular pancreas may present at birth with high-grade duodenal obstruction, it more commonly presents in the second to seventh decades with the peak incidence in the fourth decade [100,101]. Ulcers with annular pancreas are sometimes postbulbar and have been associated with basal acid hypersecretion. The mechanisms linking these various "obstructing" lesions of the pylorus and duodenum to acid hypersecretion and ulcer disease remain undefined.

Other uncommon forms

Unusual secondary forms of ulcer include postradiation therapy, infiltrating disease, connective tissue disease, and unusual neoplastic conditions. Inflammatory conditions include Crohn's disease, Wegener granulomatosis [102], and Behçet disease [103]. Neoplastic conditions that mimic peptic ulcer include lymphomatous gastric ulcers [104], primary gastric [105] and metastatic malignant melanoma [106], and metastatic renal cell carcinoma [107].

Childhood peptic ulcer

Duodenal ulcer occurring in the first two decades often presents with nocturnal pain, and one-third of patients present with hemorrhage [108]. Diagnosis is often delayed because of the low level of suspicion. Hp is responsible for most DU in children (e.g., 82%) [109–111]. However, DU in childhood may occur in the absence of Hp [110,112].

Risk factors for peptic ulcer

Cigarette smoking

Strong evidence implicates smoking as a major risk factor associated with the occurrence, persistence, recurrence, and complications of Hp-associated peptic ulcer. Epidemiological studies have shown that smokers are at increased risk for both duodenal and gastric ulcers and that the risk is proportional to the amount smoked [113–117]. Smoking impairs ulcer healing, promotes recurrences, and increases the risk of complications and the need for surgery. Of interest, following Hp eradication, smoking ceases to be an important variable related to recurrent duodenal ulcer [118,119].

There are several candidate mechanisms by which smoking exacerbates the ulcer diathesis, including increased duodenal acid load by increasing gastric acid secretion and impairing duodenal and pancreatic bicarbonate secretion [16,120].

Alcohol

Alcohol in high concentrations damages the gastric mucosal barrier to hydrogen ions and is associated with acute gastric mucosal lesions characterized by mucosal hemorrhages. Alcohol itself stimulates acid secretion [121], and the contents of alcoholic beverages other than alcohol also stimulate acid secretion. Despite these acute effects, there is no evidence that alcohol intake causes or exacerbates chronic PUD [122,123]. Modest alcohol consumption is associated with

enhanced ulcer healing [124,125]. However, alcohol misuse interferes with patient compliance and ulcer healing [126]. Furthermore, cirrhosis, regardless of etiology, is associated with increased risk of peptic ulcer (see Disease associations).

Psychological factors

The importance of psychodynamic factors in the genesis of peptic ulcer remains controversial despite decades of study [127]. Investigation of the psychodynamic aspects of ulcer disease has been hampered for a variety of reasons including:

1 It is difficult to define ulcer occurrence and recurrence and to achieve adequate sampling and blinding. Many retrospective studies are based on self-report about the ulcer history, which could lead to bias favoring association with psychosocial factors.

2 Psychological stress is difficult to measure. Stressful events are only part of the picture; the level of distress also reflects the patient's perception of the stressful event, interpretation of implications, and coping mechanisms.

3 As the pathogenesis of ulcer disease is multifactorial, psychodynamic factors need to be correlated with known pathophysiological factors, such as infection with Hp, adequate acid secretory mass, smoking, and NSAID use.

4 Psychodynamic factors are likely to play a role only in a subset of ulcer patients, which remains undefined.

Keeping these limitations in mind, two lines of evidence strongly implicate psychosocial factors in ulcer pathogenesis. First, several recent studies have used a prospective design to address methodological limitations [127]. The most useful approach has been to track ulcer incidence (new ulcers) or ulcer recurrence in a defined epidemiological cohort. Using this type of approach, studies over the last 15 years have painted a consistent picture about the importance of several forms of psychological stress as predictors of de novo ulcer incidence or ulcer recurrence. As an example, poorly tolerated stress or depressive symptoms at baseline increased the risk of ulcer development over the next 9–15 years (Fig. 40.6) [127]. Other psychosocial factors, such as work-related stress, social problems, and posttraumatic stress disorders (PTSD), were also predictive of subsequent ulcer disease. Second, several studies have established that peptic ulcer complications are more prevalent during periods of natural disaster or societal catastrophe. The first such study was the demonstration of a dramatically increased rate of ulcer perforation during the German bombardment of London [128]. Several other studies have shown similar findings following other such horrific events [127].

Stress, anxiety, and depression have been observed to impair endoscopic healing and to promote relapse of endoscopically diagnosed ulcers [127,129]. The effects of stress seem to be reversible; patients who develop an ulcer following traumatic life events, but who are psychologically stable, tend to do well after the stress has resolved. The pathophysiological mechanisms accounting for the effects of stress on ulcer formation have not been defined. These effects could be mediated by both altered behaviors and by altered physiology. Pathogenic mechanisms for peptic ulcer are multifactorial, a fact that confounds unraveling the link between psychosocial factors and ulcerogenesis. For example, stress increases acid secretion and the effects are more prominent in patients with DU than in control subjects [130]. As a result, one must consider not only the stressor, but the individual's physiological and psychosocial response to the stress. Lastly, finding a relationship between psychosocial factors and ulcer disease does not establish causality. One study found that anxiety and neuroticism were high in a group of DU patients at the outset, but normalized in relapse-free patients at the end of a 10-year follow-up period [131]. In some cases, psychological features may be the result and not the cause of the disease process in susceptible hosts.

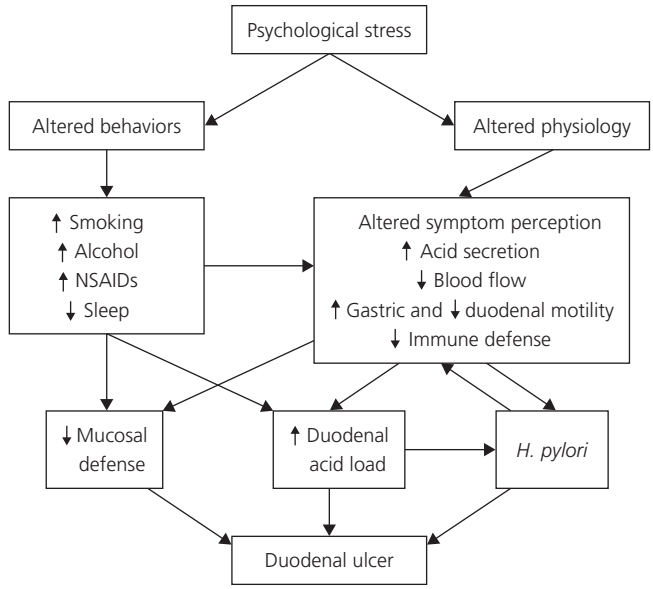


Figure 40.6 A model for the relation between psychological factors and pathogenesis of duodenal ulcer. Psychological stress can exacerbate the ulcer diathesis through altered behaviors and altered physiology. Adapted from Levenstein [127], with permission.

The genetics of peptic ulcer disease

Studies from the pre-Hp era recognized familial aggregation of PUD in a pattern suggesting polygenic inheritance. The familial aggregation of both DU and GU appeared distinct: first-degree relatives of patients with DU had a threefold increased prevalence of DU but not GU; in contrast, relatives of patients with GU had a threefold increased prevalence of GU but not DU [132]. Hyperpepsinogenemia was proposed as a marker of autosomal dominant inheritance of predisposition to peptic ulcer. Subsequent follow up of these same subjects revealed that this pattern of hyperpepsinogenemia

was in fact due to familial clustering of Hp infection [133]. Although hyperpepsinogenemia may generally be related to Hp infection, Italian investigators have identified a family in whom peptic ulcer was linked to elevated serum pepsinogen group A in the absence of Hp infection [134].

Questions were reframed for the Hp era: First, are genetic factors involved in predisposing to Hp infection or in predisposing to certain disease outcomes in infected individuals? Second, are there genetic factors that operate independently of Hp? Host factors appear to be important in predisposing to Hp infection and to disease outcomes such as DU and gastric cancer. In the setting of Hp infection, polymorphisms involving the cytokine IL-1-β [135,136], but not IL-6 [137], appear linked to DU, probably related to the effects of Hp-associated inflammation and acid secretion.

Twin studies also provide evidence for a clear genetic predisposition to PUD that is independent of any predisposition to Hp infection. An illustrative report found high concordance of a self-reported ulcer history in monozygotic compared with dizygotic twins, regardless of whether they were reared apart or together [138]. Cross-twin, cross-trait correlations for monozygotic and dizygotic twins indicated that the genetic effects for peptic ulcer were independent of the genetic influences for Hp. Another twin study also concluded that genetic factors were linked to DU independently of environmental variables [139].

There are a few rare inherited syndromes associated with PUD, such as genetic forms of gastrinoma and mastocytosis. There are other scattered reports of peptic ulcer linked to inherited syndromes, but many of these reports are dated and not well investigated and familial clustering of Hp has not been excluded.

Epidemiology of peptic ulcer

Estimates of ulcer prevalence

Prevalence is defined as the proportion of the population with a given disease at a single point in time, over a period of time, or over a lifetime. Results from the pre-Hp era are difficult to link to the current situation, in which the prevalence of Hp has markedly decreased. The prevalence of PUD must be defined as a function of Hp status and of NSAID and aspirin use. Prevalence estimates from endoscopic studies of adult volunteers indicate a prevalence rate of 1%–6% in patients who were Hp positive by at least one screening test [140], with a lifetime risk of about 10%–20% in those with Hp infection [140]. The prevalence of PUD in the subpopulation who are neither Hp infected nor NSAID users is unknown but is probably very low.

DU was infrequently recognized before the end of the nineteenth century, after which the incidence appeared to steadily increase until 1960 and then to decrease [141]. Although the pathogenesis of this phenomenon is unknown, this was also the time in history when there were marked improvements in diet, sanitation, standards of living, and household hygiene. Together these events were associated with a change in the pattern of transmission of many infectious diseases including Hp infection. The prevalence of Hp infection in 20 year olds in the United States has declined to less than 15%, which has resulted in Hp-related PUD becoming an increasingly uncommon disease. DU requires a normal or near-normal gastric corpus capable of secreting sufficient acid (e.g., 12 mmol/h) to sustain the ulcer disease. In contrast, gastric cancer and GU are associated with corpus gastritis and reduced acid secretion. It appears likely that the change in diet with increased availability of fresh fruits and vegetables, fortification of foods, and a reduction in the frequency of childhood febrile illnesses all combined to produce changes in the pattern of gastritis, which underlies the observed pattern of the appearance of DU as a "new" disease and the decline in the prevalence of gastric cancer [141,142]. The decline in Hp ulcers occurred at the same time that NSAID and aspirin use was increasing, and the current data are consistent with the notion that the rates of ulcer disease are decreasing in young subjects but are increasing in older subjects related to both residual Hp infection and use of NSAIDs and aspirin. These trends were evident in data collected in the United States from 1970 to 1990, and similar trends are also evident in data from the UK, the Netherlands, and Denmark (Fig. 40.7a,b) [143–145].

A complete understanding of the incidence and time trends in ulcer occurrence requires a detailed understanding of a number of important environmental variables including the prevalence of Hp infection, NSAID use, and cigarette smoking, all of which influence the occurrence of ulcer in an age-dependent fashion (see Etiology and pathophysiology of peptic ulcer). In addition, the epidemiological assessment of ulcer prevalence and incidence is challenging as different results may reflect different scenarios such as:

- the mode of ulcer detection (symptoms, radiography, endoscopy, hospitalization, complications, surgery, or death)
- the period assessed (the disease is intermittent)
- the care with which DU and GU are differentiated from other disorders (e.g., other peptic disorders such as esophagitis, nonulcer dyspepsia, and other causes of GI bleeding)
- the dramatic shift to outpatient management
- improved medical therapy
- changes in the criteria and coding system for mortality and hospitalization data [146].

Comorbid ulcers

Disease associations

There has been a long-standing suspicion that peptic ulcer is associated with other medical conditions [92]. However, the existence, mechanisms, and implications of these putative

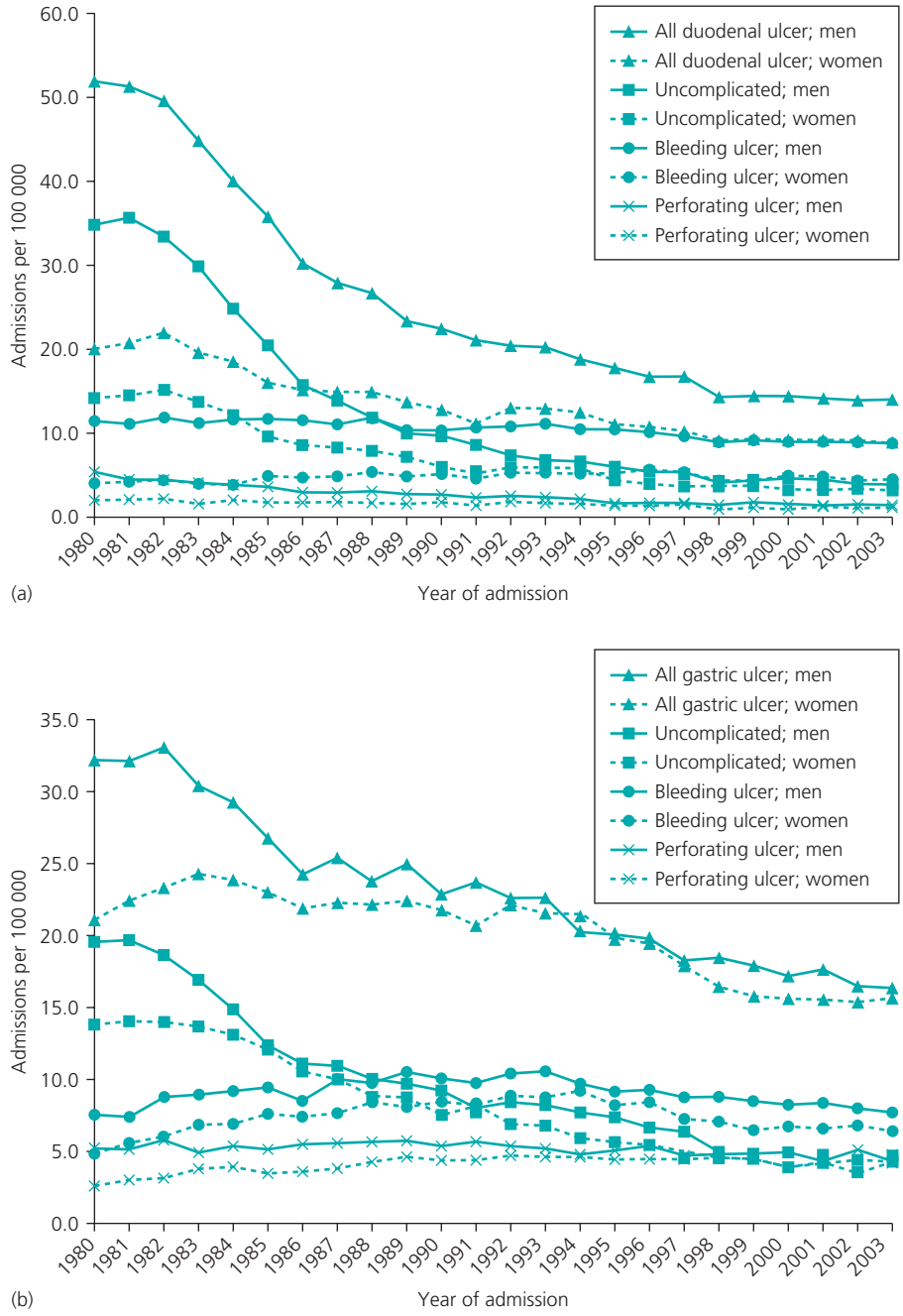


Figure 40.7 Trends in hospital admission rates in the Netherlands: **(a)** duodenal ulcer; **(b)** gastric ulcer. Data show a dramatic fall in rates of uncomplicated duodenal ulcer and gastric ulcer in men, and to a lesser extent in women. In contrast, rates of complications are relatively stable over this period. From Post et al. [145], with permission from Blackwell Publishing.

associations remain unclear and many such apparent associations may be spurious. Berkson recognized the fallacy of apparent disease association due to referral and selection bias among patients referred for a given disease [147]; studies with a hospital or referral population are likely to indicate a greater prevalence of a given disease than examining a random population sample for both diseases, alone and in combination. Furthermore, patients presenting for evaluation are more likely to be examined for other medical problems.

Any study of disease associations must also take into account known ulcer risk factors, such as Hp, NSAID use, and smoking. However, reliable detection of these factors outside of prospective studies is difficult. False-negative results for Hp are common in patients with PUD, especially if only a single test is done, and NSAID use is difficult to uncover, even with direct questioning. Furthermore, simply the presence of Hp does not guarantee that it caused the ulcer disease.

Intensive care unit stress, medical comorbidity, and peptic ulcer

The association between medical comorbidity and peptic ulcer is generally accepted for "stress ulcers" that occur in an

ICU setting. Superficial mucosal lesions are present in the majority of seriously ill patients within 18 h of admission to an ICU [148]; this finding has been called stress-related mucosal damage. However, although stress-related mucosal damage can cause low-grade GI bleeding, it rarely causes clinically significant bleeding in the absence of severe coagulopathy. Serious complications result from discrete ulcers that erode into sizable blood vessels or perforate.

The incidence of ICU stress ulcers has dramatically fallen in recent years. For example, studies of patients with severe burns three or four decades ago reported ulcer complications in more than 20% of patients, whereas the ulcer complication rate today in state-of-the-art burn units is very low. Presumably, most of this improvement is due to improved medical care, which primarily reduces pulmonary, septic, and renal complications and improves nutritional balance. These factors are probably much more important than antiulcer therapy per se. This point underlines the most important element of management of stress ulcers: treat the underlying medical condition.

The medical comorbidity of relevance to peptic ulcer occurs in two forms: chronic medical comorbidity and acute multisystem organ failure. The latter is obviously more relevant in the ICU, but we anticipate that serious medical comorbidity occurring outside of the ICU is also a very important factor in peptic ulcer pathogenesis, as considered below. We will refer to "comorbid ulcers" as those associated with either form of comorbidity. We hypothesize that comorbid ulcers are a distinct subgroup that should not be confused with idiopathic, non-Hp, non-NSAID ulcers; the epidemiology, pathogenesis, and management are likely to be very different.

There is little understanding of the epidemiology, mechanisms, and natural history of comorbid ulcers. We speculate that the most important factors are generic rather than disease specific. Probably the most important generic factors are malnutrition and a catabolic state with negative nitrogen balance interfering with tissue integrity and repair and tissue hypoperfusion and oxygenation due to hypovolemia, anemia, low vascular oncotic pressure, vasoconstriction, and poor oxygen delivery. These factors, in addition to multisystem organ failure, can be acute or subacute. As mucosal damage is very common in seriously ill patients, the impairment of tissue repair and healing may be the most critical factor in patients who make enough gastric acid and pepsin to be at risk for developing comorbid ulcers. Disease-specific factors may also be important in a subset of patients with comorbid ulcers but, with the exception, possibly, of cirrhosis, such factors have not been identified, (see section on cirrhosis).

Two recent studies of ulcer complications have observed an association with medical comorbidity that was more evident in patients with non-Hp, non-NSAID ulcers [73,149]. Hung and coworkers from Hong Kong [73] made an important observation that 16% of bleeding ulcers presenting as outpatients were Hp negative; in contrast, 42% of bleeding ulcers presenting as inpatients were Hp negative. Patients who develop ulcer bleeding as inpatients are much more likely to have serious medical comorbidity. Another study from Hong Kong also found that Hp-negative ulcers were more likely to occur in older subjects who had concomitant medical problems, malignancy, recent surgery, or sepsis [149]. We cannot answer the question of how many of these ulcers should be considered ICU ulcers related to stress of acute cormorbid disease; neither study discriminated among ulcers associated with acute organ failure, ICU admission, or stable or decompensated chronic comorbidity.

Hp-negative ulcers have been observed to have poor outcomes when compared with Hp-positive ulcers [73,149,150]. However, it is unclear whether these poor outcomes are due to the ulcer disease per se or to the medical comorbidity, which may be causing both the ulcers and the poor outcomes. Although it is certainly true that some patients die with comorbid conditions from ulcer complications for which they are probably more susceptible, we anticipate that mortality is largely due to the underlying comorbid conditions.

As noted above, studies have reported that Hp is less common in ulcers presenting in hospitalized patients than in those presenting in outpatients [73]. It is quite likely that Hp does play a role in a small number of patients, as preexisting, often clinically silent ulcers or ulcer scars may exist in a few percent of an asymptomatic Hp-positive population, especially if elderly. Such preexisting ulcers might be exacerbated with the stress of acute illness. However, we suspect that a sizable portion of patients presenting with comorbid ulcers will be Hp negative.

The following sections list several chronic conditions that have been linked to PUD. The strongest association is noted between cirrhosis and PUD, which appears to be mediated by a disease-specific mechanism. For other conditions it is unclear whether the association reflects an undiscovered disease-specific mechanism or the more generic mechanisms mentioned above. Most of the studies of this topic have only correlated the presence or absence of a given disease, rather than attempting to quantify the number and severity of comorbid conditions and overall nutritional status. Obviously, prospective studies are needed; we strongly encourage future investigators to include a more comprehensive approach to capturing comorbidity and overall status.

Chronic obstructive pulmonary disease

An association between chronic obstructive pulmonary disease (COPD) and peptic ulcers has been suspected for many years. The frequency of chronic lung disease in patients with peptic ulcer is increased 2- to 3-fold over the general population and peptic ulcers, both gastric and duodenal, occur in up to 30% of patients with chronic lung disease [92,115,151].

The association between these diseases is not influenced by the clinical severity or therapy of the lung disease. It appears

likely that the major factor underlying the association is the impact of cigarette smoking on both diseases. In one series of Japanese men living in Hawaii, cigarette smoking was associated with an increased risk of gastric and duodenal ulcer, but showed a dose–response in pack-years only for gastric ulcer [115].

Cirrhosis

The prevalence of peptic ulcer is significantly increased in patients with cirrhosis [83,152,153], with prevalence estimates ranging from 10% to 49% [154,155]. The annual incidence has been estimated to be 4.3% [153]. The ulcers are often asymptomatic but are associated with a high rate of complications and frequent recurrences.

The mechanism of this association is unknown. Alcohol ingestion is not an explanation. Although Hp has been reported to be associated with ulcers in cirrhotic patients [154,156], other studies have not found any striking association [155]. Investigators who treated Hp with antibiotics found that the high ulcer recurrence rate was not apparently different amongst the Hp-cured, persisting Hp-positive, and Hp-negative subjects [155]. The Child–Pugh class is a risk factor [154] suggesting that the severity of the cirrhosis is important.

The yearly recurrence rates are about 50%, underlining the importance of careful follow-up for patients with cirrhosis and PUD [155]. Although many of the recurrences are asymptomatic, the risk of complications is sufficiently high to warrant consideration of maintenance antisecretory therapy.

Renal failure

Earlier studies described an increased incidence of peptic ulcer in patients with chronic renal failure. However, subsequent studies have yielded conflicting data regarding the prevalence of PUD in patients with chronic renal failure [157,158]. The role of Hp eradication in the apparent change in epidemiology is unknown.

Organ transplantation

Peptic ulcer disease has been a significant cause of morbidity posttransplantation, in part because of immunosuppression, the administration of corticosteroids, and CMV infection. The use of cyclosporine (ciclosporin), antiviral therapy, and early diagnosis and treatment appear to have had beneficial effects, although the incidence of PUD remains increased and peptic ulcers are still an important source of morbidity in this population [159,160]. As noted above, some of these ulcers are stress ulcers associated with multisystem failure; the improvement reflects a general improvement in status and nutrition. Chen et al. [161] observed two risk factors for peptic ulcer in transplant patients: methylprednisolone pulse therapy and a pretransplantation history of PUD. The value of pretransplant screening is controversial and antiulcer prophylaxis is routine in most centers [162].

Clinical presentation and diagnosis of peptic ulcer

Peptic ulcer classically presents with symptoms of acid dyspepsia, which appears to reflect gastroduodenal sensitization to hydrogen ions. However, it can also present with other symptoms (Table 40.2) [163–165].

"Acid dyspepsia:" the classic ulcer symptom

In the era of classic diseases, DU was thought to be easy to diagnose [166]. The classic symptom of ulcer is characterized as a burning epigastric "hunger" pain or discomfort that tends to occur when acid is secreted in the absence of a food buffer (e.g., 2–3 h after meals) and at night, usually between 11 PM and 2 AM, when the circadian stimulation of acid secretion is maximal. Pain rarely occurs before breakfast. Alkali, food, and antisecretory agents produce relief such that "classic" patients tend to "feed" their ulcers. However, the classic presentation proved not to be specific for PUD as it occurs in nonulcer dyspepsia; in addition, many ulcer patients have "atypical" symptoms [163–165,167,168]. Despite these qualifications, food relief is more likely to occur with peptic ulcer, whereas food provocation of symptoms (postprandial pain or food intolerance) and nausea have negative predictive value for underlying PUD [169]. GU is less likely than DU to be relieved by antacids or food and more likely to display food provocation.

The discomfort occurs in the epigastrium in about two-thirds of symptomatic ulcer patients, but may occasionally localize in the right or left upper quadrants or hypochondrium [163]. Radiation of the pain to the back may also occur, but primary back pain is atypical. Clusters of pain lasting a few weeks followed by symptom-free periods of weeks or months is a pattern that is characteristic of classic DU. However, the classic symptoms of acid dyspepsia with food relief, described above, occur in only about 50% of DU patients (Table 40.2).

Unencumbered by placebo controls, Palmer [170] demonstrated that aspiration of gastric contents relieved pain and that reinfusion of the acidic (but not neutralized) gastric juice precipitated pain, supporting the concept that acid bathing an ulcer crater caused symptoms. In a double-blind protocol duodenal infusion of acid, but not saline, induced pain in about 40% of patients with active duodenal ulcers [171], whereas infusion of saline produced pain in only 10%. Pain was not produced by infusions in control subjects. Thus, in at least a portion of DU patients, symptoms are provoked by acid bathing the ulcer.

Data from numerous controlled endoscopic trials, largely in the pre-Hp era, have shown that symptoms and mucosal pathology are frequently dissociated such that ulcer healing does not guarantee disappearance of symptoms. Conversely, ulcers may also be present without producing symptoms;

Table 40.2 Symptoms of gastric and duodenal ulcers and nonulcer dyspepsia [163–165]

Symptom	Gastric ulcer (%)	Duodenal ulcer (%)	Nonulcer dyspepsia (%)
Pain/discomfort	100	100	100
Features of the pain:			
Primary pain			
Epigastric	67	61–86	52–73
Right hypochondrium	6	7–17	4
Left hypochondrium	6	3–5	5
Frequently severe	68	53	37
Within 30 min of food	20	5	32
Gnawing pain	13	16	6
Increased by food	24	10–40	45
Clusters (episodic)	16	56	35
Relieved by alkali	36–87	39–86	26–75
Food relief	2–48	20–63	4–32
Occurs at night	32–43	50–88	24–32
Not related to food or variable	22–53	21–49	22–65
Radiation to back	34	20–31	24–28
Increased appetite		19	
Anorexia	46–57	25–36	26–36
Weight loss	24–61	19–45	18–32
Nausea	54–70	49–59	43–60
Vomiting	38–73	25–57	26–34
Heartburn	19	27–59	28
Nondyspeptic symptoms	2	8	18
Fatty food intolerance		41–72	53
Bloating	55	49	52
Belching	48	59	60

15%–44% of ulcer patients who become symptom free during therapy still have an ulcer crater at endoscopy. Thus, in a largely Hp-positive group of ulcer patients, resolution of symptoms with antiulcer, but not anti-Hp, therapy does not predict ulcer healing. Complete resolution of dyspeptic symptoms may have different implications when Hp is being treated; complete symptom resolution at 3 months had a 98% positive predictive value for successful eradication of Hp infection [172]. In contrast, persisting symptoms had only a 25% positive predictive value for persisting Hp infection [172]. The presence or absence of persisting dyspepsia is an inadequate measure of Hp status posttherapy and emphasizes the need for post-Hp eradication testing (see Confirmation of *Helicobacter pylori* cure).

The dissociation between ulcers and symptoms is also evident from the impact of Hp eradication on the long-term symptomatic outcomes. In one study [173], 1–3 years after successful eradication of Hp in Hp-positive ulcer patients, 55% had resolution of their dyspeptic symptoms compared with only 18% of those with failed eradication. Although Hp cure clearly reduces symptoms in ulcer patients, excluding patients with preexisting gastroesophageal reflux disease (GERD), approximately one-third of Hp-positive ulcer patients can be expected to have persisting symptoms 1–3 years after successful cure of the Hp infection. The pathogenesis of these symptoms remains unclear. However, it is clear that the symptoms experienced by ulcer patients reflect factors more complex than simply acid bathing an ulcer crater. Symptoms may relate in part to sensitization of afferent nerves in response to tissue injury (i.e., visceral somatic hypersensitivity) [174,175]. Dyspepsia may result in an exaggerated response to superimposed chemical, mechanical, infectious, or inflammatory insults, and the gastroduodenitis associated with Hp or the inflammatory response to the peptic ulcer itself may produce this sensitization.

Gastroesophageal reflux, duodenal ulcer, *Helicobacter pylori*, and acid secretion

Gastroesophageal reflux disease evident by heartburn, acid reflux, or esophagitis occurs in 20%–60% of DU patients (Table 40.2) [173]. Both are common disorders and Berkson's fallacy deserves consideration [147] (see section on diseases associated with peptic ulcer). However, the association is strong and has a clear pathophysiological basis. With acid hypersecretion from gastrinoma or idiopathic PUD defined as basal acid output of more than 15 mmol/h, GERD was found

in 80% (65% with grade 2 or greater esophagitis and 15% with heartburn only) [176]. Furthermore, patients with esophagitis had a median lower esophageal sphincter pressure (LESP) of 15.5 mmHg compared with 23 mmHg in those without symptoms. Frequent vomiting and obesity were also identified as risk factors for esophagitis, whereas Hp was a strong negative predictor (odds ratio, 0.16) [176]. Therefore, GERD in an ulcer patient should be one clue to an underlying acid hypersecretory disorder.

This association between GERD and PUD has important implications for management. Hp-positive ulcer patients with prominent GERD symptoms are more likely to have persisting symptoms after Hp eradication [173]. In contrast, treatment of Hp in GERD patients either has no effect on requirements for therapy or is associated with an improvement [173,177].

History and physical examination

Clinical evaluation should accomplish three goals. The first is to elicit a history and findings that help discriminate between common and uncommon disorders that overlap with, obscure, or mimic ulcer disease. The second is to evaluate the severity of underlying disease if present. The third goal is to evaluate the psychosocial dimensions, including patient questions and concerns about the diagnosis, process, and prognosis. Clues to more severe forms of PUD, such as gastrinoma, include the presence of multiple duodenal ulcers, ulcers occurring distal to the duodenal bulb, a strong family history, ulcers that are refractory to medical therapy, and ulcers that recur after surgery. Additional clues to Zollinger–Ellison syndrome (gastrinoma) are peptic ulcers in Hp-negative, NSAID-negative subjects, an association with diarrhea, steatorrhea, or weight loss, and an association with hypercalcemia and renal stones.

Presentation of ulcer complications

Complications, especially in the absence of NSAID use, are usually associated with chronic peptic ulcers and are often heralded by the development of ulcer symptoms or a change in symptom pattern. However, in approximately 25% of cases in the absence of NSAID use and 50% in the presence of NSAIDs, complications are the presenting symptom of an ulcer that was otherwise clinically silent [178,179]. However, in controlled trials the new development of symptoms appears to herald ulcer formation in some patients [73,74,180]. Classically, penetrating ulcers present with a shift from the typical, vague visceral discomfort to a more localized and intense pain that radiates to the back; the expected relief from food or antacids is also diminished. A sudden development of severe, diffuse abdominal pain may indicate perforation. Vomiting is the cardinal feature present in most cases of pyloric outlet obstruction. Hemorrhage may be heralded by nausea, hematemesis, melena, or dizziness.

Unusual complications may also herald the presentation of peptic ulcer. Massive hemorrhage can occur with erosion into a major artery. Posterior perforation is usually characterized by the insidious onset of upper abdominal pain due to a sealed perforation, localized retroperitoneal abscess, or generalized contamination of the lesser sac and peritoneal cavity [181]. Pneumoperitoneum on chest roentgenogram or computed tomography (CT) is a critical diagnostic clue. Valentino syndrome is a DU presenting with retroperitoneal perforation and right lower quadrant pain. The veiled right kidney sign (retroperitoneal air obscuring the right kidney) can be a clue [182]. Penetrating ulcers can also result in fistulae. Gastrocolonic fistulae occur with anterior GU and present with halitosis, feculent vomiting, postprandial diarrhea, dyspepsia, and weight loss [183]. An apparent double pylorus has been observed to develop by endoscopy due to erosion of a GU into the duodenum [184]. Hepatic penetration has been observed with large DU or GU [185].

Laboratory evaluation

Routine laboratory studies are typically normal. Screening studies should include a complete blood count, liver chemistries, serum creatinine, and serum calcium. A fasting serum gastrin level is indicated in ulcers refractory to therapy, with a positive family history, or in cases requiring surgery. Serum salicylate levels can reveal unsuspected aspirin abuse, and are indicated in refractory and complicated cases. Surreptitious use of aspirin and NSAIDs can also be detected by testing for platelet COX activity [186].

Acid secretion

Formal gastric analysis has fallen into disfavor, exacerbated by the fact that pentagastrin is largely unavailable. However, one can easily assess basal gastric acid secretion using traditional techniques and one can measure the acid concentration in gastric fluid, including that obtained at the time of endoscopy. The presence of an elevated gastrin level should always prompt measurement of gastric acidity to exclude achlorhydria as the cause. A fasting hydrogen ion concentration of approximately 100 mmol/L or more is highly suggestive of a hypersecretory state.

Helicobacter pylori testing

Patients with peptic ulcers have high pretest probabilities for the presence of Hp. This high pretest probability means that a positive test, whether it be the presence of anti-Hp IgG antibody, a carbon 13 or carbon 14 urea breath test (UBT), or a stool antigen test, is strong evidence of the presence of infection and treatment can be initiated. In contrast, especially in the setting of ulcer disease, a negative test has a reasonably high probability of being a false-negative result, and thus negative tests must be confirmed by a second test (e.g., one designed to identify the presence of active infection such as a UBT or stool antigen test). One caveat is that the use of PPIs, bismuth, or antibiotics all reduce the bacterial load and

will result in an increased risk of a false-negative UBT, stool antigen test, histology, rapid urease test, and culture. If the patient is receiving a PPI, it should be stopped for approximately 2 weeks and an H2RA substituted before testing as it does not reduce bacterial load. Culture of Hp is generally not available nor necessary, except for antibiotic susceptibility testing in refractory cases. Another caveat is that, although experienced pathologists can diagnose Hp using hematoxylin and eosin staining, false negatives are frequent, especially when the number of organisms is low. Special stains are recommended, especially when chronic gastritis is present. As histology is more expensive than biopsy or rapid urease testing, at endoscopy it is reasonable to obtain biopsies from normal-appearing mucosa for both tests and then to discard histology specimens if the urease test is positive. Biopsies for histological testing for Hp should always include sampling of both the antrum and corpus.

Upper gastrointestinal radiography

A definitive radiographic diagnosis of peptic ulcer requires demonstration of barium within an ulcer niche, which is generally round or oval and may be surrounded by a smooth mound of edema [187,188]. Secondary changes include folds radiating to the crater, and deformities in the region secondary to spasm, edema, and scarring. The incisura found encircling a GU reflects secondary spasm of circular muscle. Secondary signs of DU include deformity of the duodenal bulb, which may be evidenced by flattening of the superior or inferior fornices, eccentricity of the pyloric channel, pseudodiverticulum, or exaggerated outpouching of recesses at the base of the bulb [187]. In the presence of deformity of the duodenal bulb, edema of the folds, or postoperative deformity, ulcer craters become much more difficult to detect. False-positive results occur when barium is trapped between folds. Although endoscopy has replaced radiology as the technique of choice for the evaluation of upper GI complaints in general and detection of cancer in particular, radiology can occasionally be useful. Radiographic signs suggesting gastric malignancy include: (1) an ulcer within a definitive mass; (2) effaced, interrupted, fused, or nodular mucosal folds as they approach the margin of the crater; and (3) negative, irregular filling defects in the ulcer crater.

Endoscopy

Endoscopy provides a sensitive, specific, and safe method for diagnosing peptic ulcers, allowing direct inspection and biopsy. Benign ulcers have smooth, regular, rounded edges, with a flat, smooth ulcer base often filled with exudate. Malignancy [189] is associated with an ulcerated mass that protrudes into the lumen; surrounding folds that are nodular, clubbed, fused, or stop short of the ulcer margin; or margins that are overhanging, irregular, or thickened. The gold standard for determining whether an ulcer is benign or malignant is biopsy. Biopsy should be performed even for gastric lesions with a benign endoscopic appearance, as benign-appearing ulcers may harbor malignancy [190]. The proper number of biopsies has been debated and depends upon technique. Four jumbo biopsies of the ulcer margin are equivalent to 6–7 adequate, regular-sized biopsies, the number shown to detect nearly all cancers. The description "adequate" biopsy warrants emphasis, because biopsies that do not provide a good sample of the lesion are useless at best and misleading at worst.

In addition to direct endoscopic inspection and biopsy, repeat endoscopy to confirm healing of gastric ulcers may be required for confirmation of benignity. Until recently, follow-up endoscopy was the rule for gastric ulcers. Currently, management is individualized based on the clinical situation, endoscopic findings, and the adequacy of initial biopsy. For example, an obviously benign ulcer in a young, NSAID user without Hp infection and with adequate initial biopsies that were negative would not mandate follow-up endoscopy to rule out malignancy. However, a large, endoscopically indeterminate ulcer in the body of the stomach of an elderly subject generally warrants repeat endoscopy and biopsy.

Computed tomography

Despite very limited data, CT is clearly the most valuable test if a penetrating or perforated ulcer is suspected. Furthermore, the much higher resolution of the newer spiral and multislice CT techniques makes them more valuable for assessing the sometimes subtle findings of complicated ulcers. Advanced CT is clearly more effective than an upright chest radiograph in detecting free air, with positive findings in all 14 consecutive patients with perforation in one surgical series [191]. In total, 10 of these 14 patients also had fluid collections and 5 had inflammatory changes in surrounding soft tissues [191]. Extravasation or sinus tracks with intralumenal contrast material, discontinuity of the bowel wall, and mucosal thickening can also be useful findings localizing the site of perforation or penetration, but these findings only appear to be reliable with the highest resolution scanning equipment [192,193]. Use of oral contrast is controversial, but also appears to be valuable with higher resolution equipment [192].

Recommendations regarding evaluation and follow-up

The goal in evaluating a patient with upper gastrointestinal symptoms is to expeditiously arrive at a definitive diagnosis without unnecessarily exposing patients to costly and potentially risky diagnostic procedures. These decisions fall within the realm of "good clinical judgment;" there is no gold standard for decisions that are both cost-effective and appropriately cautious. However, decisions must be made and data interpreted in light of specific patient characteristics and diagnosis, such as patient age, socioeconomic background, index event, "ulcer" history, and previous workup. Decisions would be very different for a 25-year-old graduate student

with uninvestigated dyspepsia compared with a 70-year-old patient with previously documented ulcer complications. Decisions would also be very different in patients with a firm recent endoscopic diagnosis of ulcer but no Hp testing compared with a remote "ulcer" diagnosis on clinical grounds.

Complicated gastric or duodenal ulcer with unknown *Helicobacter pylori* status

With all ulcers, the question is "Hp, NSAIDs, neither, or both." As noted, testing for Hp is relatively straightforward provided that the caveats for false-negative results are taken into account. The goals include healing the ulcer and preventing its recurrence. If Hp is present it should be treated and, at a suitable time (e.g., 4 weeks) after the end of therapy, cure should be confirmed (see *Helicobacter pylori* and ulcer therapy – Confirmation of Hp cure). Patients with a history of ulcer complications are at a markedly increased risk for future life-threatening events. Hp eradication essentially removes that risk, whereas a return to NSAID use is associated with a recurrent risk of a new complication.

Helicobacter pylori-negative ulcers

In ulcers that test negative for Hp, alternative etiologies (Table 40.1) deserve consideration. However, the most common etiologies for Hp-negative ulcers are false-negative testing for Hp and undiscovered consumption of NSAIDs. Once Hp and NSAID use have been firmly excluded, then alternate etiologies deserve consideration, especially for complicated or troublesome ulcers.

Atypical ulcers

Giant ulcers

Giant ulcers commonly occur in association with NSAID consumption [194]. Giant duodenal or prepyloric ulcers have also been reported in association with end-stage renal failure [195]. Giant GU may also carry an increased risk of harboring carcinoma. Crohn's and other disease processes can present with giant ulcers [196].

Atypical duodenal ulcers

Duodenal carcinoma and invading pancreatic carcinoma typically present as masses but may produce benign-appearing ulcers. Because of the very low yield, routine DU biopsies cannot be recommended unless the clinical condition warrants it, such as a refractory DU or an atypical endoscopic appearance.

Postbulbar ulcers

Generally, ulcers in the duodenum are located within 2–3 cm of the pylorus. Postbulbar ulcers are uncommon and suggest the presence of gastrinoma and other hypersecretory states. Differential diagnosis should include diverticula, adhesive bands, annular pancreas, and neoplasia of the pancreas and duodenum.

Natural history of peptic ulcer

The natural history of Hp-related DU and GU is to recur. Bardhan [197], following patients for 12 months after documented DU healing, observed that without medical treatment 26% of patients remained symptom free and 74% relapsed; 33% had one relapse, 24% had two recurrences, and 17% experienced three or more recurrences. The placebo-treated control groups drawn from numerous clinical trials of maintenance therapy indicate a similar 50%–80% recurrence rate during the 6–12 months following initial ulcer healing [198,199]. These recurrences can produce symptoms, but asymptomatic ulcers are frequently found at surveillance endoscopy. A number of factors influence the highly variable rate at which ulcers heal and recur (see Medical treatment of active peptic ulcer disease – Factors influencing healing). Successful eradication of Hp markedly changes the natural history and markedly reduces recurrences. However, after Hp eradication, erosions may occur in the duodenum that spontaneously heal and do not produce any clinical problems [200,201]. Such post-eradication erosions were likely responsible for post hoc subgroup analysis of patients participating in a US anti-Hp treatment trial reporting an unexpectedly high rate of "ulcer" recurrence after successful eradication of Hp [202].

The natural history of NSAID ulcers has not been formally studied in patients who continue on NSAIDs without ulcer treatment. It is clear that when NSAIDs are stopped, associated ulcers heal unless an independent cause of ulceration exists. As NSAIDs impair healing, it is possible that such ulcers are more persistent than Hp ulcers.

The limited data that exist for non-HP, non-NSAID ulcers also suggests that they may persist. Persistence of the ulcer diathesis is also expected for hypersecretory, idiopathic PUD [93].

Antiulcer pharmacology

Antisecretory agents

Antisecretory agents accelerate the healing of ulcers regardless of the etiology. They also relieve ulcer-associated dyspepsia. Continued use retards the rate of ulcer recurrence and reduces the risk of complications.

H_2 receptor antagonists

H_2 receptor antagonists are competitive reversible inhibitors of histamine at H_2 receptors on parietal cells. These drugs are equally efficacious and safe and differ primarily in potency. Available drugs include cimetidine (Tagamet), ranitidine (Zantac), famotidine (Pepcid), and nizatidine (Axid). These drugs are also licensed for sale over the counter at reduced dosages, including Tagamet HB, Zantac 75, Pepcid AC, and

Axid. H_2 receptor antagonists have no antibacterial activity against Hp.

H_2 receptor antagonists are well absorbed after oral dosing; absorption is inhibited by 10%–20% by concomitant antacids, but not by food. Peak serum concentrations occur 1–3 h after oral dosing. All four drugs cross the blood–brain and placental barrier and are excreted in breast milk [203]. The distribution of cimetidine in cerebral spinal fluid appears to be increased in liver failure [203].

Cimetidine is the least potent of the H2RAs and has the highest incidence of side effects and drug–drug interactions. As such, it has largely fallen into disfavor. H_2 receptor antagonists have largely been replaced by PPIs for the treatment of PUD because of their increased potency and lack of tachyphylaxis (tolerance). The only advantage of H2RAs is cost, but as PPIs come off patent, this is less of a consideration.

Proton pump inhibitors

Proton pump inhibitors block acid secretion at the hydrogen–potassium adenosine triphosphatase (H^+,K^+-ATPase) pump located on the lumenal border of the gastric parietal cell. This pump exchanges hydrogen ions for potassium ions across the parietal cell microvillus membrane, secreting hydrogen ions into the gastric lumen and creating the low pH environment characteristic of gastric secretions. Proton pump inhibitors bind to and irreversibly inhibit the ATPase. Proton pump inhibitors are the most effective antisecretory agents currently available, achieving better pH control than that provided by H2RAs. Available agents include omeprazole (Prilosec), lansoprazole (Prevacid), pantoprazole (Protonix), rabeprazole (Aciphex), and esomeprazole (Nexium). Of note, although PPIs have some direct antibacterial activity against Hp in vitro, used alone they do not cure the infection. As noted in the section on diagnosis, PPI therapy reduces the Hp load in the stomach and can result in false-negative diagnostic tests.

PPI are acid labile and are inactivated if permitted to dissolve in acidic gastric juice. Therefore, enteric coated granules that dissolve at pH6 or above or sodium bicarbonate are used to protect the drug during transit through the stomach. When given with sodium bicarbonate buffer to fasting patients, PPIs are rapidly absorbed and peak plasma levels are reached in 30 min. With the enteric coated granules the peak concentration occurs 1–3 h after dosing and the drug is detectable in serum for about 6 h (Fig. 40.8a) [204]. The effectiveness of PPIs when given by oral administration relates closely to the AUC (area under the plasma concentration–time curve) rather than to peak plasma drug levels (Fig. 40.8b) [204–209]. The prolonged duration of PPI antisecretory action reflects irreversible inactivation of the parietal cell H^+,K^+-ATPase, rather than a prolonged serum half-life. Administering PPIs with food delays absorption but does not alter the AUC [210]. Although PPIs differ slightly in metabolism and potency, clinically they are completely interchangeable and cost should be the primary consideration when choosing which to prescribe. Drug metabolism, as evident by the AUC [211], occurs via P450 (see Drug interactions via cytochrome P450), and is prolonged with liver but not renal disease (Fig. 40.8c). Delayed metabolism is also observed with genetic P450 polymorphism and in the elderly. However, in light of the wide safety margin with these drugs and the apparent absence of accumulation, there are no current recommendations to alter dose in the elderly or in the face of hepatic failure.

The dose and time dependencies of inhibition of acid secretion by PPIs are characterized as follows:

1 *A time lag* in the onset of antisecretory effectiveness is noted over the first 4–7 days of therapy, probably reflecting progressive inhibition of the H^+,K^+-ATPase and possibly increases in drug bioavailability. This lag period is inversely related to dose.

2 There is *variability in antisecretory effectiveness*, probably due to variability in absorption and clearance, as reflected in the AUC. For example, after 7 days of therapy on a dose of 10 mg omeprazole, some subjects have minimal inhibition whereas others have more than 90% inhibition [212]. At 20 mg, inhibition is less variable [213] (Fig. 40.9), with a mean inhibition of acidity (hydrogen ion concentration) of about 90% [214]. Therefore, more consistent inhibition of acid secretion is observed at higher doses (Fig. 40.9). A PPI given twice daily produces better inhibition of secretion than one given once daily.

3 *PPIs maximally inhibit stimulated parietal cells.* As PPIs must be concentrated and activated in the acidic compartments of the parietal cell, they will only inactivate the H^+,K^+-ATPase present in actively secreting membrane compartments. PPI effectiveness therefore depends on the degree of activation of acid secretion at the time of drug administration. Omeprazole action is reduced, for example, if administered while secretion is inhibited by H2RA [215], as efficacy varies depending upon whether the patient is fasting, has recently taken an H2RA, or has secretion activated by food, gastrin or vagal pathways during the time that the prodrug is circulating. PPIs are effective when taken shortly before meals.

4 *Rapid control of acid secretion* can be achieved using higher doses of PPI or more frequent administration (i.e., three or four doses of 20 mg of omeprazole in the first 24 h).

5 The *bioavailability* of PPIs administered via nasogastric tube or parenterally is comparable to that of oral dosing [15,216]. Intravenous formulations of esomeprazole and pantoprazole are available; the pharmacodynamics, duration of action, and effectiveness are essentially identical to those of oral administration. In an ICU setting with fasting patients, intravenous PPIs are most effective when given by continuous infusion of 8–10 mg/h after a bolus loading dose [217,218].

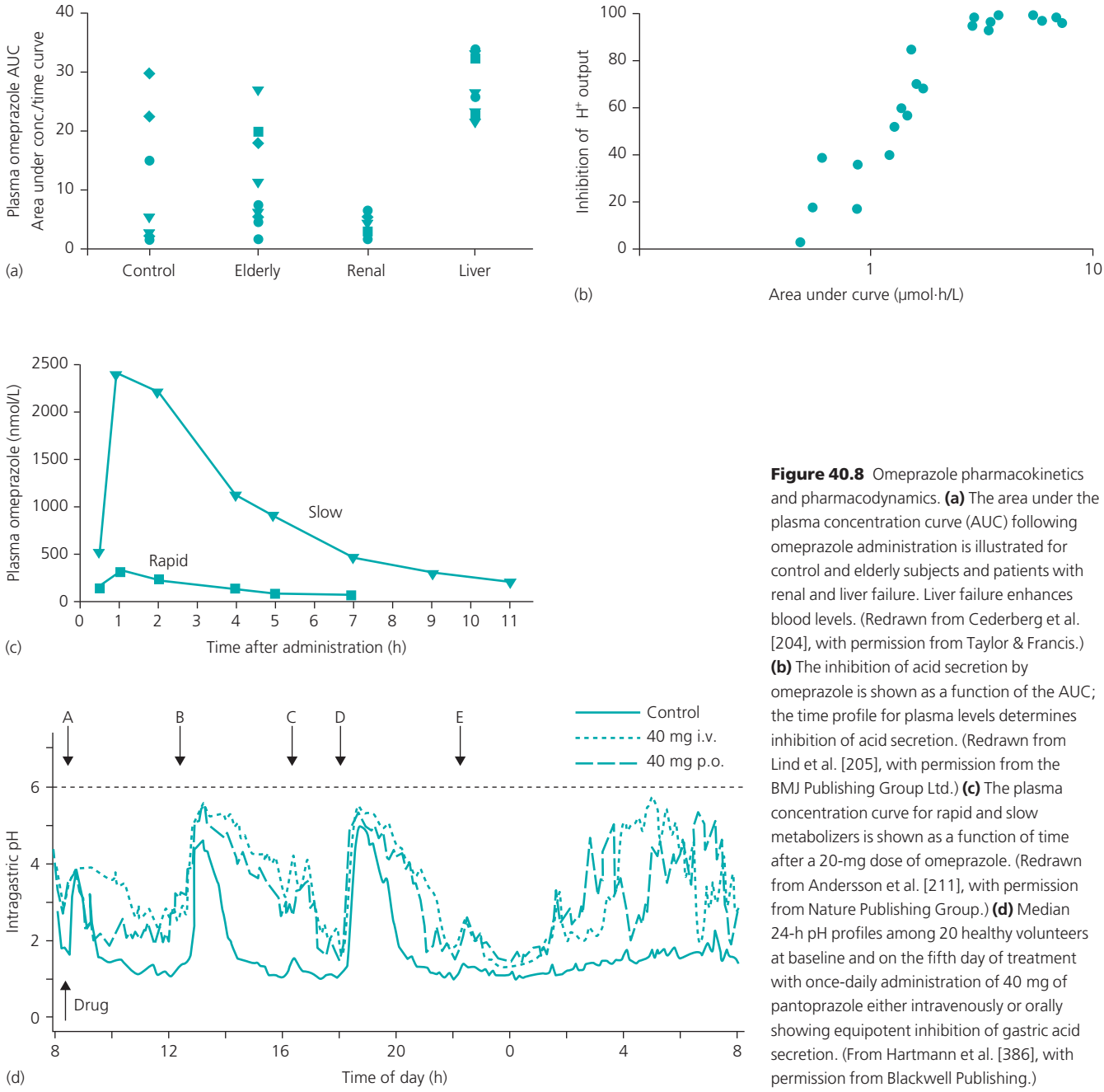


Figure 40.8 Omeprazole pharmacokinetics and pharmacodynamics. **(a)** The area under the plasma concentration curve (AUC) following omeprazole administration is illustrated for control and elderly subjects and patients with renal and liver failure. Liver failure enhances blood levels. (Redrawn from Cederberg et al. [204], with permission from Taylor & Francis.) **(b)** The inhibition of acid secretion by omeprazole is shown as a function of the AUC; the time profile for plasma levels determines inhibition of acid secretion. (Redrawn from Lind et al. [205], with permission from the BMJ Publishing Group Ltd.) **(c)** The plasma concentration curve for rapid and slow metabolizers is shown as a function of time after a 20-mg dose of omeprazole. (Redrawn from Andersson et al. [211], with permission from Nature Publishing Group.) **(d)** Median 24-h pH profiles among 20 healthy volunteers at baseline and on the fifth day of treatment with once-daily administration of 40 mg of pantoprazole either intravenously or orally showing equipotent inhibition of gastric acid secretion. (From Hartmann et al. [386], with permission from Blackwell Publishing.)

Anticholinergics

Anticholinergic medicines have weak antisecretory ability and no antibacterial activity against Hp and should be considered obsolete for treatment of ulcer disease. These agents may have some place in Hp-negative, NSAID-negative PUD.

Prostaglandins

Misoprostol (Cytotec) is a 15-deoxy-15-hydroxy-16-methyl analogue of prostaglandin E_1. It shares the properties of other E-type prostaglandins, displaying moderate inhibition of basal and food-stimulated acid secretion in humans [219]. Topical action appears critical in prostaglandin action; oral

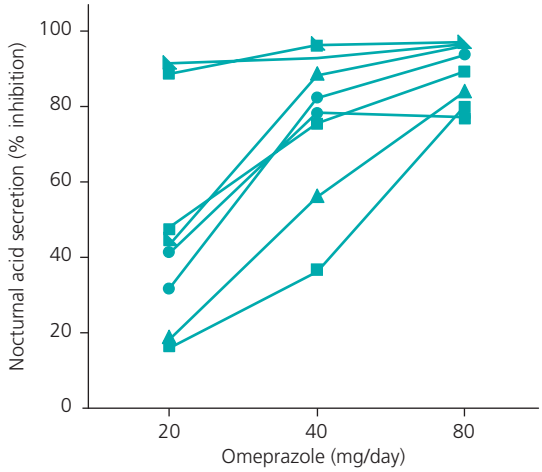


Figure 40.9 Dose–response curves for omeprazole inhibition of nocturnal acid secretion. Acid secretion was determined during four night-time sessions for seven patients with duodenal ulcer. Single doses of placebo or omeprazole (20, 40, or 80 mg) were given at 8AM. Acid secretion was then determined between 9PM and 6AM, with the data expressed as a percentage of control secretion. This study illustrates the high variability of inhibition with low but not high doses of proton pump inhibitors. Data from Shearman et al. [213].

administration gives greater antisecretory efficacy and fewer side effects than systemic administration [220] Misoprostol has no antimicrobial activity against Hp. The primary adverse effects are crampy abdominal pain and diarrhea, which are dose-dependent effects [221,222]. Diarrhea occurred in 3%–39% of patients on the 200 μg four times daily dose of misoprostol, with the range reflecting trial design and the definition of diarrhea [221,222], Diarrhea is often mild and transient, and may respond to a temporary reduction in dose. In clinical trials, usually less than 20% of subjects drop out because of diarrhea, such that the majority can take the drug without problems. The prevalence and significance of side effects have been heavily marketed by producers of antisecretory drugs competing for the same market (chronic NSAID users). In clinical trials, misoprostol, even when given at half dose, has proven superior to PPIs for the healing of endoscopic gastric ulcers and prevention of ulcer relapse among patients without concomitant Hp infections (see Prostaglandins) [223,224]. Prostaglandins of the E group are generally uterotropic and are contraindicated in women of childbearing potential who are not receiving contraception. All patients should be informed of this risk, to minimize the drug being inadvertently given by the patient to a pregnant woman.

Sucralfate

Sucralfate is a topical antiulcer drug. Sucralfate is the aluminum hydroxide salt of sulfated sucrose and has the main activity of coating ulcer sites, leading to slightly increased rates of ulcer healing. It does have an effect on Hp adherence in vitro but has not demonstrated any convincing anti-Hp effect in vitro. It is now generally considered as obsolete for the treatment of ulcer disease.

Antacids

Antacids neutralize gastric acid and have a long history in the therapy of peptic ulcer. However, they are inconvenient, requiring multiple doses per day and are outmoded for PUD.

Antimicrobials

Hp causes a bacterial infection of a mucosal surface. As with other bacterial infections, the mainstay of therapy is use of antimicrobials to which the bacterium is susceptible. Because the infection is inside the stomach, but outside the stomach body, there may be a place for topical therapy. In fact, because of the hostile acid environment of the stomach, topical therapy may greatly enhance overall effectiveness. It is not clear how much of the effectiveness of current therapies is related to the topical action of the drugs and how much is due to a systemic action, but both are probably important. There is considerable evidence that Hp also invade surface epithelial cells and thus the combination of topical and systemic actions may be best.

Topical agents

Bismuth salts have been used to treat 'gastritis' and peptic ulcers for over 200 years. Bismuth is directly bactericidal to Hp and leads to bacterial lysis. Upon ingesting bismuth, there is an immediate and marked reduction in bacterial numbers, thereby reducing the chances that an antimicrobial-resistant subpopulation will survive.

Bismuth has an excellent safety record with the most problematic side effect being temporary discoloration of the tongue and development of black stools. There is a hypothetical concern about systemic absorption of bismuth leading to bismuth toxicity but this has not proved to be a clinical problem when it is used for short duration therapies. Of note, if the common subsalicylate form of bismuth is used, a significant salicylate ingestion will occur. Each tablet of Pepto-Bismol, for example, has 225 mg of salicylate and the majority is absorbed. Patients receiving salicylate therapy should have their doses modified and patients who should avoid salicylates (e.g., children under the age of 16 years) should consider an alternate form of bismuth. In the United States bismuth is primarily available as bismuth subsalicylate (Pepto-Bismol) although bismuth citrate has become available as part of a combination therapy.

Traditional antimicrobials

Hp is susceptible to many different antibiotics and a variety of antibiotics and antibiotic combinations have been tried. The antibiotics that have proven effective include clarithromycin,

amoxicillin, metronidazole/tinidazole, tetracycline, furazolidone, fluoroquinolones, and rifabutin.

Clarithromycin

Clarithromycin (Biaxin) is a macrolide that binds to bacterial ribosomes and disrupts protein synthesis, leading to bacterial cell death. Clarithromycin is the most acid stable of the macrolides and has the lowest minimal inhibitory concentration. The major metabolite of clarithromycin is also active against Hp. Clarithromycin is generally well tolerated. Reported side effects include an altered sense of taste, frequently described as metallic, nausea, vomiting, and headache. Clarithromycin resistance is increasing; resistance predicts treatment failure.

Amoxicillin

Amoxicillin is an acid-stable semisynthetic penicillin that is bactericidal in vivo. The antimicrobial activity of amoxicillin is pH dependent with the minimal inhibitory concentration decreasing as the pH increases. Resistance to amoxicillin is rare but has recently been reported and may in part be responsible for the markedly variable success of the dual combination of a PPI and amoxicillin. Amoxicillin concentrations are highest in the antral mucosa with lower levels achieved in the corpus mucosa or the mucus layer. Overall, results with combination therapy that includes amoxicillin have been good, suggesting that sufficient levels are achieved and that it is an effective anti-Hp agent when used as part of a combination therapy.

Metronidazole/tinidazole

Metronidazole (Flagyl, Protostat) and the very similar tinidazole are nitroimidazoles that are selectively toxic to microaerophilic microorganisms. A chemically reactive, reduced form of the drug leads to generation of cytotoxic products and destruction of microorganisms. The activity of metronidazole is pH independent making it theoretically an ideal drug for the gastric environment. Unfortunately, Hp rapidly develops resistance to metronidazole and the widespread use of this drug has resulted in a high rate of primary resistance. Metronidazole resistance identified in vitro does not always predict resistance in vivo, such that this agent may remain useful despite a high level of resistance in a population or even in an individual (see Antibiotic resistance).

Tetracycline

Tetracycline hydrochloride was used in the first effective therapies against Hp and remains an important antibiotic. The activity of tetracycline is independent of gastric acidity making it a potentially very useful drug. Tetracycline is also inexpensive and resistance is rare. Success has been reported with tetracycline hydrochloride and with oxytetracycline, whereas doxycycline has produced inferior results. Tetracyclines are contraindicated in children because of the abnormal pigmentation of permanent dentition that occurs when the drug is used while teeth are being calcified.

Fluoroquinolones

Fluoroquinolones have been used increasingly for Hp eradication; they act by blocking DNA gyrase and DNA synthesis in Hp. Resistance to fluoroquinolones develops rapidly and previous use of these medications is associated with a high rate of resistance.

Furazolidone

Furazolidone is a monoamine oxidase inhibitor with broad antibacterial activity based on interference of bacterial enzymes; it has proven to be an effective part of triple therapy. Resistance to Hp is rare such that furazolidone is currently an underutilized antimicrobial.

Rifabutin

Rifabutin is a semisynthetic ansamycin antibiotic with a low minimal inhibitory concentration (MIC) level for Hp. Rifabutin use is becoming increasingly common, primarily in combination with a PPI and amoxicillin as a rescue therapy.

Drug interactions with antiulcer drugs

Altered absorption

As a result of intralumenal binding, antacids and sucralfate decrease absorption of a number of drugs [225,226] and it is best to advise separation of consumption of antacids and other drugs by an hour (Table 40.3). Bismuth may have the same effect, but data are limited and bismuth is generally given along with the other drugs used in the treatment regimen [227]. Antisecretory agents, by increasing intralumenal pH, can alter drug absorption. For example, the effect of antisecretory agents on reducing absorption of food-bound vitamin B-12 can be quite dramatic [228,229]. The effect appears to be mediated by the increase in pH and resultant decrease in peptic activity, rather than by inhibition of intrinsic factor secretion [230]. The absorption of iron and calcium can also be compromised and there is increasing evidence of an association between antisecretory drug use, especially PPIs, and the risk of hip fracture [231]. It is not clear whether this is a consequence of the effect on calcium absorption, effects on osteoclast function, or both. There is also evidence that thyroxine absorption is markedly suppressed by either the use of PPIs or in cases of Hp gastritis, requiring adjustment of the thyroxine dose [232].

The dissolution of some drugs, particularly weak bases, will be decreased with gastric neutralization. The consequences depend on the specific drug and its preparation. For example, decreased dissolution of ketoconazole, a weak base, significantly decreases absorption [203]. Alternatively, the absorption of bismuth from colloidal bismuth subcitrate is increased, presumably because decreased gastric acidity increases free

Table 40.3 Impact of acid peptic therapy on nutrient absorption and drug interactions

General mechanism	Acid peptic therapeutic agent	Drug or nutrient	Consequences
Intralumenal binding	Sucralfate, antacids, ?bismuth	Potentially binds several drugs in gastric lumen	Prevents absorption, producing lower blood levels
Decreased acid secretion	PPI,[a] H2RA	Vitamin B-12	Anemia
		Iron	Anemia
		Calcium	Osteoporosis, pathological fractures
		Thyroxine	Decreased T4 and increased TSH
		Ketoconazole, cefpodoxime, cefuroxime, protease inhibitors (atazanavir and indinavir)	Decreased absorption, particularly of drugs that are weak bases
		Aspirin	Another weak base for which gastric absorption is decreased at high pH[b]
		Colloidal bismuth	Increases bismuth absorption
P450 drug interactions	Cimetidine	Alfentanil, amiodarone, nifedipine, carbamazepine, cyclosporine (ciclosporin), citalopram, sulfonylureas, warfarin, and CYP substrates, such as 1A2 (theophylline, trifluoperazine), 2C19 (citalopram, phenytoin, propranolol, sertraline), 3A4[c]	Altered metabolism increases blood levels. Significant consequences may result when the therapeutic margin is small. Consequences are minimal for others, such as diazepam, because of the wide therapeutic margin
	Omeprazole, other PPI[d]	Carbamazepine, CYP2C9 substrates (amiodarone, fluoxetine, phenytoin, sertraline, warfarin), CYP2C19 substrates (citalopram, propranolol)[e]	Same type of impact as noted for cimetidine
	PPI in slow metabolizers	Antibiotics	Enhanced cure of *Helicobacter pylori*
	PPI in slow metabolizers or PPI in combination with multiple other drugs that share CYP sites of metabolism, especially 3A4 (e.g., omeprazole plus clarithromycin and carbamazepine)[f]	Any drug metabolized by CYP3A4[c] or possibly other CYP sites	Delayed clearance with significantly increased levels of drugs metabolized by 3A4

a Effects will be exaggerated in slow metabolizers because of genetic polymorphism at the CYP2C19 pathway.
b Decreased absorption may decrease superficial mucosal damage, but clinical consequences are minimal because intestinal absorption is so much more important and aspirin and NSAIDs have systemic actions resulting in ulcers.
c Many drugs interact with 3A4, including carbamazepine, cisapride, nifedipine, macrolide antibiotics, azole antifungals, antitumor agents, tacrolimus, psychotropics, diclofenac, doxycycline, imatinib, isoniazid, nefazodone, nicardipine, propofol, protease inhibitors, quinidine, telithromycin, and verapamil.
d Although omeprazole has been implicated for these interactions, in vitro studies suggest that lansoprazole and pantoprazole are potent in vitro inhibitors of CYP2C19 and CYP2C9 respectively [387]. Therefore, caution is appropriate and interactions may become important for other PPI, especially if combined with other factors, such as in patients who are homozygous slow metabolizers, who are taking multiple drugs that amplify interactions, and who have illnesses that impair hepatic metabolism or renal excretion.
e To the extent that these interactions are mediated at CYP2C19, they are *not* more pronounced in slow PPI metabolizers, presumably because the point of interaction is itself defective or absent.
f There may be some differences among PPI in that such effects may be less prominent with rabeprazole [388], but data are so limited that no firm conclusions can be reached.
H2RA, histamine H_2 receptor antagonist; NSAIDs, nonsteroidal antiinflammatory drugs; PPI, proton pump inhibitor, T4, thyroxine, TSH, thyroid-stimulation hormone.

bismuth concentrations [233]. Acid secretory inhibition can decrease the absorption of weak acids from the stomach and increase absorption of weak bases. For example, secretory inhibitors will reduce uptake into gastric mucosa of weak acid NSAIDs, such as aspirin, and decrease superficial damage. However, gastric absorption is usually modest compared to intestinal absorption, and systemic effects override this sparing of gastric mucosa.

Drug interactions via cytochrome P450

To some extent, all of the nine PPIs and H2RAs available in the United States for acid inhibition undergo hepatic metabolism and interact with members of the cytochrome P450 (CYP) superfamily of mixed function oxidases. This topic is inherently complex as the CYP superfamily is complex, composed of three major families (CYP1–3), a varying number of subfamilies (e.g., CYP2A–E), and numerous specific members or isoforms within these subfamilies (e.g., CYP2C1–19). For the antisecretory agents, these interactions with the CYP superfamily generally carry little clinical significance. However, there are specific circumstances in which they can become critical and potentially life-threatening (Table 40.3). These serious events are rare and occur in seriously ill subjects with impaired hepatic function and drug metabolism, often confounded by multiple (more than 2) interacting drugs and genetic polymorphism of the CYP metabolic pathways. Such factors will not be detected in routine pharmacokinetic studies in young, healthy volunteers with usually undefined CYP genetic backgrounds; therefore, such studies carry little negative predictive value for excluding uncommon, serious interactions encountered in the treatment of complex cases in practice.

Several features of P450 metabolism are important with respect to the antisecretory drugs; this list has been adapted from previous reviews [230,234]:

1 *Time and dose dependence*. Drug metabolism and drug interactions undergo dose- and time-dependent changes over the first several days of therapy. Generally, 3–5 days are needed to reach maximal inhibition of drug metabolism, although maximal effects can be further delayed in some instances.

2 *The therapeutic margin*. Drug interactions are of greatest relevance when the drugs have a narrow therapeutic range. For example, cimetidine may potentially produce significant effects with R-warfarin, theophylline, phenytoin, and nifedipine [203,230].

3 *Genetic polymorphism of drug metabolism and interactions*. Genetic polymorphism is particularly important for the PPIs, for which the CYP2C19 pathway is a primary metabolic step in degradation. Because of the wide safety margin of PPIs, the major clinical implication is that acid secretory inhibition will be modest in rapid metabolizers and exaggerated in slow metabolizers, as the degree of acid inhibition reflects the AUC. The impact in many situations is modest. For example, healing of GU is more rapid at 2 weeks in slow metabolizers, with comparable healing found at 8 weeks in rapid metabolizers [235]. In contrast, cure of Hp with omeprazole plus amoxicillin is highly dependent on CYP2C19 metabolism: slow metabolizers had a 100% cure rate, rapid metabolizers a 29% cure rate, and heterozygotes a 60% cure rate [236]. CYP2C19 is also the site of interaction between diazepam and either omeprazole or cimetidine; however, with the wide therapeutic range for diazepam and marginal effects of the interactions, clinical implications are minimal [210,237]. Omeprazole does not further delay clearance of diazepam or S-mephenytoin in slow metabolizers, probably because the point of interaction is defective or absent [234,238]. However, in slow metabolizers, delayed PPI clearance increases the potential for interactions at other CYP sites, such as 3A4 or 2C8. For example, although pharmacokinetic studies indicated that lansoprazole had only minimal P450 drug interactions, in a patient with a CYP2C19 mutation, lansoprazole caused significant increases in tacrolimus levels. These levels returned to normal after replacing lanxoprazole with famotidine [239]. Drug interactions may only show up in slow metabolizers who shift metabolism to 3A4.

4 *Variability among individuals*. In addition to genetic polymorphism, other factors can also underlie the variability of drug interactions among individuals. Age can be important on both ends of the spectrum. Elderly subjects are much more likely to have impaired drug metabolism. Infants and very young children tend to have rapid metabolism, underlying the use of higher PPI doses in the very young [240]. The relative impact of slow metabolizers is comparable in very young subjects.

5 *Multidrug (beyond 2-way) interactions*. Three-way drug interactions can produce unexpected results. All of the PPIs are metabolized by CYP3A4 and, therefore, potential interactions with other drugs metabolized by this pathway warrant consideration, particularly for drugs with a narrow therapeutic margin. Omeprazole and clarithromycin interact at 3A4 [241] with the result being an increased AUC for both. Both drugs have a wide therapeutic window and this interaction may even enhance cure of Hp infection. However, the omeprazole–clarithromycin combination considerably increases carbamazepine (Tegretol) levels [242]. Other drugs metabolized at CYP3A4 should also be used cautiously during combination therapy with a PPI and clarithromycin. Drugs interacting at CPY3A4 include drugs related to cisapride, ketoconazole, macrolide antibiotics, antitumor agents, and several psychotropics.

6 Whether interactions produce relevant effects depends upon whether alternate pathways exist for drug metabolism.

7 The antisecretory drugs interact with multiple CYP sites. Furthermore, a drug may be a substrate for and, thus, be metabolized by one or several CYP sites, or it may induce or inhibit a CYP site.

Medical treatment of active peptic ulcer disease

Factors influencing healing

Several factors potentially influence the rate of ulcer healing and the tendency for recurrence. Reversible factors should be identified and addressed, particularly Hp infection, NSAID use, and cigarette smoking.

Ulcer size influences the time to healing. Large and small GU heal at the same rate and, although effective medical

therapy results in more rapid healing, larger ulcers will still require more time to heal [243–245].

Conventional ulcer therapy

The superior potency and duration of action of PPIs have resulted in PPI therapy becoming the therapy of choice with regard to rapid relief of ulcer symptoms and acceleration of ulcer healing. Numerous marketing studies have attempted to demonstrate differences among the available PPIs. However, when the results are corrected for differences in dose, it becomes clear that the best PPI is the cheapest one (i.e., a PPI is a PPI). It is important to remember that parietal cell hyperplasia and rebound acid hypersecretion follow discontinuation of prolonged PPI therapy [246–249]. Although the rebound acid secretory response is generally small and transient, this hypersecretion may underlie transient exacerbation of acid peptic disease (e.g., the development of duodenal erosions noted above) and GERD symptoms.

Recommendations

Treatment decisions are based on the situation (e.g., presentation, complicating issues, sense of urgency) and etiology of the ulcer disease. Clearly, in the presence of a complicated ulcer (e.g., major upper GI bleeding), diagnosis of the cause can wait until the crisis has passed. For routine symptomatic ulcers, diagnosis and treatment can occur concomitantly. Symptomatic ulcers, regardless of cause, typically respond well to antisecretory drug therapy and most physicians would consider the least expensive PPI as the treatment of choice. There is no place for the routine use of antacids, sucralfate, or prostaglandins for DU, and certainly not for GU. If the ulcer is diagnosed by endoscopy, one would expect that the endoscopist would have obtained samples to diagnose or exclude Hp infection. There is no urgency to diagnosis Hp, and diagnosis or confirmation of the diagnosis can certainly be delayed until it is convenient for the patient and physician. However, as antisecretory drug therapy is typically part of anti-Hp therapy, there may be cost savings to do both together. For an uncomplicated, small, Hp-related DU, both treatment of the infection and the ulcer can be carried out in the same time frame (e.g., 2 weeks) (see next section). Treatment of large and complicated ulcers will need to be individualized. The only instance in which antiulcer therapy is required beyond the antibiotic treatment period is for larger or complicated ulcers (e.g., bleeding ulcers) or in the setting of sustained NSAID use, as considered in the following sections.

Helicobacter pylori and ulcer therapy

Drug combinations used to treat *Helicobacter pylori*-associated ulcers

Treatment of Hp infection results in acceleration of ulcer healing, prevention of ulcer relapse, and prevention of ulcer complications (Fig. 40.10) [250,251]. Successful eradication of Hp will also reduce the rate of development of gastric cancer in high-risk groups [252,253]. If the infection is cured before significant atrophy develops, it is likely that gastric cancer can be completely prevented [254]. In developed countries Hp eradication is rarely followed by reinfection. In developing countries where hygiene may be poor, reinfection is common and this remains a major problem for future worldwide eradication programs [255,256].

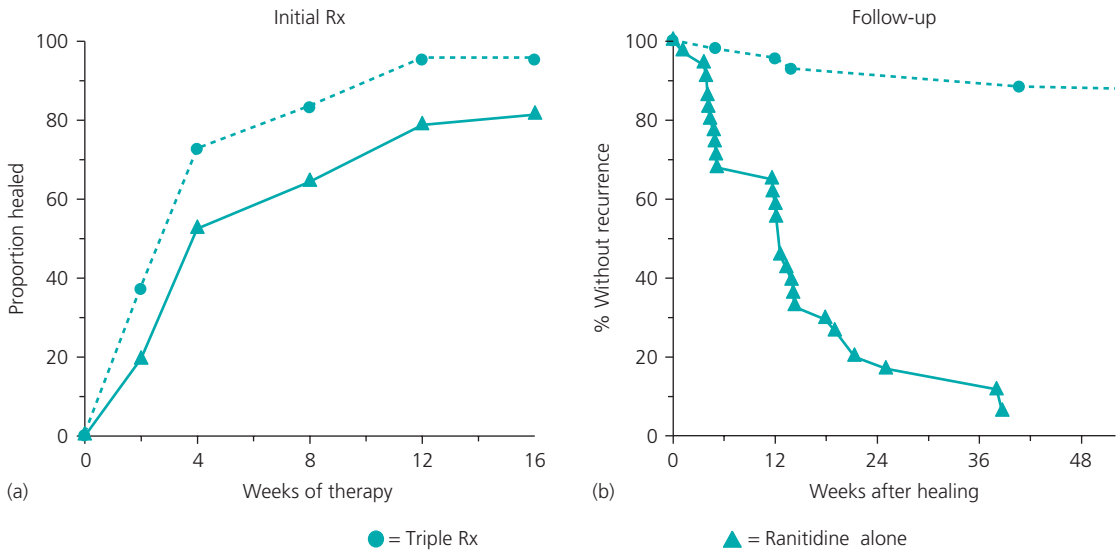


Figure 40.10 **(a)** Patients with duodenal ulcer disease received either triple therapy with ranitidine (closed circles) or ranitidine alone (closed triangles) until the ulcer was healed. **(b)** After ulcer healing patients were followed with regular endoscopic evaluation of the ulcer site. *Helicobacter pylori* (Hp) therapy resulted in enhanced ulcer healing and a reduction in ulcer recurrence. All ulcer recurrences in the antibiotic-treated group were related to failed Hp therapy, use of nonsteroidal antiinflammatory drugs, or both [250,251].

Although there are a number of drug combinations that are approved for use, none is ideal and many are outdated. A number of studies have shown that subtle changes in protocol, such as changes in drugs, drug formulation, dosing interval, dosing in relation to meals, and duration of therapy, may have profound effects on outcome [257,258]. There are a number of practical issues to be considered when selecting a regimen for treatment of Hp infection, including cost, simplicity, effectiveness, side effects, the rate of antibiotic resistance in the community, dose, duration, number of administrations per day, whether to administer with meals or fasting, and whether control of pH is needed [257,258]. None of these issues has been adequately evaluated in a systematic way and recent studies with sequential therapy have definitely shown that the outcome of therapy can be improved. The lack of such studies is possibly part of the legacy of Hp infection being a disease studied by gastroenterologists rather than infectious disease specialists, who by their training and experience are less likely to have declared "victory" or "success" at eradication rates considered unacceptable for other serious infectious diseases. The approach for other infectious diseases has been to identify a therapy that is universally effective and then to attempt to simplify it. With Hp, the approach was to simplify only partially effective therapies. To remain competitive the pharmaceutical industry did the minimum required to obtain approval and, in the absence of clear FDA-defined requirements for high eradication rates, attempted to gain marketing advantages by using shorter duration and less frequent administrations instead of attempting to identify regimens with very high and reliable cure rates. This has resulted in a number of approved but only partially effective regimens.

An evidence-based choice of the best treatment regimen is nearly impossible because large randomized trials comparing the most effective regimens have rarely been done. Metaanalyses have shown that the most commonly used therapies produce cure rates that are typically less than acceptable [259–263]. Although metaanalysis is usually a powerful tool to help understand the overall effectiveness of treatment, it is less helpful in choosing the best therapy because of the presence of considerable heterogeneity in the data, including absence of crucial data such as the presence or absence of pretreatment antimicrobial resistance. There are few head-to-head comparisons of different antibiotic dosages or therapy durations, leaving the influence of these parameters on treatment success unknown. Finally, and unexpectedly based on the responses to treatments of other infectious diseases, the cure rates often differ greatly in different countries despite using the same regimes [262,264]. All of the PPIs appear to be equally as effective as the clarithromycin–amoxicillin or metronidazole triple therapies, and they can be used interchangeably depending on local availability and cost [265]. H2RAs are approximately equally effective with the only benefit being a slight reduction in cost of therapy [266]. Healing rates and time to pain relief are better with PPIs and they are generally preferred.

Large population-based trials have also shown that the high rates of success expected from the pharmaceutical company-sponsored trials are often unobtainable in clinical practice. Part of this difference is likely to be related to patient selection. Pharmaceutical company-sponsored trials typically study patients with duodenal ulcer disease in which the cure rates are typically higher than among patients without ulcers [263,267–269]. Fundamentally, Hp is a serious chronic transmissible infectious disease that causes damage to gastric structure and function and which is a major cause of morbidity and mortality worldwide. As noted above, therapies for bacterial infectious diseases are expected to yield cure rates of nearly 100%. Although Hp proved more difficult than some, by 1989 a successful therapy for Hp had been defined as one that cured more than 80% of patients [270]. By 1995 it seemed that a 90% cure rate was achievable [271] and that 95% or greater cure rates were just around the corner. However, such cure rates have not been achieved, in part because of the failure to learn from the successes of the previous decade. Here we will use the Maastricht consensus conference definition of a useful therapy as being one with an intention to treat (ITT) cure rate of greater than 80% (i.e., those with cure rates of 80% or less would be unacceptable) [272].

Legacy-approved triple therapy (e.g., a PPI plus amoxicillin and clarithromycin) now yields unacceptably low eradication rates in Europe and the United States, typically below the minimally acceptable 80% success rate (Fig. 40.11a; Table 40.4) [268,269,273–296]. These results indicate that traditional triple therapy should no longer be considered initial therapy in Western populations unless pretreatment susceptibility is confirmed, in which case it should be used for 14 days [264,297,298].

Better *Helicobacter pylori* therapies

Because most antibiotics are more effective when given with an antisecretory drug, an antisecretory drug, typically a PPI, is used along with three or four antimicrobials. The original formulas used a PPI plus two antibiotics chosen from amoxicillin, clarithromycin, and metronidazole/tinidazole (i.e., triple therapy) or four drugs consisting of bismuth, tetracycline, metronidazole/tinidazole, and a PPI (i.e., quadruple therapy). These were administered for 7–14 days; as a longer duration of treatment generally provided higher cure rates, 14 days was the preferred treatment time. Searches for new therapies have followed two basic formulas. One was to start with a PPI plus amoxicillin and add a new drug or drugs. The other was to replace the metronidazole/tinidazole in quadruple therapy with another drug. Although triple therapy generally provides unacceptably low cure rates, cure rates with quadruple therapy have largely been maintained. In quadruple therapy, including either bismuth subcitrate or subsalicylate, tetracycline hydrochloride, metronidazole/

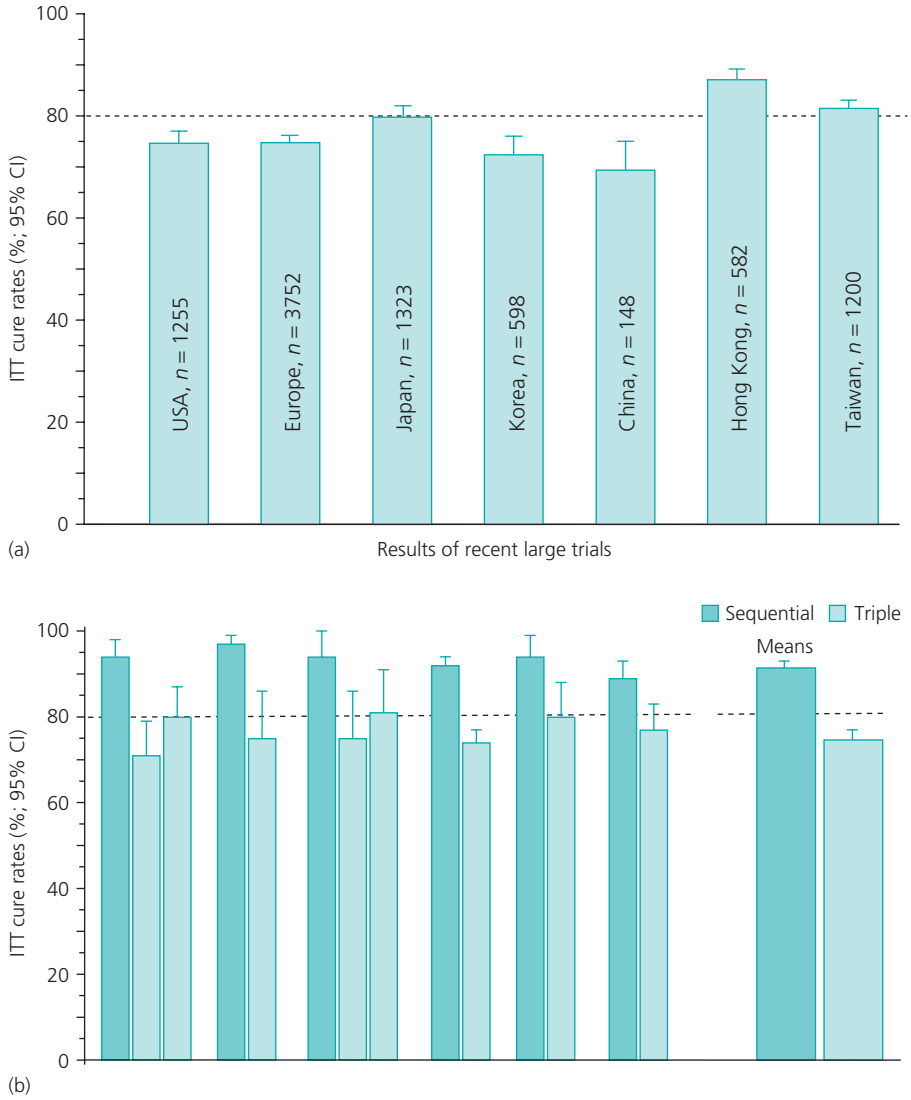


Figure 40.11 **(a)** The mean pooled results as intention-to-cure (ITT) rates and 95% confidence interval (CI) of recent large studies using a proton pump inhibitor (PPI) plus clarithromycin and amoxicillin to treat *Helicobacter pylori* (Hp) infections [268,269,273–278,280,281,283–296]. The 80% or minimally acceptable cure rate is also shown (dotted line). As seen, in the majority of areas the results are unacceptably low suggesting that this therapy should not be utilized unless clarithromycin-susceptible strains are definitely present. **(b)** The six direct comparisons of triple therapy consisting of a PPI plus clarithromycin and amoxicillin with sequential therapy. The pooled results of all of the comparisons are shown on the right. The mean cure rate (ITT) for sequential therapy was only 90.5% (95% CI = 89%–92%) suggesting that additional experiments are needed to further improve the efficacy.

Table 40.4 Regimens for the initial therapy of *Helicobacter pylori* infection

Regimen	Antisecretory adjunct	Antibiotics	Duration	Comments
Quadruple therapy	PPI b.i.d.	Tetracycline HCl, metronidazole, and bismuth	14 days	Higher doses help surmount metronidazole resistance
Sequential therapy	PPI b.i.d.	Amoxicillin	5 days	Cure rates low with dual resistance to metronidazole and clarithromycin
		Clarithromycin and metronidazole[a]	5 days	
Triple therapy	PPI b.i.d.	Clarithromycin plus metronidazole or amoxicillin	7–14 days	Avoid where clarithromycin resistance is high; 14-day treatment increased cure by 12%

a Metronidazole can be replaced by tinidazole.
b.i.d., twice a day; PPI, proton pump inhibitor.

tinidazole, and a PPI, the antimicrobials are effective in an acid environment. Effectiveness requires that the regimen be given at appropriate doses and durations (e.g., a minimum of 1500 mg of tetracycline HCl and 1200–1500 mg of metronidazole/tinidazole, and six or eight tablets of bismuth given in divided doses (e.g., three or four times daily) for 14 days.

New antibiotic combinations

Quinolones (e.g., ciprofloxacin) were tried in the early phases of searching for effective anti-Hp therapies but were abandoned because of the rapid development of resistance. Fluoroquinolones have seen a renaissance as a replacement for clarithromycin in triple therapy. However, as with legacy triple therapy, 7-day duration therapy provides an unacceptably low cure rate, which is improved by longer treatment duration [299]. However, the widespread use of these drugs for other indications has led to a rapid increase in fluoroquinolone resistance in many communities, such that it usefulness will likely be short-lived [300]. Furazolidone is an old but effective drug that can be substituted for metronidazole or clarithromycin in either legacy triple or quadruple therapy. Unfortunately, it has become difficult to obtain in the United States as the company that sold it no longer does; it is, however, easily available in Mexico. Despite the issues related to its role as a monoamine oxidase inhibitor, it is probably one of the best choices for difficult cases. Rifibutin is also commonly used as a substitution drug and, again, longer duration of treatment provides better outcomes. None of these approaches is universally satisfactory and clearly new drugs are needed.

Sequential therapies

Rather than abandoning triple therapy, Italian investigators decided to provide the drugs sequentially. In this approach a PPI and amoxicillin are given for 5 days followed by the PPI plus clarithromycin (250 or 500 mg) and tinidazole (500 mg) for an additional 5 days [282,286]. This approach has repeatedly proven to be superior to legacy triple therapy in both adults and children (Fig. 40.11b) [276,279,282,283,301,302]. Sequential administration of antibiotics has traditionally been avoided because of concern that it would promote drug resistance rather than result in increased eradication; however, this sequential therapy starts with drugs against which development of resistance is rare. This approach markedly reduces bacterial load, making the development of resistant organisms less likely [257,303]. The addition of the third or third and fourth drug would then kill the few remaining organisms. The high rate of success (e.g., reliably ~ 90% ITT) suggests that sequential therapy should supplant triple therapy in regions where the success rates have become unacceptable. However, success of sequential therapy is reduced in the face of dual resistance (clarithromycin and metronidazole) [283]. Sequential therapy can likely be improved by altering the dose, duration, frequency of administration, or order of administration (e.g., similar effects have been reported when all four drugs are given nonsequentially) [304–306].

Recommendations for therapy

In areas where legacy triple therapy no longer provides adequate cure rates (e.g., the United States, most of Europe, and much of developed Asia), one should use sequential therapy as described or add a fourth drug to legacy triple therapy (e.g., metronidazole or tinidazole to the PPI–clarithromycin–amoxicillin combination). Studies are needed to confirm that sequential therapy can be improved by changes in the dose, duration, or drugs utilized. The alternative is to use traditional quadruple therapy with a PPI, bismuth, tetracycline, and metronidazole/tinidazole (Table 40.4).

Other approaches for areas with high rates of antimicrobial resistance

Dual therapy with a PPI and amoxicillin was an early attempt at therapy but was soon abandoned because of low and variable eradication rates. In Germany this therapy is still used clinically, but with a high-dose PPI and high-dose amoxicillin (e.g., 40 mg of omeprazole and 750 mg of amoxicillin given three times daily for 14 days). Reported eradication rates range from 67% to 91% [307–309]. Our recent experience with the high-dose approach has yielded a cure rate in the range of 70%–75%, and this high-dose dual therapy has formed the basis of our approach to sequential therapy for patients who have proven, difficult-to-cure infections. Our approach has been to use 40 mg of omeprazole or esomeprazole and 1 g of amoxicillin three times daily for 5 days, followed by the addition of a third antibiotic or third and fourth antibiotics on day 6, continuing as a triple or quadruple therapy for a total of 12–14 days (e.g., ref. 300). The third and fourth antibiotics should be ones that the patient has not used before (such as tetracycline, levofloxacin, furazolidone, or rifibutin). However, therapy is best guided by pretreatment antibiotic susceptibility testing.

Probiotics and nonantibiotic additions

Probiotics are live, nonpathogenic microbial feed or food supplements that can affect the host in a beneficial manner. The best-studied probiotics are the lactic acid-producing bacteria, particularly *Lactobacillus* spp. and *Bifidobacterium* spp. Probiotics are thought to play a role in keeping the gut microbial ecosystem stable by restoring resident microflora after antibiotic treatment [310,311]. Some strains of *Lactobacillus* spp. and *Bifidobacterium* spp. have shown in vitro bactericidal effects against Hp and a protective effect has been confirmed in some animal models [312]. Clinical trials however indicate that probiotics generally do not cure Hp but may decrease the density of colonization and may decrease the mucosal gastroduodenal inflammation. Metaanalyses have suggested a slight but not dramatic improvement in cure rates by the addition of probiotics [313].

In addition to probiotics, many plant or dairy foodstuffs have been evaluated for gastroprotective and anti-Hp properties [314,315]. Turmeric, cumin, ginger, chili, borage, broccoli sprouts, black caraway, oregano, and liquorices have antimicrobial activities in vitro [316,317]. No data justify the therapeutic use of these agents, but further investigation would be of interest. Mucolytics such as *N*-acetylcysteine [318] and the proteolytic enzyme pronase [319] have additive effects to triple therapy, possibly as a result of reducing the mucosal surface gel layer and improving local antibiotic delivery.

Improving compliance with probiotics

Probiotic cotherapy with antibiotics decreases diarrhea and taste disturbances [320,321]. Additional studies are needed to determine whether such cotherapy improves the poor compliance seen with antibiotic regimens, which is a major reason for ineffective therapy.

Treatment failures

The best results after initial therapy has failed are obtained when susceptibility testing guides the choice of antibiotics. Generally, such testing is unavailable, and choices must be based on drugs that have not been administered previously [322,323]. Clearly, one should avoid drugs (e.g., clarithromycin) where resistance eliminates effectiveness during subsequent therapy. Resistance can be expected if fluoroquinolones have been given previously for any other reason. Quadruple therapy is generally recommended after triple therapies have failed [257,258,324] as this combination is effective even with metronidazole resistance [325,326]. Despite the large number of tablets and the frequent drug administrations, dropout rates and compliance of quadruple therapy in most clinical trials have been comparable to those of twice-daily triple therapy regimens. Furazolidone is often the drug of choice for multiple treatment failures (a salvage therapy).

Explanations for failure of Hp therapy include inappropriate choice of initial therapy (e.g., use of doxycycline instead of tetracycline, or erythromycin instead of clarithromycin), poor compliance with the regimen, and antibiotic resistance. Resistance is the major problem. Compliance can be improved if the patient is counseled regarding the need to continue the regimen for the prescribed duration even when symptoms have disappeared. Compliance is reduced when side effects occur.

Side effects of therapy

Side effects occur in 20%–30% of patients and are generally mild and transient. Severe side effects are rare. The most common side effect is nausea, which can result from most drugs. Clarithromycin given in doses greater than 1 g a day typically causes a taste disturbance and patients should be warned to expect it. Patients treated with bismuth salts will have dark stools and they must be warned that this is not blood [327]. Diarrhea is relatively common with antibiotic therapy, and pseudomembranous colitis is rare but does occur. Candida vaginitis may occur in women, especially following treatment with tetracycline. Alcoholic beverages should not be consumed during metronidazole therapy and for at least 1 day afterward because of an Antabuse-like reaction with abdominal cramps, nausea, vomiting, headaches, and flushing.

Antibiotic resistance

Acquired resistance has been demonstrated with all of the antimicrobial agents used to treat Hp infection, especially with nitroimidazoles, macrolides, fluoroquinolones, and rifampins (rifampicins). Resistance to tetracycline and amoxicillin has been demonstrated but is uncommon. Resistance to metronidazole in the United States is in the range of 25% and rates of metronidazole resistance in excess of 60% have been documented in some developing countries. Increasing the metronidazole dose partially overcomes acquired resistance, but high-dose quadruple therapy is more effective. Hp resistance to clarithromycin has steadily increased, especially in locations where clarithromycin use is high (e.g., Japan and in children in Western countries) [323,328]. Clarithromycin and fluoroquinolone resistance cannot be overcome by increasing the dosage [260,263,323].

Patient education

Poor compliance is the most controllable factor resulting in treatment failure. An informed, motivated patient is important for success. Patients should be instructed about dose, duration, possible side effects, and the necessity of completing the entire course of therapy, rather than stopping when they feel better [327]. Patients who use tobacco should be educated about the benefits of stopping [263]. Written instructions about how to take the pills are helpful. Gastric distress from the drugs can be reduced by instructing the patient to take them with food. Many pharmacists routinely label tetracycline to not be taken with food. We administer all drugs with food, because this greatly simplifies the treatment regimen, is not associated with a reduction in cure rate, reduces nausea, and improves mixing and retention in the stomach. This requires education of both the patient and the pharmacist, who may resist giving tetracycline or bismuth with meals.

Confirmation of *Helicobacter pylori* cure

Confirmation of cure is now the standard of care, particularly because poor outcomes accompany persisting infection and because cure rates are often below 80%. Failure to cure the infection in an ulcer patient causes recurrent ulcer disease, continuing risk of ulcer complications, and the need for more testing and treatment. The infected patient also remains a reservoir for transmission to others, especially family members.

Noninvasive, inexpensive, and widely available urea breath or stool antigen tests are typically sufficient for confirmation of cure. Whichever test is used, it is important to wait a sufficient time after stopping antibiotics to ensure

that residual Hp have multiplied to detectable levels. The data suggest that a 4-week hiatus is preferable for histology, culture, or urea breath testing. Approximately 8 weeks is required for the stool antigen test. Proton pump inhibitors should be preferably stopped for 2 weeks to avoid false-negative results for the tests that require actively growing Hp, including urea breath, rapid urease, and stool antigen tests; culture; and histology. A positive test at any time after therapy indicates treatment failure, but a negative test is difficult to interpret as long as prolonged suppression is possible. If confirmation of cure is critical, then endoscopy with biopsy or a urea breath test should be performed 8–12 weeks posttherapy.

Serological testing is not useful for confirming the outcome of therapy because antibody levels can remain elevated for a long time. A negative test correlates with cure but is unusual within 2 years after therapy. Recurrent infection is associated with a return of test positivity.

Treatment of NSAID ulcers

There are two critical steps in treating NSAID-associated ulcers. First, whenever possible discontinue or at least reduce the NSAID dose. Second, search for and cure Hp infection. If NSAIDs can be discontinued, ulcers will generally readily heal with adequate antisecretory drug therapy, although PPIs are preferred, especially for complicated or large ulcers. Continuing NSAIDs, including COX-2-selective agents, may interfere with ulcer healing; they should be continued only with great caution and with explicit and documented patient consent. The necessity and duration of cessation of low-dose aspirin in patients at high cardiac or cerebrovascular risk has been controversial. A recent controlled trial of patients randomized after endoscopic hemostasis for ulcer bleeding reported that discontinuing low-dose aspirin for 8 weeks led to significantly increased mortality compared with continuing aspirin [329]. Both groups were treated with intravenous and then oral PPI; there was no increase in recurrent GI bleeding in the aspirin-treated group. Although preliminary, this study was well carried out, statistically significant, and indicates that ulcers in high vascular risk patients should be treated aggressively with PPIs without discontinuing low-dose aspirin.

PPIs are preferred for ulcer healing in the presence of ongoing NSAIDs. However, even double-dose PPIs will fail in some patients and recurrences and complications will occur. There are inadequate data from controlled trials to guide treatment decisions in this setting, other than discontinuing NSAIDs. Patient preferences should be included in any decision to continue NSAIDs and we advise documenting that the patient has elected to continue NSAIDs despite being warned of the consequences.

Misoprostol is generally not a good alternative for the healing of NSAID ulcers; it has only been studied for prevention (see Prevention of NSAID ulcers – Prostaglandins). H2RAs, sucralfate, and antacids are inadequate for treatment of NSAID ulcers, especially if NSAIDs will be continued.

Prevention of NSAID ulcers

Earlier studies on the prevention of NSAID ulcer complications were largely short-term trials in normal subjects or patients free of active ulcer disease and well enough to qualify for study. As noted previously, "endoscopic" ulcers, defined by arbitrary size criteria that may or may not require visible depth, are found at surveillance endoscopy in 5%–25% of subjects taking NSAIDs. In contrast, "clinical" ulcers present with complications at a rate of 0.5%–2% per patient-year of NSAID use. Valid recommendations are those based on preventing complications [70] rather than "endoscopic" ulcers, most of which remain clinically silent.

Determining cost-effective prevention of NSAID ulcer complications is difficult with the available data. Although the frequency of ulcer complications from NSAIDs is very low, these drugs are widely used and so the number of patients at risk is high. Several factors impart increased risk for complications (see Risk factors for NSAID ulcers); these factors should be taken into account when deciding whether to offer a more costly regimen to reduce ulcer risk. NSAIDs carry a black box warning label regarding ulcer complications; patients should be informed of this risk and whether they are in a high-risk category and this discussion should be documented.

Prostaglandins

Misoprostol (200 mg four times daily) is approved for prevention of NSAID-induced GU and DU [222,330]. Diarrhea and abdominal cramps limit patient compliance, but these side effects are often transient and may be reduced by starting therapy with lower doses. The low-dose regimen is less effective in preventing "endoscopic" ulcers, suggesting that the higher dose is preferable if tolerated [222]. However, in clinical trials with Hp-negative NSAID users, low-dose misoprostol was superior to omeprazole for both endoscopic gastric ulcer healing and ulcer prevention [223]. Data from a randomized clinical trial with about 9000 patients indicated that misoprostol (200 mg four times daily) reduced NSAID ulcer complications by about 40% [70]. Although patients with active ulcer disease were excluded, misoprostol suppression of complications was evident in the subgroup with history of previous peptic ulcer [70]. Unfortunately, the numbers were small and, most importantly, Hp status was not determined so that it is unknown how well misoprostol works for prevention of clinical complications in Hp-negative subjects vs Hp-positive subjects.

Antisecretory agents: proton pump inhibitors

There are no approved regimens for the prevention of ulcer complications in patients who cannot tolerate or who fail misoprostol. Data from two randomized clinical trials indicate that standard doses of H2RAs do not prevent NSAID-induced

"endoscopic" GU [331,332]. One caveat is that the early studies were carried out in patients of unknown Hp status. Subsequent studies have shown a marked difference between the outcome of those with and without Hp infections [223]. PPIs were remarkably less effective in those without active Hp infection. Interestingly, misoprostol, even at a dose of 400 μg/day, was superior to PPIs for the healing of endoscopic GU and preventing ulcer relapse, and PPIs were also only marginally superior to low-dose ranitidine [223]. The superiority of misoprostol was confirmed in a separate study of full-dose misoprostol vs full-dose lansoprazole [224]. Epidemiological studies have also shown reduced mortality with the fixed combination of diclofenac and misoprostol (Arthrotec) compared with traditional NSAIDs [333,334]. It is likely that misoprostol is under utilized.

There are few data available regarding H2RA or PPI prevention of ulcer complications, particularly in Hp-negative subjects, either naturally or after Hp eradication. There are data, however, regarding the highest risk group (i.e., patients who have experienced an upper gastrointestinal bleed and who have restarted NSAIDs). In this high-risk group, the addition of a PPI to traditional NSAID therapy produced very disappointing results, showing rebleeding rates of 12.8% and 22.2% (annualized) [50,335]. The expected rate is between 24% and 36% per year, suggesting that the benefit was probably in the range of 50% protection. In contrast, the combination of a PPI and the coxib Celebrex appeared effective in preventing rebleeding, whereas the coxib alone did not [62].

Overall, PPIs do not appear to be very effective in preventing clinically important NSAID-associated upper gastrointestinal events. As was so elegantly described for gabapentin, medical opinion can be swayed via the use of marketing studies with surrogate end points, opinion leaders speakers bureaus, and regular consensus conferences, despite the lack of data [336]. As noted above, one should probably not expect more than a 50% reduction in serious events with PPI cotherapy. Importantly, there are no data suggesting a dose–response effect (i.e., the lowest PPI doses have proven equally as effective as higher doses for the prevention of endoscopic ulcers), such that the lowest dose of the least expensive PPI is probably sufficient. No data support the use of sucralfate, antacids or bismuth for the prevention or treatment of NSAID-induced ulceration.

Age alone and age plus symptoms markedly influence the baseline risk of NSAID ulcer complications [68]. The issues have become complicated by recent data suggesting that many traditional NSAIDs have significant cardiovascular risks [59,60], making the choice of therapy among the elderly even more difficult. This has led to rethinking of the role of non-drug therapy [337].

The ulcer risk with different NSAIDs

The choice of agent is dependent on the therapeutic goal. If analgesia is the primary aim, then in most cases a trial of full-strength non-NSAID pain relievers should be initiated first. As with NSAIDs, one should strive for the lowest effective dose. If the patient fails this first-line therapy (e.g., acetaminophen [paracetamol] prescribed at regular intervals for the pain of osteoarthritis or other noninflammatory conditions), the cost, safety, and efficacy of individual NSAIDs should be considered before prescription of NSAIDs.

In general, one should choose NSAID agents with the shortest half-lives for the temporary treatment of acute painful states. The dental pain model suggests that both aspirin and acetaminophen at 650 mg are equally effective as analgesics. The maximum analgesic dose of aspirin or acetaminophen is approximately 650 mg, which is equivalent to 200 mg of ibuprofen. The maximum analgesic dose of ibuprofen is 400 mg. Thus, there is no reason to give more drug when analgesia is the goal, as it only increases the risk with no additional benefits. As noted above, the issue of the cardiovascular risk of selective and nonselective NSAIDs also needs to be taken into account. The decisions regarding which NSAID for which indication are continuing to evolve.

Refractory and recurrent ulcers

With the advances made since the beginning of the 1980s, most peptic ulcers now respond to antimicrobial therapy for Hp or withdrawal of NSAIDs, or heal with usual doses of potent antisecretory drugs. However, occasional ulcers are either refractory to treatment or recur following initial healing. Careful clinical and endoscopic evaluation is essential to ensure that refractory or recurrent symptoms in fact reflect peptic ulceration and to evaluate potential causes (Table 40.5).

Ulcers are considered refractory if unhealed after 8–12 weeks of treatment. However, bigger ulcers take longer to heal. Obviously, the nature of the therapy has a big impact; patients with ulcers refractory to standard doses of H2RAs, for example, represent a different population from the much smaller number refractory to twice-daily PPIs.

Symptoms and recurrent and refractory ulceration

It is essential to differentiate between refractory symptoms and refractory ulcers. Endoscopy discriminates those patients who have intractable symptoms associated with unhealed ulcers from those without active ulceration. Persistent symptoms in the absence of active ulceration suggest that other etiologies for the symptoms should be sought.

Two lines of evidence suggest that symptoms in patients with refractory peptic ulcer often reflect more than acid bathing an unhealed ulcer crater. First, patients with refractory ulcers frequently have a return of symptoms after their ulcers have healed. This was illustrated in a surgical series of patients who underwent highly selective vagotomy for refractory ulcers that did not respond to medical management; only 3% patients developed an ulcer recurrence,

Table 40.5 Causes of refractory and recurrent ulceration

Persisting* Helicobacter pylori *infection
Poor compliance
Resistant organism
Inadequate *H. pylori* regimen
Persisting or recurrent ulcer, no apparent* H. pylori *infection
False-negative *H. pylori* testing
Continued or undiscovered NSAID use
Delayed healing:
Dense fibrosis or deformity
Heavy smoking
Giant ulcer
Serious comorbidity
Inadequate inhibition of acid secretion:
H2RA tolerance or PPI resistance (rapid metabolism)
Hypersecretory states: gastrinoma, idiopathic hypersecretory DU
Uncommon forms of peptic ulcer (see Table 40.1)
Non-peptic ulcer: cancer, Crohn's disease, other infections
Persisting symptoms, no ulcer
Another diagnosis
Irritable gut

DU, duodenal ulcer; H2RA, histamine H_2 receptor antagonist; NSAID, nonsteroidal antiinflammatory drug; PPI, proton pump inhibitor.

whereas 41% returned for recurrent symptoms [338]. In contrast, the patients who underwent surgery for recurrent, but not refractory, ulcers had a 5% ulcer recurrence rate and an 11% symptom recurrence rate.

The second line of evidence is that symptoms in DU patients often include more than classic acid dyspepsia (hunger pain with relief occurring when acid is buffered with food or antacids or inhibited by antisecretory agents) (see Table 40.2). Ulcer patients also frequently complain of symptoms of acid reflux or an irritable stomach (causing indigestion-like symptoms). There can also be considerable overlap with irritable bowel syndrome (IBS). Although complete systematic surveys of ulcer patients are limited, approximately 30%–50% experience symptoms in addition to or other than classic acid dyspepsia (Table 40.2).

On the other hand, the absence of symptoms does not exclude the presence of a persisting ulcer crater. At least 25% of refractory and recurrent ulcers are asymptomatic [339].

Etiology of refractory and recurrent ulcers

For both refractory and recurrent ulcers, the most important step in management is to consider the several elements that may influence natural history. Factors that perpetuate the ulcer itself can also retard healing and predispose to recurrence. These factors are considered in the following sections. This evaluation should exclude diseases mimicking peptic ulcer (e.g., gastric cancer mimicking GU), and causes of peptic ulcer that require specific therapy, such as Hp infection, NSAID use, and gastrinoma (Table 40.5).

Persistent *Helicobacter pylori* infection

Hp infection may have been missed because of a false-negative test or infection may not have been successfully eradicated. The latter situation occurs most frequently as a result of selection of an inappropriate antibiotic regimen, antibiotic resistance, or poor patient compliance.

Nonsteroidal antiinflammatory drugs

Continuing NSAID use is a critical factor underlying a surprising number of troublesome ulcers. Patients may not admit to the use of aspirin and NSAIDs, even after experiencing ulcer recurrences or a refractory course, or undergoing surgery [340–342]. More than 40% of NSAID use associated with refractory ulceration was initially not reported by the patient. It is essential to confidently exclude NSAID use or abuse in all patients with refractory or recurrent ulcers. This evaluation should include measurement of serum salicylate levels, platelet adhesiveness, and taking a history with family members.

Giant ulcers

Large and small GU heal at the same rate on antacids, approximately 3 mm per week [343]. Thus, larger ulcers will require more time to heal. Ulcer size also influences healing rates for DU.

Smoking

Effective antiulcer therapy usually compensates for the deleterious effects of smoking on ulcer healing. However, cigarette smoking, particularly if heavy, may contribute to impaired healing and recurrences [245]. Effects of smoking on ulcer have only been defined for Hp ulcers before eradication (see Risk factors for peptic ulcer – Cigarette smoking).

Impaired healing, scarring, and deformity

It is likely that general factors (severe comorbidity or catabolism) and local factors (dense scarring) impair healing. Specific mechanisms have not been defined, but low mucosal blood flow, which would impair angiogenesis and tissue repair, is one attractive candidate.

Duodenal deformity, probably resulting from dense scarring, has been associated with poor healing [344–346]. Local inflammation, an absence of glandular structure, and blunt or absent villi are markers of poor histological restoration and healing, which also predispose to ulcer recurrence [347].

Acid hypersecretory states

Obviously, refractory and recurrent DU can be due to gastrinoma [348]. It is not surprising that in nongastrinoma patients the degree of healing inversely reflects acid output.

In one study 55 patients with complete healing of their DU had a mean basal acid output of 6.6 mEq/h, whereas 20 patients with nonhealing DUs had a mean basal acid output of 20 mEq/h [349]. Following Hp treatment, occasional recurrences have been associated with continued high pentagastrin-stimulated peak acid output, rather than reinfection with Hp [35]. Once identified, maintenance antisecretory treatment may be necessary to prevent relapse in this subset of patients.

Impaired response to antisecretory agents

Tolerance to H2RAs that develops during sustained therapy has been hypothesized to contribute to some cases of refractory ulcers in response to these agents. Tolerance does not seem to develop with PPIs. Occasional patients fail to respond to standard doses of PPI; double doses are effective in inducing and maintaining healing in many of these individuals [350].

Diagnostic approach for refractory or recurrent ulceration

A careful clinical evaluation is needed to assess the etiology of symptoms. For DU, fasting serum gastrin should be measured to exclude gastrinoma [348], but caution is necessary because both PPI and Hp can raise gastrin levels. Endoscopy is necessary to determine if symptoms are linked to refractory or recurrent ulceration. Edema, spasm, or a deformed duodenal bulb can impair the accuracy of endoscopy, especially in inexperienced hands. If ulceration is found, biopsies of the antrum and body are indicated to assess Hp status. The presence of gastritis suggests false-negative Hp testing (see Laboratory evaluation – *Helicobacter pylori* testing). Refractory ulcers in the absence of antral gastritis are usually the result of NSAID use.

With refractory ulcers, particularly in the absence of Hp or NSAID use, endoscopic biopsy of the ulcer should be considered to exclude other benign (e.g., infectious causes) and malignant diseases that can present with ulceration. In some instances endoscopic ultrasound may be helpful, especially for the diagnosis of ulcerated smooth muscle tumors.

Refractory or recurrent GU require multiple biopsies of the ulcer margin and base to exclude neoplasia. Nonhealing GUs must be considered cancer until proven otherwise. Gastric ulceration in the presence of severe body gastritis or atrophy should heighten concern about malignancy.

Treatment of refractory ulcers

The presence of a refractory ulcer should always prompt the clinician to reconsider the diagnosis and management to date, focusing on three points. First, confirm that the patient is compliant with an adequate PPI regimen, now the mainstay of treatment. Second, ensure that continued use of gastrotoxic drugs (e.g., NSAIDs) has been confidently excluded by assessing platelet function, measuring salicylate levels, and taking a history from family members. Third, either confirm that Hp has been confidently excluded using a second, sensitive test or confirm that it has been successfully cured. Judging the appropriate length of continued therapy is an empirical process for refractory ulcers, which must be adapted to the individual patient. If the underlying cause can be reversed, then treatment usually becomes easy. However, particularly for large or densely scarred ulcers, an extended treatment period may be necessary to prevent recurrences and allow mucosal remodeling to take place.

Surgery is only rarely required for refractory or recurrent ulceration. Unless emergent, surgery should be approached very cautiously and only after carefully excluding reversible causes and allowing adequate time for healing on PPIs, assuming patient compliance can be ensured. Truly refractive ulcers are sufficiently rare that one should consider referral, especially of difficult cases, to individuals with extensive experience.

Prevention of ulcer recurrence

Maintenance therapy is indicated to prevent recurrence in high-risk subgroups, defined by a history of complications, frequent recurrences, or refractory, giant, or severely fibrosed ulcers. In patients in the high-risk subgroup who are infected with Hp, maintenance therapy should be continued at least until cure of the infection has been confirmed. Maintenance therapy is also indicated in high-risk patients who fail Hp eradication or who have Hp-negative ulcers. In the Hp–NSAID era, maintenance has received little study, except for the prevention of NSAID ulcers during continued NSAID treatment (see Prevention of NSAID ulcers). PPI therapy appears to be effective for the small set of hypersecretory, non-Hp DU [93]. When maintenance is indicated, standard doses of PPIs are reasonable choices, unless the patient has previously failed such a regimen and requires higher doses.

Complications of peptic ulcer

The complications of ulceration include hemorrhage, perforation, penetration, and obstruction, and occur with all types of ulcers. In one study the annualized incidence of complications of NSAIDs per year of NSAID use were 1% for hemorrhage, 0.3% for perforation, and 0.1% for obstruction [70]. Complications for largely Hp-positive ulcers occur at a rate of about 1%–2% per patient-year of follow-up [351].

The pathophysiological factors that distinguish patients predisposed to complications or recurrence of complications are unclear. However, it is clear that patients with complicated ulcers represent a distinct subset who are more prone to experiencing another complication than are patients without history of previous complications [351–353]. Another common feature is that most complicated ulcers are chronic and densely fibrosed ulcers, penetrating deeply into the wall to form a dense fibrotic scar that is slow to heal, involves blood vessels, or reaches the serosa. A prolonged ulcer history is also common among complicated ulcers; however,

some patients have few or no symptoms until complications develop, despite an obviously scarred, chronic ulcer. In contrast, some complicated ulcers, especially when linked to NSAID use or developing in hospitalized patients (stress ulcers), are acute and occur without dense fibrosis, previous symptoms, or ulcer history; they can develop and complicate within days.

Gastric outlet obstruction

Pathophysiology and epidemiology

Gastric outlet obstruction is the least frequent ulcer complication. Most cases are associated with duodenal or pyloric channel ulceration; gastric ulceration accounts for only 5% of cases (Table 40.6) [353]. Several elements can contribute to gastric outlet obstruction. Rapidly reversible elements include spasm, edema inflammation, and pyloric dysmotility related to the ulcer or inflammatory changes. Fibrosis, scarring, and deformity underlie slowly reversible or irreversible obstruction. Gastric atony, developing after prolonged obstruction, contributes to gastric retention.

Table 40.6 Differential diagnosis of gastric retention

Gastric outlet obstruction
Peptic ulcer: DU and channel ulcers greater than GU
Neoplasia:
Gastric carcinoma
Pancreatic cancer
Lymphoma
Benign adenomatous polyp
Inflammation:
Cholecystitis
Acute pancreatitis
Crohn's disease
Eosinophilic gastroenteritis
Miscellaneous causes:
Adult hypertrophic pyloric stenosis
Postsurgical stenosis
Pyloric, antral, and duodenal webs/diaphragms
Duodenal bands
Annular/ectopic pancreas
Caustic stricture
Gastric dysmotility (see Chapter 39)
Idiopathic
Drugs: atropine, opioids, high-dose tricyclics, etc.
Neuromuscular:
Diabetic gastroparesis
Gastric atony from prolonged obstruction
Previous gastric surgery: vagotomy, antrectomy
Collagen vascular disorders, e.g., scleroderma
Pseudoobstruction
Inflammatory: contiguous inflammation, peritoneal disease
Miscellaneous anorexia nervosa

DU, duodenal ulcer; GU, gastric ulcer.

There are few good data available regarding the frequency and natural history of obstruction. Most data come from venerable surgical series. Obstruction had accounted for 10%–30% [353,354] of patients undergoing ulcer surgery, but this number is dropping as medical and endoscopic management have become more effective and underlying causes have been identified and treated. Although there are no good statistics, it appears that obstruction from peptic ulcer has become less frequent than bleeding and perforation. Obstruction presenting in a medical setting is likely to be different from surgical data, but data are limited [70,355,356]. In a medical setting, partial outlet obstruction that resolves with medical management is more likely. Reflecting the apparent drop in obstruction due to peptic ulcer, malignancy has emerged as a prominent cause of obstruction [355,357].

Webs in the antrum [358] and to a lesser extent in the duodenum [359] can occur either as a congenital abnormality or as a sequelae of peptic or inflammatory disease. Surprisingly, symptoms can be intermittent with these lesions and in some cases it is difficult to determine if they are obstructing.

Both Hp and NSAIDs can clearly cause obstructing ulcers. As with other complications, the rate of Hp infection in patients not consuming NSAIDs is controversial [360,361]. Regardless, the bottom line is clear; both factors must be diligently excluded.

Clinical presentation

Symptoms of gastric retention include early satiety, bloating, indigestion, anorexia, nausea, vomiting, epigastric pain, and weight loss (Table 40.7) [353,356]. Although commonly absent, the presence of recognizable food more than 8–12 h after eating is indicative of gastric retention. The average duration of symptoms is 1 month, although one-third of patients have symptoms for longer than 3 months. Although these patterns are useful, they are drawn from surgical series, and presentation in a medical setting may be different. Important variables include the patient's response to these symptoms, age, comorbid conditions, and general health status. A subset of patients with chronic organic disease – and peptic ulcer in particular – have visceral hyposensitization; in these patients, high-grade outlet obstruction may be present without perceived gastric distress. Appetite and food intake may be preserved in association with delayed, large-volume vomiting of undigested food. Pain, nausea, and bloating are variable with gastric retention (Table 40.7). Conversely, other patients may have minimal or intermittent obstruction, but complain of considerable pain and indigestion.

Diagnosis and differential diagnosis

It is first important to determine whether the symptoms are due to gastric retention and whether outlet obstruction is the explanation. Clinical assessment may provide helpful clues to the presence of gastric retention [353] (Table 40.7). A high

Table 40.7 Manifestations of gastric retention

History	Vomiting (80%–90%), especially with stale food ingested > 4 h previously; projectile or nocturnal Abdominal pain (87%) Weight loss (65%) Early satiety (60%) Nausea (40%)
Physical examination	Visible gastric peristalsis Succussion splash > 4 h after eating (25%)
Gastric aspirate	Gastric residual volume > 300 mL 4 h after eating Overnight fasting gastric residual volume > 200 mL
Radiography	Retention of barium > 50% at 4 h Large, atonic stomach; retained food
Special studies	Saline load (see Table 40.8)
External scanning technique – radionuclide gastric emptying	Liquid phase: fractional emptying rate < 10% per min Solid phase: fractional emptying rate < 0.5% per min
Barium burger	Barium residue after 6 h in intact stomach and after 3 h in resected stomach

Table 40.8 Saline load test

Procedure

Place a No. 16 French nasogastric sump tube into the stomach
Position the tip 65 cm from the nares
Empty the stomach
Place patient right side down to enhance gastric emptying
Infuse 750 mL of 0.9% NaCl into the tube over 3–5 min
After 30 min use a syringe to aspirate and measure gastric residual volume

Interpretation of gastric residual volume

> 400 mL: definite gastric outlet obstruction
300–400 mL: probable gastric outlet obstruction
200–300 mL: indeterminate
< 200 mL: normal

volume of residual gastric fluid is expected and obstruction is unlikely if it is not present. After gastric decompression, the saline load is helpful to evaluate mechanical outlet obstruction [353] (Table 40.8). If gastric residual volume is found and the saline load is positive, then several days of gastric decompression are indicated, as considered below.

Definitive diagnosis of gastric pathology must await gastric cleansing, decompression, and restoration of fluid and electrolyte abnormalities (see Medical management). Endoscopic inspection and biopsy are indicated in suspected cases of outlet obstruction and usually provide definitive diagnosis of underlying pathology. Although conventional upper GI radiography can provide useful information [362], it is rarely definitive and the stomach is left with a large amount of barium residue that interferes with visualization during endoscopy.

Malignancy must be excluded in all cases of obstruction; obstruction should be assumed to be neoplastic until proven otherwise (Table 40.7). About 50% of cases of outlet obstruction are due to malignancy [355,357]. Patients with malignancy tend to be older and typically do not have a history of peptic ulcer or use NSAIDs [355]. Importantly, in about 10% of obstructed patients undergoing endoscopy and biopsy, malignancy was not suspected before operation [355,357]. Endoscopy should include careful inspection and multiple, jumbo biopsies. Endoscopic ultrasound with biopsy or needle aspiration should be considered in persisting obstruction. CT with oral and intravenous contrast may also be useful in detecting malignancy, although a negative study does not rule out malignancy.

Other causes of delayed gastric emptying require consideration (Table 40.7). Diagnosis of obstruction due to peptic ulcer has been delayed when confused with various conditions, including vomiting in pregnancy [363] or a presentation suggesting anorexia nervosa [364].

Medical management

Especially in a surgical setting, obstruction is often accompanied by severe, long-standing ulcer disease [353]. Furthermore, in the era before definitive treatment of the underlying cause was possible, obstruction frequently recurred and a large majority of cases underwent surgery within a 3-year period [365]. Although no formal trials or hard data are available, management has undergone a dramatic evolution since the mid-1990s.

In patients with severe and long-standing obstruction, the initial steps in management are to resuscitate the patient and to confirm the diagnosis, including emptying and decompressing the stomach. Depending on the condition of the patient, the diagnosis of gastric retention can be confirmed at endoscopy or by using a saline load test. If gastric retention is confirmed, the nasogastric tube should be replaced by a large-bore (e.g., Ewald) tube and the stomach lavaged to remove debris. The nasogastric tube should then be replaced, good placement and function of the tube confirmed, and intermittent suction continued for 3–5 days to decompress the stomach while intravenous fluid and electrolytes are administered. In some cases, prolonged vomiting and poor fluid intake cause prerenal azotemia, hyponatremia, or a hypokalemic metabolic alkalosis (the loss of HCl and volume leads to renal conservation of Na and loss of K). In these cases, after intravenous saline has restored volume status and urine output, K can be administered. Only in rare cases will alkalosis require direct correction with intravenous HCl. Intravenous H2RA or PPI therapy is useful to reduce volume requirements, facilitate control of gastric pH, and heal the ulcer. Careful assessment of nutritional status is required and intravenous hyperalimentation deserves consideration in malnourished patients. Repeating the saline load test (Table 40.6) can gauge progress; residual volume of less than 200 mL indicates resolution of obstruction. About half of the cases of chronic obstruction will respond to this regimen [366].

In the current era, most patients do not have chronic obstruction but rather have symptoms due to spasm, edema, inflammation, or associated pyloric dysmotility rather than to malignancy or dense scarring. Such patients respond rapidly to intravenous H2RA or PPI therapy. Some start anti-Hp therapy immediately [367] but in our experience the rapid response is actually related to the antisecretory drug therapy and not to the antibiotics and we prefer a more measured approach including endoscopy and biopsy to exclude malignancy, diagnose ulcer disease, and determine if Hp is present.

Although there are no controlled studies, obstruction definitively occurs with Hp ulcers, and case reports indicate that, as expected in the absence of dense fibrosis, cure of Hp infection is associated with resolution of the obstruction [367,368]. Since deformities of the duodenum appear to resolve slowly following cure of Hp infection, complete resolution may take considerable time following cure of Hp infection.

Although controversial in the past, NSAID ulcers also cause gastric outlet obstruction. The MUCOSA trial had three cases among 4500 NSAID users over 6 months of observation [70]. In 119 consecutive elderly patients undergoing endoscopy for peptic ulcer, 12 cases with obstruction were found and 11 were consuming NSAIDs [369]. Most importantly, these cases responded to medical management, possibly suggesting a different natural history from ordinary peptic ulcer presenting with obstruction.

Medical management should include antisecretory therapy, and full-dose intravenous antisecretory therapy is warranted during gastric decompression. After the patient resumes oral intake and the stomach is emptying adequately, oral PPI treatment can be started. If Hp is present we recommend deferring antibiotic therapy until adequate gastric emptying can be ensured. Full-dose antisecretory medication should probably be continued until successful Hp cure has been confirmed and until any antroduodenal deformity has resolved. It is reasonable to assume that the combination of curing Hp infection and continued antisecretory therapy will eventually reverse antroduodenal deformity and the tendency for recurrent obstruction.

For patients who do not respond to medical therapy alone, endoscopic balloon dilation can provide lasting benefit [356], although repeated sessions are necessary in some cases. Dilation can be accomplished at endoscopy with a through-the-scope balloon or by using a balloon placed over a guidewire positioned under fluoroscopic guidance. Advancing the wire well past the stricture and use of 6- to 8-cm balloons stabilizes balloon placement during dilation [353]. Balloon dilation should be monitored by pressure gauge, sustained for 1 min, and repeated 3–4 times. Successful dilation is usually confirmed by pulling the balloon through the strictured segment, although failure to accomplish this test does not preclude a successful procedure. Balloon dilation using dilute contrast medium allows progress to be followed fluoroscopically; a waist forms initially during dilation but is effaced during balloon distention in successful dilation. Long duodenal strictures are the most difficult to dilate. In one series of 30 patients [356], dilation to 18 mm produced perforation in two patients, who did well after prompt surgery. Although no controlled studies are available, stepwise dilation for tight obstruction will probably lower the risk of perforation. Symptoms are usually improved with successful dilation to 12 mm [353] and there may be advantages to postponing dilation beyond 15 mm until after a period of time and medical management. With the finite risk of perforation, patients should be appropriately prepared for surgery before dilation and monitored closely after dilation before resuming oral intake. A postprocedure oral contrast study will generally detect perforation in a timely fashion.

Peptic ulcer hemorrhage

The presentation and management of ulcer hemorrhage is detailed elsewhere (see Chapter 124). Key elements in management are outlined below.

Clinical assessment at triage is essential: Is bleeding life-threatening? The aggressiveness of intervention should be scaled to the severity and rapidity of bleeding. Appropriate, cost-effective management requires triage by experienced clinicians. Several factors influence prognosis: age older than 60 years, hematemesis, hematochezia from an upper GI source, shock, requirement for multiple transfusions, and

the presence of comorbid disease, particularly of cardiorespiratory, hepatic, or malignant origin. Mortality is not usually due to exsanguination but to either comorbid disease or to the surgical procedure performed to stop the bleeding. Appropriate global assessment of patient status is critical at the outset and periodically during care.

Endoscopic diagnosis and assessment of risk for continued bleeding or rebleeding has become the focal point of initial management. However, first the patient must be stabilized and the general status assessed. Then, upper endoscopy is indicated, both for acute upper GI bleeding and also for acute lower GI bleeding. Hematochezia is a trap for the uninitiated; about 10% of apparent acute lower GI bleeding is due to an upper source, most frequently duodenal ulcer. When peptic ulcer is found, endoscopic findings have been firmly established as useful indicators for the risk of recurrent bleeding (active bleeding, visible vessel, and adherent clot).

Intravenously administered high-dose PPIs along with endoscopic management are indicated for actively bleeding or high-risk lesions (visible vessel or occasionally adherent clot) [370].

Surgical intervention remains an important option for carefully selected cases (see Chapter 44). Although emergency surgery usually carries a high risk, controlled studies have indicated an excellent outcome when surgical intervention is used early in the course of management for rapid or recurrent bleeding that is not controlled by expert endoscopic intervention. Surgical risk is much higher when surgery is delayed, awaiting multiple endoscopic or radiological interventions. Optimal management entails endoscopic intervention by endoscopists experienced in management of acute bleeding; this expertise is evident by rapid and effective intervention coupled with timely hand-off to surgery in cases in which active or recurrent bleeding is not readily controlled by endoscopic intervention. The experienced endoscopist knows when to quit and send the patient to surgery. Outcomes are best when patients are managed by a team that includes experienced endoscopists and surgeons.

Without definitive treatment, the risk of recurrent bleeding is 10%–36% per patient-year [351,352,371]. Every effort should be made to identify underlying causes; eradication of Hp, if present, and discontinuation of NSAID use dramatically decrease subsequent recurrent hemorrhage. Diagnosis of Hp is more difficult in bleeding patients, in part because of obvious factors such as the reluctance to perform biopsies for definitive tests at the time of bleeding and also because of the use of Hp suppressant therapy (PPI, bismuth, and many antibiotics) [372]. In addition, active bleeding appears to cause false-negative tests dependent on Hp urease activity [189,373,374] as well as false-negative Hp stool antigen tests [375]. The UBT is typically still accurate. Because of the high pretest prevalence of Hp, negative serology or urease testing should be confirmed with histology to accurately exclude inflammation and organisms. An extra effort to exclude Hp infection can be justified in this high-risk subset of patients. It is critical to detect NSAID and aspirin use; as noted previously, surreptitious use is easily missed and must be sought. There should actually be no rush to make a definitive diagnosis as treatment of an Hp infection can be safely delayed until after the acute episode has passed and even until after the ulcer causing the bleed has healed.

Data indicate that early treatment with high-dose continuous intravenous PPIs coupled with endoscopic hemostatic therapy has a beneficial impact on ulcer rebleeding rates; decision analysis suggests that administering PPIs for the treatment of bleeding ulcers is cost-effective [376,377].

Ulcer perforation (see Chapter 44)

Duodenal, antral, and gastric body ulcers account for 60%, 20%, and 20% of perforations respectively [353,378]. One-third to one-half of perforated ulcers are NSAID associated [378], usually in elderly patients [379]. Hp infection probably accounts for the large majority of other cases but data are limited and rates of Hp infection vary considerably among available studies [380,381]. As with bleeding ulcers, surreptitious NSAID use and false Hp testing should be kept in mind, but it appears that there are other explanations for a subset of these cases. Although perforation occurs in patients without an ulcer history, when a detailed history is taken after recovery, the majority of patients report having ulcer symptoms before the perforation [382]. However, older patients who experience perforation in association with NSAID use frequently deny any preceding ulcer symptoms.

As with many acute conditions, the challenge is to consider perforation in the differential diagnosis. The highest mortality rates occur when presentation to the hospital or recognition of the problem is delayed. High mortality rates also occur when hospitalized patients suffer perforation. Perforation presenting in "inpatients" is probably due to stress ulcers. This presentation will be blurred and outcome impaired by comorbid disease. Perforation should be included among the conditions considered in the evaluation of shock and sepsis.

As detailed elsewhere (see Chapter 44), surgical management has changed since the mid-1980s; sealing the perforation with an omental patch and performing a parietal cell vagotomy is a highly effective approach with low rates of recurrence and morbidity [381]. However, simple closure followed by Hp eradication appears adequate, especially for perforated DU [383]. Nonoperative management (intravenous fluids, antibiotics, nasogastric suction) is also an alternative in cases in which the leak seals quickly in response to medical management [384]. Conservative management becomes particularly attractive because the underlying cause can be reversed by either discontinuing NSAID use or curing Hp infection.

In the past, patients with a history of ulcer perforation who received an omental patch without definitive ulcer operation had a high likelihood of experiencing another ulcer com-

plication [353]. Although no firm data are available, it is reasonable to assume that definitive therapy (cure of Hp infection or removal of NSAID exposure) will prevent the recurrences of ulcers due to these causes. It is also likely that maintenance therapy with antisecretory agents will reduce, but not eliminate, recurrence of ulcer complications. Therefore, patients with a history of ulcer perforation should be offered definitive management, as outlined previously. Patients with a history of Hp should be maintained on full dose antisecretory therapy until successful cure of Hp has been confirmed.

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B STOMACH

41 Zollinger–Ellison syndrome

John Del Valle

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"Fortunate indeed is the surgical service that does not have one or more problem cases of recurrent marginal ulceration despite the adequacy of the surgical attack," noted the opening sentence of Zollinger and Ellison's landmark description of the syndrome that today bears their names [1]. Benign ulceration of the upper jejunum associated with extremely high gastric acid secretion and the presence of a non-β-cell adenoma of the pancreas was the triad of clinical findings described in two patients who eventually required total gastrectomy for relief of their severe ulcer disease. Although earlier reports [2,3] described a similar clinical situation, it was Zollinger and Ellison who hypothesized that the components of this clinical triad were intimately related and were produced by a humoral agent that induced gastric acid hypersecretion and its sequelae. This hypothesis was confirmed by Gregory and colleagues [4,5], when they established that the tumor extract from a patient with Zollinger–Ellison syndrome contained a substance that was chemically and physiologically indistinguishable from the antral hormone gastrin.

Subsequent development of a sensitive and specific radioimmunoassay for gastrin [6,7] facilitated the measurement of this peptide in the sera of patients with Zollinger–Ellison syndrome and confirmed that their circulating gastrin concentrations exceeded by several-fold the plasma levels found in a normal control population. This sensitive assay also permitted earlier tumor detection, which has led to a steady increase in the number of patients described with gastrinoma. Potent inhibitors of gastric acid secretion and sophisticated preoperative and intraoperative diagnostic procedures have transformed Zollinger–Ellison syndrome from an illness for which total gastrectomy and tumor progression were the normal course to a condition for which a cure is feasible in many patients. Moreover, new molecular tools are leading to an improved understanding of disease pathogenesis at the cellular level, potential new markers for prognostication and novel therapeutic approaches for advanced disease.

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Epidemiology

The true incidence and prevalence of Zollinger–Ellison syndrome are unknown. Estimates of incidence have ranged from 0.1% to 1% of patients who present with peptic ulcer disease in the United States [8]. The heterogeneous clinical manifestations, the widespread use of proton pump inhibitors (PPIs) for the treatment of acid peptic disease and its sequelae [9], and the high incidence of silent pancreatic endocrine neoplasms [10] have all contributed to an underestimation of the incidence and prevalence of Zollinger–Ellison syndrome. The age range of affected persons has varied from 7 to 90 years, with the majority of patients diagnosed between the age of 30 and 50 years. The disorder is found more often in men than women, with ratios varying from 2:1 to 3:2 [8,11,12].

Gastrinomas can be subdivided into tumors that are sporadic [13] and those that are genetically transmitted and associated with *multiple endocrine neoplasia type 1* (MEN1) [14]. Sporadic gastrinomas, the more common of the two types, frequently behave as malignant tumors. Zollinger–Ellison tumors associated with MEN1 occur at a younger age than the sporadic tumors and have been characterized by some investigators as following a more benign course. These two subtypes have various distinguishing features, as discussed later in this chapter.

Pathophysiology

Gastric acid hypersecretion and severe peptic ulcer diathesis secondary to unbridled release of gastrin from a non-β-cell endocrine neoplasm are the essential elements leading to Zollinger–Ellison syndrome. The neoplastic pancreatic cells secreting gastrin are thought to arise from the ductular epithelium and not from cells of the islets of Langerhans,

despite the appellation of gastrinomas as "islet cell tumors" [1]. Normally, the adult pancreas does not secrete gastrin, but the fetal pancreas contains large quantities of this peptide [15]. After birth, the gastrin-secreting cells in the pancreas disappear and are not seen again, except as benign or malignant neoplasms in Zollinger–Ellison syndrome. The hypergastrinemia that results from the release of peptide from an endocrine neoplasm free of the usual regulatory restraints has two synergistic effects: overstimulation of gastric parietal cells to secrete acid and increased mass of parietal cells susceptible to overstimulation. The enhanced gastric acid hypersecretion that results is presumably the cause of the clinical manifestations (i.e., acid peptic disease and diarrhea) of the gastrinoma syndrome.

In contrast to many of the common malignant diseases, such as those originating in the pancreas, colon, or breast, gastrinomas are not associated with alterations in classic oncogenes such as *ras*, *myc*, *fos*, and *src* or with tumor suppressor genes such as *p53* or the retinoblastoma susceptibility gene (*Rb*) [16]. Despite this observation, studies reveal that 16%–40% of sporadic gastrointestinal neuroendocrine tumors express mutations in the *MEN1* gene, which is found to be defective in patients with MEN1. This is a 10-exon gene found on chromosome 11q13 [17–20], as further described later in this chapter.

Goebel and colleagues [21] attempted to correlate mutations of the *MEN1* gene with clinical characteristics in 51 patients with sporadic gastrinoma. This study revealed that 31% of the tumors had a mutated *MEN1* gene. Of interest, mutations are clustered within a fairly narrow region (amino acids 61–166), a finding different from that seen in MEN1-related gastrinomas, in which the mutations occur throughout the gene. The presence of a mutation in the *MEN1* gene did not appear to be useful as a marker at an early stage of disease for predicting patient outcome.

Additional studies have demonstrated a series of genetic alterations in gastrinomas including methylation and deletion of the *p16/MTS1* tumor suppressor gene [22], amplification of the *HER-2/neu* protooncogene [23], amplification of chromosome 9q [24], and deletion of chromosome 1 [25,26] or 3p [24–27]. Yu and colleagues [27] performed a detailed survey of genetic alterations in eight sporadic gastrinomas. Although the number of tumors was small, these investigators concluded that important molecular features of gastrinomas include defects in chromosome 1, aneuploidy, and mismatch repair defects and deletions in the *MEN1* gene. Serrano and colleagues [28] determined that alterations (hypermethylation of the promoter) in the tumor suppressor gene *p16INK4aCDKN2A* were present in more than 50% of gastrinomas, independent of tumor extent. This genetic alteration is the most common one found in gastrinomas to date and suggests that changes in this gene may be an early event in its development. Subsequent attempts at characterizing the molecular fingerprint of pancreatic endocrine tumors using microarray technology will be discussed later in the chapter. Although posttranslational processing variants of gastrin have been described in patients with gastrinoma [29–32], the clinical relevance of this finding has not been established.

Tumor distribution

More than 80% of gastrinomas have been localized in the anatomical area known as the *gastrinoma triangle* [33]. The boundaries of this triangle include the confluence of the cystic and common bile ducts superiorly, the junction of the second and third portions of the duodenum inferiorly, and the junction of the neck and body of the pancreas medially. The most common extrapancreatic site is the duodenum [34], wherein up to 40%–50% of sporadic gastrinomas arise [35–37]. Other less common extrapancreatic sites include the stomach, bones, ovaries, liver, heart, and lymph nodes, which together account for fewer than 10% of gastrinomas [38–40]. More than 50% of gastrinomas are considered to be malignant. Solitary pancreatic lesions were found in fewer than 30% of the earliest reported patients with gastrinoma [11]. With the trend toward earlier measurement of serum gastrin and exploratory surgery [41], the incidence of metastatic disease at the time of operation has decreased. Nevertheless, multiple tumors or metastatic lesions are still observed in 30%–55% of patients at the time of diagnosis [35,37].

The morphology of the neoplastic cells in gastrinomas is heterogeneous [42,43]. The gastrin-producing cells are generally well differentiated and contain histological markers characteristic of endocrine neoplasms in general; that is, they contain chromogranin, neuron-specific enolase, synaptophysin, and tyrosine hydroxylase. The degree of malignancy does not appear to correlate with a gastrinoma's histological appearance; however, this observation must be tempered by the knowledge that tumor aggressiveness is usually determined retrospectively.

As in the case with other endocrine neoplasms, gastrinomas have been found to express a variety of neuroendocrine peptides besides gastrin, including somatostatin, pancreatic polypeptide, adrenocorticotrophic hormone, and vasoactive intestinal polypeptide. Although the clinical manifestations are generally associated with overproduction of one hormone, case reports illustrating combined syndromes have been described [44–48]. Of these, Cushing syndrome in association with gastrinoma is the mixed syndrome most frequently described [49,50].

Clinical manifestations

More than 90% of patients with gastrinoma develop ulcers in the upper gastrointestinal tract at some point during the

disease [11–13]. Presenting ulcer symptoms are indistinguishable from those associated with benign peptic ulcer disease, but frequently they are less responsive to standard therapy. As in benign peptic ulcer disease, ulcers in patients with Zollinger–Ellison syndrome occur most frequently in the first portion of the duodenum (75%), and they are usually solitary. However, ulcers in patients with gastrinoma also may occur in the second, third, and fourth portions of the duodenum (14%), and in the jejunum (11%) [12]. The ulcers are usually less than 1 cm in diameter but can occasionally present as giant lesions. Peptic ulcer disease refractory to standard medical therapy, recurrent ulcers after prior gastric surgery, diarrhea in patients with ulcers, or peptic disease presenting with complications such as obstruction, perforation, or bleeding suggest a possible diagnosis of Zollinger–Ellison syndrome. Esophageal symptoms caused by acid reflux are seen in up to two-thirds of patients with Zollinger–Ellison syndrome [51,52]. The disease found in the esophagus may range from mild to severe esophagitis complicated by stricture formation and Barrett mucosa.

Diarrhea is the other common feature of Zollinger–Ellison syndrome; it may be present in more than half of patients, and can precede the diagnosis by many years and occur in the absence of peptic ulcer symptoms [11,13,53,54]. The pathogenesis of diarrhea is likely multifactorial, but its dependence on acid hypersecretion is demonstrated by amelioration of symptoms on continuous nasogastric suction or inhibition of acid secretion [8,55]. Added features of the diarrhea are steatorrhea and maldigestion [56,57], which result from the inactivity of pancreatic enzymes at the low pH of the duodenum caused by the excessive acid load. The acidic pH of the small bowel also may lead to damage of the intestinal mucosa, resulting in a sprue-like state, with flattened intestinal villi and accompanying malabsorption [58]. Bile acids are poorly soluble in an acid milieu; thus, Zollinger–Ellison syndrome may result in decreased micelle formation and subsequent malabsorption of vitamin B-12 and other lipid-soluble nutrients and vitamins [56]. It is possible that in the presence of markedly high serum gastrin levels, gastrin itself may increase secretion of potassium and may reduce absorption of sodium and water by the small intestine [59,60], thus potentially leading to secretory diarrhea. Secretory diarrhea also may occur in Zollinger–Ellison syndrome in association with the secretion of another hormone besides gastrin, such as vasoactive intestinal polypeptide.

Twenty-five percent of patients with gastrinoma have MEN1 syndrome [14]. The primary sites of organ involvement in this autosomal dominant genetic disorder include the parathyroid, pancreas, pituitary, and, less commonly, adrenal cortex and thyroid. In a large series by Ballard and colleagues [61], 87% of 85 patients with MEN1 had involvement of the parathyroid glands, 81% had disorders of the endocrine pancreas, and 65% had pituitary involvement. Trump and colleagues [62], reporting on their findings in 220 patients with MEN1, observed different rates of organ system involvement, with 95%, 41%, and 30% of patients having parathyroid, pancreatic, and pituitary neoplasms, respectively. This latter study also confirmed that in 87% of patients parathyroid lesions were the first indication of this genetic disorder. The clinical features of Zollinger–Ellison syndrome (ulcer complications, gastric or pancreatic surgery) account for the major morbidity and mortality related to MEN1 [63]. In up to one-third of patients, the first clinical manifestation of MEN1 may be Zollinger–Ellison syndrome, which suggests that patients with the presumptive diagnosis of sporadic Zollinger–Ellison syndrome should be monitored biochemically and, when feasible, genetically screened for evidence of MEN1 [64]. Others recommend an oral calcium tolerance test to exclude early evidence of hyperparathyroidism and MEN1 [65].

The genetic defect in patients with MEN1 has been mapped to the long arm of chromosome 11 (11q11–q13) by analysis of restriction fragment length polymorphisms in affected families [62,63]. As outlined earlier, the *MEN1* gene consists of 10 exons and encodes the protein menin. Menin is a nuclear protein of 610 amino acids that is expressed widely throughout the body and functions as a tumor suppressor [66]. It interacts with Jun D, an AP-1 transcription factor, and with a protein involved in cell growth, SMAD-3 (SMAD, small mothers against decapentaplegic) [67,68]. Subsequent work has demonstrated that menin may be involved in methylating histone H3 and enhance gene transcription [69]. In addition, studies with genetically modified mouse models suggest that the critical consequence of MEN1 mutations may be deregulation of the cell cycle, which in turn may play a central role in the pathogenesis of neuroendocrine tumors (NETs).

Hyperparathyroidism and the associated hypercalcemia have direct bearing on the gastric acid hypersecretory state found in patients with Zollinger–Ellison syndrome and MEN1. Intravenous infusion of calcium in healthy human volunteers induces gastric acid hypersecretion, and calcium has been shown both in vivo and in vitro [70] to stimulate gastrin release directly from gastrinomas. Moreover, gastrinomas express the calcium-sensing receptor [71], thus providing the mechanism by which elevated calcium levels cause gastrin release from these tumors. Indeed, previous reports [72,73] have demonstrated that resolution of hypercalcemia by parathyroidectomy reduces basal acid output (BAO) and fasting serum gastrin concentrations in patients with gastrinoma with MEN1.

One consequence of prolonged hypergastrinemia in patients with Zollinger–Ellison syndrome with MEN1 is the development of primary gastric carcinoid tumors. In a review of data from the National Institutes of Health (NIH), Jensen [74] reported that 13% of patients with Zollinger–Ellison tumors with MEN1 developed gastric carcinoids. Similar tumors have been noted previously in patients with achlorhydria and

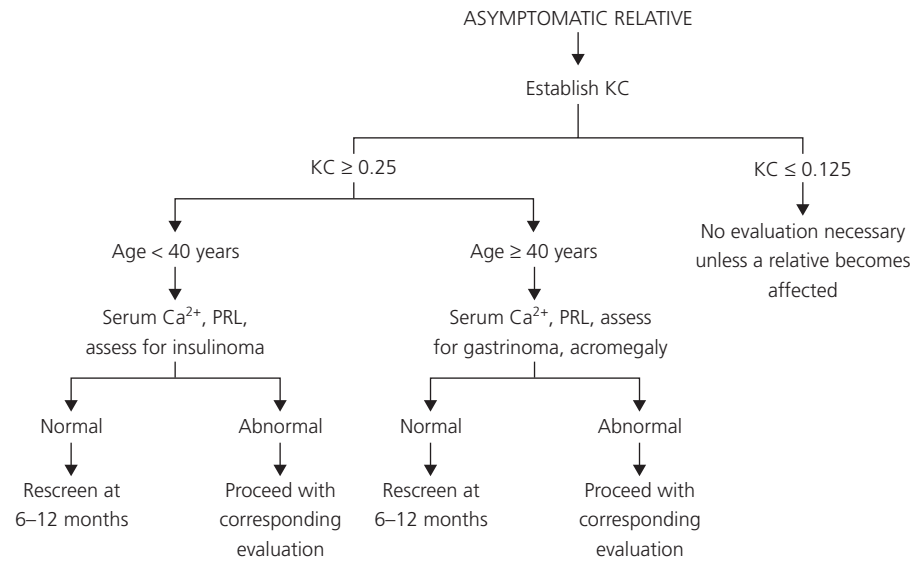

Figure 41.1 Screening protocol for relatives of patients with multiple endocrine neoplasia type 1. KC refers to the coefficient of kinship, in which known affected individuals were assigned a KC of 1.0. Asymptomatic first-degree relatives (siblings, children) were assigned a KC of 0.5, and asymptomatic second-degree relatives (grandchildren, nephews, nieces) were assigned a KC of 0.25. Spouses were also screened and were assigned a KC of 0. Adapted from Trump et al. [62].

hypergastrinemia [75], and in rats made hypochlorhydric and hypergastrinemic with potent antisecretory agents such as omeprazole. These findings presumably arise from the action of gastrin in inducing cellular proliferation. However, gastrin may not be the only cause of the carcinoids, inasmuch as in Jensen's NIH series [74] only 0.6% of patients with sporadic Zollinger–Ellison tumors developed carcinoids. In support of the hypothesis that hypergastrinemia alone is not sufficient to promote the development of gastric carcinoid tumors with enterochromaffin-like cells, it was observed that 75% (15 of 20 patients) of Zollinger–Ellison syndrome-related gastric carcinoid tumors had loss of heterozygosity at the *MEN1* gene locus (11q13) [76]. Therefore, it is postulated that the enterochromaffin-like-cell carcinoid is a tumor type independent of MEN1 but sharing a similar pathogenesis by inactivation of the *MEN1* gene.

Once a secretory endocrine tumor is diagnosed, patients with MEN1 should be evaluated carefully for the presence of other endocrinopathies. A thorough medical and family history must be obtained. Diagnostic studies should include evaluation of visual fields, fasting blood glucose, serum calcium, and prolactin levels. If there is suspicion of a pituitary lesion, a computed tomography (CT) or magnetic resonance imaging (MRI) scan of the head should be considered. The inheritance of MEN1 is autosomal dominant, with a high degree of penetrance. Children of these patients have an approximately 50% risk of developing this disorder by 20 years of age [77]; thus, genetic counseling and screening of family members are imperative. In a study evaluating a large kindred (221 members) of patients with MEN1, elevated albumin-adjusted calcium, parathyroid hormone, gastrin, and prolactin (in girls and women) were the most frequently encountered laboratory abnormalities among presumed carriers of the *MEN1* gene [78]. Trump and colleagues [62] evaluated 709 members of 62 families with MEN1 and developed a screening program based on their observations (Fig. 41.1). The genetic defect in MEN1 can be used to develop predictive testing for this syndrome [66,79,80] and may eventually serve as the basis for informed genetic counseling in families with MEN1.

A consensus statement for genetic screening and follow-up of patients with MEN1 and their family members has been published [81]. These authors recommended that mutation analysis of the *MEN1* gene be performed in patients meeting the following criteria:

- newly diagnosed neuroendocrine tumor typically found in MEN1 (primary hyperparathyroidism, pancreatic endocrine tumor, or pituitary adenoma)
- age less than 40 years
- positive family history
- recurrent or multifocal neoplasia
- involvement of two or more organ systems.

It is also recommended that genetic screening be performed in early adolescence of first-degree relatives of patients with MEN1. Persons carrying the *MEN1* mutation should undergo annual biochemical screening, as outlined earlier.

Differential diagnosis and diagnostic studies

The various clinical circumstances in which a serum gastrin level should be obtained because of a suspected gastrinoma are outlined in Table 41.1. Fasting serum gastrin levels in healthy persons and in patients with routine peptic ulcer disease are usually less than 150 pg/mL [82]. The degree of hypergastrinemia in patients with Zollinger–Ellison syndrome varies greatly. Although occasional reports of normal

Table 41.1 When to obtain a serum gastrin determination in patients with peptic ulcer

Multiple ulcers
Ulcers in unusual locations
Ulcers associated with severe esophagitis
Ulcers resistant to therapy, with frequent recurrences
Ulcer patients awaiting surgery
Extensive family history of peptic ulcer disease
Postoperative ulcer recurrence
Basal hyperchlorhydria
Unexplained diarrhea or steatorrhea
Hypercalcemia
Family history of pancreatic islet, pituitary, or parathyroid tumor
Prominent gastric or duodenal folds

Table 41.2 Hypergastrinemia: differential diagnosis

Hypochlorhydria or achlorhydria with or without pernicious anemia
Retained gastric antrum
G-cell hyperplasia
Renal insufficiency
Massive small bowel resection
Gastric outlet obstruction
Other conditions (rheumatoid arthritis, vitiligo, diabetes, pheochromocytoma)

levels [83,84], and values that vary from day to day [85,86], have been described, virtually all patients with gastrinoma have fasting levels greater than 150 pg/mL, with levels exceeding 100 000 pg/mL in some patients. A serum gastrin level greater than ten times normal is highly suggestive of Zollinger–Ellison syndrome. Two-thirds of patients will have fasting gastrin levels that overlap with levels found in the more common disorders outlined in Table 41.2 [87].

Chromogranin A is a 49-amino-acid glycoprotein constituent of the secretory granules found in many neuroendocrine cells [88], and as such it has been used as a histological and serological marker for neuroendocrine tumors [88–90]. Although serum gastrin and chromogranin A were found to be sensitive indicators for the presence of a gastrinoma, the latter had a relatively low specificity (67%) [90]. Moreover, neither of the markers correlated with tumor growth or extent [91]. It is also important to remember that chromogranin levels may also be elevated in patients with chronic atrophic gastritis (CAG), which is a common disorder in the differential diagnosis for hypergastrinemia. Campana and colleagues examined this issue further by comparing chromogranin A levels in 238 patients with NETs vs 42 patients with CAG and 48 normal volunteers [92]. Although overlap occurred amongst these groups, the highest levels were seen in NET patients. Accordingly, it is necessary to use specific cutoffs to maximize sensitivity and specificity of this marker. It appears that the best cutoff level to distinguish NET patients from normal volunteers was 19 U/L (sensitivity 75.3%; specificity 84.2%). More importantly, it is necessary to use a cutoff of 84–87 U/L to differentiate between NET patients and individuals with elevated chromogranin levels caused by nonneoplastic disorders such as CAG (sensitivity 55%; specificity 95%). This study was, however, performed with one particular commercial assay. There may be variability in results across centers because of the lack of international standardization for the different kits used for measuring chromogranin [48].

With the ever-increasing use of potent antisecretory agents such as long-acting histamine H_2 receptor antagonists and PPIs, drug-induced hypergastrinemia [93] must be excluded before proceeding with an extensive diagnostic evaluation for Zollinger–Ellison syndrome. The level of hypergastrinemia is usually less than 1.5–2 times normal levels and is reversible with drug cessation. Many patients with *Helicobacter pylori* infection and peptic disease develop hypergastrinemia and gastric acid hypersecretion [94], which are reversible with eradication of the organism. The potential for *H. pylori* infection to mimic the clinical and biochemical picture of Zollinger–Ellison syndrome was illustrated in a report [95]; thus, careful search and eradication of *H. pylori* are mandatory in any patient with peptic ulcer disease before one considers a diagnosis of gastrinoma.

The most common cause of hypergastrinemia is gastric atrophy [96]. Gastric acid is the primary inhibitor of gastrin release; therefore, its absence leads to uninhibited secretion of the hormone with concomitant hyperplasia of antral G cells and hypergastrinemia as observed in pernicious anemia [95–99]. Up to 75% of patients with pernicious anemia have substantial hypergastrinemia. The gastrin levels in these patients can approximate those found in patients with gastrinoma, reaching values greater than 1000 pg/mL. Chronic atrophic gastritis and gastric carcinoma are two other conditions associated with hypochlorhydria or achlorhydria and hypergastrinemia. Additional important causes of hypergastrinemia are outlined in Table 41.2.

To differentiate among the many causes of hypergastrinemia, various provocative tests [100–108] have been developed (Table 41.3). The *secretin test*, initially developed by Isenberg and associates [102], has proved to be the easiest and most reliable study to perform. Secretin can induce a significant early (albeit low) increase of serum gastrin levels in a few healthy persons and in patients with common duodenal ulcer disease [107], but rarely in persons with achlorhydria [108]. The mechanism by which secretin stimulates release of gastrin from endocrine neoplasms is unclear [70,109,110].

Although naturally occurring purified porcine secretin is no longer available in the United States, human synthetic secretin has been synthesized and approved by the US Food

Table 41.3 Gastrin provocative testing

Disorder	Calcium infusion	Secretin injection	Test meal
Gastrinoma	Increase in serum gastrin by > 400 pg/mL	Increase is usually > 200 pg/mL	Increase is < 50%
Antral G-cell hyperfunction	May or may not elicit increase in serum gastrin > 400 pg/mL	Decrease, no change, or slight increase in serum gastrin	Increase by > 200%
Common duodenal ulcer	Small increase in serum gastrin	Increase in serum gastrin is < 200 pg/mL	Moderate increase

and Drug Administration for clinical use. A randomized controlled crossover study of six patients with confirmed gastrinoma showed that the synthetic secretin preparations could be used to confirm the presence of a gastrinoma [111]. Based on several studies involving small numbers of patients, various criteria for establishing the positivity of a secretin provocative test have been proposed, including increases in serum gastrin of 100 pg/mL, 200 pg/mL, or 50% above basal levels. Frucht and colleagues [112] observed that the secretin test was positive in 87%–93% of all patients with Zollinger–Ellison syndrome (80 patients) and appeared to be more advantageous than the calcium infusion study (see later) because of its greater sensitivity, shorter sampling time, ease of performance, and lack of side effects. An increase in serum gastrin of 200 pg/mL or greater is highly suggestive of Zollinger–Ellison syndrome. Berna and colleagues [113] reported their results on a prospective study of gastrin provocative testing in 293 gastrinoma patients and compared their findings with the published literature (537 gastrinoma patients and 462 non-gastrinoma patients). They compared secretin stimulation with calcium infusion and meal testing. The authors confirmed the overall value of secretin stimulation above the calcium and meal studies. Secretin stimulation appears to be much more sensitive and specific than calcium infusion (94% vs 62%). Moreover, after a systematic analysis of the sensitivity and specificity of multiple criteria for a positive provocative test (secretin and calcium) the authors observed that a rise in gastrin of 120 pg/mL above baseline after secretin infusion has a sensitivity and specificity of 94% and 100%, respectively.

Calcium infusion study is a less commonly employed provocative test. More than 80% of patients with gastrinoma demonstrate an increase in gastrin levels of more than 395 pg/mL during the third hour of calcium infusion. As outlined earlier, lack of specificity, diminished sensitivity, and the potential adverse effects of administering intravenous calcium have made this provocative test less useful than the secretin test in diagnosing Zollinger–Ellison syndrome. It is usually reserved for patients with a negative secretin test in the presence of gastric acid hypersecretion and a strong clinical suspicion of gastrinoma [112,113]. False negative secretin testing is occasionally observed in a small subgroup of gastrinoma patients. Ding and colleagues suggested that certain gastrinomas expressed an abnormal secretin receptor, leading to a negative response to secretin [114]. Long and colleagues examined this question in 54 patients with Zollinger–Ellison syndrome and concluded that the variation in responsiveness to secretin was more a function of total receptor density than structurally variant receptors [115].

Standard meal studies have been employed to distinguish hypergastrinemia of gastric origin (as in G-cell hyperplasia or hyperfunction) from that of pancreatic origin (gastrinoma) [116]. Patients with Zollinger–Ellison syndrome may have less than a 50% increase in serum gastrin in response to a meal, and a postprandial increase in serum gastrin of more than 100% is characteristic of G-cell hyperplasia or hyperfunction. However, the studies of Frucht and associates [116] suggest that 50% of patients with Zollinger–Ellison syndrome have a greater than 50% postprandial increase in serum gastrin, and work by Berna and colleagues confirm the limited usefulness, if any, of meal testing in the evaluation of patients with hypergastrinemia [113].

The net effect of hypergastrinemia is increased gastric acid secretion; thus, it is not surprising that a BAO of 15 mEq/h or greater is found in as many as 90% of patients with gastrinoma [54,86,117–119]. However, 12% of patients with common duodenal ulcer disease can have similar levels of acid secretion. A BAO of greater than 5 mEq/h is found in 55% of patients with Zollinger–Ellison syndrome after surgery [117]. The widespread use of potent antisecretory agents such as the PPIs complicates the interpretation of acid secretory studies. A study by Corleto and colleagues [9] suggests that the use of PPIs may mask Zollinger–Ellison syndrome, thus leading to delay in establishing this diagnosis. To enhance the sensitivity of the gastric secretory studies, *maximal acid output* (MAO) values may also be measured. Patients with gastrinoma are presumed to have near-maximal secretion of gastric acid even under basal conditions; thus, the acid secretory response to exogenously administered secretagogue (pentagastrin) is diminished. Accordingly, a ratio of BAO to MAO greater than 0.6 is highly suggestive of Zollinger–Ellison syndrome [8], although a ratio of less than 0.6 does not exclude the diagnosis [53,86,117]. Unfortunately, pentagastrin is no longer available in the United States, making measurement of MAO virtually impossible. The other secretagogue used in gastric secretory studies was Histalog,

which has been off the market for several years. Metz and Starr [120] observed that measurement of MAO had a small but significant benefit compared with measuring BAO alone.

There has been controversy over the utility of gastric acid analysis in view of the sensitivity and specificity of serum gastrin values with or without secretin stimulation. Investigators at the NIH performed the most exhaustive analysis to date of the role of gastric secretory studies in Zollinger–Ellison syndrome [121]. They prospectively examined 235 patients and reviewed 984 cases in the literature in an effort to delineate the potential role of gastric secretory studies in the diagnosis, clinical expression, and extent of tumor growth. These authors observed that diarrhea, esophageal stricture, and pyloric scarring were associated with a higher BAO. The level of acid hypersecretion did not appear to correlate with other clinical characteristics including tumor extent or size, primary tumor location, or other signs and symptoms in patients with gastrinoma. A summary of the recommendations made for measurement of gastric acid secretion in patients with Zollinger–Ellison syndrome is presented in Table 41.4. Under optimal circumstances, acid secretory studies should be part of the diagnostic evaluation in patients suspected of having gastrinomas. If the equipment needed for this test is not available, a simple determination of basal gastric pH of greater than 3 essentially rules out the diagnosis of Zollinger–Ellison syndrome. Oh and colleagues have validated a novel endoscopic method of performing gastric analysis [122]. Although the method shows promise, further validation and cost analysis are needed.

In summary, no single diagnostic test has a sensitivity or specificity of 100% in evaluating patients for Zollinger–Ellison syndrome. A fasting gastrin level greater than 10 times normal and a BAO greater than 15 mEq/h in a patient with recalcitrant peptic ulcer is virtually diagnostic of Zollinger–Ellison syndrome. In the absence of this triad of findings, provocative testing may be required. Of these, the secretin stimulation test offers the greatest sensitivity and specificity.

Table 41.4 Recommendations for measurement of gastric acid secretion for the diagnosis of Zollinger–Ellison syndrome[a]

1. Secretory criteria for patients with no prior gastric acid-reducing surgery
1.1 BAO ≥ 15 mEq/h
1.2 BAO ≥ 18 mEq/h, in patients with known sporadic form
1.3 No pH criterion useful for diagnosis
1.4 BAC/MAC ratio ≥ 0.6
1.5 Basal gastric volume > 160 mL/h
1.6 No MAO criterion useful
2. Patients with prior gastric acid-reducing surgery
2.1 BAO ≥ 5 mEq/h
2.2 No pH criteria useful for diagnosis
2.3 BAC/MAC ratio ≥ 0.6
2.4 BAO/MAO ratio > 0.6
2.5 Basal gastric volume > 140 mL/h
2.6 No MAO criterion alone useful
3. In hypergastrinemic patients, if pH > 2, patient rarely has ZES
4. Acid measurements required only for 30 min

a All values refer to acid measurements made by nasogastric secretion with the patient not taking any gastric antisecretory medications (off proton pump inhibitors ≥ 1 week; oral histamine H_2-receptor antagonists ≥ 48 h; intravenous histamine H_2-receptor antagonists ≥ 12 h).
BAC, basal acid H^+ concentration; BAO, basal acid output; MAC, maximal acid H^+ concentration; MAO, maximal acid output; ZES, Zollinger–Ellison syndrome.
Adapted from Bonfils et al. [123], with permission of Lippincott, Williams & Wilkins.

Tumor localization

Gastrinomas are notoriously difficult to localize. Case series of patients who eventually underwent surgery for Zollinger–Ellison syndrome before the 1990s noted the inability to identify the tumor in approximately 30%–50% of patients, with more than 20% having metastatic disease at laparotomy [35,123–127]. Subsequent series, perhaps reflecting improvements in preoperative imaging modalities, have localized tumor in 85% of cases, although metastatic disease is frequently present (~50% of patients). Early intervention with resection of a localized tumor presents the only opportunity to cure this disease [127–129]. In addition, preoperative detection of metastatic disease will prevent unnecessary surgery. Accordingly, every effort should be made to localize the tumor preoperatively. A diagnostic plan for localizing gastrinomas in patients with Zollinger–Ellison syndrome is depicted in Fig. 41.2.

Many approaches have been used for the purpose of localizing gastrinomas. These include abdominal ultrasonography [130,131], CT scanning [131–135], abdominal arteriography [136–139], selective portal venous sampling for gastrin [138, 140–143], selective arterial stimulation with secretin (SASI) [144–147], endoscopic ultrasonography (EUS) [148–154], and somatostatin receptor scintigraphy (SRS) with the somatostatin analog octreotide [155–158]. The latest additions to the imaging techniques, SRS and EUS, have emerged as most useful in preoperative staging algorithms (see Fig. 41.2). The sensitivity of these modalities in localizing primary and metastatic gastrinomas is outlined in Table 41.5.

Ultrasonography: transcutaneous, endoscopic, and intraoperative

In general, transcutaneous abdominal ultrasonography is not considered useful for the diagnosis of gastrinomas. Most

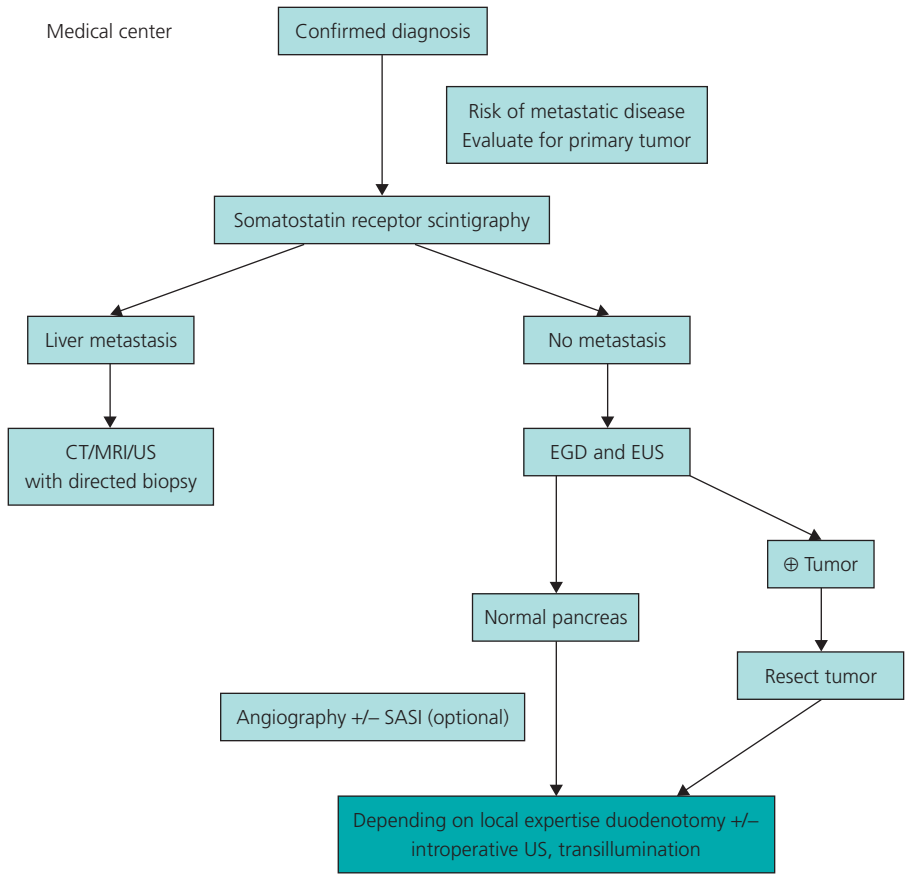

Figure 41.2 Diagnostic plan for localizing gastrinomas in patients with Zollinger–Ellison syndrome. CT, computed tomography; MRI, magnetic resonance imaging; EUS, endoscopic ultrasound; EGD, esophagogastroduodenoscopy; R/O, rule out; SASI, selective arterial secretin stimulation.

gastrinomas are small; therefore, sensitivity of this test is low, as demonstrated in a prospective study [159] in which transcutaneous ultrasonography could localize tumors in only 20% of patients later shown by surgery to have gastrinomas. Nevertheless, if a lesion is seen, it is likely to represent gastrinoma; thus, the specificity of ultrasonography was 90%–100% in this prospective series.

High-resolution imaging of the pancreas has been facilitated by EUS, permitting delineation of structures smaller than 5 mm. Because the accuracy of this procedure in diagnosing small pancreatic carcinomas approaches 100% [160,161] it is not surprising that this diagnostic modality is also useful in localizing small neuroendocrine tumors of the pancreas [148–154]. Rösch and associates [151] examined 37 patients with surgically confirmed endocrine neoplasms of the pancreas (39 tumors), in whom preoperative ultrasonography and CT scan of the abdomen yielded negative findings. Thirty-two of the tumors were diagnosed by EUS (82% sensitivity), with a specificity of 95%. Tumors varied in diameter between 0.5 and 2.5 cm. However, all seven of the gastrinomas

Table 41.5 Sensitivity of imaging studies in Zollinger–Ellison syndrome

	Sensitivity (%)	
Study	**Primary gastrinoma**	**Metastatic gastrinoma**
Ultrasound	21–28	14
Computed tomography	35–59	35–72
Selective angiography	35–68	33–86
Portal venous sampling	70–90	N/A
Selective arterial stimulation of secretin	55–78	41
Magnetic resonance imaging	30–60	71
OctreoScan	67–86	80–100
Endoscopic ultrasound	80–100	N/A

in this series were located in the pancreas, and none was located in extrapancreatic sites, such as the duodenum.

The University of Michigan group used EUS as a primary diagnostic tool to localize neuroendocrine tumors and evaluated more than 82 patients in a prospective study [162]. In their initial prospective study of 16 patients, Thompson and colleagues [153] observed that the sensitivity of a negative EUS result in predicting an extrapancreatic gastrinoma was 100%. Subsequently, their series was expanded to include 75 patients with neuroendocrine tumors with operative confirmation of the EUS findings. In 36 patients with suspected gastrinoma, these investigators confirmed a 100% accuracy in localizing pancreatic lesions and guiding the surgeon to an extrapancreatic primary tumor, findings supporting the contention that EUS was invaluable in the preoperative evaluation of the patient with Zollinger–Ellison syndrome [162]. An early EUS localization strategy was found to be highly cost-effective (and cost-saving) when this group compared this approach with that employed before the introduction of EUS, which primarily used angiographic and venous sampling techniques [163].

The sensitivity of EUS in detecting gastrinomas has been noted by other investigators [154]. In a study of 22 patients with Zollinger–Ellison syndrome, the sensitivity of EUS was 50% for duodenal wall tumors, 75% for pancreatic tumors, and 62.5% for tumor-bearing lymph nodes. These investigators observed that conventional endoscopy had a sensitivity of 40% for detecting duodenal wall lesions; thus, EUS has a small advantage over endoscopy in localizing duodenal wall lesions. Zimmer and colleagues [164] reported a similar experience, with EUS localizing 11 of 14 (79%) of all gastrinomas in 10 patients with Zollinger–Ellison syndrome. Of note, EUS localized 89% of the pancreatic lesions, and the results of conventional endoscopy are not reported. At the University of Michigan, a routine endoscopy (with a pediatric colonoscope) is performed to look for duodenal submucosal tumors, and EUS is primarily used to examine the pancreas. This strategy is based on the findings that EUS has limited value in the detection of duodenal wall lesions, with overall detection rates of 43% [165]. The advent of somatostatin receptor scintigraphy, improved CT/MRI techniques, coupled with the low sensitivity of EUS in finding duodenal wall lesions have led others to recommend the use of EUS in the preoperative evaluation of a patient suspected of having Zollinger–Ellison syndrome (sporadic) only after the octreotide and CT scan have been performed and are negative [165]. A study by Kann et al. indicated that the finding of a pancreatic nodule by EUS in a patient suspected of having neuroendocrine tumor should be interpreted with great caution [166]. In general, it is believed that EUS has greater benefit as part of the preoperative evaluation in patients with MEN1, in view of its ability to map the pancreatic lesions, which are often multiple in this subgroup of patients [167].

Other centers without EUS experience have reported the utility of intraoperative ultrasonography, which appears to be useful in the localization of small gastrinomas [168–170]. Tumors not detected by the surgeon through manual inspection may be imaged using this technique, which can achieve a sensitivity of 95% in the hands of experienced operators [170].

Intraoperative transillumination

Frucht and associates [171] prospectively examined the efficacy of intraoperative endoscopic transillumination for detecting duodenal gastrinomas in 26 patients. These investigators detected duodenal gastrinomas with 83% sensitivity, which compares well with the sensitivity of the combined preoperative studies (25%) or intraoperative ultrasonography and surgical palpation (42%). This technique remains part of surgical protocols for the management of Zollinger–Ellison syndrome [37].

Computed tomography

The diagnostic accuracy of CT scanning reflects continued innovation in techniques and instrumentation. A prospective study [135] examined the value of CT in localizing tumors in 61 consecutive patients with gastrinoma. For extrahepatic gastrinoma, CT scanning with intravenous contrast had a specificity of 95%, a sensitivity of 59%, a positive predictive value of 96%, and a negative predictive value of 54%. For gastrinomas metastatic to liver, CT scanning had a specificity of 98%, a sensitivity of 72%, a positive predictive value of 93%, and a negative predictive value of 90%. Tumor size and location appear to be the key determinants in the successful diagnosis of gastrinoma by CT scanning. Approximately 80% of tumors in the pancreas and tumors larger than 3 cm are detected. Only 40% of extrapancreatic lesions and no tumors measuring less than 1 cm in diameter are detectable by CT scanning. The sensitivity and specificity of CT do not appear to be enhanced by performing dynamic studies. Whether helical techniques have led to improved sensitivity remains uncertain, although one study (requiring further comparative study) has reported 82% sensitivity for tumor localization [172].

A study by Panzuto and colleagues suggests that helical CT was more sensitive than OctreoScan for detecting duodenal lesions [173], but this result requires confirmation.

Angiography

Diagnostic innovations have limited angiography to selected patients for whom imaging by SRS or EUS has proved unsuccessful. Maton and colleagues [139] prospectively examined the role of selective angiography in the diagnosis of 20 consecutive patients with gastrinoma. For hepatic gastrinoma, angiography had a specificity of 100% and a sensitivity of 86%, with a positive predictive value of 97% and a negative predictive value of 53%. In combination with CT scanning,

100% of the hepatic tumors were detected. Extrahepatic lesions were not as easily detected, inasmuch as CT scanning detected only 57%, angiography 70%, and the combination 73%, with a false-positive rate of 7%. This study suggests that after a CT scan, selective abdominal angiography will detect an additional 28% of patients with liver lesions and an incremental 16% of patients with extrahepatic tumors. Unfortunately, 24% of the extrahepatic lesions will be missed even when both tests are used. Nevertheless, angiography was found to be a useful adjunct to CT scanning in patients with Zollinger–Ellison syndrome who undergo surgery. This same group [174] subsequently reported less enthusiastic experience with CT (31% sensitivity for primary tumors, 42% for liver lesions) and angiography (28% and 62%, respectively). The reduced sensitivity likely reflects both increased numbers of patients referred with negative outside CT scans and angiograms and the ability of newer imaging modalities such as SRS to detect small lesions that would have been missed in the past.

Portal venous sampling

Before SRS and EUS, under the best circumstances the combination of ultrasonography, CT scanning, and angiography failed to localize tumors in up to 50% of the patients with biochemical evidence of Zollinger–Ellison syndrome. Thus, percutaneous transhepatic selective portal venous sampling for gastrin was developed as a means to improve the diagnostic yield [138,140–143]. There is general agreement on the relative sensitivity of this test in identifying the presence of gastrinomas (70%–90%), but considerable debate over its specificity, with values ranging from 33% to 90%, depending on the series. Despite the potential usefulness of the selective venous sampling technique, its complexity and invasiveness limit its availability to only a few specialized centers, where it has largely been replaced by selective arterial secretin injection (SASI); thus, it is not recommended for the evaluation of Zollinger–Ellison syndrome on a routine basis.

Selective arterial secretin injection (SASI)

Selective arterial secretin injection (SASI) has been added to the growing list of modalities used for localizing gastrinomas [145–148]. As initially described by Imamura and colleagues [144], secretin (30 U) is injected selectively into the celiac, proximal splenic, gastroduodenal, proper hepatic, and superior mesenteric arteries, and blood samples for gastrin measurement are obtained simultaneously from the hepatic vein at short intervals (30, 60, 120, and 210 seconds). Given the results of emerging imaging modalities such as EUS and SRS, it appears that SASI will be employed infrequently and will be most helpful when imaging data are conflicting [175].

Magnetic resonance imaging (MRI)

Magnetic resonance imaging [176,177] has been evaluated for the detection of both primary and metastatic gastrinoma, and initial results appear similar to those of CT scanning. In a prospective study, the NIH group found that MRI, using short-time inversion-inversion recovery sequences, was superior to CT for the detection of liver metastases (sensitivity 71% vs 42%). In the detection of primary lesions MRI was equivalent to CT, leading this group to suggest that it should replace CT as the cross-sectional imaging test of choice. For most medical centers the choice between CT and MRI for detecting liver metastases depends on the availability and local expertise. There might be instances where CT and MRI can be complementary in the evaluation of a patient with positron emission tomography (PET) [178]. For example, MRI may be used to confirm a finding noted on CT scan.

Somatostatin receptor scintigraphy (SRS)

Many gastrinomas express somatostatin receptors. Therefore, targeted nuclear scanning after injection of an isotopic analogue of somatostatin – either ^{123}I-labeled Tyr3-octreotide (Tyr3, tyrosine 3) or ^{111}In-labeled diethylenetriamine pentaacetic acid-Dphe^{1}-octreotide (Dphe^{1}, D-phenylalanine-1) [155–158,164,174,179] – has emerged as a readily available, noninvasive test to localize gastrinomas, with sensitivity and specificity of greater than 75%. The indium analogue (^{111}In-pentetreotide) is marketed as OctreoScan 111 (Mallinckrodt, St Louis, MO). In prospective studies [177], the NIH group found that the scan was more sensitive than transcutaneous ultrasonography, CT, MRI, and angiography for localizing both primary and metastatic lesions, with a sensitivity of 58% and 92%, respectively. The efficacy of EUS and SASI was not directly compared with SRS with regard to the ability to localize primary lesions. Cadiot and colleagues [179] observed that OctreoScan 111 scintigraphy was useful in detecting small duodenal lesions (3 mm) and peripancreatic lymph nodes. It also appears that the addition of single photon emission computed tomography (SPECT) enhances the sensitivity of OctreoScan over planar imaging [180,181]. In a review of 122 patients who underwent SRS to localize primary and metastatic gastrinoma lesions, the NIH group noted that SRS altered management in 47% of patients, with its greatest impact in the detection of liver metastases [182]. These investigators concluded that SRS should become the initial imaging modality for patients with gastrinoma. However, it should be noted that a study examining 64 patients for metastatic NET to the liver using a MRI, CT scan and OctreoScan suggested MRI was superior to CT scan and somatostatin scintigraphy [183]. However, a number of issues may compromise the validity of this observation: (i) only a small number of the patients evaluated had elevated gastrin levels (1/64); and (ii) 12 of the patients studied had been treated with somatostatin analogue, which may in turn have led to a false-negative OctreoScan, although this latter issue is somewhat controversial. Therefore, based on the available literature, it remains reasonable to consider somatostatin scintigraphy as the most sensitive imaging modality

for detecting metastatic gastrinomas. Among patients undergoing surgery for cure at the NIH, SRS was the most accurate preoperative imaging study, yet it still missed one-third of all lesions found at surgery [184]. The ability of SRS to detect tumors was related to size, because only 30% of tumors 1.1 cm or smaller were imaged, compared with 64% of those 1.1–2.2 cm and 96% of those larger than 2 cm. Duodenal primary tumors were most commonly missed by SRS. Because of these shortcomings, OctreoScan does not seem to change surgical cure rates [165]. This same group also noted the limitations of SRS with regard to specificity; false-positive results occurred in up to 10% of cases [185]. Knowledge of the clinical situations leading to false-positive results is essential for optimal interpretation of the OctreoScan 111 technique.

Novel nuclear imaging modalities based on positron emission tomography (PET) are being explored in the evaluation of patients with NETs in general [186]. This is based on the observation that most neuroendocrine cells have the ability to take up amine precursors and decarboxylate them (i.e., amine precursor uptake and decarboxylation, or APUD, cells). The ^{18}F and ^{11}C-labeled amine precursor L-dihydroxyphenylalanine and 5-hydroxy-L-tryptophan (5-HTP) have been used for PET imaging of NETs. Early studies suggest that ^{11}C-labeled 5-HTP-PET is promising for the detection of NETs, but studies with gastrinomas are needed.

In summary, surgical cure of Zollinger–Ellison syndrome requires accurate tumor localization. To avoid unnecessary laparotomy, OctreoScan 111 in combination with CT or MRI should be used to exclude metastatic disease. If no metastatic disease is found, the approach to localize disease in the peripancreatic region is governed by local expertise and surgical experience. Endoscopic ultrasonography (with upper gastrointestinal endoscopy to evaluate for duodenal tumors) appears to be the most cost-effective modality to evaluate the pancreas and duodenum, whereas SASI remains an alternative if EUS is not available. Occult metastasis can also be detected using SASI, which may assist in the interpretation of equivocal examinations. In other medical centers, the endocrine surgeon may go directly to exploration with intraoperative ultrasonography or transillumination. In the management of Zollinger–Ellison syndrome, it is clear that a team approach to localization and surgical exploration is essential to ensure curative resection.

Therapy

When considering the therapy of patients with Zollinger–Ellison syndrome, it is important to entertain two objectives: control of gastric acid hypersecretion and treatment of the malignant neoplasm. The emphasis placed on each of these objectives has shifted since the 1970s. Initially, total gastrectomy seemed the only option for effective treatment of the potentially lethal ulcer disease, and less attention was directed at tumor excision because many of these patients died of ulcer complications long before their tumors became problematic. The availability of highly effective antisecretory therapy such as histamine H_2 blockers and H^+,K^+-ATPase antagonists led to a significant reduction in mortality related to the complication of acid peptic disease. The major cause of morbidity and mortality in Zollinger–Ellison syndrome is widespread metastatic disease [187]; thus, early tumor detection and excision have assumed primary importance.

Medical therapy

The primary aim of medical therapy in Zollinger–Ellison syndrome is control of gastric acid hypersecretion. The development of histamine H_2-receptor antagonists was a major breakthrough toward this aim. Cimetidine, the first of these agents, proved efficacious in controlling acid hypersecretion and prompted ulcer healing and symptom improvement in more than 80% of patients with Zollinger–Ellison syndrome treated on a short-term basis [188]. However, over a longer term, these patients often required progressive increases in the frequency and dose of medication. Although H_2-receptor antagonists with greater potency and duration of action have been useful in treating patients with gastrinoma [189–191], an important newer class of drugs made available for the treatment of Zollinger–Ellison syndrome are the proton pump inhibitors (PPIs), of which omeprazole was the first of its class [192]. The efficacy of these agents in inhibiting acid secretion, relieving dyspeptic symptoms, and promoting ulcer healing in patients with gastrinoma has been established [193–196].

Although earlier studies suggested that the doses of omeprazole required for effective inhibition of acid secretion in patients with gastrinoma were significantly higher than in patients with standard peptic ulcers (Table 41.6), a study by Metz and associates [197] suggests that the recommended maintenance dose of omeprazole (~ 60 mg/day) used in these patients is too high. Accordingly, a gradual reduction of omeprazole dose is recommended once the initial dose required for adequate control of gastric acid hypersecretion (as described earlier) has been achieved. The NIH group has

Table 41.6 Median dose of antisecretory agents used in patients with gastrinoma

Agent	Median doses g/day (range)
H_2-receptor antagonists	
Cimetidine	3.6 (1.2–12.6)
Ranitidine	1.2 (0.45–6)
Famotidine	0.25 (0.05–0.8)
H^+,K^+-ATPase inhibitors	
Omeprazole	60 mg/day
Lansoprazole	60–90 mg/day

prospectively examined the effectiveness of low doses of omeprazole (20 mg/day) as initial therapy in patients with gastrinoma [198], and based on their results, the investigators continue to recommend initiating omeprazole therapy at a dose of 60 mg/day. This dose allows rapid control of gastric acid secretion, thus minimizing complications related to peptic diathesis. Once an adequate maintenance dose is achieved, tapering the medication is then indicated while following symptoms and acid output. Lansoprazole has also been found to be safe and effective in the treatment, for up to 10 years, of patients with gastrinoma [196]. Tachyphylaxis is observed less frequently with PPIs than with H_2-receptor antagonists. One concern with long-term omeprazole therapy has been its potential for inducing enterochromaffin cell hyperplasia. A prospective study following 40 patients with Zollinger–Ellison syndrome who were treated with omeprazole for a mean of 29 months (6–51 months) reported no evidence of hematological, biochemical, or gastric toxicity [199,200]. Their marked potency, prolonged duration of action, and safety profile make PPIs the treatment of choice for peptic disease in patients with Zollinger–Ellison syndrome. However, long-term hypochlorhydria or achlorhydria may lead to vitamin B-12 and iron deficiency, thus these micronutrients should be monitored in patients on long-term PPIs. The intravenous formulation of pantoprazole has become available and appears to be effective in the treatment of patients with Zollinger–Ellison syndrome [201].

Somatostatin is a known peptide inhibitor of gastric acid secretion [202] and gastrin release [203]. The two biochemically stable analogues of somatostatin in clinical use are octreotide and lanreotide. The former has been used with varying success in patients with gastrinomas [204–206]. At present, however, these compounds are available only in injectable form; thus, they are rarely used as first-line agents in the treatment of patients with Zollinger–Ellison syndrome, especially considering the overall efficacy of PPIs in this group of patients. The long-acting depot formulation of octreotide has become available and shown in preliminary studies to be beneficial in the treatment of neuroendocrine tumors of the gut [207]. Future studies will clarify the role of these compounds in the therapy of patients with gastrinoma. Side effects from somatostatin analogues are mild and usually include bloating, abdominal discomfort, and mild steatorrhea, all of which tend to subside within the first several weeks of therapy [208].

Surgical therapy

Before the advent of potent antisecretory agents, total gastrectomy was the treatment of choice in patients with gastrinoma. Although initial operative mortality was high (15%–20%) [209], total gastrectomy offered better long-term survival than more conservative approaches [11]. Increased experience and earlier diagnostic testing have reduced the operative mortality rate associated with total gastrectomy to less than 5% [210]. Nevertheless, the newer gastric antisecretory agents have greatly reduced the need for total gastrectomy, and at present this procedure should be considered only in the rare patient with nonresectable gastrinoma in whom aggressive medical therapy has failed or in those patients who cannot take oral medications.

Another operation applied in the past as an adjunct to medical therapy in patients with Zollinger–Ellison syndrome is proximal gastric vagotomy [211,212]. The rationale for this operation was the high failure rate with H_2-receptor antagonists. The advent of PPIs may obviate the need for the routine use of this procedure although some still advocate parietal cell vagotomy as part of the surgical approach in an individual who is being considered for surgical cure [165].

As discussed earlier in this chapter, the hypercalcemia found in patients with MEN1 may be associated with a gastric acid hypersecretory state that is refractory to medical therapy. A report [213] demonstrated that in addition to lowering both BAO and fasting hypergastrinemia, parathyroidectomy facilitates the management of peptic disease with H_2-receptor antagonists. Although the availability of H^+,K^+-ATPase inhibitors may make this operation unnecessary, parathyroidectomy may be a surgical option for treatment of patients with gastrinoma with MEN1 and hyperparathyroidism who are refractory to medical management.

Clearly, the appropriate surgical approach for therapy of Zollinger–Ellison syndrome is curative resection of the neoplasm. Although initial series reported a cure rate of less than 10% after tumor resection, present data indicate that cure may be possible in as many as 30% of cases. Norton and colleagues [36] published the largest experience of surgical intervention in patients with gastrinoma. This group followed 151 consecutive patients (123 with sporadic cases, 28 with MEN1) who underwent surgery between 1981 and 1998. Thirty-four percent of patients with sporadic gastrinoma were free of disease at 10 years. Unfortunately, none of the patients with MEN1 was disease free at the 10-year mark. Moreover, the overall 10-year survival observed in these patients was 94%. This same group reported a more extensive follow-up on 160 patients with Zollinger–Ellison syndrome (sporadic and MEN1) who underwent surgery, and the disease-free survival at 15 years was 98% [214]. These authors concluded that all patients with Zollinger–Ellison syndrome who do not have metastatic disease or MEN1 should be offered surgical intervention for possible cure. Improved diagnostic capabilities have led not only to a reduction in unnecessary surgery on patients with metastatic disease, but also to the identification of extrapancreatic neoplasms with increasing frequency [33,34,126,127,213–217]. Studies have reported the incidence of extrapancreatic lesions to be as high as 66% and resection of these lesions is associated with a high likelihood of long-term cure [125,218].

Patients with gastrinoma with MEN1 represent a particularly difficult problem to the surgeon. Because of the

multicentric nature and extrapancreatic location of tumors in MEN1 syndrome [219–224], curative resection is virtually impossible [142,187,210,225,226]. Moreover, studies suggest that the vast majority of gastrinomas in MEN1 patients are localized in the duodenum, which may impact the surgical approach in these patients [37]. Some surgeons advocate an aggressive approach whereby all patients without evidence of hepatic metastasis are explored, with removal of all duodenal lesions found by duodenotomy, enucleation of all tumors found in the head of the pancreas, and distal pancreatectomy [221,224]. The rationale for this approach is to effect a potential cure and, failing that, to prevent the development of future pancreatic neoplasms. The value of such an aggressive approach remains to be established. If a single tumor is found during the preoperative evaluation, an attempt at surgical resection should be undertaken [227] because larger tumors may be associated with a greater likelihood of metastasis. The role of surgery in patients with gastrinoma and MEN1 was explored in 81 consecutive patients [228]. All patients with limited disease (single tumor between 2.5 and 6 cm, $n = 17$) and advanced disease (two or more lesions > 6 cm, $n = 31$) underwent exploratory laparotomy with tumor enucleation or resection. Patients with tumors smaller than 2.5 cm ($n = 17$) and those with liver metastases ($n = 8$) were observed. Approximately 40% of patients had advanced disease without metastases. Duodenal lesions were found in 80% of patients with Zollinger–Ellison syndrome and MEN1. The authors observed that tumors could be removed in patients with advanced disease with no mortality and a complication rate similar to that observed in patients with limited disease. An additional important observation was that patients with advanced tumor who underwent resection had the same survival rate as patients with limited disease and those without identifiable tumor. As a group (nonresected and resected), 15-year survival was between 89% and 100%, whereas for patients with metastatic disease, it was only 52%. The authors of this study concluded that surgical resection should be performed in patients with gastrinoma and MEN1, even with advanced localized disease. This study, however, did not address the ideal type of operation for this group of patients. Although a subgroup of patients with gastrinoma may follow an aggressive course warranting a Whipple procedure, the tools to predict this subgroup of patients have not been developed (see later). Work by Bartsch and colleagues supports a more aggressive approach in which surgery should be provided for patients with MEN1 and pancreatic endocrine tumors greater than 1 cm without evidence of distant metastasis [229]. They examined 26 genetically confirmed MEN1 patients, 11 of whom had Zollinger–Ellison syndrome. After a median of 83 months, 7/11 patients with Zollinger–Ellison syndrome were biochemically cured. This group noted that the procedure of choice is a pylorus-sparing pancreaticoduodenectomy. A subsequent study by Tonelli and colleagues also supports the notion that a more aggressive surgical approach in MEN1 patients is warranted, adding intraoperative gastrin measurement as a tool to assess surgical cure [230].

Although laparoscopic sugery has been used successfully by some in resecting certain NETs, such as insulinoma, the experience with gastrinomas is limited. Moreover, because many gastrinomas are within the duodenum and these lesions tend to be more advanced, a laparoscopic approach is not recommended in Zollinger–Ellison syndrome patients [165].

Operative management of Zollinger–Ellison syndrome requires careful and detailed mobilization and exploration of the entire pancreas and duodenum (duodenotomy) and surrounding areas [217,221,224]. Any tumors found in the region of the pancreatic head should be enucleated, and tumors located elsewhere should be resected with great care. If a tumor is not found at surgery, distal pancreatectomy should be avoided because more than 80% of the gastrinomas are located within the gastrinoma triangle [33]. If a lesion is found in the head of the pancreas, a Whipple procedure should be considered, albeit with great care because the mortality of this surgical procedure may outweigh its potential benefits. Nevertheless, in some cases the Whipple procedure may be the only option if complete tumor excision is not possible by a less invasive procedure [231].

Pisegna and colleagues [232] examined the effects of curative gastrinoma resection on gastric secretory function. They observed that the trophic effects of long-standing hypergastrinemia are largely reversible within a short period, with a 50% and 75% decrease in MAO and BAO, respectively, within 3–6 months after curative resection. However, these observations also indicate that acid secretion must be monitored even after curative resection because as many as 55% of patients at 3–6 months and up to 67% at 4 years after gastrinoma resection remained mild hypersecretors requiring low doses of antisecretory drug.

Therapy for metastatic gastrinoma

Metastatic disease occurs in 25%–40% of patients with gastrinoma and is the most common source of morbidity and mortality in this disease. One major difficulty in evaluating the effectiveness of the various therapeutic regimens is that most clinical trials have involved small numbers of patients. The overall value of chemotherapy in gastrinomas and NETs in general remains somewhat controversial. Cytotoxic chemotherapy has been of limited usefulness in Zollinger–Ellison syndrome, because gastrinomas are less responsive than the other pancreatic NETs examined [233]. Combined treatment with streptozotocin, 5-fluorouracil (5-FU), and doxorubicin (adriamycin) has provided the most promising results. In one series of 42 patients with metastatic islet tumors of various types [234], initial response rates of 63% and complete response rates of 33% were reported with this regimen. However, gastrinomas comprised a small percentage of the cases studied, and the response to chemotherapy

may vary according to the cell type of the tumor. In another study using a similar treatment regimen, Bonfils and colleagues [235] reported the disappearance of the gastrinoma, as assessed by imaging studies, in 20% of their patients with Zollinger–Ellison syndrome with metastatic tumors. Other agents evaluated in a limited number of patients include cisplatin/etoposide and temozolomide [233].

These encouraging results of earlier studies were not confirmed in later trials. A prospective study [236] by the investigators at the NIH provided a sobering perspective on treatment of metastatic gastrinoma with chemotherapy. Ten consecutive patients with gastrinomas were treated with a regimen of streptozotocin, 5-FU, and doxorubicin, and although a modest objective response was observed initially in four patients, the response lasted for less than 10 months. All the patients were considered as treatment failures thereafter, with no significant change in short-term survival, and experienced many side effects of the therapy, including nausea and vomiting (100%), alopecia (80%), proteinuria (40%), and leukemia (20%). At 10 months after initiation of therapy, all patients had evidence of tumor growth over the preceding 6 months. The question when to treat patients having metastatic gastrinoma with chemotherapy remains unanswered. Although an occasional patient with metastatic disease may survive up to 20 years, it appears that most will die within 5 years. Two approaches to treating these patients are to begin chemotherapy when the patient is symptomatic [234], or to reassess the patient 3–6 months after initial presentation and to use chemotherapy if the metastases are increasing in size [237]. Both approaches are based on studies mostly involving relatively small numbers of patients; thus, additional data are needed before firm recommendations can be given.

In view of the limited success of chemotherapy in the treatment of metastatic gastrinomas and neuroendocrine tumors of the pancreas in general (see Chapter 000), alternative therapeutic modalities have been explored. As in the case of assessing the value of chemotherapy in patients with gastrinoma, the rarity of the disease coupled with the heterogeneous group of patients studied and the variable natural history of these malignancies make the available data somewhat difficult to interpret.

Therapy with biological agents such as the stable somatostatin analogues octreotide and lanreotide has been attempted in metastatic gastrinoma [238,239]. Although early reports suggested a significant decrease in tumor size in response to octreotide [240], subsequent studies have not been as encouraging [241,242]. Tumor stabilization may be achievable in some of the NETs, but this is unclear in the case of patients with gastrinoma.

A novel and exciting therapeutic alternative for the treatment of neuroendocrine tumors is peptide receptor radionuclide therapy [243,244]. Therapy with radiolabeled somatostatin analogues appears promising, because many NETs express a high density of somatostatin receptors (predominantly subtype 2). Several nuclides being explored with some success include indium-111, yttrium-90, and lutetium-177. Early reports have demonstrated an approximate 25% rate of tumor shrinkage of greater than 50% in a heterogeneous group of tumor types. Although this option is in the early stages of development, future studies may prove it beneficial for patients with metastatic disease.

Leukocyte interferon is another biological agent that also has been used in the therapy of neuroendocrine tumors with reported success [245,246]. Unfortunately, these encouraging results were not observed by Pisegna and associates [247] after examining the effect of human recombinant interferon-α in patients with Zollinger–Ellison syndrome. Smaller trials examining the efficacy of combining interferon with octreotide or 5-FU have been published [208,248,249], but the limited number of patients evaluated makes these data difficult to interpret. In addition, there was a substantial increase in drug toxicity when interferon was combined with 5-FU, making it a less desirable treatment option [208,249].

Vascular occlusive therapy (hepatic artery embolization [HAE] with particles and chemoembolization [HACE]) of metastatic NETs to the liver is based on the principle that these lesions receive the majority of their blood supply from the hepatic artery. By contrast, normal hepatic tissue receives its blood supply mainly from the portal vein. Ruszniewski and associates [250] examined the efficacy of chemoembolization with doxorubicin in 24 patients with metastatic tumors (five patients with gastrinoma), and tumor stabilization was observed in three patients. Subsequently, Gupta and colleagues performed a retrospective analysis comparing HAE and HACE in 69 patients with metastatic carcinoid tumor and 54 patients with metastatic NETs of the pancreas (nine gastrinomas) [251]. The authors observed that carcinoid patients did better overall and that, in addition, they appeared to have a better response to HAE compared with NET patients (who had a better outcome with HACE), with initial response rates of 67% and 35%, respectively, and overall median survival of 34 months and 23 months. Attempts at combination therapy have also produced marginal results. Moertel and colleagues [252] treated 111 patients (with carcinoid and pancreatic endocrine tumors) with systemic chemotherapy in addition to chemoembolization, and achieved an overall response rate of 80%. In view of the limited data available in patients with gastrinoma and the potential complications (10%–17%) related to HAE and HACE, including abdominal pain, fever, nausea, and even death (< 5%) [253,254], these treatment modalities should be reserved for treatment-refractory patients until further data are available.

Several other ablative treatments have been examined to a limited extent in patients with metastatic NETs. These include percutaneous ethanol injection, cryotherapy, and radiofrequency ablation [255–261]. Although initial reports

suggest these treatment options may be promising, more data are needed.

Aggressive surgical resection (debulking) [165,262–265] has been performed successfully, with 5-year survival achieved in more than 75% of patients whose tumors were amenable to complete resection [263]. Elias and colleagues reviewed their experience in 47 patients who underwent aggressive resection with intent to cure and observed that resection was curative in only 53% of the patients, despite having removed as much as 97% of tumor in each of the patients [265]. Morbidity and mortality for surgery were 45% and 5%, respectively, and median survival was 91 months, with overall survival rates at 5 and 10 years being 71% and 35%, respectively. These authors concluded that surgery for liver metastases from well-differentiated endocrine neoplasms is indicated when all visible intrahepatic and extrahepatic lesions can be resected safely. These data suggest a potential benefit of an aggressive surgical approach, and some advocate this approach in patients in whom at least 90% of the tumor can be resected based on imaging studies [165].

Orthotopic liver transplantation also has been attempted as a therapeutic option in patients with hepatic metastases. The finding that these tumors are slow growing and are often unresponsive to aggressive medical management has served as the driving force behind this treatment modality [266–268]. Unfortunately, the 4-year survival rate in patients with pancreatic neuroendocrine tumors following liver transplantation was only 8% in the largest sample of patients examined [268]. Thus, orthotopic liver transplantation is not an attractive option for these unfortunate patients.

Despite the strides made in the therapy of these rare neoplasms, the treatment of recurrent or metastatic disease leaves much to be desired. Better understanding of the basic molecular and cell biology of these rare tumors may offer new therapeutic options. Studies have demonstrated the biological importance of angiogenesis and angiogenic growth factors such as vascular endothelial growth factor (VEGF) and its receptor, insulin-like growth factor 1 (IGF-1) and its receptor, the platelet-derived growth factor receptor (PDGFR), epidermal growth factor receptor (EGFR), and the mammalian target of rapamycin (mTOR) signaling pathways. Phase II–III clinical studies in patients with NETs of agents that target various of these pathways have shown initial promising results for bevacizumab (VEGF monoclonal antibody) and everolimus (mTOR inhibitor) [269]. Larger-scale studies focusing on the use of these agents in the treatment of different subtypes of NETs are pending.

Prognosis

Zollinger–Ellison tumors can be classified as either benign or malignant [35,270] based on a series of clinical characteristics. Of these characteristics, tumor extent and rate of tumor growth are the most important prognostic indicators for patients with gastrinoma. As reviewed by Maton and colleagues [227], 5-year survival rates for all patients with gastrinoma vary between 62% and 75%, and 10-year survival rates vary between 47% and 53%. If one segregates the survival data according to extent of tumor at the time of diagnosis, patients who have a negative laparotomy or who have all their tumor removed surgically have 5- and 10-year survival rates greater than 90%. By contrast, patients with tumors that were incompletely resected have 5- and 10-year survival rates of 43% and 25%, respectively. Weber and associates [35] critically evaluated 185 consecutive patients with Zollinger–Ellison syndrome and identified the criteria that determined metastatic rate and survival in these patients. These investigators confirmed that both benign and malignant categories of gastrinomas exist. The overall 10-year survival rates were 93% for patients with MEN1 and 74% for patients without MEN1, somewhat better than rates reported previously [227], but similar to results reported by Eriksson and colleagues [271]. It was also found by these investigators that there was no difference in survival for the following groups of patients: those rendered disease free at surgery; those with residual tumor after surgery; and those with disease and negative exploratory laparotomy results. A poor clinical outcome is seen in female patients, in patients without MEN1, in patients with high serum gastrin levels, in patients with a large pancreatic tumor, and in patients with hepatic metastasis.

A detailed prospective analysis of the clinical course of 212 patients with Zollinger–Ellison syndrome [272] observed for a mean period of 15 years (range 0.1–31 years) was published. Thirty-two percent of patients died (none of acid-related complications); 50% of them had gastrinoma-related causes of death, which are outlined in Table 41.7, with the most common being progressive cachexia associated with hepatic metastases (67%). Gastrinoma-related causes of death were more commonly observed in patients with the following characteristics: shorter disease duration; higher gastrin levels; pancreatic primary and large tumor; metastatic disease to lymph nodes, liver, or bones; and ectopic Cushing

Table 41.7 Causes of death related to Zollinger–Ellison syndrome

Cause of death	Number	Percentage
Liver metastases with progressive inanition	22	67
Secondary PET syndrome	10	30
Liver metastases causing hepatic failure	4	12
Tumor-related embolism	2	6
Postoperative complications	1	3
Cardiac gastrinoma with arrhythmia	1	3

PET, pancreatic endocrine tumor.
Adapted from Seifert et al. [258].

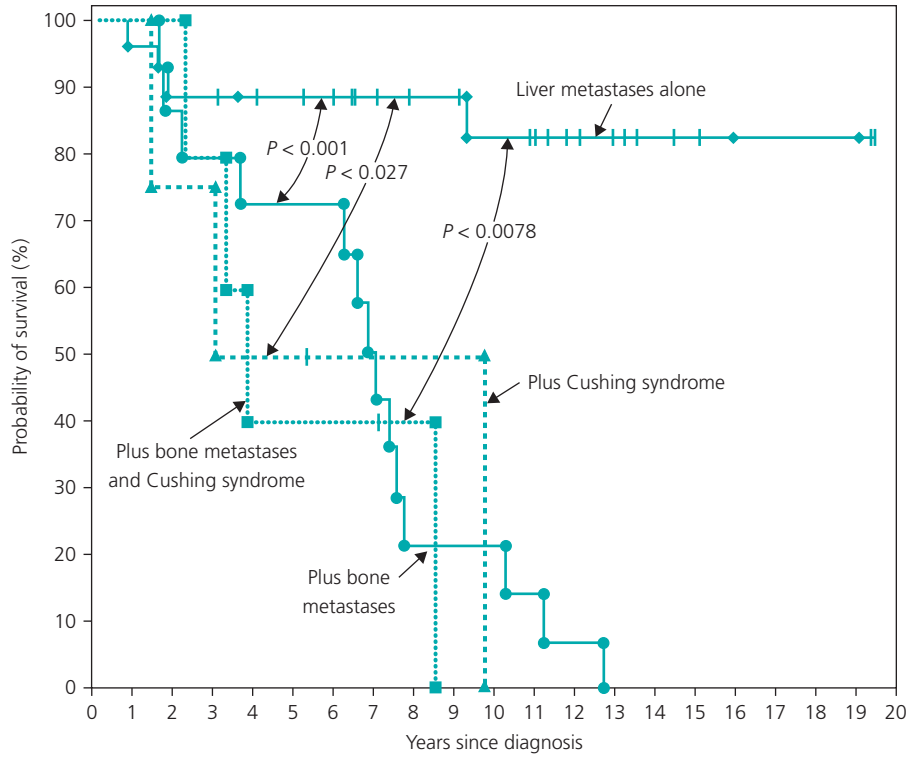

Figure 41.3 Impact of the presence of bone metastases, liver metastases, or ectopic Cushing syndrome, alone or in combination, on survival of patients with gastrinoma. Adapted from Seifert et al. [258], with permission of John Wiley & Sons.

syndrome. When considering patients with hepatic metastases, tumor-related Cushing syndrome and development of metastases to bone were independent predictors of decreased survival time (Fig. 41.3). Besides the extent of hepatic metastases, the rate at which they developed was an independent indicator of poor prognosis. From this and other studies [35], it appears that roughly 25% of patients with gastrinoma follow an aggressive pattern, with 10-year survival approaching 30%, whereas the remaining patients follow a more indolent course, with a 10-year survival of 95%.

Gibril and colleagues [273] performed a similar type of prospective study on the natural history of gastrinoma in 57 patients with MEN1. These patients were followed-up for a mean period of 8 years, with 23% having or developing liver metastases and 14% having a tumor with aggressive growth, defined as tumor growth of 25% volume increase or more per month or the appearance of any new lesion. In terms of survival, 5% of the study group, 23% of patients with hepatic metastases, and 38% of patients with aggressive tumor growth died during the study period. Factors predictive of aggressive gastrinoma growth were identified by the authors and are summarized in Table 41.8. Ultimately, further elucidation of the molecular determinants important in tumor development in MEN1 coupled with recognition of clinical features predictive of poor outcome may allow earlier identification and intervention in patients prone to developing aggressive disease.

Table 41.8 Factors predictive of aggressive growth of gastrinomas in patients with multiple endocrine neoplasia type 1

Clinical
Age at MEN1 diagnosis (years) < 35 ($P = 0.021$)
Age at ZES onset (years) ≤ 27 ($P = 0.043$)
Age at ZES diagnosis (years) ≤ 33 ($P = 0.032$)
Duration of ZES before diagnosis (years) < 2.1 ($P = 0.021$)
Laboratory
Fasting gastrin levels ≥ 10 000 pg/mL ($P < 0.0001$)
Tumor characteristics
Pancreatic tumor size > 3 cm ($P < 0.0001$)[a]
Presence of liver metastases ($P < 0.00001$)
Presence of bone metastases ($P = 0.0019$)
Presence of gastric carcinoid ($P = 0.024$)

a Data from 41 patients who underwent abdominal exploration.
MEN1, multiple endocrine neoplasia type 1; ZES, Zollinger–Ellison syndrome.
Adapted from Wessels & Schnell [259], with permission of Elsevier.

Advances in elucidating the molecular makeup or fingerprint of gastrinomas, and NETs of the gut in general, will lead to further insights into predicting not only the clinical outcome of tumor types but also potentially shed light on

optimization of treatment for these often refractory neoplasms once they are metastatic. Modern molecular tools have begun to help in this regard. For example, studies using surgically resected specimens suggest that genetic alterations accumulate during malignant transformation [274]. Specific changes noted include the loss of chromosome 1 and 11q and gain of 9q early in pancreatic endocrine tumor development, which occur with smaller more indolent tumors. By contrast, loss of chromosomal arms 3p, 6pq, and 10pq, and gains of 5q, 12q, 18q, and 20q occur with the development of a malignant phenotype [275–277]. The utilization of EUS-guided sampling of these lesions combined with modern molecular techniques has also led to several interesting observations concerning NETs that may help to understand the natural course of gastrinomas. Nodit and colleagues performed a broad panel microsatellite loss analysis of fine-needle aspirate (FNA) samples obtained by EUS and could differentiate between benign and malignant pancreatic endocrine tumors [278]. Another exciting development is the potential to perform gene expression profiling using complementary DNA microarray analysis on EUS-obtained FNA samples to identify potential therapeutic targets. Couvelard and colleagues [279] found that 72 gene transcripts were upregulated and 51 downregulated in malignant tumors. In metastatic (vs nonmetastatic tumors) they observed selective expression of CD34, E-selectin (endothelial adhesion molecule 1), MKK4 (map kinase kinase 4) and MDR1, genes involved in angiogenesis and remodeling (CD34, cadherin-5, E-selectin, semaphorin E, and fibrillin), in signal transduction through tyrosine kinases (tyrosine kinase-2), platelet-derived growth factor Rb (receptor beta), MKKR (map kinase kinase receptor), and discoidin domain receptor 1), and in calcium-dependent cell signaling. Although the number of gastrinomas studied was relatively small, future work may confirm these findings in gastrinproducing tumors. This type of biological profiling of NETs may shed light on prognosis and assist in the development of effective molecularly targeted therapy.

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B STOMACH

42 Gastritis and gastropathy

Mary Kay Washington, Richard M. Peek Jr

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Gastritis is defined as microscopic inflammation of the stomach and represents a histological not a clinical entity, as the majority of persons with gastric inflammation are completely asymptomatic. A myriad of etiological agents can induce gastritis, but the most common cause is infection by *Helicobacter pylori*, a bacterium that resides in the mucus gel layer overlying the gastric mucosa [1,2]. Barry Marshall and Robin Warren were awarded the Nobel Prize in Medicine in 2005 as the investigators who in 1983 first identified and cultured these spiral bacilli from gastric epithelium of patients with chronic gastritis [3,4]. Since that time, a strong link has been established between *H. pylori* and a diverse spectrum of gastroduodenal diseases, including gastric and duodenal ulceration, gastric adenocarcinoma, mucosa-associated lymphoid tumor (MALToma), and non-Hodgkin lymphoma of the stomach (Fig. 42.1) [1,2,5–28]. *H. pylori* colonizes the stomach for years or decades, not days or weeks, as is usually the case for bacterial pathogens, and this organism virtually always induces chronic gastritis; however, only a fraction of *H. pylori*-colonized individuals ever develop disease. Identification of mechanisms that regulate *H. pylori*–host interactions will not only improve diagnostic and therapeutic modalities, but also may provide insights into other diseases that arise within the context of pathogen-initiated inflammatory states, such as chronic viral hepatitis and hepatocellular carcinoma. In addition to *H. pylori*, the other pathogens and exogenous agents that induce gastric inflammation and mucosal injury, gastritis, and gastropathy will also be discussed in this chapter.

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Classification of gastritis using the Sydney system

The most widely used method for classification of gastritis and reporting of nonneoplastic gastric biopsies in the research setting is the updated Sydney system [29], which classifies chronic gastritis based on topography, morphology, and etiology (Table 42.1).

Biopsy protocol

The Sydney system requires histological sampling with separate submission of biopsies from five locations in the stomach: the greater and lesser curvature in the antrum, the greater and lesser curvature of the corpus, and the incisura. The incisura is a typical site for atrophy, intestinal metaplasia, and dysplasia [29], although data suggest that biopsies from endoscopically normal incisura add little additional clinical information [30]. Histological features assessed on a 0–4+ scale include *H. pylori* density, neutrophil infiltration, mononuclear cell infiltration, atrophy, and intestinal metaplasia. A series of cartoons illustrating the scoring is available to assist in standardizing histopathological grading [29]. Additional features assessed as present or absent and not otherwise graded are surface epithelial damage, lymphoid follicles, and foveolar hyperplasia. Additional findings such as granulomas are noted when present. The final diagnosis represents a synthesis of individual histological findings, topographical location, and available clinical information. Although the Sydney system is useful for research purposes, in clinical practice, failure to obtain the specified numbers of biopsies from the various regions of the stomach and the complexity of the scoring system limit its widespread use.

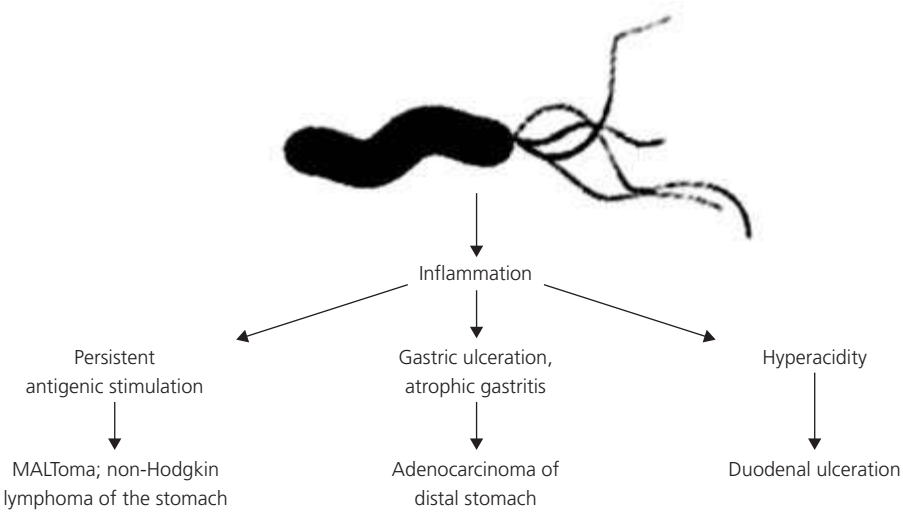

Figure 42.1 Disease outcomes that develop within the context of *H. pylori*-induced gastric inflammation.

Table 42.1 Classification of chronic gastritis based on topography, morphology, and etiology (updated Sydney system)

Type of gastritis	Etiology	Synonymous terms
Nonatrophic chronic gastritis	*H. pylori* ?Other factors	Chronic superficial gastritis Diffuse antral gastritis Chronic antral gastritis Type B gastritis
Atrophic gastritis		
Autoimmune gastritis	Autoimmunity ?*H. pylori*	Type A gastritis Diffuse corporal gastritis Pernicious anemia-associated gastritis
Multifocal atrophic gastritis	*H. pylori* Environmental factors	Type B or type AB gastritis Atrophic pangastritis
Special forms of gastritis		
Chemical gastropathy	NSAIDs Bile reflux Other chemical irritation	Reactive gastropathy Reflux gastropathy
Radiation	Radiation injury	
Lymphocytic	?Gluten ?Autoimmune mechanisms Drugs ?*H. pylori* ?Idiopathic	Celiac disease-associated Varioliform
Noninfectious granulomatous gastritis	Crohn's disease Sarcoidosis Foreign substances ?Idiopathic	Isolated granulomatous gastritis
Eosinophilic gastritis	Food sensitivity ?Other allergies	Allergic
Other infectious gastritides	Bacteria other than *H. pylori* Viruses Fungi Parasites	Phlegmonous gastritis Emphysematous gastritis Cytomegalovirus Anisakiasis

NSAIDs, nonsteroidal antiinflammatory drugs.

Histological features of chronic gastritis

Three patterns of chronic nonspecific gastritis are recognized:
- diffuse antral gastritis (nonatrophic gastritis in the Sydney system), which is associated with *H. pylori* infection
- multifocal atrophic gastritis, which is also highly associated with *H. pylori*
- diffuse corporal atrophic gastritis (autoimmune gastritis).

Nonatrophic chronic gastritis

In the nonatrophic chronic gastritis associated with *H. pylori* infection, a diffuse chronic inflammatory infiltrate composed of lymphocytes, plasma cells, and scattered eosinophils and mast cells is present in the lamina propria. The density of this infiltrate is variable and tends to be sparser in atrophic gastritis. The presence of lymphoid aggregates (Fig. 42.2), especially with germinal centers, is greatly associated with *H. pylori* infection and may persist after eradication of the organism. The presence of neutrophils indicates an "active" gastritis and is often associated with the presence of *H. pylori* organisms in the nearby superficial epithelium. Neutrophils infiltrate gastric glandular epithelium, typically in the neck area of the gland, and may form "pit abscesses" (Fig. 42.3). The foveolar epithelium often shows degenerative changes, and superficial erosions may be seen. Mucosal edema and hyperemia are common but nonspecific findings. *H. pylori* organisms

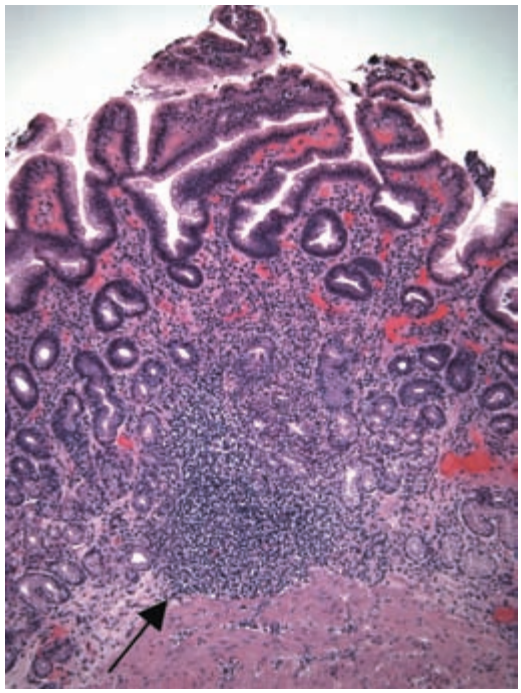

Figure 42.2 Lymphoid aggregates (arrow) are common findings in *H. pylori*-associated gastritis. The lamina propria contains a mixed inflammatory infiltrate of plasma cells, lymphocytes, neutrophils, and eosinophils.

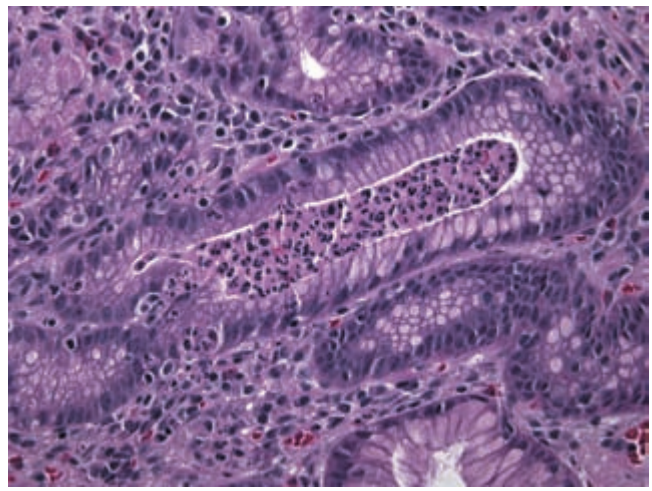

Figure 42.3 Neutrophils infiltrating in the lamina propria and foveolar epithelium and forming pit abscesses are characteristic of *H. pylori* gastritis.

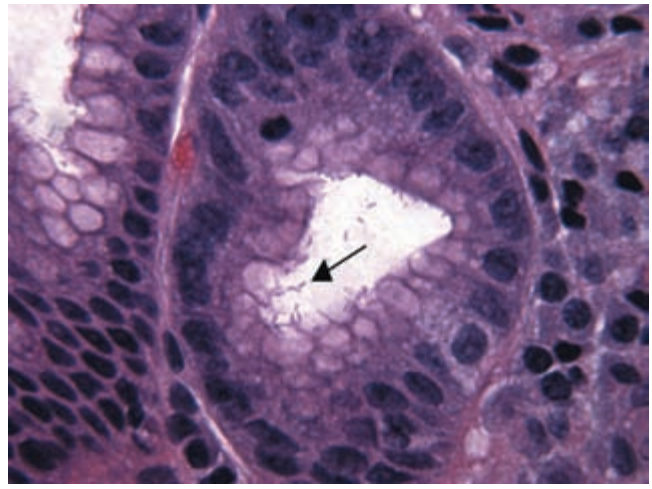

Figure 42.4 *Helicobacter pylori* organisms (arrow) are found in the surface mucous layer and extending into the lumen of the gastric pits.

are generally readily identifiable on routine hematoxylin–eosin-stained slides in the superficial mucus layer and extending into the lumen of the gastric pits (Fig. 42.4).

Multifocal atrophic gastritis

In atrophic gastritis, the specialized glands, such as chief and parietal cells in the gastric corpus and deep mucinous glands in the antrum, are lost and replaced by metaplastic epithelium or by fibrous tissue. Multifocal atrophic gastritis involves the gastric antrum and corpus and is generally patchy in distribution. Intestinal metaplasia, in which the gastric mucosa is replaced by mucosa resembling the small bowel, is common (Fig. 42.5). Pseudopyloric metaplasia, in which the oxyntic glands of the corpus are replaced by mucinous glands resembling antral mucosa, may be difficult to distinguish from gastric antral mucosa. Endocrine cell hyperplasia secondary

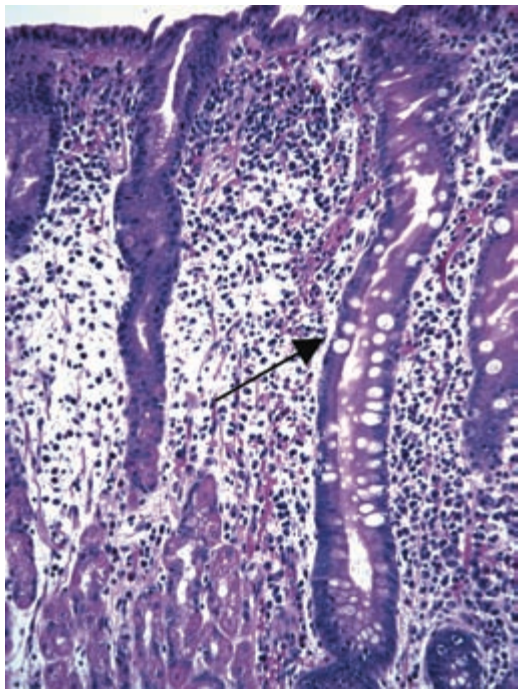

Figure 42.5 Multifocal atrophic gastritis involving the gastric corpus, showing patchy loss of oxyntic glands and intestinal metaplasia (arrow).

to hypergastrinemia is common. Because gastric acid production falls in atrophic gastritis, *H. pylori* organisms colonize more sparsely and are more difficult to detect.

Diffuse corporal atrophic gastritis (autoimmune gastritis)

The hallmark of this pattern of gastritis is the destruction of oxyntic glands. Atrophic gastritis is confined to the corpus and involves the antrum only minimally (see Autoimmune gastritis).

Helicobacter pylori-induced gastritis

H. pylori colonizes the stomachs of at least half the world's population, and persons in all geographical zones have been shown to carry the bacteria. Infection is usually acquired in childhood and, when left untreated, persists for the lifetime of the host [27,31]. The prevalence of *H. pylori* is greater in developing than developed countries [27,31,32]. In the United States, *H. pylori* is present in 10%–15% of children aged under 12 years, compared with 50%–60% of people older than 60 years [31–33]. The rate of acquisition of new *H. pylori* infections among adults in developed countries is less than 1% per year [32], and most carriers in the United States acquired *H. pylori* during childhood. Since the 1960s, however, progressively fewer children have been shown to carry *H. pylori* [1]. Risk factors for *H. pylori* acquisition include lower socioeconomic status and household crowding, and colonization rates reflect country of origin and ethnicity [31,34]. Colonization is related to intrafamilial clustering, but not to the presence of nonprimate reservoirs, favoring person-to-person transmission as the mode of infection. Experimental induction of regurgitation and catharsis increases *H. pylori* culture positivity rates from vomitus and diarrheal specimens [35], suggesting that *H. pylori* transmission may increase during childhood episodes of gastroenteritis.

Gastric colonization

H. pylori has evolved several mechanisms for eluding primary host defenses such as gastric acidity and peristalsis, to establish persistent infection within the stomach. One pH-altering mechanism is production of urease [36–38], and *H. pylori* isolates that cannot produce urease fail to colonize rodent models of infection [39,40], indicating that urease is a necessary factor for establishment of infection. To facilitate locomotion within gastric mucus and to counteract peristalsis, *H. pylori* possesses five or six polar flagella [41]. Like urease production, bacterial motility is required for persistent infection [42]. Approximately 20% of the *H. pylori* population in the stomach binds to gastric epithelial cells in vivo, and adhesion is important for acquisition of nutrients from the host and for resistance to shedding of the mucus gel layer. Several *H. pylori* and host ligands have been identified, including an *H. pylori* hemagglutinin encoded by *hpa* that binds to sialic acid-containing components of erythrocyte membranes [43], an exoenzyme S-like protein that binds phosphatidylethanolamine and gangliotetraosylceramide [44–46], and two outer membrane proteins (BabA and SabA) that bind the human blood group antigens Lewis^b and sialyl-Lewis^x, respectively [47–51].

Histopathological studies in human subjects have shown that *H. pylori* binds to specific regions of gastric epithelium, and that bacterial density is greatest in the upper portion of the gastric glands. This pattern of colonization mirrors the distribution of trefoil factor 1 (TFF1), and because *H. pylori* binds avidly to TFF1 in vitro [52], this molecule likely functions as a receptor for *H. pylori* in vivo. A specific component of gastric mucin (α1,4-linked *N*-acetylglucosamine *O*-glycan), which is confined to deep glandular regions rarely colonized by *H. pylori,* has been shown to exert antimicrobial effects against *H. pylori* by inhibiting the synthesis of cholesteryl-D-glucopyranoside, a major *H. pylori* cell wall component [53]. Thus, the host seems to have evolved mechanisms to restrict *H. pylori* to certain microniches within the gastric mucosa.

H. pylori can also limit the bactericidal effects of proinflammatory molecules, such as nitric oxide [54]. *H. pylori* expresses an enzyme called arginase, which siphons the nitric oxide substrate L-arginine away from inducible nitric oxide synthase (iNOS), limiting the production of iNOS-generated nitric oxide [54]. Another level of host defense circumvented by *H. pylori* is innate immunity. Toll-like receptors (TLRs) are a conserved family of host-cell receptors that function in innate

immunity by recognition of invariant regions in bacterial molecules termed pathogen- or microbe-associated molecular patterns [55–57]. *H. pylori* can avoid global activation of this system because *H. pylori* lipopolysaccharide and flagellin are relatively anergic compared with LPS and flagellins of other enteric bacteria [58–62]. *H. pylori* can also actively suppress the adaptive immune response [63–65] and survive within epithelial cells and macrophages [66–68]. However, despite these strategies for persistence, substantial immune activation still occurs, as evidenced by proinflammatory cytokine release, infiltration of the mucosa by inflammatory cells, and cellular and humoral recognition of *H. pylori* antigens [59].

Development of *H. pylori*-induced gastric inflammation

The gastric inflammatory response induced by *H. pylori* consists of neutrophils, lymphocytes, plasma cells, and macrophages, along with varying degrees of epithelial cell degeneration and injury (Figs 42.2 and 42.3), and the most distinctive feature of *H. pylori*-induced gastritis is infiltration of the gastric epithelium by neutrophils. Within the lamina propria, polymorphonuclear cells are admixed with lymphocytes, lymphoid follicles, plasma cells, and eosinophils, and the severity of gastritis is greatest in the superficial portions of the lamina propria. *H. pylori* colonization also induces an exuberant systemic and mucosal humoral response directed at multiple antigens [33,69–73]; however, antibody production does not result in eradication. Experimental mouse models have demonstrated that the inflammatory response to *H. pylori* is strongly dependent on the presence of T cells [74]. $CD4^+$ T lymphocytes can be broadly divided into two functional subsets, type 1 (Th1) and type 2 (Th2) T-helper cells, each of which are defined by distinct patterns of cytokine secretion. Th1 cells produce interleukin-2 (IL-2) and γ-interferon (IFN-γ) and promote cell-mediated immune responses, whereas Th2 cells secrete IL-4, IL-5, IL-6, and IL-10 and induce B-cell activation and differentiation. The type of immune response to a particular microbial agent is governed by preferential expansion of one T-helper cell subset accompanied by a relative downregulation of the other. Intracellular bacteria classically induce Th1 responses, whereas extracellular pathogens stimulate Th2-type responses. However, the majority of *H. pylori*-specific T-cell clones produce greater levels of IFN-γ than IL-4 [75], and IL-12, a cytokine that promotes Th1 differentiation [76]. These data are consistent with a model in which an inappropriate host T-cell response towards an extracellular pathogen, *H. pylori*, facilitates the development and chronicity of gastric inflammation.

Patterns of *H. pylori*-induced gastritis and disease outcome

There is a distinct dichotomy between *H. pylori*-induced duodenal ulceration and gastric cancer [77], and this depends on the topography of inflammation within the stomach. In the majority of infected persons residing in developed countries, *H. pylori*-induced gastritis is more severe in the gastric antrum, with only minimal involvement of the corpus, a pattern termed "diffuse antral gastritis" or "nonatrophic chronic gastritis" [78,79]. Gastric atrophy and intestinal metaplasia are typically absent in this type of gastritis. Persons with such corpus-sparing inflammation are at increased risk for duodenal ulcer disease, and gastric acid production in this population is either normal or increased [78,79]. By contrast, persons who progress to gastric ulcer disease and distal gastric adenocarcinoma have a different pattern of gastritis: there is involvement not only of the antrum but also the acid-secreting corpus and, as predicted, patients with pangastritis tend to have reduced acid outputs [15,80]. Corpus gastritis is often accompanied by gastric atrophy and intestinal metaplasia both in the antrum and the corpus. This pattern of gastric inflammation is most common among infected persons residing in Southeast Asia, South America, and certain regions of central, eastern, and southern Europe [81].

H. pylori virulence factors and gastric injury

H. pylori strains from different individuals are extremely diverse [82–85], and genetically unique derivatives of a single strain are present simultaneously within any individual human host [86]. The most well-studied strain-specific virulence determinant is the *cag* pathogenicity island, a 27-gene locus that is present in approximately 60% of US strains [82,83,87,88]. Although all *H. pylori* strains induce gastritis, the presence of cag^+ strains augments the risk for severe gastritis, peptic ulcer disease, and gastric cancer compared with that posed by cag^- strains [1,2,71,72,89–99]. Gene products of the *cag* island form a secretion system that functions as a molecular syringe to export proteins, and the product of the terminal gene in the island (CagA) is translocated into host epithelial cells following bacterial attachment (Fig. 42.6) [1,100–104]. Epithelial cell responses induced by the *cag* island include morphological alterations that are reminiscent of unrestrained cellular stimulation by growth factors, and production of the proinflammatory cytokine IL-8 (Fig. 42.6) [100–103,105–119].

Another *H. pylori* locus linked with disease outcome is *vacA*, which encodes a secreted bacterial toxin (VacA) [120–125]. Unlike the *cag* island, *vacA* is present in virtually all *H. pylori* strains [121,125]; however, strains vary in cytotoxin activity because of variations in *vacA* gene structure. The regions of greatest diversity are localized near the 5′ end of *vacA* (allele types s1a, s1b, s1c, or s2) and in the midregion of *vacA* (allele types m1 or m2) [125–127]. *H. pylori* strains that possess both type s1 and m1 *vacA* alleles are associated with an increased risk of peptic ulcer disease and gastric cancer [47,128–130] and enhanced gastric epithelial cell injury [131,132] compared with *vacA* s2/m2 strains. VacA also actively suppresses both T-cell proliferation and activation [63–65], which may contribute to the longevity of *H. pylori* colonization.

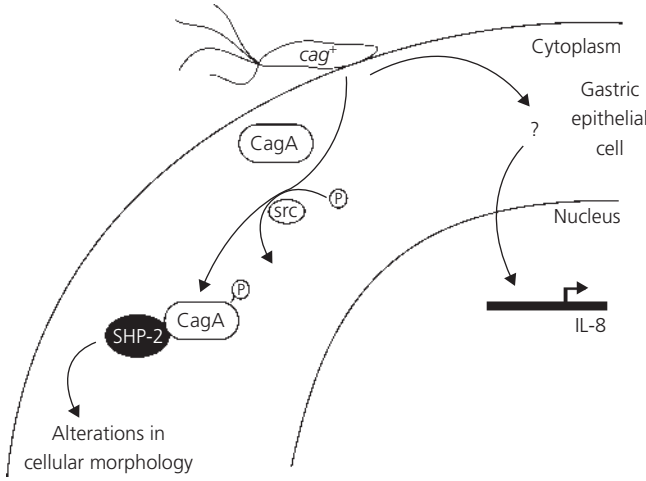

Figure 42.6 *Helicobacter pylori* affects multiple pathways in gastric epithelial cells that induce alterations in cellular morphology or production of interleukin-8 (IL-8). CagA is translocated into host cells, where it becomes phosphorylated by Src kinases, binds to a host phosphatase (SHP-2), and alters cellular morphology. An undefined factor is also translocated into host cells by the *H. pylori cag* secretion system leading to increased IL-8 expression.

Sequence analysis of the genomes from the two completely sequenced *H. pylori* strains, 26695 and J99, has revealed that an unusually high proportion of identified genes encode outer membrane proteins (OMPs) [49,51,82,83]. BabA is a membrane-bound OMP encoded by the strain-selective gene *babA2* and binds the blood-group antigen Lewis^b present on gastric epithelial cell membranes [47,48]. *H. pylori* strains possessing *babA2* are associated with an increased risk for gastric adenocarcinoma [47]. Sialyl-Lewis^x glycosphingolipid also functions as a receptor for *H. pylori* [50], and the bacterial adhesin required for binding is the OMP SabA. Sialyl-Lewis^x antigens are established tumor antigens and markers of gastric dysplasia that are upregulated by chronic gastric inflammation. Another *H. pylori* OMP is a proinflammatory protein encoded by *oipA* [133], and *H. pylori* strains that contain a functional copy of *oipA* induce more severe gastric inflammation, enhanced mucosal levels of IL-8, and duodenal ulcer disease [134]. These studies emphasize the pivotal role of *H. pylori* adherence in the induction of gastric inflammation and injury.

Human genetic polymorphisms and development of disease among *H. pylori*-infected persons

Most persons colonized with disease-associated *H. pylori* strains remain asymptomatic. *H. pylori cag*$^+$ toxigenic strains are related to both duodenal ulcer disease and distal gastric cancer, considered to be two mutually exclusive disease outcomes. The inability of bacterial virulence components completely to account for pathological outcomes has highlighted the need to explore host factors that may influence disease, particularly gastric cancer.

The cytokine IL-1β is a proinflammatory molecule whose concentration is increased within *H. pylori*-colonized gastric mucosa [135]. Interleukin-1β amplifies host inflammatory responses and potently inhibits acid secretion by parietal cells (100-fold more potent than proton pump inhibitors) [136]. These effects are biologically significant because inflammation that involves the acid-secreting gastric corpus (e.g., pangastritis) increases the risk of gastric adenocarcinoma, perhaps by inducing hypochlorhydria and atrophic gastritis leading to bacterial overgrowth [1,137]. The IL-1β gene cluster contains a number of functionally relevant polymorphisms that are associated with either increased or decreased IL-1β production, which has permitted the performing of case-control studies that relate host genotypes and disease. El-Omar and colleagues demonstrated that *H. pylori*-colonized persons with high-expression IL-1β alleles have a significantly increased risk for hypochlorhydria, gastric atrophy, and distal gastric adenocarcinoma than persons with genotypes that limit IL-1β expression (Table 42.2) [138]. These relationships, subsequently replicated in geographically distinct regions

Table 42.2 Human genetic polymorphisms that influence the development of distal gastric cancer

Genetic locus	Function of expressed protein	Genotype associated with enhanced risk
IL-1β	Induces expression of inflammatory cytokines; potently inhibits acid secretion from parietal cells	–31 C/C –511 T/T
IL-1RN	Receptor for IL-1β	Pentaallelic 86-bp tandem repeat in intron 2
TNF-α	Activates intracellular signaling pathways related to inflammation and apoptosis; inhibits acid secretion from parietal cells	–308 A/A
IL-10	Inhibits production of proinflammatory cytokines	–592 ATA/ATA –819 ATA/ATA –1082 ATA/ATA

IL, interleukin; IL-1RN, IL-1 receptor; TNF, tumor necrosis factor.

of the world, were only present among *H. pylori*-colonized persons, emphasizing the importance of host–environment interactions and inflammation in the progression to gastric cancer [139,140].

Similar to IL-1β, tumor necrosis factor α (TNF-α) is a proinflammatory, acid-suppressive cytokine that is increased within *H. pylori*-colonized mucosa [141]. Polymorphisms of the TNF-α gene that increase TNF-α expression are also associated with an increased risk of gastric cancer and its precursors (Table 42.2) [142]. Conversely, polymorphisms that reduce the production of antiinflammatory cytokines such as IL-10 increase the risk for gastric cancer (Table 42.2) [142]. The relative risk for distal gastric cancer increases progressively with an increasing number of proinflammatory polymorphisms, to the point that three polymorphisms increase the risk of cancer 27-fold over baseline [142]. *H. pylori* virulence factors synergize with host genotype to augment further the risk of cancer. In persons with high-expression *IL1B* alleles that are colonized by *H. pylori vacA* s1 strains, the relative risk for gastric cancer is 87-fold over baseline [143]. Combined bacterial and host genotyping may identify individuals at extremely high risk for gastric cancer.

Diagnosis of *H. pylori* infection

The presence of *H. pylori* should only be determined if such results will affect clinical decisions. The only persons for whom a clear indication for *H. pylori* testing exists are those with peptic ulcer disease, MALToma, prolonged use of nonsteroidal antiinflammatory drugs (NSAIDs), and possibly those with a strong family history of distal gastric cancer [144,145]. Techniques to diagnose *H. pylori* can be classified as either invasive (requiring endoscopy) or noninvasive (requiring breath tests, body fluid, or stool) (Table 42.3) [146,147]. Invasive methods require endoscopy with biopsy and include histology, rapid urease tests, culture, or polymerase chain reaction [146,147]. Although none of the available diagnostic tests is completely reliable because of heterogeneity in colonization density, most are sufficiently accurate to permit their widespread use, either for initial diagnosis or confirmation of cure (Table 42.3).

Histological detection of *H. pylori*

The presence of *H. pylori* within biopsy specimens can be determined histologically with or without the use of special stains that enhance bacterial visibility such as Warthin–Starry, Giemsa, Diff Quick, Genta, and el-Zimaity stains. The last two techniques offer an additional advantage by permitting simultaneous visualization of *H. pylori* and coexisting features of gastritis or damage, such as intestinal metaplasia. Advantages of histological diagnosis include direct visualization of the organism, assessment of the severity of gastritis, identification of more severe gastric lesions such as atrophic gastritis, intestinal metaplasia, adenocarcinoma, or lymphoproliferative disease, and accuracy for both initial diagnosis and confirmation of cure [146,147]. Although histology is considered by many to be the gold standard, its reliability depends on the number and sites of biopsy harvest, staining technique, and the experience of the histopathologist [148,149]. Evaluation of 3–5 biopsy specimens in total obtained from the greater or lesser curvature of the antrum and the corpus, respectively, in addition to the angularis, reliably distinguish infected from uninfected mucosa [149].

Rapid urease tests

Rapid urease tests take advantage of the robust production of urease by *H. pylori*. The most widely used form consists of a gel tablet containing urea and a pH indicator [146,147]. When an infected biopsy is placed into the gel, urea is converted to ammonia by urease, the pH within the tablet increases, and the pH indicator undergoes a color change (typically from yellow to red), often within 30 minutes. Additional forms of rapid urease tests have been developed,

Table 42.3 Diagnostic tests for *Helicobacter pylori*

Test	Clinical utility for:		Detects viable bacteria
	Primary diagnosis	Confirmation of cure	
Invasive			
Histology	+	+	–
Rapid urease testing	+	+	+
Culture	±	±	+
Polymerase chain reaction	–	–	–
Noninvasive			
Urea breath testing	+	+	+
Serology	+	–	–
Stool antigen tests	+	+	–

including paper and tablet tests, but their superiority to gel forms has not been established.

The advantages of rapid urease testing include ease of use, cost-effectiveness, and rapid acquisition of results, and they can be used for both initial diagnosis and follow-up of therapy. Sensitivities of rapid urease tests range from 93% to 97%, with specificities of approximately 98%; however, biological conditions can affect their accuracy [146,147]. Achlorhydria that develops as a consequence of atrophy or medication use (i.e., proton pump inhibitors) can lead to both false-positive (bacterial overgrowth with urease-producing organisms such as *Proteus*) and false-negative (decreased *H. pylori* colonization density) results [150]. False-positive results may also occur when non-*H. pylori Helicobacter* species, such as *H. heilmanni*, colonize the stomach. Heterogeneity in sites of colonization can alter the accuracy of biopsy-based rapid urease tests. For these reasons, it is recommended to wait at least 4 weeks following completion of anti-*H. pylori* therapy and at least 1 week after discontinuing proton pump inhibitors before performing a rapid urease test [146,147].

H. pylori culture

H. pylori can be grown directly from gastric tissue either by gently rubbing a biopsy specimen onto selective or nonselective media or by homogenizing gastric tissue in sterile saline and plating serial dilutions of homogenate [89]. Samples are then incubated for up to 10 days at 37°C in an environment containing 5%–12% CO_2. Similar to rapid urease testing, medications such as antibiotics or acid-suppressive drugs that alter bacterial colonization patterns in vivo may affect culture sensitivity. Despite significant advances over the past two decades, microbiological culture has not been widely accepted as a practical means to diagnose *H. pylori*, primarily due to cost, the special conditions required for specimen transport and growth, and the long interval between specimen harvest and test results, which delays treatment decisions. *H. pylori* culture is advantageous, however, if antimicrobial susceptibility patterns for specific isolates need to be determined.

Detection of *H. pylori*-specific DNA sequences in vivo

H. pylori DNA can be successfully detected in gastric tissue by the polymerase chain reaction (PCR). Using specific primers, PCR amplifies DNA sequences specific for *H. pylori* and can yield high levels of sensitivity (93%) and specificity (100%) by detecting as few as 10 *H. pylori* colony-forming units (CFU). *H. pylori* DNA has also been amplified from gastric juice, saliva, dental plaque, and stool [146,147,151–155]. Disadvantages of this technique include a high rate of false-positive results as a result of contamination, either within the clinical setting (i.e., contaminated or improperly cleaned endoscopes) or laboratory setting. Because PCR is only detecting DNA, a positive result cannot be equated with the presence of viable organisms. False-negative results can occur due to the presence of PCR inhibitors within gastric tissue, extremely low numbers of organisms, or DNA damage that occurs during specimen processing. For these reasons, plus the availability of other diagnostic techniques with comparable levels of sensitivity and specificity, PCR is still considered a research technique.

Urea breath tests

A noninvasive technique that also capitalizes on urease production is urea breath testing [146,147]. When urea labeled with either carbon-13 or carbon-14 is ingested by a patient with active *H. pylori* infection, gastric urease hydrolyzes labeled urea to ammonia and labeled CO_2, which is quickly absorbed into the bloodstream and can be detected in expired breath samples [146,147]. Urea breath tests accurately ascertain the presence of active infection, unlike PCR or serological methods, and allow a more global assessment of *H. pylori* within the stomach than can be provided by biopsy-based techniques. Breath tests also are effective for detection of *H. pylori* post-therapy, and on account of their noninvasive nature and high levels of sensitivity and specificity, they represent the gold standard for confirmation of cure.

Serological testing for *H. pylori*

The production of antibodies against *H. pylori* does not result in eradication of the infection; however, quantitating levels of anti-*H. pylori* immunoglobulins is an effective technique for primary diagnosis. A variety of serological methods have been developed, which include bacterial agglutination, complement fixation, hemagglutination, enzyme-linked immunosorbent assay (ELISA), immunofluorescence, radioimmunoassay, and latex agglutination [146,147]. Patient samples range from blood obtained by venipuncture or finger-stick, to saliva. As a rule, whole-blood tests are less accurate than serum-based tests due to the presence of chylomicrons within whole blood, and finger-stick tests have reduced sensitivity and specificity compared with venipuncture tests. Levels of anti-*H. pylori* antibodies in saliva are much more variable than in blood, and thus saliva-based tests are inferior to serum-based tests. Commercially available serology kits provide an extremely accurate means to diagnose *H. pylori* in patients who have not been previously treated, and levels of sensitivity are comparable with those obtained by histology, culture, and urea breath testing [146,147]. Additionally, there are clinical situations in which serology may be preferable to other techniques, for example with patients who present with an actively bleeding ulcer. Newer tests for detecting antibodies directed against *H. pylori* include urine assays, and sensitivities range from 82% to 94%, with specificities of 68%–92%, depending on the population under study [146,147,156–158].

The most significant limitation of serological testing is its inability reliably to confirm cure. Following treatment, anti-*H. pylori* antibody levels decrease slowly, and often 6 months

or more elapse before serum antibody concentrations decrease below the cutoff value for positivity [159,160]. In addition, serology is not cost-effective for confirming eradication in clinical practice because of the potential for multiple patient return visits and tests, and the requirement for storing pre-therapy samples as a standard against which post-therapy antibody levels must be compared. The availability, accuracy, and simplicity of urea breath tests also preclude using serology as a practical means to confirm cure.

An interesting area for future investigation is to assess the presence of *H. pylori* virulence characteristics by serology and segregate infected persons on the basis of differing levels of disease risk, such as risk for gastric cancer. CagA serology is simple to perform and reliable [161]. However, there is no convincing evidence to suggest that treatment of patients harboring $CagA^+$ strains decreases the risk for either premalignant or malignant gastric lesions. Another concern is that disease risk in *H. pylori*$^+$ $CagA^-$ subjects has not yet been established to be equivalent to that seen among uninfected persons [94]. If the prevalence of $CagA^+$ strains in a particular patient population is exceedingly high (i.e., East Asia), then screening for CagA may not be relevant. Finally, differences in serological recognition of CagA among clinical populations may not only reflect variation in the overall prevalence of *cagA*$^+$ strains, but also reflect the particular CagA antigen used in the assay, a limitation of serological tests designed to detect responses against *H. pylori* whole-cell antigens.

H. pylori stool antigen tests

An enzymatic immunoassay (HpSA, Meridian Diagnostics) that detects the presence of bacterial antigens in feces was also developed as a noninvasive diagnostic technique for *H. pylori* [162]. This test was originally formulated as a sandwich ELISA in which polyclonal anti-*H. pylori* antibody is bound to microtiter wells. Stool samples are then added and *H. pylori* antigens (if present) are "captured" by the bound antibody. A secondary antibody is then added followed by the addition of substrate, and bound *H. pylori* antigens are detected by a color change [162]. This assay has subsequently been modified into an immunocard [146].

The *H. pylori* stool antigen test is both sensitive and specific [146,147], and the US Food and Drug Administration (FDA) has approved HpSA for initial diagnosis and for monitoring post-therapy responses in *H. pylori*-infected adults [162]. Several trials have reported the sensitivity of HpSA to range from 91% to 96%, and specificities were also 91%–96% [147,162]. Concomitant use of proton pump inhibitors does not affect the accuracy of detecting *H. pylori* antigens in stool, in contrast to urea breath tests or biopsy-based techniques [162]. Following successful treatment of *H. pylori*, stool antigen levels rapidly decline, and in the majority of patients are undetectable by 5 days post-treatment [162]. Stool samples can be stored for prolonged periods of time, and multiple samples can be assayed at once. HpSA testing is safe and accurate in pediatric patients infected with *H. pylori* [162]. Finally, testing stool for *H. pylori* antigens is cost-effective. These data indicate that the *H. pylori* stool antigen test is a highly accurate technique that can identify the presence of *H. pylori* prior to and shortly following initiation of antimicrobial therapy. Its place in the *H. pylori* diagnostic armamentarium in the United States remains to be determined, especially given US patients' reluctance to obtain stool samples.

Treatment of *H. pylori* infection

The goal of *H. pylori* therapy is to eliminate the organism from the stomach, and successful eradication is defined as a negative test for the bacterium ≥ 4 weeks after the completion of therapy [145]. Treatment regimens should be straightforward, well tolerated, and cost-effective [163]. There are several different options for effectively treating *H. pylori* infections, and selection of a particular therapy is dependent on many factors, including drug availability, antmicrobial resistance patterns, and cost. First-line anti-*H. pylori* regimens may consist of a proton pump inhibitor (PPI) or ranitidine bismuth citrate (RBC), in combination with clarithromycin and either amoxicillin or metronidazole (triple therapy), for at least 7, but preferably 10 days (Table 42.4) [145,146,163–166]. There are no consistent advantages to using a particular PPI for *H. pylori* eradication [165,167]; therefore, any agent in this class can be used as a component of triple therapy. The prevalence of antimicrobial resistance in *H. pylori* is increasing, particularly for metronidazole, which reduces treatment efficacy [168]. Because the most effective second-line regimens contain metronidazole (Table 42.4), it is advisable to use clarithromycin and amoxicillin instead of clarithromycin and metronidazole in first-line regimens to optimize results should subsequent therapy be required [164,165].

Sequential therapies against *H. pylori* have also been tested [146,166]. In a randomized trial, over 1000 *H. pylori*-infected patients received either standard triple therapy for 7 days or a 10-day sequential therapy regimen consisting of a PPI daily plus amoxicillin 1 g twice a day for the first 5 days, followed by PPI therapy plus clarithromycin 500 mg and tinidazole 500 mg, each twice a day, for the remaining 5 days [146,166,169]. Higher eradication rates were found with the sequential regimen, whereas compliance rates and side-effect profiles were similar.

Second-line (rescue) therapies should be used if first-line regimens fail to eliminate *H. pylori*. The most effective salvage regimen is quadruple therapy using a PPI, bismuth subsalicylate, metronidazole, and tetracycline for 14 days (Table 42.4) [145,146,163–166]. This regimen is so effective that some experts have advocated it as first-line therapy (see Chapter 40, Peptic ulcer disease). If this regimen is used as first-line treatment, an appropriate second-line therapy would consist of a PPI, clarithromycin, and amoxicillin for 14 days [164].

Table 42.4 Treatment regimens for *Helicobacter pylori*

Therapy	Drug	Dosage	Duration (days)
First-line	Proton pump inhibitor (PPI)[a]	Twice a day[b]	7–10
	and		
	Clarithromycin	250 or 500 mg twice a day	7–10
	and		
	Amoxicillin	1000 mg twice a day	7–10
	or		
	Metronidazole	500 mg twice a day	7–10
Second-line	PPI	Twice a day[b]	14
	and		
	Bismuth subsalicylate	120 mg four times a day	14
	and		
	Tetracycline	500 mg four times a day	14
	and		
	Metronidazole	500 mg three times a day	14

a PPIs can be used interchangeably.
b Dose depends on individual PPI.

Anti-*H. pylori* therapies are also available as prepackaged formulations, such as Prevpac (lansoprazole 30 mg, clarithromycin 500 mg, amoxicillin 500 mg; each taken twice a day for 7 days) and Helidac (bismuth subsalicylate 262.4 mg, metronidazole 250 mg, tetracycline 500 mg; each taken four times a day for 14 days). Although these may be more convenient, they are not cost-effective, and the shorter duration (7 days) and lower dose of amoxicillin (500 mg) in Prevpac compared with standard triple therapy (Table 42.4), in addition to the need for combination therapy with a PPI (Helidac), preclude recommending these formulations as optimal first-line anti-*H. pylori* therapies [145].

Although eradication rates in controlled trials frequently exceed 90%, efficacy in clinical practice is often lower [145,146,166,170], primarily because of differences in patient populations, the genetic composition of infecting *H. pylori* isolates, or antibiotic resistance patterns of strains in a particular geographic locale. Because of the availability of accurate noninvasive diagnostic tests for *H. pylori*, it is recommended that urea breath testing or stool antigen tests be performed post-intervention to confirm successful cure [165].

Infectious gastritis (excluding *H. pylori*)

Bacteria

Bacterial colonization of the achlorhydric [171] or dysmotile stomach does not elicit an inflammatory response, and is generally asymptomatic. True bacterial infections of the stomach, excluding *H. pylori* gastritis, are exceedingly rare. Acute suppurative gastritis, or *phlegmonous gastritis*, is a life-threatening condition caused by infection of the submucosa and muscularis propria of the stomach by pyogenic bacteria, most often α-hemolytic streptococci. Immunocompromised patients, the elderly, and alcoholics are at greatest risk [172], and iatrogenic causes include endoscopic mucosal resection [173] and polypectomy [174]. Patients typically present with acute abdominal pain, peritonitis, fever, and hypotension. When gas-forming bacteria such as *Clostridium* species invade the stomach wall, the term *emphysematous gastritis* is used [175]. Surgical intervention with gastrectomy may be necessary, and mortality is high for both conditions. Other rare causes of gastric bacterial infections include syphilis, which may cause ulcerated lesions [176] and nodular masses [177], and *Mycobacterium tuberculosis* [178], which may present as gastric outlet obstruction. In gastric tuberculosis, biopsies show necrotizing granulomas, with acid-fast bacilli demonstrated on appropriate stains.

Viruses

Although enteric viruses cause a clinical picture of acute gastritis, biopsy is rarely performed in such cases and no distinctive findings have been reported. The most common viral infection identified in gastric biopsies is cytomegalovirus (CMV), usually seen in immunocompromised patients as part of disseminated infection, although it is occasionally found in elderly apparently immunocompetent patients [179]. Clinically, gastric involvement by CMV may be manifested by nausea and upper abdominal pain [180] or occasionally by gastric outlet obstruction [181] or hemorrhage. Endoscopically, erosions, ulcers, hypertrophic folds, and edema are identified. In children, cytomegalovirus gastritis causes massive foveolar

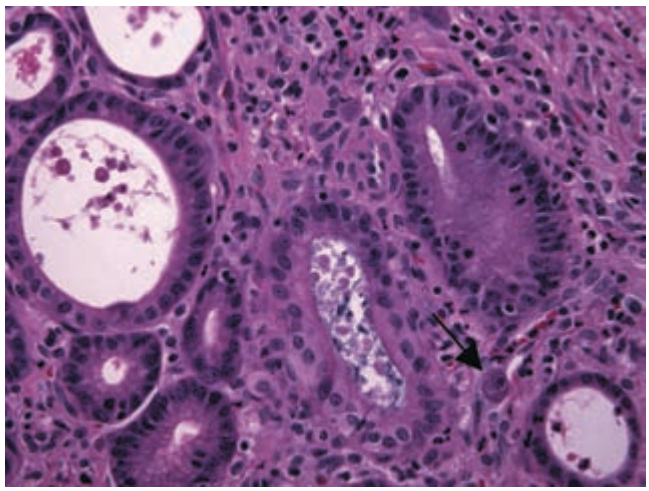

Figure 42.7 In cytomegalovirus gastritis, the inflammatory infiltrate is variable; characteristic nuclear viral inclusions may be seen in endothelial cells (arrow), stromal cells, epithelium, and smooth muscle.

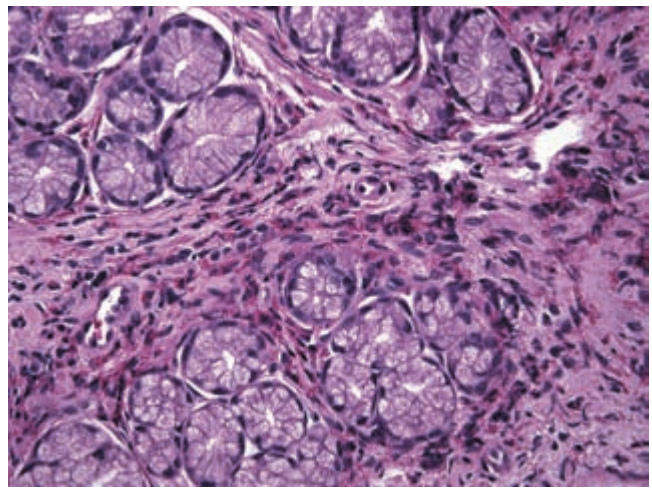

Figure 42.8 Eosinophilic gastritis is characterized by a dense but irregularly distributed infiltrate of eosinophils in the lamina propria or deeper layers of the stomach.

hyperplasia that is indistinguishable from Ménétrier disease. Nuclear cytomegalovirus inclusions may be found in enlarged epithelial, endothelial, smooth muscle, and stromal cells and range in number from numerous to undetectable on routine histology. The inflammatory response is variable; in some cases, patchy acute inflammatory infiltrates often centered about small vessels in the lamina propria and submucosa may be seen. Diagnosis is established by demonstration of the characteristic viral inclusions (Fig. 42.7), highlighted by immunohistochemical or in situ hybridization techniques. Treatment is with ganciclovir.

Fungi

Fungal colonization of gastric ulcers by *Candida* species is relatively common [182] but is of little clinical consequence. Invasive or disseminated fungal infections with *Histoplasma capsulatum* [183], *Cryptococcus neoformans* [184], and Zygomycetes species [185] (among others) involving the stomach have been reported in immunocompromised patients.

Parasites

Although involvement of the stomach by parasitic infestation is rare in Western countries, many cases of invasive anisakiasis have been reported in Japan, where raw marine fish is commonly consumed. Nematode larvae of the genus *Anisakis* may migrate into the gastric wall and cause sporadic epigastric pain. Multiple gastric erosions are a typical finding on endoscopy, and microscopically an eosinophilic granulomatous inflammatory infiltrate with abscess formation and granulation tissue is seen [186]. A small worm may be identified in some cases. Other parasites occasionally found in gastric mucosa, primarily in immunocompromised patients, include *Cryptosporidium* species [187], *Giardia intestinalis* [188], and *Strongyloides stercoralis* [189].

Eosinophilic gastritis

Small numbers of eosinophils are normally found in the gastric lamina propria, and eosinophils are a typical component of the inflammatory infiltrate in a wide range of inflammatory diseases involving the stomach. Eosinophilic gastritis, the gastric component of eosinophilic gastroenteritis, should only be diagnosed when numerous eosinophils disproportionate to the other components of the inflammatory infiltrate are present (Fig. 42.8). Eosinophilic gastroenteritis is considered a polygenic allergic disorder [190]; in the majority of cases, serum immunoglobulin E (IgE) is elevated and peripheral eosinophilia is present. Endoscopic findings are nonspecific and include friability, erythema, erosions, and enlarged gastric folds, particularly in the antrum; the endoscopic appearance may be normal even when the gastric mucosa is heavily infiltrated by eosinophils. Biopsy diagnosis may be difficult, as selective involvement of the deeper layers of the gastric wall may be seen, with minimal or only patchy mucosal collections of eosinophils [191]. Eosinophilic gastritis has also been described in the setting of collagen vascular disease [192] and parasitic infection by the fish parasite *Anisaka simplex* [193]. In noninfectious cases, the disease often responds to corticosteroid therapy [190].

Granulomatous gastritis

Granulomas may be found in the gastric mucosa in a wide variety of infectious and noninfectious inflammatory disorders. Clinical and endoscopic findings generally reflect the underlying disease, but the gastric mucosa may also appear entirely normal on endoscopy; small erosions and ulcers are common [194]. Although in most cases of granulomatous gastritis a specific diagnosis can be made by integrating histological findings with clinical data [195], in a small number of cases, termed idiopathic, no etiology can be identified.

Infectious causes of granulomatous gastritis include acid-fast bacilli (*Mycobacterium tuberculosis*), fungi (*Histoplasma capsulatum*), bacteria (*H. pylori*), and parasites (*Anisaka simplex*). Granulomas associated with *H. pylori* gastritis are generally found in the superficial mucosa in the antrum near injured gastric glands [194]. Foreign bodies such as sutures may also give rise to a granulomatous reaction. Rarely, a granulomatous response to tumor antigens may be seen in nonneoplastic mucosa or perigastric lymph nodes in patients with gastric adenocarcinomas [194,196] or gastric lymphomas [197,198].

In most series, Crohn's disease, followed by sarcoidosis, are the most common disorders associated with granulomatous gastritis [195,199], with over half of all cases identified in retrospective series attributed to upper GI involvement by Crohn's disease. A prevalence of 10%–15% for gastric granulomas (Fig. 42.9) and up to 70% for "focally enhanced gastritis" (characterized by focal periglandular mononuclear inflammatory infiltrates composed of T cells and $CD68^+$ histiocytes) has been reported in these patients [200]. It should be noted that focally enhanced gastritis has also been reported in children and adults with ulcerative colitis [201,202]. In unselected gastric biopsies, this pattern of gastric inflammation is relatively rare, and has a low positive predictive value for inflammatory bowel disease [203]. In many cases, involvement of the stomach by Crohn's disease is an incidental finding; however, severe involvement, with strictures and fistulas refractory to medical management, also occurs [204,205]. Gastrointestinal involvement by sarcoidosis is generally an incidental finding and rarely has clinical importance. In severe cases, scarring and ulcers [206] are seen; these may mimic gastric carcinoma or cause gastric outlet obstruction.

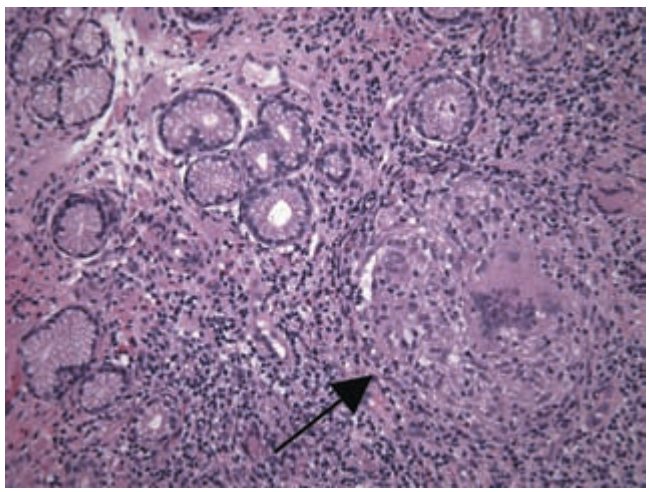

Figure 42.9 Nonnecrotizing granulomas (arrow) composed of epithelioid histiocytes with occasional giant cells are found in up to 15% of gastric biopsies from patients with Crohn's disease.

Autoimmune gastritis

Autoimmune gastritis is a chronic atrophic gastritis restricted to the body of the stomach and associated with circulating anti-parietal cell and anti-intrinsic factor antibodies, with resulting deficiency of intrinsic factor. Only a small subset of patients with autoimmune gastritis will have classic pernicious anemia.

Clinical features and pathogenesis

Patients with autoimmune gastritis may be clinically asymptomatic for many years, until parietal cell mass has decreased to the point that hypochlorhydria or achlorhydria results. Achlorhydric patients have hypergastrinemia, and injury to the chief cells results in reduced pepsin activity. Achlorhydria results in iron deficiency anemia in roughly 15% of patients [207] as a result of lack of absorption of nonheme iron. Patients who present with pernicious anemia may complain of anorexia, weight loss, sore tongue, and neurological disturbances including numbness and paresthesias in the extremities, weakness, and ataxia.

Patients with autoimmune gastritis may have other autoimmune diseases such as Graves disease, idiopathic adrenocortical insufficiency, and vitiligo. It has been hypothesized that *H. pylori* infection may trigger autoimmune gastritis in a genetically susceptible individual as a consequence of molecular mimicry between *H. pylori* and gastric mucosal antigens; this is supported by evidence that up to 80% of patients with *H. pylori* may have autoantibodies with H^+,K^+-ATPase specificity directed against the canalicular membranes of parietal cells, the same target autoantigen as occurs in autoimmune gastritis [208]. However, additional evidence is needed before concluding that *H. pylori* is the causative agent for autoimmune gastritis.

Endoscopic appearance

Progressive thinning of the mucosa in atrophic gastritis results in loss of gastric folds and increased visibility of fine submucosal vessels. The antrum is endoscopically normal in most cases of autoimmune gastritis. Multiple hyperplastic polyps may develop in advanced stages of the disease.

Pathological features

The corpus of the stomach is most severely affected in autoimmune gastritis. Characteristic histological findings include a diffuse, chronic, atrophic gastritis with variable degrees of intestinal metaplasia (Fig. 42.10). Typical features early in the disease are deep or diffuse lymphoplasmacytic infiltrates in the lamina propria with foci of epithelial infiltration and damage, intestinal or pyloric metaplasia, and linear enterochromaffin-like cell hyperplasia [209]. Pyloric metaplasia tends to predominate over intestinal metaplasia in the early

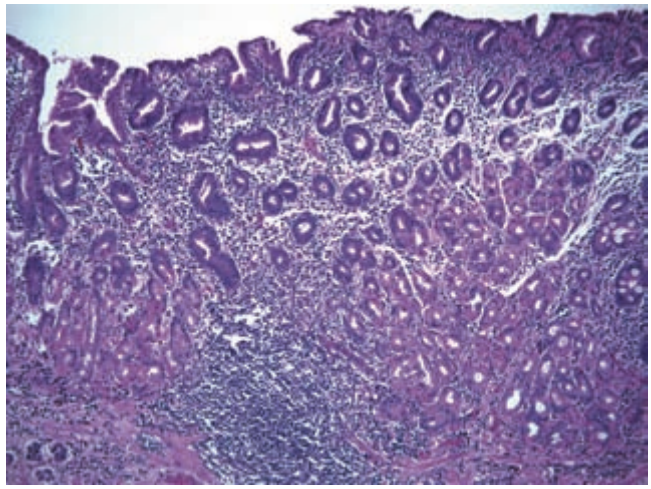

Figure 42.10 In autoimmune gastritis, loss of oxyntic glands results in atrophic gastritis primarily affecting the gastric corpus. A diffuse lymphoplasmacytic infiltrate is common.

stages. In late-stage disease, the oxyntic mucosa is largely destroyed, with replacement of chief and parietal cells by metaplastic cells; at this stage, the inflammatory infiltrate is minimal. Extreme degrees of ECL cell hyperplasia often lead to the formation of multiple microcarcinoids secondary to hypergastrinemia. The antrum may show mild chronic inflammation with intestinal metaplasia, and hyperplasia of gastric cells may be seen.

Management

The mucosal changes in autoimmune gastritis are irreversible. Although patients with multiple hyperplastic polyps and extensive metaplasia may be at increased risk for gastric adenocarcinoma, there are no standards for frequency of endoscopic surveillance. Most abnormalities resulting from cobalamin deficiency are reversible with replacement therapy, except for the neurological manifestations.

Lymphocytic gastritis

Lymphocytic gastritis is characterized by large numbers of mature lymphocytes infiltrating the surface and foveolar epithelium (i.e., intraepithelial lymphocytes in contrast to lamina propria lymphocytes). It occurs most commonly in patients with celiac disease, with up to 45% of celiac disease patients [210] showing evidence of lymphocytic gastritis on biopsy. Approximately 4% of patients with *H. pylori* gastritis [211] also have increased numbers of lymphocytes in surface epithelium, but this feature may be obscured by the presence of active inflammation. The pathogenesis of lymphocytic gastritis is unknown, but given its association with celiac disease it likely represents a response to persistent antigens.

Clinical and endoscopic features

Patients with lymphocytic gastritis may be asymptomatic, and the condition is discovered by random biopsy of endoscopically normal mucosa. In more severe disease, there may be scattered erosions and enlarged mucosal folds resembling Ménétrier disease and accompanied by anemia and hypoalbuminemia (hypertrophic lymphocytic gastritis).

Pathological features

The minimal number of lymphocytes considered diagnostic for lymphocytic gastritis is 25 per 100 epithelial cells [211]; counts of up to 65 lymphocytes per 100 epithelial cells are not uncommon. Virtually all of the lymphocytes are of T-cell origin. Lymphocytes are distributed singly (Fig. 42.11), but rarely small clusters resembling lymphoepithelial lesions may be seen. The antrum is more severely involved in patients with celiac disease compared with patients with *H. pylori* gastritis [211], in which the lymphocytic gastritis pattern is more likely to be corpus-predominant and to show concomitant infiltration of the mucosa by neutrophils. The intraepithelial lymphocytes are more numerous in hypertrophic forms of lymphocytic gastritis.

Therapy

Treatment of lymphocytic gastritis is aimed at treatment of the underlying *H. pylori* gastritis or celiac disease; for patients with lymphocytic gastritis not associated with these diseases, therapy has largely consisted of proton pump inhibitors [212].

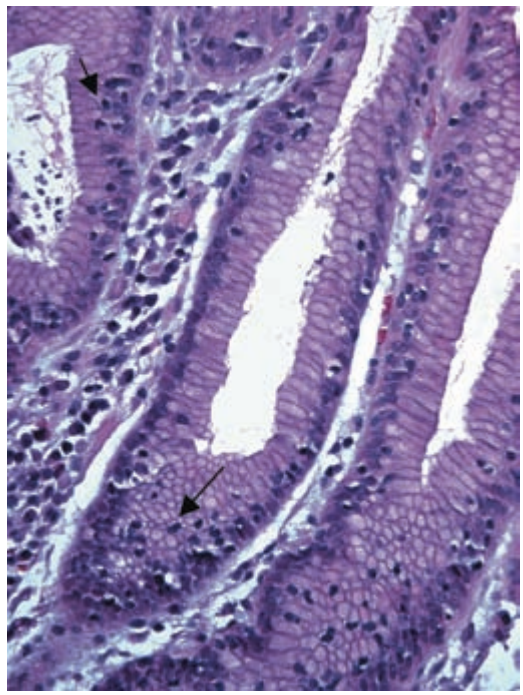

Figure 42.11 Numerous intraepithelial lymphocytes (arrows) are found in the mucosa in lymphocytic gastritis.

Collagenous gastritis

Collagenous gastritis is a rare but histologically distinctive disorder characterized by thickening of the subepithelial collagen layer in the gastric mucosa. It has been reported in association with collagenous colitis in older adults [213] and celiac disease [214]. In children and young adults, collagenous gastritis may present with disease limited to the gastric mucosa and showing a nodular pattern on endoscopy [213]. On biopsy, in addition to the markedly thickened and irregular subepithelial collagen band, the mucosa contains a variable chronic inflammatory infiltrate, and the surface epithelium shows degenerative changes and infiltration by lymphocytes and eosinophils.

Chemical (reactive) gastropathy (NSAID and bile reflux)

Chemical gastropathy (also variously referred to as reflux gastritis or gastropathy, chemical gastritis, or reactive gastritis) is a distinctive constellation of endoscopic and microscopic findings first described in the setting of bile reflux in stomachs subjected to gastric surgery, most commonly Bilroth II gastrojejunostomy. Subsequently it has been recognized that bile reflux in the intact stomach may lead to chemical gastropathy. Most cases, however, are associated with use of nonsteroidal antiinflammatory drugs (NSAIDs), with an estimated prevalence of chemical gastropathy of up to 45% in this group [215].

Clinical manifestations and pathogenesis

Chemical gastropathy may be seen in three clinical settings: postgastrectomy alkaline reflux; bile reflux secondary to gastroduodenal dysmotility; and aspirin or NSAID ingestion. Patients with bile reflux complain of burning midepigastric pain unresponsive to antacids; bilious vomiting may occur. In persons ingesting aspirin or NSAIDs on a regular basis, epigastric pain is common, but no relationship has been established between the mucosal appearance and the presence or severity of symptoms. Unless complicating erosions or ulcers are present, chemical gastropathy may be an asymptomatic lesion.

Disruption of the mucus barrier of the antrum and direct damage to surface epithelium, either from reflux of alkaline duodenal contents or reduced prostaglandin synthesis secondary to NSAID use, is a major factor in the pathogenesis of chemical gastropathy. Cyclooxygenase- and prostaglandin-independent mechanisms may also be involved in NSAID-induced gastric injury, perhaps through systemic release of TNF-α [216]. The gastric injury induced by NSAIDS is exacerbated by acid, and use of potent acid suppression therapy is a common approach to reducing NSAID-related complications.

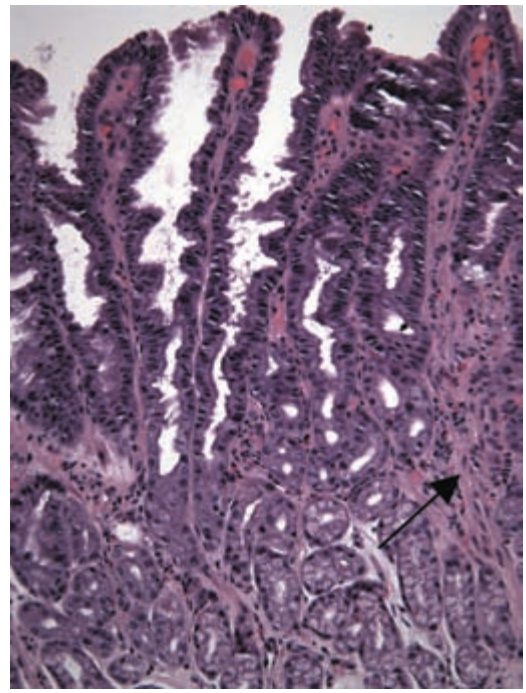

Figure 42.12 Chemical gastropathy is characterized by reactive epithelial changes out of proportion to the sparse inflammatory infiltrate, foveolar hyperplasia with corkscrew appearance to the superficial mucosa, and increased lamina propria smooth muscle fibers (arrow).

Endoscopic appearance

In patients with nonoperated stomachs, the mucosa in chemical gastropathy may show edema, vascular congestion and erythema, and erosions. In the operated stomach, the mucosa shows similar features, often primarily involving the anastomotic site. Superficial erosions may be seen in this setting but are not specific for chemical gastropathy.

Histological features

The characteristic histopathological features of chemical gastropathy are best seen in the antrum and include foveolar hyperplasia imparting a corkscrew appearance to the superficial mucosa, reactive epithelial changes (nuclear enlargement and loss of cytoplasmic mucin), increased lamina propria smooth muscle fibers, and edema of the lamina propria (Fig. 42.12). Acute and chronic inflammatory cells are typically sparse.

Hemorrhagic gastropathy

Hemorrhagic gastropathies are a multifactorial group of conditions characterized by mucosal hemorrhages and erosions. The major etiological factors are NSAID use, major physical stress, and ingestion of large quantities of alcohol. Hemorrhagic gastropathy has also been reported in the setting of crack cocaine use [217]. The most severe examples of hemorrhagic gastropathy are *stress ulcers*, originally described by Dr Thomas

Curling in 1842 [218] and therefore sometimes referred to as Curling ulcers, seen in patients who suffer major physical or thermal trauma, shock, sepsis, or head injury. Respiratory failure requiring mechanical ventilation for more than 48 hours and coagulopathy are independent risk factors for the development of bleeding from hemorrhagic gastropathy [219]. The pathogenesis of hemorrhagic gastropathy is related to disruption of the mucosal protective mechanisms secondary to compromise of gastric mucosal microcirculation in some cases; gastric acid secretion likely contributes to the injury [219], and *H. pylori* infection has been implicated as a contributing factor in some cases [220].

In hemorrhagic gastropathy, the mucosal lesions are multiple, superficial, and nontransmural. Microscopically, the superficial mucosa is eroded and edematous; a neutrophilic inflammatory response may be seen, but mononuclear inflammation is not present unless there is concomitant chronic mucosal injury, as in *H. pylori* infection. Hemorrhagic gastropathy usually produces no symptoms and blood loss is minor. However, because of the increased mortality associated with gastrointestinal bleeding in critically ill patients, prophylactic treatment with proton pump inhibitors is advocated by some investigators [219].

Vascular gastropathy

Vascular gastropathies are the result of alterations in gastric circulation; the most common are gastric antral vascular ectasia ("watermelon stomach"), and portal hypertensive gastropathy (see Chapters 111 and 123). Congestive heart failure may also cause a congestive gastropathy [221] that endoscopically resembles portal hypertensive gastropathy. Frank ischemic necrosis of gastric mucosa is rarely seen, but occasionally occurs in patients with severe atherosclerotic disease.

Portal hypertensive gastropathy

Clinical manifestations and pathogenesis

Portal hypertensive gastropathy (PHG) produces characteristic mucosal changes recognizable at endoscopy, and is a common finding in patients with portal hypertension, both with and without underlying liver disease. Although the development of PHG correlates with duration and severity of liver disease [222] and presence and size of gastroesophageal varices, the severity and presence of portal hypertensive gastropathy does not correlate in a linear fashion with severity of portal hypertension, and measurements of gastric blood flow to the stomach have shown variable results [223]. Overall, the incidence of acute bleeding is low, and blood loss is usually mild and rarely life threatening [222].

Endoscopic appearance

Portal hypertensive gastropathy is more prominent in the body and fundus of the stomach and is rare in the gastric antrum. Characteristic patterns on endoscopy are a mosaic or snakeskin pattern, scarlatina rash, and cherry-red spots or black-brown spots (or both). Endoscopic grading schemes have been proposed [224], with more severe scores correlating with increased risk for bleeding; in general, prominent mosaic pattern and confluent red marks are considered indicative of more severe changes.

Histopathology

Small veins in the mucosa and submucosa show dilation, tortuosity, and thickened walls [225] (Fig. 42.13). The mucosa is otherwise generally normal in appearance, with neither erosions, nor significant inflammation, nor thrombi. Correlation of histological findings with the endoscopic impression of PHG is poor, because the vascular changes are more pronounced in submucosal vessels, and small mucosal biopsies may not show diagnostic changes.

Management

Because clinically significant bleeding is rare, most patients require no specific treatment for PHG. Nonselective β-blockers, such as propranolol or nadalol, may be used to lower portal pressure and gastric mucosal blood flow. Endoscopic intervention is of limited value because of the mild and diffuse nature of the bleeding in most cases. Transjugular intrahepatic portosystemic shunt (TIPS) and shunt surgery have been used to treat PHG hemorrhage in refractory cases, but are generally considered a last resort [223].

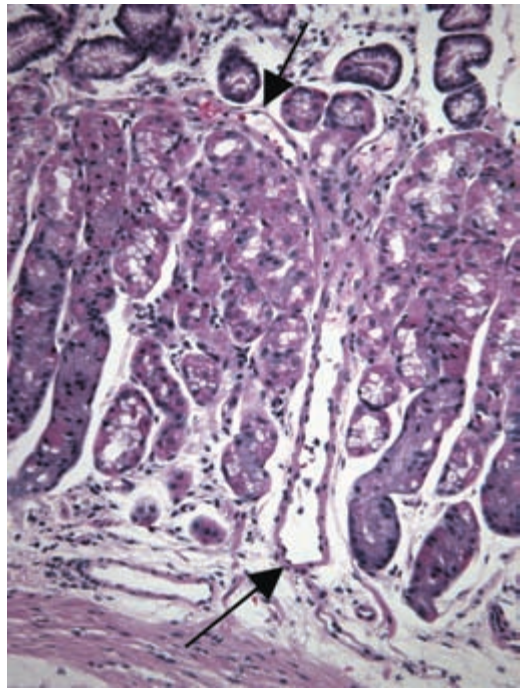

Figure 42.13 In portal hypertensive gastropathy, dilated small veins are found in the submucosa and lamina propria (arrows).

Hypertrophic gastropathy

Hypertrophic gastropathy is characterized by diffuse macroscopic enlargement of the gastric folds, primarily in the body of the stomach. These markedly enlarged folds follow the normal rugal pattern of the stomach, and may resemble cerebral convolutions at endoscopy. Three main types are recognized, according to which mucosal compartment is hyperplastic:

- foveolar hyperplasia (Ménétrier disease), usually accompanied by atrophy of oxyntic glands
- hyperplasia of oxyntic glands, without foveolar hyperplasia (Zollinger–Ellison syndrome; see Chapter 41)
- mixed foveolar/oxyntic hyperplasia, seen in a diverse group of conditions (see above), including *H. pylori* infection, cytomegalovirus gastritis in children, lymphocytic gastritis, sarcoidosis, and Cronkhite–Canada syndrome (see Chapter 55).

Ménétrier disease

Whereas enlarged gastric folds may be associated with a wide variety of inflammatory conditions, classic Ménétrier disease is rare, affecting primarily men in their 50s and 60s. Patients may present with weight loss, abdominal pain, nausea, and vomiting. Histologically, the enlarged folds in Ménétrier disease show massive expansion of the foveolar compartment, with normal or reduced numbers of chief and parietal cells (Fig. 42.14). The foveolae are tortuous and dilated, and cystic glands may be seen. Inflammation is not typically a feature, although the enlarged folds are subject to erosion, and may thus show secondary inflammatory changes in the superficial mucosa. In well-developed Ménétrier disease, severe protein loss from the abnormal gastric mucosa leads to hypoalbuminemia; low acid secretion is the result of loss of parietal cell mass.

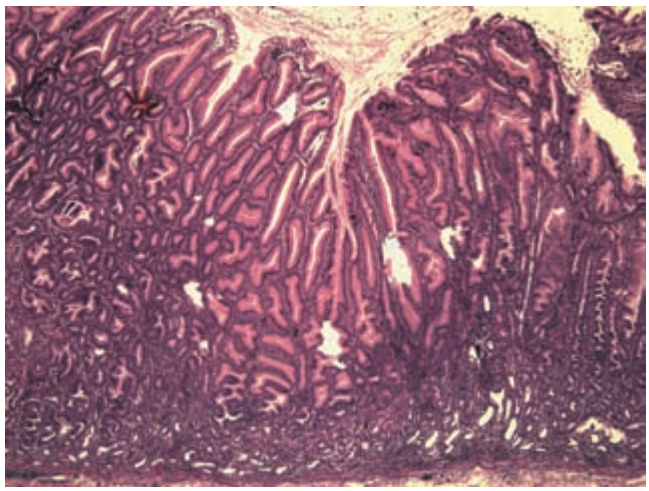

Figure 42.14 The enlarged folds in Ménétrier disease are composed of massively expanded foveolar epithelium, leading to protein loss and hypoalbuminemia.

Transient forms of hypertrophic gastropathy resembling Ménétrier disease are seen in children and are almost always associated with cytomegalovirus gastritis [226] although cow's milk protein allergy [227] and other inflammatory disorders have also been implicated. Cytomegalovirus-associated Ménétrier disease has also been reported in adults [228]. The pathogenesis of the classic form of the disorder is unknown, although overexpression of transforming growth factor-α (TGF-α), which stimulates epithelial cell proliferation and inhibits gastric acid secretion, among other functions, has been implicated [229]. Medical treatments for Ménétrier disease include antacids, corticosteroids, and proton pump inhibitors, but they have been inconsistently effective; treatment with a monoclonal antibody directed against the epidermal growth factor receptor has proven effective in a few patients treated on an experimental basis [229].

Gastritis of the cardia

The cardia is an ill-defined anatomical zone of the stomach extending 10–20 mm distal to the lower end of the esophagus. Given the rising incidence of adenocarcinoma of the proximal stomach and gastroesophageal junction, pathological changes in this region have generated intense interest. Histologically, cardiac mucosa resembles antral mucosa in that it lacks oxyntic glands. Inflammation of the cardia ("carditis") may be seen in the setting of pangastritis due to *H. pylori* infection [230]; its intensity parallels that of antral inflammation [231]. Similarly, the presence of intestinal metaplasia in the cardia is associated with multifocal atrophic gastritis in the remainder of the stomach [232]. Carditis is also reported in *H. pylori*-negative patients with reflux esophagitis and without significant inflammation elsewhere in the stomach [233], with an estimated prevalence of 23% [230]. Intestinal metaplasia of the cardia is present in roughly 3% of patients in this group [234], raising the issue of whether this finding represents short-segment Barrett esophagus. Although a distinction of gastric cardiac intestinal metaplasia vs Barrett esophagus has been proposed based on differential expression of cytokeratins 7 and 20 [235], others have reported inconsistent results [236,237]; hence no histological markers differentiate between these entities in a reliable and reproducible fashion.

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B STOMACH

43 Tumors of the stomach

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Gastric adenocarcinoma, 1026 Other gastric tumors, 1044

Gastric cancer is the second most common cause of cancer-related mortality worldwide [1]. Although relatively uncommon in the United States, gastric cancer is the commonest cancer in many Asian countries. With the rapid changes in economy and improvement in sanitary conditions, the incidence of gastric cancer is declining globally.

Because the majority of gastric tumors are adenocarcinomas, this chapter will focus on this type of cancer. Other less common gastric malignancies, such as lymphoma, mesenchymal tumors, and endocrine tumors, will be discussed briefly.

Gastric adenocarcinoma

Epidemiology

Gastric carcinoma is the second commonest malignancy, causing over 700 000 deaths per year in the world (Fig. 43.1) [1]. In 2002 it was estimated that in the United States 21 860 new cases of gastric cancer were diagnosed and 11 550 patients died from this disease [2]. In most countries, the incidence of gastric cancer has been declining over the past few decades (Fig. 43.2). Nonetheless, due to the rapidly growing populations in developing countries with high background incidence rates for gastric cancer, the total number of gastric cancer cases in the world has actually remained static [1]. Murray and Lopez projected that the annual number of new cases will continue to rise in the developing world over the next few decades as a result of population growth [3].

The overall reduction in gastric cancer incidence largely reflects a decline in incidence of cancers of the distal stomach, mostly of the intestinal type. However, since the mid-1970s there has been a steady rise in the incidence of adenocarcinoma of the proximal stomach and the gastroesophageal junction [4,5]. According to the US National Cancer Institute Surveillance, Epidemiology, and End Results (SEER) database, the annual increase in proximal gastric lesions is estimated to be 3.6%–5.6% [4]. The rise in proximal gastric cancer is alarming because on a percentage basis the increase is greater than that of lung cancer or melanoma, and proximal gastric cancer is associated with a worse prognosis than distal gastric cancer. This increase is paralleled by a rising hospitalization rate for gastroesophageal reflux disease, as noted in the US Department of Veterans Affairs database [6].

There are marked geographical variations in the incidence of gastric cancer (Table 43.1). The highest rates are observed in east Asia, where gastric cancer is still the most frequent cancer in men. The age-adjusted standardized rates in Japan and China are 62 and 41 per 100 000, respectively [1]. The corresponding rates in North America and Western Europe are 7.4 and 12.8 per 100 000, respectively. Apart from geographical variations, variations in cancer incidence and mortality can also be observed between different ethnic groups. In the United States, African Americans, Hispanic Americans and Native Americans are 1.5–2.5 times more likely to have gastric carcinoma than whites [7]. Similarly, the incidence of gastric cancer in Singapore Chinese is about twice that of Indians and five times greater than that of Malays [8]. The reason for this discrepancy is unclear, but factors including differences in socioeconomic status, and dietary and environmental conditions have been suggested. In Japan and China, there appears to be a dichotomized distribution of gastric cancers in which both incidence and mortality are higher in the northern parts than in the southern parts of the countries [9,10].

Gastric cancer has a predilection for elderly patients, with incidence peaks in the seventh decade. Men are more commonly affected than women, with male incidence rates double those for females. Besides gender differences, the incidence of gastric cancer also appears to be inversely related to social class. Rates are higher in unskilled laborers (class V) and lower in professional groups (class I) [11].

Etiology

With the global contrasts in the incidence of gastric carcinoma, tremendous effort has been made to identify the cause

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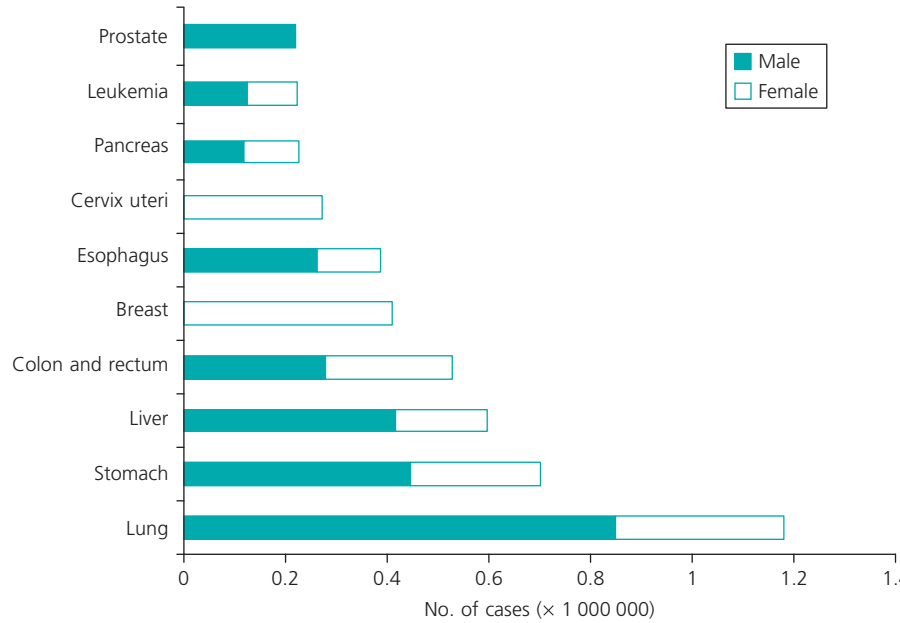


Figure 43.1 Top 10 leading cancers in the world.

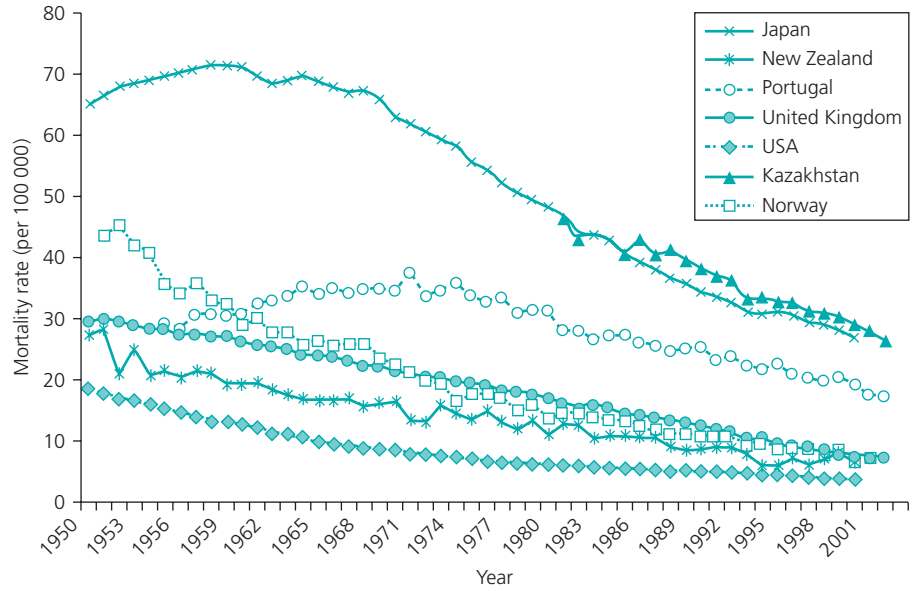


Figure 43.2 Mortality from gastric cancer in different countries (1950–2004).

for this malignancy. It is generally believed that gastric cancer has multiple etiological factors including diet, exogenous chemicals, intragastric synthesis of carcinogens, infectious agents, and the genetic make-up of the host (Table 43.2). Among various factors, *Helicobacter pylori* infection is the one most frequently examined.

Helicobacter pylori

Helicobacter pylori is a Gram-negative spiral-shaped bacterium found in the mucus layer of the human stomach. It was first successfully cultured by Marshall and Warren in 1984 [12]. This discovery dramatically revolutionized our understanding of gastroduodenal diseases, and earned Marshall and Warren the 2005 Nobel Prize in Medicine or Physiology.

Based on several large-scale epidemiological cohort studies that linked *H. pylori* to gastric carcinoma [13–16], the International Agency for Research on Cancer (IARC) classified *H. pylori* as a group 1 carcinogen in 1994 [17]. Data from 19 case–control studies have been summarized in a metaanalysis and show that the combined odds ratio (OR) for gastric cancer in *H. pylori*-infected subjects is 1.92 [95% confidence interval (CI), 1.32–2.78] [18]. Moreover, *H. pylori* infection is linked to both intestinal and diffuse-type cancers. The association between gastric cancer and *H. pylori* is found to be

Table 43.1 Age-standardized rate of gastric cancer incidence and mortality in the world (per 100 000 population)

	Male		Female	
	Incidence	**Mortality**	**Incidence**	**Mortality**
World	22	16.3	10.4	7.9
More developed regions	22.3	14.5	10	6.9
Less developed regions	21.5	17	10.4	8.3
North America	7.4	4.2	3.4	2.2
United States	7.2	4	3.3	2.2
Canada	9.1	5.9	4	2.8
South America	24.3	18.1	12.2	9.3
Chile	46.1	32.5	17.7	13.2
Colombia	36	27.8	20.3	15.7
Central America				
Mexico	13.1	9.9	9.5	7.2
Northern Europe	12.4	9.2	6	4.6
Finland	9.6	7.9	6	4.5
Sweden	8	6.8	4.3	3.8
United Kingdom	12.5	8.7	5.5	4
Western Europe	12.8	8.9	6.6	5
France	10.4	7	4.1	3.1
Germany	15.1	10.4	8.8	6.4
Central and Eastern Europe	29.6	25.2	12.8	10.8
Hungary	20.5	18.2	9.5	8.5
Poland	20.7	16.6	7.8	6.2
Southern Europe	18	12.9	8.7	6.3
Italy	18.8	12.6	9.7	6.5
Portugal	27.6	20.3	13.6	10.1
Spain	15.7	11.4	7.2	5.4
Eastern Asia	46.1	32.5	20.6	14.8
China	41.4	32.7	19.2	15.1
Japan	62	28.7	26.1	12.7
Korea	69.7	37.1	26.8	15

Data from Ferlay J, et al. [1].

Table 43.2 Possible etiological factors for gastric cancer

Diet	Consumption of salted, smoked, and poorly preserved foods Low consumption of fruits and vegetables
Infection	*Helicobacter pylori* Epstein–Barr virus
Genetic	Hereditary nonpolyposis colon cancer syndrome Hereditary diffuse gastric cancer Polymorphism in proinflammatory cytokines
Previous gastric resection	
Pernicious anemia	

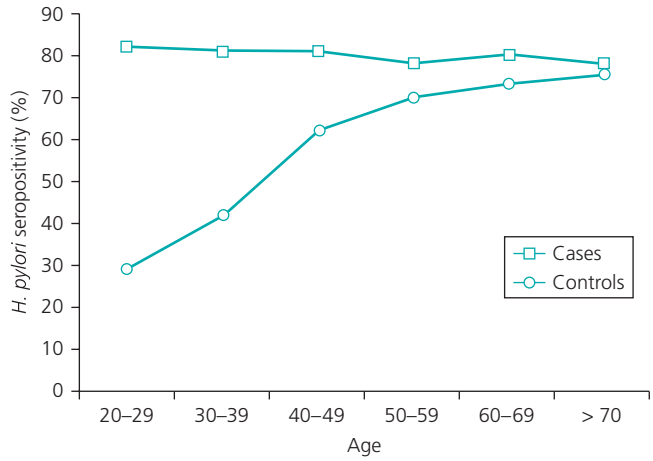


Figure 43.3 Age distribution of *Helicobacter pylori* seropositivity in gastric cancer cases and controls. Data from Huang et al. [18].

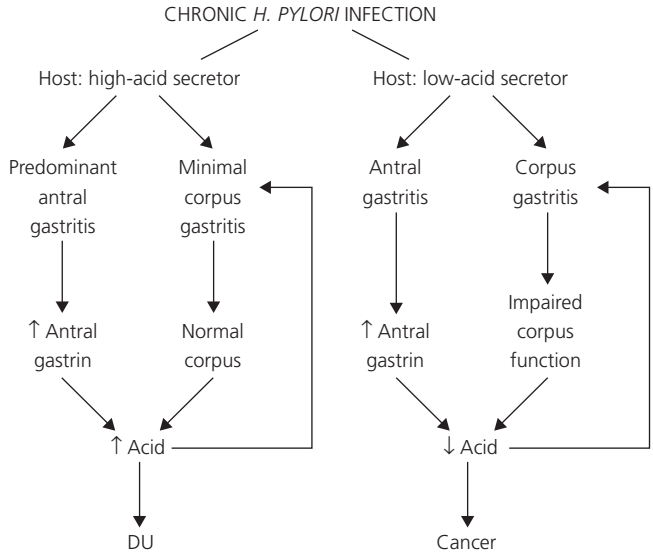


Figure 43.4 Divergent host response to *Helicobacter pylori* infection. Based on data from McColl KE, et al. *Helicobacter pylori* gastritis and gastric acid secretory function – an integrated approach. In: Hunt RH, Tytgat GN (eds). Basic mechanisms to clinical cure. Kluwer Academic Publishers, Dordrecht, the Netherlands, 1996. Copyright © 1996, Axcan Pharma Inc.

restricted to noncardiac cancers, and is stronger when blood samples for *H. pylori* serology are collected more than 10 years before cancer diagnosis (OR, 5.9). Because elderly patients with severe atrophic gastritis may have spontaneous remission of infection, the association between *H. pylori* and gastric cancer is particularly strong in young infected subjects. The OR increased from 1.05 at age ≥ 70 years to 9.29 at age ≤ 29 years (Fig. 43.3).

There are several genotypic variations among different *H. pylori* strains. For example, strains that express the cytotoxin-associated gene A (*cagA*) and secrete high amounts of the vacuolating cytotoxin (VacA) are more frequently found in patients with peptic ulcer and gastric carcinoma [19,20]. Possession of the *cagA* and *vacA* s1 genotype is therefore sometimes termed the type I strain. Moreover, strains that carry the *babA2*$^{+}$ gene are considered to be potentially more virulent [21].

In addition to epidemiological study, the causative role of *H. pylori* infection in gastric carcinogenesis was confirmed by experimental animal studies. In the absence of exogenous mutagens, Japanese investigators demonstrated that Mongolian gerbils infected with *H. pylori* developed severe active chronic gastritis, ulcers, and intestinal metaplasia within 6 months of infection [22]. Adenocarcinoma was detected in the stomach of one-third of the gerbils after 1 year of infection.

Although *H. pylori* infection is associated with noncardiac gastric cancers, there are data to suggest an inverse association between *H. pylori* infection and proximal gastric carcinoma [23]. It is believed that *H. pylori* infection leads to suppressed acid output, which paradoxically protects infected individuals from gastroesophageal reflux and hence Barrett esophagus, the precursor of adenocarcinoma of distal esophagus and proximal stomach. [24,25].

It is important to note that not all *H. pylori*-infected individuals will develop gastric cancer in their lifetime. In a large Swedish cohort study, the risk of gastric cancer among patients with duodenal ulcer was in fact significantly reduced (OR, 0.6; 95% CI, 0.4–0.7) [26]. Thus, *H. pylori* infection appears to produce two distinct phenotypes, namely duodenal ulcer or gastric ulcer/cancer [27,28]. The duodenal ulcer phenotype is characterized by the antral-predominant nonatrophic type of gastritis whereas gastric cancer patients tend to have multifocal or extensive corpus atrophic gastritis (Fig. 43.4). This hypothesis was confirmed by a prospective study in Japan showing that subjects with pangastritis and corpus-predominant gastritis have, respectively, a 16- and 35-fold increase in risk of gastric cancer compared with those with antral-predominant gastritis [29].

Interestingly, the incidence of gastric cancer is unexpectedly low in African countries despite the high prevalence of *H. pylori* infection [30]. It was found that concurrent helminth and *H. pylori* infection converts the T-helper cell type 1 (Th1) responses, which normally promote cell-mediated immune responses and tissue injury, to a less damaging Th2 response [31]. Thus, the progression of *Helicobacter*-associated gastric atrophy, a premalignant lesion, is attenuated. This finding illustrates the potential interaction between *H. pylori* infection and environmental factors that may partly explain the "African enigma."

Epstein–Barr virus

The virus being most extensively studied as an etiological factor for gastric cancer is Epstein–Barr virus (EBV). This is an icosahedral herpevirus containing linear double-stranded DNA, and is ubiquitous in all human populations. It has been

estimated that about 10% of gastric carcinomas worldwide show monoclonal proliferation of EBV-infected cancer cells [32]. In contrast to Burkitt lymphoma and nasopharyngeal carcinoma, which are endemic in Africa and southeast Asia, EBV-positive gastric carcinoma is a nonendemic disease.

Although EBV has been detected in all types of gastric cancer, it is closely linked to a form of undifferentiated gastric carcinoma called the lymphoepithelioma-like carcinoma. Histologically, these tumors resemble nasopharyngeal carcinomas. The presence of lymphoid stroma, proximal location, and predominance in males are characteristics of EBV-positive gastric carcinomas [33]. They also tend to be of diffuse histological types and have a lower frequency of lymph node metastasis [34]. The prevalence of *H. pylori* infection with this tumor is also lower than with non-lymphoepithelioma-like carcinomas [35].

Dietary factors

Dietary factors have long been suspected as an important cause of gastric cancer. However, most studies were based on retrospective data collection, which are readily subject to recall bias. In spite of these caveats, many case–control studies have shown a consistent inverse association between gastric cancer and consumption of fresh fruits or vegetables [36–40]. This observation may be partly related to the presence of vitamins such as vitamins C and E, carotenoids, and flavonoids in fruits and vegetables. These vitamins may act through various mechanisms, including antioxidant effects, inhibition of nitrosamine formation [41], and dilution or binding of carcinogens. An epidemiological study from China demonstrated that the risk of progression to gastric dysplasia and carcinoma was reduced by 80% (95% CI, 30%–90%) in subjects with high baseline ascorbic acid levels [42].

By contrast, food substances that are rich in nitrates, nitrites, and secondary amines, which combine to form *N*-nitroso compounds (NOC), are known mutagens or carcinogens. Nitrates and nitrites were used in food preservation before the era of refrigeration. Consumption of preserved meat and vegetables is consistently linked to the development of gastric cancer [37,43]. Humans are exposed not only to preformed NOCs, but also to a wide range of precursors and nitrosating agents, which can react in vivo to form potentially carcinogenic NOC. This process is facilitated in the presence of nitrate-reducing bacteria of the digestive tract. With the advent of refrigeration and the growth of the frozen food industry, consumption of preserved meats and vegetables has declined in most developed countries. This has been postulated to be a crucial factor for the reduction of gastric cancer incidence in North America and Western Europe [44].

A high-salt diet in humans and experimental animals is associated with an elevated risk of atrophic gastritis, a preneoplastic lesion [45,46]. Salt also enhances the colonization of *H. pylori* in mice and may therefore perpetuate the development of gastritis and glandular atrophy [47]. In Japan, a significant correlation was found between the amount of salt excreted in urine and stomach cancer mortality [48]. A study from 65 Chinese counties also demonstrated a significant positive association between stomach cancer mortality and consumption of salted vegetables [49]. In addition, consumption of a carbohydrate-rich diet was found to have a positive association with gastric cancer. A study from Shanghai showed a 1.5- to 1.9-fold increase in the risk of gastric cancer for the highest quartile of carbohydrate intake [40]. However, this association was not confirmed in other studies [50] and the possible role of carbohydrate in gastric carcinogenesis remains poorly defined.

The polyphenols in green tea have been postulated to have anticarcinogenic effects. Several case–control studies suggested an inverse association between consumption of green tea and the risk of gastric cancer [51]. However, the anticarcinogenic effect of green tea is refuted by a large-scale population-based prospective cohort study in Japan, which involved more than 26 000 residents [52].

Apart from case–control studies of dietary ingredients, migrant studies also provide invaluable information on the role of diet in gastric carcinogenesis. Migration is accompanied by change in lifestyle, especially diets. It is found that Japanese people who migrate to the United States have a significant reduction in mortality from stomach cancer when compared with native Japanese [53]. This observation implicates environmental factors in the pathogenesis of gastric cancer, with diet being the most likely cause.

Tobacco and alcohol

Although several studies have shown a 1.5- to 3.0-fold increase in the risk of gastric cancer among smokers, a clear–dose response relation cannot be demonstrated [54]. Moreover, smoking may be more related to cancer in the cardia than in the distal stomach. Given the rapid rise in the incidence of lung cancer and the progressive fall in stomach cancer incidence worldwide (Fig. 43.2), tobacco use is unlikely to be an important etiological factor for gastric cancer. Moreover, epidemiological studies have failed to show any significant correlation between alcohol use and stomach cancer [38,39].

Previous gastric resection

Many studies have suggested an increased incidence of gastric cancer in the stump remaining after gastrectomy. An annual incidence of 3% has been reported [55]. Rates of gastric cancer appear to remain low for 15–20 years after resection of the distal stomach and increase substantially thereafter [56,57]. Notably, Billroth II gastrectomy was associated with higher risk than Billroth I gastrectomy. The increase in cancer risk may be explained by changes in the gastric environment after acid-reducing surgery. As gastric surgery reduces acid production in the stomach and causes neutralization of the gastric milieu, the luminal bacterial content increases. Of particular interest is the presence of anaerobic bacteria in the

gastric remnant; these could convert dietary nitrates into nitrites, a potential gastric carcinogen. In addition, bile reflux after gastric surgery promotes gastric epithelial proliferation in the stomach remnant and possibly favors cancer development [58].

Genetic predisposition

Familial clustering of gastric cancer suggested a genetic basic for this malignancy. Case–control studies showed that first-degree relative of patients with gastric cancer have a two- to three-fold increase in the risk of gastric cancer [59]. However, true hereditary gastric cancer is rare except in patients suffering from germ-line mutation in the E-cadherin (*CDH1*) gene. This mutation was first identified in a large family from New Zealand with early-onset diffuse-type gastric cancer [60]. Screening for the *CDH1* mutation is recommended to patients with hereditary diffuse gastric cancer (HDGC) or even isolated cases of early-onset diffuse gastric cancer (< 35 years) [61]. It has been suggested that prophylactic gastrectomy may be indicated in young asymptomatic carriers of germ-line *CDH1* mutations because superficial infiltrates of malignant signet-ring cells are frequently found in these subjects [62]. Hereditary gastric cancers are also uncommonly seen in kindreds of hereditary nonpolyposis colorectal cancer (HNPCC) syndrome, familial adenomatous polyposis and Peutz–Jeghers syndrome.

However, familial clustering of gastric cancers may be explained by common exposure to various environmental factors, particularly diet and *H. pylori* infection [63,64] among family members. It has also been shown that gastric atrophy and intestinal metaplasia are more common in relatives of gastric cancer patients than in control populations [65,66].

The increased prevalence of gastric atrophy among family members may be related to a polymorphism in the interleukin-1β (IL-1β) gene [67]. Although IL-1β is a proinflammatory cytokine and a potent inhibitor of gastric acid secretion, inheritance of the proinflammatory polymorphism trait is associated with increased risk of gastric cancer and of gastric atrophy in different populations [67,68]. Moreover, polymorphisms in a number of other inflammatory genes, for example IL-10 and tumor necrosis factor-α (TNF-α), are found to increase the risk of gastric adenocarcinoma [69]. The combination of *H. pylori* virulent factors and proinflammatory gene polymorphism may further increase the risk of development of cancer and premalignant gastric lesions [70].

The cascade of gastric carcinogenesis

Based on extensive observational studies in populations with high incidences of the disease, gastric cancer is generally believed to be a multistep progression from chronic gastritis to gastric atrophy, intestinal metaplasia, dysplasia, and cancer [71]. This is dubbed Correa's model of gastric carcinogenesis, which is usually triggered by *H. pylori* infection [72] (Fig. 43.5).

In this context, gastric atrophy and intestinal metaplasia are regarded as preneoplastic gastric lesions, particularly for intestinal-type cancer. Gastric atrophy usually predates the development of intestinal metaplasia and can be detected in 80%–90% of patients with gastric cancer. It has been estimated that 10% of individuals with chronic atrophic gastritis will develop gastric carcinoma in a 15-year follow-up period [73]. Apart from endoscopic methods, evaluation of serum pepsinogen I/II ratios may also help to identify individuals with gastric atrophy. In a prospective study from Japan, it was found that individuals with atrophic gastritis with elevated serum pepsinogen and negative serology for *H. pylori* had the greatest risk of gastric cancer development (hazard ratio, 8.2; 95% CI, 3.2–21.5) [74]. In addition, patients with type A or autoimmune atrophic gastritis, which typically

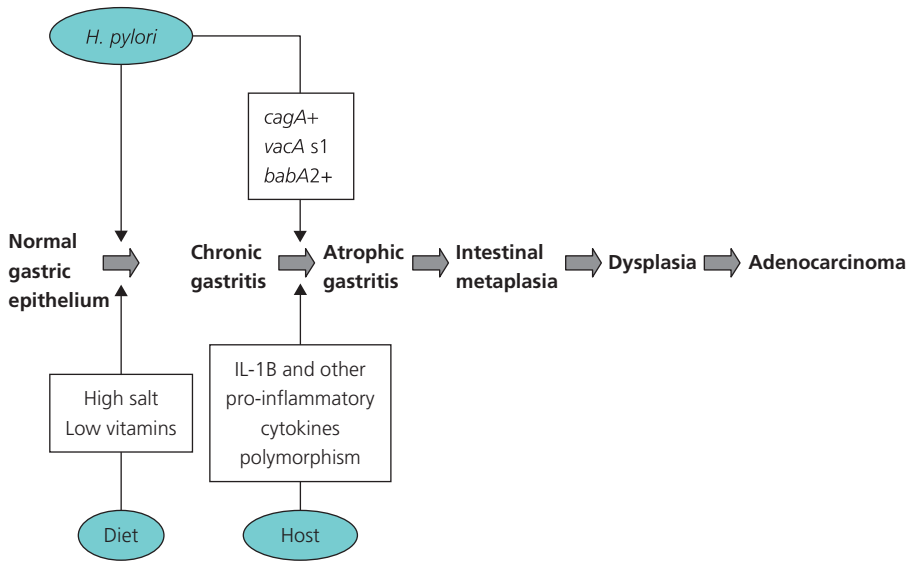


Figure 43.5 Multistep gastric carcinogenesis: the role of *Helicobacter pylori* genotypes, host proinflammatory cytokine polymorphism, and diet on the gastric carcinogenesis model.

affects the corpus, have a two- to three-fold increased risk of stomach cancer [75].

Intestinal metaplasia (IM) is recognized by the presence of goblet cells, absorptive cells, and cells resembling colonocytes or by the mucin content of the colonocyte-resembling cells. Intestinal metaplasia can be further divided into small intestinal and colonic types, or complete and incomplete types, on the basis of morphology and enzyme histochemistry [76]. Type I (complete) refers to the presence of goblet cells containing sialomucins interspersed between nonsecretory absorptive cells with well-delineated brush borders. Type II (incomplete) indicates the presence of sialomucin-containing goblet cells scattered among gastric-type cells containing either neutral mucin or sialomucins. Type III (incomplete or colonic type) is diagnosed when there are tortuous and branched crypts lined by tall columnar cells containing abundant sulfomucins. Sulfomucins can be differentiated from sialomucins by using high-iron diamine (HID)–alcian blue staining (Fig. 43.6). Complete, or type I, IM is generally believed to carry the lowest risk of gastric cancer, whereas type III (colonic) has the strongest association [77]. Although the real clinical significance of IM typing remains controversial, there is unequivocal evidence that individuals harboring IM have an increased cancer risk. In a cohort study from Shandong Province, China, involving more than 3000 subjects with a follow-up of 4–5 years, the odds ratio of progression from intestinal metaplasia to gastric cancer varied from 17 to 29. The risk was particularly high for those with more advanced baseline histological lesions, such as the presence of dysplasia [78]. Another prospective study from Japan further showed that individuals with IM had at least a sixfold increase in risk of gastric cancer [29].

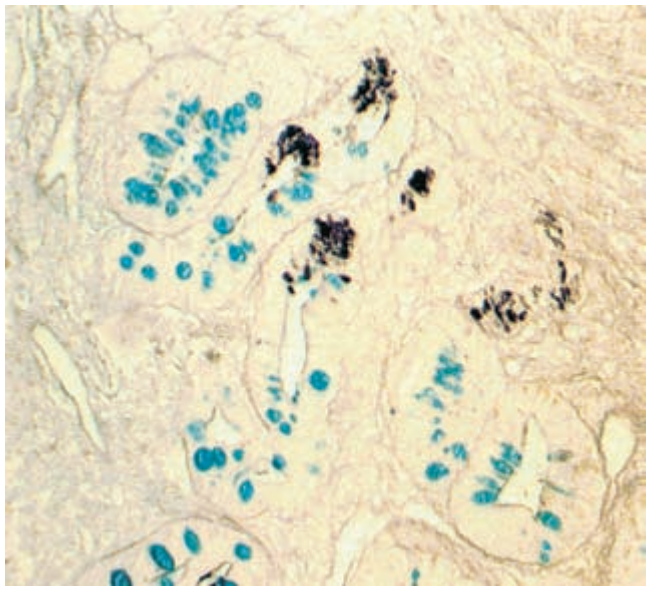


Figure 43.6 Type III intestinal metaplasia of stomach. The presence of sulfomucin in type III intestinal metaplasia is illustrated by the high iron diamine (HID)/alcian blue staining.

Molecular changes associated with gastric carcinogenesis

The molecular changes associated with gastric carcinogenesis are poorly characterized, in contrast to those of colorectal cancer. A number of genetic alterations have been identified but many are detected at a relatively low frequency [79]. Mutations in p53 are observed in up to 45% of intestinal-type cancers, with a lower frequency in diffuse-type cancer (0%–21%). Mutations in the *APC* gene are detected in more than 50% of well-differentiated gastric cancers, but not in any poorly differentiated gastric cancers [80]. Unlike hereditary diffuse gastric cancer, somatic mutations of E-cadherin are detected at a lower frequency in sporadic diffuse-type gastric cancer than in hereditary diffuse gastric cancer [81]. The *RUNX3* gene has been identified as another potential tumor suppressor gene in gastric cancer [82]. Activation of protooncogenes such as *ras* and *c-myc* is infrequently detected in gastric cancer [83].

Microsatellite instability (MSI), a form of genomic instability and a manifestation of cellular defects in DNA mismatch repair, is present in 17%–48% of gastric cancer [84,85]. Gastric cancers with MSI phenotypes tend to have specific clinicopathological characteristics, including intestinal type, prominent lymphocyte infiltration, and antral location, and are associated with a better prognosis [85,86]. Microsatellite instability can also be detected in gastric intestinal metaplasia, a preneoplastic lesion, in patients without gastric cancer [87]. In contrast to hereditary forms of colorectal cancers with MSI phenotypes, germ-line mutations of DNA mismatch repair genes are infrequent in MSI-positive gastric cancer. Instead, epigenetic inactivation of *hMLH1*, a DNA mismatch repair gene, by promoter hypermethylation was found to be the major mechanism underlying the gene silencing [88]. The E-cadherin gene is also frequently silenced by promoter hypermethylation. A second hit of promotor hypermethylation of E-cadherin in carriers of germline E-cadherin mutations leads to cancer development [89]. Gastric cancer frequently demonstrates concordant methylation in multiple tumor-related genes [90]; cancers with methylation in several specific loci are also called the CpG island methylator phenotype (CIMP) [91].

Cyclooxygenase-2 (COX-2) is an inducible form of prostaglandin synthase responsible for the conversion of arachidonic acid to prostanoids. Upregulation of the COX-2 gene and overexpression of the COX-2 enzyme are detected in approximately 70% of human gastric cancers [92–94]. Gastric carcinomas with COX-2 overexpression tend to have enhanced angiogenesis [95] and are associated with a worse prognosis [96]. Interestingly, there are overwhelming experimental data to support the potential use of COX-2 inhibitors in chemoprevention and treatment of gastric cancer. The use of celecoxib, a specific COX-2 inhibitor, has been shown significantly to reduce gastric cancer development in rats treated with mutagen [97]. Inhibition of COX-2 also was found to inhibit the growth [98] and angiogenesis [99] of gastric cancer cells.

Expression of COX-2 is also detected in *H. pylori*-associated gastritis and intestinal metaplasia [94,100], suggesting their early involvement in the gastric carcinogenesis pathway.

Chemoprevention of gastric cancer

Chemoprevention, as defined by the International Agency for Research on Cancer (IARC), refers to interventions with pharmaceuticals, vitamins, minerals, or other chemicals (natural or synthetic) at any of the multiple stages of carcinogenesis to reduce cancer incidence. Chemoprevention of gastric cancer focuses on the effect of *H. pylori* eradication or supplementary antioxidants.

Despite the strong etiological link between *H. pylori* infection and gastric cancer, it remains elusive whether eradication of *H. pylori* could prevent gastric cancer development. These chemoprevention studies are extremely difficult to perform due to the long cancer development process, which typically takes a few decades. To determine the potential benefits of *H. pylori* eradication, most studies were designed to examine the regression of presumed preneoplastic lesions – atrophy and intestinal metaplasia – as the surrogate end point of treatment success. Results of two large-scale randomized controlled studies performed in China and Colombia showed marginal benefits of *H. pylori* eradication in preventing regression of intestinal metaplasia at the end of 5–6 years [101–103]. Another study performed in southern China, which used gastric cancer incidence as primary end point, failed to show any significant benefits of *H. pylori* eradication [104]. It was only in subgroup analysis that individuals with no precancerous gastric lesions at baseline were found to have a marginally lower risk of gastric cancer development after *H. pylori* eradication.

The Colombian study by Correa et al. also examined the long-term effects of vitamin supplementation on changes in atrophy and intestinal metaplasia [102]. It was found that both β-carotene and ascorbic acid resulted in significant regression of both atrophy and intestinal metaplasia. However, there was no additional benefit of combination treatment with vitamins or *H. pylori* eradication. It remains unknown whether these findings could translate into real reductions in gastric cancer incidence.

Pathological features

Macroscopically, gastric tumors can be broadly categorized by their locations and appearances. Categorization of tumors into proximal (cardia) and distal (including body and antrum) cancers has major clinical significance in terms of etiology and prognosis. Distal cancers are more common in areas with a high incidence of gastric cancer, whereas cardia cancers are more prevalent in Caucasians with a low background gastric cancer incidence. Distal cancer is closely related to chronic *H. pylori* infection whereas cardia cancer is often the consequence of chronic gastroesophageal reflux disease and Barrett esophagus.

The macroscopic appearance of gastric cancer has been classified by Borrmann into four types (Fig. 43.7). Type I represents polypoid lesions without necrosis or ulceration; type II encompasses fungating cancers, which may contain superficial ulceration; type III represents ulcerated lesions infiltrating the gastric wall; and type IV are diffusely infiltrating tumors or linitis plastica lesions. There is considerable overlap between the different types, and this typing system does not appear to correlate with histological typing. Around

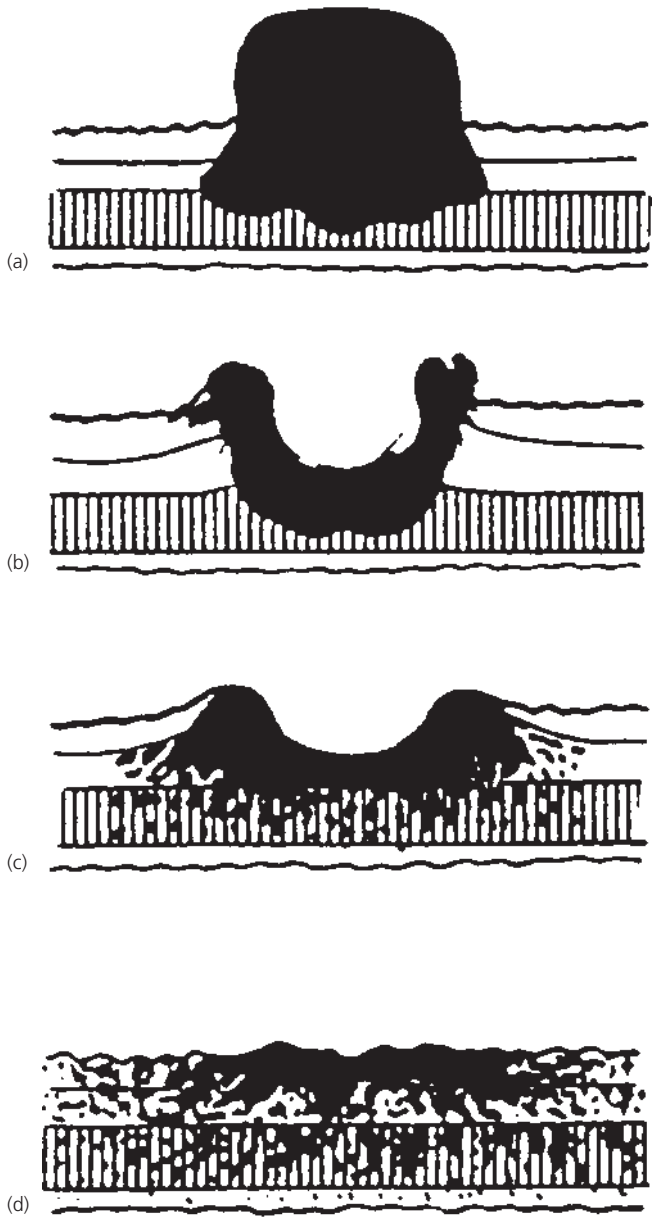


Figure 43.7 Macroscopic appearance of tumor as classified by Borrmann. **(a)** Polypoid; **(b)** fungating with surface ulceration and hemorrhage; **(c)** ulcerated without major lumen component; **(d)** infiltrative. From Borrmann R. Geshwulste des Magen und Duodenums. In: Henke F, Lunbarsch O (eds). Handbuch der Speziellen Pathologischen Anatomie und Histologie, vol. 4. Berlin: Julius Springer, 1926:812.

40%–50% of tumors are fungating or polypoid in appearance, and a similar proportion of tumors are ulcerative in nature. Linitis plastica is an aggressive infiltrating tumor consisting of high-grade tumor cells accompanied by a marked desmoplastic reaction that results in the rigid, leather-bottle-like stomach. This form of tumor spreads through the submucosa and is associated with a worse prognosis.

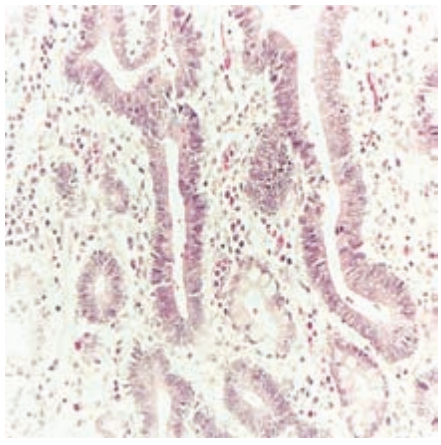
Adenocarcinoma accounts for 95% of the stomach cancers, whereas squamous cell carcinoma, carcinoid tumors, leiomyosarcoma, and lymphoma constitute the rest of the gastric tumors. Microscopically, there are several histological classifications for gastric adenocarcinoma. The most widely used classification was proposed by Lauren [105]; this broadly divides gastric cancer into diffuse and intestinal types (Fig. 43.8). Despite its simplicity, this classification categorizes tumors into two distinct types with contrasting epidemiology, etiology, and prognosis. The intestinal type is named by its histological morphology, and the diffuse type is characterized by its biological behavior. Intestinal-type cancer is characterized by cohesive neoplastic cells forming glandular tubular structures whereas diffuse-type cancer comprises sheets of epithelial cells or cells scattered in a stromal matrix without evidence of gland formation. Diffuse cancers may contain signet-ring cells. Intestinal-type cancer is more common in the distal stomach and is often preceded by preneoplastic stages such as intestinal metaplasia. It is more prevalent in regions with a high background incidence of gastric cancer and is generally associated with a better surgical outcome than the diffuse type. Approximately 5%–10% of tumors remain unclassified and are called "mixed type." These unclassified tumors are usually regarded as diffuse type during clinicopathological evaluations.

Another classification system proposed by the World Health Organization (WHO) divides gastric tumors into 10 types (Table 43.3) [106]. This classification is highly reproducible and is useful for routine pathological diagnosis of gastric cancer. Based on the degree of differentiation at architectural and cellular levels, gastric tumors can be divided into well-, moderately, or poorly differentiated types. Tubular adenocarcinoma, in which the glands form branching tubules, is the most common type of gastric adenocarcinoma. In papillary tumors there are prominent intraglandular foldings and projections, with cuboidal to high-columnar carcinoma cells lining the narrow bands of interstitial tissue in an arborizing pattern. Both types contain absorptive cell type and tend to form polypoid or fungating masses macroscopically. Signet-ring cell carcinomas are named because of the shape of the tumor cells, which contain abundant intracytoplasmic mucus. There is minimal gland formation, and this type of tumor tends to infiltrate. Mucinous adenocarcinoma, also called mucoid or colloid carcinoma, is diagnosed when mucus occupies more than 50% of tumor mass [107].

Table 43.3 World Health Organization histological typing of malignant epithelial tumors of the stomach

Adenocarcinoma:
Papillary
Tubular mucinous
Signet-ring cell
Adenosquamous
Squamous cell
Small cell
Undifferentiated
Others

There have been some controversies concerning the diagnosis of well-differentiated carcinoma between pathologists in the West and the East. According to Western criteria, diagnosis of adenocarcinoma is based on evidence of invasion. By contrast, Japanese pathologists diagnose gastric carcinoma on the nuclear and structural criteria even in the absence of invasion. Thus, dysplasia according to Western pathologists was frequently interpreted as early carcinoma in Japan [108]. In this regard, the Western policy runs the risk of overlooking cancers whereas the Japanese policy may overtreat "nonmalignant" lesions.



(a)

(b)

Figure 43.8 Lauren classification of gastric adenocarcinoma. **(a)** Well-differentiated intestinal type gastric cancer. **(b)** Diffuse-type cancer with infiltrative and discohesive tumor cells. Courtesy of Dr K.F. To, Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong.

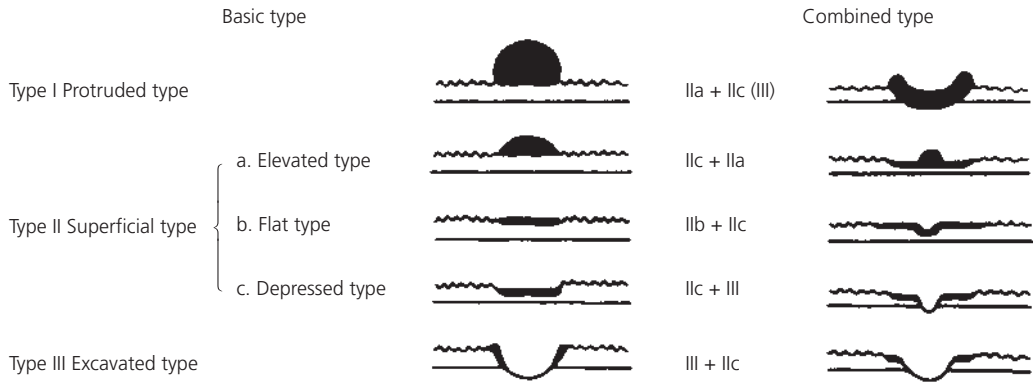


Figure 43.9 Classification of early gastric cancer by the Japanese Gastroenterological Endoscopy Society. From Japanese Research Society for Gastric Cancer. The general rules for the gastric cancer. Jpn J Surg 1973;3:61.

Early gastric cancer

Early gastric cancer (EGC) refers to tumor confined to the mucosa or submucosa, regardless of the presence or absence of metastasis. This disease is particularly prevalent in Japan, where mass screening for gastric cancer is performed. It is estimated that up to 65% of gastric cancers diagnosed in Japan belong to this entity. The prognosis for EGC is extremely good, with a more than 90% 5-year survival rate [109].

The Japanese Research Society for Gastric Cancer classified EGC into three types based on the macroscopic appearances [110] (Fig. 43.9). Type I is an exophytic lesion, type II is flat (or superficial), and type III is an ulcerated or depressed lesion. Type II is further divided into three subtypes depending on whether the lesion is elevated (IIa), flat (IIb), or depressed (IIc). In addition, combined types have been defined to include complex configurations. The protruded-type (type I and IIa), with a diameter of less than 25 mm, and the excavated type (type IIc), with a diameter less than 20 mm, seldom produce lymph node metastasis. The incidence of nodal metastasis of intramucosal and submucosal cancer is reported to be 3% and 20%, respectively [111]. Other parameters that may determine the rate of lymph node metastases are the size of the tumors and the presence of ulcerations. Distant metastasis is uncommon in EGC.

Many argue that EGC is not a genuine malignant condition because of the diagnostic criteria for cancer adopted by Japanese pathologists [112]. Tsukuma et al. reported their long-term follow-up of 56 patients with EGC diagnosed by endoscopy and confirmed by histology, and in whom surgical resection was delayed or not carried out [113]. Over a period of 6–137 months, 36 (64%) patients progressed to the advanced stage. The cumulative risk for progression to advanced cancer in 5 years was 63%, suggesting that EGC progresses at a relatively slow pace.

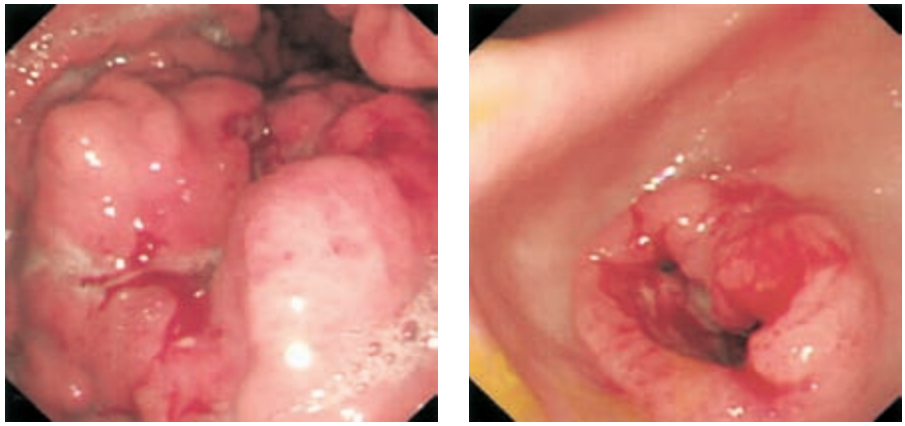
Clinical features

Early gastric cancer typically is asymptomatic and only patients with advanced disease will notice discomfort. Frequent symptoms are weight loss and abdominal pain [114]. Persistent vomiting can occur in antral tumors obstructing the gastric outlet. Dysphagia may be present with tumors obstructing the gastroesophageal junction. Early satiety, although not a common presentation of gastric cancer, may suggest a diffusely infiltrative tumor resulting in loss of distensibility of the stomach. Gastrointestinal bleeding has been reported in about 10%–15% of patients but frank bleeding is rare. Overt massive upper gastrointestinal bleeding may be more common in gastric stromal tumors.

Physical signs are usually absent in gastric carcinomas except in metastatic diseases. Palpable abdominal mass is rare before regional extension of the tumor. Gastric carcinomas spread by local extension to adjacent structures or metastasize by lymphatic, peritoneal, and hematogenous routes. Tumor may penetrate the gastric wall and directly invade omentum, pancreas, kidney, colon, spleen, and liver. Gastrocolic fistula is uncommon but may result in vomiting of fecolith materials or passage of recently ingested food into stool. Lymphatic metastasis occurs early and local lymph nodes are usually the first to be involved. Metastasis to left supraclavicular lymph node produces the so-called Virchow node. Tumor spreading along the peritoneal surfaces may result in a periumbilical nodule (Sister Mary Joseph node), ovarian mass (Krukenberg tumor), or tumor mass in the cul-de-sac (Blumer shelf). Peritoneal seeding of tumor may produce malignant ascites. Hematogenous spread usually involves the liver and occasionally the lung, bone, or brain. Patients with gastric cancer occasionally present with paraneoplastic syndromes, such as acanthosis nigricans, membranous glomerulonephritis, microangiographic hemolytic anemia, arterial and venous thrombi (Trousseau syndrome), seborrheic dermatitis (Leser–Trélat sign), and dermatomyositis.

Diagnosis

Barium meal was once the investigation of choice for all upper gastrointestinal symptoms. With the use of double-contrast techniques, better visualization of mucosal detail



(a) (b)

Figure 43.10 Endoscopic appearance of gastric cancer.

can be obtained. Malignant ulcer typically has an asymmetric ulcer crater eccentrically located in an irregular mass with distortion or obliteration of the normal mucosal fold surrounding the ulcer. Other features such as nodularity, clubbing, fusion, or amputation of radiating folds also suggest malignancy. However, false-negative rates of up to 25% have been reported in lesions between 5 mm and 10 mm in diameter [115]. Use of barium meal and radiography in the diagnosis of gastric cancer is currently limited to mass population screening.

Since its introduction in the 1960s, upper gastrointestinal endoscopy and biopsy has become the gold standard for the diagnosis of gastric cancer. However, endoscopic appearance alone is insufficient for the diagnosis of stomach cancer (Fig. 43.10). Diagnostic accuracy was reported to be 95% [115–117] and this can be further improved by increasing the number of biopsies [118]. Biopsies should be taken from the edge and the base of a malignant ulcer [119]. The additional use of brush cytology may further increase the sensitivity of endoscopic diagnosis. Identification of two of the three cytological features makes the diagnosis of gastric cancer more likely: (1) single atypical cells with intact cytoplasm; (2) eccentric nuclei; and (3) atypical naked nuclei [120]. Diagnosis of early gastric cancer can be difficult as lesions may not be conspicuous. Endeavors to improve the diagnostic accuracy of endoscopy have led to the development of new technologies. The use of a magnifying endoscope has enabled visualization of the microvascular architecture of the stomach. It was shown that the well-differentiated carcinoma may demonstrate the following findings:

- a demarcation line between the cancerous and the non-cancerous mucosa
- the disappearance of the regular subepithelial capillary network pattern
- the presence of an irregular microvascular pattern [121].

Light-induced fluorescence endoscopy (LIFE-GI) is another new technique that detects the autofluorescence from tissues after irradiation with blue or violet light. Characteristically, gastric tumors emit a different fluorescence from that of normal tissues, and this can be used as an adjunctive technique for the evaluation of presumptive neoplastic tissue [122,123].

Screening for gastric cancer

Screening for gastric cancer in asymptomatic individuals may increase the chance of detecting early cancer and hence improve overall survival. It is widely practiced in Japan. The early screening programs used double-contrast radiography [124], which has been largely replaced by endoscopy. It is estimated that 40%–60% of cancers diagnosed by the screening program are early cancer with no lymphatic metastasis [125], and that about 25% of gastric cancers in Japan are diagnosed by screening procedures [79]. Although screening is regarded as the main reason for the decline in stomach cancer mortality in Japan, a formal randomized study to evaluate the effectiveness of the screening program is lacking. Given the low incidence of gastric carcinoma in most developed countries, it is unlikely that a similar population-based screening program can be adopted. In areas with a low incidence of gastric cancer, screening may have to be targeted to high-risk individuals only, such as those with family history of gastric cancer, chronic atrophic gastritis, previous gastric surgery, or pernicious anemia.

Apart from contrast study and endoscopy, biochemical markers have been used to identify those at risk of developing gastric carcinoma. A low level of serum pepsinogen I, which correlates with corpus atrophy, has been found to be significantly associated with gastric cancer [126]. Kitahara et al. showed that a pepsinogen I concentration of less than 70 ng/mL, and a ratio of pepsinogen I:pepsinogen II of less than 3, are useful indicators, with a sensitivity of 84.6% and a specificity of 73.5% for detecting gastric cancer [127]. In this context, an elevated serum gastrin level has also been used in the screening for severe atrophic gastritis [128]. Although an elevated gastrin level of greater than 200 ng/L

appears to be specific, the sensitivity is low (30%), which precludes its use as a screening tool. The use of molecular screening techniques, such as serum protein profiling, may also help to identify patients with gastric cancer [129].

Staging

The staging of gastric carcinoma is of paramount importance for the planning of therapy, besides its prognostic implications. The most widely used staging system in most countries is the TNM (tumor, node, metastasis) classification devised by the International Union against Cancer (UICC) and American Joint Committee on Cancer (AJCC). The system used in Japan is the Japanese Classification of Gastric Carcinoma, and the second English edition was published in 1998 [110].

The acronym TNM stands for tumor invasion (T; Fig. 43.11), lymph node involvement (N), and the presence of metastasis to other organ(s) (M). The classification has been constantly revised, and the sixth edition of the TNM classification was published in 2002 [130] (Table 43.4). The lymph node staging system underwent major revision in the fifth edition, being based on the number of regional lymph nodes involved rather than the anatomical site or the distance of metastatic lymph nodes from the primary tumor, as used in the Japanese Classification (Fig. 43.12). This revised classification is easier to follow than the previous sophisticated localization of lymph nodes around the stomach, and reduces the problems involved in lymph node retrieval.

The Japanese Research Society for Gastric Cancer uses a more meticulous classification system including four categories of findings: clinical, surgical, pathological, and final [110]. In addition to TNM, the presence of hepatic metastases (H) and peritoneal metastases (P) is documented. Another important difference from the UICC/AJCC classification lies in the lymph node staging. In the Japanese classification, the regional lymph nodes, each labeled with a different station number (numbers 1–20 and 110–112), are classified into three groups depending on the location of the primary tumor. Hence, patients with N1 staging have the group 1 lymph node involved only whereas patients with N3 staging have group 3 lymph node metastasis as well.

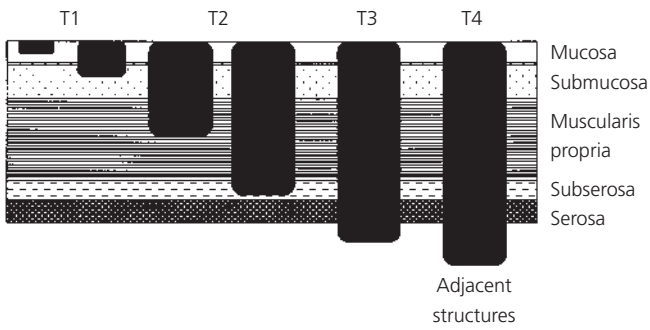


Figure 43.11 T-classification of gastric carcinoma (TNM staging system).

Table 43.4 Summary of the UICC (International Union Against Cancer) TNM classification of gastric cancer, sixth edition

T Primary tumor	
Tis	Carcinoma in situ; intraepithelial tumor without invasion of lamina propria
T1	Tumor invades lamina propria or submucosa
T2	Tumor invades muscularis propria or subserosa
T2a	Tumor invades muscularis propria
T2b	Tumor invades subserosa
T3	Tumor penetrates serosa (visceral peritoneum) without invasion of adjacent structures
T4	Tumor invades adjacent structures
N Regional lymph node	
N0	No regional lymph node metastasis
N1	Metastasis in 1–6 regional lymph nodes
N2	Metastasis in 7–15 lymph nodes
N3	Metastasis in more than 15 regional lymph nodes
M Distant metastasis	
M0	No distant metastasis
M1	Distant metastasis

Stage grouping

Stage	T	N	M
0	Tis	N0	M0
IA	T1	N0	M0
IB	T1	N1	M0
	T2a/b	N0	M0
II	T1	N2	M0
	T2a/b	N1	M0
	T3	N0	M0
IIIA	T2a/b	N2	M0
	T3	N1	M0
	T4	N0	M0
IIIB	T3	N2	M0
IV	T4	N1–3	M0
	T1–3	N3	M0
	Any T	Any N	M1

Preoperative staging

In general, no single imaging technique is accurate enough in the preoperative staging of gastric cancer. The use of multiple test modalities is therefore necessary for accurate preoperative staging. However, 25% of metastatic nodes measure 5 mm or less and are undetectable by most imaging techniques [131].

Ultrasonography (US) is a noninvasive, relatively inexpensive, and easily available imaging technique for intraabdominal malignancy. In experienced hands, US can be a useful

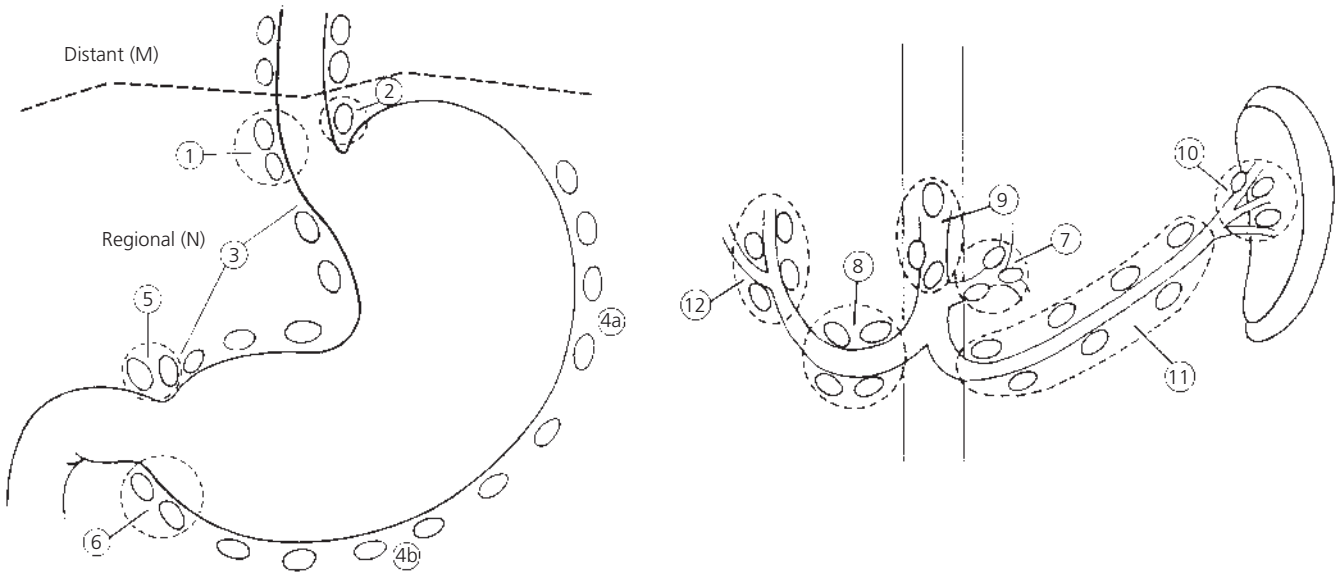


Figure 43.12 Regional lymph node stations as proposed by the Japanese Research Society for Gastric Cancer. From Hermanek P, Huttter RVP, Sobin LH, et al. (eds). TNM atlas, 4th edn. Berlin, Heidelberg: Springer-Verlag, 1997:82.

preoperative investigation for screening of solid organ metastases, enlarged paraaortic and portal lymph nodes, ascites, and pelvic deposits such as Krukenberg tumors of the ovary. Computed tomography (CT) is widely used for preoperative staging of stomach cancer [132]. Although it is sensitive in detecting direct invasion of the tumor into adjacent organs and liver metastasis [133,134], one of the major limitations of CT is its inability to differentiate between different structural layers of the gastric wall and hence to define the T stage accurately. The use of multidetector row CT gastrography with multiplanar reformation and virtual endoscopy may help to improve the accuracy of preoperative T staging [135].

For lymphatic metastasis, the sensitivity of CT varies from 48% to 91% [136,137]. The sensitivity of CT in detecting a distant metastasis is about 57% [138] but false positives are not uncommon. Finally, it is generally recognized that CT is poor in the visualization of small peritoneal nodules except in the presence of ascites. Overall, CT scan tends to understage gastric cancer and may be more useful in identifying advanced stage disease.

The role of magnetic resonance imaging (MRI) in the preoperative staging of gastric cancer has yet to be defined. It is superior to CT scan in delineating the structural layers of the stomach wall and may be used in predicting the depth of tumor invasion (T staging) [54]. However, MRI requires a longer scan time and is prone to artifact caused by breathing and bowel movements.

Positron emission tomography (PET) with ^{18}F-deoxyglucose (FDG) is being increasingly used in the evaluation of gastrointestinal malignancy. The preferential accumulation of positron-emitting FDG by tumor cells has been used in the detection of human cancers. It has been shown that PET combined with CT is more accurate for preoperative staging than either modality alone, particularly in lymph node staging [139].

Endoscopic ultrasonography (EUS) is an important preoperative staging tool for gastric cancer. With the use of a medium- to high-frequency ultrasound probe (7.5–30 MHz), the gastrointestinal tract is clearly delineated as a five-layered structure by EUS. The ability of EUS to distinguish between mucosa, submucosa, and muscularis mucosa makes it the best imaging modality to assess the depth of tumor invasion (Fig. 43.13). It is superior to conventional CT scan, with reported accuracy ranging from 71% to 92% for T-staging, and 55% to 87% for N-staging [140]. The presence of ascitic fluid on EUS may also predict the presence of peritoneal metastasis [141]. However, the presence of metastases within a normal-sized lymph node may be missed and EUS is generally unsuitable for the assessment of distant metastases due to the low penetration of high-frequency ultrasound.

Although transcelomic spread occurs in about 40% of tumors with serosal involvement (stage T3 or above), the inability of CT scan to diagnose peritoneal and omental metastases may lead to understaging of the tumors. Preoperative staging laparoscopy often detects unexpected peritoneal or liver metastases that may not be identified by other imaging techniques (Fig. 43.14). The overall rate of detecting peritoneal and liver metastasis by laparoscopy ranged from 3% to 37% and from 2% to 25%, respectively [142]. The addition of laparoscopy has modified the management plan of 25%–40% of patients who were otherwise deemed to be

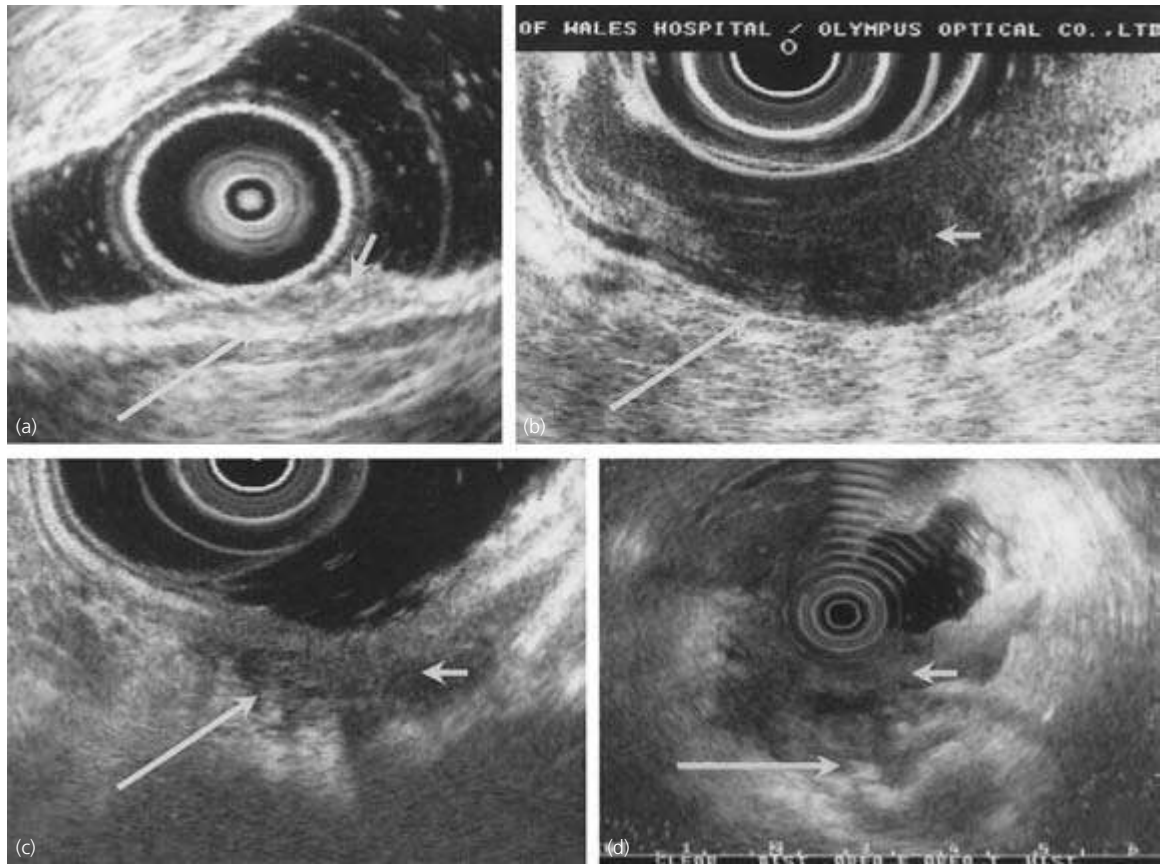


Figure 43.13 Preoperative staging of gastric carcinoma by endoscopic ultrasound. **(a)** Intramucosal early gastric cancer (T1is) with focal mucosal thickening (short arrow) and the lamina propria (long arrow) is intact. **(b)** T2 gastric cancer (short arrow) invades into the muscularis layer and the serosa (long arrow) is intact. **(c)** Small gastric cancer (short arrow) invades through the serosa (T3). **(d)** Large T4 gastric cancer (short arrow) invades into the neck of pancreas (long arrow). Courtesy of Dr Y.T. Lee, Prince of Wales Hospital, Hong Kong.

Figure 43.14 Laparoscopic view of gastric carcinoma patient with peritoneal metastasis (whitish nodule).

curable by radical surgery according to other preoperative staging investigations [143,144]. The use of laparoscopy may therefore be particularly useful in patients with locally advanced disease that may benefit from preoperative multimodality treatment [145].

Treatment

Surgery was long the mainstay of treatment for gastric cancer. Conventional treatment comprises resection of the primary tumor and control of lymph node metastasis. However, with the increasing recognition of EGC and improvement in techniques, endoscopic treatment is widely used in removing cancer confined to the mucosa.

Endoscopic therapy

Therapeutic endoscopy has three roles in gastric cancer treatment:

- to remove or obliterate the cancer
- to palliate obstruction
- to control bleeding.

Due to the low incidence of lymph node and distant metastasis (3% for intramucosal and 20% for submucosal tumors), surgical resection of the stomach for EGC (T1 cancer) is sometimes considered unnecessary. Endoscopic mucosal resection (EMR), or strip biopsy, was first described by Tada and colleagues in Japan [146], and is the standard treatment for EGC in Japan. It involves injection of saline into the submucosa beneath the lesion to lift the mucosal and submucosal layer from the muscle to avoid perforation. Methylene blue may be added to the saline to demarcate the plane of separation. The endoscope is fitted with a suction cap at its tip. The lesion is sucked into the chamber and resected by EMR snare (Fig. 43.15). Resection of the mucosa may then be achieved by using a cap-fit device at the tip of the endoscope and a mucosectomy snare. The malignant tissue can be removed en bloc or by piecemeal resection. Muscle fibers can be seen at the base of the resected area. Reepithelialization usually occurs within weeks. In contrast to ablation by laser or argon plasma, EMR can provide the original resected specimens for histological evaluation. The major complications of EMR are bleeding and perforation together occurs at a rate of about 5% [147]. According to the Japanese Gastric Cancer Association treatment guidelines [148], EMR is indicated for patients with a small mucosal cancer with no lymph node metastasis. Specifically, an intestinal-type mucosal cancer less than 2 cm in diameter is the best tumor type for EMR. In a review of the 11-year results of EMR performed at the National Cancer Center Hospital of Japan, which included 479 cancer patients, 69% of tumors were resected with clear margin and a total of 17 local recurrences were detected during subsequent follow-up [147]. There were no gastric cancer-related deaths during the follow-up period (3–120 months). Bleeding and perforation occurred in 5% of cases.

A more extensive endoscopic resection technique has been introduced in Japan, involving the use of a new device called the insulation-tipped (IT) needle knife [149]. The procedure is called endoscopic submucosal dissection (ESD), to indicate the potential depth of resection. The advantage of this procedure over EMR is the provision of a large en bloc specimen for histological staging and a greater potential to prevent recurrent disease. Bleeding (7%) and perforation (4%) are more commonly encountered after this procedure than after EMR [150].

For advanced inoperable gastric cancer with obstruction, laser or argon plasma coagulation therapy and endoscopic stenting procedures can be considered. Laser ablation of tumors requires expensive equipment, and thus argon plasma coagulation is preferred by most gastroenterologists. With the advent of self-expanding metallic stents, the palliation of malignant stricture can be achieved with minimal complications. It is particularly useful in the palliation of proximal gastric cancer as well as gastric outlet obstruction. In addition, any fistula or perforations can be sealed by the covered stent. Endoscopic stenting can also be used to relieve obstruction caused by tumor recurrence at an anastomosis after partial or total gastrectomy (Fig. 43.16). Tumor ingrowth, stent

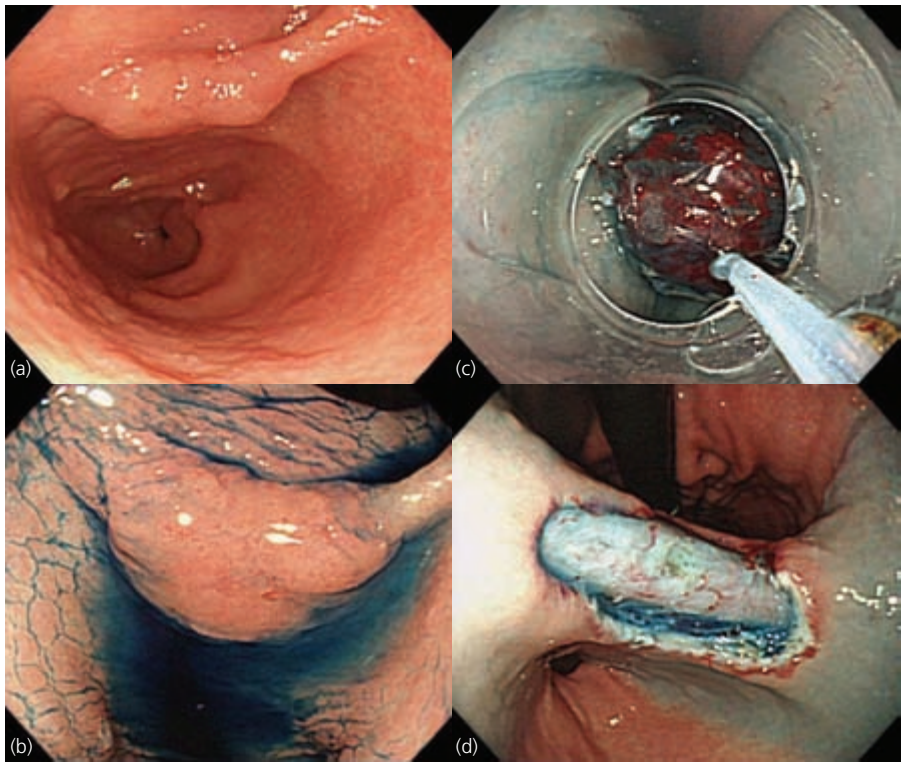
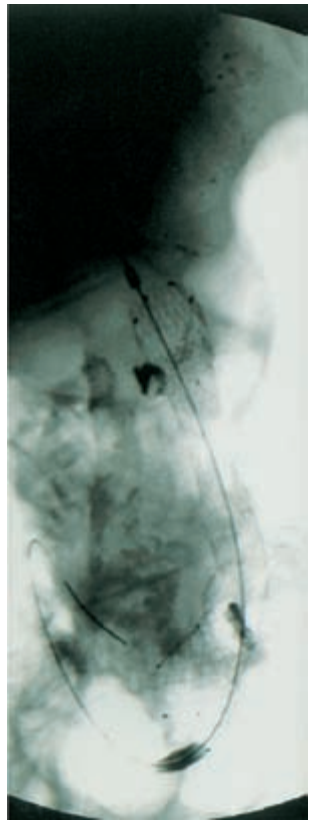
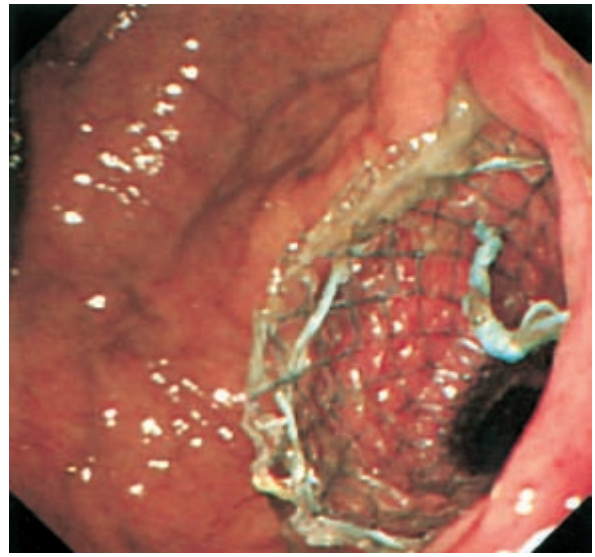


Figure 43.15 Endoscopic mucosal resection. Early gastric cancer **(a)** was first stained with indigocarmine and injected with normal saline to lift up the lesion **(b)**. The lesion was then sucked into the suction cap **(c)** and removed by endoscopic mucosal resection (EMR) snare **(d)**.



(a)



(b)

Figure 43.16 Self-expandable metal stent in patient with recurrent gastric carcinoma causing gastric outlet obstruction after subtotal gastrectomy.

migration, and gastroesophageal reflux remain problems of endoscopic stenting. Various covered stents are available to overcome the problem of tumor ingrowth, and antireflux mechanisms have been devised to avoid gastroesophageal reflux.

Surgery

Surgery can achieve the best locoregional control of gastric tumors, and it remains the standard treatment for such tumors other than EGC. It has been shown that the completeness of surgical resection is an important factor for survival [151]. Hence, patients with an R0 resection (i.e., no residual disease and clear margins) have a better survival than R1 (microscopic residual disease) or R2 (gross macroscopic residual disease). There are, however, considerable differences in the extent of gastric resection and lymph node dissection performed by Asian surgeons and Western surgeons. In the Japanese Gastric Cancer Association guidelines [148], the extent of surgical resection and lymph node dissection is dictated by the clinical staging of the patients (Fig. 43.17). By contrast, there is no similar guideline in the West.

Removal of the primary tumor

Total gastrectomy was, at one time, the recommended surgery for cancers of the stomach regardless of their location. Although the procedure is expected to provide better locoregional control of the primary tumor and remove all the remaining at-risk gastric mucosa, the resulting functional disturbance can be disabling. However, there is an increasing tendency to perform a proximal or distal gastrectomy, depending on the size and location of the tumor, instead of total gastrectomy. Two randomized trials conducted in the 1980s showed that the overall survival of patients with antral cancers after subtotal gastrectomy was not inferior to that after total gastrectomy [152,153]. A subsequent randomized trial by the Italian Gastrointestinal Tumor Study Group showed that patients with subtotal gastrectomy had a similar survival probability (65.3%) when compared with those undergoing total gastrectomy (62.4%) [154]. Subtotal gastrectomy should therefore be considered in patients with distally located tumor of intestinal differentiation. For a sizable tumor of diffuse subtype or more proximally located lesions, a total gastrectomy is advisable. Tumors involving the esophagogastric junction are better treated with esophagogastrectomy by a left thoracoabdominal approach.

Apart from curative resection, palliative surgery may still be considered in circumstances such as tumor obstruction, perforation, and bleeding. There is a reduction in the incidence of debilitating ascites after palliative gastrectomy [155]. If gastrectomy is performed, gross disease immediately adjacent

<table>
<tr><td></td><td>N0</td><td>N1</td><td>N2</td><td>N3</td></tr>
<tr><td>T1 (mucosa)</td><td>EMR or
Modified gastrectomy</td><td rowspan="2">Modified or standard gastrectomy</td><td>Standard gastrectomy</td><td rowspan="6">Extended or palliative gastrectomy or
Chemotherapy or
radiation or palliative care</td></tr>
<tr><td>T1 (submucosa)</td><td>Modified gastrectomy</td><td></td></tr>
<tr><td>T2</td><td>Standard gastrectomy</td><td colspan="2" rowspan="2">Standard gastrectomy
Adjuvant chemotherapy[a]</td></tr>
<tr><td>T3</td><td>Standard gastrectomy
Adjuvant chemotherapy[a]</td></tr>
<tr><td>T4</td><td colspan="2">Extended gastrectomy
Adjuvant chemotherapy[a]
Radiation[a]</td><td>Extended or palliative gastrectomy
Chemotherapy or
radiation or palliative care</td></tr>
<tr><td>M1
Recurrence</td><td colspan="3">Extended or palliative gastrectomy
Chemotherapy or radiation or palliative care</td></tr>
</table>

[a] This treatment modality should be reserved for prospective clinical trials.

Figure 43.17 Treatment guidelines for gastric adenocarcinoma by the Japanese Gastric Cancer Association. EMR, endoscopic mucosal resection; N, node; T, tumor.

to the stomach should be removed. Leaving behind a primary tumor mass in the stomach not only invites bleeding and perforation but also diminishes the likelihood of response to subsequent palliative chemotherapy. Alternatively, a bypass procedure or gastrojejunostomy can be performed, although this is associated with a significantly shorter palliation period compared with gastric resection [156]. There is also no signficant difference in terms of operation-related morbidity and mortality. However, gastric resection is not indicated in patients with a poor chance of survival, and the final decision is usually dictated by the overall clinical conditions of the patients.

Lymph node dissection

There is significant controversy about the extent of lymph node dissection, particularly between Western and Japanese surgeons. Japanese surgeons first advocated the use of extended lymphadenectomy in the 1960s in the hope that it might reduce the chance of nodal recurrence and improve survival. All intraabdominal lymph nodes are placed, according to location of the tumor, into groups 1, 2, or 3 [110]. In Japan, the standard practice for stage II or above cancers is standard gastrectomy with D2 dissection (removal of all group 1 and group 2 nodes). D1 dissection (removal of group 1 nodes only) is generally reserved for T1 tumors with N0 or N1 lymph node status. Although this practice is considered to be the major reason for the better outcome of Japanese cancer patients, results from two prospective randomized controlled trials performed in Western countries comparing D1 with D2 lymphadenectomy failed to demonstrate any survival benefit of the more aggressive approach using D2 dissection [157–159]. Both the British Medical Research Council (MRC) trial and the Dutch Gastric Cancer Group trial showed a significantly higher short-term morbidity and hospital mortality among patients undergoing D2 dissection. The high initial complication rate of D2 gastrectomy has been attributed to pancreaticosplenectomy and the unfamiliarity of the surgeons with this operation. In the MRC trial, participant institutes were criticized for their low case volume and lack of formal training on D2 resection [157,158]. Another controversial issue relating to these two trials was the high rate of noncompliance with either D1 or D2 gastrectomy, which may interfere with the final outcome. A Cochrane Database systemic review summarizing all trials published after 1970 with 5-year survival data [160] concluded that there was no survival benefit for extended lymph node dissection (risk ratio [RR] 0.95, 95% CI, 0.83–1.09). Extended lymph node dissection with spleen and pancreas resection significantly increased postoperative mortality associated with spleen and pancreas resection (RR, 2.23; 95% CI, 1.45–3.45). Even in experienced Japanese centers, the morbidity associated with D2 lymphadenectomy was as high as 21% [161]. However, subgroup analysis showed an apparent benefit in patients with N2 staging. To complicate the issue, an analysis of the Surveillance, Epidemiology, and End Results (SEER) database of the United States [162] showed that the overall survival was highly dependent on the number of lymph nodes examined in all stages. For every 10 extra lymph nodes dissected, there was a survival benefit of 6%–11%, which favored extended lymph node dissection. Thus, the debate is likely to continue until results from better designed and controlled clinical trials are available.

Open vs laparoscopic surgery

Laparoscopic surgery has been increasingly employed for gastric resection. Most reports of laparoscopic gastrectomy have been confined to cases of early disease for which there is no demand for extended lymphadenectomy [163,164]. Laparoscopic gastrectomy takes significantly longer in operating time but results in less wound pain in the postoperative period. However, there is no difference in the overall hospital stay and complication rate when compared with the conventional open approach.

In a small randomized study from Italy, it was suggested that the early and 5-year survival rates of open and laparoscopic-assisted radical subtotal gastrectomy were comparable [165]. More experience has subsequently been gained for laparoscopic extraperigastric lymph node dissection [166], although the long-term results are yet to be determined.

Chemotherapy

Chemotherapy can be given preoperatively (neoadjuvant), intraoperatively, or postoperatively to reduce the chance of local or systemic recurrence. The ultimate aim of giving systemic chemotherapy is to decrease the chance of tumor recurrence and increase the overall survival rate of patients.

Adjuvant chemotherapy

Adjuvant chemotherapy is generally considered as an additional treatment for patients who have already undergone potentially curative surgical procedures. Numerous chemotherapeutic agents, either given alone or in combination, have been used with variable success [167]. 5-Fluorouracil (5-FU) is the most frequently used agent, with responses ranging from 20% to 30% if given alone. Differences in effect and toxicity profile are the reasons for its application as continuous infusion in many modern protocols. Mitomycin C (MMC), commonly used in Japan, has an overall objective response rate of 30%. Other agents, with reported response rates of 19% to 26%, include epirubicin, doxorubicin, cisplatin, and hydroxyurea. In addition, a variety of combinations such as FAM (5-FU, adriamycin, mitomycin C), FAMTX (5-FU, adriamycin, methotrexate), FEP (5-FU, etoposide, cisplatin), FAP (5-FU, adriamycin, cisplatin), and ECF (epirubicin, cisplatin, infusional form of 5-FU) have been used in various studies [168]. Combination chemotherapy is the accepted standard in Europe and the United States whereas in Japan it remains unclear as to whether a single agent or combination therapy achieves better results [169].

Despite a large number of different single-agent or combination chemotherapy regimes, neither single-agent nor combination chemotherapy can be considered as an internationally accepted standard of care. A number of metaanalyses have been published since the mid-1980s addressing the role of postoperative chemotherapy [170–174]. Most of these reviews showed a small benefit in favor of chemotherapy. However, the benefits may be limited to trials conducted in Asia [174]. The controversy was summarized in the Cochrane systematic review of controlled trials [169]. When compared with patients receiving supportive care, ones receiving chemotherapy were found to have a better overall survival rate (hazard ratio [HR], 0.39; 95% CI, 0.28–0.52). Combination chemotherapy was also shown to have a survival benefit over single-agent chemotherapy (HR, 0.85; 95% CI, 0.76–0.96) but incurred increased toxicity. Among various combinations of chemotherapy regimes studied, the best survival results are achieved with regimes containing 5-FU, anthracyclines, and cisplatin.

In addition to the systemic route, antitumor drugs can be delivered intraperitoneally. The potential benefit of intraperitoneal chemotherapy lies in the ability to control local-regional recurrence after curative resection. Hagiwara evaluated the use of intraperitoneal mitomycin C adsorbed to activated charcoal, in high-risk patients following curative resection [175]. There was a highly significant difference in the 2-year survival rate (68.6% vs 26.9%) favoring the use of this regime. A prospective randomized trial of early postoperative intraperitoneal chemotherapy as an adjuvant therapy for resectable gastric cancer also showed significant improvement in the 5-year survival rate in a subset of patients with stage III disease [176].

Preoperative chemotherapy

For patients with locally advanced cancer, complete surgical resection may not be feasible. Hence, the use of neoadjuvant (preoperative) chemotherapy may reduce the size of large tumors to enable an R0 resection or reduce the chance of occult micrometastatic disease before surgery. Numerous trials have been conducted and it appears that neoadjuvant chemotherapy does not increase the postoperative morbidity and mortality [177]. Several phase II studies with neoadjuvant treatment have shown low toxicity and increased R0 resection rates for advanced disease [178,179]. However, data regarding long-term survival with the combination of neoadjuvant chemotherapy and surgery are still lacking.

Radiotherapy

Gastric carcinoma is relatively resistant to radiotherapy, including both external beam and intraoperative radiation. The British Stomach Cancer Group found that postoperative radiotherapy in addition to surgery did not confer any benefit for survival [180]. However, intraoperative radiotherapy is frequently used in Japan [181]. Although data suggest it may prolong survival for patients with stage II and III disease, this therapy needs further evaluation.

Chemoirradiation

There has been intense interest in the use of a combination of radiation for local control and chemotherapy to prevent systemic micrometastases in the postoperative treatment of gastric cancer, although early studies were largely inconclusive.

The Gastrointestinal Cancer Intergroup Trial (INT0116) [182] randomized 566 patients with resectable adenocarcinoma of the stomach or gastroesophageal junction to receive surgery alone or surgery plus postoperative chemoradiotherapy consisting of bolus 5-FU/leucovorin and irradiation. The median overall survival in the surgery-only group was 27 months, compared with 36 months in the chemoradiotherapy group. The HRs for death and relapse were 1.35 (95% CI, 1.09–1.66) and 1.52 (95% CI, 1.23–1.86), respectively. Despite the superior results, grade 4 toxicities occurred in 32% of patients receiving chemoradiotherapy. Moreover, the quality and extent of surgical resection was criticized: 54% of patients underwent a less than D1 dissection and only 10% of patients underwent a D2 resection. In subgroup analysis, there was no difference in the overall survival or disease-free survival in the D2 group of patients. It is therefore argued that the enhanced survival observed in the chemoradiotherapy group may be due to a compensatory effect for the inadequate surgical resection.

The feasibility of preoperative chemoradiotherapy has been evaluated in patients with potentially resectable gastric carcinoma [183]. In this small nonrandomized trial, the R0 resection rate was 70% and the median survival of these patients was 33.7 months. Further results of randomized controlled trials are needed to clarify the role of preoperative chemoradiotherapy.

Prognosis

The stage of the disease and completeness of surgical resection are important factors underlying patients' survival. Despite many advances in surgical techniques and chemotherapy, the mortality rate of gastric cancer in many parts of the world has not changed over the past few decades. A review from the National Cancer Data Base revealed that the overall 5-year and 10-year survival rates of patients diagnosed to have gastric cancer between 1985 and 1996 were 28% and 20%, respectively [184] (Fig. 43.18). By contrast, the overall survival rate of Asian patients, particularly in Japan and Korea, is higher than that in most Western countries. This is largely related to the use of screening procedures that detect early lesions with an excellent prognosis. However, even stage-stratified survival tends to be higher in these Asian countries [151,185]. The reasons for this discrepancy may be attributed to the more extensive lymph node dissection, which results in better locoregional control, and more precise staging of lymph node status.

In general, stage-stratified survival analysis suggests that males have a poorer prognosis than females, and proximal cancer is associated with a worse prognosis than distal cancer. Difficulty in attaining an adequate proximal resection margin and the close proximity to paraaortic lymph nodes around the hiatus may be reasons for the worse outcome in proximal gastric tumors. The predominance of distal cancer may also account for the better outcome of Asian patients [184].

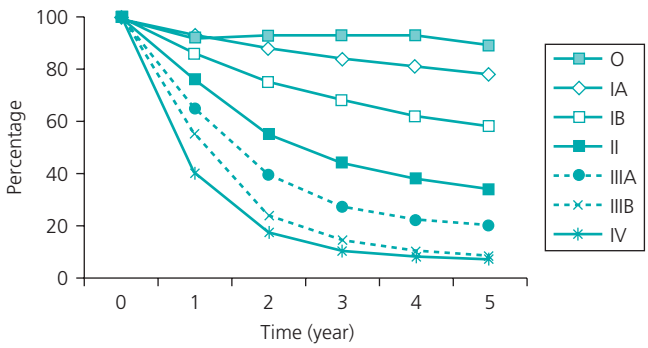


Figure 43.18 Five-year survival rates of gastric cancer patients treated by gastrectomy in the United States. Based on data from Hundahl et al. [184].

Other gastric tumors

Gastric lymphoma

The gastrointestinal tract is the most common site of extranodal lymphoma, accounting for 30%–50% of cases. Although primary gastric lymphoma is a rare malignancy, which accounts for less than 5% of all gastric malignancies, it is the commonest form of gastrointestinal lymphoma. In the WHO classification, primary gastric lymphomas are defined as lymphomas originating from the stomach and contiguous lymph nodes [186]. Lymphomas at this site are considered primary if the main bulk of disease is located in the stomach. The majority (80%) of primary gastric lymphomas are B-cell non-Hodgkin lymphoma, either low-grade mucosa-associated lymphoid tissue (MALT) lymphoma or a high-grade, diffuse, large-cell lymphoma (Table 43.5). Gastric lymphoma has a worldwide distribution. Most patients are over 50 years at presentation.

MALT lymphomas tend to arise as a polyclonal proliferation in chronically inflamed tissues, either of autoimmune or

Table 43.5 Types of primary gastrointestinal non-Hodgkin lymphoma

B-cell	MALT-type:
	Low grade
	High grade with or without low-grade component
	Mantle cell
	Burkitt
	Immunodeficiency related:
	Posttransplant
	AIDS
	Congenital
	Others
T cell	Enteropathy associated
	Others

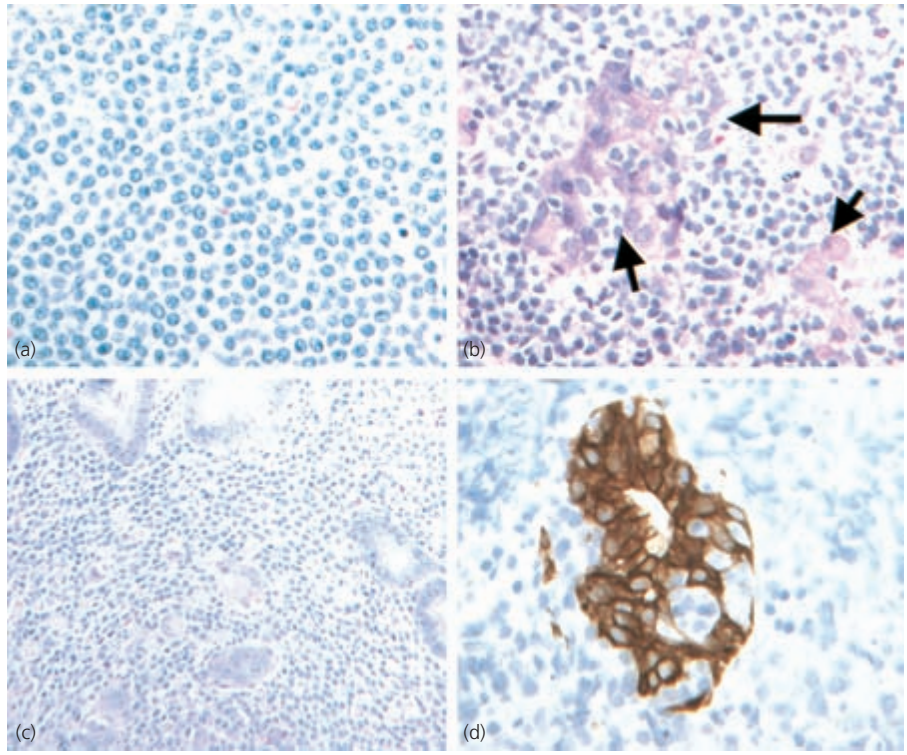


Figure 43.19 Gastric mucosa-associated lymphoid tissue (MALT) lymphoma. Characteristic features of low-grade MALT lymphoma: **(a)** centrocyte-like cells (H & E stain, original magnification × 200); **(b)** lymphoepithelial lesions (marked by arrows) (H & E stain, original magnification × 200); **(c)** plasmacytic differentiation (H & E stain, original magnification × 100); **(d)** lymphoepithelial lesions (immunostain for cytokeratin, original magnification × 200). Courtesy of Dr Wing Y. Chan, Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong.

infectious origin. Compelling epidemiological and pathological evidence suggests a link between chronic *H. pylori* infection and gastric MALT lymphoma [187,188]. More than 90% of low-grade gastric MALT lymphoma shows evidence of *H. pylori* infection, and this disease is more prevalent in regions with a high prevalence of *H. pylori* infection [189].

MALT lymphoma is characterized by the presence of plasma cells, reactive follicles, and centrocyte-like cells that tend to invade mucosal epithelium and form characteristic lymphoepithelial lesions (Fig. 43.19). The use of immunostaining for cytokeratin enhances the visibility of lymphoepithelial lesions. The detection of monoclonal rearrangements of the immunoglobulin heavy chain gene by polymerase chain reaction (PCR) or Southern blotting suggests the presence of lymphoma. The immunophenotypes of low-grade MALT lymphomas overlap with those of B cells.

Symptoms of gastric lymphoma are nonspecific, and most frequently include abdominal pain, weight loss, nausea, vomiting, and anorexia. Bleeding occurs in about 20% of cases [190]. Gastric lymphoma can be polypoid, fungating, infiltrating, or even ulcerative in appearance. Most lymphomas spread by submucosal infiltration and the muscular layer is usually spared until the late stage of the disease. Further spread to local and regional lymph nodes then follows.

Although upper gastrointestinal endoscopy is widely used in the diagnosis of gastric lymphoma, multiple and deep gastric biopsies are usually required for accurate histological diagnosis. The Musshoff modified Ann Arbor staging system is widely used for staging of gastric lymphoma (Table 43.6) [191]. Stage IE_1 refers to infiltration of the mucosa and submucosa only, whereas IE_2 lymphomas extend beyond the submucosa. Staging of disease can be done by CT scan and EUS. The latter is reliable in delineating the depth of

Table 43.6 Musshoff's modification of the Ann Arbor staging system for gastric lymphoma

Stage	Description
IE	Localized involvement of one or more gastrointestinal sites on the same side of the diaphragm without lymph node involvement
IE_1	Confined to the mucosa and submucosa
IE_2	Extending beyond submucosa
IIE	Stage IE with lymph node involvement; penetration of lymphoma through the gut wall
IIE_1	Regional lymph node involvement
IIE_2	Lymph node infiltration beyond the regional wall
IIIE	Involvement of the gastrointestinal tract or lymph nodes (or both) on both sides of the diaphragm
IVE	Diffuse or disseminated involvement of nongastrointestinal tract organs or tissues

tumor involvement and is superior to CT in distinguishing stage IE from stage IIE_1 disease [192]. To differentiate primary gastric lymphoma from systemic lymphoma with gastric involvement, other investigations, such as peripheral blood smear, bone marrow biopsy, and radiological imaging, may be required.

Eradication of *H. pylori* infection results in long-term cure of the majority of low-grade gastric MALT lymphomas [193–195]. The overall success of antibiotics in achieving complete remission in stage IE1 lymphomas is about 80%, with an annual recurrence rate of about 5% [194]. However, the time for complete tumor remission varies, and regression may not be apparent until several months after clearance of the organism [195]. Monoclonal B cells, as detected by PCR, may persist for up to several years after cure of *H. pylori* infection or complete histological and endoscopic remission [196,197]. Staging by EUS might predict the response of gastric MALT lymphoma to anti-*H. pylori* treatment. Patients with stage IE1 disease (confined to mucosa and submucosa) are more likely to have complete regression of the MALT lymphoma after anti-*H. pylori* therapy [198,199]. Patients should be followed by endoscopic examination every 6 months for the first 2 years and then yearly to monitor histological regressions. Other factors associated with a nonresponse to antibacterial therapy include *H. pylori* negativity and the presence of high-grade lymphoma features. The presence of t(11;18), which results in a chimeric transcript between the *API2* and *MLT* genes, may also predict resistance to antibiotic treatment [200]. For patients who fail *H. pylori* eradication, there is little consensus on the most appropriate management. Various treatment modalities, including expectant management, surgery, irradiation, and chemotherapy, have been used. Rituximab, a monoclonal antibody against cell surface CD20 on B cells, has been shown to result in complete pathological and clinical remission in 46% of patients with gastric MALT lymphoma resistant to or ineligible for anti-*H. pylori* therapy [201].

The emergence of clusters of large transformed "blastic" B cells reflects transformation to high-grade lymphoma. Treatment options for localized high-grade lymphoma remain controversial. Although surgery is particularly useful for local control of disease, there is an increasing tendency toward an organ-preserving approach. Surgery is associated with a favorable 5-year survival rate of 85%–100%, particularly in early-stage diseases [197]. However, aggressive surgery with radical excision is associated with high morbidity and mortality, and the extent of resection remains poorly defined. Data suggest that chemotherapy alone may be as effective as surgery plus chemotherapy in patients with localized disease [202,203]. In the prospective nonrandomized German Multicenter Study [204], patients were treated with either surgery plus radiation–chemotherapy or chemotherapy–radiation therapy alone. The 5-year survival and complete remission rates were comparable in the two groups. For chemotherapy, the combination of cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) is the standard regime used in the treatment of gastric lymphoma. One argument against nonsurgical therapy is the risk of perforation and hemorrhage. However, the overall risks of perforation and bleeding in those treated without surgery are 1.7% and 2.1%, respectively, which is not significantly different from those treated with surgery (0.9% and 2.1%) [204].

Patients with advanced stage disease (stages III and IV) are best treated with systemic chemotherapy or combination treatment. Radiotherapy is usually used as an adjuvant to surgery or chemotherapy, or to both. However, radiotherapy alone has been shown to have a reasonable outcome [205,206].

Mesenchymal tumors of the stomach

Most gastrointestinal mesenchymal neoplasms are gastrointestinal stromal tumors (GIST) or smooth muscle types. The term GIST, originally used to describe tumors that were neither leiomyomas nor schwannomas, denotes a specific group of tumors comprising the majority of gastrointestinal mesenchymal tumors including those previously called leiomyoma, cellular leiomyoma, leiomyoblastoma, and leiomyosarcoma. Approximately 60%–70% of GISTs occur in the stomach [207].

The precise cellular origin of GISTs has been proposed to be the interstitial cell of Cajal, an intestinal pacemaker cell [208,209]. This is supported by the sharing of morphological, immunohistochemical, and ultrastructural features between GIST and interstitial cells of Cajal. Mutations of c-*kit* that cause constitutive activation of the tyrosine kinase function of KIT are detectable in most GISTs and appear to play a pivotal role in the pathogenesis of these tumors [210]. The c-*kit* protooncogene encodes a type III tyrosine kinase growth factor receptor, which can be detected by immunohistochemistry for CD117. Immunoreactivity against CD117 is expressed in 80%–100% of GISTs and may be more sensitive and specific than the CD34, which is present in 60%–70% of cases [211]. In addition, a subset of GISTs contain mutations in the homologous kinase platelet-derived growth factor receptor-α (PDGFRA) [212].

GIST is an uncommon disease. The Finnish Cancer Registry estimated an annual incidence of 4 cases per million, which may be an underestimation [213]. In a review performed by the Memorial Sloan-Kettering Cancer Center, there had been only 200 patients with GIST treated during the previous 16 years [214]. The most frequent site affected is the stomach, which accounts for about 70% of the cases [215]. The peak incidence is found in adults during the fifth and sixth decades, and it is uncommon under the age of 40. Both genders are affected. Etiological agents have not been identified.

Many of these tumors are asymptomatic and are discovered incidentally but the clinical courses are highly variable.

Patients can present with nausea, vomiting, abdominal pain, gastrointestinal bleeding, and even metastatic disease. Bleeding is considered the most common presentation. Malignant tumors can metastasize to liver and peritoneum, and rarely to lymph nodes, bone, and lung [214]. Abdominal CT scan is useful in the evaluation of primary tumor and distant metastases. A GIST is usually demonstrated by intense uptake on a PET scan. The use of EUS may also help in differentiating benign and malignant tumors. Tumors less than 30 mm in diameter with regular margins and a homogeneous echo pattern are usually benign in nature [216]. Percutaneous biopsy is usually inadequate for making a proper histological diagnosis, and carries a theoretical risk of peritoneal seeding and tumor rupture.

The distinction between benign and malignant GIST is difficult. Conventionally, various parameters have been used to predict the biological behavior, including mitotic activity (> 10/high-power field), tumor size (> 5 cm), tumor necrosis, histological type/pattern, immunohistochemical profile, staining for proliferation antigens, and ploidy status [213]. Among these, mitotic activity and tumor size appear to be more reliable in categorizing tumors into benign, borderline, and malignant, but there is no agreed cutoff point for these values.

Surgical resection of the tumor was once the most important treatment for GIST. In general, a limited dissection is known to achieve comparable results to extended resection [217]. Lymphadenectomy is not routinely practiced due to the low incidence of lymph node metastasis. The availability of imatinib (formerly STI 571), a selective and competitive inhibitor of ABL tyrosine kinases, has revolutionized the treatment of GIST. Imatinib is reported to produce a partial tumor response in 45% of patients and stable disease in approximately 30% [218–220]. The role of adjuvant imatinib after complete resection of GIST is being investigated. Primary resistance to imatinib occurs in less than 15% of patients. Acquired resistance typically occurs after more than 1 year of therapy and is often due to secondary *KIT* mutation in novel sites including exons 13, 14, and 17 [221,222]. Before the era of imatinib, the median survival of recurrent GIST was only 15 months [223]. The 2-year survival of patients with metastatic disease is now about 70% [220].

Apart from GISTs, other gastric mesenchymal tumors include gastrointestinal autonomic nerve tumor (GANT), leiomyoma, glomus tumors, schwannomas, lipoma, and granular cell tumor.

Endocrine tumors of the stomach

Gastric carcinoid tumors are uncommon and account for only about 3% of all carcinoids [224]. Reviews, however, suggest that the incidence of gastric carcinoid is rising, from 3.8% in the 1970s to 8.9% in 1990s, according to the SEER database [225]. Whether this increase is the result of an increase in endoscopic detection or the use of acid suppressive therapy resulting in hypergastrinemia remains unknown at this stage.

Three distinct types of gastric carcinoid tumors with characteristic biological behavior and prognosis have been identified. Type I are the most frequent form (about 65%) of gastric neuroendocrine tumors and are localized in the oxyntic mucosa at the fundus and body. Patients usually have chronic atrophic gastritis, achlorhydria, and pernicious anemia. They often have elevated basal gastrin levels and are highly sensitive to trophic stimulus of gastrin. Type I tumors tend to be small (less than 100 mm) and multiple. They are considered to be the most benign of the three forms of carcinoid tumors, with a relatively low frequency (9%–23%) of metastasis [226–229]. Type II tumors are associated with the gastrin-producing neoplasms in Zollinger–Ellison syndrome as part of the multiple endocrine neoplasia type 1 (MEN1) syndrome. This type of carcinoid has intermediate metastatic potential. Enterochromaffin-like (ECL) cells are the main endocrine cell types in types I and II tumors. By contrast, type III, or sporadic, carcinoid lesions are encountered less frequently. They are not associated with hypergastrinemia and are frequently solitary large lesions in nonatrophic mucosa. This type of tumor behaves more aggressively, with a strong tendency for advanced local invasion and metastasis [230].

The clinical presentations of gastric carcinoids are usually nonspecific and variable, including pain, vomiting, anemia, and bleeding, or they can be discovered incidentally during endoscopy for other reasons. Presentation with the typical carcinoid syndrome of flushing, diarrhea, cutaneous edema, and bronchoconstriction is uncommon [230,231]. This is related to the secretion of histamine instead of substance P, serotonin, or 5-hydroxyindoleacetic acid. In a review of the cases identified by the SEER program from 1973 to 1991, 52.9% of the gastric carcinoids were localized disease, 10.3% regionalized, and 20.6% with distant metastasis [224].

Upper gastrointestinal endoscopy is the most useful diagnostic tool, and the typical lesions are small, rounded, submucosal masses that often are yellow [232]. There is no definite histological criterion for establishing the degree of malignant potential, and the best indicator is still the demonstration of invasive growth and presence of regional or distant metastases [224]. Indium-111 pentetreotide (OctreoScan) is increasingly used for identification of metastatic disease. The overall sensitivity is approximately 80%–90%, and it is particularly useful for lesions not apparent by conventional imaging techniques [233].

It is generally believed that endoscopic removal is appropriate for hypergastrinemia-associated lesions less than 1 cm in size and fewer than 3–5 in number [234]. Local excision, either antrectomy or fundic resection, should be considered for large and multiple lesions [231]. Antrectomy reduces the serum gastrin levels, resulting in regression of ECL cell carcinoids [226]. Fundic resection, however, removes all

ECL cells and carcinoids, which eliminates the chance of local recurrence. Surveillance endoscopy is recommended at 6-month intervals after endoscopic or surgical removal. However, a more radical approach is necessary for type III carcinoids due to their high malignant potential and strong tendency for metastasis. Complete or partial gastrectomy with regional lymph node dissection is recommended [227]. The use of long-acting somatostatin analogues has been reported to be efficacious in diminishing tumor load and decreasing ECL cell density [228]. The prognosis of gastric carcinoids is dependent on both the subtype of lesion and the extent of the disease. In general, patients with localized disease have a better survival than those with distant metastasis. The overall 5-year survival for gastric carcinoid ranges from 63% to 80% [225,229,235].

Gastric polyps

Gastric polyps are uncommon. The estimated incidence is less than 1% in autopsy or radiological survey [236]. However, up to 50% of gastric polyps are discovered incidentally during endoscopy for unrelated symptoms [237]. They may be solitary or multiple, and occasionally associated with other polyposis syndromes such as familial adenomatous polyposis, Peutz–Jeghers syndrome, Cowden syndrome and Cronkhite–Canada syndrome.

Hyperplastic polyps are among the commonest gastric polyps [236]. Hyperplastic or regenerative polyp frequently occurs in association with gastritis, particularly *H. pylori*-associated gastritis. In general, the malignant potential of hyperplastic polyps is minimal (1.5%–3%) [238,239].

Adenomas are circumscribed lesions comprising tubular or villous structures (or both) showing intraepithelial dysplasia. Adenomatous polyps account for about 10% of gastric polyps [240] and are more prevalent in areas with high incidences of gastric cancer. This type of polyp is associated with a greater risk of malignant transformation, and this risk is size dependent [241]: the risk is approximately 40%–50% in lesions greater than 2 cm.

Fundic gland polyp, also known as fundic gland hyperplasia or glandular cyst, is recognized as the most frequent type of gastric polyp in Western populations [240]. These lesions are composed of fundic glands lined with increased numbers of normal-appearing parietal and chief cells, and with a variable degree of cystic dilation. They are located exclusively in the acid-producing mucosa and thus are not found in the antrum. They are associated with the long-term use of proton pump inhibitors and with familial adenomatous polyposis syndrome.

Occasionally, misplaced gastric glands can present as a polypoid mass in the stomach. These heterotopic polyps are composed of tissue from the neighboring pancreas or duodenum. Heterotopic pancreas is frequently found in the prepyloric region and presents as a submucosal, nipple-like lesion, composed of mainly exocrine glands and ducts.

In the American Society for Gastrointestinal Endoscopy review, all polyps causing symptoms, such as bleeding and obstruction, and those greater than 2 cm, should be removed [242]. Polyps smaller than 2 cm can be initially biopsied and excised. If biopsies reveal adenomatous change, endoscopic excision should be considered. Most gastric polyps can be safely removed by snare polypectomy. Complications such as bleeding, perforation, and abdominal pain are rare. Surveillance endoscopy is only recommended in adenomatous gastric polyps 1 year after removal to assess for recurrence, or development of new polyps.

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44 Surgery for peptic ulcer disease and postgastrectomy syndromes

Robert E. Glasgow, Sean J. Mulvihill

Surgical treatment of peptic ulcer disease, 1054 Postgastrectomy syndromes, 1060

The current scope of gastric surgery encompasses four main indications: peptic ulcer disease, gastroesophageal reflux disease, neoplasms, and operations for morbid obesity. Formerly, peptic ulcer disease was the most common indication for gastric surgery; however, the introduction of antisecretory agents, the development of endoscopic therapy for complications of ulcer disease, and the recognition of the importance of *Helicobacter pylori* in the pathogenesis of peptic ulcer disease have significantly changed the spectrum of gastric surgery. These improvements in medical management have dramatically reduced the frequency of hospitalizations and operations for uncomplicated ulcer disease. However, the incidence of complications of peptic ulcer disease is unchanged, especially in the elderly population.

The changes in normal gastroduodenal anatomy and physiology resulting from gastric surgery are usually well tolerated and have no clinically relevant sequelae. However, approximately one-fourth of patients develop significant postoperative symptoms, which are severe enough in 1%–3% of patients to warrant remedial surgery [1]. In some patients the postoperative symptoms are vague or ill-defined, whereas other patients describe a more clear-cut constellation of symptoms. Collectively, these disorders of gastrointestinal function following gastric surgery are termed postgastrectomy syndromes. Although some of these postgastrectomy disorders are mechanical in origin and require surgical intervention, the majority result from functional changes in normal physiology. In the latter group, remedial surgery is reserved for patients who fail conservative measures. The goal of this chapter is to provide an overview of concepts in surgical treatment of peptic ulcer disease and management of postgastrectomy syndromes.

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Surgical treatment of peptic ulcer disease

Duodenal ulcer

Intractability

Surgical treatment of peptic ulcer disease is indicated for intractability, perforation, bleeding, and obstruction (Table 44.1). Intractability is generally defined as the failure of an ulcer to heal after 8–12 weeks of therapy or when relapse follows discontinuation of therapy. Elective definitive surgical treatment of peptic ulcer disease is rarely required today, but should be considered in the patient with intractable symptoms of duodenal ulcer despite optimal medical treatment including the eradication of *H. pylori* infection [2]. Additional indications for elective surgery may include suspicion of malignancy, intolerance to or noncompliance with medical therapy, patients at high risk for ulcer complications (transplant recipients, steroid or nonsteroidal antiinflammatory

Table 44.1 Indications for surgical treatment of peptic ulcer disease

Complications of peptic ulcer disease
Perforation[a]
Bleeding[a]
Obstruction[a]
Failure of a nonoperative treatment of an ulcer complication
Other indications
Intractability[a]
Suspicion of malignancy
Intolerance or noncompliance with medical therapy
High-risk patients (transplant, steroid or NSAID dependency, giant ulcers)
Strong ulcer diathesis
Patient preference

a Most common indications for surgery.
NSAID, nonsteroidal antiinflammatory drug.

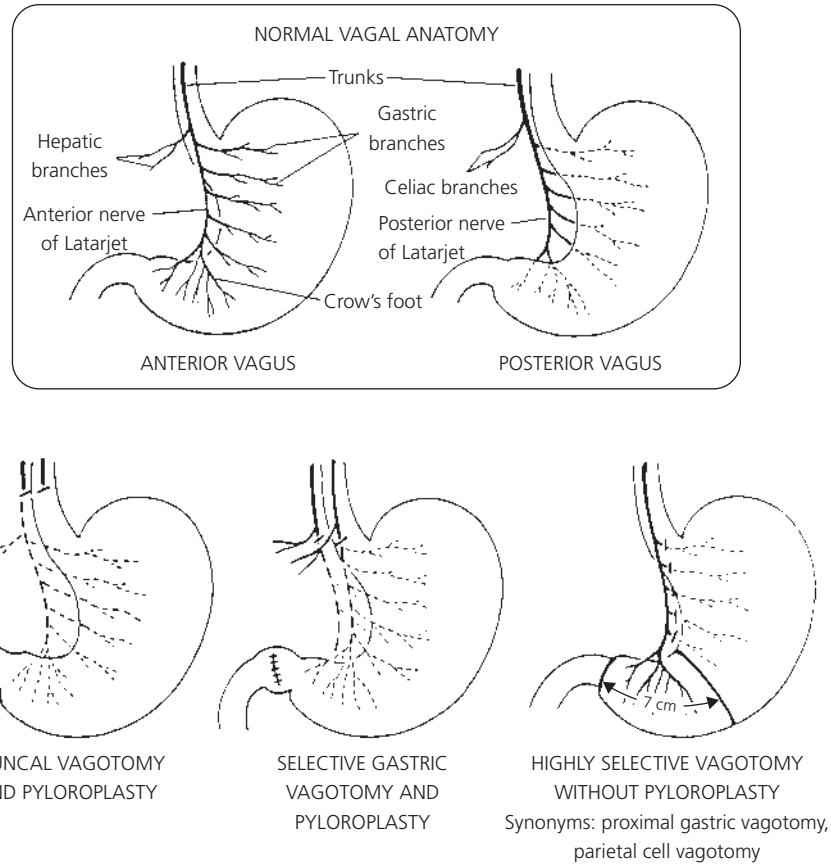


Figure 44.1 Normal vagal anatomy and types of vagotomy. Truncal and selective vagotomies are shown with a gastric drainage procedure (pyloroplasty). During truncal vagotomy, nerve trunks are divided above the celiac and hepatic branches. During selective vagotomy, the anterior and posterior nerves are divided distal to the celiac and hepatic branches, thus preserving extragastric innervation to the gastrointestinal tract. During highly selective vagotomy, individual branches of the anterior and posterior nerves of Latarjet to the body of the stomach are divided, sparing the branches to the antrum and pylorus ("crow's foot"). Pyloroantral motor function is preserved and, therefore, a drainage procedure is not indicated.

drug [NSAID] dependency, giant ulcers), a strong ulcer diathesis, patient preference, and failure of nonoperative management of an ulcer complication. The cost of long-term antisecretory therapy may also be considered when deciding on a course of antiulcer surgery. Regardless of the indication for surgery, the fundamental goals of surgical therapy are to permit ulcer healing, prevent or treat ulcer complications, address the underlying ulcer diathesis, and minimize postoperative digestive sequelae.

Surgery for peptic ulcer disease consists of two elements: vagotomy to decrease gastric acid secretion and drainage of the stomach. Three versions of vagotomy have been described: truncal, selective, and highly selective or parietal cell vagotomy. These are shown in Figure 44.1. When performing truncal or selective vagotomy, gastric emptying is impaired because of a loss of coordination of the antral mill and pyloric sphincter mechanism. Therefore, a drainage procedure is indicated. Drainage options include antrectomy, pyloroplasty, and gastrojejunostomy. The most effective operation for duodenal ulcer is truncal vagotomy with antrectomy. This operation inhibits both the cephalic and gastric phases of acid secretion, including the gastrin drive, and results in a greater decrease in acid production compared with vagotomy with pyloroplasty or gastrojejunostomy. Vagotomy with antrectomy and Billroth I or Billroth II reconstruction is shown in Figure 44.2 and vagotomy with pyloroplasty is shown in Figure 44.3.

Before the introduction of proton pump inhibitors (PPIs), several prospective trials demonstrated that vagotomy with antrectomy is superior to vagotomy and pyloroplasty, with ulcer recurrence rates of 1%–2% vs 10% respectively [3–7]. The impact of antisecretory therapy on recurrence rates following each of these procedures is not well defined. Because truncal vagotomy sacrifices vagal innervation to the pancreas, small intestine, proximal colon, and hepatobiliary tree, in addition to the stomach, a selective vagotomy was developed. Selective vagotomy spares the hepatic and celiac branches. However, compared with truncal vagotomy and drainage, selective vagotomy is inferior with regard to recurrence, dumping symptoms, diarrhea, pain, and dyspepsia, and has fallen out of favor.

Highly selective vagotomy (HSV), also known as parietal cell vagotomy or proximal gastric vagotomy, selectively eliminates the vagal innervation to acid-producing parietal cells and pepsin-producing chief cells of the fundus and body of the stomach while preserving innervation to the antrum and pylorus as well as the liver, pancreas, gallbladder, and bowel. A drainage procedure is unnecessary because the integrity of the antral mill and pyloric sphincter mechanism is preserved. Prospective randomized trials have demonstrated lower

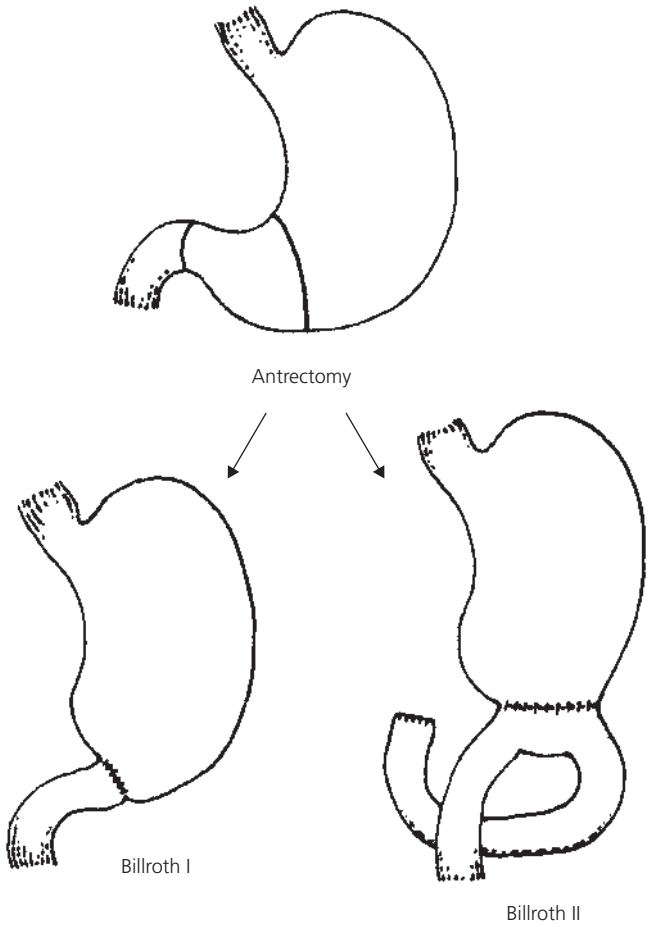


Figure 44.2 Reconstruction after gastrectomy. Billroth I gastroduodenostomy and Billroth II gastrojejunostomy.

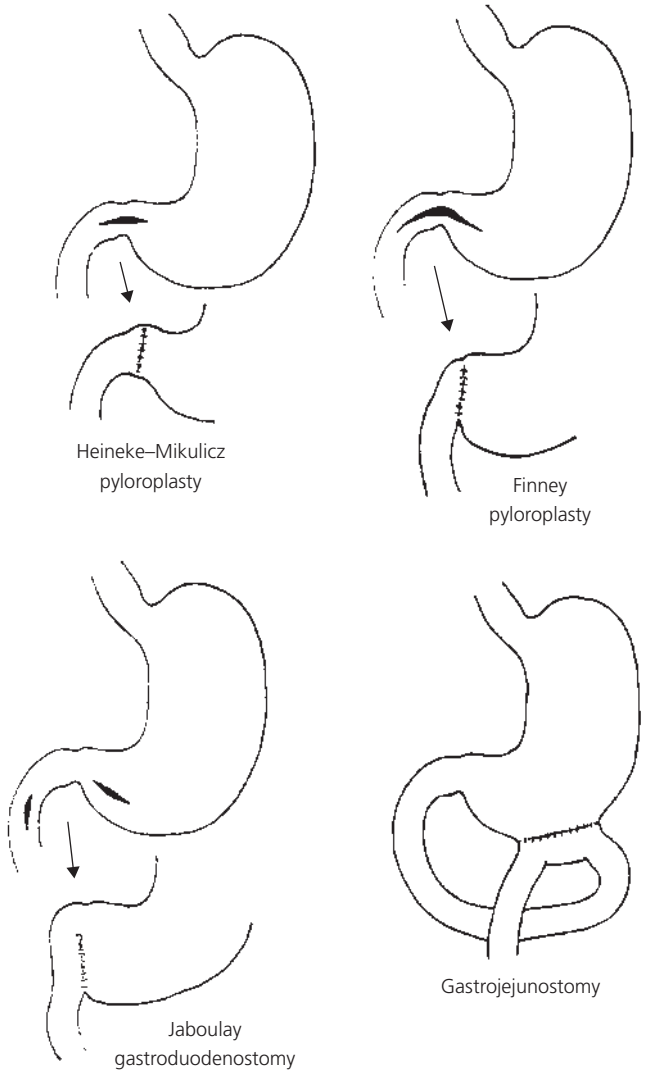


Figure 44.3 Methods of gastric drainage. The Heineke–Mikulicz pyloroplasty is most frequently used. The pylorus is opened in a longitudinal fashion and closed transversely to open and distract the edges of the pyloric sphincter. The Finney and Jaboulay gastroduodenostomy are used when the duodenal bulb is severely deformed from chronic ulcer. They are rarely indicated. A gastrojejunostomy is used when access to the duodenal area is limited.

postoperative morbidity related to postvagotomy symptoms when HSV is compared with both truncal and selective vagotomy [8,9]; however, although postoperative morbidity rates are lower following HSV, ulcer recurrence rates are higher. Studies have reported ulcer recurrence rates that range from 13% at 5 years [10] to 22% at 12 years [11]. The differences between the various ulcer operations with regard to recurrence and morbidity are summarized in Table 44.2 [12].

Laparoscopy has emerged as a surgical option not only for elective ulcer surgery but also for emergent complications of peptic ulcer disease. All of these surgical procedures, including parietal cell vagotomy and anterior seromyotomy with posterior truncal vagotomy, have been performed laparoscopically in the elective setting with patients experiencing low recurrence rates and shorter hospital stays.

Perforation

The clinical presentation of a perforated duodenal ulcer may vary according to the location of the ulcer. Posterior penetrating ulcers present with bleeding whereas anterior duodenal perforations cause the acute onset of severe, diffuse abdominal pain from free perforation into the peritoneal cavity. Patients with "kissing ulcers" of the duodenum present with both bleeding and perforation. When an anterior perforation occurs, air escapes into the abdominal cavity and may be detected by plain or upright radiographs of the abdomen and chest in approximately 70% of cases [13]. Most patients who experience a perforation will have a history of peptic ulcer disease or symptoms suggesting the diagnosis of peptic ulcer disease. However, chronically ill, elderly patients may be an exception to this presentation with vague, poorly localized abdominal pain that has developed over days to weeks.

Table 44.2 Results of duodenal ulcer surgery

	Highly selective vagotomy	Truncal vagotomy with pyloroplasty	Truncal vagotomy with antrectomy
Mortality rate (%)	0	0.5–1	2
Ulcer recurrence (%)	10–15	10	1–2
Gastric emptying			
Liquids	Accelerated	Accelerated	Accelerated
Solids	No change	Accelerated	Slowed
Dumping (%)			
Mild	< 5	10	10–15
Severe	0	1	1–2
Diarrhea (%)			
Mild	< 5	25	20
Severe	0	2	1–2

Duodenal ulcer perforation usually involves the anterior wall of the duodenal bulb whereas the lesser curvature is commonly the sight of gastric ulcer perforation [14]. The two major causes of perforated duodenal ulcers are *H. pylori* infection and NSAID use. Perforated ulcers are associated with NSAID use in one-third to one-half of patients [15,16] and more commonly occur in elderly patients. Smoking has also been implicated in peptic ulcer perforation, especially in younger patients [17].

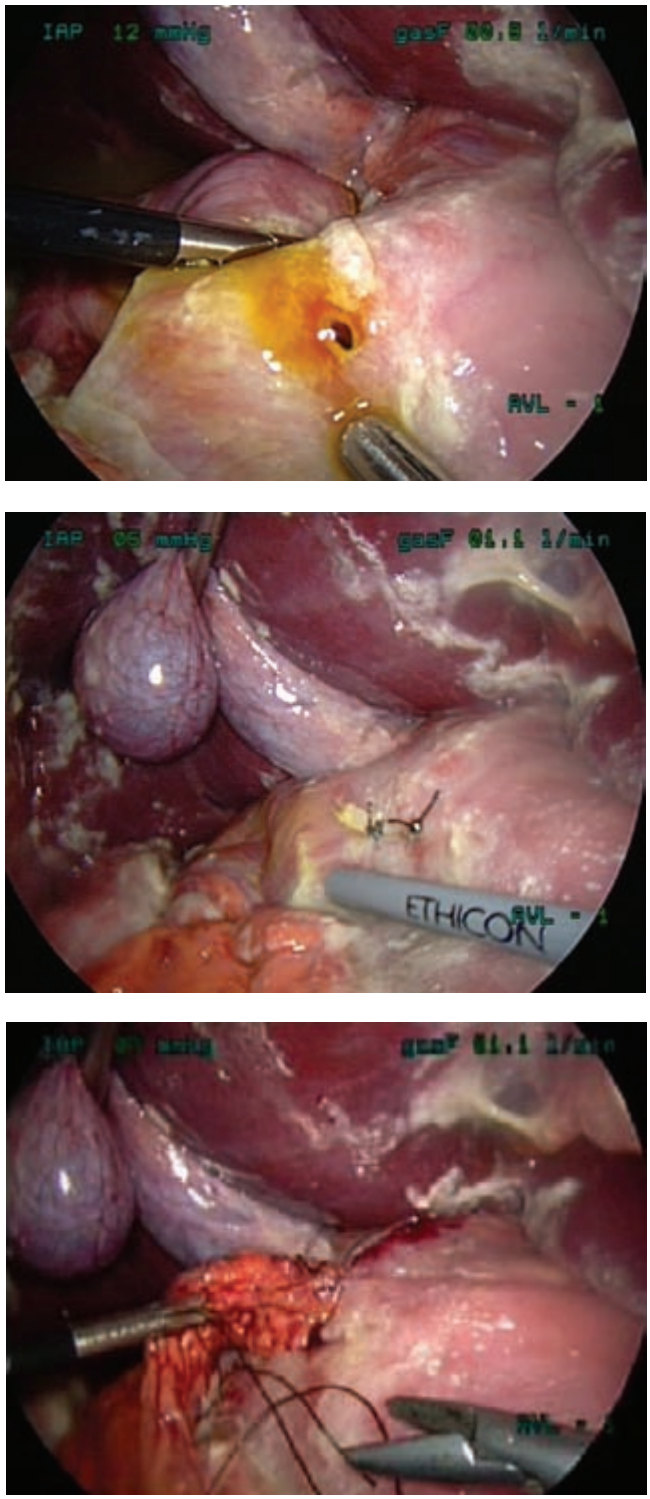
A perforated duodenal ulcer is a surgical emergency. It is most commonly managed with an omental patch (Graham patch) or truncal vagotomy with pyloroplasty, which incorporates the perforation. Simple patch closure (Fig. 44.4) is appropriate in patients who have not been previously treated for peptic ulcer disease and who can take PPIs and antibiotics for *H. pylori* infection [18,19]. Vagotomy is not necessary. Perforations related to NSAID use may also be treated with patch closure if the drug can be discontinued. Simple closure of perforated peptic ulcer has been compared with truncal vagotomy and pyloroplasty and vagotomy and antrectomy in randomized clinical trials prior to the *H. pylori* era [20–22]. Ulcer recurrence rates were 61% following simple closure compared with 6% following a definitive operation [2]. However, recurrent ulcer disease after peptic ulcer perforation occurs mainly in patients infected with *H. pylori* [23]. This is supported by several studies that demonstrated low recurrence rates following simple closure of the ulcer with *H. pylori* eradication [19,24–26]. Therefore, patients presenting with perforated duodenal ulcers should be tested for *H. pylori* and if found to be positive treated with eradication therapy. In a study by Ng et al. [19] of patients presenting with perforation, 81% were shown to be infected by *H. pylori*. Although initial ulcer healing rates were similar between patients treated for *H. pylori* compared with those treated with omeprazole, recurrence rates at 1 year were far less in the *H. pylori*-treated group (4.8% vs 38.1% respectively).

Definitive ulcer surgery in the form of vagotomy and pyloroplasty or antrectomy should be considered in patients who will require continued use of nonselective NSAIDs, who fail optimal medical treatment, or in whom compliance with *H. pylori* therapy is in doubt. One study that followed patients for more than 10 years after truncal vagotomy and pyloroplasty for perforated duodenal ulcer reported a mortality rate of 5.5% with a recurrence rate of 8.8%. Operative mortality rates from perforated duodenal ulcers range from 5% [20] to more than 30% in the elderly [27,28]. Risk factors that increase mortality include a severe comorbidity, the presence of shock on admission, and a presentation delay of more than 24 h [20,28].

The laparoscopic treatment of perforated peptic ulcers with an omental patch has been compared with open repair in randomized clinical trials [29,30]. The laparoscopic approach offers comparable efficacy, morbidity, and mortality to open surgery with a shorter hospital stay, lower analgesic requirements, and a shorter overall recovery time to normal function. The laparoscopic repair was also associated with shorter operative times. Additionally, laparoscopy has been advocated as a diagnostic technique when the diagnosis of duodenal ulcer perforation is in doubt.

Hemorrhage

Hemorrhage from a duodenal ulcer typically involves a penetrating posterior ulcer in the duodenal bulb in close proximity to the gastroduodenal artery. Bleeding from a peptic ulcer stops spontaneously in approximately 80% of cases [31]. Several stigmata predicting a higher than average risk of recurrent bleeding may be identified endoscopically. These include an actively bleeding ulcer, a nonbleeding visible vessel, and an adherent clot at the base of the ulcer. The rebleeding rate for each of these findings is 90%–100%, 40%–50%, and 20%–30% respectively [32]. The mainstay of treatment of a bleeding duodenal ulcer is endoscopic, with hemostasis



(a)

(b)

(c)

Figure 44.4 Modified Graham patch (omental) closure of a perforated duodenal ulcer. **(a)** Laparoscopic view of perforated anterior duodenal bulb ulcer. **(b)** Suture closure of the perforation. **(c)** Omental patch reinforcement of the perforation.

Table 44.3 Indications for surgical treatment of bleeding duodenal ulcer

1. Hemodynamic instability despite aggressive resuscitation (> 3 units transfused)
2. Failed endoscopic therapy
3. Recurrent hemorrhage after initial endoscopic hemostasis
4. Continued slow bleeding requiring > 3 units per day of transfusion

achieved in more than 90% of cases [33]. Thermal coagulation, injection therapy, hemostatic clips, fibrin sealant, argon plasma coagulation, and combination therapy have all been successfully used. The most commonly used endoscopic therapy is a combined modality with injection of an epinephrine (adrenaline)-containing solution followed by thermocoagulation. Patients should receive antisecretory therapy and be tested and treated for *H. pylori* infection. In patients who rebleed after endoscopic therapy the decision becomes one of repeat endoscopy or surgery. In one controlled trial, patients were randomized to repeat endoscopic therapy or surgery. Repeat endoscopy achieved long-term hemostasis in 73% of patients with similar mortality rates and lower morbidity rates compared with surgical intervention [34].

Surgery for peptic ulcer bleeding is indicated in the following scenarios: hemodynamic instability despite aggressive resuscitation (> 3 units transfused), failed endoscopic therapy, recurrent hemorrhage after initial endoscopic hemostasis, and continued slow bleeding requiring greater than 3 units per day of transfusion (Table 44.3). Surgical control of a bleeding duodenal ulcer consists of a longitudinal duodenotomy, usually encompassing the pylorus. Direct pressure is placed on the bleeding ulcer to allow resuscitation of the patient. The ulcer is then oversewn at three separate sites: cephalad and caudad to the ulcer to ligate the gastroduodenal

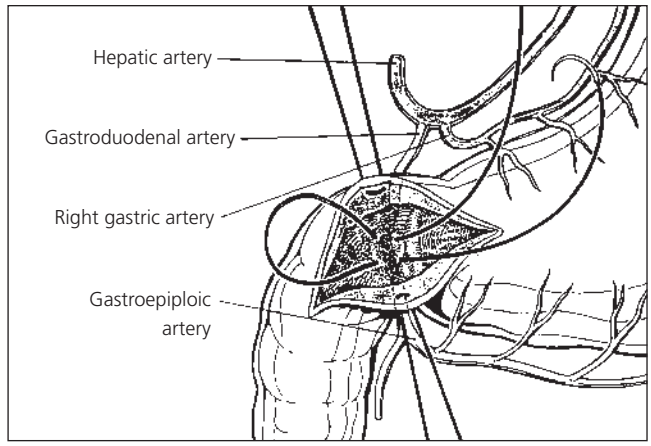


Figure 44.5 Suture closure of a posterior bleeding duodenal ulcer. Exposure is through a longitudinal gastroduodenotomy. The bleeding point within the ulcer is oversewn with monofilament sutures. Additional sutures are used above and below the ulcer to secure the gastroduodenal artery. From Seymour NE, Gastroenterology and Hepatology: The Comprehensive Visual Reference. Philadelphia, PA: Current Medicine, 1996.

artery and a U-stitch placed through the base of the ulcer to ligate the transverse pancreatic artery. This is demonstrated in Figure 44.5. Once the bleeding is controlled by these suture ligatures, a truncal vagotomy and pyloroplasty using the Heineke–Mikulicz technique should be performed [35]. The use of vagotomy in this circumstance is not universally supported, however [36]. A randomized controlled trial by Millat et al. [37] concluded that patients undergoing emergency surgery for massive, persistent bleeding or recurrent bleeding from a duodenal ulcer should undergo distal gastric resection because of a lower postoperative recurrent bleeding rate compared with oversewing the ulcer and vagotomy.

Gastric outlet obstruction

Patients with gastric outlet obstruction due to peptic ulcer disease typically present with nausea, vomiting, early satiety, bloating, indigestion, reflux symptoms, epigastric pain, and weight loss. Often patients have a hypochloremic, hypokalemic metabolic alkalosis as a result of chronic vomiting. Initial management of these patients includes nasogastric tube decompression, fluid resuscitation and correction of electrolyte abnormalities, and intravenous antisecretory agents. Approximately one-half of patients may initially respond to this regimen [38].

Two underlying mechanisms have been proposed for gastric outlet obstruction. The first is related to the inflammation, edema, and muscular spasm that may accompany an acute ulceration. The second mechanism of obstruction is the scarring and fibrosis of chronic ulceration [39,40]. Upper gastrointestinal radiography can provide useful information although endoscopic inspection is warranted for both diagnosis and to rule out malignancy. Malignant obstruction has been reported in as many as 66% of patients. Patients presenting with malignant obstruction tend to be older and do not have a history of peptic ulcers or NSAID use [41].

Patients who present with a gastric outlet obstruction secondary to peptic ulcer disease are commonly infected with *H. pylori* [42–44]. Reversal of gastric outlet obstruction has been reported following the eradication of *H. pylori* infection [45,46]; however, the time span from eradication of *H. pylori* to resolution of gastric outlet obstruction ranges from weeks to months [44]. In patients who fail to respond to initial medical therapy and in whom malignancy has been ruled out, endoscopic balloon dilation may be useful [47,48]. Patients may require multiple endoscopic sessions [49] but when *H. pylori* can be eradicated and NSAID use discontinued a good long-term result may be achieved.

Surgery is generally indicated if obstruction fails to resolve after nonoperative management [50]. These patients usually require vagotomy and antrectomy to relieve the obstruction and to rule out malignancy. A randomized controlled trial was performed to examine the outcomes of 90 consecutive patients with gastric outlet obstruction secondary to a duodenal ulcer [51]. The three operations tested were selective vagotomy with antrectomy, highly selective vagotomy with gastrojejunostomy, and highly selective vagotomy with Jaboulay (gastroduodenostomy) anastomosis. Postoperative gastric acid secretion was similar among the groups. Overall, results were best with the highly selective vagotomy and gastrojejunostomy whereas Jaboulay patients did the worst. Laparoscopic management of gastric outlet obstruction due to duodenal ulceration has also been described. In one small series of 12 patients, truncal vagotomy and gastroenterostomy was performed, with patients experiencing good results with a median follow-up of 6 months [52].

Gastric ulcer

Similar to duodenal ulcer, gastric ulcer can present with intractability, bleeding, perforation, and, less commonly, obstruction. However, fundamental differences exist between these two entities, which affect medical and surgical therapy. Only 70% of gastric ulcers are associated with *H. pylori* infection compared with 90% of duodenal ulcers [53]. In addition, reduced gastric acid secretion in gastric ulcer patients is more common, compared with normal or increased acid secretion in duodenal ulcer patients [54].

Malignant ulcers are more common in the stomach than in the duodenum and, therefore, all gastric ulcers must be excised or biopsied. Furthermore, gastric ulcers tend to affect older individuals, which may increase perioperative morbidity and mortality rates.

Gastric ulcers have been divided into five types depending on their location and specific etiology. These are summarized in Figure 44.6. Type I gastric ulcers are the most common and occur along the lesser curvature at the junction of the fundic and antral mucosa. This type of ulceration is generally associated with acid hyposecretion and can be treated with distal gastrectomy incorporating the ulcer and Billroth I or II reconstruction. A reasonable alternative is vagotomy, pyloroplasty, and local excision of the ulcer. Type I gastric ulcers are generally the consequence of inadequate mucosal defense rather than acid hypersecretion, and previous trials have shown no substantial advantage to the addition of vagotomy over distal gastrectomy alone. However, agreement has not been reached on this recommendation and, in practice, vagotomy has commonly been performed for gastric ulcer. For example, the Cleveland Clinic reviewed their experience with 349 patients operated upon between 1950 and 1979 with a mean follow-up of 11.8 years. In total, 55% of the patients had a gastric resection without vagotomy; 19.8% had gastric resection with vagotomy; and 20.3% had vagotomy, pyloroplasty, and wedge excision or biopsy of the ulcer. Operations were selected based on the type of ulcer, whether the surgeon suspected cancer preoperatively, whether the operation was elective or an emergency, and the age and general health of the patient (presence of significant comorbid disease). Long-term results were equivalent with an ulcer recurrence rate of 4% [55].

Type	Location	Symptom	Acid secretion
I	Gastric body on lesser curvature	Penetration	Normal or low
II	Gastric body and duodenal ulcer	Hemorrhage Obstruction Perforation	Elevated
III	Prepyloric	Hemorrhage Perforation	Elevated
IV	High on lesser curvature	Hemorrhage	Low
V	Anywhere on stomach		NSAID use

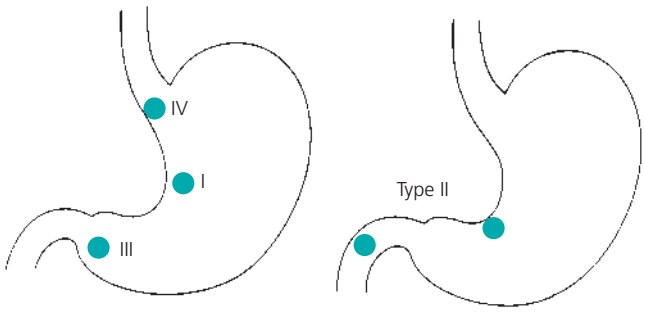


Figure 44.6 Classification of gastric ulcers according to location, symptoms, and acid secretion.

Type II gastric ulcers are found in the body of the stomach in conjunction with a duodenal ulcer whereas type III gastric ulcers occur in the prepyloric region. Types II and III are associated with increased acid secretion and should be managed similarly to duodenal ulcer with vagotomy and antrectomy. Type IV gastric ulcers are similar to type I gastric ulcers in that they tend to occur in the setting of acid hyposecretion. Type IV ulcers are located high on the lesser curve in close proximity to the gastroesophageal junction and may represent a migration of the junction of the fundic and antral mucosa to this location. Subtotal gastric resection for type IV ulcers is considered optimal and reconstruction with a Roux-en-Y esophagogastrojejunostomy (Csendes' procedure) is recommended [56]. Type V gastric ulcers are associated with NSAID use and can occur anywhere in the stomach. Operations for benign gastric ulcers are shown in Figure 44.7.

Perforation of a gastric ulcer is a morbid event as patients tend to be elderly with significant comorbidities. Mortality rates following gastric perforation range from 10% to 40% in reported series [29,57–59]. The preferred surgical treatment is distal gastrectomy incorporating the ulcer, as the risk of a malignant ulcer is high in this situation. If the patient has severe comorbid conditions or becomes unstable intraoperatively, the ulcer should be excised or biopsied and oversewn to rule out malignancy [60]. Depending on the intraoperative condition of the patient, truncal vagotomy and pyloroplasty may be added if the frozen section analysis of the ulcer biopsies shows no malignancy.

Laparoscopic repair of perforated peptic ulcers with an omental patch has been compared with open repair in randomized clinical trials [29,30]. In general, patients treated with the laparoscopic approach had decreased analgesic requirements, experienced shorter hospital stays, returned to normal activity earlier, and had no significant differences in morbidity, reoperation rates, or mortality compared with the open approach.

As with perforation, bleeding from a gastric ulcer raises the suspicion of malignancy. Hemodynamically stable patients with bleeding should undergo a definitive ulcer operation, particularly if the need for continued ulcerogenic medication such as NSAIDs is predicted. A distal gastrectomy incorporating the ulcer is preferred, with Billroth I or II reconstruction. Truncal vagotomy may be added. An acceptable alternative is excision of the ulcer, truncal vagotomy, and pyloroplasty. The unstable patient with a bleeding gastric ulcer requires ulcer excision or oversewing of the ulcer with biopsy. Excision of the ulcer is performed from within the gastric lumen through an anterior gastrostomy. In patients with a type IV bleeding gastric ulcer, the left gastric artery may be ligated and the ulcer biopsied and oversewn through a high anterior gastrostomy [61].

Postgastrectomy syndromes

Dumping syndrome

The dumping syndrome is one of the most common causes of morbidity following gastric surgery. Of all patients who undergo gastric surgery, 25%–50% have some symptoms of dumping, but these are disabling in only 1%–5%. The incidence of dumping symptoms varies according to the procedure performed. Significant dumping occurs in 5%–15% of patients following truncal vagotomy and drainage, 15%–20% of patients following partial gastrectomy, but less than 2% following highly selective vagotomy [1].

The dumping syndrome has two forms, early and late, based on the onset of symptoms. The early form occurs within 10–30 min of a meal and is characterized by both gastrointestinal and vasomotor symptoms. Gastrointestinal symptoms include postprandial fullness, crampy abdominal pain, nausea, and explosive diarrhea. Vasomotor symptoms include diaphoresis, weakness, dizziness, flushing, palpitations, and an intense desire to lie down. Expression of these symptoms is variable among patients. Most, however, exhibit both gastrointestinal and vasomotor manifestations. In the late form of dumping, symptoms arise 2–3 h following a meal and are typical of hypoglycemia. These differences are summarized in Table 44.4. Approximately 75% of patients with dumping syndrome have symptoms of early dumping and about 25% of patients have late symptoms. A few patients have both early and late dumping symptoms.

Pathophysiology

Dumping symptoms may occur following any gastric operation. The central factor responsible for the development of

Table 44.4 Forms of dumping syndrome

	Early dumping	Late dumping
Postprandial onset	10–30 min	2–3 h
Typical symptoms	Postprandial fullness	Diaphoresis
	Crampy abdominal pain	Weakness
	Nausea	Palpitations
	Explosive diarrhea	Flushing
	Diaphoresis	Dizziness
	Weakness	
	Palpitations	
	Flushing	
	Dizziness	

dumping appears to be the disruption of the normal regulation of gastric emptying. The emptying of the solid components of a meal is regulated by the coordination of antral grinding and pyloric relaxation, whereas liquid emptying is largely controlled by the pressure differential between the fundus of the stomach and duodenum. Gastric resection reduces fundic volume with a secondary increase in basal fundic tone. Vagotomy increases gastric fundic tone and limits accommodation to a meal. These operations result in more rapid emptying of liquids. Similarly, operations in which the pylorus is removed, bypassed, or destroyed increase the rate of gastric emptying of solids, both through loss of coordinated antropyloric motility and loss of duodenal feedback inhibition of gastric emptying.

Early dumping

Early dumping is thought to result from rapid emptying of hyperosmolar chyme into the small bowel. This leads to fluid shifts from the intravascular space into the intestinal lumen, resulting in small bowel distention and an increase in both the amplitude and frequency of bowel contractions. This results in bloating, crampy abdominal pain, and diarrhea. Sequestration of fluid into the bowel depletes circulating blood volume, causing hypovolemia with tachycardia and lightheadedness.

Several gut hormones have been implicated in the pathogenesis of early dumping. The postprandial concentrations of enteroglucagon, glucose-dependent insulinotropic peptide (GIP), pancreatic polypeptide, vasoactive intestinal polypeptide, gastrin-releasing peptide, serotonin, bradykinin, motilin, and neurotensin are all increased in dumping patients compared with asymptomatic patients following gastric surgery. Of these, neurotensin is the most likely agent involved in the pathogenesis of dumping. It has been shown to have both vasomotor and gastrointestinal actions that mimic those seen in early dumping. However, proof that neurotensin or other peptides cause dumping symptoms is lacking.

Late dumping

Late dumping most likely results from rapid gastric emptying leading to delivery of unusually high carbohydrate concentrations to the proximal small bowel. The carbohydrates are rapidly absorbed, resulting in hyperglycemia and excessive insulin release. The cause of this increased insulin release is unclear, but is thought to be related to the rapid absorption of lumenal carbohydrates or to an enhanced incretin effect. Several peptides may play a role in mediating this incretin effect. These include cholecystokinin, enteroglucagon, GIP, and glucagon-like peptide (GLP). The increased insulin release causes a postprandial hypoglycemia approximately 2–3 h following the meal. This reactive hypoglycemia leads to vasomotor symptoms characteristic of an insulin reaction.

Diagnosis

The diagnosis of dumping is based mainly on clinical history. A scoring system has been developed to assist in the differentiation of dumping from other postgastrectomy complications (Table 44.5). Any patient with a history of gastric surgery who develops symptoms consistent with dumping warrants further evaluation. Despite the characteristic symptomatology, other postgastrectomy complications may mimic the presentation of dumping. Patients should undergo an upper gastrointestinal contrast study to exclude stomal obstruction or afferent loop syndrome. Upper endoscopy should also be carried out to exclude recurrent ulcer, reflux alkaline gastritis, or gastric remnant carcinoma. A radionuclide-labeled gastric emptying study should be performed to document rapid gastric emptying and exclude gastric stasis. Falsely normal

Table 44.5 Sigstad clinical dumping score

Symptom	Score[a]
Preshock, shock	+5
"Almost fainting", syncope, unconsciousness	+4
Desire to lie or sit down	+4
Breathlessness, dyspnea	+3
Weakness, exhaustion	+3
Sleepiness, drowsiness, yawning, apathy, falling asleep	+3
Palpitations	+3
Restlessness	+2
Dizziness	+2
Headache	+1
Feeling of warmth, sweating, pallor, clammy skin	+1
Nausea	+1
Fullness in the abdomen, meteorismus	+1
Borborygmus	+1
Eructation	–1
Vomiting	–4

a A score of +7 or more suggests dumping; indices of +4 or less suggest other diagnoses.

gastric emptying as measured by radionuclide scanning may be observed if the test meal is of insufficient volume to reproduce the patient's symptoms.

Treatment

Prevention

As with other postgastrectomy syndromes, prevention is the best form of treatment for dumping. Of the surgical options for patients with peptic ulcer disease, highly selective vagotomy has the lowest risk of postoperative dumping syndrome. Although the long-term ulcer recurrence rate after highly selective vagotomy is higher than that after truncal vagotomy and antrectomy, management of recurrent ulcer is less problematic than management of dumping. If resection is required, some surgeons have advocated the use of Roux-en-Y gastrojejunostomy as the drainage procedure of choice to minimize the risk of dumping symptoms. Roux-en-Y gastrojejunostomy clearly decreases the rate of gastric emptying compared with pyloroplasty or loop gastrojejunostomy. However, Roux-en-Y reconstruction has its own set of problems, including Roux stasis syndrome and obstruction. When feasible, Billroth I gastroduodenostomy is the preferred reconstruction after antrectomy, as it most closely restores normal anatomy.

Medical therapy

The majority of patients with dumping syndrome have relatively mild symptoms that improve with time. Dietary modification is the mainstay of therapy and most patients intuitively modify their diet appropriately to minimize their dumping symptoms. The best diet avoids simple carbohydrates and favors complex carbohydrates and dietary fiber. The patient should eat frequent, small meals and avoid liquids during a meal. Liquids may be taken an hour or later following the "dry" meal. For severe vasomotor symptoms, lying supine for 30 min postprandially may slow gastric emptying and increase venous return, thereby minimizing the likelihood of syncope.

Octreotide acetate, a long-acting somatostatin analogue, is the most effective agent for the treatment of severe dumping symptoms. Octreotide ameliorates dumping by slowing gastric emptying, inhibiting insulin release, and decreasing enteric peptide secretion. Both anecdotal reports and randomized trials have documented the short-term efficacy of octreotide treatment in patients with severe dumping symptoms [62–65]. In general, octreotide improves the symptoms in over 90% of patients with severe dumping unresponsive to other medical therapy. In all studies, acute octreotide therapy significantly decreased the symptom score, pulse rate, and plasma insulin level compared with placebo. Long-term follow-up of patients treated with octreotide for dumping has not been reported. Steatorrhea is common with octreotide therapy because of inhibition of pancreatic exocrine secretion. This responds to oral pancreatic enzyme replacement. Other complications of octreotide treatment include hyper- or hypoglycemia, injection site pain, and cholelithiasis.

Surgical therapy

For patients with debilitating dumping unresponsive to medical therapy or patients unwilling to continue medical therapy, remedial surgery may be considered. A conservative approach is warranted, however, because most patients with dumping improve with time and remedial surgery does not cure all patients. Many remedial operations for dumping syndrome have been described, including pyloric reconstruction, jejunal interpositions, conversion of Billroth II anastomosis to Billroth I gastroduodenostomy, and conversion to Roux-en-Y gastrojejunostomy. No controlled trials comparing the relative efficacy of these operations exist. Analysis of their efficacy is difficult, as many of the procedures have good early results but are long-term failures.

For patients with dumping following pyloroplasty, pyloric reconstruction is the optimal remedial approach. In this operation, the pyloroplasty scar is identified and opened along its length. The sphincter muscle is identified and reapproximated. The incision is then closed longitudinally. This procedure has the advantages of simplicity and low risk. Interestingly, gastric stasis is unusual following pyloric reconstruction [66].

Several types of small bowel segment interposition for dumping syndrome have been described. These include an isoperistaltic jejunal segment interposed between the stomach and duodenum, creation of a 10-cm reversed jejunal segment interposed between the stomach and duodenum, and insertion of an antiperistaltic jejunal segment in the efferent limb of a Billroth II gastrojejunostomy or in a Roux-en-Y limb. Mixed results have been reported with this remedial operation, with generally favorable improvement of dumping symptoms but development of gastric outlet obstruction symptoms in some patients. It is clear that the reversed segment must be tailored to an appropriate length. Segments that are too short do not resolve the dumping symptoms, but segments constructed too long result in gastric retention. A 10-cm length appears ideal. Care must be taken to rotate the interposed segment only 180° and to avoid undue torsion of the mesentery. Mesenteric defects should be securely closed to avoid internal herniation.

Conversion of a Billroth II anastomosis to a Billroth I gastroduodenostomy improves dumping symptoms in about 75% of patients. The main advantage of this procedure is the restoration of the normal physiological delivery of a meal into the duodenum. This operation, however, is occasionally technically difficult and fails to alleviate symptoms in a substantial minority of patients. Overall, conversion of a Billroth II to a Billroth I has been eclipsed by the relative simplicity of Roux-en-Y conversion.

There is support in the literature for conversion of a Billroth I or a Billroth II to a Roux-en-Y gastrojejunostomy as

a remedial operation for dumping syndrome. In patients with previous Billroth I or Billroth II gastrectomy and medically resistant dumping, 85%–90% have favorable outcomes with Roux-en-Y conversion. In one of the larger series, Vogel et al. [67] reported that 19 of 22 patients treated with Roux-en-Y conversion had a good result. Of the three failures, only one was due to persistent dumping symptoms whereas the other two were due to the development of Roux stasis syndrome. This procedure has the advantage of simplicity when compared with jejunal interpositions, and it appears to have fewer undesirable long-term complications.

To summarize, remedial surgery for dumping syndrome should be considered for patients with medically unresponsive, severe symptoms. For patients with previous pyloroplasty, pyloric reconstruction is preferred. For patients with previous Billroth I or Billroth II gastrectomy, Roux-en-Y conversion is a simple and effective corrective operation. For patients who have previously undergone Roux-en-Y gastrojejunostomy, the best approach is construction of a 10 cm antiperistaltic jejunal segment within the Roux limb. These recommendations are summarized in Figure 44.8.

Postvagotomy diarrhea

Up to 20% of patients undergoing truncal vagotomy complain of postoperative diarrhea and it is debilitating in 1%–2% [1]. Its severe form is characterized by frequent, watery stools, which are occasionally explosive. The diarrhea may be postprandial or continuous. In some patients, nocturnal diarrhea is problematic. It is important to recognize that diarrhea following gastric surgery most commonly results from causes other than vagotomy. Thus, patients with early postoperative diarrhea should be initially investigated for causes such as bacterial overgrowth, pseudomembranous colitis, obstruction, inflammatory bowel disease, intestinal parasites, and malabsorption. The appropriate initial diagnostic tests should include fecal white blood cell count, stool culture, fecal *Clostridium difficile* titer, and, occasionally, sigmoidoscopy. For patients with persistent diarrhea, further studies should be performed to exclude steatorrhea, partial intestinal obstruction, and inflammatory bowel disease. Appropriate diagnostic studies include 3-day fecal fat quantification, colonoscopy, upper gastrointestinal barium contrast series with small bowel follow through, and barium enema. Consideration should also be given to the possibility of laxative abuse. If this is suspected, measurement of stool pH and magnesium concentration may be useful. If the workup is unrevealing, the diarrhea may be due to dumping syndrome or vagotomy. These last conditions are diagnoses of exclusion. No specific tests are available to confirm that the diarrhea is, indeed, due to the gastric surgery.

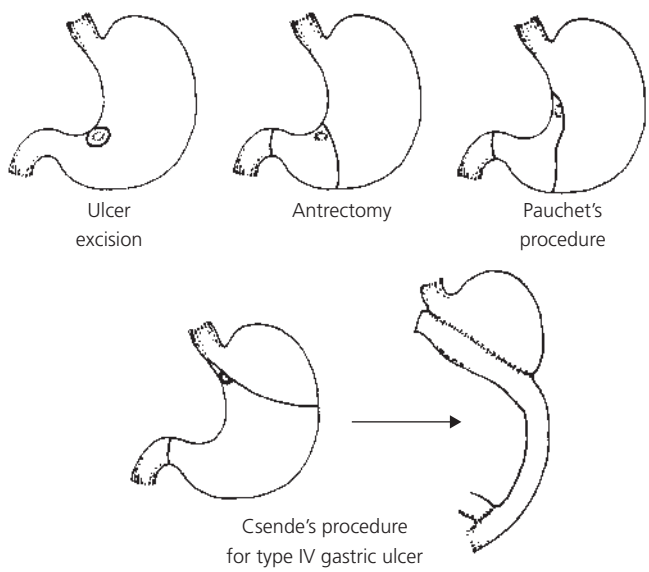


Figure 44.7 Operations for benign gastric ulcer.

Pathophysiology

The pathophysiology of postvagotomy diarrhea is unclear. Although there is no direct evidence, the most likely explanation is that truncal vagotomy interferes with central nervous system modulation of enteric nervous system control of gut motility and secretion. Rapid gastric emptying is often associated with the diarrhea but normalization of gastric emptying by remedial surgery does not always correct the problem. Impaired gallbladder emptying and increased excretion of bile salts may be involved. Supportive evidence includes the finding of higher levels of fecal bile salts in patients with postvagotomy diarrhea compared with control subjects and the beneficial effect of cholestyramine, a bile acid-binding agent, in many patients.

Treatment

Medical therapy

The medical treatment of postvagotomy diarrhea is similar to that recommended for patients with the dumping syndrome. Simple dietary changes including small, frequent, dry meals and increased dietary fiber may slow intestinal transit and therefore improve diarrhea. Opiates are beneficial through their inhibitory effect on intestinal transit. Cholestyramine improves diarrhea in the majority of patients. For patients unresponsive to these measures, octreotide, a long-acting somatostatin analogue, may be tried. Octreotide, however, has had limited success in controlling severe postvagotomy diarrhea as it may exacerbate the diarrhea by inducing steatorrhea.

Surgical therapy

For severe postvagotomy diarrhea unresponsive to medical therapy, remedial surgery may be considered. Although rapid gastric emptying is associated with the diarrhea, operations that slow gastric emptying are not usually curative. The most effective surgical strategies have focused on slowing small

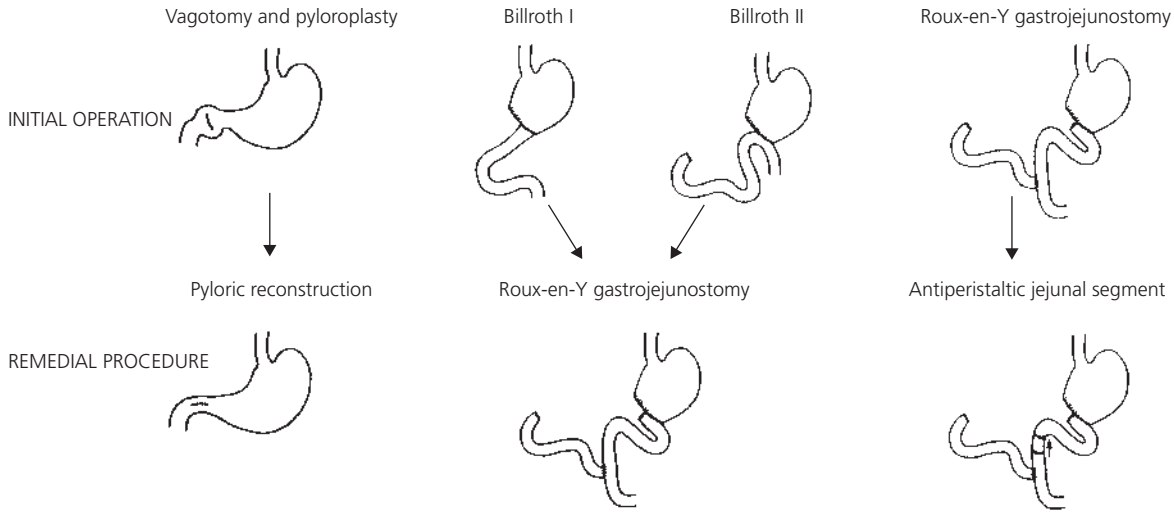


Figure 44.8 Remedial operations for dumping syndrome following gastric surgery. Although many options exist, for each initial operation, a reasonable remedial procedure is proposed. From Glasgow & Mulvihill [1].

bowel transit. Construction of a 10-cm antiperistaltic jejunal segment located 100 cm from the ligament of Treitz is effective in about two-thirds of patients. This is shown in Figure 44.9. Sawyers et al. [68], for example, reported a series of 16 patients undergoing this procedure in whom 14 had successful outcomes. On the other hand, Cuschieri [69] has reported that 12 of 13 patients in whom he performed this procedure had relief of diarrhea, but 10 required reoperation for pain and obstruction. The most important lesson learned regarding the treatment of postvagotomy diarrhea is that, like dumping, prevention is much simpler than treatment. Highly selective vagotomy minimizes the risk of postvagotomy diarrhea and should be performed when possible in lieu of truncal vagotomy for this reason.

Alkaline reflux gastritis

Alkaline reflux gastritis can occur in patients who have undergone removal, bypass, or destruction of the pyloric sphincter mechanism. Loss of pyloric function causes reflux of duodenal contents into the stomach, which may result in gastritis. Although some degree of reflux is seen in all patients without significant sequelae following gastric surgery, approximately 3% develop severe alkaline reflux gastritis. The majority of patients have a previous history of Billroth II reconstruction. However, Billroth I gastroduodenostomy and pyloroplasty are also associated with this syndrome.

Patients typically complain of persistent, burning, epigastric abdominal pain and nausea that are exacerbated by meal ingestion. Bilious emesis, often mixed with incompletely digested food, does not relieve the symptoms of pain and nausea. This feature distinguishes this condition from afferent loop syndrome in which bilious emesis, void of foodstuff, relieves the patient's discomfort. Patients develop an avoidance to food, resulting in significant weight loss. In addition, chronic low-grade gastritis results in anemia, a common finding in these patients. Alkaline reflux gastritis is the most common postgastrectomy syndrome for which remedial surgery is performed.

Pathophysiology

Patients with alkaline reflux gastritis have extensive gastritis involving the entire gastric remnant on gross examination. The gastric mucosa has an erythematous, friable, inflamed, ulcerated appearance on endoscopic examination, usually with visible pooling of bile and duodenogastric reflux. Histologically, the mucosa is void of parietal cells, hemorrhagic, and superficially ulcerated. The former observation is responsible for the achlorhydria or hypochlorhydria seen in patients with alkaline gastritis. Intestinalization of gastric glands, an increase in mucin cells, and a chronic inflammatory infiltrate are commonly observed.

Although the association between duodenogastric reflux and the symptoms of alkaline reflux gastritis is clearly established, the exact pathogenesis of the gastritis is not clear. Some degree of reflux is seen in all patients following gastric surgery; however, only a small minority develop gastritis. Several reports have suggested that patients with alkaline gastritis have increased concentrations of bile acids in the refluxed duodenal juice, increased volume and frequency of reflux, increased concentration of specific bile acids such as deoxycholic acid, or altered composition of bile acids and pancreatic enzymes. Others have suggested that prolonged mucosal exposure to bile acids resulting from gastric dysmotility may play a role in the pathogenesis. Likewise, prolonged gastritis may exacerbate gastric hypomotility. Nevertheless, it appears that the mucosal injury is most likely

caused by bile acids. Contact of bile acids with gastric mucosa results in breakdown of gastric mucosal barrier function, resulting in back-diffusion of hydrogen ions. This causes mast cell disruption with release of serotonin, histamine, and other vasoactive amines, which trigger the inflammatory changes characteristic of this condition.

Diagnosis

The diagnosis of alkaline reflux gastritis is based on a careful clinical history and endoscopic evidence of gastritis by gross and histological appearance. Radiographic studies should be performed to exclude mechanical causes of epigastric pain, nausea, and vomiting, including recurrent ulcer and afferent loop syndrome. An analysis of gastric fluid to document the presence of hypo- or achlorhydria should be performed. If hyperchlorhydria is encountered, hypergastrinemia from an undiagnosed Zollinger–Ellison syndrome, retained antrum, or incomplete vagotomy should be excluded. Bile acid composition and concentration should be determined. Technetium scintigraphy with and without cholecystokinin stimulation can be used to quantify duodenogastric reflux. Provocative testing with alkaline solutions and bile acids may reproduce symptoms in afflicted patients and serve to identify patients who may benefit from remedial surgery. In patients being considered for surgery, a solid-phase gastric emptying study should be performed to exclude significant concomitant gastric dysmotility.

Treatment

Medical therapy

The mainstays of medical therapy for alkaline reflux gastritis are prokinetic agents to improve gastric emptying and coating agents such as sucralfate to enhance gastric mucosal defense. No specific dietary or behavioral therapy is reliably successful. Attempts at binding bile acids with cholestyramine or aluminum-containing antacids have been unsuccessful.

Surgical therapy

The goal of remedial surgery in patients with alkaline reflux gastritis is to prevent pancreaticobiliary secretions from refluxing into the gastric remnant. For patients with a previous history of Billroth I or Billroth II anastomosis, the best remedial choice is conversion to a Roux-en-Y gastrojejunostomy with a Roux limb of sufficient length to prevent retrograde reflux, usually 45–60 cm. This procedure is 75%–85% effective in treating reflux symptoms [70]. However, Roux-en-Y gastrojejunostomy has two major potential complications. First, Roux-en-Y gastrojejunostomy is ulcerogenic, and therefore a vagotomy must be performed if not done so in the initial operation. Second, approximately one-fourth of patients with alkaline reflux gastritis who undergo Roux conversion will develop the Roux stasis syndrome. This underscores the importance of adequate preoperative assessment of gastric emptying in patients with alkaline reflux gastritis. If significant preoperative gastroparesis is encountered, a 90%–95% near-total gastrectomy should be performed with the Roux-en-Y gastrojejunostomy. Others have advocated a Braun enteroenterostomy in patients at risk for Roux failure. In this procedure, the afferent and efferent limbs are anastomosed 30–60 cm away from the gastroenterostomy in patients with a Billroth II anastomosis. Although the results with this procedure appear promising, it requires the presence of a long afferent limb.

For patients with reflux gastritis following truncal vagotomy and drainage, antrectomy with Roux-en-Y gastrojejunostomy should be performed. Antrectomy is necessary to minimize the risk of ulcer formation. For patients with a previous history of Billroth I gastroduodenostomy, a Roux-en-Y conversion should also be considered. Another option in this case is the Henley procedure, in which an isoperistaltic segment of jejunum is interposed between the gastric remnant and the duodenum. However, the results with this procedure have not been favorable. The duodenal switch operation described by DeMeester has also been proposed in the treatment of patients with alkaline reflux gastritis. In this operation, the duodenum is divided 5–7 cm beyond the pylorus. The distal end is oversewn and the proximal end anastomosed end to end to a Roux-en-Y limb. Long-term results with this procedure are not available, but short-term results appear promising. Remedial operations for alkaline reflux gastritis are shown in Figure 44.10.

Stasis syndromes

Stasis syndromes, including gastroparesis and the Roux syndrome, are well-recognized complications of gastric surgery. Postsurgical gastroparesis can occur following vagotomy or gastric resection. Two forms of postoperative delayed gastric emptying have been identified: an early form occurring in

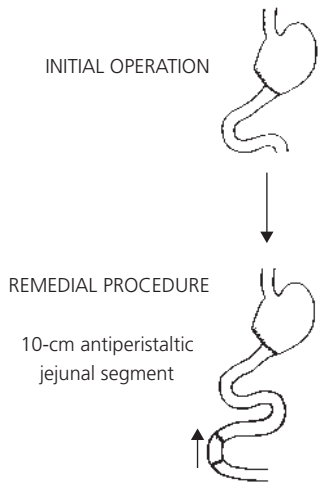


Figure 44.9 Remedial operations for postvagotomy diarrhea include construction of a 10-cm antiperistaltic jejunal segment located 100 cm from the ligament of Treitz. From Glasgow & Mulvihill [1].

the immediate postoperative period and a late form occurring from weeks to years following surgery. The incidence of gastric atony varies with the indication for surgery and type of procedure initially performed. In patients operated on for gastric outlet obstruction, for example, the incidence of postsurgical gastroparesis is as high as 27%–50%. Gastroparesis is seen in 1.4% of patients following vagotomy and drainage, in 2.4%–9% of patients after vagotomy and antrectomy, and in 3% of patients following subtotal gastrectomy without vagotomy. Overall, the incidence of postsurgical gastroparesis is 2%–3% [1].

In patients who have previously undergone Roux-en-Y reconstruction, gastroparesis or stasis in the Roux limb are termed the Roux syndrome. The incidence of this syndrome has been reported to be as high as 25%–30%. The incidence in patients who undergo Roux-en-Y diversion as a means of treating alkaline reflux gastritis is 10%–50%.

Patients with postsurgical stasis syndromes present with postprandial epigastric fullness or bloating, early satiety, epigastric pain, nausea, and vomiting of incompletely digested food. Some patients develop bezoars. Patients may attempt to compensate for their condition by consuming only soft, semisolid foods or liquids. Weight loss and nutritional deficiencies often result.

The diagnosis of postsurgical stasis syndromes is one of exclusion, as symptoms mirror those of other postgastrectomy syndromes. These include mechanical problems such as stomal obstruction from edema, kinking, or stricture, recurrent ulcer, and gastric stump carcinoma. In addition, symptoms may be confused with those of afferent or efferent limb syndrome or postoperative small bowel obstruction. Functional problems, such as gastroparesis related to diabetes mellitus, may be confused with a postoperative complication. These conditions must be excluded before instituting therapy for presumed postsurgical stasis. When surgery is necessary, a near-total gastrectomy is indicated with good long-term results [71,72].

Afferent limb syndrome

The afferent limb syndrome occurs in rare patients following Billroth II gastrojejunostomy. In this condition, the limb of duodenum and jejunum responsible for proximal intestinal, biliary, and pancreatic drainage becomes partially or completely obstructed proximal to the gastric anastomosis. The cause of this obstruction is mechanical and is usually related to excessive length. When the limb is too long, internal small bowel herniation, kinking from redundancy or adhesions, loop volvulus, and intussusception can occur. In addition, obstruction at the gastrojejunostomy from recurrent ulceration, stricture formation, and carcinoma may cause the afferent limb syndrome.

Two forms of afferent limb syndrome have been described: acute and chronic. Acute afferent limb syndrome occurs in the early postoperative period, usually within the first week. Obstruction of the afferent limb leads to accumulation of intestinal, pancreatic, and biliary secretions within the proximal jejunal lumen. As lumenal pressure increases, venous pressures are quickly exceeded, resulting in ischemia and pressure necrosis of the intestinal mucosa. Obstruction of pancreatic and biliary outflow may arise if lumenal pressures are sufficiently elevated. Disruption of the duodenal stump may result.

The symptoms of acute afferent limb syndrome are abrupt in onset. Severe epigastric pain, nausea, nonbilious vomiting, tachycardia, and fever are uniformly present. Abdominal tenderness and fullness are usually present. If uncorrected, shock may ensue. Elevated serum amylase and liver function tests often confuse the clinician into believing postoperative pancreatitis or biliary pathology is the cause of the patient's deterioration. This leads to delay in appropriate surgical management. The diagnosis is confirmed by ultrasound or computed tomography scan, which reveals a fluid-filled mass in the epigastrium. Acute afferent limb syndrome is a form of closed loop obstruction and, as such, is a surgical emergency. Mortality rates associated with acute afferent limb syndrome approach 50% [1].

Chronic afferent limb syndrome may occur at any point in time after the initial surgery. It results from intermittent, partial mechanical obstruction of the afferent limb. Patients typically present with postprandial epigastric discomfort, pain, and fullness. This results from the rapid accumulation of pancreatic, biliary, and duodenojejunal secretions within the limb in response to a meal. As intralumenal pressure exceeds the resistance to outflow caused by the obstruction, the patient experiences explosive bilious vomiting, usually void of foodstuff. This emesis relieves the patient of symptoms. These last two features distinguish chronic afferent limb syndrome from alkaline reflux gastritis, in which emesis usually contains undigested food and does not relieve the discomfort. A minority of patients develop bacterial overgrowth in the partially obstructed afferent limb, resulting in a blind loop syndrome. Plain abdominal radiographs are usually nondiagnostic. Ultrasound or computed tomography is the test of choice. Endoscopy should be performed to exclude the presence of alkaline gastritis, recurrent ulcer, anastomotic stricture, and anastomotic carcinoma.

Patients with afferent limb syndrome require remedial surgery. In the average patient, this is best accomplished via conversion to a Roux-en-Y gastrojejunostomy as seen in Figure 44.11. Alternatively, a Braun enteroenterostomy between the afferent and efferent limbs is effective in decompressing the obstructed afferent limb. In severely ill patients with acute obstruction, the afferent limb and duodenal stump must be examined for viability. In the rare patient with necrosis of the afferent limb, resection of the devitalized tissue, reconstruction of the afferent limb, bypass of the obstruction, and drainage of the duodenal stump is indicated. If extensive necrosis exists, pancreaticoduodenectomy may

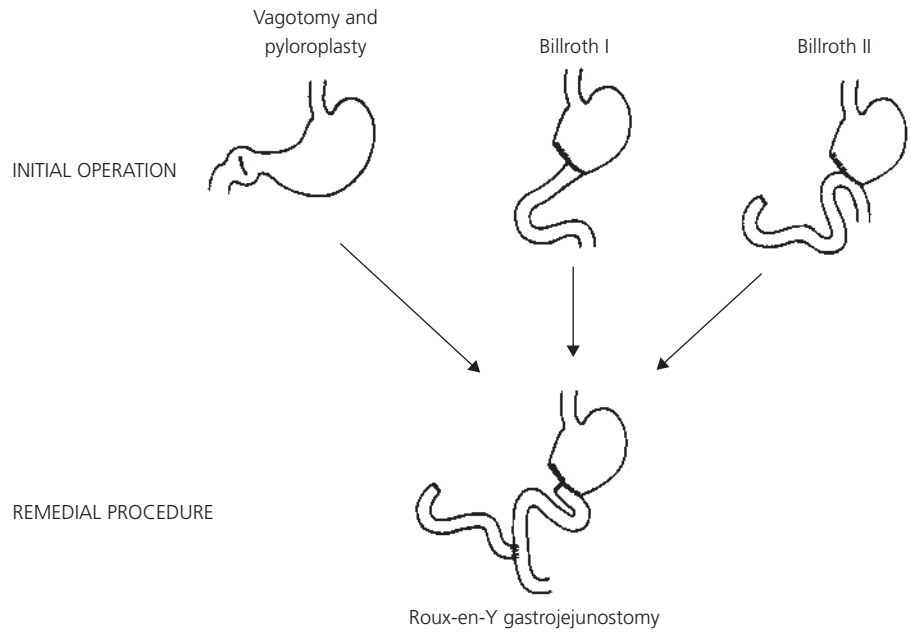


Figure 44.10 Remedial operation for alkaline reflux gastritis involves conversion to Roux-en-Y gastrojejunostomy, regardless of the initial operation. From Glasgow & Mulvihill [1].

be necessary. Afferent limb syndrome can be prevented by creation of a retrocolic gastrojejunostomy with a short (5–15 cm) afferent limb. All defects in the mesocolon or small bowel mesentery should be closed. The gastrojejunostomy should lie in a horizontal plain without kinks.

Efferent limb syndrome

In patients treated with Billroth II gastrectomy, obstruction of the gastrojejunostomy distal to the anastomosis is termed the efferent limb syndrome. Efferent limb syndrome is less common than afferent limb syndrome and is synonymous with postoperative small bowel obstruction. Efferent limb obstruction can be intermittent, partial, or complete. It can occur soon after the original surgery or years later. The causes of obstruction include postoperative adhesions, internal herniation, and jejunogastric intussusception. The last condition typically presents years after the initial surgery and results from intussusception of the efferent limb into the stomach. Less common causes include recurrent or marginal ulcer, gastric stump carcinoma, and lumenal obstruction from passage of undigested food or bezoar through the gastrojejunostomy.

Patients present with symptoms suggestive of small bowel obstruction. These include colicky abdominal pain, distension, diffuse tenderness, and frequent bilious emesis. The diagnosis is confirmed by either barium swallow or computed tomography scan with oral contrast. Upper endoscopy should be performed when recurrent ulcer, gastric stump carcinoma, or intussusception are suspected.

Most cases of efferent limb syndrome require remedial surgery. For patients with obstruction secondary to adhesions or internal hernia, lysis of adhesions, reduction of the internal hernia, and closure of the offending mesenteric defect, if present, should be performed. Anchoring the efferent limb to the anterior abdominal wall in the case of an antecolic reconstruction or to the transverse mesocolon in the case of a retrocolic gastroenterostomy may prevent recurrent herniation, kinking, or twisting of the gastroenterostomy. Most patients with jejunogastric intussusception may be managed nonoperatively. In patients failing conservative therapy, reduction of the intussusception and anchoring of the gastroenterostomy to the abdominal wall or mesocolon should be performed. Resection and revision of the gastroenterostomy or conversion to a gastroduodenostomy is occasionally needed.

Nutritional and metabolic consequences of gastrectomy

Several metabolic and nutritional abnormalities have been attributed to gastric surgery. In general, these result from alterations in the normal digestive and absorptive mechanisms responsible for nutrient uptake. Although weight loss is common following gastric resection, it is typically short-lived and seldom of significance in terms of effects on nutritional and metabolic homeostasis. However, in a minority of patients, considerable weight loss is encountered as a consequence of inadequate food intake and disabling concurrent gastrointestinal symptoms resulting from the resection. Although uncommon, the malabsorption of fat is occasionally seen following gastric resection. The normal mixing of bile acids and pancreatic lipase is disrupted following duodenal bypass in Billroth II gastrectomies or Roux gastrojejunostomies. This fat malabsorption results in steatorrhea. Other causes of fat malabsorption following gastric surgery include bacterial overgrowth or stasis of the afferent limb, pancreatic insufficiency, or intestinal mucosal dysfunction.

Deficiencies of specific nutrients are often encountered in these patients. Iron-deficiency anemia is seen in 30%–50% of patients at long-term follow-up. The cause appears to be the failure to liberate and absorb iron bound in organic molecules in food. When these patients are encountered, marginal or anastomotic ulcer and bleeding from occult gastrointestinal neoplasia must be excluded. Megaloblastic anemia following gastrectomy results from either folate or vitamin B-12 malabsorption. Intrinsic factor, required for vitamin B-12 absorption, is secreted by gastric parietal cells. Vitamin B-12 deficiency may follow gastric resection or occur because of gastric atrophy from gastritis or loss of trophic stimuli such as gastrin. Vitamin B-12 malabsorption may also result from blind loop syndrome with bacterial overgrowth. Osteomalacia from impaired calcium and vitamin D absorption has also been reported. Nutritional and metabolic deficiencies are usually best treated by replacement therapy and rarely require remedial surgery. Recommendations for postgastrectomy vitamin and mineral replacement are shown in Table 44.6.

Gastric remnant carcinoma

Several reports have suggested an association between previous gastric resection for benign disease and the development of gastric remnant carcinoma. Patients undergoing antrectomy with Billroth II reconstruction appear to have a two- to sixfold increased risk of developing gastric remnant carcinoma. However, this increased risk appears to be a late phenomenon, appearing 15–20 years after the initial surgery [73]. Patients with gastric remnant carcinomas tend to present late in their course, with more advanced disease, and tend to be elderly. Reports have suggested similar outcomes to primary gastric cancers, despite the late presentations [73]. These observations have led some authors to recommend screening following gastric resection, with radiographic or endoscopic surveillance beginning 15–20 years after the original resection, especially if the patient experiences new symptoms. However, this recommendation has not been universally endorsed. The low incidence of gastric cancer in the general population and in patients with a previous history of gastric surgery, the lack of evidence supporting a progression of gastric remnant dysplasia to cancer, and the operative risk of total gastrectomy in these high-risk patients argue against routine use of endoscopic surveillance [74,75]. Gastric remnant carcinoma usually requires completion gastrectomy with Roux-en-Y reconstruction.

Recurrent ulcer

Recurrent ulceration following acid-reduction surgery for peptic ulcer disease is an uncommon complication. The incidence varies according to the type of surgery originally performed. Patients undergoing vagotomy and antrectomy or subtotal gastrectomy have a 1%–4% recurrence rate. In contrast, this rate increases to 10% in patients undergoing truncal vagotomy and drainage and 15% or greater in patients undergoing parietal cell vagotomy [1]. Recurrence rates are higher in patients who smoke, and in those with combined duodenal and prepyloric ulcers.

Symptoms may be pain typical of ulcer, or obstructive in nature, related to anastomotic recurrence and stricture. Most commonly, patients complain of episodic midabdominal pain and burning that is often exacerbated by eating and alleviated by antacids. About one-third of patients present with upper gastrointestinal hemorrhage. Perforation is less common, occurring in fewer than 5% of patients. An unusual complication is erosion of a marginal ulcer into a neighboring viscus such as the colon, resulting in a gastrojejunocolic fistula. In this condition, severe diarrhea, weight loss, feculent emesis, fluid and electrolyte disturbances, and malnutrition accompany ulcer pain. Barium enema is the best diagnostic tool, as the fistula is often missed by barium swallow or endoscopy.

The main causes of recurrent ulcers are an inadequate initial operation, continued exposure to environmental ulcerogens, or the Zollinger–Ellison syndrome. Technical features typical of an inadequate initial operation include incomplete vagotomy, retained gastric antrum, or anastomotic stricture. Incomplete vagotomy can be confirmed with the sham feeding test, in which basal and cephalic phase gastric secretions are measured. Retained antrum and the Zollinger–Ellison syndrome can be diagnosed with serum gastrin measurements, with and without secretin stimulation. All patients with suspected recurrent ulcer should undergo gastroscopy. Whenever possible a complete diagnostic evaluation should be undertaken, so that the pathophysiology of ulcer recurrence in a given patient is completely understood.

Table 44.6 Nutrient replacement following gastric resection

Nutrient	Monitor	Sign or symptom	Replacement
Vitamin B-12	B-12	Megaloblastic anemia	Intramuscular, subcutaneous, or intranasal vitamin B-12
Folate	Red blood cell folate	Megaloblastic anemia	Oral folate
Iron	Ferritin	Microcytic anemia	Oral iron supplements
Calcium	Bone mineral density	Osteomalacia	Oral calcium supplements
Fat malabsorption	Qualitative or quantitative fecal fat	Steatorrhea	Pancreatic enzymes

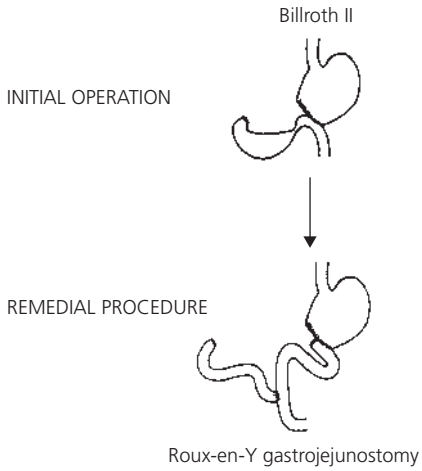


Figure 44.11 Remedial operations for afferent limb syndrome include resection of the obstructed gastrojejunostomy with Roux-en-Y reconstruction. From Glasgow & Mulvihill [1].

The initial management of patients with recurrent ulcer includes a trial of H_2 receptor or proton pump antagonists. Antibiotics are warranted if *H. pylori* infection is suspected. The majority of patients respond initially to medical therapy. Surgery is reserved for patients who fail conservative medical management, those with gastrinoma, and those who develop complications including hemorrhage, perforation, obstruction, or fistulas. Surgical planning should begin with a review of the patient's previous surgical and pathological record to help evaluate the possibility of retained gastric antrum or incomplete vagotomy. Patients with documented incomplete vagotomy can be managed with thoracoscopic truncal vagotomy with minimal morbidity. If the initial procedure was parietal cell vagotomy, reoperation should include truncal vagotomy and antrectomy. For patients previously treated with vagotomy and antrectomy or drainage, a search should be made for residual vagal trunks. In addition, a 50% distal gastric resection with gastrojejunostomy should be performed. If the problem is retained antrum, resection of the tissue at the duodenal stump down to the gastroduodenal artery is indicated. Gastrinoma is best dealt with via resection of the tumor. Results of reoperation are generally good with low rates of rerecurrence.

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B STOMACH

45 Miscellaneous diseases of the stomach

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Hiatal hernias

Under normal circumstances, the stomach is held in position by the gastrophrenic, gastrohepatic, gastroduodenal, and gastrosplenic ligaments. Furthermore, the phrenoesophageal membrane helps to prevent herniation through the diaphragmatic hiatus. This membrane attaches near the squamocolumnar junction and extends approximately 1 cm above this. During swallowing and longitudinal esophageal muscle contraction, there is "physiological" herniation of the gastric cardia through the diaphragmatic hiatus. The phrenoesophageal membrane has a recoil action to pull the squamocolumnar junction back into its normal anatomical position. When these mechanisms are defective, true herniation can develop.

Type I hernias

The most common type of hernia is the sliding, or type I, hernia, with a prevalence of 10%–80% depending on age. In one retrospective analysis, hiatal hernias were identified in 29% of patients with noncardiac chest pain and in 45% of gastroesophageal reflux disease (GERD) patients [1]. Predisposing factors include a widened hiatal tunnel and laxity of the phrenoesophageal membrane, resulting in passage of the gastric cardia into the posterior mediastinum. This herniation is often transient and asymptomatic, but larger hernias (typically defined as greater than 3 cm in length) are often nonreducing and predispose to gastroesophageal reflux. One study has suggested that patients with hiatal hernias are more than twice as likely as patients without hiatal hernias to have inguinal hernias as well [2]. Obesity was also cited as a risk factor for inguinal hernias, and the authors concluded that increased intraabdominal pressure predisposes patients to both types of herniation.

The sliding hernia is an acquired condition with a peak prevalence in the fifth decade of life [3]. Factors such as pregnancy, obesity, deep inspiration, vomiting, coughing, and the Valsalva maneuver, which cause a positive peritoneopleural pressure gradient, predispose to this type of hernia. Longstanding gastroesophageal reflux may also result in a hiatal hernia because of esophageal shortening consequent to acid-induced tonic longitudinal muscle contraction within the esophagus. Age-related degeneration of the phrenoesophageal membrane likely increases vulnerability toward herniation as well. Endoscopically, a sliding hiatal hernia results in cephalad displacement of the squamocolumnar junction above the diaphragm and widening of the angle of His [4]. The cardia, identified by gastric folds, will be seen extending above the diaphragmatic impression. While in gastric retroflexion, this herniation can be seen through the diaphragmatic hiatus because the lower esophageal sphincter has been displaced (Fig. 45.1). As a result of disruption of the antireflux barrier (which is composed of the diaphragmatic crura, phrenoesophageal membrane, and lower esophageal sphincter in anatomical unity), the distal esophagus can also be clearly seen in gastric retroflexion.

Normally, the squamocolumnar junction is located 0.5 cm below the diaphragmatic hiatus, and the gastroesophageal junction high-pressure zone extends about 1 cm distal to this [5]. With a hiatal hernia, two high-pressure zones can be found manometrically because of disruption of the antireflux barrier, one corresponding to the lower esophageal sphincter and one corresponding to the diaphragmatic hiatus. The distal high-pressure zone (at the level of the diaphragm) is augmented by inspiration and abdominal compression. With regard to gastroesophageal reflux, patients with large hernias (over 3 cm long) have weaker and shorter lower esophageal sphincters than do patients with smaller or no hernias; also, distal esophageal peristalsis and acid clearance are less effective [6]. Although esophagitis is more common in patients

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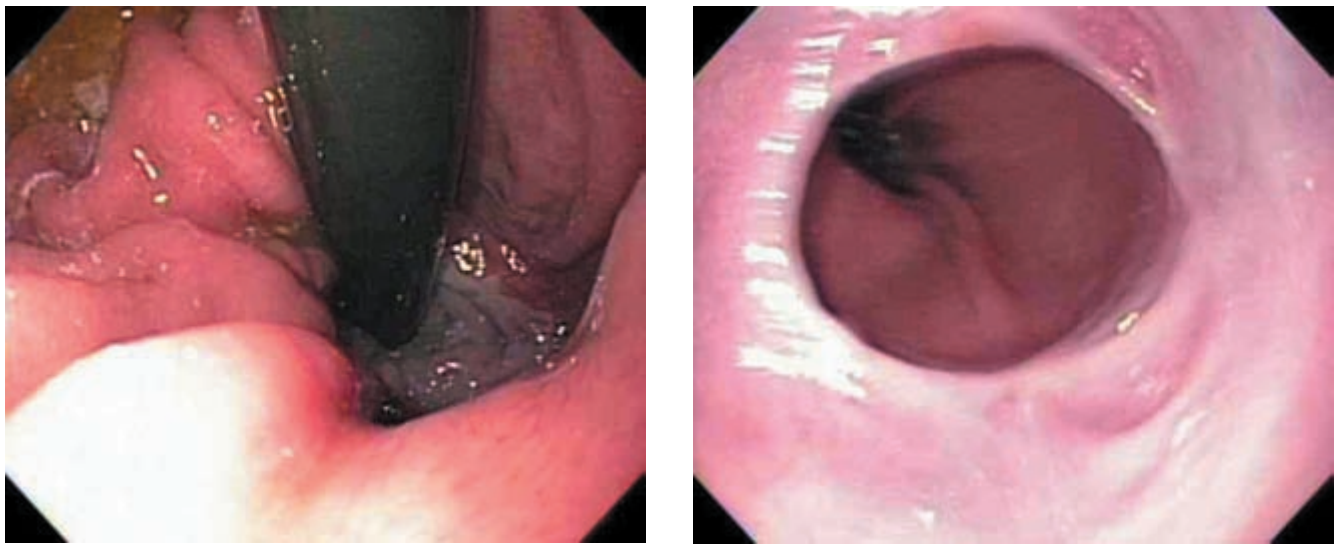

Figure 45.1 Hiatal hernia in retroflexed view.

with a hiatal hernia of any size, it is often more severe in patients with large hernias. Not surprisingly, given the correlation between GERD and Barrett esophagus, hiatal hernias are also associated with Barrett esophagus. In one series, 96% of patients with long-segment and 72% of patients with short-segment Barrett esophagus had a hiatal hernia of at least 2 cm in size, compared with 42% of controls without Barrett esophagus [7]. The mean length of the hernia in patients with Barrett esophagus was nearly 4 cm. In patients with esophagitis but no specialized columnar epithelium, 71% had a hiatal hernia as well. Gastroesophageal reflux disease and Barrett esophagus are discussed in greater detail elsewhere, but their association with gastric herniation is important to note.

Type II to IV hernias

Approximately 5% of hiatal hernias are of types II–IV, the paraesophageal hernias [8]. Unlike type I hernias, these hernias are associated with fundic herniation into the thoracic cavity (i.e., alongside the gastroesophageal junction), whereas the gastroesophageal junction may or may not be displaced as well. Although relatively uncommon, these hernias are associated with severe complications such as gastric volvulus and strangulation. They frequently develop after surgical procedures in the region of the hiatus, such as fundoplication and splenectomy. In an era where laparoscopic Nissen fundoplications are becoming more prevalent, it is important to realize that postoperative paraesophageal hernias develop in 1%–6% of cases [9–12].

In type II hernias there is a localized phrenoesophageal membrane defect allowing the gastric fundus to become the lead point of a herniation. The gastroesophageal junction is still in proper anatomical attachment to preaortic fascia and the phrenoesophageal membrane (Figs 45.2 and 45.3). As more fundus herniates into the thoracic cavity, in part because of gastrocolic and gastrosplenic ligament laxity, gastric rotation can develop along the longitudinal axis of the stomach. This organoaxial volvulus results in the greater curve lying anterior to the lesser curve. Type II hernias can also result in a mesenteroaxial volvulus where rotation is along the transverse axis, but this is much less common. Type

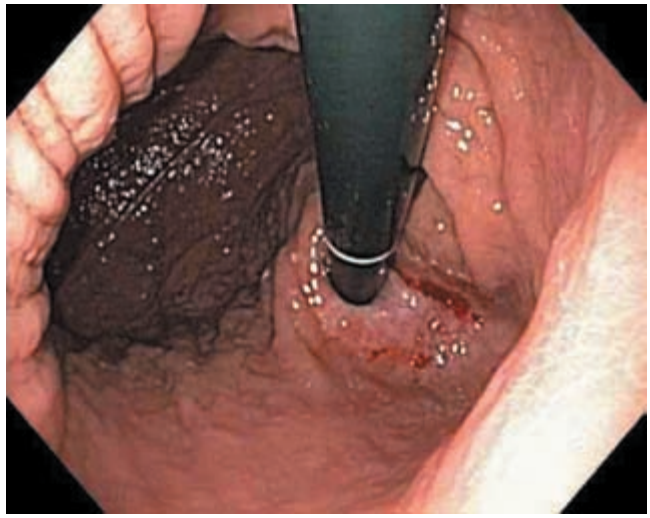

Figure 45.2 Hiatal hernia in standard view.

Figure 45.3 Radiographic view of a type II paraesophageal hernia. The arrow denotes the gastroesophageal junction, which remains below the diaphragm. There is an associated gastric volvulus with a portion of the stomach within the hernia sac.

Figure 45.4 A type III, or mixed, paraesophageal hernia where the gastroesophageal junction is markedly elevated due to gastric herniation. The leading gastric edge has also herniated through the phrenoesophageal membrane and hence is the paraesophageal component of the hernia.

III hernias are a combination of types I and II; the fundus herniates through the hiatus, but stretching of the phrenoesophageal membrane results in displacement of the gastroesophageal junction above the diaphragm as well (Fig. 45.4). Type IV hernias are rare and require a massive hiatal defect that results in herniation of abdominal organs (colon, small bowel, spleen, and pancreas) into the thoracic cavity.

Common symptoms of the paraesophageal hernias include epigastric and substernal pain, postprandial fullness, nausea, pyrosis, dysphagia, and nonproductive retching [13]. A "floppy" fundus, defined by the location of the fundus below the superior portion of herniated gastric body, has been proposed to be an indicator for hernia-related symptoms [14]. Acute bleeding from gastritis or ulceration is rare, but up to one-third of these patients may have iron deficiency anemia resulting from erosions (Cameron ulcers) or venous dilation and engorgement from hiatal constriction and ischemia [15,16] Similar to type I hernias, these hernias are also associated with a hypotensive lower esophageal sphincter, shortened intraabdominal lower eosophageal sphincter (LES) length, distal esophageal dysmotility, and impaired acid clearance [17,18]. Because of the vague nature of many complaints, the true prevalence of paraesophageal hernias is unclear. Nonetheless, up to 20% of such patients may present with acute gastric strangulation or perforation, with associated mortality rates of 50% [19,20].

As a general rule, surgical repair of paraesophageal hernias is indicated only in patients with symptoms [17,21]. Perhaps one exception to this rule is the immediate repair of paraesophageal hernias that develop after routine fundoplication. Many authors recommend obtaining routine barium esophagrams in the first week after fundoplication to rule out the development of a hernia, which is more easily repaired in the immediate postoperative period, even in asymptomatic patients [10,12]. This approach is not unreasonable because these patients are considered to have a failed surgical procedure with persistent gastroesophageal junction derangements and a high likelihood of recurrent symptoms at some point.

The risks associated with paraesophageal hernia repair include bleeding, pneumothorax, vagal injury, early satiety and bloating, diarrhea, and nausea. Up to 20% of patients will have dysphagia initially, and 6% will have chronic dysphagia [17,22]. In addition to hernia reduction and repair of the diaphragmatic defect, fundoplication is routinely performed given the gastroesophageal junction abnormalities associated with paraesophageal hernias. Swanstrom and colleagues followed 52 patients after surgery for paraesophageal

hernias, most of whom had Nissen fundoplications rather than Toupet or Collis–Nissen procedures [22]. Preoperatively, 59% of these patients had pyrosis, 50% had dysphagia, and 26% had esophageal body dysmotility. Postoperatively, pyrosis was reduced to 10%, dysphagia to 6%, and esophageal dysmotility to 13% after a mean follow-up of 18 months. In addition, more than 60% of patients had improved LES pressure after surgery, but 8% developed recurrent herniation. Laparoscopic surgery is feasible in most patients; conversion to an open procedure is necessary about 5% of the time [23]. A study of patients who had undergone laparoscopic hernia repair demonstrated a good or excellent outcome in 80% of patients based on symptoms [24]. It should be noted that the same study had a hernia recurrence rate of 30% and that one-third of the patients with recurrent hernias were asymptomatic. In a Mayo Clinic series comparing open with laparoscopic approaches to hernia repair, there was an overall rate of 17% intraoperative and 19% postoperative complications [25]. In this series, there was only a 2% incidence of hernia recurrence over 14 months.

Although there are no prospective trials with long-term efficacy data that compare open vs laparoscopic repair of paraesophageal hernias, one study has shown that the laparoscopic approach is associated with decreased operative blood loss, decreased rates of ileus, and decreased intensive care and total hospital stays [26]. Patient selection is certainly important because complication rates reportedly increase from 17% to 28% when comparing patients below the age of 65 with those over the age of 75 [27]. Interestingly, this same series reported a hernia recurrence rate of approximately 27% with no difference between various age groups. New approaches have been proposed to improve long-term outcomes. For example, one German group reports only a 1.8% hernia recurrence rate at 6 months by tailoring the type of crural closure (to include the selective use of mesh) to the size of the diaphragmatic defect [28].

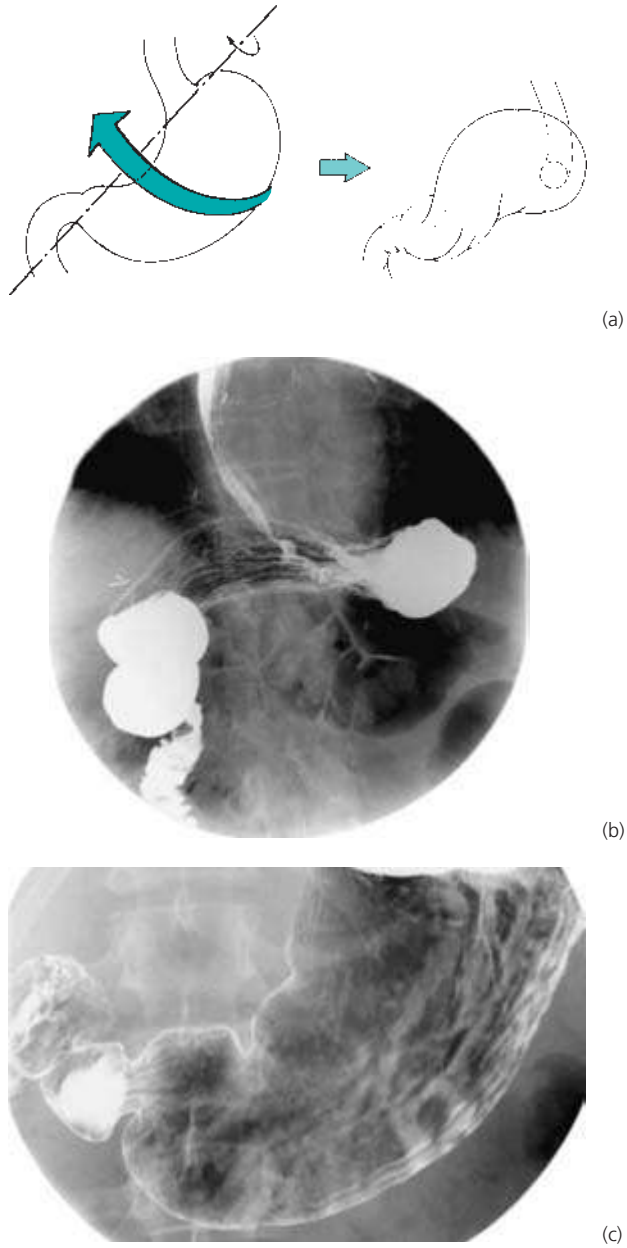

Figure 45.5 **(a)** Schematic representation of an organoaxial volvulus. **(b)** Large organoaxial volvulus with much of the stomach trapped within a hiatal hernia. **(c)** After spontaneous resolution of organoaxial volvulus.

Gastric volvulus

A volvulus of the stomach occurs when one portion of the stomach twists around another. If the twist occurs around an imaginary line between the pylorus and the gastroesophageal junction, it is an organoaxial volvulus (Fig. 45.5). Typically, the greater curve spins upward such that the stomach appears "upside-down" with the true posterior wall lying anteriorly. The antrum rotates anteriorly and superiorly while the fundus is displaced posteriorly and inferiorly. Alternatively, a mesenteroaxial volvulus develops when the distal stomach twists around an imaginary line between the center of the greater curve and the porta hepatis (Fig. 45.6). The antrum and distal body twist to the right (anteriorly and superiorly) such that the posterior wall again becomes anterior in placement; occasionally, the antrum rotates posteriorly in a mesenteroaxial volvulus, although this is rare. Approximately 60% of cases are organoaxial, 30% are mesenteroaxial, and the remainder are either mixed or unclassified [29]. There is no clear prognostic value in defining the type of volvulus, although defining the degree of the volvulus is important [30]. A volvulus may be partial with no symptoms and

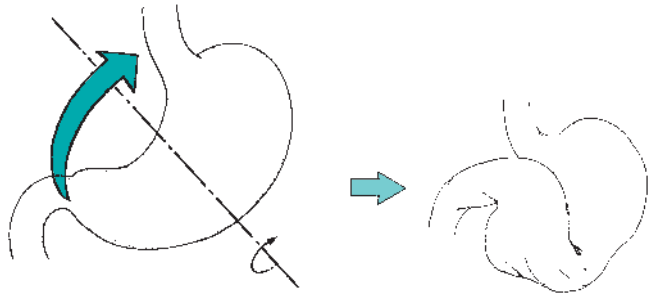

Figure 45.6 Schematic representation of a mesenteroaxial volvulus.

spontaneous resolution, but a complete volvulus can result in vascular compromise, ischemia, and gastric infarction.

The peak incidence of gastric volvulus is in the fifth decade with no clear gender predomination, but 20% of cases may occur in children, usually in the first year of life [29,31]. Predisposing factors include paraesophageal hernias (in up to 76% of cases), abnormal ligament fixation (of the gastrophrenic, gastrohepatic, gastrosplenic, gastroduodenal, and gastrocolic ligaments), prior trauma, and phrenic nerve injury [30,32–34]. Abdominal surgery is another risk factor, largely due to adhesions, but specific procedures such as Nissen fundoplications and gastrostomies are also frequently associated with gastric volvulus [35–37]. In children, ligamentous laxity, splenic mobility, congenital diaphragmatic defects, and hiatal hernias are predisposing factors [31].

Acute gastric volvulus

The acute presentation of gastric volvulus includes substernal or epigastric pain that frequently radiates to the neck and arms and may be confused with a myocardial infarction. Patients may have minimally productive emesis or dry heaves. Hematemesis is rare but suggests mucosal ischemia or tears. The upper abdomen may become tense and distended whereas the lower abdomen is soft. Tympany is not uncommon, and abdominal tenderness may not be impressive. Organoaxial rotations are much more likely than mesenteroaxial rotations to cause an acute volvulus. If the volvulus is complete, it may not be possible to place a nasogastric tube. The Borchardt triad, associated with an acute and complete volvulus, is characterized by abdominal pain, violent retching, and the inability to place a nasogastric tube [38]. In organoaxial volvulus, vascular compromise and gastric infarction occur in 5%–25% of cases, and there is a 15%–20% overall mortality rate with an acute volvulus [35]. In cases of intrathoracic volvulus resulting from a hiatal hernia, emergent surgical mortality rates are 40% but approach 60% if there is gastric strangulation [32,39].

The diagnosis is suggested by the presence of a large gas-filled viscus in the chest or upper abdomen on routine radiography, with the appropriate clinical symptoms. Plain films, however, may be difficult to interpret with an organoaxial volvulus because the stomach still lies in a horizontal position with a single air–fluid level. A nasogastric tube should be placed if possible, although this is unlikely to untwist the volvulus. Although surgery is still considered standard therapy, some have proposed endoscopic reduction as a temporary maneuver in patients where vascular compromise is not suspected. There are case reports of volvulus reduction where the endoscope tip is placed just beyond the torsion, dials are locked, and the scope is rotated 180° to untwist the volvulus [40–42]. Eventually, if not emergently, surgery should be pursued to untwist and repair the volvulus with either anterior gastropexy (where the greater curve is fixed to the anterior abdominal wall) or tube gastrostomy. A subtotal or even complete gastrectomy may be necessary in cases of necrosis. Anatomical defects that might have predisposed to the volvulus can be repaired at the same time. Laparoscopic surgery may be a viable option in many volvulus patients, although this may be a better option in cases of chronic volvulus [43,44]. Given the risk of ischemic strangulation, perforation, and death with acute volvulus, immediate surgical intervention should probably remain the initial treatment of choice, especially as endoscopic reduction does not prevent volvulus recurrence [29].

Chronic gastric volvulus

Unlike the acute volvulus, a chronic volvulus is far more likely to involve a mesenteroaxial rotation. Because this volvulus is usually incomplete and intermittent, patients are more likely to present with chronic and vague symptoms. Abdominal discomfort, dysphagia, pyrosis, fullness, bloating, and postprandial borborygmi are the primary symptoms of a chronic volvulus. Such symptoms are usually worsened after a large meal, and patients may report that changes in body position or self-induced vomiting help to alleviate the symptoms [30]. The diagnosis is often made incidentally on a chest radiograph or barium study. In a mesenteroaxial volvulus, plain films demonstrate a spherical stomach with two air–fluid levels in the upright position; the upper level reflects the antrum whereas the lower level reflects the fundus. The mortality of chronic gastric volvulus is 0%–13% [30,45]. Although surgery is again considered the mainstay of treatment for the chronic and relapsing volvulus, the endoscopic reduction of chronic gastric volvulus and placement of percutaneous gastrostomy tubes (in the body and antrum) has been reported in poor surgical candidates [40–42,46,47]. The need to pursue surgical or endoscopic procedures is unclear in patients with an asymptomatic volvulus or in those with only mild symptoms attributable to the volvulus. In such cases, the risks of surgery or endoscopy must be weighed against the risk of an untreated volvulus.

Gastric rupture

Nontraumatic tears of the gastric wall are relatively rare, but the true incidence is not well defined. In one autopsy study, 0.12% of more than 5100 patients were found to have a spontaneous gastric rupture [48]. Those affected are generally in the fourth decade of life, and women are more commonly affected than men [49]. Gastric ruptures may be as large as 15 cm, and the affected muscle may demonstrate edema and hemorrhage without an inflammatory infiltrate [50]. Initially, there is a mucosal tear followed by dissection of the deeper muscle layers and serosa.

Numerous etiologies may result in gastric rupture. Intragastric pressure and overdistention may play a role in many ruptures, especially if there are pyloric or gastroesophageal junction obstructions to prevent gastric decompression. Normally, the gastric musculature will relax in response to distention, and it has been suggested that distention does not lead to rupture until the intragastric volume is about 4 L [50,51]. Mechanical outlet obstruction, preventing decompression at either the gastroesophageal junction or the pylorus, may develop as a consequence of tumors of the esophagus, pancreas, distal stomach, or duodenum, of complicated peptic ulcer disease, of congenital duodenal obstruction, or of fundoplication [52–54]. There can also be functional obstruction at either gastric orifice. For example, increased intragastric pressure will normally result in a tightened lower esophageal sphincter and an accentuated angulation of the esophagus against the right diaphragmatic crus, both of which tend to prevent gastroesophageal reflux and gastric decompression [49,53,55]. Similarly, a distended stomach may cause angulation and obstruction at the pylorus [56]. Other causes of gastric distention and rupture include gastric lavage in the setting of balloon tamponade for bleeding varices, mouth-to-mouth resuscitation with external cardiac compressions, aerophagia, oxygen administration from nasal cannulation, gastric insufflation during general anesthesia, and fermentation of retained gastric food with associated gas production [53,57–61]. Rapid decompression in diving accidents has also resulted in profound gastric distention and rupture [62–64]. Excessive dosing of sodium bicarbonate for indigestion in the setting of existing overdistention has been reported to cause rupture, although large volumes of sodium bicarbonate would be necessary to generate significant carbon dioxide gas [51,65,66]. Spontaneous rupture of a gastrointestinal stromal tumor (GIST) has also been reported as a cause of gastric rupture [67]. Finally, neonates may be at increased risk for gastric perforation for unknown reasons; in a series of 13 newborns with gastric rupture, 45% were preterm, and mortality was 54% [68].

Trauma and vomiting can also result in gastric rupture. Surprisingly, gastric rupture from blunt abdominal trauma occurs in less than 2% of trauma cases, in part because the stomach is somewhat protected by the ribs and liver [69]. Examples of trauma-induced ruptures include coughing, grand mal seizures, exertion during heavy lifting, the Heimlich maneuver, and motor vehicle accidents with seat-belt compression of the stomach on the vertebrae [58,70–76]. Endoscopic procedures, especially when cautery or laser therapies are involved, can result in a traumatic rupture. Violent vomiting that results in a gastric tear may be seen in peripartum and postpartum emesis, in retching from pyloric stenosis, and in vomiting induced by ipecac [77–79].

In cases of gastric distention, 60% of ruptures occur along the lesser curve near the cardia or at the fundus [50]. It is much less common for such ruptures to occur along the greater curve, on the anterior or posterior walls, or near the pylorus. However, ruptures induced by vomiting typically occur on the greater curve and fundus because violent emesis results in herniation of the stomach into the chest [80]. Symptom onset is immediate, and patients frequently report a history of recent overeating and emesis [50]. Severe pain, abdominal distention, and dyspnea are typical. Shock may develop from peritonitis, blood loss, and tension pneumoperitoneum (which causes inferior vena cava compression with reduced blood return to the heart and hypotension) [56]. Subcutaneous emphysema, where abdominal free air traverses the retroperitoneum and mediastinum to the neck, has also been reported [55]. These symptoms and findings can mimic other intraabdominal catastrophes, such as ulcer perforation, pancreatitis, appendicitis, neoplastic perforation, and splenic rupture. Plain radiographs will typically demonstrate the pneumoperitoneum. Other imaging modalities are of questionable benefit because the clinical scenario typically necessitates immediate surgical attention.

Gastric rupture has a 100% mortality rate without intervention [49]. Death is caused by sepsis, bleeding, respiratory failure, and air embolism [56]. Mediastinitis may also develop with similar end reults [70]. With surgical intervention, mortality rates still exceed 60%. Larger tears, reflecting increased peritoneal contamination, contribute to the high mortality. Perforated ulcers typically have a small tear (i.e., less than 2 cm) and a better outcome, whereas gastric ruptures usually measure 5 cm or greater, and have a poorer outcome [65]. If abdominal pressure is excessive and compromises the patient's cardiorespiratory status, a decompressive paracentesis may be necessary prior to surgical correction [57]. Surgery consists of resection of necrotic gastric tissue, correction of any predisposing obstructive lesions, and peritoneal lavage. Broad-spectrum antibiotics against aerobic and anaerobic organisms are warranted perioperatively. There are case reports of treating gastric perforations with endoclip placement, although this approach is likely only feasible with small or incomplete tears [81,82]. This approach requires

further study and should not be considered the standard approach in the United States.

Foreign bodies

Foreign objects are frequently swallowed, either intentionally or accidentally; in the United States approximately 1500 persons die each year from either swallowing or aspirating foreign bodies [83]. In children, small toys are common, but coins clearly account for most accidental ingestions [84]. In adults, items may be accidentally swallowed as a result of carelessness, poor vision, or alcohol intoxication [85,86]. Such items may be articles that are frequently placed in the mouth, such as toothbrushes, dental prostheses, or nails. Patients with dementia or psychological disease are most likely to intentionally ingest a foreign body, but patients with bulimia may inadvertently swallow objects while trying to induce emesis [87–89]. Incarcerated criminals may swallow objects for secondary gain (hospitalization), whereas drug traffickers may be "body-packers," who intentionally ingest small packets of illicit drugs [85,90].

The vast majority of foreign bodies will traverse the stomach without causing gastric symptoms; symptoms induced by a gastric foreign body suggest mucosal penetration or perforation, peritonitis, or obstruction [85,87]. Mucosal tears, ulceration, perforation, abscess formation, hemorrhage, and fistula formation may all develop as a result of a retained foreign object [85,86]. Sharp objects such as splinters or animal bones are more likely to induce a perforation. When to remove foreign bodies from the stomach is a clinical uncertainty. Objects more than 2 cm in diameter or 5 cm in length should strongly be considered for removal because such items may lead to duodenal obstruction [91]. Due to the risk of perforation, sharp objects should also be removed promptly. If a sharp object is too large to reasonably retrieve endoscopically, a laparoscopic gastrostomy or other surgical methods may be necessary [92]. Blunt objects that are less than 2 cm in diameter or 5 cm in length may be followed with serial radiographs. If the object remains in the stomach for 3 weeks or if the patient develops symptoms of mucosal irritation, endoscopic retrieval of the foreign body should be pursued.

Before attempting endoscopic removal, the patient should be placed in the left lateral decubitus position, and glucagon may be administered to limit motility (although this is more likely to be beneficial for objects in the duodenum than ones in the stomach). Although there is some risk of perforation, a protective overtube may be placed in the esophagus for sharp or difficult-to-grab objects to prevent the possibility of aspiration or esophagopharyngeal trauma [93,94]. Standard biopsy forceps or snares may be used, but larger forceps are available specifically for foreign body removal. Oval or round objects may be trapped within a basket or net. Surgery should be considered before endoscopy for large, jagged objects, and for "body-packers" where there is a significant risk of death from the endoscopic rupture of a packet containing illicit drugs. With the use of commercially available forceps, snares, baskets, and nets, endoscopic retrieval of foreign bodies is successful in 94%–98% of cases [95,96].

The accidental ingestion of button batteries deserves special mention. These batteries contain alkaline compounds such as sodium or potassium hydroxide. Damage to the stomach may occur as a result of low-voltage burns, pressure necrosis, or direct corrosion [84]. Batteries lodged within the esophagus should be removed immediately by endoscopy. If the plain radiograph demonstrates the battery within the stomach and the patient is asymptomatic, the patient can be followed with serial radiographs. Endoscopic removal is warranted if the battery remains in the stomach at 48 hours, if it is greater than 15 mm in diameter, if it is a mercury-based battery, or if the patient is symptomatic with localized abdominal pain, hematemesis, or melena [97]. Ipecac syrup should never be administered in cases of battery ingestion because it has not been shown to be effective and may result in esophageal impaction of the battery [97]. If there is battery disruption, heavy metal levels (dependent on the type of battery) in the blood and urine should be measured.

Caustic ingestion injury

Often more dangerous and damaging than foreign bodies is the ingestion of caustic materials. Common household cleansers and chemicals may contain compounds such as sodium or potassium hydroxide, sodium carbonate, aluminum hydroxide, and bleaches like hydrogen peroxide. Acids tend to cause immediate pain and are quickly expelled by the patient, even in the setting of an intentional ingestion. However, alkali agents are often odorless and tasteless, leading to more significant ingestions. Furthermore, alkali agents penetrate tissue more rapidly than acidic agents because of their solvent action on lipoproteins [98]. Acute manifestations of a caustic ingestion include persistent salivation, odynophagia or even a complete inability to swallow, and the development of palatal or pharyngeal ulcers and white plaques [99,100]. Hoarseness, stridor, and hematemesis may also develop. In a series of 378 patients with acid ingestions, 3% developed massive upper gastrointestinal bleeding between 9 and 21 days after the ingestion [101]. The majority of these hemorrhages were from the stomach rather than the duodenum, and 90% of gastric bleeds required surgical intervention. The presence of peritonitis or mediastinitis suggests a perforation. Acute and chronic manifestations of a caustic ingestion are more commonly seen in the oropharynx and esophagus, but gastric damage may result in late manifestations such as early satiety, weight loss, and vomiting

(symptoms of a possible outlet obstruction). A reasonable approach to the patient with caustic ingestion should begin with abdominal and chest radiographs.

These radiographs are most useful to exclude the presence of free air or esophageal and gastric distention, which are commonly seen after caustic ingestions. If there is no free air but perforation is still suspected, barium studies or a computed tomography (CT) scan can be pursued. The patient may be sent home if there is no free air on plain films, if there are no symptoms, and if the ingestion was accidental. Obviously, admission and surgical consultation are warranted if there is evidence of perforation. If the patient has respiratory or gastrointestinal symptoms or if the ingestion was intentional, hospital admission is necessary. The patient should ingest nothing by mouth initially, and psychiatric consultation should be considered. If the patient has significant respiratory complaints, laryngoscopy may be justified to determine the need for intubation or tracheostomy. Endoscopy is warranted in all cases, but the timing of an endoscopic examination is unclear. Although some authors recommend immediate endoscopy, most recommend an examination at 24–72 hours better to define the extent of injury [99,100]. An endoscopic grading system has been proposed:

- grade I: erythema and edema
- grade II: hemorrhage, erosions, blisters, and superficial ulcers (IIA, linear; IIB, circumferential)
- grade III: multiple, deep, brown-black or gray ulcers
- grade IV: full perforation.

For grade I–IIA damage, liquids can be started with dietary advancement as tolerated. Grade IIB or III patients should have a nasogastric tube placed for gastric decompression, and acid suppression should be employed. Oral liquids may be started approximately 48 hours after endoscopy if the patient is swallowing well. Immediate surgery is warranted in patients with grade IV damage.

Acid neutralizers have been used to limit caustic damage in the past, but these are no longer recommended because heat production from the neutralization process has been shown to worsen tissue injury [102]. Owing to the risk of esophageal strictures and antral stenosis, some investigators have proposed administering oral corticosteroids, based on animal or anecdotal data only. The only randomized controlled trial performed suggested that steroids did little to prevent or limit esophageal strictures, and routine steroid use is not recommended by all clinicians [103].

In the long term, caustic gastric injury may lead to antral stenosis within 3–6 weeks of the ingestion, or it may be delayed for many years. It is not unreasonable to perform an upper gastrointestinal series at 3–6 weeks to look for this complication. Some cases of antral or pyloric stenosis may respond well to endoscopic balloon dilation, but most patients will eventually require a surgical pyloroplasty or distal gastrectomy. In patients with prior caustic injury who are undergoing endoscopy for other reasons, antral hyalinization has been reported and may mimic gastric Crohn's disease or malignant disease [104]. Corrosive damage may increase the risk for subsequent gastric cancer, but such reports are isolated. Endoscopic surveillance is not warranted, based on the available literature [105].

Gastric bezoars

Bezoars are concretions of foreign material that are retained most frequently within the stomach but have also been found in the esophagus and rectum [100]. Such matter may include plant and vegetable debris (phytobezoar), hair (trichobezoar), medications (pharmacobezoar), and persimmons (disopyrobezoar). The true prevalence of bezoars is uncertain because many patients are asymptomatic, but bezoars are uncommon in the intact stomach, with a reported prevalence of 0.4%. However, 10%–25% of patients with prior antrectomies have been found to have symptomatic bezoars [106,107].

The formation of bezoars is likely multifactorial. Altered gastric motility and emptying are the primary causes, but size and digestibility of swallowed material are factors as well. Inadequate mastication, missing teeth, and poorly fitting dentures may also contribute to bezoar formation [108,109]. Prior gastric surgery, whether it be a pyloroplasty, antrectomy, Roux-en-Y gastric bypass, or partial gastrectomy, clearly places patients at risk for phytobezoar or fungus ball formation [110,111]. Surgery can induce gastric atony and dysmotility, but outlet obstruction from stenosis and decreased secretion of acid and pepsin also play a role in bezoar formation. Furthermore, many postsurgical patients develop chronic gastropathy that leads to increased mucus production, and this mucus may act like cement for developing concretions [112]. In an era of antiobesity surgeries, Roux-en-Y gastric bypass and even laparoscopic adjustable gastric banding have been associated with bezoar formation [111,113]. In addition to surgery, gastric stasis and bezoars have been linked to diabetic gastroparesis, mixed connective tissue disease, hypothyroidism, and myotonic dystrophy [114–117].

Phytobezoars commonly comprise apples, grapes, oranges, cherries, raisins, bran, oats, cabbage, potato peels, peanuts, and celery [108,118]. Bezoars may also be composed of nonfood items such as plastic, paper, string, or Styrofoam. Disopyrobezoars develop when unripe persimmon material comes into contact with gastric acid; a tannin in the fruit forms a coagulum that acts as a base for bezoar formation. Trichobezoars may be large, depending on the amount of hair consumed. Medications have reportedly been trapped within hair fibers, and drug toxicity has been attributed to altered drug metabolism [119]. Trichobezoars may also contain food material and fat. Fungus balls, also termed yeast bezoars, are associated with prior gastric surgery and fre-

quently resolve without therapy. For symptomatic fungus balls, gastric lavage, antimycotics, and gastric outlet reconstruction have all been used [120,121].

Concretions are typically hard bezoars. Shellac, furniture polish, and concrete are classic components of such bezoars, and surgery may be necessary to remove concretions because other therapies are generally ineffective. Pharmacobezoars may develop in patients with normal or abnormal gastric physiology. Aluminum hydroxide antacids have caused bezoars in patients with renal failure, and the cellulose-based coating of enteric coated aspirin has been problematic in other patients [122,123]. In patients with achlorhydria, calcium and magnesium carbonate preparations may not dissolve normally. Sucralfate will occasionally form concretions at normal doses and in patients with gastric outlet obstruction, so this agent should be avoided in patients with outlet obstruction or significant dysmotility [124]. Other drugs with capsules that do not digest (such as certain nifedipine or mesalamine preparations) should also be avoided in patients with gastric outlet narrowing [125].

Many patients with bezoars are asymptomatic, but epigastric pain, early satiety, nausea, and vomiting are common manifestations of a bezoar [100,108]. Bloating, malaise, and weight loss may also be reported. In some patients, these symptoms may persist despite bezoar resolution; in such cases, the nonspecific symptoms may be more reflective of the gastric dysmotility than of the actual presence of a bezoar. In patients at risk for bezoar formation, symptoms of a small bowel obstruction may reflect the passage of a bezoar into the small bowel, although this is rare [126]. Gastric outlet obstruction and gastric bleeding (hematemesis or melena) can also be complications of bezoars. Given the nonspecific nature of the symptoms, the patient's history may suggest other causes such as peptic ulcer disease, gastric cancer, or many other intraabdominal processes. On physical exam, potential findings include abdominal tenderness, a palpable mass, and a succussion splash [100]. Plain radiographs may demonstrate an air shadow or a mottled-appearing mass in the stomach. Barium studies are much more likely to demonstrate a bezoar; trichobezoars "absorb" barium and have a mottled appearance whereas phytobezoars and concretions are typically impermeable to barium (Fig. 45.7). Radiographic studies may miss a bezoar, and endoscopy remains the test of choice. Although other studies such as ultrasound or CT may demonstrate a bezoar, endoscopy is best for determining the type of bezoar, for excluding cancer, and for evaluating the mucosa for associated ulceration or outlet obstruction.

Figure 45.7 Gastric bezoar on upper endoscopy examination.

Enzyme therapy

Because many bezoars contain cellulose, protein, and mucus, enzymatic degradation of phytobezoars may be possible. For bezoars containing fibrous material, papain may be a useful proteolytic enzyme. Although this enzyme can be individually purchased, papain can be obtained more cheaply by using Adolph's Meat Tenderizer [1 teaspoon in 115 mL (4 ounces) of water before each meal] [100,118]. Some caution should be used with the latter salt-containing preparation (1800 mg per tablespoon) because hypernatremia is a potential problem. Cellulase may be used for phytobezoars as well. The cellulase tablets are frequently crushed and given after each meal, or 2 L of cellulase solution may be consumed over the course of 2 days (20 g cellulase in 2 L of water) [118,127]. Biochemical supply companies can provide papain and cellulase preparations. Because many phytobezoars contain mucus as well, the mucolytic agent acetylcysteine may also be used. This agent has been reported to be effective at a dose of 15 mL in 50 mL of saline administered through a nasogastric tube two times per day for 2 days; although not specifically stated in this case report, it is presumed that a commercially available 20% acetylcysteine preparation was used and diluted to a 5% solution as recommended by the manufacturer for other indications [128]. If there is ulceration associated with the bezoar, some investigators have suggested that mucolytic and cellulolytic agents may be safer than the proteolytic regimens provided.

Mechanical therapy

Many bezoars contain insoluble agents that are not amenable to enzyme therapy, and some patients are so symptomatic that more urgent therapy is warranted. Small bezoars may be retrieved like other foreign bodies with endoscopic baskets, snares, or forceps. An esophageal overtube is frequently used to prevent inadvertent aspiration of the bezoar [129]. Similarly, many bezoars can be mechanically disrupted into small pieces with forceps such that the pieces are retrieved by suction or allowed to pass through the pylorus. For soft and permeable bezoars, a large-bore orogastric lavage tube can be placed for aggressive water lavage with subsequent

aspiration of the fragmented bezoar. Frequently, a combination of endoscopic fragmentation and subsequent orogastric lavage is used, with immediate benefits to the patient.

Other modes of mechanical disruption have been proposed. Plastic tubing has been placed through an endoscope channel and attached externally to a Teledyne Water Pik system. Water or an enzymatic solution can be pulsated against the bezoar to allow fragmentation and subsequent gastric lavage [118,130]. Neodymium:yttrium–aluminum–garnet (Nd:YAG) laser may be useful for refractory bezoars, although multiple sessions may be necessary [131]. Extracorporeal shock wave lithotripsy and mechanical lithotripsy – with an endoscopic retrograde cholangiopancreatography (ERCP) lithotriptor for hard bezoars – have also been successful [132,133]. In patients who have undergone gastroplasty and in whom the surgical stoma may be too small (predisposing to bezoar formation and not allowing passage of bezoar fragments), the stoma may require balloon dilation or even an endoscopic incision with a papillotome [134,135].

Endoscopic management is warranted for symptomatic patients only. If a bezoar is incidentally found or if symptoms are minimal, enzymatic and prokinetic therapies are likely adequate. Endoscopy is clearly indicated for more symptomatic bezoars, especially if complications such as obstruction develop.

Medical therapy

Prokinetic agents such as metoclopramide may be used on a long-term basis to aid in the prevention and recurrence of bezoars. Metoclopramide has been used alone and with concomitant endoscopic therapy in the acute setting (40 mg intravenously over 24 hours) with some success for the management of symptomatic bezoars, but patients should also be placed on a clear liquid diet under such circumstances [136]. Although liquid diets may have some benefit in bezoar dissolution, this is a slow process that is not practical as sole therapy. The prokinetic benefit of long-term oral metoclopramide use must be weighed against the severity of the patient's symptoms and the significant side-effect profile. Domperidone, not available within the United States, may be another option, with fewer side effects but similar gastric effects compared with metoclopramide. Erythromycin and tegaserod are other agents that can be used to promote gastric motility and to decrease the risk for bezoar formation, although tegaserod has been removed from the open market in the United States and is available for this indication only with a compassionate use approval from the Food and Drug Administration [137].

For patients with or at risk for the development of bezoars, prokinetic agents should be considered, based on the patient's symptom severity. Conversely, all such patients should follow basic dietary guidelines to limit bezoar formation. Specifically, smaller but more frequent meals are advisable to promote gastric emptying. Patients should chew their food adequately into small pieces. Foods that easily coalesce into a bezoar, such as persimmons, raw fruits, and stringy vegetables, should be avoided [108].

Surgical therapy

Surgery is rarely required for bezoars but may be necessary in the setting of bezoar complications such as perforation, gastric or small bowel obstruction, and uncontrollable hemorrhage. Symptomatic trichobezoars often require surgery more than other bezoar types because they are resistant to standard management techniques. Of course, other bezoars may also require surgery if they cannot be treated by the methods outlined earlier. Because surgery is unlikely to correct the underlying cause of bezoar formation and may worsen gastric motility and emptying, such a course should not be undertaken lightly.

Heterotopic pancreas

Heterotopic pancreatic tissue, also termed a "pancreatic rest," has been identified in 0.5%–14% of persons at autopsy [138]. These rests are found in the stomach, duodenum, or jejunum in 75% of cases but may also be seen in the lung, gallbladder, common bile duct, ileum, Meckel diverticula, and numerous other locations. Typically, these rests are asymptomatic, but abdominal pain, nausea, vomiting, and rarely bleeding have been attributed to rests located in the stomach. In one series of 17 patients, pancreatic rests were the presumed cause of pain in 77% of patients, of abdominal fullness in 30%, and of melena in 24% [139]. After surgical resection of the rests, significant symptom improvement was reported, but it is still somewhat controversial if rests truly do cause symptoms to warrant surgical excision. These lesions typically appear as 2–4-cm submucosal masses in the prepyloric region, and central umbilication is not unusual (Fig. 45.8). The nodules are firm and may be yellow. Because this typical appearance is not always appreciated, rests have been mistaken for malignant tumors in 67% of upper endoscopies and in 71% of barium studies [139]. If the endoscopic appearance is not diagnostic, endoscopic ultrasound may be helpful in the evaluation, especially when deep biopsies or surgical resection are being considered [140]. Surgery is indicated only in cases of symptomatic pancreatic rests.

Squamous cardiac epithelium

Squamous epithelial extension into the proximal stomach has been reported. In the earliest report, 16 patients, all male and nearly all Caucasian, were described [141]. Most of the patients had pyrosis (75%) and hiatal hernias (100%), and many had Barrett esophagus (38%). No patient had a history of caustic ingestion or prior gastric surgery. Most had solitary

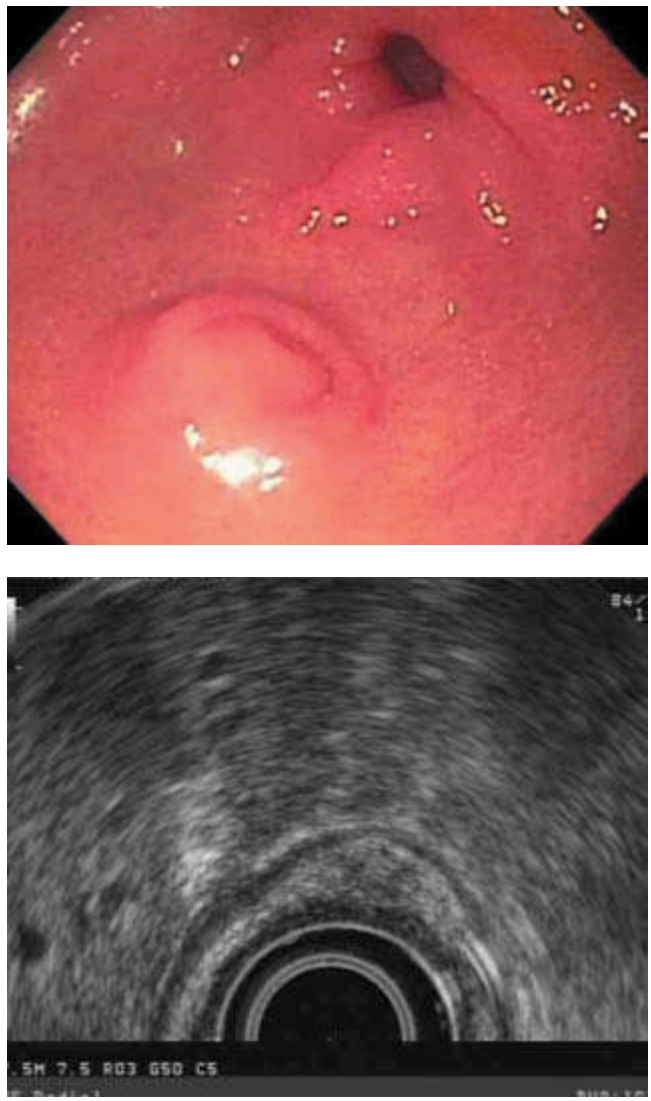

Figure 45.8 **(a)** Pancreatic rest identified in the antrum during upper endoscopy. **(b)** Endoscopic ultrasound appearance of pancreatic rest.

tongues of this squamous epithelium in the proximal stomach, but multiple tongues and islands were also identified. Squamous metaplasia of the stomach has also been described on the lesser curvature of the stomach, near the cardia [142]. Prior gastric injury, such as that from gastric ulcers or electrocautery, has been proposed as an explanation [141–143]. For example, 12 patients with previous ablation of Barrett esophagus with multipolar electrocautery have been reported to develop squamous epithelium in the gastric cardia (mean length 1.7 cm). Although some investigators have proposed that squamous epithelium in the stomach represents metaplasia from prior injury, others state that this phenomenon is a normal variant [144]. In reality, the true incidence and clinical significance of this finding are unknown.

Note

The views expressed in this article are those of the author and do not reflect the official policy or position of the United States Air Force, Department of Defense, or the US Government.

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C SMALL INTESTINE

46 Small intestine: anatomy and structural anomalies

Deborah C. Rubin

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Gross anatomy

The small intestine begins at the pyloric sphincter of the stomach and extends to the cecum. Approximately 600 cm long, the small bowel is composed of three major segments: the duodenum, the jejunum, and the ileum. The junction between the duodenum and the jejunum is anatomically demarcated by the ligament of Treitz. No similar landmark distinguishes the jejunum from the ileum, but the jejunum is usually defined as the proximal two-fifths of the small bowel after the duodenum, and the ileum as the distal three-fifths.

After the appropriate rotation of the gut and its return to the abdominal cavity in fetal life, the normal position of the small bowel loops is attained. The jejunum is generally located in the mid upper and left upper quadrants, the proximal ileum in the middle abdominal region, and the distal ileum in the right lower quadrant [1].

The first part of the duodenum, the bulb, is invested with mesentery. The descending, transverse, and ascending parts of the duodenum are retroperitoneal and partially encircle the pancreatic head (Fig. 46.1). The jejunum begins at the ligament of Treitz as the small intestine reenters the peritoneal cavity. The jejunum and the ileum are invested with mesentery. The most distal part of the ileum may occasionally be retroperitoneally located [2].

The gross appearance of the small intestine changes from the jejunum to the ileum. The wall of the jejunum generally is thicker than that of the ileum. Also, the plicae circulares, or circular folds of mucosa and submucosa that invaginate the lumen and increase the gut surface area, are prominent in the duodenum and jejunum and disappear in the middle ileum [1]. Peyer patches usually are found on the antimesenteric border of the small intestine. They are particularly prominent in childhood and atrophy with aging [1].

Extrinsic arterial, venous, and lymphatic supply

The arteries, veins, and lymphatics that supply the small bowel travel through the mesentery (see Chapter 113). The hepatic artery gives rise to the gastroduodenal artery, which branches into the anterior and posterior superior pancreaticoduodenal arteries. These anastomose around the duodenum and communicate with the inferior pancreaticoduodenal artery, which arises from the superior mesenteric artery. The superior mesenteric artery also supplies the jejunum and the ileum through a series of branches that form numerous arcades in the mesentery and then penetrate the intestine.

The superior mesenteric vein serves as the major venous conduit and joins the splenic vein to empty into the portal vein. Small lymph channels or villus lacteals drain into mesenteric lymph nodes located near the intestine and along the superior mesenteric and celiac arteries. These drain into the cisterna chyli and the thoracic duct.

Neural supply

The intestine contains an abundant, complex intrinsic neural supply that coordinates motor activities and consists of myenteric (Auerbach) and submucosal (Meissner) plexuses (see Chapter 2). The extrinsic autonomic innervation of the small intestine consists of components from the parasympathetic and sympathetic systems [3]. Postganglionic fibers arising from the superior mesenteric ganglion supply sympathetic motor innervation. These synapse with preganglionic fibers from the spinal cord in the region of the 10th and 11th thoracic roots and travel in the lesser splanchnic nerve. Adrenergic neurons innervate both the Auerbach and the Meissner plexuses. Sensory nerves arise from the dorsal root ganglia. The parasympathetic motor innervation consists of preganglionic nerve fibers that arise from the vagus nerve.

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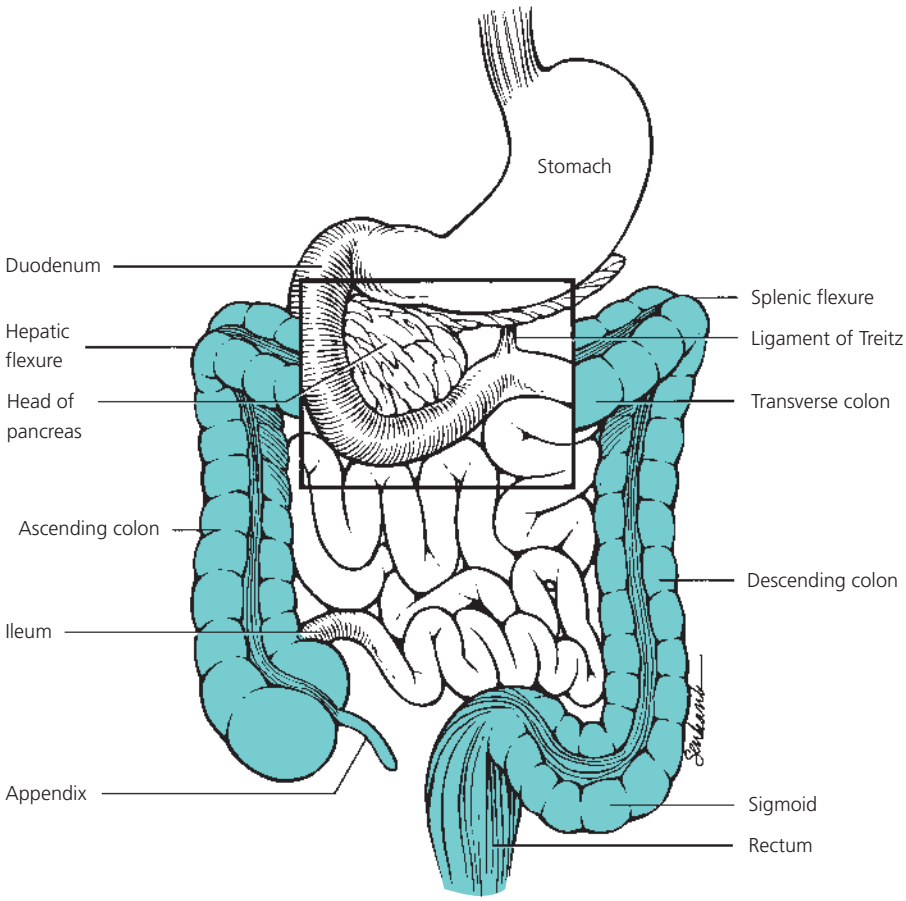

Figure 46.1 Anatomical relations of the stomach, small intestine, and large intestine. The duodenum encircles the head of the pancreas and is retroperitoneal. As the intestine reenters the peritoneal cavity at the ligament of Treitz, it becomes the jejunum. Generally, the jejunal bowel loops are located in the left and middle upper abdomen, the proximal ileum in the middle abdominal region, and the distal ileal loops in the right lower quadrant. **Inset:** Retroperitoneal duodenal loop and the ligament of Treitz, located behind the transverse colon.

Vagal fibers originate from the dorsal motor nucleus and then divide into esophageal, anterior, and posterior vagal trunks. Both the anterior and posterior trunks give rise to celiac branches that directly innervate the small bowel by communicating with the intrinsic nervous system.

The axons of a small number of intrinsic nerves project from the intestine to travel with extrinsic nerves and synapse with sympathetic postganglionic neurons at the sympathetic prevertebral ganglia.

Enhancement of small intestinal surface area: gross and microscopic features

The surface area of the small intestine is enhanced by three features that are peculiar to the gut: the plicae circulares, the villi, and the microvilli (Fig. 46.2) [2]. The plicae circulares, or circular folds, consist of visible mucosal or submucosal invaginations that are predominantly located in the duodenum and jejunum. The villi, fingerlike projections that protrude into the intestinal lumen, are approximately 0.5–1.5 mm long and cover the mucosal surface. They can be viewed by close inspection of the mucosa under low-power microscopy or as tiny mucosal protrusions at endoscopy. They consist of a layer of epithelial cells overlying the lamina propria. Their microscopic appearance varies: duodenal villi are characteristically broad and leaf-shaped, jejunal villi are tall and thin, and ileal villi are short and broad. The length and shape of the villi also vary with the geographical region; in some underdeveloped regions of the world, villi tend to be shorter than in the United States [4,5]. At the base of the villi, the epithelium enters the lamina propria and forms the crypts of Lieberkühn (Figs 46.3 and 46.4), which extend almost to the muscularis mucosae. The microvilli are sub-light microscopic tubular projections that are extensions of the apical cell membrane and compose the brush border. This complex membranous network contains the enzymes, receptors, and carriers required for terminal digestion and absorption.

Microscopic anatomy

The small intestine is composed of four concentric layers: the serosa, muscularis propria, submucosa, and mucosa.

Serosa and muscularis propria

The thin serosal lining of the small bowel consists of mesothelial cells overlying loose connective tissue. This outer layer becomes continuous with the mesentery as it joins the small bowel; many large blood vessels course through it.

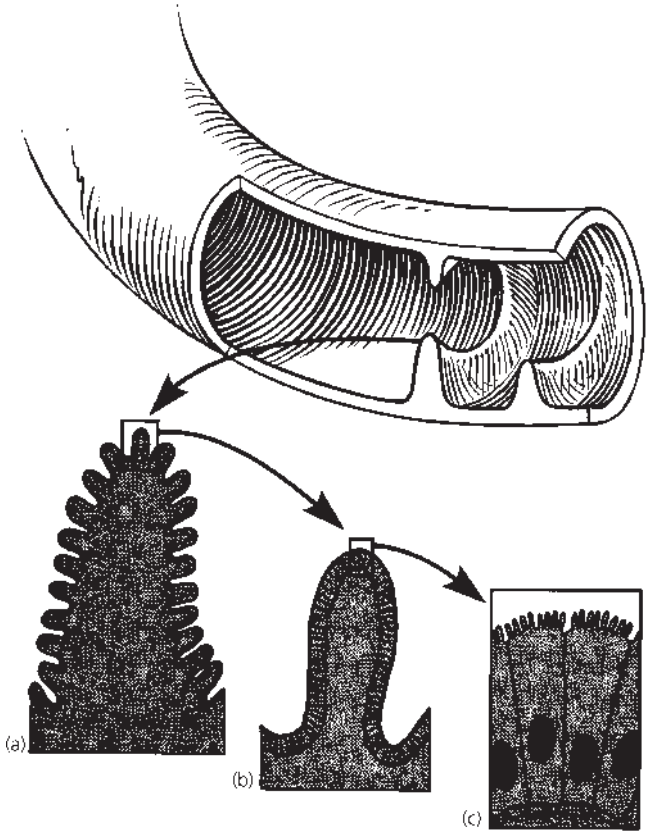

Figure 46.2 The surface area of the small intestine is enhanced by three mechanisms: the valvulae conniventes **(a)**, the villi **(b)**, and the microvilli **(c)**. Adapted from Weaver [82].

Figure 46.3 This semithin 1-μm epon section of human jejunum, stained with toluidine blue and viewed by light microscopy, depicts the mucosa. The villi are formed by a continuous sheet of columnar epithelial cells and underlying connective tissue, the lamina propria. The epithelial sheets are composed primarily of enterocytes; mucus-secreting goblet cells are also present but are much less abundant, as are rare enteroendocrine cells. At the base of the villi, the epithelial cells descend into the lamina propria to form the crypts, one of which is denoted at the left (c). The crypts are lined mainly by undifferentiated crypt cells, which can be observed in mitosis (arrows). Differentiation proceeds as these cells migrate up the villus. Paneth cells at the bases of the crypts are recognized by their dark granules. The muscularis mucosae (m), a thin layer of smooth muscle, separates the mucosa from the submucosa below. Original magnification × 150. From Rubin W. The epithelial "membrane" of the small intestine. Am J Clin Nutr 1971;24:45.

Those portions of the gut that are invested with mesentery are completely covered by serosa, whereas the retroperitoneal segments are invested only on the anterior surface. The muscularis propria consists of two muscle layers: an outer, longitudinally oriented layer and an inner, circumferential layer. Between the two layers lies the myenteric or Auerbach nerve plexus, composed of intrinsic ganglion cells and nerve fibers. The muscular layers of the gut are responsible for generating coordinated peristaltic movements.

Submucosa

The submucosa is a dense connective tissue layer containing numerous arteries as well as venous and lymphatic plexuses. Typical connective tissue cells are also present, but vascular structures predominate, consistent with the role of this portion of the gut as a conduit for absorptive and digestive products. In the duodenal bulb and descending duodenum are specialized Brunner glands that secrete mucus and bicarbonate. These glands are branched epithelial structures that fill the submucosa and are thought to be important in neutralizing stomach acid. The submucosa also contains ganglion cells and nerve fibers, termed the Meissner plexus, a collection of autonomic nerves that communicate with the Auerbach plexus. A variety of neuroendocrine substances are produced in the Meissner plexus (see Chapter 2). These plexuses interact to produce regulated, coordinated gut peristalsis.

Mucosa

The mucosal layer of the gut consists of epithelial cells overlying the lamina propria, or connective tissue core, and resting on a narrow layer of smooth muscle, the muscularis mucosae (see Fig. 46.3).

Lamina propria

The connective tissue core of the villus (lamina propria) contains a variety of cells and vascular structures (Fig. 46.5). The immune cellular component includes lymphocytes,

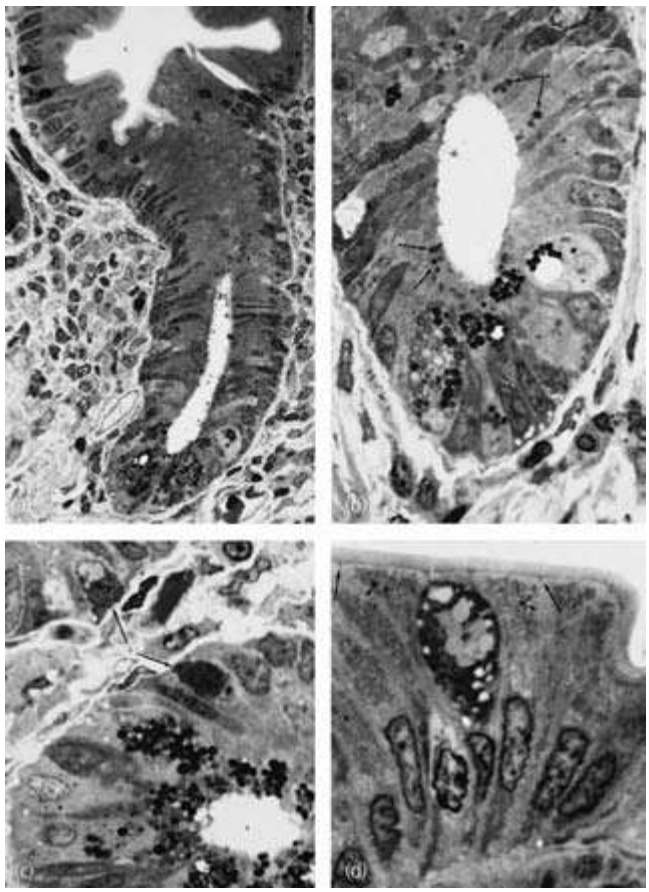

Figure 46.4 High-power views of the crypt. **(a)** A crypt is observed extending to the base of a villus. Note the prominent brush border on the differentiated cells above the villus base and the inapparent brush border on the crypt cells below. The arrow shows a short and irregular brush border on an upper crypt cell. Large, dense Paneth cell granules are evident at the crypt base; the smaller apical granules higher in the crypt are in undifferentiated crypt cells. Original magnification × 300. **(b)** The large granules in Paneth cells at the base of another crypt are located mainly in the apical cytoplasm. The scantier, lighter apical granules in cells above (arrows) are in undifferentiated crypt cells. Original magnification × 700. **(c)** The large, dense apical granules in Paneth cells at another crypt base should be contrasted with the smaller, barely resolved basal infranuclear granules observed in two endocrine cells (arrows). Original magnification × 700. **(d)** Differentiated villus epithelial cells and a goblet cell. Note the tall, prominent brush border. The narrow, light zone below the brush border represents the terminal web. The numerous light structures within the cells are mitochondria. The occasional dense apical structures are lysosome derivatives (arrowheads). The apical densities between adjoining cells at the level of the terminal web (arrows) are the terminal bars. They represent the junctional complexes of electron microscopy – the tight junctions, intermediate junctions, and desmosomes; these are specialized structures that serve to bind adjoining epithelial cells at their apices. The accumulation of mucus granules within the apical cytoplasm of the goblet cell distends it into the shape of a brandy goblet. Original magnification × 1500. From Rubin W. The epithelial "membrane" of the small intestine. Am J Clin Nutr 1971;24:45.

Figure 46.5 Upper villus region, with lamina propria, goblet cells, enterocytes, and intraepithelial lymphocytes (arrow). Hematoxylin–eosin stain; original magnification × 495. From Phillips AD. The small intestinal mucosa. In: Whitehead R (ed.). Gastrointestinal and oesophageal pathology. New York: Churchill Livingstone, 1989:29.

macrophages, granulocytes, plasma cells, and mast cells (see Chapter 7). The majority of lamina propria T lymphocytes are T-helper/inducer cells that are surface antigen $CD4^+$, although smaller numbers of T-cytotoxic/suppressor cells ($CD8^+$) are also present [6]. The intraepithelial lymphocytes (IELs) reside on the basolateral side of the villus epithelial cells, and can be classified as type a or type b IELs [7]. The small intestine IELs are type b, expressing T-cell receptor subtypes TCRαβ and TCRγδ. Type a cells are present but are more enriched in the colon, and are TCRαβ and surface marker CD4 or CD8αβ positive. Type a cells are more like peripheral spleen and lymph node T cells, and are present in the lamina propria. They are acquired memory T cells that migrate into the gut after an encounter with antigens outside the gut. Type b cells, in contrast, are permanent residents of the gut epithelium and are natural memory T cells [7,8]. The lamina propria plasma cells produce immunoglobulin, especially dimeric immunoglobulin A (IgA), which is taken up into the intestinal epithelium and secreted intraluminally after joining with secretory component [9]. A much smaller number of plasma cells produce IgM or IgG. The maturation of the B cell to an IgA-secreting plasma cell occurs during the

course of a migratory journey throughout the body's vascular system. Cells exit the Peyer patches through lymph vessels, enter the mesenteric lymph nodes, thoracic duct, and peripheral blood, and then finally "home" to the lamina propria of the gut (as well as to other organs, such as the lung, genitourinary tract, and breast) [9]. T lymphocytes similarly migrate from one lymphoid organ to another and eventually home to mucosal sites, which are determined by their interactions with specific proteins expressed on lymphoid cells as well as the high endothelial venules, specialized vessels of the lymph nodes, Peyer patches, and appendix [9]. These vascular structures can also proliferate in organs affected by chronic inflammation. Macrophages and eosinophils are present in the normal human intestinal lamina propria. However, neutrophils are primarily found in inflamed rather than normal gut.

Isolated lymph follicles are small collections of lymphocytes in the mucosa and submucosa, found scattered throughout the gastrointestinal tract [10–12]. Peyer patches, found in the mucosa and submucosa, are localized collections of lymphoid follicles, may be as large as 30 cm in diameter, and are most prominent in the ileum. The number of Peyer patches in the gut increases after birth [10]. The Peyer patch contains specialized epithelial cells, known as M cells, that overlie the lymphoid cells and allow luminal antigens to enter the mucosa [13,14]. The dome area of the Peyer patch lies above the lymph follicles and just below the M cells and contains lymphocytes, macrophages, and an extensive dendritic cell network [15]. The follicular or central region contains large numbers of precursor B cells that undergo active cell division. The interfollicular zone is populated predominantly by T cells. Peyer patches are involved in the induction of mucosal immune responses.

Other cellular components of the lamina propria include fibroblasts and smooth muscle cells. Arterioles, venules, and a central lacteal function to deliver nutrients into the vascular system (see Fig. 46.5).

Epithelium

The villi and crypts contain a complex, rapidly proliferating, and perpetually differentiating epithelium, a continuous sheet of simple columnar cells that rests on a filamentous basal lamina or basement membrane (see Figs 46.3 and 46.4). It constitutes the major barrier between the intestinal lumen and the lamina propria and regulates flux between these two compartments. The villus enterocytes vectorially transport the products of digestion into the lamina propria, where they enter the venous capillaries or lymphatic system and are transported to other areas of the body. Tight junctions (zonula occludens) bind adjacent epithelial cells tightly together near their apices to restrict flux between adjoining cells (the paracellular route) to small ions, small molecules, and water [16].

The crypt and villus form the basic structural and functional unit of the small bowel (Fig. 46.6; see also Figs 46.3 and 46.4). Anchored stem cells located in the crypts of Lieberkühn are the source of the four major terminally differentiated cell

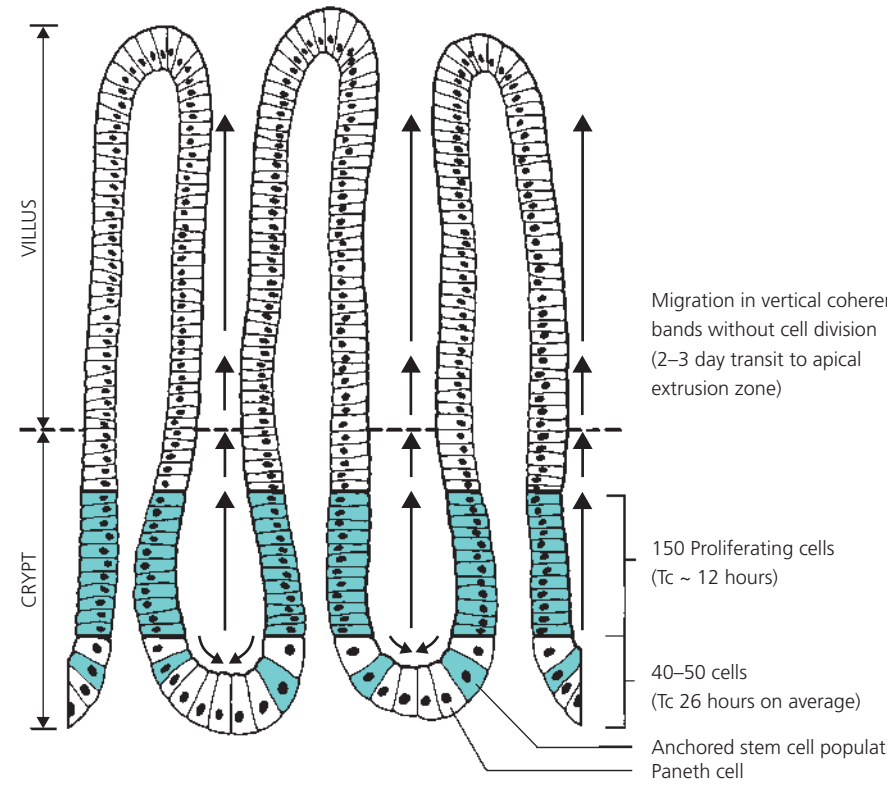

Figure 46.6 (a) Organization of the crypt–villus axis in the small intestine. The small intestinal crypt contains approximately 250 cells. The intestinal stem cell is located in the crypt base, although the precise position and identification is still controversial. The undifferentiated cells divide asymmetrically to give rise to proliferating progenitor cells that migrate up toward the villus and differentiate into enterocytes, goblet cells, and enteroendocrine cells, or migrate downward to the crypt base and differentiate into Paneth cells. Pericryptal myofibroblasts and other mesenchymal cells "condition" the crypt epithelium.

types: absorptive enterocyte, mucus-secreting goblet cell, enteroendocrine cell, and Paneth cell [17,18]. The stem cells reside in a niche composed of pericryptal fibroblasts and other mesenchymal cells [18]. Other epithelial cell types include the tuft cell (or caveolated cell), a rare constituent of the villus [19], and the M cell, a specialized epithelial cell that overlies the Peyer patch (see above). Cellular differentiation proceeds during a bidirectional migration process (see Fig. 46.6) [20]. The gut epithelial stem cells are believed to reside in the lower crypt region, approximately four to five cell positions above the crypt base [17,18,20–22]. They give rise to proliferating progenitor or transit amplifying cells, which differentiate as they migrate up the crypt onto the villus to become enterocytes, goblet cells, and enteroendocrine cells. In contrast, the Paneth cells arise as their progenitors journey to the crypt base [17,21]. Several crypts contribute cells to a single villus. The number of crypts supplying each villus varies from the duodenum to the ileum. Epithelial cell migration and differentiation occur continuously, and the process of cellular renewal and migration to the villus tip takes approximately 5 days in humans. Apoptotic cell death occurs spontaneously near the stem cell region in the crypts, presumably to regulate stem cell numbers. Effete cells are removed from the villus tip by a process known as *anoikis,* in which altered cellular adhesion results in apoptosis [22].

Cellular and topographic organization of the crypt

The present model of the organization of the crypt and its cellular kinetics is derived primarily from studies in the mouse. Crypts arise from one or perhaps a few intestinal stem cells [17]. It is postulated that between four and six stem cells are present per crypt [18,19,21–24]. The number of active stem cells in any one particular crypt is unknown [20]. In the adult gut, these cells are monoclonal [25]. The small intestinal epithelial stem cell presumably undergoes a process of asymmetric division to give rise to actively proliferating progenitor cells while the stem cell is maintained [22]. Proliferating progenitor cells or transit cells are located in the mid-crypt region, above the presumptive stem cell region. The stem cell is regulated by WNT and WNT ligands. In addition, bone morphogenetic proteins and noggin cross-talk to the WNT–β-catenin pathway through PTEN–phosphoinositide (PI) 3-kinase and regulate the production of transit-amplifying (proliferative) cells [17,26] (Fig. 46.6). The sources of these signaling molecules include crypt epithelial cells, myofibroblasts and other mesenchymal cells [17]. In mouse gut, these cells enter the cell cycle every 12 hours [27]. The progenitor cells subsequently differentiate into the four major cell types.

Undifferentiated crypt cells

Undifferentiated crypt cells have many ribosomes and polysomes but scant endoplasmic reticulum, mitochondria, and microvilli, and undeveloped terminal webs. They are basophilic because they contain large amounts of cytoplasmic RNA. Secretory granules are found near the apical surface (see Fig. 46.4b); their nature is unknown, but they stain with the periodic acid–Schiff (PAS) reaction and are not lysosomes. Undifferentiated crypt cells also synthesize secretory component (the receptor for IgA), contain chloride channels, and can secrete chloride in response to cholera toxin or other enterotoxins [28].

Absorptive enterocytes

Cellular differentiation commences during migration to the upper crypt and villus base. The production of a differentiated epithelium is regulated by Delta–Notch signaling pathways. The transcription factors Hes1 and Math1 are required for the normal production of the columnar (e.g. enterocytic) as opposed to the secretory (goblet, Paneth, and enteroendocrine) cell types [29,30]. The cells that are fated to become the absorptive enterocytes begin to express a variety of specific gene products that enable the cells to digest and absorb many different nutrients as they reach the crypt–villus junction. These include brush-border enzymes (disaccharidases, peptidases, and alkaline phosphatase); proteins involved in lipid absorption (apolipoproteins and fatty acid-binding proteins); and many receptors, carriers, and transporters [31]. Immunohistochemical and in situ hybridization analyses indicate that most major enterocyte gene products, such as disaccharidases, apolipoproteins, fatty acid-binding proteins, and the sodium-dependent glucose transporter, are expressed as cells emerge above the crypt–villus junction [32,33], but are not found in the crypts. This precise vertical differentiation continues as cells migrate up the villus. Microvilli become more prominent, and the capacity of the cell to absorb lipids, sugars, and amino acids increases.

Despite rapid cellular renewal, complex spatial differentiation in the gut is maintained from duodenum to colon. Many enterocyte genes are abundantly expressed in the proximal small bowel, but their expression decreases markedly in the distal gut [32,34–36], whereas other genes are specifically expressed in the ileum. Cloning, sequencing, and promoter analysis of these genes have provided insight into the molecular mechanisms underlying the regulation of regional differentiation in the gut [36–40]. Transgenic mouse and cell culture transfection techniques have been used to map out regulatory promoter elements [24,39–40]. In addition, transgenic mice and mice in which embryonic stem cell mutations have been created to delete specific gene products ("knockout" or null mice) have been used to determine the function of these novel regulatory genes, including the homeobox-containing Cdx family [41].

Ultrastructural features

As undifferentiated crypt cells travel up the villus, they acquire longer, more numerous microvilli, measuring 0.1 μm in width and about 1 μm in height, which produce a prominent striated (brush) border under light microscopy. The

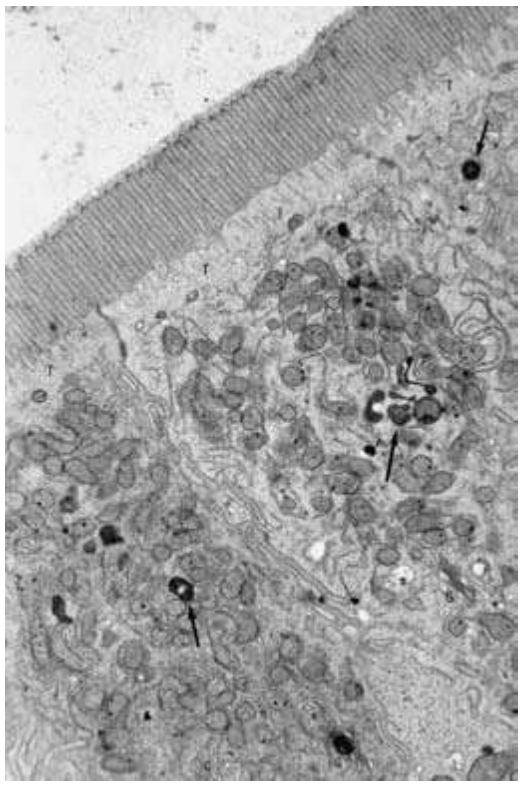

Figure 46.7 An electron microscopic picture of the apical halves of differentiated villus epithelial cells. Note the numerous tall microvilli covered with "fuzz," or glycocalyx, and an organelle-free apical zone, the terminal web (T), just below the microvilli. There are abundant mitochondria and profiles of rough endoplasmic reticulum. The irregularly shaped dense bodies (arrows) are lysosome derivatives. Supranuclear Golgi profiles are denoted by G. The intercellular space is obliterated between the apices of adjoining cells just below the level of the microvilli by the tight junction (zonula occludens), an apparent fusion of adjoining cell membranes (best seen between the two cells on the left). Transport of substances from the lumen between cells (paracellular route) is restricted to water and small molecules and ions. Most products of digestion enter the cell by crossing the microvillar membrane. Original magnification × 15 000. From Rubin W. The epithelial "membrane" of the small intestine. Am J Clin Nutr 1971;24:45.

development of these long microvilli – finger-like extensions of the apical cell membrane – markedly increases the absorptive surface of the small intestine (Fig. 46.7). The basolateral membrane is relatively smooth in comparison. These cells also develop a terminal web, an apical zone below the microvilli that contains many filaments and some vesicles. As cells migrate up the villus, they acquire more mitochondria and rough endoplasmic reticulum, lose their secretory granules, and acquire large, apical dense bodies, which represent lysosomal derivatives (Figs 46.4d and 46.7). Deep apical tubules, visualized by ruthenium red staining, are situated between microvilli and represent areas of the apical cell surface that function in endocytotic or exocytotic membrane trafficking [42]. The functional polarity of the enterocyte, with its specific apical and basolateral membrane domains, is strictly maintained, thereby ensuring the vectorial transport of a variety of nutrients and ions from the apical to the basolateral surfaces of these cells.

Filaments composed of actin extend from a dense plaque just beneath the cell membrane at the tip of the microvillus down the core of the microvillus into the terminal web [43–45]. The terminal web is a dense meshwork of filaments that contain actin, myosin, and other cytoskeletal proteins. The meshwork is oriented parallel to the surface and perpendicular to the microvillus filaments, and is attached to the lateral cell membrane at the intermediate junction (zonula adherens) [45]. The numerous filaments in the microvilli and terminal web, known as the *cytoskeleton of the brush border*, probably confer a structural rigidity to the apices of the differentiated villus cells and may provide for the movement of the microvilli [43,45,46]. This cytoskeleton probably also affects the uptake of certain nutrients and paracellular permeability [47].

Short, thin, filamentous material extends from the outer leaflet of the cell membrane covering the microvilli into the intestinal lumen, producing a surface coat, the glycocalyx (or "fuzz"). The glycocalyx represents external extensions of proteins and glycoproteins in the microvillus membrane [48]. The microvillus membrane of the differentiated villus cells contains many of the enzymes, receptors, and carriers necessary for terminal digestion and absorption, such as the disaccharidases, alkaline phosphatase, peptidases, the ileal cobalamin–intrinsic factor receptor, the bile acid receptor, and the Na^+-dependent glucose and amino acid transporters [48,49]. The hydrolytic enzymes, which are the most abundant of the microvillar membrane proteins, are localized in lipid rafts [50]. These rafts are made of glycolipids and the lectin, galectin 4, and may also represent a portal of entry for pathogens. The active portions of many of these enzymes are associated with the glycocalyx. The components of the microvillus membrane and glycocalyx turn over and are replenished by continuous synthesis, being transported from the rough endoplasmic reticulum through the Golgi apparatus to the microvillus membrane [48,51,52].

The selective vesicular transport of microvillus constituents from the Golgi apparatus to the cell apex is dependent on microtubules. Disruption of microtubules with colchicine or vinblastine results in more random routing of these components, with the incorporation of microvillus enzymes into the basolateral surface and even the formation of basolateral microvilli [51]. Some of the microvillus enzymes are finally processed in the glycocalyx by the proteolytic activity of pancreatic enzymes, especially elastase, that are adherent to the glycocalyx [48].

Paneth cells

Paneth cells are pyramid-shaped cells in the crypt base that contain large, apical eosinophilic secretory granules. They

are much longer-lived than enterocytes, with a half-life of approximately 20 days in the mouse [21]. There are approximately 5–12 Paneth cells per crypt. A role in innate immunity, host defense, and regulation of microbial density [53] is suggested by their abundant expression of lysozyme, cryptdins (see below) [54], and other antibiotic proteins, and their ability to degranulate in response to live and heat-killed bacteria [53]. Several of the defensins, a family of small peptides that are abundant in human neutrophils, are highly expressed in Paneth cells [54]. These peptides, 3–4 kDa in size, are also known as *cryptdins* (for *crypt defensins*) [54], and exhibit microbicidal activity toward many different bacterial microorganisms in vitro. Two enteric α-defensins have been identified in humans [53]. Messenger RNAs encoding tumor necrosis factor (TNF) and α_1-antitrypsin [55] have been located in human Paneth cells. In addition, Nod2 is expressed in Paneth cells, and defective Nod2 function has been implicated in the pathogenesis of Crohn's disease [56,57]. Paneth cells release defensins in response to bacteria, but not to fungi or protozoa, and the defensins have potent microbicidal activity [58]. Overexpression of human α-defensin 5 (HD-5) in the Paneth cells of transgenic mice resulted in resistance to oral *Salmonella* infection [59].

Enteroendocrine cells

The intestine contains a complex enteroendocrine cell population composed of a variety of paracrine or endocrine cell types. These cells were first characterized as argentaffin or argyrophil cells, depending on their reaction to silver staining in the presence or absence of a reducing agent. Subsequently, electron microscopy led to their classification by the appearance of granules. These cells are generally classified by immunohistochemistry, based on their primary neuroendocrine product. For example, the D cell produces somatostatin, the L cell produces glucagon-like immunoreactivity (including glicentin, glucagon-like peptide 1 [GLP-1] and GLP-2), and the enterochromaffin cell makes serotonin. A subset of enteroendocrine cells express more than one peptide or amine.

Enteroendocrine cells have two different secretory pathways, characterized by the presence of classic large, dense core vesicles, as well as a second vesicle that is smaller and synaptic-like [60]. At least 10 different enteroendocrine cell types have been identified in the small intestine. This cellular population exhibits a specific spatial distribution along the crypt-to-villus and duodenal-to-colonic axes of the gut. For example, serotonin cells are abundant throughout the gastrointestinal tract, whereas secretin and cholecystokinin cells are much more localized to the duodenum and proximal jejunum [61]. In the mouse, secretin cells are predominantly located on the villi, whereas substance P-containing cells are found primarily in the crypts. Enteroendocrine cells may also simultaneously express more than one neuroendocrine product [61,62]; this coexpression is developmentally regulated in the rodent [63].

Although early data suggested that enteroendocrine cells were derived from the neural crest, later studies indicated that they arise from the undifferentiated crypt stem cell [21,22,24]. This differentiation is hard-wired in the absence of luminal contents, including pancreaticobiliary secretions and dietary factors [62,64]. Early cell fate decisions are regulated by Notch signaling [65,66]. Inactivation of Hes1, a downstream target of Notch, results in increased enteroendocrine cell numbers. Math1 expression determines a secretory cell fate, and neurogenin 3 specifies the enteroendocrine cell lineage. The genes that regulate the appearance of each enteroendocrine cell type are still being elucidated [66]. Targeted ablation of secretin cells in transgenic mice markedly decreased the numbers of L cells as well as secretin cells, also suggesting a common developmental relationship [67].

Some of the secretory products of these cells act in a paracrine manner and function locally, whereas others are true endocrine products. The peptides and amines affect bowel motility, intestinal cellular secretion (see Chapter 4), and epithelial cell proliferation (e.g., glucagon-like peptide 2, a product of the intestinal L cell) [68]. In contrast to exocrine cells, which secrete apically into the lumen of an organ or the duct of a gland, endocrine cells are morphologically oriented toward the basement membrane. They exhibit an appreciable basal surface, and they narrow superiorly so that only narrow bands of apical cytoplasm reach the lumen. Their secretory granules are located predominantly in the basal cytoplasm below the nucleus, ready to be secreted by exocytosis through the basal membrane into the lamina propria (see Fig. 46.4c).

Goblet cells

The mucus-secreting goblet cells are present throughout the entire gastrointestinal tract but are more numerous in the ileum than the jejunum. These cells have the shape of a brandy goblet and apically located granules filled with mucin. Mucin is their primary secretory product and it presumably serves a cytoprotective and lubricant function in the gastrointestinal tract [69,70]. Biochemical differences among mucins are expressed in the stomach, small intestine, and colon, suggesting heterogeneity among goblet cell populations. The expression of specific mucins is also developmentally regulated. Intestinal trefoil factor, one of a family of small peptides that includes pS2 and human spasmolysin, is abundantly expressed in goblet cells of the small intestine and colon [71]. Intestinal trefoil factor mRNA is expressed in the crypt cells and may therefore be a marker of early goblet cell differentiation. Embryonic stem cell gene ablation experiments demonstrate a cytoprotective role for intestinal trefoil factor in the gut [72]. Goblet cells arise from the same committed stem cells as do the other principal cell types. Production of a secretory cell type (including goblet, Paneth, and enteroendocrine cells) depends on Math1 expression [30]. However, actively proliferating goblet cells have been

noted in the crypt; these cells may also contribute to the villus-associated goblet cell population.

M cells

The specialized epithelial membranous cells, or M cells, are found in the epithelium overlying Peyer patches, known as the *follicle-associated epithelium*, and may occasionally be found on the villi [14]. Unlike other absorptive cells, they lack a typical brush border, demonstrating fewer, shorter microvilli and numerous apical endocytic vesicles. Also, their cytoskeleton is less rigid and the cells can be deformed by migrating lymphocytes [11]. They serve as antigen-sampling cells and endocytose a variety of macromolecules [73], viruses, and bacteria from the lumen. These molecules are then rapidly transported across the epithelium and come into contact with immune cells, thereby initiating protective mucosal immune responses [13,74,75]. The invaginated basolateral membrane of the M cell forms an intraepithelial pocket and allows lymphocytes and macrophages to come into rapid contact with antigen [74]. The precise mechanisms involved in transepithelial transport of antigen across the M cell are still largely unknown [75]. The presumptive origin of this cell type is also the multipotent crypt stem cell [74]. M cells can be formed by culturing Peyer patch lymphocytes together with the intestinal Caco-2 cell line, or with native enterocytes [76,77]. Peyer patch development and maintenance of M cells requires B cells [78]. These and other data suggest that M-cell differentiation may be immunoregulated and that lymphoepithelial cross-talk may induce M-cell formation from follicle-associated epithelial cells. M cells have been proposed as a potential target for oral vaccines [13].

Tuft cells (caveolated cells)

Tuft, or caveolated, cells are found in the stomach, small and large bowel, pancreas, and gall bladder, as well as the respiratory tract [79]. These cells can be distinguished by unique expression patterns of villin, fimbrin, cytokeratin 18, tubulin, and ankyrin [80]. They have been postulated to act as chemoreceptors in the gut [81].

Embryology

A brief synopsis of the major events in midgut morphogenesis is presented as a basis for understanding the congenital anomalies discussed in subsequent sections of this chapter.

The primitive human gut forms when the dorsal part of the yolk sac is incorporated into the embryo at the fourth week of development, giving rise to the foregut, midgut, and hindgut [82]. The foregut is the progenitor of the esophagus, stomach, duodenum up to the biliary duct ampulla, pharynx, respiratory tract, liver, pancreas, and biliary tract. The midgut gives rise to the duodenum distal to the common bile duct, jejunum, ileum, cecum, appendix, ascending colon, and half to two-thirds of the transverse colon. The rest of the colon and the superior anal canal are derived from hindgut.

The gut endoderm is the precursor of the gastrointestinal tract epithelium. Its endothelium arises from the ectoderm of the stomodeum and proctodeum as well as the endoderm. The splanchnic mesenchyme supplies the muscular and connective tissue components of the gastrointestinal tract. The midgut first freely communicates with the yolk sac and then narrows to be connected by the omphalomesenteric or vitelline duct. The primitive gut forms a U-shaped loop; this grows so rapidly in comparison with the embryo that it herniates into the umbilical cord at the sixth week of gestation. The proximal limb of the loop elongates into multiple intestinal loops, whereas the distal limb simply develops into the cecal diverticulum. The first stage of rotation is 90° counterclockwise around the superior mesenteric artery axis. At 10 weeks, the intestines return into the abdominal cavity and rotate a further 180° counterclockwise in the second stage. Finally, the cecum and appendix descend from the right upper quadrant to the right lower quadrant, and the proximal part of the colon elongates to form the hepatic flexure and ascending colon (third stage of rotation). Fixation occurs as the ascending colonic mesentery fuses with the parietal peritoneum and becomes fixed retroperitoneally. The small bowel mesentery attains a broad-based attachment to the posterior abdominal wall, extending from the duodenal–jejunal junction to the ileocecal region. The end result of this process is a normal location of the small and large intestine (Fig. 46.1).

Congenital anomalies

Meckel diverticulum

Description and pathophysiology

During early gestation, the omphalomesenteric or vitelline duct connects the fetal yolk sac to the primitive gut. By 7–8 weeks of gestation, this duct is normally completely obliterated. A Meckel diverticulum, the most common congenital anomaly of the gastrointestinal tract, results when this structure fails to resorb completely. The duct remnant most commonly persists as a diverticular sac; alternatively, the diverticulum may be connected to the mesentery or umbilicus by a fibrous band (Fig. 46.8). Occasionally, only a thick connective tissue band remains, attaching the gut to the umbilicus. These bands may lead to volvulus or the strangulation of bowel loops. Rarely, a fistula remains patent from the ileum to the umbilicus, leading to persistent external drainage of ileal contents. Other, more unusual duct remnants include umbilical polyps and vitelline cysts.

Large autopsy series indicate a 2%–3% prevalence of Meckel diverticulum in the general population [83]. This anomaly is two to three times more common in males [84]. Meckel diverticula are true diverticula, containing all layers

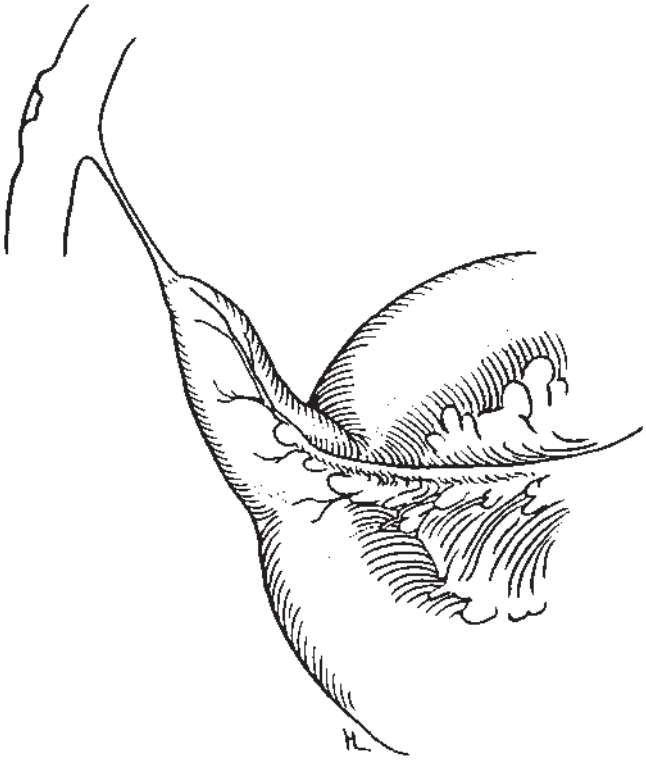

Figure 46.8 A Meckel diverticulum is attached to the umbilicus by the obliterated omphalomesenteric duct. A Meckel diverticulum may be unattached to the umbilicus or abdominal wall; alternatively, the vitelline duct may remain attached and patent, creating a fistula. Fibrous bands may predispose to volvulus and strangulation. From Johns TNP, Wheeler JR, Johns FS. Meckel's diverticulum and Meckel's diverticular disease: a study of 154 cases. Ann Surg 1959;150:241.

of the bowel from serosa to mucosa. Located on the antimesenteric border of the gut, they are most commonly found within 100 cm of the ileocecal valve but may also occur in other regions of the small bowel. Most diverticula are between 1 and 10 cm in size, although giant lesions may be as large as 100 cm in diameter. The two types of giant diverticula are: the type I lesions, which are long but of the same caliber as the ileum; and the less common type II, or ovoid, lesions [85]. Heterotopic tissue is present in approximately 50% of all diverticula [86]. Most commonly found are gastric mucosa, pancreatic tissue, or a combination of the two. Diverticula containing colonic mucosa, Brunner glands, or jejunal or hepatobiliary tissue have also been described. The presence of heterotopic mucosa correlates with an increased risk for symptomatic, complicated Meckel diverticulum. Heterotopic gastric mucosa may lead to diverticular gastrointestinal bleeding in adults and children when ileal ulceration results from acid secretion [87,88]; almost all bleeding diverticula contain gastric mucosa [89,90]. Although the data are conflicting, some studies have indicated that *Helicobacter pylori* may rarely colonize the diverticular gastric mucosa and is associated with gastritis in this site [91]. Heterotopic tissue is also associated with a modestly increased risk for other complications, including small bowel obstruction and diverticular inflammation [87].

The risk for complications dramatically decreases with age, and most adults with a Meckel diverticulum remain asymptomatic [92–94]. Patients with Meckel diverticulum have a lifetime risk of complications of ~ 6% [95]. Females are less likely than males to be symptomatic [93,94].

The complications of Meckel diverticulum include bleeding, intestinal obstruction, diverticulitis, perforation, and carcinoma. Obstruction is caused by intussusception of the diverticulum into adjacent bowel; volvulus around or herniation into a fibrous band; entrapment in inguinal, femoral, or umbilical hernia sacs (Littré hernia); or inflammation and scarring leading to blockage around the diverticular neck and adjacent ileum. The frequency of specific complications varies in adult compared with pediatric patients. The most common complications in children are gastrointestinal bleeding, most often occurring in infancy and early childhood (before 5 years of age) [89], and intestinal obstruction. In adults, intestinal obstruction is the most frequent complication. Although early studies indicated that gastrointestinal bleeding is relatively uncommon [87,88], subsequent series have indicated that bleeding from Meckel diverticulum is an important complication in adults [93]. Whereas adults most often describe melena, children usually present with painless, multiple, rapid, bright- or dark-red bowel movements. More unusual complications in adults include the development of carcinomas such as carcinoids, sarcomas, and rarely adenocarcinomas [96]. Carcinoid tumors are typically small, with biological characteristics resembling those of jejunoileal rather than appendiceal carcinoids (e.g., immunohistochemical staining and metastatic potential). Patients with carcinoid of a Meckel diverticulum are generally older (in their sixth decade) and frequently asymptomatic. Tumors larger than 5 mm in diameter have considerable metastatic potential [97]. Other complications include enteroliths, which may become lodged in the diverticulum and cause abdominal pain, vomiting, bleeding, and obstruction.

Diagnosis

The diagnosis of Meckel diverticulum remains a challenge [98,99]. Sodium pertechnetate (^{99m}Tc) radionuclide scanning is particularly useful in children. The ^{99m}Tc isotope is taken up by normal stomach tissue and by ectopic gastric mucosa in the Meckel diverticulum. Surface mucus cells from the intestine as well as the stomach accumulate and secrete this anion [100]. To enhance the sensitivity of this test, cimetidine may be administered to decrease anion secretion from the gastric mucosa [101]. Pentagastrin may also be useful, enhancing anion uptake by a poorly understood mechanism [102]. The major drawback of this detection method is that heterotopic gastric mucosa must be present in the diverticulum if a true positive result is to be obtained. In

children with lower gastrointestinal bleeding from a Meckel diverticulum, this test is sensitive and specific because almost all bleeding diverticula contain gastric mucosa, but in adults the false-positive and false-negative rates of the method are high, even in patients with bleeding [98,103]. Crohn's disease and other inflammatory disorders lead to false-positive scans. In children, a scan for Meckel diverticulum is often followed by a technetium or stannous pyrophosphate red blood cell scan to localize the site of bleeding definitively.

Small bowel follow-through is usually not useful because the diverticulum may not fill with barium and is also rapidly emptied. Enteroclysis examinations improve the sensitivity of barium studies because the contrast material under increased pressure better fills the diverticulum. Angiography can localize the source of hemorrhage and may also demonstrate the vitelline artery and its embryonic branches. This vessel arises from a distal branch of the superior mesenteric artery and ends in a characteristic blush of tortuous small vessels. Frequently, the vitelline artery involutes, and the diverticulum is directly supplied by branches from the superior mesenteric artery. Small bowel capsule endoscopy has been used successfully to detect Meckel diverticulum [104,105], and double-balloon enteroscopy may aid in the detection of obscure GI bleeding, including that resulting from Meckel diverticulum [106,107].

Management

The treatment of Meckel diverticulum complicated by bleeding, obstruction, or perforation is surgical. Diverticulectomy is performed, possibly with concurrent ileal resection if the adjacent small bowel is ulcerated, inflamed, or obstructed. These operations can be complicated, and postoperative morbidity occurs in up to 6% of all cases [87,95,108]. Laparoscopic removal has been reported [109–112]. The management of asymptomatic diverticula is controversial [99]. Because of the low risk for complications of Meckel diverticulum in adults, some authors do not recommend prophylactic removal [87,92,94,113]. However, others prefer to resect asymptomatic diverticula that are highly likely to cause complications, including: large (> 2 cm) diverticula; lesions associated with an omphalomesenteric band, which are at risk for volvulus and obstruction; lesions containing a palpable mass, which may represent tumor or ectopic mucosa; and lesions in younger male patients [90,93].

Duplications

Duplications of the gastrointestinal tract are rare congenital cystic anomalies attached to the intestinal mesenteric border (Fig. 46.9). Spherical or tubular, they are usually lined by gut mucosa, but like Meckel diverticula, they may contain heterotopic gastric mucosa or less commonly pancreatic, squamous, thyroid, or bronchial epithelium as well as lymphoid aggregates [114]. They share a blood supply with the associated native intestine and may also communicate lumen to lumen. Duplications may occur anywhere along the gastrointestinal tract from mouth to anus. Those of small bowel origin are most commonly found in the ileum [114]. The embryonic origin of gut duplications is unknown. Postulated mechanisms include aberrant recanalization of the gut lumen during morphogenesis, creating two attached yet distinct gut structures; abnormal notochord–midgut interactions; intrauterine ischemic events; and aborted (partial) twinning [115]. Intestinal atresia may be associated with duplications, supporting a possible vascular etiology. Duplications are also associated with genitourinary tract anomalies [116].

Figure 46.9 Tubular duplication of the terminal ileum in a 7-month-old boy. The duplication, lying on the mesenteric border of the bowel, was 29 cm long and communicated with the adjoining bowel near its distal end. The duplication, which is the smaller of the two cross-sections, was lined largely by gastric mucosa, and contained an ulcer near its point of communication with the bowel. Its wall contained an island of aberrant pancreas. From Arey JB, Valdes-Dapena M. Embryology and developmental disorders. In: Ming S, Goldman H (eds). Pathology of the gastrointestinal tract. Philadelphia: WB Saunders, 1992:113.

Signs, symptoms, and complications

In most patients, duplications are diagnosed in infancy or early childhood, but duplications are occasionally newly discovered in an adult. The frequency of symptoms varies inversely with age. Pediatric patients most commonly present with abdominal pain, obstructive symptoms (e.g., nausea, vomiting, pain), and hemorrhage [117]. Obstruction may result from a mass effect, from volvulus produced by a large lesion, or more rarely from inflammation of the duplication. Much less frequently, these lesions are asymptomatic and are discovered incidentally. In adults, the evaluation of relatively mild abdominal complaints by computed tomography (CT) or ultrasonography may lead to detection [118], and ultrasonography has been used for prenatal detection [119]. Intussusception may be precipitated if a duplicated gut acts as a lead point and invaginates into normal intestine. Gastrointestinal hemorrhage may result from ulceration of the duplicated or surrounding mucosa; these lesions frequently contain ectopic gastric mucosa that secretes acid and

causes ulceration. A rare complication in adults is the development of carcinoma in the duplication [120]. Carcinoid tumors, adenocarcinoma arising in ectopic gastric mucosa, and squamous cell carcinoma have all been reported.

Diagnosis

Small bowel duplications can be difficult to detect. Plain films may show a partially calcified wall of the duplication. Small bowel follow-through or enteroclysis barium examinations may reveal the duplication if it communicates with the gut lumen. Cysts that contain gastric mucosa may be detected by ^{99m}Tc abdominal scintigraphy [121], which specifically delineates gastric surface mucus cells (see "Meckel diverticulum" above). Ultrasonography (effective for prenatal diagnosis) or CT can provide clues to the diagnosis by revealing the presence of a cystic mass, and these tests are also valuable for detecting rare carcinomas, which appear as solid tissue within the cyst [120].

Management

Duplications are treated surgically. Lesions detected antenatally can be electively resected, because the majority are asymptomatic, at least for a few months after birth [122]. If small, the lesions are easily resected with the adjacent small bowel. If the duplicated bowel is extensive and resection would require the removal of too much normal bowel, the duplication is opened and the mucosa removed, with the serosa and muscular layers left intact. Alternatively, the common wall can be excised. It is important to remove all mucosa because residual ectopic gastric mucosa can lead to recurrent hemorrhage [115].

Intestinal atresia and stenosis

In intestinal atresia, the lumen of a segment of the gut is completely occluded. Stenosis is a narrowing of the gut lumen that leads to partial obstruction. Atresia is one of the most common causes of bowel obstruction in neonates [123]. Duodenal atresia and stenosis are frequently associated with Down syndrome, midgut malrotation, esophageal atresia, annular pancreas, imperforate anus, and intrauterine growth retardation (see Chapter 38) [124]. Jejunoileal atresia is much less frequently associated with other congenital anomalies. Atresias are most often single but may be multiple and are found from esophagus to rectum. The reported incidence of small bowel atresias varies but is approximately 1 in 3000 to 1 in 5000 live births [125,126]. Several types of small intestinal atresias have been described (Fig. 46.10) [127].

- Type I – a membranous septum or diaphragm of mucosa and submucosa obstructs the lumen, but the bowel wall and mesentery are intact.
- Type II – two blind bowel ends are connected by a fibrous cord, with intact mesentery in between.
- Type IIIa – two blind bowel ends are separated by a mesenteric gap.

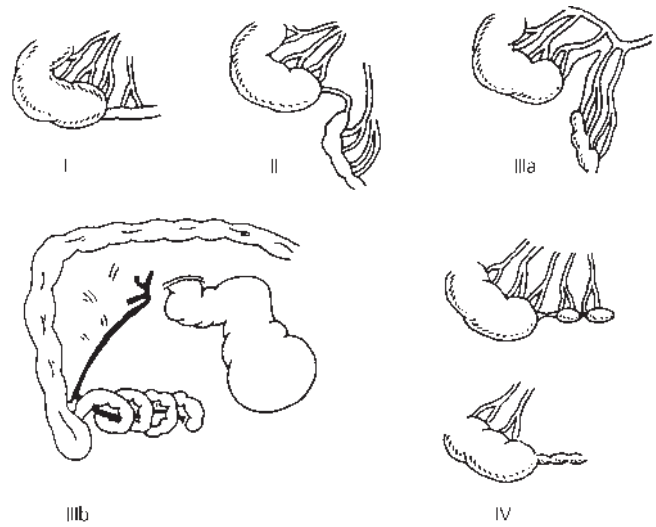

Figure 46.10 Classification of intestinal atresias. In type I, or membranous atresia, a membranous septum or diaphragm of mucosa and submucosa obstructs the lumen, but the bowel wall and mesentery are intact. In type II, two blind bowel ends are connected by a fibrous cord, with intact mesentery between them. In type IIIa, bowel lesions are separated by a mesenteric gap. Type IIIb is an "apple peel" atresia with features of proximal small bowel atresia, absence of the distal superior mesenteric artery, and coiled and foreshortened bowel. Type IV consists of multiple atresias of any type (I–IIIa). From Smith GH, Glasson M. Intestinal atresia: factors affecting survival. Aust N Z J Surg 1989;59:151.

- Type IIIb – "apple peel" atresia, characterized by proximal small bowel atresia and absence of the distal superior mesenteric artery (< 5% of all atresias) [128]. In this case, the bowel distal to the atresia is foreshortened and coiled and receives a retrograde blood supply from the ileocolic, right colic, or inferior mesenteric artery.
- Type IV – multiple atresias are present throughout the small bowel, creating the appearance of a "string of sausages;" they may be of type I, II, or IIIa.

Pathophysiology

The pathogenesis of atresia and stenosis is unknown. Duodenal atresia is thought to be caused by a failure of recanalization of the intestinal lumen after the solid cord stage, which takes place at 4–8 weeks of gestation [129]. Mutations in the α_6- and β_4-integrin genes have been reported in families affected by junctional epidermolysis bullosa and duodenal or pyloric atresia [130,131]. In mice, deletion of the fibroblast growth factor (FGF) 10 receptor, FGF2Rb, results in duodenal and colonic atresia, along with other defects such as pulmonary agenesis [132]. Ischemic intrauterine events such as midgut volvulus, arterial occlusion, intussusception, and localized volvulus secondary to meconium ileus or duplication have been implicated in the pathogenesis of small intestinal atresia, especially in single atresia and stenosis [133]. Atresia has been associated with intrauterine volvulus, gastroschisis,

cardiac and other anomalies [134], and cystic fibrosis [134,135]. A retrospective review showed a more than 200-fold increased risk for cystic fibrosis in Caucasian infants with jejunoileal atresia [136]. In a population-based study in Hawaii, small bowel atresia was associated with low birthweight, low gestational age, maternal age, and multiple births [126]. Meconium ileus is thought to predispose the bowel to ischemia or cause volvulus as a consequence of hyperperistalsis.

Pathological examination of atretic segments resected from neonates with multiple intestinal atresias suggests a role for either ischemia or a defect in recanalization of the gut lumen [137]. Rare familial cases of multiple intestinal atresias and apple peel (type IIIb) atresias have been reported [123,137–139]. An autosomal recessive inheritance pattern has been suggested [138], but variability in the types of atresia found within families and discordance in a set of monozygotic twins suggest a more complex genetic transmission [128]. Other factors implicated in the pathogenesis of atresia and stenosis are maternal ergotamine use, exposure to vasoconstrictive agents such as pseudoephedrine and amphetamine, combined with cigarette smoking [140], fetal exposure to methylene blue (which was formerly used as a marker during amniocentesis of twin pregnancies to ensure that both sacs had been sampled) [141], and toluidine blue [142]. Multiple intestinal atresias have also been reported to be associated with neonatal immunodeficiency states [143]. A new syndrome has been reported in which neonatal diabetes and hypoplastic pancreas were found in association with intestinal atresia and gallbladder hypoplasia [144].

Clinical presentation

Polyhydramnios is frequently detected in proximal gastrointestinal atresia, but the amniotic fluid may be normal in distal atresia [133]. Neonates most commonly present with signs and symptoms of obstruction within hours to 3 days after birth. Bilious vomiting early after birth is a characteristic symptom of proximal atresia, whereas abdominal distention (occasionally associated with visible bowel loops), later vomiting, and a failure to pass meconium are frequent presenting complaints of distal lesions. High atresias of the jejunum and duodenum are also associated with jaundice. The course of infants who have intestinal stenosis without atresia may be much more indolent because the obstruction is usually partial. The diagnosis may therefore be delayed.

Diagnosis

Polyhydramnios is an indication for ultrasonography, which may identify the obstructing defect [119]. Fetal echogenic bowel, enlarged stomach, and dilated loops of bowel are frequently detected [145]; the presence of fetal ascites and extralumenal calcifications may indicate aseptic perforation of the bowel, with meconium peritonitis. The intraluminal calcifications found in type IV atresias may also be seen. Ileal atresias are generally not associated with an enlarged stomach or polyhydramnios [145]. After birth, routine abdominal radiography may demonstrate multiple air–fluid levels throughout the small bowel or dilated bowel proximal to the obstruction with an absence of gas distally. A double-bubble sign on abdominal radiography or ultrasonography is classic for duodenal atresia. Barium enema shows an unused microcolon. The anatomical defect should always be definitively identified by plain films and cautious contrast radiography before surgical intervention is attempted. It is also important to identify all atretic sites, with the risk for multiple atresias kept in mind.

Important in the differential diagnosis of neonatal bowel obstruction are Hirschsprung disease, malrotation, meconium ileus associated with cystic fibrosis, duplications, Meckel diverticulum, and mesenteric bands [146]. Barium enema is recommended to rule out Hirschsprung disease and malrotation with volvulus, and to identify other atresias in the bowel distal to the primary obstruction.

Treatment and prognosis

Initial therapy consists of intravenous hydration, correction of electrolytes, and nasogastric suction to remove accumulated fluid and decompress the bowel. Surgical resection of atretic small intestine is the primary treatment, which may be challenging because bowel preservation is critical and dilation of the small bowel proximal to the obstruction makes anastomosis difficult. Several different surgical procedures have been developed, including tapering enteroplasty and the creation of an ileostomy [134]. Patients with type IIIb and type IV atresias often require extensive resective surgery, which results in short bowel syndrome and a poor prognosis. Patients with hereditary multiple intestinal atresias have particularly poor outcomes, because the extensive involvement with multiple atresias from antrum to rectum cannot be surgically corrected [139]. Small bowel transplantation in a patient with multiple intestinal atresias and immunodeficiency resulted in partial correction of immune defects, by recruitment of immune cells from the donor [147].

All patients after surgery are placed on total parenteral nutrition and monitored for a return of normal bowel function as the diet is slowly advanced. A central venous catheter should be placed early in the course of the disease. Intestinal atresia is a common prenatal cause of short bowel syndrome in children [148]. Patients with short bowel syndrome frequently require prolonged parenteral nutrition. The morbidity and mortality rates in infants have dramatically decreased since the 1950s. Postoperative survival is approximately 90%–95% [129,133]. Enhanced survival is a result of improved management of the nutritional and pulmonary status. However, overall survival has remained at approximately 84%–88% [137]. Factors adversely affecting the prognosis include the presence of other congenital anomalies

(especially cardiac), chronic pulmonary disease, or pulmonary aspiration [129,131]. Prolonged disturbance in motility is common postoperatively. The development and maturation of the enteric nervous system is impaired below the atretic segment [149].

Malrotation

Description and pathophysiology

Alterations in the normal rotation and fixation of the gut during embryogenesis result in a series of anomalies with various clinical manifestations. Malrotation or incomplete rotation is the most common disorder of rotation and fixation; it occurs when the normal 270° bowel rotation is not completed. As a result, the cecum may lie in the subpyloric region or may be in a subhepatic location. The small bowel is not attached by a broad mesentery but remains suspended from a narrow vascular pedicle. Peritoneal bands (Ladd bands) may pass from the cecum across the duodenum to the right upper quadrant or may attach to the duodenum. The two major complications of malrotation are volvulus, caused by free rotation of the midgut around the vascular pedicle, and duodenal obstruction secondary to the Ladd bands. In nonrotation, the embryonic midgut loop does not rotate and the caudal limb of the loop reenters the abdominal cavity first. Subsequently, the small intestine (jejunum and ileum) lies on the right side of the abdomen and the colon is entirely on the left. The cecum is usually located in the left iliac fossa. Frequently, the broad attachment of the small bowel mesentery does not develop, and the small bowel remains suspended by a narrow pedicle, at risk for volvulus [124,150]. In reversed rotation, clockwise instead of counterclockwise rotation of the gut leads to reentry of the colon into the abdomen first. The position of the colon is then posterior to the superior mesenteric artery and the duodenum. The placement of the duodenum is anterior to the superior mesenteric artery. The small bowel mesentery passes in front of the transverse colon, so that a mesenteric tunnel forms that causes colonic compression and obstruction. Finally, failure of the cecum to descend into its normal position in the right lower quadrant may lead to mobile cecum; the cecum remains in the right upper quadrant and is unfixed posteriorly, predisposed to cecal volvulus. The ileum may similarly be unfixed and susceptible to volvulus.

The incidence of malrotation is unknown; estimates range from 1 in 200 to 1 in 6000 live births [151].

Clinical manifestations

In approximately 50%–80% of cases of midgut malrotation, symptoms develop in infancy. Related concurrent anomalies are biliary atresia and congenital heart disease. Intestinal atresia and stenosis may result from malrotation, and omphalocele and diaphragmatic hernia may lead to malrotation secondary to abnormal placement of the bowel during rotation. In adults, malrotation is sometimes associated with absence of the superior mesenteric vein and extrahepatic biliary anomalies [152]. The most severe complications of malrotation are small bowel obstruction and volvulus, which cause recurrent bilious vomiting, passage of bloody stools or acute onset of constipation, and bowel loop distention. In patients with Ladd bands, symptoms of duodenal obstruction may develop. Patients from 1 month of age through adolescence may have symptoms that are more varied and indolent, including intermittent vomiting, failure to thrive, and recurrent abdominal pain [150]. Adults may be asymptomatic for many years [153] and then present with midgut volvulus causing abdominal pain, bloody stools, and distention [151]. Alternatively, in adult patients with a long history of recurrent abdominal pain with nausea and vomiting since childhood, the obstruction or volvulus may never have been diagnosed because it was transient and resolved before presentation [152].

Diagnosis

The diagnosis may be challenging in the older child and adult [154,155]. Plain films may reveal evidence of duodenal or small bowel obstruction. A classic double-bubble sign may be seen, indicating duodenal obstruction. However, early in the course of midgut volvulus, plain radiographs may be unrevealing, and children with bilious vomiting should undergo a contrast study to rule out volvulus [156]. Contrast radiographic studies may demonstrate anomalous small bowel, ligament of Treitz, and cecal locations. In adults with midgut volvulus, upper gastrointestinal contrast series may reveal a dilated stomach and duodenum with a typical "corkscrew" appearance of the barium in the twisted duodenum and proximal jejunum [157]. Barium enema may reveal malposition of the right colon and cecum [151,155], and CT may show reversed positions of the superior mesenteric artery and vein.

Treatment

The surgical correction of malrotation (incomplete rotation) in symptomatic patients includes relieving the obstruction caused by Ladd peritoneal bands, freeing the duodenum, reducing midgut volvulus, and broadening the mesentery to prevent recurrent rotation of the bowel around a narrow mesentery [151]. In the Ladd procedure, the bowel is placed in the position of nonrotation (small bowel on the right side of the abdomen and colon on the left). Appendectomy is performed because the cecum is incorrectly located. Variations of this operation are used to correct nonrotation and the rare reversed rotation.

In most patients, bowel function returns to normal. However, some children with recurrent and prolonged symptoms of nausea, vomiting, and pain preoperatively do not fare so well postoperatively, possibly because of persistent dysmotility syndromes associated with malrotation or damage to the small bowel resulting from long-standing, indolent obstruction [149].

Gastroschisis and omphalocele

Description and embryonic origin

Although gastroschisis and omphalocele both result from abdominal wall defects, these rare disorders are distinct entities (see Chapter 121). The incidence of gastroschisis is between 0.4 and 4 per 10 000 live births, and omphalocele is 1.5–3 per 10 000 births [158,159].

In omphalocele, the abdominal viscera herniate through the umbilical ring and persist outside the body, covered by a membranous sac but not by skin. In gastroschisis, massive evisceration of the intestines occurs through a small defect in the abdominal wall, usually to the right of the closed umbilical ring. The bowel has no membranous covering, has been exposed to amniotic fluid in utero, and is often matted, thickened, foreshortened, and covered with adhesions. It has been hypothesized that omphalocele is caused by a failure of embryonic folding at the level of the lateral folds or persistence of the body stalk [158,160]. Gastroschisis may be caused by vascular disruption in utero, leading to failure of differentiation of the somatopleural mesenchyme, or later between the time of herniation into the umbilical cord and fixation, or by disruption of the right omphalomesenteric artery. The defect is thought to occur between the fifth and tenth weeks of gestation, but perinatal insults have also been suggested in patients in whom gastroschisis was not detected sonographically [161]. Potential teratogens include tobacco, alcohol, cocaine, and other drugs [159].

Other congenital anomalies that occur in association with omphalocele are chromosomal; about one-third of cases of omphalocele with multiple anomalies have trisomy 13, trisomy 18, or the Beckwith–Wiedemann syndrome, which is characterized by macroglossia, large infant size, visceromegaly, early hypoglycemia, and increased risk of Wilms and other tumors. Gastroschisis is associated with intestinal anomalies, including atresia and malrotation, vascular disruptions, and renal and gallbladder agenesis [158]. Several mouse models of gastroschisis have been described [162–166]. In mice that are null for the bone morphogenetic protein 1 (*Bmp1*) gene, the *Alx4* gene, or the aortic carboxypeptidase-like protein gene, gastroschisis develops with early mortality. The ALX4 protein is a homeodomain transcription factor. Both BMP1 and the aortic carboxypeptidase-like protein appear to have important functions in the extracellular matrix. The relevance of these gene defects to human disease is unclear; a mutational analysis of the *BMP1* gene in human patients with gastroschisis was unrevealing [167]. Mouse models of omphalocoele have also been described [162], in mouse knockouts of insulin-like growth factor 2 (IGF-2), calreticulin, and several transcription factors. The transforming growth factor-β (TGF-β) signaling pathway has been implicated as important in ventral body wall closure [162].

Prenatal diagnosis

Elevated levels of maternal serum α-fetoprotein are associated with ventral abdominal wall defects. Gastroschisis is detected with greater sensitivity than omphalocele [160]. Improvements in the accuracy of prenatal ultrasonography have led to more frequent detection [158,159,168]. One of the major advantages of prenatal diagnosis is that the obstetrician is alerted, so that at the time of delivery the mother can be taken to a tertiary care center where appropriate surgical and neonatal intensive care support is available [160]. The role of preterm delivery to minimize intestinal damage and prevent intrauterine death is still unclear, and the factors that determine the need for this intervention are still being defined [169,170]. Intensive monitoring with ultrasonography has been utilized to determine when preterm delivery may be indicated, and this strategy appears to improve survival [170–172]. Recommendations for the appropriate mode of delivery (e.g., trial of labor vs elective cesarean section) are being debated [172–176], although it is clear that the rate of abdominal delivery is high because of peripartum complications [177].

Treatment

Surgical closure of omphalocele and gastroschisis is attempted shortly after birth. Primary closure may be possible with the use of retention sutures and stretching the abdominal wall. If this cannot be achieved, a silo or Silastic sac can be used with successive compression to reduce the herniation further. The decision to use a silo and a staged procedure may be based on intraoperative bladder or intragastric pressure measurements that reflect the intraabdominal pressure [169,178]. Careful regulation of the intraabdominal pressure is critical for successful repair; high pressures that lead to respiratory distress and intestinal and renal ischemia – the major causes of morbidity and mortality – must be avoided. The material is placed over the herniated bowel or sac and reduced manually and serially sutured to reintroduce the bowel into the abdominal cavity. A comparison of primary emergent closure vs closure following reduction with a spring-loaded silo (for gastroschisis) suggested that silos improve the overall outcome [179]. Because almost all children have bowel hypomotility, total parenteral nutrition is recommended early in the course of the illness.

Advances in surgical correction have greatly improved the prognosis of patients with gastroschisis, despite the fact that the bowel is usually damaged and slow to return to normal function. Survival rates range from 70% to more than 90% [173,180]. The presence of necrotizing enterocolitis and other anomalies (affecting other organs) or associated intestinal conditions (e.g., atresias, perforations, necrotic segments, volvulus) affects mortality rates [173,181]. In the absence of severe associated anomalies, the immediate survival and long-term outcome of patients with omphalocele have also improved in the past several years [182,183]. Survival is also related to the severity of the associated anomalies and medical conditions [158].

Structural anomalies

Volvulus

A volvulus is an abnormal twisting of the intestine around the axis of its own mesentery, resulting in obstruction of the more proximal bowel. Twisting of the mesentery may involve the mesenteric vessels and so make the involved loop particularly susceptible to strangulation and gangrene, with resulting perforation, peritonitis, and sepsis. In contrast to colonic volvulus, particularly of the cecum and sigmoid colon, small bowel volvulus is relatively rare in the United States and most of Europe [184–188]. The estimated annual incidence in the United States is 1.7–5.7 cases per 100 000 [184,185]. Small bowel volvulus occurs more frequently in parts of Africa, the Middle East, and the Indian subcontinent [184–186]. In these countries, volvulus occurs in the absence of anatomical abnormalities and is probably related to dietary factors. It has been postulated that ingesting large amounts of bulky foods after fasting may predispose to torsion of the small bowel loops. In the United States, small bowel volvulus is usually caused by a preexisting defect, such as an anomaly of rotation and fixation, postoperative adhesion, or congenital bands [189]. Other causes include jejunal diverticula and stromal tumors. Small bowel volvulus is rare in adults [186].

Clinical presentation and diagnosis

Patients present with symptoms of small bowel obstruction and an acute abdomen. Abdominal pain, nausea, and vomiting are almost always present [184–186]; the severity of the pain may be out of proportion to the physical findings. Signs include abdominal distention, rebound, guarding and rigidity, and occasionally a palpable abdominal mass.

The imaging modality of choice is CT scan [185,186]. The "whirlpool" sign, which is a whirl-like rotation of the small bowel around the axis of the superior mesenteric artery, is a sensitive indicator of volvulus [185,186]. Plain abdominal radiographs taken in supine and upright positions may demonstrate distended bowel with air–fluid levels, consistent with obstruction. Perforation may be indicated by the presence of free air. Barium studies can be useful. A typical corkscrew-like appearance of the barium in the distorted duodenum and jejunum is diagnostic. Angiography may reveal twisting of the branches of the superior mesenteric artery.

Therapy and outcome

The treatment of small bowel volvulus is surgical. Ischemic or gangrenous loops of bowel should be resected, although derotation of the bowel may be sufficient therapy in itself. Vascular compromise of the small bowel with subsequent gangrene is common and leads to increased morbidity and mortality postoperatively. Rapid recognition of volvulus and prompt surgical intervention are the keys to decreasing the fatality rate associated with this condition.

Intussusception

Description and pathophysiology

Intussusception occurs when a segment of bowel invaginates, or telescopes, into adjacent distal bowel, leading to obstruction and possible ischemic injury. The incidence, causes, clinical presentation, and therapy of intestinal intussusception are different in adult patients compared with pediatric patients [190–192]. Intestinal intussusception is one of the most common causes of small bowel obstruction in children younger than 2 years of age, but it is an unusual cause of bowel obstruction in adults. Most pediatric cases occur in children younger than 5 years of age [193]. Pediatric intussusception is most often idiopathic but may be associated with a pathological lead point in 8%–12% of cases [194,195], including: Meckel diverticulum; a variety of benign and malignant tumors, such as polyps, leiomyomas, and lymphomas; duplications; and Henoch–Schönlein purpura, in which an intramural hematoma acts as a lead point. An association between rotavirus vaccination and intussusception has been described [196,197], perhaps secondary to a lead point of vaccine-induced lymphoid hyperplasia. In idiopathic intussusception, an association with prominent Peyer patches and enlarged mesenteric lymph nodes has been observed [190]. In children, ileocolic intussusceptions are most common, followed by ileoileocolic, cecocolic, and, much less frequently, ileoileal involvement.

Among adults in the Western world, small bowel intussusception is rare, accounting for approximately 5% of all cases of intestinal obstruction [198]. A causative factor can be identified in more than 90% of adult patients [191], whereas it cannot in children [190–192]. Small intestinal intussusception may be precipitated by tumors, including leiomyomas, neurofibromas, lipomas, lymphomas, small bowel adenomatous polyps, and metastatic tumors with or without peritoneal carcinomatosis. Other causes include Meckel diverticulum or other diverticular disease and celiac disease with chronically dilated flaccid bowel. Postoperative intussusception may result from adhesions, or intussusception may follow trauma [199]. Jejunogastric intussusception may occur after Billroth II surgery, and bypassed bowel may become intussuscepted after jejunoileal bypass. Patients with AIDS and Kaposi sarcoma or diffuse enteritis are also at risk [200]. Pregnancy and the use of long intestinal or cantor tubes are other predisposing factors.

Signs and symptoms

The typical pediatric patient is a well-nourished, previously healthy child between the ages of 5 months and 5 years. The peak presenting age is 3–11 months [201]. Classic signs and symptoms include the acute onset of intermittent abdominal pain, vomiting, and hematochezia. A palpable abdominal mass, diarrhea, and somnolence are other frequent findings [194,195]. In older children, these diagnostic signs and symptoms are often not present [193]. In adults, the clinical

picture may be confusing. Abdominal pain is almost always present [202,203] but is often low-grade and chronic; patients may present after several episodes have spontaneously resolved [198,204]. A partial or complete small bowel obstruction may be present, and an abdominal mass is often palpable. Nausea and vomiting are particularly associated with small bowel compared with large bowel intussusception. Weight loss may also occur in patients with chronic, indolent symptoms.

Diagnosis and therapy

Pediatric patients

In children, an air or water-soluble contrast barium enema is performed because the ileocecal region is so frequently involved [195]. Contrast enemas demonstrate the location of the intussusception and are often successful in reducing it. Air enemas are also safe and effective in reducing intussusceptions [156,205–207]. The enema is usually administered with a surgeon in attendance in case of perforation or failure to reduce. Sonography may be used to diagnose intussusception and to monitor reduction [207]. In extremely ill patients with peritonitis, perforation, or shock, enema reduction is contraindicated. Radiological reduction is successful in 50%–90% of cases [207]. If it is not, the patient is taken to laparotomy for manual reduction. Resection is performed if ischemia or gangrene of the bowel is present. A careful search is made for a possible pathological lead point.

Plain films may reveal a crescent of gas capping the intussusceptum, outlining its leading edge, or a target sign consisting of two concentric radiolucent curvilinear lines outlining the intussusception [208]. A gasless area may also be identified, corresponding with the soft tissue mass of the intussusception. Ultrasonography may show a classical target, or doughnut, sign, characterized by multiple concentric rings of sonolucency alternating with one or two echogenic foci. The edematous outer and inner walls of the intussusception create two hypoechoic areas ringing the hyperechoic lumenal mucosa [209]. Computed tomography may similarly be useful, but it is usually avoided in children. Upper gastrointestinal contrast series may show proximal intestinal dilation and a "bird's beak" at the site of obstruction but is contraindicated if perforation or peritonitis are suspected.

Adult patients

Intussusception is diagnosed and treated differently in adult patients because its location and causes are different from those in children [190–192]. Most intussusceptions in adults are in the small bowel, although they may also occur in the colon [210]. Therefore, a combination of plain film, upper gastrointestinal series, barium enema, and CT or ultrasonography is used in adult patients. Because of the often puzzling presentation of intussusception in this age group, CT is frequently performed [191,192]. A mass of alternating high and low attenuation may be seen as a target, sausage-shaped, or bilobed lesion [204]. Mesenteric fat appears as areas of low attenuation, whereas the bowel wall itself appears as an area of high attenuation. Thickened bowel loops and an intraluminal soft tissue mass may be seen. However, this technique is not sensitive enough to determine the nature of the pathological lead point.

Because a pathological lesion is found so frequently, treatment in adults consists of surgical intervention with bowel resection. Manual reduction alone may be pursued only if it is certain that no other lesions are present [196]. With present diagnostic techniques, however, it is unlikely that a tumor or another anatomical cause can be ruled out preoperatively. Therefore, manual reduction of intussusception in adults is usually not recommended because manipulation can lead to intralumenal or intravenous tumor seeding [202]. Colonic intussusception is never treated with manual reduction because of the extremely high likelihood of malignancy [204].

Lymphangiectasia

Description and pathophysiology

Intestinal lymphangiectasia is characterized by the obstruction of lymph drainage from the small intestine and the dilation of lacteals and other intestinal lymphatics, such as those in the serosa and mesentery, depending on the level of obstruction. As a result of obstruction and increased pressure in the intestinal lymphatics, the absorption of chylomicrons and fat-soluble vitamins such as vitamin D is impaired, the reentry of intestinal lymphocytes into the peripheral circulation is impeded, and excessive amounts of intestinal lymph "leak" into the intestinal lumen. Lymphenteric fistulae may form, and intestinal lymph containing chylomicrons, protein, and lymphocytes drains directly into the intestinal lumen. Chylomicrons are sequestered in the lamina propria as well as in the distended lymphatics. Blockage of serosal and mesenteric lymphatics may lead to chylous ascites, and blockage of the thoracic duct to chylous pleural effusions.

Intestinal lymphangiectasia may occur as a primary congenital disorder or may be secondary to a disease that blocks the intestinal lymph drainage at some level. Causes of secondary lymphangiectasia include extensive abdominal or retroperitoneal carcinoma or lymphoma, retroperitoneal fibrosis, chronic pancreatitis, mesenteric tuberculosis or sarcoidosis, Crohn's disease, Whipple disease, scleroderma, celiac disease [211], constrictive pericarditis, systemic lupus erythematosus [212], chronic congestive heart failure, and following the Fontan operation (right atrium to pulmonary artery anastomosis) [213,214]. Lymphangiectasia after the Fontan procedure can occur in up to 24% of patients [214] and is thought to be caused by increased systemic venous pressure resulting in increased lymph production and decreased drainage. However, focal involvement has been reported, detectable by radionuclide studies and amenable to surgical resection [214]. Congenital intestinal lymphangiectasia

(Milroy disease) results from a malformation of the lymphatics that often affects many areas of the body.

Clinical presentation

Patients present with varying degrees of steatorrhea and malabsorption, lymphocytopenia (especially of T lymphocytes), marked hypogammaglobulinemia with impaired cell-mediated immunity, and prominent manifestations of protein-losing enteropathy. They often have edema and low serum protein levels, the reduction in serum albumin usually being the most pronounced and the only one of clinical significance. Patients with congenital disease present at any time from birth to adulthood, often with asymmetric edema of an extremity caused by peripheral lymphatic obstruction. They may also present, as do the secondary cases, with more diffuse, symmetric edema, usually the result of marked hypoproteinemia. Despite lymphocytopenia and impaired delayed hypersensitivity reactions, opportunistic infections are not common. Gastrointestinal symptoms are usually not prominent, but some patients may have diarrhea, abdominal pain, distention, nausea and vomiting, and occasionally gastrointestinal bleeding.

Diagnosis

Protein-losing enteropathy should be suspected in any patient with unexplained hypoalbuminemia, and intestinal lymphangiectasia should be considered if lymphocytopenia and steatorrhea are also present. Asymmetric lymphedema, especially dating from infancy or childhood, should suggest congenital (Milroy) disease. A diagnosis of lymphangiectasia rests on peroral jejunal biopsy demonstrating dilated lymphatic lacteals. Several specimens may be required to demonstrate the diagnostic findings because the lesions are often patchy and localized [215]. Endoscopy may reveal dilated lacteals that appear as white opaque spots or white-tipped villi, and nodular lesions and xanthomatous plaques have also been noted [216,217]. These findings aid the endoscopist in selecting appropriate regions to sample. Other pathological findings include moderate villus blunting and mild to moderate inflammatory infiltration [215]. Other tests demonstrate protein-losing enteropathy by detecting excessive enteric loss of plasma proteins. The use of radiolabeled plasma proteins, such as ^{131}I-albumin and ^{51}Cr-albumin, has been generally replaced by the measurement of gastrointestinal clearance of α_1-antitrypsin. The use of ^{99m}Tc to detect the site of protein loss appears sensitive and specific [214]. Double-contrast radiographs of the small bowel may reveal folds thickened by intestinal edema, nodular protrusions, and an absence of mucosal ulceration [218]. If secondary lymphangiectasia is suspected, appropriate tests such as CT of the abdomen should be performed to diagnose the underlying disease. The degree of malabsorption and nutritional deficiency may be assessed by quantifying stool fat and by measuring prothrombin time (which is nonspecific) and serum levels of calcium and carotene. In congenital cases, if necessary, the malformed, hypoplastic lymphatics can be demonstrated by lymphangiography. Pleural effusions and ascitic fluid may be tapped for conventional diagnostic studies and examined for chylomicrons.

Therapy

The therapy for lymphangiectasia should be directed toward treating the pathophysiological consequences, and in the case of secondary lymphangiectasia, the underlying disease (e.g., lymphoma, tuberculosis, sarcoidosis, constrictive pericarditis) should be diagnosed and treated. Substituting medium-chain triglycerides for the usual long-chain triglycerides may reduce enteric protein loss, malabsorption, and diarrhea and may improve serum albumin levels [219,220]. Medium-chain fatty acids are more water-soluble and may be more readily absorbed through portal venous channels than through the lymphatics. The concomitant reduction in dietary long-chain fatty acids presumably reduces chylomicrons in obstructed lymphatics and thereby decreases the lymphatic pressure and rate of lymph loss. Anecdotal reports have demonstrated the efficacy of octreotide [221–224]. Peripheral edema can be minimized by postural drainage and the use of elastic stockings to reduce the risk for cellulitis and lymphangitis.

Celiac artery compression

The topic of celiac artery compression is covered in Chapter 131.

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C SMALL INTESTINE

47 Dysmotility of the small intestine and colon

Michael Camilleri, Lawrence Szarka

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The functions of intestinal and colonic smooth muscle are controlled mainly by the intrinsic and extrinsic nerves of the gastrointestinal tract and, to a lesser degree, by gastrointestinal hormones (see Chapter 11). Therefore, any abnormality of smooth muscle, intrinsic or extrinsic nerves, or gastrointestinal hormones can theoretically cause intestinal or colonic dysmotility. Abnormal circulating levels of gastrointestinal hormones have not been definitely shown to result in dysmotility; however, diarrhea in patients with carcinoid syndrome and in some with irritable bowel syndrome is associated with elevated circulating levels of serotonin, and acceleration of small bowel and proximal colonic transit in these patients may at least partly reflect abnormal motor function.

The human small bowel and colon display widely divergent patterns of absorption, motility, and transit. Propulsive, peristaltic motility can rapidly move contents over long distances of the small intestine, with efficiency approaching that of the esophagus. The small intestine functions to facilitate emptying from the stomach, the mixing of chyme with digestive enzymes and bile, and ultimately delivery of residue to the colon. The ileum possesses a specialized type of contraction (giant migrating contraction) that sweeps unabsorbed residue to the colon and is analogous to the high-amplitude colonic contractions. Intermittent emptying ensures that enough time is available for salvage of the remaining nutrients in the small intestine. The small intestine and colon also set up feedback inhibitory reflexes (e.g., ileal brake) that retard proximal motor functions such as gastric emptying.

The fundamental motor programs of the colon, in contrast to those in other regions of the gut, are under strong inhibitory influences, slowing colonic transit for fecal contents to solidify. The well-developed muscular wall of the colon exhibits basal tone, but at the same time, it has a pronounced propensity to relax [1]. As the organ responsible for the final stages of absorption and elimination, the colon is highly relevant to diarrhea and constipation. Colonic fermentation of dietary residues, mainly the "unavailable carbohydrates," generates hydrogen, carbon dioxide, short-chain fatty acids, and methane through the anaerobic action of bacterial enzymes [2]; distention of the abdomen and flatulence can follow. Finally, for imagined, cultural, and psychosocial reasons, many populations focus on their habits of elimination. Constipation is thus a major concern of many individuals, and disturbances of colonic motility figure prominently in the minds of patients and physicians.

Dysmotility of the small intestine and colon has a wide range of clinical manifestations, regardless of the underlying cause of the disorder. Patients at one end of the spectrum may be asymptomatic, whereas those at the other end may have chronic intestinal pseudoobstruction, a syndrome characterized by recurrent or chronic symptoms that suggest obstruction in the absence of structural occlusion of the lumen. Between the two extremes, patients may have dyspeptic symptoms, including intermittent postprandial epigastric or periumbilical abdominal pain, bloating, nausea, vomiting, and diarrhea.

In patients with small intestinal dysmotility, motor function in other parts of the gastrointestinal tract may also be abnormal. In this chapter, we focus our initial discussion on the small intestine; motility disorders of the colon are manifested predominantly as constipation and megacolon. Motility disorders of other parts of the gastrointestinal tract are discussed in other chapters (see Chapters 59, 65, and 86).

Epidemiology

The prevalence of small intestinal dysmotility varies according to the underlying disease, and it seems to be less frequent than esophageal, gastric, or colonic dysmotility. The lack of sensitive techniques to evaluate small intestinal motility has made it difficult to estimate its prevalence. Since the early 1970s, several hereditary diseases with small intestinal

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dysmotility have been reported, including two groups of diseases associated with smooth muscle degeneration, termed *familial visceral myopathies* (FVMs) [3–29] and *childhood visceral myopathies* (CVMs) [30–56], and a group of diseases associated with myenteric plexus degeneration, termed *familial visceral neuropathies* (FVNs) [57–68]. Only a small number of families have been reported, mostly Caucasian and less often African American [9] and Latino [69]. These rare familial disorders are discussed in the first part of this chapter.

Hirschsprung disease, the prototypical congenital colonic dysmotility, affects 1 in 5000 newborns. In contrast, constipation affects about 12% to 15% of the population, with epidemiological data suggesting an equal split between functional constipation and evacuation disorders [70,71]. A telephone survey of approximately 10 000 adults assessed the prevalence of constipation (defined as an inability to have spontaneous complete bowel movements). Consistent with the results of prior studies, the overall prevalence was approximately 15%; however, only 2% of the respondents were classified as having constipation-predominant irritable bowel syndrome (IBS) [72]. In contrast to the results of prior studies, which suggested that constipation increases with advancing age, these data showed an inverse trend, with a slightly lower prevalence associated with increasing age [72]. Other studies of clinic patients have indicated an overlap in the diagnoses of constipation-predominant IBS and functional constipation despite the use of strict definitions [73,74].

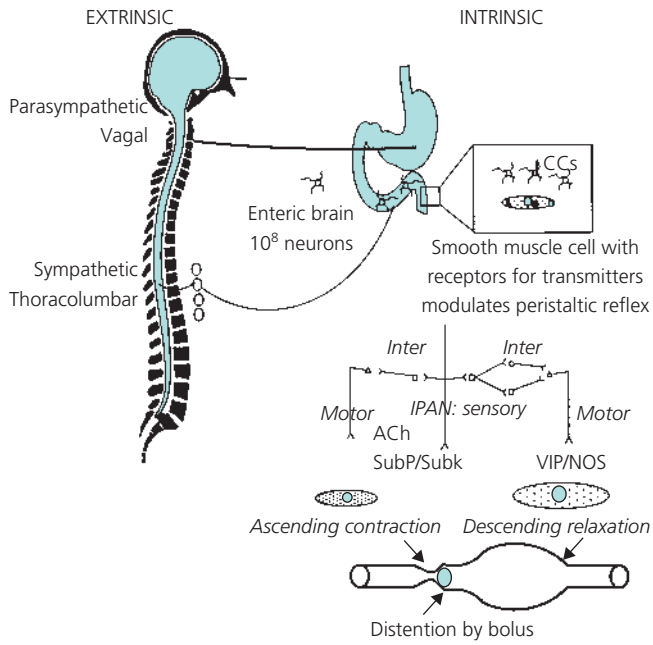


Figure 47.1 Extrinsic and enteric control of gut motility. The enteric nervous system controls stereotypical motor functions such as the migrating motor complex and the peristaltic reflex; enteric control is modulated by the extrinsic parasympathetic and sympathetic nerves, which respectively stimulate and inhibit nonsphincteric muscle. ICC, interstitial cells of Cajal; IPAN, intrinsic primary afferent neuron.

Neural control of small intestinal and colonic motility

The enteric nervous system (ENS) is a vast network of ganglionated plexuses located in the wall of the gastrointestinal tract [75,76]. Although several plexuses are identified anatomically, the most important, from a functional perspective, are the myenteric and submucosal plexuses. In association with the muscle layers, the networks of interstitial cells of Cajal are recognized as the likely pacemakers activating neuromuscular function. The ENS consists of approximately 100 million neurons in higher mammals, and this number is roughly equal to the number of neurons in the spinal cord (Fig. 47.1).

The ENS develops in utero as neural crest cells migrate to the developing alimentary canal. The migration of neural crest cells, and the sequence of innervation of different levels of the gut, are regulated by specific signaling molecules that include transcription factors (e.g., Mash-1), neurotrophic factors (e.g., the glial cell line-derived neurotrophic factor [GDNF] and its receptor subunits), and the neuregulin signaling system. These facilitate the growth, differentiation, and persistence of the migrating nerve cells once they arrive in the gut. Neuregulins are a large group of structurally related signaling proteins that are likely to have important roles in the development, maintenance, and repair of the nervous system and other selected tissues. Their receptors are the Erb-B protein tyrosine kinases, which are important in cell signaling.

Histological and electrophysiological studies [77–80] of the intestinal tract have characterized the properties of the neurons and transmitters mediating its functions, including the peristaltic reflex and the interactions between neurons and inflammatory cells [81].

Ontogeny of the enteric nervous system

Migration

The ENS cells are derived from precursor cells from three axial levels of the neural crest. These include the vagal [82], rostral–truncal [83], and lumbosacral [82,84]. levels. The enteric neurons arise mainly from the vagal neural crest of the developing hindbrain and colonize the gut by migrating in a rostrocaudal direction. Rarely, the migrating cells do not reach the entire gut; usually, the terminal portion of the bowel is affected, as in classic forms of Hirschsprung disease. Even more rarely, a zonal form of Hirschsprung disease may occur (Figs 47.2 and 47.3), which is postulated to result from the incomplete caudorostral migration of neuroblasts during embryonal development [85]. The neural crest cells that

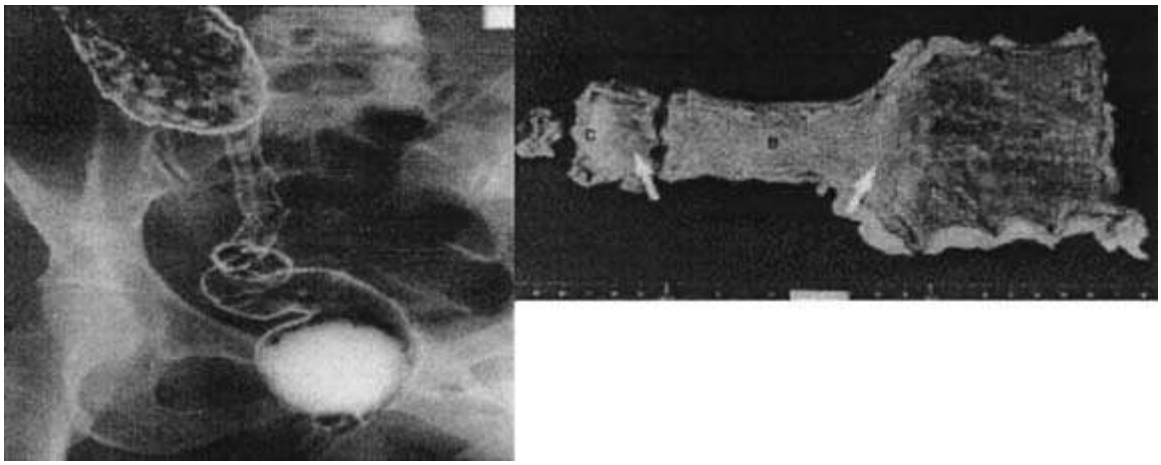
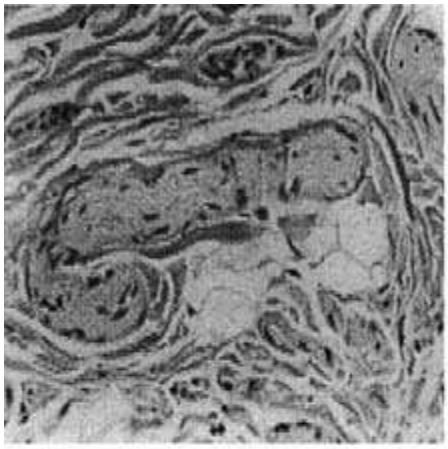
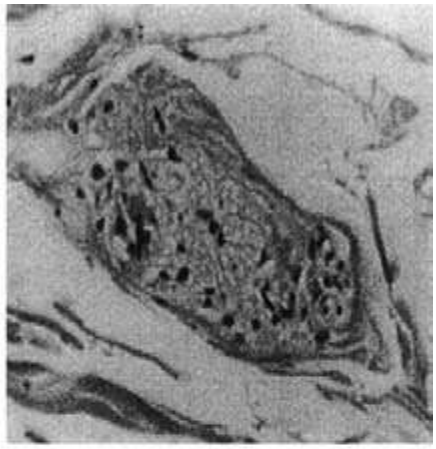


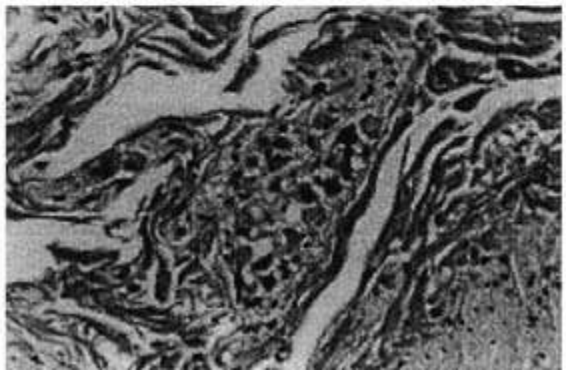
Figure 47.2 Zonal Hirschsprung disease in an adult: narrowed segment of the sigmoid colon on barium enema and gross pathological examination. From Fu et al. [85], with permission from the BMJ Publishing Group.



Aganglionic zone with hypertrophied nerve trunks (H&E × 100)



Decreased ganglion cells (arrow) in distal normal rectum (H&E × 100)



Normal ganglion cells in sigmoid (H&E × 100)

Figure 47.3 Zonal Hirschsprung disease in an adult: variation in the number of ganglion cells in different segments of the resected specimen. From Fu et al. [85], with permission from the BMJ Publishing Group.

migrate and colonize the gut become neuroblasts or neuronal support cells, glioblasts. However, *differentiation* into neurons and glial cells seems not to take place until they reach their final destinations in the gut. Movement through the gut mesenchyme, survival in the gut, and differentiation into mature cells are influenced by contacts of the precursor cells with the *microenvironment*.

The microenvironment consists of other cells in the mesenchyme, neural crest-derived cells, and the extracellular matrix. The extracellular matrix components provide direc-

tional signals to migrating neural crest cells and, together with neighboring cells, provide signals for neural crest cell differentiation. For example, the appearance of neural crest cells in the gut is preceded by the expression of extracellular matrix molecules [86], and other factors, such as GDNF, ensure the survival of committed neuroblasts [87].

Defects of the neural crest cells themselves or alterations of the microenvironment of the pathway through which the neural crest cells migrate may result in maldevelopment of the ENS. In humans, such disordered development results in congenital enteric neuromuscular diseases.

Differentiation of neurons

Migrating crest cells are multipotent [88]. The gut wall is itself a crucial site where terminal differentiation of the enteric neurons and glia occurs, and this determines what kind of nervous system arises within the bowel [89]. Combinations of enteric growth factors and their receptors influence differentiation. Combinations that enhance differentiation include the following: glial cell line-derived neurotrophic factor (GDNF)-tyrosine kinase RET (REarranged Transfection); neurotrophin 3–tyrosine kinase receptor C (NT-3–TRKC); and 5-hydroxytryptamine (5-HT, serotonin)–5-HT_{2B} receptor The RET protooncogene encodes a tyrosine kinase receptor necessary for the development of the ENS. A qualitatively different effect on nervous system development is shown by the peptide–receptor combination endothelin 3 (ET-3) and its receptor endothelin B (ET-B), which prevents the premature differentiation of enteric neurons before colonization of the gastrointestinal tract has been completed.

Several nerve growth factors are necessary, but each is insufficient individually to differentiate neurons. These include NT-3, GDNF, serotonin (5-HT), and endothelin. Enteric serotoninergic neurons appear so early that they coexist in primordial enteric ganglia with still-dividing neural precursors. Serotonin may be more than a neurotransmitter; the 5-HT_{2B} receptor in the fetal bowel is regulated and optimal at specific times, so that 5-HT strongly promotes the development of neurons at specific times and affects the development of late-arising enteric neurons [90].

These growth/differentiation factors affect virtually the entire bowel. The peptide endothelin 3 (ET3) and its receptor, ETB, play a crucial localized role in ENS development [91,92]. Examples of tyrosine kinase receptor mutations associated with specific genetic disorders, such as the multiple endocrine neoplasia (MEN) syndromes, are shown in Fig. 47.4.

Chronic small intestinal pseudoobstruction

For a review, the reader is directed to Di Lorenzo [93].

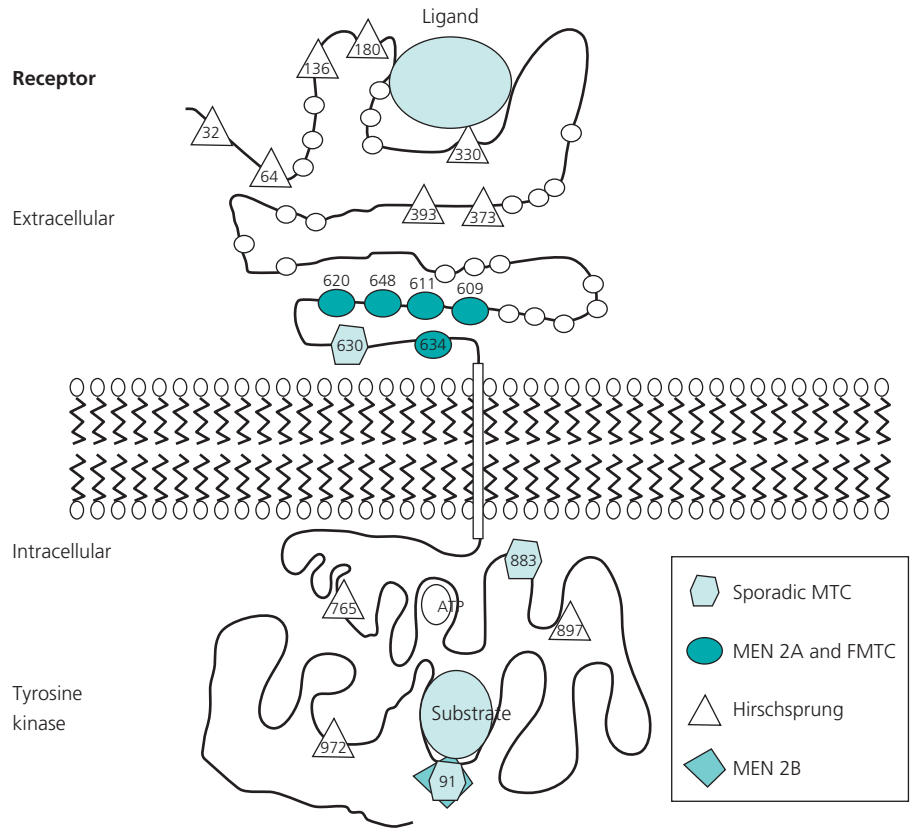


Figure 47.4 Tyrosine kinase receptor with examples of mutations associated with specific genetic disorders. [F]MTC, [familial] medullary carcinoma of the thyroid; MEN, multiple endocrine neoplasia. From Edery P, Lyonnet S, Mulligan LM, et al. Mutations of the RET proto-oncogene in Hirschsprung's disease. Nature 1994;367:378, with permission from Nature Publishing Group.

Table 47.1 Causes of small intestinal dysmotility

PRIMARY CAUSES

Familial types

Familial visceral myopathies: types I, II (MNGIE), III
Familial visceral neuropathies: types I, II
Childhood visceral myopathies: type I, type II (megacystis–microcolon–intestinal hypoperistalsis)

Nonfamilial or sporadic types

Visceral myopathies
Visceral neuropathies

SECONDARY CAUSES

Disease involving the intestinal smooth muscle

Collagen diseases (e.g., scleroderma, dermatomyositis, systemic lupus erythematosus, mixed connective tissue disease)
Muscular dystrophies (e.g., myotonic dystrophy, Duchenne muscular dystrophy)
Amyloidosis

Neurological diseases

Chagas disease, ganglioneuromatosis of the intestine, paraneoplastic neuropathy, Parkinson disease, spinal cord injury

Endocrine disorders

Diabetes mellitus, thyroid disease (i.e., hyperthyroidism, hypothyroidism), hypoparathyroidism

Pharmacological agents

Phenothiazines, tricyclic antidepressants, antiparkinsonian medications, ganglionic blockers, clonidine
Narcotics (morphine and meperidine)

Miscellaneous intestinal disorders

Celiac disease
Small intestinal diverticulosis
Radiation enteritis
Diffuse lymphoid infiltration of the small intestine
Jejunoileal bypass
After gastrointestinal viral infection

MNGIE, mitochondrial neurogastrointestinal encephalomyopathy.

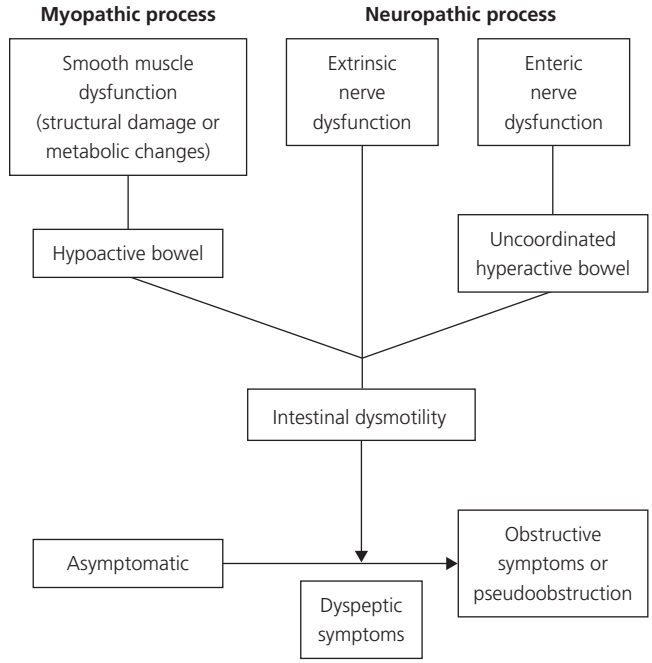


Figure 47.5 Mechanisms and manifestations of small intestinal dysmotility.

Etiology

Table 47.1 lists the causes of small intestinal dysmotility. The mechanisms and manifestations of small intestinal dysmotility are shown in Fig. 47.5. In general, small intestinal dysmotility may be secondary to neuropathy or myopathy. This is best distinguished by specialized histological and immunohistochemical study of the full thickness of the gastrointestinal wall, which is available at a few centers. Smooth muscle is best studied by sectioning across the muscle fibers and using trichrome stain [94]. The best way to evaluate the myenteric plexus is still evolving. Silver staining of sections of the gastrointestinal wall taken parallel to its long axis, described by Smith [95] and by Schuffler and Jonak [96], was state of the art but has been superseded by immunohistochemistry and confocal examination of neurons and a search for genetic markers (see below). Thus, a detailed description of phenotypes in familial cases is less pertinent than the more mechanistic or etiological descriptions that appear below.

Primary causes of small intestinal dysmotility

Primary dysmotilities are rare compared with secondary dysmotilities. They may be familial or sporadic.

Familial visceral myopathies (FVMs)

The FVMs are a group of genetic diseases characterized by the degeneration and fibrosis of smooth muscle of the gastrointestinal tract and, in one type, the urinary tract. At least three types of FVM have been reported, based on gross lesions of the gastrointestinal tract and the pattern of inheritance (Table 47.2), and, in type II FVM (also called *oculogastrointestinal neuropathy*), on documented mitochondrial DNA disorders. On routine pathology, the histological features of these three types of FVM do not differ [94]. The involved areas show a characteristic change, consisting of degenerating muscle cells and fibrosis, which may involve the full thickness of the muscularis propria but often is more prominent in or limited to the external layer (Fig. 47.6). Degenerating muscle cells appear pale, poorly defined, and fragmented. As residual thread-like remnants become surrounded by collagen, the longitudinal and circular muscles take on a vacuolated appearance that is easily recognized microscopically

Table 47.2 Classification of familial visceral myopathies

Characteristics	Type I [1–14]	Type II (MNGIE) [15–23]	Type III [24–27]
Mode of transmission	Autosomal dominant	Autosomal recessive; isolated cases	Autosomal recessive
Gross lesions	Esophageal dilation, megaduodenum, redundant colon, and megacystis	Gastric dilation, slight dilation of the entire small intestine with numerous diverticula	Marked dilation of the entire digestive tract from the esophagus to the rectum
Microscopic changes	Degeneration and fibrosis of both muscle layers	Same	Same
Clinical manifestations:			
Age at onset	After the first decade	Teens	Middle age
Percentage symptomatic	< 50%	> 75%	> 75%
Symptoms of chronic intestinal pseudoobstruction	Variable severity	Severe plus pain	Classic chronic intestinal pseudoobstruction
Extragastrointestinal manifestations	Megacystis, uterine inertia, and mydriasis	Ptosis and external ophthalmoplegia, muscle pain, peripheral neuropathy, and deafness	None observed
Treatment, prognosis	Prognosis good with or without surgery	No effective medical or surgical treatment; prognosis poor	Same as type II (MNGIE)

MNGIE, mitochondrial neurogastrointestinal encephalomyopathy.

(Fig. 47.7). Recognition of this change is greatly facilitated by the use of a trichrome stain; cytochemistry can also identify mitochondrial enzyme deficiencies in type II FVM, or mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). Because several advances in our understanding of this condition have been made, a further detailed discussion of MNGIE is warranted.

Mitochondrial DNA and myopathies

Diverse myopathies are characterized by mitochondrial abnormalities in skeletal muscle or affected bowel, which show "ragged red fibers" on a Gomori trichrome stain. The metabolic abnormality also involves cells in other, non-muscle, tissues, including central and peripheral nervous system, gut, heart, kidney, liver, thyroid, pancreas, and bone marrow. The enzymes in the respiratory chain of mitochondria are encoded by nuclear and mitochondrial DNA. The disorders may be sporadic or inherited in an autosomal dominant, autosomal recessive, or X-linked pattern; maternal inheritance is more frequent.

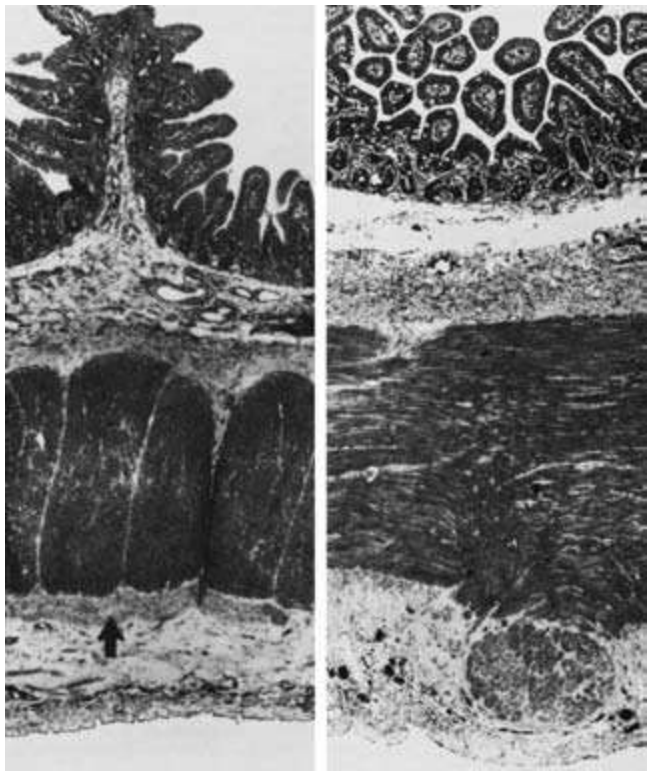
Mitochondria are involved in the production of energy (ATP), the generation of reactive oxygen species, and the initiation of apoptosis through activation of the mitochondrial permeability transition pore [97]. Mitochondrial DNA consists of two to ten double strands of circular DNA in the mitochondrial matrix. At least 37 genes have been discovered, some of which encode the respiratory chain enzymes. The transcription of mitochondrial DNA is not closely related to the cell cycle, and mitochondrial DNA is more susceptible to mutation than is nuclear DNA.

Mitochondrial disorders manifest as organ disturbances that reflect the importance of mitochondria to normal function, as in skeletal muscle and the nervous system; similarly, metabolic disorders result from deranged cellular mitochondrial functions. The locations of selected mutations in the mitochondrial 16 569-base pair genome that result in pathological disorders and syndromes have been documented [97].

The mitochondrial disorder affecting the gut is MNGIE, also referred to as FVM type II, MEPOP (mitochondrial encephalomyopathy, polyneuropathy, ophthalmoplegia, and pseudoobstruction), and oculogastrointestinal muscular dystrophy. This is an autosomal recessive condition with gastrointestinal and hepatic manifestations that may present at any age, typically with hepatomegaly or hepatic failure in neonates, seizures or diarrhea in infants, and hepatic failure or chronic intestinal pseudoobstruction in children or adults.

MNGIE is characterized clinically by severe gastrointestinal dysmotility, external ophthalmoplegia, ptosis, peripheral neuropathy, and leukoencephalopathy. The small intestine is dilated, or multiple diverticula are present (Fig. 47.8), and the amplitude of contractions is typical of a myopathic disorder [98]. Some patients have a combination of intestinal dysmotility and the Kearns–Sayre syndrome, or transfer dysphagia secondary to abnormal coordination and propagation of the swallow through the pharynx and skeletal muscle portion of the esophagus. Clearly, this becomes even more devastating when the smooth muscle portion of the esophagus is affected by the associated MNGIE.

Apart from the obvious external ophthalmoplegia, these patients manifest skeletal muscle pain and cramps, and



(a) (b)

Figure 47.6 (a) Longitudinal section of jejunum from a patient with familial visceral myopathy shows normal circular muscle layer and a prominent neural bundle (arrow). Scattered muscle fibers and collagen are observed in the longitudinal layer. (Trichrome stain; original magnification × 40.) **(b)** Cross-section of jejunum from area depicted in (a). The outer muscle layer contains one large bundle of preserved smooth muscle fibers and scattered individual fibers throughout the collagen elsewhere. (Trichrome stain; original magnification × 40.) From Mitros et al. [94], with permission from Elsevier.

systemic (lactic) acidosis. Circulating levels of muscle enzymes – creatine phosphokinase (CPK), aspartate aminotransferase (AST), alanine aminotransferases (ALT), aldolase, and others – are elevated, and muscle biopsy shows characteristic ragged red fibers on modified Gomori stain. This appearance results from the hypertrophy of mitochondria in the subsarcolemmal position in a few muscle fibers and the lack of mitochondria in other muscle fibers. Special stains for the respiratory muscle enzymes can identify the precise functional defect. For example, succinate dehydrogenase-positive fibers appear "ragged blue," and staining of the adjacent tissue section with cytochrome *c* oxidase demonstrates a deficiency of the latter enzyme and a gene defect in the control of the complex IV respiratory chain proteins (Fig. 47.9) [98]. In the intestine, hypertrophy of the circular muscle layer, atrophy of the longitudinal muscle, and megamitochondria in myenteric neurons and muscle cells are seen (Fig. 47.10) [99].

The screening tests for MNGIE are measurement of serum lactic acid, muscle enzymes, and thymidine phosphorylase

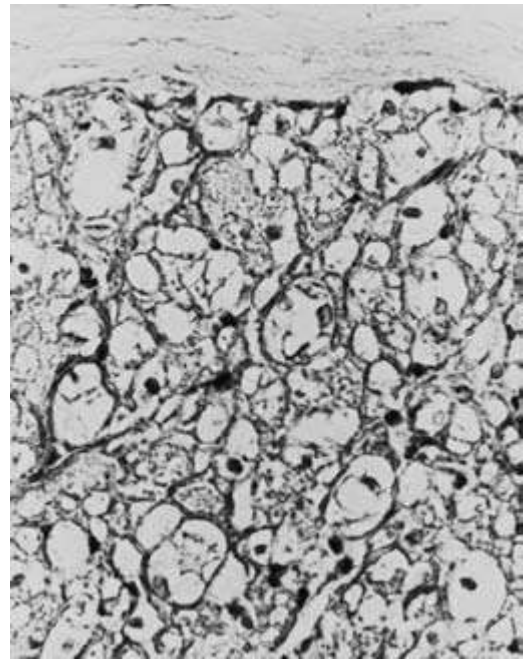


Figure 47.7 The characteristic vacuolar change, with collagen fibers encircling spaces filled by fragmented muscle cells, in a patient with familial visceral myopathy. (Trichrome stain; original magnification × 470.) From Mitros et al. [94], with permission from Elsevier.

Figure 47.8 Enteroclysis in a patient with type II familial visceral myopathy demonstrates numerous diverticula in the small intestine.

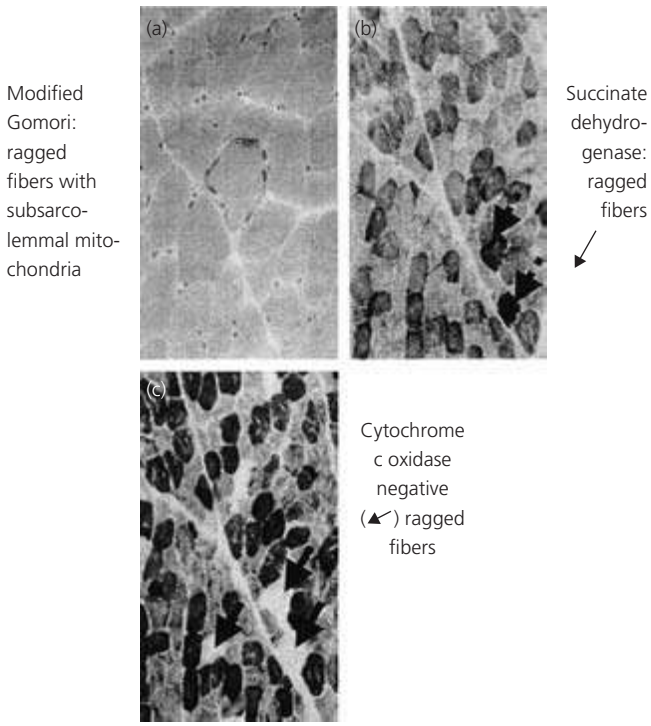


Figure 47.9 Histological and histochemical studies of a skeletal muscle biopsy specimen from a patient with mitochondrial myopathy; note the ragged red fibers, characterized by a subsarcolemmal location of giant mitochondria in a few fibers and a paucity of mitochondria in other fibers. On histochemical analysis, a few fibers are positive for succinate dehydrogenase (ragged blue appearance), but the same fibers do not express cytochrome c oxidase, suggesting a defect in the respiratory enzyme chain resulting in mitochondrial dysfunction and systemic acidosis. From Mueller et al. [98], with permission from Elsevier.

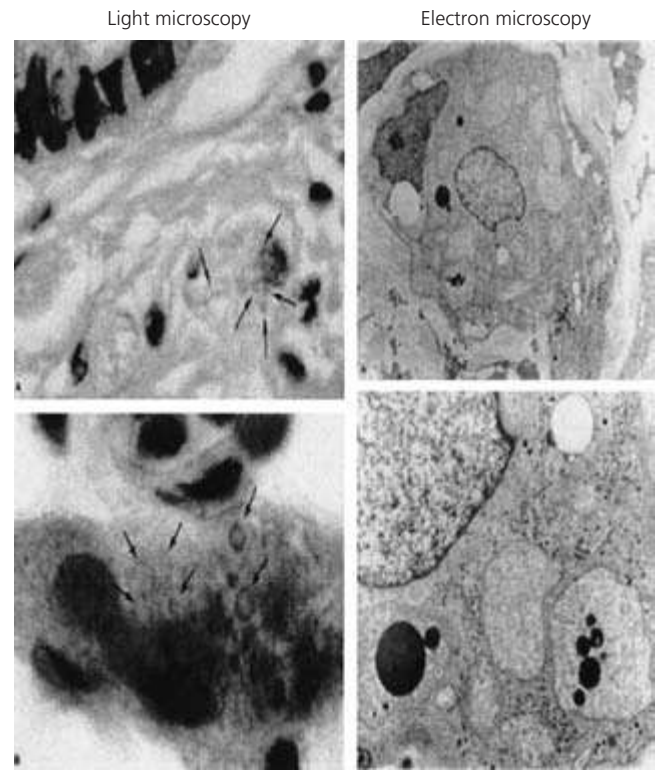


Figure 47.10 Submucosal plexus neurons showing megamitochondria in a rectal biopsy specimen from a patient with mitochondrial myopathy. From Perez-Atayde et al. [99].

in circulating leukocytes [100]. The latter test is based on mutations identified in the gene for thymidine phosphorylase in 21 probands with MNGIE [100].

Familial visceral neuropathies (FVNs)

The FVNs are a group of genetic diseases characterized by degeneration of the myenteric plexus. At least two distinct phenotypes, I and II, have been identified, and these are summarized in Table 47.3. A number of syndromic congenital neuropathies are related to disturbances in the ontogeny of the neural crest and the ENS.

Congenital neuropathic motility disorders

These disorders can be broadly classified as follows [101]:

- *Disorders of colonization* by migrating neural crest-derived neurons, as in Hirschsprung disease, related to abnormalities in the RET gene and GDNF, or disorder of ET-3 and its receptor, ET-B.
- *Disorders of differentiation* of enteric nerves, as in intestinal ganglioneuromatosis, related to a specific germ-line point mutation in RET at codon 918 of exon 16 (M918T) or at codon 883 (A883F) in MEN type 2B syndrome.
- *Disorders of survival or maintenance of enteric nerves,* as in hypoganglionosis and possibly congenital achalasia, which can result from one of several mechanistic derangements between growth factors and their receptors: $GFR_{\alpha 2}$–neurturin, $GFR_{\alpha 3}$–artemin, TRKC–NT-3, and 5-HT_{2B}–5-HT.

Hirschsprung disease is a relatively common condition causing intestinal obstruction in neonates and megacolon in infants and adults; it is often associated with mutations in genes encoding GDNF or its receptor, RET [102,103], or in ET3 or its receptor, ETB [92,104] Mutations in RET and ETB (i.e., the receptors) are much more frequently encountered than mutations in the two ligands. At least two different mechanisms can cause the terminal colon to become aganglionic. In the first, there is a deficiency of GDNF–RET, which is not so severe as to cause the entire bowel to become aganglionic. In the second, characterized by a deficiency of ET3–ETB, crest-derived cells differentiate prematurely and precursors cease dividing and migrating before the gut has been entirely colonized, leaving the last segment uncolonized. The roles of endothelins and of gene mutation M918T in the maldevelopment of the ENS and in Hirschsprung disease are discussed later.

At least six separate genetic loci are involved in the control of four different intracellular mechanisms that result in models of congenital dysmotility and their phenotypic manifestations

Table 47.3 Classification of familial visceral neuropathies

Characteristics	Type I [55–57]	Type II [58–64]
Mode of transmission	Autosomal dominant	Autosomal recessive
Gross lesions	Dilation of lengths of small intestine, often distal small bowel; megacolon; gastroparesis in ~ 25% of patients	Hypertrophic pyloric stenosis; dilated, short small intestine; malrotation of small intestine
Microscopic changes	Degeneration of argyrophilic neurons and decreased numbers of nerve fibers	Deficiency of argyrophilic neurons and increased neuroblasts
Clinical manifestations:		
Age at onset	Any age	Infancy
Percentage symptomatic	> 75%	100%
Symptoms	Chronic intestinal pseudoobstruction in ~ 67%	Chronic intestinal pseudoobstruction in all
Extragastrointestinal manifestations	None	Malformation of central nervous system; patent ductus arteriosus sometimes present
Treatment, prognosis	No effective medical or surgical treatment; prognosis fair	No effective medical or surgical treatment; prognosis poor

Table 47.4 Genetic models of Hirschsprung disease

Genetic defect	Phenotype	Associated non-gastrointestinal disease	Dysmotility: prevalence in phenotype
RET–GDNF	Hirschsprung	None in humans	20%–50% RET, 5% GDNF
ET-3–ET-B	Hirschsprung or megacolon	Waardenburg–Shah syndrome	5%–10% Hirschsprung
SOX-10	Hirschsprung	Waardenburg–Shah syndrome	?
c-*KIT*	?Chronic intestinal pseudoobstruction/ Hirschsprung	None	?

ET, endothelin; GDNF, glial cell line-derived neurotrophic factor; RET,.

in the gut and other tissues (Table 47.4). These include abnormalities of c-*Ret*, the gene that encodes the tyrosine kinase receptor; the ETB system; the SOX-10 transcription factor; and c-KIT, which is a marker for the interstitial cells of Cajal. Disturbances in these mechanisms result in syndromic dysmotilities such as Hirschsprung disease, Waardenburg–Shah syndrome (piebaldism, neural deafness, megacolon), and idiopathic hypertrophic pyloric stenosis. Figure 47.4 shows some of the mutations in the tyrosine kinase receptor that have been reported in dysmotilities associated with familial or sporadic medullary carcinoma of the thyroid, MEN types 2A and 2B, and Hirschsprung disease. Visceral neuropathy may result in bowel dilation (Fig. 47.11), although this is generally less frequent or less severe than in visceral myopathy.

Multiple endocrine neoplasia type 2B syndrome

Multiple endocrine neoplasia type 2B is a serious congenital neuropathic condition associated with tumors of the neuroendocrine system. It presents, often in infancy, with severe constipation or megacolon (Fig. 47.12), diarrhea (when associated with enterocolitis), or obstruction [105,106]. Other external stigmata of MEN 2B are a characteristic facies, "blubbery lips" resulting from mucosal neuromas, marfanoid habitus, medullated corneal nerve fibers, and medullary thyroid carcinoma [105,107]. The latter develops eventually in almost all patients.

A feature of the intestinal pathology is transmural intestinal ganglioneuromatosis, in which massive proliferations of neural tissue (neurons, supporting cells, and nerve fibers) appear as thickened nerve trunks among mature nerve cells (Fig. 47.13).

Although MEN type 2B is a dominantly inherited disorder, at least half of all patients present with a de novo mutation because only a few survive to reproductive age and are disabled by a variety of neurological symptoms that impair reproduction. The precise molecular abnormality is in the *RET* protooncogene (see Fig. 47.4), which encodes a tyrosine kinase receptor expressed particularly in neural crest-derived cells, including the enteric ganglia. A specific germ-line point mutation in *RET* in exon 16 at codon 918 (M918T) occurs in

Figure 47.11 Small bowel radiograph from a patient with type I familial visceral neuropathy shows a normal stomach, duodenum, and proximal jejunum, but a dilated distal small bowel (arrow). From Mayer et al. [58], with permission from Elsevier.

Figure 47.12 Megacolon in a patient with multiple endocrine neoplasia type 2B; note the marked distention of the colon with a relatively small rectal diameter (arrow), and the massive dilation of the transverse colon with scoliosis and deviation of the mediastinum (arrowhead).

95% of patients; the remainder have a point mutation at codon 883 (A883F) [108,109]. The M918T mutation alters RET substrate specificity and appears to act in a ligand-independent fashion (a gain-of-function mutation) [110–112]. Receptor tyrosine kinases with these mutations also bind to and phosphorylate substrates preferred by nonreceptor tyrosine kinases. The A883F RET has not been directly tested, but given its location, it is thought that kinase specificity may also be altered in this mutant [109]. The receptor is expressed in the neural crest cell-derived ENS, adrenal medulla, parathyroid, and C cells of the thyroid. The gain-of-function mutations are susceptibility factors for endocrine tumors (medullary carcinoma of the thyroid, pheochromocytoma, and parathyroid tumors). Because surgery is the only curative procedure for medullary carcinoma of the thyroid, Smith and colleagues [113] recommend that patients who present with a clinical phenotype of Hirschsprung disease undergo adequate full-thickness biopsy of the bowel at the time of the pull-through operation, and that those with transmural intestinal ganglioneuromatosis undergo molecular diagnostic testing by RET mutation analysis. If germ-line M918T or A883F mutations are found, a prophylactic thyroidectomy is indicated; furthermore, adrenal gland surveillance with abdominal ultrasonography and urinary fractionated catecholamines and metanephrine or vanillylmandelic acid are indicated because a pheochromocytoma may develop subsequently in about 50% of cases.

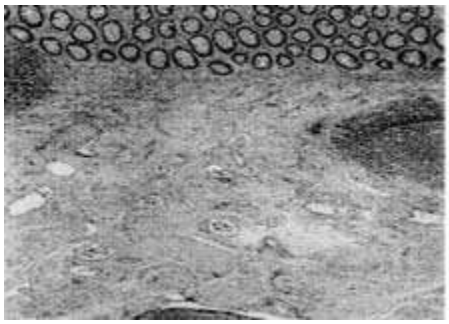
Other familial visceral neuropathies

A few reported families do not fit into the two types of FVN discussed previously [20,67,68].

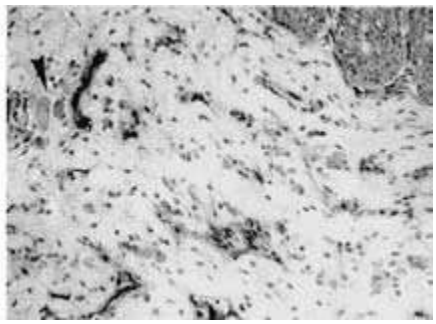
Schuffler and colleagues [67] reported two siblings who had generalized neurological disease and intestinal pseudoobstruction. One had ataxic gait; small, irregular, poorly reactive pupils; dysarthria; absent deep tendon reflexes; and impaired vibratory and positional senses. Radiographs revealed hyperactive, nonpropulsive contractions in a dilated esophagus and small intestine, and extensive colonic diverticulosis. The postmortem examination revealed degeneration of the myenteric plexuses of the esophagus, small intestine, and colon with reduced number of plexuses and round, eosinophilic intranuclear inclusions. The neurons and glial cells of the brain and spinal cord and the dorsal root and celiac plexus ganglia contained identical intranuclear inclusions. Intestinal smooth muscle was normal histologically.

In the family reported by Cockel and colleagues [68], affected members had megaduodenum, generalized dilation of the small intestine, malabsorption, redundant colon, mental retardation, and calcification of the basal ganglia. Histological studies showed degeneration of the argyrophobe cells, which produce acetylcholine. The patients became symptomatic for intestinal pseudoobstruction during late childhood.

Faber and colleagues [20] reported five patients with chronic intestinal pseudoobstruction in two families. Hypertrophy of



Extensive ganglioneuromatosis filling the submucosa × 50



Giant ganglioneuroma of myenteric plexus × 200

Figure 47.13 Histopathological features of ganglioneuromatosis in multiple endocrine neoplasia type 2B; note the extensive ganglioneuromatosis filling the submucosa and the giant ganglioneuroma of the myenteric plexus. From Smith et al. [113], with permission from the BMJ Publishing Group.

Table 47.5 Classification of childhood visceral myopathies

Characteristics	Type I	Type II (megacystis–microcolon–intestinal hypoperistalsis)
Mode of transmission	Autosomal recessive (?)	Autosomal recessive (?)
Gross lesions	Dilation of entire gastrointestinal tract	Short, malrotated small intestine and malfixation of microcolon
Microscopic changes	Degeneration and fibrosis of gastrointestinal and urinary tract smooth muscle cells	Vacuolar degeneration of gastrointestinal and urinary tract smooth muscle cells
Clinical manifestations:		
Age at onset	Infancy and young childhood	Infancy
Gender	Both	Predominantly female
Symptoms	Constipation, distention with or without chronic intestinal pseudoobstruction	Obstipation, intestinal pseudoobstruction
Extragastrointestinal manifestations	Megacystis and megaureters	Megacystis and megaureters
Treatment, prognosis	No effective treatment; prognosis poor	No effective treatment; prognosis poor

nerve fibers and an absence of ganglion cells were noted. The disease was transmitted by an autosomal recessive gene. All patients had megaduodenum; one had jejunal diverticulosis, and another dilation of the entire small intestine. Three had gastric dilation. Most patients had external ophthalmoplegia, ptosis, distal weakness of all extremities, gait disturbances, and acroparesthesia. The symptoms developed during the teen years, and the prognosis was poor.

Childhood visceral myopathies (CVMs)

The CVMs have been recognized as distinctive diseases. There are two forms of CVM (Table 47.5), the second of which is identified by the phenotype of megacystis–microcolon–intestinal hypoperistalsis. These two diseases differ from the FVMs in their clinical manifestations and modes of inheritance. Degeneration and fibrosis of gastrointestinal tract and urinary tract smooth muscle can be detected in both types of CVM [28,34,45,114] and result in bowel dilation (Fig. 47.14), ureteropelvic pyelocaliectasis (Fig. 47.15), or megacystis, which results from bladder degeneration (Fig. 47.16).

Nonfamilial visceral myopathies (sporadic hollow visceral myopathy)

Nonfamilial visceral myopathies in adults have been reported a few times [115–121]. The disease is heterogeneous, with some similarities to FVM types I and III [118]. Histologically, no difference is seen between the familial and nonfamilial types. Diffuse mixed inflammatory cell infiltration, including eosinophils, was observed in one patient [115]. In one reported case [121], acetylcholinesterase stain of the small intestine showed a considerably increased number of positive fibers, but no degeneration of neurons in the myenteric plexus. The nonfamilial type of visceral myopathy is uncommon. A careful family history must be obtained in these patients to exclude a diagnosis of FVM.

Idiopathic nonfamilial visceral neuropathies (sporadic hollow visceral neuropathy or chronic idiopathic intestinal pseudoobstruction)

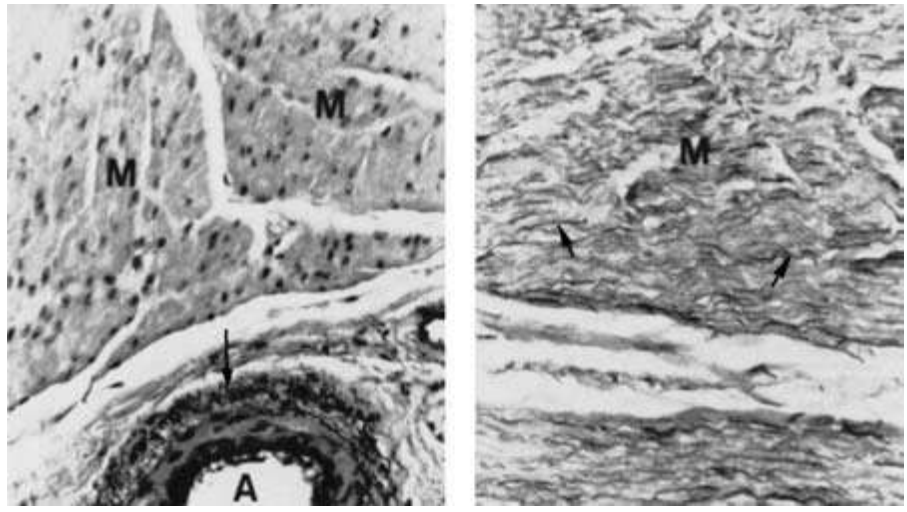
Nonfamilial visceral neuropathy or chronic idiopathic intestinal pseudoobstruction typically results from damage to the

Figure 47.14 Upper gastrointestinal radiograph from a patient with type I familial visceral myopathy demonstrates severe megaduodenum.

Figure 47.15 An intravenous pyelogram of a child with type I childhood visceral myopathy shows megacystis and bilateral ureteral pyelocaliectasis. From Bonsib et al. [114].

myenteric plexus by chemicals, drugs, or viral infections. Delayed gastric emptying has been observed in patients after viral gastroenteritis [122,123], which may cause permanent damage to the myenteric plexus in some cases.

In patients with nonfamilial visceral neuropathies [124–131] the motility of the entire gastrointestinal tract is usually disturbed, but the urinary tract is generally not affected. The intestine is dilated but shows active, nonperistaltic contractions on barium examination [130]. By contrast, patients with familial visceral myopathies have an inactive, dilated intestine [130]. In sporadic visceral neuropathy, histological examination of the myenteric plexus with the Smith silver staining technique reveals a reduction in the number of neurons, and those that remain are markedly abnormal (Fig. 47.17). The neurons are enlarged, with thick, irregular, clubbed processes. The number of Schwann cells in the nerve



(a) (b)

Figure 47.16 (a) Bladder muscularis from a control specimen demonstrates elastic fibers (arrow) in the adventitia of a small artery (A). No elastic fibers are present within muscle bundles (M). **(b)** Bladder muscularis from a patient with type I childhood visceral myopathy demonstrates numerous parallel, coarse, wavy, elastic fibers (arrows) within muscle bundles (M). (Verhoeff–van Gieson stain; original magnification × 325.) From Bonsib et al. [114].

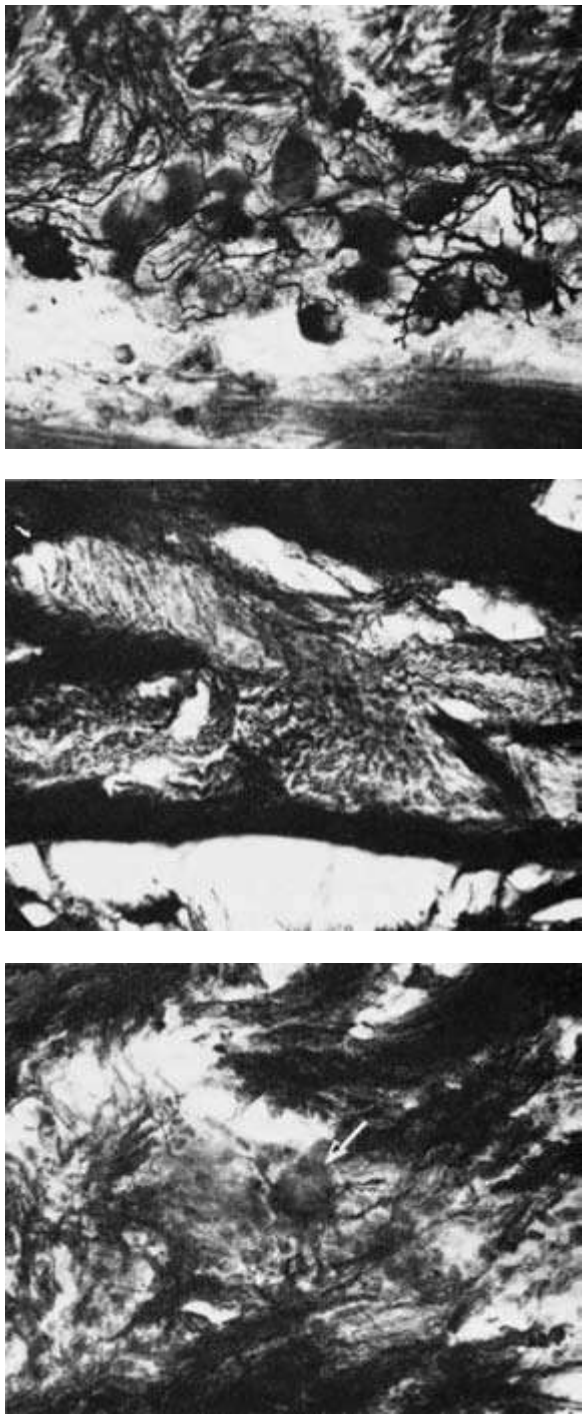


Figure 47.17 **(a)** Ganglionic area from normal control ileum demonstrates numerous argyrophilic neurons and nerve fibers. (Silver stain; original magnification × 230.) **(b)** Ganglionic area from a patient with type II familial visceral neuropathy demonstrates absence of argyrophilic neurons and few nerve fibers. There is a faint cluster of small nuclei of unknown cell type. (Silver stain; original magnification × 230.) **(c)** Another ganglionic area from the same patient shows only one poorly staining, argyrophilic neuron (arrow). (Silver stain; original magnification × 362.) From Mayer et al. [58], with permission from Elsevier.

trunks is increased, and the number of axons is reduced. Some of the remaining axons are beaded and fragmented. Hypertrophy of one or both muscle layers of the muscularis propria may also be observed. All reported cases have chronic intestinal pseudoobstruction. Infants and young children are also affected [131].

In a study of 40 patients with chronic idiopathic intestinal pseudoobstruction, the results of intestinal phasic pressure studies were abnormal, but the histological features of the gastrointestinal tract were normal in those whose tissue was available for analysis [132]. Similar findings have been reported in children with chronic idiopathic intestinal pseudoobstruction [133]. In earlier reports [134–136], histological studies of smooth muscle or myenteric plexus were not generally available for evaluation. The precise mechanism and neurotransmitter deficiencies of this disorder are unclear. Differentiation from severe IBS may be difficult if the gut is not dilated. Manometric studies may also show similar features, such as bursts or clusters of phasic pressure activity [137–139]; slow transit of chyme through the stomach or small bowel [140] provides an objective, noninvasive means by which dysmotility can be assessed.

Secondary causes of small intestinal dysmotility

The secondary causes of small intestinal dysmotility are summarized in Table 47.6 and discussed in the next sections.

Scleroderma (progressive systemic sclerosis)

The small bowel is the second most frequently involved gastrointestinal organ in scleroderma, after the esophagus. Radiographic evidence of gastric sclerosis, intestinal sclerosis, or both is found in 40% of patients with scleroderma [141–144]. Contrast studies reveal dilation of the duodenum and jejunum, delayed barium transit, and narrow valvulae conniventes, which remain tightly packed together despite the bowel dilation, producing the "hide-bound" bowel sign (Fig. 47.18) [10,144–146]. Wide-necked diverticula may be seen but are more frequent in the colon [147]. An uncommon but potentially serious finding is pneumatosis cystoides intestinalis [148].

In early scleroderma, and possibly other connective tissue diseases, antibodies that inhibit M_3 muscarinic receptors are present and may account for the defects in excitatory neurotransmission present in these conditions [149]. Later in the course of disease, degeneration of the smooth muscle and replacement with collagen is responsible for the end-stage small bowel dysmotility in scleroderma. The circular muscle layer is involved more often than the longitudinal muscle layer [10,150,151]. The submucosal and myenteric plexuses appear normal by conventional staining. Small bowel dysmotility leads to bacterial overgrowth, resulting in steatorrhea, malabsorption, and weight loss. Intestinal pseudoobstruction is a well-described gastrointestinal complication of scleroderma [10,152–155].

Table 47.6 Gastrointestinal dysmotility manifestations of some systemic diseases

Disease	Pharynx	Esophagus	Stomach	Small intestine	Colon	Anorectum
Collagenases						
Scleroderma	–	Dilation, aperistalsis of lower two-thirds, incompetent LES	Gastroparesis less frequently	Dilation with narrowed valvulae conniventes, PCI	Dilation; loss of haustra, PCI, wide-necked diverticula	Reduced internal anal sphincter tone
Dermatomyositis, polymyositis	Decreased UES tone, aspiration	Dilation, dysmotility of proximal portion	Gastroparesis rarely	Megaduodenum, distal dilation rarer	Dilation	–
SLE	–	Aperistalsis	–	–	Dilation, ischemia	–
Mixed CTD	–	Dilation lower two-thirds, aperistalsis	Not involved	Megaduodenum	Diverticula	–
Neurological						
Diabetic neuropathy	Decreased peristalsis	Decreased peristalsis, normal LES	Dilation, delayed emptying	Delayed, normal, or rapid transit	Dilation	Decreased sensation, low IAS, low EAS
Parkinsonism	Impaired peristalsis	Diffuse spasm, dilation, aperistalsis	Gastroparesis less frequently	Dilation	Dilation, sigmoid volvulus	–
Spinal cord injury	–	–	Gastroparesis infrequent	Increased gas	Delayed transit, left colon dysfunction	Pelvic floor dysfunction; sensation, IAS, EAS decreased
Neurofibromatosis	–	–	Gastroparesis infrequent	Dilation	Dilation	–
Paraneoplastic neuropathy	–	Aperistalsis/achalasia	Gastroparesis	Dilation, dysmotility	Dilation, constipation	–
Myotonic dystrophy	Decreased peristalsis, low UES tone	Decreased peristalsis, dilation	Delayed emptying, dilation	Dilation	Dilation	EAS dysfunction
Duchenne dystrophy	Decreased peristalsis	Dilation	Dilation, rare gastroparesis	Dilation	Dilation	–
Amyloidosis	–	Aperistalsis, incomplete LES relaxation	Delayed emptying	Dilation	Dilation	–
Infectious						
Chagas disease	–	Dilation, aperistalsis, incomplete LES relaxation	Dilation	Proximal dilation	Dilation	Low IAS
Endocrine						
Hypothyroidism	–	–	Delayed emptying	–	Constipation	–
Hypoparathyroidism	–	–	Delayed emptying	–	Constipation	–

CTD, connective tissue disease; EAS, external anal sphincter; IAS; internal anal sphincter; LES, lower esophageal sphincter; PCI, pneumatosis cystoides intestinalis; SLE, systemic lupus erythematosus; UES, upper esophageal sphincter.

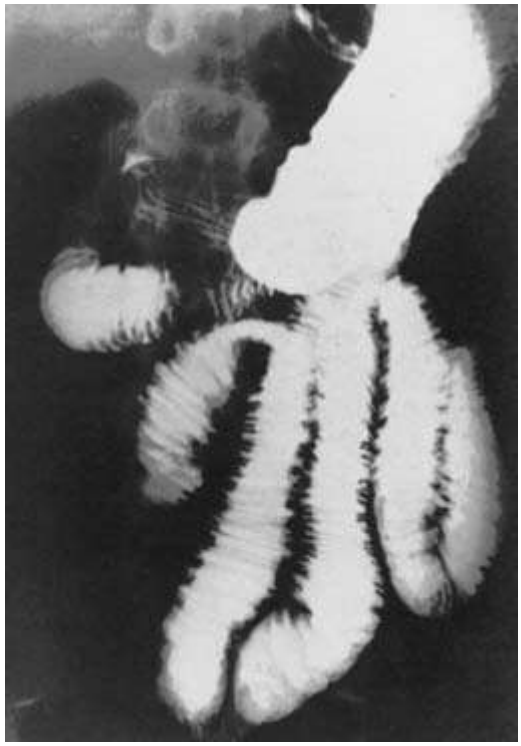


Figure 47.18 Upper gastrointestinal series in a patient with scleroderma shows dilation of the small bowel with normal appearance of the stomach. In some cases the valvulae conniventes are not thickened, as is usually seen in disorders that produce dilation, but remain narrow and crowded, producing the accordion appearance shown here. From Cohen et al. [144], with permission from Elsevier.

In patients with scleroderma and intestinal involvement, disturbances of small bowel motility [156–158] and transit [159] have been documented in several studies. Many patients have a complete absence of the interdigestive migrating motor complex (MMC); clusters of propagated and nonpropagated contractions may be the only finding in others. An abnormally prolonged MMC cycle, diminished activity of phase III activity fronts, and hypomotility of the fed pattern are other abnormal findings [156,158,160]. Antral hypomotility, when present, is characterized by low-amplitude contractions, typically below 40 mmHg; intestinal involvement usually causes the contractile amplitude to fall below 10 mmHg during fasting and postprandially [161]. In patients who have scleroderma without gastrointestinal involvement, the small bowel manometric pattern [161] and small bowel transit time are normal [162].

Oral erythromycin was effective in short-term studies that evaluated gastric emptying and symptoms in an open-label study [163]. Octreotide, a long-acting somatostatin analogue, induces phase III activity in patients with a complete absence of MMCs and may reduce bacterial overgrowth and symptoms (Fig. 47.19) [157]. More studies are required to confirm these observations and the long-term benefit of the treatments.

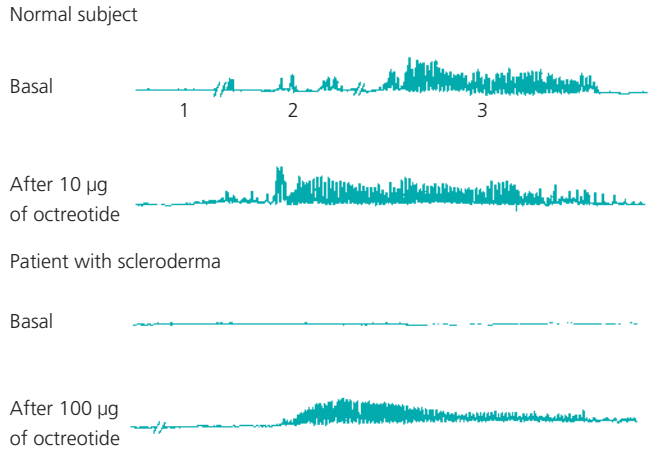


Figure 47.19 Effect of octreotide in a healthy person and in a patient with scleroderma. In the normal person, octreotide stimulates phase III activity. In the patient with scleroderma and pseudoobstruction, octreotide induces phase III activity although no migrating motor complex activity was present in the basal state. From Soudah et al. [157]. Copyright © 1991 Massachusetts Medical Society. All rights reserved.

Dermatomyositis and polymyositis

The inflammatory disorders dermatomyositis and polymyositis are characterized by weakness and atrophy of the skeletal muscles; an associated skin rash distinguishes the former from the latter. The gastrointestinal tract is involved in half of cases. Esophageal involvement causes dysphagia, which often is a presenting symptom.

Studies of the small bowel in these patients are limited mainly to radiography and histology. Megaduodenum and delayed intestinal transit of barium are prominent features [164–166]. Autopsy findings have shown atrophy and fibrosis of intestinal smooth muscle, suggesting a visceral myopathy [165,166]. Acute colonic pseudoobstruction was reported in one patient with dermatomyositis, but it was limited to the colon [167].

Systemic lupus erythematosus

Abdominal pain is the most common gastrointestinal symptom in systemic lupus erythematosus, occurring in 10% to 20% of patients. Autopsy studies disclose a 60% to 70% prevalence of previous peritoneal inflammation, but documented cases of serositis as the sole cause of abdominal pain are rare [168]. The term *lupus enteritis* is used to describe the bowel changes resulting from lupus-induced vasculitis of the small blood vessels. Ischemia leads to intestinal mucosal ulceration, edema, and hemorrhage. Smooth muscle dysfunction results in dilation of the small bowel and ileus. In severe cases, necrosis of the bowel wall develops, with perforation or infarction.

Lupus enteritis is diagnosed radiographically. Small bowel changes include dilation, coarsened folds, and "thumbprinting" secondary to submucosal edema and hemorrhage [169,170]. Diarrhea occurs in 5% to 10% of lupus patients. Unusual

intestinal manifestations can pose diagnostic difficulties. Small bowel involvement in lupus may simulate Crohn's ileitis in some cases [171,172]. A severe protein-losing enteropathy was described in one patient with lupus, who demonstrated vasculitis and basement membrane thickening on full-thickness jejunal biopsy [173]. Transit or manometric studies of the small bowel have not been performed in patients with lupus.

Mixed connective tissue disease

Mixed connective tissue disease is a clinical entity characterized by overlapping features of scleroderma, polymyositis, and rheumatoid arthritis. It has a distinct serological finding of high titers of antinuclear antibody against ribonucleoprotein. The extent of gastrointestinal involvement is unknown but appears to resemble that of scleroderma, with esophageal involvement being most common. Duodenal and jejunal dilation are seen radiographically, and pneumatosis cystoides intestinalis may complicate severe disease [174].

Diabetes mellitus

Intestinal dysmotility may contribute to features of gastroparesis in diabetes [175]. Camilleri has reviewed the clinical features and treatment of the stasis syndromes associated with upper GI motor dysfunction [176].

Diabetic patients may develop diarrhea, which has a variety of causes (e.g., bacterial overgrowth, pancreatic exocrine insufficiency, bile salt malabsorption, impaired absorption or secretion). Early radiographic studies of the small bowel demonstrated variable findings, including normal, delayed, or rapid transit. Similarly discrepant results are found when the hydrogen breath test is used, and the correlation of rapid transit, based on that test, with diarrhea is poor [177–179]. In patients presenting with the gastroparesis syndrome, the small bowel may be affected and this can be detected by manometry [175] or prolonged transit [159].

Pathologically, demyelination of the proximal vagus nerve and sympathetic nerves supplying the bowel develops in diabetes. Although thickening of the small bowel muscle layers and eosinophilic hyaline bodies in smooth muscle cells have been observed [180–182], most authorities do not believe that myopathy is a cause of gastrointestinal dysmotility in diabetic patients. A marked reduction in interstitial cells of Cajal and decreased inhibitory innervation (e.g. vasoactive intestinal peptide, nitric oxide) has been described in a patient with type I diabetes and may underlie the gastroenteropathy associated with this condition [183].

The results of motility studies of the small bowel in patients with diabetes have been mixed. The MMCs are normal in many patients with documented gastroparesis [184]. Absence of intestinal phase III activity fronts has been demonstrated in some patients [177,185,186]. Other abnormalities include MMCs originating in the distal duodenum or jejunum and uncoordinated (nonpropagated or abnormally propagated) bursts of powerful contractions lasting up to 2 minutes (Fig. 47.20) [175,177]. The clinical relevance of these abnormal

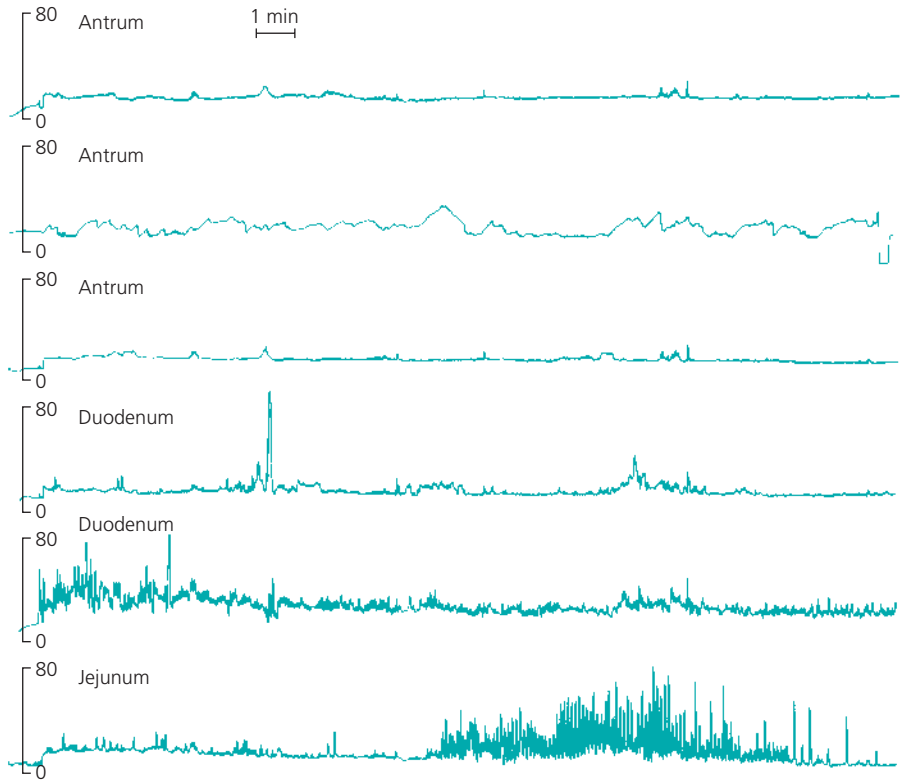


Figure 47.20 Small bowel dysmotility in diabetes. In this patient, phase III of the migrating motor complex begins in the jejunum, with no antecedent activity in the antrum or duodenum. Other patients may have a complete absence of phase III activity. From Dooley et al. [177], with permission from Taylor & Francis.

findings is uncertain. In one study, abnormal transit time and manometric abnormalities could not be correlated [177]. Hyperglycemia, which can weakly delay gastric emptying, induces intestinal phase III-like activity and alters orocecal transit time in healthy subjects [187]. However, the relevance of these findings to diabetic bowel dysmotility is unclear. Among diabetics living in the community, constipation and the use of laxatives are significantly more common than in age- and gender-matched controls [188,189]. Constipation is probably multifactorial in diabetes, as it is in nondiabetic patients [190]; acute hyperglycemia does not significantly affect colonic motor function or tone [191].

Parkinson disease

Symptoms of gastrointestinal dysmotility are common in patients with Parkinson disease. High dysphagia, nausea, bloating, constipation, and difficulty with evacuation of stool occur frequently [192]. Dilation of the small bowel may be seen radiographically [193]. Small bowel dysmotility does occur, but its frequency is unknown. Manometric studies in patients with Parkinson disease reveal infrequent MMCs (which are absent in some cases), hypomotility in the fed state, and an increased incidence of retrograde and tonic contractions in comparison with controls [194]. The contribution of these abnormalities to symptoms is not clear.

The pathogenesis of small bowel dysmotility is unknown. In patients with dysphagia and megacolon, Lewy bodies, which are neurons containing cytoplasmic hyaline inclusions, have been found in the myenteric plexus of the esophagus and colon [195,196]. Lewy bodies were originally identified in the brain and are highly characteristic of Parkinson disease. Lewy bodies have not yet been reported in the small bowel. Dopaminergic neurons are reported to be deficient in the colon of patients with parkinsonism and constipation [197].

Therapy for Parkinson disease includes drugs that adversely affect gut motility, such as dopamine agonists and anticholinergics. Symptoms are as common in untreated as in treated patients, however, suggesting that the disease itself is the primary factor producing dysmotility and resultant symptoms [192].

Spinal cord injury

Extrinsic denervation of the small bowel by spinal cord injury usually produces just mild and probably insignificant dysmotility in the small intestine. The only change revealed in manometric studies performed in a group of patients with spinal cord injury was that a greater number of phase III contractions began in the duodenum rather than the antrum in those with high spinal cord lesions [198]. Colonic dysmotility is well recognized and is responsible for the common problems of constipation and difficulty of evacuation in these patients [199]. Colonic transit is delayed at the level of the left colon and rectum, and manometric studies have shown that the baseline and postprandial colonic activity is substantially reduced in spinal cord-injured patients at the level of the descending colon, and that the postprandial response is absent at the rectosigmoid [200]. Patients with injury to the lower spinal cord demonstrated no abnormal findings, consistent with the preservation of innervation to the bowel from the vagus and third thoracic sympathetic levels. In one report, a woman with cervical spinal stenosis and paraplegia displayed normal MMCs but a two- to three-fold prolonged interval between MMCs [201].

Immediately after a spinal cord injury, a state of spinal shock develops. This is characterized by the complete loss of all sensory, motor, and reflex function below the level of injury. Paralytic ileus with abdominal distention follows and usually resolves in a few days. In the long term, postprandial abdominal distention and discomfort occur in more than 40% of patients with spinal cord injury [202]. These symptoms are likely caused by the more significant problem of constipation. In many stable patients with spinal cord injury, the amount of gas in the small and large intestine is increased on routine abdominal radiographic films (Fig. 47.21) [203].

Neurofibromatosis (von Recklinghausen disease)

Gastrointestinal neurofibromas are most commonly found in the small intestine. They are reported to occur in up to

Figure 47.21 Abdominal radiograph from a stable, healthy patient with a spinal cord injury. A nonobstructive gassy abdomen is frequently observed, even in the absence of abdominal complaints. From Gore and Mintzer [203], with permission from Elsevier.

10% of patients with neurofibromatosis; the true incidence of neurofibromas is probably underestimated, however, because they are generally asymptomatic. Small bowel neurofibromas may be single or multiple and are most frequently found in the ileum. These tumors are submucosal, and they may become large and extend to the serosa. Their presence is usually established by a small bowel series [204,205]. Ulceration through the mucosa can cause acute or chronic bleeding. Mechanical small bowel obstruction or intussusception requires exploratory surgery.

Chronic intestinal pseudoobstruction has been described in one patient with neurofibromatosis. The small bowel manometry findings were abnormal, and small bowel transit was markedly delayed [140]. Pathological studies of the nerves and muscles in the small bowel are lacking in this disease. Neurogenic changes consisting of nerve fiber proliferation in the myenteric and submucosal plexuses were found in the colon of a patient with megacolon [206].

Paraneoplastic visceral neuropathy

Chronic intestinal pseudoobstruction has been reported in association with small cell carcinoma of the lung [207–212], anaplastic lung adenocarcinoma [213], thymoma [214], epidermoid carcinoma of the lip [215], and gynecological [213] and breast cancers [216]. This phenomenon represents a paraneoplastic syndrome caused by visceral neuropathy. The small bowel, like the colon, shows widespread neuronal and axonal degeneration of the myenteric and submucosal plexuses, in addition to mononuclear cell infiltration and Schwann cell proliferation [211]. There may also be a reduction in the small intestinal network of interstitial cells of Cajal [217]. In paraneoplastic syndromes there are no metastases to the bowel.

Symptoms of pseudoobstruction are the presenting features in patients with visceral neuropathy and small cell carcinoma. The lung cancer is usually occult, missed on chest radiography, and is often found at autopsy or by chest computed tomography (CT) and mediastinoscopy. The small bowel is usually not dilated on radiographs, but barium passage through the small bowel is somewhat delayed. Small bowel manometry shows features suggestive of neuropathy, such as incoordinated bursts [210,211]. In older patients with intestinal pseudoobstruction or feeding intolerance of unknown cause and recent onset, a malignancy, especially small cell carcinoma of lung, must be suspected. A screening blood test (antineuronal enteric antibody, or anti-Hu) is highly specific [211]. If the result is positive, CT of the chest and mediastinoscopy may be necessary to confirm the diagnosis.

Myotonic dystrophy

Myotonic muscular dystrophy is a slowly progressive disease characterized by myotonia, or difficulty in muscle relaxation. Besides muscle wasting, the patient often has a nasal voice as a result of pharyngeal and palatal weakness, an early onset of cataracts, and cardiac conduction defects. Dysphagia is the most common gastrointestinal symptom resulting from esophageal involvement. Diarrhea and abdominal cramping occur in up to one-third of those affected. Malabsorption and steatorrhea have been reported in a few cases [218–220]. Constipation also is frequent and can alternate with diarrhea. Intestinal pseudoobstruction is rare [221,222]. The small bowel may demonstrate abnormal but nonspecific radiographic changes, including dilation, diminished motility, and delayed transit of barium [223].

Dysmotility of the small intestine may play a significant role in the production of intestinal symptoms. In a group of 10 patients with symptoms of intestinal dysmotility but normal findings on small bowel contrast examinations, small bowel motility was abnormal in all patients. Manometric findings included low-amplitude contractions in the fasting and fed states, retrograde propagation of phase III, interruption of phase III, and an increased incidence of tonic contractions [224]. In one patient with chronic pseudoobstruction and dilated small bowel, manometry revealed low-amplitude duodenal contractions and an increased maximal rate of contractions in the duodenum [222].

Histologically, the small intestinal smooth muscle shows changes similar to those found in dystrophic skeletal muscle; it is swollen, partially destroyed, decreased in size, and replaced by fat [225,226]. Silver stain revealed degenerative changes in the myenteric plexus of the colon in a patient with megacolon [227], indicating that intestinal dysmotility may be caused by smooth muscle as well as enteric nerve dysfunction. In most patients, the predominant cause of dysmotility appears to be smooth muscle damage.

Duchenne muscular dystrophy

Duchenne muscular dystrophy is a severe, pseudohypertrophic muscular dystrophy that follows an X-linked recessive pattern of inheritance, affecting young boys early in childhood. Skeletal and cardiac fibers are infiltrated and eventually replaced by connective and fatty tissue. Similarly, the smooth muscle of the gastrointestinal tract also is involved. Histological changes comprise degeneration and atrophy of smooth muscle fibers and separation of the fibers by connective tissue [228–230]. The myenteric plexus is not involved.

Despite typical dystrophic changes in the small bowel in most postmortem specimens, gastrointestinal symptoms are usually related to dysmotility of the esophagus and stomach, which are more severely affected than the small bowel. Dysphagia is the predominant symptom (36% in one series), followed less often by vomiting, diarrhea, and constipation [230]. The orocecal transit time is normal in asymptomatic subjects [231]; however, severe bowel dysmotility can occur. Episodes of acute intestinal pseudoobstruction, manifested by abdominal distention, persistent vomiting, and gastric or small bowel dilation, can be life-threatening [232–234].

Chronic intestinal pseudoobstruction has also been reported in Duchenne dystrophy [235].

Amyloidosis

Amyloid protein is deposited in the small bowel in all forms of amyloidosis: primary, secondary, myeloma-associated, and familial or hereditary. The degree of dysmotility is related to the amount and distribution of amyloid deposited. In all forms, the deposition of amyloid in blood vessel walls leads to ischemic ulcers, infarction, and occasionally perforation.

In primary and myeloma-associated forms of amyloidosis, the muscle layers of the small bowel are more severely affected than the mucosa, resulting in dysmotility. In the secondary and hereditary forms, the mucosa is chiefly involved, resulting in malabsorption. Amyloid deposits are infrequently found in the myenteric plexus, although in hereditary forms, familial amyloidosis neuropathy may result from deposition in major nerve trunks supplying the bowel [236,237].

Gastrointestinal symptoms include diarrhea, constipation (which is often present for years and then followed by diarrhea), malabsorption, and pseudoobstruction. Diarrhea can occur without malabsorption and is occasionally relieved by antibiotic therapy [238]. However, patients who have amyloidosis and autonomic neuropathy have been documented to have extremely rapid intestinal transit as the mechanism of their diarrhea [239]. With heavy infiltration of the small bowel muscle layers, acute intestinal pseudoobstruction is a serious and often fatal complication (Fig. 47.22) [240–242]. In patients with pseudoobstruction-related amyloidosis, radiographic features include coarsening of the small bowel mucosal pattern, dilation, and prolonged transit of barium [243].

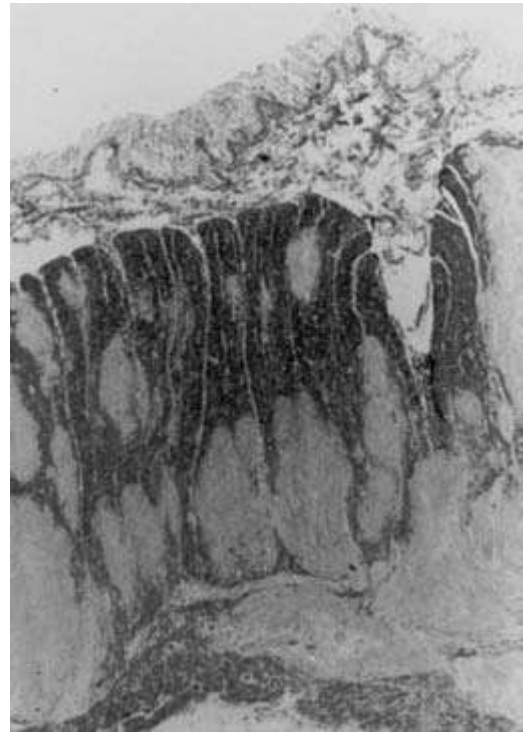


Figure 47.22 Small bowel myopathy in a patient with amyloidosis and pseudoobstruction. The large, pale areas in the muscle layer represent heavy amyloid deposition. The myenteric plexus is less frequently involved than the mucosa and muscle layers. From Mitros [242], with permission from Elsevier.

Chagas disease

Chronic infection with *Trypanosoma cruzi* leads to destruction of the submucosal and myenteric plexuses along the entire length of the gastrointestinal tract. Megacolon and megaesophagus are the most common presentations, although the small bowel may be similarly affected, with the development of megaduodenum and megajejunum. Small intestinal manometric studies in these patients demonstrate normal frequency of the MMCs, but the velocity of propagation of the activity front is significantly slowed to about half the normal value [244]. Patients with small bowel involvement may be entirely asymptomatic despite significant neuronal destruction [245]. Diarrhea, constipation, or intestinal pseudoobstruction can occur. Patients with chagasic megacolon have circulating autoantibodies that activate M_2 muscarinic acetylcholine receptors [246]. The agonist effects may result in desensitization and downregulation of the receptor, which may contribute to the development of megacolon.

Thyroid disease

Thyroid dysfunction may present primarily as gastrointestinal disease. Hyperthyroid patients may have diarrhea and mild steatorrhea, whereas hypothyroid patients frequently are constipated [247–249]. Intestinal dysmotility resulting from the altered thyroid state has been historically recognized as the cause of symptoms. This is supported by abnormalities found in the intrinsic electrical control of gut motor activity. In the duodenum, slow-wave frequencies are increased in hyperthyroid patients and decreased in hypothyroid patients [250,251]. These abnormalities return to normal after correction of the thyroid dysfunction.

Hyperthyroidism

Early radiographic studies, in which the crude barium meal test was used, reported rapid gastric emptying and rapid small bowel transit [252,253]. Later studies, in which the hydrogen breath test was used, confirmed a rapid orocecal transit time; this is a consequence of accelerated small bowel transit because the rate of gastric emptying is actually unchanged [247,254–256]. Hyperthyroid patients may present with malabsorption; more than 25% of hyperthyroid patients excrete more than 7 g of fat in their stool daily [257,258]. This is apparently caused by decreased contact time of the lumenal contents with small bowel mucosa as a result of rapid transit; the abnormality regresses after correction of the hyperthyroid state.

Hypothyroidism

In contrast to the accelerated small bowel transit in hyperthyroidism, small bowel transit is significantly slowed in hypothyroidism [247]. With adequate hormone replacement, transit may be normalized. Constipation develops in many hypothyroid patients, probably a result of colonic dysmotility. A manometric study in one patient revealed decreased amplitude of the small bowel contractions and an overall decreased motility index [259]. In severe hypothyroidism (i.e., myxedema), paralytic ileus and intestinal pseudoobstruction can occur [260–264]. Autoimmune hypothyroidism is more common in patients with celiac disease than in healthy controls, and gluten withdrawal has been reported to ameliorate the thyroid abnormality in some cases [265].

Hypoparathyroidism

The mechanism by which parathyroid hormone affects gastrointestinal motility is not known. However, calcium is essential for smooth muscle contraction, and hypocalcemia may impair gut contractile activity. Intestinal pseudoobstruction and malabsorption have been reported in association with small bowel dysmotility in hypoparathyroid subjects [266]. Barium studies reveal dilated loops of small bowel and a prolonged transit time. The symptoms are relieved by calcium administration.

Drug-induced changes in small intestinal motility

Many drugs profoundly affect gastrointestinal motility. Although the colon is usually recognized as the organ most susceptible to drug-induced dysmotility, the small bowel also is frequently affected. Phenothiazines and some antiparkinsonian drugs decrease colonic and small bowel motility and can cause constipation, colonic pseudoobstruction, and adynamic ileus [267–270]. Tricyclic antidepressants in particular are noted for causing iatrogenic ileus. The anticholinergic agents atropine and scopolamine and related belladonna alkaloids decrease intestinal tone as well as the amplitude and frequency of peristaltic contractions [271].

Opiate analgesics are well known to suppress motility throughout the gastrointestinal tract. Morphine enhances the amplitude of nonpropulsive small bowel contractions and markedly decreases propulsive contractions by its effects on μ-opioid receptors on intestinal muscle cells [272,273]. The upper small bowel is more prone to these effects than the ileum. The net effect is delayed small bowel transit. Morphine and nalbuphine significantly delay small bowel transit in comparison with placebo [274].

Loperamide, a synthetic opiate used to treat diarrhea, also reduces small bowel motility and delays transit time. This effect is antagonized by the concomitant administration of naloxone, which by itself has no effect on transit time [275,276]. Ketorolac, a nonsteroidal antiinflammatory agent used for postoperative pain, has significantly less effect on small bowel transit than morphine [277]. Whether ketorolac slows motility in comparison with controls is not known.

Calcium channel antagonists, especially verapamil, cause constipation in up to 20% of patients. The small bowel transit time in subjects taking verapamil was unchanged from pretreatment values; transit through the colon was slowed, however, and this effect likely accounts for the constipation associated with this drug [278].

Clonidine, an α_2-adrenergic agent used as an antihypertensive agent, prolongs the orocecal transit of liquids [279]; however, a comprehensive dose–response study of the transit of solids did not show any significant effects on gastric small bowel or colonic transit in healthy individuals [280]. Clonidine has been used successfully to treat diabetic diarrhea; however, its therapeutic benefit may also result from its known action of increasing fluid and electrolyte absorption from the gut [281].

Drugs that stimulate small bowel motility include erythromycin, cholinergic agonists, and tegaserod. Erythromycin binds to motilin receptors on the smooth muscle of the gastric antrum and duodenum. Motilin, in physiological doses, induces interdigestive motor complexes in the upper gut. Erythromycin has been used to treat diabetic gastroparesis; the long-term beneficial effects are not as pronounced as the short-term effects [282]. Cholinergic agonists, such as bethanechol, augment the amplitude of small bowel contractions and overall motility [283].

Octreotide is a long-acting synthetic analogue of somatostatin. In normal individuals, it increases the frequency of MMCs by significantly shortening the duration of phase II contractions [284,285]. It was shown to be useful in the short term in a small group of patients with scleroderma by inducing phase III contractions and possibly reducing small bowel bacterial overgrowth [157]. When given after a meal, intravenous somatostatin interrupts the fed pattern of motility and induces bursts of propagated activity similar to phase III activity fronts in health [286] and disease [287,288]. Octreotide retards small bowel transit in health when given before meals [289].

Tegaserod is a serotonin mimetic acting at the 5-HT_4 receptor that has demonstrated robust prokinetic effects on small intestinal and colonic transit in patients with IBS and constipation [290]. Additionally, tegaserod has been shown to accelerate the transit of intestinal gas in subjects with gas retention, and this may be one of the mechanisms by which it reduces symptoms of bloating in IBS patients [291].

Celiac disease

Intestinal pseudoobstruction occurs in association with nontropical sprue [292–294]. Dilated loops of small bowel in which the passage of barium is delayed are observed radiographically. In one patient who underwent exploratory laparotomy with full-thickness jejunal biopsy, the nerves and muscle coats appeared normal on both light and electron

microscopy [294]. Parenteral nutrition may be required during prolonged periods of pseudoobstruction.

Jejunal diverticulosis

Diverticula can occur anywhere in the small intestine, but they are most common in the jejunum. Like their counterparts in the colon, they represent herniations through the mesenteric side of the bowel and are usually acquired [242]. Jejunal diverticulosis is associated with many diseases, including scleroderma, celiac disease, Fabry disease, type II FVM, mitochondrial cytopathy, and Cronkhite–Canada syndrome [295]. Patients present with diarrhea, steatorrhea, weight loss, and megaloblastic anemia. Chronic intestinal pseudoobstruction occurs in jejunal diverticulosis and can mimic mechanical obstruction [155,296].

The concept that dysmotility causes diverticula and not vice versa is supported by a case report in which resection of the small bowel segment containing diverticula failed to relieve the patient's symptoms of pseudoobstruction [10].

In one patient, small bowel manometry showed that contractions can be antegrade, retrograde, or both simultaneously [295]. Histology of the bowel reflects the heterogeneous causes of dysmotility (e.g., visceral neuropathy, visceral myopathy) [297].

Irradiation

Ionizing radiation damages all structures of the small intestine, including the mucosa, blood vessels, connective tissue, enteric nerves, and smooth muscle. Radiation damage to the bowel can be classified as acute or chronic.

Acute injury

Above a certain threshold dose of radiation to the abdomen, symptoms of nausea, vomiting, abdominal pain, and diarrhea are common. These abate soon after exposure is discontinued. Reversible changes in small bowel absorptive function have been considered the main cause of diarrhea. Small bowel dysmotility may play a significant role in acute radiation enteropathy. In dogs, a single large dose of radiation abolishes the interdigestive MMCs. [298]. Smaller, fractionated doses, which are used for radiation treatment in humans, also result in dysmotility in the dog model. In the fasting state, manometric changes during exposure include an increased frequency of giant migrating contractions and retrograde contractions, with only a mild decrease in MMC cycle length [299]. These features revert to a normal pattern within a few days after exposure. Other studies, however, show decreased fasting and fed intestinal motility, particularly in the distal small bowel, persisting up to 1 month after the last exposure [300,301].

Motility studies have not been performed in humans in the acute phase of radiation sickness. One study demonstrated accelerated small bowel transit, compared with pretreatment values, in a group of patients undergoing abdominal and pelvic irradiation [302]. More than 75% of the patients had diarrhea during treatment.

Chronic injury

Delayed gastrointestinal complications may occur months, years, or even decades after radiation exposure. Neuronal, muscular, and vascular structures of the small bowel are affected. The resulting dysmotility leads to bacterial overgrowth, diarrhea, and malabsorption. Edema, atrophy, and fibrosis of smooth muscle fibers are characteristic histological findings [303–306]. The myenteric plexus can appear normal on routine staining; however, proliferation of the submucosal neurons with extension into the circular muscle coat has been observed [305,306]. Radiation enteropathy is also associated with microvascular endothelial dysfunction with impaired vasodilator response to acetylcholine, which may predispose to mucosal ischemia and eventual stricture formation resulting in obstruction [307].

Latent gastrointestinal symptoms resulting from previous radiation damage have been attributed to altered gut motility. Recurrent episodes of intestinal pseudoobstruction were described in two patients many years after they received abdominal radiation [306,308]. Small bowel contrast studies show dilated loops of bowel with air–fluid levels and a thickened bowel wall. Manometric evaluation in one patient revealed normal MMCs in the proximal duodenum, decreased amplitude and frequency of contractions in the distal duodenum, and an absence of peristaltic contractions in the jejunum. Subsequent histological examination of the bowel suggested smooth muscle damage as the cause of dysmotility [306].

Diffuse lymphoid infiltration

Four cases of chronic intestinal pseudoobstruction in association with diffuse lymphoid infiltration of the small bowel have been described [309]. All four patients were women who presented with diarrhea at an early age. Histology revealed lymphocytic infiltration of the lamina propria, muscularis propria, and myenteric plexus. The infiltrates were shown to be polyclonal by immunochemical stains, reflecting a pseudolymphoma rather than a true neoplasm. The myenteric plexus appeared normal with routine and silver stains. Smooth muscle cells were noted to be absent in the areas of lymphoid infiltration.

Jejunoileal bypass

Recurrent episodes of intestinal pseudoobstruction can occur in patients after jejunoileal bypass for morbid obesity [155,310]. Symptoms begin as early as 1 week postoperatively and consist of abdominal distention and pain, diarrhea, vomiting, and fever. Radiography reveals massively dilated small bowel loops and occasionally pneumatosis cystoides intestinalis. Bacterial overgrowth of the bypassed small intestine causes this enteropathy; small bowel cultures typically

grow fecal flora and anaerobes similar to those described in blind loop syndrome. Over time, either the symptoms disappear or the frequency and severity of pseudoobstruction decrease significantly. Intermittent antibiotic therapy may benefit patients with chronic symptoms.

In contrast to intestinal pseudoobstruction, mechanical obstruction secondary to ileal volvulus at the jejunoileal anastomotic site can occur and presents a difficult diagnostic challenge [311]. Contrast radiography may aid in establishing the correct diagnosis. Recurrent acute colonic pseudoobstruction also has been reported after jejunoileal bypass [312,313].

The effect on intestinal motility of bypassing a long segment of small bowel is unknown. In patients undergoing reoperation for various metabolic or hepatic complications, the bypassed segment appears normal, and normal small bowel function is restored after reversal of the bypass [314].

Anorexia nervosa and bulimia

Both anorexic and bulimic patients frequently report bloating and constipation. Delayed gastric emptying of solids is a well-established abnormality in these disorders. The orocecal transit is also modestly delayed [315,316], as is the whole-gut transit time, measured by radiopaque markers, in comparison with controls [316]. The gastric emptying abnormality and dyspeptic symptoms resolve with successful rehabilitation using nutritional therapy to achieve a target body mass index (BMI) of 18.5 kg/m^2 and cognitive-behavioral therapy [317].

Clinical manifestations

The small intestine is essential for the absorption of nutrients; therefore, individuals cannot survive without parenteral nutrition if a long segment of small intestine functions poorly. Fortunately, the small intestine is usually the last organ in the gastrointestinal tract to be affected by severe dysmotility. Severe dysmotility of the esophagus, colon, and even the stomach occur much more frequently than dysmotility of the small intestine. Isolated, severe small intestinal dysmotility is unusual; small intestinal dysmotility is generally associated with dysmotility in other parts of the digestive tract. In this chapter, the discussion is limited to the small intestine. Dysmotility in other organs of the digestive tract is discussed elsewhere in this book.

With a few exceptions, most patients with small intestinal dysmotility have similar clinical manifestations regardless of the underlying causes. The spectrum of clinical manifestations varies widely (see Fig. 47.5): at one end, a patient may be asymptomatic, whereas at the other, a patient may have recurrent symptoms and signs of small intestinal obstruction, termed *chronic small intestinal pseudoobstruction*. Between these two ends of the spectrum, a patient may have recurrent symptoms of postprandial abdominal pain – cramping, periumbilical, and epigastric – in addition to abdominal bloating, easy satiety, anorexia, weight loss, nausea, and vomiting. The symptoms are usually related to eating. Diarrhea can occur in patients with bacterial overgrowth and malabsorption. In severe cases, the patients have episodes of chronic intestinal pseudoobstruction syndrome. Plain abdominal radiographs of patients with intestinal pseudoobstruction during exacerbations show multiple air–fluid levels and dilation of the small intestine. The prevalence and severity of recurrent obstructive episodes vary from patient to patient and from episode to episode in the same patient.

The findings on physical examination vary according to the severity of the symptoms. Patients may be cachectic and malnourished because they are unable to take in adequate nutrients, or they may have malabsorption as a consequence of bacterial overgrowth in the small intestine. The abdomen may be distended and mildly tender. The bowel sounds are inactive and infrequent in patients with smooth muscle dysfunction, but they are hyperactive and high-pitched in those with myenteric plexus dysfunction. Borborygmi may be detected in some cases. In less symptomatic patients, the abdominal examination findings may be normal. In those with chronic intestinal pseudoobstruction, during an obstructive episode the abdominal examination findings may be indistinguishable from those of true mechanical obstruction.

As mentioned previously, patients with small intestinal dysmotility usually also have symptoms of dysmotility in other parts of the gastrointestinal tract. Therefore, patients with chronic intestinal pseudoobstruction may have symptoms and signs of multiple-organ dysmotility.

Extragastrointestinal manifestations may be detected in some patients, depending on the underlying disease. Megacystis and megaureter are common in type I FVM and CVM and may be associated with urinary retention and infection. Mydriasis, ptosis, and external ophthalmoplegia may be seen in certain forms of FVM, and ataxia, dysautonomia, and neurological symptoms in some forms of visceral neuropathy.

In the secondary forms of small intestinal dysmotility, patients may also have systemic manifestations of the underlying disease (e.g., scleroderma, muscular dystrophies, autonomic neuropathy). These are important clinical clues in patients whose underlying disease has not yet been diagnosed.

Complications

Malnutrition

Malnutrition develops in patients with severe dysmotility of the small intestine as the result of an inadequate intake of food and of vomiting. Postprandial abdominal pain and bloating limit oral intake. Anemia may develop secondary to iron, folate, and vitamin B-12 deficiency. Serum cholesterol, calcium, and albumin levels may be low. Supplemental formulas are useful to improve nutrition. In severe cases, small bowel transplantation or long-term parenteral nutrition may be the only methods to provide adequate nutrients, particularly to patients with familial or sporadic forms of hollow visceral myopathy.

Bacterial overgrowth in the small intestine

Bacterial overgrowth in the small intestine may complicate severe intestinal dysmotility [30,117,135,318]. The small intestine of these patients may be structurally abnormal, with dilation, diverticulosis, or strictures, or there may be only a motility abnormality. Small intestinal bacterial overgrowth causes malabsorption and steatorrhea, which result in additional weight loss. The diagnosis can be made by culturing intestinal aspirates for both aerobic and anaerobic bacteria. A number of different species are found, and the total bacterial concentration generally exceeds 10^5 aerobic organisms per milliliter or 10^3 anaerobic organisms per milliliter. Another approach to diagnosing bacterial overgrowth is the timed analysis of breath excretion of volatile metabolites produced by intralumenal bacteria. The measurement of both expired [^{14}C]carbon dioxide after the oral administration of ^{14}C-labeled substrates (e.g., D-xylose) and expired hydrogen after the administration of nonlabeled substrates has been used. This method is less accurate than culture of duodenal aspirates [319]. The patients may have macrocytic anemia secondary to vitamin B-12 deficiency. Doxycycline, metronidazole, ciprofloxacin, ampicillin, rifaximin, and other antibiotics can be used intermittently to treat intestinal bacterial overgrowth. Treatment with antibiotics results in improvement in bloating and diarrhea after 1–2 days, and the improvement persists for up to 2 weeks after a 7-day course of antibiotics [320]. In practice, different antibiotics may be rotated and given for 1 week of every month. The possibility that bacterial overgrowth occurs in the distal small bowel and may cause symptoms in IBS has been suggested [321,322], but the appropriate experiments to confirm or refute this have yet to be performed.

Pneumatosis cystoides intestinalis

Pneumatosis cystoides intestinalis is a rare condition characterized by multiple, gas-filled cysts in the wall of the small and large intestine. Pneumoperitoneum may occur. Pneumatosis cystoides intestinalis can develop in patients with small intestinal dysmotility and dilation. In most cases, the condition is found incidentally on radiographs. The pathogenesis is unknown, and no specific treatment is available. In one patient, the use of chloral hydrate was implicated as the cause of pneumatosis cystoides, based on controlled challenges of the drug [323]. Chloral hydrate is metabolized to alkyl hydrates, which potentially can inhibit bacterial consumption of hydrogen leading to overproduction of hydrogen. It has been recommended that surgery be avoided except in the cases of a complication such as an abscess or peritonitis, because surgery usually causes the patient's condition to deteriorate [154].

Diagnostic studies

The diagnostic studies for patients with small intestinal dysmotility are outlined in Table 47.7.

Table 47.7 Diagnostic studies for patients with small intestinal or colonic dysmotility

Blood tests
Complete blood cell count
Blood chemistries, including blood sugar
Antinuclear antibody
Thyroid functions: triiodothyronine, thyroxine, thyroid-stimulating hormone
Serum creatine phosphokinase and isoenzymes
Hemagglutination and complement fixation for Chagas disease
Antineuronal nuclear antibody or antititer for paraneoplastic process
Radiological studies
Plain abdominal radiography in patients with abdominal distention
Radiopaque marker transit
Upper gastrointestinal and small bowel radiography
Enteroclysis or small bowel radiography with fluoroscopy
Barium enema
Scintigraphic gastric, small bowel, and colonic transit
Intravenous pyelography
Computed tomography of chest and abdomen
Other studies
Skeletal muscle biopsy
Cultures of small bowel aspirates
Small intestinal or colonic manometry
Anorectal manometry and balloon expulsion
Histological studies of full-thickness biopsy specimens to examine the smooth muscle and myenteric plexus by special silver stain, immunohistochemistry, confocal microscopy of enteric plexuses

Blood tests

A complete blood cell count may reveal anemia and macrocytosis secondary to malnutrition and bacterial overgrowth. Blood chemistries also reflect malnutrition and malabsorption. Diabetic patients have hyperglycemia, and hypoparathyroid patients may have hypocalcemia. Patients with connective tissue disease are positive for antinuclear antibody and/or the autoantibody SCL-70. Patients with thyroid disease have altered serum levels of triiodothyronine, thyroxine, and thyroid-stimulating hormone. Patients with muscular dystrophy may have elevated levels of CPK and isoenzymes. The results of hemagglutination testing and complement fixation for Chagas disease may be positive in patients who have lived in Central or South America.

Antineuronal nuclear antibody testing is required in patients with a history of smoking to exclude paraneoplastic pseudoobstruction. Determinations of blood lactate, pyruvate (signs of acidosis), CPK, AST and ALT (muscle damage), and leukocyte thymidine phosphorylase are used to screen for mitochondrial cytopathy.

Radiological studies

Plain abdominal radiographs

These are useful in patients with abdominal distention and bloating because they may show gaseous distention of the gastrointestinal tract. Upper gastrointestinal and small bowel radiographs may show lesions typical of certain diseases. Areas affected by severe dysmotility usually are dilated.

Enteroclysis

Enteroclysis, or small bowel enema [324], is useful to detect lesions in the small intestine and rule out mechanical obstruction. Experienced radiologists can identify structural lesions in the small intestine in up to 98% of patients with mechanical obstruction. Barium enema and intravenous pyelography may be useful in detecting abnormalities of the colon and urinary tract, respectively.

Computed tomography enterography (CTE)

Unlike conventional abdominal CT, CTE utilizes thin sections and large volumes of neutral contrast such as water or low-density barium, combined with intravenous contrast to evaluate the entire thickness of the small intestinal wall, and permits better visualization of deep ileal loops that are difficult to image with conventional small intestinal radiographs [325]. In addition, CTE permits the evaluation of extralumenal disease.

Whole-gut transit with radiopaque markers

Discrete, barium-impregnated, polythene markers that move with the colonic contents have become simple but robust measures of transit. Hinton and colleagues [326] measured gastrointestinal transit times using small solids (2–5-mm polythene pellets) containing barium sulfate. These inert radiopaque nonabsorbable markers, which have a specific gravity similar to that of gut contents, were completely recoverable in the stool. Followed by either abdominal or stool radiography, the movement of such markers was taken to represent the transit of meal residues through the gut (Fig. 47.23). Of 25 normal male subjects, all but one had passed 80% of the markers by the 5th day after ingestion, but none had passed 80% by the end of the 1st day.

Segmental colonic transit

The rate of disappearance of radiopaque markers from colonic segments was monitored with daily abdominal radiographs obtained after the administration of a single dose of radiopaque markers [327,328]. Right and left colonic, sigmoid, and rectal segments were defined simply by drawing lines between bony landmarks of the vertebral column and pelvis. Methods were then developed to calculate actual segmental transit times, rather than the rate of disappearance of markers from colonic segments [329].

Metcalf and colleagues [330] simplified this method so that only one radiograph, obtained with a fast, high-kilovolt technique, was necessary; radiation exposure was therefore minimal. By this method, a fixed number of radiopaque markers were taken (24 per day) at the same time each day (0900 hours arbitrarily) for 4 days. On day 5, again at the same time, a radiograph was obtained. The method works on the assumption that a 24-hour sampling interval approximates continuous observation. In rapid transit, all the markers can be lost in the feces before radiography on day 4; conversely, in slow transit, all 96 markers may be present on the single radiograph. A film obtained on day 7 can then provide more information.

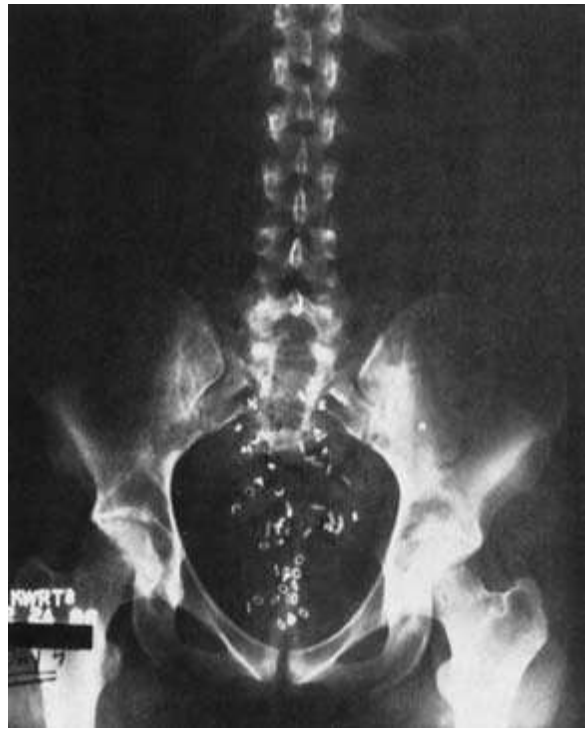


Figure 47.23 Radiograph of radiopaque markers as a measure of colon transit. Most of the markers are in the rectosigmoid colon of this patient.

Radioactive isotope transit by scintigraphy

The transit of gamma ray-emitting radioactive isotopes through the colon can be quantified with a gamma camera linked to a computerized recording and processing system [331]. The isotope can be monitored for long periods without increasing radiation exposure; this is not possible with methods using X-rays. The transit of solids and liquids can be measured simultaneously [332] if different isotopes are used to label liquid and solid bowel contents, and segmental regions of interest in the colon can be easily defined. A pH-sensitive polymer that dissolves at the pH of the ileum is used to coat capsules containing isotope-labeled beads [331]. With this approach, it has been possible to image the unprepared colon without the use of intubation; it is likely that such methods allow more "physiological" measurements of colonic transit.

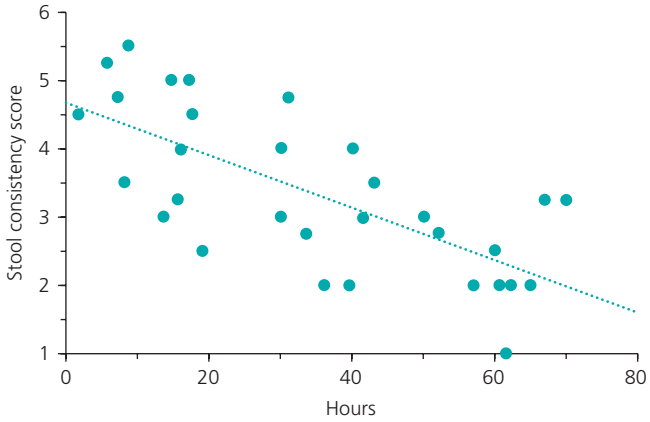


Figure 47.24 Correlation between mean colonic transit time (radiopaque markers) and physical appearance of stools (ranges from 1, small and hard, to 6, loose and semiliquid). From O'Donnell et al. [333], with permission from the BMJ Publishing Group.

The net effects of fast or slow colonic transit are most clearly seen in the consistency of the stools. O'Donnell and colleagues [333] developed a 7-point scale for describing stool form and consistency, ranging from liquids to hard solids; they were able to relate stool consistency to the overall mouth-to-anus transit time. Faster transit was associated with looser stools. Stool form was then related specifically to colonic transit [332] after colonic transit times had been altered experimentally by rapid cecal infusions of fluid. When this noninvasive approach was used [334] a good correlation was found between stool consistency and colonic transit; however, the correlation is clearly influenced by the extremes of transit and form, with little predictive value for stool that is not watery or extremely hard (Fig. 47.24).

Other studies

A biopsy of skeletal striated muscle may be required for patients suspected of having muscular dystrophy or mitochondrial cytopathy. Cultures of small bowel aspirates should be obtained from patients with clinically suspected bacterial overgrowth (e.g., steatorrhea, bloating). Small intestinal manometry is indicated if no known underlying disease is present and the nature of the dysmotility (myopathy vs neuropathy) is unclear.

If a full-thickness biopsy specimen of the dysfunctional part of the intestine is available, careful pathological examination for abnormalities in the smooth muscle or myenteric plexus with special trichrome and silver stains must be carried out. The most informative evaluation includes assessment of the neurotransmitters by immunohistochemistry and morphological assessment of the interstitial cells of Cajal with confocal microscopy.

Small intestinal manometry

A low-compliance water infusion technique is the method most often used for manometric studies of the small intestine, but ambulatory studies with miniature transducers and radiotelemetry are also used. Impedance measurements have the potential to provide pressure profiles from closely spaced sensors, although it is unclear whether these aid in the diagnosis.

The small intestine has a unique pattern of motility. During fasting, a cyclic pattern of motility, the MMC, is seen [335]. The MMC is divided into three phases. Phase I is a quiescent period that lasts for about 15 to 30 minutes. Phase II, which lasts for about 60 minutes, is a period of intermittent contractions that increase in frequency with time until phase III is initiated. During phase III, intense contractions propagate aborally from the duodenum to the ileum. Phase III lasts for 2 to 10 minutes. After phase III, the small intestine become quiescent again (i.e., enters phase I) to start a new MMC cycle. This cyclic pattern continues until the subject eats. After eating, the MMC is replaced by frequent, intermittent, irregular contractions – the fed pattern. The fed pattern usually lasts for 2 to 6 hours, depending on the size of the meal; after it is complete, the fasting pattern, or MMC, returns.

Small intestinal manometry has been used to study patients with various types of small intestinal dysmotility [132,133,139,157,160,175,177,184,224,318,336–343]. However, motility patterns are not diagnostic for any specific diseases. Many studies have been performed in patients with various clinical syndromes rather than specific diseases, which make the results difficult to interpret. Small intestinal motility studies have been performed in patients with small bowel bacterial overgrowth secondary to scleroderma [318], chronic neuropathic intestinal pseudoobstruction [132,139], autonomic disorders [344], FVM [5,17,27,338–340], nonfamilial visceral myopathy [30,340], FVN [58,59], nonfamilial visceral (paraneoplastic) neuropathy [209,211], scleroderma [157,159], myotonic dystrophy [224], and diabetes mellitus with gastroparesis [178,342,343]. Three important patterns have been recognized from these studies.

Visceral myopathic pattern

In patients with smooth muscle dysfunction or degeneration, manometry demonstrates a decrease in the frequency and amplitude of contractions in the affected segment, generally during both the fast and fed periods. During fasting, the MMC is usually present but is diminished in amplitude. Myoelectric recordings may reveal abnormalities of the slow wave, such as variability in rate or rhythm, and abnormalities of propagation, such as loss of coupling or shortening of the length or even changes in the direction of propagation. These recordings are characteristic of diseases involving the smooth muscle, such as FVM, advanced scleroderma, dermatomyositis, the muscular dystrophies, and amyloidosis [236,243,344]. Weston and associates [161] showed that in myopathic disorders, antral amplitudes are usually less than 40 mmHg and duodenal amplitudes less than 10 mmHg.

Visceral neuropathic pattern

Neurological disorders tend to produce disorganization and incoordination of motor activity. The MMC is often absent or abnormal. An abnormal rate of migration, as well as retrograde propagation of the activity front (phase III), also may be noted. Activity fronts may appear normal proximally and then become arrested or disappear in the more distal segments of the small intestine. In neurological disorders, the normal fed pattern may not replace the fasted pattern; MMC-like activity persists postprandially, and the frequency of distal antral contractions in the first hour is typically less than 1/min [161]. These abnormalities occur because the motility patterns are regulated by the ENS. The amplitude and frequency of contractions are usually normal when the inhibiting innervation and the coordinating function of the ENS are perturbed. Many of these abnormalities are seen best during sleep, when motor activity is stereotyped and normal phase II activity is suppressed [345]. The neurological disorders that have been described as producing manometric abnormalities include diabetes mellitus, Chagas disease, early scleroderma and amyloidosis, neurofibromatosis, paraneoplastic neuropathy, primary autonomic neuropathy, Parkinson disease, brain tumors, and multiple sclerosis [58,175,177, 194,244,346,347].

Mechanical obstruction pattern

Recurring episodes of intense contractions followed by periods of rest have been observed radiographically in the part of the intestine proximal to an obstruction. This phenomenon can be detected manometrically as clustered runs [139] or simultaneous prolonged contractions in conjunction with periods of intervening quiescence lasting longer than 1 minute (Fig. 47.25) [348,349]. Manometry is not the usual way to establish the diagnosis of mechanical obstruction, but these patterns should alert the physician to the possibility of mechanical obstruction and mandate careful small bowel barium radiography (e.g., enteroclysis).

Overall, small intestinal manometry lacks the specificity to diagnose the underlying disease other than suggesting the pathophysiological process. The relative merits of a laboratory-based study [350] and ambulatory manometry [351] have been discussed in the literature. From a diagnostic standpoint, the latter does not significantly enhance the results of the shorter laboratory-based studies, which have the added advantage of examining antral motor function more accurately; however, the ambulatory study is more practical and probably less costly.

Colonic manometry

Pressure waves recorded manometrically are generally equated with contractile forces. Colonic contractions are often irregular, of varying frequency and amplitude, and subject to the emotional state of the subject, the type of meal eaten, and the composition of chyme entering the colon from the ileum [352–356]. The colon is often "quiet" or exhibits isolated contractions (perhaps reflecting the dominant inhibitory control). At times, pressure waves are recorded in continuous bursts ranging in duration from 10 to 30 minutes [354], with a dominant frequency of approximately six contractions per minute in the right side of the colon and approximately three to seven per minute in the distal colon.

Phasic colonic contractions are stimulated by meals, beginning 20 to 30 minutes after a meal and lasting for up to 3 hours; sometimes, a second peak of activity occurs approximately

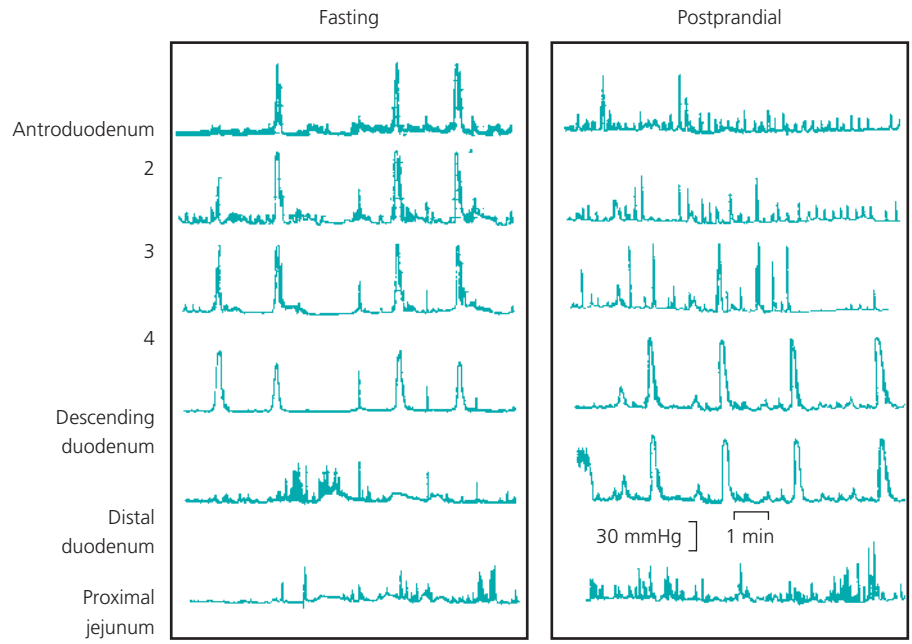


Figure 47.25 Gastroduodenal motility tracing demonstrating simultaneous prolonged contractions during fasting **(left)** and postprandial **(right)** phases. Contractions were present in the duodenum but not in the proximal jejunum (bottom tracing), a suggestion of mechanical obstruction in the distal duodenum or the proximal jejunum. From Loftus EV, Farrugia G, Donohue JH, Camilleri M. Duodenal obstruction: diagnosis by gastroduodenal manometry. Mayo Clin Proc 1997;72:130, with permission from Mayo Clinic Proceedings.

70 to 90 minutes postprandially [352–356]. This response to eating (gastrocolic reflex) remains after gastrectomy and after vagotomy [357]. If the stomach is intact, gastric distention and chemical stimulation by nutrients elicit a comparable response; ingestion of lipids or a total caloric intake of more than 500 kcal (2.1 MJ) are the most potent stimuli [1,358].

Control of the colonic response to eating involves neural and possibly hormonal mechanisms. The part of the response mediated by gastric mechanoreceptors is sensitive to blockade by atropine, but the part originating in the small intestine is only partially under muscarinic control. Cholecystokinin (CCK), which is normally secreted after meals, has been thought to play a role in the response because high blood concentrations of CCK stimulate contractile activity in both the small and large intestine. However, loxiglumide, a CCK A receptor antagonist, did not block the gastrocolic response to food [359]. Nitric oxide synthase blockade significantly increases the proximal colon propagating motor activity, suggesting that removal of nitrergic tone might be the physiological stimulus for proximal colonic emptying [360]. Low-amplitude irregular phasic activity does not propagate and probably equates with segmentation; on the other hand, high-pressure propagating waves are the equivalent of peristalsis. Isolated, high-amplitude (~ 200 mgHg) peristaltic contractions (Fig. 47.26), propagating at about 1 cm/s over long distances, are thought to be important for transit or "mass movement" in the colon. These occur infrequently (mean of six per day in healthy humans) and are most frequent after waking and meals [361]. They can be reliably induced by instilling irritants like bisacodyl [362] or fatty acids [363] into the colon. Endoluminal administration of 10 mg of bisacodyl is a potent stimulant capable of promoting high-amplitude peristaltic contractions (HAPCs) even in some patients who have manometric features of an inert colon [364].

An important variant of manometry uses the electromechanical barostat [1,365–367]. Originally developed to detect volume changes in the gastric fundus, the methodology is well suited for identifying relaxation in any hollow viscus. The 8–10-cm-long polyethylene bag used in humans is of large volume and "infinite compliance" until it reaches a volume of 500–600 mL. Air is introduced or withdrawn from the bag by a servomechanism to maintain a preset low pressure in the bag. Changes in volume are reflections of changes in wall tone. Basal tone in the proximal and distal colon exhibits rhythmic fluctuations [1], and food induces an immediate and prolonged increase in tone. The postprandial response is inhibited by 5-HT_3 blockers [368] and modified by hyperglycemia [369]. The rectum also shows a tonic response to eating [370], one that is impaired in patients with chronic slow-transit constipation [371]. The rectum also demonstrates prominent relaxation during distention [372–374].

Differential diagnosis of chronic intestinal pseudoobstruction and mechanical obstruction

In patients with severe dysmotility of the small intestine, chronic intestinal pseudoobstruction may develop. Partial small bowel obstruction from adhesions, tumors, intussusception, or stricture can mimic chronic intestinal pseudoobstruction. The features listed in Table 47.8 can be used to differentiate between these two problems. Enteroclysis, CT enterography, or careful small bowel radiography with fluoroscopy are probably the most helpful ways to differentiate

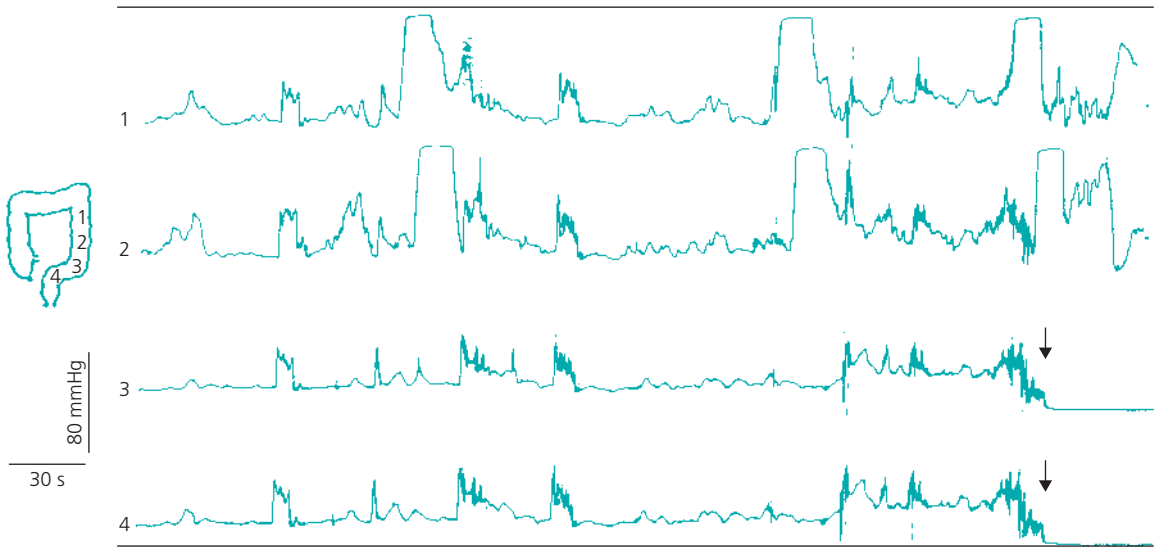


Figure 47.26 High-pressure waves recorded sequentially from four sites in the human colon. These contractions (giant migrating contractions) are thought to correspond to mass movements of colonic content. Their frequency is reduced in patients with constipation. From Narducci et al. [361], with permission from the BMJ Publishing Group.

Table 47.8 Differentiation of chronic intestinal pseudoobstruction from true mechanical obstruction

Feature	Chronic pseudoobstruction	Mechanical obstruction
Bowel symptoms	Diarrhea or constipation	Constipation and obstipation
Other gastrointestinal symptoms	± Dysphagia or symptoms of gastric atony	No esophageal or gastric problems
Between attacks	Abdominal pain, nausea, vomiting, or dysphagia	Usually symptom-free
Appearance	Cachectic	Seldom cachectic
Urinary symptoms	May have retention and infection	No urinary symptoms
Systemic disease	Symptoms and signs of systemic disease	No underlying systemic disease
Family history	Positive or negative	None
Abdominal radiography	Air throughout small bowel and colon	No air beyond point of obstruction
Esophagram	± Esophageal aperistalsis and dilation	Normal
Barium: stomach and small bowel	Gastric atony and megaduodenum	Bowel dilated above obstruction
Enteroclysis	No obstructing lesion	Obstructing lesion
Barium enema	± Redundant colon or wide-mouthed diverticula	Obstructing lesion
IVU	Megacystis or megaureter	Normal
Esophageal manometry	Diminished LES tone and low-amplitude contractions distal two-thirds (myopathy); incoordination (neuropathy)	Normal
Jejunal manometry	Fasting: absence of MMCs, and low-amplitude contractions (myopathy); fed: antral < 1/min, inactive fed pattern with MMCs (neuropathy)	Clusters, or simultaneous prolonged contractions fasting and fed periods, but MMCs during fasting
Exploratory laparotomy	No obstructing lesion found	Obstructing lesion

IVU, intravenous urography; LES, lower esophageal sphincter; MMC, migrating motor complex.

chronic intestinal pseudoobstruction from mechanical obstruction. In our experience, however, enteroclysis may not conclusively differentiate chronic intestinal pseudoobstruction from mechanical obstruction in some patients. Manometry proved useful in one series [349]; however, in most cases, exploratory laparoscopy may be necessary to rule out an obstructing lesion.

Treatment

Drug therapy to improve small intestinal motility

Theoretically, it is difficult to envision a drug that can either stimulate damaged muscle to contract effectively or normalize the coordinating functions of a damaged myenteric plexus. Many drugs that stimulate intestinal motility in normal subjects (e.g., bethanechol, neostigmine, metoclopramide, erythromycin, prucalopride) have no beneficial effects in patients with small intestinal dysmotility. No information is available on the use of domperidone for small bowel dysmotility. Tegaserod accelerates small intestinal transit, proximal colonic emptying, and overall colonic transit in constipation-predominant IBS [375].

In a small, short-term study, octreotide, a somatostatin analogue, stimulated intestinal motility, possibly reduced bacterial overgrowth, and relieved abdominal symptoms in patients with scleroderma [157]. Other open-label studies confirmed the long-term effectiveness of octreotide with erythromycin in the treatment of chronic intestinal pseudoobstruction [288].

Symptomatic and supportive treatments

Abdominal pain, bloating, nausea, and vomiting in patients with small intestinal dysmotility are often related to eating. Most of these symptoms can be minimized by manipulating the size, nature, and frequency of meals. The important point to keep in mind is to provide sufficient calories without overloading the inefficient bowel. A rule of thumb is to give approximately 25 kcal (105 kJ/kg) of the patient's ideal bodyweight per day. Adult patients should consume 1500 to 1800 kcal (6.3–7.5 MJ.day), divided into three or four equal feedings. A low-residue diet may reduce obstructive episodes. At least half of the calories should come from supplemental formulas such as Ensure because a liquid meal empties faster from the stomach and probably progresses more easily through the small bowel than a solid meal. These formulas are lactose free and contain the daily requirements of vitamins and minerals. When patients still feel full several hours after the first meal, it is important that they do not force themselves to eat subsequent meals to avoid aggravating their symptoms, and they that restrict their oral intake to fluids for the rest of the day.

In patients with chronic intestinal pseudoobstruction, recurrent symptoms and signs of intestinal pseudoobstruction may occur despite implementation of the previously described regimens. In these situations, nasogastric suction and intravenous fluids are needed when obstructive symptoms develop. When obstructive symptoms and pain persist or occur several times a week despite dietary manipulation,

long-term parenteral nutrition or small intestinal transplantation are the only treatments that will relieve symptoms and improve nutrition.

Abdominal pain unrelated to eating is uncommon in patients with small intestinal dysmotility, and if a patient has pain predominantly unrelated to meals, questions should be raised about the diagnosis. During episodes of obstruction, parenteral administration of narcotics such as morphine or meperidine may be required. Long-term narcotic use should be discouraged because addiction is possible, and narcotics can further disturb gastrointestinal motility.

Constipation is common in patients who also have colonic involvement. It is important to make certain that the patient has a good bowel movement at least once every few days because constipation tends to increase the symptoms of intestinal dysmotility. We recommend a daily osmotic laxative, such as magnesium hydroxide or polyethylene glycol, and a bisacodyl suppository if the patient has no bowel movement for 3 days. Those with severe small intestinal dysmotility should avoid bulk-forming laxatives because they increase the load on an inefficient intestine and exacerbate symptoms.

Treatment of secondary causes

A few types of secondary small intestinal dysmotility, such as those associated with myxedema, celiac sprue, and drug-induced dysmotility, can be treated with thyroid replacement, a gluten-free diet, and discontinuation of the offending drug, respectively. No specific treatments are available for most of the secondary types of small intestinal dysmotility.

Surgical treatment

Patients with dysmotility limited to short segments of the small intestine, such as those with megaduodenum, have a better prognosis than those with dysmotility throughout the length of the bowel because the dysfunctional segment can be resected or bypassed [376]. Megaduodenum, which is commonly seen in FVM type I and scleroderma, has been drained with a side-to-side duodenojejunostomy, which usually relieves symptoms. In some patients with a massively dilated duodenum, a side-to-side duodenojejunostomy may be inadequate to drain the duodenum. In these cases, resecting as much of the duodenum as possible with preservation of the papilla of Vater and anastomosing the opened jejunum to the cut edge of the duodenum to create a small conduit may be required as treatment [13,377].

For patients with long segments of small intestinal dysmotility (i.e., > 1.2 m), no effective surgical treatment is available. Any unnecessary surgery should be avoided in these cases because it can create adhesions and additional difficulties. Venting decompression by percutaneous jejunostomy or minilaparotomy or laparoscopy relieves symptoms and reduces the rate of hospitalization for recurrent exacerbations of pseudoobstruction [378,379].

Small bowel transplantation

Small bowel transplantation has become a reality, with acceptable benefit-to-risk ratio and outcomes with the introduction of isolated small bowel transplants, judicious timing of transplant, and a team approach. There is no definite consensus on indications but experienced teams recommend that those adult and pediatric patients with no jejunoileum who have guaranteed lifelong parenteral dependence, should be immediately referred to a transplant center because of the high likelihood of the development of liver disease. Similarly, patients with metastatic infectious complications from catheter sepsis, or cholestasis seen intermittently with sepsis episodes, progressive thrombocytopenia, and those with deteriorating motility disorder should be referred to an intestinal rehabilitation and transplant program. The objective of evaluation at such centers is to maximize the opportunities for rehabilitation (e.g. relief of adhesive obstruction or other "surgical complications" that compromise bowel function) while not missing the critical window of opportunity for successful transplantation when needed. Psychological preparation for transplantation and other options, while not rushing to judgment, will avoid a premature transplant [380,381]. Graft and patient survival are expected to be 80% at 3 years in a successful program. Survival has been enhanced by effective immunosuppression (chiefly tacrolimus and mycophenolate) and the less frequent need for immunosuppression by avoiding unnecessary grafts when the recipient's organ is not failing. Thus, outcomes have improved with increased experience, the avoidance of splenectomy, optimization of immunoregulatory regimens (administration of interleukin-2 receptor antagonists), and improved controls of viral infections and rejection [382]. In reaching a decision about transplantation, it is important to recall that the 3-year survival of patients on home total parenteral nutrition (TPN) is 85% [383].

Several factors limit the success of small bowel transplantation and may result in death: infection (including cytomegalovirus and Epstein–Barr virus, which together account for approximately 50% of deaths); rejection (approximately 8% of deaths); technical problems; and lymphoproliferative disease (20%–40% of deaths in different programs). The latter is particularly linked to Epstein–Barr virus infection, the use of OKT3 (muromonab-CD3) immunosuppression, and steroids [384,385].

In general, small bowel transplantation is restricted to life-threatening situations, such as necrotizing enterocolitis, intestinal failure (short bowel syndrome) in patients who cannot be maintained on TPN, and rarely chronic intestinal pseudoobstruction in young patients who have TPN-related or other liver failure and lack intravenous access for TPN.

In the future, gastroenterologists may be involved in the management of patients after small bowel transplantation. Observations that are likely to affect this practice include the following:

- zoom videoendoscopy is capable of showing the enlarged crypt areas and villus blunting or flattening that correlate with histological evidence of rejection [386]
- severe rejection (mucosal necrosis with loss of villi) is of grave prognostic significance (11% graft survival, 18% patient survival) [387] and, pending newer immunosuppressive regimens, may be an indication for removal of the graft
- living-related small bowel transplantation is technically feasible and has been reported to be followed by complete functional adaptation of the graft [388].

It is clear that survival after this formidable procedure depends on the experience of the entire transplant team and center, and aggressive treatment of infection and rejection. The rate of mortal complications, including lymphoproliferative disease, remains high [389]. The use of tacrolimus in immunosuppression is associated with a fivefold increase in lymphoproliferative disease relative to cyclosporine (ciclosporin) in children receiving liver transplants [390]. Molecular methods to screen for Epstein–Barr virus infection, improved immunosuppression, and possibly bone marrow transplantation to enhance chimerism may change the biology or prevalence of lymphoproliferative complications in the future.

Colonic dysmotility (pseudoobstruction) and megacolon

Interstitial cells of Cajal in maldevelopment and acquired diseases of the colon

The significance of the interstitial cells of Cajal (ICCs) was established during the mid-1970s and 1980s through the careful histological and electrophysiological observations of Faussone-Pellegrini and colleagues [77,78] and Thuneberg [79]; the latter established the candidacy of ICCs as the intestinal pacemaker cells. The protooncogene c-*Kit* encodes a tyrosine kinase receptor that facilitates the development of the ICCs [391]. Furthermore, mice with mutations in the dominant white spotting (*W*) locus, which have cellular defects in hematopoiesis, melanogenesis, and gametogenesis as a result of mutations in the c-*Kit* gene, also lack the network of ICCs and intestinal pacemaker activity [392]. In the 1990s, the role of ICCs became more clearly understood through studies of a mutant strain of mice (w/w^v) that lack c-KIT-positive cells and in which gastrointestinal dilation and failure of peristalsis develop [393–397].

Typically ICCs are surrounded by collagen fibers and form close contacts (Fig. 47.27) with smooth muscle cells [393]. These gap junctions with smooth muscle cells allow transmission of the pacemaking activity, which is unaffected by 10^{-6}-M verapamil, an L-type calcium channel blocker [393]. When studied in short-term primary culture, ICCs assume a triangular shape with three to four branches that are in apposition with cocultured smooth muscle cells (Fig. 47.28). Huizinga, Ward, Sanders, and their colleagues [394–396] have characterized the expression profiles of pacemaker ICCs isolated from the murine small intestine and of ICCs and smooth muscle cells involved in neurotransmission in the gastric fundus. All cell types express muscarinic receptor types M_2 and M_3, neurokinin receptors NK_1 and NK_3, and the inhibitory type 1 vasoactive intestinal peptide receptor (VIP_1) [396].

In clinical studies, a relative deficiency of c-KIT-positive cells has been reported in Hirschsprung disease, chronic intestinal pseudoobstruction [397,398], gastrointestinal stromal tumors, multiple gastrointestinal autonomic tumors [399,400], and idiopathic slow-transit constipation [401–403].

Delayed maturation or maldevelopment of ICCs is well documented (Fig. 47.29) [404]. At 1 month of age, a child with chronic colonic pseudoobstruction underwent biopsy of the affected colon, which showed no peristaltic activity and no c-KIT immunoreactivity within the circular muscle layer or the submuscular layer (see Fig. 47.29) but a normal ICC population in the myenteric plexus. At 6 months of age, the peristaltic activity was normal, and the ICCs were fully developed in all layers of a full-thickness biopsy specimen of the colon. This careful case study illustrates the following points:

- the concept of postnatal maturation of ICCs
- the importance of the conservative management of children with neonatal megacolon or pseudoobstruction
- the importance of ICCs to the overall peristaltic function of the colon.

Little is known about the transmitters expressed by ICCs in disease. However, immunoreactivity to the NK_1 receptor has been detected in ICCs and may be used as a marker of the ICCs at the deep muscular plexus; these cells may participate in the actions exerted by tachykinins on muscle cells [405].

Megacolon in an adult has also been associated with abnormal morphology and ultrastructure of ICCs. Faussone-Pellegrini and colleagues [406] demonstrated ICCs with several branches in the dilated transverse colon, but abnormal ICCs with a paucity of mitochondria, filaments, and caveolae in the nondilated descending colon.

In patients with acquired slow-transit constipation not associated with colonic dilation, the number of ICCs in the different layers of the sigmoid colon is lower (Fig. 47.30) than in controls (specimens resected for other indications). Moreover, the confocal images show irregular surface markings and a paucity of branches of the cells (Fig. 47.31) [401]. Staining of myenteric plexus for c-KIT is reduced, consistent with a reduction of ICCs or their tyrosine kinase content. In addition to the reduction of ICCs, there also appears to be a significant decrease in other neuroenteric elements, including gangliar cells, glial cells, and enteric neurons in the colons of patients with idiopathic slow-transit constipation [407].

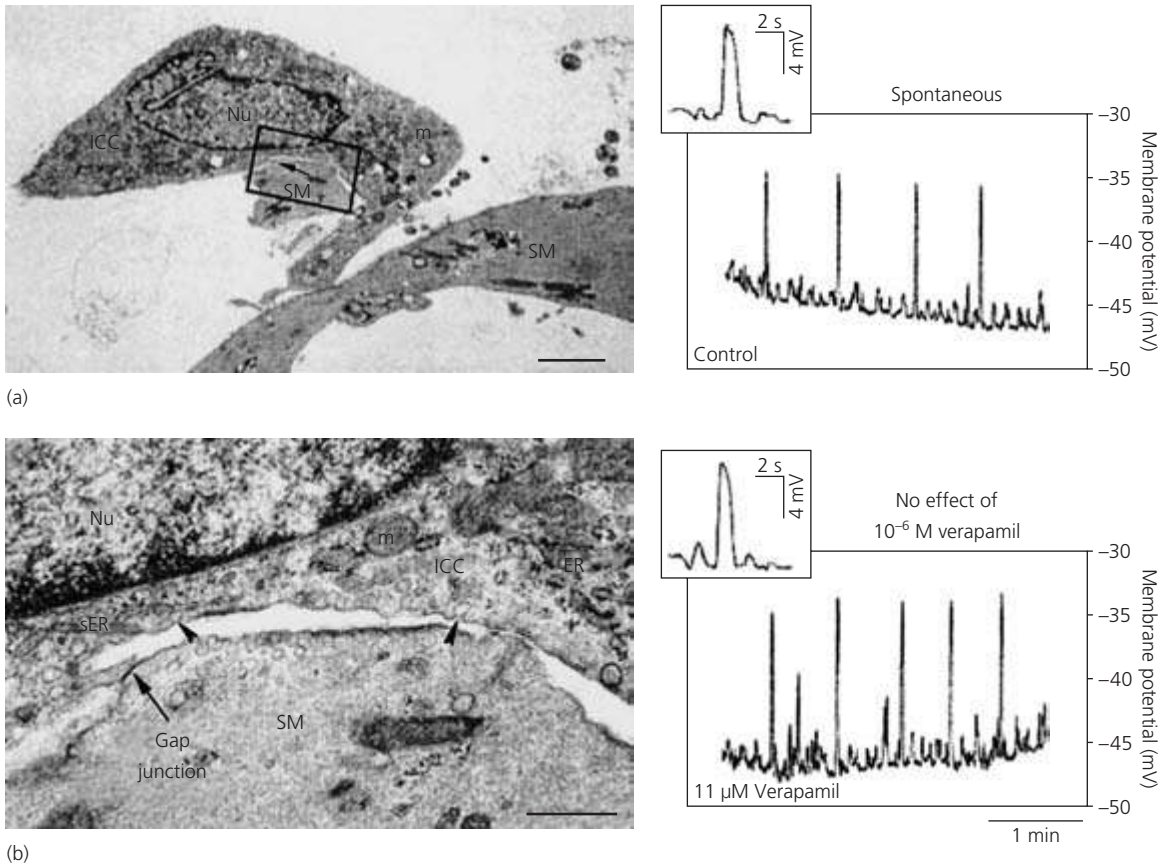


Figure 47.27 Gap junctions between interstitial cells of Cajal and smooth muscle cells; note the spontaneous electrical oscillations of the resting membrane potential of the interstitial cells of Cajal and the lack of inhibition by the L-type calcium channel blocker verapamil. From Lee et al. [393], with permission from the American Physiological Society.

Syndromes generally attributed to disorders of colonic motility

Constipation

Patients consider themselves to be constipated when they pass stools more infrequently, require more effort for passage ("straining"), or experience more pain or discomfort during passage than they think appropriate. Difficult evacuation of feces, especially when the consistency of stools is softer than normal, is more likely to be caused by disorders of the pelvic floor or anorectum than by slow colonic transit. Disorders of the pelvic floor are disturbances of evacuation (dyschezia, obstructed defecation) and are not considered here (see Chapters 43 and 92).

Slow transit through some or all segments of the colon leads to the formation of hard fecal masses that are passed infrequently and often with great difficulty. This condition, slow-transit constipation (STC), should be considered as separate from IBS and should be diagnosed only if abdominal pain is not a prominent feature. However, STC certainly merges with IBS when pain, abdominal distention, excessive rectal mucus, and intermittent episodes of frequent, looser stools also occur (see Chapter 86). STC is considered in the next section.

Slow-transit constipation (STC)

Figure 47.23 shows radiopaque markers retained predominantly in the distal colon (rectosigmoid segment) 96 hours after ingestion [326]. This technique is simple, inexpensive, and of proven value in the documentation of STC [326–330]. Gamma scintigraphy [331] offers another means of testing that can be of shorter duration (24 or 48 hours). Whatever method is used, the implications for therapy are potentially important. Most patients with STC respond to laxative programs, suppositories, or enemas. Resective surgery of the colon (usually subtotal colectomy with ileorectostomy) is occasionally necessary for severe STC. When tests of pelvic floor function identify a clinically relevant abnormality, colectomy is not indicated, even in the presence of slow transit. Retraining programs for the pelvic floor should first be used to correct the defect in expulsion, after which transit can be retested.

Manometry

In STC, the normal high-amplitude pressure (peristaltic) waves in the colon are reduced. Hirschsprung disease, or congenital megacolon (see "Hirschsprung disease: congenital

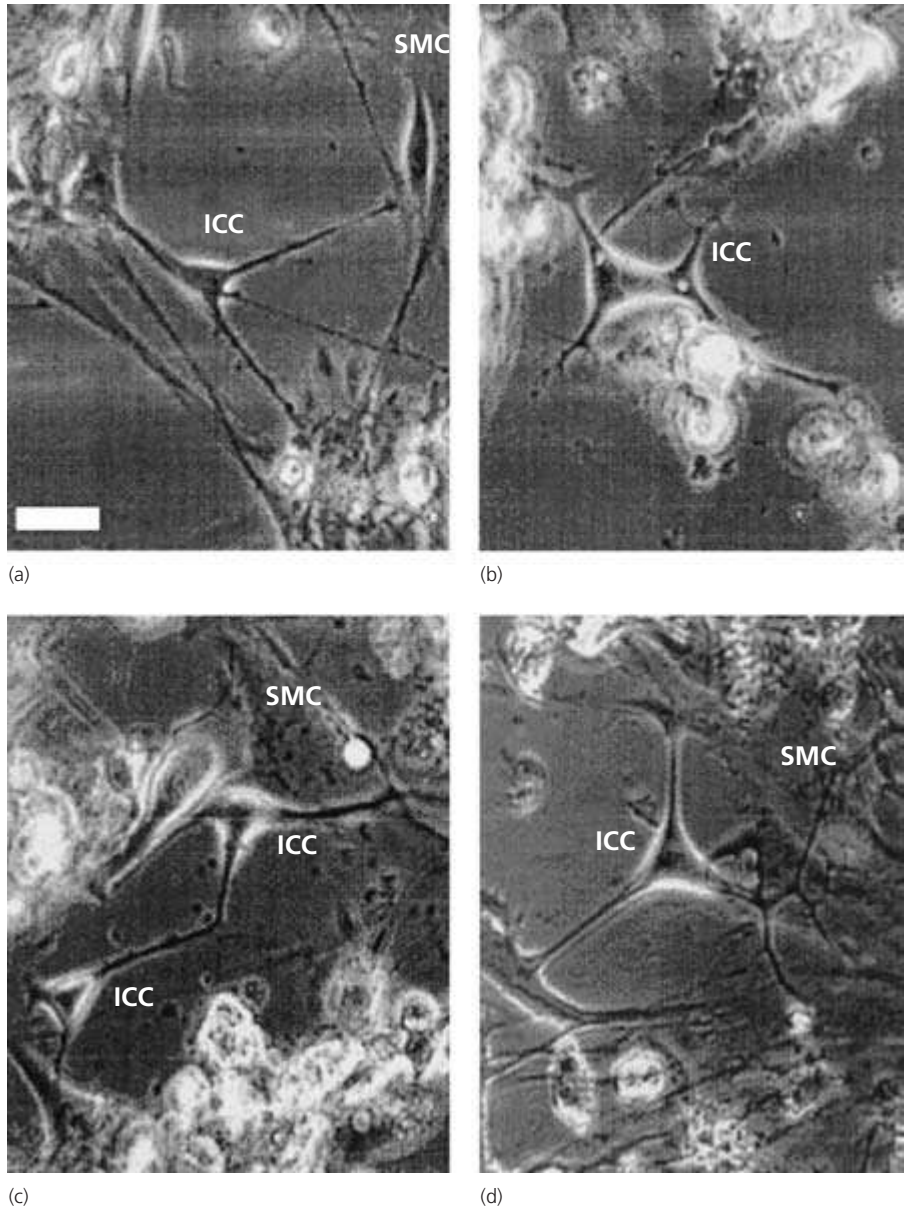


Figure 47.28 In short-term culture, interstitial cells of Cajal assume a triangular shape and have three to four branches that establish contact with cultured smooth muscle cells. From Lee et al. [393], with permission from the American Physiological Society.

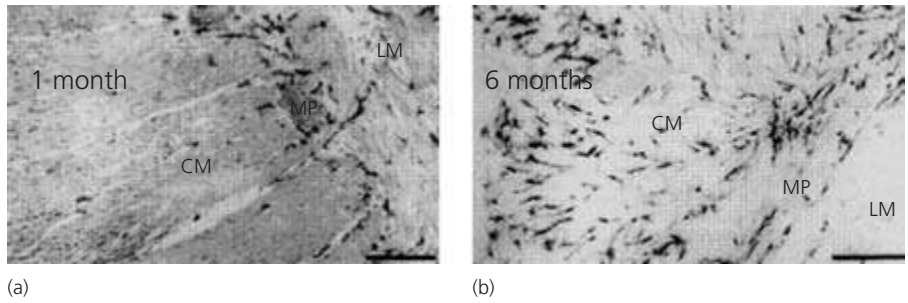
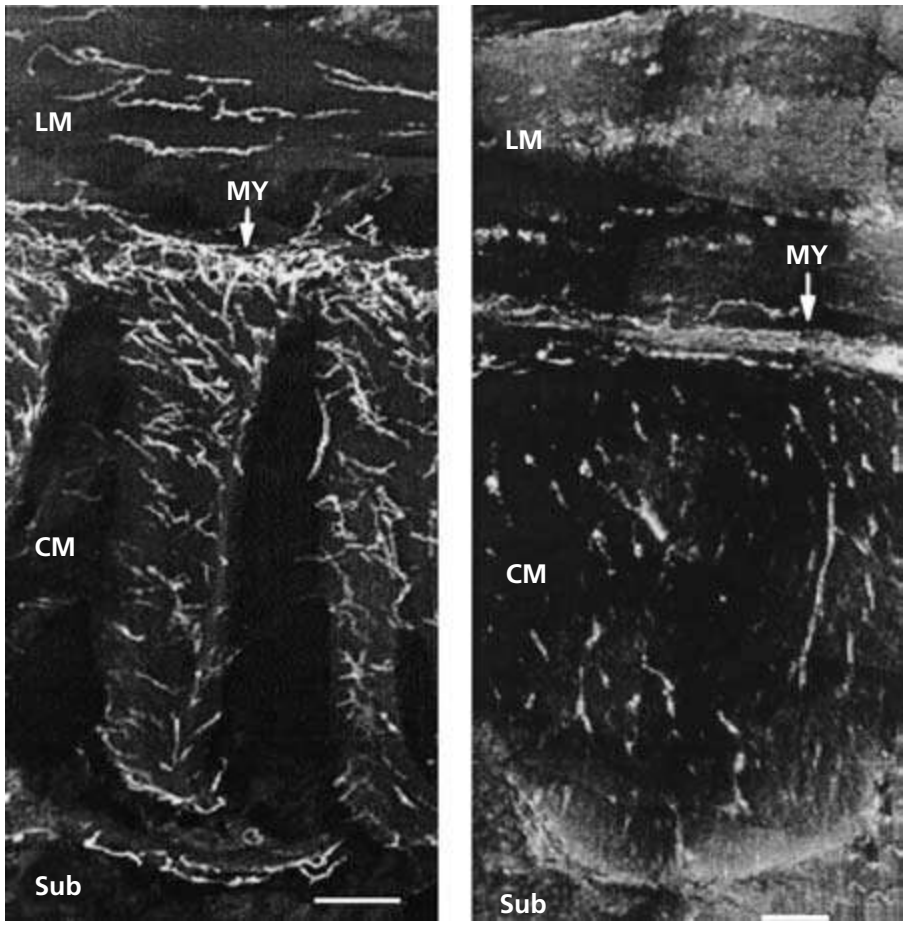


Figure 47.29 Full-thickness biopsy specimens from the colon of an infant with neonatal chronic colonic pseudoobstruction demonstrating delayed maturation of interstitial cells of Cajal at 1 month and subsequent expression of the cells at 6 months of age. From Kenny et al. [404], with permission from Elsevier.

megacolon" below), is a special case in which the pathophysiological abnormality is absence of relaxation of the internal anal sphincters, manifested by absence of the rectoanal inhibitor reflex.

Myoelectric patterns

Myoelectric patterns have confirmed the concept of "paradoxical colonic motility:" patients with diarrheal disease often have a colon that demonstrates relatively little overall



(a) (b)

Figure 47.30 Distribution of interstitial cells of Cajal in whole transverse mounts of **(a)** a normal-appearing control section of the sigmoid colon and **(b)** the sigmoid colon of a patient with slow-transit constipation. From He et al. [401], with permission from Elsevier.

myoelectric activity, whereas those with constipation often have active motor patterns that favor "holding up" contents.

Histopathology

Many patients with STC severe enough to warrant subtotal colectomy display histological changes in the ENS of the resected colon on silver staining. Table 47.9 summarizes the information derived from a number of studies in the literature [401,408–418]. In general, it appears that reduced levels of substance P and increased numbers of nitrergic neurons are associated with constipation that leads to surgical resection of the colon. In Parkinson disease, evidence is found of a reduction in the dopamine content of the myenteric plexus and a significant reduction in the dopamine-positive neurons of whole mounts of the plexus [419].

Ultrastructural quantitative studies have demonstrated abnormalities of the morphology and numbers of ICCs [401]. It is assumed, therefore, that these patients have a morphological abnormality of the neural control of colonic motility. These clinical and histopathological features appear to merge with the syndromes of intestinal pseudoobstruction (see "Colonic pseudoobstruction and megacolon" below). The treatment of constipation is addressed in Chapter 000.

Colonic pseudoobstruction and megacolon

Megacolon (Table 47.10) and *megarectum* are descriptive terms without etiological or pathophysiological implications. *Megacolon* has been defined as a diameter of the rectosigmoid region or descending colon, on radiography, of more than 6.5 cm, of the ascending colon of more than 8 cm, or of the cecum of more than 12 cm [420]. Megacolon can be caused by aganglionosis (Hirschsprung disease), may be idiopathic (complicating chronic constipation of any cause), or may be a manifestation of diffuse gastrointestinal dysmotility (intestinal pseudoobstruction). *Acute megacolon* is often designated as *Ogilvie syndrome*. Rectal dilation, or megarectum, may be an isolated finding or, more often, associated with megacolon or simply prolonged chronic constipation, particularly in children with functional fecal retention. Note that megacolon does not include simple elongation of the colon (dolichocolon).

In *congenital megacolon* (Hirschsprung disease), colonic dilation results from functional obstruction (usually of the

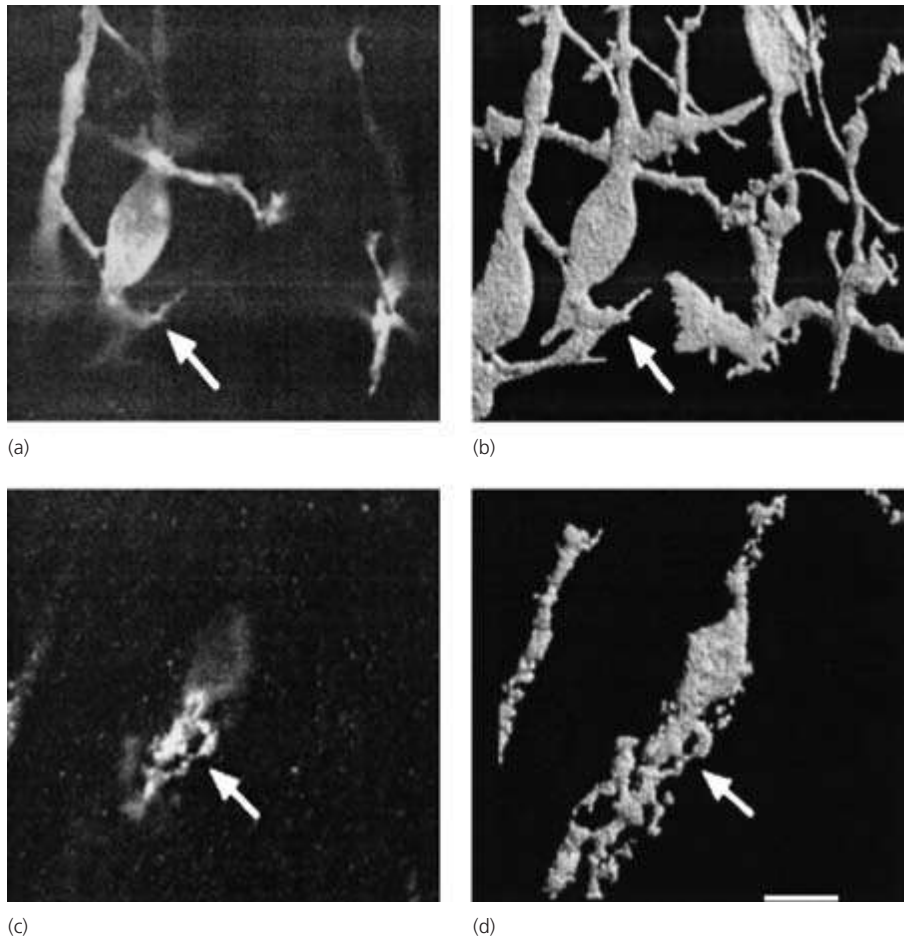


Figure 47.31 High-magnification confocal microscopy of the interstitial cells of Cajal in human sigmoid colon. **(a)** and **(c)** are single slices; **(b)** and **(d)** are reconstructions of 20 consecutive single slices. **(a)** and **(b)** are from a healthy-appearing disease control colon; note the multiple fine processes and the network of interconnecting interstitial cells of Cajal. **(c)** and **(d)** are from a patient with slow-transit constipation. Note the irregular markings and loss of fine processes. Bar = 10 μm. From He et al. [401], with permission from Elsevier.

rectum) caused by a congenital absence of the intramural neural plexuses that mediate relaxation (aganglionosis). This leads to a narrow segment of the large intestine – that is, one that fails to relax.

Acquired megacolon can complicate any of the many causes of constipation, and it can be assumed that megacolon is acquired when it can be ascertained that colonic dilation was not present at some earlier examination. A common background for acquired megacolon is colonic inertia, which occurs frequently at both extremes of life. In children, this form can be confused with the congenital condition.

Worldwide, infection with *Trypanosoma cruzi* (Chagas disease) is the most common form of acquired megacolon. In this condition, the dilated segment of colon is abnormal because of destruction of the ENS by the neurotoxin of the infecting organism. Although infection was originally confined to the South American continent, it is estimated that there are 350 000 seropositive persons in the United States; among these, one-third are thought to have chronic Chagas disease [421]. Some patients acquire megacolon as part of a generalized intestinal pseudoobstruction.

When colonic pseudoobstruction is acute and associated with another medical condition (abdominal or orthopedic surgery, spinal cord injury, serious cardiovascular or other medical problems), the term Ogilvie syndrome is often applied. Toxic megacolon is a life-threatening complication of inflammatory bowel disease and infectious colitides (see Chapters 000 and 000).

Hirschsprung disease: congenital megacolon

Pathophysiology

Aganglionosis is caused by the arrested migration of cells caudad from the neural crest; these are the cells destined to develop as the intramural plexuses of the gut. In Hirschsprung disease, the aganglionic segment always extends from the internal anal sphincter for a variable distance proximally, but in most instances it remains within the rectum and sigmoid colon. The involvement of short segments, affecting only the anal sphincters, has also been described. The aganglionic segment is permanently contracted, causing dilation proximally. Longer aganglionic segments occur in fewer than 20% of affected individuals; involvement of the entire colon is infrequent, and aganglionosis extending proximally into the small intestine is rare. Thus, the hallmark of diagnosis is the absence of ganglion cells from the myenteric and submucosal plexuses, as seen on a full-thickness or suction

Table 47.9 Colonic neuropathology in slow-transit constipation

Histological and immunohistochemical findings	References
Decreased number or abnormal appearance of silver-staining neurons or axons; increased number of variably sized nuclei within ganglia	Krishnamurthy et al., 1985 [408]
Decreased colonic VIP nerves	Koch et al., 1988 [409]
Decreased neurofilament staining in myenteric plexus in 75% of patients; 17 of 29, entire colon affected; 12 of 29, segmental involvement	Schouten et al., 1993 [410]
Increased number of PGP 9.5-reactive nerve fibers in muscularis layer of ascending and descending colon	Park et al., 1995 [414]
Decreased total nerve density in myenteric plexus	Cortesini et al., 1995 [413]
Decreased VIP and increased NO-positive neurons	
Decreased substance P nerves in 7 of 10 patients, decreased VIP nerves in 4 of 7 patients	Hutson et al., 1997 [415]
Decreased substance P in mucosa and submucosa of rectal biopsy specimens	Tzavella et al., 1996 [412]
Increased VIP, substance P, and galanin in ascending colon	Sjolund et al., 1997 [416]
Increased VIP and galanin in transverse colon	
Increased VIP and neuropeptide Y in descending colon myenteric plexus	
Decreased VIP in submucosa	
Decreased tachykinin (substance P) and enkephalin fibers in circular muscle	Porter et al., 1998 [411]
Decreased colonic total neuron density	Faussone-Pellegrini et al., 1999 [418]
Decreased VIP and NO neurons in myenteric, decreased VIP neurons in submucous plexus	
Decreased enteroglucagon and 5-HT cells in mucosa	El-Salhy et al., 1999 [417]
Decreased cell secretory indices of enteroglucagon and somatostatin cells	
Decreased volume of interstitial cells of Cajal and neurons in circular muscle	He et al., 2000 [401]

5-HT, 5-hydroxytryptamine (serotonin); NO, nitric oxide; PGP, protein gene product; VIP; vasoactive intestinal polypeptide.

Table 47.10 Clinical classification of megacolon

Congenital megacolon (Hirschsprung disease)
"Classical" type
Short segment
Ultrashort segment
Total colonic aganglionosis, zonal loss of ganglia, and other variants

Acquired megacolon (associated with constipation)
Idiopathic
In children (associated with encopresis)
Acute form in adults (Ogilvie syndrome)

Secondary to neurological diseases
Chagas disease
Parkinson disease and central nervous system dysfunction; myotonic dystrophy
Diabetic neuropathy; others (ganglioneuromatosis, familial autonomic dysfunction)
Intestinal pseudoobstruction ("neurogenic" forms)

Secondary to diseases of intestinal smooth muscle
Scleroderma and other "collagen diseases;" amyloidosis
Intestinal pseudoobstruction ("myogenic" forms)

Secondary to metabolic diseases
Hypothyroidism, hypokalemia, porphyria, pheochromocytoma (with ganglioneuromatosis)

Drugs

Mechanical obstruction

(mucosal–submucosal) biopsy specimen of the rectum. Proximal contents fail to enter the unrelaxed aganglionic segment.

Morphologically, ganglion cells are absent from the narrowed segment and for a short distance (usually 1–5 cm) into the dilated segment. In contrast, the nerve fibers are hypertrophic, with abundant, thickened bundles. Specific stains for acetylcholinesterase highlight the abnormal morphology. Adrenergic denervation of the dilated segment is another inconsistent finding, as is a decreased supply of peptidergic nerves (containing VIP, substance P, enkephalins, and other peptides).

The most characteristic functional abnormality of aganglionosis is a failure of the internal anal sphincter to relax following rectal distention [422]. Transient distention of a balloon in the rectum causes the intralumenal pressure at the level of the internal anal sphincter to drop; the drop is often accompanied by a reflex contraction of the external sphincter. Up to 20% of normal children may have a falsely absent reflex, especially if they are premature or of low birthweight, but a positive response is strong evidence against Hirschsprung disease.

Incidence and genetics

The defect occurs once in each 5000 live births and is in some cases familial, with an overall incidence of 3.6% among siblings of all index cases [423]. Because the disease was lethal until the introduction of curative surgery in the 1950s, accurate assessment of the incidence in the offspring of

successfully treated patients is incomplete. Consanguinity of parents is exceptional, and the condition is reported to be discordant in dizygotic twins and concordant in monozygotes.

The association of congenital aganglionosis of the colon with Down syndrome is ten times more frequent than would be expected by chance [423]; approximately 2% of patients with congenital megacolon have Down syndrome. A number of other congenital anomalies have been reported: hydrocephalus, ventricular septal defect, cystic deformities and agenesis of the kidney, cryptorchidism, diverticulum of the urinary bladder, imperforate anus, Meckel diverticulum, hypoplastic uterus, polyposis of the colon, ependymoma of the fourth ventricle, the Laurence–Moon–Bardet–Biedl syndrome, and congenital central hypoventilation syndrome (Ondine curse).

The genetic disorders resulting in disordered development of the neural crest in Hirschsprung disease are discussed in detail above (see "Ontogeny of the enteric nervous system").

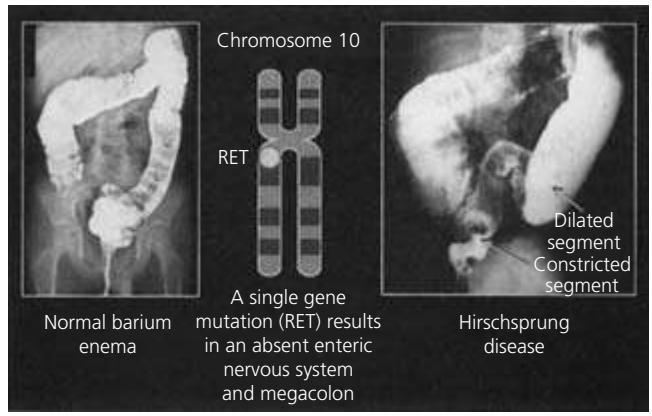


Figure 47.32 Barium enema in a normal child contrasted with a study showing megacolon and a narrow segment affected with Hirschsprung disease. The genetic defect involves a mutation of the *RET* protooncogene on chromosome 10q11.2.

Clinical features

Hirschsprung disease should be suspected shortly after birth when the infant passes little meconium and the abdomen is distended. Digital examination of the rectum, insertion of a rectal tube, or administration of a small enema causes retained fecal material to gush forth, with apparent relief of the symptoms. However, the respite is often short-lived; signs of partial intestinal obstruction return, with persistent vomiting and distention as the major features. In about 20% of patients, diarrhea persists; it is caused by pseudomembranous enterocolitis, which develops as a complication of the obstruction.

Later in life, the presentation is often less dramatic and may not mimic an acute intestinal obstruction. Severe constipation and recurrent fecal impactions are more common. Children occasionally show evidence of anemia, malnutrition, and even hypoproteinemia resulting from protein-losing enteropathy; their resistance to infection can also be impaired. Although difficulties develop in most children before the second month of life, extremely-short-segment aganglionosis may not cause severe symptoms until after infancy.

Differential diagnosis

Hirschsprung disease must be distinguished in the neonate from other developmental causes of intestinal obstruction, such as atresias and imperforate anus. Later in life, acquired (secondary) megacolon is the other major consideration. The diagnosis of congenital megacolon is usually not difficult beyond the immediate neonatal period, and the availability of improved diagnostic methods enables a positive diagnosis in most cases. Obstipation, with infrequent spontaneous passage of stool, dates from infancy, and the rectal examination reveals an empty ampulla. Overflow incontinence is not a feature of Hirschsprung disease.

A barium enema radiograph (Fig. 47.32) confirms the diagnosis if the characteristic transition from the narrowed, distal rectum or rectosigmoid to the dilated proximal colon is seen. However, when the aganglionic segment is extremely short, a narrowed segment is not seen radiologically. In patients with acquired megacolon, encopresis is common, dilation extends all the way to the anus, and a narrowed zone is not seen.

Proctosigmoidoscopy reveals a normal but empty rectum. The dilated proximal bowel, if within range of the scope, is easily traversed except for abundant feces in the lumen; occasionally, stercoral ulcers are noted. The key findings are the empty lower segment and the absence of evidence of organic obstruction.

The diagnosis is best substantiated by a full-thickness biopsy of the rectum. The presence of normal numbers of ganglion cells excludes the diagnosis. Mucosal suction biopsy, satisfactory in many instances, is the initial procedure of choice because it is performed easily and requires no anesthesia. If the depth of the examination is sufficient to show the presence of ganglia in the Meissner (submucosal) plexus, the classic form of Hirschsprung disease is excluded. However, the absence of ganglion cells does not establish the diagnosis, and a full-thickness biopsy specimen should be obtained at least 3 cm proximal to the pectinate line. A diminution or absence of ganglion cells distal to this point is difficult to interpret. Careful histology proximal to the internal sphincter reveals that myenteric ganglia may be absent from normal infants over a distance of 4 to 5 mm in this segment; ganglia may be absent from the deep and superficial submucosal layers for even longer distances. Immunohistochemical techniques can highlight the morphological abnormalities, showing an abundance of hyperplastic axons but an absence of ganglion cells. Several approaches employing antibodies to acetylcholinesterase, neuron-specific enolase, neurofilament, and neuropeptides have been described [424,425].

Physiological tests complement the diagnosis in doubtful cases, and they may be crucial when the aganglionic segment is short. Such cases are less easily detected by radiography and are also likely to be missed by biopsy. The most important pathophysiological test is the response of the anal sphincters to distention of the rectum [422]. In contrast to the internal sphincter in normal individuals and in patients with acquired megacolon, the internal sphincter in patients with congenital aganglionosis fails to relax (or contracts even further) after the rectum is distended. The most common cause of a false-positive test result is a capacious rectum in constipation or megarectum; under these circumstances, distention of the rectal balloon does not stimulate the reflex. Therefore, an enlarged rectum should be excluded before Hirschsprung disease is diagnosed.

Treatment of congenital megacolon

Definitive surgical cure is the treatment of choice. Preliminary decompression by colostomy is still sometimes necessary to relieve obstruction, or it may be necessary in some infants when it is decided to postpone definitive surgery. However, the goal should be early diagnosis and a one-stage surgical approach.

The main goals are to establish regular and spontaneous defecation, to maintain normal continence, and not to interfere with sexual potency. The surgical procedure should cause essentially no mortality and minimal morbidity. A number of different operations have been used to remove successfully or to counterbalance the obstructing effect of the aganglionic segment. Long-term results are good in the great majority of patients, but 10% to 20% have residual problems, usually with fecal soiling [426].

Variants of congenital megacolon

The spectrum of Hirschsprung disease has widened considerably. Patients with a compatible clinical picture may have an ultrashort segment of aganglionosis, involving only the internal anal sphincter [427]. Morphological confirmation of the diagnosis may be difficult, and physiological testing becomes even more important. Patchy or zonal loss of ganglia (ladder pattern) and dysplastic neurons have also been described. Cases classified as acquired aganglionosis have been reported. In these, ganglia were seen in tissues removed at an initial operation, but when clinical failure led to further surgery, an aganglionic segment was clearly demonstrable. Rather than these being cases of acquired disease, it seems more likely that a short aganglionic segment was missed on the initial evaluation. With greater awareness of the more subtle morphological and physiological abnormalities, Hirschsprung disease is being detected more often in adults [428]. The clinical, physiological, and morphological features in adulthood are usually similar to those of the milder form of the disease when recognized earlier in life. Thus, congenital megacolon (Hirschsprung disease) can be subdivided into the classic form, short-segment types, ultrashort-segment types, and other variants (see Table 47.10).

Acute megacolon

Acute toxic megacolon occurs in patients with severe, fulminant inflammatory bowel disease or infectious colitis (see Chapters 83 and 88); the colon also dilates in response to acute distal obstruction (e.g., volvulus or carcinoma). However, pseudoobstruction and acute megacolon can occur without evidence of intrinsic colonic disease or mechanical obstruction. In this latter instance, the patient has a form of acute colonic pseudoobstruction, named after Ogilvie [429]. In fact, the condition he described in two patients was longstanding and caused by malignant infiltration of mesenteric nerves. Regardless of the misnomer, the syndrome, given Ogilvie's name, should be considered as a form of pseudoobstruction localized to the colon; the characteristic time course is acute or subacute. Moreover, most cases are clearly associated with an underlying disease; the most common predisposing conditions described in a metaanalysis of 400 cases were trauma, orthopedic surgery, obstetrical procedures, pelvic and abdominal surgery, metabolic imbalance, and neurological conditions [430].

Pathophysiology

The precise cause of acute colonic pseudoobstruction is unknown. However, Ogilvie syndrome is the clearest example of the human colon dilating in response to nonmechanical factors. Ogilvie proposed that an imbalance of intrinsic and autonomic neural control is the basis for the syndrome [429]. This propensity of the human colon to relax and dilate has been studied experimentally with the barostat [1]. In these experiments, wall tone decreased markedly during sleep and in response to glucagon; a model of colonic dilation during acute distention of the ileum has been described in dogs [431]. This concept, that the wall of the colon can relax readily in response to physiological and pharmacological stimuli, may be an important basis for megacolon.

Clinical presentation

The typical patient is a middle-aged or older person who is recovering uneventfully from surgery performed a few days previously and who is already on a general diet. In one report [432], more than half the patients had undergone recent surgery or manipulation of the spine or retroperitoneum. The abdomen becomes grossly distended and breathing becomes labored, but early in the course no peritoneal signs are present and the white blood cell count is normal. An abdominal film shows massive gaseous distention of the colon with air distributed throughout, including the rectum. Usually, the small bowel is not seen. The diameter of the cecum at this point is often 9–10 cm.

Initial management

Oral feedings should be stopped, parenteral fluids started, and a nasogastric tube passed; all nonessential drugs should be discontinued. A Hypaque (water-soluble contrast) enema is administered; in this way, mechanical obstruction can be excluded and pseudoobstruction confirmed. As a side benefit, hyperosmolar Hypaque usually evacuates the colon during the diagnostic maneuvers. Once confirmed, acute colonic pseudoobstruction should be treated aggressively, with a rectal decompression tube and enemas. Any associated metabolic abnormalities or electrolyte disorders (e.g., hypokalemia) must be corrected; these are prominent in 20% to 30% of cases [432]. Efficacy has been reported for pharmacological blockade of ganglia with guanethidine followed by cholinergic stimulation with neostigmine [433], although it must be emphasized that in many cases the condition resolves with simple measures [434]. Intravenous neostigmine (up to 2 mg) was effective in acute colonic pseudoobstruction in a randomized controlled trial [435].

Subsequent management

Most of the patients who do not respond to enemas, nasogastric decompression, and drug therapy continue to have a normal white blood cell count, and fever or peritoneal signs do not develop. If the cecum measures more than 11 cm in diameter, the next step is colonoscopic decompression. The previous enema often empties the colon, facilitating endoscopy. Gas and liquid stool are aspirated while small amounts of additional air (or carbon dioxide) are insufflated. The mucosal detail is often obscured by the contents, but an obstructing lesion can usually be seen. It is not necessary to reach the cecum to accomplish adequate decompression; positioning the colonoscope at the hepatic flexure with aspiration of the distal contents usually collapses the right colon. An abdominal film should then be obtained; the collapsed cecum can be documented and the patient kept on enemas until stool and flatus pass spontaneously. Although colonoscopic decompression may have to be repeated, more than 80% of patients respond to colonoscopic decompression and require no further management. Administration of polyethylene glycol, 29.5 g daily, following successful resolution of colonic pseudoobstruction with either neostigmine or colonic decompression, was reported to be effective in preventing recurrence [436].

An operation is advisable for patients with an exceedingly large cecum (> 14 cm) and for those whose condition remains intractable to medical and endoscopic management. The most useful and efficacious approach is tube cecostomy, which is possible by laparoscopic techniques. Moreover, if at any time a patient manifests fever, leukocytosis, or peritoneal signs, abdominal exploration is mandatory. In these situations, the right side of the colon is frequently nonviable or is already perforated. For perforation, right hemicolectomy with ileostomy and a mucous fistula is the operation of choice. In patients with nonviable bowel but without perforation, a right hemicolectomy and primary anastomosis can be performed with little risk for serious complications.

For patients with megacolon, a normal-sized rectum, and a normal mechanism of defecation as determined by tests of pelvic floor function, ileorectostomy is the operation of choice; these patients usually establish a relatively normal bowel habit. Patients with megarectum, a normal-sized colon, and a defecation abnormality are candidates for proctectomy with coloanal anastomosis. The enlarged rectum is resected in its entirety; therefore, fecaliths in the rectal remnant, so common in patients after Duhamel procedures, are avoided.

Syndromes partly attributed to disorders of colonic motility

Chronic diarrhea

Diarrhea is defined as defecation that is more frequent or the passage of stools that are less well formed than thought normal by the individual. In patients with IBS and diarrhea, transit through the proximal colon is accelerated, and the rates at which the ascending and transverse portions of the colon empty are related to fecal weight [437]. Stools that are less solid (fragmented, semisolid, or liquid) result from more rapid transit through the colon; however, the most characteristic bowel pattern in IBS is one of wide fluctuations of stool frequency and consistency. Presumably, colonic transit changes dramatically in IBS, from slow to fast (or vice versa), to produce these stool patterns.

The scintigraphic method has been used to demonstrate rapid small bowel and colonic transit in the diarrhea of carcinoid syndrome [438]. A five-fold increase in the emptying rate of the ascending and transverse parts of the colon was noted; this was associated with storage of a reduced volume in the ascending colon, as measured by the scintigraphic method. The rapid transit and reduced accommodation of the proximal colon were associated with increased postprandial tone as measured by the barostat. A serotoninergic mechanism has been implicated, and in carcinoid diarrhea, octreotide and alosetron ($5\text{-}HT_3$ antagonists) reversed the abnormalities [439].

Irritable bowel syndrome: motor dysfunction

Irritable bowel syndrome (IBS) is defined usually by the criteria of an international panel that evaluated the symptomatic features of this extremely common condition. The major features are altered stool frequency or consistency and abdominal pain that is directly related to a change in the pattern of bowel movements. Thus, it is not surprising that the colon has been incriminated as the origin of these symptoms.

Colonic transit was evaluated in patients with IBS and diarrhea [437,440], and rapid transit of solids through the

proximal colon was documented. It had earlier been reported that small bowel transit also was rapid in IBS with diarrhea [440]. The variable consistency of the stools that is so characteristic of IBS presumably is the result of slow transit of some of the fecal residue and rapid transit of other portions.

The constipation experienced by some patients with IBS appears to blend imperceptibly into the syndrome of idiopathic constipation. Not surprisingly, then, the transit of radiopaque markers is prolonged in constipated patients with IBS. In a group of nearly 300 persons referred to a tertiary center with a major complaint of constipation [441], the majority had only a modest slowing of colonic transit (it was sometimes normal) and were considered to be examples of IBS.

Inflammatory disease of the colon

The diarrhea of inflammatory colitis is primarily caused by the exudation of fluid and mucus, bleeding, and impaired absorption; however, disordered motility and transit should also be considered. Connell first proposed that the colon in advanced ulcerative colitis may behave as a semirigid tube, showing fewer phasic pressure waves than is normal [442]; moreover, it is well recognized clinically that haustral contractions are absent in the late stage of colitis. These radiological phenomena (haustra) are thought to be produced by repetitive, nonpropagated, phasic contractions. Reddy and colleagues used combined manometric and scintigraphic techniques to study transit in ulcerative colitis [443]. They confirmed the paucity of phasic pressure activity in the fasting state. Postprandially, however, low-amplitude pressure waves were more frequent in colitis, and when these were propagated, they always moved contents in an antegrade direction. Thus, movement of isotope from the splenic flexure to the sigmoid colon was usually rapid, perhaps reflecting the absence of retarding pressure waves [443].

Another important disorder of motility is seen in ulcerative proctitis, in which a heightened sensitivity of the rectum to distention has been reported [444,445]. Clinically, such patients are often sensitive to small amounts of feces or mucus in the rectum. Pressure waves of high amplitude in the rectum have been recorded in proctitis and implicated in the symptom of tenesmus.

Response of the colon to drugs

Laxatives

The most complete study of laxative agents and drugs that cause diarrhea as a major side effect was that of Karaus and Sarna [446]. They studied the effects of hypertonic glucose, castor oil, neostigmine, and guanethidine in dogs with strain gauges sewn onto the colon. In all instances, when the agents induced diarrhea, powerful contractions migrated around most or all of the colon and led to an evacuation. On the other hand, a local stimulus to the rectum (distention) could provoke expulsion of the balloon without participation of the whole colon in the motor event. The motor effects of the local instillation of stimulant laxatives have been recorded from the human colon [362], and the myoelectric responses to oral administration of senna have also been reported [447]. The effects of laxative doses of castor oil on the colon should be similar to those reported for oleic acid [363]; oleic acid and the active principle of castor oil (ricinoleic acid) are both C_{18} aliphatic fatty acids. Lactulose has also been used experimentally as a stimulant of colonic transit; when it was administered in doses that induced a modest increase in stool frequency, transit through the right side of the colon was hastened [448].

Antidiarrheal agents

The most effective antidiarrheal agents are those related to opioids. Opiates are said to augment the mixing contractions that inhibit transit and to increase colonic tone, both of which might be expected to favor absorption. These actions should retard transit and help alleviate diarrhea. The effects of morphine on colonic tone have also been explored in dogs and humans using the electromechanical barostat. Under baseline conditions in the dog, the drug increased tone sharply [365] in association with an increase in phasic contractions. In the human colon [449], the effects of morphine were more complex; it relaxed a segment showing high postprandial tone and caused a late (40 minutes after injection) decrease in spontaneous tone. Thus, the effects of opiates on the colon are complex, and perhaps species dependent.

Alosetron is a 5-HT_3 receptor antagonist that has been shown to decrease colonic transit, improve stool consistency, and decrease sensation and discomfort thresholds in the rectum in patients with IBS [450–452]. Alosetron has also been shown to be clinically effective in treating females with diarrhea-predominant IBS [453,454]. However, an increased incidence of drug-related ischemic colitis has resulted in restricted availability of the drug [455].

Colonic prokinetic agents

Tegaserod is a selective 5-HT_4 receptor partial agonist. It has been shown to enhance the peristaltic reflex and increase chloride secretion and water movement into the bowel lumen and accelerate colonic transit in health [456–461]. Multiple studies have demonstrated efficacy in patients with constipation-predominant IBS and idiopathic constipation [462–464]. Longer-term studies over 13 months have also confirmed efficacy in this setting [465]. Nevertheless, the availability of tegaserod has become restricted because of a possible increase in drug-related ischemic cardiovascular events [466].

Novel approaches to treating constipation with specific secretagogues have the potential advantage of specific targeting of the secreting epithelium in the small intestine and colon, and this, coupled with low bioavailability, enhances the safety of compounds such as lubiprostone, a chloride channel agonist [467], and linaclotide, a guanylate cyclase C agonist [468].

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C SMALL INTESTINE

48 Bacterial, viral, and toxic causes of diarrhea, gastroenteritis, and anorectal infections

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Gastrointestinal infections, frequently manifest as diarrhea or gastroenteritis, continue to plague humanity. The World Health Organization (WHO) estimates that diarrhea causes 27% of all childhood deaths beyond the neonatal period [1]. The Centers for Disease Control and Prevention (CDC) reports that there are 1.4 episodes of diarrhea per person per year in the United States [2]. Diarrhea impairs daily activities at least once annually in most citizens of the world. Because of their impact on morbidity and mortality worldwide, and especially in less advantaged countries, gastroenteritis and diarrhea, no matter how defined, are undeniably important disorders.

Diarrhea and gastroenteritis diagnosis and management pose considerable clinical and economic challenges. Decisions depend on local epidemiology, resources, microbiological expertise, and the likelihood that outcome will be affected by diagnosis or treatment. Moreover, there is the reality that diarrhea is usually self-limited, whether or not an etiological agent is identified and whether or not specific or non-specific therapies are used. Therefore, resources are often, and more appropriately, devoted to supportive care and prevention and not to determining specific etiologies in individual cases. Symptomatic relief is often used, and empiric antibiotics are often prescribed, but the value and risks of these interventions are subjects of debate.

In this chapter, we will provide an overview of diagnostic considerations for all patients with diarrhea in terms of microbiological assessments, specific and non-specific therapies, and general preventive measures. We will then discuss the history, epidemiology, pathogenesis, clinical aspects, complications, diagnosis, and treatment of a diversity of bacterial and viral gastrointestinal pathogens. Major gastrointestinal infections that are often not accompanied by diarrhea or vomiting, such as parasitic infestations of the gastrointestinal tract (Chapter 106), infectious esophagitis (Chapter 34), and *Helicobacter pylori* infections (Chapter 40), are covered in other chapters. Although many episodes of gastroenteritis go undiagnosed, and we are unable to address thoroughly all of the multidimensional aspects of the myriad agents that cause gastroenteritis and diarrhea, we believe that this orthogonal approach to gastrointestinal infections will be helpful in general and in specific situations.

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Clinical considerations at presentation

Microbiological diagnosis

Comprehensive microbiological diagnosis of enteric infections, usually by examination of stool from symptomatic patients, employs diverse, pathogen-specific tests, including culture, microscopy, antigen detection, nucleic acid hybridization, and serology. This technology can be an impediment to clinical care and epidemiological surveillance. These multiple modalities are expensive and, for many, interpretation of results is "operator dependent." Results are often returned to the provider too late to be useful or are not clinically actionable. Some tests are of questionable sensitivity, and, in the case of bacterial culture, utilize technology developed in the late 1800s. Despite these limitations, good gastrointestinal practice often critically relies on the accurate identification of pathogens or on the exclusion of known infectious agents. Moreover, public health authorities depend on accurate and rapid diagnosis of enteric pathogens to halt outbreaks and to monitor incidence trends. Admittedly, the microbiologically and physically complex matrix of stool poses difficult

challenges for pathogen detection. Nonetheless, it is important to recognize that major technological advancements will be required to better diagnose gastrointestinal infections, and that new technologies which identify multiple pathogens in the same testing format, accurately, economically, and rapidly, are urgently needed.

Diagnosis is often tailored to the presentation and symptoms, with local epidemiology, custom, and laboratory practices helping to guide the tests requested and performed. The test categories can be grouped into those that identify bacterial, parasitic (eukaryotic, largely protozoal), and viral diarrheagenic agents. Within each group, a diversity of methods, reagents, and detection systems can be applied, which further complicates decisions.

Bacterial pathogen testing consists of plating stool specimens on selective agar plates and inoculating stools into various broths. The media serve several purposes. They can be variably selective (diminishing or preventing growth of nontarget organisms) or differentiating (exploiting a phenotype of a target organism to identify it among co-isolated flora), or both. Most laboratories will seek, without special requests, *Salmonella* and *Shigella* using one or more of these media (e.g., MacConkey, Hektoen, *or Salmonella/Shigella* agar, often in combination with selenite-F broth). Laboratories also usually routinely seek *Campylobacter* using selective media and incubation at 42°C under microaerophilic conditions to suppress the growth of ambient flora, which do not grow as well as *Campylobacter* at this temperature. Special requests are often needed to detect *Yersinia* (using *Yersinia* agar), *Escherichia coli* O157:H7 (using sorbitol–MacConkey agar), and other Shiga toxin-producing *E. coli* (using a broth culture and a Shiga toxin enzyme immunoassay [EIA]) [3]. Laboratories will occasionally culture for vibrios using blood or thiosulfate–citrate–bile salts–sucrose (TCBS) agar, and *Staphylococcus aureus* using blood agar plates. Laboratories sometimes screen colonies on blood agar plates by sweeping the growth onto oxidase paper and determining if oxidase producers are vibrios, *Aeromonas*, or *Plesiomonas.* In reality, most oxidase-positive colonies are *Pseudomonas aeruginosa* and not enteric pathogens. It is prudent to confirm that the laboratory to which a specimen is sent performs an appropriate array of cultures, and knowledge of local epidemiology is essential. Unfortunately, many physicians do not really know their laboratory's practices [4].

Viral studies rarely help manage individual cases of gastroenteritis. Specific therapies do not exist, and finding a viral pathogen does not alter one's approach to a patient, which is (rightly) focused on addressing hydration status. Viral enteric diagnosis, at least in North America, consists of antigen detection for rotavirus and adenovirus types 40 and 41. Tests for noroviruses (caliciviruses) and astroviruses are less often applied or are unavailable except in research settings. Viral culture of the stool does not help in the diagnosis of gastroenteritis or diarrhea.

Parasite evaluations consist of microscopy of wet mount specimens, and special additional stained slides of fresh or preserved specimens, to detect diverse definite (*Cryptosporidium parvum*, *Entamoeba histolytica*, *Giardia lamblia*, *Hymenolepsis nana*, *Isospora*, and microsporidia) and candidate (*Blastocystis hominis* and *Dientamoeba fragilis*) diarrheagenic protozoa. Antigen detection will identify *C. parvum* and *G. lamblia*, but not other parasites, and microscopy is necessary for other agents. There is considerable debate about the utility of the classically applied three ova and parasite examinations. Some authorities promote one microscopic examination [5–9], whereas others advocate the long-standing practice [10] of triplicate submissions [11–14]. Pooling of specimens from the same patient prior to examination has been proposed as a cost-saving measure and does not diminish recovery rates [15,16]. Antigen testing has been widely utilized but there are concerns about its specificity [17]. In cases in which it is important to diagnose an enteric parasite, stools should not be considered to be negative until they have been subjected to a thorough microscopic evaluation at an experienced laboratory.

Several screening tests have been employed in an attempt to improve the detection rate of bacterial pathogens in stool cultures by restricting processing to those specimens with reasonable likelihoods of containing a pathogen. These include testing for fecal occult blood, leukocytes, lactoferrin, and calprotectin [18]. Although positive tests might increase the yield of a stool culture, negative tests are not adequately predictive to be used as exclusion criteria [19]. If there is clinical suspicion of a possible bacterial enteric infection, a culture should be obtained, even if a surrogate test is negative.

We also wish to stress that clinical microbiologists are underutilized resources for many gastroenterologists. These individuals are considerably more aware of the latest diagnostic technology and protocols in enteric microbiology than are practicing gastroenterologists. We often find it helpful to confer with clinical microbiology staff on difficult cases to increase the likelihood that specimens will be handled appropriately and to ensure that the best evaluations are performed. We also encourage regular communication to ensure that specimens which are obtained by clinicians are put into the appropriate transport media and that appropriate testing is requested.

Finally, in many jurisdictions, it is the responsibility of the requesting provider to notify the local health department of reportable enteric infections. The diagnosing microbiology laboratory can usually provide contact information and a list of reportable pathogens. Even though regulations require reporting within 7 days of notification, it is important to make the report immediately, to accelerate disease control measures. A 2006 nationwide outbreak of *E. coli* O157:H7 transmitted by contaminated fresh spinach exemplifies the importance of rapid reporting and transfer of pathogens to disease control authorities as soon as possible, so that matching isolates can be further investigated [20].

Adjunct evaluations

Patients with severe gastroenteritis and diarrhea sometimes present with such profound and confusing symptoms, or with symptoms of sufficient duration, that adjunctive tests and evaluations are considered and requested. It is important to be strategic in the selection of these evaluations, because the likelihood of obtaining misleading information sometimes exceeds the likelihood of obtaining helpful results. Unfortunately, we are not aware of any rigorous analysis of the utility of such determinations. However, we wish to make several observations on commonly obtained tests, based on our experience caring for such patients.

Blood count

Although a blood count is rarely helpful in nonbloody diarrhea, several situations do arise in which this test is valuable. First, in patients presenting with acute bloody diarrhea, we find it useful to obtain a complete blood count so that we may more accurately assess trends in the platelet count if the patient is subsequently demonstrated to be infected with an enterohemorrhagic *E. coli* (EHEC) (see Enterohemorrhagic *E. coli*, Clinical aspects). Abnormally high hemoglobin concentrations could indicate dehydration, and profound left shifts without marked leukocytosis (relative bandemia) could represent *Shigella* or *Salmonella* infection.

Blood culture

We encourage blood cultures in patients with acute gastrointestinal infections that could be of bacterial origin if they are at the extremes of age, are immunocompromised, or if they might be infected with *Salmonella* Typhi. The value of blood cultures in other situations is not well established and is probably considerably less.

Urinalysis

In febrile children with diarrhea, and especially infants, it is difficult to know if a urinary tract infection is also present. However, the decision to obtain a urinalysis must be balanced by the realization that there is a high likelihood of receiving inaccurate data because the risk of fecal contamination is considerable and that the resulting administration of antibiotics might be contraindicated. We also strongly discourage obtaining catheterized specimens during acute gastroenteritis because of the risk of introducing fecal bacteria into the urinary system. There is no role for a urinary assessment if there is no fever or if the illness most closely resembles bacterial infection (sudden onset, bloody diarrhea).

Imaging studies

Abdominal ultrasonography might help clarify distal small bowel involvement during bacterial infections by demonstrating evidence of bacterial ileocecitis, especially if a patient has right lower quadrant tenderness [21]. Air- or barium-contrast enemas, or computed tomography, are occasionally warranted during acute bloody diarrhea to exclude the possibility of a surgically remediable lesion, in particular an intussusception. Contrast studies demonstrating colonic edema (e.g., "thumbprinting" of the mucosa) should raise the possibility of an EHEC infection. Computed tomography in infectious colitis generally demonstrates a diffusely inflamed colon [22]. Dilated colons can be seen on plain abdominal radiographs in a variety of infections and are non-specific. Nonetheless, imaging studies can help exclude the possibility of an obstructing intussusception.

Chemistry studies

Dehydrated patients should undergo evaluations to identify electrolyte abnormalities. We also recommend daily electrolyte, urea, and creatinine determinations in patients who are possibly or definitely infected with an EHEC (see specific recommendations below under Enterohemorrhagic *E. coli*, Treatment).

Surgical consultation

Many patients with severe gastroenteritis will have signs or symptoms suggesting an acute abdomen, most particularly acute appendicitis. Indeed, diarrhea is present in bona fide non-infectious appendicitis [23], and bacterial gastroenteritis can be associated with appendicitis [24,25] and colonic perforation [26,27]. Experienced surgical consultation should be pursued if the clinical situation warrants, even if there is a high likelihood of a patient being infected with an infectious agent.

Specific therapy

Specific therapy for enteric infections, defined as administration of a medication to eradicate the infecting agent, is best guided by microbiological results. We do not believe that it is appropriate to administer antibiotics empirically at the time of presentation because of the potential for side effects of the antibiotics, their expense, the possibility that such agents could be contraindicated, and the risk that antibiotics will select for antimicrobial resistance. We do acknowledge that the practice of treating patients empirically is not uncommon, and that several studies have demonstrated that the administration of antibiotics at the time of presentation with diarrhea to adults does shorten the duration of illness, whether or not a specific bacterial pathogen is identified [28–32]. However, the attributed benefit was only one or two fewer days of diarrhea during the illness in these studies, and not avoidance of serious complications. Additionally, in some circumstances, antibiotics are harmful, such as in *E. coli* O157:H7 infections, in which their use is associated with a higher frequency of development of the hemolytic uremic syndrome (HUS), and in uncomplicated *Salmonella* infections in otherwise healthy hosts, in which use of antimicrobials is associated with prolonged carriage [33].

Table 48.1 Composition of oral rehydration solutions

Component	Standard WHO (1975)	Reduced osmolarity WHO (2002)	Glucose plus glycine[a]
Glucose (mmol/L)	111	75	110
Sodium (mEq/L)	90	75	120
Potassium (mEq/L)	20	20	15
Chloride (mEq/L)	80	65	72
Base	10[b]	10[b]	48[c] (15[d])
Glycine (mmol/L)			110
Osmolarity (mOsm/L)	311	245	510

a See Nalin et al. [54] and Patra et al. [55]. See also Patra et al. [53].
b Citrate (mmol/L).
c The base was bicarbonate (mEq/L) in the original study [54].
d Potassium citrate (mmol/L).
WHO, World Health Organization.
From Nalin et al. [49]. Copyright © 2004, American Medical Association.

Rifaximin, a non-absorbable antibiotic derived from rifampin (rifampicin), has shown considerable promise in the treatment of traveler's diarrhea [34], but its appropriateness in other bacterial enteric infections is not yet established.

Symptomatic measures

The value and safety of therapies used to ameliorate the symptoms of acute gastrointestinal infections are subject to debate. Antimotility agents, such as loperamide, are controversial because studies, often small case series, have reported that these agents are associated with complications, such as toxic megacolon or ileus [35–37]. Other studies, however, attest to their safety and partial efficacy [38,39]. We are least comfortable administering antimotility agents to children, in whom safety has been less well established. In view of the fairly small benefits conferred by antimotility agents, and in consideration of their potential risks, especially in children, we urge caution in using them in cases of acute diarrhea, unless and until more substantial data emerge in support of their safety and efficacy. Oral ondansetron given in an emergency department to children with diarrhea diminished their time in the facility (though it did not affect need for hospitalization or need to return for evaluation) and is a potential adjunct to oral rehydration in situations in which vomiting might hinder this intervention [40]. However, it should be noted that ondansetron during gastroenteritis is associated with increased postadministration diarrhea [41].

Prevention

Prevention of diarrhea is an ideal goal. Vaccination has been successful only for typhoid fever, rotavirus, cholera (reviewed in [42]), and gastroenteritis necroticans [43]. Travelers should minimize risks whenever possible. Assiduous hygiene should be applied to acutely infected patients to diminish spread in the home or health-care setting. Food safety measures should be practiced by all individuals, especially those whose underlying illness or age render them more susceptible to the stress or sequelae of enteric infections. Successful prevention of morbidity and especially mortality of enteric infections is likely to follow improvements in economies and infrastructure, as demonstrated in Mexico during the 1990s [44–46].

Hydration

Maintenance of circulating volume is of paramount importance in all forms of diarrhea and gastroenteritis. Generally, oral rehydration is adequate to treat gastrointestinal fluid losses, but the ideal composition of such fluids remains subject to debate [47–49]. Parenteral fluid management is needed in children and adults who are severely dehydrated, who are unable to tolerate oral rehydration because of vomiting or abdominal pain, or who are medically fragile. Again, the ideal composition of intravenous volume expansion is also controversial, especially in children [50–52]. Reduced osmolarity solutions (Table 48.1) have been recommended by WHO in an attempt to diminish stool volumes and the need for intravenous volume replacement in patients with diarrhea. However, they carry the risk of inducing hyponatremia, especially in illnesses such as cholera in which fecal sodium loss exceeds the amount of sodium likely to be imbibed in these solutions [49].

Syndromic management, including diagnosis and therapy, of community-acquired diarrhea and gastroenteritis

Patients with diarrhea and gastroenteritis rarely present initially to consultant gastroenterologists. Therefore, experience accrued, and recommendations offered, by subspecialists is often removed from a point in these illnesses at which critical

diagnostic and therapeutic decisions are made. Nonetheless, we think it is helpful to review some considerations that we believe are pertinent to the initial presentation, in terms of syndromic profiling, because gastroenterologists are often asked to make recommendations to their colleagues in primary care about these situations.

In profiling these syndromes we recognize that purely upper or lower gastrointestinal presentations of enteric infections are rare; however, some categorizations might be instructive.

Illnesses in which vomiting predominates

Patients of all ages with illness characterized by vomiting and little or no diarrhea often are infected with a virus, in particular a norovirus, or have ingested a preformed toxin, such as staphylococcal enterotoxin. Diagnosis is often elusive, and attempts to find an etiological agent in individual clinical situations are often futile, but fortunately such illnesses resolve rapidly and spontaneously, although sometimes parenteral hydration is helpful. However, as with any form of acute vomiting illness, careful assessments should be made to confirm the absence of important noninfectious processes, such as pancreatitis. Cohorts with similar symptoms increase the likelihood that there is a point source (i.e., infectious or food-borne toxic cause) for the illness.

Nonbloody acute diarrhea

Nonbloody diarrhea often remains undiagnosed and the likelihood of recovering a pathogen from stool cultures is low in these situations, especially if there is no abdominal pain or tenderness, recent foreign travel, fever, or multiple stools in the 24 h preceding presentation [19,56]. These illnesses can usually be treated with oral rehydration and occasionally intravenous fluids, especially if the illness is accompanied by vomiting. Ensuring follow-up and that patients reseek medical care for nonimprovement or clinical deterioration are good medical practices in these situations, because assessments provide only a brief period of observation during an illness that will evolve over several more days. If microbiological diagnosis is considered, we encourage a stool culture to identify all actionable pathogens and seek, at a minimum, *Campylobacter*, *E. coli* O157:H7, *Salmonella*, and *Shigella*. Ideally, the evaluation should also include a toxin immunoassay to detect non-O157:H7 Shiga toxin-producing *E. coli* and culture for *Yersinia*. The value of testing for *Clostridium difficile* in these circumstances has not been established and is controversial [57,58], but in view of data that antibiotics have often not been recently taken by patients infected with *C. difficile*, such testing should not necessarily be limited to patients with antibiotic exposure.

Bloody acute diarrhea

Bloody acute diarrhea is a medical emergency because it is highly likely to represent an infectious colitis, is often associated with considerable patient discomfort, and is frequently amenable to intervention. Typically, in all forms of bacterial colitis, there are several days of nonbloody diarrhea before gross blood is observed, and pain is quite prominent. Fever is variable and, when it is absent on medical evaluation, suggests the possibility of an *E. coli* O157:H7 infection. Cultures should be obtained for a diverse panel of bacterial enteric pathogens (*Campylobacter*, *E. coli* O157:H7 and non-O157:H7 Shiga toxin-producing *E. coli*, *Salmonella*, and *Shigella*). Ideally, *Yersinia* should also be sought. Shiga toxin immunoassays should be requested in addition to, but not in lieu of, cultures for *E. coli* O157:H7. We also find it helpful to obtain baseline blood counts. Tests for *C. difficile* should also be requested, although, in our experience, community-acquired colitis caused by *C. difficile* usually has a period of onset that is more gradual than is seen in other causes of community-acquired bloody diarrhea. There is no role for viral testing in this setting. Similarly, in most of the developed world, parasites are very unlikely to cause acute bloody diarrhea. If parasites are sought we discourage reliance on antigen tests, because these investigations seek protozoa (*G. lamblia* and *C. parvum*) that do not cause bloody diarrhea; microscopy is preferred.

We discourage using empiric antibiotics unless and until it is known that such patients are not infected with an organism for which antibiotics are contraindicated. We strongly encourage admission to hospital and intravenous volume expansion for children and many adults with acute bloody diarrhea.

Bloody diarrhea can also be caused by non-infectious illnesses. If the age and setting are appropriate, one should consider a diagnosis of intussusception, ischemic colitis, and inflammatory bowel disease (IBD). However, sometimes these conditions can be complicated by bona fide enteric infections.

Chronic or persistent nonbloody diarrhea

Chronic or persistent diarrhea (arbitrarily defined as diarrhea of ≥ 2 weeks duration) is not very likely to be attributed to an easily diagnosed bacterial enteric pathogen and so the value of stool cultures in this setting is minimal. We encourage focusing the assessment of such patients on testing for *C. difficile* and parasites. The ideal parasite assessment combines antigen testing for *G. lamblia* and *C. parvum*, with microscopy. If testing is negative, and the patient's symptoms suggest the possibility of infection with a diarrheagenic parasite, we do think that it is appropriate to provide empiric antiparasitic therapy, usually metronidazole.

Chronic or persistent bloody diarrhea

In most of the developed world, chronic or persistent bloody diarrhea (arbitrarily defined as bloody diarrhea of ≥ 2 weeks duration) is likely to represent IBD. However, an infectious etiology should at least be considered, and workup, if it is pursued, should consist of a stool culture seeking *Campylobacter*, *E. coli* O157:H7, *Salmonella*, and *Shigella*; antigen or cytotoxicity assay for *C. difficile*; and microscopic evaluation of the stool

Table 48.2 Differentiating characteristics of acute self-limited colitis and inflammatory bowel disease (IBD)

Feature	Infectious colitis	Acute onset IBD
Distribution of lesions	Focal and segmental	Diffuse and general
Crypt architecture	Preserved	Distorted, atrophic
Mucus cell depletion	Absent or mild	Pronounced
Inflammatory cells	Mainly polymorphs in focal collections; crypt abscess formation	Chronic inflammatory cells (especially plasma cells)
Distribution of cells	Mainly mucosal	Submucosa may be involved
Epithelioid granulomas		Often present
Basal lymphoid aggregates		Present
Isolated giant cells	Absent	Basally located
Relapse	Very unusual	Present
Onset	Acute	Usually subacute, with prodromal symptoms, including extraintestinal symptoms, lasting at least several weeks
Cultures	Usually positive	Usually negative
Microcytic anemia	Usually absent	Frequently present

A panel of histological and clinical features can help differentiate acute self-limited colitis (which is presumably infectious) from acute IBD [59–63]. It is important to note that the gross appearance of the colonic mucosa cannot differentiate infections from IBD, and in a subset of cases there is a need to observe the course over time, with repeat biopsies [64].

for parasites, especially *E. histolytica*. However, these agents are usually not found, especially if patients have microcytosis, anemia, hypoalbuminemia, or elevated erythrocyte sedimentation rates or C-reactive protein [59–62], and plans should be made to make an expeditious diagnosis of IBD by colonoscopy.

Table 48.2 demonstrates criteria that help distinguish IBD from acute, self-limited, plausibly infectious colitis. In reviewing these differentiating characteristics, it is noteworthy (and troubling) that documented bacterial enteric infections can be a prelude to subsequent hospitalizations for IBD [27].

Returned traveler, or newly arrived immigrant, with acute or chronic nonbloody diarrhea

In this scenario we recommend a broad assessment, including bacterial cultures to rule out *Campylobacter*, *E. coli* O157:H7, *Salmonella*, and *Shigella*, and a thorough examination of the stool for diarrheagenic parasites. The value of empiric treatment of such cases is not known, but often performed. In view of the fact that enterotoxigenic *E. coli*, a leading and treatable cause of traveler's diarrhea, will not be detected with the available diagnostic testing, empiric antibiotic treatment is probably appropriate.

Anorectal symptoms

Patients with symptoms confined largely to the distal gastrointestinal tract (tenesmus, blood or pus in stools, pruritus ani), and who have recently had anal receptive intercourse, could have a sexually transmitted infection. Evaluation should consist of rectal swabs to seek a broad range of plausible pathogens (*Chlamydia*, *Neisseria gonorrhoeae*, and herpes simplex virus). A serological test for syphilis should also be performed. Sigmoidoscopy should be performed before starting treatment, and, ideally, treatment should be guided by culture, microscopy, or serology.

Persistent diarrhea in resource-poor settings

Many residents of resource-poor, largely tropical regions, and especially children in these populations, probably have abnormal small bowel structure and function. Changes include crypt hyperplasia, villous stunting, hypercellular lamina propria, decreased mucosal surface, and increased gut permeability. The net result of this syndrome in residents of these areas is stunting. This disorder is termed tropical enteropathy [65], tropical malabsorption [66], or tropical sprue [67]. Definite causative agents include the parasites *G. lamblia*, *C. parvum*, *Isospora belli*, *Cyclospora cayetanensis*, and the microsporidia; a strong bacterial candidate for persistent diarrhea in children in the tropics is enteroaggregative *E. coli* [68]. This condition is sometimes seen in travelers returning from the tropics. The optimal way to diagnose persistent diarrhea has not yet been found [69], although we recommend, subject to resource availability, a thorough microscopic examination of the stool, and consideration of empiric treatment with antiparasitics and antibiotics, and endoscopy. Even though it is not likely to contribute to mortality, persistent diarrhea in resource-poor countries is not a benign disorder. It is associated with, and probably a cause of, stunting, susceptibility to other diseases, and cognitive impairment [70–73]. The detection and eradication of this entity should be a priority for future research and development [74].

Diarrhea in the immunocompromised

Immunocompromised transplant recipients can be infected with a diversity of diarrheagenic agents, and so a broad approach to evaluation (including stool cultures, viral studies, and, if the patient has had environmental exposures, parasitic evaluation) is warranted. However, a limited repertoire of causative agents (*C. difficile* and cytomegalovirus) has been identified in patients who have been studied systematically and thoroughly [75,76]. It should be emphasized that it is not possible to differentiate infectious diarrhea from graft-versus-host disease in such patients, and histopathological evaluation of the intestinal mucosa is often quite helpful.

Traveler's diarrhea

Traveler's diarrhea is often brought to the attention of gastroenterologists. It has been estimated that 30%–40% of the 50 million people who travel to developing countries every year from the developed parts of the world will experience classic symptoms of traveler's diarrhea. Traveler's diarrhea has been defined as three or more loose bowel movements within a 24-h interval, accompanied by cramps, nausea, fever, blood in the stool, or vomiting, or a combination of these symptoms [77]. Interestingly, antibiotics were demonstrated to prevent traveler's diarrhea well before the recognition that bacteria were major causes of this disorder [78]. The risk of acquiring traveler's diarrhea varies from country to country (see Fig. 48.1).

A subset of patients with traveler's diarrhea will experience dysenteric symptoms, defined as diarrhea with fever, with or without visible blood in the stool, and these rates, too, vary from country to country [77]. Acute traveler's diarrhea usually occurs during the first week overseas, the average duration of untreated illnesses in travelers is approximately 4 days, and many patients have symptoms that resolve considerably sooner [79,80]. Approximately one-fifth of all patients with traveler's diarrhea experience incapacitation such that they are unable to take part in their planned activities. There is a possibility that traveler's diarrhea precipitates persisting symptoms, including chronic diarrhea and irritable bowel syndrome [81–83], although some investigators have failed to associate traveler's diarrhea with irritable bowel symptoms [84]. Also, there have been concerns that IBD could be precipitated by a recent visit to the tropics [85,86].

In view of the morbidity of traveler's diarrhea, considerable effort has been devoted to its prevention. Certainly, it is important to promote good hygiene, although data supporting

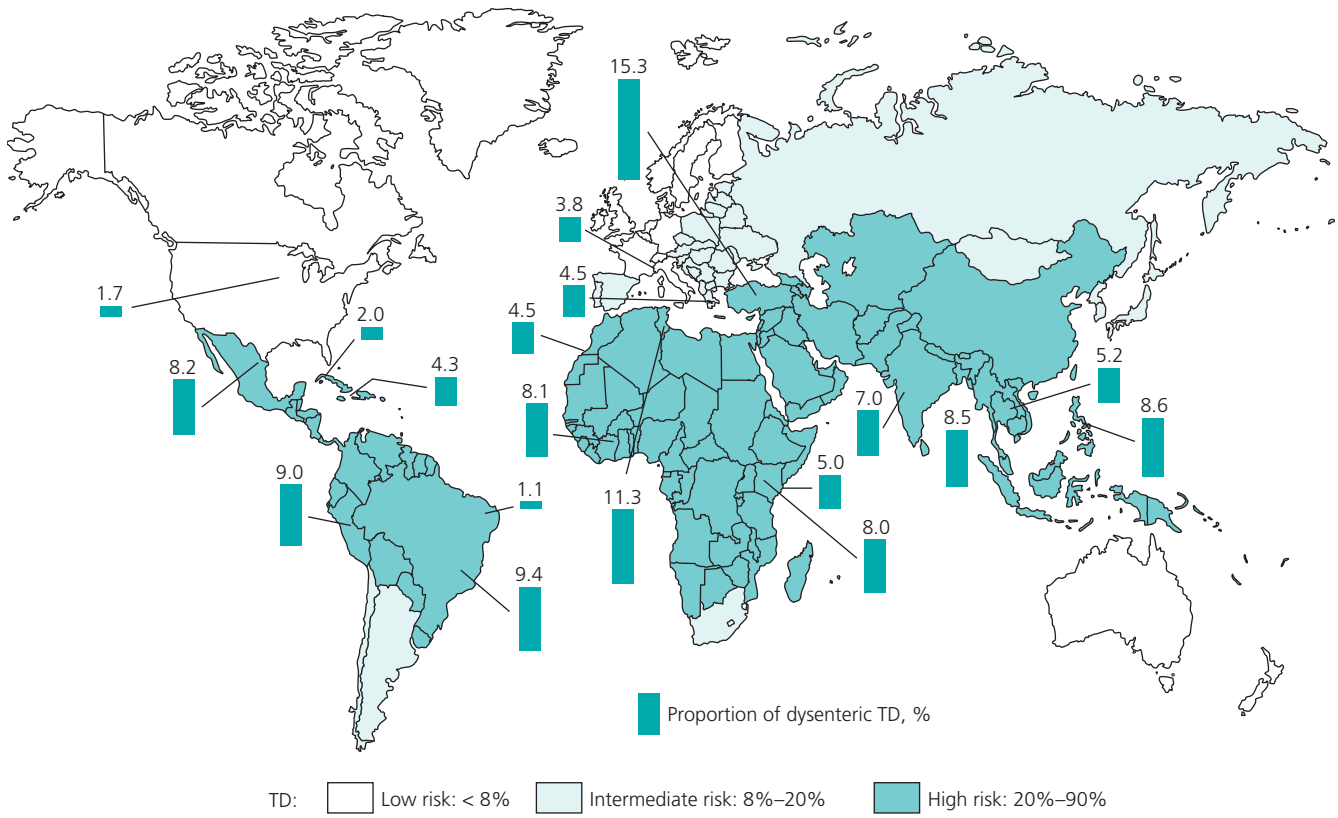


Figure 48.1 Country-specific risk of acquiring traveler's diarrhea. Percentages indicate the likelihood that travelers to specific countries will acquire diarrhea within 2 weeks of arrival. Percentages demonstrate frequency of blood in the stools and/or fever, representing "dysenteric" traveler's diarrhea. From Steffen [77], with permission from University of Chicago Press.

the efficacy of hygienic precautions are scarce [87,88]. Various pharmacological approaches have been tried including nonantimicrobial agents, most particularly bismuth subsalicylate, tannin albuminate plus ethacridine lactate, and *Lactobacillus* GG (reviewed in [89]). Agents demonstrated to be effective in treating traveler's diarrhea include antimotility/antisecretory agents such as loperamide, bismuth subsalicylate, the calmodulin inhibitor zaldaride maleate, tannin albuminate plus ethacridine lactate, and attapulgite. Racecadotril (an enkephalinase inhibitor), probiotics, tincture of opium, kaolin–pectin, and diphenoxylate ameliorate the symptoms of acute diarrhea, but have not been specifically or thoroughly evaluated in traveler's diarrhea (reviewed in [90]). A variety of antibacterial agents has been subject to placebo-controlled trials for the chemoprophylaxis of traveler's diarrhea, including doxycycline [91–93], trimethoprim–sulfamethoxazole [94,95], and the fluoroquinolones, in particular norfloxacin [96–98] and ciprofloxacin [99,100]. This topic has been extensively reviewed [90]. Interest has also been prompted by the efficacy of rifaximin, which is a poorly absorbed oral analogue of rifampin (rifampicin), in preventing traveler's diarrhea [34]. Rifaximin was comparable to ciprofloxacin in treating traveler's diarrhea caused by presumably non-invasive pathogens, but was less effective than ciprofloxacin for treating traveler's diarrhea caused by invasive pathogens [101]. Considerations regarding the use of rifaximin in the future will depend on its aftermarket side-effect profile (presumably minimal for this non-absorbed antibiotic, compared with the somewhat higher risk of fluoroquinolone-related side effects), the theoretic emergence of resistance, and cost. A National Institutes of Health (NIH) consensus conference recommended against using antibiotics to prevent traveler's diarrhea, in view of the potential side effects from these drugs, but did recommend that symptoms should be treated when they occur, quite often with antibiotics [102]. Certainly, antibiotics should be considered when traveler's diarrhea ensues, and, depending on the patient's age, medical conditions, and history of allergies, a fluoroquinolone or azithromycin [103] should be considered. An antimotility agent taken at the same time might provide additive benefit [104] but blanket use of antimotility agents is problematic, because such a recommendation would not be made for traveler's diarrhea caused by invasive bacteria, and such invasive infections often begin as nonbloody diarrhea. If a traveler develops bloody diarrhea, then antibiotics should certainly be taken.

In the returned traveler, the use of antibiotics in what appears to be persistent diarrhea is probably appropriate although not entirely risk free. Nonetheless, it is common practice. The diagnostic considerations in returned travelers also pose challenges because many of the diarrheagenic *E. coli* associated with traveler's diarrhea are not detected by routine stool cultures, and classic bacterial pathogens are found in only a small minority. Similarly, such patients could be infected with a parasite. In a returned traveler, we do think it is appropriate and advisable to obtain a thorough microbiological evaluation, including bacterial culture and microscopic evaluation of the stool and antigen studies for *Giardia* and *Cryptosporidium* before beginning therapy.

Genetic susceptibility or resistance to infections

Multiple studies have suggested that there can be a genetic predilection for acquiring certain gastrointestinal infections or for experiencing particular complications of these infections. Children with sickle cell anemia are at increased risk of extraintestinal *Salmonella* infections [105]. Patients with iron overload are prone to *Yersinia* infections [106]. Patients with HLA-B27 are at risk for postinfectious Reiter syndrome following a variety of enteric infections [107]. Patients with polymorphisms in the interleukin (IL)-8 promoter are at increased risk of acquiring clinically apparent traveler's diarrhea attributable to enteroaggregative *E. coli* [108]. Blood group O residents of a cholera-endemic area were less likely to become infected with *Vibrio cholerae*, but more likely to experience severe disease if infected [109]. Heterozygotes for the cystic fibrosis transmembrane conductance regulator (CFTR) have been postulated to be relatively resistant to cholera and enterotoxigenic *E. coli* [110,111]. Polymorphisms in the CFTR also influence susceptibility to typhoid fever [112]. Even though host genetics probably play important and underestimated roles in susceptibility to gastrointestinal infections and their sequelae, such associations are not absolute and should not, at present, be used to make diagnostic or therapeutic decisions about managing individual patients.

Specific agents

In this section, we will describe common and instructive bacterial and viral agents of gastroenteritis, listed alphabetically. We exercised some discretion regarding the breadth and depth of the discussion of each agent, because it is not possible to review every candidate etiological agent of gastroenteritis and diarrhea, and, as we note above, the etiologies of most cases encountered remain clinically undetermined. However, in deciding which pathogens to include, we believed that it would be instructive to discuss pathogens that are likely to be encountered worldwide (e.g., *Salmonella*) or that have well-defined pathogenetic mechanisms even though their epidemiology and the spectrum of illnesses that they cause are not yet well delineated (e.g., enteropathogenic *E. coli*), or agents whose role in human disease is newly emerging (e.g., *Bacteroides fragilis*). We also believed that it was noteworthy to review situations in which gastroenteritis precedes or accompanies multisystem, often overwhelming, illnesses (e.g., anthrax). Finally, we provide an overview of infections largely confined to the anorectum. We have attempted to reduce redundancy when multiple classes of

agents possess the same virulence mechanism by elaborating them only once, such as discussing Shiga toxins predominantly in the section on EHEC and providing less information in the section on *Shigella dysenteriae* serotype 1. Also, we wish to note that we do not usually provide specific dosages or durations of therapies because of the likelihood that there will be interedition changes in recommendations and because we cannot cover all dosing contingencies that pertain to patients of different ages and with different renal and hepatic function.

Bacterial pathogens

Bacteroides fragilis

History

Bacteroides fragilis has long been considered to be a member of the commensal anaerobic gastrointestinal flora. However, in the 1980s, some strains of *B. fragilis* were proposed to cause diarrhea by virtue of their ability to produce an enterotoxin in a ligated intestinal loop assay [113]. These enteroxigenic *B. fragilis* strains were isolated from infant lambs with acute diarrhea. The enterotoxin has been cloned, and several case–control studies have associated enterotoxigenic *B. fragilis* with diarrhea in children [114] and adults [115].

Pathogenesis

The only known virulence trait of diarrhea-associated *B. fragilis* is the ability to produce the *B. fragilis* enterotoxin (BFT). BFT is a 20-kDa metalloprotease, which stimulates secretion of IL-8 by epithelial cells [116]. BFT can also induce c-Myc expression and activate cellular proliferation via the mitogen-activated protein kinase p38 and β-catenin nuclear signaling [117], raising the possibility that this molecule can induce oncogenic transformation [118]. BFT also increases intestinal permeability [119].

Epidemiology and prevention

The extent of disease caused by enterotoxigenic *B. fragilis* is uncertain. Available data suggest a global distribution of enterotoxigenic *B. fragilis*, with most reports focusing on young children [120–125]. Because detection of enterotoxigenic *B. fragilis* is technically complex, extensive knowledge regarding epidemiology and prevention does not exist.

Clinical manifestations

Children and adults infected with enterotoxigenic *B. fragilis* have generally had nonbloody diarrhea; very few have fever [114,115].

Diagnosis

The ability to identify enterotoxigenic *B. fragilis* rests on the use of selective media and 48 hours of anaerobic culture [114], followed by genetic or biological assays to detect enterotoxin production. Detection of enterotoxigenic *B. fragilis* in stool is not performed outside of research studies.

Treatment

The optimal treatment for enterotoxigenic *B. fragilis* infection is not known. Patients are generally not diagnosed during their acute illness, and no therapeutic trials have been conducted.

Complications

Complications of acute enterotoxigenic *B. fragilis* gastrointestinal infections have not been described.

Campylobacter species

History

Although Theodor Escherich possibly described *Campylobacters* in the colons of infants dying of "cholera infantum" in 1886 [126], *Campylobacter* were not recognized as important human pathogens until the late 1970s [127]. What is retrospectively considered the initial *Campylobacter* outbreak occurred in Illinois in 1938, when 355 inmates of two adjacent state institutions suffered diarrhea associated with the ingestion of milk [128]. *Vibrio jejuni*, as it was then termed, was cultured from the blood of several of the patients, because methodology for recovering these organisms from stool had not yet been defined. It was not until 1968 that Dekeyser et al. [129] recovered *Campylobacter jejuni* from human feces and confirmed the intestine as the origin of the bacteremia demonstrated previously. Antibiotic susceptibility testing led to the recognition that erythromycin was effective for treatment [130]. In the 1980s, with routine microbiological culturing, *C. jejuni* came to be recognized as the leading cause of bacterial enterocolitis in humans. *Campylobacter*, and most particularly *C. jejuni*, remain the most common bacterial enteric pathogens in developed countries [131], including the United States [132].

Epidemiology and prevention

The CDC reports that *C. jejuni* caused nearly two million illnesses in the United States in 1999, comprising 14.2% of all foodborne infections [132–134]. *Campylobacter* infections are estimated to cost US$1.3–6.2 billion each year in the United States alone, in medical costs and hours lost from work [135,136]. Individuals of any age can be infected, but children under 4 years old are most susceptible, with a second peak incidence of infection occurring in those between 15 and 44 years old [128]. People with AIDS are at greater risk for infection and often exhibit more severe disease. The peak incidence of infection occurs in the summer.

Because *Campylobacter* do not multiply in contaminated foods or the open environment, large outbreaks are rare. Foods of animal origin are the most common sources of pathogenic *Campylobacters*, because *Campylobacter* spp. colonize the gastrointestinal tracts of many animals including chicken, sheep, swine, oxen [137], and cattle [138]. Various species of *Campylobacter* were found in 73% of retail meat samples in the United Kingdom [137]. The highest contamination rates (14%–98%) were in chicken [139,140]. Infections can also

be acquired from water, unpasteurized milk, or contact with infected humans or pets [131]. Undercooked poultry was responsible for 10%–50% of *Campylobacter* infections in case–control studies. Freezing diminishes *Campylobacter* viability. *Campylobacter* also accounts for 3%–50% of cases of travel-related diarrhea [128].

Of the 18 species and subspecies of *Campylobacter*, *C. jejuni*, *Campylobacter coli, Campylobacter lari, Campylobacter upsaliensis, Campylobacter hyointestinalis, Campylobacter concisus, Campylobacter mucosalis*, and *Campylobacter fetus* are confirmed or postulated pathogens of the human gastrointestinal tract. *C. jejuni*, *C. coli*, and *C. fetus* are most commonly associated with human disease, but newer diagnostic techniques have identified *C. hyointestinalis*, *C. concisus*, and *C. upsaliensis* at an increasing rate in human specimens [134,141]. In clinical laboratories, *Campylobacters* are rarely identified at the species level. The advisability and feasibility of identifying *Campylobacter* other than *C. jejuni* await further studies defining their roles in disease and their epidemiology.

The single most important source of *Campylobacter* is poultry. Proper food handling, preparation, and handwashing will reduce the incidence of these infections. The standard recommendations for avoiding traveler's diarrhea will reduce infection by this agent as well. Vaccine strategies have included the use of a killed whole-bacterial preparation combined with *E. coli* heat-labile toxin as an adjuvant [142], and a *C. jejuni* flagellar vaccine administered intranasally [143], but have not advanced to efficacy trials. Subjects have at least temporary immunity to disease (although not infection) following experimental challenge [144].

Pathogenesis

The pathogenesis of *Campylobacter* infections remains poorly defined. Four major virulence properties of *C. jejuni* and *C. coli* have been recognized: motility, adherence, invasion, and toxin production [145]. Motility enables *Campylobacter* to move to host cells so that they can adhere to and invade, and flagella are required for this property [145]. Adherence of *Campylobacter* to host intestinal epithelial cells permits colonization and enhances concentrations of secreted bacterial factors encountered by these cells. Adherence is presumed to be a prerequisite for invasion. The rates of invasiveness of *Campylobacter* spp. vary considerably [146], but might be as low as 1% of the initial inoculum [147]. Internalization into host cells requires the concerted effort of actin microfilaments and microtubules [148,149]. Once intracellular, *Campylobacters* reside in membrane-bound vacuoles where replication appears to be limited and survival is aided by the expression of catalase, which protects the organism from the oxidative stress of lysosomes [149,150].

Functional studies suggest that *C. jejuni* and *C. coli* express a cholera toxin (CT)-like enterotoxin [145], but no genes similar to CT have been identified in the *C. jejuni* genome [151]. *Campylobacter* spp. can produce several distinct cytotoxins [145], the best defined of which is the multisubunit cytolethal distending toxin (CDT). This toxin causes cell cycle arrest, leading to a characteristic elongation and swelling of susceptible cells. Although intoxicated cells remain viable for up to 72 h, they eventually die. The CdtB subunit of CDT has DNase activity [152,153]. However, the role of CDT in *Campylobacter* pathogenesis is unclear. Other organisms, including pathogenic *E. coli*, *Shigella* spp., and certain *Helicobacter* spp., also express CDT, but the contribution of CDT to fluid secretion is variable. For example, *E. coli* CDT does not stimulate intestinal secretion in rabbit ileal loops [154]. The role of the CDT family of toxins in enteric bacterial pathogenesis warrants further elucidation.

Clinical aspects

As few as 500 ingested *C. jejuni* can cause disease [155,156], suggesting that this organism possesses mechanisms of protection against gastric acid. The typical course of *Campylobacter* infection is depicted in Figure 48.2. The incubation period is from 18 h to 8 days (average 3.2 days) [157]. The onset

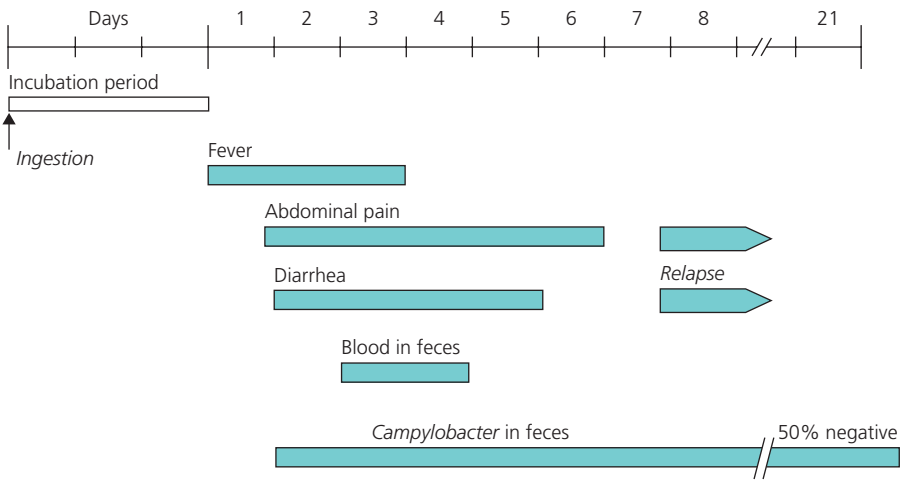


Figure 48.2 Typical course of human infection with *Campylobacter jejuni*. Redrawn by V.K. Viswanathan from Greenwood et al. [159].

of symptoms can be abrupt, with prodromal abdominal cramps, fever, headache, malaise, and myalgia, followed by diarrhea. Febrile rigors can occur. An influenza-like prodrome for 2–3 days before gastrointestinal symptoms caused by *Campylobacter* predicts more severe illness. *Campylobacter*-associated diarrhea (up to 10 stools per day) is watery initially and progresses to bloody diarrhea 1–2 days later in about 15% of patients [157]. This might indicate extension of infection to the colon. The colonoscopic appearance ranges from mild mucosal hyperemia to severe mucosal destruction with friable, edematous, and even ulcerated epithelia, but such findings are nonspecific. Histologically, there is acute inflammation, with predominant neutrophilic infiltration and crypt abscesses. The absence of distorted crypts and a normal mucus cell population can aid the differentiation of infection from IBD, but in chronic infections the distinction becomes less clear. Further blurring the boundary between *Campylobacter* infection and IBD is the potential for relapse of this infection, reported in 15%–25% of infected patients, and the possibility that IBD patients can be infected with *C. jejuni* [158].

The abdominal pain caused by *Campylobacter* can be so severe and localized that it mimics appendicitis. This is usually caused by ileocecitis and mesenteric adenitis, but *Campylobacter* has also been isolated from inflamed appendices [160]. Other symptoms include nausea and vomiting (15% of patients) and significant weight loss. Most patients begin to improve after 3–4 days, but they can excrete organisms for extended periods (mean 38 days, maximum 69 days) [161].

Circulating antibodies against *Campylobacter* antigens can be detected within 5 days of illness onset, peaking in 2–4 weeks [155]. Infection confers at least short-term protection against homologous strains, but the duration and breadth of protection are undefined. The incidence and severity of *Campylobacter* infections decrease in at-risk groups over time, suggesting some level of long-term protection in populations (or less adventurous eating habits).

Complications

Intestinal complications of *Campylobacter* infection include hemorrhage [162], ulceration of ileostomy stomas [163,164], pancolitis [165], and toxic megacolon. Extraintestinal complications include erythema nodosum [166], urticaria [167], bacteremia [168], cholecystitis [169], pancreatitis [170], abortion [171], nephritis [172], and cardiac complications [173]. More common complications include reactive arthritis, Reiter syndrome (for which HLA-B27 tissue antigen gives a strong predisposition), and Guillain–Barré syndrome (GBS) [174].

GBS is defined by flaccid paralysis, areflexia, and albuminocytological dissociation in the spinal fluid. At least four subtypes have been described [175]. It is caused by autoimmune-induced inflammation of peripheral nerves, which disrupts their function. GBS is the leading cause of acute paralysis, but has an annual incidence of only one to two cases per 100 000 population [175]. One-third of GBS patients have positive stool cultures and/or circulating antibodies to *C. jejuni* antigens [176]. Serum antibodies to *C. jejuni* lipopolysaccharide react with peripheral and central nervous system gangliosides [177]. Because antiglycoconjugate antibodies are present in GBS patients [174], molecular mimicry of neural oligosaccharides by microbial pathogens might underlie GBS pathogenesis.

Diagnosis

Campylobacters are long (0.5–5.0 μM), S-shaped, or spiraled gram-negative bacilli (rods). They have also been likened to "seagull wings." Immediate transfer into special transport medium such as Cary-Blair and inoculation onto plating medium enhances the isolation of this sometimes fastidious organism from stool. Although suboptimal, samples can be held at 4°C in transport medium for up to 24 h. The visualization of spiral organisms that rapidly dart and spin in diarrhea stools suggests *Campylobacter*, but a definitive diagnosis of *Campylobacter* infection can be made only using time-consuming bacteriology. We do not believe that a patient should be started on antibiotics in advance of positive culture results. Several rapid diagnostic tests have been developed, including latex agglutination tests to detect *Campylobacter* antigens in stool [128,178] and PCR-based tests, which provide same-day detection but which are expensive, do not allow antibiotic susceptibility testing [179], and are not widely available. Serological testing should be performed in patients with GBS, reactive arthritis, or erythema nodosum if stool cultures are negative for *C. jejuni* and such an antecedent infection is suspected [180].

Treatment

Specific therapy is not required for most patients because symptoms have usually resolved or are resolving by the time a microbiological diagnosis is made [181]. However, severely affected patients, i.e., those with dysenteric symptoms, relapsing symptoms, systemic infection, immunosuppression, or who are institutionalized, might benefit from antibiotic treatment. *C. jejuni* and *C. coli* are widely resistant to penicillins, cephalosporins, trimethoprim–sulfamethoxazole, rifampin and vancomycin [182]. Resistance rates of *Campylobacter* to fluoroquinolones are high (40%–88%) in many centers [183,184], and have been steadily increasing in the United States. Resistance is possibly caused by the administration of fluoroquinolones to animals, in particular commercial poultry [181]. Although these drugs are no longer administered to commercial flocks, resistant strains persist in the absence of selective pressure. In fact, coadministration of fluoroquinolone-resistant and -susceptible strains to the same chickens demonstrated a competitive advantage in the resistant ones, suggesting a survival advantage in the

resistant strains even in the absence of the antibiotic [185]. Erythromycin is the preferred drug for *Campylobacter* infection when antibiotics are indicated [186]. *Campylobacters* are also susceptible to the newer macrolides (azithromycin and clarithromycin), which might be better tolerated than erythromycin [181]. The resistance rates to erythromycin are up to 11% for *C. jejuni*, and up to 68% for *C. coli*. Erythromycin stearate, which is acid resistant, stable, and incompletely absorbed, offers a theoretical advantage over erythromycin ethylsuccinate (EES), but clinical evidence of superiority is lacking. EES is also the recommended treatment for children infected with *Campylobacter*. When resistance or other reasons preclude the use of erythromycin, tetracyclines and chloramphenicol are reasonable alternatives, but the prevalence of tetracycline resistance is around 60% and this drug should not be used in children in whom dental development is not yet complete. Azithromycin is an effective alternative in areas where fluoroquinolone resistance is high [187], and *C. jejuni* that are resistant to erythromycin are generally susceptible to azithromycin [188]. For systemic infections, an aminoglycoside or a carbapenem might be warranted; a simultaneous oral macrolide might aid eradication of the intestinal infection.

Clostridium infections

Clostridium difficile

History

Clostridium difficile gastrointestinal infections frequently come to the attention of consultant gastroenterologists. This organism was initially termed *Bacillus difficile* because of difficulties in culturing it; it was first described as part of the intestinal flora of newborns in 1935 [189]. Early in the antibiotic era, pseudomembranous colitis was attributed to *S. aureus*, until Bartlett et al. implicated *C. difficile* as a cause of this disorder in 1978 [190]. *C. difficile* is now the most common cause of nosocomial infectious diarrhea because of the widespread use of antibiotics and the high colonization rates in hospitalized patients. More recently, variant strains of *C. difficile* have emerged, including one that is toxin A negative/toxin B positive, at times presenting a detection challenge if cytotoxicity assays are the sole diagnosing modality [191], and one that produces the binary toxin CDT, has increased levels of toxins A and B, and is resistant to fluoroquinolones [192,193], making it potentially more virulent. Continuing studies will enable the delineation of the extent of the problems caused by this variant.

Epidemiology and prevention

Clostridium difficile is a gram-positive, anaerobic rod that is fastidious in its vegetative state but which sporulates in conditions that fail to support its growth. Sporulation allows *C. difficile* to persist for long periods in harsh environments, including dry surfaces and soil. This capacity for survival contributes to making *C. difficile* the leading infectious cause of health-care-associated diarrhea [194]. In addition, hospitals and long-term health-care facilities are often reservoirs of this pathogen, because establishment of infection usually depends on disruption of the indigenous intestinal microflora by antibiotics. Although *C. difficile* is recoverable from the stool of less than 3% of healthy individuals, it is found in approximately 20% of those hospitalized for longer than 1 week [194,195]. *C. difficile* can be cultured from the stool of up to 80% of healthy neonates and infants, but fails to produce symptoms, possibly from lack of expression of tissue receptors for toxins A and B by these children.

The incidence of *C. difficile* nosocomial infectious diarrhea in adults ranges from 20–60 cases per 100 000 patient days [195,196] and has significantly increased over the past few years [197]. There is evidence that the incidence of *C. difficile* infection in hospitalized patients has increased recently [197,198]. Interestingly, and of particular concern, the incidence of community-acquired *C. difficile*-associated diarrhea or disease (CDAD) has also increased [199]. In 1994, the number of cases of *C. difficile* infection was less than one per 100 000 persons; this had increased to 22 per 100 000 in 2004. Independent traditional risk factors for community-acquired CDAD include gastric acid suppression (proton pump inhibitors more than H_2 receptor antagonists), use of nonsteroidal antiinflammatory drugs, renal failure, IBD, malignancy, and infection with methicillin-resistant *S. aureus* [199]. In this study the role of antibiotics was questioned as only 37% of individuals had received antibiotics in the 90 days preceding the diagnosis of *C. difficile* infection. In addition, over the same interval, prescriptions for antibiotics actually decreased while those for proton pump inhibitors increased. Other studies have published similar data regarding the lack of association with antibiotic use [200], suggesting that, although certainly important, antibiotics are not a prerequisite for CDAD. The majority of studies carried out on CDAD are hospital-based, potentially exaggerating the role of antibiotics in CDAD because most patients have received such drugs. Additional investigation will be required to confirm the role of acid suppression and NSAIDs as risk factors for community-acquired CDAD.

Antibiotics predispose to CDAD by disrupting the normal colonic flora, allowing colonization after the patient is exposed to *C. difficile* or its spores, or expansion of resident *C. difficile* in response to selective pressures by antibiotics. Clindamycin is highly associated with CDAD [201] and its removal from hospital formularies has controlled outbreaks [202]. Some antibiotics, especially ampicillin and cephalosporins, often predispose to CDAD whereas others, such as parenteral aminoglycosides, vancomycin, and metronidazole, are less frequently implicated. For unclear reasons, third-generation cephalosporins are more commonly associated with *C. difficile* infection than are other broad-spectrum agents [203], and alterations in the use of these agents have decreased the occurrence of CDAD [204]. More recently, fluoroquinolones,

especially levofloxacin, have been associated with CDAD [205], which can be related to the emergence of a potentially more virulent strain [192,193].

Hospitalization is another risk factor for CDAD. There is a direct correlation between length of hospital stay and risk of becoming infected. Among patients hospitalized for greater than 4 weeks, 50% acquired *C. difficile* according to colonization analysis [206]. *C. difficile* has been isolated from numerous sites within hospital rooms, including telephones, call buttons, bed rails, toilets and bathroom floors, and from stethoscopes and the hands of health-care workers [195,207]. Patient-to-patient spread also occurs, particularly if a room is shared with an infected patient [207].

Although antibiotic administration and hospitalization set the stage for CDAD, the majority of patients exposed to these two risk factors do not manifest symptoms. Additional factors might be needed for clinical disease, such as alterations in host immunity, as suggested by the observations that patients with CDAD have lower levels of antibodies against *C. difficile* and its toxins than patients who are colonized with *C. difficile* [208]. Other possibilities include nonimmune host susceptibility and strain-specific virulence.

Outbreaks of CDAD within hospitals are often caused by a single or a related group of strains. Investigations of strains responsible for CDAD outbreaks in both the United States and Canada led to the identification of a toxin gene-variant strain of *C. difficile* [192,193]. Specifically, analysis of this strain demonstrated the presence of the gene encoding the binary toxin CDT, which is present in only 6% of *C. difficile* clinical isolates. In addition, this strain also contained a deletion in the *tcdC* gene, which is believed to negatively regulate toxin A and B expression; hence, deletion of this gene is associated with increased production of both of these toxins. Together, these genetic changes result in a more virulent strain of *C. difficile*. Hospitals worldwide have reported an increase in both the frequency and the severity of *C. difficile* infections [209–212]. Of additional concern is the resistance of this strain to fluoroquinolones; the increased use of these agents is likely responsible for the emergence of this strain.

Guidelines for preventing and controlling *C. difficile* infection have been published by the American College of Gastroenterology [213] and the Society for Healthcare Epidemiology of America [214] (Table 48.3). These guidelines have been consolidated and summarized [215]. Because the most likely method of transfer of *C. difficile* to patients is via the hands of health-care workers, good handwashing is the most important step in decreasing infection. Wearing gloves when handling *C. difficile*-infected patients or working in their environment might further reduce transmission; gowns should be worn if contamination of clothing is likely. The use of chlorine-containing disinfectants (1:100 dilutions or unbuffered hypochlorite or phosphate-buffered hypochlorite [1600 ppm]) significantly decreases environmental contamination [214]. Disinfection of thermometers and stethoscopes is also important.

Pathogenesis

The pathogenesis of *C. difficile* colitis is largely related to the production and secretion of two potent toxins, A and B [217,218]. These are high-molecular weight proteins (308 and 270 kDa respectively) that enter intestinal epithelial cells via specific, but as yet unidentified, receptors expressed on the apical surface. Once inside the host cell, these distinct but homologous (49% amino acid identity) toxins exert similar

Table 48.3 Practice guidelines for the prevention and control of *Clostridium difficile* infection

American College of Gastroenterology recommendations[a]	Society for Healthcare Epidemiology of America recommendations[b]
1. Limit the use of antimicrobial drugs	1. Antimicrobial use restriction is indicated if a specific antimicrobial, particularly clindamycin, is identified as a risk for *C. difficile*-associated diarrhea (CDAD)
2. Wash hands between contact with all patients	2. Handwashing with either an antimicrobial agent or soap is recommended after contact with patients, their body substances, or environmental surfaces
3. Use enteric (stool) isolation precautions with *C. difficile* diarrhea	3. Isolation of patients with CDAD in private rooms is recommended if private rooms are available; priority should be given to patients unable to maintain bowel continence and good hand-washing hygiene
4. Wear gloves when in contact with patients who have *C. difficile* diarrhea/colitis or with their environment	4. Glove use by personnel for the handling of body substances of all patients is recommended to reduce the rate of CDAD
5. Disinfect objects contaminated with *C. difficile* with sodium hypochlorite, alkaline glutaraldehyde, or ethylene oxide	5. Replacement of electronic thermometers with disposable thermometers is recommended if CDAD rates are high
6. Educate the medical, nursing, and other appropriate staff members about the disease and its epidemiology	

a From Fekety [213].
b From Gerding et al. [216].

biochemical effects by inactivating Rho proteins (a family of small GTP-binding proteins) that regulate actin polymerization. Rho inactivation is accomplished by toxin-induced glucosylation of a specific threonine residue in the Rho proteins, for example Rho_{ABC}, Rac, and Cdc42 [219,220]. This modification depolymerizes actin filaments, which leads to collapse of the cytoskeleton and, subsequently, the characteristic cell rounding that is the basis of the diagnostic tissue culture assay for *C. difficile* toxin. The physiological effects on the intestinal epithelial cell are to increase tight junction permeability [221,222] and fluid secretion [223,224]. In addition to their direct effects on intestinal epithelial cells, toxins A and B elicit profound inflammation, contributing further to disruption of the tight junction barrier and increased lumenal fluid. Moreover, colonic damage is caused by both proteins [224]. The binary toxin CDT is similar to the iota toxin produced by *Clostridium perfringens*. The role of CDT in *C. difficile* pathogenesis is unclear because toxin A- and B-negative strains colonize hamsters but do not induce diarrhea, but instillation of purified CDT into closed intestinal loops of animals does induce fluid secretion [225].

The marked inflammation that accompanies *C. difficile* infection is a multifactorial response. One early event is activation of the inflammation-associated transcription factor NF-κB. Inhibitors of cytokine synthesis attenuate toxin A effects [226]. Toxin A also causes monocytes to release IL-1β and IL-6 [226]. These effects recruit neutrophils to the intestine [227,228]. Induction of neutropenia significantly reduced fluid secretion [229], and inhibition of neutrophil infiltration of the intestine by specific antibodies [227,230], and inhibition or depletion of mast cells, attenuated neutrophil activation, fluid secretion, and host injury in experimental models of infection [231,232]. These effects can follow initial disruption of the intestinal epithelial barrier, allowing direct interactions between toxin A and inflammatory cells residing in the lamina propia.

Enteric nerves and neuropeptides are also involved in CDAD. Substance P (SP) expression by nerve cells in spinal dorsal root ganglia is immediately up-regulated following exposure of the intestine to toxin A. SP binds to neurokinin-1 (NK-1) receptors that are expressed by macrophages, releasing tumor necrosis factor (TNF)-α, a potent cytokine [233]. NK-1-deficient mice show diminished responses to intestinal toxin A [230,234]. Neurotensin (NT) is also involved in *C. difficile* colitis [235]. Both NT and SP receptors, whose expression is NF-κB dependent, are markedly up-regulated within 15–30 min of exposure to toxin A. An NT receptor antagonist reduces the intestinal effects of toxin A, including mucosal mast cell activation [235].

Clinical features

The clinical manifestations of *C. difficile* infection are broad, encompassing a carrier state devoid of symptoms, colitis without or with pseudomembranes, and fulminant colitis with megacolon or perforation. However, it should be noted that antibiotics commonly cause diarrhea independently of *C. difficile* infection [236]. The mechanisms underlying the broad range of symptoms associated with *C. difficile* are unclear but are likely attributable to host rather than bacterial factors; they include toxin receptor density, status of the resident intestinal flora, and concentration of antibodies against bacterial/toxin antigens. Hospitalized patients receiving antibiotics but who are not colonized with *C. difficile* have no immune protection against *C. difficile* colonization [208]. Following colonization, high levels of circulating immunoglobulin (Ig)G antibody against toxin A were strongly correlated with asymptomatic carriage of *C. difficile*, whereas low serum levels of toxin A IgG antibody were correlated with a much greater risk of *C. difficile* diarrhea. There was an eightfold increase in the risk of *C. difficile* diarrhea in patients with severe underlying illnesses [237]. These data support the development of passive or active immunization to control nosocomial *C. difficile* diarrhea.

Symptoms of *C. difficile* infection typically begin 4–9 days after initiation of antibiotic therapy, but diarrhea can develop even after antibiotic treatment has been halted in up to 30% of patients. This delay complicates the diagnosis of CDAD unless the clinician is aware of this previous risk factor.

C. difficile can affect the small bowel. Additional noncolonic manifestations of *C. difficile* infection include metastatic infection and postinfectious arthritis [238].

Asymptomatic carriage of C. difficile

Although hospitalization is a risk for infection with *C. difficile*, most colonized patients are free of symptoms [195,207]. This subset of *C. difficile*-infected individuals serves as a reservoir of this organism, which can contaminate the hospital environment. Asymptomatic carriers of *C. difficile* should not, however, be treated with antibiotics in an attempt to eradicate this organism.

Nonpseudomembranous C. difficile *colitis*

About half of those with diarrhea who are confirmed as positive for the *C. difficile* organism or toxin have clinical symptoms that are similar to but less severe than those associated with pseudomembranous colitis. Typical manifestations include abdominal pain, cramps, watery diarrhea, malaise, nausea, and possibly dehydration and fever. Laboratory tests commonly demonstrate a neutrophilic leukocytosis. In fact, the sudden onset of leukocytosis in hospitalized patients who have recently received, or who are receiving, antibiotics should immediately trigger testing for *C. difficile* infection [239]. Sigmoidoscopy can show only friable and erythematous mucosa but no pseudomembranes.

Pseudomembranous colitis

Pseudomembranous colitis is the prototypic entity associated with *C. difficile* infection but occurs in less than 25% of

diagnosed cases [215]. Symptoms, including diarrhea and abdominal pain, usually begin within a week of antibiotic administration, but might not appear until even 6 weeks after antibiotics have been discontinued [228]. Fever, chills, nausea, vomiting, dehydration, and tenesmus can accompany the diarrhea and abdominal cramping. Physical findings are nonspecific and include diffuse abdominal tenderness and distention. Occult blood can be present in the stool but hematochezia is rare. Fecal leukocytes are detected in about half of patients but peripheral leukocytosis is common and can serve as a surrogate marker of this infection [239]. Three patterns of leukocytosis have been reported: a sudden increase in the white blood cell (WBC) count correlating with symptoms of *C. difficile* colitis; leukocytosis preceding *C. difficile*-associated symptoms; or exacerbation of preexisting leukocytosis plus nonspecific symptoms of *C. difficile* disease [239]. In the last two scenarios, the diagnosis of *C. difficile* colitis is generally delayed. Consideration of *C. difficile* infection as a cause of unexplained leukocytosis is therefore recommended.

Although rarely necessary, the diagnosis of pseudomembranous colitis can be confirmed rapidly by sigmoidoscopy. Characteristic yellowish-white, raised plaques, 2–10 mm in diameter, are seen. These small plaques can coalesce and cover the entire mucosa (Fig. 48.3). Microscopic analysis of biopsies from pseudomembranes shows the typical summit or "volcano" lesion in which inflammatory cells, fibrin, and mucus exude from microulcerations in the surface epithelium (Fig. 48.4).

Although sigmoidoscopy usually demonstrates such findings in up to 30% of patients, pseudomembranes can be restricted to the more proximal colon [241] or even be absent altogether. Colonoscopy is not recommended, however, unless other diagnostic measures have failed.

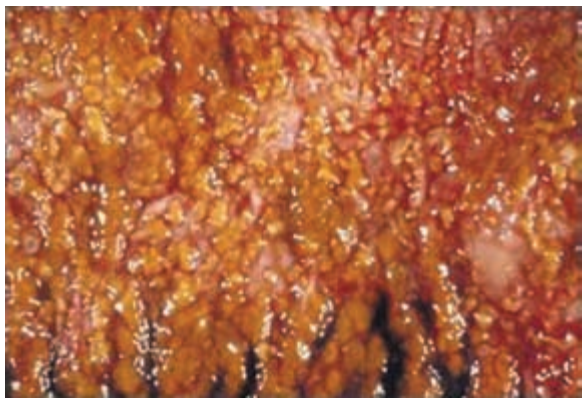


Figure 48.3 Colonoscopic appearance of mucosa with pale or yellow plaques (pseudomembranes) in patient with *Clostridium difficile* colitis. The nearly confluent light-colored pseudomembranes are typical of severe *C. difficile* pseudomembranous colitis. The pseudomembranes are composed of purulent debris. From Bartlett [240], with permission from the Massachusetts Medical Society.

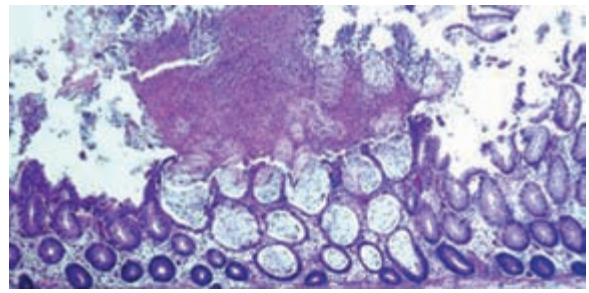


Figure 48.4 Colonoscopic biopsy of *Clostridium difficile* pseudomembranous colitis. The photomicrograph shows a classic volcano-like eruption with a "cloud" of adherent inflammatory exudate. Courtesy of Dr Jerrold R. Turner, University of Chicago, Chicago, IL.

Fulminant colitis

In fulminant *C. difficile* colitis, transmural inflammation can lead to the most serious complications associated with this infection. These include ileus, perforation, megacolon, and death [242]. This scenario occurs in approximately 3% of patients but might be more common in those infected with the toxin gene-variant strains that express the binary toxin and higher concentrations of toxins A and B [192]. Fulminant colitis can present acutely or evolve from a milder infection. The symptoms are typically more severe with signs of toxemia, constant, localized abdominal pain with guarding, and decreased bowel sounds. Fever, tachycardia, and ascites can also be present. Severe leukocytosis (up to 40 000 WBC/μl) can occur. Hypoalbuminemia can result from severe protein-losing enteropathy [243]. Diarrhea can actually decrease as severe colitis evolves as a result of acute colonic dilation and paralytic ileus. The diagnosis of toxic megacolon is defined as dilation of the colon to more than 7 cm in diameter and severe toxemia, manifest as fever, chills, leukocytosis, and dehydration. An abdominal flat plate can demonstrate a dilated colon and thickening of the colonic wall can be seen by computed tomography, although these findings are nonspecific. Dilation of the small bowel with air–fluid levels can accompany large bowel findings and mimic intestinal obstruction or ischemia [243]. If perforation has occurred, abdominal guarding, rebound tenderness, rigidity, and diminished bowel sounds are typical. An acute abdomen in a patient with a history of current or recent antibiotic therapy requires the urgent exclusion of *C. difficile* colitis to prevent unnecessary surgical intervention [244]. In such cases, a limited and cautious sigmoidoscopy with minimal air introduction should be considered. The finding of pseudomembranes will establish the diagnosis of *C. difficile* colitis. If, however, more severe signs, such as extreme leukocytosis (> 30 000 WBC/μl), hypotension, or metabolic acidosis are present, laparotomy and colectomy might nevertheless be indicated to treat impending or existing colonic perforation (Fig. 48.5) [244].

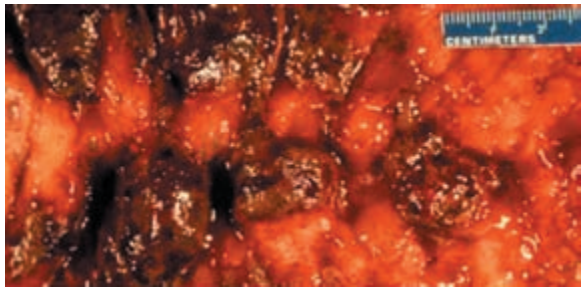


Figure 48.5 Resected colon of patient with fulminant colitis caused by *Clostridium difficile.* The photograph shows the nearly confluent light-colored pseudomembranes that are typical of severe *C. difficile* pseudomembranous colitis. The pseudomembranes are composed of purulent debris and contrast sharply with the edematous interposed darkly colored mucosa.

Diagnosis

Unlike the diagnostic strategy for other enteric pathogens, stool culture for *C. difficile* is rarely used because both toxigenic and nontoxigenic strains (i.e., nonpathogens) are detected by culture. Isolates must then be tested for toxin production, further delaying the diagnosis. Moreover, the technology needed to recover this anaerobic organism from the polymicrobial matrix of human stool is more complicated than the techniques employed in most clinical diagnostic laboratories. For these reasons, the gold standard for diagnosing *C. difficile* infection has been a tissue culture assay that seeks the presence of toxins A and B in the stool [245,246]. Diarrheal stool is filtered and applied to cultured human fibroblasts that are examined 24–48 h later for cytotoxic effects (cell rounding or detachment). Incubating the stool sample with neutralizing antitoxin antibody (antibody to *Clostridium sordellii*) and assessing for protection against cytotoxic changes confers specificity of effect. Test results are reported as positive or negative because titers do not correlate with disease activity [246]. Although this test is highly sensitive (94%–100%) and specific (99%) [227,228], it is expensive and is performed in a diminishing number of centers. Because toxins A and B each possess cytotoxic activity, this assay detects both. On a molar basis, however, toxin B is much more potent than toxin A; thus, the stool cytotoxic effect is due primarily to toxin B, which is classically (though erroneously) considered to be the sole *C. difficile* cytotoxin.

Several EIAs detect *C. difficile* products, including toxins [247] and a nontoxin *C. difficile* antigen [248]. Although these tests are somewhat less sensitive than cell culture, they are equally specific (99%), provide same-day results, and are widely utilized by laboratories. It is imperative, however, that assays capable of detecting both toxin A and toxin B be employed before calling a specimen negative, in view of the emergence of strains that are toxin A negative but toxin B positive [191]. Sigmoidoscopy or colonoscopy is rarely required to diagnose *C. difficile* colitis, but if a diagnosis is urgently required in a seriously ill patient, or if the diagnosis is in doubt, these tests can be performed. The presence of pseudomembranes (Fig. 48.3) essentially establishes the diagnosis of *C. difficile* colitis, although illness without severe colitis will not be discerned.

Treatment

Because CDAD is often a consequence of antimicrobial therapy, the first step in treatment is to discontinue such drugs [249]. However, if this is not possible, or if symptoms persist following cessation of antibiotics, then a series of therapeutic options should be pursued (Table 48.4). Antiperistaltic drugs should be avoided. This measure will resolve symptoms in 15%–23% of patients [250]. Indications for treatment of CDAD with antibiotics include severe or persistent diarrhea following discontinuation of the causative antibiotic, and clinical evidence of colitis including fever and leukocytosis. Metronidazole and vancomycin are the mainstays of therapy for *C. difficile* colitis. Oral metronidazole is the initial drug of choice because of its lower cost, excellent clinical efficacy, and rising concern regarding glycopeptide resistance in other organisms such as enterococci [249]. The efficacy of this therapy is greater than 95% [214]. The oral adult dosing regimen is 250 mg four times a day or 500 mg three times a day for 10 days. Symptomatic improvement is typically seen within 2–4 days and resolution of diarrhea by 7–10 days [251]; treatment should not be considered to have failed before 7 days of therapy. Although metronidazole is absorbed in the small bowel, it subsequently gains access to the colonic lumen by diffusing from the blood across the inflamed intestinal mucosa. Excretion into bile also contributes to the intestinal concentration [252]. Metronidazole causes a metallic aftertaste, can induce alcohol intolerance, might cause nausea, and should not be used in pregnant or lactating women.

Oral vancomycin is an alternate treatment for CDAD and its efficacy is equal to or better than metronidazole [254]. Vancomycin is not well absorbed, accounting for high fecal levels and rare side effects. Low doses (125 mg p.o. four times a day for 10 days in adults) are as effective as higher doses [255]. The expense of vancomycin and the emergence of resistant organisms have led to vancomycin being recommended for patients who fail to respond to, or who do not tolerate, metronidazole treatment [213]. For severe or fulminant pseudomembranous colitis, however, initial treatment with vancomycin is recommended. The rationale is the superb delivery of vancomycin to the colon and a slightly, but potentially important, greater efficacy in the treatment of pseudomembranous colitis [251]. In the case of ileus or toxic megacolon, however, intravenous metronidazole should be given either alone or possibly in combination with vancomycin administered by nasogastric tube or rectally.

Table 48.4 Treatment recommendations for first-occurrence *C. difficile*-associated diarrhea (CDAD) and CDAD recurrences

	Agent	Adult dose[a]	Duration	Side effects
First-line treatment, first occurrence	Metronidazole	250 mg t.i.d.	10 days	Disulfiram-like reaction, nausea, vomiting, metallic taste in mouth, peripheral neuropathy (rare during short-course therapy). Should not be used during pregnancy
	Vancomycin	125 mg p.o. q.i.d.	10 days	None
First recurrence (confirm diagnosis with antigen test for all recurrences; consider withholding treatment if symptoms are not severe)	Metronidazole	250 mg t.i.d.	14 days	Disulfiram-like reaction, nausea, vomiting, metallic taste in mouth, peripheral neuropathy (rare during short-course therapy). Should not be used during pregnancy
	Vancomycin	250 mg p.o. t.i.d.	14 days	None
7-week taper	Tapering course of vancomycin	125 mg p.o. q.i.d. 125 mg p.o. t.i.d. 150 mg q.d. 125 mg q.o.d. 125 mg q3days	1 week 1 week 1 week 2 weeks 2 weeks	
Third and subsequent recurrences	Tapering vancomycin as above, plus:			
	Saccharamyces boulardii	500 mg p.o. b.i.d.	30 days started during the last week of the taper	None
	or			
	Cholestyramine	4 g p.o. per day	Starting during the last week of the taper	Bloating, constipation, medication interactions

a Dosage recommendations are for adults and might need adjustment based on body habitus and other factors.
b.i.d, twice a day; p.o., orally; q.d., once a day; q.i.d., four times a day; q3days, every 3 days; q.o.d., every other day; t.i.d., three times a day.
Recommendations abstracted from Maroo & Lamont [253].

Relapsing C. difficile *colitis*

A minority (5%–30%) of patients develop recurrent symptoms 1–2 weeks following the discontinuation of antibiotics for *C. difficile* treatment [214], caused either by relapse from the original infecting organism or by reinfection with a different strain, especially if the patient remains in hospital. A screen of 93 hospitalized patients with recurrent *C. difficile* diarrhea demonstrated that nearly half (48%) of these cases were caused by reinfection with a different strain [256]. If only mild diarrhea is experienced, consideration should be given to symptomatic treatment without antibiotics. However, if treatment is warranted on clinical grounds, and because recurrence of CDAD is not a result of antimicrobial resistance [250], the first approach should be to administer another 10- to 14-day course of the antibiotic used initially (Table 48.4). There is no need to use higher doses of metronidazole or vancomycin to treat recurrent CDAD.

For multiple relapses, various treatment options exist and often depend on relapse severity [253]. Possibilities include a 4-week course of either metronidazole or vancomycin or a tapering schedule of vancomycin over several weeks. This regimen was curative in all patients tested with no relapses seen during follow-up ranging in duration from 2 to 12 months [257]. Rare cases in which symptoms recur following cessation of therapy and toxin assays remain positive or revert back to positive after they became negative on therapy can require tapering vancomycin over months or even years. Bacitracin is rarely used to treat CDAD today. An anion-binding resin, such as cholestyramine, which theoretically binds the toxins of *C. difficile*, can be added to this regimen. Because cholestyramine can bind to and neutralize vancomycin, patients must not take these agents simultaneously but, instead, separate the doses by 3 h. The combination of vancomycin plus rifampin has also proven effective for relapses [258], and rifaximin has been proposed to treat this infection in difficult cases [259].

Novel strategies to treat *C. difficile* infection, including the use of probiotics, are being investigated [260,261]. *Saccharomyces boulardii*, a nonpathogenic yeast, has been used to prevent antibiotic-associated diarrhea [262]. *S. boulardii* also decreased significantly the number of relapses in patients treated with high-dose vancomycin (2 g/day) but not those treated with

lower doses (500 mg/day) or metronidazole [263]. Other strategies to reestablish normal flora include the administration of *Lactobacillus* species [264] and rectal infusions of feces with or without antecedent bowel irrigation [240,265,266]. Intravenous immunoglobulin and passive oral immunotherapies, such as bovine colostrum from cows immunized with *C. difficile*, have produced increased serum antitoxin levels or resolution of symptoms [267–270].

The possibility that recurrent CDAD symptoms represent IBD should also be considered.

Clostridium perfringens

History

Clostridium perfringens was initially implicated as a cause of food poisoning in the mid-1900s [271,272]. Studies subsequently demonstrated that *C. perfringens* causes two distinct illnesses in humans: type A food poisoning [273] and gastroenteritis necroticans (also termed Darmbrand or Pig Bel) [274]. Because microbiological tests to detect this organism are rarely applied outside of epidemic settings, and because *C. perfringens* food poisoning, although common, is self-limited, gastrointestinal infections caused by *C. perfringens* are rarely diagnosed.

Epidemiology and prevention

A diarrheal syndrome resembling type A *C. perfringens* food poisoning is often identified in outbreaks and diagnosed if appropriate microbiological evaluations are applied. Meat and poultry have been the food vehicles most frequently implicated [275]. A relatively high proportion of foodborne outbreaks have been attributed to *C. perfringens* in the United States [275] and Australia [276]. Type A food poisoning from *C. perfringens* is most common in the summer [277], but infections can occur year round. The considerably rarer gastroenteritis necroticans has been limited to particular regions of the world. It was first described in post-World War II Germany, where malnourished populations suffered necrotizing enteritis following the consumption of pork. It remained endemic in the Papua New Guinea highlands until the 1980s when a vaccine was introduced that dramatically diminished its incidence and lethality [43]. Gastroenteritis necroticans is quite likely present and under-recognized in nonendemic areas [278,279].

Infections with *C. perfringens* type A are best prevented by keeping foods refrigerated [273]. Gastroenteritis necroticans is prevented by appropriate cooking of food that is likely to be contaminated, in particular pork products, and also by vaccination with a toxoid in endemic areas [280].

Pathogenesis

Clostridium perfringens is a gram-positive rod that is ubiquitous in soil and is also a normal inhabitant of human and animal gastrointestinal tracts. *C. perfringens* is characterized by its toxin-producing profiles (toxinotypes), which group these organisms into five categories (A, B, C, D, and E) (reviewed in [281]). *C. perfringens* type A, which produces alpha toxin as well as the *C. perfringens* enterotoxin (CPE), is the organism that is the chief cause of food poisoning in humans. Alpha toxin is a chromosomally encoded phospholipase C/sphingomyelinase, which disrupts membranes by hydrolyzing phospholipids. Although alpha toxin might mediate extraintestinal injury, such as myonecrosis (gas gangrene), it is not believed to play a major role in gastrointestinal illnesses.

CPE is believed to be the most important *C. perfringens* toxin in the pathogenesis of type A food poisoning. CPE is a 35-kDa protein, which, in human volunteer studies, is sufficient to produce diarrhea and cramping, symptoms that are prominent in *C. perfringens* type A food poisoning [282]. Moreover, *C. perfringens* from which *cpe*, the gene encoding CPE, has been deleted do not cause secretion in a rabbit ligated ileal loop model [283]. In type A *C. perfringens* food poisoning that is associated with human foodborne intoxications, the *cpe* gene is usually encoded on the chromosome, but sporadic cases are often associated with strains that have plasmid-encoded *cpe* [284,285]. CPE is secreted into the intestinal lumen by *C. perfringens* after sporulation is complete. It then binds to a receptor, probably a member of the claudins [286], a family of tight junction transmembrane proteins that are important for tight junction integrity and function; it is possible that the efects of CPE on the gut relate to its actions on claudins. In rabbits, CPE shortens villi and disrupts epithelial integrity [287,288]. When CPE binds to host cell plasma membranes, a so-called "small-complex" forms, which probably represents the attachment of CPE to its receptor or receptors (reviewed in [289]). In vitro, small complexes coalesce into large CPE complexes within plasma membranes, which then correlate with changes in membrane permeability in cultured eukaryotic cells. Pores permeable to water and ions are formed in the membranes. CPE can induce apoptosis via caspase 3/7 activation [290]. Additional mechanisms of cell death from CPE include calcium-mediated oncosis [291].

Toxinotype C *C. perfringens* causes gastroenteritis necroticans. Toxinotype C strains produce beta toxin, which is believed to be the critical virulence factor. An interesting hypothesis emerged in the 1970s, which proposed that dietary trypsin inhibitors (such as are found in sweet potatoes, a dietary staple in the Papua New Guinea highlands) inactivate this toxin [292]. Beta toxin is encoded on a large plasmid and forms pores within membranes [293]; its additional intracellular targets remain unknown [294]. Its role in human disease pathogenesis is established by the fact that a vaccine against a toxoid of beta toxin largely eliminated disease in an endemic area [43].

Clinical manifestations

Type A food poisoning is usually recognized as a clustered infection and consists of 1–2 days of painful, nonbloody,

afebrile diarrhea [295]. It strikes people of all ages. Preformed toxin in food can cause symptoms. Vomiting is rare [296]. Gastroenteritis necroticans generally presents with a distended, painful abdomen and might or might not be accompanied by diarrhea. Often the adult with gastroenteritis necroticans has a serious underlying disorder, including alcoholism and occasionally fecal impaction.

Treatment

Clostridium perfringens type A infections are self-limited, short-lived, and rarely warrant intervention. Gastroenteritis necroticans is considerably more serious; affected patients are treated with antibiotics and resection of the affected areas of the bowel.

Diagnosis

Clostridium perfringens type A food poisoning is best diagnosed by EIA of the stool. This test is rarely available outside of research studies or public health laboratory investigations. Gastroenteritis necroticans is best diagnosed clinically at laparotomy and confirmed by sampling enteric contents and by PCR and phenotypic analysis of recovered anaerobes.

Sequelae

There are no known sequelae to either infection, but the mortality rates from both forms of *C. perfringens* infection can be considerable in fragile hosts.

Escherichia coli

Background

Escherichia coli are typically harmless commensals that colonize the gastrointestinal tracts of humans and mammals, with the initial colonization within the first day of life. They are gram-negative bacilli and the major component of the facultative anaerobic flora. However, a subset of *E. coli* cause a variety of human intestinal illnesses, in particular diarrhea. These diarrheagenic *E. coli* often cause signature clinical manifestations and host injury patterns. Here, we will discuss the currently accepted major diarrheagenic *E. coli* groupings: enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC), enteroinvasive *E. coli* (EIEC), EHEC, enteroaggregative *E. coli* (EAEC), and diffuse adherent *E. coli* (DAEC). It is likely that additional categories of diarrheagenic *E. coli* will be recognized in the future.

There are three major challenges pertaining to the diagnosis of diarrheagenic *E. coli*. First, diagnosis at presentation is rarely possible because, except for *E. coli* O157:H7, diarrheagenic *E. coli* have few or no phenotypes that enable them to be distinguished using standard or widely available microbiological techniques. The detection of diarrheagenic *E. coli* largely relies on cumbersome phenotypic determinations (cell invasion, adherence, toxicity assays) and DNA hybridization assays (colony hybridizations or PCR). Identifying the O (somatic) antigen can suggest categorizations of diarrheagenic *E. coli*, but such tests are rarely performed in clinical laboratories. Because most illnesses caused by diarrheagenic *E. coli* are self-limited, it is difficult to make a compelling case for diagnosis. However, the failure to detect diarrheagenic *E. coli* has hindered attempts to study their epidemiology and the diseases that they cause, and their importance is almost certainly underappreciated. Furthermore, this inability to identify the patients who are infected with diarrheagenic *E. coli* at the point of presentation thwarts our ability to determine if antibiotics can change the clinical course of such infections.

Second, diarrheagenic *E. coli* exemplify the difficulty of defining an enteric pathogen. Pathogenicity among *E. coli* is derived from multiple virulence genes, which encode molecules that overcome or subvert host defenses and enable the pathogen to adhere to, injure, and invade host cells or tissues. These molecules include adhesins, polysaccharides, toxins, invasins, and siderophores (iron acquisition proteins). However, virulence loci are often found on mobile elements (i.e., bacteriophages and plasmids) and can be horizontally transferred to and from nonpathogens and even between different bacterial species. A repertoire of virulence determinants is necessary for pathogenicity; allelic variation, synergy between virulence factors, and well-performed epidemiological studies all need to be considered before designating an organism as a pathogen merely based on the presence of one or more pathogenicity-associated loci.

Third, people without diarrhea can excrete putatively diarrheagenic *E. coli* at variable frequencies, which is frequently not the case with pathogens in other genera. To address this problem, a "pathogenicity index" was proposed, which takes into account excretion rates in people with and without diarrhea [297].

Enterohemorrhagic E. coli *(*E. coli *O157:H7 and non-O157:H7 Shiga toxin-producing* E. coli*)*

History

In 1983, two nearly simultaneous publications introduced the concept of cytotoxin-producing *E. coli* to the medical community. Karmali et al. [298] associated fecal *E. coli* that could produce a toxin lethal to cultured eukaryotic cells with childhood HUS, a disorder that often follows bloody diarrhea. Riley et al. [299] described clusters of cases of bloody diarrhea that were attributable to *E. coli* expressing O antigen 157 and H antigen 7, and which were associated with the consumption of undercooked hamburgers. Soon after it was demonstrated that *E. coli* O157:H7 produced a cytotoxin neutralizable by antibodies to the principal toxin produced by *S. dysenteriae* serotype 1 [300], and in 1987 the Shiga toxin (Stx or STEC) of *E. coli* O157:H7 was cloned and sequenced [301]. *E. coli* O157:H7, and, to lesser extents, non-O157:H7 Stx-producing *E. coli*, have subsequently been isolated worldwide. They have also been associated with HUS, epidemics, and sporadic infections. *E. coli* containing genes encoding Stx

are quite common in food and the environment; the subset that causes human disease is typically referred to as EHEC. Their virulence probably results from their ability to produce factors other than Stx, of which intimin and other molecules encoded by genes in the locus of enterocyte effacement (LEE) pathogenicity island are the best studied.

Epidemiology and prevention

Escherichia coli O157:H7 infections have most notoriously been associated with the consumption of poorly cooked ground beef and have occurred in the context of large outbreaks. However, in reality, a wide range of food and environmental vehicles have been implicated in the transmission of *E. coli* O157:H7 [302]. Furthermore, most infections occur sporadically or in conjunction with another infected member of the household and not as large epidemics [302]. EHEC have been found in resource-poor countries [303] suggesting that they are not solely a problem of industrialized countries, even though that is where they have been most frequently detected. The epidemiology and clinical aspects of non-O157:H7 EHEC infections, and the vehicles that transmit these agents, are considerably less well delineated than are *E. coli* O157:H7 infections.

Consumers and the food industry can prevent *E. coli* O157:H7 infections by thoroughly cooking food of animal origin, hygienic food preparation practices, and consuming pasteurized milk and milk products. Control of this pathogen in the environment and on fresh produce is more challenging and probably more important, because *E. coli* O157:H7 infections are at least likely to be caused by rural, environmental, and agricultural exposures, and consumption of contaminated produce, as they are to the consumption of contaminated beef. A nationwide epidemic of *E. coli* O157:H7 infections disseminated by raw spinach attests to this risk [20].

Vaccination of humans against *E. coli* O157:H7 is potentially feasible, and a purified lipopolysaccharide vaccine is immunogenic and safe in children and adults [304,305]. A plant-based vaccine strategy using Stx epitopes as the immunogen was successful in mice [306]. Challenges to the immunoprophylaxis of human infections include the need to test effectiveness for a disorder with a low incidence, duration of immunity, and lifelong risk of acquiring infection (i.e., persons of all ages are susceptible). Vaccination of, or use of probiotics in, cattle, has been proposed as a mechanism to control this pathogen in this reservoir, but efficacy will depend on a variety of ecological factors (discussed in [307]).

Pathogenesis

The cardinal virulence trait of EHEC is the ability to produce one or more variants of Stx. Stx1, and its closely related allelic forms, are nearly identical to Stx produced by *S. dysenteriae* serotype 1 [301]. Stx2 and its variants (which are more diverse than those of Stx 1) are more distantly related to Stx/Stx1. Stxs are typical A/B toxins. There are five B subunits for each A subunit in the holotoxin, resulting in an AB_5 structure.

The B subunit forms a pentamer and binds to a receptor on eukaryotic cell surfaces, namely globotriaosylceramide (Gb_3) [308]. The toxin is then internalized and the A subunit is probably the most important effector (active) subunit.

Stxs have multiple effects on eukaryotic cells [309]. The A subunit resembles ricin in structure and it functions as an N-glycosidase, cleaving the eukaryotic large ribosomal subunit at a specific adenine residue, thereby halting protein synthesis. Purified B subunit, in the absence of the A subunit, causes apoptosis [310,311]. EHEC rarely cause fatal infections as would be expected in systemic intoxications by a ricin-like molecule. Indeed, sublethal concentrations of Stx induce expression of endothelin mRNA, which could contribute to the thrombotic response to intestinal infections with EHEC [312].

Stxs typify the conundrum posed by virulence loci and their allelic variants. In animal [313] and *in vitro* [314] studies, Stx2 is more potent than Stx1. In epidemiological and cohort studies, EHEC containing Stx2 [315,316] are more often associated with extraintestinal complications and with bloody diarrhea than are organisms containing Stx1. Interestingly, and counterintuitively, *E. coli* O157:H7 that contain genes encoding Stx1 and Stx2 are less often associated with HUS than are *E. coli* O157:H7 that contain Stx2 but which lack Stx1. Additional complexities emerge when examining allelic forms of Stxs. Stx2, and to a lesser extent Stx1, belong to larger families of toxins. Some Stx2 variants (Stx2 and Stx2c) have strong associations with human disease, whereas others (Stx2d and Stx2e) are less firmly associated with pathogenicity [317].

EHEC possess a variety of putative or confirmed non-Stx virulence loci. The best studied of these is intimin. In human pathogenic EHEC, intimins usually exist as either intimin-γ (found in *E. coli* O157:H7) or intimin-β (found in many non-O157:H7 EHEC). EHEC intimins are similar to the various intimins found in EPEC and they mediate the intimate attachment of EHEC to epithelial cells. The gene encoding intimin, *eae*, is also located in the LEE pathogenicity island, which encodes many proteins that are similar to those encoded by the LEE pathogenicity island of EPEC, including proteins that are necessary for the assembly and function of the structures and molecules of the type 3 secretion system (T3SS) (described in Enteropathogenic *E. coli*). With few exceptions [318], almost all pathogenic EHEC (which have a variety of Stx alleles) contain an intimin variant. Eukaryotic nucleolin can also serve as a receptor for intimin [319], although it is likely that EHEC Tir, which is injected by the organisms into the host cell, is also a ligand for intimin; EHEC Tir, unlike its counterpart in EPEC, is not tyrosine phosphorylated [320].

Two strains of *E. coli* O157:H7 have been sequenced in their entirety [321,322]. Approximately one-fourth of the

chromosomes of these organisms are not found in *E. coli* K-12. Except for Stxs, intimin, and several other members of the LEE pathogenicity island, few loci have been examined in great depth as candidate virulence loci. Of those that have, several emerge as potentially important in virulence. StcE is a secreted protein that potentiates the effect of C1 and which might protect *E. coli* O157:H7 from the lytic effects of complement [323]. Additional interesting loci include those encoding molecules that mediate adherence of *E. coli* O157:H7 to epithelial cells, such as long polar fimbriae [324], Iha, a demonstrated virulence factor in uropathogenic *E. coli* [325], Efa1/Lif1 [326], ToxB [327], and $EspF_U$ [328], which is important for epithelial cell pedestal formation.

Clinical aspects

Escherichia coli O157:H7 infections display a spectrum of severity, including rare cases of asymptomatic carriage [329], nonbloody and bloody diarrhea [316,330,331], and HUS. HUS, a nonimmune thrombotic microangiopathy, occurs in approximately 15% of diagnosed *E. coli* O157:H7 infections in children under 10 years of age. Typically, a patient infected with *E. coli* O157:H7 will experience 1–3 days of nonbloody diarrhea, which then becomes grossly bloody diarrhea in about 80% of microbiologically diagnosed cases (Fig. 48.6). About half of all infected patients report a fever early in illness, but very few patients are febrile when they are initially evaluated, usually at the onset of bloody diarrhea. Abdominal pain, which can be quite severe, and vomiting can precede the diarrhea. The clinical manifestations of non-O157:H7 EHEC infections are less well delineated, but, as a group, they are less likely to cause HUS [3] or to be associated with bloody diarrhea [332]. The colon can be quite severely affected (Fig. 48.7).

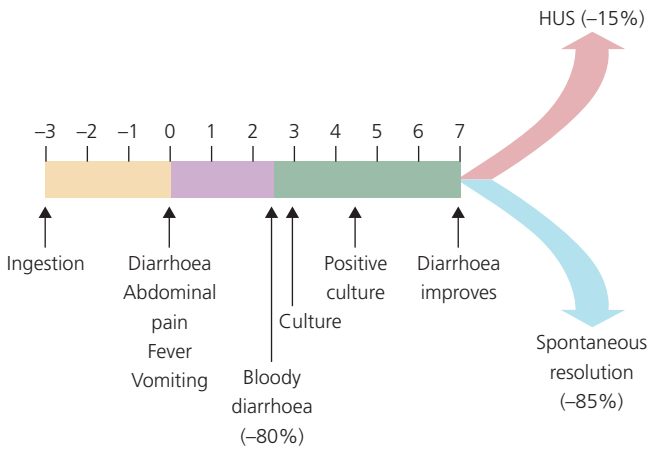


Figure 48.6 Progression of *Escherichia coli* O157:H7 infections in children. *E. coli* O157:H7 infections tend to follow a stereotypical pattern [343], and it is helpful to make clinical judgments based on stage of illness, in consideration of trends in laboratory tests, as portrayed in Figure 48.8. From Tarr et al. [302], with permission from Elsevier.

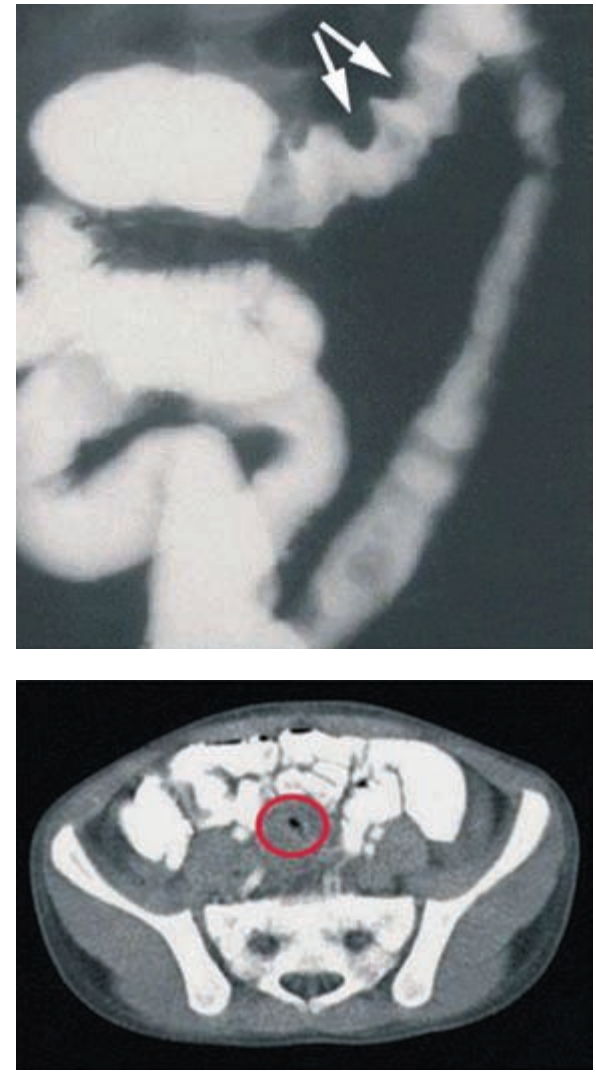


Figure 48.7 Radiographic features of *Escherichia coli* O157:H7 infection. **(a)** Radiograph after barium enema with thumbprinting appearance of mucosa (arrows), suggesting colonic edema, in a patient who subsequently developed hemolytic uremic syndrome (HUS). **(b)** Computed tomography of the pelvis of an 8-year-old boy on the eighth day of an *E coli* O157:H7 infection. Note the severely thickened colon (circled). This infection did not progress to HUS. From Tarr et al. [302], with permission from Elsevier.

E. coli O157:H7 infections follow a fairly predictable sequence of events to either resolution or development of HUS (Fig. 48.6); the course is usually apparent by the tenth day of illness (or sooner if there is no evidence of a falling platelet count). There is a variable pattern to the vascular lesions in *E. coli* O157:H7 infections (Fig. 48.8). The trend in the platelet count is the most useful indicator of deterioration into HUS or resolution of the infection without development of this complication. It should be determined daily until the trend is apparent. It need not be assessed daily if it is rising or if it is stable and the abdominal symptoms are resolving.

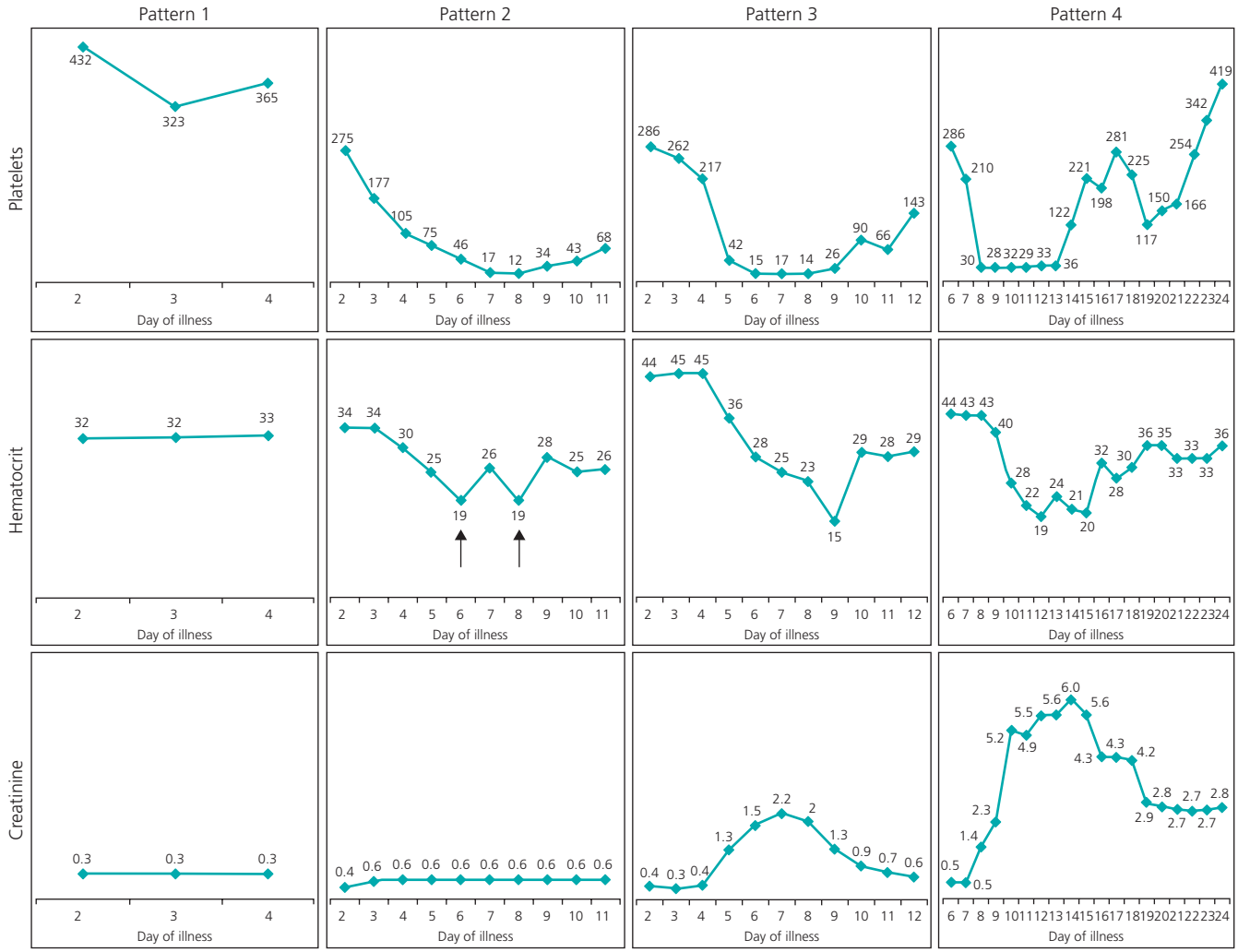


Figure 48.8 Patterns of laboratory abnormalities in human *Escherichia coli* O157:H7 infections. Pattern 1 (uncomplicated infection) occurs in approximately 70% of cases diagnosed microbiologically early in illness. In this patient the platelet count falls between the first and the subsequent determination, and then rises. In about 10% of patients the platelet count does not fall, or actually rises, between the first and subsequent assessments. On other occasions the platelet count falls quite markedly (with a nadir of less than 150 000/mm^3) but there is a minimal decrement in the hematocrit. Pattern 2 infections display substantial vascular injury in the absence of azotemia, a situation that occurs in 5%–10% of patients who we have diagnosed early in illness. This patient received vigorous intravenous volume expansion on the second day of illness because her diarrhea became bloody on the first day; her culture was presumptively positive one day later, at which time she was admitted. Hemolysis was sufficiently severe to require two erythrocyte transfusions (arrows). This pattern is sometimes termed partial hemolytic uremic syndrome (HUS), but in the absence of any evidence of renal insufficiency we do not believe that this designation is appropriate, especially because there is no risk of chronic renal sequelae if patients do not have renal failure during their acute infection [333]. Pattern 3 exemplifies nonoligoanuric HUS. This form of HUS occurs in slightly less than half of all patients who develop HUS. Patients meet the case definition of HUS (thrombocytopenia [platelet count < 150 000/mm^3], hemolytic anemia [hematocrit < 30% with evidence of intravascular erythrocyte destruction on peripheral blood smear] and renal insufficiency [creatinine > upper limit of normal for age]) between 5 and 13 days after the onset of diarrhea. These patients continue to urinate and rarely need dialysis. Pattern 4 is anuric HUS, which occurs in slightly more than half of all patients with HUS. This patient's severe course has been published previously [334]. Anuric HUS is considerably more severe than nonoligoanuric HUS, and has a worse prognosis [335,336]. Note that in patterns 3 and 4 a decrement in the platelet count precedes other laboratory abnormalities. These patterns are also seen in adults, but the proportion of patients over the age of 10 years who develop azotemia is lower, probably around 5%. Courtesy of Dr Jerrold R. Turner, University of Chicago, Chicago, IL.

Complications

HUS is the most common and serious complication of *E. coli* O157:H7 infections. Patients meet the case definition of HUS between 5 and 13 days after the onset of diarrhea, but the course is usually suggested by thrombocytopenia that occurs by day 8 or 9 of illness (Figs 48.6 and 48.8). On rare occasions, infected patients will develop HUS without antecedent diarrhea. Most infected patients will experience some degree

of diminution in their platelet counts, whether or not HUS develops. Infected patients should undergo daily assessment of their blood counts until it is clear that the platelet count is rising [302]. In some cases, enteric symptoms resolve without a rising platelet count. Nonrenal complications almost always occur only in those patients who develop HUS and include intussusception, appendicitis, intestinal perforation, pancreatitis, stroke, coma, seizures, gallstones, small bowel and colonic strictures, and sudden death [337–340], although in rare cases serious complications such as pancreatitis [341] and colonic perforation [26] can occur without HUS. *E. coli* O157:H7 infections are a risk factor for subsequent development of diarrhea-predominant irritable bowel syndrome [342].

Diagnosis

The ideal approach to diagnosing EHEC infections is to employ methods that detect O157:H7 as well as non-O157:H7 EHEC. However, it is important to not employ algorithms that delay detection of *E. coli* O157:H7, such as plating stools on MacConkey agar only if a toxin assay is positive. This goal is best achieved by initially plating all stool specimens on MacConkey agar that contains sorbitol but not lactose as its carbon source. Screening for this organism should not be limited to physician request or to stools containing only gross blood. Because *E. coli* O157:H7 fail to ferment sorbitol after overnight incubation, they can be distinguished from other enteric flora as colorless colonies on this agar. If resources permit, an additional approach would be to screen for Stx by inoculating stool into a broth and assaying an overnight culture using commercial EIAs for Stx to detect non-O157:H7 EHEC (see Non-O157:H7 pathogenic enterohemorrhagic *E. coli*). Stx assays should not replace plating of the stool on sorbitol MacConkey agar as the sole screen for the detection of pathogenic EHEC, because many of the positive results in this assay do not signify the presence of organisms that pose a substantial likelihood of leading to HUS or of causing epidemics. Moreover, a clinician needs to know that *E. coli* O157:H7 is present or absent in a stool culture when a report of a positive EIA is made. Utilization of sorbitol MacConkey agar only after an STEC EIA is positive is an undesirable and inferior practice; the two tests should be employed in parallel. Use of toxin detection methodologies leads to a delay in diagnosis or to misdiagnosis [344] if such assays are used as a screen in lieu of sorbitol MacConkey agar screening [344].

Treatment

Escherichia coli O157:H7 are usually susceptible to antibiotics. However, antibiotics should not be given to patients who are possibly or definitely infected with *E. coli* O157:H7 or other EHEC. Infected children [331,345] and adults [346,347] treated with antibiotics experience higher complication rates, in particular HUS, than patients who are not treated. A metaanalysis [348] suggesting that there was no harm accruing to patients infected with *E. coli* O157:H7 who were treated with antibiotics was erroneous, chiefly because it mischaracterized a major series from Japan in which a nontreatment group was not included [349]. There are no data on the risks or benefits of treating non-O157:H7 EHEC infections with antibiotics, but there is no reason to believe that such therapy would be useful.

We strongly recommend hospitalization and intravenous isotonic volume expansion for patients who are possibly or definitely infected with *E. coli* O157:H7 for two main reasons. First, such patients are highly infectious to others and infection control is often more difficult to practice in the community [302] than in the hospital. Second, intravenous volume expansion with isotonic crystalloid appears to confer nephroprotection [350]. Antimotility agents, nonsteroidal antiinflammatory drugs, and opioid narcotics should be avoided [302]. Monitoring of such patients must be performed assiduously, especially as the creatinine level is rising. However, if a patient is still urinating and there is no evidence of hypertension or pulmonary overload, there is no need to restrict fluids merely because the creatinine level is rising. The goal of volume expansion is to avert anuria, because anuric HUS is a categorically different and worse form of HUS than nonanuric HUS [335,336]. It is helpful in managing infected patients, especially in managing their fluids as azotemia develops, to note that if a patient does not become anuric within 10 days of the onset of diarrhea, it is very unlikely that this complication will occur.

HUS treatment is reviewed in several publications [302,351]. Transfusion might be needed in some patients manifesting a pattern B clinical course (see Fig. 48.8), or even prior to the onset of azotemia in patients who develop HUS. We use clinical indicators (especially tachycardia) to prompt red cell transfusions. Platelet transfusions should be avoided unless a surgical procedure is planned, to avoid contributing to the underlying thrombotic lesion.

Non-O157:H7 pathogenic enterohemorrhagic E. coli

This class of organisms bears mention because they are overlooked on routine culture of stool on sorbitol MacConkey agar. However, as a group, they are considerably less likely than *E. coli* O157:H7 to cause HUS or outbreaks. Even when sought appropriately, they are recovered less frequently than are *E. coli* O157:H7 [3,352]. Usually, patients whose stools produce a positive signal in assays that detect Stx but who are not infected with *E. coli* O157:H7 have considerably milder illnesses. However, some serogroups, especially EHEC expressing O antigens 26, 45, 103, 111, 113, 145 and an unusual sorbitol-fermenting clone of *E. coli* O157:H^- [353] can cause severe illnesses, outbreaks, and HUS. *E. coli* O113 produces a novel subtilase toxin [354] in addition to Stx. This toxin is a serine protease that cleaves BiP, which is a multifunctional endoplasmic reticulum chaperone protein [355]. Detection of Stx production by EIA is the best methodology to identify non-O157:H7 EHEC, but false-positive signals can occur

[344,356]. Non-O157:H7 EHEC have not emerged as large public health or clinical problems in North America, but have in Australia [357].

Enterotoxigenic E. coli

History

In Calcutta in 1956, De et al. [358] demonstrated that *E. coli* from children and adults with watery diarrhea elicited fluid secretion in ligated rabbit ileal loops, similar to the effects of cholera toxin, thereby introducing ETEC as causes of human illness. In 1968, Sack [359] demonstrated that this effect was attributed to bacterial products. In the mid-1970s, Gorbach et al. [360] and Merson et al. [361] reported that ETEC were the predominant causes of diarrhea in travelers to the developing world. Interestingly, the ability of antibiotics to prevent traveler's diarrhea, a practice that is currently recommended only in special circumstances, was demonstrated in advance of identifying the major etiological role played by ETEC in this disorder [78].

Pathogenesis

ETEC produce one or both of two distinct families of enterotoxins, which promote secretion of chloride and water into the small bowel, and inhibit reabsorbtion of fluids (reviewed in [362]). Heat-labile toxins (LTs) are similar in structure and function to cholera toxin; LT-I is the most common LT in human ETEC isolates. LTs are AB_5 toxins, with the B subunit binding GM1 and GD1bp gangliosides, and the A subunit being the enzymatically active moiety. The A subunit ADP-ribosylates, and thereby irreversibly activates, the G (stimulatory) protein. This enzyme activates adenylate cyclase and leads to increased intracellular levels of cAMP. Elevated intracellular cAMP levels activate cAMP-dependent kinases, which then stimulate chloride secretion via CFTR, leading to net secretion of chloride ions from intestinal crypt cells. These changes also inhibit sodium chloride reabsorbtion in the villus tip cells. Similar to cholera toxin, LT is an adjuvant, augmenting mucosal immune responses to coadministered antigens. LT is delivered in a polar fashion by adherent ETEC [363]; even though it is immunogenic, it might be sequestered from the immune response, accounting for discordance between circulating antibodies to LT and protection [364].

ETEC also produce heat-stable toxins (ST). STa (also termed ST-h), the predominant ST in human ETEC, is an 18- or 19-amino-acid peptide that binds reversibly to guanylate cyclase, leading to increased levels of intracellular cyclic GMP [365]. Increased cGMP levels activate cGMP- and cAMP-dependent kinases, which results in an increase in net intestinal secretion, as does LT. STa has homology to guanylin, a host molecule that binds to epithelial guanylate cyclase.

ETEC employ a wide range of adherence factors to colonize the gut. These factors were previously designated colonization factors. More recently, these largely fibrillar molecules have been termed coli surface antigens (except for CFA/I, which retains its initial designation) [366] and grouped into families that contain conserved epitopes. A subset of colonization factors is overrepresented among human ETEC, but the adherence mechanisms of many strains warrant additional characterizations [367].

Epidemiology and prevention

ETEC are probably the most common bacterial cause of acute diarrhea worldwide. In resource-poor countries, ETEC infections have their highest incidence in children under the age of 2 years. The incidence rises again in the mid-teen years. In case–control studies, non-diarrheal control subjects excrete ETEC but at lower rates than patients with diarrhea. ETEC are the leading, although by no means the exclusive, cause of diarrhea in international travelers. ETEC are spread via consumption of food or water that has not undergone reliable bactericidal processes. Person-to-person spread is also possible. Endogenous North American ETEC infections have occurred, usually from a contaminated point source or food [368,369]. Malnourished children seem to be predisposed to ETEC infection [370,371]. Moreover, repeated attacks of diarrhea, caused by ETEC as well as by other agents, could underlie malnutrition [372,373]. The infectious dose is high, estimated at greater than one million organisms [374]. Prevention is directed at avoiding food and water that has not been subjected to a reliable bactericidal process, and avoidance of street-vended food in areas of the world where ETEC infections are endemic.

Vaccination against diseases caused by ETEC has considerable appeal because of the high frequency of disease and the delineation of several potentially immunogenic virulence factors such as toxins and colonization factors. However, this approach faces challenges from antigenic variation in the colonization factors, and would be aided by a search for a common antigen [367]. Killed ETEC comprising a range of colonization factors, combined with recombinant cholera toxin B subunit, elicits, after oral challenge, a systemic response. Live vaccines have consisted of ETEC from which ST and LT genes have been deleted. Efficacy studies demonstrate immune responses, but protection of populations of children in endemic areas has been more difficult to demonstrate. Another approach is to express ETEC virulence factors in an attenuated *Shigella* vector. These strategies have been summarized [42,375].

Clinical manifestations

ETEC cause secretory, noninflammatory diarrhea. The severity of the diarrhea ranges from asymptomatic to cholera-like shock. Fever is rare. Vomiting is common among infected children [362].

Diagnosis

Identification of ETEC requires DNA hybridization studies or other analyses (EIAs or cell culture assays) that detect ST, LT,

or colonization factors. However, these technologies are not employed outside research settings. The diagnosis is usually based on syndromic recognition, taking into account the likelihood on epidemiological grounds that cholera is or is not a consideration.

Treatment

Fluid management underlies the treatment of ETEC infections. Volume replenishment and fluid maintenance can often be addressed by oral rehydration with glucose/electrolyte solutions. Antibiotics do not clearly benefit ETEC-infected children or adults in the developing world, but are used (successfully) in the empiric treatment of diarrhea in international travelers. Antibiotic therapies have tended to evolve, as has resistance to specific antimicrobials. Therapeutics have included tetracyclines, trimethoprim–sulfamethoxazole, fluoroquinolones, azithromycin, rifamycin, and, most recently, rifaximin [34,376]. Antibiotics are generally not recommended to prevent traveler's diarrhea because of their potential side effects, their effectiveness in treating traveler's diarrhea if administered soon after symptoms begin, and the desire to avoid selective pressures leading to antimicrobial resistance in the countries visited. Rifaximin, a non-absorbable derivative of rifampin, has shown promise in preventing and treating traveler's diarrhea [34,376], but sufficient experience has yet to accumulate to determine if this agent will retain its safety and efficacy profiles.

Complications

Most ETEC complications can be related to acute dehydration. However, repeated cycles of diarrhea, many of which are probably caused by ETEC, almost certainly contribute to malnutrition spirals in children in developing countries [372,373]. From an evolutionary standpoint, ETEC have been proposed to be an infectious agent that exerted selective pressure in humans in favor of heterozygous carriage of the mutated CFTR gene [111]. It is likely that ETEC precipitate at least some cases of irritable bowel syndrome that follow an episode of traveler's diarrhea [83].

Enteropathogenic E. coli

History

In 1945, Bray et al. [377] described severe epidemic infantile diarrhea in the United Kingdom associated with specific O serogroups of *E. coli*. Similar outbreaks have occurred in the United States [378]. During subsequent decades, these organisms have been demonstrated to adhere to epithelial cells in localized microcolonies and form characteristic "attaching and effacing (AE)" lesions [379], which are also visible on electron microscopy of biopsies obtained from children [380]. Profound cytoskeletal rearrangements underlie the AE lesions; the most notable phenotype is the aggregation of actin filaments under the membrane where the microcolonies are located. Furthermore, the molecular mechanisms that EPEC employ to injure host cells, especially those mediated via a T3SS, are being elucidated at an accelerating rate. However, there is discordance between the emerging knowledge of EPEC pathogenesis and the paucity of data surrounding epidemiology, clinical manifestations, and diagnosis of infections caused by EPEC.

Pathogenesis

EPEC adhere to host cells in characteristic microcolonies (Figs 48.9 and 48.10). The initial mechanism of adherence is not certain and plausibly involves proteins other than those that are more directly involved in the AE lesion.

Candidate adhesins for this initial attachment include the bundle-forming pilus [383], EspA [384], flagella [385], and LifA/Efa 1 [386]. After initial adherence, intimin is the molecule through which EPEC bind to host cell structures, by interacting with the bacterial-encoded and translocated intimin receptor (Tir) described below. Bfp is responsible for the formation of EPEC microcolonies [387].

The most characteristic trait of EPEC infections is their utilization of an intricate T3SS encoded by the LEE pathogenicity island. T3SSs are utilized by many enteric pathogens to translocate virulence factors into host cells via needle-like bacterial organelles. The prototypic EPEC LEE region is a 35-kilobase pair island, inserted into the chromosome at *selC* (the same site as the insertion of the *E. coli* O157:H7 T3SS) [388]. The transcription of open reading frames within the LEE region is promoted by intra-LEE positive regulators (LEE-encoded regulator and global regulator of LEE activator) and by a plasmid-encoded positive regulator (*per*); transcription is negatively regulated by the global regulator of LEE repressor.

When EPEC contact a host cell, a LEE-encoded syringe-like structure, largely composed of polymerized EspA filaments, translocates additional effector molecules into the eukaryotic

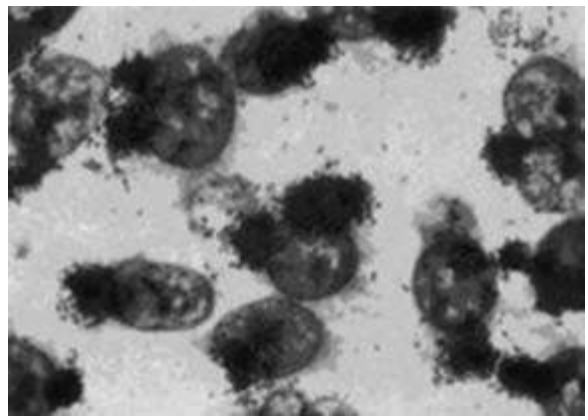


Figure 48.9 Enteropathogenic *Escherichia coli* (EPEC) adherence to cultured epithelial cells. Typical EPEC form characteristic microcolonies (arrow) at points of adherence. From Campos et al. [381], with permission from Memórias do Instituto Oswaldo Cruz.

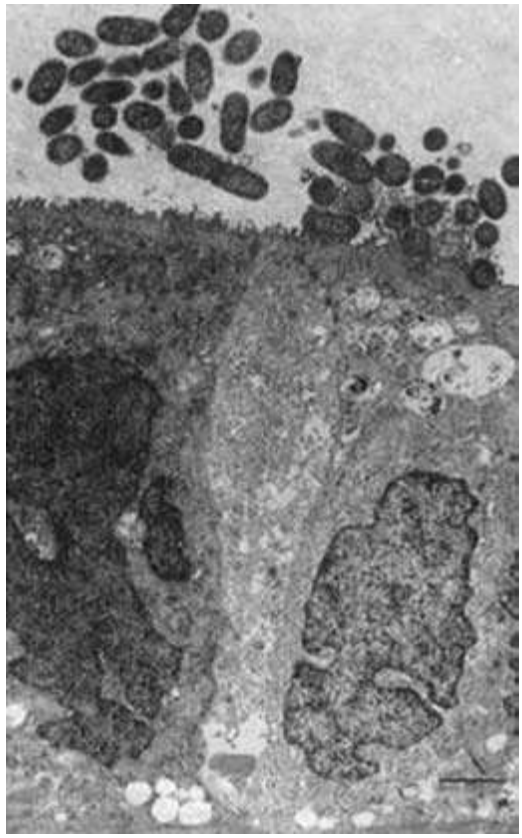


Figure 48.10 Enteropathogenic *Escherichia coli* (EPEC) attaching to epithelial cells. Transmission electron micrograph of T84 cells that have been infected with EPEC. The characteristic attaching and effacing lesions are associated with intimate bacterial attachment to the apical enterocyte membrane. Loss of microvilli is apparent adjacent to the adherent microcolony. There is also microvillus elongation, which precedes effacement. Cup-like pedestals with underlying condensation of cytoskeletal elements are also seen. The intestinal epithelial cells are intact and tight junctions are closely approximated, but electrophysiological studies demonstrate enhanced tight junction permeability. From Spitz et al. [382], with permission from the American Physiological Society.

cell, including EspB and EspD. These effectors form pores in the host cell plasma membrane. These actions allow delivery of a panel of bacterial proteins directly into the host cell cytoplasm where they participate in AE lesion formation or disrupt normal physiological functions such as intestinal epithelial barrier function (reviewed in [389]). Tir is one EPEC protein that is injected into the host cell, where it is phosphorylated by host kinases and then serves as the ligand for the attachment of intimin. Tir is required for the formation of a classic pedestal on which the EPEC sit, and for the underlying actin polymerization and aggregation at this site. Additional translocated molecules disrupt intestinal barrier function (EspF, Map) [390,391], damage mitochondria, and activate NF-κB [392]. However, it should be noted that some of the injected proteins can attenuate host cell inflammatory responses [393]. Data suggest that several EPEC effector proteins (Map, EspF, and Tir) inactivate the sodium-D-glucose cotransporter SGLT-1 [394]. The activities of other intestinal epithelial transporters (MCT-1 and NHEs) are also altered by EPEC infection [395,396].

Proteins encoded by loci outside of the LEE region also exploit the LEE-encoded T3SS to injure host cells. The best studied of these is the plasmid encoded regulator (*per*), which is encoded on the large plasmid common to "typical" EPEC [397]. EspG2, a homologue of EspG and which disrupts microtubules, is another example [398,399].

Epidemiology and prevention

Typical EPEC have been incriminated as causes of epidemic infantile diarrhea with severe illnesses in North America [378]. However, such clusters in the developed world are rare, and typical EPEC are believed to be problems of children in underprivileged settings. However, several studies have suggested that "atypical" EPEC, which consist of *E. coli* containing the LEE region but lacking the large EPEC plasmid, are also pathogenic in children [400–404]. Adults can occasionally be affected [405]. Because typical and atypical EPEC are only detected for the purposes of research studies, their epidemiology is not well delineated. Community-based preventive measures, beyond encouragement of good hygiene whenever possible, are not well formulated. If an institutional outbreak is recognized, contact precautions and cohorting should be considered. Vaccination strategies have not been well developed.

Clinical manifestations

The inability to diagnose EPEC has hampered characterizations of diseases caused by EPEC. Generally, typical and atypical EPEC are associated with nonbloody, and often protracted, diarrhea in infants and children [352,400,404].

Diagnosis

Detection of EPEC requires DNA probing or adherence assays. Serotyping of isolates has also been used but is of questionable value compared with genetic characterizations. These determinations are not performed outside research settings.

Treatment

It is reasonable to consider treatment if an EPEC is suspected on empiric grounds. Oral, nonabsorbable aminoglycosides have been used in small series with success [406], but large-scale randomized controlled studies, using rigorous definitions of EPEC, are lacking.

Complications

Infected children can present with intestinal failure accompanied by nutritional consequences and growth retardation [406]. Long-term complications in the developing world consist of the initiation or perpetuation of infection/malnutrition cycles.

Enteroaggregative E. coli

History

EAEC were first associated with human diarrhea in 1987 [407–409] in Chilean children, when *E. coli* with characteristic adherence patterns (a stacked brick appearance) were found more frequently in patients than in control subjects. Subsequent studies have identified EAEC as etiological agents of diarrhea in patients infected with HIV [410]. They are found in children and adults in developed as well as in developing countries [297,400,411].

Pathogenesis

EAEC, like other diarrheagenic *E. coli*, appear to cause disease via sequential, as well as parallel, processes. The prevailing pathogenetic paradigm suggests that initial colonization of the mucosal surface is followed by elaboration of cytotoxic, secretory, and proinflammatory factors. EAEC adhere to intestinal epithelial cells via aggregative adherence fimbriae (AAFs), for which several distinct alleles exist. EAEC are covered by a surface protein called dispersin, which is postulated to neutralize the negative surface charge conferred by lipopolysaccharides, thus freeing the positively charged AAFs of their attraction to lipopolysaccharides and enabling the fimbriae and bacteria to splay out over epithelial surfaces. EAEC also produce a mucinase (Pic), an enterotoxin termed *Shigella* enterotoxin 1 (ShET1) [412], and a homologue of ETEC ST termed EAST1 [413], which is a toxin elaborated across a broad panel of potentially diarrheagenic *E. coli* [414]. ShET1 elicits increased conductance (ion secretion) across intestinal tissue in Ussing chambers [415], as does EAST1 [416]. EAEC also frequently secrete a toxin termed Pet, which results in rounding of cultured epithelial cells, possibly by dissolving the cellular cytoskeleton [417]. EAEC infection elicits intestinal inflammation, probably by inducing release of IL-8 from epithelial cells, possibly in response to production of flagellin [418], a defined ligand of toll-like receptor 5 (TLR5), as well as of other factors [419]. EAEC pathogenesis has been thorough reviewed [420].

Epidemiology and prevention

The full spectrum of EAEC-associated diseases is beginning to emerge in North America [297,400,403], Europe [421,422], and developing regions of the world [423,424], with the most sophisticated studies having been performed in Brazil. EAEC are also associated with traveler's diarrhea [420,425]. Most studies have focused on the role of EAEC in children, but a study from Maryland and Connecticut has demonstrated its association with adult diarrhea as well [297]. Specific prevention measures cannot be proposed at this time [411].

Clinical manifestations

Volunteer challenges and cohort studies suggest that EAEC are associated with nonbloody, usually afebrile diarrhea. Prolonged illness is seen in HIV/AIDS patients [426]. A provocative study from Brazil [68] associated fecal EAEC excretion with persistent diarrhea, markers of intestinal inflammation (lactoferrin and IL-8), and growth impairment, whether or not diarrhea was present. This raises the possibility that intestinal colonization with EAEC might underlie stunting in children in resource-poor environments.

Treatment

Because EAEC are not detected in clinical laboratories, the role of antimicrobial therapy in the treatment of these infections remains poorly studied and so evidence-based recommendations cannot be made. Moreover, published studies need to be considered in the context of definitional issues: EAEC have been variably defined, using nucleic acid characterization with or without adherence studies. Ciprofloxacin has been used to treat human EAEC infections in the setting of HIV infection or in traveler's diarrhea [427,428], but many Thai isolates were resistant to a diverse panel of antibiotics [429,430]. In Bangladesh, similar high rates of resistance were noted in collections from the 1980s [431]. EAEC serogroup O44 (a serogroup of EAEC that is found worldwide) in Kenya were resistant to tetracycline, ampicillin, erythromycin, trimethoprim–sulfamethoxazole, and amoxicillin clavulanate, but susceptible to chloramphenicol, nalidixic acid, azithromycin, and cefuroxime [432]. It is concerning that EAEC from returned travelers in Spain demonstrated resistance to fluoroquinolones [433]. Rifaximin shows promise in the treatment of EAEC infections in travelers [434–436]. EAEC were moderately resistant to rifaximin but were inhibited by the high intralumenal concentrations that this agent can achieve [437].

Diagnosis

EAEC are best identified using an epithelial cell adherence assay (typically with Hep-2 cells) [438] (Fig. 48.11) or by DNA hybridization (reviewed in [420]), including several multiplex PCR assays [439,440]. Diagnosis is hampered by the specialized nature of the assays required to detect EAEC and the lack of consensus regarding sensitive and specific target loci that define these organisms. Moreover, 1%–3% [400,441] of control subjects excrete probe-positive EAEC, making it difficult to assign pathogenicity when EAEC are detected in individual cases. Phenotype (adherence assay) positive rates in control subjects can exceed 20% [400].

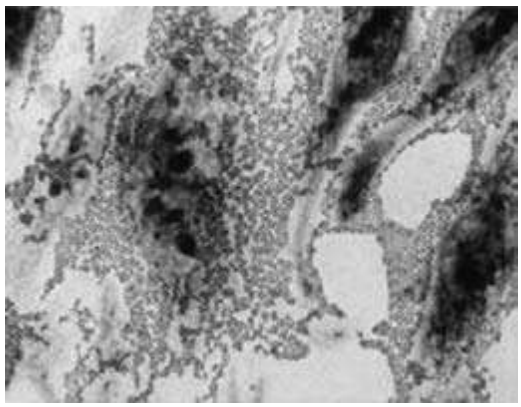
Complications

The complications of EAEC infections, beyond the acute diarrhea, appear to be related to stunting in children in developing countries [68], and irritable bowel syndrome [82]. These consequences of persistent infection warrant further investigation.

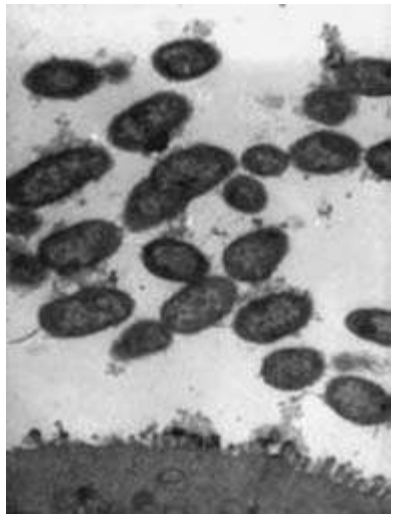
Enteroinvasive E. coli

History

Microbiologically, EIEC resemble *Shigella*, and, in fact, they



(a)



(b)

Figure 48.11 **(a)** Enteroaggregative *Escherichia coli* (EAEC) adhering to epithelial cells. Note the agglomeration of bacteria around the epithelial cells (arrow), which has also been called the "stacked brick" adherence pattern, after 6 h of incubation of bacteria and cells. Also note the accumulation of bacteria at some distance from the eukaryotic cells. From Campos et al. [381], with permission from Memórias do Instituto Oswaldo Cruz. **(b)** Human intestinal in vitro organ culture and EAEC. EAEC adherent to intestinal epithelial cells removed by biopsy from patient undergoing endoscopy. Electron micrograph courtesy of Dr Alan D. Phillips.

were initially considered to be *Shigella*. These organisms were first associated with human illnesses by Ewing and Gravatti [442] in the Mediterranean among Allied soldiers who were treated for presumptive bacillary dysentery. Some strains biochemically atypical for *Shigella* were subsequently demonstrated to be *E. coli*, but the differentiation between *Shigella* and *E. coli* is artificial [443]. Similar organisms were isolated in 1947 from British schoolchildren [444], and these isolates caused diarrhea in human volunteers [445].

Epidemiology and prevention

Most EIEC infections that have been described have been associated with outbreaks. Modes of transmission include foodborne vehicles [446], and waterborne [447] and person to person [448] transmission. In developing countries, EIEC are most frequently identified in children [449]. Specific prevention measures cannot be recommended at this time. There are no vaccines available.

Pathogenesis

The principle virulence property of EIEC is the ability to invade epithelial cells, a trait shared with *Shigella*. EIEC penetrate epithelial cells, lyse the endocytic vacuole, multiply, and extend into adjacent cells [450]. Intracytoplasmic movement is mediated by host cell actin. Most effectors of the invasion phenotype are mediated by a T3SS encoded on a 140-MDa plasmid that is found in EIEC and *Shigella* [451]. Most notably, the IpaB and IpaC proteins are injected into host cells, create pores, trigger cytoskeletal rearrangements, lead to apoptosis, and release inflammatory cytokines. Actin is polymerized via IpaC and translocated IpgD, an inositol 4-phosphatase that dephosphorylates phosphatidylinositol 4,5-bisphosphate into phosphatidylinositol 5-monophosphate, which then accumulates in the host cell. This accumulation uncouples the host cell's plasma membrane from the actin cytoskeleton, leading to extrusions (blebbing) [452]. IpaA binds to vinculin, which induces the polymerization of actin fibers. Once polymerized, the extensions induced by IpaC are transitioned into structures that permit bacterial entry into the eukaryotic cells. EIEC also possess iron acquisition systems [453].

Clinical manifestations

The full spectrum and frequency of EIEC infections are unknown. Although some cases present with a dysenteric picture [442], EIEC cause dysentery only in a small subset of patients in outbreaks [444,446,454] and in experimentally challenged subjects [445].

Treatment

Human EIEC infections are rarely diagnosed and therefore the efficacy of antibiotics is not known.

Diagnosis

The identification of an EIEC is sometimes prompted by the presence of lactose nonfermenting *E. coli* on MacConkey agar. Subsequent methods of assessment of such strains include nucleic acid hybridization, seeking loci associated with invasion, and phenotypic assays, assessing invasiveness. In reality, such testing is not performed in clinical laboratories.

Complications

Complications of EIEC infections, beyond the acute episode, are not well described.

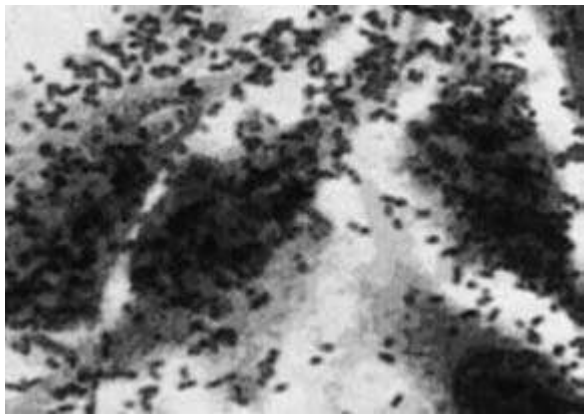


Figure 48.12 Adherence of diffuse adherent *Escherichia coli* (DAEC) to cultured epithelial cells. DAEC adhere at all regions of epithelial cells. From Campos et al. [381], with permission from Memórias do Instituto Oswaldo Cruz.

Diffusely adhering E. coli

History

DAEC characteristically adhere to epithelial cells in a diffuse pattern (Fig. 48.12). This organism was initially associated with diarrhea in case reports [455]. Subsequent work demonstrates a statistically significant association with patients with diarrhea compared with control subjects.

Epidemiology and prevention

DAEC have been implicated as causes of diarrhea in the developed [400,456] and developing world [457]. Specific preventive measures are not available.

Pathogenesis

DAEC are a genetically diverse group and the pathogenetic mechanisms that cause human disease are poorly delineated. The best characterized virulence factors are the Dr family of fimbrial adhesins expressed by many DAEC (and by some uropathogenic *E. coli* as well). These adhesins mediate binding of *E. coli* to a number of cell surface proteins including decay accelerating factor, carcinoembryonic antigen and related cell adhesion molecules, and β1 integrins [458–460]. Dr adhesin-mediated epithelial cell binding is associated with the microtubule-dependent internalization of a small proportion of adherent bacteria, but the relevance of this finding to disease has not been established [461]. Following binding, cytoskeletal alterations are mediated by activation of signal transduction cascades [462], the structures of epithelial brush borders and tight junctions are deranged [460,463], and a proinflammatory response is induced [464,465]. The epidemiology of DAEC infections is emerging. In one study in Chile, DAEC were not associated with diarrhea [457], but in studies from France [456] and Cincinnati [400], DAEC were statistically associated with diarrhea. In the most recent North American study [400] there was an appreciable carriage of DAEC in control children, but these organisms were more often excreted by ill children. The risk of DAEC diarrhea in children appears to increase with age, with stronger associations seen in children over 2 years of age [466]. The characteristics of diarrhea associated with DAEC, however, are not well established.

Clinical manifestations

DAEC tend to be isolated from children with nonbloody diarrhea. Additional details such as association with fever and duration of illness are incompletely identified.

Diagnosis

DAEC diagnosis largely relies on phenotype (adherence to epithelial cells) and DNA hybridization assays. Because these organisms can be carried by control subjects, and because detection methodologies have been exclusively formulated in research studies, useful diagnostic strategies have not emerged.

Treatment

It is not known if antibiotics diminish the duration or severity of illnesses caused by DAEC.

Complications

It is not known if DAEC infections have long-term consequences.

Listeria monocytogenes

History

Listeria monocytogenes was first described as a human pathogen in 1929 when it was recovered from a blood culture and was mistakenly thought to cause infectious mononucleosis [467]. It is typically considered to be an invasive (i.e., systemic) pathogen. When gastrointestinal symptoms occur, they usually occur during, or before, extraintestinal dissemination. During the past 15 years, multiple episodes of gastroenteritis have been attributed to infection with *L. monocytogenes* [468–473].

Pathogenesis

Listeria monocytogenes invades and replicates in epithelial cells and macrophages. It has been used extensively to study actin-mediated motility in eukaryotic systems. *L. monocytogenes* permeates the intestinal barrier via the internalin family of bacterial surface proteins [474], which bind to E-cadherin [475]. Following invasion, *L. monocytogenes* reside in vacuoles and utilize host cell actin polymerization for transport. This organism has served as a model for dissection of the mechanisms of gram-positive pathogenesis (reviewed in [476]), but the intestinal phases of infection are less well delineated. *L. monocytogenes* can survive gastric acidity, but acid suppression increases infectivity [477,478]. After exposure to acid, *L. monocytogenes* undergoes additional invasive

adaptations, a process that possibly increases virulence [479]. Osmotic forces also increase the ability of *L. monocytogenes* to achieve full virulence [480]. *L. monocytogenes* is variably resistant to the bactericidal action of bile [481]. Hsp60 has been proposed to be the epithelial cell receptor for *L. monocytogenes* [482] before the pathogen is internalized into the eukaryotic cell. However, it is not known to what extent these mechanisms contribute to the gastrointestinal symptoms observed in infected patients.

Epidemiology and prevention

Outbreaks of *L. monocytogenes* gastroenteritis have all been foodborne, and implicated vehicles include rice, chocolate milk, vegetables, smoked trout, corned beef, ham, delicatessen meat, and cheese. Risk factors for nonepidemic cases are not known because sporadic cases of gastroenteritis are rarely investigated and *L. monocytogenes* is rarely identified outside of outbreaks. *L. monocytogenes* is ubiquitous in food [483] and human infection is probably a function of inoculum size, *L. monocytogenes* serotype, and host susceptibility. In outbreaks, attack rates often exceed 50%. Inoculum density in the source is variable but usually exceeds 10 000 colony-forming units per gram of ingested vehicle. Serotypes 1/2a and 1/2b predominate in outbreaks. *L. monocytogenes* can be excreted in the stools of humans in the absence of disease [478].

Consumers can diminish the risk of listeriosis by observing food safety guidelines, which are focused on storage temperatures and ready-to-eat foods, including unpasteurized cheeses and milk, ready-to-eat delicatessen foods, and frankfurters [484]. Pregnant women should be especially vigilant. A novel mechanism of control is the addition of *L. monocytogenes*-specific bacteriophages to ready-to-eat foods that might contain this pathogen [485].

Diagnosis

Because *L. monocytogenes* can be carried by healthy people, and in consideration of the labor involved in recovering this organism from the polymicrobial milieu of stool, routine cultures for this agent in sporadic cases are not recommended [486,487]. If *L. monocytogenes* gastroenteritis is suspected, evaluation of stool for the presence of this agent should be discussed with the involved health departments and clinical microbiologists, to assure optimal specimen processing. It is important to inoculate the stool into the appropriate *Listeria* selective media, but clinical and reference or public health laboratories will often need to coordinate efforts to isolate this organism from patient stools and from suspected vehicles of transmission. Recovery of *L. monocytogenes* from sites that are otherwise sterile, such as blood or the central nervous system, would be an alternate way to make a diagnosis, but such cultures are rarely performed in cases of isolated gastroenteritis and probably would not be positive. Culture of the stool can be performed using *Listeria* selective enrichment broths, supplements containing cycloheximide, colistin, acriflavin, cefotetan, and fosfomycin, or PALCAM selective media [488]. For retrospective (i.e., postclearance) diagnosis, antibodies to listeriolysin O might identify recently infected individuals.

Clinical manifestations

The most prominent characteristic of *L. monocytogenes* gastroenteritis is fever. Most patients also have headache. Additional accompanying symptoms include musculoskeletal pain, nausea, and vomiting. In only a small subset is the diarrhea grossly bloody. Diarrhea need not be present in all cases [486]. The clinical characteristics of sporadic cases remain uncharacterized because such cases are rarely diagnosed.

Treatment

Listeria gastroenteritis is usually self-limited and lasts several days. The efficacy of antibiotics in treating infections limited to the gastrointestinal tract is not demonstrated, but some authorities recommend that antibiotics should be given to individuals in outbreaks who are at high risk of disseminated infection or complicated outcomes (such as pregnant women) [486]. By the time outbreaks come to light and the etiological role of *L. monocytogenes* is known, most cases have resolved. For uncomplicated gastroenteritis, trimethoprim–sulfamethoxazole or oral ampicillin or amoxicillin is recommended if one is compelled to prescribe an antibiotic. For invasive cases an aminoglycoside should be combined with ampicillin or amoxicillin. The efficacy of fluoroquinolones has not been established [489].

Complications

The vast majority of cases of gastrointestinal infection with *L. monocytogenes* resolve without dissemination. However, gastroenteritis is a common prelude to systemic infection with *L. monocytogenes*.

Salmonella infections

History

Salmonella were named after United States Department of Agriculture pathologist Dr Daniel E Salmon, who first isolated *Salmonella choleraesuis* from porcine intestine [490]. PCA Louis [491] differentiated typhoid fever as a separate entity based on pathological changes in the intestinal lymph nodes and spleen, and further described the association with rose spots, intestinal perforation, and bleeding [491]. Typhoid fever was further distinguished from typhus by William Jenner, who recognized that Peyer patches and mesenteric lymph nodes were enlarged in typhoid, and that previous infection protected against subsequent attacks in the former condition [492]. The organism responsible for typhoid fever was isolated in 1884 from the spleens of infected patients [493], and in 1896 a heat-killed vaccine was developed [494]. The serotyping of *Salmonella* is based on the presence of specific surface antigens detected by agglutination in response to the

presence of reactive antibodies. The classification system developed by Kaufmann and White in the mid-1900s [495] continues to be used today.

Classification

The genus *Salmonella* is a member of the Enterobacteriaceae family of gram-negative bacilli. The genus is divided into two species, *Salmonella enterica* (formerly *S. choleraesuis*) and *Salmonella bongori*. *S. enterica* is further divided into six subspecies: I – *enterica*; II – *salamae*; IIIa – *arizonae*; IIIb – *diarizonae*; IV – *houtenae*; and VI – *indica*. V is now obsolete as a subspecies of *S. enterica* and is currently designated as a separate species, *S. bongori*. *S. bongori* does not cause disease in humans. Over 2400 serovars have been reported. Serotype designation is based on the presence of specific antigens: O (somatic/cell wall) antigen and flagellar H antigens. Strains are therefore named, for example, as *Salmonella enterica* subspecies *enterica* serotype Typhimurium. The CDC recommends that the species designation be simplified to the genus and serovar only, i.e., *Salmonella* Typhimurium.

Epidemiology and prevention

Nontyphoid Salmonella

The burden of nontyphoid *Salmonella* infection is huge. According to US FoodNet data, the incidence of *Salmonella* infection was 13.4 cases per 100 000 population from 1996 to 1999 [496]; however, a population-based survey demonstrated that reporting was underestimated by a factor of 38.6, translating into an estimated 1.4 million cases of *Salmonella* illness per year. Higher incidences of infection are reported outside the United States. Of greatest concern has been the increase in the number of infections caused by *S. enterica* serovar Typhimurium definitive phage type 104 (DT104). DT104 is typically resistant to ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline and can acquire resistance to ciprofloxacin and ceftriaxone [497]. Reports from around the world indicate that the number of *Salmonella*-induced illnesses, including those caused by DT104, has significantly decreased [498,499]. For over three decades, the incidence of infection by nontyphoidal *Salmonella* has increased but there is now a reversal of that trend [499]. Unfortunately, rates of antibiotic resistance and multidrug resistance are rising. Although over 2000 serovars of *Salmonella* exist, a small minority cause most human illness, predominantly *Salmonella* Enteritidis and *Salmonella* Typhimurium. The predominant serovars isolated from infected humans vary from year to year and country to country [500].

Most *Salmonella* infections are self-limited, but hospitalization might be necessary and death can result. Between 1996 and 1999, the hospitalization rate of *Salmonella* infections was 20% and the mortality rate was 0.6% [496]. In 2000, however, the hospitalization rate decreased to 10.1% but the death rate remained at 0.8% [501]. *Salmonella* causes approximately one-third of the deaths related to foodborne illness, making it the leading cause of such mortality [501]. Salmonellosis in patients with malignancies, immunosuppression, alcoholism, sickle cell disease, cardiovascular dysfunction, and hemolytic anemias is more aggressive and if associated with bacteremia can result in mortality rates of 3% or higher [499].

Salmonella infection is usually acquired by consuming contaminated raw or undercooked food, especially meat, poultry, eggs, and milk. *Salmonella* can be commonly isolated from poultry and various types of farm animals, and is part of the normal flora of reptiles and amphibians. Most infections (85%) are associated with outbreaks, but person-to-person transmission can occur. The highest incidence of infection occurs at the extremes of life; the annual incidence in early childhood is approximately sixfold higher than the average incidence but there is a low incidence in young adulthood [499]. There is seasonal variation with the peak incidence occurring in summer and fall. Proton pump inhibitor use has been associated with an increased risk of infection with DT104 [502].

Nontyphoidal *Salmonella*, in contrast to typhoid, is rarely transmitted from person to person. The indiscriminate use of antibiotics in poultry and farm animals, as well as in humans, needs to be addressed, and mechanisms of antibiotic resistance transfer between microbes more carefully explored to control this problem.

Pathogenesis

The key feature of *Salmonella* pathogenesis is uptake by host cells. Epidemiological studies suggest that as few as 100 viable *Salmonella* can cause clinical infection [503]. The absence of gastric acid is a risk factor for illness because it reduces further the inoculum required for establishment of an active infection. Once past the stomach, *Salmonella* interact with both enterocytes and M cells and are internalized via a complex series of events triggered in the host cell. Important aspects of *Salmonella* pathogenesis are being unraveled. The virulence genes of this pathogen are localized to *Salmonella* pathogenicity islands (SPIs), of which five have been identified. The sequencing of the complete genome of *Salmonella* identified five potential additional SPIs [504]. SPI-1 encodes a T3SS that allows delivery of bacterial effector proteins into host cells. Some SPI-1 effectors include SopE and SopE2, which activate host cell Rho GTPases leading to actin rearrangement and uptake of bacteria (for a detailed review of this process, see [505]). SPI-2 also encodes a T3SS that permits *Salmonella* to survive in host epithelial cells and macrophages. Genes encoded in SPI-3 are believed to permit growth within phagosomes, where concentrations of magnesium are low [506], and SPI-4 is needed for survival within macrophages [507]. Evidence suggests that genes in SPI-5 contribute to intestinal secretion and inflammation. Other *Salmonella* virulence factors have also been reviewed [508]. Of such factors, flagellin deserves mention here. Flagellin,

which comprises bacterial flagella, is now recognized as a major proinflammatory stimulus via its interaction with TLR5 [509,510]. *Salmonella* flagellin is translocated across intestinal epithelial cells to the basolateral surface where it interacts with its receptor TLR5 and triggers the inflammatory cascade by eventually activating NF-κB. In addition, the secreted *Salmonella* effector SipA induces the expression of the eicosanoid hepoxilin A3, which is secreted apically and serves as a chemoattractant for polymorphonuclear leukocytes across the intestinal epithelial layer, resulting in the formation of crypt abscesses [511,512]. The inflammatory response is a major factor in *Salmonella*-induced enteritis; it is now clear that several factors contribute to this response.

Clinical manifestations

Salmonella infection can result in gastroenteritis, enteric fever, bacteremia, or asymptomatic carriage. The symptoms of *Salmonella* gastroenteritis are indistinguishable from those caused by other enteric pathogens and range from a few loose stools to a cholera-like illness with profuse diarrhea and significant dehydration or even dysentery. Symptoms including fever and chills, abdominal cramps, nausea, and vomiting typically develop within 48 h following ingestion of contaminated food or water. *Salmonella*-associated fever usually resolves within 2–3 days but diarrhea persists for 3–7 days. Nontyphoidal *Salmonella* typically persist in the stool for 4 or 5 weeks following resolution of gastroenteritis [513]. Bacteremia occurs in 2%–4% of patients [514] but is more frequent in children under the age of 1 year, adults over 50 years, and patients with underlying health problems including cancer, lymphoproliferative disorders, HIV/AIDS infection, valvular heart disease, hemoglobinopathies, transplant recipients, and those taking steroids.

Complications

Serious complications most commonly occur in infected patients with underlying disorders. Bacteremia and focal abscesses, which can result in septic shock, are complications seen in patients with AIDS and those with malignancies or receiving therapy for malignancies. Finally, infected aortic or iliac aneurysms in patients with preexisting atherosclerotic vascular disease can result in mortality rates of over 60%. Pleuropulmonary complications, including empyema, have also been reported and again occur most commonly in the immunocompromised [515].

Diagnosis

The diagnosis of *Salmonella* as the etiological agent of gastroenteritis is made by identifying this organism in fecal cultures or, on rare occasions, cultures of the blood or other extraintestinal sites. Frequently, local laboratories will determine that *Salmonella* is or is not *Salmonella* Typhi, but more extensive classifications of isolates are usually performed in public health reference laboratories.

Treatment

Antibiotic therapy is not recommended for otherwise healthy individuals with uncomplicated *Salmonella* gastroenteritis. Antibiotics can not only prolong the carrier state or even cause relapse but also they typically do not abbreviate the duration of diarrhea [514]. In contrast, those with predisposing factors that are associated with higher incidences of complications of *Salmonella* infection should receive antimicrobial therapy. This includes individuals under 1 and over 50 years of age and those with artificial joints or vascular grafts, degenerative joint disease, valvular heart disease, or immunocompromising conditions. Patients demonstrating signs or symptoms of sepsis or bacteremia should also receive antibiotic therapy for *Salmonella* infection as should those with local extraintestinal infections such as osteomyelitis and pleuropulmonary infection. Treatment with trimethoprim–sulfamethoxazole or fluoroquinolone, if susceptibility is demonstrated, is recommended; ceftriaxone is also effective [514]. Infants with *Salmonella* infection who are under the age of 3 months should be treated with antibiotics [516].

Drug resistance among *Salmonella* species has been increasing [517]. In particular, DT104 (see Epidemiology and prevention above) has emerged as a significant problem in North America and elsewhere. It is therefore important to determine the antibiotic susceptibility of strains isolated from infected individuals instead of resorting to empiric therapy, although antibiotics sometimes must be started before the results of susceptibility testing are known.

Salmonella *Typhi (typhoid [enteric] fever)*

Typhoid fever, a systemic disease caused by infection with *S. enterica* serotype Typhi, is more appropriately referred to as enteric fever, because this category also includes disease caused by serotype Paratyphi A, B, and C. Although *Salmonella* Typhi is responsible for 80% of cases in endemic countries, infection by *Salmonella* Paratyphi is more common among travelers [518], probably because vaccination is available and effective only for *Salmonella* Typhi. Humans are the sole reservoir for these organisms, thus the disease is transmitted from person to person or by ingestion of food that has been contaminated by an acutely infected individual or a chronic carrier. Drinking water contaminated by feces of infected individuals is also a source of infection.

Epidemiology and prevention

The worldwide prevalence of enteric fever might be as high as 30 million cases [519]. The epidemiology of this infection in developed countries, where it was once endemic, has changed dramatically over the past 50 years with a marked decrease in the incidence and a shift to cases now being associated with travel to specific geographic regions. In the 1940s the incidence of this infection was 7.5 per 100 000, but this fell to 0.2 per 100 000 in the 1990s [520]. In the late 1960s and early 1970s only 33% of cases were associated with

travel, but by the late 1990s, 80% of cases were related to foreign travel [520]. Travel to six countries accounts for 80% of enteric fever cases: India, Mexico, Philippines, Pakistan, El Salvador, and Haiti [520]; however, the risk of acquiring enteric fever is 18 times higher with travel to the Indian subcontinent than with travel to any other area [521]. Risk factors for acquisition of enteric fever by travelers include travel to rural areas, failure to adhere to food and water precautions, and the lack of education regarding such issues prior to travel. Increased age and length of stay are also associated with enhanced risk of infection [522]. Immigrants returning to their homeland are also at increased risk. They may not be aware of this and are thus less inclined to follow food and water precautions and receive vaccination for typhoid and are more likely to travel to rural areas.

In endemic areas, children and adolescents are the primary targets of disease, accounting for 90% of cases, but there is no age specificity amongst travelers. Travelers typically ingest less of an inoculum as a result of hygiene precautions; in addition, travelers are more likely to seek and have access to medical care and hence receive treatment early in illness.

Food and water precautions when traveling are crucial for preventing this infection. In addition, enteric fever caused by *Salmonella* Typhi, but not *Salmonella* Paratyphi, can be prevented by immunization. There are two types of vaccine, an injectable vaccine, which is a conjugate of *Salmonella* Typhi and recombinant *Pseudomonas* exotoxin A [523], and a live attenuated oral vaccine, which requires four doses taken every other day for 4 days [524]. The parenteral vaccines provide a circulating antibody response whereas the oral live vaccine induces a secretory IgA response and cell-mediated immunity. The efficacy of these vaccines in travelers is difficult to establish and so most trials have been performed in endemic countries, indicating approximately 55%–75% efficacy [521]. It should be noted, however, that ingesting a large inoculum of viable organisms can overcome typhoid immunity, underscoring the need to avoid contaminated food and water. A booster series of four capsules is recommended for the oral vaccine every 5 years and for the parenteral vaccine every 2 years.

Pathogenesis

Following ingestion, *Salmonella* Typhi survive gastric acid by means of a two-component system that includes induction of a pH homeostasis system and expression of acid shock proteins; together, these systems permit survival in low pH [525,526]. Once in the small intestine, *Salmonella* Typhi invade M cells, are engulfed by mononuclear cells in the underlying lymphoid tissue, and disseminate via lymphatics or the bloodstream. Intracellular multiplication proceeds in reticuloendothelial cells and macrophages in the liver, spleen, lymph nodes, and bone marrow. Infected individuals are asymptomatic during this phase. Once the concentration of *Salmonella* Typhi in these tissues is sufficient, organisms gain access to the systemic circulation resulting in the production of cytokines and symptoms in the host. It is during this phase of persistent bacteremia that extraintestinal organs are seeded and complications ensue.

Clinical manifestations

The typical incubation period of *Salmonella* Typhi is 7–14 days but it can range from 3 to 60 days. Clinical manifestations can be protean and include anorexia; abdominal pain, tenderness, and bloating; nausea and vomiting; diarrhea; and bloody diarrhea [527]. Enterocolitis, with diarrhea lasting several days, can develop but usually resolves before the onset of fever. The bacteremic phase follows this asymptomatic incubation phase and is manifest as an acute febrile illness accompanied by flu-like symptoms including frontal headache, general malaise, prostration, confusion, anorexia, and abdominal discomfort and bloating. Some individuals present with upper respiratory symptoms, such as dry cough, coryza, and sore throat, thus delaying the diagnosis. Constipation can occur early in illness but generally is followed by diarrhea in the late phase of the illness. The fever pattern is interesting in that it can increase over the initial 5–7 days with body temperatures peaking in the afternoon. Relative bradycardia for the degree of fever is considered common for typhoid fever but is certainly not specific for this infection. Blood cultures are positive in more than 90% of patients during this initial phase of fever and nonspecific symptoms. A longer period, 10–14 days, of sustained high fevers (39°C–41°C) can follow but medical attention is usually sought earlier in the course of infection, particularly by travelers, and so this phase is typically not seen in this group. The liver and spleen are frequently enlarged and abdominal tenderness is often present, particularly over the lower quadrants (at times mimicking appendicitis) and liver. Rose spots, which are 2- to 4-mm erythematous maculopapular lesions that blanch, have been reported in up to 30% of patients and occur within the first 1–2 weeks but last only 3–4 days. These typically are seen on the chest and abdomen but can be missed in individuals with darker skin. Symptoms begin to abate after the third week of untreated illness in the 90% who survive. Relapse has been reported to occur in 3%–13% of untreated patients and about 20% of treated patients, on average after 2 weeks have elapsed. The illness associated with relapse tends to be milder and shorter than the initial manifestations.

Complications

Intestinal hemorrhage and perforation related to hyperplasia of the lymphoid tissue in the ileocecum can occur. This is typically observed in untreated individuals in the third to fourth week of illness. The extraintestinal complications associated with *Salmonella* Typhi can target almost any organ system including the central nervous system (3%–35%), cardiovascular system (1%–5%), pulmonary system (1%–6%), bones

and joints (< 1%), hepatobiliary system (1%–26%), genitourinary system (< 1%), soft tissues, and hematological system (rarely). This topic has been extensively reviewed [519]. Specific complications include encephalopathy, cerebral edema, meningitis, seizures, GBS, endocarditis, myocarditis, pneumonia, empyema, osteomyelitis, septic arthritis, cholecystitis, hepatitis, hepatic abscess, splenic abscess, peritonitis, urinary tract infection, renal abscess, testicular abscess, prostatitis, and epididymitis.

The chronic carrier state is defined as excretion of the organism for 1 year or longer and occurs in 2%–5% of patients with *Salmonella* Typhi and *Salmonella* Paratyphi infections. Risk factors for becoming a chronic carrier include age less than 1 year, age older than 60 years, concomitant biliary disease, and presence of schistosomiasis. The biliary tract commonly serves as a focus of *Salmonella* organism multiplication in typhoid carriers, especially if concomitant abnormalities, such as stones or obstruction, are present.

Fatality rates can be as high as 30% in individuals from endemic countries; the fatality rate in travelers, however, is reported as less than 0.4% [520].

Diagnosis

Enteric fever is diagnosed by isolating the causative agents from blood or stool. Culture of the bone marrow is successful in recovering these pathogens from 90% of individuals who have received antibiotics [528]. *Salmonella* Typhi and *Salmonella* Paratyphi are also quite frequently isolated from rose spots.

Treatment

Ceftriaxone is currently the recommended first-line therapy for *Salmonella* Typhi and *Salmonella* Paratyphi infections [519,521]. If fluoroquinolone susceptibility is demonstrated than these agents can be used as well, but they are not approved for use in children in the United States. Alternative therapies are based on antibiotic susceptibility data of recovered strains. The frequency of multidrug-resistant *Salmonella* Typhi in the United States has increased significantly over the past two decades, with 30% being resistant to at least one antibiotic and 12% demonstrating resistance to chloramphenicol, ampicillin, and trimethoprim–sulfamethoxazole. Such strains require longer treatment with expensive agents that can be less effective, thus increasing the risk of carrier status.

High-dose dexamethasone should be given to individuals with severe typhoid fever. Perforation is one of the most serious intestinal consequences of typhoid fever and warrants surgery [529].

Shigella species

History

Shiga bacillus, now known as *Shigella dysenteriae* type 1, was originally isolated and described by Kiyoshi Shiga in 1898 [530]. As a young physician, Shiga investigated the etiology of the *sekiri* (red diarrhea) epidemic of 1897 in Japan, which affected over 90 000 individuals and had a mortality rate of greater than 20% [531]. He isolated a gram-negative bacillus from the stools of afflicted patients that, when subcultured and orally administered to dogs, caused diarrhea. Initially named *Bacillus dysenteriae*, this organism was also determined by Shiga to produce toxic factors including the now well-characterized Shiga toxin. Following the initial report of this organism, others identified related strains now known as *Shigella flexneri*, *Shigella boydii*, and *Shigella sonnei* for those responsible for their description – Flexner, Boyd, and Sonne respectively [531]. The genus was renamed in 1930. It is plausible that the "emerods" that "smote" the encampment of Philistines at Ashdod [532] were rectal prolapses secondary to shigellosis, which is notorious for epidemics appearing during military campaigns through the ages. However, some scholars believe that emerods were the buboes of epidemic *Yersinia pestis* infection [533].

The genus *Shigella* belongs to the tribe *Escherichia* in the family Enterobacteriaceae and consists of four species or groups (A–D) of gram-negative, aerobic rods: *S. dysenteriae* (group A), *S. flexneri* (group B), *S. boydii* (group C), and *S. sonnei* (group D). Each species, except for *S. sonnei*, is divided into several serotypes, defined by the somatic O antigen. Only *S. flexneri* is further divided into subtypes. The distribution of *Shigella* species worldwide correlates with the level of development and hygiene, the basis of which is not well understood. *S. dysenteriae* serotype 1, a particularly virulent serotype associated with significant morbidity and mortality, occurs in less developed countries and has pandemic potential. *S. flexneri* are also most commonly isolated in developing countries, especially those with tropical climates. *S. sonnei*, which causes mild cases of watery diarrhea, accounts for most cases of *Shigella* infection in industrialized nations. *S. boydii* is rarely isolated; however, analysis of isolates from Canada and the United States in 2005 showed that they were a new *S. boydii* serotype [534].

Epidemiology and prevention

Humans are the only known reservoir of *Shigella*. Although this single reservoir should offer an advantage for their control, *Shigellae* remain one of the most common causes of bloody diarrhea worldwide. This paradox is likely explained by their exquisitely low infectious dose. Contributing to their survival is the emergence of antibiotic-resistant strains and their resistance to gastric acid. Transmission is predominantly fecal–oral, but person-to-person transmission via contaminated hands and drinking water and food can occur through fomites, food, and water [535]. The common housefly can spread *Shigella*, particularly in areas where the disposal of human waste is inadequate [536]. Approximately 165 million cases of *Shigella* infection are estimated to occur annually worldwide [537], 163 million of which occur in developing countries. In the United States, approximately 15 000 cases

are reported annually [538], but the actual number is likely to be higher. Untreated *Shigella* can be severe and fatal [539], although the mortality rate is falling [540]. Most infections occur in children under 5 years of age (69%), and most deaths (61%) also occur in this age group [537].

In industrialized countries, shigellosis is primarily a problem in populations exposed to substandard hygiene in group settings, in particular day-care centers, custodial institutions, and nursing homes. Travelers to developing countries are also at greater risk for infection. *Shigella* causes an estimated 4% of traveler's diarrhea [541,542]. Although much less common than infections by ETEC, traveler's diarrhea caused by *Shigella* is more severe.

Shigella is highly contagious, requiring a minute inoculum to establish infection. Ingestion of as few as 180 organisms caused diarrhea in 22% of healthy volunteers [543]. *Shigella* persists as a pathogen because fecal organisms in patients suffering acute shigellosis are abundant (approximately 10^9 per gram of stool).

Frequencies of infection by *Shigella* spp. vary by country and population. In a study from Calcutta, India, *S. flexneri* was the most commonly isolated species (60%), followed by *S. sonnei* (24%), *S. dysenteriae* (10%), and *S. boydii* (6%) [544]. An important observation from this study was the reemergence of *S. dysenteriae* serotype 1 after a 14-year absence; this strain exhibited multidrug resistance including to fluoroquinolones. Increased drug resistance in *S. flexneri* type 2a was also noted. In contrast, in Sweden, *S. sonnei* was the most commonly isolated species (67%), with *S. flexneri* accounting for 26%, *S. boydii* 5%, and *S. dysenteriae* 3% of infections [545]. Of greatest concern is *S. dysenteriae* serotype 1 because of the severity of the associated clinical symptoms, potential for serious complications, and the propensity of this strain to cause pandemics. In the face of emerging antibiotic resistance these traits present a major health concern [546], as highlighted by epidemics of resistant dysentery in Asia and Africa.

The major route of *Shigella* transmission is person-to-person. This is in large part because of poor handwashing and toileting practices and inadequate sanitation. Together, these can lead to the contamination of drinking water and food. Preventive interventions include the use of soap and water for handwashing, decreased contact with human excreta, disinfection of water, and overall improved sanitation. Although a *Shigella* vaccine is needed, the antigenic complexity of this species and the poor understanding of protective immunity for *Shigella* have made this a difficult task. Nonetheless, the safety and immunogenicity of serotype-specific protection with conjugate vaccines consisting of *S. flexneri* and *S. sonnei* lipopolysaccharide coupled to succinylated mutant *Pseudomonas aeruginosa* exotoxin A have been demonstrated in children and adults [547,548]. This approach has considerable promise in situations in which diseases are caused by a limited number of pathogenic serotypes. *Shigella* vaccine strategies have been reviewed [549].

Pathogenesis

The pathogenesis of *Shigella* is multifactorial and includes the production of enterotoxins (important but not essential) and its ability to invade and destroy host tissues, resulting in large part from the extensive inflammatory response associated with this infection. *Shigella* are believed to penetrate the intestinal epithelial barrier through M cells that overlie lymphoid follicles, allowing them to reach the basolateral aspect of intestinal epithelial cells where they can invade. Once internalized, the bacteria multiply rapidly and disseminate to adjacent cells. The host protein Nod1 recognizes peptidoglycan fragments of *Shigella*, leading to activation of the proinflammatory transcription factor NF-κB [550]. Approximately 30 bacterial gene products are involved in *Shigella* invasion and its intercellular spread, and these proteins are encoded on large virulence plasmids present in all *Shigella* species [551]. Some of these encoded proteins comprise a T3SS, a multiprotein complex that allows direct delivery of bacterial proteins into host cells. Recognition of host cell components by bacterial sensors can trigger the secretion of bacterial factors through this apparatus. A complex series of host cell signals ultimately leads to bacterial invasion and spread. The ability of *Shigella* to invade and colonize the intestinal epithelium is crucial to the establishment of disease and associated morbidity.

Shigella species also elaborate toxins that play a role in disease including enterotoxins, cytotoxins, and neurotoxins. The classic *Shigella* toxin, Shiga toxin (Stx), is produced by *S. dysenteriae* serotype 1. The 70-kDa Stx consists of one catalytic A subunit and five B subunits. As with EHEC Stxs, the B subunits bind the toxin to host receptors, a process that is common to many bacterial toxins. The Stx receptor, globotriaosylceramide (Gb_3), is internalized by receptor-mediated endocytosis following toxin binding [552]. Once internalized, the catalytic A subunit is released, leading to cytotoxicity via inhibition of protein synthesis. The absorptive capacity of the intestinal epithelium is impaired, contributing to diarrhea. Stx does not activate adenylate or guanylate cyclase, as other bacterial enterotoxins do, but it is biologically similar to Stxs secreted by EHEC. Similarly to these toxins it can affect renal endothelial cells, resulting in HUS, although the risk of HUS is lower than with EHEC [553].

Other *Shigella* enterotoxins have been identified. ShET1, a 55-kDa holotoxin that also has the A–B subunit structure, is unique to the *S. flexneri* 2a serotype among *Shigellae*. As with Stx, the gene encoding this protein is located on the chromosome. The presence of such a toxin was predicted by studies in monkeys showing that small intestinal secretion accounted for the watery diarrhea seen early in shigellosis [554]. ShET2, a 64-kDa holotoxin expressed by 80% of diverse *Shigella* serotypes, also contributes to the watery diarrhea associated with infection. Unlike Stx and ShET1, ShET2 is encoded by a gene on the large virulence plasmid that confers invasion [555]. Another *Shigella* secreted protein,

designated SigA, has both cytopathic and enterotoxic activity [556]. SigA, a 103-kDa product encoded within the chromosomal *she* pathogenicity island of *Shigella*, has serine protease activity, likely accounting for its toxic effects.

Invasion is more important than toxin production in the pathogenesis of shigellosis. Infection of macaques with isogenic mutants of *S. dysenteriae* that lacked either the invasion plasmid or the Stx gene demonstrated that the invasive but nontoxigenic strain induced disease which was indistinguishable from wild-type *Shigella* except that the diarrhea caused by the Stx-expressing strains was bloodier [557]. A correlation between the level of Stx production and gastrointestinal bleeding has been suggested in humans [558]. However, non-Stx producing *Shigella* can also cause notable extraintestinal complications, including seizures [559].

The cardinal feature of *Shigella* is its ability to invade the host intestinal epithelium. This is initially achieved by invasion of M cells, the specialized epithelial cells strategically positioned over lymphoid follicles. After entering M cells, the internalized organisms traverse these cells and emerge into the gut-associated lymphoid tissues (GALT) of the terminal ileum and colon where they are engulfed by macrophages. In contrast to the usual scenario whereby engulfed organisms are destroyed, *Shigella* induce macrophage apoptosis [560], releasing IL-1β [561] and thereby recruiting neutrophils to and across the intestinal epithelial layer. Intercellular tight junctions are breached and lumenally situated *Shigella* directly gain access to the subepithelial space and basolateral pole of the host intestinal epithelial cell, the only membrane domain through which these microbes can invade [562,563]. *Shigellae* multiply intracellularly and then disseminate into adjacent cells throughout the intestine and destroy the epithelium [564]. This marked inflammatory infiltrate and tissue destruction characterize shigellosis.

Clinical features

The incubation period for *Shigella* is 1–4 days and can be up as long as 8 days for *S. dysenteriae*. Most individuals infected with *Shigella* have a self-limited course lasting 5–7 days in adults and 2–3 days in children. Some adults, however, experience a subacute presentation characterized by 2–3 weeks of waxing and waning diarrhea. Such a presentation can mimic new-onset ulcerative colitis, especially because the endoscopic appearance of the mucosa (erythema with small ulcers) is quite similar [531]. In such cases it is prudent to perform stool cultures and, if *Shigella* is isolated, treatment with appropriate antibiotics should rapidly resolve the symptoms. A long-term carrier state for *Shigella* has been described, defined as fecal shedding following the resolution of symptoms from acute dysentery caused by *S. sonnei* and *S. flexneri,* but is rare [565,566].

Shigella dysentery is characterized by acute diarrhea containing blood and mucus accompanied by fever and abdominal pain. The presentation can occur in two phases, the initial phase characterized by the abrupt onset of frequent (every 20–30 min) watery diarrhea, which is followed by the second, or dysenteric, phase 12–72 h later. In this phase the passage of small amounts of blood-tinged mucus with or without blood clots occurs. Approximately half of all patients infected with *Shigella* experience grossly bloody diarrhea. Fever develops 1–3 days postinfection and is then often followed by abdominal cramps and diarrhea within 24 h. In most patients the diarrhea resolves after 7 days but symptoms can persist for up to 30 days. A much more severe clinical course can be seen in children, including fever, abdominal pain, malaise, vomiting, watery or bloody diarrhea, and tenesmus [567].

The physical examination often demonstrates only lower abdominal tenderness, and bowel sounds are normal or increased. More alarming physical findings, such as rebound tenderness or ileus, are not typical of *Shigella* infection and should suggest an alternate diagnosis. Dehydration can occur in young children, especially with prolonged (48 h) diarrhea and vomiting; in general, however, the fluid loss in shigellosis is not voluminous. Leukocytosis is unusual but is more likely to be seen in patients, both adults and children, with severe rectal histology [568]. The differential white cell count frequently demonstrates a relative "bandemia."

Complications

Shigella bacteremia is an uncommon but severe complication of *Shigella* dysentery, occurring most frequently in malnourished children, the immunocompromised, or patients over 65 years of age. *Shigella* bacteremia is probably underappreciated and usually is not associated with metastatic infections [569]. The mortality rate of shigellosis is falling, even in disease caused by *S. dysenteriae* serotype 1 [553].

Reiter syndrome, a triad of arthritis, urethritis, and conjunctivitis, occurs most often after bacillary dysentery in men aged 20–40 years who express the human leukocyte antigen (HLA)-B27 phenotype. Approximately 1%–2% of infected individuals develop Reiter syndrome [566,570,571], 80% of whom are HLA-B27 positive [571]. Of the HLA-B27-positive individuals who suffer bacillary dysentery caused by any agent, approximately 20% develop Reiter syndrome.

The clinical presentation of Reiter syndrome can be quite variable, with mild forms being common [572] and genitourinary symptoms being absent or minimal. Patients often present with asymmetric lower extremity arthritis or periarthritis 2–4 weeks following shigellosis or other bacterial dysenteries. By this point in the illness, intestinal symptoms have usually resolved and antibiotic treatment is not indicated. The arthritic symptoms tend to be chronic and relapsing, even in the absence of dysentery. Reiter syndrome is treated symptomatically with nonsteroidal antiinflammatory drugs.

HUS, most often associated with EHEC infections, is also a recognized complication of *S. dysenteriae* serotype 1 infection;

it does not follow infections with non-*dysenteriae* species because they do not make Stx. The leukemoid reaction during the diarrheal phase of *S. dysenteriae* serotype 1 infections can be a harbinger of HUS [573]. Shiga toxin and Stx1 of *E. coli* are nearly identical structurally. HUS following *S. dysenteriae* serotype 1 infection has not been as extensively studied as HUS following *E. coli* O157:H7 infection, but it is likely that there is a similar series of pathophysiological events leading to microangiopathic hemolytic anemia, thrombocytopenia, and renal failure.

Additional complications of all forms of shigellosis include hyponatremia, hypoglycemia, toxic megacolon, seizures, and rectal prolapse. *Shigella* can infect a number of extraintestinal organs, including the eyes, joints, heart, liver, skin, and nervous system [535], and can cause Ekiri syndrome, which is a *Shigella*-associated encephalopathy [574]. Unlike EHEC infections, currently available data strongly suggest the safety and efficacy of antibiotics in *Shigella* infections [553].

Diagnosis

Confirmation of *Shigella* infection requires isolation and identification of the pathogen by stool culture. Stool cultures were positive as early as 3 days following oral challenge in volunteer studies and, if untreated, remained positive for an average of 27 days [575,576]. Interestingly, not all patients with positive stool cultures suffered symptoms. Conversely, some volunteers with classic symptoms of *Shigella* dysentery had negative cultures; thus, a negative stool culture does not exclude the diagnosis. To optimize the isolation of *Shigella*, liquid stool should be collected in a sterile container and inoculated as soon as possible on media selective for *Shigella* and *Salmonella*. The presence of fecal leukocytes and gross blood greatly enhance the likelihood that a stool specimen will be positive for a bacterial pathogen. Only 20% of stool cultures from adults with acute diarrhea were positive if performed nonselectively whereas the yield increased to 75% or more if the specimen contained leukocytes [577]. A report of bloody diarrhea should also prompt a search for *E. coli* O157:H7 and other bacterial enteric pathogens.

Treatment

Antibiotics shorten shigellosis and reduce the duration of excretion of the organism. Antibiotics, therefore, are usually indicated, especially if the patient is still symptomatic when the culture results are reported back as being positive or if the patient is likely to spread infection in the convalescent phase (e.g., a child returning to day care, health-care workers, or food handlers) or is medically fragile (e.g., the elderly, malnourished, and those with comorbidities including HIV). In all cases, treatment of dehydration with either oral rehydration solutions or intravenous fluids in cases of mild to severe diarrhea is important. Antidiarrheal medications, on the other hand, can prolong symptoms and delay clearance of the organism and are not recommended [578].

Treatment recommendations for shigellosis change frequently because of the emergence of multidrug resistant strains. *Shigellae* are frequently resistant to ampicillin, nalidixic acid, sulfonamides, tetracyclines, and trimethoprim–sulfamethoxazole, hence the choices for the treatment of shigellosis are severely limited. Ideally, selection of antibiotic therapy should be dictated by culture and antibiotic susceptibility data, but such testing requires at least 2 days. Therefore, antimicrobial choices are usually made empirically but this requires an informed decision based on local antibiotic resistance patterns. A workshop organized by the WHO and a number of other international agencies recommended that ciprofloxacin be the first-line agent for the treatment of shigellosis. In one study, ciprofloxacin led to the resolution of disease or significant symptomatic improvement in 95% of patients with severe disease [579]. Of concern, however, is the report that resistance to fluoroquinolones has also emerged following the extensive use of these drugs for shigellosis. In Calcutta, for example, resistance of *S. dysenteriae* serotype 1 to fluoroquinolones increased from 15% in 2003 to 25% in 2004 [544]. Azithromycin and pivmecillinam have been reported to be effective against fluoroquinolone-resistant *S. dysenteriae* type 1 [580–582], and the third-generation cephalosporins, ceftriaxone and cefixime, are safe and effective in children [579], although concerns have been raised about treating shigellosis with cefixime [583]. If susceptibility to trimethoprim–sulfamethoxazole or ampicillin is demonstrated, these agents can also be used. For mild infections, shorter courses of therapy may be adequate, especially for non-*dysenteriae Shigellae* [584]. Recommendations for treatment of *Shigella* infections warrant frequent review given the rapidity of change in patterns of antimicrobial resistance.

Vibrio infections

Vibrio cholerae

History

Vibrio cholerae is an ancient scourge [585,586]. Initial descriptions arose in southern Asia, which is a region in which epidemics persist. Cholera is also prone to cause pandemics that extend beyond the endemic region. Cholera was the basis for the classic 1854 epidemiological study of John Snow, which demonstrated its strong association with contaminated drinking water at the Broad Street pump [587], but its causative agent, *V. cholerae*, was not identified until 1884. North America was not protected from cholera; cholera was the first reportable disease mandated by the New York Board of Health in the mid-1800s [588]. The world is in the midst of the seventh pandemic of cholera, which began in 1960. This particular pandemic strain is a biotype of *V. cholerae* serogroup 01, first isolated in 1905 in El Tor, Egypt and termed the El Tor strain [586]. A new serogroup of *V. cholerae* emerged in 1992, designated O139 Bengal, which has coexisted with the present El Tor strain in Asia.

Epidemiology and prevention

Cholera is typically a waterborne infection. Foodborne transmission can occur but such episodes are usually related to contamination of food with water that contains *V. cholerae*. Cholera occurs in one or two peaks per year in endemic areas, usually coincident with torrential rains [589–591]. Its interepidemic reservoir is unknown. One novel mechanism of pathogen control might be environmental cholera-specific bacteriophages, which reside in the lysogenized state in nonpathogenic aquatic vibrios [592]. The infectious dose needed to cause cholera is generally higher than that of other bacterial enteric pathogens. Children are disproportionately affected in endemic areas, but, when cholera is newly introduced into a population, it affects persons of all ages similarly [590]. Because *V. cholerae* is transmitted chiefly via water, reducing exposure to contaminated water and ensuring that all water undergoes a reliable bactericidal process before consumption are especially important control measures. However, infrastructures in countries where *V. cholerae* is prevalent are often insufficient to ensure that these important measures are adhered to.

Several oral cholera vaccines are available or under consideration. In the Dukoral vaccine (SBL Vaccine AB, Sweden), four different heat-inactivated *V. cholerae* strains are coadministered with a recombinant cholera toxin (CT) B subunit (which is a potent mucosal adjuvant); this vaccine requires two doses for optimal efficacy [593]. The second available vaccine is a live, attenuated strain (CVD103HgR) [594], which can be given as a single dose (Orochol, manufactured by CSL, Australia). These oral vaccines do not confer absolute protection and new formulations are being considered (reviewed in [595]).

Pathogenesis

Vibrio cholerae has two circular chromosomes and all known virulence factors are located on chromosome I, which is 3 Mb in size [596]. The principal virulence trait of *V. cholerae* is its ability to produce CT. CT is a classic AB_5 toxin, with the B subunit homopentamer mediating cellular binding. The 27.5-kDa A subunit is actually a disulfide-linked dimeric moiety. The CT A_1 fragment possesses ADP-ribosylating activity, whereas the CT A_2 fragment associates the CT A subunit with the B subunit pentamer. The 84-kDa holotoxin attaches via its B subunit pentamer to the GM_1 ganglioside (Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-1ceramide) on eukaryotic cell surfaces, which is concentrated in lipid rafts [597], and endocytosis follows binding of the holotoxin. It is possible that various cell types utilize different internalization mechanisms [595,598]. After internalization, CT undergoes retrograde transport to the endoplasmic reticulum via actin-associated lipid rafts [599]. After reaching the endoplasmic reticulum, the A subunit dissociates from the pentameric B subunit. The A subunit is relatively resistant to degradation and, after it arrives in the cytoplasm, it catalyzes ADP-ribosylation of a G-stimulatory component of adenylate cyclase. Adenylate cyclase then remains bound to GTP and its activity is thereby enhanced. The resulting elevated levels of cAMP augment chloride secretion by enterocytes and diminish sodium uptake. The chloride and accompanying bicarbonate secretion also cause sodium outflow accompanied by water secretion, resulting in the profound net secretory injury that typifies cholera. The augmentation of chloride secretion is most prominent in crypt cells, and inhibition of sodium chloride reabsorption predominates in villus cells. The resulting watery diarrhea contains excessive sodium, chloride, bicarbonate, and potassium, but is not an inflammatory process.

CT is also postulated to have effects independent of its role as a secretory toxin, the most notable of which is its effect on enterochromaffin cells, releasing serotonin, which increases secretion of vasoactive intestinal polypeptide [600,601].

V. cholerae has additional virulence mechanisms. The toxin-coregulated pilus (TCP) permits colonization of the small bowel by allowing the formation of microcolonies [602]. Genes encoding TCP, and the cholera toxin operon, are located on the same bacteriophage. This bacteriophage can infect nontoxigenic *V. cholerae*, rendering the recipient organism toxigenic. However, among the large number of *V. cholerae*, only strains expressing O antigens 1 and 139 are major human pathogens. The genetic complement of the O1 and O139 strains are largely similar, suggesting commonality of virulence loci. Non-O1 or non-O139 *V. cholerae* can occasionally be pathogenic but cause neither epidemics nor pandemics.

V. cholerae produces several additional toxins that might play roles in pathogenicity. Accessory cholera enterotoxin [603] stimulates calcium-dependent chloride and bicarbonate secretion [604]. Zonula occludens toxin [605] disrupts intestinal tight junctions via protein kinase C-dependent reorganization of actin [606].

Treatment

The mainstay of cholera treatment is correcting the often profound hypovolemia and replenishing continuing fluid losses. Usually, this can be performed by oral rehydration using fluids that contain carbohydrates and electrolytes, the efficacy of which was first reported nearly four decades ago [48]. The exact composition of the rehydration fluid with respect to osmolarity [47,49] is controversial (see Table 48.1 for concentrations of oral rehydration solutions that have been used). Fluids administered intravenously or via nasogastric tubes can be utilized if oral intake is inadequate. Severely dehydrated patients can require replacement of 10% of their body weight within the first few hours of treatment; the extent of dehydration in this illness should not be underestimated. Hydration should be maintained until fluid losses abate. An oral antibiotic should be administered to patients as soon as vomiting stops. Doxycycline administered as a single dose to adults often suffices [607]. Novel

future therapeutics might include small molecular interventions that target the expression of one or more virulence traits [608].

Diagnostic microbiology

Vibrio cholerae is a gram-negative organism that is best detected by plating on TCBS agar. It can also be detected by oxidase screening of blood agar plate growth. Media that support the growth of vibrios but which suppress most other flora, such as alkaline peptone water, can be used as a preplating enrichment step. Resulting colonies are characterized as expressing the O1 or O139 antigens.

Noncholera vibrios

Vibrios other than *V. cholerae* can cause human gastroenteritis. *Vibrio vulnificus*, which usually causes extraintestinal infections such as bacteremia or skin ulcerations, can cause gastroenteritis without or before other symptoms [609]. *Vibrio parahaemolyticus* has been the most common cause of seafood-associated gastroenteritis in the United States [610]. In an outbreak of diarrhea caused by *Vibrio parahaemolyticus* in Alaska, additional signs and symptoms included abdominal cramps (82%), chills (44%), myalgias (36%), self-reported fever (34%), headache (32%), vomiting (29%), and bloody stools (7%). Patients were ill for about 5 days [611]. These organisms are isolated from stool by plating fresh specimen on TCBS agar.

Yersinia infections

History

The genus *Yersinia* consists of three species that infect humans: *Yersinia enterocolitica*, *Yersinia pseudotuberculosis* (which are predominantly gastrointestinal pathogens), and *Yersinia pestis*, the causative agent of human pulmonic and bubonic plague. *Yersinia* were overlooked as agents of abdominal illnesses until *Y. pseudotuberculosis* was isolated from mesenteric lymph nodes in patients with mesenteric adenitis, and *Y. enterocolitica* was recovered from stools of patients with diarrhea who had no other enteric pathogens [612,613].

Epidemiology and prevention

Yersinia infections are rarer than other bacterial enteric infections in North America [614]; the infection is better studied in Europe [615]. The most common source of infection is pork, but raw milk and contaminated water are additional vehicles. Most cases are sporadic and not epidemic. In the United States, African Americans are disproportionately infected by *Yersinia* [616]. *Yersinia* infections occur considerably more frequently in children than in adults [27], although adult infections with *Y. enterocolitica* [617] and *Y. pseudotuberculosis* [618] occur, and adults without diarrhea can excrete *Yersinia* [619]. *Yersinia* infections often cluster in the winter, possibly related to food preparation mishaps during the holidays [620,621]. *Y. enterocolitica* infections are considerably more common than *Y. pseudotuberculosis* infections. Iron overload syndromes, diabetes, alcoholism, and malnutrition [622,623] appear to predispose to *Yersinia* infections, and in these disorders case fatality rates are higher.

Yersinia gastrointestinal infections are best prevented by attention to adequate cooking of foods that might contain this pathogen, in particular pork products. Their tendency to cluster at end-of-year holidays provides a particular opportunity to focus on education about domestic food preparation practices.

Pathogenesis

The cardinal feature of *Yersinia* pathogenesis is cellular invasion. *Yersinia* enter M cells via the binding of bacterial YadA to β1 integrins [624,625], and then multiply in underlying Peyer patches. *Yersinia* has a well-studied plasmid-encoded T3SS, which mediates its invasiveness [626]. A unique additional feature of this system is the ability of secreted proteins of *Yersinia* to down-regulate inflammation, leading to increased intracellular survival [627]. *Yersinia* also critically depend on iron for their virulence, using their own iron uptake system [628].

Clinical manifestations

Most clinical data pertain to infections caused by *Y. enterocolitica*. Most infected individuals have abdominal pain, with fever and diarrhea being somewhat less frequent. Diarrhea is visibly bloody in up to one-fourth of all infected patients [612]. Accompanying signs and symptoms include nausea, vomiting, pharyngitis (quite common), arthralgia, erythema nodosum, and abdominal tenderness, especially in the right lower quadrant. Regional lymphatic spread occurs after invasion of Peyer patches, and this extension causes a clinical picture of pseudoappendicitis or mesenteric adenitis. Occasionally, disseminated bacteremia and extraintestinal suppuration can occur. The pseudoappendicular syndrome is more frequent in teenagers and young adults, whereas enteritis and colitis predominate in younger children [629].

Infections caused by *Y. pseudotuberculosis* are rare and can manifest as granulomatous colitis [630].

Treatment

Yersinia are generally resistant to ampicillin and variably resistant to other β-lactam antibiotics, but susceptible to fluoroquinolones, trimethoprim–sulfamethoxazole, chloramphenicol, and gentamicin [631]. However, these susceptibilities have not translated into unequivocally effective therapy. For example, in one randomized controlled trial, trimethoprim–sulfamethoxazole was no better than placebo in ameliorating disease course, although treatment was administered over a week after symptoms began [632]. In view of the potential seriousness of these infections, however, most clinicians, after establishing a diagnosis of *Yersinia* gastrointestinal infection, will prescribe an antimicrobial, usually

trimethoprim–sulfamethoxazole or a fluoroquinolone [633]. In cases of extraintestinal infection, and in particular bacteremia, antimicrobial treatment is warranted. If doubt exists whether a patient has mesenteric adenitis or pseudoappendicitis from *Yersinia* infection or true appendicitis, it is prudent to err on the side of an exploratory laparotomy.

Diagnosis

Yersinia is detected in stool using selective enrichment techniques, in particular *Yersinia* selective agar, which contains cefsulodin, irgasan, and novobiocin to suppress non-*Yersinia* flora. Cold enrichment, exploiting the preferential growth of *Yersinia* at 25°C, has been largely abandoned. Unfortunately, many clinical microbiology laboratories do not routinely seek *Yersinia* in stool cultures [634], so it is important to request that this genus be sought if there is any question of its presence. There is no need to request special techniques for *Yersinia* when submitting lymph nodes or blood for culture, because there is little likelihood of competing flora being present in these specimens.

Complications

Yersinia cause several autoimmune postinfectious sequelae, including sterile arthritis, occurring 1–2 weeks after acute enteric infection and attributed to *Yersinia* antigens in affected joints [635], Reiter syndrome, myocarditis, glomerulonephritis, and thyroiditis [612] *Y. pseudotuberculosis* has been suggested to be a precipitant of Kawasaki disease in children [636].

Miscellaneous, unusual, and controversial bacterial causes of diarrhea and gastroenteritis

Aeromonas *species*

Several studies suggest that *Aeromonas hydrophila*, and, to lesser extents, *Aeromonas caviae* and *Aeromonas sobria*, cause human diarrhea, although it is not at all clear that isolation of this genus from stool means that *Aeromonas* is the cause of diarrhea [637,638]. These organisms tend to be associated with sporadic human illnesses and not epidemics. Their reservoirs are probably contaminated food and water, including food of aquatic origin. They are detected using blood agar plates and are susceptible, if one chooses to treat them, to fluoroquinolones, aminoglycosides, third-generation cephalosporins, carbapenems, aztreonam, and, to a lesser extent, trimethoprim–sulfamethoxazole [639].

Bacillus anthracis

Infection with *Bacillus anthracis*, the cause of human anthrax, can present with prominent gastrointestinal manifestations [640] after transmission from contaminated food. It can be recovered by stool culture [640] but is more commonly isolated from extraintestinal sites, such as cultures of blood, pulmonary tissue or secretions, and skin. Anthrax can cause intestinal ulcerations, severe abdominal pain, intestinal edema, ascites, vomiting, hematemesis, diarrhea, and bloody diarrhea. There can also be prominent gastrointestinal symptoms in inhalation anthrax [641].

Bacillus cereus

Bacillus cereus is a spore-forming anaerobe that causes disease by producing toxins. *B. cereus* causes two distinct syndromes in humans. The first syndrome is an emetic disorder without diarrhea, which is attributable to cereulide, a small cyclic dodecadepsipeptide that resists heat and acid [642]. Diarrhea caused by *B. cereus* has been attributed to heat-labile enterotoxins [643], which can be divided into at least three distinct types, including a hemolysin [644], a nonhemolytic enterotoxin [645], and a cytotoxin [646]. Most cases resolve within 1–2 days but *B. cereus* emetic toxin can occasionally cause fulminant hepatic failure [647]. Treatment of all illnesses caused by *B. cereus* is supportive. Diagnosis is made by isolating the organism from food or emesis. In reality, this is performed only by public health authorities in outbreak settings. *B. cereus* infections can possibly be prevented by adequate refrigeration of prepared foods, because toxin production appears to be optimal at 12–15°C [648].

Helicobacter pylori

Helicobacter pylori have been proposed to cause diarrhea. *H. pylori* can be excreted in the absence of gastroenteritis [649] and, although their role in either acute or persistent diarrhea did not emerge in several case–control studies [650–654], other studies have demonstrated an association with these disorders [655]. Other *Helicobacter* species have been suggested as causes of acute diarrhea [656]. *Helicobacter canadensis* [657] and *Helicobacter pullorum* [658] have been reported to cause an acute gastroenteritis resembling that caused by *C. jejuni* and *Campylobacter coli*, and *Helicobacter fennelliae* and *Helicobacter cinaedi* appear to cause gastroenteritis and invasive disease in immunocompromised and immunocompetent patients [659–661]. The recognition of these organisms as pathogens has been hampered by the fact that the routine clinical microbiological techniques developed to isolate *C. jejuni* and *C. coli* are not ideal for the growth of these *Helicobacter*.

Plesiomonas shigelloides

Plesiomonas shigelloides enteric infections are diagnosed even less commonly than those associated with *Aeromonas* species. *P. shigelloides* were associated with pathogenicity in the 1970s in southern Asia [662]. Cases have been identified in North America, usually associated with travel or with the consumption of shellfish [663]. *P. shigelloides* are detected using blood agar plates. They are susceptible to a wide range of antibiotics [664], including fluoroquinolones and third-generation cephalosporins. Many isolates are resistant to trimethoprim–sulfamethoxazole. Prevention includes care in handling and preparing seafood.

Staphylococcal food poisoning

Staphylococcus aureus produces a family of superantigens, including enterotoxins A, B, C, D, E, G, H, I, K, L, and M, exfoliative toxins A and B, and toxic shock syndrome toxin. These molecules activate T lymphocytes, although not by typical antigen presentation, and cause exuberant production of cytokines. Toxemia from any of these proteins can be associated with diarrhea, usually as part of a systemic constellation of symptoms [665]. Pure staphylococcal food poisoning usually begins abruptly, and vomiting is more prominent than diarrhea. The toxin is heat stable and therefore foods that have been properly heated can still be vehicles for disease. The incubation period is short, usually within several hours of ingestion. Treatment is supportive.

Additional bacterial causes of gastroenteritis and diarrhea

Myriad extraintestinal infections can result in prominent gastrointestinal infection [666] (Table 48.5). Streptococcal enterotoxins, which are closely related to staphylococcal enterotoxins, can cause similar toxemic symptoms and be associated with vomiting and diarrhea [667]. *Legionella* infections are also often accompanied by diarrhea [668,669].

Diarrheal syndromes caused by yet to be discovered bacterial agents

Several syndromes are believed to be of bacterial origin, even though, despite thorough investigations, monomicrobial etiologies have not emerged.

*Antibiotic-associated, non-*Clostridium difficile *diarrhea*

Mild diarrhea during antibiotic therapy is common and only a minority (approximately 20%) [243] of these cases is caused by *C. difficile*. In general, antibiotics interfere with the digestion of carbohydrates by eliminating colonic bacteria that metabolize this energy source. Decreased carbohydrate metabolism can then lead to osmotic diarrhea, diminished

Table 48.5 Frequency of diarrhea in systemic illnesses

Cause of diarrhea	Percentage of patients with diarrhea (%)	References
Viral		
HIV	15–60	Schmitz et al. [670]
Dengue virus	37	Pancharoen et al. [671]
Influenza A virus (children)	8–18	Wang et al. [672]
Avian influenza virus	7	Tran et al. [673]
Hantavirus	25–40	Chapman et al. [674]
SARS coronavirus	38–74	Leung et al. [675]
Ebola virus	86–96	Ndambi et al. [676]
Bacterial		
Community-acquired pneumonia (non-*Legionella*)	6	Sopena et al. [668]
Legionella spp.	25	Sopena et al. [668]
Pneumococci	6–8	Fernandez-Guerrero [677]
Meningococci	Rare	Hussein et al. [678]
Bacteremia	20–40	McCabe and Jackson [679], Ribeiro and Moreira [680]
Leptospira spp.	58	CDC [681]
Yersinia pestis	23–51	Hull et al. [682]
Brucella spp.	6–16	Bwaka et al. [683]
Borrelia burgdorferi	2	Zaidi and Singer [684]
Borrelia hermsii	19	Zaidi and Singer [684]
Ehrlichia spp.	10	Zaidi and Singer [684]
Francisella tularensis	40	Dienst [685]
Rickettsia rickettsii	19–45	Zaidi and Singer [684]
Protozoan		
Plasmodium falciparum	5–38	Prasad and Virk [686]
Fungal		
Candida spp.	Rare	Krause et al. [687]
Others		
Antibiotic-associated diarrhea	5–15	Hogenauer et al. [688]

From Reisinger et al. [666], with permission from Macmillan Publishers.

production of short-chain fatty acids, and disruption of water and electrolyte absorption [236]. Bile acid metabolism may also be diminished causing intestinal ion secretion. In these cases, evidence of *C. difficile* infection is absent and the colonic mucosa appears normal or mildly hyperemic. Discontinuation of antibiotics resolves symptoms. In some cases of antibiotic-associated diarrhea, *S. aureus* is recovered in high proportions in the stool and has been proposed to play an etiological role [689,690]. Other enteric bacteria that have been proposed to play roles in non-*C. difficile* antibiotic-associated diarrhea include *Klebsiella oxytoca*, enterotoxin-producing *C. perfringens*, and *Candida* [691].

Brainerd diarrhea

A cluster of nonbloody, chronic (lasting over a year in many patients) diarrhea epidemiologically associated with the consumption of raw milk was identified in Brainerd, Minnesota in 1983 [692]. Its etiology remains undetermined despite years of investigation and the appearance of similar clusters of cases [693].

Bacterial overgrowth

Data suggest that chronic abdominal pain syndromes, and especially those with diarrhea, could be related to intestinal bacterial overgrowth and be responsive to antibiotics [694,695]. In these situations, bacterial communities, and not specific pathogens, are likely to be the causative agents. Interestingly, rifaximin had a beneficial effect on irritable bowel syndrome in a randomized controlled trial [696].

Viral agents of gastroenteritis

Viral infections of the intestinal epithelium are the most common causes of acute diarrhea and gastroenteritis. Four viruses are well-established causes of acute gastroenteritis: rotavirus, calicivirus, astrovirus, and enteric adenoviruses. All four are nonenveloped icosahedral viruses, affording them great environmental stability and subsequent enhanced ability to spread in human communities. All except adenovirus are RNA viruses that lack proofreading ability in their genomic replication. This feature facilitates very high rates of mutation and enables populations of these viruses to respond quickly to selection pressures such as immune responses. Our knowledge of the assembly and structure of viruses that cause gastroenteritis in humans greatly exceeds our understanding of their pathogenesis in the infected host.

Viruses were long suspected to cause gastroenteritis but efforts to demonstrate specific agents were not successful until the 1970s when the Norwalk agent (a calicivirus) was first observed by electron microscopy in negatively stained stool suspensions [697]. Since then, agents of viral gastroenteritis have been extensively characterized and effective vaccines for rotavirus are on the threshold of widespread use.

In the vast majority of situations, viruses cause self-limited illnesses and require only supportive therapy. However, when viral gastrointestinal infections occur in children in resource-poor environments, most particularly in circumstances in which oral rehydration is not used and referral centers are not accessible, these common infections can be lethal.

Rotavirus

History

In 1973, Bishop and colleagues [698] identified round viral particles termed "rotavirus" by electron microscopy in duodenal biopsies of infants with gastroenteritis in Melbourne. Soon after, additional groups demonstrated agents with similar morphological and serological properties in other centers [699–702]. Multiple subsequent studies have demonstrated the unequivocal predominance of rotavirus as a cause of infantile and childhood diarrhea worldwide.

Rotaviruses are double-stranded RNA viruses that belong to the Reovirus family. They are encapsidated in a characteristic round triple-shelled virion [703]. There are four major groups (A–D), defined by their middle capsid layer. The vast majority of human disease is caused by group A rotaviruses, but groups B and C can cause outbreaks and sporadic human cases. Within group A rotaviruses, serotypes are determined by the outer capsid antigens VP7 (the variants of which assign G type) and VP4 (the variants of which assign P type). G types 1–4 and 9 are the most common serotypes [704], although geographic differences exist. G types 1–4 predominate in North America, Europe, and Australia, but are less common in South America, Asia, and Africa [705,706]. The G type forms the basis for serotyping and serves as the foundation for many seroepidemiological studies.

Epidemiology and prevention

Rotavirus is a major human problem. Rotavirus is one of the most important gastrointestinal causes of morbidity and mortality in all countries, and the most common cause of severe dehydrating pediatric diarrhea worldwide. It accounts for approximately 600 000 deaths each year, mainly in children in developing countries, and numerous hospitalizations throughout the world [707]. As such, rotavirus is responsible for the loss of a massive number of years of life. Although oral rehydration plays an important role in individual cases and in public health programs, there is considerable residual mortality, especially in resource-poor areas of sub-Saharan Africa and south Asia (Fig. 48.13) [707].

It has been estimated that one in 65 children born in the United States will be hospitalized for rotavirus disease in the first 5 years of life [708], and rotavirus disease is also a considerable burden on the health system in Europe [709]. Virtually all children experience rotavirus infection by the age of 5 years; the most severe illnesses occur in children under 2 years of age. Subsequent infections are milder than initial infections [710], but severe illnesses in the elderly have been reported [711,712]. Asymptomatic infection

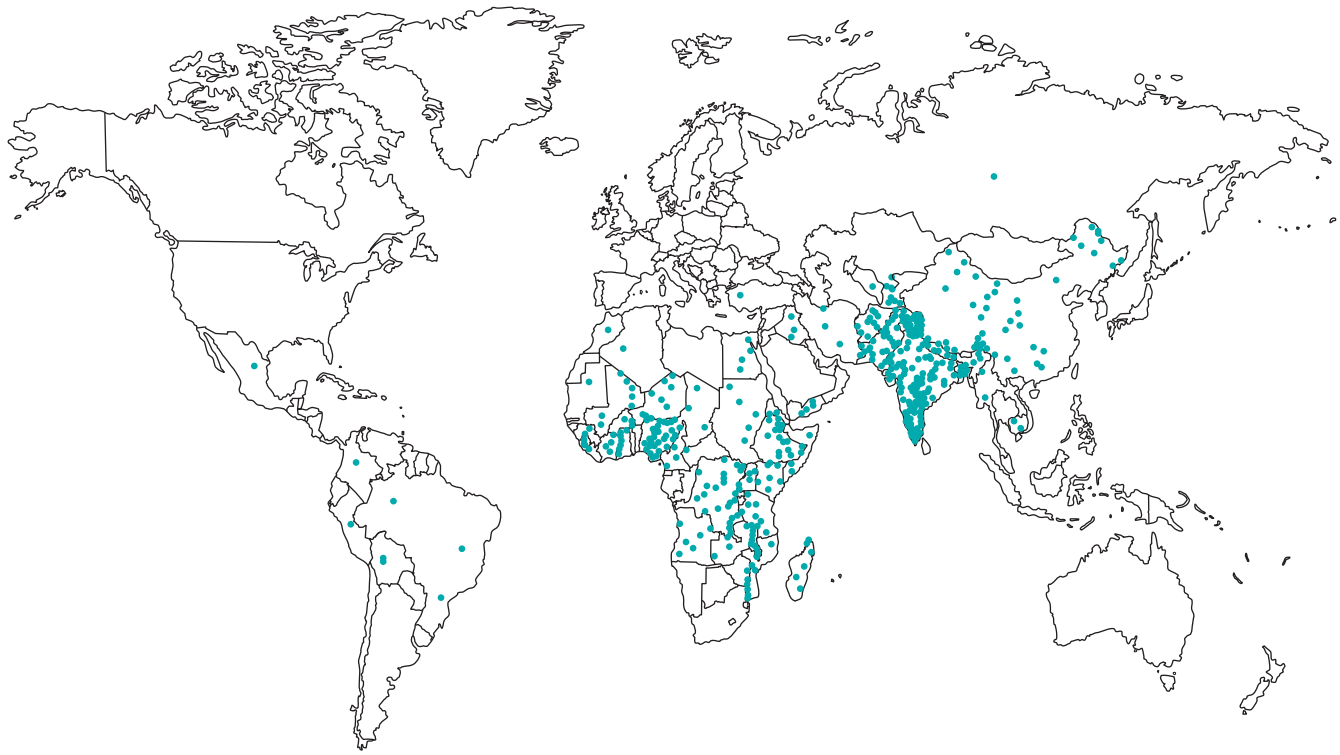


Figure 48.13 Estimated global distribution of rotavirus-related deaths. Each dot represents 1000 rotavirus-related deaths.

is quite common in neonates, older children, and adults. Rotavirus spreads by the fecal–oral route and is quite contagious. Rotaviruses can resist desiccation and persist on inanimate objects; outbreaks can have characteristics of airborne spread and can occur quite easily in hospitals [713]. In temperate climates, rotavirus infections have strong peaks in the winter [708].

An effective multivalent live attenuated rotavirus vaccine, Rotashield, was used in the United States in 1998 and 1999, but an association with intussusceptions forced its withdrawal [714]. Two new oral attenuated vaccines that appear to be free of such concerns after extensive safety trials have considerable promise as interventions to greatly reduce the morbidity and mortality caused by rotavirus. One is a monovalent attenuated human strain [715] and the other is a pentavalent bovine recombinant preparation [716]; both have received FDA approval. Because of the age group being immunized there will still be cases of intussusception temporally, but not causally, related to rotavirus vaccination, and it will be important to maintain this perspective in analyzing such associations [717]. Although there are considerable challenges to universal vaccination as a strategy to prevent rotavirus infections, for the first time we now have an opportunity to dramatically reduce the burden imposed by this worldwide gastrointestinal infection [710]. Hygienic precautions should be implemented to the extent possible to prevent nosocomial spread.

Pathogenesis

Rotaviruses replicate selectively in absorptive villous epithelial cells of the small intestine, sparing the crypts and colonic epithelium. Infected villi atrophy and shorten. There is a mononuclear cell infiltrate in the lamina propria [718–720]. *In vitro*, rotavirus inhibits eukaryotic cell RNA, DNA, and protein synthesis [721–723]. Resulting cell injury and death diminish the absorptive and hydrolytic capacities of the small bowel, leaving secretory crypt function intact. Thus, rotavirus disease has been considered to be a combination of secretory and osmotic diarrhea.

Rotaviruses possess double-stranded genomes with 11 open reading frames, with each encoding at least one protein [724] (Fig. 48.14). Antibodies to VP4 and VP7 neutralize infection [713]. This immunological protection, combined with the observation that second and subsequent infections beyond the neonatal period are milder than the initial infection, justify vaccination as a disease control strategy. The ability of rotavirus to cause disease is possibly attributable to other mechanisms in addition to direct cellular morphological injury. Rotavirus nonstructural protein 4 (NSP4) may be important in pathogenesis [725]. This 20-kDa protein undergoes glycosylation and serves several roles in viral morphogenesis [724], including facilitation of viral budding of the double-layered particles into the lumen of the endoplasmic reticulum. Most intriguingly from a pathogenesis perspective, NSP4 may function as a viral enterotoxin that promotes

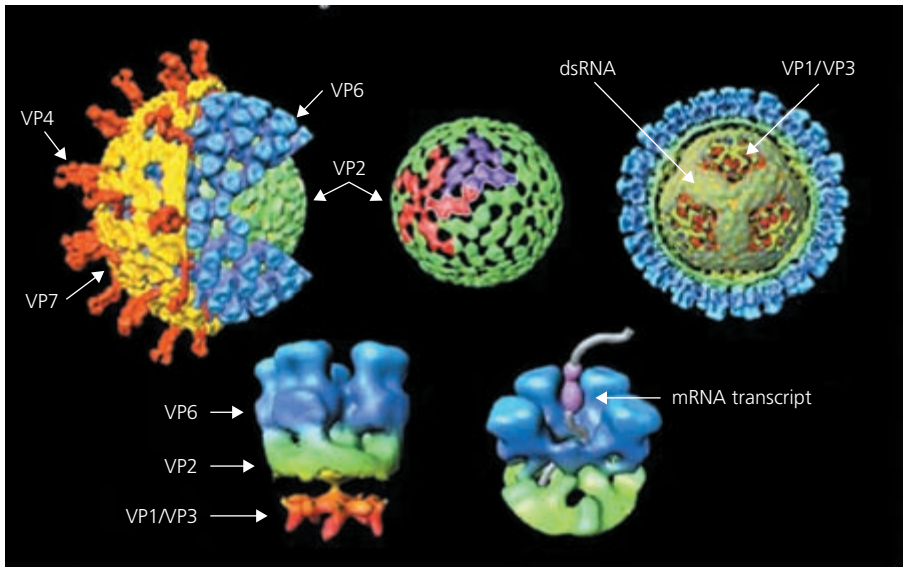


Figure 48.14 The triple-layered architecture of rotavirus; three-dimensional reconstructions of cryoelectron photomicrographs. Intact triple-layered rotavirus particle partially uncoated to reveal the outer layer (VP4 and VP7) and the middle layer (VP6) (top left), core particle consisting of mainly VP2 (center), and cross-sectional view (top right) showing the relative positions of viral double-stranded RNA (dsRNA) and the VP1/VP3 polymerase complex within the core. The lower right image shows extrusion of newly synthesized mRNA through the pore in the middle VP6 layer. Courtesy of Dr B.V. Prasad.

chloride secretion and raises intracellular ionized calcium concentrations [726,727]. Other possible mechanisms of host injury include transcellular flux caused by damaged epithelial junctions [728], effects on the enteric nervous system [729], impaired transport of sucrase–isomaltase to the enterocyte apical surface [730], and villous ischemia [731]. These proposed mechanisms are not mutually exclusive and it is possible that no single process is responsible for human enteric illnesses caused by rotaviruses.

Clinical manifestations

In a typically severe primary infection, symptoms begin 2–7 days after exposure. In hospitalized children infected with rotavirus in Washington, DC [732], vomiting occurred in almost all, and fever in approximately half. Associated symptoms include dehydration (83%) and irritability (47%). Respiratory symptoms and signs are also common, with pharyngeal erythema, rhinitis, signs of otitis media, or palpable lymph nodes occurring in about one-fourth of all patients. Fever and vomiting, if they occur, are followed typically by profuse watery diarrhea that contains no blood or leukocytes. Vomiting abates after 1 or 2 days, while diarrhea persists for 4–7 days. Mild elevations of transaminases without cholestasis are common and of no clinical significance [733]. Infants beyond the neonatal period are the most severely affected by rotavirus diarrhea [734].

Diagnosis

Group A rotavirus infection is easily detected by EIAs of stool. Non-group A infections can be identified by electron microscopy or RNA electrophoresis of stool samples [735].

Treatment

The mainstay of treatment is managing dehydration. A reduced sodium (75 mEq/L) oral rehydration solution might be somewhat more effective in noncholera diarrhea than the standard WHO formulation (containing 90 mEq of sodium/L) [736]. Oral rehydration is adequate in most cases. Encouraging adequate nutritional intake can shorten the course of rotavirus disease and is especially important in children with baseline compromised nutrition. Clear liquid and bland carbohydrate diets do not improve outcomes despite their long tradition in both alternative and mainstream medicine. Food should not be withheld because of concern about the effect of rotavirus infection on gut function in the presence of enteral feeds. Enteric administration of immunoglobulin has been reported to reduce the duration of severe disease [737,738]. Probiotics (especially *Lactobacillus* GG) have been shown to reduce the duration of mild [739] but not severe acute disease [740]. Antimotility and antidiarrheal agents, opiates, and atropine-containing compounds are contraindicated in rotavirus and all other acute diarrheal illnesses in children [741]. In rare cases of prolonged or severe rotavirus infection, orally administered passive immune therapy has been used [742].

Human caliciviruses (noroviruses and sapoviruses)

History

These viruses were first observed in stools during a school outbreak of vomiting in Norwalk, Ohio [743] and were subsequently shown by volunteer inoculations to be pathogenic [744]. Initially, different strains were named for the sites of outbreaks, such as Norwalk, Snow Mountain, Hawaii, etc. Genomic sequencing has lead to the creation of two genera: norovirus and sapovirus. Both genera consist of small (28 nm) single-stranded RNA viruses with genomic and physical structures that are somewhat similar to picornaviruses (Fig. 48.15). The genome is approximately 7.7 kilobases in length and is a positive-sense single-stranded RNA.

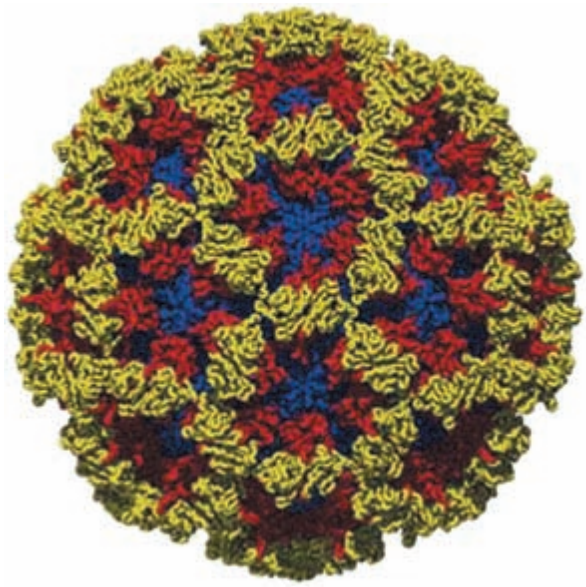


Figure 48.15 Norwalk agent particle. X-ray structure of recombinant Norwalk virus capsid showing the S (blue), P1 (red), and P2 (yellow) domains. From Estes et al. [745], with permission from Lippincott Williams & Wilkins.

Epidemiology and prevention

Noroviruses are best known for causing fulminant outbreaks of gastroenteritis in confined settings such as schools, camps, and cruise ships. All ages are affected and common-source food items or water sources can often be identified. The CDC has shown that more than 95% of such outbreaks are caused by caliciviruses [746]. Some data suggest that norovirus is an important agent of endemic pediatric gastroenteritis worldwide, accounting for up to 8% of cases [747], although other studies have shown lower rates of infection [403]. There is an increased incidence of norovirus pediatric gastroenteritis during winter. Group 2 noroviruses (a serological grouping) predominate in epidemics [748]. Norovirus outbreaks tend to affect more people than outbreaks caused by bacterial agents [749]. Syndromic recognition using the criteria of Kaplan et al. [750] is reasonable for identifying clusters, even without viral testing [751].

Because of the limited duration of natural immunity after infection, an effective vaccine would probably require frequent booster doses. A transgenic potato that expresses Norwalk capsid antigen elicits an immune response after oral administration to human volunteers [752]. In the interim, disease control measures largely consist of ensuring safe water supplies and good personal hygiene in closed populations.

Pathogenesis

Like rotavirus, norovirus appears to cause infection by replicating within small intestinal epithelium. Human volunteer studies have shown that, following challenge with norovirus, there is small bowel villous blunting, crypt hypertrophy, and mild inflammatory infiltrates [744]. Attempts to gain detailed knowledge of norovirus pathophysiology have been hampered by the lack of animal models demonstrating gastrointestinal pathogenicity and by difficulties growing norovirus in tissue culture, although success in culturing a murine norovirus has been reported [753]. Studies suggest that blood group determinants confer susceptibility to specific caliciviruses [754]. These observations explain previous enigmatic observations that adult volunteers who have no antibody response to some of these agents are not susceptible to experimental infection whereas those who do have measurable antibody often are. Several mature norovirus proteins have been identified [755]. Major capsid protein VP1 is implicated in ABO blood group susceptibility, and several nonstructural proteins are postulated to play roles in viral assembly and physiology.

Clinical manifestations

In older children and adults, symptomatic disease typically begins with the rapid onset of vomiting after 24–48 h of incubation. High fever is unusual but myalgia and headache are common. Diarrhea is often not prominent if it is even present. Symptoms typically resolve within 1–2 days. Attack rates often approach 50% of exposed populations. In young infants, diarrhea is more common and the illness resembles rotavirus infection.

Diagnosis

Because of the large number of antigenically diverse strains of human calicivirus, a simple stool EIA is not yet available in the United States. In a research setting, electron microscopy or PCR testing of stools can be performed. Most outbreaks can be identified syndromically [751].

Treatment

Specific therapy does not exist. Treatment consists of measures to ensure adequate hydration.

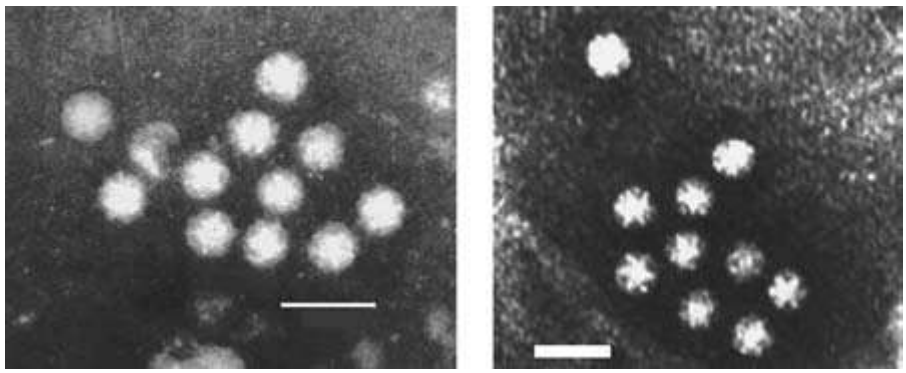
Enteric adenoviruses

History

Adenoviruses were suggested as a cause of acute diarrhea in the 1960s [756], but most fecal adenoviruses are actually respiratory pathogens. However, two serotypes (40 and 41) proposed to be causes of diarrhea in 1983, based on analyses of stools from children in the Netherlands and German [757], remain consistently associated with human gastroenteritis, although there is considerable interpopulation variability in the frequency of enteric infections caused by these serotypes [758]. Adenoviruses are 80-nm nonenveloped double-stranded DNA viruses.

Epidemiology and prevention

Enteric adenoviruses have a worldwide distribution and cause disease mainly in children under 2 years of age.



(a) (b)

Figure 48.16 Electron microscopic appearance of astroviruses. **(a)** Tissue culture-grown astrovirus particles visualized by negative-stained electron microscopy. The atypical "star" morphology represents intact virions. **(b)** Astrovirus particles from a fecal sample visualized by negative-stained electron microscopy (bar = 50 nm). The star morphology actually represents partial degradation of virions. Courtesy of Dr B.V. Prasad.

Adenovirus gastroenteritis occurs throughout the year without the seasonality noted in other enteric viral infections. Adenoviruses account for 3%–5% of pediatric gastroenteritis cases [19,403]. Adenoviruses are presumably acquired via the fecal–oral route. Although very large numbers of adenovirus particles are shed by infected children, large outbreaks are uncommon.

No vaccine is available. Hygienic measures probably suppress nosocomial spread.

Pathogenesis

Most adenoviruses replicate in the gastrointestinal tract, but symptomatic disease is largely restricted to serotypes 40 and 41. Disease manifestations (i.e., diarrhea) are presumably related to infection of villous epithelium; however, most research has focused on the pathogenesis of respiratory adenovirus infections and not gastrointestinal adenovirus illnesses.

Clinical manifestations

The main distinguishing feature of adenovirus gastroenteritis is its longer duration (often 2 weeks) compared with diarrhea caused by other agents [759].

Diagnosis

A commercial EIA is available for adenovirus serotypes 40 and 41.

Treatment

Adenovirus infections are treated with supportive measures. Ribavirin [760] and cidofovir [761,762] have been used in immunocompromised patients with severe adenoviral enteric illnesses, with improvement in diarrhea.

Astrovirus

History

Astroviruses were proposed as causes of human diarrhea in 1975, when they were identified by electron microscopy in the stools of children with gastroenteritis [763,764]. Astroviruses are small, 28- to 34-nm RNA viruses that infect a variety of mammalian and avian species [765]. They are named for their appearance on negatively stained electron microscopy preparations, appearing as five- or six-point stars (Fig. 48.16). They are a common cause of mild to moderate gastroenteritis in young children. There are eight human serotypes with serotype 1 being the most common cause of human gastroenteritis.

Epidemiology and prevention

Astrovirus accounts for 7%–15% of cases of infantile diarrhea [19,766–769]. Infants under 1 year of age are most likely to be symptomatic. The institutionalized elderly can be affected by astrovirus [770,771], as can other ambulatory adults [772]. Astrovirus is probably more likely to cause disease in immunocompromised hosts. Astrovirus serotype 1 infections are most common [773,774], although outbreaks involving less common serotypes have been reported in military personnel and nursing home residents [712,775,776]. The seasonal pattern resembles rotavirus (winter or rainy season predominance). Astroviruses are transmitted via the fecal–oral route [765].

No vaccine is available. Enteric precautions might contain or prevent institutional outbreaks.

Pathogenesis

Similar to rotavirus, astrovirus infects the small intestinal villous epithelium [777], but detailed studies of the gut in human infections have not been performed. Lambs challenged with an ovine astrovirus sustained viral infection of apical villous cells, and underwent short-lived villous atrophy and crypt hyperplasia. The histological changes occurred within a day in some animals, and resolved within 5 days [778]. Astroviruses enter cultured epithelial cells via endocytic pathways [779].

Clinical manifestations

Incubation ranges from 1 to 4 days. Astrovirus infections resemble rotavirus gastroenteritis but are usually considered to be milder. However, severe illness can result [780]. Astrovirus infections are frequently accompanied by vomiting and abdominal pain [765,781,782].

Diagnosis

Astroviruses are detected in the stool by electron microscopy, EIA, or PCR. These tests are not available for routine clinical use in the United States, but EIAs are available in Europe.

Treatment

Ensuring adequate hydration is the mainstay of therapy. Specific treatments do not exist, although immunoglobulin has been used to treat severely ill immunocompromised hosts infected with astroviruses [783,784].

Other viral agents

A number of systemic viral infections such as HIV, cytomegalovirus (CMV), Epstein–Barr virus (EBV), and measles can present with diarrhea [785–788]. Other viruses such as picobirnavirus [789], torovirus [790], and Aichi virus [791] are likely, but not proven, agents of human gastroenteritis. SARS coronavirus, the causative agent of severe acute respiratory syndrome, can have diarrhea as an initial manifestation [792,793].

Many other enteroviruses, reoviruses, and nonenteric adenoviruses are found frequently in stool but are not associated with any gastrointestinal disease. Available data do not support the use of viral cultures to detect diarrhea etiology in any clinical setting.

Marine intoxications with gastrointestinal effects

A variety of marine foods cause gastrointestinal symptoms [794], and affected patients might present to gastroenterologists. These illnesses are the consequences of ingesting preformed neurotoxins and not the result of consuming pathogens that then replicate in the human host. In the absence of readily available tests these disorders are likely underdiagnosed, and syndromic recognition is critical [795].

Ciguatera fish poisoning is the most common marine poisoning. Ciguatera poisoning had been largely confined to South Pacific and Caribbean fish species [796], although with globalization of the food supply this intoxication can now be acquired worldwide [794]. Finfish from the tropics and subtropics are the vehicles of transmission of ciguatoxins to humans. This toxin originates as a precursor toxin (gambiertoxins) in the aquatic dinoflagellate *Gambierdiscus toxicus*. These dinoflagellates (and their gambiertoxins) are consumed by small herbivorous fish, which then convert the gambiertoxins to ciguatoxins before they are subsequently consumed by larger finfish. When these heat-stable sodium channel poisons are consumed by humans, their net effect is to enable spontaneous firing of tetrodotoxin-sensitive channels, resulting in a variety of neurological (especially myalgias, paresthesias, and cold allodynia) and gastrointestinal (diarrhea, abdominal pain, nausea, and vomiting) effects [794]. Ciguatera poisoning is diagnosed on clinical grounds. Chronic sequelae consisting of fatigue, myalgia, headache, pruritus, depression, and hypersensitization to subsequent exposures have been reported [797,798]. Management of the acute episode is supportive, and early reports of the efficacy of mannitol have not been confirmed in randomized controlled trials [794].

Paralytic shellfish poisoning occurs worldwide after consuming shellfish, generally bivalve species such as mussels, oysters, and clams, that contain saxitoxins synthesized by dinoflagellate algae. These algae are the cause of "red tide" waters, although shellfish can assimilate this toxin in waters that are not noticeably discolored. Bivalve species consume the poison-producing algae and concentrate saxitoxins by filtration. Saxitoxins block tetrodotoxin-sensitive sodium channels and inhibit nerve conduction. Symptoms occur sequentially and consist of dizziness, paresthesias, weakness, ataxia, nausea, and vomiting. Hypersalivation and diaphoresis can also be present. Consciousness is not impaired. Symptoms begin within 3 h of ingestion. Treatment is supportive [794].

Neurotoxic shellfish poisoning resembles ciguatera poisoning. It produces a neuroexcitatory picture and not a paralytic syndrome. It occurs in selected regions (west coast of Florida, North Carolina, and New Zealand) and is the consequence of ingesting brevetoxins concentrated in filter-feeding shellfish. Brevetoxins, which originate in the dinoflagellate *Gymnodinium breve*, enhance sodium entry into cells, as do ciguatoxins [799]. The gastrointestinal effects are nausea, vomiting, and diarrhea, and the neurological effects resemble those of ciguatera poisoning. The clinical picture is usually fairly mild and treatment is supportive [794].

Amnesic (encephalopathic) shellfish poisoning is caused by the consumption of shellfish containing domoic acid. Domoic acid is produced by *Nitzschia* algae. It is an excitatory toxin that causes symptoms about 5 h after ingestion. Its most distinctive effect is its ability to induce amnesia, among other neurological (headache, weakness, confusion, aberrant eye movements, seizures, myoclonus, loss of consciousness) and nonneurological (shock, arrhythmias, increased respiratory secretions) symptoms. Gastrointestinal symptoms consist of vomiting, spasmodic abdominal pain, and diarrhea [794,800,801]. Cases can be fatal. Diagnosis is by clinical picture, with amnesia being a prominent clue, although incriminated vehicles, if available, can be tested for the presence of domoic acid. Treatment is supportive [794].

Tetrodotoxin poisoning results from the ingestion of fish, usually the Asian puffer fish, and occasionally other marine life [794]. Fillets of fish (*fugu*) containing preformed tetrodotoxin cause a gradation of intoxications, ranging from mild parethesias and perioral numbness ensuing as early as 5 min following ingestion (grade 1), to paralysis, cardiopulmonary collapse, and coma occurring between 1 and 24 h after ingestion (grade 4) [794,802,803]. The gastrointestinal symptoms of tetrodotoxin poisoning are mild and consist of nausea and vomiting [794]. Tetrodotoxin blocks sodium channels at the nodes of Ranvier and inhibits conduction. Diagnosis is by

high performance liquid chromotography (HPLC) analysis of the urine of patients or of the uneaten suspected vehicle. Treatment is supportive. Because of the serious and sudden consequences of ingesting tetrodotoxin, prevention of these illness through the education of food preparers is critically important and has been quite effective in Japan [794,804].

Diarrhetic shellfish poisoning is acquired from consuming shellfish that have sequestered dinoflagellate-synthesized okadaic acid and its dinophysitoxin analogues [805]. Mussels are the most frequently implicated vehicle, but other mollusks can transmit these toxins to consumers. This disorder occurs worldwide. Okadaic acid and its analogues are lipid soluble and survive heating; they inhibit serine and threonine protein phosphatases, especially the PP2A class of phosphatases [806]. Diarrhea begins within a day of ingestion and sometimes as soon as 30 min after ingestion [807]. There are no neurological accompaniments, unlike other toxic shellfish poisoning.

An additional rare marine toxic cause of nausea, vomiting, and diarrhea is clupeotoxism [794,803], acquired from consumption of contaminated plankton-eating fish such as herring and sardines. There is an accompanying sharp metallic taste. The mortality rate can be high. Treatment is supportive [794].

Scombroid fish poisoning (scombrotoxism, also referred to as "scombroid") is caused by systemic histamine toxicity following the consumption of dark meat of bony fish (e.g., tuna, mackerel) [808]. Bacterial decomposition of the flesh of these fish results in an increase in histidine decarboxylase activity and excessive production of histamine in the fish meat. Symptoms can begin within an hour of ingestion and resemble an allergic reaction (urticaria, flushing), but scombroid is really a histamine intoxication and not a true allergy. Diarrhea can be severe but all symptoms are short-lived. Scombroid fish poisoning is treated with antihistamines [809].

Anorectal infections

The populations at greatest risk for acquiring anorectal infections are men who have sex with men, and heterosexual women who engage in anorectal intercourse. The most commonly occurring infections in women are *Chlamydia trachomatis*, *N. gonorrhoea* and *Treponema pallidum*. Infections with *E. histolytica*, herpes simplex virus (HSV), and human papillomavirus (HPV) are endemic in men who have sex with men. Infection with multiple pathogens occurs in 22% of this population who are symptomatic [810] and is associated with high-risk behaviors.

Bacterial infections of the anorectum

Chlamydia trachomatis

Chlamydia trachomatis, a gram-negative, obligate intracellular bacterium, is the leading bacterial cause of sexually transmitted disease (STD) in the United States. *C. trachomatis* is represented by three biovars, two of which cause human disease: trachoma and lymphogranuloma venereum (LGV). The replication of trachoma is restricted to columnar epithelial cells, whereas LGV can also proliferate in macrophages, explaining the differences in symptomatology.

The results of infection by *C. trachomatis* range from asymptomatic proctitis, caused by non-LGV biovars, to severe granulomatous proctitis, usually caused by LGV biovars. Sigmoidoscopic examination of individuals infected with non-LGV biovars can demonstrate normal mucosa or erythema, friability, and erosions. Histological examination of these tissues shows neutrophilic infiltration of the lamina propria [811]. In contrast, LGV biovars induce severe proctitis or proctocolitis manifested as pruritus, purulent rectal discharge, hematochezia, and diarrhea or constipation. Physical examination might demonstrate lymphadenopathy and lower abdominal tenderness. The sigmoidoscopic findings are more impressive and demonstrate friable or bloody mucosa with multiple ulcerations [811]. The infiltrate has variable content, with eosinophils, mononuclear cells, and plasma cells in addition to neutrophils. Crypt abscesses and granulomas with giant cells can also be present and might lead to a mistaken diagnosis of Crohn's disease. Anorectal LGV biovars can also cause strictures, fistula formation, and perirectal abscesses if left untreated, further confusing the diagnosis with Crohn's disease.

Chlamydia infection is diagnosed by culturing samples obtained by rectal swab in McCoy cells. Calcium alginate swabs are less inhibitory to *Chlamydia* than wooden swabs. Sigmoidoscopic biopsies can also be sent for culture. Infected cells are identified by immunofluorescent staining, using a monoclonal antibody against *Chlamydia* antigens, which has a sensitivity and specificity of 90% and 100% respectively [812]. Uncomplicated chlamydial infection of the rectum can be treated with either a single oral dose of 1 g of azithromycin or 100 mg of doxycycline twice a day for 7 days [813]. Alternate therapies include erythromycin, ofloxacin, or levofloxacin. Surgical resection might be required to alleviate strictures.

Neisseria gonorrhoeae

Neisseria gonorrhoeae is a gram-negative intracellular diplococcus that typically infects the epithelial lining of the endocervix and urethra but which can also infect other sites including the rectum. A robust inflammatory response including submucosal abscesses occurs following attachment, internalization, and subepithelial delivery of the organism. Up to half of homosexual men assessed in STD clinics are infected with this organism, with 40% manifesting only rectal involvement. Of women with gonococcal proctitis, only 20% had rectal infection alone [814].

Most gonorrheal anorectal infections are asymptomatic; when present, symptoms include purulent, creamy, and sometimes bloody discharge with pruritus ani, tenesmus, and even constipation. Symptoms typically appear 5–7 days

after exposure. Rare complications include stricture formation, abscesses, and fistulae. The sigmoidoscopic findings are nonspecific and include friable, erythematous mucosa with superficial erosions most prominent at the anorectal junction. Culture of material obtained via rectal swab plated on selective Thayer-Martin media is the recommended diagnostic test. Gram stain of this same material is also suggested but is only positive in about half of those infected and should not replace culture. Sigmoidoscopic biopsies can also be sent for microbiology.

Anorectal gonorrhea is best treated by a single injection of intramuscular ceftriaxone, which cures more than 98% of such infections [813]. Cefixime (400 mg orally in a single dose) is reported to cure 97.4% of uncomplicated cases of anorectal infection but does not provide the same levels of bactericidal therapy as injected ceftriaxone. Fluoroquinolone-resistant *N. gonorrhoeae* have become more prevalent in Asia, the Pacific islands, and California, and fluoroquinolones are no longer universally effective for gonococcal infections in the United States [813]. The isolation of these resistant strains from men having sex with men has also increased and therefore the CDC does not recommend fluoroquinolones as the treatment of choice in this group [815]. Because of a high coinfection rate with *Chlamydia* species, it is recommended that all *N. gonorrhoeae*-infected individuals also receive 100 mg of doxycycline twice a day for 7 days [813].

Treponema pallidum

Treponema pallidum is a spirochete capable of infecting damaged skin or mucosa. Anorectal syphilis is primarily diagnosed in men who have sex with men. Primary anorectal syphilis presents as a chancre of the squamous epithelial lining of the anal canal or in the rectum. These lesions, which develop 2–6 weeks postexposure, appear as 1- to 2-cm indurated lesions with raised borders. "Mirror" or "kissing" lesions can be present [816]. Anorectal chancres are often asymptomatic unless superinfected.

The anorectal presentation of secondary anorectal syphilis is condyloma lata. This stage usually develops 6 weeks to 6 months from the time of the initial infection. Condyloma lata are smooth, wart-like lesions near or in the rectum. Colitis associated with secondary syphilis can also occur in the distal 15–20 cm of the colon. This lesion spontaneously resolves after 3–4 weeks. Intestinal lesions are rarely associated with tertiary syphilis but rectal gummas have been reported.

Anorectal syphilitic lesions show a chronic inflammatory infiltrate of lymphocytes, histiocytes, and plasma cells [817]. Dark-field microscopic examination demonstrates spirochetal organisms, but nonpathogenic spirochetes can also be present in the rectum [818].

Anorectal syphilis should be treated with a single intramuscular injection of 2.4 million units of benzathine penicillin. If the patient is allergic to penicillin, 100 mg of doxycycline twice a day or 500 mg of tetracycline four times a day can be given orally for 2–4 weeks for early and latent syphilis respectively. Proof of eradication should be sought 3 and 6 months posttreatment by serological testing and clinical response. Persistence or recurrence of symptoms or a sustained fourfold increase in nontreponemal test titer compared with the baseline or maximum titer likely indicates treatment failure or reinfection, and retreatment is indicated [813].

Viral infections of the anorectum

Herpes simplex virus

Herpes simplex virus 2 accounts for 90% of cases of anorectal herpes. The virus, for which humans are the sole reservoir, is transmitted by direct contact with an infected individual who is actively shedding viral particles. Symptoms of the primary infection begin 4–12 days after contact and spontaneously resolve after 3 weeks, although they can recur. Symptoms associated with subsequent bouts range from none to severe, painful ulcerative proctitis. Other symptoms include tenesmus, pruritus ani, constipation, inguinal adenopathy, sacral and posterior thigh paresthesias, and urinary difficulty. The symptomatic triad of constipation, urinary retention, and anorectal pain strongly suggests anorectal HSV infection.

Anoscopy typically demonstrates vesicles, pustules, or shallow ulcers depending on the stage of the lesions. Biopsies of these lesions show acute and chronic inflammation, superficial ulcerations, and microabscesses [819]. Perirectal lesions are relatively uncommon, occurring in 19% and 27% of infected women and men respectively [820]. Viral cultures should be performed to confirm the diagnosis. PCR can also be used to detect HSV DNA in clinical samples [821]. Depending on the severity of the symptoms one can choose to treat mildly symptomatic individuals with only stool softeners, sitz baths, and analgesics or with acyclovir (aciclovir; 400 mg orally three times a day for 7–10 days). This approach diminishes viral shedding and the duration of symptoms [813]. Other recommended antivirals include famciclovir or valacyclovir (valaciclovir) [813].

Human papillomavirus

Human papillomavirus enters basal germinal epithelial cells and replicates in the nucleus resulting in increased cell proliferation. This response accounts for the skin and mucosal lesions that appear as irregular, verrucous lesions called anal warts or condyloma accuminata. These lesions must be distinguished from the condyloma lata in syphilis, which are more moist and smooth. Histological examination of condyloma accuminata shows irregular, thickened epithelium containing areas of koilocytotic cells with perinuclear cavitation and nuclear atypia. Some HPV strains cause neoplasias including cervical and anal cancer [822].

The virus is transmitted through anal intercourse or autoinoculation to the perianal area and anus. Latency ranges from 1–6 months. If condyloma accuminata are present in

the perianal area, anoscopy must be performed to determine whether lesions also exist inside the anal canal.

Individuals with anal warts can be asymptomatic or complain of rectal discharge, bleeding, or pruritus ani. Diagnosis is by clinical observation, and anorectal syphilis and squamous cell carcinoma should be excluded. This is accomplished by histological examination to distinguish between benign and malignant lesions, and dark-field microscopy to exclude syphilis. Although curative treatment is still lacking, lesions can be controlled with either cryotherapy [823–825] or a 20% solution of podophyllin in tincture of benzoin [824,825]. This solution is applied for up to 12 h and this can be repeated once or twice weekly until all lesions are gone. Its caustic nature precludes intraanal use. Trichloroacetic acid or bichloroacetic acid (80%–90%) can also be used to treat anal warts. Small amounts should be applied to warts and allowed to dry, at which time a white frosting develops; weekly treatment can be used if necessary [813]. Other therapeutic options include electrocautery, electrodessication, CO_2 laser, and surgical removal. Recurrence rates vary depending upon the treatment used; in one study, 29% of warts recurred postsurgery and 65% recurred following podophyllin treatment [826].

Multiple serotypes of HPV can cause anogenital warts, and therapeutic vaccination does not add to the benefit of standard treatments [827]. It is possible that vaccination to prevent HPV infection of the cervix might, over time, diminish the prevalence of anogenital warts, even though vaccine formulations have a limited range of antigens that might not cover all serotypes that cause noncervical disease [828].

Sexual transmission of intestinal pathogens

In addition to anorectal transmission of *Chlamydia*, *N. gonorrhoeae*, *T. pallidum*, HSV, and HPV, *E. histolytica*, *G. lamblia*, *Shigellae* and *Campylobacter* species can also be transmitted sexually. The signs and symptoms associated with infection by these pathogens in men who have sex with men are the same as those experienced by individuals who acquire these organisms by ingestion (i.e., they are not confined to the anorectum).

Summary

Enteric infections are major, and unacceptable, causes of human morbidity and mortality. Children and members of less-advantaged populations disproportionately suffer from these illnesses. Multiple different agents cause diarrhea and gastroenteritis, but diagnostic approaches to these infections are Balkanized, expensive, slow, and suboptimal. There are few immunoprophylactic control measures and treatments are either nonspecific or administered too late in the course to alter outcomes. Preventing and treating gastrointestinal infections worldwide should receive high priority by the specialty of gastroenterology.

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C SMALL INTESTINE

49 Chronic infections of the small intestine

George T. Fantry, Lori E. Fantry, Stephen P. James

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Whipple disease

Whipple disease, first described by George Hoyt Whipple in 1907 [1], is a rare, chronic, systemic infection caused by *Tropheryma whippelei*, a member of the diverse family of actinomycetes usually found in soil. Before 1985, it was estimated that 2000 patients had been afflicted since 1907 [2], and even with the advent of sensitive and specific diagnostic tests, the disease remains rare. The clinical manifestations vary widely and include malabsorption and extraintestinal symptoms, such as seronegative arthritis, fever, or neurological symptoms, which may predominate or predate the gastrointestinal symptoms by years. The disease may involve any organ system, with the predominant pathological changes occurring in the gastrointestinal tract and central nervous system. In most cases, the small intestine is involved, resulting in malabsorption. Before the availability of antibiotic treatment, Whipple disease uniformly progressed to a fatal outcome. Important advances included the identification of periodic acid–Schiff (PAS)-positive macrophages in biopsy specimens of the small bowel [3,4] and other organs [5–7], the discovery that antibiotic therapy could induce remission of the disease [8–16], and the observation by electron microscopy of the characteristic rod-shaped bacillus in mucosa and nonintestinal tissues [12,17–23]. The seminal advance in this disease occurred in 1992 when the uncultured bacillus of Whipple disease, which was named *Tropheryma whippelii* [24], was identified using polymerase chain reaction (PCR) techniques. In 2000, the bacterium was first successfully cultured in vitro, permitting antibiotic susceptibility testing [25]. These findings have provided a basis for more specific diagnostic testing [26,27].

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Epidemiology

Epidemiological data for this rare disease are limited to case series and individual reports. The disease is most common between the fourth and sixth decades of life, with a mean age at onset of 50 years [15,16,28,29], but it can occur at nearly any age. The male-to-female ratio is approximately 8:1 [15,28–31]. Whipple disease occurs predominantly in Caucasians and has rarely been described in persons of African descent and other ethnic groups. No clearly defined geographical or environmental risk factors have been established, except that the disease appears to be more common in farmers and individuals involved in farm-related trades [15], consistent with the causative agent being related to soil organisms. *T. whippelii* DNA has been found in samples of waste water [32], and in the gastric juice and saliva of persons without clinical evidence of Whipple disease [33–36], suggesting that *T. whippelii* may be an environmental or commensal organism that is ubiquitous. Humans are an unlikely reservoir as *T. whippelii* rarely is found in normal intestinal mucoa [37]. Direct person-to-person transmission has not been documented, although the disease has been reported in brothers on two occasions [38,39] and in a father–daughter pair [40]. These observations are also consistent with a common environmental exposure or shared genetic susceptibility. The effectiveness of disinfection methods for endoscopes has been questioned [41], although nosocomial transmission has not been documented.

Etiology and pathogenesis

Although an infectious etiology of Whipple disease has long been suspected, early reports of successful culture of the organism were not reproducible. A major advance was the use of PCR amplification of 16S ribosomal RNA (rRNA) [42] to identify and classify unculturable bacteria, which was successfully applied to tissues from patients with Whipple disease to identify unique bacterial sequences [24,43]. Phylogenetic analysis of the sequences suggested that the Whipple disease bacillus is a gram-positive bacterium closely related to the actinobacilli, and the bacterium was given the designation

T. whippelii. Actinobacilli are actinomycetes, a diverse group of bacteria, most of which are aerobic soil organisms. Detailed analysis has more precisely placed the organism in the phylogenetic position of the actinomycetes [44]. Subsequently, *T. whippelii* was identified by PCR in patients in peripheral blood mononuclear cells, peripheral blood, pleural effusion cells [45,46], and in the vitreous aspirate of a patient with uveitis whose intestinal biopsy specimens had appeared normal by light microscopy [47]. Simplified and rapid real-time PCR techniques have been shown to have similar sensitivity and specificity compared with earlier more complex PCR methods [48,49].

In 1997, the Whipple bacillus was isolated and grown in human macrophages treated with interleukin-4 (IL-4) [50], and subsequently subcultures were established in human fibroblast cell lines [25]. Several additional strains of *T. whippelii* have been cultured from human samples, including the cerebrospinal fluid (CSF) [51–54]. In 2001, the definitive name *Tropheryma whipplei* was accepted [55]. The complete genome sequence of *T. whipplei* is available [56], and this predicts a condensed genome with a lack of key biosynthetic pathways and a reduced capacity for energy metabolism. In addition, both known and novel mechanisms for elaboration and variation of surface structures were found, suggesting that immune evasion and host interaction play an important role in the persistence of infection. Phylogenetic analysis of non-rRNA genes of *T. whipplei* confirmed its placement within the class Actinobacter [57]. With the availability of the *T. whipplei* genome, PCR targeting of repeated sequences enhances the sensitivity of organism detection [58].

Because the distribution of *T. whipplei* in the environment is unknown, it is uncertain to what extent the disease may be caused by exposure to an unusual pathogen. However, the finding of *T. whipplei* DNA in the gastric juice and saliva [33–36] of people without Whipple disease and the striking clinical features of the disease, with the persistence of intracellular bacteria in macrophages, strongly suggest that a defective host immune response may play a central role in pathogenesis. Dobbins [59] reported that the frequency of HLA-B27 was higher (28%) than would be expected in the ethnic groups affected by Whipple disease, which is of interest because HLA-B27-associated spondyloarthropathies may be caused by abnormal interactions of the host with enteric bacterial flora. However, the incidence of spondyloarthritis does not appear to be increased in patients with Whipple disease. Furthermore, one small study was unable to replicate the increased frequency of HLA-B27 [60].

Numerous studies strongly suggest that host defense defects are the basis for Whipple disease, although no single defect has definitively been shown to be the primary, rather than a secondary abnormality. Patients generally exhibit lymphopenia, diminished skin test responses, and decreased mitogen-induced lymphocyte responses in vitro, and these changes may persist after treatment [61]. The abnormality of cellular skin test responses was confirmed and extended [62]. In a study of 20 patients with Whipple disease, none had skin test responses to streptococcal antigens, whether they were untreated or in remission. Because it is known that streptococcal antigens may cross-react with bacterial antigens of actinomycetes, the authors suggested that patients with Whipple disease may have selective defects in their ability to respond to certain types of bacterial antigens. It has been shown that the expression of CD11b, a complement receptor on circulating lymphoid cells involved in bacterial phagocytosis [63], is decreased in patients with Whipple disease, suggesting that there may be a molecular basis for defective macrophage function in this disease. Subsequently, in vitro studies of stimulated monocytes from patients with Whipple disease demonstrated an impaired type 1 T-helper cell (Th1) response with decreased IL-12 production leading to reduced T-cell production of interferon-γ (IFN-γ) [64–66]. In addition, decreased concentrations of IL-12p40 and tumor necrosis factor-α (TNF-α) were found in the serum of patients with Whipple disease [67]. Furthermore, a study of the gene expression profile of macrophages from intestinal lesions in a patient with Whipple disease revealed a pattern corresponding to M2/alternatively activated macrophages suggesting that a Th2 response in the intestine may play an important in the pathophysiology of Whipple disease [68]. It has been demonstrated that IL-16 is critical for *T. whipplei* replication, with enhanced *T. whipplei* replication in patients with Whipple disease related to high levels of circulating IL-16 [69]. These studies suggest that underlying defects of monocyte/macrophage function play an important pathophysiological role, which may lead to the inability of the host response to eliminate the causal bacteria. This may have important clinical implications in patients with Whipple disease because immunomodulating therapy with IFN-γ may be beneficial [70], whereas immunosuppressive therapy for chronic inflammatory arthropathy may be associated with the appearance or exacerbation of gastrointestinal symptoms [71,72].

Histopathology

The histopathological appearance of the small bowel mucosa in Whipple disease is distinct, unique, and usually diagnostic when present, although small bowel disease may not be identified until many years after the onset of other systemic manifestations. The lamina propria is infiltrated by large foamy macrophages that grossly distort normal villous architecture, so that the villi take on a blunted, club-like appearance. The cytoplasm of these macrophages is filled with large glycoprotein granules that stain with PAS (Fig. 49.1). The lymphatic channels in the mucosa and submucosa are dilated, and fat droplets may be seen in the extracellular spaces within the lamina propria, reflecting lymphatic obstruction by enlarged mesenteric lymph nodes.

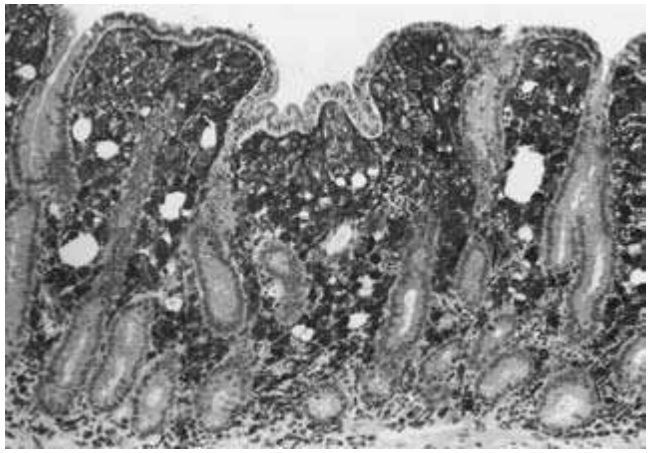

Figure 49.1 Light microscopic appearance of the intestinal mucosa in a patient with Whipple disease. The villi are clubbed, and the lamina propria is extensively infiltrated with periodic acid–Schiff (PAS)-positive macrophages. (PAS and hematoxylin stain; original magnification × 250.) From Dobbins [15].

With electron microscopy, many of the characteristic rod-shaped bacillary bodies can be seen in the lamina propria; they are most abundant just beneath the absorptive epithelium. The fine structure of the bacilli is identical in different patients. The bacilli are 0.25 μm wide and 1–2.5 μm long, with a characteristic cell wall and pale central nucleoid [10,17,18,73–75]. The bacilli are often seen within the PAS-positive macrophages (Fig. 49.2). Most of the PAS-positive glycoprotein within the macrophages likely represents remnants of the cell wall of the phagocytosed bacilli. Unlike the *Mycobacterium avium* complex, the Whipple bacillus is acid-fast-negative.

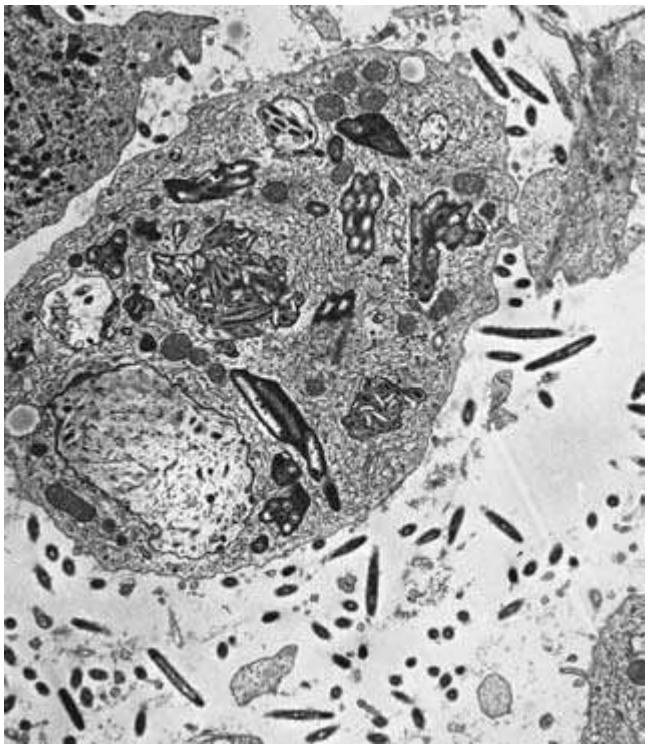

Figure 49.2 Electron micrograph showing numerous bacilli in the extracellular spaces and ingested bacilli in varying stages of breakdown within a macrophage. (Original magnification × 11 000.) From Dobbins [15].

The PAS-positive macrophages and characteristic bacilli have been identified in many nonintestinal tissues, including heart [19,76], lung [20], liver [12], and brain [21], documenting the systemic nature of the disease.

The evolution of the histology of intestinal Whipple disease after therapy has been described in a study of 48 patients [77]. Treatment is associated with a continuous decrease in PAS-positive macrophages and a change in the pattern of mucosal infiltration from diffuse to patchy. A few PAS-positive macrophages commonly persist at long-term follow-up of treated patients. Because of this finding, it is recommended that multiple biopsies be obtained to evaluate the response to treatment or a clinical suspicion of relapse and that electron microscopy be performed to detect intact bacilli, because the persistence of PAS-positive material following therapy is not diagnostic for active infection.

Clinical manifestations

The clinical features and presentation of Whipple disease are highly variable and include symptoms suggestive of malabsorption, such as steatorrhea, abdominal bloating, and weight loss (Table 49.1). Occasionally patients present only with extraintestinal symptoms, such as arthritis and fever. Extraintestinal symptoms may precede gastrointestinal symptoms by many years. Given the nonspecific nature of the symptoms, there is often a delay of months to years prior to diagnosis. Diarrhea is the most common presenting complaint. In a study of 29 patients with Whipple disease, diarrhea was the chief complaint in 39% [16], and initially present in 75% of patients, similar to observations in other reports [28–30]. Thus, although diarrhea affects most patients with Whipple disease, it is not invariably present. When present, diarrhea symptoms are usually suggestive of steatorrhea; multiple large, watery, or semiformed stools are passed daily. Other intestinal symptoms include abdominal bloating, cramps, or distention, anorexia, and occasionally melena and hematochezia [7,78,79], although bleeding, when present, is most often occult [30]. Weight loss, the second most common presenting complaint in patients with Whipple disease, is present before the initial evaluation in 89%–100% of patients [16,28–30]. Severe cachexia may result from anorexia and malabsorption. Fatigue and generalized weakness are also common. Untreated malabsorption may result in specific nutritional deficiencies and their attendant symptoms. With the loss of albumin into the intestinal lumen as a consequence of lymphatic obstruction, ascites and peripheral edema may develop.

Arthritis is the most common extraintestinal symptom and affects the majority of patients [16,28–30]. It often develops several months to years before the initial diagnosis of Whipple

Table 49.1 Symptoms and physical findings in patients with Whipple disease

Gastrointestinal	Extraintestinal	Neurological
Symptoms		
Common	**Common**	**Common**
Diarrhea	Arthritis	*Central nervous system:*
Weight loss	Arthralgias	Dementia
Anorexia	Fever	Myoclonus
		Insomnia
Less common	**Less common**	Lethargy
Abdominal bloating	*Pulmonary:*	Headache
Abdominal cramps	Chronic cough	Muscle weakness
Hematochezia	Pleuritic pain	Ataxia
	Cardiac:	*Cranial nerves:*
	Congestive heart failure	Deafness
	Symptomatic pericarditis	Dysarthria
	Symptomatic valvular disease	Facial numbness
		Diplopia
	Ocular:	
	Decreased visual acuity	
Physical findings		
Common	**Common**	**Common**
Weight loss	Lymphadenopathy	Physical findings associated with
Abdominal distension	Hyperpigmentation	manifestations above
	Fever	
	Arthritis	
	Heart murmurs	
Uncommon	**Uncommon**	
Ascites	Peripheral edema	
Hepatomegaly	Pleural friction rub	
Splenomegaly	Ocular findings	

disease and is typically an intermittent, migratory arthritis of both the large and small joints. Some patients may have only arthralgias. Permanent joint deformity is rare. Fever, usually low grade and intermittent, is the second most common extraintestinal symptom of Whipple disease [16]. Numerous other extraintestinal symptoms develop reflecting the systemic nature of the infection. Pulmonary involvement is often manifested by chronic cough or pleuritic chest pain [1,16,20,80]. Cardiac involvement [81–84] may be manifested as congestive heart failure, valvular lesions [81,82], or pericarditis [16,19,80,84]. Numerous reports of endocarditis secondary to *T. whipplei* infection in the absence of overt gastrointestinal disease have been published [85–88].

Although central nervous system (CNS) involvement is thought to be common, symptoms related to CNS Whipple disease may be present in only 10%–20% of patients (Table 49.1) [89–94]. Neurological symptoms may occur in association with gastrointestinal symptoms or as isolated symptoms [15,21,95,96]. The most common CNS symptoms are dementia, paralysis of gaze, and myoclonus [15]. Hypothalamic symptoms such as insomnia, hyperphagia, and polydipsia are also frequently present [15].

The physical findings in patients with Whipple disease are variable, depending on the organ systems involved (Table 49.1). Frequently, the findings are related to severe malabsorption and the loss of fluid and electrolytes, such as emaciation, muscle wasting, peripheral edema, purpura, glossitis, muscular irritability, and peripheral neuropathy. Hyperpigmentation and peripheral lymphadenopathy is the most common physical findings [16,29,30], seen in more than 50% of patients with Whipple disease. The palpable lymph nodes are usually firm, nontender, and mobile. Abdominal findings often include mild distention and tenderness. Abdominal fullness or mass may be caused by marked enlargement of the mesenteric lymph nodes. Ascites is uncommon but may be seen in association with severe hypoalbuminemia. Hepatomegaly and splenomegaly are uncommon [16,30]. Additional well-recognized physical findings in patients with Whipple disease

include fever, peripheral arthritis, heart murmurs, pleural or pericardial friction rubs, and ocular abnormalities, such as uveitis [47]. The neurological examination may reveal findings suggestive of CNS or cranial nerve involvement, such as dementia, ataxia, muscle weakness, sensory loss, and ophthalmoplegia.

Laboratory findings

Laboratory abnormalities, usually related to severe malabsorption, are common in patients with Whipple disease. Most have steatorrhea [16,30] in addition to low serum carotene levels [16,30] and hypoalbuminemia [16,29,97]. In patients with severe diarrhea and malabsorption, electrolyte disturbances such as hypokalemia, hypomagnesemia, and hypocalcemia may be seen. Anemia is a common finding [15,16,28–30]. The anemia is usually normochromic–normocytic, suggestive of chronic disease. Occasionally, it is hypochromic–microcytic, related to iron deficiency; however, macrocytosis secondary to folate or vitamin B-12 deficiency is unusual. Many patients have an elevated erythrocyte sedimentation rate, and the prothrombin time is often prolonged secondary to malabsorption of vitamin K [16].

Radiological and endoscopic findings

Radiological evaluation can be helpful, particularly a barium contrast study of the small intestine, the results of which are abnormal in most patients with Whipple disease [16,30,98]. The most characteristic finding is marked thickening of the mucosal folds, suggestive of an infiltrative process. This finding is usually more prominent in the duodenum and proximal jejunum, with decreased involvement distally and sparing of the ileum. In addition to the marked thickening of the proximal small bowel, abdominal computed tomography (CT) often reveals massive paraaortic and retroperitoneal adenopathy [16,99].

Gross endoscopic lesions in the duodenum have been described in Whipple disease [100,101]. The characteristic finding is pale, shaggy, yellow mucosa in the postbulbar duodenum. Yellow plaques or erosions may also be seen. Wireless capsule endoscopy revealed disease affecting the entire small intestine in a patient with refractory Whipple disease [102]. Although involvement of the esophagus and colon is unusual in Whipple disease, endoscopic lesions similar to those seen in the duodenum, consisting of pale, white, plaque-like nodules, have been described [103].

Diagnostic evaluation

The most important step in the diagnosis of Whipple disease is to consider this rare disease in the differential diagnosis of the much more common conditions that cause the many symptoms and findings associated with the disease. Thus, for example, Whipple disease should be considered in the differential diagnosis of unexplained gastrointestinal symptoms or malabsorption, weight loss, seronegative arthritis, culture-negative endocarditis, and fever of unknown origin

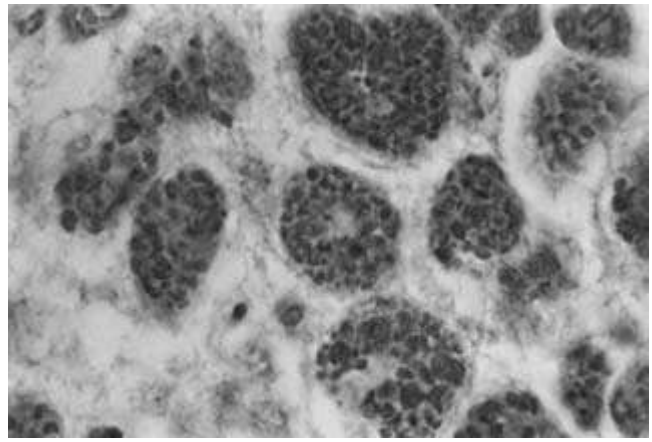

Figure 49.3 Light microscopic appearance of macrophages in the intestinal lamina propria in Whipple disease. Note that most of the periodic acid–Schiff (PAS)-stained inclusions are round or sickle-shaped. This appearance is virtually diagnostic, especially in the intestinal mucosa. (PAS and hematoxylin stain; original magnification × 1000.) From Dobbins [15].

Small intestinal mucosal biopsy is the diagnostic test of choice and is required for a definitive diagnosis. The histological finding of infiltration of the small intestinal lamina propria by PAS-positive macrophages containing gram-positive, acid-fast-negative bacilli accompanied by lymphatic dilation is specific and diagnostic of Whipple disease (Fig. 49.3). Immunohistochemical analysis has also been shown to be a sensitive and specific method for the diagnosis of Whipple disease [104,105]. Electron microscopy should also be routinely performed on the biopsy specimens to verify the presence of the characteristic bacillus. This is particularly important during the follow-up of patients treated for Whipple disease in whom PAS positivity persists [10,16,106], often for many years; electron microscopy in successfully treated patients shows the disappearance of the Whipple bacillus.

Rarely, the diagnosis of Whipple disease is established in the absence of intestinal involvement by the identification of bacilli in involved tissues, including the brain and lymph nodes. Although the colonic mucosa may occasionally be involved in Whipple disease, caution is required in the interpretation of rectal biopsy specimens, because PAS-positive macrophages may be found in normal rectal and colonic biopsies [107].

Molecular diagnosis using PCR-based diagnostic tests has become useful in confirming the diagnosis of Whipple disease and in monitoring the response during antibiotic treatment [24,43,45–47]. In two retrospective studies of intestinal biopsies, PCR was shown to be a highly sensitive and specific test to confirm the diagnosis of Whipple disease [26,27]. Additional studies have confirmed the value of PCR for diagnosis and monitoring bacterial elimination during

therapy [108–110] and in diagnosis of extraintestinal Whipple disease [111–114]. A PCR assay on stool specimens may provide a diagnostic tool that does not require endoscopy [115]. Although previous reports suggested that PCR-based tests of peripheral blood mononuclear cells or whole blood were useful [45,46], subsequent studies have not confirmed the sensitivity of this approach compared with testing of duodenal biopsy specimens [108,116].

Differential diagnosis

The differential diagnosis of Whipple disease includes other malabsorptive diseases with diffuse small intestinal involvement, such as celiac disease, and infiltrative diseases of the small intestine, such as intraabdominal lymphoma. These diseases can be easily and reliably differentiated by small intestinal mucosal biopsy.

Of particular importance, given the increasing incidence of acquired immunodeficiency syndrome (AIDS), Whipple disease must be distinguished from *Mycobacterium avium* complex (MAC) infection. The latter infection causes infiltration of the lamina propria with PAS-positive macrophages (Fig. 49.4), as in Whipple disease [117,118], but MAC bacilli are acid-fast, whereas the Whipple bacillus is not. In addition, MAC organisms can be cultured and have an electron microscopic appearance markedly different from that of Whipple bacilli. Systemic histoplasmosis and macroglobulinemia are also conditins in which PAS-positive macrophages can be seen in the intestinal lamina propria; however, in systemic histoplasmosis, large PAS-positive, rounded, encapsulated *Histoplasma* organisms are easily seen in macrophages, and the faintly staining, homogeneously PAS-positive macrophages of macroglobulinemia are distinctly different from Whipple disease.

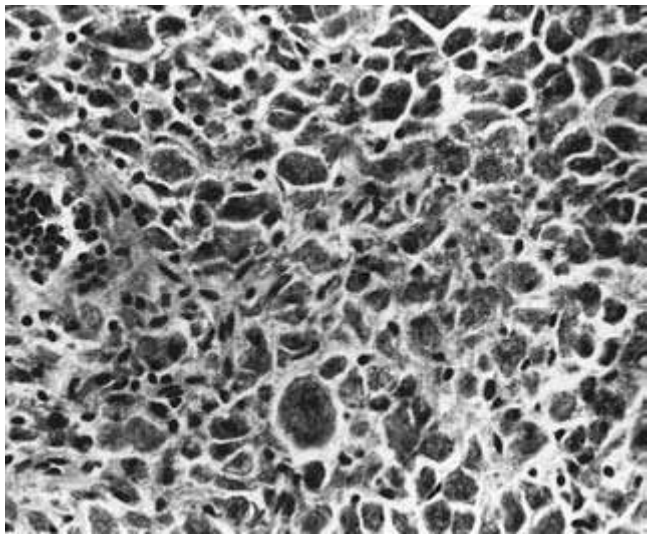

Figure 49.4 Light microscopic appearance of macrophages in the intestinal lamina propria of a patient with AIDS and *Mycobacterium avium* complex involvement of the gut. Note the easily identified bacilli within the macrophages. (Acid-fast stain; original magnification × 500.) From Dobbins [15].

Treatment and prognosis

Although controlled treatment trials have not been conducted in Whipple disease, there is substantial observational evidence to guide antibiotic thereapy, which usually results in dramatic improvement. Numerous antibiotic regimens effective against gram-positive organisms and given for variable lengths of time have been used successfully, including penicillin, penicillin and streptomycin, erythromycin, ampicillin, chloramphenicol, tetracycline, and trimethoprim–sulfamethoxazole (TMP–SMX) [8–16]. Empirical observations are supported by the development of techniques to culture *T. whipplei* [51–54], enabling antibiotic susceptibility testing [119–121]. Doxycycline, macrolides, aminoglycosides, penicillin, rifampin, chloramphenicol and TMP–SMX were found to be active in vitro against three strains of *T. whipplei*. There was heterogeneity in susceptibility to imipenem and cephalosporins. Aztreonam and fluoroquinolones were not active. Further study revealed that *T. whipplei* is naturally resistant to fluoroquinolones [119] and that the susceptibility to TMP–SMX is due to SMX alone [120]. The combination of doxycycline and hydroxychloroquine was bactericidal suggesting that this combination should be evaluated in the treatment of patients with Whipple disease [121].

Despite the initial response to antibiotic therapy, relapses are common. Relapses can occur during treatment or months to years after completion of treatment [10,14–16,92]. In a review of the long-term follow-up of 88 patients with Whipple disease who had initially responded successfully to antibiotic therapy, 31 patients eventually relapsed [14]. The mean time to relapse was 4.2 years. Of the 31 patients who relapsed, 21 had received initial therapy with tetracycline alone. Clinical relapses, consisting of a recurrence of gastrointestinal symptoms and arthritis, occurred both early and late and responded favorably to further antibiotic treatment, whereas CNS relapses tended to occur late and responded poorly to additional antibiotic therapy.

Given the concern about CNS relapses [14,64,92,95] it is reasonable to assume that all patients may have CNS involvement and to treat initially with an antibiotic that readily crosses the blood–brain barrier. One double-strength tablet of TMP–SMX (160 mg of TMP and 800 mg of SMX) given twice daily for 1 year is the best long-term option (Table 49.2) [14]. The TMP–SMX penetrates the blood–brain barrier and should effectively eradicate CNS involvement. Although shorter courses of antibiotic therapy may be effective, because of the concerns about CNS relapse and its poor response to additional therapy, it is appropriate to treat patients for 1 year [14,16,122]. Expert opinion suggests that initial therapy with parenteral penicillin G (1.2 million U/day) and streptomycin (1.0 g/day) for 10–14 days may be of additional benefit [14,16,122], resulting in the lowest

Table 49.2 Treatment of Whipple disease

	Medication	Duration
Treatment of choice	Trimethoprim–-sulfamethoxazole	1 year
	or	
	Procaine penicillin G/streptomycin (parenteral)	2 weeks
	plus	
	Trimethoprim–sulfamethoxazole	1 year
Alternatives	Penicillin or ampicillin[a]	1 year
	Chloramphenicol[b]	1 year

a May be used in patients with sulfa allergy without central nervous system symptoms.
b May be used in patients with sulfa allergy with central nervous system symptoms.

Table 49.3 Infectious diseases mimicking tuberculous enteritis

	Primary risk group(s)	Primary gastrointestinal site	Distinguishing clinical features	Diagnosis
Mycobacterium tuberculosis infection	AIDS immunosuppression	Ileum	Abdominal mass	Caseating granulomas
		Cecum		AFB in mucosa
MAC infection	AIDS	Ileum Cecum	Prominent fevers	AFB in blood
Aspergillosis	Neutropenic patients	Multiple sites	Widely disseminated symptoms	Y-shaped branching, septate hyphae in mucosa
Histoplasmosis	AIDS	Ileum Cecum	Pulmonary symptoms	Intracellular oval yeast
Mucormycosis	Malnourished patients	Multiple sites	Bleeding from multiple sites	Large, nonseptate, thick-walled hyphae in mucosa
Yersinia enterocolitis	Children	Terminal ileum	Shorter disease course	Gram-negative coccobacilli in stool
Cryptosporidiosis	AIDS	Small intestine	Diarrhea, often voluminous	Oocytes within epithelial cells or in stool
CMV infection	AIDS Transplant patients	Colon	Bloody diarrhea	Inclusion bodies in submucosal or mucosal crypts

AFB, acid-fast bacilli; CMV, cytomegalovirus; MAC, *Mycobacterium avium* complex.

relapse rate. This should be considered in all patients with Whipple disease, particularly because compliance with the prescribed regimen is often limited (Table 49.3). In patients who are allergic to or cannot tolerate TMP–SMX, parenteral penicillin and streptomycin for 10–14 days followed by oral penicillin VK or ampicillin for 1 year is reasonable. In these patients, if the development of CNS symptoms is a concern, chloramphenicol is an option. Oral chloramphenicol, like TMP–SMX, achieves relatively high CNS concentrations. In addition to antibiotic treatment, in patients with severe malabsorption and malnutrition, appropriate supplementation of specific nutrients, such as folic acid, vitamin B-12, fat-soluble vitamins, and iron, should be given to prevent deficiency.

At the conclusion of 1 year of antibiotic therapy, a small intestinal mucosal biopsy should be repeated to document the absence of residual bacilli before treatment is discontinued. Although PAS-positive macrophages may be present in the lamina propria for many years in patients treated for Whipple disease, the presence of bacilli on electron microscopy suggests inadequate treatment. At this time, PCR for *T. whipplei* in intestinal biopsy specimens is not routinely recommended; however, if available, it may provide useful

information regarding the adequacy of therapy and the likelihood of relapse. Positivity on PCR suggests inadequate treatment, whereas PCR negativity predicts a low likelihood of clinical relapse but does not guarantee an absence of future CNS relapse [26,27].

The prognosis for patients with Whipple disease who receive appropriate and effective antibiotic therapy is excellent. Extraintestinal symptoms such as fever and arthritis often disappear within a few days. Gastrointestinal symptoms frequently resolve within 1 month. Most patients are asymptomatic within a few months. After therapy has been discontinued, patients must be carefully observed for evidence of relapse, including clinical evaluation and laboratory studies. Endoscopy with intestinal biopsy should be performed at 1 year to confirm the response to treatment, but routine invasive diagnostic studies, such as annual endoscopy with intestinal biopsy, are not warranted. Relapse may be signaled by a recurrence of gastrointestinal or extraintestinal symptoms. If relapse is suspected, small intestinal biopsy should be repeated and the specimen examined for the presence of free bacilli. The treatment of a relapse of Whipple disease consists of a repetition of the initial therapy. Gastrointestinal relapse responds well to further antibiotic therapy. If the patient does not respond to TMP–SMX, then a trial of oral penicillin VK or tetracycline is reasonable. Patients with CNS relapse have a relatively poor prognosis. If the patient does not respond to a repeated course of the initial therapy, then a course of chloramphenicol is warranted. Combination therapy with antibiotics and IFN-γ may be beneficial in patients with refractory Whipple disease [70].

Tropical sprue

Tropical sprue, first reported in 1759 in Barbados, is a syndrome occurring in individuals residing in certain tropical locations [123]. The cause is presumed to be bacterial overgrowth of the small intestine. The essential clinical features of the disease are chronic diarrhea and malabsorption, with resultant weight loss. Megaloblastic anemia secondary to folate and vitamin B-12 deficiency is usually present. The diagnosis is made by small intestinal biopsy and confirmed by prompt clinical and laboratory response to treatment with folic acid and broad-spectrum antibiotics.

Epidemiology

Tropical sprue is found in many tropical locales, including India, Cuba, the Dominican Republic, Haiti, Puerto Rico, northern South America, and a number of other countries, including the Philippines. The disease is primarily found in adults, occurring only rarely in children. Tropical sprue seems to occur mostly as sporadic isolated cases, but epidemics have been described among British forces serving in Burma during World War II, American military personnel serving in the Philippines, and villagers living in southern India [124–126]. In both sporadic cases and epidemics, a peak seasonal occurrence of tropical sprue has been observed that is unrelated to the rainy season [127].

Although tropical sprue can afflict visitors within weeks or months after their arrival in a region of endemicity, it rarely occurs in short-term travelers, usually developing in persons who have lived in a region for more than 6 months to 1 year [128]. The prevalence of tropical sprue appears to be decreasing, possibly related to the frequent self-medication with antibiotics. Tropical sprue is more common where sanitary conditions are poor [129]. Rarely, tropical sprue becomes clinically apparent months or even years after individuals have moved to a temperate climate [130].

Etiology and pathogenesis

Tropical sprue is presumed to result from chronic bacterial overgrowth of the small intestine by toxigenic strains of coliform bacteria. An infectious etiology is supported by documented epidemics and seasonal variation and the response to antibiotic treatment.

Most individuals with tropical sprue exhibit bacterial overgrowth of the jejunum with strains of *Klebsiella pneumoniae* or, less often, *Enterobacter cloacae* or *Escherichia coli* [131–133]. Eradication of these bacteria with antimicrobial therapy results in cure of the intestinal abnormalities [131,133]. The organisms that overgrow the small bowel of patients with tropical sprue are facultative anaerobes, typically consisting of only one or a few species, serotypes, or biotypes in each individual patient [132]. There are no anaerobes as classically seen in diseases manifested by small bowel stasis and resultant bacterial overgrowth, such as blind loop syndrome. The organisms isolated from patients with tropical sprue have been shown to have pathogenic effects in animal models, but specific pathogenic factors have not been identified [134,135]. In most nonnative residents, tropical sprue develops after an attack of acute watery diarrhea. Acute infectious diarrhea, caused by a variety of enteric pathogens, may be associated with transient jejunal contamination by coliform bacteria [136]. Multiple factors have been suggested to contribute to the disease, including increased bacterial adherence, immune dysfunction, dietary fat, and altered motility, but no definitive primary abnormalities have been identified [137–146]. The unique role of folate deficiency in the pathogenesis of tropical sprue is not well understood. Folate deficiency leads to a decreased number of intestinal epithelial cells and atrophy of villi and is associated with a number of intestinal structural and functional alterations, including crypt hypertrophy, blunting of villi, and less efficient absorption of water, electrolytes, and carbohydrates. Treatment with pharmacological doses of folic acid results in structural and functional improvement in the small bowel of patients with tropical sprue [128].

The role of coliform bacteria has been questioned, because they have been isolated from the proximal small intestine in healthy asymptomatic controls in tropical locations such as India and South Africa [147,148], and these bacteria have not been found in all patients with tropical sprue.

Histopathology

The histopathological finding in patients with tropical sprue is partial atrophy of the villi in the small intestine. A completely flat mucosa, as is seen in untreated celiac sprue, is found in fewer than 10% of patients with tropical sprue. The histological changes consist of lengthening of the crypt area, broadening and shortening of the villi, chronic infiltration of the lamina propria by inflammatory cells, and variable degrees of abnormality of the surface epithelium (Fig. 49.5) [138,149]. The basement membrane usually appears thickened, staining as collagen on light microscopy [149]. Structural abnormalities are more prominent in the jejunum during the early phase of tropical sprue; however, eventually the entire small bowel is equally affected.

Clinical manifestations

Many individuals can identify the onset of their disease as an episode of acute diarrhea. Less commonly, the onset of tropical sprue is insidious. The chronic symptoms, including prolonged diarrhea, abdominal cramping, nausea, and anorexia with associated weight loss, are nonspecific and related to malabsorption. Fever may be present at the onset of the disease but rarely persists. Lactose intolerance often develops early in the course of tropical sprue, caused by a reduction in brush-border disaccharidase enzyme activity. Exocrine pancreatic insufficiency caused by intestinal damage may develop and contribute to the malabsorption [150]. Within a few months, malabsorption results in folate deficiency. After a variable period of time, usually about 6 months but sometimes earlier in conjunction with poor nutrition, severe depletion of folate coupled with the development of vitamin B-12 deficiency causes symptoms associated with megaloblastic anemia, such as fatigue and weakness. Later in the course of the disease, peripheral neuropathy and confusion may occur. High-output heart failure may occur as a result of severe anemia. These deficiencies and their manifestations can develop in about 10% of patients with tropical sprue who have never experienced any gastrointestinal symptoms. The physical findings include manifestations of nutritional deficiencies such as pallor, glossitis, and edema and typically appear late [151,152]. Stomatitis, hyperpigmentation, and neurological manifestations of vitamin B-12 deficiency may occur but are rare.

Laboratory findings

Megaloblastic anemia, caused by combined deficiencies of folate and vitamin B-12, is the hallmark of tropical sprue. Steatorrhea and reduced xylose absorption are frequently present. Malabsorption of fat-soluble vitamins often occurs, resulting in low serum concentrations of vitamin A (and β-carotene), 25-hydroxyvitamin D, and calcium. Marked elevation of the prothrombin time is unusual. Hypoalbuminemia and hypocholesterolemia are also seen.

Radiological and endoscopic findings

Contrast radiography of the small bowel may reveal findings suggestive of tropical sprue such as prominent, thickened mucosal folds with a coarsened or irregular contour involving the jejunum and ileum and lumenal dilation and flocculation of the barium [153]. Although these findings are nonspecific, ileal involvement is characteristic of tropical sprue and relatively uncommon in celiac sprue, Whipple disease, and other small bowel enteropathies. Endoscopic visualization of the small bowel may reveal scalloping of the valvulae conniventes and a mosaic pattern of the mucosa [154], findings frequently described in celiac sprue.

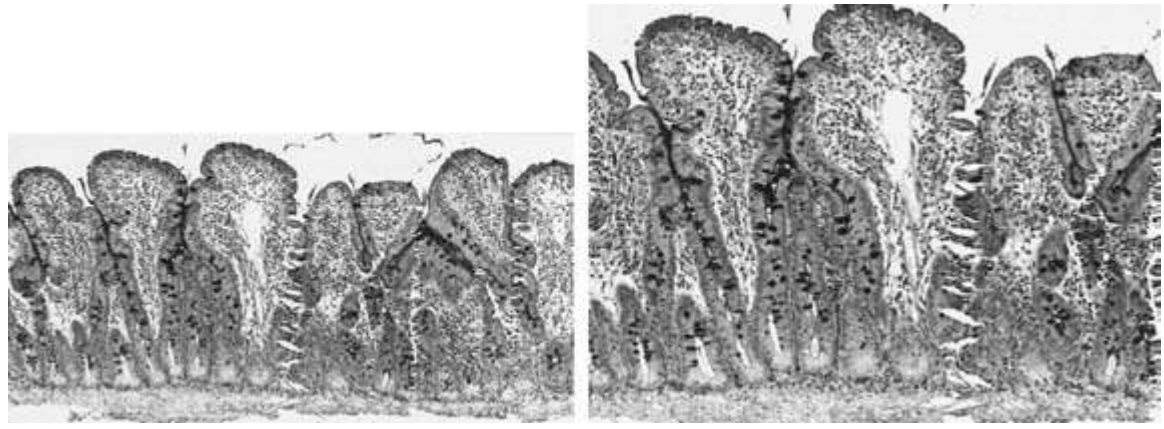

Figure 49.5 **(a)** Light microscopic appearance of the moderate villus abnormality of tropical sprue acquired by a North American during a 2-week visit to the Philippines. Note the broadened villi, infiltration of the lamina propria by plasma cells and lymphocytes, and lymphocytic infiltration of the epithelium. (Periodic acid–Schiff and hematoxylin stain; original magnification × 80.) **(b)** Enlargement of a portion of (a) reveals the epithelial changes. (Original magnification × 132.)

Diagnostic evaluation

When the diagnosis of tropical sprue is suspected, endoscopy with small bowel biopsy should be performed. The demonstration of characteristic changes in the architecture of the villi, including partial atrophy, supports the diagnosis. The diagnosis of tropical sprue is confirmed by a prompt clinical and laboratory response to the institution of appropriate treatment. Stool culture is of no diagnostic value in tropical sprue, but the stool should be examined for ova and parasites to rule out other infectious causes of chronic diarrhea, such as *Giardia*.

Differential diagnosis

The differential diagnosis includes other malabsorptive diseases and enteropathies, including celiac disease, Whipple disease, HIV enteropathy, infection of the small intestine with parasites such as *Cryptosporidium, Giardia,* and *Strongyloides*, and bacterial overgrowth caused by diseases that alter intestinal motility, such as amyloidosis, pseudoobstruction, scleroderma, and blind loop syndrome. Primary intestinal lymphoma may present with chronic diarrhea, often with significant associated abdominal pain. Intestinal tuberculosis may occur in some tropical countries but it is much less common than tropical sprue. Finally, tropical sprue occurs in areas such as Haiti, where another entity, usually referred to as *tropical enteropathy,* is prevalent [155]. Tropical enteropathy is generally associated with milder abnormalities of intestinal structure and function, and clinically significant malabsorption or nutritional deficiencies do not develop in affected persons. Unlike the abnormalities of tropical sprue, the intestinal abnormalities of tropical enteropathy resolve within a year after return to a temperate climate, even without specific therapy [156]. It is thought that the intestinal abnormalities of tropical enteropathy are a manifestation of repeated, transient episodes of intestinal infection caused by various enteric pathogens.

Treatment and prognosis

Combination therapy with tetracycline and folate is the recommended approach to the treatment of tropical sprue. This consists of 250 mg of tetracycline four times a day and 5 mg of folic acid once daily. Although 1 month of therapy has been effective in travelers with tropical sprue, treatment for up to 6 months is often required for those with chronic disease to achieve complete remission of the malabsorptive syndrome and to prevent recurrence [157–159]. Although not necessary for the acute phase of therapy, vitamin B-12 (1000 μg), administered parenterally once a week for several weeks, should also be given to replete tissue stores. Despite treatment with combination therapy for 6 months, intestinal abnormalities may persist, and clinical relapse, requiring additional courses of therapy, may occur in 10%–20% of patients who remain in tropical locations of endemicity [160].

Tuberculosis

Mycobacterium tuberculosis, the primary cause of tuberculosis (TB), infects a third of the world's population and causes more deaths than any other infectious agent except HIV [161]. It is found primarily in developing countries, where overcrowding and poor sanitation contribute to its spread. In the United States, the number of reported TB cases reached an all time low of 4.9 per 100 000 persons in 2004, and slightly more than half were among foreign-born persons [162]. Although pulmonary manifestations predominate in most cases of TB, gastrointestinal involvement can be part of a disease process affecting multiple organs; less commonly, gastrointestinal involvement in the absence of other identifiable sites of infection (so-called primary gastrointestinal TB) may be associated with symptoms and even be the primary cause of death [163–165].

Epidemiology

Before the development of effective anti-TB chemotherapy, gastrointestinal TB was seen in about 70% of persons with advanced pulmonary disease [166]. Since the advent of effective chemotherapy, gastrointestinal TB has become much rarer, and in the United States and Canada it is reported in fewer than 1% of all cases [167,168]. In developed countries, it is most commonly found in association with immunosuppression, especially in patients with AIDS [169]. In developing countries, AIDS and immunosuppression, in combination with limited access to anti-TB therapy and inadequate sanitation, appear to have increased the incidence of gastrointestinal TB. In one series from South Africa, 46% of patients with smear-positive, cavitating pulmonary TB had gastrointestinal involvement [170]. Some data suggest that the risk for gastrointestinal disease is directly related to the severity of pulmonary disease, although not all persons with gastrointestinal TB have evidence of current or past pulmonary disease [164,170,171]. Rare cases of congenital necrotizing enterocolitis have been reported in infants born to mothers with miliary TB [172].

Etiology and pathogenesis

Gastrointestinal TB is usually caused by *M. tuberculosis,* but in developing countries it may also rarely be caused by a closely related species, *M. bovis* [161]. *M tuberculosis* is an acid-fast, curved bacillus that can infect certain primates, including humans, although humans are the only known reservoir. It spreads from human to human by aerosolization. It is thought that gastrointestinal disease arises when organisms are swallowed and survive destruction by gastric acid; the source of the organisms can be either dietary, such as infected milk in the rare cases of *M. bovis* infection, or more commonly pulmonary secretions. Lesions may also occur as a result of disseminated miliary disease.

Infection of the upper gastrointestinal tract is exceptional, and lesions are more often found in areas characterized by high lymphoid density and stasis, such as the distal small bowel, cecum, appendix, and, less commonly, colon and rectosigmoid [164,166,171,173,174]. Gastrointestinal TB is thought to arise by the same pathophysiological sequence as pulmonary infection: initial infection of macrophages is followed by multiplication of the organisms in macrophages, caseation necrosis, and a secondary host immune response. The immune response is characterized by either cell-mediated immunity, with a marked influx of activated myeloid and lymphoid cells, or a delayed hypersensitivity reaction accompanied by cell death and caseation necrosis [175]. The defects of cell-mediated immunity seen in patients with AIDS and other immunocompromised hosts result in failure to contain the organism, so that these patients are predisposed to the development of clinical disease once infected.

Pathology

The pathological lesions of gastrointestinal TB are characterized by ulcers with granulomas and later extensive fibrosis [164,166,171,176]. The ulcerations presumably develop as a result of infection of gastrointestinal macrophages, with subsequent caseation necrosis and intense inflammatory changes. Multiple focal areas of involvement are typical [166,176]. Fistulae and anorectal lesions may form [164]. Histologically, in the early stages, the ulcerative lesions are characterized by granulomas, usually with caseation and larger numbers of acid-fast organisms [171]. Involvement of draining lymph nodes is common [164]. With more chronic inflammation, the bowel wall may become markedly fibrotic and stenotic, with the formation of mass lesions (tuberculomas). Strictures are typically short but may be greater than 10 cm long and involve multiple areas of the intestinal wall [164]. The number of organisms may be markedly diminished in the fibrotic stages, or organisms may be absent in patients who have received treatment.

Clinical manifestations

The symptoms of gastrointestinal TB are nonspecific, and in the absence of pulmonary TB, the diagnosis may be difficult. The most common symptom of gastrointestinal TB is abdominal pain [164,171,173,177]. Others include fever, anorexia, diarrhea, weight loss, constipation, bloating, and hemorrhage [164,166,171,173,177].

If disease is limited to the duodenum, the symptoms may be primarily those of peptic ulcer disease [178]. The physical findings may include cachexia and, in patients with an acute pulmonary process, pulmonary manifestations [179–181]. The abdominal examination may reveal a palpable mass, which in various series is the abnormality most commonly found [171,173]. Other physical examination findings include evidence of complications such as perforation, obstruction, fistulae, massive hemorrhage, and malabsorption [163,164,174,178].

Diagnostic evaluation

Barium contrast studies may demonstrate ulcerations, spasticity, strictures, fistulae, and thickening, deformity, and incompetence of the ileocecal valve; evidence of mass lesions similar to those of Crohn's disease may be seen (Fig. 49.6) [182–184]. Computed tomography and ultrasonography both show mural thickening of the bowel at sites that correlate with the pathological findings at colonoscopy [185]. Mesenteric lymphadenitis, omental thickening, and ascites are frequently associated with tuberculous enterocolitis [180]. Peritonitis is found in about 20% of patients [186]. Lesions may also be found in liver, spleen, or pancreas. Fine-needle aspiration, either CT-guided or ultrasonographically guided, has been used successfully for diagnosis [187].

The diagnostic procedure of choice is usually colonoscopy with biopsy because it does not involve surgery, and the most common site of involvement is the ileocecal region

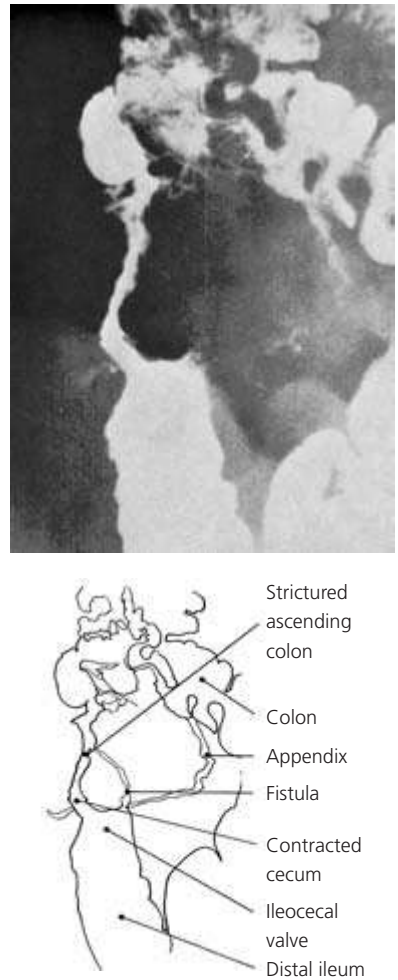

Figure 49.6 Barium study in a patient with hypertrophic tuberculosis shows a dilated distal ileum leading into a contracted cecum and strictured ascending colon. From Misiewicz JJ, Forbes A, Price A, et al. Atlas of Clinical Gastroenterology, 2nd edn. London: Wolfe, 1994:5.4, with permission from Elsevier.

[171,172,176]. Visual inspection of the involved bowel most commonly reveals multiple areas of nodular mucosa with areas of ulceration [173,176]. The ulcers are typically irregular; circumferential, in contrast to the linear ulcerations of Crohn's disease; and have a necrotic base [171,188]. Upper gastrointestinal endoscopy with jejunal or duodenal biopsy is indicated less commonly, when disease at these sites is suspected [177]. In addition, paracentesis, laparoscopy, and laparotomy can also be used to diagnose TB.

Histological studies of biopsy material showing caseating granulomas, or Ziehl–Neelsen- or Kinyoun-stained specimens showing acid-fast, beaded, slightly bent rods, may provide immediate answers, but often are nondiagnostic. Typical caseating granulomas are found less frequently in biopsy material from lymph nodes than from the intestine and may be found in only half of specimens [176]. Potentially, PCR can provide a rapid diagnosis for extrapulmonary tuberculosis, with studies showing sensitivities between 64% and 86% and specificities of 100% [189–191]. When ascitic fluid is present, a high ascitic fluid adenosine deaminase may greatly aid in the diagnosis, with multiple studies showing greater than 90% sensitivity and specificity for TB [192–195]. However, in nonendemic areas of the world, such as the United States, the sensitivity is lower, especially in patients with cirrhosis [196].

Culture of biopsy material or ascitic fluid remains the gold standard but requires at a minimum 1–2 weeks for results. The diagnostic yield is best with material obtained from peritoneal biopsies, with one case series showing positive results for 87% of patients with peritoneal biopsies compared with 54% of patients who underwent colonoscopy [177,197].

Differential diagnosis

Tuberculosis should always be considered in the differential diagnosis of patients with obscure abdominal symptoms or physical findings, especially those who are immunocompromised or living where TB is endemic. Crohn's disease often presents similarly to TB but more commonly begins earlier in life, and granulomas, when present, are poorly organized, less than 400 μm in maximum dimension, and more often located in the rectosigmoid [176]. In addition, severe active Crohn's disease is usually not found in patients with advanced HIV infection. Other entities in the differential diagnosis of TB enteritis include lymphoma, carcinoma, diverticular disease, appendicitis, and other infections of the gastrointestinal tract such as *Yersinia*, histoplasmosis, MAC enteritis, and cytomegalovirus infection (Table 49.3) [166,181,185].

Treatment and prognosis

The prognosis of a patient with gastrointestinal TB depends primarily on their immune status and the institution of the appropriate therapy in a timely manner. In untreated HIV-infected patients, the disease progresses rapidly and is almost always fatal [169]. However, the results of therapy are usually satisfactory, even in immunocompromised hosts, when therapy is started sufficiently early in the course of disease. The difficulty lies in making a timely diagnosis, and in many series significant numbers of patients die before the disease is diagnosed [165,170].

If any indication of TB is noted, such as a compatible clinical picture in a high-risk patient, a positive acid-fast stain, or histological evidence of granulomas, therapy should be started immediately pending the results of culture and susceptibility testing. The regimen of choice is 300 mg isoniazid, 600 mg rifampin, 15–30 mg/kg pyrazinamide, and 15 mg/kg ethambutol [198]. If susceptibility testing reveals that the organism is sensitive to all drugs, then ethambutol can be stopped immediately and pyrazinamide continued for only 2 months. Isoniazid and rifampin must be continued for an additional 4 months, for a total of 6 months of therapy.

If the organism is resistant to isoniazid (the most common form of resistance), then treatment should be started with or changed to rifampin, pyrazinamide, and ethambutol and continued for 6 months [198]. In cases with suspected multidrug-resistant TB, isoniazid, rifampin, and pyrazinamide plus an additional two or three agents should be started until in vitro sensitivity tests are complete. Additional agents include a fluoroquinolone (levofloxacin, moxifloxacin, or gatifloxacin), an injectable agent such as streptomycin 15 mg/kg/day (if not used previously and susceptibility to streptomycin had been established), amikacin or kanamycin 15 mg/kg/day (maximum dose 1.0 g/day), or capreomycin, with or without an additional oral drug. Adverse effects of these second-line agents include ototoxicity, neurotoxicity, and nephrotoxicity. Two to four additional second-line agents should also be used if drug malabsorption is suspected.

Surgical intervention is usually not necessary and is reserved for complications such as obstruction, perforation, fistula, or a mass that does not resolve with medical therapy [165,174,179]. In most cases, a trial of medical therapy should be undertaken before surgical intervention. Complications can be fatal, even with surgical intervention, and may occur after the initiation of anti-TB medications [163–165,199].

Mycotic infections

Mycotic infections are most often a result of dissemination from a pulmonary source and usually occur in immunocompromised hosts, although they may also occur in normal hosts. The etiological agents of aspergillosis, candidiasis, histoplasmosis, cryptococcosis, and mucormycosis have all been cultured from the small intestine. Although *Cryptococcus* has been cultured from the small intestine of patients with AIDS, it has only rarely been associated with clinical disease so it will not be discussed further here [200,201]. The differential

diagnosis for all mycotic infection of the small bowel is similar and includes other mycotic infections, Crohn's disease, TB enteritis, disseminated *Mycobacterium avium* complex (MAC), bacterial typhlitis, ischemic bowel, and carcinoma [202,203].

Histoplasmosis

Epidemiology

Histoplasmosis is a common infection in the midwestern and south central regions of the United States, where 80% of the inhabitants are infected [204]. Infection, however, rarely equates with disease. When symptoms do occur, they most commonly involve the lungs, the primary portal of entry. Gastrointestinal disease occurs usually, but not always, as a result of widespread dissemination in an immunocompromised host. Infants and children, the elderly, patients on immunosuppressive therapy, including steroids, and persons with immunosuppressive diseases (especially AIDS) are all at greater risk than the general population.

Etiology and pathology

Histoplasmosis is disease due to infection by *Histoplasma capsulatum*, a fungus that exists in the mycelial form at ambient temperature (30°C) and in the yeast form at body temperature (37°C) [204]. This dimorphic fungus produces both macroconidia and microconidia in clinical isolates. Visual inspection of the involved bowel may reveal entirely normal tissue or a variety of lesions. The most common lesions are single or multiple ulcers and tissue necrosis [202,205,206]. Other lesions include flat elevations and polypoid protrusions. Microscopic examination of pathological specimens, after staining with methenamine silver, hematoxylin and eosin (H & E), periodic acid–Schiff (PAS), or Wright–Giemsa stain, reveals tiny (2–4 μm in diameter) oval yeast forms inside macrophages [204,207]. Pathological specimens may also show granulomatous inflammation if the patient is not severely immunocompromised [204,205].

Clinical manifestations

Although involvement of the small and large bowel is common in disseminated disease, symptomatic disease is much less common. In one series, only 50% of persons with gastrointestinal ulcerations had diarrhea or abdominal pain [206]. In addition, persons with disseminated disease who have little or no gastrointestinal pathology may have diarrhea or abdominal pain. Furthermore, even when gastrointestinal symptoms are present, they are often overshadowed by systemic or pulmonary complaints [203]. Occasionally, however, gastrointestinal complaints predominate. In one series, over 40% of patients presented with gastrointestinal complaints rather than pulmonary complaints [205].

The symptoms most commonly associated with small intestinal histoplasmosis include diarrhea, weight loss, fever, abdominal pain, nausea, vomiting, lethargy, and fatigue [202,207,208]. The stools are typically liquid or semiliquid and on occasion are bloody, especially if the colon is also involved. Weight loss of up to 13.5 kg (30 lb) has been reported. The abdominal pain is usually cramping and may be diffuse or localized, depending on the underlying pathology. Other reported presentations include edema of the legs and eyelids caused by protein-losing enteropathy [202] or malabsorption with steatorrhea [203,207].

The most frequent complication and often a cause of death is perforation of the small bowel [202,209]. In one series, this was the terminal event in 11% of persons with disseminated histoplasmosis [202]. Obstruction by ileocecal masses or enlarged retroperitoneal lymph nodes can also occur, as well as intussusception in association with pseudopolypoid or polypoid lesions [202,206].

Diagnostic evaluation

Involvement of the gastrointestinal tract should be suspected in the setting of blood cultures positive for *H. capsulatum*, or a positive test result for *H. capsulatum* antigen in the blood or urine [208]. For a definitive diagnosis of gastrointestinal involvement it is necessary to perform upper gastrointestinal endoscopy with duodenal or jejunal biopsy, or colonoscopy with terminal ileum biopsy or, in rare cases, surgery. The choice of biopsy site should be guided by the symptoms and the knowledge that the ileocecal region is most commonly involved; all specimens should be cultured for diagnosis [202,206].

Treatment and prognosis

In the past, cases of disseminated disease, in which small intestinal involvement is frequent, have gone on for more than 10 years without specific therapy [202]. However, this situation is less likely today because of the increased association of the disease with immunosuppression, in particular AIDS. Immunosuppressed persons often have more severe disease that progresses relatively rapidly, and death is inevitable without treatment.

Because gastrointestinal histoplasmosis usually implies widely disseminated disease, adults with gastrointestinal disease should be with treated with amphotericin B deoxycholate 0.5 mg/kg/day intravenously until clinical improvement occurs, usually in 1–2 weeks, and then they can be switched to itraconazole for the remaining course of therapy [210]. Therapy should be continued with itraconazole for 6–18 months. Patients with AIDS require maintenance therapy for life with 200 mg of itraconazole per day [211]. If amphotericin needs to be used for the duration of therapy because of the patient's inability to tolerate oral medications, the total dose of amphotericin should be 35 mg/kg given over 2–4 months. Liposomal preparations can be used instead of amphotericin B deoxycholate if the patient is refractory or intolerant to amphotericin B deoxycholate [211].

Aspergillosis

Epidemiology

Unlike histoplasmosis, which can develop in immunocompetent hosts, aspergillosis affects only severely immunocompromised patients. Factors that have contributed to an increase in the prevalence of aspergillosis include AIDS, organ transplantation, immunosuppressive chemotherapy, and the improved survival of premature infants [212]. The small intestine is involved in about 5% of patients with disseminated aspergillosis [212,213].

Etiology and pathology

Aspergillosis is a disease caused by infection with *Aspergillus*, a mold that grows by branching and longitudinal extension of wide (2–5-μm), Y-shaped, branching, septate hyphae [214]. The most common clinical isolate is *Aspergillus fumigatus*, followed by *Aspergillus flavus*. Clinical disease is produced by vascular invasion and necrosis. Lesions seen in pathological specimens or during endoscopy include linear ulcers, hemorrhagic infarcts, and necrotic mucous plaques [212,213,215].

Clinical manifestations

The data on the clinical manifestations of aspergillosis of the gastrointestinal tract are sparse because much of the information has been derived from autopsy series. The most commonly reported symptom is gastrointestinal bleeding [212,213]. Small intestinal perforation may also occur [215,216].

Diagnostic evaluation

The diagnosis is especially difficult because *Aspergillus* is not easily cultured in the laboratory, and even when it is cultured, the culture may signify only contamination or colonization [212,214]. Biopsy with histological evidence of tissue invasion is essential to verify that *Aspergillus* is the cause of the disease process. *Candida* may also be seen on histological specimens and may contribute to the disease process [213,214].

Treatment and prognosis

Disseminated aspergillosis, regardless of whether or not small intestinal involvement is present, carries a uniformly poor prognosis unless the underlying immunosuppression can be reversed [214]. It is critical for successful treatment that antibiotic therapy be started early in the course of disease. Based on a clinical trial showing improved survival with voriconazole compared with amphotericin B for the treatment of invasive aspergillosis, the recommended treatment for small intestinal aspergillosis is voriconazole 4–6 mg/kg every 12 hours i.v. followed by 200 mg twice a day by mouth once oral medication is tolerated [217]. Therapy should be continued until a clinical response occurs and, if the patient is temporarily neutropenic, until neutropenia has resolved [214]. Amphotericin B deoxycholate and lipid formulations of amphotericin are alternative therapies. In addition, caspofungin, a new echinocandin antifungal agent for salvage therapy, is also licensed in the United States for treatment of aspergillosis in patients refractory to or intolerant to the above-mentioned therapies [210].

Candidiasis

Epidemiology

Candida species are the fourth most common organisms isolated from the blood of hospitalized patients [218] and are normal colonizers of the gastrointestinal tract [219]. The organisms are commonly associated with esophageal disease in immunocompromised patients but rarely cause disease of the small intestine. Persons at highest risk include those with AIDS, chronic mucocutaneous candidiasis, or malignancies, and those taking immunosuppressive agents.

Etiology and pathology

Candida is a yeast-like fungus that reproduces by budding small (4–6-μm), thin-walled, ovoid cells. Yeast forms, pseudohyphae, and hyphae can all be found in tissue specimens. *C. albicans* is the species most often associated with disease of the small intestine. The most common lesions associated with *Candida* are single or multiple ulcerations [215,219]. Superficial erosions, bloody masses, and pseudomembranes also occur, but with less frequency. White plaques and thickened mucosal folds may also be seen on endoscopy [215].

Clinical manifestations

Candidiasis of the small bowel is rarely diagnosed antemortem, so which symptoms are attributable to fungal invasion is often unclear. The most common symptom associated with intestinal candidiasis is diarrhea [215]. Other reported symptoms include nausea, vomiting, flatulence, abdominal pain, abdominal distention, melena, and hematochezia [215,219]. Complications include perforation and penetration of intestinal ulcers [215].

Diagnostic evaluation

Because *Candida* is a normal colonizer of the entire gastrointestinal tract and even more abundant in persons at risk for disease because of decreased immune function and antibiotic treatment, a diagnosis of small intestinal candidiasis is not easily made. The mere presence of the organism on stain or culture of specimens from the small intestine does not establish the diagnosis. A biopsy specimen of the small intestine with histological evidence of invasion is necessary. *Aspergillus* may also be found on histological sections and may be part of the disease process [213,215].

Treatment and prognosis

Therapy for *Candida* infection continues to evolve, with Food and Drug Adminstration (FDA) approval of two echinocandin antifungal agents. Because of its comparable efficacy and far fewer adverse events when compared with amphotericin

in the treatment of invasive candidiasis, we recommend caspofungin 70 mg i.v. loading dose followed by 50 mg i.v. daily [220]. Therapy should continue for at least 2 weeks and until a clinical response occurs. In patients with temporary neutropenia caused by chemotherapy, treatment should not be stopped until the neutrophil count rises above 500/mm^3. Alternative therapies include: 400 mg/day of fluconazole for stable patients who are not suspected of having azole resitance; 0.6–0.7 mg/kg/day of amphotericin B; or lipid formulations of amphotericin B for patients who do not respond or have adverse reactions to amphotericin B deoxycholate. Resection of the diseased bowel or maintenance therapy may be required, especially in patients who are permanently immunosuppressed.

Mucormycosis

Epidemiology

Like gastrointestinal aspergillosis and candidiasis, small intestinal mucormycosis is most commonly found in persons with impaired immune defenses. These include patients treated with deferoxamine mesylate and immunosuppressive medications, and patients with diabetes mellitus, malnutrition, chronic renal insufficiency, burns, aplastic anemia, solid and bone marrow transplantation, malignancy, and AIDS [221–223]. Injecting drug users and premature infants may also have significant gastrointestinal mucormycosis [223,224]. Small intestinal infection is usually acquired through dissemination from a pulmonary source, although some authors have suggested that fungi can enter the body with food [221].

Etiology and pathogenesis

The usual pathogens, *Rhizopus*, *Absidia*, and *Mucor*, are ubiquitous in nature and are inhaled as spores that produce infectious particles called sporangiospores. Organisms produce disease by invading blood vessel walls, causing infarction and necrosis [222]. They can be identified by their large, nonseptate, thick-walled hyphae with right-angled branching in tissue sections [222]. In culture, they grow as fluffy molds. Mucormycosis has a predilection for certain anatomical sites, including the sinuses, orbits, brain, and lungs. Isolated gastrointestinal lesions are uncommon. Involvement of the gastrointestinal tract may occur as a part of disseminated disease. Mucormycosis may cause mass lesions resembling carcinoma, cause infarction and perforation, or involve the ileocecal region in a pattern resembling perforated appendicitis. In neonates, small bowel infarction resembling necrotizing enterocolitis has also been reported. Mucormycosis can lead to superinfection of preexisting lesions of the gastrointestinal tract, including peptic ulcers, penetrating wounds, and TB [225].

Clinical manifestations

Fever, abdominal pain, and distention are the most common symptoms [221,222]. Nausea, vomiting, hematemesis, hematochezia, diarrhea, and melena are seen less commonly. Because multiple sites of the gastrointestinal tract are often involved, it is difficult to determine which symptoms are attributable to small intestinal involvement. In one reported case with only small intestinal involvement, the only symptom was abdominal distention [221]. The physical findings may include abdominal mass lesions or signs of bowel necrosis or perforation. Intestinal perforation is the most common complication [221,222].

Diagnostic evaluation

As in other small intestinal mycoses, disease is more commonly diagnosed postmortem than antemortem [222]. A definitive diagnosis requires biopsy for culture and histological examination for evidence of tissue invasion [226]. Endoscopy may reveal ulcerations, especially in the ileum [221,227]. Culture is frequently unsuccessful, and even when it is successful, it may signify only colonization because the agents of mucormycosis are saprophytic and ubiquitous in nature.

Treatment and prognosis

In one series, 85% of patients with gastrointestinal mucormycosis died during the course of mycosis and the majority of deaths were due to bowel perforation [223]. The treatment of choice is 1.0–1.5 mg/kg/day of amphotericin B deoxycholate combined with surgical resection [228]. Lipid formulations of amphotericin B can be used in higher doses to treat patients with renal insufficiency or those not responding to initial therapy. If a clinical response to amphotericin B occurs, dosing frequency can be decreased to every other day for an unknown duration and, in fact, may be lifelong. A newly approved azole, posaconazole, is active against many of the agents of mucor and may provide another therapeutic option [228].

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C SMALL INTESTINE

50 Celiac disease

Peter H.R. Green, Anne R. Lee

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Definition

Celiac disease is a gluten-sensitive enteropathy. Characteristic, although not specific, abnormalities found in the small intestine are reversed by withdrawal of gluten from the diet. The disease is defined in those in whom both clinical and pathological abnormalities are reversed by gluten elimination [1]. Gluten is the term for the storage proteins of wheat. The alcohol-soluble fraction of gluten, gliadin, is toxic in celiac disease, along with similar proteins in barley (hordeins) and rye (secalins). Although the primary disease event occurs in the small intestine, the disease clinically resembles a multisystem disease [2].

Dermatitis herpetiformis (DH), an intensely pruritic, vesicular rash, is the dermatological manifestation of celiac disease. The rash is usually symmetrical, occurring on the elbows, knees, buttocks or scalp, although it can occur anywhere on the body.

Historical aspects

The word "celiac" comes from the Greek word for abdomen. Celiac disease has been known for about two thousand years, with accounts found in the writing of Aretaeus the Cappadocian in the first century AD [3].

The first modern description of celiac disease was by Gee [4] from London. In 1888 he described the disease as occurring in all age groups, although mainly in young children. He also recognized that treatment was dietary in nature. Subsequently, medical literature reflected the restriction of certain carbohydrates and use of bananas in the diet [5]. Andersen [6], in New York, determined that some children with the celiac syndrome in fact had cystic fibrosis. However, it was the Dutch physician, Dicke, who in the 1930s recognized wheat as the cause of celiac disease [7], publishing the work in Dutch in 1941 [8]. He subsequently determined that it was the protein or gluten component of wheat which was injurious [9–11].

The gluten-free diet was first used in adults in 1952 [12], although it was not uniformly accepted in the United States as a necessary therapy [13]. Greater understanding of the nature of the disease came with the recognition of the pathological finding of villous atrophy in the small intestine [14,15]. Subsequently, the development of peroral biopsy instruments in the 1950s [16] allowed widespread use of this technique to determine that the disease was the same in children and adults [17] and that the villous atrophy improved on the gluten-free diet [18]. Booth and colleagues [19] in London, in a follow-up study of children as adults, showed persistence of the intestinal lesion into adulthood, indicating that therapy of the disease should be lifelong.

It was also noted that patients may present in subtle ways, without diarrhea or the malabsorption syndrome, increasing the clinical spectrum of the disease [20]. Marsh [21] subsequently emphasized the histological spectrum of the disease.

In the last two decades the development of very specific and sensitive antibody tests (to endomysial tissue and the enzyme tissue transglutaminase) allowed the epidemiological studies that have demonstrated the worldwide occurrence and the high prevalence (approaching 1%) of the disease [22,23]. In addition these tests have allowed the diagnosis to be considered by any physician.

The immunological mechanism of the disease has also been elucidated [24], providing the potential for the development of drugs that may be used in addition to the gluten-free diet [25].

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Epidemiology

Serological screening studies have revealed that celiac disease is very common, with an incidence of nearly 1% [26–30]. It is recognized in every continent including Asia [31,32], the Middle East [28,33,34], North Africa [35–37], and South America [38] and Cuba [39]. The majority of those with celiac disease are undiagnosed [26], although the rate of diagnosis is increasing [40].

Genetic factors

Celiac disease is a polygenic disease. Concordance in monozygotic twins (70%) is much higher than in major histocompatibility complex (MHC)-identical siblings (30%). Overall about 10% of first-degree relatives of affected individuals have celiac disease. This increases to 20% if there are affected sib pairs with celiac disease in the family, and even extends to second-degree relatives [41].

Human leukocyte antigen (HLA) genes confer up to 40% of the genetic risk, the rest being attributable to non-HLA genes [42]. The human major histocompatibility molecules DQ2 and DQ8 are essential genetic factors for the development of celiac disease, with the majority of patients carrying DQ2 (DQA1*05–DQB1*02). In the remaining patients, an association with DQ8 (DQA1*0301–DQB1*0302) is found [42]. A very few patients may lack DQ2 or DQ8 (DQ2 or DQ8 negative), but carry half of the DQ2 alleles, either DQA1*05 or DQB1*02.

Among Caucasians, 30%–40% carry DQ2 or DQ8 but less than 3% will develop celiac disease, suggesting that, although necessary, DQ2 and DQ8 molecules are not sufficient to mediate the disease. Non-HLA genes have been sought and candidate genes identified. There is evidence for strong linkage at 5p31-33 [43] and also, albeit to a lesser degree, at 19p13.1 [44] and 11q [45]. A small and controversial effect of the non-HLA gene *CTLA4* located on chromosome 2q33, which encodes a molecule involved in the inhibition of T-cell activation, has been reported [46,47]. Because of the linkage disequilibrium effect it is difficult to identify associations with non-MHC class II genes that are encoded within the HLA locus. However, there are indications that the *MICB*10* gene coding for MIC B molecules is associated with celiac disease [48]. There is also evidence of an association with tumor necrosis factor (TNF)2, also encoded within the HLA locus in the MHC class III region [49]. In addition, an association with a common variant located in intron 28 of the gene myosin IXB (*MYO9B*), which encodes an unconventional myosin molecule that has a role in actin remodeling of epithelial enterocytes, has been identified [50].

Environmental factors

Considerable knowledge concerning environmental factors important in the development of celiac disease was obtained from studies of an epidemic of infantile celiac disease in Sweden in the early 1980s [51]. Lack of breast-feeding, a large amount of gluten in infant formula, and ≥ 3 infections markedly increased the risk of celiac disease [52,53]. The greatest protection from celiac disease occurred when a small amount of gluten was ingested while breast-feeding was undertaken. The timing of gluten ingestion was also important in the development of celiac disease in a cohort of at-risk children studied in the United States [54]. These data form the basis of recommendations regarding breast-feeding and the timing of gluten ingestion [55]. For at-risk children, for example children with celiac disease in other family members, breast-feeding for at least 6 months is recommended, with the introduction of gluten between the fourth and sixth months. As well as protecting against the development of celiac disease, breast-feeding delays the onset and alters the clinical presentation of celiac disease in children [56,57].

Pathogenesis

Gluten is not fully digested by humans. A 33-amino-acid peptide (33-mer) and probably other immunogenic peptides remain after the action of gastric, duodenal, and pancreatic enzymes [58]. This 33-mer is considered to be immunogenic in celiac disease.

Gliadin enters the mucosa, probably during gastrointestinal infections or because of alterations in tight junction permeability by up-regulation of zonulin, a protein that induces tight junction disassembly and a subsequent increase in intestinal permeability [59]. The gliadin-induced T-cell response comprises an adaptive and an innate component [24]. The former is a DQ2/DQ8-restricted CD4 T-cell response in the lamina propria. Gliadin is a good substrate for the enzyme tissue transglutaminase [60], which can transform glutamine residues into negatively charged glutamate residues by deamidation. In this process gliadin becomes conformationally altered, facilitating binding to the groove on the surface of DQ2 and DQ8 molecules on antigen-presenting cells. Deamidation markedly enhances the CD4 antigluten T-cell response [61,62]. Interferon-γ is the main cytokine responsible for villous atrophy (Fig. 50.1). However, the role of other cytokines in the intestinal inflammatory response is not fully defined [63].

As the antigliadin response develops in the gut, antitransglutaminase antibodies appear. Their role in the pathogenesis of the disease is not clear; however, serum IgA antibodies do inhibit crypt epithelial cell differentiation [64].

The second component of the antigluten T-cell response is the intraepithelial CD8 T-cell response involving the innate immune system [25]. The model proposed for the CD8 T-cell response in the epithelium is one directed against stressed epithelial cells [25]. There are several lines of evidence sup-

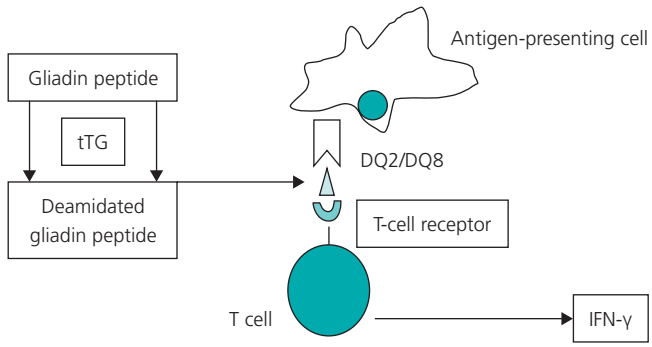

Figure 50.1 Immunological mechanism occurring in the lamina propria. Gliadin enters the lamina propria where it is deaminated by tissue transglutaminase (tTG), allowing the gliadin molecule to bind to the DQ2 or DQ8 molecule on antigen-presenting cells. Gliadin is then presented to gliadin-restricted CD4 T cells, which release interferon (IFN) and other cytokines that cause villous atrophy.

porting this model. First, intraepithelial CD8 T cells, which express the natural killer receptor NKG2D, can kill epithelial cells expressing the stress-induced MIC molecules [65,66]. Second, studies suggest that peptides not recognized by CD4 T cells can induce early epithelial changes [66,67] and induce IL-15 and MIC molecules on epithelial cells which arm the cytolytic NKG2D pathway to kill stressed epithelial cells [65,66].

It is unclear how gluten triggers the expression of stress molecules and IL-15 on epithelial cells and how the CD4 T-cell response in the lamina propria relates to the CD8 T-cell process in the epithelium.

Clinical presentation

The clinical classification of celiac disease is based on the presence of gastrointestinal symptoms. *Symptomatic* or *classical* celiac disease refers to presentations with diarrhea, with or without a malabsorption syndrome, whereas in *asymptomatic*, *atypical* or *silent* celiac disease gastrointestinal symptoms are lacking or not prominent. Females predominate over males, with a ratio of 2:1 or 3:1 [68]; however, men may have a more severe form of the disease at presentation [69]. Although the peak age of diagnosis is in the fourth and fifth decades [68], population-based screening studies from the United Kingdom revealing that 1% of both 7 year olds and adults have celiac disease suggest that celiac disease occurs in children and may remain undetected until adulthood [26,30].

The majority of those diagnosed with celiac disease have Caucasian genetic traits, although the disease has been diagnosed in Chinese people living in Canada [70], in African Americans [71], in a North American native [72], and in West Indian children living in England [73].

Younger children present with diarrhea and failure to thrive, whereas older children are more likely to present with anemia, short stature, neurological problems, and other atypical symptoms such as constipation [57].

Patients have a long duration of symptoms before diagnosis [68]. The delay in diagnosis has been attributed to physician delay rather than delay in patients seeking health care [74]. Diarrhea is the most frequent presenting symptom in adults, although the numbers of patients presenting with diarrhea has been decreasing [40,75]. Some patients present with the classical malabsorption syndrome with steatorrhea, weight loss, edema secondary to hypoalbuminemia, and hypocalcemia.

Other modes of presentation include iron deficiency [76], osteoporosis [77], and the recognition of mucosal changes in patients undergoing endoscopy for either esophageal reflux or dyspeptic symptoms [78,79], as well as screening high-risk groups including relatives of patients with celiac disease [29], type I diabetics [80], and those with Down syndrome [81]. There is increasing recognition of celiac disease as a cause of various neurological syndromes including small fiber peripheral neuropathy [82,83], epilepsy with occipital calcifications in childhood [84], and ataxia [85]. A previous diagnosis of irritable bowel syndrome is common [68], and in one study 5% of those fulfilling strict criteria for irritable bowel syndrome had celiac disease [86]. In addition, atypical presentations include rheumatological symptoms, abdominal pain, macroamylasemia, hypoalbunimemia, abnormal liver tests, and evidence of hyposplenism [2].

Abdominal pain may be severe and mimic an acute surgical condition [87]. In fact, patients diagnosed with celiac disease have a greater prevalence of surgery compared with controls. This includes a greater frequency of appendectomy, tonsillectomy, hernia repair, and cosmetic surgery. These increased surgical rates have been attributed to misdiagnosis or poor health status as well as to an impaired psychological profile [88].

Diagnosis

Traditionally, the diagnosis of celiac disease is made in an individual in whom a biopsy of the upper small intestine demonstrates the characteristic findings of villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis, and in whom there is an unequivocal clinical response to gluten withdrawal [1]. A gluten-free diet is therefore initiated when a provisional diagnosis of celiac disease is made. Patients come to biopsy because of a clinical suspicion of the disease, the presence of positive serological tests, or the recognition of abnormalities in the duodenum at endoscopy [89] (Fig. 50.2).

Serological testing for celiac disease should occur under various clinical settings (Table 50.1).

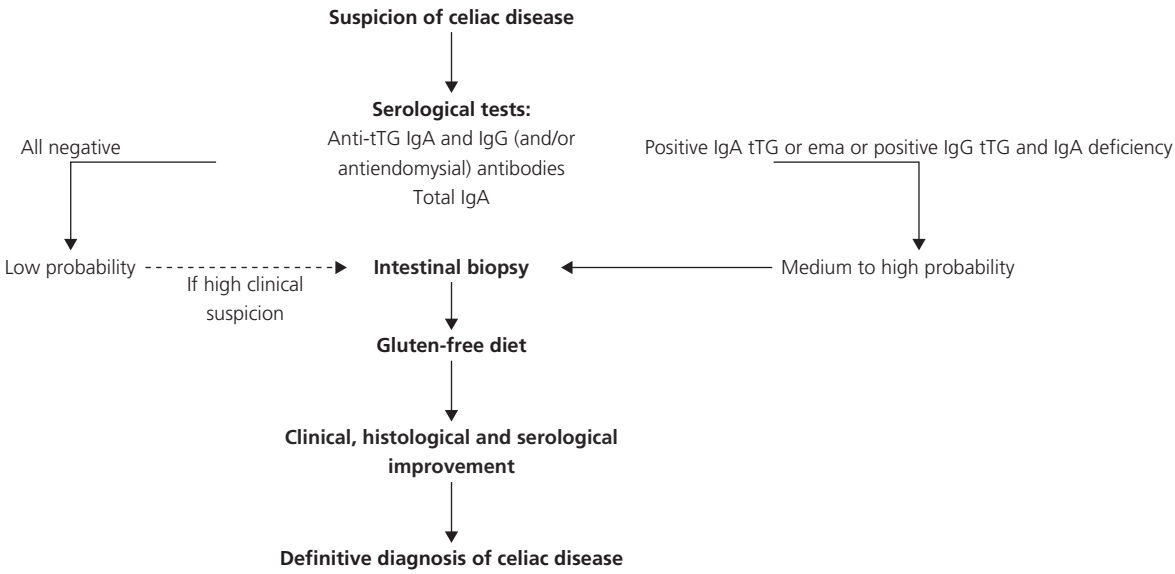

Figure 50.2 An algorithm for the evaluation of a patient for celiac disease. ema, endomysial antibody; tTG, tissue transglutaminase.

Table 50.1 Clinical indications for serological testing

Chronic diarrhea with and without malabsorption
Irritable bowel syndrome
Unexplained weight loss
Iron-deficiency anemia
Folate deficiency
Vitamin E or K deficiency
Osteoporosis
Hypocalcemia or vitamin D deficiency, secondary hyperparathyroidism, persistently low urinary calcium excretion
Unexplained elevation of transaminases
First-degree relatives of patients with celiac disease
Associated autoimmune diseases: type I diabetes, Sjögren syndrome, primary biliary cirrhosis
Down and Turner syndromes
Neurological disorders: unexplained peripheral neuropathy, epilepsy, and ataxia

Serological testing

The most sensitive tests are based on the use of IgA isotypes. The available tests include antigliadin antibodies as well as connective tissue antibodies: endomysial and tissue transglutaminase antibodies. The antigliadin antibodies have been available for many years; however, their lower sensitivity and specificity compared with the tissue transglutaminase and endomysial antibodies has resulted in their use being called into question for the diagnosis of celiac disease [90]. The role of newer generation antigliadin antibodies, specific for immunodominant epitopes of gliadin, is yet to be determined [91].

The standard test uses the IgA endomysial antibody (EMA). This is based on its very high specificity, which approaches 100%. Overall, the sensitivity of the EMA is excellent, with the majority of reports indicating greater than 90% sensitivity [90]. The EMA is detected using immunoflouresence techniques requiring either monkey esophagus or human umbilical cord as the substrate.

The recognition of the enzyme tissue transglutaminase 2 (tTG) as the autoantigen for the EMA [60] allowed the development of enzyme-linked immunoassays that are less expensive and less observer dependent than the EMA test [23]. Different commercial kits for assaying tTG have different characteristics and resultant sensitivities and specificities [92]. Overall, the sensitivity of IgA anti-tTG is greater than 90% [90]. Although the specificity of the EMA is virtually 100%, the tTG test does not achieve the same degree of specificity. There are many reports of positive tTG results in the absense of celiac disease [93–95].

Selective IgA deficiency occurs more commonly in patients with celiac disease than in the general population [96]. To detect celiac disease in those with selective IgA deficiency, a total IgA level should be incorporated into the testing for celiac disease, as well as an IgG antibody-based test, preferably IgG-tTG [97].

Several studies have revealed a lack of sensitivity of the serological tests in the clinical practice setting [86,98,99]. This is more than likely due to the inclusion of patients with lesser degrees of atrophy, a situation in which patients may not express EMA or tTG [100,101].

Antigliadin antibodies and EMA and tTG antibodies may be recovered in duodenal secretions, stool, and saliva of patients with celiac disease as well as in normal individuals and

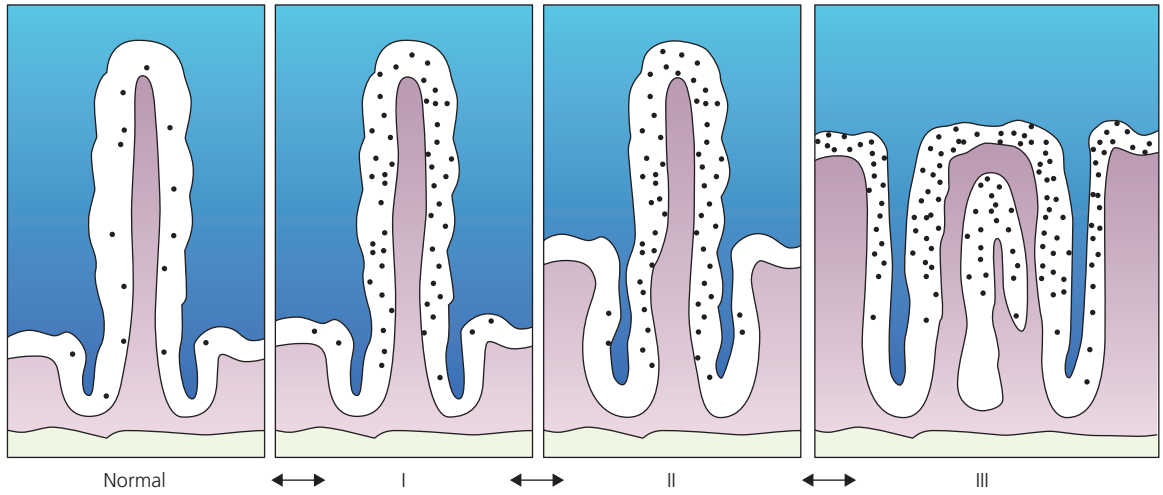

Figure 50.3 The spectrum of histological findings in celiac disease is shown diagramatically. Marsh 0 is normal with a crypt–villous ratio of 1:4. Marsh I consists of normal crypt/villous architecture together with an intraepithelial lymphocytosis (> 20 lymphocytes per 100 epithelial cells). Marsh II has, in addition to an intraepithelial lymphocytosis, crypt hyperplasia. Marsh III lesions include villous atrophy together with crypt hyperplasia and an intraepithelial lymphocytosis. Marsh III a, b and c correspond to partial, subtotal and total villous atrophy.

those with small intestinal bacterial overgrowth [102–105]; however, their value in the diagnosis of celiac disease is limited [104,106].

Biopsy and histology

Biopsy of the small intestine remains the gold standard in the diagnosis of celiac disease [107]. Biopsies of the descending duodenum rather than the more distal intestine are sufficient for the diagnosis of celiac disease [108]. The recognition of the spectrum of histological changes in celiac disease, as classified by Marsh [21], has provided a major advance in the diagnosis of celiac disease (Fig. 50.3).

The earliest lesion, Marsh I, is characterized by normal villous architecture with an intraepithelial lymphocytosis. A duodenal intraepithelial lymphocytosis is, however, not specific for celiac disease. It may be identified in giardiasis, tropical sprue, autoimmune diseases, *Helicobacter pylori* gastritis, Crohn's disease, and bacterial overgrowth [109–112]. A Marsh II lesion is identified when the intraepithelial lymphocytosis is accompanied by crypt hypertrophy. The majority of patients diagnosed with celiac disease (90%) fall into the category of Marsh III, which includes partial, subtotal, and total villous atrophy. Villous atrophy is not specific for celiac disease and may be seen in a variety of other conditions (Table 50.2).

Pitfalls in the diagnosis of celiac disease include both over- and underinterpretation of villous atrophy because of poorly oriented biopsies. Reasons for histological findings that are milder than expected include a reduced amount of gluten in the diet, as often happens in a family in which there are members with celiac disease, or the use of immunosuppressant medications.

Table 50.2 Causes of villous atrophy

Giardiasis
Tropical sprue
Collagenous sprue
Eosinophilic gastroenteritis
Combined variable immunodeficiency
HIV enteropathy
Autoimmune enteropathy
Tuberculosis
Radiation enteritis
Zollinger–Ellison syndrome
Whipple disease
Intestinal lymphoma
Crohn's disease
Other food intolerances (milk, soy, chicken, tuna as isolated case reports)

Role of HLA DQ2/DQ8 assessment

HLA DQ2 is found in up to 90%–95% of patients with celiac disease, with HLA DQ8 found in most of the remaining patients [113]. Because these HLA alleles occur in up to 30%–40% of the general population, the role of determining whether an individual carries HLA DQ2 or DQ8 rests in their high negative predictive value [114]. It is therefore of value in determining which family members should be screened, in excluding the disease when patients are already on a gluten-free diet, and when the diagnosis of celiac disease is unclear.

Associated diseases

There are many conditions associated with celiac disease (Table 50.3). These include autoimmune diseases, which occur three to ten times as frequently in those with celiac disease as in the general population [69,115,116].

Autoimmune diseases

The increased frequency of autoimmune diseases in patients with celiac disease may occur because of a common genetic and immunological mechanism as well as because of the presence of celiac disease itself. Gluten withdrawal does not protect from the development of autoimmune diseases [115], although diabetes and thyroid-specific autoantibodies may disappear in children and adolescents after starting a gluten-free diet, suggesting a relationship between the autoimmune process and gluten exposure [117]. Improvement of cardiomyopathy [118], hypothyroidism [119], or peripheral neuropathy [83] may occur on a gluten-free diet; however, generally, the associated autoimmune disorders do not improve after the diagnosis of celiac disease and treatment with a gluten-free diet. Associated autoimmune diseases include Sjogren syndrome [120] and alopecia areata [121].

Malignancy and celiac disease

Various malignancies are associated with celiac disease. These include esophageal and head and neck squamous carcinoma, small intestinal adenocarcinoma, and non-Hodgkin lymphoma [122–124]. DH also carries an increased rate of non-Hodgkin lymphoma [125]. The non-Hodgkin lymphomas are of both T- and B-cell type, occurring at both intestinal and extraintestinal sites [124–126]. A large population-based study from Sweden showed elevated risks for malignant lymphomas and small intestinal, oropharyngeal, esophageal, large intestinal, hepatobiliary, and pancreatic carcinomas [127]. A smaller study has demonstrated an increased risk for melanoma and papillary thyroid cancer [124].

The gluten-free diet is considered to be protective against the development of malignancy. Several studies have demonstrated that the increased risk for the development of lymphoma is less than previously thought [128–130], only two- to fourfold [131]. There is a reduced risk of breast cancer in celiac disease [127,132].

Hematological associations

Oxentenko et al. [76] found a high incidence (15%) of celiac disease among patients undergoing endoscopy for evaluation of iron-deficiency anemia. The same authors detected celiac disease among 8.7% of 103 patients subjected to routine duodenal biopsy at endoscopy for iron-deficiency anemia [133]. Karnam et al. [134] found a lower incidence (2.8%) of celiac disease in their prospective study of iron-deficient patients who underwent both endoscopy and colonoscopy. They concluded that all patients with iron deficiency should have duodenal biopsies routinely as celiac disease is a common etiology which is treatable [134]. In total, 5% of another series of iron-deficient anemic patients had celiac disease; all those with celiac disease had been refractory to oral iron therapy [135].

Anemia was present in 90% of children with celiac disease in an Indian series [136]. Hematological manifestations among 22 children with celiac disease included anemia alone in 19 patients (86.3%), leukopenia in two patients (9%), and thrombocytopenia in one patient (4.5%). In total, 12 patients had an iron-deficiency anemia that coexisted with zinc and vitamin B-12 deficiency [137]. Despite iron supplementation and a gluten-free diet, abnormal hematological parameters may persist [138].

Vitamin B-12 deficiency was considered rare in celiac disease. However, studies have revealed that it is common, occurring in 12% and 40% of two case series respectively [139,140].

Osteoporosis

Reduced bone density is common in patients with celiac disease [77]. A few studies have shown that screening osteoporotic patients with celiac antibodies is beneficial in diagnosing patients with silent celiac disease [141]. The prevalence of celiac disease in patients with osteoporosis has been found to be 2%–7%. Worsening T scores have also been found to correlate with more severe celiac disease [77,142]. Among 431 patients presenting for bone density determination, the prevalence of celiac disease was 1.2% in patients with osteopenia and 2.1% in those with osteoporosis [143]. The yield from screening postmenopausal patients with osteoporosis for celiac disease is low and not considered worthwhile [144].

Rheumatological manifestations

Among the extraintestinal manifestations of celiac disease, arthritis is common. There have been many case reports of arthritis being the presenting symptom in both adults and children [145–149]. In one study, systematic examination of patients with celiac disease revealed arthritis to be common; arthritis was present in 26% of 200 celiac patients compared with 7.5% of control subjects. There was also a higher incidence of arthritis among untreated celiac patients. The arthritis was seronegative and oligoarticular, similar to the other enteroarthritides [150]. Bone scintigraphy was carried out to detect early acute inflammatory lesions in 22 adult celiac patients and was positive for sacroiliitis in 14 cases (63.6%) [151]. An increased prevalence of celiac disease in juvenile chronic arthritis is controversial [152,153].

Luft et al. [154] tested the sera of patients with various rheumatological disorders including Sjögren syndrome, rheumatoid arthritis, systemic lupus erythematosus (SLE), and systemic sclerosis for the presence of tissue transglutamin-

ase antibodies and found an increased incidence (10%) of celiac disease only among patients with Sjögren syndrome. Similarly, Rensch et al. [155] screened patients with SLE for antigliadin and antiendomysial antibodies and found no patients with endomysial antibodies. They did find that 23% of patients were positive for antigliadin antibodies but that they had no evidence of celiac disease [155].

Neurological disorders

Neurological symptoms as a presenting feature of celiac disease have become increasingly recognized. The associations discussed in this chapter are not the result of vitamin or mineral deficiency states. When neurological symptoms occur in those with celiac disease, vitamins E and B-12 deficiency need to be excluded as well as copper deficiency.

The most well-documented neurological associations are with peripheral neuropathies, ataxia, and epilepsy. The celiac neuropathy is typically a sensory, small fiber neuropathy. One study reported that 23% of patients with celiac disease who were well controlled by diet had peripheral neuropathy compared with a 4% occurrence rate in the control group [156]. At a tertiary care referral center, celiac neuropathy was found in 2.5% of the patients evaluated for neuropathy [82]. Furthermore, celiac disease was found in 5% of patients with symptoms of neuropathy *and* normal electrodiagnostic studies, making celiac disease an important diagnostic consideration in the evaluation of small fiber or idiopathic sensory neuropathies [82,83].

Ataxia is one of the neurological syndromes more frequently associated with celiac disease [157,158] – it is often not suspected because of a lack of gastrointestinal symptoms or signs of malabsorption. The frequency of biopsy-confirmed celiac disease in patients with ataxia of unknown origin is about 12% [159]. One series, however, found no celiac disease-associated antibodies in 32 patients with idiopathic cerebellar ataxia [160]. Celiac disease-associated ataxia often lacks particular clinical features that would distinguish it from other forms of cerebellar ataxia [161]. Severe ataxia in association with celiac disease may respond to intravenous gamma globulin [85].

Epilepsy is recognized more in association with childhood celiac disease than with adult celiac disease [162–164]. Two large studies of adults with celiac disease failed to detect an increased association with epilepsy [165,166]. The syndrome of occipital calcification, epilepsy, and celiac disease may be reversed with a gluten-free diet [84,167,168].

Blood test abnormalities

Various blood test abnormalities have been associated with celiac disease. The more common ones include hyperamylasemia, abnormal liver function tests, and hypocholesterolemia.

Elevated serum amylase, due to macroamylasemia, has been found more commonly among untreated celiac patients than among those on a gluten-free diet [169]. In cases of recurrent pancreatitis, abnormalities of cholecystokinin, impaired secretin release, and papillary stenosis have been proposed [170].

Abnormal liver tests have been frequently associated with celiac disease. In particular, hypertransaminasemia (aspartate aminotransferase, alanine aminotransferase) may be present in up to 40% of patients, both adults and children, and is usually reversible with a gluten-free diet [171–174]. Celiac disease is detected as the cause of about 10% of cases of cryptogenic hypertransaminasemia [175,176]. The exact etiology of the liver abnormalities is unknown. Biopsies have shown nonspecific hepatitis as well as steatosis.

It is not a surprising finding that patients with celiac disease often have low cholesterol levels. Ciacci et al. [177] reviewed 100 patients with hypochromic anemia who did not have diarrhea and found an inverse correlation between cholesterol level and prevalence of celiac disease. All patients with celiac disease were found to have cholesterol levels below 156 mg/100 mL. They suggested that, among patients with hypochromic anemia, high or high–normal cholesterol could possibly be used to exclude celiac disease [177]. Lower low-density lipoprotein (LDL) levels have been found in patients with celiac disease and these levels did not necessarily rise on a gluten-free diet [178].

Oral and facial manifestations

Celiac disease may be manifested in the mouth by Sjögren syndrome [120], recurrent aphthous stomatitis [179–182], and dental enamel defects [183,184].

A large forehead has been proposed to be a sign of celiac disease [185]. This is due to failure of growth of the visceral cranium after weaning, resulting in the middle third of the face being proportionally smaller than the forehead.

Inflammatory bowel disease

Celiac disease is associated with both ulcerative colitis and Crohn's disease [186–188]. Inflammatory bowel disease was a major cause of death in a population-based study of causes of death of patients with celiac disease in Sweden [189].

It may be difficult to distinguish celiac disease from duodenal involvement by Crohn's disease because of similar endoscopic and biopsy appearances [190,191]. In addition, serological tests considered specific for Crohn's disease may be seen in celiac disease and vice versa. Anti-*Saccharomyces* antibodies can be seen in celiac disease and antitissue transglutaminase antibodies in Crohn's disease [94,192,193].

Liver disease

As well as causing an elevation of serum transaminases in adults and children [171,174,194], celiac disease is associated with primary biliary cirrhosis [195], autoimmune hepatitis [196], and autoimmune cholangitis [197]. Celiac disease identification and treatment in liver transplantation candidates reverses liver failure [198]. Hepatitis C is also associated

with celiac disease and may become evident after treatment with interferon [199,200].

Endocrine disorders

There is a well-established association of celiac disease with type I diabetes mellitus, such that many centers regularly screen for celiac disease [80]. Autoimmune thyroid disorders [119] and Addison disease are also associated with celiac disease [201].

Therapy of celiac disease

The medical treatment for celiac disease is lifelong adherence to a gluten-free diet. Patients are advised to avoid all gluten; however, there is some controversy over what this entails. Wheat is now ubiquitous in the Western diet. The amount of gluten in a slice of bread is considered to be 2.25 g [21] and the mean daily gluten intake in the general population in Western Europe is 13 g [202]. Washed wheat starch, which contains trace amounts of gluten, is allowable in the gluten-free diet in some European countries [203], but not in the United States [204] or in Australia where the standard is "no detectable gluten" [205]. In Finland studies have demonstrated that even naturally occurring gluten-free foods may have traces of detectable gluten [206].

There may be differing degrees of sensitivity or tolerance levels among different patients with celiac disease. However, inflammatory changes occur rapidly, within hours, of gluten instillation into the duodenum [207]. Infusion of gliadin into the small intestine of those with celiac disease indicates that that there is a dose response, with 10 mg of gliadin not eliciting an inflammatory reaction but 100 mg resulting in damage [208]. In Finland, where the gluten-free diet is considered to contain the equivalent of 30 mg of gluten daily, most patients do exceptionally well. Biopsies normalize [203] and, overall, patients who follow a gluten-free diet do not have an increased mortality rate [209]. The basics of the diet are shown in Table 50.3.

There is considerable confusion among some patients and their dietary advisors about the safety of some foods. Vinegars and distilled alcohol are safe. Grains such as corn, rice, quinoa, buckwheat, and millet are not related to wheat, rye, or barley and therefore are considered safe. These grains are an important cornerstone of the gluten-free diet. They provide texture, fiber, iron, and B vitamins. Flour from wheat, rye, and barley is usually fortified with iron, thiamin, riboflavin, and niacin; however, the gluten-free substitutes are frequently rice based and are not usually fortified. As a result the gluten-free diet is low in B-complex vitamins and iron [210] and after 10 years on a gluten-free diet patients frequently have evidence of poor B-vitamin status [211]. A gluten-free diet may also be low in fiber [210]. Inclusion of gluten-free alternative grains provides both variety to the diet as well as texture, fiber, and nutrients.

Table 50.3 Fundamentals of the gluten-free diet

Safe grains	Toxic grains
Amaranth	Wheat (includes spelt, kamut, semolina, triticale)
Buckwheat	Rye
Corn	Barley (including malt)
Millet	
Quinoa	
Sorghum	
Teff	
Oats	

Gluten-free starch (flour) alternatives

Cereal grains (seeds of cultivated grasses): amaranth, buckwheat, corn (polenta), millet, quinoa, sorghum, teff; rice (white, brown, wild, basmati, jasmine); Montina (Indian ricegrass)
Tubers (swollen underground plant stem): arrowroot, jicama, taro; potato (white, sweet); tapioca (cassava, manioc, yucca)
Legumes (edible seeds from a pod): beans including chickpea, lentil, kidney, navy, peas, peanuts, soybean
Nuts (edible kernel of a hard shell): almonds, walnuts, chestnuts, hazelnuts, cashews
Seeds: sunflower, flax, pumpkin

Problem area: food additives (label reading)

Additives to avoid	Safe additives
Wheat starch	Citric acid
Modified food starch (from wheat)	Maltodextrin
Malt, malt flavoring	Dextrin
Malt vinegar	Vinegar
Hydrolyzed vegetable or plant protein	Mono- and diglycerides
Soy sauce	Artificial colors and flavors
	Natural colors and flavors
	Monosodium glutamate
	Carmel color

Oats remain a dilemma for patients. Multiple studies have demonstrated that most patients with celiac disease or DH tolerate oats [212–214]. However, a few patients (only three in total) with celiac disease have been shown to mount an immune response to oats [215,216]. One problem is that oats may be contaminated with other gluten-containing grains, even brands considered to be gluten free [217].

Nutrition counseling of an individual with celiac disease must be an all-inclusive event and is probably the most important aspect of the diagnosis. Also important are a review of eating and shopping behaviors, a social/emotional assessment, and an in-depth educational component. Knowledge of the diet is gradually acquired and, as a result, follow-up nutritional counseling is important, as is follow-up medical care [218].

Patients are faced with many problems including inadequate food labeling for wheat or gluten, lack of information while eating out of the home in restaurants, use of gluten-containing products in medications, and conflicting information available from physicians, nutritionists, support groups, and the internet [219,220].

Vitamin supplementation with gluten-free forms is reasonable, especially because the major gluten-free flours are not fortified with vitamins. The routine use of pneumococcal vaccination is not established, although the high rate of hyposplenism argues for its use [221]. Bone mineral density assessment is standard as is the use of calcium supplements. If osteoporosis or hypocalcemia is present, measurement of fat-soluble vitamins, serum ferritin, vitamin B-12, and serum parathyroid hormone are necessary.

Follow-up management includes monitoring of serum antibodies, which usually normalize between 6 and 12 months after commencement of the gluten-free diet. Follow-up biopsy, although not necessary for diagnosis, is reasonable to assess the response to the diet. This is often performed too early. If the serum antibodies remain elevated, further dietary counseling is necessary. Repeat biopsy should be performed after 1–2 years. Follow-up dietary/nutritional counseling should be an annual event.

Refractory celiac disease/sprue

Before considering refractory sprue, a systematic approach to the evaluation of the poorly responsive patient needs to be undertaken [222]. Table 50.4 provides a list of the causes of a poor response to the diet. The most important part of the evaluation of the poorly responsive patient is a thorough dietary evaluation for both intentional and unintentional gluten ingestion. The presence of positive antibodies argues for persistent gluten ingestion.

Table 50.4 Causes of poorly responsive celiac disease

Incorrect diagnosis
Gluten ingestion (intentional or unintentional)
Microscopic colitis
Lactose intolerance
Pancreatic insufficiency
Bacterial overgrowth
Other food intolerances (fructose, milk, soy)
Inflammatory bowel disease
Irritable bowel syndrome
Anal incontinence
Collagenous sprue
Autoimmune enteropathy
Refractory sprue (+/– clonal T cells)
Enteropathy-associated T-cell lymphoma

Bacterial overgrowth [223], pancreatic insufficiency [224], and microscopic colitis are important causes of treatment failure in celiac disease [225]. Thorough assessment with breath tests for bacterial overgrowth and lactose intolerance, colonoscopy to rule out inflammatory bowel disease and microscopic colitis, and computed tomography (CT) scanning of the abdomen are part of the evaluation. The last will exclude the presence of significant lymphadenopathy or the rare lymph node cavitation syndrome [226].

Refractory celiac disease or sprue is considered to occur if patients have persistent diarrhea and villous atrophy despite a gluten-free diet for at least 6 months [227]. The term refractory sprue was coined because it was unclear whether all patients had celiac disease; some lack the crucial component of the diagnosis of celiac disease, that is a response to the gluten-free diet. Studies have demonstrated that some patients who are refractory to the diet have an aberrant intraepithelial T-cell population which lacks normal lymphocyte surface markers and exhibits restricted T-cell receptor (TCR) gamma gene rearrangements [228]. These patients are considered to have a type 11 refractory sprue with clonal intraepithelial T cells, whereas type 1 patients have polyclonal (normal) T cells [229]. The type 11 patients are considered to harbour a cryptic T-cell lymphoma and have a high mortality rate and a high rate of progression to enteropathy-type T-cell lymphoma [229,230]. Therapy of patients with refractory celiac disease may include steroids, either alone or in combination with azathioprine [231,232], cyclosporin (ciclosporin) [233], and infliximab [234]. Innovative therapies have been tried in refractory celiac disease, including an elemental diet [235] and recombinant IL-10 [236]. Because of the poor response of patients with refractory sprue to the above therapies, the use of stem cell transplantation is under trial [229]. The approach that we use is that of steroids and azathioprine as the initial therapy. Cyclosporin and infliximab are used as second-line therapies.

Quality of life issues

Because of the restraints encountered with adherence to a gluten-free diet, quality of life is an important issue. Those surveyed in the United States reported an improvement in quality of life after diagnosis of celiac disease and commencement of a gluten-free diet [68]. Even those diagnosed in Finland as asymptomatic, screened relatives reported an improvement in quality of life [237]. However, other studies report an impact on aspects of the quality of life. The disease and diet impact on the quality of life of females rather than males [238], those with gastrointestinal symptoms [239], and those who are less compliant [240]. An impact was also reported on activities such as dining out, social functions, and travel [241,242]. As a result, compliance with the diet remains an issue, especially in the social setting [68].

Conclusions

Celiac disease is common. The only therapy currently available is the gluten-free diet. This diet is burdensome and the development of medications that help with the diet is both feasible and attractive to patients [25].

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C SMALL INTESTINE

51 Disorders of epithelial transport in the small intestine

Martín G. Martín, Ernest M. Wright

Disorders of carbohydrate assimilation, 1260
Disorders of amino acid and peptide assimilation, 1266
Disorders of fat assimilation, 1269
Disorders of mineral and electrolyte absorption and secretion, 1272
Disorders of vitamin absorption, 1277

Disturbances in intestinal nutrient and ion assimilation can lead to clinical symptoms that range from severe life-threatening diarrhea in a neonate to neurological deficits in adulthood. With the exception of lactose intolerance, most disorders of nutrient assimilation are both exceedingly rare and inherited in an autosomal recessive manner. Milder forms of all these disorders may be subtle and not diagnosed until well into adulthood. Given their genetic basis, disorders of nutrient assimilation should be entertained particularly when there is a history of consanguinity, even in the absence of other similarly afflicted family members.

Chronic diarrhea is the most common clinical manifestation of disorders that are secondary to abnormalities of nutrient digestion and absorption (Fig. 51.1) [1]. Categorizing the type of diarrhea into either malabsorptive or secretory is a useful first step in the evaluation of any patient with chronic diarrhea. Subclassifying the diarrhea may be done with either a trial of fasting, or by analysis of stool electrolytes while the patient is on a full diet. To calculate the stool osmotic gap, either cations such as Na^+ and K^+ or anions such as Cl^- and HCO_3^- may be measured in liquid fecal samples [2,3]. If the sum of twice the value of cations (or anions) is lower (< 50 mOsm/L) than the predicted osmolarity (290 mOsm/L), an osmotic gap is present, and the patient is malabsorbing dietary nutrients. In contrast, a low osmotic gap (< 50 mOsm/L) is seen in secretory diarrheas, which are generally large volume (1–3 L) and fail to abate with fasting.

Nutrient-specific malabsorption is an important characteristic of all inherited transport disorders but is generally not a feature of some of the more common chronic diarrheal disorders. However, defects of pancreatic and brush-border

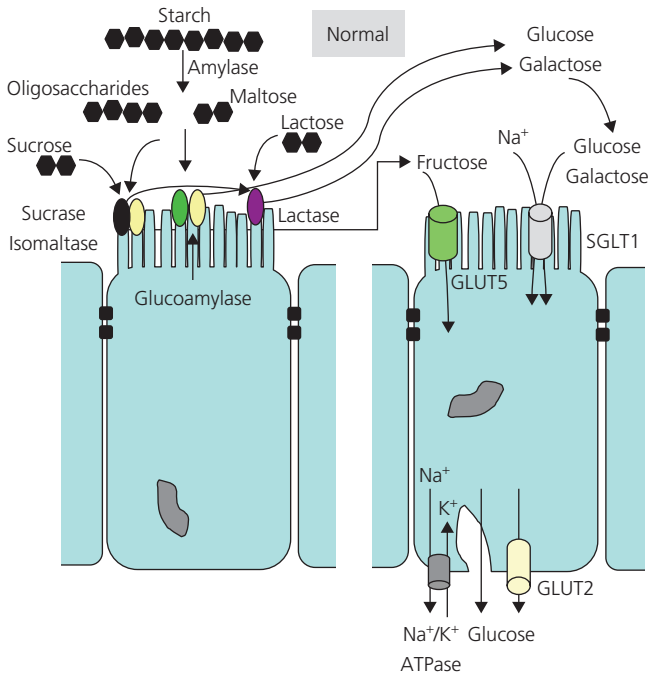

Figure 51.1 Normal assimilation of carbohydrates in the intestine. ATPase, adenosine triphosphatase; GLUT2, glucose–galactose–fructose uniporter; SGLT1, Na^+-dependent glucose–galactose cotransporter.

enzyme function share many of the clinical characteristics seen in transport defects and will also lead to maldigestion and diarrhea of specific complex nutrients. In contrast, generalized malabsorption (i.e., not nutrient specific) occurs in the clinical setting where the overall intestinal absorptive capacity is insufficient adequately to handle a dietary load that is required to maintain appropriate growth and development [4]. Nonspecific abnormalities of intestinal absorption most often occur when either small bowel surface area is inadequate (i.e., short bowel syndrome) or when intestinal transit time is too short to allow for adequate nutrient assimilation. For instance, enteropathies such as hypersensitivity

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to cow's milk proteins, celiac disease, and even more unusual disorders, such as autoimmune enteropathy, microvillus inclusion disease (Mendelian Inheritance in Man [MIM] #251850), and immunodysregulation-polyendocrinopathy-enteropathy (MIM #304790), generally present with features of nonspecific malabsorption [5,6]. In fact, the majority of patients with abnormal intestinal histology have a mixed form of diarrhea that has both osmotic and secretory components. Therefore, the identification of normal small bowel histology is an essential part of the evaluation of all patients with presumed defects of nutrient assimilation.

Secretory diarrhea is due to an imbalance between absorption and secretion of electrolytes, and the resulting elevated lumenal concentration of ions drives the influx of water to reach isoosmolar conditions with serum. During early infancy, chronic diarrhea that is purely secretory in nature is the result almost exclusively of a small number of transport disorders and is not secondary to a defect of enzymatic function, as is frequently seen in maldigestive diarrhea. Several hormone-secreting tumors may present with severe secretory diarrhea and need to be distinguished from two disorders of intestinal transport, namely congenital chloride diarrhea (MIM #214700) and congenital sodium diarrhea (CSD) (MIM #270420) [7,8].

Disorders of carbohydrate assimilation

Complex sugars are digested initially by salivary and pancreatic α-amylases and further by brush-border enzymes into monosaccharides, which are absorbed in the small intestine (Fig. 51.1). Enterocytes, which are renewed every 6 days, express the enzymes and transporters needed for carbohydrate digestion and absorption. The apical brush-border membrane of enterocytes contains various saccharidases including glucoamylase, sucrase-isomaltase, and lactase-phlorhizin hydrolase (LPH), the Na^+-dependent glucose–galactose cotransporter (SGLT1), and the fructose uniporter (GLUT5). The basolateral membrane contains the glucose–galactose–fructose uniporter (GLUT2) and the Na^+,K^+-ATPase, which maintains the low intracellular Na^+ concentration by extruding Na^+ into the blood in exchange for K^+ [9]. This Na^+,K^+-ATPase provides the potential energy required for the movement of sugars and amino acids up their steep concentration gradients across the apical membrane of the enterocyte. For instance, the low intracellular concentration of Na^+ provided by the pump enables the influx of Na^+ to be coupled to the movement of glucose up its own osmotic gradient by the active transporter SGLT1. By contrast, fructose is passively transported, moving down its own osmotic gradient, across the intestine by the brush-border uniporter (GLUT5) and by the basolateral GLUT2 uniporter.

Disturbances of carbohydrate assimilation may be secondary to defective enzymatic processing of complex sugars and disaccharides or failure to transport monosaccharides across the apical membrane (Fig. 51.1). Carbohydrate intolerance is characterized by diarrhea that subsides shortly after carbohydrates are reduced or eliminated from the diet. Therefore, abnormal carbohydrate assimilation results in the presence of major osmotic forces in colonic lumenal fluid derived from oligosaccharides, lactose, sucrose, or glucose. Unabsorbed carbohydrates are fermented by the resident colonic microflora to gas (hydrogen, methane, and carbon dioxide) and volatile short-chain fatty acids (SCFAs) such as acetate, butyrate, and propionate. A proportion of these SCFAs are absorbed across the colonic epithelium by poorly defined diffusion and transporter-mediated mechanisms [10]. The absorbed SCFAs are metabolized preferentially by colonic epithelia, where they are an important energy source and also facilitate the absorption of NaCl. Therefore, SCFA absorption leads to a reduction of lumenal osmolarity and thereby facilitates the colonic absorption of water. Nevertheless, diarrhea may result when the ileal effluent exceeds the salvaging capacity of the colon. Specifically, the passage of unabsorbed and unfermented carbohydrates and SCFAs into the distal colon provides an additional osmotic load that results in the retention of water in the gut and subsequent diarrhea.

Lactose intolerance

Abnormalities of carbohydrate assimilation can be categorized into four general forms:

- ontogenic forms that result from the normal immaturity of digestive functions during the early stages of life
- developmental forms that change at the time of weaning
- genetic forms that are congenital conditions
- acquired forms that are preceded by a variable period of normal function – particularly true for lactose intolerance resulting from either the synthesis of defective lactase-phlorhizin hydrolase or reduced expression, which may be secondary to a developmental stage or an inflammatory or infectious condition.

Lactase-phlorhizin hydrolase (LPH) (Enzyme Commission [EC] 3.2.1.108) is a large 1972-amino-acid protein whose short hydrophobic carboxyl terminus spans the brush border membrane once, with the majority of the protein situated lumenally (Fig. 51.2). Details of the synthesis and processing of the polypeptide as it proceeds from the endoplasmic reticulum to its final destination as an active hydrolase on the apical membrane have been thoroughly studied [11]. The precursor molecule is *N*- and *O*-glycosylated and eventually proteolytically cleaved at two locations, yielding a mature 145-kDa LPH enzyme protein with two distinct enzymatic sites for lactase and phlorizin hydrolysis [12]. The enzyme hydrolyzes the β-1,4-glycosidic linkage of lactose to yield glucose and galactose.

Lactase-phlorhizin hydrolase is produced predominantly in the small intestine, where it has a distinct pattern of expression along the proximo-distal axis of the intestine. Mature

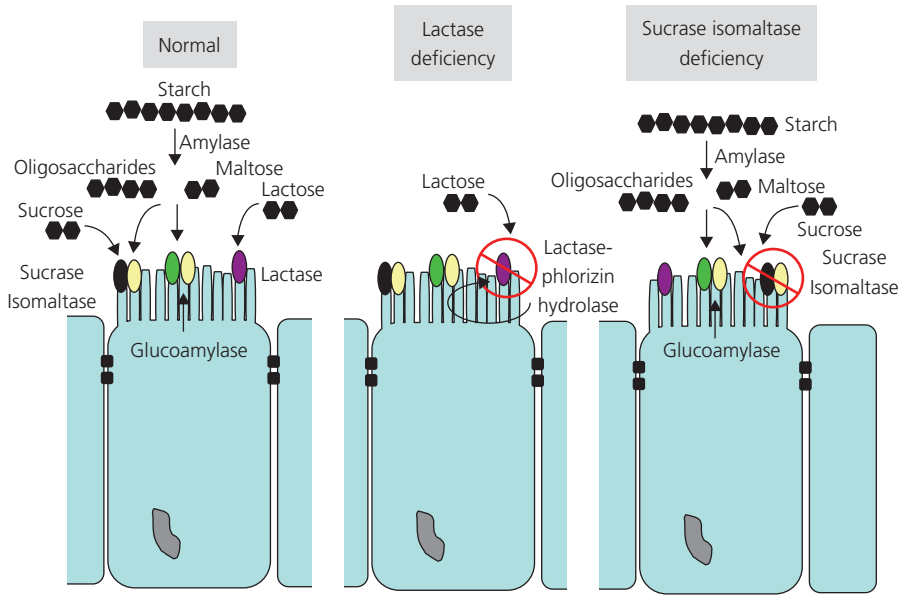

Figure 51.2 Disorders of disaccharidase deficiency.

protein is localized to enterocytes along the entire length of the villus, and is most abundant along the distal duodenum and jejunum [13]. The spatial and developmental pattern of LPH expression is regulated mainly at the level of transcription and appears to be orchestrated largely by several transcription factors that bind specific elements located in the immediate promoter region of the human and rodent lactase gene (*LCT*) promoters, including caudal-related homeodomain proteins (Cdx-2), hepatocyte nuclear factor 1 (HNF-1), and GATA-4/5/6 [14,15].

Genetics and molecular pathophysiology

Hypolactasia

In all mammals, lactase activity dramatically declines at the time of weaning, spurring the drive toward a more diversified diet depleted of lactose [16]. Several studies have indicated that the developmental regulation of lactase expression is hard-wired, and is not significantly modified by exposure to lactose-containing dietary load [17]. Hypolactasia (MIM #223100), or lactase nonpersistence, results from this hard-wired developmental decline of LPH activity that occurs in the majority of humans at a prevalence that varies based on race and ethnicity [18,19]. Lactase levels decline by as much as 90% as early as 3 years of age in children from Thailand and Bangladesh, and 10 years of age for others of Asian, African, and Latin descent [20].

By contrast, the continued presence of high levels of lactase activity throughout adulthood has been called lactase persistence, and is generally limited to descendants of people originating from northwest Europe and certain tribal regions in Africa, and is believed to result from the ability to bypass the normal developmental decline in LPH activity [16]. Several kindreds and isolated inhabitants within either lactase-persistent or lactase-nonpersistent populations have been carefully characterized and the findings suggest that lactose tolerance is determined by a homozygous inheritance of an autosomal recessive allele.

Several single-nucleotide polymorphisms along various regions of the *LCT* gene have been identified; however, no polymorphisms have been identified in either the vicinity of the *LCT* gene or its immediate promoter region that could be associated with either lactase persistence or nonpersistence. Therefore, it is generally assumed that the normal decline in LPH activity at weaning is secondary to changes in the abundance of the transcriptional factors that bind the immediate promoter region of LCT and alter its rate of transcription.

The long-sought breakthrough in understanding the molecular basis of lactase nonpersistence was made with linkage disequilibrium analysis of nine well-characterized Finnish families [19]. Sequence analysis revealed two distinct areas of sequence variability that are precisely associated with low levels of lactase activity. Specifically, C/T-13910, a DNA variant located 13 910 base pairs upstream from the *LCT* gene, and a G/A-22018 polymorphism located 8 kb further upstream, showed complete cosegregation with the nonpersistence trait. The C/T-13910 variant was also associated with hypolactasia in a large cohort of non-Finnish subjects in four different populations.

The T-13910 polymorphism is associated with lactase persistence in the European population but not in African descendants, whereas the C variant is limited to the nonpersistence population [16]. These polymorphisms are located in a potent enhancer whose transcriptional activity is augmented by the T variant, which binds Oct-1 isoform of the octamer family of transcription factors with greater avidity than the C variant [21,22]. Binding sites for other transcription

factors, including HNF-4, GATA, and Fox, were identified in the vicinity of the -13910 polymorphism. It is believed that in the presence of the T variant, this transcriptional enhancer is of sufficient potency to sustain LPH activity despite the natural age-dependent decline that is driven by elements residing in the gene's immediate promoter region.

Congenital lactase deficiency

The rarest form of lactose intolerance is the autosomal recessive congenital form (MIM #223000), which presents during the immediate postnatal period as severe watery diarrhea while the infant is on a lactose-containing diet [18,19]. This form can be distinguished clinically as a selective lactase deficiency because symptoms subside on a non-lactose-containing diet. Mucosal lactase levels are exceedingly low in the presence of normal intestinal histology. Five distinct mutations have been identified in the coding region of the *LCT* gene, including three nonsense mutations and two missense mutations that alter highly conserved residues that may be important for either the protein's catalytic activity or its three-dimensional structure [23].

Developmental or neonatal lactase deficiency

Activity of LPH is detectable as early as 26 weeks of gestation and increases dramatically in full-term neonates. Relative lactase deficiency and lactose intolerance has been described in premature infants less than 34 weeks of gestation, because lactase is the final disaccharidase to develop in premature infants. In a prospective randomized study, administration of lactase to this population was shown to result in improved weight gain and was without complications [24]. Similarly, a low-lactose diet has been shown to improve feeding tolerance, including weight gain and caloric intake, in premature infants [25].

Secondary lactase deficiency

Lactase deficiency that results from a concomitant condition is considered secondary. Common causes of secondary lactase deficiency are an assortment of infectious, allergic, and inflammatory conditions. Rotavirus is a common cause of short-term selective lactose intolerance that results from injury to lactase-containing enterocytes residing in the tips of the villi. Parasitic infections, including giardiasis, are also common causes of this form of lactose intolerance. Celiac and Crohn's disease may have lactose intolerance as one of their prominent signs and should be considered when intolerance is identified with other symptoms (e.g., weight loss, hematochezia).

Clinical manifestations

The most common symptoms associated with lactose intolerance are nausea, crampy abdominal pain, distention, excessive flatulence, and diarrhea, which begin as early as 30 min following the ingestion of lactose. The severity of symptoms depends on both the lactose load and the residual absorptive capacity of the intestine, which may vary depending on the subject's age and ethnicity.

Diagnostic criteria or laboratory investigations

The diagnosis of lactose intolerance is suggested by the resolution of clinical symptoms when subjects are placed on a strict lactose elimination diet for 2 weeks, and the recurrence of symptoms once lactose is reintroduced. However, it should be recognized that dietary history is frequently unreliable as a means of establishing the diagnosis of lactose intolerance [26]. The inconsistency of dietary history has been attributable to several factors including the patient's age, dose of lactose, and confounding clinical conditions.

A stool test that can be used to assess for carbohydrate malabsorption includes pH and the presence of reducing substances (Clinitest). Carbohydrate malabsorption and subsequent colonic bacterial fermentation result in the synthesis of high concentrations of SCFAs, which leads to the acidification of stool (pH < 5.3) [2]. Small bowel biopsies should be considered if there is a high suspicion for secondary lactose intolerance in the presence of diarrheal symptoms lasting longer than 4 weeks. Mucosal histology may assist in identifying the cause of the secondary lactose intolerance whereas disaccharidase enzyme activity can be used to assess whether there is a selective lactase deficiency or a general decline in some or all disaccharidases.

Carbohydrate breath hydrogen testing is an easily performed test that may assist in the diagnosis of several types of carbohydrate malabsorption disorders, including lactose, sucrose, glucose, and fructose malabsorption. The test relies on the colonic fermentation of unabsorbed carbohydrate by bacteria that produce methane, hydrogen, and carbon dioxide gas. This gas chromatographic analysis quantifies the level of hydrogen gas in exhaled air following the administration of either lactose at 2 g/kg bodyweight or a maximum of 25 g. An elevation of more than 20 p.p.m. above the fasting baseline is consistent with the diagnosis of lactose intolerance [27]. Genetic tests have been developed to assess for the homozygote CC (-13910) genotype that is associated with hypolactasia. Several studies have confirmed that genotyping is as reliable as breath hydrogen testing in adults and older children [25].

Treatment

As with all other disorders of carbohydrate assimilation, symptoms resulting from lactose intolerance may be minimized by the avoidance of milk and dairy products. Commercially available milk products that are either lactose-free or contain a reduced lactose content, including rice and soy-based products, may be used as substitutes. Because the malabsorptive symptoms are dose-dependent, the consumption of small quantities 100–200 g (4–8 oz) may be encouraged, either alone or with the use of oral lactase replacement. Milk

and other dairy products are rich dietary sources of calcium and vitamin D, hence alternative sources of these vital nutrients should be recommended to patients with lactose intolerance that severely limits their intake of dairy products, to reduce the long-term risk of abnormal bone density and fractures.

Sucrase-isomaltase deficiency

Sucrase-isomaltase (SI) (EC 3.2.1.48/10) is a type II integral membrane glycoprotein that forms a noncovalent homodimer complex. The human isoform is an 1827-amino acid polypeptide that undergoes posttranslational glycosylation prior to sorting to the apical membrane, where it is further processed by lumenal proteases to sucrase and isomaltase (Fig. 51.2). These functional subunits remain associated by means of hydrostatic interactions, with the isomaltase site hydrolyzing the α-1,4-glycosidic linkage of maltose and the α-1,6 glucose bonds of α-limit dextrins and isomaltose. By contrast, the sucrase site cleaves the α-1,4-glucose bonds of maltotriose and maltose and the identical linkage of sucrose into glucose and fructose. Redundant hydrolysis of the α-1,4-glucose linkage is provided by maltase-glucoamylase; however, 80% of the intestinal maltase activity is provided by sucrase-isomaltase.

Extensive studies done primarily in rodents have successfully defined the transcriptional basis of the spatial, developmental, and dietary regulation of SI [28]. The 5′-flanking region of the *SI* gene contains many functional cis-acting DNA elements that are sufficient to drive SI expression in the appropriate tissue and developmental-specific manner. The transcriptional factors that bind to the immediate promoter region of the SI gene include HNF-1, Cdx2, and GATA-4, which work in a combinatory manner to drive appropriate expression [29].

Sucrase-isomaltase expression changes during development and is modulated by diet. In humans, SI expression is measurable in the small bowel by the end of the first trimester, whereas in rodents it remains undetectable until the time of weaning, when it is dramatically induced [28]. This developmental pattern of expression is hard-wired and is controlled by cis-elements within the 5′-flanking region of the *SI* gene. Expression of SI is also induced by both corticosteroids and the introduction of either sucrose or starch to the diet [30].

Genetics and molecular pathophysiology

A deficiency of sucrase-isomaltase may result from either a selective congenital deficiency (MIM #222900) or as a global decline in disaccharidase activity that occurs with infection and inflammation. Secondary SI deficiency may result from the general decline in small bowel surface area associated with villus blunting, or because of transcriptional repression that can be induced by cytokines such as interleukin-6 (IL-6) and γ-interferon (IFN-γ) [31].

Congenital sucrase-isomaltase deficiency (CSID) is a rare autosomal recessive disorder that leads to malabsorptive diarrhea with the ingestion of sucrose and oligosaccharides [32]. Sucrase-isomaltase deficiency is particularly common among Eskimos in Greenland and Alaska, being estimated to occur in about 10% of this population. Multiple germ-line mutations have been identified in patients with CSID that result in a decline in sucrase or isomaltase activity (or both). Specifically, mutations have been characterized that result in either a selective decline in enzymatic activity, or abnormalities in the processing and sorting of the SI protein [33]. Mutations that alter protein targeting will result in a combined decline of sucrase and isomaltase activity [34].

Diagnostic criteria or laboratory investigations

The resolution of chronic diarrheal symptoms in a patient on a strict sucrose and glucose polymer-free diet is suggestive of the diagnosis of CSID. Diarrhea induced by large complex carbohydrates is usually an indication of abnormal exocrine pancreatic function, such as is seen with cystic fibrosis, but may also be attributed to a deficiency of glucoamylase synthesis or CSID [35,36]. Distinguishing between these various disorders can be accomplished by analyzing small bowel disaccharidase activity and with carbohydrate-specific breath hydrogen testing [27]. A selective deficiency of mucosal sucrase activity would be consistent with the congenital form of this disorder, whereas a general decline in multiple disaccharidases would suggest a secondary form of SI deficiency. Breath hydrogen testing can also be performed with sucrose; however, evidence of malabsorption should be followed by testing with each monosaccharide (glucose and fructose) to confirm the specific disaccharidase deficiency.

Clinical manifestations and treatment

The majority of patients will present in infancy with chronic malabsorptive diarrhea and failure to thrive. Because most neonates either breast-feed or consume a lactose-containing formula, diarrheal symptoms generally begin at about 4 months of age when the dietary repertoire is advanced to contain either sucrose or glucose polymers. An invertase (β-fructofuranosidase) preparation that hydrolyzes sucrose has been shown to be effective in reducing the severity of symptoms in patients with CSID on a sucrose-based diet [37].

Glucose-galactose malabsorption

Glucose-galactose malabsorption (GGM) is a rare disorder that causes severe life-threatening diarrhea and dehydration during the neonatal period (MIM #182380). GGM was first established when investigators demonstrated evidence of an inherited disorder of active glucose and galactose transport [38]. Confirmation was provided by in vitro studies on GGM duodenal biopsies, which demonstrated that enterocytes were unable to accumulate galactose owing to a reduction in the number of transporters in the brush-border membrane [39–41] (Fig. 51.3). Expression studies confirmed that glucose and galactose are transported by the sodium-dependent

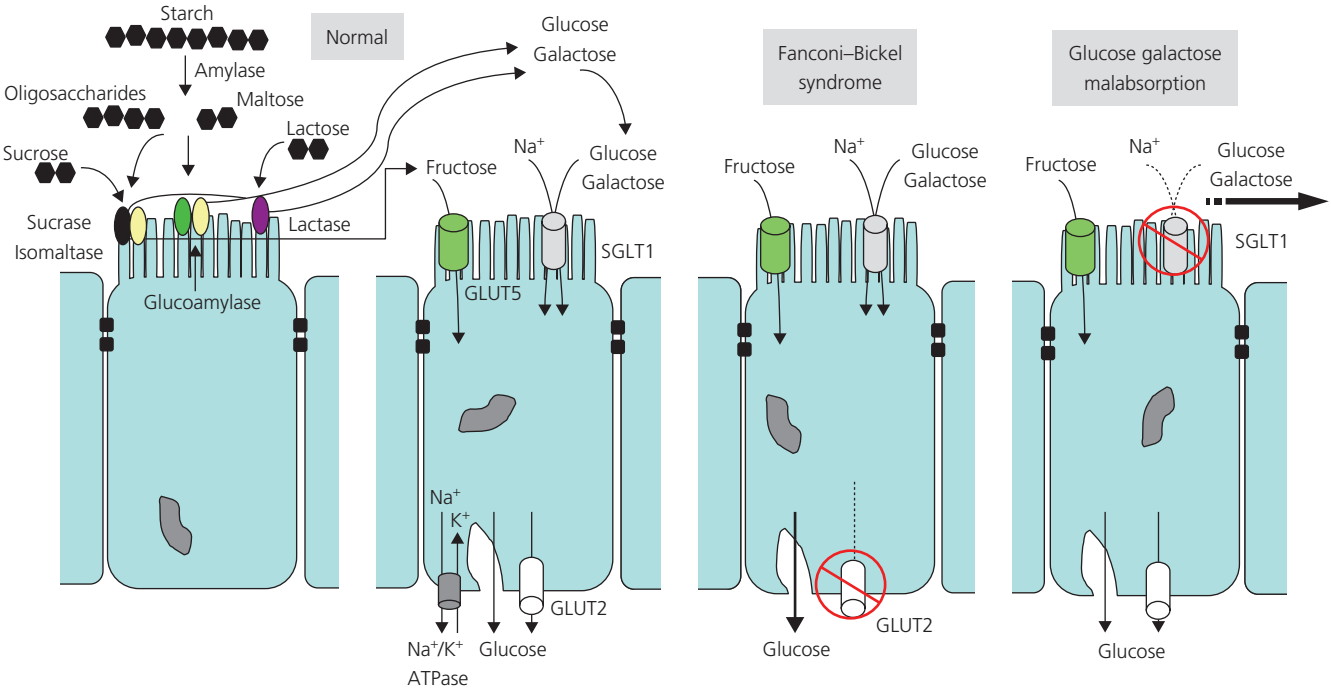

Figure 51.3 Carbohydrate absorption in normal subjects, in Fanconi–Bickel syndrome and in glucose-galactose malabsorption. ATPase, adenosine triphosphatase; GLUT2, glucose–galactose–fructose uniporter; GLUT5, fructose uniporter; SGLT1, Na^+-dependent glucose–galactose cotransporter.

transporter, SGLT1, which is defective in patients with GGM [42].

Genetics

The relative abundance of GGM in consanguineous unions and the absence of vertical transmission suggest an autosomal recessive pattern of inheritance. Genetic testing performed on 82 patients in 74 unrelated families identified 46 mutations in the *SGLT1* gene of patients with GGM [43–46]. The majority of the mutations are missense; however, six nonsense and seven frameshift mutations that produce truncated nonfunctional proteins have been identified. All missense mutations that caused a defect in sugar transport were in amino acids that were conserved within the 18 closely related genes, including eight *SGLT1* genes from different species. However, the two missense mutations that do not impair sugar transport, Phe405Ser and His615Gln, are not conserved and rarely occur in the general population. In contrast, the Asn51Ser variant occurs in the general population at a frequency of 4% in over 552 alleles analyzed.

Molecular pathophysiology

The primary carbohydrate present in breast milk is lactose, which is hydrolyzed by lactase on the external surface of the intestinal brush border. The liberated glucose and galactose are then transported across the brush-border membrane by SGLT1 and accumulated within the enterocyte (Fig. 51.3). Complex carbohydrates provide the major source of glucose in older children and adults. The facilitated carrier GLUT2 was believed to be responsible for transport of glucose and galactose across the basolateral membrane; however, studies on humans and mice with mutations of GLUT2 but no evidence of osmotic diarrhea have cast doubt on this [47,48].

The SGLT1 transporter is responsible for the tight coupling of two Na^+ ions and one sugar molecule across the membrane during one catalytic turnover. The basolateral Na^+,K^+-ATPase maintains the low intracellular sodium concentration by pumping sodium out of the cell, and this results in the net absorption of sodium (chloride and bicarbonate), sugar, and water across the epithelium [49]. Because the osmolarity of stool in both secretory and malabsorptive diarrhea is always isomolar to that of the serum, the addition of undigested solutes (glucose and galactose) and SCFA that reach the colon of patients with GGM will result in an excessive amount of water loss to maintain the obligatory fecal osmolarity.

Glucose stimulates salt and water absorption, and this is generally explained by the coupled transport of Na^+ and glucose across the brush border, and the subsequent pumping of Na^+ out of the enterocyte across the basolateral membrane by the Na^+,K^+-ATPase. It is thought that water follows solute absorption by osmosis. However, it has been postulated that there is a more direct link between sodium, sugar, and water transport across the brush-border membrane, namely water cotransport, but this is not widely accepted [50]. This link between salt, sugar, and water absorption provides the rationale for the use of oral rehydration therapy in patients

with various forms of acute diarrhea including those of infectious origin.

Clinical manifestations

The majority of patients with GGM present clinically with severe life-threatening diarrhea during the neonatal period; however, on several occasions a diagnosis has not been established until adulthood [51]. Diarrhea is most frequently detected during the first week of life, and will result in a severe metabolic acidosis and hyperosmolar dehydration. If not properly diagnosed in a timely manner and if dietary management is not implemented, GGM is frequently fatal. Isolated and profuse carbohydrate malabsorption is distinctly unusual during the first several days of life, and its presence should initiate the workup for both GGM and primary lactase deficiency.

Diagnostic criteria or laboratory investigations

Three key features are necessary to establish the diagnosis of GGM:

- elimination of glucose and galactose (and lactose) from the diet results in the complete resolution of diarrheal symptoms
- a normal intestinal biopsy
- an abnormal glucose breath hydrogen test.

When considering the diagnosis of GGM, several tests should be performed. Patients should have standard stool analysis, including pH, occult blood, and leukocyte analysis and evaluation for the presence of reducing and nonreducing sugars.

Small bowel biopsies should be performed early as they provide several important clues in establishing the diagnosis. Patients with GGM should have normal-appearing microvilli on electron microscopy, with normal villus architecture on light microscopy, including the normal distribution and number of enterocytes, Paneth cells, goblet cells, and enteroendocrine cells [52]. Mucosal biopsies can also be used to assess lactase and sucrase activity, which may be helpful in distinguishing GGM from primary lactase or sucrase deficiency.

Breath hydrogen testing is easily performed and may assist in the diagnosis of GGM. This gas chromatographic analysis quantifies the level of hydrogen gas in exhaled air following the administration of either glucose (or galactose) at 2 g/kg bodyweight or a maximum of 50 g for patients who weigh more than 25 kg. Although an elevation of more than 20 p.p.m. above the fasting baseline is consistent with the diagnosis of glucose malabsorption, most patients with GGM have levels that are frequently higher than 100 p.p.m. (MG Martin, EM Wright, unpublished observations) [53]. The specificity of an abnormal glucose breath hydrogen test may be confirmed by performing a fructose breath hydrogen test, which should be normal in GGM patients because fructose is absorbed by the unaffected uniporter, GLUT5 (Fig. 51.3). However, although slight fructose malabsorption is particularly common in younger children, breath hydrogen levels in a GGM patient will be substantially higher with glucose compared with fructose administration [54].

Treatment

With GGM, as with other disorders of carbohydrate malabsorption, symptoms resolve on an elimination diet free of glucose and galactose. Several formulas are available for managing GGM patients during early infancy, for example, Ross Carbohydrate Free Formula (Ross Products, Columbus, OH) and Galactomin 19 (Nutricia, Holland). Care should be exercised with formulas containing fructose as fructose absorptive capacity is rather limited in young children. The dietary management becomes particularly challenging as children enter late infancy and begin to exert more independence. Guidance from a well-trained dietitian is frequently invaluable. Highly motivated parents will generally benefit from dietary manuals that list the sugar content of selected foods. Although the obligatory lifelong glucose- and galactose-free diet may have significant long-term renal and cardiovascular consequences, none have been reported in several adult patients.

Fructose malabsorption

Fructose malabsorption has been implicated in both toddler's diarrhea and in a rare and poorly defined autosomal recessive disorder named isolated fructose malabsorption (IFM). Distinguishing between the more common toddler's diarrhea and IFM is particularly difficult, and it is unclear if they are distinct disorders. The clinical and genetic features of IFM are not as well defined as its counterpart carbohydrate transport disorder, GGM. Although the ability of GLUT5 to transport fructose has been demonstrated, there remains insufficient information confirming that GLUT5 represents the main fructose facilitative carrier in the intestine (Fig. 51.3) [55]. Studies have identified GLUT7 as a high-affinity glucose and fructose carrier on the brush-border membrane of enterocytes located in the distal intestine [56]. In rats studies imply that brush border GLUT2 plays a major role in the absorption of fructose [57].

Genetics and molecular pathophysiology

Eight patients with clinical evidence suggestive of IFM were evaluated for mutations in the *GLUT5* gene [58]. Analysis of both the coding region and intron and exon boundaries of the *GLUT5* gene failed to identify a deleterious mutation that could account for defective transport.

Data suggest that fructose absorption in the small intestine is accomplished by GLUT5, because the latter has a much higher Michaelis constant (K_m) (and thereby higher capacity) for fructose than GLUT7 [59,60]. Fructose absorption is independent of glucose absorption and remains intact in patients with GGM. Studies performed primarily in rodents have demonstrated that GLUT5 expression is regulated in a tissue- and development-specific manner and is induced by dietary fructose [61]. Specifically, GLUT5 messenger ribonucleic acid (mRNA) and protein levels are found at low levels prior to weaning and increase dramatically in the proximal small

intestine in weaned animals. An analysis of the age dependency of GLUT5 levels in human intestine has not been performed; however, fructose malabsorption as determined by the breath hydrogen test is certainly more common during early infancy when compared with adults [54]. Moreover, whether or not the fructose malabsorption in toddler's diarrhea is secondary to inadequate GLUT5 expression has yet to be formally established.

Clinical manifestation

Fructose is the primary monosaccharide found in fruits and fruit juices. Fruit juices that contain a high proportion of fructose relative to glucose, or an excessive amount of the nonabsorbable carbohydrate sorbitol, have been associated with infant diarrhea and abdominal pain [62,63]. Malabsorption of fruit juices is dose-dependent, and diarrheal symptoms are associated with a daily intake of juice exceeding 15 mL/kg. Whether children with toddler's diarrhea have a reduced capacity to absorb fructose compared with other similarly aged children has yet to be defined. Intolerance to dietary fructose has also been identified in infants with colic and adults with irritable bowel syndrome [64,65].

Diagnostic criteria or laboratory investigations

Fructose malabsorption can be assessed by either placing the patient on a fructose elimination diet or by performing a fructose breath hydrogen test. Fructose given at 2 g/kg bodyweight or a maximum of 50 g can be used to perform these studies [27]. Concomitant administration of glucose can attenuate the malabsorption of fructose as assessed by breath testing, presumably by improving solvent drag [66]. To determine whether the malabsorption is fructose-specific, intestinal biopsies should reveal normal intestinal histology, and malabsorption should be limited to fructose and not other monosaccharides.

Treatment

Patients experiencing significant fructose-induced diarrhea should either reduce or eliminate their dietary fructose load to resolve symptoms. It has been established in rodent models that GLUT5 levels are dependent on the chronic load of dietary fructose; therefore, it is conceivable that fructose malabsorption may resolve by initiating a feeding regimen of incrementally increasing amounts of fructose. Because fructose malabsorption is generally limited to early infancy, attempts to reintroduce fructose should be considered as patients enter into the school-age years.

Fanconi–Bickel syndrome

Fanconi–Bickel syndrome (FBS; MIM #227810) is a rare autosomal recessive disorder that is characterized by carbohydrate malabsorption, tubular nephropathy, hepatomegaly and abnormal glycogen accumulation, failure to thrive, and fasting hypoglycemia [67,68].

Genetics and molecular pathophysiology

Homozygous mutations in the facilitative glucose transporter gene, *GLUT2*, have been identified in over 60 patients with FBS (Fig. 51.3) [48]. Although most of the mutations are nonsense and result in a truncated transporter, several missense mutations were also identified. In vitro functional studies have not been performed to confirm that these mutations adversely alter the transport capabilities of the GLUT2 protein.

The mechanism by which mutation in the facilitative carrier, GLUT2, alters the fasting- and feeding-induced changes in serum glucose is not well understood; however, impaired hepatocyte transport and glucose-sensing mechanism of pancreatic β cells are possible explanations [68]. In the small intestine, GLUT2 is localized on the basolateral membrane and was previously believed to be the primary method of monosaccharide exit from the enterocyte (Fig. 51.3). However, severe osmotic diarrhea is generally not associated with FBS, and mice containing a targeted deletion of *Glut2* also retain monosaccharide transport across the basolateral membrane, suggesting that a GLUT2-independent pathway for glucose transport exists in enterocytes [69].

Clinical manifestations

The diagnosis of FBS should be considered in patients with hepatomegaly and evidence of a renal tubulopathy. Although carbohydrate malabsorption has been reported in some but not all cases of FBS, it is certainly not a dominant feature of the disorder; however, failure to thrive, rickets, and osteoporosis are clinical characteristics that are found in most patients with FBS [68].

Diagnostic criteria or laboratory investigations and treatment

Urine analysis generally shows evidence of glycosuria, a generalized aminoaciduria, and excessive urinary losses of phosphate and calcium. Histological analysis of the small bowel, liver, and kidney generally reveals excessive glycogen stores. Abdominal ultrasonography is useful to confirm the relative enlargement of the liver and kidney. Management is primarily focused on supplementing urinary electrolyte losses. Uncooked cornstarch has been used to prevent hypoglycemia and to minimize postprandial hyperglycemia [70].

Disorders of amino acid and peptide assimilation

The complex process of dietary protein assimilation begins with proteolytic hydrolysis by multiple gastric, pancreatic, and brush-border proteases to form small peptides and amino acids. Small peptides (di- and tripeptides) and amino acids are transported into enterocytes across the brush-border membrane through a series of transporters with over-

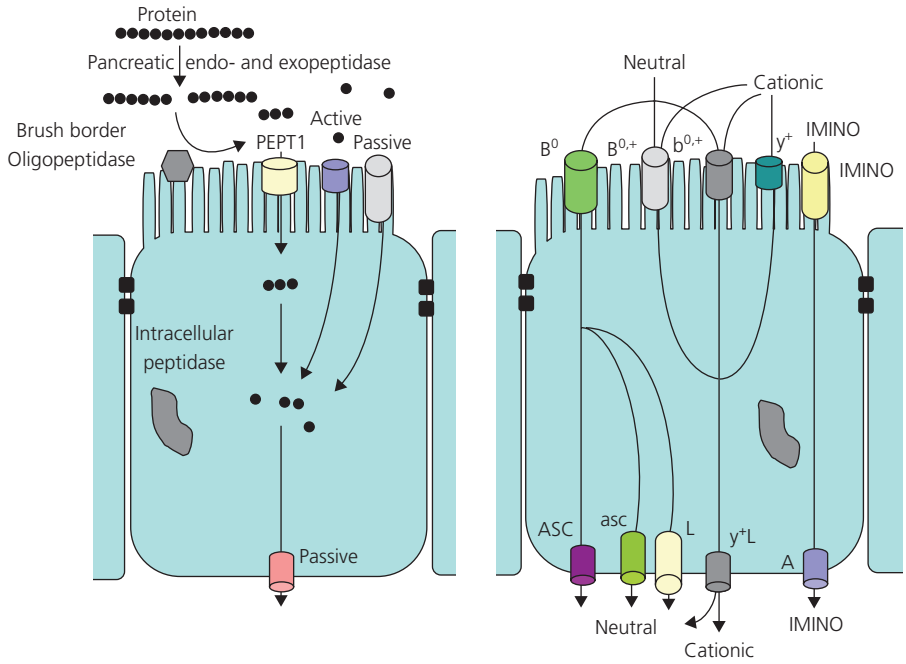

Figure 51.4 Protein and amino acid assimilation in the small intestine. A, neutral amino acid and IMINO acid carrier system; ASC, small neutral amino acid carrier; B^0 and $B^{0,+}$, Na^+-dependent transport systems for neutral amino acids; $b^{0,+}$, Na-independent transport system for cationic and neutral (cysteine) amino acids; L, large intracellular amino acid carrier; PEPT1, H^+–oligopeptide cotransporter; y^+, cationic amino acid transport system; y^+L, Na^+-dependent cationic amino acid transport system.

lapping specificities based on the characteristics of substrate size and charge (Fig. 51.4). Di- and tripeptides are hydrolyzed by intracellular peptidases to single amino acids, which efflux across the basolateral membrane through a second series of transporters. The 21 common amino acids add to the diversity of dietary proteins whose digestion requires a host of endopeptidases and exopeptidases with specific yet overlapping specificities. Passive and ion-mediated transepithelial absorption is mediated by a diverse group of transporters with broad amino acid specificities [71–73]. The redundant function of these enzyme and transport systems minimizes the physiological consequences of a single deficiency in almost any component of this process.

Protein digestion is initiated in the stomach by a family of pepsin proteases that are secreted in the inactive form of pepsinogen by chief cells (Fig. 51.4). Pepsinogen becomes activated by the acidic content of the stomach, where it produces nonabsorbable peptides. Under most circumstances, however, gastric hydrolysis is not essential for protein digestion. Dietary proteins that reach the intestine are further hydrolyzed into smaller peptides by a family of endopeptidases (trypsin, chymotrypsin, and elastase) and exopeptidases (carboxypeptidase A and B) that are secreted in their inactive form by the exocrine pancreas.

Enterokinase, a serine protease located on the brush-border membrane of enterocytes in the duodenum, converts the proenzyme trypsinogen to trypsin. Trypsin subsequently cleaves the precursor forms of other endo- and exopeptidases to their active equivalents. The oligopeptides undergo further hydrolysis by another large family of oligopeptidases (aminopeptidases and carboxypeptidases) located on the brush-border membrane. These proteolytic enzymes have well-defined substrate specificity and hydrolyze the dietary protein into either dipeptides or single amino acids, which are subsequently transported by a group of uniporters.

Disorders of protein digestion are rare but should be considered when evaluating a patient for a potential defect of amino acid transport. The best characterized disorder of specific protein malabsorption is a deficiency of enterokinase synthesis (MIM #226200) [74]. Individuals with enterokinase deficiency present with diarrhea, failure to thrive, and hypoproteinemic edema when on a diet of intact dietary proteins; these symptoms resolve when on an amino acid-based diet. Primary trypsinogen deficiency (MIM #276000) has also been reported and results in clinical symptoms that resemble enterokinase deficiency [75]. Nearly 90% of patients with cystic fibrosis (MIM #219700) have clinical evidence of exocrine pancreatic dysfunction that results in the impaired digestion of dietary protein, fat, and carbohydrates [35,36]. A similar type of generalized malabsorptive condition also occurs in the late stages of other disorders that result in chronic pancreatitis, including hereditary pancreatitis (MIM #167800) [76].

A large group of amino acid transporters have been isolated and characterized and these are expressed either on the apical or basolateral membrane of the enterocyte (Fig. 51.4) [71,72]. The brush-border membrane contains the B^0, B^0AT1, and $B^{0,+}$ Na^+-dependent systems that transport neutral amino acids [77]. Anionic and cationic amino acids are selectively transported by systems x_c^- and y^+, respectively. Proline and hydroxyproline are selectively transported by the IMINO system SIT1 [78]. By contrast, cationic and

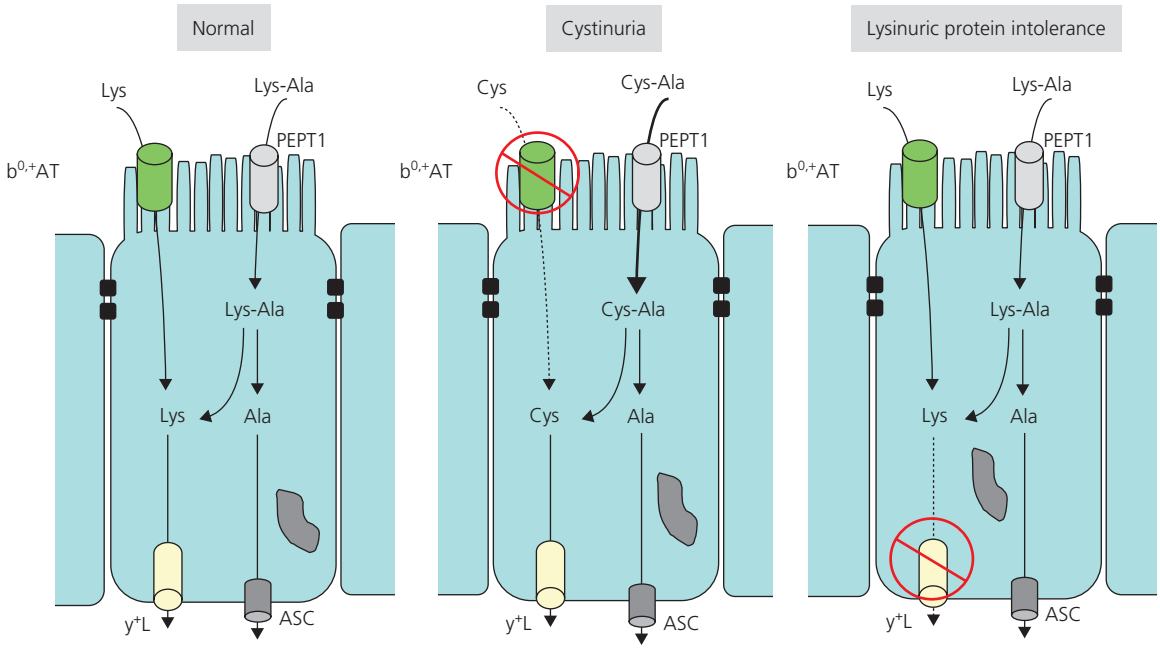

Figure 51.5 Amino acid transport in the small intestine of normal subjects, in lysinuric protein intolerance, and in cystinuria patients. Ala, alanine; ASC, small neutral amino acid carrier; $b^{0,+}AT$, ; Cys, cysteine; Lys, lysine; PEPT1, H^+–oligopeptide cotransporter; y^+L, Na^+-dependent cationic amino acid transport system.

neutral (cysteine) amino acids are transported by the Na-independent system $b^{0,+}$, which is defective in cystinuria (MIM #220100), an autosomal recessive aminoaciduria that is not associated with a significant gastrointestinal phenotype (Fig. 51.5) [79]. Although the $b^{0,+}$ system is expressed in the kidney and intestine, in patients with cystinuria dietary cysteine may still be absorbed as a component of oligopeptides by the H^+–oligopeptide cotransporter (PEPT1) [80]. Proton-coupled amino acid transporters, PAT1 and PAT2, have been identified and PAT1 is found expressed in the small intestine brush border [81,82]. The relative contributions of SIT1 and PAT1 to imino acid absorption and iminoaciduria have yet to be resolved.

Intracellular neutral amino acids efflux across the basolateral membrane in a size-dependent manner, with system L carrying the larger and system ASC the smaller amino acids (Fig. 51.5). Small neutral amino acids also cross the basolateral membrane by a Na^+-dependent ASC system, whereas the A system is selective for both neutral and imino acids. By contrast, cationic amino acids are transported by the Na^+-dependent system y^+L, which is defective in lysinuric protein intolerance (LPI). Although the redundancy in the transport specificity of these various systems essentially minimizes the gastrointestinal and nutritional consequences observed when an individual transport system is disrupted, LPI patients do experience significant gastrointestinal symptoms.

Lysinuric protein intolerance

Lysinuric protein intolerance (LPI) (MIM #222700) is an autosomal recessive disorder that results from mutations in the *SLC7A7* gene, which encodes for the cationic amino acid transporter y^+LAT-1 (Fig. 51.5) [83,84]. The y^+LAT-1 transporter is located on the basolateral membrane of the enterocyte and renal tubules, and transports cytoplasmic dibasic amino acids such as lysine, arginine, and ornithine in exchange for Na^+ and neutral amino acids [85]. Several gastrointestinal symptoms, including failure to thrive, diarrhea, vomiting, and pancreatitis, are the main clinical symptoms associated with LPI.

Genetics and molecular pathophysiology

The *LPI* gene was discovered by positional cloning, and approximately 30 mutations have been identified in more then 100 patients with LPI [83,84,86,87]. Founder mutations have been identified in Finnish, Japanese, and Italian kindreds in whom the incidence of LPI is particularly common. Ornithine and arginine are important urea cycle intermediates that fail to exit the intestinal and renal epithelium of patients with LPI. Their relative deficiency, particularly during a meal on a high-protein diet, results in urea cycle dysfunction that leads to hyperammonemia and subsequent alterations in mental status [88].

Clinical manifestations

Undiagnosed patients with LPI generally present with failure to thrive and a self-imposed restriction of dietary protein. Although diarrhea, emesis, and abdominal pain are frequent symptoms, the distinguishing feature of LPI is that high-protein diets result in hyperammonemia and dramatic changes in mental status, and chronic exposure to dietary protein is

usually associated with various degrees of mental retardation. Marked hepatosplenomegaly and frequent bone fractures are also characteristic of LPI, and life spans are generally limited by complications associated with alveolar proteinosis and osteoporosis [89,90].

Diagnostic criteria or laboratory investigations and treatment

Quantitation of plasma amino acids and urinary organic acids provides useful clues in the detection of LPI because plasma levels of diamino acids are low, whereas urinary levels of lysine and orotic acid are elevated [85]. The diagnosis can frequently be established by challenging with an enteral bolus of dietary protein. This would result in the rapid impairment of motor function and alteration of mental status resulting from hyperammonemia. Citrulline supplements (200 mg/kg/day) and dietary protein restriction (1.5 g/kg/day) are used to manage patients with LPI [88]. Mealtime supplements of citrulline are readily absorbed and reduce postprandial hyperammonemia following a protein meal. Oral carnitine supplementation has been shown to be important in a subset of patients described with LPI and hypocarnitinemia [91].

Hartnup disease

Hartnup disease (MIM #234500) and iminoglycinuria (MIM #242600) are two other autosomal recessive disorders that have been well characterized clinically [92,93]. Hartnup disease is characterized by a malabsorption and renal excretion of neutral amino acids (neutral aminoaciduria) and a heterogeneous set of symptoms ranging from a pellagra-like rash to neurological disorders [77]. Through linkage analysis a candidate gene, *B0AT1* (SLC6A19), was identified and sequencing of affected individuals found splice-site variants, deletions, and nonsense mutations that produce a truncated protein, and missense mutations. Testing of the missense mutant proteins expressed in *Xenopus laevis* oocytes eliminated amino acid transport [94,95]. B^0AT1 is a sodium-dependent and chloride-independent neutral amino acid transporter that is expressed in the intestine and kidney.

Other defects of amino acid transport

A second disturbance of amino acid transport has been characterized: system $b^{0,+}$, which is defective in cystinuria (MIM #220100) and is selective for cationic and neutral amino acid transport across the brush border membrane (Fig. 51.5) [79]. The diagnosis of this disorder can be established with analysis of urine amino acid profiles, and both disorders present with either neurological or renal symptoms and an absence of significant gastrointestinal complications. There is only one report of impaired proline absorption, which may be due to mutations in the imino transporter SIT1 [93,96]. This involved a severely retarded patient who also had familial iminoaciduria.

Disorders of fat assimilation

Assimilation of dietary fats requires hydrolysis and subsequent repackaging of lipids by a complex group of intralumenal and intracellular events [97]. The clinical consequences of disorders of fat assimilation include failure to thrive, steatorrhea, and neurological deficits that result from the malabsorption of fat-soluble vitamins. Lipolysis is initiated by gastric lipase in the stomach and concludes in the intestine through the action of pancreatic lipase. Because pancreatic exocrine function is particularly immature during early infancy, fat absorption in this age group primarily depends on the action of gastric lipase [98]. A congenital deficiency of pancreatic lipase (MIM #246600) is an exceedingly rare autosomal recessive disorder that leads to fat malabsorption [99].

Fatty acids and diglycerides are the products of triglyceride hydrolysis, and their solubilization to the aqueous phase of lumenal content requires adequate levels of conjugated bile salts; therefore, various disorders of intrahepatic and extrahepatic cholestasis will impede the delivery of lumenal bile salts and result in fat malabsorption. Bile salts are particularly important in the emulsification of long-chain fatty acids because of the lower aqueous solubility of the latter compared with their medium-chain (6–12 carbons) counterparts.

A fatty acid transport protein (FATP4) that has been localized to the brush-border membrane appears to play an essential role in the absorption of long-chain fatty acids [100,101]. Analysis of *Fatp4*-null mice demonstrates a 40% reduction in fatty acid uptake and a neonatal lethal restrictive dermopathy [102–104]. In contrast, the apical cholesterol transporter named Niemann–Pick C1-like protein (NPC1L1) was identified using a genomic-bioinformatics approach [105], and *Npc1l1*-null mice absorbed only 30% of lumenal cholesterol [106]. Ezetimibe is a potent selective inhibitor of NPC1L1, and may have an important role in curbing the absorption of dietary cholesterol in patients with hypercholesterolemia [107,108]. It has been established that cholesterol is exported across the epithelial layer by an adenosine triphosphate (ATP)-binding cassette transporter, ABCA1. The role of ABCA1 in cholesterol and phospholipid transport was confirmed when linkage analysis established it as the gene defective in Tangier disease (MIM #205400) [109,110]. Tangier disease patients have a deficiency of high-density lipoproteins, which predisposes to the development of premature coronary heart disease despite hypocholesterolemia; in addition, there is accumulation of cholesterol in tissues such as liver, spleen, lymph nodes, and small intestine. Patients with sitosterolemia (MIM #210250) have the peculiar ability to absorb an excessive amount of the main plant sterol, sitosterol. Mutations in the ABC transporter, ABCG5 and ABCG8, have been associated with sitosterolemia [111]. These proteins form a heterodimer that serves as

the primary sterol efflux mechanism in the intestine. Despite defective processing of cholesterol and sterol, patients with Tangier disease and sitosterolemia have no significant gastrointestinal phenotype except hepatosplenomegaly. By contrast, disorders that result in defective packaging of reesterified lipids into apolipoprotein-rich chylomicrons and very-low-density lipoprotein (VLDL) particles generally lead to fat malabsorption and failure to thrive.

Abetalipoproteinemia

Abetalipoproteinemia (ABL), or Bassen–Kornzweig syndrome (MIM #200100), is the classic and best-characterized disorder of fat absorption and results from failure to reassemble dietary fat in the form of β-lipoproteins. Triglyceride and cholesterol esters are primarily transported in the plasma as β-lipoproteins; therefore, ABL patients have a severe deficiency of both these neutral lipids. Patients generally present shortly after birth with failure to thrive and steatorrhea, and if, untreated, will develop irreversible neurological problems in late infancy. However, milder variants have been identified in asymptomatic adult patients who were identified by routine cholesterol screening.

Genetics and molecular pathophysiology

Wetterau and colleagues determined that the microsomal triglyceride transfer protein (MTP) was not detectable in the small bowel enterocytes of ABL patients and described homozygous mutations in the *MTP* gene [112]. The MTP protein is a 97-kDa subunit that forms a heterodimer with a 55-kDa protein named disulfide isomerase (PDI) [113]. Only a handful of missense mutations have been identified in ABL patients, and the majority involve a truncated or nonfunctional MTP; no mutations have been reported in the gene encoding the PDI protein [114]. Serum lipoproteins in patients with ABL are deficient in triglycerides and cholesterol-rich apolipoproteins B-100 and B-48, which form VLDL and chylomicrons. Apolipoprotein B-100 is synthesized by hepatocytes that produce VLDL, whereas the truncated protein (apolipoprotein B-48) is formed by enterocytes and is essential for chylomicron formation.

The role of MTP in chylomicron formation has been definitively established by investigating the consequences of ABL-causing mutations [113,115–117]. These studies demonstrate that apolipoprotein B alone is incapable of initiating lipoprotein formation and that MTP is specifically required to complete proper lipoprotein assembly. The protein component of apolipoproteins B-100 and B-48 is synthesized in the endoplasmic reticulum and must fold correctly to form a complex with lipids. Therefore, MTP has two distinct roles in lipoprotein assembly: first, to move lipids such as cholesterol and triglycerides into the lumen of the smooth endoplasmic reticulum and, second, to transfer small quantities of these lipids to the newly formed and unstable apolipoprotein B protein in a process that has been termed "lipidation" [116]. In the absence of lipoprotein assembly, lipid droplets accumulate in the endoplasmic reticulum and are not transported to the trans-Golgi apparatus in the enterocyte and hepatocytes [118].

Clinical manifestations

The typical clinical presentation is failure to thrive, emesis, and low-volume diarrhea. Poor growth despite adequate caloric intake is an early clinical feature that should hasten the evaluation of a possible defect in fat malabsorption. In the long term, patients may develop an aversion to fatty meals as a way to minimize their diarrheal symptoms. The first evidence of neuromuscular abnormalities is usually the loss of deep tendon reflexes, which results from prolonged vitamin E deficiency; other neuromuscular manifestations, including retinitis pigmentosa, ataxia, and spinocerebellar degeneration, may be mistaken for various forms of Friedreich ataxia.

Diagnostic criteria or laboratory investigations and treatment

Serum samples should be analyzed for evidence of β-lipoprotein (VLDL and chylomicron) deficiencies. Specifically, plasma triglyceride levels are generally low (< 10 mg/dL), whereas cholesterol levels range from 25 to 40 mg/dL. Acanthocytosis is usually seen in peripheral blood smears and results from abnormal lipoproteins in the plasma membrane of erythrocytes. Endoscopic evaluation of the small bowel can show yellow discoloration, and biopsies have characteristic fat-laden enterocytes located in the upper portion of the villus. Fat droplets within the cytoplasmic compartment of the enterocyte may be confirmed by electron microscopy.

To minimize diarrheal symptoms, ABL patients may be managed on a low-fat diet (~ 15 g/day), and serum levels of fat-soluble vitamins should be monitored periodically. Adequate serum levels of vitamins A and K can be achieved with supplementation of moderate oral doses; however, absorption of tocopherol is severely impaired and may require massive doses (2000 mg in infants, and 10 000 mg in older children and adults) of tocopherol to diminish the neuromuscular manifestations of vitamin E deficiency.

Hypobetalipoproteinemia

Hypobetalipoproteinemia (HBL; MIM #107730) is another well-characterized disorder of fat absorption that results from the formation of abnormally truncated forms of apolipoprotein B [119,120]. The heterozygous state has been estimated to occur in approximately 1 in every 3000 Americans [120].

Genetics and molecular pathophysiology

The primary distinction between ABL and HBL is that ABL heterozygotes have normal cholesterol and triglyceride levels, whereas HBL heterozygotes have low lipoprotein levels [121]. Therefore, HBL is inherited in an autosomal dominant

manner; however, patients with only a single defective allele generally have no gastrointestinal and only minimal, if any, neuromuscular abnormalities. In contrast, individuals with two defective alleles are indistinguishable from ABL patients based on both physical and laboratory evaluation.

Many mutations have been described in the *APOB* gene in patients with HBL [122]. The mutations all result in proteins that are truncated because of a nonsense or frameshift mutation that creates a premature downstream stop codon. Several kindreds have been described that are clinically indistinguishable from HBL and ABL, but in which mutations in either the apolipoprotein B or MTP alleles have not been identified. Various models have shown that truncation of the carboxy terminus of the apolipoprotein B protein influences the process of lipidation, as described earlier. Therefore, the inadequate addition of neutral lipids to the shortened apolipoprotein B protein leads to instability of the protein and its eventual degradation.

Clinical manifestations

The clinical manifestations of patients with homozygous mutations of the *APOB* gene are identical to those described above for abetalipoproteinemia. By contrast, defects in a single allele (heterozygotes) may cause subtle neurological findings indicative of mild vitamin E deficiency.

Diagnostic criteria or laboratory investigations and treatment

In heterozygous patients, cholesterol, triglyceride, and low-density lipoprotein (LDL) levels are lower than in other unaffected members of the same kindred. By contrast, patients with homozygous mutations of the *APOB* allele have cholesterol, triglyceride, chylomicron, and VLDL levels similar to those previously described for ABL patients. Moreover, characteristic findings such as acanthocytosis and fat-laden enterocytes are also seen. Homozygous HBL patients should be managed as described in the ABL section, including the restriction of dietary fat and the supplementation of vitamin E. By contrast, heterozygous patients should be given moderate doses of vitamin E.

Chylomicron retention disease

Chylomicron retention disease (CMRD; MIM #246700), or Andersen disease, is the least common of the classic disorders of lipid packaging in the intestine [123,124]. It is an autosomal recessive disorder in which apolipoprotein B and MTP are produced, but there is failure to secrete chylomicrons across the basolateral membrane of the enterocyte.

Genetics and molecular pathophysiology

It has been shown that CMRD is secondary to a defect in a gene named *SARA2* that encodes for the SAR1B protein, which is a member of the SAR1-adenosine diphosphate (ADP)-ribosylation factor family of small guanosine triphosphatases [125]. This family of proteins has an important role in intracellular trafficking; it is suggested that when these proteins are defective, chylomicron trafficking to the trans-Golgi apparatus may be disrupted [126]. Although some nonsense mutations have been identified, all the missense mutations alter a highly conserved residue in the guanine nucleotide-binding motif, which would presumably interfere with guanosine triphosphate (GTP) binding. This region of the SAR1B protein interacts with a series of Sec proteins (including Sec12 and Sec23) that eventually leads to membrane deformation, budding, and the eventual transport of chylomicron through the secretory pathway [127].

Clinical manifestations, diagnostic criteria or laboratory investigations, and treatment

Patients have been reported to present as neonates with severe diarrhea but may also first come to medical attention in early adolescence with neuromuscular manifestations of vitamin E deficiency. However, the retinitis pigmentosa and neuromuscular manifestations are less severe when compared with either HBL or ABL. Unlike patients with HBL and ABL, triglyceride apolipoprotein B100 levels are normal in this condition. Enterocytes are also laden with fat, as is seen in HBL and ABL. Treatment consists of restricting dietary fats and supplementing the diet with vitamin E.

Primary bile acid malabsorption

Efficient digestion and absorption of dietary lipids and lipid-soluble vitamins require their emulsification by bile salts (taurocholate) within the intestinal lumen [128]. The majority of dietary fats are absorbed in the jejunum, and residual bile salts are reabsorbed primarily by ileal enterocytes that express a Na^+-dependent bile salt transporter, ASBT (encoded by the *SLC10A2* gene), on its apical membrane. Primary bile acid malabsorption (PBAM; MIM #601295) is a rare autosomal recessive disorder that results from an impairment of bile acid reabsorption secondary to a defect in the function of the ASBT transporter [129,130]. Taurocholate malabsorption more frequently results from the loss of an adequate amount of ileum, which may occur either because of surgical resection or because of extensive inflammation secondary to disorders such as Crohn's disease [131,132]. The diarrhea that accompanies both primary and secondary forms of bile acid malabsorption may be attributed to both fat malabsorption (steatorrhea) and bile acid stimulation of colonocyte secretion of ions and fluid. Idiopathic adult-onset bile acid malabsorption has also been described in patients with presumably normal ileal function [133].

Genetics and molecular pathophysiology

Dawson and colleagues identified three missense (Ala171Ser, Leu243Pro, Thr262Met) and a splice-site mutation of the *SLC10A2* gene in patients with PBAM [129]. In vitro analysis of these missense mutations failed to alter the

proper targeting of ASBT to the cell membrane; however, the Leu243Pro and Thr262Met mutations impaired taurocholate transport [134]. The Ala171Ser mutation was found in 28% of asymptomatic individuals and appears to represent a benign polymorphism because in vitro analysis showed no impairment of bile acid absorption. A missense mutation (Pro290Ser) was identified on a single ASBT allele (in a heterozygote), a patient with Crohn's disease [135]. The Pro290Ser mutation abolishes taurocholate transport and in heterozygous carriers may influence cholesterol homeostasis and bile acid metabolism; however, the frequency and role of this and other polymorphisms in the general population await further investigations. No significant genomic mutations were identified in a large cohort of adult patients with idiopathic bile acid malabsorption [133].

The Na^+-dependent bile salt transporter ASBT is expressed on cholangiocytes and ileocytes and therefore plays an essential role in the enteric and hepatic phases of the enterohepatic circulation. Taurocholate is excreted in the biliary tree, where it is initially stored in the gallbladder and eventually enters the duodenum after cholecystokinin-induced gallbladder contraction in response to a meal. A small fraction of taurocholate is passively absorbed in the jejunum and colon. Along the horizontal axis of the intestine, ASBT expression is limited to the ileum, where it transports taurocholate by an electrogenic, Na^+-dependent mechanism. The reabsorption of taurocholate is about 95% efficient, and the 5% loss is replaced by hepatocyte conversion of cholesterol to bile salts.

Differential expression of orphan solute transporters in *SLC10A2*-null mice was used to identify the putative basolateral taurocholate transporter [136]. The heteromeric organic solute transporter, α–β, Ostα–Ostβ, is expressed in the kidney and intestine and localized by immunohistochemistry at the basolateral surface. Both proteins are required to get correct targeting of the complex to the basolateral membrane to facilitate taurocholate efflux in the ileum. Eventually, taurocholate enters the portal circulation and is transported across the hepatocyte's sinusoidal membrane by a Na^+-dependent taurocholate cotransport polypeptide, NTCP (*SLC10A1*). Transport of recycled and newly formed taurocholate out of the hepatocyte occurs across the canalicular membrane by an ATP-dependent bile salt export pump, BSEP.

Clinical manifestations

Patients with PBAM present during infancy with diarrhea, steatorrhea, failure to thrive, and low plasma levels of LDL cholesterol [129,130]. The diarrhea is generally secretory in character (persists while fasting) and is exacerbated by the addition of dietary fats.

Diagnostic criteria or laboratory investigations and treatment

The small intestine of patients with PBAM should be normal in length and architecture, and the extent of the diarrhea should improve with a trial of cholestyramine and a low-fat diet. Bile acid absorption by the ileum may be measured using the bile acid analogue ^{75}Se-homocholic acid–taurine test.

Disorders of mineral and electrolyte absorption and secretion

Congenital chloride diarrhea

The most common cause of congenital secretory diarrhea in the presence of normal intestinal mucosa is the autosomal recessive disorder congenital chloride diarrhea (CCD; MIM #214700) (Fig. 51.6) [137]. Nearly half of the reported cases have been confined to Finland, where it has been estimated to occur in 1 in 20 000 of the population; in the Persian Gulf it has been estimated to occur in 1 in 32 000 live births [138]. An earlier linkage analysis of several families identified the CCD locus to a region of chromosome 7q31 located in the vicinity of the cystic fibrosis gene, cystic fibrosis transmembrane regulator (*CFTR*) [139,140]. The defective gene in CCD is *SLC26A3*, which encodes a Na^+-independent Cl^-/HCO_3^- exchanger that is expressed primarily in the apical brush-border membrane of enterocytes (Fig. 51.6) [141].

Genetics and molecular pathophysiology

Höglund and colleagues identified several mutations in the *SLC26A3* gene in patients with CCD, including the prevalent Val317del mutation, which was found in a Finnish population and impaired chloride transport [137,141,142]. Over 30 disease-related mutations have been identified in more than 100 patients studied with CCD [137]. One-third of the mutations are either microdeletions or missense and nonsense mutations. Two other prominent founder mutations have been identified that account for the majority of cases of CCD in Poland (Ile675-676ins), and in Saudi Arabia and Kuwait (Gly187X) [138]. A small number of patients have been identified that fit the clinical characteristics of CCD but lack detectable mutations in the *SLC26A3* gene.

The main role of the SLC26A3 protein as a Cl^-/HCO_3^- exchanger is to reabsorb chloride along the length of the distal small bowel and colon (Fig. 51.6) [139]. In the distal intestine and colon, salt (NaCl) absorption is secondary to SLC26A3 transport of chloride and Na^+–H^+ exchange (NHE-3) transport of sodium. In normal physiological conditions, the expression of NHE-3 in the proximal small intestine is low, and the Cl^-/HCO_3^- exchanger secretes bicarbonate into the lumen while absorbing chloride produced by gastric acid [143,144]. Thus, the exchanger neutralizes the acidity of gastric secretion once it reaches the proximal small bowel. The CFTR protein is a chloride channel whose primary role in the small intestine is to secrete chloride, which is then absorbed in the distal intestine and colon by the Cl^-/HCO_3^- exchanger. Thus, impaired Cl^-/HCO_3^- exchanger function in CCD patients would disrupt the neutralization of gastric acid in the

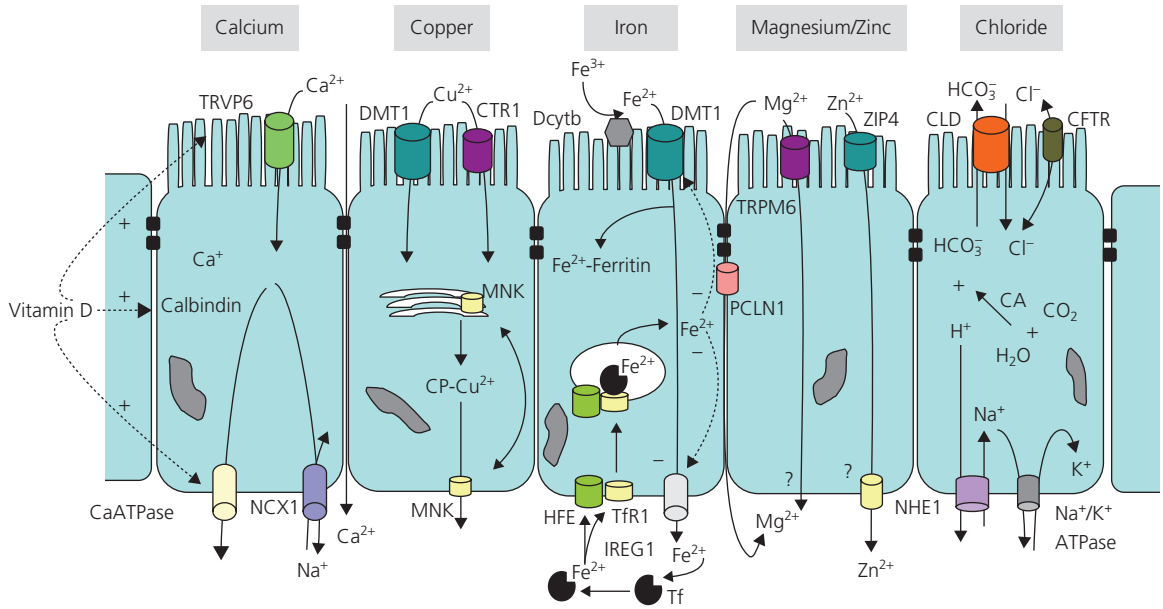

Figure 51.6 Ion and metal transport in the small intestine. ATPase, adenosine triphosphatase; Ca, calcium; CFTR, cystic fibrosis transmembrane regulator; CLD, congenital chloride diarrhea; Cp, copper; CTR, copper transporter; Dcytb, duodenal cytochrome b; DMT, divalent metal transporter; FPN, ferroportin; HFE, hemochromatosis protein; IF, intrinsic factor; IREG, ferroportin 1; MNK, Menkes syndrome transport protein; NCX, Na^+–Ca^{2+} exchanger; NHE, Na^+–H^+ exchange; PCLN, paracellin; TfR, transferrin receptor; TRPM6, transient receptor potential cation channel subfamily M, receptor 6; TRVP, transient receptor potential vanilloid; ZIP, zinc- and iron-regulated protein.

proximal small bowel and impair lumenal chloride reabsorption in the distal small intestine and colon.

Clinical manifestations

The earliest clinical symptoms may occur in utero with severe polyhydramnios and dilated loops of small bowel detectable by ultrasonography and possibly resembling a distal intestinal obstruction [145]. Patients with CCD generally present during the first week of life with severe life-threatening secretory diarrhea. The profile of serum electrolytes prior to treatment is unique among the various congenital diarrheal disorders and manifests metabolic alkalosis, hypochloremia, hypokalemia, and hyponatremia.

Diagnostic criteria or laboratory investigations and treatment

The diagnosis of CCD is suggested if fecal chloride concentration exceeds the concentration of cations (Na^+ and K^+). This classic characteristic of CCD suggests that fecal HCO_3^- levels are particularly low and that another cation, presumably accounted for by H^+, contributes to the excessive electroneutral transfer of HCl. The mainstay of therapy is the oral or parenteral administration of KCl supplements. Although oral administration is the preferred route of electrolyte supplements, they are not well absorbed and may therefore contribute to the diarrhea. The administration of proton pump inhibitors slightly diminishes the severity of diarrhea, presumably by inhibiting meal-induced gastric acid secretion [146]. Orally administered butyrate has been shown to improve dramatically stool volume and electrolyte depletion in a single patient with CCD in a dose-dependent manner [147]. Lumenal butyrate can increase NaCl absorption by activation of the Cl^-–butyrate exchanger and by reducing both basal and cyclic adenosine monophosphate (cAMP)-mediated chloride secretion.

Congenital sodium diarrhea

Congenital sodium diarrhea (CSD, MIM #270420) is an exceedingly rare autosomal recessive disorder that presents with secretory diarrhea and many of the same clinical characteristics seen in patients with CCD [148,149]. Only six cases of CSD have been described in the literature, and the molecular basis of this condition has not been fully established [150]. Nevertheless, the available information suggests that the disorder results from impaired function of the intestinal Na^+–H^+ exchanger (Fig. 51.6). The gastrointestinal tract expresses primarily three Na^+–H^+ exchangers: NHE-2, -3, and -4 [151]; NHE-4 is expressed largely by gastric mucosa, whereas both NHE-2 and -3 are present in the small intestine.

Genetics and molecular pathophysiology

A study of two large Austrian kindreds with five affected members failed to identify linkage to any of the known Na^+–H^+ exchangers [150]. Vesicle transport studies performed on brush-border membrane samples confirmed that

CSD is the result of a faulty Na^+–H^+ exchanger [148]. The NHE-3 exchanger is considered to be the primary transporter responsible for Na^+ absorption because deletion of the *Nhe-3* gene in mice results in sodium secretion that resembles CSD [152,153]. These mice also had a mild proximal tubular acidosis that was secondary to the role of NHE-3 in renal Na^+ and HCO_3^- reabsorption. Although mild diarrhea was reported in these mice, the primary finding in the gut was an excess amount of lumenal fluids and an expanded surface area.

Clinical manifestations

Patients with CSD have high-volume secretory diarrhea that is exceedingly alkaline and contains high concentrations of Na^+. Therefore, patients generally have hyponatremia, low or normal excretion of urinary Na^+, and metabolic acidosis. Polyhydramnios resulting from in utero diarrhea is also associated with CSD, and a report described choanal atresia in two patients with CSD [150]. Although the intestinal biopsies of patients with selective impairment of transport function are generally considered to be normal, biopsies from three cases that fit the clinical characteristics of CSD showed partial villous atrophy and a decreased villus-to-crypt ratio, suggesting the possibility of a distinct disorder. Electron microscopy showed no evidence of microvillus atrophy or inclusion disease but demonstrated unexpected membranous whorls located in lysosomal bodies, vacuoles, and mitochondria.

Diagnostic criteria, laboratory investigations, and treatment

The diagnosis of CSD should be considered in patients who present as newborns with high-output secretory diarrhea and can be established by confirming the presence of elevated levels of fecal Na^+ and HCO_3^-. Unlike patients with CCD, who have metabolic alkalosis, metabolic acidosis is typically found in CSD and in all other forms of congenital diarrhea. The medical management of CSD patients is to maintain their fluid and electrolyte balance with the use of oral or parenteral fluids and salts.

Acrodermatitis enteropathica

Primary acrodermatitis enteropathica (AE; MIM #201100) is a rare autosomal recessive disorder of impaired intestinal absorption of zinc (Fig. 51.6) [154,155]. Although secondary deficiencies in trace elements such as zinc and selenium are a common complication of chronic diarrhea, patients with AE present in early infancy with profound depletion of total body zinc.

Genetics and molecular pathophysiology

Genome-wide screening for the AE allele was performed on two large consanguineous families, which led to the identification of a candidate gene that encodes a member of the zinc- and iron-regulated transporter family of proteins (ZIP4; Fig. 51.6) [156,157]. Sequencing of the *ZIP4* gene identified five missense mutations that altered highly conserved and critical amino acid residues in the family of zinc transporters in patients with AE [158,159]. ZIP4 is expressed on the apical membrane of epithelia in the intestine, colon, stomach, and kidney, and is a member of a family of proteins that control the transport of zinc into the cytoplasm of various cells [160]. During periods of zinc deficiency, the ZIP4 content of the brush border increases as expression is augmented and the protein is recruited to the apical membrane [161]. Although patients with AE have a markedly reduced capacity to absorb lumenal zinc, their responsiveness to high-dose oral zinc supplements raises the possibility that they retain a redundant but less efficient zinc transport mechanism in the intestine (Fig. 51.6).

Clinical manifestations

Zinc deficiency may develop either because of a primary defect in absorption or secondary to either inadequate intake or excessive losses [154]. Zinc deficiency resulting from excessive fecal losses of zinc is a common finding in patients with acute and chronic diarrhea, and short-term oral supplementation of zinc has been shown to reduce the duration and severity of diarrhea [162]. Severe zinc deficiency can present with anorexia, diarrhea, and severe failure to thrive [154]. The dermatitis located on the hands, feet, and perirectal and oral regions has a vesicobullous character, and the alopecia of the scalp and face is also a striking feature that occurs in patients with prolonged zinc deficiency. Humoral and cell-mediated immunodeficiencies have been reported in association with AE and may contribute to both poor wound healing and recurrent infections. Poorly characterized neurological features such as mental lethargy and neurosensory abnormalities may also be present.

Diagnostic criteria, laboratory investigations, and treatment

Zinc deficiency results in low levels of serum alkaline phosphatase, a zinc-dependent metalloenzyme. Alkaline phosphatase levels decline with age, and lower than expected levels should raise the possibility of zinc deficiency. Both serum and urinary zinc concentrations are significantly reduced in untreated AE patients. Radiolabeled zinc has been used to assess the absorptive capacity of the intestine using both mucosal biopsy samples and in vivo uptake studies [154]. Histology of the small intestine has shown several abnormalities that may represent the consequence of inadequate zinc concentrations in patients with AE. Paneth cells and enterocytes of the small bowel have been shown to have inclusion bodies and abnormal intracellular organelles; villus atrophy has also been reported [154]. Patients with AE can be managed with oral zinc supplementation, using ~ 1 mg elemental zinc/kg/day. Chronic supplementation with ade-

quate doses of zinc will resolve the clinical and laboratory features of this disorder.

Calcium absorption

Calcium absorption in the small intestine occurs by both active and passive transport mechanisms [163]. Passive absorption of dietary calcium occurs paracellularly along the entire length of the small bowel, and this pathway is predominant when the calcium supply is abundant. By contrast, the active calcium transport system is located primarily in the duodenum and is particularly important when the level of dietary calcium is low. The active transcellular transport system has been identified as a member of the transient receptor potential cation channel 6, or TRPV6, and is expressed primarily on the brush border membrane of enterocytes located in the proximal small bowel (Fig. 51.6) [164,165]. Once within the cytoplasm, calcium forms a complex with a calcium-binding protein called calbindin D9k, or CaBP, and calcium efflux out of the enterocyte takes place by either the CaATPase (PMCA1b) or by the Na^+–Ca^{2+} exchanger NCX1 (Fig. 51.6). Vitamin D (1,25-dihydroxyvitamin D-3) directly influences the expression of both TRPV6 and CaBP by directly activating vitamin D-responsive elements in the immediate promoter region of both genes and thereby increasing transcription [164]. Congenital disorders that impair calcium absorption include pseudovitamin D deficiency rickets (MIM #264700), which is secondary to a defective synthesis of 25-hydroxyvitamin D-3 1α-hydroxylase [166]. Specific inherited defects in the transporters that control dietary calcium absorption have yet to be described [167].

Disorders of magnesium transport

Magnesium absorption in the gut occurs primarily in the small intestine by two different pathways: a nonsaturable paracellular passive process and a saturable active transcellular transport process. Primary hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC, MIM #248250) is a rare autosomal recessive disorder that is associated with impaired absorption of dietary magnesium and a renal tubular defect of magnesium transport (Fig. 51.6) [168]. Hypocalcemia secondary to low levels of parathyroid hormone and 1,25-dihydroxyvitamin D-3 is common and may result in tetany. The molecular basis of primary hypomagnesemia was elucidated when linkage analysis led to the identification of a tight junction protein named paracellin 1 (PCLN1) [169]. Mutations in the gene encoding the PCLN protein (also called Claudin 16) were identified in ten kindreds, and the protein was located in the tight junction region of the thick ascending limb of Henle. Whether paracellin is expressed by small intestinal epithelium has not been reported, yet it accounts for the nonsaturable paracellular magnesium transport system that is particularly active at high intralumenal concentrations. Although paracellin may serve as a selective paracellular transporter of magnesium, it may also represent a sensor of magnesium concentration.

Active transcellular transport of magnesium occurs by means of a transient receptor potential (TRP) family of cation channels named TRPM6. Various missense and nonsense mutations of the *TRPM6* gene have been identified in patients with hypomagnesemia with secondary hypocalcemia (HSH, MIM #602014) [170,171]. The TRPM6 protein is located along the entire gut and in the distal tubule cells of the kidney, where immunofluorescence studies have localized it to the apical membrane [172].

Disorders of copper transport

Copper is a critical cofactor required for the synthesis and function of numerous proteins whose roles range from antioxidants to enzymes required for mitochondrial function. The diet is an important source of copper, and our understanding of the complex process of copper absorption, intracellular processing, and efflux has improved by investigating the basis of two disorders of copper transport, Menkes syndrome and Wilson disease [173,174]. Menkes syndrome (MIM #309400) is a rare (1 in 100 000) X-linked recessive disorder of copper deficiency resulting from defective synthesis of the Menkes syndrome transport protein (MNK) (Fig. 51.6) [175]. By contrast, Wilson disease (WD; MIM #277900) is a more common autosomal recessive disorder that is the consequence of copper accumulation secondary to malfunctioning of the WD transporter [176]. Both transporters have been localized to the membranes of the trans-Golgi network (TGN), where they modulate copper transport into the network. When intracellular copper levels increase, both transporters redistribute to small vesicles and the plasma membrane, where they presumably control the efflux of copper out of the cell. Expression of MNK is primarily by enterocytes and cells of the placenta and central nervous system, whereas expression of the WD transporter is limited mainly to hepatocytes. Reflecting the tissue-specific expression pattern of the respective proteins, defects in the function of MNK result in inadequate efflux of copper from the gut, whereas an alteration of WD results in failure to excrete copper to bile, which subsequently accumulates in the liver [173].

The mechanism of dietary copper absorption has not been definitively established; however, two active transporters located on the apical membrane of the enterocyte have been implicated (Fig. 51.6). The H^+-divalent metal transporter (DMT1) has a broad range of substrates, including copper, iron, manganese, and cobalt [177]. A more specific larger family of high-affinity copper transporters, CTR1 and CTR2, has been isolated and is expressed in a broad range of tissues, including the intestine [178]. Within the intracellular compartment of the enterocyte, copper may be either incorporated into copper-containing proteins or conveyed into the TGN by means of the MNK transporter [173,174]. Copper

binds ceruloplasmin within the TGN and subsequently enters the systemic circulation bound primarily to ceruloplasmin. Although copper levels do not influence the production of ceruloplasmin, the latter has a shorter half-life during copper-deficient states, resulting in low ceruloplasmin levels. Because defects of both the MNK and WD transporters lead to impaired trafficking of copper across the TGN membrane, Menkes syndrome and WD are both associated with hypoceruloplasminemia.

Genotype–phenotype relationships have been established in Menkes syndrome, and the clinical consequences of mutations in MNK are primarily the result of a copper-deficient state that leads to improper synthesis of copper-containing proteins. Patients generally present during early infancy with failure to thrive, various neurological symptoms, hyperpigmentation, and morphological changes of the hair. Most children with Menkes syndrome die during early infancy, and although copper supplementation has not been shown to be efficacious, anecdotal reports suggest that copper-histidine may delay the onset of various symptoms [179]. In contrast, WD generally presents during early adolescence with either neurological or hepatic consequences of copper overload. Chelation therapy with D-penicillamine or ammonium tetrathiomolybdate augments renal excretion of copper while improving its positive balance in the liver and brain [180]. Oral administration of zinc has been shown to inhibit dietary intake of copper, presumably by competing for transport into the enterocyte by the DMT (Fig. 51.6) [177,180].

Disorders of iron transport

Our understanding of the mechanism of iron absorption has improved dramatically over the last decade with the discovery of the molecular basis of hereditary hemochromatosis (HH; MIM #235200) [181,182]. This is an autosomal recessive disorder characterized by excessive intestinal iron absorption and secondary multiorgan failure related to excessive tissue iron content. Hemochromatosis is the most common genetic disorder among individuals of European ancestry, with an estimated carrier frequency of about 1 in 8 [181,183]. The most common form of HH is secondary to mutations in the hemochromatosis gene *HFE*, which encodes a major histocompatibility class I-like protein. Several mutations, including Cys282Tyr, His63Asn, and Ser65Cys, account for the majority of abnormal alleles of the *HFE* gene [181,182].

The role of HFE in iron absorption was elucidated when HFE was found to interact with the transferrin receptor (TfR) with an affinity comparable with that of diferric transferrin (Fig. 51.6). Immunohistochemical analysis also confirmed that HFE and TfR were localized to both the basolateral membrane and the intracellular compartment of crypt cells of the intestine. It is believed that HFE facilitates the interaction of diferric transferrin and TfR, resulting in endocytosis by clathrin-coated vesicles and the eventual release of iron into the cell cytoplasm.

The primary apical iron transporter, DMT1, was isolated by expression cloning; it also transports copper, another divalent cation (Fig. 51.6) [177]. Because the absorption of dietary iron is limited to the reduced ferric (Fe^{2+}) form, the reduction of the oxidized form (Fe^{3+}) by the brush-border enzyme duodenal cytochrome *b* (CYBRD1) prepares it for transport (Fig. 51.6). Within the enterocyte, iron can be stored in ferritin, or effluxed across the basolateral membrane by the transporter ferroportin 1 (IREG1) [184]. At the basolateral membrane, hephaestin facilitates the export of iron with its ferroxidase activity. Both apical and basolateral iron transporters are expressed primarily in duodenal enterocytes located along the upper portion of the villus, and their expression is dramatically upregulated in iron-deficient states.

The common HFE mutations appear to inhibit its interaction with its heterodimeric partner, β_2-microglobulin, and diminish the level of HFE on the basolateral membrane. Because endocytosis of the transferrin–TfR–Fe^{2+} complex requires adequate levels of HFE, mutations of the HFE protein would reduce cytoplasmic iron levels while augmenting the expression of DMT1 and FPN1 and increasing iron intake [185,186]. By contrast, in iron-abundant states associated with wild-type HFE, endocytosis of the Fe^{2+}-containing complex would increase the cellular stores of iron and thereby reduce the synthesis of DMT1 and FPN1.

Deficiencies of proteins other than HFE have been implicated in the pathogenesis of other forms of hemochromatosis. Mutations in the gene encoding ferroportin 1 have been identified in an autosomal dominant form of hemochromatosis (MIM #606069) [187]. Hepcidin, TfR2, and hemojuvelin are three proteins expressed by hepatocytes that regulate the intestinal absorption of iron [188]. Defects in TfR2 have also been identified in several kindreds (MIM #604250), whereas a juvenile form of hemochromatosis (MIM #602390) has been associated with mutations of either hepcidin (an antimicrobial peptide synthesized by hepatocytes that appears to negatively modulate intestinal absorption of iron) or hemojuvelin [189–192]. Hepcidin responds to iron body stores and inflammation and regulates iron efflux by binding to the iron exporter ferroportin [193]. Moreover, hepcidin production is increased by IL-6 and appears to explain the anemia of chronic inflammation [194].

The mechanism by which heme-containing iron is transported in the intestine has been elucidated. Heme carrier protein 1 (HCP1) is located on the apical membrane of enterocytes of the duodenum and transports the heme–iron complex intact [195]. Once within the enterocyte the complex dissociates, probably by heme oxygenase, releasing iron, which is transferred to the circulation by the basolateral transporter ferroportin. Whereas the abundance of HCP1 was not altered by the iron status of the host, its expression was dramatically upregulated by hypoxia. Interestingly, in iron-depleted states, HCP1 occupies the apical brush-border

membrane, whereas in iron-plentiful conditions it moves to internal cellular compartments and thereby interrupts lumenal absorption [195].

Disorders of vitamin absorption

Folate malabsorption

Dietary folate is found primarily in green leafy vegetables, organ meats, and grains as polyglutamylated folates. Glutamate carboxypeptidase II (GCP-II), expressed on the brush-border membrane of small bowel enterocytes, hydrolyzes folylpolyglutamates to a form (monoglutamyl folates) that can be transported by the reduced folate transporter 1 (RFC-1) (Fig. 51.7). Once within the enterocyte, folylmonoglutamate is converted to 5-methyltetrahydrofolate (5-MTHF), which exits the basolateral membrane by means of the RFC-1 transporter. The folate-binding protein mediates the transport of 5-MTHF into hepatocytes, where it is stored in the form of polyglutamylated folates; these are essential cofactors for nucleic acid and amino acid synthesis.

Folate deficiency generally results in hyperhomocysteinemia because 5-MTHF is required to synthesize methionine from homocysteine. A sufficient level of methionine is required for the synthesis of *S*-adenosylmethionine, an enzyme critical for DNA methylation. Congenital folate malabsorption (MIM #229050) is a rare autosomal recessive disorder associated with the clinical symptoms of diarrhea, glossitis, and seizures in conjunction with pancytopenia [196]. The underlying mechanism of the congenital form of folate deficiency has not been identified. Folic acid deficiency has been definitively associated with neural tube defects in neonates and an increased risk of cardiovascular disease in adults with elevated levels of homocysteine [197]. Serum folate and homocysteine levels are readily available assays that may be used to assess adequate folate stores.

Vitamin B-12 malabsorption

The absorption of vitamin B-12 (cobalamin) is a complex process that is initiated by gastric acidity, which removes cobalamin from dietary proteins and transfers it to a binding protein called haptocorrin [198]. Intrinsic factor (IF), produced by parietal cells, binds cobalamin after pancreatic proteases hydrolyze the cobalamin–haptocorrin complex in the duodenum. The enterocytes of the ileum express cubulin, which forms a heterodimer with amnionless, forming a receptor for the cobalamin–IF complex (Fig. 51.7) [198]. The role of amnionless is to direct the receptor–vitamin complex to the endosomes, where it encounters a second receptor, megalin [199]. The cobalamin–IF complex is cleaved within the endosome, resulting in the formation of a cobalamin–transcobalamin 2 complex, which traverses the enterocyte and enters the systemic circulation.

The clinical consequences of vitamin B-12 and folate deficiency overlap because cobalamin is a cofactor for methionine synthase, which uses a methyl group from 5-MTHF to convert homocysteine to methionine [200]. Hyperhomocysteinemia is thus the consequence of both vitamin B-12 and folate deficiency. Defects of cobalamin absorption are, however, uniquely associated with methylmalonicacidemia because

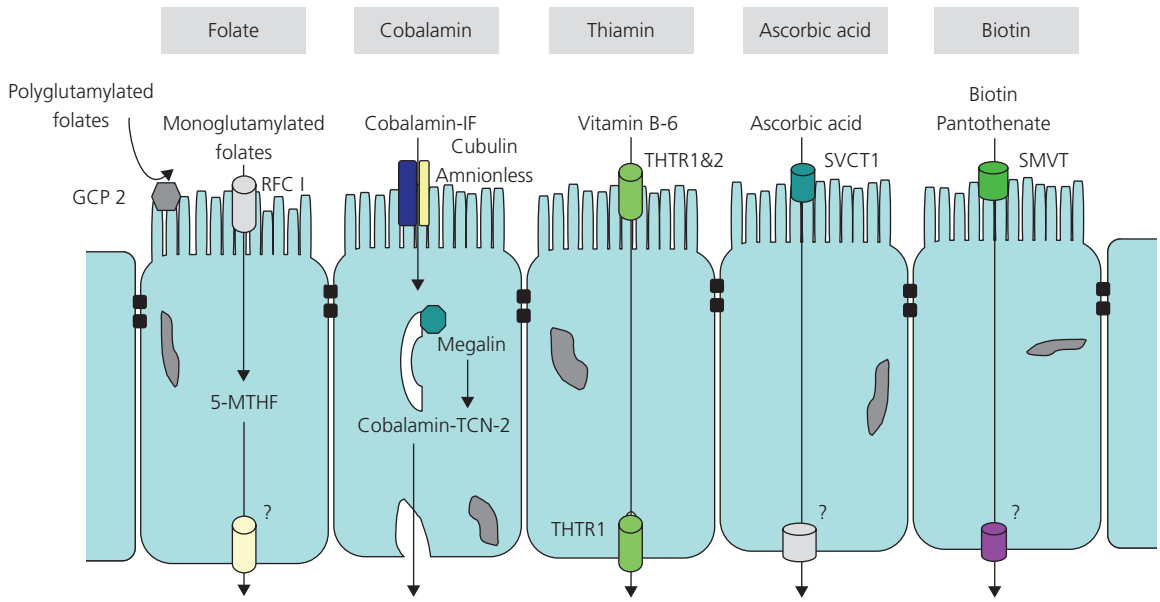

Figure 51.7 Water-soluble vitamin absorption in the small intestine. GCP, glutamate carboxypeptidase; IF, intrinsic factor; 5-MTHF, 5-methyltetrahydrofolate; RFC, reduced folate transporter; SMVT, sodium multivitamin transporter; SVCT, sodium vitamin cotransporter; TCN, transcobalamin; THTR, thiamine transporter protein.

adenosylcobalamin is required for appropriate mitochondrial fatty acid metabolism [201].

Because of the complexity of vitamin B-12 assimilation, deficiency may be secondary to numerous problems, ranging from inadequate intake to disruption of any of the various steps required for absorption [200]. Primary or secondary achlorhydria is commonly associated with cobalamin deficiency because gastric acidity is required for the formation of the cobalamin–haptocorrin complex. Bacterial overgrowth in the small intestine may also disrupt the cobalamin–IF complex and lead to a decline in cobalamin levels. Because cobalamin is absorbed exclusively in the ileum, disorders resulting in an inadequate ileal absorptive capacity will also result in vitamin B-12 deficiency.

There are three rare yet well-described autosomal recessive disorders associated with congenital forms of cobalamin deficiency. Congenital pernicious anemia (MIM #261000) is associated with an absence of IF, which facilitates the binding of cobalamin to the cubilin–amnionless receptor [202]. Imerslund–Grasbeck syndrome (MIM #261100) was originally described in both Finland and Norway. Linkage analysis of the Finnish patients identified mutations in the cubilin receptor [203], whereas the Norwegian cases were identified as secondary to mutations in the amnionless gene [198,204]. Finally, congenital defects of transcobalamin 2 (MIM #275350) have also been shown to result in inadequate levels of vitamin B-12 and in both bone marrow failure and a range of neurological deficits [205]. Measurement of serum vitamin B-12 levels and evidence of homocysteine and methylmalonic acid may be used to assess vitamin B-12 stores. The Schilling test is particularly useful in assessing the various origins of cobalamin deficiency [201].

Absorption of various water-soluble vitamins

The molecular mechanisms responsible for the absorption of several water-soluble vitamins have been elucidated. Vitamin C (L-ascorbic acid) has been found to be transported across the intestinal epithelial layer by the Na^+-dependent vitamin C transporter, SVCT1 (Fig. 51.7) [206,207]. Ascorbic acid transport by SVCT1 appears to be critical during the perinatal period because *Svct1*-null mice succumb immediately after birth [208]. Whereas dietary deficiency of ascorbic acid is common, inherited forms of a congenital deficiency have not been reported. Although the pyridoxine (vitamin B-6) transporter has not been identified, various characteristics of this pH-dependent carrier-mediated system have been determined [209].

Expression cloning techniques were used to identify the biotin transporter. The Na^+-dependent multivitamin transporter SMVT was also identified as transporting pantothenate (vitamin B-5) and lipoate with high affinities (Fig. 51.7) [210]. An abnormality of biotin transport was described in an encephalopathic child; however, the molecular mechanism has not been described [211]. Thiamin (vitamin B-1) absorption across the intestinal brush-border membrane is facilitated by two thiamin transporter proteins (THTR-1 and THTR-2). Both transporters are abundantly expressed in enterocytes, and whereas THTR-1 has been localized to both the apical and basolateral membranes, THTR-2 is restricted to the apical surface. The thiamin transporters resemble the structure of the folate transporter RFC-1, and mutations of THTR-1 have been associated with a thiamin-responsive megaloblastic anemia syndrome (MIM #249270) [212,213]. This is a rare autosomal recessive disorder that is associated with megaloblastic anemia, diabetes mellitus, and deafness.

Absorption of various fat-soluble vitamins

The most common causes of a generalized deficiency of fat-soluble vitamins include specific dietary restrictions and a wide assortment of gastrointestinal disorders that impair fat absorption. All fat-soluble vitamins are absorbed by enterocytes primarily by passive diffusion, a process that is facilitated by the emulsification of fats by bile salts. Selective deficiencies are certainly more unusual and may suggest a defect in the interaction of the vitamin with a specific transfer protein. For instance, vitamin A (retinol) is packaged in chylomicrons in the enterocyte and is eventually taken up by hepatocytes, where it is either stored or remains bound to retinol-binding protein for transfer to other cells. A rare inherited disorder of retinol-binding protein deficiency (MIM #180250) has been described and leads to an assortment of ophthalmological findings and a low serum level of vitamin A [214]. A selective vitamin E deficiency (MIM #277460) has also been identified that is secondary to mutations in the α-tocopherol transfer protein and results in a form of spinocerebellar ataxia and undetectable serum vitamin E levels [215].

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C SMALL INTESTINE

52

Bacterial overgrowth

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Bacterial overgrowth of the small bowel is a syndrome in which nutrient malabsorption is associated with excessive numbers of bacteria in the small intestine [1,2]. Other terms that have been used to describe the syndrome of bacterial overgrowth are blind loop syndrome, contaminated small bowel, small bowel stasis, and stagnant loop syndrome. Recognition dates back to 1897, when the association between pernicious anemia and strictures of the small intestine was first reported [3]. In 1939, the association of macrocytic anemia with intestinal strictures or anastomoses occurring as the result of bacterial overgrowth, or "putrefaction," was initially postulated [4].

Patients with a variety of structural or functional disorders of the intestinal tract are predisposed to the development of small bowel bacterial overgrowth [1,2]. Evidence suggests that bacterial overgrowth may develop in the absence of an anatomic lesion or a severe motility disorder, particularly in the elderly and children [5,6]. Bacterial overgrowth should be considered as a possible cause of diarrhea, malabsorption, or abdominal pain in these clinical settings. As the diagnosis of bacterial overgrowth presents many difficulties and limitations, a course of empiric treatment is often employed in clinical practice.

Conditions favoring bacterial overgrowth

The conditions favoring bacterial overgrowth can be classified according to the mechanisms involved in the normal control of the enteric microflora (Table 52.1). Furthermore, the presence of a single condition may not result in clinically significant small intestinal overgrowth until another condition favors bacterial overgrowth.

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Stasis of the small intestine

Intestinal peristalsis plays a major role in preventing bacterial overgrowth. Anatomic or structural disorders that interfere with gut peristalsis frequently lead to small bowel bacterial overgrowth. These include, for example, chronic small intestinal obstruction secondary to intestinal strictures, such as those caused by Crohn's disease, lymphoma, or radiation injury [7–9]. Surgical alterations of the intestinal anatomy that create blind pouches or divert long segments of small bowel, such as end-to-side enteroenteric anastomosis, the Billroth II anastomosis, jejunoileal bypass, and the Koch distal ileal pouch (for continent ileostomies), have been associated with clinically significant bacterial overgrowth [10–12].

Table 52.1 Conditions favoring bacterial overgrowth

Intestinal stasis
Anatomic: strictures (e.g., Crohn's disease, radiation enteritis); diverticulosis of the small intestine; surgical operations
End-to-side enteroenteric anastomoses: Billroth II anastomoses; jejunoileal bypass; Koch distal ileal pouches
Small intestinal motility disorders
Scleroderma: idiopathic intestinal pseudoobstruction; diabetic autonomic neuropathy
Abnormal connection between proximal and distal bowel
Fistulae (secondary to peptic ulcer disease, carcinoma)
Gastrocolic: gastrojejunocolic
Resection of the ileocecal valve
Hypochlorhydria
Chronic atrophic gastritis
Hypochlorhydric medications
Surgical therapy for peptic ulcer disease
Immunodeficiency
Primary immunodeficiency states
Acquired immunodeficiency syndrome
Malnutrition
Age

Diverticula of the small intestine can serve as pockets of stagnation that can become clinically significant, particularly in the setting of hypochlorhydria or achlorhydria [10]. Generalized impairment of small intestinal motor function (see Chapter 47) may also lead to small bowel bacterial overgrowth. Malabsorption resulting from small intestine bacterial overgrowth has been documented in patients with intestinal hypomotility in association with scleroderma, idiopathic intestinal pseudoobstruction, and diabetic autonomic neuropathy [13–15]. Absent, or disordered, interdigestive migrating motor complex has also been associated with bacterial overgrowth [16]; in one patient, the return of the phase III complex of migrating motor complexes preceded the resolution of bacterial overgrowth [17].

Abnormal connections between proximal and distal portions of the bowel

Bacterial overgrowth of the small intestine may arise secondary to abnormal connections between the proximal bowel and the colon, which leads to seeding of the proximal bowel with colonic flora. Resection of the ileocecal valve frequently causes retrograde seeding of the small intestine with colonic flora and contributes to the development of small intestinal bacterial overgrowth [18,19]. Resection of the ileocecal valve (as well as a portion of the terminal ileum and cecum) also results in alterations of motility that may well play a contributory role in the development of bacterial overgrowth [20]. Gastrocolic or gastrojejunocolic fistulae arising in patients with gastric ulcers and ulcerating carcinomas of the stomach or colon can cause malabsorption resulting from the reflux of colonic contents, including lumenal bacteria, into the proximal bowel [21].

Gastric acid is responsible for decreasing the bacterial inoculum reaching the proximal small intestine. The proximal gastrointestinal bacterial concentration is markedly increased in patients with achlorhydria secondary to atrophic gastritis compared with healthy control subjects [22]. Gastric acid secretion is also impaired as a result of medical and surgical therapy of peptic ulcer disease. Increased bacterial counts and overgrowth have been reported in association with acid suppression therapies [23]. Gastric surgery affects bacterial proliferation by impairing gastric acid secretion and by altering gastrointestinal motility. The results of studies of both animal models and humans after surgery indicate that impaired gastric acid secretion alone is not sufficient to cause clinically significant bacterial overgrowth [11,24]. Hypochlorhydria, however, may promote intestinal overgrowth in patients who also have intestinal disorders associated with stasis (e.g., diverticula, delayed intestinal transit).

Immunodeficiency

Chronic diarrhea, malabsorption, and weight loss are observed in a number of immunodeficiency states (see Chapter 105). For example, more than half of patients with common variable immunodeficiency have chronic diarrhea. Overgrowth of bacteria is a common finding in these patients; however, the bacterial counts (particularly those of anaerobic organisms) are lower than those in cases associated with intestinal stasis [25]. Bacterial overgrowth may also be related to decreased acid secretion in some of these patients [25]. Chronic diarrhea is a prominent symptom in patients with the acquired immunodeficiency syndrome (AIDS), who may have multiple opportunistic infections (see Chapter 104); however, the cause of the diarrhea and malabsorption frequently cannot be identified. Bacterial colonization of the proximal bowel is observed in some patients with AIDS, but the role it plays in the pathogenesis of AIDS-related diarrhea remains unclear because the concentration of anaerobic organisms is lower than that usually observed in bacterial overgrowth associated with gut stasis [26].

Age

The prevalence of small bowel bacterial overgrowth increases with age, and overgrowth can occur in the elderly even in the absence of an anatomic defect of the small bowel [5,27–30]. Possible explanations are reduced gastric acidity, impaired intestinal motility, or both. As bacterial overgrowth is not associated with altered anthropomorphic measurements or bowel habits, the clinical significance of bacterial overgrowth in the elderly is unclear. However, in patients with evidence of malabsorption and bacterial overgrowth, treatment with antibiotics may prove of clinical benefit [30,31].

Pathogenesis

The pathogenesis of small bowel bacterial overgrowth can be considered in terms of the consequences of misplacing the normal colonic lumenal microbiota into the small intestine. The enteric flora, which normally is located principally within the colon, plays an important role in the metabolism of ingested substances (Table 52.2). In cases of small bowel bacterial overgrowth, microorganisms have greater access to nutrients. Mucosal injury also results in the malabsorption of fats, carbohydrates, and proteins [32,33]; however, bacterial invasion and translocation appear not to be the usual consequences of small bowel bacterial overgrowth [34].

The surgical creation of a self-filling blind loop (Fig. 52.1) in animals results in marked proliferation of both aerobic and anaerobic bacteria and reproduces the manifestations of overgrowth observed in humans [35,36]. Much of the experimental work that has defined the pathogenesis of small bowel bacterial overgrowth is based on the use of this model.

Malabsorption of fats and fat-soluble vitamins

Bacterial deconjugation of primary bile acids is a mechanism accounting for the malabsorption of dietary fats and fat-soluble vitamins [37,38]. Normal absorption of long-chain

Table 52.2 Pathogenesis of bacterial overgrowth

Bacterial metabolism of intralumenal substances
Impaired deconjugation of bile acids
Impaired vitamin B-12 metabolism
Impaired metabolism of carbohydrates
Malabsorption of fats and vitamins A, D, and E; generation of secretagogues
Malabsorption of vitamin B-12
Intestinal gas (H_2, CO_2), osmotic diarrhea
Mucosal injury
Decreased brush border enzyme activity: lactase, enterokinase, peptidase
Impaired uptake of monosaccharides
Impaired uptake of peptides
Impaired uptake of lipids
Lactose intolerance
Hypoproteinemia
Carbohydrate intolerance
Fat malabsorption

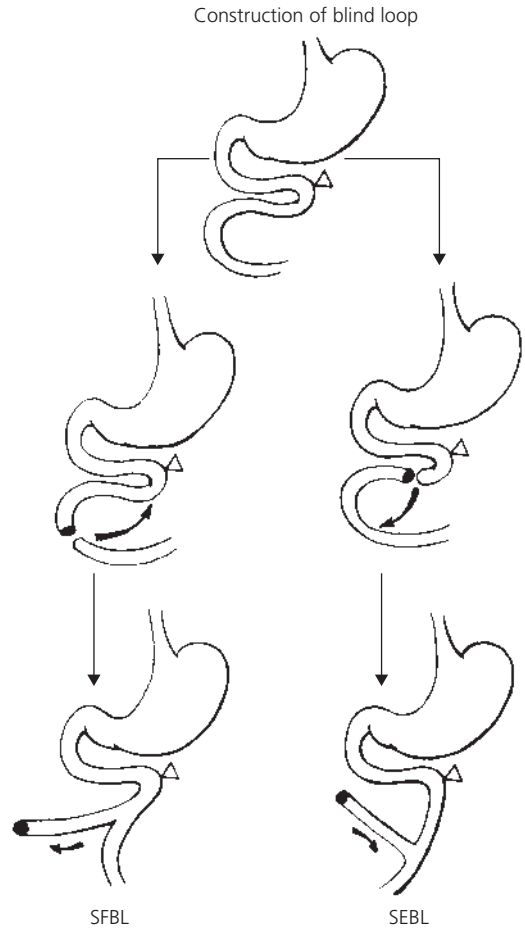

Figure 52.1 Animal model of small bowel bacterial overgrowth. Diversion of an isoperistaltic segment of jejunum results in the formation of a self-filling blind loop (SFBL). The SFBL is often used to characterize the pathophysiology of bacterial overgrowth in the small bowel because it results in the proliferation of colonic-type bacterial flora and sequelae that mimic those observed in human disease. A short, patent, antiperistaltic segment of jejunum, referred to as a self-emptying blind loop (SEBL), serves as an operated control because bacterial overgrowth and its sequelae do not develop postoperatively. From Sherman and Lichtman [45], with permission from S. Karger AG, Basel.

triglycerides requires a critical concentration of conjugated bile acids for the assembly of mixed micelles. Deconjugation of bile acids by lumenal bacteria, particularly anaerobic organisms [39], reduces the level of conjugated bile acids below the critical micellar concentration. Steatorrhea in patients [37] and experimental animals [38] with bacterial overgrowth is relieved by the administration of conjugated bile acids; however, the observation that complete biliary diversion in animals does not produce the steatorrhea seen in bacterial overgrowth indicates that other factors are also likely to play a role [19].

Deficiencies of vitamins D, A, and E have each been reported as complications of small bowel bacterial overgrowth [40,41]. The synthesis of vitamin K by lumenal bacteria, however, accounts for the absence of coagulopathy in patients with bacterial overgrowth. Bacterial metabolites, such as hydroxylated fatty acids and unconjugated bile acids, may have a direct toxic effect on the intestinal mucosa, resulting in the malabsorption of fats, carbohydrates, and proteins. These metabolites also act as secretagogues that can contribute to the development of diarrhea.

Carbohydrate intolerance

Bacterial overgrowth results in carbohydrate intolerance secondary to a reduction of brush border disaccharidases and decreased uptake of monosaccharides [42–45]. Lactase activity is the first hydrolase to be reduced and is the last disaccharidase activity to recover after antibiotic therapy is administered [43,45]. Anaerobic organisms produce proteases that either cleave or destroy hydrolases on the apical brush border of enterocytes [42,43,46]. Carbohydrate malabsorption and maldigestion result in increased delivery to the colon of osmotically active sugars, which then further contribute to the development of the diarrhea associated with bacterial overgrowth. Bacterial metabolism of unabsorbed carbohydrates to hydrogen and carbon dioxide plays a role in the development of abdominal pain and serves as the basis of the various breath tests that have been employed as indirect assays of the presence of small bowel bacterial overgrowth.

Hypoproteinemia

Hypoproteinemia is common in bacterial overgrowth, although severe protein undernutrition is rarely seen [47]. Normal protein assimilation is disrupted by multiple factors: bacteria

compete with the host for protein substrates [44]; decreased amino acid and peptide uptake is demonstrated in experimental models reflecting mucosal injury [47]; decreased levels of enterokinase are noted in patients with bacterial overgrowth, which could impair the activation of pancreatic proteases [48]; and, finally, a protein-losing enteropathy has been described both in patients and in experimental animals with small bowel bacterial overgrowth [49].

Malabsorption of water-soluble vitamins

The association of macrocytic anemia with bacterial overgrowth is the result of direct competition between the intestinal flora and the host for vitamin B-12. Anaerobic organisms are primarily responsible because only anaerobicidal therapy reverses vitamin B-12 deficiency in an experimental model of stasis [19]. Anaerobic organisms, in contrast to aerobic bacteria, use vitamin B-12 either in its free form or when it is complexed with intrinsic factor [50,51]. When bacteria take up the vitamin, not only does it become unavailable to the host but also inactive metabolites are produced that compete with normal vitamin B-12 binding and absorption [52]. In contrast, folate levels are either normal or elevated in bacterial overgrowth because bacteria synthesize folate that subsequently is absorbed by the host. Levels of thiamin [53] and nicotinamide [54] have also been reported on occasion to be low in patients with small bowel bacterial overgrowth.

Although iron-deficiency anemia is not commonly observed with small bowel bacterial overgrowth in humans, increased intestinal losses of iron and blood are documented in animal models [55]. Mineral and trace element micronutrient deficiencies have not been reported in patients with bacterial overgrowth.

Liver disease

Hepatobiliary injury is observed in experimental models of bacterial overgrowth in susceptible rat strains [56,57]. The injury can be prevented by antibiotic treatment [58]. Bacterial cell wall polymers derived from the blind loop have been identified as the underlying cause of the abnormalities identified in the liver and bile ducts [56].

A high prevalence of small bowel bacterial overgrowth is reported in patients with nonalcoholic steatohepatitis (NASH). It has been proposed that bacterial overgrowth plays a role in the pathogenesis of NASH by triggering the release of proinflammatory cytokines, including, for example, elevated levels of tumor necrosis factor-α [59,60]. Small bowel bacterial overgrowth also occurs with increased frequency in patients with cirrhosis [61,62]. The underlying mechanisms are likely to be multifactorial, including both gastric hypoacidity and delayed small bowel transit. The consequences of these changes are not fully understood, but it has been suggested that patients with cirrhosis and small bowel bacterial overgrowth are predisposed to the development of spontaneous bacterial peritonitis [61–63].

Pathology

Lumenal bacteria can clearly influence intestinal morphology. Many of the morphological characteristics of the normal intestinal epithelium are seen only in the presence of a resident microbiota (see Chapter 25). In the germ-free state, villi are thinner and longer, and the crypts are shallower. Enterocytes are cuboidal rather than columnar in shape. The lamina propria consists of a sparse stroma infiltrated with few lymphocytes and macrophages, plasma cells are absent, and the Peyer patches are smaller, with fewer germinal centers. Under light microscopy, the histological appearance of the small intestine in the setting of bacterial overgrowth usually is not markedly different from that of the normal small bowel. The main purpose of obtaining a mucosal biopsy specimen in the evaluation of these patients, therefore, is to rule out mucosal enteropathies such as celiac sprue.

Histological evidence of mucosal damage, however, has been identified in some cases of bacterial overgrowth, including subtotal villous atrophy and increased numbers of inflammatory cells within the lamina propria [33]. Focal areas of ulceration and erosion are observed in some instances and may be important in understanding the symptoms of "pouchitis" associated with bacterial overgrowth after the surgical creation of a continent ileostomy (Koch pouch) or ileoanal anastomosis [64,65]. Ultrastructural studies in experimental models of bacterial overgrowth reveal vacuolization of microvillus membranes and swelling of the mitochondria (Fig. 52.2), suggesting that bacterial overgrowth does result in damage to the enterocyte [32].

Clinical manifestations

Many of the clinical features of bacterial overgrowth can be predicted based on the pathogenesis of the disorder (Table 52.3). Diarrhea and weight loss are prominent symptoms and are largely a consequence of the malabsorption and maldigestion of fats, carbohydrates, and proteins. In addition, many patients with bacterial overgrowth curtail their dietary intake to reduce symptoms [2]. Using lactulose and confirmatory glucose breath testing, one study found that 20% of 145 consecutive patients with Crohn's disease had evidence of small bowel bacterial overgrowth [66]. Although not compared in a placebo-controlled fashion, apparent improvements in symptoms of bloating, abdominal pain, and consistency of the bowel motions were observed in the 29 subjects with bacterial overgrowth receiving oral antibiotics. Abnormal results of breath testing also returned to normal values in 27 of 29 patients treated with either metronidazole or ciprofloxacin. In one study, small bowel bacterial overgrowth was identified as the underlying cause of symptoms in 48% of 87 consecutive children evaluated for chronic diarrhea [67].

Figure 52.2 Electron micrograph of columnar cells in the midvillus area of a self-filling blind loop. In the cell at the lower left, the microvilli are regular, and the mitochondria and endoplasmic reticulum are unaltered. In the cell at the upper right, the microvilli are altered, and the mitochondria and endoplasmic reticulum are swollen. Alterations of the microvilli include blunting, swelling, budding, and ballooning. (Original magnification × 22 000). From Toskes et al. [32], with permission from Elsevier.

Table 52.3 Clinical manifestations of bacterial overgrowth

Diarrhea
Steatorrhea
Malnutrition
Macrocytic anemia
Abdominal pain
Peripheral neuropathy
Tetany
Osteomalacia
Night blindness
Dermatitis, hepatic injury, nephrotoxicity, and arthritis (observed in jejunoileal bypass)

The association of macrocytic anemia with intestinal strictures is a manifestation of vitamin B-12 deficiency. Patients also may present with the neurological changes associated with vitamin B-12 deficiency. Recurrent abdominal pain may be reported, particularly among young children [6]. Other presentations include night blindness, osteomalacia, tetany, peripheral neuropathy, and edema [68,69], reflecting malabsorption of micronutrients such as vitamins A, D, and E, as well as thiamin and nicotinamide.

It is possible that the diagnosis should also be considered in patients with symptoms of irritable bowel syndrome [70]. A high prevalence of bacterial overgrowth has been reported in patients in whom irritable bowel syndrome has been diagnosed. For instance, one report describes a prevalence of abnormal lactulose breath hydrogen tests in more than 80% of patients with irritable bowel syndrome [71]. Furthermore, treatment of the bacterial overgrowth reduced intestinal symptoms in three-fourths of the subjects. A randomized, placebo-controlled trial by other investigators is clearly warranted to confirm these provocative findings [72], as others have not been able to confirm the reported association between bacterial overgrowth and irritable bowel syndrome [73]. It is possible that the variable findings simply may be related to the method that is employed to establish the diagnosis of small bowel bacterial overgrowth [74]. In the setting of irritable bowel syndrome, lactulose breath hydrogen testing may provide false-positive test results. Alternative methods to establish the diagnosis (e.g., culture of duodenal fluid) and confirmation of the consequences of bacterial overgrowth other than carbohydrate malabsorption (including steatorrhea and vitamin B-12 malabsorption) in subjects with irritable bowel should be undertaken to clarify the current controversy in the field [75].

Extraintestinal manifestations of bacterial overgrowth have been observed, especially in patients who have undergone jejunoileal bypass. These immune complex-mediated disorders include dermatitis, hepatic injury, nephrotoxicity, and arthritis [76].

Diagnosis

The diagnosis of small bowel bacterial overgrowth should be considered in patients with chronic diarrhea who have the predisposing conditions discussed previously in this chapter. The differential diagnostic considerations include other causes of malabsorption and maldigestion: intestinal mucosal diseases (e.g., gluten-sensitive enteropathy), infections (particularly parasitic diseases such as giardiasis), and exocrine pancreatic insufficiency. In patients with underlying diseases that favor bacterial proliferation, such as Crohn's disease or scleroderma, it may be difficult to distinguish whether clinical deterioration is caused by the development of bacterial overgrowth or worsening of the primary intestinal disease.

Microbiological culture of small bowel aspirates is considered the most direct method for diagnosing bacterial overgrowth (Table 52.4). In general, the diagnosis of bacterial overgrowth is confirmed if the count exceeds 10^5 colonies per mL after duodenal intubation. A variety of techniques have been reported: the use of sterile and nonsterile tubes, the capsule method, direct needle aspiration of gut contents, and the string test [77,78]. The feasibility of collecting aspirates from the proximal small bowel under direct visualization at the time of fiberoptic endoscopy has been demonstrated and can facilitate the collection of appropriate samples [79].

Table 52.4 Diagnostic tests for bacterial overgrowth

Test	Limitations
Culture of small intestinal aspirate	Invasive, possible contamination with oral flora, difficulty in transportation of anaerobic cultures, distal intestinal overgrowth can be missed
^{14}C-xylose breath test	Radioactivity, decreased sensitivity
Hydrogen breath tests:	
Glucose–hydrogen	20% of population unable to produce hydrogen, decreased sensitivity
Lactulose–hydrogen	Distal intestinal overgrowth can be missed because of overlap between early and late peak

Small intestinal aspirates are obtained by placing a sterile suction catheter inside a sterile overtube that is passed through the suction channel of the endoscope after the desired collection site is reached. Aspirates should be transferred immediately to an anaerobic transport vial and contents plated onto agar plates for the growth of both aerobic and anaerobic organisms [79]. Small bowel bacterial overgrowth usually is associated with the detection of anaerobic organisms. However, because of the technical difficulties related to the successful culturing of fastidious anaerobic bacteria, the presence of more than 10^5 colony-forming units per mL in the duodenum is considered diagnostic for small bowel bacterial overgrowth. Analyses of unconjugated bile acids [80] and short-chain fatty acids [81] in the fluid have also been performed but remain largely investigational tools. In addition to the technical difficulties involved in collecting the specimen properly (e.g., without contamination by organisms resident as part of the normal oral microflora) and in transporting and culturing lumenal fluids, an aspirate of the proximal bowel may not accurately reflect bacterial overgrowth in the distal small intestine.

Breath tests were devised as a less invasive alternative to intubation of the proximal bowel. These tests measure the breath excretion of carbon dioxide or hydrogen produced during the intralumenal bacterial metabolism of an administered substrate. The bile acid or carbon 14-cholylglycine breath test was one of the first breath tests developed, but it does not distinguish bacterial overgrowth from ileal malabsorption [82]. Because of its relatively poor sensitivity and low specificity, this test has been largely abandoned. A radioactively labeled pentose, ^{14}C-D-Xylose, is a more suitable substrate for use in breath testing because it is minimally metabolized by the host. D-Xylose is absorbed in the proximal bowel, whereas bile acids are absorbed in the ileum, so the chance of obtaining a false-positive measurement – as a result of colonic metabolism – is reduced [19]. The sensitivity of this test ranges between 30% and 100% [31,83]. The specificity of the test appears to be high (89%–100%) [31,83]. To avoid the administration of radioactive substrates, employing stable isotopes in combination with mass spectrometry, such as the ^{13}C-xylose breath test, is likely see more widespread use in the future [84].

The hydrogen breath tests also have the advantage of not involving radioactive substrates. However, a limitation with the use of these tests is that 15%–20% of the human population harbor a resident microflora that does not produce hydrogen [85]. Lactulose and glucose are two substrates often used for the purpose of diagnosing small bowel bacterial overgrowth. Patients with bacterial overgrowth who are administered lactulose exhibit an early breath hydrogen peak from small intestinal bacterial fermentation, followed by a prolonged peak from colonic bacterial metabolism [85]. In a case of distal intestinal overgrowth, however, it may be difficult to resolve the two peaks. In a study from Burma comparing the 10-g lactulose–hydrogen breath test with the Enterotest string test, only 2 of 15 children with more than 10^5 organisms per mL had a positive breath test result [86]. In another study the glucose–hydrogen and lactulose–hydrogen tests showed sensitivities of 62% and 68%, respectively, and specificities of 83% and 44%, respectively, in comparison with jejunal culture [87]. An elevated level of fasting breath hydrogen has been observed in some patients with small bowel bacterial overgrowth, but this is a relatively insensitive indicator [88]. Analysis of the presence of unconjugated bile acids in serum may provide another noninvasive test for the diagnosis of small bowel bacterial overgrowth [89].

In summary, each of the currently available diagnostic tests has limitations. Many of the tests are still used primarily as investigational tools and are not widely available in the clinical setting. Barium studies may be useful for documenting an anatomic defect or suggesting the presence of a severe motility disorder. In certain clinical situations, such as the need to document fat malabsorption or vitamin B-12 malabsorption in the setting of an anatomic defect or severe motility disorder, it is reasonable to consider a course of empiric antibiotic therapy without further confirmation of the diagnosis. In the absence of a documented predisposing abnormality of the gastrointestinal tract, however, it is recommended that further diagnostic testing be performed. Routine upper gastrointestinal endoscopy with small bowel biopsy and culture of an aspirate obtained by means of a sterile overtube [69] may be the most efficient means of confirming the diagnosis and excluding other causes of malabsorption due to an enteropathy, such as celiac sprue, giardiasis, and previously

unrecognized infections that can mimic bacterial overgrowth syndrome [90].

Treatment

Initial management consists of fluid and nutritional support, including the correction of documented vitamin deficiencies (Table 52.5). After small bowel bacterial overgrowth has been diagnosed, an attempt should be made to identify an underlying cause. Surgical correction of an anatomic cause of intestinal stasis should be considered. Severe motility disorders are more difficult to manage. Administration of low doses of octreotide to patients with scleroderma for 3 weeks has been reported to clear bacterial overgrowth, as demonstrated by a reversal of abnormal breath hydrogen excretion [91]. At higher doses, however, octreotide can cause steatorrhea secondary to hypomotility and bacterial overgrowth [92]. The prokinetic cisapride may help to normalize intestinal motility in patients with motor disorders and has been efficacious in the treatment of bacterial overgrowth in patients with cirrhosis [63,93]. However, access to this therapeutic agent is limited in some countries, including the United States. The efficacy of motilin receptor agonists in the treatment of dysmotility-associated bacterial overgrowth remains to be tested [94].

Often, the underlying lesion predisposing to small bowel bacterial overgrowth cannot be corrected, and the primary treatment is directed at suppressing the lumenal flora by using oral antibiotics. Although numerous antibiotics have been reported to be effective, few controlled trials have formally studied the choice of antimicrobial agent. In a randomized crossover trial, both norfloxacin and amoxicillin–clavulanic acid given for 7 days were effective in treating diarrhea and in reducing the level of breath-excreted hydrogen in subjects with bacterial overgrowth [95]. However, diarrhea often recurred within a week after the antibiotics were discontinued. This observation has prompted practitioners to employ either repeated or rotating courses of antimicrobial therapy. A study of the relative efficacy of two antibiotics that were given in sequence to seven patients with bacterial overgrowth indicated that metronidazole is more effective in improving relevant clinical symptoms than the nonabsorbable antibiotic rifaximin, even though both agents reduced levels of hydrogen excretion in breath samples after the ingestion of a glucose load [96]. Another study testing three doses of rifaximin [97] showed a dose-dependent reduction in breath-excreted hydrogen following glucose ingestion in 90 subjects with small bowel bacterial overgrowth; however, this study did not compare its efficacy in symptom resolution with another antimicrobial agent.

Although the precise cause of pouchitis is not known [98], mucosal inflammation in surgically fashioned ileal neoreservoirs has been associated with the overgrowth of anaerobic bacteria. In many cases there will be a positive response to therapy with broad-spectrum antibiotics, including metronidazole. In vitro studies support the efficacy of metronidazole in reducing the proinflammatory effects of sonicated lumenal contents derived from patients with documented pouchitis [99].

Table 52.5 Treatment of bacterial overgrowth

Nutritional supplementation
Increase calorie intake
Correct micronutrient deficiencies (e.g., vitamin B-12)
Correction of intestinal stasis
Surgery (e.g., resection of strictures)
Prokinetic agents
Low-dose octreotide (in progressive systemic sclerosis)
Antibiotics

Prebiotics and probiotics to alter the intestinal microflora

Details of the normal colonic microbiota are considered in Chapter 25. Probiotics refers to live microbes that confer health benefits to the host. Prebiotics is used to refer to nondigestive carbohydrates, such as inulin and fructooligosaccharides, which in a selective manner promote the growth of probiotic agents (primarily bifidobacteria) but not strict anaerobic organisms such as *Bacteroides* and *Clostridium* species [100,101]. Synbiotics defines the mixture of prebiotics and probiotics when employed together as combination therapy. Interest has focused on the potential of probiotics as therapeutic agents in the prevention and management of a variety of disease states, including small bowel bacterial overgrowth [102,103,104].

A variety of nonpathogenic bacteria and fungi have been employed as probiotic agents. Although primarily lactic acid-producing gram-positive bacteria (lactobacilli, bifidobacteria and *Streptococcus salivarius* subspecies *thermophilus*) have been used [105], the gram-negative organism *Escherichia coli* strain Nissle 1917 and the yeast *Saccharomyces boulardii* have also been investigated in both animal and human studies. Interest has focused on the potential benefit of employing a combination of probiotic agents [106]. The need for large numbers of viable bacteria to provide greater efficacy has also been emphasized [107].

Probiotic strains exert their beneficial effects through a variety of mechanisms that appear to be unique to each strain [108]. Whether or not living microorganisms are required or even if oral administration is necessary for clinical benefit is still uncertain and might well depend on the clinical effect and the bacterial strain. For instance, several

studies in animal models have demonstrated that nonviable bacterial components could have beneficial effects. Bacterial DNA derived from a probiotic mixture attenuated colitis, an effect that was dependent on Toll-like receptor 9 (TLR-9) [109,110]. Probiotics can also impact on other Toll-like receptors (e.g., TLR-2, TLR-4), particularly in the small bowel [111]. Probiotics also modulate intestinal epithelial barrier function [112], possibly through interactions with TLR-2 [113]. Furthermore, probiotic compounds may not have to be taken orally to have benefits. One study showed, for instance, that subcutaneous delivery of *Lactobacillus salivarius* attenuated colitis and proinflammatory cytokine production in a mouse model [114]. These reports suggest that, in the future, it may be possible to use nonviable and purified probiotic components for the prevention and treatment of human diseases. By contrast, other reports [115,116], albeit using different experimental models and approaches, indicate the need for viable organisms.

Probiotics may prove useful in prolonging remission after antibiotic therapy of bacterial overgrowth. Probiotics are also reported to have efficacy as primary therapy in the treatment of small bowel bacterial overgrowth, but the data are not entirely convincing. Treatment with *S. boulardii* for 7 days showed no efficacy in one trial [95]. On the other hand, probiotic therapy with *Lactobacillus plantarum* 299V and *Lactobacillus* GG was reported as helpful in treating children with small bowel bacterial overgrowth complicating short bowel syndrome [117]. An apparent, but modest, benefit was observed using a single probiotic strain (*Lactobacillus acidophilus*) in an open uncontrolled trial to treat eight subjects with small bowel bacterial overgrowth developing as a complication of chronic renal failure [118].

Probiotics have proven to be effective in the management of ileal inflammation following colectomy and ileal pouch formation (referred to as pouchitis). Randomized controlled trials have shown that the administration of a high dose of a combination preparation of probiotics (5×10^{11} viable lyophilized bacteria per gram containing four strains of lactobacilli, three strains of bifidobacteria, and *Streptococcus salivarius* subspecies *thermophilus*) is effective in the maintenance of antibiotic-induced remission of pouchitis and in postsurgical prevention of pouchitis [119,120]. Trials using a fermented milk product containing lactobacilli and bifidobacteria have also shown some benefit [121]. A reduction in the level of proinflammatory cytokines in the neoreservoir appears to provide an explanation for the mechanism underlying the reported benefits of probiotics in the setting of both preventing and treating pouchitis [122]. By contrast, the use of *Lactobacillus* GG for the treatment of active pouchitis did not demonstrate efficacy [123].

One advantage of using probiotics as therapeutic agents is that the organisms do not permanently reside in the gut. On the other hand, the potential for adverse effects when providing large numbers of viable bacteria, even those that only transiently colonize the gastrointestinal tract, especially to undernourished and immunocompromised patients, cannot be overlooked [124,125]. Nevertheless, the mechanisms underlying the observations that probiotics may be employed as therapeutic agents in a variety of diseases are the subject of intensive research [126,127]. This knowledge could be used to define novel intervention strategies for future use in a variety of disease states, including the treatment of small bowel bacterial overgrowth syndrome and its varying clinical manifestations.

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C SMALL INTESTINE

53 Short bowel syndrome

Richard N. Fedorak, Lana Bistritz

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The term short bowel syndrome covers the symptoms and pathophysiological disorders associated with a malabsorptive state resulting from the removal of a large portion of the small or large intestine. The extent of intestinal resection that will produce this syndrome varies from one person to another. In children, survival without enteral supplements or total parenteral nutrition (TPN) is generally possible if there remains more than 40 cm of the small intestine (i.e., at least 20% of the normal length), measured distally from the ligament of Treitz. In adults, survival without enteral or parenteral nutrition is generally possible if the residual length of the small intestine, measured distally from the ligament of Treitz, is more than 150 cm (i.e., 25% of the normal length). In general, if the ileocecal valve is removed, longer lengths of residual bowel may not prevent short bowel syndrome.

Short bowel syndrome refers only to a well-organized clinical pattern that is sometimes seen in patients with intestinal resection and is not necessarily related to the length of intestine removed. The clinical consequences of removing a portion of the small intestine are extremely variable and depend on a number of factors, including the extent of resection, the site of resection, and subsequent adaptive processes. Thus, it is possible that the removal of identical lengths of small intestine may cause short bowel syndrome to develop in one person but not in another. Due to this confusion, consensus definitions do not include the length of residual intestine. Rather, short bowel syndrome is defined as "result[ing] from surgical resection, congenital defect or disease-associated loss of absorption and is characterized by the inability to maintain protein-energy, fluid, electrolyte or micronutrient balances when on a conventionally accepted, normal diet" [1]. A broader term, *intestinal failure*, encompasses short bowel syndrome, but also includes disease states where the intestine is not reduced in length but is functionally impaired. According to the consensus definitions, "intestinal failure results from obstruction, dysmotility, surgical resection, congenital defect, or disease-associated loss of absorption and is characterized by the inability to maintain protein-energy, fluid, electrolyte, or micronutrient balance" [1].

Significant confusion exists regarding the actual length of the small intestine. Autopsy studies report the length of the small intestine as the distance from the pylorus to the ileocecal valve. Because it is difficult to measure the length of the duodenum, however, measurements made during operations often report the length of the small intestine as the distance from the ligament of Treitz to the ileocecal valve. The mean length of the small intestine at autopsy (from pylorus to ileocecal valve) has been reported as 630 cm in men and 592 cm in women; the mean colonic length is 150 cm in both sexes. These lengths are likely to be greater than the lengths measured in vivo because tone is lost in the postmortem state. Furthermore, small intestinal lengths probably differ according to racial and genetic factors. Full-term newborns have about 250 cm of small intestine and 50 cm of colon. The small intestine develops to its adult length by about 9 years of age.

Although clear functional distinctions exist between the proximal jejunum and the distal ileum, it is difficult to determine the exact normal ratio of jejunal length to ileal length because no clear demarcation exists between the two. In general, in North American adults, the proximal two-fifths (~ 240 cm) of the small bowel are referred to as the *jejunum,* and the distal three-fifths (~ 360 cm) are called the *ileum* [2,3].

Incidence and prevalence

The overall incidence and prevalence of short bowel syndrome are extrapolated from the incidence and prevalence of home parenteral nutrition, for which short bowel syndrome is the most prevalent indication. A 1997 survey indicated

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that the incidence of home TPN was 3 per million and the prevalence was 4 per million [4]. A retrospective review from a major pediatric referral center estimated the overall incidence of short bowel syndrome in neonates was 22 per 1000 neonatal intensive care unit admissions and 25 per 100 000 live births [5]. The small bowel syndrome case fatality rate in this cohort was 37.5%.

Etiology

Adults

Several conditions are associated with short bowel syndrome in adults (Table 53.1) [6]. The most common of these are intestinal resection following a vascular insult to the small intestine (thrombosis or embolism of the superior mesenteric artery, thrombosis of the superior mesenteric vein, volvulus, strangulation) and Crohn's disease, with or without multiple intestinal resections. Less common causes of short bowel syndrome are jejunoileal bypass for obesity, abdominal trauma with resultant intestinal resection, inadvertent gastrocolic fistulization at the time of operation, radiation enteropathy, and neoplasms involving the gastrointestinal tract.

Infants and children

Most of the underlying causes of short bowel syndrome in children develop during intrauterine life (Table 53.2) [6]. Prenatal vascular accidents presenting as intestinal atresia or volvulus that result from malrotation are a particularly common cause of short bowel syndrome in this age group. Less common postnatal causes include extensive aganglionosis, vascular insults, surgery for necrotizing enterocolitis or Crohn's disease, and trauma.

Table 53.1 Causes of short bowel syndrome in adults

Vascular insults
Thrombosis or embolism of the superior mesenteric artery
Thrombosis of the superior mesenteric vein
Volvulus
Strangulation
Postoperative
Jejunoileal bypass for obesity
Abdominal trauma necessitating bowel resection
Inadvertent gastrocolonic/ileal anastomosis
Miscellaneous
Crohn's disease, with or without surgical resection
Intestinal fistula
Radiation enteropathy
Primary or secondary neoplasms involving the gastrointestinal tract

Table 53.2 Causes of short bowel syndrome in infants and children

Prenatal
Vascular insults
Intestinal atresia
Midgut volvulus (malrotation)
Abdominal wall defects
Gastroschisis
Postnatal
Arterial thrombosis or embolism
Venous thrombosis
Necrotizing enterocolitis
Trauma
Crohn's disease
Volvulus
Hirschsprung disease
Radiation enteropathy
Complicated intussusception

Factors influencing short bowel syndrome [7–10]

Extent of intestine removed

Generally, the more extensive the resection of the small intestine, the greater the loss of absorptive surface area that transports nutrients, water, and electrolytes. It is clear, however, that the length of residual intestine and its function are more important prognostically than the extent of intestine removed. Short bowel syndrome can result from either a single massive intestinal resection or repeated lesser resections. Mesenteric vascular disease is more prevalent among patients who have undergone massive resection, whereas Crohn's disease is more prevalent in patients who have undergone repeated surgical resections leading to short bowel syndrome [11,12]. Although the 30-day mortality rate is higher in patients who have had a massive resection, those surviving longer than 30 days have similar survival rates at 5 years [12].

The amount of small intestine remaining after resection determines the surface area available for absorption, as well as the transit time and subsequently the time during which nutrients and nonnutrient fluids and electrolytes are in contact with the absorptive surface of the intestine. The retention of the ileocecal valve will have a marked effect on slowing transit time and can significantly improve absorption. By contrast, the presence of residual disease in the remaining intestine can also adversely affect absorption of nutrients and electrolytes.

About 50% (~ 300 cm) of the small intestine usually can be resected without the development of the significant nutrient or electrolyte losses that lead to short bowel syndrome. The removal of more than 75% (~ 450 cm) of the small intestine almost invariably results in fluid or electrolyte

malabsorption that requires enteral or parenteral replacement therapy.

Site of intestine removed

Jejunum

The jejunum, in its intact state, absorbs a significant fraction of the nutrients and water required by the body, but removal of the jejunum alone generally causes only a limited defect in the absorption of macronutrients, electrolytes, and free water. This limited effect is a consequence of two factors: first, the jejunum is "leaky," and thus a significant back diffusion of transported material into the lumen occurs, causing water and electrolyte absorption in this region of the bowel to be inefficient (~ 40% efficient) when compared with the ileum (~ 75% efficient); second, the ileum is the part of the intestine with the greatest capacity for adaptation, and it can compensate for almost all the absorptive functions of the jejunum [13]. Thus, jejunal resections are often reasonably well tolerated.

Removal of the jejunum reduces the secretion of cholecystokinin and secretin, which may lead to subsequent decreases in biliary and exocrine pancreatic secretions; in turn, these decreases compromise nutrient absorptive capacity. In addition, cholecystokinin, vasoactive intestinal peptide, gastric inhibitory peptide, and serotonin, all of which are distributed mainly in the jejunum, inhibit gastric secretion. Thus, gastric hypersecretion is more likely to occur after resection of the jejunum [14,15]. Increased serum gastrin levels and gastric hypersecretion also are seen more frequently after jejunal resection; these increases probably are also related to the length of bowel resected [16,17].

Ileum

The ileum, which is relatively impermeable compared with the jejunum, remains critically important in the active transport of nutrients and fluid and electrolytes. Removal of the ileum alone generally results in adequate nutrition (maintained by jejunal absorption) but results in fluid and electrolyte deficiencies. With a resected ileum the colon is often incapable of handling the large volume of chyme delivered from the jejunum, and fluid loss is exacerbated by bile acid and fatty acid-induced diarrhea. For this reason, patients usually poorly tolerate extensive ileal resections.

The ileum is the only site of active bile acid absorption from the intestine, although small amounts of bile acids are absorbed by passive diffusion in the jejunum and colon. Therefore, a loss of more than 100 cm of ileum usually precludes the active intestinal absorption of bile acids; thereafter, the passive absorptive capacity of the rest of the small intestine is rapidly overwhelmed, and bile acids are left in the lumen and spill into the colon [8]. These bile acids are deconjugated by colonic bacteria. The deconjugated bile acids directly stimulate the colon to secrete fluid and electrolytes, causing a secretory watery diarrhea and thereby aggravating the short bowel syndrome [13,14]. Bile acids stimulate active anion secretion in the colon through several mechanisms:

- increasing cytosolic calcium levels within colonocytes through a calcium ionophore effect
- increasing prostaglandin synthesis in lamina propria cells
- stimulating excitatory enteric neurons, which then stimulate active anion secretion and propulsive motor contractions
- increasing tight junction permeability to fluid and electrolytes.

During intestinal adaptation, the body compensates for bile acid loss into the colon by maintaining the size of the bile acid pool through an up to eightfold increase in hepatic bile acid synthesis. The more extensive losses of ileum, however, result in severe bile acid malabsorption wherein loss exceeds synthesis. A reduction in the circulating pool of bile salts follows, leading to impaired intestinal micelle formation and the spillage of fat (steatorrhea) into the colon. Fat within the colon is hydroxylated by bacteria into hydroxy fatty acids. Hydroxystearic acid is the hydroxy acid most often associated with steatorrhea, and it has inhibitory effects on colonic fluid and electrolyte absorption. Hydroxy fatty acids stimulate net secretion through several mechanisms: changing net absorption to net secretion, increasing mucosal permeability, and increasing propulsive motor activity. These effects are similar to those of bile acids and may be related to the detergent properties of both hydroxy fatty acids and bile acids.

The ileum is the primary site of vitamin B-12 absorption. Malabsorption of vitamin B-12 occurs after the resection of more than 60 cm of ileum. The intestine appears to be largely unable to recruit new vitamin B-12 receptors into residual ileum or jejunum.

Resection of the distal ileum also affects nutrient absorption in the jejunum. A 50% distal ileal resection in one rat model decreased transmucosal jejunal calcium absorption by unknown mechanisms that did not appear to involve the brush-border membrane [18].

Whereas the mucosal morphological and functional absorptive response to distal ileal resection has been well documented, little is known about the motor response. In dogs, initial motor responses to resections of the distal small intestine of various lengths were dominated by the development of abnormal motility patterns, with prominent cluster activity noted in the intestinal remnant [19]. The intact ileum markedly slows intestinal transit [20]; thus, ileal resection decreases the intestinal transit time significantly, impairing the ileal brake phenomenon thought to be controlled by peptide YY [21].

Colon

Patients with short bowel syndrome who retain their colon and ileocecal valve in continuity have the best prognosis. The colon becomes an important digestive organ in these patients, with its ability to absorb some fluid and electrolytes and amino acids as well as salvage energy from absorbed

short-chain fatty acids. Removal of the colon in conjunction with the jejunum or ileum (or both) often results in life-long parenteral nutrition.

Throughout the intestine, motility is slowest and absorptive capacity is highest in the colon (~ 90% efficient). The absence of the colon significantly increases the rate of transit and the liquidity of stools. The primary function of the colon is to absorb the 1–2 L of fluid it receives daily from the ileum. The liquidity of the feces and the extent of fluid and electrolyte loss in the colon depend on the total volume of fluid and its rate of arrival at the cecum, and also on the presence of unabsorbed solutes such as bile salts. The removal of both ileum and colon obviously drastically increases fluid loss, dehydration, volume depletion, hypocalcemia, and hypomagnesemia. Preservation of the colon in cases of major small intestinal resection appears to lessen the severity of short bowel syndrome because the colon not only provides an absorptive surface area but also exerts a braking effect on early gastric emptying [21]. Preservation of even 50% of the colon is associated with significantly lower morbidity and mortality rates [22].

Although retention of the colon improves fluid and electrolyte absorption, colonic conservation also can have maladaptive consequences. Colonic bacteria deconjugate and hydroxylate unabsorbed bile salts and fats, respectively, creating solutes (bile acids and fatty acids) that limit water absorption in the colon through direct stimulation of the secretion of colonic fluid and electrolytes and through osmotic effects. In addition, the combined effects of massive small bowel resection, fat malabsorption, and an intact colon lead to the formation of calcium oxalate renal stones as free fatty acids in the colonic lumen preferentially bind calcium, leaving oxalate unbound and free to be absorbed systemically through the colonic mucosa.

Presence of an ileocecal valve

The ileocecal valve functions to prolong the intestinal transit time, thereby increasing the duration of contact of the lumenal contents with the small intestinal mucosal surface and enhancing the absorption of nutrients, fluids, and electrolytes [7,8]. If the ileocecal valve is removed during an intestinal resection, the likelihood of the development of short bowel syndrome, often in a severe form, is increased [22,23]. In an outcome study of neonatal short bowel syndrome, the presence of an ileocecal valve was not significantly correlated with successful weaning from parenteral nutrition, but it did reduce the mean duration of parenteral nutritional therapy from 20 to 7 months [24]. By contrast, in a pediatric population the presence of the ileocecal valve was a strong predictor of successful weaning from parenteral nutrition; however, its presence was not a predictor of survival [25]. In addition, the ileocecal valve serves as a barrier that prevents the migration of lumenal colonic microorganisms into the distal small bowel [14,26]. Removal of the ileocecal valve may result in bacterial overgrowth within the small intestine. These bacteria deconjugate bile salts within the small intestinal lumen; impaired micelle formation follows, so that the absorption of fat and fat-soluble vitamins is reduced. Furthermore, deconjugated bile salts spill into the colon and directly stimulate the colonic secretion of fluid and electrolytes, further aggravating the short bowel syndrome. Bacterial overgrowth associated with enteritis in children with short bowel syndrome prolongs the need for parenteral nutrition [27]. Finally, the bacteria use intralumenal vitamin B-12 for their own metabolic purposes, limiting the availability of vitamin B-12 for absorption by the host and exacerbating vitamin B-12 deficiencies.

Intestinal adaptation

Following the development of a short bowel the intestine adapts to ensure more efficient absorption per unit length. Unfortunately, most of the evidence for adaptation comes from rodent and swine animal models of short bowel syndrome, and there are limited human data to support a similar extent of adaptation [28–33]. The adaptive process evolves over 1 or 2 years and is heavily dependent on enteral nutrients to maintain small intestinal structure and function [34–36]. Patients may be taught to adapt to their decreased absorption also by dramatically increasing their food intake.

Jejunum and ileum

In experimental animal models, following resection of a major portion of the small bowel, adaptive changes affect all layers of the remaining intestine. Lumenal dilation with thickening and lengthening of the gastrointestinal tract develops in association with hyperplasia of the crypt–villus axis, and the mucosal surface area increases significantly [28–33]. These effects are more noticeable in residual ileum (i.e., after jejunal resection) than in residual jejunum (i.e., after ileal resection). The number of cells in the proliferative zone of the crypts increases, and these cells rapidly migrate up the villi, causing villus hyperplasia and a parallel increase in the production of brush-border membrane enzymes. This increase in mucosal mass is confirmed by increases in mucosal wet and dry weight, protein, and RNA and DNA contents [37]. An attempt to identify the molecular mechanism behind the observation of ileal hyperplasia in the rodent model of short bowel syndrome demonstrated the expression of a variety of nutrient absorptive and heat-shock proteins in the residual intestine early in the adaptive process [38].

In addition to morphological changes, an upregulation of specific functional absorptive activity occurs in these animal models [39,40]. The absorption of glucose, sucrose, bile acids, vitamin B-12, and calcium is increased in animal models of short bowel syndrome [41]. This increase is related to an increase in the number of absorptive cells rather than to an increase in the capacity of individual cells [33,42]. In contrast to the plethora of findings in animal models of short

bowel syndrome, few data are available regarding functional absorptive adaptation following the resection of human intestine. Dowling and Booth [43] reported an increase in jejunal glucose absorption in patients with short bowel syndrome that was proportional to the amount resected; furthermore, intestinal adaptive responses in humans most often required more than 1 year to reach maximum development [14,16,17,43].

Colon

The colon also undergoes adaptive dilation, lengthening, and mucosal proliferation. These forms of adaptation result in an increased capacity to absorb fluid and electrolytes [44]. Several animal studies suggest that the colon also may be able to upregulate the absorption of glucose and dipeptides through increased expression of mucosal transporters [16,37,45].

Mechanisms of intestinal adaptation

Direct effect on epithelial cells

Enteral nutrients appear to stimulate intestinal adaptation through at least three major mechanisms: direct contact with epithelial cells, stimulation of trophic gastrointestinal hormone secretion, and stimulation of pancreatic and biliary secretions trophic to the small intestine [16]. Again, most experiments on potential stimuli and mechanisms of intestinal adaptation focus on animal, rather than human, models. Evidence that intralumenal nutrients have a direct effect on intestinal adaptation comes from animal studies in which segments of ileum were transposed into the proximal jejunum. The mass of ileal mucosa in this position increased markedly, and transposed villi became larger than those in the adjacent jejunum. In these experiments, the concentration of intralumenal nutrients, rather than simple osmolality, stimulated lengthening of the relatively short ileal villi [7,8]. Without exposure to intralumenal nutrients, intestinal adaptation did not occur; rather, hypoplasia developed [7,8]. In addition, nonnutritive agents did not stimulate mucosal growth, implying that the absorption or metabolism of lumenal nutrients is necessary for the process of adaptation to take place [8,46]. The extent to which enteral nutrients stimulate intestinal adaptation also depends on the type and complexity of the nutrient administered [46,47]. Disaccharides are more potent stimulants of adaptation than monosaccharides [47]. In addition, a nutrient need not always be metabolized for it to induce adaptation: 3-*O*-methyl-D-glucose, a nonmetabolizable glucose analogue transported by the sodium–glucose cotransport system, stimulates intestinal adaptation in a rat model of short bowel syndrome [48,49]. Fats that are highly unsaturated appear to be more effective in inducing intestinal adaptation than fats that are less unsaturated [50].

In animal models of short bowel syndrome, morphological and functional intestinal adaptation following intestinal resection generally does not occur in the absence of enteral nutrition or oral feeding; in fact, a significant degree of mucosal atrophy results if all nutrients are provided parenterally [16,37,51]. Whether it is appropriate to extrapolate findings in rodent models to human short bowel syndrome has been questioned [49,52]. Jejunal biopsy specimens taken from eight normal volunteers before and after 14 days of parenteral nutrition therapy demonstrated decreased villus height, a decreased villus cell count, and increased intestinal permeability during parenteral nutrition, but no changes in DNA, RNA, and protein content in comparison with controls, suggesting that the total enterocyte number did not change [53,54]. In a study of similar design, parenteral nutrition did not affect immunoglobulin-producing lymphocytes in the lamina propria, intestinal permeability, or bacterial translocation [55]. These results suggest that, in contrast to the findings in animal models, depriving the human intestinal lumen of enteral feeding does not lead to intestinal hypoplasia or immune dysfunction. Additional human studies of longer duration and with functional absorptive measurements must be conducted before the direct effects of enteral feeding on intestinal adaptation, identified in the rodent models of short bowel syndrome, can be confirmed [56].

Stimulation of trophic gastrointestinal hormones

The second mechanism by which enteral nutrients stimulate intestinal adaptation is stimulation of the secretion of trophic gastrointestinal hormones. Thiry–Vella fistula models have been used to demonstrate this mechanism of action. Thiry–Vella fistulae are segments of bowel in which the blood supply is intact; however, because of the creation of proximal and distal ostomies, they are excluded from the flow of enteric contents. Animals fed orally after the creation of such a fistula exhibit greater adaptation in both intact intestine and in the Thiry–Vella fistula than do animals fed intravenously [57–59]. Several different hormones are under consideration as candidate trophic hormones. Gastrin appears to have a trophic effect only in the duodenum. Secretin and cholecystokinin stimulate intestinal mucosal growth; however, their effects seem to be exerted through their actions on biliary and pancreatic secretions rather than through direct trophism. Other hormones, including corticosteroids, prostaglandins, epidermal growth factor, enteroglucagon, insulin, and growth hormone-releasing factor, appear to stimulate epithelial cell proliferation in the small intestine [33,60–64]. The role of these hormones in the intestinal adaptation that occurs following intestinal resection remains to be clarified.

Gastrin

Gastrin produces mucosal hyperplasia in the rat stomach and duodenum. When the major source of endogenous gastrin is removed by antrectomy, the resulting gastric and duodenal hypoplasia can be prevented through pentagastrin treatment. Gastrin does not promote adaptive changes in the jejunum or ileum. Furthermore, the relationship between gastrin levels and gastroduodenal adaptation is variable [65].

Cholecystokinin

Cholecystokinin exerts a consistent trophic action on the small intestine. When infused into dogs or rats maintained on TPN, cholecystokinin completely prevented villus hypoplasia in one study [66], but other investigators have been unable to substantiate this effect. Cholecystokinin probably exerts no direct trophic effect on the intestinal mucosa; instead, it likely influences the adaptive process by stimulating pancreatic or biliary secretions [66].

Insulin

Insulin stimulates intestinal mucosal proliferation in a rat model of short bowel syndrome [60]. Parenteral insulin administered twice daily increased jejunal and ileal mucosal weights as well as crypt depth. The use of insulin in humans with short bowel syndrome has not been described.

Enteroglucagon and polyamines

Enteroglucagon-producing cells are found primarily in the terminal ileum. Polyamines (putrescine, spermidine, spermine) are found in all tissues of the body and directly stimulate tissue growth. Both hormones increase RNA and DNA synthesis, which results in epithelial hyperplasia [59,67,68]. Blocking polyamine synthesis negatively affects the adaptation process, whereas blocking polyamine degradation increases intestinal adaptation [68,69]. Enteroglucagon-producing cells are stimulated by carbohydrates and long-chain triglycerides to release preformed enteroglucagon, which stimulates ornithine decarboxylase, increasing polyamine production and, thus, cellular hyperplasia and intestinal adaptation. Changes in enteroglucagon levels correspond closely to changes in the crypt cell production rate, and this peptide remains a favorite candidate as the humorally mediated trophic agent for the small bowel [65,70]. Evidence suggests that precursors of enteroglucagon, such as proglucagon-derived peptides, may be responsible for some of the activities previously attributed to enteroglucagon. Ileal proglucagon messenger RNA levels increase rapidly after intestinal resection [71]. Glucagon-like peptide 2, a product of proglucagon, is capable of inducing marked villus hyperplasia within 4 days after administration, and this change provides compelling evidence that proglucagon plays a significant role in the growth and adaptation of intestinal epithelium [72–74]. The mechanisms responsible for the upregulation of proglucagon in short bowel syndrome are not yet known; however, the fact that dietary fiber fermentation modulates intestinal proglucagon expression may explain why intestinal adaptation requires oral intake [75].

Elevated fasting and meal-stimulated plasma levels of glucagon-like peptides 1 and 2 have been detected in patients with short bowel syndrome [76,77]. The rise in glucagon-like peptide 2 correlates with the amount of intestine resected, nutrient malabsorption and crypt cell proliferation, indicating a trophic effect during the process of intestinal adaptation. Conversely, in a separate study, the meal-stimulated increase in plasma glucagon-like peptide 2 in patients with short bowel syndrome was smaller than that in control patients [78]. Although these findings may simply represent an epiphenomenon of glucagon-like peptides, the results of previous animal studies suggest that these peptides do indeed play a significant role in the intestinal adaptation of short bowel syndrome (see "Glucagon-like peptide 2 therapy" below).

Somatostatin

Somatostatin reduces the crypt cell production rate and hyperplasia in the small intestine after resection; it also inhibits the rise in enteroglucagon level that normally follows jejunal resection [79]. Therefore, somatostatin may modulate mucosal cell turnover by influencing glucagon release. Somatostatin causes a number of competing effects on the intestine. It increases intestinal transit time and inhibits gastric secretions, both of which would benefit the patient with short bowel syndrome; in contrast, somatostatin-induced reductions in pancreatic and biliary secretions can lead to frank fat malabsorption and steatorrhea.

Neurotensin

In rats with 80% of their intestine resected, the administration of exogenous neurotensin increased villus length in both jejunum and ileum. Increases in circulating enteroglucagon observed after the administration of neurotensin suggest that the trophic effect of neurotensin may be mediated, at least in part, by enteroglucagon [80].

Growth factors

Epidermal growth factor (EGF), a potent stimulant of gastrointestinal epithelial proliferation, stimulates DNA synthesis and polyamine synthesis in the small intestine of experimental animals [81,82]. In animals with 70%–80% of their intestine resected, EGF receptor activity was increased, and EGF augmented not only intestinal size but also expression of the sodium–glucose cotransport carrier [82–86]. Insulin-like growth factors (IGFs) also stimulated intestinal adaptation and increase in bodyweight when used immediately after small intestinal resection in the rat [87–89]. Insulin-like growth factor type I transgenic mouse models have been used to demonstrate that IGF regulates intestinal mass and augments colonic fluid and electrolyte absorption [74,90,91]. Nevertheless, there is no evidence for any beneficial effects of the administration of EGF at the time of resection, or to support the use of EGF in well-adapted short bowel syndrome [84], which suggests the existence of an optimal window for growth factor augmentation of adaptation.

Prostaglandins

Prostaglandins of the E_2 class stimulate mucosal hyperplasia in the proximal small bowel of rats [85,92]. Hollwarth and

colleagues [86], however, found that prostaglandins have a strong trophic effect not only in the small bowel but also in the cecum and colon.

Glutamine

Glutamine "deficiency" and subsequent supplementation have been the focus of extensive investigation during short bowel syndrome. However, solid evidence to support a major role remains elusive [93]. Glutamine is an important fuel for enterocytes and colonocytes, in the fed animal [94]. In humans who have undergone intestinal resection, however, the turnover of glutamine is markedly diminished and a relative glutamine deficiency exists [95]. Glutamine also appears to play an important role in maintaining the structural integrity of the small intestine. Indeed, in animal studies, glutamine-enriched parenteral nutrition prevented the deterioration of gut permeability and preserved mucosal structure [96,97]. By contrast, rat enteral diets supplemented with 2% glutamine had no beneficial effect on intestinal adaptation following massive small bowel resection [98]. In the setting of short bowel syndrome, intestinal use of glutamine is increased during adaptive hyperplasia following intestinal resection [99,100]. Glutamine is unstable, however, and it is not generally available in parenteral form except as alanyl-glutamine. Furthermore, to be effective, glutamine must be administered in pharmacological doses of at least 0.5 g/kg daily.

The requirements for large doses of glutamine and the variable results have raised the question of whether the role of glutamine alone, as a fuel for the enterocyte, has been exaggerated. In initial studies, the intestinal metabolism and oxidation of lumenal glutamate and glutamine were identical, with 60% of the carbon atoms of both precursors oxidized to carbon dioxide, which led to the hypothesis that perhaps both glutamate and glutamine are important amino acids in intestinal adaptation [101]. In an infant swine model in vivo, glutamate rather than glutamine appeared to be the preferred source for mucosal glutathione synthesis [102,103] and a preferred substrate for intestinal proline synthesis [104]. Taken together, these results highlight the relative importance of enteral and systemic glutamate and glutamine as key precursors for the intestinal synthesis of amino acids, which in turn may become essential during conditions of short bowel syndrome. However, no controlled human clinical trials have thus far demonstrated that glutamate or glutamine supplementation directly enhances morphological or functional improvement in the small intestine of patients with short bowel syndrome [105].

Arginine

The intestine is an important site for the synthesis of citrulline, which then enters the circulation to act as the primary precursor for renal arginine synthesis. Dietary arginine thus becomes nutritionally indispensable in rats with massive small intestinal resection [106]. The results of studies in pigs suggest that intestinal enterocytes are capable of turning on the synthesis of arginine, which in turn induces intestinal polyamine synthesis for maintenance and growth of the intestinal mucosa [107–111]. Thus, dietary and synthesized amino acids may play an important role in intestinal adaptation following intestinal resection.

Short-chain fatty acids

Acetate, propionate, and butyrate are the principal short-chain fatty acids produced by bacterial fermentation of the carbohydrates and fibrous polysaccharides that spill into the colon [112]. These short-chain fatty acids are absorbed by the colon and metabolized in the colonic epithelial cells as a source of fuel; carbon atoms are salvaged that would otherwise be lost. It has been estimated that up to 3 kJ of energy can be absorbed each day by the human colon in the form of short-chain fatty acids [113]. Supplementation of an elemental diet with pectin, a fiber completely fermented to short-chain fatty acids in the colon, improved adaptation of the ileum, jejunum, and colon after massive small bowel resection [114]. Subsequent studies on the supplementation of parenteral nutrition with short-chain fatty acids [115] and the intracecal infusion of short-chain fatty acids [116] confirmed that supplementation reduces the mucosal atrophy and intestinal immune dysfunction [117] associated with TPN following massive small bowel resection in animal models [118].

In addition to their local effects, systemic short-chain fatty acids can affect the motility of both the stomach [119] and the ileum [120] through neuroendocrine mechanisms, likely through the expression of proglucagon and peptide YY [121–123]. Furthermore, both systemic and enteral short-chain fatty acids exert a trophic effect on the jejunum [124] by increasing mucosal mass, DNA, and villus height. These morphometric effects were associated with an increase in amino acid absorption in a porcine model of small bowel resection [118]. The effects are yet to be confirmed in human short bowel syndrome. It is important to remember that excess amounts of intraluminal short-chain fatty acids, when exceeding the concentrations that can be absorbed by the colon, aggravate diarrhea by directly stimulating fluid and electrolyte secretion and by exerting an osmotic effect that limits water absorption from the colon.

Stimulation of pancreatic and biliary secretions

The third mechanism by which lumenal nutrients stimulate intestinal adaptation is the production and stimulation of pancreatic and biliary secretions. Nutrients that stimulate biliary and exocrine pancreatic secretions produce mucosal hyperplasia, especially when such nutrients are present in concentrations higher than usual in the residual ileum. Ileal mucosal growth is markedly enhanced when biliary and pancreatic ducts are transplanted to the residual ileum of

otherwise normal rats [33]. The presence of food is likely essential for these gastrointestinal secretions to exert their trophic effect [16].

Clinical manifestations

As a result of gradual intestinal adaptation, the clinical course of a patient with short bowel syndrome passes through a number of stages [125–127].

The first stage usually lasts 1–2 weeks and is characterized by a period of substantial fluid and electrolyte loss resulting from overwhelming diarrhea. During this stage, fluid, electrolytes, and nutrition are usually supplied by the parenteral route. The intensity of the watery diarrhea generally decreases during the next several months. Oral intake and subsequent gastric hypersecretion can aggravate fluid and electrolyte loss. Parietal cell hyperplasia, hypergastrinemia, and hypersecretion of gastric acid also can alter the duodenal pH and inactivate pancreatic lipase, contributing to steatorrhea.

The second stage is the period of intestinal adaptation, during which oral intake is initiated and gradually increased. Full or partial enteral or parenteral supplementation often is required to maintain optimal nutritive and fluid and electrolyte status. This stage may last from several months to longer than a year.

During the third stage, maximal intestinal adaptation is achieved, and a relatively normal oral intake is usually possible. Whereas some patients never reach the stage of full oral nutrition, life at home can continue with the patient supported by a combination of enteral or parenteral nutrients and electrolytes.

Diarrhea

Many factors make diarrhea inevitable in patients who have undergone extensive small intestinal resection, including:

- reduction of the absorptive surface area
- decrease in intestinal transit time
- humorally mediated gastric, small intestinal, and colonic hypersecretion
- increase in the osmolality of colonic contents with subsequent osmotic diarrhea secondary to malabsorption of fat and carbohydrates.

Bacterial overgrowth in the small intestine and associated structural damage to enterocytes (increased permeability, decreased brush-border membrane function) has been detected by increased breath hydrogen levels compared with healthy volunteers, and remits with antibiotic therapy [128]. It may be more common in patients with a resected ileocecal valve. Colonic hypersecretion is also a common cause of diarrhea in the short bowel syndrome patient, resulting from increased levels of dehydroxylated bile acids and hydroxylated fatty acids associated with the spillage of bile acids and fat, respectively, into the colon, where bacteria metabolize them into their secretory solutes. Because the diarrhea in short bowel syndrome is complex, it is critical to evaluate its possible causes so that management is approached correctly.

Gastric hypersecretion

Gastric hypersecretion secondary to hypergastrinemia occurs within 24 hours after intestinal resection and can result in excessive fluid and electrolyte loss, peptic ulcer disease, and compromised intestinal absorption [129–131]. Absorptive compromise occurs as a consequence of reduced lipase activity (as a result of spillage of gastric acid into the duodenum and subsequent lowering of the duodenal pH), which leads to impaired micelle formation and reduced intralumenal lipid absorption. Gastric hypersecretion responds well to histamine receptor antagonists or proton pump inhibitors; furthermore, it usually abates with time. Acid suppression during the early post-resection phase significantly decreases stool volumes and electrolyte losses [132]. A continuous infusion of proton pump inhibitor is usually administered until the patient is tolerating an oral diet [133]. This beneficial effect of acid inhibition is no longer apparent with the initiation of oral intake [132]. The cause of gastric hypersecretion remains unclear because serum gastrin levels have been found to be both elevated [129] and unchanged [134] in patients with gastric hypersecretion following intestinal resection. Normal acid secretion also has been noted in patients with hypergastrinemia, and in some patients, hypochlorhydria develops after intestinal resection.

Steatorrhea

Several mechanisms are responsible for the steatorrhea of short bowel syndrome. Following surgical resection, gastric hypersecretion can alter the pH in the duodenum and inhibit the action of pancreatic enzymes, particularly lipase (see "Gastric hypersecretion" above). In the case of ileal resection and loss of the bile salt-reabsorbing region of the intestine, depletion of the bile salt pool can lead to insufficient micelle formation and steatorrhea (see "Site of intestine removed" above).

Carbohydrate malabsorption

After extensive resection, carbohydrates pass from the small intestine into the colon, where they are metabolized by bacteria into short-chain fatty acids. These short-chain fatty acids (butyric acid, propionic acid, acetic acid) cause diarrhea through two mechanisms (see "Mechanisms of intestinal adaptation" above). First they cause an osmotic diarrhea, and then they directly stimulate the colon to secrete fluid and electrolytes. Changes in the osmotic load and malabsorption of carbohydrates are probably the most important causes of diarrhea in short bowel syndrome. Increases in intestinal permeability and further carbohydrate and fluid malabsorption have been demonstrated in patients with short bowel syndrome who have episodes of sepsis [135], and these may

help explain the intermittent and rapid deterioration that sometimes occurs in patients with short bowel syndrome with seemingly minor infections.

In children with short bowel syndrome, the development of clinically significant D-lactic acidemia and D-lactic aciduria is well described and leads to increased serum levels of lactate, acidosis, compensatory hyperventilation, and neurological symptoms including encephalopathy [136]. The D-lactic acid is derived predominantly from malabsorbed fermentable carbohydrates that are delivered to the colon, where lactobacilli abundantly metabolize them to D-lactic acid. Treatment includes reduction in dietary carbohydrates, antibiotic therapy, and possibly probiotic administration [137].

Protein malabsorption

Protein is usually well tolerated as a caloric source in short bowel syndrome and contributes relatively little to the osmotic load. However, protein is metabolized by colonic bacteria in the same way as malabsorbed carbohydrates and can produce a similar osmotic type of diarrhea, although it is consumed in much smaller quantities than carbohydrates and so usually causes much less severe diarrhea.

Fluid and electrolyte malabsorption

Fluid and electrolyte losses can be enormous and are common during the first few weeks after intestinal resection. Daily fecal effluents larger than 5 L can be seen. It is during this time that prompt replacement therapy is important to prevent severe hypovolemia, hyponatremia, and hypocalcemia.

Vitamin and micronutrient deficiencies

Vitamin B-12 deficiency is likely after ileal resection because the recruitment of vitamin B-12-intrinsic factor receptors into the jejunum is extremely limited. In addition, colonic or small intestinal bacteria can metabolize vitamin B-12, further aggravating vitamin B-12 deficiency. Vitamin B-12 deficiency (as well as folate deficiency) leads to hyperhomocysteinemia and has been associated with venous thrombosis in patients with short bowel syndrome [138]. Other water-soluble vitamins are well absorbed throughout the entire small intestinal tract and are generally well preserved except in the shortest intestines [139,140].

Mineral deficiencies, including those of calcium and magnesium, are common. They occur secondary to the malabsorption of fatty acids, which form soap complexes with these divalent cations [139]. The administration of vitamin D and calcium supplements is usually helpful in correcting calcium deficiency, but magnesium deficiency may be more difficult to manage because the enteral administration of most magnesium salts results in osmotic diarrhea. Magnesium gluconate is less likely to cause diarrhea than is magnesium oxide or magnesium sulfate. Calcium depletion can be exacerbated by malabsorption of vitamin D. Although calcium deficiency normally results in an increased secretion of parathyroid hormone, in patients with concomitant calcium and magnesium depletion, the release of this hormone is decreased because of the magnesium deficiency [139].

Steatorrhea also is associated with the decreased absorption of fat-soluble vitamins, particularly vitamin D, but also vitamins A, K, and (rarely) E.

The absorption of major trace elements (chromium, copper), like that of water-soluble vitamins, is usually sufficient after intestinal resection [139,140]. Clinical zinc (12 mg lost/L small intestinal fluid and 16 mg lost /L stool) and selenium deficiency resulting from diarrhea can develop fairly rapidly and have been described to impair intestinal epithelialization and adaptation and wound healing.

Systemic manifestations

Renal

Renal dysfunction, both permanent and intermittent, can occur as a consequence of hypovolemia. This complication often develops soon after intestinal resection, but the fluid balance of some patients with short bowel syndrome is so precarious that short episodes of viral gastroenteritis can "tip" them into renal failure.

An increased incidence of renal calculi in association with ileal resection or ileal disease is well documented [141–144]. Hyperoxaluria is frequent in these patients, and the calculi are predominantly formed from calcium oxalate [144]. The hyperoxaluria appears to be secondary to an increased absorption of dietary oxalate, particularly in the colon. The increase involves two mechanisms. First, steatorrhea contributes to the increased absorption of oxalate through an increase in lumenal fatty acids, which preferentially bind to calcium, leaving free oxalate in the lumen that can readily be absorbed. Second, bile salts that enter the colon of these patients appear to increase the colonic mucosal permeability, thereby allowing increased oxalate absorption in the colon [143,144]. In addition to hyperoxaluria, other mechanisms may be responsible for increases in renal calcium oxalate and calculi [145]. These include a decreased urinary concentration of calcium-binding anions, such as phosphate and citrate, and diminished urine flow. Conjugated bile acid replacement therapy (9 g daily) with the aim of increasing fat absorption in the proximal small bowel reduced fecal fat excretion, urinary oxalate excretion, and oxalate nephrolithiasis in patients with the colon in continuity. This may represent a treatment option for patients with a history of oxalate stone formation [146].

Hepatobiliary

Patients with short bowel syndrome are at significant risk for cholelithiasis if the length of the intestinal remnant is less than 120 cm, if TPN is required, or if the terminal ileum is resected. The incidence of gallstones increases threefold after ileal resection [147,148]. Interruption of the enterohepatic circulation of bile and the subsequent bile salt malabsorption

lead to an increase in the hepatic synthesis of cholesterol; bile becomes supersaturated with cholesterol, and gallstones develop. A significant number of patients with ileostomies have calcium-containing radiopaque gallstones rather than radiolucent cholesterol gallstones [147]. Some other mechanism, therefore, must be involved. It also appears that interruption of the intrahepatic circulation predisposes humans to an increase in pigment stones [149–151]. With this understanding of the pathophysiology of gallstone formation, prophylactic cholecystectomy should be considered in all patients who have undergone extensive intestinal resection [152].

In addition to the increased risk for cholelithiasis, the incidence of intestinal failure-associated liver disease (intrahepatic cholestasis, hepatic steatosis, and hepatic dysfunction) is up to 65% in patients with short bowel syndrome on long-term TPN; these conditions are usually associated with parenteral nutrition therapy and sepsis [153,154]. Vascular factors may also play a role in hepatic dysfunction in these patients. In a neonatal piglet model, activation of stellate cells and subsequent hepatic fibrosis is preceded by a decrease in portal blood flow [155]. Hepatic fibrosis is not seen in animals with intestinal bypass and normal portal flow, implicating altered portal blood flow as one factor in the development of liver disease in short bowel syndrome.

Liver disease in the setting of short bowel syndrome is multifactorial (Table 53.3). Hepatic steatosis has been attributed to excess lipid content in TPN, and to essential fatty acid deficiency. Cholestasis is associated with prematurity and low birthweight in infants, and the extent of bowel resected in adults. It may be caused by lack of enteral intake and reduced gastrointestinal hormone secretion or by episodes of sepsis, including bacterial translocation across a permeable intestinal epithelium. Deficiency of amino acids such as taurine in TPN may play a role, as may excess TPN lipid infusion or toxicity from manganese infusion [154].

Increasing severity of cholestatic jaundice and portal hypertension in children not only carries its own risks, but also impairs intestinal adaptation and increases the duration of parenteral nutrition 2.5-fold [156]. Hepatic dysfunction, particularly in children with short bowel syndrome, results in liver failure, cirrhosis, and the need for liver transplantation. In fact, the presence of cholestasis was the strongest predictor of mortality in a large series of pediatric short bowel syndrome patients [25].

Table 53.3 Etiology of short bowel syndrome-associated liver disease

Duration of parenteral nutrition
Length of bowel remnant
Reduced enterohepatic circulation
Lack of enteral feeding
Recurrent sepsis
Protein undernutrition
Fatty acid deficiency
Excess dextrose and lipid emulsion (> 1 g/kg/day)

Treatment of intestinal failure-associated liver disease includes the introduction of enteral nutrition, reduction or cycling of TPN, control of bacterial overgrowth (with probiotics, fiber, or glutamine supplementation), and avoidance of septic complications with fastidious central line care. Oral ursodeoxycholic acid in doses of 15–20 mg/kg has also been recommended for cholestasis related to TPN. Nonetheless, liver disease may be an indication for small bowel transplantation, with or without concomitant liver transplantation [154].

Wound healing and infection

As a result of malabsorption of macro- and micronutrients, wound healing is impaired in short bowel syndrome. In animal models, rats with extensive intestinal resections show poor anastomotic collagen synthesis and reduced anastomotic strength resulting in dehiscence or stenosis [157]. Efforts to maximize nutrition in the perioperative period may decrease postoperative complications.

An increased risk for infectious complications, mainly caused by the egress of lumenal intestinal bacteria to distant organs – a process termed *bacterial translocation* – has been described in pediatric, but not adult, patients with short bowel syndrome [49,135]. In animal models of short bowel syndrome, rates of bacterial translocation were increased to an overall incidence rate of 87% as a consequence of intestinal bacterial overgrowth in the residual bowel. Loss of epithelial barrier integrity and bacterial translocation were positively correlated with removal of the ileocecal valve [158]. *Bifidobacterium lactis*, a probiotic bacterium, decreased the rates of bacterial translocation in a rat model by 43%, raising intriguing possibilities for human trials [159].

Quality of life

Not surprisingly, patients with short bowel syndrome report a lower health-related quality of life compared with healthy controls. Patients on chronic TPN rate their quality of life lower than those who are able to maintain enteral nutrition. This is further incentive to strive for complete weaning of TPN whenever possible.

Medical management

The nutritional and medical management of short bowel syndrome has been the subject of excellent reviews [160–165].

Nutritional management

The nutritional management of short bowel syndrome is a dynamic process that follows the evolution of the clinical and adapted state of the bowel. Depending on the length of

resection and the postsurgical adaptation, the process will result in one of four nutritional outcomes:

- maintenance of a balanced nutritional status on a normal or modified oral diet
- maintenance through the use of defined enteral formula diets
- maintenance through enteral intake with parenteral electrolyte and fluid supplementation
- maintenance through total or partial parenteral nutritional intake supplemented by variable amounts of enteral intake.

Whatever the source of nutrition, the caloric and protein intake should be increased slowly and progressively until a target is reached of about 100–125 kJ/kg (25–30 kcal/kg) and 1.0–1.5 g/kg of ideal bodyweight per day, respectively. This targeted intake, in general, will compensate for the increased losses that result from inefficient and inadequate absorption.

Parenteral therapy

In the immediate postoperative period, all patients who have undergone extensive small intestinal resection require total parenteral nutrition at 100–125 kJ/kg (25–30 kcal/kg) daily. Sodium losses in early secretions are often high (80–120 mmol/L), and electrolyte losses must be monitored carefully. In general, gastric losses are replaced 1:1 with 154 mmol/L sodium chloride; pancreatic and upper small bowel losses are replaced with 4:1 with 154 mmol/L sodium chloride, sodium lactate, or sodium acetate. In addition, 20 mmol/L potassium and 10 mmol/L of magnesium sulfate are added. Dextrose is provided at a rate of 5–7 mg/kg/min. Lipids are used to provide 20%–30% of calories. Protein is delivered at 1.0–1.5 g/kg daily.

As the amount of enteral nutrition is gradually increased, the duration and intensity of parenteral nutrition infusion can be reduced. Initially, parenteral nutrition can be stopped for one or two nights each week. Eventually, administration every other night or every third night is possible. This entire process of parenteral nutrition weaning may take years. If more than 25% of the patient's intestine remains, it should be possible to stop parenteral nutrition completely. Some patients, however, require parenteral therapy, particularly fluids and electrolytes, for extended periods. In a study of 135 patients with short bowel syndrome, small intestinal length (> 35 cm with an ileojejunal anastomosis), preservation of the colon, and an ileocolonic anastomosis were the features most predictive of the ability to wean from parenteral nutrition [166]. Serum levels of citrulline, an amino acid synthesized by the intestine, also predict successful weaning from parenteral therapy, with a sensitivity of 94% and specificity of 67% [167].

Oral therapy

A balanced solution containing carbohydrates and electrolytes can be given orally once stool output is less than 2 L daily. As previously noted, early oral feeding is important to intestinal adaptation. Clear fluid diets are not useful because their nutritional value is inadequate; they are also severely hyperosmolar and likely to provoke osmotic diarrhea (Table 53.4). Because the tight junctions of the jejunum are extremely permeable, supplements high in glucose and low in sodium often promote sodium and water losses and aggravate short bowel syndrome. To optimize oral fluid and electrolyte absorption, a balanced oral replacement solution is necessary. A solution that contains an isosmotic sodium and glucose mixture takes advantage of the small intestinal sodium–glucose cotransport carrier to enhance salt and water absorption. These solutions can be purchased ready-made (Table 53.5) or mixed at home (Table 53.6). The patient should sip the solution at frequent intervals throughout the day to prevent nausea and to avoid passing a large bolus of fluid rapidly through the short intestine [168]. The oral replacement solution also can be used instead of parenteral electrolytes at a later stage to reduce parenteral requirements. Patients with short bowel syndrome, in general, require an oral intake with a higher sodium concentration. Those with less than 100 cm of residual jejunum are usually net sodium secretors [169] and can achieve an optimal sodium balance with an intake of 120–160 mmol/L/day [168], whereas oral replacement solutions with lower sodium concentrations of 90 mmol/L do not maximize sodium balance [170]. Sports drinks like Gatorade are too low in sodium

Table 53.4 Composition of some common household beverages

Liquid	Glucose (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	HCO_3^- (mmol/L)	Osmolality (mOsm/kg)
Jello	888	20	0	0	1200
Apple juice	666	2	35	0	690
Ginger ale	333	3	3	0	540
Cola	590	2	0	0	600
Chicken broth	0	250	8	0	450
Tea	0	0	0	0	5

Note: Because of their nonphysiological osmolarity and inappropriate sodium and glucose concentrations, these beverages are inappropriate as oral replacement solutions.

Table 53.5 Commercially available oral replacement solutions

Solution	Glucose (mmol/L)	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Base (mmol/L)	Osmolarity (mmol/L)
World Health Organization	111	90	20	80	30 (bicarbonate)	331
Pedialyte	139	45	20	35	30 (citrate)	269
Gastrolyte	100	50	20	52	18 (bicarbonate)	240

Pedialyte, Abbott Laboratories, Columbus, OH. Gastrolyte, Sanofi-Aventis, Macquarie Park, NSW, Australia.

Table 53.6 An isosmotic oral replacement solution that can be mixed at home

Solution (amount)	Osmorality (mOsm/L)	Sodium (mmol/L)	Potassium (mmol/L)	Glucose (mmol/L)
Table salt (2.5 mL, i.e., ½ tsp)	91.2	45.6	0	0
Baking soda (5 mL, i.e., 1 tsp)	92.6	46.3	0	0
Light corn syrup (10 mL)	50.9	0.2	0.5	50.6
Frozen concentrate orange juice (150 mL)[a]	83.1	0.5	6.2	76.9
Water (1 L)	0	0	0	0
Total	317.8	92.2	6.3	127.5

a Dilute with three parts water by volume.

to be of use as oral replacement solutions in patients with short bowel syndrome. They are designed to replace fluid losses and prevent dehydration from perspiration, not intestinal efflux.

For patients with more than 60–80 cm of small bowel, oral feeding should be reintroduced slowly until a normal or modified oral diet is reached. Because gastric emptying is slower for solids, patients have been advised to consume dry solids at meals and take only isotonic fluids 1 h later. However, this regimen has not consistently been shown to improve the absorption of nutrients [171]. Diarrhea that occurs as a consequence of oral feeding usually can be managed with an antidiarrheal agent, which should be taken on a regular basis 1 hour before meals and snacks. Waiting to take an antidiarrheal agent until after a meal has been started and diarrhea has occurred is not effective (see "Management of diarrhea" below). Fluids sweetened with disaccharides such as fruit juices will exacerbate osmotic diarrhea and should be avoided. Care must be taken to limit the amount of lactose in the diet because the initial disease, gastric hypersecretion, or surgical therapy can cause lactose intolerance. It may be useful to test the lactose tolerance of patients to provide them with specific guidelines about lactose intake. The lactose tolerance of patients with short bowel syndrome and a jejunocolonic anastomosis is greater than that of patients with a terminal jejunostomy; the lactose provided in yogurt is generally better tolerated than the lactose in milk [172]. Nonetheless, most patients with short bowel syndrome are able to tolerate at least one cup of milk per day, and should not restrict this important source of dietary calcium unless lactose intolerance has been formally diagnosed [173,174].

Patients with significant ileal resection and an intact colon in whom steatorrhea is expected to develop may require monitoring of their oxalate secretions (see "Systemic manifestations" above). A low-oxalate diet is difficult to follow, but guidance can be provided regarding which foods are high in oxalate. Oral calcium decreases oxalate absorption because of the formation of insoluble salts. A 4-g dose of cholestyramine given three times daily reduces bile salt-induced diarrhea and also binds intralumenal oxalate, further decreasing oxalate absorption.

Attention to vitamin and mineral supplementation is critical. In general, a multiple-vitamin preparation with minerals will suffice (see "Vitamin and mineral supplementation" below). For patients with ileal resection, vitamin B-12 should be administered once monthly.

Traditionally, low-fat diets (often with medium-chain triglycerides) and high-carbohydrate diets have been used to treat patients with short bowel syndrome. This practice was based on an understanding that steatorrhea has many adverse effects on fluid absorption, that dietary fat induces the secretion of gastrointestinal hormones, and that carbohydrates malabsorbed in the small bowel undergo fermentation in the colon to short-chain fatty acids. These short-chain fatty acids are easily absorbed across the colonic mucosa, so that carbohydrate energy is salvaged that would otherwise have been lost in the feces. However, this practice has been challenged. Cross-over studies have demonstrated that high-fat diets were comparable with low-fat diets with regard to total fluid, energy, nitrogen, sodium, potassium, and divalent ion absorption and ileostomy output [175–178]. Emerging

evidence from animal studies indicates that low-fat diets impede intestinal adaptation and reduce weight gain through a mechanism related to decreased cell proliferation [179]. Management thus supports the use of high-fat diets as a caloric source in patients with short bowel syndrome.

Divalent cations, including calcium, magnesium, zinc, and copper, may bind to fatty acids in the stool, and excessive fecal losses of these minerals have been documented in patients with steatorrhea [177,180]. Steatorrhea also exacerbates malabsorption of the fat-soluble vitamins A, D, E, and K. Increased fecal losses of fat also enhance dietary oxalate absorption, oxaluria, and renal stone formation. Recommendations regarding dietary fat intake therefore must be balanced between caloric requirements and fecal loss.

Medium-chain triglycerides can be used as caloric supplements for patients with short bowel syndrome. Medium-chain triglycerides are hydrolyzed in the intestinal lumen to water-soluble components, which are absorbed in the absence of bile salts [181]. These medium-chain fatty acids do not require resynthesis in the enterocyte and can be absorbed directly into the portal circulation. Thus, medium-chain triglycerides may serve as a nutritional source for patients with significant long-chain triglyceride malabsorption. However, medium-chain triglycerides have an unpleasant taste, their osmotic load in the proximal small intestine sometimes causes diarrhea (in doses of more than 35 g/day), and they do not provide essential fatty acids. Furthermore, they have a slightly lower caloric density than long-chain fatty acids – 35 kJ/g (8.3 kcal/g) compared with 37.5 kJ/g (9.0 kcal/g).

High-fiber diets remain controversial. Their use is based on observations that some types of dietary fiber, such as wheat bran, increase intestinal transit times and fecal weight [182]. It is clear, however, that dietary fiber has variable effects on the intestine [183]. Soluble fibers such as pectin do delay gastric emptying and increase intestinal transit time [184–186]. However, they also bind bile salts and have a significant water-retaining capacity. Soluble fiber is not digested or absorbed and is passed into the colon, where bacterial fermentation produces short-chain fatty acids. In high concentrations, the short-chain fatty acids cause osmotic diarrhea and colonic secretion, but in low concentrations they are absorbed as an energy source, provide fuel for colonocytes, and help to heal and prevent inflammation.

Enteral therapy

Patients who cannot tolerate a normal oral diet and those with a short bowel (< 60–80 cm) often benefit from an enteral formula. If a chemically defined formula is used, it is important to control the rate of infusion to match osmotic inflow with osmolar absorption. Rates of full-strength infusion usually begin at 25 mL/h and gradually increase to 125 mL/h. The rate is modulated according to the tolerance displayed by the small intestine. If a polymeric formula is used, it should be lactose free because most patients with a massive small intestinal resection do not have an adequate reserve of lactase.

Elemental or semielemental diets require a minimal absorptive surface [125,187]. These diets may be useful during the early adaptive stage for persons with a short gut [188]; they also decrease the secretion of digestive juices [189] and gastric hypersecretion. Nevertheless, significant evidence suggests that elemental diets are not absorbed more readily than polymeric diets and have no additional beneficial effect on nitrogen balance or intestinal permeability [175,190–192]. Elemental or semielemental formulas contain amino acids in a free form as well as dipeptides and tripeptides. Because separate carrier mechanisms exist for free amino acids and dipeptides, a mixture of amino acids and dipeptides results in increased protein absorption [193–195]. Many enteral feeding formulations have a high content of carbohydrates in the form of sucrose or glucose polymers. These high levels lead to carbohydrate malabsorption and spillage of carbohydrates into the colon, with resultant osmotic diarrhea. Elemental formulas are low in fat, often providing the minimal amounts to meet essential fatty acid requirements. The formulas are effectively absorbed within the first 100 cm of jejunum [196] and stimulate adaptation of the proximal small intestine [197]. The unpleasant taste of elemental or semielemental formulas, however, often limits oral intake, and because they are hyperosmolar, they may considerably worsen diarrhea. The formulas therefore should be provided by slow, constant infusion.

Polymeric diet supplements generally provide about 30% of calories as fat and contain intact protein sources. They are more palatable than elemental diets; they are also less hyperosmolar and considerably less expensive.

Patients with short bowel syndrome absorb less than 100% of oral calories and protein. Many of them therefore need to consume calories and protein in excess of the normal estimated requirement [171]. Nevertheless, overfeeding must be avoided because it can induce a loss of endogenous fluid and electrolytes into the gut. Excess consumption of fats, fiber, concentrated sweets and sugar, milk and milk products, caffeine, and alcohol may increase normal outputs [198–201]. High enteral loads of carbohydrate should be avoided. Alterations of the bacterial flora in the colon, particularly overgrowth of *Lactobacillus acidophilus*, may result in the excessive production of D-lactate from unabsorbed carbohydrates, and severe metabolic acidosis and encephalopathy have been described [202–204].

Vitamin and mineral supplementation

In general, vitamin and mineral supplements are included in enteral and parenteral solutions. As patients are weaned from these solutions, and once they have stopped them completely, vitamin and mineral deficiencies may slowly develop because of inadequate intake (as patients try to prevent

Table 53.7 An oral vitamin and mineral supplement for use in selected patients with short bowel syndrome

Component	Dose regimen
ADEKs multiple vitamin	Four tablets per day, chewed or crushed thoroughly; each tablet contains 4000 IU of vitamin A, 3 mg of β-carotene, 400 IU of vitamin D, 150 IU of vitamin E, 0.15 mg of vitamin K, 60 mg of vitamin C, 0.2 mg of folic acid, 1.2 mg of thiamin, 1.3 mg of riboflavin, 10 mg of niacin, 1.5 mg of vitamin B-6, 12 μg of vitamin B-12, 50 μg of biotin, 10 mg of pantothenic acid, and 7.5 mg of zinc
Calcium gluconate liquid	500 mg p.o. twice daily = 448 mg of elemental calcium twice daily
Ferrous sulfate liquid	300 mg p.o. twice daily = 60 mg of elemental iron twice daily
Vitamin D-2 capsule	50 000 IU once per week
Phosphate-Novartis (tablet dissolved)	500 mg p.o. twice daily = 500 mg of elemental phosphorus twice daily
Potassium liquid	60 mL p.o. twice daily = 40 mEq of potassium chloride twice daily
Magnesium glucoheptonate liquid	30 mL p.o. twice daily = 150 mg of magnesium twice daily[a]
Zinc gluconate	15–20 mg per liter of output, or 50–100 mg/day
Vitamin B-12	200 μg s.c. monthly
Follow-up blood work (every 4–6 months): vitamins A, B-12, D, and E; prothrombin time and international normalized ratio; zinc, magnesium, phosphorus, calcium, albumin, red blood cell folate, iron, total iron-binding capacity, ferritin; complete blood cell count; electrolytes.	

Note: Exact doses will vary depending on the requirements of individual patients.
a Patients not tolerating oral magnesium will require intravenous magnesium therapy on a regular basis.

diarrhea), excess nutrient losses (from the short bowel), or a combination of both. Table 53.7 presents an example of a liquid vitamin and mineral oral supplementation regimen that could be considered for some patients as they are weaned from parenterally supplied vitamins and minerals. Liquid supplements are preferable to solid pills because hard outside matrices often do not dissolve during rapid transit through a short bowel.

Bile supplementation

Bile acid replacement with oral conjugated bile acids (2 g per meal in the form of ox bile or synthetic cholylsarcosine supplementation) did not worsen diarrhea in a patient with an ileostomy, and caused fat absorption to increase by 40 g/day, eventually leading to a significant weight gain [205]. This carefully conducted balance study suggests that conjugated bile acid replacement therapy can be considered for some patients with short bowel syndrome. Conjugated bile acid replacement therapy (9 g daily), with the aim of increasing fat absorption in the proximal small bowel, reduced fecal fat excretion, urinary oxalate excretion, and oxalate nephrolithiasis in patients with the colon in continuity. This may represent a treatment option for patients with a history of oxalate stone formation [146].

Glutamine supplementation

Six studies (four with glutamine alone and two with glutamine and growth hormone) of patients with short bowel syndrome provided glutamine supplementation (range 0.63–0.21 g/kg/day) by mouth or intravenously for 28–56 days and found no significant changes in the parameters assessed [93]. A workshop on intestinal failure, sponsored by the National Institutes of Health (NIH), concluded that "there are no current data in humans showing that glutamine availability is rate limiting for regulation of protein metabolism in acute illness" [93]. Although glutamine supplementation is theoretically attractive its general use will need to await larger controlled clinical trials.

Medication absorption

Medication absorption is often impaired in short bowel syndrome. The oral and enteral route for medication delivery should be used whenever possible to avoid additional manipulations of the central venous catheter. Most medications are absorbed in the proximal jejunum; however, the degree to which medication malabsorption occurs depends on the intestinal site removed and the rapidity of transit. Liquid or sublingual formulations will thus provide best pharmacokinetic absorptive results.

Management of diarrhea

In general, the cause of the diarrhea must be identified before appropriate treatment can begin (e.g., bile acid-induced diarrhea, steatorrhea, osmotic diarrhea, bacterial overgrowth; see Chapters 51 and 52). Nevertheless, in the absence of sufficient intestinal adaptation, debilitating diarrhea develops simply because the intestinal surface area is inadequate for absorption to take place, and antidiarrheal therapy becomes an integral part of the management of short bowel syndrome.

Opiates are the most widely used products in the treatment of diarrhea associated with short bowel syndrome. Clinically available opiates include natural substances, such

as paregoric and opium alkaloids, and synthetic preparations, such as codeine, diphenoxylate (Lomotil), and loperamide (Imodium). These agents relieve diarrhea by reducing intestinal secretion or by promoting intestinal absorption and reducing intestinal motility. For the diarrhea of short bowel syndrome, these agents should be taken on a regular basis 1 h before meals, as the liquid formulation when available: 30–150 mg of codeine daily, 7.5–20 mg of diphenoxylate daily, or 6–20 mg of loperamide daily. As-needed dosing, rather than regular dosing, often results in unnecessary diarrhea and loss of the nutrient value of a meal before the opiate has had time to exert a therapeutic effect.

α_2-Adrenergic agonists are potent stimulators of intestinal absorption. These agents stimulate sodium and chloride absorption and inhibit bicarbonate and chloride secretion in the colon and small intestine. In addition, α_2-adrenergic agonists reduce intestinal motility. A dose of clonidine, 0.1–0.2 mg, taken with each meal, can act as an adjunct to opiates, helping to prevent the difficult-to-control diarrhea of short bowel syndrome [206–208]. Despite the fact that clonidine has profound antidiarrheal effects in experimental conditions, its centrally mediated hypotensive and sedative effects limit its use as an antidiarrheal agent.

Octreotide, a long-acting somatostatin analogue, has been used to treat diarrhea of both neuroendocrine and nonneuroendocrine origin. The response in cases of short bowel syndrome-associated diarrhea has been variable, and although the diarrhea may respond initially, tachyphylaxis has been known to occur [200]. In animal models of massive intestinal resection, physiological and pharmacological doses of octreotide reduce intestinal secretion, but paradoxically impair intestinal growth and adaptation by reducing splanchnic protein synthesis and thus mucosal protein incorporation and villus growth rate [201,209]. This important negative growth effect has not been confirmed in humans, nevertheless, octreotide should be administered cautiously in short bowel syndrome and limited to patients with a high-output jejunostomy and significant fluid and electrolyte losses [173]. The standard initial therapy is 50–100 μg given subcutaneously over an 8–12-h period; titration is based on the clinical and biochemical effects, with the dose escalated by 50 μg per week up to a maximal dose of 200 μg. The dose can be increased in 100-μg increments from 200 to 500 μg every 8 h. Once the effectiveness of octreotide has been demonstrated in an individual patient, the patient can be switched to a comparable dose of the long-acting release depot octreotide preparation, which has similar beneficial effects of decreasing fecal output [210].

The adverse effects of octreotide are usually mild and depend on the duration of therapy. Short-term side effects include pain at the injection site and transient nausea and bloating. However, octreotide predisposes to gallstone formation through inhibitory effects on gallbladder contraction, which may pose a significant risk in this group of patients who are already prone to gallstone formation [173,211]. Lanreotide, a new somatostatin analogue, significantly prolongs duodenal-to-cecal transit time in healthy male patients; however, it concomitantly decreases carbohydrate and fat absorption and thus may worsen diarrhea. It is possible therefore that somatostatin analogues have mixed effects in the management of diarrhea associated with short bowel syndrome [212].

Recombinant human growth hormone therapy

The results of six clinical trials with recombinant growth hormone therapy have been variable, with three reporting negative results and three reporting positive results [213]. The effect of low-dose growth hormone (0.024 mg/kg daily) on body composition and absorptive capacity in patients with short bowel syndrome resulting from Crohn's disease were examined in a placebo-controlled cross-over clinical trial. Whereas bodyweight and lean body mass increased there was no effect on nutrient absorption, indicating that the increased body mass may reflect water retention as a side effect of growth hormone therapy [214]. A double-blind cross-over controlled study of low-dose growth hormone (0.05 mg/kg daily) in TPN-dependent short bowel syndrome patients taking an oral diet was positive and showed increased energy and macronutrient absorption [215]. The effect of growth hormone was seen only while patients were taking the active drug, indicating a possible need for long-term drug therapy.

The combination of growth hormone and oral glutamine supplementation with a high-carbohydrate, low-fat diet was also positive and superior to either agent alone [216]. In an uncontrolled study of eight patients on this regime, protein absorption increased by 40% and stool output decreased by 30%; of the patients, 40% were weaned from TPN within 1 year and another 40% were able to decrease their parenteral nutrition requirements when they followed the diet [216]. The same investigators, a decade later, compared oral glutamine (30 g/day), high-dose growth hormone (0.1 mg/kg/day) and combined glutamine and growth hormone therapy in a randomized controlled trial of patients requiring chronic parenteral nutrition for short bowel syndrome [217]. All patients received a high-protein, high-carbohydrate diet and were closely monitored in a hospital setting for 4 weeks. Patients receiving growth hormone injections had greater reductions in volume and frequency of parenteral nutrition administration than those receiving glutamine. Furthermore, patients receiving combined growth hormone and glutamine treatment had the most marked reductions in volume and frequency of parenteral nutrition. Unfortunately, no growth hormone-treated patient was able to stop parenteral nutrition and peripheral edema occurred in 94% of growth hormone-treated patients [217].

However, these results with growth hormone therapy could not be confirmed in two other trials [218]. In a randomized,

double-blinded, controlled trial, no benefit after 3 weeks of high-dose growth hormone (0.14 mg/kg/day) and glutamine therapy on intestinal morphology, stool losses, or macronutrient absorption could be demonstrated in patients with short bowel syndrome [219]. Similarly, in a randomized, double-blinded, placebo-controlled cross-over study, a combination of high-dose growth hormone (0.12 mg/kg/day) with oral (28 g/day) and parenteral (5.2 g/day) glutamine for 28 days did not improve intestinal absorption in patients with short bowel syndrome on their usual diet [218], nor did it affect body weight or composition [220].

The importance of a multidisciplinary approach when using growth hormone is demonstrated in the review by Wilmore [221] encompassing more than 300 patients with short bowel syndrome who received glutamine, growth hormone, and a dietary regimen optimized to enhance absorption. After 2 years of long-term follow-up, 40% of the group could be weaned completely from parenteral nutrition, and 40% were able to reduce their parenteral nutrition requirements.

A meta-analysis of 13 trials involving 258 patients examined the outcome of growth hormone in combination with glutamine supplementation and a high-carbohydrate, low-fat diet; it found a significant increase in weight, lean body mass, and carbohydrate and protein absorption [222]. This combination therapy also decreased stool output and decreased reliance on parenteral nutrition. The use of growth hormone alone was not assessed in the meta-analysis.

The cost-effectiveness of growth hormone was modeled in one study, and was found to be cost-effective compared to standard TPN over 2 years of treatment. However, the model assumed that 96% of patients receiving 4 weeks of growth hormone would reduce or eliminate their need for parenteral nutrition, which is optimistic [223]. Further studies are needed to determine whether the short-term cost of growth hormone is offset by a reduction in TPN costs.

Whether growth hormone treatment will yield better outcomes in pediatric than in adult patients with short bowel remains to be determined. Indeed, case reports have been published describing particularly impressive results in children using a combination of surgical lengthening and growth hormone administration [224]. These findings may imply different intestinal responses in children and adults to trophic growth hormones. Case reports of growth hormone administration, in combination with glutamine, to children who had short bowel syndrome for 6 years or more have shown continued benefit in terms of growth and weight gain [225]. In fact, a 4-week course of bowel rehabilitation therapy consisting of enteral nutrition, oral glutamine, and growth hormone administered within 2 years of the onset of short bowel syndrome resulted in 57% of patients discontinuing parenteral nutrition after 2 years of follow-up [226].

In summary, our understanding of the role of growth hormone in treating short bowel syndrome continues to evolve. The high-dose growth hormone studies have produced conflicting results and appear to have significant adverse events (edema, arthralgia, and carpal tunnel syndrome). The low-dose trials, metaanalysis, and pediatric results appear encouraging but need replication before growth hormone can become standard therapy. Nonetheless, growth hormone has received approval from the Food and Drug Administration (FDA) for use in short bowel syndrome.

Glucagon-like peptide 2 therapy

Glucagon-like peptide 2 (GLP-2) is an enteroendocrine peptide that is released in response to lumenal nutrients, has unique trophic actions in the gastrointestinal tract, and has been postulated as a mediator of intestinal adaptation. In animal models of short bowel syndrome, basal and postprandial levels of GLP-2 correlated with the magnitude of intestinal resection and nutrient malabsorption. Furthermore, the increase in crypt cell proliferation was associated with ongoing elevation in GLP-2 release [76,77]. These findings suggest that GLP-2 is important in initiating and maintaining the small intestinal adaptive response to resection. Using a rat model of short bowel syndrome, infusion of GLP-2 in addition to TPN was shown to cause increased intestinal villus height and crypt cell proliferation as well as reduced intestinal permeability and increased transport protein expression compared with animals receiving TPN alone [227]. Markers of apoptosis were not different, indicating that GLP-2 likely exerts its trophic effect by induction of cell proliferation rather than suppression of apoptosis. The pattern of response to GLP-2 differs in the jejunum compared with the ileum, with an increase in jejunal crypt apoptotic index and absence of upregulation of the Na^+-dependent glucose–galactose cotransporter SGLT1, and the fructose uniporter, GLUT-5 [228]. The clinical importance of this difference remains to be seen.

The use of GLP-2 in humans has been reviewed [229]. In a human clinical trial, 400 μg of GLP-2 was administered subcutaneously twice a day for 35 days to eight patients with short bowel syndrome. Treatment with GLP-2 improved the intestinal absorption of energy and nitrogen, increased bodyweight, decreased stomal output, and slowed the gastric, but not the small intestinal, emptying time [230]. A subsequent open-label trial administered teduglutide, a dipeptidyl peptidase IV-resistant GLP-2 analogue, to 16 short bowel syndrome patients for 21 days. Teduglutide decreased fecal wet weight and fecal energy excretion as well as increasing villus height, crypt depth, and mitotic index in small intestinal biopsies [231]. Although the clinical effects of GLP-2 analogues in these pilot studies were modest, they are encouraging and a controlled study is underway to determine the optimal dose and duration of therapy.

Epidermal growth factor therapy

The role of epidermal growth factor in epithelial proliferation makes it an attractive candidate for augmenting intestinal

adaptation in short bowel syndrome. Animal studies have been encouraging, showing less weight loss, increased carbohydrate absorption, and reduced intestinal permeability compared with placebo [232]. A pilot trial of enterally administered epidermal growth factor in five children with short bowel syndrome showed an increase in carbohydrate absorption and increased proportion of nutrition from enteral feeds [233].

Gene therapy

Preliminary animal research has demonstrated successful plasmid transfer of a sodium–glucose transporter into colonic epithelial cells to expand the absorptive capacity of remaining colon by nearly 200% [234]. This is an exciting possible therapeutic avenue but research in this area remains in its infancy.

Surgical management

Many of the surgical approaches require technical perfection and careful patient selection, and they have yet to be well tested in clinical trials; however, along with intestinal transplantation, they will likely provide the future foundation of short bowel syndrome therapy.

Techniques designed to slow transit time

Antiperistaltic segments act like physiological valves by slowing intestinal transit and altering myoelectric activity [235,236]. These reversed segments increase the absorption of water, nitrogen, and fat [237–239]. In animal studies, antiperistaltic segments seem to induce intestinal adaptation by increasing intestinal diameter both upstream and downstream from the anastomosis, as well as increasing villus height and crypt depth [240]. The treatment of more than 50 patients with short bowel syndrome by this method has been reported thus far, with good results in about 70% of them. The ideal length for a reversal segment is 10–15 cm of distal small bowel in an adult and about 3 cm in an infant [241–247]. Reversal may provide temporary relief while intestinal adaptation is taking place [248]. Patients with an extremely short intestine may not have the 10 cm of intestine required to fashion the reversal segment. Furthermore, small bowel obstruction at the antiperistaltic site is always a risk and may necessitate additional intestinal resection.

Peristaltic activity in the colon differs from that in the small intestine; it is slower and segmental and progresses by means of infrequent peristaltic rushes [249]. In dogs and rats, isoperistaltic interposition of a segment of colon between jejunal or ileal segments prolongs the intestinal transit time without causing obstruction [249,250]. The isoperistaltic colon is placed proximally and functions by allowing the slow delivery of chyme to the distal intestine. Despite slowed transit, however, the interposed colonic segment does not show evidence of small bowel intestinalization [251] and appears to have no significant effect on the absorption of nutrients [252]. In case reports, the length of the interposed colon, which does not appear to be critical, varied from 8 to 24 cm.

The colon also can be interposed in an antiperistaltic fashion. The antiperistaltic colon is placed distally. In one study, this procedure significantly improved the absorption of fat and xylose in monkeys [253]. Other authors, however, did not identify significant benefits to intestinal absorptive function [254–256].

Various techniques are used in the construction of valves to replace lost ileocecal valves. Nipple valves can be inserted at an ileostomy site [257]. In general, excision of the outer longitudinal muscle layer allows the inner circular muscular layer to contract without opposition, so that submucosal tunneling and the development of a nipple-like valve are possible [258,259]. Distal bowel also can be everted and telescoped into the proximal bowel [260]. These techniques of constructing an artificial valve do not modify the intestinal motor response to intestinal resection but simply create a low-grade mechanical obstruction [261]. In both experimental animal models and in humans with short bowel syndrome, such valves have reduced the number of evacuations and the rate of small bowel bacterial overgrowth [262–264]. However, despite a significantly prolonged transit time, the operation has not yet been shown to result in increases in absorptive surface area, functional absorptive capacity, or bodyweight [265].

In principle, recirculating loops should prolong the transit time by promoting repeated exposure of the intestinal mucosal surface area to lumenal contents. However, this procedure has been associated with high morbidity and mortality rates in experimental animals as a consequence of extensive bacterial overgrowth [266].

Retrograde electrical pacing is designed to slow and reverse the flow of intestinal contents, so that chyme pools and absorption is increased through prolonged mucosal contact. In dog jejunum, retrograde electrical pacing increased water, sucrose, and sodium absorption [267].

In animal studies, intrinsic myenteric denervation of the ileum, in an attempt to slow the forward flow of intestinal contents, led to dilation of the residual small and large intestine in rats that had been subjected to massive intestinal resection and resulted in an increase in absorptive surface area and postoperative weight gain [268].

Techniques designed to taper and lengthen intestine

Adaptive dilation of the small intestine causes ineffective peristalsis and stasis. Under normal circumstances the dilated segment is simply excised. However, in cases of short bowel syndrome excision is not often a valid option and in this circumstance remodeling is the treatment of choice. The diameter of the dilated intestine can be reduced through

intestinal plication or tapering enteroplasty. This simple and safe technique allows the mucosal surface to be preserved [269]. Ramanujam [270], using mongrel puppies, demonstrated that plication is a remodeling technique that is superior to tapering enteroplasty.

Bianchi [271] described a technique for lengthening the bowel of pigs by using the dilated segment, and has reviewed the human trials of this technique [272]. The intestine is tapered, and the redundant small intestine is used for lengthening purposes. Since the first clinical application, this has been the surgical technique most frequently used in attempts to reverse short bowel syndrome in children [273,274]. Although the intestinal length is increased, mucosal function may be compromised. In one study, sucrose transport was found to be decreased across the segment involved in the intestine lengthening [39]. In dogs with a 75% distal ileal resection, tapering and lengthening impaired nutritional status and intestinal absorption and adaptation. The changes in absorption and transit appeared to be related to altered intestinal transit rates, and the impaired adaptation was related to decreased enteroglucagon levels and increased somatostatin levels [275]. These findings are not surprising given that intestinal lengthening does not provide new mucosa. Any benefit is likely to be attributable to the mechanical effect of slowed intestinal transit.

In a series involving five human subjects, isoperistaltic tapering and lengthening (Bianchi procedure) resulted in improvement of intestinal function. At 1 and 6 months postoperatively, stool output was decreased, D-xylose absorption was increased, and fecal fat was decreased in comparison with preoperative measurements [276]. Bianchi reported that isoperistaltic tapering and lengthening resulted in survival off parenteral nutrition in 45% of 20 pediatric patients; however, successful weaning from parenteral nutrition was related to an initial intestinal length of more than 40 cm [277]. Similar results have been reported by separate German and American pediatric surgical groups [278,279]. The length of intestine after the Bianchi procedure seems to correlate with the potential for weaning from parenteral nutrition [280].

Georgeson and associates [281] combined several different surgical techniques. If the intestine was not sufficiently dilated, a nipple valve was constructed distally to provide partial small bowel obstruction and so induce dilation. Following this, the Bianchi technique was used to lengthen the intestine. In a small series of case reports, length was increased on average by a factor of 2.5 during 1 year; enteral intake averaged 66% of the total requirement, in comparison with less than 10% before the operation.

Serial transverse enteroplasty (STEP) is a lengthening procedure performed by stapling of dilated small intestine perpendicularly to the longitudinal axis of the bowel sequentially along the intestine. This creates a longer, zigzag-shaped bowel. In animals, this procedure increases intestinal length, and improves carbohydrate and fat absorption, as well as increased body weight [282]. A case series of five children who underwent the STEP procedure showed increased intestinal length and weight, and increased enteral intake in all the children, with discontinuation of parenteral nutrition in one patient [283]. In addition, one patient who had been listed for a combined liver and small bowel transplant due to severe TPN cholestasis had normalization of bilirubin levels after STEP. This procedure may also reduce small bowel bacterial overgrowth through improved intestinal motility, and was used to treat bacterial-induced refractory D-lactic acidosis in one case report [282,284]. A web-based registry of outcomes from the STEP procedure, the International STEP Data Registry (www.stepoperation.org), was initiated by the study authors to ascertain multicenter outcomes.

Intestinal regeneration takes place in full-thickness intestinal defects patched with a variety of substances, including small intestinal submucosa and prosthetic materials [285,286]. The new intestinal tissue, which develops by lateral growth, is functionally similar to normal intestinal mucosa [287].

Intestinal transplantation

As outcomes in intestinal transplantation continue to improve the role of this therapy is being clarified; for reviews see Fryer [288] and Abu-Elmagd [289].

Natural history of short bowel syndrome treated with parenteral nutrition

The long-term rates of survival and dependence on parenteral nutrition of adult [290] and pediatric [290] patients with short bowel syndrome have been reviewed. Rates of 5-year survival and dependence on parenteral nutrition in adults are 55% and 45%, and in children 73% and 75%, respectively. Survival is negatively related to end-enterostomy, ileocecal valve removal, colonic resection, small bowel length of less than 50 cm (adult) and 15 cm (children), advanced age, early persistent cholestatic jaundice, and arterial infarction as a cause of the short bowel syndrome [25,290–292]. In one small series, patients with less than 50 cm of small intestine without a colon had 100% mortality at 1 year due to multiorgan failure, compared with 50% mortality in patients with a colon and 50–100 cm of small intestine, indicating the need for earlier consideration of transplant in patients with extensive intestinal resections [293]. Dependence on parenteral nutrition is positively related to a small bowel length of less than 100 cm and to the absence of a terminal ileum, ileocecal valve, or colon. After 2 years of parenteral nutrition, the probability of a permanent need for parenteral nutrition is 95%. Children with short bowel syndrome may have slightly better outcomes, as series show 5-year survival rates of more than 70%, with 64%–77% of children free from parenteral nutrition [25,291].

Long-term home parenteral nutrition, although it can maintain normal nutritional parameters in 80% of patients, results in frequent hospitalizations, predominantly for catheter-related problems, a 30% rate of regular opiate use, and an inability to achieve complete rehabilitation [294]. The Oley foundation registry of home parenteral nutrition described hospital admission rates of 0.70–2.23 times per patient years on TPN, mostly due to septic complications, with a mortality rate ranging from 5% to 25% per year [295]. Liver disease related to TPN develops at a rate of 15% in patients receiving TPN for over 1 year, and carries a mortality of up to 100% within 2 years of diagnosis [296]. Given the risks inherent in any transplant procedure, less invasive therapeutic modalities must be exhausted prior to transplantation. This was demonstrated by a series of 13 children with short bowel syndrome who were referred for consideration for combined intestinal and liver transplantation due to TPN-related cholestatic liver disease. These patients showed significant reduction in bilirubin levels and TPN requirements through treatment at a multidisciplinary intestinal rehabilitation program with the goal of optimizing management through diet, antibiotic therapy, and selected nontransplant bowel lengthening surgery [297]. These complex patients are best managed by a dedicated multidisciplinary team approach.

Indications

Intestinal transplantation has become a life-saving treatment that can be considered for patients with irreversible intestinal failure who cannot be maintained on parenteral nutrition. Life-threatening complications of parenteral nutrition that warrant a consideration of intestinal transplantation include: parenteral nutrition-associated liver disease; recurrent sepsis; recurrent dehydration; and loss of central venous access. The indications are similar for children and adults. The fact that intestinal failure itself can lead to the development of profound neuropathological abnormalities in the brain may be a reason to consider earlier transplantation [298]. Patients with a high likelihood of progressing to intestinal transplantation will also benefit from early referral. These patients include those with no jejunoileum, patients with short bowel syndrome who are not able to wean from TPN and show progressive thrombocytopenia, and patients with deteriorating motility disorders – all have a high likelihood of lifelong TPN dependence and ultimate indications for intestinal transplantation [299]. Their care can be optimized at a referral center and appropriate timing for small intestinal transplant considered.

Also, although waiting times for intestinal transplant are comparable with those for other organs, the mortality on the waiting list is greater for patients awaiting intestinal transplant than for any other organ [173]. The waiting list mortality is largely due to patients requiring combined intestinal and liver transplant. Other factors prolonging the wait for intestinal transplant are the need for cytomegalovirus (CMV)-negative donors to decrease the risk of CMV enteritis, and the need for smaller donors due to restricted peritoneal cavity size in the short bowel syndrome patient [173]. Therefore, it is important that patients with life-threatening complications of intestinal failure and parenteral nutrition therapy be identified and referred for transplantation early [298,300,301]. Case reports of intestinal transplants from a living-related donor are available, and this may be one way to overcome the limitation of donor availability [302]. Because the survival rate of isolated intestinal transplants is higher than that of combined intestinal–liver transplants, patients should be considered for transplantation before bridging fibrosis and cirrhosis develop. Finally, an appreciation of the unique and specific nutritional needs of the recipient and the donor is important in maximizing survival [303].

Results

Intestinal transplantation is an evolving procedure with survival rates comparable, at some larger centers, with those for parenteral nutrition [304]. The Intestinal Transplant Registry (www.intestinaltransplant.org) provides updated data for graft and patient survival from intestinal transplant centers across the world. Sixty percent of the recipients are children or adolescents. Short bowel syndrome with a failure of maintenance on parenteral nutrition is the most common indication for transplantation [305]. The types of grafts are different in the adult and pediatric population. In pediatric recipients, 50% require combined intestine and liver transplantation, 37% require intestine alone, and 13% receive a multivisceral transplant, which includes portions of stomach, duodenum, or pancreas [306]. By contrast, in adults only 21% require combined intestine and liver transplantation, 55% require intestine alone, and 24% require multivisceral transplants.

A 2003 review of an international small intestinal transplant registry showed 1-year graft/patient survival rates of 65%/77% for small intestinal transplant, 59%/60% for combined small bowel and liver transplant, and 61%/66% for multivisceral transplant [306]. These results are similar for both adult and pediatric transplantations. The registry data also demonstrated that outcomes of intestinal transplantation have improved over the past decade. Indeed, one site has shown 1- and 3-year patient survival of 94%/73% for transplants between 1999 and 2003 compared with survivals of 77%/52% between 1991 and 1998 [307].

The single most important factor in both graft and patient survival is the number of transplants a center completes each year. Rates of intestinal transplantation are increasing as outcomes improve, from 11 cases annually worldwide in 1990 to 140 cases annually in 2003 [306]. However, the majority (75.5%) of intestinal transplants were performed in the United States, and 83% were performed at 10 highly specialized centers. In a retrospective review from a single center

performing a large number of intestinal transplants, the 1-year patient/graft survival was reported as 93%/71% [308]. Evidence of a threshold effect, where a certain volume of procedures is required for optimal outcomes, is lacking but centers with greater than 10 transplants have improved survival.

Increased graft and patient survival is also seen in patients waiting for transplant at home compared with hospitalized recipients, emphasizing the need for early referral for transplant assessment. Other predictors of a positive outcome are the use of induction immunosuppressive treatment with antiinterleukin-2 (anti-IL-2) or antibodies to T-lymphocytes and B-lymphocytes, and the use of maintenance immunosuppressive treatment with rapamycin and tacrolimus [306,309]. Anti-IL-2 antibody use increased 1-year survival from 33% to 62.5% in one series [310]. Combined liver and small intestinal transplant may improve survival of the intestinal graft and decrease acute rejection episodes [310]. The mechanism behind this effect is unclear. Donor type, donor organ irradiation, recipient diagnosis, and maintenance immunosuppression do not affect survival [306]. Approximately 80% of survivors of transplantation have been able to discontinue parenteral nutrition and resume oral nutrition, 10% have a partially functioning or nonfunctioning transplant in place, and 10% have had their transplant removed. Physical and psychosocial quality of life indicators as determined by the child recipient 1 year posttransplant show no difference from those of normal schoolchildren [311,312]. By contrast, parental proxy assessments perceived decreased general health and physical functioning [311].

Rejection remains the single most important factor leading to graft failure and removal, accounting for 60% of graft failure. The donor intestine carries a large lymphoid mass, the gut-associated lymphoid tissue (GALT), which expresses MHC class I and class II antigens. The immunogenicity of the intestine may contribute to the high rates of organ rejection traditionally associated with small intestinal transplantation [288]. However, advances in immunosuppression have significantly reduced rejection rates. Part of the challenge in managing these patients is maintaining the delicate balance between immunosuppression, which is necessary to prevent graft-versus-host disease and rejection, and immunosuppression-related adverse effects. The required degree of immunosuppression is associated with a high risk for lymphoproliferative disorders and intestine-derived sepsis. Rejection may also increase the risk of sepsis and subsequent multiorgan system failure through increased intestinal permeability leading to bacterial translocation, further complicating the issue of immunosuppression after intestinal transplantation. The incidence of lymphoproliferative disease after intestinal transplantation is 15%; it appears to be linked to Epstein–Barr virus (EBV) infection, OKT3 (anti-CD3) induction therapy, and corticosteroids [313]. Epstein–Barr virus-associated lymphomas are treated with reduction of immunosuppression and anti-CD20 monoclonal antibodies. The risk of organ rejection and infection necessitates a thorough posttransplant protocol. This may include enteral decontamination with antibiotics for the first posttransplant month, with weekly quantitative stool cultures, prophylactic ganciclovir, weekly polymerase chain reaction (PCR) for EBV, and frequent biopsies of the intestinal graft [310]. Additional reasons for graft failure are thrombosis, ischemia, and bleeding (25%), multiple-organ failure (10%), sepsis (6%), and lymphoma (1%).

Sepsis is the most frequent cause of death in this transplant population (46%) [306]. Up to 92% of intestinal transplant recipients show evidence of bacterial infection postoperatively, with an average of 2.6 episodes per patient [314]. The most common sources are catheter-related infection (29.5%), intraabdominal infection (16.5%), and respiratory infection (5.7%) [314]. Reduced rates of sepsis and improved survival rates have been described following living-related transplantation [315–317]. Investigations of bacterial translocation following intestinal transplantation have suggested that cold ischemia time, not rejection or the associated transplantation of the colon, influences bacterial translocation [318]. Animal studies show a decrease in bacterial translocation after intestinal transplant when nitric oxide donors and various apoptosis inhibitors are administered, but no human trials have examined this effect [319]. Additional causes of death are multiorgan failure (2.5%), respiratory failure (6.6%), lymphoma (6.2%), graft thrombosis (3.2%), technical problems (6.2%), and graft rejection (11.2%) [306].

Intestinal transplantation has gone from being an experimental procedure to one that is mainstream, albeit at highly selected sites. Nevertheless, it has not replaced parenteral nutrition for short bowel syndrome but instead should be reserved for the patient in whom parenteral nutrition has reached its limits, with life-threatening complications, loss of vascular access, or liver disease.

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C SMALL INTESTINE

54 Tumors of the small intestine

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Small intestinal tumors (tumors of the duodenum, jejunum, and ileum) are uncommon compared with those occurring elsewhere in the gastrointestinal tract, and fewer than 2% of malignant gastrointestinal tumors are derived from this organ [1–6]. Primary small intestinal tumors are derived from both epithelial and mesenchymal components of the small bowel (Table 54.1). Primary malignant tumors include adenocarcinomas, carcinoids, lymphomas, and sarcomas (i.e., gastrointestinal stromal cell tumors). Approximately 6100 new cases of primary small intestinal cancer occurred in the United States in 2008 (equally distributed between men and women), with 1100 estimated cancer deaths [1]. The diverse nature of small intestinal tumors makes it difficult to generalize about the group as a whole. Some tumors represent distinct entities, whereas others share overlapping features.

Descriptive epidemiology

The frequency of small bowel tumors varies among different populations [7]. Reviews of the incidence and natural history of small bowel tumors comprise small series of patients, and some may include both primary or secondary (e.g., lymphomas) tumors. Some series include periampullary tumors and tumors of the ampulla of Vater, whereas others do not [2]. The relative frequency of primary tumors ranges in the literature, with adenocarcinoma (24%–52%), malignant carcinoid (17%–41%), lymphoma (12%–29%), and sarcomas (11%–20%) the most frequent [2–6,8–14]. The pattern of distribution of tumors in the small intestine is dependent on histological type. In Western countries, adenocarcinomas are most commonly found in the duodenum. In the setting of Crohn's disease, adenocarcinomas may occur distally in the ileum. Carcinoids and primary lymphomas occur predominantly in the ileum and jejunum. Sarcomas are found more evenly distributed. Metastases to the small bowel from adenocarcinomas and sarcomas account for approximately 50% of all small bowel tumors.

An overall age-adjusted incidence of primary small bowel cancers of 1.4 per 100 000 was reported in a US population-based study [5]. Small but significant increases in the incidence of adenocarcinomas and carcinoids have been noted since the 1970s [15], with a slight male predominance. The incidence of small bowel cancers rises with age, beginning at 30 years, with a mean age of diagnosis of approximately 60 years [16,17]. Incidence rates for adenocarcinomas and malignant carcinoids are higher in African Americans than in whites (6.4% and 1.4% increase per year, respectively) [15].

Etiology and pathogenesis

Why are small bowel tumors rare?

Several hypotheses have been proposed to explain this phenomenon, but objective data are mostly lacking [17].

- Rapid transit time and liquid lumenal contents reduce physical damage and mucosal contact with putative carcinogens; moreover, the latter are detoxified by mucosal enzymes such as benzpyrene hydroxylase.
- Bacterial enzymes have been postulated to play a role in colonic carcinogenesis. When the bacterial flora is altered, as in bacterial overgrowth syndromes, small intestinal carcinomas develop with a higher frequency than expected. The small intestine, conversely, has low bacterial counts, with an absence of anaerobes under normal conditions.
- Stem cells located at the crypt base lie deeper in the small intestine than their counterparts in the colon, perhaps reducing contact with lumenal carcinogens. The rapid differentiation of stem cells into mature, nonproliferating enterocytes and goblet cells may also protect against factors that deregulate cell growth and promote cancer development.

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Table 54.1 Classification of small intestinal tumors[a]

Benign epithelial tumors
Brunner gland lesions[b]
Benign intestinal epithelial polyps
Adenomas
Hamartomas (Peutz–Jeghers syndrome, Cronkhite–Canada syndrome, juvenile polyposis, Cowden disease, Bannayan–Riley–Ruvalcaba syndrome)
Malignant epithelial lesions
Primary adenocarcinomas
Secondary carcinomas (metastases)
Carcinoid tumors (neuroendocrine tumors)
Lymphoproliferative disorders
B-cell:
Diffuse large cell lymphoma
Small noncleaved cell lymphoma
MALT cell lymphoma
Mantle cell lymphoma (multiple lymphomatous polyposis)
Immunoproliferative small intestinal disease
T-cell:
Enteropathy-associated T-cell lymphoma
Mesenchymal tumors[c]
Gastrointestinal stromal tumors (benign and malignant)
Fatty tumors (lipoma, liposarcoma)
Neural tumors (gut autonomic tumors, schwannomas, neurofibromas, ganglioneuromas, granular cell tumors)
Paragangliomas
Smooth muscle tumors (leiomyoma, leiomyosarcoma)
Vascular tumors (hemangioma, angiosarcoma, lymphangioma, Kaposi sarcoma)

a This is a partial list of tumors found in the small intestine. Whereas the overall incidence of small intestinal tumors is low, many different benign and malignant lesions have been described in this organ.
b It is unclear whether these lesions should be classified as hyperplasias, neoplasia, hamartomas, or adenomatous proliferations.
c Some mesenchymal tumors represent clear-cut diagnostic entities, whereas many are more difficult to classify into any specific cell lineage. The latter are designated gastrointestinal stromal tumors.
MALT, mucosa-associated lymphoid tissue.

- Based on preclinical data from murine models [18], apoptosis involving damaged cells and potentially tumorigenic clones differs between the small and large intestine.
- Lymphoid tissue in the lamina propria and Peyer patches of the ileum may provide immune surveillance against neoplastic cells through IgA-rich secretions.

Diet

The limited descriptive epidemiology of small intestinal tumors has not provided strong evidence for environmental influences on tumor formation, in contrast to studies of the large intestine. Weekly or more frequent consumption of red meat or salt-cured foods has been associated with a two- to threefold increase in risk of small intestinal cancer [19]. Another study [20] reported odds ratios (OR) for cigarette smoking of 4.6 for adenocarcinomas and 4.2 for carcinoids and an OR for alcohol consumption of 4.0 for adenocarcinomas and 3.1 for carcinoids. A population-based case–control study [21] observed a significant threefold increased risk of adenocarcinoma of the small bowel in heavy drinkers (≥ 80 g/day of ethanol) relative to moderate drinkers or nondrinkers. Frequent intake of foods rich in heterocyclic amines (fried bacon, ham, barbecued or smoked meat and fish) was associated with a significant four- to fivefold increased risk of adenocarcinoma in men, but not in women. There was also a consistent and significant trend in risk with increasing sugar intake, with an OR of 3.8 in the highest intake category. In a hospital-based case–control study [22], alcohol and tobacco did not increase the risk of adenocarcinoma of the small intestine. Risk was positively related to intake of bread, pasta, or rice (OR = 3.8), sugar (OR = 2.9), and red meat (OR = 4.6), and it was inversely related to coffee (OR = 0.4), fish (OR = 0.3), vegetables (OR = 0.3), and fruit (OR = 0.6).

Molecular biology and biochemical changes

In comparison with colorectal cancer, relatively little is known about the molecular genetic events associated with the evolution of small bowel tumors.

Cellular protooncogenes are evolutionarily conserved human genes that play a role in signal transduction and normal regulation of cell growth. Human *RAS* genes encode guanine nucleotide-binding proteins that regulate intracellular signaling pathways. Point mutations in *KRAS* (K-*ras*), especially at codon 12, have been reported in 14%–53% of primary small intestinal adenocarcinomas [23,24] but not in carcinoids. Mutations of *KRAS* were common in sporadic and Crohn's disease-associated adenocarcinomas of the small intestine [25]; *KRAS* mutations were present in all four sporadic carcinomas with contiguous adenomas, in 2 of 11 (18%) without adenomas, and in 4 of 7 (43%) of Crohn's disease-associated carcinomas.

Allelic losses involving tumor suppressor genes play major roles in the genesis of intestinal tumors. The *APC* gene on the long arm of chromosome 5 (5q21) is mutated in the germ line of patients with familial adenomatous polyposis (FAP), and somatic mutations of *APC* occur in 60%–80% of sporadic colorectal carcinomas and adenomas (see Chapters 000, 000). Mutation of the mouse homologue of *APC* (*Apc*) by chemical carcinogenesis or by genetic manipulation [26] results in the development of polyps in both the large and the small intestine. Small intestinal and periampullary adenomas and carcinomas are common in patients with FAP. Mutations in *APC* have also been reported in sporadic small intestinal adenocarcinoma [24], but they appear to occur at a much lower frequency than in sporadic colorectal cancer.

Deletions of chromosome 17p involve the *p53* tumor suppressor gene, whose product normally prevents cells with damaged DNA from progressing from the G1 phase to the S phase in the cell cycle and regulates apoptosis. Mutations in *p53* were found in 27% of small bowel adenocarcinomas in one series [24], whereas alterations in the p53 gene product and allelic loss of chromosome 17p were present in 47% of sporadic small bowel adenocarcinomas, 33% of contiguous adenomas, and 71% of Crohn's disease-associated carcinomas in a second series [25].

Candidate tumor suppressor genes on chromosome 18q include the *DCC* (deleted in colon cancer) gene and *DPC4* (*SMAD4*). The *DPC4* gene belongs to the SMAD gene family, which is involved in signal transduction pathways activated through the transforming growth factor β (TGF-β) family receptors. Loss of 18q has been reported in intestinal tumors from the Apc 1638N mouse model [26], but rarely in human small intestinal adenocarcinomas [25]. However, chromosome 18 deletions appear to be common events in classical midgut carcinoids of the small intestine [27,28].

Alterations in genes that help to maintain DNA fidelity during replication (mismatch repair, or MMR, genes) are characteristic of patients with hereditary nonpolyposis colorectal cancer (HNPCC). Mismatch repair gene mutations in *hMLH1* and *hMSH2* were present in 15 of 42 (36%) of HNPCC-associated small bowel carcinomas in one series [29]. Pathogenic germ-line mutations in genes including *MLH1, MSH2,* and *MSH6* were identified in 81% of individuals with HNPCC and small bowel tumors in another large series [30]; 89% of these cases had loss of MMR protein expression. DNA replication errors characterized by microsatellite instability were also reported in 13% of sporadic small bowel carcinomas [25].

Most gastrointestinal stromal cell tumors (GISTs) contain gain-of-function mutations in the c-*KIT* oncogene resulting in activation of the KIT tyrosine kinase [31]. Some tumors contain mutations in the platelet-derived growth factor α (PDGFRA) receptor tyrosine kinase. Activation of *KIT* is a crucial oncogenic mechanism in sporadic and familial GISTs. Mutations in *KIT* or *PDGFRA* are found in over 90% of adult GISTs, and predict response to therapy with tyrosine kinase inhibitors.

Risk factors and associated conditions

Numerous risk factors and associated conditions have been described with relation to tumors of the small intestine (Table 54.2).

Table 54.2 Conditions associated with an increased risk of primary small intestinal neoplasia

Adenocarcinoma
Familial adenomatous polyposis
Hereditary nonpolyposis colorectal cancer
Peutz–Jeghers syndrome
Juvenile polyposis syndrome
Crohn's disease
Previous history of cholecystectomy
Long-standing ileostomy (especially with Crohn's disease)
Ileal loop conduits, ileal pouches, ileal cystoplasty
Duplication cysts and Meckel diverticula
Gluten-sensitive enteropathy
Non-Hodgkin B-cell lymphoma
Immunoproliferative small intestinal disease
Gluten-sensitive enteropathy
Nodular lymphoid hyperplasia
Acquired immunodeficiency syndrome
Crohn's disease

Familial adenomatous polyposis (FAP)

Periampullary duodenal carcinoma is the most common extracolonic malignant tumor in patients with FAP. There were 11 cases of duodenal and ampullary adenocarcinoma reported among 1391 patients with FAP [32], representing a relative risk (RR) of duodenal adenocarcinoma of 331, and an RR of 124 for ampullary carcinoma compared with the general population. Mean ages of diagnosis were 53 years for duodenal carcinoma and 49 years for ampullary carcinoma. Duodenal adenomas are common in patients with FAP, and they have been reported in up to 90% of such patients.

Hereditary nonpolyposis colorectal cancer (HNPCC)

Hereditary nonpolyposis colorectal cancer is an inherited disease in which colon cancers arise in discrete adenomas, but polyposis (i.e., hundreds of polyps) does not occur (see Chapter 65). Forty-two members of 40 families with HNPCC developed 42 primary and 7 metachronous small bowel tumors [29]. There were 46 adenocarcinomas and 3 carcinoid tumors. The median age at diagnosis of the index small bowel tumor was 49 years. The small bowel was the first site of carcinoma in 24 (57%) patients. The relative risks of small bowel cancer in families that fulfilled the Amsterdam II and Bethesda criteria for HNPCC (see Chapter 65) were 36.1 and 5.75, respectively, in a report from a Swedish registry [33].

Hamartomatous polyposis syndromes

Hamartomatous polyposis syndromes are a group of disorders marked by the finding of multiple hamartomatous polyps in the gastrointestinal tract (see Chapter 64). The *Peutz–Jeghers syndrome* is an autosomal dominant disease, which in most families has been mapped to chromosome 19p13.3 and the *STK11* (serine threonine kinase 11) gene. Adenomatous changes have been reported in 3%–6% of hamartomas. Extracolonic cancers are common, occurring in

50%–90% of patients (small intestine, stomach, pancreas, esophagus, ovary, lung, uterus, breast). The RR for all cancers is 15.2 compared with the general population [34]. The small intestine represents the most frequent site of cancer development, with an RR of development of adenocarcinoma of 520 compared with the general population. Patients with Peutz–Jeghers syndrome have characteristic mucocutaneous pigment spots, which are frequent in the perioral region and across the vermilion border. Polyps are distinctive, with an arborizing pattern of growth of the muscularis mucosa extending into the branching fronds of the polyp. Patients often present with obstructive symptoms and may develop intussusception of the small bowel.

Juvenile polyposis syndrome is an autosomal dominant disease in which hamartomatous polyps may be limited to the colon, or to the stomach, or occur throughout the gastrointestinal tract. Mixed juvenile and adenomatous polyps have been reported. The RR for small intestinal cancer is unclear, but cancers of the duodenum have been reported. Patients may present with bleeding, intussusception, and obstruction. A life-threatening protein-losing enteropathy has been reported in some patients. Germ-line mutations in the *SMAD4* (chromosome 18q21.1) and *PTEN* (chromosome 10q23) genes have been reported in separate kindreds with juvenile polyposis syndrome [35].

Crohn's disease

An increased incidence of both small intestinal adenocarcinoma and lymphoma has been reported to occur in Crohn's disease. The RR of small bowel carcinoma in Crohn's disease (mostly ileal) has been estimated to be 60–100 times that of the general population [36,37]. A dysplasia–carcinoma sequence occurs in the small intestine in Crohn's disease similar to that of the colon and rectum [25,38]. Crohn's disease-associated small bowel carcinomas and dysplasias are accompanied by frequent genetic alterations in *KRAS* and *p53*.

Diversion of bile (previous cholecystectomy)

Diversion of bile to the lower small intestine either surgically or by feeding cholestyramine increases the yield of intestinal carcinomas in carcinogen-treated animals. Thus, cholecystectomy, which increases delivery of bile to the proximal bowel, could theoretically lead to an increased risk of small and large bowel cancer. Indeed, patients who underwent cholecystectomy had an increased risk of proximal intestinal adenocarcinoma that declined with increasing distance from the common bile duct [39]. The risk was significantly increased for adenocarcinoma of the small bowel (RR = 1.71) and the right-sided colon.

Gluten-sensitive enteropathy (celiac disease)

An increased risk of both small intestinal adenocarcinoma and non-Hodgkin lymphoma has been reported to be associated with celiac disease (gluten-sensitive enteropathy) [40,41] (see Chapter 50). The overall incidence of malignant disease in this condition has been reported to be 11%–13%, with an incidence of small bowel lymphomas of 4%–7%. In some reported cases, it is not clear whether the primary diagnosis is actually celiac sprue or whether the histological changes are secondary to lymphoma per se. Lymphomas in the setting of celiac disease are usually of T-cell origin (enteropathy-associated T-cell lymphomas, or EATLs). Adenocarcinomas may arise in focal areas of dysplasia [42].

Other conditions may carry an increased RR for small bowel neoplasia (see Table 54.2), but they are relatively rare. Patients with urinary diversion to the small bowel (ileal loop conduits) appear to have an increased risk of carcinoma. Adenocarcinomas arise in the mucosa of ileostomies and ileal pouches in patients with inflammatory bowel diseases and FAP [40], although this event is probably rare [43,44].

Clinical picture

The clinical signs and symptoms of small bowel neoplasms may have some common features, but for the most part they depend on the individual tumor type (Table 54.3). Abdominal pain may result from partial or complete small bowel obstruction, intussusception, or ischemia. Obstruction may be the result of the physical bulk of the tumor, infiltration, or annular constriction. An intense dermoplastic reaction by carcinoids causes intestinal buckling or kinking. Intussusception may occur with benign polypoid lesions (which account for half of adult cases of intussusception) and is common in patients with GISTs. Occult gastrointestinal blood loss is another common feature of benign and malignant small bowel tumors. However, bleeding may be acute and brisk in the case of ulcerated GISTs. Weight loss is especially common and pronounced in patients with small bowel lymphoma.

Adenocarcinoma

Epidemiology and risk factors

Adenocarcinoma is the most common primary malignant disease of the small intestine. The incidence of primary small bowel adenocarcinoma ranges between 3.0 and 6.5 per million, representing approximately 40% (range 24%–42%) of primary small bowel tumors in most population-based studies [17]. Small bowel adenocarcinoma accounts for approximately 2% of gastrointestinal tumors and 1% of gastrointestinal cancer deaths. Fifty-five percent of these tumors occur in the duodenum, 18% in the jejunum, 13% in the ileum, and 14% in nonspecified sites [2]. The overall 5-year survival is 30.5%, with a median survival of 19.7 months. A slight male predominance is usual. The incidence of small bowel adenocarcinoma is slightly higher in black people compared with white people [15].

Table 54.3 Clinical features of small bowel tumors

Symptom	Benign	Malignant
Abdominal pain	Most frequent symptom	Most frequent symptom: one-third of patients develop partial or complete obstruction
Intussusception	Most common cause of intussusception in adults, lipomas the leading cause	Rare
Occult blood loss	25%–50% of cases	Up to 50% of cases
Frank bleeding	Rare	Rare; GIST most likely tumor
Weight loss	Exceedingly rare	Up to 50% of cases, most severe with lymphoma
Palpable abdominal mass	Rare	40% of cases
Perforation	Exceedingly rare	About 10% of cases, almost all of which are lymphomas or GIST
Jaundice	Rare occurrence with benign periampullary tumor	Occurs with about 80% of malignant periampullary tumors
Flushing	Metastatic spread of carcinoid tumor required for carcinoid syndrome to occur	Occurs in a few cases of metastatic carcinoid tumor
Diarrhea	Exceedingly rare	Common with lymphoma and can occur with carcinoid syndrome

GIST, gastrointestinal stromal cell tumor.

Adenocarcinomas arise from adenomas or dysplastic changes of the small intestine (see "Pathology, natural history, and staging" below). Adenomas and adenocarcinomas of the small intestine, and especially of the duodenum and ampulla of Vater, are most frequently encountered in the setting of FAP. Untreated duodenal and ampullary adenomas in patients with FAP were followed in the context of an endoscopic surveillance program [45]. Duodenal polyps progressed in size in 26% (25 of 95), in number in 32% (34 of 106), and in histology in 11% (5 of 45). Morphology and histology of ampullary adenomas progressed in 14% (15 of 110) and 11% (12 of 105) of patients, respectively. One patient developed a periampullary carcinoma.

Pathology, natural history, and staging

Adenocarcinomas of the small intestine arise in discrete adenomatous polyps, in adenomatous changes involving the ampulla of Vater, or in dysplasia associated with inflammatory bowel disease. In hamartomatous polyposis syndromes, carcinomas may arise from discrete adenomas or mixed polyps containing adenomatous components [32]. Dysplasia is associated with familial juvenile polyps, but not with solitary, sporadic juvenile polyps. Dysplastic polyps contain markers suggesting a loss of proliferative control within the epithelium and mutations in *APC* [46].

Adenomas in the small intestine display the same gross and microscopic features as those in the large intestine. They may be pedunculated or sessile. Tubular adenomas tend to be small, whereas those with villous architecture tend to be larger. The presence of multiple duodenal adenomas or adenomatous changes in the ampulla of Vater suggests the diagnosis of FAP. Tubular adenomas contain tall, columnar, pseudostratified epithelial cells. Villous adenomas contain finger-like villous or papillary processes similar to those in the colon. Small intestinal adenomas exhibit a spectrum of dysplasia ranging from mild dysplasia to intramucosal carcinoma and invasive cancer. Glandular crowding ("back to back" glands), loss of epithelial polarity, an increased nuclear-to-cytoplasmic ratio, and increased mitoses characterize high-grade dysplasia. These changes, accompanied by invasion into the lamina propria, define *intramucosal carcinoma.*

Invasive carcinoma may be found in one-fourth to half of resected villous adenomas in the setting of FAP [46], but most duodenal adenomas appear to progress slowly to carcinoma [45,47], and the actual risk of malignant degeneration is probably considerably lower. Adenomatous polyps of the small intestine do occur outside the setting of FAP, but even less is known about their natural history.

Small intestinal adenocarcinomas may appear grossly as flat, stenosing, ulcerative, infiltrating, or polypoid lesions. Most are moderately differentiated tumors with gland formation and variable degrees of mucin secretion. Poorly differentiated tumors occur in approximately 20% of cases. The presence of *signet cells*, in which a large vacuole of mucin displaces the nucleus to one side, is a feature of some of these tumors. Rare adenosquamous carcinomas of the duodenum have been reported.

Adenocarcinomas are staged depending on the extent of bowel wall invasion, the presence or absence of invasion into adjacent structures, and the presence or absence of lymph node or distant metastases (Table 54.4) [48]. The prognosis of patients with adenocarcinomas of the small bowel is poor. In one report, the overall 5-year disease-specific survival in the United States was 30.5%, with a median survival of 19.7 months [2]. Factors significantly correlated with survival included age (poorer prognosis for patients > 75 years), tumor site (duodenum worse than jejunum or ileum), disease stage, and whether cancer-directed surgery was performed.

Table 54.4 Staging of small intestinal adenocarcinoma: American Joint Committee on Cancer (TNM classification)

Stage 0
Carcinoma in situ: TIS N0 M0
Stage I
Tumor invades lamina propria or submucosa: T1 M0 N0
Tumor invades muscularis propria: T2 N0 M0
Stage II
Tumor invades muscularis propria into the subserosa or into the nonperitonealized perimuscular tissue (mesentery or retroperitoneum[a]) with extension 2 cm or less: T3 N0 M0
Tumor perforates the visceral peritoneum or directly invades other organs or structures, includes other loops of small intestine, mesentery, or retroperitoneum more than 2 cm, and the abdominal wall by way of the serosa; for the duodenum only includes invasion of the pancreas: T4 N0 M0
Stage III
Any degree of bowel wall perforation with regional lymph node metastasis; any T N1 M0
Stage IV
Any degree of bowel wall perforation with or without regional lymph node metastasis, with distant metastasis

a The nonperitonealized perimuscular tissue is, for the jejeunum and ileum, part of the mesentery, and for the duodenum, in areas where serosa is lacking, it is part of the retroperitoneum.
T, tumor; N, node; M, metastasis.
From American Joint Committee on Cancer [48].

Five-year survival rates were 65% for stage I, 48% for stage II, 35% for stage III, and 4% for stage IV tumors. Similar trends have been reported from Europe [6,49,50]. Adenocarcinomas arising in the setting of HNPCC have a better prognosis than those occurring in FAP or in the general population [29]. Patients with tumors arising at or near the ampulla of Vater may do better than those with more distal tumors, perhaps because they become symptomatic earlier. Five-year survival rates approaching 50% have been reported for patients with node-negative cancers of the ampulla after radical resection.

Clinical picture

The mean age of presentation for adenocarcinomas is approximately 65 years, although the range of age at presentation is wide [2]. Less than 1% of tumors occur before the age of 30 years, and approximately 85% occur after the age of 50 years. Symptoms relate to tumor size, location, and blood supply. Small tumors are asymptomatic or may present with anemia secondary to chronic blood loss, but for the most part they are indolent and difficult to diagnose. Abdominal pain and other obstructive symptoms such as nausea and vomiting are common late symptoms as tumors obstruct from infiltration with lumenal narrowing or mass effect. Anorexia and weight loss are also common symptoms. Ileal tumors may present uncommonly with intussusception. Tumors located in the periampullary duodenum or ampulla of Vater may cause obstruction of the common bile duct, presenting with jaundice and other signs of biliary obstruction.

Diagnosis

Endoscopy (with forward and side viewing) may be used to examine the duodenum where a lesion is suspected or in families with FAP. Screening for duodenal adenomas and lesions of the ampulla of Vater, plus surveillance following endoscopic removal of adenomas, should be performed in persons exhibiting the phenotype of FAP or in family members in whom genetic testing is positive for mutations in the *APC* gene. Individual polyps may be examined by biopsy or, if possible, removed by snare cautery and the ampulla examined by biopsy as necessary. There is a high incidence of adenomatous change in the papilla, even if the appearance is normal. It has been suggested that the slow evolution of duodenal and papillary lesions may justify an endoscopic surveillance interval of 3 years for most untreated patients with FAP [43]. Upper endoscopic surveillance should begin at age 20 years, and biopsies should be taken of any suspicious lesions and of the papilla, even if normal appearing. Push enteroscopy is of use in identifying and biopsying small bowel lesions seen on small bowel barium studies or computed tomography (CT), in evaluating occult gastrointestinal bleeding, and in surveillance of patients with Peutz–Jeghers syndrome, in whom polyps occur throughout the gastrointestinal tract [51]. Capsule endoscopy has been demonstrated to be effective in diagnosing small bowel adenocarcinomas in patients with obscure gastrointestinal bleeding [52]. Colonoscopy with intubation of the terminal ileum may be helpful in examining the terminal ileum in patients with Crohn's disease or in biopsy of suspicious lesions suggested by radiographic procedures.

Small bowel follow-through is 70%–80% accurate for detection of duodenal lesions [53]. Accuracy can be improved by the use of hypotonic duodenography. Barium studies, however, are not as accurate in detecting more distal tumors. The tumor detection rate of enteroclysis (95%) is greater than that for small bowel follow-through (61%) [54]. The radiological appearances of small bowel adenocarcinomas are similar to those of the colon, including annular narrowing or stricture formation (Fig. 54.1) and filling defects comprising polypoid or ulcerated masses. Seventy percent of tumors in the duodenum are polypoid. Large duodenal lesions are ulcerative in 20% of cases. Adenocarcinomas of the jejunum are primary annular-constricting lesions (75%)

Figure 54.1 Annular constricting adenocarcinoma of the jejunum. This upper gastrointestinal barium study demonstrates an "apple-core" lesion (arrow).

Figure 54.2 Computed tomography scan demonstrating intussusception in the terminal ileum resulting from a small intestinal cancer (asterisk). Intussusception gives the impression of a soft tissue "mass" or "bowel within bowel" surrounded by contrast. Layers of serosal fat are seen in the periphery and centrally.

and may be partially ulcerated or fungating. Differentiation of the infiltrating form of adenocarcinoma from stenosing Crohn's disease may be difficult.

Increasingly, CT is used in demonstrating small bowel tumors and their complications, and is often preferable to small bowel follow-through [55]. Intussusception and obstruction may be associated with polypoid adenocarcinomas that are detectable on CT examination (Fig. 54.2), and CT can be helpful in guiding the surgeon to the source of obstruction. Endoscopic ultrasonography can also be used for diagnosing and staging small bowel tumors.

Therapy

Pedunculated adenomas of the duodenum may be removed by endoscopic snare polypectomy. It is often difficult to remove villous adenomas of the small intestine in this manner. These lesions are often sessile and contain foci of invasive carcinoma. Local mucosal resection can be performed surgically for some lesions, but wide excision is indicated for villous adenomas containing invasive cancer. Endoscopic resection of benign tumors of the duodenal papilla with and without intraductal growth using endomucosal rection (EMR) may be performed in some cases [56].

Surgical resection is the treatment of choice for adenocarcinoma of the small intestine. Resection of the tumor with adjacent mesentery and lymph nodes with a wide margin (≥ 10 cm) of normal bowel on either side of it is practical for adenocarcinomas of the jejunum. Malignant tumors of the terminal ileum are treated as cecal tumors with terminal ileal resection and right hemicolectomy. Villous adenomas of the duodenum containing invasive carcinoma and carcinomas of the ampulla of Vater usually require pancreatoduodenectomy (Whipple procedure) with curative intent. Disease-specific survival for primary small bowel adenocarcinomas confined to the small intestine (local disease) was 47%, for regional disease it was 33%, and in distant disease it was 3.9% [2].

The role of postoperative chemotherapy, either in the adjuvant setting or for unresectable lesions, has not been defined in patients with adenocarcinoma of the small intestine. Small series have claimed some benefit for 5-fluorouracil (5-FU) [57], and combination chemotherapy with 5-FU and other agents such as platinum compounds and irinotecan has been advocated for advanced disease [58]. Objective evidence of an impact on survival is lacking. Palliative radiation may have some role in controlling chronic blood loss associated with duodenal tumors, but it is difficult in more distal small bowel tumors because of a mobile mesentery and difficulty in localizing the target field. Chemoprevention with aspirin and other nonsteroidal antiinflammatory drugs (NSAIDs) and selective cyclooxygenase-2 (COX-2) inhibitors may be useful in the care of patients with FAP. Whereas several short-term studies have demonstrated the ability of these agents to reduce the size and number of colonic polyps in FAP, data concerning small intestinal tumors are scant [59].

Carcinoid tumors

Epidemiology

Carcinoid tumors, or argentaffinomas, belong to a family of rare neuroendocrine neoplasms known also as amine

precursor uptake and decarboxylation tumors. This entire family of neoplasms has in common the ability to secrete amines and polypeptides, which produce the characteristic clinical syndromes with which they sometimes present. Seventy-four percent of carcinoid tumors occur in the gastrointestinal tract [60]. Most appear in the appendix, followed in frequency by the small bowel and rectum. Most of the clinically significant carcinoid tumors are located in the small bowel, i.e., small bowel carcinoids. Most (87%) small bowel tumors are in the ileum, especially within 60 cm of the ileocecal valve. The incidence rate of small bowel carcinoids is estimated to be 1.2–6.5 cases per million persons [5,16,17,60–64]. Autopsy studies cite a tenfold higher rate of carcinoids that were not evident clinically [65]. The median age at diagnosis is 60 years, but carcinoids have been detected in a wide range of ages (22–84 years). In the United States, the incidence of small bowel carcinoids is somewhat higher in men and in African Americans [60].

No specific risk factors for carcinoids have been proven. Whereas the role of alcohol is controversial, cigarette smoking has been associated with an increased risk of developing small bowel carcinoids [21]. The incidence rates of carcinoids in first-degree relatives of patients with carcinoid tumors was higher than the expected population incidence rates [66]. The calculated cumulative probability that a first-degree relative will develop a carcinoid tumor by age 80 years was 1.5%. Persons with carcinoid tumors, particularly tumors of the small bowel, may have an increased incidence of coexisting noncarcinoid malignant diseases [60,65,67], although a lack of association has also been reported [64,66]. The noncarcinoid neoplasms are mostly adenocarcinomas of the gastrointestinal tract (50%), followed in frequency by lung, cervix, and prostate carcinomas [65].

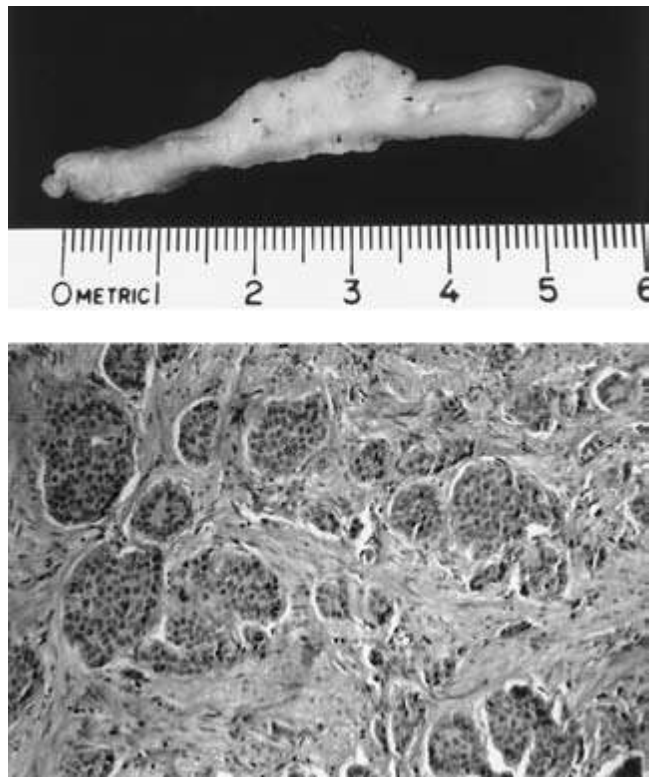

Figure 54.3 Carcinoid tumors of the small intestine. **(a)** A gross specimen of a nodular small intestinal carcinoid is demarcated by arrowheads. Carcinoid tumors develop deep in the mucosa and grow slowly, extending into the underlying submucosa and the overlying mucosa. **(b)** Histological section shows a small intestinal carcinoid tumor characterized by closely packed, round, regular, and monomorphous cell masses, buds, and islands. Lumena and rosette-like structures are also present. A desmoplastic reaction is responsible for many of the clinical findings of small intestinal carcinoid tumors.

Pathology, natural history, and staging

Carcinoid tumors are malignant, despite the indolent course associated with some tumors. Small bowel carcinoids, in particular, are associated with locoregional spread or metastases at the time of diagnosis. The tumors are usually intramucosal, and they rarely ulcerate to the lumen of the bowel. Small bowel carcinoids spread locally. When serosal breach has occurred, an intense local fibroblastic reaction is commonly seen [68,69]. The desmoplastic reaction is responsible for many of the clinical findings in patients with small bowel carcinoid. Another characteristic feature of small bowel carcinoids is multicentricity: at the time of diagnosis, 35% of patients will have more than one tumor in the small intestine [65,70]. Tumor size varies in several series, but most are 2 cm or less in diameter. Only 6% of small bowel carcinoids smaller than 1 cm have been associated with metastases, whereas 80% of patients with tumors larger than 2 cm will present with metastases [67–73]. Microscopically, sheets of uniform cells with hyperchromatic nuclei are arranged in characteristic clumps (Fig. 54.3). Light microscopy of metastases to the liver cannot differentiate a carcinoid from an islet cell tumor.

Carcinoid tumors can be classified in terms of the substances they secrete, and over 40 different secretory products have been identified [74,75]. "Typical" small bowel (midgut) carcinoids secrete serotonin (5-hydroxytryptamine, 5-HT), and they contain the enzyme dopa decarboxylase, which converts 5-hydroxytryptophan (5-HPT) to 5-HT. The 5-HT is further metabolized and is excreted in the urine as the metabolite 5-hydroxyindoleacetic acid (5-HIAA). "Atypical" carcinoids (mostly foregut and hindgut) lack dopa decarboxylase and do not secrete 5-HT [76]. 5-Hydroxytryptophan is released into the blood and can be measured in platelets and urine. Some 5-HPT is decarboxylated in the kidney before excretion, and 5-HT can thus be detected in the urine, but not 5-HIAA [77].

Carcinoid tumors do not have a specific staging system. Most authors describe small bowel carcinoids in terms of size, degree of bowel wall invasion, presence or absence of

lymph node involvement, and presence or absence of distant metastases.

The natural history of small bowel carcinoids is more favorable than that of adenocarcinomas of comparable stage, with overall 5-year survival rates of 60% [78]. However, the 25-year survival of patients with regional spread is reported to be less than 20% [65]. Patients with liver metastases and the carcinoid syndrome have a prognosis similar to that of patients with metastases who do not have the syndrome, but survival is lower for patients with carcinoid heart disease who develop valvular dysfunction [65,77].

Clinical picture

Patients with small bowel carcinoids can present with nonspecific gastrointestinal symptoms, small bowel obstruction, intestinal ischemia, intussusception, gastrointestinal hemorrhage, hepatomegaly, or symptoms of the carcinoid syndrome [79]. Many of the signs and symptoms of small bowel carcinoids result from the intense desmoplastic reaction of the mesentery in proximity to the tumor, leading to partial small bowel obstructions, weight loss, and intermittent crampy abdominal pain [80]. Approximately 50% of patients with metastatic carcinoid initially present with intestinal obstruction requiring surgery [81]. Nodal involvement causes compression of the main mesenteric arteries, leading to small bowel infarction [82]. Regional vascular thickening of medium-sized arteries has been described with carcinoid, and this may act synergistically in the development of bowel ischemia [83,84]. Gastrointestinal hemorrhage and intussusception are uncommon presentations of small bowel carcinoids [85]. Asymptomatic hepatomegaly from metastases is more common with carcinoids and islet cell tumors than with neoplasms of epithelial origin, so transaminases may not be abnormally elevated despite hepatomegaly and a large tumor burden [86].

The most common clinical situation is a prolonged history of nonspecific abdominal pain that did not much alter the patient's lifestyle. The median time from onset of symptoms to diagnosis is reported to be 2 years, and it may be as long as 20 years [65,87]. Investigation is not always sought by the patient, or has a low yield, thus prolonging the interval to diagnosis and treatment.

Patients with liver metastases from carcinoids may exhibit symptoms and signs of the *carcinoid syndrome*, caused by a variety of mediators. Overall, 10%–18% of patients with small bowel carcinoid tumors will present initially with the carcinoid syndrome [88–90]. Flushing of the face and neck occurs in most patients, and it may be episodic or permanent. Flushing is most commonly transient and simulates a mild sunburn involving the face, neck, and upper trunk (the *midgut flush* associated with ileal carcinoids). The so-called *foregut flush* is more intense, frequently associated with conjunctival injection and edema, and has a greater tendency to involve the body more diffusely [65]. Diarrhea is seen in 75% of patients and manifests as intermittent episodes of explosive, watery diarrhea resulting from intestinal hypermotility. Steatorrhea is rare. Abdominal cramping may accompany the diarrhea episodes about 50% of the time. Dyspnea can result from advanced carcinoid heart disease or, less frequently, from pulmonary fibrosis, bronchoconstriction, and asthma [91]. Two-thirds of patients with carcinoid syndrome will present with a large liver or an abdominal mass; 40% will have heart valve abnormalities that can be auscultated at presentation. Other less common signs include cyanosis (25%), peripheral edema, arthritis, and pellagra (2%–7%) [88,90].

Diagnosis

Carcinoid tumors of the small intestine frequently do not manifest clinically until regional spread has occurred or metastatic disease is present. Small, localized tumors are most often diagnosed incidentally during endoscopic or radiological examinations performed for unrelated reasons, or at the time of surgery for other abdominal disorders.

Biochemical markers

Urinary 5-HIAA is the most common test used to screen for carcinoid tumors. Most small bowel carcinoids secrete 5-HT, and urinary 5-HIAA is consequently high [92]. Unfortunately, it is not highly specific or sensitive for localized tumors because other neuroendocrine tumors can secrete 5-HT, and small carcinoids may secrete minute amounts of 5-HT. In contrast to localized tumors, urinary 5-HIAA is highly sensitive and specific for the carcinoid syndrome because of the large tumor burden necessary to produce the features of the syndrome [93]. Elevated levels of more than 30 mg of 5-HIAA in 24 h with provocative testing are diagnostic of the syndrome. False-positive 5-HIAA measurements can occur in diseases such as celiac disease, Whipple disease, and tropical sprue, and when 5-HT-rich foods such as walnuts, bananas, and avocado are ingested [94], but these levels are lower than those associated with carcinoid tumors. False-negative 5-HIAA determinations have been reported with the use of aspirin, levodopa, and phenothiazines. Overproduction of 5-HT can also be assessed by measuring platelet and urinary 5-HT levels, which are not affected by food or medications [26]. Urinary 5-HIAA, platelet 5-HT, and urinary 5-HT levels are elevated in all patients with small bowel carcinoids [76].

Chromogranin A is another useful biochemical marker of carcinoid tumors found in the serum. Chromogranin A is produced by most neuroendocrine tumors, and it has a high sensitivity for all types of neuroendocrine tumors. The sensitivity of this substance for carcinoid tumors approaches 80% [95]. It can be used to monitor treatment response and recurrence or for diagnostic purposes in patients who do not have elevated 5-HIAA urine measurements. Other biochemical markers such as bradykinin, substance P, neurotensin,

neuropeptide PP, neuropeptide K, and human chorionic gonadotropin (HCG), have been described; however, none is as specific or predictive as chromogranin A or 5-HIAA [96].

Radiological imaging

Conventional barium studies of the small intestine may identify the primary lesion as a smooth, semilunar filling defect in the lumen or, more often, as wall thickening, lumen angulation, and nonspecific mass effect. Enteroclysis is generally more successful than conventional small bowel barium studies. [55,97]. Computed tomography and magnetic resonance imaging (MRI) can provide initial localization of carcinoid tumors or their metastasis; however, their detection rates and sensitivities are lower than hormone-based imaging with indium and octreotide. Radiographic findings include mass lesions as well as calcification and fibrosis. Radiating strands of fibrosis and spiculation are characteristic. Mesenteric lymph node metastases are seen on CT in 91% [98,99]; CT scans are also useful to detect hepatic metastases but are somewhat less sensitive than MRI examinations [100]. Positron emission tomography (PET) may be useful in assessing the response of metastatic carcinoid to treatment, but it has not been shown to be greatly superior to CT or MRI in detecting tumors [99]. Scintigraphy has long been used for detecting neuroendocrine tumors. Radioiodine – ^{131}I- or ^{123}I-labeled metaiodobenzylguanidine (I-MIBG) – has a sensitivity of 55%–70% and a specificity of 95% [101,102]. Somatostatin receptors are expressed by 87% of carcinoid tumors and are therefore ideal for detection using radiolabeled somatostatin analogs. Indium-labeled (^{111}In) pentetreotide has a sensitivity of 80%–90% for detection of primary carcinoid and metastatic lesions [103–105]. [^{111}In]pentetreotide is more sensitive than [^{123}I]MIBG and conventional CT scanning [106]. Octreotide-labeled scintigraphy is useful for diagnosis, to predict who will respond to octreotide therapy and to locate tumors before surgical debulking [105,106]. Positron emission tomography with octreotide labeled with copper tetraazacyclotetradecane is as sensitive as indium-labeled pentetreotide scintigraphy for detecting metastatic carcinoids [107].

Endoscopy

Small bowel carcinoids have been detected endoscopically in the duodenum, proximal jejunum, and terminal ileum. The tumors generally appear as nodular, submucosal protuberances that are yellowish and shiny. Ulcerated lesions and pedunculated lesions have been reported as well [108]. Endoscopic biopsies of lesions are often unrevealing, because the tumors are frequently subepithelial. Snare polypectomy or endoscopic mucosal resection of the lesion is much more successful for obtaining a histological diagnosis [109]. Duodenal carcinoids as small as 2–3 mm [110] can also be diagnosed by endoscopic ultrasound. Small noninvasive lesions usually arise from the deep mucosa or submucosa, and at endosonography appear as moderately hypoechoic masses in the second or third echo layers [111–114]. Determination of invasion with endoscopic ultrasound can be useful for staging purposes [111], and to gauge the appropriateness of endoscopic resection as a therapeutic measure [113,114,115]. Capsule endoscopy has been shown to have utility in surveying the small intestine for carcinoid tumors and has superseded enteroscopy in this role [116].

Treatment

Localized tumors

Localized small bowel tumors should be resected completely [71,74,117]. Duodenal carcinoids are less likely to metastasize, and they can often be removed endoscopically, preferably after endosonographic verification that there is no deep tumor invasion. Pedunculated carcinoids can be removed by snare polypectomy, but subepithelial sessile growths will require endoscopic mucosal resection after submucosal injection of saline [109,115,118–120]. Duodenal carcinoids that show spread beyond the submucosa and carcinoids of the jejunum or ileum should undergo surgical resection. Surgery is effective for both local tumor manifestations (e.g., obstruction, bleeding) and symptoms caused by secretory agents. The 5-year survival of patients with local disease who undergo wide excision and removal of regional lymph nodes approaches 75% [121].

Tumors with regional spread

Small bowel carcinoids with regional spread should also undergo wide surgical resection [71,75,86,117]. In some case series, the 5-year survival of patients for whom careful excision of all peritoneal and nodal metastases was possible was 65%–71% [122,123], compared with a 5-year survival of 38% for patients in whom regional tumor spread could not be completely excised [123]. If residual tumor is present after surgery, long-acting somatostatin analogues have proven effective in symptom management [124].

Distant metastases

The approach to patients with metastatic small bowel carcinoids is multimodal [125]. The goals of therapy are to suppress the symptoms of carcinoid syndrome, to improve quality of life, and, if possible, to prolong survival.

Patients with symptomatic bowel obstruction can undergo segmental bowel resection or enteroenteric bypass and, if possible, surgical debulking of tumor deposits to decrease symptoms related to tumor burden [65,77]. Targeted scintigraphic therapies may be more successful when the tumor burden is small [126–128]. Wedge resection or lobectomy of liver metastases can reduce symptoms and possibly improve survival [129].

Hepatic artery occlusion (e.g., ligation, embolization) has been shown to decrease symptoms of carcinoid syndrome and produce tumor regression in 65% of patients [130]. Embolization combined with sequential chemotherapy has

been even more successful, with a reduction in tumor size noted in 78% of subjects [131]. The mortality from hepatic artery embolization or chemoembolization is 0%–2%. Common complications include fever, abdominal pain, nausea and vomiting, and transient transaminase elevations. Less commonly, myelosuppression, arterial thrombosis, liver abscess, and cardiac dysrhythmias may occur [132,133]. Cryosurgical debulking and radiofrequency ablation of hepatic metastases have been described, but have not been well studied [134].

Receptor-targeted therapy with [^{131}I]MIBG and [^{111}In]pentetreotide is variably successful for controlling the symptoms of metastatic carcinoid syndrome [75,135–137]. These therapies require patient isolation during treatment but have few adverse effects, produce some disease stabilization, and decrease octreotide requirements [138,139].

Symptom control is best achieved with octreotide, a somatostatin analogue that inhibits 5-HT release. Administered subcutaneously every 6–12 h, octreotide is effective in resolving symptoms of flushing and diarrhea in 70%–80% of patients. It decreases urinary 5-HIAA levels in about 70% of patients, and it may prevent further tumor growth in patients who respond symptomatically to octreotide [140]. The long-acting somatostatin analogue, lanreotide, can be injected once every 2–4 weeks, with comparable efficacy to that of daily administered octreotide [141,142]. Complications of prolonged high-dose octreotide therapy include steatorrhea, gallstone formation, and development of resistance to the medication.

The role of systemic cytotoxic chemotherapy (5-FU, doxorubicin, actinomycin D, streptozocin, dacarbazine) in the management of metastatic carcinoid is debated. Toxicity is significant and response rates have ranged between 10% and 40% [143–150]. At present, chemotherapy is reserved for patients who do not respond to the other treatment modalities described earlier.

A combination of chemotherapy and immunotherapy with interferon has been studied but has not been shown to be beneficial [151,152]. The addition of interferon to octreotide monotherapy may improve symptom control [153], or it may lead to stabilization of tumor progression [153]. Combination interferon therapy and hepatic chemoembolization has been shown to improve the median survival of patients treated with interferon alone [154].

Mesenchymal tumors

Of the mesenchymal small intestinal tumors, the most common variant is the *mesenchymal spindle cell tumor* (Table 54.1). Some mesenchymal tumors represent clear-cut diagnostic entities (e.g., lipoma, ganglioneuroma), whereas most are more difficult to classify into any specific cell lineage. Tumors may share overlapping features of several diagnostic entities, or they may be histologically heterogeneous. Thus, the general term *gastrointestinal stromal cell tumors* (GISTs) was coined to describe the group. Most GISTs have a spindle cell appearance, but electron microscopy and immunostains indicate that most GISTs are not true smooth muscle tumors [155,156]. It has been suggested that up to 94% of "smooth muscle" tumors in the older literature are actually not of muscle origin and would be classified today as GISTs [155,157].

Epidemiology

Approximately 60% of GISTs are found in the stomach, 20%–30% occur in the small bowel, and the remainder are scattered in the esophagus, colon, and mesentery [156]. Benign GISTs are three to four times more common than malignant GISTs [155,158,159]. Malignant GISTs are gut-specific sarcomas and represent 11%–12.7% of all small bowel malignant tumors, with an incidence of 1.2–1.5 cases per million persons [5,16,160]. Malignant GISTs are somewhat more common in male than in female patients, and they can be found in all ages, but the most common age range at the time of diagnosis is 50–59 years [17,161].

Kaposi sarcoma is thought to result from a herpes-type virus [162,163]. Small bowel schwannomas can be seen in patients with neurofibromatosis [164]. Small bowel ganglioneuromas have been reported in association with the multiple endocrine neoplasia type 2 syndrome [165].

Pathology

In the small bowel, GISTs occur most frequently in the jejunum, followed by the ileum, and then the duodenum. Benign GISTs are generally less than 5 cm in diameter, but tumors larger than 20 cm have been reported. Malignant GISTs are greater than 5 cm at time of diagnosis in 80% of patients [161,166,167]. In most cases, GISTs arise from the muscularis propria and generally grow extramurally, but benign GISTs of the duodenum are more likely to grow intralumenally or intramurally [161]. The histological appearance of GISTs differs from that of true leiomyomas, leiomyosarcomas, and schwannomas, with more cellularity and less cytoplasmic eosinophilia. Small bowel GISTs generally have a spindle cell-like appearance, but infrequently they can appear epithelioid [155]. Ultrastructural characteristics can be used to classify GISTs as myoid, neural, or ganglionic phenotypes, but the frequent presence of several different ultrastructural phenotypes suggests a common origin to all subtypes [157].

Ninety-four percent of GISTs express CD117, the product of the *KIT* protooncogene and a transmembrane receptor for the stem cell growth factor [168]; and 70%–80% of GISTs express CD34, the human progenitor cell antigen [169,170]. Less frequently, GISTs stain positive for actin and desmin (implying myogenic differentiation), but seldom are they S100 positive (a protein found in neuron-differentiated cells) [155]. True leiomyomas and leiomyosarcomas are positive

for actin and desmin, but do not express CD117 or CD34; and schwannomas express S100, but not CD117 or CD34. When spindle-cell tumors are excised, immunohistochemical markers should be used to determine whether the neoplasm is a GIST or not [156].

Observations that normal intestinal Cajal cells also express CD117 and CD34 suggest that GISTs are of the same embryonic origin as Cajal cells. This would explain the similar morphology and intramural location of GISTs and true smooth muscle tumors [155]. The *KIT* gene on chromosome 4 is most commonly mutated at exon 11, especially in malignant tumors [171,172]. Some malignant GISTs also display loss of 14q and chromosome 22 on comparative genomic hybridization [173]. These genetic changes are not found on true smooth muscle tumors.

Natural history and prognosis

Small bowel GISTs tend to invade locally, and they frequently present with peritoneal seeding or direct invasion to adjacent organs [161,167]. Lymph node metastases are uncommon, but 31%–41% of patients with small bowel GISTs will present with liver metastases.

Overall, 5-year survival rates are variable, ranging between 7% and 56% [161,167,174–176]. In general, survival chances are most favorable in patients without extralumenal tumor invasion or metastases and for tumors smaller than 2–5 cm.

Many factors have been suggested as prognostic indicators for GISTs (Table 54.5) [167,176–178]. Unfortunately, except for tumor size and the number of mitotic figures, most of these factors are not found consistently. The most useful indicators of survival and the risk of metastases are the size of the tumor at presentation, the mitotic index (the number of mitotic figures per 50 high-power fields), and histological evidence of tumor invasion into the lamina propria [156]. None of the other factors in Table 54.5 has consistently been shown to predict prognosis accurately. Classification of GISTs should not be in terms of "benign" or "malignant," but rather by the risk for malignant behavior.

Table 54.5 Gastrointestinal stromal cell tumors: prognostic factors[a]

	Risk of malignancy	
Factor	**Low risk**	**High risk**
Size	< 2 cm	> 5 cm
Mitosis	0–1 per 30–50/ high-power field	> 5 per 10–50/ high-power field
Cellularity	Low	High
Necrosis	Absent	Present
KIT exon 11 deletion	Absent	Present
Anatomic site	Rectum	Jejunum
Infiltration into lamina propria[b]	Absent	Present
Cystic spaces[b]	Absent	Present
Irregular borders[b]	Absent	Present

a Size and mitosis are the only factors consistently reported.
b Endoscopic ultrasound criteria.

Clinical picture and diagnosis

Most GISTs less than 5 cm in diameter are discovered incidentally at endoscopy, by barium radiography, or in gastrointestinal specimens resected for other reasons. The small tumors are generally asymptomatic. By contrast, at least 80% of patients with larger tumors will have symptoms related to the GIST [161,167,174,175,178]. More than 50% of patients with tumors greater than 5 cm will have either a palpable abdominal mass or gastrointestinal hemorrhage. Bleeding from ulcerated small bowel GISTs is usually acute, and it may be brisk [179]. Thirty to forty percent of patients with small bowel GISTs will present with abdominal pain, nausea and vomiting, or weight loss, and 40% of patients with ileal tumors may present with intussusception [161].

Small bowel barium studies can detect GISTs 53%–72% of the time [180]. Computed tomography has been reported to detect 89%–98% of large GISTs [181]. Angiography may also be useful because of the characteristic "tumor blush" noted in more than 85% of tumors.

Endoscopic diagnosis of small bowel GISTs is difficult because most tumors are submucosal. If ulceration is present, biopsies can identify the tumor as a GIST. On endoscopic ultrasound GISTs appear as hypoechoic masses arising from the fourth echo layer (muscularis propria). Putative endosonographic criteria to differentiate benign from malignant GISTs include tumor size greater than 4 cm, irregular extralumenal margins, and cystic spaces [182–184]. The positive predictive value for malignancy approaches 100% when any two of these findings are present.

Immunohistochemical staining should be performed on resected spindle-cell tumors and biopsies of tumors suspected to be GISTs. Positivity for CD117 confirms that the tumor is a GIST.

Response to targeted therapies (see "Treatment" below) may be monitored with CT or [^{18}F]deoxyglucose positron emission tomography (FDG-PET) [185].

Treatment

Segmental bowel resection, and not simple enucleation, is the treatment of choice for GISTs [157–161]. Aggressive resection of organ segments that have been invaded with tumor and of hepatic metastases appears to confer some improvement in survival [176,178,186–189]. Despite complete resection with negative margins, 44%–80% of patients will suffer local or peritoneal recurrence. Most recurrences will occur within 2 years, but lag times of 10 years have been reported [190,191].

Chemotherapy and radiation therapy have not been beneficial for patients with unresectable metastases or for patients with tumor recurrence [192,193]. Imatinib mesylate is an inhibitor of the KIT, ABL, BCR–ABL, and PDGFR tyrosine kinases. A growing body of evidence indicates that imatinib and several newer tyrosine kinase inhibitors that target KIT and PDGFR are effective in the control of metastatic GISTs [194,195]. The 1-year progression-free survival has increased from 10% with chemotherapy to 70% with imatinib. Specific mutations in the *KIT* gene may affect prognosis and response to targeted therapy [196,197]. Although the accepted treatment of small bowel GISTs larger than 4–5 cm is surgical resection, it is unclear what to do with smaller tumors. This gap in our knowledge is important because as the use of endosonography becomes more prevalent, more small GISTs will likely be diagnosed.

Lymphomas

Primary small bowel lymphoma (PSBL) is characterized by the following:

- absence of palpable peripheral lymphadenopathy
- normal peripheral leukocyte count and differential
- no mediastinal lymphadenopathy on a chest radiograph
- involvement of only the organs of the gastrointestinal tract and proximal regional lymph nodes
- no involvement of the liver or spleen, unless by direct extension from the primary gastrointestinal tumor [198–200].

Extranodal lymphomas of the intestine can be classified according to the cell lineage (Table 54.1) or according to their clinical characteristics. The latter method categorizes small bowel lymphomas into either PSBLs, which tend to be focal, or immunoproliferative small intestinal disease (IPSID), which is diffuse.

Epidemiology

Small bowel lymphomas represent 1%–10% of all extranodal lymphomas [198] and 7%–25% of all small bowel tumors [5,16]. Incidence rates of small bowel lymphomas range from 0.9 to 3.6 cases per million [5,16,63,198,201,202]. In the United States, small bowel lymphomas are more common in whites than African-Americans. Population registries from the Middle East suggest a higher incidence than in Europe or the United States [198] due to the predominance of IPSID. Studies from the United Kingdom [201], Germany [63], Denmark [202], Ireland [203], and the United States [17] show an increase in incidence of 30%–50% since the early 1970s. Primary small bowel lymphoma is typically diagnosed in the sixth aand seventh decades of life and is more common in men than in women, at a ratio of 1.5:1; IPSID, however, is generally diagnosed in the second or third decades of life and affects men and women equally [204].

Risk factors

Several diseases and immunodeficiency states are associated with an increased risk of developing PSBL. Patients with long-standing celiac disease have a 7% incidence of enteropathy-associated T-cell lymphoma (EATL) [205,206]. Nodular lymphoid hyperplasia is usually an incidental finding in the terminal ileum. Diffuse nodular lymphoid hyperplasia is seen in primary immunodeficiencies, and it is associated with intestinal lymphomas [207]. Rarely, diffuse nodular hyperplasia and intestinal lymphoma can occur in the absence of immunodeficiency [208]. Some authors report an increased incidence in patients with inflammatory bowel disease using immunosuppressive medications [209]; some report an increased risk only in male patients with Crohn's disease [210]; and others report no increased risk at all [211,212]. Small bowel lymphoma has been associated with congenital and acquired immunodeficiency states [213]. Patients with AIDS have a 30% risk of developing lymphomas, of which 10%–25% will be PSBLs [214]. Immunosuppression after solid organ transplantation has been associated with an increased risk of lymphoma. Most extranodal lymphomas are PSBLs. Risk seems to be correlated with the nature and dose of immunosuppression. For example, 30% of small bowel, heart, or lung recipients will develop lymphoma, whereas only 5% of kidney recipients will acquire the disease [215].

Immunoproliferative small intestinal disease is considered an infectious-pathogen-related lymphoma. Although the causative agent remains a subject of debate, molecular and immunohistochemical studies have suggested an association with *Campylobacter jejuni* [216]. The disease is most prevalent in the Middle East, Africa, Southeast Asia, and South America, regions in which poor hygiene and malnutrition are endemic. Malnutrition can impair the normally vigorous defenses of the bowel in a variety of ways [217]. In response to the abnormal antigenic presentation, specific clonal lines of IgA-secreting B cells proliferate in the lamina propria [158]. The α-heavy chains can be detected in the blood of up to 69% of patients with early-stage IPSID [218].

Pathology

The ileum is the site most frequently affected by PSBLs, followed by the jejunum and then the duodenum [5,16,63]. They are generally localized to one segment of the bowel, except in the case of *mantle cell lymphoma* (*multiple lymphoid polyposis*; MLP). The tumors have many different appearances. They may be large exophytic masses (Fig. 54.4a), polyp-like (Fig. 54.4b), ulcer-like, or nodular [219]. Tumor growth and extension are frequently intramural for a prolonged period. Involvement of regional lymph nodes is present in approximately 50% of patients with most types of PSBL, except low-grade *mucosa-associated lymphoid tissue (MALT) lymphomas,* in which lymph nodes are involved in only 30% of patients [219–222]. Immunoproliferative small

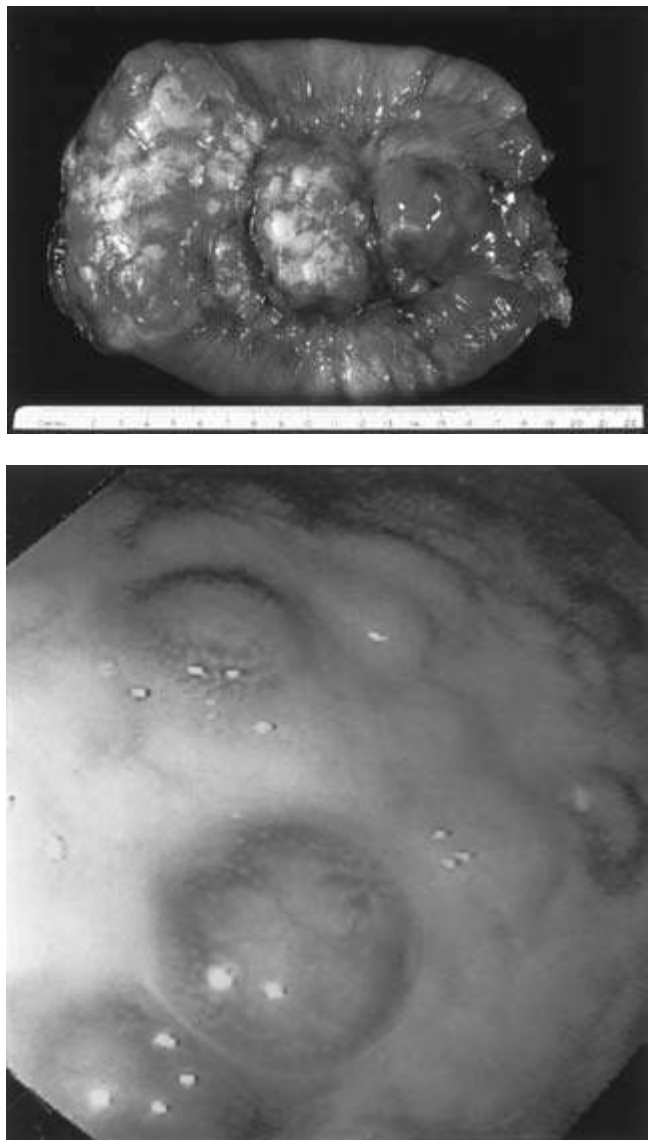

Figure 54.4 Primary small intestinal lymphomas. **(a)** A gross specimen of a primary lymphoma extensively involving the small intestine. **(b)** Mantle cell lymphomatous polyposis of the intestine seen endoscopically. The mucosa is studded with elevated polypoid nodules.

intestinal disease tends to be a diffuse jejunal disease. Gross findings can range from thickened folds to discrete masses [223,224] affecting a significant portion of the intestine in a contiguous fashion [225]. This feature probably explains the different clinical presentation of IPSID from PSBL, which usually has normal mural architecture in bowel segments not affected.

With the exception of EATL, most small bowel lymphomas are B-cell derived. The different histological patterns of small bowel lymphomas are summarized in Table 54.6. The diffuse large-cell type – the most common intestinal lymphoma – and the small noncleaved cell type are similar histologically to their nongut counterparts [205]. Mucosa-associated lymphoid tissue lymphomas occur in nongut sites, but they are most common in the gastrointestinal tract. The characteristic cells are centrocyte-like cells that resemble the small cleaved centrocyte cells of nodal follicular cell lymphomas [226]. Mantle cell lymphomas (MLP) show monotonous small cells with irregular nuclei arranged in a pattern similar to the mantle zone of lymph node follicles. The mucosa and submucosa are most often involved. Despite the lack of deep invasion, MLP is an aggressive lymphoma that presents frequently with distant lymph node and bone marrow involvement [222,227]. Enteropathy-associated T-cell lymphoma is a high-grade lymphoma composed of large cells resembling histiocytes. The adjacent mucosa displays the characteristic findings of celiac disease: villous atrophy, crypt hyperplasia, and abundant intraepithelial lymphocytes. Epithelial invasion is common, and the subsequent mucosal ulceration displays an intense inflammatory infiltrate [228]. The distinctive histological appearance of IPSID is characterized by a dense mucosal and submucosal cellular infiltrate throughout the length of the affected intestine. In the early phase, plasma cells and small lymphoid cells predominate. In the late phase, dysplastic changes in the small cells are noted, with an increase in the proportion of large cells evolving into a diffuse large cell lymphoma [223,224].

Staging and prognosis

Traditionally, a modified version of the Ann Arbor system was used for staging primary gastrointestinal (GI) lymphomas [229]. This system did not account for tumor histology, bulk, and other prognostic factors, and was replaced by a version of the Blackledge system (Table 54.7) [230].

Of the intestinal lymphomas, MLP, EATL, and IPSID (immunoblastic stage) tend to have the worst prognosis [231–235]. Patients presenting with stage I PSBLs or low-grade MALT PSBLs have median 5-year survivals of 50%–60%, and 44%–76%, respectively, and they have the best chance of cure [236]. The median survival of patients with stage II disease is only 20% [231–235].

In addition to the stage at presentation and the cell type, many criteria have been reported as being adverse prognostic factors for PSBL. These include a tumor larger than 7 cm, poor performance status, age greater than 70 years, the presence of B symptoms (fever, night sweats, and weight loss), mural invasion beyond the muscularis propria, distant lymph node involvement, unresectability of the tumor, elevated serum lactate dehydrogenase, elevated serum β_2-microglobulin, and the presence of *BCL-2* genomic alterations [231–235,237–242].

The clinical course of IPSID is different from that of other intestinal lymphomas and rarely involves extraabdominal sites. Hence, two different staging systems have been proposed for IPSID. One stages IPSID by the histological findings of prelymphomatous and lymphomatous stages [224], and the other stages the disease by the bulk of tumor and extent

Table 54.6 Features of the major gastrointestinal lymphomas

Type	Histology	Immunohistochemistry	Genotype
Diffuse large B-cell lymphoma	Diffuse growth of large lymphoma cells, often with necrosis	B markers (B1), $^{+}$sIG	Ig gene rearranged
Small noncleaved-cell lymphoma (includes Burkitt)	Medium-sized cells with many mitoses, "starry sky" appearance from histiocytes	B markers, $^{+}$sIG, CD10^{+}	Chromosomal translocation involving *MYC*
Low-grade B-cell MALT lymphoma	Centrocyte-like cells with formation of lymphoepithelial lesions	B markers, CD22^{+}, LN-1^{+}, CD5^{-}, CD10^{-}, CD23^{-}	Ig genes rearranged, trisomy 3 common
Multiple lymphoid polyposis (mantle cell lymphoma)	Monotonous small cells with irregular nuclei; epithelial invasion uncommon	B markers, CD5^{+}, CD23^{-}, cyclinD1^{+}, often IgM^{+}, IgD^{+}	t(11:114) translocation with implication of *bcl-1* oncogene
Enteropathy-associated T-cell lymphoma	Large cells with intense surrounding inflammation; adjacent atrophic mucosa	T-cell markers, CD103^{+}, HML-1, CD4^{-}, CD8^{-}	T-cell receptor genes rearranged
IPSID	Early: plasma cells, superficial Late: atypical large lymphoma cells with bowel wall invasion	B markers, KB61^{+}, α-heavy chain paraprotein	

CD, cluster of differentiation; HML-1, ; Ig, immunoglobulin; IPSID, immunoproliferative small intestinal disease; KB61, ; LN-1, ; MALT, mucosa-associated lymphoid tissue; sIG, secretory immunoglobulin.

Table 54.7 Staging system of primary gastrointestinal lymphoma

Stage I
Tumor confined to the gastrointestinal tract
Single primary lesion or multiple noncontiguous lesions

Stage II
Tumor extending in the abdomen from a primary gastrointestinal site
Nodal involvement
II1: local (paragastric or paraintestinal)
II2: distant (mesenteric, paraaortic, paracaval, pelvic, inguinal)

Stage IIIE
Penetration of serosa to involve adjacent organs or tissues

Stage IV
Disseminated extranodal involvement or a gastrointestinal tract lesion with supradiaphragmatic nodal involvement

of distant nodal and organ involvement [225]. Neither system has been validated in large survival cohorts.

Clinical picture and diagnosis

The symptoms of PSBL are usually nonspecific and may continue for 4–18 months before a diagnosis is rendered [231,233]. Abdominal pain, often described as crampy, is reported in 65%–87% of patients with PSBL, and weight loss is seen in approximately 50%. The remaining symptoms are present in fewer than 30% of patients and include gastrointestinal hemorrhage, malaise, night sweats and fatigue, or acute abdomen resulting from bleeding or perforation [225,231,232,234,235,239]. Small bowel obstruction is the presenting symptom in 5%–12% of patients. Diarrhea and malabsorption are common in EATL. In fact, patients with celiac disease who become unresponsive to a gluten-free diet should be investigated aggressively for small bowel lymphoma [228]. The most common physical finding is a palpable abdominal mass, present in 30%–50% of patients.

The symptoms of IPSID differ from those of PSBL. Nearly all patients with IPSID have diarrhea, weight loss, anorexia, and abdominal pain. Emesis and fever occur in 50% of patients. The physical examination in IPSID reveals clubbing of the fingers and ankle edema in 50%–75% of patients. Later in the disease, ascites, hepatomegaly, and a palpable mass may be present [223–225]. Up to 70% of patients will have the α-heavy chain paraprotein in the serum.

Small intestine barium studies, especially enteroclysis, are fairly accurate for detecting small bowel neoplasms [180]; CT is less sensitive for small lesions, but it detects most lesions larger than 5 cm. Small bowel lymphomas are categorized by CT as follows:

- primary, divided into circumferential and cavitary
- diffuse, usually EATL
- mesenteric [181].

Capsule endoscopy has been evaluated in patients with known small bowel lymphomas; however, diagnostic accuracy rates have not been determined [243]. Because of their location, most small bowel lymphomas require laparotomy for tissue diagnosis. Those located within the proximal 60 cm of the jejunum can usually be identified and sampled for biopsy at enteroscopy [108]. The accuracy of endoscopic

biopsy for diagnosing lymphomas is 82% on the first attempt, with an additional 10% on the second attempt [164]. Tissue should be sent both in formalin for standard studies and in saline for flow cytometry analysis. Characteristic findings at endosonography of an irregular hypoechoic mass disrupting the normal echo architecture may suggest the presence of a PSBL despite normal mucosal biopsies [112]. Unlike primary gastric lymphomas (especially MALT lymphoma), the role of endoscopic ultrasound in staging of duodenal lymphomas is not clear. Laparotomy for a diagnosis of lymphoma should be performed if the suspicion for IPSID is high despite normal endoscopic biopsies.

Patients with a confirmed diagnosis of small bowel lymphoma should undergo several studies for staging purposes, including a physical examination, indirect laryngoscopy to exclude involvement of Waldeyer ring, CT scan of the abdomen and chest to assess distant lymph node sites, bone marrow aspiration and biopsy, and lactate dehydrogenase and β_2-microglobulin determinations [215].

Treatment

The mainstay of treatment for PSBL is surgical resection. Complete resection with lymph node sampling is recommended to avoid a further operation [215]. Complete resection is associated with an improved survival compared with retained tumor after the surgical procedure [244,245], and may improve response to chemotherapy and radiotherapy [245–247].

Primary small bowel lymphoma that cannot be completely resected is usually treated by chemotherapy and sometimes with the addition of radiotherapy. The chemotherapeutic regimen usually includes anthracycline, but there is no consensus on the best combination of drugs [248–251]. Aggressive chemotherapy causes perforation of the small bowel in 5%–15% of patients. For some patients with diffuse MLP and EATL, chemotherapy is the only option, but response to chemotherapy is usually poor. The 5-year survival for patients with stage I and II MLP and EATL is approximately 20%–25% [219,233,234,252].

Immunoproliferative small intestinal disease is less amenable to surgical resection than PSBL because of the diffuse nature of the tumor and the low performance status of patients at the time of presentation. Nutritional support with total parenteral nutrition is essential, because malnutrition is severe and likely will not improve unless significant tumor regression is possible. Patients with IPSID in the prelymphomatous stage can be offered a trial of tetracycline or metronidazole for 6–24 months. Complete remission rates of 33%–70% have been reported [204]. Patients who do not respond to antibiotics or patients already in the lymphomatous stage may benefit from anthracycline-based chemotherapy [253]. A few patients with resectable stage I and II disease have a 5-year survival of 40%–47%, whereas most other patients whose tumors are unresectable have a 5-year survival of only 0%–25% [254]. A complete response rate of 62%–69% and a 3.5-year survival rate of 58%–70% have been reported in small groups of patients receiving anthracycline-based combination chemotherapy with or without tetracycline [255–257].

Secondary tumors

Metastatic tumors represent the most common tumors involving the small intestine in many series. Grossly, secondary tumors often present as submucosal nodules or plaques, and they may grow to form intramural masses that cause obstruction, intussusception, or perforation. Often, tumors present as stenotic lesions or infiltrative lesions simulating Crohn's disease. Metastases to the small intestine from melanoma and from carcinomas of the lung, testes, adrenal, ovary, stomach, large intestine, uterus, cervix, liver, and kidney have all been reported. Metastatic melanoma accounts for one-third of small bowel metastases [258]. Lesions are typically multiple and present as polypoid lesions that may cause obstruction or intussusception. Tumors of the pancreas, stomach, colon, or mesentery may also involve the small bowel by contiguous spread.

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C SMALL INTESTINE

55 Miscellaneous diseases of the small intestine

Marc S. Levin, C. Prakash Gyawali

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Drug-induced small bowel disease, 1352
Necrotizing enterocolitis, 1356
Protein-losing gastroenteropathy, 1357

The diseases discussed in this chapter should be considered in patients presenting with gastrointestinal hemorrhage, abdominal pain, diarrhea, intestinal obstruction, or perforation that eludes diagnosis by routine gastroduodenal endoscopy and radiographic studies. Topics include ulceration of the small intestine, small intestinal complications of drug therapy, necrotizing enterocolitis (NEC), and protein-losing gastroenteropathy (PLGE). Although most of these entities are infrequently encountered in clinical practice, differentiating them from diseases that are more common can yield important therapeutic benefits.

Advances in diagnostic techniques over the past few years have allowed improved visualization of the small bowel mucosa, leading to more frequent identification of small bowel pathology. Push enteroscopy is a useful adjunct for the diagnosis of small intestine lesions because of improvements in equipment and technique [1–6]. With the advent of wireless capsule endoscopy, visual images can be captured from the entire small bowel, although localization of abnormal findings is not always accurate [7–13]. Wireless capsule endoscopy is superior to push enteroscopy and enteroclysis for the detection of small intestinal ulcers in that it allows evaluation of almost the entire small bowel mucosa [12–16]. When findings on wireless capsule endoscopy need further evaluation, double-balloon enteroscopy is a novel endoscopic procedure that may allow endoscopic inspection or intervention within the small bowel [17–21]. Preliminary results indicate a new diagnosis can be made with double-balloon enteroscopy in as many as two-thirds of patients with undiagnosed small bowel disorders including obscure gastrointestinal (GI) bleeding [21–23]. Intraoperative enteroscopy with laparotomy or laparoscopy also can be a valuable adjunct in difficult cases, or when findings on other tests suggest the need for resection of a diseased small bowel segment [24–26].

Advances in radiological imaging techniques have also provided greater detail in evaluating the small bowel. Enteroclysis, involving instillation of contrast agent into the duodenum through a nasoduodenal tube, has a better sensitivity and specificity for lumenal small bowel abnormalities compared with oral small bowel follow-through imaging [27]. Multidetector row computed tomography (CT) enteroclysis demonstrates an even higher degree of diagnostic accuracy in characterizing lumenal, mucosal, intramural, and extramural abnormalities in small bowel inflammatory diseases [28–30]. Magnetic resonance enteroclysis is another emerging imaging technique, and has been mostly studied in the evaluation of small bowel Crohn's disease. Using this imaging modality, deep ulcers and wall thickening can be easily identified in the small bowel in patients with active Crohn's disease, suggesting that other small bowel mucosal inflammatory or neoplastic lesions can be similarly identified [31–33].

Ulcers of the small intestine

Many causes of small intestine ulcerations distal to the duodenum have been identified (Table 55.1). Most of these produce solitary ulcers with sharp borders and normal surrounding mucosa. With surgical resection or removal of offending agents, these ulcers seldom recur and are associated with a benign clinical course. The mortality from multiple ulcers occurring in an abnormal mucosa, as is occasionally seen with gluten-sensitive enteropathy (GSE) or unclassified sprue, chronic ulcerative (nongranulomatous) jejunoileitis (CUJ), or lymphoma, can be as high as 75% [34]. Attempts to define the underlying cause of small intestine ulcers are therefore crucial for their management.

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Table 55.1 Causes of small intestine ulceration

Category	Causes
Infectious	Tuberculosis, typhoid, cytomegalovirus, syphilis, parasites, strongyloidosis hyperinfection, *Campylobacter*, yersiniosis
Toxic	Acute jejunitis (β-toxin-producing *Clostridium perfringens*), arsenic
Inflammatory	Crohn's disease, systemic lupus erythematosus with high serum antiphospholipid levels, diverticulitis
Mucosal	Gluten-sensitive enteropathy (jejunoileitis)
Neoplastic	Malignant histiocytosis, lymphoma (primary), adenocarcinoma, melanoma, Kaposi sarcoma (secondary)
Vascular	Mesenteric insufficiency, giant cell arteritis, vasculitis, vascular abnormality, amyloidosis (ischemic lesion)
Hyperacidic	Zollinger–Ellison syndrome, Meckel diverticulum, stomal ulceration
Metabolic	Uremia
Drug-related	Potassium chloride, nonsteroidal antiinflammatory drugs, antimetabolites
Radiation-induced	Therapeutic, accidental
Idiopathic	Primary ulcer, Behçet syndrome

Primary (idiopathic) small bowel ulcers

Epidemiology and etiology

The diagnosis of primary small bowel ulcers is made after identifiable causes of small bowel ulcers have been eliminated and thus is unlikely to represent a distinct etiological entity. With increasing recognition of secondary causes of small intestine ulceration, fewer patients fall into this diagnostic category. The incidence of idiopathic small bowel ulceration is unknown. A review based on 59 patients (6 were taking enteric potassium preparations) found an incidence of four small bowel ulcers per 100 000 new patients [35]. Men and women were affected equally, and all age groups were affected (Table 55.2) [35–40]. These data suggest that primary small bowel ulceration is an uncommon disorder. However, because asymptomatic and uncomplicated intestinal ulcers are rarely detected, the true incidence of this entity may be higher than estimates.

Clinical features

Symptomatic complications of small bowel ulcers include bleeding, perforation, and obstruction. The incidence of these complications is not known. Intestinal obstruction appears to occur more frequently than with duodenal ulcers. Intermittent crampy abdominal pain resulting from partial small bowel obstruction is the most frequent presenting complaint (63%–100% of patients; see Table 55.2). Symptoms related to intermittent partial small bowel obstruction have been reported for periods ranging from 3 days to 20 years preceding

Table 55.2 Profile and presenting symptoms of primary small bowel ulcers

Study and year	Number of patients	Gender (male to female) ratio	Age	Small bowel obstruction (%)	Bleeding (%)	Acute abdomen (%)	Duration of symptoms
Davies and Brightmore, 1970 [36]	8[a]	5:3	16–62 years	63	38	25	No data
Boydstun et al., 1981 [35]	59[b]	31:28	17–77 years	78	25	12	3 days–20 years
Reid et al., 1982 [37]	3	1:2	12–64 years	0	100	0	6 months–30 years
Glynn et al., 1984 [38]	2	1:1	57–71 years	0	100	0	No data
Thomas and Williamson, 1985 [39]	6	4:2	4 months–76 years	66	83	17	No data
Ballantyne et al., 1986 [40]	3[c]	1:2	6 weeks–12 years	100	100	0	6 weeks–12 years
Matsumoto et al., 2004 [41]	9	2:7	12–53 years	No data	89	No data	12 years (median)

a Included two subjects taking potassium chloride.
b Included six subjects taking potassium chloride.
c Included one subject taking nonsteroidal antiinflammatory drugs.

presentation [35]. Patients with small intestine obstruction or perforation also can present with an acute abdomen. Although ulcers are more commonly seen in the ileum, perforated ulcers are more common in the jejunum (78% vs 11%) [35]. Because ileal ulcers, which are less likely to perforate, are more prevalent, the overall incidence of perforation is only 22%. Gastrointestinal bleeding is also common, especially in younger patients, who present with intestinal hemorrhage or with occult blood loss. In a series of nine patients with idiopathic small bowel ulcers undergoing surgical resection for gastrointestinal bleeding, multicentric stenoses were seen, with multiple, sharply demarcated ulcers without much inflammatory infiltrate [41]. In addition, patients presenting with gastrointestinal bleeding often have symptoms of intermittent intestinal obstruction. Significant weight loss leading to emaciation may also be seen [41]. The diagnosis of small intestine ulceration should be considered if intestinal bleeding and evidence of small intestine obstruction coexist.

Pathology

Most primary small intestine ulcers (75%) are located in the middle to distal ileum [34]. In one report, a solitary ulcer was found in 70%, two or three ulcers in 20%, and more than three in 10% of patients [35]. Ulcers typically have sharply demarcated borders and vary in diameter from 0.3 cm to 5 cm [35,41]. The pathological features are nonspecific and are identical to those of peptic ulcerations [42]. A marked eosinophilic or lymphoplasmacytoid infiltrate is sometimes present [43,44], but a nonspecific pattern of inflammatory cells may be also seen [41]. The surrounding mucosa is usually normal. Granulomas, sinus tracts, and pathological vascular changes are not seen. Granulation tissue and fibrosis that extend deep into the bowel wall may produce intestinal stenosis. Normal surrounding mucosa and absence of granulomas are features that help to distinguish idiopathic ulcers from those seen in Crohn's disease or CUJ.

Diagnosis

Both evidence of intestinal ulceration and exclusion of known etiological agents are required to support a diagnosis of primary small bowel ulcers. A careful history is necessary to identify exposure to potentially ulcerogenic substances such as nonsteroidal antiinflammatory drugs (NSAIDs) and enteric-coated potassium preparations. Prior abdominal surgery and other risk factors that predispose to intestinal ischemia should be identified. Assessment of immune status also may be useful. It is important to exclude cytomegalovirus infection in immunocompromised patients because of the propensity for cytomegalovirus ulcers to bleed or perforate. Other infectious causes, including *Salmonella typhi*, need to be excluded [45,46].

There are no pathognomonic biochemical abnormalities, and the only laboratory tests that are useful for diagnostic purposes are those that help to establish an alternative diagnosis. Small intestine ulcers can be detected radiographically, endoscopically, or at laparotomy.

Although small intestine ulcers cannot be detected by plain abdominal radiographs, small bowel dilation suggestive of obstruction and pneumoperitoneum indicative of perforation may be detected. Conventional contrast radiography of the small intestine rarely identifies primary ulcers of the small intestine [35]. Enteroclysis is a superior technique for demonstrating small intestine mucosal lesions (see Chapter 128), and this can be performed using CT or magnetic resonance (MR) imaging with improved sensitivity. Ileal ulcers can, on rare occasions, be detected if contrast material is refluxed into the terminal ileum; however, the yield from barium enemas does not justify their use for this purpose. Wireless capsule endoscopy complements contrast radiography in the evaluation of mucosal lesions and ulceration in the small bowel. The small risk of capsule obstruction at the site of a stricture makes the use of contrast studies a prerequisite in patients with obstructive symptoms [8,47]. Other radiographic techniques, such as technetium 99m (^{99m}Tc)-labeled red cell scanning and mesenteric angiography used for the evaluation of gastrointestinal bleeding, or ^{99m}Tc pertechnetate for the diagnosis of a Meckel diverticulum, may lead indirectly to the detection of small intestine ulcerations.

Despite the refinement of enteroclysis and enteroscopy, many symptomatic idiopathic small intestine ulcers are not detected without surgical intervention. In the patient with persistent gastrointestinal blood loss and iron deficiency anemia or abdominal pain with evidence of small bowel obstruction, advanced imaging or endoscopic techniques should be given serious consideration prior to proceeding to surgical exploration with or without intraoperative enteroscopy (see Chapter 119) [48,49].

Therapy

The therapy for primary small intestine ulcers is dictated by the severity of complications. Emergency surgical intervention is required if an acute abdomen or pneumoperitoneum indicative of intestinal perforation is evident. Life-threatening intestinal bleeding is an indication for diagnostic and therapeutic mesenteric angiography or surgical exploration (see Chapter 135). The best therapy for ulcers that present with less serious complications is also surgical resection. Detection of small bowel ulcers is usually readily accomplished at laparotomy, which can be facilitated by advanced imaging and endoscopic studies preoperatively. Intraoperative enteroscopy may be a useful adjunct. Most ulcers can be managed by surgical resection with end-to-end anastomosis, providing adequate material for pathological analysis with a low incidence of complications or ulcer recurrences. Simple oversewing of primary small bowel ulcers is not recommended. With such an approach, the amount of material available for pathological analysis is limited, and the risk of ulcer recurrence is high. Medical management with antiinflammatory

or immunosuppressive agents is not effective therapy for intestinal ulcers or strictures. Furthermore, unsuspected perforation is often found at surgery [35].

Drug-induced small bowel ulcers

The entrapment of a pill or capsule before dissolution or the release of large concentrations of a drug in a segment of intestine can result in localized ulceration because of physical pressure or specific cytotoxic effects. The most common cause of drug-induced small intestine ulcers are NSAIDs. In the 1960s, enteric-coated potassium chloride (KCl) was introduced and was soon recognized to be an intestinal ulcerogen. Ferrous salts, digoxin, corticosteroids, zirconium, and clofazimine have all been implicated as ulcerogens as well [50].

Nonsteroidal antiinflammatory drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) are a heterogeneous group of organic acids that inhibit prostaglandin synthesis and have analgesic, antiinflammatory, and antipyretic properties [51]. They have been used for decades in the treatment of inflammatory arthropathies, and lately also for many nonrheumatological problems [51]. Many generic NSAIDs are available without prescription. As a result, NSAIDs are among the most commonly used drugs worldwide. Numerous publications have addressed the magnitude and cost of NSAID-associated gastrointestinal complications, which have been described as an emerging epidemic [52–62].

Because of the increased use of NSAIDs, the introduction of more potent NSAIDs, and improvements in small intestine diagnostic methods, small bowel enteropathy is a more frequently diagnosed complication of NSAID use. Multiple ulcerations, mucosal diaphragms, ileal strictures, and perforations have all been associated with NSAID use [56,63–67]. Small bowel ulcers related to NSAID use can occur even in the absence of similar lesions elsewhere in the gut [68]. An ulcerating ileitis resembling Crohn's ileitis has also been described [69]. Cyclooxygenase-2 (COX-2)-selective inhibitors reduce the incidence of gastroduodenal ulcers. A prospective randomized comparison by wireless capsule endoscopy of healthy adults treated for 2 weeks with celecoxib, naproxen with omeprazole, or placebo, suggests that the protection extends to the small intestine [70]. In this study the percentage of patients with mucosal breaks and the number of breaks per patient were significantly reduced in the celecoxib group compared with the naproxen with omeprazole group. However, both of these parameters were significantly increased compared with the placebo group.

The epidemiology of NSAID-induced enteropathy is not well characterized. Up to 70% of patients who take NSAIDs have evidence of increased intestinal permeability or inflammation [56,71–75], but most are asymptomatic. In a small study of 41 patients, wireless capsule endoscopy detected visual evidence of mucosal injury to the small bowel in as many as 71% of NSAID users, compared with only 10% of controls [76]. An autopsy study showed the presence of nonspecific small intestine ulcers in 8.4% of 249 patients who had NSAIDs prescribed during the 6 months before death, compared with an incidence of 0.6% in 464 patients who had not used NSAIDs [77]. Three of the long-term users of NSAIDs were found to have died of perforated nonspecific small intestine ulcers. Small intestine erosions or ulcerations were detected by small bowel enteroscopy in 7 of 15 iron-deficient patients receiving long-term NSAID therapy for rheumatoid arthritis [78]. All the patients had negative results of upper and lower endoscopy. These studies indicate a high prevalence of unsuspected gastrointestinal lesions in patients taking NSAIDs. A metaanalysis of studies examining the association between NSAIDs and serious adverse gastrointestinal events indicated that NSAID users are at three times greater risk than nonusers [77,79]. Independent risk factors seem to be age over 60 years, previous NSAID complications, and concomitant steroid use. This study did not analyze small bowel effects independently, so it is not certain whether this risk profile is applicable to NSAID-induced small intestine enteropathy.

Pathophysiology

Increased intestinal permeability, which enhances susceptibility to lumenal macromolecules and toxins, has been implicated in the pathogenesis of NSAID-induced enteropathy [80]. This defect in permeability is evident within hours after NSAID administration [56,81], and in rats it may be ameliorated by pretreatment or simultaneous treatment with glucocorticoids, sulfasalazine, or tetracycline [82]. The mechanisms by which NSAIDs injure the small intestine mucosa are unknown but are thought to include roles for enteric bacteria, the enterohepatic circulation, neutrophil infiltration, and suppression of prostaglandin synthesis [83,84]. Because COX-1-mediated synthesis of prostaglandins appears to be less important as a cytoprotective mechanism in the small intestine, NSAID-mediated COX inhibition is not thought to be the major pathogenic mechanism in NSAID-induced enteropathy [85,86]. Furthermore, COX-1 knockout mice had no apparent intestinal disorders [87]. Although nitric oxide derivatives of NSAIDs are equally potent as their parent compounds as suppressors of prostaglandin synthesis, the magnitude of small intestinal injury is markedly attenuated with these agents [88–91].

In rats, diclofenac, but not nitrofenac (a nitric oxide derivative that does not undergo extensive enterohepatic circulation), caused increases in epithelial permeability, gram-negative bacterial colonization, and intestinal ulceration [92]. In these rats, the increased bacterial load, which occurred after the changes in intestinal permeability, may have exacerbated the NSAID injury [83]. These studies suggest that NSAIDs primarily alter intestinal permeability through local rather than systemic effects, and suggest a pathogenic role for enteric bacteria. Additional studies indicate that NSAID-induced

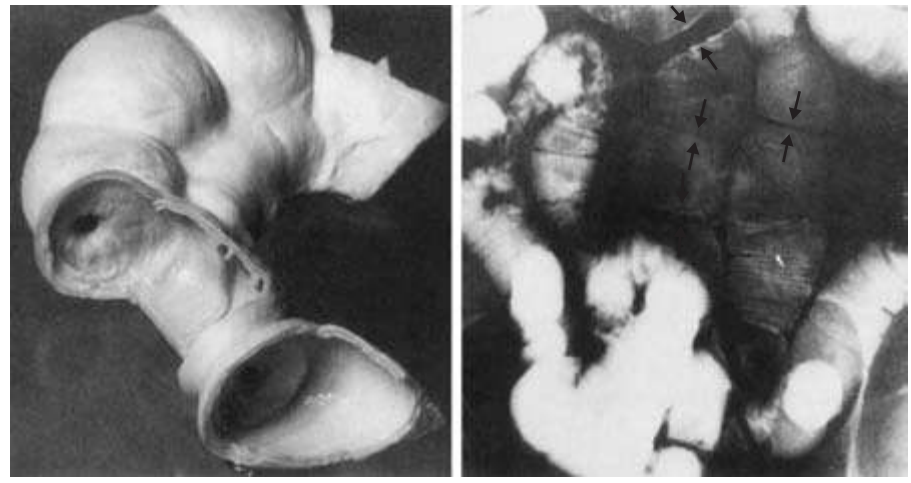
microvascular leakage also contributes to NSAID-induced enteropathy [93]. In rats, NSAID-induced plasma leakage requires induction of calcium-independent nitric oxide synthase and occurs only in the presence of gut bacteria [94]. The relative importance of local and systemic effects in the pathogenesis of NSAID-induced enteropathy was also addressed in human volunteers who received either indomethacin (indometacin) or the prodrug nabumetone. Because only indomethacin increased intestinal permeability, local effects of NSAIDs appeared to be more important [95]. Another prodrug, sulindac, was compared with indomethacin, with similar results [96]. To date, clinical trials comparing COX-2-selective inhibitors with nonselective COX inhibitors have not had the power to address the comparative magnitudes of small intestinal enteropathy. A proposed COX-independent direct mechanism for NSAID damage is inhibition of glycolysis and the tricarboxylic acid cycle, leading to reduced cellular adenosine triphosphate (ATP) production and uncoupling of oxidative phosphorylation [85,97,98]. Coadministration of glucose and citrate with indomethacin to humans was shown to prevent the increased permeability seen with indomethacin alone [97], a finding compatible with this hypothesized mechanism.

Intestinal injury due to NSAIDs primarily affects the distal small intestine. Intestinal ulcers induced by NSAIDs are pathologically identical to primary ulcers and to other drug-induced ulcers. Strictures are seen in up to 5% of patients receiving long-term NSAID therapy for arthritis [56]. In addition to strictures that are indistinguishable from those seen in Crohn's disease and other disorders, apparently unique, diaphragm-like strictures have been described (Fig. 55.1) [63,65,99–101]. These strictures encroach on and narrow the lumen to an opening as small as 1 mm in diameter [99]. They usually occur as multiple strictures separated by only a few centimeters of normal bowel [99]. Submucosal fibrosis replacing or merging with the muscularis mucosae is characteristic [63,99]. Mild inflammation of the overlying mucosa is invariably present, with or without shallow ulceration [63]. The adjacent mucosa is usually completely normal. Despite the strong association with NSAID use, a single case report without apparent NSAID use suggests that diaphragm disease may be a nonspecific response to intestinal injury [102]. Patients with this "diaphragm disease" present with weight loss, hypoalbuminemia, anemia, and intermittent vague abdominal pain [99]. The diaphragm-like strictures are difficult to detect radiologically and can be missed at laparotomy unless an enterotomy is performed [99–101]. Small bowel enteroscopy, which is useful for detecting intestinal ulcers [78], may also be useful for detecting NSAID-related intestinal abnormalities [103].

Morphologically, the presentation of NSAID-related enteropathy is often similar to that of Crohn's disease, with transmural injury that can cause stricture formation [85,98]. Some patients receiving long-term NSAID therapy do present with clinical features of Crohn's disease, although most patients with NSAID-associated small intestine enteropathy are asymptomatic [51]. In a prospective study, 10% of patients with colitis not due to Crohn's disease were thought to have NSAID-induced colitis [104]. There is also evidence that NSAIDs may exacerbate Crohn's disease and ulcerative colitis [54,105–111]. These studies emphasize the importance of obtaining an accurate drug history for all patients presenting with an illness resembling Crohn's disease. Reports have also associated NSAIDs with a variety of other intestinal disorders, including collagenous colitis [112], nonceliac flattening of small intestinal mucosa [113–115], celiac sprue, intestinal malignant diseases [115], and perhaps diverticulitis [85]. However, the available data are not sufficient to establish a true cause and effect relationship between NSAID use and any of these conditions [98].

Therapeutic considerations

Wireless capsule endoscopy and double-balloon endoscopy are newer investigative techniques that may have diagnostic



(a) (b)

Figure 55.1 Nonsteroidal antiinflammatory drug-induced small intestine strictures. **(a)** Diaphragm strictures of the small intestine are apparent in resected intestine. **(b)** Enteroclysis study in a patient taking nonsteroidal antiinflammatory drugs. Diaphragm strictures (arrows), which were found at surgery, are difficult to appreciate and resemble exaggerated plicae circulares. From Bjarnason et al. [85], with permission from Elsevier.

utility in assessing the effects of NSAIDs on the small intestine, as an alternative to endoscopy or surgery [13,67,68,76]. Most patients who take NSAIDs appear to have intestinal inflammation as assessed by indium 111 leukocyte scans and fecal excretion [72], and increased intestinal permeability as assessed by chromium 51 (^{51}Cr)-ethylenediaminetetraacetate (EDTA) absorption. These abnormalities usually resolve after the medications are discontinued, but inflammation may persist for up to 16 months [56]. Discontinuation of NSAIDs should be sufficient for treatment of uncomplicated ulcers. Surgical intervention is required for therapy of intestinal perforation or symptomatic strictures.

The optimal regimen for patients for whom there is no good alternative to continued NSAID use has not been established. Based on the observation that nabumetone and sulindac did not increase intestinal permeation to [^{51}Cr]EDTA, switching to these or other prodrugs may be beneficial. Observations in rats indicate that switching to NSAIDs that are less potent inhibitors of COX also may be helpful [116,117]. The hypothesis that COX-2-selective NSAIDs, by preserving the cytoprotective effects of prostaglandins synthesized by COX-1, may be less enteropathic than nonselective NSAIDs has not been directly addressed by the clinical trials completed to date. A double-blind cross-over study demonstrated that unlike indomethacin, COX-2 inhibition with rofecoxib did not increase intestinal permeability in healthy subjects [118]. In another double-blind cross-over study, nimesulide, another COX inhibitor, did not increase intestinal permeability or excretion of calprotectin, a marker of intestinal inflammation [119].

Delivery of NSAIDs encapsulated in liposomes was protective in a rat study [120]. The degree of ulceration in rats was related to the amount of active drug excreted in bile [116,117,121]. Human studies have not established that the incidence of small intestine enteropathy or upper gastrointestinal bleeding can be reduced by measures such as using prodrugs, NSAIDs with decreased biliary excretion of active drug, enteric coatings, or slow-release formulations [67].

Germ-free rats are resistant to indomethacin-induced intestinal lesions [122], and antibiotics can reduce the incidence of indomethacin-induced small intestine ulcers in rats [123]. In humans, metronidazole [98] and sulfasalazine [124] reduce intestinal inflammation, a response not yet proven to correspond to prevention of or recovery from NSAID-induced complications.

In studies using rats, pretreatment or concurrent treatment with glucocorticoids, sulfasalazine, tetracycline, sucralfate [125], pentagastrin [126], naloxone (tested because morphine potentiates the ulcerogenic effect of indomethacin) [127], clonidine [128], or thromboxane synthetase inhibitors [82] reduced indomethacin-induced permeability and inflammatory changes in rats. Drugs that exacerbated the intestinal toxicity of indomethacin in rats include cyclosporine [129] and morphine. Although prostaglandin E analogues such as misoprostol and enprostil prevent NSAID-associated injury of the stomach and duodenum [130–134], their effects in the remaining small intestine are not known. Protection was demonstrated in animal models [135–137]. In humans, high-dose misoprostol prevented NSAID-induced permeability changes in one study [138], but therapeutic doses did not [95].

Potassium chloride

The introduction of enteric-coated potassium chloride (KCl) tablets in the 1960s was associated with an unacceptably high incidence of small intestine ulceration, attributed to high lumenal concentrations of KCl from rapid dissolution of the tablets in the small intestine. Slow-release formulations using a wax-polymer matrix (dissolution in approximately 4 h) or microencapsulation (dissolution in 8–10 h) were developed to eliminate this adverse effect; gastrointestinal ulceration was reduced in incidence but not eliminated. Two cases of small intestinal ulceration occurred from 1970 through 1983 (3 in 100 000 patient-years of slow-release wax-matrix tablet use), compared with 56 cases from 1960 through 1965 (65 in 100 000 patient-years of enteric preparations) [139]. Regardless of the preparation used, the risk of ulceration is increased if intestinal transit is delayed by small bowel disease, concurrent medications, general debility, or advanced age [140].

The clinical presentation, diagnosis, and treatment of patients with KCl-induced small intestine ulcers or strictures are the same as those for patients with primary or NSAID-induced ulcers. Although the ulcers can occur anywhere along the gastrointestinal tract, they are most common in the distal ileum [141].

Other drugs

Parenteral gold therapy also has been associated with enterocolitis, in which edema and ulceration are limited to the ileum [142–144]. This rare complication, most common in middle-aged women, can occur shortly after the initiation of therapy and has a high mortality rate; thus, prompt cessation of gold therapy is essential. Therapy includes bowel decompression and antibiotics. Steroids are frequently used, although efficacy has not been proven. Octreotide was used successfully in one case [145].

Other drugs reported to cause small intestine ulcers or perforation include corticosteroids [146], cytarabine [147] and other chemotherapeutic agents, digoxin [148], ferrous sulfate preparations [50], and kayexalate [149].

Small bowel ulcers associated with systemic disorders (see Chapter 109)

Behçet syndrome

Behçet syndrome is a systemic disease that affects the skin, joints, vascular system, central nervous system, and intestinal tract. Chromosomal trisomy 8 has been reported in asso-

ciation with intestinal Behçet disease, suggesting that this chromosomal anomaly may play a role in some forms of Behçet disease [150]. Oral and genital ulcers are a common feature; intestinal ulceration has been described in fewer than 1% of all patients with Behçet syndrome [151]. The ileocecal region is most frequently involved [152]. Multiple deep ulcers in a background of minimally inflamed mucosa are characteristic; fold thickening, granular mucosa, and pseudopolyp formation have also been described [153]. The lesions predominate at sites coinciding with intramucosal lymphoid tissue [154]. The ulcers bleed easily and are often penetrating [155,156]. The abnormalities are readily differentiated from those of Crohn's disease or ulcerative colitis [152,153,157].

The optimal treatment of Behçet syndrome has yet to be established. No medical treatment significantly alters the natural course of this syndrome. Immunomodulation with corticosteroids, methotrexate, colchicine, thalidomide [158,159], cytotoxic agents, cyclosporine (ciclosporin), and tacrolimus (FK506) have been used for treatment of multisystem Behçet syndrome [160]. Recombinant α-interferon may benefit patients with systemic involvement including intestinal ulcers [161–164]. Surgery with wide resection margins should not be delayed in patients with complicated intestinal cases, although ulcer recurrence is common postoperatively [152,165,166]. Most recurrences occur within 2 years of surgery and consist of one or two deep ulcers at or near the anastomotic site [158].

Collagen vascular diseases

Patients with *systemic lupus erythematosus* (SLE) can develop intestinal ulceration as an uncommon complication, thought to be secondary to microthrombosis and vasculitis [167]. Mesenteric vasculitis can result in enteritis, which may present with nausea, vomiting, fever, diarrhea, and hematochezia [168,169]. This can progress to bowel infarction, which is associated with high mortality. Mesenteric vasculitis presenting with small bowel ischemia and stricture has also been reported in patients with other inflammatory disorders [168], including rheumatoid arthritis, scleroderma, polyarteritis nodosa, Henoch–Schönlein purpura, Wegener granulomatosis, giant-cell arteritis, Churg–Strauss syndrome, and Sézary syndrome [170]. Spondylarthropathies have also been associated with an increased incidence of ileal ulceration, although this is most often caused by concurrent inflammatory bowel disease [171]. Patients with acquired antiphospholipid antibodies and SLE or related autoimmune diseases are at increased risk of developing intestinal ischemia and hemorrhage related to mesenteric arterial thrombosis [172,173].

Cryptogenic multifocal ulcerous stenosing enteritis is an idiopathic syndrome consisting of intermittent bouts of intestinal obstruction and ulcerative stenosis, and characterized by steroid responsiveness [174–176]. Patients present with multiple jejunal and proximal ileal stenoses associated with superficial ulcers. They often have a chronic and relapsing course but share no other characteristics of Crohn's disease. Iron deficiency anemia has also been reported [176]. The presence of systemic symptoms (weight loss, fever, malaise) in most patients, responsiveness to steroids, and demonstration of C2 deficiency in one reported case suggest that this syndrome is a variant of polyarteritis nodosa or an unclassified independent vasculitis [174,175], although overt signs of vasculitis are not always present [175].

Diffuse small bowel ulceration and concurrent malabsorption

A rare clinical syndrome consisting of malabsorption, abdominal pain, and multiple nonmalignant ulcers of the small intestine has been described [177]. Typically, these patients are in the sixth or seventh decade of life and have long-standing GSE. In such patients, the disorder is characterized by extensive villus atrophy and small bowel ulcerations that fail to improve on gluten withdrawal. There is also a distinct subgroup of patients who do not have GSE. The term *chronic ulcerative (nongranulomatous) jejunitis* (CUJ) – and synonyms such as idiopathic mucosal enteropathy, idiopathic chronic ulcerative enteritis, unclassified sprue, malignant histiocytosis, and enteropathy-associated T-cell lymphoma (EATL) – have been applied to this latter group of patients. The nomenclature reflects the poor understanding of the etiology and pathophysiology of CUJ [177,178]. For the purposes of this chapter, CUJ is used to describe this disease in patients with and without evidence of GSE.

The typical histological features of CUJ are partial or total villus atrophy, mucosal ulceration, crypt hyperplasia, and an inflammatory cell infiltrate [179,180]. The loss of intestinal villi results in a marked decrease in the absorptive area of the intestine, and the aborted maturation of the enterocytes results in reduced levels of digestive enzymes on the intestinal brush border. The effects of these disturbances are malabsorption of all dietary components and a variable degree of PLGE. Although the mechanism is poorly understood, villus atrophy appears to be related to infiltration by activated T cells [181].

Etiology

Chronic ulcerative jejunitis without a prior history of GSE is usually more extensive in distribution and fails to respond to gluten withdrawal. Both CUJ and GSE are associated with splenic atrophy of unknown origin, and most cases of CUJ are preceded by a variable period of suspected or proven GSE [34,182,183]. Nevertheless, it seems clear that CUJ can develop without GSE [184,185].

Although small bowel lymphoma can resemble GSE clinically [186], there is strong evidence that lymphomas arise in chronic GSE [187–191]. The incidence of malignant degeneration may be decreased by a strict gluten-free diet [192].

In contrast to primary small intestine lymphomas, the typical lymphoma associated with GSE is derived from T cells [193–195]. These lymphomas, which are referred to as *enteropathy-associated T-cell lymphomas* (EATLs), are difficult to differentiate from those of CUJ. Histologically, both EATLs and CUJ exhibit villus atrophy, ulceration, and mucosal infiltration by activated T cells. Both disorders usually present with splenic atrophy. The major diagnostic difficulty results from the observations that EATL may be indolent in its early stages and patients with CUJ can develop lymphoma several years after diagnosis. In some cases, retrospective examination of biopsy samples obtained at the time of diagnosis of CUJ has revealed typical malignant cells [179,182,196]. Using the polymerase chain reaction, clonal rearrangements of the T-cell receptor were found in tumor specimens and, from the same patients, in enteropathic bowel without evidence of tumor involvement [197,198]. Analysis of intestinal biopsies from patients with CUJ and refractory GSE revealed that the monoclonal cytologically normal, noninvasive intraepithelial T lymphocytes share an aberrant immunophenotype with EATL [199]. In one series, all patients with CUJ had metastatic disease at the time of diagnosis [179], but in another retrospective study, no evidence of preexisting lymphoma was detected [200]. Chromosomal analysis in patients with EATL has demonstrated loss of heterozygosity at chromosome 9p21 (a frequent site of deletions in patients who develop lymphomas), suggesting that genetic abnormalities may participate in the development of EATL [201]. These data indicate that CUJ is a significant risk factor for the development of lymphoma and suggest that this development may be invariable.

Non-T-cell lymphomas, such as immunoproliferative small intestinal disease and Mediterranean (B-cell) lymphoma, can also be accompanied by mucosal atrophy and ulceration and may have all the features of CUJ (see Chapter 54). This group may have a dramatic response to antibiotics or cytotoxic agents [202–205]. These lymphomas cannot be differentiated solely based on morphology; thus, it is important to determine cell surface markers for proper diagnosis.

Clinical features

The age at diagnosis of CUJ tends to be older than it is for typical GSE. Childhood cases have not been reported. The onset of CUJ is indolent. Patients with CUJ with and without known GSE were symptomatic for a mean of 13 and 4.5 years, respectively. Most patients present with chronic symptoms that are typical of malabsorption syndromes. Midepigastric pain and weight loss associated with steatorrhea and diarrhea are common [200]. Shortly after presentation, 20%–30% of patients have ulcer complications such as small bowel obstruction (30%), melena (25%), and perforation (22%). Along with symptoms caused by these complications, patients with CUJ may have low-grade fever and signs of malnutrition.

Pathology

Multiple superficial or deep ulcers are invariably present in the stomach, small bowel, and colon [200,206]. Splenic atrophy is often present. Lymphomatous infiltration of lymph nodes, liver, spleen, and bone marrow is common with concurrent lymphoma [179].

According to Isaacson [180], there are "variable degrees (usually severe) of villus atrophy, crypt hyperplasia, intraepithelial lymphocytic infiltration, irregularity of surface enterocytes, and a dense lamina propria infiltrate consisting almost solely of plasma cells with a variable number of eosinophils and occasional neutrophils." Invasion of the epithelial layer may cause ulceration (Fig. 55.2). Subepithelial collagen deposition, which is also seen in GSE, and marked fibrosis of the ulcer base also may be present. Crypt hyperplasia and villus atrophy are seen in the adjacent mucosa, and the remaining mucosa is usually normal. It is imperative that multiple sections be exhaustively examined for malignant cells to avoid missing the diagnosis of lymphoma. Neoplastic cells are derived from T cells [193–195,207]. They resemble histiocytes, with minimal or no atypia, and the presence of intracellular erythrocytes, platelets, and cell debris is a common feature (Fig. 55.3) [179]. Confusion with Hodgkin disease may occur because of similarities with Reed–Sternberg cells [186,187,208].

Diagnosis

The diagnosis of CUJ should be considered in patients with established or apparent GSE who present with worsening malabsorption despite continued compliance with gluten-free diets. Essentially CUJ is a diagnosis of exclusion, whether or not a history of celiac disease is present, unless lymphoma is detected. Disorders presenting with malabsorption and intestinal ulceration that must be excluded include Crohn's disease, ischemic enteritis, radiation enteritis, lymphoma,

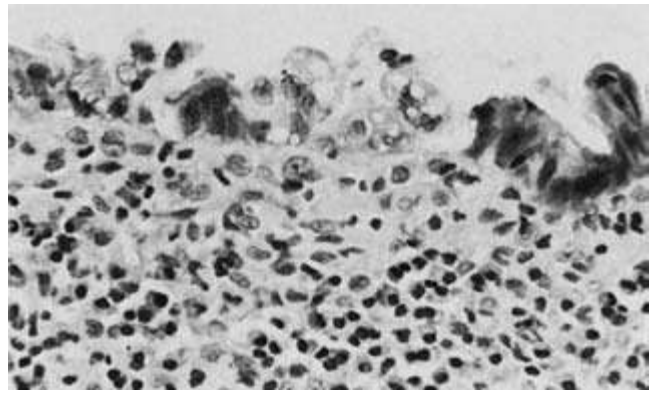


Figure 55.2 The malignant chronic ulcerative nongranulomatous jejunoileitis cells have invaded the surface epithelium and produced an ulcer. An exuberant plasma cell infiltrate is seen in the subepithelial tissue (original magnification × 400). From Isaacson [180], with permission from the BMJ Publishing Group.

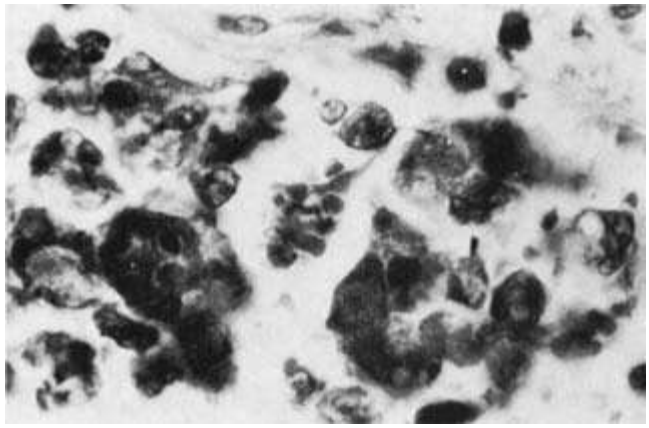


Figure 55.3 Chronic ulcerative nongranulomatous jejunoileitis tumor cells show typical erythrophagocytosis (original magnification × 1000). From Isaacson & Wright [179], with permission from Elsevier.

drug-induced ulceration, vasculitis, Zollinger–Ellison syndrome, and Whipple disease.

The patient with suspected CUJ should be assessed for malnutrition, and a careful evaluation of the extent of disease should be undertaken. Malabsorption and PLGE account for hypoalbuminemia, and hypoglobulinemia and deficiencies of divalent cations, iron, and folate are common. Hyposplenism is often present [183]. Intestinal perforation should be ruled out if leukocytosis, with or without fever and peritoneal signs, is present.

The diagnosis of CUJ is rarely made radiographically. Evidence of bowel thickening and of complications such as small bowel perforation or obstruction may be present on plain films. Demonstration of small bowel strictures by contrast studies (Fig. 55.4) or by CT helps to distinguish CUJ from uncomplicated GSE: small bowel dilation is typical of the latter [209–211]. Bowel thickening, mesenteric lymphadenopathy, and splenic atrophy are consistent with CUJ. However, lymphadenopathy in patients with GSE is not diagnostic of lymphoma.

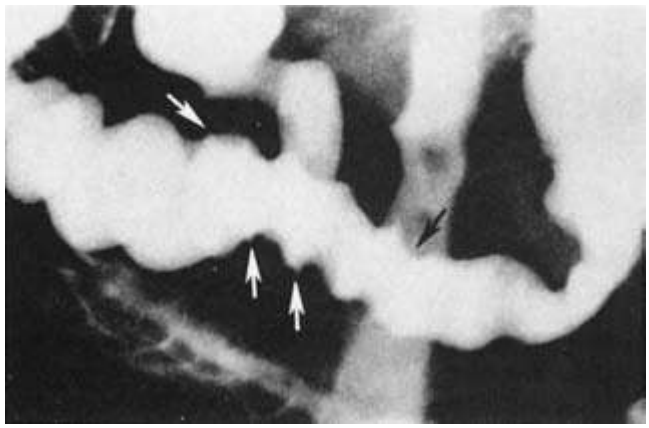


Figure 55.4 Chronic ulcerative nongranulomatous jejunoileitis (CUJ). Thirty-minute small bowel follow-through shows prominent intestinal folds, thickening of the bowel wall, diffuse lumenal narrowing, and mucosal ulceration (arrows). Dilation, which is typical of gluten-sensitive enteropathy, is not a feature of CUJ. From Freeman & Cho [211], with permission from Blackwell Publishing.

Small bowel biopsies are essential for establishing the presence of CUJ. Although the yield from blind biopsies is poor, improvements in the ability to obtain endoscopic biopsy samples should markedly reduce the need for surgery to establish the diagnosis [211]. Biopsy samples obtained from ulcerated regions are the most desirable. It is essential that adequate tissue is obtained, sectioned extensively, and examined by an experienced pathologist before excluding the diagnosis of EATL; immunohistology and T-cell flow cytometry may be useful adjuncts [180,212]. A careful search for disseminated lymphoma at the time of presentation is essential, using imaging techniques such as CT, MR, and positron emission tomography (PET) scanning [179,212]. It is also important to assess for the presence of B-cell surface markers because B-cell lymphomas and immunoproliferative small intestinal disease may be associated with villus atrophy. Polymerase chain reaction has been used to detect T-cell receptor gene rearrangements in biopsies [202].

Therapy

No specific therapy has been shown to modulate the course of CUJ. Most patients have unresponsive malabsorption, and life expectancy at diagnosis is less than 2 years. Surgical resection of severely affected bowel has been of benefit in some cases [196,200]. Although patients with CUJ are at increased risk of intestinal perforation, prednisone therapy should be considered in all symptomatic patients with no evidence of lymphoma. The dose should be reduced to the minimum necessary to maintain a symptomatic response with tolerable side effects. Azathioprine has been studied in small series, one study reporting clinical improvement in five of seven patients at doses of 2 mg/kg/day, used in conjunction with oral prednisone (1 mg/kg/day) for a mean of 11 months [213].

Although CUJ does not respond to removal of dietary gluten, it is possible that this measure may impede the development of lymphoma [192,214]. If a localized lymphomatous infiltrate is found, local excision is mandatory. Although some patients have been cured by resection alone [215], most remain symptomatic after the surgical procedure. Mortality in these patients is high, presumably because of progression of the lymphoma. The impact of chemotherapy with or without adjuvant radiation has not been evaluated in clinical trials. Disseminated T-cell lymphoma should be treated with aggressive chemotherapy using combinations, such as cyclophosphamide, hydroxydoxorubicin, vincristine, and prednisone, that are used in the treatment of other lymphomas. In a retrospective study of 33 patients treated with

high-dose chemotherapy regimens, the 5-year survival rate was 19.7% and the disease-free rate was 3.2% [216].

Autoimmune enteropathy

Autoimmune enteropathy is another rare malabsorptive disorder that can present with chronic diarrhea and weight loss, and has been described in both children and adults [217–220]. Diagnosis is based on the presence of small intestinal villous atrophy, and circulating antienterocyte antibodies, which are pathognomonic for this disorder [219–221]. Associations include immunoglobulin deficiencies and thymomas; autoantibodies to other gut organs may coexist, with gastritis, hepatitis, colitis, pancreatitis, and pernicious anemia in some instances. Autoimmunity against multiple sites in the gut has led to suggestions that this may represent a generalized autoimmune gut disorder resulting from inappropriate T-cell activation and increased tumor necrosis factor α (TNF-α) production [222]. The mainstay of therapy is immunosuppression, using corticosteroids as first-line therapy; alternate agents that have been successfully used for maintenance of immunosuppression include azathioprine, cyclosporine, and tacrolimus [222–225]. Two case reports outline the use of infliximab infusions to induce and maintain remissions with success [219,226].

Drug-induced small bowel disease

Many contemporary drugs mediate various mechanisms of injury, including the following:

- erosive damage by NSAIDs and KCl
- ischemic damage by cardiac drugs, cocaine, ergotamine alkaloids, and oral contraceptives
- hematomas from anticoagulants
- motility disorders from opiates
- malabsorption
- inhibition of epithelial cell turnover by chemotherapeutic agents.

These adverse reactions may be reversible if the drug is discontinued, but some medications produce permanent effects. This section focuses on drugs that have a profound or permanent effect on the small intestine, except for those causing erosive damage, as discussed earlier. The pathological and radiological features of drug-related lesions of the intestinal tract have been reviewed [227–230].

Drugs causing ischemia

Mechanisms by which drugs and toxins can produce occlusive or nonocclusive intestinal ischemia include induction of arterial vasoconstriction, systemic or splanchnic hypotension, and thrombosis of mesenteric vessels (see Chapter 113). Regardless of cause, the mortality rate from small bowel ischemia progressing to bowel infarction ranges from 70% to 90% [231,232]. Delays in presentation and diagnosis and coexistent health problems account for this high mortality. Patients with drug-induced mesenteric ischemia usually present with severe, poorly localized abdominal pain, low-grade fever, and hematochezia. Signs of peritonitis or abdominal distention secondary to ileus may be present. Evidence of intestinal perforation, obstruction, or ileus can be obtained by radiographic studies. The demonstration of bowel wall edema and thumbprinting, pneumatosis intestinalis, or portal venous gas are consistent with mesenteric infarction (Fig. 55.5) [231]. Computed tomography is a reliable diagnostic method [233,234]. Radiographic studies may be normal in patients with nonocclusive ischemia, such as occurs with digitalis-induced mesenteric spasm and antihypertensive medications [235,236]. Mesenteric angiography may be needed to document digitalis-induced ischemia [237].

Besides measures specific to the offending agent, general supportive measures include administration of fluids and oxygen, correction of electrolyte and acid–base disorders, and antibiotic therapy [231]. Surgical resection of infarcted small bowel is necessary, although most patients are high-risk surgical candidates.

Cardiac drugs

Intestinal ischemia or infarction may result from drugs that produce or potentiate arterial vasoconstriction or hypotension [238]. The incidence of these side effects is greatest when the splanchnic circulation is already compromised, such as in congestive heart failure. Antihypertensive agents and diuretics can induce intestinal ischemia secondary to hypotension and hypovolemia. Drugs with direct vasoconstrictive effects, such as norepinephrine (noradrenaline), dopamine, and intraarterial or intravenous vasopressin, reduce intestinal blood flow and can cause mesenteric infarction [239,240]. Digitalis glycosides can further reduce splanchnic flow in patients with congestive heart failure. Intestinal ischemia resulting from digoxin use may be reversed with nitroprusside and calcium channel blockers [237,241].

Cocaine

Cocaine is a lipophilic drug that is readily absorbed by mucosal surfaces, including the intestinal mucosa. It has potent stimulatory effects on the central nervous system because of inhibition of presynaptic reuptake of norepinephrine, dopamine, and serotonin (5-hydroxytryptamine, 5-HT), in addition to causing increased synthesis of norepinephrine and dopamine [242,243]. Excessive sympathetic stimulation produces peripheral vasoconstriction and tachycardia, which can cause hypertension and nonocclusive intestinal ischemia [244]. Rebound vasodilation after intense vasoconstriction can further compromise intestinal perfusion.

Gastrointestinal manifestations of cocaine use include appetite suppression, nausea, vomiting and hyperperistalsis,

(a) (b) (c)

Figure 55.5 Acute spontaneous mesenteric venous thrombosis. **(a)** Involved jejunum shows separation of loops and thumbprinting. **(b)** Blurring of mucosal folds is seen in a distal zone of involvement. **(c)** Barium study of a surgical specimen shows thickening of mucosal wall and folds, thumbprinting, and pseudomembrane formation (arrows). From Pringot & Bodart [440], with permission from Elsevier.

perforated gastroduodenal ulcers (most common in crack users), enterocolitis, and hepatotoxicity [245,246]. Intestinal ischemia is a serious but rare complication of cocaine and crack abuse, and carries a 50% mortality if peritonitis ensues [246–252]. Life-threatening gastrointestinal problems are more likely to be encountered by "body packers." Such persons transport illicit drugs by ingesting drug-containing packets or condoms or by inserting them in the rectum [244,253,254]. Complications include mechanical intestinal obstruction and rupture of a packet, which can release a lethal quantity of cocaine [254,255]. Signs of drug toxicity, mechanical intestinal obstruction, or marked leukocytosis, which may indicate the presence of gangrenous bowel, are indications for laparotomy [239]. Asymptomatic patients should be treated with activated charcoal to bind any leached drug and with mild cathartics to stimulate gastrointestinal transit [239,253]. Abdominal radiographs may reveal evidence of intestinal obstruction or ileus as well the pathognomonic "double-condom sign" [256].

Ergotamines

Ergot alkaloids such as ergotamine, dihydroergotamine, methylergonovine, and methysergide are used in the symptomatic treatment or prevention of migraine headaches. However, due to side effects, numerous contraindications, and the introduction of more efficacious drugs, their clinical use has been sharply reduced [257]. Common intestinal complications of ergots include abdominal pain, nausea, and vomiting. There are several reports of bowel infarction after administration of large doses of ergots [200,201].

Oral contraceptives

Oral contraceptives have been reported to cause small intestine infarction and, more commonly, segmental colitis [258]. The mechanisms are not known, although mesenteric arterial occlusion [259–262] or mesenteric venous thrombosis [263–267] is often present. Progestins cause arterial occlusion, whereas estrogens can produce both arterial and venous occlusion [268,269]. Mechanisms that have been implicated include hypercoagulability, reduced mesenteric venous blood flow, and endothelial proliferation [261,270]. The risk is increased by ancillary factors such as collagen-vascular disease, hypercoagulable states, smoking, and hypertension [271]. The duration of contraceptive use is not an independent risk factor and does not correlate with the severity of symptoms [261].

The diagnosis should be considered in contraceptive users presenting with poorly localized colicky abdominal pain, bloody diarrhea, or hematochezia. Fever is often present, and presenting symptoms may also include nausea and vomiting [262]. Although abdominal pain may be present for several weeks or months, pain serious enough to warrant hospitalization is usually of recent onset. Results of laboratory studies, including platelet count, prothrombin time, and partial thromboplastin time, are usually normal [262]. The diagnosis depends on the demonstration of intestinal ulceration or ischemia in contraceptive users. Abdominal radiographs may demonstrate bowel wall thickening or ileus. Ulceration, which may be detected with contrast studies, is usually continuous in involved segments [262]. The diagnosis of small intestinal infarction may rarely be made at exploratory laparotomy for an acute abdomen [267].

Oral contraceptives should be discontinued and supportive therapy initiated. Although mesenteric ischemia may be reversible, the mortality rate in severe cases is almost 50% [262]. The risk of thromboembolic complications decreases to normal levels within 1 month of discontinuation of oral contraceptives [270].

Anticoagulants

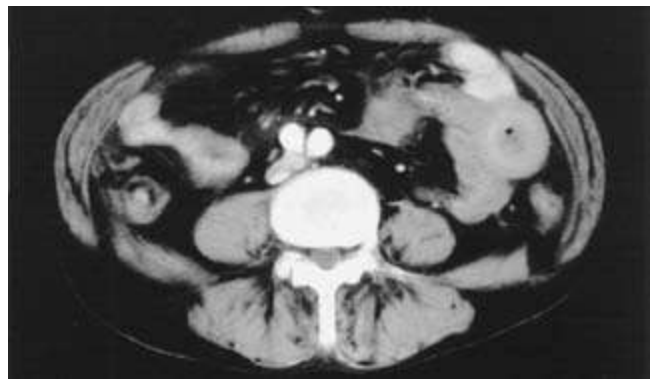
Gastrointestinal hemorrhage is a common complication of anticoagulant therapy with heparin or warfarin. Antiplatelet

therapy with ticlopidine has been associated with chronic severe diarrhea that is related to altered motility but not to hemorrhagic complications [272,273]. The incidence of bleeding depends on the intensity of therapy, the adequacy of monitoring and dosage adjustment, the route of administration, concurrent drug therapy, and the gender, age, and underlying condition of the patient. In the Boston Collaborative Drug Surveillance Program the incidence of gastrointestinal bleeding requiring transfusion for medical inpatients without predisposing illness was 1.2% and 0.2% for heparin and warfarin, respectively [274]. Minor gastrointestinal bleeding occurred in 8.3% and 3.3%, respectively. Gastrointestinal bleeding should be thoroughly investigated, even if it is a minor symptom, and regardless of the prothrombin time [275–279]. Most episodes of gastrointestinal bleeding in patients who are taking anticoagulants are attributable to a demonstrable gastric or intestinal lesion [278,279].

The most common hemorrhagic small intestine complication is spontaneous or trauma-induced intramural hematoma [280], usually in the jejunum, presenting as intestinal obstruction [281–283]. This has been reported with both oral and parenteral anticoagulants in the setting of supratherapeutic coagulation parameters. The relative fixation of the descending duodenum is responsible for a higher susceptibility to intramural hematoma in response to blunt abdominal trauma [280]. Hematomas may cause colicky abdominal pain and symptoms secondary to intestinal obstruction. Biliary symptoms or pancreatitis may result from obstruction of the biliary or pancreatic ducts. Minor intestinal bleeding is detected in 25%, and major bleeding occurs in 3.5% [284]. Patients may present with an acute abdomen that can be differentiated from other causes by the more gradual onset of symptoms and the acute fall in hematocrit [285].

Abdominal tenderness, low-grade fever, and a palpable abdominal mass are common physical findings. Evidence of proximal small bowel obstruction may be present on plain abdominal radiographs. An upper gastrointestinal series may show the classic coiled-spring, picket-fence, or stack-of-coins sign (Fig. 55.6), caused by extravasation of blood into the valvulae conniventes, narrowing the lumen with spike-like projections of barium outlining the normal caliber of the lumen [275,280]. Accurate diagnosis is provided by CT scans, and findings may include circumferential wall thickening, intramural hematomas, and lumenal narrowing [282]. Serial ultrasound examinations can be useful for monitoring the behavior of the hematoma [286].

Conservative medical management is usually sufficient because most hematomas undergo spontaneous reabsorption, with resolution of symptoms within 3 weeks. Reversal of anticoagulation with fresh-frozen plasma and vitamin K, intravenous hydration, and continuous nasogastric suction are sufficient for management of most patients. Surgical intervention may be required if there is complete intestinal



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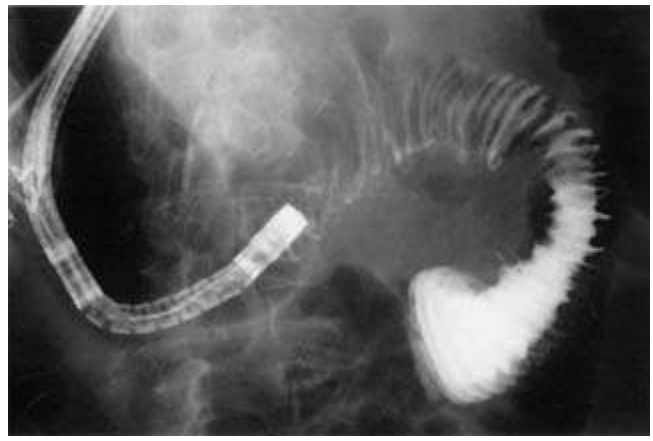

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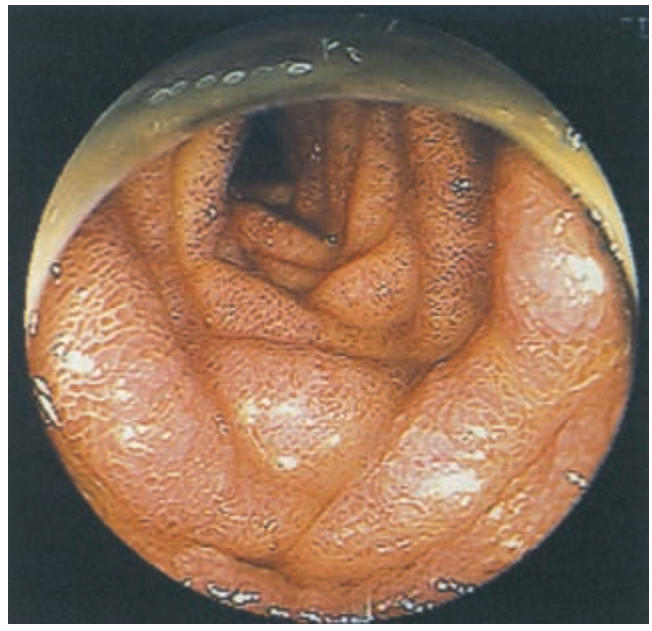

(c)

Figure 55.6 Small intestine intramural hematoma in a patient with a prothrombin time internationalized ratio of 14.6 as a result of coumadin (warfarin) therapy. **(a)** Abdominal CT scan demonstrating circumferential and homogeneous jejunal wall thickening. **(b)** Jejunal contrast study obtained 5 days later demonstrating the "stack of coins" appearance due to thickened mucosal folds. **(c)** Endoscopic view obtained using double-balloon enteroscopy demonstrates the erythematous and edematous jejunal segment corresponding to the images in (a) and (b). From Shinozaki et al. [439], with permission from Springer Science and Business Media.

obstruction or if conservative medical management fails to resolve intestinal bleeding, obstructive symptoms, or fever [277,284].

Drugs causing motility disorders

Drug-induced intestinal pseudoobstruction can occur as follows: with use of anticholinergic drugs; with use of drugs, such as phenothiazines and tricyclic antidepressants, opioids, verapamil, and clonidine, with anticholinergic effects; and, occasionally, with initiation of cyclosporine therapy. Neurotoxicity induced by such drugs as vincristine can also produce intestinal pseudoobstruction [287], which frequently appears within 3 days of the initiation of therapy and resolves within 2 weeks of cessation of therapy. Direct toxicity to the enteric nervous system is probable because evidence of peripheral nerve dysfunction is not always present in patients with this complication.

Persistent use of narcotic analgesics can produce the *narcotic bowel syndrome*, characterized by abdominal pain, intermittent vomiting, weight loss, and other symptoms suggestive of intermittent pseudoobstruction [288]. Poorly localized chronic abdominal pain that is colicky, with acute exacerbations, is typically the major symptom. The pain is initially responsive to narcotic analgesics, but, with continued narcotic use, progressively larger doses are required for pain relief. Although the differential diagnosis includes biliary colic, pancreatitis, peptic ulcer disease, and renal calculi, the history, physical examination, and laboratory tests are usually not consistent with these entities. Abdominal radiographs typically are consistent with ileus and, on rare occasions, with mechanical small bowel obstruction [288]. The diagnosis of narcotic bowel syndrome is readily made with an intake history of more than 2 weeks of moderate to heavy doses of narcotics and a workup that excludes more serious disorders [288].

Narcotic withdrawal is essential for treatment of narcotic bowel syndrome. On withdrawal of these drugs, the patient may experience vomiting, diarrhea, and cramping resulting from increased intestinal motility [288]. These symptoms can be markedly reduced or abolished by the α_2-adrenergic receptor agonist, clonidine [289,290]. Opiates are discontinued, and clonidine is gradually increased from 0.1 mg twice daily to 0.1 mg four times daily [289]. After 1 week of therapy, the dose is tapered over 3 days. Narcotic bowel syndrome and withdrawal symptoms can be successfully treated in 90%–95% of patients with this regimen. Metoclopramide, narcotic antagonists, and rotating opioid use have been used with variable benefit in patients with cancer [291,292].

Drugs causing malabsorption

Drug-induced malabsorption of nutrients, electrolytes, and concurrently used medications occurs by several different mechanisms [235,262,293,294]. These include the following:

- intralumenal interactions that interfere with the solubilization, digestion, or transport of nutrients
- increased rate of intestinal transit
- mucosal injury
- direct inhibition of absorptive processes
- inhibition of gastric, biliary, or pancreatic secretions.

Drugs that impede nutrient assimilation by direct interaction include the following: tetracycline, which chelates calcium ions; cholestyramine, which binds to iron and vitamin B-12; mineral oil, which reduces the solubilization of β-carotene and fat-soluble vitamins; and aluminum and magnesium hydroxide, which precipitate calcium and phosphate ions. Prokinetic agents and cathartics can impair fat absorption by increasing intestinal transit [295]. Mucosal injury resulting in diminished nutrient absorption has been reported with drugs such as colchicine, neomycin, and methotrexate. Colchicine inhibits mitosis and brush-border disaccharidases and impairs absorption of fat, vitamin B-12, β-carotene, D-xylose, lactose, bile salts, and steroids [296]. Neomycin is thought to cause brush-border damage by inhibiting enterocyte protein synthesis [297,298], and to impair micellar solubilization of bile salts, cholesterol, fatty acids, and fat-soluble vitamins by directly binding to bile salts. Azotorrhea and decreased D-xylose absorption can also occur with neomycin. The malabsorption varies in degree according to the dose of neomycin, and can be seen with as little as 3 g/day [297]. Discontinuation of the drug typically reverses the malabsorption and diarrhea.

Methotrexate decreases the height of intestinal microvilli and brush-border membrane protein and lipid content. Other drugs that produce histological changes in the jejunal mucosa associated with fat malabsorption include methyldopa [299], allopurinol [300], and mefenamic acid [301]. The histological features of mefenamic acid enteropathy closely resemble those associated with celiac disease. Sodium aminosalicylate [302] and thiazide diuretics, which impair ileal vitamin B-12 and sodium transport, respectively, are examples of drugs that directly inhibit nutrient transport. A reported case study convincingly showed that azathioprine therapy induced small bowel villus atrophy and associated severe diarrhea; the malabsorption was reversed with drug discontinuation [303].

Clofazimine, when used in high doses for the treatment of leprosy, can cause a distinctive enteropathy characterized by red-brown birefringent crystals in the small bowel mucosa and submucosa as well as in the mesenteric lymph nodes [304]. Clofazimine enteropathy is associated with nonspecific clinical and radiological signs including diarrhea, abdominal pain, anorexia, vomiting, diarrhea, and weight loss but not clinically significant malabsorption [305].

The clinical significance of drug-induced malabsorption is influenced by such variables as baseline nutritional status and dietary intake, underlying disorders that interfere with intestinal function, and pharmacological considerations

such as drug dosage and schedule and duration of therapy. Awareness of the nutritional consequences of drug therapy facilitates the prevention and early detection of these disorders. For some drugs, prophylactic nutritional supplementation is reasonable. For example, patients treated on a long-term basis with sulfasalazine, phenytoin, or colestipol should receive folic acid, and patients receiving the latter two drugs should also receive vitamin D supplementation.

Chemotherapeutic agents

Most chemotherapeutic agents produce cytotoxic effects in normal cells because they have low therapeutic indices. Cells that have a high turnover rate, such as those of the small intestine crypt epithelium, are particularly vulnerable to drugs that inhibit cell proliferation. The magnitude of gastrointestinal toxicity is highly variable among patients and is affected by dose, duration of treatment, specific agents used, concurrent radiotherapy, and nutritional status of the patient. Nausea and vomiting are common acute side effects of antineoplastic drugs and are mediated in part by the chemoreceptor trigger zone in the brain. These symptoms predominate in the early stages of therapy. After a single course of therapy, intestinal mucosal damage is usually evident within the first 3 days. Regeneration and repair occur rapidly after cessation of therapy, with grossly normal mucosa present by 14 days, although inflammatory changes may be present for several weeks [306].

Erosive enteritis, with or without stomatitis, presents with abdominal pain, bleeding, vomiting, ileus, or diarrhea. It is most common with methotrexate therapy but is also seen with 5-fluorouracil, irinotecan, actinomycin D, doxorubicin, cytarabine (cytosine arabinoside; Ara-C), bleomycin, and vincristine. Other complications result from these chemotherapeutic agents. Methotrexate is also associated with malabsorption (see above). Irinotecan also causes cholinergically mediated diarrhea within hours of infusion in ~ 50% of patients [307], in addition to late-onset diarrhea resulting from secretory and exudative mechanisms [308]. The erosive enteritis associated with cytarabine when used in combination regimens occurs within 8–11 days of the start of therapy and is associated with hypokalemia, hypocalcemia, PLGE, and a syndrome characterized by telangiectasis and intramural hematomas [309]. Recombinant interleukin-2 (IL-2), which is used for treatment of advanced renal cell carcinoma, has been associated with intestinal ischemia, necrosis, perforation, and diffuse bowel ulceration requiring surgery [310]. These complications have occurred with IL-2 alone or in combination with γ-interferon (IFN-γ) or lymphokine-activated killer cells. Cyclosporine-induced vasoconstriction can contribute to the development of nonocclusive mesenteric ischemia in renal transplant recipients [311]. Vincristine can induce acute intestinal pseudoobstruction (as already discussed). Preconditioning for bone marrow transplantation with cyclophosphamide and total-body irradiation produces diffuse intestinal injury. Symptoms, including crampy abdominal pain, watery diarrhea, and anorexia, are common within the first 3 weeks after transplantation. Symptoms that persist beyond 3 weeks should be investigated to rule out enteric infection or other intestinal disorders. Neutropenic enterocolitis or typhlitis is most frequently encountered after chemotherapy for leukemia or lymphoma. Diffuse patchy mucosal necrosis involving the ileocecal region is associated with nonspecific symptoms and fever, nausea, vomiting, right lower quadrant pain, and hematochezia. Colon complications resulting from these drugs are discussed in Chapter 59.

Necrotizing enterocolitis

There are several synonyms for nectrotizing enterocolitis (NEC), including pigbel, enteritis necroticans, darmbrand, nonspecific jejunitis, epidemic regional jejunitis, Pasini regional jejunitis, and necrotizing jejunitis. Although there were numerous European outbreaks during and after World War II [312], acute jejunitis is largely a problem in nonindustrialized nations [313–315]. Outbreaks are most frequent in communities where protein deprivation and poor food hygiene are prevalent [316]. The disease was a major cause of illness and death among children in the highlands of Papua New Guinea until immunization against the β-toxin of the causative organism, *Clostridium perfringens,* was begun in 1980 [317]. Isolated cases continue to be reported from industrialized nations, although detection of the causative organism is not always reported [318–321]. Well-documented North American cases include four previously healthy adult males [321] and a 12-year-old boy with poorly controlled diabetes who had eaten pig intestines (chitterlings) [322].

C. perfringens type C (initially identified as *C. welchii* type F) is a heat-resistant bacterium that can often be isolated from the tissues, stool, and food of affected patients [323–326]. One-sixth of healthy persons have been shown to harbor a less pathogenic strain. A similar illness attributed to pathogenic strains of this organism has been described in certain nonhuman animal species [327,328]. Immunization against the β-toxin resulted in a reduction in hospital admissions for acute jejunitis to less than one-fifth of previous figures [317]. These observations have firmly established that this organism is a causative agent of acute jejunitis. The agent has also been isolated from children in Bangladesh with nonnecrotizing diarrheal illnesses [329]. Nevertheless, there are patients who have an identical syndrome without evidence of *C. perfringens* type C infection or the β-toxin [330]. *C. perfringens* type A, which does not produce the β-toxin, was implicated as the causative agent in adult patients with an NEC-like

illness involving the small bowel and colon [322]. Another cytotoxin, isolated from *Bacillus cereus*, has been identified as a putative cause of acute jejunitis [331]. Additional factors associated with development of acute jejunitis include ingestion of foods containing trypsin inhibitors (e.g., sweet potatoes), reduced gastric acidity, and reduced gastrointestinal motility [321].

Acute jejunitis, with or without colonic involvement, occurs sporadically and in epidemics. Illness is characterized by bloody diarrhea, fever, and abdominal pain, with a high mortality rate (58%) [316]. Nonocclusive small intestinal ischemia resulting in necrosis of varying severity, with areas of full-thickness necrosis, is usually found. Early recognition, treatment with antibiotics, and surgical resection are essential for successful treatment.

Neonatal NEC is a disease of focal or diffuse small intestine ulceration and necrosis that is seen almost exclusively in premature infants and low-birthweight neonates who have been fed enterally. Similar pathophysiological mechanisms may operate in adult necrotizing enteropathies. The putative causes, clinical presentation, differential diagnosis, pathology, and management of NEC are discussed in several excellent reviews [332–341].

The cause of NEC is unknown, but several factors appear to be implicated, including prematurity, intestinal ischemia, infectious agents and bacterial colonization, and the initiation of enteral nutrition [342,343]. However, a randomized trial and subsequent reviews [344,345] are at odds with earlier case–control studies suggesting that stressed infants are at greater risk of developing NEC if enteral feedings are advanced rapidly or if excessive volumes are used [346–349]. The pathogenesis of NEC is clearly multifactorial, and susceptibility of individual patients to NEC is probably also dependent on unidentified host factors [342,343]. For example, prospective studies that reported a reduced incidence and severity of NEC in neonates randomized to receive probiotics support a role for alterations of the gut flora in the pathogenesis and treatment of this disorder [350,351]. Successful delineation of these factors and other pathogenic mechanisms should improve the ability to prevent and treat NEC and should provide insight into the mechanisms of similar disorders seen in adults.

Protein-losing gastroenteropathy

Definition

Protein-losing gastroenteropathy (PLGE) is a syndrome characterized by enteric loss of plasma proteins in abnormal amounts. Many intestinal and extraintestinal diseases may be associated with PLGE, and treatment is aimed almost exclusively at the underlying disorder. The recognition of PLGE mandates a careful search for an associated illness.

The defining characteristic of PLGE is hypoproteinemia resulting from gastric or intestinal losses. Studies of iodine 131-labeled albumin secretion identified the stomach as the site of excessive protein loss in patients with hypertrophic gastritis and atrophic gastritis, and the intestine as a site of loss in patients with inflammatory bowel disease.

Under physiological conditions, sloughed enterocytes and pancreatic and biliary secretions account for almost all the endogenous protein that is found in the intestine. Most of these proteins are digested, and the constituent amino acids are reabsorbed [352,353]. Gastrointestinal loss of serum proteins, as indicated by measuring serum albumin kinetics, accounts for less than 10% of daily protein catabolism [354–358]. This may increase four- to fivefold in patients with PLGE. Hypoproteinemia results if the capacity to increase protein synthesis is not sufficient to compensate for increased intestinal protein losses. For example, albumin synthesis is increased no greater than two-fold in patients with PLGE [359].

Etiology

Most gastrointestinal disorders associated with PLGE cause exudative protein losses secondary to ulcerative or nonulcerative enteropathies or secondary to lymphatic obstruction or increased lymphatic hydrostatic pressure (Table 55.3). Enteropathies resulting in PLGE include ulcerating gastrointestinal carcinomas and inflammatory disorders such as Crohn's disease and ulcerative colitis. Increased enteric protein loss and increased catabolism are the primary contributors to the hypoalbuminemia and reduced protein pools that are seen in these disorders. In the case of inflammatory bowel disease, the magnitude of enteric protein loss is most significant, and it is directly related to the extent and activity of mucosal inflammation [360,361].

Nonulcerative diseases such as Ménétrier disease [362], atrophic gastritis, tropical sprue, celiac disease, allergic gastroenteritis, eosinophilic gastroenteritis, collagenous colitis, and polyposis syndromes (including Cronkhite–Canada syndrome, juvenile polyposis, and Peutz–Jeghers syndrome) have also been associated with PLGE [362–371]. In addition to neurological symptoms, defects in glycoprotein biosynthesis (the carbohydrate-deficient glycoprotein syndromes) can also present with severe PLGE [372].

Acute and chronic intestinal infections can result in PLGE, including acute staphylococcal, *Salmonella* [352], and *Shigella* [373] infections. The incidence of PLGE is species and strain dependent; for example, *Shigella dysenteriae* type 1 infections cause greater loss of enteric protein than do other strains [373]. Both pseudomembranous colitis and asymptomatic colonization by *C. difficile* can result in enteric loss of protein [374,375]. In a study of elderly nursing home patients, PLGE was documented in all 12 patients with pseudomembranous colitis, in 6 of 14 with *C. difficile* diarrhea, and in 6 of 12

Table 55.3 Causes of protein-losing gastroenteropathy

Increased interstitial pressure
Congenital intestinal lymphangiectasia, mesenteric lymphatic obstruction, tuberculosis, sarcoidosis, lymphoma, retroperitoneal fibrosis, increased central venous pressure, constrictive pericarditis, congestive heart failure, Fontan procedure
Ulcerative diseases
Erosive gastritis or enteritis, *Helicobacter pylori*-associated gastritis, neoplasia (carcinoma or lymphoma), Crohn's disease, pseudomembranous enterocolitis, acute graft-versus-host disease
Nonulcerative diseases
Giant hypertrophic gastropathy (Ménétrier disease), viral enteritides, bacterial overgrowth, parasitic diseases (malaria, giardiasis, schistosomiasis, helminth infections), cystic fibrosis, Whipple disease, allergic enteritis, eosinophilic gastroenteritis, gluten-sensitive enteropathy, tropical sprue, systemic lupus erythematosus

colonized with *C. difficile* without toxin or diarrhea [375]. Parasitic diseases with documented PLGE include *Strongyloides stercoralis* infection [376], schistosomiasis [377,378]. and giardiasis [379–381]. Acute viral illnesses including cytomegalovirus, measles, and varicella also have been linked to PLGE [382–386]. Small bowel bacterial overgrowth can also be associated with PLGE [387].

It has been well documented that PLGE occurs in patients with SLE [388–395], most commonly in young women; moreover, it may be the initial clinical manifestation of SLE. Fifty percent of these patients present with steroid-responsive diarrhea without steatorrhea [396]. Mixed connective tissue disease [397,398], Henoch–Schönlein purpura [399], and other collagen-vascular diseases also can be complicated by PLGE [400]. Another cause of PLGE is secondary amyloidosis complicated by small intestinal tract ulcers, resulting from arteriole and lymphatic infiltration with amyloid deposits [401,402]. Thee is also an association of PLGE with mesenteric vascular changes resulting from neurofibromatosis [403] and idiopathic mesenteric thrombophlebitis [404].

Diseases that produce disruption of intestinal lymphatic vessels or obstruction of lymph flow are an additional cause of PLGE. Tortuous, dilated mucosal and submucosal lymphatic vessels are the hallmark of primary intestinal lymphangiectasia (see Chapter 46). Most patients with this disease present by 30 years of age with edema, diarrhea, hypoproteinemia, and lymphocytopenia resulting from epithelial leakage and lymphatic rupture [405]. A similar lesion has been implicated as the mechanism of enteric protein loss in some patients with systemic sclerosis and SLE [406,407].

Inflammatory granulomatous diseases such as sarcoidosis and mesenteric tuberculosis cause PLGE [408,409]. By producing retroperitoneal lymph node enlargement or fibrosis, Whipple disease [410,411], lymphoma [412], endometriosis of the small intestine [413], and Crohn's disease [360,414] impair lymphatic egress by causing mechanical obstruction or increased hydrodynamic pressure. Additional retroperitoneal processes, including pancreatitis, pancreatic cancer, and other retroperitoneal tumors, also can present with PLGE. Right-sided congestive heart failure, constrictive pericarditis, superior vena caval obstruction, and other causes of elevated central venous pressure [415,416] commonly produce PLGE.

Clinical features

The major clinical manifestation of PLGE is dependent edema resulting from decreased plasma oncotic pressure. Although all plasma proteins are usually lost, selective loss of low-molecular-weight proteins may occur without lymphatic obstruction. Proteins such as albumin, immunoglobulins (IgM, IgG, and IgA but not IgE), fibrinogen, ceruloplasmin, and α_1-antitrypsin that have long circulating half-lives and a limited capacity for increased synthesis are most likely to exhibit depressed levels. Decreased levels of proteins other than albumin are rarely symptomatic, although in the setting of lymphatic obstruction, symptomatic lymphopenia and steatorrhea may be present.

Diagnosis

Edema and hypoproteinemia resulting from enteric protein loss are presenting manifestations of disease, but they are often minor aspects of the underlying disease. The diagnosis should always be considered in hypoalbuminemic patients if other causes such as malnutrition or protein loss from other sites are not apparent. If it is necessary to document intestinal protein losses, the preferred method is to quantitate fecal α_1-antitrypsin concentration or clearance. Alternative methods employing radiolabeled substrates for quantitating enteric protein loss remain useful research tools, but they are seldom used for clinical purposes. Clinical studies indicate that PLGE can be diagnosed and the sites of albumin loss can occasionally be determined scintigraphically using ^{99m}Tc-labeled albumin or dextran [417–422].

α_1-Antitrypsin is particularly valuable because it constitutes approximately 4% of serum proteins, has a molecular weight similar to that of albumin, is resistant to proteolysis, is not actively absorbed or secreted, is normally present in low quantities in stool, and is easy to assay. For detecting protein-losing gastropathy, particularly in patients with Zollinger–Ellison syndrome, acid secretion must be suppressed because α_1-antitrypsin is degraded at a pH below 3 [423–425]. The α_1-antitrypsin concentration in stool and the plasma clearance of α_1-antitrypsin were compared in healthy persons and

in consecutive patients with chronic diarrhea, malabsorption, or unexplained hypoalbuminemia [426]. The plasma clearance is the product of daily stool volume and stool α_1-antitrypsin concentration divided by the serum α_1-antitrypsin concentration [427]. In contrast to earlier studies [428–430] there is ample evidence that analysis of fecal α_1-antitrypsin concentration is not a reliable substitute for measurement of α_1-antitrypsin clearance [426]. There is a highly significant correlation between α_1-antitrypsin clearance and serum albumin concentration and fecal loss of radiolabeled albumin. When the α_1-antitrypsin clearance was elevated more than three-fold, serum albumin levels were less than 3.0 g/dL. α_1-Antitrypsin clearance levels may be increased in otherwise healthy persons with diarrhea secondary to ingestion of lactulose, sorbitol, sodium sulfate, or phenolphthalein [426]. Clearance levels also may be overestimated if hematochezia or meconium is present.

Therapy

There is no specific therapy for PLGE. Optimal therapy of the primary illness is the only effective remedy. For example, PLGE associated with autoimmune diseases (e.g., CREST syndrome [calcinosis, Reynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasia] and SLE) has been successfully treated by aggressive immunosuppressive regimens including steroids, cyclosporine, azathioprine or cyclophosphamide [388,431,432]. Octreotide and medium-chain triglycerides were beneficial when added to immunosuppressive therapy in a patient with SLE [389]. Severe PLGE with intractable diarrhea due to systemic AA amyloidosis has also been successfully treated with corticosteroids and octreotide [433]. In the case of Ménétrier disease, octreotide and eradication of *Helicobacter pylori* may reduce enteral protein losses [434,435]. Prophylactic measures to avoid complications resulting from peripheral edema should be instituted. In the setting of lymphangiectasia, reduction of lymphatic transport may reduce enteric protein loss. Low-fat diets supplemented with medium-chain triglycerides may be beneficial in this setting [436,437]. Surgical drainage of dilated lymphatic channels (lymphovenous anastomosis) is occasionally helpful [414,438].

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Anatomy of the colon

The colon is a tubular organ that extends from the ileocecal valve to the proximal rectum. It is approximately 90–150 cm (3–5 feet) in length. Its principal functions are absorption of water and electrolytes as well as storage of intralumenal contents to permit controlled elimination of the feces. The terminal ileum enters the cecum on its posteromedial border at the ileocecal valve. The cecum is a large, blind pouch, approximately 7.5–8.5 cm in diameter, which projects from the antimesenteric side of the ascending colon. The colon progressively diminishes in size toward the sigmoid colon, which is approximately 2.5 cm in diameter and is the narrowest portion of the colon. This size discrepancy accounts for the fact that cecal tumors often grow to be large and bulky before the onset of symptoms but sigmoid tumors are symptomatic at smaller sizes. In addition, tension on the bowel wall is directly proportional to the diameter of the bowel. This is explained by the law of LaPlace: $T = PR$, where T is tension in the wall of the bowel, P is internal pressure, and R is the radius of the bowel. Because the cecum has the largest diameter, it is usually the first part of the bowel to rupture as a result of distal obstruction.

The omentum is attached to the transverse colon on its anterior superior edge. The ascending colon, descending colon, rectum, and posterior surface of the hepatic and splenic flexures are fixed retroperitoneal structures. The cecum, transverse colon, and sigmoid colon are intraperitoneal and are prone to volvulus because of their location and relative lack of fixation.

The longitudinal muscle is an incomplete layer and is seen as three bands of muscle, called taeniae coli, located 120° apart around the circumference of the colon. The taeniae converge proximally at the appendix as distinct bands and disappear at the level of the sacral promontory. Haustra coli are sacculations between the taeniae and are separated by crescent-shaped folds called plicae semilunares. Appendices epiploicae are fatty appendages covered by peritoneum and have no anatomical or pathological significance.

The rectal wall consists of mucosal, submucosal, inner circular, and outer longitudinal muscular layers (Fig. 56.1). There is no serosal layer in the rectum. The rectum is approximately 12–15 cm in length and extends from the sigmoid colon to the anal canal, following the curve of the sacrum. The proximal rectum begins at the sacral promontory (third sacral vertebra), at which point the taeniae fan out to form a complete layer of longitudinal muscle.

The anal canal is approximately 4 cm long and appears as a collapsed anteroposterior slit in the normal patient (see Fig. 56.1). The anal verge is the junction between anal and perianal skin. Anal epithelium (anoderm) lacks hair follicles, sebaceous glands, and sweat glands. The dentate line is a true mucocutaneous junction located 1–1.5 cm above the anal verge. A 6–12-mm transitional zone exists above the dentate line, in which the squamous epithelium of the anoderm becomes cuboidal and then columnar epithelium. The columns of Morgagni are 8–14 mucosal folds located just above the dentate line that are surrounded by anal crypts. Small rudimentary glands open into some of the crypts. These glands go through the internal sphincter into the intersphincteric groove but do not penetrate the external sphincter. The anorectal ring is 1–1.5 cm above the dentate line and is the palpable upper border of the anal sphincter complex. The anatomical anal canal extends from the anal verge to the dentate line. The surgical anal canal extends from the anal verge to the anorectal ring.

Arterial supply

The superior mesenteric artery arises from the ventral surface of the aorta below the celiac axis, passes behind the pancreas, and crosses in front of the third portion of the duodenum

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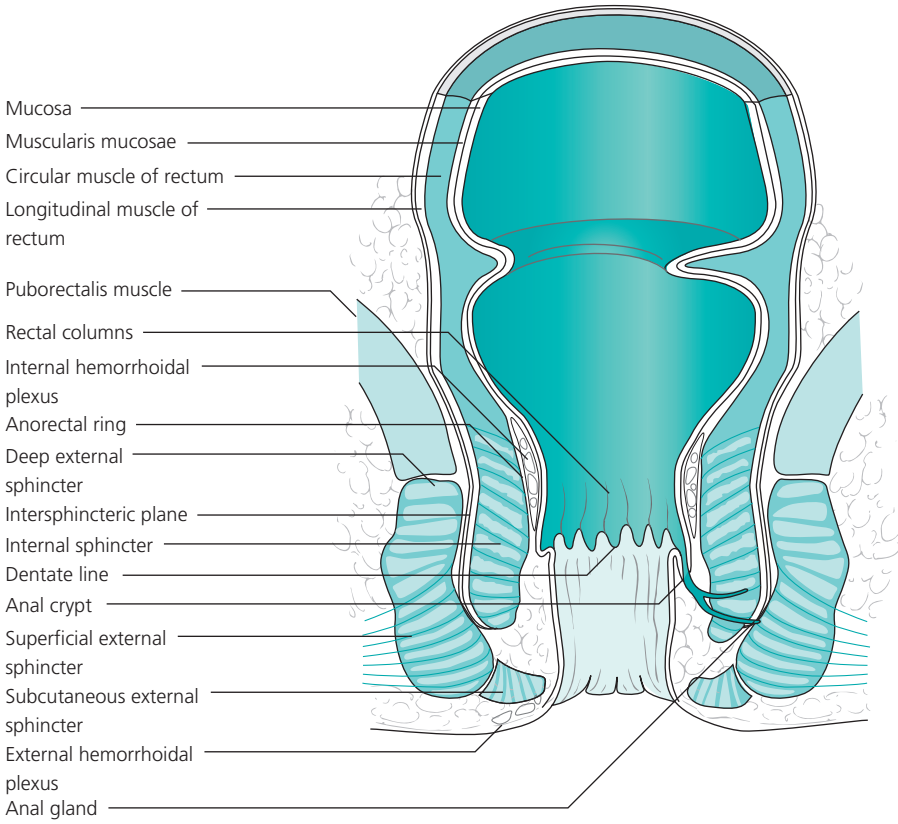

Figure 56.1 Anorectal anatomy. Illustrated by John A. Craig. From Clinical Symposia 1985;37/6.

(Fig. 56.2). The cecum, ascending colon, and transverse colon are supplied through its ileocolic and middle colic branches. Cadaver studies have shown that the right colic artery arises from the ileocolic artery in the majority of patients and from the superior mesenteric artery in less than 15% of patients [1]. The inferior mesenteric artery arises from the infrarenal aorta and supplies the descending colon, sigmoid colon, and upper rectum through its left colic, sigmoidal, and superior rectal branches.

Collaterals exist between the superior and inferior mesenteric arteries in the region of the splenic flexure [2]. The arcades of the ileocolic, right, middle, and left colic arteries are peripherally connected by the marginal artery of Drummond, which runs along the mesenteric border of the colon and provides the vasa recta to the colon. The arc of Riolan is a tortuous, inconstant vessel that exists between the left colic branch of the inferior mesenteric artery and the middle colic branch of the superior mesenteric artery. It is frequently referred to as the meandering mesenteric artery and is best seen if there is an occlusion of either the inferior or superior mesenteric artery.

The terminal branch of the inferior mesenteric artery becomes the superior rectal artery, which descends in the sigmoid mesocolon and bifurcates at the level of the third sacral vertebra (Fig. 56.3a). Branches of the superior rectal artery supply the upper and middle rectum. The middle rectal arteries arise from the internal iliac arteries, and the inferior rectal arteries are branches of the internal pudendal arteries. These arteries supply the lower two-thirds of the rectum [3]. The middle sacral artery arises just before the aortic bifurcation and provides little blood supply to the rectum. Anastomosis exists between the middle and superior rectal arteries; there are no anastomoses with the inferior rectal arteries. Preservation of the middle rectal arteries is necessary to maintain viability of the rectal stump after high ligation of the inferior mesenteric artery [3].

Venous drainage

The veins draining the colon follow the same course as the corresponding arteries except for the inferior mesenteric vein. The inferior mesenteric vein drains the descending colon, sigmoid colon, and proximal rectum and runs in a retroperitoneal location to the left of the ligament of Treitz, at which point it enters the splenic vein. The superior mesenteric vein drains the cecum, ascending colon, and transverse colon and joins the splenic vein to form the portal vein.

The venous drainage of the rectum enters the portal or systemic (caval) system. The upper and middle rectum are drained by the superior rectal vein, which enters the portal system through the inferior mesenteric vein (Fig. 56.3b). The

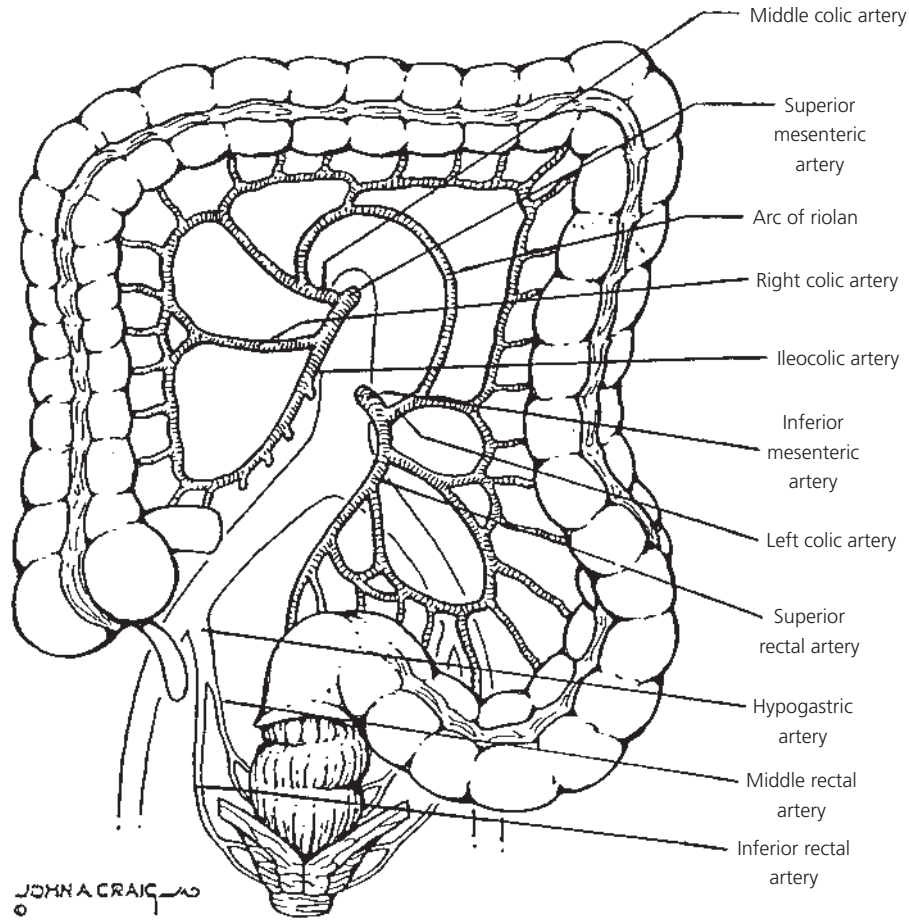

Figure 56.2 Arterial blood supply of colon and rectum. From Kodner IJ, Fry RD, Fleshman JW, Birnbaum EH. Colon, rectum and anus. In: Schwartz SI (ed.). Principles of Surgery, 6th edn. New York: McGraw-Hill, 1993, with permission from the McGraw-Hill Companies.

lower rectum and upper anal canal are drained by the middle rectal vein, which empties into the internal iliac veins and then into the caval system. The inferior rectal veins drain the lower anal canal and empty into the pudendal veins, which empty into the caval system through the internal iliac veins. Rectal tumors can metastasize through venous channels into either the portal or systemic venous systems.

Three submucosal internal hemorrhoidal complexes (normal hemorrhoidal tissue) are located above the dentate line. Hemorrhoidal tissue receives its blood supply from the superior, middle, and inferior rectal arteries. The left lateral, right posterolateral, and right anterolateral internal hemorrhoids drain into the superior rectal vein (Fig. 56.3b). The external hemorrhoids are located below the dentate line and drain into the pudendal veins. There is communication between the internal and external plexi, and mixed internal–external hemorrhoids result if these communications become engorged (see Chapter 66).

Lymphatic drainage

The colonic mucosa has rich vascular plexi but no lymphatics. Lymphatic capillaries encircle the colon in the submucosal and muscularis mucosae layers. This segmental architecture limits longitudinal intramural extension of tumors, and circumferential extension results in annular lesions. Lymphatic vessels follow the blood supply of the colon.

Lymph nodes are located on the bowel wall (epicolic), along the inner margin of the bowel (paracolic), around the named mesenteric arteries (intermediate), and along the origin of the superior and inferior arteries (main) (Fig. 56.4). Lymph from the upper and middle rectum drains into the inferior mesenteric nodes. The lower rectal lymphatics follow the superior rectal artery and enter the inferior mesenteric nodes. Lymph from the lower rectum can also flow laterally along the middle and inferior rectal arteries, posteriorly along the middle sacral artery, or anteriorly through channels in the rectovesical or rectovaginal septum [4]. These channels drain to the iliac nodes and subsequently to periaortic lymph nodes.

Lymphatics from the anal canal above the dentate line drain through the superior rectal lymphatics to the inferior mesenteric lymph nodes or laterally to the internal iliac lymph nodes. Below the dentate line, the lymphatics drain primarily to the inguinal nodes but they can drain to the inferior or superior rectal lymph nodes as well [4].

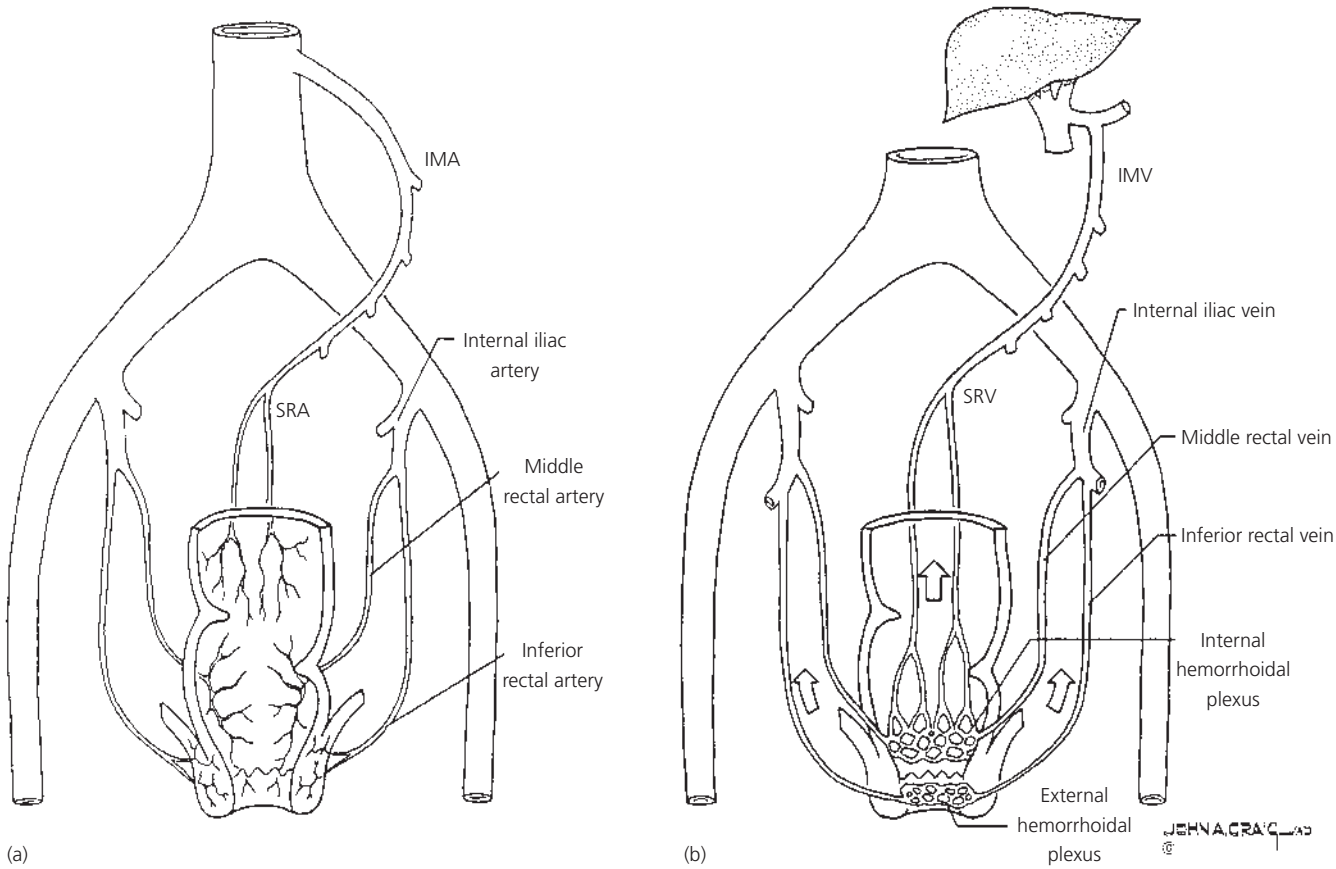

Figure 56.3 Vascular supply of the anus and rectum. **(a)** Arterial supply. **(b)** Venous drainage. IMA, inferior mesenteric artery; IMV, inferior mesenteric vein; SRA, superior rectal artery; SRV, superior rectal vein. From Kodner IJ, Fleshman JW, Fry RD. Anal and rectal cancer: principles of management. In: Schwartz SI, Ellis H (eds). Maingot's Abdominal Operations, 9th edn. Norwalk, CT: Appleton & Lange, 1989, with permission from the McGraw-Hill Companies.

Nerve supply

The sympathetic and parasympathetic nerves to the colon follow the course of the blood vessels. Sympathetic nerves inhibit and parasympathetic nerves stimulate peristalsis. Sympathetic fibers to the right colon originate in the lower six thoracic segments and travel in the thoracic splanchnic nerves to the celiac plexus and then to the superior mesenteric plexus. The sympathetic supply to the left colon and rectum originates in the first three lumbar segments. These nerves join the preaortic plexus and become the inferior mesenteric plexus below the bifurcation of the aorta. The parasympathetic supply to the right colon is presumed to come from the right vagus. The parasympathetic nerves to the left colon ascend from the pelvis, pass through the sigmoid mesocolon, and spread out toward the sigmoid and the descending colon.

The rectum is innervated by both sympathetic and parasympathetic nerves [5]. Sympathetic nerves from thoracolumbar segments unite below the inferior mesenteric artery, forming the inferior mesenteric plexus, and descend to the superior hypogastric plexus below the aortic bifurcation. The nerves bifurcate and descend in the pelvis as the hypogastric nerves, supplying sympathetic innervation to the lower rectum, bladder, and sexual organs, in both males and females. Injury to the inferior mesenteric plexus can result during ligation of the inferior mesenteric artery at its origin.

Parasympathetic fibers from the second, third, and fourth sacral roots (the nervi erigentes) unite with the hypogastric nerves anterior and lateral to the rectum, forming the pelvic plexus [5]. The periprostatic plexus arises from the pelvic plexus. Mixed fibers from these plexi innervate the rectum, internal anal sphincter, prostate, bladder, and penis. The pudendal nerve (originating from the second, third, and fourth sacral nerves) mediates sensory stimuli from the penis or clitoris through the dorsal nerve. Damage to the periprostatic plexus may occur during dissection of the rectum. Injury to the pelvic autonomic nerves may result in bladder dysfunction, impotence, or both.

Below the dentate line, afferent fibers of the inferior rectal and perineal branches of the pudendal nerve convey cutaneous sensations of temperature, pain, and touch. Numerous free nerve endings make this area sensitive to sensations [6]. Above the dentate line, a poorly defined dull sensation, experienced if the mucosa is pinched or if internal hemorrhoids are ligated, is probably mediated by parasympathetic fibers.

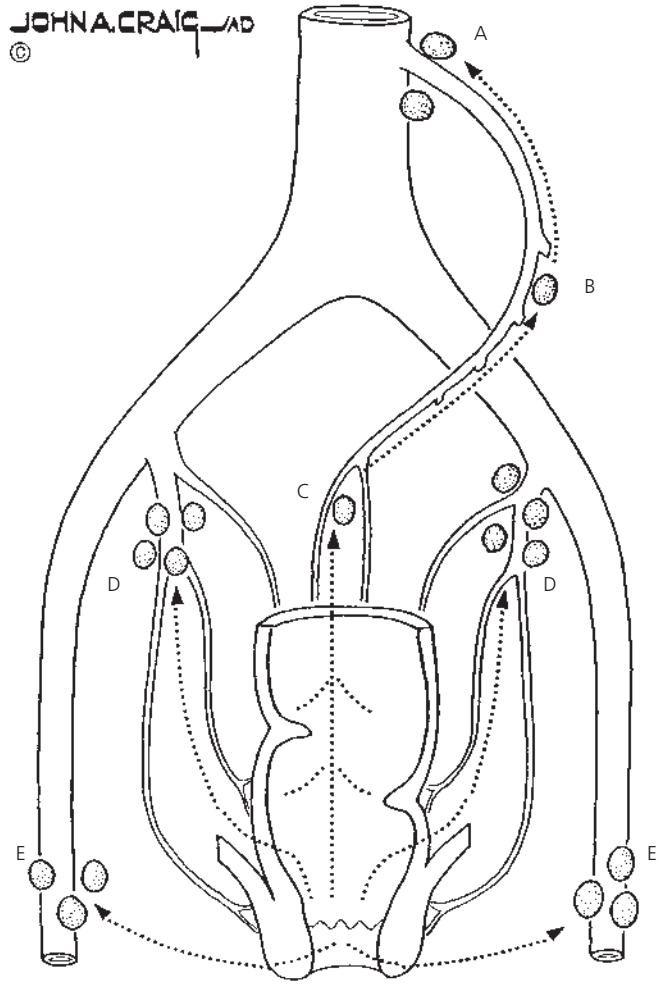

Figure 56.4 Lymphatic drainage of the rectum and anus. **(A)** Nodes at origin of inferior mesenteric artery; **(B)** nodes at origin of sigmoid branches; **(C)** sacral nodes; **(D)** internal iliac nodes; **(E)** inguinal nodes. From Kodner IJ, Fleshman JW, Fry RD. Anal and rectal cancer: principles of management. In: Schwartz SI, Ellis H (eds). Maingot's Abdominal Operations, 9th edn. Norwalk, CT: Appleton & Lange, 1989, with permission from the McGraw-Hill Companies.

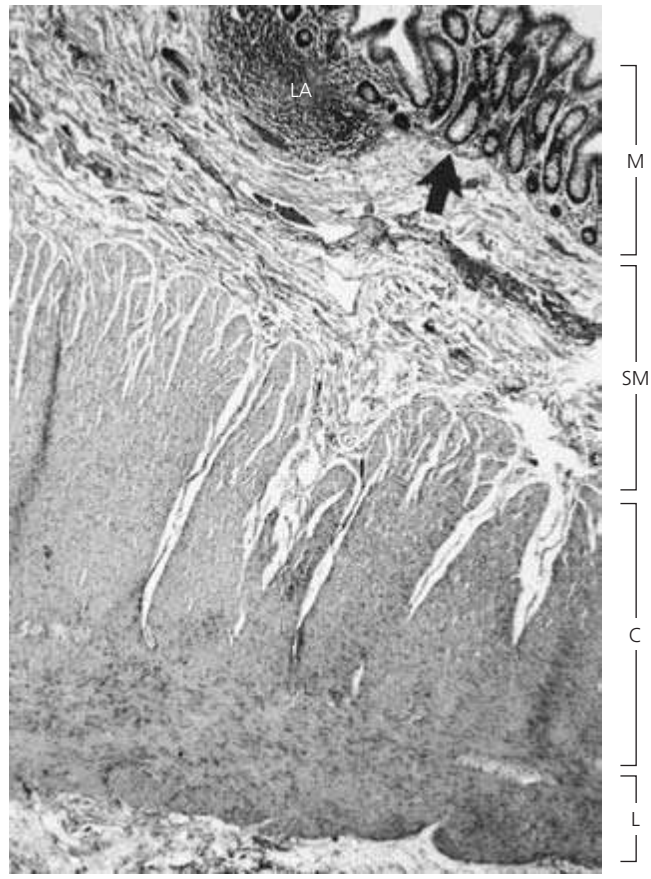

Figure 56.5 Anatomy of the colonic wall. The muscularis mucosae is indicated (arrow). C, circular smooth muscle layer; L, longitudinal smooth muscle layer; LA, lymphoid aggregate; M, mucosa; SM, submucosa. (Paraffin-embedded 4-μm H & E stained section, original magnification × 40.)

Histology

The colonic wall consists of mucosa, submucosa, inner circular muscle, outer longitudinal muscle, and serosa (Fig. 56.5). The colonic mucosa is similar in organization to the small intestine mucosa except that it lacks villi. The mucosa is lined by a simple columnar epithelium that forms straight tubular crypts that are about 0.5 mm in length (Fig. 56.6). The lamina propria extends from the simple columnar epithelium to the muscularis mucosae and contains many cells involved in immunological functions of the gut (see Chapter 7). Numerous immunoglobulin-secreting plasma cells, macrophages, and lymphocytes are present, in addition to abundant lymphoid nodules that often extend through the muscularis mucosae

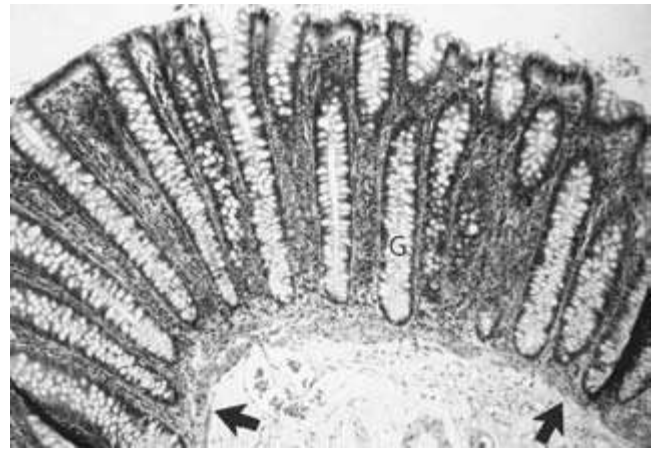

Figure 56.6 Histology of the colonic mucosa. Straight, simple colonic glands (G) extend from the muscularis mucosae (arrows) to the lumenal surface of the colon. Note the numerous small arteries, veins, and lymphatics coursing through the loose connective tissue stroma in the submucosa. (Paraffin-embedded 4-μm H & E stained section, original magnification × 100.)

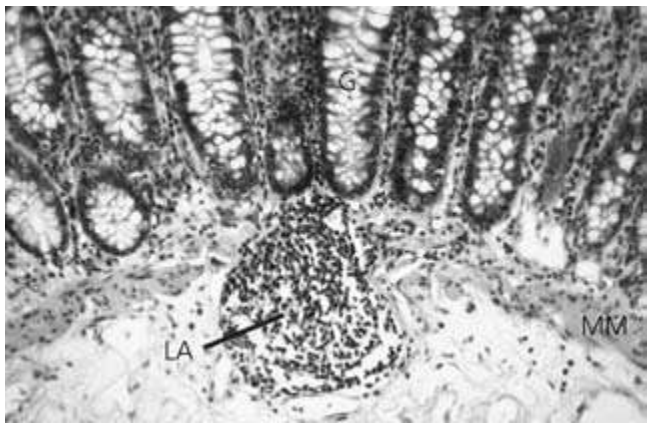

Figure 56.7 A lymphoid aggregate (LA) arises in the lamina propria and extends through the muscularis mucosae (MM) into the submucosa. (Paraffin-embedded 4-μm H & E stained section, original magnification × 200.)

into the underlying submucosa (Fig. 56.7). Subepithelial fibroblasts surround each crypt and produce the collagen table as well as many of the extracellular matrix components that underlie the basal lamina of the epithelium (Fig. 56.8). These fibroblasts and the extracellular matrix they secrete appear to have an important role in regulating cellular proliferation and differentiation within the overlying epithelium [7–11]. The submucosa contains numerous small veins, arteries, and lymphatic channels surrounded by loose connective tissue. The inner circular muscle fibers form a continuous layer around the colon. The outer longitudinal smooth muscle fibers are condensed into three bands (taeniae coli) equidistant around the circumference of the colon. Haustra are the bulging sacculations that form between adjacent taeniae coli. The serosa is a mesothelial-derived cell layer that covers the peritoneal surface of the colonic wall. Therefore, regions of the ascending colon, the descending colon, and the rectum that lie outside the peritoneal cavity have no outer serosal layer (see "Anatomy of the colon" above).

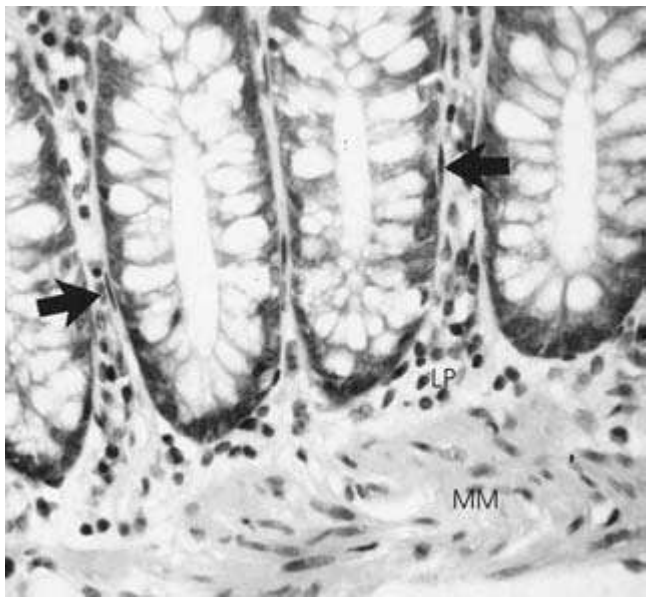

Figure 56.8 Colonic crypts. Note the subepithelial fibroblasts (arrows). Numerous plasma cells, macrophages, and lymphocytes are seen in the lamina propria (LP). MM, muscularis mucosae. (Paraffin-embedded 4-μm H & E stained section, original magnification × 400.)

The colonic epithelium undergoes continuous and rapid renewal; epithelial cells are sloughed from the flat lumenal surface of the colon and are replaced by replication of the descendants of epithelial stem cells located in the bottom one-third of each crypt. There are three principal differentiated epithelial cell types present in the adult colonic epithelium: absorptive colonocytes, goblet cells, and enteroendocrine cells. All these cell lineages appear to be derived from a common epithelial cell stem cell precursor. Undifferentiated cells, replicating cells, and enteroendocrine cells predominate near the base of each colonic gland (crypt) [12]. Cells belonging to each of the principal cell lineages differentiate during a highly ordered migration away from the zone of proliferation. The average life span of goblet cells and absorptive cells, from their birth deep in the crypt to the point where they are sloughed into the lumen, is approximately 6 days [13]. Some enteroendocrine cell subtypes appear to have a much longer life span, suggesting that their migration up the crypt to the surface epithelium must be uncoupled from that of adjacent epithelial cells [13]. The epithelial cell population of each small intestine or colonic crypt is derived from a single stem cell or, at most, a small number of stem cells [14–18]. Although the mechanisms that regulate epithelial cell renewal have yet to be fully elucidated, a number of peptide growth factors are emerging as important potential regulators of the dynamic balance between cell proliferation and cell loss in the intestinal and colonic epithelium (see Chapter 23) [19].

The principal function of absorptive columnar cells is the regulation of water and electrolyte content of the feces. The mature adult absorptive columnar cell in the colon absorbs sodium, chloride, and water; potassium and bicarbonate are secreted (see Chapter 14) [20]. In contrast to the enterocytes of the small intestine, however, colonocytes cannot absorb significant amounts of glucose or amino acids. Under normal conditions, lipid absorption is complete in the small intestine, but under pathological conditions in which lipid absorption in the small intestine is incomplete, the surface columnar cells of the colon have a limited capacity for uptake of fatty acids and subsequent packaging of them into chylomicrons [21]. As these cells differentiate during their migration up the crypt, they develop microvilli and clear, apically oriented vesicles containing a fibrillar, glycoprotein-rich secretory product that may contribute to the glycocalyx [21].

Goblet cells are flask-shaped cells with large apical vesicles that store and secrete mucus [22]. Mucus secreted by the goblet cell forms a viscous gel that functions as a lubricant

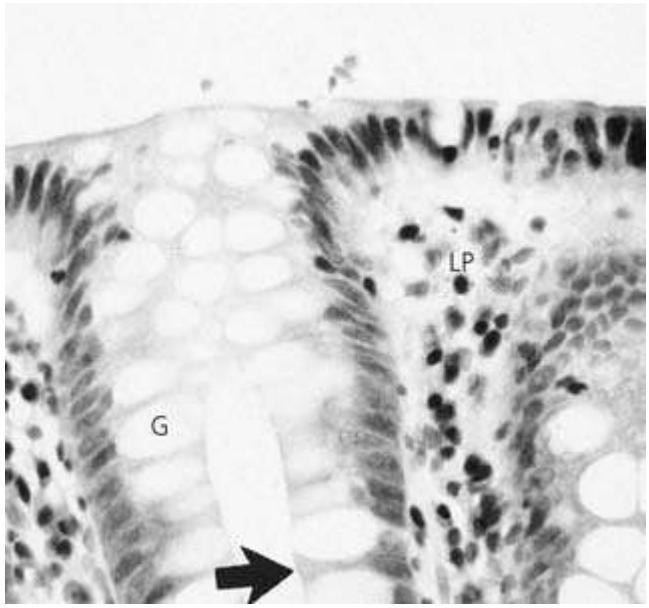

Figure 56.9 Surface epithelium of the colon. The mature absorptive columnar epithelial cells (arrow) lose their apical vesicles as they migrate into the upper regions of the colonic glands and emerge onto the lumenal surface. Goblet cells (G) elongate as they emerge onto the surface epithelium because of accelerated secretion of their mucus granules. LP, lamina propria. (Paraffin-embedded 4-μm H & E stained section, original magnification × 400.)

and also protects the surface epithelium against adherence of invading pathogens. Mucins are a family of large, sulfated glycoproteins that are synthesized in the rough endoplasmic reticulum (Fig. 56.9). Glycosylation and sulfation of these proteins occur posttranslationally in the Golgi apparatus. The composition of mucus shows regional variability along the cephalocaudal axis of the gut [22]. Mucus granules accumulate during the migration of goblet cells toward the lumenal surface of the colon, distending the apical aspect of the cell and giving it its broad, flask-like appearance. As goblet cells emerge onto the surface epithelium, the secretion of mucus occurs more rapidly, depleting the apically stored granules and causing the goblet cells to elongate (Fig. 56.9). Increased cholinergic stimulation can cause accelerated secretion of mucus from crypt-associated goblet cells, leading to deep invaginations of the apical surface. Certain components of mucus may become depleted in pathological conditions, such as ulcerative colitis (see Chapter 57).

Originally thought to be of neuroectodermal origin, the enteroendocrine cells found in the intestinal and colonic epithelium in fact arise from the same common stem cell precursor as the other principal cell lineages found in the gut epithelium. A number of different enteroendocrine cell types are found within the colonic epithelium [23,24], including:

- L cells, which contain both enteroglucagon and peptide YY (PYY)
- cells that secrete only PYY
- EC1 cells, which secrete serotonin, substance P, and [Leu]enkephalin
- pancreatic polypeptide-secreting cells
- rare somatostatin-secreting cells.

These enteroendocrine cells are characterized by their polygonal shape, their broad base, and the numerous membrane-bound secretory granules present in their basilar portions. Enteroendocrine cells are identified in pathological specimens using argentaffin or agyrophilic silver staining techniques or through immunohistochemical methods using antibodies to the particular neuroendocrine products stored in each enteroendocrine cell subtype. The narrow apical process of the enteroendocrine cell communicates with the lumen of the crypt and may serve to sense changes in the lumenal environment. Each enteroendocrine cell is joined to other adjacent cells in the epithelium through junctional complexes located near the apical pole [13]. The regulatory peptides or bioamine products stored in the basally located secretory granules are secreted through the basolateral membrane and act through paracrine or endocrine mechanisms as mediators of gastrointestinal secretion, absorptive function, and motility in response to lumenally or basolaterally derived signals (see Chapter 8). Enteroendocrine cells are more numerous in the appendix and the rectum than in the rest of the colon. This may explain, in part, the more frequent occurrence of certain subtypes of neuroendocrine tumors at these sites.

The appendix is similar in histological organization to the rest of the colon. The mucosa of the appendix consists of deep folds lined by a columnar epithelium forming simple tubular or forked glands [21]. This epithelium contains abundant goblet cells and enteroendocrine cells. Numerous lymphoid nodules are found in the lamina propria. The normal histological architecture of the adult appendix is often replaced by fibrous scar tissue as a result of subclinical bouts of appendicitis.

Colonic development

The primitive foregut, midgut, and hindgut develop during the fourth week in utero. The midgut lengthens, herniates out of the abdominal cavity, and rotates 90° counterclockwise around the superior mesenteric artery during the sixth week in utero. By the tenth week, the midgut rotates an additional 180° as it returns to the abdominal cavity. The cecum descends to the right iliac fossa, where it becomes fixed. The base of the small bowel mesentery then runs from the ligament of Treitz in the left upper quadrant to the ileocecal valve in the right lower quadrant [25,26].

The small intestine (distal to the entrance of the common bile duct), cecum, appendix, ascending colon, and proximal transverse colon all arise from the midgut and are supplied by the superior mesenteric artery. The distal transverse colon, descending colon, sigmoid colon, rectum, upper portion of

the anal canal, and part of the lower urogenital tract are derivatives of the hindgut and are supplied by the inferior mesenteric artery. The lower third of the anal canal is ectodermal in origin and is supplied by branches of the internal pudendal artery.

The cloaca is located at the distal end of the hindgut; by the end of the sixth week it is separated by the anorectal septum into a ventral urogenital sinus and a dorsal rectum [27]. The anal membrane covering the cloaca ruptures at the end of the eighth week, forming the anal canal. The dentate line is the approximate location of the transition from endoderm to ectoderm, at the junction of the upper two-thirds of the anal canal with the lower one-third.

The enteric nervous system forms during early fetal life. Neural crest cells, precursors of ganglion cells, migrate from cephalad to caudad during the first 12 weeks in utero [28]. The migration from the midtransverse colon to the anus takes 4 weeks to complete. Smooth muscle cells begin to appear within the mesenchymal layer around the tenth week of gestation. The developing circular muscle layer spreads cranially. The outer longitudinal bands of smooth muscle (taeniae coli) and haustra begin to appear between the 10th and 11th weeks of gestation [29]. By the 12th week of gestation, Auerbach and Meissner plexuses and the circular and longitudinal muscle layers of the colon are well formed, and colonic motility may be observed in isolated fetal colon as early as this developmental stage [29,30].

Failure of the neural crest cells to complete their cephalocaudal migration results in a congenital absence of the myenteric plexus in a distal segment of bowel and is thought to be the cause of Hirschsprung disease (see "Structural and congenital abnormalities" below). Qualitative differences in laminin and type IV collagen have been found in some patients with Hirschsprung disease, suggesting a potential role for abnormalities in fetal extracellular matrix components in the pathogenesis of this disease [31]. Studies using the (ls) mouse, a model for Hirschsprung disease, confirm the importance of mesenchymally derived nonneural crest cells in directing appropriate migration of ganglion cell precursors during normal embryogenesis [32,33]. A number of genes involved in the susceptibility and development of Hirschsprung disease have been identified. A recessive susceptibility locus for Hirschsprung disease that encodes the endothelin-B receptor has been mapped to human chromosome 13q22.3, and mutations of the mouse endothelin-B receptor or endothelin-3 ligand genes also result in aganglionosis and a megacolon phenotype [34–36]. Over 30 distinct missense and deletion mutations in the *RET* receptor tyrosine kinase gene located on human chromosome 10q11.2, distributed along the entire length of the receptor, have been identified in autosomal dominant forms of Hirschsprung disease [37–42]. Mutations of RET are found in approximately 10%–20% of sporadic cases and 50% of patients with a familial history of Hirschsprung disease [39–41]. Most RET mutations associated with Hirschsprung disease result in loss of receptor function [39]. Segregation analysis in a Mennonite family has indicated the existence of other major loci for susceptibility to Hirschsprung disease [34]. These findings confirm the multigenic nature of this disease.

The development of the colonic epithelium parallels that of the small intestine epithelium (see Chapter 46). At 8–9 weeks of gestation, the developing colon consists of a pseudostratified columnar epithelium several cell layers deep, with a slit-like lumen surrounded by mesenchymal tissue forming a simple tubular structure [43–45]. Epithelial ridges form between 10 and 11 weeks of gestation, giving the colonic lumen a stellate appearance. Small secondary lumens form at the base of the pseudostratified epithelium and subsequently extend to the primary lumen, resulting in the formation of broad primary villi. Between the 13th and 15th weeks of gestation, the formation of cyst-like spaces within these primary villi and the accompanying upward growth of mesenchymal tissue result in the division of primary villi into numerous secondary villi. During this developmental stage, epithelial cells near the bases of the primary villi undergo rapid proliferation and bud into the surrounding mesenchyme to form the developing colonic crypts. Numerous goblet cells, columnar epithelial cells with well-developed microvilli, and enteroendocrine cells can be observed in the epithelium by this time [23,46]. The fetal enteroendocrine cell population has been found to secrete a variety of neuroendocrine mediators, some of which may have important trophic effects on the developing epithelium. The mesenchymal tissue underlying the developing colonic epithelium appears to play a crucial role in the induction of appropriate regional differentiation in the gut epithelium [47,48–50].

Colonic villi are present through at least week 29 of gestation but are not found in the colon of full-term infants [46,52]. Prominent supranuclear inclusion bodies, originally termed meconium corpuscles, can be observed in the principal epithelial cells lining the fetal colon. These membrane-bound inclusion bodies are the result of apoptosis, emphasizing the role of programmed cell death in the morphological restructuring of the epithelium that occurs during fetal life [51]. During fetal life, the colonic epithelium is similar to the developing small intestine in its biochemical characteristics, including the expression of many brush-border hydrolases also found in the developing small intestine [43,52–55]. By full term, however, the activities of these brush-border hydrolases have declined to levels similar to those found in the adult colon.

Structural and congenital abnormalities

Congenital abnormalities of the intestine are common; most arise from errors in rotation or fusion of the mesenteries or from failure of neural crest cells to complete their migration

during fetal colonic development. Imperforate anus, duplication, and malrotation are usually identified in the neonatal period. Hirschsprung disease may present in the neonatal period, in childhood, or, more rarely, in the young adult.

Omphaloceles and gastroschisis

Abdominal wall defects are present at birth. Omphaloceles are identified by a mass of bowel and viscera in the central abdomen covered by a translucent membrane. The size of the defect varies, and it can be associated with other congenital anomalies. Gastroschisis is usually a small defect that occurs at the junction of the umbilicus and normal skin. Although no membrane covers the bowel, there are usually no associated anomalies. Both omphaloceles and gastroschisis require immediate surgery. Only in gastroschisis are reduction of the bowel and primary closure of the defect usually possible. If the bowel is edematous and thickened or the defect is too large to close primarily, a Silastic silo can be constructed to achieve gradual reduction of the bowel (see Chapter 46 for a more complete discussion).

Hirschsprung disease and congenital megacolon

Hirschsprung disease is a result of the failure of neural crest cells (precursors of ganglion cells) to complete their caudal migration during normal colonic development (see section "Colonic development" above). The aganglionic segment does not relax and causes a functional obstruction. The normal proximal bowel hypertrophies and eventually dilates. The rectosigmoid is involved in approximately 75%–80% of cases [56]. The entire colon and various lengths of small bowel are aganglionic in 5%–10% of cases [57]. A short aganglionic segment is the typical finding in adult Hirschsprung disease.

Delayed passage of meconium and abdominal distention are often the presenting symptoms in the newborn [58]. Chronic constipation, abdominal distention, volvulus, or perforation may be symptoms of the disease in a child. Hirschsprung disease in the adult is rare but must be considered in the evaluation of patients with chronic constipation dating back to childhood [59–62]. Patients with total colonic aganglionosis may present with less dramatic intermittent symptoms of constipation [57].

Digital rectal examination aids in the diagnosis. The rectal vault is empty in patients with Hirschsprung disease and is full of stool in patients with idiopathic megacolon. Abdominal radiographs usually show colonic dilation and a paucity of gas in the rectum. Barium enema may suggest the presence of a short, narrow segment or transition zone (Fig. 56.10). Patients with total aganglionosis or short segment Hirschsprung disease may have a normal-appearing barium enema. Newborns may have a normal-appearing barium enema because the proximal bowel has not had time to dilate.

Anorectal manometry may be useful in the evaluation of patients with suspected Hirschsprung disease but is difficult to perform in the newborn and is reserved for older infants and children. Typically a normal sphincter profile (resting pressure of 40–80 mmHg and squeeze pressure of 80–160 mmHg) and an abnormal rectoanal inhibitory reflex are seen in patients with Hirschsprung disease. The rectoanal inhibitory reflex is the normal relaxation of the internal sphincter in response to rectal distention. The internal sphincter does not relax in response to rectal distention in patients with Hirschsprung disease, and an abnormal reflex during manometry aids in the diagnosis [63,64]. This reflex is easily identified using anal manometry with a manometric catheter and a balloon to distend the rectum. When the rectal balloon is distended, the manometry tracing will reveal a normal external sphincteric contraction but no compensatory relaxation of the internal anal sphincter. The rectoanal inhibitory reflex may be normal in patients with short-segment Hirschsprung disease. These patients may have a short segment as their primary disease or the short segment may be residual disease after surgery. High anal resting pressures and impaired rectal emptying may be seen.

Histological evaluation is necessary to make the diagnosis of Hirschsprung disease. Hyperplasia or hypertrophy of nerve fibers can be seen, but ganglion cells are absent in the submucosa and myenteric plexus of these patients. Mucosal

Figure 56.10 Radiological study from a patient with Hirschsprung disease. The short, collapsed, aganglionic segment (arrows) is evident on this radiograph of a barium enema performed on a 28-month-old infant with constipation. The colon proximal to the aganglionic segment is often dilated and filled with stool.

pinch biopsy specimens are not adequate for diagnosis because sufficient submucosa must be present in the biopsy specimen to conclude that ganglion cells are absent. Suction biopsies are simple to perform, but the mucosa and submucosa must be identifiable to have an adequate specimen. Full-thickness rectal biopsy samples may be necessary for confirmation. Routine hematoxylin–eosin staining misses ganglion cells in almost 40% of normal patients [65]. Acetylcholinesterase staining has been found to be more accurate in identifying patients with Hirschsprung disease. Acetylcholinesterase-positive nerve fibers are increased in number in the lamina propria and muscularis propria in these patients. A positive diagnosis may be obtained even by evaluation of superficial suction biopsy samples.

Colostomies are usually performed after the diagnosis is made in newborns. This allows a period of stabilization and growth before definitive repair. A colostomy may be used in the older child or adult to allow hypertrophied bowel to resume a more normal caliber. Pull-through operations (Swenson technique, Duhamel procedure, or Soave procedure) have been devised to anastomose normally innervated bowel to the anus with or without resection of the abnormal bowel [58–61]. Intraoperative biopsy results must demonstrate the presence of ganglion cells at the level of the colostomy and the proximal margin of the colonic resection. Rectal myectomy may be curative in patients with short-segment Hirschsprung disease.

Intestinal duplications

Intestinal duplications are rare congenital anomalies that can occur anywhere along the gastrointestinal tract. There are many theories regarding the cause of intestinal duplication, including failure of the bowel to recanalize in utero, caudal twinning, and incomplete separation of the notochord from the entoderm. The resultant structures may either be tubular, communicating with the intestinal lumen, or, more commonly, closed cystic duplications. These cysts are lined with gastrointestinal mucosa and contain a layer of smooth muscle within their walls. Duplications are usually found on the mesenteric side of the intestine. They frequently share a common wall and blood supply with the involved bowel. All regions of the gastrointestinal tract may be involved; the esophagus and ileum are the most commonly involved sites. The colon and rectum account for 5%–10% of all gastrointestinal duplications [66].

Duplications are usually symptomatic during childhood. They can produce symptoms of obstruction, volvulus, or hemorrhage. Duplications in adults are often asymptomatic and may be discovered incidentally. They can be complicated by infection, bleeding, or malignant degeneration. Plain abdominal radiographs, barium enemas, and computed tomography (CT) scans may reveal soft tissue masses or the point of communication with normal bowel. Evaluation of the genitourinary tract should be performed to rule out associated anomalies.

Resection is indicated for rectal duplications because of the risk of neoplasia, even for asymptomatic duplications. Cystic duplications can often be excised. Dividing the common wall is the treatment for tubular duplications. Mucosal excision may be necessary if heterotopic gastric mucosa is present [66].

Malrotation

Malrotation occurs if the midgut fails to complete the 270° counterclockwise rotation as it returns from herniation (10–12 weeks of gestation) [26,67]. The base of the small bowel mesentery normally stretches from the ligament of Treitz in the left upper quadrant to the ileocecal valve in the right lower quadrant. In malrotation, the entire midgut and its vascular pedicle have a narrow-based mesenteric attachment in the right upper quadrant. The duodenojejunal junction (ligament of Treitz) comes to lie to the right of the spine. The cecum and right colon overlie the duodenum in the right upper quadrant, and Ladd bands form over the duodenum in an attempt to peritonealize the cecum. Associated anomalies, such as small bowel atresias, intussusception, Hirschsprung disease, and abdominal wall defects, have been reported in 30%–60% of patients [67,68].

Most infants present with evidence of malrotation within 1 month of birth [67,68]. The clinical picture is one of a high small bowel obstruction, volvulus, or ischemia. One should suspect the diagnosis in an infant with bilious vomiting and a flat abdomen. Bloody stools indicate vascular compromise. Less severe forms of malrotation can present in childhood or into adulthood.

Plain abdominal radiographs may show evidence of a complete gastric or duodenal obstruction with a paucity of gas in the distal small bowel. A barium upper gastrointestinal series shows the ligament of Treitz to the right of midline. A barium enema is rarely needed, but it may demonstrate the cecum in the right upper quadrant.

The treatment of malrotation is operative. Most neonates with an obstruction from malrotation have a midgut volvulus [68]. The volvulus is reduced by untwisting it in a counterclockwise rotation [69]. Ladd bands overlying the right colon and duodenum are lysed. The duodenum is placed in the right upper quadrant and the cecum in the left upper quadrant. An appendectomy is performed because the alteration in anatomy would make future diagnosis of appendicitis difficult [68].

Imperforate anus

Imperforate anus occurs in 1 in 20 000 live births and is associated with other congenital anomalies in 50% of cases. Genitourinary anomalies and sacral abnormalities are frequently seen. Cardiac and gastrointestinal anomalies (primarily esophageal atresia) are also associated with imperforate anus.

Imperforate anus is classified according to the relationship of the rectum to the levator ani muscles [70]. High lesions are those in which bowel of normal or greater caliber terminates above the levator muscle complex. The rectum often ends in the prostatic urethra in males. In low lesions, the rectum terminates distal to the levators, often ending in a fistula. Low lesions are more common in females. Fistula tracts may connect the distal rectum with the perineum, vagina, or other hollow organs. Intermediate lesions are found within the levator complex and may be associated with a partial anal canal or perineal fistula.

The diagnosis is usually made by physical examination at birth. Most infants with imperforate anus fail to pass meconium at birth unless a fistula is present. Radiography can be used to determine the type of defect. Air invertograms can be used to identify the location of the rectum in relation to the perineum but are difficult to interpret. Fistulography may be performed, and CT and magnetic resonance imaging (MRI) are useful in evaluating high lesions. The urinary tract should be evaluated with an intravenous pyelogram and voiding cystourethrogram because of the associated urogenital anomalies.

Treatment depends on the type of lesion [70]. Low lesions can be treated by dilation of fistula tracts with or without anoplasty. Most healthy infants with low lesions do not require colostomies. Intermediate and high lesions are treated with an initial diverting colostomy to allow a period of stabilization and growth. A number of pull-through operations have been advocated. The posterior sagittal approach developed by Peña and deVries [71] identifies and divides the levator muscle complex. The rectal segment is then identified and brought through the center of the divided muscles. A coloanal anastomosis is performed in the location of the anal dimple. Most series have claimed a 70%–82% success rate with intermediate and high lesions [72]. A greater success rate can be expected with low lesions.

Volvulus

Volvulus occurs whenever an air-filled segment of bowel twists about its mesentery; it almost never occurs if the colon is filled with solid stool. A dilated, redundant colon with a narrow-based mesocolon is a prerequisite for colonic volvulus. In the United States, fewer than 10% of colonic obstructions are caused by colonic volvulus. In countries with high-fiber diets, volvulus has been reported as the most common cause of intestinal obstruction [73]. Volvulus is classified and treated according to its location in the colon.

Sigmoid volvulus accounts for approximately 60% of all volvulus seen in the United States [74]. It usually occurs in older adult or institutionalized patients or in patients with various neuropsychiatric disorders. Chronic constipation, laxative abuse, and colonic atony have been implicated as the causes of the dilated, redundant sigmoid colon.

Patients usually present with abdominal pain, distention, and obstipation. Evidence of peritoneal irritation on physical examination, fever, or an elevated leukocyte count indicates gangrenous bowel. Plain abdominal radiographs showing an inverted, U-shaped, sausage-like loop with a dense line running toward the point of torsion are diagnostic for sigmoid volvulus. In questionable cases, radiological enema examination with barium or with water-soluble contrast may be diagnostic. The "bird's beak" deformity forms where the contrast tapers to a point at the site of obstruction (Fig. 56.11). Reduction of the volvulus can occur during these examinations. If the patient has signs of peritoneal irritation or gangrene is suspected, barium or Gastrografin enema (Bracco Diagnostics, Inc., Princeton, NJ) should not be attempted, and the patient should undergo emergency exploration.

If peritonitis is not present, sigmoidoscopy should be performed. Inserting a soft rectal tube past the point of obstruction can often reduce the volvulus. This results in dramatic decompression, and the rectal tube is left in place to assist with further decompression. Volvulus beyond the limits of the rigid sigmoidoscope may be reduced by flexible sigmoidoscopy or colonoscopy [75]. A 43% recurrence rate after nonoperative reduction can be expected [76]. Therefore, after reduction, medically stable patients should undergo mechanical bowel preparation and an elective resection. Evidence of mucosal ischemia, bloody discharge, or unsuccessful detorsion indicates strangulation and possibly gangrene. If this occurs, sigmoidoscopy should be terminated,

Figure 56.11 Sigmoid volvulus. Hypaque enema shows tapering of the column of contrast at the site of torsion ("bird's beak" sign) of the sigmoid colon.

and the patient should undergo an emergency exploration. Mortality is related to the presence of gangrenous bowel [76].

Cecal volvulus accounts for less than 20% of all cases of colonic volvulus and is generally seen in younger patients [75]. It is thought to be caused by anomalous fixation of the right colon, leading to a freely mobile cecum [77]. Other precipitating factors include adhesions from previous surgery, pregnancy, and obstructing lesions of the left colon. Ninety percent of patients with cecal volvulus have a full axial volvulus twisting the associated mesentery and blood vessels [73]. In the remaining patients, the cecum folds in an anterior cephalad direction (cecal bascule). The cecal bascule is not a true volvulus around a mesentery, but gangrene can result from tension on the bowel wall.

Abdominal pain, nausea, vomiting, and obstipation are common symptoms of cecal volvulus. Patients with cecal volvulus appear clinically to have a small bowel obstruction. Many patients give a history of similar, chronic, intermittent symptoms [73]. A plain abdominal radiograph may show the cecum as a kidney-shaped, air-filled structure in the left upper quadrant. Multiple air-filled levels may be seen and indicate a distal small bowel obstruction. Radiological examination with barium or with a water-soluble contrast enema may show obstruction of the column of contrast at the level of the volvulus. The tapered edge of the contrast points toward the site of torsion. Barium studies should not be routinely performed if the diagnosis is clear. Colonoscopy has been used to treat cecal volvulus, but with limited success, and should rarely be attempted [75,78].

Operative detorsion alone, cecopexy, and cecostomy are associated with variable rates of recurrence [73,79]. Right hemicolectomy with primary anastomosis or cecopexy and cecostomy is recommended if there is no evidence of gangrenous bowel. Resection, ileostomy, and mucous fistula are indicated if there is evidence of gangrene. Mortality is increased in the presence of gangrenous bowel.

Volvulus of the transverse colon is rare because the mesentery is short and broad-based. Failure of the mesentery to fuse normally or narrowing of the mesenteric attachments may predispose the transverse colon to volvulus. Patients present with a clinical picture of large bowel obstruction. Barium enema is diagnostic and reveals the point of obstruction. Although successful attempts at colonoscopic detorsion have been reported, most patients require operative detorsion and resection of the redundant transverse colon [80,81].

Surgically altered anatomy

Many diseases of the colon and rectum will require surgery. In general, surgery of the colon and rectum involves a segmental resection of the colon and the restoration of intestinal continuity. However, when the restoration of intestinal continuity is either not possible or desirable, a surgical ostomy may be created. These procedures will alter normal anatomy. An understanding of surgically altered anatomy is important as physicians treat patients who have had previous colon and rectal surgery.

Right colectomy

A *right colectomy* involves the resection of the proximal one-third to one-half of the intraabdominal colon. The resected specimen will include the ileocecal valve, a variable length of ileum, and the appendix. Ligation of the ileocolic and right colic arteries is nearly always performed. A surgical anastomosis is then created between the ileum and the transverse colon.

Colonic neoplasia is the most common indication for a right colectomy. Tumors involving the cecum or ascending colon are treated with a standard right colectomy. To ensure the complete removal of the associated lymphatics, the ileocolic and right colic vessels are ligated at the origin of the superior mesenteric artery. However, tumors located at the hepatic flexure will also have lymphatic drainage associated with the middle colic artery. Under these circumstances an *extended right colectomy* is performed, which includes ligation of the ileocolic, right colic, and middle colic arteries at their origins, requiring additional resection of the transverse colon. The blood supply of the remaining colon is provided solely by the inferior mesenteric artery.

Crohn's disease is the most common benign indication for removal of a portion of the right colon. Because the terminal ileum is usually involved, a variable length of terminal ileum is resected in addition to a portion of the right colon, usually the cecum. Because more ileum is resected than with a standard right colectomy, this resection is termed an *ileocolic resection.* Ligation of the colonic vessels can be done closer to the colon, because removal of the associated lymphatics is not necessary with benign disease.

Left and sigmoid resection

A true *left colectomy* involves the complete resection of the descending and sigmoid colon as well as the distal one-third to one-half of the transverse colon. The left colic and superior rectal arteries are ligated and the transverse colon is then anastomosed to the top of the rectum. The most common indication for a left colectomy is colonic neoplasia. To ensure adequate lymphatic removal, the inferior mesenteric artery may be ligated at the aorta.

A *sigmoid resection* involves just the sigmoid colon. The superior rectal artery is ligated and the distal descending colon is anastomosed to the top of the rectum. For cancer, ligation of the superior rectal artery is done just distal to the takeoff of the left colic artery, whereas for diverticulitis the vasculature can be ligated closer to the colon.

Low anterior and abdominal perineal resection

A *low anterior resection* is generally performed for a neoplasia involving the upper and middle thirds of the rectum. The

inferior mesenteric artery is ligated just distal to the takeoff of the left colic artery, and the descending colon is anastomosed to the rectum. For tumors of the upper third, about one-half of the rectum is resected. However, for tumors of the middle and lower thirds the entire rectum and the associated mesorectum is removed, leaving intact, when possible, the anal sphincter mechanism. A hand-sewn or stapled anastomosis is then performed. When the tumor invades the anal sphincter than an *abdominal perineal resection* (APR) is performed. Under these circumstances, the rectum is resected as described above and then the anus is also completely removed using a wide elliptical excision. A permanent end colostomy is then created using the descending colon.

Total proctocolectomy

A *total proctocolectomy* involves the simultaneous removal of the entire colon and rectum. The most common indication is chronic ulcerative colitis. Other indications include familial adenomatous polyposis (FAP) and Crohn's colitis. Often, the anus is also resected and a permanent end ileostomy is created.

In selected patients who do not want a permanent ileostomy, an *ileal pouch anal anastomosis* (IPAA) can be performed. This is most commonly performed using a stapling technique. The distal rectum is transected, using a transverse stapling device, about 1–2 cm above the anal sphincter. A 20-cm J-pouch is created from the terminal ileum and then a stapled end-to-end anastomosis is performed. When performed in this way it is important to understand that about 1–2 cm of rectal mucosa is left in situ. This mucosa is susceptible to the development of rectal cancer [82]. Therefore, continued surveillance is necessary. A *mucusectomy* can be performed to remove the last 1–2 cm of rectal mucosa. When this is done then a hand-sewn anastomosis must be performed. A mucusectomy is technically more difficult and the functional results of the pouch may be inferior [83,84]. Therefore, a stapled technique is far more common and, although controversial, is even performed for FAP [84,85]. More importantly, a mucusectomy does not completely remove the risk of developing subsequent rectal cancer, so continued surveillance may still be necessary [86,87].

Anastomoses

Once a portion of intestine has been removed, intestinal continuity is restored by reconnecting the two ends of the intestine, thus creating a surgical *anastomosis*. Although the purpose of all intestinal anastomoses is the same, there are several different techniques used for their construction. A general understanding of these techniques can be helpful when trying to interpret the endosocopic or fluoroscopic appearance of an anastomosis.

Originally all intestinal anastomoses were hand sewn, using a variety of suture materials. Over the past several decades surgical stapling devices have been introduced, and many anastomoses are created with a combination of linear and circular staplers. Although stapling is generally quicker than hand sewing an anastomosis, there is no evidence to suggest staplers create a more secure anastomosis [88,89]. Anastomotic leaks, although uncommon, still occur in about 3%–15% of patients having a colectomy and seem to be independent of surgical technique. Therefore, the choice of sewing or stapling is a surgeon's preference. Regardless of the technique, it is not uncommon to visualize endoscopically either suture material or staples at an anastomotic site. Staples are nonreactive and therefore cause little surrounding inflammation of the mucosa. However, permanent suture material, especially silk, can be reactive and cause mucosal inflammation around the anastomosis, which can be easily misinterpreted as recurrent disease [90].

When the two ends of the intestine are directly connected, either by staples or suture, the anastomosis is referred to as an *end-to-end anastomosis*. Similarly, when the two pieces of intestine are laid side by side and then connected in this manner the anastomosis is referred to as a *side-to-side* or *functional end-to-end anastomosis*. End-to-end anastomoses are more common on the left-hand side (e.g., left colectomy, sigmoid colectomy, low anterior resection) whereas side-to-side anastomoses are more common on the right-hand side (ileocolectomy, right colectomy). Endoscopically, an end-to-end anastomosis can be subtle and difficult to detect. However, a side-to-side anastomosis is generally recognizable because the lumen of the intestine will take a sharp turn. In fact, it can often be difficult to intubate the anastomosis when they are constructed in a side-to-side fashion. This can easily be misinterpreted as a stenotic anastomosis. When there is doubt about the patency of a side-to-side anastomosis, a barium enema can be extremely helpful.

Although schematic drawings of anastomoses are precise it is important to remember that an actual anastomosis may not have an ideal appearance. Not uncommonly, a real anastomosis has numerous "nooks and crannies" because overlapping of staple lines can cause distortions. Therefore, endoscopy of an anastomosis must be done carefully to ensure that all of the mucosa in the various "nooks and crannies" has been adequately visualized.

Colostomy, ileostomy, and mucus fistula

Although the restoration of intestinal continuity is desirable it is not always possible. Under these circumstances, a surgical ostomy is created to divert the fecal stream. An *ileostomy* refers to stomas created using the ileum, whereas a *colostomy* is constructed using the colon. Ostomies are frequently performed during emergency colon surgery, such as perforated diverticulitis, when the creation of a primary anastomosis is considered dangerous. Generally, the diseased portion of the colon is resected, the end of the proximal colon is pulled through the rectus sheath, and a surgical colostomy is

created at the skin level. The distal segment is closed and left in situ as a *Hartmann pouch.* Because the Hartmann pouch is in continuity with the anus, any residual stool or mucus can readily pass. However, under circumstances when the distal colon or rectum is completely obstructed, decompression of the Hartmann pouch through the anus is not possible and residual stool or mucus can leak through the proximal end of the pouch, leading to life-threatening sepsis. Therefore, in the presence of complete distal obstruction, the surgeon may chose not to close the Hartmann pouch but instead to pull also the proximal portion of the pouch through the abdominal wall and open the colon at the skin level. This will allow the obstructed Hartmann pouch to decompress. This is referred to as a *mucus fistula*, because mucus will continue to drain from this opening.

Most colostomies and ileostomies are *end ostomies*, because the end of the ileum or colon is used to create the surgical opening. On examination of an end ostomy only one lumen should be present. However, *loop ostomies* are also commonly constructed. Loop ileostomies and colostomies are most commonly created when fecal diversion is desired but the distal intestine remains in continuity with the anus. Common clinical examples include perineal sepsis from Crohn's disease or a distal obstructing rectal cancer, when fecal diversion is necessary but a primary resection is not desirable. Under these circumstances, a loop of proximal intestine is pulled through the rectus sheath. The intestine is then opened and secured to the skin, creating the stoma. Examination of a loop colostomy or ileostomy should demonstrate two lumens; the proximal one allowing for the egress of stool and the distal opening allowing for the decompression of the distal intestine, similar to a mucus fistula.

An understanding of surgically altered anatomy is critical in treating patients with previous colon and rectal surgery. A basic understanding of common surgical procedures should help the treating physician, especially during endoscopic evaluation. If a second colonic surgical procedure is necessary a precise understanding of the existing anatomy is imperative to ensure adequate blood supply for the remaining colon and a successful outcome. Although most surgical procedures are performed in a similar fashion the details of any procedure can only be ascertained by the actual operative notes. Obtaining these records can be extremely helpful in delineating the altered anatomy of any given patient.

Functional results

Because the primary purpose of the colon is water absorption, partial resection of the colon can alter daily function. Fortunately, in most patients, a segmental colonic resection does not significantly alter clinical function. Up to 75% of the abdominal colon can be resected with little impact. However, complete removal of the abdominal colon with an ileorectal anastomosis will alter daily function. Patients having this operation can expect approximately five loose bowel movements per day [91]. Because the rectum has been preserved there is little urgency or incontinence [91]. Furthermore, other complications associated with rectal dissection, such as impotence and infertility, are generally avoided.

Nutritionally, segmental colon resection has little long-term impact on patients. However, with both right colectomy and ileocolectomy, a variable length of ileum is also resected. When done for cancer only about 5–10 cm of terminal ileum is removed, which is generally not enough to cause nutritional deficiencies. However, for Crohn's disease the length of the terminal ileum resection is highly variable, depending on the extent of disease. This can have an impact on vitamin B-12 absorption and may result in the sequelae of vitamin B-12 deficiency [92,93]. In addition to vitamin B-12, bile salt absorption can also be impaired and may contribute to the postoperative diarrhea often seen in patients with Crohn's disease who have had an ileocolic resection [94].

In contrast to segmental colon resection, removing the rectum does have a functional impact on the patient. The rectum is an effective storage device. Stool can be stored in the rectal vault and most people can defer the call for evacuation until an appropiate time. However, after the rectum has been resected, as is done with a low anterior resection, a neorectum is created using intraabdominal colon. Unfortunately, the neorectum has less storage capacity, which often results in urgency, increased stool frequency, and occasional incontinence. Collectively this has been referred to as the anterior resection syndrome [95]. There can be functional improvement for up to 1 year after the operation, but rarely does function return to preoperative levels. Because of these functional difficulties some surgeons create a small colonic J-pouch or perform a coloplasty to increase neorectal capacitance [95,96]. Although short-term results seem better with these techniques, long-term results appear to be similar to the straight low anterior resection [97,98]. Even with suboptimal results, most patients still prefer to have their intestinal continuity restored if the only other option is a permanent colostomy. However, in frail elderly patients or in patients who have preoperative incontinence, the low anterior syndrome may be debilitating and, therefore, a colostomy may be more appropriate.

In addition to affecting gastrointestinal function, rectal resection can be associated with a number of other functional issues. The pelvis contains numerous sympathetic and parasympathetic nerves, damage to which can cause both sexual and urinary dysfunction. Men are at risk for impotence or retrograde ejaculation whereas woman may have dyspareunia [99,100]. The rates of these problems increase considerably with age and are more common in patients with cancer, who often receive pelvic irradiation as well [101]. Fortunately, sexual dysfunction in young men with benign disease remains uncommon [99]. In addition to dyspareunia, women who have had rectal surgery have a higher rate of infertility [102,103]. This seems to be secondary to pelvic

adhesions, because assisted reproduction is still possible. In light of these potential devastating side effects, rectal resection should be avoided until it is clearly medically indicated.

Total proctocolectomy with an IPAA is an effective treatment for patients with severe chronic ulcerative colitis. Following this procedure patients can expect about 6–7 loose bowel movements per day. Not infrequently nighttime evacuation may be necessary. Although continence is generally good, some patients will experience leakage and incontinence. This is more common at night when patients are sleeping [104]. Ironically, because most patients who have this surgery have severe ulcerative colitis, this level of function is a considerable improvement and enhances their quality of life [105,106]. Patients who have normal preoperative function, such as those with FAP, are less pleased with the functional results [107], but still prefer this procedure to the alternative, which is a permanent ileostomy. Because a total proctocolectomy does include the rectum, patients are at risk for the associated complications of rectal dissection, such as sexual dysfunction and infertility (see above). Interestingly, because of the severity of the patient's disease, there may actually be an overall improvement in sexual activity, although a small number of patients do experience true sexual dysfunction [99,100,108].

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57 Inflammatory bowel disease

William F. Stenson, Stephen B. Hanauer, Russell D. Cohen

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Ulcerative colitis and Crohn's disease, chronic inflammatory diseases of the gastrointestinal tract, are identified and diagnosed by the appearance of a set of clinical endoscopic, and histological characteristics [1–3]. The inflammatory response in ulcerative colitis is largely confined to the mucosa and submucosa, but in Crohn's disease inflammation extends through the intestinal wall from mucosa to serosa. Ulcerative colitis is confined to the colon, and colectomy is a curative procedure. Crohn's disease, in contrast, has the potential to involve the patient's entire gastrointestinal tract, even through only a small segment is involved initially. Resection of the inflamed segment is not curative in Crohn's disease, and inflammation is likely to recur in the gastrointestinal tract sometime after resection. Despite these differences in distribution, no single finding is absolutely diagnostic of one disease or the other. Moreover, a group of patients, accounting for 5%–15% of those with inflammatory bowel disease (IBD), have characteristics of both diseases and cannot be cleanly classified; these patients are considered to have indeterminate colitis.

The genetic basis of these diseases is being elucidated. Both appear to be multigenic diseases. Patterns of familial aggregation suggest that the genetic influences at least partially overlap. Relatives of patients with each disease have an increased risk of that particular disease as well as of the other form of IBD [4]. Consequently, not only is there an increased incidence of Crohn's disease in relatives of those with Crohn's disease but their relatives also have an increased incidence of ulcerative colitis. This pattern holds for patients with ulcerative colitis as well.

In view of the similarities in clinical and histological presentation and the shared genetic background, these two diseases are presented in a single chapter. In the absence of established etiological agents or definitive markers, it is possible that what is designated as ulcerative colitis or Crohn's disease may in fact be a mixture of diseases with diverse causes but a common clinical presentation. Actually, more than two diseases may exist under the name of inflammatory bowel disease. Presentation in a single chapter emphasizes the common characteristics of these diseases and minimizes splitting them into groups that may not be valid. This combined presentation allows the areas of similarity to be detailed without repetition and allows comparisons in areas of dissimilarity.

Epidemiology

The incidence and prevalence of Crohn's disease (1–6 and 10–100/100 000, respectively) and of ulcerative colitis (2–10 and 35–100/100 000, respectively) vary greatly with geographic location. These rates are for Caucasian populations in northern Europe and North America. The countries of greatest incidence and prevalence encompass the industrialized world. Rates in central and southern Europe are somewhat lower. In South America, Asia, and Africa these diseases remain uncommon but appear to be increasing. Within geographic areas, ethnic and racial variations exist in the incidence of IBD [5]. Crohn's disease is three to eight times more common in Jewish than in non-Jewish persons. Similarly, the incidence of ulcerative colitis is two to four times higher among Jews. The incidence among Israeli Jews, however, is much lower than among American and European Jews. Furthermore, in Israel the incidence is lower among Sephardic or Oriental Jews of Asian or African origin than among Ashkenazi Jews of European or American origin [5]. In the United States, the incidence of both ulcerative colitis and Crohn's disease in the African-American population has

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been one-fifth to one-half that in the Caucasian population, but in recent years that gap appears to be narrowing [6].

Some of the apparent increases in incidence of IBD over time may reflect greater awareness of these diseases, better diagnostic studies, and improved reporting. Moreover, some patients diagnosed as having Crohn's colitis may have been diagnosed as having ulcerative colitis in the past. Despite these ambiguities, certain trends are apparent [7,8]. In geographic areas where the incidence of these diseases has been slight, it is increasing. In northern Europe and North America, where the incidence of both diseases has been substantial, the incidence of ulcerative colitis has leveled, but that of Crohn's disease is still increasing.

The peak age of onset for both diseases is between 15 and 25 years. In some but not all series, a second, lesser peak of incidence occurs between 55 and 65 years [6]. Both diseases occur in childhood, although the incidence is much lower before the age of 15 years than after. Ulcerative colitis is more common than Crohn's disease in children younger than 10 years. Most series show an approximately equal incidence of both diseases in males and females, but other studies show an incidence of Crohn's disease that is greater in females by up to 30% and an incidence of ulcerative colitis that may be greater among males [6]. These gender differences of disease distribution may represent confounding environmental factors such as use of tobacco. The similarities between ulcerative colitis and Crohn's disease in geographic distribution, racial and ethnic distribution, distribution by sex, and age of onset support the contention that the two diseases are related.

One fascinating but unexplained difference between patients with these two diseases is the incidence of smoking. Among patients with ulcerative colitis, the incidence of smoking is less than that in the general population. In several studies, the risk of developing ulcerative colitis was increased among nonsmokers compared with current smokers and increased even further among former smokers compared with lifetime nonsmokers [9–12]. Quitting smoking can provoke the emergence of ulcerative colitis, although this is still much less of a risk than smoking on overall health [13,14]. In contrast, the incidence of smoking among patients with Crohn's disease is higher than in the general population [10]. Smokers have a more severe course of Crohn's disease, require more immunosuppressive medications, and demonstrate a faster recurrence after surgery [15,16]. Cessation of smoking may moderate the clinical course although the greatest benefit of cessation is seen with the heaviest smokers [17]. The active ingredients in cigarette smoke that increase the risk of Crohn's disease or decrease the risk ulcerative colitis have not been defined, nor have mechanisms of action. On the assumption that nicotine is the active ingredient in cigarette smoke, trials of transdermal nicotine and nicotine gum for the treatment of ulcerative colitis have been performed; in two trials transdermal nicotine was somewhat effective [18,19].

Appendectomy strongly protects against the development of ulcerative colitis, while not having any influence on Crohn's disease [20]. This effect in ulcerative colitis is noted particularly when appendectomy is performed for an inflammatory problem, such as lymphadenitis or appendicitis and if done before the age of 21 years [21]. Poor sanitation in childhood is a protective factor against the development of Crohn's disease, although not against ulcerative colitis [22,23].

Etiology and pathogenesis

Genetics

Any theory of the pathogenesis of IBD must deal with the evidence for a genetic basis of the disease. The most firmly established and quantitatively greatest risk factor for developing IBD is a positive family history [24]. Approximately 15% of patients with IBD have first-degree relatives who also have IBD [25]. The lifetime risk of developing IBD among first-degree relatives of affected individuals is 8.9% for offspring, 8.8% for siblings, and 3.5% for parents [26]. The incidence of disease among first-degree relatives is higher in Crohn's disease than in ulcerative colitis. The incidence of IBD among first-degree relatives of patients with IBD is 30–100 times that of the general population. Moreover, although the relatives of patients with Crohn's disease are more likely to have Crohn's disease than ulcerative colitis, the incidence of ulcerative colitis in this group is also higher than in the general population [4]. Similarly, relatives of patients with ulcerative colitis have a higher incidence of both ulcerative colitis and Crohn's disease than the general population. These data support the contention that ulcerative colitis and Crohn's disease are related diseases. The incidence of IBD is not increased in spouses of patients.

Studies of twins also support the presence of a genetic basis for these diseases. A study of unselected twins from a Swedish twin registry demonstrated that dizygotic twins have the same rate of concordance as would be expected for siblings. There was concordance for Crohn's disease in eight of 18 monozygotic twin pairs; whereas there was concordance for ulcerative colitis in only one of 16 monozygotic twin pairs [27]. No case has been reported of monozygotic twins in which one twin had Crohn's disease and the other ulcerative colitis, suggesting that these diseases have a similar but not identical genetic background.

No clear-cut mendelian pattern of inheritance in IBD has been established; however, specific mutations have been associated with IBD. Work by two different groups has led to the identification of NOD2 mutations associated with Crohn's disease [28,29]. NOD2, a cytosolic protein expressed in monocytes, functions as an intracellular receptor for a bacterial product, muramyl dipeptide [30,31]. NOD2 is a component of the innate immune system. Crohn's disease is

associated with frameshift mutations in NOD2 resulting in a truncated and nonfunctioning protein. Although NOD2 mutations are seen much more commonly in Crohn's disease than in the general population, only a small portion (~ 15%) of all patients with Crohn's disease have a mutation in NOD2. This demonstrates that Crohn's disease is genetically heterogeneous and suggests that other mutations in other genes may contribute to Crohn's disease in other populations. Genome-wide association studies have identified other novel genetic associations with Crohn's disease including a subunit of the receptor for the proinflammatory cytokine interleukin-23 (IL-23R) and the ATG16 autophagy-related 16-like 1 (ATG16L1) [32,33]. The association of Crohn's disease with mutations in NOD2 and IL-23R suggests that both the innate and adaptive immune systems participate in its pathogenesis.

It has become increasingly clear over the last few years that Crohn's disease is clinically heterogeneous and that there is a genetic basis for the clinical heterogeneity. Not only are patients with Crohn's disease more likely to have relatives with Crohn's disease, but those relatives are likely to have Crohn's disease that is similar to that of the proband in terms of anatomical location, age of onset, and disease behavior (inflammatory vs. fistulizing vs. stenotic) [34–37]. In affected parent–child pairs, the age of onset for the parents was significantly higher. In contrast, affected siblings were highly concordant for age of onset [35]. It is not clear whether these genetically based clinical patterns reflect differences in the genes associated with Crohn's disease or the presence of another set of genes that affect the timing and location of the appearance of Crohn's disease in patients who are genetically disposed. Genetic markers have been implicated as predictive of disease severity in ulcerative colitis [38]. Human leukocyte antigen (HLA) *DRB1*0103* and allele 2 of the IL-1 receptor antagonist are associated with extensive disease.

Another approach to understanding the genetic basis of IBD is the search for subclinical markers, i.e., parameters used to detect the abnormal genotype in the absence of the full clinical phenotype. The two subclinical markers that have received the most attention are increased intestinal epithelial permeability in Crohn's disease and antineutrophil cytoplasmic antibodies (ANCA) in ulcerative colitis. There is a report of increased intestinal permeability to PEG-400 in patients with Crohn's disease and their first-degree relatives [39]. A genetic defect of intestinal epithelial cells allowing permeation of lumenal antigens across the epithelial monolayer could result in nonspecific activation of the intestinal immune system; however, subsequent studies have yielded inconclusive data [40,41]. The other subclinical marker that has received considerable attention is the presence of ANCA in the sera of patients with ulcerative colitis and Crohn's disease [42]. Two groups of ANCA-positive patients have been defined based on this intracellular staining pattern: pANCA positivity reflects a perinuclear pattern, whereas cANCA reflects a diffuse cytoplasmic pattern. About 70% of patients with ulcerative colitis are pANCA positive, with no correlation with disease extent or activity. Fewer than half of patients with Crohn's disease are ANCA positive and these patients are almost evenly divided between cANCA-positive and pANCA-positive. Many patients with Crohn's disease who are pANCA-positive have left-sided colitis and a clinical picture similar to ulcerative colitis [43]. The role, if any, of ANCA in the pathogenesis of IBD is unknown; however, clinically healthy relatives of patients with ulcerative colitis have an increased incidence of ANCA positivity compared with the general population, suggesting that ANCA is more than just a marker for colonic inflammation [44].

Potential etiological agents and antigenic triggers

Clear evidence exists for the activation of the immune response in IBD. The lamina propria is infiltrated with lymphocytes, macrophages, and other cells of the immune system. A gene chip analysis of gene expression in ulcerative colitis mucosa demonstrated up-regulation of many genes associated with immune activation [45]. In any immune response, a specific antigen serves as a trigger for the response and as a target for the effector arm of the response. Over the past 30 years, an intensive search has been conducted for the antigens that trigger the immune response in IBD. Immune activation in IBD is largely confined to the gastrointestinal tract; therefore, the search for the antigenic trigger has focused on the intestinal lumen, where most of the antigens are of microbial or dietary origin. Three major hypotheses as to the antigenic triggers in IBD have been postulated.

One hypothesis is that the antigenic triggers are microbial pathogens that have not yet been identified because of fastidious culture requirements. According to this hypothesis, the immune response in IBD is an appropriate but ineffective response to these pathogens. Various viruses and bacteria have been proposed as candidate organisms, but little evidence has been found to support any of these organisms as having a causative role in IBD. One organism that created some interest is a species of mycobacteria similar to or identical with *Mycobacterium paratuberculosis*; however, there are few data to support this organism as a causal agent in Crohn's disease [46–48].

The second (and best supported) hypothesis as to the antigenic trigger in IBD is that it is some common dietary antigen or usually nonpathogenic microbial agent against which the patient mounts an abnormal immune response. In healthy persons, a finely tuned, low-grade chronic inflammation is present in the intestinal lamina propria. Presumably, this chronic inflammation is a product of chronic exposure of the lamina propria to lumenal antigens. Failure to suppress this inflammatory response could result in the uncontrolled immune activation seen in IBD. As a result of failure of normal suppressor mechanisms, immune activation in IBD may be an inappropriately vigorous and prolonged response to

some normal lumenal antigen. The genetic basis of IBD may relate to a genetically determined ability to mount an immune response to a specific lumenal antigen. It may be that patients with IBD are genetically programmed to mount an intense immune response to some common lumenal antigen – either dietary or microbial – to which most people do not respond. In genetically based mouse models of colitis, defects in T-cell function or cytokine production result in uncontrolled immune responses to normal colonic bacteria [49]. In IL-$10^{-/-}$, IL-$2^{-/-}$, and T-cell receptor double-negative ($TCR^{-/-}$) mice inflammation only develops in the presence of normal colonic flora. If these mice are grown under germ-free conditions they do not develop inflammation. These studies demonstrate that normal colonic flora can stimulate a pathological inflammatory response in the presence of a defect in immune regulation. Moreover, a number of different defects in immune regulation result in a mucosal inflammatory response to normal colonic flora. This has two potential implications for IBD. The first is that IBD may result from the initiation of an immune response to normal flora as a result of a defect in immune regulation. The second is that ulcerative colitis and Crohn's disease may be a group of diseases resulting from a number of defects in immune regulation but all manifesting as a mucosal inflammatory response to normal flora. The demonstration that a fraction of patients with Crohn's disease have mutations in *NOD2*, a gene that codes for a protein involved in the response to bacterial products, supports both the suggestion that Crohn's disease involves a dysregulated immune response to bacteria and the suggestion that Crohn's disease is genetically heterogeneous [32,33].

The third hypothesis (autoimmunity) as to the antigenic trigger in IBD is that it is one expressed on the patient's own cells, particularly intestinal epithelial cells. In this theory, the patient mounts an appropriate immune response against some lumenal antigen, either dietary or microbial; however, because of similarities between proteins on the epithelial cells and the lumenal antigen, the patient's immune system also attacks the epithelial cells. Under the autoimmune theory, the immune response is directed specifically toward the epithelial cell, and the epithelial cell is destroyed by one of two immune effector mechanisms, either antibody-dependent cellular cytotoxicity, which requires an antibody directed against an epithelial cell surface antigen, or direct cell-mediated cytotoxicity. Although little evidence exists to support a role for autoimmunity in Crohn's disease, several investigators have reported the presence of anticolon antibodies in the sera of patients with ulcerative colitis [50,51]; however, similar antibodies have been found in the sera of healthy persons and in patients with systemic lupus erythematosus in whom there was no intestinal inflammation [52]. Das and colleagues [53,54] demonstrated the presence of a tissue-bound antibody that could be eluted from surgical resections performed for ulcerative colitis; this antibody is directed against tropomyosin. Whether this antibody participates in the pathogenesis of ulcerative colitis or is just a marker for immune activation is not clear. The presence of an anticolon antibody does not necessarily mean that the antibody plays a pathogenetic role. A more convincing case for a pathogenetic role could be made by the demonstration that this antibody participates in antibody-dependent cellular cytotoxicity.

Immune response

The first step in the immune response to an antigen is the uptake and processing of the antigen by macrophages or other antigen-presenting cells (Fig. 57.1). CD4 (helper/inducer) T cells recognize soluble antigens in conjunction with HLA class II molecules (HLA-DP, -DQ, and -DR) present on dendritic cells and macrophages. Class II antigens on intestinal epithelial cells also may play a role in antigen processing within the intestinal immune compartment. Class II molecules are present on epithelial cells of the normal human small intestine but not on those of the colon. Class II antigen expression on small intestine epithelial cells is retained in patients with Crohn's disease. HLA-DR-like molecules on human intestinal epithelial cells may be involved in antigen-induced triggering of intraepithelial lymphocytes. Marked enhancement of colon epithelial cell HLA-DR staining occurs in active ulcerative colitis and Crohn's colitis, and intestinal epithelial cells from patients with IBD are capable of processing and presenting antigens in vitro [55].

The activated macrophage, in addition to presenting antigen to the T cell, releases IL-1, which activates the T cell. The activated T cell releases IL-2, which promotes the clonal expansion of cytotoxic T cells and increases the function of helper T cells and B cells. Cell-mediated immune responses may be involved in the pathogenesis of IBD. There is a finely balanced chronic low-grade immune response in the normal intestine and colon. The immune response is a product of $CD4^+$ T-cell activation by bacteria in the lumen, as the response is largely absent in germ-free animals. This activation is promoted by interferon-γ (IFN-γ) and is down-regulated by IL-10 and transforming growth factor-β (TGF-β) produced by regulatory T cells (see Fig. 57.1). The current data suggest that IBD is a product of genetically determined immune dysregulation to lumenal antigens. Whether the immune dysregulation produces an overly active up-regulation of the immune response or an inadequate down-regulation is not clear. It is also not clear whether this dysregulation is specific to certain antigens.

There are three T-cell effector phenotypes: T helper type 1 (Th1), Th2, and Th17 subsets. Th1 and Th17 effector cells mediate Crohn's disease whereas Th2 effector cells are thought to play a role in ulcerative colitis. The Th17 effector cells, the most recently described effector phenotype, are associated with chronic inflammation [56]. Th17 cells express IL-23R and produce IL-17 in response to IL-23. The association of Crohn's disease with a mutation in IL-23R suggests a role for Th17 cells in the pathogenesis of Crohn's disease.

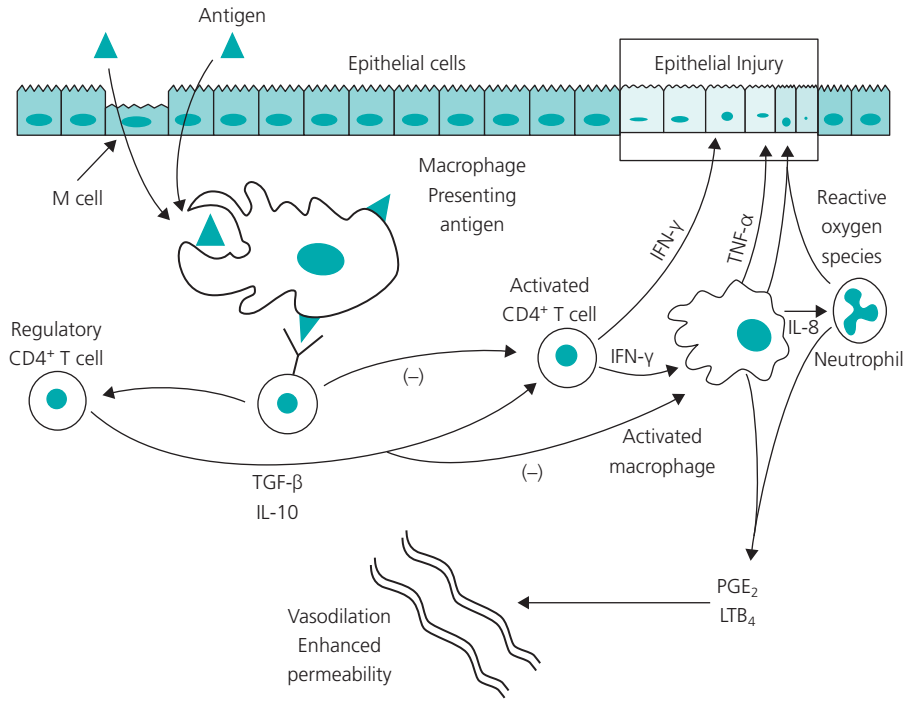


Figure 57.1 An immunological sequence postulated to account for the inflammatory response observed in inflammatory bowel disease starts with an exogenous sensitization to lumenal antigens, presumably bacterial, facilitated by genetic influences. The first cell population involved is the dendritic cell, which phagocytoses and processes antigen and presents it to a $CD4^+$ T cell. Two populations of $CD4^+$ T cells are produced: one an activated effector population and the second a population of regulatory T cells. The activated T cells produce interferon-γ (IFN-γ), which activates macrophages and participates in epithelial injury. The regulatory T cells produce transforming growth factor-β (TGF-β) and interleukin-10 (IL-10), which down-regulate both the activated $CD4^+$ T cells and the activated macrophages. Activated macrophages make IL-12, which further promotes the differentiation of $CD4^+$ T cells towards a T helper type 1 phenotype. Activated macrophages participate in epithelial injury by producing tumor necrosis factor-α (TNF-α) and reactive oxygen species and by recruiting neutrophils, which also produce reactive oxygen species. Macrophages and neutrophils also produce prostaglandin E_2 (PGE_2) and leukotriene (LTB_4), which contribute to the vasodilation and enhanced vascular permeability that is characteristic of inflammatory bowel disease.

Not only T-cell effector phenotypes but also regulatory cells (Tregs) are important in regulating the intestinal immune response [57,58]. The Tregs play important roles in self-tolerance and in the response to lumenal antigens. They come in two varieties, natural Tregs (nTregs), which are generated in the thymus and have high-affinity TCR to self antigens, and adaptive Tregs, which are generated outside the thymus in response to antigens encountered in organs or secondary lymphoid tissue. The role of Tregs in regulating mucosal inflammation is best demonstrated by the decrease in colonic inflammation associated with the adoptive transfer of Tregs in mouse models of colitis.

Antibody secretion

In view of the increased access of lumenal antigens to the immune cells of the lamina propria, it is not surprising that there is increased antibody secretion by intestinal mononuclear cells in IBD. Not only is the number of antibody-secreting cells increased, but also the distribution of immunoglobulin classes is changed. In healthy persons, the vast majority of the immunoglobulin secreted by intestinal mononuclear cells is immunoglobulin A (IgA). In IBD, there is markedly increased production of IgM and IgG. IgA does not fix complement, whereas IgM and some IgG subclasses do. There are subclasses of IgG that are defined by the structure of their constant regions. There is some subclass specificity in the increase in IgG production seen in IBD. Ulcerative colitis is associated with increased production of IgG1 and IgG3, whereas Crohn's disease is associated with increased production of IgG2. IgG1 and IgG3 antibodies account for the predominant IgG response to proteins and T-cell-dependent antigens. IgG2 provides the predominant IgG response to carbohydrates and many bacterial antigens. Delineation of the stimuli and antigens that induce the increased secretion of IgG subclasses may provide insight into the etiology and pathogenesis of IBD.

Cytokines

Cytokines are glycosylated proteins that mediate potent biological function at low concentrations in a hormone-like fashion (see section on cytokines, Chapter 7). Like hormones, they act through receptors, but unlike hormones, which act

on cells at a distance from their cell of origin, cytokines act on neighboring cells. Originally, cytokines were described as being produced by immune cells and acting on immune cells, but it is clear that they are both produced by and act on non-immune cells. Cytokines almost certainly play important regulatory roles in IBD, as they do in other chronic inflammatory states [59]. The best-developed data are those demonstrating increased production of most of the major proinflammatory cytokines (IL-1, IL-6, IL-8) and tumor necrosis factor-α (TNF-α). The proinflammatory cytokines contribute to many of the characteristic attributes of IBD: neutrophil infiltration (IL-8), T-cell activation (IL-1), expression of adhesion molecules (IL-1 and TNF-α), and enhanced epithelial permeability (IFN-γ). TNF-α and IFN-γ may contribute directly to epithelial injury (see Fig. 57.1). The natural inhibitor of IL-1, IL-1 receptor antagonist, is a protein produced by macrophages in parallel with IL-1. The ratio of IL-1 receptor antagonist to IL-1 is lower in IBD mucosa than in normal mucosa. Other cytokines, including IL-10 and TGF-β serve to down-regulate the immune response (Fig. 57.1). Genetic mouse models in which IL-10 or TGF-β is disrupted have colitis as their phenotype. These models demonstrate that failure of the regulatory processes that down-regulate the immune response can lead to chronic inflammation.

$CD4^+$ T cells have been divided into subsets based on the profile of the cytokines produced. IL-12, which is produced by macrophages, causes naive lymphocytes to differentiate to Th1 lymphocytes. The cytokines produced by Th1 lymphocytes, IL-2 and IFN-γ, are associated with cellular immune responses and increased IgG2 production. IL-4, which is produced by macrophages, causes naive lymphocytes to differentiate to Th2 lymphocytes. The cytokines produced by Th2 lymphocytes, IL-4, IL-5, IL-6, and IL-10, are associated with hypersensitivity reactions and increased IgG1 production. Crohn's disease appears to be associated with activation of Th1 lymphocytes as demonstrated by increased levels of IL-2, IL-18, IFN-γ, and IgG2 [60–63]. Th17 effector T cells make IL-17 (or IL-17A), IL-17F and IL-6 [56]. Crohn's disease is associated with a mutation in IL-23R and increased levels of IL-17, suggesting an association with Th17 cells [32,64]. Ulcerative colitis is associated with activation of Th2 lymphocytes as demonstrated by increased IL-5, IL-10, and IgG1 [60]. These findings indicate that Crohn's disease is characterized by dysregulated Th1 and Th17 responses and ulcerative colitis is characterized by a dysregulated Th2 response but they do not reveal the mechanisms involved in the induction of these dysregulated responses. Genetically based animal models of colitis are also characterized by dysregulated Th1, Th2, and Th17 responses. In these animal models it is clear that an abnormal immune response is mounted against normal bacterial flora, raising the possibility that the antigenic trigger for the dysregulated immune response in IBD is the normal flora. The evidence for dysregulation of the Th1, Th2, and Th17 systems in IBD is strong. A number of therapeutic strategies that target these pathways are sure to be tested [65].

The major cell source of the proinflammatory cytokines is almost certainly activated macrophages in the lamina propria, although other cell types may make important contributions. Cytokines, particularly IL-1, TNF-α, and IFN-γ, stimulate epithelial, endothelial, and mesenchymal cells and activate immune cells. They also are involved in the regulation of wound healing and fibrosis [66]. Cytokines may be useful as markers of disease activity, especially tissue IL-1 levels in ulcerative colitis and serum IL-2 receptor levels in Crohn's disease [67,68].

The demonstrated efficacy of infliximab, a chimeric antibody to TNF-α, in Crohn's disease supports the key role of cytokines in IBD pathogenesis [69]. TNF-α is produced in Th1-mediated immune responses and the effectiveness of infliximab in Crohn's disease supports the suggestion that Th1 lymphocytes are important in mediating immune activation in Crohn's disease. The success of infliximab will certainly lead to other attempts to treat Crohn's disease by modulating Th1 cytokines.

Genetically based animal models of colitis

A number of genetically based animal models of IBD have been developed [49]. In most cases, these models were developed to study the effects of specific genetic perturbations of the immune system rather than as models of colitis. A surprising product of these manipulations of the immune system was the discovery that many genetic abnormalities in the systemic immune system have as their only phenotype the development of colitis. Furthermore, the development of colitis in these genetic models requires the presence of intestinal bacteria. The genetic defects that have colitis as their phenotype in animals with conventional bacterial flora have no phenotype in germ-free animals.

These genetically based models of colitis fall into three groups. One group of models includes three cytokine knockouts. Mice with disruption of the genes for IL-2, IL-10, and TGF-β all develop colitis [70–72]. The second group of models includes mice or rats with T-cell defects. Among these are mice with defects in the T-cell receptor and transgenic rats into which the human *HLA-B27* gene has been introduced [73,74]. In the *HLA-B27* transgenic rats, colitis is not the only phenotype; these animals also have gastritis and arthritis. An especially interesting model is the severe combined immunodeficiency disease (SCID) mouse repleted with CD45RBhi $CD4^+$ T cells [49]. SCID mice lack all $CD4^+$ cells yet they develop no colitis; however, when these mice are repleted with a subset of $CD4^+$ cells, they develop colitis. The third group of models includes mice lacking $G\alpha_{12}$, a subunit of a G-protein [75]. Whether T cells are the important targets in this genetic model is not clear.

It is possible that all of these models relate to T cells and their regulation by cytokines. One could imagine a model in

which immune activation in the intestinal lamina propria in response to lumenal bacteria is kept in check by a population of regulatory T cells through the secretion of down-regulatory cytokines, such as IL-10 or TGF-β (see Fig. 57.1). Defects in that T-cell population or in its ability to produce down-regulatory cytokines would remove the brake on immune activation induced by bacterial stimulation of other T-cell populations. The interaction of T cells and cytokines was demonstrated further in studies with the CD45RBhi $CD4^+$ T-cell repleted SCID mice. Administration of a monoclonal antibody against IFN-γ to these animals completely eliminated the colonic inflammation, and administration of a monoclonal antibody against TNF-α reduced it significantly. These studies raise the possibility that the intestinal damage caused by unrestrained T-cell activation is mediated by IFN-γ and TNF-α.

In general the animal models that most resemble Crohn's disease are those associated with abnormalities in Th1 cytokines. Colitis induced by rectal administration of trinitrobenzene sulfonic acid has a histological picture consistent with Crohn's disease. There are high levels of IFN-γ in the colons of mice receiving trinitrobenzene sulfonic acid and the colitis can be abrogated with antibodies to IL-12 [76]. Both these findings are consistent with inflammation mediated by Th1 lymphocytes. There is an especially intriguing spontaneous mouse model, called the SAMP1/Yit mouse, that closely resembles Crohn's disease [77]. Adoptive transfer studies demonstrate that $CD4^+$ T cells that produce a Th1-like profile of cytokines mediate intestinal inflammation in this model. In contrast animal models that most resemble ulcerative colitis are those associated with elevated levels of Th2 cytokines. A histological picture similar to ulcerative colitis develops in mice with a mutant T-cell receptor alpha gene [73]. Analysis of spontaneous cytokine expression by mesenteric lymph node cells showed a decrease in IL-2 and an increase in IL-4 in the $TCR\alpha^{-/-}$ mice with colitis. The decrease in IL-2 and increase in IL-4 is consistent with a Th2-mediated response.

Two of the major themes in the pathogenesis of IBD have been the importance of the lumenal bacteria driving the immune response and the importance of Th1 and Th2 T cells in mediating the immune response. These two themes were combined in a study of a spontaneously occurring model of colitis in the C3H-HeJBir mice [78]. Adoptive transfer of bacterial antigen-activated $CD4^+$ T cells from colitic C3H/HeJBir mice to SCID mice induced colitis. This study demonstrated that T cells reactive with conventional antigens of the enteric flora can mediate IBD.

Although most animal models of colitis involve defects in lymphocyte regulation, enhanced Th1 activity and chronic colitis occur in mice with a cell type-specific disruption of the *Stat-3* gene in neutrophils and macrophages [79]. *Stat-3* codes for a protein involved in the signal transduction of the receptor binding of a number of cytokines and growth factors including IL-6, IL-10, and granulocyte colony-stimulating factor. There are other genetic models of intestinal inflammation based on defects in epithelial cells. N-cadherin is a protein involved in the attachment of adjacent epithelial cells. Mice expressing a dominant-negative N-cadherin along their crypt-villus axis develop IBD [80]. Keratin 8 is a cytoskeletal molecule in epithelial cells. Mice in which the keratin 8 gene is disrupted develop crypt abnormalities and inflammation of the lamina propria and submucosal [81].

The finding that a wide variety of defects in the systemic immune system and epithelial cells results in colitis is informative in defining the regulation of the mucosal immune system. It is less informative in defining the defects in immune regulation or epithelial cells that may be involved in the mediation of human IBD. The pathogenesis of IBD may involve one or more of these regulatory systems or, more likely, may involve other regulatory systems.

Inflammation

IBD is considered a disorder of chronic inflammation, a view reinforced by the large numbers of lymphocytes and histiocytes in the diseased mucosa and submucosa. Ulcerative colitis and Crohn's disease also have histological characteristics of acute inflammation, including an intense infiltration of the mucosa and submucosa with neutrophils. Large numbers of neutrophils leave the bloodstream and enter the inflamed mucosa and submucosa of the bowel [82]. Some neutrophils migrate across the epithelium into the lumen and are passed in the stool, and others are destroyed in the inflamed tissue before they have a chance to migrate into the lumen. This constant flux of neutrophils is mediated by the expression of adhesion molecules on circulating neutrophils and vascular endothelial cells, which allow the neutrophils to bind to the endothelium before migrating into the tissue. Both the expression of adhesion molecules and the migration of neutrophils are regulated by inflammatory cytokines (IL-8 and TNF) and lipid mediators of inflammation, including platelet activating factor and leukotriene B_4 (LTB_4). Neutrophil activation in the inflamed gut results in the release of granule-bound proteases and the production of superoxide and other reactive oxygen species (see Fig. 57.1) [83]. These products of activated neutrophils probably play a role in the destruction of epithelial cells in IBD.

Some of the functional and macroscopic changes seen in IBD, including mucosal hyperemia and edema, are typical of changes seen in any inflammatory state, no matter which organ system is involved. These changes are the products of soluble mediators released in the process of inflammation. Mediators cause tissue edema by increasing vascular (postcapillary venule) permeability to albumin and other macromolecules; hyperemia results from mediators that induce vasodilation. Progress has been made in characterizing the soluble mediators of inflammation and their role in the amplification of the immune response in IBD. There are two reasons for defining their pathogenic role. First, the soluble

mediators appear to be largely responsible for the clinical and histological changes seen in the disease. Second, the drugs that have proved to be beneficial for ulcerative colitis and Crohn's disease appear to exert their therapeutic effects by blocking the synthesis of those mediators. Corticosteroids down-regulate the expression of proinflammatory cytokines and block the migration of neutrophils out of the bloodstream. 5-Aminosalicylate (5-ASA) appears to act farther downstream; it is an antioxidant and may act to block the tissue damage induced by reactive oxygen species. Corticosteroids and sulfasalazine block the synthesis of prostaglandins and leukotrienes in vivo and in vitro [84,85]. Until the etiological agents of ulcerative colitis and Crohn's disease are identified, it is likely that advances in medical therapy will be in the area of regulation of the synthesis of soluble mediators of inflammation.

Almost all mammalian cells, including intestinal epithelium and cells associated with inflammatory events (e.g., mast cells, macrophages, platelets), produce prostaglandins. Prostaglandins, particularly those of the E series, have biological properties that are proinflammatory, including enhanced vascular permeability, vasodilation, and production of pain. Prostaglandin levels are elevated in mucosa and serum in IBD and correlate with disease activity [86]. There are two cyclooxygenases that synthesize prostaglandins. Cyclooxygenase-1 (COX-1) is a constitutive enzyme, whereas COX-2 is induced by proinflammatory cytokines. COX-2 is not expressed in colonic epithelial cells in the absence of inflammation but is expressed in epithelial cells from areas of inflammation in ulcerative colitis [87]. Nonsteroidal antiinflammatory drugs (NSAIDs), which are potent inhibitors of prostaglandin production through both pathways, reduce inflammation in chronic inflammatory diseases, such as rheumatoid arthritis, but are not effective in IBD. Small trials of indomethacin administered orally and rectally revealed no improvement in ulcerative colitis; there was even some suggestion that indomethacin causes clinical deterioration despite decreasing prostaglandin production [88,89]. Prostaglandin E_2 produced through COX-2 appears to be important in gastrointestinal wound healing [90]. The negative effects of NSAIDs in IBD may be related to impaired wound healing.

The mucosa in IBD contains large amounts of LTB_4 [91], a neutrophil chemotactic factor [92]. The concentrations of LTB_4 are markedly higher in rectal dialysates from patients with ulcerative colitis than from controls and in vitro studies have identified LTB_4 [93] as a major neutrophil chemotactic agent in IBD mucosa. Despite the strong evidence that LTB_4 [92] is the major neutrophil chemotactic factor in IBD, clinical trials of inhibitors of LTB_4 synthesis in ulcerative colitis have failed to demonstrate clinical efficacy [94]. Dietary supplementation with fish oil containing eicosapentaenoic acid, which reduces LTB_4 production by acting as a competitive substrate for 5-lipoxygenase, results in significant but modest improvements in ulcerative colitis symptoms [95].

Nitric oxide is found in large amounts in the mucosa in IBD, and inducible nitric oxide synthase is expressed in epithelial cells in patients with IBD but not in epithelial cells from healthy persons [96]. Nitric oxide may contribute to vasodilation and mucosal edema. Whether the net effect of nitric oxide in IBD is to promote tissue injury or tissue repair is not clear.

The inflammatory response can be viewed as a component of the larger process of wound healing. The inflamed mucosa in IBD is characterized by the activation not only of typical inflammation-associated genes, such as COX-2 and IL-8, but also by the activation of genes associated with wound healing including growth factors and metalloproteinases [97,98].

Ulcerative colitis

Clinical findings and natural history

Ulcerative colitis is characterized by diffuse, superficial inflammation of the colonic mucosa that begins in the rectum and extends proximally to involve any contiguous length of colon. The small bowel is not involved, although the distal ileum may exhibit similar superficial inflammation, usually in the setting of extensive colitis, termed backwash ileitis [99]. The extent of colitis usually remains constant from the onset with the length of colonic involvement defining the classification of ulcerative colitis: proctitis (limited to the rectum), proctosigmoiditis or left-sided colitis (extending up to the splenic flexure), or pancolitis/extensive colitis (extending into the transverse colon) (Fig. 57.2) Proximal extension

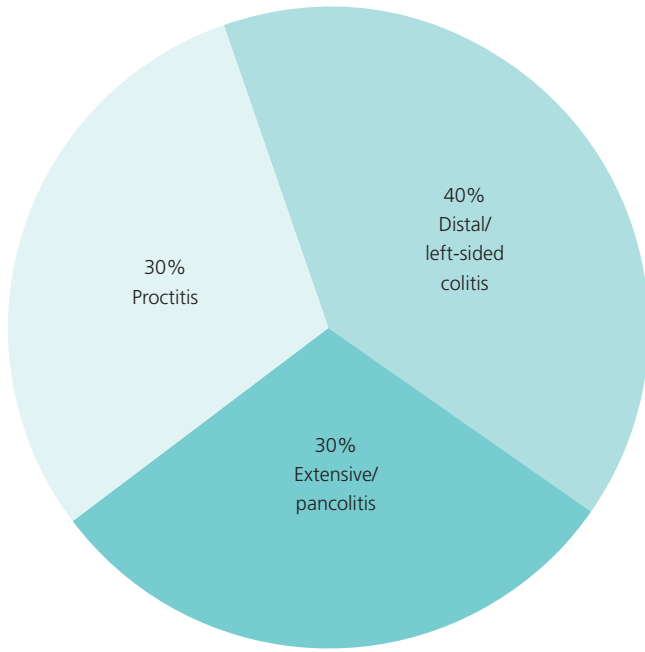


Figure 57.2 Extent of ulcerative colitis at time of initial disease presentation.

occurs in approximately one-third of patients with distal disease, and regression from pancolitis is also possible [100–102]. The extent of involvement does not necessarily imply severity but does impact both prognosis (e.g., the risk of cancer) and treatment selection [103]. The symptoms and course of ulcerative colitis relate to both the extent and the severity of inflammation within the involved segment of colon.

Clinical manifestations

The presenting symptoms of ulcerative colitis are, typically, insidious rather than abrupt; although the disease occasionally presents acutely after infectious colitis or traveler's diarrhea [104]. The presence of rectal inflammation makes bleeding the most consistent presenting feature and this may be gross, or associated with evacuation of mucopus. Symptoms of rectal urgency and tenesmus are related to diminished compliance of the rectum [105]. Increased stool frequency is, likewise, related to decreased rectal compliance and proximal fecal stasis in patients with distal colitis [106] and "bowel movements", per se, should distinguish passage of mucopus without stool. The greater the length of inflamed colon, the more likely patients will present with diarrhea and, conversely patients with proctitis often present with constipated bowel movements with interim passage of blood or mucus [106]. Colicky abdominal pain or cramps preceding bowel movements are common, whereas localized abdominal pain or tenderness (related to transmural inflammation) signifies progressive, severe disease. In the setting of severe or fulminant colitis, systemic symptoms including night sweats, fever, nausea and vomiting, and weight loss accompanied by diarrhea. Most often, nausea and vomiting occur while patients are on the toilet associated with tenesmus. Extraintestinal manifestations (see below) can include inflammation of the eyes, skin, joints, and liver.

Ulcerative proctitis is defined by inflammation limited to the distal 15–20 cm of rectum and is the most common, and usually the mildest presenting extent of ulcerative colitis; accounting for approximately 25%–30% of cases [101,107]. Patients with ulcerative proctitis typically present with hematochezia, a sense of rectal urgency, and constipated bowel movements because of delayed transit of fecal material in the right colon [106]. Systemic manifestations are uncommon with proctitis, but skin or joint symptoms can occur [107]. The disease usually remains confined to the rectum but may advance proximally in as many as 30%–40% of patients [101,102,107].

Proctosigmoiditis or left-sided colitis, accounting for about 40% of ulcerative colitis cases, is an intermediate syndrome in which patients may present with either constipation or diarrhea accompanied by tenesmus, urgency, and rectal bleeding. Colicky left lower quadrant pain is more common than with proctitis, as are extraintestinal symptoms. The proximal disease margin usually remains fixed throughout the course but can spread more proximal or even retract distally [101,102,107].

Extensive colitis (pancolitis) is diagnosed when inflammation extends into the transverse or right colon. In this setting patients are more likely to present with diarrhea as a result of the diminished absorptive capacity of the colon [106], accompanied by rectal bleeding, urgency and tenesmus. Cramping abdominal pain may be diffuse or localized, and patients are more likely to have weight loss, systemic or extraintestinal symptoms, and anemia [107].

Toxic megacolon is the most severe manifestation of ulcerative colitis, diagnosed when the inflammation extends from the superficial mucosa into the submucosal and muscular layers of the colon [108]. Toxic megacolon occurs more commonly in the setting of extensive colitis but can also occur with severe distal colitis. The manifestations of toxic megacolon include: fever, prostration, severe cramps, abdominal distention, and abdominal tenderness that may be localized, diffuse, or associated rebound tenderness. These *toxic* manifestations result from transmural extension of inflammation producing circular muscle paralysis and precipitating dilation along with a "tissue-paper" thin colonic wall.

The clinical severity of ulcerative colitis depends on both the length of colon involved and the severity of colonic inflammation. The symptomatic criteria most commonly used to define the severity of disease were established by Truelove and Witts [109] to assess improvement in the first clinical trial of cortisone for ulcerative colitis and remain useful to classify severity in clinical practice [110–112]. The criteria define *mild* and *severe* disease activity with *moderate* activity being present when there are intermediate symptoms. These criteria may also be modified to include *fulminant* colitis (Table 57.1). Most patients present with *moderately* severe symptoms of ulcerative colitis [113] and the course is, typically, intermittent with the majority of patients maintained in clinical remission or having mild symptoms despite a 90% likelihood of relapse if medical therapy is not maintained.

Table 57.1

Criteria	Mild	Severe	Fulminant
Stools (no. per day)	< 4	> 6	> 10
Blood in stool	Intermittent	Frequent	Continuous
Temperature (°C)	Normal	> 37.5°C	> 37.5°C
Pulse (beats/minute)	Normal	> 90	> 90
Hematocrit (%)	Normal	< 75%	Transfusion
ESR* (mm/h)	< 30	> 30	> 30

ESR, erythrocyte sedimentation rate.
Modified Truelove and Witts' Clinical Classification of Ulcerative Colitis Severity. Adapted from Truelove SS, Witts LI. BMJ 1955;2:1041.

Mild symptoms are defined as patients with fewer than four bowel movements daily and minimal cramping and urgency. Typically, most of the bowel movements occur early in the day, and after the morning evacuations patients are able to proceed with the activities of daily life.

Moderate ulcerative colitis severity is defined by symptoms of four to eight bowel movements daily, typically in the early morning and after meals, associated with rectal urgency. Blood or mucopus are present with most evacuations and nocturnal awakening for bowel movements is common. The disease can interfere with daily work or school activities and social life [113].

Severe ulcerative colitis is manifest by more than eight bowel movements daily, nocturnal bowel movements, severe urgency with or without incontinence, and systemic signs including low-grade fever, night sweats, weakness, and weight loss. Abdominal tenderness, tachycardia, anemia, leukocytosis, and hypoalbuminemia are common [107].

Although not included in Truelove and Witts original classification, patients with *fulminant* colitis have more than 10 bowel movements a day, nocturnal bowel movements, severe abdominal pain or relentless tenesmus, rebound tenderness or distention with tympanic bowel sounds. They also have prostration, high fever, and hypotension. Radiographic studies show evidence of mucosal edema, intramural air (pneumatosis coli), colonic dilation (toxic megacolon), or free abdominal air (perforation) (see below).

Disease course

The natural history of ulcerative colitis is modified by medical therapy. In a population series, the average duration of symptoms before diagnosis was 4.5 months [114] and less than 10% of patients required surgery (colectomy) in the first year (Table 57.2). Untreated, more than 90% of patients will have intermittent or relapsing disease activity [113] and about 50% will have a relapse in any year although only a small minority of patients have chronic, continuous disease activity. More than 90% of patients are able to maintain the ability to work or attend school [113]. Ultimately, up to a third of patients with extensive colitis will require a colectomy at some time through the disease course and up to 30% of patients with disease severe enough to require corticosteroids will undergo colectomy within the subsequent year [115]. Mortality rates, however, for ulcerative colitis are not significantly different from those of the general population [116,117].

Table 57.2 Clinical course in ulcerative colitis

Clinical course	Number of patients	Percentage
Acute fulminating	20	8.0
Chronic intermittent	161	64.4
Chronic continuous	18	7.2
One attack only	45	18.0
Total colectomy in first attack	2	0.8
Died in first attack of other causes	1	0.4
Unknown	2	0.8
Total	249	100.0

Adapted from Edwards and Truelove [791].

Diagnosis

In the absence of pathognomonic features, the diagnosis of ulcerative colitis is made on the basis of clinical, endoscopic, and histological findings. There may also be overlapping pathophysiology between ulcerative colitis and postinfectious irritable bowel syndrome [118]. Thus, the first order of diagnostics is to determine whether there is an inflammatory process or whether symptoms (diarrhea and abdominal cramps) are related to irritable bowel syndrome. Subsequently, the differentiation between ulcerative colitis and other forms of inflammatory bowel disease is the priority for patients presenting with diarrhea and, despite all efforts, we continue to be confronted with patients whose inflammatory pattern remains "indeterminate" between ulcerative colitis and Crohn's disease. At presentation, stool examination for fecal leukocytes confirms the presence of an "inflammatory bowel disease" and cultures should be performed to rule out infectious diseases that may mimic or complicate ulcerative colitis, such as infection with *Salmonella*, *Shigella*, *Campylobacter*, hemorrhagic *Escherichia coli*, and, in particular, *Clostridium difficile* [99].

In contrast to patients presenting with diarrhea, alone, the presence of rectal bleeding should raise the suspicion of ulcerative colitis in individuals below the age of 40. Most often the presentation is subacute or chronic, but it may also be intermittent or progressive [99].

Ulcerative colitis always involves the rectum so inflammatory changes should be visible with a limited examination. The easiest way to exclude ulcerative colitis is by direct examination of the rectosigmoid colon with a proctoscope or flexible sigmoidoscope. Radiographic examinations (e.g., barium enemas), aside from plain abdominal radiographs in patients with severe presentations, have been almost completely supplanted by more sensitive endoscopic examinations.

History and physical examination

A full history should include recent travel; recent infections; use of medications (in particular antibiotics and NSAIDs); past and current smoking history, and family history of inflammatory bowel disease, other immune-mediated inflammatory diseases (psoriasis, eczema, arthritis, liver disease, multiple sclerosis), and colon polyps or cancer. While not of immediate necessity, these can give clues to potential long-term complications and risk factors (e.g., colon cancer). In addition to symptoms of bleeding and diarrhea the history should

determine the assessment of severity related to nocturnal bowel movements, incontinence, weight loss, fevers, and other extraintestinal symptoms such as arthritis, back pain, skin lesions, eye inflammation, and oral ulcerations. In women a menstrual history is relevant related to disease activity and potential therapies.

In most patients with ulcerative colitis, the physical examination is normal, including the abdominal examination. There may be mild abdominal tenderness to deep palpation, particularly in the left colon, but significant abdominal findings are limited to patients with moderate to severe disease, in whom tenderness is more prominent. Abdominal distention, tympany, diminished bowel sounds, or rebound tenderness are ominous signs of fulminant colitis or toxic megacolon. Despite frequent diarrhea, perianal manifestations are absent in ulcerative colitis. Any significant perianal findings (e.g., large hemorrhoids, skin tags, fissures, abscesses, or fistulae) suggest Crohn's disease rather than ulcerative colitis.

Conjunctival or nail bed pallor are common signs of anemia. Patients may present with ocular inflammation, erythema nodosum, pyoderma gangrenosum, or arthritis of larger joints [119]. Low back pain with diminished range of motion, or sacroiliac tenderness, is uncommon. Hepatomegaly, splenomegaly, or evidence of chronic liver disease is rare and limited to patients with end-stage primary sclerosing cholangitis.

Laboratory investigations

Investigations for patients with suspected ulcerative colitis should include a complete blood count and differential. Assessment of liver enzymes is relevant regarding potential hepatic complications and baseline kidney function is necessary for any patient for whom an aminosalicylate test is planned. Microbiological testing should test for potential causes of infectious diarrhea including ova and parasites, enteric pathogens, and, in particular, *C. difficile* [99].

Fecal markers

A hallmark of ulcerative colitis is the presence of neutrophilic infiltration into the epithelial crypts and lamina propria in conjunction with exudation of fecal leukocytes into the colonic lumen. The absence of fecal leukocytes rules out the presence of an inflammatory diarrhea and focuses subsequent diagnostic strategies toward irritable bowel or structural abnormalities of the colon. In addition to direct examination of stool for fecal leukocytes with methylene blue, several fecal tests have become available to assist with the diagnosis of inflammatory bowel disease and have the potential of contributing to quantification of disease activity [120]. Calprotectin is a granulocyte-derived, calcium-binding protein that has been evaluated as a diagnostic assay to identify inflammatory diarrhea [121–123] and to quantify "inflammatory activity" [124–126]. Similarly, lactoferrin, a neutrophil granulocytic protein quantified by a latex agglutination assay [127] or by a polyclonal antibody-based enzyme linked immunoassay has been evaluated to discriminate between active IBD and irritable bowel syndrome [128].

Serological markers

There has been considerable interest in identifying serological markers for diagnostic and prognostic utility in IBD [129,130]. Advances in techniques have improved the sensitivity and specificity of ANCA and pANCA determinations pertaining to the diagnosis of ulcerative colitis [131]. Progress has been made regarding the targeted antigen(s) for pANCA. Both the nuclear heterochromatin DNA-bound protein H1 [132] and protein epitopes expressed by colonic bacteria [133], as well as cathepsin G, elastase, lysozyme, lactoferrin, catalase, enolase, and high-mobility group nonhistone chromosomal proteins [130] have been recorded as cross-reacting with pANCA.

The prevalence of pANCA in patients with ulcerative colitis ranges from 50% to 80% and levels usually remain stable throughout the course of the disease. Differences between various populations may be related to ethnic differences, referral bias, or methodological differences between assays [131,134]. Unfortunately, pANCA can also be identified in up to 31% of patients with an "ulcerative colitis-like" pattern of Crohn's disease [130,135,136].

In contrast to the predominance of pANCA in ulcerative colitis, antibodies to the baker's yeast *Saccharomyces cerevisiae*, (ASCA) are more specific for patients with small bowel Crohn's disease [130] and are present in 40%–60% of patients [137]. The specificity of ASCA appears to relate to small bowel pathology as between 40% and 60% of patients with celiac disease also have ASCA present [137].

Additional serological markers identified in patients with IBD react with the outer membrane of *E. coli* cell wall (OmpC) or an RNA-derived immunologically associated bacterial sequence of *Pseudomonas fluorescens* (I2 peptide) and have been associated more with Crohn's disease than ulcerative colitis and have not been useful in discriminating between the IBD subtypes [131]. The identification of serological markers to microbial antigens provides additional evidence for the loss of tolerance to normal commensal bacteria that has been postulated to occur as an initiating event in the failure to down-regulate the inflammatory cascades in IBD [138–140].

Potential roles for assessing serological assays in IBD include: as markers of disease specificity or prognosis, as a screening tool to discriminate IBD from other digestive disorders, and to assist in the understanding of disease mechanisms or immune interactions. At present, the primary utility of these markers has been to identify disease subgroups with similar immune interactions [131]. In addition, there is evidence that high titers of pANCA correlate with the risk of chronic pouchitis after colectomy and ileoanal anastomosis [141] whereas high titers of ASCA have been associated with intestinal complications and the need for surgery in Crohn's

disease [136,137,142]; the presence of anti-OmpC has been associated with internal penetrating Crohn's disease, and anti-I2 with stricturing Crohn's disease [137].

In contrast, the utility of screening for IBD has been limited to the pediatric population where invasive testing (i.e., colonoscopy) is less acceptable and more costly [143,144]. In adult populations the ability to diagnose ulcerative colitis by flexible sigmoidoscopy or colonoscopy is less of an issue and offers immediate confirmation and access to histology such that serological markers are not necessary to screen for or exclude ulcerative colitis compared to conventional (endoscopic) investigations.

Finally, the potential for serological studies to discriminate between ulcerative colitis and Crohn's disease, particularly for patients with "indeterminate colitis", has been evaluated. The presence of pANCA is not helpful to discriminate between ulcerative colitis and Crohn's disease in patients with indeterminate colitis. In contrast, the presence of ASCA is more helpful, but the sensitivity in indeterminate colitis is so low that a positive or negative predictive value will be low [145]. The serological pattern of $pANCA^+$ $ASCA^-$ is more common in ulcerative colitis compared to $pANCA^-$ $ASCA^+$ in (classic, i.e., small bowel involvement) Crohn's disease with sensitivity for the former of 44%–58% and specificity of 81%–98%. In contrast, the pattern of $pANCA^-$ $ASCA^+$ has a sensitivity of 30%–64% and specificity of 92%–97% for (classic) Crohn's disease [137]. In the future, the addition of OmpC or I2 peptide may help to further discriminate between ulcerative colitis and Crohn's disease in the setting of indeterminate colitis [131].

Table 57.3 Frequency of clinical features in Crohn's disease

	Disease location (%)		
Clinical feature	**Ileitis (%)**	**Ileocolitis (%)**	**Colitis (%)**
Diarrhea	~ 100	~ 100	~ 100
Abdominal pain	65	62	55
Bleeding	22	10	46
Weight loss	12	19	22
Perianal disease	14	38	36
Internal fistulae	17	34	16
Intestinal obstruction	35	44	17
Megacolon	0	2	11
Arthritis	4	4	16
Spondylitis	1	2	5

Adapted from Farmer et al. [191].

Endoscopy

Ulcerative colitis can almost always be diagnosed by endoscopic examination of the rectum and sigmoid colon [99,146]. The disease presents as diffuse, continuous inflammation beginning in the rectum, with proximal extension that varies among individual patients. In most cases it is advisable to determine the proximal margin of disease, from the standpoints of prognosis and therapy [110,111,146]. To avoid confusion with trauma or inflammation from administration of an enema or oral laxatives the initial examination can be performed without enema preparation as patients with active mucosal inflammation rarely have any fecal material in the involved lumen. Complete colonoscopy into the cecum and terminal ileum is most helpful in distinguishing ulcerative colitis from Crohn's disease [147] (Table 57.3).

Healthy colonic mucosa is smooth and glistening, reflects light back from the endoscope, and demonstrates a branching mucosal vascular pattern. With inflammation, the mucosa becomes edematous, erythematosus, and more granular, breaking apart the light reflection (Fig. 57.3). Mucosal granularity

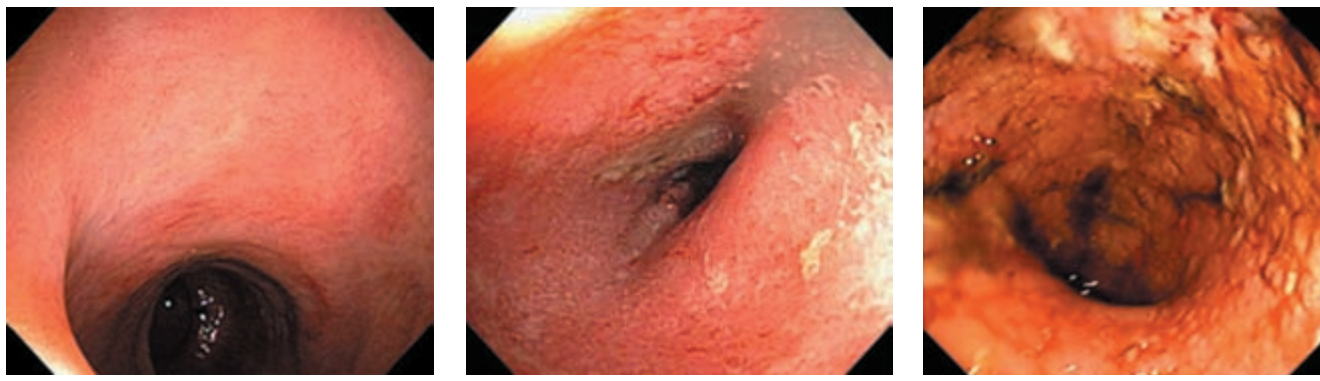


Figure 57.3 Endoscopic severity index for ulcerative colitis. Modified from Sutherland et al. [790].

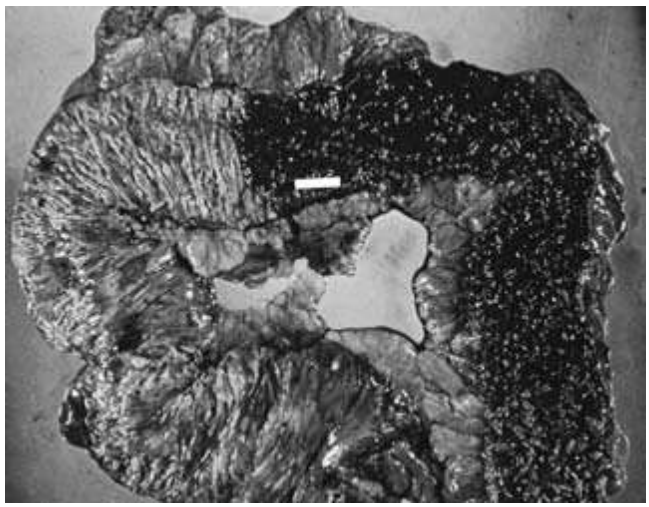


Figure 57.4 Colectomy specimen from patient with ulcerative colitis demonstrates sharp demarcation in the midtransverse colon between involved and uninvolved mucosa. Courtesy of Dr Ira Kodner, St Louis, MO.

Figure 57.5 Filiform pseudopolyps. Courtesy of Dr Ira Kodner, St Louis, MO.

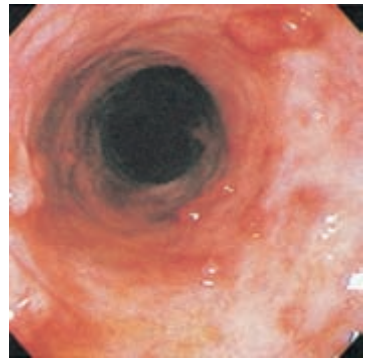


Figure 57.6 Healing ulcerative colitis with residual edema, absent vasculature, and postinflammatory polyps in a tubular, ahaustral lumen. Courtesy of the Crohn's and Colitis Foundation of America, New York, NY.

may be fine or coarse; the latter represents microscopic or pinpoint ulcerations and is associated with friability (either spontaneous or scope-induced hemorrhage from the mucosa). Exudates of mucopus are a common associated finding whereas gross ulcerations represent more severe disease and, although usually shallow, can progress to a total denudation of the mucosa with exposure of the underlying circular musculature. Before treatment, these changes are continuous up to a distinct margin of normal appearing mucosa (Fig. 57.4), or they may extend diffusely to the cecum and, occasionally, into the distal ileum (backwash ileitis). Examination and biopsies of the ileum in the setting of backwash ileitis can differentiate ulcerative colitis from Crohn's disease [146,147]. In the setting of pancolitis, the ileocecal valve is usually patulous, allowing easy entry into the terminal ileum. Limited inflammatory changes in the cecum, around the appendix (peri-appendiceal or cecal red-patch), may be present in patients with distal disease, in which case the focality is not assumed to be related to Crohn's disease [148].

As ulcerative colitis heals, the mucosal changes may become more focal [149,150]. The colonic mucosa regenerates with granularity replacing ulceration, and eventual restitution of the mucosal vascular pattern, which is chronically "distorted" with less distinct branching or irregular, vessels that appear "pruned". In areas that had been more severely inflamed, granulation tissue may protrude and become reepithelialized as postinflammatory, "pseudopolyps" (Figs 57.5, 57.6). These postinflammatory changes can arise in a variety of sizes and shapes and often become fingerlike projections, or even mucosal bridges, in areas that had severe, undermining ulcerations. Pseudopolyps have no neoplastic potential but can be difficult to differentiate from adenomatous polyps. When extensive, pseudopolyps can totally carpet the mucosa, making it impossible to discern distinct, potentially neoplastic polyps.

In the setting of severe colitis, colonoscopy can be performed safely, *in experienced hands*, to assess the severity of mucosal changes as the presence of deep ulcerations bode a poor prognosis for medical treatment [151–153]. Colonoscopy and with biopsies can also assist in the exclusion of complicating infections, including *C. difficile* [146] and cytomegalovirus [154]. However, in the setting of severe colitis it may be difficult to differentiate infectious from idiopathic colitis [155].

Histology

The histological features of ulcerative colitis parallel the endoscopic appearance of a diffuse, continuous pattern of superficial inflammation within the involved mucosal segments and normal proximal mucosa. The principle components are disruption of glandular architecture and an inflammatory infiltrate [156,157]. While there are no pathognomonic histological criteria for the diagnosis of ulcerative colitis [103] a

Table 57.4 Colonoscopic mucosal features and their diagnostic specificity in inflammatory bowel disease

Lesion	Ulcerative colitis	Crohn's disease
Inflammation		
Distribution		
Colon		
Contiguous	+++	+
Symmetric	+++	+
Rectum	+++	+
Friability	+++	+
Topography		
Granularity	+++	+
Cobblestoned	+	+++
Ulceration		
Location		
Overt colitis	+++	+
Ileum	0	++++
Discrete lesion	+	+++
Features		
Size > 1 cm	+	+++
Deep	+	++
Linear	+	+++
Aphthoid	0	++++
Bridging	+	++

Specificity index range: 0 (not seen) to ++++ (diagnostic).
From Hogan [792].

primary distinction between chronic inflammatory bowel disease such as ulcerative colitis and acute self-limited (infectious) colitis is architectural distortion (Table 57.4). In ulcerative colitis, the normal vertical ("test-tube") alignment of glands is distorted and, often branched or irregularly shaped (Fig. 57.7). The glands are separated by expanded lamina propria lymphocytes, plasma cells, and eosinophils, as well as by neutrophils, which are normally sparse. The neutrophilic infiltrate is localized to the base of the glandular crypts and invades the crypts, producing crypt abscesses (Figs 57.8, 57.9) In more severe disease, the epithelial lining is destroyed, with denuding ulcerations over the lamina propria. The

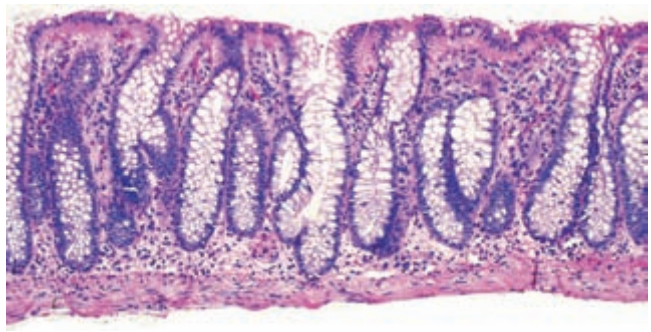


Figure 57.7 Histology of quiescent ulcerative colitis, with branched colonic crypts but no active inflammatory infiltrate. Courtesy of Dr John Hart, Chicago, IL.

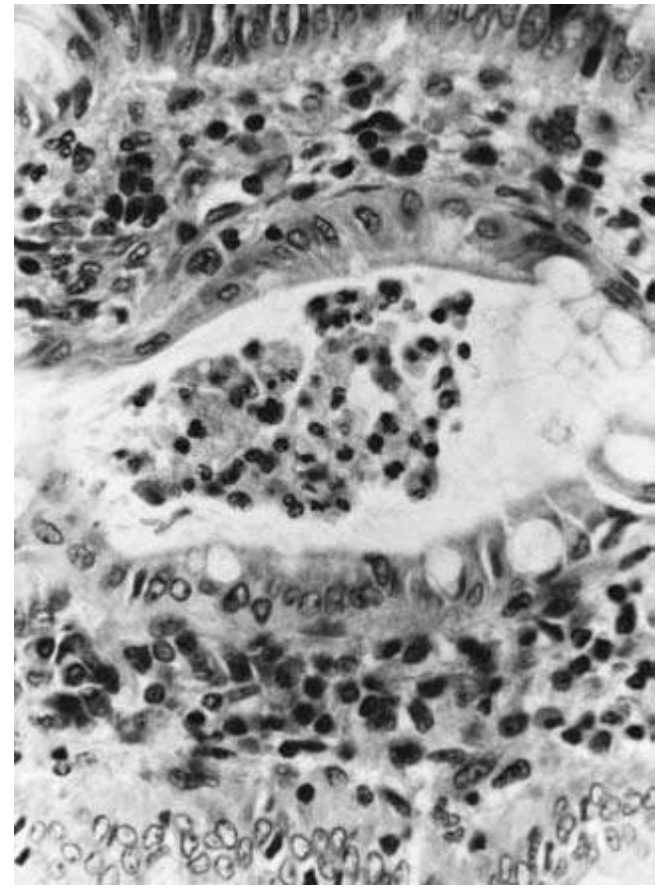


Figure 57.8 Crypt abscess from the specimen shown in Fig. 57.10. Courtesy of Dr David Lacey, St Louis, MO.

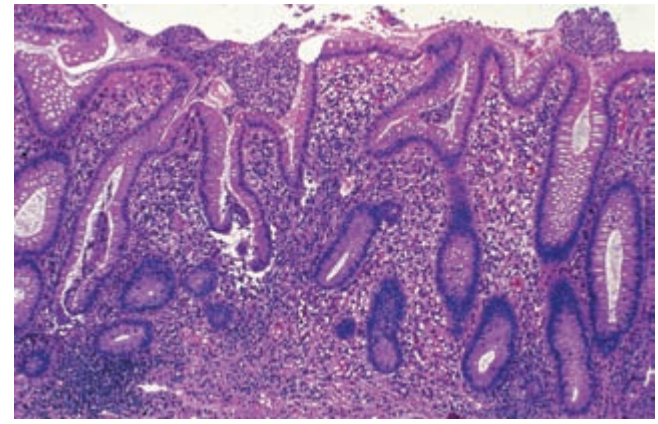


Figure 57.9 Histology of active ulcerative colitis, with distorted colonic crypts, inflammatory infiltrates, and crypt abscesses. Courtesy of Dr John Hart, Chicago, IL.

Figure 57.10 Ulcerative colitis in the appendix showing mucosal inflammation with crypt abscesses. Note the absence of inflammation in the muscularis propria. Courtesy of Dr David Lacey, St Louis, MO.

Figure 57.11 Ulcerative colitis in the appendix showing mucosal inflammation with crypt abscesses. Note the absence of inflammation in the muscularis propria. Courtesy of Dr David Lacey, St Louis, MO.

inflammatory changes are usually superficial, limited by the muscularis mucosae (Figs 57.10, 57.11). In fulminant colitis, the muscular layers are breached by expanding inflammatory ulceration that can leave the bowel wall tissue-paper thin and protected only by the serosa.

As the mucosa heals, the glandular crypts may become atrophied or shortened and irregularly shaped, with a thinned-out lamina propria. Inflammatory polyps comprise vascular granulation tissue with a thin colonic epithelium. In quiescent colitis, the architectural distortion is present but acute inflammation (neutrophils and crypt abscesses) is absent. Both acute inflammation and regeneration of the colonic epithelium produces cellular atypia that must be distinguished from epithelial dysplasia [158,159]. In regenerating mucosa, the glandular epithelium becomes irregularly shaped and hyperchromatic, with depletion of normal apical mucus. Stratification of nuclei or loss-of-polarity are manifestations of neoplastic transformation, i.e., dysplasia (see below).

Imaging studies

Conventional barium radiography has largely been supplanted by endoscopic examinations for the diagnosis and assessment of ulcerative colitis, but radiological examinations may be a valuable adjunct to endoscopy in specific clinical situations [160]. Plain abdominal radiographs are useful in the setting of severe colitis where outlines of the air-filled colon can demonstrate the presence or absence of haustrations or dilation of the colon (to rule out toxic megacolon) (Fig. 57.11). Extralumenal gas under the diaphragm (free air), pneumatosis coli, and evidence of an ileus pattern are additional features of severe colitis. Plain abdominal radiographs also provide a view of the sacroiliac joints and lumbosacral spine as a gross assessment of sacroiliitis or ankylosing spondylitis for patients presenting with low back pain.

Contrast barium studies, once the primary diagnostic modality, are less sensitive and specific than endoscopic examinations that also permit histological sampling [110,111]. Air contrast barium enemas demonstrate the fine or coarse mucosal granularity of microscopic ulcerations or the diffuse, continuous, and symmetric pattern of ulceration involving the rectum to the proximal extent of disease (Fig. 57.12). Other features of ulcerative colitis that may be visible on barium enemas are the loss of haustration in inflamed segments, foreshortening of the colon, and an increase in the space between the sacrum and rectum (Fig. 57.13). Barium enema examinations are contraindicated in severely ill patients because of the potential for perforation or the induction of a toxic megacolon.

Scintigraphic scanning with indium-labeled or technetium-labeled leukocytes has, occasionally, been used to assess severely ill adults or children for relatively rapid determination of the extent, severity, and continuity of inflammation

Figure 57.12 Mild to moderate, left-sided, ulcerative colitis shows granularity of mucosa without ulceration. Courtesy of Dr Dennis Balfe, St Louis, MO.

Figure 57.13 Full-column barium enema in a patient with ulcerative colitis shows shortening of the colon and loss of haustral markings. Courtesy of Dr Dennis Balfe, St Louis, MO.

when the extent of colitis is uncertain or when small bowel disease has not been excluded [161]. Scintigraphy is noninvasive and occasionally helpful in discriminating ulcerative colitis from Crohn's disease.

Computed tomography (CT) enterography has also been evolving as a diagnostic technique for patients with IBD. The technique uses a negatively charged or neutral contrast agent (e.g., water, isoosmotic mannitol, or methyl cellulose) to enhance mucosal viewing and allow improved ability to diagnose small bowel disease [137]. Routine CT scanning can be useful in patients with severe disease or potentially complicating abdominal complications such as an abscess or perforation.

Magnetic resonance imaging of the colon is also an evolving, potentially helpful modality to detect histological inflammation in patients with suspected IBD [162,163] but the sensitivity and specificity are lower than with colonoscopy [164].

Wireless capsule endoscopy is a potential tool to exclude the presence of Crohn's disease in patients with indeterminate colitis [137]. The superiority of wireless capsule endoscopy has been demonstrated compared with enteroscopy [165] and small bowel radiography [166,167] at identifying small intestinal "lesions". However, the differentiation between "normal" small ulcerations or aphthae, and true Crohn's disease lesions, has not been well defined [168–170]. In addition, the utility of wireless capsule endoscopy to identify the etiology of chronic abdominal pain has not been established [171]. The development of an endoscopic classification of small bowel lesions is necessary and minimal prospective "endoscopic" criteria for the diagnosis of Crohn's disease will be required before this novel procedure gains utility in patients with ulcerative colitis [131].

Crohn's disease

Crohn's disease is manifest by focal, asymmetric, and transmural inflammation of the digestive tract that may, or may not, be accompanied by granuloma formation. In contrast to the diffuse, continuous, superficial (mucosal) inflammation limited to the colon in ulcerative colitis, the inflammation of Crohn's disease is patchier (focal), may be transmural, and can involve any segment of the gastrointestinal tract from mouth to anus. The transmural nature of Crohn's disease can lead to intestinal complications of stenoses (strictures) and fistulae. The histological finding of noncaseating granulomas is a hallmark of Crohn's disease, but these lesions are identified in only about 30% of patients; and are not necessary to make the diagnosis.

Crohn's disease has the potential to involve any segment of the gastrointestinal tract so the presentations are more heterogeneous than those of ulcerative colitis: yet they are still are determined by the location, extent, and severity of inflammation as well as the inflammatory pattern. Despite the concept of "skip areas," the location and pattern of intestinal inflammation tends to remain constant for each individual [100]. The spectrum of inflammatory patterns in Crohn's disease ranges from superficial inflammation similar

to ulcerative colitis, to formation of fibrostenosing strictures, to penetration of the bowel wall and fistula formation accompanied by a mesenteric inflammatory mass or perienteric abscess. An attempt to classify Crohn's disease on the basis of inflammatory patterns [172] has been compromised by the tendency towards the development of stenoses over time [173]. In contrast to the potential of curing ulcerative colitis with a colectomy, Crohn's disease is usually not cured by surgery; intestinal resection and anastomosis is almost inevitably followed by recurrence of the disease involving the anastomotic site and proximal intestine [174].

The instrument most commonly used to quantify disease activity has been the Crohn's Disease Activity Index (CDAI) [175], however, because of its complex derivation and lack of discrimination between symptoms and inflammation, the CDAI is not used in clinical practice. Numerous alternative indices have been used in clinical trials, however, to date, none have superseded the CDAI as an investigative tool or for regulatory approval [176]. Instead, physicians perform individualized assessments of the severity of disease according to inflammatory symptoms, obstruction, fistulization, abscess formation, systemic complications, and effect on the patient's quality of life [177].

Diagnosis

Similar to ulcerative colitis, there is no pathognomonic clinical, endoscopic, or pathological marker, or a biomarker for the diagnosis of Crohn's disease which, instead, is diagnosed on the basis of composite clinical, radiographic, endoscopic (Table 57.3), and histological criteria (Table 57.4). Both macroscopic (endoscopic or radiographic) and microscopic (biopsy or surgical specimen) evidence of discontinuous intestinal inflammation with or without granulomas is a hallmark of Crohn's disease. It is not uncommon, particularly in children [178,179], to have inflammation confined to the colon with features that cannot be categorized as ulcerative colitis or Crohn's disease (indeterminate colitis) [180]. Serological studies are becoming more specific for Crohn's disease but, as yet, they do not have sufficient positive or negative predictive values to suffice as confirmatory studies [137], in particular for patients with low, or high, pretest probabilities [181]. At present, the clinical presentation and key features of the history, physical examination, in combination with laboratory studies (including serologies) confirm the presence of IBD and determine a diagnostic workup, to differentiate Crohn's disease from ulcerative colitis and other enterocolonopathies [99].

Clinical manifestations and natural history

Crohn's disease most commonly involves the terminal ileum and cecum. However, the pattern of Crohn's disease can be varied. The presentation is determined by the site, extent, severity, and complications of intestinal and extraintestinal disease [99,182] (Table 57.5). Patients usually present with chronic disease with more than 6 weeks of symptoms, but Crohn's disease can be acute, presenting with severe abdominal pain, intestinal blockage, or hemorrhage. Chronic diarrhea is the most common presenting complaint and abdominal pain and weight loss are present in over 60% of patients at diagnosis. Abdominal pain is a more common feature of Crohn's disease than ulcerative colitis because of the transmural extension of inflammation that results in stimulation of pain receptors in the serosa and peritoneum. Abdominal cramping and postprandial pain are common symptoms that often are accompanied by diarrhea, rectal bleeding, nocturnal bowel movements, fevers, night sweats, and weight

Table 57.5 Histological features that allow the physician to distinguish among acute self-limited colitis (ASLC), ulcerative colitis (UC), and Crohn's disease (CD)

ASLC Feature	UC ($n = 44$)	ASLC vs. UC ($n = 56$)	CD (P[a])	ASLC vs. CD ($n = 26$)	UC vs. CD (P[a])	(P[a])
Distorted crypt architecture	0	32	< 0.00001	7	< 0.0005	0.01
Mixed lamina propria inflammation	0	15	< 0.001	7	< 0.001	NS
Villous surface	0	22	< 0.00001	3	< 0.047	< 0.11
Granuloma	1[b]	3†	NS	16	< 0.0017	< 0.00001
Crypt atrophy	0	16	< 0.0001	3	< 0.048	< 0.047
Basal lymphoid aggregates	0	12	< 0.0011	3	NS	NS
Surface erosions	4	21	< 0.0011	2	NS	< 0.007
Superficial isolated giant cell	7	3	NS	0	NS	NS
Basal isolated giant cell	1	6	NS	3	NS	NS
Polymorphonuclear cells in surface epithelium	9	19	NS	3	NS	< 0.04

a From Surawicz and Belic [793].

b Rectal biopsy information was not used in the clinical categorization of cases. NS = not significant.

loss [182]. Nausea and vomiting occur in the presence of intestinal strictures that produce partial or complete bowel obstructions. Transmural disease commonly manifests in the perianal region as skin tags or perirectal abscesses or fistulae that occur in approximately 10% of patients at the time of diagnosis [183], but also can present as an inflammatory mass in the right lower quadrant. Extraintestinal manifestations including musculoskeletal symptoms with peripheral or axial arthritis, or skin lesions, primarily erythema nodosum, may precede, or accompany, intestinal symptoms and are most common when the colon is involved [119]. In children and adolescents, the presentation often is more insidious, with weight loss, failure to grow or to develop secondary sex characteristics, arthritis, or fevers of undetermined origin [184,185].

Crohn's disease of the esophagus, stomach, and duodenum

Crohn's disease involving the upper gastrointestinal tract is most commonly accompanied by small or large bowel involvement [186,187]. Infrequently, Crohn's disease involving the esophagus, stomach, or duodenum presents with symptoms that mimic gastroesophageal reflux or peptic ulcer disease. Heartburn, dysphagia, nausea, dyspepsia, epigastric pain, and early satiety or postprandial vomiting typically accompanies other systemic inflammatory symptoms such as fever, night sweats, or rectal bleeding. Often, esophageal symptoms and findings resolve with treatment for intestinal disease activity [188]. Duodenal Crohn's disease may present with obstruction, fistula formation, or biliary obstruction [189,190] (Fig. 57.14).

Figure 57.14 Crohn's disease involving the antrum and duodenum. Courtesy of Dr Dennis Balfe, St Louis, MO.

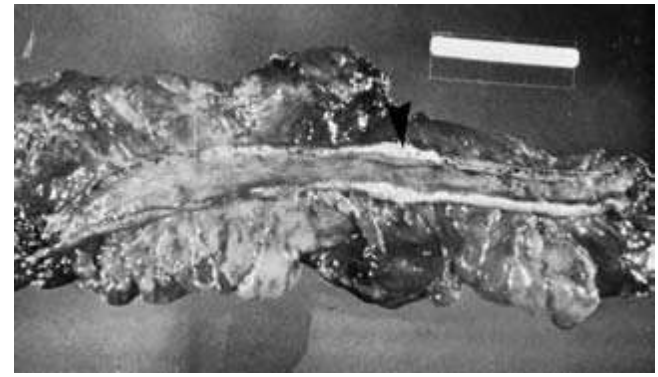


Figure 57.15 Crohn's disease of the small intestine showing lumenal narrowing and thickening of the intestinal wall (arrowhead). There is also a large amount of fat on the serosa. Courtesy of Dr Ira Kodner, St Louis, MO.

Jejunoileitis

Crohn's disease involving, primarily, the proximal small bowel most often presents with vomiting and diarrhea, cramping abdominal pain, and weight loss [189, 191]. The presentation typically includes borborygmi related to focal, segmental strictures compromising the passage of enteric contents. Diarrhea is multifactorial and can be secondary to malabsorption as a consequence of inflammation, protein-losing enteropathy, or stasis and small bowel bacterial overgrowth proximal to strictures.

Ileitis and ileocecal Crohn's disease

Crohn's disease most commonly presents as right lower quadrant abdominal pain and tenderness (often accompanied by an inflammatory mass), diarrhea with or without rectal bleeding, weight loss, fevers, chills, and night sweats. The pain is often described as cramping and usually occurs after meals related to partial or intermittent obstruction of the narrowed intestinal lumen, in which case abdominal distention, nausea, and vomiting may accompany the pain (Fig. 57.15). An acute presentation may mimic appendicitis; occasionally, Crohn's ileitis will be diagnosed at exploratory laparotomy for presumed appendicitis.

Crohn's colitis

Approximately 15% of Crohn's disease cases are limited to the colon [191]. Distinguishing these cases from ulcerative colitis can be difficult, because the clinical manifestations – diarrhea, rectal bleeding, and urgency – overlap with those of ulcerative colitis [103,192]. However, Crohn's disease of the colon is more likely than ulcerative colitis to be accompanied by perianal manifestations (skin tags, perirectal abscess or fistulae) [100] and the rectum is often spared, whereas ulcerative colitis always involves the rectum. In approximately 10%–20% of patients presenting with colitis, the classification may be indeterminate in the setting of diffuse or severe inflammation, or questionable focal inflammation [192] (Fig. 57.16).

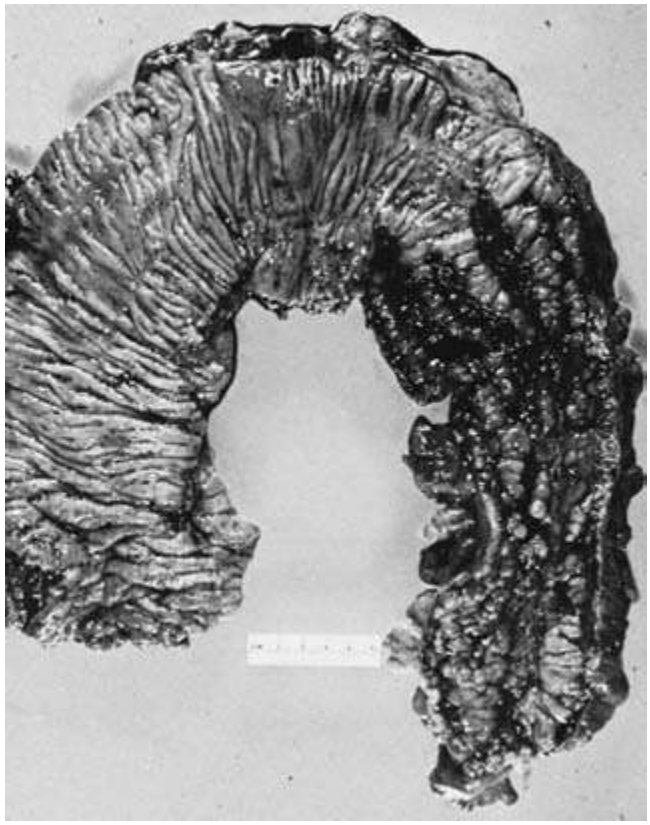


Figure 57.16 Crohn's colitis colectomy specimen shows deep, clawed linear ulcers in the transverse and descending colon. Courtesy of Dr Ira Kodner, St Louis, MO.

Perianal Crohn's disease

Perianal involvement in Crohn's disease develops at some point in the course of Crohn's disease in approximately 25% of patients and most often accompanies colonic disease [193]. Perianal inflammation arises from the anal crypts at the anorectal junction and progresses through or around the anal sphincter, presenting as perirectal abscesses or fistulae. Often, perianal tissue becomes hypertrophied, producing skin tags that may be misdiagnosed, or attributed to hemorrhoids. At times, perianal manifestations are the primary presentation and in extreme situations, the anal sphincter and perineum can become grossly deformed [183,193].

Disease course

Crohn's disease, like ulcerative colitis, has been described as a relapsing and remitting disease. However, such a description takes into account, primarily, symptomatic activity rather than disease progression and does not take into account differing genetic patterns [194], disease location [195], or the nature of medical or surgical therapy [196]. Furthermore, there are environmental factors [197], such as cigarette smoking [198], NSAIDs [199], intercurrent infections [200] and stress [201–203], that may impact on symptoms or inflammatory features.

At any time the majority of patients are either in a symptomatic remission or having mild disease activity [204,205]. The long-term "prognosis" of Crohn's disease depends on the definition of the end point. Mortality is not substantially greater than for the general population [206]. Most deaths are related to either severe disease [207] that may be complicated by infectious complications related to therapy [208]. The association between Crohn's disease and smoking may also contribute to long-term mortality risks, particularly in women [209]. The disease "course" does not "burn out" as similar proportions of patients from cohort studies have evidence of clinical activity or the need for surgery over several decades [210].

The course, as defined as the need for surgery, is impacted by genotypic/phenotypic correlations. Patients with *NOD2/CARD15* mutations are more likely to develop fibrostenosing ileal disease [211,212] that has a high risk of evolving to a need for surgery [195]. Similarly, ileal disease and ileocolonic disease are risk factors for surgical resections [195]. Early onset Crohn's disease is also a risk factor for the need for surgery [136,213,214]. The presence of perianal disease is a risk factor for a more complicated (stricturing, penetrating) course of Crohn's disease [213,214] as is the presence of multiple, or high-titer antimicrobial antibodies [137], particularly in pediatric patients [136,142]. Cigarette smoking is a modifiable risk [215] related to the need for surgery [198] and of post-operative recurrence [216]. Additional factors that have been associated with disease flares are similar to those in ulcerative colitis including: intercurrent infections, in particular *C. difficile* [200,217]; concurrent use of NSAIDs [199,218], and, possibly, stress in selected individuals [201]. The latter concept of stress-induced flare-ups is obfuscated by the possibility of stress-induced symptoms or coexistent irritable bowel syndrome [118] rather than increased inflammatory activity.

Medical therapy also impacts on the disease course and interpretation of effects requires comprehension of specific therapeutic end-points [112,176]. There is a hierarchy of end points according to requisite changes in the CDAI (e.g. 70- to 100-point decrease vs remission defined as a CDAI < 150) and, most recently, both maintenance of clinical remissions and the achievement of "mucosal healing" with the use of biological agents have been correlated with reductions in the need for hospitalizations and surgery [219,220]. Conversely, patients who are treated with acute courses of corticosteroids have a poor, long-term prognosis [115,221] and a likelihood of developing more refractory disease or steroid-dependence. Early introduction of immune-modulators (see section on medical therapy) with corticosteroids improves 1-year remission rates in adults [222] and children [223]. However, the increasing use of thiopurines has not altered the composite risk for surgery [224], possibly because of the delay in introduction of therapy.

Despite the low mortality, Crohn's disease can have a substantial impact on quality of life [225]. Of greatest concern to

patients is the possible need for an stoma, the uncertain nature of disease, and the lack of energy [226]. Patients have problems with employment, social activities, and interpersonal relationships. The quality of life in Crohn's disease is, on average, less good than in ulcerative colitis [227]. The majority of patients are employable but disease activity has a substantial impact on employment that improves with clinical remissions [228,229]. There is also a higher incidence of depression as a comorbid condition with Crohn's disease that diminishes the quality of life [227].

Physical examination

The key findings on physical examination of patients with Crohn's disease include both abdominal and general systematic abnormalities. The abdominal examination may be significant for distention and tympany with high-pitched, abnormal bowel sounds in the presence of intestinal strictures producing partial intestinal obstruction. Tenderness in the area of involvement and the presence of an inflammatory mass are common. It is important to examine the perianal region for evidence of abscess, fistula, skin tags, or anal stricture.

Patients with Crohn's disease often are chronically ill and can present with weight loss and pallor. The eye examination may demonstrate episcleritis or uveitis. Aphthous ulcerations in the mouth are common and in extreme cases patients may exhibit evidence of profound weight loss (temporal and interosseous wasting) and nutritional deficiencies (e.g., cheilosis, tongue atrophy).

Examination of the musculoskeletal system may demonstrate swelling or redness of large joints (e.g., knees, ankles, wrists) or clubbing of the fingers. Skin examination can reveal erythema nodosum or, rarely, pyoderma gangrenosum [119].

Laboratory studies

Laboratory studies in Crohn's disease are largely nonspecific [110]. Anemia is common and can result from deficiencies of iron, vitamin B-12, or folic acid or from chronic disease. Serum ferritin levels correlate better than iron and iron-binding protein levels with bone marrow iron stores in IBD [230]. Leukocytosis is common, depending on the severity of inflammation and the presence of suppurative complications. Thrombocytosis also is common and is related to inflammation or iron deficiency [231]. Elevated erythrocyte sedimentation rates and C-reactive protein levels reflect nonspecific acute-phase reactions [232,233]. Electrolyte disturbances depend on the severity of diarrhea and dehydration. Serum albumin often is reduced as a result of malnutrition and enteric protein losses [234]. Patients with severe weight loss may have prolonged clotting times because of vitamin K deficiency [235]. Urinalysis commonly demonstrates calcium oxalate crystals or red blood cells related to nephrolithiasis.

Quantitative stool examinations are useful in the setting of diarrhea to assess fecal leukocytes (confirming inflammatory diarrhea), stool volume, and fecal fat. Quantification of either fecal calprotectin [236] or lactoferrin [128] are surrogates for the presence of fecal leukocytes [137,237]. As discussed above, the presence of the serological markers anti-*Saccharomyces cerevisiae* antibody and an antibody to the outer core membrane of *E. coli* (OmpC) have high specificity for Crohn's disease and the addition of an RNA-derived immunologically associated bacterial sequence of *Pseudomonas fluorescens* (I2 peptide) improves the sensitivity and specificity of the serological studies for Crohn's disease [137,237]. Nevertheless, these markers have primarily been evaluated and correlated with known diagnostic patterns of Crohn's disease and are less sensitive for the diagnosis in the case of a low pretest probability and their specificity has not been sufficiently evaluated compared to other enteropathies such as celiac disease or NSAID-induced enterocolitis [137].

Imaging studies

There is an evolution of imaging studies to evaluate patients with Crohn's disease [137]. The decision as to which imaging modality to use is made on the basis of the question being addressed because they are complementary techniques with their own strengths and weaknesses. Air-contrast radiography is better for assessing mucosal detail, colonic distensibility, and the presence of strictures. However, in an acutely ill patient with Crohn's disease, a CT scan is often the preferred initial diagnostic test to answer urgent clinical questions concerning the presence of extralumenal complications such as an abscess, or possible obstruction.

For the most part, air-contrast or double-contrast studies are preferred to single-contrast studies because they give much better definition of fine mucosal detail and can even assess the depth of ulcerations. Although contrast studies are usually well tolerated, some precautions should be observed. Barium enema is contraindicated in patients with severe colitis because injection of air and barium into the inflamed colon may precipitate toxic megacolon. In addition, nonsoluble barium contrast agents should be used with caution when obstruction is suspected.

Radiographic findings do not correlate well with symptomatic disease activity [232]. In particular, asymptomatic patients may have markedly abnormal radiographic studies because of fibrosis of the bowel wall with resultant loss of distensibility. A peroral pneumocolon, a regular air-contrast, small bowel follow-through in which air is insufflated in the colon, can assist in determining distensibility of the terminal ileum and can improve the visualization of the ileum. However, air-contrast studies are frequently unhelpful in distinguishing whether a narrowed segment is the result of fibrosis or inflammation.

The earliest form of Crohn's disease detectable by air-contrast barium studies is the presence of aphthous ulcers, which appear as small discrete collections of barium surrounded by radiolucent halos of inflammatory infiltrate

Figure 57.17 Crohn's colitis with numerous aphthous ulcers (arrowhead). Courtesy of Dr Dennis Balfe, St Louis, MO.

Figure 57.18 In Crohn's ileitis with a long segment of involved ileum, the barium columns are widely separated as a result of wall thickening. Courtesy of Dr Dennis Balfe, St Louis, MO.

(Fig. 57.17). These small ulcers are usually multiple, and the intervening mucosa is normal. Aphthous ulcers are not, pathognomonic to Crohn's disease; they are seen in amebiasis, Behçet syndrome, shigellosis, and other conditions. As Crohn's disease becomes more severe, the aphthous ulcers enlarge, deepen, and connect with one another to form stellate and linear ulcers; the intervening mucosa develops a nodular appearance on radiograph, a process termed cobblestoning.

Progressive deepening of ulcers leads to formation of abscesses or fistulae and transmural inflammation and fibrosis induce limited distensibility, with decreased lumenal diameter and stricture formation and a potential "string sign" or "chain of lakes" appearance in the setting of multiple strictures with intervening prestenotic dilation. Thickened bowel wall and adjacent mesentery induce a separation of bowel loops or the appearance of a mass (Fig. 57.18). Formation of fistuliae can also be identified.

Small bowel Crohn's disease can be evaluated by small bowel follow-through or by enteroclysis with the evolving potential to utilize either CT (Fig. 57.19) or magnetic resonance imaging (MRI) enteroclysis (Fig. 57.20) [160,238]. These techniques combine CT scanning with the ability to

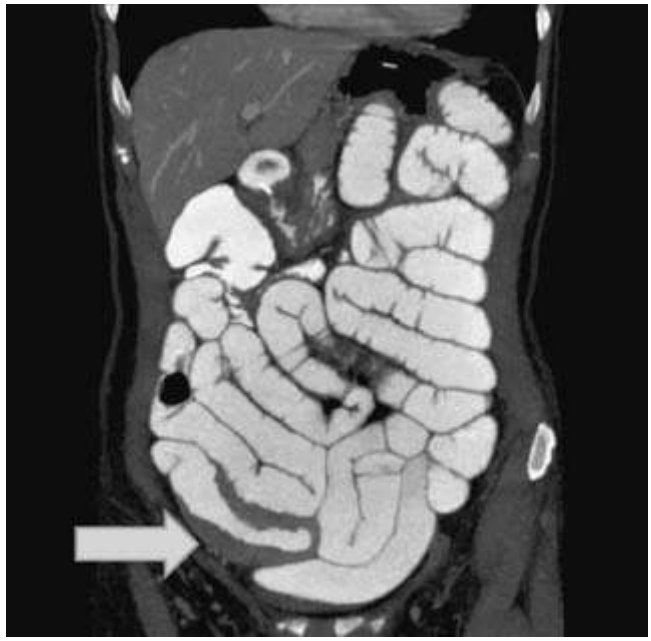


Figure 57.19 Computed tomographic enterography showing thickened terminal ileum (arrow) in a patient with Crohn's disease.

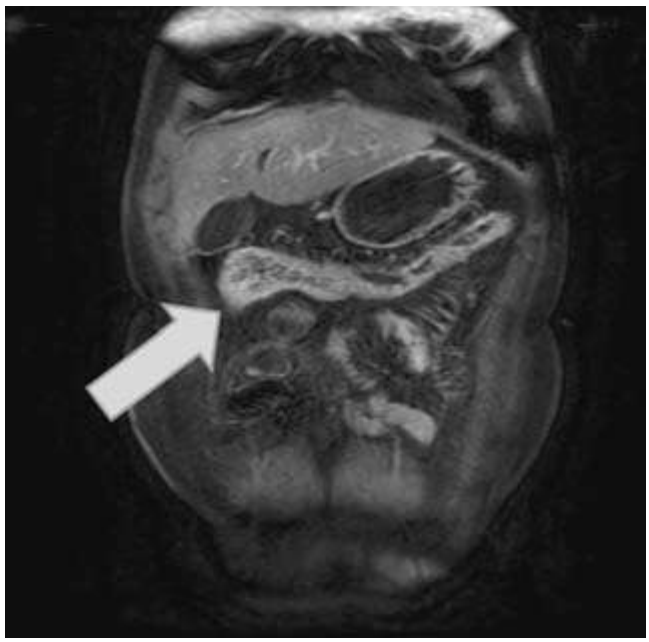


Figure 57.20 Magnetic resonance enterography showing long ileal stricture (arrow) in a patient with Crohn's disease. Courtesy of Dr David Rubin, Chicago, IL.

assess a wall-thickening, inflammatory reaction in adjacent fat ("creeping fat"), and extralumenal complications such as abscesses or a mesenteric inflammatory mass allowing a rapid assessment and helping to guide therapy such as percutaneous drainage of an abscess.

CT is particularly critical in evaluating a patient for intestinal complications of Crohn's disease, most notably fistulae, abscesses, and obstruction (Fig. 57.21). The iodinated contrast used in CT scans is safer in the presence of an intestinal obstruction than barium. In addition, extraintestinal manifestations of Crohn's disease (including hydronephrosis, sacroiliitis, gallstones, and renal calculi) can be identified by CT.

Other imaging modalities occasionally may have a role in IBD, including ultrasonography and MRI. Ultrasonography can be used to complement CT scans for localization and percutaneous drainage of abdominal abscesses whereas MRI examinations are sensitive tools to measure inflammatory activity [165] and to identify perianal disease [193]. Perianal complications can also be identified by transrectal ultrasound [193]. Occasionally, scintigraphy using leukocytes labeled with indium or technetium can be helpful to define locations of intestinal inflammation when barium studies are not possible or their results are indeterminate [161,239].

Endoscopy

Endoscopy and radiology serve complementary roles in the diagnosis and management of patients with Crohn's disease. Endoscopy serves to diagnose Crohn's disease involving the terminal ileum or colon, to distinguish between ulcerative colitis and Crohn's disease, to determine the extent and severity of disease, to assess response to treatment, and to screen for dysplasia. With its ability to visualize the mucosa directly and obtain biopsies, endoscopy has become established as the primary diagnostic tool. In contrast to the characteristic findings of rectal involvement with a pattern of diffuse, continuous inflammation, absent mucosal vasculature with friability, granularity and superficial ulcerations in ulcerative colitis; the distinguishing features of Crohn's disease include rectal sparing, areas of active disease interspersed with normal mucosa; sharp, punched-out ulcerations surrounded by normal mucosa; and "cobblestoning," or nodular mucosa often intersected by crossing linear ulcerations [161,240] (Table 57.4).

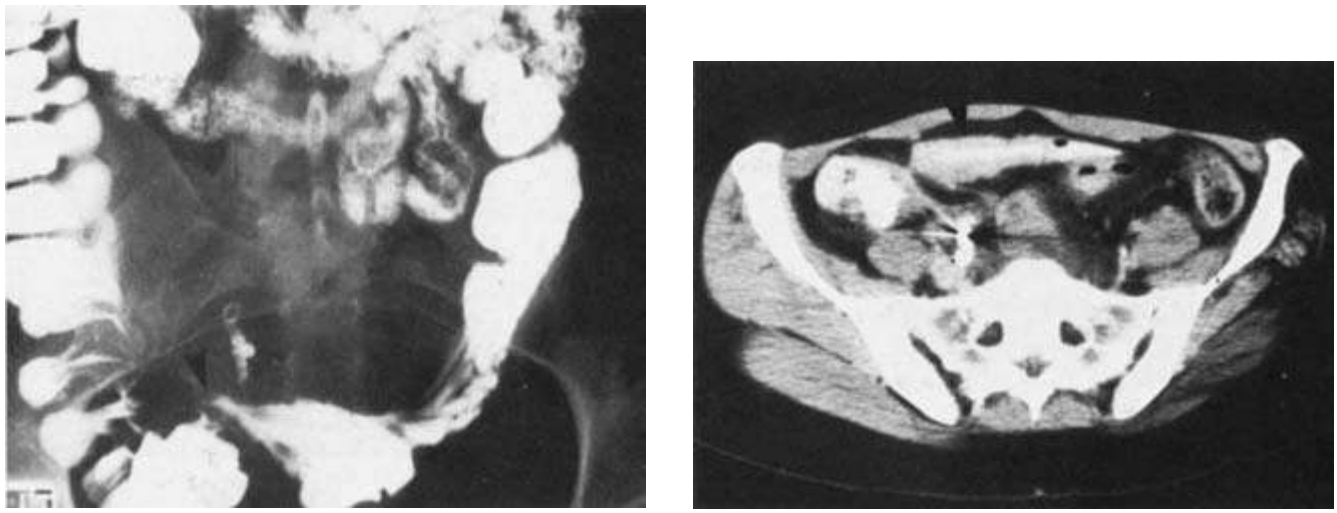


Figure 57.21 Crohn's disease of the terminal ileum (arrows) on small bowel follow-through **(left)** and computed tomography (CT) scan **(right)** in the same patient. Thickening of the intestinal wall is easily appreciated on the CT scan. On small bowel follow-through, wall thickening is indicated by the separation of the columns of barium. Courtesy of Dr Dennis Balfe, St Louis, MO.

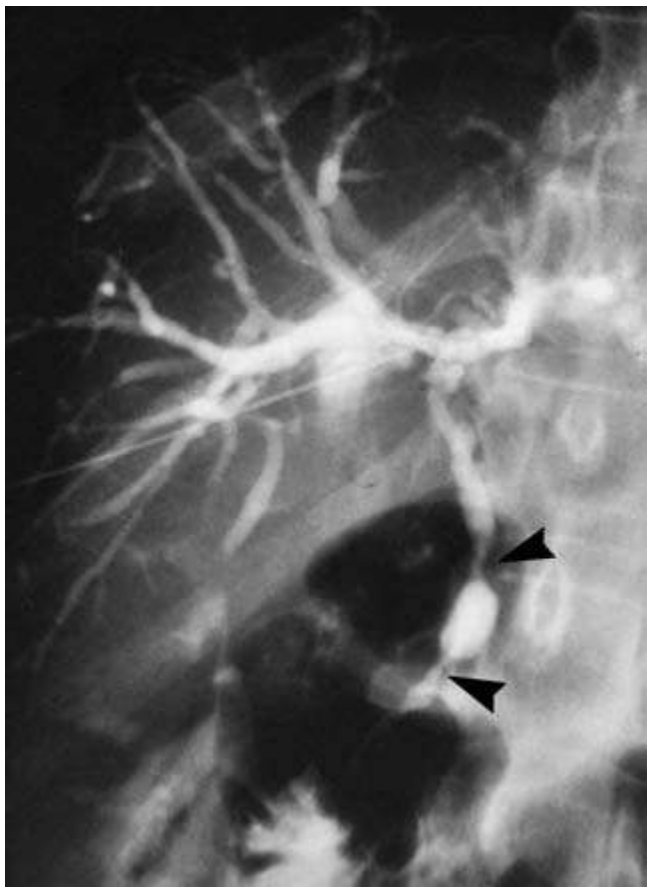


Figure 57.22 Early Crohn's disease with aphthoid ulcers. Courtesy of the Crohn's and Colitis Foundation of America, New York, NY.

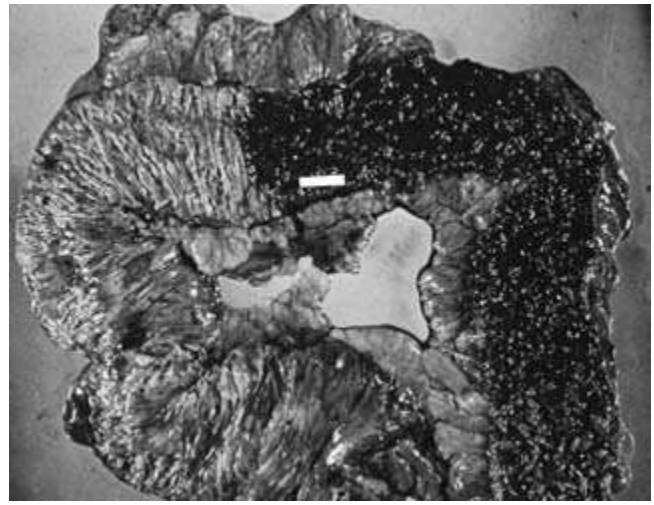


Figure 57.23 Crohn's ileocolitis with rounded, punched-out ulcers around the ileocecal valve. Courtesy of the Crohn's and Colitis Foundation of America, New York, NY.

The earliest endoscopic manifestations of Crohn's disease are aphthous ulcers (Fig. 57.22), small discrete ulcers a few millimeters in diameter surrounded by a thin red halo of edematous tissue. Aphthous ulcers are usually multiple, and the intervening mucosa is normal. They can grow to form rounded, punched out ulcers (Fig. 57.23), or long serpiginous or large stellate ulcers (Fig. 57.24). Longitudinal and transverse ulcers may intersect to form a grid with intervening cobblestone-like areas of nonulcerated mucosa. The diffuse mucosal irregularities of erythema, edema, and granularity, which are prominent in ulcerative colitis, occur less commonly in Crohn's disease. Areas of involvement can be interspersed with normal "skip areas". In a prospective endoscopic study of patients with Crohn's disease who were about to start a course of prednisone, the incidence of endoscopic findings was as follows: superficial erosions, 93%; deep erosions, 74%; mucosal edema, 48%; erythema, 44%; pseudopolyps, 41%; aphthous ulcers, 10%; ulcerated stenosis, 8%; and nonulcerated stenosis, 2% [241]. An endoscopic scoring system has been developed and validated [242] to assess the severity of ileal and colonic disease, but as a result of the complexity of assessing multiple aspects of lesions it has been simplified and based on ulcer size, ulcerated surface, affected surface, and lumenal narrowing [243]. In recent clinical trials with biological agents, an even more simplified assessment of mucosal healing was defined as the absence of mucosal ulceration in all examined segments of the colon and ileum [220].

Colonoscopy has also been used to assess postoperative recurrence of Crohn's disease at ileocolonic anastomoses. Given the high likelihood of recurrent endoscopic and clinical disease [244] the severity of lesions at the anastomosis predicts the likelihood of clinical relapse [245].

Similar endoscopic features may be present in the esophagus, stomach, or duodenum [246] that usually accompany small or large bowel involvement [247]. In addition, the finding of granulomas or focal "enhanced" or "active" gastritis, in the absence of ulceration or *Helicobacter pylori*, at gastric biopsy can help to establish a diagnosis of Crohn's disease [247].

Wireless capsule endoscopy is being used to diagnose Crohn's disease allowing access to parts of the small bowel that cannot be reached by standard endoscopy (Fig. 57.25) [247]. Capsule endoscopy may be more sensitive than conventional imaging studies, including CT enterography or small bowel enteroclysis, for identifying subtle lesions [248]; however, the specificity of small lesions such as "mucosal breaks" identified by capsule endoscopy to diagnose or attribute findings to Crohn's disease activity is compromised by the presence of these lesions in 14% of normal controls [137,249]. Furthermore, there is a significant risk of capsule "retention" in patients with known or asymptomatic strictures [137]. Therefore, patients need to be informed of the risk of capsule retention and the potential need for surgical removal; although some clinicians claim therapeutic benefit from surgical treatment of strictures identified at capsule

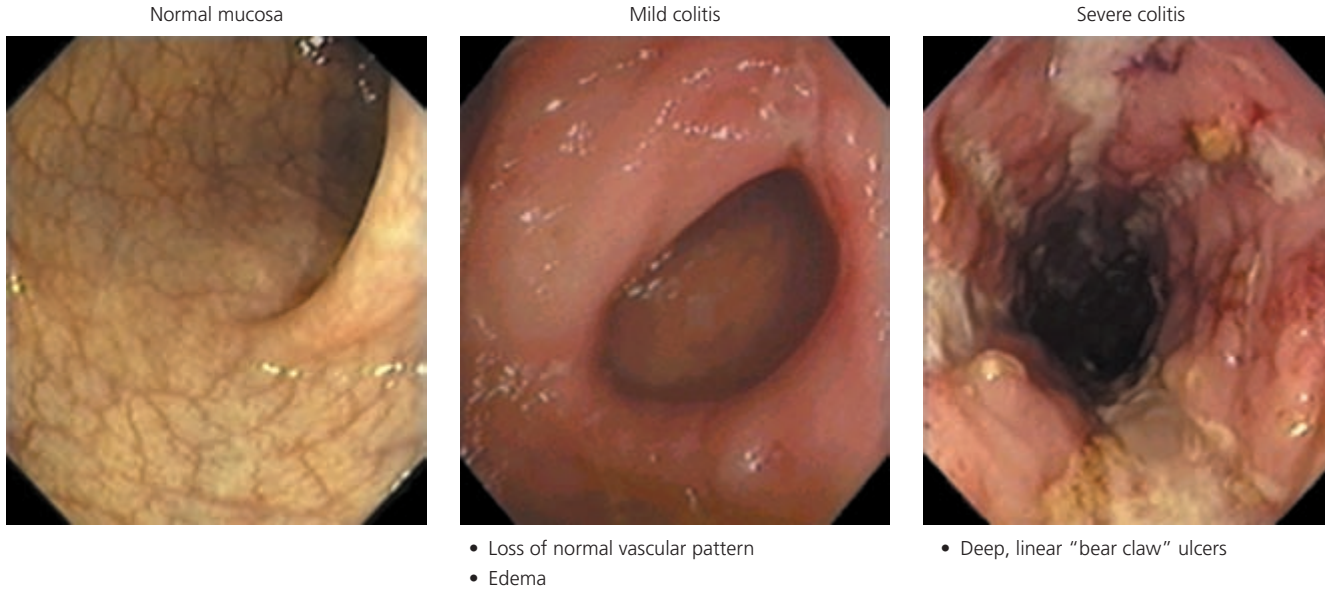


Figure 57.24 Endoscopic appearance of Crohn's colitis.

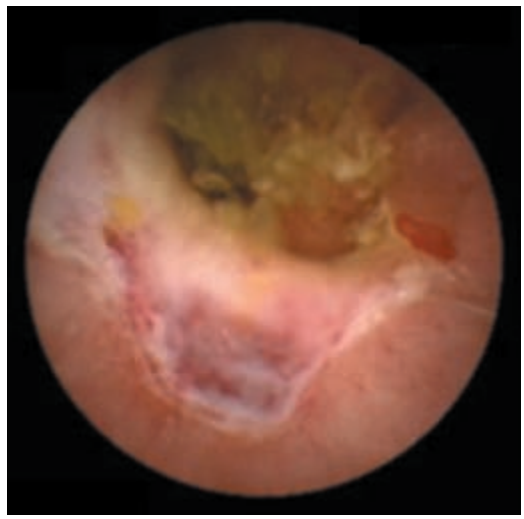


Figure 57.25 Wireless capsule endoscopy image of a small bowel stricture in a patient with Crohn's disease. Courtesy of Dr David Rubin, Chicago, IL.

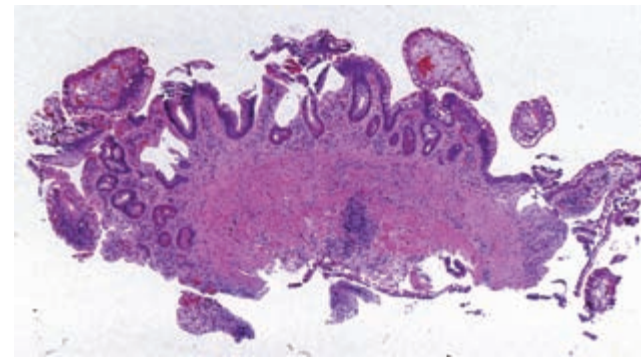


Figure 57.26 Histology of Crohn's ileitis, with distorted villous architecture. Courtesy of Dr John Hart, Chicago, IL.

endoscopy the appropriate algorithm for diagnosis of Crohn's disease supports the use of conventional imaging studies before capsule endoscopy [137] because of the risks and the lack of specificity [249].

Histology

The pathological findings in Crohn's disease reflect the gross pattern of focal and asymmetric intestinal involvement [156] (Fig. 57.26). The primary lesion is an aphthous ulcer; minute erosions overlying lymphoid aggregates [250,251]. As the microscopic ulceration extends in either a linear or a transmural pattern, the histological changes that develop include a mixed acute and chronic inflammatory cell infiltrate comprising lymphocytes, plasma cells, and neutrophils (Table 57.5). Crypt abscesses are common, and the inflammatory infiltrates are often located adjacent to normal epithelium. Noncaseating granulomas, which may be identified in mucosal biopsies or in resected specimens, are characteristic of Crohn's disease (Fig. 57.27); however, the presence of granulomas is not necessary to confirm the diagnosis [179] because they only occur in biopsy specimens from approximately one-third of patients [252]. However, the finding of granulomas in a mucosa that appears grossly normal, such as the "spared" rectum, can help to support the diagnosis of Crohn's disease [253,254]. Granulomas also can be found in lymph nodes, mesentery, peritoneum, and liver [255]. Inflammatory "pseudopolyps" can also be observed in areas of acute or previously inflamed intestine in Crohn's disease [256]. Also, similar to ulcerative colitis, medical therapy can affect the

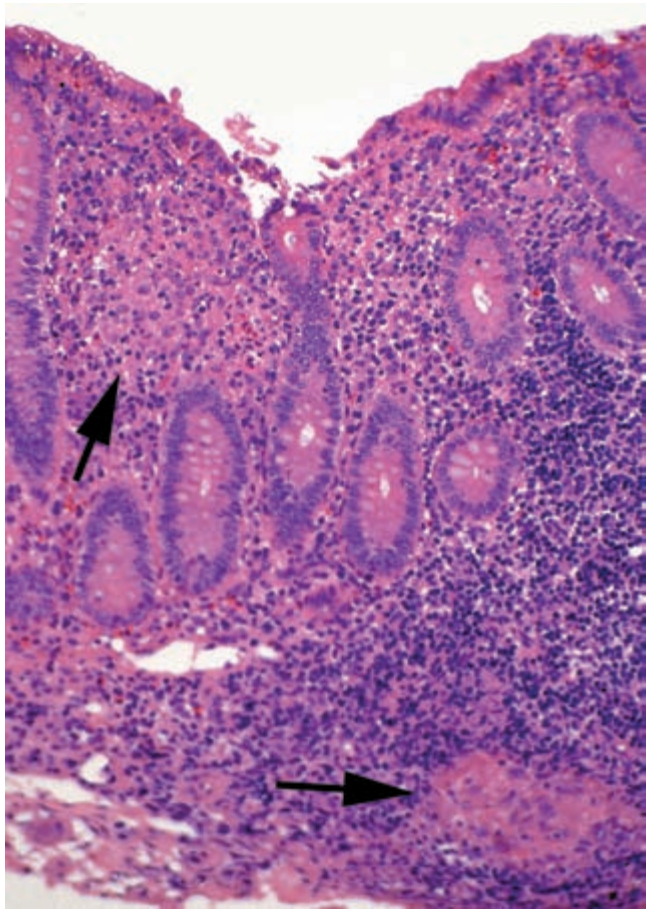


Figure 57.27 Histology of Crohn's colitis. Arrows indicate noncaseating granulomas. Courtesy of Dr John Hart, Chicago, IL.

Figure 57.28 Fissure in a patient with Crohn's disease. A fissure and a chronic inflammatory infiltrate are present, both of which extend into the muscularis propria. Courtesy of Dr David Lacey, St Louis, MO.

histological appearance of the mucosa with focal, or near complete resolution of acute inflammatory changes [150].

Gross examination of the intestine from resected specimens in Crohn's disease shows the transmural nature of inflammation producing thickening of the bowel wall [255]. The adjacent mesentery is thickened, edematous, and contracted, which fixes the intestine in one position. Adipose tissue extends from the mesentery and "creeps" over the serosal surface of the intestine. Transmural inflammation may cause loops of intestine to be matted together. All layers of the intestine are thickened, and the lumen is narrowed (Fig. 57.15). There may be paradoxical involvement of the deeper layers of the bowel wall with lymphoid aggregates overlying normal-appearing epithelium. Submucosal fibrosis, deep fissuring ulcerations, and fistulizing ulcerations communicate between loops of bowel or into the adjacent mesentery (Fig. 57.28). Deep linear ulcers typically are present along the mesenteric surface of the mucosa and contract the lumen in an asymmetrical pattern [257]. Linear ulcerations surrounding "islands" of normal mucosa produce a "cobblestone" appearance whereas parallel deep linear ulcerations can produce a "bear-clawed" appearance (Fig. 57.16).

Extraintestinal manifestations

Although ulcerative colitis and Crohn's disease primarily involve the bowel, both are associated with manifestations in other organ systems. For some patients, especially those with sclerosing cholangitis or ankylosing spondylitis, the extraintestinal manifestations may be more troublesome than the bowel disease. The extraintestinal manifestations can be divided into two major groups: those in which the clinical activity follows the activity of the bowel disease and those in which the clinical activity is unrelated to the clinical activity of the bowel disease. Most extraintestinal manifestations occur more commonly with ulcerative colitis or Crohn's colitis than with Crohn's disease confined to the small intestine.

Peripheral arthritis

The most common extraintestinal manifestation of IBD is arthritis [258–260]. The arthritic complications of IBD are colitic or peripheral arthritis and ankylosing spondylitis or axial arthritis. Peripheral arthritis can be either panciarthitis (four or fewer joints) or polyarthritis (five or more joints) [261].

Panciarthritis is typically asymmetric and predominately affects larger joints (elbow, knees, and wrist) whereas polyarthritis, which is less common, is symmetric and predominately affects smaller joints. The activity of panciarticula arthritis is more likely to follow the activity of the bowel disease whereas in polyarticular arthritis the joint pain can be independent of the activity of the bowel disease. Peripherial arthritis is more common with Crohn's colitis than ulcerative colitis and is uncommon with Crohn's disease confined to the small intestine. Most arthritic flares last only a few weeks, although recurrences are common with renewed disease flares. Deformity with radiological changes occurs in fewer than 25% of cases. Serology for rheumatoid factor is negative in these patients. Peripheral arthritis, uveitis, and erythema nodosum often are seen together. Acetaminophen can be used for joint discomfort. NSAIDs are generally avoided because they may induce a flare or worsening of the colitis. Infliximab is useful in peripheral arthritis [262].

Axial arthritis

Ankylosing spondylitis is seen in patients with IBD, but its activity does not follow that of the bowel disease, and treatment of the bowel disease does not affect the spondylitis [259,260]. Ankylosing spondylitis presents with morning stiffness, low back pain, and stooped posture. Radiography of the spine in ankylosing spondylitis shows squaring of the vertebrae and straightening of the spine. Syndesmophytes appear along the lateral and anterior portions of the intervertebral discs, giving the picture of "bamboo spine" (Fig. 57.29).

Patients with ulcerative colitis have a 30-fold increase in the incidence of ankylosing spondylitis compared with the general population. Even though there is no increased incidence of HLA-B27 in ulcerative colitis, 80% of those with both ulcerative colitis and ankylosing spondylitis are B27-positive [261]. IBD-associated ankylosing spondylitis occurs frequently in females (perhaps up to 40%), whereas, ankylosing spondylitis unassociated with intestinal disease occurs at a male-to-female ratio of nearly 9 : 1. In contrast to peripheral arthritis, which is episodic and usually nondeforming, ankylosing spondylitis can be relentlessly progressive and crippling. The results of medical management of ankylosing spondylitis in IBD are poor. Physical therapy plays a major role in maintaining function. Pain management is a problem, and narcotic addiction is common. NSAIDs, although they reduce inflammation and pain in spondylitis and in colitic arthritis, do not halt the progression of the disease and may exacerbate intestinal inflammation. Medical treatment of IBD and colectomy are not helpful in managing the ankylosing spondylitis. A report of successful treatment with infliximab suggests a hopeful, possibly effective option [263].

Sacroiliitis, inflammation of the joint between the sacrum and the ileum, can be seen in conjunction with ankylosing spondylitis but more often is seen alone. Over 20% of patients with IBD, most of whom have no back pain, have radiographs consistent with sacroiliitis [264,265]. Some will present with low back pain which can be slowly progressive, not related to intestinal inflammation. Radiographs of the pelvis reveal blurring of the margins of the sacroiliac joints, with patchy sclerosis.

Figure 57.29 Sacroiliitis and ankylosing spondylitis demonstrating obliteration of the sacroiliac joints (large arrow) and bamboo spine with syndesmophytes (small arrow). Courtesy of Dr Dennis Balfe, St Louis, MO.

Osteoporosis

Osteoporosis or osteopenia occurs in as many as half of patients with IBD [266]. The incidence of fractures in an IBD population was 40% higher than in a cohort population [267]. In Crohn's disease osteopenia may be present at diagnosis. Numerous factors may contribute including malnutrition, malabsorption, and smoking. Persistent inflammation and steroid use may be the most important factors; TNF enhances bone resorption. Although osteopenia may occur independent of steroid use, steroids can still worsen the problem. In ulcerative colitis, the loss of bone density occurs more directly as a result of steroid use. Patients with primary sclerosing cholangitis, particularly with longer standing disease, can have more severe osteoporosis. The therapeutic options include estrogen replacement therapy, raloxifene, calcitonin; bisphosphonates; alendronate (Fosamax), and risedronate (Actonel) [268]. Screening patients using dual photon absorptiometry is recommended. Guidelines for the management of osteoporosis in IBD have been developed by the British Society of Gastroenterology and the American Gastroenterological Association [269,270].

Renal complications

The incidence of kidney stones in patients with IBD, with estimates ranging between 1% and 25%, is several times that in the general population [271]. Calcium oxalate stones are seen in patients with small intestine Crohn's disease. Ileal resections or ileal disease can lead to fat malabsorption, and unabsorbed fatty acids bind calcium in the lumen. In healthy persons, oxalate in the lumen is bound to calcium. Calcium oxalate is poorly soluble and poorly absorbed. If calcium is bound to malabsorbed fatty acids, however, oxalate combines with sodium to form sodium oxalate, which is soluble and is absorbed in the colon [272]. The development of calcium oxalate stones in Crohn's disease requires an intact colon to absorb oxalate; patients with ileostomies do not develop calcium oxalate stones. However, the presence of an ileostomy predisposes to other kinds of kidney stones, particularly urate stones, because of the diminished urine volumes in patients with significant ileostomy volume losses [273]. Measurement of 24-h urinary oxalate, uric acid, and citrate may be useful to guide therapy. Dietary restriction of oxalate and citrate supplementation can help prevent recurrence of oxalate stones.

Other urinary tract complications are associated specifically with Crohn's disease. Inflammation from the bowel extending into the retroperitoneal space can occlude the ureters, leading to obstruction and hydronephrosis [274]. This process most commonly extends from the area of the cecum and terminal ileum and involves the right ureter. Also in Crohn's disease, fistulae can form between an inflamed bowel and the urinary bladder, leading to urinary tract infections [275]. Amyloidosis is seen in patients with Crohn's disease and has caused death from renal insufficiency [276]. Renal amyloidosis does not respond to conventional medical therapy for Crohn's disease and, for the most part, does not improve after the inflamed section of intestine is resected.

Dermatological manifestations (see Chapter 110)

The two common dermal complications of IBD are pyoderma gangrenosum and erythema nodosum. Pyoderma gangrenosum is seen with colitis and ileocolitis and is associated with extensive long-standing disease. The incidence of pyoderma in ulcerative colitis is low (1%–5%), and it is even lower in Crohn's disease; however, 36%–50% of all patients with pyoderma gangrenosum have IBD [277,278]. The typical lesion is a discrete ulcer with a necrotic base, usually found on the lower extremities (Fig. 57.30) but may occur elsewhere, particularly the face. Lesions often occur at the site of previous injury (pathergy) and are seen around stomas. The ulcers may drain purulent material, but the drainage is sterile on culture. These ulcers can become large and deep, with destruction of surrounding soft tissues. Activity of pyoderma gangrenosum may or may not follow the activity of the bowel disease, but these lesions almost always develop during a bout of acute colitis. Usually, the lesions resolve with control of the colitis by use of oral corticosteroids. Alternatively, corticosteroids can be injected directly into the skin lesions. Therapies with Periactin, dapsone, and azathioprine have been reported, with cyclosporine (ciclosporin [rINN]) increasingly recommended [279–281]. Infliximab has also been reported to be dramatically effective in some cases [282]. Topical tacrolimus has also been effective [283].

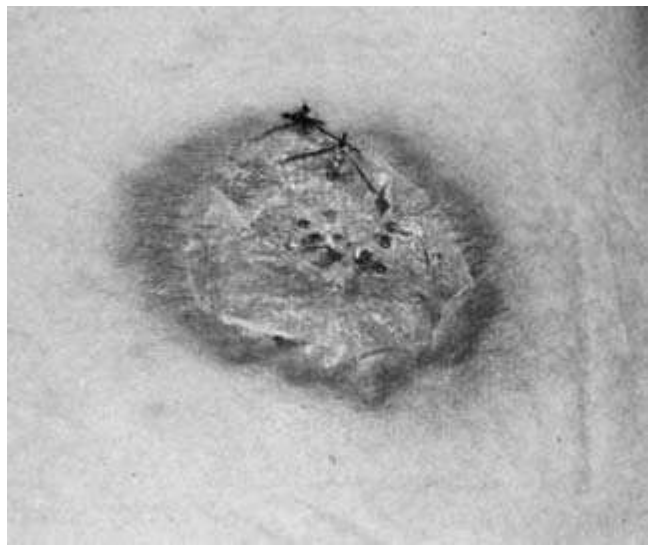


Figure 57.30 Pyoderma gangrenosum (with stitches after biopsy). Courtesy of Dr Ira Kodner, St Louis, MO.

Erythema nodosum is seen particularly in association with Crohn's disease in children [284]. The lesions are raised, tender nodules, usually found over the anterior surface of the tibia. Activity of erythema nodosum follows the activity of the bowel disease and responds to treatment of the bowel disease. Most patients have only a single episode of erythema nodosum. Sweet syndrome (acute febrile neutrophilic dermatosis), which has some similarity to erythema nodosum, is another dermatological condition associated with IBD [285].

Eye complications

The ocular complications of IBD are uveitis and episcleritis [286]. Uveitis (iritis) is an inflammatory lesion of the anterior chamber, presenting with blurred vision, headache, eye pain, photophobia, and conjunctival injection. Diagnosis is made by slit-lamp examination, which demonstrates perilimbic edema and cells in the anterior chamber. Scarring and blindness can be prevented with local therapy of corticosteroids and atropine or other agents that dilate the pupil. Episcleritis is a less serious problem, presenting with burning eyes and scleral injection. Treatment with topical steroids is effective. Infliximab can be useful for refractory uveitis [287].

Thromboembolic complications

Thromboembolic complications, with arterial as well as venous events, occur in 1%–2% of patients with ulcerative colitis

and Crohn's disease [288]. Nonetheless, in half of patients with IBD with thrombotic events no clotting defect can be identified [289]. Deep vein thrombosis and pulmonary emboli affect patients with active disease. In a population study there was a three- to four-fold increase in the incidence of deep venous thrombosis in pulmonary embolism in patients with IBD compared with a control group [290]. Thromboembolic events in the eye and in intracranial vessels also have been described [288,291]. These thrombotic events should be treated with anticoagulants just as they would be in patients without IBD. Activation of clotting factors and thrombocytosis are common. In patients with active disease, procoagulant factors tend to be increased and fibrolytic factors are decreased, potentially generating a hypercoaguable state. In both Crohn's disease and ulcerative colitis increased levels of factor V, factor VIII, and fibrinogen and decreased levels of antithrombin III have been demonstrated [292,293].

Hepatobiliary complications

The hepatic complications of IBD include fatty liver, pericholangitis, chronic active hepatitis, and cirrhosis. The biliary tract complications are sclerosing cholangitis and gallstones. Cholesterol gallstones occur in patients with ileal disease or ileal resections owing to malabsorption of bile salts and the resultant decrease in the size of the bile salt pool. Pericholangitis is the most common hepatic complication of IBD, with prevalences as high as 50%–80% reported [294,295]. Patients with pericholangitis are usually asymptomatic. Elevations of alkaline phosphatase are seen frequently; elevations of bilirubin are less common. Histologically, there is inflammation of the portal tracts, with lymphocyte and eosinophil infiltrates and degenerative changes of the bile ductules [296]. In more advanced cases, progressive fibrosis can develop. The relationship between pericholangitis and primary sclerosing cholangitis is uncertain. Some studies have suggested that pericholangitis may be a part of the spectrum of sclerosing cholangitis, and the histological manifestations of sclerosing cholangitis and pericholangitis on liver biopsy may be indistinguishable [297]. Patients diagnosed on liver biopsy as having pericholangitis may need to undergo endoscopic retrograde cholangiopancreatography (ERCP) to rule out sclerosing cholangitis. There is no effective therapy for pericholangitis.

Sclerosing cholangitis is a chronic cholestatic liver disease marked by fibrosing inflammation of the intrahepatic and extrahepatic bile ducts; it occurs in 1%–4% of patients with ulcerative colitis and with lower frequency in Crohn's disease [298,299]. Most patients with sclerosing cholangitis have IBD. The prevalence of IBD is so high in patients with sclerosing cholangitis that colonoscopy should be performed even on patients who are without intestinal symptoms. Endoscopic and histological evidence of IBD are commonly found in patients with sclerosing cholangitis without intestinal symptoms. Patients with ulcerative colitis and sclerosing cholangitis have a higher risk of colon cancer than those with ulcerative colitis alone and should undergo more intensive screening [300]. Sclerosing cholangitis can affect intrahepatic bile ducts, extrahepatic bile ducts, or both. In patients with IBD and sclerosing cholangitis, usually both intrahepatic and extrahepatic ducts are affected. Early in the disease, liver biopsy shows enlargement of the portal tracts, with edema and bile duct proliferation. Later, biopsies show extension of fibrosis out of the portal space, eventually leading to cirrhosis.

Bile duct strictures are the major clinical problem in sclerosing cholangitis; strictures can occur in both intrahepatic and extrahepatic bile ducts (Fig. 57.31). Patients may be asymptomatic until the disease is far advanced and then present with fever, right upper quadrant pain, and jaundice. More often, sclerosing cholangitis is first recognized in the evaluation of abnormal laboratory studies: elevated alkaline phosphatase, bilirubin, and aminotransferases. The differential diagnosis of sclerosing cholangitis includes biliary tumors and common duct gallstones; diagnosis is made by ERCP or

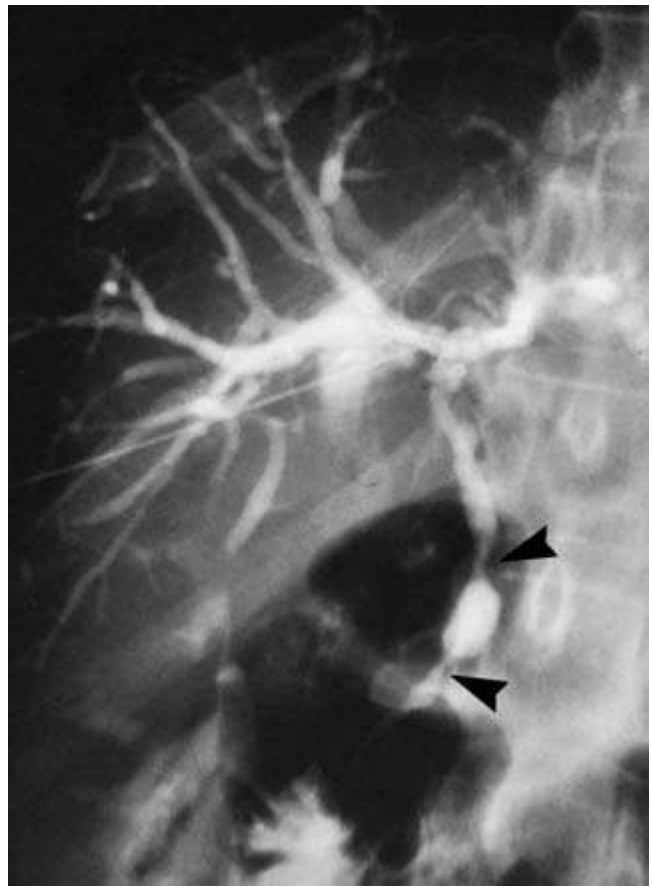


Figure 57.31 Sclerosing cholangitis with segmental strictures (*arrows*) in common bile duct. There are also strictures in the intrahepatic bile ducts. Courtesy of Dr Dennis Balfe, St Louis, MO.

transhepatic cholangiography. Sclerosing cholangitis progresses to cirrhosis, hepatic failure, and death in 5–10 years [301]. Clinical features at presentation associated with a poorer prognosis include hepatomegaly, splenomegaly, elevated serum alkaline phosphatase, advanced histological stage, and age [302]. Endoscopic balloon dilation or stenting of extrahepatic and intrahepatic strictures may be palliative. Cholestyramine, a bile-salt-binding resin, may help to relieve pruritus. Colectomy and medical therapy of the bowel disease are without benefit in the management of sclerosing cholangitis. Ursodeoxycholic acid can decrease alkaline phosphatase and aminotransferases but does not alter histological progression or influence the occurrence of clinically important events such as time to transplant or death [303]. Liver transplantation has been used with success, and sclerosing cholangitis is one of the most common indications for liver transplantation in adults. Cholangiocarcinoma develops in 10%–15% of patients with IBD with long-standing sclerosing cholangitis. Differentiating cholangiocarcinoma from the benign strictures of sclerosing cholangitis is difficult. ERCP with brushing or cholangioscopy with biopsy may help to make this distinction.

Differential diagnosis

IBD should be considered in any patient who presents with rectal bleeding or chronic diarrhea. Identification of fecal leukocytes is the simplest means of discerning an inflammatory process of the intestine. Other causes of rectal bleeding are either traumatic or neoplastic. Diarrhea is nonspecific and has a large differential schema. The primary chronic diarrheal illness that requires differentiation from ulcerative colitis or Crohn's disease is irritable bowel syndrome (IBS). However, IBS is never a cause of rectal bleeding and nocturnal symptoms or associated weight loss is rarely seen in IBS, alone. The presence of occult blood or fecal leukocytes excludes IBS. Although there is no evidence that IBS predisposes to IBD [304,305], the two conditions not, infrequently, coexist and may be associated with depressive symptoms [306]. In addition to IBS, diarrhea and rectal bleeding secondary to NSAIDs is a common mimic of IBD and the recent advent of wireless capsule endoscopy has made differentiation difficult between NSAID enteritis (or colitis) and Crohn's disease [307–309].

The most common problem in the differential diagnosis of an acute presentation of IBD (including flare-ups of quiescent disease) is ruling out infectious colitis (see Chapter 48) [99,104,217,310,311]. An important distinction between acute bacterial infections and IBD is that the diarrhea in the infectious diseases tends to be limited to a period of days to a few weeks, whereas the diarrhea of IBD is typically of longer duration. In patients who present with prolonged diarrhea, the diagnosis of other protozoal diseases, such as giardiasis, must be considered. Stool cultures for enteric pathogens, ova, and parasites and serological tests for amebiasis help to distinguish infectious diarrhea from IBD. Infections with *Shigella*, ameba, *Giardia*, *E. coli* O157:H7, and *Campylobacter* organisms all cause symptoms similar to those of ulcerative colitis and the endoscopic findings of infections with *Shigella* or *Campylobacter* organisms can be identical to those seen in ulcerative or Crohn's colitis. In contrast to bacterial infections of the colon, protozoal infections can produce more chronic symptoms. Amebiasis can cause bloody diarrhea with an endoscopic appearance resembling ulcerative or Crohn's colitis, but in amebiasis the ulcerations are usually scattered, 5–15 mm in diameter, and covered with a yellow exudate that contains organisms. These ulcers may occur anywhere in the colon but are most common in the cecum and ascending colon. Diarrhea secondary to *Giardia* infection is not associated with rectal bleeding but, like Crohn's disease, can present with cramping abdominal pain, weight loss, and lactose intolerance.

Sexual transmission of enteric pathogens by anal intercourse can lead to proctitis resembling idiopathic IBD [312]. *Neisseria gonorrhoeae*, chlamydial organisms, herpes simplex virus, *Treponema pallidum*, and lymphogranuloma venereum can all cause proctitis. The increased prevalence of the human immunodeficiency virus (HIV) has broadened the number of potential enteric pathogens to include *Cryptosporidium* and *Isospora* species, *Mycobacterium avium* complex, and cytomegalovirus (see Chapter 104) In patients without risk factors for HIV infection, the primary diagnostic studies needed to rule out infectious colitis are stool cultures for enteric pathogens, examination of stool for ova and parasites, and stool examination for *C. difficile* toxin (on three separate days). Patients at risk for HIV infection require a more elaborate evaluation with viral culture and histological examination of biopsy specimens to identify potential cytomegalovirus and herpesvirus. In addition, patients who are immunosuppressed as the result of therapy for IBD may have cytomegalovirus complicating their IBD.

Intestinal tuberculosis resembles Crohn's disease both clinically and pathologically (see Chapter 49). *Mycobacterium tuberculosis* most commonly involves the ileum and cecum and is not difficult to diagnose in the presence of pulmonary tuberculosis [313], but can occur more insidiously without pulmonary involvement, including the development of intestinal strictures [314], perianal abscesses, and fistulae [313,315]. Although colonoscopic features overlap, significantly, with Crohn's disease including ulcerations, nodularity, and an inflammatory mass, histological findings that help to distinguish tuberculosis from Crohn's disease include: confluent granulomas, more than 10 granulomas per biopsy site, and caseous necrosis in tuberculosis as well as ulcers lined by conglomerate epithelioid histiocytes and disproportionate submucosal inflammation [316]. Although Crohn's disease may be increasing in the developing world, intestinal tuberculosis remains a higher diagnostic probability among recent emigrants from developing countries and should be

ruled out among this population before a presumptive diagnosis of Crohn's disease is made.

Much has been written about the necessity for differentiating the initial episode of ileocolonic Crohn's disease from acute appendicitis; however, in practice, this distinction is seldom a major problem [317]. In contrast to patients with Crohn's disease, who usually provide a history of more chronic diarrhea before the episode of severe pain, a more acute presentation of severe pain, guarding, and a more rapid course is indicative of appendicitis. A CT scan is likely to help sort out this dilemma preoperatively. Another consideration in this differential diagnosis of right lower quadrant abdominal pain accompanied by diarrhea and fever is acute ileitis caused by *Yersinia* infection. *Yersinia* infection may present with a clinical picture resembling appendicitis or the initial onset of appendicitis and there have been reports of *Yersinia* infections evolving into Crohn's disease [318]. At laparotomy, the acute *Yersinia* ileitis may be indistinguishable from Crohn's ileitis. The diagnosis of this infection is made by stool culture, and antibiotic therapy is curative.

Whipple's disease can also mimic Crohn's disease with the common presenting symptoms of diarrhea, occult bleeding, weight loss, and arthritis associated with anemia, leukocytosis, thrombocytosis, elevated acute-phase reactants, and laboratory evidence of malabsorption. The diagnosis is made by endoscopy of the duodenum and jejunum, which demonstrates focal changes of the small bowel mucosa with erythema, friability, and erosions with associated histopathological findings of magenta-stained inclusions in macrophages of the lamina propria with periodic acid–Schiff staining [319].

Intestinal lymphoma can produce symptoms similar to those of Crohn's disease – fever, weight loss, diarrhea, rectal bleeding, and abdominal pain [320,321]. Small bowel radiographs in lymphoma may show diffuse involvement with masses in the bowel wall, but in Crohn's disease there is usually more localized involvement of the ileum, with ulceration and narrowing of the lumen.

Collagenous or lymphocytic colitis are chronic inflammatory diseases marked pathologically by the presence of a thick collagen deposition in the subepithelial layer of the colonic mucosa, lymphocyte infiltration, or both [179,322–324]. The clinical presentation is chronic watery (secretory) diarrhea that most commonly occurs in middle-aged women. These conditions are often associated with the use of NSAIDs and may be seen in conjunction with other "autoimmune" conditions such as celiac disease, rheumatoid arthritis, and thyroiditis. The endoscopic appearance of the colon is usually normal or mildly edematous and the histological findings provide the diagnosis. Collagenous colitis is discussed more fully in Chapter 59.

Intestinal ischemia may also mimic Crohn's disease and should be considered in older adults or others at particular risk for ischemic disease [325] (Chapter 113). Bloody diarrhea and abdominal pain are common with ischemic colitis. Colonoscopy reveals mucosal edema and erythema, and there may be discrete ulcers similar to those seen in Crohn's colitis. Usually, ischemic colitis spares the rectum because of its extensive collateral circulation. Differentiation of ischemic colitis from IBD, especially Crohn's colitis, may be impossible on clinical, histological, or endoscopic grounds [326]. In contrast to IBD, ischemic colitis usually resolves spontaneously over a few weeks. However, as many as 10% of patients with acute ischemic disease can continue into a chronic picture resembling Crohn's disease, distinguished only in its failure to respond to treatment. If the mesenteric vasculature is involved, systemic vasculitis can have small intestinal manifestations similar to Crohn's disease [247]. Small intestinal ulcerations, perforation, and hemorrhage are seen in systemic lupus erythematosus, polyarteritis nodosa, rheumatoid arthritis, and dermatomyositis but almost exclusively in the setting of active vasculitis outside the gut. Similarly Henoch–Schönlein purpura presents with rectal bleeding and abdominal pain before the development of the characteristic rash on the legs and buttocks (see Chapter 110).

Behçet syndrome is marked by oral and genital ulcers with uveitis. There is an associated inflammation of the ileocecal area with aphthous ulcers and a lymphocytic infiltrate; these lesions can resemble Crohn's disease [99]. Moreover, Behçet syndrome and Crohn's disease share a number of extraintestinal manifestations, including arthritis, erythema nodosum, and iridocyclitis (see Chapter 109).

Inflammatory diverticular disease (diverticulitis or diverticular colitis) (see Chapter 61) may be difficult to separate from both ulcerative colitis and Crohn's disease [99,179] especially in older adult patients. There may also be shared pathogenesis as some cases of diverticular colitis evolve into a more chronic pattern of IBD that can mimic either ulcerative colitis or Crohn's disease, including the development of fistulae tracking along the sigmoid colon [328–330]. Often diverticular colitis presents with hematochezia or gross rectal bleeding, with or without abdominal pain, fever, and diarrhea. The presence of inflammation away from diverticula or the presence of perianal disease suggests Crohn's disease whereas extension of sigmoid colitis into the rectum determines an evolution to ulcerative colitis.

Diversion colitis occurs in the presence of colon excluded from the fecal stream and is the result of a lack of nutrients provided by bacterial production of short chain fatty acids to feed colonocytes is a nonspecific inflammation of segments of colon excluded from the fecal stream [331] and can occurs in patients who have had surgery for Crohn's colitis with creation of a Hartmann pouch or in patients undergoing staged colectomy for ileoanal anastomoses [332]. In these cases, diversion colitis may be difficult to differentiate from either ulcerative colitis or Crohn's disease in the excluded segment [179]. Many patients with diversion colitis have severe endoscopic findings with minimal symptoms. A clinical response

to antibiotics or to enemas of short-chain fatty acids should differentiate diversion colitis from active Crohn's disease [333].

It is not uncommon for symptoms of mild ulcerative colitis or proctitis in which rectal bleeding is the primary manifestation to be attributed to hemorrhoids or anal fissures. The presence of urgency or diarrhea is more consistent with ulcerative colitis or proctitis. Sigmoidoscopy should easily differentiate ulcerative colitis from these perianal problems. Solitary rectal ulcer is another mimic of idiopathic proctitis that typically presents in young individuals with rectal urgency, bleeding, and passage of mucus. Most often the patient presents with a history of constipation or straining on the toilet and, at sigmoidoscopy there is a semicircumferential or circumferential ulceration, often with heaped-up margins along the first rectal fold [334]. Histological features are those of mucosal prolapse [335].

Nutritional management

Malnutrition is an important clinical problem in IBD, particularly in Crohn's disease. At diagnosis, most patients with Crohn's disease have lost weight. Hypoalbuminemia, anemia, and vitamin D deficiency can all result from malnutrition in Crohn's disease.

Five factors lead to malnutrition in Crohn's disease. The first, diminished oral intake primarily as a result of loss of appetite, is the most important cause of malnutrition in patients with Crohn's disease [336]. Anorexia may result from inflammation, depression, or the side effects of medication. Even in the absence of anorexia, many patients diminish their food intake because eating causes pain, cramps, and diarrhea; this problem is seen particularly in patients with small bowel obstruction. The second factor is the increase in caloric requirements because of the catabolism associated with fever and inflammation; however, this factor makes a relatively minor contribution to malnutrition in Crohn's disease [327]. The third factor is malabsorption of nutrients as a result of a reduction in the absorptive area by extensive surgical resections or involvement of large areas of small bowel with inflammation. Rarely, fistulae can result in malabsorption as a result of nutrients bypassing absorptive epithelium. The nutrients most likely to be affected are calcium, magnesium, fats and fat-soluble vitamins, and vitamin B-12. Bile salts and vitamin B-12 are selectively absorbed in the distal ileum, the area of the small intestine most commonly involved in Crohn's disease and the area most commonly resected. Malabsorption of bile salts leads to a decrease in the total bile salt pool and so to fat malabsorption. Bacterial overgrowth in the small intestine proximal to strictures leads to deconjugation of bile salts by bacteria. The deconjugated bile salts are absorbed passively and so are not retained in the lumen to participate in the absorption of fat and fat-soluble vitamins. The fourth factor is the loss of proteins and electrolytes in the intestinal lumen in areas of inflammation. Bleeding into the intestinal lumen results in iron deficiency. Magnesium and zinc can be depleted by losses into the intestine. The fifth and last factor is drug therapy. Sulfasalazine impairs folate absorption but uncommonly leads to anemia. Corticosteroids induce negative nitrogen balance and decrease intestinal calcium absorption.

For most patients with IBD, the only nutritional therapy required is the instruction to eat adequate amounts of a well-balanced diet. Patients look for a nutritional basis to their disease and for nutritionally based therapy. These expectations lead many to decide that exacerbations of pain or diarrhea were caused by the foods they ate before the exacerbation. They exclude from their diets a lengthy list of foods that were associated temporally with an exacerbation of disease. As a result, these patients place themselves on a diet that has limited variety and is likely to be nutritionally unbalanced. Foods should be eliminated from the diet only if they consistently and reproducibly result in symptoms. Some patients have lactose intolerance and should avoid dairy products or use lactase-containing products, such as Lactaid. Patients with strictures should avoid fibrous, high-residue foods such as nuts, popcorn, bean sprouts, and celery.

More involved nutritional therapy in Crohn's disease can be directed toward one of two goals: treating nutritional deficiencies or reducing inflammation. Deficiencies of specific nutrients usually can be managed by supplementation. Oral or parenteral iron supplementation may be necessary in patients with significant intestinal bleeding. Specific supplementation with calcium, magnesium, zinc, vitamin B-12, vitamin D, and vitamin K may be required if clinical or biochemical manifestations of deficiency occur (see Chapter 101). These nutrients may be malabsorbed if there is extensive involvement of the small bowel mucosa. Bone densitometry will reveal evidence of calcium loss from bone caused by calcium malabsorption or steroid therapy. Even in the absence of clinical or biochemical evidence of micronutrient deficiency, it is reasonable to give multivitamin supplements to patients with small bowel Crohn's disease. Extensive (> 100 cm) resections of the terminal ileum or lesser resections combined with active disease or scarring in the remaining ileum lead to vitamin B-12 malabsorption. Some clinicians evaluate patients with serum vitamin B-12 levels, but others empirically treat all patients with significant ileal resections with parenteral vitamin B-12 (1000 μg once a month). Ileal resection and ileal disease also lead to malabsorption of bile salt and fat. Diarrhea induced by bile salts can be managed with cholestyramine, a resin that binds bile salts and prevents them from inducing water and electrolyte secretion from the colonic mucosa. Cholestyramine therapy, however, further depletes the bile salt pool and can worsen fat malabsorption. Unabsorbed fatty acids entering the colon are hydroxylated by bacteria. Hydroxylated fatty acids induce colonic water and electrolyte secretion. Fatty acid-induced

diarrhea is managed by reducing the fat content of the diet. Caloric deficiencies are made up by increasing the intake of complex carbohydrates. Alternatively, medium-chain triglycerides, which do not require bile salts for absorption, can be substituted for part of the conventional long-chain triglycerides in the diet. The dose of medium-chain triglycerides is limited to about 3 tablespoons per day (300 kcal) because larger amounts cause diarrhea.

Anorexia leads to inadequate nutritional intake and malnutrition in many patients with IBD, even if absorptive function is normal. Supplementation of the patient's oral intake with defined formula diets can prevent or reverse malnutrition. Defined formula diets can be taken orally or administered by nasogastric tube, gastrostomy tube, or jejunostomy tube (see Chapter 21). Most patients with IBD who require nutritional supplementation can be managed with enteral therapy. In some cases, however, parenteral nutrition is required: in the presence of chronic small intestine obstruction that is not amenable to surgical resection; after massive small bowel resection, especially if the amount of remaining small intestine is insufficient for absorption of daily caloric needs; or if enteral nutrition results in severe, uncontrollable diarrhea.

The use of total enteral administration of an elemental diet as specific therapy in IBD is controversial (see Chapter 101). Elemental diets consist of amino acids, monosaccharides, vitamins, minerals, and essential fatty acids. The results of controlled trials comparing elemental diets with drug therapy have been inconsistent [338–341]. In Crohn's disease, elemental diets have been used more extensively in children than adults. Providing adequate nutrients can reverse growth failure in Crohn's disease. Elemental diets taken orally or by nasogastric tube enhance linear growth in children with Crohn's disease [342]. In these studies, enteral nutrition was superior to steroids in promoting growth. In several studies of active Crohn's disease in adults enteral nutrition was found to reduce clinical activity. This has been the subject of a Cochrane review [343]. There is less experience of enteral nutrition as a maintenance therapy in Crohn's disease; however, long-term maintenance therapy with enteral nutrition plus mesalamine was found to be superior to mesalamine alone [344]. In this study the patients took 50% of their calories as oral nutrition and 50% as enteral nutrition. Therefore, the beneficial effects of the enteral nutrition probably do not relate to the diminished dietary antigen exposure.

Malnutrition is less common in ulcerative colitis than in Crohn's disease. Malabsorption of nutrients is not a problem in ulcerative colitis; however, diminished food intake is common. Patients with ulcerative colitis may decrease their food intake because of diminished appetite or because eating worsens their diarrhea. Nutritional requirements are increased in severe ulcerative colitis because of the catabolism associated with fever and inflammation. Total parenteral nutrition (TPN) has been used to improve the nutritional status and reduce the symptoms of patients with severe ulcerative colitis [345]. This approach has been especially successful in improving the nutritional status of patients before colectomy is undertaken. Improved nutritional status decreases the likelihood of complications for surgical procedures in general and, presumably, for IBD surgery in particular. No evidence has been found that TPN reduces the necessity for colectomy or that it is an effective primary therapy for the disease. Two studies compared the rates of colectomy in patients with ulcerative colitis receiving TPN with those on regular hospital food. In both studies, the rates for colectomy were the same in the two groups [346,347]. Dietary supplementation with fish oil may have antiinflammatory activity because the *n*-3 fatty acids in fish oil compete in the substrate pool for the enzymes that synthesize prostaglandins and leukotrienes. Supplementation with high-dose fish oil over a period of months reduced steroid requirements in ulcerative colitis [348]. Fish oil supplementation was tested in placebo-controlled trials as maintenance therapy in Crohn's disease. In one trial it was effective [349], but in the other it was not [350].

Drugs used in inflammatory bowel disease

Aminosalicylates

Formulations of 5-aminosalicylic acid (5-ASA, mesalamine, mesalazine) have been the primary therapies to treat mild to moderately active ulcerative colitis and to maintain remission [110,111] (Table 57.6). Their efficacy in the treatment of Crohn's disease has become somewhat more controversial [110,351–354]. These agents have a long history of clinical use and have been extensively studied in clinical trials for both ulcerative colitis [355,356] and Crohn's disease [357,358]. Sulfasalazine, the prototype aminosalicylate formulation, was developed with the concept of providing both an antibacterial (sulfapyridine) and antiinflammatory (5-aminosalicylic acid, mesalamine, mesalazine) agent into the connective tissues [359]. Sulfasalazine had been the benchmark agent for the initial comparison trials with alternative 5-ASA agents, but it is recognized that the 5-aminosalicylic acid moiety accounts for the primary therapeutic benefits whereas sulfapyridine causes the majority of side effects [354,360]. This recognition has allowed the development of a series of sulfa-free aminosalicylates that can be targeted to specific sites along the gastrointestinal tract [361,362] (Fig. 57.32).

Sulfasalazine

Sulfasalazine (salazopyrine) is a compound linking sulfapyridine with 5-ASA via an azo-bond. With oral administration the compound remains, mostly, intact with minimal enteric absorption until reaching the colon where the azo-bond is cleaved by colonic bacteria. The sulfapyridine is almost completely absorbed from the colon and undergoes a genetically

Table 57.6 Currently available mesalamine (5-ASA) preparations in the United States

Product	Formulation	How supplied	Typical dosing	Site of action
Canasa	5-ASA suppository	1000 mg	q.d.–b.i.d.	Rectum
Rowasa	5-ASA enema	4 g (60 mL)	q.h.s.	Rectum; distal colon
Azulfidine (sulfasalazine)	5-ASA linked to sulfapyridine	500 mg tablet	3–4 g; b.i.d.–q.i.d.	Colon
Asacol	5-ASA coated with acrylic resin; release at pH ≥ 7	400 mg tablet	2.4–4.8 g; q.d.–b.i.d.	Distal ileum; colon
Lialda	5-ASA coated with acrylic resin and multi-matrix; release at pH ≥ 7	1.2 g capsules	2.4–4.8 g; b.i.d.–t.i.d.	Distal ileum; colon
Pentasa	5-ASA in ethylcellulose	500 mg capsules microgranules	3–4 g; b.i.d.–q.i.d.	Small bowel; colon
Dipentum	5-ASA dimer	250 mg capsules	1–2 g; b.i.d.	Colon
Colazal	5-ASA linked to inert carrier	750 mg capsules	6.75 g; b.i.d.–t.i.d.	Colon

5-ASA, 5-acetylsalicylate; b.i.d., twice a day; t.i.d., three times a day; q.d., every day; q.i.d., four times a day; q.h.s., every bedtime.

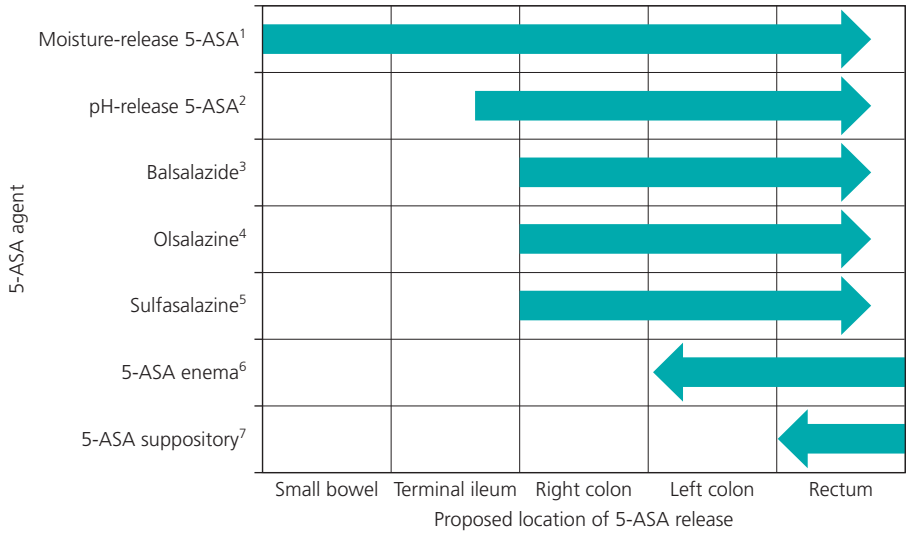


Figure 57.32 Proposed location of release of 5-aminosalicylate (5-ASA; mesalamine) preparations available in the United States. 1, Pentasa; 2, Asacol or Lialda; 3, Colazal; 4, Dipentum; 5, Azulfidine; 6, Rowasa; 7, Canasa.

determined hepatic acetylation and subsequent renal excretion [360]. Slow acetylators achieve higher plasma concentrations of sulfapyridine than rapid acetylators, which translate into a greater or lesser likelihood of intolerance to the compound. In contrast, once delivered to the colon 5-ASA is acetylated by colonic bacteria or within the colonic epithelium and is poorly absorbed. The majority of 5-ASA is eliminated as acetylated 5-ASA in the feces [363]. The small amount (~ 15%) of 5-ASA absorbed from the colon is acetylated within the colonic mucosa or liver and is excreted from the kidneys, again primarily as acetylated 5-ASA. Thus, sulfasalazine functions, primarily, as a carrier of 5-ASA to distal sites in the intestinal tract.

A specific mechanism of action for sulfasalazine and 5-ASA has yet to be established [360,364] and novel antiinflammatory [354] and antineoplastic [365] effects continue to be elucidated. There are multiple antiinflammatory effects including inhibition of the arachidonic acid cascade involving the cyclooxygenase, lipoxygenase, and platelet-activating factor pathways [360]. In addition, the aminosalicylates are inhibitors of oxygen radical production and scavengers of free radicals [340]. They also inhibit lymphocyte and monocyte function as well as plasma cell production of immunoglobulin. Sulfasalazine also has been shown to inhibit IL-1 production and nuclear factor-κB [366] and mesalamine has been shown to activate the γ form of peroxisome proliferator-activated receptors involved in the control of inflammation, cell proliferation, apoptosis, and metabolic function [365]. A basic premise regarding the aminosalicylates is that the effects of 5-ASA are topical (mucosal), not systemic, and that the active moiety needs to be delivered to the site of intestinal inflammation [354].

Side effects of sulfasalazine are relatively common and the majority of adverse reactions are related to plasma sulfapy-

ridine concentrations [367]. In general, the side effects can be divided into intolerance or allergy, although there are also direct toxic effects on spermatozoa. Intolerance side effects from sulfasalazine (e.g., nausea, vomiting, malaise, anorexia, dyspepsia, and headaches) are dose-related and worse in patients who are genetically slow acetylators. Administration with meals or gradually increasing the dose can minimize these symptoms, as can administration of an enteric-coated formulation. Most of these side effects occur within the first few days or weeks of therapy and resolve with discontinuation.

In contrast to the dose-related intolerance side effects, allergic or hypersensitivity reactions including rash, fever, hemolytic anemia, agranulocytosis, hepatitis, hypersensitivity pneumonitis, pancreatitis, or worsening of colitis, are not related to sulfapyridine concentrations and are far less frequent [368–370]. Idiosyncratic pancreatitis, pneumonitis, and worsening of colitis are related to the 5-ASA moiety while reversible sperm abnormalities and folate malabsorption are complications related to sulfasalazine. Sulfasalazine interferes with folate absorption and may contribute to anemia, leading to the recommendation of folate supplementation during therapy [371]. Additional drug interactions identified with sulfasalazine include the potential for warfarin resistance [372] and inhibition of thiopurine methyl transferase that clinically has been shown to increase 6-thioguanine nucleotide levels with coadministration of sulfasalazine, mesalamine, or balsalazide leading to a risk of leucopenia [373] as well as lowering of 6-thioguanine nucleotide levels when a sulfasalazine or alternative aminosalicylates is withdrawn [374].

Non-sulfa containing aminosalicylates

In contrast to sulfasalazine, 5-ASA (mesalamine) is rapidly absorbed from the proximal gastrointestinal tract depending on local concentrations and lumenal pH [363]. A variety of approaches have been followed to deliver 5-ASA to distal sites along the intestine with the intent of "protecting" 5-ASA from proximal absorption without exposure to the toxicities associated with sulfapyridine (Table 57.6). Since initial studies demonstrated that mesalamine is the active ingredient of sulfasalazine and that it works when topically applied to the rectum [375] an initial approach was to formulate 5-ASA into suppositories [376,377] or enemas [378] for treatment of colitis confined to the rectum or the left colon. Subsequently, oral formulations of 5-ASA have been developed to deliver sufficient lumenal concentrations at distal sites in the small and large intestine (Fig. 57.32). The latter include mesalamine formulations that are "protected" against proximal absorption or mesalamine compounds with 5-ASA linked by the same azo-bond present in sulfasalazine, but instead, to an alternative carrier.

The oral formulations of mesalamine vary in their coating mechanisms to prevent proximal absorption and, hence, inactivation as 5-ASA is considered a topically active anti-inflammatory [379]. Although all of these formulations deliver "pure" mesalamine, there are pharmacological differences in the release and intestinal delivery [380] although no distinct clinical differences between the pure mesalamine agents have been identified when they are administered for ulcerative colitis on a gram for gram basis [354,363,381]. The Pentasa formulation of 5-ASA uses ethylcellulose-coated granules that behave as a semipermeable membrane to release 5-ASA at pH 6 and above, thus delivering approximately 50% of 5-ASA into the small bowel and the remainder into the colon. Because 5-ASA is a zwitter ion and its absorption depends on both local concentrations and pH, "release" does not equate with "absorption" and only about 35% of mesalamine is absorbed from this formulation, independent of intestinal transit time [363]. Another formulation of mesalamine (Lialda) uses a "matrix" combination of pH-resistant coatings and semipermeable ethylcellulose to deliver 5-ASA primarily into the colon [382,383]. Other pure mesalamine formulations use Eudragit resins to provide more of a bolus-release of 5-ASA targeted according to intestinal pH. Asacol uses Eudragit-S, dissolves at pH 7 or above, and releases 5-ASA primarily in the colon and terminal ileum whereas Salofalk (Mesasal) is formulated with Eudragit-L, which releases at pH 6. The latter, because of the proximal bolus release, leads to slightly more absorption and renal elimination [363].

The azo-compounds of 5-ASA include olsalazine and balsalazide. Olsalazine (Dipentum) is a dimer of two 5-ASA molecules linked covalently through an azo-bond similar to that in sulfasalazine. Each mole of olsalazine provides two moles of 5-ASA [384]. The second azo-compound is balsalazide (Colazal), in which 5-ASA is linked via an azo-bond to 4-aminobenzoyl-β-alanine, an inert carrier [385]. Similar to sulfasalazine and olsalazine, balsalazide releases 5-ASA entirely into the colon. In contrast to the pure mesalamine compounds, the azo-compounds have the potential to induce chloride secretion in the small intestine, resulting in watery diarrhea in 5%–10% of patients treated with olsalazine at dosages of 1–2 g/day. Sulfasalazine and balsalazide are less likely to induce diarrhea at the prescribed dosage, which provide smaller amounts of 5-ASA (e.g., 4 g sulfasalazine provides 1.6 g 5-ASA whereas 6.75 g balsalazide provides approximately 2.4 g 5-ASA) [386].

In contrast to sulfasalazine, the pure mesalamine and azo-compounds have relatively few side effects and in trials using up to 4.8 g/day of delayed-release formulations there have not been any dose-related toxicities [367,370]. Indeed, the primary advantage of the non-sulfa-containing aminosalicylates is the improved side effect profile compared with sulfasalazine [355,356]. Eighty percent of patients who are unable to tolerate sulfasalazine can tolerate a non-sulfa-containing 5-ASA [387,388]. Rare, idiosyncratic reactions including pericarditis, myocarditis, pancreatitis, pneumonitis,

hepatitis, nephritis, and worsening of colitis have been reported. In contrast to the idiosyncratic hypersensitivity reactions related to mesalamine, diarrhea related to olsalazine is dose-dependent, improved by administration with food, and is less prevalent in patients with inactive colitis.

Two potential side effects of all 5-ASA products should be mentioned. Worsening of colitis has been described with all formulations and is a rare, presumably hypersensitivity reaction [388]. Increasing symptoms of colitis (bleeding, diarrhea, rectal urgency) can be seen with administration of oral or rectal aminosalicylates. On occasion, 5-ASA-induced colitis may be masked by corticosteroid therapy and can become symptomatic as corticosteroids are tapered. Therefore, for patients with steroid-refractory ulcerative colitis who have never been in remission with 5-ASA agents, a trial off of 5-ASA, with an alternative maintenance agent should be considered.

The other recognized, but rare, consequence of 5-ASA is an idiosyncratic, presumed hypersensitivity, form of interstitial nephritis [389]. Nephrotoxicity is not dose-related, has been described with all 5-ASA agents (overall estimated risk ~ 0.26% per patient year), and often presents gradually over months or years. The labeling of all 5-ASA agents warns against the potential for nephrotoxicity and recommends monitoring of kidney function. However, the optimal means of monitoring has not been established. As a result of the frequency of abnormal urinary sediment findings in patients with IBD, measurement of baseline or follow-up urinary sediments is not adequate and monitoring of the blood urea nitrogen and serum creatinine is warranted at baseline, after 3–6 months of therapy, and yearly, thereafter [368,389,390]. Clinicians should be aware of mild elevations of serum creatinine in young individuals that can reflect significant reductions in renal function. 5-ASA agents should be discontinued in the presence of rising serum creatinine and referral to a nephrologist is warranted to determine the etiology of abnormal kidney function [389].

Aminosalicylates in ulcerative colitis

Mild or moderate ulcerative colitis

Sulfasalazine and all of the oral aminosalicylates have been efficacious in the treatment of mild to moderate ulcerative colitis as long as the active component (5-ASA) is delivered to the site of disease activity [110,111,356,391]. Oral aminosalicylates are effective for both proximal and distal colitis and the topical agents are efficacious for distal colitis as long as the formulation reaches the proximal extent of disease [392]. The dose–response for sulfasalazine is often compromised by dose-related toxicity [356,369,370]; the dose–response for mesalamine formulations is not associated with dose-related toxicities up to the tested dosage of 4.8 g/day [393,394]. Whereas a dose–response for the oral aminosalicylates is well defined for the treatment of active disease [356] there do not appear to be increasing benefits from topical formulations (suppositories, foams, or enemas) above 1 g daily [392,395,396]. In comparison trials between sulfasalazine and the alternative agents there is no advantage of one compound over another from a therapeutic standpoint as long as equal amounts of 5-ASA are delivered [356]. Similarly, there does not appear to be a significant difference between the oral aminosalicylates regarding their efficacy in extensive or distal ulcerative colitis [362,380] or in the pharmacokinetic profiles [363].

The clinical response to sulfasalazine or an alternative aminosalicylate for active (mild-to-moderate) ulcerative colitis averages between 40% and 80%, but varies considerably between clinical trials because of differing patient populations and end-point definitions of response [380,391]. There is a dose–response for sulfasalazine between 1 and 4 g daily and in reports dosages up to 6 g/day have been tested. Mesalamine is equally effective with a dose–response between 1.5 g/day and 4.8 g/day, the latter being the highest dose evaluated in clinical trials [356,393,394]. The azo-bond compounds olsalazine and balsalazide have been tested in dosages up to 3 g/day and 6.75 g/day (comparable to 3 g and 2.4 g of mesalamine, respectively) [356]. Whereas increasing doses of sulfasalazine (intolerance) and olsalazine (diarrhea) are associated with incremental dose-related side effects, an advantage for mesalamine compounds and balsalazide is that, within the tested therapeutic range, the dose–response is not compromised by dose-related side effects [356,369,370].

Rectal formulations of mesalamine have been highly efficacious for the treatment of distal colitis [392]. Mesalamine suppositories reach the upper rectum (approximately 15–20 cm), and enemas or foams up to the splenic flexure or distal transverse colon according to volume and disease activity [397]. In the setting of distal colitis topical (rectal) mesalamine has been more effective than corticosteroid enemas or equivalent doses of oral mesalamine [392] and combination treatment with oral and topical mesalamine is superior to either single approach [397]. Mesalamine enemas can also be used as adjunctive therapy for patients with extensive colitis to accelerate healing [398].

Severe ulcerative colitis

The aminosalicylates are not regarded as effective when ised as single agents for severe colitis and because of the frequency of gastrointestinal intolerance, the small risk of worsening colitis, and their inferior antiinflammatory properties compared to corticosteroids, they are not recommended until patients have stabilized. These agents can be added or resumed when the patient has responded to treatment for severe disease with resumption of normal bowel habits and consumption of a full oral diet [399]. Unlike corticosteroid enemas, rectal mesalamine formulations have not been evaluated in severe disease and clinical experience has shown that in this setting they may be less well tolerated than rectal corticosteroids.

Maintenance of ulcerative colitis remission

Prevention of relapse and the prolongation of remission have been primary indications for all of the aminosalicylates [110,111,355]. In contrast to the evidence base for mild to moderate active ulcerative colitis there are fewer data regarding a dose–response for the aminosalicylates for maintaining remissions [355,400,401]. Nevertheless, in the setting of quiescent colitis there is likely to be a similar dose–response although there is no consensus on an "optimal" maintenance dose [401]. Similar to the setting in active disease, with maintenance therapy all of the non-sulfa-containing formulations provide comparable efficacy to sulfasalazine when equimolar doses of 5-ASA are administered [355,363,401]. Historically, the dose-related side effects of sulfasalazine led to the compromise in maintenance dosing based on the efficacy and side effects and recognition that many patients are not compliant with prescribed doses, the suggested maintenance dose for sulfasalazine has been 50% of the dose for active disease [402]. However, this historical "dogma" does not apply to the non-sulfa-containing aminosalicylates that are devoid of dose-related side effects and clinical experience has dictated that a substantial proportion of patients who require higher doses of an aminosalicylate to achieve remission will, similarly, benefit more from maintaining the same induction dose to maintain remissions [362,380].

Topical mesalamine formulations are also effective at preventing relapse in the setting of ulcerative proctitis and distal colitis and are more effective than oral treatment [403,404]. Many patients with distal colitis who require topical therapy to achieve remission will require maintenance therapy with a rectal formulation [380,392,405].

Aminosalicylates in Crohn's disease

Mild to moderate active Crohn's disease

The role of aminosalicylates in the setting of Crohn's disease has become more controversial [352,353,357,362,380,406]. Numerous factors contribute to the debate regarding the role of aminosalicylates in Crohn's disease including: the heterogeneous presentations (disease location, severity, complications), transmural nature of Crohn's disease (vs the mucosal activity of aminosalicylates), definition of disease activity and response, dose and delivery systems of varied formulations, clinical trial design and evolution of clinical trials over the past two decades. Compared to the evidence base for ulcerative colitis, the efficacy of sulfasalazine in Crohn's disease is less definitive and more dependent on location of disease activity [357,362,380,406]. The necessity for colonic bacterial azo-reductase activity to liberate 5-ASA implies that sulfasalazine is unlikely to benefit proximal (small intestinal) Crohn's disease and, in two early clinical trials evaluating more than 500 patients with active Crohn's disease sulfasalazine at dosages of 3–5 g/day were superior to placebo only for patients with ileocolonic or colonic disease but not for patients with disease confined to the small intestine [407,408]. Although sulfasalazine has been commonly used to prevent relapse for patients with quiescent Crohn's disease, neither the National Cooperative Crohn's Disease Study nor the European Cooperative Crohn's Disease Study demonstrated maintenance benefits at (low) dosages of 1–3 g/day. While one trial utilizing a 3 g/day dosage of sulfasalazine demonstrated efficacy at reducing the likelihood of postoperative clinical recurrence [409] there remains little evidence of a maintenance benefit after medically-induced clinical remissions [358].

The non-sulfa aminosalicylates have also been evaluated in clinical trials for the treatment of mild to moderately active Crohn's disease. Overall, clinical remission rates range between 40% and 50% [357,406] but the heterogeneity of clinical trials and high placebo response [410] in this population makes it difficult to confirm a reasonable number needed to treat in specific scenarios of active disease. Clinical trials evaluating non-sulfa-containing aminosalicylates as maintenance agents for Crohn's disease have also produced conflicting results [353,358,406]. Again, the heterogeneity of maintenance scenario has also hampered an interpretation of their potential. Mesalamine agents may have maintenance benefits when initiated immediately on induction of remission [411] but have not been effective after corticosteroid-induced remissions in Crohn's disease [412].

Maintenance of Crohn's disease in remission

Clinical trials have yet to evaluate mesalamine as a maintenance agent for individuals who have responded to mesalamine as an inductive agent. Mesalamine agents have also been evaluated in a series of trials to delay postoperative recurrence after resection in CD. Although initial metaanalysis suggested benefits at reducing both endoscopic and clinical evidence of disease [413] the inclusion of a subsequent (negative) large trial [414] would have invalidated the overall positive results. Nevertheless, there does appear to be a modest, overall (~ 10%) benefit for mesalamine at reducing the likelihood of recurrent Crohn's disease after an ileocolonic resection [227,415].

Antibiotics

The unknown etiology of the inflammatory bowel diseases, along with the expanded understanding of the gut immune system's interactions with the intestinal flora [416], have led not only to investigations into the role of infectious agents as initiators or instigators of disease flares, but also into antimicrobials as potential therapies. Most of the attention has been directed towards antibiotics targeting bacterial species.

Antibiotics in ulcerative colitis

There are no well-designed clinical trials or even large case series that suggest a convincing therapeutic role for antibiotics in the treatment of ulcerative colitis, although some practitioners advocate them in the setting of severe

colitis to potentially decrease sepsis and other complications [417–419]. Combined therapy with intravenous tobramycin and metronidazole showed no benefit over placebo in clinical response, incidence of complications, or need for emergent colectomy in patients with severe colitis [420]. Oral vancomycin [421], oral ciprofloxacin [422], and intravenous metronidazole [422] were found to be not effective in these patients.

There may be a role of antibiotics for patients with less-severe colitis. A recent metaanalysis of 10 randomized, placebo-controlled trials (530 patients), however, suggested that antibiotics used as an adjunct to corticosteroids or mesalamine yielded a pooled odds ratio of 2.14 (confidence interval [CI] 1.48–3.09) favoring antibiotics [423]. Clinical remission was higher in a pooled analysis of short-term therapy with antibiotics (5–14 days), odds ratio 2.02 (CI 1.26–3). Short-term symptomatic remission rates were better with the addition of oral tobramycin (vs placebo) to corticosteroids in 84 patients with acute relapsing ulcerative colitis (74% vs. 43%, respectively) [421], the durability of these responses is unknown. The addition of ciprofloxacin (vs placebo) to a regimen of corticosteroids and mesalamine resulted in lower 6-month failure rates in the patients receiving the antibiotic (21% vs. 44%) [424]. Rifaximin has also shown promise in some patients with corticosteroid-refractory disease [425].

Antibiotics are first-line therapy in the treatment of *pouchitis* for patients with ulcerative colitis who have undergone an ileopouch anal anastomosis. Ciprofloxacin and metronidazole are the two antibiotics with the most use and success; often one is started and the other is added in refractory cases [426,427]. For refractory cases, adding antibiotics such as rifaximin [428–430] or combination therapy with ciprofloxacin and tinidazole [431] has proven efficacy. Prophylaxis against pouchitis onset [432] or prevention of relapse of chronic pouchitis [433,434] has been shown in placebo-controlled trials using a probiotic cocktail containing viable lyophilized bacteria of four strains of lactobacilli, three strains of bifidobacteria, and one strain of *Streptococcus salivarius* subsp. *thermophilus*.

Antibiotics in Crohn's disease

There is also a surprising absence of controlled data supporting the use of antibiotics in patients with Crohn's disease [435]. Multiple studies of metronidazole, either as sole-therapy or as a combination with other antibiotics, have failed to show convincing remission data, although in a randomized, placebo-controlled trial, patients receiving high-dose metronidazole (20 mg/kg) had improved disease activity, with the greatest benefit seen in colonic or ileocolonic disease [436]. That same dose was also shown to be initially effective as postoperative prophylaxis in a placebo-controlled trial of 60 patients undergoing curative ileocolonic resections for Crohn's ileitis. Twelve weeks of therapy decreased severe endoscopic recurrence rates at the time of therapy discontinuation (13% vs. 43% for placebo); the benefit over clinical recurrence rates was maintained at 12 months 4% vs. 25%), but not afterwards [437]. Limited by a high incidence of neuropathy, a subsequent similar study randomized 80 patients with Crohn's disease undergoing curative ileocolonic resection to either ornidazole (1 g/day) or placebo for 1 year [438]. Clinical recurrence rates at 1 year were lower with ornidazole (8% vs. 38%), as were endoscopic recurrence rates (54% vs. 79%). Side effects, however, limit long-term use.

Ciprofloxacin is often used in patients with Crohn's disease, despite the absence of benefit from large controlled trials. Smaller trials or case series have suggested that ciprofloxacin may be superior to placebo [439] and comparable to mesalamine [440]. Combination therapy with metronidazole has been effective in open-label experience [441], showed efficacy similar to corticosteroids in one trial [442], but adding the same combination to budesonide proved no better than adding a placebo in another trial [443].

Many other antibiotics have been studied, either alone or in combination, with varying results [444–446]. A recent metaanalysis [447] of the use of broad-spectrum antibiotics in Crohn's disease calculated a pooled odds ratio of 2.257 (CI 1.68–3.04) favoring the antibiotics over placebo.

Antibiotics are typically used in perianal or enterocutaneous fistulous Crohn's disease, although there is little published literature regarding the topic. One study showed a benefit of combined therapy with metronidazole and ciprofloxacin in patients, some of whom were already receiving or initiating therapy with azathioprine [448]. The presence of draining pus in many of these patients makes it advisable to use antibiotics, especially if also initiating immunosuppressive therapy.

Corticosteroids

Corticosteroids have been a mainstay of acute therapy for moderate to severe, and fulminant ulcerative colitis and as a first-line agent for moderate to severe Crohn's disease. In addition, nonsystemic corticosteroids (e.g., ileal-release budesonide) have become a therapeutic option for patients with mild to moderate Crohn's disease involving the ileum or right colon.

The mechanisms of action of corticosteroids continue to be elucidated [449–453]. Glucocorticoid receptors are present on most human cells and their concentration is autoregulated. The ubiquitous distribution accounts for the systemic effects and side effects on most organ systems. By binding to intracytoplasmic glucocorticoid receptors found in most cell types, glucocorticosteroids activate glucocorticoid-responsive elements that influence multiple signal transduction pathways. An important action is the switching off of multiple activated inflammatory genes through the inhibition of histone acetylase activity and recruitment of histone deacetylases

to the inflammatory gene transcriptional complex resulting in transrepression of proinflammatory transcription factors (e.g., nuclear factor-κB) and apoptosis of inflammatory cells and activated lymphocytes [450]. Glucocorticoids also activate several antiinflammatory genes and increase the degradation of messenger RNA encoding inflammatory proteins, resulting in a broad spectrum of effects on the immune system including inhibition of the recruitment and proliferation of lymphocytes, monocytes and macrophages, migration of neutrophils to sites of inflammation, and decreased production of soluble inflammatory mediators including cytokines, leukotrienes, and prostaglandins [451,452].

There is a large variation in plasma concentrations of glucocorticoids after oral administration in both normal subjects and patients with inflammatory bowel disease and the biological half-life of glucocorticoids is 18–36 h compared with a 2- to 4-h plasma half-life [451]. The most commonly used oral glucocorticoids are prednisone (or prednisolone) and methyl-prednisolone. The potencies of prednisone/prednisolone and methyl-prednisolone compared to hydrocortisone are 4 : 1 and 5 : 1, respectively [451,452]. In Europe, prednisolone metasulfobenzoate has been used to minimize systemic absorption with oral delivery and enema formulations [370]. The bioavailability after rectal administration of hydrocortisone is dependent on disease activity and retention time, but can be up to 80% of an administered dose [451]. Nonsystemic corticosteroids have been developed for both topical and rectal administration [370,452]. The most commonly used oral and rectal formulation of a nonsystemic steroid is budesonide, which has been targeted in an oral formulation for ileal and right colonic delivery [454].

There have been conflicting reports describing glucocorticoid receptors in patients with inflammatory bowel disease but it appears that mucosal glucocorticoid receptors are decreased in patients compared with healthy controls [452]. Glucocorticoid-resistance has also been described [455] related to ligand-induced down-regulation of the receptor, the dominant-negative inhibition by the β-isoform of the receptor, or repression by the transcription factor nuclear factor-κB [453]. Other potential mechanisms of steroid-resistance include: decreased cytoplasmic glucocorticoid concentration secondary to increased P-glycoprotein-mediated efflux from target cells secondary to overexpression of the multidrug resistance gene (*MDR1*) [456]; impaired glucocorticoid signaling because of dysfunction at the level of the glucocorticoid receptor; and constitutive epithelial activation of proinflammatory mediators, including nuclear factor-κB, resulting in significant inhibition of glucocorticoid signaling, and decreased efficacy of glucocorticoids [457].

The ubiquitous side effects of corticosteroids are the major factor limiting their use in IBD [370,449,451]. Although the majority of corticosteroid side effects are dose dependent, there is a wide range of patient susceptibility such that some patients tolerate prolonged courses of high-dose corticosteroid therapy without ill effects, but others develop devastating side effects with short courses of low-dose therapy. Patients taking more than 10 mg prednisone daily for longer than 3 weeks should be viewed as having a suppressed hypothalamic–pituitary–adrenal axis for 1 year after cessation of therapy and should receive supplemental glucocorticoids for surgery or severe illness. Patients should be instructed about adrenal suppression and the need for glucocorticoid supplementation for surgery and severe illness even after cessation of therapy.

Patients also need to be educated about the benefits and toxicities of glucocorticoids. Common predictable side effects include increased appetite, central obesity, moon facies, acne, insomnia, increased risk of infection, and, in children, growth retardation. Less common problems requiring patient monitoring include hypertension and glucose intolerance. Steroid-induced glucose intolerance may require reatment with insulin or oral hypoglycemic agents and in patients with diabetes; steroids may make blood-sugar control more difficult. Sleep disorders are common along with mood swings. More profound psychiatric problems, including depression and psychosis, may become unmasked or exacerbated by steroid therapy. Osteonecrosis, particularly of the femoral heads, can be related to corticosteroid therapy or the underlying disease [458,459].

Osteoporosis is a common and potentially devastating side effect of corticosteroid therapy when fractures occur. Virtually every patient treated with glucocorticoids develops negative calcium balance although there is a variation in risk of bone loss that typically occurs within the first months of therapy [449,460]. The effects of corticosteroids on bone include both enhanced bone resorption and diminished bone formation. Bone loss characteristically involves trabecular bone in ribs, vertebrae, and distal radius. Rib fractures and vertebral compression fractures are common. Patients with small intestinal Crohn's disease are at greater risk for steroid-induced bone loss because of malabsorption of calcium and vitamin D. The incidence of symptomatic bone loss also is higher in children and postmenopausal women. To minimize bone loss, the management of patients on corticosteroids should include the following [460]:

- use of the lowest possible dose of corticosteroids
- smoking cessation, limitation of alcohol consumption, and 30–60 min of weight-bearing exercise per day
- history, physical examination, and laboratory evaluation, including bone mineral densitometry
- dietary or supplemental calcium (1500 mg/day)
- vitamin D (50 000 IU 1–3 times/week) or calcitriol (0.5 μg/day)
- consideration of hormone (estrogen) replacement therapy where appropriate
- consideration of a bisphosphonate antiresorptive agent.

Posterior subcapsular cataracts occur frequently with corticosteroid therapy; also related to dose and duration of

therapy and increased intraocular pressure can occur in up to 40% of patients and can rarely lead to irreversible glaucoma and blindness. Periodic ophthalmological examinations with measurements of intraocular pressure and slit-lamp examination should be performed for patients on long-term corticosteroid management.

While budesonide is generally associated with fewer adverse events than prednisone, and in short-term trials has a similar side effect profile to placebo [461], monitoring for steroid-related side effects is warranted, particularly in long-term use. With long-term use budesonide at doses up to 9 mg daily is unlikely to induce osteoporosis [462], however, because patients with ileal Crohn's disease are already at increased risk, bone density monitoring and calcium and vitamin D supplementation are warranted.

Corticosteroids in ulcerative colitis

Oral corticosteroids

Oral corticosteroids are indicated for the treatment of outpatients with moderately severe ulcerative colitis [110,111,449,463]. Although the original trials of corticosteroids in ulcerative colitis evaluated 100 mg oral cortisone daily [109], there has since been a general consensus that prednisone or prednisolone are favored preparations related to their pharmacokinetic profiles and ease of administration on a regimen from once a day to four times a day. There is a dose–response for prednisone from 20 mg to 60 mg daily, although the modest benefits of the higher dose are offset by increasing steroid side effects [464]. Similarly, there is scant evidence regarding dosing schedules with only a single small trial that compared once daily dose of 40 mg prednisone with 10 mg administered four times a day [465]. In the 45-patient trial there was no difference in the clinical outcomes over 2 weeks, leading to the trend to use single daily dosing. Recognizing that there have been considerable differences in outcome measures in clinical trials of ulcerative colitis [466], overall oral corticosteroids have been effective at inducing remission in approximately 70% of patients with a number needed to treat of 2 [391]. Similarly, in population series approximately 50% of patients achieve a remission with 4 weeks of treatment and an additional 30% attain a partial response [115]. Unfortunately, once initiating steroid therapy 16% of patients failed to respond and, after 1 year, less than one-half were in remission and over 20% of patients required a colectomy (Fig. 57.33).

Oral corticosteroids have neither been effective nor indicated for maintaining remissions of ulcerative colitis. In distinction, steroid-dependence is common [115]. Neither cortisone 50 mg daily [467] nor prednisone up to 15 mg daily [468] was effective at preventing relapse. While high-dose (40 mg) alternate-day prednisone in addition to sulfasalazine did reduce relapse rates in a small, 24-patient trial, side effects of oral corticosteroids rarely justify their long-term use [110,111,391,463,469].

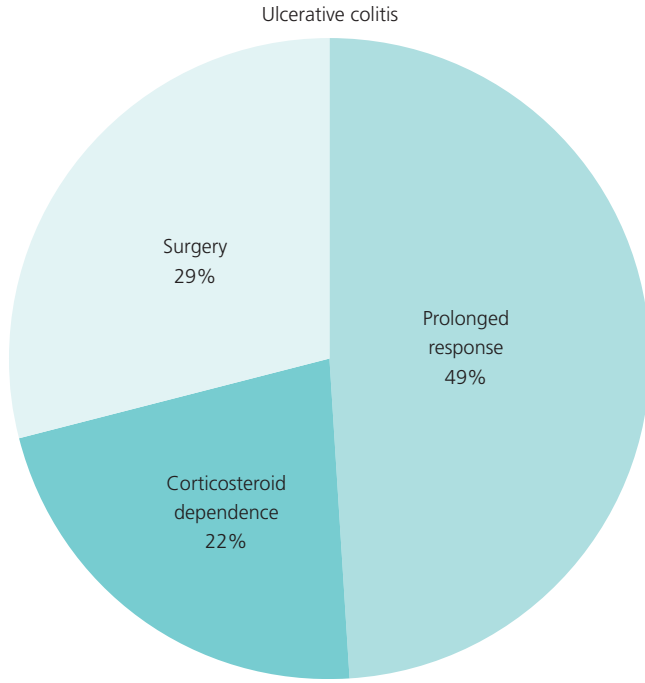


Figure 57.33 One-year outcomes after the first course of corticosteroids in 63 patients with ulcerative colitis. Data from Faubion et al. [115].

Topical corticosteroids

Topical corticosteroids (i.e. formulations applied per rectum) have long been recognized as an important therapeutic approach in patients with ulcerative colitis, as either first-line therapy in patients with disease limited to the distal colon or rectum, or as combination therapy in patients with more extensive colitis [470,471]. A recent metaanalysis described pooled odds ratios for symptomatic and endoscopic improvement at 0.21 (95% CI 0.07–0.71) and 0.27 (95% CI, 0.10–0.77), respectively [472]. For disease located solely in the rectum, corticosteroid-containing suppositories or foam are preferable. Patients with proctosigmoiditis may find improvement with a foam or liquid enema preparation, while those with inflammation extending past the sigmoid colon are best treated with the liquid enema formulation. Corticosteroid enemas are also useful in as adjunctive therapy in patients with severe colitis [418]. Estimates of systemic absorption of the corticosteroid from a corticosteroid enema preparation range from 40% [473] to 75% or 80% [451,474]. As a result, long-term use of topical corticosteroids is discouraged; patients should preferentially be treated with mesalamine-based products when possible, which comparative analysis has shown to be more efficacious than the corticosteroid-containing agents [392,472]. Novel topical corticosteroid preparations, such as budesonide [475], beclomethasone dipropionate [476], and others, allow for targeted mucosal release and effect with minimal systemic side effects, but have yet to achieve Food and Drug Administration approval in the United States.

Parenteral corticosteroids

Parenteral corticosteroids have been a mainstay of therapy for hospitalized patients with severe-fulminant ulcerative colitis despite an absence of controlled trial data in this complicated group of patients [449]. While placebo-controlled trials would be unethical in this setting, several studies have compared parenteral hydrocortisone with adrenocorticotropic hormone (ACTH; corticotropin) but variations in response between formulations failed to define an "optimal" approach between hydrocortisone and ACTH. Although the latter may have a small advantage for steroid-naïve patients [477], the majority of hospitalized patients will have been hospitalized as the result of the failure of outpatient corticosteroid treatment. In addition, ACTH has been associated with adrenal hemorrhage and is no longer marketed as a therapy in the United States. Most clinicians have therefore moved to equivalent doses of prednisolone, methylprednisolone or hydrocortisone, although the last of these is less preferred because of higher mineralocorticoid effects and greater potassium losses. A dose–response for parenteral steroids has not been clarified in clinical trials although there is no evidence to favor significantly higher doses than 48 mg/day of methylprednisolone [478]. Furthermore, it does not appear to matter whether intravenous steroids are administered as a bolus infusion or as a 24-h continuous infusion [479].

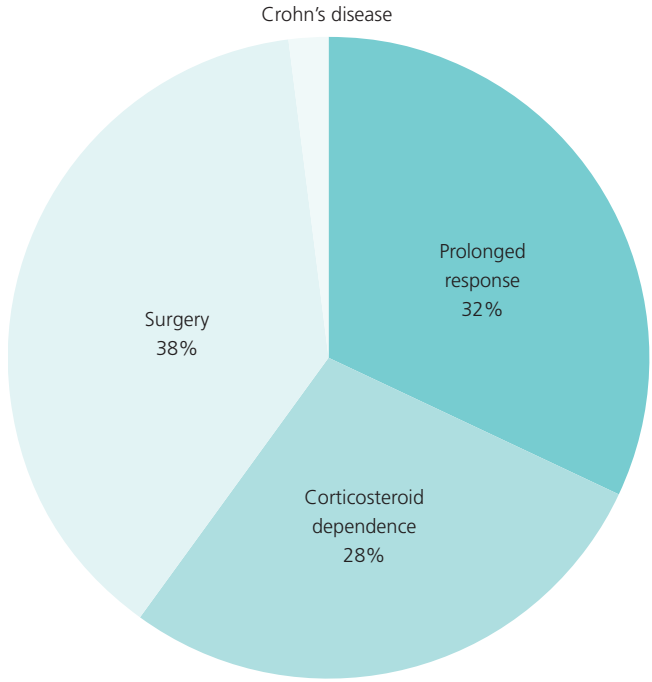


Figure 57.34 One-year outcomes after the first course of corticosteroids in 74 patients with Crohn's disease. One patient was lost to follow-up. Data from Faubion et al. [115].

Corticosteroids in Crohn's disease

Oral corticosteroids

Oral corticosteroids are indicated for the induction of a clinical response and remission in patients with moderately or severely active Crohn's disease (Fig. 57.34). Dosed within a range of 0.25–0.75 mg/kg with subsequent taper after remission induction, prednisone or prednisolone were shown to be superior to placebo in both the National Cooperative Crohn's Disease Study [480] and the European Cooperative Crohn's Disease Study [408]. Studies of higher doses (1 mg/kg of prednisolone) showed superior remission rates, from roughly 60% to over 90% [241]. However, because of prohibitive side effects at the higher doses, many clinicians treat at an initial dose of 40 mg/day, in either single or divided doses. Once remission is attained, doses are tapered, although no discrete tapering regimens have been established. In one study, 6-month outcomes were similar with a 4-week vs. 12-week taper of methylprednisolone from an initial dose of 40 mg daily [481].

Clinical trial outcomes in Crohn's disease are superior to sulfasalazine [407,408] and to elemental diets (with and without antibiotics) [482,483]. Remission rates with oral corticosteroids were better than for combination therapy with metronidazole and ciprofloxacin [442]. Coadministration of prednisone (30 mg/day) or 6-methylprednisolone (48 mg/day) with sulfasalazine therapy (3–6 g/day) showed improved efficacy [484] the reverse, however, was not seen [485], suggesting that the corticosteroid is the more effective agent in remission induction [408].

Oral budesonide has proven an effective alternative to traditional corticosteroids for patients with Crohn's disease affecting the small intestine and right colon (the location of release from the drug's pH-dependent coating) [454]. Controlled ileal release (CIR) budesonide was superior to placebo in the treatment of active Crohn's disease, with an optimal dose of 9 mg daily (8-week remission rates 51% vs. 20%, $P < 0.001$) [486]. Budesonide 9 mg/day was more effective than mesalamine 4 g/day at both 8 weeks (69% vs. 45%, $P = 0.001$) and 16 weeks (62% vs. 36%, $P < 0.001$) of therapy, with minimal impact on the adrenal–pituitary axis [487].

Comparisons of efficacy between budesonide and "traditional" oral corticosteroids have had mixed results; most have had similar outcomes between regimens, with a tendency for the budesonide to have slightly lower remission rates, but with far fewer corticosteroid-related side effects and less measured impact on the adrenal–pituitary axis [488–490]. Metaanalysis has shown budesonide to be more likely to induce remission in Crohn's disease than placebo (relative risk [RR] = 1.82, 95% CI 1.15–2.88) or than mesalamine (RR = 1.73, 95% CI 1.26–2.39), but less frequently than conventional corticosteroids (RR = 0.87, 95% CI 0.76–0.995). It was also less likely to cause corticosteroid-associated adverse events than conventional corticosteroids (RR=0.65, 95% CI 0.53–0.80) [461]. Optimal dosing of

budesonide is 9 mg once daily [490]; higher doses have not shown greater efficacy, but are associated with higher rates of adrenal suppression [486].

Topical corticosteroids

Topical corticosteroids have not been well studied in Crohn's disease; many practitioners use the same enemas, foams, and suppositories that are successful in ulcerative colitis for patients with Crohn's disease with distal disease, or for Crohn's disease of an ileoanal pouch [491].

Parenteral corticosteroids

Parenteral corticosteroids have been reserved for patients with Crohn's disease who are refractory to oral corticosteroids. Intravenous prednisolone is more effective than oral delivery; continuous infusion of intravenous prednisolone provides higher mean serum concentrations than bolus administration [492]. No large controlled trials of intravenous corticosteroids in Crohn's disease have been conducted, and open-label studies are few in number. One group reported a 76% response rate in 49 patients, with the lowest rates in patients with ileocolonic disease [493]. A double-blinded randomized trial comparing intravenous administration of either 120 units/day of ACTH or 300 mg/day of hydrocortisone revealed similar 10-day response rates (82% vs. 93%, respectively). As a result of the risk of adverse events from ACTH, methylprednisolone is preferentially used, typically at doses initially ranging from 40 mg/day to 60 mg/day, with subsequent transition to oral corticosteroids in patients who respond adequately to the intravenous therapy.

Immunomodulators

The term "immunomodulators", often used interchangeably with "immunosuppressants", refers to nonbiological therapy that directly targets the immune systems' cellular processes. These agents are often loosely grouped into families based on their primary proposed mechanisms of action. The common families include the antimetabolites (azathioprine, 6-mercaptopurine, and methotrexate), and the calcineurin inhibitors (cyclosporine, tacrolimus). The immunomodulators have traditionally been used in patients who are corticosteroid-dependent, or refractory, although there has been a recent emphasis on starting these therapies earlier in patients with IBD, particularly those with an initial moderately severe or severe presentation. These agents require frequent safety monitoring, and so are only suitable for use in patients who are compliant with the necessary medical testing, and should not be prescribed by practitioners unfamiliar with their use and risks.

Azathioprine and 6-mercaptopurine

Often used interchangeably in the management of IBD, azathioprine is a prodrug which undergoes initial conversion to 6-mercaptopurine. Further hepatic metabolism results in the formation of a variety of metabolites; the 6-thioguanine nucleotides are believed to be the primary nucleotides that account for the drugs' efficacy. Increased intracellular concentration of these nucleotides and other metabolites inhibits purine metabolism, and in turn DNA synthesis and repair, inhibiting cellular proliferation [494]. There are multiple cell-types whose activities are either directly or indirectly inhibited by these compounds, including lymphocytes [495] plasma cells [496], and natural killer cells [497].

Neither azathioprine nor 6-mercaptopurine is a therapeutically active agent; rather, it is the various 6-thioguanine nucleotide metabolites of these drugs that subsequently impact the body's biological systems. One of the critical enzymes necessary for the metabolism of the parent drugs is thiopurine methyltransferase (TPMT) [494,498] (Fig. 57.35). It is important that the genetic variations in the presence and activity of this enzyme are understood when dosing these drugs. Approximately 88% of the population is a wild-type for both TPMT alleles; another 11% is heterozygous, and 0.3% is homozygous-deficient for either allele [499,500].

Furthermore, mutant alleles can account for variation in the enzyme activity. Absence or low activity of the TPMT enzyme shunts much more of the parent drug towards the production of 6-thioguanine (6-TG) nucleotides, leading to toxicity at seemingly very low doses of the parent compounds. Conversely, there is little or no production of the 6-methylmercaptopurine (6-MMP) nucleotide, which at high levels has been associated with elevation of liver function tests.

Erythrocyte TPMT has been shown to be an accurate reflection of the enzymatic activity in other tissue, allowing for commercially available measurement of TPMT genotype and enzymatic activity from a simple blood sample [500]. This may be an important tool in determining the appropriate initial dosage, and subsequent evaluation of changes in hematological or hepatic parameters after therapy has been initiated. For example, patients who are wild type for the TPMT enzyme, or who have high levels of TPMT activity, may be eventually dosed with azathioprine at 2.0–3.0 mg/kg/day, or 6-mercaptopurine at 1.0–1.5 mg/kg/day. However, those who are heterozygous for the TPMT genotype, or have

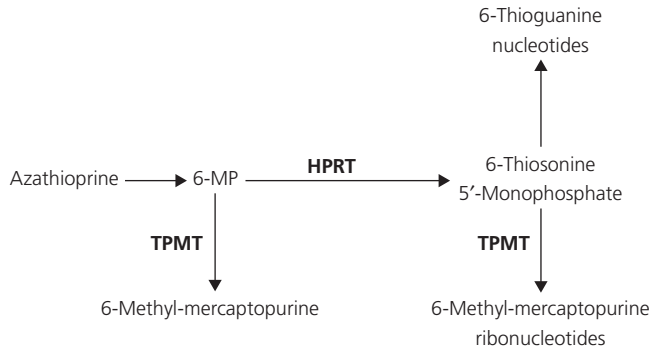


Figure 57.35 Metabolism of azathioprine and 6-mercaptopurine.

only intermediate levels of enzyme activity, are typically targeted at 50% of the full dose. Patients who are homozygous deficient for both alleles, or in whom enzyme activity is very low, should not be treated with either agent [501,502].

It is recommended to check TPMT enzyme genotype or phenotype before or shortly after initiating therapy, and to adjust the initial and subsequent dosing appropriately [503]. However, it is important to realize that nearly two-thirds of leukopenic events on these medications result from events that are *independent* of the TPMT enzymes. As a result, it is prudent to start patients at low doses of these drugs (1.0 mg/kg of azathioprine; 0.5–0.75 mg/kg of 6-mercaptopurine) and monitor complete blood counts, platelet count, and differential, and liver function tests every 2–3 weeks when initiating therapy and following a dose increase [502,504]. Once the doses are stable, it is advisable to follow the hematological studies every 3–4 months, and the liver function tests at least twice yearly.

The utility of monitoring levels of the 6-TG and 6-MMP is less clear. Retrospective studies have suggested that patients with IBD with 6-TG levels of 235–250 or greater are more likely to be in remission than those with levels below that limit, although there were clearly patients in remission with "subtherapeutic" levels and others who still had evidence of active disease with levels exceeding that limit [502–505]. The 6-MMP nucleotide, while in some instances shown to also correlate with drug efficacy, has been more directly linked to a higher risk of toxicity [506] and developing increased liver function test abnormalities, particularly at levels greater than 5600. However, hepatotoxicity can occur at lower levels, and many patients have 6-MMP levels far higher than this limit without evidence of hepatic compromise. Until well-designed prospective trials reveal the true clinical relevance of monitoring these metabolite levels, which would justify measurement in all patients receiving these agents, there are a few scenarios where one could gain valuable information from these tests [503]. The first is ensuring patient compliance (or absorption) of these oral agents; low levels of both metabolites suggest that an adequate dose is either not being taken or not being absorbed. The second scenario is the patient in whom there is no apparent response to therapy; in this instance, patients who preferentially shunt towards the production of 6-MMP at the expense of a low 6-TG have been shown to be ill-served by dose increase, which simply drives up the 6-MMP with little effect on the 6-TG. Experimental investigations into the controversial administration of low-dose allopurinol (100 mg) while drastically decreasing the dose of the purine analogue to 25–50 mg/day has shown some exciting results, with increases of the 6-TG nucleotide, low levels of 6-MMP, and patient responses. However, strict monitoring of cell counts must maintained, as there is a risk of profound pancytopenia in some individuals.

A third scenario whereby one should consider the 6-TG and 6-MMP levels is when a patient's aminotransferases increase while on therapy. Finding a 6-MMP level > 5600 suggests that simple dose reduction might be sufficient, although repeating the hepatic enzyme levels to ensure normalization is important. In this particular patient scenario, if the patient has a "low" 6-TG level, and active disease, one should consider switching to a different class of immunomodulator [504].

A few novel approaches to patients who are either intolerant to the purine analogues, or preferentially shunt metabolism away from the active 6-TG nucleotide towards the 6-MMP nucleotide have been tried. The first is to substitute therapy with another agent in the purine metabolic chain, 6-TG. Therapeutic studies of 6-TG have shown the agent to be effective in Crohn's disease and ulcerative colitis [507–509]. However, overriding safety concerns, especially the development of nodular regenerative hyperplasia in 76% of patients with liver function test abnormalities, and 33% of those with normal liver chemistries [510], has relegated this agent to experimental trials, or dosing under strict safety parameters, if at all [511,512]. A different approach in patients who preferentially are shunting metabolism towards the 6-MMP nucleotide has been experimental combination therapy with very low doses of azathioprine and a low dose of allopurinol [513]. Previously considered to be a contraindicated therapeutic approach, very low doses seem to shift the metabolism of the purine analogue towards the 6-TG nucleotide, as well as achieving clinical efficacy. Very careful safety monitoring is required; this is not an approach advocated for general use at this time.

The safety of the purine analogues when used appropriately in patients with inflammatory bowel disease has generally been at a level acceptable to support their use, particularly as therapies sparing patients from the toxicities of long-term corticosteroids [514–519]. Side effects noted on initial introduction of therapy are often a reflection of allergic sensitivities, or idiosyncratic reactions, such as fevers, rash, back pain, headaches, diarrhea, pancytopenia, and pancreatitis [516,520,521]. Some of these reactions may be seen with azathioprine but not 6-mercaptopurine, and vice versa. Some patients may be successfully switched to the other agent without redevelopment of side effects [522–524], although pancreatitis and pancytopenia almost always recur in this setting, and should not lead to rechallenge with the other agent.

Adverse events that may develop after the initial dosing period are expanded to include cytopenias, hepatitis, infections, and neoplasms (particularly lymphomas) [501,516,520,525]. As the mechanism of action of these agents interfered with the purine synthesis pathway, any of the major blood cell lines may be adversely impacted by these drugs, most commonly the white cell lineage [525]. Bone marrow toxicity has been thought to be primarily due to the activity of the TPMT enzyme; however, the implication that a normal TPMT level allows for unfettered higher dosing in patients without concern for cytopenias is incorrect, and potentially dangerous.

One investigation into myelosuppression in patients with IBD treated with the purine analogues determined that 73% of the patients who experienced myelosuppression had no known TPMT mutation, with cases reported as late as 87 months after initiation of therapy [501]. Hepatic toxicity is most commonly thought to be related to elevated levels of the 6-MMP nucleotide (> 5700 pmol/8 × 10^8 erythrocytes) [502], although drug-induced cholestatic hepatitis [527] nodular regenerative hyperplasia [528,529] and venoocclusive disease [527] have been reported.

Concerns about infections with the use of the purine analogues are greatest for opportunistic infections; serious infections are rare [504,516,520] and may not be any greater than those seen in sick patients with IBD treated with corticosteroids or other agents [208]. However, patients must be monitored while on therapy, especially if on concomitant immunosuppressive agents. Concerns over the development of Epstein–Barr virus-related non-Hodgkin lymphomas [530,531] has raised concern over the need for better dosing guidelines with these agents, and discouraged the use of concomitant immunosuppressants. It is still not clear whether overall cancer and lymphoma rates are higher in patients with IBD on the purine analogues [532,533], especially those neoplasms that are not virally mediated. A larger retrospective series suggested an overall neoplasm rate of 3.1% [520]. Another review compared cancer rates between 626 patients with IBD treated for a mean duration of 27 months with azathioprine to 1578 patients with IBD who did not receive the drug [534]. Rates of all cancers (4.5% vs. 4.5%, respectively), colon cancers/high-grade dysplasias (2.2% vs. 2.8%), and lymphomas were similar between the two groups. Sustained leukopenia has been identified as a risk factor for the development of neoplasms in patients with IBD treated with these agents [535]. Most recently, rare instances of hepatosplenic T-cell lymphomas have been reported in young, almost exclusively male, patients with Crohn's disease receiving concomitant therapy with a purine analogue and infliximab [536]. This rare, typically fatal lymphoma has been reported previously in patients solely receiving the purine analogues [537], and raises further concerns over the use of multiple immunosuppressants in these patients [538].

Azathioprine and 6-mercaptopurine in ulcerative colitis

Although these agents are mainstays of therapy in the treatment of patients with corticosteroid-dependent or refractory IBD, the literature is surprisingly sparse in well-designed studies supporting their efficacy [539]. This may be partly the result of their delayed time-to-efficacy, shown by metaanalysis to be at least 17 weeks [540], and initial overlap in many studies with a tapering course of corticosteroids to induce the initial remission. As a result, the drugs' efficacy is typically not reflected in the outcomes at 1-month, but rather as a maintenance drug of a corticosteroid-induced remission, with a nearly 50% benefit over placebo [541]. Double-blinded withdrawal trials have shown azathioprine to be beneficial compared to placebo in preventing relapse (relapse rates 36% for azathioprine vs. 59% for placebo), with subgroup analysis showing the benefit extended to those previously in remission for more than 6 months (relapse rates 31% vs. 61%, respectively) [542]. When given in a placebo-controlled trial as combination therapy with sulfasalazine and an induction course of corticosteroids in patients with severe ulcerative colitis, remission induction rates were similar, but relapse rates were much lower with azathioprine than placebo (23.5% vs. 55.6%) [543,544]. The impact of the aminosalicylates on azathioprine's success in maintaining remission has been subsequently shown to be minimal, with relapse rates of 0.21/year for combination therapy vs. 0.19/year for those on azathioprine alone [545].

Open-label long-term retrospective analyses also yield information that supports the role of the purine analogues in inflammatory bowel disease. A 30-year survey of 662 patients treated with azathioprine in the Oxford IBD clinic reported remission rates of 45% for Crohn's disease, and 58% for ulcerative colitis; those rates improved to 64% and 87%, respectively, in patients receiving at least 6 months of therapy [518]. The proportions of patients remaining in remission wre 95% at 1 year, 69% at 3 years, and 55% at 5 years. These rates were notably lower in patients who stopped their azathioprine (63%, 44%, and 35%, respectively). Similar success with 6-mercaptopurine was reported in a 20-year retrospective survey from the Mount Sinai Hospital in New York, with a complete clinical remission rate of 65% and an additional 24% of patients achieving partial remission [515]. While 35% of patients subsequently had a relapse, remission was successfully restored in 88%, while relapse rates in those who stopped therapy were reported at 87%. These high success rates, and subsequently high relapse rates when the medications are discontinued, have strongly supported the use of these purine analogues as long-term therapy in ulcerative colitis [514].

Frustrated by the long delay until these therapies become efficacious, attempts at hastening onset of action by intravenous loading have been unsuccessful, and potentially toxic [546]. As a result, most of the studies mentioned above have relied on corticosteroids to induce an initial remission. For patients who are refractory or intolerant of high-dose corticosteroids, the purine analogues have proved successful in maintaining a remission induced by cyclosporine [547–549] or tacrolimus therapy [550,551].

Azathioprine and 6-mercaptopurine in Crohn's disease

There have been multiple placebo-controlled trials of the purine analogues in patients with Crohn's disease [221,480,552–556] most of which support the use of these agents in the induction or maintenance of remission, and in the closure of Crohn's fistulae. A metaanalysis calculated an odds ratio for response (vs. placebo) of 3.09 for patients with

active disease, 2.27 for quiescent disease, and 4.44 for fistulous disease [540]. A subsequent Cochrane analysis of the efficacy with azathioprine in the maintenance of remission reported odds ratios of 2.16 (CI 1.35–3.47) for maintenance of remission with azathioprine, 5.22 (CI 1.06–25.68) for corticosteroid-sparing, and 4.36 (CI 1.63–11.67) for withdrawal because of adverse events [557].

The success of these agents has led to subsequent studies of early intervention in patients with newly diagnosed moderate to severe Crohn's disease. One group randomized 55 children within 8 weeks of initial diagnosis to receive 6-mercaptopurine (1.5 mg/kg/day) or placebo, along with a tapering dose of prednisone (initially 40 mg/day). The children who received the purine analogue had a shorter duration of corticosteroid use, lower cumulative corticosteroid dose, and far lower relapse rates (9% vs. 47%) [223]. Unfortunately, intravenous loading of azathioprine was not shown to be successful in hastening time to response or remission in Crohn's patients [558].

The purine analogues have also been evaluated in fistulous Crohn's disease [559]. In a study of Crohn's fistulae with an 8-week treatment regimen of ciprofloxacin and metronidazole, patients who were already receiving azathioprine (2.0–2.5 mg/kg) at study initiation were more likely to achieve a response than those who were not on the purine analogue (48% vs. 15%) [448]. Outcomes were also notable in pediatric Crohn's patients, with 67% of patients improving and 40% closing their fistula with either azathioprine or 6-mercaptopurine. The purine analogues also are effective in maintaining fistula closure in patients whose fistulae were initially closed with cyclosporine [560] or tacrolimus [561].

There is also a growing body of literature supporting the use of the purine analogues as a postoperative preventative therapy in patients with Crohn's disease [562,563]. A multicenter, double-blind, double-dummy trial comparing daily doses of 6-mercaptopurine (50 mg) or mesalamine (3 g) to placebo reported lower 24-month rates of clinical (50% vs. 77%) and endoscopic (43% vs. 64%) recurrence with 6-mercaptopurine compared to placebo [562]. In another study, azathioprine (2 mg/kg) was also shown to be superior to mesalamine (3 g) in nearly halving surgical recurrence rates, with the greatest benefit seen in patients who had more than one previous surgery [563].

Methotrexate

Methotrexate is a competitive inhibitor of dihydrofolate reductase, preventing DNA synthesis and purine production [564]. Methotrexate therapy decreases levels of many of the proinflammatory cytokines found to be elevated in patients with inflammatory bowel disease, such as IL-1 [565], TNF, IFN-γ [566], and others. Methotrexate dosing in IBD was "borrowed" from that used in psoriasis and rheumatoid arthritis [567]; unlike those diseases, however, only parenteral methotrexate has been shown to be effective in clinical trials in IBD. This may be the result of variable bioavailability when dosed orally, as compared with over 90% bioavailability when dosed subcutaneously or intramuscularly [568]. Methotrexate is delivered weekly, to minimize side effects and hepatotoxicity [569], with folic acid supplementation (1 mg daily).

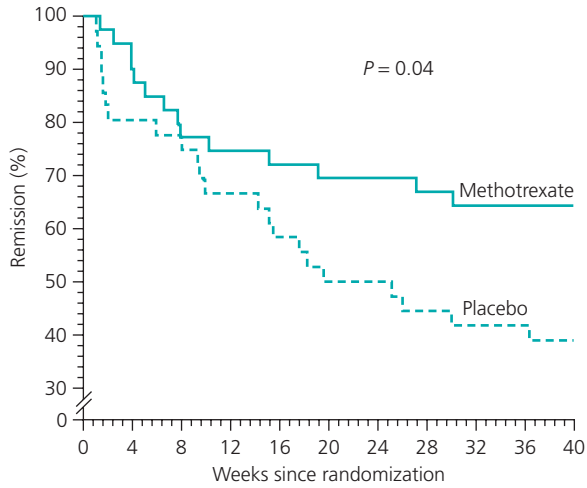
Toxicity is not uncommon with methotrexate, although the lower doses used in autoimmune diseases make the agent safer than when used for hematological malignancies. Side effects have been reported in nearly one-half of the patients with Crohn's disease treated in some series [570], leading to withdrawal of therapy in nearly 18% [570,571]. While most adverse events are the result of allergic reactions or intolerance, some are the result of the antimetabolic effects, similar to those seen in patients receiving chemotherapeutic agents [572]. Hepatic fibrosis and cirrhosis are rarely seen, leading to a withdrawal of the recommendation for a surveillance liver biopsy at 1.5 g cumulative dose [573], unless repeatedly elevated aminotransferases or decreased serum albumin are detected [574].

Methotrexate in ulcerative colitis

Although advocated by some clinicians, there have yet to be convincing clinical trials proving methotrexate's efficacy in ulcerative colitis. Although five of the seven patients in the initial experiment reported by Kozarek had a clinical response, none had mucosal healing, although the histological scores did improve [567]. While there are individual centers and case reports of success with methotrexate [575,576] a recent Cochrane analysis did not find convincing support for use of the drug, in part because of a lack of suitable randomized controlled trials in ulcerative colitis [577]. The only placebo-controlled trial found nearly identical remission rates with oral methotrexate 12.5 mg daily (46.7%) and placebo (48.6%). Larger trials with parenteral therapy are needed.

Methotrexate in Crohn's disease

Methotrexate has been shown to be efficacious in the induction and maintenance of remission in Crohn's disease, when given either intramuscularly [571] or subcutaneously [570,575]. Data on oral dosing have been disappointing [578]; a Cochrane Database review concluded that there was insufficient evidence supporting oral delivery in Crohn's disease [579]. The pivotal induction trial for methotrexate dosed 141 patients with corticosteroid-refractory Crohn's disease with either methotrexate 25 mg intramuscularly weekly or placebo in a 2 : 1 ratio, along with prednisone 20 mg which was tapered over 10 weeks [571]. Remission rates were higher with the methotrexate (39% vs. 19%), while total prednisone received was less in that same group. A subsequent "continuation" remission maintenance study in this group randomized 76 of the patients who had achieved remission to either intramuscular methotrexate (15 mg/week)



No. at risk

Methotrexate	40	36	30	29	28	27	27	26	25	24	19
Placebo	36	29	28	24	21	18	18	16	15	15	12

Figure 57.36 Estimated time to relapse in patients with Crohn's disease who entered into remission after 16–24 weeks of treatment with intramuscular methotrexate. From Feagan et al. [580], with permission from the Massachusetts Medical Society.

or placebo [580]. Week 40 remission maintenance rates were higher with methotrexate (65% vs. 39%), with comparable safety rates (Fig. 57.36). The University of Chicago group subsequently published open-label data showing a remission rate of 37% at a mean dose of 20 mg/week, with maintenance of remission in 46% with parenteral (but not oral) methotrexate [570].

Cyclosporine and tacrolimus

Cyclosporine (a polypeptide produced by the fungus *Tolypocladium inflatum*) and tacrolimus (a macrolide antibiotic) both bind specific proteins which, in turn, inhibit calcineurin, an enzyme required for activation of T lymphocytes [581–583]. Both are metabolized by the cytochrome P450 system, and care must be taken when dosing these agents with other medications or foods that interact on the same pathway.

Cyclosporine is typically administered intravenously to achieve a rapid response, and then patients are converted to oral dosing. There are two oral formulations that differ greatly in intestinal absorption and bioavailability: the cremaphor-based Sandimmune and a microemulsion Neoral [584,585]. It is important to identify which agent the patients are receiving, as accurate dosing by blood levels is critical to allow for efficacy and to avoid toxicity [586]. One of the main advantages of cyclosporine is the rapid onset of response (typically within a few days), even in the most severely ill patients. Used mostly for corticosteroid-refractory ulcerative colitis, cyclosporine is typically added on to the corticosteroids as a continuous intravenous infusion at 2–4 mg/kg/day, with dose titration to achieve whole blood adjusted radioimmunoassay or high-performance liquid chromatography levels of 250–400 ng/mL [111,463,547,587,588]. Dose adjustments are often necessary because of side effects. Patients who are successful in achieving a complete clinical response are transitioned to an oral formulation of cyclosporine (twice the daily dose as was given intravenously, dosed twice a day), oral prednisone, and initiated on long-term therapy with either 6-mercaptopurine or azathioprine. Over the subsequent 2–3 months, the purine analogue dose is optimized, and the other agents tapered off.

Tacrolimus is more potent than cyclosporine, and has been used intravenously in the pediatric population in the same setting of severe refractory colitis [550], but more commonly is dosed orally [550, 551,589]. Initial doses are 0.10–0.15 mg/kg twice daily, titrated to trough levels of 10–20 ng/mL. Transitioning patients to the longer-acting, safer purine analogues is also typically the goal of therapy.

Cyclosporine and tacrolimus have formidable toxicities that limit their use to selected patients, and for limited periods of time. Most concerning is the nephrotoxic impact of these agents, with decreases in creatinine clearance of approximately 20% with chronic use of cyclosporine [590]. The most common side effects reported in center series of patients with IBD treated with cyclosporine were paresthesias, hypertension, hypertrichosis, renal insufficiency, infections, gingival hyperplasia, and seizures [547,591]. Rare opportunistic infections, including *Pneumocystis carinii*, have been reported, as well as patient deaths. Large-scale experiences with tacrolimus in IBD have not been reported, but the literature links the drug to higher rates of neurotoxicity, diarrhea, diabetes mellitus, pruritis, and alopecia [592]. For both agents, prophylaxis against *Pneumocystis carinii* is advocated.

Cyclosporine and tacrolimus in ulcerative colitis

Cyclosporine's major role in IBD has been its use as an intravenous infusion in patients with severe ulcerative colitis that is refractory to intravenous corticosteroids. In a small placebo-controlled trial conducted by the University of Chicago and New York's Mount Sinai Hospital, 82% of the patients who received cyclosporine (4 mg/kg/day delivered as a continuous infusion) responded to therapy at a mean of 7 days, as compared to 0% of the placebo-treated patients [593]. Of note, all of the patients were still receiving intravenous corticosteroids and corticosteroid enemas; it was clearly the cyclosporine that made the difference. Retrospective open-label reports found similar initial response rates of 76%–86% [547,594,595]. It may be possible to use lower dose intravenous cyclosporine; in one study, patients who were randomized to receive 2 mg/kg had nearly identical response rates to those who received 4 mg/kg (85% vs. 84%, respectively), although only 55%–60% of the study participants were true failures of high-dose corticosteroids

[596]. Lower response rates have been reported with cyclosporine monotherapy (64%) [597], suggesting that this might be suitable in patients for whom corticosteroids are contraindicated, or poorly tolerated [598].

It quickly became apparent that while intravenous cyclosporine was effective in "putting out the fire" for patients with severe refractory ulcerative colitis, maintenance therapy with oral cyclosporine was ineffective [597,599,600]. Relapse rates resulting in surgery were high, except in patients who were transitioned to one of the purine analogues. Treatment paradigms with cyclosporine quickly adapted to use the agent solely as a "bridging therapy" to the slower acting but effective azathioprine or 6-mercaptopurine [463,547,587] (Fig. 57.37). The use of oral cyclosporine to induce a response or remission has not been formally investigated in clinical trials, although open-label experiences suggest that it is an effective short-term strategy [584,601,602].

Tacrolimus has not been studied in large controlled trials. Open-label experience in severe pediatric ulcerative or Crohn's colitis showed an initial response rate of 69%, although only 38% of patients avoided colectomy at 12 months, despite the institution of concomitant therapy with azathioprine or 6-mercaptopurine [551]. Small studies in ulcerative or indeterminate colitis have shown initial response rates of 47%–66%, with colectomies in approximately one-third of patients [550,589].

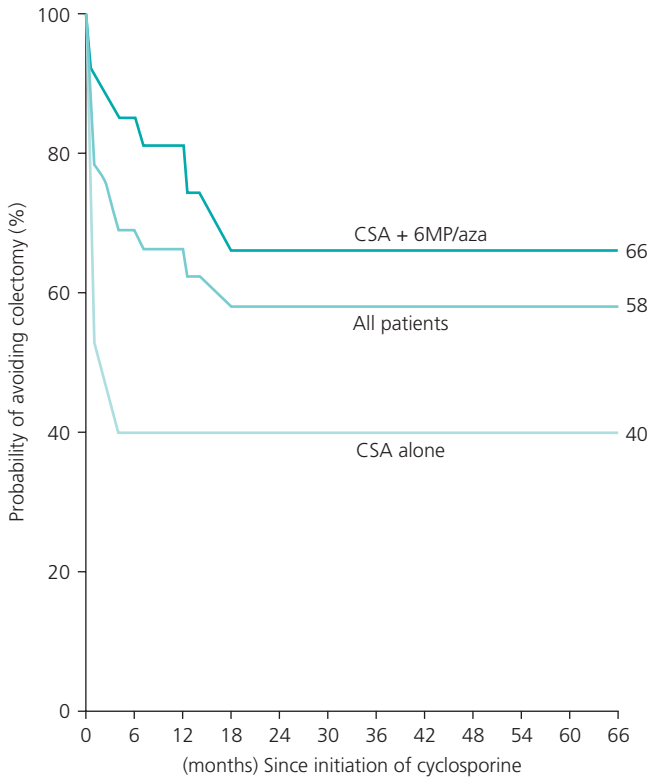


Figure 57.37 Predicted avoidance of colectomy over time in 42 patients with severe intravenous corticosteroid refractory ulcerative colitis treated with intravenous cyclosporine, followed by short-term oral cyclosporine and either long-term therapy with 6-mercaptopurine or azathioprine (6MP/aza) vs. no long-term purine analogue. ($P = 0.04$ Mantel–Haenszel χ^2; $P = 0.045$ log rank χ^2) From Cohen RD et al. [547].

Cyclosporine and tacrolimus in Crohn's disease

Cyclosporine use for Crohn's disease has been mostly abandoned, partly because of the lack of successful controlled trials, the toxicity of the drug, and the introduction of infliximab as a therapeutic agent for Crohn's disease in the late 1990s. Studies in lumenal Crohn's disease have been disappointing, with only high-dose (7.6 mg/kg/day), and potentially toxic, cyclosporine proven effective in clinical trials [603–605]. While still used in some instances of refractory fistulous disease, there are no published controlled clinical trials, or even large case series that utilize cyclosporine in these patients. Although initial fistula closure rates range from 88% to 90% in small case series [606,607] relapse rates were high (82%), in the absence of continued therapy with azathioprine [560].

Oral tacrolimus (0.2 mg/kg/day) has been shown in one randomized placebo-controlled trial to improve draining perianal or enterocutaneous Crohn's fistulae (43% vs. 8% for placebo), although complete fistula closure rates were similar between the groups [608]. Other open-label experiences have had mixed results [561,609].

Anti-tumor necrosis factor agents

The introduction of therapies that have activity against TNF has opened up an exciting new pathway in the treatment of inflammatory bowel disease. While most of these agents are still under investigation, the anti-TNF antibodies infliximab and adalimumab are commercially available, and certolizumab is anticipated to follow. Thalidomide is also an agent believed to work through similar pathways [610,611] but its use is extremely limited because of toxicity. Clinical trials with etanercept were not successful [612], and some of the other investigational agents (onercept [613], CDP571 [614]) also failed in clinical trials and are unlikely to come to market yet.

One of the overriding issues when using the biological anti-TNF agents is the awareness that certain rare opportunistic infections have been directly linked to their use [615–619] the most concerning of which is reactivation of *Mycobacterium tuberculosis* [620]. Screening of all patients using the most appropriate tests for their population (purified-protein derivative, chest radiograph, or QuantiFERON test) is important before starting therapy. Patients who test positive, or in whom history or physical examination suggests possible tuberculosis infection should not receive therapies directed against TNF until they have completed therapy. Anti-TNF therapies should not be used in patients with known or suspected infections, neoplasms, demyelinating disease, or moderate to severe heart failure. Lupus-like events and other rare disorders have also been reported with the anti-TNF agents [621].

Infliximab

Infliximab is a monoclonal mouse/human chimeric IgG_1 antibody that targets TNF-α. It binds both free and membrane-bound TNF, and causes apoptosis of TNF-producing cells [622]. Immune processes initiated by complement fixation and antibody-mediated cellular cytotoxicity are also possibly mediated by interaction with infliximab and TNF [623]. Infliximab is delivered intravenously; IBD patients typically receive an initial loading dose regimen of three infusions at weeks 0, 2, and 6, and subsequently receive maintenance infusions every 8 weeks (Fig. 57.38). The standard dose is 5 mg/kg; higher doses up to 10 mg/kg have proved effective in patients who have lost response to the 5 mg/kg dose. Lower doses have not been shown to be effective.

Loss of efficacy, and some adverse events, have been linked to the development of antibodies against infliximab [624], referred to as "HACAs" (human anti-chimeric antibodies), or more appropriately, "ATI" (antibodies to infliximab), as they do not cross-react with other chimeric antibodies, or other anti-TNF antibodies. The likelihood of HACA formation is lower in patients receiving maintenance infusions of infliximab [624,625] or those with either pretreatment with hydrocortisone [626], or concomitant treatment with azathioprine, 6-mercaptopurine, or methotrexate [627]. Coadministration with another immunosuppressant had been strongly advocated; recent safety concerns have led some experts to advocate switching to monotherapy after maintenance therapy has been established [538] (see below). Long hiatuses of therapy are discouraged [628], because of the development of a "serum-sickness-like" delayed hypersensitivity reaction in 25% of patients, characterized by severe arthralgias and loss of drug efficacy, along with very

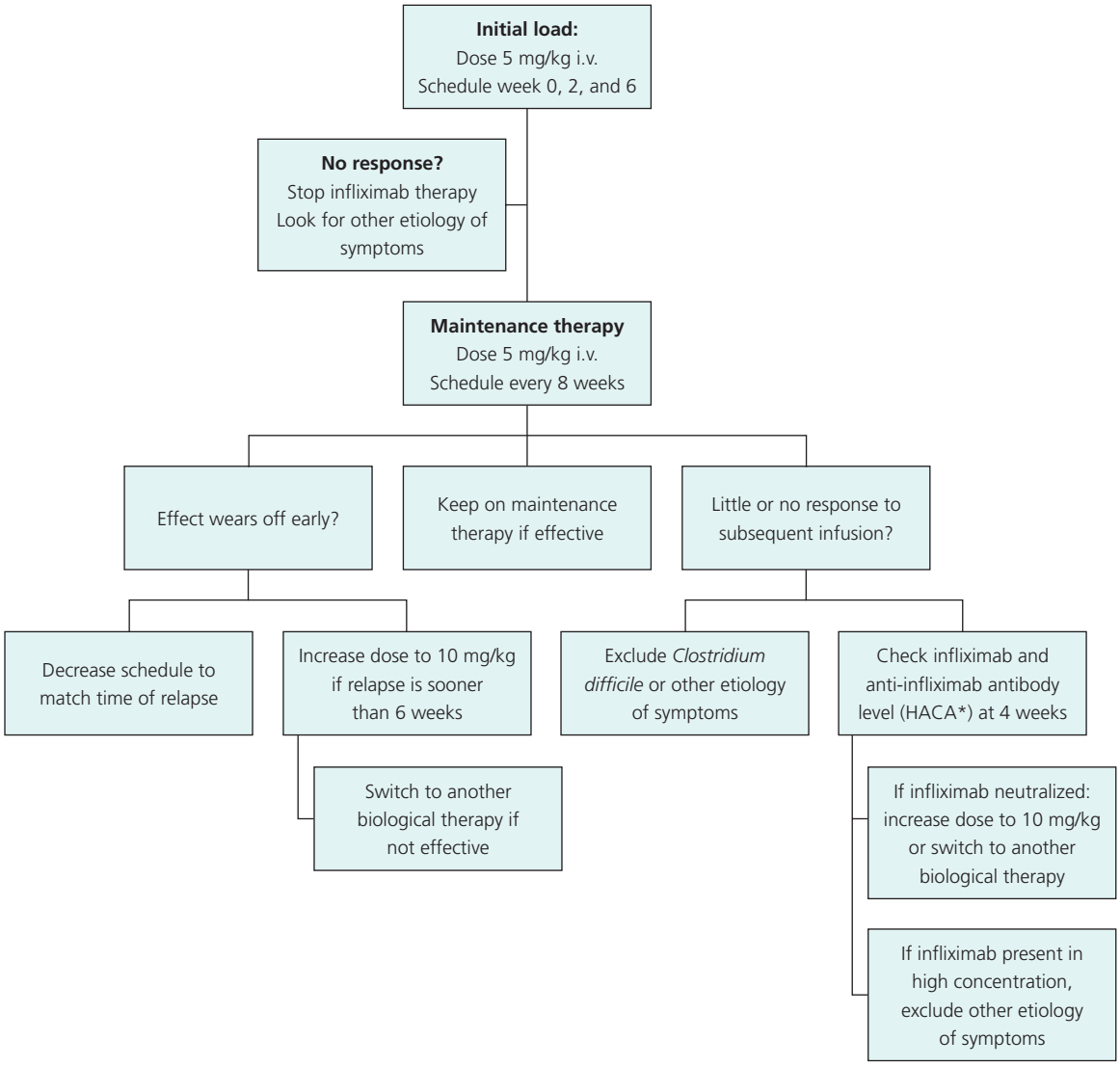


Figure 57.38 Dosing regimen for infliximab in patients with Crohn's disease or ulcerative colitis. *HACA = human antichimeric antibodies; more accurately described as antibodies to infliximab (ATI).

high titers of the "HACA" [629]. Maintenance infusions are typically given every 8 weeks; patients may be infused sooner if needed, but extending beyond 8 weeks has been associated with worse outcomes, and is discouraged.

Adverse events to infliximab can be categorized as those related to the infusion itself, and those thought to be independent of the infusion process. Infusion-related reactions were reported in 20% of all patients (all indications) in clinical trials (the rate was 10% in patients receiving placebo infusions) [621]. Among all infliximab infusions, 3% were accompanied by nonspecific fevers or chills, 1% by cardiopulmonary reactions (chest pain, blood pressure changes, dyspnea), and less than 1% by pruritus, urticaria, or a combination of symptoms [621]. Serious reactions, such as anaphylaxis, convulsions, and hypotension occur in less than 1%. The most common side effects in all clinical trials, similar in occurrence between patients receiving infliximab and those who received placebo, were upper respiratory infections, nausea, headache, and other gastrointestinal and respiratory complaints [621].

Infections requiring antibiotic treatment were reported in 32% of patients by the end of the 52-week ACCENT I Crohn's disease trial; serious infections were seen in 4% [625]. In the ACT I ulcerative colitis trial, serious infections were seen in 4.1% of the patients receiving placebo, 2.5% of the patients receiving infliximab at 5 mg/kg, and 6.6% of the patients receiving infliximab at 10 mg/kg. Regression analysis of the date from the TREAT registry (an industry-sponsored safety registry enrolling over 6000 Crohn's patients, half of whom have received infliximab) linked serious infections with the use of prednisone and of narcotics, but not with infliximab usage [630]. Mortality rates were also higher with prednisone use, but not with infliximab.

The rates of neoplasms in patients treated with infliximab are similar to those in the general population [621]. Lymphoma rates are higher with the immunosuppressants than in the healthy population; it is unclear whether the rates are elevated over patient cohorts matched for severity and duration of disease [621]. Rare instances of hepatosplenic T-cell lymphomas have been reported in young patients with Crohn's disease receiving concomitant therapy with a purine analogue and infliximab [536], although not in patients treated with infliximab alone. As a result, there has been a change in practice patterns to avoid long-term use of multiple immunosuppressants in patients receiving infliximab [538].

Infliximab in ulcerative colitis

Although a small clinical trial of infliximab in severe ulcerative colitis was promising [631], subsequent open-label experiences had mixed results [632–634] and infliximab was not widely used in ulcerative colitis until pivotal clinical trials were conducted. The ACT-1 and ACT-2 (Active Ulcerative Colitis Trials 1 and 2) trials each randomized 364 patients to receive intravenous infusions of either placebo or infliximab (dosed at 5 or 10 mg/kg) at weeks 0, 2, and 6, and then every 8 weeks [635]. The ACT-1 study continued for a total of 52 weeks, and boasted clinical response rates at week 8 of 37%, 69%, and 61% for placebo, 5 mg/kg infliximab, and 10 mg/kg infliximab, respectively, while week 54 rates were 20%, 55%, and 45%, respectively (Fig. 57.39). The ACT-2 study continued for 30 weeks, with week 8 response rates of 29%, 64%, and 69%, respectively, and week 30 rates of 26%, 47%, 60% (Fig. 57.39). The infliximab-treated patients also had superior mucosal healing rates, greater decreases in median corticosteroid dosage, and comparable safety results to patients given placebo. These studies ultimately led to the approval of infliximab for the induction and maintenance of remission in patients with moderate to severely active ulcerative colitis who were refractory to other therapies. The role of infliximab in patients whose severe ulcerative colitis is refractory to corticosteroids has been less clear. In a randomized blinded placebo-controlled trial, infliximab 5 mg/kg was shown to be an effective salvage therapy, with lower 3-month colectomy rates (29% vs. 67% for placebo) [636]. The utility of infliximab as a successful long-term option in the severe colitis patient refractory to intravenous corticosteroids has still yet to be determined.

Infliximab in Crohn's disease

There are multiple randomized placebo-controlled clinical trials that have established infliximab as an effective therapy in the treatment of moderate to severe lumenal Crohn's disease. The initial large trial was a 12-week study where patients received a single dose of infliximab at 5, 10, or 20 mg/kg or placebo. Clinical response rates at 4 weeks were 81%, 50%, 64%, and 17%, respectively. Overall, 12-week response rates were 41% for infliximab and 12% for placebo [637]. Retreatment of the responders with four infusions of infliximab 10 mg/kg at 8-week intervals maintained patient response and remission rates, while those patients randomized to placebo tended towards relapse [638].

The ACCENT-I trial treated 573 patients with moderate to severe Crohn's disease with a single infusion of infliximab at 5 mg/kg; responders at week 2 where then randomized to receive subsequent infusions of placebo, of infliximab 5 mg/kg at weeks 2 and 6 and then every 8 weeks, or of infliximab 5 mg/kg at weeks 2 and 6 and then infliximab at 10 mg/kg every 8 weeks [625]. Fifty-eight percent of patients responded at week 2; week 30 remission rates were 21% (placebo), 39% (5 mg/kg), and 50% (5 mg then 10 mg/kg group) (Fig. 57.40). There were also benefits seen in median time to loss of response, and in sparing of corticosteroids. Patients who lost response to their therapy were allowed a one-time change to increase the dose of the subsequent infusions by 5 mg/kg.

There were separate trials for patients with fistulous Crohn's disease. In the first trial, 94 patients with draining

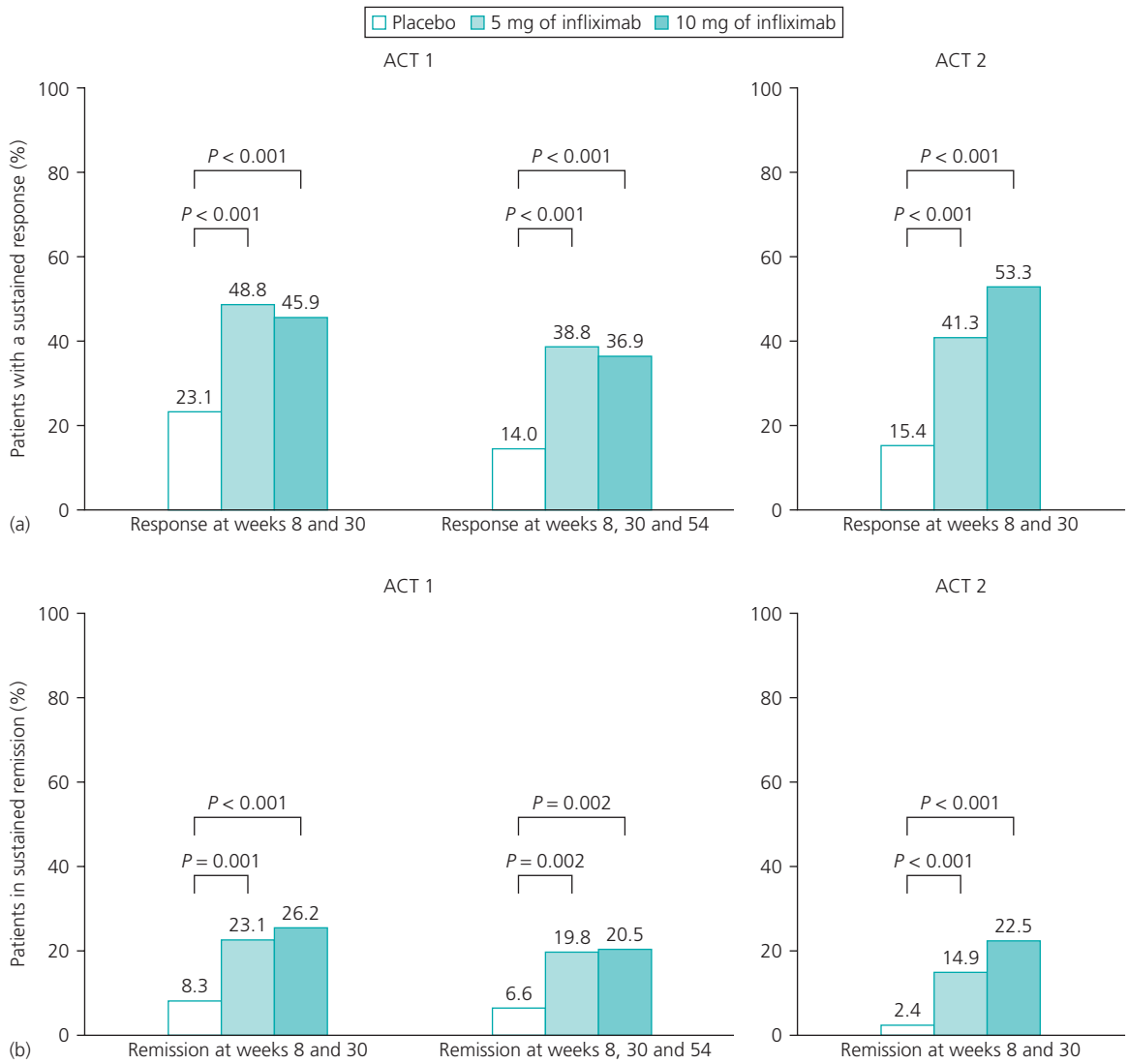


Figure 57.39 **(a)** Percentage of patients with moderate to severe ulcerative colitis who achieved a sustained response or **(b)** sustained remission after receiving randomized therapy with placebo, infliximab 5 mg/kg, or infliximab 10 mg/kg at weeks 0, 2, 6, and then every 8 weeks. From Rutgeerts et al. [635], with permission from the Massachusetts Medical Society.

abdominal or perianal fistulae were randomized to receive three infusions of placebo, infliximab 5 mg/kg, or infliximab 10 mg/kg at weeks 0, 2, and 6 [639]. The success rates of decreasing the number of draining fistulae by 50% or more were 26%, 68%, and 56%, respectively. Complete closure of all fistulae was seen in 13%, 55%, and 38%, respectively. The median time of fistula closure was 3 months. Subsequently, the ACCENT-II trial infused 306 adult patients with Crohn's disease and draining abdominal or perianal fistulae with infliximab 5 mg/kg at weeks 0, 2, and 6 [640] (Fig. 57.41). Responding patients were then randomized to receive either infliximab 5 mg/kg or placebo every 8 weeks. Time to loss of response was longer with infliximab (greater than 40 weeks) than placebo (14 weeks). At week 54, fistula closure rates were 36% and 19%, respectively.

Adalimumab

Adalimumab is a monoclonal human IgG_1 antibody that targets TNF-α. It binds both free and membrane-bound TNF, and causes apoptosis of TNF-producing cells [641]. Adalimumab is delivered subcutaneously in either a prefilled syringe or a self-injectable pen. Either device contains 40 mg adalimumab, which is delivered as a single dose. Adalimumab has many of the same safety concerns as infliximab, with the exception of infusion-related complications. Injection site reactions were reported in 20% of patients in the clinical trials (14% in placebo patients); other common adverse events in the clinical trials (similar in occurrence between patients receiving adalimumab and those who received placebo) were upper respiratory infection, injection site pain, headache, and sinusitis [642]. Whether antidrug antibody

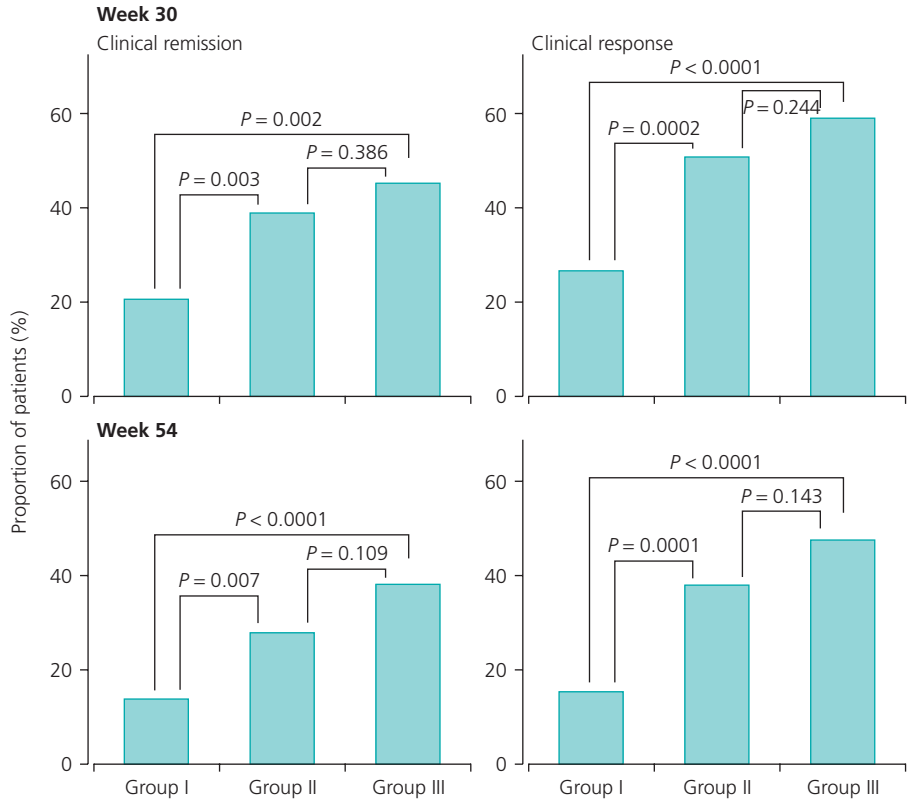


Figure 57.40 Clinical remission and response rates in patients with moderate to severe Crohn's disease who responded to an initial open-label infusion of infliximab 5 mg/kg at week 0, and then were randomized to receive week 2, week 6, and then 8-weekly doses of placebo (Group 1), infliximab 5 mg/kg (Group 2), or infliximab 5 mg/kg for week 2 and week 6 followed by infliximab 10 mg/kg every 8 weeks (Group 3). From Hanauer et al. [625].

levels are clinically relevant has yet to be fully determined. To date, there have been no published clinical trials of adalimumab in patients with ulcerative colitis; one clinical report showed short-term efficacy in four of 10 patients who had lost response or become intolerant to infliximab [643].

Adalimumab in Crohn's disease

There have been multiple clinical trials of adalimumab in Crohn's disease. While patients treated with adalimumab for other disorders are not started on an initial higher load, adalimumab must be given with a loading dose of 160 mg (four injections) at week 0, followed by 80 mg (two injections) at week 2, and then 40 mg every other week starting at week 4. Patients who lose response are switched to weekly dosing (Fig. 57.42).

The induction regimen of 160 mg at week 0 and then 80 mg at week 2 was established in two clinical trials. In the CLASSIC I trial, 299 patients with moderate to severely active lumenal Crohn's disease were randomized to receive two injections (week 0 and 2) of either placebo, adalimumab 40 mg/20 mg, adalimumab 80 mg/40 mg, or adalimumab 160 mg/80 mg [644]. The 4-week remission rates were statistically higher for only the highest dose (36%) than for placebo (12%). The same 160 mg/80 mg loading dose was used in the GAIN trial, with a 4-week remission rate of 21% compared with 7% for placebo [645].

The maintenance regimen of 40 mg given every 1–2 weeks was established in two other clinical trials. In the CLASSIC II trial, 55 patients (20% of induction population) who had achieved remission after receiving two open-label injections of 40 mg adalimumab at weeks 0 and 2 were randomized at week 4 to receive subsequent injections of either adalimumab 40 mg every other week, adalimumab 40 mg weekly, or placebo for the remainder of the year [646]. Remission maintenance rates were 68%, 83%, and 39%, respectively. In the CHARM study, 778 patients who had achieved a clinical response after receiving open-label adalimumab 80 mg at week 0 and 40 mg at week 2 were randomized to the same three maintenance groups as in CLASSIC II [647]. Their 1-year remission maintenance rates were 36%, 41%, and 12%, respectively (Fig. 57.43).

Adalimumab is effective in some patients with Crohn's disease who previously responded to infliximab, but then either lost response, or had an allergic reaction to either the infliximab or the infusion and could not continue therapy. In the GAIN trial (Gauging Adalimumab Efficacy in Infliximab Nonresponders), 325 such patients with moderate to severely active Crohn's disease were randomized to receive two injections of either placebo or adalimumab 160 mg at week 0, followed by 80 mg at week 2. Week 4 remission rates were 7% and 21% for placebo and treatment, respectively. Remission rates were similar in patients who had lost response to infliximab (8% vs. 20%) and those who had a previous intolerance to infliximab (5% vs. 22%).

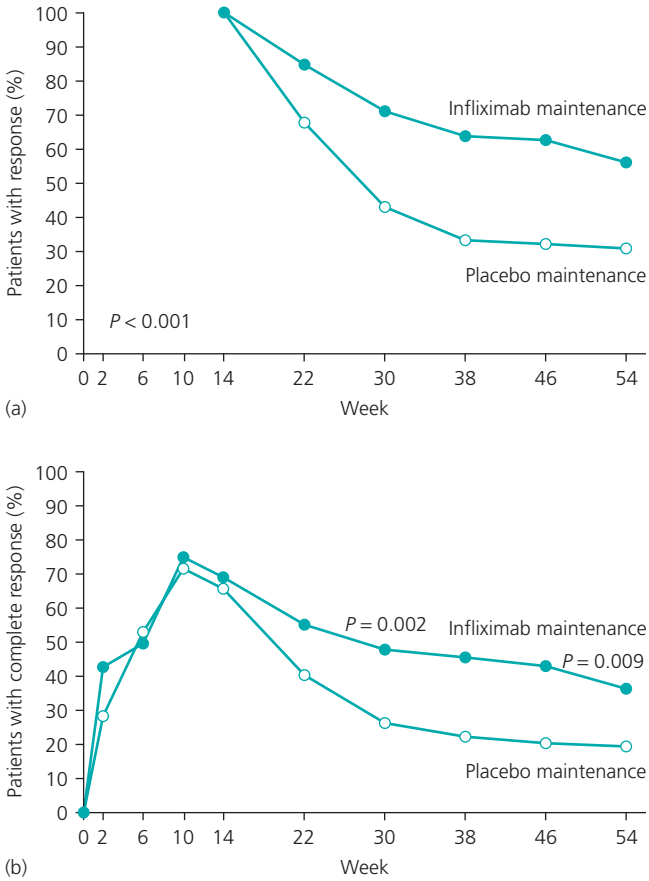


Figure 57.41 **(a)** Time to loss of response in patients with fistulous Crohn's disease who responded to an initial open-label induction therapy with infliximab 5 mg/kg at weeks 0, 2, and 6 and were then randomized at week 14 to receive maintenance infliximab 5 mg/kg or placebo every 8 weeks. **(b)** Percentage of patients with a complete response (absence of draining fistulas). From Sands et al. [640], with permission from the Massachusetts Medical Society.

Other investigational agents

The "biological revolution" that has descended on the field of medicine has resulted in an ever-expanding list of agents that target, duplicate, or otherwise influence the immune cascade. This list is constantly changing; many products that seem promising never see the light of day, and are quickly replaced by others. There are two agents that have completed multiple large-scale Phase III clinical trials in IBD that will be briefly mentioned here.

Certolizumab

Certolizumab pegol is a pegylated humanized Fab' fragment that binds TNF-α [648]. Early studies have been promising in Crohn's disease [649]. A recent randomized, double-blind, placebo-controlled trial of 662 adults with moderate to severe Crohn's disease stratified patients to receive either 400 mg of certolizumab pegol or placebo subcutaneously at weeks 0, 2, and 4 and then every 4 weeks [650]. There were superior response rates with certolizumab than placebo, but remission rates were similar between the two groups. In a separate maintenance trial, 668 adults with moderate to severe Crohn's disease all received open-label certolizumab pegol 400 mg at weeks 0, 2, and 4 [651]. The responders (64%) were then randomized to receive either certolizumab pegol 400 mg or placebo as a monthly injection for 6 months. Of the initial responders, 26-week response rates were 63% for treatment compared with 36% for placebo, while remission rates were 48% and 29%, respectively. Subsequent studies have been completed and are awaiting publication.

Natalizumab

Natalizumab is a humanized antibody directed against the integrin α_4, an important mediator of cellular trafficking in various tissues. There has been early data suggesting natalizumab may be an effective therapy for ulcerative colitis [652], as well as multiple large Phase III studies in Crohn's disease, with convincing evidence of a role for natalizumab in the induction and maintenance of remission in Crohn's disease [653–655]. Natalizumab is also effective in patients who are intolerant to infliximab, or who have lost response to that agent.

An unfortunate safety issue arose after natalizumab was released for the treatment of multiple sclerosis, with three confirmed cases of progressive multifocal leukoencephalopathy as the result of the JC human polyomavirus out of the roughly 3000 patients in the original clinical trials [656–658]. Extensive safety evaluations have failed to uncover other cases, and the drug is back on the market for multiple sclerosis under a strict registry program. It is anticipated that its initial use in Crohn's disease will be limited to patients who have failed anti-TNF therapies, and concomitant immunosuppressants will be disallowed [659].

Medical management of ulcerative colitis

Medical therapeutic decisions in treating patients with ulcerative colitis should be based on a few important issues. The first thing to determine is the extent of the colitis. It is helpful to determine from your colonoscopy whether the patient has inflammation limited to the rectum only (proctitis), to the rectum and sigmoid (proctosigmoiditis), extending up to 60 cm from the anal verge (left-sided colitis), or colitis extending beyond that boundary (extensive or pancolitis). It is also helpful to know if the normal-appearing colonic mucosa that is proximal to the area of colitis is indeed histologically normal, or if there is evidence of mild or quiescent colitis. One might choose to use more than just a topical therapy in patients whose colitis reaches beyond the proximal extent of a rectally applied therapy, to help maintain quiescence in that area (although this is a theoretical and not yet proven strategy).

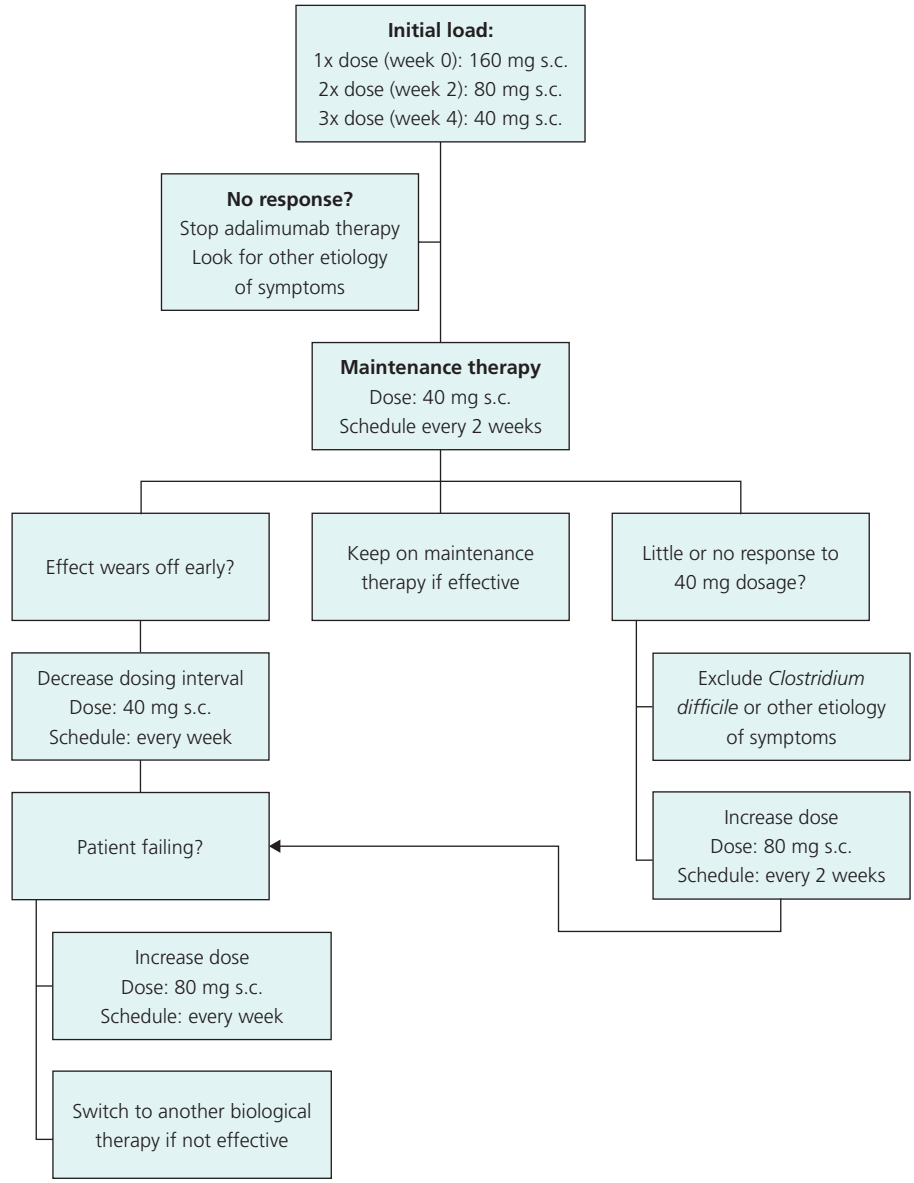


Figure 57.42 Dosing regimen for adalimumab in patients with Crohn's disease.

The second point to consider is the severity of the colitis, both clinically and endoscopically. The mesalamine-based therapies have their greatest success in patients with mildly active colitis, as well as some patients with moderately active disease (Table 57.1; Fig. 57.3). Corticosteroids are often held back in patients with mild disease, but play an important role in the sicker patient. Although the purine analogue immunomodulators do not work quickly, it may be reasonable to start these in patients who are likely to be beyond the first-line agents, to avoid longer than necessary illness or corticosteroid exposure. Earlier use of infliximab may also be appropriate, particularly if the patient is ill and is either refractory to, or has a contraindication to, corticosteroid therapy. In the case of patients with fulminant colitis, immediate surgical consultation is required and cyclosporine should be considered.

A patient's prior clinical course, previous response (good or bad) to therapies, and concomitant medical conditions are also factors that should be weighed when designing the safest, most efficacious therapeutic approach. Unfortunately, some of the medical and surgical decisions may be swayed by a patient's willingness or ability to commit to a medical or surgical treatment plan, and by the financial constraints that may exist in certain circumstances.

Whether one should treat the patient's symptoms alone, or include the endoscopic findings in determining long-term therapeutic decisions has been a hotly debated topic. The relevance of mucosal healing is implied, but not proven. Recent studies linking mucosal healing to better patient outcomes, fewer hospitalizations and surgeries, and possibly lower colorectal cancer rates may change these attitudes and guide therapy [220,660–663].

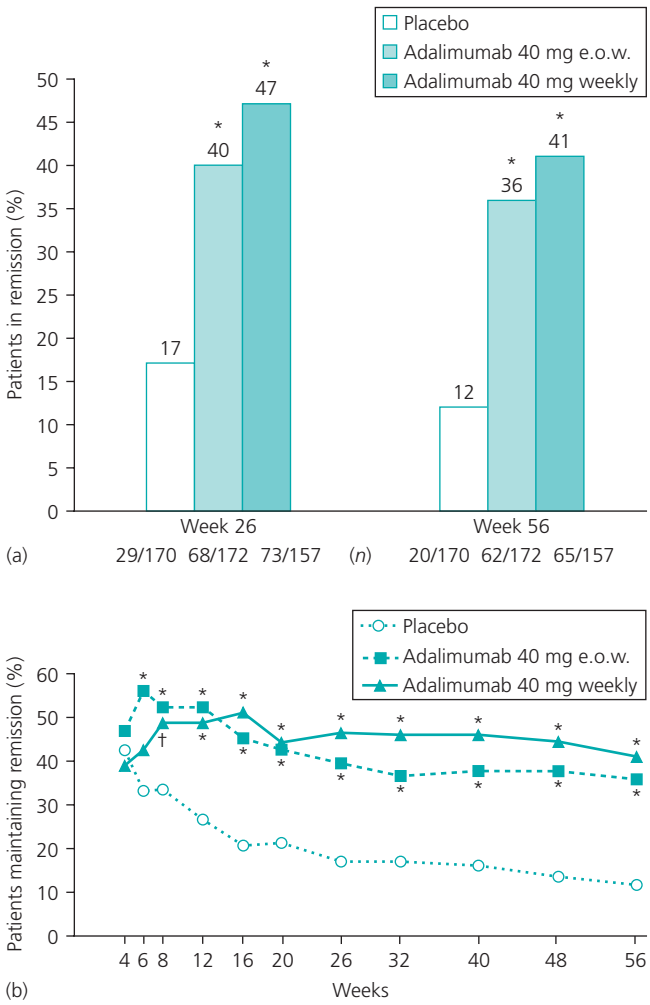


Figure 57.43 **(a)** Clinical remission rates in patients with moderate to severe Crohn's disease who responded to an initial open-label induction therapy with adalimumab 80 mg (week 0) and 40 mg (week 2) who were then randomized to either placebo, adalimumab 40 mg every other week (eow) or weekly. *$P < 0.001$ for pairwise comparison between each adalimumab treatment group and placebo e.o.w., every other week. **(b)** Clinical remission rates over time in each group. *$P = 0.001$ vs. placebo. †$P = 0.005$ vs. placebo. From Colombel et al. [647].

Patients with colitis who are not responding to therapy, or who develop new diarrhea (sometimes with blood) that differs from their usual disease presentation should be ruled out for *C. difficile* infection. Coinfection with this organism is endemic in colitis patients [664], and repeated checks may be necessary with any relapse in the treatment course. Other microorganisms should also be excluded, as well as other mitigating factors that might cause a colitis flare, or similar symptoms, such as NSAIDs, new diets, sorbitol, or new prescription medications or "alternative" therapies. Sicker patients should have full blood panels (complete blood and platelet counts, differential, C-reactive protein, comprehensive metabolic panel, coagulation factors, thyroid-stimulating hormone, cholesterol) and multiview abdominal radiographs to rule out megacolon or obstruction. It may be helpful to do a limited endoscopic examination into the rectum and sigmoid to document the severity of disease and exclude cytomegalovirus and other infections. Care should be taken to ensure a gentle examination, with limited insufflations, decompression of the bowel on withdrawal, and minimal (if any) narcotics.

The inflammation in patients with ulcerative colitis is limited to the mucosal, and sometimes submucosal, layers. While patients with long-standing colitis may develop a tubular, featureless colon, or one filled with inflammatory pseudopolyps, these patients should not be forming true stenotic strictures of the colon. The finding of strictures signals either that the patient likely has Crohn's colitis, or that they have developed a possible colonic neoplasm. In the absence of evidence of Crohn's disease, a strictured colon in ulcerative colitis should be considered a likely colon cancer until proven otherwise by surgical removal (colectomy, proctocolectomy) [665,666].

The use of narcotic pain relievers is controversial in patients with ulcerative colitis. Studies in toxic megacolon have implicated narcotic use as an inciting event [667], as well as other anticholinergics [668] and colonoscopy [108]. Therapy should be directed to decreasing inflammation, not covering up warnings of a more severe colitic process. Patients with active colitis who are having severe pain should undergo a CT scan to exclude fulminant colitis, abscess, vascular event, or Crohn's disease, as well as evaluation by a surgeon. Patients with pain severe enough to require narcotics require hospitalization and intensification of therapy, or surgery. There is no role for narcotics in the outpatient management of ulcerative colitis.

Patients with mild to moderate disease may benefit from the use of antispasmotics, antidiarrheals, and simethicone. While a low-residue diet is advised for patients with diarrhea and colitis, some patients find that fiber or cholestyramine provides more form to their stool. Patients with distal disease with constipation may benefit from fiber in their diets, non-fermentable fiber-based therapy, or polyethyleneglycol-based laxatives. A multivitamin with iron (if needed and tolerated), calcium supplementation, and avoidance of NSAIDs is recommended for all patients. A helpful website for all patients with IBD is the Crohn's and Colitis Foundation of America (www.ccfa.org), which provides useful information and links covering a wide range of topics of interest to health-care professionals, patients, their families, and caregivers.

Ulcerative proctitis

Patients with ulcerative proctitis have inflammation limited to the rectum; as a result, it makes good clinical sense to utilize topical therapies in the induction and maintenance of response and remission in these individuals, even if they are

also being treated with other agents. Mesalamine suppositories have been the mainstay of treatment in these patients [669], with efficacy and safety data superior to corticosteroid preparations [392]. Suppository dosing is typically nightly (twice or three times a day in more refractory patients) (Table 57.6). Once in remission, many patients can taper to alternate-day or twice-a-week dosing, although many stop therapy altogether. Subsequent relapse in symptoms often identifies those patients whom might benefit from maintenance dosing. Mesalamine foams, gels, and liquid enemas are also efficacious in patients with ulcerative proctitis. The choice of an agent may be based on patient tolerance or preference.

Topical corticosteroids are also effective in ulcerative proctitis, and sometimes less irritating than the mesalamine products. Systemic absorption (with subsequent corticosteroid side effects) is most pronounced for the liquid enema formulations. Adequate studies detailing the absorption of the various corticosteroid gels, foams, or suppositories have not been performed. Nevertheless, one might favor one of the newer corticosteroid formulations with fewer systemic side effects, such as budesonide, beclomethasone, or prednisone-metasulfobenzoate, although they are not available in many countries.

Although many patients, and perhaps physicians, prefer to use oral mesalamine agents as a first-line attempt for treating active ulcerative proctitis, they are not as effective as the topical agents [392]. Many clinical trials of ulcerative colitis therapies exclude patients who have only proctitis, and adequate comparative studies of oral agents in treating proctitis have not been published. The role of oral mesalamine agents in maintaining a remission that was achieved with a topical agent also has not been thoroughly investigated. Although on achieving remission with topical therapy (either alone or as a combination with oral therapy) many physicians switch patients to an oral mesalamine agent alone in an attempt to maintain remission, placebo-controlled trials have not been published that prove this to be an effective therapeutic approach.

Patients who have more severe or refractory proctitis are treated with a therapeutic approach that is similar to the one outlined below for patients with more extensive colitis, although one should keep patients on topical therapies (if effective) while pursuing other agents, as they help to decrease the tenesmus, urgency, mucus discharge, and blood loss that plague many of these patients.

Ulcerative proctosigmoiditis

There are not many trials that separate out patients with proctosigmoiditis for treatment, or separate out the results for this patient subpopulation. Many of the same principles that were discussed for proctitis can be applied to patients with proctosigmoiditis, with the topical therapies being the mainstay of the medical approach, either alone or in combination with oral agents. Since suppositories are not believed to adequately reach above the rectum, mesalamine enemas, foams, and gels are the preferred first-line agents. The enemas are typically given at bedtime (Table 57.6); patients should be encouraged to empty their bladder before instilling the liquid formulation, and lie with their left-side down for at least 20–30 min to let the enema have a chance to coat the sigmoid and left colon. The mesalamine enemas are retention enemas; that is, the intent is to retain them as long as possible (numerous hours, if not all night) before expelling. Some patients find it helpful to take an antidiarrheal (such as loperamide) or even a sleeping aid to assist in this endeavor. Foams and gels may be used multiple times a day; it is difficult for many patients to hold the liquid enema unless they are lying down. Combination therapy with a nighttime enema, and morning mesalamine suppository is helpful in some patients.

The corticosteroid-based enemas, foams, and gels are also useful in these patients, but because of systemic absorption, they are typically used in patients who are allergic to or intolerant of the mesalamine-based products. These steroid formulations may be easier for the sick colitis patient to hold; one approach is to induce a remission with the steroid-based products, and then transition to the mesalamine compounds. Further treatment approaches to patients with proctosigmoiditis are similar to those discussed below in the section on left-sided ulcerative colitis.

Left-sided ulcerative colitis

Seventy percent of patients with ulcerative colitis have their inflammation limited to the distal colon or rectum; as a result, most of the patients a physician will treat will benefit from the therapies discussed here and in the previous two sections [101,102,107]. Once again, topical mesalamine agents are the most efficacious, quickest, and, unfortunately, most often over-looked therapies for these patients (Table 57.6). Mesalamine enemas are superior to oral mesalamine, as well as to corticosteroid enemas [392]. Combination therapy with oral mesalamine is superior to oral mesalamine alone in the induction and maintenance of treatment response or remission [397,403]. Often the patients are weaned off the topical therapy once they are are well, but some patients will require maintenance enema therapy. Dosing mesalamine enemas every second or third night is also an effective maintenance strategy.

Corticosteroid enemas are also highly effective in patients with left-sided ulcerative colitis, but because of systemic absorption, estimated at 40%–80%, they are suitable only for short-term use [451]. They are particularly useful in patients with active colitis who cannot hold the mesalamine enemas, although transition back to the mesalamine compound should be attempted once the patient is well. Corticosteroid enemas should be tapered off when used; abrupt cessation is not recommended in patients who have been on long-standing therapy because Addisonian crisis may result.

Oral mesalamine agents are typically used in patients with left-sided ulcerative colitis, either as sole-therapy or in combination with a topical agent. Sulfasalazine and all of the mesalamine-based therapies (mesalamine, balsalazide, olsalazine) are effective in the treatment of left-sided ulcerative colitis. Further differentiation between the agents, and treatment of refractory colitis, will be discussed in the next section on the treatment of pancolitis.

Pancolitis

Roughly 30% of patients with ulcerative colitis have inflammation extending proximal to the splenic flexure [101,102,107]. Although all of them might not have truly "pan-" ulcerative colitis, patients with extensive colitis and pancolitis have the same treatment protocols. These are the patients most likely to undergo surgery, and who are at a higher risk for colorectal cancer; new data suggest that this risk may be decreased with medical therapies that decrease inflammation, particularly mesalamine.

Mild disease

First-line therapy for patients with pancolitis are oral mesalamine agents; combined therapy with topical agents is often helpful. Although inexpensive, many practitioners have shied away from using sulfasalazine because of high rates of patient allergy or intolerance. All of the mesalamine-based therapies are effective in the treatment of acute colitis, and are used for maintenance therapy. There are high-relapse rates in patients that stop therapy so long-term maintenance therapy should be the rule when treating these patients.

The mesalamine agents are typically categorized by their release mechanism (Table 57.6, Fig. 57.32), as detailed earlier in this chapter. Head-to-head blinded randomized studies with equimolar doses of azo-bond release balsalazide and pH-release mesalamine have shown superior remission rates, earlier time to complete absence of symptoms, as well as other outcomes favoring balsalazide in some, but not all, studies [670–672]. Whether this benefit extends to other azo-bond release agents has not been firmly established [381,673]. Patients with an inadequate response to a "standard" daily dose of mesalamine-equivalents (2–3 g) are often subjected to a dose escalation to 4.0–4.8 g. Adequate clinical trials subjecting such patients to a higher dose have not been published; in fact, there are few data supporting a role of oral mesalamine in daily doses higher than 2.4 g; some trials have shown that sicker patients (i.e., those with moderately severe colitis) may have better improvement rates (but not remission rates) and endoscopic outcomes at a dose of 4.8 g [393,394,674]. Some patients who are not responding (or possibly worsening) with one mesalamine-based therapy may have success with another agent. Others are either intolerant, or their disease is too advanced, for these agents to work.

Moderate disease

Some patients will have success with high-dose oral mesalamine, or combination therapy with oral and topical mesalamine agents. Corticosteroid enemas are also helpful in patients who cannot hold, or who are intolerant to, mesalamine enemas. Those for whom this therapy fails are acutely treated with corticosteroids. Prednisone 40 mg daily (or equivalent) is a typical starting dose; some patients who do not respond may require 60 mg, although the side effects are much higher at this level. The goal of corticosteroid therapy is to induce remission, and then slowly taper therapy while maintaining patients on their oral or topical mesalamine, or the purine analogues (6-mercaptopurine or azathioprine). Patients who repeatedly relapse on corticosteroid taper will need initiation or optimization of their purine analogue therapy, or infliximab should be started (Figs 57.37, 57.38). Patients who are not adequately responding to high-dose oral corticosteroids should be considered for treatment with infliximab or hospitalization for intravenous corticosteroids (see below). Oral tacrolimus or cyclosporine is another option, but can only be used with strict monitoring, and with the intent of maintaining the patients on safer therapies.

Severe disease

Patients with severe ulcerative colitis need immediate evaluation and treatment to avoid progression to a fulminant or toxic state. Full assessment of laboratory parameters, serial abdominal films to exlude megacolon, and CT scan if pain is a dominant factor should be performed. Hospitalization, aggressive repletion of fluid and electrolyte losses, and stool cultures to exclude pathogens and *C. difficile* should be performed (and repeated if symptoms or laboratory parameters worsen). While many of these patients may have normal white blood cell counts, worsening left-shift towards band forms is an indication for reevaluation (examination, abdominal radiograph, stool examination for *C. difficile*). Intravenous corticosteroids in divided doses or continuous infusion (methylprednisolone 40–60 mg; hydrocortisone 200–300 mg) should be started after an initial bolus dose. Oral mesalamine agents are typically held; topical therapy with hydrocortisone enemas is encouraged if helpful. Surgical consultation is recommended, as well as nutritional assessment.

Patients who have initial signs of colonic dilation, but are otherwise not toxic (i.e., febrile, hypotensive) need to have serial abdominal films, correction of fluid and electrolytes, and may benefit from "rolling" from decubitus positions to the prone position, to decompress the colon [675]. Surgical consultation is necessary; any worsening should lead to urgent colectomy. Cyclosporine has been used successfully in some cases.

Although initially patients are kept nil-by-mouth, those who are improving and able to eat without a worsening of symptoms are encouraged to take a low-residue diet, often

after an initial trial of clear liquids. Parenteral nutrition is recommended for patients who are nutritionally depleted, but line-related thromboses are common and precautions must be taken. All hospitalized IBD patients should receive higher-dose prophylaxis with heparin 5000 IU delivered three-times daily, or enoxaprin 40 mg subcutaneously daily.

Patients who do show any response to corticosteroids within 3 days have a poorer prognosis for avoiding surgery [676]. Reassessment of clinical status with radiographs, stool studies excluding *C. difficile*, and gentle unprepped sigmoidoscope without insufflation into the rectum and rectosigmoid to exclude infections such as cytomegalovirus (biopsies taken of ulcers) is recommended.

Reconsultation with surgery, and consideration of the addition of infliximab (Fig. 57.38) or intravenous cyclosporine (Fig. 57.37) should be given. Before infliximab, exposure to tubercuolosis should be excluded by careful patient history, purified protein derivative placement, QuantiFERON test (in patients who have already had bacillus Calmette–Guérin), or chest radiograph. Before cyclosporine use, serum cholesterol must be checked, with a dosage decrease to 1 mg/kg for levels around 100 mg/dL, and avoidance of the drug for levels below 100 mg/dL. Long-term results with cyclosporine have been disappointing in patients already established on adequate doses of the purine analogues [677]; the drug should not be used in patients with renal disease, neurological disorders, hypertension, or who are noncompliant with therapy [587]. Patients with severe colitis should have near complete, if not complete, resolution of signs and symptoms of severe disease before discontinuation of intravenous therapy. Careful monitoring as outpatients is mandatory because relapse is not uncommon. Surgical intervention should not be withheld in the hope of an unlikely "miraculous" cure.

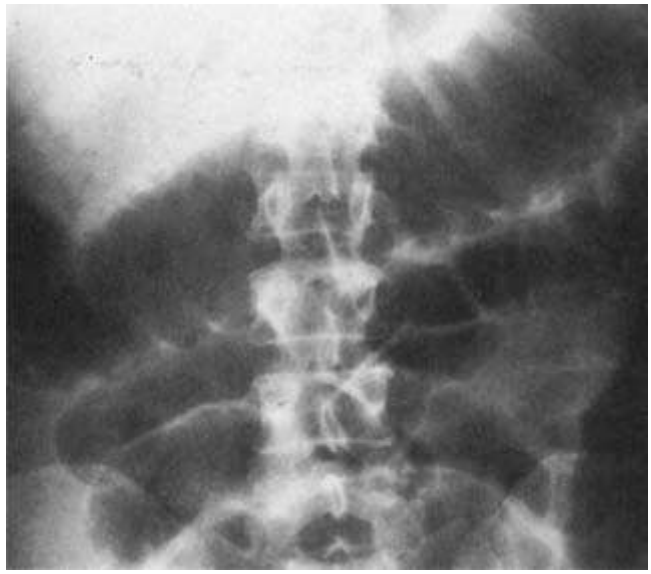
Fulminant colitis and toxic megacolon

Dilation of the colon on radiograph (Fig. 57.44) with signs of systemic compromise (fever, tachycardia, hypotension) is a surgical emergency; patients should be rescuscitated and taken for an emergency total abdominal colectomy with ileostomy and Hartman's pouch. Often intravenous broad-spectrum antibiotics are given, as well as blood products and other supportive therapies. Subsequent surgeries for completion proctectomy and creation of an ileoanal pouch anastomosis (if desired) can be performed months later after full rehabilitation of the patients. Narcotics, anticholinergics, and colonoscopies are contraindicated. Rolling the patient from side-to-side and onto their stomach has been described as a technique to decompress the dilated colon [675].

Maintenance of remission

The agent used to induce remission is often the one to initially try to maintain remission, with the exception of corticosteroid-containing therapies. In those instances, the appropriate maintenance therapy is started, while the patient is weaned off corticosteroids. The rate of weaning is dependent on the duration and dose of therapy, experience with previous attempts at weaning, extraintestinal manifestations (especially joint pains) patient's age, and speed at which the maintenance therapy is expected to work. For example, if the intent is to maintain a corticosteroid-induced remission with a slow-acting agent such as azathioprine or 6-mercaptopurine, a more gradual taper will be used compared with the patient who is started on infliximab and may experience benefit within days. Some extraintestinal conditions such as erythema nodosum may suddenly appear with a quick corticosteroid taper. Caution should also be exercised in patients with asthma, who were deriving pulmonary benefit from

(a)



(b)

Figure 57.44 Toxic megacolon. **(a)** Plain film radiograph shows colonic dilation. **(b)** Contrast radiography reveals large ulcerations. Courtesy of Dr Dennis Balfe, St Louis, MO.

the steroid therapies. Corticosteroid enemas should also be tapered, rather than stopped abruptly, if the patient has been using them for more than a few weeks.

Maintenance with oral mesalamine-based therapies should initially be at the same dose used to induce remission. Some patients may be able to subsequently decrease the dose to roughly one-half to two-thirds of the induction dose; relapse may occur, and patients who initially failed those lower doses, or who were difficult to induce into remission should be advised to stay at or near their induction doses. Rectum-based mesalamine therapies should be continued if needed, although many patients fare well with dosing every second or third night. Abrupt cessation is not recommended because of "rebound" relapse, which has been noted by physicians experienced in the use of these products. Combination therapy with oral and topical mesalamine is superior to maintenance therapy with oral agents alone, at least in patients with distal colitis [403].

The recommended maintenance dosing with the purine analogues is the same as the final induction dose, adjusted for any safety-related matters. Routine dose reduction is not recommended in patients with normal blood counts and liver chemistries, in the absence of other side effects. Prescribers need to be aware that purine-analogue-related cytopenias and blood dyscrasias have been reported years after initiation of therapy; as a result, patients need blood tests and office examinations every few months, even on stable-dose therapy.

Infliximab should be given as a maintenance infusion every 8 weeks (earlier if necessary) at the same dosage necessary for induction of remission [625] (Fig. 57.38). Some patients require dose escalation to 10 mg/kg; if this was performed because of a loss of response and the formation of antibodies then it is recommended that subsequent maintenance doses stay at this higher level. There are anecdotal experiences of successful dose reduction back to the 5-mg level in some patients who had experienced a flare-up of symptoms during standard therapy and regained remission in the absence of neutralizing antibodies.

Complications of ulcerative colitis

General

Patients who are acutely ill with colitis must be adequately monitored for dehydration, electrolyte abnormalities, anemia, and the related physiological processes that can result from these conditions, such as renal insufficiency, kidney stones, cardiac arythmias, ischemic events, and others. Rapid identification and replacement of fluids, electrolytes, and blood products is essential. Chronically ill patients may suffer from similar consequences, as well as nutritional deficiencies, acquired coagulopathy defects, bone mineralization deficiencies, and hair and skin changes. Additional complications are discussed in the section on Crohn's disease.

Toxic megacolon

One of the most feared complications of severe colitis is the development of a toxic megacolon. In this situation, the colonic lumen dilates to a diameter of 6 cm or more, there is evidence of toxicity (dehydration, hypotension, mental status change, or electrolyte abnormalities) and the patient has at least three of the following:

- fever above 38.6°C
- tachycardia above 120 beats per minute
- leukocytosis above 10 500 cells/mm^3, or
- anemia [108,678,679].

Physical examination often reveals a distended, tympanitic, diffusely tender abdomen, with minimal or absent bowel sounds. Radiographic evidence of the dilation may be pancolonic or limited to segments (Fig. 57.44). The transverse colon is traditionally involved, perhaps because of the distribution of the bowel gas when in the supine position. In fact, rolling the patient into the right-lateral decubitus and subsequently prone position has been shown to temporarily "deflate" the distended colon [675].

Conditions that may initiate or exacerbate a toxic megacolon include the use of narcotic or anticholinergic drugs, electrolyte abnormalities, discontinuation of corticosteroids, barium enema, or colonoscopy [108]. These patients needs aggressive repletion of fluids and electrolytes, blood products, discontinuation of any inciting medications, and complete bowel rest. Gastrointestinal pathogens, especially *C. difficile*, should be excluded. Decompression is recommended by both nasogastric and rectal tube, as well as by repositioning the patient as detailed above. Intravenous corticosteroids and broad-spectrum antibiotics should be initiated and there should be a surgical consultation for urgent colectomy. Serial abdominal radiographs (every 6–12 h) should be performed; signs of progressive dilation or toxicity require surgery. Patients should not undergo colonoscopic examination, or have barium (or air) instilled rectally. Patients who are not initially taken to surgery should undergo colectomy if they do not improve within 24–48 h [680]. Mortality rates as a result of perforation fall from 44% to only 2% with appropriately timed surgery [681].

Patients with colonic dilation but without signs of true toxicity can be managed as above; worsening of their intestinal or physiological parameters are indications for surgery. Cyclosporin has been used successfully in select cases.

Perforation

Colonic perforation in severe colitis can occur in the absence of a megacolon [682]. Free perforations are often of abrupt onset, with evidence of peritoneal signs, fever, and subsequent decompensation. Perforations may be seen in patients with new onset colitis that follows a fulminant course, as well as in patients with chronic colitis, where suspicion for a colonic neoplasm is heightened [683].

Subacute perforations, often sealed off at the serosal surface by a forming phlegmon or abscess, are more commonly seen in Crohn's disease or in associated with diverticular disease, but may also occur in patients with ulcerative colitis. Prompt rescucitation of the patient, plain film radiographs (and often CT scan), and surgical evaluation are required. Broad-spectrum intravenous antibiotics are advised, as are repletion of other fluids, electrolytes, and blood products. In the instance of a sealed off abscess cavity, radiographic drainage of the abscess cavity may allow for improved surgical outcomes. Colonoscopy is contraindicated in patients with a presumed perforation or diverticulitis; single-column gastrograffin enema evaluation may be helpful in selected cases, after consultation with the radiologist. The vast majority of these patients will require surgery so additional testing should only be conducted if it were to reasonably change treatment course of action.

Stress-dose corticosteroids should be given to patients who are already on these agents, or at risk for adrenal insufficiency because of recent corticosteroid use. Patients who are not on corticosteroids should not start them, unless there is a need for decreasing inflammation in a patient with a sealed off perforation who is not a surgical candidate at the time.

Colonic stricture

Often considered a finding more consistent with Crohn's colitis, colonic narrowing or strictures have been reported in up to 12% of surgical specimens [684]. Since ulcerative colitis by definition is a disease only affecting the mucosal or submucosal linings, true strictures should raise suspicion for colonic neoplasm, Crohn's disease, or, in selected patients, diverticular or ischemic disease. Patients often have longstanding colitis that has been characterized by either continuous or repeated episodes of active colonic inflammation. Symptoms suggestive of a possible stricture may include focal pain, unpredictable diarrhea, and, in the case of rectal strictures, fecal incontinence. Ironically, some patients may enjoy an initial period of improvement in previously difficult to control diarrhea.

Medical management of Crohn's disease

Most of the recommendations in the preceding section on Medical management of ulcerative colitis are carried over to patients with Crohn's disease, especially Crohn's colitis. It is recommended that the reader refer first to the previous section, and then refer back here for special circumstances that are more unique for patients with Crohn's disease.

The location of disease in the patient with Crohn's disease, and the severity of disease in each area, helps to guide therapeutic management. Distribution of Crohn's disease is typically broken down into lumenal and fistulous disease. Lumenal disease categories are upper gastrointestinal (esophageal, stomach, duodenum), small bowel, ileocolonic, colonic, and perianal. The perianal designation implies penetrating (fistulous, abscess) disease, but fistulae can occur in other locations as well. There are rare patients with oral Crohn's disease, as well as very rare "metastatic" Crohn's disease, with an autoimmune inflammatory disease lesion appearing in a location discontiguous with the bowel (usually the skin). Many of these less common entities have not been adequately studied; the literature, if any, consists of single or a few case reports that detail the particular clinical course and response to therapy.

Lumenal disease is often classified as "inflammatory", "stricturing", or "penetrating" [172,173]. Epidemiological studies suggest that there may be a gradual transition from an inflammatory to stricturing to a penetrating state, with surgery required eventually. Crohn's disease recurs postoperatively in most settings so the patients may start the cycle again. Defining the phenotype of disease may guide therapeutic choices; those patients with more advanced disease are the ones in whom systemically acting immunomodulators may be started earlier, even as first-line treatment. Patients with penetrating disease may benefit from cotherapy with an antibiotic, at least for antiinfective if not antiinflammatory benefits. Surgical therapy is turned to sooner in patients who already have obstructive strictures or penetrating disease that is unlikely to respond to medical therapies.

Patients with stricturing Crohn's disease often need to avoid dietary intake of materials that are poorly digested by humans, which might create an obstruction at the stricture point, depending on the lumenal diameter. Foods that should be discouraged include raw fruits, vegetables, salads, and whole grains. It is wise to entirely avoid nuts, seeds, corn, popcorn, and similar products. Attempts at endoscopic dilation or stenting of strictures pose a substantial risk for bowel perforation [685–687]. Endoscopically directed injection of corticosteroids into the strictures rarely provides long-term relief [688,689]; infliximab injections have also been reported to have short-term success [690].

Mechanisms of diarrhea in patients with Crohn's disease can be multifold and complex. Small bowel disease (and resection) can lead to reduced absorptive surface, as well as secretion or weeping of fluid. Roughly 2.54 m (100 inches) of healthy small bowel is needed to avoid "short-bowel syndrome" and the potential need for parenteral nutrition, hydration, or electrolyte repletion. A diseased or removed terminal ileum results in disruption of the enterohepatic recirculation of bile salts and can result in a coloretic diarrhea, fat malabsorption, and vitamin deficiencies. The diarrhea that results is often alleviated by bile-acid-binding resins, such as cholestyramine, colestipol, or colesevelam. The latter two are available in pill form, given as one or more

pills before meals, and are better tolerated by patients. These agents should not be used in patients with a short bowel because they can exacerbate fat-soluble vitamin deficiency. Patients may develop oxalate kidney stones, and need oral calcium therapy to bind the oxalate in the lumen. Another mechanism of diarrhea is "dumping" from the loss of the "ileal brake" following ileocolecomy. Patients with Crohn's colitis can suffer from diarrhea as the result of a diseased large bowel, and the condition can be exacerbated by perianal disease that limits fecal continence. Antidiarrheals (loperamide, diphenoxylate, and even tincture of opium) are often required in patients with Crohn's disease who have diarrhea. Infection with *C. difficile* should be excluded in patients with diarrhea that is unexplained, relapsing, or refractory to treatment.

Mild to moderate Crohn's disease

First-line therapies in patients with mild Crohn's disease consist of the mesalamine agents and antibiotics. There is a running debate on the overall efficacy of mesalamine in Crohn's disease [413,691,692]; however, removed from the limitation of clinical trials, there are some patients who benefit from therapy. The guidelines are the same as laid out in the previous section on Medical management of ulcerative colitis, although many practitioners use a higher dosage in the patients with Crohn's disease (3–4 g daily) (Table 57.6, Fig. 57.32). For patients with small bowel Crohn's disease, moisture-released mesalamine (Pentasa) is the preferred choice [693,694], because it is not dependent on a pH-based system, and the azo-bond agents are thought to be released almost exclusively in the colon. Maintenance therapy with the same agent (and dose) that induced remission is advised. Patients with distal disease may benefit from mesalamine enemas and suppositories.

The data supporting the use of antibiotics in lumenal Crohn's disease is sparse; clinical trials that have found an effect have been more convincing in patients who have solely colitis, or ileocolitis. Recommended doses are ciprofloxacin 1000 mg or metronidazole 10 mg/kg to 20 mg/kg daily. Long-term use of metronidazole is discouraged because of the risk of neuropathy, sometimes permanent, which mandates cessation of the drug therapy. High-dose rifaximin (1600 mg daily) is effective in some patients as well, without evident toxicity [445]. Maintenance therapy has not been studied; nevertheless, in the absence of other effective therapies, it is reasonable to continue patients on the antibiotics long-term (other than metronidazole); an appreciable rate of emergence of resistant organisms has not been widely reported.

Patients with Crohn's disease affecting the small bowel or right colon in whom the first-line therapies are ineffective, or who have more moderately active disease, can be successfully treated with budesonide-CIR 9 mg once daily for 8–12 weeks [486,695], with a taper by 3 mg every few weeks at the latter part of the treatment course. Maintenance therapy with 6 mg (but not 3 mg) has been proven for 3–6 months, but not longer [696,697]. For those patients who fail on 9 mg budesonide, or who require more than 9 mg equivalent doses of budesonide, treatment with conventional steroids can be used. Although budesonide has far fewer corticosteroid-related side effects than traditional steroids, long-term use can lead to suppression of the pituitary–adrenal axis, and steroid-related effects can be seen. As a result, maintenance therapy should be attempted with one of the other first-line therapies discussed above, or with long-term immunomodulators.

Azathioprine and 6-mercaptopurine can be used to induce remission in Crohn's disease, although the clinical studies typically used a corticosteroid induction regimen, and then a maintenance dosing strategy with the purine analogues [540,557]. Another option in Crohn's disease is injectable methotrexate for the induction or maintenance of remission. Methotrexate is administered at 25 mg intramuscularly or subcutaneously once weekly, with oral folic acid (1 mg daily) supplementation [571]. Dose reduction is necessary if side effects occur. Maintenance doing in the clinical trials was 15 mg [580] but other clinical results maintained the patients at their highest tolerated dose [570]. Oral dosing has not been shown to be effective in Crohn's disease.

Moderate to severe disease

Patients with moderately severe, or severe, Crohn's disease can be given budesonide 9 mg (if they have small bowel or right colon disease), prednisone (or its equivalent) 40–60 mg orally four times a day, infliximab (Fig. 57.38) or adalimumab (Fig. 57.42). The last two agents mentioned are typically used only in patients who are relapsing while still on corticosteroids, who have had repeated corticosteroid courses or relapses despite the appropriate use of azathioprine, 6-mercaptopurirne, or methotrexate, or who have a contraindication to receiving corticosteroids. Patients not responding to oral prednisone are hospitalized for intravenous corticosteroids (40–60 mg intravenous methylprednisolone or equivalent) or initiated on anti-TNF therapy. Short-term use of the calcineurin inhibitors tacrolimus or cyclosporine may be appropriate in individual cases, but not as long-term maintenance. The other drugs are used as maintenance therapies, with the exception of corticosteroids.

Hospitalized patients with severe Crohn's disease

Many of the same warnings and recommendations for this patient population appear in the previous sections on severe and fulminant ulcerative colitis, as detailed above. Intravenous cyclosporine is rarely given for Crohn's disease; use of infliximab or adalimumab has supplanted that drug's role. Complete bowel rest with TPN supplementation is necessary in patients with refractory pain, diarrhea, or abscesses; extended therapy may be necessary in some patients. Ruling out infection with *C. difficile*, cytomegalovirus, or other

opportunistic organisms is necessary. Many patients are hospitalized for obstructive Crohn's disease; failure to respond to conservative medical management results in surgery. CT scans are helpful in excluding inflammatory phlegmonous masses, abscesses, and fistulae; abscess cavities may be drained under radiographic guidance, but many of the patients will subsequently require surgical resection. Patients who have suspected abscesses or other infectious processes should not be treated with anti-TNF therapies, or other potent immumosuppressants. Small bowel series, CT enterography (Fig. 57.19), or MRI enterography (Fig. 57.20) may be helpful in locating the level of a bowel obstruction, and determining whether it is related to disease, infiltrative processes, or simply adhesions from a previous surgery. Capsule endoscopy is discouraged in patients with Crohn's disease who have not had a small bowel radiographic test to exclude strictures; however, there are patients whose strictures are missed on the examinations, so precapsule testing with the "patency capsule" is advised.

Fistulous Crohn's disease

Crohn's fistulae affect an estimated 30% of patients; most commonly they are in or near the perianal area (including the perineum, vagina, and scrotum), although fistulae to the abdominal wall (often following an abdominal surgery, or tracking along a previous scar or drain site), and internal fistulae to surrounding organs can be seen. The fistulae that affect the perianal area often originate from the dentate line, and repeated abscesses and surgeries can lead to compromise of the anal sphincters and fecal incontinence.

Management of perianal disease (fistulae, abscesses, inflammatory skin tags) often involves a combined medical and surgical approach. Suspicion of an abscess should lead to prompt initiation of antibiotics (typically ciprofloxacin 1 g and metronidazole 10–15 mg/kg, or equivalent) surgical evaluation, and in some cases, pelvic CT scan (for abscesses) or MRI (for fistulae). It is important to instruct the radiology staff to extend the scanning to the mid-thighs, to ensure that the perineum is fully visualized. Soaking the area multiple times a day (sitz baths) in very warm water may promote drainage. The finding of an abscess cavity on digital rectal examination, fever, or other signs of systemic illness should prompt a surgical examination and drainage. Marsupialization of the abscess cavity may occur; in other instances, the drainage tracts from the abscess cavity are intentionally kept open with a seton or purse-string to allow drainage while the tract closes. Unfortunately, many fistulous tracts close only intermittently or recur, so effective medical therapy is also necessary.

If antibiotic therapies alone are not sufficient, then initiation of azathioprine, 6-mercaptopurine, or perhaps methotrexate should follow, although the literature supporting these therapies is sparse. For patients already on these agents, or in whom it is not desirable to wait for the slow (if any) response, infliximab has emerged as a highly effective therapy (Fig. 57.41). After the initial loading dose of 5 mg/kg at weeks 0, 2, and 6, maintenance therapy with infusions every 8 weeks is recommended. Failure to continue infliximab often leads to reopening of the fistulae. Continuing antibiotic therapy until the fistula is closed and not tender is advisable to potentially decrease the risk of abscess. Adalimumab has also been shown to be effective in some patients with Crohn's fistulae, although formalized fistula trials have not yet been published. Tacrolimus and cyclosporine are also effective in closing fistulae, but neither is safe, nor effective, as maintenance therapy.

Postoperative recurrence prophylaxis

Crohn's disease recurs after most bowel resections with reanastomosis at rates approaching 50%–75% in 10 years [247,698–700]. Numerous therapies have been studied in an attempt to decrease the incidence of disease relapse. The data with mesalamine have been mixed [701–704]; a metaanalysis suggested that 2.4–3 g mesalamine decreases the risk of relapse by 13% [413] – the evidence was strongest in patients with ileal disease. Controlled trials with either metronidazole (20 mg/kg/day) [437] or ornidazole (1 g/day) [438] showed efficacy in decreasing endoscopic and clinical relapses, although the benefit faded on discontinuation of the antibiotic therapy. Neither budesonide (6 mg/day) [704] nor Lactobacillis GG proved effective in preventing relapse. Low-dose 6-mercaptopurine (50 mg daily) was superior to placebo in preventing clinical and endoscopic relapse [562], and azathioprine (2.5 mg/kg) almost halved the surgical relapse rate compared with 3 g mesalamine [705]. Infliximab is anticipated to effectively decrease relapse rates, although data on the drug's efficacy has yet to be published.

At the current time, it is reasonable to assess the risk relapse, the impact of relapse, and the options feasible for each individual when deciding on a postoperative strategy. Patients deemed at lower risk for early relapse (nonsmokers and those with nonpenetrating disease, first surgery, or long-duration of disease before surgery) might be offered no therapy, mesalamine (2.4 g or higher daily), or antibiotics. One treatment approach is to perform a colonoscopy at 6–12 months following surgery [245]; signs of more aggressive disease, such as more than five aphthous lesions or an already ulcerated anastomosis (Rutgeerts' Grade II) [244], would suggest that a more aggressive approach was necessary.

Those at higher risk, who have already had previous surgeries, have failed the first-line therapies, have lost substantial amounts of bowel, and who might not be able to weather another recurrence, let alone a resection, should be treated with azathioprine or 6-mercaptopurine. If they were already on these agents immediately after their previous surgery, at an adequate dose, and nevertheless developed new disease, then escalation to an anti-TNF therapy is reasonable. Patients who "failed" on anti-TNF (but had an initial

prolonged response) should be checked for neutralizing antibodies; if present, an alternative anti-TNF agent should be used.

Complications of Crohn's disease

General

Patients with Crohn's disease who suffer from diarrhea or vomiting require repletion of fluids and electrolytes. Those with anemia, either from blood loss or from iron-deficiency, may require transfusion of blood products. Patients with small bowel Crohn's disease with malabsorption often need correction of deficiencies of iron, folate, vitamin B-12, zinc, the fat-soluble vitamins (A, D, E, and K), as well as other trace minerals and vitamins (by supplementation with a multivitamin formulation).

Patients with small bowel malabsorption of fat are at higher risk of developing kidney stones comprised partially of oxalate; dehydration can also lead to other kidney stone compositions. Gallstones are more common in patients with Crohn's disease, and certain medications may cause pancreatitis or hepatitis.

There are numerous systemic events that are seen more commonly in patients with acutely or chronically active Crohn's disease or ulcerative colitis. They may develop a hypercoagulable state, which may lead to venous thromboses and related complications. Pericardial and pleural effusions have been linked to the disease state or medications, and patients with severe metabolic disorders may develop cardiac arrhythmias or tetany. Bone diseases such as osteoporosis, osteomalacia, osteonecrosis, and avascular necrosis must be monitored. Psychiatric disorders are also prevalent in this population, although often as a result of, rather than as a cause of, the physical disease states. A myriad of extraintestinal complications are covered elsewhere in this chapter.

Abscesses and fistulae

Crohn's disease that penetrates through the bowel lining can result in the formation of a sealed off cavity containing succus, stool, or pus (an abscess) or extension of the inflammatory process to the surrounding viscera or through the skin's surface (a fistula). Roughly one-third of patients with Crohn's disease are estimated to suffer from these penetrative complications. Abscesses and fistulae that result from anorectal Crohn's disease are diagnoses, are treated differently, and will be discussed separately.

Abscesses

Abscesses have been reported in up to 20% of patients with Crohn's disease [706]. Intraabdominal abscesses are located adjacent to the inflamed bowel, most typically the terminal ileum. Suspicion for an abscess to the ileopsoas area can be detected on physical examination by flexion of the right hip (psoas sign), or localized severe abdominal pain [707]. While patients with abscesses classically have pain, fever, chills, or rigors, these findings may be absent or masked in patients on immunosuppressants or narcotics. While one would expect that a perforation through the intestinal wall would result in obvious peritonitis, the chronic nature of Crohn's may result in an inflammatory phlegmon or a sealed off abscess, with minimal symptoms other than an abdominal mass.

Laboratory findings in patients with abscesses may include leukocytosis, acidosis, mild elevations of aminotransferases, and often greatly elevated erythrocyte sedimentation rates or C-reactive protein levels. Diagnosis is confirmed by an abdominal–pelvic CT scan with oral and intravenous contrast; in some cases, abdominal or transvaginal ultrasound, MRI, or radionucleotide scanning may suggest an abscess [708–710].

Drainage of an abscess cavity under CT or ultrasound guidance is often the first step in reducing the immediate pain and infection; surgical resection of the defective bowel area is ultimately required (and advised) in most cases to avoid recurrence and further complications. The infectious agents are often polymicrobial; broad-spectrum intravenous antibiotics (including coverage for anaerobes) should be started immediately and continued until definitive surgery is performed. Blood cultures should be checked, and antibiotic treatment should be tailored to the results of the culture from the blood or abscess cavity. Corticosteroid use is initially discouraged in the setting of acute abscess and infection until after drainage and once sepsis has been excluded, although stress doses may be needed for those on chronic corticosteroid therapy. Patients with an inflammatory phelgmon, evidence of surrounding bowel inflammation, and with a drained abscess often benefit from the addition of corticosteroids to their regimen. Allowing time for the abscess cavity to be drained, and for the intravenous antibiotics (and in some cases corticosteroids) to decrease the associated infection and inflammation may help the surgeon limit the resection to only the diseased portion of bowel, and enable a primary anastomosis with a primary closure of the abdominal incision, without the need for a temporary diverting ostomy. Keeping the patient absolutely nil by mouth with TPN support may be beneficial. Anti-TNF therapies or other potent immunosuppressants should not be used (or continued) until the abscess area is resected.

Fistulae

Fistulae have been reported in 20%–40% of patients with Crohn's disease [706]. Often associated with a strictured area of diseased bowel, fistulae can originate in any portion of the bowel affected by Crohn's disease. Their destination can be a nearby organ, the skin, or a convoluted course to seemingly nowhere, ending in an inflammatory collection. Depending on their location, they may be asymptomatic (as is the case with many enteroenteric fistulae), or result in physical findings specific for the location. For example, patients with gastrocolic or enterosigmoid fistulae often have profound diarrhea.

Enterovesicular or colovesicular fistulae may be characterized by pneumaturia or fecaluria. Those existing cutaneously present initially with painful swelling at the skin site, followed by breakthrough and subsequent drainage.

Diagnostic studies for internal fistulae are also specific for their location. Enteroenteric fistulae may be shown on small bowel series or enterography, colic fistulae with barium enema, and vesicular fistulae by cystography. Fistula entrance or exit sites may be seen endoscopically or with cystoscopy. The course of cutaneous fistulae may be determined by cannulation of the fistula exit point at the skin and instillation of contrast material to locate the source in the bowel. Patients with multiple fistulae often have just a single originating defect in the affected bowel that drains to a sealed of inflammatory "pool" from which multiple fistulae may branch out.

Therapies that are effective in the treatment of fistulous Crohn's disease are discussed in the preceding section on medical therapy. Enterocutaneous fistulae are often responsive to these therapies. There are no controlled trials of medical therapies for the treatment of internal fistulae. Asymptomatic enteroenteric fistulae do not necessarily require a change in medical management, although their associated bowel inflammation may require more effective therapies. Fistulae that threaten other body organs or systems, such as enterovesicular fistulae that lead to repeated infections or pyelonephritis, should be managed surgically. In many cases, the fistulae arise just proximal to or within an area of severely diseased or strictured bowel that will ultimately require resection. Complete bowel rest with TPN repletion may be required in patients with complicated fistulous disease that is not amenable to medical or surgical therapy.

Postoperative fistulae are often a confusing issue in patients with Crohn's disease. Often assumed to be the result of active Crohn's disease, fistulae that arise from a recent surgical anastomosis are more likely to be the result of a leak at the sight, rather than de novo Crohn's disease. Anastomotic leaks may be more common in patients on corticosteroids, in those with poor nutritional status, or in patients whose connected bowel loops contain active disease. Surgical exploration and revision of the defective anastomosis is often required. Crohn's disease typically recurs in the area of previous resection so patients who had an uncomplicated postoperative course yet subsequently present with anastomotic fistulae after 6 or more months should be evaluated for recurrent disease as the etiology.

Anorectal abscesses and fistulae

The diagnosis and treatment of Crohn's disease of the anorectal area often involves a multidisciplinary approach between the gastroenterologist, surgeon, and radiologist. The fistulae typically originate in the anorectal glands at the dentate line, and can drain into a blind cavity (forming an abscess), or the nearby skin, genitals, or urinary system (Fig. 57.45). Multiple fistula tracts are not uncommon; they often originate from one or two initial sources. Dedicated CT, MRI, endoscopic ultrasound, and dynamic proctography may each be helpful investigations. The course of cutaneous fistulae may be shown by injection of contrast material directly into the exiting orifice. Fistulae to the vagina may be heralded by vaginal pain, bacterial vaginosis, passage of air, mucus, or feces from the vaginal vestibule. Sometimes difficult to find, and initially misdiagnosed as Bartholin gland cysts, these fistulae are often inferoposterior, and the fistula tract can be discerned by digital examination of the anterior anorectal canal and vaginal vestibule.

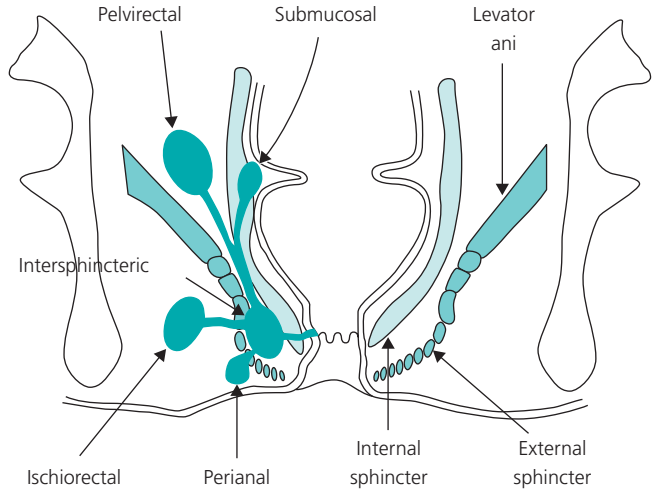


Figure 57.45 In Crohn's disease perirectal abscesses develop from fistulous tracts that begin in rectal ulcers of anorectal glands. These fistulae extend into the intersphincteric space and then follow tissue planes to a variety of pelvic locations where abscesses may form.

Surgical examination under anesthesia with incision and drainage of abscess cavities, fistulectomy, or seton placement is recommended in patients with acute abscesses and refractory fistulae. Antibiotics (typically ciprofloxacin and metronidazole) should be promptly initiated and continued until the abscess is draining and the area is free of inflammation, and while the fistula is draining. Chronic antibiotic administration (usually with ciprofloxacin or a derivative) is common in patients with persistent or recurrent disease, and should also be used in patients with fistulae who are undergoing treatment with anti-TNF therapy. Corticosteroids have not been shown to be efficacious in closing the fistulae per se, but in the case of patients with active Crohn's disease in the area of fistula origination, may be helpful in decreasing the inflammation in the bowel that is feeding the process. Topical use of mesalamine or corticosteroids is helpful in some patients.

Medical therapies directed at fistula closure are discussed in the preceding section. Cessation of therapy often leads to fistula recurrence; maintenance therapy is required. Patients with complicated or recurrent fistulae may benefit from the initiation of infliximab therapy (Fig. 57.41), following initial

surgical therapy. The surgical management of complicated fistulae, especially rectovaginal fistulae, is best covered in a dedicated text. Although fecal diversion with colostomy or ileostomy often leads to improvement of the downstream bowel and perianal area, relapse on reversal of the ostomy is common; whether use of the anti-TNF drugs can change the course of disease remains to be seen.

Obstruction

The nature of Crohn's disease with stricture leads to obstruction of the bowel in many cases of long-standing disease [191,711]. As is the case with other types of bowel obstructions, signs and symptoms depend on the location of the blockage. Pyloric or duodenal obstructions are characterized by early satiety, gastric distention, belching, and vomiting during or immediately after eating. Patients with jejunal obstruction often have pain and vomiting shortly after meals; ileal obstructions will present with pain, while vomiting may occur long after meals are completed and may be feculant. Patients with small bowel obstructions will often first notice a cessation of flatus and then bowel movements; an impending obstruction may be heralded by increasing pain, early satiety, and a change in bowel patterns. Colonic obstructions are more insidious and may present with increased diarrhea or constipation with pain. Anorectal obstructions are seen in patients with distal disease, are often associated with local fistulous disease, and may be characterized by fecal incontinence and worsening of fistula symptoms.

Patients who present with acute obstructions need intravenous hydration, and often nasogastric suctioning, while being kept nil by mouth. Intravenous corticosteroids are used to quickly control the inflammatory components of the disease process, although they may ultimately be of little or no use in patients with fibrostenotic disease. Anti-TNF agents are discouraged in the setting of obstructive strictures because there have been reports of worsening symptoms; retrospective analyses have not shown such an association, although some patients may have been excluded from receiving these therapies and not included in the analyses [712]. Adhesive disease as the result of previous abdominal or pelvic surgeries (or inflammation) can be a particularly troublesome cause of repeated bowel obstructions, and will often require surgical intervention.

Abdominal radiographs (multiple view), CT scan, or MRI may be helpful in assessing the location and etiology of the obstruction. Small bowel series, enteroclysis, or enterography may be performed to further localize the obstructive point, after the bowel is decompressed and vomiting has ceased. Patients with high-grade obstructions that do not quickly decompress require surgical intervention; otherwise, the conservative management described above is usually successful. Patients who have repeated obstructions, strictures that are not responsive to medical therapy, or who do not completely resolve their obstructions will likely require surgery.

The role of endoscopy in patients with Crohn's disease with stenoses is primarily to exclude obvious neoplasms, although many of the neoplasms are deeper than can be determined by mucosal biopsies, and adequate prospective trials have not been conducted (nor are they likely to be). Patients in whom the strictures cannot be adequately traversed and biopsied require surgical resection. The inability to adequately survey the colon proximal to an obstruction to rule out dysplasia and neoplasm is another controversial issue that may prompt surgical intervention. Attempts at endoscopic dilation, in some instances with direct injection of corticosteroids into the strictures, have been reported [685–689]. High perforation rates, and early restenoses argue against this approach in the majority of patients.

Dietary indiscretion is a frequent cause of obstructions in patients with small bowel stricturing disease. Strict adherence to a low-residue diet is advised in all such patients, avoiding poorly digested foods such as seeds, nuts, corn, popcorn, raw or slightly cooked fruits and vegetables, and whole grain products. Patients with gastric or duodenal Crohn's disease should be encouraged to also avoid food that interferes with gastric emptying, or is poorly absorbed.

Surgical management (see also Chapter 58)

Ulcerative colitis

About 20%–25% of patients with extensive ulcerative colitis eventually undergo colectomy, usually because their disease has not responded adequately to medical therapy [713,714]. For some, the indications for colectomy are urgent and compelling, but more commonly the decision for or against colectomy is made at a time when the disease is active but stable. The decision between surgery and continued medical therapy is often not clear, and in many cases arguments can be made for either course. It is important in these cases to attempt to balance the risks and benefits of the two approaches. Excessive enthusiasm for and avoidance of operative therapy are both inconsistent with the patient's best interests. At the time of first diagnosis, the patient may reject even the consideration of eventual colectomy. However, a single prolonged episode of severe colitis or multiple recurrent episodes of more moderate severity increase the patient's desire to eliminate this debilitating disease with its restrictions on activity, and the prospect of colectomy becomes less intolerable.

In ulcerative colitis, colectomy is a curative procedure, in contrast to Crohn's disease, in which there is a significant likelihood of recurrence sometime after colectomy [715]. The assurance of a permanent cure increases the appeal of colectomy in ulcerative colitis. The development of the ileoanal anastomosis, eliminating the need for a permanent ostomy, has made the idea of colectomy more tolerable for many patients. The patient's age, social circumstances, and

duration of disease all influence the decision for or against colectomy. The morbidity of severe ulcerative colitis in childhood and the morbidity of corticosteroids in childhood combine to lead to early consideration of colectomy as the definitive therapy. For young adults with moderately severe disease, colectomy may be more acceptable for those in stable marriages than for those who are single. The risk of developing malignancy enters into the equation when considering colectomy in those with long-standing ulcerative colitis; if the other indications are equivocal, the risk of malignancy may tilt the balance in favor of colectomy.

Emergency colectomy may be required in toxic megacolon or in a severe fulminating attack without toxic megacolon [716]. Although colectomy for acute attacks of ulcerative colitis is more commonly associated with toxic megacolon, it is clear that acute fulminating ulcerative colitis in the absence of colonic dilation can also proceed to perforation. The goal is to recognize the necessity for surgical intervention before perforation occurs. If perforation does occur, it is an absolute indication for surgery. Perforation in this circumstance is a medical disaster, with a 40% mortality rate [717]. For fulminant colitis, in the absence of toxic megacolon, the general indications for operation are similar. Before the patient undergoes colectomy for intractable ulcerative colitis, it is important to rule out complicating conditions that can be treated. The possibilities of infection with *Giardia* spp., amoebae, *C. difficile*, and cytomegalovirus should be considered and, if indicated, testing for these organisms should be performed. The presence of worsening colitis and especially worsening systemic signs (e.g., fever, prostration, tachycardia, hypotension) in the face of aggressive medical therapy is a sign that surgical intervention may be indicated. Severe hemorrhage is another indication for emergency colectomy.

The failure of medical therapy to control the activity of ulcerative colitis, resulting in an unacceptable lifestyle, is the most common indication for colectomy in ulcerative colitis. There are several varieties of intractability. In one variety, the disease enters a chronic continuous phase in which the patient no longer achieves remission even with maximal medical therapy, and chronic diarrhea is severe enough that the quality of life is severely compromised. Frequent bowel movements prevent the patient from moving far from a bathroom and do not allow normal travel or employment. In some cases, the patient's or the physician's aversion to colectomy results in months or years of functional disability far worse than would have existed if a colectomy had been performed. Another type of intractability occurs when adequate control of the patient's symptoms requires high doses of corticosteroids over a long period. It is appropriate to attempt to reduce steroid requirements by administering azathioprine or 6-mercaptopurine. The side effects of corticosteroids are dose and time dependent and vary considerably from patient to patient. Unacceptable side effects (e.g., psychosis, accelerated hypertension) occur in some patients even with modest doses given over a short period. In these cases, the decision for surgery is clear.

A more difficult and more common problem is the patient who requires moderate to high doses of corticosteroids to control disease activity but who has had no specific problem with the corticosteroid treatment. Although this is a decision in which factors specific to the individual patient play a large role, some general guidelines may be useful. In patients who have taken large doses of prednisone (> 15 mg/day) for more than 6 months, even if no specific corticosteroid side effects are present, the morbidity of continued steroid therapy must be weighed against the morbidity of surgery. Ophthalmological examination for cataract and bone densitometry may help to identify patients with clinically silent corticosteroid side effects. The identification of asymptomatic but clinically important corticosteroid side effects may make the decision for colectomy easier. Growth failure in children is another form of intractability. Adequate nutritional support helps in most young patients, but some require colectomy. Surgical intervention should be strongly considered if growth retardation persists despite maximal nutritional and medical therapy [718]. In such cases, it is important to initiate therapy before puberty and the closing of epiphyses.

Surgery plays a relatively small role in the management of extraintestinal manifestation in ulcerative colitis. Uveitis, pyoderma gangrenosum, and arthritis usually resolve with colectomy. Some other extraintestinal manifestations, including ankylosing spondylitis and sclerosing cholangitis, do not. The extraintestinal manifestations that respond to colectomy usually respond to medical management as well. Extraintestinal manifestations are an infrequent indication for colectomy.

In the past the standard operation for ulcerative colitis was a proctocolectomy and Brooke ileostomy. This procedure is definitive and curative and additional surgery is seldom required. The patients' functional status can be predicted with confidence. Most patients make a remarkably good adjustment to the ileostomy and lead full, useful lives. The difficulty with this procedure is the perminate ileostomy with the requirement for an appliance. Although the proctocolectomy and ileostomy is still a reasonable choice for some patients, the more commonly performed procedure is the proctocolectomy with an ileoanal anastomosis, a procedure that does not require a permanent appliance [719–721]. In this procedure, the colon is removed completely, but the mucosa and submucosa of the rectum are dissected from the muscularis; the mucosa and submucosa are removed and the muscularis, including the internal and external sphincters, is left in place. A pouch is constructed from the terminal 30 cm of ileum. The distal end of the pouch is pulled through the anal canal and the ileal mucosa is sewed to the dentate line. The advantage of the ileoanal anastomosis is that the patient has no ostomy and no appliance; however, a proximal temporary diverting loop ileostomy commonly is used to protect the ileoanal anastomosis until it heals. For several months

after the creation of an ileoanal anastomosis, the patient has numerous bowel movements [722]. The number gradually declines so that, at 12 months, most patients are having five or six bowel movements per day. Twelve months after operation, 75% of patients have complete daytime continence, 23% have daytime seepage (minor staining of underclothes), and 2% have daytime incontinence [723]. Twelve months after the operation, 48% of patients are completely continent at night, 47% have nighttime seepage, and 5% have nighttime incontinence. Results are better for patients younger than 50 years than for older patients. An in-depth discussion of these surgical alternatives is presented in Chapter 58.

Crohn's disease

Within 10 years of diagnosis, about 60% of patients with Crohn's disease undergo surgery for their disease [724]. The surgical approach to Crohn's disease differs markedly from the approach to ulcerative colitis. Surgical resection is not curative in Crohn's disease as colectomy is in ulcerative colitis. As a consequence, there has been a more conservative approach to the amount of tissue removed, in the knowledge that recurrences are likely and that additional surgical resections may be necessary. A controlled trial comparing limited resection (narrow margins) and extended resection (wide margins) found no difference in the incidence of recurrence [725]. Intractability, or failure of medical management, is a common cause for resection in Crohn's disease, as it is in ulcerative colitis, but complications (e.g., obstruction, fistula, abscess) are often indications for resection in Crohn's disease. The line between failure of medical management and a more defined complication, such as obstruction, is often indistinct. If a patient with a radiographically identifiable stricture has symptoms consistent with intermittent partial obstruction (i.e., diarrhea and pain brought on by eating) and does not respond to medical management, it is difficult to say whether the indication for surgery is obstruction or failure of medical management. Indications for surgery have been used for defining two subsets of patients with Crohn's disease, those with perforating indications (perforation or fistula) and those with nonperforating indications (obstruction or stricture) [726]. If a patient's initial surgery was for a perforating indication, it is likely that subsequent surgeries also will be for perforating indications; similar results are seen with surgery for nonperforating indications.

Defining the point at which medical management has failed in Crohn's disease is somewhat more involved than in ulcerative colitis, because in Crohn's disease symptoms are not always caused by disease activity. Diarrhea and weight loss may be the result of fatty-acid malabsorption, bile-salt-induced colonic secretion, or small bowel bacterial overgrowth. Pain may not be the result of obstruction. Optimal medical management requires recognition and treatment of these disorders. If these problems are not identified, patients may be subjected to unnecessary surgery.

As in ulcerative colitis, surgery for failure of medical management in Crohn's disease falls into two groups. In the first group, surgery is required because the progression of the disease has led the patient to a condition of unacceptable disability and invalidism despite optimal medical management. Whereas in ulcerative colitis the major source of disability is diarrhea, in Crohn's disease there is more commonly a combination of diarrhea, pain, weight loss, and malnutrition. In evaluating the possibility of surgical intervention in ulcerative colitis, the patient's current morbidity from disease activity is balanced against the relatively predictable morbidity of a colectomy. In Crohn's disease, however, the current morbidity must be balanced against the less well-defined morbidity of more limited surgery and also against the projected long-term morbidity as the disease progresses. Another distinction between surgical therapy in ulcerative colitis and Crohn's disease is that surgery for ulcerative colitis is almost always performed to remove actively inflamed tissue, but surgery for Crohn's disease may be required to remove actively inflamed tissue or to remove the sequelae of inflammation. For example, strictures requiring surgical intervention may occur in Crohn's disease at the site of a surgical anastomosis where there is considerable scarring with only a modest amount of active inflammation. This distinction is important because there is no point in treating a stricture with a therapeutic trial of steroids if scarring and fibrosis have caused the stricture. In addition, if the problem is known to be largely scar tissue and fibrosis, a stricturoplasty can be performed or the extent of the surgical resection can be limited to the area of narrowing without worry about maintaining adequate uninflamed margins.

The second group of patients who undergo surgery for failure of medical management in Crohn's disease includes those suffering side effects from drugs, usually corticosteroids. The guidelines for surgical intervention in patients with Crohn's disease with complications of medical therapy are exactly the same as for patients with ulcerative colitis. A problem frequently faced by physicians treating Crohn's disease is the patient whose disease is controlled with corticosteroids but flares if the dose of corticosteroids is reduced below a certain level. The level varies from patient to patient, but one study put the average at 0.15 mg prednisone per kilogram per day [727]. Several options are available in this situation. One approach is to maintain the patient at a steroid dose above the critical level and to monitor the patient carefully for manifestations of steroid toxicity. This follow-up includes bone densitometry and ophthalmological examinations. A second approach, not exclusive of the first, is the use of immunomodulators to achieve a steroid-sparing effect. This approach trades potential complications of corticosteroids for those of immunomodulators. A third approach is to resect the involved segment, knowing that the chance of eventual recurrence is high. Each of these approaches

has advantages and disadvantages, all of which should be explained to the patient before a decision is made.

The incidence of recurrence after ileal resection for ileitis or ileocolic resection for ileocolic disease is about 50% after 10 years and 75% after 15 years, as assessed by rates of reoperation [728]. If the presence of recurrence is assessed endoscopically, it is clear that recurrence after resection occurs more rapidly than was previously appreciated [729]. Routine colonoscopic surveillance 1 year after ileocolic resections revealed a 72% incidence of recurrence, as manifested by the presence of aphthous ulcers on the ileal side of the ileocolic anastomosis [728]. Many patients who undergo ileocoloic resection for Crohn's disease will later require another resection. Predicting which patients will require a second surgery and how long after the first resection that will be is an area of active investigation [730]. Reliable predictors of time to reoperation have not been established. Whether the time to initial operation or reoperation can be prolonged with maintenance medical therapy is under investigation. Studies of azathroprine and 6-mercaptopurine in preventing relapse after operation and in prolonging time to initial operation have given inconsistent results [730–733].

A surgical alternative to resection for strictures in the small intestine, termed stricturoplasty, involves longitudinal incision of the strictured segment, followed by transverse closure [734,735]. This method preserves bowel and has a low rate of complications [736,737].

Surgical approaches to Crohn's colitis include segmental resection, subtotal colectomy with ileoproctostomy, and total colectomy with ileostomy. Segmental resection of Crohn's colitis is appropriate if a relatively short segment of colon is involved. Recurrence rates are high, approximating those described for ileal and ileocolic disease. Subtotal colectomy with ileoproctostomy should be considered only in a patient with an absolutely normal rectum [738]. Even in selected patients, the recurrence rate after this procedure is high, about 75% at 10 years [739]. For patients with extensive disease including the rectum, the procedure of choice is total proctocolectomy with a Brooke ileostomy. Total colectomy with ileoanal anastomosis is not appropriate in Crohn's colitis because recurrence of Crohn's disease in the ileal segment forming the new pouch would require a repeat operation and loss of a long segment of ileum.

Colon cancer, dysplasia, and colonoscopic surveillance

Patients with extensive ulcerative colitis have an increased risk for colon cancer compared with the general population. The molecular pathway to the development of colon cancer in ulcerative colitis is different from that seen in sporadic colon cancers [740]. In the development of most sporadic colon cancers the loss of function of the suppressor gene adenomatosis polyposi coli (APC) is an early event and is associated with the formation of adenomatous polyps. However, in ulcerative colitis the loss of APC is a late event in carcinogenesis and polyps do not typically develop. In contrast p53 mutations typically occur late in the adenoma–carcinoma sequence in sporadic colon cancers but occur early in the development of colon cancer in ulcerative colitis. These differences in the molecular events leading to colon cancer are associated with differences in presentation. Ulcerative colitis cancers are more likely to be submucosal, more likely to occur in flat mucosa, and less likely to be associated with polyps. In sporadic colon cancers multicentric cancers are seen in 2%–3% of individuals but more than 20% of ulcerative colitis cancers are multicentric [741].

The magnitude of the risk of colon cancer in ulcerative colitis is uncertain. Early reports of extremely high incidences of colon cancer among patients with ulcerative colitis (50% cumulative risk at 30 years) were biased by the use of referral-based populations that were not representative of the general population [742]. Data from a metaanalysis suggest that the risk of colon cancer in ulcerative colitis is 2% after 10 years of disease, 8% after 20 years of disease, and 18% at 30 years [745]. Some populations of patients with chronic ulcerative colitis do not have an increased risk of colorectal cancer compared with the general population. In a population-based cohort from Olmsted County, Minnesota the cumulative probability of colorectal cancer was 0% at 5 years, 0.4% at 15 years, and 2% at 25 years after the diagnosis of ulcerative colitis [744]. It is possible that the lower rate of colorectal cancer seen in certain ulcerative colitis populations may relate to improved medical management, increased use of chronic 5-ASA therapy, higher rates of surveillance colonoscopy with proctocolectomy for dysplasia, and higher rates of proctocolectomy for medical treatment failures. Each of these interventions might decrease the risk of colorectal cancer in a population of patients with chronic ulcerative colitis.

The identified risk factors for colorectal cancer in patients with ulcerative colitis include duration and extent of disease and the presence of primary sclerosing cholangitis. A family history of colorectal cancer is an independent risk factor as well. The risk of developing colon cancer becomes appreciable 8–10 years after diagnosis of ulcerative colitis and increases with time. Age of onset of ulcerative colitis seems to have little impact on the incidence of colon cancer. The risk of malignancy, however, is a larger issue for those who develop ulcerative colitis earlier in life because they have a longer period at which they are at risk and thus a higher cumulative incidence of cancer. Colon cancer in the general population is a disease of late middle and older age, but in ulcerative colitis colon cancer develops earlier and is commonly seen in the fourth decade of life. The risk of malignancy is also a function of the anatomic extent of disease; the risk is much greater with pancolitis than with left-sided colitis or proctosigmoiditis.

Patients with long standing ulcerative colitis are at risk for developing cancer even if their symptoms have been relatively mild [742,745]. Patients are seen with colon cancer whose ulcerative colitis has been quiescent for 10–15 years. The risk of colon cancer is much higher in individuals with ulcerative colitis and primary sclerosing cholangitis than in those with ulcerative colitis alone [746]. Although the risk of colon cancer is increased in ulcerative colitis it is not high enough to recommend proctocolectomy for all patients at a certain time after diagnosis. However, the risk is high enough to consider screening patients with ulcerative colitis to identify those at particular risk for colon cancer. In the general population colon cancer commonly develops in polyps and screening colonoscopy looking for cancer and polyps is recommended for people over 50 years of age. This approach is less effective in ulcerative colitis where colon cancer usually develops in flat mucosa rather than in polyps.

In ulcerative colitis the tumor may be indistinguishable from the surrounding mucosa; it may appear as a flat, plaque-like lesion, or it may present as a stricture. The large exophytic masses with sharp margins seen in colon cancer in the general population are less common in ulcerative colitis.

An effective approach to colonoscopic surveillance in ulcerative colitis came from the observation that colon cancer in ulcerative colitis is associated with dysplasia elsewhere in the colon. Dysplasia is an unequivocal neoplastic transformation in the epithelium without penetration to the lamina propria. It is marked by nuclear stratification, loss of nuclear polarity, and nuclear and cellular pleomorphism. In 1967, Morson and Pang [747] reported on a group of nine patients with ulcerative colitis who were found to have dysplasia on rectal biopsy and underwent colectomy. Of these nine patients, five were found to have malignancies elsewhere in the colon. Other groups have confirmed these observations and it is clear that the vast majority of colons resected for malignancy in ulcerative colitis have dysplasia somewhere in the specimen. These findings suggest that dysplasia may precede the development of carcinoma and that dysplasia may be a marker to identify patients with ulcerative colitis who have developed or are at risk of developing carcinoma [748]. Dysplasia should therefore be viewed as not a marker for future cancer but an indicator of a high risk of concurrent cancer in the colon at that point in time. These findings have led to the suggestion that patients with long-standing ulcerative colitis undergo periodic colonoscopy with biopsies and, if the biopsies show dysplasia, then patients should undergo prophylactic colectomy.

This plan of action has several problems. The first is that dysplasia is not easy to read on colonic biopsy, and interobserver variation is considerable. A group of pathologists have developed a classification of dysplasia that should help to resolve this problem [749]. This classification divides biopsies into negative, indefinite, and positive groups; the positive group is further divided into those of high-grade and low-grade dysplasia. Even in major academic centers, considerable interobserver variation occurs in diagnosing low-grade dysplasia. A second problem is that the presence of inflammation often makes it effectively impossible to determine if dysplasia is present. For this reason surveillance colonoscopy should be performed when the patient's disease is quiescent. A third problem is that, although dysplasia may be extensive, it is more often patchy and can be missed entirely by standard screening protocols. Although the vast majority of carcinomas in ulcerative colitis are associated with dysplasia elsewhere in the specimen, this does not mean that colonoscopy with biopsies will always find the dysplastic area.

Despite these limitations a consensus guideline for colonoscopic screening in ulcerative colitis has been developed [750]. The recommendation is that screening should begin 8–10 years after the onset of symptoms in patients with pancolitis or left-sided colitis. Surveillance should be repeated at 1- to 2-year intervals. For patients with only proctosigmoiditis the risk of colon cancer is considerably lower than that for patients with left-sided disease or pancolitis and in this population the recommendation for colon cancer screening is the same as for the general population. For patients who have ulcerative colitis and primary sclerosing cholangitis there is a markedly increased risk of colon cancer compared with patients who have ulcerative colitis alone and yearly colonoscopic examinations are recommended beginning as soon as the diagnosis of primary sclerosing cholangitis is made. The consensus recommendations for surveillance colonoscopy include four-quadrant biopsies taken every 10 cm, beginning at the proximal extent of disease. The data supporting these recommendations are thin. There are no large-scale studies comparing different schedules for colonoscopies or different protocols for the number or distribution of biopsies. There are some data suggesting that in patients with dysplasia the larger the total number of biopsies the more likely dysplasia will be found.

Guidelines have been developed for decision-making based on the results of the surveillance biopsies [751]. A patient with low-grade or high-grade dysplasia found in a discrete adenoma-like polyp, but nowhere else, can be safely managed with polypectomy and accelerated surveillance. When a polyp is removed, separate biopsies should be taken from the flat areas around the base of the polyp. If dysplasia is found in the area around the polyp then colectomy is recommended because of the high association with synchronous cancer. Dysplasia of any grade found in an endoscopically unresectable polyp or high-grade dysplasia found in flat mucosa are both strong indications for proctocolectomy. High-grade dysplasia in a flat area is associated with a high risk of synchronous colorectal cancer elsewhere in the colon and a colectomy is recommended. The management of low-

grade dysplasia found in flat mucosa is controversial. Some investigators recommend colectomy whereas others recommend repeat colonoscopy within 6 months. The controversy about the management of low-grade dysplasia relates to the widely varying reports of the predictive value of low-grade dysplasia. In a review of 10 surveillance studies low-grade dysplasia was associated with synchronous cancer in 19% of patients [752]. In patients who were followed after a diagnosis of low-grade dysplasia a significant number (16%–29%) progressed to high-grade dysplasia, dysplasia-associated lesion or mass, or cancer. However, in other studies only 3%–10% of patients with low-grade dysplasia progressed to more severe disease [751,753]. In any case in which the diagnosis of dysplasia will influence management, it is reasonable to have the slides reviewed by a second expert pathologist.

Assessing the value of surveillance colonoscopy is a difficult issue. No randomized prospective control trials have been carried out, nor are they likely to be performed. In one retrospective study variables associated with colorectal cancer were assessed in 188 patients with ulcerative colitis-related cancer and a similar number of matched patients with ulcerative colitis but without colorectal cancer [755]. Fifty-five of the patients with colon cancer had undergone surveillance colonoscopy whereas 97 of the controls had undergone surveillance colonoscopy. These data suggest that the surveillance colonoscopy reduces but does not eliminate the risk of colon cancer. The largest prospective study of the efficacy of colonoscopic biopsies followed 600 patients with ulcerative colitis who underwent 2627 colonoscopies during 5932 patient years of follow up [756]. Seventy-four patients (12.3%) developed neoplasia, including 30 colorectal cancers. Sixteen of the 30 cancers were interval cancers, i.e., they either presented after an initial negative colonoscopy or presented as advanced cancers at surveillance. The cumulative incidence of colorectal cancer by colitis duration in this group of individuals undergoing surveillance was 2.5% at 20 years, 7.6% at 30 years, and 10.8% at 40 years. Although this is the largest prospective study of the utility of colonoscopic surveillance in ulcerative colitis the results are not easy to interpret. There was no control group and it is difficult to use historical controls because of the wide range of reported rates of colorectal cancer in populations where surveillance was not carried out. This study does demonstrate that even in a program where colonoscopic surveillance is well organized and where expert pathological services are available the incidence of colorectal cancer in patients with ulcerative colitis undergoing surveillance is still substantial.

Chronic use of 5-ASA compounds as maintenance therapy for ulcerative colitis is associated with a decreased risk of developing both dysplasia and colorectal cancer [757,758]. The relative risk reduction for colorectal cancer seen with 5-ASA use is 46%–81% in a series of retrospective studies. The chemopreventive effects of 5-ASA are dose-related, with at least 2 g/day being required for any effect. The reported benefits of 5-ASA are for individuals who have not already progressed to dysplasia. There is no evidence that initiating 5-ASA affects the progression of low-grade dysplasia.

Much less information is available on cancer in Crohn's disease than in ulcerative colitis [759]. For those with Crohn's colitis, the incidence of colon cancer is higher than in the general population; the risk of colorectal cancer in extensive Crohn's colitis appears to be similar to that of ulcerative colitis [760–762]. As with ulcerative colitis, the incidence of colon cancer in Crohn's colitis increases with the duration of the disease and is associated with dysplasia. Also, as in ulcerative colitis, colon cancers occur in patients with Crohn's disease at a younger age than in the general population. A similar surveillance strategy has been suggested with extensive Crohn's colitis as with ulcerative colitis [762].

An association between small intestine Crohn's disease and adenocarcinoma of the small intestine has been found [763]. Most adenocarcinomas occur in areas of the small intestine actively involved with Crohn's disease. Adenocarcinoma of the small intestine occurs at an earlier age in patients with Crohn's disease than in the general population. There is also an association of small intestine adenocarcinoma with surgically bypassed loops of small bowel. It is not clear whether the high incidence of cancer in bypassed bowel is a product of the bypass procedure or merely reflects the long duration of the disease.

Pregnancy and inflammatory bowel disease

Because IBD affects many women in early adulthood, the effect of IBD on pregnancy is an important clinical issue [764,765]. Fertility in women with IBD is normal or only minimally impaired [766,767]. Fertility in men taking sulfasalazine is diminished but returns to normal a few months after cessation of the drug [768,769].

Most pregnancies in women with IBD produce healthy babies. The incidences of stillbirth and developmental defects are similar to those of the general population [770]. The children of women with Crohn's disease have a slight increase in the incidence of prematurity and a slight decrease in birth weight [771]. The incidence of spontaneous abortion is higher in women with IBD (12.2%) than in the general population (9.9%). There is some suggestion that the incidence of fetal complications is somewhat higher in cases in which the mother's disease is clinically active, irrespective of drug therapy [770]. The incidences of prematurity and spontaneous abortion are both higher in patients with more active disease. Proctocolectomy with the creation of a J-pouch and an ileoanal anastomosis is associated with markedly decreased fertility [772,773].

Many women with IBD take medication on a chronic basis, and the possibility of the fetus suffering undesirable effects from the drugs is a matter of concern [774,775]. Many women have taken sulfasalazine throughout the course of pregnancy, and there is no evidence of harm to the fetus or newborn. In one series of 174 patients, sulfasalazine had no effect on spontaneous abortion, prematurity, or fetal weight [776].

Pregnant women have an increased requirement for folic acid, and sulfasalazine interferes with folate absorption. Women taking sulfasalazine who are pregnant or considering pregnancy should receive folate supplements (1 mg twice daily) to ensure that the fetus receives amounts that are adequate for normal development. Up to 1 mg daily of folate supplementation is recommended for all pregnant women to prevent congenital neural tube malformations. It seems reasonable, therefore, that all women taking sulfasalazine who are pregnant or contemplating pregnancy should take 2 mg folate daily to ensure absorption of adequate amounts, as the period surrounding conception is the most critical for neural tube development. The use of corticosteroids by pregnant women with IBD is not associated with an increased rate of fetal complications [776]. In general, it appears that the risks to the pregnancy of treatment with sulfasalazine or corticosteroids are less than the risks of allowing disease activity to go untreated.

Most data on azathioprine and 6-mercaptopurine in pregnancy come from the transplant literature and involve higher doses than are commonly used in IBD. Reported fetal effects in the transplant population include congenital malformations, immunosuppression, prematurity, and growth retardation. However, surveys of women with IBD taking azathioprine or 6-mercaptopurine during pregnancy have demonstrated no increase in the number of birth defects [777]. It is reasonable for a patient to continue maintenance therapy with azathioprine as 6-mercaptopurine during pregnancy. Breast-feeding is not recommended for women taking these drugs. Methotrexate induces abortions and should not be used in women at risk for pregnancy. Metronidazole, in the short courses used to treat trichomoniasis, appears to be safe in pregnancy; however, there are no data for the longer courses commonly used in IBD. The available data suggests that infliximab is safe in pregnancy but the number of patients is small [778].

A number of studies have assessed the effects of pregnancy on disease activity in IBD. If the patient's disease is inactive at the time of conception, it is likely that it will remain inactive during the course of the pregnancy [779]. If the disease is active at the time of conception, the course is harder to predict. Ulcerative colitis that is active at the time of conception tends to worsen. In two-thirds of patients with Crohn's disease that is active at conception, the degree of activity remains the same; among the other third, some improve clinically and others deteriorate.

Inflammatory bowel disease in childhood and adolescence

Although the peak incidence of IBD is in young adulthood, 15% of patients with ulcerative colitis and 25%–33% of patients with Crohn's disease present before 20 years of age. For the most part, IBD presenting in childhood is similar to the disease in adults [780,781]. The clinical presentation of ulcerative colitis in childhood, as in adulthood, is marked by diarrhea and rectal bleeding. The clinical presentation of Crohn's disease, however, is somewhat different in childhood [782]. Abdominal pain, weight loss, and diarrhea are common in both adults and children, but extraintestinal manifestations (e.g., arthritis, iridocyclitis, clubbing, erythema nodosum) are more likely to be major components of the initial clinical presentation in children than in adults. Abdominal pain in Crohn's disease tends to be periumbilical and colicky and is confused with the pain of functional disease. Psychosocial issues are an important part of the morbidity of pediatric IBD. Anxiety, depression, and antisocial behavior are common. Delayed growth and maturation and the cosmetic effects of steroid therapy contribute to the psychosocial burden of IBD in childhood and adolescence.

Whether cytokines and other factors produced during inflammation contribute to the growth retardation is unknown. Corticosteriod therapy also may contribute to the growth failure. Growth retardation is less common in children with ulcerative colitis than in those with Crohn's disease. Bone demineralization is also a bigger problem in the pediatric IBD population than in adult patients. Decreased activity, corticosteroid therapy, and decreased intake and absorption of calcium and vitamin D all contribute to bone demineralization.

Medical management of IBD in childhood is largely the same as in adulthood. All the major controlled studies of therapy in IBD purposefully excluded children. Treatment in children is extrapolated from those studies. The indications for sulfasalazine therapy in children are the same as in adults. In children, sulfasalazine is begun at 25–40 mg/kg daily and increased to 70 mg/kg daily. The indications for corticosteroid therapy are also the same as for adults with IBD. Typical starting doses for moderate to severe disease would be 1–2 mg prednisone per kilogram per day. Continuous therapy with prednisone in doses as low as 10 mg/day can inhibit normal growth. After acute symptoms are under control, an attempt should be made to convert the patient from prednisone to an immunomodulator.

Immunomodulators, specifically 6-mercaptopurine and azathioprine, have been used in childhood Crohn's disease much as they have been used in the adult disease [783–785]. Over the last few years there has been a trend to introduce azathioprine and 6-mercaptopurine earlier in themanagement of IBD in children with some practitioners using them as primary therapy [786,787].

Indications for colectomy in ulcerative colitis in childhood are the same as for adults, with the addition of growth failure as an indication. Growth failure may be the result of either disease activity or corticosteroid administration. Surgical resection of localized areas of disease activity may be useful in children with Crohn's disease; however, the success of surgical resections in reversing growth retardation has been disappointing [788].

Growth retardation is a common element in pediatric Crohn's disease; corticosteroid therapy may contribute to growth retardation but the major cause is uncontrolled disease activity. In two randomized controlled trials of enteral feedings with elemental diets compared to corticosteroids the enteral feeding groups had improved weight gain [789].

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D COLON

58 Surgical treatment of inflammatory bowel disease

Emily Finlayson, Fabrizio Michelassi

Crohn's disease, 1473
Ulcerative colitis, 1490
Indeterminate colitis, 1500

Crohn's disease, ulcerative colitis, and the rare forms of true indeterminate colitis represent a disease spectrum with protean manifestation and complications. Although as many as half of the patients with inflammatory bowel disease require at least one surgical procedure during their lifetime, the decision to operate is rarely an easy one. Such a decision should be the result of a thorough collaboration between the gastroenterologist and the surgeon, assisted by the radiologist and the pathologist. Factors to take into consideration are the age and the general conditions of the patient; the extent of the disease; the duration of the disease and the treatment offered to that point; and the specific complication in regard to the available treatment options. Once the need for surgical intervention has been established, other factors, specific to each individual presentation, must be considered to choose the most appropriate surgical procedure. This chapter addresses surgical options and their results in view of the different indications for surgical treatment of inflammatory bowel disease. Emphasis is given to new options offered by advances in laparoscopic surgery.

Crohn's disease

Crohn's disease is a heterogeneous entity, which requires individual approaches for management due to the variety of intestinal segments affected and the different clinical manifestations of the disease. The first attempt to classify patients with Crohn's disease was based on the anatomical locations of the disease [1]. Subsequently Greenstein and Greenstein [2] proposed that perforating and nonperforating presentations might separate patients into two different clinical forms of Crohn's disease. Indeed, recurrent postoperative disease usually follows the pattern of the initial presentation [2,3]. The Vienna Classification divides patients with Crohn's disease into groups based on age at diagnosis, location of involved segment, and clinical behavior (nonstricturing and nonpenetrating, stricturing and penetrating) [4]. These distinctions have proven implications with respect to medical therapy, indication for surgery, and risk of postoperative recurrence.

The majority of patients presenting with uncomplicated active disease are initially treated with medical therapy. Although medical therapy has become more sophisticated with the adoption of new biological agents, it still fails significantly to change the natural history of the disease [5,6]. Patients still tend to require surgery as time progresses: after 20 and 30 years of symptoms, 78% and 90% of patients, respectively, require surgery [7].

Indications for surgery in Crohn's disease

The chronic, unrelenting, and recurrent nature of Crohn's disease brings these patients to the attention of the gastroenterologist during the early phases of the disease [1]. In the absence of complications, the initial management is medical, until the treatment fails or a complication ensues. Table 58.1 lists the indications for surgical treatment.

A significant number of patients with perforating Crohn's disease present with septic complications as the first presentation or recurrence [2]. Not all the septic complications are

Table 58.1 Indications for surgery in Crohn's disease

Sepsis:
Inflammatory masses or abscesses
Fistulae
Perforation
Failure of medical therapy
Obstruction
Hemorrhage
Cancer
Fulminant colitis with or without toxic megacolon

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an absolute indication for surgery; rather they are a marker of a severe and aggressive form of the disease. Fistulae are identified in one-third of patients with Crohn's disease [8], but they are rarely the primary indication for surgery. Specific indications for surgical treatment include:

- enterocutaneous and enterovaginal fistulae, where the enteric drainage becomes a matter of personal embarrassment for the patient [9]
- enterovesical or colovesical fistulae, where the connection of the intestine to the genitourinary system causes repeated urinary tract infections and eventually impairment of renal function [10]
- enteroenteric fistulae that produce functional and anatomical bypass of a major segment of intestine with consequent malabsorption or profuse diarrhea.

Inflammatory masses and abscesses occur in as many as 20% of patients [8,11]. These patients should be treated with antibiotics, the abscesses drained percutaneously (if feasible), and surgery postponed until a definitive procedure can be safely performed. Primary free perforation is an unusual complication of Crohn's disease: usually the severity of the transmural inflammation leads to the formation of adhesions, which wall-off the perforation resulting in an abscess. The secondary rupture of an abscess into the abdominal cavity requires prompt surgical intervention.

Medical treatment has failed and surgery is necessary:

- when maximal medical therapy proves inadequate
- for those patients who may be asymptomatic while on maximal medical therapy, but develop recurrence of symptoms with tapering of the medications
- when the disease progresses with worsening symptoms or a complication arises while the patient is receiving maximal medical therapy
- in the presence of significant treatment-related complications, including steroid-induced Cushingoid features, cataracts, glaucoma, hypertension, aseptic necrosis of the head of the femur, myopathy, vertebral body fractures, myelosuppression, anaphylactic reaction, or growth retardation in children [12]. In one series, failure of medical therapy was the primary indication for surgery in 33.6% of patients [8].

Up to 22% of patients present to the surgeon with worsening obstipation [8]. Symptoms are caused by single or multiple strictures or a lengthy diseased segment and differ depending on the location of the disease in the gastrointestinal tract: delayed gastric emptying in gastroduodenal disease; postprandial cramps in jejunoileitis; distention, pain, and diarrhea in colonic disease; laborious defecation in perianal Crohn's disease. Intestinal obstruction, even when complete, is rarely an indication for urgent surgery. In general, it is advisable to let the obstruction, even if complete, resolve with nasogastric decompression, intravenous hydration, and medical therapy, and to postpone surgery until after resolution of the obstruction to allow for a definitive procedure (i.e., strictureplasty or resection).

Other less frequent complications requiring surgical treatment include gastrointestinal hemorrhage, cancer, and toxic megacolon. Massive hemorrhage is an extremely rare complication occurring in less than 1% of patients [1,13]. Preoperative localization studies are mandatory to minimize the length of intestine resected. Cancer has been described in the small bowel [14] and the colon [15] and in a previous strictureplasty site [16]. The risk of cancer correlates with duration of the disease and may be higher in bypassed loops. Surgery for cancer in patients with Crohn's disease follows the same oncological principles as for sporadic cancers. Fulminant colitis with or without toxic megacolon is a known entity in inflammatory bowel disease [17,18]. The surgical treatment of this complication will be discussed in more detail under "Ulcerative colitis" below.

Crohn's disease of the stomach and duodenum

Crohn's disease may involve any portion of the gastrointestinal tract, from the mouth to the anus. About 2%–4% of patients with Crohn's disease present with involvement of the stomach or duodenum [19]. The most common indications for surgical treatment include hemorrhage and obstruction [20] (Fig. 58.1). Indeed, in a review of 108 patients, 83% underwent surgery for this complication [21]. Multiple surgical procedures have been advocated for the treatment of gastroduodenal Crohn's disease. Resectional antiulcer procedures have been performed mostly in situations in which an ulcer was misdiagnosed, and they have been associated with high morbidity and mortality rates [19]. Bypass procedures, such as gastrojejunostomy with or without vagotomy, have been the procedures most often advocated. Based on the location of the disease, gastroduodenostomy or duodenojejunostomy have also been advocated. These procedures are associated with good short-term outcome and acceptable morbidity rates, although delayed gastric emptying occurs in up to 24% of patients [22], occasionally requiring reoperation [23]. Strictureplasty has been advocated as an alternative to bypass procedures in selected patients. Only two retrospective reports are available that compare strictureplasty with bypass surgery for treatment of gastroduodenal Crohn's disease [22,23]. With a follow-up of between 42 and 192 months the strictureplasty-treated group had a lesser rate of delayed gastric emptying (15% vs 24%), but suffered more anastomotic dehiscences (12% vs 6%) and had a higher rate of repeated surgical interventions: 11 of 26 patients (42%) required a second operation after strictureplasty, 9 of them due to recurrent Crohn's disease, compared with 24% in the bypass-treated group [22,23]. Based on these limited data, both bypass and strictureplasty procedures appear to have merit in appropriately selected patients.

Crohn's disease of the small bowel

The jejunum and ileum, not including the terminal ileum, are affected by Crohn's disease in 3%–10% of patients

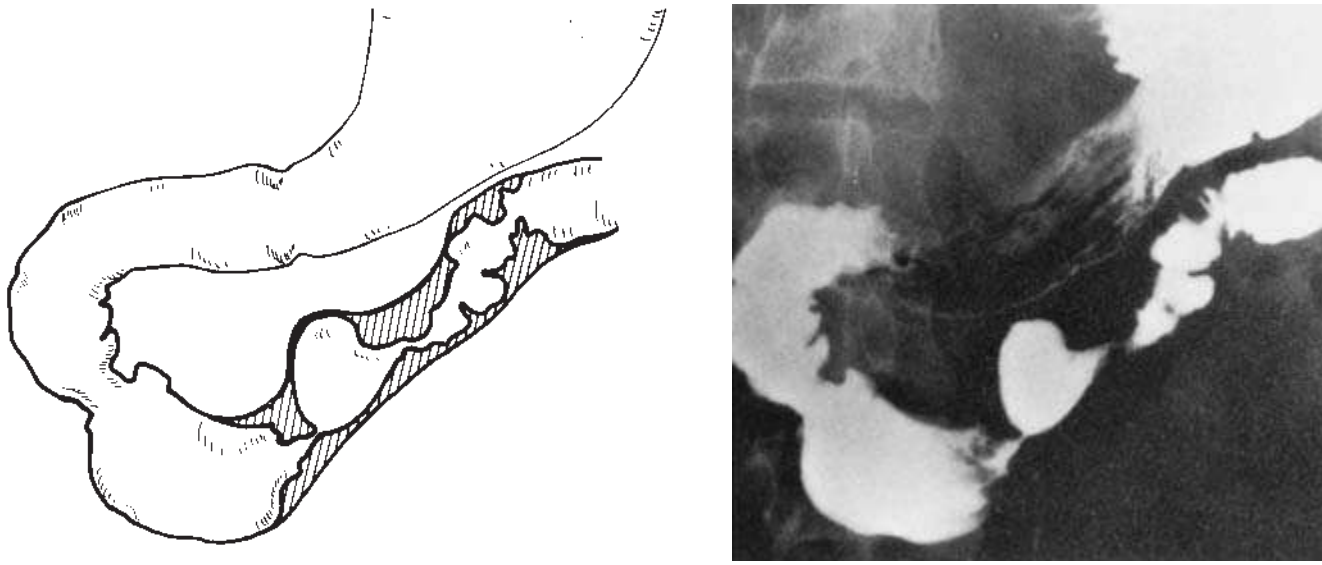


Figure 58.1 Upper gastrointestinal barium study showing Crohn's disease of the third and fourth portions of the duodenum. From Block GE, Michelassi F, Tanaka M, et al. Crohn's disease. Curr Probl Surg 1993;30:173.

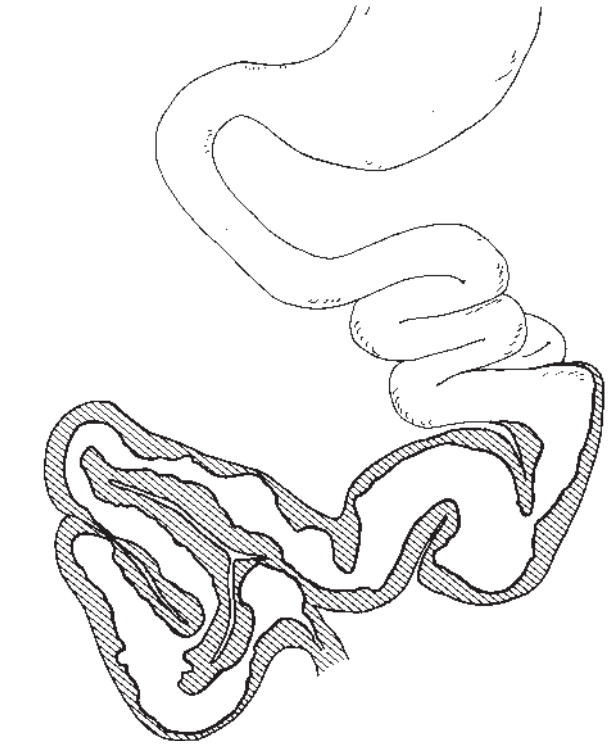
[8,24]. The two most common indications for surgical treatment are obstruction and sepsis; massive hemorrhage and carcinoma are much less common. Chronic, high-grade small bowel obstruction may be caused by single or multiple, short or long strictures. These patients present with postprandial abdominal pain, nausea, and vomiting and often progress to a high-grade obstruction. When multiple tight strictures are present, the small bowel is transformed into a sequence of dilated saccular segments separated by tight, ring-like strictures (Fig. 58.2). The dilated segments, which contain partially digested food particles, become the ideal environment for bacterial overgrowth. Because of bacterial overgrowth and stagnation, patients report symptoms of occasional diarrhea, malabsorption, and vitamin B-12 deficiency.

Patients with this clinical picture have traditionally undergone resection of long segments of small bowel. Considering that Crohn's disease is a recurrent disease and up to 30% of patients require a second operation [25], short bowel syndrome may eventually be precipitated in 1.5%–12.6% of cases [26,27]. In an attempt to preserve bowel length, Lee and Papaioannou in 1982 [28], and subsequently Alexander-Williams and Haynes in 1985 [29], described the use of strictureplasty, which had been previously described in India to correct tubercular stricture of the terminal ileum and cecum [30].

Many different strictureplasty techniques have been described. The most popular are the Heineke–Mikulicz, the Finney, and the side-to-side isoperistaltic strictureplasties [31–39]. Generally for short strictures, less than 10–12 cm in length, a Heineke–Mikulicz strictureplasty is performed (Fig. 58.3); for longer strictures either a Finney type (Fig. 58.4) or a side-to-side isoperistaltic strictureplasty (Fig. 58.5) is indicated. Prior to performing a strictureplasty, it is advisable to perform biopsy on all suspicious areas to rule out the presence of malignancy. Strictureplasty is contraindicated in the presence of active sepsis (peritonitis, abscess, or acute fistula), when the bowel wall is thickened and unyielding, when the stricture has undergone malignant degeneration, or in patients with severe weight loss and marked hypoalbuminemia [40].

The Cleveland Clinic group reviewed their experience of 1124 strictureplasties in 314 patients with a median follow-up of 7.5 years [40]. The overall morbidity rate was 18%, including a 2% rate of anastomotic dehiscence and 7% postoperative bleeding from the suture line requiring transfusion [41]. These investigators reported a 34% surgical recurrence rate, which compares favorably with the results of resective surgery [42]. The authors identified age and weight loss as the only factors associated with postoperative morbidity, and younger age at diagnosis as the primary factor associated with early recurrence [40]. These results confirm the safety and efficacy of strictureplasty in selected patients. Yet, as a word of caution, one of their patients developed adenocarcinoma at the strictureplasty site 7 years after the index operation. Negative results of biopsies of the stricture had been obtained at the time of the strictureplasty [43].

Septic complications, such as an inflammatory mass, abscess, and, occasionally, fistula, are also considered indications for surgical treatment of small bowel Crohn's disease. As already discussed, fistulae usually reflect severe disease



(a)

(b)

Figure 58.2 Small bowel barium study showing extensive jejunoileal disease with strictures and saccular dilations. From Block GE, Michelassi F, Tanaka M, et al. Crohn's disease. Curr Probl Surg 1993;30:173.

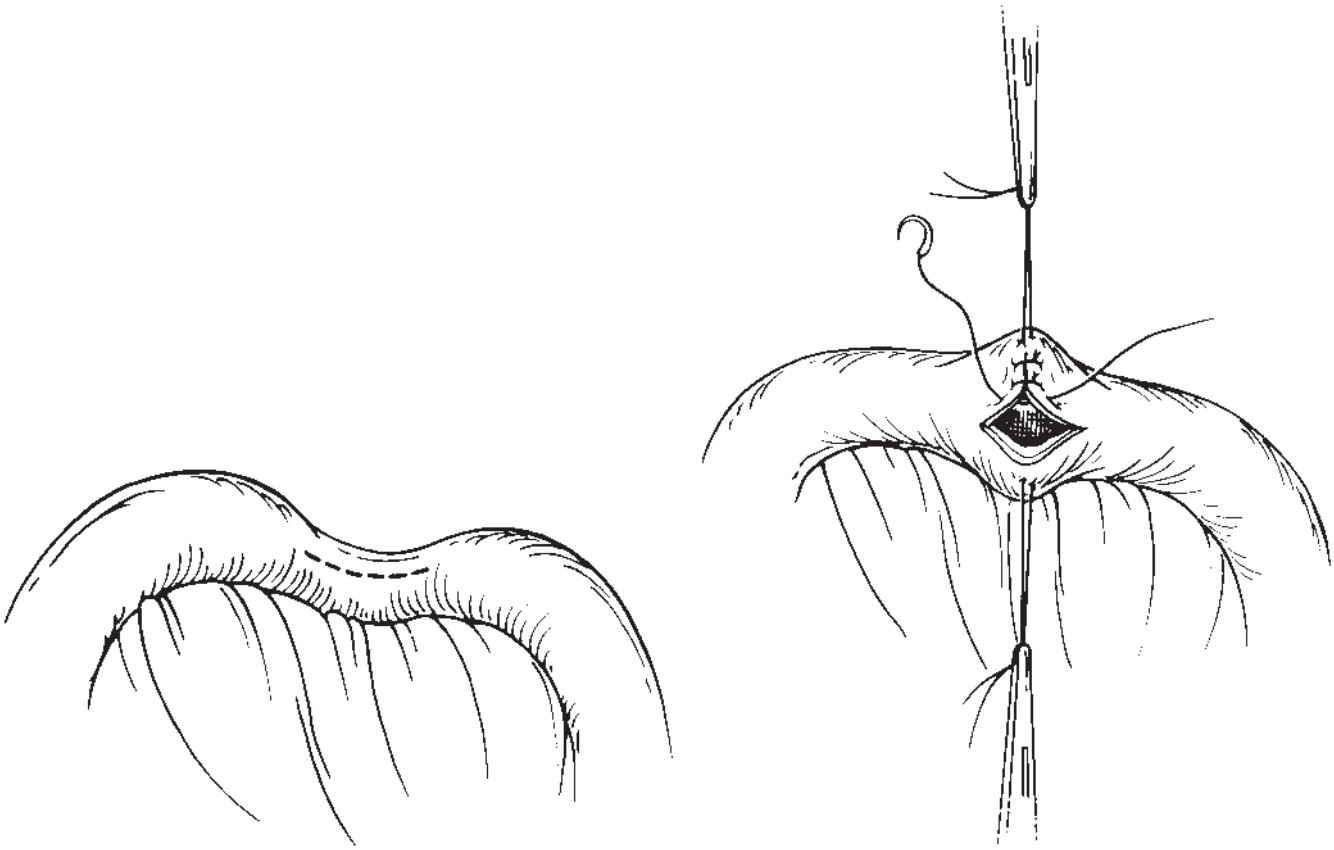


Figure 58.3 Heineke–Mikulicz strictureplasty for short stenosis (up to 7 cm). From Fazio VW, Galandiuk S, Jagelman DG, et al. Strictureplasty in Crohn's disease. Ann Surg 1989;210:621.

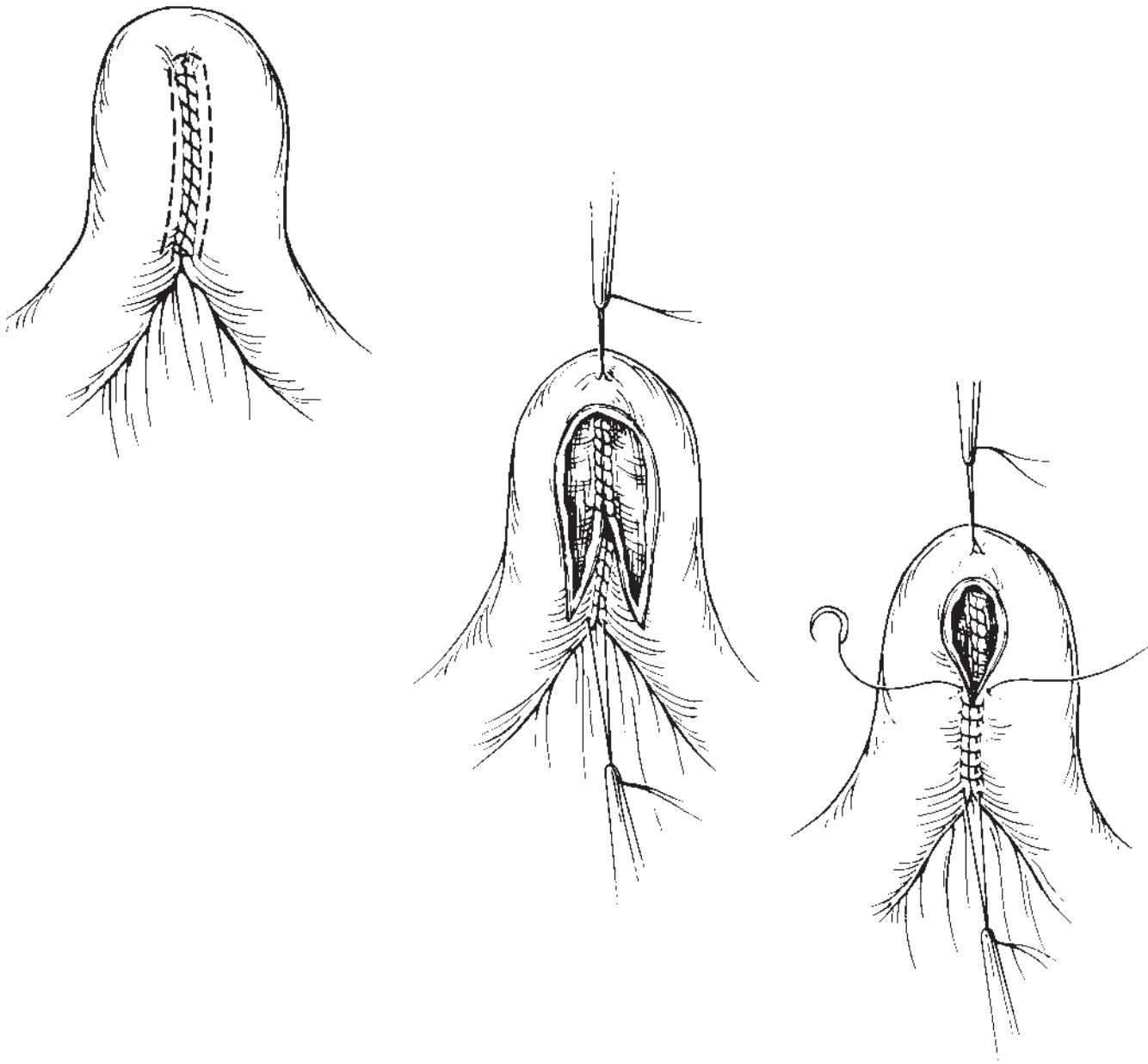


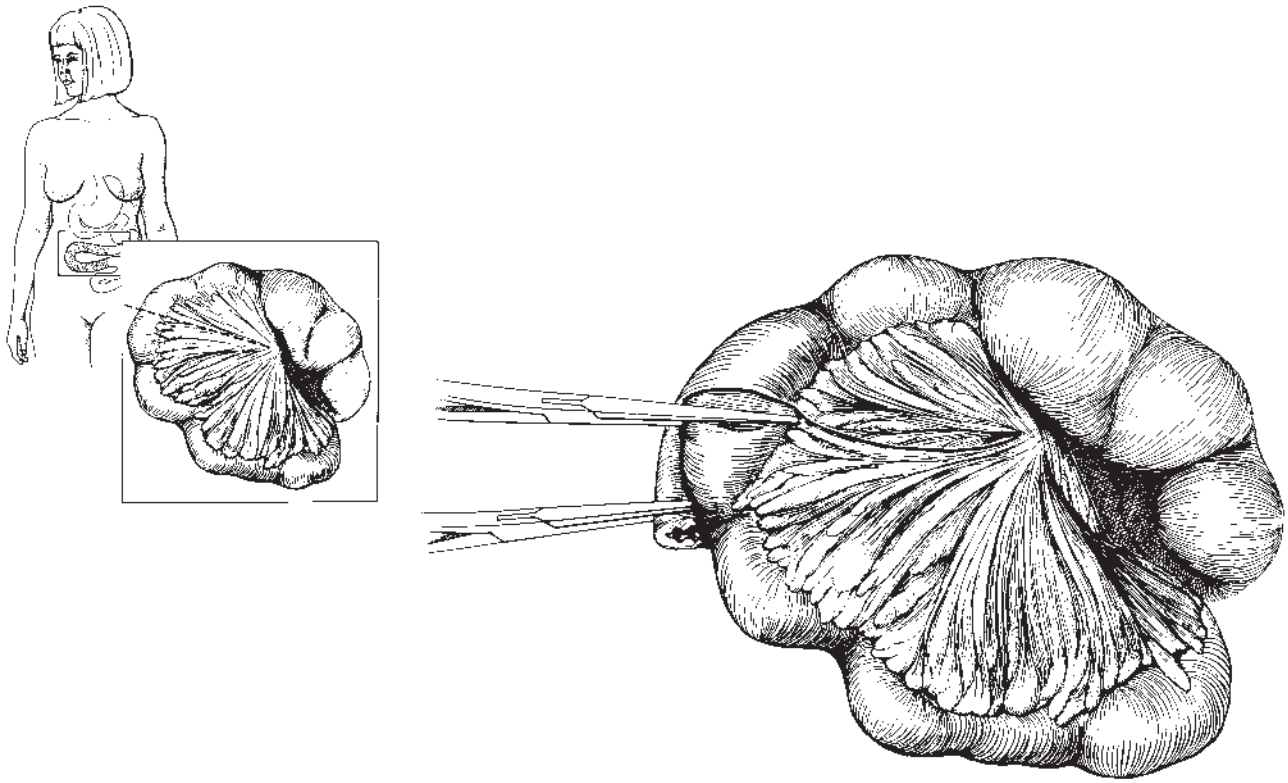
Figure 58.4 Finney strictureplasty for single stenosis longer than 7 cm. From Shahrif H, Alexander-Williams J. The role of strictureplasty in Crohn's disease. Int Surg 1992;77:15.

rather than an absolute indication for surgical treatment. Septic complications are discussed in further detail under "Crohn's disease of the terminal ileum" below.

The first case of a carcinoma of the small bowel in Crohn's disease was described by Leon Ginzburg in 1956 in a case of stricturing small bowel Crohn's disease [44]. Since then there have been more than 100 cases described in the literature [16,43–51]. Most cancers are adenocarcinomas [46], but they differ from de novo cancers in many respects: they present at a younger age, are more common in the distal small bowel, and may be multifocal or diffuse [47].

Cancer may occur in association with chronic perineal fistulae and excluded loops of small bowel. Several cases of small bowel adenocarcinoma in bypassed loops have been reported [47–50]. The increased risk of cancer development in a bypassed loop and the inability to image such a loop has deterred surgeons from performing intestinal bypass or allowed them to consider leaving excluded intestinal segments in situ for a long time.

The surgical treatment of small bowel carcinomas in Crohn's disease is segmental radical resection, when feasible. The prognosis is poor, with survival rates reported between



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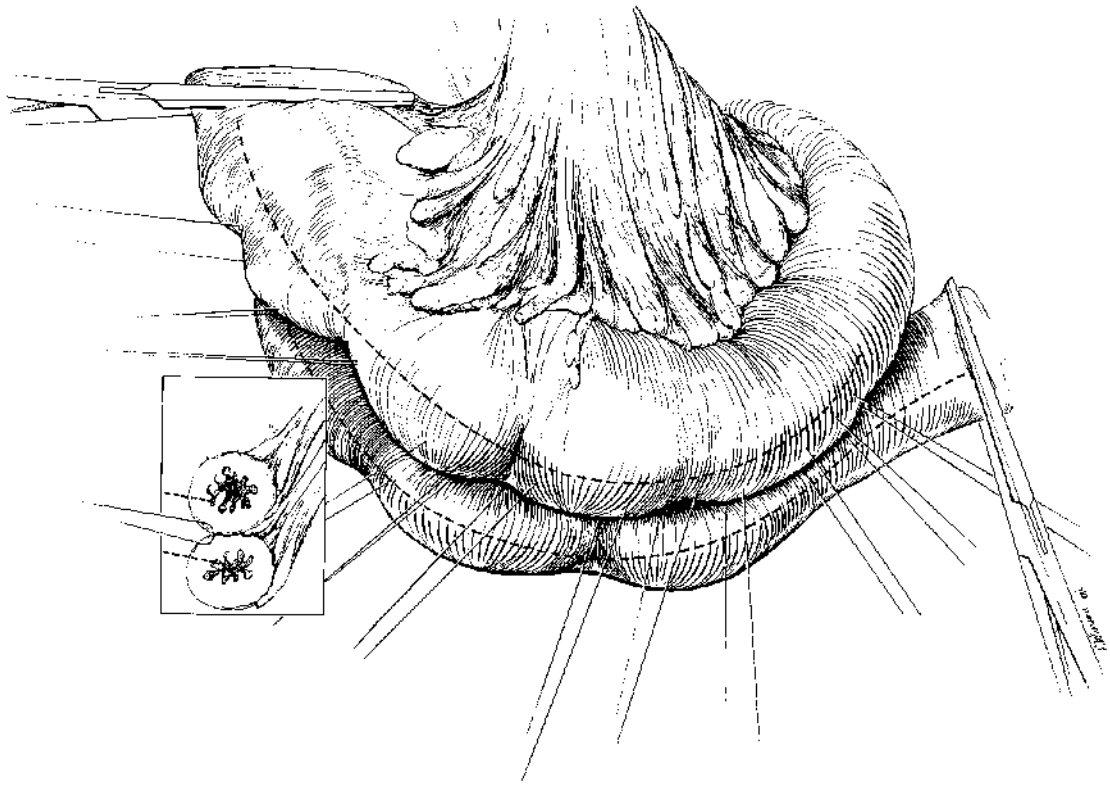
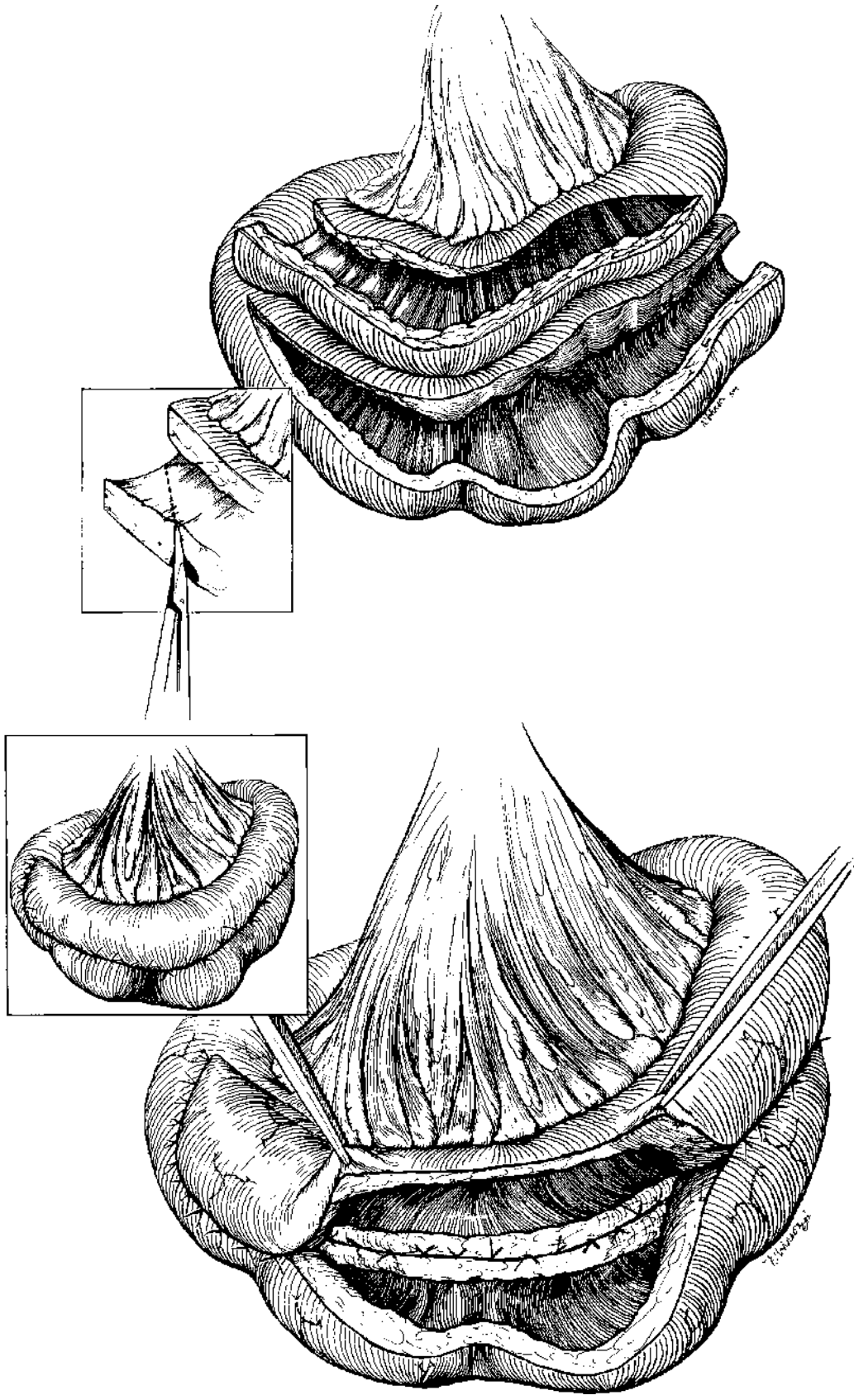


(b)

Figure 58.5 Isoperistaltic side-to-side strictureplasty for multiple sequential strictures and extensive jejunoileitis. **(a)** The mesentery of the diseased loop is divided at its midpoint, and the small bowel is severed between atraumatic intestinal clamps. The proximal intestinal loop is moved over the distal one in a side-to-side fashion. **(b)** The two loops are approximated by a layer of interrupted seromuscular Cushing stitches using nonabsorbable sutures. **(c)** A longitudinal enterotomy is performed on both loops and the intestinal ends are spatulated to avoid blind stumps (inset). **(d)** Both outer and inner suture lines are continued and finished anteriorly. The completed side-to-side isoperistaltic strictureplasty is shown in the inset. From Michelassi et al. [33].



(c)

(d)

23% at 3 years [49] and 5% at 5 years [51]. Patients with cancers in a bypassed loop usually do not survive longer than 18 months [49].

Crohn's disease of the terminal ileum

The terminal ileum is the most common gastrointestinal location requiring surgery in Crohn's disease and accounts for approximately 40% of patients with Crohn's disease referred to the surgeon [1]. Classically, patients present with obstructive symptoms or with septic features suggesting either a contained perforation or an abscess with or without a fistula. Cancer and hemorrhage are infrequent indications to surgical resection.

For obstructive disease of the terminal ileum secondary to strictures (Fig. 58.6) or an inflammatory mass (Fig. 58.7), resection is the treatment of choice. An ileocecectomy usually suffices; if the disease extends into the colon, an ileocolectomy may be necessary.

Abscesses (Fig. 58.8) and fistulae are two relatively common complications of Crohn's disease of the terminal ileum. Their occurrence may influence the timing of surgery and significantly increase its complexity. When feasible, these patients should be managed with image-guided drainage and antibiotics, with elective resection at a later time. This approach is feasible in approximately 50% of patients [52]. If percutaneous drainage is not successful or in the presence of a secondary free rupture of the abscess in the peritoneal cavity, open surgical exploration is necessary. The procedure should aim at draining the abscess, clearing the sepsis, and resecting the diseased intestinal segment. A primary anastomosis can then be fashioned if the patient's hemodynamic and nutritional conditions allow it and if the final suture line can be placed away from the abscess cavity. It is also advisable to lay an omental flap on the residual wall of the abscess cavity to quarantine any residual infection from the abdominal cavity and the anastomosis. If a primary anastomosis is not advisable, a stoma needs to be brought out as a temporary end ileostomy. When the extent of the inflammatory reaction is massive and involves several adjacent loops of bowel, the procedure should be limited to draining the abscess and placing a proximal diverting stoma. In due time, after most of the acute inflammatory reaction has subsided, a limited resection of the diseased bowel can be performed, saving a significant amount of bowel that otherwise would have been resected at the time of the first exploration.

Psoas abscess occurs as a result of a retroperitoneal perforation of the ileocecal region. Clinical manifestations vary from mild sepsis to severe psoas spasm with hip pain, flexion, and external rotation of the thigh associated with an abdominal mass. The retroperitoneal process can compress the ureter

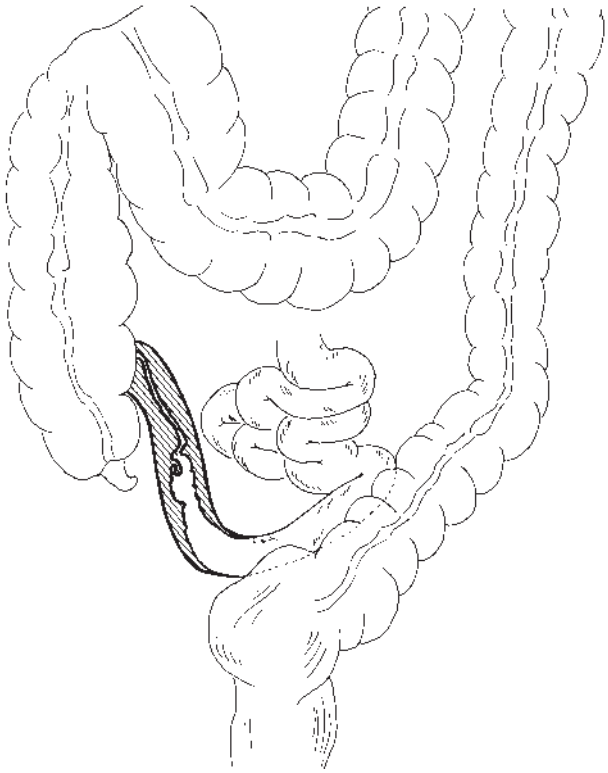


Figure 58.6 Barium enema with reflux in the terminal ileum showing long stenosis, referred to as the "string sign." From Michelassi F. Crohn's disease. In: Bell RH Jr, Rikkers LF, Mulholland MW (eds). Digestive Tract Surgery: a Text and Atlas. Philadelphia: Lippincott-Raven, 1996:1201.

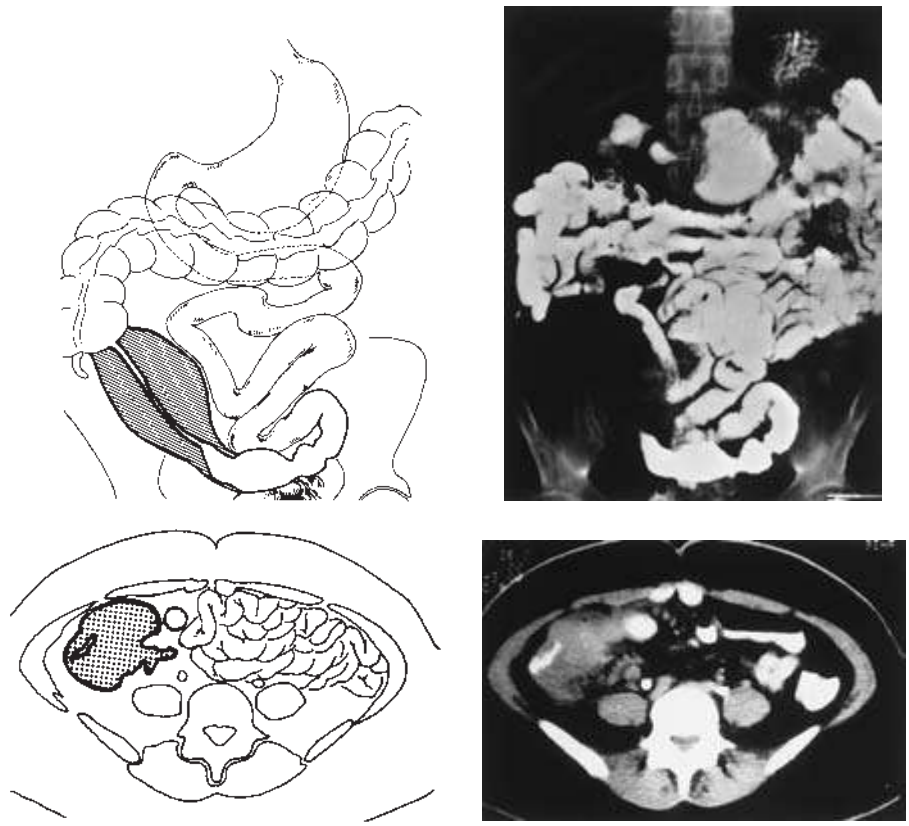


Figure 58.7 Upper gastrointestinal series and computed axial tomography showing a large right lower quadrant inflammatory mass in a patient with disease confined to the terminal ileum. Note the displacement of several loops of small bowel in the right lower quadrant. From Michelassi et al. [8].

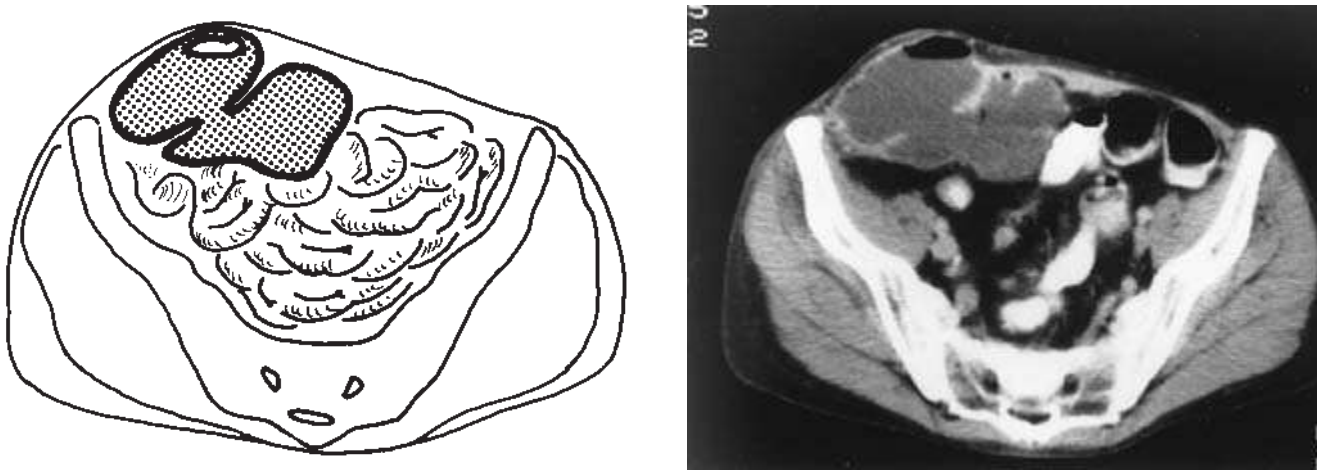


Figure 58.8 Computed axial tomography showing a right lower quadrant abscess originating from Crohn's disease of the terminal ileum and perforated through the abdominal wall into the subcut. Air–fluid level is evident. From Michelassi et al. [8].

and cause right hydronephrosis (Fig. 58.9). Resection of the inflammatory mass and drainage of the abscess usually relieves the compression on the ureter and cures the hydronephrosis.

Fistulae are common in Crohn's disease of the terminal ileum, but they rarely represent the only indication for surgical treatment. In one series of 639 patients undergoing surgery for Crohn's disease, 331 patients had disease in the terminal ileum. Of these, 217 patients (65.6%) were found to harbor 285 intraabdominal fistulae; yet these fistulae represented the primary indication for surgical treatment in only 6.3% of our patients [53]. Enteroduodenal, enteroenteric, and enterocolic fistulae usually are asymptomatic and often discovered only during a careful abdominal exploration or at inspection of the resected specimen [54]. These fistulae require surgical treatment only if they cause massive diarrhea because of the bypass of a sizable length of intestine.

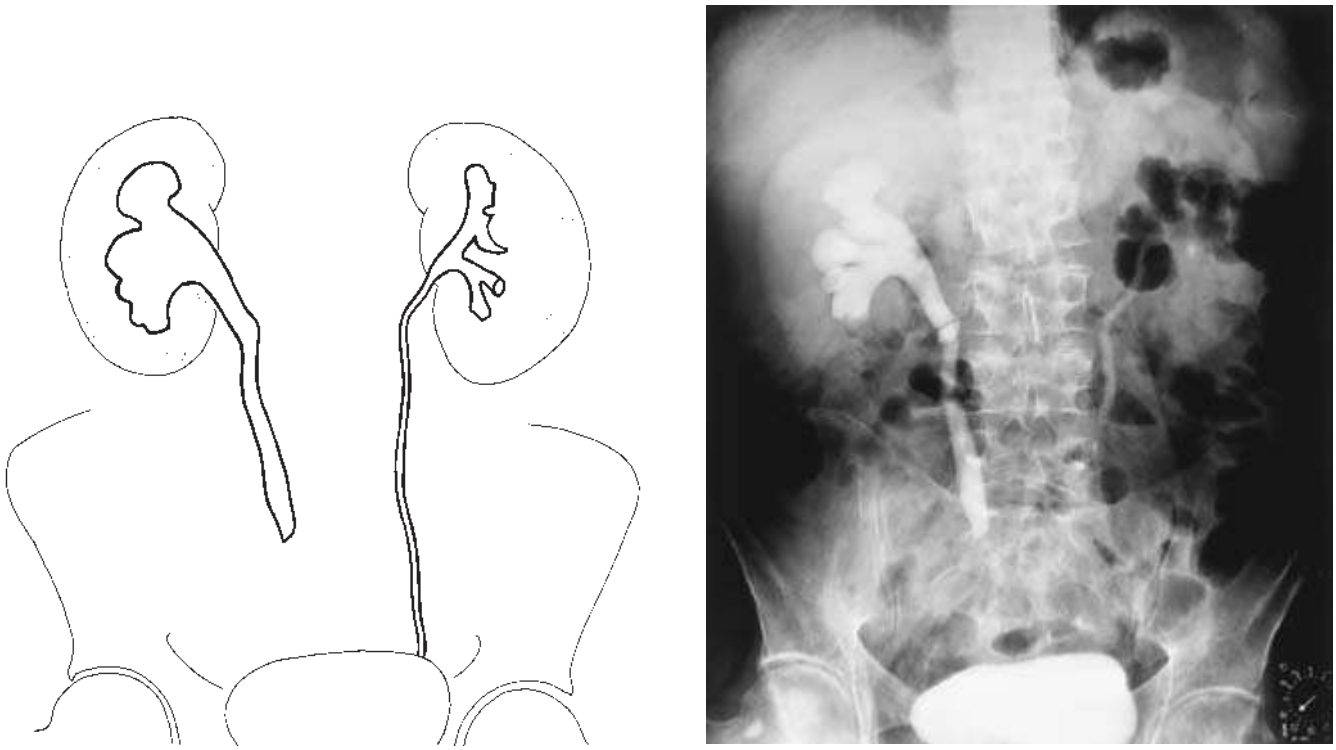


Figure 58.9 Complete right ureteral obstruction with hydronephrosis. From Michelassi et al. [8].

Enterocutaneous fistulae usually drain through a previous abdominal scar or through the umbilicus [55]. At times they result from surgical incision and drainage of a subcutaneous abscess complicating severe intraabdominal disease or from percutaneous drainage of an abdominal abscess [52]. The presence of an enterocutaneous fistula does not necessarily entail immediate surgical intervention [9,56]. Patients may be reluctant to undergo surgical treatment when the enterocutaneous fistula has a minimal output and the underlying disease is under satisfactory control. However, in most cases the difficulty in maintaining personal hygiene, the fear of social embarrassment, the bothersome symptoms associated with the severely diseased segment that led to the formation of the fistula, and the skin excoriation, which invariably forms around the cutaneous opening of the fistula, become factors in indicating the need for surgical treatment.

Enterovesical fistulae occur in 2%–5% of patients with Crohn's disease [57,58]. Some controversy exists regarding the timing of surgical intervention in the presence of these fistulae. Nevertheless, most surgeons and gastroenterologists agree that the consequences of chronic urinary tract infections on renal function, in addition to the symptoms affecting the intestinal tract itself, represent an indication for operation. Enterovaginal fistulae are rare complications of Crohn's disease and most often occur in women who have undergone a previous hysterectomy. The vaginal discharge is cause for discomfort, social and sexual embarrassment, and difficulty in maintaining personal hygiene. Most patients readily accept surgical intervention.

Massive intestinal hemorrhage is a rare complication of terminal ileitis [59]. Because the hemorrhage originates from active disease, patients with Crohn's disease are likely to have repeated episodes ultimately requiring surgical intervention. If the extent of the disease is limited, as assessed by contrast radiography, an elective resection should be considered after two episodes of self-limited hemorrhage. In the presence of an ongoing, unrelenting hemorrhage, an angiogram should be obtained to localize the source of bleeding, especially in the presence of multiple sites of disease involvement [13]. The possibility of a life-threatening hemorrhage in patients with Crohn's disease should not be underestimated. Five cases of exsanguinating gastrointestinal hemorrhage have been reported in the literature [60].

Malignant transformation of the terminal ileum is, fortunately, rare. Its treatment is based on oncological principles and it has been described in the previous section.

Controversies still persist about the timing of the operation. In view of the fact that 70%–90% of these patients require a resection in their lifetime [61,62], some authors have advocated early resection to avoid possible septic complication associated with recurrent attacks and the side effects of aggressive medical treatment [63,64]. However, in general,

most physicians and surgeons recommend a resection only after medical treatment has failed or a complication of the disease has arisen.

Overall, the results of resection are excellent. Large series have reported a surgical recurrence rate between 31% and 36% at 10 years, with 69% of patients requiring only one resection [61,63]. Several disease-related and patient-related factors associated with high risk of recurrence have been identified. The presence of perianal disease, extensive ileal involvement, and cigarette smoking seem to be significantly associated with a high recurrence rate after ileocolic resection [61,65,66]. When Crohn's disease recurs, it usually involves the ileum just proximal to the anastomosis; less frequently the colon just distal to the anastomosis or the anastomosis exclusively are involved. Strictureplasty has been used, with excellent results, for the treatment of anastomotic stricture secondary to recurrent Crohn's disease after ileocolic resection [67,68].

Crohn's colitis

The colon is affected by Crohn's disease in up to 30% of patients [1,8,69]. The involvement can be limited to a segment or extend to the entire colon and rectum; furthermore, patients may present with associated anorectal or small bowel disease. In the presence of pancolitis without perineal or small bowel manifestations of the disease, the differential diagnosis between Crohn's disease and ulcerative colitis may be difficult, and many patients end up carrying a diagnosis of indeterminate colitis as a result of diagnostic uncertainty [70] or the wrong diagnosis [71–74]. With the advent of ileoanal pouch procedures this differentiation has become crucial because pouch reconstruction in patients with Crohn's disease is contraindicated, with a pouch failure rate of up to 40% [71–74].

The most common indication for surgery in patients with disease localized primarily to the colon is failure of medical therapy [8,75]. These patients present with persistent and often bloody diarrhea and abdominal pain not responding to medical therapy. With worsening conditions, toxic colitis with or without megacolon may develop. This complication is less frequently associated with Crohn's disease than with ulcerative colitis, but it carries similar mortality rates (14%–16%) in both conditions. Factors affecting mortality include age (30% for patients older than 40 years old vs 5% for those younger than 40), gender (21% in women vs 13% in men), and the occurrence of colonic perforation (44% for cases with perforation vs only 2% in those without perforation) [18]. In the absence of overt perforation, initial therapy consists of high-dose steroids, bowel rest, and antibiotics. Lack of improvement over a short period of time or any signs of worsening condition are indications for an urgent operation to avoid colonic perforation.

There is a 4–20 times increased risk of colorectal cancer in Crohn's colitis [76,77], with an incidence between 1.4% and 1.8% [15,44–46,78,79]. Carcinoma can arise in a long-standing benign stricture, probably due to chronic inflammation [80].

Routine surveillance colonoscopy should be considered after 7–10 years of disease duration, with the recommendation to proceed with resection in the presence of dysplasia. Prophylactic colectomies should be performed for: tight strictures not allowing passage of the endoscope and, therefore, interfering with a complete colonoscopy; a stricture difficult to survey; multiple pseudopolyps rendering surveillance difficult; and, as mentioned earlier, the presence of an excluded intestinal segment (retained rectal stump). Surgery for adenocarcinoma in Crohn's colitis should be based on oncological principles and on the extent of the lumenal disease.

Septic complications occur in the form of inflammatory masses or, more infrequently, fistulae or abscesses. Inflammatory masses can be found in any segment of the colon and rectum with equal frequency. Transverse colon disease occasionally fistulizes into the stomach. A common location for abscesses to occur is the descending colon at the junction with the sigmoid colon. These abscesses, which form in the left parietocolic gutter and lie over the psoas muscle, may be confused with a complication of diverticular disease. Usually, the age of the patient and other manifestations of Crohn's disease help in arriving at the correct diagnosis.

Several different surgical approaches are available for the treatment of patients with Crohn's colitis. The surgical plan depends on the location of the disease, the urgency of intervention, the presence of complications, and the general condition of the patient. In Crohn's colitis the need to preserve bowel length is less pressing than in small bowel Crohn's disease, but still the extent of surgery should be carefully planned by the surgeon in conjunction with the gastroenterologist, the pathologist, the radiologist, and, foremost, the patient.

Surgery for Crohn's disease of the colon must encompass the entire gross disease. In patients with disease limited to the right colon, a right hemicolectomy will suffice (Fig. 58.10); if the disease extends beyond the mid-transverse colon, an extended right hemicolectomy may be necessary; for patients with disease involving the entire abdominal colon (Fig. 58.11), an abdominal colectomy will be needed. All these procedures can be performed in one stage. In the past, a diverting ileostomy was widely used as the sole initial step in patients too ill to tolerate definitive surgery [81–85]. A more contemporary approach consists of the appropriate colon resection with an end ileostomy and delayed reconstruction of the gastrointestinal continuity. This procedure is also indicated in patients with a diagnosis of indeterminate colitis to preserve the option of an ileal pouch procedure if the diagnosis of ulcerative colitis is later confirmed or established.

Abdominal colectomy and ileorectal anastomosis, in one or two stages, is ideally suited for young patients with no sigmoid or rectal involvement and normal anal sphincter function [86]. In older adult patients, the decision in favor

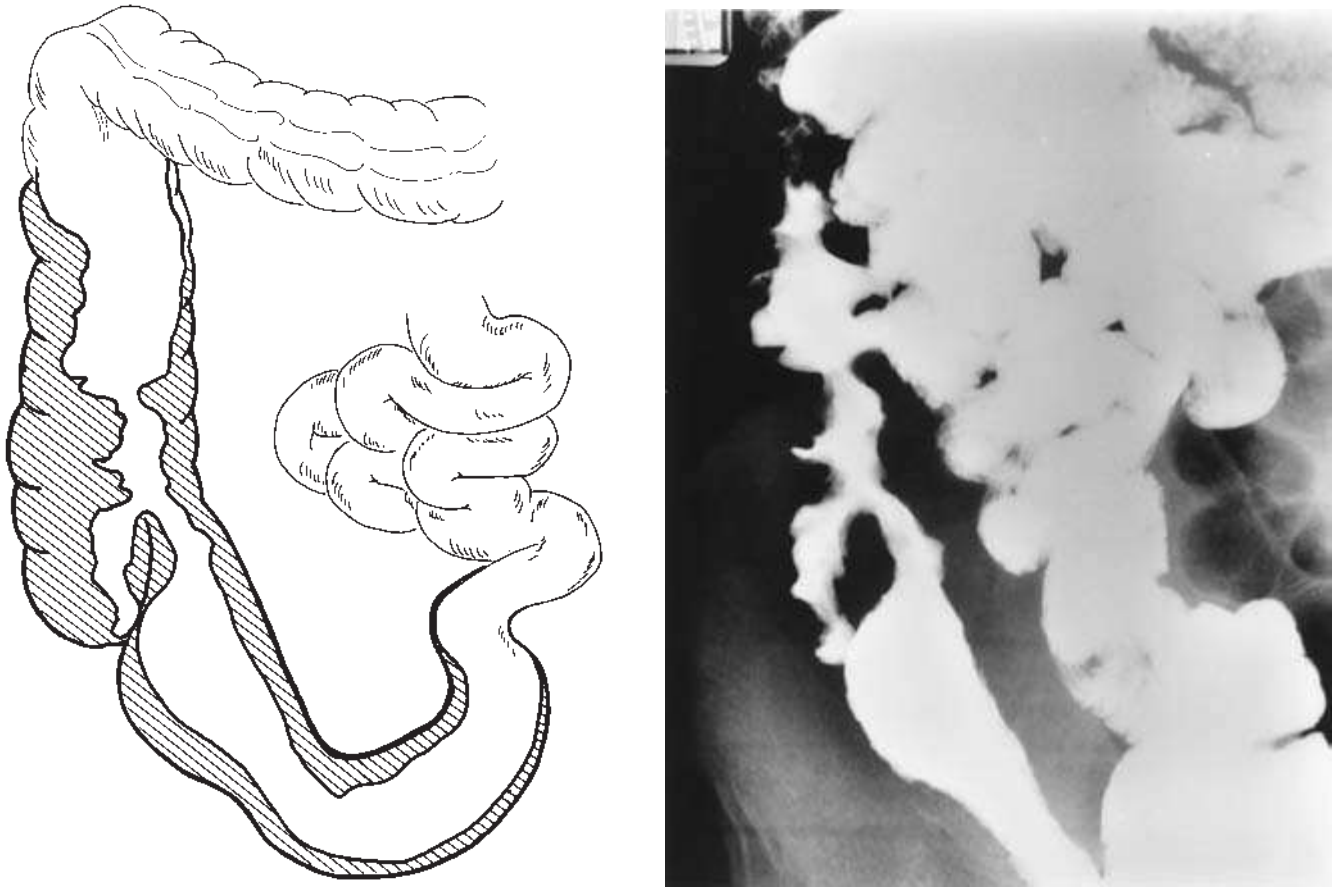


Figure 58.10 Small bowel follow-through series showing Crohn's disease of the ileal cecal valve and right colon. From Michelassi F. Crohn's disease. In Bell RH Jr, Rikkers LF, Mulholland MW (eds). Digestive Tract Surgery: a Text and Atlas. Philadelphia: Lippincott-Raven, 1996:1201.

of an abdominal colectomy with ileorectal anastomosis is a difficult one and needs careful consideration of the degree of anal sphincter function and the possible debilitating high fecal frequency.

Total proctocolectomy and end ileostomy, in one or two stages, has been and still is the surgical therapy of choice in patients with Crohn's pancolitis or in Crohn's colitis with extensive anorectal involvement or anal incontinence [87]. Recurrence of Crohn's disease in the small bowel after total proctocolectomy has been reported in 3%–46% of patients [88,89], and it involves the distal 25 cm of ileum in up to 90% of patients [90]. If Crohn's disease is limited to the rectum (Fig. 58.12) or the patient is affected by severe anorectal disease or incontinence, an abdominoperineal resection with a left-end colostomy is the procedure of choice.

In the presence of severe perineal sepsis, patients undergoing a proctocolectomy or an abdominoperineal resection are at risk of developing perineal wound problems. Large series have reported up to 34.4% of nonhealing perineal wounds after proctectomy in Crohn's disease [90–92]. A subtotal colectomy or an abdominal proctectomy with colostomy may be indicated as the first step to allow for appropriate drainage and healing of the perineal sepsis. A perineal proctectomy at a later time is still necessary to avoid the risks of a retained rectal stump. The use of myocutaneous flaps may further decrease the incidence of nonhealing perineal wounds [93].

For left-sided Crohn's colitis (Fig. 58.13), segmental resection is a valuable alternative. It allows for restoration of bowel continuity and maintenance of the absorptive mucosa of the right colon, but patients are at risk of recurrence and additional surgery [94]. Small series have reported a recurrence rate of 62% at 5.5 years, requiring further surgery [95], but up to 86% of patients maintaining bowel continuity at 14 years [96].

Ileal pouch surgery is contraindicated for patients with Crohn's colitis [72,74]. Despite the effort correctly to diagnose patients before surgery, some patients undergo surgery with a preoperative diagnosis of indeterminate colitis or ulcerative colitis and are found to have Crohn's disease on final pathological evaluation of the specimen [97]. Sagar and colleagues [98] and Deustch and associates [71], in two separate unselected series, reported a pouch failure rate of 45% at 10 years in a total of 46 surgical patients with a preoperative diagnosis of mucosal ulcerative colitis who were subse-

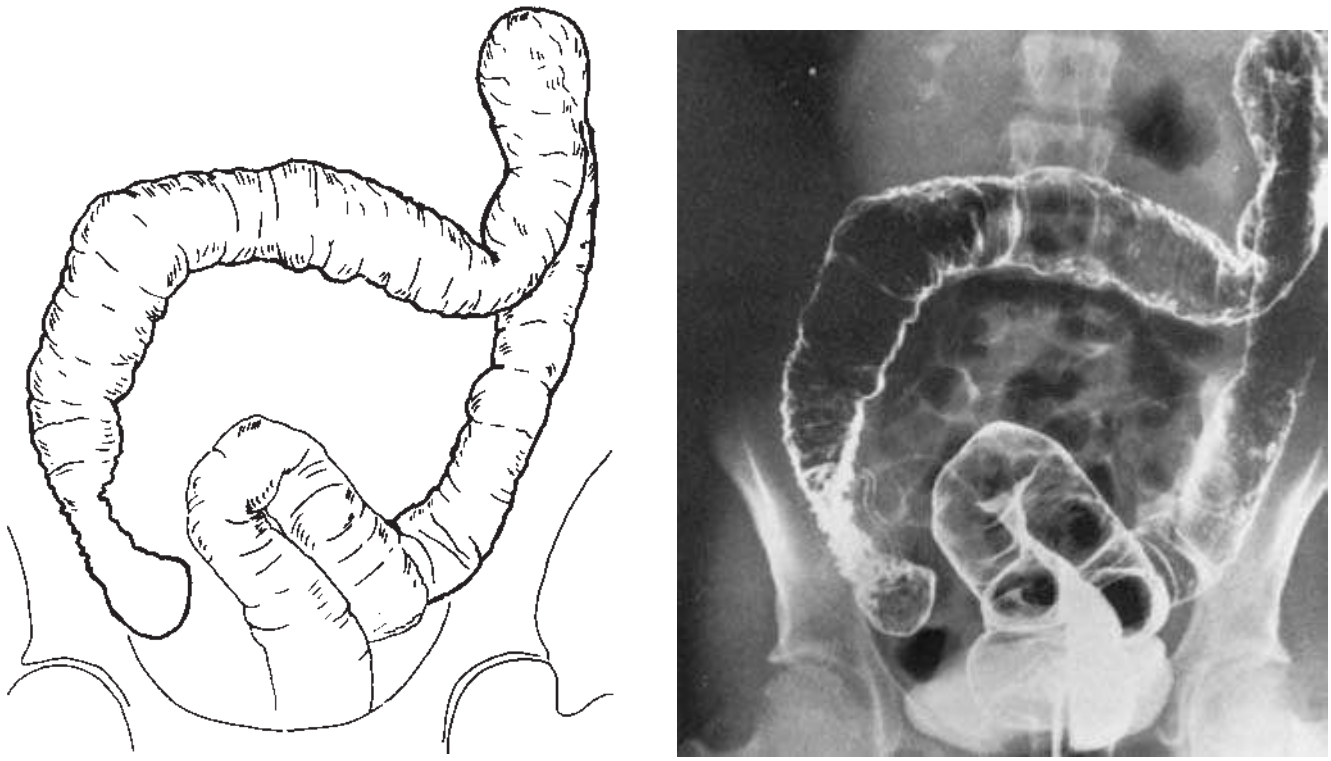


Figure 58.11 Barium enema showing Crohn's colitis with sparing of the rectum and rectosigmoid. From Michelassi F. Crohn's disease. In: Bell RH Jr, Rikkers LF, Mulholland MW (eds). Digestive Tract Surgery: a Text and Atlas. Philadelphia: Lippincott-Raven, 1996:1201.

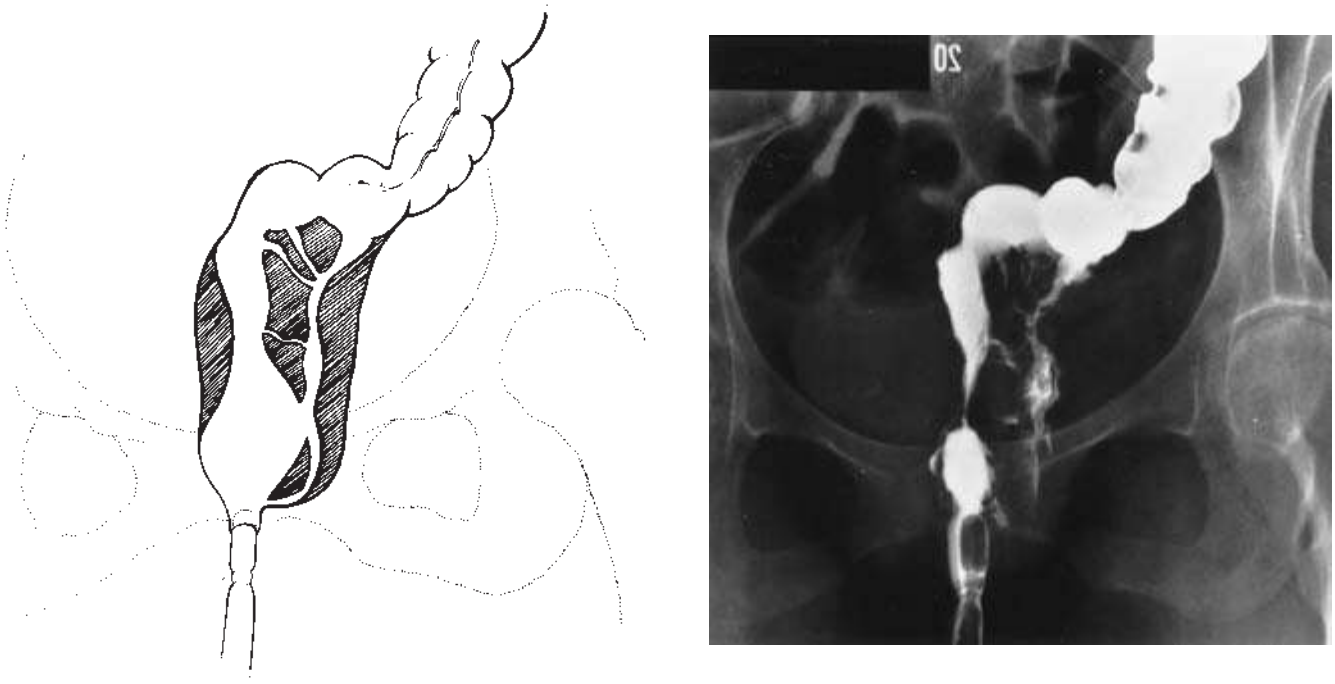


Figure 58.12 Barium enema showing Crohn's proctitis with loss of the rectal ampulla, several rectosigmoid fistulae, and an intervening perirectal abscess. From Michelassi F. Crohn's disease. In: Bell RH Jr, Rikkers LF, Mulholland MW (eds). Digestive Tract Surgery: a Text and Atlas. Philadelphia: Lippincott-Raven, 1996:1201.

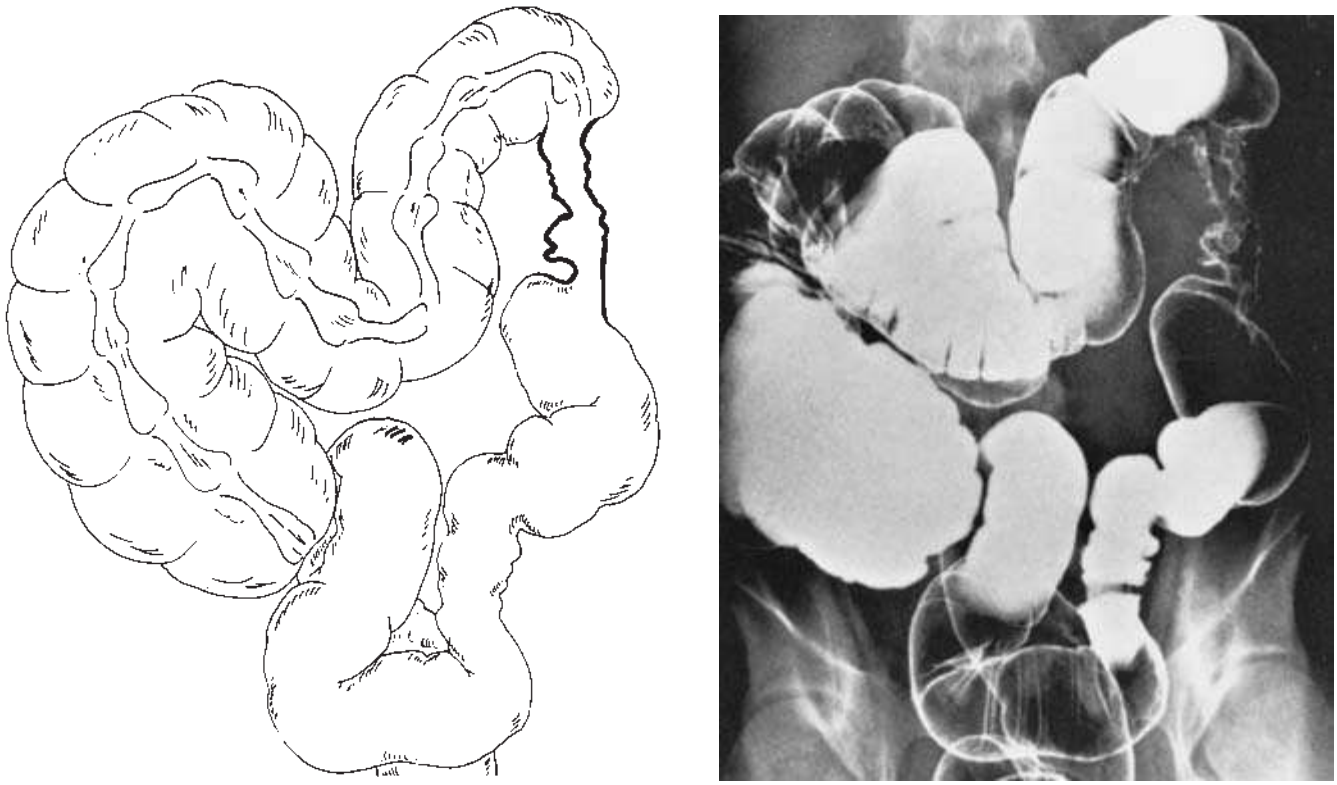


Figure 58.13 Barium enema showing Crohn's colitis limited to the proximal portion of the descending colon. From Michelassi F. Crohn's disease. In: Bell RH Jr, Rikkers LF, Mulholland MW (eds). Digestive Tract Surgery: a Text and Atlas. Philadelphia: Lippincott-Raven, 1996:1201.

quently proven to have Crohn's disease. Hyman and colleagues [73] analyzed the outcome of this procedure on 25 patients: 16 patients, at a mean follow-up of 38 months, had a functioning pouch, 7 had required pouch excision, 1 had a diverting stoma, and 1 had died. Only one of nine patients with preoperative clinical features suggestive of Crohn's disease had a functioning pouch, with complications uniformly occurring within months of ileostomy closure. In contrast, 15 of 16 patients without preoperative features of Crohn's disease had maintained their pouch, generally with good results. These studies suggest that the pelvic pouch procedure should not be performed in patients with clinical features of Crohn's disease because of the high associated complication rate. Yet, there might be a subgroup of patients with Crohn's colitis who could ultimately be recognized as possible candidates for an ileal pouch procedure. To this extent Panis and colleagues [99] reported a series of 31 patients with Crohn's disease with no evidence of perineal or small bowel disease. These patients were specifically selected for ileoanal pouch as an alternative to ileostomy. Their results were compared with those obtained from patients with ulcerative colitis undergoing the same procedure. Of the 31 patients with Crohn's disease, six (19%) experienced specific complications 9 months to 6 years after surgery: three had pouch–perineal fistulae, which required pouch excision in two cases. At the 5-year follow-up, there was no significant difference between patients with Crohn's disease and patients with ulcerative colitis with a functioning pouch, in terms of stool frequency, continence, gas/stool discrimination, leak or need for protective pads, and sexual activity. It is probable that in the future more sophisticated diagnostic tests may allow selection of a subgroup of patients with Crohn's disease appropriate for ileal pouch procedures.

Perianal Crohn's disease

Anorectal Crohn's disease may manifest with edematous skin tags, fissures, ulcers, abscesses, fistulae, strictures, and, as a complication of chronic, long-standing inflammation, anal cancer. The reported incidence of perianal Crohn's disease requiring surgery varies between 25% and 34% [93,100]. Crohn's colitis is much more frequently associated with anal lesions than Crohn's disease of the small bowel (52% vs 14%). When an anal lesion is the manifesting sign, Crohn's disease will soon develop elsewhere in the intestine [100]. Because these lesions frequently herald the onset of intestinal Crohn's disease, the physician must always be aware of the possibility of inflammatory bowel disease when dealing with a suspicious anal lesion.

Skin tags, fissures, and ulcers rarely require surgical treatment. The most common indications for surgery in perianal

Crohn's disease are septic in nature. For these complications the treatment plan should be based on the patient's general condition, anal continence, and degree of colorectal involvement. In patients with a grossly normal rectal mucosa, the anal stenosis can be dilated, ischiorectal abscesses can be drained, appropriate fistulectomies can be performed, and repair of complex fistulae or low rectovaginal fistulae can be attempted with every expectation of successful outcome. In patients with mild to moderate involvement of the rectal mucosa, treatment of the rectal disease with topical anti-inflammatory agents may occasionally effect dramatic improvement. If the improvement persists, surgical repair of even complicated defects may result in complete healing and normal function. In patients with severe perineal disease or incontinence, only a proctectomy with a permanent stoma may be expected to bring relief. Extensive perineal disease, although not a sole indication for proctectomy, usually is associated with destruction of the sphincter mechanism and incontinence, often dictating a resection with a permanent stoma.

Patients presenting with an ischiorectal abscess require prompt incision and drainage. In the majority of patients, the incision will heal by secondary intention without any need for further intervention. In 35% of patients, a fistula-in-ano will develop [93].

Many surgeons mistakenly believe that perineal wounds do not heal in patients with Crohn's disease and therefore have adopted a philosophy of therapeutic nihilism even in the presence of simple fistulae-in-ano [101–105]. This attitude may result in the patient's continued suffering and may lead to the development of new abscesses and fistulae, progression to a "watering-pot" perineum, and destruction of the sphincter mechanism. Patients with Crohn's disease with symptomatic low anal fistula involving minimum sphincter musculature can be treated safely with fistulotomy [106], with healing rates up to 85% [107,108]. In patients with horseshoe abscesses and high fistulae, aggressive local surgical intervention aimed at drainage of sepsis and appropriate usage of setons permit preservation of the sphincter and good postoperative function [106,107,109,110].

Rectovaginal fistulae occur as a complication of anorectal Crohn's disease in about 10% of patients [111,112] and require special mention. Most fistulae are truly anovaginal, with the internal opening located at the dentate line. These fistulae result from deep anterior anal ulceration and less commonly from a cryptoglandular source. These patients may be treated with a mucosal advancement flap [113–115]. This procedure entails performing a semicircular incision at the dentate line, with the internal opening of the fistula at its center (Fig. 58.14). A 3–4-cm flap of mucosa, submucosa, and smooth muscle is elevated in a proximal direction. The tract is curetted and closed and the flap is advanced to the anoderm and sutured without tension. Several variations have been proposed when the rectal mucosa is significantly diseased, including performing the repair from the vaginal side [116,117] and using an anocutaneous flap from the perianal skin [118]. Success rates with this approach have been reported around 70%–75% in relatively small series [113–115]. In the event of a failure, the advancement can be repeated, as reported by Joo and colleagues [115]. In their series, four of five patients who did not heal with the first procedure were successfully closed after a second attempt.

Anorectal complications also include stenosis and cancer. In the absence of severe rectal disease, anal stenosis can be dilated to achieve prolonged symptomatic relief. Cancer may develop from the diseased anorectal mucosa, a chronic fistula-in-ano, or a retained rectal stump. In all cases, an oncological resection needs to be carried out. In the presence of a resulting large soft tissue and cutaneous perianal defect, the use of myocutaneous flaps may be the only way to achieve primary closure of the perineal wound (Fig. 58.15).

All told, an aggressive surgical approach should allow healing of the Crohn's disease perineal septic complication with sphincter preservation in 62%–86% of patients [112,119]. In our series of 224 consecutive patients, the reasons for proctectomy in 85 patients included aggressive disease not responding to conservative measures in 66 patients, extensive fistular disease in 15 patients, fecal incontinence in 2 patients, and tight anal stenosis in 2 patients. We found that patients with rectal disease had a significantly higher rate of proctectomy than patients with rectal sparing (77.6% vs 13.6%), and that in the absence of rectal involvement, patients with multiple complications had a significantly higher rate of proctectomy than patients with single complications (23% vs 10%) [119].

Laparoscopic surgery for Crohn's disease

Laparoscopic techniques have become standard approaches to the treatment of several upper abdominal pathologies (i.e., gallbladder disease and reflux esophagitis) and several colorectal conditions (i.e., diverticulitis, colectomies for polyps and cancer, endometriosis involving the intestine). Many of the unique features of Crohn's disease, such as the intense inflammation, thickened mesentery, enteric fistulae, inflammatory masses or abscesses, and the multiplicity of areas of intestinal involvement, have deterred some surgeons from considering a laparoscopic approach. The natural history of the disease, however, often leads to the need for repeated operations, and a less invasive approach is appealing. Additionally, patients are generally young and interested in undergoing procedures that involve minimal scarring and prompt recovery. Numerous early clinical reports described laparoscopic-assisted resections in Crohn's disease as safe and feasible [120–127].

Laparoscopic-assisted ileocolic resection (Fig. 58.16) is the most commonly performed laparoscopic procedure for Crohn's disease [120,125]. Laparoscopic fecal diversion and closure of stomas may obviate a laparotomy and allow for a

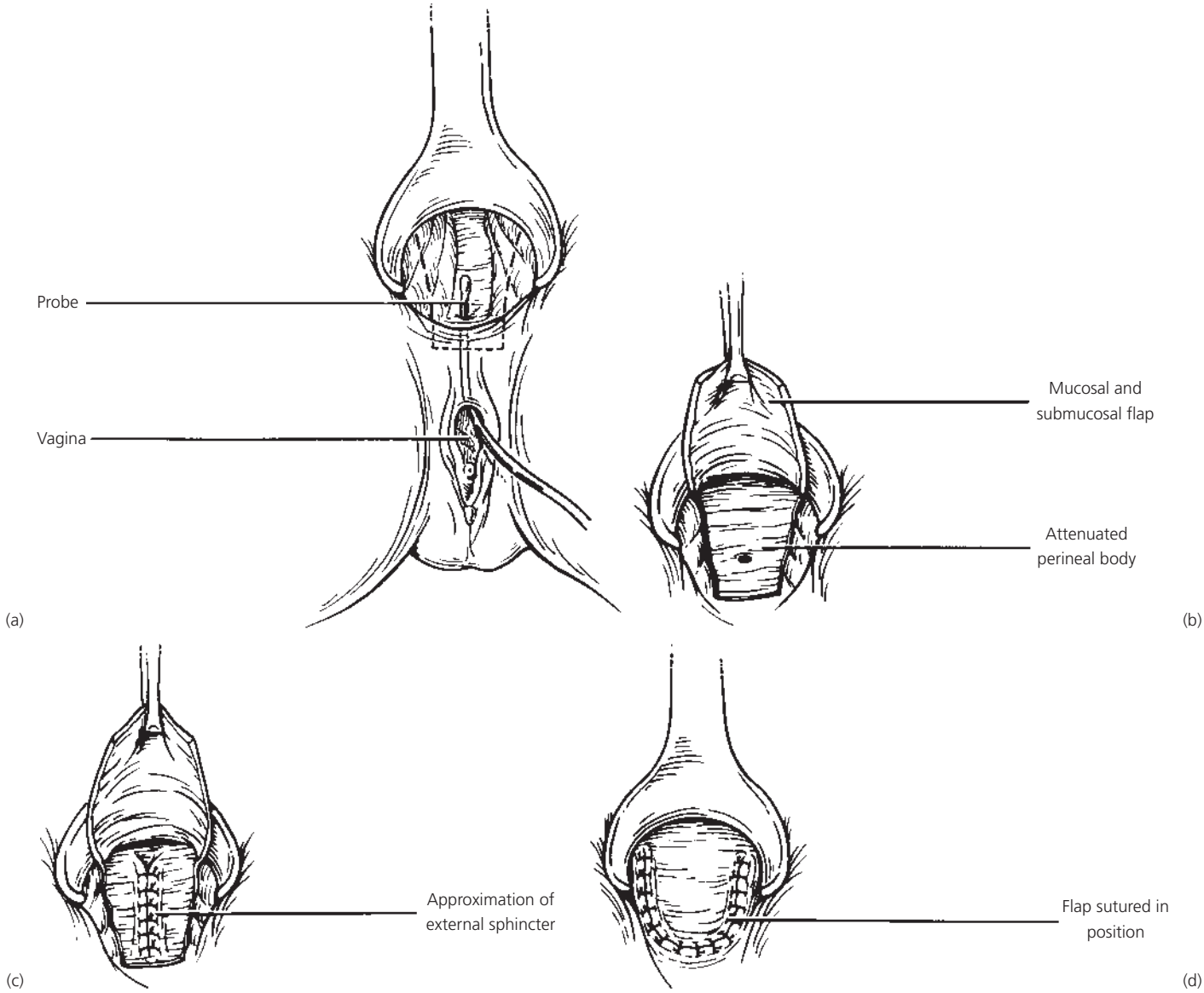


Figure 58.14 Endorectal advancement flap for low rectovaginal fistula. **(a)** The patient is in the jack-knife prone position and the probe identifies the rectovaginal fistula. **(b)** The rectal wall is elevated from the external sphincter, starting at the dentate line. **(c)** Plication of the external sphincter with closure of fistula. **(d)** The rectal wall flap is advanced over the plicated external sphincter; the tip of the flap with the fistula opening is excised; the flap is then sutured in position with interrupted sutures. From Michelassi F. Crohn's disease. In: Bell RH Jr, Rikkers LF, Mulholland MW (eds). Digestive Tract Surgery: a Text and Atlas. Philadelphia: Lippincott-Raven, 1996:1201.

full evaluation of the gastrointestinal tract. Laparoscopic-assisted small bowel resection or strictureplasty may be performed as the index procedure or when associated with another bowel resection, usually an ileocolic resection. The diseased area or areas can be marked with intracorporeal sutures, exteriorized through a small abdominal incision needed for removal of the resected specimen, and the strictureplasty performed extracorporeally in a standard fashion. Segmental or total colectomies with or without anastomosis can be performed by the laparoscopic-assisted method.

Several studies from tertiary referral centers have reported improved outcomes and reduced costs with laparoscopic ileocolic resection [127–130]. A prospective randomized trial comparing open and laparoscopic-assisted ileocolic resections in 60 patients [128] showed a faster postoperative recovery of respiratory function (measured as recovery of 80% of forced expiratory volume and forced vital capacity), a lower incidence of minor complications, and the need for a shorter abdominal incision in the laparoscopic-limited group. With a follow-up of 20 months there were no recurrences in either group.

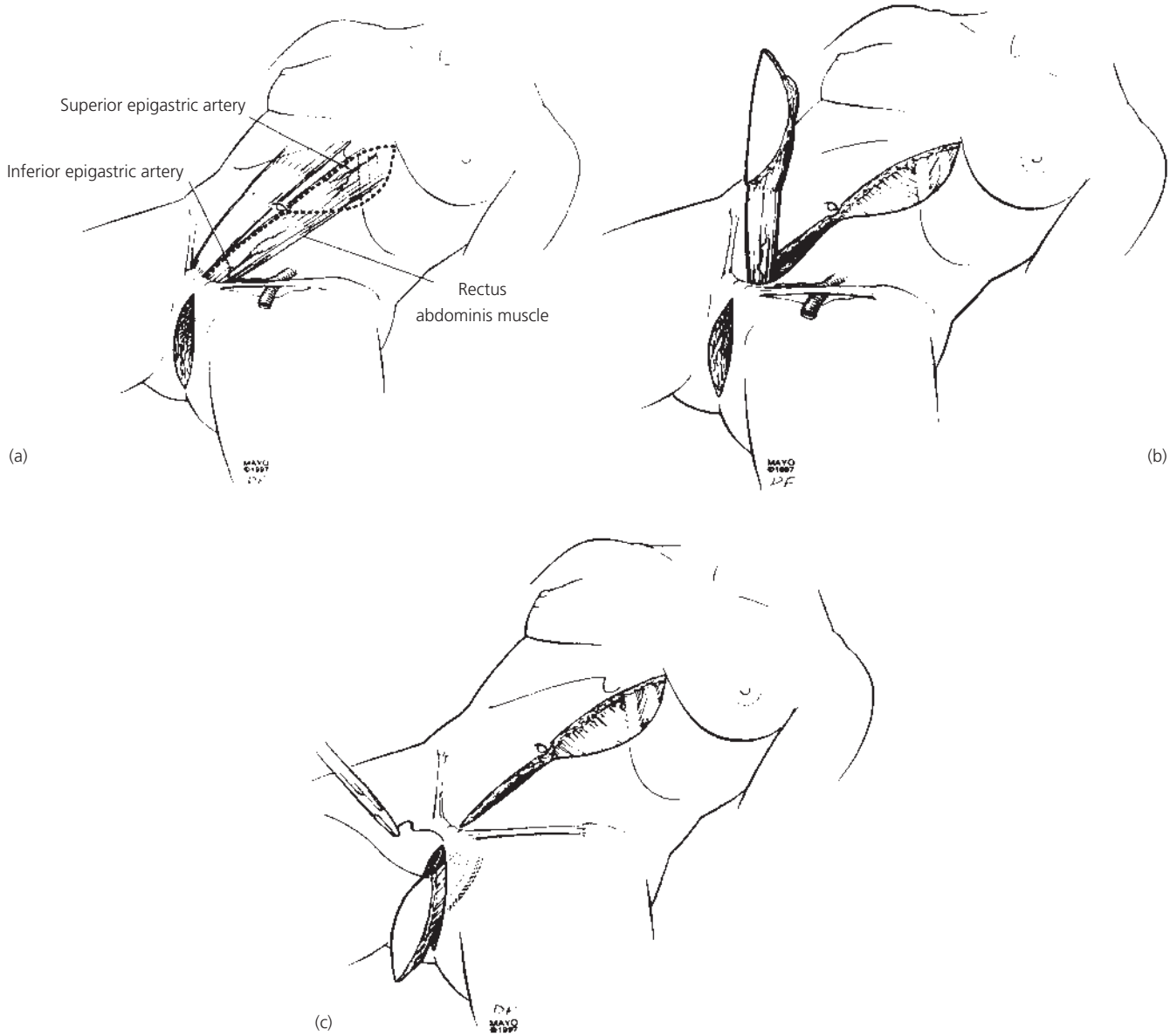


Figure 58.15 **(a)** The superior epigastric vessels supply the rectus abdominis myocutaneous flap. **(b)** The flap includes skin, anterior fascia, and the rectus muscle. **(c)** The flap is delivered through the pelvis to the perineal defect. From Hurst RD, Gottlieb LJ, Crucitti P, et al. Primary closure of complicated perineal wounds with myocutaneous and fascia cutaneous flaps after proctectomy for Crohn's disease. Primary myocutaneous flap closure following resection of locally advanced pelvic malignancies. Surgery 2001;130:767; originally from Radice E, Nelson H, Mercill S, et al., Br J Surg 1999;86:349.

Case-matched retrospective studies have also shown small but measurable advantages for the laparoscopic approach [129,130]. In an analysis of 66 patients undergoing laparoscopic or open ileocolonic resection, Young-Fadok et al. [129] found that patients undergoing laparoscopic resection had a more rapid return of bowel function, shorter duration of narcotic use, and shorter hospital stay (4 days vs 7 days). In addition, costs – both direct and total – were significantly lower in the laparoscopic group. A metaanalysis of randomized and nonrandomized studies comparing open and laparoscopic surgery for Crohn's disease found in pooled analysis that whereas operative times were an average of 27 minutes longer in the laparoscopic group, laparoscopy resulted in improved short- and long-term outcomes. Patients undergoing laparoscopic resection had a faster return of bowel function, shorter hospital stay, fewer postoperative bowel obstructions, and decreased surgical recurrences [131]. Whether these results can be reproduced outside specialized centers is unclear.

In summary, laparoscopy in Crohn's disease may allow for faster recovery and reduced surgical trauma; morbidity

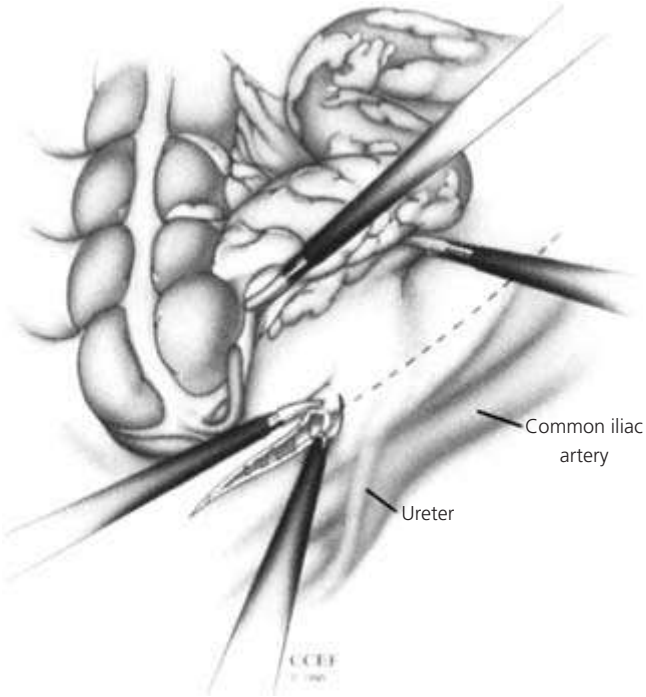


Figure 58.16 Laparoscopic ileocecectomy. From Kim SH, Milsom JW. Laparoscopy in inflammatory bowel disease. In: Michelassi F, Milsom JW (eds). Operative Strategies in Inflammatory Bowel Disease. New York: Springer-Verlag, 1999:326.

associated with laparoscopy performed by experienced hands compares favorably with the conventional open approach. With the continual improvement of laparoscopic instruments and the refinement of laparoscopic surgical techniques, this approach will likely be used increasingly in patients with more challenging and extensive complications of Crohn's disease.

Ulcerative colitis

There is a major difference between the management of ulcerative colitis and Crohn's disease. In Crohn's disease the surgical approach is palliative, with the more limited objectives of relieving the complications of the disease to improve the patient's clinical status and preserving bowel length. In ulcerative colitis, removal of the colon and rectum eliminates and cures the disease.

Ulcerative colitis is a disease confined to the colon and rectum and the presence of intestinal involvement outside these boundaries must be ruled out to confirm the diagnosis. Anamnestic evidence of recurrent episodes of perianal sepsis or bowel obstruction should suggest the possibility of Crohn's disease. On physical examination, the presence of active perianal sepsis and fistulae, healed scars, or anal stenosis should raise doubts about the diagnosis of ulcerative colitis. A complete preoperative evaluation should include: stool cultures to rule out an infectious etiology; small bowel radiographic series to exclude involvement of the small intestine; and colonoscopy to evaluate the extension of the disease, rule out the presence of malignancy, and obtain histological confirmation.

The majority of patients with ulcerative colitis present with bloody diarrhea, urgency, and fatigue; during an acute flare, they may present with fever and, possibly, abdominal distention. In absence of absolute indications for immediate surgery, such as perforation or carcinoma, the patient is treated initially with medical therapy [132–134]. However, a definitive treatment plan should be formulated after complete diagnostic evaluation, taking into account the extent and duration of the disease, the presence of dysplasia or cancer, medical therapy used, presence of side effects due to the medical treatment, and the patient's general condition and expectations. As in Crohn's disease an optimal treatment plan requires input from the patient as well as the surgeon and the gastroenterologist, assisted by the radiologist and pathologist.

Indications for surgery

The most common indication for surgical treatment of ulcerative colitis in our experience is the failure of medical therapy [135], as in the following scenarios:

- when maximum medical therapy proves inadequate
- patients who may be asymptomatic while on maximum medical therapy, but develop recurrence of symptoms with tapering of the medications
- when the disease progresses with worsening symptoms while the patient is receiving maximum medical therapy
- when the patient develops significant treatment-related complications.

Other indications for surgical treatment include the presence of a mass at colonoscopy, dysplasia on colonic biopsy, and dysplasia-associated lesions or masses (DALM). Colonoscopic detection of mucosal dysplasia is considered the best available surveillance tool for the detection of cancer. The presence of dysplasia has been reported in a much higher percentage of specimens of ulcerative colitis complicated by cancer than in ulcerative colitis without malignant transformation. Because the correlation between dysplasia and cancer is not constant, when dysplasia is found on surveillance colonoscopy, some suggest immediate colectomy, whereas others opt for continued surveillance. Gorfine and colleagues [136] reviewed the pathology reports of 590 patients who underwent total proctocolectomy or restorative proctocolectomy for ulcerative colitis and found that cancers were significantly more common among specimens with dysplastic changes. Specimens with dysplasia of any grade were 36 times more likely to harbor invasive carcinoma. Stage III disease was found in association with indefinite or low-grade dysplasia in 19.2% of cases. More important, tumor stage did

not correlate with dysplasia grade. These investigators concluded that although dysplasia is an unreliable marker for the detection of synchronous carcinoma, when dysplasia of any grade is discovered at colonoscopy, the probability of a coexistent carcinoma is high. Many believe that a histopathological diagnosis of dysplasia is an indication for colectomy.

Carcinoma was the indication for surgery in 2% of patients in one series [135]. Patients with ulcerative colitis are known to have an increased risk of colorectal cancer [137,138]. The risk of cancer increases with the extent and the duration of the colitis. Gyde and associates [139] reported an overall eight-fold increase in the risk of cancer in these patients, with a four-fold increase in the left-sided colitis and proctitis group and a 19-fold increase in the pancolitis group. In addition, the pancolitis group had a cumulative risk of 7.2% at 20 years and 16.5% at 30 years from diagnosis.

Acute fulminant colitis with or without an acute abdomen develops in about 13% of patients [139]. About 60% of these patients fail to respond to medical therapy [132,140] or develop persistent abdominal pain, distention, diffuse abdominal tenderness, rebound tachycardia, and fever, suggesting an acute abdomen. The acute abdomen may be due to the development of a toxic megacolon or a walled-off or free perforation. These patients should undergo emergency surgical intervention after appropriate volume resuscitation.

Massive hemorrhage is also another indication for urgent surgery. Persistent hemorrhage despite maximal medical treatment is a manifestation of severe disease. The hemorrhage originates from extensive mucosal ulcerations, rather than a definite arterial source. As such, an abdominal colectomy is necessary to remove the majority of the bleeding surface. A primary anastomosis should be avoided in favor of an ileostomy and closure of the rectal stump. With fecal diversion obtained by placing the ileostomy, postoperative rectal bleeding is rare. When it happens it usually responds to increased doses of systemic steroids, or administration of topical steroids or intrarectal tamponade with gauzes soaked in a diluted epinephrine (adrenaline) solution. Retention of the rectal stump maintains the option of a subsequent restorative procedure.

Proctocolectomy with Brooke ileostomy

Proctocolectomy with Brooke ileostomy was the gold standard for the treatment of ulcerative colitis until the early 1980s when Utsonomiya popularized the ileo pouch–anal anastomosis [141]. Since then the role of proctocolectomy with Brooke ileostomy in the surgical treatment of ulcerative colitis has become more limited, although it is still valuable in selected patients [142]. By removing all diseased epithelium, a proctocolectomy cures patients of the disease, eradicates the risk of malignancy associated with it, and eliminates the need for costly medications and time-consuming lifelong follow-up. The disadvantages of this operation include the presence of a permanent ileostomy, the potential for nerve injury during pelvic dissection, and the risk of perineal wound healing problems, albeit significantly less than in Crohn's disease.

A proctocolectomy is indicated in patients who are not candidates for an ileal pouch–anal anastomosis or a Kock pouch, either because of advanced age, the presence of poor anal sphincter function, or obesity. The operation may also be indicated if other medical problems make a more complex, longer operation too risky [143], or in the presence of a low rectal cancer in need of resection of the anal sphincter. Finally, proctocolectomy should be considered in patients who desire a single operation for cure or whose work makes it easier to handle an appliance rather than frequent bowel movements. There are no absolute contraindications to this procedure, although, in the emergent setting, it is advisable to stage the procedure with an initial abdominal colectomy. This strategy avoids the morbidity associated with the rectal dissection, which can be potentially difficult and time consuming in an unstable patient, and maintains the option of an ileal pouch–anal anastomosis as a second stage.

Preoperatively, the surgeon is well advised to have a thorough discussion with the patient so that the latter has a full understanding of this procedure and its implications on body image and self-perception. In addition, a stoma therapist, in charge of the postoperative care of the stoma, should mark the abdominal site for the ileostomy (Fig. 58.17). The appropriate site is the least inconvenient for the patient – generally away from bony prominences and previous scars, and not prone to interfere with the patient's clothing. The site is usually just lateral and inferior to the umbilicus midway between the midline and the right anterior iliac spine.

The proctocolectomy is associated with low postoperative morbidity. Delayed healing or nonhealing of the perineal wound is rare if the perineal dissection is performed in the intersphincteric plane, between the internal and external anal sphincter. Primary closure is associated with an 80%–90% success rate [144–146]. In the event of perineal wound infection, drainage of the wound abscess and debridement usually are sufficient for secondary healing. Rarely, myocutaneous flaps or skin grafts are necessary to achieve closure of the perineal wound. Impotence is reported in 2%–3% of male patients after proctectomy for benign disease [146,147]. Impotence is secondary to injury of the autonomic nerves during pelvic dissection, and its occurrence is minimized by keeping the dissection close to the rectum and by choosing an intersphincteric plane for the most distal anorectal resection. In women, dyspareunia may occur due to the posterior displacement of the vagina. Fortunately, it is usually temporary [146,147]. Late complications are largely related to the stoma and include retraction, prolapse, parastomal hernia, bleeding, stenosis, and varices. They usually require surgical revision. Other late complications are intestinal obstruction, urinary stone formation, and gallstone formation.

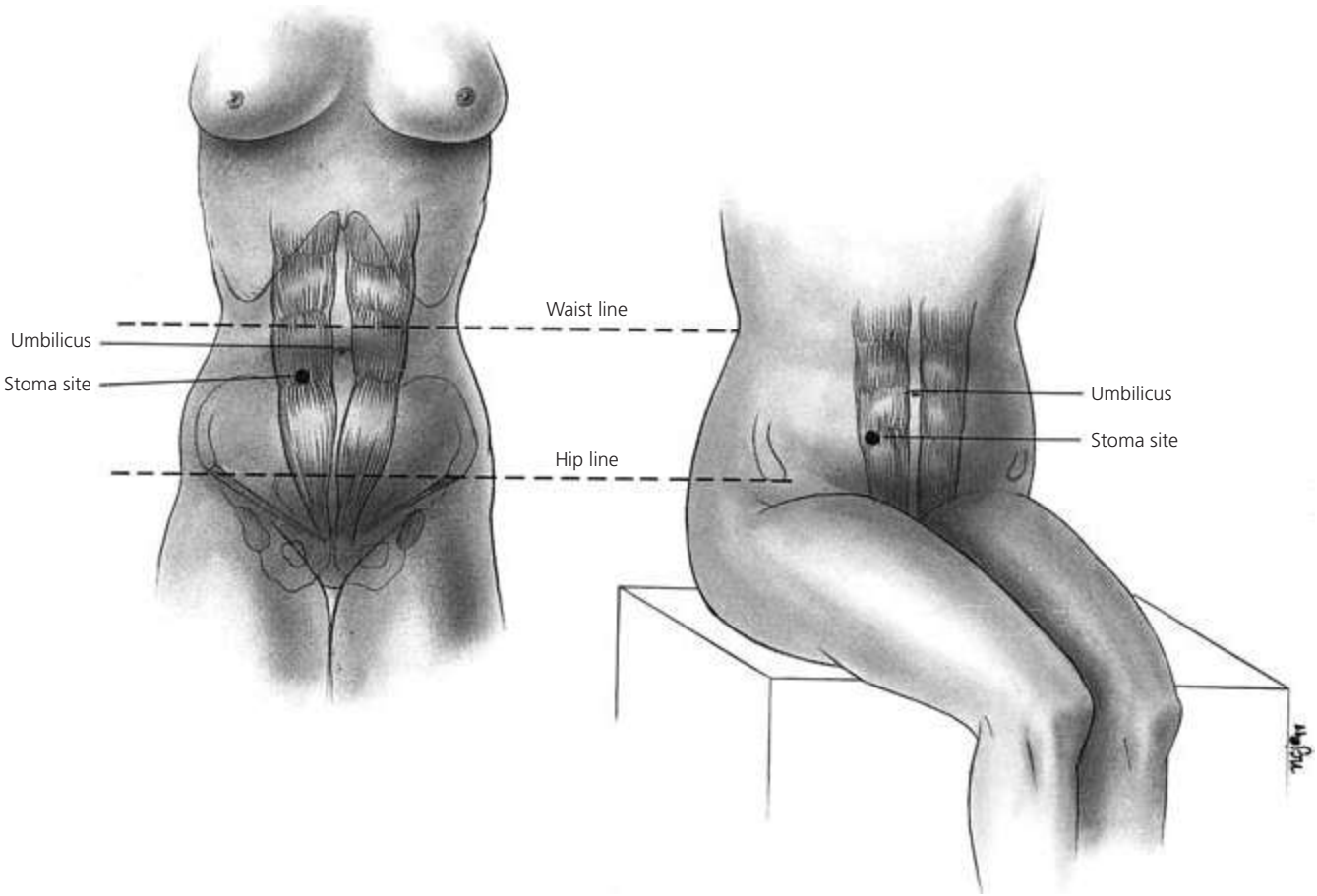


Figure 58.17 The stoma site is marked prior to surgery. The ileostomy should be located over the right rectus abdominis muscle on a flat area away from skin folds and bony prominences. From Hurst R. Proctocolectomy with ileostomy, abdominal colectomy with ileostomy, and abdominal colectomy with ileoproctostomy. In: Michelassi F, Milsom JW (eds). Operative Strategies in Inflammatory Bowel Disease. New York: Springer-Verlag, 1999:157.

Restorative proctocolectomy with ileal pouch–anal anastomosis

Most patients with ulcerative colitis who are candidates for elective operation are suitable for proctocolectomy with ileal pouch–anal anastomosis. Exceptions include patients with low rectal cancers, patients with fecal incontinence, or patients who, for work-related or personal reasons, prefer not to have a pouch [143].

Obesity is considered a relative contraindication. A fatty ileal mesentery may cause the pouch to be rather bulky and ill fitting in a narrow pelvis; furthermore, the ileal pouch may not reach the upper anal canal despite operative techniques designed to lengthen the ileal mesentery [148]. A stapled ileoanal anastomosis, as described later in the chapter, offers advantages over a conventional hand-sewn anastomosis in these challenging patients. Alternatively, patients may need to undergo an initial colectomy with ileostomy to allow for cessation of steroid therapy and a successful weight reduction program.

The presence of cancer of the colon or proximal rectum does not rule out the possibility of an ileal pouch–anal anastomosis, provided that the cancer can be completely resected during the operation [149–152]. In the presence of metastatic cancer and a short life expectancy, less extensive procedures, such as colectomy and ileorectal anastomosis or Brooke ileostomy should be considered. The Mayo Clinic group presented their experience with 77 patients with carcinoma complicating ulcerative colitis. Although pouch failure was more common in patients with cancer than in the overall registry (16% vs 7%), there were no differences between cancer and noncancer groups in operative complications, median stool frequency, incontinence, pad usage, or pouchitis [149]. A study from Toronto that reviewed function and quality of life after ileal pouch–anal anastomosis for colitis-associated neoplasia confirmed the functional results reported by the Mayo Group and showed that quality of life of these patients is excellent and equal to that of patients who have a pouch for failure of medical therapy [149].

Patients with sclerosing cholangitis and cirrhosis are at increased risk of chronic pouchitis and pouch loss after an ileal pouch–anal anastomosis, but the operation usually can be performed successfully and with morbidity rates similar

to that in patients without hepatobiliary complications of ulcerative colitis.

In the past, patient age exceeding 50 years was considered a contraindication to the procedure. However, this is no longer true. The clinical results in fit patients over the age of 50 are similar to those in younger patients [153–157]. Takao and colleagues [156] have shown that there are no significant differences among age groups relative to manometric results, frequency of bowel movements, incontinence scores, or overall patient satisfaction. The same results were confirmed by others [153,155]. Jorge and associates [154] showed that the effect of ileoanal reservoir on anal sphincter function in patients older than 50 years is similar to that in younger patients. Although impairment of internal anal sphincter function is more pronounced after ileoanal reservoir in older patients, this appears to be transient, as it is in younger patients.

Although there are small series showing that an ileal pouch–anal anastomosis is feasible in fulminant ulcerative colitis [158], most patients with emergent indications to surgical treatment should undergo an initial colectomy with ileostomy followed by ileal pouch–anal anastomosis [159] several months later.

The ileal pouch–anal anastomosis is most commonly performed in two stages. The colon and the rectum are excised to the pelvic floor, an ileal pouch is constructed with the terminal ileum (Fig. 58.18), an ileal pouch–anal anastomosis is constructed, and a diverting loop ileostomy is fashioned. The loop ileostomy is closed at a second operation about 3 months later. The operation, or portions of it, can be accomplished by using laparoscopic techniques.

Occasionally a diverting ileostomy can be omitted after construction of the ileal pouch. Omitting this transforms an elective ileal pouch–anal anastomosis into a single-stage procedure and avoids the need for a third stage in those patients who underwent an initial colectomy and ileostomy in the emergent setting or in elective circumstances (indeterminate colitis, obesity).

Several large series examining complications and long-term outcome have been published [160–163]. The Cleveland Clinic group reported their experience with 1005 ileal pouch–anal anastomoses, 858 of which had been performed for ulcerative colitis [161]. One death (0.1%) was reported, related to pouch necrosis and sepsis. The overall morbidity rate was high, with 630 patients (62.7%) suffering 1218 complications. Septic complications and reoperation occurred in 6.8% and 24%, respectively. The ileal pouch was removed in 34 patients (3.4%) overall, but in only 15 patients (1.8%) with ulcerative colitis. Approximately 25% of patients developed pouchitis. Functional results and quality of life were good to excellent in 93% of patients. The Mayo Clinic series [160,163] of more than 1300 patients showed similar results. Three patients died as a consequence of the procedure. Postoperative pelvic sepsis rates decreased from 7% in the 1981–85 period to 3% in the 1991–94 period. After mean follow-up of 6.5 years, the mean number of stools was five during daytime and one at nighttime. Frequent daytime and nighttime incontinence occurred in 7% and 12% of patients, respectively, and did not change over a 10-year period. The cumulative probability of suffering at least one episode of clinical pouchitis was 18% and 48% at 1 and 10 years, respectively, and the cumulative probability of pouch failure at 1 and 10 years was 2% and 9%, respectively [163]. The most common

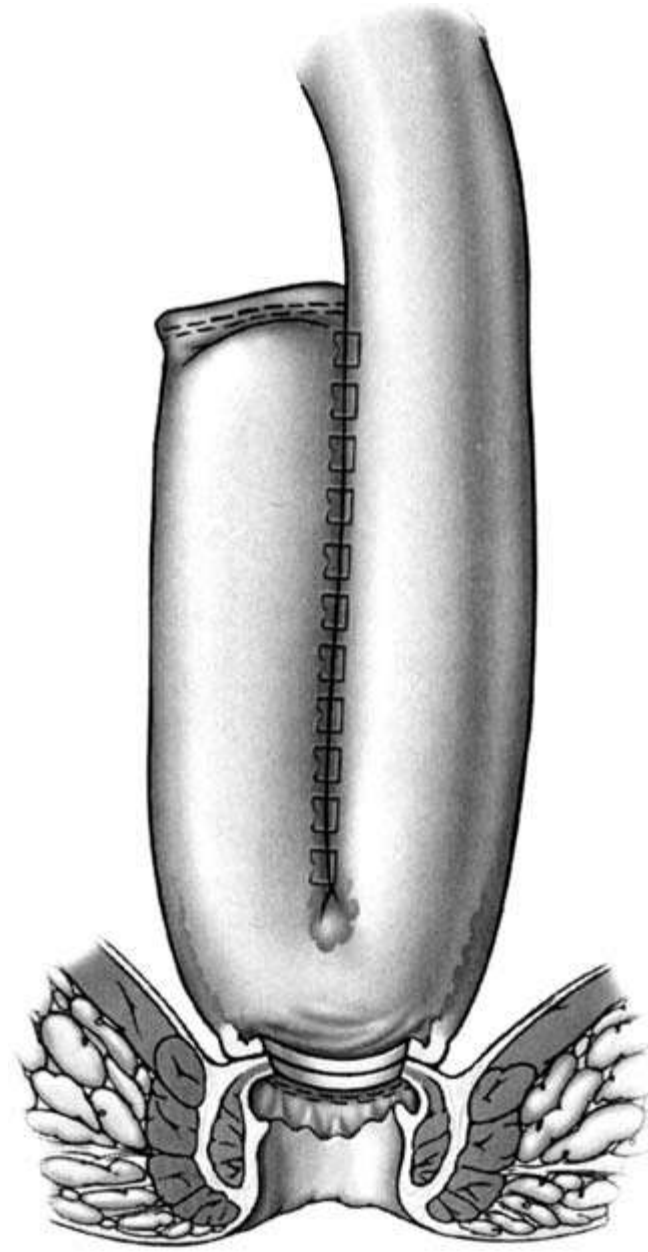


Figure 58.18 Stapled J-pouch ileoanal anastomosis. From Michelassi F, Takanishi D, McLeod RS, et al. Ileal reservoirs. In: Michelassi F, Milsom JW (eds). Operative Strategies in Inflammatory Bowel Disease. New York: Springer-Verlag, 1999:186.

early postoperative complications were bowel obstructions (13%), pelvic sepsis (5%) and wound sepsis (3%), transient urinary dysfunction (7%), and large enteric losses from the temporary loop ileostomy. Postoperative impotence and retrograde ejaculation was noted in up to 3% of men; transient dyspareunia occurred in 11% of patients after operation [160]. Pregnancy and delivery are well tolerated by women with a pouch [160].

Late complications of the procedure include: anal fistulae and abscesses, which may need drainage and possibly temporary fecal diversion; stricture of the anastomosis, which can be repeatedly dilated or treated with pouch advancement in presence of dense scar tissue or inflammation of the mucosal cuff; and pouchitis, which is the most frequent late complication. Patients with pouchitis present with abdominal cramps, frequent watery stools, urgency, incontinence, malaise, and fever. Of 120 patients who underwent ileal pouch–anal anastomosis for ulcerative colitis at the University of Chicago, 50 suffered at least one episode of pouchitis. Two-thirds of patients had multiple episodes. Chronic pouchitis occurred in six patients, necessitating pouch removal in two [164]. In a prospective evaluation of 149 patients in Sweden [165], the risk of pouchitis was highest during the initial six postoperative months. The cumulative risk leveled off after 2 years but was substantial (51%) at 4 years. Similar to our data and those of the Mayo Clinic, fewer than 10% of the Swedish patients had severe chronic pouchitis, and only two patients (1.3%) had their pouch removed because of pouchitis.

The etiology of pouchitis remains unknown. It may be due to bacterial overgrowth secondary to poor pouch emptying, or to an immunological reaction to bacterial products or a chemical injury; ischemia and reperfusion injury has also been postulated; or it may be a novel manifestation of inflammatory bowel disease. Patients who are positive for antineutrophil cytoplasmic antibody (ANCA) are more likely to develop pouchitis than those who are not [166]; also, patients with primary sclerosing cholangitis and extraintestinal manifestation of ulcerative colitis are at a higher risk of developing pouchitis, especially of the chronic type [167]. Backwash ileitis, however, does not predispose to pouchitis. Pouchitis is rare in patients who have undergone ileal pouch–anal anastomosis for familial polyposis, an observation that seems to suggest that whatever causes colitis may predispose to pouchitis.

The activity of pouchitis can be scored using a Pouchitis Activity Index [168], which uses clinical, histological, and endoscopic criteria. The activity index may help with the medical treatment of pouchitis, which is based on oral antibiotics (metronidazole and ciprofloxacin) in the majority of cases. Interestingly, smoking seems to prevent pouchitis [169].

The risk of cancer in the ileal pouch is unknown. Although no reports have appeared describing the development of carcinoma in the mucosa of a pelvic pouch, the pouch mucosa undergoes structural transformation: initially the mucosa appears normal or with mild villous atrophy and, possibly, mild inflammation. Then the mucosa shows transient atrophy with temporary moderate inflammation followed by normalization of architecture. Finally, it develops a pattern of persistent atrophy with severe inflammation [170]. These changes can progress to dysplasia [171,172]. The continual contact with a noxious medium and changes in bacterial flora are hypothesized as causes of these phenomena. The large number of proliferating cells and the frequent presence of inflammation provide fertile ground for carcinogenesis. Pouch surveillance is probably indicated, as the natural history of the pouch mucosal change remains unknown.

Controversies in ileal reservoir surgery

The technique of restorative proctocolectomy and ileal pouch–anal anastomosis for adult patients was originally described by Parks and Nicholls [173] and Utsunomiya and colleagues [141]. Since these initial descriptions several modifications have been introduced into clinical practice; these mainly involve the configuration of the pouch and the method of anastomosing the pouch to the anal canal (Table 58.2).

In the initial description by Parks and Nicholls [173], the ileal reservoir was fashioned as a triple-loop S-pouch (Fig. 58.19) with a 5-cm-long exit conduit, which created problems with evacuation. Subsequently the limb was shortened to less than 2 cm with significant functional improvement [174]. The two other pouch designs that are in use are the double-loop J-pouch [141] and the quadruple-loop W-pouch [175,176]. The lateral isoperistaltic H-pouch (Fig. 58.20) is no longer used and it is of historical interest only [177]. Each pouch design has its own advantages, and no single configuration is ideal for every patient. The S-pouch with the long exit limb allows for further reach in tall male patients with short mesentery [178]. The W-pouch (Fig. 58.21) has larger capacity and better compliance [175] and is ideal when part of the terminal ileum has been or needs to be resected. The J-pouch is technically easier and faster to perform [179,180]. When comparing the W-pouch with either the S-pouch [178] or

Table 58.2 Controversies in pouch surgery

Pouch configuration
J
W
S
The anal transition zone
Mucosectomy and hand-sewn pouch–anal canal anastomosis
Stapled pouch–distal rectal anastomosis
Diverting loop ileostomy
One-stage procedure
Multiple-stage procedure

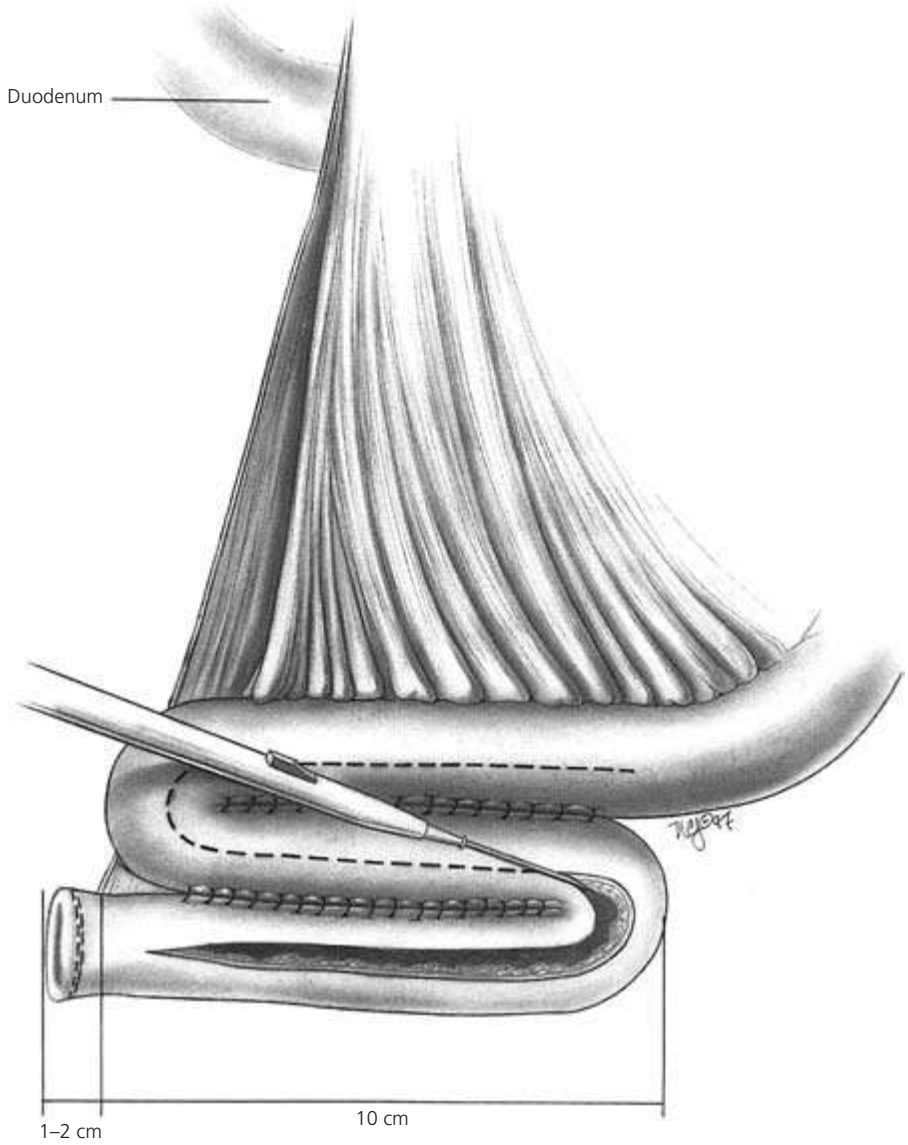


Figure 58.19 Construction of a triple loop S-pouch. From Michelassi F, Takanishi D, McLeod RS, et al. Ileal reservoirs. In: Michelassi F, Milsom JW (eds). Operative Strategies in Inflammatory Bowel Disease. New York: Springer-Verlag, 1999:186.

the J-pouch [176,181,182], patients with the W-pouch have significantly fewer bowel movements compared with the S-pouch or J-pouch group during the first 6–12 months after closure of the ileostomy. However, Keighley and colleagues [183], comparing the J-pouch with the W-pouch in a randomized prospective trial, did not confirm those findings, and Johnston and colleagues [184] showed that both types of pouches conferred the same functional results at 1 year.

We elect to perform a J-pouch for the vast majority of our patients because of the lower complication rate and the excellent functional results [135,164,179,180,185]. We reserve the S-pouch for patients in whom we suspect a difficult "reach" to the anal canal, and the W-pouch for patients in whom a substantial portion of terminal ileum has been or needs to be sacrificed.

Controversy still exists on whether the ileoanal anastomosis should be stapled or hand sewn. The initial descriptions of ileal pouch–anal anastomosis included a mucosectomy to the dentate line [141,173,186]. The anal dilation necessary for a complete mucosectomy [187] or the eversion of the anorectum advocated by some authors [188,189] caused significant decrease in the maximum resting pressure [190] and increase of the threshold sensation, which correlated with increased number of episodes of incontinence [188,189]. Furthermore, removal of the most distal lower rectal–upper canal epithelium, the anal transitional zone (ATZ), resulted in the loss of the anoderm sensory capacity, which, together with the rectoanal inhibitory reflex, allows for sampling of rectal contents and contributes to continence [191]. The technique of stapled ileal pouch–anal anastomosis was introduced to

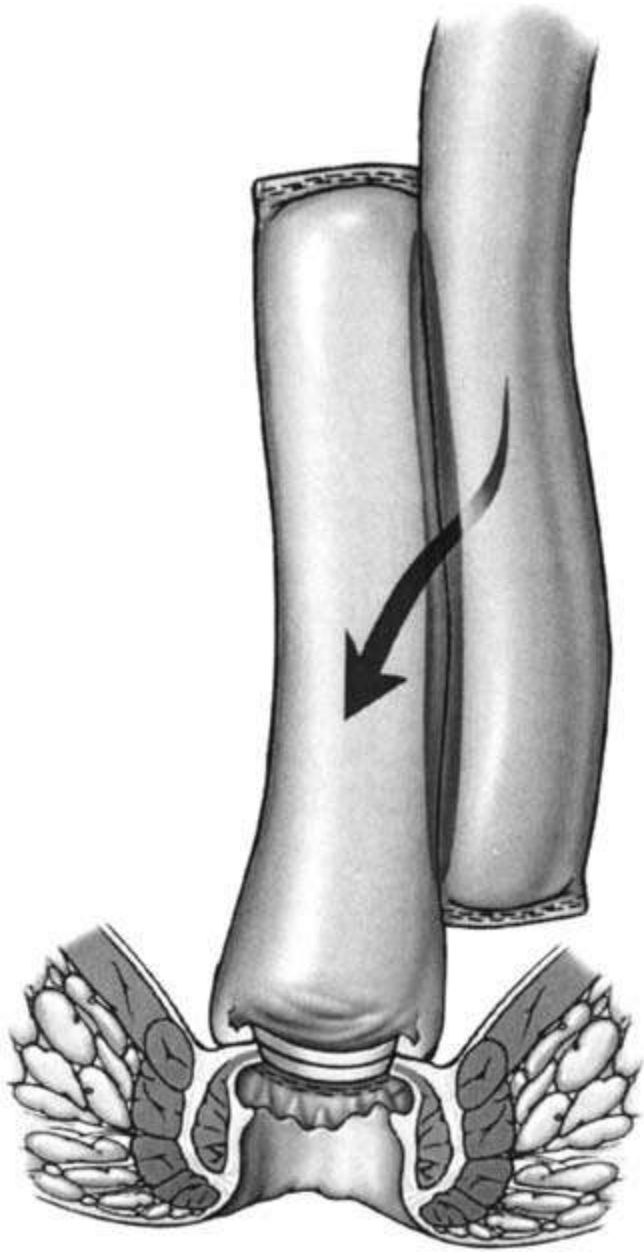


Figure 58.20 Isoperistaltic H-pouch. From Michelassi F, Takanishi D, McLeod RS, et al. Ileal reservoirs. In: Michelassi F, Milsom JW (eds). Operative Strategies in Inflammatory Bowel Disease. New York: Springer-Verlag, 1999:186.

Figure 58.21 Quadruple loop W-pouch. From Michelassi F, Takanishi D, McLeod RS, et al. Ileal reservoirs. In: Michelassi F, Milsom JW (eds). Operative Strategies in Inflammatory Bowel Disease. New York: Springer-Verlag, 1999:186.

address some of the shortcomings of the hand-sewn technique. The potential advantages of the stapled technique include less tension on the anastomosis, ease of construction, preservation of the lower rectal–upper anal canal, and less trauma to the sphincter mechanism. These advantages translate to a shorter operation with fewer anastomotic complications and, potentially, better long-term functional results.

Several comparative long-term studies have reported better functional results for the stapled ileal pouch–anal anastomosis [190,192–195]. Gemlo and associates [190] evaluated 235 patients with a mean follow-up of 70 months and found that elimination of a mucosectomy dramatically reduced nocturnal minor and major incontinence, daytime minor incontinence, and daytime protective pad use. Sagar and colleagues [195] studied anal physiological results in 20 patients up to 12 months after stapled ileal pouch–anal anastomosis. Resting anal pressure, which decreased immediately after the surgical procedure, was almost back to normal 12 months later, the rectoanal inhibitory reflex was present in 19 patients, and sampling was observed in 17 patients. The ability to discriminate flatus from feces was associated with the return of the rectoanal reflex and sampling. The functional results obtained following a stapled or hand-sewn ileal pouch–anal

anastomosis were evaluated by several prospective randomized studies [196–198]. No statistically significant differences were detected, although the validity of the results of these studies is compromised by their small size and by the short follow-up.

Several studies have looked at the rate of anastomotic complications after mucosectomy and hand-sewn ileal pouch–anal anastomosis. Preserving a short rectal cuff with the stapled technique lessens the tension on the anastomosis and reduces anastomotic complications. In a retrospective study, the Cleveland Clinic group evaluated 692 ileal pouch–anal anastomoses, 238 hand-sewn and 454 stapled. In the hand-sewn ileal pouch–anal anastomosis group, 25 patients (10.5%) had 32 septic complications, and 24 required 89 reoperations. In seven patients, the pouch was excised. In the stapled ileal pouch anal anastomosis group, 21 patients (4.6%) had 23 septic complications, and 14 required 40 reoperations. One patient needed pouch excision. Patients with hand-sewn anastomoses experienced more early septic complications and more pouch excisions related to those complications than patients with stapled ileal pouch–anal anastomosis [199]. Yet, when these complications were evaluated in prospective randomized studies, no difference was noted [196–198].

The potential disadvantages associated with preserving the ATZ include persistence of ulcerative proctitis symptoms and risk of malignant degeneration. The Cleveland Clinic [200,201] noticed that symptomatic inflammation of the retained mucosa occurred in 14.7% of their patients; 4.1% of patients had inflammation of the anal canal alone, and 10.6% had pouchitis in addition. Surgical treatment was needed in 12.9% of patients with anal canal inflammation, and in 10.6% of patients with anal canal inflammation plus pouchitis [200,201]. Such patients are usually treated with topical steroids or 5-aminosalicylate (5-ASA), and when that fails and surgical treatment is needed, a transanal mucosectomy with ileal pouch advancement is performed, with excellent results [201].

Advocates of the mucosectomy argue that the entire diseased anorectal mucosa, including the ATZ, must be removed to eliminate the risk of future dysplasia and cancer. To determine the long-term risk of dysplasia and cancer in the retained mucosal cuff after stapled ileal pouch–anal anastomosis, the Cleveland Clinic group published their series of 210 patients with at least 5 years (median 77 months) of follow-up [202]. Dysplasia developed in seven patients (3.3%) at a median of 11 months postoperatively. Patients with history of cancer or dysplasia in the colon or rectum were at a higher risk of developing dysplasia. Two patients, each with low-grade dysplasia detected on three separate occasions, underwent mucosectomy 29 and 38 months after detection of low-grade dysplasia and, fortunately, no cancer was found. The five other patients with dysplasia on one or two occasions were treated expectantly and were apparently dysplasia-free for a median of 72 months. More important, preservation of the ATZ did not lead to the development of cancer after 5–10 years of follow-up.

The actual extent of the ATZ is highly variable and mucosectomy does not ensure its complete removal. Fenger [203], using Alcian blue staining, found that the mean span of the ATZ was 8.9 mm (range 0–20 mm), starting up to 6 mm below the dentate line. By contrast, Thompson-Fawcett and colleagues [204] measured the ATZ by two techniques: whole-mount Alcian blue staining and a computer map of the histological findings based on longitudinal sections taken every 3 mm. They found that the Alcian blue technique overestimates the length of the ATZ, which usually commences just above the dentate line. The median length of the ATZ measured from computer maps of the histology was only 4.5 mm. Due to this variability, mucosectomy does not reliably remove the entire rectal mucosa [205]. Small islets of residual rectal mucosa have been identified in up to 14% of patients; in 7% at the ileoanal anastomosis [205].

This probably explains why the reported cases of cancer after a pouch procedure are all in patients who had a mucosectomy [206–208], except in one case, where the cancer was detected 16 months after stapled ileal pouch–anal anastomosis following long-standing ulcerative colitis complicated by a cancer in the upper rectum [209]. It is likely that this cancer represents a local recurrence rather than a metachronous cancer originated in the ATZ.

No hard data exist to support the superiority of one technique over the other, and there is a role for both procedures in clinical practice [135,179]. There is an advantage in preserving the ATZ in older patients with borderline sphincter function, and in tall or obese patients to decrease tension on the anastomosis. Mucosectomy may be advisable in presence of dysplasia or cancer in the colon or rectum in the pediatric population [210] and in patients with primary sclerosis cholangitis, known to have a high risk of dysplasia and cancer [211]. If the ATZ is preserved, surveillance anoscopies with biopsies are performed on an annual basis to detect dysplasia and indicate the need for a completion mucosectomy.

With the perception that anastomotic complications are reduced by the stapled ileal pouch–anal anastomosis technique, some authors have questioned the need for a diverting ileostomy [212]. The Medical College of Virginia group started to perform stapled ileal pouch–anal anastomosis without diverting ileostomy in the early 1980s [213,214]. Their series of 193 patients who underwent a stapled ileal pouch–anal anastomosis as a one-stage procedure without ileostomy, was reported with a mean follow-up of 5.1 years [214]. They noticed an anastomotic leak rate of 12% (23 patients), with 5% (9 patients) requiring a diverting ileostomy; ileal pouch–anal anastomosis function was excellent in 19 of these patients, although 2 required permanent ileostomies. Daytime stool control was 95% and nighttime control was 90%. In patients taking steroids, there was no significant difference in complication rate.

Similar results were reported by Mowschenson and colleagues [215]. Among 102 patients they reported an anastomotic leak and subsequent diverting ileostomy in 9.8%. Overall 74.8% of their patients reported total satisfaction, and 84.7% regarded themselves as being in perfect health. Once again patients on steroids did not experience a higher complication rate. These results are in contrast with several studies where patients on steroids either had a higher incidence of anastomotic complication [216–218] or were not even considered candidates for a one-stage procedure [219].

The Mayo Clinic group [160,216] and the Cleveland Clinic group [161,217] are strong advocates of a two- or three-stage operation, especially in patients who are taking more than 20 mg of prednisone. When comparing the two procedures these studies reported septic complications in up to 22% of the nondiverted group [216], and it was noted that half of the nondiverted patients with septic complications required relaparotomy for diversion and drainage, whereas none of the patients in the diverted group with septic complications required surgery of any kind [217]. A select group of patients may benefit from the avoidance of a temporary stoma and its associated complications [217]. Healthy young patients, not on immunosuppressants or steroids, whose surgery was uneventful and who show minimal tension on the anastomosis, may be better served by a one-stage procedure. Leaving a rectal tube in these patients for a few days avoids pouch distention and perianal skin irritation due to the initial diarrhea.

Proctocolectomy with continent ileostomy

The proctocolectomy with continent ileostomy, or Kock pouch, offers an alternative to an ileal pouch–anal anastomosis for a few selected patients with ulcerative colitis. Specifically, this operation should be offered in specialized centers to:

- patients with a locally advanced low rectal cancer who will need adjuvant therapy postoperatively
- patients who already have a Brooke ileostomy, after proctocolectomy, and wish to have a continent ileostomy
- patients who are not candidates for an ileal pouch–anal anastomosis because of poor sphincter function
- patients who prefer a continent ileostomy to an ileal pouch–anal anastomosis as a personal choice
- patients who have failed an ileal pouch–anal anastomosis but prefer a continence-preserving procedure to a Brooke ileostomy [220,221].

Contraindications to this procedure include older age, Crohn's disease, obesity, critical illness, and poor psychological fitness. This procedure has also been performed in the pediatric population with satisfactory results [222,223].

The distal 30 cm of the terminal ileum are used to form the internal Kock pouch. Intussuscepting the terminal ileum backward into the pouch and anchoring the intussusceptum in place with staples creates a valve that prevents distal outflow from the pouch. The end of the terminal ileum is brought to the skin surface as a flash stoma (Fig. 58.22). The patient empties the pouch intermittently by passing a catheter

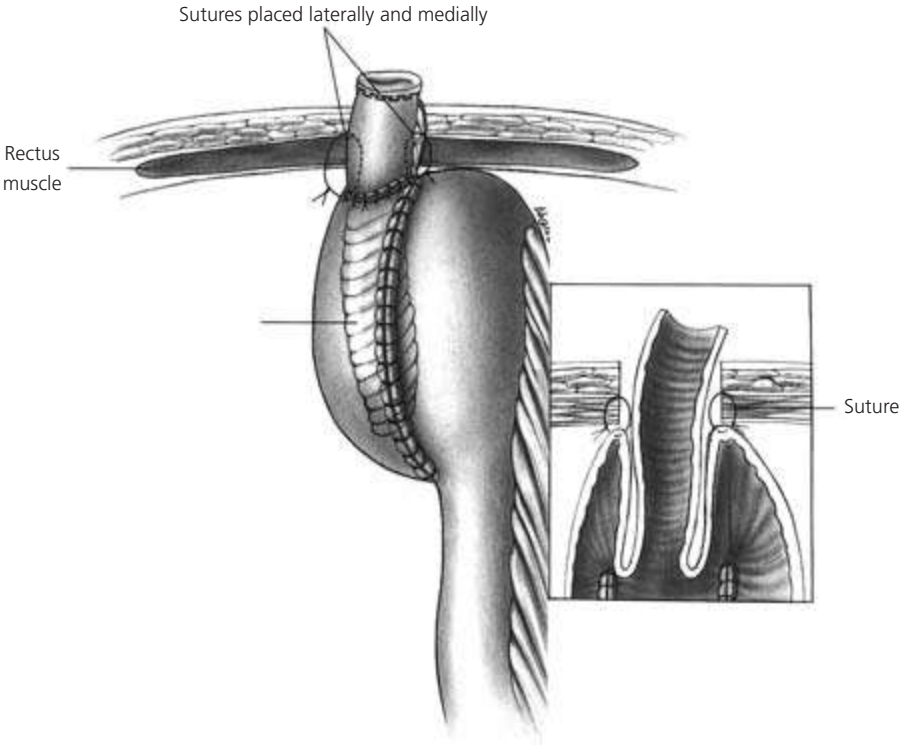


Figure 58.22 Kock pouch. From Cohen Z, McLeod RS. Proctocolectomy with Kock pouch. In: Michelassi F, Milsom JW (eds). Operative Strategies in Inflammatory Bowel Disease. New York: Springer-Verlag, 1999:215.

through the stoma and valve into the pouch. Between intubations, the pouch is continent to both gas and stool.

Kock pouch procedures have fallen out of favor because the majority of patients with ulcerative colitis undergo an ileal pouch–anal anastomosis. The largest series reporting on the Kock pouch were published in the late 1970s [220,224,225]. Overall long-term follow-up showed excellent results, between 70% and 89% continence for gas and stool, with 95% of these patients free from wearing an appliance [220,224,225]. The two main long-term problems with continent ileostomy are malfunction of the valve and pouchitis. Malfunction of the valve causes incontinence and difficult intubation of the pouch, resulting in reoperation. Between 11% and 20% of patients developed this problem [220,224,225], precipitating the need for valve revision. The incidence of pouchitis is nearly identical to that after ileal pouch–anal anastomosis, and management is similar.

Kohler and colleagues [226] evaluated quality of life in 406 patients with Brooke ileostomy, 313 with Kock pouches, and 298 with ileal pouch–anal anastomosis. They found that patients with ileal pouch–anal anastomosis had fewer restrictions in sports and sexual activity than the Koch pouch group, who in turn had fewer restrictions in these activities, but more travel restrictions than patients with Brooke ileostomy. Overall, as regards social life, recreation, work, and family activities, there was no difference between those groups and more than 90% of patients in each group were satisfied with the results [226].

Total abdominal colectomy with Brooke ileostomy

In patients who are too ill to withstand a definitive procedure for ulcerative colitis, a total abdominal colectomy with end ileostomy is warranted [227,228]. Acute toxic colitis nonresponsive to medical therapy [229] with or without megacolon or perforation or hemorrhage is the primary indication for a total abdominal colectomy–end ileostomy, and either a rectal or rectosigmoid Hartmann stump or mucous fistula. This procedure eradicates the disease, requires no bowel anastomosis or pelvic dissection, and allows the patients to be weaned off medications.

Potential candidates for a total abdominal colectomy are those patients who carry a diagnosis of indeterminate colitis. This preliminary step allows the pathologist to obtain a final diagnosis after reviewing the resected colon, thus avoiding performance of an ileal pouch–anal anastomosis in a patient with Crohn's disease. As mentioned earlier, this procedure may also be indicated in the obese patient as a first step toward an ileal pouch–anal anastomosis. In these patients, a total abdominal colectomy with Brooke ileostomy eradicates most of the active disease, allows for decrease and eventual cessation of any steroid therapy, and for the possibility of successful weight reduction.

The abdominal colectomy with ileostomy entails resection of the entire colon with exteriorization of the terminal ileum as an end ileostomy. Distally the colon is usually transected at the level of the distal sigmoid or upper rectum, based on the surgeon preference. Controversy persists regarding the management of the rectal stump. Some authors prefer to place it as a closed rectal stump into the subcutaneous tissue of the midline abdominal incision, whereas others leave it as an open mucous fistula through a separate abdominal wound [227]; the majority prefer closure of the intestinal stump and intrapelvic placement (Hartmann pouch) [227,229]. Only small comparative studies have examined this issue. Carter and associates [230] suggested that exteriorization of the closed rectal stump following total abdominal colectomy is associated with fewer pelvic septic complications and minimal local morbidity, facilitates subsequent pelvic dissection, and is not associated with increased disease activity in the retained rectum. Yet, the increased incidence of wound infections mitigates against this strategy as routine. A surgeon will be well served by choosing appropriately from the different options after having evaluated the patient's general condition, the presence or absence of generalized peritonitis, and the local status of the rectosigmoid.

The removal of the vast majority of the diseased intestine and the diversion of the fecal stream from the residual disease allow these patients to recover from the debilitating effects of high-dose medications [229], malnutrition, and sepsis. Delaying the ileal pouch–anal anastomosis does not affect postoperative functional results [228].

Colectomy with ileorectal anastomosis

Colectomy with ileorectal anastomosis is performed in only a few selected patients. The obvious advantages of this procedure are its relative simplicity, the preservation of the anal route of defecation, and the maintenance of fecal continence in all but the most debilitated patient. In addition, the risk of pelvic nerve dysfunction and perineal wound problems is virtually eliminated. Yet, this operation does not excise the diseased rectum, which maintains a risk of neoplastic transformation and may continue to cause symptoms. Indications for this conservative approach include patients with relative rectal sparing at high medical risk or older patients who are not good candidates for ileal pouch–anal anastomosis; in addition, patients with metastatic colon carcinoma in ulcerative colitis are good candidates due to their limited life expectancy [86].

Contraindications include severely diseased rectum, dysplasia or nonmetastatic rectal carcinoma, and poor sphincter function; noncompliant patients are subject to lifelong surveillance [143].

Based on the patient's medical condition and presentation, colectomy and ileorectal anastomosis may be performed as a one-stage procedure or as a two-stage procedure in those requiring emergency surgery. This operation can also be done by laparoscopic techniques.

The presence of pelvic anastomosis puts these patients at risk of septic complications due to an anastomotic leak, which occurs in about 4% of patients [84]. Dehiscences can manifest themselves in the immediate postoperative period as diffuse peritonitis, or later as abscess or fistula. When diffuse peritonitis occurs, patients need to be emergently reexplored, drained, and diverted; if the dehiscence manifests itself as a pelvic abscess, this can be treated by percutaneous, transrectal, transvaginal, transabdominal, or transperineal drainage, depending on the location of the abscess, and, possibly, proximal fecal diversion. Patients who have suffered an anastomotic dehiscence have worse functional results, with more than eight stools per day, continued need for steroids, and incontinence. Eventually, 12%–56% of these patients require proctectomy within a few years [82,231,232].

Long-term poor function can also be due to recurrent or worsening proctitis. Endoscopy and biopsy can guide in the diagnosis and treatment. Persistent disease requires either topical or systemic therapy and, ultimately, proctectomy may be necessary [144].

The risk of cancer in the rectal remnant has been reported to be 15.3% at 12 years [82], but it varies considerably between series [231,232] due to the difference in follow-up rates. The risk, however, is major, considering that most of these patients are relatively young.

More than 90% of patients with a functioning ileorectal anastomosis considered their health status to be better than before surgery [82]. Comparative studies, however, have shown no major benefit of colectomy and ileorectal anastomosis over ileal pouch–anal anastomosis in terms of postoperative morbidity, mortality, and long-term function [233,234].

Laparoscopic surgery for ulcerative colitis

As experience in laparoscopy has increased, both technique and instrumentation have improved. Laparoscopic-assisted restorative proctocolectomy has been shown by various authors to be technically safe and feasible [235–237]. In a case-matched series comparing laparoscopic and open restorative proctocolectomy procedures, Marcello and colleagues [237] have shown that return of intestinal function and length of hospital stay are reduced in the laparoscopic group without increase of morbidity or mortality. Patients undergoing laparoscopic restorative proctocolectomy have been found to have functional outcomes similar to patients undergoing open surgery and are more satisfied with the cosmetic result of the scar [238]. The laparoscopic approach has also been shown to be safe and feasible for colectomy in the setting of severe acute colitis [239].

Different techniques have been proposed for laparoscopic-assisted restorative proctocolectomy [235–237]. In general, the entire colon is mobilized intracorporeally. The mesenteric vessels are divided either intracorporeally or extracorporeally through a periumbilical or Pfannenstiel incision, based on surgeon preference and skills. The rectal dissection can be accomplished intracorporeally. Some authors, however, argue that because a Pfannenstiel incision is often used to extract the specimen, the dissection should be safely and expeditiously performed through that approach [236]. Once the specimen has been delivered through the incision, the rectum is divided at the top of the anal canal using a linear stapler, and an ileal pouch is constructed. A double-stapled anastomosis is then fashioned in a standard procedure. The use of a diverting loop has been advocated after laparoscopic-assisted ileal pouch–anal anastomosis [235–237]. The role of laparoscopy in the surgical treatment of patients with ulcerative colitis is likely to expand in the future.

Indeterminate colitis

A definitive pathological diagnosis is not feasible in 5%–20% of patients with pancolitis as the only manifestation of inflammatory bowel disease, even after the entire proctocolectomy specimen has been analyzed [66,240–243]. This group of patients has been classified as having indeterminate colitis. The fear that many of these patients with indeterminate colitis may actually harbor Crohn's disease [244] has created uneasiness about the ultimate treatment recommendation [66]. The largest series of patients with indeterminate colitis with a long-term follow-up after ileal pouch–anal anastomosis is from the Mayo Clinic [243]. The authors evaluated 82 patients with indeterminate colitis with a median follow-up of 83 months. They noticed that 15% of patients with indeterminate colitis and only 2% of patients with ulcerative colitis, as a control group, had their original diagnosis changed to Crohn's disease. At 10 years, patients with indeterminate colitis had significantly more episodes of pelvic sepsis, pouch fistula, and pouch failure (27%) than patients with ulcerative colitis [243]. Marcello and colleagues [240] also reported a 13% incidence of Crohn's disease in patients with an initial diagnosis of indeterminate colitis and a 37% pouch failure rate in this group.

Considering that approximately 15% of patients with indeterminate colitis will develop Crohn's disease over time, Bodzin and colleagues [245] advocated ileorectal anastomosis over ileal pouch–anal anastomosis in patients with indeterminate colitis. Yet, because approximately 85% of patients with indeterminate colitis will remain free of Crohn's disease, unless there are preoperative signs suggestive of Crohn's disease [93], ileal pouch–anal anastomosis, rather than ileorectal anastomosis, should be considered as an option in the treatment of these patients. Patients should be informed preoperatively that they face a higher risk of development of Crohn's disease, but should also be reassured that, after ileal pouch–anal anastomosis, they will experience long-term outcomes identical to those of patients with ulcerative colitis [243], in the absence of development of Crohn's features.

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D COLON

59 Miscellaneous inflammatory and structural disorders of the colon

Meredythe A. McNally, Thomas C. Smyrk, William J. Tremaine

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Microscopic colitis

Microscopic colitis is a cause of chronic watery diarrhea that may be accompanied by abdominal pain and weight loss. Severity can range from mild, self-limited episodes to severe, medically refractory symptoms [1]. "Microscopic colitis" was proposed in 1980 as a descriptive term for patients with chronic diarrhea and normal findings on radiographic and endoscopic examination [2]. Two subtypes of microscopic colitis are described: collagenous colitis and lymphocytic colitis. The term collagenous colitis was first used by Lindstrom in 1976 to describe a woman with chronic watery diarrhea with normal proctoscopic findings but rectal mucosal histological changes of subepithelial fibrosis and chronic inflammation [3]. In 1989, the term lymphocytic colitis was introduced by Lazenby and colleagues to describe an entity with a clinical presentation similar to collagenous colitis, yet distinguishable by the histological changes of intraepithelial lymphocytosis and expanded lamina propria without an abnormally thickened subepithelial collagen band [4]. Although both subtypes have a similar clinical presentation, they are distinguishable by these histological characteristics (Fig. 59.1a–d). The relationship between collagenous colitis and lymphocytic colitis is not clear, and it is unknown if these conditions are two separate disease entities or part of a spectrum of a single disease.

Prevalence and incidence

Microscopic colitis is found in about 10% of patients with chronic diarrhea [5,6]. Prevalence and incidence vary between collagenous and lymphocytic colitis. In North America, the prevalence of collagenous colitis is 36/100 000 population whereas the prevalence of lymphocytic colitis is 64/100 000 population [7]. The incidence of microscopic colitis in North America is increasing significantly with time (0.8/100 000 population between 1985 and 1989 compared with 19.1/100 000 population for the period 1998–2001) [7]. As with prevalence, incidence also differs between collagenous colitis and lymphocytic colitis in North America, namely 5.1/100 000 population and 9.8/100 000 population, respectively [7]. Individuals with microscopic colitis are most often in their sixth or seventh decades, and incidence increases with age. Pediatric cases and familial occurrences, although reported in the literature, are uncommon [8–13]. Historically, women reportedly have been chiefly affected, especially for collagenous colitis, with female-to-male ratios as high as 20:1 [14–17]. However, a comprehensive review of all published microscopic colitis cases through 1994 demonstrated no significant gender predominance for either collagenous colitis or lymphocytic colitis [18]. Pardi et al. found no significant gender difference in lymphocytic colitis patients [7]. An association between microscopic colitis and autoimmune disorders (e.g., celiac disease, thyroid disorders, and diabetes) and rheumatological disorders (e.g., rheumatoid arthritis) has been reported [13,15,16,18,19]. Up to one-third of patients with celiac disease may show histological changes consistent with microscopic colitis [20,21]. If patients with celiac disease do not improve on a gluten-free diet, one should consider microscopic colitis as a cause of the diarrhea [22].

Clinical features and natural history

The predominant symptom for both lymphocytic and collagenous colitis is chronic nonbloody diarrhea, with stool volumes ranging from 300 to 1700 g/24 h, with occasional fecal incontinence and decreased stool volume when fasting

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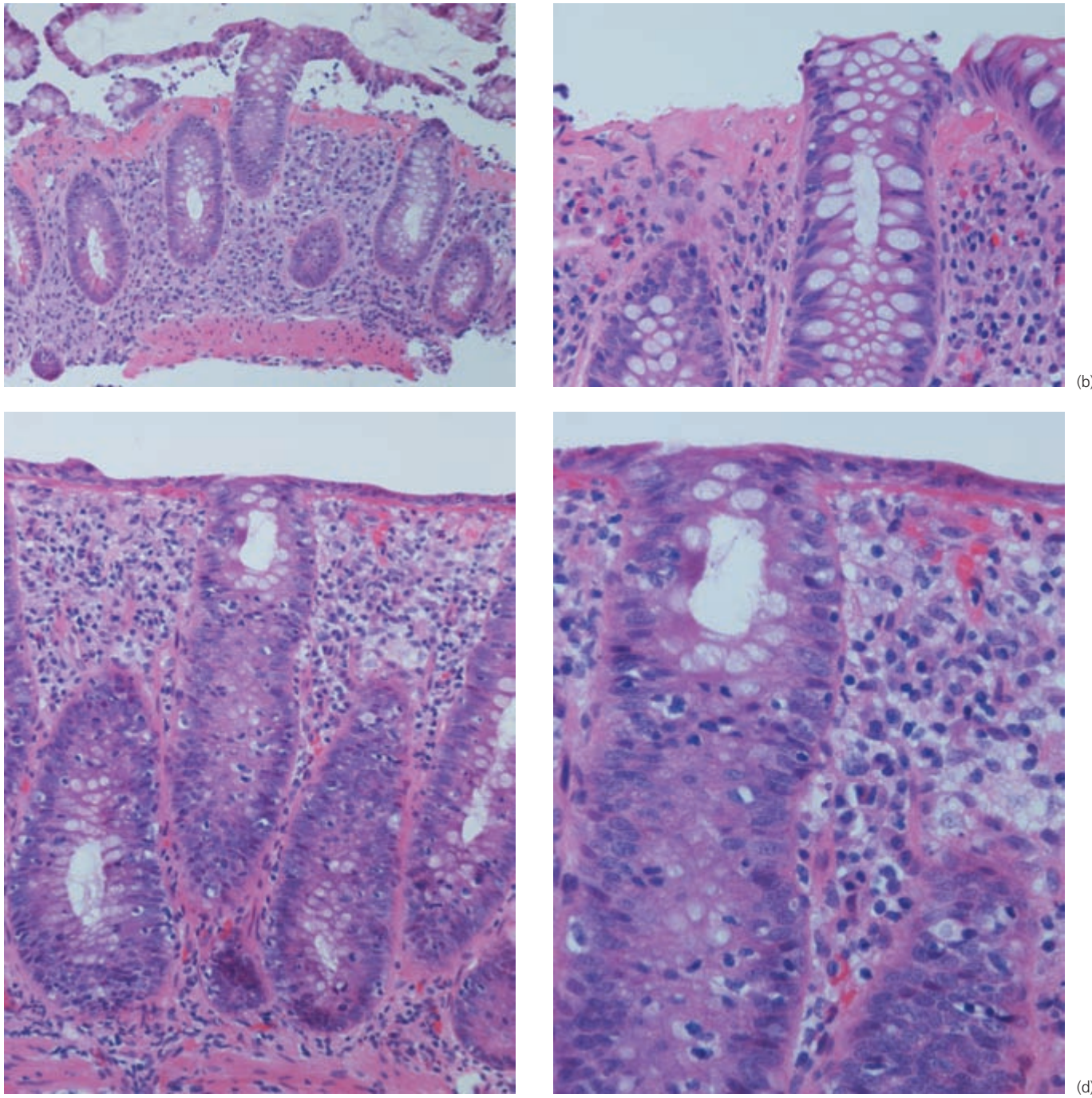


Figure 59.1 Microscopic colitis. **(a)** Lymphocytic colitis features preserved crypt architecture, lamina propria inflammation, and increased epithelial lymphocytes in surface and crypt epithelium. **(b)** The surface epithelium is often markedly flattened, as shown here. The subepithelial collagen band is not thickened. Collagenous colitis features straight crypts, slight lamina propria inflammation, and a markedly thickened subepithelial collagen band. **(c)** Stripping of the surface epithelium is characteristic. **(d)** The collagen band often contains entrapped capillaries, a helpful finding when the thickening is less dramatic than shown here.

[23]. In a study of 72 patients, in which acute onset was defined as symptoms for less than 1 week's duration, subacute as symptoms for 1–4 weeks, and chronic as persistent symptoms for which the patient could not recall a definite starting point, the onset of diarrhea in microscopic colitis was acute in 33%, subacute in 42%, and chronic in 25% [24]. There was no significant difference in the duration of the symptoms with collagenous colitis compared with lymphocytic colitis [24]. In microscopic colitis, stool frequency averages six bowel movements daily, but more than 20 bowel

movements daily is not unusual [16,25]. Other symptoms include weight loss (41%), abdominal pain (40%), urgency (29%), and nocturnal stools (22%) [26]. The presence of nocturnal stools is a feature distinguishing between microscopic colitis and irritable bowel syndrome. Fever, nausea, vomiting, and hematochezia are atypical features of microscopic colitis. Although routine laboratory tests are usually normal in microscopic colitis, electrolyte abnormalities (hypokalemia), positive serum autoimmune markers (antinuclear antibody), and elevated inflammatory markers (erythrocyte sedimentation rate) may be present. Fecal leukocytes have been noted in up to 55% of cases [27]. Malabsorption with steatorrhea or protein-losing enteropathy has been noted in rare cases [28,29].

In most cases, the colonic mucosa appears endoscopically normal, but mild edema, erythema, or a slight decrease in the usual mucosal vascular pattern have all been described [16,19,24]. Colonic ulcerations and perforation have been reported in microscopic colitis and are most likely related to the use of medications such as nonsteroidal antiinflammatory drugs (NSAIDs) [30]. Rarely, pseudomembranes may be present, giving rise to the term "pseudomembranous collagenous colitis" [31]. It is unclear if the pseudomembranes are the result of a superimposed *Clostridium difficile* infection in the setting of underlying collagenous colitis-like changes, or if the *C. difficile* infection causes both the pseudomembranes and the thickening of the subepithelial collagen band [32].

The natural history of microscopic colitis is variable. The pattern of symptoms can range from intermittent and relapsing to chronic and continuous [16]. Remission rates vary both within and between the two subtypes of microscopic colitis, and spontaneous as well as treatment-induced remission have been reported. In one study, 34% of patients with collagenous colitis and 59% of those with lymphocytic colitis remitted at 6 months [33]. Because of the high rate of spontaneous remission and the lack of long-term random maintenance studies, the optimal treatment duration remains unclear [34]. Although there have been case reports of microscopic colitis evolving into chronic ulcerative colitis and Crohn's disease and, alternatively, cases of inflammatory bowel disease (IBD) evolving into microscopic colitis, it is unclear if a true association between microscopic colitis and these other forms of IBD exists [16,35–37]. In contrast to chronic ulcerative colitis and Crohn's disease, microscopic colitis has not been identified with an increased risk of colon cancer [38,39]. In a series of 117 patients with a mean follow-up of 7 years, no cases of colorectal cancer were found and the relative risk of overall malignancy and overall mortality did not differ from the general population [38,39].

Histology

The classic histological changes in lymphocytic colitis include increased surface intraepithelial lymphocytes, preserved crypt architecture with increased crypt intraepithelial lymphocytes, and increased mixed inflammatory infiltrate, consisting of lymphocytes, eosinophils, plasma cells, and macrophages within the lamina propria [40]. Normal colon epithelium contains less than 5 intraepithelial lymphocytes per 100 epithelial cells. The intraepithelial lymphocyte count in lymphocytic colitis is 20 or more per 100 surface epithelial cells [4,33] (Fig. 59.1a,b) In collagenous colitis, the classic histological findings are essentially identical to those in lymphocytic colitis, with the exception of the presence of the thickened subepithelial collagen band, which is generally thicker than 10 µm, normal being less than 5 µm [4,41]. The thickened collagen band is most prominent beneath the surface epithelium between crypts, and frequently contains entrapped capillaries. The overlying epithelium is often detached from the collagen table. Distribution of the thickened collagen band is spotty and is most often found in the cecum (82%) and the transverse colon (83%) and least likely to be found in the rectum (72%) [42] (Fig. 59.1c,d).

There are a number of variations in the histopathological appearance of lymphocytic and collagenous colitis. Active crypt inflammation accompanied by architectural distortion and Paneth cell metaplasia [43] may be seen in otherwise typical microscopic colitis (Fig. 59.2). These findings may make it difficult to distinguish microscopic colitis from IBD. In one study, cryptitis and crypt abscesses were noted in 38% of lymphocytic colitis patients and 30% of collagenous colitis patients, whereas Paneth cell metaplasia was seen more often in collagenous colitis compared with lymphocytic colitis (44% vs 14%, respectively) [43]. Multinucleated giant cells [41], granulomas [44,45], and pseudomembranes [31] have also been described (Fig. 59.3; see also Fig. 59.2).

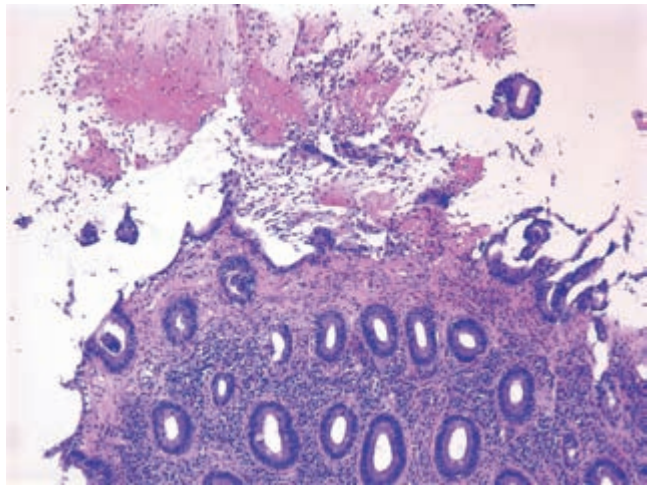


Figure 59.2 Pseudomembranous collagenous colitis. The subepithelial collagen band is thickened. The mucosal surface is covered by a mixture of fibrin and inflammatory cells in the "volcano-like" pattern typical of pseudomembranous colitis.

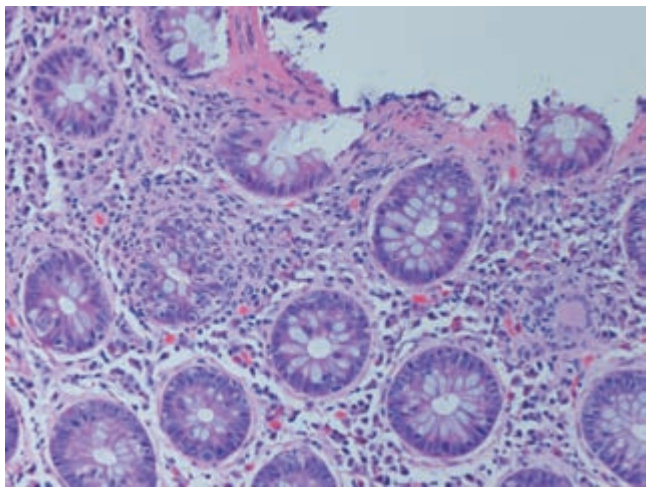


Figure 59.3 Atypical changes in collagenous colitis. There is active cryptitis on the far left and a multinucleated giant cell left of center. Neither of these changes should alter the diagnosis if the biopsy is otherwise typical of collagenous colitis.

Etiology

The etiology of microscopic colitis remains unknown. Autoimmune dysregulation, abnormal response to lumenal factor(s), drug effects, and infection have all been proposed as causes of microscopic colitis. The abnormal response to lumenal antigens hypothesis is supported by the finding that ileostomy with diversion of the fecal stream induces clinical and histological remission, and restoration of intestinal continuity results in clinical and histological relapse [46,47]. In addition, fecal extracts from patients with collagenous colitis are cytotoxic for McCoy cell cultures, and treatment with cholestyramine eliminated the cytotoxic effect [48]. Lumenal bacterial antigens may be important, and antibiotics have been therapeutic in some patients with microscopic colitis [16,49]. Given the increased infiltration and degranulation of eosinophils found in collagenous colitis, the possibility of an allergic reaction to a lumenal antigen has been proposed [50]. Indeed, dogs and cats exhibiting a disorder similar to microscopic colitis have resolution of the colonic mucosal abnormalities after being placed on a hypoallergenic diet [51]. So far, no causal antigens for the inflammatory process have been identified [52,53].

Lymphocytic colitis-like changes can be induced by administration of a gluten enema in some patients with celiac sprue [54]. Both lymphocytic and collagenous colitis can be associated with typical or refractory celiac disease. Approximately 20%–30% of patients with typical celiac disease have lymphocytic colitis on investigation by colonic biopsies [20,21,55]. Those patients diagnosed with both celiac disease and microscopic colitis are approximately 10 years younger than patients suffering only from microscopic colitis [21]. The prevalence of celiac disease in microscopic colitis patients with the reported range is from 2% to 40% [15,16,18,19,56–59]. Whether lymphocytic colitis or collagenous colitis has a stronger association with celiac disease is controversial [24,59]. Moreover, colonic inflammation not primarily caused by celiac disease responds at variable rates to a gluten-free diet, and the majority of lymphocytic colitis and collagenous colitis patients do not have malabsorption. Collagenous gastroduodenitis, collagenous enterocolitis, collagenous sprue, and autoimmune enteropathy have been reported in association with microscopic colitis [60,61].

In addition to lumenal antigens, genetic factors have been implicated in the development of microscopic colitis. Some investigators have linked celiac disease and microscopic colitis to similar HLA loci (HLA-DQ2, HLA-DQ1,3, and subtypes HLA-DQ1,7, HLA-DQ1,8, and HLA-DQ1,9), suggesting that similar immune mechanisms are involved in the development of both disease entities, but these findings have not been replicated [15,58,62]. In animal models, HLA-B27 rats developed the histological changes of collagenous colitis only in the presence of colonic bacteria [49], but this finding has not been observed in human studies. HLA-A1 was noted to be increased with diminished HLA-A3 in lymphocytic colitis, with no HLA associations noted for collagenous colitis [63], but others have not demonstrated any HLA associations [56]. Because of the inconsistent findings no definitive conclusions can be drawn regarding HLA associations in microscopic colitis.

Another hypothesis is that microscopic colitis is a stereotypical response by the gastrointestinal immune system to multiple unidentified antigens [64]. An autoimmune etiology is suggested by the findings of arthritis, thyroid abnormalities, asthma, diabetes, celiac sprue, rheumatic diseases, and hepatitis that frequently accompany microscopic colitis [16,59,65]. Concomitant autoimmune disorders are thought to be more prevalent with collagenous colitis (53%) compared with lymphocytic colitis (26%) [59]. Antiendomysial antibodies are found in 2%–4% of patients with microscopic colitis, whereas antigliadin antibodies are found in 5%–17% of microscopic colitis patients, even in the absence of celiac disease [57,58]. The antibody titers tend to be lower in microscopic colitis than in celiac disease. Perinuclear antineutrophil cytoplasmic antibodies (p-ANCA) are found in 20% of microscopic colitis patients, and the frequency of an elevated antinuclear antibody titer is mildly increased [57,66]. The roles of estrogen and progesterone have been considered as contributory or causal factors in the development of microscopic colitis, and, in one case, in the resolution of the disease with pregnancy [16].

The strong association of arthritis [44] with these disorders raises the possibility that nonsteroidal antiinflammatory drugs (NSAIDs) may play an etiological role. In some studies NSAIDs have been implicated in the development of microscopic colitis, whereas other studies have refuted this finding

[13,33,46,59,67,68]. In a well-controlled study, significantly more collagenous colitis patients (61%) had used NSAIDs for more than 6 months versus age- and gender-matched controls (13%; $P < 0.02$) [67]. By contrast, a separate case–control study found that use of NSAIDs in microscopic colitis patients (38%) did not differ significantly from age- and gender-matched controls (33%) [59]. In uncontrolled studies, 21% and 35% of patients in two collagenous colitis registries had NSAIDs [16,68]. It has been shown that NSAIDs cause gastric, small bowel, and colonic inflammation in patients and experimental animals, and can exacerbate IBD [65,68,69]. Despite these conflicting results, histological and clinical remission can occur with the discontinuation of NSAIDs, and some patients may redevelop histological changes and clinical symptoms consistent with microscopic colitis when rechallenged with NSAIDs [67,68,70–72]. Because of these findings, patients with microscopic colitis should avoid NSAIDs. Other medications, such as ticlopidine, lansoprazole, ranitidine, simvastatin, selective serotonin reuptake inhibitors, and carbamazepine, have been implicated as the cause of microscopic colitis, especially lymphocytic colitis [13,26,33,59,73–75].

In some cases of microscopic colitis, patients have a preceding acute intestinal infection, but no single pathogen has been identified. The abrupt onset of symptoms in up to 33% of patients [24] and the response in some to antibiotics [16,18,76] supports the hypothesis that an acute enteric infection is the inciting event. In addition, elevated titers of antibodies to *Yersinia enterocolitica* have been noted in patients with collagenous colitis, and the development of collagenous colitis has occurred after infection with *Y. enterocolitica* and *C. difficile* [77–80]. A chronic watery diarrhea, known as "Brainerd diarrhea," has many clinical and histological features in common with microscopic colitis. Brainerd diarrhea is associated with ingestion of raw milk, and epidemiological data indicate an infectious etiology [81]. The similarities between Brainerd diarrhea and microscopic colitis support the hypothesis for an infectious etiology for microscopic colitis.

Another theory is that the presence of lumenal bile acids leads to the colonic epithelial injury [16]. Because some patients with microscopic colitis demonstrate histological changes in the ileum as well as in the colon, this does provide a plausible explanation for why bile acid malabsorption might occur [82,83]. Investigations have noted variable frequencies of bile acid malabsorption in collagenous colitis patients as determined by an abnormal selenium-labelled homocholic acid-taurine (SeHCAT) test. Some of these patients, as well as those with a normal SeHCAT, had clinical improvement with the use of bile acid binding resins [84]. Bile acid malabsorption is only partially responsible for the development of collagenous colitis, because patients with symptomatic improvement did not always have histological improvement.

Diarrhea in microscopic colitis appears to be secretory in nature, and in a small number of patients is caused by defective active and passive absorption of sodium and chloride with reduced chloride–bicarbonate exchange [23,85–87]. Two of six patients displayed coexisting abnormal small intestinal fluid and electrolyte absorption. Active colonic chloride secretion associated with increased lumenal prostaglandin E_2 concentrations and prevention of diarrhea with a histamine H_1-receptor antagonist in a patient with increased numbers of mast cells suggest that soluble mediators produced by activated immune and mesenchymal cells mediate epithelial cell absorptive dysfunction [87–89]. This hypothesis is supported by the direct relationship between the severity of diarrhea and the numbers of mucosal inflammatory cells, and the lack of correlation with the thickness of the submucosal collagen band [90]. The latter observation argues against the hypothesis that subepithelial collagen blocks electrolyte and water absorption. Also noted in collagenous colitis are derangements in lumenal nitric oxide levels and nitric oxide synthase activity. Expression of inducible nitric oxide synthase is upregulated in the colonic mucosa of collagenous colitis patients [91], and elevated levels of nitric oxide have been noted when compared with healthy controls [92,93]. Nitric oxide acts as a secretagogue in the colon and inhibits fluid absorption by the colon. Therefore, it may play a role in the secretory diarrhea seen in microscopic colitis.

The cause of the collagen deposition in collagenous colitis is unknown. Collagen typing studies have yielded conflicting results. In some studies type I and type III collagen predominate, with an absence of type IV in the subepithelial band, but other studies have shown primarily type IV collagen deposition or a mix of collagen types [9,85,94–96]. These studies have not resolved the issue of whether the abnormal collagen band in collagenous colitis is normal collagen that is overproduced as part of the repair process, or if it is an abnormal collagen. Type III collagen is typically found when tissues are repairing after an injury, whereas type IV collagen indicates a primary abnormality of collagen production [97]. Myofibroblasts within the pericryptal fibroblast sheath, which surrounds and gives support to the crypt epithelium, may become sequestered within the collagen band and be responsible for the increased collagen production deposition [46,98]. Transforming growth factor-β (TGF-β) is overexpressed in patients with collagenous colitis, which may lead to collagen accumulation in tissues, and there is evidence to suggest that the rate of collagen degradation is decreased [95,96]. It remains unknown why this abnormality in the sheath is not present in lymphocytic colitis. This gives reason to consider that the collagen deposition is not the primary defect, but occurs in response to the inflammatory infiltrate. This hypothesis is supported by the fact that the diarrhea in collagenous colitis does not seem to be related to the thickness of the collagen band but rather the degree of inflammatory infiltrate, as noted above [90].

Diagnosis

A minority of patients (24%–29%) with microscopic colitis, collagenous colitis in particular, have nonspecific endoscopic abnormalities including erythema, edema, or abnormal vasculature [16,24]. In a study of 362 patients who underwent colonoscopy for evaluation of diarrhea, significant histopathology was noted in 18 (5%), with a significantly higher prevalence of microscopic colitis in patients over 60 years of age (7% vs 0.4%) [99]. Multiple biopsies must be taken from different colonic segments to diagnose microscopic colitis [27,42,100]. Colonoscopy with biopsy of multiple segments demonstrated only one site positive in 5 of 17 patients, and a greater than 50% variability of the collagen band thickness in multiple samples taken simultaneously [42]. Flexible sigmoidoscopy is an adequate screening exam, with approximately 95% of pooled biopsies from the descending colon, sigmoid colon, and rectum showing changes of chronic inflammation or a thickened collagen plate [27,42]. However, if suspicion remains high for microscopic colitis despite negative left colon biopsies, one should consider a colonoscopy with proximal colon and random biopsies. Ileoscopy should be undertaken, if possible, as it may identify up to 5% more abnormalities than colonoscopy to the cecum [99]. Biopsies of a normal-appearing ileum have a low yield and typically are not useful. Proper orientation of the histological specimen is essential to prevent artifactual thickening of the subepithelial basement membrane caused by tangential sectioning. The presence of associated lamina propria inflammation is an important co-diagnostic feature of collagenous colitis [4].

Stool weight in microscopic colitis is usually 500–750 g/day, with an electrolyte pattern consistent with secretory diarrhea [101]. Crohn's disease and chronic ulcerative colitis can be differentiated from microscopic colitis by diagnostic radiography and colonoscopy as well as by histology. This can become more difficult in those microscopic colitis patients who exhibit "atypical" morphological features, often associated with chronic ulcerative colitis and Crohn's disease [43]. Crypt abscesses, crypt architectural abnormalities, the presence of neutrophils, lymphocytes within the crypts, giant cells, and the presence of granulomas have been reported in microscopic colitis [40]. Pericrypt eosinophilic enterocolitis is characterized by chronic watery diarrhea and normal endoscopy, but may be differentiated from lymphocytic colitis by the absence of increased numbers of intraepithelial and superficial lamina propria lymphocytes and the presence of eosinophilic infiltration, crypt foreshortening, and inflammation in the deep lamina propria and muscularis mucosa [102]. Infectious agents may be excluded by stool examination for ova and parasites, standard bacterial stool cultures, and *C. difficile* toxin assays. Jejunal aspirates can be performed to rule out bacterial overgrowth and *Giardia* infection. Given the increased occurrence of celiac disease in microscopic colitis patients, small bowel biopsies can be performed to rule out both celiac disease and Whipple disease. Irritable bowel syndrome can typically be differentiated by normal stool volume and normal or nearly normal colonic biopsies. Malabsorption, although uncommon in microscopic colitis, may be present. Hormone-producing tumors, hyperthyroidism, and laxative abuse could be considered if the history or physical examination are suggestive. Ischemic colitis should be considered if clinically relevant. Histologically, the fibrosis that occurs with ischemic colitis, radiation injury to the bowel, and solitary rectal ulcer syndrome (SRUS) is diffuse within the lamina propria. The collagen deposition that accompanies progressive systemic sclerosis occurs along the basement membrane and may be transmural throughout the colonic wall. Amyloidosis can be a cause of chronic watery diarrhea, and amyloid deposition occurs along the basement membrane of the crypts, blood vessels, and surface epithelium. Histochemical staining can be used to diagnose amyloidosis.

Treatment

There are few prospective controlled trials for the treatment of microscopic colitis. Clinical symptoms of microscopic colitis tend to wax and wane in a relapsing remitting fashion. Many cases spontaneously improve. This makes it difficult to interpret the results of the small, mostly retrospective, therapeutic studies. Most agree that initial therapy should focus on the discontinuation of agents and substances that are potential causes of diarrhea, such as dairy products, excess alcohol intake, and excessive caffeine intake [32]. If possible, NSAIDs and other potential causative medications should be stopped. Antidiarrheal therapies are well-tolerated and can be effective [16,76]. Bismuth subsalicylate has been noted to be helpful in some patients with microscopic colitis. Bismuth possesses antiinflammatory, antidiarrheal, and antimicrobial properties. Fine and Lee reported that 11 out of 13 patients with microscopic colitis responded to oral bismuth subsalicylate, eight tablets (262 mg each) per day for 8 weeks; there was a durable remission with complete resolution of histological changes in 9 subjects [103]. Pardi et al. noted that bismuth subsalicylate at a dose of eight tablets (262 mg each) per day taken in 3–4 divided doses led to a complete response in 23% of patients and a partial response in 50%, with only 4% of patients intolerant to the medication [76]. In one small randomized controlled trial of bismuth subsalicylate, a regimen of three 262-mg tablets, administered orally three times a day for 8 weeks, was tested versus placebo. Of the nine patients, four showed clinical and histological improvement with bismuth subsalicylate, whereas the remaining five assigned to placebo did not improve ($P = 0.003$) [104]. Improved symptoms and histology have been noted following the use of 5-aminosalicylate (5-ASA) products and sulfasalazine. Whereas one review noted improvement in the majority of patients using 5-ASA products, other studies have noted less promising remission rates [13,16,18,76]. In one large retrospective review, 15% of patients reported a complete remission of diarrhea and another 27% reported

a partial improvement in diarrhea [76]. In general, response rates of 40%–50% are seen with mesalamine (rINN mesalazine) and sulfasalazine. Bile acid-binding drugs have been used with some success, both clinically and for mucosal healing, in patients with microscopic colitis [105]. This is particularly true in those patients who demonstrated evidence of bile acid malabsorption by the SeHCAT retention test [105]. Traditional corticosteroids, such as prednisone and prednisolone, have been used for microscopic colitis. In a retrospective study, 82% of collagenous colitis patients treated with prednisolone had a clinical response, whereas 59% and 50% responded to sulfasalazine and mesalamine, respectively [16]. In addition, response to metronidazole or erythromycin was 55% and 67%, respectively [16]. Although corticosteroids have been shown to be effective in uncontrolled series, only one randomized controlled trial of prednisolone has been completed [16,19,76,106]. Prednisolone 50 mg per day for 2 weeks was compared with placebo in the treatment of 12 patients, 11 of whom had collagenous colitis [106]. Complete remission was noted in only two patients. This was not significantly different from placebo although prednisolone decreased the mean stool weight and reduced the frequency of bowel movements from six per day to three per day in the treatment group [106]. A longer course of therapy may be necessary to see improvement both clinically and histologically. In a separate controlled trial, six patients with collagenous colitis (43%) had a significantly decreased number of stools ($P = 0.03$) and a decrease in the degree of mucosal inflammation (not quantitated) after 3 months of prednisolone therapy. Stool frequency returned to pretherapy levels within 2–4 weeks after cessation of therapy; however, the thickness of the collagen band was unchanged by treatment in five of six patients [107]. Patients treated with steroids tend to relapse on withdrawal of the steroid medication, and many are left steroid dependent [16,19]. Because the side effects of traditional corticosteroids can lead to short-term and long-term health concerns, budesonide has been utilized. Budesonide is a synthetic corticosteroid with low systemic bioavailability as a result of its metabolic characteristics, and is one of the most extensively studied drugs in the treatment of microscopic colitis. Budesonide primarily acts locally in the distal ileum and right colon with some minor absorption in the transverse and descending colon. Due to the high first-pass metabolism, the side-effect profile of budesonide is preferable to that of traditional corticosteroids [108–110]. In a Cochrane review of three randomized controlled trials of budesonide versus placebo in the treatment of collagenous colitis, the pooled odds ratio for response to therapy was 12.32 [95% confidence interval (CI): 5.53–27.46]. The number needed to treat (NNT) was 2 patients. It should be noted that treatment regimens differed between the three budesonide studies. The dose varied from 9 mg/day for 8 weeks [111], 9 mg/day for 6 weeks [112], and 9 mg of budesonide for 4 weeks with a 3-mg taper every 2 weeks for a total duration of therapy of 8 weeks [113]. Interestingly, all studies showed a benefit in clinical symptoms over that seen by placebo, with response rates ranging from 57% to 100% [111–113]. The time to clinical response was between 12 and 14 days [111,112,114]. The rates of histological remission were significantly superior to placebo in all three randomized controlled trials of budesonide, with rates ranging from 54% to 100% [111–114].

Immunomodulators have shown efficacy in the treatment of microscopic colitis patients who are intolerant of, refractory, or dependent on steroid therapy. Among nine steroid-dependent or steroid-intolerant microscopic colitis patients treated with a median azathioprine dosage of 2 mg/kg/day, five (56%) experienced resolution of symptoms and were successfully tapered off steroids, three (33%) were able to taper or discontinue steroids but were left with mild diarrhea, and one patient required an ileostomy for persistent severe diarrhea [115]. These patients maintained remission or improvement in diarrhea for a median follow-up of 26 months. However, adverse events such as infection, nausea, mild leukopenia, and liver abnormalities required four patients to be successfully switched to 6-mercaptopurine (6-MP). As the prognosis of microscopic colitis is good and the rate of adverse events small, with no risk of increased mortality, use of these medications should be reserved for those patients who face severely debilitating symptoms or severe side effects from their current therapy [115].

Uncontrolled studies have reported symptomatic benefit with antibiotics, methotrexate, cyclosporine (ciclosporin), octreotide, and calcium channel blockers [16,19,76,116,117]. In the rare situation when all medical options have failed, surgery is a consideration. Ileostomy without colectomy, ileostomy with colectomy, and ileal pouch–anal anastomosis have been shown to be effective in controlling diarrhea [46,76,115,118].

The following progressive evidence-based approach is recommended for treatment of microscopic colitis [32]. Initial therapy should focus on withdrawal of agents possibly responsible for diarrhea and implicated in the development of microscopic colitis. Symptomatic control should be attempted with antidiarrheal therapy, such as loperamide or diphenoxylate hydrochloride/atropine. If symptoms continue and are mild, bismuth subsalicylate, eight or nine 262-mg tablets, taken in three or four divided doses daily, can be added. In the event that no improvement or remission occurs, the use of cholestyramine or 5-ASA products should be considered. For more severe symptoms and in patients with mild symptoms who have failed symptomatic measures, budesonide, 9 mg per day for 6–8 weeks, is appropriate. In patients who are refractory to other medical interventions, azathioprine and 6-MP are options. In refractory patients with severe symptoms, other options are octreotide, methotrexate, and cyclosporine, or surgical intervention. When a medication leads to clinical remission or improvement,

it should be continued for 6–8 weeks and then stopped, given that spontaneous remissions occur and treatment can lead to a durable remission. If symptoms recur on discontinuation of the treatment, the initial treatment should restarted, as patients have been shown to respond favorably when rechallenged with the same initial medical therapy [103,104,119,120].

Distinct entities or not?

There is controversy as to whether lymphocytic colitis and collagenous colitis are two distinct entities or represent a continuum of the same disorder or different phenotypic manifestations of the same disorder. Both lymphocytic and microscopic colitis share similar clinical, demographic, and histological characteristics and differ primarily according to the the presence or absence of a thickened subepithelial collagen band. The etiology of the collagen band remains unknown. Serial biopsies have shown both resolution and appearance of the collagen band with time [15]. However, other investigators have demonstrated that the collagen band has a patchy distribution and this calls into question the theory that lymphocytic colitis and collagenous colitis are distinct clinical entities. Further investigations into the cause of microscopic colitis are necessary before this debate can be put to rest.

Drug- and chemical-induced colonic injury

Colonic injury and inflammation has been attributed to a number of orally and rectally administered substances. These agents may cause colonic injury through a number of mechanisms, including direct mucosal injury and ischemic injury. This section will review the most common types of drug- and chemical-induced injury, with an emphasis on pathogenesis and clinical findings.

Syndromes associated with enema use

Various rectally administered substances are common causes of toxic colitis. Traditional soapsuds enemas, radiographic contrast media, hydrogen peroxide, herbal enemas, and residual disinfecting agents on the colonoscope have been shown to be associated with toxic colitis. Mechanisms of injury include direct mucosal toxicity from corrosive acids, bases, and caustic agents; intracellular explosive oxygen production (hydrogen peroxide); osmotic shifts due to hypertonicity of the enema solution (radiographic contrast agents); and cross-linking of extracellular and intracellular proteins (formalin) [121]. Risk factors include radiographic contrast media enemas, detergent enemas, and a history of self-mutilation. The severity of the colonic injury depends on the type of substance, the concentration of the substance, and the duration and extent of mucosal contact. Affected individuals typically present with abdominal distention, pain, bloody or nonbloody diarrhea, tenesmus, and possibly fever. Physical examination may reveal abdominal tenderness, possibly with peritoneal signs, and rectal tenderness. Laboratory investigation may demonstrate leukocytosis and elevated serum inflammatory markers [122]. On physical examination there may be perianal excoriation. Endoscopy may reveal friable mucosa with or without ulceration or pseudomembranes, or segmental distal colitis. Treatment is supportive with bowel rest, intravenous fluid administration, and, if clinically indicated, broad-spectrum antibiotics. Surgery may be necessary in more severe cases with necrosis and perforation [122].

Water-soluble contrast media

Hyperosmolar water-soluble contrast media, such as Gastrografin (Bracco Diagnostics Inc., Princeton, NJ), Hypaque (Nycomed Inc., Princeton, NJ), and Renografin (Bracco Diagnostics Inc., Princeton, NJ), are often used to relieve fecal impactions and to opacify partial colon obstructions, avoiding the potential complications of barium enema. Although these agents are generally safe, a mild to severe colitis can result from their use [123]. Because these contrast agents are hypertonic, they facilitate passage of stool by drawing liquid into the lumen of the colon. The addition of the detergent Tween 80 may also aid in passage of stool. Yet, the same properties that help to facilitate stool passage, namely the hypertonicity of the contrast media and the presence of an agent with detergent properties, are thought to contribute to the mucosal damage in the colon. It may be that lumenal distention leads to decreased blood flow to the colonic mucosa [124]. In most reports, contrast media-induced colitis has occurred proximal to an obstruction, chiefly in the cecum and ascending colon [121].

Sodium phosphate solutions

Their advantages of cost-effectiveness and the need to ingest smaller volumes mean that sodium phosphate preparations are being used more commonly for colonoscopy. In an outpatient setting in Canada, one group noted that sodium phosphate was used more often than the traditional polyethylene glycol (PEG) preparation in the outpatient setting (46% vs 35%, respectively; $P < 0.02$) [125]. However, sodium phosphate bowel preparations are not suitable for use in some patient groups, namely those with renal failure, heart disease, and those individuals prone to electrolyte disturbances, because this type of preparation has been reported to cause hyperphosphatemia, hypokalemia, and hypocalcemia [126]. Indeed, there is one case report of acute renal failure induced by use of sodium phosphate bowel preparation in a previously healthy patient with no history of renal failure [127]. In addition, sodium phosphate preparations have been shown to lead to more episodes of dehydration when compared

with PEG preparations (9% vs 3%, respectively; $P < 0.02$) [125]. Variable rates of colonic mucosal injury have been reported with the use of sodium phosphate bowel preparations. Zwas et al. randomized 97 patients to sodium phosphate or PEG prior to colonoscopy, and noted the occurrence of aphthoid lesions of the colonic mucosa in 25% and focal active colitis in 6% of patients receiving sodium phosphate as compared with 2% of patients who received PEG [128]. However, in a study of 687 consecutive patients receiving sodium phosphate as colonic preparation, a 6.1% incidence of any type of colonic mucosal abnormality was noted, which included both endoscopically apparent lesions, such as aphthoid ulcerations, which were noted in 2.6% of patients, and microscopically apparent lesions, such as focal active colitis and aphthoid lesions, which were noted in 3.5% of patients [129]. Lesions are usually found in the left colon but can occur at any location. Endoscopically, the mucosa may appear edematous and erythematous, with the appearance of aphthoid lesions ranging from petechial markings on the mucosa to discrete superficial ulcerations surrounded by a circumferential halo of edema and normal adjacent colonic mucosa [130]. Histologically, sodium phosphate-induced colonic injury demonstrates focal neutrophilic cryptitis and the presence of apoptotic bodies within the crypts (Fig. 59.4a–c). The surrounding mucosa may or may not demonstrate neutrophils in the lamina propria [129].

The mechanism of injury induced by sodium phosphate bowel preparation is unknown. It has been hypothesized that direct mucosal injury, apoptosis of crypt cells, increased epithelial proliferation, and oxidative damage may play a role in colonic injury [129,131]. Biopsies of sodium phosphate-induced mucosal lesions demonstrate an increased number of apoptotic bodies when compared with biopsies of normal mucosa in a non-sodium phosphate-treated bowel [129].

Diagnosis is made by endoscopic and histological appearance in conjunction with the clinical history of the patient. It is important to distinguish these lesions and ulcerations from those of IBD, namely Crohn's disease.

Treatment is simply supportive, and the lesions resolve on cessation of exposure to the offending agent.

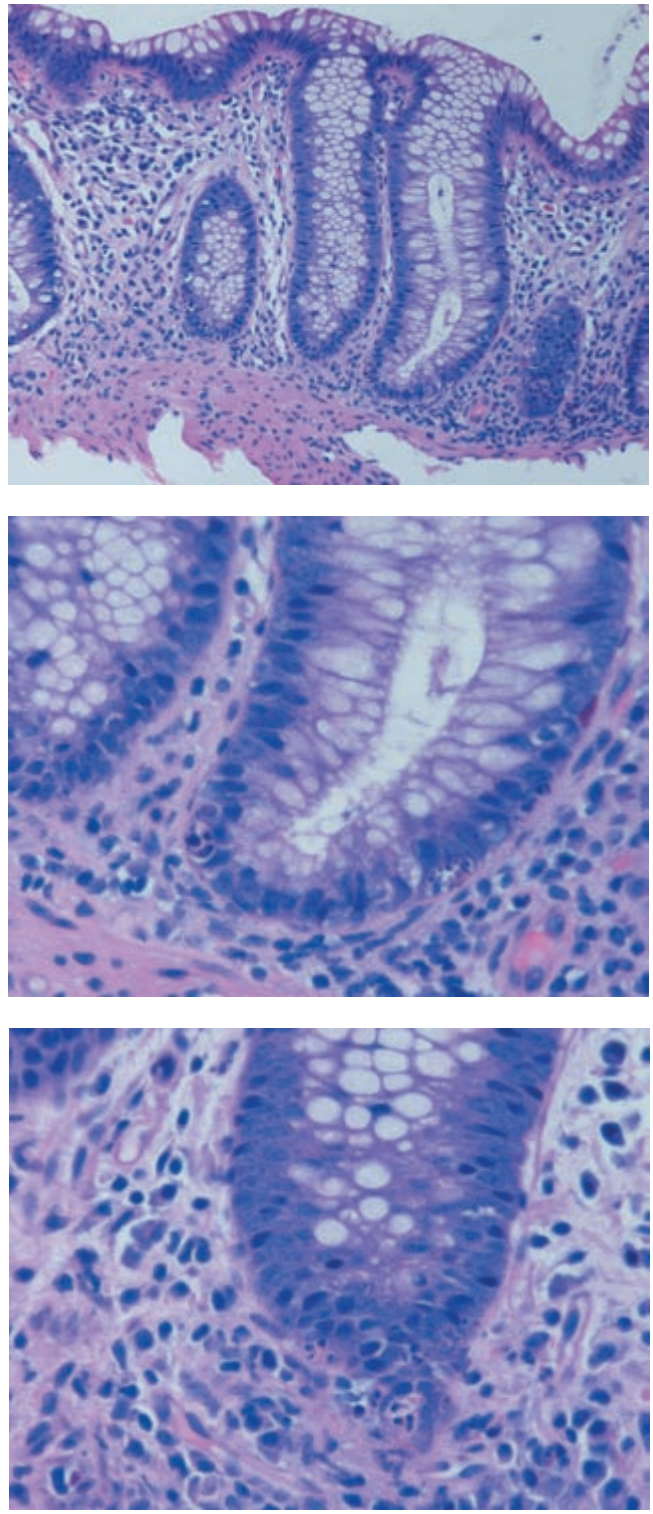
Other miscellaneous substances

Many other substances, when introduced into the rectum, can result in extensive tissue damage to the colonic mucosa. Enemas consisting of vinegar [132], ethanol [133], hydrofluoric acid, potassium permanganate [132], and chloroxylenol (Dettol) [132] are just a few of the agents that can result in variable degrees of colonic inflammation and necrosis.

Syndromes associated with laxative abuse

Melanosis coli

First described in 1835 by Billiard and named by Virchow in 1857, melanosis coli is characterized by a brown to black



(a) (b) (c)

Figure 59.4 Mucosal changes produced by sodium phosphate bowel preparation solution. **(a)** The crypts are straight, the lamina propria is not inflamed, and the surface epithelium is normal. **(b)** There is apoptotic debris at the crypt base. **(c)** Occasionally, neutrophils accompany the apoptosis. The latter can be mistaken for Crohn's disease.

pigmentation of the colonic mucosa that is associated with chronic laxative use. It is typically found in patients who chronically use anthranoid laxatives, such as sennosides, cascara frangula, rhubarb, and aloe, but may be seen in patients who do not use laxatives and suffer from constipation [134]. Up to 73% of patients using anthranoid laxatives develop melanosis coli [135], and a study noted a three-fold increased risk of melanosis coli with anthranoid laxative use [136]. Changes of melanosis coli can occur as early as 4 months after beginning ingestion, and generally regress within 9–12 months of stopping the anthranoid laxative [137]. Patients with endoscopic or histological findings of melanosis coli are not always laxative users. Pardi and colleagues noted melanosis coli in 25 patients with underlying IBD; however, only 20% admitted to use of laxatives [134]. This retrospective investigation highlights the fact that not all cases of melanosis coli are associated with the use of laxatives, and also raises the possibility that IBD is a risk factor for development of melanosis.

Because the pigmented changes initially were thought to be due to deposition of melanin or a melanin-like pigment, the disorder was named "melanosis coli." However, studies have revealed that the pigmentation is due to lipofuscin and partially digested cell organelles originating from apoptotic enterocytes [138]; therefore, this disorder is more correctly known as "pseudomelanosis coli." The pathogenesis of pigmentation is thought to involve anthranoid-induced apoptosis of colonic epithelial cells, which leads to phagocytosis and degradation of these apoptotic bodies by macrophages, leading to the formation of pigment-laden macrophages. These macrophages then migrate deep into the lamina propria and into lymph nodes [138]. This hypothesis is supported by the finding of significantly increased apoptotic bodies in those patients treated with an anthranoid laxative 6 h prior to colonoscopy and in those with severe melanosis coli when compared with controls ($P = 0.001$ and $P = 0.02$, respectively) [139].

Endoscopic evaluation may demonstrate findings of a brown to black pigmented colon, which can involve the entire length of the large intestine. Colonic adenomas and adenocarcinomas lack pigmentation and may appear as islands of unpigmented mucosa in a background of a darkly pigmented colon. This is thought to occur because the apoptotic bodies of adenomatous or neoplastic tissue tend to stay within the epithelial layer instead of migrating to the lamina propria, the location where macrophages typically ingest and degrade these bodies into lipofuscin-like pigment [138]. Therefore, these nonpigmented areas should be biopsied. Histologically, the macrophages within the lamina propria are increased in number and size, and are situated between the crypts. These macrophages are filled with a brown granular pigment, and it has been noted that the most pigment is found in those macrophages farthest from the lumen. This is thought to be the result of increased degradation of intracellular material as the macrophage migrates downward toward the lymph vessels [138]. The colonic epithelium appears normal under light microscopy, but electron microscopy demonstrates decreased numbers of microvilli, vacuolization of the apical cell surface, and small vesicles near the lateral cell membrane [140].

Many regard anthranoid laxatives as harmless, "herbal" laxatives, but reports have linked melanosis coli to an increased incidence of colonic adenomas [141] and colonic adenocarcinomas [142]. However, a case–control study found no association between the occurrence of preneoplastic lesions of the colonic mucosa, known as aberrant crypt foci, and the presence of melanosis coli or laxative use [136]. Overall, melanosis coli is generally regarded as a benign condition that remits with cessation of the laxatives.

Cathartic colon

Heilbrun in 1943 described the first case of cathartic colon when he described markedly abnormal radiographic studies in a young female with a 20-year history of laxative use [143]. Cathartic colon is an uncommon disorder that occurs in individuals, frequently middle-aged women (mean age 55), who have abused irritant laxatives for an average of over 26 years [144]. Interestingly, cathartic colon appears to have dramatically decreased in recent years.

Drugs resulting in ischemic colonic injury

Ischemic injury of the colon results from compromised mucosal blood flow, which can occur for a number of reasons. Most commonly, it is a disorder of the elderly population, brought about by atherosclerotic disease, arrhythmias, hypotension, hypercoagulopathy, and valvular heart disease. The occurrence of this condition in younger patients should prompt a thorough investigation into risk factors, including medication usage. Ischemia of the bowel typically occurs in watershed areas, such as the splenic flexure of the colon. Medications can induce ischemic colitis by a number of mechanisms: vasospasm, thrombosis, decreased blood delivery to the mesenteric vessel, extrinsic compression of the vessels as a result of fibrosis, and idiopathic mechanisms [121]. Patients with ischemic colitis present similarly, although the severity of the symptoms depends on the severity of the ischemia and which mesenteric vessel is involved. Abrupt onset of severe abdominal pain, tenesmus, bloody or nonbloody diarrhea, nausea, and fever are all possible presenting symptoms. Hypoactive to absent bowel sounds and rebound or guarding may be present, although classically the clinical symptoms are out of proportion to the physical examination findings. Leukocytosis is not always present. Abdominal plain films may show dilated bowel, "thumbprinting," pneumatosis coli, or pneumoperitoneum. Endoscopic investigation may reveal variable degrees of mucosal edema, erythema, friability, granularity, ulceration with or without pseudomembranes, and pale or dusky mucosa.

Ergotamine

Ergotamine is a medication indicated for the treatment of migraine headaches. The use of ergotamine can lead to vasospasm-induced ischemic colitis. The first case reported involved a 50-year-old woman using ergotamine tartrate who developed acute colitis of the splenic flexure, which resolved after discontinuation of the medication [145]. In addition, patients may develop proctitis after use of ergotamine suppositories [121,145,146]. Endoscopy may demonstrate shallow geographical rectal ulcers that resolve on withdrawal of the medication [121]. Histologically, obliteration of the small blood vessels with vascular wall thickening is noted [147]. Other migraine therapies, such as sumatriptan, have also been reported to cause ischemic colitis [148].

Oral contraceptives

The association between ischemic colitis and hormones such as estrogen and progesterone has been known for decades. Both estrogen and progesterone, in their various forms, can lead to a hypercoagulable state, vasospasm in the mesenteric vessels, and endothelial proliferation with subendothelial fibrosis, all of which may play a role in oral contraceptive-induced ischemic colitis [122,149]. The dose of the estrogenic factor correlates with the degree of thrombogenicity [122]. Both venous and arterial mesenteric thrombosis have been associated with the use of oral contraceptives. Mesenteric arterial thrombosis occurs less often but carries twice the mortality of venous thrombosis. In a single-institution review of 39 adults aged under 50 years and diagnosed with ischemic colitis over a period of 9 years, a strong female predominance was noted and 52% of the women were taking oral contraceptives at the time of diagnosis [150]. The rectosigmoid and the splenic flexure are the most commonly affected sites. Withdrawal of the oral contraceptive is indicated if ischemic colitis develops while on therapy.

Cocaine

Cocaine leads to intense vasoconstriction as a result of catecholamine release and has been linked to the occurrence of severe ischemic colitis. Intestinal ischemia can develop after intranasal, intravenous, or oral administration of cocaine [151]. A review noted that the majority of cases affect the proximal colon [152]. Many patients require resection of gangrenous bowel.

5-HT$_3$ receptor antagonists

Alosetron is a selective serotonin (5-hydroxytryptophan, or 5-HT) antagonist that targets 5-HT$_3$ receptors. It is indicated for the treatment of females with severe refractory diarrhea-predominant irritable bowel syndrome (IBS). In post-market monitoring of adverse reactions, a number of patients developed ischemic colitis, and these cases were reported to the US Food and Drug Administration, which resulted in the subsequent withdrawal of the drug from the US market [153]. Also, eight cases (six women and two men) of ischemic colitis have been reported with another 5-HT$_3$ antagonist known as cilansetron [154]. Therefore, it appears that ischemic colitis may be a side effect common to all drugs of this class. A number of hypotheses exist regarding the mechanism by which alosetron leads to ischemic colitis: vasoconstriction as a result of 5-HT$_1$ and 5-HT$_2$ receptor activation, and hypoperfusion of the intestinal mucosa resulting from increased intralumenal pressure in patients with constipation or fecal impaction [121,155]. Although alosetron has been removed from the regular prescription market, it is available through a prescription program for use in female patients with severe refractory cases of diarrhea-predominant IBS.

Sodium polystyrene in sorbitol

Used to treat severe hyperkalemia, sodium polystyrene in sorbitol acts as a cation-exchange resin to decrease the serum potassium level. Rarely, ischemic colitis has been reported with both orally and rectally administered forms of the medication. It has been proposed that the hypertonicity of the sorbitol leads to rapid shifts of electrolytes, and both of these factors may be responsible for the colonic injury. Histologically, sodium polystyrene-in-sorbitol crystals are found adjacent to areas of necrosis in the colonic mucosa. However, the majority of patients are severely ill with separate risk factors for ischemic colitis, including renal failure and uremia [156,157].

Dextroamphetamines

Dextroamphetamine and other amphetamine medications, such as methamphetamine, are used in the treatment of attention deficit disorders and can lead to ischemic colitis [121]. Because amphetamines are sympathomimetic agents they can cause potent vasoconstriction, which is thought to be responsible for amphetamine-induced ischemic colitis [158]. The colitis typically resolves with withdrawal of the drug.

Pseudoephedrine

A sympathomimetic commonly used as a nasal decongestant, pseudoephedrine has been associated with ischemic colitis [159]. Colonic ischemia that results from use of pseudoephedrine is the result of vasoconstriction from its α-adrenergic agonist activity.

Vasopressin

Vasopressin and its analogues are potent vasoconstrictors traditionally used in the management of bleeding esophageal varices, and also employed for resuscitation following sepsis-induced shock. In its intravenous form, it acts by reducing splanchnic blood flow through vasoconstriction, which can induce colonic ischemia [160].

Digitalis

Digitalis is used in the treatment of cardiac arrhythmias and cardiac failure. Although digitalis has been noted to lead to

mesenteric vasoconstriction, it is also used in the treatment of conditions that predispose to an underlying low-perfusion state of the intestine, making it difficult to determine the true cause of the intestinal ischemia.

Neuroleptics

Case reports have associated neuroleptic medications, including tricyclic antidepressants, with the development of ischemic colitis [161].

Miscellaneous drug-induced colitides

Nonsteroidal antiinflammatory drugs (NSAIDs)

Nonsteroidal antiinflammatory drugs (NSAIDs) have long been associated with the development of intestinal pathology, most especially of the upper intestinal tract. The recognition of NSAID-induced colon toxicity has also increased but is still frequently underestimated. Up to 10% of all newly diagnosed colitis may be related to NSAIDs [162]. In one case–control study, 74% of patients with newly diagnosed colitis had a recent history of exposure to NSAIDS compared with 30% of controls [163]. The development of colitis in previously asymptomatic individuals has been associated with numerous NSAIDs such as ibuprofen, indomethacin (indometacin), diclofenac, enteric-coated aspirin, naproxen, phenylbutazone, piroxicam, and mefenamic and flufenamic acids [164]. Sustained-release formulations tend to lead most often to NSAID-induced colopathy. With rectal preparations, proctitis, rectal ulcers, and anorectal stenosis may result. Colitis induced by NSAIDs typically affects older adults, and no gender difference has been noted [164]. The most common presenting symptoms are diarrhea with or without occult bleeding or hematochezia; however, others may present with weight loss, fever, iron-deficiency anemia, elevated sedimentation rate, and hypoalbuminemia [165]. A rare few will experience symptoms of obstruction due to stricture or edema, which may lead to perforation. An increased risk of colonic diverticular perforation and the development of appendicitis has been associated with NSAIDs [67,164]. Ulceration and other inflammatory changes may be present in a focal or diffuse pattern throughout the colon, making it difficult to distinguish NSAID-induced colonic injury from IBD. Usage of NSAIDs may precipitate the initial flare of or worsen existing IBD [164,166,167]. Relapse of quiescent IBD was reported in 19 patients, the majority of whom had underlying ulcerative colitis, and relapse typically occurred within 1 week of NSAID ingestion [166].

The mechanism of injury to the intestinal mucosa may be due to multiple mechanisms. First, direct toxicity of the NSAID on the colonic mucosa may lead to increased intestinal permeability. Secondly, NSAIDs inhibit cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2), thereby decreasing prostaglandin synthesis and increasing lipoxygenase production [67,168]. Because prostaglandins have cytoprotective and vasodilating effects on the intestinal lining, whereas lipoxygenase metabolites are proinflammatory and lead to vasoconstriction of the mesenteric blood vessels, increased mucosal inflammation and ulceration may result [67,168]. Both direct and indirect injury lead to increased intestinal permeability, which allows lumenal bacteria to translocate across the bowel wall and induce the production of inflammatory cytokines and nitric oxide [169,170]. Because evidence exists demonstrating that deficiency of prostaglandin production is not sufficient to alter short-term intestinal permeability or integrity, a combination of factors is thought most likely to lead to NSAID-induced colitis.

Endoscopic findings vary from mild mucosal erythema with granularity, erosions, or flat ulcerations to severe pancolitis [165]. Diaphragm-like strictures are common in the small bowel with NSAID use but may be seen in the colon as well, typically in the right colon, and can lead to obstruction [171]. Histology is nonspecific and similar to that seen in ischemic colitis.

Treatment consists of withdrawal of the NSAID and supportive care, which usually leads to healing. Surgery may be necessary in patients presenting with severe bleeding, obstruction, and perforation. Anecdotal reports of antibiotics and steroids causing improvement exist [165]. Rechallenge with NSAID therapy typically induces recurrence of colonic injury [164]. Patients with underlying bowel disease should avoid NSAIDs.

Gold

Gold salts have been used to treat collagen-vascular diseases, especially rheumatoid arthritis. Use of this agent has been associated with the development of colitis, likely caused by local drug toxicity. Up to 95% of gold is excreted in the stool, and biopsy specimens have demonstrated gold in the tissues [172]. Gold-induced colitis appears to have a female predominance. Presenting symptoms include watery or bloody diarrhea, abdominal pain, tenesmus, fever, and rash. Laboratory investigation may reveal leukocytosis, eosinophilia, anemia, and hypoproteinemia. Endoscopic studies reveal mucosal edema and friability, ulceration, and hemorrhage, usually in the rectosigmoid colon [172]. Histology may demonstrate acute and chronic inflammatory infiltrate, possibly with eosinophils. Treatment consists of withdrawal of the medication and supportive care [173].

Isotretinoin

An analogue of vitamin A used in the treatment of cystic acne, isotretinoin has been associated with acute colitis and reactivation of quiescent IBD. Cessation of the medication has been reported to result in improvement, and rechallenge causes recurrence [174]. The mechanism of injury is unknown.

Antibiotics

Ampicillin, amoxicillin, and erythromycin have been associated with a hemorrhagic colitis with bloody diarrhea, right-

sided colitis, absence of pseudomembranes, and absence of *C. difficile* toxin [175]. The pathogenesis is thought to involve overgrowth of intestinal flora other than *C. difficile*, such as *Klebsiella oxytoca* [176]. The colitis typically resolves rapidly on withdrawal of the offending agent.

Chemotherapeutic agents

Intestinal epithelial cells have a rapid turnover, which is disrupted by the administration of cytotoxic chemotherapeutic agents. The majority of chemotherapeutic medications, including cytosine arabinoside, methotrexate, 5-fluorouracil, cyclophosphamide, and cisplatin, have the potential to induce colonic mucosal injury and inflammation [177]. The severity of the colitis ranges from mild segmental colitis to fulminant ulcerative colitis with toxic megacolon [178]. If the cecum is involved, it is known as "neutropenic typhlitis." Most cases of chemotherapeutic-induced colonic injury occur in patients with severe leukopenia, specifically neutropenia, which allows bacteria to translocate across the intestinal mucosa. The presenting symptoms include fever, diarrhea, and abdominal pain, possibly with the presence of rebound abdominal wall tenderness and guarding [179]. Laboratory studies reveal leukopenia with neutropenia and, possibly, bacteremia. Treatment is supportive with cessation of chemotherapy, antibiotic therapy, and bowel rest. Use of granulocyte colony-stimulating factor (G-CSF) may aid in the reduction of neutropenia. When the neutropenia resolves, the colitis will improve. Complications can be severe and life-threatening and include hemorrhage, shock, and perforation.

Methyldopa

Methyldopa is an antihypertensive medication that can induce colitis [180]. All reported cases have improved with withdrawal of the medication, and readministration led to development of colitis in most cases.

Flucytosine

Flucytosine is an antifungal medication that has been associated with severe colitis, often complicated by perforation [181]. The pathogenesis is unknown but may be related to intestinal conversion of flucytosine to 5-fluorouracil, a metabolite used as a chemotherapeutic agent.

Diversion colitis

Diversion colitis is an inflammatory process that develops in segments of the colon that have been excluded from the fecal stream by surgical bypass, such as with a Hartmann pouch or mucous fistula. First noted by Morson in 1972 and named by Glotzer et al. in 1981, diversion colitis has since been noted in patients undergoing surgical diversion for IBD, cancer, diverticulitis, Hirschsprung disease, and trauma. The condition may develop months to years after diversion has occurred, and clinical, endoscopic, and histological findings appear to be progressive, with mild changes observed at 3 months [182] and more severe changes noted at 6–9 months [183,184] after diversion of the fecal stream has occurred. The majority of patients are asymptomatic but presenting symptoms can include mucoid or bloody discharge, abdominal cramping, and rectal pain [183]. Symptoms have been reported to be more prevalent in patients with IBD (89%) compared with cancer patients (23%) and patients with nonmalignant lesions (50%) [185]. The inflammatory process typically resolves in 3–6 months after restoration of the fecal stream [186].

Endoscopic evaluation may disclose mucosal edema, friability, granularity, mucous plugs, nodularity, and erosions with or without exudate [183]. In one prospective study, endoscopic evidence of inflammation was seen in 91% of patients, with mild changes noted in 52%, moderate changes in 44%, and severe changes in 4% [187]. Despite the large percentage of patients with moderate to mild changes, only 6% were symptomatic. With longer periods of diversion, inflammatory polyps, aphthous ulcerations, and strictures may develop [188]. Although the majority of patients are asymptomatic, up to 100% of patients exhibit histological changes of diversion colitis [182–185]. Within 3 months the majority of patients demonstrate lymphoid follicular hyperplasia with frequent germinal centers and a lymphoplasmacytic infiltrate within the lamina propria; in 60% of patients there is also mild crypt architectural distortion [187,189] (Fig. 59.5a,b). In more severe cases, crypt abscesses, Paneth cell metaplasia, aphthous erosions, ulcerations, mucinous granulomas, and occasional epithelioid granulomas may be noted [190]. However, transmural inflammation, large ulcers, and major crypt architectural distortion are rarely seen in diversion colitis.

Multiple theories exist for the development of diversion colitis. However, the most widely accepted hypothesis maintains that a lumenal deficiency of short-chain fatty acids (SCFAs) results in a lack of fuel for the distal colonocytes, which leads to a decrease in epithelial cell proliferation and resultant inflammation [191]. Roediger demonstrated that SCFAs, the metabolic products of carbohydrate and peptide fermentation by anaerobic bacteria, are the main fuel for the distal colonocyte [192]. There are multiple beneficial effects SCFAs on colonic epithelium, including downregulation of tumor necrosis factor-α (TNF-α)-induced cytokine expression [193]. Harig et al. demonstrated that excluded colonic segments contained minimal amounts of SCFAs and that infused carbohydrates were not metabolized to SCFAs [194]. This may be a consequence of the reduced number of anaerobic bacteria within the excluded segment of bowel [195]. Instillation of SCFA enemas once or twice daily induced remission, both clinically and histologically, in patients with diversion colitis [196]. It should be noted that SCFA therapy of diversion colitis is not always successful; therefore, other

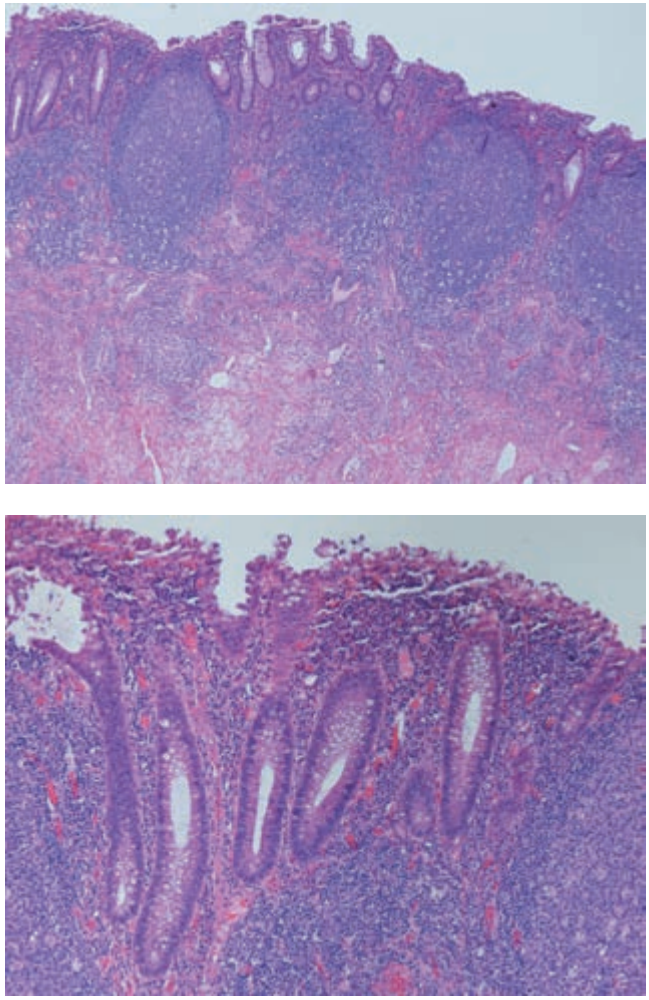


Figure 59.5 Diversion colitis is characterized by marked lymphoid hyperplasia, often compressing the crypt architecture **(a)**. There is no active cryptitis **(b)**.

hypotheses have been proposed regarding the pathogenesis of diversion colitis. A bacterial pathogenesis has been suggested but no single pathogenic organism has been isolated. An increase in nitrate-reducing bacteria (NRB) has been found in the defunctionalized colon, and a link between NRB and nitric oxide synthase-induced inflammation has been proposed [197]. Other possible mechanisms that have been suggested include the presence or lack of certain dietary factors, lumenal growth factors, or other bacterial metabolic products that may be necessary for optimal colonocyte function.

Diagnosis of diversion colitis is relatively straightforward in patients without presurgical intestinal inflammation, typically from underlying IBD. Flexible sigmoidoscopy with biopsies and lumenal aspirates for bacterial cultures, ova, parasites, and *C. difficile* toxin are useful to determine the extent of intestinal inflammation and to rule out other common causes of intestinal inflammation. Unless fistula or abscess is suspected, computed tomography (CT) and other radiographic examinations are not helpful. After resection of a colonic adenocarcinoma, the excluded segment of bowel must be included in regular colon screening, as polyps and recurrent adenocarcinoma may be a source of bleeding from the excluded bowel [185].

Differential diagnosis is typically straightforward. Diversion colitis lacks major crypt architectural distortion and is generally milder than IBD.

The majority of patients with diversion colitis are asymptomatic, therefore, therapy is rarely indicated. In the symptomatic patient, the preferred treatment is surgical restoration of intestinal continuity if possible. In those patients where intestinal restoration is not possible, treatment with SCFA enemas (60 mmol/L acetate, 40 mmol/L butyrate, 30 mmol/L propionate) administered twice daily per rectum or mucus fistula for 4 weeks and then decreasing to once or twice weekly may induce remission [194]. Mesalamine enemas have been reported to be helpful in a small number of patients [198].

Diverticular colitis

Diverticular disease-associated colitis (DAC) occurs in patients with diverticulosis of the sigmoid colon and is characterized by a chronic focal inflammation of the interdiverticular lumenal mucosa. First recognized in 1983, it is also known as diverticular colitis, crescentic fold disease, isolated sigmoiditis, and segmental colitis associated with diverticula (SCAD) [199]. Because the macroscopic and microscopic changes of diverticular colitis may be indistinguishable from those seen with IBD [200], it can be difficult to separate DAC from coexistent diverticulosis and IBD. Reports of coexistent disease are not uncommon. Diverticulosis is highly prevalent in Western populations, affecting up to 60% of people aged over 70 years [201], and Crohn's disease has a bimodal distribution, with a second peak occurring later in life [202]; therefore, the association of these two disorders is not unexpected [203]. The majority of patients with diverticulosis and IBD-related segmental colitis also have IBD elsewhere within the digestive tract. Resection of the segment demonstrating diverticular involvement effectively cures diverticulosis in such patients, but will not protect against further flares of the underlying IBD [204]. Nevertheless, there are patients with segmental colitis localized to an area of sigmoid diverticulosis in whom colitis does not recur after resection of the involved areas of colon. McCue and colleagues, for example, reported four patients who underwent segmental resection of the sigmoid colon for diverticulosis and Crohn's disease limited to the sigmoid colon. No patient developed recurrent Crohn's disease during a mean follow-up period of 22 months [205]. Gledhill and Dixon reported 11 patients diagnosed with both Crohn's disease and sigmoid diverticulosis or diverticulitis [206]. Three of the patients had Crohn's disease elsewhere in the digestive tract, but the remaining eight patients remained

free of recurrence over a follow-up of 1–10 years. In a prospective study of 23 patients with the endoscopic and histological diagnosis of segmental sigmoid colitis, only 3 patients developed unequivocal features of ulcerative colitis, whereas the remaining 20 patients had no extension of colitis over a 4-month to 8-year follow-up [203]. Goldstein et al. reported 29 patients who underwent sigmoid resection for Crohn's disease with diverticular disease [207]. Four had Crohn's prior to development of diverticular disease, but only 2 of the remaining 25 patients developed Crohn's disease over a median follow-up of 6 years. These observations suggest that colitis accompanying sigmoid diverticular disease is not likely to be classic IBD and is a separate disorder. The incidence of DAC ranges from 0.3% to 1.4% [203,208], and affected patients have a mean age at diagnosis of 63–69 years [203,206,208]. No gender predominance has been observed in diverticular colitis. Although some patients may be asymptomatic, with inflammation noted incidentally at the time of endoscopy or biopsy, up to 83% of patients may present with hematochezia [203], and other symptoms include cramping abdominal pain, diarrhea, and tenesmus. As stricture formation is rare, it is unusual for patients to present with obstruction.

The etiology of diverticular colitis is unknown. Left-sided diverticula are typically pseudodiverticula (false diverticula), which are outpouchings of the colonic mucosa and submucosa through the muscularis propria at points where blood vessels penetrate to supply the mucosa and submucosa [209]. Fecal stasis within and around the diverticula may alter local bacterial flora, contributing to the inflammation in diverticular colitis [200]. Peridiverticular mucosal prolapse may lead to diminished blood flow to segments of the colon affected by diverticulosis, initiating mucosal injury and allowing bacterial translocation across the mucosa. Intimal hyperplasia of blood vessels could limit local blood supply and contribute to a low-perfusion state, leading to ischemic injury of the mucosa surrounding the diverticula. Finally, activated immune cells in diverticulitis produce nitric oxide and could damage the intestinal mucosa by formation of oxygen radicals [210]. Complications of diverticulosis, such as subserosal peridiverticulitis and abscess formation, may lead to mass effect on the colonic wall and induce a localized inflammatory response.

Grossly, diverticula are universally present. Colonoscopic findings with diverticular colitis include mucosal edema, erythema, and friability confined to the distal descending and sigmoid colon [203]. The terminal ileum and remainder of the colon are unaffected; in particular, the rectum is spared and may aid in differentiating diverticular colitis from untreated chronic ulcerative colitis. Distribution of mucosal inflammation can be patchy or diffuse. The affected length of colon may range from 5 cm to 25 cm [203]. Focal bowel wall thickening, stricture formation, cobblestoning of the mucosa, and creeping serosal fat have been noted on resection specimens [206,208]. Histologically, diverticular colitis may be indistinguishable from IBD. Cryptitis, crypt abscess formation with distortion of crypt architecture, mononuclear infiltrate within the lamina propria, transmural lymphoid aggregates, transmural granulomatous inflammation, and granulomatous vasculitis have all been described. Makapugay and Dean, for example, found plasmacellular and eosinophilic expansion of the lamina propria in 100% of patients, neutrophilic cryptitis in 100%, crypt abscesses in 61%, distorted crypt architecture in 87%, surface epithelial sloughing in 61%, Paneth cell metaplasia in 48%, and granulomatous cryptitis in 26% [203]. The granulomatous inflammation is often a foreign-body reaction to mucin extrusion due to crypt rupture [203], although others have observed that many of the granulomas are found not near inflamed diverticula but throughout the entire intestinal wall and in the serosal fat, indicating that they represent "true" granulomas [206,208]. In one series, regional lymph node granulomas were noted in 75% of patients, effectively ruling out foreign body reaction as a cause of the granuloma [208].

Appropriate treatment rests on ruling out complications of diverticular disease, such as abscess formation and perforation, and ruling out other causes of segmental colitis, such as IBD, infection, and medication-induced colitis. Diverticular colitis can be distinguished from untreated chronic ulcerative colitis by the sparing of the rectal mucosa, which should be biopsied in all patients presenting with left-sided colitis [203]. Because diverticular colitis may exhibit the "classic" findings of Crohn's disease – transmural inflammation, fissures or fistulae, and granulomas – it may be impossible to distinguish diverticular colitis from Crohn's disease on endoscopic biopsies as well as segmental colectomy specimens [207]. As noted above, many patients exhibiting changes consistent with Crohn's disease only in the area involved with diverticulosis will not experience a recurrence of colitis after surgical resection, suggesting that the majority of patients with a dual diagnosis probably have diverticular colitis not Crohn's disease [207,208]. In this setting, unless the patient has involvement of the perianal area or other segments of bowel usually involved with Crohn's disease, the diagnosis of IBD should not be made because this may have greater implications for the patient, such as increased risk of colorectal cancer. Conservative treatment has been shown to be effective in up to 60% of patients and consists of a high-fiber diet, antibiotics, sulfasalazine, 5-aminosalicylic acid, and, in some cases, corticosteroids [203]. If diverticulitis is present, immunosuppression may be contraindicated, and bowel rest, intravenous hydration, and antibiotics may be warranted. Surgical therapy is warranted in those patients with complicated diverticulitis, such as perforation and intraabdominal sepsis. Segmental resection of the colon may also be warranted in patients with obstruction, stricture of unknown etiology, and refractory symptoms, such as hematochezia with anemia [203].

Colonic ulcers

Colonic ulceration occurring in otherwise healthy colonic mucosa may be associated with a number of conditions. Although the underlying etiology may vary, the presenting symptoms are strikingly similar and include abdominal pain, hematochezia, and mucopurulent rectal discharge. Diarrhea can occur if a large amount of colonic surface area is affected, but is rare with isolated ulceration. A careful history and physical combined with endoscopic and histological evaluation will aid in the differentiation of these conditions.

Isolated nonspecific colonic ulceration

First described by Cruveilheir in 1832, benign nonspecific colonic ulcerations occur infrequently. Ona and colleagues reported 127 patients with nonspecific colonic ulceration and determined that onset occurs at an average age of 45 years (range 8–84 years) and noted a slight female predominance (55%) [211]. Although nonspecific colonic ulcers may occur anywhere in the colon, they are most often found in the cecum (67%) and ascending or transverse colon (18%). These ulcers are usually solitary, round, or oval with sharply demarcated margins surrounded by normal colonic mucosa, and are located on the antimesenteric side of the intestinal lumen [211–214]. Biopsies are nonspecific and demonstrate both acute and chronic inflammation [211].

The pathogenesis of nonspecific colonic ulceration remains unknown, and other causes of colonic ulceration, such as inflammatory bowel disease, infection, medication-induced ulceration, vasculitis, fecal stasis, rectal prolapse, renal failure, and malignancy, must be carefully ruled out before a diagnosis of nonspecific colonic ulceration can be made. In a case series of 26 patients with nonspecific erosions and ulcerations of the colonic mucosa, the most common causes were previous abdominal irradiation and ischemia [215]. Vascular abnormalities, including thickening of the vascular walls and microvascular thrombosis, have been described in the area surrounding the ulceration, which may implicate a localized form of ischemia [212–214]. The ischemic hypothesis is supported by the location of the ulcers on the antimesenteric border, the shallow granulation base, and the sometimes circumferential orientation of the ulcers. However, ischemia typically occurs in the watershed area of the colon, usually in the descending colon and sigmoid colon. This would fail to explain why the majority of nonspecific colonic ulcerations are right-sided. Cecal diverticulosis has been proposed as a cause of nonspecific colonic ulceration as a result of the observation that 5 out of 10 cases of cecal ulceration had cecal diverticula [216]. However, cecal diverticula typically occur on the mesenteric border whereas ulcerations are most often found on the antimesenteric border of the cecum, which casts doubt on this hypothesis. In addition, the sigmoid colon has a much higher prevalence of diverticula when compared with the cecum, yet is not a common site for nonspecific colonic ulceration. It has also been proposed that a disturbance in acid–base balance in the ileocecal region is involved in the pathogenesis of nonspecific cecal ulcers or that digestive enzymes presented to the cecal mucosa lead to ulceration [212]. Medications such as NSAIDs, corticosteroids, and oral contraceptives [211,214], as well as foreign body trauma [211], infection [212,217], and toxins, have each been implicated in the formation of colonic ulcers. It is likely that multiple causes exist for the development of nonspecific colonic ulceration.

The most common presenting symptoms are abdominal pain in the right lower quadrant (50%) and hematochezia (33%) [211]. Other features include fever and leukocytosis. A palpable abdominal mass was noted in up to 16% of patients and was present with transverse- or left-sided colonic ulcerations, presumably due to associated edema of the colon. Such an inflammatory mass has potential to obstruct the lumen of the intestine and may mimic a malignancy on diagnostic work-up. Perforation presenting with an acute surgical abdomen occurred in about one-fifth of patients [211].

A history is typically unhelpful unless the patient has overt rectal bleeding or signs and symptoms of obstruction. Physical examination may be normal or may disclose abdominal pain or mass on rectal examination. In the past, this diagnosis was made at the time of surgery for a perforating complication, which was noted in 82% of patients in a retrospective review [213]. Colonoscopy, the diagnostic test of choice, has facilitated early diagnosis and more aggressive medical management.

The differential diagnosis for nonspecific colonic ulceration is broad and the diagnosis is one of exclusion. An infectious cause of ulceration must be ruled out, especially in immunocompromised hosts, although typically an infectious etiology leads to the formation of multiple ulcerations. Infections that can cause colonic ulceration include cytomegalovirus, tuberculosis, *Yersinia enterocolitica*, *Entamoeba histolytica*, and *Salmonella typhi*. Tuberculosis of the colon usually causes nodularity and deformity of the ileocecal valve whereas nonspecific colonic ulceration usually does not produce these findings [214,218]. Inflammatory bowel disease should be excluded and other clinical features, such as the involvement of other areas of the intestinal tract and the appearance of the ulcers, may be helpful in differentiating nonspecific colonic ulceration from Crohn's disease [214]. Ischemic ulcers are usually located within the watershed areas of the colon, namely the rectosigmoid junction and the splenic flexure [211]. Solitary rectal ulcers are not found in the right colon and have a distinct microscopic appearance, which is described later in this chapter. Stercoral ulcers are normally caused by fecal impaction with resultant pressure necrosis and are most commonly found within the sigmoid area. The diagnosis of malignancy may be difficult to distinguish on investigations from nonspecific colonic ulcers, especially

if a mass effect is associated with the ulcer, and must be ruled out histologically. Vasculitis due to Behçet disease, systemic lupus erythematosus, and Churg–Strauss syndrome [211,219] have rarely been associated with nonspecific colonic ulcers and, if the clinical scenario is fitting, should be ruled out.

Uncomplicated colonic ulcers may be followed endoscopically every 4–6 weeks, with multiple biopsies being taken at the time of each endoscopy to document healing and rule out malignancy and infection. Surgery remains the mainstay of therapy for nonspecific ulcers complicated by bleeding, failure to heal on endoscopic examination, abscess formation, and perforation. Oversewing of the ulcer, local excision of the ulcer, and right hemicolectomy have been performed for surgical therapy of nonspecific colonic ulceration.

Dieulafoy-type colonic ulceration

In 1897 Georges Dieulafoy described three otherwise asymptomatic patients who exsanguinated due to a minute gastric ulceration overlying a submucosal artery. He termed this lesion *exulceratio simplex*. Although this type of ulceration most commonly occurs in the upper third of the stomach, lesions virtually identical to Dieulafoy's initial description have been reported to occur throughout the intestine [220,221]. Colonic Dieulafoy lesions are exceedingly rare. In the largest published series [221], only five colonic Dieulafoy lesions were noted among 3059 patients presenting with gastrointestinal bleeding. Presentation is usually one of acute massive rectal bleeding accompanied by severe hemodynamic instability. Pathophysiology is not known, although it has been proposed that pulsations of the large-caliber submucosal artery lead to erosion of the overlying mucosa [220]. Diagnosis may prove problematic as lesions are notoriously difficult to see on endoscopy because of the intermittent nature of the arterial bleeding coupled with the lack of inflammation surrounding the mucosal ulceration. Often, the lesion may not be bleeding at the time of the endoscopy and it may resemble a pseudopolyp, although once the extruded tissue is removed, the resulting hemorrhage is characteristic of a Dieulafoy lesion. Doppler-endoscopy may be helpful to identify arterial flow within a lesion [222]. A pulsating red spot covered with clot should raise suspicion for a Dieulafoy lesion of the colon [217]. If endoscopic localization of the lesion is unsuccessful, selective mesenteric angiography may be helpful [223]. Angiography is most useful when the patient is actively bleeding. Colonic lesions are grossly and histologically identical to classic gastric Dieulafoy lesions, demonstrating a single mucosal defect extending no deeper than the upper submucosa. Endoscopic therapies such as sclerotherapy, electrocautery, and hemoclipping have led to successful control of bleeding in a number of reports, and endoscopic intervention should be attempted if the condition of the patient allows [221,224]. Surgical resection of the affected area remains the definitive therapy.

Stercoral ulcer (Huntley syndrome)

A stercoral ulcer is a colonic ulceration caused by ischemic pressure necrosis resulting directly from a fecal (stercoraceous) mass or impaction. This ulceration may lead to perforation and peritonitis. Risk factors for the development of both stercoral ulceration and perforation include: chronic constipation; factors or medical conditions that predispose to constipation, such as confinement to a long-term care facility; use of constipating medications, such as narcotics; hypothyroidism; colonic strictures; neoplasms; renal failure; and transplantation. In these patients, fecalomas (masses of hard stool) may have been present for years and may have laminations and calcification [225]. Less than 100 cases have been reported in the literature. Most stercoral ulcers occur in the rectosigmoid colon [225,226]. Perforating stercoral ulcers are typically located on the antimesenteric border of the sigmoid or rectosigmoid colon, and the diameter of the perforation usually exceeds 1 cm [225,226].

Surgical resection is the definitive therapy of both perforated and nonperforated stercoral ulcers. The major cause of mortality is uncontrolled sepsis [225]. Resection of the involved area with primary anastomosis may be performed in the absence of perforation, whereas resection with end ileostomy and Hartmann pouch or mucous fistula may be performed for those with stercoral perforation.

Solitary rectal ulcer syndrome and colitis cystica profunda

Solitary rectal ulcer syndrome

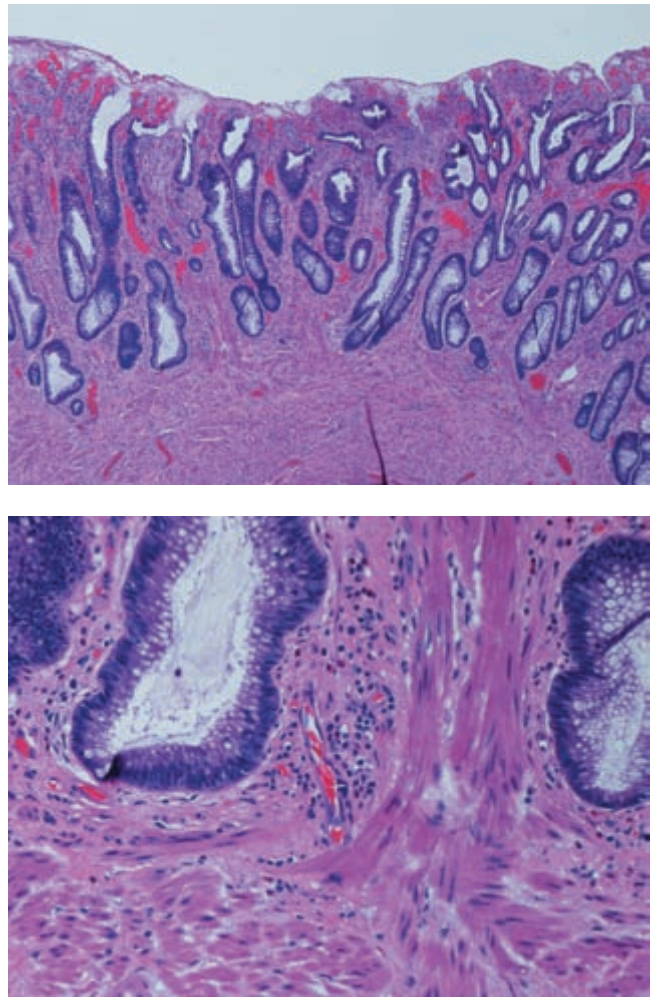
Solitary rectal ulcer syndrome (SRUS) was first described in 1830 by Cruveilheir, but it was not until the 1969 review by Madigan and Morson, which reported on 68 cases and established diagnostic criteria, that the knowledge of this disease entity became widespread [227]. An uncommon disorder, it affects 1 in 100 000 [228]. It typically affects young adults, presenting in the third or fourth decades [229].

Presenting symptoms are alteration of bowel habits, difficult evacuation, rectal bleeding, and mucous discharge. Tenesmus, constipation, straining, incomplete evacuation, and lower abdominal pain have been reported in 70%–90% of patients [230,231]. Rectal bleeding occurs in up to 89% of patients [230] and can range from minimal to massive blood loss [232]. Overt rectal prolapse and fecal incontinence are less common. Interestingly, in one large review, 26% of patients with endoscopic discovery of a solitary rectal ulcer were asymptomatic [230].

The etiology and underlying pathophysiology of SRUS are not clear. Local trauma due to prolapse of rectal mucosa, self-digitation, and stool impaction, as well as ischemic injury have been implicated in the pathogenesis of SRUS [233]. However, 50% or more SRUS patients deny digital manipulation of the rectum and, at times, the ulcer is located in an area too proximal in the rectum to be reached by insertion of a finger. The incidence of mucosal or full-thickness rectal

prolapse ranges from 13% to 100% in published series of SRUS patients [230]. Salzano et al. noted that 52% of patients experienced either mucosal prolapse or external rectal prolapse [234]. Ischemic injury can occur as a consequence of impacted stool, pressure on the rectal mucosa due to contracted puborectalis muscle or overactivity of the external anal sphincter [235], use of suppositories such as ergotamine [236], and a history of radiation to the rectal area [237]. A combination of rectal prolapse and a high fecal voiding pressure (caused by overactivity of the external anal sphincter or failure of puborectalis relaxation during defecation) has been theorized to cause both trauma and localized ischemic injury to the rectal mucosa resulting in ulceration [235]. It appears that SRUS is a benign condition but, rarely, an underlying carcinoma may be present with SRUS.

Diagnosis is based on appropriate symptomatology combined with endoscopic and histological findings consistent with SRUS. Obtaining a proper history is crucial in excluding more serious or potentially life-threatening disorders, such as malignancy, infection (amebiasis, lymphogranuloma venereum, syphilis), and chronic intestinal ischemia. Other diagnoses that may present in a similar fashion to SRUS include inflammatory bowel disease, NSAID-induced or ergotamine-induced ulcerations, and endometriosis. Despite the term solitary rectal ulcer syndrome, patients may present with multiple ulcers, polypoid lesions, or simply erythematous mucosa [228]. Therefore, it is not surprising that its endoscopic appearance is variable. In classic SRUS, a single, well-demarcated ulcer measuring up to 5 cm will be located at 6–10 cm from the anal verge on the anterior rectal wall in 68%–95% of cases [228,230]. However, given the variable endoscopic appearance, it is imperative to obtain biopsies of the affected area to rule out other disorders, such as malignancy. Defecography can be helpful in demonstrating the presence of internal and external rectal prolapse as well as colonic intussusception, incomplete rectal emptying, and nonrelaxing puborectalis. This examination is typically utilized when operative intervention is considered [238]. Barium enema and anorectal manometry do not appear to be useful in the diagnosis of SRUS. Transrectal or endoscopic ultrasound may be used to rule out invasive malignancy and assess the extent of disease in SRUS patients [233]. Specific histological criteria as defined by Madigan and Morson are used to establish the diagnosis of SRUS [227]. Fibroblasts, smooth muscle, and collagen replace the lamina propria with associated hypertrophy and disorganization of the muscularis mucosa, termed fibromuscular obliteration of the lamina propria (Fig. 59.6). Erosion of the mucosal surface as well as distortion and displacement of the mucosal glands into the submucosa may be seen. Diffuse collagen infiltration of the lamina propria is seen almost exclusively in SRUS and can be used to differentiate this disorder from inflammatory bowel disease and chronic ischemic colitis [239]. Occasionally, cystic submucosal glands have been noted, and this has led to speculation that colitis cystica profunda and SRUS are identical or closely related syndromes.



(a)

(b)

Figure 59.6 Solitary rectal ulcer syndrome (prolapse-related injury). **(a)** Thickened mucosa with surface inflammation and erosion. **(b)** The base of the mucosa shows thickened, frayed bands of smooth muscle typical of prolapse injury.

Treatment of SRUS depends on the presence or absence of symptoms, the presence or absence of rectal prolapse, and the type of lesion in the rectum. Treatment can be divided into medical and surgical options, although there are few data regarding the efficacy of medical therapies. Treatment may not be necessary in asymptomatic patients, and spontaneous resolution of SRUS has been reported. Patients with polypoid lesions are usually younger and less symptomatic and often respond to conservative medical therapy [230]. Bowel retraining, high-fiber diet, and bulk laxatives have all been used with variable success rates. Fibrin glue has shown promise with topical application to the ulcer leading to healing in 2 weeks [240]. Surgery with abdominal rectopexy is advised for patients with significant rectal prolapse or those refractory to medical therapy. One group demonstrated

complete resolution of rectal prolapse in 95% of patients with both prolapse and SRUS [238].

Colitis cystica profunda

Colitis cystica profunda is a rare condition in which variably sized mucus-filled cysts are found within the submucosa of the lower intestinal tract, most often as a solitary lesion in the rectum [241]. The cysts are typically large, located below the muscularis mucosae, and may be mistaken for a malignant process based on presentation of the patient and radiographic and endoscopic appearances. Similar cysts may be found in the small bowel, where the entity is then termed "enteritis cystica profunda." The distribution of colitis cystica profunda may be localized, segmental, or diffuse [241,242]. Presenting symptoms are most commonly diarrhea, hematochezia, and mucus discharge per rectum [241]. Bulky cysts or fibrotic lesions may lead to intestinal obstruction, although this is rare. Colitis cystica profunda does not have proven malignant potential but adenocarcinoma has been found adjacent to the submucosal cysts [243]. The pathogenesis is unknown.

Physical examination is variable and depends on the distribution of the disease. The localized form can usually be detected at the time of digital rectal exam, which reveals one or more smooth, firm masses not adherent to the underlying muscle layer, thickened rectal wall, or stenosis. Lesions are usually found within 12 cm of the anal verge on the anterior rectal wall. At colonoscopy, findings range from normal to mucosal edema, erythema, friability, or ulceration overlying a polypoid submucosal mass. An umbilication may be noted on the lumenal surface of the cyst as these lesions typically communicate with the lumen through mucosal openings. Large cysts may be present, and multiple cysts can coalesce to form a mass measuring up to 3 cm. Rarely, this mass may obstruct the lumen of the bowel. Rectal stricture or prolapse may be noted. It can be difficult to differentiate endoscopically the findings of colitis cystica profunda from other disorders such as IBD, pseudopolyps, submucosal lipomas, and adenocarcinoma. Because the mucosal biopsies obtained by endoscopy are shallow, they may be inadequate for definitive diagnosis of colitis cystica profunda. Surgical excision may be necessary to make a definitive diagnosis. Biopsy demonstrating the typical histological features and the absence of malignant findings is necessary to make a definitive diagnosis of colitis cystica profunda. Microscopically, the submucosal layer is thickened by cysts, which occasionally extend into the muscularis propria and serosa (Fig. 59.7). Care must be taken to differentiate this disorder from well-differentiated mucin-producing adenocarcinoma. Cysts might be lined with a well-differentiated epithelial lining or have no lining at all. The lamina propria is usually replaced by collagen and disoriented smooth muscle cells. The cysts are typically bordered by normal colonic mucosa, but adjacent mucosal edema, superficial ulceration, acute and chronic inflammation, pseudomembranes, and distorted crypt architecture may be present.

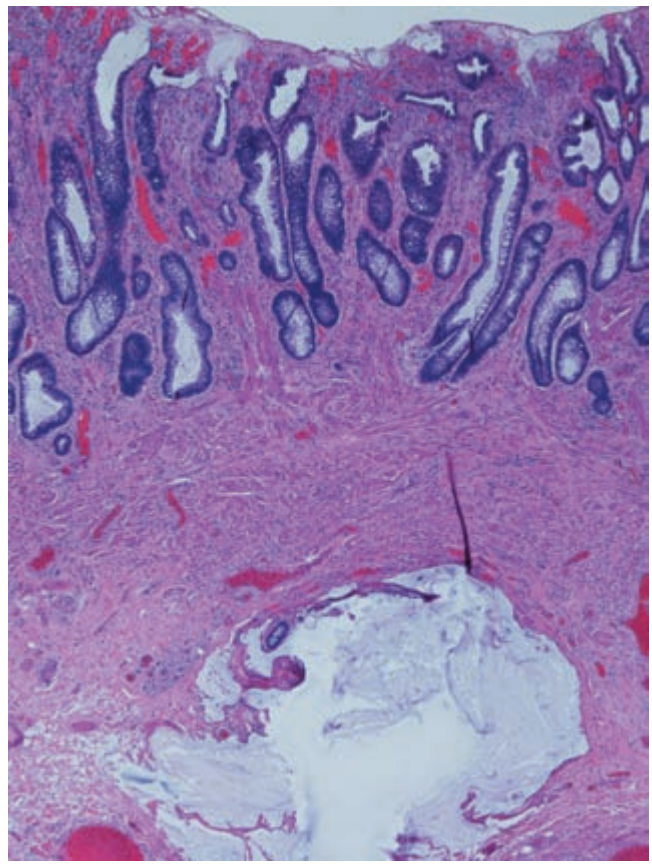


Figure 59.7 Colitis cystica profunda. The mucosa shows prolapse-related injury. There is displaced mucin and epithelium in the submucosa.

The majority of symptomatic patients have been treated with local surgical excision of cysts and ulcers. If localized colitis cystica profunda is present, transanal excision is usually adequate [241]. In the setting of rectal prolapse, repair may lead to resolution of the cysts. Although colonic resection is usually unnecessary, it may be appropriate for cases of diffuse involvement, severely symptomatic cases, and those cases where cysts lead to intestinal obstruction.

Pneumatosis cystoides intestinalis

Pneumatosis cystoides intestinalis is an uncommon condition characterized by gas-filled cysts within the wall of the intestine, typically within the subserosa and submucosa of the small and large bowel. Cysts occur most commonly in the small bowel (42%), in the large bowel less often (36%), and diffusely in both the small and large bowel in the remainder (22%) [244]. These gas-filled cysts are also rarely found in the stomach and other extraintestinal structures such as the omentum, mesentery, and lymph nodes [245]. Pneumatosis is a sign not a disease. Clinical presentation and history should be considered when attempting to determine the etiology

of the pneumatosis. Pneumatosis has been associated with more fulminant conditions such as bowel infarction, perforation, necrotizing enterocolitis, and pseudomembranous colitis. Pneumatosis cystoides intestinalis can be classified as either primary (idiopathic) or secondary. Secondary pneumatosis has been associated with both primary gastrointestinal disorders and extraintestinal conditions, most commonly with chronic obstructive pulmonary disease, collagen vascular diseases (mixed connective tissue disease, scleroderma, systemic lupus erythematosus, dermatomyositis, polymyositis), cystic fibrosis, intestinal obstruction, pseudoobstruction, pyloric stenosis, inflammatory bowel disease, status post-jejunoileal bypass, sigmoid volvulus, and iatrogenic induction by endoscopy or surgery [246–249]. Pneumatosis has occurred in patients with late-stage AIDS, which appears to be associated with cryptosporidial diarrhea, and tends to involve the right colon and cecum [250]. Reports of association with rotavirus in children and the presence of spirochetes within the cysts can be found in the literature [251,252]. Patients with hematological malignancies undergoing chemotherapy have developed pneumatosis during times of leukopenia, thought to be due to damage of the intestinal mucosa by chemotherapeutic agents or steroids [247]. Medications have also been associated with secondary pneumatosis. Chloral hydrate medications and exposure to trichloroethylene, both of which are chemically similar to chloroform, have been implicated in the development of pneumatosis cystoides intestinalis [253]. In patients with IBD and transplantation, steroids have been linked to the development of pneumatosis intestinalis [248]. A case–controlled study was performed comparing 50 patients with Crohn's disease who underwent CT scanning to rule out abscess, with 50 control patients without inflammatory bowel disease [248]. In the Crohn's disease group, 12% were found with pneumatosis whereas none in the control group had evidence of pneumatosis by CT scan. Corticosteroid treatment was significantly related to development of pneumatosis ($P = 0.025$) [248]. Pneumatosis occurs in patients with a history of cardiac, liver, renal, or bone marrow transplantation [254,255]. In the setting of systemic illness or complication, it is a poor prognostic sign [256]. Only 15% of cases are idiopathic [245]. Pneumatosis can occur at any time from infancy to adulthood, but the majority of cases occur between 30 and 50 years of age [257].

Pneumatosis is typically found incidentally on radiographs, endoscopic evaluation, or at the time of laparotomy as the majority of patients are asymptomatic or minimally symptomatic. The most common symptoms are diarrhea, rectal bleeding, and mucous discharge [246]. Patients have also identified constipation, abdominal pain, weight loss, and tenesmus as presenting symptoms of the condition [246]. Secondary pneumatosis intestinalis typically affects the small bowel and ascending colon, whereas the left colon is typically affected in idiopathic cases [258]. The sigmoid colon has been found to be redundant in 80% [246], and it remains unknown if this redundancy plays a role in the formation of pneumatosis. Infrequently, the rectum is involved [257]. Cysts can vary in size from millimeters to a few centimeters, and are typically found in clusters. Complications are rare and include pneumoperitoneum, obstruction, volvulus, intussusception, hemorrhage, and perforation [259].

In up to two-thirds of patients, diagnosis can be made on the basis of plain abdominal films, in which the cysts appear as curvilinear, linear, cystic, or grape-like clusters within the bowel wall [260]. In addition, free intraperitoneal air and retroperitoneal air may be noted. Decubitus and upright positions may help to distinguish intralumenal air from pneumatosis intestinalis. Barium contrast studies show multiple radiolucent lumenal filling defects interposed between the barium column and the outer bowel, and can help confirm location within the bowel wall [261]. However, on barium enema, pneumatosis can be misdiagnosed as intestinal polyps, pseudopolyposis, and intramural hematomas [262]. CT scanning is reported to be more sensitive in the detection of intramural gas than plain abdominal radiographs [263,264]. On CT the intramural gas of pneumatosis appears parallel to the bowel wall and can be located on the dependent aspect of the bowel and does not contain air–fluid levels. In addition, CT demonstrates thickened bowel wall, free intraperitoneal air, hepatic portal venous gas, intralumenal gas, and mesenteric gas. The presence of portal venous gas or mesenteric gas with pneumatosis is highly suggestive of bowel infarction. Pneumatosis may be misdiagnosed as stool mixed with colonic gas. Magnetic resonance imaging (MRI) has been used to identify pneumatosis, which is suggested by finding circumferential collections of air adherent to or within the bowel wall. Endoscopic evaluation with flexible sigmoidoscopy or colonoscopy reveals multiple, round, pale to bluish, submucosal polypoid masses covered with normal mucosa protruding into the bowel lumen [265]. Diagnosis can be confirmed by collapse of the cysts on attempted biopsy or puncture, which often produces a hissing or popping sound [256]. Caution must be exercised when attempting to biopsy one of these nodules as perforation of the bowel has been reported [266]. Endoscopic ultrasound has been utilized in a small number of patients to confirm the presence of submucosal cysts [267]. Histology of these cysts demonstrates the absence of a true epithelial lining but instead they are partially lined with histiocytes and foreign-body giant cells [268]. At times, the cysts have been found to be lined with a single layer of mesothelium or endothelium [268]. Inflammation with neutrophils, eosinophils, plasma cells, lymphocytes, and epithelioid granulomas has been noted as well.

The significance of pneumatosis is directly related to the overall clinical condition of the patient. In asymptomatic patients, treatment is indicated only for any associated illness(es) as the cysts typically resolve spontaneously. In fact,

no treatment is necessary in 85% of asymptomatic patients, and up to 50% of cases will resolve spontaneously [245,269]. High-flow oxygen and hyperbaric oxygen have been used with success in the treatment of pneumatosis cystoides intestinalis. Oxygen therapy is based on the hypothesis that the high concentration of inhaled oxygen increases the partial pressure of oxygen in the bloodstream relative to hydrogen and nitrogen, thus causing resorption of the gases from the cysts into the bloodstream [257]. High-flow oxygen, delivering 55%–75% oxygen by a venturi mask and used to achieve an arterial oxygen partial pressure of 200–350 mmHg, has been shown to lead to resolution of cysts. One report showed that oxygen delivered at lower concentrations (40%) was also successful [270]. Treatment should be continued for at least 5–7 days and for 2 days after resolution of symptoms [270,271]. Unfortunately, relapse is common with the use of oxygen (50%–78%), although patients do appear to respond to a repeated course of oxygen therapy [246]. Hyperbaric oxygen administered at 303.975 kPa for 45–60 min twice daily for up to 30 days has been successfully used as well [272,273]. Metronidazole has been used in the treatment of pneumatosis on the premise that anaerobic bacteria are responsible for the formation of the gas-filled cysts [257,274]. Use of an elemental diet has also been shown to be helpful in the treatment of pneumatosis [275]. Although most patients with benign pneumatosis can be managed medically, a rare few will require surgical resection for symptomatic relief. Surgery typically involves a partial colectomy, yet this does leave a potential for recurrence. There are reports of successful management with ileal pouch–anal anastomosis [276]. Surgical intervention is typically warranted in those patients with fulminant pneumatosis.

Endometriosis

Endometriosis is a common disorder that occurs in approximately 15% of menstruating women [277], although the prevalence may be higher due to the presence of nonpigmented endometriosis [278]. Endometriosis can involve sites distant from the uterus and ovaries. Endometrial involvement of the intestinal tract occurs in 15%–37% of patients with pelvic endometriosis and is often asymptomatic [279–281]. Within this group, the rectosigmoid colon is most commonly affected (95%), with more proximal locations, such as the appendix (10%) and the terminal ileum (5%), involved less frequently [280]. In a review of 7200 women with endometriosis, the small bowel was affected in only 38 patients [282]. The mean age of diagnosis is between 25 and 29 years [277], and most patients who undergo surgical resection of intestinal endometriosis are between the ages of 28 and 56 [283]. The usual indication for intestinal resection is chronic partial obstruction, due to the chronic inflammation and fibrosis from endometrial intestinal implants or due to a mass effect of the submucosal implant causing compression of the lumen [284]. Colonic obstruction due to endometriosis is rare and usually occurs in postmenopausal women treated with hormone replacement therapy or tamoxifen [285].

Symptoms of pelvic endometriosis, such as dysmenorrhea, dysfunctional uterine bleeding, dyspareunia, and infertility, are more prominent than the intestinal symptoms. Those patients with serosal implants may present with abdominal pain or cramping, abdominal bloating, low back pain, localized tenderness, palpable nodules on rectovaginal exam, change in stool caliber, diarrhea, and rectal bleeding [283]. Hematochezia is noted in 3%–33% of patients undergoing surgical resection of the intestine due to endometriosis [284,286]. Intestinal symptoms do not follow the cyclic changes with the menstrual cycle, primarily because the intestinal lesions of endometriosis are not strongly influenced by hormonal changes (i.e., fibrostenosis of the lumen, and compression of the lumen due to the endometrioma) [284]. Acute appendicitis due to intussusception of an endometrioma has been reported [282].

Grossly, endometrial implants may be described as bluish-gray nodules resembling "powder burns" ranging from 2 mm to 2 cm in diameter [277]. Adhesions and mural cysts surrounded by dense fibrosis may be noted in the area surrounding implantation [283]. Histologically, aggregates of secretory glands lined by cuboidal endometrial epithelial cells and embedded in endometrial stroma with surrounding fibrosis are seen. These glands can be cystic or filled with blood. The mucosa of the intestine is rarely involved but can demonstrate changes more often found with IBD, such as crypt architecture distortion and branching lymphoplasmacytic infiltration, and Paneth cell metaplasia [283], confined to the mucosa adjacent to the invading endometrial nodules. Uncommon features include mucosal ulcers, and polypoid masses [287]. Endometrial tissue is typically deep within the intestinal wall and it may not be present in endoscopic biopsies.

The classic clinical presentation is partial obstruction of the colon in an infertile woman of reproductive age with dysmenorrhea and dyspareunia. On pelvic examination there may be palpable nodules in the rectovaginal septum. Barium enema and CT scanning are usually nondiagnostic, but may demonstrate strictures, compression, obstruction, or polypoid masses of the intestine. However, the lesions of intestinal endometriosis are typically too small to exclude malignancy based on these imaging studies. Endoscopic evaluation may be normal in the presence of noninvasive serosal implants. If hematochezia is present, invasion of the mucosa by endometrial implants may be noted on endoscopy or, in the presence of obstruction, strictures or extrinsic compression of the lumen may be noted. Endorectal ultrasonography may be helpful in detecting intestinal wall invasion by endometrial implants [288]. Diagnosis is confirmed by laparoscopy or

laparotomy, with biopsy demonstrating the histological findings of endometriosis.

The differential diagnosis includes irritable bowel syndrome, IBD, intestinal ischemia, gastrointestinal or gynecological malignancy, and lymphoma.

Although medical therapies such as danazol and medroxyprogesterone are beneficial in the treatment of pelvic endometriosis, these compounds have not proved effective in the treatment of symptomatic intestinal endometriosis [289]. Laparoscopic carbon dioxide laser is used to ablate endometrial implants [290]. If the intestine is partially obstructed by an implant or fibrosis, segmental resection is recommended to exclude carcinoma [285].

Typhlitis

Typhlitis is an acute necrotizing inflammation of the colon, primarily involving the cecum, terminal ileum, and ascending colon, associated with chemotherapy-induced neutropenia. Also known as necrotizing enterocolitis, neutropenic enterocolitis, and ileocecal syndrome, it can also involve the small bowel, appendix, and other areas of the colon [179,291]. Typhlitis has also been noted in a number of immunosuppressed adult patients, such as those receiving chemotherapy for hematological and solid malignancies, transplantation patients, cyclic neutropenia, aplastic anemia, drug-induced granulocytopenia, and AIDS [179]. Typhlitis has been estimated to occur in 2.6%–33% of those at risk [179,292]. The incidence of typhlitis may be increasing with the increasing use of more aggressive chemotherapeutic and immunosuppressive regimens. Various types of chemotherapy may lead to typhlitis [293], and it occurs more commonly with induction chemotherapy than with consolidation chemotherapy. Patients classically present 10–14 days after initiation of chemotherapy with fever, diarrhea, and abdominal pain, which may localize to the right lower quadrant (60% of patients) in the presence of neutropenia [293,294]. Patients may also present with nonspecific findings of stomatitis, nausea, emesis, abdominal distention, and bloody stools. Rarely, signs of peritonitis and shock are present; however, perforation or sepsis should also be considered in the differential diagnosis of these patients. Occasionally, neutropenic patients will present with a septic picture but nonlocalizing signs or symptoms. Blood cultures may be positive in up to 50% of patients and usually grow enteric organisms, such as *Pseudomonas* spp., *Escherichia coli*, *Staphylococcus aureus*, *Clostridia* spp., and *Klebsiella* spp. [179]. Fungal organisms, such as *Candida* spp. and *Aspergillus* spp., were found in 14% of cases prior to death but in 53% of cases after death [179]. The pathogenesis of typhlitis remains unknown.

Typhlitis may be difficult to diagnose given the nonspecific signs, symptoms, and radiographic findings that accompany the disorder. The physical exam is typically unrevealing, although a boggy cecum may be palpable as a mass in the right lower quadrant [293]. Laboratory examination is most remarkable for severe neutropenia, usually with an absolute neutrophil count less than 500 [179]. Blood cultures may be positive for bacterial infection in 50%. Radiological investigation with plain films of the abdomen is rarely helpful but may demonstrate findings of dilated bowel, dilated cecum with a decrease in gas in the right lower quadrant, thumbprinting, or, in the case of perforation, free air. Barium enema is avoided because of the possibility of perforation. Ultrasound may demonstrate the nonspecific findings of a thickened bowel wall, intraabdominal abscess, and an enlarged cecum. Cartoni and colleagues demonstrated a 60% mortality in patients with a bowel wall thickness greater than 10 mm, compared with only 4% for patients with a bowel wall thickness of less than 10 mm [295]. Computed tomography scan is more sensitive than plain radiography and ultrasound for typhlitis, and may demonstrate findings of thickened bowel, thickened fascial planes, pneumatosis intestinalis, ascites, bowel distention, and mesenteric stranding [291]. Instillation of air by endoscopy may be hazardous and induce or worsen perforation.

Typhlitis must be considered in any neutropenic patient with fever, diarrhea, and abdominal pain. Appendicitis, diverticulitis, IBD, enteric infection, intussusception, intramural hemorrhage, leukemic infiltrate, and Ogilvie syndrome should all be considered in the neutropenic patient presenting with fever, diarrhea, and abdominal pain.

Survival depends on prompt recognition of this disorder, appropriate management, and return of the neutrophil count to normal. Both conservative medical therapy and more aggressive surgical management have been advocated but study results have been conflicting. Conservative management may be better for those patients with a more indolent course, whereas surgery is best reserved those with persistent abdominal pain, pneumatosis intestinalis, sepsis, and persistent small bowel obstruction. Medical therapy consists of bowel rest with nasogastric suction if necessary, intravenous hydration, and broad-spectrum antibiotics with *Pseudomonas* coverage. If the patient remains febrile after 72 h of antibiotics, antifungal therapy should be added and further investigations undertaken to rule out abscess or other complications. Granulocyte colony-stimulating factor (G-CSF) may be helpful in bringing about an increase in the neutrophil count of these patients, which typically leads to resolution of the colitis [296]. Close observation of patient clinical status and radiographic progression is necessary to determine if conservative management is successful. Surgery is indicated for clinical deterioration despite medical therapy, free intraabdominal perforation, persistent gastrointestinal bleeding after improvement of neutropenia, thrombocytopenia, and coagulopathy [292,293]. The preferred procedure is right hemicolectomy with ileostomy and stoma formation, because any anastomosis may break down in the presence of

neutropenia. Resection of all necrotic foci is imperative for the survival of the patient [292]. Mortality from typhlitis is high, ranging from 8% to 60% [294,295], whether treated medically or surgically. Patients who develop typhlitis during induction chemotherapy and are medically treated are prone to relapse during subsequent chemotherapy (up to 67%) [297].

Malacoplakia

This is a rare chronic granulomatous inflammatory disorder predominantly affecting the genitourinary tract (60%–75% of all cases) [298]. Involvement of the gastrointestinal tract ranks second in frequency, and the most common site of intestinal involvement is the rectosigmoid colon [298–300]. The average age of affected adults is 57 years, with an age range of 18–88 years. The majority of patients are asymptomatic although some patients have abdominal pain, diarrhea, hematochezia, malabsorption, weight loss, and occasionally fistulae or obstruction from an associated mass or stricture [298,300,301]. Nearly one-third of patients with gastrointestinal malacoplakia were found to have associated colorectal carcinoma [298,302]. Colonic malacoplakia associated with carcinoma is usually found adjacent to the tumor [299,303]. Malacoplakia is associated with a number of other conditions including chronic infection with coliform bacteria (especially *E. coli*) [304], chronic granulomatous processes such as sarcoidosis and tuberculosis [298], immunosuppressed states (HIV/AIDS, posttransplantation, hypogammaglobulinemia, during chemotherapy or treatment of IBD) [305,306], and malignancy.

Pathogenesis of malacoplakia is not well understood. The lesions of malacoplakia are soft plaques or polypoid lesions, measuring 1–20 mm or larger, yellow-tan to yellow-gray in color, usually elevated, at times with surrounding hyperemia and a central depression [302]. They can be single or multiple, segmental or diffuse, and can involve adjacent structures such as lymph nodes, mesentery, retroperitoneum, and bladder [307].

Physical examination findings may include abdominal pain, tenderness, abdominal mass, rectal tumor, or weight loss [301,308]. Diagnosis depends on the presence of classic histological findings of malacoplakia in a biopsy specimen or fine-needle aspirate (FNA) [309]. Because of the rarity of this condition, there is no accepted medical therapy.

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D COLON

60 Irritable bowel syndrome

Chung Owyang

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Definition

Irritable bowel syndrome (IBS) is a functional bowel disorder in which abdominal pain or discomfort is associated with defecation or a change in bowel habit, and with features of abnormal defecation. This occurs in the absence of organic disease. No clear diagnostic markers exist for IBS so the diagnosis is based on clinical presentation. The diagnostic criteria have undergone three modifications since they were proposed in 1989. In 2006, the Rome criteria for the diagnosis of IBS were revised [1] and are summarized in Table 60.1. Supportive symptoms that are not part of the diagnostic criteria include abnormal stool frequency ([a] three or fewer bowel movements per week or [b] more than three bowel movements per day), abnormal stool form ([c] lumpy/hard stool or [d] loose/watery stool), [e] defecation straining, [f] urgency, or also a feeling of incomplete bowel movement, passing mucus, and bloating. Based on the predominant stool pattern, IBS can be subtyped clinically, as shown in Table 60.2, into:

- IBS with constipation (IBS-C)
- IBS with diarrhea (IBS-D)
- mixed IBS (IBS-M)
- unsubtyped IBS.

However, the validity and stability of such subtypes over time is unknown and is a subject of future research.

Table 60.1 Functional gastrointestinal disorders

Functional bowel disorders
Irritable bowel syndrome
Functional bloating
Functional constipation
Functional diarrhea
Unspecified functional bowel disorder

From Longstreth et al. [1], with permission from Elsevier.

Societal impact of irritable bowel syndrome

Irritable bowel syndrome is the most prevalent digestive disease, accounting for 12% of visits to primary-care physicians and 28% of referrals to gastroenterologists [2]. It is the seventh most common outpatient diagnosis with a rate of 10.6 visits per thousand population per year [3]. Over 2 million prescriptions are written for IBS [4]. Although usually an outpatient disorder, IBS was the major discharge diagnosis in 96 000 inpatients as recently as 1976 [5]. As awareness of the disorder has increased and hospital admission criteria have become stricter, admissions for IBS have markedly decreased [6]. In a survey in the United States, the total prevalence of IBS was 14.1% although more than three-fourths of the patients were undiagnosed but fulfilled the IBS diagnostic criteria of Rome II [7]. Abdominal pain/discomfort was the most common symptom prompting consultation. Most sufferers also reported alternating constipation and diarrhea. The patients with IBS had more days off work (6.4 vs 3.0) and days in bed, reducing activities to a greater extent than nonsufferers. Health-care costs for the management of IBS are high. In a survey of health-service utilization, the odds of a patient with IBS incurring charges were 1.6-fold greater than for a similar asymptomatic individual, which translated into overall median yearly charges of $742 (in 1992 dollars) for each patient with IBS compared to $429 for controls [8].

Irritable bowel syndrome produces health consequences in addition to its economic costs. Outcome studies reported

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Table 60.2 Subtyping of irritable bowel syndrome (IBS) by predominant stool pattern

IBS with constipation (IBS-C)	Hard or lumpy stools[a] ≥ 25% and loose (mushy) or watery stools[b] < 25% of bowel movements.[c]
IBS with diarrhea (IBS-D)	Loose (mushy) or watery stools[b] ≥ 25% and hard or lumpy stool[a] < 25% of bowel movements.[c]
Mixed IBS (IBS-M)	Hard or lumpy stools[a] ≥ 25% and loose (mushy) or watery stools[b] > 25% of bowel movements.[c]
Unsubtyped IBS	Insuffiencient abnormality of stool consistency to meet criteria for IBS-C, IBS-D, or IBS-M.[c]

a Bristol Stool Form Scale 1–2 (separate hard lumps like nuts [difficult to pass] or sausage-shaped but lumpy).
b Briston Stool Form Scale 6–7 (fluffy pieces with ragged edges, a mushy stool or watery, no solid pieces, entirely liquid).
c In the absence of use of antidiarrheals or laxatives.
From Longstreth et al. [1], with permission from Elsevier.

greater impairments in health-related quality of life in patients with IBS than in the US general population [9,10]. In a recent health-related quality of life survey it was reported that patients with functional bowel disorder experienced functional limitations in social interactions, home management, and recreational activities, felt helpless, out of control, depressed and irritable, and perceived restrictions in lifestyle relating to toilet accessibility and eating [11]. In fact, IBS produces disability rates equal to or greater than severe organic gastrointestinal disease and is the second leading cause of absenteeism after the common cold, with patients with IBS missing three times as many workdays yearly as persons without IBS [12–14]. Doctor visits for IBS are associated with high medical costs. In a recent US study 49% of IBS patients sought medical care for abdominal symptoms [15]. Health-care seeking did not differ between males and females although the reasons for seeking medical attention differed by gender. Older age (55 years or older), fear of serious underlying disease, decreased bowel movements, dyspepsia, and pelvic pain were associated with seeking care in females. Among males, being disabled and abdominal cramping were the primary reasons for seeing a physician. Neither pain severity nor mental health status was associated with seeking care. The impact of IBS is emphasized by a British study reporting that 8% of patients with IBS retire early as a result of their symptoms [16].

Epidemiology of irritable bowel syndrome

Irritable bowel syndrome is a disorder of young people, with most new cases presenting before age 45 [17]. However, some reports suggest that the elderly are troubled by symptoms of IBS up to 92% as often as middle-aged persons [14,18–21]. Indeed, patients over 65 years old make 500 000 physician visits per year for IBS [22]. Many of the diagnoses of "painful diverticular disease" given to elderly patients may represent IBS [23]. Functional gastrointestinal disorders also are prevalent in pediatric populations. IBS symptoms are reported by 14% of high-school students and 6% of middle-school students in one study and in 16% of students aged 11–17 years in another [24–26]. Women are diagnosed with IBS two to three times as often as men (Fig. 60.1) [17,27,28]. Moreover, women make up 80% of the population with severe IBS [29]. Recent studies indicated that a history of abdominal pain or irregular bowel habits in first-degree relatives was significantly associated with IBS and dyspepsia [30]. Whether the familial associations may represent similar exposures in a shared environment, heightened familial awareness of gastrointestinal symptoms, or genetic factors is unclear. A recent study of monozygotic and dizygotic twins showed no difference in carewise concordanced rates between them (28% vs 27%) arguing against the importance of genetic factors in IBS [31]. Logistic regression analysis revealed that decreasing age and increasing psychosomatic score were independently associated with IBS. Multifactorial liability threshold modeling suggested that a combination of unique and shared environmental factors provided the best model for IBS. Fewer Asians and Hispanics appear to develop the syndrome relative to other ethnic groups [29,32]. The epidemiology of IBS outside the Western world is poorly characterized.

The number of patients with IBS seeking care is dwarfed by the number of individuals who experience symptoms of IBS. Using strict criteria, 9%–22% of the population experience symptoms diagnostic for IBS, yet only a small fraction of these (9%–33%) seek medical attention, suggesting that other factors are important in the decision to obtain medical care [18,19,33,34].

Clinical features of irritable bowel syndrome

Gastrointestinal symptoms of irritable bowel syndrome

Irritable bowel syndrome is a heterogeneous disorder with distinct symptom presentations. Different subsets of IBS can be defined based on the dominant symptom. (Table 60.2).

Abdominal pain

According to the new Rome criteria, abdominal pain or discomfort is a prerequisite clinical feature of IBS. The intensity

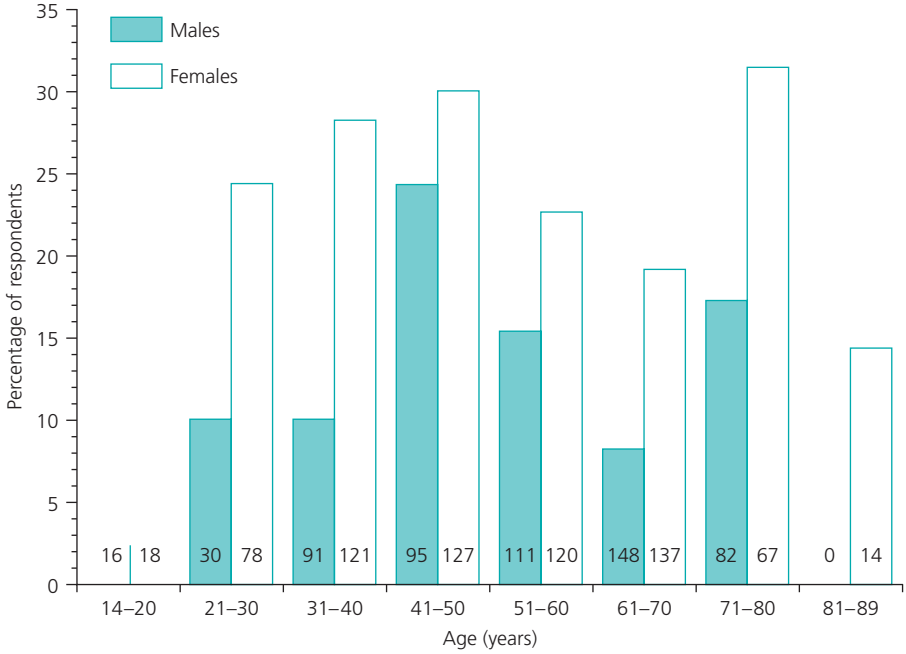

Figure 60.1 The age and gender distributions of individuals with symptoms of irritable bowel syndrome (IBS) are shown. The peak age range of symptom reports is in the 40s, although both younger and older persons experience symptoms. Women report IBS-like symptoms significantly more than men across all ages. From Longstreth & Wolde-Tsadik [29], with kind permission from Springer Science & Business Media.

and location of abdominal pain in IBS are highly variable, even within an individual patient. Abdominal pain in IBS is localized to the hypogastrium in 25%, the right side in 20%, to the left side in 20%, and the epigastrium in 10% of patients [35]. The pain is generally crampy or achy, although sharp, dull, gaslike, or nondescript pains are also common [36]. It is frequently relieved to some extent with defecation. Pain may be mild enough to be ignored or it may interfere with daily activities. Despite this, malnutrition as the result of inadequate caloric intake is exceedingly rare with IBS. Sleep deprivation is also unusual because abdominal pain is usually present only during waking hours. However, a recent study indicated that patients with severe IBS frequently wake repeatedly during the night and more than half of these patients reported that they were awakened by their abdominal pain [37]. Hence nocturnal pain is a poor discriminating factor between organic and functional bowel disease.

Several factors exacerbate or reduce pain in IBS. Many patients report increased symptoms during periods of stress or emotional turmoil as with job or marital difficulties. Defecation may provide temporary relief from the pain of IBS, whereas meal ingestion may exacerbate the discomfort usually 60–90 min postprandially. This is especially true after consuming foods rich in carbohydrates and fat [38]. In addition, female patients with IBS commonly report worsening symptoms during the premenstrual and menstrual phases [39]. Pain that is progressive, prevents sleep, leads to anorexia or inability to eat, or is associated with weight loss warrants exclusion of organic disease.

Alteration in bowel habits

Patients with IBS present with constipation (IBS-C), diarrhea (IBS-D), or constipation alternating with diarrhea (IBS-M) (Table 60.2). In some patients, the abnormality of stool consistency is not specific enough to meet criteria for subtyping (Unsubtyped). With constipation, stools are usually hard and may be scybalous or pellet-like. Long periods of straining may be required for fecal evacuation both in constipation- and diarrhea-predominant patients [40]. Constipation can persist for weeks to months, interrupted by brief periods of diarrhea. Feelings of incomplete fecal evacuation may lead to multiple attempts at stool passage daily. In diarrhea-prone patients, stools characteristically are loose and frequent but of normal daily volume. Generally, diarrheal stools occur only during waking hours, often early in the day, and an urgent desire to defecate after a meal is reported by 36% of patients [41]. Mucus discharge has been reported in up to 50% of patients with IBS [41]. As with abdominal pain, fecal urgency and loose stools may develop during stress. Early descriptions of IBS observed patient populations who presented with painless diarrhea, representing 17% in one report [36]. However, disorders such as lactase deficiency were less readily appreciated and others such as microscopic and collagenous colitis were undefined; it is conceivable many patients with functional diarrhea had one of these entities. In fact painless diarrhea does not strictly fulfill the current Rome criteria to be classified as IBS. A recent survey revealed that women with IBS report mucus discharge, a sensation of incomplete fecal evacuation, pellet-like stools, and distention more commonly than men [42]. It is important

to note that the symptoms of IBS typically wax and wane over time. Patients may have some days with symptoms and some days without. In a 12-week trial study of 59 patients with IBS, Hahn and colleagues [43] found that the population reported pain or discomfort on 50% of days. Bloating was reported on 33% of days and altered stool formation and passage were found in 25% and 18% of days respectively; 50% of the patients had at least one symptom for more than half of the study period. The symptoms lasted on average for 5 days or less although pain/discomfort and bloating tended to last the longest. It is not uncommon for patients with IBS to change their predominant bowel pattern from diarrhea to constipation or vice versa. Here the stability of each subtype over time is unknown. Symptoms that are not associated with IBS and that warrant exclusion of organic disease include nocturnal diarrhea, rectal bleeding, malabsorption, and weight loss.

Other gastrointestinal symptoms

Upper gut symptoms are common in IBS, with 25%–50% of patients reporting heartburn, early satiety, nausea, and vomiting; up to 87% note intermittent dyspepsia [44].

Evidence points to a significant overlap between IBS and gastroesophageal reflux disease (GERD). In a review of 15 studies published from 1966 through to January 2005, the weighted mean prevalence of GERD in patients diagnosed with IBS was 30.3%, whereas the mean prevalence of IBS in subjects with known GERD was 80.5% [45]. Based on the prevalence of IBS (12.1%) and GERD (19.4%) in the community, the rate of IBS in the non-GERD community was calculated to be only 5.1%. This suggests that IBS is relatively uncommon among patients without GERD.

It also appears that there is a great deal of overlap between dyspepsia and IBS. A community-based study in Spain reported that the prevalence of IBS was higher among subjects with dyspepsia (31.7%) than among those who reported no symptoms of dyspepsia (7.9%) [46]. Conversely among the subjects with IBS, 55.6% reported symptoms of dyspepsia. Other studies indicated that functional abdominal symptoms could change over time [47]. Those with predominant dyspepsia or IBS can fluctuate between the two. In contrast, those with predominant reflux symptoms seldom change to dyspepsia or IBS. It is conceivable that functional dyspepsia and IBS are two manifestations of a single, more extensive digestive system disorder. The symptoms of IBS are prevalent in patients with noncardiac chest pain, but not in patients with cardiac chest pain, suggesting overlap with other functional gut disorders [48].

Many IBS patients report the sensation of bloating as being the most bothersome symptom. This may be frequently accompanied by abdominal distention and increased gas production [49]. The relationship between bloating and actual distention manifest as an increase in abdominal girth is controversial. In a study of patients with IBS with diarrhea, with constipation, and with alternating bowel habits it was found that all patients irrespective of bowel habit reported greater bloating than controls, but only 48% showed distention as measured by abdominal inductance plethysmography [50]. Bloating correlated strongly only with distention in patients with IBS-C. Hence bloating and distention may differ pathophysiologically and this appears to be reflected in the bowel habit subtype.

A recent study indicated that the majority of patients with IBS have impaired transit and tolerance of intestinal gas loads [51]. The malfunctioning of colonic–intestinal or intestinal–intestinal reflex may contribute to gas retention and the symptoms of gas and bloating in patients with IBS. In contrast to healthy controls, rectal distention in patients with bloating failed to reduce lipid-induced gas retention [52]. Other patients with IBS may demonstrate a maximum rate of gas excretion greater than healthy subjects [53]. Although their total gas production was not increased, hydrogen production was higher. In these patients an exclusion diet reduced symptoms and produced a decrease in maximum gas excretion [53]. In a recent study it was demonstrated that the perception of intestinal gas accumulation depends on the mechanism of retention [54]. Obstructed evacuation increased symptom perception whereas gas retention in a hypotonic gut was virtually unperceived. Computed tomography has shown increased lateral profiles in patients with IBS, which was postulated to result from altered gastrointestinal motility or tone [55]. Another study has reported that those patients with significant bloating were most likely to have gained weight and more commonly had weak abdominal muscles [56]. This observation, however, was not confirmed by a subsequent study, which showed no anterior abdominal muscle weakness in patients with IBS with gas and bloating [57]. Hence the pathomechanism of bloating in IBS still remains undefined and is likely to be multifactorial.

Extraintestinal symptoms of irritable bowel syndrome

Although gastrointestinal symptoms predominate, extraintestinal complaints are common in IBS. Patients with functional gut disorders visit primary-care physicians three times as often for nongastrointestinal problems as do healthy persons and undergo more appendectomies and hysterectomies [27,58–60]. Chronic pelvic pain is much more commonly reported by IBS patients than patients with inflammatory bowel disease [61]. Genitourinary dysfunction, including dysmenorrhea, dyspareunia, impotence, urinary frequency, nocturia, and a sensation of incomplete bladder emptying, is prevalent [36]. Impaired sexual function is reported by 83% of patients with IBS compared with 30% of patients with inflammatory bowel disease and 16% of patients with peptic ulcers [62]. An association between IBS and primary fibromyalgia has been noted, with two-thirds of patients with IBS reporting rheumatological symptoms. Conversely, IBS

symptoms are found in 42% of patients with fibromyalgia [63]. Patients with both IBS and fibromyalgia have worse scores on health-related quality of life indices than patients with either disorder alone [64]. The presence of fibromyalgia in IBS patients seems to be associated with severity of bowel symptoms [65]. At the same time comorbidity with fibromyalgia results in somatic hyperalgesia in IBS patients [66]. Similarly, 63% of patients with chronic fatigue syndrome report symptoms of IBS [67]. Patients with functional bowel disorders have higher incidences of peptic ulcer disease, hypertension, low back pain, headaches, and rashes than the general population and more commonly report fatigue, loss of concentration, insomnia, palpitations, and unpleasant tastes in the mouth [35,68,69].

Pathophysiology of irritable bowel syndrome

The pathogenesis of IBS is poorly understood, although roles for abnormal gut motor and sensory activity, central neural dysfunction, psychological disturbances, stress, lumenal factors, and colonic microflora have been proposed. Unfortunately, there is no single pathophysiological abnormality that clearly separates IBS from organic gastrointestinal disease or from normality.

Gastrointestinal motor abnormalities in irritable bowel syndrome

Role of abnormal colonic motor activity

The myoelectrical activity of the colon is comprised of slow waves, which regulate the frequencies of some phasic contractions, and spike potentials, which elevate the membrane potential above a contractile threshold [70]. Spike potentials may occur in the form of short-spike bursts (SSB) of 5–15 s duration, which are temporally in phase with the slow-wave activity. Long-spike bursts (LSB) of 15–60 s duration are unrelated to the slow wave [28,71,72]. The SSBs are responsible for the generation of short-duration contractions, which are nonpropagative and segmenting in character leading to fecal mixing and resistance to stool passage [70]. The LSBs produce long-duration contractions, some of which are nonpropagative and some of which may propel feces in either an oral or aboral direction. Finally, high-amplitude propagated contractions are intensely propulsive, beginning in the right colon and occurring once or twice daily, and produce mass movements of feces and defecation [70].

Studies of colonic myoelectrical and motor activity under unstimulated conditions have not shown consistent abnormalities in IBS. A predominant slow-wave frequency of three cycles per minute (c.p.m.) with increased 3 c.p.m. motor activity was observed in both patients with diarrhea-predominant and constipation-predominant IBS in an older study that has not been confirmed by more recent studies [73,74]. Furthermore, similar slow-wave abnormalities are demonstrable in psychoneurotic patients without bowel symptoms, suggesting that the myoelectric disturbance is neither diagnostic for IBS nor pathogenic of symptoms [75]. Constipation-predominant patients were reported to exhibit increased numbers of SSBs whereas diarrhea-prone patients had reduced SSB activity [71]. Most investigators have observed no colonic motor abnormalities under basal conditions although others have reported increases in basal motility [73–77]. Increased numbers of high-amplitude propagated contractions have been noted in patients with diarrhea-predominant IBS compared to healthy volunteers [78]. Using scintigraphic assessment of colonic transit, selective acceleration in right colon transit has been shown in some patients with IBS-D [79]. It is unknown if these are pathogenic motor abnormalities in IBS or if all patients with diarrhea exhibit these findings. Similar studies have not been reported in constipation-predominant IBS, although patients with idiopathic constipation exhibit delayed right colon transit [80]. Recent dual barostat studies showed that there was attenuation of the colorectal tonic reflex in female patients with IBS-D [81]. These patients displayed significantly less than normal increase in rectal tone in response to colonic distention under fasting and postprandial conditions. How this abnormality contributes to the symptoms of IBS-D is unclear.

In contrast to the unstimulated colon, colonic motor abnormalities are more prominent under stimulated conditions in IBS. In normal volunteers, meal ingestion induces a rapid increase in spike potential and contractile activity that peak 20–30 min after eating, decrease to a basal level after 50–60 min, and may exhibit a second increase at 80 min [71]. Conversely, patients with IBS may exhibit increased rectosigmoid motor activity for up to 3 h after eating [72,82]. Although this response is most prominent in diarrhea-prone patients, patients with IBS-C also show an exaggerated gastrocolonic response albeit to a lesser degree [83]. In contrast to healthy volunteers, sham feeding increases rectosigmoid motility in IBS that is suggestive of a cephalic phase of the gastrocolonic response in affected patients [84–86]. Provocative stimuli induce exaggerated colonic motor responses in patients with IBS compared to healthy volunteers. Inflation of rectal balloons both in patients with diarrhea-predominant and constipation-predominant IBS leads to marked distention-evoked contractile activity that may be prolonged [72,87]. Patients with painful IBS exhibit greater increases in rectosigmoid motility after cholinergic stimulation than patients with painless diarrhea [88]. Intravenous cholecystokinin induces painful rectosigmoid contractions that reproduce the presenting symptoms in patients with IBS with postprandial pain [89]. An exaggerated motor response is demonstrable in patients with IBS after colonic perfusion of deoxycholic acid [90]. In contrast, it has been reported that rectal motor characteristics in response to isobaric distentions were not different between patients with constipation-predominant IBS,

patients with slow transit constipation, and healthy controls [91]. On the other hand, sensations of urge were reduced in slow transit constipation and sensations of pain were increased in IBS.

Until recently most of the colonic motility studies in IBS were performed in the rectosigmoid colon. Recording from the transverse, descending, and sigmoid colon, Chey and colleagues [92] reported that the motility index and peak amplitude of high-amplitude propagating contractions in diarrhea-prone patients with IBS were greatly increased compared to healthy subjects. These were associated with rapid colonic transit and accompanied by abdominal pain. Similar findings were made by another group who reported increased numbers of high-amplitude propagated contractions and reduced duration of increased colonic tone postprandially in diarrhea-prone patients with IBS [93].

Role of abnormal small bowel motor activity

Small intestinal motor patterns under unstimulated conditions are organized and well characterized. During fasting, the migrating motor complex (MMC) cycles every 90–120 min to clear the proximal gut of undigested debris. Prolonged manometric studies suggest subtle abnormalities in fasting small intestinal motility in IBS. Decreases in MMC contractile amplitudes in patients with constipation-predominant IBS and reductions in MMC cycle length in diarrhea-predominant individuals have been characterized, although these abnormalities have not been universally described [94,95]. These findings have not been observed during sleep [94–96]. Studies involving nonlinear analysis of 24-h jejunal motility revealed that patients with IBS had increased irregularities for the intercontractile intervals during phase II of MMC [97]. Other physiological motor patterns include discrete clustered contractions (DCC), which are bursts of phasic contractions occurring every minute in the duodenum or jejunum, and prolonged propagated contractions (PPC), which are intense ileal complexes that evacuate the ileum and prevent coloileal reflux. In patients with IBS, DCCs and PPCs have been reported to make up a larger fraction of fasting recording time; however, this has not been confirmed by recent investigations [40,98–101]. It is unlikely that these complexes have pathogenic significance because their occurrences are similar in patients with constipation and with diarrhea.

As in the colon, small intestinal motor abnormalities under stimulated conditions have been described in IBS. The fed motor pattern is a period of intense irregular phasic contractions that occurs for 2–3 h after eating. In both constipation-predominant and diarrhea-predominant IBS, the duration of the fed pattern is shorter than in healthy volunteers [40]. Increases in the postprandial intestinal contraction frequency have been observed in diarrhea-predominant and constipation-predominant IBS [101], although the contractile amplitudes were lower in constipation-predominant patients [102]. Recent studies [103] further demonstrated that increased frequency of retrograde propagation of duodenal pressure waves occurred postprandially in both constipation-prone and diarrhea-prone patients with IBS. This abnormal propagation pattern of duodenal pressure waves was associated with symptoms in this group of patients. Intravenous cholecystokinin evokes exaggerated high-amplitude ileal contractions in diarrhea-predominant IBS patients, whereas the anticholinesterase neostigmine induces frequent DCCs in both diarrhea-prone and constipation-prone patients [104]. Small intestinal transit of a meal is reportedly delayed in patients with IBS with constipation or distention plus abdominal pain whereas transit is characteristically accelerated in diarrhea-predominant patients [23]. Ileocecal function in IBS is variable with one study demonstrating delayed ileal emptying and decreased ileocecal clearance compared to healthy individuals and a second study reporting accelerated ileocecal transit both in constipation- and diarrhea-predominant patients [105,106].

Other motor abnormalities in irritable bowel syndrome

In addition to colonic and small intestinal motility disturbances, IBS is associated with motor abnormalities in other smooth muscle sites. Decreases in lower esophageal sphincter pressures and abnormalities of esophageal peristalsis have been found in IBS including increased spontaneous motor activity, repetitive contractions, and simultaneous waves in the esophageal body [72,107]. In contrast, the upper esophageal sphincter, which is striated muscle, exhibits no manometric abnormalities in IBS. Gastric slow-wave dysrhythmias (tachygastria, bradygastria) have been reported in dyspeptic patients with IBS symptoms [72]. Fasting gallbladder volumes and residual volumes after contraction are greater in patients with IBS, whereas gallbladder emptying is impaired, although there is no increase in gallbladder disease with IBS [108,109]. However, another study has reported that constipation-predominant patients exhibit enhanced gallbladder contraction, whereas patients with diarrhea exhibit impaired contraction compared to controls [110]. Sphincter of Oddi dysfunction has been described in association with IBS with a demonstration of impairment of the sphincter relaxation in response to exogenous cholecystokinin [111]. Detrusor instability of the bladder has been demonstrated in 10 of 30 patients with IBS compared to 1 of 10 controls [112]. Similarly, patients with IBS exhibit hyperreactive airway function after administration of the smooth muscle stimulant methacholine [113].

Visceral sensory abnormalities in irritable bowel syndrome

A major current focus of investigation is to define the visceral sensory abnormalities that may be responsible for sensations of pain, gas, or bloating in IBS. Perception of abdominal symptoms is mediated by afferent neural pathways, which

are activated by visceral stimuli acting on chemoreceptors (which sense osmolarity, temperature, pH), mechanoreceptors (which are located in the gut wall and discharge in response to changes in tension), and receptors in the mesentery, which may play a role in painful stimulation of the gut [114]. Information from these activated receptors is carried in spinal afferent nerves, which synapse in the dorsal horn of the spinal cord. From there, the impulses are transmitted to the brain where conscious perception occurs. It is postulated that IBS results from sensitization of afferent pathways such that normal physiological gut stimuli not perceived by healthy individuals induce pain in the patient with IBS. The sensitizing event responsible for induction of symptoms in IBS is unknown.

Disturbed sensation under basal conditions

The temporal association of symptoms with gut myoelectric and motor events provides evidence for disturbed visceral sensation under unstimulated conditions. Myoelectric recordings show correlations between the appearance of intense, fused colonic SSB and abdominal pain in patients with constipation-predominant IBS [72]. Irregular jejunal motor activity has been described coincident with the development of abdominal pain although other investigators have detected no abnormal motor patterns [95,115,116]. In one study, 61% of PPCs were associated with symptoms in patients with IBS compared to 17% in controls [98]. Similarly, DCCs were correlated with abdominal pain reports in four of 12 patients with IBS but in no healthy volunteers [98] (Fig. 60.2). Another investigation has shown that nearly half of patients with IBS perceive different phases of the MMC as discomfort, a tugging sensation, or frank pain [40]. In contrast to healthy individuals, patients with IBS possess the ability to sense physiological motor events in the gastrointestinal tract.

These findings may provide an explanation for older studies postulating a role for intestinal gas in the generation of abdominal symptoms in patients with functional bowel disorders. It was once believed that affected patients had abnormal amounts or distributions of intestinal gas. Early investigators described an entity called the splenic flexure syndrome, which consisted of severe left upper quadrant abdominal pain associated with apparent gas collections in the splenic flexure on abdominal radiographs [117]. Relief could be obtained by defecation, passage of flatus, or administration of an enema, and symptoms were reproducible by inflation of a balloon in the splenic flexure. Subsequent studies have shown that the absolute volume of gas in patients who complain of gas and bloating is normal. Using a washout technique, total volumes and transit times of intestinal gas were similar in asymptomatic individuals and in subjects complaining of excess gas [49]. However, patients with complaints of excess gas refluxed more gas from the intestine to the stomach and complained of distention and pain at low volumes of gas infusion that did not affect the controls. In addition to showing abnormal propulsion of intestinal gas, abnormal sensitivity to physiological amounts of intestinal gas is also demonstrable in patients with functional symptoms. Recent studies reported that in a subgroup of patients with IBS who were low hydrogen producers, lactulose ingestion lowered sensitivity thresholds for perception of bloating even though breath hydrogen excretion and mouth-to-cecum transit were similar to healthy controls [118]. It is conceivable that this group of patients might have hypersensitivity to products of colonic fermentation.

Disturbed sensation under stimulated conditions

As with studies of motor activity, patients with IBS frequently exhibit exaggerated sensory responses to visceral stimulation. Postprandial symptoms in IBS are associated with characteristic motor patterns. Postprandial pain has been temporally related to entry of the food bolus into the cecum in 74% of patients [23]. Some patients with concurrent

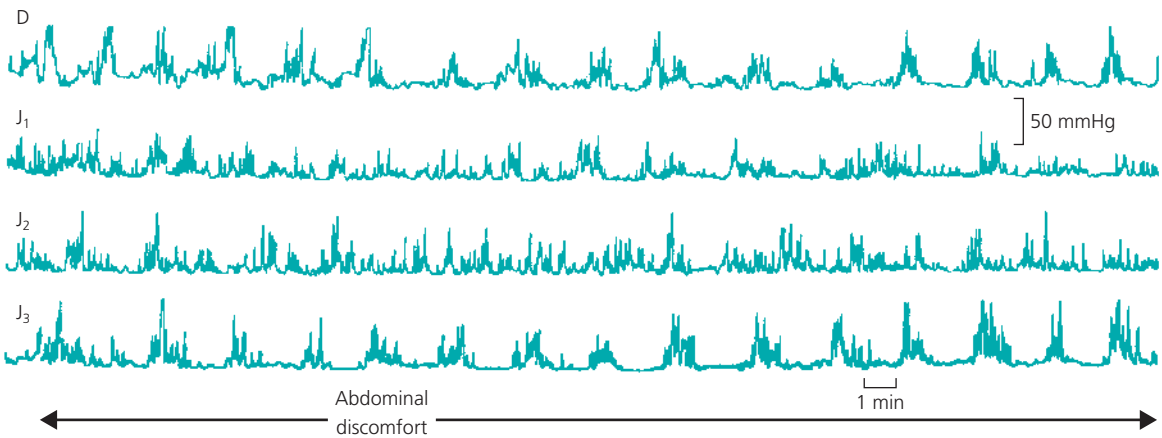

Figure 60.2 A manometric recording of small intestinal motility from a patient with irritable bowel syndrome (IBS) shows 30 min of discrete clustered contractions in the duodenum and jejunum which are associated with discomfort. D, duodenal pressure recording; $J_{1,2,3}$, jejunal pressure recordings. From Kellow & Phillips [98], with permission from Elsevier.

nonulcer dyspepsia and IBS experience postprandial pain in association with increased rectosigmoid pressure [119]. Similarly, food has been shown to induce intestinal bursts that correlate temporally with pain [90]. A human clinical study showed that lipids lowered the thresholds for the first sensation of gas, discomfort, and pain in patients with IBS [97]. Furthermore, patients with IBS had an increased area of referred pain after lipid intake that was not observed in healthy subjects. The lowered threshold of discomfort evoked by intraduodenal lipid fusion was observed in both IBS-D and IBS-C patients whose major symptoms were rectal urgency and abdominal pain respectively [120]. These symptoms were reproduced during intraduodenal lipid infusions, suggesting that lipids may be responsible for postprandial symptom exacerbation in IBS. The rectal sensitivity to intraduodenal lipid was nutrient-specific as it was not observed with carbohydrates infusion [121]. This abnormal response was largely unaffected by psychological factors, sex or predominant bowel habit [122].

Exaggerated symptoms can be induced by visceral distention in patients with IBS. Rectal balloon inflation produced nonpainful and painful sensations at lower volumes in patients with IBS than in healthy controls without altering rectal tension, which was suggestive of visceral afferent dysfunction in IBS [123] (Fig. 60.3). This effect persisted even after the exclusion of psychological factors such as neuroticism. However, more recent studies suggested that psychological factors influenced pain thresholds in patients with IBS as stress altered sensory thresholds [102,103]. When perception tests that minimize psychological influences were used, many patients with IBS showed a normal sensory threshold. In fact, there are studies suggesting that the increased colonic sensitivity in IBS was strongly influenced by a psychological tendency to report pain and urge rather than actual increased rectal sensitivity [124]. On the other hand, sexual abuse was not associated with a lower pain threshold in patients with IBS. The visceral hyperalgesia of IBS appears to be selective for mechanoreceptor-activated stimuli as perception of intestinal mucosal electrical stimulation is normal in IBS [125]. In patients with IBS visceral hypersensitivity is not limited to the colon and rectum. Enhanced sensitivity to distention of the small bowel has been demonstrated. Jejunal sensitivity is increased after feeding in patients with IBS [126]. Furthermore, nonperceived rectal stimulation appears to modify the intensity of jejunal perception to a greater extent in patients with IBS than in healthy controls. Similar studies showed gastric and esophageal hypersensitivity in patients with nonulcer dyspepsia and noncardiac chest pain, respectively [123,127]. Furthermore, patients with IBS, nonulcer dyspepsia, or both all exhibited similar hypersensitivity to small intestinal distention, raising the possibility that these conditions have a similar pathophysiological basis [128]. Other studies of patients with IBS and nonulcer dyspepsia report enhanced sensitivity to esophageal distention, which is suggestive of a generalized visceral hyperalgesia in the functional bowel disorders [129,130]. One group has categorized patients with IBS into three groups based on the response to rectal distention [131], including:

- those with a sensitive rectum, with a low sensory threshold to distention, and normal to low intrarectal pressures
- those with a stiff rectum, with normal to low sensory thresholds, and high pressures
- those with an insensitive rectum, with a high sensory threshold, and normal or high pressures.

It should be noted that the sensitive rectum subtype was found in 57% of diarrhea-predominant patients compared with only 7% of patients with constipation. Seventy-five percent of diarrhea-predominant patients exhibited abnormal balloon distention responses compared with 30% of patients with constipation, suggesting that visceral hypersensitivity is not universal in IBS. This has been questioned in a study involving both rectal and sigmoid distention in patients with IBS [132]. In an investigation of patients with IBS with normal perception of rectal distention, all of them exhibited abnormal responses to sigmoid distention including alterations in colonic compliance and reflex relaxation. The duration of symptoms does not seem to be a factor in the degree of visceral hypersensitivity [133]. Finally, some investigators have postulated that IBS symptoms may result from interaction of visceral sensory dysfunction and abnormal gut motor patterns. In a study of women with IBS, those who exhibited enhanced perception of small intestinal distention had abnormal postprandial intestinal motor patterns [134].

The relationship between symptoms of IBS and hypersensitivity to rectal distention was examined in a recent study [135]. The proportions of IBS-D, IBS-C, and the alternating type were similar in hypersensitive and normosensitive

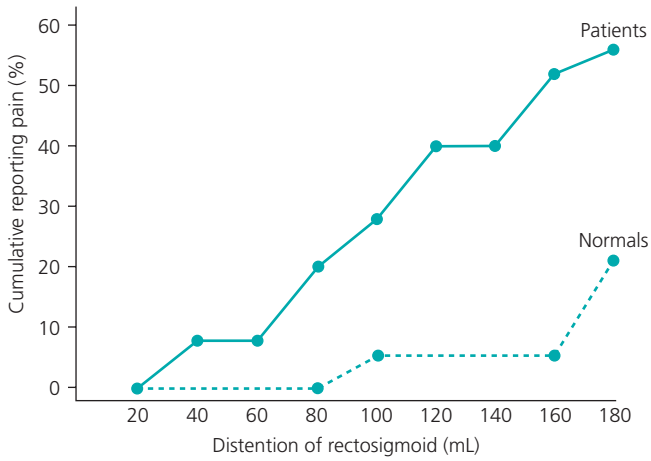

Figure 60.3 Perception of rectal balloon inflation is compared in healthy volunteers (dashed line) and patients with irritable bowel syndrome (IBS) (solid line). More patients with IBS reported pain with rectal distention. Furthermore, they experienced pain at much lower volumes than the healthy volunteers. From Whitehead et al. [123], with kind permission from Springer Science & Business Media.

subgroups. No individual IBS symptom could accurately predict the presence of hypersensitivity. Hence selection based on clinical parameters is unlikely to discriminate individual patients with IBS with visceral hypersensitivity from those patients with normal visceral sensitivity reflecting the complexity of the pathogenesis of IBS.

In addition to visceral hyperalgesia, patients with IBS exhibit abnormal pain referral patterns with balloon inflation of the stomach, small intestine, or colon [70,136,137]. In contrast to healthy controls who perceive discomfort in only one quadrant with colonic distention, IBS patients experience pain diffusely in the right upper and lower quadrants, right flank, hypochondrium, and epigastrium as well as in the back, shoulders, thighs, and chest [136] (Fig. 60.4). One group has proposed that, when alterations in viscerosomatic referral patterns are coupled with hypersensitive perception of rectal distention, visceral sensory abnormalities can be detected in nearly all IBS patients and so represent objective markers for the disorder [138].

In contrast to enhanced gut sensitivity, patients with IBS do not exhibit heightened sensitivity elsewhere in the body. Patients with IBS and Crohn's disease exhibit higher touch and pain thresholds with electrical stimulation of the skin than normal volunteers [139]. Furthermore, patients with IBS, lactose intolerance, and healthy volunteers exhibit similar perceptions of ice water immersion of the hand [140]. The afferent pathway disturbances in IBS therefore appear to be selective for visceral innervation with sparing of somatic pathways.

Visceral and somatic sensitivity disturbances were investigated in patients with IBS and fibromyalgia [141]. Patients with IBS and with IBS with fibromyalgia demonstrated rectal hypersensitivity in comparison to controls whereas patients with fibromyalgia tolerated rectal distention without discomfort. On the other hand, patients with fibromyalgia with or without IBS demonstrated somatic hypersensitivity involving all layers of the bodywall (skin, subcutis and muscle). These observations suggest that although IBS and fibromyalgia share a common hypersensitivity background, other mechanisms may modulate perceptual somatic and visceral responses in these two groups of patients.

Central nervous system factors in the pathogenesis of irritable bowel syndrome

The role of central nervous system factors in the pathogenesis of IBS is strongly suggested by the clinical association of emotional disorders and stress with symptom exacerbation and the therapeutic response to therapies that act on cerebral cortical sites.

Central neural dysfunction in irritable bowel syndrome

It is not known whether IBS represents a primary gut disturbance with inappropriate input from the central nervous system or a central nervous system disorder with centrally directed changes in gut motor and sensory activity [142]. It is further undefined if the peripheral afferent nerve pathways that project from the gut to the brain exhibit abnormalities. New technologies are being developed to study this issue. Using cerebral evoked potential recordings, investigators reported that afferent pathways stimulated by esophageal distention are not abnormal in noncardiac chest pain and concluded that perceptual disturbances result from abnormalities in the central nervous system [143]. Conversely, evoked potential recordings in patients with nonulcer dyspepsia and with IBS show abnormal waveform latencies and amplitudes suggestive of visceral afferent dysfunction [144,145]. The presence of peripheral afferent dysfunction in IBS is uncertain. Recent studies indicated that visceral hypersensitivity in IBS appears to be related to alterations in the

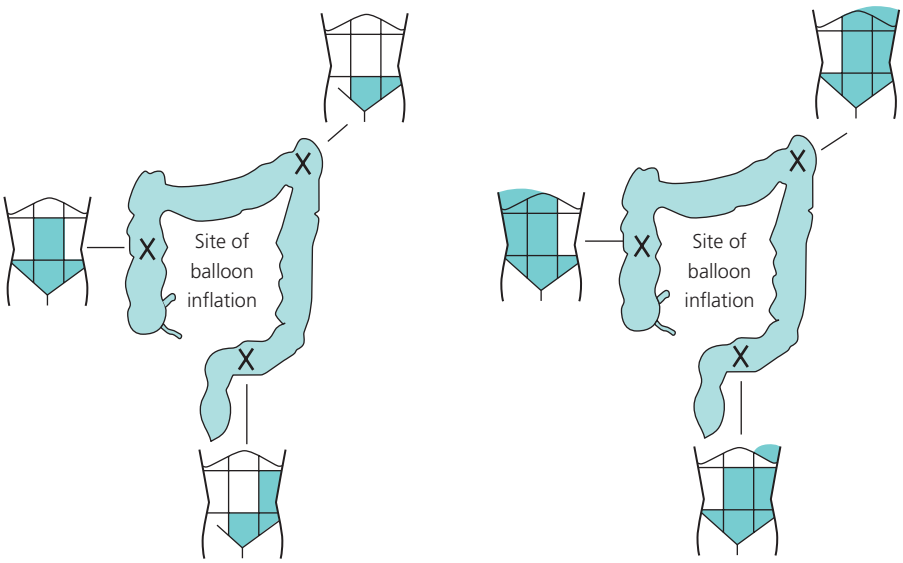

Figure 60.4 Pain referral patterns on inflation of colonic balloons is compared in healthy volunteers **(left)** and patients with irritable bowel syndrome (IBS) **(right)**. Colonic distention in healthy volunteers produced discomfort generally localized to a single abdominal quadrant whereas inflation in patients with IBS produced diffuse abdominal pain, providing evidence for altered viscerosomatic pain referral in IBS. Adapted from Mayer & Gebhart [454], with permission from Elsevier.

nervous system rather than to biochemical parameters such as the tension and strain of the gut wall [146].

Cerebral functioning in IBS is poorly understood; however, methodologies to assess neurological disease are being used to address this issue. Both mental stress and administration of the cholinesterase inhibitor neostigmine evoke increases in colonic motility and changes in electroencephalographic waveforms, which are exaggerated in patients with IBS compared to healthy volunteers, suggesting that both the gut and brain are hypersensitive in IBS [147]. Functional brain imaging using positron emission tomography (PET) and functional MRI (fMRI), based respectively on regional blood flow or degree of oxygenation, demonstrated differences in central pain modulatory systems between IBS patients and healthy controls. These studies showed that visceral stimulation such as rectal distention activated corticolimbic modulatory systems including the insular cortex, thalamus and hypothalamus, right ventrolateral prefrontal cortex, amygdale, and anterior cingulated cortex (ACC). Many of these brain regions are associated with unpleasant affect and autonomic responses [148]. Among these, the ACC is the region of particular interest with regard to pain regulation, stress response, and IBS. It consists of several structurally and functionally heterogeneous regions as shown in Fig. 60.5. This area processes information on stimulus intensity, emotion, mood, and attention, and is also the area involved with unpleasant affect and autonomic responses. The ACC consists of two subregions (Fig. 60.5):

- an anterior perigenual ACC (pACC)
- a more dorsal and caudal portion located in the middle of the cingulated gyrus, the mid-cingulated cortex (MCC).

With painful visceral stimulation, there is an increase in activity of the pACC associated with emotional distress and an increase in activity of the MCC where pain is coupled with fear and with increased attention to the stimulus and inhibition of motor activity [149]. Despite inconsistencies across the studies, in general there is an association of ACC activation to rectal distention in IBS relative to controls [150]. Both fMRI and PET showed enhanced activities of unspecified areas of the ACC among patients with IBS [151]. Subsequent studies demonstrated more intense activation of the MCC [148,152–154] or pACC [155] in patients with IBS compared to controls, thus linking emotion or fear with visceral pain in these patients. This heightened pain sensitivity of the brain–gut axis may contribute to abnormal processing of sensory information in IBS resulting in visceral hypersensitivity.

Visceral hypersensitivity and abnormal brain activation in patients with IBS may occur as a result of enhanced sensitivity of visceral afferent pathways or abnormal cortical modulation related to cognitive or emotional factors. To draw a distinction between cognitive and non-cognitive processes, fMRI was performed in patients with IBS in response to rectal stimulation at distention pressure below conscious perception [156]. Patients with IBS showed a several fold higher activation at 10 mmHg, which remained at a similar higher level at 15 and 20 mmHg (all subliminal distention pressures), suggesting the presence of hypersensitivity of the neural circuitry in IBS irrespective of stimulus-related cognitive processes. This is in agreement with work from Verne and colleagues [157] who came to a similar conclusion based on the presence of increased activations in primary pain processing areas of the thalamus and insula in patients with IBS. Other groups, however, have suggested that differences in central pain modulation by emotional and cognitive factors may be responsible for some IBS-specific alterations in visceral perceptin and associated altered autonomic response [152,158]. In fact, a recent fMRI study showed that placebo

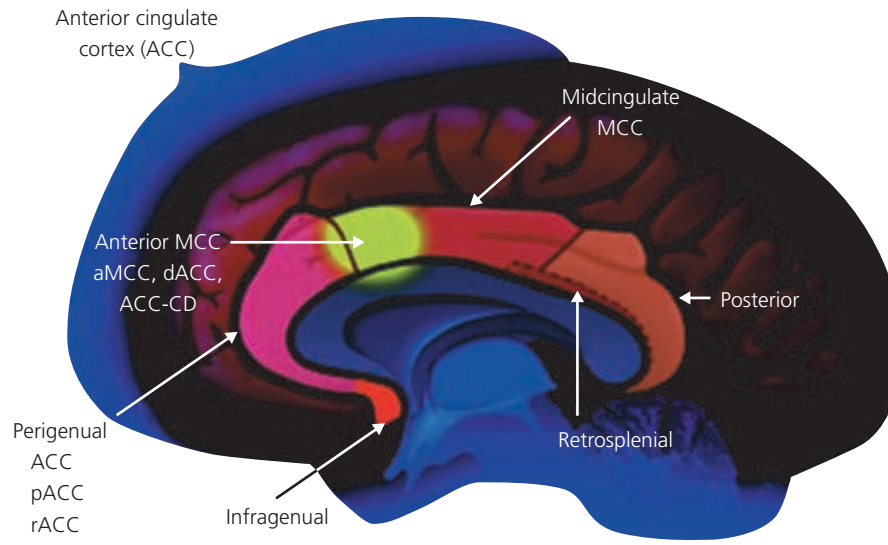

Figure 60.5 Structure of the cingulated cortex. The anterior region of the mid cingulated cortex (MCC, shown in green) is a subregion of the anterior cingulated cortex. Painful visceral stimulation caused an enhanced activation of this region in irritable bowel syndrome, which is coupled with fear and increased attention to the stimulus. From Drossman [455] from BMJ Publishing Group Ltd.

analgesia was accompanied by large reductions in pain-related brain activity (thalamus, somatosensory cortices, insula, and anterior cingulated cortex) in patients with IBS [159].

Abnormal psychological features in irritable bowel syndrome

Abnormal psychiatric features are recorded in up to 80% of patients with IBS, especially in referral centers; however, no single psychiatric diagnosis predominates [17,123,160,161]. The lifetime incidences for major depression, somatization disorder, generalized anxiety disorder, panic disorder, and phobias are higher in patients with IBS than in healthy controls [162,163]. An early investigation observed that 80% of patients with IBS had underlying depression or anxiety, whereas another study reported hysteria or depression in 72% of patients with IBS compared to 18% in normal volunteers [36,161,164]. Conversely, when depressed patients are examined, 25% meet the criteria for IBS compared to 2.5% of nondepressed controls [165]. Similarly, IBS is found in 59% of dysthymic patients [166]. A significant overlap of panic disorder and IBS has been observed, with similar demographic and clinical characteristics [167]. In one study, 46% of patients with panic disorder met criteria for IBS vs 2.5% of controls [168]. Increases in neuroticism and decreases in extroversion are reported in IBS [164]. Additional abnormalities associated with IBS include hostility, hypochondriasis, and increased interpersonal sensitivity [123]. In an illness attitudes assessment, three abnormalities specific to IBS were bodily preoccupation, hypochondriacal beliefs, and disease phobia [169]. Psychiatric features predate or occur simultaneously with the onset of bowel symptoms in 67%–85% of patients, suggesting that it is not the symptoms of IBS that induce psychiatric disease [123,170–172].

An association between prior sexual or physical abuse and development of IBS has been reported [12,173]. Rates of severe lifetime sexual trauma, severe childhood sexual abuse, and any lifetime sexual victimization are significantly greater in patients with IBS than individuals with inflammatory bowel disease [174]. Forms of sexual abuse associated with IBS include verbal aggression, exhibitionism, sexual harassment, sexual touching, and rape [175]. Sexually traumatized patients experience significantly higher rates of lifetime development of depression, panic disorder, phobia, somatization, ethanol abuse, and sexual dysfunction. Furthermore, they exhibit higher rates of physician visits and more gastrointestinal and nongastrointestinal symptoms, and they undergo more surgeries [173]. Another study has reported histories of sexual abuse in 40% of patients with functional lower gastrointestinal disease both in tertiary referral centers and in private gastroenterology practices [176]. Prior sexual abuse is more common in constipated patients and is not prevalent in patients with functional upper gut symptoms. In a complementary study, patients with prior abuse were more likely to report symptoms of IBS than those without abuse histories [177]. From a pathophysiological standpoint, sexual abuse was not associated with lower pain threshold in IBS patients [178]. The prevalence of functional dyspepsia and IBS was examined in 70 females reporting domestic violence to the police [179]. Seventy-one percent of women who experienced domestic violence have functional dyspepsia (67%) or IBS (47%). In two-thirds of the cases, onset of gastrointestinal symptoms occurred simultaneously with the abuse or soon after the abuse began.

A recent prospective study examined whether development of abdominal pain and IBS occurs following gynecological surgery [180]. Among women undergoing gynecological surgery for nonpain indications, the development of IBS was not significantly greater than controls. Abdominal pain not related to IBS did develop in 17% of women in the surgical group, suggesting that surgery facilitated its development. Only psychosocial variables predicted pain development, suggesting that pain development is associated with central registration and amplification of the afferent signal by way of cognitive and emotional input.

Only a small fraction of individuals with symptoms of IBS present to a physician. Investigators have attempted to characterize differences between symptomatic individuals who seek medical attention and those who do not, known as nonreporters. Patients with IBS have been shown to exhibit more personality abnormalities and greater illness behavior than nonreporters [58]. Similarly, patients with IBS possess psychiatric disturbances greatly exceeding the general population; however, nonreporters are not different from asymptomatic individuals [170]. How a person responds to his or her symptoms determines whether he or she will present to a physician. Patients with IBS have histories of more frequent and serious childhood illnesses resulting in school absences and pediatrician visits and report that gifts were provided during childhood illness, suggesting that their expectations for illness behavior involve a reward [68,181].

Role of stress on visceral function in health and irritable bowel syndrome

The role of the central nervous system in modulating gastrointestinal function has intrigued researchers for decades. Early investigations of the effects of stress reinforce the importance of the brain–gut axis in the regulation of colonic activities. Painful somatic stimuli, such as ice water immersion of the hand or tightening of a circumferential band around the head, were shown to produce rectal spasm and mucosal engorgement [182]. Psychological stressors have been shown to have similar effects. One hoax involved telling a medical student during proctoscopy that a rectal cancer had been found, after which he was shown a piece of potato that he was told was a biopsy of the alleged malignancy [183]. As with the painful stimuli, the rectum exhibited intense contractions and mucosal reddening, which disappeared when the hoax was revealed. Similarly, in seven

constipated subjects and eight with diarrhea-prone IBS, changes in rectosigmoid motility were inducible with stressful interviews [184]. Hostile or aggressive reactions provoked increased motor activity, whereas passive reactions or dejection led to motor quiescence. Other stressors such as anger, ball sorting, and stimulus differentiation testing increase colonic motility or spike potential activity in patients with IBS and controls [76,77,185]. The central nervous system also modifies visceral perception that suppresses or enhances afferent impulses from the gastrointestinal tract [114]. Hypnosis reduces perception of rectal distention in patients with diarrhea-predominant IBS with a lesser effect in constipated patients [186]. The lack of effect of hypnotherapy on rectal compliance or motor activity is indicative of active suppression of visceral afferent function by central nervous system activity.

Stressful stimuli also induce physiological responses elsewhere in the gastrointestinal tract. Vertigo induced by cold caloric stimulation of the external ear delays gastric emptying and converts the fed motor pattern to fasting complexes [187]. Barium radiography of the small intestine performed on subjects during stressful interviews show segmentation, spasm, widening of the mucosal folds, and areas of distention [188,189]. Psychological stress, as with intense participation in a video game or driving in rush hour traffic, decreases cycling of the intestinal MMC in healthy volunteers [190]. The relative effects of stress on small intestinal motility in patients with IBS compared to healthy individuals are controversial. Using radiotelemetry capsules in the small intestine, long periods of mental stress induced motor abnormalities in 19 of 22 patients with IBS compared to one of 10 controls [116]. In seven patients, MMC cycling was eliminated and in 18 patients there were episodes of irregular motor activity lasting 1–6 h. However, another study showed equivalent suppression of MMC activity in patients with IBS and controls as well as inhibition, rather than induction, of DCCs in both subject groups, suggesting that the intestine in patients with IBS may not be more susceptible to stress than those of asymptomatic controls [191].

Current stressful events may exacerbate symptoms in a large subset of patients with IBS, although they are not likely to be the cause of the IBS. A strongly positive relationship was reported between daily stress and daily symptoms in women with IBS [192]. Disturbances of sleep have been correlated with symptom development in IBS, especially in the morning [193]. In addition to current stressors, a history of severe emotional upheaval in the distant past is often elicited on careful questioning. Loss of a parent, either through death or marital dissolution, has been reported to be an important stressor in one-third of patients [194–196]. A strong association between IBS and posttraumatic stress disorder was noted [197]. Furthermore, military veterans returning from the Gulf War presented with gastrointestinal complaints and exhibited similar visceral hyperalgesic properties to patients with IBS, suggesting that a subset of persons with Gulf War syndrome may in fact have IBS [198].

Other studies on the pathogenesis of irritable bowel syndrome

Induction of irritable bowel syndrome by gastrointestinal infection

A number of studies correlate development of IBS with a prior gastrointestinal infectious illness. In an investigation of 544 patients with confirmed bacterial gastroenteritis, one-fourth reported persistently abnormal bowel habits 6 months later [199]. Seven percent developed IBS with a 3.4-fold increased risk in women compared to men. A similar study of 75 patients with acute gastroenteritis requiring hospital admission recorded that 20 had persistent symptoms for more than 6 months compatible with a diagnosis of IBS [200]. Since these two earlier reports [199,200], several other studies have also identified IBS as a sequela of infectious gastroenteritis with reported prevalence ranging from 4% to 31% and relative risk from 2.5 to 11.9 [201–204]. A metaanalysis of eight studies showed a median prevalence of IBS to be 9.8% compared with 1.2% in the control group. The pooled odds ratio was 7.3 (95% confidence interval 4.7–11.1). Conversely Chaudhary and Truelove reported that about one-third of IBS patients experienced an acute "gastroenteritis-like" illness at the onset of their chronic IBS symptomatology [36]. The above-cited studies have shown that IBS following an enteric infection occurred more commonly in females, affected younger rather than older patients, and those who had a protracted acute diarrheal illness. Prior gastrointestinal morbidity and prolonged use of antibiotics are also risk factors for developing postinfectious IBS [202]. Psychometric assessment at the time of acute infection found that those who developed IBS had higher scores for anxiety, depression, somatization, and neuroticism compared to those who recovered. Increases in ileal mast cell density are reported in IBS, raising the possibility that subtle inflammation is pathogenic [205].

In addition to IBS, other functional gastrointestinal symptoms may occur after infectious gastroenteritis [203,204]. In a prospective study of dyspepsia and IBS after an outbreak of salmonella gastroenteritis [203], it was reported that at 1 year, the relative risk for development of dyspepsia was 5.2 and for IBS was 7.8. Prolonged abdominal pain and vomiting during acute gastroenteritis were positive predictors for dyspepsia.

The microbes involved in the initial infection were *Campylobacter* sp., *Salmonella* sp., and *Shigella* sp. [199,200,206]. Those patients with *Campylobacter* sp. infection who were toxin-positive were more likely to develop postinfectious irritable bowel syndrome [207]. Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability were acute changes following campylobacter enteritis, which could persist for more than a year and may contribute to postinfectious IBS [208].

Postinfectious IBS has also been reported to occur after a food-borne outbreak of acute gastroenteritis attributed to the norovirus [209]. After a 3-month interval, 23.6% reported symptoms consistent with postinfectious IBS compared with 3.4% who remained well (odd ratio 6.9). After 6 months the prevalence of IBS was similar among exposed and nonexposed individuals. In multiple logistic regressions, vomiting during the acute illness independently predicted risk of postinfectious IBS at 3 months. Hence IBS is common after presumptive viral gastroenteritis but might be more transient than after bacterial dysentery.

Altered gut flora in irritable bowel syndrome

In a provocative study, Pimentel and colleagues [210] reported that of the 202 patients with IBS, 78% had small intestinal bacterial overgrowth diagnosed using the lactulose hydrogen breath test. Forty-eight percent of the subjects who had eradication of bacterial overgrowth experienced symptomatic improvement. The validity of this interesting finding has been challenged by a number of other studies. Walters and Vanner [211] found no significant difference in the incidence of abnormal lactulose hydrogen breath test between patients with IBS and healthy controls. Furthermore, using a ^{14}C-D-xylose test, a test with acknowledged greater specificity for bacterial overgrowth, the incidence of positive tests among patients with IBS was much lower compared to the lactulose hydrogen breath test. Using jejunal aspirate to diagnose small bowel bacterial overgrowth, no increased incidence of bacterial overgrowth was found in patients with IBS compared to control subjects [212]. Therefore it appears that the lactulose hydrogen breath test may not be a reliable method to detect small bowel bacterial overgrowth. There are no convincing data to support the proposal that small bowel bacterial overgrowth plays an important role in the pathogenesis of IBS.

Studies of germ-free and colonized animals have indicated that the microbiota influences the structure and function of the gastrointestinal tract. It appears that signaling from the enteric bacteria is required for mucosal homeostasis with regulatory effects not only on the epithelium and immune system but also on the enteric neuromuscular system [213]. This has led to the proposition that the gut microbiota may contribute to the onset and maintenance of IBS. A number of studies examined the composition and temporal stability of the fecal microbiota in patients with IBS and healthy controls [214–216]. Kessinen and colleagues found significant differences between the molecular profile of the fecal microbiota of patients with IBS compared with that of control subjects [216]. Several bacterial genera with *Lactobacillus* sequences appear to be absent from IBS and *Collinsella* sequences were greatly reduced in IBS. These intriguing results require confirmation. Furthermore, whether such changes are causal, consequential, or merely the results of constipation and diarrhea is unclear. In addition, the stability of the changes in the microbiota needs to be demonstrated.

Role of malabsorption or intolerance to specific foods

The role of carbohydrate malabsorption as a cause of bloating, pain, and diarrhea in IBS has been extensively evaluated. Lactase deficiency is a well-described syndrome where ingestion of milk products causes symptoms similar to those in IBS. Using hydrogen breath testing, 25.8% of one group of patients with presumed IBS exhibited lactase deficiency but 52% of these individuals did not relate their symptoms to lactose ingestion [205]. Another study of lactose-intolerant patients with IBS reported that symptoms were independent of lactase supplementation when given in a placebo-controlled fashion, suggesting that lactase deficiency was not pathogenic of IBS in these patients [217]. Conversely, a recent hydrogen breath test analysis of patients with presumed IBS identified a subset of patients with lactase deficiency unsuspected from their clinical history, who responded to dietary lactose exclusion [218]. This observation was confirmed in a more recent study [219], which reported that for the majority of patients with IBS with lactose malabsorption, which was previously clinically unrecognized, a lactose-restricted diet improved symptoms markedly both in the short term and the long term. These studies suggest that lactose malabsorption should be excluded before diagnosing IBS.

Malabsorption of other sugars has been proposed as a cause of symptoms in some patients with IBS. In one study, fructose and sorbitol, which are found in fruits as well as soft drinks and candy, produced prominent symptoms in most IBS subjects; however, dietary exclusion of these sugars was not performed [220]. A more recent investigation reported increased symptoms in patients with functional gastrointestinal disease after lactose or fructose plus sorbitol compared to healthy controls [221]. In this study, 40% of patients experienced symptom reduction after restriction of the offending carbohydrate. A recent study in Israel indicated that combined carbohydrate malabsorption was common in IBS and may contribute to symptomatology in many of the patients investigated [222]. However, the weight of available evidence suggests that carbohydrate malabsorption is not the cause of symptoms in most patients with IBS in the United States, although intolerance to certain sugars probably plays a role in some individuals.

Food hypersensitivity likely plays only a minor role in most patients, although some individuals with IBS may have a true allergic component to their symptoms [223]. In a study of 14 patients with diarrhea-predominant IBS who noted symptoms after eating wheat, dairy products, coffee, tea, or citrus fruits, a blinded challenge with the offending food led to worsening of the symptoms in six patients; this was associated with elevations in rectal prostaglandin E_2 [224]. Subsequent studies, however, have reported that only a small fraction of diarrhea-prone patients with IBS improve on an exclusion diet, an effect which may not be greater than

with placebo [225,226]. Even those individuals with positive skin test responses to particular foods do not show symptom resolution on dietary exclusion of those foods [227]. In a recent food hypersensitivity study, serum immunoglobulin G4 (IgG4) antibodies to common foods like wheat, beef, pork, and lamb were elevated in all patients with IBS including IBS-D, IBS-C and alternators [228]. No correlation was seen between the pattern of elevated IgG4 antibody titers and patients' symptoms and so the clinical significance of this observation is unclear. In another study comprising 25 patients with IBS, IgG4 antibodies to milk, eggs, wheat, beef, pork, and lamb were found to be commonly elevated. Foods with IgG4 titers greater than 250 μg/L were excluded for 6 months and this resulted in a significant improvement in pain severity and frequency, bloating severity, bowel habits, and effect of IBS on life in general [229]. However, this study did not include a control group and so a placebo effect cannot be ruled out.

Disturbed neurohumoral activity

Systemic disturbances in neural and hormonal activity have been explored as pathogenic factors in IBS. Efferent vagal dysfunction is suggested by demonstrations of reductions in vagally mediated increases in lower esophageal sphincter pressure after abdominal compression, impaired pulse variability with inspiration, and reduced ratios of insulin-induced peak gastric acid output to pentagastrin-stimulated maximal acid output in subsets of patients with IBS [230]. Decreases in digital temperatures and enhanced peripheral electromyographic activity have led investigators to postulate a diffuse autonomic neural disturbance in IBS [231]. A quantitative assessment of sympathetic adrenergic and cholinergic and cardiovagal cholinergic function revealed abnormalities in nine of 33 patients with IBS [232]. Abnormalities of the viscerosensory–cardiovascular reflex exist in some patients with IBS. Arterial baroreflex sensitivity was found to be significantly higher in patients with IBS at baseline and increased during mild rectal distention in both patients with IBS and healthy controls. This increase persisted in controls during intense stimulation, but baroreflex sensitivity returned to baseline in the patients with IBS, suggesting altered viscerosensory processing in the central nervous system [233]. Recent studies indicate that sex differences exist among patients with IBS in terms of alterations in autonomic function [234]. Decreased parasympathetic tone and increased sympathetic activity in response to sigmoid colon distention were prominent in male patients with IBS but not in female patients. This may be the result of gender differences in corticolimbic processing of visceral stimuli. Another investigation suggested that patients with constipation-predominant IBS exhibit abnormalities of cholinergic function whereas diarrhea-prone individuals have evidence of adrenergic neural dysfunction [235]. Parasympathetic tone was significantly lower in the constipation-predominant compared to the diarrhea-predominant patients [236]. Prolongation of rapid eye movement sleep with episodes of sleep apnea has been reported in IBS, raising the possibility that the central nervous system exhibits abnormalities outside the brain–gut axis [237,238]. Blunting of α-adrenergic pathways in the central nervous system is suggested by the observation of impaired growth hormone release in patients with IBS, but not in patients with ulcers or in healthy controls, in response to α_2-adrenergic receptor stimulation [239]. Furthermore, patients with IBS appear to have an exaggerated response to corticotropin-releasing hormone (CRH) [240]. Exogenous CRH not only induced higher plasma adrenocorticotropic hormone levels in patients with IBS, it also produced abdominal symptoms, which were accompanied by a greater motility index in the colon as well as duodenal dysmotility in this group of patients. Intravenous infusion of CRH induced rectal hypersensitivity to repetitive painful rectal distention, suggesting a potential role of CRH in the pathogenesis of visceral hypersensitivity in IBS [241]. Patents with IBS in which diarrhea is predominant showed a significant postprandial increase in cortisol that was not evident in healthy subjects or in patients with constipation-predominant IBS [242]. Hormonal disturbances have been postulated to be pathogenically important in patients with IBS who experience symptoms during menses [60,243]. Gut hormones exert a wide range of effects on gastrointestinal functions. Abnormal circulating pancreatic polypeptide, neurotensin, insulin, motilin, cholecystokinin, and gastrin levels are reported in some patients with IBS [244–247]. Patients with IBS maintained the cyclic variation of plasma motilin during the occurrence of interdisgestive migrating motor complex although the fasting and postprandial motilin levels were higher compared to those in healthy controls, independent of predominant bowel habits [248]. In women with IBS, higher urinary norepinephrine, epinephrine, and cortisol levels have been observed [249]. The roles of these neurohumoral disturbances in the pathogenesis of IBS are unclear but they are likely to be epiphenomenal.

Nitric oxide (NO)-containing neurons are found in abundance in the myenteric plexus. Although NO does not seem to play a major role in normal rectal sensation or tone in health, administration of nitro-L-arginine methyl ester (L-NAME), a specific NO synthase inhibitor, significantly increased the threshold for discomfort/pain in patients with IBS, suggesting that abnormal release or action of NO may contribute to visceral hypersensitivity in IBS [250].

Protease-activated receptor-2 is present in a variety of sensory neurons. They are proalgesic receptors and when activated, may contribute to visceral hypersensitivity. Trypsin and tryptase expression and release were increased in colonic biopsies from patients with IBS [251]. These biopsies released mediators that sensitized murine sensory neurons in culture. Sensitization was prevented by a serine protease inhibitor and was absent in neurons lacking functional protease-activated receptor-2 [251]. Furthermore, supernatants from

colonic biopsies of patients with IBS caused visceral hyperalgesia and allodynia in mice when administered in the colon. These studies demonstrated that proteases are released in IBS and they can directly stimulate sensory neurons and generate hypersensitivity symptoms through activation of protease-activated receptor-2.

Serotonin (5-HT) is present in abundance in the gastrointestinal tract. It may play an important role in the regulation of gastrointestinal motility and visceral perception. A clinical study indicated that 5-HT containing enterochromaffin cells in the colon were increased in patients with diarrhea-predominant IBS compared to healthy subjects or patients with ulcerative colitis [252]. Furthermore, postprandial 5-HT plasma levels were significantly higher in patients with diarrhea-predominant IBS compared to healthy controls [253]. These observations were confirmed in another study showing that patients with IBS-C with slow colonic transit had impaired postprandial 5-HT release whereas the patients with postinfectious IBS-D had higher peak levels of 5-HT and shorter colonic transit time [254]. Similar observations were made by Atkinson and colleagues [255], who further demonstrated that IBS-C was associated with a decreased 5-hydroxyindole acetic acid (5-H1AA) : 5-HT ratio whereas patients with IBS-D have normal plasma 5-H1AA concentrations but a reduced 5-H1AA : 5-HT ratio. These findings support the concept that patients with IBS-D may have reduced 5-HT uptake and that IBS-C may be associated with impaired release of 5-HT. Further in vitro metabolic studies using colonic biopsies from patients with IBS-C and IBS-D would help to clarify this issue. Overall, this group of studies suggests a possible role for 5-HT in the symptom generation of these patients and provides a rationale for the use of 5-HT agonists and antagonists in the treatment of IBS.

Immune activation and mucosal inflammation

Some patients with IBS, especially those with postinfectious IBS, display persistent signs of low-grade mucosal inflammation with activated lymphocytes, mast cells, and enhanced expression of proinflammatory cytokines [208]. These abnormalities may contribute to abnormal epithelial secretion and visceral hypersensitivity. In a recent study mast cells were found to be greatly increased in the terminal ileum, ascending colon, and rectum of patients with IBS-D and these patients showed visceral hypersensitivity in response to rectal distention [256]. Mucosal mast cells from patients with IBS secreted histamine and tryptase. These mediators markedly enhanced the firings of mesenteric nerves and stimulated mobilization of Ca^{2+} in dorsal root ganglia neurons [257]. The latter was inhibited by histamine H_1 receptor blockade and serine protease inactivation. Hence mucosal mast cells in patients with IBS may contribute to visceral hypersensitivity in this group of patients.

Abnormal immune activation occurs in some patients with IBS with augmental cellular immune responses and enhanced production of proinflammatory cytokines [258]. In a recent study, peripheral blood mononuclear cells from patients with IBS-D and with postinfectious IBS showed higher baseline interferon-α, interleukin-1β (IL-1β), and IL-6 levels and lipopolysaccharide-induced IL-6 secretion whereas colonic biopsy specimens did not reveal any inflammatory changes. These proinflammatory cytokines may alter mucosal barrier function. Interestingly, clinical studies have shown increased intestinal permeability in patients with IBS-D [259].

Psychological stress and anxiety can increase the release of proinflammatory cytokine [260]. This may contribute to dysregulation of the hypothalamic–pituitary–gut axis which has been reported to occur in patients with IBS [261]. Following intravenous infusion of CRH, an exaggerated release of both adrenocorticotrophic hormone and cortisol was observed in patients with IBS. This was accompanied by an increase of plasma levels of IL-6 and IL-8. Both IL-1β and tumor necrosis factor-α (TNF-α) synergistically stimulated the hypothalamo–pituitary–adrenal axis either directly or indirectly through activation of nociceptive, visceral, and somatosensory afferents [260]. Hence this series of observations provide a functional link between psychological stress, immune activation, and symptom generation in patients with IBS (Fig. 60.6).

Genetic indicators

Aggregation of IBS symptoms has been described in families, suggesting a possible contribution by genetic factors to the pathogenesis of this disorder. Studies on monozygotic and dizygotic twins, however, do not support a key role for genetic factors in IBS. The predominant influences appear to be environmental [31]. In recent years a number of biological molecules have emerged as potential genetic markers for IBS. These include molecules involved in serotonin metabolism [262], G protein translation of ligand receptor activation [263], and factors controlling production of pro- and antiinflammatory cytokines [264]. The *GN beta 3* gene regulates G protein translation of a majority of ligand–receptor activations and homozygous genotypes of the C825T polymorphism in the *GN beta 3* gene has been associated with functional dyspepsia [265] but not with IBS [263]. Imbalances in the genetically controlled pro- and antiinflammatory cytokine production may promote the low-grade mucosal inflammation observed in some patients with IBS. Studies by Van der Veek and colleagues [266] showed that the heterozygous (G/A) genotype that encodes for high secretion of the proinflammatory cytokine TNF-α was significantly more prevalent in the IBS group compared to controls (41% vs 26%), whereas the frequency of the A/A genotype that encodes low secretion of this cytokine was similar in both groups. However, the combination of the genotypes encoding low secretion for the counterinflammatory cytokine IL-10 (-1082 A/A) and high secretion of TNF-α (-308 A/A or G/A) was significantly more prevalent in the IBS group (9%) compared to controls (3%).

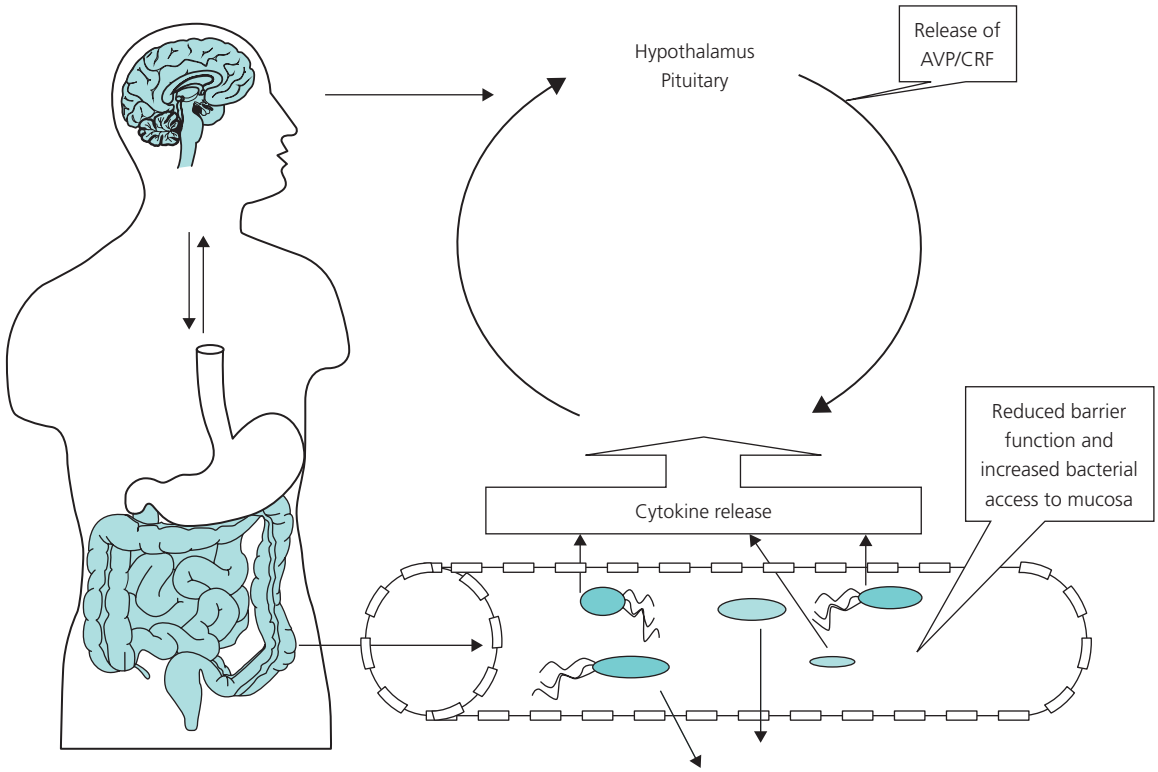

Figure 60.6 Functional link between psychological stress, immune activation, and irritable bowel syndrome (IBS) symptoms. Anxiety and stress induce an exaggerated release of corticotrophin-releasing hormone (CRH) in IBS. CRH infusion stimulates the secretion of proinflammatory cytokines, which may alter motor and secretory function and increase mucosal permeability. Cytokines such as interleukin-1β and tumor necrosis factor-α can synergistically activate the hypothalamo–pituitary–adrenal axis either directly or indirectly through stimulation of nociceptive, visceral, and somatosensory afferents. This positive feedback pathway provides a link between psychological stress, immune activation, and altered gut functions in IBS. From Shanahan [456], with permission from Elsevier.

Abnormalities in 5-HT metabolism have been implicated in the pathogenesis of IBS. Two proteins that influence the function of 5-HT and 5-HT receptors are serotonin transporter protein (SERT) or soluble carrier protein (SLC [6A4]) and p11 (S-180 A10, or calpactin I right chain). Camilleri and colleagues [262] found no difference in the messenger RNA for SERT compared with reference genes between any of the groups in the sigmoid or rectal mucosa. In contrast, they found a significant increase of p11 in the sigmoid mucosa, especially in those patients with IBS-C. Given that overexpression of p11 can increase serotonergic receptor functions (e.g., 5-HT_{1B} receptors) [267,268], it may influence the functions of the 5-HT pathways in the gastrointestinal tract. Furthermore, p11 increased surface expression of Nav 1.8 [269], $\text{TRVP}_{5\text{and}6}$ [270], and TASK-1 [271] in sensory nerves resulting in enhanced excitability of these ion channels, which may cause visceral hypersensitivity.

These new findings may provide new insight into the pathophysiology of the disorder and possibly suggest new targets for drug development. However, given that genetic polymorphisms are rather common and IBS is a heterogeneous group of disorders with very few components that are genetically determined, it is unlikely that genetic testing will help in the diagnosis of IBS.

Abnormal stool characteristics

Abnormal characteristics of the fecal material have been postulated as symptom inducers in IBS. As bile acids induce colonic contractions, investigators have proposed that patients with diarrhea-predominant IBS have bile acid malabsorption [71]. Although bile acid binders such as cholestyramine are used in IBS with anecdotal reports of success, this may be because of the general constipating effects of the resin. A recent study reported that about one-third of patients with IBS had bile acid malabsorption and 70% of these patients responded to bile acid sequestrants whereas only 15% responded to conventional therapy [272]. Others have proposed that elevated levels of fecal short-chain fatty acids may cause diarrhea in IBS; however, symptoms do not correlate with fecal levels of these compounds [273]. A clinical investigation revealed that diarrhea-prone patients have lower fecal concentrations of total short-chain fatty acids, acetate, and propionate, and higher levels of *n*-butyrate than controls [274]. The pathophysiological significance of these findings remains to be determined.

Diagnostic approach to the patient with presumed irritable bowel syndrome

History and physical examination in irritable bowel syndrome

Confident diagnosis of IBS relies on recognition of characteristic symptom profiles as well as the detection of alarm findings (weight loss, bleeding, fever, or palpable masses), which are more suggestive of organic disease. The symptoms that define IBS are prominent complaints of many other conditions including malignancies, inflammatory diseases, infections, and ischemic diseases of the gastrointestinal tract as well as some nongastrointestinal conditions. Several investigators have attempted to ascertain if specific symptom patterns can distinguish IBS from more severe illnesses. In a questionnaire survey, Manning and colleagues [275] defined four symptoms that were significantly more common in IBS than organic disease including relief of abdominal pain on defecation, looser stools with onset of pain, more frequent stools with the onset of pain, and abdominal distention. Ninety-one percent of patients with IBS had at least two of these symptoms compared with 30% of patients with organic disease. Other symptoms which were common but which did not distinguish IBS from organic disease included passage of mucus, a sensation of incomplete evacuation, fecal urgency, harder or less frequent stools at pain onset, pain eased by passage of flatus, and defecation before breakfast, at night, or between meals. In a study by Kruis and colleagues [276], an absence of organic disease was predicted by:

- the combination of pain, flatulence, and irregular defecation
- symptoms persisting for more than 2 years
- pain described as burning, cutting, strong, or terrible
- a defecation pattern of alternating defecation and constipation
- pencil-like or pellet-like stools
- the passage of mucus in the stools.

These initial investigations have been followed by international assemblies that have devised the commonly employed Rome I, and most recently Rome III, criteria [1,277].

More recent studies have quantified the reliability of symptom-based criteria in the diagnosis of IBS. An early evaluation observed that the Manning criteria could distinguish IBS from organic disease with a sensitivity of only 58% and a specificity of 74% [278]. Other investigators found that the Manning criteria were able to discriminate IBS from organic disease with a sensitivity and specificity of 90% and 87%, whereas the Kruis system exhibited a sensitivity of 81% and a specificity of 97%, respectively. When employed together, the two sets of criteria exhibited 80% sensitivity and 97% specificity for the diagnosis of IBS [279].

Clinical studies suggest that the positive predictive value of Rome II criteria for diagnosing IBS can be enhanced by excluding red flag symptoms suggestive of organic diseases [280]. The six red flags used by the studies were blood in stools, nocturnal symptoms, documented weight loss, antibiotic use, family history of colon cancer, and relevant abnormalities on physical examination. This recommendation was not supported by a subsequent study of 1434 patients with clinical diagnosis of IBS who completed questionnaires to identify Rome II criteria and red flag symptoms [281]. Red flag symptoms have modest but significant predictive value for identifying patients with functional gastrointestinal symptoms who are at risk for gastrointestinal cancers, IBD, or malabsorption. However, the fact that 84% of patients with functional gastrointestinal symptoms endorsed one or more red flag symptoms, significantly reduced the utility of these red flag symptoms as screening factors. The general consensus is that red flags may be useful for identifying patients who require additional diagnostic evaluation, but incorporating them into the Rome criteria would not improve sensitivity and would result in too many missed IBS diagnoses.

The physical examination of the IBS patient is usually normal. Abdominal compression may elicit tenderness, which is vague and poorly localized. Tender bowel loops are commonly palpable [27]. Masses, adenopathy, hepatosplenomegaly, ascites, blood in the stool, or autonomic or peripheral neuropathy are suggestive of organic disease and are not consistent with IBS.

Laboratory and structural findings in irritable bowel syndrome

A minimal laboratory and structural evaluation has been advocated as a screen for organic disease in the patient with presumed IBS. Basic testing includes a complete blood count to assess for anemia, leukocytosis, or leukopenia, a sedimentation rate to screen for inflammation, thyroid chemistries to exclude hormonal causes of altered bowel function, and serum electrolytes and stool studies for ova and parasites or *Giardia* antigen in patients with diarrhea. For diarrhea-predominant individuals, hydrogen breath testing to exclude lactose intolerance should be considered [282]. Empiric dietary lactose restriction is an alternative when breath testing is not available. For pain-predominant patients, plain abdominal radiography during an episode of pain can exclude intermittent bowel obstruction [282]. Sigmoidoscopy is recommended for most young individuals (< 50 years old) to rule out distal obstruction in patients with constipation and inflammatory diseases in those with diarrhea. In diarrhea-predominant patients, random biopsies are obtained to search for microscopic colitis. Sigmoidoscopy should reveal normal anatomy in IBS, although increased mucus secretion, mucosal engorgement, and pain with air insufflation are commonly noted [27]. In patients over 50 years of age, colonoscopy or air-contrast barium enema radiography are indicated because of the prevalence of colonic neoplasm in this age group. A recent technical review for practice guideline development by the American Gastroenterological

Association delineated factors to be considered when determining the aggressiveness of the diagnostic evaluation [282]. These include the duration of symptoms, the change in symptoms over time, the age and sex of the patient, the referral status of the patient, prior diagnostic studies, a family history of colorectal malignancy, and the degree of psychosocial dysfunction. A younger individual with mild symptoms would therefore require a minimal (or in select cases no) diagnostic evaluation whereas an older person or an individual with rapidly progressive symptoms should undergo a more thorough exclusion of organic disease.

The validity of this approach has been the subject of recent scrutiny. In the absence of alarm findings, a normal complete blood count and sedimentation rate provide 83% sensitivity and 97% specificity for the diagnosis of IBS [35]. However a study of 196 patients satisfying the Rome criteria I found no diagnostic yield from sedimentation rates, thyroid profiles, and stool examinations in the absence of specific historical or physical examination findings [20]. Furthermore, barium enema radiography or lower endoscopy revealed only one case with colon cancer, nine with polyps, and one with colitis out of a sample size of 196 patients [205]. In data from two other multicenter trials, lactose intolerance was diagnosed in 23% of patients, colonic structural abnormalities were found in 2%, abnormal thyroid chemistries were observed in 6%, and positive fecal ova and parasites were detected in 2% [283]. Nevertheless, a thorough initial evaluation of the patient with presumed IBS precludes the need for further diagnostic testing years later. In 112 patients with IBS followed for a median of 29 years, organic disease developed in only 10 patients a median of 15 years after the original diagnosis of IBS [284]. Some patients with diarrhea-predominant IBS may have undiagnosed celiac sprue. The symptoms of celiac sprue respond to a gluten-free diet so testing for celiac sprue in IBS may prevent years of morbidity and attendant expense. Decision analysis studies show that serology testing for celiac sprue in patients with diarrhea-predominant IBS has an acceptable cost when the prevalence of celiac sprue is above 1% and is the dominant strategy when the prevalence exceeds 8% [285]. In patients with concurrent symptoms of dyspepsia, upper gastrointestinal radiographs or esophagogastroduodenoscopy may be advisable. In patients with postprandial right upper quadrant pain, an ultrasonogram of the gallbladder should be obtained. Laboratory features that argue against IBS include evidence of anemia, elevated sedimentation rate, presence of leukocytes or blood in stool, and stool volume between 200 and 300 mL/day. These findings would necessitate other diagnostic considerations.

Treatment of irritable bowel syndrome

The treatment of IBS ranges from patient education and dietary modifications to medications or even psychological intervention. Some patients, especially those with new onset symptoms, will express relief that they do not have a serious condition such as cancer. Lifestyle modification with active patient participation may be helpful. In a randomized controlled trial involving 420 patients with IBS, it was reported that the introduction of a self-help guide book resulted in a 60% decrease in primary-care consultations, a perceived reduction in symptoms, and significant health-service savings [286]. Increased physical activity has been reported to enhance intestinal gas clearance and reduce symptoms in patients with IBS complaining of bloating [287]. Whenever possible, the physician should resist the temptation to rely on drugs which might provide only limited efficacy and which could provoke unacceptable side effects. Nonetheless, medications are prescribed at 75% of outpatient visits for IBS [17]. High placebo response rates ranging from 30% to 70% have diminished the ability of controlled studies to detect therapeutic benefits of drug therapies of IBS [288]. Reviews have judged that few studies offer convincing evidence of drug efficacy, although some benefit for antispasmodics in reducing pain and for loperamide in controlling diarrhea can be concluded [289,290]. Critiques of published IBS drug trials raised methodological concerns about the peer-reviewed literature including studies which were too short in duration to detect efficacy, poorly designed cross-over protocols in which washout periods between active drug and placebo were inadequate, inappropriate statistical methods, and large placebo effects which would mandate the enrollment of hundreds of patients to detect a statistically significant drug effect [288]. In the design of new studies, they recommended careful description of the randomization method, use of international diagnostic criteria, use of placebo control and double-blinding, and definition of clear outcome measures.

Dietary measures and fiber supplements in irritable bowel syndrome

Dietary modifications are commonly recommended in IBS, although little controlled investigation has been performed. It is reasonable to limit fat intake as lipids most potently activate exaggerated motor reflexes such as the gastrocolonic response. Patients with bloating and diarrhea should restrict their intake of poorly digestible sugars such as fructose and sorbitol. A subset of patients with excess gas may derive benefit from additional dietary modification. Certain foods promote intestinal gas production. Pork and bean meals increase colonic gas passage from 15 to 176 mL/h as a consequence of maldigestion of oligosaccharides such as stachyose and raffinose [291]. Fiber content in the diet also can contribute to gas generation. A high-fiber diet with beans produces 49.4 mL gas per hour vs 10.9 mL/h on a liquid low-fiber diet [292]. However, most IBS patients who complain of excess flatus in fact produce normal amounts of gas. Exclusion of foods associated with increased flatulence including beans, onions, celery, carrots, raisins, bananas, apricots, prunes,

Table 60.3 Foods and flatus production

Normoflatulogenic foods
Meat, fowl, and fish
Vegetables (e.g., lettuce, cucumber, broccoli, pepper, avocado, cauliflower, tomato, asparagus, zucchini, okra, olives)
Fruits (e.g., cantaloupe, grapes, berries)
Carbohydrates (e.g., rice, corn chips, potato chips, popcorn, graham crackers)
Nuts
Miscellaneous (e.g., eggs, non-milk chocolate, flavored gelatin, fruit ice)
Moderately flatulogenic foods
Pastries
Potatoes
Eggplant
Citrus fruit
Apple bread
Extremely flatulogenic foods
Milk and milk products
Vegetables (e.g., onions, beans, celery, carrots, brussel sprouts)
Fruit (e.g., raisins, bananas, apricots, prune juice)
Miscellaneous (e.g., pretzels, bagels, wheat germ)

Adapted from Van Ness & Cattau [293].

brussel sprouts, wheat germ, pretzels, and bagels has been reported to reduce flatus expulsions in uncontrolled reports of patients with excess gas (Table 60.3) [293].

Fiber supplements are widely recommended for treating IBS and exhibit several properties which theoretically should be beneficial in IBS, including enhanced water retention and bulking of the stool, formation of gels to provide stool lubrication, and binding bile acids which could cause symptoms in some cases [294]. Notably, patients with IBS do not consume less dietary fiber than healthy individuals [295]. Studies in animal models have observed induction of propagative colonic motor activity with augmentation of dietary fiber content [296]. In a review of human investigations, bran increased fecal weight in 18 of the 20 studies and accelerated fecal transit in 16 [297]. Bran accelerated colonic transit in healthy volunteers with an initial colonic transit time of at least 3 days, whereas it retarded propulsion in those with a transit time of 1 day, suggesting that dietary fiber promotes a more regular bowel pattern in normal individuals [298]. Psyllium reduces perception of rectal distention, indicating that fiber may have positive effects on visceral afferent function as well [299].

Fiber supplements that have been studied in IBS in a placebo-controlled fashion include bran, psyllium, processed flea seed husk (ispaghula), and calcium polycarbophil. Because of high placebo response rates and the enrollment of small numbers of patients, studies have failed to document a therapeutic benefit of fiber supplementation in the IBS population as a whole [288]. Most investigations report increases in stool weight, decreases in colonic transit times, and improvement in constipation [300–303]. Others have noted benefits in patients with alternating diarrhea and constipation, pain, and bloating; however, most studies observe no responses in patients with diarrhea-predominant or pain-predominant IBS [304]. It is possible that different fiber preparations may have dissimilar effects on selected symptoms in IBS. One study observed symptom exacerbation in 55% of patients with IBS with bran but only 10% experienced symptom improvement [305]. A cross-over comparison of different fiber preparations found that psyllium produced greater improvements in stool pattern and abdominal pain than bran [306]. Furthermore, bran exacerbated complaints of abdominal distention but psyllium supplements reduced such complaints. A third study reported increases in flatulence with bran therapy [307]. Patients with IBS were noted to benefit from being placed on a fiber-restricted diet but with the use of commercial bulking agents as needed, suggesting that low-fiber diets may be advisable in some patients [308].

Medication treatments in irritable bowel syndrome

Osmotic laxatives

For patients with constipation-predominant IBS who do not respond to fiber, osmotic laxatives are often recommended to effect defecation. Available agents include hypertonic salt solutions such as milk of magnesia, poorly absorbable sugars such as lactulose and sorbitol, and isotonic electrolyte solutions containing polyethylene glycol. There is little controlled literature to support the use of osmotic laxatives specifically in IBS, although several studies do report benefits for polyethylene glycol which is a large polymer that is poorly absorbed and metabolically inert, in accelerating colonic transit and increasing stool frequency in patients with idiopathic slow transit constipation [309]. There are multiple placebo-controlled clinical trials of polyethylene glycol compounds showing this compound to be superior to placebo in increasing stool frequency and stool consistency in patients with chronic constipation [310,311]. The use of polyethylene glycol solution to relieve functional chronic constipation is generally safe and no blood electrolyte changes have been reported. Some patients may experience bloating or nausea.

Chloride channel activators

Lubiprostone, a member of a new class of compounds of bicyclic fatty acids called prostones, selectively activates type 2 chloride channels in the enterocytes without affecting the cystic fibrosis transmembrane conductance regulator [312]. As the Cl^- leaves the cell to enter the lumen, Na^+ and H_2O are also secreted into the lumen paracellularly to maintain the electrical gradient within the cell (Fig. 60.7). In this manner lubiprostone increases intestinal fluid secretion and facilitates intestinal transit, thereby increasing the passage of stool and alleviating symptoms associated with chronic constipation [313]. Clinical studies support the safety and efficacy of

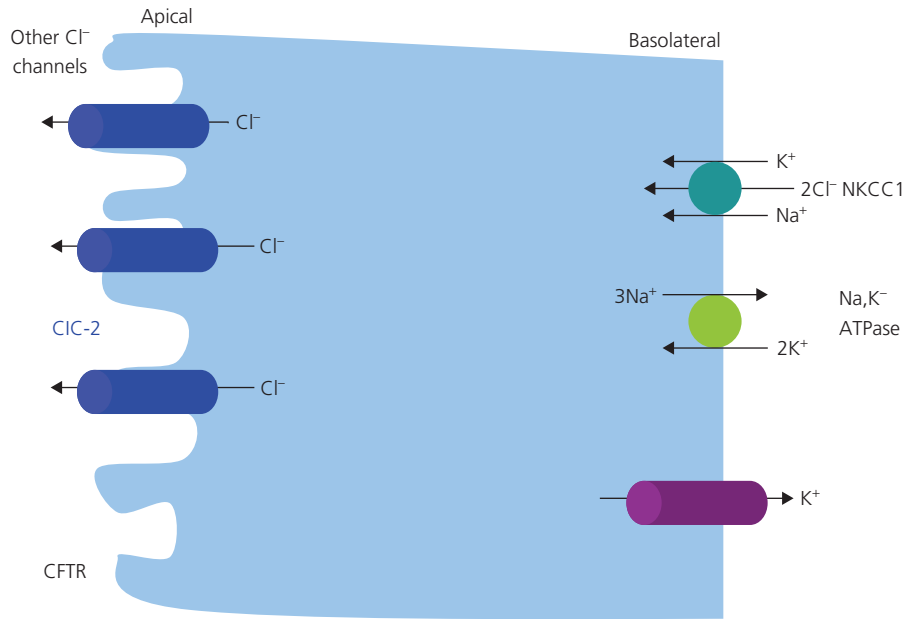

Figure 60.7 Chloride channels are located in the apical and serosal membranes of the enterocyte and facilitate Cl^- transport. Lubiprostone selectively activates type 2 Cl^- into the lumen. To maintain the electrical gradient Na^+ and H_2O are also simultaneously secreted into the lumen paracellularly.

lubiprostone for treatment of functional constipation. In a randomized, double-blind, placebo-controlled trial involving a total of 129 patients, it was demonstrated that lubiprostone 24 μg given twice daily for 21 days was more effective than placebo in inducing spontaneous bowel movement, decreasing straining and abdominal discomfort, and improving stool consistency [314]. Long-term studies indicate that efficacy was maintained for up to 48 weeks of treatment. The most commont side effects were nausea, diarrhea and headache.

Antidiarrheal agents

Peripherally acting opiate-based agents are the initial therapy of choice for diarrhea-predominant IBS. Physiological studies demonstrate increases in segmenting colonic contractions, delays in fecal transit, increases in anal pressures, and reductions in rectal perception with these drugs. Placebo-controlled investigations of loperamide report decreases in stool frequency and urgency, improved consistency, reduced borborygmi, and benefits for well-being in patients with diarrhea or alternating diarrhea and constipation [315]. Other agents have been proposed for use in IBS patients with diarrhea. An uncontrolled study of diarrhea-predominant IBS patients noted a 70% response to bile-acid-sequestering drugs [272]. Acid-suppressing drugs in the H_2-receptor antagonist and proton pump inhibitor classes have been reported in an uncontrolled trial to reduce diarrhea in IBS [316].

Antispasmodic agents

Antispasmodic agents are the most commonly prescribed agents for IBS and are the initial recommended therapy for pain-predominant IBS [282]. Included in this category are agents that block cholinergic nerve function, drugs that prevent calcium flux, direct gut smooth muscle relaxants, and agents that act by way of unknown pathways. From a physiological standpoint, anticholinergic drugs and calcium channel antagonists are potent inhibitors of the gastrocolonic response [317,318]. A metaanalysis of 26 double-blind clinical trials of antispasmodic agents in IBS reported better global improvement (62%) and abdominal pain reductions (64%) compared to placebo (35% and 45%, respectively), although none of the medications analyzed (cimetropium, pinaverium, trimebutine, octylonium, and mebeverine) are approved for use in the United States [319]. A second metaanalysis of controlled trials of cimetropium, hyoscine, mebeverine, otylonium, pinaverium, and trimebutine quantified improvements in global well-being in 56% vs 38% on placebo and reductions in pain in 53% compared to 41% on placebo [320].

Anticholinergic agents are the major class of antispasmodics prescribed in the United States. In small trials, dicyclomine, prifinium, and cimetropium have been reported to reduce symptoms including fecal urgency and pain in IBS compared to placebo [321,322]. However, issues of blinding, study duration, and statistical analysis raise questions as to the validity of these conclusions [288,321]. The gut-selective anticholinergic M_3 muscarinic receptor antagonist zamifenacin was demonstrated to blunt the colonic motor response to meal ingestion and shows promise as therapy for pain-predominant illness [322]. In animal models, M_3 receptor antagonists inhibit diarrheal responses to stress, serotonin, prostaglandins, and castor oil [323].

Calcium channel blockers have been proposed for use in IBS because of their smooth muscle relaxant properties and their inhibitory effects on the gastrocolonic response. A

double-blind trial of diltiazem did not show global improvement, but did record trends to reduced diarrhea and abdominal pain [324]. In a randomized, placebo-controlled trial, pinaverium reduced the duration of abdominal pain in IBS [325]. Octylonium produced significant improvements in the number of pain episodes, the severity of distention, and global assessments in a placebo-controlled trial in 325 patients [326]. Peppermint oil relaxes gastrointestinal smooth muscle through reduction of calcium influx [327]. In a recent large trial, 79% experienced pain reduction, 83% reported less abdominal distention, 83% had reduced stool frequency, and 79% passed less flatus, values which were all superior to placebo [328]. A metaanalysis of eight randomized, controlled trials reported a trend toward therapeutic benefit of peppermint oil in IBS, however this could not be definitively concluded because of methodological concerns [329]. Peppermint oil may also be of benefit in children with IBS, as the agent has been shown to reduce abdominal pain severity in 75% of cases [330].

Other antispasmodics unavailable in the United States that do not act by inhibition of cholinergic activity or calcium flux have been used for IBS. Mebeverine is a smooth muscle relaxant similar to papaverine that inhibits ileal and colonic motility in patients with IBS [331]. Two small studies have reported benefits for mebeverine over placebo whereas a third investigation observed no improvement [331,332]. Both the short- and long-acting forms of mebeverine were reported to be "effective" or "very effective" by more than 80% of patients with IBS in a non-placebo-controlled trial [333]. Trimebutine is an antispasmodic which stimulates small intestinal motility by acting on peripheral opiate receptors but inhibits colonic motility by way of a naloxone-insensitive pathway [334]. In the largest placebo-controlled trial involving 60 patients with IBS, there were no differences in pain relief with trimebutine vs placebo [335].

Antidepressant agents

Antidepressant agents in the tricyclic class have demonstrated clear efficacy in selected IBS subsets (Fig. 60.8), but the mechanisms of their beneficial effects are uncertain. In diarrhea-predominant IBS patients, imipramine slows propagation of jejunal pressure waves and delays orocecal and whole gut transit indicative of a motor inhibitory effect [336,337]. Some investigations have reported reduced perception of lumenal distention suggestive of inhibition of visceral afferent transmission, although other studies have shown no effects on visceral perception [338,339]. The effects of tricyclic antidepressants on central neural processing of painful viscera were studied. In a fMRI sudy of 19 females with painful IBS randomized to amitriptyline 50 mg or placebo for 1 month, rectal pain induced significant activation of the perigenual ACC, right insula, and right prefrontal cortex, and treatment led to reduced pain activation of the perigenual ACC and left posterior parietal cortex

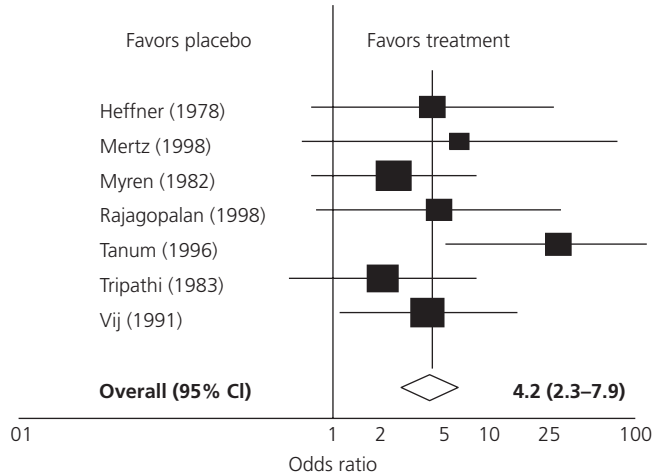

Figure 60.8 The results of metaanalysis of antidepressant medications on the overall improvement in gastrointestinal symptoms are shown. The boxes represent the odds ratios favoring active drug or placebo and the horizontal lines show the 95% confidence intervals. The box sizes are proportional to each study's weight in the analysis. When the data are pooled, the odds ratio for a benefit of antidepressant treatment is 4.2 with a 95% confidence interval ranging from 2.3 to 7.9. From Clouse et al. [357], with permission from Elsevier.

only during stress [340]. Since the perigenual ACC is part of the corticolimbic network that modulates the affective and cognitive components of pain perception during stress, amitriptyline appears to exact its beneficial effects on IBS by uncoupling the negative effects of stress on pain by way of the ACC. A controlled trial of amitriptyline reported benefits in global well-being, abdominal pain, and bowel pattern and identified young age and increased extroversion as predictors of drug response [341]. Studies of trimipramine have reported improvements in vomiting, depression, sleeplessness, and fecal mucus with variable reductions in pain compared to placebo [342]. Placebo-controlled trials of desipramine recorded decreases in pain, stool frequency, and depression which were confined to diarrhea-predominant patients [343]. Nortriptyline given in combination with fluphenazine was reported to reduce pain and diarrhea compared to placebo [344]. A large placebo-controlled, randomized study on desipramine (a tricyclic) showed that the medication (50–150 mg) was effective over placebo in treating women with moderate to severe IBS [345,346]. A clinical response appeared to be dependent on the presence of detectable blood levels of the medication. The general consensus is that patients with predominant diarrhea or pain may experience benefit with the tricyclic antidepressants. Additionally it is likely that patients with IBS who have well-defined depression will experience clinical improvement on tricyclic therapy.

The efficacy of antidepressant agents in other classes for treating IBS is less clear [328–330]. In contrast to tricyclic agents, the selective serotonin reuptake inhibitor (SSRI)

paroxetine accelerates orocecal transit raising the possibility that this drug class may be useful in constipation-predominant patients [337]. There is a paucity of randomized placebo-controlled trials of SSRI in IBS [347]. In one trial of patients who had failed to respond to a high-fiber diet, Tabas and colleagues [348] found that a low dose of paroxetine was superior to placebo in terms of overall well-being, IBS-related anxiety, and less food avoidance. These effects were observed even among nondepressed patients. The study by Kuiken and colleagues [349] found that in 40 nondepressed patients with IBS, fluoxetine did not significantly alter rectal sensitivity and symptoms in the patients in a double-blind, randomized placebo-controlled study. On the other hand, the trial by Tack and colleagues [350] showed that citalopram, an SSRI, was significantly superior to placebo in reducing the number of days with abdominal pain and the number of days in which IBS impacted on daily life. Again the improvement in IBS symptoms was not associated with improved mood. Based on the available studies reported so far, it appears that SSRI antidepressants do promote general well-being in some patients with IBS and possibly improvements in abdominal pain and bowel symptoms, and this effect is independent of improved depression. There are a number of possible mechanisms that might explain the beneficial effect of SSRI on IBS. In contrast to the tricyclic agents, paroxetine accelerates orocecal transit in both healthy subjects and patients with IBS, raising the possibility that this antidepressant class may have fewer deleterious effects in patients with IBS-C [318]. However, this mechanism is unlikely to be the principle therapeutic action of SSRI. The SSRI such as citalopram decrease the sensitivity of the colon to distention in healthy subjects [351] but in clinical trials, the result of intravenous citalopram did not predict outcome [350]. There is some evidence that SSRIs have an analgesic effect. This is not as strong as that of tricyclic antidepressants when used in IBS [337], or on neuropathic pain and back pain [352] but SSRI appear to be effective in patients with somatoform disorder [353]. Perhaps this action may be more important than any of the possible effects on the gut as citalopram may reduce the reporting of multiple bodily symptoms or "somatization" [354].

An investigation of mianserin, with serotonin 5-HT_2 and 5-HT_3 receptor antagonist and α_2-adrenergic receptor antagonist effects, reported reductions in pain, distress, and functional disability compared to placebo [355]. A metaanalysis of tricyclic and nontricyclic antidepressants reported an odds ratio for clinical improvement of 4.2 (confidence interval 2.3–7.9) (Fig. 60.8) [356]. On average, 3.2 patients needed to be treated to produce symptom improvement in one patient. A second metaanalysis of all antidepressant trials in IBS reported an odds ratio for improvements in pain of 8.0 and for global improvement of 4.4 [367].

Serotonin receptor agonists and antagonists

Serotonin exists in abundance in the enterochromaffin cells of the gastrointestinal mucosa. It is a paracrine messenger which functions as sensory transducer [358]. Because 5-HT activates intrinsic and extrinsic primary afferent neurons to initate peristaltic and secondary reflexes, and to transmit sensory information to the central nervous system (Fig. 60.9), serotonin receptor agonists and antagonists have been developed to treat pain and motility abnormalities in IBS.

The 5-HT acting on 5-HT_3 receptors enhances the sensitivity of afferent neurons projecting from the gut to the central nervous system (Fig. 60.9) [359]. In humans, 5-HT_3 receptor antagonists such as alosetron reduced the perception of

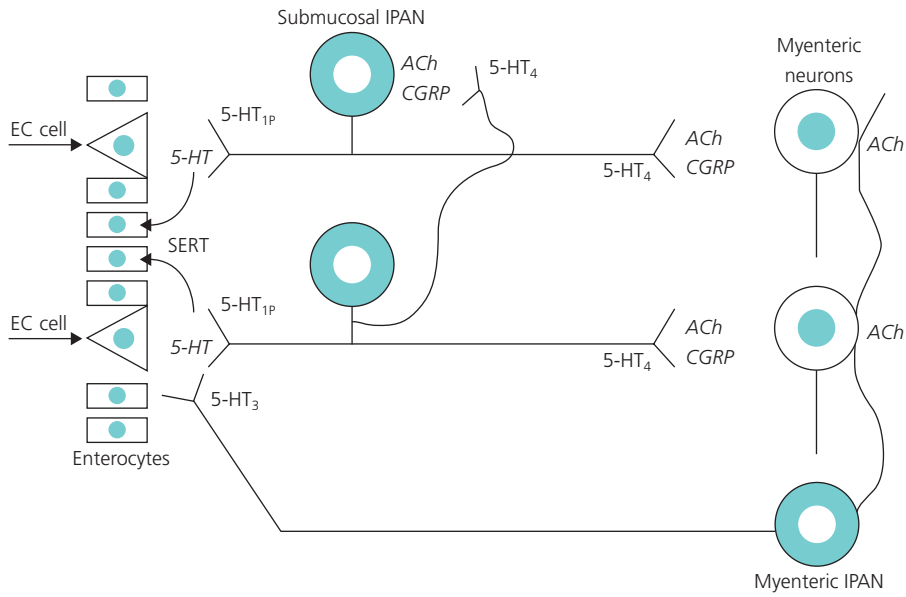

Figure 60.9 Major serotonin (5-HT) receptor subtypes in the enteric neural circuits. The 5-HT secreted from epithelial 5-HT containing enterochromaffin (EC) cells can stimulate extrinsic (not shown) or intrinsic primary afferent neurons (IPAN), which are found in the submucous and myenteric plexus. Submucosal IPANs are activated by 5-HT_{1P} receptors, whereas myenteric IPANs are activated by 5-HT_3 receptors. Release of acetylcholinesterase and calcitonin gene-related peptide from IPANs is amplified by 5-HT_4 receptors, which are presynaptic. Myenteric IPANs have multiple projections that terminate on numerous other cells. They are activated by 5-HT_3 receptors. Submucosal IPANs mediate peristaltic and secretory reflexes. Myenteric IPANs are associated with giant migrating contractions. Adapted from Gershon & Tack [358], with permission from Elsevier.

painful visceral stimulation in IBS [360–362]. It also induced rectum relaxation, increased rectal compliance and delayed colonic transit [363,364]. A number of placebo-controlled trials of alosetron reported reductions in discomfort and improvements in stool frequency, consistency, and urgency in nonconstipated patients with IBS [365–368]. A follow-up study of 48 weeks duration confirmed the long-term efficacy of alosetron. For unclear reasons, women with IBS derived greater benefit than men. However, in postrelease surveillance, 84 cases of ischemic colitis were observed including 44 cases that required surgery and four deaths. As a consequence, the medication was voluntarily withdrawn by the manufacturer in 2000 [369]. Alosetron was reintroduced under a new risk management program where patients have to sign a patient–physician agreement. This has significantly limited its usage.

The 5-HT_4 receptor agonists stimulate motility and secretion through enhanced release of acetylcholine from excitatory motor neurons and interneurons (Fig. 60.9) [370]. In this manner they strengthen cholinergic neurotransmission in enteric neural circuits in the myenteric plexus and so are prokinetic. In patients with IBS who have constipation, tegaserod (a 5-HT_4 agonist) accelerated intestinal and ascending colon transit. Clinical trials involving more than 1200 patients with constipation-predominant IBS have reported that tegaserod given at 2 mg and 6 mg twice daily produced a dose-dependent improvement in constipation and bloating in 40% of patients compared to 27% on placebo [371,372]. Symptom improvements were maintained in an open-label 13-month extension study [373]. Diarrhea and headache were the only significant side effects observed in these studies; however, there were a few cases of ischemic colitis reported in the postmarketing surveillance [374]. The drug was withdrawn by the manufacturer because of concerns with coronary and cerebrovascular events.

Serotonin plays an important role in modulating gut motility, sensation, and secretion so the 5-HT receptor agonists and antagonists are attractive candidates for the treatment of functional bowel diseases such as IBS. However, the reported vascular complications associated with the use of 5-HT_3 antagonists and 5-HT_4 agonists raise some safety issues. More selective 5-HT receptor agonists and antagonists are being studied for the treatment of functional gastrointestinal disorders.

Modulation of gut flora

Probiotics, which are defined as live or attenuated bacteria or bacterial products that confer a significant health benefit to the host, have been used to treat patients with IBS. There are a number of theoretical therapeutic benefits in IBS.

- Many probiotics exert antibacterial and antiviral effects and could prevent or modify the course of postinfectious IBS [375,376].
- Probiotics may have antiinflammatory effects on the mucosal surface [377,378] and so reduce immune-mediated activation of enteric motor and sensory neurons and modify neural traffic between the gut and the central nervous system.
- Probiotics could alter volume or composition of stool and gas [379] and increase intestinal mucus secretion [380]. This may help to modulate symptoms such as constipation and diarrhea.

Clinical evidence of benefit of probiotics in IBS, however, has been less than compelling. Until recently, the best documented double-blind, randomized, placebo-controlled study in 60 patients with IBS reported that flatulence was the only symptom improved with probiotics [381]. A follow-up study using the same *Lactobacillus plantarum* preparation failed to show any change in colonic fermentation, nor did it confirm any clinical benefit [382]. *Lactobacillus plantarum* was also used in another study, which showed a 100% response rate vs just 16% in the control [383]. This remarkable response rate is an outlier among all IBS studies and needs confirmation. A more recent study using VSL #3, a probiotic mixture including lactobacilli and bifidobacteria, in 48 patients with IBS showed that VSL #3 reduced flatulence and retarded colonic transit without altering other gastrointestinal symptoms such as abdominal pain, bowel movements, and bloating scores [384]. Perhaps the most encouraging study to date is that by O'Mahony and colleagues [377], who compared the effect of two probiotics, *Lactobacillus salivarius* against *Bifidobacterium infantis*, in 77 patients with IBS in a double-blind, randomized, placebo-controlled trial. The treatment phase was 2 months, preceded by a 4-week run-in period and followed by a 4-week washout period. Patients taking *B. infantis* experienced a greater reduction in symptom scores for abdominal pain, bloating/distention, and bowel movement difficulty for every week during treatment and during the 4-week washout period, whereas the *L. salivarius* group appeared no different from placebo. This beneficial effect appeared within the first week of treatment and reached its maximum after 2 weeks. In addition, for the first time, a marked immunological difference between IBS and controls was reported. Patients with IBS demonstrated an abnormal IL-10 : IL-12 ratio in the blood, indicative of a proinflammatory T helper type 1 state. This ratio was normalized by *B. infantis* feeding alone, suggesting an immune-modulating role for the organism. This exciting finding requires confirmation with a larger study involving patients with IBS stratified on the basis of inflammatory and immune markers before and after treatment.

Other medication treatments of irritable bowel syndrome

Because IBS symptoms are often associated with menstrual cycling, investigators have searched for hormonal treatments for IBS. Continuous gonadotropin-releasing hormone administration reduces synthesis of the gonadotropins, follicle-stimulating hormone, and luteinizing hormone, with inhibition of cyclic gonadal hormone variations. Small trials

of the gonadotropin-releasing hormone analogue leuprolide in patients with functional gastrointestinal symptoms showed improvements in nausea, abdominal pain, early satiety, bloating, and altered bowel habits for up to 1 year [360,361]. A large multicenter trial of leuprolide reported improvements in abdominal pain, nausea, altered bowel habits, and quality of life in patients with functional bowel disease [362]. The mechanisms of action of leuprolide are uncertain. In an immunosuppressed patient with intestinal pseudoobstruction, leuprolide evoked intense, propagative upper gut motor activity [363]. It is unlikely that this action is responsible for the clinical benefits of the drug in IBS because most patients with IBS do not exhibit pronounced dysmotility. As it induces amenorrhea and osteoporosis, leuprolide is unlikely to achieve widespread long-term use in IBS.

Several agents have been promoted to reduce gas and bloating. Anticholinergics blunt the gastrocolonic response to a meal and reduce the increase in flatus caused by ingestion of beans [293]. Uncontrolled studies using silicone agents such as simethicone show reduction in excess bloating and flatus [364]. Activated charcoal has a large surface area to mass ratio (450–1800 m^2/g) which allows it to be an excellent adsorbent of gas [385]. Small doses of activated charcoal decrease the number of flatus passages after ingestion of beans in healthy volunteers. The microbial α-galactosidase agent Beano reduces hydrogen production after black bean ingestion by healthy volunteers. In a controlled trial in healthy volunteers, pancreatic enzymes reduced bloating, gas, and fullness after eating high-fat, high-calorie meals compared to placebo [386]. No trials of Beano or pancreatic enzymes have been reported in IBS. In a recent study, the nonabsorbable antibiotic rifamaxin reduced hydrogen production, flatus emissions, and abdominal distention to greater degrees than activated charcoal in healthy volunteers and patients with functional bowel disease [387].

Mast cell degranulation inhibitors have been proposed as treatments for diarrhea-predominant patients by investigators who postulate a role for food allergy in IBS. One study evaluated 101 patients with diarrhea-predominant IBS with evidence of food hypersensitivity on the basis of exclusion diets [388]. Of those with positive skin prick tests for food allergy, 67% reported improved symptoms on sodium cromoglycate whereas only 41% with negative skin tests experienced relief. In a second uncontrolled report, sodium cromoglycate produced improvement in 67% of patients with diarrhea-predominant IBS when given for 1 month, especially in those with food allergy [389]. The response to sodium cromoglycate may be more impressive in pediatric populations with IBS symptoms where true food intolerance may be more prevalent. A multicenter trial reported that 97% of children (mean age 4 years) exhibited a clinical response to the drug over a 1-month period [390]. These results, however, require confirmation in placebo-controlled trials.

Tranquilizers have been used for decades to treat IBS. Older studies reported benefits for phenaglycodol, meprobamate, heteronium plus amobarbital, propantheline plus phenoarbital, chlordiazepoxide, diazepam, medazepam, and aprazolam; however, many of these studies were small or poorly designed [391–393]. Buspirone increases colonic compliance; however, its efficacy in reducing symptoms has not been convincingly demonstrated in IBS [394]. Coupled with their abuse potential and development of tolerance, the use of sedatives is not indicated in most patients with IBS.

Potential future therapies for irritable bowel syndrome

Recent investigation has focused on medications which blunt visceral hyperalgesia in IBS. Such "antiafferent" agents might act by way of one or more mechanisms including:

- modification of release of pain-inducing mediators in the gut wall
- blockade or activation of peripheral afferent nerve receptors
- inhibition of afferent nerve transmission
- modification of afferent activity in the central nervous system [114].

Some available drugs, including calcium channel antagonists and opiate agents, reduce visceral perception in association with reducing gut motor function or altering central nervous system processing of pain [395].

κ-Opioid compounds inhibit somatic pain through their action on peripheral sensory nerve endings [396]. In patients with IBS, the κ-opioid analogue fedotozine increases the thresholds to colonic distention for first perception and pain without affecting compliance [397]. It also modifies neural reflexes in experimental gut inflammation, chemically induced peritonitis, and postoperative ileus [398]. In a double-blind, placebo-controlled, multi-center trial in 238 patients with IBS, fedotozine reduced pain and overall disease severity over a 6-week period [399].

α_2-Adrenergic receptor agonists also have been proposed to reduce perception of visceral pain. In healthy volunteers, the α_2-adrenergic receptor agonist clonidine increased colonic and rectal compliance and decreased tone, urgency, and pain during balloon distention [400]. This suggests possible utility for this class of drugs in pain-predominant IBS. Conversely, the α_2-adrenergic receptor antagonist yohimbine increased tone and perception of colorectal distention. Another α_2-adrenergic receptor agonist lidamidine reduced perception of rectal distention compared to placebo in 50 patients with IBS [401]. This drug has been reported to have benefit in treating patients with IBS [402].

Substance P has been postulated to participate in the mediation of visceral pain through activation of neurokinin receptors. In animal models, neurokinin NK_1 receptor antagonists decrease stress-induced defecation as well as reduce the colonic response to the central nervous system stress mediator CRH [403]. Preliminary studies of the NK_1 receptor

antagonist CJ-11,974 have reported benefits in reducing symptoms in patients with IBS with trends to reductions in visceral sensitivity [404].

Physiological studies suggest that other agents may have visceral analgesic effects. The somatostatin analogue octreotide reduces the perception of rectal distention through inhibition of visceral afferent pathways in healthy volunteers [405]. Patients with IBS who have fecal urgency were shown to exhibit a similar response to octreotide [406]. Additionally, octreotide reduced the increased pressures that resulted from rectal distention in these patients. In a second study, octreotide blunted perception of rectal distention without altering lumenal compliance in an IBS population with more constipation-predominant patients [407]. Subsequent work using cortical and spinal evoked potentials showed that octreotide specifically inhibits spinal afferent transmission from the rectum, documenting a peripheral site of action for the somatostatin analogue [408]. Finally, intravenous oxytocin increases the thresholds for perception of colonic distention by way of opiate-independent pathways without affecting lumenal compliance [409]. To date, no placebo-controlled trials of the clinical efficacy of these agents have been performed, so their utility in IBS is unproved.

Psychological therapy of irritable bowel syndrome

Psychological therapy has been most commonly employed for patients with IBS with psychosocial features who do not respond to standard therapies. A systematic review of controlled psychological treatments in the literature from 1966 to 1994 uncovered only 14 studies that exhibited appropriate study design [410]. Eight studies reported that psychological treatment was superior to control treatment whereas six observed no benefits. Because of methodological inadequacies, it was felt that the efficacy of psychological therapy of IBS was not established and that further trials were needed. With recent advances in psychological treatments, better results have been obtained [411–413]. Some studies report that improvements achieved with psychological treatments are similar or even greater than the improvement obtained with gut-directed pharmacological treatment (e.g., tegaserod and alosetron) [414,415]. A metaanalysis of cognitive behavior therapy for IBS reported efficacy data (> 50% reduction of gastrointestinal symptoms) from 17 studies yielded an odds ratio of 12 (5.6–26). The average number of patients needed to treat with cognitive behavior therapy or hypnotherapy for one patient to achieve improvement over the waiting list or other control groups was estimated to be 2 [416]. These outcomes compared favorably with those obtained using alosetron [415] and tegaserod [414]. As a result, many investigators have proposed a range of treatment for IBS including psychotherapy, cognitive therapy, biofeedback, and hypnosis.

Psychotherapy has shown promise in patients who do not respond to medications. In 101 patients with IBS, 3 months of dynamically oriented psychotherapy plus medications provided greater improvements in somatic symptoms than medical treatment alone [417]. A second psychotherapy study of medically refractory patients reported reductions in abdominal pain and diarrhea, but not constipation, as well as improvement in depression [418]. A long-term follow-up of patients undergoing psychotherapy showed maintenance of symptom control at 1 year, whereas individuals who declined psychotherapy had relapsed. Group psychotherapy, including techniques such as psychodrama in which the patient involves other group members in his or her bowel symptoms, does not reduce abdominal pain or improve bowel habits although anxiety levels may decrease [419].

Cognitive therapy produces improvements in bowel symptoms in some patients with IBS. When compared with daily symptom monitoring, cognitive therapy produced clinical improvement in 80% of patients vs 10% of the symptom-monitoring group [420]. Similarly, patients with IBS undergoing cognitive therapy experienced greater reductions in bowel symptoms than did patients assigned to a self-help support group or to a symptom-monitoring wait list [421]. The benefits of cognitive therapy have been reported to persist in long-term follow-up after completion of the treatment [422]. Although multiple clinical trials support the efficacy of psychological treatments for reducing IBS symptoms, the mechanisms responsible for symptomatic improvement remains unclear. To address this issue, Lackner and colleagues [423] applied a mediation analysis to a subsample of data from a randomized clinical trial of group-based cognitive behavior therapy for IBS [411]. These investigators reported that group-based cognitive behavior therapy in comparison to a psychoeducational group, as an attention-based placebo control condition, reduced gastrointesintal symptoms including abdominal pain, bowel dysfunction, nausea and belching independent of its effects on psychological distress and health-related quality of life. This provocative finding suggests that cognitive behavior therapy may have a direct effect on global IBS symptoms that is not related to its beneficial effect on psychological distress. In a brain imaging study using PET [424], the same group of investigators demonstrated that neural activity in the parahippocampal gyrus and inferior portion of the right cortex cingulate gyrus was reduced in the postcognitive therapy scan, compared to the pretreatment scan. The changes in limbic activity were accompanied by improvements in gastrointestinal symptoms, whereas the left pons showed decreased neural activity, which was correlated with the posttreatment anxiety score. This suggests that changes in neural activity of corticolimbic regions, which subserve hypervigilance and emotion regulation, may be responsible for the symptom improvement with cognitive therapy. It is conceivable that strengthening of corticolimbic–pontine inhibition circuits by this form of psychotherapy directly reduces central pain amplication mechanisms [425]. A recent trial compared therapeutic

responses on standard medical therapy to standard medical therapy plus a multicomponent program consisting of IBS education, muscle relaxation, cognitive coping strategies, and assertiveness training [426]. The combination therapy group reported superior reductions in symptoms and improved quality of life, although perception of rectal distention was unaffected by either therapy. Another study suggested that listening to a therapeutic audiotape on IBS provided results that were nearly as good as those achieved with hypnotherapy [427].

Biofeedback and stress reduction techniques have been employed by some clinicians. Muscle relaxation training and stress-reduction education reduced bowel symptoms in an uncontrolled study, especially in patients who exhibited improved depression and anxiety [428]. When followed up at 4 years, 50% reported sustained improvements in pain, diarrhea, nausea, and flatulence. Similarly, in another study, the frequency and severity of painful IBS attacks were decreased by relaxation treatment compared to antispasmodic drugs [429]. More recently, relaxation treatment was shown to reduce primary gastrointestinal symptoms to a greater degree than symptom monitoring [430]. However, other studies have shown no improvement in bowel symptoms. A recent 3-month relaxation response meditation program produced significant improvements in flatulence, belching, bloating, and diarrhea [431]. In a novel study, patients were trained to reduce symptoms by listening to their bowel sound pattern through a stethoscope and attempting to modify their borborygmus [432]. This bowel-sound biofeedback led to improved symptomatology in three of five patients enrolled, with long-term success in two of them. More recently, computerized biofeedback video games have been found in uncontrolled studies to reduce stress and improve relaxation in a significant fraction of patients with IBS [433].

Hypnosis has been proposed for refractory cases of IBS. In a study of 30 patients with IBS, hypnosis provided better improvements in abdominal pain, distention, and well-being but not bowel pattern after 3 months of treatment compared to psychotherapy [434]. When followed for 18 months after being instructed in autohypnosis, sustained remission was reported by most individuals although patients over 50 years old or with underlying psychopathology were less likely to respond. Subsequently the authors reported a success rate of 85% in more than 200 patients with IBS treated with hypnosis. Another controlled trial of hypnosis also reported improvements in abdominal pain, constipation, and flatulence [435]. No correlation was observed between initial susceptibility to hypnosis and treatment gain. In addition to improving symptoms of pain, bloating, bowel habit, nausea, and flatulence, one clinical trial of hypnosis reported beneficial effects on quality of life measures including physical and psychic well-being, mood, and work attitude [436]. Furthermore, patients treated with hypnosis missed less work and visited their physicians less frequently than control patients. An anorectal manometric study of 15 patients with IBS demonstrated reduced perception of rectal distention with hypnosis suggesting that hypnosis may inhibit afferent pathways mediating perception of visceral discomfort [186].

Alternative medicine in irritable bowel syndrome

Over the last 10 years, a wide variety of complementary and alternative medical practices and therapies including hypnotherapy, some forms of herbal therapy and specific diets have been used to treat IBS [437]. Among these, acupuncture is frequently used despite the fact that its efficacy has not been convincingly demonstrated. Human studies have shown that acupuncture modulates the endorphin system [438] by way of central processing pathways [439]. In a study by Chan and colleagues [440], immediate improvement of symptoms was observed in seven patients but this pilot study did not include a control group. A randomized controlled study in 25 patients did not find acupuncture to be effective but it employed atypical acupuncture treatment using a single acupuncture point [441]. In a double-blind, controlled study involving 43 patients with IBS, Schneider and colleagues [442] reported that both the acupuncture and sham acupuncture groups improved significantly in global quality of life (QOL) as assessed by the functional digestive diseases quality of life questionnaire (FDDQOL) at the end of treatment with no difference between the groups. It was concluded that acupuncture in IBS is primarily a placebo response. Further randomized control trials involving large number of patients stratified by sex and subgroup are needed to assess the therapeutic effects of acupuncture in IBS.

In Asian countries, traditional Chinese herbal medicine is used with increasing frequency, especially to treat IBS. In a large placebo-controlled, double-blind, multiarmed trial of a number of herbal remedies, measures of pain, stool function, or quality of life were not better with *Fumaria officinalis*, *Curcuma xanthorrhiza*, Ayurvedic, or spagyric remedies compared to placebo [443]. Similary, a randomized double-blind placebo-controlled trial involving 119 Chinese patients with IBS with predominantly diarrhea symptoms showed no significant difference in individual symptom score and the quality of life assessment between those receiving a standard preparation of traditional Chinese medicine extracts that contained 11 herbs and placebo [444]. Although complementary and alternative medical therapies are widely employed in the treatment of IBS, their efficacies remain to be proven.

Approach to different subsets of patients with irritable bowel syndrome

The management of the patient with IBS is dependent on the predominant symptom. In the technical review for practice

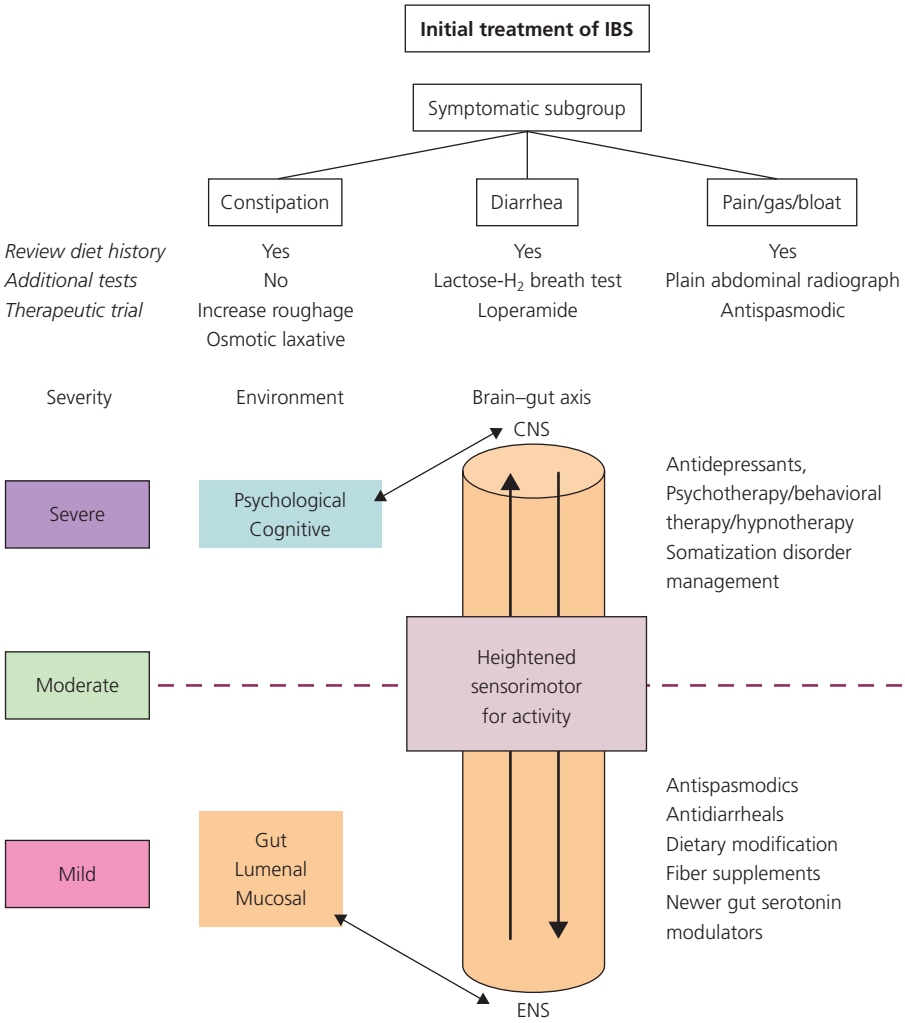

Figure 60.10 Recommendations of a technical review for practice guideline development for the initial management of irritable bowel syndrome (IBS) are shown based on the dominant symptom subtype. From Drossman et al. [282], with permission from Elsevier.

Figure 60.11 Therapeutic targets for irritable bowel syndrome. Patients with mild to moderate symptoms usually have intermittent symptoms that correlate with altered gut physiology. Treatments include gut-acting pharmacological agents such as antispasmodics, antidiarrheal, fiber supplements, and gut serotonin modulators. Patients who have severe symptoms usually have constant pain and psychosocial difficulties. This group of patients is best managed with antidepressants and other psychosocial treatments.

guideline development published by the American Gastroenterological Association, specific therapeutic trials of 3–6 weeks duration were proposed for each symptom subtype before embarking on a more extensive diagnostic evaluation (Fig. 60.10) [282]. Therapeutic targets for IBS are determined by severity of symptoms and their perceived pathomechanisms (Fig. 60.11). Patients with mild to moderate symptoms usually have intermittent symptoms that correlate with altered gut physiology. Treatments include gut-acting pharmacological agents such as antispasmodics, antidiarrheals, probiotics, fiber supplements, and osmotic laxatives. Patients with more severe IBS frequently have constant pain and psychological difficulties. They are best managed with tricyclic antidepressants and psychological therapy. An approach to different subsets of patients with IBS is detailed below.

The constipation-predominant patient

The management of the patient with constipation-predominant IBS involves measures to increase stool water and bulk and reduce the effort of defecation. The most widely used and efficacious compounds for this purpose are bulking agents in concert with a high-fiber diet. Patients should be cautioned that fiber supplements may take several weeks to produce satisfactory results and should be introduced gradually to prevent excess gas. Additionally, any medications that slow colonic transit should be discontinued. Patients with an inadequate response should be given an osmotic agent such as milk of magnesia or an isotonic solution containing polyethylene glycol in addition to the fiber program.

The chloride channel activator, lubiprostone, has been shown to be effective for functional constipation. This medication may be used in patients who fail to achieve satisfactory results. In patients with refractory constipation, further diagnostic testing may be needed to rule out other functional or structural diseases. Using radiopaque markers or colonic scintigraphy, generalized delays in colonic transit indicate the presence of slow-transit constipation (also known as colonic inertia) whereas localized delays suggest functional outlet obstruction in the distal colon. Defecography (cinefluoroscopy of the defecation process) can identify puborectalis muscle dysfunction, rectocele, or rectal prolapse as independent causes of constipation. In IBS, defecography

studies have shown an inability to widen the rectoanal angle as well as impaired perineal descent during simulated defecation indicative of diffuse alterations in pelvic floor mobility [445]. Anorectal manometry, in addition to demonstrating sensory abnormalities, can screen for Hirschsprung disease. These techniques are useful for detecting patients who might be better treated surgically or with a biofeedback program of bowel retraining. Laboratory studies can screen for porphyria or lead toxicity.

The diarrhea-predominant patient

Controlling diarrhea in IBS centers on reducing defecation frequency and urgency and improving stool consistency. Opiate agents such as loperamide are most effective as the initial therapy of diarrhea in IBS. Individuals with prominent meal-related symptoms may achieve benefit from antispasmodic agents in the anticholinergic and calcium channel antagonist classes. Tricyclic antidepressants and bile-acid-binding agents may also be useful in some diarrhea-prone patients. A careful dietary history should screen for foods containing lactose, sorbitol, or fructose. Other dietary manipulations are usually without benefit and are not encouraged in most patients. Use of sodium cromoglycate for food hypersensitivity is unsubstantiated in most diarrhea-prone patients.

Patients not responsive to these therapies may be considered for additional testing. Duodenal aspiration is rarely required to exclude giardiasis. Endomysial antibodies can screen for celiac sprue. Small intestinal biopsy and barium radiography may be performed to rule out Crohn's disease or celiac sprue. Stool volume and fat quantification can assist in the diagnosis of secretory processes or malabsorption. Laxative screens are indicated for the evaluation of refractory or atypical symptoms.

In patients who are resistant to antidiarrheal therapy, a colonic biopsy should be performed to rule out microscopic colitis because the clinical-symptom-based criteria for IBS are not specific enough to exclude the diagnosis of microscopic colitis [446].

The patient with painful irritable bowel syndrome

Controlling abdominal pain in IBS can be challenging. Narcotics should be avoided because of the risks of tolerance and dependence. Antispasmodic drugs are the preferred initial agents for pain-predominant IBS. In tertiary referral centers, antidepressants such as tricyclics have shown good results in those patients who have painful IBS and they may be the drug of choice for a subset of patients who have severe pain and diarrhea. The gonadotropin-releasing hormone analogue leuprolide can be considered for rare patients with refractory symptoms, especially those whose symptoms are prominently associated with menstrual cycling.

Further evaluation to investigate the cause of pain in medication-refractory cases should be directed at objective historical and examination findings. Upper endoscopy may detect acid peptic injury in cases of severe dyspepsia or heartburn. Liver and pancreatic chemistries and imaging tests such as abdominal ultrasonography or computed tomography are obtained if hepatobiliary or pancreatic disease is suspected. Computed tomography enterography or capsule endoscopy can detect atypical presentations of Crohn's disease, whereas gastric scintigraphy or gastrointestinal manometry may be obtained in the patient with associated nausea and vomiting. Exclusion of intestinal bacterial overgrowth may be obtained with hydrogen breath testing. Rarely, patients will need laboratory testing to exclude lead toxicity or porphyria.

Patient outcome in irritable bowel syndrome

Most long-term studies of IBS report that symptoms persist for more than 5 years in more than 75% of patients despite appropriate therapy. Only 34 of 103 patients were symptom-free on long-term follow-up in one study [447]. A second investigation followed 50 patients with IBS at 2-month intervals for 12–31 months [448]. In 44 patients, the qualitative nature of the symptoms did not change with long-term follow-up although symptom severity was variable. More recent studies indicate that not infrequently patients with IBS may change their bowel habits from diarrhea to constipation or vice versa. A follow-up of 4581 individuals with IBS reported that only 5% were symptom-free after 5 years [449]. This lack of significant improvement in patients with IBS may not hold true for all populations with IBS symptoms. In a study of elderly Danes, 50%–79% of subjects suffering from IBS on initial inquiry no longer experienced symptoms at the 5-year follow-up [450].

Even though symptoms persist in many patients with IBS, their quality of life can be enhanced by appropriate physician intervention. In one study, 18 of 44 patients noted reduced symptom intensity after 12–31 months of continued medical follow-up [448]. Of 34 fully employed patients, 20 lost no work in the first year after diagnosis. Only seven patients lost more than 2 weeks because of their symptoms, as a consequence of improved coping skills. A second investigation of 43 patients with IBS reported that abdominal pain and the overall sense of well-being were reduced after 5 years of follow-up but other symptoms were unchanged [451]. Those who had improved exhibited reduced anxiety compared to those who had not. Symptom resolution was observed in 20% of 97 patients with IBS who were studied for 5–7 years on a treatment program of antispasmodic drugs, bulking agents, and high-fiber diets and significant symptom reduction was reported in an additional 42% of individuals [447]. Patients who were male, had a short history of symptoms, had a history of recent acute onset of symptoms, exhibited predominant constipation, and noted a good initial response to treatment were most likely to achieve good long-term

improvement. Other studies have found that reductions in anxiety and fear of cancer correlate with improvement over a 6-month period [452]. On the other hand, a recent retrospective study found no independent association between a negative colonoscopy and reassurance or improved health-related quality of life in patients with IBS younger than 50 years [453]. In the longest follow-up reported to date (median 29 years), fewer return visits to physicians were reported for patients who had undergone inquiry about psychosocial issues and precipitating factors and who had been given detailed discussions about their diagnosis and treatment [284]. These studies suggest that counseling, reassurance, and education provided by the physicians, judicious use of medications, and continued interest in the patients' well-being may promote successful outcomes in many patients with IBS.

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D COLON

61 Diverticular disease of the colon

Tonia M. Young-Fadok, Michael G. Sarr

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A diverticulum (plural: diverticula) is a sac-like protrusion of the colonic wall. The spectrum of diverticular disease encompasses diverticulosis, diverticulitis, and diverticular bleeding. *Diverticulosis* describes the presence of diverticula. *Diverticulitis* refers to symptomatic inflammation of a diverticulum. "Complicated" diverticulitis refers to a diverticular abscess, fistula, small bowel or colonic obstruction, or free intraperitoneal perforation; "simple" diverticulitis is inflammation in the absence of these complications.

This chapter initially discusses the epidemiology, etiopathogenesis, pathophysiology, and natural history of diverticular diseases of the colon. A detailed discussion of the clinical presentation, diagnosis, and treatment follows, concentrating on the specific diverticular entities and addressing special situations dictating a more individualized approach. Attempts have been made to identify and discuss controversies.

Epidemiology

The epidemiology of diverticular disease of the colon has changed markedly since its initial recognition as a disease entity at the beginning of the 20th century. Indeed, the first reported surgical resection for complicated diverticulitis was performed by W. J. Mayo and colleagues in 1907 [1]. The apparent prevalence of diverticulosis increased from 5%–10% in 1918 [2] to 35%–50% in an autopsy series from 1969 [3]; there are no good recent, population-based studies. The prevalence of colonic diverticula increases with age from about 5% at 40 years of age, to 30% at age 60, and 65% by age 85 [4,5].

The predominance of males reported in early series has changed to either equal distribution or to a slight female preponderance in more contemporary studies [4]. In a study of 934 patients admitted to a single major medical center between 1988 and 1999, 63% were female [6]. The sex distribution appears also to vary with age. In one series, 60% of symptomatic patients were women [7] but, in patients under 50 years, men predominated with a male to female ratio of 2.2 : 1. In several other reports, male preponderance has also been noted in patients less than 40 years of age [8,9].

Geographic variations also exist both in the prevalence and anatomic pattern of diverticulosis. Westernized countries have prevalence rates of 5%–45%, depending on the method of diagnosis and age of the population [3,10]. Diverticular disease in these countries is predominantly left-sided, with right-sided diverticulitis present in only about 2% of symptomatic patients [11]. This distribution differs markedly in Africa and Asia [2,12], where the prevalence of diverticulosis is less than 0.2%, and diverticulitis is usually right-sided. With Japan and Singapore adopting a Western lifestyle, these cultures have experienced an increase in the prevalence of diverticulosis [13,14]. A review of 13 947 barium enema examinations performed over a 15-year period in Tokyo, Japan, revealed a steady increase in the incidence of diverticulosis of the right colon [15]. Somewhat surprisingly it remains right-sided [3]; moreover, 70% of symptomatic patients are less than 40 years old [14]. In Hong Kong, diverticulosis involves the right colon in 76% of patients [16], and right-sided diverticulitis accounts for 17% of patients with acute diverticulitis [17], a much greater percentage than in Western cultures.

Etiopathogenesis

Painter and Burkitt first speculated that low dietary fiber is a major factor predisposing to development of diverticulosis [5,18]. They recognized that where African natives had a diet high in dietary fiber and produced a large volume of daily stool output, diverticulitis was distinctly unusual. This concept, however, remains controversial [12,19,20]. One study of a cohort of 47 000 men provided strong evidence for a

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primary role of lack of fiber in the etiopathogenesis of left-sided diverticulosis [21]; a diet low in fiber content increased the risk of symptomatic diverticular disease. Diverticular disease is also less common in vegetarians than nonvegetarians [22]. Of related interest, a low-fiber diet may be a common etiological factor in the development of both diverticulosis and colon cancer. In one report of 7000 patients, an excess number of colon and rectal cancers was noted in the first 2 years after diagnosis of diverticular disease [23], an incidence not explained solely by investigations performed for the evaluation of diverticular disease.

Other dietary factors have been examined. There is no substantially increased risk associated with smoking or caffeine or alcohol intake [24]. The recognized association of acute diverticulitis with obesity in men under 40 years of age may be associated with observations that a high intake of total fat or red meat plus a diet low in fiber particularly augments the risk of symptomatic diverticular disease [21]. Whether fat and red meat are independent risk factors or merely markers of a lower fiber diet remains speculative.

Pathophysiology

Pathology

The typical colonic diverticulum is a false or pulsion diverticulum; that is, it does not contain all layers of the wall, as is the case in a true (congenital) diverticulum. Mucosa and submucosa herniate through the muscle layer of the colon, separated from the intraperitoneal space only by the serosa. Diverticula develop at the four constant points of the colonic circumference where the vasa recta penetrate the circular muscle layer [25]. These vessels enter the wall of the colon on each side of the mesenteric taenia and on the mesenteric border of the two antimesenteric taeniae (Fig. 61.1). Diverticula do not develop in the rectum; presumably this is in some way related to the coalescence of taeniae into a longitudinal muscle layer.

Diverticula are distributed unevenly throughout the colon. In Western cultures, 95% of patients with diverticula have sigmoid diverticula. Diverticula are limited to the sigmoid colon in 65% of patients, whereas, in 24%, diverticula involve predominantly the sigmoid but with other segments of the colon involved to a lesser degree; in 7% of patients the distribution is equally divided throughout the colon, whereas in only 4% are diverticula limited to a segment proximal to the sigmoid [26]. Similarly, 95% of all operations for diverticular disease are directed at the sigmoid colon [7].

Pathophysiology of diverticular disease

Diverticulosis

The points at which diverticula develop, i.e., where the vasa recta penetrate the colon wall [27], are considered to be areas of potential weakness within the wall of the colon. Most patients with sigmoid diverticula exhibit grossly evident myochosis, that is, thickening of the circular muscle layer, shortening of the taeniae, and lumenal narrowing. Although there is no apparent hypertrophy or hyperplasia of the muscular layer of the colonic wall, there is increased deposition of elastin in the taeniae [28]. Structural changes in collagen

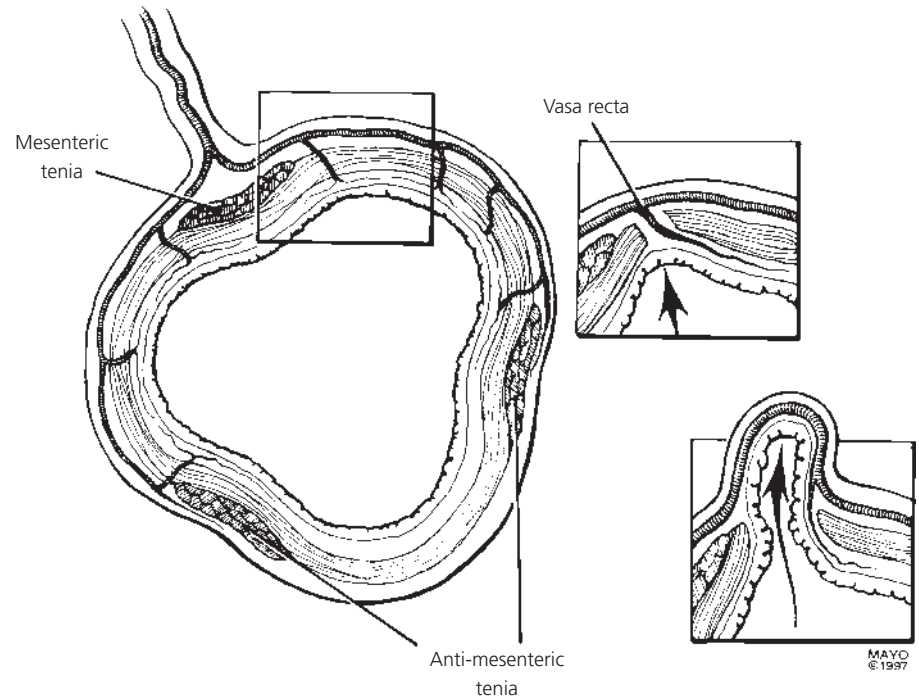


Figure 61.1 Cross-section of the sigmoid colon. The main illustration indicates the points of penetration of the vasa recti around the bowel circumference. **Inset:** The development of a diverticulum at one such point of weakness. By permission of Mayo Foundation for Medical Education and Research. All rights reserved.

content of the wall of the sigmoid colon are similar to those resulting from aging but are greater in magnitude [29] and may decrease the resistance of susceptible segments of the wall to abnormal increases in intralumenal pressure. Reduced levels of mature collagen type I and increased levels of collagen type III have been demonstrated in tissue from individuals with diverticulosis, in addition to reduced expression of matrix metalloproteinase-1 compared with normal controls [30]. Changes in the tissue collagen levels of patients with connective tissue disorders, such as the Ehlers–Danlos and Marfan syndromes, may explain the appearance of diverticula at an early age.

Application of the principles of the law of Laplace may explain, in part, the development of colonic diverticula. The law states that pressure (P) is proportional to wall tension (T) and inversely proportional to radius (R) of the colon – that is, $P = kT/R$ (where k is a conversion factor). Normally intralumenal pressure is the same throughout the colon. However, segmentation of the colon (a motility process in which proximal and distal segmental muscular contractions separate the lumen into isolated chambers) is believed to be exaggerated in diverticulosis. Strong occlusive contractions at both ends of the chamber lead to marked segmental increases in intralumenal pressures (Fig. 61.2) [31], which may predispose to herniation of mucosal diverticula at the sites where the blood vessels penetrate the muscular layers of the colon. Because the sigmoid colon is the segment of the colon with the smallest diameter, it may also be the site of the greatest intralumenal pressure. Whether the thickened taeniae of the sigmoid colon are a result of the environmental milieu, such as a lower fiber diet and less bulky stools (and a resultant less wide colonic diameter), a response to increased intralumenal pressure, a primary genetic variation in muscle structure and function, or an acquired abnormality from some other as yet undefined cause, remains unknown.

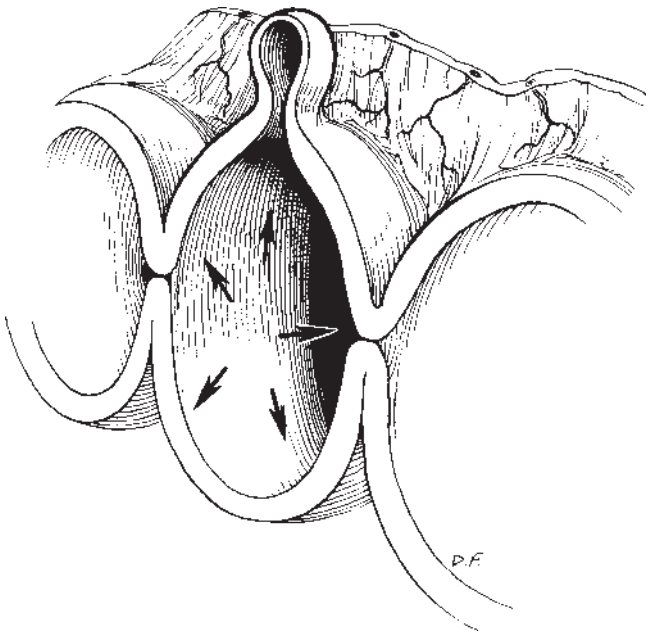


Figure 61.2 The concept of Painter and Burkitt of segmentation causing formation of pulsion diverticula.

The development of diverticula may result from disorders of both bowel-wall structure and motility. Golder and colleagues suggested a mechanism whereby structure and function may be tied together [32,33]. They described reduced smooth muscle choline acetyltransferase activity, up-regulation of M3 receptors, and increased in vitro sensitivity to acetylcholine in the sigmoid colon of patients with diverticulosis, i.e., the possibility of a cholinergic defect in diverticulosis. They postulated that increased expression of M3 receptors is a response to reduced cholinergic innervation and that this increased expression maintains normal tone, until there is external stimulation resulting in increased motility responses. Differential deposition of elastin in the taeniae may result in an inability of this layer to contract in response to acetylcholine, leading to uncoordinated contractions between circular and longitudinal muscle layers and the subsequent outpouching of diverticula.

Diverticulitis

Inflammation of the wall of the diverticulum and the surrounding peridiverticular tissue has a range of clinical manifestations from subclinical inflammation to generalized peritonitis. The underlying cause of the inflammation is thought to involve a micro- or macroscopic perforation of this thin-walled diverticulum. It was believed previously that obstruction of the ostia of the diverticula, for example, by fecoliths or lumenal material, led to increased intradiverticular pressure and subsequent perforation; this occurrence is thought to be rare [12]. Any physician who performs flexible sigmoidoscopy or colonoscopy routinely has seen numerous diverticula containing fecoliths – not one of which is inflamed. Increased intralumenal pressure in the involved segment of the colon secondary to abnormal motor activity or local trauma of inspissated fecoliths may erode the wall of the diverticulum. Inflammation and focal necrosis result in perforation. Frequently, a small perforation becomes walled off and contained by pericolic fat and mesentery, resulting in the formation of a localized abscess or resolution without further consequence. In contrast, with a more extensive inflammatory reaction, surrounding hollow organs adhere to the inflamed segment of colon, and intestinal obstruction or an internal fistula may develop. Poor containment, as may occur in antimesenteric diverticula, results in free intraperitoneal perforation and peritonitis.

In summary, diverticula occur at weakened points in the intraabdominal, taenia-bearing colon where intramural vessels penetrate the muscular layer. Development of diverticula appears to be multifactorial. Localized increases in intralumenal pressure may be caused by abnormal motor

patterns that predispose to exaggerated segmentation and are likely exacerbated by a low-fiber diet. Morphological changes in the muscle and collagen content of the bowel wall lead to decreased tensile strength of the wall; these changes are similar to, but more pronounced than, those that occur with age. Local trauma to the wall of the diverticulum may produce inflammation, resulting in either localized perforation with peridiverticulitis or free intraperitoneal perforation with generalized peritonitis. Similarly, local trauma to the vasa recti within the diverticula can predispose to hemorrhage.

Natural history

Understanding the natural history of diverticular disease is critical in determining which patients will develop complications if left untreated (and who are therefore appropriate candidates for operative intervention) and those who are best served by a more conservative approach.

Diverticulosis

Of patients with significant diverticulosis, 70% remain asymptomatic and never develop complications related to their diverticulosis. With long-term follow-up, however, as many as 15%–25% of patients with diverticulosis will develop acute diverticulitis and 5%–15% will exhibit diverticular bleeding (Fig. 61.3). As the majority of patients remain asymptomatic, the mere presence of multiple diverticula does not indicate the need for intervention other than possibly recommending a high-fiber diet in an attempt to decrease the incidence of complications.

Diverticulitis

Diverticulitis may present with manifestations ranging from mild abdominal discomfort to perforated diverticulitis with fecal peritonitis, sepsis, and even death. Diverticulitis can be divided into simple and complicated presentations (Fig. 61.4). Complicated diverticulitis describes localized or free intraperitoneal perforation, obstruction, abscess, or fistula, which arise in 25% of patients with their first episode of acute diverticulitis; nearly all of these patients will eventually require operative treatment. Simple diverticulitis accounts for 75% of episodes and refers to inflammation alone in the absence of complications; the majority of these patients respond rapidly to medical therapy.

Of those who require operative intervention, uncomplicated diverticulitis is the indication in approximately 40%, and the various manifestations of complicated diverticulitis account for the other 60% [6]. In a study of 934 patients hospitalized between 1988 and 1998 at a single medical center with proven diverticular disease requiring operative resection, the following were the indications for operative intervention: chronic diverticulitis 35%, perforation 18%, fistulae 16%, abscess 8%, obstructive symptoms 6.5%, stricture 6%, massive rectal bleeding 5%, and acute diverticulitis 4% [6].

Simple diverticulitis

Most patients with simple diverticulitis are initially treated conservatively (see Fig. 61.4); 85% respond rapidly, but 15% will require operative intervention [34]. Long-term follow-up of patients after their first episode of acute diverticulitis suggests a readmission rate of 2% per patient year [34] so elective colectomy is not necessary for all patients who respond to medical therapy. Indeed, after successful conservative therapy of the first attack of diverticulitis, 30%–40% of patients remain asymptomatic, 30%–40% have episodic abdominal cramps without frank diverticulitis, and about one-third proceed to a second attack of acute diverticulitis [4].

Older data suggest that the prognosis is worse with a second attack, because the rate of complicated diverticulitis

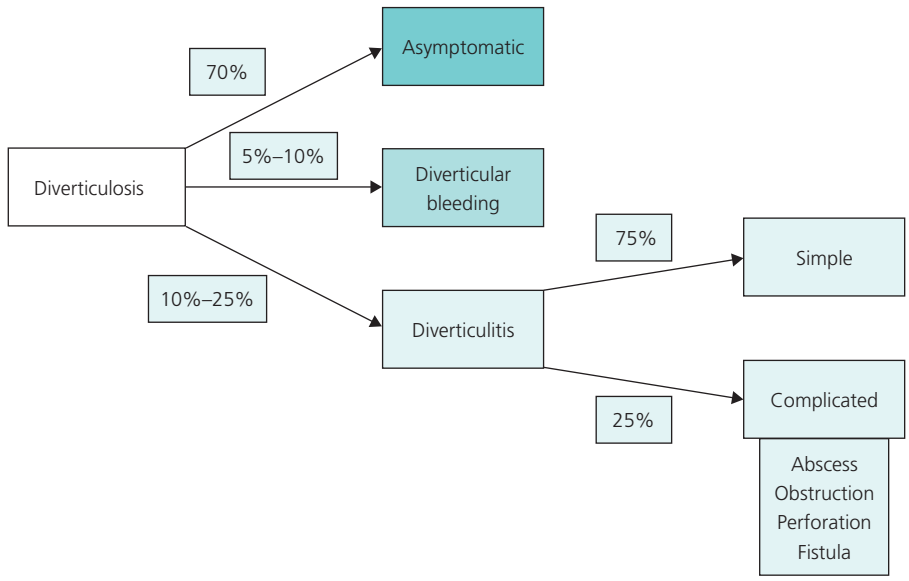


Figure 61.3 The natural history of diverticulosis. From Young-Fadok & Pemberton [149].

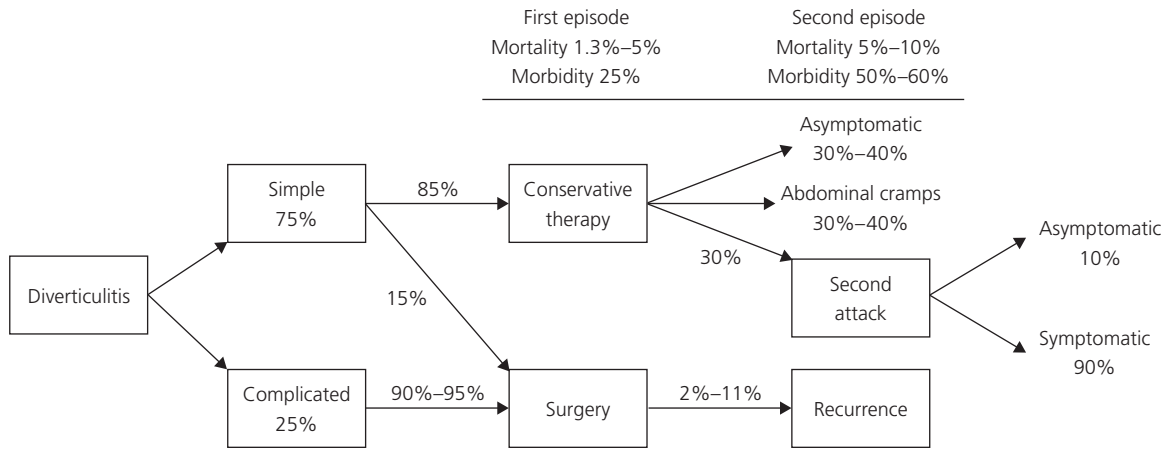


Figure 61.4 The natural history of diverticulitis. From Young-Fadok & Pemberton [149].

approached 60%, and the mortality rate doubled [4,35]. Only 10% of these patients remained asymptomatic after nonoperative resolution of a second attack of acute diverticulitis. These older studies form the basis for the current recommendation that resection should be recommended after two confirmed attacks of diverticulitis, with one episode of sufficient severity to merit hospitalization. This dogma has been challenged. Chautems and colleagues reported on 110 patients treated conservatively for a first episode of diverticulitis; 83 had complete follow-up, and 15 patients (18.3%) had one recurrence and only six (7.3%) had two or more episodes of recurrence [36]. Nine of these patients required inpatient therapy. Two patients underwent operation, and no emergency procedure was required. The authors concluded that recurrent diverticulitis may be treated in a similar fashion to a first episode and that medical therapy remains a valuable alternative to resection. This study was useful in having long median follow-up of 10.5 years, but the conclusions were hampered by a high death rate of 33.7% from unrelated causes during follow-up. Anaya and colleagues employed a statewide database to identify all patients hospitalized nonelectively for diverticulitis [37]. A total of 25 058 patients were hospitalized with an initial attack of diverticulitis. Of 20 136 not requiring initial operation, 19% had recurrences. Patients younger than 50 years were more likely to have a recurrent attack (27% vs 17%). Young age and increasing number of recurrent events were associated with the risk of emergency colectomy or colostomy after initial successful nonoperative treatment. Similarly, a study of 3165 patients confirmed that after nonoperative management of an initial episode the recurrence rate was only 13%, and age less than 50 years and a first recurrence were factors that predicted further recurrence [38].

Complicated diverticulitis

Most patients with complicated diverticulitis require operative intervention (Fig. 61.4). With the first attack of acute diverticulitis, 15%–29% require operative treatment [34,39], most of whom have complicated diverticulitis. The mortality rate is 1.3%–5% [34]. Indications for emergency or early operative intervention are generalized peritonitis, nonresolving intestinal obstruction, abscess that is not amenable to radiologically guided percutaneous drainage, clinical deterioration, or failure to improve with appropriate conservative management (Table 61.1). Indications for elective operative intervention are recurrent or intractable symptoms, persistent mass, inability to exclude carcinoma, functional colonic obstruction, presence of a fistula, and previous percutaneous drainage of an abscess. Several unique situations are discussed below.

Those who are treated operatively are generally thought of as cured even though the standard extent of resection may leave diverticula in the remaining proximal colon. Diverticulosis progresses in the remaining colon in about 15% of patients [40], and future operative treatment is required in as many as 2%–11% [40–42]. Up to 27% of patients after a colectomy may describe abdominal pain postoperatively in the same location; these persistent symptoms, however, may be secondary to coexistent irritable bowel syndrome rather than to recurrent diverticulitis.

Table 61.1 Indications for operative treatment of acute diverticulitis

Absolute	Relative
Complications of diverticulitis	Symptomatic stricture
Peritonitis	Immunosuppression
Abscess (failed percutaneous drainage)	Right-sided diverticulitis
Fistula	? Young patient
Obstruction	
Clinical deterioration/failure to improve	
Recurrent episodes	
Intractable symptoms	
Inability to exclude carcinoma	

Diverticular diseases

Diverticulosis

Diverticulosis in the majority of patients is asymptomatic. Some patients with uncomplicated diverticulosis have symptoms of cramping, bloating, flatulence, and irregular defecation; it is unclear if these symptoms are related directly to the underlying diverticulosis or to the coexistence of irritable bowel disease. Older patients with this symptom complex are investigated with a contrast enema, and the prevalence of diverticulosis in the elderly is so common that this ostensible association of symptoms and diverticulosis has often been made, but a true causal relationship is unproven.

Diverticulitis

Presenting symptoms

Constant, noncolicky, left lower quadrant pain is the most common complaint occurring in 70% of patients. The pain has often been present for several days before presentation, which helps to differentiate diverticulitis from other causes of an acute abdomen; only 17% of patients have symptoms for less than 24 h [7]. When carefully questioned, almost half the patients describe previous episodes of similar, although less intense, pain. Nausea and vomiting occur in 20%–62%, diarrhea coexists in one-fourth to one-third of patients [43], and recent obstipation has been present in one-half. Urinary symptoms, such as dysuria, urgency, and frequency, coexist in 10%–15%.

Presenting signs

Physical examination reveals abdominal tenderness, usually in the left lower quadrant. A tender mass is palpable in 20% [4]. Right lower quadrant tenderness may result from a redundant inflamed sigmoid colon lying to the right of the midline or, less frequently, from right colonic diverticulitis. Generalized peritoneal signs suggest free perforation and peritonitis. Abdominal distention occurs in two-thirds of patients. Low-grade fever and mild leukocytosis are common but not always present; 14% of patients have no fever, and 45% have a normal white count [44]. Urinalysis may reveal a sterile pyuria apparently secondary to adjacent inflammation; mixed colonic flora, fecaluria, or pneumaturia are pathognomonic of a colovesical fistula. Similarly, fecal discharge from the vagina is strongly suggestive of a colovaginal fistula.

Diagnosis

The diagnosis of diverticulitis should be suspected and made primarily on the basis of the history and physical examination. In the acute stage, when complicated diverticulitis is suspected, imaging studies can confirm the diagnosis and exclude other sources of acute abdominal signs; in contrast, elective evaluation after resolution of an acute episode of diverticulitis should evaluate the whole colon.

Diagnosis in the acute setting

Abdominal radiographs are useful and appropriate in excluding other causes of acute abdominal pain, rather than in making the diagnosis of diverticulitis. In addition, the presence of free air on the abdominal radiograph signifies an abdominal emergency and obligates further evaluation. The diagnosis of acute diverticulitis can be made frequently on the basis of the history and physical examination alone. Older published guidelines recommended that when the clinical picture was clear then additional tests were not necessary to make a diagnosis [45–47]. This may, however, have been a disservice to the patient. A clinical diagnosis alone may be incorrect in up to one-third of patients [48]. In addition, should there be additional unconfirmed attacks, the patient may potentially be put at risk when surgical management is refused, whereas had there been evidence of an earlier attack, an operative resection might have been prudent and the decision to proceed with resection would have been possible. In the patient who has symptoms of sufficient severity to merit hospitalization, our preference is to obtain radiographic confirmation with a computed tomography (CT) scan [49]. This is reinforced by more recent guidelines which suggest that CT of the abdomen and pelvis is usually the most appropriate imaging modality in the patient with suspected diverticulitis [50]. After an episode of uncomplicated acute diverticulitis, complete evaluation of the colon can be performed electively.

The immediate use of CT is not controversial if the diagnosis is not secure or if complicated acute diverticulitis is suspected. Computerized tomography has become the investigation of choice for diagnosis, recognition of complicated disease (abscess, fistula, obstruction), potential therapeutic intervention, and quantifying resolution of disease. Ambrosetti and colleagues performed a prospective comparison of CT and water-soluble contrast enema in 420 patients over an 11-year period [51]. Among 136 patients who subsequently underwent colonic resection, 132 had histologically proven diverticulitis. The CT was more sensitive than contrast enema (98% vs 92%) and identified all of those patients who had an associated abscess, whereas contrast enema only indicated indirect evidence of complicated diverticulitis in 29% of these patients. Helical CT with rectal contrast has been shown to have a sensitivity of 97% and specificity of 100%, similar to studies using oral contrast [52]. This technique may avoid the risks, discomfort, and costs of oral and intravenous contrast and allow for immediate scanning.

The CT features of acute diverticulitis (Fig. 61.5) include increased soft tissue density in pericolic fat secondary to inflammation in 98%, air-filled diverticula in 84%, bowel wall thickening in 70%, soft tissue masses representing a pericolic phlegmon, and pericolic (usually intramesenteric) fluid collections representing abscesses in 35% [53,54]. In 10% of patients, acute diverticulitis cannot be distinguished from carcinoma, as both may show focal thickening of the bowel wall; features more suggestive of acute diverticulitis

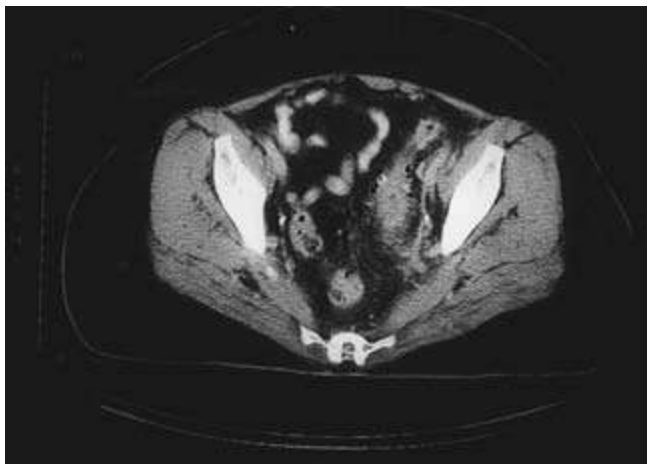


Figure 61.5 Computed tomography with oral and intravenous contrast in a 59-year-old woman with increasing abdominal pain for 3 weeks. Mild changes of diverticulitis are seen with narrowing of colonic lumen, bowel wall thickening in the midsigmoid, tissue stranding in pericolic pelvic fat, and diverticula. There is no pericolic fluid collection.

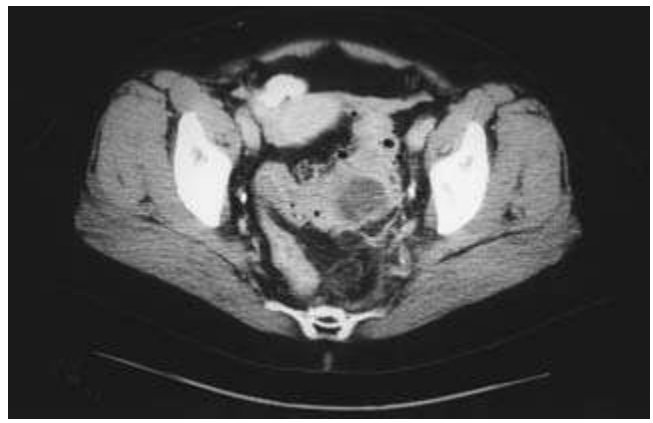


Figure 61.6 Computed tomography with oral and intravenous contrast in a 60-year-old woman with a 2-week history of crampy lower abdominal pain, fever, and chills showing a low-density mass adjacent to the sigmoid in the left pelvis. The central, low-density region within the mass, the pockets of surrounding gas, and inflammatory changes in pericolic tissues are consistent with diverticular disease.

are fluid at the base of the mesentery, edematous thickening of the mesentery, and mesenteric vascular engorgement [55]. Others have found additional features more suggestive of diverticulitis than carcinoma: presence of noninflamed diverticulum, inflamed diverticulum, mild degree of enhancement, and small lymph node size; when colonic obstruction is present colon cancer is more likely than diverticulitis [56]. A CT can identify complicated diverticulitis – i.e., peritonitis (diffuse inflammatory changes and scattered, loculated fluid collections), fistula formation (usually inferred from air or rectally administered contrast in bladder, vagina, or abdominal wall, rather than direct visualization of the fistulous tract), and obstruction. It correctly predicts Hinchey stage in more than 90% of cases [57], because it stages more clearly the extent of extralumenal inflammation, which is underestimated by contrast enema [51,54]. Findings on CT can be classified as mild disease (localized colonic wall thickening and inflammation of pericolic fat [see Fig. 61.5]) or severe (abscess, extralumenal air- or water-soluble contrast [Fig. 61.6]); the latter findings have been employed as criteria for elective resection in patients responding initially to medical therapy with resolution of acute clinical symptoms [58]. In addition, CT functions as a therapeutic modality when combined with percutaneous drainage of localized abscesses in selected patients; this approach avoids the need for emergent operative intervention and permits an elective, single-stage resection [59]. Computed tomographic colonography may also indicate the presence of the characteristic muscular thickening of the bowel wall associated with diverticulosis, and also demonstrates significantly less sigmoid colon distensibility compared with normal colons [60].

Contrast radiography or colonoscopy generally should be avoided in patients with suspected acute diverticulitis because of concerns that overzealous infusion of even water-soluble contrast or insufflation of air may lead to rupture of previously contained diverticulitis. However, a single-contrast enema using water-soluble contrast is safe in the acute phase if performed carefully. Pneumoperitoneum or evidence of generalized peritonitis should be absolute contraindications to the use of barium as the contrast agent [61]. Some physicians prefer a water-soluble contrast enema, even in the absence of these findings, in any patient suspected of having acute diverticulitis in the event that there is an unsuspected leak (Fig. 61.7). Contrast enema has the advantage of evaluating the colonic lumen, is less expensive and more readily available than CT, and in some practices is the initial procedure of choice; in the patient in whom the diagnosis of uncomplicated acute diverticulitis is secure, however, contrast enema examination adds little therapeutically useful information.

Magnetic resonance colonography may offer an alternative means of diagnosis. Features suggestive of diverticulitis are increased wall thickness, focal uptake of contrast material, and pericolonic reaction including mesenteric infiltration on T1-weighted images [62]. It has been found to be equally helpful in establishing the diagnosis of right-sided diverticulitis and may be a valuable alternative to CT in young or pregnant patients [63].

High-resolution, graded-compression ultrasonography may offer an innovative method to evaluate acute diverticulitis in experienced hands. Visualization of an abnormal colonic segment, as evidenced by mural thickening greater than

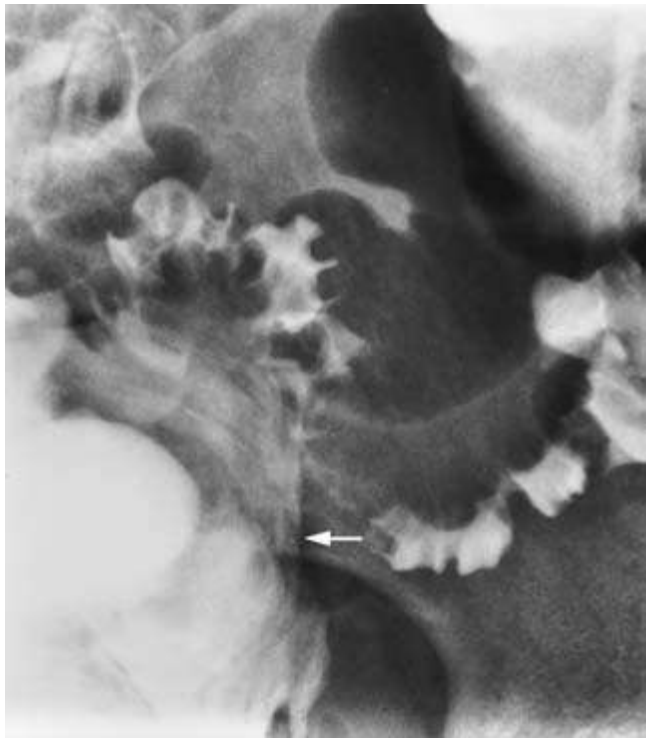


Figure 61.7 Hypaque enema in a 79-year-old woman shows nonanatomic distribution of contrast (*arrow*) around the rectum, demonstrating perforation.

4 mm and involving a segment 5 cm or longer at the point of maximal tenderness, is found in 85% of patients and represents the most specific sonographic feature [64]. In cross-section, the thickened colon has the appearance of a target (Fig. 61.8). Inflamed diverticula, mural abscess, extraluminal gas bubbles, peridiverticular abscess, and inflammation may be visualized. Reported sensitivities of ultrasonography range from 85% to 98%, with specificities of 80%–98% [64,65], but these values are undoubtedly operator dependent. Although ultrasonography is less widely accepted and validated in the evaluation of diverticulitis than CT, it may be a useful additional tool – for instance in following the resolution of an abscess, or in allowing percutaneous, transrectal, or transvaginal drainage of an abscess.

Diagnosis in the elective setting

After resolution of acute diverticulitis, evaluation requires imaging of the entire colon to determine the extent of disease and to rule out coexistent lesions, such as polyps or carcinoma. Pancolonic imaging may be achieved with colonoscopy or flexible sigmoidoscopy plus barium enema. An investigational method being developed, virtual colonoscopy (also colography or colonography) (Fig. 61.9), may prove to be useful in the elective setting. This technique employs air insufflation of the colon and rapidly acquired CT images, which allow computer generation of a three-dimensional image of the colonic lumen. On occasion, diverticulitis may lead to stricture formation with the characteristic appearances of carcinoma; this finding requires further intervention as for suspected colon cancer.

Treatment

Decision to observe or operate

Treatment must be individualized for each patient; correct initial therapy depends on clinical acumen (see Table 61.1). The spectrum of disease can be divided into mild symptoms,

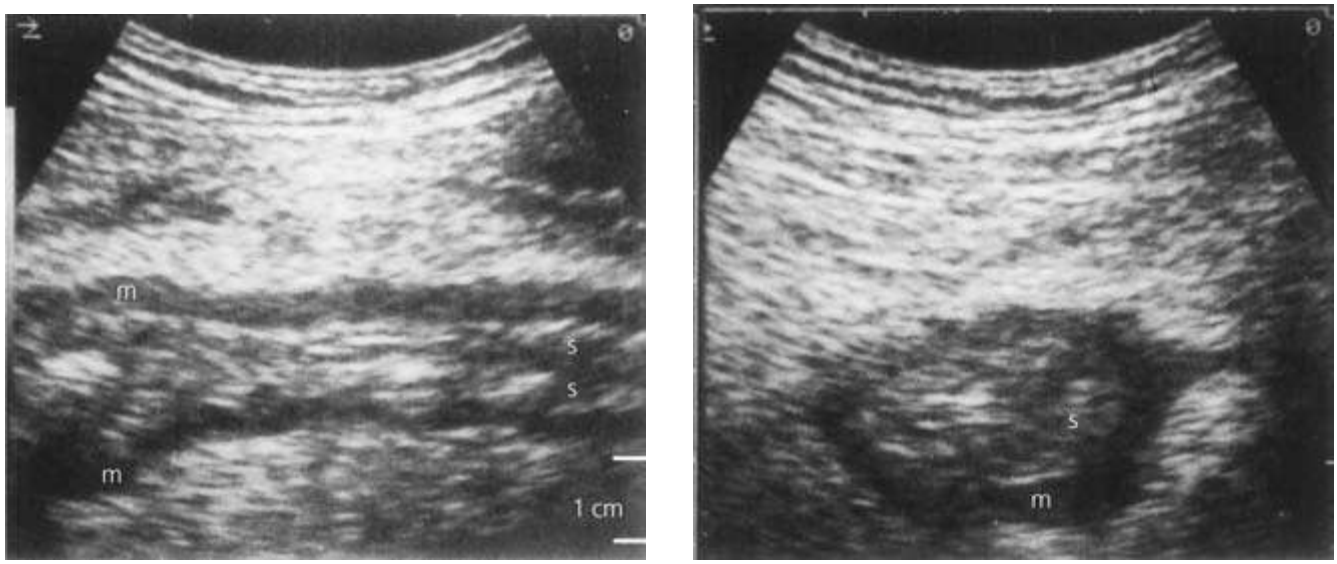
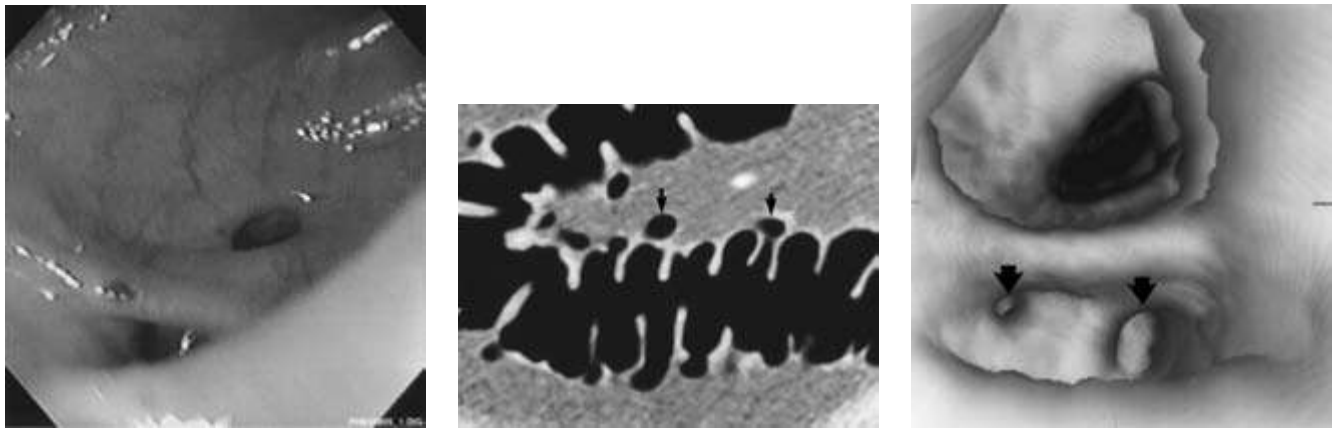


Figure 61.8 Ultrasonographic findings of sigmoid diverticulitis. Longitudinal and transverse sections showing thickening of the entire wall and the hypoechoic muscularis propria (m), with lumenal narrowing. s, mucosa/submucosa. From Schwerk et al. [65], with kind permission of Springer Science and Business Media.



(a) (b) (c)

Figure 61.9 Virtual colonoscopy. **(a)** View of diverticula as seen on routine colonoscopy. **(b)** Two-dimensional image of diverticula (*arrows*) obtained by spiral computed tomography scan. **(c)** Three-dimensional reconstruction (virtual colonoscopy) illustrating diverticula (*arrows*). From Young-Fadok & Pemberton [149].

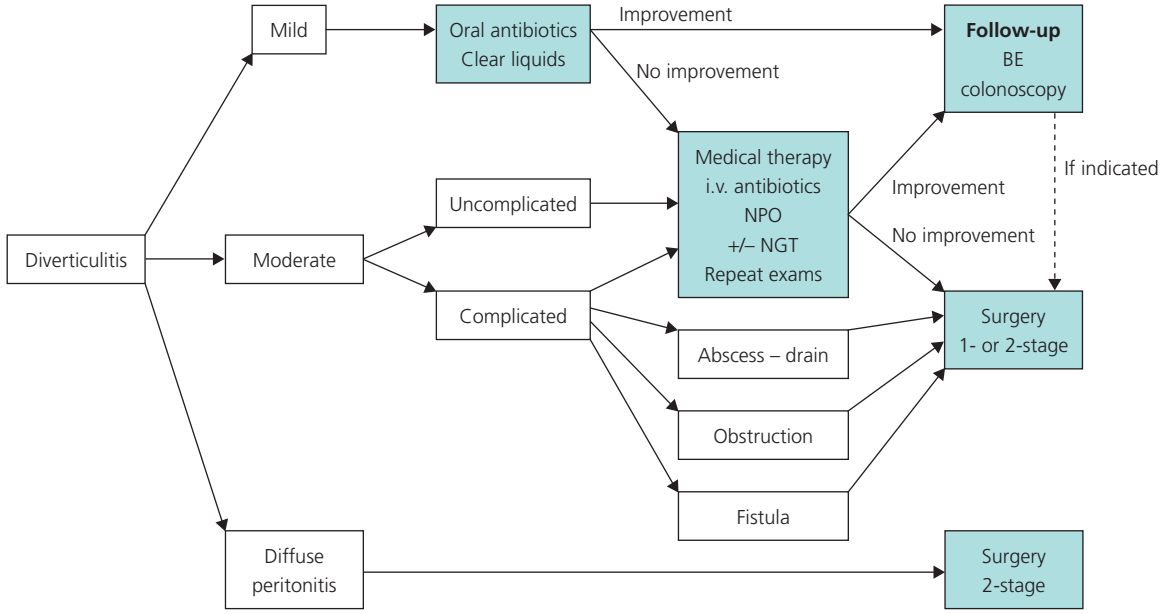


Figure 61.10 Algorithm for treatment of acute diverticulitis.

moderate to severe symptoms, and diffuse peritonitis (Fig. 61.10). The first and last groups require little decision-making regarding operative intervention; the second group is the more challenging.

Mild symptoms

Patients with mild diverticulitis are characterized by left lower quadrant pain, low-grade fever, and minimal physical findings without peritonitis. These patients are best treated as outpatients with a liquid diet and broad-spectrum oral antibiotics such as ciprofloxacin or metronidazole. Exceptions include immunosuppressed patients and other high-risk groups, such as patients with diabetes or the elderly. Patients can be followed for increasing fever, pain, or inability to tolerate oral intake. Hospitalization is only necessary if the patient fails to improve. Diet is reintroduced as symptoms resolve. Once the episode has resolved, the colon should be evaluated, usually 6 weeks later to allow residual inflammation to resolve. After symptoms have resolved, long-term therapy with fiber is generally recommended as one of the few interventions that may alter the risk of recurrence. There has also been some interest in the use of mesalazine to reduce the risk of recurrent symptoms. One study, using a scoring system that included such variables as abdominal pain, bloating, tenesmus, diarrhea, tenderness, and fever, found that mesalazine reduced symptoms at 3 months follow-up

compared with rifaximin [66]. A randomized trial from Italy comparing long-term use of rifaximin alone with rifaximin plus mesalazine, found that the latter combination was more effective in producing resolution of symptoms and prevention of recurrent episodes [67]. Another interesting randomized study has indicated that administration of an oral immunostimulant, a highly purified, polymicrobial lysate, to patients with at least two attacks of diverticulitis was effective compared with placebo in reducing the risk of recurrent attacks [68]. The combination of mesalazine and *Lactobacillus casei* has been shown in a randomized trial to be effective in preventing recurrence of symptomatic uncomplicated diverticulitis [69].

Moderate to severe symptoms

This patient group can be distinguished by the presence of more severe, left-sided abdominal pain, fever, chills, obstipation, and left lower quadrant peritoneal irritation. Classic symptoms do not initially require advanced diagnostic imaging, and medical therapy is instituted on the basis of a clinical diagnosis [70]. When the diagnosis or extent of disease is in doubt, CT or water-soluble contrast enema should then be considered. In stable, immunocompetent patients, initial treatment is conservative with bowel rest, intravenous fluids, and broad-spectrum parenteral antibiotics. Antibiotics need to be active against gram-negative rods and anaerobes; a single antibiotic active against both aerobes and anaerobes appears to be as effective as combination therapy, for example, a second-generation or third-generation cephalosporin, or an aminoglycoside combined with an anaerobically active agent such as metronidazole or clindamycin [47,71,72]. The aim is to obtain a clinical response to avoid the need for early, nonelective operative intervention. Except for patients with generalized peritonitis, this approach is usually successful. A CT may help to identify patients who are likely to respond to medical therapy [59,73].

Three outcomes are possible: improvement, failure to improve, or deterioration. Clear improvement should occur within 24–48 h, with a decrease in pain, fever, tenderness, and leukocytosis [7,12,74]. Oral intake can be resumed once abdominal tenderness has resolved. Workup is completed 2–6 weeks after the acute phase has settled either with colonoscopy or flexible sigmoidoscopy combined with barium enema. Fiber supplementation of the diet may prevent recurrence in 70% of patients [74].

If there is no objective improvement, the diagnosis may be incorrect or complicated diverticulitis is present. A CT should be obtained. Abscesses amenable to treatment should be drained percutaneously at the time of the CT. Operative intervention is necessary if there is no reversible condition, if abscesses cannot be drained, or if drainage does not result in improvement. If a patient's condition deteriorates and there is no treatable condition visible on CT, operative exploration should proceed without waiting.

Peritonitis

Diffuse peritonitis mandates initial resuscitation, broad-spectrum parenteral antibiotics, and operative intervention; diagnostic studies are rarely necessary. Perforated diverticulitis is a serious disease carrying a mortality rate of 6% for purulent peritonitis and 35% for fecal peritonitis [75].

Which operation – surgical principles

The surgeon's aims are to remove the septic focus by resecting the involved segment of colon, to treat the associated obstruction or fistula, and, if possible, to restore bowel continuity, while minimizing morbidity and mortality.

Preoperative preparation

In nonemergency situations, mechanical bowel preparation with or without oral antibiotics is usually possible and may decrease the rate of wound infection. Patients should be warned of the possibility of a stoma and the abdomen should be marked for optimal stoma placement preoperatively, taking into consideration body habitus, belt line, and so forth. Preoperatively placed ureteral stents are usually unnecessary but may be helpful if there is intense pericolonic inflammation. Positioning the patient in a modified lithotomy position allows a circular stapler to be passed transanally if anastomosis is possible; this positioning also allows intraoperative proctoscopy to assess and empty the rectum if necessary.

Intraoperative assessment

Under emergency conditions, the abdomen is explored through a midline incision; a laparoscopic approach may be possible electively at least 6 weeks after the episode of diverticulitis has resolved. Assessment of peritoneal contamination, proximal obstruction, and fecal load determine the advisability of a primary anastomosis (Hinchey's classification; Table 61.2) [76]. For instance, class I and II disease may still allow a primary anastomosis, depending on whether the abscess is removed en bloc with the resected segment (as with an intramesenteric abscess), or whether the abscess has been well-controlled by the peritoneum, can be safely drained without local contamination, and is distant from the anastomotic site.

Table 61.2 Hinchey's classification of diverticulitis

Stage	Characteristics
I	Pericolic or mesenteric abscess
II	Walled-off pelvic abscess
III	Generalized purulent peritonitis
IV	Generalized fecal peritonitis

Adapted from Rao et al. [52].

In elective or semielective situations, resection and primary anastomosis are usually possible because the inflammatory condition is well localized or has resolved substantially. After resection, the ends of the bowel for anastomosis must be well vascularized, nonedematous, and tension free. The distal margin of resection should be in the upper third of the rectum, because there are no diverticula distal to this point. A multivariate analysis of factors involved potentially in recurrent diverticulitis after prior sigmoid resection indicated that the level of the anastomosis was the single predictor of recurrence. Patients with a colosigmoid anastomosis had a four times higher risk of having a recurrence than patients with a colorectal anastomosis [77]. The proximal resection should extend to where the colon becomes soft and nonedematous. It is not absolutely necessary to remove all diverticula-bearing colon proximal to the intended anastomosis [42], but attempts should be made to remove the majority of diverticula on the left side of the colon. Absolute contraindications to primary anastomosis are fecal or purulent peritonitis; relative contraindications include severe comorbid conditions that might jeopardize blood flow, poor nutrition, and immunosuppression [69].

In emergency situations with peritoneal contamination, a two-stage procedure is appropriate (Fig. 61.11). The most common two-stage approach involves resection of the diseased colon, oversewing of the rectal stump, and an end colostomy – a Hartmann procedure. In patients with fecal or purulent peritonitis, this approach is the preferred method. Creation of a mucous fistula from the rectal stump is not possible after resection of the entire sigmoid. Many surgeons mark the rectal stump with a long, nonabsorbable suture and tack it to the anterior abdominal wall or sacral promontory for ease of location in the future. The colostomy can be closed 3 months later. Cleveland Clinic has developed a propensity score that incorporates independent predictors of the decision to proceed with Hartmann resection: body mass index at least 30, Mannheim peritonitis index more than 10, operative urgency (emergent, urgent vs elective), and Hinchey stage [78].

The Hartmann procedure removes the source of sepsis, but reversal of the colostomy is often difficult because of adhesions and difficulty identifying the rectal stump [7,12]. Up to one-third of patients are left with a permanent stoma [79], and this percentage has remained surprisingly stable over the last 10 years. Of 11 582 admissions for diverticulitis in California over a 1-year period, 1176 underwent a Hartmann procedure, and over 35% had not had the colostomies reversed at 4-year follow-up [80]. Those patients who do undergo reversal have a higher rate of postoperative complications than patients undergoing primary resection and anastomosis (53% vs 31%) [81]. Relative contraindications to an unprotected anastomosis include the presence of a chronic abscess cavity and mild systemic illness, in which case the patient may be better served by creation of a protected

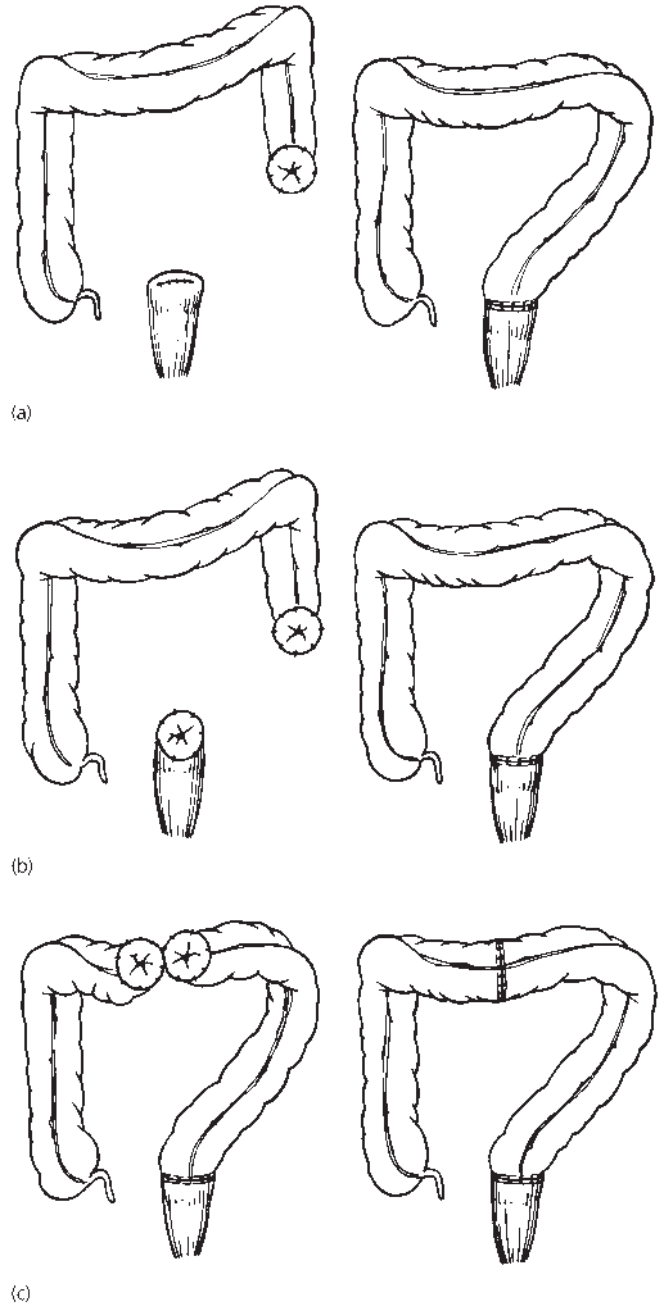


Figure 61.11 Two-stage approaches to resection of diverticular disease. Common among the approaches is that the offending segment of diverticular disease is resected at the first operation. **(a)** Stage 1: The Hartmann procedure. Diseased sigmoid colon is removed, the fecal stream is diverted, and the rectum is oversewn. Stage 2: Intestinal continuity is reestablished by a descending colorectostomy. **(b)** Stage 1: Diseased sigmoid colon is removed and both ends of bowel are brought to the surface, one as an end colostomy and the other as a mucous fistula. Stage 2: Intestinal continuity is reestablished as in **(a)**. **(c)** Stage 1: Diseased sigmoid colon is resected and a colorectostomy is constructed and protected by a diverting transverse colostomy or diverting loop ileostomy. Stage 2: Closure of the transverse colostomy is achieved.

primary anastomosis. This increasingly popular two-stage approach entails resection, primary anastomosis, and proximal diverting stoma; the second stage then involves a simple stoma closure. This approach is employed when there are relative contraindications to primary anastomosis, but no purulent or fecal peritonitis, and the bowel is nonedematous. This option has advantages because the second-stage stoma closure may often be accomplished through the stoma site alone, especially if a diverting loop ileostomy (our preference) is used. Another factor in the emergent setting is that bowel preparation is not possible, and a large fecal load precludes a primary anastomosis. Consideration may be given to intraoperative lavage in selected patients to permit primary resection and anastomosis. In addition to mobilization of the affected sigmoid, mobilization of the splenic and sometimes the hepatic flexure may help [49]. Bowel proximal to the sigmoid is occluded with tape, and immediately above this, the lumen is cannulated with large-bore, corrugated plastic tubing (anesthesia ventilator tubing). The end of the tube is passed off the field. The distal cecum, or appendix base, is cannulated with a Foley catheter through which warm saline is infused until the efflux is clear. Lavage was performed in 33 of 62 patients undergoing nonelective operation for diverticulitis: 18 Hinchey stage I, 10 stage II, and five stage III disease [82]. Only one anastomotic complication occurred.

The outdated classic three-stage procedure (Fig. 61.12) is no longer indicated [70] because it has a mortality rate of 26% vs 7% after a two-stage resection [75]. In rare and exceptional cases, if mesenteric inflammation precludes a safe dissection of the involved segment from the iliac vessels and ureters, or if the patient is unstable, it may be necessary simply to drain the area and create a proximal stoma. Rarely, optimal patient care in areas lacking a well-qualified abdominal surgeon is best served by drainage and diversion, which allows stabilization and early transfer to a large center for definitive therapy.

Laparoscopic vs open approaches

Laparoscopic techniques may be valuable in several facets of diverticular disease [83]. These include diagnosis, diversion, resection of the affected segment of colon, and restoration of colonic continuity (either as a staged procedure or primarily). Absolute contraindications to the laparoscopic approach are free perforation with fecal peritonitis or extensive purulent peritonitis because of the limitations on complete exploration and clearance of contaminated material. Both single institution [84–86] and multicenter [87] studies have demonstrated benefits of this approach in terms of reduced ileus and hospital stay, with similar or even reduced [88] complication rates compared with laparotomy. This approach can be achieved with reduced total direct costs for the laparoscopic cases [89]. Laparoscopic techniques can adhere to the same principles as open operations, with the ability to construct the anastomosis at the equivalent level in the rectum, resulting in no differences in rates of recurrent attacks of diverticulitis, although the latter may be biased by fewer follow-up periods in the laparoscopic group [90]. Hand-assisted vs pure laparoscopic techniques may offer shorter operative times and lesser conversion rates, although most studies are biased by selection based on time period (the hand-assisted device being utilized in more recent cases) or perceived difficulty of the case resulting in the decision to use a hand-assisted approach [91]. Randomized trials confirming superiority of the approach, however, have not been performed.

The inflammation that may accompany diverticulitis is often challenging even in open procedures. Resolution of the

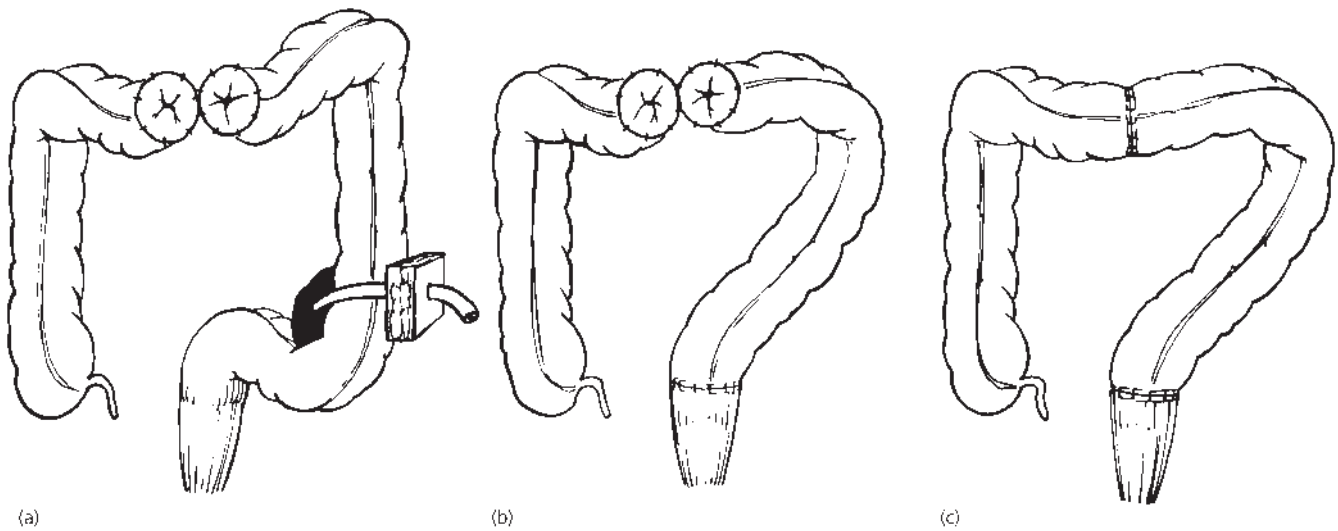


Figure 61.12 Three-stage resection of diverticular disease. **(a)** Stage 1: Diverticular abscess is drained and the fecal stream is diverted by a double-barreled colostomy. **(b)** Stage 2: Sigmoid diverticular disease is resected and a colorectostomy is constructed. **(c)** Stage 3: The colostomy is closed to restore intestinal continuity.

inflammatory component permits a successful laparoscopic approach in most cases [83]. The presence of a colovesical, colovaginal, or coloenteric fistula reduces the chances of completing the procedure laparoscopically, as does previous use of a drain, but is not an absolute contraindication to this approach. Even in open cases, the bladder side of a colovesical fistula frequently requires nothing more than pinching off the fibrous fistula track and leaving the bladder decompressed with catheter drainage, and the same principles apply to the laparoscopic approach. Ureteral stents may be helpful, and the firm tubular structure of the stent in the retroperitoneum can usually be detected with the laparoscopic instruments, making lighted stents unnecessary. As with open procedures, it is helpful to approach the mass in the sigmoid from both cephalad and caudad directions having identified normal tissue planes away from the phlegmon. Substantial experience is required to identify these planes. A low threshold for conversion to laparotomy should be maintained to avoid damage to vessels and ureter. The same extent of resection should be accomplished laparoscopically as for an open procedure, namely proximal resection of the sigmoid back to soft, pliable tissue, and distal resection to a point where the taenia have coalesced, usually to a point below the sacral promontory. This precludes an extracorporeal anastomosis and mandates an intracorporeal stapled anastomosis. One study of 79 laparoscopic sigmoid resections vs 79 open procedures, showed lesser rates of splenic flexure mobilization in the laparoscopic group (19% vs 41%) but also lesser rates of finding taeniae coli at the resection margin (4% vs 53%), suggesting a more distal anastomosis in the laparoscopic group [92]. With a minimum follow-up of 5 years in each group, there was no significant difference in the rate of recurrence (three vs seven patients). In patients who did have a recurrence, the rate of taeniae coli present at the distal resection margin was significantly increased, suggesting a potential advantage of the laparoscopic approach, which appeared to achieve a more distal margin.

Figure 61.13 Contrast enema revealing picket fence appearance of the sigmoid colon, associated with symptoms of obstruction.

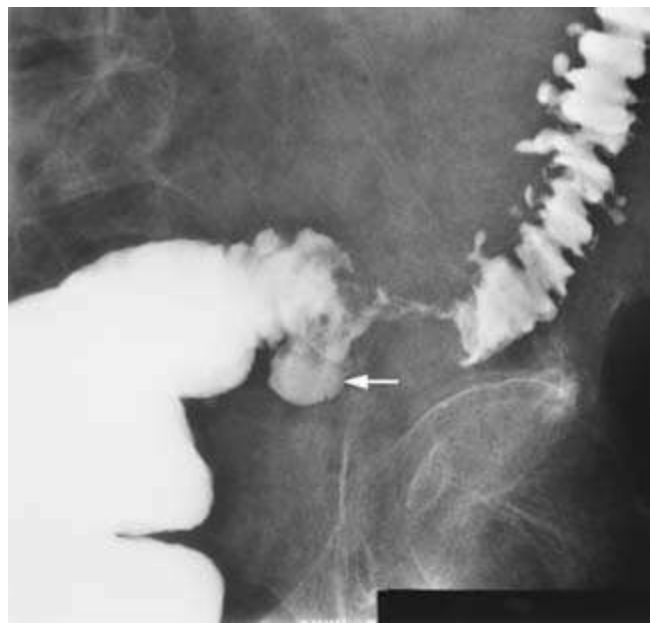


Figure 61.14 Barium enema demonstrating constricting lesion in sigmoid colon in a region of diverticulosis. There is also a 3-cm localized collection of barium (*arrow*) off the lateral colonic wall consistent with an abscess cavity. The most likely radiological diagnosis is diverticulitis but carcinoma cannot be excluded.

Complications

Obstruction

Diverticular obstruction of the colon is rarely a true or complete mechanical obstruction; however, obstipation as the result of functional obstruction is a common presenting complaint. Evaluation often shows a ratty stricture of the involved segment with a picket fence appearance (Fig. 61.13). The primary concern is differentiation from carcinoma (Fig. 61.14); even with negative biopsies, resection is usually unavoidable for this suspicious appearance. Resection with primary anastomosis is often possible, with colostomy if the preoperative bowel preparation is inadequate. In rare situations, acute diverticulitis leads to complete mechanical large bowel obstruction; this situation requires an emergent exploration. The inflammatory reaction may also involve secondarily the small bowel, leading to a small bowel obstruction; if this fails

to respond to nonoperative treatment, operative intervention may be necessary.

Perforation

Free intraperitoneal rupture of diverticulitis is unusual but is associated with extremely high mortality rates of 20%–30% [75,93] and appears to be more common in severely ill or immunocompromised patients. Treatment involves an emergent, two-stage procedure with resection of the involved segment and proximal diversion.

Abscess

Abscesses occur in 16% of patients with acute diverticulitis presenting without peritonitis [58] and in 31%–56% of patients requiring early operative intervention for diverticulitis [7]. Before the introduction of radiologically guided, percutaneous techniques of drainage, abscesses mandated operative intervention, often requiring a two-stage procedure. Percutaneous drainage permits a future, elective, single-stage operative approach in 60%–80% of patients [94–96]; indeed, in selected patients with severe comorbid conditions, which contraindicate operative therapy, catheter drainage alone may relieve symptoms and provide definitive treatment [95]. The catheter should remain in place until drainage is less than 10 mL in 24 h; this approach may take up to 30 days [95]. Contrast radiographs through the drainage catheter (sinograms) can show persistent communication between the bowel and the abscess cavity and may be one way to follow resolution of the abscess. The route of drainage is preferably through the anterior abdominal wall; abscesses deep in the pelvis or obscured by other organs may be accessed transgluteally or through the rectum or vagina [97,98], but these approaches are less optimal for patient comfort and catheter care. Operative intervention is indicated if improvement does not occur.

Fistula

Incidence

Fistulae account for up to 20% of all indications for operative intervention in complicated diverticular disease [99]. Because 90% of diverticular inflammation involves the sigmoid colon, fistulization most commonly arises from this segment. Colovesical fistulae (Fig. 61.15) comprise 65% of diverticular fistulae, colovaginal fistulae comprise 25%, and coloenteric and colouterine fistulae are less common [99], but fistulae can form with any hollow organ and they have been described in many unexpected sites (Fig. 61.16).

Colovesical fistulae are the most common form of diverticular fistula and, conversely, diverticulitis is the commonest cause of colovesical fistula, accounting for 40%–89% [100]. Although acute diverticulitis has an overall slight female predominance, males comprise the majority (66%–78%) of patients with colovesical fistulae secondary to diverticulitis [99,101]. The uterus appears to protect the bladder from the inflamed sigmoid; indeed, 50%–68% of women with colovesical fistulae and 83% of with colovaginal fistulae have had a previous hysterectomy [99,100,102].

Figure 61.15 Barium enema in a 54-year-old woman with pneumaturia and left lower quadrant pain. A fistulous tract (*closed arrow*) arises in the mid sigmoid passing into an abscess cavity (*arrowhead*) and then into the bladder, where contrast is also seen (*open arrow*). From Young-Fadok & Pemberton [149].

Clinical features and diagnosis

Colovesical fistulae are illustrative of most forms of diverticular fistulae. Only about 50% of patients will give a history of diverticulitis; in the remainder, diverticulosis is diagnosed when the fistula manifests clinically [101]. Patients describe passage of feculent matter or gas during urination; this manifests as pneumaturia in 67%–75%, dysuria in 56%–94%, or fecaluria in 50%–76% [99–101]. Crampy abdominal pain, diarrhea, hematuria, and passage of urine per rectum occur in a much lower percentage of patients [99,101]. Physical examination is characteristically unremarkable. Urinalysis is invariably abnormal with pyuria or fecal particulate matter; cultures reveal a polymicrobial growth. Such findings suggest a fistula, but not the etiology, which may be diverticulitis, Crohn's colitis, or carcinoma.

Computed tomography, barium enema, colonoscopy, cystoscopy, cystogram, and intravenous urography have all been used for the diagnosis of colovesical fistula. The reported

Figure 61.16 Barium enema in a 64-year-old man with 3 weeks of right hip pain. There is extravasation of barium from a perforated sigmoid diverticulum with collection of contrast material in an abscess cavity (*white arrow*) adjacent to the diverticulum. A fistulous communication extends from the abscess cavity to the right lower quadrant and extends to the region of the right hip (*black arrow*). From Young-Fadok & Pemberton [149].

diagnostic yield of each modality varies widely in different reports. The diagnostic yield from contrast enema and colonoscopy tends to be low (20%–26% and 0%–3%, respectively) [100,101], but these studies are valuable in assessing the proximal colon. Although the actual fistula is rarely demonstrated, abnormalities suggestive of the diagnosis are present in up to 100% of barium enemas and 25% of colonoscopies. If the diagnosis remains questionable after these studies, or if further anatomic delineation is required, CT is the most accurate and sensitive test. Occasionally, the fistula itself is opacified but, more commonly, colonic thickening adjacent to an area of thickened bladder, associated diverticula, and the presence of rectally administered contrast material or more commonly gas in the bladder (before instrumentation of the urinary tract) are present and are highly sensitive and specific for colovesical fistulae [103]. Gas bubbles within organs other than the bowel are highly suggestive of fistulization [53]. Although CT may not actually delineate the fistula tract, associated findings predict the location of the involved segment of colon and provide important information regarding extralumenal inflammation and its relationship to the ureters.

Cystoscopy, lauded by some authors, identifies directly only 20% of fistulae but strongly suggests the diagnosis by localizing inflammation and bullous edema of the bladder mucosa in the vast majority (96%) of patients [100]. Others consider such changes too nonspecific and find cystoscopy useful in only 37%–46% of patients [99,101]. Similarly, retrograde cystography and intravenous urography objectively demonstrate the fistula in less than 30% of patients and generally are of little clinical benefit.

Treatment

In general, diverticular fistulae do not close spontaneously, but also the presence of a fistula is rarely an indication in itself for urgent operative intervention; indeed, the rare instance of an acute initial presentation of a fistula may be an indication to postpone operative correction until the acute inflammation inducing the fistula resolves. As most patients present electively and can tolerate a bowel preparation, a one-stage procedure with resection and primary anastomosis is usually feasible and is associated with decreased morbidity and hospital stay [100]. If extensive inflammation is present and operative intervention is required to treat nonresolving acute diverticulitis, a two-stage procedure may be necessary.

The portion of the colon from which the fistula arises should be resected as described previously (see section on treatment). Management of the fistula site at the bladder depends on the size of the fistula, the degree of local inflammation, and the anatomic site. In most patients, the fistula can be pinched off, and either no visible defect in the bladder wall or a small defect will be evident. Management of the site of the fistula in the bladder may include merely leaving an indwelling bladder catheter for 7–10 days with or without closing the bladder defect with a few absorbable sutures over the site and leaving a closed suction drain in the region of the bladder defect. With a larger defect, simple closure is almost always preferred to formal resection, which is almost never necessary because the cause of the fistula originates in the colon and the bladder itself is normal; any local induration usually settles rapidly once the inciting cause (the colon) has been removed [69]. These simple principles of minimal intervention apply to the distal aspect of most types of diverticular fistula, be they in small bowel, vagina, or uterus.

Although operative management is usually recommended, in highly selected patients, especially with severe comorbid conditions, there may be a rationale for conservative nonoperative management, but no good follow-up studies are available to support this approach in most patients. In one series, six patients were managed nonoperatively for 3–14 years, treated only with prophylactic antibiotics; there were no complications and specifically no impairment of renal function and no urinary sepsis [104]. In the absence of urinary tract obstruction, observation appears safe in patients

with significant contraindications to operative management; this nonoperative approach may, however, be unacceptable if the symptoms are bothersome.

Special situations

Diverticulitis in the younger patient

Management of patients with their first episode of acute diverticulitis at less than 40 years of age is controversial. The current standard of therapy is to recommend resection after one episode based on two premises: first, these patients suffer a more virulent disease; and, second, patients responding to medical therapy are much more likely to have recurrent diverticulitis and ultimately require operative intervention. However, controversy exists as to the true virulence and natural history in this age group. Young patients comprise 12%–29% of the larger series of patients with acute diverticulitis requiring operative treatment [8,9,339,69,105]. Common findings are a male predominance of from 2 : 1 to 4 : 1 [8,9,39,69] and the coexistence of obesity in 84%–96% [9,69].

The concept that diverticulitis is more virulent in the young arises from follow-up studies of younger patients with their first episode of acute diverticulitis; 48%–88% of this group will require operative treatment either acutely or in the future [9,69,105]. Others describe much lower rates of operative intervention (15%–41%) and a more successful nonoperative management approach [8,39]. These inconsistencies may arise, because the younger patient may undergo operation because of an incorrect diagnosis [106]; some series have high rates of incorrect diagnoses approaching 50% [9,69]. This inordinately high rate is probably because the diagnosis of diverticulitis is not entertained in this young population, and the presence of localized peritonitis is interpreted as an abdominal catastrophe requiring emergent operative exploration. If CT or contrast studies are used more widely, then the operative rate in young patients is actually only 15% compared with as high as 33% in older patients [39]; however, the diagnosis of acute diverticulitis must first be entertained.

The natural history of acute diverticulitis in this young population after initially successful medical management is disputed. Some studies report readmission rates of 55% [107] and subsequent operative rates of 20%–41% [106–108]; others report no need for operation during a mean follow-up of more than 4 years [109]. When the total operative rates are examined with long-term follow-up, data are more consistent with an ultimate operative risk of about 50%. Some series have high initial operative rates [9,69,105], while others with a lower rate of acute operation (15%–25%) have higher rates at later times (29%–32%) [39,108]. In one study of 762 patients admitted with diverticulitis, 259 (34%) were younger than 50 years [110]. The risk of requiring operative intervention during the first hospitalization was similar between patients younger than 50 years and patients older than 50 years (24% vs 22%). However, although the younger patients were more likely to be treated operatively during the study period, this was because of an increase in elective resections, not further episodes of disease. These authors espoused a selective approach in young patients. This study differs from that of Chautems and colleagues who evaluated the long-term natural history of sigmoid diverticulitis in 144 patients treated nonoperatively for a first acute episode [111]. Young age and severe diverticulitis (by CT criteria) were both statistically significant factors of poor outcome (persistent or recurrent diverticulitis), but age was no longer significant after stratification for disease severity as graded on CT (but the analysis did suggest that more severe disease was linked to a younger age).

Although there are no reliable convincing arguments that mandate elective resection in this younger population after resolution of *uncomplicated* diverticulitis, in patients with no comorbid conditions, early elective colectomy after one episode of acute diverticulitis seems the most reasonable recommendation. It does seem clear, however, that the issue is one for individualized frank discussion with each patient to evaluate their risk and lifestyle concerns.

Immunocompromised patient

Pharmacological or functional immunosuppression is associated with an increased risk of severe diverticulitis. This patient group includes those patients treated with chemotherapy, corticosteroids, and medications such as azathioprine or cyclosporine, but the phenomenon is also apparent in diabetics and those with chronic renal failure. These patients may present with minimal symptoms and unimpressive abdominal findings even when they have advanced peritonitis; so the diagnosis is frequently delayed. In patients who have undergone heart or lung transplantation, the risk of severe diverticulitis requiring operative intervention has been estimated to be over 20 times greater than in the general population [112]. While aggressive medical therapy is successful in 75% of normal patients with acute diverticulitis, operative intervention is necessary in most immunocompromised patients [113]; early operative intervention must be entertained in these patients, especially if they fail to show an objective response early during aggressive medical treatment. Patients who respond to medical therapy should be considered for operative intervention after just a single episode and appear to tolerate operative intervention well [110]. Patients with connective tissue disorders, such as systemic lupus, appear to have an increased risk of complicated diverticulitis over and above that related to immunosuppression from use of steroids [114].

Right-sided diverticulitis

Right-sided diverticula comprise 5% of diverticulosis in Western countries [3] but up to 20% in Asia [13,14]. Most are wide-mouthed, false diverticula [16]. Patients tend to be younger than those with left-sided disease [13,14]. Because

right-sided acute diverticulitis presents clinically as acute appendicitis, the correct preoperative diagnosis is made in only 4%–16% of patients.

If an objective diagnosis can be secured preoperatively, then aggressive medical management should be undertaken. More commonly, the diagnosis is made or suspected only intraoperatively. If there is no peritoneal contamination, an obviously normal or absent appendix, and no worry of colonic malignancy, then appropriate management involves parenteral antibiotics and an appendectomy if the appendix is present [13,14]. If the diverticulitis is limited, consideration can be given to simple diverticulectomy [14] (and appendectomy). However, if there is diverticular perforation, extensive inflammation, or a colonic malignancy that cannot be confidently excluded, then some form of right colectomy is justified. Primary anastomosis is usually possible, but one should not hesitate to construct an ileostomy and distal mucous fistula in the presence of severe peritonitis or in the compromised host [13,17].

Diverticular bleeding

Incidence

Diverticular bleeding is the most common cause of acute massive colonic blood loss, accounting for 30%–50% of massive bleeding [115,116]; although angiodysplasia makes up an additional 20%–30% [116], it is a more common cause of chronic, intermittent, low-grade lower gastrointestinal bleeding in patients over 65 years old [117,118].

Pathophysiology

One explanation of the pathophysiology of diverticular bleeding is the hypothesis that as a diverticulum herniates, the vessel responsible for the weakness in the wall of the colon at that point becomes draped over the dome of the diverticulum, separated from the bowel lumen only by mucosa (see Fig. 61.1); with time, the artery is exposed to repeated local injury along its lumenal aspect secondary to inflammation or increased intralumenal pressure. Eccentric intimal thickening and thinning of the media occur in the vasa recta along the side adjacent to the lumen [27]. These localized changes in vessel wall structure may result in segmental weakness of the artery and rupture into the lumen. Right-sided diverticula tend to have wider ostia and domes than those in the left colon, possibly exposing vasa recta to injury over a greater length of the vessel wall and hence accounting for the higher incidence of hemorrhage recognized from right-sided colonic diverticula [27]. Others believe that the thinner wall of the right colon predisposes to diverticular bleeding [35].

Natural history

Bleeding will develop in 15% of patients with extensive diverticulosis; in a third of these patients (5%) the bleeding is massive and can potentially lead to cardiovascular instability [35]. Many patients are elderly (mean age, 68–77 years) [115,116] with comorbid conditions that contribute to morbidity and mortality rates of 10%–20% [116,119]. Several studies have suggested that management with a specialized bleeding team may decrease mortality [115]. Bleeding stops spontaneously during 75% of episodes; a requirement for blood transfusion of less than 4 units in 24 h is associated with spontaneous cessation of bleeding in 99% of patients [120]. The risk of rebleeding, however, is substantial, ranging from 14% to 38% [115,120]; with a second episode, the risk of further hemorrhage approaches 50% [120]. The right colon is the source of diverticular bleeding in 48%–90% of these patients [27,115,121] despite the fact that 75% of all diverticula are left-sided, and when right-sided diverticula occur, they are usually associated with left-sided diverticula [12].

Clinical presentation

The hallmark of diverticular bleeding is painless, bright red rectal bleeding. Most episodes are minor (< 1 unit of blood), and up to 50% of patients [121] give a history of intermittent passage of maroon or bright red blood. Abdominal pain is usually absent; indeed, it is extremely rare for bleeding to coexist with acute diverticulitis [27]. Physical examination is usually unremarkable without abdominal tenderness or mass. Less than 5% of patients with bleeding diverticulosis present with massive hemorrhage requiring transfusion; this bleeding is usually not of an acute exsanguinating form but rather as a persistent bleed.

Management

Management of massive colonic bleeding has three components: resuscitation, diagnosis and localization of the site of bleeding, and subsequent management. A coordinated approach by gastroenterologists, radiologists, and surgeons as members of a specialized bleeding team has been shown to decrease mortality [122]. The American College of Gastroenterology has published Practice Guidelines addressing lower gastrointestinal bleeding [123].

Resuscitation and initial assessment

After aggressive fluid resuscitation and cross-matching of blood, blood loss must be estimated, an assessment must be made of hemodynamic stability, and the source should be sought. The patient's age is not a great help in diagnosis nor is the physical examination. Rectal examination, anoscopy, and proctoscopy must be part of the initial assessment; massive hemorrhoidal bleeding, although rare, can be easily excluded. If there is a strong suspicion of bleeding from a lower gastrointestinal source, the appropriate diagnostic workup is initiated. If assessment does not indicate if bleeding is proximal or distal to the ligament of Treitz, an extended upper endoscopy should be performed because massive hemorrhage from the upper gut is statistically more likely than from the lower gastrointestinal tract (82% vs 18%)

[115,124]; if this investigation is negative, then evaluation of the lower gastrointestinal tract should begin.

Diagnostic modalities

The aim of the diagnostic evaluation is twofold: to localize the site of bleeding should operative treatment become necessary, and to determine the cause of bleeding, as treatment of diverticular bleeding differs from that of other causes. Radionuclide bleeding scan, mesenteric angiography, and colonoscopy have all been employed; the appropriate order in which they are utilized depends on the rate of bleeding and on available expertise. A combination of diagnostic modalities can identify successfully the source preoperatively in 90% of patients with massive bleeding [125].

Colonoscopy

The role of colonoscopy in acute diverticular bleeding is evolving but, in some centers, it has become the preferred initial approach. The first report of endoscopic management of a bleeding diverticulum with injection of 1 : 1000 epinephrine was in 1985 [126]. Success has since been reported with the heater probe [127], bicap probe [128], injection therapies [129,130], and fibrin sealant [131]. Endoscopic "stigmata" with prognostic value have been reported [132]. Cumulative results of small studies suggest a 95% rate of hemostasis [133]. Some proceed with emergent colonoscopy in unprepared bowel, as blood is cathartic [134]. Others use enemas or rapid preparation with balanced electrolyte solutions given orally or through a nasogastric tube [135,136]. When performed successfully (i.e., satisfactory visualization of the mucosal surface), colonoscopy has a diagnostic accuracy of 72%–86% [134–136]. Advantages include precise localization and the potential for therapeutic intervention. Only a select few patients, however, are realistically amenable to colonoscopy – those with low volume, intermittent colonic bleeding.

Radionuclide imaging

Two types of radionuclide bleeding scans have been employed. The first utilizes a sulfur colloid labeled with technetium (^{99m}Tc). Scans obtained shortly after intravenous injection can image the area of bleeding even if the rate of bleeding is as low as 0.1 mL/min. This method is exceedingly sensitive but the short half-life of the marker within the intravascular space (the sulfur colloid is rapidly cleared by the spleen and liver) limits the usefulness of this imaging modality to patients who are actively bleeding during the short duration that the label remains in the vascular space. Because of this exceedingly short functional half-life of efficacy, this imaging modality has been replaced with the so-called tagged red cell scan, which uses ^{99m}Tc pertechnate to label or tag autologous red blood cells removed from the patient before reinjection into the patient (Fig. 61.17). Abdominal images are obtained frequently during the first 30 min, and then every few hours for up to 24 h. The major advantage of this label is that patients may be scanned several times over 24 h and so it is possible to image the site of intermittent bleeding or localize the appropriate quadrant of the abdomen.

Radionuclide bleeding scans have advantages and disadvantages. Advantages are their noninvasiveness and sensitivity. One series demonstrated a sensitivity of 97%, specificity of 83%, and positive predictive value of 94% [137]. The disadvantages of radionuclide scans are that they localize

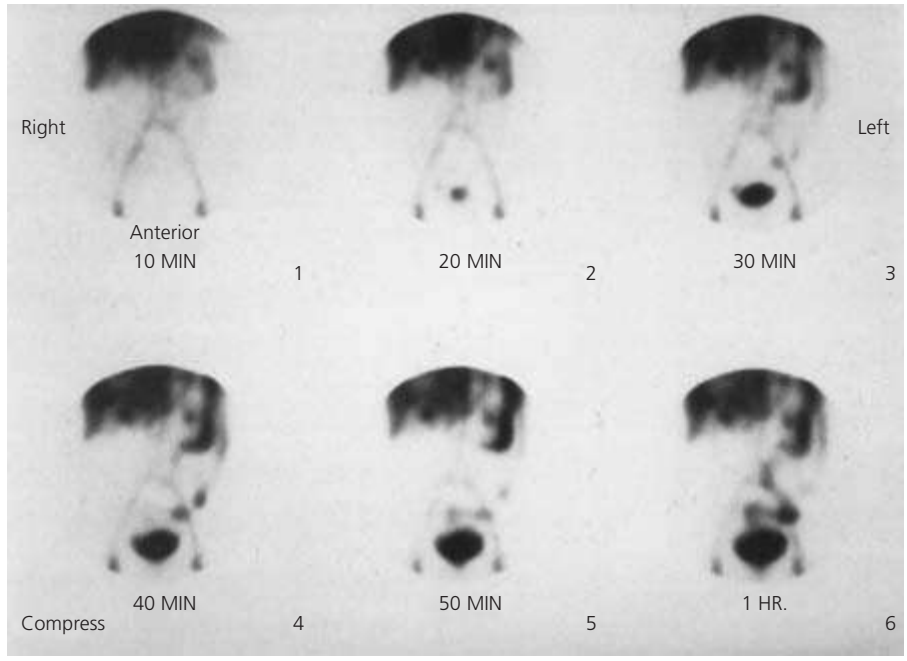


Figure 61.17 Technetium (^{99m}Tc) -labeled red blood cell scan for evaluation of lower gastrointestinal bleeding in a 63-year-old woman with a 24-h history of bright red blood per rectum. Increased activity in the distal transverse colon near the splenic flexure progresses towards the descending colon and sigmoid colon by termination of the examination at 1 h. Findings are consistent with a bleeding source in the distal transverse colon near the splenic flexure. From Young-Fadok & Pemberton [149].

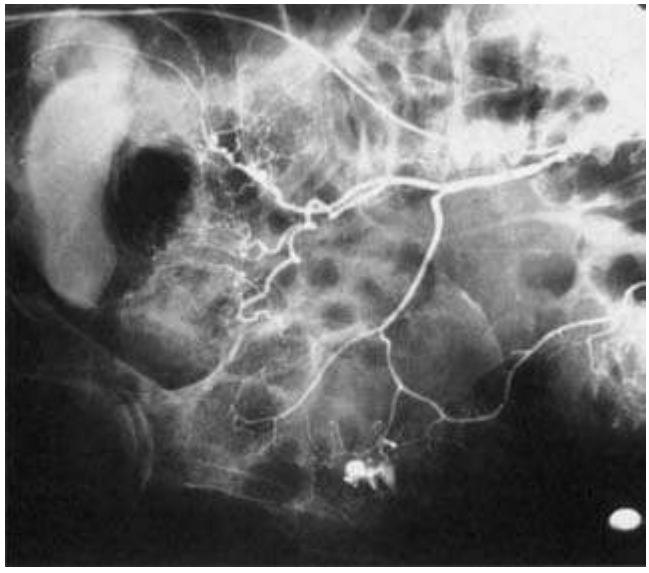
bleeding to a general area of the abdomen without necessarily localizing the bleeding source to a specific organ, for example, colon vs small bowel, and so accuracy rates range from 24% to 91% [35]. Poor organ localization occurs, because the extravasated blood may move within the hollow organ in either a peristaltic or antiperistaltic direction, and, in addition, localization to an area of the abdomen cannot objectively define a site in the colon; for example, bleeding from a redundant sigmoid colon may appear as extravasation in the right lower quadrant suggesting bleeding either from the right colon or the distal small bowel. Hence, radionuclide imaging is often used before angiography to confirm bleeding rates sufficient to justify the use of the more invasive selective angiographic studies.

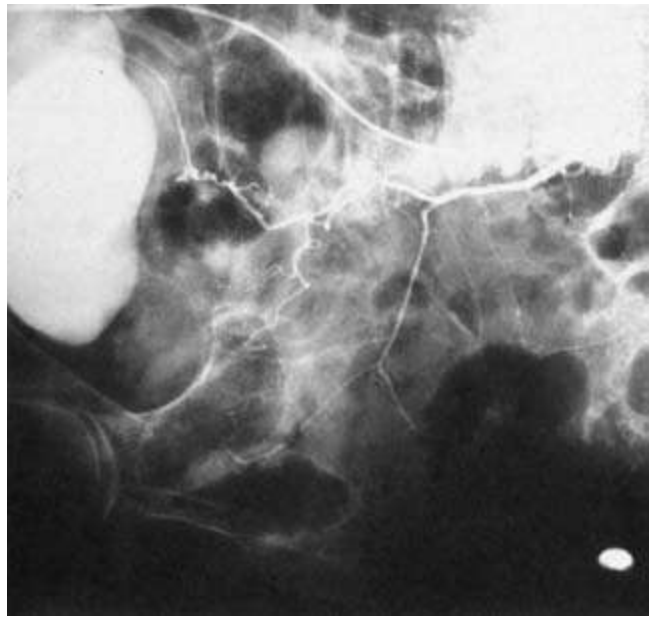
Angiography

In the absence of a likely source in the distal colon or rectum, the first mesenteric vessel examined is the superior mesenteric artery, as 50%–80% of diverticular bleeding and virtually all bleeding from angiodysplasia occur in segments of the colon and bowel supplied by this artery [138]. If no source is found, the inferior mesenteric and celiac vessels are studied. Successful localization of the anatomic site of bleeding varies from 14% to 72% [116,119,135,139] and depends on the rate of bleeding (sensitivities are as low as 0.5 mL/min), timing (the patient must be bleeding actively at the time of the intravenous contrast administration), and the expertise of the angiographer. Angiographic localization is 100% specific, but sensitivity varies with the pattern of bleeding; with acute bleeding, sensitivity approaches 50% but decreases to 30% with a pattern of recurrent hemorrhage [140]. A positive angiogram is associated with an 86% likelihood of operative treatment [140]. The use of radionuclide imaging to screen for active bleeding may decrease the frequency of negative arteriograms [124], but some groups maintain that the incidence of negative arteriograms is increased by the delay inherent in performing radionuclide scans [116]. Complications of urgent arteriography occur in about 9% of patients and include arterial thrombosis, mesenteric or peripheral embolization of atherosclerotic debris from the aorta, and renal failure [135].

The advantages of angiography are that bowel preparation is not required, and the anatomic localization provided is organ-accurate, especially in the colon. Moreover, angiographic techniques may allow therapeutic intervention with selective infusion of vasoconstrictor agents or vascular embolization. Vasopressin can be infused directly into the artery supplying the segment of colon bleeding by a subselectively positioned intraarterial catheter (Fig. 61.18). Up to 91% of patients receiving intraarterial vasopressin will stop bleeding; however, as many as 50% will rebleed on cessation of vasopressin [116]. In these patients, however, vasopressin may permit resuscitation, stabilization, and bowel preparation with the goal of resection and primary reanastomosis [116]. Transcatheter embolization is a more definitive means of controlling hemorrhage. Early studies were associated with an incidence of colonic infarction in up to 20% of patients embolized. Attempts to reduce this risk have included use of

(a)





(b)

Figure 61.18 Arteriogram of the inferior mesenteric artery in a patient with diverticular bleeding. **(a)** Extravasation of contrast medium into the lumen of the descending colon. **(b)** Arteriogram of the same patient after infusion of vasopressin. Note the markedly reduced flow in the left colonic and sigmoidal branches.

temporary occluding agents or have employed a "superselective" catheterization technique [141]. Investigational studies in a canine model showed that when medium to large (420–1000 μm) polyvinyl alcohol particles were used, smaller vessels were preserved, maintaining intramural collateral microcirculation. Studies in both canine and porcine models have demonstrated no histological evidence of bowel infarction when microcoils are used to perform superselective embolization [142]. In humans, cumulative retrospective results have also confirmed that use of microcoil embolization minimizes ischemic complications [143].

Management approach

During resuscitation, the management rationale is determined by categorizing patients into one of three groups: hemodynamically unstable with active bleeding, continuing bleeding but hemodynamically stable, and hemodynamically stable with apparent cessation of bleeding (Fig. 61.19).

Hemodynamic instability

Persistent instability despite aggressive resuscitation requires emergency operative intervention in 18%–25% of patients with diverticular bleeding who require blood transfusion [120,144]. Every attempt should have been made to exclude an upper gastrointestinal source of bleeding (e.g., nasogastric intubation or even emergency gastroscopy), as the operative approach is markedly different for the surgeon. At operation, a careful exploration is undertaken looking for an obvious source or a mass. Because diverticular bleeding is not associated with acute diverticulitis, the involved diverticulum is not immediately evident externally. Indeed, most patients are elderly and would be expected to have colonic diverticula. Without preoperative localization, the decision of what or where to resect becomes problematic. Multiple techniques have been described, including dividing the colon into sections by applying occlusive clamps across the bowel and determining which section fills with blood; this technique,

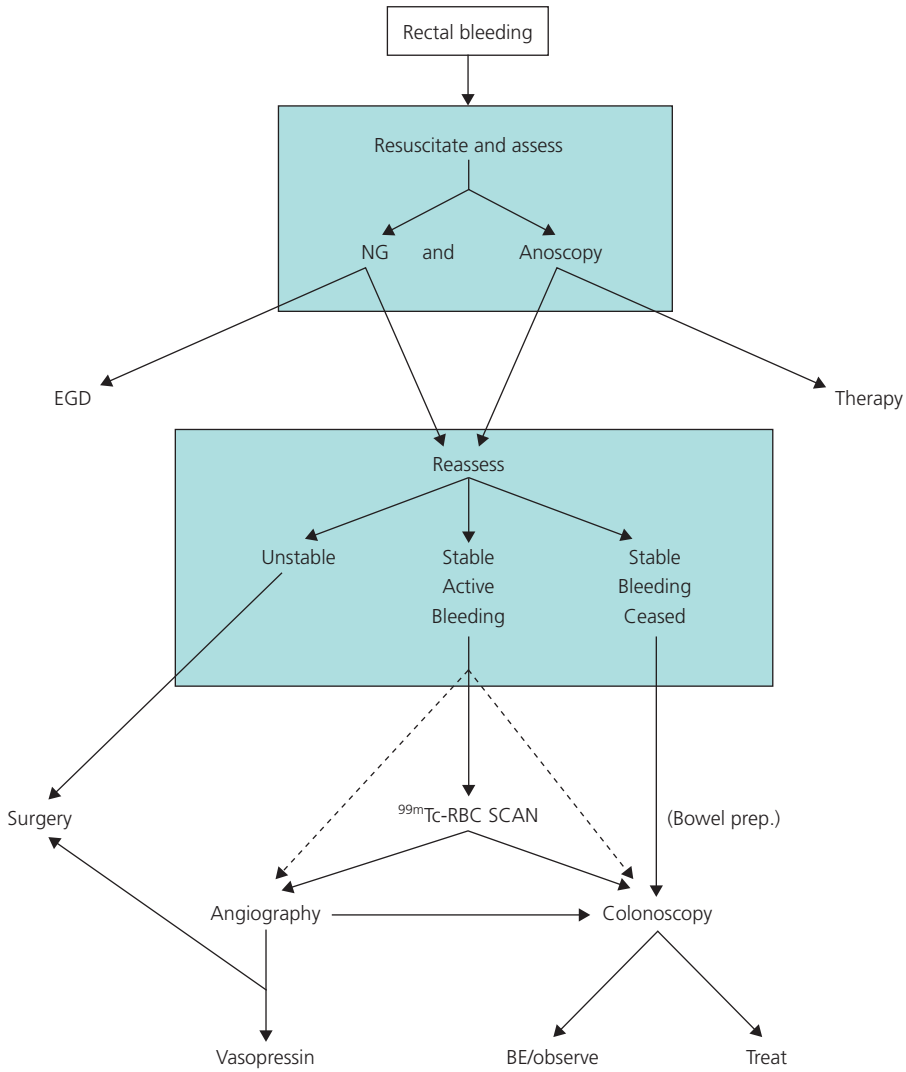


Figure 61.19 Algorithm for management of diverticular bleeding.

which requires the presence of ongoing massive bleeding, is generally unsatisfactory. A much better approach involves intraoperative colonoscopy [125], possibly after a rapid, on-the-table intraoperative bowel preparation using an infusion catheter placed into the cecum, through which a warmed electrolyte solution is infused rapidly and placement of a transanal catheter to control the efflux of infusate from below. This approach allows the possibility of a localized, segmental colectomy involving only the site of bleeding. If, however, no obvious colonic site can be identified, and the bleeding is massive and life threatening, the surgeon may need to perform an abdominal colectomy resecting the entire colon susceptible to diverticular and angiodysplasia. All possible attempts at specific localization preoperatively and intraoperatively are, therefore, important to limit the extent of the colectomy required. A source of bleeding is identified ultimately in 78% of patients undergoing emergent operation for lower gut bleeding [125].

Hemodynamically stable with continued active bleeding

The most straightforward approach is to start with a tagged red blood cell scan. If positive, it can serve as a useful screen before mesenteric arteriography, which may permit infusion of vasopressin to stop the bleeding and allow bowel preparation for future operative treatment should the bleeding recur. Infusion begins at 0.2 units/min, with repeat angiography after 20 min; if bleeding persists, the rate is increased to 0.4–0.6 units/min [145]. If the bleeding is controlled, then the infusion should be continued in an intensive care setting for 24–36 h and then tapered over 24 h [145]. In the high-risk patient who has failed the initial vasopressin trial but has substantial contraindications to operative intervention, embolization may be used followed by close observation for ischemic complications. If angiography is negative, the next step in the diagnostic algorithm (see Fig. 61.19) is colonoscopy, which may identify a source or localize the site of active bleeding. Diverticular bleeding is observed rarely at colonoscopy and is often a diagnosis of exclusion. If the radionuclide scan does not demonstrate bleeding, angiography is unlikely to be helpful. Colonoscopy is then performed after bowel preparation. Some institutions proceed directly to angiography, especially for massive bleeding, whereas others use a rapid bowel preparation before colonoscopy. Of note is that barium enema (vs water-soluble contrast) has no role in the management of acute colonic bleeding, as it prevents angiography because of retained barium.

Hemodynamically stable, bleeding stopped

The patient who has stopped bleeding provides a diagnostic challenge but also allows a semielective evaluation, which involves bowel preparation and colonoscopy in preference to contrast enema.

Operative treatment

The need for operative intervention varies with the degree of bleeding. With severe or massive rectal bleeding, 24%–78% will require operative intervention [116,120,125,135]. Preoperative localization of the site of bleeding and the use of vasopressin as a temporizing measure have decreased operative morbidity from 37% after emergency resection to 9% after segmental colectomy. The operative mortality of 9%–11% reflects comorbid conditions [116,125,144].

When the source of bleeding has been localized, a segmental colectomy is performed, and the rate of rebleeding is 0%–14% [116,120,146]. Blind segmental resection of the most involved diverticula-bearing segment carries a rebleeding rate as high as 42% [146] and an ultimate morbidity and mortality of 83% and 57%, respectively; it is, therefore, contraindicated [146]. Even in patients with extensive diverticular disease, if the site of bleeding has been localized objectively, a segmental resection eradicating the bleeding site is adequate [116]. Abdominal colectomy is reserved for the patient who continues to bleed without a documented site of bleeding but who has typically been associated with a relatively high morbidity and mortality (37% and 11%–33%, respectively) [116,120,147]. More recent studies, however, would suggest that subtotal colectomy can be performed with no excess morbidity or mortality compared with segmental resection [148]. Although the rebleeding rate after abdominal colectomy approaches 0% [146], abdominal colectomy can be associated with relatively disabling diarrhea in the elderly in about 15% and socially unsatisfactory diarrhea in a larger percentage. Therefore, this extent of operation should not be undertaken lightly.

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62 Colorectal cancer screening

Graeme P. Young, James E. Allison

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Screening for colorectal cancer can be defined as the testing of asymptomatic people to classify them as likely or unlikely to have the disease [1]. The World Health Organization (WHO) has described the requirements that should be met before it is appropriate to screen [2]. Colorectal cancer meets all these criteria:

- it is a major health problem in many countries
- it has a latent stage
- there are appropriate proven screening tests
- testing is feasible
- treatment can lead to cure.

WHO recommends screening using a simple test. The result of the screening test determines who undergoes a diagnostic test.

Goals of screening

The goal of screening for colorectal cancer is to reduce mortality from or morbidity due to the disease. The natural history and the impact of early detection methods on outcome can be conceptualized as shown in Fig. 62.1. Presymptomatic detection of localized cancer (point S3) reduces morbidity or mortality. If screening detects preinvasive lesions, namely dysplasia or adenoma (points S1 or S2), it will reduce cancer incidence.

Ideally, screening is an ongoing process, which is directed at asymptomatic individuals who are at average risk for developing colorectal cancer [2]. Average risk is defined as age at least 50 years, absence of high-risk factors such as family or personal history of colorectal neoplasia, resident in a developed or developing country where the lifestyle is changing to that of Western countries, and absence of symptoms suggestive of colorectal cancer [3]. Screening should perhaps be avoided in those individuals where screening is not a health priority, such as when active major illness is present [4]. When high-risk factors are present, an individualized surveillance program is indicated based on specific circumstances.

The most immediate impacts of screening will be adenoma detection, down-staging of the detected cancers and prolonged survival after treatment. The longer-term impacts will be reduction in overall population mortality and incidence.

The contexts of screening

Screening for colorectal cancer is usually undertaken in either personalized or population settings [5]. The health imperatives driving each are different.

In some countries the traditional approach has consisted of a face-to-face meeting between the individual and a physician – sometimes referred to as *case-finding* or *individualistic* screening. It may be initiated by the individual or the physician. Here, the person can be assessed for symptoms and level of risk. What is offered is individualized and done in the context of counseling. Duty of care and doing what is best for the person drive the decision-making – cost-effectiveness tends not to be a consideration, although affordability and access are.

Population or *mass screening* involves an organized and systematic approach aimed at maximum participation in screening within a population. It seeks, through a standardized and often impersonal approach, to engage individuals in at least some form of preventive activity – in effect, doing something is better than doing nothing. Outcomes at the population level such as acceptability, feasibility, and low initial cost with proven cost-effectiveness are key issues. Many thousands will be tested in a short time frame, often without ascertainment of presence of symptoms or without profiling risk through a counseling process.

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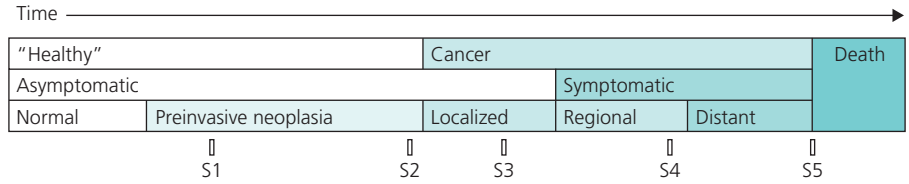

Figure 62.1 Schematic showing stages of colorectal oncogenesis as they relate to different points of early detection as might occur during screening (S1, S2, S3). Relative durations are meant to serve only as a guide. The impact of detection at each point is as follows: S1, detects many adenomas, a proportion of which might not be precursors of cancer but removal should lead to reduced incidence of and mortality from cancer; S2, detects fewer inconsequential adenomas but will remove adenomas and prevent cancer, thereby reducing incidence and mortality; S3, down-stages detected cancers and so will lengthen survival and reduce mortality through increased rate of cure; S4, lesser impact than at S2 but likely to reduce mortality to a degree; S5, probably no impact.

The nature of screening

No matter what the context, screening is by its nature a process that aims to improve the likelihood that affected people, while at a curable and usually unsuspecting stage, receive effective diagnosis and treatment. Screening is, therefore, a process with multiple phases as depicted in Fig. 62.2 [6]. Screening is only beneficial to the individual if the screening test is performed, if diagnosis is accurate, and if treatment is effective.

There are two main choices at the phase where a test is offered (Fig. 62.2).

- *One-step testing* The diagnostic test, colonoscopy, is offered. Selection for colonoscopy is based solely on age and many people will be found to have no neoplasia.
- *Two-step testing* A simpler test is offered first, e.g., a fecal occult blood test (FOBT) or flexible sigmoidoscopy, then those with a positive result proceed to colonoscopy. A simple screening test calls attention to the likelihood of disease being present and serves to direct resources to those most likely to benefit from diagnostic and therapeutic procedures [6].

Operational phases of the screening process proceed as follows:

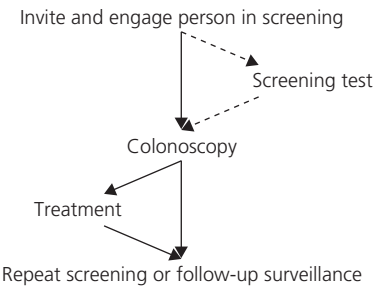

Figure 62.2 Diagrammatic representation of the screening process to demonstrate the main phases involved in the screening process and the options of one-step and two-step screening.

- *Invite and encourage* participation in a screening program.
  - Use either a personalized approach within a health-care environment, or
  - Use a mass population approach with wide coverage of the population.
- Where a person is found to have a *high-risk* factor (e.g., colorectal symptoms, family history or personal history of relevant colorectal disease), provide access to an individualized prevention strategy appropriate to that risk.
- Where a person is found to have *symptoms*, offer the appropriate diagnostic test.
- For those who are confirmed *average risk*, provide a safe, effective, acceptable, and affordable screening test in either of two main ways:
  - *two-step testing*: perform a simple test such as a fecal occult blood test (FOBT) to establish risk and select who should proceed to colonoscopy, or
  - *one-step testing*: perform colonoscopy.
- Facilitate *compliance* with any necessary diagnostic and therapeutic follow-up.
- Offer *rescreening* at the appropriate interval, having identified risk status.
- *Monitor* adequacy of participation, quality of tests and clinical procedures, availability of necessary resources, and the outcomes of the program.

The nature of screening tests

The nature of the major screening tests, either established or under study, and their place in the testing phase of the screening process, are summarized in Table 62.1. In general, the efficacy of screening for colorectal cancer is supported by the highest level of evidence, namely randomized controlled trials, at the population level. However, as summarized in Table 62.1, this is not the case for all of the test options. As the evidence is strongest for tests that detect fecal blood or for those that endoscopically visualize lesions, we will critically evaluate these approaches in detail.

Table 62.1 Overview of available screening tests describing basis for detection of neoplasia, strength of evidence for efficacy, and factors that limit sensitivity and specificity

Detection goal	Technology	Strongest evidence for benefit	Place in screening process	Sensitivity determinants	Specificity determinants
Fecal blood	Guaiac-based FOBT	Population RCT – reduced incidence and mortality	Two-step	Amount of fecal heme	Dietary heme; bleeding nonneoplastic lesions
	Fecal immunochemical test (FIT)	Comparative cohort – better sensitivity or specificity	Two-step	Amount of fecal globin	Bleeding nonneoplastic lesions
Fecal neoplasm-derived DNA	Multitarget fecal DNA test	Comparative cohort – assessing sensitivity and specificity	Two-step	Spectrum of mutations in lesion	Unclear
Visualize lesion	Colonoscopy	Case–control and cohort – reduced incidence and mortality	One-step	Quality of procedure	Histopathology
	Flexible sigmoidoscopy	Case–control – reduced mortality	One- or two-step	Quality of procedure; limited depth of insertion	Histopathology
Imaging of lesion	Barium enema	None	Two-step	Quality of procedure	Lumenal artifact; nonneoplastic pathology
	CT colonography	Comparative cohort – assessing sensitivity and specificity	Two-step	Quality of procedure; precision of technology	Lumenal artifact; nonneoplastic pathology

CT, computed tomography; FOBT, fecal occult blood test, RCT, randomized controlled trial.

Evidence of effectiveness

As screening is a process where the goal is reduction in population mortality, randomized studies at the population level where mortality has been shown to be reduced even when analyzed on an intention-to-screen basis, i.e., regardless of whether people offered the test actually took it, is the most reliable indicator of effectiveness. We present the evidence available for the best-studied screening tests – namely FOBT, flexible sigmoidoscopy and colonoscopy – with the intention of developing an evidence-based guide for how to undertake screening.

Fecal occult blood tests

The fact that microscopic bleeding may arise from curable cancers and adenomas, provides the basis for screening using an FOBT [7,8]. However, the biology of bleeding is complex and for FOBT screening to be effective, these tests must be used properly in a way that maximizes their ability to detect bleeding from neoplasia.

Types of fecal occult blood test: guaiac and immunochemical tests

Available FOBTs are based on two principal technologies: chemical and immunochemical. The major biological and technological features of these tests are outlined in Table 62.2 [5,6,8,9].

Chemical fecal occult blood test

The chemical tests (e.g., Hemoccult II) detect the peroxidase activity of heme. The majority use guaiac as the reagent. These guaiac-based FOBT (GFOBT) react to any peroxidase in feces (e.g., plant peroxidases or heme in red meat) and are affected by certain chemicals (e.g., vitamin C). Because heme is relatively stable in the gut (although slow degradation occurs in the colon), GFOBT may detect bleeding from any site in the gastrointestinal tract, including the stomach [11]. Development and interpretation of GFOBT results seem simple but in practice the test is not reliable without training [12]. Most tests employ a wooden spatula for sampling stools, which should ideally be kept clear of the toilet bowl water to avoid leaching of hemoglobin from the stool [13].

Other GFOBT more sensitive than Hemoccult, such as HemoccultSensa, have been developed although at the cost of worsened specificity, especially if red meat has not been excluded from the diet before a sample is collected [9,10]. HemoccultSensa is also more readable [14]. Some studies indicate that if development of GFOBT is delayed 72 h after sampling, the problem of plant peroxidase interference is minimized [14] and dietary restrictions for controlling GFOBT specificity can be simplified such that only red meat and very high-peroxidase foods such as horseradish need be excluded. Unfortunately, unpredictably high positivity rates still occur in several ethnic communities [10,15], resulting in GFOBT not being of practical use.

Table 62.2 Nature, usage, and performance issues for different types of fecal occult blood tests (FOBT) [5, 6]

Type of FOBT	Chemical basis	Diet restrictions	Drug interference	Site of occult bleeding detected	Specificity for neoplasia[d]	Sensitivity for cancer[d]
Chemical (GFOBT)	Guaiac detects peroxidase activity of heme	Required: Red meats Possibly certain raw plant foods[a]	Vitamin C Possibly NSAIDs[b]	Rectum > colon > stomach (in decreasing order of sensitivity)	90%–98% depending on test brand, and usage	35%–67% with one-time testing Over 80% with repeated testing
Immuno-chemical (FIT)	Anti-human hemoglobin antibody detects globin	None required	None	Colon and rectum	Around 95% depending on sensitivity level chosen[c]	65%–90% with one-time testing Unclear for repeated testing

a Delaying development for 72 h minimizes interference from plant foods and avoids the need for their restriction with standard Hemoccult II. Red meats must be restricted when using a more sensitive GFOBT [9].
b Low-dose aspirin is not a problem, but therapeutic doses, such as those used for rheumatic disorders, may be a problem.
c Tests generally provide a qualitative result, but some newer FIT can be quantifiable.
d Indicative estimates only derived from Tables 62.3 and 62.4 and reference [10].
FIT, fecal immunochemical tests for hemoglobin; GFOBT, guaiac-based FOBT; NSAIDs, nonsteroidal antiinflammatory drugs.

Before the availability of more sensitive GFOBT, rehydration of Hemoccult test cards was applied to increase sensitivity [16], but this has an adverse effect on specificity by activation of plant peroxidases [14]. Guidelines to standardize development have never been established and rehydration is no longer recommended [9,14,17].

To summarize, while of proven efficacy in decreasing colorectal cancer mortality and incidence, chemical FOBT, i.e., GFOBT, has the following shortcomings [8].

- Restrictions of diet and drugs are needed to optimize specificity, especially for the sensitive GFOBT.
- GFOBTs generally use spatula sampling of stools, and stools need to be kept out of contact with toilet bowl water.
- GFOBTs are not selective for colorectal bleeding.
- The end point can be difficult to read and is transient.
- Sensitivity for cancer is suboptimal for less sensitive GFOBT brands.
- More sensitive GFOBTs are subject to unpredictably high false-positive rates.
- GFOBTs are not suitable for automated mass development or reading of the result.

Fecal immunochemical tests

Fecal immunochemical tests for hemoglobin (FIT) use antibodies that are specific for human globin and, therefore, these tests are not affected by diet [11,18,19]. They are subject to less variability in positivity rate than the sensitive GFOBT [15]. Because globin is rapidly degraded by digestive enzymes, these tests are highly selective for occult bleeding of colorectal origin [11].

Depending on the version of FIT and the manufacturer, improvements in fecal sampling have been made; these are discussed elsewhere in detail [8,9,18,20]. The tests may be developed in the office or laboratory and quality assurance of test development is much improved. It is beyond the scope of this chapter to further describe the technological details but laboratory development is preferred in many countries, especially for mass screening when many tests must be performed and quality assurance is vital [5]. Most branded versions of FIT require fewer than three fecal samples, the recommended number for GFOBT.

Several of the latest FIT allow quantification of fecal hemoglobin. The advantage of fecal hemoglobin measurement is that it returns full control of sensitivity and specificity to the end-user [6,8] who can establish the level of fecal hemoglobin that would trigger colonoscopy. At the population level, such an approach allows the test specificity–sensitivity ratio to be maximized, so adapting the colonoscopy rate to the facilities and funds available for screening.

Table 62.2 provides estimates of sensitivity of the different types of the FOBT, i.e., GFOBT and FIT. As a general rule, FITs are at the more sensitive end of the range while GFOBT tests vary widely across the range. A detailed comparison of the different FOBTs has been reported [9] and a recent study has shown a brush-sampling FIT to be more sensitive for cancers and advanced adenomas than even the more sensitive GFOBT [18]. A large-scale study of over 7000 people undergoing screening in California is informative in this respect [10]. It shows that an FIT achieves the same sensitivity as a sensitive GFOBT but with better specificity, i.e., fewer colonoscopies. FITs overcome most of the disadvantages presented by GFOBT and it has been concluded that FIT should replace GFOBT [9,18,20].

Table 62.3 Nature of randomized controlled trials of two-step screening for colorectal cancer by guaiac-based fecal occult blood test

Site	Funen [21–23]	Gothenburg [24,25]	Minnesota [16,26,27]	Nottingham [28–30]	Burgundy [31]
Design					
Status	General population	General population	Volunteers	General population	General population
Age (years)	45–70	60–64	50–80	45–74	45–74
Randomized by:	Individual/household	Individual/household	Individual/household	Individual/household	District
Screen group size	30 967	34 144	15 570 (annual) 15 587 (biennial)	76 466	45 642
Control group size	30 966	34 164	15 394	76 384	45 557
GFOBT used	Hemoccult	Hemoccult – rehydrated	Hemoccult (17.5%) Hemoccult – rehydrated (82.5%)	Hemoccult	Hemoccult
Program					
Period	1985–1995	1982–1984	1975–1992	1981–1991	1988–1998
Rounds of screening	5	2	Up to 10	5	6
Frequency	Biennial	18 months	Annual or biennial	Biennial	Biennial
Cumulative endoscopic follow-up[a]	5.3% (after 9 rounds)	6%	Annual – 38% Biennial – 28%	Unclear	3.7%

a Proportion of people undergoing colonoscopy after a positive GFOBT at least once during the trial.
GFOBT, guaiac-based fecal occult blood test.

Screening by fecal occult blood test: evidence of effectiveness

Six controlled trials of screening based on GFOBT have been reported [16,21–32]. The five of these where there was no planned intervention in the control arm [16,21–31] are summarized in Tables 62.3 and 62.4. Follow-up ranged from 8.3 to 18 years for these trials. In all of these trials invitees were asked to sample three separate stools for testing by the GFOBT, as it has been shown that when a cancer is present bleeding may be intermittent [33].

Intermediate outcomes

All studies reported a favorable shift in stage of diagnosed cancers, with more stage A cancers or fewer stage D cancers detected in the screened compared with control groups (Table 62.4).

Test positivity rates, and hence colonoscopy rates, varied greatly between the studies mainly because of the type of test used, but also because of the frequency of testing and the length of the trial (Table 62.3). In the studies where the GFOBT Hemoccult was used without rehydration, positivity rates were low and colonoscopy intervention rates fell in the range 2%–4% [21–23,28–31]. Where the fecal sample was rehydrated before development, the proportion of people undergoing colonoscopy at least once during the trial period was as high as 38% (Table 62.3) [16].

Complication rates for follow-up of positive screening tests are available for several of the trials (Table 62.4). In the Minnesota trial there were 12 serious complications (perforations or major bleeds) per 10 000 offered screening [16]. No complications were reported in the Burgundy trial [31]. Higher rates were observed in Gothenburg [24]. Bowles and colleagues [34] reported a perforation rate alone of 12 in 9223 colonoscopies examined as part of a prospective audit and six deaths were considered as possibly being a result of the procedure.

Behavioral outcomes

In the four true population studies (Funen, Gothenburg, Nottingham, and Burgundy), participation in the first round varied between 53% and 67% (Table 62.4). Participation fell off in the subsequent rounds where only initial participants were reoffered screening but it was maintained when nonparticipants were reoffered screening (e.g., Burgundy) [31]. When the study groups consisted of volunteers, as in the Minnesota study, initial participation was higher.

Impact on mortality from colorectal cancer

All of the trials offered FOBT on a *repeated* basis. The Gothenburg trial offered screening only twice, i.e., two rounds, and did not see a significant impact on mortality (Table 62.4). All other trials offered more than two rounds of screening and all showed a significant impact on colorectal cancer-related mortality based on an intention-to-screen basis. Analysis on the basis of intention-to-screen takes into account acceptance of screening, not just test performance, and is the public health outcome of greatest impact.

Table 62.4 Outcomes in randomized controlled trials of two-step screening for colorectal cancer by guaiac-based fecal occult blood test

Site	Funen [21–23]	Gothenburg [24,25]	Minnesota [16,26,27]	Nottingham [28–30]	Burgundy [31]
Intermediate measures					
Participation by round	First – 67% All – 46%	First – 63% Any – 68%	Any – 90%	Any – 60%	First – 52.8% Any – 69.5% All – 38.1%
Proportion of cancers at stage A	Screen[a] – 36% Control – 11%	Screen[a] – 26% Control – 9%	Annual[b] – 33% Biennial[b] –29% Control – 25%	Screen[a] – 21% Control – 12%	Screen[a] – 42% Control – 17%
Sensitivity for cancer	46%	81%	Hemoccult – rehydrated – 92% Hemoccult – 81%	59%	40.1% (computed from screen-detected plus interval cancers)
Main colorectal cancer measures					
Mortality on intention-to-screen basis, OR (CI) (follow-up duration)	0.82 (0.68–0.99) (10 years)	0.88 (0.69–1.12) (8.3 years) [25]	Annual – 0.67 (0.51–0.83) (13 years) Biennial – 0.79 (0.62–0.97) (13 years)	0.86 (0.74–0.99) (7.8 years)	0.84 (0.71–0.99) (11 years)
Participant mortality, OR (CI)	0.66 (0.54–0.81) (7 rounds)	Not stated	Not stated	0.61 (0.50–0.74)	0.67 (0.56–0.81)
Incidence of cancer, OR (CI)			Annual – 0.80 (0.70–0.90) (18 years) Biennial – 0.83 (0.78–0.94) (18 years)		1.01 (0.91–1.12) (11 years)
Major complications – colonoscopy		3/2296	15/12 246	"No deaths"	"None"

a Screen-detected lesions not whole screening cohort.
b Whole screening cohort.
CI, confidence interval; OR, odds ratio.

From Table 62.4 it can be seen that biennial screening with Hemoccult (not rehydrated) led to reductions in mortality of 14%–18% (Funen, Nottingham, and Burgundy trials [21,28,31]). A meta-analysis of the early results indicated a reduction in colorectal cancer mortality of 16% [25].

This effect on mortality seems modest but randomized controlled trials tend to underestimate the actual benefit of screening because of nonparticipation in the screened group and uncontrolled access to screening in the control group [35]. A better guide for magnitude of effect comes from the Funen, Nottingham, and Burgundy trials of actual participation in biennial screening with Hemoccult; mortality was reduced by 33%–41% in participants.

The Minnesota trial screened annually and used rehydration of Hemoccult to improve sensitivity [16]; it observed the greatest reduction in mortality on an intention-to-screen basis of 33%. The relative contribution of each is unclear because biennial screening in the Minnesota trial using rehydrated Hemoccult achieved a 21% reduction in mortality compared to the 14%–18% achieved with biennial Hemoccult in Europe. However, the Minnesota trial is the most informative for the magnitude of benefit because it had the best participation rate and the longest follow-up.

Impact on incidence (Table 62.4)

Cumulative incidence rates for colorectal cancer did not differ between the controls or screened groups in any of the trials by 13 years of follow-up. However, the Minnesota study by 18 years of follow-up (the longest) showed a significant impact on incidence whether screening was annual or biennial [27]. It seems likely that the higher sensitivity of rehydrated Hemoccult and the higher colonoscopy rate have resulted in a better detection (and thus removal) of adenomas.

Doctor's office or home-based fecal sampling?

Performing an in-office GFOBT as part of a digital rectal examination has been common practice [36]. However, usually only one stool sample, rather than the recommended

three, is obtained. The patient has not usually undertaken dietary preparation, which increases the risk of falsepositives with GFOBT [14], and there is always the concern that digital rectal examination will generate minor trauma [37]. FOBT as part of a digital rectal examination was only recently evaluated, in 2005, in a large group of asymptomatic subjects [38]. It was found that sensitivity for neoplasia was much better if 3-day sampling was performed compared to a single in-office test and a negative test did not reduce the odds of cancer being present. Screening based on digital sampling of stool as part of a rectal examination is not recommended.

Endoscopic screening tests: evidence of effectiveness

While the largest population screening trials have been undertaken using FOBT, the use of flexible sigmoidoscopy and colonoscopy for screening is also supported by evidence of impact on mortality of colorectal cancer.

Screening by flexible sigmoidoscopy

The evidence for efficacy of screening sigmoidoscopy comes from case–control studies, the design and outcomes of which are summarized in Table 62.5. In a case–control study of 66 subjects dying from colorectal cancer, the risk of dying was reduced by a single-screening flexible sigmoidoscopy (odds ratio [OR] 0.21; confidence interval [CI] 0.08–0.52) [39]. A similar study involving 261 cases of distal colorectal cancer found an adjusted OR, i.e., risk of death, of 0.41 (CI 0.25–0.69) in those who had had a rigid sigmoidoscopy within 10 years [40]. In both studies, the reduction in risk was limited to rectal and distal colonic lesions, i.e., to lesions within reach. More recent studies of flexible sigmoidoscopy (Table 62.5) continue to support the association [41,42] with similar risk reductions and benefit lasting 10 years or more after the procedure. Mandel [35] provides a critique of such case–control studies, pointing out that all such studies are liable to biases that overestimate the effect relative to randomized trials.

Screening sigmoidoscopy is challenged on the grounds that it represents an incomplete examination of the colon, albeit in the region where cancers are more likely [43] and if one is going to the trouble of performing an endoscopic examination, why not perform colonoscopy. Others see it as

Table 62.5 Outcomes in case–control and cohort studies addressing impact of endoscopic screening on colorectal cancer

Country	Design	Population	Intervention	Result	Duration of benefit	Reference
USA	Case–control	Dead from CRC, $n = 66$	Single flexible sigmoidoscopy	Dying from distal CRC, OR 0.21 (CI 0.08–0.52)	10 years	[39]
USA	Case–control	Dead from distal CRC, $n = 261$	Rigid sigmoidoscopy	Dying from distal CRC, OR 0.41 (CI 0.25–0.69)	10 years	[40]
USA	Case–control	"Incident CRC"	"Sigmoidoscopy"	Sigmoidoscopy done, OR 0.56 (men, CI 0.44–0.77). Similar in women, minimal impact on proximal CRC		[41]
USA	Case–control	Cases of distal CRC, $n = 1026$	Screening sigmoidoscopy	Sigmoidoscopy done, OR 0.24 (CI 0.17–0.33)	16 years	[42]
Germany	Case–control	Cases of CRC, $n = 320$	Sigmoidoscopy or colonoscopy	Endoscopy done, OR 0.28 (CI 0.16–0.48)	> 10 years	[50]
Israel	Case–control	Cases of CRC, $n = 40$ high-risk surveillance	Colonoscopy	Colonoscopy done, 48.7% of controls compared with 2.5% cases ($P < 0.05$)		[51]
Norway	Cohort	Screened vs not screened, $n = 400$ and $n = 399$, randomized	Flexible sigmoidoscopy initially, subsequent colonoscopy if neoplasia found	Developing CRC, RR 0.20 (CI 0.03–0.95)		[52]
Finland	Cohort	HNPCC complying vs refusing surveillance, $n = 133$ and $n = 119$	Colonoscopy at 3-yearly intervals	Developing CRC, 6% of surveillance vs 16% refusers ($P = 0.014$)		[53]

CI, confidence interval; CRC, colorectal cancer; HNPCC, hereditary nonpolyposis colorectal cancer; OR, odds ratio; RR, relative risk.

an efficient way to access a majority of colonic neoplasia at low cost, and include polypectomy as part of the procedure [44]. There are concerns about feasibility in terms of adequate numbers of proceduralists and inadequacy of reimbursement [45].

Until the randomized population studies under way in the UK, Italy, and the USA (the PLCO study) [46] are complete and adequate cost-effectiveness studies are undertaken, it is not possible to reach a firm conclusion on the place for flexible sigmoidoscopy in screening.

Screening by colonoscopy

Colonoscopy has long been established as a powerful diagnostic tool for colorectal neoplasia. It is also therapeutic in that successful removal of many adenomas and even early malignant polyps can be undertaken during the procedure. Cohort studies have demonstrated that polypectomy in people prone to develop polyps reduces the risk of cancer [47]. The technology is well established and the reader is referred to other relevant chapters for further discussion on its implementation and practice. Colonoscopy as a screening test is often justified on the basis that it is the most accurate test. Accuracy, however, should not be the sole criterion for choosing a screening test [6,48,49].

The evidence for efficacy of screening colonoscopy for reducing risk of or mortality from colorectal cancer comes from a few case–control studies and small randomized studies [50–53], summarized in Table 62.5. The magnitude of benefit seems large but the studies are small and there are several biases that magnify the effect [35]. The largest, a case–control study of 320 cases, found an OR of 0.28 (0.16–0.48) for diagnosis of colorectal cancer where screening sigmoidoscopy or colonoscopy had been undertaken [50].

Further evidence to support the use of screening colonoscopy comes from the FOBT trials because without colonoscopic follow-up of a positive test, these trials would not have shown benefit. Some argue that further proof of efficacy, in the form of randomized controlled trials, is unnecessary [54] because colonoscopy is more accurate.

Colonoscopic screening has been calculated to be a cost-effective strategy. Several studies have examined effectiveness and incremental cost-effectiveness of multiple screening strategies [48] and two found colonoscopy every 10 years to be the most effective [55,56]. In contrast, a model developed by Lieberman [57] showed that fecal occult blood testing alone was the most cost-effective strategy, but colonoscopy every 10 years became the most cost-effective strategy if the cost for diagnostic colonoscopy was under US$ 750.

How often should endoscopic screening be performed?

For flexible sigmoidoscopy, 5-yearly screening intervals are recommended based on the apparent protection for at least 5 years shown in the case–control studies. Screening flexible sigmoidoscopy may be performed in combination with GFOBT for better detection of proximal lesions although there is little evidence to indicate how much gain is achieved.

For screening colonoscopy, the question of how often to do it in people with average risk remains unanswered. There has been no large prospective study reported that addresses this. If screening colonoscopy is to be a once-only event, then the age at which it is performed is critical [58]. Once-only screening is only sound if we can be confident that most cases destined to develop colorectal cancer in their lifetime will have a "sentinel" lesion at the time the colonoscopy is performed.

When choosing an interval for screening by colonoscopy, there are clues from the few case–control studies where intervals of at least 5 years, and sometimes 10 years, seem justified [50–52]. Some consensus recommendations, e.g., that of the USPSTF [48], recommends 10 years in average risk people. However, cancers may be missed and interval cancers occur in surveillance programs in high-risk individuals [59,60] Without evidence from formal randomized trials, it is not possible to be confident about the appropriate interval.

Is colonoscopy the screening test of first choice?

There are several perspectives to this question and they arise from the two main contexts of screening discussed above.

In the context of individualized screening, differential sensitivity of colonoscopy against FOBT or flexible sigmoidoscopy becomes a consideration in practice, because people often want what is perceived as being best. Colonoscopy is clearly more sensitive [61–63].

On the other hand, some argue that there is a strong case for caution before colonoscopy is accepted as the ideal screening test [48], especially as part of a programmed public-health initiative. Many points must be considered.

- The sensitivity of colonoscopy compared to once-only sigmoidoscopy or FOBT overestimates the benefit. Repeated application of FOBT results in compounding of sensitivity, probably because the dwell time of a curable lesion is longer than the screening interval, meaning that curable lesions may still be detected in a subsequent round of testing. This is borne out in practice: sensitivity of once-only Hemoccult has been shown to be less than 40% [10] yet cumulative sensitivity of unhydrated Hemoccult in the Minnesota trial was 80% (Table 62.4).
- Is the lower sensitivity of the simpler screening tests for advanced neoplasms truly unacceptable? For flexible sigmoidoscopy, the deduced miss rate for advanced proximal neoplasms has been estimated at 24% [61] but the risk of advanced proximal neoplasms progressing to fatal cancer is relatively low, estimated at 1% per year [64–66].
- Colonoscopy is not perfect in practice and lesions are missed [59, 60, 67–69]. Flat lesions in particular are easy to miss during conventional endoscopy [44] and flat or depressed adenomas may progress more rapidly to cancer

than polypoid adenomas. If the cancers that are most likely to be fatal are those that develop and grow rapidly [48, 70], there is an advantage to a screening program that allows multiple chances to detect an existing lesion rather than testing just once every 10 years [48,70].

- The serious complication rate with colonoscopic screening is not trivial. One study, where all procedures were carried out by skilled operators, reported a rate of 33 per 10 000 people screened [34]. This is over twice the rate with FOBT screening (Table 62.3).

Colonoscopy may uncover the largest number of neoplasms and minimize the likelihood of missing any colorectal neoplasia but screening policy is not decided by effectiveness alone. If alternative screening strategies can significantly reduce the death rate from colorectal cancer but at an acceptable or lower cost and risk than those associated with colonoscopy, these strategies would be appropriate. The incremental effectiveness of colonoscopy needs to be large enough to justify its disadvantages (considerable manpower and financial resources, bowel preparation, conscious sedation, and serious potential complications). The millions (i.e., almost 95% of the general population) who will undergo colonoscopic screening for no apparent gain are subject to harms that could cumulatively outweigh the benefit to the much smaller number found to have significant colorectal neoplasia [49].

Summary of the effectiveness of available screening tests

Guaiac-based fecal occult blood tests and fecal immunochemical tests

It can be concluded from this evidence that screening of the general population, most of whom will lack high-risk factors, using a GFOBT is justified. The data show that screening should be repeated, with intervals of 1 year possibly giving a better effect than biennial screening. It should be noted that participants in the GFOBT trials were asked to sample three stools in the home environment. There is no evidence that collecting fewer samples by digital rectal examination in the physician's office is effective. Use of a sensitive GFOBT is preferred to rehydration of Hemoccult. Overall, FITs overcome most of the disadvantages presented by GFOBTs. Based on performance, FIT should replace GFOBT in screening for colorectal cancer [18].

Such recommendations are consistent with consensus recommendations from many national or professional groups [17,48]. Most bodies recommend that screening starts in the general population (i.e., undefined or average risk) at age 50 years, because around 90% of colorectal cancer occurs after that age. Cost-effectiveness of screening the general population at a younger age is of questionable value.

Colonoscopy and flexible sigmoidoscopy

On the basis of the studies described above it can be concluded that endoscopic screening will be effective. However, compared to GFOBT, we lack evidence from randomized controlled trials that address acceptability, incremental benefit and complication rates – it is important to understand the balance of risks and benefits. Because case–control studies tend to overestimate benefit, we cannot be certain of the magnitude of incremental benefit provided by endoscopic screening compared with FOBT screening.

Recent developments in screening tests

A recent American Gastroenterological Association Future Trends Committee report on emerging screening and diagnostic technologies for colorectal cancer [19] identified a range of tests and procedures that might be appropriate. These include proteomics or the analysis of broad protein patterns, making it possible to assess small amounts of protein for the presence of identified cancer markers using new protein assessment tools and computerized artificial intelligence analysis. In addition, high-magnification chromoscopic endoscopy [71], spectroscopy, and optical coherence tomography show promise as adjuncts to traditional colonoscopy. Fecal DNA testing also provides new options. The better studied advances, such as stool DNA testing and computed tomography (CT) colonography, will be discussed in some detail.

Fecal DNA tests

Cells shed from cancers and adenomas are detectable in the stools [72]. Detection of molecular or genetic events that either cause cancer or else reflect development of neoplasia could theoretically be useful in selecting who undergoes colonoscopy. Because of the molecular heterogeneity of DNA in neoplasms, selecting the best panel of markers represents a challenge.

The first large-scale evaluation of the value of fecal DNA testing (using a 21-mutation multitarget panel) as a possible first-step test in two-step screening for colorectal cancer has recently been reported [73]. The present version of the stool DNA test is costly (in excess of US$ 400) and requires a cumbersome stool collection that needs to be rapidly delivered to the processing laboratory [74]. The comparators were colonoscopy as the diagnostic reference standard and Hemoccult II (unhydrated) as the proven first-step screening test. A cohort of 4404 subjects completed all three tests and their results were analyzed in a subgroup of 2507, which included all subjects found to have neoplasia. The detection rate of cancer using the fecal DNA panel was 16 of 31 (52%) compared with 4 of 31 (13%, $P = 0.003$) for Hemoccult II. The reported sensitivity of Hemoccult II was substantially lower than that reported in other studies for one-time testing [10] and so it cannot be concluded that Hemoccult, a much cheaper test, is necessarily inferior. The performance of both tests for detecting advanced adenomas was similarly disappointing; 61 of 403 (15.1%) for fecal DNA compared with 43

of 403 (62.7%) for Hemoccult II. In those with negative colonoscopy, 5.6% had tested positive on fecal DNA compared with 4.8% on Hemoccult II (specificities of 94.4% and 95.2%, respectively). Fecal DNA testing did not identify the majority of neoplastic lesions found at colonoscopy and in this format it does not yet represent an advance over the much cheaper FOBT types [74].

It is expected that within the next decade, this innovative technology will eventually lead to more specific and simple tests that will provide an effective first test in a two-step screening process. The newer stool molecular tests might be based on RNA or protein markers that are subject to lesser heterogeneity than DNA tests. A more detailed review is presented by Young [20].

Computed tomography and magnetic resonance imaging colonography

Virtual colonoscopy, also known preferably as computed tomographic colonography (CTC) was first described in 1994. It is an imaging method for detecting pathology in the colon and rectum that is less invasive than colonoscopy [75].

It has been pointed out that CTC avoids some of the drawbacks of colonoscopy, such as the need for intravenous sedation, recovery time, and assistance with transport [76]. The risks of bleeding, perforation, and side effects of sedatives will be much less than with colonoscopy. As the majority of screening colonoscopies identify no clinically significant pathology, CTC, as with other first-step tests such as FOBT, should reduce unnecessary colonoscopies.

With the latest equipment, CTC takes 10–15 min. Advances in software now allow for typical reading times of 10 min or less and final reports can be issued within 2 h. It might eventually prove possible to avoid colonic cleansing using "preparation-less" CTC where an ingested contrast material is used to "tag" lumenal content. Imaging software digitally subtracts tagged material by a process known as electronic cleansing [77].

Performance characteristics of screening CTC have been difficult to assess because CTC is a technology undergoing rapid improvement. In a metaanalysis of 33 studies providing data on 6393 patients [78], sensitivity was 48% for polyps less than 6 mm, 70% for polyps 6–9 mm, and 85% for polyps larger than 9 mm. Specificity also varied according to size: 92% for detection of polyps less than 6 mm, 93% for polyps 6–9 mm and 97% for polyps larger than 9 mm. A weakness of the Mulhall analysis was that only a few studies included in the metaanalysis used the latest technology.

The use of the latest technology including electronic cleansing and multidetector CT scanners which permit faster higher resolution imaging than single detector scanners has been reported on by Pickhardt and colleagues [68]. They prospectively evaluated 1233 average-risk asymptomatic adults who underwent CTC and same-day conventional colonoscopy. The sensitivity of CTC for adenomatous polyps was 93.8% for polyps at least 10 mm in diameter, 93.9% for polyps at least 8 mm in diameter, and 88.7% for polyps at least 6 mm in diameter. The sensitivities of optical colonoscopy for adenomatous polyps were 87.5%, 91.5%, and 92.3% for the three sizes of polyps, respectively.

While such results are excellent, the practical application of screening CTC requires resolution of the following factors [76,79,80].

- Results in practice are not comparable to those of specialized centers.
- Can some polyps be safely left in place because chance of progression is very low. In other words, which types of lesions require immediate follow-up colonoscopy?
- CTC would ideally be coordinated with follow-up colonoscopy on the same day.
- The workload generated by false-positive CTC, caused by fecal matter, nonadenomatous polyps, or insignificant adenomas, remains unclear.
- Detection rate of flat adenomas by CTC is unclear [81].
- Population acceptance of a procedure that is uncomfortable and that requires time and possibly bowel preparation remains unclear. "Preparation-less" CTC is an unknown at present.
- The impact of finding extracolonic pathology is unclear. One study has undertaken a cost-effectiveness analysis [82]; 118 of 432 subjects (27.3%) had extracolonic lesions. In 7.4% it was felt that findings were clinically relevant and in 2.1% it was felt that subjects may derive a clinical benefit from treatment. Clinical follow-up added just 14.2% to the cost. Such findings suggest that extracolonic lesions might not pose a major problem.
- The cost-effectiveness of population screening with CTC is unclear.

Computed tomographic colonography has the potential to be a valid first-step screening test for colorectal cancer. It can accurately exclude the presence of large polyps and, by inference, cancer with high confidence. Technology improvement is likely to make it more sensitive for target lesions and perhaps more acceptable to those being screened, in the not too distant future. If shown to be cost-effective and applicable to large populations of average risk individuals, it may stand beside other established first-step screening tests such as FOBT, flexible sigmoidoscopy, and colonoscopy.

Other considerations in screening practice

As screening is a process, it is important that each step is feasible, otherwise benefit will not be obtained. Sustainability is also important because screening is unlikely to have a major impact if it is a one-time event in a person's lifetime [74].

Without participation there can be no detection [6]. Removal of dietary restrictions in several populations has been shown to enhance participation in screening with FOBT

[83], in one study by 28% [84]. The brush sampling method also simplifies the process and enhances it by 30%. Together, these two advances increase participation by 66% [84]. Obviously, FIT is superior to GFOBT in terms of both participation and performance and should replace GFOBT in two-step screening.

Capacity to undertake colonoscopy, whether as a follow-up assessment of those with a positive FOBT or as a one-step colonoscopic screening test, is critical. Many countries, perhaps all, are unable to comprehensively resource one-step colonoscopic screening. Evidence suggests that the manpower necessary to provide a skilled colonoscopic examination for all age-eligible people in the United States is insufficient [85,86].

Managing risk and duty of care

It is of paramount importance that risks as well as benefits are carefully explained to those being invited to take part. Informed consent must be obtained and the patient must be told that even with colonoscopy, it is possible that polyps or cancers may be missed. As two-step screening is effective and directs colonoscopy to those more likely to have neoplasia [6], there is a duty of care to offer this option. It is important to indicate that better sensitivity of colonoscopy is at the cost of more procedures and higher risk of complication to the screened person.

Conclusions

Screening for colorectal cancer detects unsuspected neoplasia and leads to its removal such that death or suffering from colorectal cancer is avoided. Proof of the value of screening is available even when studies are evaluated at a population level on an intention-to-screen basis where nonparticipating invitees are included in the analysis.

Two-step screening based on FOBT as the initial test is proven to be effective by population randomized controlled trials. FIT has emerged as the FOBT of choice because of a better sensitivity/specificity balance than GFOBT and better population acceptance. Collection of two or three samples for FOBT testing is necessary and there is no evidence that a single fecal sample collected by digital examination in the office is an effective screening strategy. Flexible sigmoidoscopy, while effective as judged from case–control studies, is initially invasive, inconvenient, and uncomfortable compared to FOBT. It will miss proximal lesions although misses can be reduced by coupling with FOBT. The results of the randomized trials of flexible sigmoidoscopic screening are needed before a clear conclusion can be reached on its role.

One-step screening by colonoscopy is likely to provide benefit but incremental cost–benefit and risk–benefit relative to FOBT screening is not clear. Colonoscopy is more likely to be accepted in the context of individual screening where there is face-to-face discussion with a physician. Acceptance at the population level where the offer of screening is more impersonal but also more consistent with the modern lifestyle, is unclear and not likely to be good. Estimates of the workforce required to deliver comprehensive colonoscopic screening raise doubt as to the practicality of using colonoscopy as a public-health initiative.

Fecal DNA tests and CTC are potential additions to FOBT in two-step screening but much more data on their effectiveness, acceptability, and cost–benefit are needed before they can be accepted for routine use. Stool molecular tests hold great promise for the future, especially if they can serve as sensitive and specific tests to identify those who have adenomas.

Screening should not be seen as merely the performance of a one-time test. Repeated testing improves benefit.

On balance, screening of average-risk subjects by the one-step or two-step approach is appropriate practice, starting at age 50 years. An FOBT, preferably of the FIT type, should be undertaken at least as frequently as 2-yearly (biennial), while the maximum safe interval for screening by flexible sigmoidoscopy and colonoscopy remains to be ascertained from prospective studies.

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D COLON

63 Neoplastic and nonneoplastic polyps of the colon and rectum

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A *polyp* is any protrusion above the mucosal surface. Polyps may take various sizes and shapes, including sessile or pedunculated. They can also be described according to texture of the mucosal surface, position, color, ulceration, bleeding, and histological type. Polypoid lesions of the large bowel are normally classified into three main subgroups (Table 63.1): epithelial polyps, subclassified into neoplastic polyps (consisting of adenomas or carcinomas [1,2]) and nonneoplastic polyps; and submucosal lesions (which produce a polypoid appearance).

Colorectal polyps are important for several clinically relevant reasons. First, they are easily detected and removed with modern flexible endoscopes (see Chapter 126). Largely because of this, our understanding of their role as precursors of colorectal carcinoma has increased. Ready access to polyps has led to the elucidation of the genetic basis of the major inherited polyposis syndromes. Finally, intervention trials aimed at removal of adenomas or reduction of metachronous (newly developing) adenomas have demonstrated polypectomy to be valuable for controlling colorectal cancer [3–5] (see Chapters 65 and 126).

The natural history of the adenomatous polyp is variable, but it seems that when progression to carcinoma occurs it generally takes 3–7 years, although the progression of adenomas in patients with hereditary nonpolyposis colorectal cancer (HNPCC) appears to be faster.

Management of the patient with a colorectal polyp requires an understanding of the pathology, epidemiology, etiology, clinical manifestations, and course as well as the appropriate diagnostic studies and therapeutic options [1,2].

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Epidemiology

Prevalence

Information on the prevalence of polyps comes from autopsy studies and from colonoscopic surveys, which are of a much smaller scale. Autopsy studies generally show a higher prevalence of polyps and adenomas than endoscopic studies. Prevalence rates vary considerably between gender (males more common), ethnic groups, and countries, and reflect the rates of colorectal cancer in those population groups (see Chapter 63). In the United States, autopsy studies suggest an overall polyp prevalence of up to 50% [6,7], but colonoscopic surveys suggest no more than 40% [8–15]. In autopsy studies, fixation of the colon and careful use of a magnifying glass allow detection of small polyps that are more readily missed during colonoscopy [16–20].

More than 90% of polyps detected at colonoscopy are epithelial in origin, that is, adenomas or hyperplastic polyps, and 75% of these are adenomas [8–13]. In the case of diminutive epithelial polyps (less than 5 mm), about half are hyperplastic [14,15].

Age is the major determinant of adenoma prevalence [6,7]. Size at detection also increases with age [20]. Colorectal adenomas are uncommon in patients younger than 30 years of age.

Anatomical distribution

Studies show that colorectal adenomas are more common in the distal colon and rectum, similar to the distribution of colorectal cancer (see Chapter 63). Distribution, however, relates to size. Small adenomas are more uniformly distributed throughout the entire colon, whereas large adenomas (greater than 1 cm) show a distal predominance [7,8,21,22].

Table 63.1 Classification of colorectal polyps according to tissue plane and pathology

Epithelial		
Neoplastic	**Nonneoplastic**	**Submucosal**
Premalignant (adenomas)	**Inflammatory**	Lymphoid collection
Tubular	Hyperplastic	Pneumatosis cystoides intestinalis
Tubulovillous		Colitis cystica profunda
Villous		Lipoma
Low-grade dysplasia		Carcinoid
High-grade dysplasia (intraepithelial carcinoma)		Metastatic lesions
Serrated		Hemangioma
		Fibroma
		Endometriosis
		Leiomyoma
Malignant (carcinomas)	**Hamartoma**	
Carcinomatous or malignant polyp	Juvenile	
	Peutz–Jeghers	

Association of adenomas with other diseases

Associations between colonic polyps and other diseases, apart from the polyposis syndromes discussed in Chapter 63, are infrequent. In a prospective study of acromegalic patients, nearly 50% had adenomas, increasing in prevalence with age [23].

Nonneoplastic polyps

The epidemiology of nonneoplastic polyps is not well studied except for the hyperplastic polyp, the second most common colorectal polyp [14,24]. Hyperplastic polyps usually are less than 5 mm in diameter and are most commonly found in the rectosigmoid area. Some studies have found an association between distal hyperplastic polyps and more proximal adenomas and colorectal cancer [25–30] but others have not [31–33], especially in the asymptomatic screening setting [33–37].

The predominant evidence from the larger and better controlled studies would suggest that hyperplastic polyps are not premalignant and do not signify a risk for colorectal neoplasia. In the US National Polyp Study, individuals with distal hyperplastic polyps were no more likely than those without to have more proximal colorectal neoplastic polyps [31]. Where associations are found, the relative risks are not clinically significant, and should therefore not determine clinical practice. Risk factors for hyperplastic polyps and adenomas are, however, surprisingly similar, complicating the interpretation of studies of associations with cancer to each type of polyp [38].

Hyperplastic polyposis and serrated adenomas

Individuals with *multiple* hyperplastic polyposis are clearly at increased risk of colorectal cancer [38]. Perhaps relating to this, molecular studies indicate that large right-sided sessile "serrated" hyperplastic polyps are prone to oncogenetic and epigenetic changes leading to genetic instability and neoplasia [39–41]. The descriptor "serrated" refers to the distinctive sawtooth architecture on histopathology. Serrated adenomas have the additional feature of neoplastic nuclei, that is, dysplasia (see Features of neoplastic polyps: Serrated adenomas and mixed hyperplastic–adenomatous polyps).

Clinically, sessile serrated polyps and serrated adenomas are associated with the syndrome of hyperplastic polyposis. This association might represent an extreme end of a clinical spectrum, driven perhaps by an as yet undiscovered germline predisposition. The syndrome and its inherent risk for malignant disease should be considered when hyperplastic polyps are numerous (more than 20, i.e., polyposis), are large (> 1 cm), are proximally located (especially if more than five are proximal to sigmoid colon), and especially when there are serrated adenomas. A family history of hyperplastic polyposis is uncommon (suggesting a recessive disorder) but colorectal cancer in a first-degree relative of a patient with hyperplastic polyposis is common and reflects inherited increased risk, perhaps associated with the putative heterozygous state [40].

Etiology and pathogenesis

The etiology of colonic polyps varies with histological type. For instance, inflammatory polyps result from the inflammatory and regenerative responses to injury whereas neoplastic polyps result from DNA aberrations. The remainder of this section focuses largely on neoplastic polyps because of their clinical significance and prevalence.

Adenomas are benign neoplasms; all show dysplasia (by definition). Dysplasia is characterized by cells with enlarged,

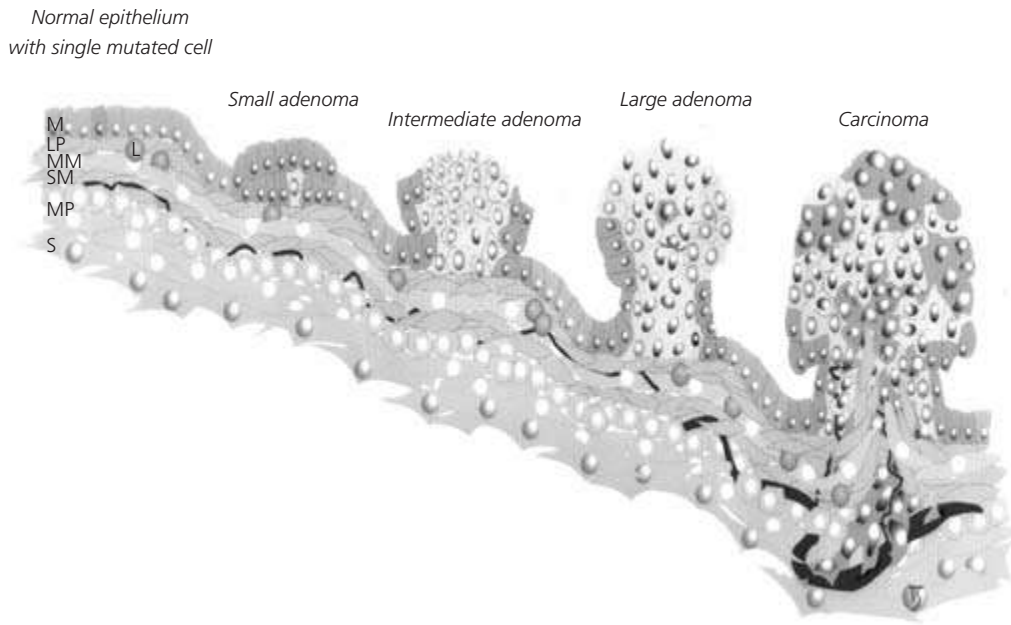

Figure 63.1 Model of development of a malignant colorectal carcinoma by a series of phenotypic changes and related genetic alterations. **Normal epithelium:** A single cell, frequently with a mutation in the *APC* gene (on chromosome 5), will give rise to the small adenoma in the next stage. **Small adenoma:** At this stage, when dysplasia is usually mild, both alleles of *APC* are affected by deletion or truncation (chromosome 5q). Changes in the level of methylation of DNA also may be observed. Note that a single cell in the clone has acquired an additional mutation in its *KRAS* gene (chromosome 12p). **Intermediate adenoma:** The cell with the *APC* and *KRAS* mutations has given rise to a new clone, forming an intermediate adenoma. An additional mutation in one of the cells, such as loss of heterozygosity (LOH), followed by deletion of a tumor suppressor gene (such as *DCC* [deleted in colorectal cancer] on chromosome 18q), gives this cell an additional survival advantage. **Large adenoma:** The cell with mutations and deletions in the *APC* and *KRAS* genes and LOH on chromosome 18q has clonally expanded into a large adenomatous polyp that has not invaded underlying mucosal layers. One cell of this clone acquires an additional mutation in the *p53* gene (chromosome 17p). **Carcinoma:** The cell that has accumulated mutations in the *APC KRAS* and *p53* genes and developed LOH on chromosome 18q has grown into a clone of malignant cells that penetrates through the lamina propria and invades underlying tissues. Note how cells are capable of lysing capillary walls and entering the vessels to be transported to distant sites where they form metastatic tumors. (Additional changes to proteolytic enzymes such as urokinase or telomerase may be required for metastatic behavior.) M, mucosa; LP, lamina propria; MM, muscularis mucosae; SM, submucosa; MP, muscularis propria.

hyperchromatic, elongated, and less polar nuclei often arranged in a picket-fence pattern (i.e., somewhat stratified), with variably decreased cytoplasmic volume, stroma, apoptosis, and cellular mucin, and variably increased numbers of mitotic cells. These changes result in a more basophilic appearance to the adenomatous epithelium in hematoxylin–eosin-stained sections [42,43]. Dysplasia ranges from mild to severe (Fig. 63.1). The terms *severe dysplasia* or *high-grade dysplasia* have been used synonymously with, and are preferred over, the terms *carcinoma in situ* or *intraepithelial cancer* when the severely dysplastic cells are confined within the basement membrane [44,45]. High-grade dysplasia is characterized by marked cytological change with disordered crypt architecture. Adenomas may be classified by predominant histology; tubular adenomas are the most common, constituting 80%–85% of all adenomas [46,47]. Tubulovillous adenomas constitute about 10%, with villous adenomas making up the remaining 5% [47].

Increasingly, studies refer to "advanced" adenomas. Definitions vary, but usually this refers to adenomas that are either 1 cm or larger in any dimension, or have a villous component (> 25% villous) or have high-grade dysplasia. Some reports also include the setting where a patient has multiple (three or more) adenomas even if the above features are not present [48]. This concept has evolved to identify patients at greater risk of developing cancer, with the implication that prevention or detection of advanced adenomas is a more useful target than detection of small tubular adenomas without severe dysplasia. There is empirical evidence to support this, both from observations of rates of malignancy associated with these features, and follow-up studies of patients with these adenomas previously removed (see Predicting further development of adenomas).

Cell kinetics in normal epithelium and polyps

The healthy colonic epithelium is characterized by constant cell proliferation and differentiation, resulting in total renewal of the surface epithelial cells about every 3–6 days. The proliferative compartment is situated in the basal third of the crypt. Daughter cells migrate up the crypt and out onto

the surface. As the cells migrate, proliferation ceases, and maturation begins – goblet cells become common [49,50]. The process of cell proliferation, maturation, and death is highly regulated, resulting in a homeostatic balance between production and loss [51].

In adenomas, the normal homeostatic process is disordered. Cell proliferation extends up the crypt. Differentiation is delayed (with fewer goblet cells), and apoptosis is reduced [42,49,52]. This enhanced proliferation and retarded cell maturation results in an increased number of cells. This abnormal cellular load downward infolding of the crypts, which interpose and branch between the normal crypt elements [42,52–54]. Mesenchymal proliferation is also thought to play a role in the formation of adenomas, especially when villous change occurs [53–55]. Although there is still some controversy about the dynamics of adenoma formation, most evidence points to the "bottom up" theory of adenoma formation, from cells situated toward the base of the crypt [56]. Crypt fission is involved in the formation of both types of polyps, but proliferation leads to infolding and the typical sawtooth appearance of hyperplastic polyps [57].

The entire colonic mucosa of patients with adenomas may show increased epithelial cell proliferation, an observation that qualifies epithelial proliferation as a biomarker for neoplasia in the colon [50].

Hereditary factors and adenomas

Hereditary factors obviously play the key role in familial adenomatous polyposis (FAP) and HNPCC [58]; adenoma biology is characteristically disturbed in each condition, and is well characterized at the molecular level (see Chapters 63 and 65). Screening programmes show higher cancer rates in those with a family history of adenomas compared with those without [58,59].

Analysis of family pedigrees in the state of Utah has identified an autosomal dominant pattern of inheritance of adenomas. About 200 members of a large pedigree with familial clustering of colorectal cancer, without evidence of the known inherited syndromes of colorectal cancer, were systematically examined by flexible sigmoidoscopy [60]. Twenty-one percent of family members, but only 9% of spouses, were found to have one or more adenomas. In comparison, hyperplastic polyps were found in 24% of family members and 29% of spouses [60,61]. It was found that an autosomal dominant inheritance of susceptibility to adenoma (and colorectal carcinoma) best explained the pattern of adenoma and carcinoma occurrence in the pedigrees [60–62]. In addition, the gene frequency was estimated to be 19% in that population. Subsequent analyses of additional pedigrees of colorectal cancer patients continue to demonstrate a two-fold to three-fold increase in adenomas in family members compared with spouse controls [58,61–64].

Considered together, these results suggest that familial or hereditary factors may play a role, perhaps as much as 30%, in the causation of adenomas [58,60–64]. If it is the adenoma rather than cancer that signals the inherited risk then there are substantial implications for screening based on family history and the complexity, cost, and magnitude of these programs will increase dramatically as adenomas are so common. Some authorities, but not all, advocate such screening. More information is needed on the risk to other family members when an adenoma is detected.

Environmental factors

Epidemiological studies suggest that environmental factors play an important role because worldwide variation in incidence is not fully explained by genetic variations [65]. Diet is probably the most important environmental factor. The major related factors will be discussed.

Obesity

Weight gain is relevant to adenoma occurrence [66,67], as is obesity. Whereas the evidence linking obesity with neoplasia is based on observations on cancer, there is an implied link to adenoma unless the effect is stage specific. Obesity, particularly central obesity, is an independent factor, but not as strong as weight gain per se, for colorectal cancer and adenomas [66–69]. Obesity interacts with other lifestyle variables in a manner that is difficult to disentangle [65].

The 2002 International Agency for Research on Cancer (IARC) report summarizes the relationship of colorectal cancer risk and body mass index (BMI), especially in men, concluding that there is sufficient evidence to support a link between weight gain and cancer of the colon, and certain other types of cancer, but inadequate evidence to show that intentional weight loss reduces cancer risk for any site [69]. The report also concluded that regular physical activity reduces the risk of colorectal cancer, independently of weight control.

The American Cancer Society Cohort Study of over 900 000 adults identified relative risks of cancer death in the morbidly obese compared with those of normal weight of 1.52 (confidence interval [CI] 1.13–2.05) for men and 1.62 (CI 1.40–1.87) for women. There was also a significant association between BMI and death rates for both colon cancer and rectal cancer [70]. In a Canadian prospective study, the greater risk associated with obesity was found mainly in premenopausal women [71]. Intentional weight loss was associated with a reduction in colorectal cancer risk [68,72].

Physical activity

The evidence that physical activity protects against colorectal cancer is strong but varies by site in the bowel. It is strongest for colonic rather than rectal cancer, again with inferred importance for adenomas. Some of these studies showed statistical benefit restricted to one gender, specific age ranges, intense but not moderate levels of exercise, or only with one of either recreational or occupational exercise categories [66].

Levels of activity needed to protect against colon cancer are 30–60 minutes per day of moderate to vigorous physical activity, and the relationship is dose-dependent [72,73]. Physical activity (> 2 h/week) protects against advanced adenomas more than nonadvanced adenomas, suggesting stage-specific effects [74]. Confirmatory studies suggest physical activity can modify adenoma risk except in the severely obese [75]. In a colonoscopy controlled case–control study, the odds ratio was lowest when physical activity was combined with low waist:hip measurement ratio [76].

Various mechanistic hypotheses are postulated. These include reduced bile acid secretion, stimulation of intestinal transit, immune and hormonal changes, and a reduction in levels of insulin-like growth factor (IGF, trophic to the colonic mucosa) [66,77,78].

Alcohol

With some exceptions, total alcohol intake is more consistently associated with risk for colorectal cancer than specific types of alcoholic beverages [79]. In cohort studies, significant associations between alcohol intake and risk have been found in four of five colon cancer studies; three of three rectal cancer studies; and two of three studies of colorectal cancer (subsite not distinguished) [65]. A US cohort study of men showed a particularly strong association of alcohol intake with the development of colorectal cancer and adenomas in individuals with low folate or methionine intakes [80].

In case–control studies, significant associations between alcohol intake and increased risk have been found in 9 of 18 studies of colon cancer and 9 of 17 studies for rectal cancer [65]. This effect is generally stronger in men than women. Australian studies have found a similar positive association with beer drinking in men [81,82].

A pooled analysis of eight cohort studies reports a modest increase in risk for colorectal cancer with consumption of three or more drinks per day (relative risk [RR] 1.41, CI 1.16–1.72), a risk that was not site specific within the large bowel [79]. In a French case–control study heavy drinking was also related to advanced adenomas [83]. Direct observation of alcohol intake on adenoma growth in vivo showed a low effect at low alcohol intakes over 3 years [84].

Colorectal adenomas, especially advanced adenomas, also are related to high alcohol intake [83]. There may be a particular risk for adenomas in people with certain alcohol dehydrogenase genotypes [85].

Tobacco smoking

Of 51 case–control or cohort studies of colorectal cancer, 22 show a 50% increase in risk from tobacco smoking, and 10 out of these 22 are statistically significant. The evidence is stronger for rectal than colonic cancer, and risk is increased for those who have smoked for a longer period [86,87]. Data are even more consistent for adenomas, with 22 of 27 case–control or cohort studies showing at least a 50% increase in risk. Nineteen are significant and 9 of 10 have a positive dose response [66]. Thus, the main effect of smoking seems to occur early in the process, during adenoma formation, and applies especially to distal sites in the large bowel [88]. Smoking is also recognizable as a risk factor in vegetarians [89].

Smoking may cause specific mutations [90] and may induce activation of CYP1A2 enzymes in the liver to metabolize heterocyclic amines to mutagenic substances [91], particularly with reference to cancers progressing through the microsatellite unstable pathway [92].

Energy intake

Case–control studies consistently show a positive association between energy intake and colorectal cancer risk. As fat intake is closely associated with energy intake, it has been difficult to differentiate between the two [93]. A metaanalysis of 13 case–control studies of diet and colorectal cancer found little evidence of an energy-independent effect on risk of either total or saturated fat intake [94].

A role for insulin or IGF-1 as an endogenous hormone trophic to colorectal neoplastic lesions has been suggested. Epidemiological studies provide mixed evidence to support this. The Women's Health Study of 39 876 health professionals reported a positive association (RR 2.85, 95% CI 1.40–5.80) for dietary glycemic load ($P = 0.004$ for trend across quintiles), supporting this hypothesis [95]. The PLCO (Prostate, Lung, Colorectal and Ovarian) Cancer Screening study has also found an association with IGF-1 and insulin for all adenomas and advanced adenomas [78]. Total carbohydrate intake was also significantly related (RR 2.41, CI 1.10–5.27), especially with respect to nonfiber carbohydrate, namely sugar (RR 2.60, CI 1.22–5.54). However, a US prospective cohort study of 49 124 women showed no association between colorectal cancer incidence and glycemic load or total carbohydrate or sugar consumption [96].

Dietary fat

Although multivariate analyses show no energy-independent effect of fat on colorectal cancer risk, direct relationships between adenoma risk and fat intake are evident, and mechanisms of risk appear more plausible relating to fat than energy, although either is possible. Prevalence of colorectal adenomas, as with cancers, has been correlated with high dietary fat intake (animal fat intake greater than 40% of total caloric intake) [97] (Table 63.2). Conversely, dietary fat intake of less than 15% of total caloric intake has been associated with a low frequency of colorectal adenomas and cancers [98–103].

Increased dietary fat has been shown in experimental animals to lead to increased hepatic synthesis and presence in colonic contents of cholesterol and bile acids. Bacterial flora in the colon might convert these sterols into cholesterol metabolites and oxidized bile acids, which have tumor-promoting activity [104]. Higher fecal concentrations of bile

Table 63.2 Values for relative risk for colorectal adenoma in relation to dietary intake

Dietary component	Quantile of intake: Low 1	2	3	4	High 5	95% CI	*P* value for trend
Energy	1.0	0.81	0.69	0.72	0.85	0.54–1.34	NS
Fat, total	1.0	1.99	1.54	2.29	2.29	1.35–3.88	0.003
Animal	1.0	1.48	1.99	1.17	1.97	0.90–2.24	0.03
Saturated	1.0	1.48	1.79	1.62	1.97	1.20–3.22	0.006
Protein, total	1.0	0.70	0.67	0.98	0.88	0.42–1.07	NS
Animal	1.0	0.80	0.89	0.79	1.02	0.65–1.60	NS

Data relate to a cohort of 7284 males examined within the US Health Professionals Study, 170 of whom had adenomas. Data are adjusted for age, family history of colorectal cancer, and total energy intake.
CI, confidence interval.
Data adapted from Giovannucci et al. [97].

acids and oxidized bile acids have been found in populations at greater risk for colorectal adenomas and cancers, compared with those at lower risk. Subjects living in areas with a high prevalence of colorectal adenomas have greater concentrations of fecal bile acids. Similarly, subjects in these geographical areas have been found to have higher numbers of fecal anaerobic bacteria. There is also an association between high numbers of fecal anaerobic bacteria, increased amounts of metabolized cholesterol and oxidized bile acids in the feces, and the high prevalence of colorectal neoplasms in different populations [104,105]. Oxidation seems a critical step, and efforts to reduce, scavenge, or correct for oxidative damage and free radical production have been proposed, but to date have not proved to be useful preventive measures. A likely exception to the relationship between dietary fat and colorectal cancer risk concerns fish oil. Intakes of fish and fish oil correlate inversely with colorectal cancer incidence [101–106]. Fish oil consumption (rich in omega-3 fatty acids) has been shown to reduce rectal epithelial proliferation [101–107]. Studies supporting this protection include a large endoscopy-controlled study showing a trend for an inverse association of marine *n*-3 fatty acids and large adenomas [108]. The mechanism may implicate an increase in apoptosis, at least as judged from tissues in the "normal" mucosa of adenoma patients challenged with *n*-3 fatty acids [109].

Dietary fiber

Dietary fiber is a heterogeneous mix of plant-derived components, specifically nonstarch polysaccharides as well as undigested starch polysaccharides and noncarbohydrates that are resistant to digestion in the upper digestive tract; this heterogeneity complicates interpretation of epidemiological data. Nevertheless, subjects consuming diets high in fiber that produce bulky stools tend to have a lower frequency of colorectal adenomas and cancers [97,98,100,102,110]. Case–control studies of dietary fiber and colorectal neoplasia have shown more consistent protection from vegetable than cereal fiber sources, although with a notable exception [102,107,110–114]. However, animal studies focus on the relative solubility of different cereal fibers [115] and support the hypothesis that poorly soluble fibers, such as wheat bran, continue to be fermented throughout the colon, releasing metabolic products of fermentation along its length, including the distal colon, which is the region of greatest risk for colorectal cancer in humans [110]. Among these products are the short-chain fatty acids: acetate, propionate, and butyrate. Apart from altering the pH to a more favorable acidic state, short-chain fatty acids are an important fuel for the colonocyte.

Attention has focused particularly on butyrate, which is capable of inducing a more differentiated phenotype, reducing cell proliferation, inhibiting tumorigenesis, and regulating gene expression by inhibition of histone deacetylase [115,116]. In carefully controlled animal experiments, tumorigenesis is inhibited, with one-third of the protective effect accounted for by the measured stool parameters of fecal pH and butyrate production [117]. Butyrate also induces apoptosis in vitro in colon cancer cell lines by activating the caspase cascade [118] and blocks the cell cycle.

The role of dietary polysaccharides has been comprehensively reviewed [119]. It was concluded that the case for their protective effect remained strong but still unproven in intervention trials, probably due largely to issues of trial design and end points. Pooled analyses of prospective trials show a protective effect, and the largest contemporary prospective cohort study (the European EPIC study) also shows protection for both colon and rectal cancer [120,121].

Table 63.3 Recurrence of adenomas ≥ 1 cm at 2 and 4 years of follow-up in the Australian Polyp Prevention Project

Intervention		Adenoma recurrence (≥ 1 cm, number of patients)			
		1-year follow-up		4-year follow-up	
Bran	Low-fat	Absent	Present	Absent	Present
No	No	93	6	74	6
No	Yes	93	5	72	4
Yes	No	89	7	66	7
Yes	Yes	97	0*	75	0[a]

a Significant reduction ($P \leq 0.03$) compared with the groups consuming high-fat diets or no added bran.
Results are shown only for subjects randomized to low-fat or wheat-bran interventions.
Data adapted from MacLennan et al. [123].

Several of the critically reviewed randomized controlled trials will be briefly mentioned here. A study in New York of patients with FAP showed that wheat bran reduced adenoma formation in compliers, although not when analyzed on an intention-to-treat basis [122]. The Australian Polyp Prevention Project [123] was a factorial randomized controlled trial in 424 subjects with recently removed adenomas, testing the effect of a low-fat diet (< 25% energy as fat, red meat retained), a 25-g fine-particle *unprocessed* wheat-bran supplement, and a 20-mg capsule of β-carotene on adenoma occurrence at 4 years. None of the subjects randomized to the combination of low fat and wheat bran developed adenomas more than 9 mm in size, a result that was significant ($P < 0.03$) at both 2 and 4 years (Table 63.3). Although the proportion of patients with large adenomas was not the primary outcome parameter used to establish the sample size in the Australian Polyp Prevention Project, adenoma size was always considered an important outcome variable. Furthermore, pathological [46], epidemiological, anatomical [21,22], and molecular biological [41,124] considerations all point to the importance of large adenomas in the pathogenesis of colorectal cancer. The study demonstrated the feasibility of compliance with dietary change in a high-risk group of patients (those with a history of adenomas).

A larger randomized controlled trial testing 13.5 g of *processed* wheat-bran fiber vs placebo bran, not associated with fat reduction, showed no effect on adenoma recurrence of any size [124]. The National Cancer Institute Polyp Study also yielded a null result with a low-fat diet (< 20% calories as fat) in combination with 3.5 servings per 1000 calories per day when compared with a no-change diet [125]. Neither of those latter studies combined a low-fat diet with *unprocessed* wheat bran supplementation as in the Australian Polyp Prevention Project, but they have been influential in reducing enthusiasm for the preventive effects of dietary fiber.

Resistant starch, by definition, also escapes digestion in the small bowel and may benefit colonic physiology in similar fashion to the nonstarch polysaccharides [119,126,127]. Resistant starch is being tested in a randomized controlled trial of high-risk individuals in HNPCC families (the CAPP-2 study).

Dietary carcinogens

Studies have demonstrated increased fecal mutagenic activity in the stools of patients at risk for development of colorectal adenomas and cancers [104,128,129]. Whether the increased fecal mutagenic activity is related to dietary fat intake, fecal bile acids, or bacteria is still unclear. One interesting line of investigation relates to metabolic activation of dietary procarcinogens such as heterocyclic amines formed in high-temperature cooking; the risk for adenomas was demonstrable with well-done red meat especially in the distal colon and several heterocyclic amines [130]. Acetylator status has been shown to correlate with risk for colorectal cancers and adenomas [129–131]. Attention has also focused on fecal *N*-nitroso compounds, which are formed in the colon after meat ingestion and which may cause DNA adducts and be mutagenic [132].

Dietary carcinogens may be detoxified by hepatic enzymes (n-acetyl transferase [NAT] 1 and 2), which are themselves polymorphic and have variable activity, thereby providing another explanation of familial risk [133]. Glutathione *S*-transferase *M1* and *T1* genes are also involved in the metabolism of polycyclic aromatic hydrocarbons, carcinogens found in tobacco smoke and the diet [134].

Micronutrients

Studies using antioxidants, such as vitamins A, C, and E and β-carotene, have attempted to decrease the production of mutagens in the stool as a way of decreasing colorectal adenomas and cancers. Antioxidants are an attractive intervention as oxidative damage to DNA is thought to be a common final common pathway of damage induced by many carcinogens. However, randomized controlled trials of these and other antioxidants in subjects with previous adenomas have universally failed to show a reduction in the risk for metachronous adenomas or cancer [122,123,135,136]. However, β-carotene does reduce cell proliferation in the colon [137,138]. This dichotomy highlights the multistage nature of colorectal carcinogenesis, with the opportunity for preventive agents to intercept the process at different stages. Because the actions of dietary agents may be stage specific, the final marker of benefit should be cancer, if this can be demonstrated.

Calcium, perhaps by binding fecal fat, bile acids, and cholesterol, may confer a protective effect [139,140,141]. A meta-analysis of several randomized controlled trials of calcium

supplementation (around 1200 mg of elemental calcium daily) reports a modest but significant effect on adenoma occurrence [140], also supported, although less conclusively, by a rigorous Cochrane review [142]. Calcium (such as in calcium-fortified low-fat dairy products) also reduces cell proliferation in randomized controlled trials and inhibits tumorigenesis in animals [143]. The effect is more evident for supplemental, rather than dietary calcium [144], especially in those replete with vitamin D [145], and may be more important for prevention of large adenomas [146].

Selenium levels in serum and soil have an inverse relationship to adenoma prevalence in case–control studies, and this factor is under active investigation [147,148]. Furthermore, serum selenium levels are inversely related to adenoma development [149,150]. Again, the protection may be greatest against large adenomas [151].

Molecular genetics of adenomas

Multiple alterations in DNA have been noted in adenomas. Chromosomal instability characterized by aneuploidy, tetraploidy, and allelic deletions, and global DNA hypomethylation have been observed in sporadic adenomas and in FAP [124,152–156]. Altered patterns of expression of, or mutations in, oncogenes, such as *FOS*, *MYC*, and *RAS*, and deletions of the *APC* gene [152–154] (see Fig. 63.1) may also be seen. Increasing data from studies of small (2 mm) adenomas highlight the importance of *APC* mutation as the initiating (gate-keeping) event, especially in the chromosomal instability pathway [155,156].

In the microsatellite instability pathway characteristic of HNPCC, replication errors occur during mitosis and mutations occur in other genes, including *TGFβRII*, *MSH6*, and IGF receptor type II [41,156]. Inactivation of promoter regions by methylation (the CpG island methylator phenotype) of tumor suppressor and other genes (*p16*, *THBSI*, E cadherin, and *HPP1*) or mismatch repair genes (*hMLH1* and *MGMT*) is another important molecular mechanism that may be evident in adenomas [41,157]. In the microsatellite instability pathway, *KRAS*, *DCC*, or *p53* are less often involved [158]. Up to 15% of cancers show microsatellite stable (MSI), although only about 1%–5% are due to a germ-line mutation of one of the mismatch repair enzyme loci (*MLH2, MSH1, MSH6, PMS2*). In the balance of microsatellite unstable tumors, mismatch repair deficiency (usually loss of function of *hMLH1*) develops sporadically in the tumor itself as a consequence of silencing of the promoter by methylation. Comprehensive sequential data on genetic changes during adenoma development are still lacking.

A third molecular mechanism, estimated to account for up to 30%–50% of colorectal cancers [39,159], involves silencing by methylation with subsequent loss of expression of genes, including the *HPP1* gene [157], the DNA repair gene O^6-methylguanine DNA methyltransferase (*MGMT*) [160], and the *SLC5A8* gene [161]. Methylation of promoter DNA, characterized by CpG dinucleotides, is a common theme in this pathway, a phenomenon known as CpG-island-methylator phenotype (CIMP) [39]. These changes may relate to progression from small to large hyperplastic polyps through to the serrated adenoma, and ultimately to cancers with variable MSI status [157,162]. This pathway is becoming referred to as the "serrated neoplasia pathway" [163]. It is more common in females, more usually involves proximal tumors, and the cancers are usually mucinous and poorly differentiated. A particular feature of this pathway is an activating mutation in the *BRAF* gene and, in some series, frequent *KRAS* mutations [164]. Interestingly, *BRAF* and *KRAS* mutations in most series are mutually exclusive [165,166]. Other investigators have not shown consistent molecular changes relating to the serrated morphology, indicating a more heterogeneous molecular pathogenesis [167,168]. Expression of *hMLH1* may also be lost in serrated adenomas and even in nondysplastic crypts within such polyps, although this usually leads to microsatellite unstable cancers, often also with mutations in short mononucleotide repeats within their coding regions, for example, *BAX, TGFBR2, IGF2R, MSH3*, and *MSH6* [39]. These are not molecular features of traditional adenomas. This variability might be explained by which gene promotor is methylated: methylation of *MGMT* may predispose to mutations in *KRAS* and *TP53*, and thus chromosomal instability with CIMP-positive MSI or MSI-Low cancers. On the other hand, methylation of *hMLH1* would lead to CIMP-positive MSI-H cancers [39]. The CpG island methylator phenotype (CIMP) may therefore overlap with both pathways.

Molecular changes in hyperplastic polyps

A range of genetic abnormalities has been described in sporadic distal hyperplastic polyps, including oncogenes and tumor suppressor genes, especially abnormalities in *KRAS* and *TGFBR2* and also loss of chromosome 1p. Mutations of *APC*, however, are rare. These changes suggest a need to reevaluate the premalignant status of hyperplastic polyps, which at least on clinical observation are considered unrelated to cancer risk. Correlation of the clinical outcomes and molecular pathology of hyperplastic polyps may teach us further lessons about the pathogenicity of certain accumulated mutations. The morphological correlates of hyperplastic polyps with oncogenic mutations are emerging and should allow colonoscopists to target those hyperplastic polyps developing along the serrated neoplasia pathway. The finding of large right-sided sessile "floppy" polyps on haustral folds (sessile serrated polyps) is one characteristic phenotype with malignant potential [169].

Natural history and the adenoma–carcinoma sequence

It is generally accepted that most colorectal carcinomas arise in one way or another from dysplastic epithelium. The fre-

quency, inevitability, and time course of this neoplastic progression is not clearly defined, as prospective observational studies of adenomas left in patients are unethical. The evidence for the adenoma–carcinoma sequence is thus derived from epidemiological association and morphological, biological, anecdotal, clinical [170], and therapeutic data.

Morphological evidence

If the sequence is correct, adenomas and carcinomas should be closely associated. Some 30%–50% of patients with adenomas have at least one other (synchronous) adenoma [170,171]. Furthermore, having once had an adenoma, the patient's likelihood of a future (metachronous) adenoma is increased [3,171–174]. In those with two or more synchronous cancers, 50%–85% have one or more synchronous adenomas. Adenomas found in those with colorectal carcinoma are more advanced [171,173–175].

If the adenoma–carcinoma sequence is correct, one would expect to find small adenocarcinomas arising within adenomas, but few or no small adenocarcinomas arising de novo. Systematic morphological studies of resected specimens show that when minute adenocarcinomas occur, they are frequently found within adenomas; de novo minute carcinomas are rare [170,174].

The relative frequency of the de novo versus adenoma pathway to cancer development is, however, debated. Japanese investigators claim that a sizable minority of cancers do develop without recognizable adenomatous copathology [175]. Japanese investigators studying Western patients have identified similar phenomena, indicating a need to review this question further in Western societies with high colorectal cancer incidence [176–179].

There is a plethora of reports of "flat adenomas" in Western populations, implying a change in nomenclature rather than epidemiology or pathogenesis [176–181]. Studies using standardized pictures and Western and Japanese colonoscopists show significant interobserver variability in reporting the same polyp. Nevertheless, careful reporting using magnifying colonoscopy and dye spray clearly shows small, flat cancers (with malignant invasion), especially where the central area is depressed [182]. Flat morphology carries greater malignant potential [183], although molecular accompaniments of this have been less evident [184].

If the adenoma–carcinoma sequence is correct, then carcinomas arising within adenomas should gradually increase in size and ultimately replace all the adenomatous elements with carcinoma. Indeed, the frequency of finding adenomatous tissue in a carcinoma varies inversely with the size and extent of invasion of the carcinoma. Associated adenomatous tissue is found in more than 50% of carcinomas with only submucosal invasion, in less than 20% of carcinomas with invasion of the muscularis propria, and in less than 10% of carcinomas with serosal invasion. Furthermore, the proportion of adenomas showing severe dysplasia, which is cytologically tantamount to cancer, is proportional to the size of the adenoma. These observations are founded on extensive experience with large series of adenomatous polyps from many centers, but they were initiated by the studies of Morson [170].

The frequency of finding carcinoma within an adenoma also increases proportionally with increasing size, more severe dysplasia, and greater villous component of the adenoma [170,185,186]. Increasing number of adenomas is associated with increasing risk for future carcinoma [170].

Evidence from progressive genetic changes

Molecular analysis of colorectal neoplasia at all putative stages of development reveals an apparent progressive accumulation of genetic abnormalities [155,156,185] (see Fig. 63.1). In the chromosomal instability pathway, a prevalent molecular genetic finding in the earliest identifiable neoplastic pathology – aberrant crypt foci or microadenomas – is that of mutations in the *KRAS* oncogene. By the time these microadenomas have progressed to a size of 1 cm, about half have *KRAS* gene mutations, primarily in the codon 12 [152–181,183–186]. Mutations or deletions in the tumor suppressor *APC* gene on chromosome 5, which lead to a variety of neoplastic pressures in the cell, including activation of the WNT pathway after translocation of β-catenin from the cell membrane to the nucleus [187], appear to be another early molecular event associated with small adenoma development. These are usually associated with aneupoidy and signal entry into the chromosomal instability pathway [185–189]. A range of other effects on cell biology are related to APC function [190].

About one-third of large adenomas and carcinomas have lost one of their chromosome 5 pair and half have allelic loss of chromosome 18, reflecting progressive genomic instability [156]. Loss of function of the tumor suppressor gene *TP53* on chromosome 17 occurs late in the adenoma stage and seems pivotal to the cancer phenotype [191,192]. See Chapters 63 and 65 for a more detailed discussion.

Not all the molecular carcinogenesis is genomic and some is epigenetic. Silencing of promoters of a range of genes through methylation is frequent and leads to a classification of tumors based on methylation status, that is, CIMP status (see "Molecular genetics of adenomas" above). Methylation occurs as frequently in adenomas as in cancers [193].

Evidence from familial disorders

Familial adenomatous polyposis

The increasing risk for occurrence of colorectal carcinoma with increasing numbers of adenomas is well established in the polyposis syndromes. Classic FAP is characterized by the development of more than 100 adenomas; the risk for colorectal carcinomas is virtually 100% if the colon is not removed. In addition, adenomas precede the appearance of carcinoma by about 5–7 years. Mutations in the *APC* gene

occur constitutionally in FAP, whereas in sporadic colorectal cancers the same gene becomes mutated in the tumor cells (only) [194–197].

Hereditary nonpolyposis colorectal cancer (HNPCC)

In the HNPCC (Lynch) syndromes, carcinomas may arise through small precursor adenomas and flat adenomas [158,182,198]. This progression may occur rapidly, as a result of an accumulation of oncogene mutations or suppressor gene deletions [199]. Thus, the morphological sequence of dysplasia to cancer is retained in the HNPCC pathway, but the molecular pathway is different. There are also subtle differences in pathology: more mucinous tumors at a more proximal location and a more prominent lymphoid reaction with tumor-infiltrating lymphocytes. The hallmark feature of this sequence is MSI due to DNA mismatch repair; the frequency of MSI in adenomas is not yet clear.

The serrated neoplasia pathway and hyperplastic polyposis

The molecular evidence relating to cancer development through this pathway is described above (see "Molecular genetics of adenomas"). Up to 30% of colorectal cancers pass through this pathway [39].

Evidence from clinical observations

The average age at which adenomas develop, 50 years, appears to precede the age of development of colorectal carcinomas, 57 years [171]. Available evidence suggests that progression of adenoma to cancer is the exception rather than the norm. If one compares adenoma prevalence data in autopsy studies (40%–50% prevalence; see "Prevalence" above) with lifetime cumulative incidence of colorectal cancer (about 4%–6% in most Western countries), after allowing for multiplicity of adenomas, it can be deduced that only about 1 in 20 adenomas progresses to malignancy.

In a Norwegian study, small polyps were allowed to remain in situ, tattooed for later localization, and then followed for potential progression. Progression was slow and sometimes regression was observed. A study using serial sigmoidoscopy to follow asymptomatic tattooed rectal polyps smaller than 1 cm (not all of which would have been adenomas) found that over the course of 3–5 years, only 4% of the polyps increased in size, 70% remained unchanged, 8% were smaller, and 18% disappeared spontaneously [200,201]. Studies using serial barium enemas also suggest that most polyps (undefined type) grow slowly, if at all [202,203].

However, progression to cancer has been documented. Adenomas have occasionally been left untreated because of patient noncompliance or technical difficulties; invasive carcinomas have been found to develop at the site of these adenomas [170,185,204]. Follow-up studies of patients who have had polyps smaller than 1 cm in diameter demonstrated on barium enema in the precolonoscopic era, when lesions of that size were not surgically removed, have shown that cancers subsequently developed at this site (15%) or elsewhere in the colon (25%) [204].

Using the available data on the growth rate of adenomas and carcinomas and including information on average age of diagnosis of adenomas by size and level of dysplasia and cancer [170,200], a mathematical model has suggested that for those that grow, it takes 2–3 years for a small adenoma (< 0.5 cm) to grow to 1 cm and another 2–5 years for the 1-cm adenoma to progress to carcinoma [205,206]. Invasion and metastasis probably takes another 2 years, and on average, death occurs 2 years after this. These results, when taken together, suggest that those adenomatous polyps that do enlarge, do so only slowly, and that those most likely to progress to malignancy are those that have reached 1 cm in size.

Evidence from therapy

In a large, uncontrolled study, patients underwent periodic sigmoidoscopies, and all polyps that were found were removed. Most polyps removed were found to be adenomatous. The frequency of carcinomas occurring in a subsequent 7–14-year period was found to be about one-third of the expected rate in an untreated population [207]. This has been confirmed in a retrospective study [208] and in the prospective National Polyp Study [3]. In the study by Murakami et al. [208], risk for subsequent cancer was highest in the patients who had biopsy alone and lowest when polypectomy was performed. In the National Polyp Study, patients underwent colonoscopy and polypectomy on enrollment with repeat colonoscopy at 1 year (in half) and at 3 and 6 years. No symptomatic cancers and five asymptomatic malignant polyps were found after almost 6000 patient-years of follow-up over more than 4 years [3]. This represents a 58%–85% reduction in mortality rate from colorectal cancer, depending on whether the reference population is of average risk or includes adenomatous patients. In a Chinese cohort study including nearly 250 000 participants, rectal cancer incidence was reduced by 31% and mortality by 18% by polypectomy, over a 20-year period of observation [5]. In a study of patients with FAP who had colectomies and ileorectal anastomoses, it was found that periodic removal of adenomas from the rectal stump prevented the subsequent development of rectal carcinoma [171].

Randomized controlled trials demonstrating cancer outcomes in those with adenomas left in situ relative to those who have had polyps removed, will never be done for ethical reasons. This precludes the highest level evidence for the benefit of adenoma detection and removal.

Exceptions in the evidence

There are apparent exceptions to this adenomatous polyp–carcinoma sequence. There are rare reports of small carcinomas developing de novo in apparently nonadenomatous

epithelia [209–211]. Nonpolypoid dysplasia is particularly difficult to detect and manage in ulcerative colitis [212]. The frequency with which this nonpolypoid progression occurs is important because effectiveness of strategies to prevent colorectal cancer through detection and removal of adenomatous polyps will be limited to the proportion that evolve through this pathway, explaining in part the cancers that occur despite close surveillance and polypectomy [182,211,213].

Conclusions

Overall, it would appear from these lines of evidence that most carcinomas arise from polypoid or flat dysplastic change in the colonic epithelium, although different molecular biological pathways may be responsible.

Primary prevention

A 20-fold change in risk for colorectal cancer has been observed in individuals at the extremes of the dietary spectrum [98]. If populations can be convinced to reject unhealthy eating habits and change to a healthier lifestyle, substantial control of colorectal cancer incidence could follow. Unfortunately, the prospect for primary prevention by dietary means has suffered setbacks with the publication of the negative findings in various interventional studies including the Arizona wheat bran trial and the National Cancer Institute study of low-fat plus fresh fruit and vegetables diet [124,125]. Much more work is needed to determine feasible diet-based strategies that can be confidently expected to affect incidence.

The option of chemoprevention, especially by nonsteroidal antiinflammatory drugs, may have benefit and is discussed later (see "Therapy" below).

Signs and symptoms of polyps

Colorectal polyps are usually asymptomatic [214,215]. Most often, the polyps are discovered as an incidental finding during examination for intestinal or nonspecific abdominal complaints or other disorders, or during screening endoscopy. Even in the case of FAP, in which adenomas number in the hundreds to thousands, symptoms occur late or result from development of colorectal carcinomas.

In a report of more than 800 hospitalized patients with colorectal polyps, 32% were found to be asymptomatic [215]. When symptoms occur, they tend to be in patients who have colorectal polyps measuring 1 cm or larger. The most common symptoms are rectal bleeding, both overt and occult, change in bowel habit, abdominal pain, and rectal prolapse. Of course, the association of these with a polyp may be coincidental. Furthermore, symptoms have little correlation with histological status, pathological condition, or location [216].

Histological studies have shown that compared with carcinomas, adenomas tend to have minimal ulceration and an intact lumenal surface. Overt rectal bleeding tends to be intermittent and small in amount, only rarely resulting in anemia, with the exception of FAP [217]. Early studies using measurement of total gastrointestinal bleeding demonstrated that only patients with polyps measuring 1.5 cm or larger had consistently higher amounts of (usually occult) fecal blood loss than healthy control subjects [218]. Subsequent studies, based on measurement of immunoreactive hemoglobin from stool samples, have shown that even people with small adenomas have, as a group, higher fecal hemoglobin levels than normal controls [219].

Less than 20% of patients presenting with frank rectal bleeding are found to have colorectal adenomas as the only obvious pathology. Rectal bleeding, especially of recent onset and in patients older than 40 years of age or those with other risk factors such as family history, should not be ascribed to coexisting hemorrhoids without a thorough evaluation of the anus, colon, and rectum [220].

A syndrome of profuse, watery diarrhea, resulting in massive fluid and electrolyte depletion, has been described for large villous adenomas, especially those located in the distal colon and rectum [221]. Colorectal polyps, especially juvenile polyps, may occasionally undergo autoamputation, resulting in rectal bleeding. The presence of recognizable polyp tissue in the feces is extremely rare [222]. Colocolic intussusception due to large polyps has been found in children [223]. Diffuse, nonspecific abdominal pain has been at times attributed to intussusception, particularly of larger polyps. Rectal prolapse of larger polyps also has been reported.

Physical examination rarely reveals signs of colorectal polyps. A digital examination by an experienced person is important as part of all anorectal and colonic evaluations, especially because flat villous lesions close to the dentate line are easily missed at endoscopy, and the digital examination frequently reveals the diagnosis most easily.

Features of neoplastic polyps

The initial diagnosis is usually made by direct endoscopic visualization of the polyp, which should then undergo biopsy or be removed by polypectomy to determine its histological type. At the time of endoscopic examination, certain morphological features may help classify the polyp, although these features can be misleading [224,225].

Because the chance of malignancy in an adenoma is proportional to the size of the adenoma, its villous component, and the degree of dysplasia, these parameters should be described by the pathologist [191,226]. It also partly determines the risk for future advanced adenomas in that individual, thereby guiding decisions on surveillance.

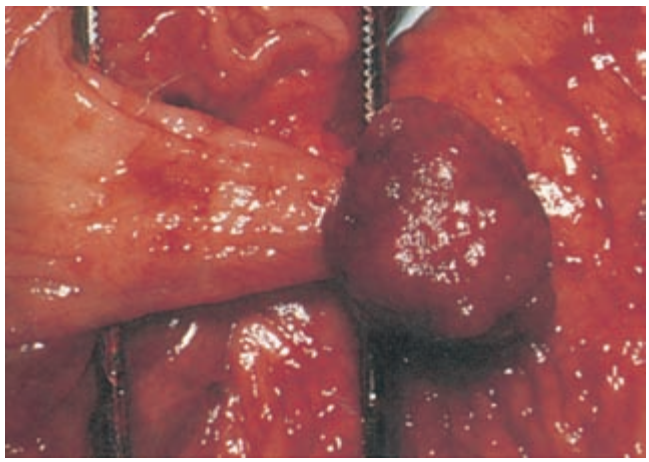

Figure 63.2 Macroscopic appearance of a pedunculated tubular adenoma. The forceps mark the base and the neck of the stalk.

Figure 63.3 A synchronous pedunculated tubular adenoma adjacent to a sessile tubulovillous adenoma. Synchronous adenomas not infrequently are found adjacent to each other. In this instance, a 1-cm pedunculated tubular adenoma is found about 5 cm from a 3-cm sessile tubulovillous adenoma. Note the smooth surface of the tubular adenoma and the smooth, lobulated surface of the tubulovillous, primarily tubular adenoma.

Polypoid adenomas

Tubular adenomas (Figs 63.2 and 63.3) usually are darker red than the surrounding mucosa, have a smooth surface, and tend to be smaller than villous adenomas. Villous adenomas constitute about 5% of all adenomas and occur even more frequently in patients older than 60 years of age [226,227]. When seen during endoscopy, they are usually larger and typically sessile compared with tubular adenomas. The surface has a shaggy, cauliflower-like or frond-like appearance, may be soft and velvety, and is often friable. Villous adenomas tend to be distal in location, large, and bulky.

Tubulovillous adenomas contain at least a 25% villous component and at least a 25% tubular component, without either architectural type predominating. Their clinical behavior tends to be intermediate between that of tubular and villous adenomas [46].

Flat adenomas

Nonpolypoid adenomas, or flat adenomas (localized dysplasia with minimal macroscopic protrusion), tend to be minimally raised (if at all), small (usually < 1 cm) discoid plaques, and they are often erythematous. Because they are not polypoid, they may be missed during colonoscopy [174,178,209,211,227,228]. Judicious use of air insufflation during colonoscopy is needed to detect these lesions because overinflation makes them difficult to detect at colonoscopy. Chromoendoscopy enhances their detection [229].

Despite the small size of these flat adenomas, more than one-third have foci of high-grade dysplasia, especially if centrally depressed [230–233]. In addition, Japanese studies have observed that flat adenomas, when multiple, are often associated with small adenocarcinomas [231,233], although observations in Western studies are less convincing [234].

Serrated adenomas and mixed hyperplastic–adenomatous polyps

Serrated adenomas have the architectural features of hyperplastic polyps but the cytological features of adenomas (dysplasia) [235–238] (Fig. 63.4). They account for less than 1% of colorectal polyps [236]. They are usually polypoid. As noted above, they may be distinguished from sessile, serrated polyps that appear as floppy redundant folds in the right colon, usually having no dysplastic epithelium; these may progress to dysplastic cytology (sometimes referred to as "superficial serrated adenomas") [39,239]. Serrated adenomas are associated with a high frequency of high-grade dysplasia (up to 40%). Mixed hyperplastic–adenomatous polyps show the two types of histology in distinctly separate regions; they should also be treated with caution.

Figure 63.4 Histological section of a serrated adenoma showing intermingling of dysplastic and hyperplastic cytology, the latter being responsible for the serrated appearance at a lower power of magnification. Goblet cell density is highly variable. Typically, the hyperplastic cytology is more prominent toward the surface. Note the substantial architectural disturbance and the great variation in nuclear-to-cytoplasmic ratio between the different types.

Features of nonneoplastic polyps

The nonneoplastic polyps consist of distinct and unrelated groups: simple mucosal, hyperplastic, inflammatory (including pseudopolyps), hamartomatous (including juvenile polyps), and other submucosal polypoid lesions. By definition dysplasia does not occur.

Simple mucosal polyps

Simple mucosal polyps are small, pearl-like excrescences with coloration and appearance similar to adjacent normal mucosa. They are usually smaller than 5 mm, consist of normal colonic mucosa, and have no known clinical significance [46].

Hyperplastic polyps

Hyperplastic polyps are usually small (< 5 mm) and sessile, are found predominantly in the distal colon, and are pale in color. The frequency of hyperplastic polyps appears to increase with age [16]. They are characterized by elongated glands with papillary infolding and increased mucus content (Figs 63.5 and 63.6), which often gives a serrated appearance to the surface of the polyp [43,53,239,240]. The cribriform crypt pattern of adenomas is not usually seen. Such changes may be more easily discerned by using chromoendoscopy. The variant sessile serrated polyp has special molecular significance (see "Hyperplastic polyposis and serrated adenomas" above [39].

Usually, hyperplastic polyps should be removed because one cannot be certain at endoscopy that they are not adenomas [224,225].

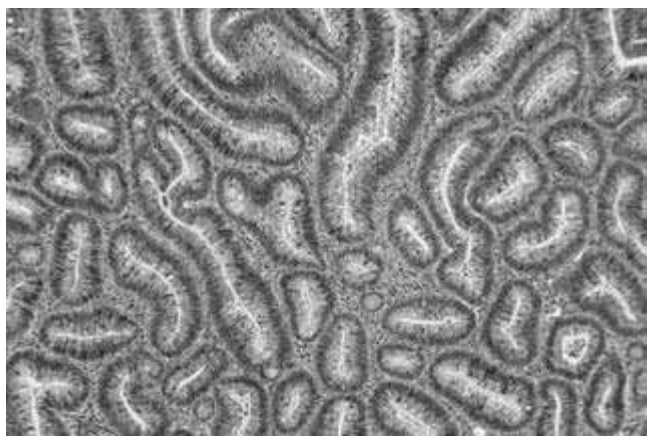

Figure 63.5 Histological appearance of a hyperplastic polyp, characterized by the typical elongated glands with papillary infolding, which produces a serrated surface appearance. The epithelial cells are well differentiated, with abundant cytoplasm, a normal nuclear-to-cytoplasmic ratio, and abundant goblet cells. The nuclei are not hyperchromatic, retain their basal polarity, and show no dysplasia. The starfish appearance of the hyperplastic crypts seen in cross-section is characteristic.

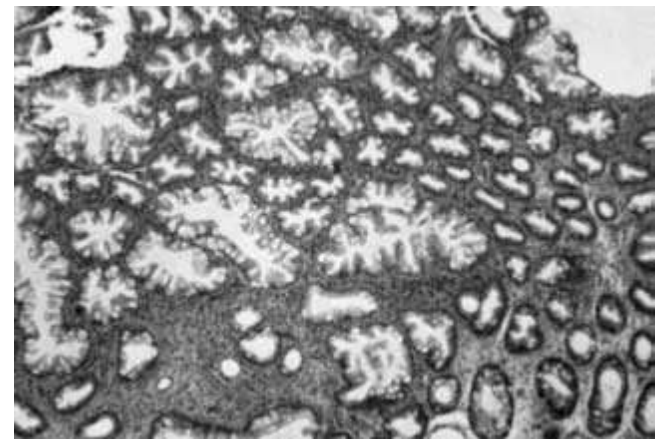

Figure 63.6 Histological appearance of adenomatous epithelium in a predominantly hyperplastic polyp. This polyp should be managed as if it were an adenoma.

Inflammatory polyps

Inflammatory polyps are usually found in the setting of severe inflammatory diseases of the colonic mucosa: ulcerative colitis, Crohn's disease, amebiasis, strongyloidiasis, tuberculosis, solitary rectal ulcer syndrome, ischemic colitis, severe diverticular disease, or schistosomiasis [241]. When a polyp consists of residual islands of intact colonic epithelium in an area denuded of mucosa, the term *pseudopolyp* is often applied. Inflammatory polyps can also consist of exuberant masses of granulation tissue. Inflammatory polyps may be large and solitary, with marked inflammation and ulceration, and can thus appear to be malignant at endoscopy [242–245]. In schistosomiasis, the inflammatory polyp may contain schistosome eggs or adult worms.

Inflammatory polyps may produce symptoms of pain and obstruction [246] and may be pedunculated or filiform. They may occur in settings of minimal inflammation, such as ulcerative colitis in remission, or in a familial setting [247]. Inflammatory polyps must be carefully distinguished from dysplastic mass lesions [243].

The inflammatory "cap" polyp, found in the rectal area, is thought to be a result of mucosal prolapse. It is covered by a cap of inflammatory, hemorrhagic, or granulation tissue. Associated symptoms include mucous diarrhea, tenesmus, and rectal bleeding. Symptoms resolve after polypectomy [248,249]. There have been anecdotal reports of cure following antibiotic therapy, suggesting an infective etiology [250,251]. An unusual inflammatory polyp arising in the anorectal mucosa, and often presenting with rectal bleeding, is the inflammatory cloacogenic polyp [252,253].

Another inflammatory polyp has been termed the *inflammatory myoglandular polyp* because of the presence of inflammatory and granulation tissue in the lamina propria

with abundant smooth muscle proliferation and hyperplastic glands with occasional cystic dilation. When symptoms are present, they consist of overt or occult rectal bleeding [254].

Carcinoma can occur in inflammatory polyps; unusual inflammatory polyps in complex formations should be sampled [243].

Hamartomatous polyps

Juvenile polyps

Juvenile polyps, also known as *retention polyps* because they contain mucin cysts, are so termed because three-fourths of them are found in children younger than 10 years [255,256]. They tend to be single, pedunculated, cherry-red, and round, and possess a smooth surface. They are often friable and ulcerated, bleed frequently, and tend to prolapse because of their pedunculated nature [256–260]. They are also liable to volvulus with resultant ulceration, hemorrhage, autoinfarction, and autoamputation. Histologically, juvenile polyps are hamartomas with distended mucus-filled glands, often with cystic dilation and edematous lamina propria containing abundant vasculature.

Juvenile polyps are rare in the first year of life and are thus presumed to be acquired and not congenital. They occur rarely in the second decade of life but become common again in adulthood. The true prevalence is difficult to determine. In one study, a frequency of about 2% was found in asymptomatic children [261]. In series of children seen for rectal bleeding, 30% had juvenile polyps, with up to half of these being multiple [258,262]. Of all polyps found in children aged under 15 years, up to 97% can be juvenile polyps [257]. Isolated pure juvenile polyps usually are not dysplastic and have no intrinsic malignant potential.

Juvenile polyps also may occur in a familial setting [263,264], with an autosomal dominant pattern of inheritance of polyposis [265] (see Chapter 64). In familial juvenile polyposis, adenomatous and carcinomatous changes are frequent throughout the gastrointestinal tract and in family members [263–270]. Jass [271] has provided guidelines for the diagnosis of juvenile polyposis, describing three criteria:

- more than five juvenile polyps in the large bowel
- juvenile polyps throughout the gastrointestinal tract, or
- any number of juvenile polyps with a family history of juvenile polyposis.

Because solitary juvenile polyps occasionally have associated adenomatous changes, all juvenile polyps should be totally excised. After removal, juvenile polyps recur only infrequently, with a rate of about 3% in 3 years [261]. If extensive adenomatous changes or severe dysplasia are seen, the patient may be considered for surveillance [268–271].

Two genes responsible for familial juvenile polyposis have been reported: the *PTEN* gene [272] and the *SMAD4*/*DPC4* gene [273], on chromosomes 10 and 21, respectively. Another locus, the bone morphogenetic receptor 1A gene (*BMPR1A*), has also been described in juvenile polyposis [274]. About half the families with juvenile polyposis have mutations in one or other of these genes [275].

Peutz–Jeghers polyps

Multiple hamartomatous polyps are found throughout the gastrointestinal tract in Peutz–Jeghers syndrome [276], and usually show prominent branching of smooth muscle. This is a rare autosomal dominant syndrome of gastrointestinal hamartomatous polyposis associated with melanin spots of the lips, buccal mucosa, and extremities [277] (see Chapter 64). Peutz–Jeghers polyps are not considered premalignant, although the syndrome is associated with an increase in carcinomas, mostly extracolonic [278–281]. Syndrome-associated cancers include testicular cancers, breast cancer, and hepatobiliary cancers. Surveillance should be for gastrointestinal polyps, as well as clinical examination of the breast and scrotum. Capsule endoscopy is defining a role for itself in the small bowel surveillance of these familial syndromes, with double-balloon enteroscopy reaching well into the small bowel for polypectomy [282–285]. The genetic locus for Peutz–Jeghers syndrome is the serine-threonine protein kinase gene (*STK11*) on chromosome 19p [285]. Unusual hamartomas with abundant adipose tissue have been described [286,287]. A review of the polyposis syndromes has been published [288].

Submucosal polyps

Submucosal polyps represent elevations of the mucosal surface by submucosal lesions. They are diverse in origin.

Lymphoid hyperplasia

When a focus of diffuse lamina propria lymphocytes becomes hyperplastic, diffuse nodular lymphoid hyperplasia may result [289]. Typically, the normal colonic (or more usually, small intestinal) mucosa is covered with polypoid nodules, generally measuring 5 mm or smaller [289,290]. Discrete lymphoid polyps, which appear pale-yellow or white, may also occur but these do not produce symptoms [291,292]. Diffuse nodular lymphoid hyperplasia occurring in the large bowel needs to be distinguished from FAP; biopsy with histopathological diagnosis is important [292,293]. Familial adenomatous polyposis requires surgery, whereas diffuse nodular lymphoid hyperplasia is usually coincidental and requires no intervention.

Distinguishing between malignant lymphoma and multiple polyps can be difficult [294–296]; lymphoma has developed in a few patients with apparent nodular lymphoid hyperplasia [297,298]. It is unclear whether the malignant lymphoma represents a progression from the lymphoid hyperplasia or a de novo development of lymphoma.

Pneumatosis cystoides intestinalis

Pneumatosis cystoides intestinalis refers to multiple air-filled cysts found within the submucosa of large or small intestine.

The air-filled cysts are easily recognizable by radiographic examination as well as endoscopy. At endoscopy, they are seen as multiple polypoid lesions in the colonic mucosa, which on closer examination appear to be translucent polyps that may look like air blebs just underneath the mucosa. On biopsy, there is often a release of the submucosal air and deflation of the polypoid lesion [299,300].

Pneumatosis intestinalis is generally asymptomatic but can also be associated with pneumoperitoneum [301,302]. Occasional cases associated with scleroderma or chronic obstructive pulmonary disease have been seen [300]. In these cases, the air-filled cysts may resolve after treatment with oxygen [302]. Pneumatosis intestinalis may also complicate ischemia, necrosis, or necrotizing enterocolitis, when it is often fatal [298].

Colitis cystica profunda

Colitis cystica profunda is usually a manifestation of rectal prolapse. The radiographic appearance of colitis cystica profunda is virtually indistinguishable from that of solitary or multiple colonic polyps. The endoscopic appearance can be alarming, mimicking a polypoid cancer. The polyps are sessile and consist of dilated, epithelium-lined mucous cysts within the muscularis mucosae. They are found most often in the rectum in association with inflammatory colitis, or after surgery [303–305]. When seen within an adenoma, colitis cystica profunda has been referred to as *pseudoinvasion, epithelial displacement*, or *misplaced epithelium* [306,307].

Lipomas

Lipomas within the gastrointestinal tract are found most commonly within the colon, usually in the right colon on or near the ileocecal valve. These lesions tend to be solitary but may be multiple in 20% or more of cases. Radiographically, they often show up as slightly radiolucent submucosal lesions. At colonoscopy, they appear yellow with a normal mucosa overlying the polyp continuous with surrounding nonpolypoid mucosa. They are easily identifiable on probing with the biopsy forceps (the "pillow" sign). Polyps with these characteristics need neither biopsy nor polypectomy [308,309].

Other submucosal lesions

A number of other rare submucosal lesions also may present radiographically or endoscopically as polypoid lesions. Biopsies of the surface reveal normal colonic epithelium. Deeper biopsies, or biopsy after submucosal saline injection to elevate the mass, may be required to reach the submucosal mass. Endorectal ultrasonography is particularly useful in diagnosing the origin of a submucosal tumor and whether invasion is present.

Malignant submucosal lesions include carcinoids; metastatic tumors including malignant melanoma and lymphoma; gastric cancer; Kaposi sarcoma; and plasma cell leukemia. Benign lesions include lymphangiomas, fibromas, neurofibromas, leiomyomas, myoblastomas, hemangiomas (including the blue rubber bleb syndrome), and endometriosis [241].

Other colorectal lesions may occasionally masquerade as polyps. Such lesions include hemorrhoids, condylomas, enlarged anal papillae, fibrous anal polyps, inverted diverticula [308], enteroenteric and ureteroenteric anastomoses, and the residual stalk of a pedunculated polyp after polypectomy [241]. Inverted diverticula are important to recognize and not misdiagnose because "polypectomy" can result in perforation. They are rather flat and a little distensible, often with a slight central umbilication.

Diagnosis of adenomas

Colonoscopy is the procedure of choice because of its diagnostic and therapeutic potential. Digital rectal examination is a poor method for diagnosing adenomas except for villous lesions low in the rectum, which may be missed if care is not taken to examine the anorectal region, for example with retroversion of the flexible endoscope. The role of sigmoidoscopy is discussed in Chapter 120. Guaiac or immunochemical fecal occult blood tests are aids to detection as part of a screening program and are discussed in Chapter 62.

Barium enema radiography

Single-contrast barium enema has been shown to have about 75% sensitivity and specificity for detection of polypoid lesions [309,310]; double-contrast (air-contrast) barium enema improves on this [311,312]. Even so, most studies have found that double-contrast barium enemas miss a proportion of polypoid lesions subsequently detected at colonoscopy [311,313,314].

Colonoscopy

When a patient is being assessed for the possibility of having a colorectal neoplasm, colonoscopy is the procedure of choice (see Chapter 120). Colonoscopy allows detection, and either removal of polyps (in most instances) or biopsy. Although colonoscopy carries some risk, the risk is small relative to benefit [314–316]. Polypectomy increases the risk, but is intrinsic to most of the benefit.

Colonoscopy is highly accurate for polyp detection but is not infallible [317–319]. Miss rates for adenomas of any size have been estimated at 17% from studies of paired colonoscopies performed within 120 days [320]. In a back-to-back tandem colonoscopy study, there was a 24% miss rate overall, with 6% of the adenomas larger than 1 cm being missed [12,321]. In a study at St Mark's Hospital, London, colonoscopy and double-contrast barium enema were performed in tandem; 92% of polyps larger than 7 mm were detected at colonoscopy, compared with 74% by double-contrast barium enema [322]. In the dietary polyp prevention trial,

13 cancers in 2079 participants were detected over the intervention period; four were considered as incomplete removal from a preceding polypectomy, and three were considered missed at previous colonoscopy [323]. The sensitivity of colonoscopy for neoplasia is further discussed in Chapter 63.

Computed tomography colonography (virtual colonoscopy)

Studies of CT colonography report widely different accuracies when compared with colonoscopy. The best studies report impressive levels of sensitivity and specificity for adenomas over 1 cm in size and for cancers, and CT colonography can detect polyps missed by colonoscopy [324]. However, experienced radiologists and state-of-the-art software and hardware are all needed to optimize performance, and these are not widely distributed even in Western countries. The better studies do provide a benchmark for performance that is likely to be replicated as better equipment and training diffuse through the radiology community. The value of this procedure is discussed in further detail in Chapter 131.

Biopsy and histopathological examination

Biopsy or polypectomy of a polypoid lesion is essential because histopathological analysis is the definitive diagnostic test. It also informs assessment of future risk. Pathology review with the pathologist [325] is necessary to optimize biopsy and polypectomy procedures including handling of colonoscopically derived specimens. Separate specimen containers should be used when specimens are obtained from different sites, otherwise lesions may be interchanged and it may be difficult or impossible to determine which of several anatomically distinct lesions is the one showing adenomatous changes or malignancy on histological examination. Sampling of every polyp is becoming more important as the patient phenotype is characterized by the number of each specific type of polyp; this information is critical when deciding on which gene to test in a mutational analysis for cancer predisposition. Marking the site of a polyp with sterile india ink may help subsequent localization, especially if surgery is needed for malignancy within a polyp. Alternatively, a metal clip can be attached to the site.

Screening for adenomas

Strategies for screening for colorectal adenoma and cancer are critically presented in Chapter 62. Adenoma detection is important in the context of screening if the goal is to reduce the incidence of cancer. On the other hand, many adenomas are not destined to progress to cancer and the ideal strategy for adenoma detection – that which achieves reliable detection of advanced lesions – is not yet available.

Therapy

Therapy falls into two contexts: treatment of existing lesions and prevention of lesions especially in high-risk settings. Such approaches may be using agents capable of affecting precancer biology, or excisional removal.

Chemoprevention of adenomas

Therapy may be directed at preventing adenoma recurrence in the case of sporadic adenomas or FAP, or at regressing existing adenomas in FAP when removal or resection is not feasible, such as with small duodenal polyps or polyps in retained rectum after colectomy.

The potential value of dietary factors has been discussed under "Primary Prevention" above. At this stage, the most promising agents for chemoprevention are the nonsteroidal antiinflammatory drugs (NSAIDs).

Nonsteroidal antiinflammatory drugs

Most evidence indicates that use of nonsteroidal antiinflammatory drugs, specifically aspirin, is associated with reduced mortality from colorectal cancer [326–331]. Two large trials of aspirin intervention have shown reductions in adenoma occurrence: in one, conducted in patients with previous adenomas, 81 mg (but not 325 mg) aspirin daily (RR 0.81; 95% CI 0.69–0.96) reduced new adenoma occurrence by 19% [332]; the other, in patients with previous colorectal cancer, tested 325 mg aspirin only and also found benefit (RR 065; 95% CI 0.46–0.91) [333]. The dosage discrepancy remains difficult to explain. A Cochrane review has concluded that there is evidence for prevention of adenomas resulting from aspirin use, but not (yet) for cancer prevention, and for incomplete elimination of adenomas in FAP [334]. Pharmacogenomic research suggests that the benefit may be genotypically determined [335]. This effect extends to adenomas, as seen in the Nottingham screening study [329]. Sulindac, a nonselective NSAID, has been shown to be effective in reversing rectal adenoma growth in patients with FAP after ileorectal anastomosis [336–338]. The European CAPP-1 and CAPP-2 trials are evaluating aspirin in randomized controlled trials as therapy for FAP and HNPCC, respectively. The cyclooxygenase 2 (COX-2) selective NSAID celecoxib is effective in FAP [339].

Evidence to date does not support routine chemoprevention with aspirin or sulindac in patients with sporadic (common) adenomas because, while they might be effective, the risk:benefit ratio is unclear. The dose needed to achieve prevention has been estimated from cohort studies to be 600 mg aspirin daily [340]. The risk versus benefit of long-term dosage at this level has not been established, especially in average risk persons.

Inhibition of COX-2 is considered an attractive approach to chemoprevention, as many adenomas express COX-2, espe-

cially if large and distal [341]. Use of rofecoxib and celecoxib for chemoprevention of sporadic adenomas has been confounded by significant cardiovascular and cerebrovascular morbidity and mortality in the active compared with placebo arms of the trials [342], leading to the complete withdrawal of rofecoxib from the market. The situation for celecoxib is under intense debate, but cardiovascular risk was also statistically significant at high doses (800 mg daily) in the large APC chemoprevention trial [343]. Whether this cardiovascular risk exists for all nonaspirin NSAIDs is uncertain. Nevertheless both rofecoxib and celecoxib have been shown to reduce the incidence of metachronous small and large adenomas in the APPROVe and APC trials respectively.

Polypectomy as therapy

Endoscopy and snare polypectomy provide a simple and cost-effective means of managing colorectal polyps [8,344–346]. Cold snare polypectomy or garroting of small polyps is effective [347]. In practice, all polyps should be considered for removal because it is not possible to determine the histological type by their visual appearance. The number of polyps that can safely be removed by polypectomy depends on their location, morphology, and size. In principle, histopathology should be obtained on all polyps. There are two exceptions: multiple polyps (as discussed below) and perhaps where there is the pale, flat morphology typical of hyperplastic polyps in the distal colon [348,349]. In the case of multiple polyps, for practical purposes, at least three of the largest polyps should be removed for confirmatory histological examination [346,347]. The remainder should ideally be subsequently removed if any is found to be an adenoma. However, it should be noted that with this policy, the exact polyp phenotype of the patient will not be ascertainable, and this information may prove critical in any subsequent assessment for genotyping, both with respect to whether it should be done and on which gene to focus. Although hot biopsy [348,349] and bipolar electrocoagulation [350] have been advocated for eradication of diminutive polyps, these techniques can leave behind residual polyp (adenomatous) tissue [351–353] and are potentially dangerous in the thin-walled cecum.

Polyps larger than 1 cm are usually adenomatous and should be removed in toto if pedunculated. If sessile, they may need to be removed piecemeal [354,355]. Histological evaluation of the presence or degree of invasion of any carcinomatous foci is critical because the chance of malignancy increases with increasing size [170]. If there is any concern that residual polyp tissue may have been left, the polypectomy site should be tattooed with sterilized india ink to facilitate follow-up evaluation [356]. This should also be done if there is a high index of suspicion that a polyp may be malignant.

If partially removed polyps are found to be inflammatory, hyperplastic, or otherwise nonadenomatous by histological examination, subsequent complete removal is unnecessary but a sampling of several polyps from each cluster is required.

Multiple polyps require particular care. In patients with newly diagnosed FAP, colectomy, not polypectomy, is indicated. For extensive multiple adenomatous polyps not due to FAP, the colon should be endoscopically cleared of polyps a section at a time to reduce the chance of complications. Note that the thinner wall of the cecal area and right colon requires care when removing polyps by cautery [355,357,358].

Polypectomy is generally safe in adults [315,316] and children [359] when performed by experienced operators. Complications are generally related to polypectomy and the major complication rate is less than 2%. The requirement for hospitalization or surgical intervention (usually as a result of hemorrhage or perforation) is less than 0.3%, and death is rare. Polypectomy should be deferred in patients on anticoagulation and in those with severe bleeding diatheses, unstable cardiac arrhythmias, recent myocardial infarction, acute colitis, pregnancy (second or third trimester), recent colonic surgery, or abdominal abscess or perforation [358]. Some consider that administration of aspirin should cease 1 week before colonoscopy in case polypectomy is required, but others do not; although the issue is unresolved, the risk would appear to be low [360].

Removal of sessile polyps can be assisted by submucosal injection of saline (2–10 ml) to lift the polyp from the muscular wall and minimize the risk for perforation. Kits to facilitate this are commercially available [361,362]. Variations on this technique using sodium hyaluronate to raise the polyp, and needle knife dissection have been developed [363].

The procedural aspects of colonoscopy and polypectomy, including patient preparation, premedication, antibiotic prophylaxis, polypectomy, and polyp retrieval techniques, are discussed in Chapter 126.

Laser ablation and argon plasma coagulation

Ablation with the neodymium : yttrium–aluminum–garnet (Nd : YAG) laser or argon plasma coagulation are alternative methods for the rapid removal of large numbers of small polyps from segments of the colon [364–368]. In experienced hands, these techniques are efficacious, rapid, and relatively safe; complications occur in about 5% of cases [368]. Perforation is rare, but extra care is needed in the thin-walled cecal area [367]. Such ablation should be restricted to the removal of multiple polyps after the histological diagnosis has been confirmed by previous biopsies or polypectomies. Argon plasma coagulation has appeal because the energy can be applied tangentially to the lesion, and the depth of ablation is limited to 3–5 mm, adding a potential increment of safety over lasers. Argon plasma coagulation has been useful for completing removal of large sessile lesions primarily treated with endoscopic mucosal resection [369,370]. The disadvantages are that these methods are destructive, do not yield pathology specimens, and so a focus of invasive malignancy in the polyp could be missed.

Surgical resection

In cases in which a polyp is large and sessile or otherwise cannot be removed by endoscopic polypectomy, surgical resection may be necessary. Surgical resection may also be indicated for those polyps where invasive carcinoma is found (see "Malignant polyps" below) [371–373]. Large rectal adenomas may be extirpated by transanal excision using an operating sigmoidoscope and rigid snare under anesthesia or with an operating microscope in association with a sigmoidoscope. Other large polypoid lesions and malignant polyps need to be removed at laparotomy or by laparoscopy [374]. Once laparotomy is indicated, many surgeons advocate the use of a formal segmental colectomy, including lymph node dissection. The extra resection adds little anesthesia time or perioperative mortality and morbidity [375].

Severe dysplasia and intraepithelial carcinoma

Severe dysplasia and intraepithelial carcinoma are nonmalignant lesions and can be cured by adequate polypectomy. Such change in a polyp is not considered to be cancer because it does not breach the muscularis mucosae. Lymphatics in the colon do not extend beyond the muscularis mucosae into the lamina propria, and hence the possibility of spread is negligible. There have been no confirmed reports of lymphatic invasion resulting from severe dysplasia or intraepithelial carcinoma [185,373–378].

The possibility of progression of severe dysplasia to invasive malignancy demands careful management and complete removal. Evaluation of a polyp removed by fragmental biopsy or piecemeal polypectomy is difficult [45,170,371,375]. The presence of cancerous cells on a biopsy or cytology specimen, without the architectural information provided by a single polypectomy specimen, may not reveal the malignant nature of a polyp.

Malignant polyps

Evaluation of invasiveness comes from careful pathological examination of a completely removed polyp and serial sectioning of it; otherwise, malignancy may be missed [186]. If the neoplastic lesion invades through the muscularis mucosae (with exposure to the lymphatics), the lesion is then termed a *carcinomatous* or *malignant polyp* [377,378].

Misplaced epithelium (pseudoinvasion) may be difficult to distinguish from invasive carcinoma [307,379]. In general, the displaced epithelial cells are surrounded by lamina propria and not by desmoplastic reaction and are similar to the overlying adenomatous epithelium [306,379]. Hemosiderin deposition in the polyp indicates torsion.

The management of malignant polyps remains controversial [344]. This is largely because although the risk of metastasis is small, the studies examining this have also been small. There is general agreement that when a malignant polyp has been identified, poor prognostic features include incomplete polypectomy as judged by the endoscopist or pathologist, poor differentiation on histopathology, and neoplastic cells within 2 mm of the polypectomy margin [380–382]. The presence of venous or lymphatic invasion has been shown to be an independent poor prognostic factor in some series [383–385]. Absence of such risk factors is associated with a low chance of nodal involvement [383,384]. Lymphatic invasion is, however, subject to observer variability [386]. Malignant polyps with venous (or lymphatic) invasion are almost always associated with poor tumour differentiation, but when venous or lymphatic invasion occurs in isolation from the other prognostic variables, they need to be taken into account within the overall context of the patient's comorbidity [387,388].

Controversial prognostic factors are the replacement of the bulk of the adenoma by carcinoma, sessile nature, and large polyp size. Sessile malignant polyps are generally considered to have a worse prognosis, and most usually have other markers of poor risk [389].

Results of removal of malignant polyps

All studies have been small. Of 62 evaluable patients with malignant polyps who had endoscopic polypectomy alone, 4 (7%) had subsequent recurrence [384]. In other studies, patients who underwent endoscopic polypectomy for pedunculated malignant polyps did much better [390]. In a retrospective review of 43 patients with pedunculated malignant polyps who were followed for almost 5 years, 19 patients initially underwent colonic resection, but none from either treatment group had a recurrence [391]. Data indicate that even polypoid carcinomas (where the entire polyp has malignant architecture and cytology) removed by polypectomy do not spread and therefore do not require segmental resection *unless* poor prognostic features are present [392].

Practice recommendations

For the individual patient, it is necessary to weigh the estimated risks of surgery against the risks of leaving residual malignancy in situ after endoscopic polypectomy of a malignant polyp. The presence of poor prognostic features listed above is generally accepted as a reason for surgical resection [45,174,186,380–393], but age of the patient and any coexistent morbidity should always be considered. The decision depends on an estimate of the risk that residual cancer is present compared with the risk for perioperative mortality and morbidity in the individual patient [394]. Operative mortality rates range from 2% to 10% and increase with the age of the patient [373,394]. Because most patients with malignant polyps are in their seventh and eighth decades of life, the risk of colectomy is significant and perhaps greater than the risk of spread.

Based on the evidence [45,186,170,373,392] the following recommendations for management of malignant polyps can be made (Fig. 63.7):

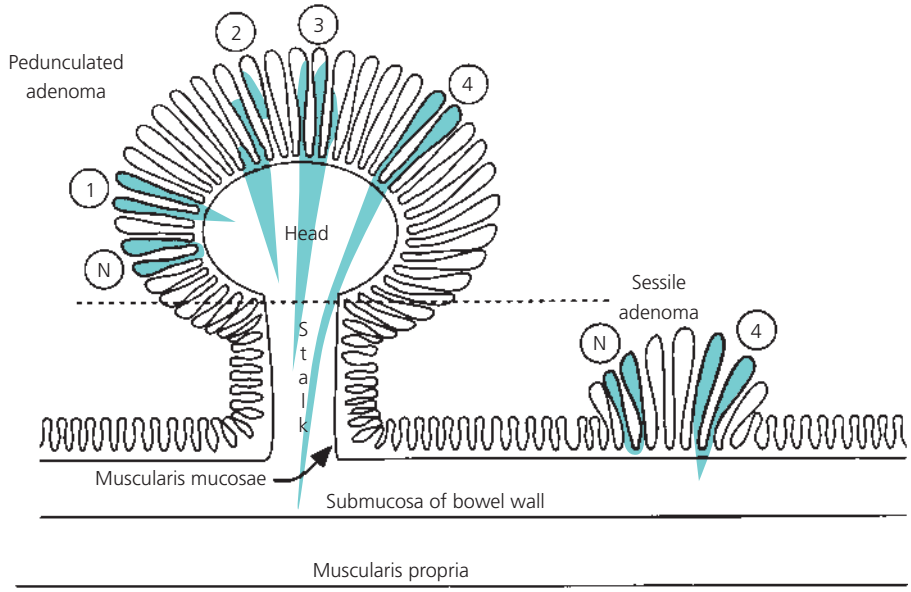

Figure 63.7 Levels of carcinomatous extension in malignant polyps – pedunculated versus sessile adenomas. When carcinomatous cells are contained within the mucosa, without breaching the muscularis mucosae and so extending into the "head" of the stalk, the lesions are known as noninvasive carcinomas, otherwise referred to as severe focal atypia or intramucosal carcinoma (N). Once cancer has invaded through the muscularis mucosae, the polyp is then termed a malignant polyp. Because lymphatics are present in the submucosa, metastatic spread is then possible. Increasing levels of invasion of the carcinoma, to the head (1), neck (2), stalk (3), and base of the stalk or submucosa (4), may carry worsening prognostic implications. A malignant sessile adenoma has, by definition, invaded the submucosa of the bowel wall (4). Lesions with severe dysplasia sometimes described as carcinoma in situ or intraepithelial cancer (N) require no additional therapy after polypectomy. Usually, malignant polyps with invasions only of the head, neck, and stalk (1, 2, 3) and with clear resection margins (at least 2 mm) and no poor prognostic features are adequately treated by polypectomy alone. In malignant polyps with invasion of the submucosa of the bowel wall (4) or with poor prognostic features, colectomy should be seriously considered for potential cure.

- A pedunculated malignant polyp with invasion of the head, neck, or stalk is adequately treated by polypectomy alone if the polypectomy is not piecemeal, is judged completely removed by the endoscopist, the cancer is not poorly differentiated, and the resection margin is free of cancer for 2 mm.
- Malignant polyps with involvement within 2 mm of the margin of resection or with nonassessable margins, polyps with invasion of the submucosa of the bowel wall (especially if there is less than 2 mm margin of clearance), or polyps containing poorly differentiated invasive carcinoma should be treated by surgical resection unless the surgical risk is prohibitive. For sessile malignant polyps, one or more of these criteria are usually present, rendering surgery usually advisable. Blood vessel and lymphatic involvement may influence a decision to perform colectomy.
- Malignant polyps treated by polypectomy alone should be followed by repeat colonoscopy within 3–12 months to assess for recurrence at the polypectomy site.

In all cases, patients must be informed of the rationale behind the physician's therapeutic decision and should participate in that decision.

Follow-up for metachronous adenomas and cancers

Recommendations for following patients with adenomatous polyps vary [344] and several prospective studies are addressing this issue.

Predicting further development of adenomas

In the initial St Mark's Neoplastic Follow-up Study, age, multiplicity of previous adenomas, and size of largest adenoma were independent risk factors for further development of clinically significant adenomas or cancer [395].

These prognostic risk factors for future adenoma occurrence, that is, metachronous adenomas, have been confirmed in colonoscopic follow-up studies such as the US National Polyp Study. In addition to multiplicity (more than two adenomas), the US study also identified family history in association with older age (greater than 60 years) at diagnosis of adenomas as a risk factor for future advanced neoplasms. In Erlangen, the risk for metachronous advanced adenomas was found to relate to parental history of colorectal cancer, entry polyps

with villous or tubulovillous histology, or larger adenomas (> 10 mm) [396].

The US National Polyp Study found that a follow-up interval of 3 years after complete polypectomy for adenomas was as effective as follow-up colonoscopy after 1 year [3]. Longer intervals might be adequate, especially in those patients with small tubular adenomas in the rectosigmoid region [396]. Although definitive data are not available, follow-up intervals extending to 10 years are being recommended for low-risk adenomas [174,344,395,396]. The yield in such patients appears to be less rewarding than in subjects without prior adenomas who are older than 60 years of age [395,397–400].

Rectal epithelial proliferation has been shown in some studies to predict future adenomas [401].

The role of fecal occult blood test (FOBT) in adenoma surveillance remains unclear. However, FOBT is better than no follow-up at all and has been shown to be useful in the intervening years between colonoscopies to maintain contact with patients and aid detection of missed or rapidly developing interval cancers or large adenomas [402].

Predicting future development of cancer

Factors predicting future cancer are similar to those predicting adenoma. In 1618 patients followed for a mean period of 14 years after initial sigmoidoscopy and polypectomy for rectal adenoma, the risk for subsequent rectal cancer depended on adenoma size, histological type, and multiplicity, with a size greater than 1 cm and villous architecture being the strongest risk factors (Table 63.4). Conversely, patients with small tubular rectal adenomas without severe dysplasia had no increased risk for colorectal cancer [174].

Patients who have undergone surgical resection of a primary colorectal cancer are not only at risk for recurrence of their first cancer but also at a three-fold to four-fold increased risk for metachronous adenomas or cancer [173,403,404]. Patients with microsatellite unstable cancers, whether sporadic or HNPCC, are at particularly high risk. Therefore, follow-up should seek not only evidence of metastatic disease and recurrent disease at the surgical anastomosis but also development of a second primary cancer or an adenoma. If the entire length of the colon was not completely visualized before surgery (owing to tumor obstruction or poor preparation), a complete colonoscopy to look for synchronous lesions is indicated after 3–6 months. After the colon has been documented to be free from neoplasms, initial follow-up may be at 3 years, as in the follow-up after polypectomy for adenomatous polyps [405]. Examining the anastomosis for local recurrence in the first 2 years after left-sided cancer is also advisable.

Table 63.4 Risk for colorectal cancer after polypectomy from rectum or sigmoid colon

	Relative risk[a]	95% CI
Single tubular adenoma < 1 cm	0.5	0.4–1.3
Single TVA, VA, or TA > 1 cm	3.4	2.3–4.8
Multiple adenomas	7.7	3.7–14.2

a Risk assessed from 1618 patients at St Mark's Hospital, diagnosed in the precolonoscopy era.
CI, confidence interval; TVA, tubulovillous adenoma; VA, villous adenoma; TA, tubular adenoma.
Data adapted from Atkin et al. [174].

Surveillance recommendations

When a significant adenoma is found (> 10 mm or with villous or tubulovillous histology, or severe dysplasia), surveillance colonoscopy after 3 years suffices provided that the colonoscopist has reasonable confidence that polypectomy is complete and that the entire colon has been cleared of polyps [397,398,405]. The same is appropriate for patients with multiple (> 2) adenomas. If the colon has not been cleared of polyps, colonoscopy should be repeated within 3–6 months to ensure clearance. If the repeat surveillance colonoscopy is clear of adenomas after 3 years, many consider that the interval may be lengthened to 5 years. For patients at higher risk for colorectal cancers, such as those at risk because of HNPCC, more frequent examinations may be in order. However, patients with only small tubular adenomas in the rectum are not at increased risk for colorectal cancer (provided that the adenomas have been removed) and can be dismissed from follow-up or offered repeat examination at 5 or 10 years [174].

Impact of polypectomy on cancer

Polypectomy should reduce the incidence and mortality from colorectal cancer. This has been, to date, difficult to document in national statistics. Trends in colorectal cancer incidence vary around the world, with an upward trend appearing in countries that are adopting a Western lifestyle. Despite the availability of screening and colonoscopic surveillance, the trend continues upward in some countries. One such country is Australia, where the age-adjusted upward trend has been 2% per annum in men and 1% per annum in women between the 1970s and early 1990s [406]. Later data do not show a downward trend. The continuing increase may be due to environmental factors that drive up the incidence despite polypectomy, or may be due to an increase in early diagnosis of cancers ahead of an anticipated later decrease in cancer diagnoses.

In the United States, however, improvements in national mortality statistics and incidence are evident [407]. This improvement is multifactorial, and is a consequence of earlier diagnosis; better surgery, anesthesia, and postoperative care;

Table 63.5 Relative risk for colorectal cancer in subjects undergoing surveillance for adenomas, compared with age- and sex-matched control groups

Study	*n*	Observed	Expected	O/E	95% CI
London [396]	555	4	2.56	1.56	0.43–4.00
Funen, Denmark [409]					
Males	614	6	5.36	1.12	0.41–2.44
Females	442	4	2.60	1.54	0.42–3.94
US National Polyp Study [3]	1422	5	11	0.45	
Japan [208]	206	8	1	8	1.7–78.4
Australia [410]	645	3	3.75	0.8	NS

CI, confidence interval; *n*, number in study; O/E, ratio of observed to expected.

and the impact of adjuvant therapy. The reduction in incidence precedes widescale polypectomy.

Five large-scale adenoma follow-up studies have examined the impact of colonoscopic surveillance on subsequent cancer development [3,5,208,395,409,410] (Table 63.5). The London, Danish, and Australian studies each showed that the incidence of cancer during follow-up was not significantly different from the incidence in age- and sex-matched population controls, implying a reduction from a higher risk if surveillance was not undertaken. Unfortunately, without control groups, the real significance is unclear. The US National Polyp Study demonstrated a statistically significant reduction in risk in participants within the study compared with either population controls or an independent (but historical) population of adenoma patients who did not have colonoscopic follow-up [3]. This is the best study demonstrating effectiveness.

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D COLON

64 Polyposis syndromes

Randall W. Burt, Russell F. Jacoby

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MUTYH gene-associated polyposis, 1651
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Polyposis syndromes with neural polyp histology, 1658
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Miscellaneous noninherited polyposis syndromes, 1661

The polyposis syndromes are a group of conditions in which multiple gastrointestinal (GI) polyps occur in the lumen of the gut (Table 64.1). Most of these syndromes are inherited, and most exhibit an increased risk for colon cancer. Many benign and malignant extraintestinal manifestations are also observed. The polyposis syndromes have traditionally been categorized according to polyp histology. However, elucidation of the responsible genes has allowed refinement of the categorization as well as a more precise understanding of the pathogenesis, phenotypes, and cancer risks. The clinical importance of the syndromes relates to their inheritance as well as to their benign and malignant manifestations. Cancer prevention is often possible with proper management of patients and their families. The syndromes are also of great importance to biological investigation as understanding of their genetic and molecular etiologies has contributed immensely to the knowledge of cancer pathogenesis and cell biology.

Table 64.1 Polyposis syndromes

Familial adenomatous polyposis (FAP)
Gardner syndrome (GS)
Two-thirds of Turcot syndrome families
Attenuated familial adenomatous polyposis (AFAP)

***MYH* gene-associated polyposis (MAP)**

Syndromes with hamartomatous polyps
Peutz–Jeghers syndrome (PJS)
Juvenile polyposis syndrome (JPS)
Cowden syndrome (CS)
Bannayan–Riley–Ruvalcaba (BRR) syndrome
Hereditary mixed polyposis syndrome (HMPS)
Gorlin syndrome

Polyposis syndromes with neural polyp histology
Neurofibromatosis type I (NFI)
Multiple endocrine neoplasia type IIB (MEN IIB)
Isolated intestinal neurofibromatosis of the colon

Polyposis syndromes of uncertain etiology
Cronkhite–Canada syndrome (CCS)
Hyperplastic polyposis syndrome (HP)

Polyposis syndromes with inflammatory polyps
Inflammatory bowel disease
Devon polyposis
Cap polyposis

Polyposis conditions arising from lymphoid tissue
Nodular lymphoid hyperplasia (NLH)
Multiple lymphomatous polyposis (MLP)
Immunoproliferative small intestinal disease (IPSID)

Miscellaneous noninherited polyposis syndromes
Leiomyomatosis
Lipomatous polyposis
Multiple lymphangiomas
Pneumatosis cystoides intestinalis

Familial adenomatous polyposis (FAP)

Familial adenomatous polyposis (FAP) is the first described, the most common, and the best characterized of the polyposis syndromes. The first reports of FAP appeared in the medical literature in 1861 and 1873 [1,2]. The hereditary nature of this disease was recognized by 1882, and cases of associated malignancy were published in 1887 and 1890. The autosomal dominant inheritance pattern of the disease was clearly established by 1934 [3].

It is known that FAP is an inherited condition characterized by hundreds to thousands of colonic adenomatous

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polyps that typically begin to emerge in the second and third decades of life. Colon cancer is inevitable if the colon is not removed. The condition includes Gardner syndrome, about two-thirds of Turcot syndrome families, and attenuated FAP (AFAP). Familial adenomatous polyposis and its variants arise from mutations of the adenomatous polyposis coli (*APC*) gene. Inheritance is autosomal dominant with nearly complete penetrance. Synonymous historical terms for FAP include *adenomatous polyposis coli* and *familial polyposis coli*.

Epidemiology

Estimates of the prevalence of FAP vary from 1 in 6850 to 1 in 31 250 live births (2.29 to 3.2 cases per 100 000 population) [1,4–8]. The frequency is fairly constant throughout the world, with men and women being affected equally. Up to one-third of newly diagnosed cases not belonging to previously identified families appear to represent new mutations [1,9]. About 0.5% of all colon cancer cases historically have arisen in the setting of FAP [4], although the figure may be as low as 0.07%, possibly owing to screening in identified FAP families [5].

Genetics

APC gene

Familial adenomatous polyposis arises from germ-line mutations of the *APC* gene on chromosome 5q21. The *APC* gene is a tumor suppressor gene; thus gene inactivation occurs only after both alleles are mutationally damaged. In FAP, one allele is inherited in a mutated form. Adenoma formation is initiated when the second allele is damaged or lost by a somatic event. The progression from adenoma to carcinoma after *APC* inactivation is similar in both FAP and the sporadic setting, with accumulation of mutations in additional relevant genes, including *KRAS*, *p53*, a gene or genes on chromosome 18, and possibly others [10].

APC gene function

The APC protein is a scaffolding protein that is directly or indirectly involved with a number of cellular functions including cell proliferation, apoptosis, differentiation, adhesion, migration, cell-cycle control, stabilization and assembly of microtubules, and maintenance of cell polarity [10–12]. It is part of the WNT signaling pathway in which activation favors cell growth, suppression of apoptosis, loss of differention, and loss of cell-cycle control. Loss of APC function causes this activation to be constant and unregulated, that is, constitutive. The APC protein also binds to microtubules and can promote microtubule assembly, which is part of the normal mechanism by which colonocytes migrate up the colonic crypt [11,12]. Mutated *APC* thus causes disruption of normal cellular positioning in the crypt, consistent with present models of carcinogenesis. Moreover, APC appears to play a role in cell polarity, which is important for proper cell growth, differentiation, and migration [13,14].

APC mutations in familial adenomatous polyposis

More than 90% of *APC* germ-line mutations that cause FAP give rise to a truncated protein product. The causative mutations (Fig. 64.1) introduce a premature stop codon by nonsense mutations (30%), frameshift mutations (68%), or large deletions (2%) [11]. The actual frequency of large deletions, however, may be as high as 15% because many studies have not included detection methods for large deletions [15–17]. More than 825 germ-line mutations have been found in families with FAP [18], of which over 80% are distinct for each family. The most common germ-line *APC* mutation observed in multiple unrelated families is a 5-bp deletion that results in a frameshift mutation at codon 1309. Although mutations have been found scattered throughout the gene, most are located in the 5′ end of exon 15, often called the *mutation cluster region* [19,20].

The number of colonic adenomas in FAP correlates to some extent with the location of the *APC* germ-line mutation. Profuse or dense polyposis (up to 4000 colonic adenomas) has been reported with mutations in codons 1250–1464, with less dense polyposis proximal and distal to this location [21–23]. Whole gene deletions likewise cause profuse polyposis [24]. As stated previously, the most frequent FAP mutation is at codon 1309. One study found that patients with mutations at that codon presented with symptoms at an average age of 20 years, whereas patients with mutations between codons 168 and 1580, excluding 1309, presented with symptoms on average 10 years later. Certain gene modifiers may also be associated with the severity of polyp expression [25]. Smaller numbers of polyps occur in AFAP, which has been associated with mutations in codons 1–163, 329–338, and 1596–2644 [26]. Disease-causing *APC* mutations are only found in 8.5% of persons with multiple adenomas, defined as 3–100 [27]. A missense *APC* polymorphism, E1317Q, has been postulated to account for some of the remainder of those with multiple adenomas, but this mutation remains of questionable significance [26]. It remains unknown why mutations in certain areas of the gene give rise to AFAP, but there is evidence of functional protein production on the basis of internal translation initiation in AFAP alleles [28].

Correlations have also been found between the locations of the mutations in the *APC* gene and extracolonic manifestations of FAP. Congenital hypertrophy of the retinal pigment epithelium (CHRPE) is observed with mutations between codons 463 and 1387 [29], although mutations between codons 1444 and 1578 are not associated with CHRPE expression [30,31]. Other extracolonic lesions can be found with mutations anywhere in the *APC* gene, but are more common when mutations occur in certain regions of the gene [23,32]. Papillary thyroid cancer is most common with *APC* mutations affecting codons 140–1309 [26]. A higher incidence of desmoid tumors is found with mutations in codons 1445–1580 [26], and in a slightly different region in another

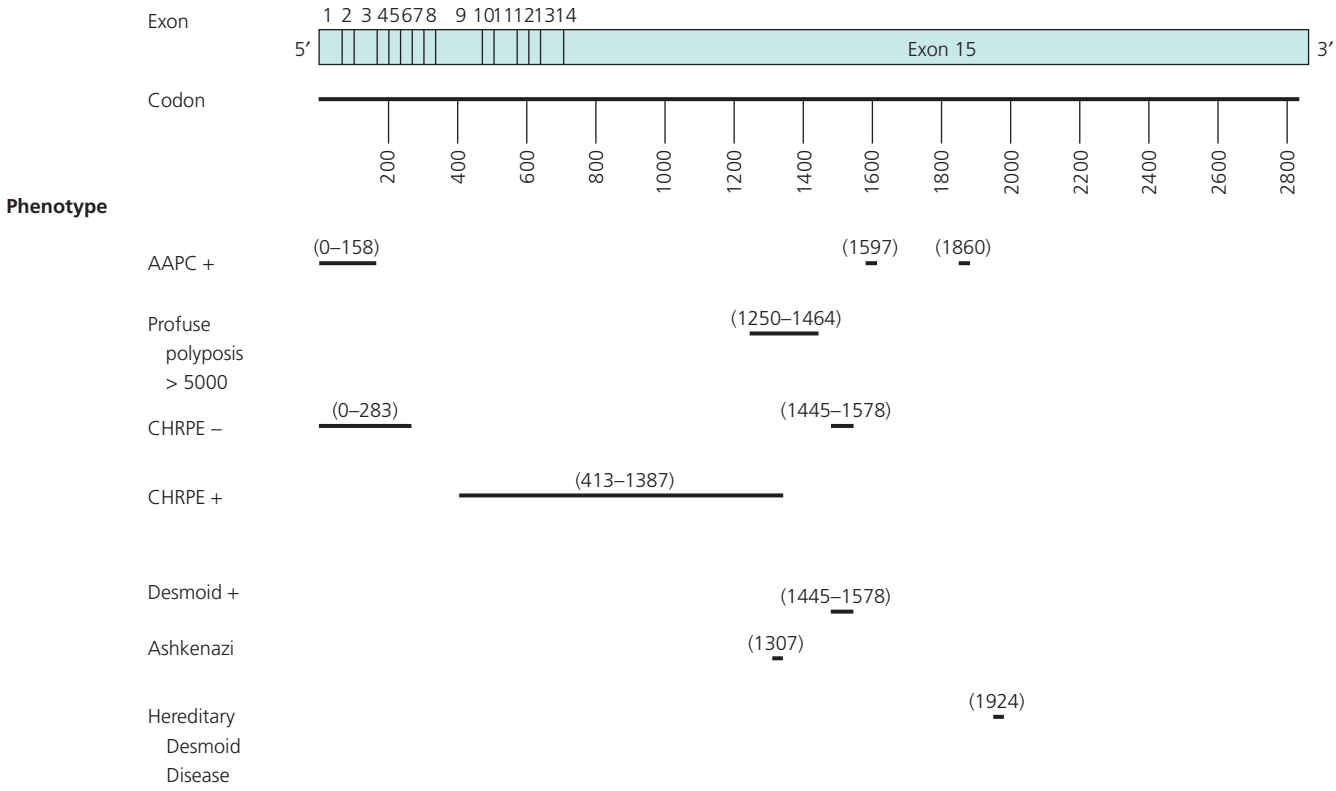

Figure 64.1 Location of disease-causing mutations in the *APC* gene correlated with phenotypic manifestations. Note that thyroid cancer, hepatoblastoma, desmoid tumors, and duodenal adenomas may occur with mutations anywhere in the gene, but are most common in the areas indicated. From Galiatsatos & Foulkes [26], with permission from Blackwell Publishing.

study [33]. Osteomas are more common with mutations beyond codon 1444 [23,31,33]. Hepatoblastoma occurs more frequently with mutations affecting codons 457–1309, whereas duodenal adenomas are more common with mutations between codons 976 and 1067.

A polymorphism of the *APC* gene unique to people of Ashkenazi Jewish descent has also been identified [34]. This polymorphism, I1307K, is present in 6% of Ashkenazi people but in 12% with colon cancer, and in 29% with colon cancer and a family history of colon cancer. It confers an approximate 1.5- to 2-fold increased risk for colon cancer compared with the general population and is believed to cause disease by increasing the mutability of the *APC* allele on which it resides [34–37]. The age of colon cancer onset or adenoma number does not differ in persons with I1307K mutations compared with sporadic cancer and adenomas [38].

Biallelic *MUTYH* gene mutations can give rise to a phenotype that simulates FAP or AFAP, although it more often results in less than 100 adenomatous polyps (see "*MUTYH* gene-associated polyposis" below).

Laboratory genetic testing methods

The laboratory methods used for genetic testing in FAP include protein truncation testing (PTT), gene sequencing, and linkage testing [39–42]. Protein truncation testing is successful in identifying evidence for a disease-causing mutation in 80%–90% of families with clinically defined FAP.

DNA sequencing is more accurate than PTT, with the specific disease-causing mutation being identified in the majority of families with known FAP. Mutations that delete the entire allele or large portions of it will not be detected by DNA sequencing so Southern blotting and multiplex ligation-dependent probe amplification (MLPA) are applied for this purpose [42–44]. Up to 30% of clinically typical FAP patients will not be found to have *APC* mutations by standard methods [45]. In a study of FAP subjects without a disease-causing mutation detectable by standard methods, 14% were found to have large *APC* deletions, 24% had unbalanced allelic mRNA expression of *APC*, 11% had germ-line mutation in other genes, including *AXIN2* and *MUTYH*, whereas 52% remained unexplained [43]. A technique to separate *APC* alleles and test them separately may help further in finding disease-causing mutations [46].

Perhaps the most useful application of genetic testing is mutation-specific testing. Once a specific disease-causing mutation has been identified in an index case in a family, other family members can be tested for the presence or absence of that specific mutation by one of a number of highly accurate

polymerase chain reaction (PCR)-based methods. The accuracy for mutation finding in relatives at this stage is near 100%.

Linkage testing employs DNA markers to track the segregation of a purported mutation through a family. The sensitivity of linkage in identifying genetically affected individuals approaches 99% because of the highly informative markers available. Linkage is limited, however, in that two or three known affected people involving two generations must be available for successful application of the method. Linkage is thus usually applied only when PTT and sequencing fail.

Clinical process of genetic testing

Genetic testing is done on DNA obtained from the white blood cells of peripheral blood. A number of laboratories perform genetic testing for FAP and other inherited conditions. These laboratories can be identified at the GeneTests website (www.genetests.org). Genetic counseling is an essential prelude to genetic testing [47]. Important medical and psychosocial issues surround asymptomatic genetic testing [48–50]. Children's rights and psychological issues are of particular concern when testing of children is being considered [51]. All of these issues must be thoroughly understood by the physician and patient when genetic testing is anticipated. Informed consent for testing is required before testing is done [52]. The physician must either be familiar with the issues or use trained genetic counselors.

There are four settings in which to consider genetic testing for FAP [40–42,53]:

- testing a person with some features of FAP, but where the clinical diagnosis is not certain
- testing a person with clinically defined FAP, but the mutation is not known in the family
- testing relatives in an FAP family once the mutation is known
- embryo testing for preimplantation genetic diagnosis.

The first setting is usually defined as a person with at least 10–20 cumulative adenomas but no known family history of FAP. Finding a mutation confirms the diagnosis of FAP and allows relatives to be tested with high accuracy. Failure to identify a mutation does not rule out FAP, because initial mutation finding is 70% successful at best. Genetic testing in the second situation is similar, but the patient has more than 100 adenomas. Failure to find a mutation means that all close relatives must still be screened as if they have by FAP. In the third setting, relatives of a person with a known APC disease mutation are tested for the presence or absence of that mutation. A positive test indicates the diagnosis of FAP, whereas a negative test (absence of mutation) essentially rules it out. Such individuals then need only average risk screening for colon cancer. The fourth type of testing has been approved in the United Kingdom for prospective biological parents affected by a mutation known to cause FAP [54].

Clinical manifestations

Colonic polyposis

The clinical diagnosis of FAP is made with the presence of at least 100 colonic adenomatous polyps or fewer polyps and an immediate relative with FAP [1] (Figs 64.2 and 64.3). The average number of adenomatous polyps in a person with fully expressed FAP averages 1000, with some people exhibiting more than 5000 polyps. Fewer adenomas are seen early in the development of the disease and in AFAP (see "Attenuated familial adenomatous polyposis" below), with further subdivisions of expected polyp number known to correlate with *APC* mutation location (see "*APC* mutations in familial adenomatous polyposis" above).

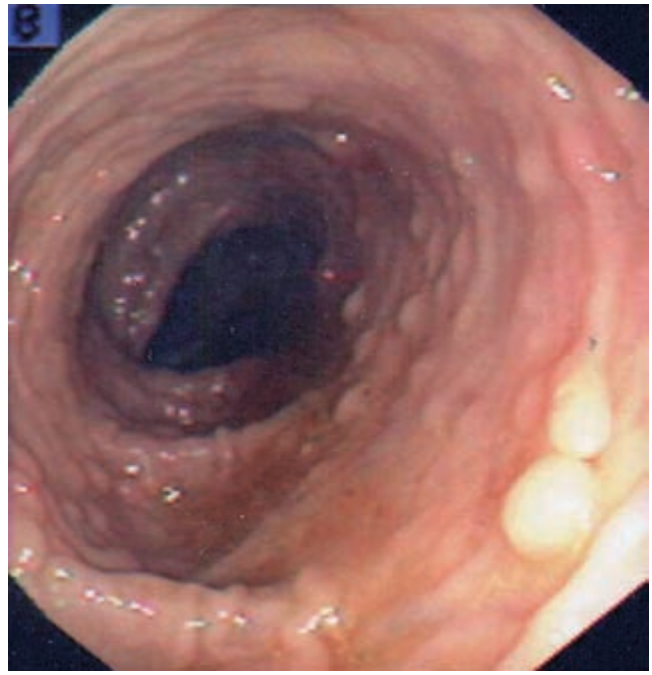

Figure 64.2 Early colonic polyposis in familial adenomatous polyposis.

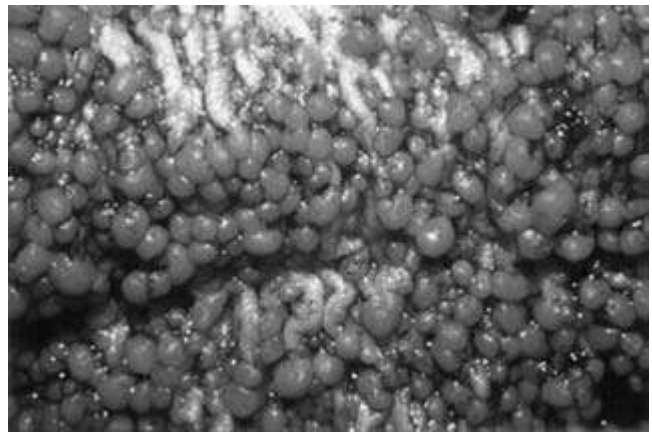

Figure 64.3 Fully developed colonic polyposis in familial adenomatous polyposis. Courtesy of Karen Kim.

Polyps most often begin to appear in the second or third decade of life. The mean age of polyp occurrence assessed by prospective rigid sigmoidoscopy is 15.9 years (range 8–34 years) [55], although occurrence of polyps at younger and older ages has been reported. The average age of FAP diagnosis in patients presenting with symptoms is 35.8 years (range 4–72 years) in the St Marks FAP Registry in London [1]. Similar finding are described in the Danish and Japanese FAP registries [7,8]. The age of symptom presentation correlates with gene mutation location, which, in turn, correlates with colonic polyp density [22,56].

Adenomatous polyps are usually distributed evenly throughout the colon, with a slight excess in the distal colon. The size of the polyps depends on the stage at which the patient is examined. Even in fully developed cases, however, 90% of adenomas are less than 0.5 cm in diameter, and less than 1% of polyps are larger than 1 cm. Polyps may either carpet the colon with myriad small lesions or occur as more distinct and somewhat larger lesions. These patterns appear to have a genetic origin (see "APC mutations in familial adenomatous polyposis" above), although striking heterogeneity of polyp number and growth rate also has been observed [57].

Histopathology demonstrates tubular adenomas, indistinguishable from common or sporadic adenomas. Villous and tubulovillous histologies are also seen, but much less frequently and usually in larger polyps. A histological feature of FAP not observed in the general population is dysplastic or adenomatous epithelial cells in single crypts or even portions of single crypts. These are called *microadenomas* and are often seen in biopsy specimens of normal-appearing flat mucosa [1]. Budding of dysplastic epithelium from normal crypts is also observed. Aberrant crypt foci also have been reported to occur with increased frequency in FAP [58]. These lesions are similar to microadenomas but are identified with methylene blue staining of the colonic mucosal surface.

Colon cancer

Adenocarcinoma is the inevitable consequence of FAP unless the colon is removed. In the St Mark's series, the average age at cancer diagnosis was 39 years. By 45 years of age, 87% had developed cancer, and by 50 years, 93%. Multiple colonic malignancies were present in about 48% of those with cancer (41% synchronous, 7% metachronous). Eighty-four percent of malignancies were at or distal to the splenic flexure, a fraction almost identical to that found in the series of random colorectal malignancies observed by the same authors at that time. Average life expectancy after diagnosis of cancer was 2.6 years. Survival is remarkably improved in relatives of probands who undergo screening [4,59]. Similar observations have been made in other registries [7,8]. Colon cancer has been reported as early as 9 years of age, although the occurrence of malignancy before adolescence is unusual. Polyp number correlates with cancer risk. A study found a 2.3-fold increase in cancer risk at diagnosis for those with more than 1000 colonic polyps compared with those with fewer than 1000 polyps [60]. Each 10-year older age group also exhibited a 2.4-fold increase in cancer risk. There is a 25% incidence of colon cancer in newly diagnosed FAP patients, who remain common because of the high frequency of new mutations [9,61].

Upper gastrointestinal polyps and cancer

Stomach

Gastric polyps occur in 23%–100% of FAP patients [20,62,63] (Fig. 64.4). In the gastric fundus and body, the polyps are most often fundic gland polyps, considered hamartomas. These polyps are histologically seen to consist of simple hyperplasia of the fundic glands with microcysts. Endoscopically, the polyps are multiple sessile lesions, 1–5 mm in diameter, and the same color as surrounding mucosa. They are sessile, hemispherical elevations, occasionally with the appearance of a "waist" but not a stalk [64]. At endoscopic biopsy the polyps usually "chunk off" or detach entirely at the base, unlike other gastric polyps. Considerable variation in size and number is observed. The polyps are sometimes so numerous that they coalesce, forming areas of irregular, matted surface mucosa. Fundic gland polyps rarely cause symptoms.

Fundic gland polyps have been observed to occur in patients as young as 8 years of age. Size and number may gradually increase, remain static, or even decrease. Although they are considered nonneoplastic, fundic gland polyps may rarely progress to cancer [65–67]. Furthermore, up to 25% of fundic gland polyps in FAP patients may exhibit dysplasia, and up to 44% of FAP patients with fundic gland polyps may have dysplasia in some of the polyps. Dysplasia sometimes becomes severe and progresses to gastric cancer [65,68,69]. Similar polyps, although usually fewer in number, also are observed in up to 2% of the general population sporadically and sometimes with chronic use of proton pump inhibitors [62]. Sporadic fundic gland polyps arise from mutations of β-catenin, whereas those in FAP arise from *APC* inactivation

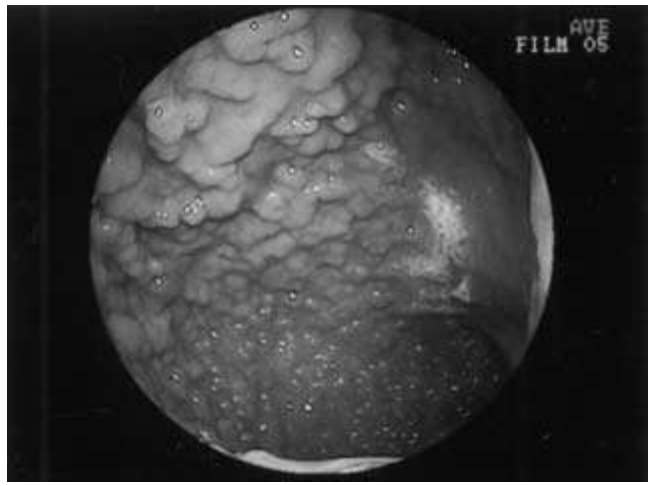

Figure 64.4 Fundic gland polyposis in familial adenomatous polyposis.

[70–72]. *Helicobacter* infection with resultant atrophic gastritis may be protective of fundic gland polyps in FAP [73].

Adenomatous polyps occur in the stomach of about 10% of patients with FAP. They are most often confined to the antrum but are occasionally found in the body and fundus [74,75]. The lifetime risk for gastric cancer in FAP is about 0.6%, believed in part to be from adenomatous polyps.

Duodenum and small bowel

Endoscopically visible duodenal adenomas are found in 50% to more than 90% of FAP patients, and microadenomas are frequently observed [20,63]. The polyps are most commonly 1–5 mm in diameter and multiple. Most patients have numerous small adenomas scattered throughout the duodenum, particularly in the second and third areas, whereas in others the polyps are restricted to the periampullary area. Adenomas may progress, often slowly, and there is evidence of an adenoma–carcinoma sequence similar to that observed in the colon [20]. Adenomas may sometimes grow large, exhibit villous histology and increasing degrees of dysplasia, and may cause symptoms. A scoring system has been developed to evaluate the severity of duodenal polyposis and is widely applied as the Spigelman staging system [76] (Table 64.2). The risk for duodenal cancer increases to 36% within 10 years for Spigelman stage 4 patients [77]. The risk of exhibiting Spigelman stage 4 duodenal polyposis is 43% by age 60 years and 50% by age 70 years [78].

The lifetime risk for duodenal cancer, which most often occurs in the periampullary area, is 3%–5% but in some series has been higher [1,20,63,79]. The age at diagnosis of duodenal cancer ranges from 17 to 81 years, with a mean between 45 and 52 years. Duodenal cancer is one of the leading causes of death in FAP patients who have had prophylactic colectomy.

The presence or severity of duodenal polyposis has been associated with germ-line and somatic mutations between codon 1400 and 1580 [20], although a more proximal mutation region has been suggested in another study [33]. Mutations downstream from codon 1051 have also been assocated with severe periampullary lesions [79]. The frequent clustering of adenomas and cancer in the periampullary area suggests that bile or pancreatic secretions may play a role in carcinogenesis, and a number of mechanisms have been suggested [20,75].

Adenomas may occur throughout the small bowel but are concentrated for the most part in the proximal jejunum (50% of cases) and distal ileum (20% of cases) [80,81]. Malignancy is unusual, but small bowel cancer has been reported. Adenomatous polyps are also known to occur, possibly with increased frequency postoperatively, in the distal ileum after subtotal colectomy, colectomy with ileostomy, and colectomy with ileoanal pull-through. Distal ileal cancer also has been reported in these settings, but it is unusual. Prominent lymphoid polyps may occur frequently in the terminal ileum of younger patients with FAP and should be differentiated from adenomas by biopsy.

Gallbladder, bile ducts, and pancreas

Both adenomatous change and cancer have been reported in the gallbladder, bile ducts, and pancreas [26,80,82–84]. Biliary and pancreatic duct obstruction have arisen from both benign and malignant lesions. The cancer risks are shown in Table 64.3.

Benign extraintestinal growths

Osteomas and dental abnormalities

Multiple osteomas were the first extracolonic lesions to be associated with FAP [1,85,86]. These lesions are benign bone growths found most commonly on the skull and mandible, but they may occur on any bone of the body [87]. The size ranges from imperceptible to several centimeters in diameter, and they number from one to dozens. Osteomas may occur in children who are at risk for FAP before the onset of colonic polyposis [88], but they can continue to occur throughout life [89]. Osteomas have no malignant potential and are not a clinical problem except for occasional cosmetic concern. Subtle radiopaque jaw lesions are often evident by panoramic dental radiographs in patients with FAP and no other apparent extraintestinal lesions of Gardner syndrome

Table 64.2 Duodenal adenomatosis staging system

Polyps	1 Point	2 Points	3 Points
Number	< 4	5–20	> 20
Size	0–4 mm	5–10 mm	> 10 mm
Histology	Tubular	Tubulovillous	Villous
Dysplasia	Mild	Moderate	Severe

Stage I, 4 or fewer points; stage II, 5–6 points; stage III, 7–8 points; stage IV, 9–12 points.
Adapted from Spigelman et al. [76].

Table 64.3 Extracolonic cancer risks in familial adenomatous polyposis (FAP)

Malignancy	Relative risk	Absolute lifetime risk (%)
Desmoid	852.0	15.0
Duodenum	330.8	3.0–5.0
Thyroid	7.6	2.0
Brain	7.0	2.0
Ampullary	123.7	1.7
Pancreas	4.5	1.7
Hepatoblastoma	847.0	1.6
Gastric	–	0.6

From Galiatsatos and Foulkes [26], with permission from Blackwell Publishing.

[90,91]. Various dental abnormalities also have been described in FAP, including unerupted teeth, supernumerary teeth, dentigerous cysts, and odontomas [86,88,92,93]. These all may precede the development of colonic polyposis, and the prevalence is estimated at 17%, compared with 1%–2% in the general population.

Cutaneous lesions

Epidermoid cysts, sebaceous cysts, fibromas, and lipomas have all been described as a part of FAP [87,88,94–98]. Epidermoid cysts occur most commonly on the legs, face, scalp, and arms, in that order, but they may appear anywhere on the body surface [96]. They range in size from millimeters to several centimeters. Although common in the general population, they often occur in FAP before puberty, may precede the onset of polyposis, and occur at an average age of 13 years. Fibromas, ranging in size from millimeters to centimeters, are found most commonly on the cutaneous surface of the scalp, shoulders, arms, and back. The cutaneous lesions may cause cosmetic problems, and cysts occasionally become infected.

Desmoid tumors

Desmoid tumors (also called *desmoid fibromatosis*) are benign fibrous growths that occur rarely in the general population (5–6 per 1 million per year [99]) but frequently in FAP, affecting 3.6%–20% of patients [100–103]. About 2% of all desmoids arise in patients with FAP. The relative risk for FAP patients compared with the general population is 825 [104]. The peak incidence of desmoids in FAP is between 28 and 31 years, although desmoids may occur at any age. Independent predictors for desmoids include an *APC* mutation 3′ of codon 1444 (see "Genetics" above), a family history of desmoids, female gender, and the presence of osteomas [33,105]. They most commonly occur following surgery after an average interval of 4.6 years [106]. Desmoid tumors are one of the most common causes of mortality in FAP patients who have had prophylactic colectomy, with a 10%–50% mortality rate from the tumor in those with desmoids. The 10-year survival rate is 63%.

Desmoids may arise in musculoaponeurotic structures throughout the body, although they are most common in the abdomen [102]. They do not metastasize but may infiltrate adjacent structures, extend along facial planes, attach to and erode bones, and engulf and compress blood vessels, nerves, ureters, and other hollow organs of the abdomen. Severe and even fatal clinical problems are sometimes caused by these tumors, especially if mesenteric vessels or other abdominal organs are obstructed. Other complications include bowel perforation, fistulization, bleeding, and ureteral obstruction [100,107]. In the abdomen of FAP patients, mesenteric plaque-like lesions occur that may progress to mesenteric fibromatosis and finally desmoid tumors, often stimulated by surgery [108]. Desmoid tumors usually enlarge gradually and sometimes stop growing altogether. Only about one-third of abdominal desmoids cause pain, although abdominal pain is the most common symptom. Intraabdominal desmoids sometimes become massive, occupying much of the abdominal cavity and encasing many segments of viscera.

These tumors may be the first manifestation of FAP in some patients and families. Furthermore, some families with *APC* mutations exhibit desmoids as their only disease manifestation [109,110]. A distinctive histopathology has been demonstrated in desmoid tumors of young FAP patients, whose first manifestation of the condition was often this lesion [111]. Desmoids in FAP are monoclonal growths of hyperproliferative fibroblastic cells [112]. Excess cell growth appears to occur as a consequence of *APC* gene inactivation and accumulation of β-catenin in the cells [113]. The relationship of desmoids in FAP to sporadic cases is uncertain in view of the distinctive early histopathology [111] and the observation that *APC* gene mutations are uncommon in sporadic desmoids [114].

Congenital hypertrophy of the retinal pigment epithelium (CHRPE)

Multiple and bilateral patches of CHRPE, also called *pigmented ocular fundus lesions*, have been described as a common manifestation of FAP [115]. The lesions are discrete, darkly pigmented, round, oval, or kidney shaped and range in size from 0.1 to 1.0 disc diameters. The presence of bilateral or multiple (more than four) lesions is highly specific (94%–100%) but only moderately sensitive (58%–84%) for FAP [116]. The lesion appears to be congenital, has been detected in a 3-month-old at-risk infant, and may be the earliest clinically detectable lesion of FAP. Careful slit-lamp examination is often required for detection. The presence of CHRPE correlates closely with mutations in specific areas of the *APC* gene (see "Genetics" above).

Adrenal adenomas

Adrenal adenomas have been reported in FAP [117]. Two studies found their occurrence to be significantly higher than in the general population, at 7% and 13%, respectively, versus 3% [118,119]. Several cases of functioning adrenal adenomas and adrenal carcinomas have also been noted in these same series, but any association is uncertain. Most adrenal masses in FAP are found incidentally on computed tomography (CT). It has been recommended that adrenal lesions be managed similarly to those in the general population, which generally entails hormonal investigation if relevant symptoms are present, and needle biopsy if a simple adenoma is not indicated by CT or the lesion is 5 cm or larger [118,119].

Nasopharyngeal angiofibroma

Nasal angiofibromas have been correlated with FAP in a number of patients [120]. The *APC* gene acts as a tumor suppressor gene in nasopharyngeal angiofibromas [121].

Extracolonic malignancies

A number of malignancies outside the colon are known to exhibit an increased lifetime risk in patients with FAP, as shown in Table 64.3. There have been case reports of other rare tumors such as myoepithelial carcinoma and intraductal papillary and mucinous pancreatic tumour [122,123].

The mean age of diagnosis of thyroid cancer is 28 years, ranging from 12 to 62 years [124–126]. A female preponderance is observed, and the histology is predominantly papillary, commonly with a cribriform pattern. Familial aggregation has been observed, and mutation analysis has revealed that most mutations are identified outside the mutation cluster region [126].

Hepatoblastoma exhibits a male predominance and associates somewhat with mutations in the 5′ end of the *APC* gene. This malignancy most often occurs in the first 5 years of life, with some risk up to 15 years of age [127]. The initial presentation of polyposis in a family may be in a young patient with a relatively rare tumor like hepatoblastoma or periampullary adenocarcinoma [128,129].

Syndromes related to familial adenomatous polyposis

Gardner syndrome

In the early 1950s, Gardner and colleagues described a large kindred that exhibited typical FAP, but also prominent extracolonic growths, especially osteomas, epidermoid cysts, and fibromas [86]. All of the other extracolonic lesions noted previously were eventually added to the phenotype of Gardner syndrome. Discovery of the *APC* gene led to the knowledge that FAP and Gardner syndrome arise from mutations of that gene. Further, the expression of extracolonic manifestations correlates to some extent with location of the mutation (see "Genetics" above). The term *Gardner syndrome* is thus mainly of historical interest but will likely continue to be used for families with frequent and obvious extraintestinal manifestations of the condition [32].

Turcot syndrome

Turcot syndrome is characterized by colonic polyposis and central nervous system (CNS) tumors [130]. About two-thirds of Turcot syndrome cases arise from mutations of the *APC* gene and exhibit medulloblastoma-type CNS tumors as well as some anaplastic astrocytomas and ependymomas [131,132]. These cases usually exhibit typical colonic polyposis. The other one-third of cases come from mutations of the mismatch repair genes and are thus a part of hereditary nonpolyposis colorectal cancer (HNPCC). The CNS malignancies in HNPCC-related Turcot syndrome are glioblastoma multiforme tumors, and affected patients also exhibit fewer adenomatous polyps, typical of HNPCC [132]. The risk for cerebellar medulloblastoma in FAP is calculated to be 92 times that of the general population [131]. The occurrence of CNS malignancies is not a completely random event in FAP, however, because 40% of families with brain tumors include more than one affected person. Correlations have not been made between the locations of *APC* mutations and CNS tumors.

Attenuated familial adenomatous polyposis (AFAP)

Attenuated FAP (AFAP), also called attenuated APC, arises from *APC* mutations at either the extreme proximal (5′) region of the *APC* gene, or at the extreme distal (3′) portion of the gene, or in certain parts of exon 9 [133]. Patients with AFAP have many fewer adenomas than those with typical FAP, averaging 25 (range 0–470) in one study and exhibiting a proximal colonic preponderance [133–135]. Equally notable, however, is that the polyp number is extremely variable in many kindreds, with some members of the same kindred having a small number of adenomas and others having numbers approaching that of typical FAP. The cumulative risk of colorectal cancer by age 80 in two large carefully studied kindreds was estimated to be 69%, with an average age at diagnosis of 58 years (range 29–81 years) [133]. In another study, the average age at symptomatic presentation was 52 years [56]. The emergence of adenomas is also delayed by about 10 years. The expression of upper GI polyps, both gastric and duodenal, does not appear to be attenuated in number, age at emergence, or cancer risk [67,135]. It may be difficult to diagnose AFAP without genetic testing because some patients exhibit few adenomas even at advanced ages [133].

Differential diagnosis

All of the polyposis syndromes reviewed in this chapter are included in the differential diagnosis of a patient found to have GI polyposis. Hereditary nonpolyposis colorectal cancer must occasionally also be distinguished, particularly from AFAP [136]. Histology is paramount to specific diagnosis because most of the syndromes have histologically characteristic polyps. The colonoscopic appearance of myriad 1–5-mm polyps is characteristic of FAP, although lymphoid hyperplasia and hyperplastic polyposis (HP) may mimic this appearance. Additional parameters to be considered in differentiating the various polyposis conditions include: polyp distribution throughout the GI tract; polyp number and size; the presence of extraintestinal growths, lesions, or malignancy; and the family history of any of these features. Finally, genetic testing is a powerful tool that can be used to make a definitive syndrome diagnosis in most cases, enabling further genetic testing in family members when the relevant mutation is found. Each of the polyposis syndromes in this chapter is characterized in terms of these parameters. *MUTYH*-associated polyposis (MAP) is often only distinguishable from FAP or AFAP by genetic testing, as many MAP patients present as typical FAP or AFAP. Of note, however, the MAP phenotype consists of multiple to hundreds of adenomatous polyps, but not thousands.

Clinical course and complications

Most individuals with FAP remain asymptomatic until colon cancer occurs. Adenomatous polyp development is slow and insidious. There are usually only a few minute polyps when adenomas are first detected. Polyp number and size increase gradually over ensuing years. Symptoms from the disease are seldom experienced if a timely colectomy is performed. Left untreated, nonspecific symptoms eventually develop from the polyposis or colon cancer. The most common symptoms include rectal bleeding (79%), diarrhea (70%), and abdominal pain (40%) [1]. The average age at onset of symptoms is 32.8 years. Sixty-six percent of individuals who present with symptoms have already developed cancer. Presymptomatic screening and appropriate prophylaxis comprise the favored approach in FAP.

Upper GI polyps are usually asymptomatic unless cancer occurs, with the exception of polyps at the duodenal papilla or in the bile or pancreatic ducts, which may cause obstructive jaundice or pancreatitis. Symptoms from polyps and cancer are nonspecific and include abdominal pain, weight loss, GI blood loss, palpable mass, and lumenal obstruction.

The average life expectancy of patients with untreated FAP has been estimated to be 42 years, but it approaches that of the general population for up to 18 years after colectomy [137]. The major causes of death after colectomy are duodenal cancer (particularly periampullary), desmoid tumors, and rectal cancer if a subtotal colectomy was preformed [138–140]. Other cancers are occasionally observed, such as pancreatic, biliary, adrenal, and small bowel. The cumulative probability of an FAP patient developing extracolonic cancer of any type after colectomy in the St Mark's Registry was found to be 11% by 50 years of age and 52% by 75 years, although most of the malignancies, like in other series, were in the periampullary region [141]. This overall risk was significantly greater than the comparable risk in the general population of about 4% and 22% at ages 50 and 75 years, respectively.

Management

Diagnosis

The diagnosis of FAP is made by:

- finding characteristic clinical manifestations, specifically more than 100 colonic adenomas at any age
- finding adenomas in a younger first-degree relative of a person with known FAP
- finding a disease-causing mutation in the *APC* gene in a person suspected of having FAP [41,42].

Many fewer polyps can give rise to suspicion of AFAP [133]. A constellation of other findings, such as typical upper GI polyps and osteoma, can lead to a high suspicion of FAP. Thus, diagnosis virtually always involves colonoscopic examination with biopsy of the colon and frequently genetic testing.

Screening and surveillance

The primary goal of FAP management is to prevent cancer. In view of the extreme cancer risk in the colon and emerging knowledge of other cancer risks, empirical screening guidelines have been suggested on the basis of these risks and the likely ages of cancer development (Table 64.4).

Colon screening

Colon screening with subsequent surgery has been shown definitively to decrease mortality from large bowel malignancy. A study from the Swedish National Polyposis Registry that included 431 FAP patients from 145 families found a mortality rate of 44% in self-presenting FAP patients compared with 1.9% in relatives who were called up for screening [142]. The Danish Polyposis Registry found colon cancer was diagnosed in 170/252 probands (67%) but only 5/182 screened patients (3%) [59]. Cancer and mortality rates in

Table 64.4 Screening recommendations in familial adenomatous polyposis (FAP)

Cancer	Age to begin screening (years)	Screening interval (years)	Screening procedures
Colon	10–12 Late teens if AFAP	1	Sigmoidoscopy or colonoscopy if AFAP
Duodenal or periampullary	20–25	1–3	EGD also with "sideviewing" exam of the duodenal papilla
Pancreatic (possibly)	20	1–3	Abdominal ultrasound
Thyroid	10–12	1	Physical examination, possibly ultrasound
Gastric	20–25	1–3	Same as for duodenal
Central nervous system, usually cerebellar meduloblastoma (Turcot syndrome)	First decade	1	Annual physical examination, possibly periodic MRI scan of head in affected families
Hepatoblastoma	3 months	3 months	Possibly liver palpation, hepatic ultrasound, α-fetoprotein during first decade of life

AFAP, attenuated familial adenomatous polyposis; EGD, esophagogastroduodenoscopy; MRI, magnetic resonance imaging.

FAP declined dramatically in Denmark after 1975 because screening for polyposis has been successful in leading to colectomy in most patients before cancer could develop [59]. The Finnish polyposis registry found survival in postcolectomy FAP patients who had undergone screening because of a relative with known FAP to be equivalent to that in the general population and statistically much lower than that in FAP patients presenting with symptoms up to 18 years after colectomy [137].

Colon screening should be performed in those with a genetic diagnosis of FAP or in first-degree relatives of those with FAP if genetic testing is uninformative or has not been done. The generally accepted colon screening guideline for typical FAP is annual sigmoidoscopy beginning at 10–12 years of age [39,143,144]. Those initially screened at an older age should probably have colonoscopy for the first examination. If surgery is delayed longer than a year after polyps emerge, annual colonoscopy should be used for surveillance. For AFAP, colonoscopy should always be used for screening in view of the polyp distribution, but can reasonably be delayed until the patient's late teens to mid-20s.

Upper gastrointestinal screening

Upper GI screening has not been demonstrated to improve prognosis but is nonetheless recommended in view of the cancer risk and expectation that mortality can be reduced [63,78,79,145]. The most common recommendation is to begin screening at the age of 20–25 years, although some recommend an initial examination at the time of diagnosis and earlier screening if other family members have exhibited advanced duodenal disease at an earlier age [20]. Standard upper endoscopy should be supplemented with a side-viewing instrument to visualize the duodenal papilla. Endoscopic ultrasound or endoscopic retrograde cholangiopancreatography may also be indicated, depending on endoscopic findings or symptoms. A 1–3-year interval for reexamination is given as follows [20] (see Table 64.2):

- every 3 years for Spigelman stages I and II disease
- every 2 years for stage III disease
- every 6–12 months for stage IV disease.

Another approach is triennial endoscopy if adenomas are not found and annually if they are. It would certainly seem reasonable to examine the stomach during endoscopy and to biopsy any polyps of concern because of size, color, or gross appearance. A pediatric study concluded that all pediatric patients should have upper GI screening at the onset of colonic screening [146].

Screening for other malignancies and desmoids

Screening for the other associated cancers and for desmoids is empirical. The thyroid should be examined annually, starting at age 10–12 years, in view of risk and ease of examination. Some argue that other sites should be evaluated only for symptoms or if these cancers have occurred in relatives. Screening is not performed for desmoids, and evaluation is done only for palpable masses or symptoms. Patients may be given the option of abdominal CT with oral contrast every 3 years to examine the pancreas, small bowel, and adrenal glands, starting at age 20–25 years. Careful questioning is undertaken for CNS symptoms, and periodic magnetic resonance imaging (MRI) of the head is recommended if any family member has had CNS malignancy. Screening pediatric patients for hepatoblastoma is debated but if done should include three-monthly α-fetoprotein determination, liver ultrasound, and liver palpation, probably from birth and continuing throughout the first decade. Specific biliary evaluation is done only for abnormal laboratory tests or symptoms. The small bowel should be examined periodically by radiography or push enteroscopy if upper endoscopy demonstrates severe duodenal polyposis. Capsule endoscopy is also available for small bowel screening [147] but a question remains as to whether the examination is sufficiently complete for larger lesions.

Ileal and rectal screening after colectomy

Adenomas may develop in the ileal pouch after colectomy with ileoanal pull-through surgery, or they may develop from rectal epithelium left with restorative proctocolectomy [148,149]. Whether there is a risk for cancer in ileal pouch adenomas is uncertain, although cases have been reported. Advanced dysplasia and cancer are, however, seen at the anal transition zone, either from rectal tissue unexpectedly remaining with ileoanal anastomosis or from the short segment of rectum remaining with restorative proctocolectomy [150,151]. Screening of the pouch should be done within 2 years after surgery and then every 6 months to 3 years, depending on the presence and number of adenomas.

When subtotal colectomy with ileorectal anastomosis is performed, rectal adenomas often regress [152]. However, they almost always recur, and are associated with a substantial cancer risk [153]. One study of cancer risk in the rectum after subtotal colectomy indicates a 40-year risk of 32% [154]. Chronological age was the only independent risk factor, but cancer was more common when *APC* mutations occurred in gene areas that give rise to dense polyposis. Sigmoidoscopy with ablation of recurrent adenomas is believed to minimize and delay cancer occurrence and is suggested to be done every 3–6 months, depending on the rate of adenoma recurrence [81].

Treatment

Colon and rectum

An appropriately timed colectomy remains the keystone of cancer prevention in FAP. It should be considered once adenomas emerge. Most centers prefer to wait until after the patient has completed high school if colonic adenomatosis remains minimal, although delay has been associated with some cancer occurrence, particularly in FAP patients older than 20 years of age [155]. Surgical options include:

- subtotal colectomy with ileorectal anastomosis
- total colectomy with mucosal proctectomy, ileal pouch construction, and ileoanal pull-through (also known as ileal pouch–anal anastomosis, or IPAA) and restorative proctocolectomy, in which an ileal pouch is also constructed and a 1–2-cm cuff of rectal tissue (the anal transition zone) is left for continence.

The latter procedure is the preferred option in many cases [150]. Colectomy with ileostomy is rarely needed. Subtotal colectomy is a single-stage procedure with slightly less morbidity than the pull-through surgery, but some cancer risk remains and regular follow-up must be assured. This is most commonly done for AFAP and when rectal polyp sparing is present [61,154]. Unfortunately, some cancer risk remains even with the IPAA and restorative proctocolectomy [150,151].

The ileal pouch will fail and require excision or permanent diversion in fewer than 5% of patients after surgery [156,157]. There is a low rate of incontinence in the daytime (5.6%) or nighttime (13.3%), more commonly in older patients [158]. Fecundity is lower in women, but in total only 14% of patients complain of social, sexual, or work restrictions after IPAA [159]. Mesenteric desmoid tumors (MDT) are the principal cause of obstruction and death in FAP patients after colectomy [160]. Laparoscopic restorative proctocolectomy is a minimally invasive technique that aims to reduce the scarring that may trigger MDT, but a 4–7-cm periumbilical incision is still required for vascular dissection and pouch formation [161]. Mutation location may help in determining choice of procedures, but adenoma expression is variable, even with identical mutations, making clinical expression of polyps the determining factor in surgical decisions [56]. Despite rectal screening, the cumulative risk for secondary proctectomy to control polyposis or treat cancer in those with a remaining rectum was 0.70 at 40 years of follow-up in one study, although it was never necessary in patients with AFAP [154].

Some patients with AFAP can be managed for many years with colonoscopic polypectomy of millimeter-sized polyps and may possibly never need colectomy [133]. In general, this approach should only be considered with AFAP patients who exhibit only small numbers of millimeter-sized adenomas. After subtotal colectomy in a large series of patients with AFAP, an average of 3.4 recurrent polyps (range 0–29) and only one rectal cancer was found in the postcolectomy rectal remnant over a mean follow-up of 7.8 years (range 1–34 years) [133].

A number of studies have demonstrated that nonsteroidal antiinflammatory drugs (NSAIDs) and selective cyclooxygenase-2 (COX-2) inhibitors induce colonic adenoma regression in FAP [162–169]. It is presumed but not proven that cancer risk is also diminished. Equally important, polyp ablation is considerably easier in patients with a remaining rectum because NSAID therapy often minimizes the number of new adenomas encountered during follow-up examinations [167]. The use of NSAIDs to prevent the emergence of adenomas in patients with a genetic diagnosis of FAP or to delay colectomy in those with polyposis is not yet recommended [170].

The COX-2 inhibitor rofecoxib (Vioxx) induces polyp regression [168,169] but has been withdrawn from the market because of increased cardiovascular and cerebrovascular events associated with its use [171]. The selective COX-2 inhibitor, celecoxib (Celebrex), has been approved by the US Food and Drug Administration (FDA) and the analogous regulatory agencies in Europe and Japan for polyp regression therapy in FAP patients who have had a subtotal colectomy with a remaining rectum [166]. However, celecoxib has also been found to have similar side effects to refecoxib, but remains available [172]. There are no studies directly comparing celecoxib with sulindac, but sulindac causes anastomotic erosions or ulcerations leading to discontinuation in about half of the patients treated and its efficacy is variable in different studies [167,170]. Interindividual differences in chemopreventive responses are large, and appear to be correlated with the amount of reduction in mucosal prostaglandin levels [173]. Polyp regression has also been reported with use of estrogen/progesterone compounds [174].

Upper gastrointestinal tract

Intervention for gastric polyps is unusual. Discrete adenomas should be endoscopically resected if possible. The development of high-grade dysplasia in multiple fundic gland polyps can lead to the consideration of partial or total gastrectomy.

Duodenal adenomas, particularly ampullary and periampullary ones, more often need therapy [20]. Possible therapies include endoscopic ablation or polypectomy, surgical polypectomy, endoscopic and surgical papillectomy, surgical duodenectomy, and pancreaticoduodenectomy (Whipple procedure) [175]. In view of their malignancy potential, removal of duodenal adenomas should be considered if they become large (probably at least 2 cm) or exhibit villous histology or advanced dysplasia. Single, large, pedunculated adenomas can usually be removed endoscopically, whereas large ones, especially if they are sessile with advanced dysplasia, may require surgical duodenotomy. Severe, diffuse duodenal adenomatosis may require duodenectomy or the Whipple procedure, particularly if severe dysplasia is present. Unexpected cancers are often found in the surgical specimen, although it is still debatable whether radical surgery is appropriate for benign lesions in view of possible complications and morbidity. There is a need for development of more effective chemotherapy for duodenal polyps [175]. The chemopreventive drugs that are effective in the colon – NSAIDs such as sulindac and the COX-2 selective inhibitors celecoxib and rofecoxib – are not as effective in the duodenum and are not FDA-approved for treatment in the small bowel [176]. Ursodeoxycholic acid decreases duodenal adenomas in mice with polyposis, but has not yet been studied in humans [177].

Endoscopic papillectomy is indicated for ampullary adenomas that become symptomatic (including laboratory cholestasis) or develop villous change or advanced dysplasia [20,79,178–181]. The recurrence rate for ampullary adenomas is high, however, and multiple treatment sessions or surgical therapy may be needed in selected cases. The Whipple procedure is most often used if surgery becomes necessary, although an increase in diarrhea often results in patients with previous colectomy [153]. Pylorus-sparing pancreaticoduodenectomy is also an option [182].

The Heidelberg Polyposis Register study is particularly demonstrative of the various options [20]. In that registry, endoscopic follow-up of the duodenum of FAP patients was accomplished in 135 patients, with an average follow-up of 55.6 months (range 6–172 months). One hundred and thirteen of the patients (83.7%) needed no therapy of the duodenum during the follow-up period, whereas 22 (16.3%) did. Endoscopic polypectomies were accomplished in four cases, whereas surgical duodenotomy was needed because of polyp size (large and sessile) in five. Endoscopic papillectomy was done in six cases because of symptomatic adenomas or severe dysplasia. Whipple procedures were needed in seven patients for severe diffuse duodenal adenomatosis, with cancer being unexpectedly found in four of these surgical specimens. Two of these involved the papilla, whereas two were in the distal duodenum.

Other therapies are under investigation for duodenal adenomas, including photodynamic therapy and various NSAIDs. Trials of both have been inconclusive thus far. Studies with higher doses and longer duration of NSAID therapy, or of other drugs such as ursodeoxycholic acid or polyamine analogues, are needed because of the known effect of these agents on colonic adenomas and the need for effective chemoprevention in the duodenum [173].

Desmoid tumors

Magnetic resonance imaging and CT and are equally effective in following desmoid tumors [183]. Treatment is undertaken for symptoms, cosmetic issues, functional disruption, or imminent risk to adjacent structures. Extraabdominal and abdominal wall desmoids are best treated by surgery with adequate resection of margins [100–102]. Less than half of tumors so treated recur, and further therapy with radiation and repeat excision is usually successful. Surgery is difficult, and sometimes impossible, in cases of intraabdominal desmoids but remains as an important option in selected cases [100,184,185].

Because intraabdominal desmoids often involve the mesentery or encase vessels or organs, medical therapies are often first attempted. The most consistent initial therapies for abdominal desmoids appear to be the NSAID sulindac and the antiestrogen tamoxifen. Both give a better than 50% response after 3–6 months of therapy for FAP [186–188]. The response may be even better with a combination of the two drugs. Combination chemotherapy is effective and can be offered for unresectable cases that fail to respond to sulindac and tamoxifen [189–191].

Other therapies have also been used with desmoids with variable success. A combination of low-dose methotrexate and vinblastine demonstrated stabilization of tumor growth or shrinkage in 60% of patients with sporadic desmoids at various stages [99]. This therapy has not yet been reported in FAP desmoid cases. Tyrosine kinase inhibition has also shown some response [192]. Intestinal transplantation has been used successfully in a number of cases of unresectable intraabdominal desmoids [193].

MUTYH gene-associated polyposis

Epidemiology and clinical characteristics

An attenuated polyposis syndrome with a recessive pattern of inheritance has been described involving biallelic mutations in the base excision repair gene *MUTYH*, a gene involved in repair of DNA oxidative damage [194,195]. The clinical condition is characterized by multiple adenomatous polyps and an increased risk for colorectal cancer. Referred to as *MUTYH-associated polyposis* (MAP), the condition is often indistinguishable from classical FAP or AFAP except that the family history will demonstrate recessive inheritance. The inheritance pattern may not be helpful, however, because of the substantial frequency of de novo *APC* germ-line mutations causing FAP, and because many small families will not exhibit a recessive pattern even if MAP is present in the family.

MUTYH-associated polyposis accounts for 7.5%–12.5% of FAP patients in whom a disease-causing *APC* mutation is not found [195,196], and from 16% to 40% of patients with 15–100 colonic adenomas but not FAP [195,197–200]. Patients with MAP do not appear to have more than 500 adenomas but may exhibit only small numbers of adenomas [196,197]. In a study from the Mayo Clinic, biallelic *MUTYH* mutations were not found in any patients with fewer than three adenomas, with a colon cancer diagnosed when over 50 years of age, or with a microsatellite instability (MSI)-positive colon cancer. In the absence of *APC* mutations, biallelic *MUTYH* mutations were found in 1.7% of colorectal cancer patients aged up to 50 years, in 13.6% of patients with 20–49 adenomas, in 20% of patients with 50–99 adenomas, in 20% of patients with 100–499 adenomas, but in no patients with more than 500 adenomas.

MUTYH-associated polyposis appears to be an unusual cause of colon cancer, even early-onset colon cancer [201], but the risk of this malignancy in those who have biallelic *MUTYH* mutations is high. In a study from Finland, 0.4% of 1042 population-based colorectal cancer cases had biallelic *MUTYH* mutations [202]. All those with mutations were found to have adenomas, ranging in number from 3 to 100. In a large (2239 cases, 1845 controls) population-based case–control study from Scotland, 0.8% of colorectal cancer cases aged

under 55 years, and 0.54% of all cases, had biallelic mutations [203]. Biallelic mutation carriers had a 93-fold excess risk of colorectal cancer. Significantly more biallelic carriers exhibited adenomatous polyps, although 36% had no polyps. There was also a slight (1.68-fold) but significant (95% CI 1.07–2.95) increased risk of cancer among heterozygous mutation carriers among patients aged under 55 years, with a population attributable risk of 0.93% (95% CI 0%–2.0%) in this group. A Canadian case–control study with 1238 colorectal cancer cases and 1255 controls found 2.34% of cases and 1.67% of controls to be heterozygous for *MUTYH* mutations [204]. This represents an odds ratio of 1.4 (95% CI 0.8–2.5), so statistical significance was not reached. Thus the cancer risk for biallelic mutation carriers is high, accounts for almost 1% of colon cancer cases, and is usually, but not always, associated with multiple adenomas. There appears to be a slight colorectal cancer risk associated with heterozygous mutation carriers but further investigation is needed to verify this.

MUTYH-associated polyposis syndrome is different from the syndromes of AFAP and FAP, but it is impossible to distinguish from these conditions in individual patients [205]. The similarity of MAP is more with AFAP, in that it presents at later ages, typically mid-50s (but sometimes as young as 16) [205] and exhibits relatively fewer polyps. Polyp numbers from 5 to 750 have been reported, but as studies above demonstrate, many biallelic *MUTYH* mutation carriers have no polyps. Gastric cancer, duodenal adenomas and CHRPE have been reported [205], but uncommonly enough that their association remains unclear. Reported colorectal cancers in MAP have been predominantly left-sided [205].

Genetics and pathophysiology

MUTYH-associated polyposis arises from biallelic inactivation of the *MUTYH* base excision repair gene. The DNA glycosylase encoded by this gene accomplishes DNA oxidative damage repair. Inactivation of the *MUTYH* gene by mutation gives rise to frequent G:C to T:A transversions, which are thought to affect the *APC* gene somatically and thereby give rise to polyp and cancer predisposition [194,195,205]. Predisposition to polyps and cancer occurs with germ-line *MUTYH* mutations, but somatic *MUTYH* mutations do not seem to play a role in the pathogenesis of colon cancer [206]. And although mutations of *APC* are frequent, and *APC* is probably the first mutated gene in *MUTYH*-associated tumor pathogenesis, there appear to be some differences in the molecular pathways involved [207]. A specific and characteristic *KRAS* mutation is frequently observed in MAP adenomas and cancers. Neither microsatellite instability nor chromosomal instability is observed in MAP, but instead a near-diploid karyotype with overall low levels of loss of heterozygosity.

The two most prevalent *MUTYH* mutations, occurring in more than 80% of persons of European ancestry with MAP, are Y165C and G382D [194,195,198,199,202,206]. A population frequency for each of these alleles in the white population has been estimated at 0.005 [194]. An E446X nonsense mutation is found in Indian families [198,199], a 1295delGGA in affected Italian patients [208], a Y90X mutation in Pakistani families [198], and an nt1395–7delGGA in a Greek patient [205]. Mutations other than the common ones sometimes occur on the second allele, thereby causing gene inactivation. Thus in a person undergoing genetic testing, the common mutations are first examined. A diagnosis of MAP is made with the presence of one of the common mutations on each of the alleles. If only one allele is affected by one of the common mutations, sequencing is then done to determine if an inactivating mutation is present on the second allele.

Diagnosis, screening, and treatment

There are no distinguishing clinical or pathological characteristics for MAP. In fact the individual phenotype cannot be distinguished from patients with multiple adenomas, attenuated FAP, or classical FAP. The natural history of adenoma occurrence and progression is not well understood but MAP cancers occur predominantly in the left colon, with stage and grade similar to that observed in sporadic colorectal cancer [205]. Adenomas in MAP are also histologically indistinguishable from sporadic or FAP adenomas. Without further characterization of MAP, likely be accomplished in the near future, genetic testing appears to be the only method to identify accurately this condition.

MUTYH-associated polyposis is sufficiently common among patients with multiple adenomas that any such patient, particularly one with more than 15–20 adenomas, should be considered for a diagnosis of MAP and thus be considered for *MUTYH* genetic testing [196,198,200,202]. Similarly, patients with early-onset colorectal cancer not explained by other genetic syndromes could be considered for *MUTYH* genetic testing [196,202]. Some MAP patients will still be missed with these criteria, and thus family history and other elements of the Bethesda criteria have also been used to identify MAP patients [197]. Finally, those in whom AFAP or classical FAP is being considered, but in whom no *APC* mutations are found, should also have testing for *MUTYH* mutations, particularly if no vertical transmission of colorectal polyps or cancer is apparent. It may, in fact, be more efficient to begin with *MUTYH* testing rather than *APC* testing in multiple adenoma patients with no obvious vertical transmission of disease [200]. The only other known genetic condition possible in this setting is AFAP, which may be less common than MAP.

Surveillance and disease management of the colon should be similar to those performed for patients with multiple adenomas, AFAP, and FAP until further information is available concerning the natural history of MAP [205]. Thus colonoscopy should be used for screening, surveillance, and polyp removal. Colonoscopy intervals of 1–3 years would seem appropriate, depending on the adenoma number and rate of recurrence. If polyps cannot be managed colonoscopically,

or multiple advanced adenomas (> 1 cm, villous histology or severe dysplasia) are encountered, then colectomy should be considered. As with to AFAP, FAP, and some adenoma patients, subtotal colectomy with close subsequent surveillance would seem to be the best option if relative rectal sparing is present, and restorative proctocolectomy undertaken if the rectum is substantially involved. It is not known if upper endoscopic surveillance should be performed. The subset of multiple adenoma patients with MAP might be an attractive group in which to investigate antioxidant therapy as a preventive option in view of the defective oxidative repair mechanism in MAP.

Syndromes with hamartomatous polyps

Peutz–Jeghers syndrome

Epidemiology and clinical characteristics

Peutz–Jeghers syndrome (PJS) is an autosomal dominantly inherited syndrome of histologically distinctive hamartomatous polyps of the GI tract and characteristic mucocutaneous pigmentation [209]. Its incidence is estimated at 1 in 120 000 births. A high risk for both GI and non-GI cancers is known to be part of this condition [210].

The mucocutaneous melanin pigment spots are seen in more than 95% of cases (Fig. 64.5). They are 1–5 mm in diameter and most commonly occur in the perioral and buccal areas (94%). Pigment spots on the lips are distinctive in that they cross the vermilion border and are often much darker and more densely clustered than common freckles. These spots also occur on the face, forearms, digits, palms, soles, perianal area, and rarely the intestinal mucosa. The pigment appears in infancy and may fade with age, less so on the buccal mucosa.

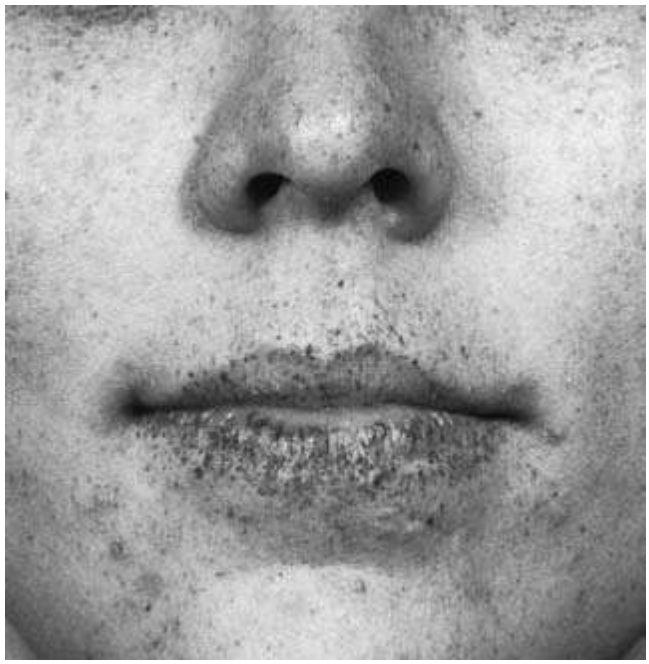

Figure 64.5 Typical perioral melanin pigmentation in Peutz–Jeghers syndrome.

The GI polyps occur in 88%–100% of patients. Their frequency by segment is: stomach, 24%; small bowel, 96%; colon, 27%; and rectum, 24% [211,212]. Between 1 and 20 polyps are usually found per GI segment. Polyps range from 0.1 to 3 cm in diameter and have a coarsely lobulated surface. Polyp growth begins in the first decade of life, but patients typically do not develop symptoms until the second or third decade [213]. Symptoms arise from larger polyps, which may infarct, ulcerate, bleed, and cause intestinal obstruction and intussusception. Peutz–Jeghers polyps are histologically distinct. They are nondysplastic, have normal overlying epithelium specific to the GI site in which they are found, and exhibit an arborizing pattern of growth with muscularis mucosae extending into branching fronds of the polyp. Epithelial infolding may result in what is termed *pseudoinvasion*, which can lead to an incorrect diagnosis of cancer. Adenoma and cancer may occur in Peutz–Jeghers polyps, which exhibit monoclonality, and are thought to be the cause of intestinal cancer in this syndrome [213]. This has been referred to as the hamartoma–adenoma–carcinoma sequence.

Benign complications from the polyps predominate in the first three decades of life. The average age at diagnosis is 22 years in men and 26 years in women [211]. One-third of patients experience symptoms in the first decade of life, and 50%–60% before the age of 20 years. The clinical course of PJS is characterized in many patients by repeated surgeries for complications of small bowel polyps. Malignant complications become the dominant cause of clinical problems after 30 years of age [211], with an average age of 43 years for cancer diagnosis of any type. The malignant risk in PJS includes colorectal, breast, pancreatic, gynecological, small bowel, lung, and gastroesophageal cancers in that order of risk [210]. The overall risk of developing cancer at ages 20, 30, 40, 50, 60, and 70 years, is 1%, 3%, 19%, 32%, 63%, and 81%, respectively. The combined risk for gastroesophageal, small bowel, colorectal, and pancreatic cancers at 20, 30, 40, and 60 years is 1%, 10%, 18%, and 42%, respectively. Individual site cancer risks are given in Table 64.5, along with screening recommendations.

Distinctive tumors in women with this condition include ovarian sex cord tumors with annular tubules (SCTAT) (which are benign although approximately 20% become malignant); mucinous tumors of the ovary; and well-differentiated adenocarcinomas of the uterine cervix, called adenoma malignum [211]. Nine percent of males develop large-cell-calcifying Sertoli cell tumors resembling SCTAT, which have a 10%–20% chance of becoming malignant. Feminization may occur with the benign testicular tumors.

Table 64.5 Cancer risks and screening recommendations for Peutz–Jeghers syndrome

Cancer	Cancer risk (%) to age 64 years	Average age of diagnosis (years)	Age to begin screening (years)	Screening interval (years)	Screening procedures and comments
Colon	39	46	25 or with symptoms	2	Colonoscopy
Stomach	29	30	10	2	EGD
Small bowel	13	42	10 or with symptoms	1	Hemoglobin, small bowel radiography or possibly capsule endoscopy
Pancreas	36	41	30	1–2	Endoscopic ultrasound or Transabdominal ultrasound
Breast	54	37	20 20	2 1	Mammography Self breast examination
Ovarian	21	28	20	1	Pelvic exam and pelvic or transvaginal ultrasound
Uterus	9	37	20	1	Pelvic exam and pelvic, transvaginal, or endometrial ultrasound
Cervix (adenoma malignum)	Unusual	34	20	1	Pap smear
SCTAT	20	40	20	1	Same as uterine and ovarian; almost all women develop SCTAT, but 20% become malignant
Testicular (Sertoli cell tumor)	9 (benign tumor)	9 (benign tumor)	10	1	Physical exam, ultrasound if indicated; 10%–20% of the benign Sertoli cell tumors become malignant
Lung	15	51	–	–	No screening recommendations given

EGD, esophagogastroduodenoscopy (or upper gastrointestinal endoscopy); SCTAT, sex cord tumor with annular tubules.
Adapted from Schreibman et al. [212], with permission from Blackwell Publishing.

Genetics and pathophysiology

Peutz–Jeghers syndrome arises from mutations of the *STK11* gene, also called *LKB1*, a tumor suppressor gene on chromosome 19p that encodes a seronine/threonine protein kinase [212]. Possibly up to 96% of PJS families have mutations of *STK11*, when methods to detect large deletions are added to standard mutation detection [214]. A few remaining families do not link to the *STK11* locus, suggesting that other genes may give rise to this condition. Up to 25% of mutations represent de novo mutations [212].

The *STK11* gene affects p53-dependent apoptosis, suppression of cell proliferation, β-catenin phosphorylation (thus the WNT signaling pathway), and cell polarity [215]. Loss of the wild-type allele of *STK11* by mutation, loss of heterozygosity, or promoter hypermethylation has been found in PJS polyps and cancers, verifying the central role of *STK11* as the disease-causing gene of PJS. Genetic testing is commercially available and is performed similar to testing in FAP. A person known to have the clinical syndrome is first tested. If a relevant mutation can be found in the *STK11* gene, other family members can be tested with near 100% accuracy.

Diagnosis, screening, and treatment

Diagnosis is based on the finding of typical melanin pigment spots and characteristic GI polyps. Management involves screening (as outlined in Table 64.5) and removal of all polyps larger than 1 cm in diameter to prevent both benign and malignant complications. Screening guidelines are empirical and based on the risk for GI complications and cancer. Colectomy is sometimes necessary to control colonic polyps and should be considered, especially if neoplastic change is found in the colon. When small bowel polyps become large or symptomatic, surgery is often necessary and includes careful examination of the entire small bowel to eliminate all significant polyps. Intraoperative endoscopy may be a helpful adjunct in this regard, and this is also an appropriate time to examine and remove gastric and duodenal polyps of significant size [216]. The advent of capsule endoscopy and double-balloon enteroscopy may revolutionize the management of PJS [217–219]. Capsule endoscopy may miss polyps but it appears to be more effective than than radiological approaches for small bowel polyp screening [218,220]. Double-balloon enteroscopy has been shown successfully to

remove small bowel polyps in PJS without surgery [219]. Overexpression of COX-2 has been observed in PJS polyps and cancers, and selective suppression of COX-2 has been found to suppress polyposis in PJS [221]. This promising development must await further studies in view of the cardiovascular and cerebrovascular risks identified with COX-2 inhibitory agents [170,171].

Juvenile polyposis syndrome

Epidemiology and clinical characteristics

Juvenile polyposis syndrome (JPS) is an autosomal dominantly inherited condition arising from mutations of the *SMAD4* gene, the *BMPR1A* gene, and rarely of the *ENG* gene. Multiple juvenile polyps are found in the colorectum (98%), stomach (14%), jejunum and ileum (7%), and duodenum (7%) [212,222,223]. There is also a high risk for colon cancer and an increased risk for gastric, duodenal, and pancreatic cancers. The incidence of JPS is about 1 in 100 000 individuals.

The generally accepted clinical criteria for JPS include the following [224]:

- at least five juvenile polyps in the colorectum
- juvenile polyps throughout the GI tract, or
- any number of juvenile polyps in a person with a known family history of juvenile polyps.

Inherited syndromes that exhibit the GI phenotype of JPS must also be ruled out, including Cowden syndrome (CS), Bannayan–Riley–Ruvalcaba (BRR) syndrome, and Gorlin syndrome (see below).

The polyps in JPS vary in size from small sessile nodules to pedunculated lesions measuring 3 cm or larger in diameter. Most large polyps are pedunculated, but small polyps, especially those in the stomach, are sessile. Grossly, most polyps exhibit a surface that is smooth, rounded, reddish-colored, and without fissures or lobulations, except for large polyps, which may appear to be multilobulated. A white exudate is often seen on the polyp surface. On cut section there are cystic spaces filled with mucin. Microscopically, there is abundant lamina propria with benign but often elongated and cystically dilated glands and lack of a smooth muscle core. Excess chronic inflammatory cells are sometimes present. The epithelial lining of the surface and cysts is nondysplastic and reflects the area of the GI tract where the polyp is located [225].

Polyps begin to appear in the first decade of life, and dozens to many hundreds of polyps are present in the fully developed syndrome. Most patients develop symptoms in the first two decades of life. The average age at diagnosis is 18.5 years [226] but may be later [227]. Rectal bleeding with anemia is the most common presenting symptom, followed by abdominal pain, diarrhea, passage of tissue per rectum, and intussusception [212,222]. A rare and often lethal form of the disease can be observed in infancy. It includes large numbers of juvenile polyps, GI bleeding, protein-losing enteropathy, and significant morbidity and even mortality [222]. In some cases JPS is known to co-occur with hereditary hemorrhagic telangiectasia, thus including a risk for aortic aneurysm, pulmonary thrombosis, digital telangiectases, arteriovenous malformations, and digital clubbing [228]. This association is observed only in cases with *SMAD4* causative germ-line mutations. Gastric polyposis is known to occur most commonly with mutations of *SMAD4*, with the phenotype sometimes of massive gastric polyposis [229,230]. Other associated congenital birth defects are found in 15% of cases, most commonly in those with no family history, and include malrotation of the gut, cardiac and cranial abnormalities, cleft palate, polydactyly, and genitourinary defects [222].

The risk of colon cancer is 17%–22% by age 35 years and approaches 68% by age 60 years [212]. The mean age at diagnosis of colon cancer is 34 years, with a range of 15–68 years. The risk of gastric cancer is 21% in those who have gastric polyps [212,224,231]. Pancreatic and periampullary cancers are also reported [222]. The average age at diagnosis of upper GI carcinoma is 58 years, with a range of 21–73 years [232,233]. The cancer risk in JPS is believed to arise from adenomatous tissue within the juvenile polyp, as up to 50% of juvenile polyps in JPS contain areas of adenomatous change. Villous and malignant foci may be seen in larger polyps [234,235]. Cancers of the duodenum, pancreas, and biliary tree have also been reported in JPS [234].

Genetics and pathophysiology

About 15% of clinically defined JPS families are found to have disease-causing mutations of the *SMAD4* gene. Another approximately 25% have mutations in the *BMPR1A* gene, and rare JPS cases have mutations in the *ENG* gene [212,222,236–239]. All three genes are tumor suppressor genes involved in the transforming growth factor-β (TGF-β) signaling family, and directly or indirectly affect cell growth inhibition and apoptosis. Numerous ectopic crypts with frequent intraepithelial neoplasia are observed when bone morphogenetic protein (BMP) signaling is inhibited in the mouse model of JPS [240]. There is some evidence that normal BMP signaling also suppresses WNT signaling to ensure a balanced control of stem cell self-renewal [241]. Biallelic gene inactivation has been noted both in stromal cells and epithelial cells of polyps [222,242,243]. The type of disease mutations usually found in the genes suggests that direct sequence analysis is the most efficient method for mutation detection in this disease [238].

Diagnosis, screening, and treatment

Diagnosis is based on recognition of the disease characteristics and on genetic testing, especially in families. Genetic testing is particularly important in JPS, both to confirm the diagnosis in a proband and to test relatives. Testing is also important to separate JPS from other conditions in which juvenile polyps form, especially Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome. Genetic testing has also been shown to increase markedly the screening compliance

in relatives of those with JPS [246]. Genetic testing in those with clinical JPS will find a disease-causing mutation in 40%–60% of families. Approximately 25% of newly diagnosed cases are sporadic, that is, represent new or de novo mutations, whereas 75% will have a family history [212].

Colorectal screening should consist of colonoscopy, starting with the onset of symptoms or at age 15 if no symptoms have occurred. It should be repeated every 1–2 years until polyps are clear, and then every 3 years [212,222,244,245]. Upper endoscopy should be performed every 1–2 years beginning at age 25 years or possibly earlier in families with *SMAD4* mutations. The small bowel past the duodenum should be examined if duodenal polyposis is present, or if there is an unexplained anemia or protein-losing enteropathy. Periodic small bowel examination by radiography, push enteroscopy, capsule endoscopy, or even double-balloon enteroscopy may also be appropriate in selected patients. Patients with limited numbers of polyps in any area of the gastrointestinal tract can usually be managed with endoscopic polypectomy.

In JPS patients with large numbers of colonic polyps, anemia or protein-losing enteropathy, polyps with advanced dysplasia, or the presence of invasive adenocarcinoma, subtotal colectomy with ileorectal anastomosis or total restorative proctocolectomy is indicated, depending on the number of rectal polyps [212,222,244]. Subsequent surveillance of the remaining rectum or pouch should be assured [246]. Furthermore, fully half of patients with rectal-preserving surgery require later proctectomy [246]. Complete or partial gastrectomy may also be necessary for patients with advanced dysplasia, gastric cancer, or even massive gastric polypsis that cannot be effectively controlled endoscopically [212,222].

Overexpression of COX-2 has been observed in juvenile polyps of JPS patients, suggesting that selective COX-2 inhibition may be a medical approach to the control of polyposis in this condition [247].

Cowden syndrome

Epidemiology and clinical characteristics

Cowden syndrome (CS), also called *multiple hamartoma syndrome*, is an autosomal dominantly inherited condition characterized by multiple hamartomas of the skin, mucous membranes, GI tract, and other organs, and a high risk for cancer of the breast, thyroid, and perhaps other sites [212,248,249]. This rare disease occurs in about 1 in 200 000 individuals but is believed to be underdiagnosed.

An international consortium has developed diagnostic criteria for CS [249] (Table 64.6). The hallmark of the syndrome is the presence of multiple facial trichilemmomas. Verrucous skin lesions of the face and limbs and cobblestone-like hyperkeratotic papules of the gingiva and buccal mucosa are common. Biopsy examination has demonstrated facial lesions most often to be trichilemmomas, oral mucosal lesions to be fibromas, and all hand and foot lesions to be hyperkeratoses.

Table 64.6 Diagnostic criteria for Cowden syndrome

Pathognomonic criteria
Mucocutaneous lesions:
- Trichilemmomas, facial
- Acral keratoses
- Papillomatous papules
- Mucosal lesions

Major criteria
Breast carcinoma
Thyroid carcinoma (nonmedullary), especially follicular
Macrocephaly (megalencephaly, at least 95th percentile)
Lhermitte–Duclos disease (LDD)
Endometrial carcinoma

Minor criteria
Other thyroid lesions (e.g., adenoma or multinodular goiter)
Gastrointestinal hamartomas
Fibrocystic disease of the breast
Lipomas
Fibromas
Genitourinary tumors (renal cell or endometrial cancer, uterine fibroids) or malformation

Operational diagnosis in a person
1. Mucocutaneous lesions alone if:
   - **a.** There are at least six facial papules, of which at least three must be trichilemmoma, or
   - **b.** Cutaneous facial papules and oral mucosal papillomatosis, or
   - **c.** Oral mucosal papillomatosis and acral keratoses, or
   - **d.** At least six palmoplantar keratoses
2. Two major criteria, but one must include macrocephaly or LDD
3. Two major and three minor criteria
4. Four minor criteria

Operational diagnosis in a family with one member diagnosed with Cowden syndrome
1. One or more of the pathognomonic criteria
2. Any one major criterion with or without minor criteria
3. Two minor criteria

Adapted from Eng [248] and Pilarski and Eng [249].

By the third decade of life, 99% of affected patients have developed mucocutaneous stigmata of the disease [249].

Other common manifestations of the disease include multinodular goiter (50%), follicular (sometimes papillary) thyroid carcinoma (10%), thyroid adenoma, fibrocystic breast disease and breast fibroadenomas (up to 75%), and breast cancer (25%–50%), with a median age at diagnosis of 38–46 years. Gastrointestinal hamartomatous polyps, particularly in the colon, stomach, and duodenum, occur in about 60% of cases. Also observed are multiple early-onset uterine leiomyomas, macrocephaly, and mental retardation. Visceral arteriovenous malformations have also been reported [250]. Other related cancers include those of the endometrium (lifetime risk 5%–

10%), kidney, and possibly colon, ovary, melanoma, lung, and retinal glioma. Male breast cancer has been reported in patients with CS. Additional benign soft-tissue and visceral tumors that have been reported include hemangiomas, lipomas, lymphangiomas, neurofibromas, and meningiomas. Developmental or congenital abnormalities also occur and include hypoplastic mandible, a prominent forehead, and a high-arched palate.

The GI hamartomas occur throughout the GI tract. A review of the reported cases in Japan found polyp distribution among 93 cases to be as follows: esophagus, 66%; stomach, 75%; duodenum, 37%; and colon, 66% [251]. Polyps of the esophagus are actually glycogenic acanthosis, and appear as whitish flat elevations in the esophagus [212]. In the stomach, small bowel, and colon, a number of different types of hamartomas are observed, including juvenile polyps (by far the most common), lipomas, inflammatory polyps, ganglioneuromas, and lymphoid hyperplasia. Juvenile-like polyps that contain some neural elements are considered characteristic of this disease. The GI cancer risk has generally been considered not elevated, although a study from Japan found 9.6% of 93 patients to have colon cancer [251].

Related syndromes

Lhermitte–Duclos disease

The rare occurrence of a benign hamartomatous overgrowth of ganglion cells in the cerebellar cortex (called *dysplastic gangliocytoma of the cerebellum*) is called Lhermitte-Duclos disease (LDD). This same tumor is a known manifestation of CS. It has been found that the large majority of cases of LDD occur as a part of CS. (250, 253, 254).

Bannayan–Riley–Ruvalcaba (BRR) syndrome

This syndrome is characterized by macrocephaly, delayed psychomotor development, lipomatosis, hemangiomatosis, and pigmented macules of the glans penis [212,249,254,255]. Similar to CS, intestinal polyps are primarily juvenile polyps, and some contain ectopic ganglion cells [256]. The majority of BRR syndrome patients mave germ-line mutations of the *PTEN* gene, and an identical mutation can lead to either BRR or CS [249,254,257]. It has been suggested that CS and BRR syndromes be considered together as the *PTEN* hamartoma tumor syndromes.

Genetics

Up to 85%–90% of CS cases and 65% of BRR cases arise from mutations of the *PTEN* gene, when analysis includes large deletions and promoter mutations in addition to standard sequencing analysis [249,258]. The *PTEN* gene encodes a ubiquitously expressed dual-specificity phosphatase, which has tumor suppressor activity. It regulates cellular processes, such as cell cycling, translation, and apoptosis, by blocking activation of AKT, a serine/threonine kinase involved in cell growth and survival [249]. Only 10%–15% of diagnosed cases have an affected parent [212]. There has been a suggestion that typical CS arises more often from 5′ *PTEN* mutations whereas BRR arises more often from 3′ mutations [259].

Surveillance and management

Management of CS involves prevention of the associated cancers. The screening recommendations for CS are given in Table 64.7. Gastrointestinal screening recommendations are given in view of the malignancy risk of other syndromes in which juvenile polyosis is found and because of the survey from Japan that demonstrated an increased risk for colon cancer [251]. It is suggested that individuals with BRR and LDD should have similar surveillance [249,254]. Gastric polyposis has been reported to regress with treatment for *Helicobacter pylori* [260].

Hereditary mixed polyposis syndrome

Three families were described in which the polyposis phenotype links to a locus on chromosome 15, called the

Table 64.7 Cancer risks and screening in Cowden syndrome

Cancer	Cancer risk	Age to begin screening (years)	Screening interval (years)	Screening procedures and comments
Colon	Possibly 9%	15	2	Colonoscopy, intervals may increase or decrease, depending on findings
Upper GI tract and small bowel	Possibly increased	15	2	EGD, examination of the small bowel if duodenal polyposis is present, repeat depending on number of polyps
Thyroid	3%–10%	Adolescence	1	Thyroid exam and baseline ultrasound
Breast	25%–50%	25 30	Monthly 1	Self breast exam Mammography
Uterine	5%–10%	35	1	Blind repel suction biopsies
Renal cell	Possibly increased	18	1	Urine analysis with cytology and possibly renal ultrasound

EGD, esophagogastroduodenoscopy (or upper gastrointestinal endoscopy).
Data from Eng [248], Schreibman et al. [212], and Pilarski and Eng [249].

HMPS gene, which appears to overlap with the colorectal adenoma and carcinoma (*CRAC1*) gene [212]. Affected patients exhibited mixed juvenile–adenomatous polyps and also adenomatous polyps, hyperplastic polyps, serrated adenomas, and mixed hyperplastic–adenomatous polyps and adenocarcinomas, with the polyps occurring in one family at a mean age of 28 years [261].

Gorlin syndrome

Gorlin syndrome, also called *nevoid basal cell carcinoma syndrome*, is an autosomal dominant disorder with an occurrence of 1:55 00 [212,262]. It accounts for about 0.5% of patients with basal cell cancer and occurs secondary to mutations of the *PTCH* gene on chromosome 9q. The primary clinical findings include multiple basal cell carcinomas, mandibular bone cysts, characteristic pits in the skin of the palms and soles, intracranial calcification, large head circumference, and congenital skeletal anomalies. Gastric hamartomatous polyps have been reported as a part of the disease but gastrointestinal findings are often not present.

Polyposis syndromes with neural polyp histology

Neural elements can be observed in the juvenile polyps of CS and BRR, and when found, are somewhat distinctive for those diagnoses. Multiple GI polyps with histological neural elements can also be found in neurofibromatosis type I, in multiple endocrine neoplasia type IIB (MEN IIB), and independently in families without any known syndromes.

Neurofibromatosis type I (NF1)

This disease, also called *von Recklinghausen disease*, is defined by the presence of more than five cutaneous café-au-lait spots together with frequent neurofibromas of the skin [262]. The incidence is 1 in 3000, and one-third to one-half of cases are new mutations. The condition is autosomal dominantly inherited and arises from mutations of the tumor suppressor gene *NF1*, on chromosome 17q. The *NF1* gene encodes neurofibromin, which is a negative regulator of RAS signaling [263,264]. About 25% of affected patients exhibit multiple intestinal polypoid neurofibromas and, less commonly, ganglioneuromas [265,266]. The small bowel is affected most commonly, followed by the stomach and then the colon. Gastrointestinal symptoms are unusual but may include GI bleeding, intussusception, or obstruction [267,268]. Neurofibrosarcoma has only rarely been reported.

In addition to neuromas, other GI tumors associated with NF1 include ampullary carcinoid, pheochromocytoma, and gastrointestinal stromal tumors (GISTs) [264]. In fact it has been suggested that GISTs are the most common NF1-associated GI tumors. Furthermore, most GI tract mesenchymal tumors previously interpreted as other tumor types probably represent GISTs [264]. In NF1 GISTs are most commonly found in the jejunum and ileum, followed by the duodenum and then stomach. Malignant behavior of the GISTs is associated with tumors measuring more than 5 cm or having a high mitotic rate, but not with multiple small tumors. The most common presenting symptoms were intermittent gastrointestinal bleeding and anemia. Amost none of the GIST tumors in NF1 demonstrates *KIT* or *PDGFRA* mutations, which are usually observed in sporadic GISTs. Instead, somatic inactivation of the wild-type *NF1* allele appears to be the pathogenetic basis of GISTs in NF1 [263].

A syndrome of early-onset colon cancer, hematological malignancies, and features of NF1 is known to occur with inherited homozygous mutations of mismatch repair genes associated with HNPCC [269,270]. There is evidence that the *NF1* gene is a mutational target in mismatch repair dysfunction [270].

Multiple endocrine neoplasia type IIB (MEN IIB)

Also known as the *mucosal neuroma syndrome*, MEN IIB is characterized by the presence of multiple tumors, including medullary thyroid carcinoma at a young age (almost all patients), pheochromocytoma (40%–50%), enlarged and nodular lips (from ganglioneuromas), a marfanoid habitus, and ganglioneuromatosis of the GI tract (40%) [212,271]. Ganglioneuromatous polyps occur throughout the GI tract, from lip to anus, but most commonly in the colon and rectum [272]. Gastrointestinal symptoms, including diarrhea and constipation, are common (more than 90% of cases) and arise from dysmotility of the GI tract secondary to the underlying often transmural ganglioneuromatoses [212]. Symptoms often begin in the first months of life and may present as megacolon. A single mutation at codon 918 in the tyrosine kinase domain of the RET receptor (encoded by the *RET* protooncogene on chromosome 10q) is found in up to 95% of cases [212]. About half of cases are considered to represent new mutations.

Isolated intestinal neurofibromatosis of the colon

Multiple colonic ganglioneuromas have been observed in families and isolated cases unrelated to NF1 or MEN IIB [273]. The observed ganglioneuromas are both microscopic and polypoid. There may be dozens of visible colonic polyps ranging up to 0.5 cm in diameter. The distribution of affected individuals in the one family described was typical of autosomal dominant inheritance.

Polyposis syndromes of uncertain etiology

Cronkhite–Canada syndrome

Cronkhite–Canada syndrome (CCS) is an acquired condition characterized by generalized GI polyposis, cutaneous hyperpigmentation, hair loss, and nail atrophy [274–276].

The syndrome has a worldwide distribution. Sixty percent of reported cases occur in men, and the average age at symptom onset is 59 years, with a range of 31–86 years. No familial occurrences have been observed. Nutritional, infectious, and immunological associations have been discussed, but the cause of CCS remains elusive.

The polyposis of CCS is present throughout the GI tract except for the esophagus [276]. Hundreds of polyps are observed, with polyp size ranging from a few millimeters to 3 cm in diameter. Colonic polyps are almost always sessile, even when large, which can often differentiate them from juvenile polyps. Ulceration and friability are surface features of some of the polyps. Microscopically, the polyps are almost identical to juvenile polyps. In contrast to JPS, however, the mucosa between polyps is also histologically abnormal, with edema, congestion, and inflammation of the lamina propria and focal glandular ectasia. Polyps may appear over several years or several months. They usually resolve after remission of symptoms, but they may persist for years. Adenomatous change and colon cancer have been reported in the hamartomatous polyps of this syndrome [274]. The overall incidence of colon cancer appears to be about 12%–15% [277]. Carcinoma of the stomach is also reported as a consequence of CCS.

A number of extraintestinal, ectodermal manifestations are observed in almost all patients with CCS. Nails of the fingers and toes exhibit various degrees of dystrophy, described as thinning, splitting, and partial separation from the nail bed (i.e., onycholysis). Hair loss occurs over a few weeks on the scalp, eyebrows, face, axillae, pubic area, and extremities. Hyperpigmentation is described as dark, brownish macules, ranging from a few millimeters to 10 cm in diameter, and it occurs over the upper extremities, followed by the lower extremities, face, palms, soles, neck, back, chest, and scalp, in that order. The nail, hair, and pigmentation abnormalities are all reversible on remission of the disease.

Hypogeusia is the dominant initial symptom in most patients, which is soon followed by diarrhea, abdominal discomfort, anorexia, and weight loss [277]. Variable degrees of fat, protein, and disaccharide malabsorption occur because of a decreased absorptive surface. Other common symptoms include fatigue, weakness, edema, nausea, and vomiting.

The disease often exhibits a fairly acute onset and a rapidly progressive course. Weight loss and edema ensue, and ectodermal changes occur in a few weeks to a few months. Gastrointestinal bleeding also may occur and can be severe in some cases. Intussusception from small bowel polyps has been reported but is unusual. The diarrhea and protein-losing enteropathy may be extremely severe, resulting in profound malnutrition. Complications resulting from malnutrition are a major cause of morbidity and mortality in this syndrome. They include severe cachexia, anemia, congestive heart failure, and impaired immunity, resulting variously in pneumonia, sepsis, and septic shock. The disease may be fatal within a few months, although a more protracted course is also possible, especially if the patient responds to therapy or remits spontaneously.

A number of spontaneous remissions have been reported in CCS, and partial or complete remissions have resulted from several different interventions [275]. Therapies have included corticosteroids, antibiotics, colectomy, parenteral nutrition, and combinations of these, each with variable success. Recommendations are for aggressive supportive therapy, including enteral or intravenous alimentation. Corticosteroid therapy is undertaken if deterioration continues, and antibiotic therapy may be attempted, although its usefulness is questionable. Surgery is most often used to treat complications, including bleeding, malignancy, intussusception, and sometimes protein-losing enteropathy. Colonoscopy should be performed, if feasible, to consider the possibility of malignancy. Periodic examination of the colon and stomach seems to be indicated in long-term survivors with persistent polyps to screen for adenomatous change and colon cancer.

Hyperplastic polyposis syndrome (HP)

Rare patients are described with large numbers of hyperplastic polyps distributed throughout the colon [278–281]. In such patients, polyps are most often sessile, 1–7 mm in diameter, but larger, sessile or pedunculated hyperplastic polyps may also be observed. Smaller polyps are distributed equally throughout the colon, whereas larger lesions often have a proximal colonic distribution [279]. Some adenomas and serrated adenomas are observed in the majority of hyperplastic polyposis cases. Colon cancer has often been reported with HP although its exact risk is unknown [279–281]. Familial cases have been reported, albeit rarely. Germ-line mutations for HP have not been identified, but chromosome 1p allelic loss has been observed in large polyps of HP patients [282].

Working criteria have been suggested to assist with the diagnosis of HP:

- at least five hyperplastic polyps proximal to the sigmoid colon with two or more of them larger than 10 mm in diameter
- any number of hyperplastic polyps proximal to the sigmoid colon in an individual who has a first-degree relative with HP, or
- more than 30 hyperplastic polyps of any size, but distributed throughout the colon [278].

As adenomas are present in the majority of cases and some cancer risk is likely, surveillance of patients with HP is indicated. Management includes colonoscopic polyp removal, every 1–3 years, depending on the number and size of hyperplastic and adenomatous polyps. Subtotal colectomy should be considered if polyposis cannot be controlled endoscopically or if there is a diagnosis of histologically advanced polyps or colon cancer.

Polyposis syndromes with inflammatory polyps

Inflammatory bowel disease

Multiple inflammatory polyps are frequently found in both ulcerative colitis and Crohn's disease. The polyps represent remaining normal tissue, with inflammatory elements that persist during the healing phases of the diseases. The polyps themselves have no malignant potential, although both ulcerative colitis and Crohn's disease have a colon malignancy risk that parallels the extent of colonic involvement and the duration of the disease.

Devon polyposis

Devon polyposis, also known as *Devon family syndrome*, was described in a family in which three generations of females were found to have multiple inflammatory fibroid polyps of the ileum [283]. The polyps varied in size from 0.5 to 8 cm, and each affected person experienced intussusception or small bowel obstruction. Similar polyps were found in the gastric antrum of one patient. Histologically, the polyps were benign proliferations of histiocytes.

Cap polyposis

Cap polyposis is a rare syndrome of multiple inflammatory polyps in the rectosigmoid, although it may occasionally be observed higher in the colon and even in the stomach [284,285]. Histologically, the polyps are inflammatory, with elongated tortuous crypts covered by a "cap" of granulation tissue. There is also smooth muscle and elastin in the mucosa, suggesting that cap polyposis, together with solitary rectal ulcer syndrome, inflammatory cloacogenic polyps, and gastric antral vascular ectasia, is part of a group of conditions referred to as "mucosal prolapse syndrome" [284,286]. Indeed, in solitary rectal ulcer syndrome, multiple 1–3-mm polypoid lesions in the distal rectum can also be observed.

Clinically, patients may have diarrhea, rectal bleeding, and protein-losing enteropathy [286]. A history of straining at stool is often elicited [284]. Endoscopically there may be from 1 to over 100 polyps, which are small, red, sessile, and covered with a layer of fibrinopurulent exudates or with an eroded surface [284,285]. Medical treatment, with variable success, has included metronidazole, immunosuppressive agents, and avoidance of intralumenal trauma [284,287]. Once case of cap polyposis of the stomach and colon responded to eradication of *H. pylori* [288], whereas another colonic case responded completely to infliximab therapy [289]. Solitary polyps can be removed endoscopically, whereas patients with multiple polyps may require surgical resection of the involved colonic segment if medical therapies are unsuccessful [284].

Polyposis conditions arising from lymphoid tissue

Nodular lymphoid hyperplasia

Nodular lymphoid hyperplasia (NLH) is a rare polyclonal lymphoproliferative disorder of unknown cause that, in most cases, is not related to a distinct disease [290]. It is found in the terminal ileum of some patients with Gardner syndrome, in about 20% of patients with common variable immunodeficiency syndrome, as a rare association with intestinal lymphoma [291], and in some otherwise healthy children and some adults [292,293]. It has been suggested that hyperplasia of the lymphoid nodules occurs as a local immune response to antigens in the gut lumen. It has also been suggested that cellular immune dysfunction, as well as immunoglobulin deficiency, must be present for NLH to be present [294].

The hyperplastic lymphoid nodules of NLH are most often found in the small bowel but may also occur in the stomach and colon. They are described as numerous, 3–6 mm in diameter, and exhibiting the same color as surrounding mucosa. Occasionally, nodules may reach 10 mm or more in diameter. The enlarged lymphoid follicles are morphologically indistinguishable from lymphoid follicles that occur normally in the GI tract. The nodules themselves do not usually cause symptoms, which are more likely related to the underlying conditions.

The primary clinical issue is to distinguish NLH from normal lymphoid tissue, follicles of which range from 0.6 to 3 mm in diameter and occur throughout the gut [295]. Barium enema may reveal lymphoid follicles without evidence of disease in up to 50% of individuals younger than 30 years of age and in up to 17% of those older. Normal lymphoid tissue is also frequently observed endoscopically, especially with melanosis [296]. Lymphoid hyperplasia of the terminal ileum is found in almost half of patients examined by colonoscopy [297]. Follicles range in size from 1 to 10 mm or more, and are larger, more numerous, and more frequent in younger individuals. Immunohistochemical staining and tissue genotyping can be used to distinguish malignant changes of lymphoid tissue and NLH [298]. No therapy is merited for NLH per se.

Multiple lymphomatous polyposis

About 30% of primary extranodal lymphomas occur in the GI tract. Most of these present as a single lesion, although about 10% exhibit GI polyposis, including multiple lymphomatous polyposis (MLP) and immunoproliferative small intestinal disease (see below) [299,300].

Multiple lymphomatous polyposis is a rare intestinal malignancy characterized by the presence of numerous GI polypoid lesions of malignant lymphoma. A non-Hodgkin B-cell lymphoma that is the GI counterpart of mantle cell

lymphoma [301,302], MLP arises from a malignant transformation of lymphocytes that exhibit homing receptors for lymphoid tissue of the GI tract, thus giving rise to a diffuse polyposis presentation [303]. The polyps measure from a few millimeters to several centimeters, involve the small and large bowel in 80%–90% of cases, and the stomach or duodenum in 50% of cases [304]. This condition occurs predominantly in men (88%), with reported cases ranging in age from 43 to 82 years at the time of diagnosis [305]. Symptoms are nonspecific, including weight loss, fatigue, diarrhea, abdominal pain, anemia, and occult GI bleeding. The condition often exhibits extraabdominal dissemination, especially to peripheral lymph nodes. Chemotherapy is the treatment of choice, although the overall prognosis is poor [306].

Mucosa-associated lymphoid tissue (MALT) lymphomas, follicular lymphomas, and primary T-cell lymphomas have also been described that are morphologically similar to MLP in that they exhibit a multiple polypoid appearance in the GI tract and are thus considered types of MLP [301,307–312].

Immunoproliferative small intestinal disease

Immunoproliferative small intestinal disease (IPSID), previously called both *Mediterranean-type lymphoma* and *α-heavy chain disease*, can also exhibit multiple nodular lesions of the small bowel [312]. It is a MALT lymphoma characterized by infiltration of the bowel wall with a plasma-cell population that secretes a monotypic truncated immunoglobulin α heavy chain lacking an associated light chain [313,314]. Evidence indicates that most cases arise as a result of infection with *Campylobacter jejuni* [314]. Those most commonly affected by IPSID are people in their second or third decade of life living in the Mediterranean region. Patients present with a malabsorption syndrome lasting months to years that includes chronic diarrhea, weight loss, and abdominal pain. Small intestinal endoscopy may variously show thickened mucosal folds, diffuse nodularity, ulcers, a mosaic pattern, and nondistensibility from infiltration.

Histologically, IPSID begins as an intense proliferation of plasma cells in the lamina propria. It eventually proceeds to an overt malignant lymphoma with extension beyond the lamina propria and sometimes the occurrence of multiple nodular lesions. The process may remain in deeper intestinal layers, however, requiring full-thickness biopsy for diagnosis. An abnormal paraprotein is frequently present, which represents the α-chain fragment of immunoglobulin A. Unlike MLP, IPSID is confined to the gut and is most often present in the small bowel, particularly the proximal small bowel.

Early-stage IPSID responds to antibiotics, resulting in a 30%–70% rate of complete remission [313]. Treatment has included tetracycline or metronidazole and ampicillin or tetracycline. Early-stage patients who do not respond to antibiotic therapy after 6 months should then receive combination chemotherapy. Antibiotics and chemotherapy should be given together for more advanced cases. Most untreated IPSID patients progress to lymphoplasmacytic and immunoblastic lymphoma invading the intestinal wall and mesenteric lymph nodes, and also possibly invading distant organs. This type of lymphoma shares clinical, morphological, and molecular features with MALT lymphoma, lymphoplasmacytic lymphoma, and plasma cell neoploasms [313]. Although unusual, the treatability of this condition in its early stages, but the poor outcome in later stages, require that gastroenterologists be familiar with this condition as a cause of proximal small bowel nodularity.

Miscellaneous noninherited polyposis syndromes

There have been a number of reports of leiomyomatosis of the colon and other segments of the GI tract [315–317]. A multinodular submucosal tumor appearance is observed lumenally, which reflects single or multiple benign growths of smooth muscle of the bowel wall.

Lipomatous polyposis is also rarely observed [318]. In one case, between 700 and 1000 lipomatous polyps were observed, but about 60 adenomatous polyps were also present. Polyps were 2–50 mm in diameter, were found throughout the colon, but were more dense in the left colon [318]. In another case, dense colonic lipomatous polyposis was observed together with lipomas of the peritoneum [319]. A third patient was found to have multiple lipomas throughout both the small and large intestine and a presentation of intussusception [320].

Lymphangiomas are unusual solitary lesions in the colon, representing dilation or overgrowth of lymphatic channels. Clusters of elevated but apparent submucosal colonic polypoid lesions were observed colonoscopically in a case in which the lesions were found to be multiple lymphangiomas [321].

Finally, pneumatosis cystoides intestinalis, which is characterized by multiple air-filled cysts of the GI tract wall, can have the appearance of polyposis on barium enema and colonoscopy [322]. The diagnosis is made by abdominal radiography or colonoscopic biopsy.

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D COLON

65 Malignant tumors of the colon

Francis M. Giardiello, Stanley R. Hamilton

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Colorectal adenocarcinoma is a major human health problem. Worldwide this malignancy affects 1 million individuals each year and causes 500 000 deaths annually. Although colorectal cancer occurs mainly in Western and industrialized countries, since 1950 the incidence of this neoplasm has also increased in traditionally low-incidence regions. In United States, each citizen has a 6% lifetime risk of colorectal cancer, and, strikingly, this tumor is the second leading cause of cancer-related death after lung cancer. In 2005, 145 000 persons were diagnosed with and 56 000 individuals died of colorectal cancer in the United States.

Since the early 1990s a significant basic science and clinical research effort has focused on colorectal cancer. This investigation has resulted in a series of seminal discoveries, both generally in the understanding of tumor biology and specifically in the pathogenesis of colorectal cancer. In some individuals with this malignancy, germ-line mutations are the readily identifiable cause. But in most, the development of cancer appears to be a complex interaction between the host genome and environmental factors.

Advances in knowledge of colorectal cancer have resulted in improvements in surgical/endoscopic techniques, epidemiology, screening, and surveillance of high-risk groups. Moreover, in the past decade, several new chemotherapeutic agents have been developed as a result of our basic scientific understanding of tumor biology. Yet, despite these advances, surgery still remains the treatment offering the greatest hope for cure. Because colorectal cancer usually arises over a prolonged period and is accessible to screening techniques, secondary prevention remains the best way to decrease death from this malignancy.

This chapter provides an up-to-date description of the state of knowledge regarding the epidemiology, etiology, pathogenesis, clinical features, pathology, and treatment of colorectal cancer.

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Epidemiology

Statistics: incidence and mortality

Colorectal cancer is an important global health problem. In 2000, an estimated 944 717 incident cases of colorectal cancer were diagnosed worldwide, with almost equal gender distribution: 498 754 cases in males and 445 963 in females [1]. Globally, about 500 000 individuals die annually from this malignancy [2]. Worldwide, colorectal cancer is the third most commonly diagnosed malignancy after lung and breast cancer [3].

The United States is a high-risk country for colorectal malignancy (Fig. 65.1). Statistics from 2005 estimate 145 290 incident cases of colorectal cancer annually, of which 40 340 are rectal malignancies [4]. Colorectal cancer accounts for about 56 290 deaths per year, or roughly 10% of all US cancer-related mortality annually, and ranks as the second leading cause of cancer death after lung cancer. The lifetime risk for colorectal cancer in the United States is 6% for both genders, and the lifetime risk of death from this tumor is 2.6% in men and 2.7% in females [3]. In unscreened individuals over 50 years of age, prevalence studies estimate one or more neoplastic lesions in 37.5% of individuals, an adenoma of 1.0 cm or greater in 7.9%, an adenoma with high-grade dysplasia in 1.6%, and invasive cancer in 1.0% [5].

The incidence of colorectal cancer is influenced more heavily by age than any other demographic variable. Sporadic colorectal cancer is rarely diagnosed before the age of 40. The incidence of this malignancy increases dramatically between 45 and 50 years of age, with 90% of cases occurring after the age of 50 years [6,7]. Consequently, deaths from colorectal cancer begin to increase slowly in the fifth decade of life, rising steeply with advancing age [6,7].

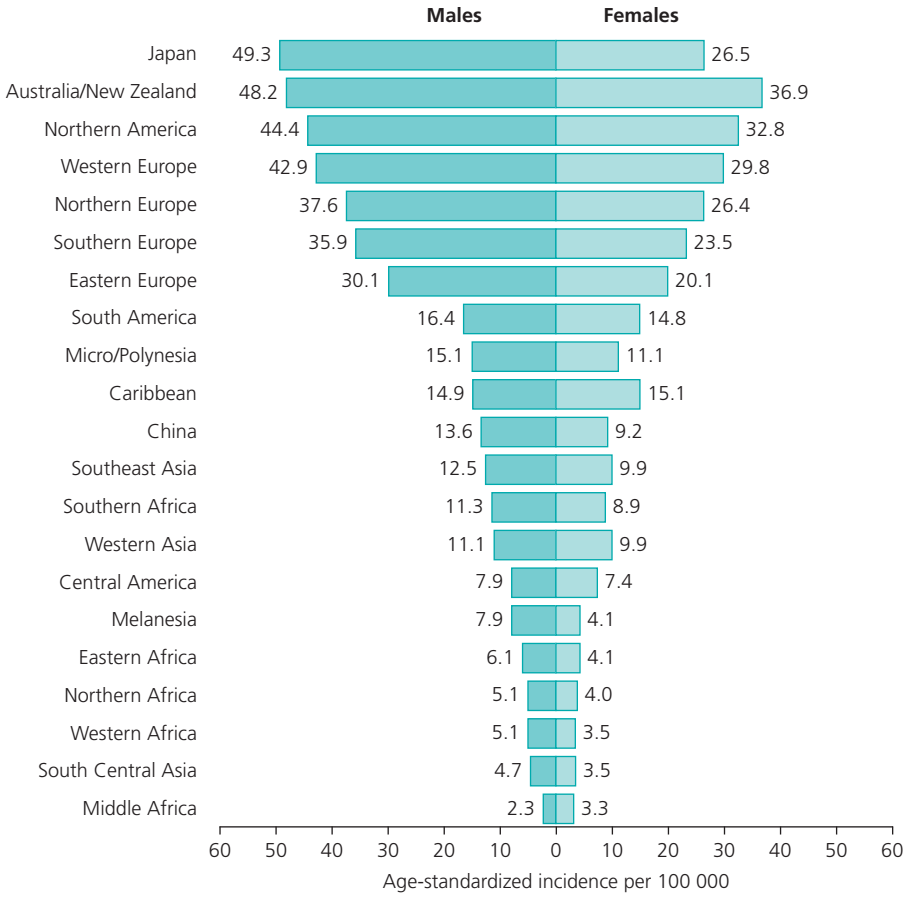


Figure 65.1 Age-standardized incidence rates for colorectal cancer. Data are shown per 100 000 by gender. From Parkin et al. [3], with permission from Lippincott, Williams and Wilkins.

Trends

In general, the incidence of colorectal cancer continues to increase rapidly in countries with formerly a low risk (particularly Japan but also Asian countries). In high-risk countries, the trends are either gradually increasing or stabilizing (north and west Europe) or declining with time (North America) [3]. In the United States (as noted by the annual age-adjusted rate calculated for the past 30 years) the high point of colorectal cancer incidence for both genders occurred the mid-1980s. Since then, the rate has steadily declined, with colorectal cancer incidence decreasing 20% from 1985 to 1995. In 2001 the annual age-adjusted incidence rates of colorectal cancer for men and women were approximately 44.4 and 32.8 cases per 100 000 population, respectively [4]. In the United States, death rates from colorectal cancer have been declining in men since the early 1980s and in women since the late 1940s [4]. From 1985 to 1995 mortality from colorectal cancer decreased by 23%. In 2001, the annual age-adjusted colorectal cancer death rate for men and women was approximately 25 and 22 per 100 000 population, respectively.

Geographical variation

The occurrence of colorectal cancer varies greatly worldwide, with an almost 25-fold difference between specific populations in high- and low-risk regions (Fig. 65.1) [3]. The highest annual incidence rates occur in Australia and New Zealand, followed by North America and Japan (75.8 to 85.1 per 100 000). Incidence tends to be lowest in middle, south central, and western Africa (5.8 to 8.6 per 100 000). The geographical distribution of colon and rectal cancer is similar although the variation between countries is more striking for colon cancer. In high-risk countries like the United States, the ratio of colon to rectal cancer is about 2:1, and in low-risk areas it is closer to 1:1 [3]. This marked variation in geographical incidence for colorectal cancer is largely ascribed to differences in environmental factors, including diet (ingestion of meat, animal fat, and fiber), physical activity, and body weight.

Geographical differences also occur within the United States. For example, the annual age-adjusted morality rates for white males vary three- to four-fold according to region [8]. Mortality rates for colon cancer are the highest in the northeast quadrant of the United States, which includes New England, mid-Atlantic and Midwestern states. In contrast, rates across the South and West have continued to remain relatively low. Similarly, for rectal cancer higher rates are noted in the northeastern, mid-Atlantic, and Midwestern areas, with low rates in the southern United States. Explanations for increased rates of colorectal cancer in the northeastern US

have focused on increased risks for urban populations with a high socioeconomic level [9], but dietary and nutritional factors are likely involved [10–12].

Race/ethnicity

In the United States, African Americans have the highest incidence of colorectal cancer of any racial or ethnic group [13,14]. Since 1985, incidence rates of colorectal cancer have decreased for whites of both genders but, by contrast, have increased for African American men and stabilized for African American women. From 1996 to 2000, incidence rates in African American men were 12.3% higher than in white men and 17.5% higher in African American women than in white women [14].

Also, the mortality rates by gender in African Americans are higher than in whites [8]. Moreover, from 1992 to 1999, the 5-year survival rate from colorectal cancer was 53% in African Americans and 63% in white Americans [14]. This difference in survival also persists when adjusted for stage of colorectal cancer [15]. The cause of the higher incidence of colorectal cancer in African Americans is unknown, but numerous environmental factors have been cited, including increased smoking rates, diet, and physical inactivity, as well as variability in screening frequency and lower use of diagnostic testing [16–19]. The poorer survival in African Americans has been ascribed to lower screening rates, less use of diagnostic tests, and more restricted access to health care [17,18,20].

Ethnic differences can also account for inherited predilection to colorectal cancer. An example is the inherited I1307K mutation of the adenomatous polyposis coli (*APC*) gene, which is noted in approximately 6% of the Ashkenazi Jewish population and less frequently in Sephardic Jews but not found in non-Jewish Caucasian or African American populations [21,22]. This germ-line mutation roughly doubles the lifetime risk of colorectal cancer.

Migration studies

Underscoring the role of environmental factors in the pathogenesis of colorectal cancer are studies of migrating populations. In essence, immigrants adopt the incidence of colorectal cancer in their new homeland. The classic study of this phenomenon documented progressively increasing rates of colorectal cancer in Japanese immigrants to Hawaii and then to the continental United States [23]. Additional examples are the higher subsequent rates in persons moving from China to the United States [24], and from Europe to Australia after World War II [25]. Conversely, people from Scotland, a relatively high-risk area, show a reduction in risk after emigrating to Australia, a relatively low-risk area [25]. These studies highlight the potential for large benefit through primary prevention of colorectal cancer by alteration of diet and lifestyle.

Anatomical trends

Analysis of US colorectal cancer incidence data reveals that about 40% of malignancies occur in the right colon (cecum, ascending colon, hepatic flexure, and transverse colon), 31% in the left colon (splenic flexure, descending colon, and sigmoid colon), and 29% in the rectum (Fig. 65.2) [26]. Several investigators have reported a shift in the incidence of cancer from the left to right colon since the early 1980s [27–30]. Previously, equal proportions of cancers were found in the three anatomical locations noted above [26]. The proximal migration of colorectal cancer over time is probably not attributable to a true increase in the incidence of right-sided colorectal cancer but to a decrease in the incidence of distal colorectal cancer and aging of the population with older persons having more right-sided tumors [26].

Socioeconomic cost

An analysis of the economic burden of colorectal cancer treatment in the United States estimated the cost at $6.5 billion per year, or 12% of the $40 billion annually spent on cancer care [31]. This figure is second only to breast cancer and greater than the cost of lung or prostate cancers. Examination of US hospital discharge data in the 1990s revealed the average number of hospital admissions for colorectal cancer was 237 000 per year, with total mean hospital charges of $4.6 billion dollars annually [32]. Based on census projections, the annual number of colon cancer admissions is estimated to increase 70% in the next 40 years [32]. Dramatic future increases in the cost of treatment of colorectal cancer are foreseen with the advent of monoclonal antibody chemotherapy, which quintuples therapeutic costs [33]. Evidence suggests that the attributable cost of care for colorectal cancer can be significantly reduced by screening the population at age 50, because most hospital admissions involve those aged over 60 years [34].

Etiology

In a minority of cases, the occurrence of colorectal adenocarcinoma is directly attributable to a definable, usually inherited, molecular cause. However, in the vast majority of patients, this tumor results from a complex interaction between environmental factors and genetic predisposition. The role of environmental determinants, especially diet, is strongly highlighted by studies of colorectal cancer incidence in migrant populations, as noted above. Moreover, an analysis of a cohort of US men concluded that six modifiable factors (body mass index, physical activity, alcohol consumption, folic acid supplementation, smoking, and red meat ingestion) resulted in a population attributable risk for colorectal cancer of 71%, suggesting that most of colon cancer in this group

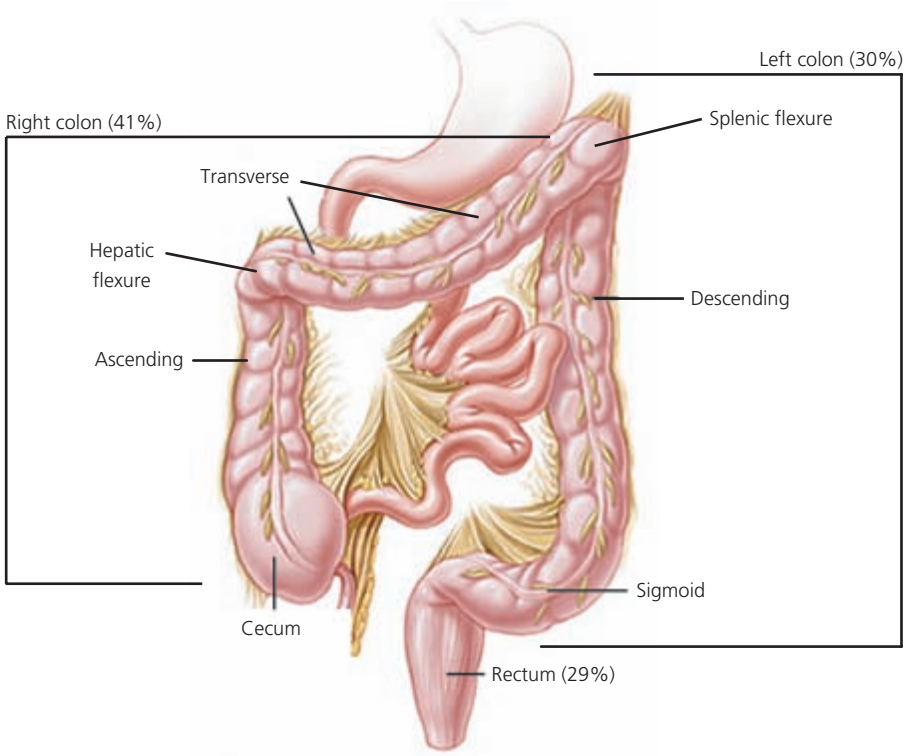


Figure 65.2 Location of colorectal cancer in the large intestine. Adapted from Johns Hopkins Gastroenterology and Hepatology Resource Center (http://hopkinsgi.org), with permission.

Table 65.1 Environmental risk factors in the causation of colorectal cancer

Risk factors	Increased risk (RR[a])
High red meat intake	1.5–3.6
Obesity	2.0
Cigarette smoking	1.9
High calorie intake	1.7
Alcohol intake	1.4–1.7
High fat intake	1.2
Protective factors	**Decreased risk (RR[a])**
Physical activity	0.70–0.80
High calcium intake	0.54–0.85
Folate intake	0.25–0.66
Selenium intake	0.58
Estrogen/progesterone hormone treatment	0.63–0.82
Nonsteroidal antiinflammatory drugs	0.58–0.60
Dietary fiber intake	0.58–0.65
Vegetable and fruit intake	0.48

a RR, relative risk estimates.

was potentially avoidable [35]. In the following section, the environmental (Table 65.1), genetic (Table 65.2), and other factors (Table 65.3) that appear to influence the risk of human colorectal cancer are described.

Environmental factors

Energy balance, obesity, and body habitus

Literature from animal studies suggests that the most powerful and consistent dietary influence on carcinogenesis is energy intake [36]. Indeed, cohort and case–control human studies note a relationship between high levels of energy intake and increased risk of colorectal cancer (odds ratio [OR] 1.70) [37,38]. In one analysis, the risk of colorectal cancer increased 2.3% per 100 calories per day [38].

The study of energy intake in humans is complex and often inaccurate. Nevertheless, indirect indicators of energy balance, such as bodyweight, also support this relationship. A positive association between adiposity and risk of colorectal neoplasia (adenoma and cancer) is noted in numerous case–control and prospective cohort studies [39–48]. Overall a modest two-fold increased risk of colorectal neoplasia is seen with a body mass index greater than 30 [46]. Also, body fat distribution may be an important factor. Case–control studies generally reveal that higher waist-to-hip ratios (central obesity) are associated with increased prevalence of colorectal adenomas and carcinomas [39,42,45–47].

Meat

Concepts about the role of meat, specifically red meat, have been constantly evolving. In the past, consumption of animal meat has been linked to colorectal cancer. This notion is supported by several key studies. The Nurses' Health Study reported that comparison of women who consumed red

Table 65.2 Hereditary factors that cause colorectal cancer

Syndrome	Genotype	Inheritance	Phenotype	Colorectal cancer risk (%)
Familial adenomatous polyposis (FAP)	*APC* gene mutation	AD	≥ 100 colorectal adenomas	~ 100
Attenuated FAP (AFAP)	*APC* gene mutation	AD	< 100 colorectal adenomas	~ 100
Crails syndrome	*APC* gene mutation	AD	Polyposis and medulloblastoma	~ 100
MYH-associated polyposis (MAP)	*MYH* biallelic mutation	AR	< or ≥ 100 colorectal adenomas	?
MYH colorectal cancer	*MYH* monoallelic mutation	AD	Colorectal cancer	~ 9
Hereditary nonpolyposis colorectal cancer (HNPCC)	MMR gene mutation	AD	Colorectal cancer and extracolonic cancers	~ 80
Turcot syndrome	MMR gene mutation	AD	HNPCC and glioblastoma	~ 80
Muir–Torre syndrome	MMR gene mutation	AD	HNPCC and sebaceous neoplasms	~ 80
Trimbath syndrome	MMR biallelic mutation	AD	Early-onset HNPCC, oligopolyposis lymphoma, café-au-lait spots, glioblastoma	> 80
Attenuated HNPCC	*MSH6* gene mutation	AD	Less colorectal cancer, increased uterine cancer	30–69
Peutz–Jeghers syndrome	*STK11* (*LKB1*) mutation	AD	Peutz–Jeghers polyps predominantly small intestine	~ 39
Juvenile polyposis	*SMAD4* and *BRPR1A* mutations	AD	Juvenile polyps predominantly colon	17–63
Cowden syndrome	*PTEN* mutations	AD	Hamartomatous polyps GI tract	~ 9
Bannayan–Riley–Ruvalcaba syndrome	*PTEN* mutation	AD	Hamartomatous polyps GI tract	~ 9
I1307K *APC* gene mutation	*APC* gene mutation in codon 1307	AD	Colorectal cancer	~ 20
Family history of adenoma/carcinoma	?	?	Colorectal cancer	11–26
Mixed polyposis	CRCA1	?	Colorectal neoplasia	?

AD, autosomal dominant; AR, autosomal recessive; GI, gastrointestinal; MMR, mismatch repair.

meat frequently with those who consumed red meat rarely showed a statistically significant 2.5-fold increase in risk of colorectal cancer for the former [49]. Data from a cohort study of over 47 000 US male health professionals revealed that men consuming five or more servings per week of beef, pork, or lamb had a relative risk of colorectal cancer of 3.57 compared with those eating these foods less than once a month [50]. Other studies raise concerns about the processing and cooking of meat, particularly the oncogenic nature of heterocyclic amines produced when meat is heated. One report noted an OR for colon cancer of 2.7 (6.0 for rectal cancer) for the most frequent consumers of fried meat with a heavily browned surface, and a 3.5-fold increase in risk for those preferring well-done meat [51].

When long-term risk of meat consumption was evaluated in a cohort of over 148 000 adults, persons in the highest tertile of consumption had a higher risk of distal colon cancer associated with processed meat (relative risk [RR] 1.50), and the ratio of red meat to poultry and fish was 1.53 relative to the consumption pattern of those persons in the lowest tertile. Also, long-term consumption of poultry and fish was inversely associated with risk of both proximal and distal colon cancer [52]. Moreover, in a metaanalysis of cohort studies, an overall positive association was seen, which was strongest for processed meats [53]. By contrast, five groups of vegetarians, totaling 76 000 individuals, originating from three different countries with socially matched controls were analyzed. Mortality from colorectal cancer was not distinguishable between vegetarians and controls (nonvegetarians) [54].

At present, it appears that increased risk exists from red meat ingestion, possibly from meat cooked at high temperatures [55]. Potential mechanisms for this risk include heterocyclic amines formed in cooking, nitroso compounds in processed meats, or ingestion of heme iron [56].

Fat

A moderately strong association between dietary fat and colorectal cancer risk is suggested by animal and epidemiological

Table 65.3 Other factors in causation of or association with colorectal cancer

Factor	Increased risk (RR[a])
Ureterosigmoidostomy	> 4.0
Previous colorectal cancer	> 3.0
Inflammatory bowel disease	> 3.0
Uterine cancer – early onset[b]	> 3.0
Ovarian cancer – early onset[b]	> 3.0
Acromegaly	2.1–4.9
Streptococcus bovis infection	3.0
Dermatomyositis	2.5
Radiation to prostate[c]	1.7
Diabetes mellitus	1.4
Cholecystectomy	1.2

a RR, relative risk estimates compared with general population.
b Early onset before 50 years of age.
c Risk is for rectal not colon cancer.

data. In animal models of colorectal cancer, high-fat diets are associated with increased cancer development [57]. Older epidemiological data note a good correlation between national consumption of fat (grams of fat per person per day per country) and national age-adjusted mortality from colorectal cancer. Countries with a high fat consumption have six times the quantity of fat ingestion and roughly six times the age-adjusted death rate compared with the lowest fat-ingestion nations [58]. Further epidemiological studies examining the association between self-reported dietary fat intake and colorectal cancer risk note that high levels of dietary fat, particularly animal fat, are usually associated with an increase in colorectal cancer [59]. Also, evidence for a stronger risk with saturated and animal fat compared with total fat exists, but the data are not convincing [60]. Not all studies support the role of fat in the genesis of colorectal cancer. A combined analysis of 13 case–control studies involving 5287 cases and 10 478 controls from various populations failed to show any increased risk with any dietary fat variable after adjustment for total energy intake [61]. However, a review of 19 case–control and cohort studies that analyzed colorectal cancer or adenoma risk and dietary intake revealed that over half showed positive correlation, with a relative risk of over 1.2 in patients with the highest dietary fat intake [62]. In summary, analysis of the data suggests a positive but mild elevation of risk for colorectal neoplasia with high-fat diets.

A potential mechanism by which high-fat diets enhance tumor production appears related to stimulation of colonic epithelial proliferation by bile acids. In humans, increasing dietary levels of animal fat produces more total fecal bile acid and fatty acid excretion [63]. Colonic bacteria convert these compounds to secondary bile acids, cholesterol metabolites, and other possibly toxic substances. In animal models, rectal instillation of bile acids increases markers of cellular proliferation [64]. Additionally, bile acids can generate reactive oxygen radicals within the colon, independently stimulating mucosal proliferation.

Vegetables and fruits

Of all dietary associations with colorectal cancer, the correlation of high vegetable and fruit intake with low colorectal neoplasia risk appears the most convincing. About 75% of published case–control and cohort studies reveal a relative risk of less than 0.8 for colorectal neoplasia in subjects with the highest intake of vegetables and fruits [62]. An analysis of 37 observational epidemiological studies and metaanalysis from 16 case–control studies of vegetable intake and colon cancer found a combined OR of 0.48 (95% confidence limits [CI] 0.41–0.57) for the highest versus lowest quintiles of consumption [65]. A lowered risk of colon cancer has been particularly associated with raw, green, and cruciferous vegetables.

Vegetables and fruits contain a host of potentially antimutagenic compounds. These include isothiocyanates, monocyclic monoterpenoids (limonene, methol), retinoids, and flavonoids, which modulate xenobiotic metabolizing enzymes and inhibit promutagen activation. Retinoids and flavonoids also enhance cellular apoptosis. Provitamins and vitamins (i.e., β-carotene, ascorbic acid) are effective in scavenging reactive oxygen species. Dietary fiber is thought to inhibit mutagen uptake. Phenols, including cinnamic acid and chlorogenic acid, suppress endogenous formation of mutagens by inhibiting nitrosation. Other vitamins, including folic acid and B-12, and minerals such as selenium and zinc are thought to maintain genomic stability [66].

Fiber

The effect of dietary fiber on colorectal cancer risk is unclear. Fiber can be defined as ingested plant material that resists gastrointestinal digestion and is not absorbed or useful as a calorie source. Dietary fiber can be divided into four groups: cellulose, hemicelluloses, pectins, and lignins. The first three types are carbohydrates, whereas lignins are noncarbohydrate complex polymers that bind cellulose and strengthen plant cell walls. High-fiber foods include vegetables, fruits, grains, and nuts.

The first association between dietary fiber intake and decreased cancer risk was suggested by Burkitt in 1969 [67]. He proposed this hypothesis based on the observation that large bowel cancer, universally prevalent in the civilized world, was almost unknown in rural Africa. He connected this fact with differences in diet between these populations, particularly the removal of unabsorbable fiber by modern food processing. He further demonstrated that large bowel cancer was rare in communities whose members consumed high-fiber diets but lived in countries where a low-residue diet was the norm [68].

Numerous cohort and case–control studies have evaluated the hypotheses of an inverse relationship between dietary fiber intake and colorectal neoplasia. In general, about 60% of investigations provide support for this concept [62]. An analysis of 13 case–control studies, which estimated risks from pooled data, found a reduction in colorectal cancer risk with increasing intake of dietary fiber [69]. Another meta-analysis of 16 case–control studies reported similar findings [65]. In these studies, those taking the highest amounts of fiber had about a 45% lower risk of colorectal cancer compared with those taking the lowest amounts. A significant drawback of most investigations is the inability to adjust for other nonfiber dietary constituents, which may be confounding the interpretation. A large European prospective study of over 519 000 individuals found significant protection by high fiber intake [70]. The RR for colorectal cancer was 0.58 (95% CI 0.41–0.85) for the highest (32–36 g/day) versus lowest quintile (13.0 g/day) of fiber intake.

The proposed chemoprotective mechanisms of fiber include lumenal dilution and binding of toxic substances, decrease of colonic transit time with less mucosal contact time for fecal mutagens, and decreased colonic pH with potential inhibition of carcinogens. Also, fiber reduces the concentration of secondary bile acids, which have been noted to increase colorectal mucosal proliferation.

However, the inverse relationship between dietary fiber and colorectal cancer has been challenged by several large studies [49,71]. A prospective study of the association of fiber intake with the incidence of colorectal cancer included US data on over 76 000 women and 47 000 men, with repeated measures of diet. After adjustment for confounding variables, no association was noted [71].

Also, clinical trial data exist from two large prospective randomized controlled studies that attempted to decrease the incidence of recurrent colorectal adenomas in patients with previously removed adenomas using dietary fiber or dietary fiber plus low fat. In both trials no statistically significant effect was noted in the treatment versus the control groups [72,73].

Calcium

The concept of a protective effect of dietary calcium for colorectal cancer was first suggested by Newmark et al. in 1984 [74]. Since then, evidence from in vitro and animal investigations has supported this concept. In colon cell culture, increasing levels of calcium decrease cell proliferation and induce cell differentiation [75]. In numerous murine studies, calcium decreases markers of colorectal mucosal proliferation, decreases colonic mucosal hyperproliferation, and inhibits colonic tumor development [76].

Initially, epidemiological studies of calcium intake and colorectal cancer suggested little or no protection against colorectal cancer from this dietary constituent [77,78]. Subsequently, several prospective cohort and large case–control studies have reported a 15%–46% decrease of colorectal cancer in individuals in the highest versus the lowest intake groups [79–83]. Moreover, metaanalysis of three randomized clinical trials of calcium supplementation to prevent recurrent colorectal adenomatous polyps revealed an overall RR for recurrent polyps of 0.80 (95% CI 0.68–0.93) with the use of calcium [84]. The largest of these studies, involving 930 patients randomized to 3.0 g of calcium carbonate daily (1.2 g of elemental calcium) or placebo, demonstrated a 19% lower risk of recurrent polyps in those given calcium [85]. The protective effect of calcium is thought secondary to the ability of this element to inactivate injurious lumenal substances, including fatty acids and bile acids, by forming insoluble compounds with these agents. Lumenal calcium may affect colorectal tumors by means of the calcium-sensing receptor (CASR) located in the plasma membrane [86].

Folate

Folate is a B vitamin found in dark-green leafy vegetables (i.e., spinach and turnip greens), fruits (i.e., citrus fruits and juices), and dried beans and peas. In both in vitro and animal studies, folic acid has been implicated in DNA synthesis and repair, DNA methylation, and modulation of cell proliferation [87,88]. In murine models of colorectal cancer, folate administration has chemopreventive effects [89,90]. Generally, case–control studies evaluating the association between folate and risk for colorectal cancer (in which folate intake was assessed by food frequency questionnaire) give weak inverse associations. In some the protective effect of folate is greatly decreased or disappears when the analysis is adjusted for other nutrition factors [91–93].

Several large prospective cohort studies have evaluated the influence of folate on colorectal neoplasia. In the Nurses' Health and Health Professional follow-up studies, a decreased RR to develop colorectal adenomas was noted in both men (RR 0.63; 95% CI 0.41–0.98) and women (RR 0.66; 95% CI 0.46–0.95) with high versus low intake of folate [94]. Also, the risk of colorectal neoplasia was inversely related to folate intake for men (RR 0.40; CI 0.18–0.88) in the National Health and Nutrition Epidemiologic follow-up study [95], and in women (RR 0.25; 95% CI 0.13–0.51) in the Nurses' Health Study [96]. In the latter investigation, the marked risk reduction occurred in those taking folate supplements for at least 15 years.

Selenium

Selenium is a trace mineral required in small amounts for human health. Selenium is incorporated into proteins to make selenoproteins, which are antioxidant enzymes. Plant foods are the major dietary sources of selenium; the concentration of selenium in food correlates with the selenium content of the soil where plants are grown. In ecological studies selenium intake is associated with a protective effect against colorectal cancer [97]. Selenium inhibits growth in colorectal

cancer cell lines [98] and, in animal studies, this trace mineral consistently shows activity against colorectal cancer [99–102]. In the laboratory, selenium is noted to have effects on key cellular processes in oncogenesis including cell proliferation and apoptosis. Moreover, selenium may be involved in mechanisms of gene promoter methylation and polyunsaturated fatty acid metabolism [103].

The association between selenium and colorectal adenomas or cancer from epidemiological studies has been inconsistent. Some investigations note a statistically significant or suggestive protective relationship [104–110], and others a null or harmful correlation [111–116]. The strongest evidence for a colorectal chemopreventive effect of selenium comes from a randomized, placebo-controlled trial of 1312 individuals utilizing selenium to prevent skin cancer. This trial showed a decreased RR of the secondary endpoint, colorectal cancer (RR 0.58; 95% CI 0.18–0.95), in individuals in the selenium group [117]. A combined evaluation has been conducted of blood selenium concentrations from 1763 participants from three randomized colorectal adenoma prevention trials [118]. This analysis revealed that individuals whose blood selenium values were in the highest quartile had lower odds of developing a new adenoma compared with those in the lowest quartile (OR 0.66; 95% CI 0.50–0.87).

Vitamins

Vitamins A, β-carotene, and vitamin E have powerful antioxidant activity, and retinoids function in laboratory models as differentiating agents. Nevertheless, research provides no evidence that these vitamins reduce the risk of colorectal cancer [119]. Among the data is a large placebo-controlled trial of β-carotene, vitamin C, and vitamin E showing a lack of effect of these agents in preventing colorectal adenomas [120].

Hormones

Endogenous and exogenous sexual hormones play a role in the etiology of colorectal tumors. Observational evidence for this association includes the finding that nuns and nulliparous women are more susceptible to colorectal cancer development compared with women of higher parity, early age at first birth, and regular users of oral contraceptives [121,122]. There are mounting data to suggest that estrogen/progesterone treatment reduces the risk of colorectal cancer. A metaanalysis evaluated the association of oral contraceptives and colorectal cancer risk [123]. The pooled RR showed an 18% decreased risk of colorectal cancer for those ever using oral contraceptives (RR 0.82; 95% CI 0.74–0.92). Also, epidemiological data suggest a 20% reduction in the risk of colorectal cancer in women who had had hormonal treatment [124]. In addition, a randomized placebo-controlled trial of healthy women by the Women's Health Initiative revealed a 37% reduction in colorectal cancer risk in those on hormonal compounds (estrogen and progesterone) compared with the placebo group [125]. A follow-up publication by this group and data from others suggests that the progesterone rather than estrogen may be the more important factor in colorectal neoplasia reduction [126,127].

Nonsteroidal antiinflammatory drugs

Strong support for the protective effect of nonsteriodal antiinflammatory drugs (NSAIDs) against colorectal cancer is provided by four lines of evidence: in vitro experiments, animal studies, epidemiological observation, and clinical trials. This class of drugs, including sulindac sulfide, piroxicam, and indomethacin (indometacin), inhibit the proliferation of colon cancer cells in vitro [128–130]. Also, multiple experiments have been conducted with a variety of NSAIDs in carcinogen-induced and genetic murine models of colorectal cancer [131]. These studies reveal dramatic reductions in the multiplicity (size and number of tumors per animal) and incidence of intestinal neoplasia in the NSAID-treated animals compared with placebo controls.

Almost all case–control and cohort studies find an inverse relationship between NSAID use and colorectal cancer incidence or mortality [131]. Among these are several seminal publications [132,133]. Thun et al. evaluated the prospective mortality from colorectal cancer of 662 424 adults from an American Cancer Society database who provided information on their frequency and duration of aspirin usage [132]. The RR of colorectal cancer death was 0.60 in men (95% CI 0.40–0.89) and 0.58 (95% CI 0.37–0.90) in women who used aspirin 16 or more times a month compared with those without aspirin use. A dose–response relationship to colorectal cancer protection was also noted. Similar protective estimates of RR for colorectal incidence were reported in a study of a cohort of 89 446 women from the Nurses' Health Study [133]. This investigation adjusted for multiple variables including age, history of polyps, prior endoscopy, family history, diet, smoking, alcohol, and physical activity.

A number of clinical trials support the chemopreventive effect of NSAIDs against colorectal neoplasia. Initially, in a randomized double-blind placebo-controlled trial, sulindac, a commonly used NSAID, regressed existing colorectal adenomas in patients with familial adenomatous polyposis [134]. This finding was supported by additional similar studies utilizing sulindac [135,136] and celecoxib, a selective cyclooxygenase-2 (COX-2) inhibitor [137]. Subsequently several large randomized placebo-controlled trials of aspirin to prevent recurrent colorectal adenomas in those with a history of a sporadic adenoma [138,139] or previous colorectal cancer [140] have been conducted. All show a moderate (19%–35% reduction of recurrent adenomas) chemopreventive effect of aspirin on large bowel adenoma recurrence. By contrast, a large randomized placebo-controlled trial of 22 071 male physicians evaluated aspirin to prevent myocardial infarction and β-carotene to prevent cancer. Because of the marked effect of aspirin in decreasing the incidence of myocardial infarction the study was terminated prematurely.

Secondary analysis revealed no effect of aspirin, 325 mg every other day, on colorectal cancer incidence [141]. Reasons for a lack of a protective association in this trial may be the small dose and short duration of aspirin usage. Also, primary chemoprevention in familial adenomatous polyposis utilizing sulindac to prevent the development of the phenotypic emergence of polyposis was ineffective [142].

Nonsteroidal antiinflammatory drugs have inhibitory effects on the COX-1 and COX-2 enzymes, which are key in the conversion of arachidonic acid into prostaglandins (PGs). Most tissues contain COX-1, whereas COX-2 is expressed in response to growth factors, lipopolysacharide, cytokines, mitogens, and tumor promoters [143]. Prostaglandins are involved in cellular functions including angiogenesis and cell proliferation, and inhibition of PG synthesis could partially explain the antineoplastic effects of NSAIDs. Also, COX-2 inhibition has antiangiogenic [144,145] and apoptotic effects [146].

Several COX-2-independent targets for NSAIDs have been proposed. These include β-catenin, because both indomethacin and exisulind reduce β-catenin expression in colorectal cancer cells [147,148]. Also, NSAIDs induce apoptosis through both the membrane-bound and the mitochondrial pathway. High doses of aspirin antagonize the transcription factor NF-κB [149], which regulates expression of antiapoptotic genes encoding proteins such as TRAF, c-IAP, c-FLIP, Bcl-X_L and A1. Proteins of the Bcl-2 family and the membrane death receptor apoptotic pathway may also be involved in response to NSAIDs [150]. Furthermore, transforming growth factor-β (TGF-β) signaling is implicated in NSAID chemoprevention [151]. Also, NSAIDs affect cell adhesion [152] and lipoxygenase metabolism [153], which reduces cell invasion of colorectal cancer cells. Finally, it appears that the members of the peroxisome proliferator-activated receptor (PPAR) family, PPARδ and PPARγ, are directly targeted by NSAIDs and PGs [154].

Alcohol

A multitude of studies evaluating the role of alcohol in colorectal cancer reveal a positive association between colon cancer and alcohol drinking [62,155]. A pooled analysis of eight cohort studies from North America and Europe indicated that daily consumption of 45 g alcohol from any type of beverage increased the risk of colorectal cancer by 41% (RR 1.41; 95% CI 1.16–1.72) [156]. Data were published from the NHANES Epidemiologic Follow-up Study (NHEFS) consisting of 10 220 participants prospectively followed over a decade [157]. This study revealed that consumption of one or more alcoholic beverages a day was associated with about a 70% greater risk of colorectal cancer (RR 1.69; 95% CI 1.03–2.79), with most risk occurring in those drinking for more than 34 years. Compared with other investigations, this study noted increased risk only in those drinking liquor versus wine or beer.

Mechanisms by which alcohol might increase carcinogenesis include enhancing gastrointestinal cell regeneration, and depressing immune responses. Also, in the absence of adequate folic acid stores, ethanol enhances DNA methylation [157].

Smoking

Numerous studies of various designs reveal a positive association between smoking and the occurrence of colorectal cancer [158]. Almost all note a statistically significant association between colorectal adenomas – the precursor lesion for colorectal cancer – and long-term smoking. Although studies evaluating smokers in the 1950s and 1960s with short smoking exposure found no correlation with colorectal cancer, all investigations with follow-up times of four or more decades report an association. A dose–response relationship between colorectal cancer and cigarette smoking has consistently been reported. Evaluation of pooled data reveals that long-term heavy cigarette smokers have a two- to three-fold elevated risk for colorectal adenomas [158]. In a study from the Chicago Heart Association evaluating a cohort of 39 299 individuals, the RR of fatal colorectal cancer was 1.87 (95% CI 1.08–3.22) for those smoking more than 20 cigarettes a day compared with nonsmokers [159].

Physical activity

Physical activity is consistently associated with reduced risk of colon cancer. Data from a metaanalysis of 19 cohort studies showed a statistically significant reduction in the risk of colon cancer in physically active males (RR 0.79; 95% CI 0.72–0.87) and females (RR 0.71; 95% CI 0.57–0.88) [160]. Evaluation of case–control studies in this analysis showed significantly reduced risks of colon cancer in both genders, but no protection against rectal cancer in either gender. An evaluation of physical activity and colorectal cancer in the Cancer Prevention Cohort Study II Nutrition Cohort analyzing over 150 000 individuals supported this conclusion [161]. In this investigation, colon and rectal cancer risk decreased significantly with increasing total hours of physical activity.

Obesity

Literature from case–control and prospective cohort studies reveal a positive association between body mass index (BMI) and risk of colorectal neoplasia. A modest increased risk of colorectal cancer exists with a BMI above 30, associated with a two-fold increased risk of colorectal adenomas [162]. Central obesity, as measured by waist-to-hip ratio, is associated with an increased prevalence of colorectal adenomas and cancer.

Other

Among other proposed causes of colorectal cancer is the JC (John Cunningham) virus (JCV) [163]. This virus, a member of the polyomavirus family, contains one of the most potent oncogenes discovered, encoding a version of the T-antigen

(transforming antigen). Commonly present in the DNA extracted from neoplastic human colonic tissues, JCV is a transforming virus in several laboratory models, forms tumors when infected into the brains of rodents and monkeys, and is a potent cause of chromosomal instability (CIN), a common phenomenon in colorectal cancer. Humans are ubiquitously infected with JCV at a young age. The proposed carcinogenic mechanism is that the virus remains latent until some stimulus prompts viral activation with associated T-antigen stimulation, CIN, and genesis of colorectal cancer.

Inherited factors

Inherited predisposition is an important factor in a significant number of patients developing colorectal cancer. Although the majority (80%) with colorectal malignancy have sporadic disease with no evidence of an inherited disorder, the remaining 20% have a potentially definable heritable genetic abnormality. There are several well-described forms of inherited colorectal cancer including familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) (Table 65.2). Also, germ-line mutations causing the harmatomous polyposis syndromes predispose individuals to colorectal cancer. Finally, additional germ-line mutations confer less dramatic but still increased risk to carriers.

Familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is an autosomal dominant syndrome caused by germ-line mutation of the *APC* (adenomatous polyposis coli) gene [164]. This disorder is estimated to occur in at least 1 in 14 000 live births, has worldwide distribution, and affects both genders equally [165]. Affected individuals characteristically develop colorectal adenomatous polyposis (> 100 colorectal adenomas) in their teenage years. The mean age of polyposis development is 15 years [166,167]. If colectomy is not performed the lifetime risk of colorectal cancer is virtually 100% at an average age of diagnosis of 35–43 years [166,168].

In addition to colorectal adenomatous polyposis, patients with FAP can develop a host of benign extracolonic manifestations. These include cutaneous lesions (lipomas, fibromas, sebaceous and epidermoid cysts) [169], desmoid tumors [170], osteomas [171], occult radiopaque jaw lesions [172], dental abnormalities [173], pigmented ocular fundic lesions (congenital hypertrophy of the retinal pigment epithelium) [174], and nasopharyngeal angiofibroma [175]. Also, patients can have extracolonic polyps (adenomas of the small intestine and stomach, fundic gland retention polyps of the stomach) [176,177]. Extracolonic malignancies that can occur in those affected with FAP include hepatoblastoma [178], upper gastrointestinal tract malignancies (duodenum and periampullary area, rarely jejunum) [177,179], thyroid gland, biliary tree, pancreas [180], and brain [181].

The *APC* gene is located on chromosome 5q21, and mutations at this locus account for almost all cases of FAP [182]. The *APC* gene is a tumor suppressor gene comprising 15 exons encoding a 2843-amino acid protein. The APC protein has many important functions including cell adhesion, signal transduction, and transcriptional activation [183]. Although more than 300 mutations of the *APC* gene causing FAP have been reported, 10% of FAP patients have a mutation in codon 1309. Commercial genetic testing of the *APC* gene is the test of choice for confirming the diagnosis and screening family members for this syndrome.

Several genotype–phenotype correlations occur in this disorder. The most important is that mutations between codon 169 and 1393 result in classic FAP whereas mutations in the more 5′ and 3′ regions of the gene (at the extremes of the coding region) predispose to a milder phenotype called attenuated familial adenomatous polyposis (AFAP) [184–186]. The key characteristic of AFAP is oligopolyposis (< 100 adenomatous polyps), rather than polyposis, noted at clinical presentation. However, marked phenotypic variation in polyp number can exist within a pedigree. Also, these patients have a delayed onset of colorectal cancer, on average 12 years later than observed in classic FAP. Also, a plethora of upper tract lesions has been noted in some AFAP patients. The clinical management of FAP and AFAP is discussed in Chapter 63.

MYH-associated polyposis

MYH-associated polyposis is an autosomal recessive syndrome caused by biallelic germ-line mutation of the *MYH* gene [187–190]. Affected individuals can develop colorectal adenomatous polyposis or oligopolyposis (< 100 adenomas at presentation) at a young age, phenotypes indistinguishable from classic FAP or AFAP [190]. Also, extracolonic manifestations like those in FAP or AFAP can be noted in these patients. Biallelic mutations of the *MYH* gene may also be a cause of early-onset colorectal cancer in the absence of multiple colorectal adenomas [191]. The *MYH* (mutY homologue) gene is located on chromosome 1p and encodes a component of the base excision repair pathway involved in repair of oxidative damage to DNA [192]. Two specific mutations, Y165C and G382D, causing the MYH protein to have amino acid substitutions, are known to be associated with this disorder.

Although increased risk of colorectal cancer and polyposis with biallelic *MYH* gene mutations has been confirmed, the risk of colorectal neoplasia among monoallelic carriers is unclear. A Canadian population-based series of 1238 colorectal cancer patients and 1255 healthy controls noted an increased risk of colorectal cancer in monoallelic carriers (RR 1.54; 95% CI 1.10–2.16) [193].

Hereditary nonpolyposis colorectal cancer

Hereditary nonpolyposis colorectal cancer (HNPCC) is a syndrome known by several other appellations. These include Lynch syndrome, after Dr Henry Lynch who detailed this condition in 1966, and cancer family syndrome, the term he

Table 65.4 Features of hereditary nonpolyposis colorectal cancer

Accounts for 1%–3% of all colorectal cancers
Autosomal dominant inheritance
Caused by mutation in a mismatch repair gene (*hMLH1*, *hMSH2*, *hMSH6*, *hPMS2*)
Eighty percent lifetime risk of colorectal cancer in mutation carriers
Risk of colorectal cancer in second decade, mean age at diagnosis 44 years
Predominantly right (proximal to splenic flexure) colon cancers (60%–80%)
Absence of multiple colorectal adenomas (nonpolyposis)
Cancer histology poorly differentiated, abundant extracellular mucin and lymphoid peritumoral host response
Cancer DNA with high microsatellite instability (90% of cancers)
Extracolonic cancers:
Endometrial, stomach, small bowel, biliary tract, ovary, brain glioblastoma
Transitional cell carcinoma of ureter, urethra, bladder and renal pelvix, skin
Sebaceous neoplasms, keratoacanthomas
Diagnostic criteria – Amsterdam I and II criteria
Treatment with colectomy and ileorectal anastomosis
Improved colorectal cancer survival, stage for stage, than sporadic colorectal cancer
Metachronous risk of cancers 3%–5% per year

Table 65.5 Amsterdam I and II criteria for the diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC)

Amsterdam I criteria

1. Three or more relatives with histologically verified colorectal cancer, one of whom is a first-degree relative of the other two: familial adenomatous polyposis should be excluded
2. Colorectal cancer involving at least two successive generations
3. One or more colorectal cancer cases diagnosed before the age of 50

Amsterdam II criteria

1. Three or more relatives with histologically verified HNPCC-associated cancer (colorectal, endometrial, small bowel, ureter, or renal pelvis), one of whom is a first-degree relative of the other two: familial adenomatous polyposis should be excluded
2. HNPCC-associated cancer involving at least two successive generations
3. One or more cancer cases diagnosed before the age of 50

used to describe the phenotype of the families in his publication [194]. Unlike FAP, in which colorectal cancer arises from colorectal adenomatous polyposis, in HNPCC colon malignancies arise usually from one or a few adenomas – hence the term "nonpolyposis" (Table 65.4).

It is an autosomal dominant condition caused by mutation of one of the mismatch repair (MMR) genes, and accounts for about 1%–3% of all colorectal cancers [195–197]. The lifetime risk of colorectal cancer in persons with an MMR gene mutation approaches 80%. The mean age of diagnosis of colorectal cancer in this syndrome is 44 years, but a wide range of age of diagnosis is noted; patients not infrequently present with colorectal malignancy in the second and third decades of life. The majority (60%–80%) of tumors in this condition are found in the right colon (proximal to the splenic flexure). By comparison, only 41%–44% of sporadic colorectal cancers are right-sided. Also, the adenoma–carcinoma sequence appears more rapid than in sporadic colorectal cancer. In those affected with HNPCC, the risk of metachronous colorectal cancer approaches 40% at 10 years from the diagnosis of the first colorectal malignancy; synchronous colorectal cancer is seen in 18% of patients [198]. Histologically, HNPCC colorectal cancers tend to be poorly differentiated, abundant in extracellular mucin, and distinguished by a lymphoid (peritumoral lymphocytes) host response to the tumor [198,199]. Some investigators report that HNPCC patients have improved 5-year survival from colorectal cancer, stage-for-stage, compared with individuals with sporadic colorectal malignancy.

Patients with HNPCC and their family members can develop a wide range of extracolonic malignancies, with the highest risk for endometrial cancer (40%–70% lifetime risk) [200,201]. Also, these patients are at increased risk for adenocarcinomas of the stomach, small bowel, biliary tract, ovary, glioblastoma of the brain, and transitional cell carcinoma of the ureter, urethra, bladder, and renal pelvis (lifetime risks for malignancies ranging from 2% to 13%). Most reported studies have not noted increased risks for lung, breast, hemopoietic, prostate, or larynx cancers in HNPCC kindreds. Café-au-lait spots, sebaceous gland tumors (adenomas and carcinomas), and keratoacanthomas (the latter two findings are hallmarks of the Muir–Torre variant of HNPCC) are the only reported clinical signs in HNPCC patients [202].

In 1990, the International Collaborative Group on Hereditary Nonpolyposis Colorectal Cancer established research (ICG) criteria for HNPCC [203] (Table 65.5). Better known as the Amsterdam I criteria, these standards are used for the clinical diagnosis of HNPCC. All of the following elements are required to diagnose HNPCC:

- one or more colorectal cancer cases diagnosed before the age of 50
- colorectal cancer involving at least two generations
- three or more relatives with histologically verified colorectal cancer, one of whom is a first-degree relative of the other two (FAP should be excluded).

Some physicians felt these standards were too stringent for clinical application, and alternative criteria (Amsterdam II criteria) were proposed (Table 65.5). Amsterdam II criteria include extracolonic tumor types commonly observed in HNPCC, such as cancer of the endometrium, upper gastrointestinal tract, and urinary tract [204].

The cause of HNPCC is mutation in one of four DNA mismatch repair (MMR) genes. The MMR genes, first discovered in yeast, maintain fidelity of DNA during cellular replication by correcting nucleotide base mispairs and small insertions or deletions generated by misincorporations or slippage of DNA polymerase. Mutations in the following MMR genes cause HNPCC: *hMSH2* (human mutS homologue 2) on chromosome 2p16 [205,206]; *hMLH1* (human mutL homologue 1) on chromosome 3p21 [207,208]; *hPMS2* (human postmeiotic segregation 2) on chromosome 7q11 [209]; and *hMSH6* (human mutS homologue 6) on chromosome 2p16 [210,211]. Germ-line mutations of *hMSH2* and *hMLH1* account for more than 90% of the mutations found in HNPCC families [212]. Insufficient evidence exists to implicate mutations in *hPMS1* or *hMLH3* as causing HNPCC.

Microsatellite instability (MSI) affects the DNA of colorectal cancer cells of individuals with germ-line MMR gene mutations but not in the patient's adjacent normal colorectal mucosa. In HNPCC patients diagnosed by Amsterdam I criteria, MSI is found in 90% of colon tumors, compared with 15%–20% of individuals with sporadic colorectal cancers [213]. Microsatellite instability is characterized by expansion or contraction of short repeated DNA sequences (i.e., microsatellite repeats) as a result of insertion or deletion of repeated base pairs. The presence of this phenomenon indicates probable defects in the MMR genes because of somatic changes, or it can suggest a germ-line mutation, that is, the diagnosis of HNPCC [214]. Developed in 1997 and revised in 2004, the Bethesda [215] and Revised Bethesda criteria [216], respectively, propose indications for the clinical use of MSI tumor analysis (Table 65.6). It has been reported that MSI, as a marker for the MMR pathway of colorectal neoplasia, is associated with improved survival, stage-for-stage, in sporadic patients with MSI-positive tumors [217].

Several genotype–phenotype correlations occur in HNPCC. First, Muir–Torre syndrome, a rare variant of HNPCC, is diagnosed in patients with HNPCC and skin sebaceous gland neoplasms (sebaceous adenomas, epithelioma, carcinomas) or keratoacanthomas. Microsatellite instability can be found in the DNA of skin tumors and colorectal tumors of affected patients [218]. Mutations of *hMSH2* are most often found in those with identified germ-line alterations of the MMR genes [219]. Second, patients with mutation of the *MSH6* gene have an "attenuated" form of HNPCC. Carriers of *hMSH6* mutations appear to have less risk for all HNPCC-related tumors (with one exception) than patients with alterations of the *MLH1* or *MSH2* genes. In one study of *hMSH6* carriers, the cumulative risk for colorectal cancer was 69% in males and 30% in females (80% in other MMR gene mutation carriers) at 70 years of age. However, endometrial cancer risk was higher (71%) in those with *MSH6* mutations (40% in others MMR gene carries) [220]. Third, Trimbath syndrome defines patients with homozygous mutations of the MMR genes, such as *hPMS2* or *hMLH1*. These patients are characterized by café-au-lait spots, early onset of colorectal neoplasia (in teenage years), oligopolyposis, glioblastoma at a young age, and lymphoma [202].

Table 65.6 Revised Bethesda guidelines for microsatellite instability testing of colorectal tumors

1. Colorectal cancer diagnosed in a patient less than 50 years of age
2. Presence of synchronous or metachronous colorectal, or other HNPCC-associated tumors, regardless of age[a]
3. Colorectal cancer with the MSI-H[b] histology[c] diagnosed in a patient less than 60 years of age
4. Colorectal cancer diagnosed in one or more first-degree relatives with an HNPCC-related tumor, with one of the cancers being diagnosed under age 50 years
5. Colorectal cancer diagnosed in two or more first- or second-degree relatives with HNPCC-related tumors, regardless of age

a Colorectal, endometrial, stomach, small bowel, ovarian, pancreas, ureter and renal pelvis, biliary tract, and brain (usually glioblastoma) cancers, and sebaceous gland neoplasms (carcinomas, adenomas) and keratoacanthomas.
b MSI-H, microsatellite instability-high; tumors with changes in two or more of the five National Cancer Institute-recommended panels of microsatellite markers.
c Presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.
From Umar et al. [216], with permission from Oxford University Press.

Screening

Consensus opinion has formulated recommendations for colorectal screening in HNPCC. In the absence of genetic testing, first-degree relatives of affected individuals are advised to undergo colonoscopy every 1–2 years, starting between 20 and 30 years of age, and annually after 40 years of age [221], or alternatively every 1–2 years, beginning at age 20–25 years, or 10 years younger than the youngest age at colorectal carcinoma (CRC) diagnosis in the family, whatever comes first [222]. Individuals with germ-line mutations are recommended to have colonoscopy annually. Because of associated risk for endometrial and ovarian cancer, annual screening in women beginning at age 25–35 years is recommended, with consideration of transvaginal ultrasound (with or without CA-125) and endometrial biopsy [222]. The efficiency of gynecological screening remains uncertain. In families with a predilection for gastric and urological tumors, consideration should be given to upper endoscopy, urine analysis, and urine cytology.

When possible, genetic testing for HNPCC is recommended for screening individuals over the age of 18 years [222–224]. Screening begins by testing an affected member of the family to identify the pedigree mutation. Microsatellite testing is indicated for patients who meet the Revised Bethesda criteria.

Microsatellite instability testing or immunohistochemical (IHC) analysis for MMR gene proteins is performed on the tumor tissue of individuals putatively affected with HNPCC. A result of MSI-high (two or more microsatellite markers positive out of five tested) or absence of a specific gene protein in tumor DNA IHC analysis suggests the presence of a germ-line MMR gene mutation and prompts patient germline testing. Commercial testing by sequencing and analysis for large gene segment rearrangement is available for the *hMSH2*, *hML1*, and *hMSH6* genes.

Treatment

Because of the high rate of metachronous colorectal cancer, subtotal colectomy with ileorectal anastomosis (and postsurgical endoscopic rectal surveillance) is recommended when colorectal cancer develops in the setting of HNPCC [223,225]. There is no consensus regarding prophylactic surgery of the colon, uterus, and ovaries for MMR gene mutation carriers, but this should be considered on an individual patient basis [225].

Other genetic factors

Discovered in 1997, the I1307K mutation of the *APC* gene is one cause of familial colorectal cancer [21]. This mutation forms an unstable polyadenine tract in the *APC* gene that confuses the DNA replication machinery, resulting in susceptibility to additional somatic mutations elsewhere on the same allele of the *APC* gene during cell division. Found in 6%–8% of Ashkenazi Jews, and in 1%–4.7% of non-Ashkenazi Jews, this mutation doubles the lifetime risk of colorectal cancer in gene mutation carriers [21,226].

In 1999, genetic linkage and mutational analysis was performed in investigating a pedigree with multiple members with colorectal adenomas, serrated colorectal adenomas, or cancer. Evidence was uncovered for a new colorectal cancer susceptibility gene, *CRAC1* (colorectal adenoma and carcinoma), mapping to chromosome 15q14-q22 [227]. Further investigation is necessary to understand the clinical significance of this genetic locus.

Additional candidates for low-penetrance susceptibility alleles for colorectal cancer include the protooncogene Harvey Ras-1 variable number tandem repeat (HRAS1-VNTR) and the methylation gene 5,10-methylenetetrahydrofolate reductase (*MTHFR*) [228]. Also, epigenetic abnormalities associated with increased cancer risk include loss of imprinting of the insulin-like growth factor gene 2 (IGF2), which has been associated with a 22-fold increased risk of colorectal cancer in those affected [229].

Other syndromes associated with colorectal cancer

(see also Chapters 63 and 64)

Peutz–Jeghers syndrome (PJS) is an autosomal dominant syndrome characterized by labial melanin pigmention and hamartomatous Peutz–Jeghers polyps primarily in the small intestine. This syndrome is cause by mutation of the *STK11* (*LKB1*) gene in up to 70% of affected individuals. Patients with PJS have a strikingly high (39%) lifetime risk of colorectal cancer and of a wide variety of other gastrointestinal and nongastrointestinal tumors [230,231].

Juvenile polyposis primarily affects the large intestine and is diagnosed in patients with five or more colorectal juvenile polyps, but affected individuals can have polyps throughout the gastrointestinal tract. Mutations of the *BMPR1A* and *SMAD4* genes are found in 25% and 20% of cases, respectively. The lifetime risk of colorectal cancer in these patients has been estimated by case series to be 17%–68% [232], and in a person-year analysis to be 39% [233].

Cowden syndrome and a variant, Bannayan–Riley–Ruvalcaba syndrome, are two additional inherited conditions associated with the development of colorectal hamartomatous polyps. Up to 80% of individuals with these syndromes have mutation of the *PTEN* (*MMAC1*) gene found on chromosome 10. The risk of colorectal cancer in these syndromes is thought to be slightly higher (9%) than that in the general population [234].

Family history of colorectal neoplasia

Of all patients with colorectal cancer, the known genetic syndromes constitute 3%–5%. However, familial clustering of colorectal cancer is noted in the pedigrees of about 20% of affected patients, suggesting that other inherited genetic abnormalities have yet to be discovered. Systematic reviews of studies of family history (excluding known genetic syndromes) and colorectal cancer risk note several findings. First, the risk of colorectal cancer is closely related to younger age at diagnosis and greater number of affected relatives. Second, familial risk of colorectal cancer is greater in relatives of colon cases rather than rectal cases. Finally, colorectal cancer in these familial colorectal cancer pedigrees has no predominant location, compared with HNPCC, which is characterized by right-sided lesions.

Over 30 case–control and cohort studies have evaluated family colorectal cancer risk. Metaanalysis of these reports estimated the RR for colorectal cancer in an unaffected family member with one affected first-degree relative to be 2.25 (95% CI 2.00–2.53) [235]; the RR for colon cancer was somewhat higher than for rectal cancer, at 2.42 (95% CI 2.20–2.65) versus 1.89 (95% CI 1.62–2.21), respectively. Both types of first-degree relatives were found to be at increased risk, but the RR for siblings, at 2.57 (95% CI 2.19–3.02), was somewhat higher than the 2.26 (95% Cl 1.96–2.59) for parent–child relationships. The RR for individuals with a first-degree relative diagnosed before age 45 was 3.87 (95% CI 2.40–6.22), between ages 45 and 59 was 2.25 (95% CI 1.85–2.72), and after age 59 was 1.82 (95% CI 1.47–2.25). The RR for colorectal cancer when two or more first-degree relative were affected was 4.25 (95% CI 3.01–6.02).

The increased risk of CRC in an unaffected family member is also associated with adenoma in a first-degree relative, with a RR of 1.99 (95% CI 1.55–2.55), and is influenced by their age at diagnosis of the adenoma. The risk of colorectal cancer among family members increases with decreasing age at diagnosis of adenoma in the index case (2.6–4.4-fold higher if the adenoma is diagnosed before age 60 rather than at or after 60 years of age) [236,237].

Other risk factors

Previous history of colorectal cancer or adenomas

Patients with colorectal cancer have increased risk for synchronous (simultaneous) or metachronous (subsequent) colorectal malignancies. Prospective and retrospective studies in patients with sporadic colorectal cancer estimate the frequency of synchronous lesions at 3.5% [238–240]. Metachronous tumors, defined as cancer diagnosed more than 6 months after surgery for the index cancers, occurs at about a 0.6% per year. About 50% of metachronous lesions arise within 5–7 years of the first lesion.

Inflammatory bowel disease

Patients with inflammatory bowel disease (IBD), both ulcerative colitis and Crohn's disease (specifically Crohn's colitis), are at increased risk for colorectal cancer [241–244]. Although IBD patients account for a small percentage (1%–2%) of all cases of colorectal cancer diagnosed annually, this neoplasm is the cause of death in one of six persons with IBD [243,245].

The risk of colorectal neoplasia is best described in ulcerative colitis. Nevertheless, estimates of colorectal neoplasia in this population have varied secondary to the population studied (community vs referral center), rate of surgical resection, and use of antiinflammatory agents that may be chemopreventive. Most data suggest that the risk of colorectal cancer increases by 0.5%–1.0% yearly, starting 8–10 years after diagnosis of IBD [245,246]. Also, the mean age of colorectal cancer diagnosis in IBD patients is younger (40–50 years old) than for sporadic colorectal cancer (64 years old). In a metaanalysis of 116 studies, the prevalence of colorectal cancer in any ulcerative colitis patient was estimated to be 3.7% (95% CI 3.2%–4.2%) overall and 5.4% (95% CI 4.4%–6.5%) for those with pancolitis. The cumulative probabilities of colorectal cancer were 2%, 8%, and 18% after 10, 20, and 30 years of disease, respectively [246]. Factors that increase CRC risk include long duration of colitis, extensive colonic involvement, family history of colorectal cancer, primary sclerosing cholangitis, and young age at IBD diagnosis [247]. Other possible risk factors include the presence of backwash ileitis and the severity of inflammation [247]. Of note, patients with active colitis and those in remission are equally at risk for colorectal cancer.

Patients with Crohn's colitis are also at increased risk for colorectal cancer compared with the general population. In one study, the RR of colorectal cancer in patients with extensive Crohn's colitis was 18 times that of the general population and similar to an identically selected cohort of ulcerative colitis patients (19 times the general population risk) [248]. The absolute cumulative frequency of risk for developing CRC in Crohn's was 8% at 22 years from onset of symptoms. Other studies of relative risk of colorectal cancer in Crohn's disease have ranged from 0.89 to 5.6 times the general population [241,249]. Previous investigations noting lower colorectal cancer risks have included patients with ileitis or failed to account for extent or duration disease. Several studies have noted an increased risk of small bowel cancer in Crohn's disease with RRs up to 66 times that in the general population [250].

The characteristics of colorectal cancer are similar in ulcerative colitis and Crohn's disease. Respectively, cancers in both disorders occurred at a median of 15 and 18 years after onset of IBD, were multiple in 11% and 12%, occurred in the setting of dysplasia in 72% and 79%, and 5-year survival was 46% and 50% [243].

The risk of colorectal cancer in IBD can be minimized by two main strategies: colectomy and lifelong surveillance. Colectomy is more appropriately applied to patients with ulcerative colitis, and total protocolectomy is the usual surgical recommendation.

Chemoprevention may also be a useful tactic to decrease the risk of colorectal neoplasia in IBD patients. Several compounds have been investigated. First, NSAIDs decrease the risk of sporadic colorectal neoplasia (see "Nonsteroidal antiinflammatory drugs" above). Some studies [251,252], but not all [253], have noted a protective effect of 5-acetylsalicylic acid (5-ASA) compounds against colorectal neoplasia in ulcerative colitis patients. Second, low folate intake has been associated with an increased risk for sporadic colorectal adenomas and carcinomas. Patients with IBD are prone to folate deficiency as a consequence of inadequate nutritional intake and intestinal losses in the setting of active inflammation. Also, sulfasalazine, often used as a chemotherapeutic agent, is a competitive inhibitor of folate absorption. Three studies report protection against CRC in folate users, although none had statistically significant results [253–255]. Finally, ursodeoxycholic acid is a dihydroxy bile acid found in small quantities in human bile. Use of this agent was associated with a decreased prevalence of colonic dysplasia in patients with primary sclerosing cholangitis and ulcerative colitis [256]. Moreover, in this patient population, a randomized placebo-controlled trial of ursodeoxycholic acid noted a 73% decrease in colorectal cancer and dysplasia in the treatment group compared with the control group [257].

Diabetes

Investigators have noted that obesity, a Western-style diet, and lack of physical activity are common risk factors for both type 2 diabetes and colorectal cancer. Accumulating evidence suggests that type 2 diabetes is associated with an

increased risk of colorectal cancer [258,259] A study of the Iowa Cancer Registry noted, after adjustment for age, body mass index, and other potential confounders, a RR of colorectal cancer of 1.4 (95% CI 1.1–1.8) for women with diabetes compared with nondiabetics. An elevated risk was noted for tumors of the proximal colon, whereas risks for the distal colon and rectum were not different from unity [260]. Interestingly, no association has been found between colorectal cancer and type 1 diabetes or gestational diabetes. Potential mechanisms of increased risk include hyperinsulinemic stimulation of cell proliferation by activation of the insulin receptor or insulin-like growth factor (IGF) receptor, or through inhibition of IGF-binding proteins resulting in increased bioavailability of IGF-1 to the IGF-1 receptor [261].

Acromegaly

The association between acromegaly and colorectal neoplasia has been a subject of debate for many years. However, almost all of the colonoscopy-based prevalence studies reveal an increased risk of colorectal adenomas in these patients. An Italian multicenter colonoscopic screening and follow-up study reported that patients with acromegaly had an OR of 1.7 (95% CI 1.1–2.5) for colonic adenomas and 4.9 (95% CI 1.1–22.4) for colorectal cancer [262]. Also, a metaanalysis of three population-based studies estimated a pooled risk ratio of 2.14 (95% CI 1.32–3.14) for colorectal cancer [263].

Streptococcus bovis

The association between *Streptococcus bovis* bacteremia and colonic neoplasia has been documented by case reports and a case–control study [264–266]. The point prevalence of colorectal neoplasia in patients with *S. bovis* bacteremia ranges from 6% to 71%, with most of the 21 individual studies reviewed reporting a greater than 25% risk. One case–control series found that colonic adenomas were present in twice as many cases as controls, and colorectal cancer was present approximately three times as often. The reason for this association is unclear. One theory is that colonic neoplasia enables overgrowth and translocation of these organisms [265]; other theories suggest that *S. bovis* is a causative factor [267]. On the basis of the above data, several investigators have recommended that all patients with *S. bovis* infection have evaluation of the colon.

Dermatomyositis

Dermatomyositis and polymyositis appear to be associated with an increased risk of a wide range of malignancies [268,269]. The concurrent onset and parallel course of these rheumatological disorders and colorectal cancer suggests a close relationship. Moreover, a pooled analysis of published national data from Sweden, Denmark, and Finland revealed that individuals with dermatomyositis had a 2.5-fold (95% CI 1.4–4.4) increased risk of colorectal cancer compared with the general populations of those nations [269].

Gynecological cancers

Evaluation of the risk of subsequent colorectal cancer in patients with cervical, uterine, and ovarian cancer was accomplished by retrospective cohort analysis of the SEER (Surveillance, Epidemiology, and End Results) database from 1974 to 1995 [270]. In this study the overall risk for colorectal cancer was elevated not only in patients with ovarian cancer diagnosed before the age of 50 years (standardized incidence ratio [SIR] 3.67; 95% CI 2.74–4.80) but also for those who were 50–64 years old at diagnosis (SIR 1.25; 95% CI 1.25–1.83). Patients with prior onset of uterine cancer before the age of 50 had an elevated risk for colorectal cancer (SIR 3.39; 95% CI 2.73–4.17), but not those older when diagnosed with uterine cancer. There was no increased risk for colorectal cancer in those with cervical cancer.

Therapeutic interventions

Therapeutic interventions have been implicated in the genesis of colorectal cancer. Radiation therapy for prostate cancer was noted to increase the risk of rectal cancer by 70% (hazard ratio 1.7; 95% CI 1.4–2.2) in a retrospective cohort analysis of the SEER registry data [271]. Most work describes a modest association between cholecystectomy and colorectal cancer. After adjustment for other risk factors, RR for the association of cholecystectomy and colorectal cancer was 1.21 (95% CI 1.01–1.46) in an evaluation of the Nurses' Health Study cohort [272]. Often forgotten is the risk of neoplasms at the anastomosis of the ureter and large bowel in patients with ureterosigmoidostomies or one of the variations such as the Mainz II or Mansoura operations. The risk of neoplasia appears to increase 10 years after the operation, and is estimated as high as 24% at 20 years of follow-up [273].

Pathogenesis

General concepts

Since the 1990s, intense investigation of colorectal carcinogenesis has led to the discovery of seminal concepts concerning the mechanism of oncogenesis. Summed up in a single sentence, "Cancer is, in essence, a genetic disease" [274]. Colorectal cancer arises through a process beginning with normal colorectal mucosa, then progressing to single histological crypt lesions (aberrant crypt foci), then colorectal adenoma, and finally colorectal adenocarcinoma, by a multistep process driven by mutations at multiple chromosomal loci (Fig. 65.3). In sporadic colorectal cancer, no one gene mutation causes malignancy, and consequently mutations contribute to rather than cause colorectal cancer. In general, solid tumors accumulate between three and eight key mutations in the progression to malignancy. Evidence shows that nearly all colorectal cancers arise from adenomatous polyps. In sporadic colorectal cancer, the dwell time – time from

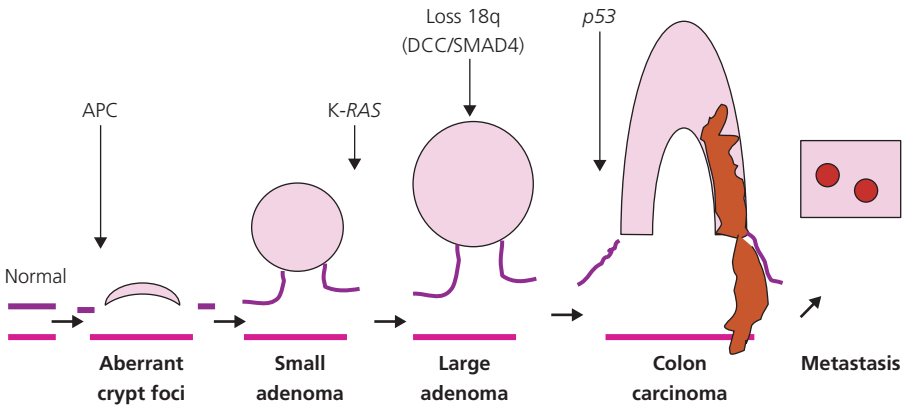


Figure 65.3 Chromosomal instability pathway of the adenoma–carcinoma sequence with particular genetic alterations arising at specific points.

adenoma to carcinoma – is estimated to be about 10 years. As acquired mutations accumulate in colorectal epithelium, they affect growth-regulating genes, thereby enhancing cell survival by increasing cell proliferation or preventing programmed cell death (apoptosis). The prevailing concept involves mutations occurring over time, with each engendering a clonal expansion resulting in large number of cells that form a substrate for subsequent mutations [274,275]. This paradigm of progression from adenoma to colorectal carcinoma driven by multiple somatic genetic mutations was first espoused by Fearon and Vogelstein in 1990 [276].

Alterations in several types of genes, including oncogenes, tumor suppressor genes, and stability genes, are responsible for colorectal carcinogenesis. Oncogenes generally drive the cell cycle, and when mutated become constitutively active or active under conditions in which the wild-type gene is quiescent. A somatic mutation in one allele of an oncogene is generally sufficient to give a selective growth advantage to the cell. Tumor suppressor genes normally function to retard tumor development. When these genes are mutated the activity of the gene product is reduced. Usually mutations in both alleles of the tumor suppressor gene are required to produce a selective growth advantage in the cell. Stability genes are responsible for repairing mistakes made during normal DNA replication or induced by mutagens. When these genes are inactivated, mutations in other genes occur at a higher rate. As with tumor suppressor genes, both maternal and paternal alleles must be inactivated before an aberrant effect is noted. The process of carcinogenesis is initiated when a competent stem cell acquires a mutation in a gatekeeping gene the endows the cell with a selective growth advantage.

Mechanisms of colorectal carcinogenesis

Genetic alterations can occur through several mechanisms including chromosomal instability, microsatellite instability, and methylation (Fig. 65.4). Chromosomal instability involves

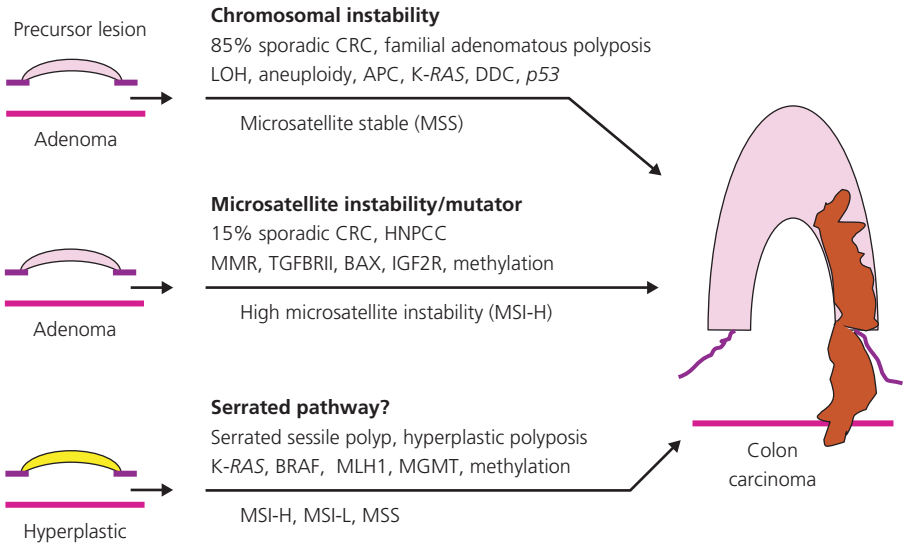


Figure 65.4 Postulated molecular pathways to colorectal cancer: chromosomal instability pathway; microsatellite instability/mutator pathway; and possibly the serrated pathway. Each pathway starts with a precursor lesion (adenoma or serrated sessile polyp), features a particular molecular mechanism (chromosomal instability, microsatellite instability, methylation), involves particular genes, and may be associated with a specific human phenotype of disease. The presence or absence of microsatellite instability in tumor tissue is noted for each pathway.

the loss of entire portions of a chromosome leading to alternations of specific genes and abnormal amounts of DNA per cell (aneuploidy). Microsatellite instability, a process in which there are mutations in the linear extent of tandemly repeated DNA sequences, is also evident in colorectal tumor tissue. Methylation is an epigenetic phenomenon that alters gene transcription.

Chromosomal instability (CIN)

The prototype pathway for colorectal carcinogensis is typified by chromosomal instability (CIN). Chromosomal instability is typified by losses and gains of whole chromosomes or large chromosomal regions in tumor tissue leading to alterations of specific genes and abnormal amounts of DNA per cell (aneuploidy) (Fig. 65.4). If the other, wild-type, allele of a corresponding gene is affected by somatic mutation, the result is inactivation of both copies of a particular gene (loss of heterozygosity, or LOH). Chromosomal instability results in a series of somatic mutations in key genes, as discussed below, and, thereby drives colorectal carcinogenesis. This mechanism seems to operate in a majority (80%) of colorectal tumors. Interestingly, CIN can be transferred in a dominant manner in cell fusion experiments and is associated with functional defects of mitotic checkpoints [277,278].

Although there are several inherited predispositions to colorectal cancer (including germ-line mutations in the MMR genes causing HNPCC, and in the *APC* gene leading to FAP), the majority of mutations contributing to colorectal cancer are acquired (somatic) in the tumor cell. Over a dozen genes are noted to be somatically mutated in colorectal cancer, but four specific gene alterations are particularly common in colorectal tumorigenesis. These include activation of RAS oncogenes and inactivation of tumor suppressor genes on chromosome 5q (*APC*), 17p (*p53*), and 18q (*DCC*, *SMAD4* [*DPC4*], and *SMAD2*) (Fig. 65.3).

APC *gene*

The *APC* gene is considered the gatekeeper gene in the colorectal adenoma–carcinoma sequence. This gene was discovered through investigation of pedigrees with FAP in which germ-line mutation of the *APC* gene caused the syndrome. Mutations in the *APC* gene are the earliest genetic event identified in the adenoma–carcinoma sequence, being found in microscopic aberrant crypt foci and in up to 80% of adenomas and carcinomas [279]. The *APC* gene is a tumor suppressor gene that plays a critical role in the WNT signal pathway [280]. The normal wild-type *APC* gene facilitates the degradation of β-catenin and, thereby, inhibits β-catenin/T-cell factor (TCF)-mediated transcription. However, mutation of the *APC* gene results an ineffective APC protein, which fails to degrade β-catenin, resulting in persistently elevated levels of cytoplasmic and nuclear β-catenin, which in turn provoke transcription of growth-promoting genes including the *MYC* oncogene. Mutations that activate the β-catenin gene are noted in the majority of colorectal tumors lacking an *APC* gene mutation, emphasizing the importance of the WNT pathway in colorectal carcinogenesis [281]. Other potential functions of the wild-type *APC* gene include regulation of cell apoptosis and cell–cell adhesion.

RAS oncogenes

The RAS genes were initially identified as the transforming components of the Kirsten and Harvey rat sarcoma virus genomes. Later, RAS genes with mutations were noted to transform cells in tissue culture [282,283]. RAS mutations are acquired during an intermediate stage of the colorectal adenoma–carcinoma progression. Mutations of *KRAS* or *NRAS* are rarely found in adenomas less than a 1.0 cm in size but appear in about 50% of all adenomas greater than 1.0 cm, and in adenocarcinomas [279]. About 85% of mutations identified are in codons 12 and 13 of the *KRAS* (Kirsten RAS) protooncogene, with some in the *NRAS* (neuroblastoma RAS) gene. The *KRAS* gene encodes a GTP-binding protein ($p21^{RAS}$), which is involved in the transduction of mitogenic signals across the cell membrane. When activated, KRAS exchanges guanosine diphosphate (GDP) for guanosine triphosphate (GTP) and initiates a signal cascade that triggers specific transcriptional factors. Mutation in the gene results in unchecked continued signal transduction and concomitant cellular growth advantage. Investigation reveals that colorectal tumors can develop in the absence of RAS mutations, or fail to progress in the presence of RAS mutations, and that preexisting *APC* mutations are important for neoplastic progression in the setting of a *KRAS* mutation.

Chromosome 18q

Initial studies revealed that about 70% of primary colorectal cancers had LOH for chromosome 18q, suggesting the existence of a novel tumor suppressor gene(s) at this location [279,284]. Further investigation noted that allelic losses of 18q were infrequent in small adenomas but common in colorectal carcinomas and almost all hepatic metastatic lesions [279,285]. This implied that LOH of 18q contributes to progression and acts at the intermediate to late adenoma stage rather than at the initiation of colorectal cancer. Investigators subsequently identified the *DCC* (deleted in colorectal cancer) gene, a large gene comprising 1.2 million base pairs, on chromosome 18q21 [284]. The DCC protein is a transmembrane receptor for netrins, key factors in axon guidance in the developing nervous system. Additional data suggest that DCC may be important in colorectal epithelial development by activating downstream signaling pathways involving the RAC1 protein and the extracellular signal-regulated kinase (ERK-1/2)-dependent pathways [286]. Also, DCC can induce active signaling of cell death by means of the caspase cleavage of DCC and the interaction of caspase-9 [286]. In several studies, somatic loss of *DCC* expression in colorectal cancers has been linked with poor patient prognosis and

increased risk of metastatic disease, suggesting the importance of this gene [287,288]. Additional candidate suppressor genes mapped to chromosome 18q include the *SMAD4* (mothers against decapentaplegic homologue 4) gene (formerly known as *DPC4*, deleted in pancreatic cancer 4), and *SMAD2* [289,290]. The *SMAD4* gene encodes a protein implicated in the signaling pathway of the TGF-β superfamily of signaling polypeptides [291]. However, based on present knowledge, one would argue that more tumor suppressor genes on chromosome 18q await discovery.

The p53 *gene*

Identified in 1979, the *p53* (*TP53*) gene was discovered following the observation that it was expressed in cells infected with tumor virus [292]. Located on chromosome 17p, the wild-type *p53* gene is a tumor suppressor gene with the function of stopping cell growth, when induced by stress conditions, through mechanisms including cell-cycle arrest or apoptosis. The *p53* gene encodes a 393-amino acid phosphoprotein that binds to specific sequences within the promoters of growth-inhibiting genes and activates their transcription [293]. Loss of function of this gene appears to conform to the Knudson two-hit hypothesis of gene inactivation [294]. One copy of the *p53* gene becomes defunct by loss of chromosome 17p13, the other by somatic mutation [295]. Loss of *p53* activity leads to a subsequent decrease in expression of growth-inhibiting genes, and hence a selective cellular growth advantage. The *p53* gene is thought to be involved in the transcriptional regulation of over 20 different genes with functions including apoptosis and cell-cycle arrest. Among the best described mechanisms of p53 action is the induction of expression of $p21^{WAF1/CIP1}$, which in turn causes G1 and G2 cell-cycle arrest [296]. The *p53* gene is commonly mutated in a variety of other human tumors including lung, breast, brain, bladder, and liver, emphasizing a central role for this wild-type gene in the prevention of carcinogenesis [297].

Chromosome 17p

Loss of chromosome 17p sequences are the most consistently observed molecular abnormality in colorectal carcinogenesis. Although seldom found in adenomas, loss of 17p is noted in 75% of carcinomas [279]. Also, *p53* mutations are noted in 50% of adenomas with high-grade dysplasia. These findings and others suggest that loss of *p53* gene function is crucial to the transformation from premalignancy (adenoma) to malignancy (adenocarcinoma), and occurs late in this sequence.

Microsatellite instability

Another mechanism of genetic instability is microsatellite instability (MSI) [298]. This phenomenon is noted in DNA analysis of approximately 15%–20% of all colorectal tumors [197] (Fig. 65.4). Microsatellite instability is characterized by unusual expansion or contraction of short repeated DNA sequences (i.e., microsatellite repeats) that are normally found in DNA. These changes in microsatellite length are caused by insertion or deletion of repeated units. This instability, known as "mutator phenotype," "replication error," or "microsatellite instability," indicates probable defects in the MMR genes. The MMR genes (*hMLH1*, *hMSH2*, *hMSH6*, *PM1S*, *hPMS2*) function to maintain fidelity of DNA during replication by correcting nucleotide base mispairs and small insertions or deletions generated by misincorporations or slippage of DNA polymerase. About 11% of patients with MSI found in tumor analysis have a germ-line mutation of a MMR gene and, thereby, are affected with HNPCC (see "Hereditary nonpolyposis colorectal carcinoma" above) [197]. However, the majority of individuals with this tumor phenotype have somatic inactivation of the MMR genes, usually methylation of the promoter region of the *MLH1* gene [299,300]. Molecular genetic characteristics of MSI tumors include diploid DNA content and lack of LOH at *APC*, *p53*, and 18q and lower frequencies of *APC* and *p53* mutations [301–303]. Other tumor suppressor genes such as *TGFBRII*, *IGF2R*, and *BAX* may replace *APC* and *p53* function [304–306]. Another molecular alteration found in some MSI-positive tumors is extensive methylation of the CpG islands in the gene promoter regions (see "Methylation" below). Compared with microsatellite-stable colorectal cancers, MSI tumors tend to be in the proximal colon, occur in women, present at an earlier age, have a more favorable prognosis, and histologically are more mucinous, poorly differentiated, and show lymphocytic infiltration [307–309].

Methylation

Methylation is an epigenetic change found in a variety of tumors. Alterations in gene methylation noted in colorectal tumors likely result in significant changes in epithelial cell biology. For example, decreased methylation could allow expression of genes that normally are silent, and conversely increased methylation could lead to silencing of genes that are important for growth suppression. Some colorectal cancers have been described as being CIMP (CpG island methylator phenotype)-positive [310]. CpG islands are aggregates of cytosine–guanine dinucleotide sequences that occur in the promoter regions of genes. Extensive methylation of these cytosine bases results in gene silencing. Cancers with methylation and silencing of multiple genes are termed CIMP positive. Methylation has been implicated in the silencing of the *MLH1* mismatch repair gene and of the *p16* gene in colorectal cancer [311,312]. A variety of other genes have been noted to be methylated, including TMEFF2 (also known as *HPP1* and *TPEF*) and *MGMT* [313,314]. Some investigators have postulated a "serrated" pathway of colorectal carcinogenesis driven by inactivation of DNA repair genes due to promoter methylation. This pathway is distinguished from others by the precursor lesion being a hyperplastic polyp (serrated sessile polyp) rather than an adenoma, and with hyperplastic polyposis as the archetypical phenotype [315].

Other signaling pathways

The TGF-β/SMAD signaling pathway includes TGF-β receptor types I and II (TFG-βRI, TFG-βRII) and SMAD proteins. Binding of TGF-β to TGF-βRII ultimately phosphorylates SMAD2 and results in formation of a complex with SMAD4. This complex then induces synthesis of p15 and p21 leading to growth arrest. Mutations in *SMAD4*, *SMAD2*, and *TGFBR2* have been identified in a minority of colorectal cancers with some alterations at the transition from adenoma to colorectal cancer. Of note, germ-line mutations in *SMAD4* are among the abnormalities responsible for juvenile polyposis, an autosomal dominantly inherited disorder characterized by a high risk of colorectal cancer.

Clinical presentation and diagnosis

Presentation

Patients with colorectal cancer can present with a variety of symptoms (Table 65.7). The most common is abdominal pain (noted in 44% of patients), followed by change in bowel habits (43%), hematochezia or melena (40%), weakness (20%), asymptomatic anemia (11%), and weight loss (6%) [316,317]. Unusual presentations include diverticulitis, fever of unknown origin secondary to abdominal abscesses, enteroentero or enterovesicular fistula formation, *S. bovis* bacteremia, or *Clostridium septicum* sepsis. Also, primary colorectal cancer is the cause of 6% of metastatic adenocarcinomas of unknown origin. About 15% of patients present with acute complications of colorectal cancer including abdominal obstruction, severe abdominal pain, bowel perforation, and massive bleeding necessitating emergent hospitalization. Others can be asymptomatic and diagnosed after work-up of iron deficiency anemia.

Patient symptomatology is dependent on the size, location, and rate of growth of the malignancy. Calculation of the growth rate of a colorectal neoplasm is imprecise, because observation of lesions in the postcolonoscopy era is unacceptable. Nevertheless, several investigators have estimated the polyp dwell time. Portrait studies, which compare mean average patient age for adenoma development with age for cancer diagnosis, calculate this period to be 15–18 years [318]. Direct observation of benign polyp-to-cancer development supports a time frame of 10–15 years [319,320]. Also, polyp dwell times ranging between 18 and 23 years are noted in FAP. Taken together, sporadic colorectal cancer is thought to develop slowly over years. However, in inherited conditions such as HNPCC a significantly accelerated adenoma–carcinoma sequence has been described.

Obstructive symptoms of colicky abdominal pain, abdominal distention, constipation, change in bowel habits, and pain with meals are more often noted in patients with left-sided versus right-sided colonic tumors. These symptoms result from solid feces attempting to pass a narrower left colon lumen compromised by a circumferentially growing tumor. These tumors are sometimes termed "apple-core lesions" after their appearance on radiological studies (Fig. 65.5). By contrast, the cecum and ascending colon are relatively capacious, and liquid feces found in the right colon can traverse areas of significant lumenal compromise without provoking symptoms. Consequently, large right-sided tumors can develop before the patient seeks medical attention.

Rectal bleeding, either gross or occult, is a common symptom of colorectal neoplasia and increases in occurrence and frequency with the size and ulceration of the lesion.

Table 65.7 Common and unusual presentations of colorectal cancer

Common presentations	
Presentation	**Percent affected**
Abdominal pain	44
Change in bowel habits	43
Hematochezia/melena	40
Weakness	20
Asymptomatic anemia	11
Unusual presentations	
Diverticulitis	
Fever of unknown origin (abdominal abscess)	
Fistula (entero-entero, entero-vesicular)	
Septicemia (*Streptococcus bovis*, *Clostridium septicum*)	
Metastatic cancer of unknown origin	

Figure 65.5 Barium enema–air contrast study with hepatic flexure colorectal cancer (bounded by circle) with the classic "apple core" lesion.

Colorectal cancer or adenoma is the second most frequent cause (accounting for 19% of all cases) of overt lower gastrointestinal tract bleeding after diverticular disease [321]. In general, mean rates of bleeding are modest, typically less than 10 mL/day with cecal and ascending tumors and less than 2 mL/day for tumors in other parts of the colorectum [322]. With increased emphasis on colorectal cancer screening, asymptomatic patients with colorectal neoplasia are presenting with occult bleeding found on stool guaiac analysis. Another common presentation is chronic occult blood loss identified by laboratory evidence of iron deficiency anemia (microcytic, hypochromic red blood cell indices and low percent saturation of iron). Bright-red blood per rectum or coating the stool is often a sign of rectal or left colon lesions. By contrast, mahogany-colored stool is a sign of right-sided cancer. Melena, although usually originating from lesions in the upper gastrointestinal (GI) tract, can be caused by cecal tumors with small amounts of bleeding in patients with slow colonic transit. Evaluation of the lower GI tract should be undertaken in patients with no cause of melena found in upper GI tract examination.

In addition to discomfort from intestinal distention, colorectal cancer can generate pain resulting from invasion of tumor into surrounding structures. Acute severe abdominal pain can develop from bowel wall perforation with accompanying peritonitis. Rectal cancer can cause back pain from invasion of tumor into perineal or sacral nerves, tenesmus, pelvic pain from invasion into bladder or vaginal wall, and urinary complaints.

Patients with colorectal cancer can also present with cachexia plus weight loss and loss of appetite and strength. Other constitutional symptoms, including fatigue, shortness of breath, and angina, can occur from anemia secondary to blood loss.

At diagnosis of colorectal cancer, asymptomatic patients have a better 5-year survival rate than symptomatic patients, respectively 71% vs 49% [323,324]. Also, patients presenting with bowel obstruction or bowel perforation have a statistically significantly poorer prognosis than those without these symptoms as adjusted for Dukes stage. Other presenting complaints, including nausea and vomiting, change in bowel habits, and abdominal pain, are not independent prognostic factors [317].

Findings indicative of colorectal cancer can be appreciated on physical exam. On palpation of the abdomen, masses are noted most commonly in the right lower quadrant corresponding to cecal tumors. These lesions can grow to a detectable size secondary to the capacious nature of the cecum. Also, hepatomegaly or liver nodularity could indicate metastatic spread to the liver. Sister Mary Joseph sign (nodular umbilicus from periumbilical lymphadenopathy) can indicate the spread of colon and rectal cancer [325]. Digital rectal exam may reveal masses or gross blood, and stool guiaic analysis may test positive for occult blood. Rarely, jaundice or pulmonary findings from metastatic disease are noted.

The symptoms of colorectal cancer – rectal bleeding, abdominal pain, and change in bowel habits – are occasionally misdiagnosed. Rectal bleeding can be excused as hemorrhoidal disease with deferral of diagnostic evaluation. In fact, failure to diagnose colorectal cancer in those with rectal bleeding is a common cause of action for malpractice suits. In general, patients over the age of 40 with complaints of lower gastrointestinal bleeding should have complete evaluation of the colon. Consideration should be given for a similar workup in younger individuals with a family history of colorectal neoplasia. A change in bowel habits can be dismissed as irritable bowel syndrome (IBS). However, IBS is a diagnosis of exclusion requiring thorough diagnostic workup before this conclusion is reached. This is especially true for atypical patients including males, middle-aged patients with a sudden change in bowel habits, and those without a many-year history of gastrointestinal complaints. Diverticular disease can be confused with the presentation of colorectal cancer, because symptoms and radiological findings can be similar. Consequently, endoscopic evaluation of the colon is recommended after acute diverticulitis, and sooner if the patient is not responding to treatment.

Diagnosis

A variety of tests are available for the assessment of a patient with possible colorectal cancer. The evaluation should begin by obtaining a history, family history (including colorectal and other cancer), physical examination with a digital rectal examination, and laboratory testing with complete blood count (CBC) and comprehensive metabolic panel. Abnormalities in CBC may reflect iron deficiency anemia, electrolyte perturbations may be present, and elevated liver function tests can result from hepatic metastases. Carcinogenic embryonic antigen (CEA) is not useful as a diagnostic or screening test but is drawn preoperatively in colorectal cancer patients to monitor for recurrent tumor postoperatively.

Examination of the colon is best done by colonoscopy (Fig. 65.6). Colonoscopy employs patient sedation and uses a 180-cm endoscope, which can visualize the entire colon 90%–95% of the time. Colonoscopy has superior sensitivity and the ability to perform therapy including polypectomy. In one study, the sensitivity of colonoscopy for polyps measuring 6 mm or greater was 92% [326]. Tandem colonoscopy studies reveal a miss rate for polyps greater than 1 cm of 0%–6% [327,328]. When colonoscopy cannot be performed because of patient factors such as colonic tortuosity or patient infirmity, a double-contrast barium enema – barium enema–air contrast (BAE–AC) – study is an alternative (Fig. 65.5). This procedure involves rectal infusion of both barium contrast and air. In a retrospective study, the sensitivity for colorectal cancer diagnosis for BAE–AC was 85% vs 95% for colonoscopy [329]. In a paired study of BAE–AC and colonoscopy (with colonoscopy as the gold standard), BAE–AC enema identified only 32% of polyps less than 0.5 cm, 53% between

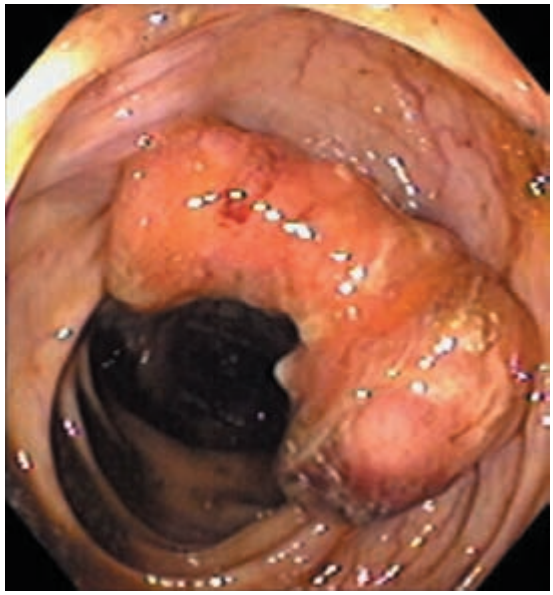


Figure 65.6 Colonoscopy view of colorectal cancer occupying 50% of the circumference of the colon. Courtesy of Dr William Ravich.

0.6 and 1.0 cm, and 48% of polyps greater than 1.0 cm [330]. Single-contrast BAE is a less sensitive technique than BAE–AC and is no longer used for this indication. Barium studies have particular difficulty in visualizing the rectum and sigmoid, so flexible sigmoidoscopy is also required in conjunction for better inspection of these areas. In patients diagnosed with colorectal cancer by radiography or flexible sigmoidoscopy, complete examination with colonoscopy is recommended prior to surgery. This strategy is employed because of the high rate of synchronous (two or more primary) colorectal lesions, which could alter the intended surgical management. Investigators found synchronous cancers in 2.1%–11% [331–334] and synchronous polyps in 24%–28% [333,335] of patients with colorectal cancer. The role of virtual colonoscopy in evaluation of a patient with signs and symptoms of colorectal cancer is unclear, but it may replace barium enema as an alternative to colonoscopy. In one study of virtual colonoscopy in an average-risk screening population, the sensitivity for colorectal polyps measuring 0.6–1.0 cm was equivalent to that of colonoscopy [326]. In patients with colon obstruction obviating complete colonic evaluation preoperatively, colonoscopy should be performed shortly after surgery to evaluate for synchronous tumors.

Preoperative staging

Preoperative cancer staging should be performed on patients with colorectal cancer to enable planning of appropriate management (Table 65.8). History and physical examination with evaluation for metastatic disease is the initial step. Digital rectal examination in patients with rectal cancer helps in planning the appropriate operation by evaluating the distance of the tumor from the dentate line and tumor involvement of the rectal sphincter. Complete visualization of the colon, preferably by colonoscopy, is necessary (see "Diagnosis" above) to exclude synchronous colorectal lesions. Other testing recommended by the National Colorectal Cancer Network and other authors includes CBC, platelet count, and comprehensive metabolic panel including liver and renal function, and carcinoembryonic antigen (CEA) and chest radiography [337,337]. Routine blood testing is relatively insensitive for cancer staging but is important for assessing the patient's ability to receive various treatment options. A high CEA level is a preoperative marker of poor prognosis in patients with stage II disease and can be used to monitor for recurrent cancer postoperatively in those with elevated preoperative levels [338,339]. A chest radiograph is obtained to assess for pulmonary metastasis.

Table 65.8 Preoperative work-up of a colorectal cancer patient

History and physical examination
Digital rectal examination
Complete blood count, platelet count, comprehensive metabolic panel (liver and renal function tests)
Colonoscopy
Carcinoembryonic antigen (CEA)
Chest radiograph
CT scan or MRI scan of the abdomen and pelvis
Rectal endoscopic ultrasound or MRI with endorectal coil (in patients with rectal cancer)

CT, computed tomography; MRI, magnetic resonance imaging.

Imaging the abdomen and pelvis with computed tomography (CT) or magnetic resonance imaging (MRI) to assess for extracolonic malignancy is also recommended. Traditionally CT scanning is used, but MRI is performed in some institutions with local expertise and advanced technology. Scanning by CT has a sensitivity of 81% and a positive predictive value of 100% in detecting Dukes D lesions [340] (Fig. 65.7). Studies show that CT scans provide information used in treatment planning in up to one-third of patients, and definitively alter the mode of treatment in one in six patients [341]. Magnetic resonance imaging appears to have superior specificity for hepatic lesions compared with CT scanning [342]. Positron emission tomography (PET) or PET in combination with CT (PET/CT) is not advocated in preoperative staging because few data regarding the use of this technique in this setting exist to date. Positron emission tomography for malignant disease with fluorine 18-labeled fluoro-2-deoxy-D-glucose (^{18}FDG) is based on the increased glucose demand of malignant cells (Fig. 65.8). The ^{18}FDG is administered intravenously and taken up by malignant cells, which convert the compound to a monophosphate. The monophosphate is trapped in the cell allowing in vivo mapping of the glucose-based tracer. Positron emission tomography is utilized to detect and evaluate the extent of

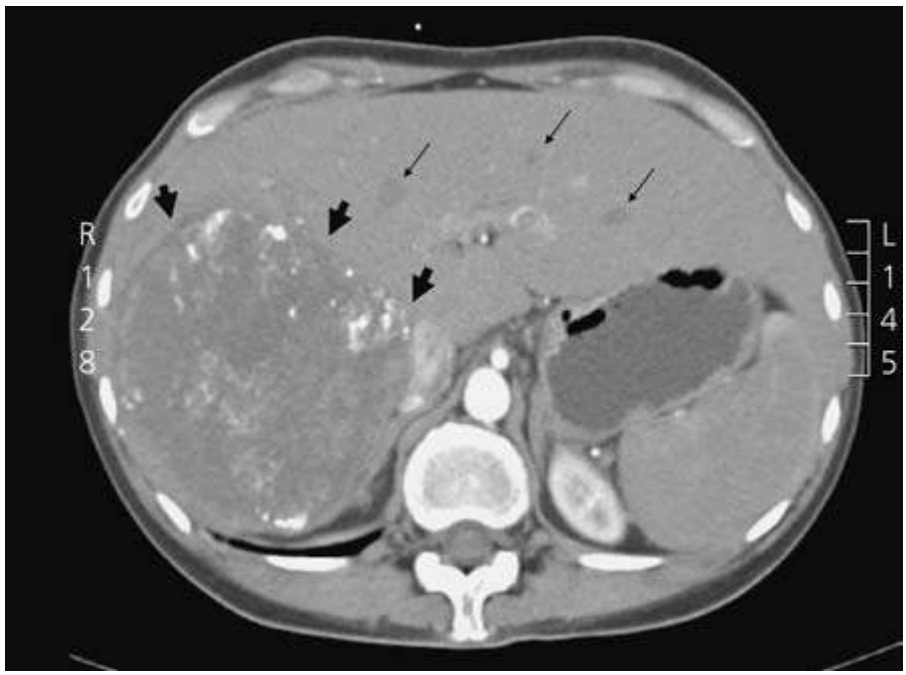


Figure 65.7 Abdominal computed tomography (CT) scan revealing hepatic metastatic lesions from colorectal cancer. Short arrows outline large hepatic metastatic lesion. Long arrows point to smaller additional metastatic lesions.

recurrent disease [343], colorectal liver metastases [344], and undiagnosed metastatic foci in the presence of rising CEA levels [345].

In rectal cancer preoperative determination of level of tumor invasion (T stage) and presence of lymph node involvement (N stage) are of prime importance in management planning.

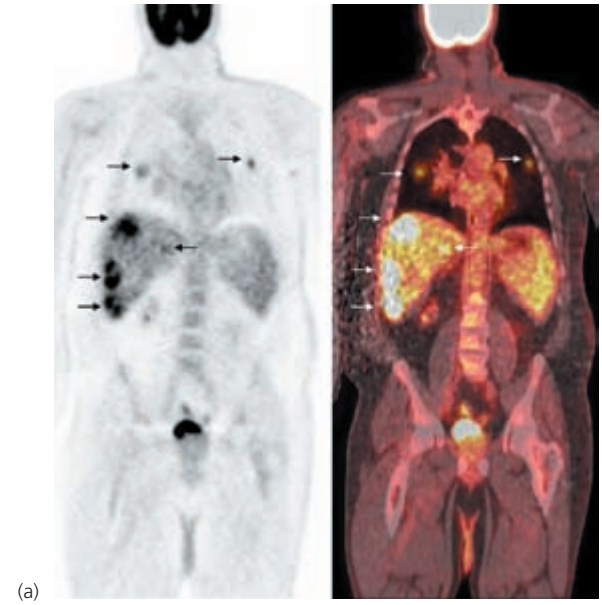


Figure 65.8 Patient with liver and lung metastases from colorectal cancer. **(a)** Positron emission tomography (PET) scan with arrows showing a pulmonary metastasis in each lung and multiple hepatic metastases in both the right and left lobes of the liver. **(b)** PET/CT fusion scan of patient with metastatic lesions marked by the arrows. Courtesy of Dr Christopher Mosley.

Traditionally, rectal endoscopic ultrasound (EUS) has been used for this purpose, but endorectal MRI is increasingly popular. In EUS a rectal endoscopic probe emits ultrasound waves to delineate five layers of the large bowel anatomy, evaluating for depth of tumor invasion and lymph node involvement (Fig. 65.9). Accuracy rates of rectal EUS for the evaluation of the depth of transmural tumor invasion are 82%–88% [346,347], and this technique has been described as superior to others for preoperative staging [348–350]. However, technological advances have meant that the accuracy of thin-section MRI performed with a phased-array coil inserted rectally rivals that of rectal EUS [351,352].

Pathology

Histopathology

Most colorectal cancers are adenocarcinomas arising from the conversion of adenomatous polyps to colorectal carcinoma. Other histological types of large bowel malignancy, including squamous cell cancer at the anorectal junction, cloacogenic cancers, melanoma, lymphoma, and carcinoids, account for less than 3% of colorectal cancers.

Colorectal adenocarcinomas typically form glandular structures (Fig. 65.10). Most have well-differentiated (25%) or moderately well-differentiated (60%) glands. The remainder (15%) are poorly differentiated or undifferentiated [353]. In poorly differentiated cancers, gland formation is less prominent. A histological grading system of differentiation by Compton designates tumors as follows [354]:

- grade1 – well differentiated
- grade 2 – moderately well differentiated

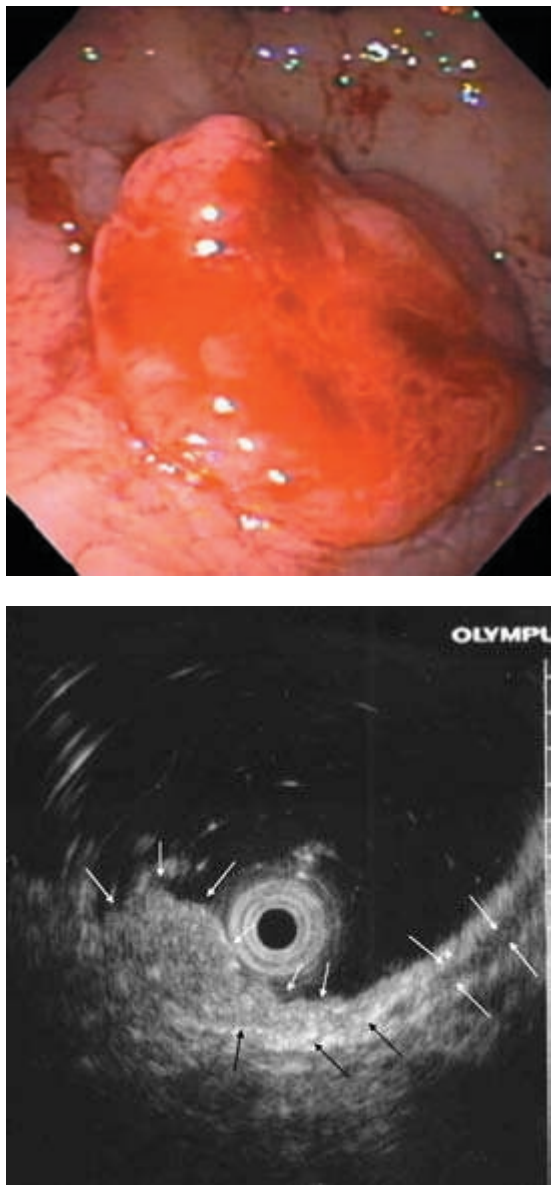


Figure 65.9 Endoscopic ultrasound. **(a)** Endoscopic view of a 3-cm rectal cancer localized 1 cm from anal verge. **(b)** Endoscopic ultrasound of same lesion showing stage II (T3, N0, M0) colorectal cancer. The rectal mass is demarcated by the upper white arrows. Between the white arrows is the dark muscularis propria layer. The rectal cancer invades through the muscularis propria with disruption and loss of this layer as noted by the black arrows. Courtesy of Dr Sergey Kantsevoy.

- grade 3 – poorly differentiated
- grade 4 – undifferentiated.

Several studies have observed that poorer differentiation is a stage-independent factor for poorer prognosis [355,356]. Undifferentiated small cell cancers of the colorectum have been identified as endocrine tumors with a poor prognosis.

Both normal and neoplastic glands secrete mucin, a glycoprotein formed by intestinal goblet cells and identified on routine pathology staining. Most large-bowel cancers secrete little or moderate amounts of mucin. A minimum of 25% of mucin compared with tumor mass is required to describe a cancer as having a mucinous component. Cancers producing abundant mucin (mucin accounts for at least half of the tumor mass) have a grossly gelatinous appearance and are designated as mucinous or colloid (Fig. 65.10). Patients with mucinous tumors tend to be diagnosed at an advanced stage, are less amenable to surgical resection, and have a poorer prognosis than others [357].

Signet-ring cell carcinoma is another rare (0.2%–1%) histological type of colorectal adenocarcinoma. These tumors are identified by signet-ring (i.e., resembling a finger ring bearing an engraved seal) cell morphology with an eccentric crescent-shaped nucleus pushed by intracytoplasmic mucin against the cell wall. Patients with this type of large-bowel cancer have a particularly poor 5-year survival rate compared with others [358].

Gross morphology and spread

Most colorectal cancers develop from colorectal adenomas protruding from the mucosal lining. In Asian populations, particularly the Japanese, investigators have described the frequent development of colorectal adenocarcinomas arising from nonpolypoid or flat lesions, an observation rarely made in Western countries [359].

Colorectal cancers can take several gross pathological forms:

- polypoid – exophytic intralumenal mass without ulceration, more commonly seen in the right colon
- fungating – polypoid cancer with prominent ulceration
- ulcerative – the surface of the lesion on a level with or ulcerating below the normal mucosa, more often found in the left and sigmoid colon
- infiltrative – infiltration of the intestinal wall, often circumferentially forming a constricting mass.

At the time of diagnosis multiple primary cancers (synchronous cancers) can be found in 3%–6% of patients with presumed sporadic colorectal cancer, and in 18% and 21% of patients with ulcerative colitis and FAP, respectively [360,361].

Colorectal cancer usually arises as a small focus of neoplastic cells in an adenoma. With time the malignancy invades perpendicularly to the intestinal lumen through various layers of the large bowel, and penetrates the bowel wall to involve neighboring organs and the peritoneal cavity. Once the tumor penetrates the muscularis mucosae layer of the intestine, it can access the lymphatic and vascular system and spread to regional and extraregional lymph nodes, hence forming distant metastases. The liver is the most common site of metastasis because venous drainage from the large bowel is directed through the portal circulation. Pulmonary metastases occur especially in tumors of the lower third of rectum, which is also drained by veins emptying into the inferior vena cava. Rectal cancer is characterized by involvement of local structures because of the lack of a serosal lining and

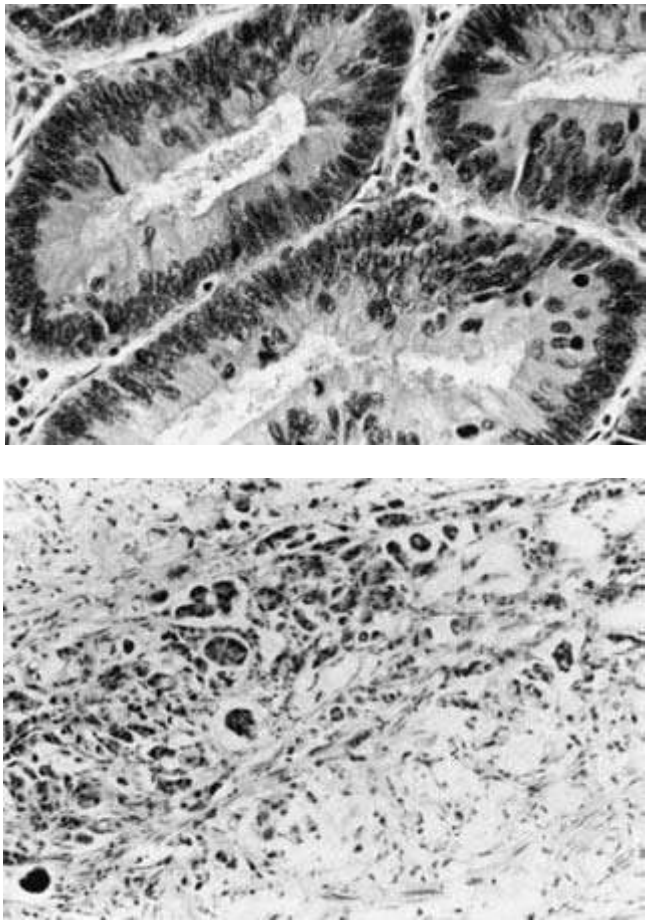


Figure 65.10 **(a)** Well-differentiated adenocarcinoma of the colon with characteristic gland-like formation. In each gland, the nuclei are large, the chromatin is poorly condensed, the nuclei are stratified away from the basement membrane, and mitoses are common. **(b)** Mucinous or colorectal cancer with cancer cell suspended in pools of mucin (dark nuclei) and typical glands are not seen.

close confinement in the pelvis. Also, vertebral metastasis can occur through communication of the portal and vertebral venous circulation through the Batson plexus.

Staging

An understanding of intestinal histology is crucial for accurate pathological staging of colorectal cancer. The large bowel is organized into four layers: mucosa, submucosa, muscularis propria, and serosa (Fig. 65.11). The innermost layer, the mucosa, has three parts: the epithelium, which lines the intestinal lumen; the lamina propria, containing connective and smooth muscle fibers; and the muscularis mucosae, a thin double layer of smooth muscle separating the mucosa from the submucosa. The submucosa contains lymphatics, blood vessels, and nerve fibers. The muscularis propria is composed of an inner circular smooth muscle and an outer layer of longitudinal muscle. The serosa is formed of mesenchymal elements and is lined externally by a single layer of mesothelial cells. Importantly, colorectal malignancy is defined by aberrant mucosal cells penetrating the muscularis mucosae and escaping confinement by gaining access to submucosal lymphatic and vascular channels.

The first system of colorectal cancer staging was devised in 1932 by Cuthbert Esquire Dukes, a Scottish pathologist who proposed a method of rectal cancer classification based on surgical resection specimens [362]. Dukes divided staging into three stages:

- stage A – tumor restricted to but not through the bowel wall
- stage B – tumor through the bowel wall
- stage C – tumor spread to lymph nodes.

Stage D to denote metastatic disease was added later. The Dukes classification has been adapted several times producing, among others, the Astler–Coller and the Dukes–Turnbull classifications. The Dukes–Turnbull is perhaps the most commonly used system and is diagrammed and explained in Fig. 65.11.

Today, colorectal cancer is classified using the TNM (tumor, node, metastasis) scheme discussed below. However, familiarity with Dukes classifications is still relevant because these systems stubbornly persist in clinical practice and understanding of them is necessary to interpret historical therapeutic trials.

TNM staging

To provide a more uniform pathological classification, the American Joint Committee on Cancer (AJCC) and the Union Internationale Contre Le Cancer (UICC) developed a system based on evaluation of the extent of the primary tumor (T), extent of local and regional lymph node involvement (N), and presence or absence of metastatic disease (M) [363]. TNM staging has replaced the Dukes classifications and is summarized as follows:

T stage

- T0 – no evidence of primary tumor
- Tis – in situ adenocarcinoma (high-grade dysplasia, carcinoma in situ); tumor confined to the mucosa and not breaching the muscularis mucosae
- T1 – tumor invades the submucosa but not the muscularis propria
- T2 – tumor invades into but not through the muscularis propria
- T3 – cancer invades through the muscularis propria into subserosa or nonperitonealized extramural tissues
- T4 – cancer directly invades other organs or structures (T4a) or perforates visceral peritoneum (T4b).

N stage

- Nx – lymph nodes cannot be assessed
- N0 – no lymph node involvement

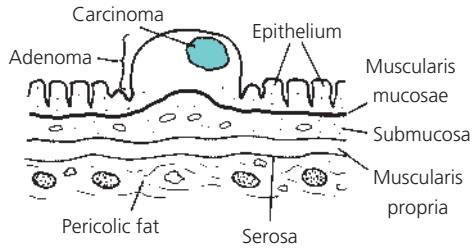
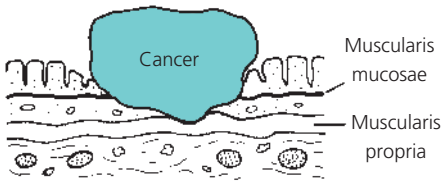
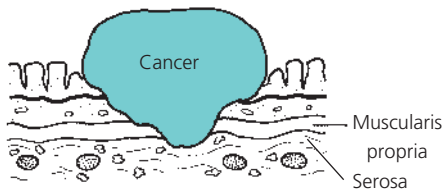
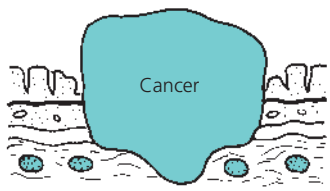
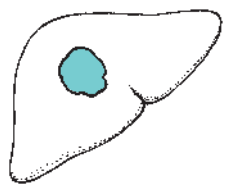
Stage		Depth of invasion	Frequency among invasive cancers	Observed 3-year survival	Actuarial cancer-free 5-year survival
Carcinoma in situ		Noninvasive			100%
A(I)		Penetrates the muscularis mucosae; invades the submucosa but does not penetrate the muscularis propria	15%	83%	95%–100%
B(II)		Penetrates muscularis propria and may extend through the serosa into pericolic fat (B1 into the muscularis propria; B2 through the serosa)	31%	79%	80%–85%
C(III)		Any invasion, plus regional lymph node metastases (C1 = ≤ 4; C2 = > 4)	23%	56%	50%–70%
D(IV)		Distant metastases	30%	5%	5%–15%

Figure 65.11 Dukes–Turnbull classification A through D with TNM stage in parentheses. The 3-year survival and actuarial cancer-free 5-year survivals for each stage are shown.

- N1 – metastasis in one to three regional lymph nodes
- N2 – metastasis in four or more regional lymph nodes.

Note: prognosis correlates with the number of lymph nodes inspected in the pathological resection specimen. Consequently the AJCC/UICC mandates that a minimum of seven lymph nodes should be inspected.

M stage

- MX – distant metastasis cannot be assessed
- M0 – no distant metastasis
- M1 – presence of distant metastasis.

Involvement of nonregional lymph nodes including common iliac, external iliac, paraaortic, and supraclavicular, is considered distant metastasis.

R stage

This refers to residual tumor at the margins of resected specimens and is designated as follows:

- R0 – complete surgical resection without microscopic evidence of tumor
- R1 – complete resection with microscopically positive margins for tumor
- R2 – incomplete surgical resection with grossly positive margins for tumor.

Patients with a more advanced R stage have poorer prognosis and are considered ineligible for adjuvant chemotherapy.

The AJCC/UICC system has eight stages (stage 0, I, IIA, IIB, IIIA, IIIB, IIIC, IV). Table 65.9 gives the 5-year survival rates for colon cancer when patients are staged according to this scheme [364]. Table 65.10 compares the original Dukes stages and the Astler–Coller modification of Dukes stages with the TNM stages.

Table 65.10 Dukes staging and Dukes modified Astler–Coller staging compared with TNM stages

Dukes stage	TNM stages
A	T1 or T2, N0, M0
B	T3 or T4, N0, M0
C	T1–4, N1 or N2, M0
D	M1
Astler–Coller stage	**TNM stages**
A	T1, N0, M0
B1	T2, N0, M0
B2	T3 or T4, N0, M0
B3	T4, N0, M0
C1	T2, N1 or N2, M0
C2	T3, N0, M0
C3	T4, N1 or N2, M0

Treatment

Surgery

Principles

Surgery is the treatment of choice for most patients with colorectal cancer. Surgical resection provides the best chance for long-term cure of the primary tumor, isolated recurrence at the colorectal anastomotic site, and metastatic lesions in the liver and lung. The basic surgical approach is an anatomical en bloc resection of the primary tumor with adequate margins and removal of the regional lymph nodes. This strategy stems from the finding that pericolic mesenteric lymph nodes are the initial site of metastatic spread, and node status carries diagnostic and therapeutic significance. The blood supply and lymph node chains follow parallel courses facilitating en bloc resection. Colorectal resection should include the tumor and 5-cm margins on either side of the cancer. When colorectal cancer has invaded or attached to other organs, en bloc resection including these organs is necessary to achieve a curative resection [365]. Previously, a no-touch surgical technique of tumor isolation and resection was encouraged to prevent detachment and circulation of tumor cells. However, subsequent evidence suggests that this technique is not justified because of the absence of escaping tumor cells in the circulation [366].

The surgical approach for stage I, II, and III colorectal cancer is based on the size and location of the tumor.

Table 65.9 Staging system recommended by the American Joint Commission on Cancer and the Union Internationale Contre Le Cancer, and corresponding 5-year survival rates

Stages	T (primary tumor)	N (lymph nodes)	M (metastatic disease)	5-year survival (%)
0	Tis	N0	M0	
I	T1–T2	N0	M0	93.2
IIA	T3	N0	M0	84.7
IIB	T4	N0	M0	74.2
IIIA	T1–T2	N1	M0	83.4
IIIB	T3–T4	N1	M0	64.1
IIIC	Any T	N2	M0	44.3
IV	Any T	Any N	M1	8.1

Adenocarcinoma is encountered in the cecum and ascending colon 15% of the time, in the transverse colon in 8% of cases, and in the splenic flexure and descending colon on 5% of occasions. The majority of sporadic cancer is found in the sigmoid and rectum.

Colonic cancers

Cecum and ascending colon

Cancers in the right colon require a right hemicolectomy (Fig. 65.12a). This procedure involves resection of the intestine from several centimeters proximal to the ileocecal value to one-third of the distance along the transverse colon.

Transverse colon

Proximal transverse lesions near the hepatic flexure are treated with an extended right hemicolectomy. Intestine is removed from several centimeters proximal to the ileocecal valve to the middle of the transverse colon. Distal transverse colon lesions are resected with an extended left hemicolectomy.

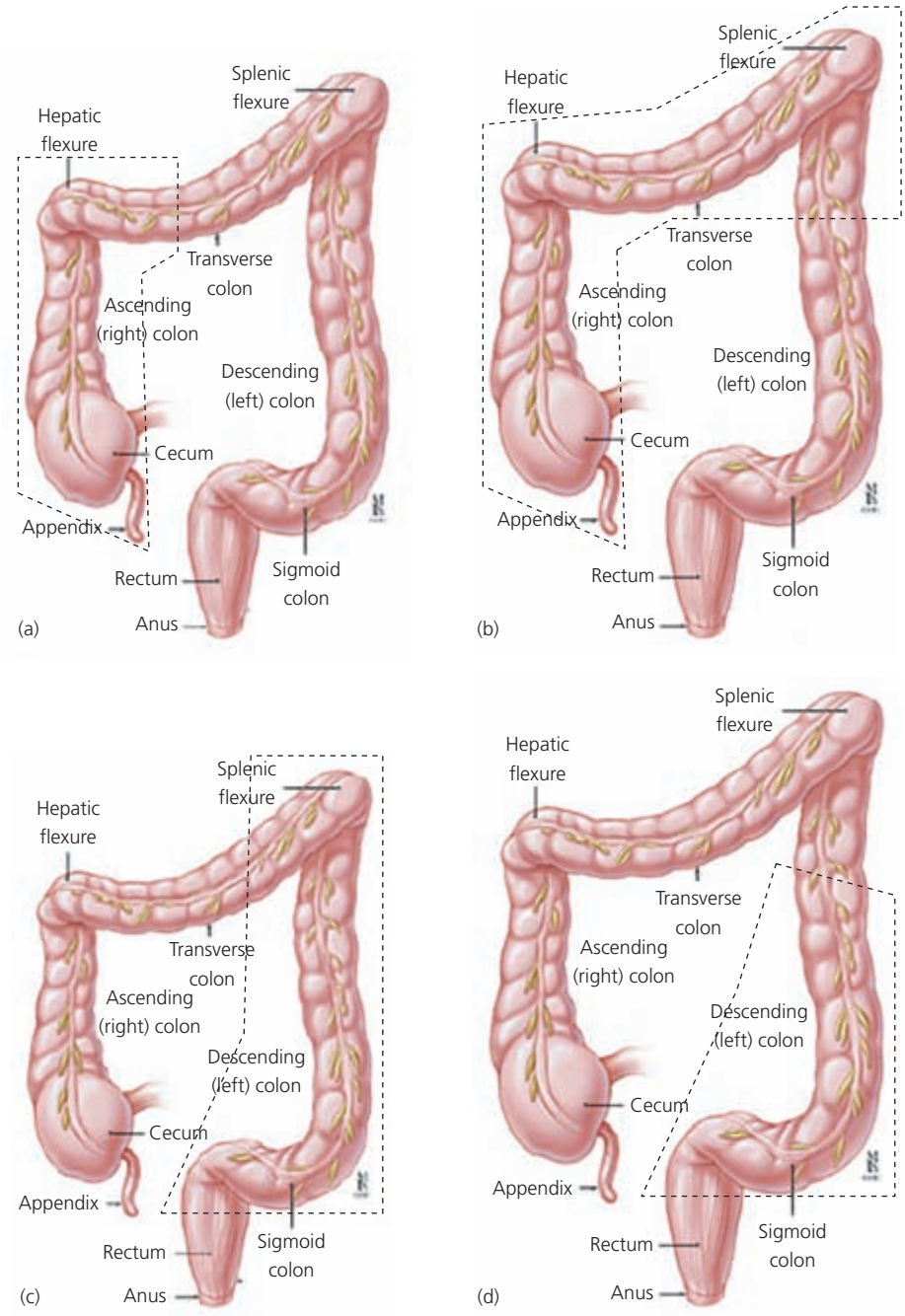


Figure 65.12 Types of surgical resection of colorectal neoplasia. **(a)** Right hemicolectomy. **(b)** Extended right hemicolectomy. **(c)** Left hemicolectomy. **(d)** Sigmoid resection. Adapted from the Johns Hopkins Gastroenterology and Hepatology Resource Center (http://hopkinsgi.org), with permission.

Mid-transverse lesions involve resection of the colon from several centimeters proximal to the ileocecal valve to approximately one-third the way along the descending colon (Fig. 65.12b).

Descending and sigmoid colon

For lesions in the proximal half of the descending colon a left hemicolectomy is performed (Fig. 65.12c). This involves removal of the colon from halfway along the transverse colon to the region of the sigmoid colon. For tumors in the sigmoid colon, data suggest that a more limited operation, a sigmoid colectomy, has comparable survival and less morbidity and mortality than left hemicolectomy [367] (Fig. 65.12d). A sigmoid colectomy involves the removal of bowel from halfway down the descending colon to the peritoneal reflection in the rectum.

Total abdominal proctocolectomy

This procedure involves removal of the entire colon and rectum with ileostomy or construction of a continent pouch from terminal ileum. This operation is indicated for those with FAP and ulcerative colitis. Several studies reveal little difference in functional outcome, particularly bowel frequency, between ileorectal anastomosis and restorative proctocolectomy with an ileal pouch [368,369].

Total abdominal colectomy

Total abdominal colectomy involves removal of the colon with preservation of the rectum and ileorectal anastomosis. This operative procedure is indicated for patients with a high risk of colorectal cancer including those with AFAP or HNPCC, and some with FAP. Postoperative endoscopic surveillance of the retained rectum for metachronous adenomas and cancers every 6–12 months is mandatory.

Rectal cancer

The management of stage II and III rectal cancer usually involves three modalities of treatment – surgery, chemotherapy, and radiation. The surgical approach to rectal cancer depends on tumor stage and location within the rectum. Most patients with stage I, II, and III rectal cancers undergo rectal excision, although some stage I patients are treated with more limited transanal resection. The rectum can be divided into three parts:

- the lower rectum – from the dentate line to the left lower valve of Houston (approximately 0–7 cm from the anal verge)
- the middle rectum – from the left lower valve of Houston to the peritoneal reflection or right middle valve of Houston (approximately 7–11 cm from the anal verge)
- the upper rectum – from the peritoneal reflection to the left upper valve of Houston (approximately 11–15 cm from the anal verge).

For stage I rectal cancers (T1 or T2, N0), some surgeons have utilized transanal excision rather than radical rectal excision for rectal tumors that invade into but not through the submucosa (T1) or invade into but not through the muscularis propria (T2) in the absence of lymph node involvement (N0). This procedure removes rectal tumors with surrounding mucosa through the anus, sparing the patient the morbidity of an extensive rectal resection. Patients typically selected for this procedure include those with tumors within 8–10 cm of the anal verge, in which the tumor encompasses less than 40% of the circumference of the bowel, with well-differentiated histology, and no lymph or blood vessel involvement. However, despite stringent selection criteria, the local recurrence rates of tumor after local excision can be as high as 18% for T1 and 37% for T2 tumors [370,371]. Consequently, the role of local excision as a curative procedure remains controversial [372].

Upper rectal cancers

The upper third of the rectum is intraperitoneal. The usual surgical approach in this location is to perform a low anterior resection (LAR), which preserves the internal and external rectal sphincters and creates an end-to-end anastomosis to restore intestinal continuity. This operation is usually performed through an anterior incision.

Middle rectal cancers

The middle rectum transitions between an intraperitoneal and extraperitoneal structure. Tumors in this area are usually removed by a LAR. At the time of the LAR a temporary ileostomy or colostomy is created to allow for wound healing of the low anastomosis.

Distal rectal cancers

Most patients with distal rectal tumors have abdominal peritoneal resections (APR). This operation involves removal of the rectosigmoid, rectum, and anus by a combined abdominal and peritoneal approach and leaves most patients with a permanent colostomy. Such a procedure is necessary because surgical margins of 2 cm from the tumor are required for a curative operation. Margins greater than 2 cm appear unnecessary because less than 6% of tumors demonstrate intramural spread beyond 2 cm; the majority of patients with tumor invasion beyond 2 cm have advanced stage disease, and distant metastases are the life-limiting issue, not local recurrence [373]. In some patients in whom at least 2 cm of distal margin can be resected, intestinal continuity can be restored by a coloanal anastomosis suturing the distal colon to the anus. Some surgeons make an anal colonic pouch prior to anastomosis to the anus. Also, in some cases preoperative chemoradiation may downsize the tumor allowing for less radical surgical approaches.

Total mesorectal excision

The mesorectum is a structure surrounding the rectum that contains the blood supply and the lymphatics for the upper,

middle, and lower rectum. Microscopic spread of tumor to the mesorectum is noted in almost 40% of patients undergoing surgery for rectal cancer [374]. Conventional rectal excision involving blunt technique along indefinable tissue planes has been supplanted by total mesorectal excision (TME). In this surgical technique, under direct visualization, sharp dissection along definable planes is done of the mesorectum posteriorly and laterally down to the pelvic floor and midline anococcygeal raphe. This technique accomplishes complete resection of an intact package of the rectum and surrounding mesorectum, enveloped within the visceral pelvic fascia with uninvolved circumferential margins. Multiple studies report that this procedure decreases local recurrence and increases overall survival. With TME, 5-year survival figures have risen from 45%–50% to 75%, local recurrence rates have declined from 30% to 5%–8%, sphincter preservation has risen by at least 20% for mid- and lower rectal cancers, and the rates of impotence and bladder dysfunction have declined from 50%–85% to 15% or less [375]. Consequently, TME has become the standard for surgery of lower and middle rectal cancer.

Laparoscopic colonic resection

Unlike the rapid acceptance and use of laparoscopic cholecystectomy, few colorectal surgeons practice laparoscopic colonic resection, although both techniques were introduced into surgical practice at about the same time [376]. Reluctance to adapt this procedure comes from initial concerns of inadequacy of resection margins, inadequate node sampling, and seeding of port sites. However, four randomized studies of laparoscopic versus open laparotomy revealed comparable numbers of lymph nodes retrieved, adequate surgical resection margins in both groups [377–380], and port site recurrence in one study [379]. No difference in disease-specific survival was noted between groups in the two large studies [379,380], and a third smaller study demonstrated a difference in favor of the laparoscopic group [378]. Patients undergoing laparoscopic procedures have less pain, a lower requirement for postoperative narcotics, reduced ileus [377,381], and possibly less postoperative morbidity than patients having open procedures [377,378,382]. Barriers to further acceptance of this procedure include a steep learning curve and increased operative time for laparoscopic colectomy.

Palliative surgery

Surgical management for stage IV colorectal cancer by resection of the primary tumor or diversion for symptoms of obstruction or bleeding is almost always indicated and provides significant palliation. In patients with intraperitoneal disease with obstruction, palliation can be done with surgical bypass or ostomy. Recurrence at the site of prior anastomosis can potentially be cured. In patients with multiple medical problems or at high surgical risk, palliation with fulguration of the rectal tumors using snare polypectomy or laser ablation is possible.

Liver metastasis

Liver metastasis from colorectal cancer is a common occurrence, with 50 000 Americans presenting annually with this condition [383]. After resection of the primary tumor, the liver is the most frequent site of relapse (33%) of colorectal cancer [384]. Approximately 70%–80% of hepatic metastases appear within 2 years of primary tumor resection. Nearly half of all patients dying with colorectal cancer will have liver-limited disease at autopsy. Surgical resection is the best treatment for long-term outcome, but only a minority (about 25%) of patients have resectable metastatic liver disease. The major reasons for unresectability include the number of lesions, proximity to major vascular structures, and preexisting liver disease. Of those undergoing curative resection, the 5-year survival rate is about 25%–35% [383,385]. The mortality of hepatic resection ranges from 1% to 4%, and the morbidity is 8%–12% [386], but without aggressive intervention the average survival of patients with liver metastases is 8 months [387,388]. Traditional criteria for liver resection include patients with three or fewer metastatic lesions in one lobe of the liver, lesions less than 5 cm in size, the ability to leave margins of greater than 1 cm, and the absence of extrahepatic disease. Over time surgeons have liberalized eligibility for surgery. It is noteworthy that patients who have undergone previous hepatic resection benefit from repeat resection if possible, with the 5-year survival in these individuals as high as 41% [387]. Intraoperative ultrasound is necessary to increase the ability to detect metastatic lesions deep within the liver and to identify and avoid vascular structures.

Hepatic lesions not amenable to surgical resection are often treated with ablative therapies. These include intraarterial chemoembolization, injection of ethanol, cryotherapy, and radiofrequency ablation. The latter two are most commonly used for patients with colorectal cancer metastasis. Cryotherapy employs rapid freezing of tissue causing crystal formation and cell death. Radiofrequency ablation uses heat to cause tumor necrosis. Both techniques are done under ultrasound guidance at the time of surgery, and radiofrequency ablation can be accomplished percutaneously. The effectiveness of these ablative therapies is difficult to evaluate because they are often performed in combination with other treatment modalities. A summary of radiofrequency ablation reported a 2%–39% recurrence of tumor at the ablated site [389].

Another treatment modality for unresectable liver metastases is hepatic arterial infusion (HAI). A pump containing a chemotherapeutic agent is surgically placed subcutaneously and an attached catheter is implanted into the hepatic artery to deliver the agent directly into the liver. In HAI, agents taken up by the liver by the first-pass effect deliver a significant

concentration of drug to the tumor while minimizing systemic toxicity. A number of randomized trials evaluating the use of HAI chemotherapy versus systemic chemotherapy have demonstrated a significant advantage in terms of response rate and progression-free survival for HAI. However, the overall advantage is small. For example, a study of HAI therapy versus systemic 5-fluorouracil (5-FU) and leucovorin in 135 patients with unresectable liver metastases showed survival of 24.9 months with HAI and 20.0 months with systemic chemotherapy, and response rates of 47% and 24%, respectively [390]. Subsequent studies utilizing HAI and systemic chemotherapy in patients with unresectable liver disease have produced response rates of greater than 70% [391,392]. Also, adjuvant therapy with HAI and systemic chemotherapy may be useful after liver resection [393].

Postoperative surveillance

The 5-year survival rate after surgical resection of the primary tumor is 90% for those with localized disease but drops to 65% in individuals with metastases in regional lymph nodes [394]. The most frequent sites of relapse after surgery are the liver (33%), lungs (22%), locoregional sites (21%), and intraabdominal sites (18%) [384]. Rectal cancers, when compared with colonic cancers, have proportionally more local recurrences and less involving retroperitoneal nodes [384]. The median time to recurrence for patients is 16.7 months, and the majority of recurrences occur within 3 years.

After surgical resection of colorectal cancer, the standard of care is surveillance to improve survival through earlier detection of cancer recurrence and metachronous colorectal neoplasia (Table 65.11). The effectiveness of various strategies of surveillance is difficult to evaluate, because different publications utilize different outcome variables and surveillance protocols. However, three independent metaanalyses of randomized studies all noted a reduction in death from all causes with more intensive follow-up, with a decrease in relative risk and absolute risk of 20% and 7%, respectively [395–397].

Evidence-based colorectal cancer surveillance guidelines have been published by the American Society of Clinical Oncology (ASCO) [398] and the National Comprehensive Cancer Network (NCCN) [399,400]. Both groups recommend periodic postoperative history-taking and physical examination, CEA testing, and colonoscopy, but differ on the frequency of these examinations. These groups do not advocate regular fecal occult blood, CBC, liver function testing, chest radiography, MRI, CT, or ultrasound scans of the abdomen or pelvis in the absence of clinical signs or symptoms [401].

Adjuvant chemotherapy

After years of minimal progress, six new chemotherapeutic agents for the treatment of colorectal cancer have been introduced since the mid-1990s [402]. The evolving use and indications for these agents necessitates that health-care providers continually review the primary medical literature for the latest strategies of therapy.

The primary treatment of stage II and III colorectal cancer is surgical resection. Despite removal of the primary tumor and accompanying lymph nodes in these patients with cancer invading the serosa or lymph nodes, high recurrence rates are noted. Of all patients resectable for cure at presentation, about 37% will fail primary therapy with recurrence of

Table 65.11 Surveillance guidelines after primary surgery with curative intent

Test or procedure	NCCN[a]	ASCO[b]
History and physical examination	Every 3 months for 2 years, then every 6 months for 3 years; counseling for family history	Every 3–6 months for the first 3 years, annually thereafter
Carcinoembryonic antigen test	Every 3 months for 2 years, then every 6 months for 3 years for T2 or higher-grade lesions if patient is a potential candidate for aggressive curative surgery	If resection of liver metastases is clinically indicated, postoperative testing may be performed every 2–3 months in patients with stage II or stage III disease for 2 years or longer after diagnosis
Flexible sigmoidoscopy and endoscopic ultrasound	Not addressed	For patients who have not received pelvic irradiation, direct imaging of the rectum at periodic intervals is suggested For patients who have received pelvic irradiation, direct imaging of the rectum (except colonoscopy at 3–5 years) is not suggested
Colonoscopy and barium enema	Colonoscopy 1 year after primary therapy; repeat in 1 year if abnormal or every 3 years if negative for polyps If no preoperative colonoscopy is performed because of obstructing lesion, perform colonoscopy in 3–6 months	Colonoscopy postoperatively if not yet done; if high-risk polyps present, excise and repeat colonoscopy annually as long as polyps found; if low-risk polyps, repeat colonoscopy in 3–5 years

a NCCN, National Comprehensive Cancer Network.
b ASCO, American Society of Clinical Oncology.
Adapted from Pfister et al. [401].

tumor [403]. The cause of recurrence is likely residual microscopic tumor foci not removed by initial surgery. The role of adjuvant chemotherapy is to eradicate postoperative residual microscopic cancer in those without known metastatic disease. Adjuvant treatment is most effective if given soon after surgery (within 8 weeks) when tumor burden is minimal. Adjuvant chemotherapy is advocated for patients with stage III colon cancer, and is controversial for those with stage II. In the United States and Western Europe about 29% of all patients have stage III disease (about 90 000 annually) and 26% have stage II.

Colon cancer

For almost the past half century, chemotherapy of colorectal cancer has centered on the use of 5-fluorouracil (5-FU) [404]. Patented in 1957, this agent requires intracellular activation to exert its antitumor activity. It kills cancer cells by disrupting DNA and protein synthesis through inhibition of thymidylate synthetase and the incorporation of metabolites as false nucleotides into DNA and RNA. This drug was utilized in the first large trial to show benefit of adjuvant chemotherapy for colorectal cancer in 1988 [405]. This study randomized 1166 Dukes B and C colorectal cancer patients to curative surgery followed by either no treatment; chemotherapy with 5-FU, semustine, and vincristine; or bacille Calmette–Guérin (BCG). Results revealed an overall improvement in disease-free survival ($P = 0.02$) and survival ($P = 0.05$) in favor of the chemotherapy-treated group.

In 1990, positive results of adjuvant chemotherapy were published in a smaller [406] and then a larger study (INT 0035) of 5-FU with levamisole [407]. Levamisole is an anthelmintic drug used by veterinarians to deworm cattle and sheep, and thought to have anticancer activity due to its enhancement of the immune response. The INT 0035 trial included 1293 patients with either locally invasive (stage B2) or with regional node (stage C) involvement. These individuals were randomized to either observation or a 1-year course of 5-FU combined with levamisole. Stage C patients could also be randomized to levamisole alone. At a median follow-up of 3 years, stage C patients treated with 5-FU and levamisole had their risk of cancer recurrence reduced by 41% ($P < 0.001$) and of death by 33% ($P = 0.006$). Treatment with levamisole alone had no detectable effect. The results in stage B2 patients were equivocal. Subsequently, longer-term follow-up with a median of 6.5 years confirmed these results [408]. This 1990 study prompted a consensus statement from the NCI establishing adjuvant chemotherapy as the standard of care for colorectal cancer patients with stage C disease [409].

The efficacy of the combination of 5-FU with leucovorin (folinic acid), a biomodulator that accentuates the cytotoxic effect of 5-FU, was first noted in the treatment of metastatic colorectal cancer. Consequently, this combination was used as adjuvant therapy in stage II and III colorectal cancer in two small studies and one medium-sized study, all with virtually identical protocols. Pooled analysis from these trials of 1493 patients randomized to either 6-month chemotherapy or observation revealed a statistically significant reduction of mortality and increased 3-year event-free survival in those receiving chemotherapy [410]. Subsequently, evaluation of 5-FU and leucovorin revealed a slight but statistically significant advantage of this regimen compared with 5-FU and levamisole in 5-year disease-free survival. Also, levamisole added to 5-FU and leucovorin provided no advantage [411]. Consequently, 6 months of treatment with 5-FU and leucovorin became the new standard of care for stage III disease adjuvant chemotherapy at the end of the 20th century [412–414].

Investigators have attempted to improve standard adjuvant treatment with 5-FU and leucovorin (Table 65.12). Oxaliplatin improves the efficacy of this combination in those with metastatic colorectal cancer. In an evaluation of adding oxaliplatin to standard adjuvant treatment, a total of

Table 65.12 Results of adjuvant chemotherapy for stage II and stage III colon cancer

Treatment	Ref.	Stage	3-year disease-free survival (%)	5-year disease-free survival (%)	5-year survival (%)
Observation	[419]	II	79	73	80
	[408]	III	52	45	54
5-FU and levamisole	[411]	II		71	81
	[411]	III		53	63
5-FU and leucovorin	[411]	II		75	84
	[411]	III		57	67
5-FU and leucovorin	[415]	II	85		
	[415]	III	66		
5-FU, leucovorin, and oxaliplatin	[415]	II	88		
	[415]	III	73		

5-FU, 5-fluorouracil.

2246 patients who had undergone curative resection for stage II or III colon cancer were randomized to receive 5-FU and leucovorin alone or with oxaliplatin for 6 months [415]. The results of this trial revealed an improvement in the standard adjuvant treatment with the addition of oxaliplatin, with disease-free survival at 3 years of 78.2% in the triple drug arm compared with 72.9% with standard adjuvant treatment ($P = 0.002$). In this study no significant interaction between the stage of disease and the treatment was noted, indicating that 5-FU and leucovorin plus oxaliplatin benefited both stage II and stage III colorectal cancer patients, although no statistically significant improvement in outcome was noted in stage II patients. However, the use of oxaliplatin adds significant sensory neuropathy to the adjuvant regimen. Although the incidence of serious grade 3 sensory neuropathy declined from 12.4% during treatment to 1.1% at 1 year of follow-up in this study, long-lasting adverse effects with grade 1 neuropathy were reported in 19% of patients at 18 months of follow-up.

Compared with intravenous 5-FU, oral 5-FU prodrugs such as capecitabine and tegafur–uracil appear to have comparable efficacy and favorable toxicity in some 5-FU and leucovorin regimens [416,417]. Other combinations being studied and contemplated are use of standard adjuvant therapy with irinotecan, and the monoclonal antibody-based agents cetuximab and bevacizumab.

Adjuvant chemotherapy for patients with stage III colorectal cancer is the standard of care. However, whether adjuvant therapy is appropriate for stage II patients is controversial. The debate was initially generated by opposite conclusions of two large groups of investigators. The National Surgical Adjuvant Breast and Bowel Project [418] reported benefits for stage II and III patients, whereas the International Multicentre Pooled Analysis of B2 Colon Cancer Trials (IMPACT B2) [419] found no statistically significant advantage in treating stage II patients. However, a pooled analysis of 3300 patients from five randomized trials [420] concluded that stage II individuals had a small but statistically significant benefit from surgery followed by adjuvant 5-FU-based chemotherapy compared with surgery alone (5-year disease-free survival 76% vs 72%, respectively), and that stage III patients had greater benefit. This study did not factor in the analysis a host of prognostic factors in stage II patients including colonic obstruction, total lymph nodes examined, or preoperative CEA levels. Treatment of stage II patients with adjuvant chemotherapy remains controversial, and decisions are made by physicians and patients, balancing risk and benefit on a case-by-case basis. A model assessing individual patient 5-year disease-free survival and overall survival [420] is available for physicians on the Mayo Clinic website (http://www.mayoclinic.com/calcs). In practice, adjuvant treatment is being administered to a significant proportion (27%) of stage II patients as reported in a Medicare population-based survey [421].

Rectal cancer

The anatomy of the rectum, confined between bony structures of the pelvis, makes surgical excision of rectal cancer difficult. Even with optimal operative technique employing total mesorectal excision, up to 15% of patients with tumors that infiltrate into the perirectal fat or spread to local lymph nodes develop local recurrence [422]. In addition to limitation of overall survival, local recurrence can lead to pelvic pain and urological disturbances.

To improve local recurrence rates, radiotherapy with or without chemotherapy before or after surgery has been employed. Studies reveal that rectal surgery followed by chemoradiation decreases local recurrence rate and improves overall survival in patients with stage II and stage III rectal cancer compared with surgery alone or surgery and radiotherapy [423,424]. Consequently, a 1990 National Institutes of Health consensus statement recommended postoperative adjuvant chemoradiotherapy as standard treatment for patients with rectal cancer classified as TNM stage II (tumor penetrating the rectal wall, without regional lymph node involvement) or stage III (any tumor with regional lymph node involvement) [425]. Subsequent trials have attempted to define the optimal timing of chemoradiation. A metaanalysis concluded that the combination of preoperative radiotherapy and surgery compared with surgery alone significantly improved local control and overall survival [426]. Subsequently, prospective evaluation of preoperative adjuvant treatment (neoadjuvant treatment) versus postoperative chemoradiation was reported [427]. Preoperative treatment with radiation and 5-FU did not improve overall survival but was associated with reduced toxicity and improved local control (6% vs 13% recurrence rate, respectively) compared with postoperative adjuvant treatment. The main disadvantage of the preoperative approach is that optimal selection of patients most likely to benefit from adjuvant treatment is impossible, because there is no resection specimen from which to assess the initial cancer stage; hence, some patients may be unnecessarily treated. However, surgeons favor preoperative treatment for several reasons: it avoids radiation toxicity to loops of small intestine postoperatively fixed to the pelvis; it can occasionally downstage the tumor prior to surgery allowing sphincter-saving procedures; and it avoids damage and complication to the reconstructed neorectum. Consequently, emphasis is shifting in the practice community toward preoperative (neoadjuvant) rather than postoperative adjuvant treatment. Adjuvant chemotherapy with 5-FU and 5-FU with leucovorin is superior or equivalent to other regimens tried.

Metastatic disease

About 22% of patients presenting with colorectal cancer have stage IV disease, defined as those with distant metastases regardless of depth of tumor invasion into the bowel wall or lymph node status. The 5-year survival rate for

stage IV disease is 5%–7% [428]. Patients with unresectable metastatic disease have incurable cancer, and subsequent treatment must be viewed as palliative care. The potential benefits of chemotherapy – including control of symptoms, lengthening of progression-free and overall survival – must be balanced against the risks of these toxic agents on a case-by-case basis. To date, the value of chemotherapy in this setting is disappointing; metaanalysis of systemic chemotherapy trials in patients with metastatic colorectal cancer reveals an improvement in 1-year survival from 34% to 50%, and an improvement in median survival of 3.7 months [429].

The cornerstone of treatment of patients with stage IV colorectal cancer is 5-FU, which remains central to all effective chemotherapeutic regimens in metastatic colorectal cancer. Given as a single agent, 5-FU has superior efficacy compared with a multitude of other agents evaluated. Combination with leucovorin enhances 5-FU efficacy by stabilizing the binding of fluorouracil to thymidylate synthetase [430]. For many years this combination was the standard of care for metastatic disease treatment, with prospective studies revealing reduced tumor size in 20% of patients and prolongation of median survival, from 5 months without chemotherapy to 11–12 months with chemotherapy [431–433]. Addition of other agents to 5-FU such as methotrexate or interferon failed to showed superiority to 5-FU and leucovorin [432–434]. Several trials to determine the optimal dosing, administration, and schedule of 5-FU and leucovorin have been conducted. Infusional administration of 5-FU has less toxicity than bolus administration, with equivalent efficacy. Capecitabine (Xeloda), an oral precursor of 5-FU, appears to have favorable safety and efficacy compared with some parenterally administered 5-FU regimens and is under study as an alternative dosing method for use in combination regimens.

After 30 years of little progress, the past decade has seen the introduction of several new drugs for the treatment of colorectal cancer (Table 65.13). Irinotecan (Camptosar, CPT-11), an analogue of camptothecin approved in 1996, is a chemotherapeutic agent that inhibits topoisomerase I activity leading to DNA fragmentation and cell death [435]. Addition of irinotecan to 5-FU and leucovorin chemotherapy significantly improves tumor response rate (13%–18%), and increases time to progression (median of 2.3–2.7 months) and survival (median of 2.2–3.3 months) [436,437]. The commonly used regimen developed in France, called FOLFIRI, consists of leucovorin (known also as folinic acid [FOL]), 5-FU (F), and irinotecan (IRI), with 5-FU given as an infusion and the other drugs given as bolus therapy [438]. Oxaliplatin (Eloxatin), approved for use in 2002, is a platinum analogue that exerts chemotherapeutic effect by the formation of platinum–DNA adducts that block DNA replication and transcription; it also possibly interferes with RNA synthesis [439]. The combination of oxaliplatin with 5-FU and leucovorin, with 5-FU infused over 46 h – (FOLFOX) = leucovorin (or folinic acid, FOL), 5-FU (F), and oxaliplatin (OX) – provides superior response rates and times to disease progression compared with 5-FU and leucovorin alone [440–442]. Most evaluations of irinotecan, 5-FU, and leucovorin compared with regimens based on oxaliplatin, 5-FU, and leucovorin suggest equivalence between these treatments [443,444], although one study shows an advantage with oxaliplatin [445].

Since 2004, three new biological agents that interrupt cellular pathways have become available for the treatment of metastatic colorectal cancer. Bevacizumab (Avastin) [446] is a humanized monoclonal antibody targeted against vascular endothelial growth factor (VEGF). The interaction of VEGF with its receptors leads to endothelial cell proliferation and new blood vessel formation in in vitro models of angiogenesis.

Bevacizumab binds VEGF and prevents the interaction of VEGF with its receptors on the surface of endothelial cells, with resultant inhibition of tumor angiogenesis. Cetuximab (Erbitux) [447] is a chimeric monoclonal antibody that binds to epithelial growth factor receptors preventing the activation of tyrosine kinase and affecting pathways regulating cell proliferation and apoptosis. In clinical studies bevacizumab

Table 65.13 Chemotherapy for stage IV colorectal cancer

Treatment	Reference	Response rate (complete and partial) (%)	Progression-free survival (months)	Overall survival (months)
FOLFIRI	[443]	56	8.5	21.5
FOLFOX6	[443]	54	8.1	20.6
IFL	[448]	35	6.2	15.6
IFL plus Bev	[448]	45	10.6	20.3

FOLFIRI = 5-fluorouracil + leucovorin + irinotecan (every 2 weeks).
FOLFOX6 = 5-fluorouracil + leucovorin + oxaliplatin.
IFL = 5-fluorouracil + leucovorin + irinotecan (every 6 weeks).
Bev = bevacizumab.
From ref. [452].

leads to a prolongation of overall survival when added to irinotecan, 5-FU, and leucovorin (IFL) [448] or when added to FOLFOX [449] in patients intolerant to irinotecan. Consequently, bevacizumab has been FDA approved in combination with any intravenous fluorouracil-containing regimen as initial therapy for patients with advanced colorectal cancer. The application and benefit of cetuximab is less clear. Cetuximab used in combination with irinotecan in patients with metastatic colorectal cancer previously refractory to irinotecan showed an improved tumor response rate and median time to tumor progression compared with patients treated with cetuximab alone [450]. Trials of this drug in 5-FU-based regimens are under way. In 2006, panitumumab (Vertibix) was approved by the FDA for treatment of patients with metastatic colorectal cancer refractory to standard chemotherapy. Panitumumab as a monotherapy significantly improved progression-free survival in these patients compared with supportive care [451]. Panitumumab, a fully human monoclonal antibody, targets epidermal growth factor receptors, similarly to cetuximab.

Three combinations of chemotherapy have shown efficacy as first-line treatment for patients with stage IV colorectal cancer: FOLFOX, FOLFIRI, and IFL with bevacizumab. Clinical superiority has not been established among these regimens and the treatment choice is left to the physician and his or her assessment of the patient's comorbidites [452]. However, IFL with bevacizumab is a less favored option because of greater toxicity than the other two chemotherapeutic regimens. Although patients with metastatic disease have been treated with continuous chemotherapy there appears no benefit to this strategy versus a planned duration and restarting with recurrent disease. Other agents under study include cyclooxygenase inhibitors in combination with conventional chemotherapy, inhibitors of VEGF receptors, tubulin binders, and topoisomerase inhibitors, to name a few.

Prognostic factors

Introduction

A variety of clinical, pathological, serological, and molecular variables have been evaluated as prognostic (linked to outcome) and predictive (predicting response to therapy) factors in patients with colorectal cancer (Table 65.14). A consensus

Table 65.14 Prognostic factors in colorectal cancer

Worsens prognosis	Improves prognosis	No proven association
Clinical factors		
Bowel perforation at diagnosis	Asymptomatic at diagnosis	Nausea, vomiting at diagnosis
Bowel obstruction at diagnosis		Abdominal pain at diagnosis
Diabetes mellitus		Bowel habits change at diagnosis
		Red cell transfusions
Pathological factors		
Increasing extent of local invasion (T stage) (I)[a]	MSI histology (intratumoral and peritumoral lymphocytes) (IIB)	Tumor size (IV)
Lymph node metastasis (N stage) (I)	Tumor grade, well-differentiated (IIA)	Tumor gross configuration (IV)
Blood or lymphatic invasion (I)		
Residual tumor following surgery with curative intent (R stage) (I)		
Residual tumor in margin after neoadjuvant chemotherapy (IIA)		
Signet-cell histology (IIB)		
Small-cell histology (IIB)		
Serological factors		
Elevated carcinoembryonic antigen levels preoperatively (I)		Tumor-associated proteins or carbohydrates (III)
Elevated postoperative carcinoembryonic antigen levels (I)		
Molecular factors		
18q loss (IIB)	MSI (IIB)	
p53 gene mutation (III)		
Thymidylate synthase overexpression (III)		
KRAS mutation (III)		

a Parentheses are American College of Pathology categories.
MSI, microsatellite instability.

statement of the College of American Pathologists [453] rates many of these as:

- Category I – definitely proven and generally used in patient management
- Category IIA – repeatedly shown to have value and should be noted in the patient report but need validation in statistically robust studies
- Category IIB – promising but present investigation lacks sufficient data for clinical use
- Category III – indeterminate value
- Category IV – well studied with no value.

Clinical factors

At diagnosis of colorectal cancer, asymptomatic patients have a better 5-year survival rate than symptomatic patients, 71% vs 49%, respectively [323,324]. Also, patients presenting with bowel obstruction or bowel perforation have a statistically significantly poorer prognosis than those patients without these symptoms as adjusted for Dukes stage. Other presenting complaints, including nausea and vomiting, change in bowel habits, or abdominal pain, are not independent prognostic factors [317]. Patients with stage II or stage III colorectal cancer and diabetes mellitus have a 42% increased risk of death from any cause, and a 21% increased risk for cancer recurrence compared with those without diabetes [454]. Long debated has been the influence of blood transfusion on colorectal cancer, thought to cause increased tumor recurrence as a consequence of immunosuppression of the patient. However, data reveal no impact on disease recurrence with blood components/red blood cell transfusions [455].

Pathological factors

Among all prognostic factors, the pathological TNM stage of colorectal cancer at diagnosis is the most important [456]. The local extent of the tumor (T), regional lymph node metastasis (N), blood or lymphatic vessel invasion, and residual tumor following surgery with curative intent are category I factors. With increasing pathological stage comes decreasing 5-year survival rate (Table 65.14). Also, interaction between tumor invasion and lymph node positivity with regard to prognosis occurs. In patients with one to three lymph nodes positive on pathological review (N1), the depth of invasion influences prognosis. Stage IIIA (T1/T2, N1) patients have a better 5-year survival (59.8%) than stage IIIB (T3/T4, N1) patients (42.0%). Moreover, those with stage IIIC (any T, N2) with 4–12 lymph nodes positive have a poorer 5-year survival (27.3%) regardless of tumor invasion compared with those with less lymph node involvement [456]. The accuracy of staging is related to the number of lymph nodes reviewed; high miss rates of lymph node metastasis occur if fewer than 15 nodes are examined. Consequently, pathology societies recommend that at least 12 lymph nodes be examined to provide accurate staging [457]. Lymph nodes are examined by microscopic review after traditional hematoxylin–eosin staining. More sophisticated evaluation for micrometastases by immunohistochemistry or molecular techniques is not recommended [456]. The prognostic importance of tumor involvement with blood or lymphatic vessel invasion is well documented with respect to malignant polyps, and found to be associated with regional lymph node involvement. Also, microscopic residual tumor left after attempted curative surgery is highly predictive of recurrence.

Histological features found to have value but needing validation are histological grade and type. The assessment of histological grade (well differentiated, moderately differentiated, poorly differentiated, and undifferentiated) is hampered by lack of a uniform grading system. Certain histological types of tumors, including signet ring [458] and small cell tumors [459], carry an adverse prognosis. In a review of 2995 serial colorectal cancers, signet cell pathology was found in 1.1% of cancers, and the 5-year survival of these patients was only 9.1% [458]. By contrast, the histology noted with MSI-H tumors with intratumoral lymphocytes, peritumoral lymphoid reaction, and transmural Crohn's-like lymphoid reaction is associated with a more favorable prognosis [460]. Interestingly, the tumor size and location are not of prognostic significance.

Serological factors

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen is a high molecular weight glycoprotein with multiple subtypes consisting of a series of immunoglobulin-like domains [461,462]. It has many putative functions including cell adhesion, immunity, and apoptosis, and is thought to enhance metastatic potential [462,463]. The initial study of CEA touted this compound as a serum marker with high sensitivity and specificity for colorectal cancer [463]. Subsequent reports noted a sensitivity for Dukes A and B colorectal cancer of 30%–40%, and estimated that for every valid colorectal cancer detected by CEA analysis there would be 250 false-positive tests [464]. Consequently, multiple consensus committees have recommended against using CEA as a screening test for colorectal cancer [465,466]. Likewise, CEA is not a diagnostic test for colorectal cancer, again because of low sensitivity and elevation of this marker in patients with liver disease and normal cigarette smokers.

Although not beneficial as a screening or diagnostic test, CEA levels are useful for assessing prognosis and postoperative surveillance. Colorectal cancer patients with high preoperative levels of CEA have a worse prognosis than those with low levels independent of tumor staging [467,468]. The recurrence rate of colorectal cancer was higher in those with CEA levels greater then 5 ng/mL, with a linear inverse correlation between preoperative levels (2–70 ng/mL) and time to recurrence (30–9.8 months) with Dukes B and C lesions. Also, two prospective studies confirmed the prognostic value

of CEA in Dukes B (stage II) colorectal cancer patients [461,469]. In one large investigation of 572 node-negative patients (T1,2,3, or 4, N0, M0) multivariate analysis concluded that the preoperative CEA level independently predicted survival. Consequently, high CEA preoperative levels may define a subset of stage II patients with poor prognosis that benefit from adjuvant chemotherapy. Also, the failure of an elevated preoperative CEA level to return to normal within 6 weeks after surgery suggests residual or metastatic disease [470]. Finally, postoperative surveillance with serial monitoring of CEA detects recurrent or metastatic disease with a sensitivity of about 80% and provides an average lead time of 5 months. Metaanalysis of intensive versus nonintensive postoperative follow-up in which only CEA and physical examinations were utilized in the intensive arm, revealed significantly better survival in the intensively followed group [471]. Most consensus groups examining this issue recommend CEA testing every 2–3 months for the first 3 years after surgery and then every 6 months for the next 2 years [466,472]. A CEA rise of 30% confirmed with repeat testing in 1 month or rises of 15%–20% persisting over 3 months are considered significant and an indication for prompt work-up for recurrent disease.

In summary, CEA for prognosis of colorectal cancer is rated as a category I factor by the College of American Pathologists [453] and recommended by the American Society of Clinical Oncology [473] and the European Group on Tumour Markers (EGTM) [472]. The latter two groups recommend CEA for postoperative surveillance for recurrent cancer. No consensus group recommends CEA levels for screening or diagnosis of colorectal cancer.

Others

A series of other serum markers have been investigated as prognostic markers in colorectal cancer. These include cancer antigen (CA) 19-9, CA 242, CA 72-4, CA 50, TPA (tissue polypeptide antigen), TPS (tissue polypeptide-specific antigen), and tissue inhibitor of metalloproteinase 1 (TIMP-1). Insufficient data exist to recommend the clinical use of these for the detection of early colorectal cancer or for prognosis or surveillance of recurrent tumor.

Molecular factors

Microsatellite instability (MSI)

Microsatellites are regions of DNA in which short sequences of nucleotides, most often dinucleotides, are commonly repeated. Microsatellite instability is the phenomenon of gain or loss of repeated nucleotides in these microsatellite regions. The presence of high-frequency microsatellite instability (MSI-H) (instability at 40% or greater of microsatellite loci tested) in the DNA of a colorectal cancer but not seen in the adjoining normal colonic mucosa can suggest functional loss in one of the MMR genes. This can result from inactivation of both alleles of the gene by a combination of coding region mutations, LOH, or promoter methylation. Microsatellite instability is noted in about 20% of patients with sporadic colorectal cancer [474], and in 90% of those with HNPCC. In the former, MSI is usually caused by somatic methylation of the promoter region of the *MLH1* gene, and in the latter by germ-line mutation of one of the MMR genes.

Patients with MSI-H colorectal cancers have better survival adjusted for stage than those with microsatellite-stable tumors [460,475,476]. In a study of 607 patients with colorectal cancer (17% with MSI-H tumors), MSI was an independent prognostic factor of survival; overall 5-year survival was 22% greater for those with MSI-H compared with those with microsatellite-stable (MSS) tumors; moreover, this improved outlook applied to patients at any stage of disease [460]. The association between tumor MSI status and response to chemotherapy is unclear. Four of six published studies correlating microsatellite status and adjuvant chemotherapy report improvement in overall survival in MSI-H patients with 5-FU-based therapy, but questions have been raised concerning the methodology in some of these reports [477–480]. By contrast, one trial analyzing 570 tissue blocks from patients with stage II and stage III colorectal cancer found that 5-FU-based adjuvant chemotherapy benefited patients with MSS or MSI-low tumors but not MSI-H malignancies [481] (17% lower 5-year survival in patients with MSI-H tumors taking adjuvant chemotherapy compared with patients not receiving chemotherapy). With respect to patients with stage IV disease, two retrospective studies report higher response rates in patients with MSI-positive tumors [482,483]. In summary, MSI is considered a prognostic factor with promise but there are insufficient data for it to be used in patient management (category IIB).

Chromosome 18q

Alterations of chromosome 18q (location of the *DCC*, *SMAD2*, and *SMAD4* genes) by allelic loss or LOH appear important in the clinical outcome of colorectal cancer patients. In stage II colorectal cancer the status of 18q appears to have strong prognostic value. The prognosis in those with chromosome 18q allelic loss is similar to stage III cancer, and therefore these patients may benefit from adjuvant chemotherapy. By contrast, those without loss at 18q have a survival rate similar to stage I disease and may not benefit from treatment [484]. In stage III patients with MSS tumors the 5-year overall survival rate after 5-FU-based chemotherapy was almost three-fold higher in patients with tumors with LOH of 18q compared with those with retained 18q alleles [485]. The College of American Pathologists considers LOH of 18q a category IIB prognostic factor.

p53 gene

The *p53* tumor suppressor gene located on chromosome 17p is frequently somatically mutated (40%–60%) in sporadic colorectal cancers. In the presence of cell damage, the p53

protein functions to activate DNA repair or, if not possible, to eliminate the damaged cell by apoptosis. In the pooled analysis of 28 studies, *p53* overexpression in patients undergoing surgery alone appeared to be a significant factor predicting poorer prognosis compared with those having wild-type *p53* function [486]. Mutation of *p53* has also been associated with a higher risk of cancer-related death in stage III colorectal cancer patients treated with 5-FU-based chemotherapy [487]. Mutation of *p53* is considered a category III prognostic factor.

Thymidylate synthase

Thymidylate synthase (TS) is a target enzyme of 5-fluoropyrimidines. Inhibition of TS leads to interference with DNA synthesis and repair and induction of DNA strand breaks. The promoter region of the TS gene has tandem repeated regulatory sequences that are polymorphic in humans and appear to affect the tissue expression of this enzyme [488]. A number of preclinical studies report that overexpression of TS correlates with insensitivity to 5-FU. However, studies in the clinical setting have been inconsistent regarding the ability of TS expression to predict response of patients to 5-FU-based therapy used in adjuvant or metastatic treatment [489]. Additional study with attention to assay methodology and patient group may further clarify the value of this marker (category III).

Other molecular markers

Because of conflicting evidence, the value of *KRAS* mutations in predicting survival and response to adjuvant treatment is unclear. Other factors such as DNA content, ploidy, or proliferative indices of the tumor are factors not yet sufficiently studied to determine prognostic value (category III).

Other malignant large bowel cancers

Malignancies other than adenocarcinomas rarely arise from the large bowel and account for less than 3% of colorectal tumors. The most common are carcinoid, lymphoma, and Kaposi sarcoma. Others include leiomyosarcoma, melanoma, squamous cell cancer at the anorectal junction, and cloacogenic cancers. Carcinoid tumors most commonly occur in the appendix, rectum, and cecum as mucosal or submucosal nodules, several millimeters in size, and pursue an indolent course (see Chapter 54). Kaposi sarcoma in those with human immunodeficiency virus (HIV) disease manifests as red- or violet-colored mucosal lesions (see Chapter 104).

Colorectal lymphoma is rare, representing only 0.9%–1.2% of all colon cancers and occurring in about 14% of all cases of gastrointestinal lymphoma [490–492]. Abdominal pain and weight loss are the most common presenting complaints, occurring in 40%–62% of cases; approximately 50% of patients have a palpable mass on abdominal examination [493]. Findings on CT scan can include either discrete bulky masses, focal mural infiltration, or diffuse colonic invasion [494]. The most common site of colorectal lymphoma is the cecum (73%), followed by the rectum. Histological review shows that most patients (40%–60%) have either diffuse large B-cell lymphoma or Burkitt lymphoma. Additional reports note an increase in incidence of other subtypes including peripheral T-cell, indolent (mucosa-associated lymphoid tissue, or MALT), and Hodgkin lymphoma. Of note, the lymphoma subtype called mantel cell lymphoma has been associated with an 88% rate of colonic involvement, prompting the need for colonic evaluation in these patients [495].

An increased risk of gastrointestinal lymphoma is noted in several autoimmune disorders including Sjögren syndrome, rheumatoid arthritis, systemic lupus erythematosus, Wegener granulomatosis, congenital immune deficiency syndromes, and AIDS. Patients with organ transplants and subsequent immunosuppressive therapy have an increased risk of lymphoma, which more often presents as a single discrete lesion than diffuse colonic involvement [496].

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66 Anorectal diseases

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Anorectal examination

A careful anorectal examination is an important part of the gastrointestinal evaluation, particularly in patients with constipation or anorectal complaints. Step-by-step explanation, reassurance, and gentle technique help to minimize patient embarrassment and discomfort. The patient should be placed in the left lateral decubitus position with the hips and knees flexed and the buttocks protruding just beyond the edge of the examining table. The prone jackknife position is ideal but requires a specialized examining table and places the patient in a somewhat unfamiliar position. The buttocks, sacral region, and thighs are observed for signs of pilonidal disease, dermatological conditions, and infections. The buttocks are then firmly retracted with both hands to permit inspection of the perianal region. Fistulous openings, fecal or mucus soiling, and excoriations or chronic skin changes give clues to underlying disease processes. Anal pathology such as tumors, skin tags, anal fissures, and prolapsing hemorrhoids should be described with regard to their anatomical location.

Perianal digital palpation may reveal induration, tenderness, or the cord of a fistulous tract. Thereafter, the lubricated index finger is gently inserted into the anus. Anal resting tone can be assessed when the patient relaxes after insertion of a finger into the anal canal. A patulous sphincter raises the possibility of neurogenic injury. Puborectalis function is assessed by palpating the muscle with the ventral aspect of the examining finger; when patients squeeze (or contract) this muscle, the examining finger is lifted upward and anteriorly. Conversely, when subjects are asked to expel the examining finger, the puborectalis should relax and the perineum should descend by approximately 2–3 cm. The digital examination should also evaluate for rectal polyps, tumors, feces, and foreign bodies. The presacral area, the prostate, and even the cul-de-sac region may be palpated. The impression of the cervix or an indwelling tampon on the anterior rectum should not be confused with pathology. The anal "wink" reflex is assessed by observing for sphincter contraction (i.e., a "wink"), in response to gentle stroking of the perianal region or to perianal pinprick.

Findings in fecal incontinence may include reduced anal resting tone, weak squeeze pressures or a reduced puborectalis "lift" when patients squeeze this muscle. Defecatory dysfunction may be suggested by reduced (i.e., < 2 cm) or increased (> 4 cm) perineal descent during simulated defecation. Moreover, the puborectalis or anal sphincter muscle may contract (i.e., dyssynergia) or fail to relax when patients try to expel the examining finger. The anal "wink" reflex may be absent in patients with a large fecal impaction, but persistence of this finding after disimpaction suggests nerve impairment. If the history or examination raise the possibility of a rectal prolapse, pouch of Douglas hernia, or excessive perineal descent that is not apparent in the lateral recumbent position, patients should be examined in the seated position on a commode. Occasionally, adequate examination is impossible except under anesthesia.

Anoscopy is the best means of examining the anal canal. Fistulous openings and anal canal lesions can be identified, and internal hemorrhoids may be visible just above the dentate line, particularly when the patient is asked to bear down. Fissures and perianal lesions may be appreciated as the instrument is withdrawn below the dentate line. Rigid

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proctosigmoidoscopy completely evaluates the distal sigmoid colon and rectum. The rigid instrument is particularly useful for preoperative documentation of rectal cancer, obtaining large-bite rectal biopsy specimens for diagnosis of Hirschsprung disease, removal of foreign bodies, and evaluation of the mucosa if fiberoptic or video endoscopic equipment is unavailable. For most other indications, the flexible instrument is preferred.

Hemorrhoids

Anatomy and epidemiology

Hemorrhoids result from dilation of the superior and inferior hemorrhoidal veins. These veins form hemorrhoidal plexuses, or cushions, in the submucosal layer of the lower rectum and are essential to the normal function of the anal canal. In the United States, estimates of symptomatic hemorrhoids range from 4.4% of the general population to as high as 50% of adults over the age of 50 years [1]. Symptoms of constipation (i.e., excessive straining at defecation, sense of incomplete evacuation, and repeated toilet visits), but not self-defined constipation, are associated with hemorrhoidal disease [2,3].

Hemorrhoids are either internal or external. Internal hemorrhoids arise from the superior hemorrhoidal venous plexus above the mucocutaneous junction, or dentate line, of the anorectum. They are covered with rectal mucosa and occur in three primary locations: right anterior, right posterior, and left lateral, although anatomical variability is common. The end branches of the superior and middle hemorrhoidal arteries terminate in the submucosa above the dentate line, with an anterior and posterior branch on the right and a single lateral branch on the left. The right posterior and left branches give off two end branches to form potentially secondary hemorrhoids. External hemorrhoids arise from the inferior hemorrhoidal venous plexus below the mucocutaneous junction and are covered by squamous epithelium. The perianal squamous epithelium contains numerous pain receptors, so that thrombosis of external hemorrhoids causes significant pain. Internal and external hemorrhoidal plexuses freely communicate to drain into the inferior vena cava through the internal pudendal veins.

Pathogenesis

Histologically, hemorrhoids consist of three important parts: the lining (rectal mucosa or squamous epithelium), stroma (blood vessels, smooth muscle, supporting connecting tissue), and anchoring connective tissue, which secures hemorrhoids to the sphincter apparatus. With age or other aggravating factors, the anchoring and supporting connective tissue deteriorates, causing hemorrhoids to bulge and descend, leading eventually to symptoms [4]. This sequence of events is supported by the increased incidence of hemorrhoids in patients with chronic constipation, diarrhea, pregnancy, or pelvic tumors – conditions that chronically or recurrently increase pelvic venous pressure. In some individuals, the internal anal sphincter becomes hypertrophic and the anal outlet becomes functionally narrowed. During straining, the fecal bolus acts as an obturator in forcing the hemorrhoidal cushions to descend through the hypertrophic sphincter, enlarge, and become symptomatic.

Definitions

External skin tags are redundant folds of skin that arise from the anal verge when thrombosed external hemorrhoids resolve spontaneously. Internal hemorrhoids may be classified as follows:

- Grade I hemorrhoids bulge into the lumen of the anorectal canal on anoscopy but do not prolapse.
- Grade II hemorrhoids prolapse out of the anus with defecation or straining but reduce spontaneously.
- Grade III hemorrhoids prolapse with defecation or straining and require manual reduction.
- Grade IV hemorrhoids are irreducibly prolapsed and are at risk for strangulation [5].

The clinical presentations and recommended therapies for these groups of hemorrhoids are discussed later.

Diagnosis and treatment

External skin tags

Following thrombosis of an external hemorrhoid, the overlying skin becomes redundant and this excess skin remains after the underlying clot resolves. Skin tags may also arise de novo in cases of inflammatory bowel disease. Symptoms include a palpable growth near the anus and difficulty with anal hygiene, which may be associated with pruritus ani. Skin tags are relatively easy to distinguish from anal cancer or condylomata acuminata by their gross appearance as normal skin and their soft, fleshy texture on palpation. Treatment is conservative and surgical excision is considered only in cases of poor hygiene or because of patient anxiety.

External hemorrhoids

Thrombosis of an external hemorrhoid can cause extreme pain due to distention of overlying perianal skin and inflammation associated with the process of thrombosis. Bleeding may occur after the overlying perianal skin ulcerates and the resolving liquefied hematoma extravasates. External hemorrhoids should be distinguished from strangulated internal hemorrhoids, which tend to be larger and more circumferential, encompassing the entire anus.

Many thrombosed external hemorrhoids can be treated with warm sitz baths two to three times per day. Stool-softening agents, such as psyllium, synthetic mucilloides, and the sodium or calcium salts of dioctyl sulfosuccinate, can minimize straining at stool and prevent aggravation of the pain and thrombosis [5]. Topical anesthetic ointments and witch hazel-impregnated pads may provide additional relief.

Attribution of clinical improvements to medical therapy must be tempered by the knowledge that most thrombosed external hemorrhoids resolve within 48–72 h.

If pain is severe and the patient is seen within 72 h of symptom onset, surgical evacuation or excision of the thrombosed external hemorrhoid can be performed under local anesthesia or occasionally in the operating room; it offers the most rapid relief from symptoms, a lower incidence of recurrence, and longer remission intervals than conservative therapies [6]. After the thrombus has organized or if more than 72 h have elapsed, it cannot be evacuated and conservative therapy is required.

Internal hemorrhoids

Internal hemorrhoids may be associated with discomfort, pruritus ani, fecal soiling, or varying degrees of prolapse. Bleeding, however, is the typical complaint that brings patients to the physician. Hemorrhoidal bleeding is described as bright-red spotting on the toilet tissue or dripping into the toilet bowl. It most often occurs at the end of defecation and is separate from the stool. Rarely, acute severe bleeding requires transfusion and, occasionally, ongoing chronic losses cause iron deficiency anemia [7]. Importantly, hemorrhoids should not be considered the source of hematochezia until other potential bleeding sources in the colon and rectum have been investigated. With the possible exception of a young patient with a bleeding pattern typical of hemorrhoids, a flexible sigmoidoscopy or, if clinically appropriate, a full colonoscopy should be performed. Occult bleeding should never be attributed to hemorrhoids.

Prolapse of hemorrhoids manifests along a continuum of symptoms ranging from difficulty with anal hygiene to painful strangulation. Prolapsed hemorrhoids should be distinguished from rectal mucosal prolapse and from prolapse of rectal polyps or cancer. Sigmoidoscopy with biopsy of suspicious lesions should be carried out if appropriate. Anal condylomas and anal cancer are easily differentiated from prolapsing internal hemorrhoids, and thrombosed external hemorrhoids cause pain as a presenting symptom.

All degrees of internal hemorrhoids may be associated with mild discomfort, but only strangulated hemorrhoids cause significant pain. Strangulation occurs secondary to prolapse with subsequent lack of blood supply. Progression to gangrene with resultant infection is life threatening and will occur if immediate surgical therapy is not instituted. In contrast to complete rectal prolapse, strangulated hemorrhoids lack concentric mucosal folds and cannot be reduced.

Topical agents are effective for most first- and second-degree hemorrhoids, including those that bleed intermittently. A high-fiber diet and adequate fluid intake helps to promote passage of soft bulky stools to minimize straining [5]. Some patients benefit from the addition of hydrophilic bulk-forming agents such as psyllium extracts or mucilloids. Excessive and prolonged defecatory straining should be discouraged. Many patients also benefit from warm sitz baths twice daily and attention to proper anal hygiene. A number of proprietary agents, such as suppositories, ointments, and hydrocortisone or anesthetic-containing preparations (benzocaine, lidocaine, pramoxine [pramocaine], dibucaine) offer short-term relief from pruritus, burning, and soreness, although firm efficacy data are lacking. Purified flavonoids derived from citrus fruits or as a traditional medicine derived from the ginkgo tree have been reported to be effective for the short-term relief of acute bleeding [8]; they are advocated as a short-term bridge to more definitive therapies.

If conservative therapy does not suffice, a definitive procedure is necessary (Table 66.1). These are classified into office-based treatments and surgical hemorrhoidectomy. In general, grade I and II hemorrhoids can be treated without removal of redundant hemorrhoidal tissue, whereas grade III and IV hemorrhoids usually require a form of therapy that results in both thrombosis of the hemorrhoid and removal of redundant tissue.

Rubber-band ligation

First described as a rapid, simple, effective device for the treatment of internal hemorrhoids in the outpatient setting [9], rubber-band ligation (RBL) is associated with both

Table 66.1 Nonoperative procedures for internal hemorrhoids

Modality	Grade	Advantages	Disadvantages
Rubber band ligation (RBL)	I, II, some III	Lowest recurrence rate Removes redundant tissue	Discomfort. Rare local complications especially if immunocompromised
Injection sclerotherapy	I, II, some III	Minimal discomfort Technically easy	Rare local complications. Poor long-term benefits
Infrared coagulation	I, II, III	Minimal discomfort	Several applications required. More expensive than RBL
Laser photocoagulation	I, II, III	Minimal discomfort	Expensive
Electrocoagulation	I, II, III		Long application time. Recurrence rates: 25%–35%
Cryosurgery	I, II, III	None	Pain and complications common. Less patient satisfaction

thrombosis of the hemorrhoid and removal of redundant mucosal tissue. By inducing submucosal scarring, development of new hemorrhoidal tissue is prevented. This procedure is effective for refractory grade I hemorrhoids, all grade II, and selected grade III hemorrhoids. Grade IV hemorrhoids are not well treated by this method.

After an anorectal evaluation, the anoscope is inserted and a hemorrhoidal cushion is selected for band application. No anesthetic is required if care is taken to place the band at least 0.5 cm above the dentate line. Some authors recommend banding of only one column per session to minimize tissue necrosis, but up to four ligations may be performed with acceptable morbidity.

In a metaanalysis of 18 prospective randomized trials, RBL was the most effective of the office-based procedures, with a lower recurrence rate than other procedures without anesthesia [10]. The ability to perform RBL in the clinic setting makes this a popular treatment option for patients with hemorrhoids. Inexpensive, plastic, single-handed ligators have been developed to capture the hemorrhoidal tissue for band placement [11,12]. A Cochrane review concluded that RBL is the treatment of choice for grade II hemorrhoids, while reserving hemorrhoidectomy for grade III hemorrhoids or for recurrences after RBL [13].

Injection sclerotherapy

The use of sclerosants, such as sodium morrhuate or sodium tetradecyl sulfate, is an accepted treatment for grade I and II bleeding hemorrhoids. The sclerosant is injected into the submucosal space but not into symptomatic hemorrhoids. An intense inflammatory reaction results in fixation of the mucosa to the underlying muscle, thus obliterating the submucosal layer where hemorrhoids form. Complications are mucosal slough, prostatic infection, contact hypersensitivity, and rectal infection. A refinement of sclerotherapy uses a flexible endoscope to inject saline through a sclerotherapy needle into the hemorrhoidal cushion. Sclerotherapy provides good short-term benefits but with long-term benefits in only 28% of patients [14]. Thus, RBL appears to be a superior choice for grade II hemorrhoids.

Cryosurgery

Special cryoprobes activated by liquid nitrogen, carbon dioxide, or nitrous oxide have been used to destroy local tissue by freezing and subsequent necrosis. If deep freezing of the submucosal hemorrhoidal cushions does not occur, symptoms persist. Tissue damage is uncontrolled and wound healing is accompanied by prolonged anal drainage, late bleeding, and pain. Compared with RBL, patient satisfaction is less and local complications more frequent [5].

Electrocoagulation

Both direct and bipolar current are particularly useful in grade I, II, and III hemorrhoids. The advantage of bipolar current (Bicap) is that applications last only several seconds (vs 8–10 min with direct current) and thermal injury to remote sites is avoided. Both techniques are associated with minor complication rates of 10% and recurrence rates of 25%–35% [15].

Photocoagulation

Infrared light and lasers have been used to treat symptomatic hemorrhoids by stimulating fibrosis of the submucosal layer to cause tissue coagulation and necrosis. Advocates of photocoagulation cite the lack of electrical contact with the patient's body and a more controlled application of destructive force compared with electrocoagulation. Infrared devices are much less expensive than laser devices but both are more expensive than equipment for RBL or injection sclerotherapy. The incidence of discomfort and complications compares favorably with those of RBL and injection sclerotherapy. Rubber-band ligation produces more posttreatment pain than infrared photocoagulation, but fewer patients are likely to need retreatment for symptomatic recurrences, especially in the presence of substantial prolapse.

Hemorrhoidectomy and hemorrhoidopexy

Fewer than 10% of patients with symptomatic hemorrhoids require surgical intervention. Hemorrhoidectomy is reserved for patients who relapse or fail office procedures, as well as most grade III and all grade IV and strangulated hemorrhoids. Hemorrhoidectomy is the most effective of available treatments but has the highest complication rate and the most postoperative disability [5].

The development of stapled hemorrhoidopexy [16] has been a major advance in the treatment of symptomatic internal hemorrhoids. Its advantages include less postoperative pain, shorter recovery times, and long-term outcomes similar to those of surgical hemorrhoidectomy [17,18]. It is therefore considered by some to be the procedure of choice for symptomatic internal hemorrhoids and prolapse [5]. It is not effective for large external hemorrhoids and should be avoided in patients who engage in anoreceptive intercourse, because staples occasionally remain in the area and may cause discomfort [19].

Ablation of the internal anal sphincter

Two strategies have been employed to decrease the abnormally high resting anal pressure found in some patients with hemorrhoids. Forceful anal dilation under general anesthesia (Lord procedure) causes variable and uncontrolled damage to the anal sphincters with resulting fecal incontinence. It is less optimal than the more controlled disruption achieved with lateral internal sphincterotomy (see "Anal fissure" below.)

Anorectal varices

Histological studies have shown that anorectal varices and hemorrhoids are unrelated [20]. Rectal varices represent

enlarged portosystemic collaterals that correlate with portal hypertension and develop from hepatofugal portal venous flow through the inferior mesenteric vein to the superior hemorrhoidal veins [21]. Rectal varices represent the communication between the superior hemorrhoidal veins (portal circulation) and the middle and inferior hemorrhoidal veins (systemic circulation), which in turn connect by the femoral vein to the inferior vena cava. The distinction of anorectal varices from external hemorrhoids may be difficult. Often, patients with anorectal varices have other manifestations of portal hypertension such as esophageal varices and ascites. Anorectal varices are usually discrete, serpentine, submucosal veins, which, in contrast to external hemorrhoids, are compressible and refill rapidly. They extend from the squamous portion of the anal canal across the dentate line and into the rectum. Nearly 45% of patients with cirrhosis have anorectal varices on endoscopic examination, and the prevalence increases to 75% using rectal endosonography [22]. Bleeding may occur from any portion of the varix and can be massive and life threatening [23].

The optimal management of anorectal varices is unknown. Treatment by underrunning the variceal columns with absorbable suture achieves primary control in most cases and has a low rate of morbidity [23]. Inferior mesenteric vein embolization or ligation has been reported [24]. Ultimately, surgical or transjugular intrahepatic portosystemic shunting may be required [25,26].

Anorectal abscess and fistula

Anorectal abscess and fistula represent the acute and chronic manifestations of suppurative anorectal infection, respectively. An anorectal abscess is an undrained collection of perianal pus, whereas a fistula is an abnormal communication between the anorectal canal and the perianal skin. In most cases, the underlying pathophysiology is thought to be the same and the treatment is essentially surgical.

Etiology and differential diagnosis

The most common cause of anal sepsis is an infection of the anal glands and ducts, perhaps secondary to fecal bacterial plugging of the ducts and obstruction (i.e., the cryptoglandular theory). These glands arise from the anal canal at the level of the crypts of Morgagni, and often penetrate into the intersphincteric space (Fig. 66.1) [27]. Anal trauma, diarrhea, hard stools, or foreign bodies may predispose to glandular obstruction or sepsis. Anal sepsis most frequently affects people in the third and fourth decades [28].

The most common bacterial isolates are *Escherichia coli*, *Enterococcus* species, and *Bacteroides fragilis*. Histological specimens from patients with anorectal fistula revealed infected anal glands 70%–90% of the time. Other conditions associated with anal sepsis include Crohn's disease, malignancy, tuberculosis, actinomycosis, lymphogranuloma venereum, radiation-induced proctitis, leukemia, and lymphoma. Other disease states in the differential diagnosis of suppurative anorectal conditions are infected presacral epidermal inclusion cysts, hidradenitis suppurativa, pilonidal disease, and Bartholin abscesses.

Classification

Anorectal abscesses may be classified according to the site of the suppurative process. Perirectal abscesses inferior to the puborectalis and levator ani are classified as low intermuscular abscesses (i.e., perianal, submucosal, intersphincteric, and ischiorectal abscesses) [29]. Deep postanal and supralevator abscesses are categorized as high intermuscular abscesses. A suppurative process may involve multiple perirectal spaces. For example, the classical "horseshoe" abscess originates in the posterior midline and extends through the intersphincteric and deep postanal spaces to one or both ischiorectal spaces.

The Parks classification system is derived from the cryptoglandular hypothesis and is the system most widely used to classify anorectal fistulae [29] (Fig. 66.1). Based on the course taken by the main tract, fistulae are characterized as inter-, trans-, supra-, or extra-sphincteric. The most common type is intersphincteric, in which the fistula ramifies between the internal and external anal sphincters. Transsphincteric fistulae pass from the intersphincteric plane through the external sphincter and into the ischiorectal fossa. Suprasphincteric fistulae pass upward in the intersphincteric plane, over the puborectalis muscle, and into the ischiorectal fossa. Extrasphincteric fistulae pass from the perianal skin through the ischiorectal fat and levator muscles into the rectum. Division of the entire tract of either suprasphincteric or extrasphincteric fistulae results in total division of the muscles of continence.

Clinical manifestations

Anorectal abscess

Common symptoms include the slow, gradual onset of pain, increasing in intensity to the sensation of pressure and fullness. The pain may be exacerbated by sitting, movement, or defecation. Constitutional symptoms include malaise and fever. Approximately 20%–33% of all patients will have had a previous episode of anorectal sepsis [30].

The physical findings depend on the location of the abscess. An examination under general anesthesia may be necessary to confirm the diagnosis. A perianal abscess generally manifests with localized swelling, hyperemia, induration or fluctuance, and perianal tenderness. Purulent discharge reflects spontaneous drainage. Patients may have fever or malaise, but usually do not have systemic symptoms. Point tenderness and poorly demarcated induration are the most frequent findings. Because of profound granulocytopenia, fluctuant masses are not usually seen in leukemic patients

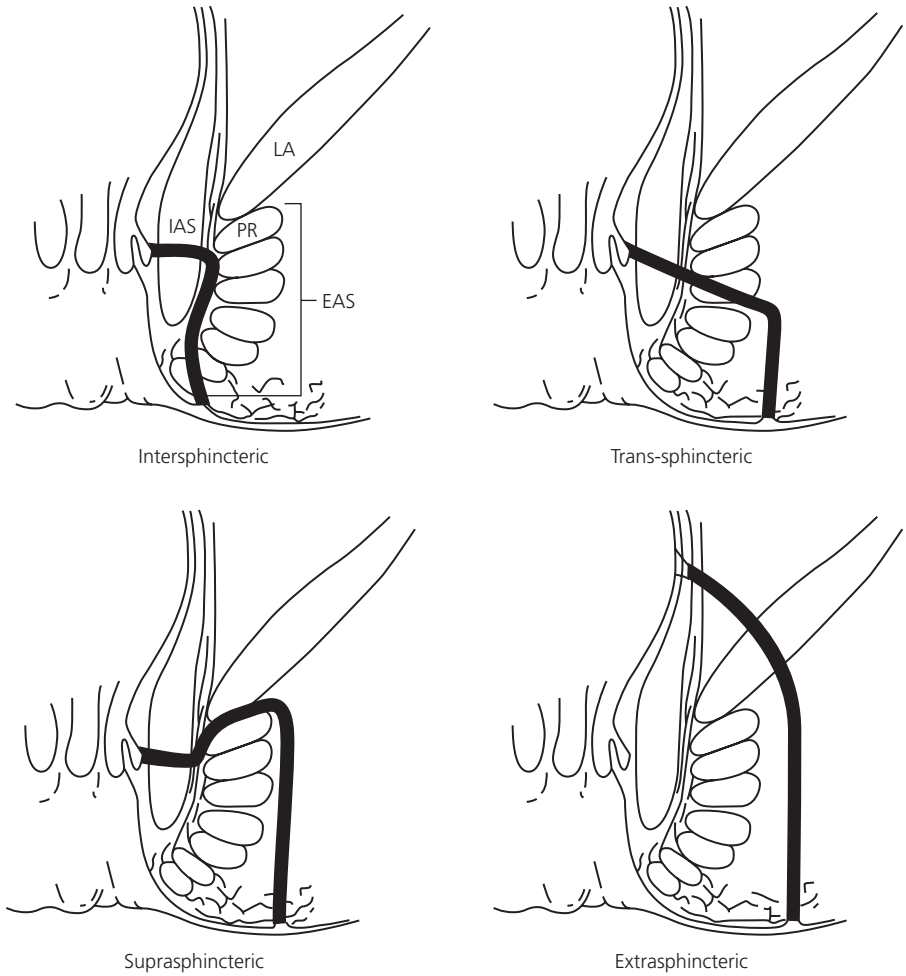

Figure 66.1 Classic location of several fistulas and their relationship to pelvic musculature depicted schematically. Anorectal anatomy: PR, puborectalis; LA, levator ani; IAS, internal anal sphincter; EAS, external anal sphincter.

receiving chemotherapy. Necrosis and tissue breakdown may proceed rapidly with extension into the genitalia and pelvis.

Ischiorectal abscesses may present as small localized swellings. More frequently, the ischiorectal space may accumulate a large volume of purulent material manifest as an erythematous and indurated mass in the buttock. Fever and leukocytosis are common. A large ischiorectal abscess frequently represents a horseshoe extension.

Supralevator abscesses may be caused by an upward extension of a collection in the distal anal canal, or a pelvic abscess secondary to intraabdominal or pelvic pathology (i.e., diverticulitis, appendicitis, or pelvic inflammatory disease). The patient may be systemically ill. A pelvic mass may be identified by rectal or vaginal examination.

Intersphincteric and postanal abscesses may be difficult to diagnose because they are frequently not associated with inflammatory skin changes. An examination under general anesthesia may be required to diagnose these abscesses, particularly because intersphincteric abscesses cause considerable discomfort.

Anorectal fistulae

Patients usually present with intermittent or persistent purulent or serosanguinous discharge from an external opening in the perianal area. The classical symptoms are increasing pain, slight fever, and pain on defecation followed by mucopurulent drainage and a reduction in pain. Chronic discharge may irritate the skin and cause pruritus. Most patients have a previous history of anorectal suppuration.

Fistulous tracts are fibrous inflammatory tubes lined by granulation tissue, with a diameter of 5–7 mm. The assessment should characterize internal and external openings, the course of primary and any secondary tracts, and presence of underlying disease. The external opening is typically a small, perhaps draining pit located close to (i.e., for intersphincteric tracts) or further away from (i.e., for transsphincteric tracts) the anal verge. Multiple external openings suggest multiple complex tracts, often caused by Crohn's disease or hidradenitis suppurativa. The internal opening may be felt as an indurated nodule, most often at the dentate line. Most fistulae can be delineated by gentle probing with anorectal probes

or crypt hooks, preferably in the operating room, and with caution to avoid creation of false tracts, which can complicate evaluation and management.

Diagnostic tests

All patients with anorectal fistulae should undergo sigmoidoscopy to identify the internal opening and associated disease (i.e., inflammatory bowel disease or neoplasms).

Pelvic magnetic resonance imaging (MRI), endoanal ultrasound, and surgical examination under anesthesia were of comparable accuracy (87%–91%) for classifying perianal fistulae in a study of 34 patients with perianal Crohn's disease [31]. When any two modalities were combined, the accuracy increased to 100%. Whereas the 7-MHz linear scanning probe is particularly useful for imaging perianal fistulae, radial scanning provides complementary information and rarely may reveal fistulae not identified by the linear probe. For MRI, the phased-array coil provides better resolution than the body coil. The choice among these modalities is primarily guided by the need for a surgical therapeutic intervention. If, as is often the case, surgical intervention is required, then surgical evaluation combined with ultrasound or MRI is appropriate. The choice between ultrasound and MRI depends on local institutional expertise. When the suspicion of a perianal lesion is low, combining ultrasound and MRI may prevent an invasive exam under anesthesia.

Imaging of perianal fistulae with fistulography in patients with fistula-in-ano, or with pelvic computed tomography (CT) in patients with Crohn's disease, is not sufficiently accurate to be clinically helpful.

Treatment

Anorectal abscesses are surgical emergencies and should be drained expeditiously. Depending on the patient's condition or preferences, this can be done in the office, emergency room, or operating room. The need for drainage is underscored by the observation in one series that the time interval from onset of primary anorectal abscess to necrotizing anorectal infection was up to 5 days, with mortality in excess of 50% [32]. Patients who are neutropenic are particularly susceptible to serious anorectal infection; early diagnosis and aggressive surgical drainage may be life-saving, with improvement in mortality rates to below 10% [33,34]. With the exception of intersphincteric and postanal abscesses with horseshoe extensions, anorectal abscesses may be drained by a simple drainage procedure or by a fistulotomy. Perianal and ischiorectal abscesses are drained externally, whereas submucosal and intersphincteric abscesses are drained internally. For postanal abscesses with horseshoe extensions or intersphincteric abscesses, the drainage technique involves sphincterotomy and fistulotomy in which the infected anal gland is destroyed. Drainage of supralevator abscesses is guided by the track taken by the abscess. Those that result from upward extension of an intersphincteric process should be drained transrectally. By contrast, supralevator collections resulting from cephalad extension of a transsphincteric fistula or an ischiorectal collection should be drained transperineally through the ischioanal fossae.

After a simple drainage procedure, the risk of a recurrent abscess and subsequently an anorectal fistula varies from 11% to 66% [35]. A primary fistulotomy might avoid the need for a second procedure in patients who develop a recurrent abscess or fistula after a simple procedure, with a slightly increased risk for fecal incontinence. Perhaps fistulotomy should be considered in patients who have a history of previous anorectal sepsis or who present with an ischiorectal abscess with an obvious internal opening. If the physician waits for an abscess to point or become ripe, he or she must realize that the risks of necrotizing infection and extension into previously uninfected spaces rise dramatically.

Antibiotics should be used as adjunctive therapy in special circumstances only (i.e., valvular heart disease, immunosuppression, extensive associated cellulitis, and diabetes). Antibiotics that provide gram-negative aerobic and anaerobic coverage (i.e., ticarcillin/clavulanate, piperacillin/tazobactam, or a broad-spectrum cephalosporin) are recommended. Wound isolates are usually polymicrobial with *Escherichia coli, Proteus vulgaris, Bacteroides* species, streptococci, and staphylococci predominating [36]. A high proportion of necrotizing anorectal infections contain *Clostridium* species [32]. Immunosuppressed patients often demonstrate *Pseudomonas aeruginosa* [34].

Anorectal fistulae should be treated surgically because they rarely heal spontaneously. Conservative surgical techniques are usually adequate to drain abscesses, reduce inflammation, and provide relief of symptoms [37]. Surgical diversion of the fecal stream, usually in conjunction with resection of diseased intestine or a local anorectal procedure, is used in selected instances (i.e., in patients with a rectovaginal fistula or complex multiple anal fistulae with considerable inflammation) [38].

For patients with Crohn's disease, medical measures intended to heal perineal fistulae and for more proximal lumenal disease are often used (see Chapter 85). The three essential aspects of surgical management for anorectal fistulae are fistulotomy, use of a seton, and endorectal advancement flaps. Most anorectal fistulae may be adequately treated by a fistulotomy. If the fistulous tract is distal to a majority of the external sphincter, the tract is opened by cautery. However, fistulotomy should be avoided if it is likely to damage a significant portion of the sphincter, and particularly for anterior fistulae. In these circumstances, or when there is an appreciable risk of incontinence or poor healing (i.e., patients with Crohn's disease, chronic diarrhea, incontinence, or compromised immunity), setons are useful. A seton refers to any foreign material (i.e., silk, Penrose drains) that can be inserted into the fistula tract to encircle the sphincter muscles [39]. Setons may be used for marking,

cutting, draining, or staging a fistula. A marking seton allows the surgeon to assess the amount of muscle located above the fistula tract, thereby guiding the decision to proceed with fistulotomy. A draining seton may be used as a bridge to or constitute definite surgical therapy. In the latter circumstance, setons may be left in place for long periods of time permitting epithelialization of tracts. A cutting seton is used gradually to transect the sphincter muscle promoting fibrosis of the tissue surrounding the seton. The muscle is divided by gradually tightening the seton, thereby subjecting the muscle to ischemic necrosis. By dividing the proximal portion of the tract, a "high" fistula may be converted to a "low" fistula leaving the distal tract encircled with a seton for division at a later date.

An advancement flap consists of mucosa, submucosa, and part of the internal sphincter, which after debriding the underlying fistula tract is sutured over the internal defect. Advancement flaps offer a one-stage procedure with quicker healing, limited damage to the sphincter, and minimal risk of anal canal deformity. Fibrin glue has also been used to plug fistula tracts, primarily long fistulous tracts with an external opening amenable to closure, and not caused by Crohn's disease [40].

Postoperative management consists of frequent wound inspection, warm sitz baths, measures to facilitate soft, formed stools – fiber supplementation, osmotic laxatives (e.g., sorbitol) – and judicious analgesia. Wound healing after fistulotomy usually takes 4–8 weeks. The incidence of recurrent fistula is about 3%–7% [28,41], and the incidence of anal incontinence after fistulotomy is 18%–52% [42].

Management of anal fistulae in Crohn's disease requires a judicious blend of surgical and medical therapy (see Chapter 57). Metronidazole (typically 750–1500 mg/day) or ciprofloxacillin (1000 mg/day) for up to 3–4 months are often prescribed for Crohn's disease, based only on uncontrolled case series [43,44]. Uncontrolled data and metaanalysis suggest that azathioprine (2–2.5 mg/kg) and 6-mercaptopurine (1.5 mg/kg) are modestly effective, reasonably safe, and relatively inexpensive [44]. One approach is to treat with antibiotics and azathioprine for 8 weeks in patients who are not toxic or miserable, adding infliximab at 8 weeks if fistulae have not closed completely. For markedly symptomatic patients, infliximab may be started sooner (i.e., at 4 weeks). A controlled trial using infliximab (5 mg/kg) (biweekly [BW]) at 0, 2, and 6 weeks improved 68% and healed 55% of patients compared with those receiving placebo (26% and 13%, respectively) [45]. Thereafter, maintenance infliximab (5 mg/kg every 8 weeks) was more effective for keeping fistulae closed [46]. At 54 weeks, fistulae remained closed in 19% of patients who received placebo and in 36% of patients who received infliximab. The coadministration of azathioprine reduces the incidence of human antichimeric antibodies (HACA) to infliximab. Measures to reduce the incidence of HACA include:

- a three-dose induction regimen followed by systematic maintenance dosing at 8-week intervals
- coadministration of immunosuppressive therapy (azathioprine, 6-mercaptopurine, methotrexate)
- pretreatment with 200 mg of intravenous hydrocortisone [47–50].

Rectal prolapse

Rectal mucosal prolapse refers to abnormal descent of the rectal mucosa (Fig. 66.2). Complete rectal prolapse, or procidentia, indicates intussusception of all layers of the rectal wall through the anus. Occult rectal prolapse refers to internal intussusception without protrusion through the anus. A pouch of Douglas hernia originates in the cul de sac of Douglas and protrudes, via the anterior rectal wall, through the anus.

Mucosal prolapse–hemorrhoids

Symptoms

Initially, the mucosal prolapse is small, occurs only after defecation, and reduces spontaneously. Later, the protrusion becomes larger and may require manual reduction or becomes irreducible, causing seepage of mucus and staining of the underclothes. Erosion and ulceration of the protruding mucosa lead to bleeding. The protruding mass has radiating furrows, which are often associated with internal hemorrhoids. By contrast, a true procidentia has circular furrows. Proctosigmoidoscopic examination is required to exclude tumor, polyps, intussusception, or procidentia.

Treatment

Predisposing or aggravating factors (i.e., constipation, diarrhea, polyps, obstructive uropathy) should be addressed. Rubber-band ligation is effective when prolapsing hemorrhoids are not responsive to medical management (see "Hemorrhoids" above). In the presence of anal incontinence, the results of hemorrhoidectomy are unsatisfactory.

Procidentia

Etiology and pathophysiology

Rectal procidentia begins with circumferential intussusception of the rectum that progressively increases over time. Cinedefecographic observations suggest that the leading edge of the intussusception may be located at 6–8 cm or at 15–18 cm from the anal verge. Factors that may predispose to procidentia include excessive straining during defecation, perhaps due to pelvic floor dysfunction, and anatomical factors (i.e., abnormally low descent of the peritoneum covering the anterior rectal wall, loss of posterior fixation of the rectum to the sacral curve, and lengthening and downward displacement of the sigmoid and rectum) [51]. Other conditions associated with procidentia include pelvic neuropathies. The

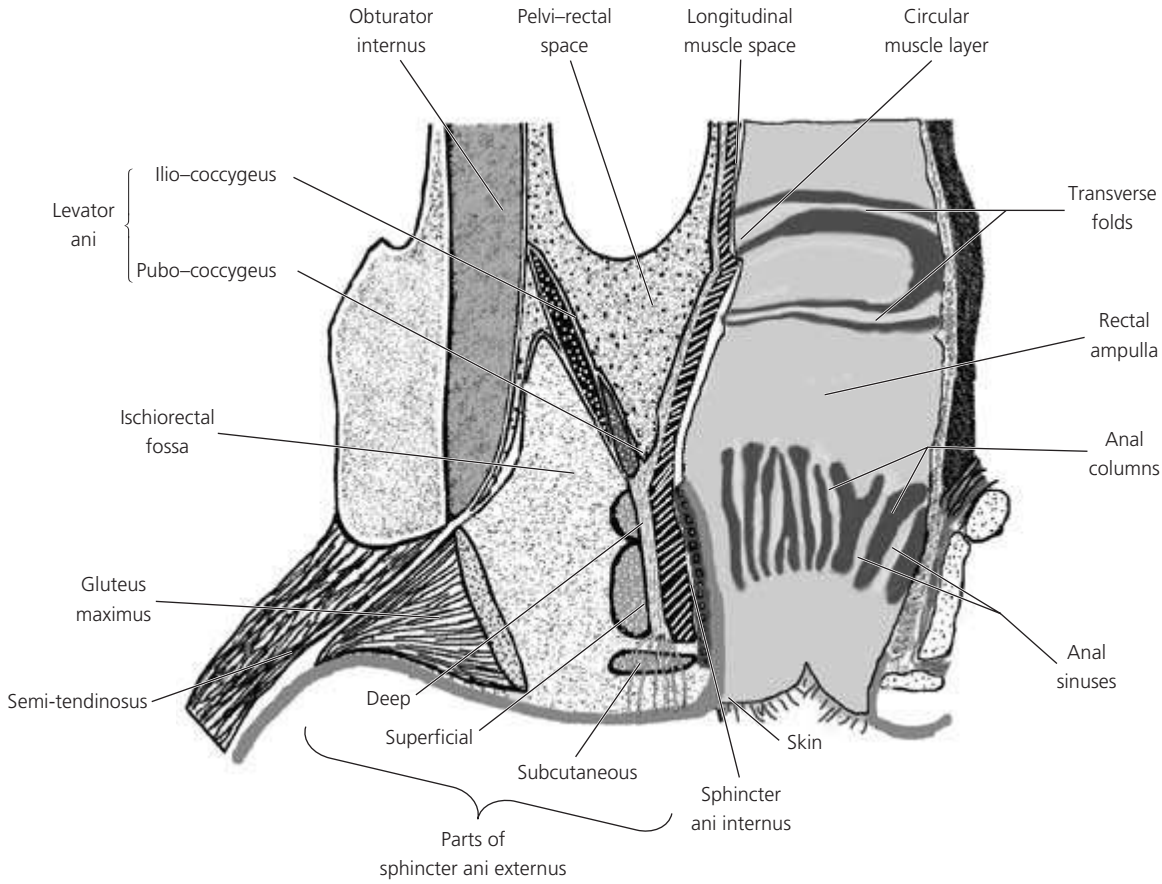

Figure 66.2 Diagram of a coronal section of the rectum, anal canal, and adjacent structures. The pelvic barrier includes the anal sphincters and pelvic floor muscles. From Bharucha [100], with permission from Elsevier.

role of obstetric trauma is uncertain because 40%–50% of women requiring surgery for this condition are nulliparous [52]. Rectal procidentia is rare in men older than 45 years and in women younger than 20 years [53].

Symptoms and diagnosis

The prolapsing mass is initially noticeable with straining only, but over time the prolapse tends to occur with minimal effort. There is usually a feeling of bearing down, or of incomplete evacuation, and often of passage of mucus and bleeding. A majority of patients have constipation, diarrhea, or both. At physical examination, a full-thickness prolapse can be identified by observation and palpation. Although anal resting tone is generally preserved in patients with mucosal prolapse, it is often reduced in patients with procidentia [54]. Denervation of striated musculature on electromyogram, perineal descent, and absence of the anocutaneous reflex are also common findings. If the prolapse is not evident in the left lateral decubitus position, the patient should be examined when straining while seated on the toilet. Proctosigmoidoscopy reveals congestion and edema of the distal rectal mucosa, sometimes associated with a rectal ulcer (see "Solitary rectal ulcer" below).

Attempts should be made manually to reduce a persistently prolapsed rectum to avoid potential complications such as strangulation, ulceration, bleeding, and perforation. Intravenous sedation may facilitate manual reduction. Placing granulated sugar on the prolapsed mucosa often eliminates edema and allows reduction [55]. Gangrene of the anterior rectal wall, a surgical emergency, is occasionally associated with evisceration of small bowel onto the perineum.

Treatment

Because rectal prolapse is generally secondary to excessive straining, and often secondary to defecatory dysfunction, conservative measures (i.e., pelvic floor retraining by biofeedback therapy, a bowel-care regimen) often suffice to ameliorate symptoms of occult rectal prolapse and should be integrated with surgical management. Surgical treatment of rectal prolapse is necessary to avoid complications and ongoing

damage to the pelvic floor and sphincter muscles. The surgical approach is guided by the severity of prolapse, surgical risk, and the surgeon's expertise. Combined abdominal proctopexy and sigmoid resection eliminates two of the presumed causes of rectal prolapse by fixing the rectum to the sacral hollow by means of nonabsorbable sutures and removing the redundant sigmoid colon [53]. Although internal anal sphincter pressures and continence may improve after rectopexy [54], about 40% of patients do not regain continence [52]. A systematic review suggested that residual fecal incontinence was less common after abdominal (vs perineal) approaches, and that division (vs preservation) of the lateral ligaments during rectopexy was associated with less recurrent prolapse but at the price of more constipation [56].

Anal fissure

An anal fissure is a tear in the lining of the distal anal canal below the dentate line, occurring most commonly in the posterior midline. It can affect individuals of all ages and both genders. Clinical symptoms include anal pain during and after defecation, often accompanied by bright-red rectal bleeding and pruritus ani. Acute fissures have sharply demarcated, fresh mucosal edges and may have granulation tissue in the base. When fissures fail to heal within 6 weeks, they are generally designated as chronic. Chronic fissures have raised edges, characteristically exposing the horizontal fibers of the internal sphincter muscle at its base. Other findings associated with chronic fissures are external skin tags, hypertrophied anal papillae, and, occasionally, anal stenosis. Fissures may also occur secondary to an underlying disease such as inflammatory bowel disease (especially Crohn's disease), HIV/AIDS, leukemia, anal carcinoma, and, rarely, syphilis or tuberculosis. Secondary fissures are usually found in a more lateral "off the midline" position [57].

Pathophysiology

The elliptical arrangement of the anal sphincter fibers offers less muscular support to the anal canal posteriorly. This predisposes the posterior anal canal to traumatic tears during passage of a large, hard stool. Many fissures heal rapidly but others do not heal and therefore become chronic.

Patients with chronic anal fissure have increased anal resting pressure. Moreover, spontaneous relaxation of the internal anal sphincter occurs less frequently [58]. As the anoderm receives part of its blood supply from arterioles that cross the internal anal sphincter, it was hypothesized that elevated anal resting pressure could reduce perfusion to the anoderm and lead to local ischemia [59,60]. Doppler ultrasound techniques to measure blood flow in the anodermal area demonstrated that the posterior midline of the anal canal had the lowest perfusion of all four quadrants [61]. Subsequently, it was shown that lateral sphincterotomy normalized both elevated sphincter pressures and blood flow to the anodermal area in these patients [62].

Clinical manifestations

Severe pain associated with scant, bright-red rectal bleeding is the hallmark of anal fissure. Pain occurs both during and after defecation ("like passing razor blades or broken glass") and seems disproportionate to the clinical findings. Severe pain may make anoscopy impossible, and even digital examination is difficult without topical anesthesia. The fissure is best identified by simple inspection after spreading the buttocks. Acute fissures are small linear tears oriented perpendicular to the dentate line in the posterior or anterior midline. Off the midline fissures, especially multiple painless fissures, should prompt a search for a secondary etiology including an examination under anesthesia with biopsy and cultures as appropriate [57].

If an acute anal fissure does not heal promptly, certain characteristic secondary features develop, which include a proximal hypertrophic papilla and a sentinel pile or fibrotic nubbin of skin found at the anal verge (i.e., the classic triad of chronic anal fissure).

Treatment

Acute anal fissures

More than 90% of acute anal fissures quickly heal with simple measures. High-fiber diet, stool softeners, and warm sitz baths following bowel movements may provide symptomatic relief [63]. Although topical anesthetic creams have been used to relieve pain, they may delay healing when compared with bran supplements or topical hydrocortisone [64]. In a randomized trial comparing three treatments (sitz baths with bran supplement, 2% hydrocortisone cream only, and 2% lignocaine [lidocaine] ointment only) in patients with acute posterior midline fissures, initial healing rates at 3 weeks were 88% for the sitz bath and bran group, 82% for the hydrocortisone group, and 60% for the lignocaine group [64].

Chronic anal fissures

The development of a chronic anal fissure with its associated anal hypertonicity requires measures designed to reduce the tone of the internal anal sphincter (IAS). This is because less than 10% will heal with conservative measures. Theoretically, reduction of IAS tone permits easier passage of the fecal bolus through the anal canal to decrease repetitive tearing of the fissure, but the primary rationale is to improve vascular perfusion to the anoderm near the fissure. These treatments involve either pharmacological agents or surgical procedures.

Topical nitrates

The use of topical nitrates is predicated on the principle that nitric oxide is a critically important nonadrenergic, noncholinergic neurotransmitter that mediates relaxation of the IAS. Nitrates are donors of nitric oxide, and topically applied

nitrates have been shown to decrease resting anal pressures and increase anodermal blood flow [65].

Although several studies have shown that 0.2% glycerol trinitrate ointment is superior to placebo [66], other studies have been less impressive [67], and several methodological concerns have been raised [57]. Moreover, headaches and light-headedness are common, and some patients cannot tolerate treatment. Overall success rates range from 27% to 85% with follow-ups of 1–29 months [57]. Interestingly, the lowest success rates were reported when topical nitrates were compared with lateral sphincterotomy [68]. Long-term follow-up has been lacking in many studies, and there is no commercially available glyceryl trinitrate product in the United States.

Calcium channel blockers

Calcium channel blockers also reduce IAS tone but have fewer side effects than do topical nitrates. Two randomized (but not placebo-controlled) trials reported healing rates of 95% over 3 weeks for 0.2% nifedipine [69] and 65% after 8 weeks for 2% diltiazem [70]. In another study, the healing rate was higher with nifedipine (89%) compared with glyceryl trinitrate (58%) [71]. Treatment side effects (headache, flushing) were more frequent with glyceryl trinitrate (40%) compared with nifedipine (5%). At 3–4 months, the recurrence rate (approximately 30%–40%) was similar for both agents.

Botulinum toxin

In a randomized placebo-controlled study, 30 patients with chronic anal fissure received two injections of botulinum toxin (20 units into the IAS), which inhibits acetylcholine release from nerve endings, or saline injection [72]. After 2 months, patients who received botulinum toxin showed significantly greater healing rates (73% vs 13%). Four patients in the botulinum toxin arm who did not heal were retreated, and they then healed within 2 months. No recurrences were observed at 16 months of follow-up. Botulinum toxin (20 units injected into the IAS on each side of the anterior midline) was also compared with topical glyceryl trinitrate (0.2% b.d. [twice daily]) in a randomized trial of 50 patients with chronic anal fissure [73]. Healing rates were significantly higher in the botulinum toxin group (96% vs 60%), and resting anal pressures decreased to a greater extent in the botulinum toxin group than in the glyceryl trinitrate group (29% vs 14%) at 2 months. Approximately 20% of patients in the glyceryl trinitrate group reported moderate to severe headaches associated with treatment, but no side effects were noted in the botulinum toxin group. Long-term results suggest that treatment with botulinum toxin is both successful and durable in many patients [74], although in one study the healing rate at 3 years was only 47% [75].

Botulinum toxin injections are generally well tolerated but are contraindicated in pregnancy and during breast-feeding and should be used with caution in patients with neuromuscular disorders [76]. Some believe that botulinum toxin, because it is less expensive and easier to perform than surgical treatment and does not require anesthesia, should be a first-line treatment for chronic anal fissure [76].

Surgery

Surgery is often reserved for patients with persistent symptoms who have failed medical therapy; but in fact it has the highest cure rate of any form of therapy available [77]. The most common procedure is lateral internal anal sphincterotomy (LIS), involving division of the IAS from its most distal end for a distance equal to that of the fissure or to the dentate line. More than 90% of patients heal after LIS, and recurrence rates of anal fissures are between 0% and 25% [57]. The most important determinant of outcome appears to be the extent of sphincterotomy. One study used anal ultrasonography to show a high rate of incomplete sphincterotomy in those patients who developed symptomatic recurrence [78]. Open and closed techniques produce similar reductions of anal pressure and rates of healing [57,79]. The major concern with LIS is the development of fecal incontinence. Approximately 45% of patients experience some degree of incontinence immediately after surgery – flatus (31%), mild fecal soiling (39%), and gross incontinence (23%). However, in a follow-up study, only 6% had minor fecal soiling and 1% experienced loss of solid stool 5 years after surgery [80].

Anal dilation was initially advocated for anal fissures in 1964, and success rates of 87%–100% have been reported [81]. It has been criticized for being poorly controlled and capable of causing significant anal sphincter damage; others report better outcomes using carefully standardized dilation under local sedation [82]. The consensus is that LIS is safer, and at least as effective as dilation.

Anal stenosis

Anal stenosis is defined as narrowing of the anal canal. This narrowing may result from a true anatomical stenosis (stricture) or a functional (i.e., muscular) stenosis. The most common cause for anal stenosis, accounting for 88% of cases in one series, is hemorrhoidectomy [83]. Excessive excision of anoderm and hemorrhoidal rectal mucosa during hemorrhoidectomy leads to scarring and a progressive stricture. When the mucosa is anchored to the anal verge, the stricture is accompanied by a mucosal ectropion, referred to as the "Whitehead deformity." However, the incidence of stenosis with almost all techniques of hemorrhoidectomy is low (i.e., 0% to < 1.5%) [84]. Other causes of benign anal stenosis include recurrent anal fissure, recurrent abscess and fistula requiring surgical treatment, anal Crohn's disease, radiation, excision of perianal skin lesions as in Paget or

Bowen disease, tuberculosis, actinomycosis, lymphogranuloma, and congenital abnormalities [83]. Malignant causes of anal stenosis include anal and rectal cancers, and, less commonly, transmural invasion of the anorectum by a urogenital malignancy.

Difficult evacuation of stool is the most troublesome symptom. Other symptoms include narrow stools, painful defecation, anal digitation during defecation, bleeding from recurrent anal tears, and constipation or obstipation. Digital rectal examination may reveal the stricture, or cause considerable discomfort to the patient. Under the latter circumstance, examination under anesthesia may be necessary. Functional but not structural anal stenosis will open easily under anesthesia alone.

Treatment is based on the severity and location of the anal stenosis. Medical therapy combines bulking of the stool with anal dilation in the office or at home using the finger or calibrated rubber dilators and topical anesthetics. Dilation is an ideal treatment for patients with Crohn's disease or high-risk patients with otherwise weakened external sphincters [84]. Repeated dilation and steroid suppositories may prevent early recurrence, but there are no well-controlled trials addressing this approach. Surgical treatments of anal stenosis include LIS, an advancement flap, or a colostomy [85]. It has been suggested that LIS is used to treat mid and low anal stenosis. However, it does not treat the ectropion, and the procedure may have to be repeated. A variety of flaps, formed with mucosa or skin, are used to manage anal stenosis. Some of these flaps also address the problem of ectropion. Cure rates in excess of 90% are usually seen [83].

Solitary rectal ulcer syndrome

Solitary rectal ulcer syndrome (SRU) is an uncommon and often underdiagnosed chronic benign disorder related to abnormal defecation. The usual presenting symptoms are excessive straining at defecation (often with the passage of mucus and blood) and feelings of incomplete evacuation. The classic description is that of a shallow, discrete ulcer with a hyperemic margin located 7–10 cm from the anal verge on the anterior rectal wall. However, ulcers may be located between a few and 13 cm from the anus, and are occasionally found on the posterior wall. Furthermore, there may be multiple ulcers or only a localized erythematous or nodular area of mucosa rather than a discrete ulcer. Initial misdiagnosis is common as lesions may be mistaken for inflammatory bowel disease or neoplasia [86]. Characteristic histological changes seen on biopsies taken from abnormal mucosa or ulcer margins include fibrous obliteration of the lumina propria, disorientation and thickening of the muscularis mucosae, and regenerative changes in the crypt epithelium [87]. Although most commonly seen in adults, this syndrome also occurs in children [88].

Pathogenesis

Prolonged straining at stool forces the anterior rectal mucosa into the anal canal leading to mucosal congestion, edema, and ulceration. Although patients with SRU infrequently have clinically apparent rectal prolapse, sigmoidoscopy or defecography demonstrates subtle forms of mucosal prolapse and delayed evacuation during defecation in 90% of these patients [89,90]. Patients with SRU have higher sphincter pressures and thicker rectal walls (primarily muscularis propria) than do patients with complete rectal prolapse [91,92]. The anterior wall mucosa (7–10 cm proximal to the anal margin) is often the first and largest part of the intussusceptum as it descends into the anal canal. This corresponds to the usual location of ulceration seen clinically.

Treatment

Complications of massive bleeding, rectal stricture, and even ulcer penetration into the prostate gland have been reported, but are so uncommon that patients should be reassured that the condition is benign [93,94]. Because the prolapsing mucosa is repeatedly traumatized during defecation, it is appropriate to begin treatment by counseling against anal digitation, eliminating straining, and improving bowel habits by increasing dietary fiber intake or administering osmotic laxatives such as polyethylene glycol [95]. Biofeedback therapy to improve pelvic floor coordination during defecation should be considered as first-line therapy for those who fail conservative measures. For patients with associated rectal prolapse, rectopexy or other procedures to eliminate prolapse may be considered [96]. For patients with occult prolapse, surgery is problematic and should be considered only for those with severe complications [97,98].

Fecal incontinence

Introduction

Fecal incontinence (FI) is defined as recurrent uncontrolled passage of fecal material in an individual with a developmental age of at least 4 years. The prevalence of FI in the community ranges from 2% to 15%. Differences among surveys are perhaps explained by differences in the definition of FI, survey techniques, and the age distribution of the population. The prevalence is similar in men and in women [99], but higher in the elderly and in nursing home residents [100]. Fecal incontinence can substantially impair quality of life and contribute to institutionalization [101,102]. Unfortunately, physicians may not always appreciate the devastating consequences of FI because patients are often embarrassed to discuss their symptoms.

Physiology of continence and defecation

Several factors act in concert to maintain continence. Anatomical factors include the anal sphincters and levator

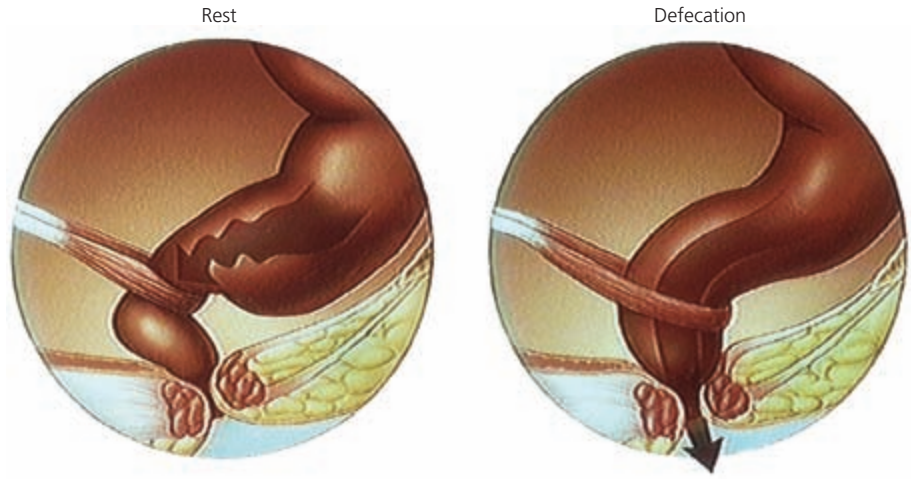

Figure 66.3 Anorectum and puborectalis muscle at rest **(left panel)** and during defecation **(right panel)**. The puborectalis and anal sphincters relax allowing opening of the anal canal and perineal descent during defecation.

ani (i.e., the pelvic floor), rectal curvatures, and transverse rectal folds (Fig. 66.2). The anal canal is 3–4 cm long and normally remains closed as an anteroposterior slit by tonic contractions of the surrounding musculature. Spongy vascular tissues within the canal play a minor, fine-tuning role in fecal continence by assisting with anal closure. The IAS comprises smooth muscle and maintains 70% of the anal resting tone. The external anal sphincter (EAS) comprises striated muscle, which also contributes to resting sphincter tone. The puborectalis maintains the rectoanal angle at rest and contracts further to reduce the angle when subjects squeeze. Stool is often transferred into the rectum by colonic high-amplitude propagated contractions, which tend to occur after awakening or meals [103]. Rectal distention induces reflex relaxation of the IAS. If defecation is convenient, the rectum is evacuated by increased intrarectal pressure coordinated with relaxation of the anal sphincters and pelvic floor muscles (Fig. 66.3). The relative importance of rectal contraction and abdominal wall contraction to increased intrarectal pressure is unclear. Conversely, if defecation is inconvenient, the desire to defecate prompts voluntary contraction of the EAS and pelvic floor, particularly the puborectalis muscle. The desire to defecate dissipates as the rectum accommodates to hold more stool.

Table 66.2 Common causes of fecal incontinence

Anal sphincter weakness
Traumatic: obstetric, surgical (e.g., hemorrhoidectomy, internal sphincterotomy)
Nontraumatic: scleroderma, internal sphincter degeneration of unknown etiology, pudendal neuropathy
Disturbances of pelvic floor
Rectal prolapse, descending perineum syndrome
Inflammatory conditions
Radiation proctitis, Crohn's disease, ulcerative colitis
Central nervous system disorders
Dementia, stroke, brain tumors, multiple sclerosis, spinal cord lesions
Diarrhea
Irritable bowel syndrome, postcholecystectomy diarrhea
Other
Fecal retention with overflow, behavioral disorders

Pathophysiology of fecal incontinence

Fecal incontinence is a heterogeneous disorder, and patients often suffer from more than one deficit of various factors maintaining continence [104,105]. Besides disordered anorectal functions, other factors contributing to FI include disturbed stool consistency, impaired cognition, and decreased mobility.

Impaired pelvic barrier

Anal resting or squeeze pressures may be reduced in FI. Common causes of anal sphincter weakness include sphincter damage, neuropathy, or reduced input from the cerebral cortex or spinal cord (Table 66.2). Although there is a tendency to focus on the anal sphincters, many patients with FI also have generalized pelvic floor weakness, particularly affecting the puborectalis [105–107].

Another manifestation of pelvic floor weakness is the descending perineum syndrome, which results from a combination of obstetric trauma, chronic straining, or neuropathy [106]. The clinical manifestations of the descending perineum syndrome are characterized initially by constipation-predominant symptoms and subsequently by FI. Excessive straining during evacuation may contribute to pelvic floor weakness [108]. Ultimately, excessive descent causes a stretch-induced pudendal neuropathy and widens the anorectal angle, impairing the flap valve that normally maintains continence

when intraabdominal pressure increases. Although sphincter pressures are lower in incontinent compared with continent patients with descending perineum syndrome [109], sphincter pressures do not always distinguish continent from incontinent subjects (see below), underscoring the contributions of disordered rectal compliance, rectal sensation, and stool consistency to FI.

Rectal compliance and sensation

Several studies have demonstrated reduced rectal sensation in some patients with FI. Reduced sensation allows stool to enter the anal canal and perhaps leak before the EAS contracts [104,110]. Conversely, other patients with FI have increased rectal sensation [104,105]. Increased sensation may be attributable to a less compliant or smaller rectum as a consequence of radiation or ulcerative proctitis, but is also found in some women with idiopathic FI. The rectal capacity (i.e., the balloon volume at the maximum imposed pressure), is also reduced in a subset of women with idiopathic FI [105]. Moreover, a smaller rectal reservoir (i.e., reduced capacity or compliance) is associated with the symptom of urgency and with rectal hypersensitivity in FI [111–113]. In irritable bowel syndrome (IBS), increased rectal sensation has been associated with an exaggerated contractile response to rectal distention [114–116]. These observations support in vitro studies indicating that perception of distention is related not to distention but to the contractile response induced by distention [117].

Transient anal sphincter relaxation during or independent of rectal distention enables the anal lining to periodically "sample" and ascertain whether rectal contents are gas, liquid, or stool [118]. There is some evidence to suggest that anal sampling is impaired in FI [118].

Etiology of fecal incontinence

A variety of diseases affect continence mechanisms to cause FI (Table 66.2). In women, FI is often imputed to obstetric anal sphincter injury when endoanal ultrasound reveals IAS or EAS lesions (i.e., scars, thinning, or defects). However, the causal relationship between structural abnormalities and FI is unclear because such abnormalities are often observed in asymptomatic subjects. For example, up to one-third of all women have anal sphincter defects after vaginal delivery [119], but the incidence of postpartum FI is low, ranging from 0% to 10% [100,119]. Clinical observations suggest that many patients with longstanding anal sphincter weakness are asymptomatic until they develop diarrhea. Indeed, among women living in the community, the median age of onset of FI is 55 years [101]. Among community women with FI, 38% had one or more bowel disturbances (i.e., IBS) without rectal urgency, and 14% had rectal urgency with or without bowel symptoms [120]. Disordered bowel habits are more important risk factors for FI than a history of obstetric or surgical anorectal trauma [120]. Although diarrhea is a stronger risk factor for FI, severe constipation with fecal retention may lead to overflow FI. It is conceivable that other factors (i.e., aging, menopause, and chronic straining) may also predispose to FI in this population.

In men, FI is frequently attributable to local causes (i.e., hemorrhoids, fistula, poorly healed surgical scars, sphincterotomy, or proctitis after radiotherapy for prostate cancer). Male FI may also reflect seepage of stool retained in the rectum secondary to a rectal evacuation disorder [121,122] or associated with isolated IAS dysfunction. Several neurological disorders are associated with FI, including diabetic neuropathy, multiple sclerosis, scleroderma, and spinal cord lesions at or below T12. Although anal squeeze pressures are often reduced in multiple sclerosis and diabetic neuropathy, resting pressures are more likely to be reduced in diabetic neuropathy than in multiple sclerosis [123].

Clinical evaluation

A carefully elicited history can help to identify the etiology and pathophysiology of FI, establish rapport with the patient, and guide diagnostic testing and treatment. Bowel habits, stool form, and consistency may be characterized by pictorial stool scales [124]. Semiformed or liquid stools pose a greater threat to continence than do formed stools.

The terms staining, soiling, and seepage may be used to characterize FI [99]. Soiling indicates more leakage than staining of underwear, whereas seepage refers to leakage of small amounts of stool. The circumstances surrounding FI may be characterized as "urge" and "passive" FI. Exaggerated ("urge" FI) or no awareness of the desire to defecate ("passive" FI) before the incontinent episode are associated with more severe weakness of the EAS and IAS, respectively [125]. In addition, rectal urgency may be associated with reduced rectal capacity and increased rectal sensitivity [105,113,126].

The severity of FI can be measured by scales that quantify frequency of stool loss [127]. Because severity may not correlate with quality of life, separate scales have been devised to address these issues. A population-based study demonstrated that the severity of FI can be rated by a simple scale (Table 66.3). Symptom severity rated by this scale strongly correlated with the impact of FI on quality of life [101,102].

The digital rectal examination assesses for anal sphincter defects, anal resting pressure, and voluntary contraction of the EAS and puborectalis when subjects squeeze. Conversely, the puborectalis should relax, and the perineum should descend by 2–4 cm when patients try to expel the examining finger. Examination in the seated position on a commode may reveal rectal prolapse or excessive perineal descent that is not evident when the patient is supine.

Diagnostic testing

Diagnostic testing is tailored to probable etiological factors, response to conservative medical management (i.e.,

Table 66.3 Assessing symptom severity in fecal incontinence

	Symptom severity score			
Symptoms	**1**	**2**	**3**	**4**
Frequency	< 1/month	> 1/month to several times/week	Daily	
Composition	Mucus/liquid stool	Solid stool	Liquid and solid stool	
Amount	Small (i.e., staining only)	Moderate (i.e., requiring change of underwear)	Large (i.e., requiring change of all clothes)	
Urgency or passive incontinence	Neither	Passive incontinence	Urge incontinence	Combined (i.e., passive and urge) incontinence

From Bharucha et al. [101], with permission from Elsevier.

loperamide or stool softeners), and availability of tests. The strengths and limitations of these tests [100,128] are discussed briefly below.

Endoscopy

Endoscopy to evaluate the rectosigmoid mucosa should be considered in most patients, particularly those with constipation or diarrhea; a colonoscopy may be desirable in certain circumstances and after age 50 for screening purposes.

Anal manometry

Anal pressures at rest and during squeeze are measured with perfused or solid-state transducers using the station pull-through method (Fig. 66.4). Anal pressures should be compared with normal values obtained in age- and gender-matched controls using the same technique [128]. Other factors (i.e., disordered stool consistency, rectoanal sensation, or rectal compliance) should be considered in incontinent patients with normal sphincter pressures.

Endoanal ultrasound

Endoanal ultrasound (US) identifies sphincter thinning and defects that are often clinically unrecognized or amenable to surgical repair (Fig. 66.5, right panel). Interpretation of EAS images is more subjective, operator-dependent, and confounded by normal anatomical variations; and there is substantial interobserver variability. The extent to which clinically occult isolated EAS defects are responsible for fecal incontinence is uncertain.

Evacuation proctography (defecography)

During evacuation proctography, anorectal anatomy and pelvic floor motion are recorded on video with the patient at rest, coughing, squeezing, and straining to expel barium from the rectum. This allows identification of excessive perineal descent, internal rectal intussusception, rectoceles, sigmoidoceles or enteroceles, and puborectalis dysfunction [129]. However, methodology and interpretation are incompletely standardized, and some abnormalities (i.e., a small

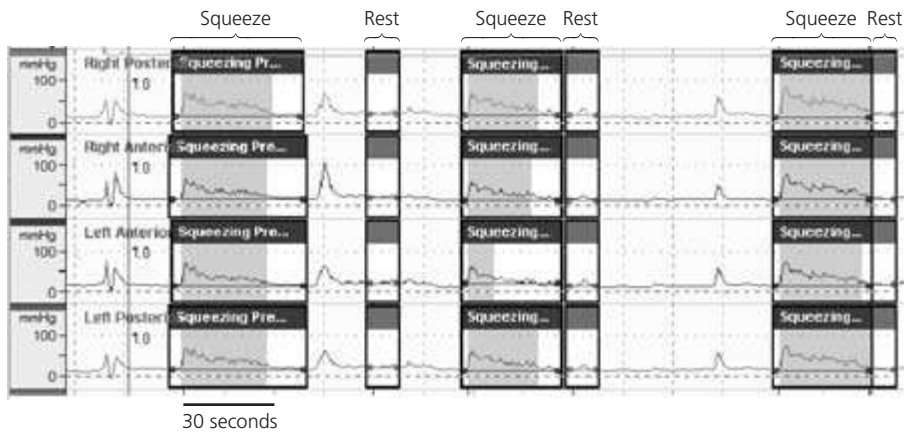

Figure 66.4 Anal sphincter pressures assessed on three separate occasions by four circumferentially oriented transducers stationed 1 cm from the anal verge; transducers were located in separate quadrants. The maximum squeeze pressure is the highest pressure recorded by all four transducers during one of three maneuvers; the average squeeze pressure is calculated by averaging pressures across all four maneuvers. In this example, resting and squeeze pressures were comparable in all four quadrants. From Bharucha AE. Outcome measures for fecal incontinence: Anorectal structure and function. Gastroenterology 2004;26:S90, with permission from Elsevier.

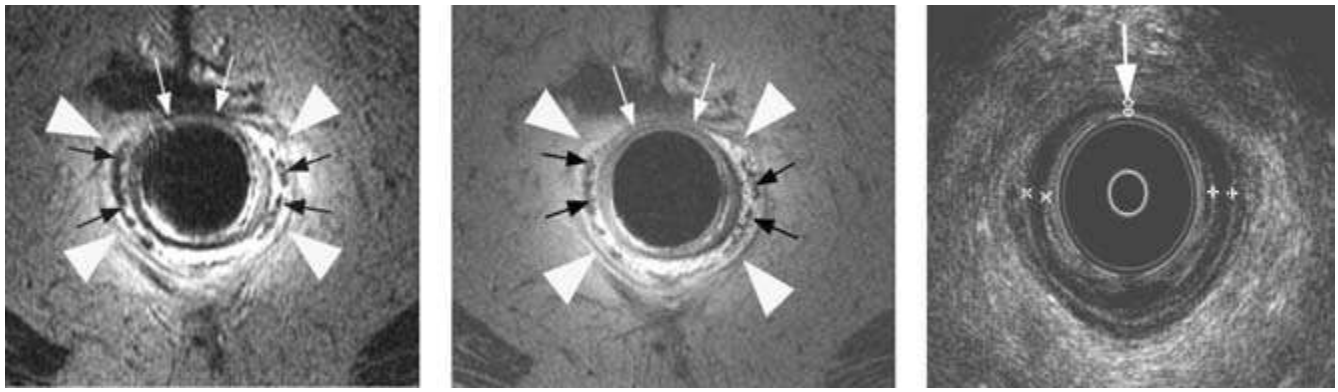

Figure 66.5 Endoanal fast spin-echo T2-weighted **(left)** and spin-echo T1-weighted **(center)** magnetic resonance (MR) images demonstrate marked atrophy of the external anal sphincter (arrowheads) in a 75-year-old incontinent patient, making the internal anal longitudinal muscle prominent (black arrows). Corresponding endoanal ultrasound images **(right)** identified patchy thinning of the internal sphincter also seen on the MR images (white arrows), but not external sphincter atrophy. From Bharucha et al. [105], with permission from BMJ Publishing Group.

rectocele) are relatively common in asymptomatic older women [128,130]. Despite these limitations, evacuation proctography may be useful in some incontinent patients.

Pelvic magnetic resonance imaging (MRI)

With rapid imaging sequences, MRI can visualize both anal sphincter anatomy and global pelvic floor motion in real time, without radiation exposure [105,131]. Dynamic images are acquired as patients squeeze their sphincters or try to expel ultrasound gel from the rectum, providing a unique assessment of global pelvic floor motion. In contrast to IAS evaluation, MRI performed the same or better than ultrasound for the assessment of the EAS [132]. In one study, 25% of patients, but only 5% of controls, had EAS atrophy [105]. Atrophy of the puborectalis muscle was also more frequent in fecal incontinence (16%) compared with controls (5%). In a small series, patients with EAS atrophy did not fare as well as patients without atrophy after undergoing anal sphincteroplasty for sphincter defects [133].

Pudendal nerve terminal motor latency (PNTML)

Delayed pudendal nerve terminal motor latency (PNTML) is widely used to ascertain if anal sphincter weakness is associated with pudendal nerve injury [128]. However, the validity of measuring PNTML is questionable. There are inadequate normative data, test reproducibility is unknown, and sensitivity and specificity are poor. A consensus committee of the American Gastroenterological Association recommended that PNTML not be used to evaluate for neurogenic injury in FI [128].

Needle electromyography (EMG)

Electromyographic (EMG) mapping of the EAS has been supplanted by endoanal US for identifying sphincter defects. Anal sphincter EMG is useful for identifying neurogenic injury in the EAS, particularly for patients in whom the clinical features suggest a proximal neurogenic process (i.e., in the spinal cord or sacral roots) [105,134].

Rectoanal sensation and compliance

Rectal sensation and compliance can be assessed by progressively distending a rectal balloon. Most laboratories assess sensation by manually distending a latex balloon. Rectal pressure–volume relationships are more accurately assessed by barostat-driven distention of a polyethylene balloon, a technique that is available at selected centers only [135].

In FI, rectal sensation may be normal, reduced, or increased [104,105]. When rectal sensation is reduced, EAS contraction in response to rectal distention may be delayed; these deficits are amenable to biofeedback therapy. Conversely, about 45% of patients with FI have increased rectal sensation [104], which may be partly attributable to reduced rectal capacity and explain the symptom of urgency [105,113,126].

Recommendations

Endoscopy to identify mucosal pathology is probably necessary for incontinent patients with recent-onset diarrhea or constipation; the extent of examination (i.e., sigmoidoscopy or colonoscopy) is also guided by the patient's age and comorbidities. The indications for and extent of diagnostic testing in incontinent patients are evolving. For ambulatory, otherwise healthy patients, anorectal manometry and endoanal ultrasounds can document the severity of weakness and identify abnormal sphincter morphology. Pelvic MRI, combining static and dynamic imaging without radiation exposure, is particularly useful for identifying external sphincter atrophy and pelvic organ prolapse but is relatively expensive and not widely available. Anal sphincter EMG should be considered for incontinent patients with an underlying disease

associated with a neuropathy (i.e., diabetes mellitus, clinical suspicion of a proximal neurogenic process, or sphincter weakness unexplained by morphology as visualized by ultrasound). Pudendal nerve latencies are not useful for investigating FI [128].

As advanced tests (i.e., pelvic MRI, anal sphincter EMG, assessment of rectal compliance and sensation) are not widely available, independently living patients with moderate–severe fecal incontinence should be referred to specialist centers under the following circumstances:

- when symptoms cannot be explained by routine diagnostic tests
- prior to repair of external sphincter defects in older women because surgical repair is not always successful; careful consideration of other factors contributing to incontinence, perhaps supplemented by pelvic MRI to identify external sphincter atrophy may be useful
- for patients who have symptoms of an evacuation disorder and fecal incontinence, biofeedback therapy to improve coordination between abdominal contraction and pelvic floor relaxation is necessary
- for patients who have combined fecal and urge urinary incontinence; sacral nerve stimulation is an FDA (Food and Drug Administration)-approved procedure for urge urinary incontinence, and bowel symptoms may also respond to sacral nerve stimulation [136].

Management

The management of FI must be tailored to clinical manifestations and includes treatment of underlying diseases.

Bowel habit modification

Modifying bowel habits is often central to managing FI effectively. A detailed dietary history may identify excessive ingestion of natural (i.e., prunes) and processed foods (i.e., beverages) that contain fructose and sorbitol and may cause or aggravate diarrhea [137].

Loperamide reduces diarrhea and slightly increases IAS tone, thereby reducing FI [138]. It is important that adequate doses are given (i.e., 2–4 mg, 30 min before meals, up to 16 mg/day). Taking loperamide prior to social occasions is helpful to avoid an accident outside the home. In patients with both constipation and diarrhea, finding the optimal dose to reduce diarrhea but avoid constipation can be challenging. Diphenoxylate is an alternative option for diarrhea [139]. Cholestyramine resin may improve postcholecystectomy diarrhea [140]. A small open-label trial showed that amitriptyline (20 mg p.o. daily) was effective in decreasing FI scores, frequency of bowel movements, and amplitude of rectal motor complexes [141]. The serotonin (5-hydroxytryptamine) 5-HT_3 receptor antagonist alosetron may reduce diarrhea and improve fecal continence in patients refractory to other measures [142]. Patients with constipation, fecal impaction, and overflow incontinence often benefit from a regular evacuation program, using digital stimulation or bisacodyl/glycerol suppositories, fiber supplementation, and selective oral laxatives [143].

Direct pharmacological approaches

Topical application of the α_1-adrenergic agonist phenylephrine to the anal canal increases anal resting pressure. However, in a randomized double-blind placebo-controlled cross-over study of 36 FI patients, phenylephrine did not significantly improve FI or resting anal pressure compared with placebo [66].

Biofeedback therapy

Biofeedback is based on the principle of operant conditioning [144]. Using a rectal balloon–anal manometry device, patients are taught to contract the EAS when they perceive balloon distention; perception may be reinforced by visual tracings of balloon volume and anal pressure, and the procedure is repeated with progressively smaller volumes. A systematic review of 566 patients treated in numerous studies found that 72% of patients resolved or improved symptoms of FI after biofeedback therapy [145]. These studies used heterogeneous methods and outcome measures, and the vast majority were not controlled. A controlled trial randomized 171 patients with FI to four groups: standard medical/nursing care (i.e., advice only); advice plus verbal instruction on sphincter exercises; hospital-based computer-assisted sphincter pressure biofeedback; or hospital biofeedback plus use of a home EMG biofeedback device [146]. Overall, there was no significant difference between the groups, with 54% reporting improved symptoms in the biofeedback groups and 53% in the advice only group. Improvement was sustained at 1 year after therapy; symptoms, resting, and squeeze pressures improved to a similar degree in all four groups. These results emphasize the utility of conservative measures (i.e., patient teaching, emotional support, lifestyle modifications including diet and fluids) and management techniques such as improvement of evacuation, a bowel training program, and antidiarrheal medication for FI. Controlled studies to assess whether biofeedback therapy is efficacious for patients who do not respond to conservative measures alone are in progress.

Surgical approaches

Sphincteroplasty is reserved for a small number of patients with FI, optimally when anal sphincter injury is recognized shortly after an obstetric event. Consensus-based guidelines recommend surgical repair of incidentally recognized anal sphincter defects, often found by imaging years after the presumed obstetric insult. In the short term, overlapping anterior sphincteroplasty improves FI in up to 85% of patients with sphincter defects. However, continence deteriorates thereafter, and far less than 50% of patients remain continent up to 5 years after the operation [147].

Dynamic graciloplasty involves continuous electrical stimulation of the gracilis muscle transposed around the anal canal; electrical stimulation facilitates anal tone by converting type II (fast-twitch, fatigue-prone) to type I (slow-twitch, fatigue-resistant) muscle fibers. Dynamic graciloplasty is approved in Europe but not in the United States. Although FI was significantly improved in roughly 60% of implanted patients, this procedure was associated with significant morbidity, including infections (28%), device problems (15%), and leg pain (13%), which may require reoperation [148,149]. Moreover, up to 50% of patients with functioning graciloplasties may have clinical features of obstructed defecation.

Experience with the artificial anal sphincter is similar; 99 out of 112 implanted patients from a US multicenter report had device-related adverse effects [150,151]. Fifty-one patients required 73 operations to revise the device, and it was explanted in 41 patients (37%). Fecal incontinence scores and quality of life improved; the outcome was successful in 85% patients with a functioning device, and the intention-to-treat success rate was 53%. A systematic review of the artificial sphincter observed that most studies were case series with little or no follow-up of patients in whom the device failed [151]. However, most patients with a functioning device reported clinically significant improvement in continence and quality of life [151]. It is conceivable that the utilization and outcomes with both procedures will increase over time.

A colostomy is the last resort for patients with severe FI; the impact of a colostomy on quality of life in FI has not been formally assessed.

Incontinence products

A Cochrane review concluded that disposable bodyworns with superabsorbent materials (i.e., diaper-type garments or pads) performed better than nondisposable bodyworns in preventing skin condition problems, but these studies focused mainly on urinary incontinence rather than FI [152].

Anal plugs are used for FI in Europe but are not commercially available in North America [153]. In one study, 64% of patients were continent for feces when they used the plug [153]. However, the plug occasionally was displaced in 43%, and 71% experienced discomfort to a varying degree. Plugs need to be removed before bowel movements and are probably not suitable for patients with frequent bowel movements. An inflatable plug with an infrared sensor that detects stool has also been described [154].

New approaches

Predominantly uncontrolled studies suggest that sacral nerve stimulation improves FI, augments anal squeeze pressures more than resting pressure, and may also modulate rectal sensation in incontinent patients [136,155,156]. Sacral stimulation is conducted as a staged procedure; patients whose symptoms respond to temporary stimulation for about 2 weeks proceed to permanent subcutaneous implantation of the device. The procedure for device placement is technically straightforward, and device-related complications are less frequent or significant relative to more invasive artificial sphincter devices discussed above. A multicenter US study assessing sacral nerve stimulation for FI is in progress.

Application of radiofrequency energy to the anal canal (the Secca procedure) improved fecal continence but not anal sphincter pressures in an uncontrolled study of 10 women with FI; the effects were maintained at 2 years thereafter [157]. Less optimistic results were reported in a larger multicenter trial [158].

Injectable sphincter bulking agents are effective for urinary incontinence [159]. Uncontrolled, predominantly small series have used autologous fat [160], collagen [161], implantable microballoons [162], carbon-coated beads [163], and silicone [164] to augment the pelvic barrier, with variable efficiency for FI. The largest study to date, using injectable silicone in 82 patients with severe FI and low anal resting pressure, was uncontrolled. Moreover, improvement in symptoms and resting anal pressures at up to 6 months after the procedure were modest, with limited follow-up thereafter [164]. The long-term efficacy and safety of these agents are unknown, and none are as yet approved by the FDA for use in FI.

Pruritus ani

Pruritus ani is an annoying itchy sensation of the anus and perianal skin that is often associated with burning and soreness. It may be transient or chronic, unrelenting, and difficult to treat. Periods of intense itching are characteristically most disturbing at night when daytime distractions are removed. It affects 1%–5% of the population, with a male-to-female predominance of 4:1 [165].

Pathogenesis

Pruritus ani may occur as a symptom of a specific disease affecting the perianal skin or, more commonly, as an idiopathic condition possibly related to residual fecal material (Table 66.4). A number of anorectal diseases, including fissures, fistulae, and hemorrhoids, make local hygiene especially difficult, and irritated macerated skin may develop. Pruritus, discomfort, and mucus drainage are more common in those suffering from hemorrhoids than in a control population, and often resolve with treatment of the hemorrhoids [166]. Anal malignancies, including Bowen disease, epidermoid cancers, and extramammary Paget disease, may rarely present as pruritus ani and should be considered even if symptoms have existed for years [167].

Candida albicans and dermatophytic infections appear as characteristic localized erythematous rashes but may also be cultured from the perianal area even if skin lesions are

Table 66.4 Causes of pruritus ani

Anorectal disorders
Diarrhea
Anal incontinence
Hemorrhoids
Fissures
Fistulae
Rectal prolapse
Malignancy: Bowen disease, epidermoid cancer, perianal Paget disease
Fungal: candidiasis, dermatophytes
Parasitic: pinworms, scabies
Venereal: herpes, gonococcus, syphilis, condylomata acuminata
Local irritants
Moisture: obesity, excessive perspiration
Soaps: hygiene products
Toilet paper: perfumed, dyed
Underwear: irritating fabrics, detergents
Anal creams, suppositories
Dietary: coffee, beer, acidic foods
Drugs: mineral oil, ascorbic acid, hydrocortisone sodium succinate, quinidine, colchicine
Dermatological diseases
Psoriasis
Atopic dermatitis
Seborrheic dermatitis

absent. *C. albicans* is more likely to be identified by random cultures, but dermatophyte infections tend to be associated with pruritus ani [168]. Pinworm (*Enterobius vermicularis*) causes pruritus ani that is characteristically nocturnal. Children are most commonly affected, but adults, especially those exposed to children, may occasionally become infected. Diagnosis is best made by applying adhesive cellophane tape to the perianal skin early in the morning in an effort to detect eggs. Scabies (*Sarcoptes scabiei)* and pubic lice (*Phthirus pubis*) can also cause pruritus ani. Itching in the genital region and identification of organisms or eggs on pubic hair lead to the proper diagnosis.

A controlled study failed to provide a bacteriological basis for this condition [169]. Sexually transmitted diseases associated with pruritus ani include herpes simplex, gonorrhea, syphilis, condyloma acuminatum, and molluscum contagiosum [170]. Dermatological conditions, such as psoriasis, eczema, and seborrheic dermatitis, must also be considered. One series of patients referred to a combined colorectal and dermatological clinic had a dermatosis, particularly psoriasis, as the most common cause of pruritus ani [171]. A variety of local irritants, allergens, and chemicals that contact the perianal skin may cause pruritus. Perfumes and dyes in toilet paper and soaps, personal hygiene products, irritating fabrics, laundry detergents, and even topical anal creams or suppositories are examples. Even certain chemotherapeutic agents have been implicated [172]. Clinical experience and elimination diets have implicated certain dietary products such as coffee, cola, beer, tomatoes, chocolate, tea, and citrus fruits, although the mechanism is unknown [173]. Food products such as lactose that cause diarrhea may lead to pruritus ani simply because of frequent fecal contamination of the perianal region.

Idiopathic pruritus ani is usually caused by a combination of perianal skin fecal contamination and trauma. Ambulatory monitoring of the anorectum has shown exaggerated transient internal sphincter relaxations, which likely predispose to subtle fecal leakage in idiopathic pruritus ani [174]. Acute diarrhea in an otherwise healthy person may irritate the perineum because of frequent wiping. Tiny amounts of feces repeatedly contaminate the perianal skin leading to further irritation. An itch–scratch cycle develops, which perpetuates skin trauma. Patients with chronic pruritus ani often suffer from frequent loose stools or some degree of fecal incontinence or seepage. The perianal area is repeatedly traumatized from vigorous wiping or scratching, and adequate healing can never occur. Pruritus is further exacerbated by inadequate perianal hygiene or excessive moisture caused by perspiration, airtight clothing, severe obesity, or a particularly deep-set anus.

Treatment

Most causes of pruritus ani can be successfully treated. Dermatological diseases and infectious causes should be treated in the usual manner. Anorectal disorders that impede good perianal hygiene, such as prolapsing internal hemorrhoids or anal fissures, should be corrected. Fecal leakage or incontinence must be aggressively managed to avoid soiling of perianal skin. For the same reason, frequent loose stools may be minimized with antidiarrheal agents if appropriate. Foods or beverages that produce diarrhea or pruritus in a particular individual should be discontinued. Blanket recommendations to discontinue or curtail coffee, beer, citrus fruits, and other foods reported to cause pruritus ani lack firm support, but elimination diets may be appropriate in particular cases.

The key to management of pruritus ani is to keep the anal area clean and dry while minimizing trauma caused by wiping and scratching [175]. The patient should be instructed gently to cleanse the anal skin with a premoistened pad or tissue after defecation. Witch hazel preparations or soothing lanolin-containing lotions always remove more residual feces than does dry wiping alone. The area should then be dried with a blow dryer or soft tissue using gentle dabbing without rubbing. Those with small amounts of fecal discharge are instructed to insert a thin cotton pledget into the anal canal, which should be changed frequently throughout the day. Excessive perspiration is corrected with application

of baby powder and avoidance of tight, nonporous clothing. Irritating fabrics, perfumed toilet paper, hygiene products, and soaps should be avoided. A 1% hydrocortisone cream may be applied sparingly twice daily during the acute phase of pruritus ani but should not be used for longer than 2 weeks to avoid skin atrophy. Applying a protective ointment (zinc oxide) over the antiinflammatory agent may facilitate healing. For severe nocturnal symptoms, a nighttime dose of a systemic antipruritic such as diphenhydramine is appropriate. Capsaicin cream was effective in 70% of patients with chronic pruritus ani in a randomized, double-blind, placebo-controlled cross-over study [176].

Proctalgia syndromes

Anorectal pain (proctalgia) is associated with a variety of organic conditions but also occurs in patients in whom organic conditions are not identified. The two common functional disorders – chronic proctalgia and proctalgia fugax – are distinguished by the duration and frequency of painful episodes and by the absence of physical findings in asymptomatic patients with proctalgia fugax [177]. Chronic proctalgia must also be distinguished from other disorders of chronic anorectal pain (i.e., coccygodynia, rare genetic syndromes of familial rectal pain, and other gynecological causes of chronic pain). In both entities, reports of effective therapy are largely anecdotal, and therapeutic modalities have not been examined rigorously with controlled trials.

Chronic proctalgia

The diagnosis of chronic proctalgia is made by the clinical history, physical examination, and exclusion of other disorders that can produce chronic anorectal pain. The pain is described as a dull or pressure-like discomfort in the rectum that lasts for several hours or longer. Both prolonged sitting and defecation may precipitate the pain; in addition, symptoms of difficult defecation or a sense of incomplete evacuation have been described [178,179]. A common clinical finding is palpable tenderness of overly contracted levator ani muscles as the examining finger moves from the coccyx posteriorly to the pubis anteriorly. The tenderness is often asymmetric and is most commonly found on the left [178]. An international committee has proposed diagnostic criteria, consisting of chronic or recurrent episodes of rectal pain or aching lasting for 20 min or longer for at least 3 months in the absence of other causes, such as ischemia, inflammatory bowel disease, cryptitis, intersphincteric abscess, anal fissure, hemorrhoids, and coccygodynia [179].

Chronic proctalgia may be further characterized as levator ani syndrome if posterior traction of the puborectalis muscle elicits tenderness, whereas if there is no tenderness, a diagnosis of "possible" levator syndrome or unspecified pain is considered. It has been estimated that 6%–7% of the general population have symptoms consistent with levator ani syndrome, but only 29% of affected individuals consult a physician [177].

Pathophysiology

Levator ani syndrome is hypothesized to result from overly contracted pelvic floor muscles. One study suggests that elevated anal canal pressures or EMG levels may constitute a useful quantitative sign for the diagnosis [180]. Grimaud and colleagues found elevated anal resting pressures in all 12 of their patients with levator ani syndrome [180]. Moreover, anal canal pressures normalized in association with pain relief after biofeedback therapy. These observations need to be confirmed in a controlled study with a larger number of patients. The pathophysiology of unspecified functional anorectal pain is poorly understood.

Treatment of chronic proctalgia

Treatments claimed to be effective are those that are directed towards reducing tension in the striated pelvic floor muscles. These include electrogalvanic stimulation [181], biofeedback training [182], muscle relaxants such as methocarbamol, diazepam, and cyclobenzaprine, digital massage of the levator ani muscles, and sitz baths [63]. A double-blind placebo-controlled study showed no efficacy of intrasphincteric injection of botulinum toxin A in levator ani syndrome [183].

Except as noted above, these therapeutic trials were uncontrolled, and patient selection criteria were variable. If circumstances require that treatment be undertaken, the only advice that can be offered is to do no harm, that is, to select a treatment, such as biofeedback, which has no significant adverse consequences. Surgical procedures such as division of the puborectalis muscle should be avoided because they do not correct the functional disorder and may lead to fecal incontinence [184].

Proctalgia fugax

Proctalgia fugax is characterized by sudden, episodic, severe pain in the rectal area that lasts for several seconds or minutes before disappearing completely. The pain has been described as aching, gnawing, cramping, or stabbing. In contrast to levator ani syndrome, patients who are asymptomatic during examination exhibit no characteristic physical findings. As a result, the diagnosis relies entirely on the clinical description, and the exclusion of other diseases that can produce proctalgia. In one survey, about 14% of a healthy population reported at least one episode of proctalgia fugax, and 5% reported having at least six episodes yearly. Less than 20% of affected individuals had sought medical attention [185].

Pathophysiology

The pathophysiology of the pain in proctalgia fugax is unknown. Early reports of anal sphincter spasm or contractions of the puborectalis and external anal sphincter

muscles [186] have never been substantiated. An autosomal dominant inherited myopathy of the IAS associated with both proctalgia fugax and constipation in members of two affected families has been described [187,188] but cannot be assumed to be the cause of most cases of proctalgia fugax. The potential role of psychological factors in proctalgia fugax is uncertain.

Treatment of proctalgia fugax

Treatment consists of an explanation of the disorder and reassurance, as episodes of pain in most patients are brief and infrequent. There have been scattered reports of benefits with clonidine [189], inhaled salbutamol [190], nitrates and diltiazem [191], and caudal epidural blocks [192].

The psychological findings in patients who consult for proctalgia include anxiety, depression, and a tendency towards hypochondriasis. When these symptoms are present, antidepressant or anxiolytic medications or behavioral therapies may be indicated. However, there are no studies on the outcome of such treatments for proctalgia fugax.

Coccygodynia

Coccygodynia refers to rectal pain that arises from the coccyx [193]. It is often described as an aching or gnawing that is sometimes sharp and may radiate into the buttocks. Coccygodynia may be caused by known causes (i.e., dislocation, fracture, arthritis, presumed injury during vaginal delivery), or it may be functional [177]. Pain may also be referred to the coccyx from the lumbosacral spine, sacrum, anus, rectum, pelvis, and genitourinary tract. The key to diagnosis is the reproduction of pain when the coccyx is manipulated; this distinguishes coccygodynia from other chronic proctalgia disorders. Lateral radiographs have shown two distinct findings in many patients with coccygodynia [194]. In the "luxation" group, posterior luxation occurs during sitting and disappears while the patient is standing. In the "hypermobility" group, the coccyx flexes abnormally while the patient is sitting.

Treatment

The initial treatment of coccygodynia is conservative and includes moist heat and analgesics. The painful coccyx should be protected by having the patient sit in a position that lessens pressure on the coccyx or on a doughnut-shaped pillow [195]. Local injection of corticosteroids into the area may benefit those who do not respond to conservative therapy. Manipulation of the coccyx under anesthesia to stretch the ligaments has been reported to be beneficial. Cryoanalgesia of the posterior rami of the lower sacral nerve roots and the coccygeal nerve has been advocated for patients who obtain temporary pain relief with local anesthetic injections [196]. Rarely, coccygectomy may be performed for severe and unresponsive organic coccygodynia [195].

Rectal foreign bodies and trauma

The insertion and incarceration of foreign objects into the anus and rectum is a well-described phenomenon and can no longer be considered rare [197,198]. These objects are inserted for purposes of medical treatment, concealment, assault, or most commonly, eroticism. In an attempt to cope with an embarrassing situation, patients may claim the object was inserted to obtain relief from a particular discomfort or symptom. Frequently, the patient may complain of pain or bleeding without admitting to a foreign body insertion [199]. A directed interview and careful abdominal and rectal examination are necessary to identify the foreign body and the potential risk of rectal trauma. Anteroposterior and lateral radiographs should be obtained to determine the object's outline and location and to detect pneumoperitoneum if present. Less commonly, a patient may unwittingly swallow a toothpick or piece of bone that later impacts in the anal canal and may mimic an abscess or fissure.

Management involves the safe retrieval of the object. Many can be removed transanally without resorting to surgery. Even if the object is easily palpable, removal under direct vision is the best way to avoid iatrogenic injury [200]. The location of foreign bodies may be classified as "low" if in the rectal ampulla or "high" if at or proximal to the rectosigmoid junction [200]. Many small, low-lying objects can be retrieved transanally using an operative anoscope. Removal of larger objects, such as vibrators or rubber phalluses, may require regional anesthesia, anal dilation, and a grasping forceps. Large, bulky objects, such as glass bottles and lightbulbs, require special care. After adequate anesthesia, several Foley catheters are inserted past the object. Air insufflation through the catheters relieves the proximal vacuum effect that may occur during attempted withdrawal. Gentle manipulation coupled with slow traction using the inflated Foley balloons successfully extricates the bulky object in most cases. An obstetrical vacuum extractor has also been used to remove a glass foreign body. If a glass object is broken accidentally during attempted removal, laparotomy is required.

High-lying foreign bodies are best managed using spinal anesthesia and the lithotomy position. By palpating the abdomen, the object may be pushed distally and then simultaneously grasped with forceps through the sigmoidoscope to coax it into the rectal vault, from which it may be removed. If the object cannot be delivered to within reach of the rigid sigmoidoscope manually or after 12 h of observation, laparoscopic assistance can be used to push the object distally to assist with removal [201]. In a large review, 76% of patients with objects in the rectum had a successful bedside extraction, whereas 55% of patients with foreign objects in the sigmoid colon required laparotomy [200]. Proctosigmoidoscopy should be performed after all foreign body retrievals to rule

out retained objects, lacerations, hematomas, and perforations. If peritonitis develops, urgent laparotomy is required.

Other causes of rectal trauma include penetrating injuries (usually gunshot wounds), blunt trauma (motor vehicle accidents), impalement injuries (criminal assaults), homosexual activities (fist fornication), and iatrogenic injuries (endoscopy, enemas, surgical procedures) [202]. Surgical management of major rectal trauma usually includes a diverting colostomy, presacral drain placement, distal rectal irrigation, and maximum preservation of sphincter musculature [203].

Anal intraepithelial neoplasia

Anal intraepithelial neoplasia (AIN) is believed to be a precursor of anal squamous cell cancer. Its incidence is rising in high-risk groups, particularly those infected with the human immunodeficiency virus (HIV). This condition is subclassified into low-grade anal squamous intraepithelial lesion (LSIL), equivalent to AIN grade 1, and high-grade anal squamous intraepithelial lesion (HSIL), equivalent to AIN grades 2 and 3. The term Bowen disease of the anus is sometimes used to denote AIN grade 3.

Etiology

These tumors are strongly associated with receptive anal intercourse in homosexual men and with sexually transmitted diseases, especially genital warts, in both genders [204]. Anal cancer shares several similarities with cervical cancer. First, the incidence of anal cancer in men who practice anoreceptive intercourse is 35 per 100 000, which is higher than the incidence of cervical cancer of 8 per 100 000 [204,205]. Second, human papillomavirus infection, particularly type 16, plays a causal role in both cancers [206,207]. Third, both originate from a precursor lesion (i.e., squamous intraepithelial lesions). Smoking, HIV infection, multiple sexual partners, cervical neoplasia, and immunosuppression after organ transplantation are other important risk factors [208].

Palefsky et al. characterized the natural history of AIN in 346 HIV-positive and 262 HIV-negative men who had sex with men [209]. Among HIV-positive men who were normal at baseline, 52% progressed to anal squamous intraepithelial lesions (ASIL) within 2 years compared with 17% of HIV-negative men. An even higher proportion of HIV-positive and HIV-negative men (70% and 31%, respectively) progressed to ASIL when diagnosed with atypical squamous cells of uncertain significance at baseline. A high proportion of those with LSIL at baseline progressed to HSIL (62% and 36%, respectively.) Other studies have shown that AIN grade 2 or 3 rarely regresses, even in HIV-negative individuals [210], and can progress to invasive cancer. In two studies, approximately 5% of AIN grade 3 lesions became malignant over two decades [210–212]. Anal cytology screening may be cost-effective in homosexual and bisexual men [213].

Clinical features, diagnosis, and management

Anal intraepithelial neoplasia is often subclinical and diagnosed when high-risk patients are screened with cytology or anal colposcopy [214]. The AIN lesions do not have a specific appearance; suspicious areas are raised, scaly, white, erythematous, pigmented, or fissured. Anal cytology has a 92% sensitivity for distinguishing benign from malignant lesions. However, cytology was only 50% specific for distinguishing benign from malignant lesions. Although colposcopy is useful for detecting AIN, colposcopy and histopathology are necessary to define the site and extent of AIN. Because the natural history and malignant potential of AIN are uncertain and because an effective therapeutic option is not available, management is not standardized. Patients with AIN should be monitored, perhaps every 12 months for HIV-negative patients with AIN grades 1 or 2, and every 4–6 months for HIV-positive patients with AIN grades 1 or 2, as also for patients with AIN grade 3 [214]. All suspicious lesions should be biopsied or excised. Unfortunately, HIV-positive patients frequently have persistent or recurrent high-grade AIN after treatment of the initial lesion. The roles of photodynamic therapy and argon laser therapy require further investigation [214].

Anal carcinoma

Anal carcinomas comprise 1%–2% of all colonic cancers. A variety of histological types have been described, including: squamous cell (70%–80%); basaloid, also known as transitional cell or cloacogenic (20%–30%); mucoepidermoid (1%–5%); and small cell anaplastic type (< 5%) [215]. All can be broadly classified as epidermoid carcinomas for purposes of clinical discussion. The mean age at presentation of patients with anal cancer is approximately 60 years [216]. Perianal and rectal carcinomas are more common in men, but anal canal tumors are nearly twice as common in women [216].

Clinical manifestations

Bleeding, pain, pruritus, and discharge are the most common symptoms of anal cancer. These relatively nonspecific symptoms are often attributed to common benign anorectal disorders, as previously discussed. Consequently, the tumor is often diagnosed when patients develop additional symptoms such as incontinence, change in bowel habits, pelvic pain, or a rectovaginal or rectovesical fistula, which suggest an advanced malignancy. The tumor may be discovered incidentally in specimens obtained for benign anorectal disease [217,218]. However, benign anal lesions do not appear to cause anal cancer [219]. At presentation, 15%–30% of patients have metastases to pelvic or inguinal lymph nodes, and 10% have distant spread to the liver or lungs [215]. Rarely, a palpable metastatic inguinal lymph node is the sole

presenting manifestation. High-grade dysplasia identified by screening cytology must be confirmed by biopsy and may be present before visible lesions exist [220].

The physical examination and clinical investigations are directed toward establishing the diagnosis and staging. For small lesions, it is important to ascertain the site of origin (i.e., the anal canal or anal margin). For large lesions that overlap the canal and the margin, this distinction may not be possible and is probably less important because therapy is guided by sensitivity to radiotherapy and chemotherapy as determined by histological type. The assessment, if necessary under general anesthesia, includes digital examination to evaluate the size and mobility of the mass, invasion of surrounding structures, and the presence of pararectal or inguinal lymph nodes; biopsies are also obtained. A colonoscopy is recommended to exclude colorectal carcinoma. Paraaortic lymph nodes can be identified by a CT scan of the abdomen and pelvis whereas pararectal nodes can be biopsied by endoanal ultrasound. Lesions originating from the anal canal tend to be undifferentiated and more aggressive, in contrast to those arising from the anal margin [221]. A poor prognosis is portended by squamous cell tumors greater than 2 cm in size (especially those > 5 cm), basaloid or anaplastic carcinomas, sphincter muscle invasion, or spread to regional pelvic or inguinal lymph nodes [215].

Treatment

Local excision is the primary modality for carcinoma at the anal margin. In the largest series, the 5-year survival was 65% [222]. Large or infiltrating lesions require an abdominoperineal resection for radical excision of structures, including the sphincters, levator muscles, and rectum. Prophylactic inguinal lymph node resection is not indicated. Among 11 patients treated by abdominoperineal resection with curative intent, the 5-year survival rate was only 36%. Another study reported a recurrence rate of 63% after excision of lesions at the anal margin [223]. However, recurrent lesions often respond to further local treatment [224].

Chemoradiotherapy has replaced surgery as the primary therapeutic modality for anal canal carcinoma [225–227]. Anal squamous cell carcinoma (SCC) is more radiosensitive than SCC arising in other locations. Chemotherapy enhances the effect of radiotherapy, improves tumor control, and minimizes severe complications. Even large, locally advanced tumors are amenable to initial chemoradiotherapy. Prospective randomized or multicenter trials demonstrate that compared with radiotherapy alone, chemoradiotherapy with 5-fluorouracil (5-FU) and mitomycin-C (MMC) provides superior local control, colostomy-free rate, progression-free survival, and cancer-free survival without significantly increasing late complications and non-cancer-related deaths. In large studies, 5-year disease-free survival rates after combined therapy range from 60% to 86%. The addition of MMC to RT and 5-FU improves local control, colostomy-free rate, tumor progression, and cancer-free survival. The incidence of acute hematological toxicity is greater with combined chemoradiotherapy using 5-FU and MMC than with radiotherapy (RT) plus 5-FU. Anal cancers regress slowly after chemoradiotherapy. Six months after therapy, a large part of the residual mass contains nonmalignant tissue. Frequent, multiple biopsies should be performed to identify residual malignant tissue, which is eminently curable with radical surgery.

Nonepidermoid anal malignancies

Adenocarcinomas may arise in the distal rectum and invade the anal canal. Rarely, adenocarcinomas may arise in the anal canal, either from the anal glands or from malignant transformation of a long-standing fistula-in-ano, such as in Crohn's disease [216]. Despite radical abdominoperineal resection, recurrences are typical.

Paget disease is an extremely rare lesion of the perianal skin. Older patients (mean age 60s) commonly complain of pruritus, occasionally with bleeding, a lump, soiling, or a change in bowel habits. Examination reveals an erythematous, well-demarcated eczematoid plaque with well-demarcated edges, and occasionally with ulceration. The characteristic Paget cells are large, rounded epithelial cells of apocrine origin with pale cytoplasm and a large nucleus. The presence or absence of apocrine markers on staining is used to differentiate between low-grade and high-grade malignant lesions respectively. The workup for an associated malignancy includes at least a colonoscopy. Treatment is by surgical excision guided by the extent of disease; the role of chemoradiation therapy is unclear [228].

Melanomas account for 1% of all anal tumors and most commonly present with rectal bleeding. The lesion often mimics a thrombosed external hemorrhoid. However, up to 25% are amelanotic. These tumors tend to metastasize early with 5-year survival rates of only 15%–20% [229]. Radical resection offers no clear advantage over wide local excision [230,231].

Basal cell carcinoma of the perianal skin is a rare lesion that produces rolled skin edges with central ulceration. The prognosis is good after adequate local excision or radiotherapy [232].

Miscellaneous conditions

Pilonidal disease

This is a common skin lesion of the gluteal cleft seen most frequently in young men, who complain of recurrent drainage, swelling, or pain [233]. Although once thought to be congenital, it is an acquired disorder in which vacuum and pulling forces in the natal cleft are thought to draw hair into the follicles, which in turn become blocked by the hair and

keratin. Subsequently, a foreign body reaction, abscess, and rupture of follicles begin the process by which pilonidal sinus disease forms [234].

The first line of treatment is incision and drainage of any abscesses followed by routine wound care until healing is complete. This is followed by regular and meticulous shaving and hair removal from the midline area of involvement until the wound has closed [235]. Conservative treatment alone is effective if patients present with pain and occasional drainage but no infections or abscess. The use of antibiotics and drains has not been shown to be helpful. If conservative measures fail, multiple surgical options have been reported, none of which has been demonstrated to be completely definitive [236]. One study reported that rhomboid excision and Limberg flap closure was superior to simple excision and primary closure [237]. Other treatments include the addition of a gentamycin collagen fleece to aid healing after primary closure [238] and fibrin tissue glue injection after curettage [239].

Hidradenitis suppurativa

This is a chronic suppurative condition of the follicular epithelium most often involving the axillary and inguinoperineal skin regions. Hormonal influences may be significant as the disease is not seen before adolescence and rarely begins after middle age [240]. Genitofemoral and perineal involvement are seen in nearly one-third of those with the disease, especially in women. Recognized risk factors are female gender, obesity, and acne, although perspiration and mechanical trauma to the skin and hair of the anogenital region may also predispose to perianal hidradenitis [240].

Warm wet compresses, systemic antibiotics, and topical antiseptics are appropriate for early disease. Surgical treatment is the most effective treatment modality for those who do not respond to conservative therapy [240]. Infliximab, a chimeric monoclonal anti-tumor necrosis factor (TNF) antibody, has been reported to be effective in several patients [241]. Other treatments reported to have had some success are combined antiandrogen and antiestrogen therapy and the 5-alpha reductase inhibitor, finasteride [240].

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E PANCREAS

67 Pancreas: anatomy and structural anomalies

David G. Heidt, Michael W. Mulholland, Diane M. Simeone

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Cross-sectional imaging techniques have greatly increased the understanding of pancreatic anatomy, and the ability to manipulate the pancreas surgically, endoscopically, and percutaneously has made it necessary for a wide variety of physicians to become familiar with pancreatic anatomical variations. The anatomy of the pancreas has been described in many standard texts, but the functional and therapeutic implications of its anatomical relations often are underemphasized.

Diseases of the pancreas are often more difficult to manage medically or surgically than those of other abdominal viscera. The pancreas lies hidden in the deeper recesses of the retroperitoneal space of the upper abdomen, is largely protected by the lower rib cage anteriorly, and is almost completely covered by the stomach, transverse colon, and transverse mesocolon. At laparotomy, the pancreas cannot be properly visualized or palpated without extensive dissection, mobilization, and retraction.

The central position of the pancreas provides for lymphatic drainage along several major routes, namely, the splenic, hepatic, and superior mesenteric nodal systems as well as the aortocaval and other posterior abdominal wall lymphatics. Moreover, the intimate anatomical association of the pancreas with vital major vessels of the epigastrium at once limits the extent of any surgical procedure and also dictates what must be removed. Thus, when a tumor spreads a short distance to involve the superior mesenteric vein, the portal vein, or the celiac axis, it usually becomes incurable. Similarly, if the gland is removed in radical fashion, the need to excise the vessels and lymph nodes associated with it frequently makes necessary the removal of the duodenum, gallbladder, distal bile duct, spleen, upper jejunum, and part of the stomach. Finally, the vascular nature of the pancreas and the adjacent organs makes it easy to understand why the most common intraoperative and postoperative complication of pancreatic resection is hemorrhage.

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Embryological development

The pancreas develops from two primordial outpouchings of the duodenum and is first apparent at 4 weeks of gestation. The dorsal pancreatic bud grows more rapidly than the ventral pancreas and by the sixth week extends into the dorsal mesentery. The ventral pancreas remains smaller and is carried away from the duodenum by the development of the hepatic rudiment into the biliary system. Investigations have shown that sonic hedgehog (SHH) expression in the embryonic foregut suppresses pancreatic differentiation of the stomach and duodenum. In the dorsal bud, signals from overlying notochord and dorsal aorta result in downregulation of SHH expression and development of pancreatic tissue. The ventral endoderm closer to the developing major papilla differentiates into pancreatic tissue whereas signals from the septum transversum lead to upregulation of SHH in the nascent hepatic bud [1]. Differential growth of the duodenum and axial rotation of the gut result in the dorsal pancreas being carried to the left and the ventral pancreas being carried to the right of the duodenum (Fig. 67.1). Migration of the distal common bile duct behind and to the left of the duodenum causes the ventral pancreas to lie below the dorsal pancreas, forming the uncinate process of the pancreas. The common bile duct lies posterior to the dorsal pancreatic duct. Fusion of the two parts of the pancreas occurs during the seventh week of gestation. Fusion of the ventral duct with the dorsal duct results in the formation of the main pancreatic duct. The proximal end of the dorsal pancreatic duct usually does not communicate with the main duct and forms the accessory pancreatic duct.

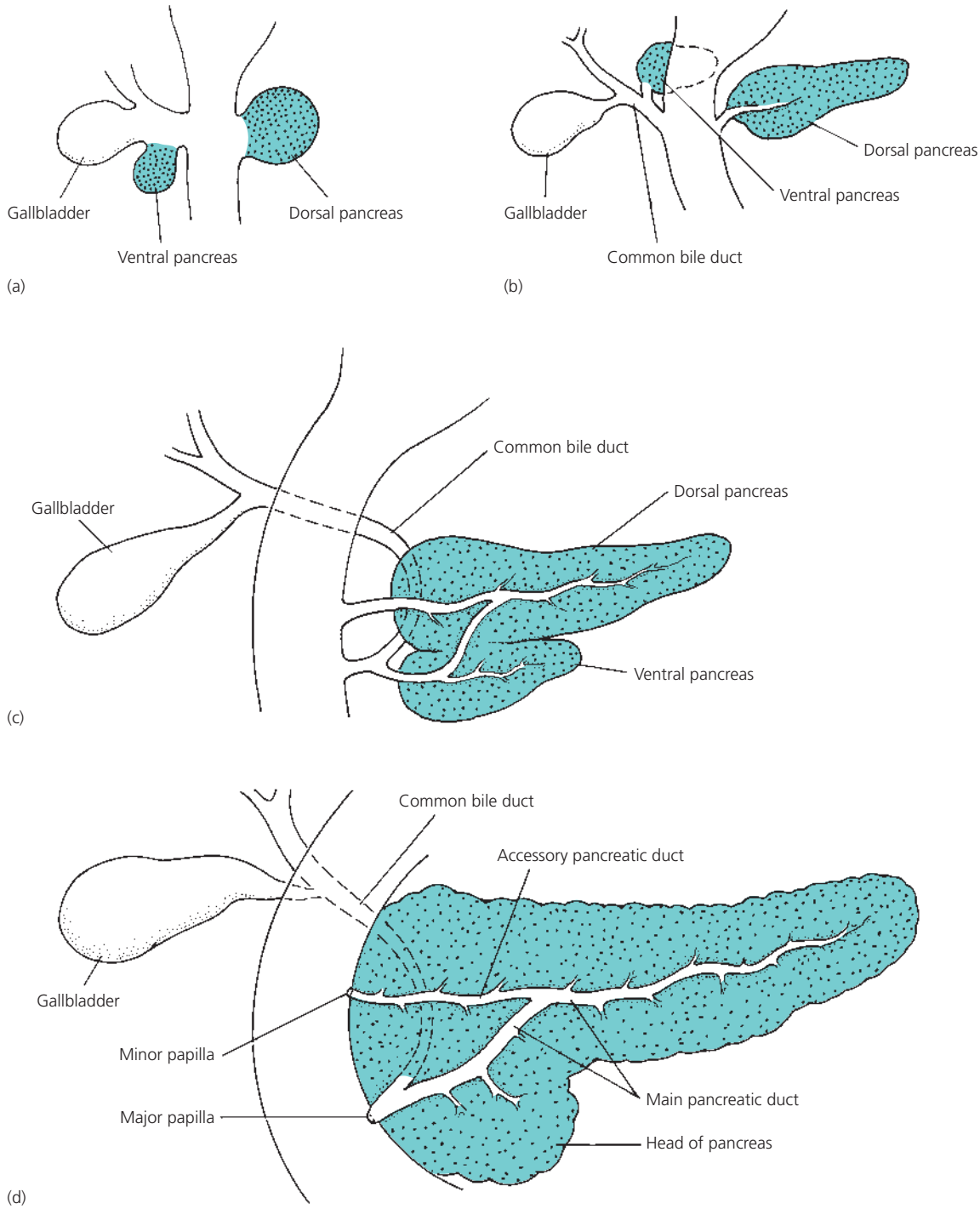

Figure 67.1 Embryological development of the pancreas. The various stages of intrauterine pancreatic growth are shown at 4 weeks **(a)**, 6 weeks **(b)**, 7 weeks **(c)**, and birth **(d)**. Adapted from Arey LB. Developmental Anatomy, 7th edn. Philadelphia: WB Saunders, 1974:259, with permission from Elsevier.

The pancreatic acini and the first zymogen granules appear at 12 weeks of gestation. Groups of endocrine cells develop from multipotential stem cells in the ductular epithelium (nesidioblasts) at 9 weeks of gestation. Discrete islets of Langerhans cells can be identified at 12 weeks. Most of the islet cells develop within the tail of the pancreas and the dorsal pancreas. The first cells to produce granules are the alpha cells, soon followed by the beta and delta cells. Complete maturation of the pancreatic gland does not occur until sometime after the end of gestation. The smooth muscle of

the sphincter of Oddi develops independently of the duodenal musculature, and only later does it become incorporated into the duodenal wall.

Most evidence indicates that islet cells arise from stem cells that appear in pancreatic ducts during the third month of gestation. Insulin-containing granules may be demonstrated immunocytochemically by the end of the third month. Islet cells migrate away from the ducts in which they arise and move into the interlobular connective tissue. Mature islet morphology is established before birth.

Gross anatomy

The pancreas is a soft, flattened, elongated gland, 12–20 cm long in the adult, that lies behind the peritoneum of the posterior abdominal wall and is obliquely rather than transversely oriented (Fig. 67.2; see also Fig. 67.1). The pancreas has a lobular structure and, although totally invested in fine connective tissue, does not have a true fibrous capsule. The adult gland weighs 85–95 g. Because of its oblique orientation, a transverse section or computed tomography (CT) scan normally does not pass through the entire length of the gland. The head of the pancreas is on the right side and lies within the C-shaped concavity of the duodenum at the level of the body of L2. The tail of the gland is to the left, lies between the two layers of peritoneum that form the lienorenal ligament, and is located at the level of the body of L1. The first portion of the duodenum is suspended in front of the head of the gland. The lesser curvature of the second part of the duodenum and the upper aspect of the third part of the duodenum intimately invest the head of the pancreas. Superiorly, the head is related to the gastroepiploic foramen and the structures that form the contents of the free border of the lesser omentum. Anteriorly, the first portion of the duodenum covers the superior part of the pancreatic head, and below this the right side of the transverse mesocolon is attached transversely. Posteriorly, the head of the pancreas contacts the right renal hilum, both renal veins, the inferior vena cava, and the termination of the right gonadal vein as well as the right side of the aorta [2].

The distal end of the common bile duct passes behind the upper border of the head of the pancreas. The bile duct grooves the posterior aspect of the head of the gland before passing through the substance of the head to reach the duodenal papilla of Vater. From the posterior aspect of the head, a tongue of pancreatic tissue of variable size, the uncinate process, extends to the left to occupy the concavity formed by the third and fourth parts of the duodenum. The uncinate process lies anterior to the inferior vena cava and aorta and is covered superiorly and anteriorly by the superior mesenteric vessels as they emerge below the neck of the pancreas.

From the head of the pancreas, a constricted part of the gland, the neck of the gland, extends toward the left. It is 3–4 cm wide and joins the head of the gland to the body of the gland on the left. The pancreatic neck lies behind the posterior peritoneum of the lesser sac, and its inferior border is covered by the attachments of the transverse mesocolon and the root of the small bowel mesentery. The neck of the pancreas lies anterior to and is closely related to the confluence of the superior mesenteric and splenic veins, which form the lowest part of the portal vein.

In front of the aorta in the midline, the body of the pancreas continues its retroperitoneal course toward the left side,

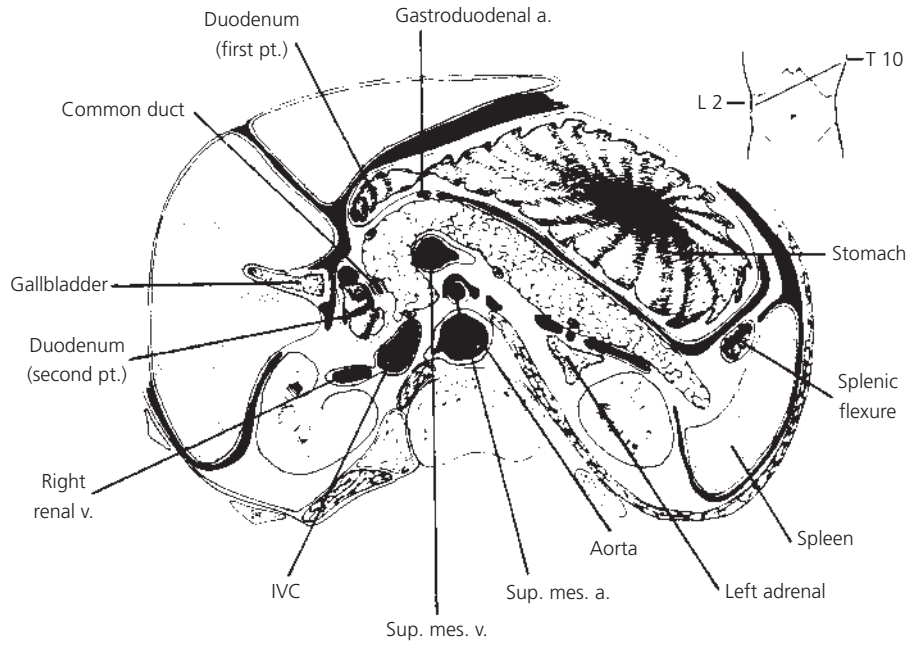

Figure 67.2 Oblique transverse cross-section of the upper abdomen viewed from below. The section passes through the long axis of the pancreas at approximately the level indicated in the inset. The disposition and relations of structures shown approximate those seen on oblique transverse ultrasonic scanning. a., aorta; mes., mesenteric; sup., superior; v., vein.

held securely against the aorta and the posterior parietes by the posterior peritoneum of the lesser sac. The antrum and the body of the stomach contact the body of the pancreas anteriorly. Posteriorly, the left renal vein, passing between the aorta and the pancreas, is separated by the latter from the first part of the superior mesenteric artery. At a slightly higher level, elements of the celiac and superior mesenteric plexus ramify between the pancreas and the aorta. The midline part of the body of the pancreas is pushed anteriorly by the bodies of L1 and L2 and, therefore, lies closest to the anterior abdominal wall. Because of its prominence and fixity in this region, this area of the pancreas is most vulnerable to blunt abdominal injuries. In addition, a tumor in this region may be palpated as a mass that does not move with respiration and that strongly transmits the aortic pulsation. The celiac axis, surrounded by the celiac plexus, divides into its major branches between the superior border of the body of the pancreas and the crura of the diaphragm.

The body of the pancreas passes laterally, posteriorly, and slightly cephalad behind the posterior peritoneum of the lesser sac and merges imperceptibly with the tail of the gland. The exact junction of the body and tail is not discernible anatomically. The posterior relationships of the body and tail of the pancreas include the posterior attachment of the left crus of the diaphragm, the left suprarenal gland, the upper pole of the left kidney, and hilum of the spleen. The splenic vein above and the left renal vein below lie close to one another behind the body and tail of the gland.

At the upper border of the body and tail of the pancreas, the splenic artery courses to the left from its origin at the celiac axis. The transverse mesocolon is attached to the anterior part of the lower border of the gland. The splenocolic ligament attaches the splenic flexure of the colon to the hilum of the spleen and brings it adjacent to the tail of the pancreas.

The shape and disposition of the pancreas varies, as demonstrated by pancreatography. In 57% of people, the bulk of the head of the gland is to the right of the spinal column. In about 38% of cases, it lies directly over the spine, but only rarely (5%) does the head of the pancreas lie to the left of the spine. There is a tendency in elderly people for the pancreas to be ptotic. The duodenal opening of the main pancreatic duct lies at the level of L2–L4 in 92% of cases, but rarely it has been described at a lower level.

Surgical exposure

Although the pancreas is located retroperitoneally, it must be approached by way of an anterior laparotomy incision. Little of the gland can be seen or palpated at laparotomy without dissection. In a thin patient, small areas of the head may be seen directly behind the peritoneum of the supracolic and right infracolic compartments, and the inferior border of the body and tail may be seen from the left infracolic compartment at the root of the transverse mesocolon. These limited views, however, usually are obscured by omental, mesocolic, and retroperitoneal fat. The neck of the pancreas may be felt from above by a finger passed through the gastroepiploic foramen of Winslow.

The head of the pancreas may be inspected and palpated more closely by performing two maneuvers [3]. First, the hepatic flexure of the colon is mobilized downward and medially, dividing the attachments of the transverse colon to the front of the duodenum and pancreatic head as far as the origin of the middle colic vessels. In addition, the attachments of the right side of the greater omentum to the transverse colon are divided. Thus, the lesser sac can be widely exposed on the right side of the middle colic vessels. Second, the peritoneum lateral to the second part of the duodenum is incised, and the duodenum and pancreatic head may be elevated and swept to the left by blunt dissection, exposing the right renal vein, the inferior vena cava, the right gonadal vein, the origin of the left renal vein, and the retroduodenal and retropancreatic portions of the distal common bile duct. After this mobilization (Kocher maneuver), the head of the pancreas may be palpated anteroposteriorly between the thumb and fingers. The mesenteric vessels are obscured from view by the uncinate process.

Limited sight of the superior part of the body of the pancreas may be obtained by opening an avascular part of the lesser (gastrohepatic) omentum and retracting the lesser curvature of the stomach inferiorly. This maneuver also brings the celiac axis into view. A much better sight of the body of the pancreas may be obtained by widely opening the lesser sac, which can be achieved by dividing the gastrocolic omentum at its attachment to the transverse colon. Extending this opening to the right into the subpyloric region allows visualization of the anterior aspect of the head and neck of the pancreas, especially if the right gastroepiploic vessels are ligated and divided. Extending the opening to the left and dividing the short gastric (gastrosplenic) vessels in the gastrosplenic ligament superiorly and the relatively vascular splenocolic ligament inferiorly will give complete visualization of the anterior surface of the body and tail of the pancreas. The spleen, splenic vessels, and tail of the pancreas may be mobilized medially and anteriorly en bloc, allowing inspection of the posterior aspect of the tail and body of the gland. All these surgical maneuvers may be carried out quickly and safely with little risk of damage to vital structures or troublesome bleeding. By this means, all except the region of the neck and uncinate process of the pancreas may be evaluated fully.

Arterial blood supply

The pancreas derives its blood supply from the celiac axis and the superior mesenteric artery (Fig. 67.3). These major

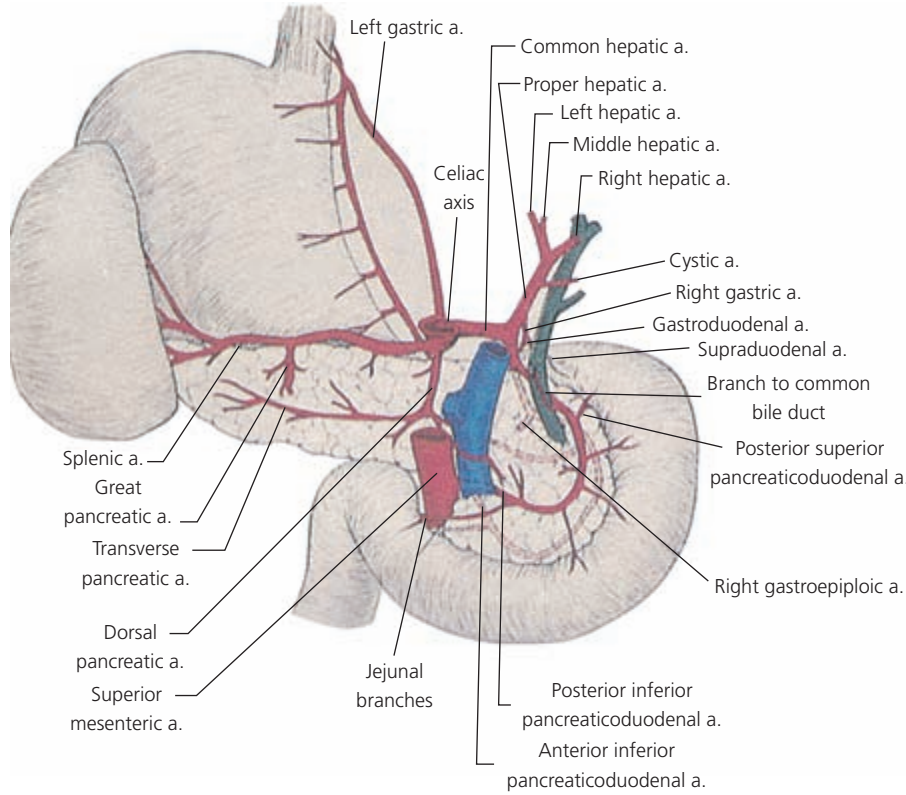

Figure 67.3 Blood supply of the pancreas. The pancreas, duodenum, stomach, and spleen are viewed from their posterior aspects. a., aorta.

vessels and their branches also provide the blood supply to other vital organs adjacent to the pancreas.

Typically (in 90% of people), the celiac axis divides into the common hepatic, the splenic, and the left gastric arteries. The common hepatic artery passes to the right, anterior to the portal vein, to reach the free border of the lesser omentum and usually lies to the left of the common bile duct. After giving off the gastroduodenal artery, the hepatic artery turns upward toward the porta hepatis and divides into the left and right hepatic arteries. The middle hepatic artery, supplying the caudate lobe of the liver, is usually a branch of one of these two vessels. This arrangement of the hepatic arterial blood supply, however, occurs in only 55% of people. Replaced or accessory hepatic arteries are frequent, occurring in as many as 30% of people. The most frequent variation from normal is the right hepatic artery branching off from the superior mesenteric artery and coursing behind the uncinate process and the head of the pancreas to reach the free border of the lesser omentum [4].

The gastroduodenal artery usually originates from the hepatic artery. The gastroduodenal artery courses from its origin behind the first part of the duodenum and, after giving off the posterior superior pancreaticoduodenal artery, lies on the anterior surface of the head of the pancreas. At the lower border of the first part of the duodenum, it branches into the right gastroepiploic artery and the anterior superior pancreaticoduodenal artery.

For the most part, the head of the pancreas and the duodenum have a common blood supply: the anterior and posterior pancreaticoduodenal arcades. The anterior arcade gives off the only arteries that enter the head of the gland from its anterior aspect. This arcade is formed by the anastomosis of the anterior superior pancreaticoduodenal artery, which is a terminal branch of the gastroduodenal artery, and the anterior inferior pancreaticoduodenal artery, which is usually a branch of a common inferior pancreaticoduodenal artery, arising from the superior mesenteric artery.

The posterior pancreaticoduodenal arcade is formed by the anastomosis of the posterior superior pancreaticoduodenal artery, usually a branch of the gastroduodenal artery, and the posterior inferior pancreaticoduodenal artery, which arises in a way analogous to the anterior pancreaticoduodenal artery. Of surgical importance is the relation of the posterior superior pancreaticoduodenal artery to the common bile duct. Typically, this vessel arises from the gastroduodenal artery and passes to the right, anterior to the common bile duct, contributing the major source of the structure's blood supply. The supraduodenal portion of the common bile duct also may be crossed anteriorly by the right hepatic artery, the right gastric artery, and the supraduodenal artery, making mobilization of the lower end of the common bile duct more difficult.

Because of the shared blood supply of the duodenum and pancreatic head, extensive interference with the pancreaticoduodenal arcades in the course of a 95% pancreatectomy

may compromise the blood supply of the duodenum [5]. In addition, because the duodenojejunal flexure and the first part of the jejunum may derive their blood supply from branches of the inferior pancreaticoduodenal artery or the pancreaticoduodenal arcades, ligation of these vessels in the course of a resection may render the proximal jejunum ischemic.

The body and tail of the gland derive their blood supply chiefly from branches of the splenic artery. This vessel, the largest branch of the celiac axis (5–11 mm in diameter), courses laterally at the upper border of the pancreas; its characteristic marked tortuosity appears to be related to age: It is often absent in infants and is most marked in the elderly. The splenic artery gives multiple side branches to supply the neck, body, and tail of the pancreas. The termination of the splenic artery passes between the layers of the lienorenal ligament and, at the hilum of the spleen, four or five branches enter the splenic hilum separately.

Venous drainage

The general pattern of veins draining the pancreas is the same as that of the arterial blood supply (Fig. 67.4). Blood from the pancreas drains ultimately into the portal vein, which is formed by the junction of the superior mesenteric vein and splenic vein behind the neck of the gland. The close relationship of the portal and superior mesenteric veins to the head, neck, and uncinate process of the pancreas is of vital surgical importance; in determining the resectability of a pancreatic lesion, a key step is assessment of involvement of these veins. The portal vein originates from behind the neck of the pancreas as a continuation of the superior mesenteric vein after it becomes confluent with the splenic vein. The portal vein passes upward to the right to gain access to the free border of the lesser omentum, where it lies posterior to the hepatic artery and the common bile duct. At the porta hepatis, it divides into a short, broad right branch and a longer, narrower left branch. In adults, the portal vein is about 8–10 cm long and 8–14 mm wide. Its average length is about 8.4 cm [6].

The splenic vein originates at the hilum of the spleen by the confluence of five or six tributaries draining the spleen. It receives the basal brevia (short gastric veins) and left gastroepiploic veins at the hilum. It passes through the lienorenal ligament behind the tail of the pancreas and below the splenic artery and is the large, straight vein that courses to the right in contact with the posterior surface of the pancreas. It receives many tributaries from the tail, body, and neck of the gland.

Below the pancreas, the ileocolic vein passes directly, and usually without tributaries, from the ileocecal region to join the right side of the superior mesenteric vein in the midline just above the inferior border of the third part of the duodenum. At about the same level on the left side, the superior mesenteric vein is joined by the jejunointermediate vein. The

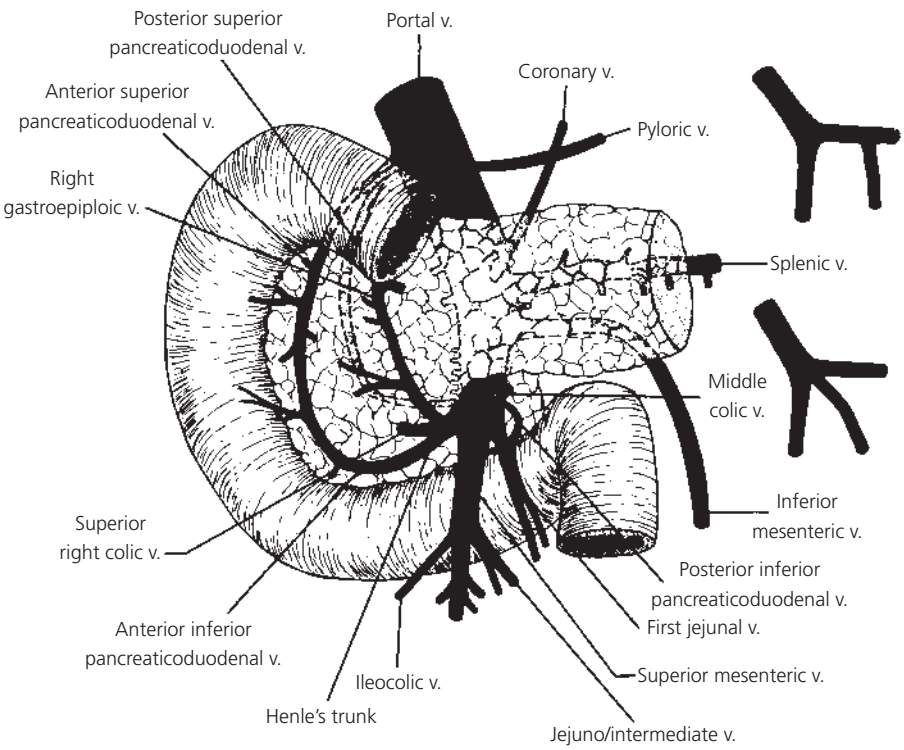

Figure 67.4 Venous drainage of the pancreas. The pancreas and duodenum are viewed from their anterior aspects. **Insets**: normal variations in the termination of the inferior mesenteric vein. v., vein.

confluence of these three veins forms the lower limit of what has been termed the surgical trunk of the superior mesenteric vein. Above this point, about two-thirds of the superior mesenteric vein lies below the inferior border of the neck of the pancreas, with the remaining one-third retropancreatic. The superior jejunal vein joins the left side of the superior mesenteric vein. The middle colic vein usually joins the superior mesenteric vein immediately below the inferior border of the pancreatic neck.

The inferior mesenteric vein typically joins the splenic vein vertically behind the body of the pancreas. The latter passes horizontally and combines with the superior mesenteric vein at a right angle to form the portal vein, which turns obliquely to the right to pass into the free edge of the lesser omentum. Small veins draining the head of the pancreas pass directly into the portal vein. Similarly, small veins draining the uncinate process terminate in the right and posterior aspects of the retropancreatic superior mesenteric vein. The anterior aspects of the major veins are usually free from tributaries. From the left side, the coronary vein joins the retropancreatic portal vein, and the pyloric vein joins the suprapancreatic portal vein. The superior and posterior pancreaticoduodenal veins drain into the portal vein from the right side, deep to the first part of the duodenum and opposite the pyloric vein. The coronary, or left gastric, vein usually (in 60% of cases) drains into the left side of the retropancreatic portal vein, but it may have a high termination well above the neck of the pancreas.

The close anatomical relationship of the splenic vein with the pancreas often leads to splenic vein occlusion in inflammatory or neoplastic diseases involving the body and tail of the gland. Retrograde venous drainage toward the splenic hilum and then by way of the short gastric veins and the left gastroepiploic vein creates the syndrome of left-sided portal hypertension and gastric varices.

Lymphatic drainage

The lymphatic vessels of the pancreas conform to the general pattern of deep lymphatic drainage and accompany the arterial supply (Fig. 67.5). The duodenum and head of the pancreas have a common lymphatic drainage; lymph from the foregut and midgut structures, including the pancreas, liver, stomach, spleen, small bowel, and proximal large bowel, flows eventually into the celiac and superior mesenteric groups of paraaortic nodes and into the cisterna chyli [7]. The lymphatics of the tail of the pancreas pass to the splenic nodes at the hilum of the spleen, and those of the body of the pancreas pass upward to the pancreaticosplenic nodes lying along the superior border of the gland. These nodes, along with the retropancreatic nodes, drain into the celiac nodes. Lymphatics of the upper anterior part of the head of the pancreas pass through the subpyloric nodes lying behind the first part of the duodenum. Obstruction of these pancreatic lymphatic drainage pathways by tumor may result in the shunting of

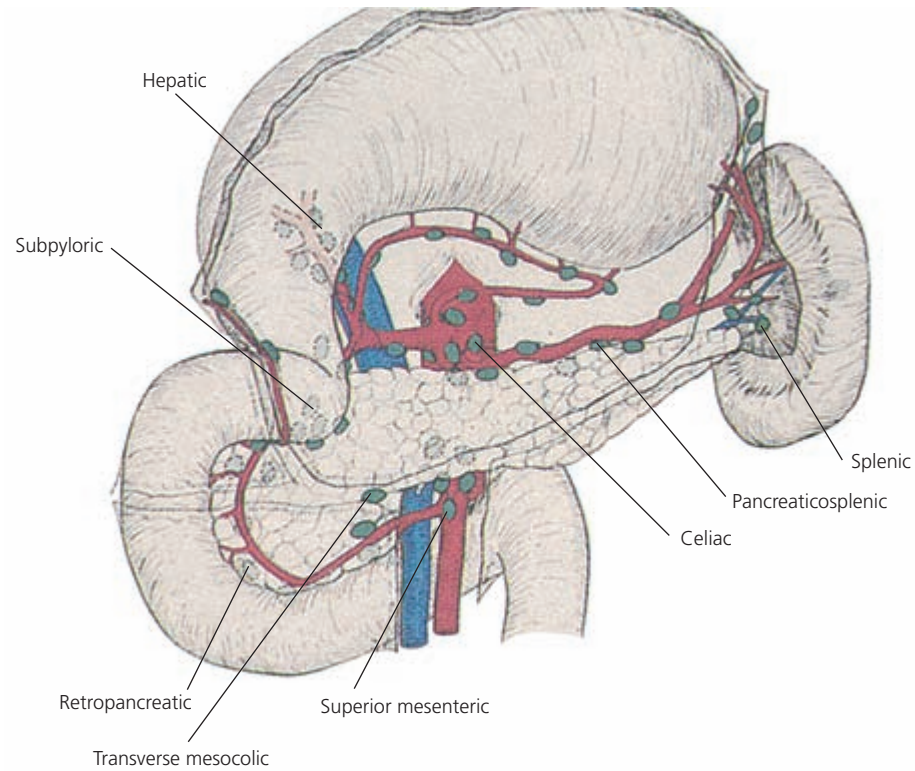

Figure 67.5 Lymphatic drainage of the pancreas. The pancreas is viewed from its anterior aspect. The gastrocolic ligament has been divided along the greater curvature of the stomach, which has been retracted anterosuperiorly. The transverse mesocolon has been detached from the peritoneum of the posterior abdominal wall. Labels indicate representative lymph nodes in the major regional nodal groups.

lymph through local collateral channels, resulting in the involvement of nodes primarily concerned with hepatic or gastric drainage. Inferiorly, the retropancreatic and antepancreatic group of nodes drain into the superior mesenteric nodes, and lymph from the latter also may pass into nodes in the root of the transverse mesocolon. The absence of fascial or retroperitoneal coverings on the posterior aspect of the pancreas allows easy communication between lymphatics of the pancreas and those of neighboring retroperitoneal tissues and organs. The lymphatic network is so rich that a thorough lymphadenectomy en bloc with a pancreatic resection frequently produces a profuse postoperative chylous leakage [8].

Nerve supply

The sympathetic efferent innervation of the pancreas is derived from the greater, lesser, and least splanchnic nerves. The bodies of the preganglionic sympathetic neurons originate in the lateral gray matter of thoracic spinal segments 5 through 10. After traversing the sympathetic trunks, presynaptic nerve fibers synapse with postganglionic sympathetic neurons within the celiac plexus, although there is some minor distribution to the pancreas through the hepatic and superior mesenteric plexuses. The celiac ganglion consists of two masses that lie on either side of the aorta, anterior to the crura of the diaphragm and close to the adrenal glands. The right celiac ganglion is partly covered by the inferior vena cava, and the left celiac ganglion is covered by the peritoneum of the lesser sac close to the upper border of the pancreas. The cell bodies of afferent sympathetic neurons are located in dorsal root ganglia. Afferent fibers often cross the midline in celiac ganglia before projecting centrally so that sympathetic afferent innervation is bilateral.

The parasympathetic innervation of the pancreas is derived from the vagal nerves. The cell bodies for efferent vagal fibers are located in the medulla in the dorsal motor nucleus. Efferent fibers pass to the pancreas by way of the celiac division of the posterior vagal trunk. No synaptic connections are made within the celiac ganglia; postsynaptic neurons are located within the pancreatic parenchyma. Afferent vagal fibers also pass through the celiac ganglia; afferent cell bodies are located within the nucleus ambiguus [9].

The ultrastructure of pancreatic innervation has been studied extensively in several animal species in addition to humans. Myelinated fibers usually are not found within the parenchyma. Intrapancreatic ganglion cells are seen within the interlobular tissues, with nonmyelinated fibers passing to both exocrine and endocrine portions of the gland. The sites of nerve termination may be generally grouped as blood vessels, pancreatic acinar cells, ductal cells, and pancreatic islets. Functional correlates suggest that pancreatic nerves may modulate the function of each of these pancreatic elements. In addition to the classic cholinergic and adrenergic neurons, evidence suggests that a large number of peptide-containing neurons also exist within the pancreas. Immunocytochemical methods have demonstrated the presence of fibers or cell bodies containing vasoactive intestinal polypeptide, substance P, cholecystokinin-8, gastrin-releasing peptide, enkephalin, galanin, neuropeptide Y, and calcitonin gene-related peptide [10,11]. The physiological actions of many of these peptidergic nerves within the pancreas remain to be defined, but anatomical studies suggest that modulation of release of acetylcholine and catecholamines from autonomic nerve terminals within the pancreas may be important. Regulation of pancreatic secretion and blood flow is also an important function of intrapancreatic neurons.

Pain fibers from the pancreas travel through the celiac ganglia and by way of the sympathetic splanchnic nerves and the thoracic sympathetic chain to reach the spinal root ganglia. Pain from the head of the pancreas tends to be broadly localized in the midepigastrium, and pain from the body and tail tends to be localized in the left upper quadrant. Visceral pain from the pancreas usually is sensed as a severe, constant discomfort in the epigastrium. Because the pancreas does not contact the somatically innervated parietal peritoneum, sharply localized pain usually does not occur. Radiation of pancreatic pain to the back in the area of the lower thoracic vertebrae is common.

Studies suggest that pancreatic innervation may be altered in disease states such as chronic pancreatitis. In patients with chronic pancreatitis, the number and diameter of intralobular and interlobular nerve bundles have been reported to be increased relative to normal. Pancreatic nerves in these patients demonstrated increased immunostaining for substance P and calcitonin gene-related peptide [12]. Because these peptides are known to be expressed in afferent neurons, the changes have been postulated to be responsible for the pain syndrome associated with chronic pancreatitis. Alterations in peptidergic pancreatic innervation also have been reported in animal models of chronic pancreatitis [13]. The hypertrophy of pancreatic nerves seen in chronic pancreatitis has been associated with increased expression of nerve growth factor (NGF) by acinar and islet cells. Expression of NGF results in increased expression of its receptor in neural tissue, and an increase in receptor expression has been shown to correlate with pain intensity [14,15]. Interactions of nerves with pancreatic inflammatory cells have been proposed as a cause [16].

Ductal system

The main pancreatic duct of Wirsung extends from the tail of the pancreas to the major duodenal papilla or ampulla of Vater (Figs 67.6 and 67.7). The average diameter of the duct in the adult tapers from 4 to 2 mm, and it is widest in the head of the gland [17]. The main duct is close and almost parallel to the distal common bile duct for 2–3 mm, then

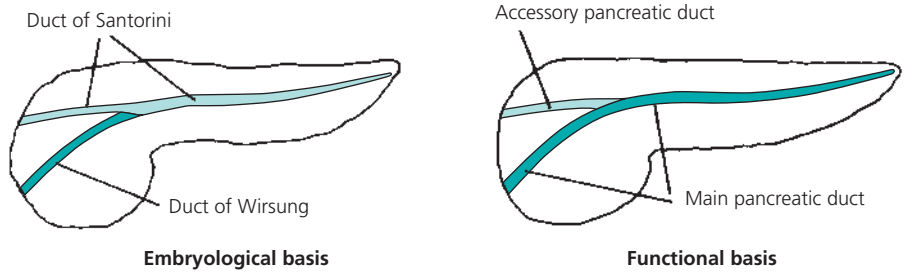

Figure 67.6 Terminology variously applied to describe the pancreatic duct system. One system indicates an understanding of ductal embryology, particularly with regard to the development of the more unusual variations **(left)**. For clarity and practicality, however, the terms given on the **right** are preferred.

combines to form a common duct channel before opening into the duodenum. The accessory pancreatic duct of Santorini, which is present in 40%–70% of people, usually communicates with the main duct and passes transversely to the right in the upper part of the head of the pancreas. The duct of Santorini lies anterior to the intrapancreatic common bile duct and usually opens into the proximal portion of the second part of the duodenum at the minor papilla, proximal to the ampulla of Vater.

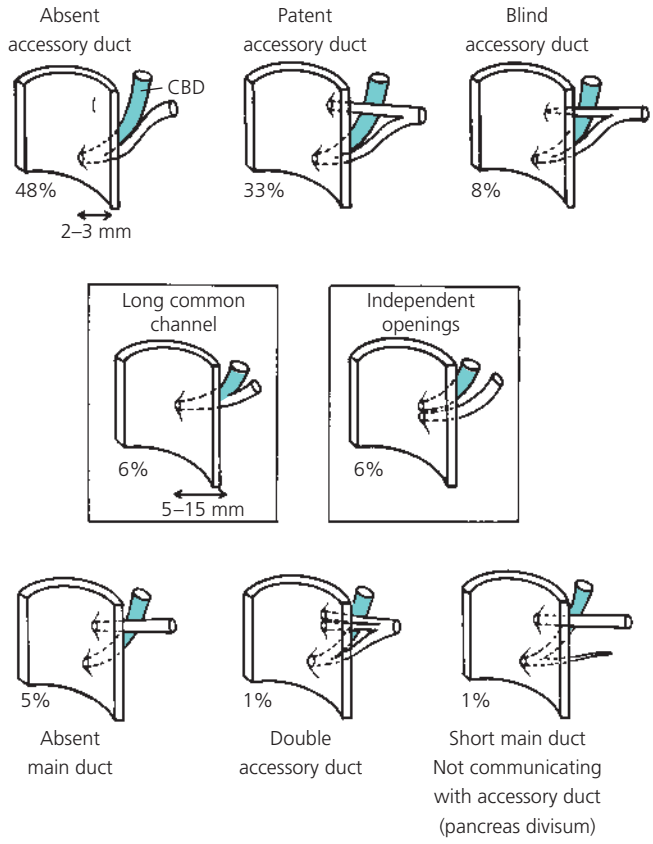

Figure 67.7 Variations of main and accessory pancreatic ducts, and their relation to the common bile duct (CBD) from a series of 143 postmortem preparations. Accessory duct variations and usual short common channel for CBD and main pancreatic duct **(top)**; variations of CBD and main pancreatic duct terminations **(middle)**; miscellaneous variations **(bottom)**. Adapted from Berman et al. [17], with permission from the Journal of the American College of Surgeons.

This "typical" ductal anatomy actually may be present in as few as 33% of people [18]. Important variations include nonpatency of the accessory duct (8%), independent openings of the common bile duct and the main pancreatic duct (6%), an absent main duct (5%), and patent double accessory ducts (1%). Noncommunication between the main duct and the accessory duct, with the body and tail of the gland draining exclusively by way of the duct of Santorini, results in the anatomical variant known as *pancreas divisum*, which occurs in 2%–6% of otherwise healthy people.

The sphincter of Oddi consists of circular smooth muscle that surrounds the common channel of the common bile duct and the main pancreatic duct at the ampulla of Vater. The muscle fibers of the sphincter of Oddi extend around the common bile duct just distal to the latter's oblique entry into the wall of the duodenum to form the choledochal sphincter. A short region of circular smooth muscle also surrounds the pancreatic duct just before its termination to form the pancreatic duct sphincter.

Ultrastructure

The pancreas is a mixed endocrine and exocrine gland. Its glandular constituents by weight are 80% exocrine tissue, 18% ductular system, and 2% endocrine tissue. Also, the pancreas is a lobulated organ with lobular subunits composed of acini; the latter are rounded or have a short tubular form and consist of single rows of epithelial cells lying on a basal lamina. Lining the lumen of the acinus are pyramidal acinar cells and pale-staining centroacinar cells, which are unique to the pancreas. The acinar lumen connects with the intralobular ducts to form the interlobular ducts, which, in turn, coalesce to form the main pancreatic duct. Lining the ductules are columnar cells, goblet cells, and occasional argentaffin cells. The large ducts are surrounded by thick layers of connective tissue and elastic fibers.

In the resting state, the basal portion of the acinar cell contains a centrally located spherical nucleus lying within a highly basophilic cytoplasm secondary to the large number of ribosomes in the rough endoplasmic reticulum. The abundant ribosomes attest to the high protein-synthesizing capacity of the acinar cells. A clear region containing the Golgi

complex separates the nucleus from numerous eosinophilic zymogen granules, each about 1 μm in diameter, lying at the apex. The acinar cell has short microvilli, averaging 0.2 μm in length, which protrude into the acinar lumen. At the apical portion, the cells are held together by tight junctions, which prevent reflux of lumenal contents. Laterally, the cells are connected by gap junctions, which permit intercellular communication.

The thin basal lamina on which the acini rest is supported by collagen fibers. A rich capillary plexus surrounds the acinus in this connective tissue, and this plexus is penetrated by numerous nerve fibers, which reach the acinar cells [19].

The endocrine portion of the pancreas consists of about one million islets of Langerhans, which are distributed throughout the gland but are relatively concentrated in the tail of the pancreas. The islets are about 200 μm in diameter, and each is associated with a prominent capillary plexus. The islets contain several cell types. About 75%–80% of islet cells are beta cells (or B cells), which secrete insulin, and 10%–20% are alpha cells (or A cells), which contain glucagon. Delta cells (or D cells) constitute about 5% of the islets and contain somatostatin [20]. The beta cells occupy the center of the islets, whereas the perimeter is lined by alpha cells. Delta cells are dispersed between these groups. Other peptide-secreting cells that may be associated with the islets include enterochromatin cells, containing 5-hydroxytryptamine, and pancreatic polypeptide cells, containing pancreatic polypeptide.

Congenital anomalies

Agenesis or hypoplasia of the pancreas

Agenesis of the pancreas is a rare and, at one time, universally fatal condition [21]. Failure of the pancreas to develop may occur as an isolated anomaly, or it may be associated with other congenital defects, such as absence of the gallbladder [22]. Although the cause is not known, pancreatic agenesis has been associated with retarded intrauterine growth, presumably resulting from failure of the endocrine pancreas to produce insulin [23]. Partial agenesis of the pancreas results from incomplete formation of either the dorsal or ventral pancreas and has a more favorable outcome than complete agenesis. Involvement of the dorsal segment appears to be more common than involvement of the ventral pancreas. These glands possess normal exocrine and endocrine function.

Hypoplasia of the pancreas is a congenital disease involving exclusively the exocrine pancreas and has been referred to as lipomatous pseudohypertrophy of the pancreas [24]. Pathologically, the gland appears enlarged and of normal shape but of a fatty consistency. The major pancreatic ducts are developed, and islets of Langerhans are present; however, secondary pancreatic ducts and acinar lobules are absent or underdeveloped, and fatty tissue replaces normal acinar cells [25]. The finding that the gland is of normal shape, but missing differentiated cellular components, led investigators to postulate that embryological development is normal in this condition. It is proposed that an intrauterine insult occurs, such as infection, causing hypoplasia of the exocrine gland [26]. Although this disease usually is diagnosed in infants, it has been reported to occur in adults sometimes in association with malignancy [24,25,27]. It is also seen in two autosomally recessive diseases, Shwachman–Diamond syndrome and Bannayan–Riley–Ruvalcaba syndrome [28,29]. Manifestations result from severe pancreatic exocrine insufficiency.

Annular pancreas

Annular pancreas is an unusual complication of disturbed embryological development in which the head of the pancreas surrounds the duodenum, often resulting in duodenal obstruction. Annular pancreas frequently is associated with other congenital defects, including Down syndrome, Meckel diverticulum, malrotation of the intestine, duodenal atresia and bands, intestinal webs, tracheoesophageal fistulas, imperforate anus, absence of the gallbladder, and certain types of cardiac defects [30–34]. Annular pancreas has been described in association with pancreas divisum (see "Pancreas divisum" below) [35]. Men appear to be affected more commonly than women. The occurrence of annular pancreas is usually sporadic, although several reports describe a familial association and apparent autosomal dominant transmission [36–39].

Typically, the annulus is a band of pancreatic tissue completely encircling the second portion of the duodenum (Fig. 67.8). The ring usually lies proximal to the major papilla, and

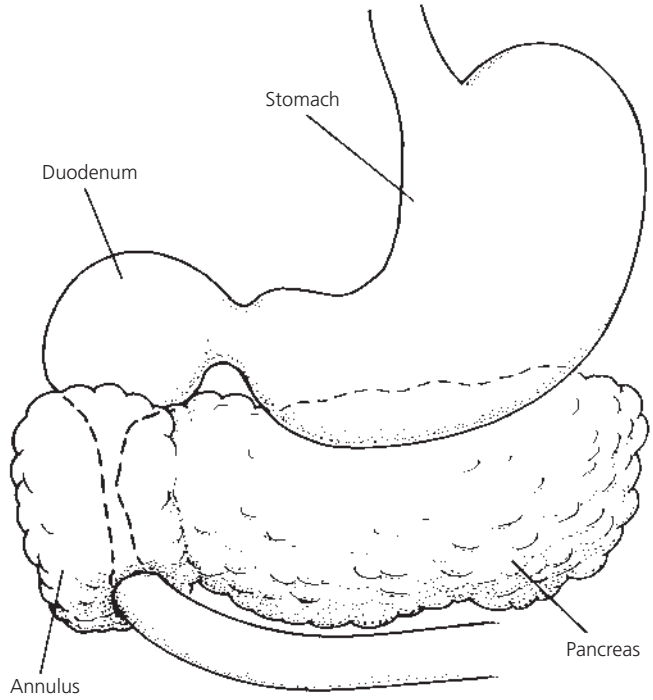

Figure 67.8 Annular pancreas. A band of pancreatic tissue surrounds the midportion of the duodenum.

in a few cases the annulus involves the first or third portion of the duodenum. Histologically, pancreatic tissue frequently invades the muscularis layer of the duodenum [40].

The most popular etiological theory suggests that the ventral pancreatic bud becomes fixed; as the pancreas and duodenum rotate, a band of pancreatic tissue is left encircling the duodenum [41]. Supporting this theory is the report that a high concentration of pancreatic polypeptide cells, which is characteristic of the ventral pancreas, was found in annular tissue [42]. Other theories propose that hypertrophy of the dorsal and ventral ducts or ectopic pancreatic tissue causes the annulus [43].

More than half of all cases are diagnosed in the first year of life. Severe duodenal stenosis is usually apparent within the first few days of birth. Because pancreatic tissue encircles the duodenum, causing obstruction, newborns and infants are intolerant of oral feedings and have vomiting, often of bilious material. Upper abdominal distention and visible peristalsis are often present on physical examination [44]. Although vomiting is the usual presenting symptom in children, adults often complain of postprandial colicky abdominal pain, bloating, and fullness that also may be associated with nausea and vomiting [45–48]. Upper gastrointestinal bleeding and duodenal ulcer disease occur in one-third of adults, and acute pancreatitis is a common associated finding in this disorder.

The radiographic signs of annular pancreas are those of duodenal obstruction. Plain radiographs of the abdomen often show a "double-bubble" sign resulting from dilation of the duodenum and stomach. With high degrees of obstruction, a duodenal cutoff sign also may be seen. In infants, these findings are the only necessary radiographic tests. Radiographic findings are not specific in older children and adults, and it is usually necessary to document duodenal obstruction by upper gastrointestinal contrast studies. Constricting bands, 0.8–5 cm long, around the duodenum have been detected radiographically [49]. Other signs include dilation and reverse peristalsis of the proximal duodenum. Endoscopic evaluation of annular pancreas usually is not helpful, although endoscopic ultrasound will occasionally reveal a circumferential band-like structure with the same echo pattern as the pancreas surrounding the duodenum [50]. The mucosa usually is normal, and it is difficult to appreciate mild narrowing of the duodenum. In severe cases, concentric narrowing of the duodenum or stenosis and associated peptic ulcer disease are suggestive of the diagnosis. Endoscopic retrograde cholangiopancreatography (ERCP) has been used to visualize the ductal system in annular pancreas and is diagnostic of the disorder [51–54]. Magnetic resonance (MR) pancreatography is being used with increasing frequency as a noninvasive alternative to diagnostic ERCP in the evaluation of pathological conditions of the pancreas such as annular pancreas and pancreas divisum [55]. Characteristic features can also be determined by CT [56]. In symptomatic patients, treatment of annular pancreas is surgical alleviation of the obstruction. Division of the annulus is not recommended because of the high incidence of pancreatitis and pancreatic fistulae complicating that procedure, and bypass of the obstructed intestinal segment is preferred [57].

Heterotopic pancreas

Aberrant localization of pancreatic tissue, also known as heterotopic pancreas or pancreatic rests, refers to segments of pancreatic tissue not in continuity with the main body of the pancreas. The condition is usually asymptomatic, and therefore its true incidence is difficult to determine. Heterotopic pancreas has been identified in 1 of every 500 laparotomies, and autopsy studies have reported frequencies ranging from 0.6% to 15% [58,59]. Seventy percent of such rests are found along the upper gastrointestinal tract, with the stomach (25.5%), duodenum (27.7%), and jejunum (15.9%) representing the most frequent locations. Other intraabdominal locations include the gallbladder, liver, small intestine, colon, appendix, omentum, and Meckel diverticulum [60–62]. In the stomach, pancreatic rests are found primarily in the prepyloric region along the greater curvature. Extraabdominal sites include the lung and umbilicus [63–65]. Histologically, heterotopic pancreas can contain acinar tissue, islets, ducts, or any combination thereof. Seventy-five percent of rests are located in the submucosa of the stomach or intestine and appear as firm, yellow nodules, 2–4 cm in diameter, underlying the mucosa. These often have a central mucosal depression, thought to represent the presence of a vestigial duct, that may be recognized endoscopically or by radiographic contrast studies. The origin of heterotopic pancreas is not known, and it is likely that several abnormalities of embryological development account for the locations in various sites. As pluripotential cells, pancreatic rests may represent abnormal differentiation of endodermal stem cells. Conversely, disturbances in migration of the pancreas as it rotates around the gut may account for some heterotopic locations [66].

Heterotopic pancreas is usually an asymptomatic condition that is an incidental finding at surgery or autopsy. Symptoms attributed to this disorder include abdominal pain, nausea, vomiting, and gastric outlet obstruction [67,68]. Peptic ulcer disease and upper gastrointestinal bleeding also have been described. Because of the varied locations of pancreatic rests, involvement of other organs can occur. Pancreatitis, biliary obstruction with jaundice, intestinal obstruction, and intussusception all have been associated with heterotopic pancreas [69–75]. As a result of the combined exocrine and endocrine function of the pancreas, any pathological change in the normal pancreas also can occur in ectopic tissue [76]. Therefore, malignant degeneration, cyst formation, and islet cell tumors also may be found in pancreatic rests [77–83].

Pancreatic rests involving the upper gastrointestinal tract often are detected initially by contrast radiography or endoscopy as a submucosal bulge with central umbilication [84,85]. These lesions are covered by normal mucosa, making routine

endoscopic biopsy impossible. Heterotopic pancreas may be difficult to differentiate from leiomyomas, fibromas, carcinoid tumors, or other malignant tumors. Endoscopic ultrasound (EUS) has become an increasingly useful modality in the evaluation of these submucosal gastrointestinal masses. It not only can distinguish between an intramural lesion and compression from an extralumenal mass, but also allows identification of the wall layer from which the mass originates. For example, pancreatic rests, located in the submucosal layer, can be differentiated from leiomyomas, the most commonly encountered submucosal masses, which are located in the layer of the muscularis propria [86]. In other cases in which a definitive diagnosis cannot be made based on EUS appearance, EUS-guided fine-needle aspiration may be used to obtain a histological diagnosis [87,88].

Treatment of ectopic pancreatic tissue is indicated only for those who have significant symptoms or complications, such as recurrent upper gastrointestinal bleeding, biliary or intestinal obstruction, or malignant degeneration. Definitive treatment is surgical removal of the ectopic tissue. Asymptomatic people with incidental discovery of heterotopic pancreas do not require further evaluation or treatment [89]. If unsuspected pancreatic rests are found at surgery, excision prevents the possible complication of malignant degeneration and eliminates confusion if subsequent symptoms develop [89,99].

Pancreas divisum

Pancreas divisum, the most common congenital anomaly of the pancreas, is caused by failure of the ducts of the dorsal and ventral anlagen to fuse during the fifth and sixth weeks of gestation. Normally, the proximal one-third of the dorsal pancreatic duct regresses as it fuses with the ventral duct, forming the main pancreatic duct. In pancreas divisum, the ventral duct of Wirsung empties into the duodenum through the major papilla but drains only a small portion of the pancreas. Secretions from the tail, body, neck, and remainder of the head of the pancreas drain into the duodenum through the minor papilla by way of a persistent duct of Santorini. In 2%–3% of cases in which the ducts do not fuse, the ventral duct may not be demonstrable. Normally, drainage of the pancreas can occur by either duct, depending on fusion of the two ductal systems and the degree of patency of each. Most pancreatic drainage is through the duct of Wirsung, and although some drainage occurs through the duct of Santorini, this volume is relatively small. Occasionally, the duct of Santorini ends blindly in the duodenal wall.

Although pancreas divisum is a long-recognized entity, only with the advent of ERCP has its clinical significance become apparent. In autopsy series, pancreas divisum has an incidence of 5%–10% [90]; as an ERCP finding, its incidence is 4% [91–93]. In a series of patients with pancreatitis, however, there was a 16% incidence of pancreas divisum, and the incidence of the abnormality increased to 25% in idiopathic pancreatitis [91]. Therefore, it appears that pancreas divisum is associated with pancreatitis. It has been postulated that pancreatitis may result from a combination of pancreas divisum and stenosis at the level of the accessory papilla, with impediment of pancreatic secretory flow. As a congenital defect, clinical manifestations of pancreas divisum may occur at any age but are uncommon in childhood. Symptoms may be mild with only occasional epigastric pain occurring postprandially, but more often episodes of severe acute pancreatitis occur. Chronic pancreatitis also may develop, with all of its associated sequelae. Changes in the pancreatic ducts characteristic of chronic pancreatitis may be detected on ERCP.

Pancreas divisum is diagnosed by pancreatography (Fig. 67.9). On ERCP, the major papilla is often difficult to cannulate, but on injection with contrast fluid, the duct of Wirsung appears shortened and of small diameter. There is rapid filling of small accessory ducts, and too much contrast can be injected before it is realized that the duct of Wirsung is not in communication with the main pancreatic duct. As a result, the patient may experience sudden pain, and pancreatitis of the ventral pancreas can develop. What appears as an abrupt cutoff of the duct of Wirsung must not be confused with a mass lesion such as a malignancy or pancreatic pseudocyst. The delicate nature of the accessory ducts on the pancreatogram is a helpful indicator of pancreas divisum. If possible, the accessory papilla should be cannulated, which should reveal a duct of Santorini running the entire length of the pancreas that is not in communication with the duct of Wirsung. Although ERCP remains the gold standard for diagnosis of pancreas divisum, MR pancreatography is an emerging, noninvasive radiographic technique that is being used to define ductal anatomy (see "Annular pancreas" above). If stenosis of the papilla is not demonstrated radiographically, pancreatic manometry and secretin ultrasound scanning may be useful in identifying patients who may benefit from endoscopic or surgical intervention [94,95].

Figure 67.9 Pancreas divisum. An endoscopic retrograde pancreatogram performed through the accessory papilla shows the dorsal duct in pancreas divisum. Courtesy of Peter B. Cotton, Durham, NC.

Patients with mild symptoms can be managed conservatively. Patients with recurrent episodes of acute pancreatitis or chronic pain require intervention to alleviate accessory ductal stenosis. Surgical accessory duct sphincteroplasty has been successful in treating patients, with the best results observed in patients with recurrent episodes of acute pancreatitis [96–98]. Endoscopic therapy with a combination of minor duct sphincterotomy and stenting has achieved similar rates of success; however, the follow-up period in these patients has been less than in the surgical groups [99–101], and long-term stenting of the pancreatic duct has been reported to result in chronic ductal changes [102]. Determination of which patients with acute recurrent pancreatitis would be served best by surgical or endoscopic treatment awaits further studies. Patients with chronic pancreatitis have a poor response to both surgical and endoscopic treatment modalities. In cases in which sphincterotomy has failed or the ductal involvement is more extensive, direct ductal drainage or resection of the involved pancreas is necessary [103,104].

Congenital cysts

Cysts of the pancreas are distinguished from more common pseudocysts by the presence of an epithelial lining. Pseudocysts often follow bouts of acute pancreatitis, with trauma being a major precipitant. Identification of true columnar or cuboidal epithelium in congenital cysts may be difficult, and it is not always possible to distinguish a congenital cyst from a pancreatic pseudocyst. In cysts located in the peripancreatic space, identification of pancreatic enzymes in the cyst fluid is helpful in determining that it is of pancreatic origin; however, not all pancreatic cysts contain fluid rich in pancreatic enzymes. Solitary congenital cysts are rare [105]. They are more commonly diagnosed in early childhood but may remain asymptomatic and undetected into adulthood. Although other congenital anomalies have been reported with pancreatic cysts, these are more often isolated defects [106].

The most common presentation of a solitary pancreatic cyst is as an abdominal mass, which may be associated with abdominal pain or complications resulting from expanding size, such as gastroduodenal obstruction. Encroachment on the intrapancreatic or extrapancreatic common bile duct may cause biliary obstruction [107]. Subcutaneous fat necrosis and osteolytic lesions secondary to pancreatic enzymes leaking into the circulation from a developmental pancreatic cyst have been described [108].

The presence of a pancreatic cyst may be suggested by a plain radiograph of the abdomen or an upper gastrointestinal radiographic series demonstrating a mass displacing a portion of the stomach, duodenum, or colon. The best diagnostic test is CT, which demonstrates a fluid-filled cystic lesion in the pancreas and its relationship to surrounding structures. In considering the differential diagnosis, it is important to consider other possible cystic lesions of the pancreas, including pancreatic pseudocysts, cystadenomas, and cystadenocarcinoma.

Cystic neoplasms of the pancreas are rare, representing about 10% of pancreatic cysts and 1% of pancreatic carcinomas [109]. Abdominal or back pain, the most common symptom, is present in about half of patients. One-third of patients have an abdominal mass or weight loss, and one-third of patients are asymptomatic, with the cyst discovered during evaluation of other complaints. There is a clear predilection for women, with a female-to-male ratio of 8:1. Computed tomography is the most useful diagnostic modality and typically reveals a multiloculated lesion measuring 3–6 cm, located in any region of the pancreas. Two major histological variants exist: serous cystadenomas and mucinous cystic neoplasms. Mucinous cystic neoplasms of the pancreas have clear malignant potential. Serous cystadenomas, by contrast, are almost invariably benign. Preliminary experience with biochemical and cytological analysis of cyst fluid to distinguish serous and mucinous neoplasms has been disappointing. Carcinoembryonic antigen (CEA) has been the most useful marker of malignancy in cyst fluid obtained by endoscopic ultrasound-guided fine needle aspiration, but it still has an accuracy of only 79%. The accuracy of cytology has been shown to be poor (59%), and no combination of tests, including EUS appearance, has been shown to be more accurate than CEA alone. Work is underway to determine the utility of proteomic and DNA analysis of cyst fluid in the differentiation of benign from malignant cystic neoplasms [110–112]. The differentiation of congenital cysts, acquired pseudocysts, and cystic neoplasms is crucial. The operative therapy for chronic pseudocysts is nonresectional, whereas therapy for cystic neoplasms requires resection [113].

Symptomatic congenital cysts should be removed whenever possible, which can be done by enucleation of the cyst if it is located in the body or head of the pancreas, or by resection of the cyst with adjacent pancreatic tissue if it is confined to the distal pancreas. If either of these approaches is precluded by the location of the cyst, such as a cyst in the head of the pancreas, drainage into the stomach or jejunum can be performed. With surgical drainage of the cyst, pathological examination should be done to exclude malignancy.

Multiple cysts

Multiple congenital pancreatic cysts are rare and usually associated with other congenital anomalies, most frequently polycystic kidney disease and cystic fibrosis. Other clinical syndromes involving multiple pancreatic cysts include von Hippel–Lindau syndrome, Ivemark syndrome, and Bruber syndrome, in which cysts of the lung, liver, and central nervous system also are found [114,115]. Many of the associated anomalies are lethal, although patients may have no symptoms referable to the pancreatic cysts. Because of the numerous cysts and the involvement of several organ systems, no treatment is necessary unless specific symptoms or a

particular complication, such as infection of a cyst, dictates surgical excision or drainage.

The reader is directed to Chapter 15 (Pancreatic secretion) and Chapter 72 (Hereditary diseases of the pancreas).

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E PANCREAS

68 Acute pancreatitis

Mark Topazian, Stephen J. Pandol

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Definition

Acute pancreatitis is a disorder primarily of the exocrine pancreas that involves varying degrees of acute inflammation associated with parenchymal injury of the gland. In its most severe forms the parenchymal injury is manifest as necrosis of pancreatic tissue and dysfunction of other organs both adjacent to and distant from the pancreas. The clinical syndrome resulting from an episode of acute pancreatitis includes the rapid onset of abdominal pain with variable abdominal findings ranging from mild tenderness to peritoneal signs. The disease is often accompanied by vomiting, fever, tachycardia, leukocytosis, and increased levels of pancreatic digestive enzymes in the blood and urine. In severe cases there is dysfunction of other organ systems (i.e., pulmonary, renal, cardiovascular, hepatic, central nervous system, and adipose tissue).

Epidemiological studies demonstrate that approximately 70% of cases of acute pancreatitis are related to biliary stones or alcohol abuse [1]. Numerous other etiologies account for another 20%, and about 10% of cases are idiopathic despite extensive evaluation. Although there are many potential etiologies for acute pancreatitis, only a few of the etiologies (i.e., alcohol abuse, genetic disorders, and hyperlipidemia) lead to chronic pancreatitis. The mechanisms underlying the transition from acute pancreatitis to chronic pancreatitis are unknown.

The first systematic description of acute pancreatitis was published by Reginald Fitz in 1889 [2]. The paper described the clinical and pathological findings in 53 patients with this disorder. The descriptions included the pattern of abdominal pain as well as clinical observations such as fever, jaundice, and abdominal findings. Fitz's paper established the initial diagnostic criteria for pancreatitis. The report presented evidence for etiological factors such as gallstones, alcohol abuse, trauma, and penetrating peptic ulcers; and pathological findings of pancreatic pseudocysts, pancreatic abscess, splenic vein thrombosis, and disseminated fat necrosis. Thus, the description of acute pancreatitis by Fitz set the stage for mechanistic and clinical studies designed to improve prevention, diagnosis, and treatment of this disorder.

Incidence

The incidence of acute pancreatitis ranges from 5 to 73 per 100 000 per year [3–8]. This incidence is an approximation for several reasons. First, the incidence is subject to variations between populations and ethnic groups as a function of the prevalence of precipitating factors such as alcohol abuse and biliary stone disease. Second, histological confirmation is available for only a few patients with acute pancreatitis. The diagnosis is often presumptive and based on clinical assessment with accompanying laboratory and imaging tests, all having limited sensitivity and specificity. Finally, some patient groups may not come to medical attention because of mild disease or because of limited access to medical care.

Reports from some countries [1,3–6,8] show a slightly greater incidence of acute pancreatitis in males compared with females. Further, in reports where precipitating factors are considered, alcohol abuse is more commonly associated with male cases than female cases, whereas gallstone disease is more commonly associated with female cases than male cases [3,5,6]. Thus, the total incidence and the gender distribution of cases of acute pancreatitis in different areas of the world are greatly influenced by the incidence of alcohol abuse and gallstone disease.

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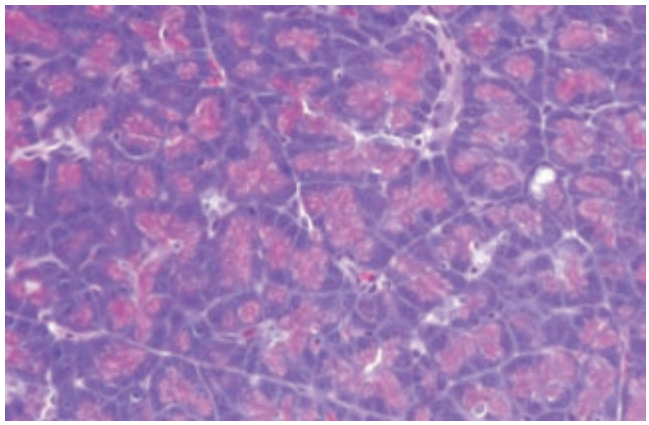
Classification

The simplest way to classify acute pancreatitis is by histology. Mild interstitial pancreatitis and severe necrotizing pancreatitis (Fig. 68.1) represent the two extremes of the disease. However, histological examination of the tissue is rarely possible. Considering this situation, other classification systems of acute pancreatitis have been devised that consider the potential causative factor(s) or severity of illness. These systems use sets of information from clinical observations, laboratory tests, and imaging studies. Several of these systems are discussed in more detail later in the chapter.

A sensible and useful approach, proposed by an international symposium convened in Atlanta [9], separates acute pancreatitis into mild and severe disease based on a combination of clinical findings, laboratory tests, and imaging studies. Mild pancreatitis is not associated with local or systemic complications and recovery is usually uneventful without need for medical or surgical interventions other than accepted supportive care. However, severe pancreatitis is associated with impaired pancreatic function, local or systemic complications, the need for medical or surgical interventions, and a significant mortality rate. In cases of severe pancreatitis, the Atlanta classification provides definitions for both systemic and local complications.

Careful determination of the local and systemic complications in cases of severe pancreatitis promotes optimal care of such patients. Cases of severe acute pancreatitis require the collaboration of gastroenterologists, intensivists, radiologists, and surgeons accurately to diagnose complications and to apply appropriate treatments.

(a)



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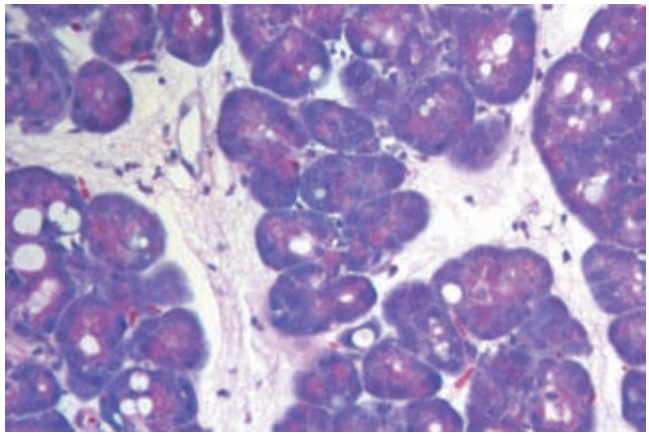

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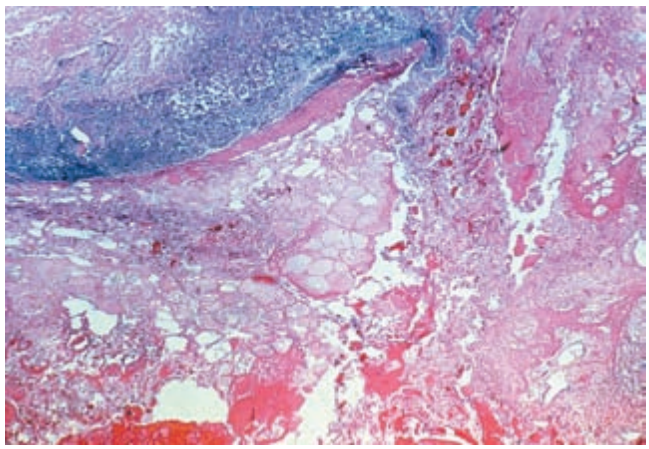


Figure 68.1 **(a)** Histology of the normal exocrine pancreas. This histological (H & E stain) image of pancreatic tissue features mostly pancreatic acini. Of note, pancreatic acinar cells are arranged in a spherical or tubular structure, the acinus, with basally located nuclei and apical lumen. The red (eosinophilic) staining in the apical area results from acidic zymogen granules. The blue staining in the basal area results from basic endoplasmic reticulum and the nucleus. The dark nuclei are located at the base of the acinar cells. There is a small pancreatic ductule in the upper right center part of the image adjacent to an acinus. There are no islets of Langerhans in the photomicrograph. **(b)** Histology of mild acute pancreatitis. Mild (edematous) pancreatitis is associated with edema, mild to moderate pancreatic inflammation, and acinar cell vacuolization with preservation of pancreatic architecture. At stages later than shown in this micrograph there is mild necrosis, especially of fat (not shown). **(c)** Histology of severe acute pancreatitis. Severe disease is associated with loss of pancreatic architecture, severe inflammation, edema, necrosis of fat and parenchymal tissues, vascular injury, and hemorrhage. Courtesy of K. Barwick, Jacksonville, FL. From Gorelick et al. [312], with permission. Copyright, American Gastroenterological Association Institute, Bethesda, MD.

Pathobiology

Overview

As indicated above, the clinical associations accounting for 70%–80% of the cases of acute pancreatitis are alcohol abuse and gallstone disease. How these two associations are involved in the causation or initiation of pancreatitis is not established. However, significant progress has been made in identifying

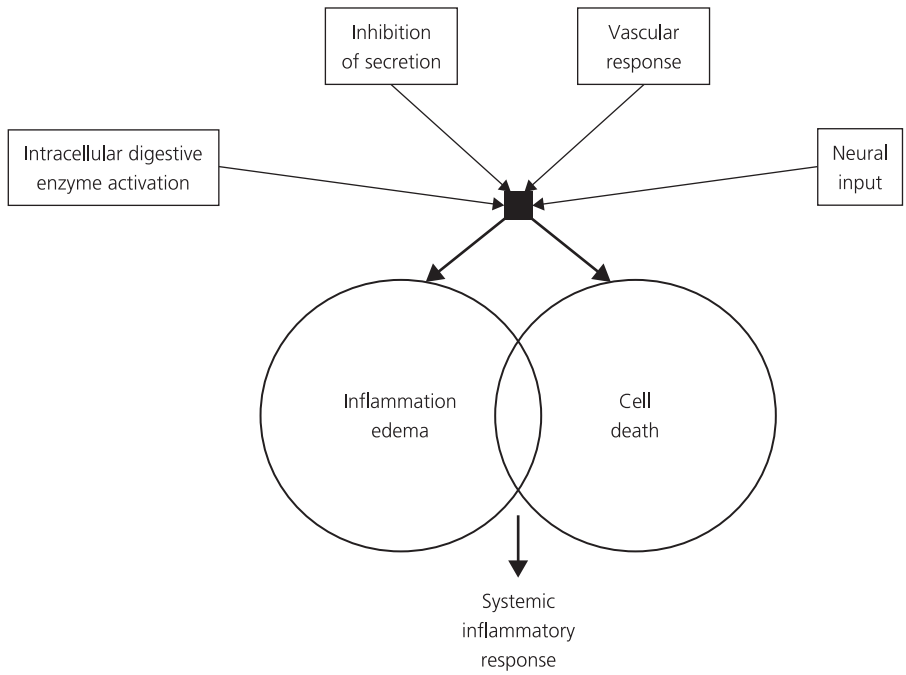


Figure 68.2 Schematic showing the major pathobiological processes of acute pancreatitis, namely inflammation/edema and cell death. The interactions between these processes are discussed in the text. Regulators of these processes include intracellular digestive enzyme activation, inhibition of secretion, and vascular and neural inputs. In severe acute pancreatitis the pancreatic pathobiological responses lead to the systemic inflammatory response.

key steps that mediate the major pathobiological processes (i.e., inflammation/edema and cell death) using experimental animal models of pancreatitis. Such investigations are leading to the identification of molecular targets for therapy.

The major pathobiological processes of acute pancreatitis are influenced by the participation of unique pancreatic intracellular and extracellular events or factors. Examples of intracellular events are activation of digestive enzymes and inhibition of secretion that occur during pancreatitis. Examples of extracellular factors include vascular and neural participation in pancreatitis. The following sections describe the experimental models of pancreatitis and the mechanisms of the major pathobiological processes occurring during pancreatitis, including participation of intra- and extracellular events and factors. Figure 68.2 provides a conceptual framework for considering the relationships between the major pathobiological processes; modulation of these processes by unique pancreatic factors; and the effects on other organ systems through the systemic inflammatory response that occurs with severe pancreatitis.

Experimental models

There are two principal functions for animal model research in acute pancreatitis. First, the models provide the ability to reveal the sequence of molecular steps resulting in the pathobiological processes of acute pancreatitis. Human pancreatic tissue is rarely available for such investigations. Animal models can be used both to identify early signals leading to disease, and to demonstrate roles for each signal in the mechanism of each of the pathobiologic processes using pharmacologic and genetic strategies to specifically alter the activity of these signals.

The second principal use for animal models is to evaluate potential therapies. There are considerable difficulties in designing human clinical trials because the disease varies so widely in its course and severity. In addition, there is a low incidence of the most severe forms of pancreatitis, where testing agents for therapeutic benefit would have the most value. Because of the low incidence of severe forms of acute pancreatitis, multicenter collaborative studies are usually required for definitive evaluation of novel therapies. Animal models of acute pancreatitis can assist by providing information to help select the most promising therapeutic agents for testing.

There are several animal models of acute pancreatitis [9–18]. The more commonly used models are listed in Table 68.1. Which animal model to use for a particular experiment depends first on the purpose of the experiment. For example, for studies testing a potential therapy, models with more severe pancreatitis and systemic inflammation should be used because the severity of pancreatitis in both animals and humans is directly related to the amount of necrosis [11,12,18–21]. For studies designed to reveal early signals underlying a pathobiological response, such as inflammation or cell death, the most important features are uniform severity and time course of the disease in the animal model chosen. It is of note that with the wider availability of genetically modified mice, experimental models of acute pancreatitis are often used to test the participation of specific biochemical pathways [15].

Table 68.1 Models of experimental acute pancreatitis

Models	Species	Features
Cholecystokinin analogues (parenteral)	Rat	Pancreatic inflammation, apoptosis, mild necrosis. Systemic inflammation
Cholecystokinin analogues (parenteral)	Mouse	Pancreatic inflammation, severe necrosis. Systemic inflammation
Pancreatic duct obstruction	Rat	Pancreatic apoptosis
Pancreatic duct obstruction	Opossum	Pancreatic inflammation, severe necrosis. Systemic inflammation
Bile acid perfusion of pancreatic duct	Rat	Pancreatic inflammation, severe necrosis. Systemic inflammation
Choline-deficient, ethionine-supplemented diet	Mouse	Pancreatic inflammation, severe necrosis. Systemic inflammation
Arginine (parenteral)	Rat	Pancreatic inflammation, severe necrosis. Systemic inflammation
Alcohol diet and cholecystokinin analogues (parenteral)	Rat	Pancreatic inflammation, mild necrosis. Systemic inflammation

Alcohol metabolism in the pancreas

The liver is the major ethanol-metabolizing organ in the body. There ethanol is metabolized mainly by an oxidative system converting ethanol first to acetaldehyde and then to acetate using the enzymes alcohol dehydrogenase and acetaldehyde dehydrogenase, respectively [22]. An alternative pathway for ethanol metabolism is through the transient formation of fatty acid ethanol esters (FAEEs). Several organs including the pancreas use this pathway in ethanol metabolism [23–25]. The esterification of ethanol with fatty acids probably requires more than one enzyme system in the pancreas [26]. Compared with the liver, the activity of FAEE synthesis is greater in the pancreas [23,26–29]. By contrast, oxidative metabolism of ethanol in the pancreas is much less than that in the liver.

The formation of FAEEs may have significant pathological effects in the pancreas [27,30–32]. These include activation of proinflammatory transcription factors that participate in the inflammatory response; increased lysosomal fragility, which enhances cell death pathways through intracellular activation of digestive enzymes; and increased cytosolic and mitochondrial calcium levels, which also activate cell death pathways. Thus, the formation of FAEEs in the pancreas during alcohol abuse represents one way in which alcohol sensitizes the pancreas to the development of acute pancreatitis.

Inflammation/edema

An early event in the mechanism of acute pancreatitis is the activation of inflammation-mediating signals originating in the pancreatic parenchymal cells (i.e., the pancreatic acinar cell). For example, the acinar cell is capable of responding to noxious stimuli by activation of signals such as nuclear factor-κB (NF-κB) and activation protein-1 (AP-1) as well as p38 mitogen-activated protein (MAP) kinase to increase the expression of various cytokines and chemokines and other inflammatory molecules [18,33,34] (Fig. 68.3). The

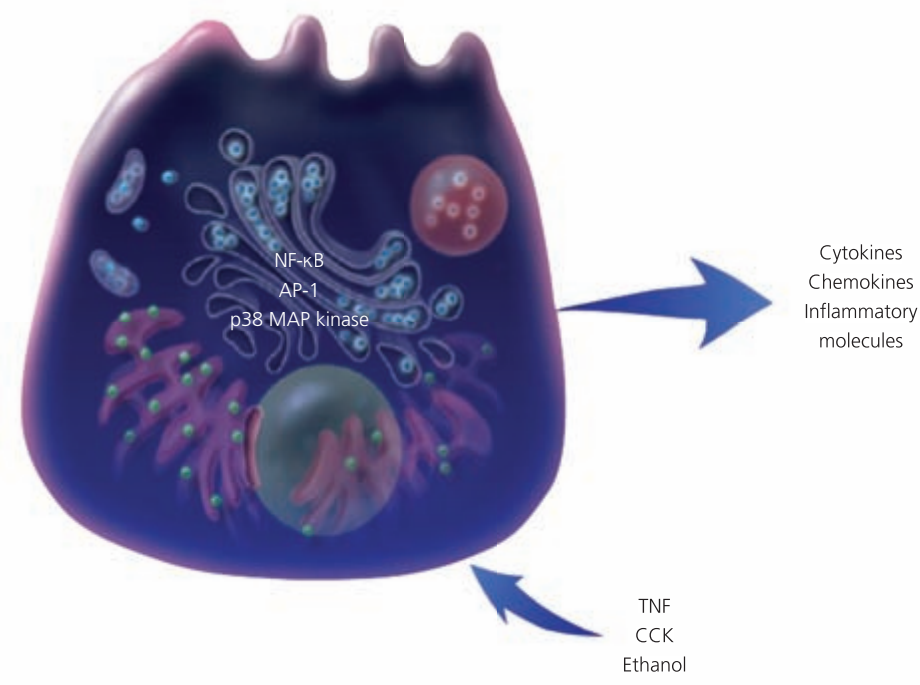


Figure 68.3 Pancreatic intracellular inflammatory response. Insults to the pancreatic acinar cell lead to upregulation of intracellular signaling systems such as nuclear factor-κB (NF-κB), activating protein-1 (AP-1), and p38 mitogen-activated protein (MAP) kinase. The signaling systems upregulate the production from the pancreatic acinar cell of cytokines, chemokines, and growth factors, which mediate the inflammatory response of acute pancreatitis. The activation of the intracellular signals can occur with supraphysiological doses of certain forms of cholecystokinin (CCK) hormone and with tumor necrosis factor-α (TNF-α). Ethanol treatment and bile acids also stimulate this inflammatory signaling or sensitize the cell to CCK and TNF-α. Adapted from Gorelick et al. [312], with permission. Copyright, American Gastroenterological Association Institute, Bethesda, MD.

proinflammatory cytokines/chemokines include tumor necrosis factor-α (TNF-α), platelet-activating factor (PAF), interleukin-6 (IL-6), IL-8, IL-1β, monocyte chemotactic protein-1, and macrophage migration inhibitory factor, among others. Other inflammatory molecules upregulated by these signaling systems include nitric oxide synthase (NOS) and intercellular adhesion molecule-1 (ICAM-1). Furthermore, ethanol feeding sensitizes the pancreas to this inflammatory response, representing another way in which alcohol abuse results in acute pancreatitis [35].

The importance of the production of the various cytokines/chemokines and inflammatory molecules in the progression of pancreatitis comes from studies in experimental models using strategies to prevent the upregulation of a specific molecule and hence determine the effect on outcome (i.e., severity of pancreatitis; Fig. 68.4). A good example comes from an experiment in animals with a genetic deficiency in ICAM-1 [33]. This molecule, which is expressed on the vascular endothelial cells and becomes activated during inflammation, is necessary for migration of neutrophils into the parenchyma during an inflammatory process. In animals with genetic deletion of ICAM-1, pancreatitis severity is markedly decreased. In a like manner, removal of neutrophils before the induction of pancreatitis results in marked reduction in the severity of pancreatitis [36]. Further, other experimental pancreatitis studies demonstrate that the recruitment of inflammatory cells into the pancreas results in both intracellular activation of digestive enzymes and necrosis [36,37]. Finally, cytokines such as TNF-α can have a direct effect on the pancreatic cells to induce cell injury and death [38].

In addition to causing pancreatic parenchymal cell injury and necrosis, the pancreatic inflammatory response is also central to the systemic inflammatory response, such as the adult respiratory distress syndrome (ARDS) that occurs in severe acute pancreatitis (Fig. 68.5) [39–44]. Chemokines/cytokines from the pancreas lead to ARDS by acting directly on lung tissue and through activation of neutrophils in the general circulation that lodge in the lung.

Treatments designed specifically to inhibit a cytokine/chemokine are unlikely to be of benefit because of the redundant nature of the system. This consideration has led to investigations in experimental models using agents known to inhibit the key signaling systems, such as those involved in production of the inflammatory response, showing potential benefit of this approach [34,45,46].

Although pancreatic edema is clearly part of the inflammatory response, the best evidence for the coordination of inflammation and edema comes from reports investigating the role of the neural system in both inflammation and edema. This evidence is discussed below (see "Neural input").

Cell death (necrosis and apoptosis)

Mechanisms of cell death in acute pancreatitis have emerged as a critical topic for investigation (Fig. 68.6). This is important because pancreatic cell necrosis is associated with severe pancreatitis and poor outcome [11,12,18–21]. Strategies aimed at inhibiting or preventing necrosis during pancreatitis may improve outcome in patients with severe pancreatitis.

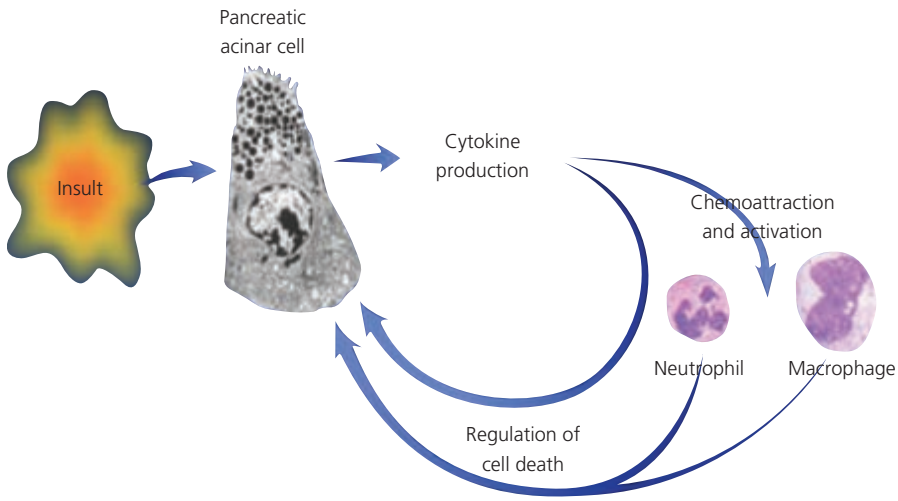


Figure 68.4 A model for the relationship between the inflammatory response and cell death response in acute pancreatitis. An insult to the pancreatic parenchymal cell, the acinar cell, leads to production of cytokines and chemokines that, in turn, have direct effects in the pancreatic parenchymal cells thereby causing a death response. Furthermore, the cytokines/chemokines recruit an inflammatory response that, in turn, mediates cell injury and death responses in the pancreatic parenchymal cells. Tumor necrosis factor-α (TNF-α) is an example of a cytokine that is produced by pancreatic acinar cells and can mediate a cell death response in the pancreatic acinar cell in an autocrine fashion. Examples of cytokines/chemokines produced by the pancreatic acinar cell that can mediate the inflammatory response include TNF-α, interleukin-6 (IL-6), IL-8, and monocyte chemotactic peptide-1 (MCP-1). Adapted from Gorelick et al. [312], with permission. Copyright, American Gastroenterological Association Institute, Bethesda, MD.

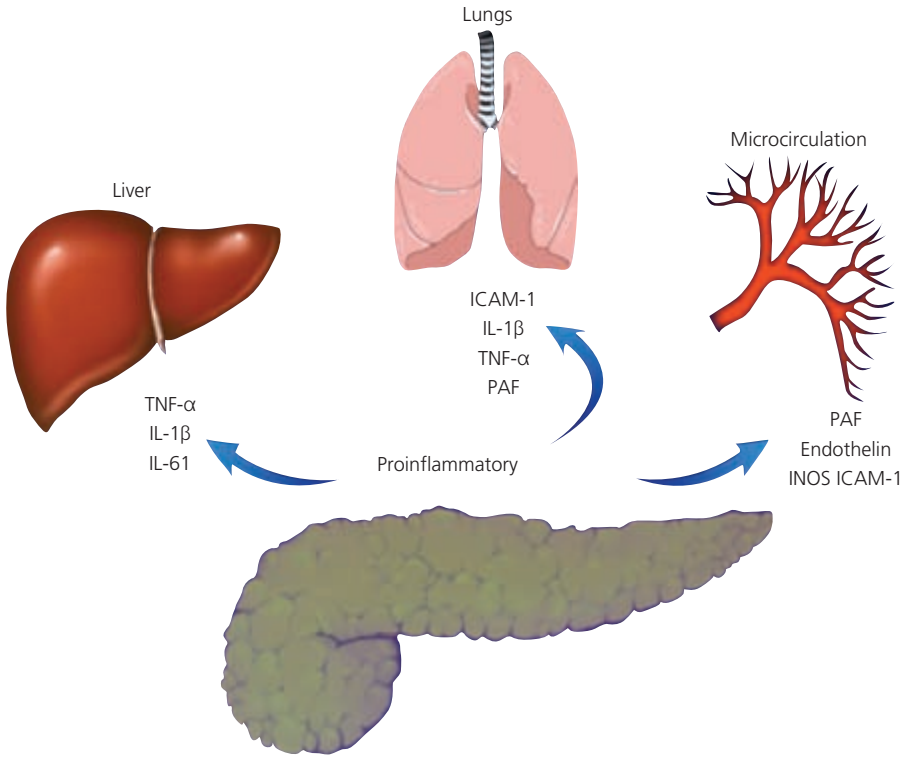


Figure 68.5 Severe pancreatitis results in liver, lung, and vascular dysfunction mediated by cytokines that are produced in the pancreas. These pancreas-derived cytokines act both directly on target tissue causing cellular injury and organ dysfunction, and indirectly by activating neutrophils in the circulation that lodge in the organ and mediate the deleterious response in the distant organ. ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1β; IL-61, interleukin-61; INOS, inducible nitric oxide synthase; PAF, platelet-activating factor; TNF-α, tumor necrosis factor-α. Adapted from Gorelick et al. [312], with permission. Copyright, American Gastroenterological Association Institute, Bethesda, MD.

There are two major categories of cell death in experimental acute pancreatitis models – apoptosis and necrosis. Apoptosis, also called programmed cell death, is required for normal development and tissue homeostasis, but can also occur in pathological conditions [47–50]. An apoptotic cell undergoes a cascade of molecular events ultimately leading to its total removal. These events result in morphological changes that include shrinkage of the cell and its organelles and condensation of nuclear chromatin. The molecular signals of apoptosis also lead to translocation of phosphatidylserine from the inner leaflet of the plasma membrane to the outer leaflet. The shrunken apoptotic cell, often referred to as an apoptotic body with its externally located phosphatidylserine, is recognized by macrophages, which phagocytose the apoptotic body and remove it from the tissue. With apoptosis there is minimal inflammation.

Necrosis occurs exclusively in pathological situations and represents a severe cellular response to injury [48,51,52]. The events of necrosis include mitochondrial swelling and dysfunction; rupture of the plasma membrane; and release of intracellular constituents to the extracellular space resulting in an acute inflammatory response.

The details and signaling mechanisms underlying apoptosis and necrosis have been intensively investigated using mostly cell lines [47,48,50–54]. In general, apoptosis in pancreatic tissue (acinar cells) as well as nonpancreatic tissues is mediated mainly by activation of a family of cysteine proteases called caspases. Caspases are synthesized as inactive precursors and are activated by cleavage induced by proteases including the caspases themselves. Caspases are divided into two major groups: the "effector," or "executioner," caspases, which are responsible for most of the cleavages that disassemble the cell; and "initiator" caspases, which trigger cleavage and activation of the "executioner" caspases. There is mitochondrial involvement in apoptosis as well. Stresses to the cell can act directly on the mitochondria or through initiator caspases to cause specific mitochondrial changes, including depolarization of the mitochondrial membrane potential, permeabilization of the outer mitochondrial membrane, and release of factors into the cytoplasm that interact with the caspase system (apoptogenic factors). A major mitochondrial apoptogenic factor is cytochrome c. Cholecystokinin-octapeptide at supraphysiological doses, tumor necrosis factor-α, and lipopolysaccharide have been shown to cause apoptosis of pancreatic acinar cells in vitro [18,38,48,55–57]. Of note, proteins of the Bcl-2 family regulate mitochondrial permeability [47,50,58–60]. Some, such as Bcl-2 and Bcl-xL, inhibit mitochondrial permeability, whereas others, such as Bax, Bak, Bad, Bid, and Bcl-xS, enhance permeability. Although roles for these regulators in the mechanism of cell death in acute pancreatitis remain largely unknown, there is upregulation of Bcl-xL and Bax in the pancreas during acute pancreatitis [61,62].

There is a general consensus that the level of ATP in the cell can determine whether a cell will die by apoptosis or necrosis [48,63–66]. That is, with a given stimulus for cell

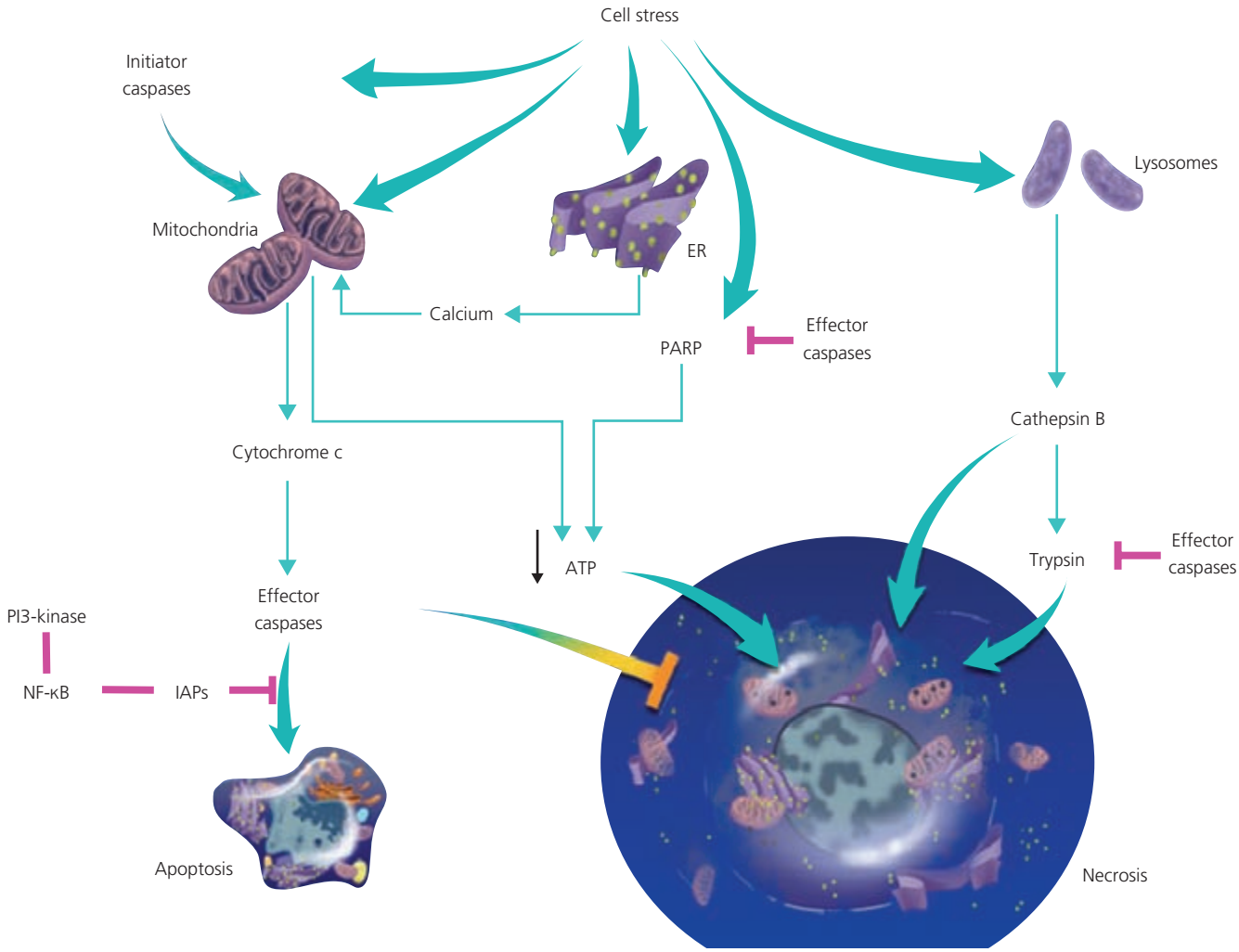


Figure 68.6 Cell death pathways in acute pancreatitis, showing the interrelationship between apoptosis and necrosis pathways and various regulatory systems that may alter the outcome of cell death. In addition to caspase and mitochondrial pathways mediating cell death, endoplasmic reticulum (ER) stress pathways can mediate cell death, at least in part, through release of its stored calcium, which can activate mitochondrial death pathways. Lysosomal pathways are involved through their cathepsins, which can mediate cell death in many tissues. In the pancreas, cathepsins can also activate digestive enzymes in the cell, which can augment cell damage. Severe mitochondrial dysfunction can lead to ATP depletion, and this deenergization of the cell can lead to necrosis. In this case, apoptosis will not occur because ATP is necessary for the activation of effector caspases. As shown, the enzyme poly(ADP-ribose) polymerase (PARP) is upregulated in cell injury; it uses ATP in performing DNA repair, thereby depleting ATP further increasing cell vulnerability to necrosis. Phosphatidylinositol 3-kinase (PI3-kinase), nuclear factor-κB (NF-κB), and inhibitors of apoptosis (IAPs) can inhibit apoptosis, a mechanism of importance in cancer as well as pancreatitis. This inhibitory system represents one pathway for cancer cell resistance to death. Finally, effector caspases can have several effects that modulate the cell injury and death response. Adapted from Sawicki et al. [313], with permission. Copyright, American Gastroenterological Association Institute, Bethesda, MD.

injury, greater levels of ATP depletion will result in necrosis whereas lesser amounts of ATP depletion will result in apoptosis. Because mitochondria are the principal generators of ATP production, necrosis results when mitochondrial injury is greater. One reason why low levels of ATP lead to necrosis is that ATP is necessary for key steps in the caspase pathway. If the caspase pathways and apoptosis are blocked the cell will likely proceed to necrosis. Another important aspect of ATP is that it is necessary for maintaining ion pumps that are essential for cell integrity [48,63–66]. Thus, mitochondrial function is a key arbiter of cell injury. Another pathway that mediates cellular ATP depletion involves the nuclear enzyme, poly(ADP-ribose) polymerase (PARP). This enzyme catalyzes polyADP-ribosylation of DNA-binding proteins such as histones and is involved in DNA repair [67,68]. Breaks in DNA activate PARP, and excessive activation results in ATP depletion because ATP is needed to replenish NAD^+, the substrate for PARP [69,70]. The importance of PARP in acute pancreatitis comes from studies in experimental models demonstrating that PARP activity markedly increases

during acute pancreatitis and pharmacological blockade of PARP decreases necrosis [71,72].

As illustrated in Fig. 68.5, there are several other potential inputs that can determine the type of cell death during pancreatitis, both dependent and independent of mitochondria and ATP. These include release of calcium from the endoplasmic reticulum, which in turn can lead to mitochondrial dysfunction [73–77]; lysosomes and their content of cathepsin B, which can mediate intracellular activation of digestive enzymes such as trypsinogen [78,79]; and intracellular signaling systems such as NF-κB and phosphatidylinositol (PI) 3-kinase, which can regulate expression of proteins that can inhibit apoptosis and thus promote necrosis. The proteins that inhibit apoptosis are called inhibitors of apoptosis (IAPs). Studies in experimental models have shown that activators of apoptosis decrease the severity of pancreatitis, suggesting that manipulating pathways that promote apoptosis during acute pancreatitis might decrease necrosis and the severity of the disease and thus improve outcome [18,48,80,81]. For example, alcohol feeding in rats has been demonstrated to decrease the expression of caspases in the pancreas and thus may reduce apoptosis and enhance necrosis [82,83]. This effect of alcohol may explain why alcohol represents the major risk factor for pancreatic necrosis [84]. Further, alcohol is metabolized in the pancreas to FAEEs, which have been shown to release calcium from endoplasmic reticulum stores, resulting in mitochondrial uptake, ATP depletion, and necrosis [31,85–87].

Experimental models of acute pancreatitis indicate that there is a close interaction between inflammation and cell death response, as depicted in Fig. 68.4 [37,38,45,88]. Removal of neutrophils from animals during experimental pancreatitis markedly decreases pancreatic necrosis and the severity of pancreatitis while increasing pancreatic apoptosis. The effect of neutrophils is likely mediated by both their production of reactive oxygen species and secretion of elastase [37,89]. Further, inhibition of intracellular inflammatory signaling in acinar cells by inhibition of NF-κB activation results in increased caspase activity and apoptosis in the pancreas during experimental pancreatitis [45]. The inflammatory response also increases intracellular activation of digestive enzymes suggesting possible relationships between the inflammatory response, digestive enzyme activation, and necrosis [37]. The signaling mechanisms underlying the effect of the inflammatory response on cell death are not well understood but probably include regulation of expression of IAPs, which inhibit executioner caspase activation, because the expression of these inhibitors is regulated by NF-κB activation [90–93]. Further, inhibitors of PI3-kinase may have an effect on IAPs because of the known ability of PI3-kinase to activate NF-κB [94]. Inhibition of both NF-κB and PI3-kinase activation have been demonstrated to decrease pancreatitis severity in pancreatitis models [95,96]. The importance of understanding the regulation of cell death by the inflammatory response comes from clinical observations that necrosis develops a few days after the initiation of acute pancreatitis when inflammation is the predominant process [21]. Thus, treatments designed to attenuate inflammation in pancreatitis will have the secondary benefit of decreasing necrosis.

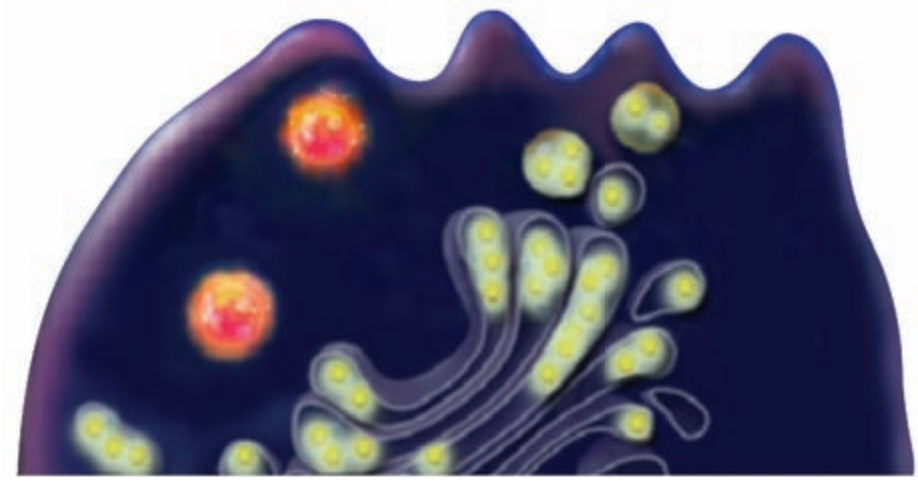
Intracellular activation of digestive enzymes

Intracellular digestive enzyme activation represents an early event in the course of both experimental and human pancreatitis [97]. In the normal state, the pancreatic acinar cell protects itself from the potential damaging effects of the digestive enzymes by several mechanisms, as listed in Fig. 68.7. More specifically, many of the digestive enzymes are synthesized and stored in zymogen granules as inactive proenzymes. Such proenzymes include trypsinogen, chymotrypsinogen, procarboxypeptidases, proelastase, and prophospholipases. In normal physiology, these proteins are delivered to the duodenum, where the brush border enzyme enterokinase cleaves an amino-terminal portion of the trypsinogen molecule resulting in an "opening" of the catalytic site of trypsin. Trypsin then catalyzes the activation of the other proenzymes and autocatalyzes activation of itself.

In contrast to normal physiology, in pancreatitis there is activation of the proenzymes in the pancreatic acinar cell. The importance of intracellular digestive enzyme activation is underscored by findings that mutations in the trypsinogen gene lead to a form of hereditary pancreatitis [98,99]. The mutations cause an increase in activated trypsin in the pancreatic acinar cell because of changes in protein folding that reduce the interaction of trypsin with pancreatic trypsin inhibitor resulting in decreased degradation and increased accumulation of active trypsin. In addition, there are mutations in the pancreatic trypsin inhibitor that also appear to be predisposed to developing pancreatitis [100].

The mechanisms of intracellular digestive activation, including the location within the cell where this occurs, are under intensive investigation. The prevailing hypothesis is that activation is a result of abnormal sorting of lysosomal cathepsin B and digestive enzymes into a common compartment [101]. There cathepsin B converts trypsinogen into trypsin. Trypsin then catalyzes the activation of itself and other zymogens as happens in the intestine with enterokinase. Support for this hypothesis comes from experiments showing that genetic and pharmacological inhibition of cathepsin B decreases activation of intracellular trypsinogen and the severity of pancreatitis in experimental models of pancreatitis [78,79,102]. There is also a line of work suggesting a key role for proelastase conversion to active elastase independent of trypsinogen activation [103,104].

Digestive enzyme conversion takes place in vacuoles that develop in the apical portion of the pancreatic acinar cell during experimental pancreatitis [37,105,106]. The mechanism of formation of these vacuoles, which is promoted by ethanol feeding and the inflammatory response, is also under investigation [37,107].



- Synthesis of enyzmes as inactive zymogens
- Trypsin inhibitor packaged in zymogen granule
- Segregation of enzymes in membrane-bound compartments
- Enterokinase restricted to small intestine

Figure 68.7 Protective mechanisms in the pancreas. The cartoon indicates zymogens that have been pathologically activated in an acinar cell. Listed are the protective mechanisms present in the pancreas to prevent activation of zymogens in the pancreatic cell and thus prevent the damaging effects of zymogen activation. Adapted from Gorelick et al. [312], with permission. Copyright, American Gastroenterological Association Institute, Bethesda, MD.

Inhibition of secretion

A common feature of many experimental models of pancreatitis is inhibition of enzyme secretion from the pancreas. This is likely to be due to derangements of the apical actin cytoskeleton or the SNARE protein machinery responsible for exocytosis at the apical pole of the acinar cell [108–110]. Inhibition of secretion may lead to pancreatitis because it prevents removal of activated intracellular enzymes from the cell [111].

Vascular response

The role of vascular responses in determining the severity of acute pancreatitis in humans is underscored by a report from Sendai, Japan [21]. A long-term (1988–2003) study in Japan showed significant vascular changes occurring early in the course of cases that went on to develop severe necrotizing pancreatitis. Specifically, the investigators compared early angiographic abnormalities with subsequent contrast-enhanced computed tomography (CE-CT) studies during the course of pancreatitis. The remarkable finding was a high incidence of vasospasm detected by angiography (both intrapancreatic and extrapancreatic) occurring in patients who went on to have necrotizing pancreatitis. Furthermore, the areas of necrosis as detected by CE-CT over subsequent days occurred in regions downstream of the vasospasms observed early in the course with angiography.

The microvascular circulation is also significantly affected in acute pancreatitis. Studies of microcirculation of the exocrine pancreas show that in the normal pancreas, the acinus is surrounded by a large number of small capillaries that form a network around the acinus [112,113]. With the onset of experimental pancreatitis there are a number of changes in the microvascular circulation; these include increased and homogeneous microperfusion in mild edematous pancreatitis; increased vascular permeability and edema formation; and a progressive decrease in capillary perfusion despite stable hemodynamics in necrotizing pancreatitis [21,112–120]. The decreased microperfusion is generally considered to be a key underlying event leading to necrosis.

In addition to the perfusion changes, the microvasculature plays an essential role in the inflammatory response [120–122]. That is, the inflammatory response requires the participation of postcapillary venules, where the expression of adhesion molecules such as ICAM-1 results in adhesion and migration of leukocytes from the intravascular space into the parenchyma. Regulation of the vascular responses is less well understood and likely involves several participants including endothelin-1, several cytokines/chemokines, and endothelial nitric oxide synthase [123–129].

Neural input

The role of neural inputs in the genesis of acute experimental pancreatitis has been demonstrated in a number of animal studies. It was reported that in experimental pancreatitis several factors activate the transient receptor potential vanilloid-1 (TRPV-1) in the pancreas. The activation of these receptors stimulates release of substance P and calcitonin gene-related peptide (CGRP) from neural endings, which in turn mediate the development of pancreatitis [130–135].

Specific etiologies

Knowledge of the cause of acute pancreatitis may guide treatment and allow prevention of further episodes. Many patients have an easily identifiable and treatable cause such

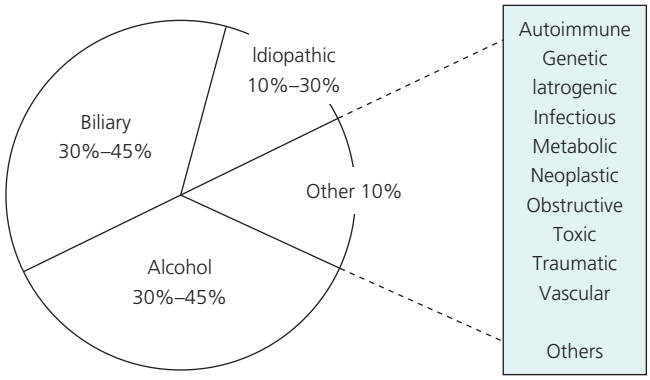


Figure 68.8 Etiologies of acute pancreatitis.

as gallstones or hyperlipidemia. Less common causes should be considered in those with no obvious precipitant, especially in patients with recurrent, unexplained bouts of pancreatitis. An overview of various etiologies is shown in Fig. 68.8.

Obstructive

Many forms of pancreatic duct obstruction have been associated with pancreatitis, including gallstones, sphincter of Oddi stenosis, ampullary or pancreatic tumors, pancreas divisum, duodenal diverticula, annular pancreas, and parasites, clots, or foreign bodies lodged in the ampulla. Gallstones are the commonest cause of acute pancreatitis in many parts of the world.

Gallstones

Pancreatitis develops in only a small percentage of patients with gallstones. In a cohort of persons with gallstones followed longitudinally, the incidence of acute pancreatitis was 0.17% per year [136]. The presence of gallstones, however, increases the relative risk for acute pancreatitis 14- to 35-fold in men and 12- to 25-fold in women [137]. The presence of small gallstones (< 5 mm), a larger cystic duct, and a long common channel in the ampulla all predispose to gallstone pancreatitis. Metabolic factors also may increase the risk for development of pancreatitis from gallstones. For example, underlying hyperlipidemia manifest by delayed chylomicron clearance may make patients more susceptible to gallstone pancreatitis [138].

Over a century ago, on the basis of autopsy findings, Opie proposed that a gallstone obstructing the ampullary orifice causes pancreatitis by promoting reflux of bile into the pancreatic duct through the common channel. Several observations have challenged that theory: patients without a common channel can have gallstone pancreatitis [139], and retrograde flow of bile into the pancreatic duct at physiological pressures does not induce pancreatitis in animals [140].

Opie also proposed that pancreatic duct obstruction alone might be the mechanism by which stones cause pancreatitis. In general, simple obstruction of the pancreatic duct in animals has not generated pancreatitis. In the American opossum, however, pancreatic duct obstruction alone does generate acute pancreatitis [141]. This animal model supports the "ductal obstruction theory," as does the occurrence of human pancreatitis in other forms of pancreatic duct obstruction.

Most biliary pancreatitis is precipitated by the transient or persistent obstruction of the papilla by a stone. When patients are selected based on hyperbilirubinemia and pancreatitis, gallstones can be recovered from the stool in up to 85% [142], proving that pancreatitis is usually accompanied by passage of a gallstone into the lumen of the intestinal tract. Stone passage into the duodenal lumen probably occurs on the first day of the attack in most patients with gallstone pancreatitis, and in many patients it may already have occurred by the time the patient reaches the hospital emergency department. Persistence of stones in the bile duct or ampulla is associated with more severe disease [143,144].

If gallstones predispose to acute pancreatitis, removal of the gallbladder should decrease the risk. In one large series, cholecystectomy in patients without a history of acute pancreatitis reduced the risk for the subsequent development of pancreatitis at least 10- to 20-fold [136]. Despite the increased risk for development of acute pancreatitis in those with gallstones and the success of cholecystectomy in decreasing the risk, the incidence of the disease is too low to warrant prophylactic cholecystectomy in patients with asymptomatic gallstones.

Microlithiasis

Crystals or aggregates of crystals in bile, referred to as microlithiasis, may cause recurrent bouts of pancreatitis in the absence of gallstones [145,146]. In some series microlithiasis is a leading cause of otherwise unexplained acute pancreatitis.

Microlithiasis may be identified during abdominal or endoscopic ultrasound as amorphous, layering, nonshadowing material (sludge) or brightly echogenic cholesterol crystals within the gallbladder (Fig. 68.9). Sonographically apparent sludge may be associated with prolonged fasting, rapid weight loss, total parenteral nutrition, gastric surgery, and administration of some drugs including octreotide and ceftriaxone.

Microlithiasis can also be diagnosed by examining bile for birefringent cholesterol monohydrate crystals or reddish brown calcium bilirubinate granules. Bile for microscopy can be obtained from the bile duct during endoscopic retrograde pancreatography (ERCP) or aspirated from the duodenum after slow intravenous administration of cholecystokinin (CCK) octapeptide; dark gallbladder bile should be collected and examined. Endoscopic ultrasound may be more sensitive than bile microscopy for detection of cholesterol crystals in the gallbladder [147].

Crystals, like stones, cause pancreatitis that begins abruptly, often with an initial rise in serum levels of both pancreatic and liver enzymes. Several treatment modalities have been

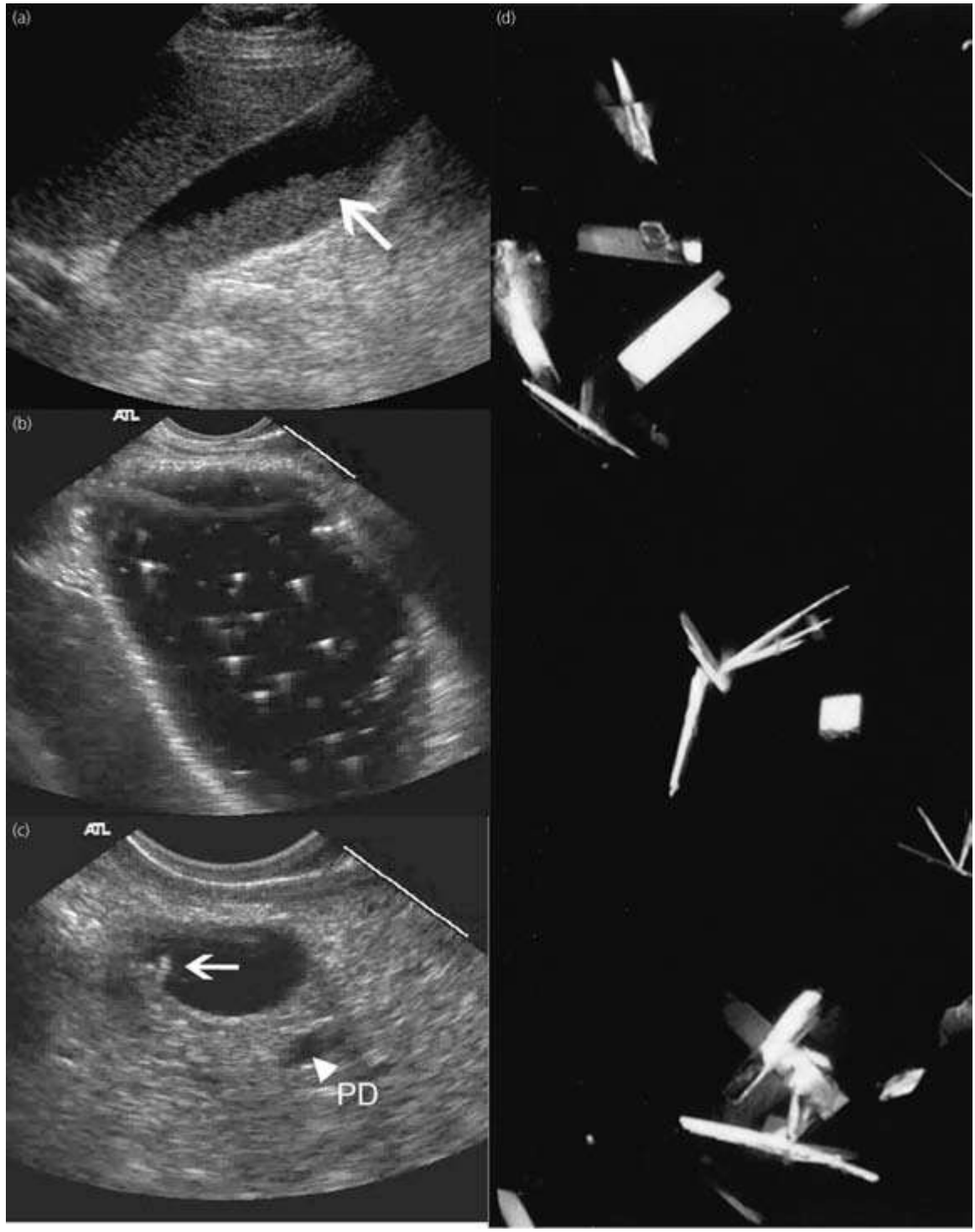


Figure 68.9 Biliary sludge and microlithiasis. **(a)** Transcutaneous ultrasound demonstrates gallbladder sludge (arrow). Courtesy of Carl Reading, Mayo College of Medicine. **(b)** Endoscopic ultrasound demonstrates floating, echogenic cholesterol crystals in gallbladder. **(c)** Endoscopic ultrasound demonstrates cholesterol crystals in the common bile duct (arrow). PD, pancreatic duct. **(d)** Cholesterol crystals in gallbladder bile demonstrate with polarized light microscopy.

used, including ursodeoxycholic acid, cholecystectomy, or endoscopic sphincterotomy. Each form of intervention has been reported to reduce the frequency of attacks by at least two-thirds.

Stenosis and spasm of the sphincter of Oddi

The sphincter of Oddi is a complex muscular structure that regulates the flow of pancreatic secretions and bile into the duodenum. The sphincter acts as a variable resistor. Abnormalities of the sphincter have been identified in patients with recurrent abdominal pain after cholecystectomy, presenting with recurrent acute pancreatitis.

The biliary and pancreatic portions of the sphincter of Oddi are partly independent anatomically and functionally. Acting indirectly through inhibitory neural pathways, CCK causes both components of the sphincter to relax, facilitating the flow of bile and pancreatic juice into the duodenum after a meal. Cholecystokinin may also act directly on the sphincter and cause contraction [148]. This paradoxical dysfunction of the sphincter may occur after biliary tract surgery (perhaps because the innervation of the sphincter is interrupted), vagotomy, or gastric resection, and has been demonstrated to occur in both components of the sphincter after intravenous administration of CCK octapeptide to patients during sphincter of Oddi manometry.

Other secretagogues and transmitters including vasoactive intestinal peptide (VIP), glucagon, and nitric oxide also appear to mediate relaxation of the sphincter [149]. Secretin normally induces an initial increase in phasic contractions of the sphincter, followed by relaxation [150].

Obstruction to flow through the ampulla may be caused by dysfunction of the sphincter or by scarring and stenosis of the sphincter and the ampullary outlet. Stenosis of the sphincter is most commonly the result of previous trauma from stone passage, surgical manipulation, or endoscopic sphincterotomy, but may also be due to a primary inflammatory or fibrosing process in the ampulla of Vater. Stenosing papillitis may be caused by chronic pancreatitis, sclerosing cholangitis, or by opportunistic infections associated with AIDS. Sphincter dysfunction may be associated with medications (such as narcotics) and may possibly follow acute pancreatitis regardless of its etiology [151].

Criteria for the clinical diagnosis of sphincter of Oddi stenosis or dysfunction are well established for biliary sphincter disease, as described elsewhere in this text. The diagnosis and role of sphincter disease in pancreatitis is less clear. Pancreatic sphincter stenosis may be suspected in patients with a dilated pancreatic duct, poor pancreatic duct drainage (identified during ERCP or secretin-MRI), and difficulty in passing ERCP catheters through the papilla over a deeply placed pancreatic guidewire. Pancreatic sphincter hypertension can be identified using sphincter of Oddi manometry performed during ERCP. Manometric abnormalities of the pancreatic sphincter have been reported in a high percentage of patients with idiopathic recurrent pancreatitis [152]. The presence of stenosis or manometric sphincter abnormalities does not prove that these findings are the primary cause of pancreatitis. Limited, uncontrolled data suggest that surgical or endoscopic treatment of a hypertensive pancreatic sphincter prevents recurrent pancreatitis in some patients [153]. Endoscopic pancreatic sphincterotomy opens the pancreatic sphincter incompletely in many patients, and symptomatic stenosis of a pancreatic sphincterotomy or sphincteroplasty may occur in up to 25%. Stenosis may recur after treatment and can be difficult to treat effectively.

Alternatives to sphincter ablation are of unclear value in treating pancreatic sphincter hypertension. Medical therapy with calcium channel blockers or intraampullary botulinum toxin injections have been used in patients with biliary sphincter dysfunction and may be effective in pancreatic sphincter dysfunction as well. Pancreatic stenting has questionable utility as a therapeutic trial, because stents can cause pain in some patients, and may induce changes of chronic pancreatitis when left in place for more than a short time [154] despite advances in stent technology [155].

Pancreas divisum

During fetal development the dorsal and ventral pancreatic buds each form ducts that enter the duodenum. The ventral pancreatic bud rotates around the duodenum and fuses with the dorsal bud; typically the ducts within the buds join as well. The ventral duct drains the pancreas through the major papilla (duct of Wirsung) and the remnant opening of the dorsal duct forms the minor papilla (duct of Santorini). When fusion between the two ducts is partial or absent, the dorsal duct drains most of the pancreas through the minor papilla. This anatomical variant is known as pancreas divisum.

Pancreas divisum can be subdivided into complete divisum, in which the ventral and dorsal ducts do not communicate, and incomplete divisum, in which a diminutive duct joins ventral and dorsal pancreas, but the bulk of exocrine secretion still occurs through the minor papilla. In both situations there is dominant dorsal duct drainage.

The relationship of pancreas divisum to pancreatitis has been debated. Pancreas divisum is a common abnormality, with autopsy series reporting an incidence of about 5%. The incidence as assessed by ERCP ranges from 0.3% to 8%. This variability reflects differences in patient populations and definitions of the anomaly. For example, some studies include only patients with a complete separation between the dorsal and ventral ducts. Although an initial ERCP study reported that pancreas divisum was more common in patients with unexplained recurrent pancreatitis than in other patients undergoing ERCP, subsequent studies have not confirmed this association [156]. Proponents of the pathological association have suggested that resistance to the flow of secretions through the duct of Santorini causes ductal hypertension and pancreatitis. Studies of experimental

pancreatitis have shown that obstruction of the opossum pancreatic duct may result in pancreatitis [141]. Although the vast majority of individuals with pancreas divisum are asymptomatic, it is likely that a small number experience recurrent pancreatitis related to relative obstruction of the minor papilla. These may be individuals with a particularly diminutive minor papilla orifice.

Proposed diagnostic tests for minor papilla stenosis rely on pancreatic duct imaging before and after intravenous secretin administration. In normal persons the pancreatic duct dilates transiently after secretin is given, returning to within 1 mm of its baseline diameter within 15 min. In some persons with pancreas divisum prolonged ductal dilation following secretin administration has been demonstrated with transabdominal ultrasound, endoscopic ultrasound, or MRI [157–159]. Magnetic resonance imaging may also demonstrate a dilated terminus of the dorsal duct, or "Santorinicele," after secretin administration [158]. The bulk of available uncontrolled data suggests that prolonged ductal dilation after secretin administration, or appearance of a Santorinicele, is a predictor of response to endoscopic treatment of pancreas divisum.

Treatment studies also suggest that obstruction to flow through the minor papilla can cause pancreatitis. A small, blinded, randomized trial showed that endoscopic stenting of the minor papilla prevented recurrent pancreatitis when compared with sham therapy in patients with pancreas divisum [160]. A larger body of uncontrolled surgical and endoscopic experience supports this conclusion, and suggests that improved dorsal duct drainage is most likely to help patients with pancreas divisum and otherwise unexplained recurrent acute pancreatitis. Early studies indicated that 50%–70% of such patients will improve substantially after minor papilla sphincterotomy, although one report found that many patients who initially respond subsequently relapse [161]. Patients with chronic pancreatitis are less likely to respond, and those with chronic abdominal pain but no history of pancreatitis will have short- to medium-term symptomatic improvement only 30% of the time [162]. A randomized study of minor papilla sphincterotomy in patients with pain alone, reported in abstract only, did not show a statistically significant benefit from endoscopic therapy [163].

The major difficulty in patients with pancreas divisum is selecting those who will benefit from endoscopic treatment. In some, divisum is an incidental finding, and treatment will be of no benefit. Secretin-stimulated pancreatic duct imaging may be useful, as failure of the duct to dilate for more than 15 min following secretin administration may predict a lower likelihood of response to invasive therapy [158,159]. Cystic fibrosis transmembrane receptor (CFTR) mutations are more common in patients with pancreas divisum and idiopathic pancreatitis than in controls [164], and divisum patients with CFTR dysfunction may be less likely to respond to endoscopic interventions [165]. Endoscopic interventions are best reserved for those with otherwise unexplained recurrent acute pancreatitis, and are best avoided in patients with pain alone. Botulinum toxin injections into the minor papilla have been reported to predict response to endoscopic sphincterotomy in patients with divisum and recurrent pancreatitis [166].

Toxins

Alcohol

Alcoholic pancreatitis may present as an acute bout of pancreatitis. These episodes are not always associated with established, chronic, underlying pancreatic damage. In one series of patients who died of acute alcoholic pancreatitis, almost 40% demonstrated little histological evidence of chronic pancreatitis [167]. The incidence of pancreatitis is surprisingly low in alcoholic patients. Dreiling and Koller reported that among 100 alcoholic patients, clinical pancreatitis developed in only 5%, cirrhosis in 15%, both in 1%, and neither in 80% [168]. Although these data may underestimate the true prevalence of disease, they indicate that in addition to the amount of alcohol ingested, unknown genetic or environmental factors affect a person's susceptibility to disease.

Several mechanisms may contribute to the development of alcoholic pancreatitis. These include sphincter of Oddi spasm, obstruction of small ductules by proteinaceous plugs, and direct toxic and metabolic effects [169]. Moreover, alcohol has also been found to stimulate pancreatic secretion through cholinergic pathways. Some have proposed that the combination of alcohol-induced sphincter of Oddi spasm and stimulated secretion is responsible for pancreatitis. Chronic ethanol exposure may also sensitize the pancreas to other insults by effects on the production of proinflammatory cytokines; conversion of digestive enzymes to their activated forms; and activation of intracellular death pathways, as described earlier.

Ethanol may also change the concentration and composition of proteins secreted by the pancreas, resulting in the formation of protein plugs within small pancreatic ductules. The protein plugs may obstruct pancreatic ducts and promote pancreatitis. Increased viscosity and elevated protein concentrations found in pancreatic secretions from alcoholic patients are likely to contribute to plug formation. By forming a rigid, insoluble protein structure, specific secretory proteins such as lithostathine and glycoprotein-2 may contribute to the formation of these protein plugs and to stone formation [170]. The failure to alkalinize the acinar lumen in alcoholics may also contribute to plug and stone formation. Increased amounts of lysosomal enzymes and an increased trypsinogen:pancreatic trypsin inhibitor ratio have been reported in pancreatic juice from alcoholic patients.

Drugs

A variety of drugs have been associated with pancreatitis [171]. With few exceptions, the mechanisms of drug-induced disease are unknown and most are idiosyncratic reactions.

Table 68.2 Examples of drug-induced pancreatitis

Proven	Probable	Possible/questionable
L-Asparaginase	Protease inhibitors	Carbamazepine
Azathioprine	Acetaminophen	Corticosteroids
Didanosine	5-Aminosalicylic acid	Cimetidine
Estrogens	Ergotamine	Furadantin
ACE inhibitors	Furosemide	Metronidazole
6-Mecaptopurine	Isoniazid	Minocycline
Pentamidine	Procainamide	Piroxicam
Sulfasalazine	Rifampicin	Ranitidine
Valproate	Thiazides	Tetracycline

ACE, angiotensin-converting enzyme.

Most drug-induced pancreatitis occurs within 2 months of drug initiation or an increase in drug dose. Drug-induced pancreatitis may be classified by the certainty of its relationship to disease. Rechallenge is taken as the gold standard of proof, but such information is often unavailable. A partial listing of medications grouped according to probability that they cause pancreatitis is presented in Table 68.2.

Among immunosuppressants, azathioprine and its major metabolite, 6-mercaptopurine, are the chief offenders; in one series of 396 patients taking these medications, pancreatitis was induced in 3.3% [172]. Symptoms developed in all patients within the first month of therapy. Rechallenge with either azathioprine or 6-mercaptopurine was invariably associated with a rapid recurrence of pancreatitis. Furthermore, attempts to desensitize with slowly increasing doses of 6-mercaptopurine were unsuccessful. Both cyclosporine (ciclosporin) and tacrolimus have been rarely associated with acute pancreatitis. The rare (~ 2%) but severe (~ 50% mortality) pancreatitis associated with renal or liver transplantation may be linked to drug therapy or to infection.

Pancreatitis can also be caused by some antiinfectives. Tetracycline has been linked to pancreatitis in patients with fatty degeneration of the liver, particularly during pregnancy, but may also precipitate pancreatitis in the absence of underlying liver disease. Tetracycline pancreatitis may occur after years of administration. Trimethoprim–sulfamethoxazole can precipitate pancreatitis, albeit uncommonly. Intravenous or aerosolized pentamidine causes pancreatitis depending on the cumulative dose of the drug. Another cause of pancreatitis in patients with AIDS is the antiviral agent, 2′,3′-dideoxyinosine (DDI). The onset of DDI-associated pancreatitis is typically delayed for several months after the initiation of therapy. Although the mechanism of DDI-induced pancreatitis is unknown, patients with a previous history of pancreatitis and those with advanced infection seem to be at highest risk for this complication. Decreasing the dose of DDI reduces the incidence of this serious complication. Pancreatitis may be caused indirectly by HIV protease inhibitors by elevating serum triglyceride levels. Stavudine and lamivudine have also been associated with pancreatitis [173].

Antiinflammatory agents may cause pancreatitis. Included are sulfasalazine and, in a small number of patients, oral 5-aminosalicylic acid [174]. Early reports of corticosteroid-induced pancreatitis are difficult to interpret because most patients had other possible causes of disease. Corticosteroids may directly precipitate pancreatitis as demonstrated by rechallenge, but this appears to be a rare event [175].

Antihypertensives and diuretics are widely used and alhough the incidence of pancreatitis is low with these drugs, they constitute an important family of disease-producing agents. Included on the list are furosemide, thiazide diuretics, and angiotensin-converting enzyme (ACE) inhibitors. A few agents induce metabolic abnormalities that predispose to pancreatitis. For example, therapy with estrogens or the retinoid derivative, isotretinoin, is associated with a dose-dependent increase in triglycerides and pancreatitis [176].

Agents that overstimulate neural pathways may cause acute pancreatitis. In animal models, neural stimulation can initiate pancreatitis or augment the disease caused by other agents. Parathion, an alkylphosphate cholinesterase inhibitor, is a widely applied agricultural insecticide. Ingestion or absorption of parathion through the skin may induce injury. Almost 50% of patients with toxic exposure to this agent will exhibit biochemical evidence of pancreatitis. Although parathion-induced pancreatitis is usually mild, fatal hemorrhagic pancreatitis has been reported. Acting through neural pathways, ergotamine poisoning decreases blood flow and may cause ischemic pancreatitis and hepatitis [177].

Scorpion venom

The venom of the scorpions *Tityus trinitatis* and *T. serrulatus*, found in Trinidad and Brazil, respectively, may induce a form of hyperstimulation pancreatitis. The toxic polypeptides in tityustoxin, acting through cholinergic pathways, are potent stimulants of pancreatic secretion in vitro and induce pancreatitis in animals [178]. In addition to causing pathological enzyme activation, tityustoxin may stimulate pancreatic secretion, induce sphincter of Oddi spasm, and alter blood flow.

Metabolic

Hyperlipidemia

Although hyperlipidemia may be caused by a bout of pancreatitis, it may also precipitate acute or chronic pancreatitis [179]. The breakdown products of triglycerides are probably responsible for inducing pancreatitis. When lipase in the pancreatic capillary bed acts on the high levels of triglycerides in serum, toxic free fatty acids are generated. The endothelial lining of small pancreatic blood vessels is the first site of injury. Damage of small blood vessels leads to recruitment of inflammatory cells and thrombosis. Hyperlipidemic pancreatitis may be associated with normal serum amylase determinations but elevated serum lipase levels.

Although triglyceride levels greater than 2000–3000 mg/dL usually are required for development of pancreatitis, a few patients seem to manifest this condition when serum levels are lower, and pancreatitis induced by intravenous lipid administration has been reported with serum triglyceride levels slightly greater than 500 mg/dL. In general, a level of greater than 1000 mg/dL should prompt suspicion of an underlying hyperlipidemic cause for pancreatitis. Some forms of hypertriglyceridemia show striking increases in serum triglycerides after a fatty meal, with lower values while fasting. It is important to measure serum triglycerides on the first serum sample obtained from a patient with otherwise unexplained pancreatitis. Measuring postprandial triglyceride levels after recovery may also be required to detect markedly elevated values.

In addition to patients with primary hyperlipidemia, elevated triglycerides may result from therapy with estrogen, retinoid derivatives, and HIV protease inhibitors. Typically, estrogen-related pancreatitis occurs within the first months following initiation of medication. Patients who are obese and have underlying glucose intolerance or fasting hypertriglyceridemia are at greater risk.

Treatment of acute hyperlipidemic pancreatitis includes standard supportive care. If parenteral nutrition is given, serum triglyceride levels should be monitored. Occasional patients with acute pancreatitis and severe hyperlipidemia (serum levels > 10 000 mg/dL) may benefit from plasmapheresis. Once the patient recovers from the acute symptoms, the cornerstone of therapy for hyperlipidemic pancreatitis is a low-fat diet. Attention must also be given to underlying diabetes or thyroid disease, as well as alcohol use, all of which can elevate serum triglycerides. In some cases, treatment with lipid-lowering drugs may be indicated.

Hypercalcemia

Experimentally, acute calcium infusion causes increased pancreatic duct permeability that may lead to a nonspecific increase in serum pancreatic enzyme levels. In animals, acute hypercalcemia may induce a mild form of pancreatic injury [180]. Virtually all causes of hypercalcemia, including hyperalimentation, infusions associated with cardiac bypass, immobilization, multiple myeloma, and hyperparathyroidism, have been linked to hyperamylasemia. These studies indicate that hypercalcemia is associated with an increase in serum enzymes, but probably causes pancreatitis infrequently and severe pancreatitis rarely.

Infectious

Viral including HIV/AIDS

The most common viral infections that may cause pancreatitis are mumps and Coxsackie B virus. These are self-limited and may account for lone bouts of pancreatitis. Viral hepatitis and fulminant hepatitis, especially hepatitis B, has been associated with pancreatitis. Influenza A and B, enterovirus, cytomegalovirus, and rubella may cause acute pancreatitis.

Hyperamylasemia has been reported in up to 40% of AIDS patients [181] and has remained common in the HAART (highly active antiretroviral therapy) era [173]. Clinical pancreatitis, however, probably occurs in less than 10% of all patients with AIDS, and in even fewer of those not receiving toxic medications. The diagnosis of pancreatitis should be based on bedside findings and imaging tests in combination with elevated enzyme levels, and not on the basis of elevated pancreatic enzymes alone. In addition to drugs and the other common causes of pancreatitis, at least three pathological processes contribute to pancreatitis in AIDS [173]:

- HIV may infect the pancreas, which may cause the elevated serum amylase often seen in AIDS, and may account for the changes of otherwise unexplained chronic pancreatitis that have been seen at autopsy of AIDS patients. However, HIV infection alone seems rarely to lead to clinical pancreatitis.
- Opportunistic infections may involve the pancreas. Most infections are caused by cytomegalovirus or *Mycobacterium avium*. *Cryptococcus*, *Toxoplasma gondii*, *Histoplasma*, *Mycobacterium tuberculosis*, and *Candida* species have been reported. Opportunistic infections involving the pancreas usually involve other organs as well. Although histological evidence of pancreatic opportunistic infection is common at autopsy, it is unusual for these opportunistic infections to produce clinical pancreatitis during life.
- Pancreatic neoplasms develop in up to 5% of patients with AIDS. Kaposi sarcoma and lymphoma are the most common and usually involve the pancreas in the setting of widely disseminated disease. Pancreatitis, however, is not a common manifestation of this tumor involvement.

Bacterial and parasitic

The list of bacteria associated with acute pancreatitis includes *Salmonella* species, *Shigella* species, *Campylobacter* species, hemorrhagic *Escherichia coli*, *Legionella* species, *Leptospira* species, and even *Brucella* species [182,183]. Pancreatitis associated with these infections is most likely secondary to released toxins. Usually, acute pancreatitis is not the primary manifestation of these infections. Direct involvement of the pancreas or ampulla with parasites is a common cause of pancreatitis in some populations. Almost 15% of cases of biliary pancreatitis in a series from the Orient could be ascribed to *Ascaris lumbricoides* infection [144]. Biliary and hepatic parasites, including *Fasciola hepatica* and *Clonorchis sinensis*, may also cause acute pancreatitis.

Autoimmune

Lymphoplasmacytic sclerosing pancreatitis

Lymphoplasmacytic sclerosing pancreatitis (LPSP), also called "autoimmune pancreatitis," has distinctive clinical, laboratory, imaging, and histological features (as shown in Fig. 68.10), and responds to corticosteroid therapy. Patients classically present with subacute symptoms of pancreatitis and frequently have obstructive jaundice; a minority present

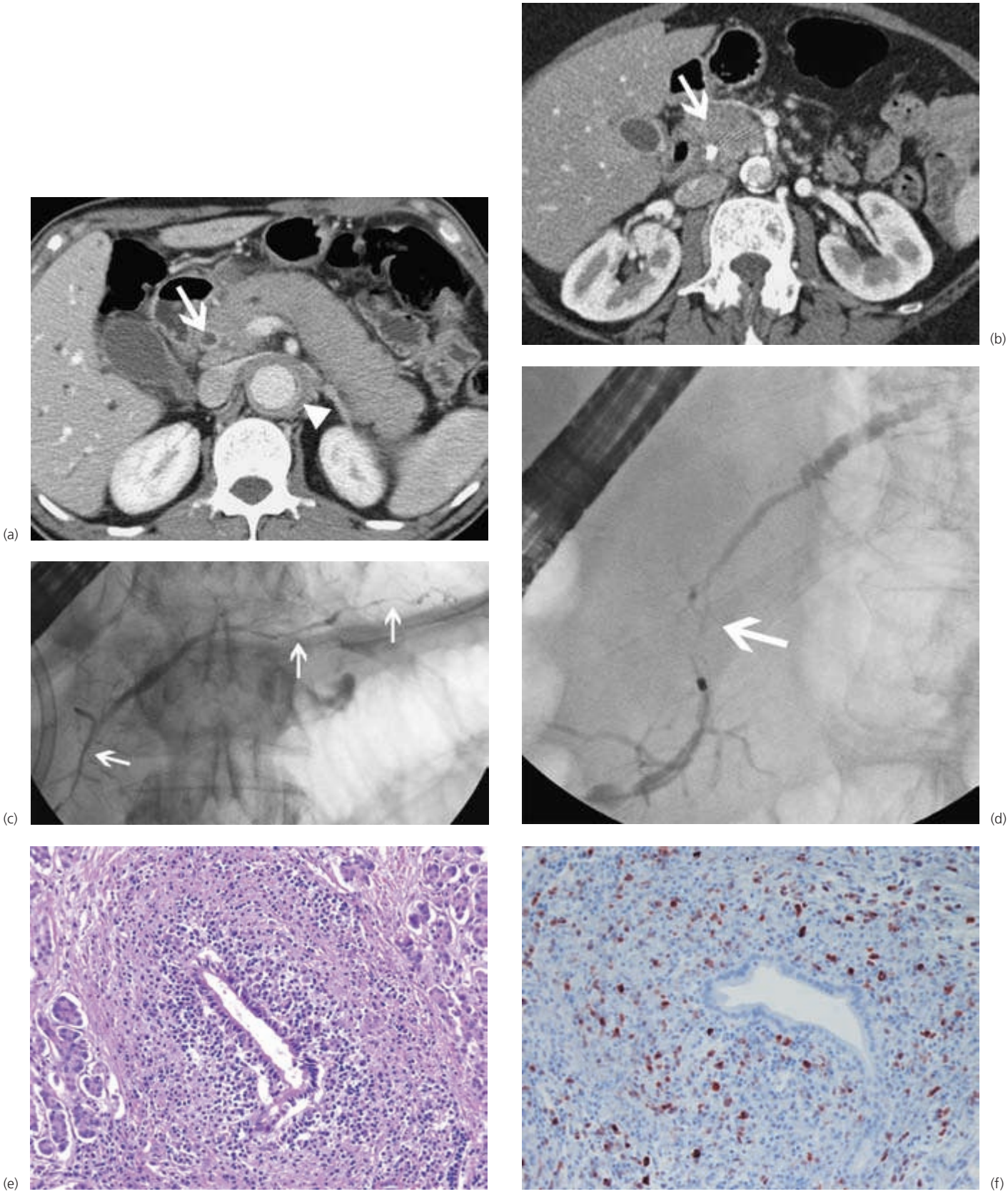


Figure 68.10 Autoimmune pancreatitis. **(a)** Diffuse pancreatic enlargement with a thick-walled common bile duct (arrow) and periaortitis (arrowhead). Courtesy of Suresh Chari, Mayo College of Medicine. **(b)** A focal pancreatic head mass (arrow). A plastic biliary stent is present in the common bile duct. **(c)** Multifocal, long pancreatic duct strictures (arrows). **(d)** Isolated pancreatic duct stricture (arrow). **(e)** Perivenular lymphoplasmacytic infiltrate. Courtesy of Thomas Smyrk, Mayo College of Medicine. **(f)** Perivenular infiltrate stained with antibody to IgG4. Courtesy of Thomas Smyrk, Mayo College of Medicine.

with recurrent acute pancreatitis or pancreatic insufficiency. Some patients also have other autoimmune illnesses. Cross-sectional imaging in the acute phase of the illness may show a diffusely enlarged, "sausage-shaped" pancreas, or a focal pancreatic mass, usually without marked peripancreatic inflammatory changes. The gland may appear to have a capsule-like edge on CT or MRI, and there may be delayed enhancement with intravenous contrast [184]. Pancreatography classically shows diffuse irregular narrowing of the main pancreatic duct, but may also demonstrate a focal dominant pancreatic duct stricture simulating malignancy. Biliary involvement is not unusual, and may include strictures of the intrapancreatic bile duct, hilar strictures simulating malignancy, or intrahepatic ductal changes reminiscent of sclerosing cholangitis (Fig. 68.11). Histology shows a pathognomonic lymphoplasmacytic infiltrate involving small ducts and venules, and sparing arterioles; the inflammatory infiltrate stains positive for IgG4 (Fig. 68.10). Pancreatic calcification is uncommon.

A variety of laboratory abnormalities have been described, including elevated autoantibody levels and serum IgE concentrations, but the most characteristic laboratory findings appears to be an elevated serum IgG4 level [185], alone or together with generalized hypergammaglobulinemia. Elevations of serum IgG4 are present in about 75% of patients. Autoimmune pancreatitis may be one presentation of a systemic IgG4 disease, which can also manifest as retroperitoneal fibrosis, periaortitis, and biliary, ocular, or renal disease.

The differential diagnosis of autoimmune pancreatitis often includes pancreatic neoplasm. Patients may have involvement of adjacent vessels on cross-sectional imaging studies or new-onset hyperglycemia, further increasing the clinical suspicion of malignancy. Diagnostic criteria for autoimmune pancreatitis are debated; classically the diagnosis has been made on the basis of elevated serum IgG4 levels, diffuse pancreatic enlargement on CT or MRI, and diffuse narrowing and irregularity of the main pancreatic duct on pancreatography, but many patients do not have all of these findings. Elevation of the serum IgG4 is suggestive but not specific, as it has been reported in pancreatic malignancy. Other organ involvement is a useful clue in some patients. Intraductal brushings and biopsies obtained during ERCP, or endoscopic ultrasound (EUS)-guided needle aspiration of a mass are appropriate in some cases but generally do not establish a definite diagnosis of autoimmune pancreatitis. Endoscopic ultrasound-guided Tru-Cut biopsy of the pancreas can make the diagnosis by showing pathognomonic histological features, or suggestive histological features together with an IgG4-positive inflammatory infiltrate (Fig. 68.10). Pancreatic biopsy is appropriate in some patients, particularly if pathognomonic histology will prevent pancreatic resection. A key feature of the disease is the good response to oral glucocorticoids with resolution of pancreatic swelling and biliary obstruction, and a decrease in IgG4 levels [185]. Impaired pancreatic endocrine function may also improve [186]. Recurrence after a slow taper of oral steroids appears to be uncommon. The natural history of autoimmune pancreatitis is unclear, but some patients appear to develop subsequent pancreatic atrophy.

Inflammatory bowel disease

Pancreatitis in patients with inflammatory bowel disease may be related to drugs, structural and inflammatory changes, or may be idiopathic in nature. Most medications used for the treatment of inflammatory bowel disease have been reported to precipitate pancreatitis. This list includes azothioprine, 6-mercaptopurine, sulfasalazine, 5-aminosalicylic acid, and (rarely) corticosteroids. Sclerosing cholangitis is occasionally associated with clinical pancreatitis. This may be due to passage of small pigment stones through the ampulla, or to a sclerosing process affecting the pancreatic ducts as well as the bile ducts [187]. When pancreatic duct abnormalities are seen together with sclerosing cholangitis-like changes, the diagnosis of autoimmune pancreatitis should be considered.

Patients with pancreatitis and Crohn's disease may have inflammatory bowel disease of the duodenum. The inflammatory process may be associated with stenosis of the ampulla or a fistula into the pancreatic duct. The increased incidence of gallstones in patients with Crohn's disease should prompt a search for biliary causes of pancreatitis. In a small number of patients, however, no etiology is identified.

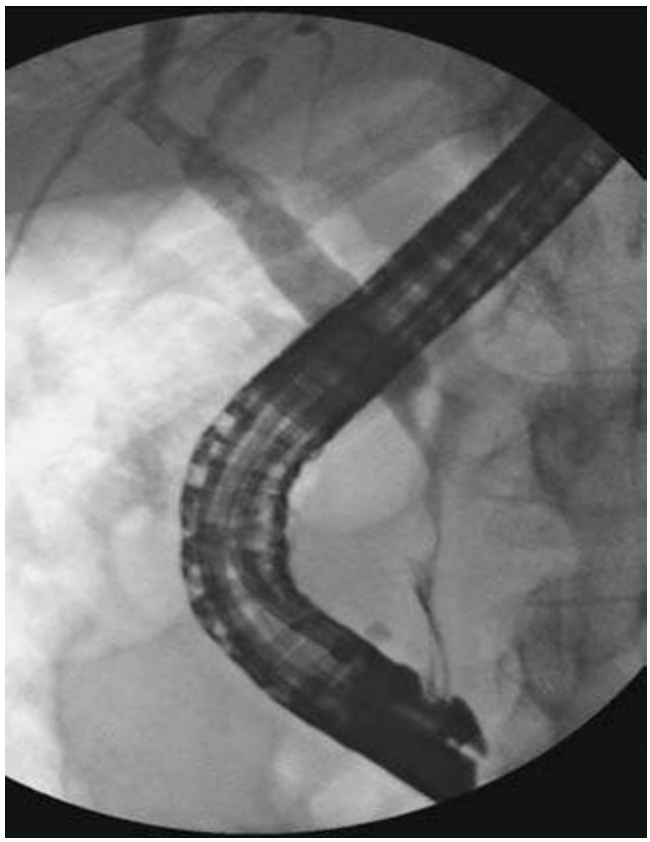
Systemic autoimmune diseases

Pancreatitis may occur in the setting of a systemic autoimmune disorder. Vasculitis affecting the pancreas has been described in systemic lupus erythematosus, periarteritis nodosa, Wegner disease, and Behçet disease. Pancreatitis may respond to successful treatment of the underlying vasculitis.

Genetic

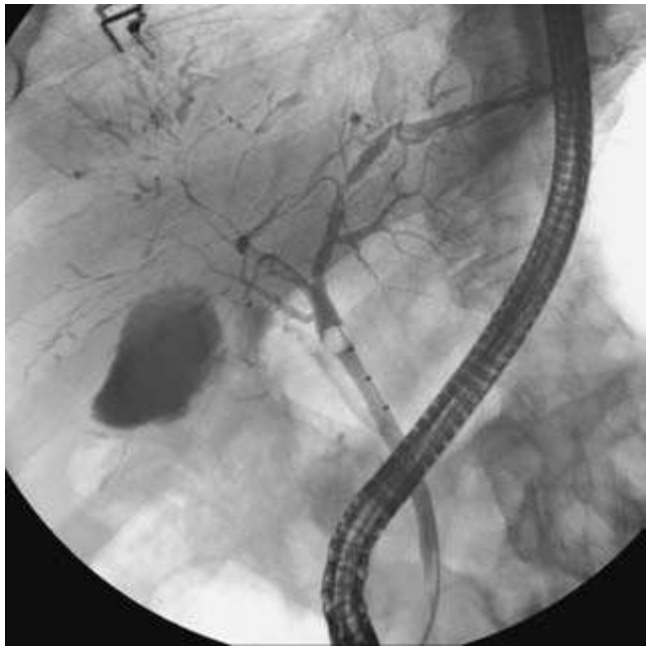
The best described form of familial pancreatitis is the autosomal dominant disorder known as hereditary pancreatitis. Penetrance approaches 80%. Affected family members have bouts of pancreatitis that often begin in childhood and typically progress to chronic pancreatitis (see Chapter 69). The course of the disease appears to be accelerated by alcohol. Many of these patients have been found to have mutations in the cationic trypsinogen (*PRSS1*) gene. The most common mutation (at Arg117) may make trypsin resistant to proteolytic degradation [98]. Patients with hereditary pancreatitis have a dramatically increased risk of pancreatic cancer [188]. Mutations in the pancreatic secretory trypsin inhibitor (*SPINK1*) gene also predispose to disease [189,190].

Mutations in the cystic fibrosis transmembrane regulator gene (*CFTR*) cause two patterns of pancreatic disease. First, classic cystic fibrosis results in pulmonary disease and pancreatic insufficiency, but rarely pancreatitis. These patients have elevated sweat chloride levels and markedly diminished



(a)

(b)



(c)

Figure 68.11 Biliary findings in autoimmune pancreatitis. **(a)** Distal common bile-duct stricture. **(b)** Strictures of the biliary confluence simulating a Klatskin tumor. **(c)** Multifocal intrahepatic duct strictures simulating sclerosing cholangitis.

CFTR function in the nasal mucosa. Second, a pancreatitic phenotype is recognized that is not associated with pulmonary disease but can likely cause both acute and chronic pancreatitis [191,192]. These patients have normal sweat chloride values, and the CFTR function in the nasal mucosa is diminished but present. Although these patients exhibit mutations in the *CFTR* gene, they are not homozygous for the mutations that result in classic cystic fibrosis. Partial loss of CFTR function has been associated with other adult-onset, single-organ diseases. The prevalence of CFTR-related dis-

ease in patients with idiopathic pancreatitis and divisum-associated pancreatitis appears to be increased compared with that in the general population. Thes contribution of CFTR-related disease to alcoholic pancreatitis remains unclear.

Iatrogenic

Endoscopic retrograde cholangiopancreatography (ERCP)

Acute pancreatitis is the commonest complication of ERCP. Prospective studies with careful patient follow-up report an incidence of about 5%. In most cases the pancreatitis is mild and resolves within several days; complications requiring surgery are infrequent. Diabetes can rarely result from an episode of severe post-ERCP pancreatitis, and deaths due to pancreatitis have been reported in 0.02% of patients undergoing the procedure.

The chance of developing post-ERCP pancreatitis varies depending on both patient-related and procedure-related factors. The skill and experience of the endoscopist also affect the chance of pancreatitis, with low case volume increasing the risk for the patient [193,194]. The risk of post-ERCP pancreatitis is substantially higher when the pancreatic duct is visualized [195]. The number and hydrostatic pressure of pancreatic duct injections are probably also important, as well as the volume of fluid injected. In one study, the administration of more than four pancreatic duct injections was associated with an increased risk. Acinarization (visualization of the pancreatic parenchyma with injected contrast) is associated with subsequent pancreatitis about one-third of the time. In addition to filling and overfilling of the pancreatic duct, pancreatic outflow obstruction due to ampullary edema or spasm is a likely contributor to post-ERCP pancreatitis. Repeated attempts at bile duct cannulation may cause postprocedure pancreatitis even in the absence of pancreatic duct cannulation [194]. Pancreatitis has been reported after percutaneous biliary drains are advanced through the ampulla into the duodenum, with no injection or manipulation of the pancreatic duct.

Endoscopic sphincterotomy further increases the risk of pancreatitis, and may do so both by thermal injury to the pancreas and by provoking ampullary edema. Pre-cut sphincterotomy appears to increase the risk in some centers, but not in others where this technique is used routinely. Sphincter of Oddi manometry has been thought to increase the risk; however, a large study of postsphincterotomy complications found that the performance of manometry did not affect the incidence of pancreatitis in patients with suspected sphincter of Oddi dysfunction [194].

Patient factors also affect the chances of ERCP-induced pancreatitis. About 20% of patients with suspected sphincter of Oddi dysfunction undergoing sphincterotomy without prophylactic pancreatic duct stenting develop pancreatitis, and these patients may be at increased risk for severe pancreatitis. The risk is especially high in patients with small bile ducts [194]. Younger age and obesity probably also predispose to pancreatitis after ERCP. Conversely, patients with a chronically diseased or obstructed pancreatic duct appear to be at decreased risk for pancreatitis from ERCP.

The presence of acute biliary pancreatitis does not seem to increase the risk of the procedure. A history of contrast allergy has been reported not to increase the risk of post-ERCP pancreatitis, but many endoscopists favor pretreatment with steroids or use of nonionic contrast media in such patients [196]. Pretreatment may be especially important in patients with a history of serious allergic reactions to topical or ingested iodine.

Diagnosis

Symptoms of post-ERCP pancreatitis typically develop within 2–6 h of the procedure, although occasionally patients will first develop symptoms the following day. The presence of abdominal symptoms shortly after the procedure is a poor predictor of subsequent pancreatitis. Consensus criteria for diagnosis of pancreatitis after ERCP require elevation of serum amylase or lipase to three-fold the upper limit of normal on the day after the procedure, together with compatible clinical symptoms [196]. Early predictors of post-ERCP pancreatitis have been identified. Although elevation of the serum amylase is a nonspecific finding in the hours following ERCP, the height of amylase elevation may be useful. A normal serum amylase is a strong negative predictor of pancreatitis when measured 2 h after ERCP, at least in patients who have not received a prophylactic pancreatic duct stent [197].

Post-ERCP pancreatitis may occur even if the procedure is easily accomplished and all identified risk factors are avoided. Patients sent home after ERCP should be instructed to seek help promptly if pain or vomiting develop. It is our practice to use a clear liquid diet until the morning following ERCP to limit pancreatic stimulation in the postprocedure period.

Prevention

The best method of preventing ERCP-induced pancreatitis is to avoid ERCP when possible. Less invasive imaging modalities have decreased the need for diagnostic ERCP. When ERCP is required, both pharmacological and endoscopic strategies for the prevention of post-ERCP pancreatitis have been proposed. Some, such as use of low-osmolarity contrast media or pretreatment with calcium channel blockers, octreotide, aprotinin (a protease inhibitor), nifedipine, allopurinol, and IL-10, have proven to be ineffective. Reports of benefit from prophylactic diclofenac adminstration require larger, multicenter confirmatory studies [198].

Gabexate mesilate is a protease inhibitor that inhibits proteolytic activity of pancreatic enzymes. The few individual trials of gabexate for prevention of post-ERCP pancreatitis have all shown a benefit, although not statistically significant in most cases. A metaanalysis demonstrated a statistically significant benefit with gabexate pretreatment, which decreased the incidence of pancreatitis from 6.5% to 1.6%

[199]. Pretreatment of 27 patients would prevent one episode of pancreatitis. This agent is not licensed for use in the United States, and enthusiasm for gabexate has been tempered by its cost and its adminstration as a prolonged intravenous infusion.

Somatostatin also appears to decrease the incidence of post-ERCP pancreatitis. Numerous controlled trials of somatostatin have shown a benefit, often not statistically significant, but in metaanalysis a significant benefit was found [199]. Taken together, the incidence of pancreatitis was decreased from 13.5% to 5.6% in these studies. Pretreatment of 13 patients would prevent one episode of pancreatitis. Somatostatin appears to be effective when given either as a 12-h intravenous infusion or as a bolus intravenous injection immediately before cannulation of the papilla [200]. Somatostatin is not licensed for use in the United States. The lack of benefit of octreotide, a somatostatin analogue, may be due to its effects on sphincter of Oddi function and pancreatic blood flow.

Temporary pancreatic duct stenting dramatically decreases the risk of postsphincterotomy pancreatitis in some patients, including those with pancreatic sphincter hypertension or difficult biliary cannulation [201,202]. This finding highlights the usefulness of sphincter of Oddi manometry in patients with suspected sphincter dysfunction. However, a controlled trial of pancreatic duct stenting immediately after biliary sphincterotomy in a mixed group of patients did not show a reduction in postprocedure pancreatitis [203].

Postoperative

In the postoperative period there is an increase in the incidence of hyperamylasemia, even after extraabdominal procedures [204]. It appears that in many cases these increases in serum amylase levels are nonspecific and unrelated to clinically overt pancreatitis, although severe pancreatitis may ensue and can be related to hypotension, medication, or pancreatic trauma. In a prospective study, 32% of patients had hyperamylasemia after cardiac bypass, but only 2.7% had overt pancreatitis [205]. A direct correlation has been demonstrated between the elevation of pancreatic enzymes and the amount of calcium administered to patients during cardiac bypass, suggesting a nonspecific effect of calcium on pancreatic duct permeability. A severe form of pancreatitis sometimes does occur after cardiac bypass, and may be related to pancreatic ischemia.

Neoplastic

Pancreatitis is associated with both primary pancreatic tumors and metastases. In one series, 3% of patients with pancreatitis admitted to a university hospital were found to have pancreatic malignancy [206]. Pancreatitis has been reported in up to 10% of patients with pancreatic cancer, but it is usually mild [207]. Cancer can mimic chronic pancreatitis, presenting with a dilated pancreatic duct, or a pseudocyst that forms because of downstream ductal obstruction by the tumor. Cancer can also mimic acute pancreatitis, especially when bleeding into the pancreatic duct (hemosuccus pancreaticus) temporarily obstructs the duct. As clots pass through the papilla the serum liver enzymes may rise together with the amylase. Intraductal papillary mucinous neoplasm (IPMN) of the pancreas can similarly precipitate bouts of acute pancreatitis related to passage of mucus.

Frequently, symptoms of pancreatitis are associated with an early stage pancreatic neoplasm and may precede the diagnosis of pancreatic cancer by months. Older patients with unexplained pancreatitis should undergo a careful evaluation for occult malignancy (often including EUS), looking for a minute pancreatic cancer amenable to resection [208]. Positron emission tomography (PET) scanning may be useful for diagnosis of underlying pancreatic cancer in patients whose pancreatitis obscures a neoplastic mass on CT or EUS. Mature cystic lesions present on the patient's first CT scan may represent cystic neoplasms such as branch duct IPMN lesions rather than pseudocysts; EUS-guided fine-needle aspiration or Tru-Cut biopsy may be useful in differential diagnosis of such lesions.

Vascular

Ischemia is an uncommon cause of acute pancreatitis. Pancreatic infarcts may occur in patients with underlying atherosclerotic vascular disease, but are unusual because the pancreas is richly perfused from several different arterial sources. Cholesterol emboli may cause pancreatitis, cholecystitis, or bowel ulceration or infarction, and should be suspected when acute pancreatitis occurs after vascular interventions such as cardiac catheterization [209]. Patients may have associated evidence of renal, gut, or peripheral cholesterol emboli. Ischemic pancreatic and hepatic injury may be associated with malignant hypertension, low flow states due to severe heart failure, or administration of potent vasoconstrictors. Vasculitis may cause pancreatitis associated with systemic autoimmune diseases, as noted above.

Other

Trauma

Traumatic pancreatitis caused by penetrating or blunt injury is an important cause of disease in both adults and children. The symptoms of pancreatic trauma may be difficult to elicit in the severely ill patient and suspected only by the location of the injury. On occasion, recurrent bouts of pancreatitis may develop months to years after injury. The mid-pancreatic duct as it crosses the vertebral column is particularly susceptible to fracture from blunt trauma. In the acute setting, CT is useful in detecting a disrupted pancreatic duct, focal pancreatitis, and pancreatic necrosis (Fig. 68.12). Similarly, ERCP has been used to demonstrate persistent duct disruption, ductal stricture, and focal pancreatitis. Traumatic pancreatitis has been treated with stenting of the disrupted duct, pancreatic resection, or drainage [210].

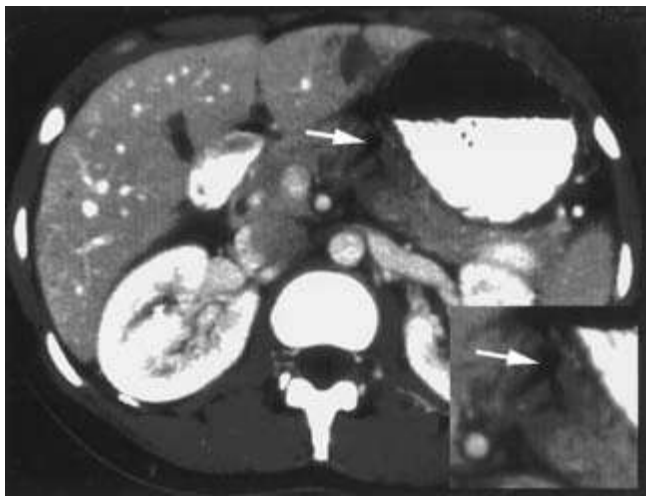


Figure 68.12 Traumatic pancreatitis. There is disruption of the pancreatic body anterior to the spine (arrow). The left lobe of the liver is also injured. Courtesy of Jeffrey Neitlich, Yale University.

Peptic ulcer

Ulcers located at the posterior duodenal bulb may penetrate into the head of the pancreas. Pancreatitis associated with duodenal ulcers is rarely severe and is usually not associated with dramatic increases in the serum amylase. This diagnosis should be considered in patients with ulcer disease when the typical epigastric burning pain of an ulcer begins to radiate to the back and is less responsive to ulcer medications.

Childhood

Pancreatitis may first present in childhood. Many cases of childhood pancreatitis are associated with multisystem disease such as sepsis, viral infections, hemolytic-uremic syndrome, and Reye syndrome. Blunt trauma caused by accidents or child abuse is the single most common cause of acute childhood pancreatitis, followed in decreasing order of incidence by congenital defects (including biliary cysts, anomalous pancreaticobiliary junctions, and pancreas divisum), metabolic diseases, and drugs. Children with hemolytic disorders such as sickle-cell anemia commonly form pigment stones in the gallbladder or bile ducts. Rarely, annular pancreas, duodenal diverticulum, ectopic pancreatic tissue, and duodenal duplication cysts may cause pancreatitis.

Miscellaneous

An increased incidence of pancreatitis has been reported during pregnancy and the early postpartum period. The increased incidence of hypertriglyceridemia and biliary stone formation during pregnancy may contribute to this predisposition. Thee is also an increased risk of acute pancreatitis reported in patients undergoing renal dialysis. The risk may be limited to those treated with chronic peritoneal dialysis and not hemodialysis [211]. Refeeding after long periods of fasting is said to precipitate pancreatitis, but this has not been well characterized. A few patients with possible food allergy-associated pancreatitis have been reported [212].

Idiopathic

The number of patients having unexplained bouts of pancreatitis has diminished in large part because of improvements in imaging tests and greater insight into genetic abnormalities in unexplained pancreatitis. The compelling evidence that biliary microlithiasis causes pancreatitis, the recognition of autoimmune pancreatitis, as well as the wider use of ERCP, EUS, and genetic testing, have further decreased the size of the idiopathic group.

Approach to unexplained pancreatitis

In 10%–20% of patients, pancreatitis is idiopathic despite careful evaluation of the history and physical exam, routine laboratory tests, transabdominal ultrasound, and CT. An etiological diagnosis can be made in most of these patients with further evaluation. A thorough diagnostic approach should be taken, including use of the medical history, physical examination, laboratory tests, and additional imaging studies. An algorithm outlining a general approach to such patients is shown in Fig. 68.13.

The patient's history should be reviewed, particularly with regard to alcohol and medication use, family history of pancreatic disease, and prior abdominal trauma. A history of male infertility or the physical examination finding of testicular atrophy may raise the possibility of congenital absence of the vas deferens, which is associated with decreased CFTR function and may signal a genetic basis for pancreatitis. Metabolic disease should also be considered. Serum triglycerides decrease with fasting, and a hyperlipidemic cause of pancreatitis may be missed if triglycerides were not measured soon after the patient's symptoms began. Hypercalcemia may not be apparent until after the patient has recovered from pancreatitis, because acute pancreatitis often lowers serum calcium levels.

Repeat abdominal imaging studies may be indicated in unexplained pancreatitis, as the quality of imaging studies may vary significantly based on the equipment used, the operator, the acuity of illness at the time the study was performed, and the use and timing of intravenous contrast. In addition, gallbladder sludge can come and go on serial abdominal ultrasound exams.

When there is evidence of chronic pancreatitis in the absence of alcohol use or familial pancreatitis, further evaluation can include laboratory tests for α_1-antitrypsin deficiency and hemochromatosis. Mutations of the *PRSS1* or *SPINK1* genes associated with hereditary pancreatitis may be present without a family history of pancreatitis as a result of a new mutation or low penetrance. Patients with *CFTR* mutations and pancreatitis usually do not have a family history of disease. Genetic testing for these disorders can be performed;

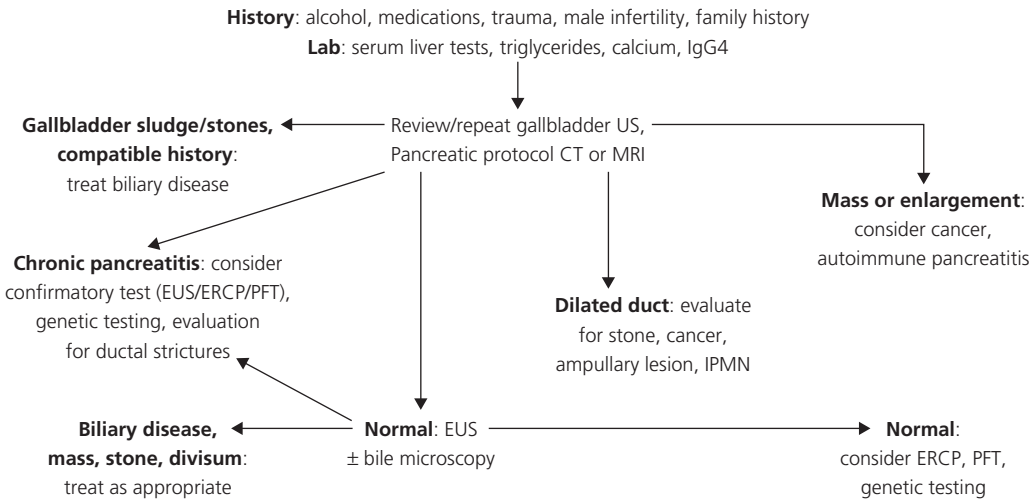


Figure 68.13 Algorithmic approach to unexplained pancreatitis. CT, computed tomography; ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasound; IPMN, intraductal papillary mucinous neoplasm; MR, magnetic resonance (imaging); PFT, pancreatic function test; US, ultrasound.

however, enthusiasm for this testing must be tempered by the absence of effective specific therapy, the lack of effective pancreatic cancer screening for patients with hereditary pancreatitis, and the possible psychological and insurability problems that may arise from the diagnosis of a genetic disorder. Genetic diagnosis may nevertheless be desirable, particularly for patients of child-bearing potential. In general it is wise to refer the patient for genetic counseling before obtaining these tests.

Biliary microlithiasis is a leading cause of recurrent acute pancreatitis and should be actively pursued in patients with unexplained disease. Endoscopic ultrasound and bile microscopy appear to play a complementary role in diagnosis, and microlithiasis is unlikely if both of these investigations are negative. Nevertheless, empirical cholecystectomy may be a reasonable option in some patients, especially if the clinical features of their acute attacks suggest a biliary source, and ampullary disease has been excluded.

Abnormalities of pancreatic duct drainage, including pancreas divisum, ampullary lesions, and sphincter of Oddi dysfunction, should be excluded in patients with recurrent acute attacks and no evidence of microlithiasis. Whereas EUS can diagnose ampullary masses and divisum, ERCP is particularly useful for diagnosis of sphincter dysfunction and incomplete divisum, in which the dorsal and ventral ducts communicate but dominant drainage is through the minor papilla. Such patients may present with recurrent mild pancreatitis [213]. Endoscopic retrograde cholangiopancreatography is also useful for diagnosis of intraductal papillary mucinous neoplasms of the pancreas, which often present with pancreatic symptoms and serum enzyme elevations. It may miss branch duct IPMN lesions, however.

It is generally our practice to use EUS instead of ERCP as the first endoscopic test in unexplained pancreatitis. Endoscopic ultrasound is particularly useful in patients with intact gallbladders, who may have microlithiasis, and in those over 40 years old, in whom the possibility of an early pancreatic malignancy or IPMN should be considered. Missed chronic pancreatitis, pancreas divisum, and ampullary lesions can be diagnosed during EUS as well. A diagnosis is made by EUS in about two-thirds of patients [214]. When disease remains idiopathic and is recurrent, ERCP (with the capability to perform sphincter of Oddi manometry) or a pancreatic function test (for diagnosis of chronic pancreatitis) can be pursued.

Diagnosis

The diagnosis of acute pancreatitis is almost always based on the presence of severe abdominal pain together with biochemical evidence of pancreatic injury. Rarely, patients with severe acute pancreatitis present without complaints of abdominal pain, but with respiratory failure or changes in mental status. The diagnosis of pancreatitis may be missed entirely when organ failure dominates the clinical picture. For example, in studies published during the 1980s, fatal pancreatitis went undetected until postmortem in 22%–42% of cases.

Symptoms

Pain is the most common symptom of acute pancreatitis and occurs in 95% of patients. It is often located in the epigastric and umbilical region and may radiate to the low thoracic region of the back. The pain usually does not reach its peak for 30 minutes to several hours and may last for hours to days. This deep, visceral pain is among the most severe and often leaves even the medicated patient in discomfort. In some, pain will decrease when sitting and leaning forward or

curling up on the left side, compared with lying flat. This finding is due to the retroperitoneal position of the pancreas and may be produced by other retroperitoneal processes. Nausea and vomiting are present in 85% of patients and may occur without an ileus or gastric outlet obstruction. Unlike patients with ulcer disease, vomiting does not relieve pain.

Signs

Low-grade fevers are reported in 60% of patients, and high-grade fevers may indicate the presence of cholangitis or either sterile or infected necrosis. Tachycardia and hypotension are found in up to 40% of patients and may result from intravascular volume depletion, enhanced vascular permeability, vasodilation, and hemorrhage. Abdominal tenderness and guarding is common, and bowel sounds are often decreased or absent. Pleural effusions are most often found on the left, but may be bilateral. Mild jaundice is not unusual, but bilirubin levels greater than 4 mg/dL suggest extrahepatic obstruction or underlying liver disease. Dark discoloration in the back, flank, or the paraumbilical region may arise from any retroperitoneal hemorrhage, including hemorrhage from pancreatitis.

Laboratory tests

Although the gold standard for diagnosing acute pancreatitis is a direct histological examination of the pancreas, this is rarely available. The goals of diagnostic tests for pancreatitis are to noninvasively establish the diagnosis and cause in patients with abdominal pain and to determine the prognosis. Clinicians should be mindful that a number of factors influence the level of serum markers of pancreatitis. First, serum levels of pancreatic enzymes are the sum of tissue production, release into the blood, and clearance. Thus, in patients with renal failure, the serum amylase may increase because the kidney does not clear the enzyme. Second, the measured enzyme activities may be influenced by a number of "serum factors" and may not reflect absolute enzyme levels. For example, hyperlipidemia is known to reduce routine serum amylase determinations to give an erroneous reading. Third, enzymes may be produced from nonpancreatic tissues, and this may limit the specificity of serum enzyme assays. In general, the higher the enzyme levels the better the chance that one is dealing with pancreatitis. Finally, standard enzyme assays, such as the amylase and lipase assays, provide no information on the severity of the pancreatitis.

Amylase

The serum amylase level has been the biochemical standard for diagnosing acute pancreatitis. Many organs generate amylase, an activity that hydrolyzes the internal 1–4α-linkages of starch. Because there are no circulating inhibitors of amylase activity, measurements of amylase activity in serum are reliable. Lactescent sera may inhibit amylase, but this effect can be eliminated by sample dilution. Although many tissues synthesize amylase, most of the serum activity originates from the pancreas (~ 40%) and the salivary glands (~ 60%). P-isoamylase arises from the pancreas and S-isoamylase from the salivary glands as well as other tissues such as the fallopian tubes and lung. Pancreatic amylase enters the blood through an unknown pathway and has a serum half-life of about 2 h. Although the major portion of serum amylase and other pancreatic enzymes is probably cleared by the reticuloendothelial system, about one-fourth of serum amylase is excreted in its intact form by the kidney.

Increased serum amylase during acute pancreatitis results from both enhanced release into the blood and decreased renal clearance. Serum amylase levels increase within the first hours after the onset of pancreatitis and parallel serum lipase levels. After an attack, however, the serum amylase falls much more rapidly than lipase, and may return to normal within 24 h. Lipase levels are more reliable indicators of pancreatitis in patients who are first seen several days after the onset of their illness (Fig. 68.14). In most situations, the degree of amylase elevation does not identify the cause or severity of pancreatitis. Elevations of serum amylase levels tend to be less in patients with alcoholic pancreatitis, pancreatic cancer, or penetrating ulcers, compared with patients with gallstone disease.

Defining abnormal values influences the sensitivity and specificity of the total serum amylase. Cutoff values of just above the normal range have been estimated to yield sensitivities greater than 90%, but specificities of less than 70% for acute pancreatitis. If the cutoff value is increased to three times the upper limit of normal, the specificity is near 100%, but the sensitivity may fall below 60%. Nonspecific increases of one- to two-fold in the serum amylase have been reported after cigarette smoking [215].

In an attempt to enhance the specificity of amylase measurements, amylase isoforms may be measured. P-isoamylase

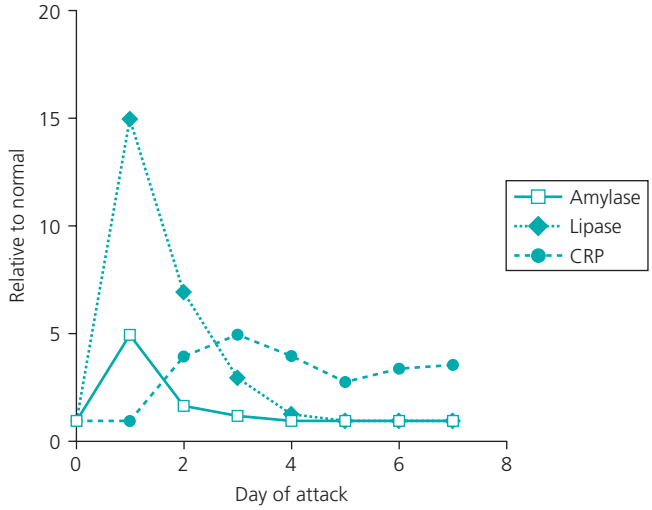


Figure 68.14 Serum markers of pancreatic injury.

is probably best used to confirm that an elevated total amylase level arises from the pancreas [216]. The clinical value of measuring P-isoamylase may be compromised by the inability of the assay accurately to measure small amounts of this isoform. Furthermore, P-isoamylase levels may increase in the absence of pancreatitis in patients with biliary tract disease, intestinal perforation, intestinal obstruction or ischemia, and ruptured abdominal aortic aneurysms.

Diseases of the salivary glands may lead to an elevated level of S-isoamylase; however, S-isoamylase levels may increase in the absence of salivary disease in chronic alcoholics, postoperative states, lactic acidosis, patients with anorexia nervosa and bulimia, after esophageal perforation, and in neoplasms such as pulmonary, gastric, and breast carcinoma, and myeloma.

Macroamylasemia

On occasion, a persistently elevated serum amylase is encountered in the absence of symptoms referable to the pancreas. An uncommon condition that can lead to such an elevated serum amylase is macroamylasemia. Amylase is normally found in the bloodstream as a molecule with a molecular weight of 45 kDa, but macroamylase often has a molecular weight of greater than 200 kDa. The normal ratio of pancreatic to salivary amylase is maintained in macroamylasemia. The high-molecular-weight macroamylase may result from antibodies complexed to amylase or from amylase polymers [217]. Unlike normal amylase, the macroamylase complex cannot enter the renal tubule for excretion and accumulates in the serum. Several methods can be used to diagnose macroamylasemia. First, amylase-to-creatinine clearance ratios of less than 1% strongly support the diagnosis. To confirm the diagnosis in patients with a low clearance, the clinical laboratory may determine the molecular weight of the serum amylase. Alternatively, immunological assays also may be used. Acquired macroamylasemia may occasionally signal the presence of a lymphoproliferative disorder.

Lipase

Rapid and reproducible methods for measuring serum lipase are available. Lipase activity is generated by a number of gastrointestinal tissues, including the pancreas, intestine, and liver and biliary tract, and from lingual and gastric sources. In some laboratory assays, the serum lipoprotein lipase activity is also detected [218]. The contribution of these various sources of lipase to serum lipase activity is not clear. Furthermore, the different substrates used in the various commercial assays may affect the specificity of the lipase measurement for pancreatic lipase. The least specific seems to be the Ektachem test, which has been found to have a specificity as low as 25% [219]. For many clinicians, the serum lipase has supplanted the amylase as the single test of choice for the diagnosis of pancreatitis. In most series, it is as sensitive and more specific than the total serum amylase, and promises to improve further when the L2 pancreatic lipase is measured. This enhanced specificity of the lipase level compared with the amylase level also has been reported in alcoholics [220]. Because lipase levels increase in parallel with serum amylase levels, lipase is a useful early marker of pancreatitis. The lipase level remains elevated longer than the amylase level, and thus may help to diagnose pancreatitis after an acute attack has passed (Fig. 68.14). Another advantage of lipase is that levels are normal in diabetic ketoacidosis and macroamylasemia.

Other pancreatic enzymes

Presumably all pancreatic enzymes are released into the blood during acute pancreatitis. Serum trypsin, chymotrypsin, elastase, ribonuclease, and phospholipase A2 have all been reported to be elevated in acute pancreatitis, but the specificity of these tests has not been sufficiently defined.

Urinary enzyme levels

Measurements of urinary enzyme levels have been used to diagnose acute pancreatitis. These include urinary trypsinogen 2 [221] and urinary levels of activation peptide of carboxypeptidase B. Both use a simple, rapid dipstick assay, and the former has reported sensitivities of 65%–95% and specificities above 90% for diagnosis of acute pancreatitis [222,223]. These findings require further confirmation. Urinary amylase levels, at one time advocated for improved sensitivity of diagnosis of acute pancreatitis, reflect the patient's intravascular volume status and renal function and probably do not improve the accuracy of diagnosis compared with serum enzyme levels.

Trypsinogen activation peptide

Premature activation of trypsinogen within the pancreas is thought to be an early step in pancreatitis. The small peptide removed during activation of trypsinogen to trypsin is known as trypsinogen activation peptide (TAP). Levels of TAP in the blood or urine reflect trypsinogen conversion to trypsin. Although no more accurate than serum amylase for diagnosis of acute pancreatitis, determinations of serum and urine levels of TAP have been reported to separate severe from mild pancreatitis when measured within the first 36 h of disease [224].

Inflammatory markers

The inflammatory response is a prominent component of pancreatitis and increases in parallel with its severity. Leukocytosis is common, and severe pancreatitis has been associated with leukemoid reactions even in the absence of infection. Several serum markers have been used to quantitate the inflammatory response. Neutrophil-specific elastase is released by inflammatory cells, and elevated serum levels are detected within the first 12–24 h of pancreatitis. Interleukin-6, released by macrophages, also increases during the acute phases of pancreatitis. Acute-phase react-

ants such as C-reactive protein (CRP) are induced by IL-6 and increase by the second day after the onset of acute pancreatitis [41]. Inflammatory markers may increase during any inflammatory process, and are not specific for diagnosis of pancreatitis. In contrast to amylase and lipase measurements, however, some of these markers have prognostic value as discussed below.

Markers of biliary tract involvement

Differentiation between biliary and nonbiliary forms of pancreatitis has important implications for treatment. Both biochemical and radiological tests are useful, but in some patients identification of a biliary cause may require EUS, bile microscopy, or empirical cholecystectomy. The timing of laboratory tests and ultrasound also affects their interpretation, especially when biliary tract obstruction is early in its course or when it is intermittent.

Most patients with gallstone pancreatitis have elevations of the serum liver tests, and the alanine aminotransferase (ALT) is the single most sensitive liver test for acute biliary obstruction by a stone. A rapid rise and fall in the serum liver tests is characteristically seen in gallstone pancreatitis in association with transient biliary obstruction at the papilla. Fluctuation is especially helpful in patients with chronic liver disease who have stable baseline elevations in aspartate aminotransferase (AST) and ALT levels. In addition, marked elevations of the amylase concentration of greater than 2000 IU/L also favor a biliary source [225]. The ratio of lipase to amylase may be greater in alcoholic than in biliary pancreatitis.

Noninvasive indicators of persistent bile-duct stones are useful in the workup of patients with gallstone pancreatitis. Laboratory scoring systems, such as the Ranson or modified Glasgow criteria, can identify patients whose biliary pancreatitis is predicted to be severe; these patients, in turn, are more likely to harbor persistent ductal or ampullary stones. Hyperbilirubinemia is associated with a higher incidence of retained bile-duct stones in gallstone pancreatitis [226,227]. Early trends in the serum liver tests and pancreatic enzyme levels are useful. Patients in whom all these values decrease over the first 48 h of hospitalization have a low (8%) risk of a persistent stone, whereas those in whom any of these lab values increase have a moderate (30%) risk of a persistent bile-duct stone [228]. None of these predictors is accurate enough to replace direct bile-duct imaging, but may be useful in selecting patients for EUS, magnetic resonance cholangiopancreatography (MRCP), or ERCP rather than elective intraoperative cholangiography.

Imaging

Abdominal radiographs

To exclude nonpancreatic diseases such as intestinal perforation, standard and upright chest and abdominal radiographs are recommended in evaluation of severe abdominal pain. Plain radiographs may be omitted if CT is available immediately.

A variety of radiographic findings have been associated with pancreatitis. Pleural effusions are most common on the left, but may be bilateral and rarely are limited to the right pleural space [229]. Intestinal gas patterns may demonstrate an ileus pattern or an isolated dilated loop of small bowel overlying the pancreas known as the sentinel loop. Colonic obstruction may result in air in the right and transverse colon that abruptly terminates at the splenic flexure, the so-called "colon cutoff sign." Loss of the psoas margins and increased separation between the stomach and colon suggest pancreatic inflammation. Pancreatic calcification or calcified gallstones may suggest an alcoholic or biliary etiology, respectively.

Sonography

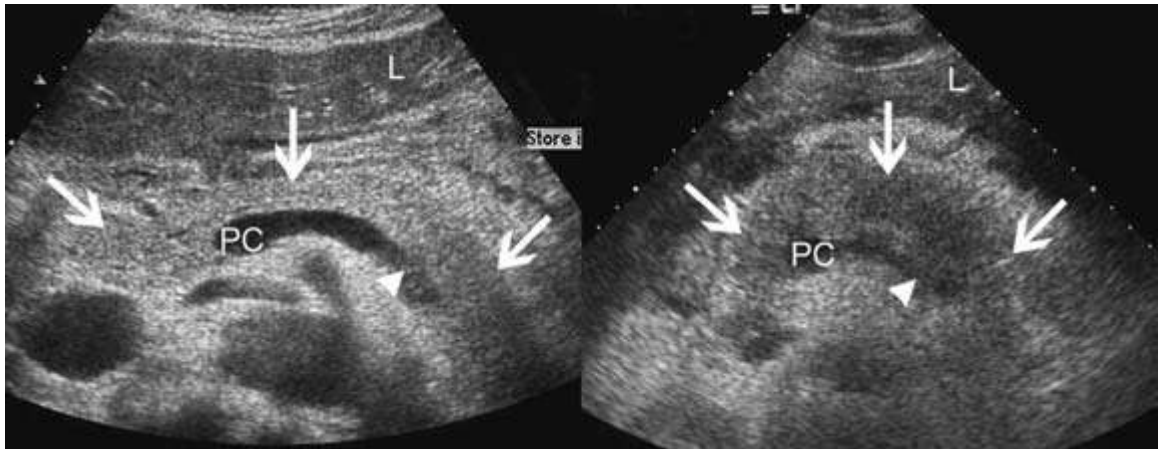
When the pancreas is inflamed it appears hypoechoic on sonography, because of increased water in the parenchyma (Fig. 68.15). Ultrasound is thus a relatively sensitive test for the diagnosis of acute pancreatitis, when the pancreas is seen. Although ultrasonography can be performed rapidly and at the patient's beside, pancreatic visualization is limited by intestinal gas or adipose tissue in 30%–40% of patients. This is especially common in patients with ileus due to their pancreatitis. Ultrasonography may miss focal masses or fluid collections, especially in and around the pancreatic tail. Its greatest value is in evaluating the biliary tract for gallstones, sludge, or ductal dilation [145,146]. Ultrasound is the single best noninvasive test for the detection of gallbladder stones, with a sensitivity of about 95%. It is much less reliable for direct visualization of a bile-duct stone, because the common bile duct is often obscured by overlying bowel gas.

Computed tomography

Computed tomography is a valuable tool for pancreatic imaging. In contrast to ultrasound, which demonstrates changing echogenicity in the parenchyma of the gland, the CT diagnosis of pancreatic inflammation is made based on pancreatic enlargement, effacement of the usual lobulated contour of the pancreas, inhomogeneity of the pancreatic parenchyma, or fluid infiltrating the peripancreatic fat (Fig. 68.16). Although CT and ultrasound are equally sensitive for diagnosis of pancreatitis, CT is better than ultrasound for detection of focal pancreatic masses or fluid collections because it reliably visualizes the entire gland.

Dynamic CT performed during bolus administration of intravenous contrast can demonstrate areas of poor perfusion or nonperfusion in the pancreas [19]. This finding is typically interpreted as necrosis of the poorly perfused segment; estimates of pancreatic necrosis by dynamic CT correlate with observations made at surgery. The CT finding of necrosis identifies patients who are at higher risk for pancreatic infection and death.

Investigators have debated whether early administration of bolus intravenous contrast worsens pancreatitis. Studies in rats have shown decreased pancreatic capillary flow rates



(a) (b)

Figure 68.15 Transabdominal ultrasound imaging of acute pancreatitis. **(a)** Normal pancreas (arrows). **(b)** Acute pancreatitis: pancreas is enlarged, hypoechoic, and heterogeneous (arrows). L, liver; PC, portal confluence; arrowhead indicates splenic vein. Courtesy of Carl Reading, Mayo College of Medicine.

and decreased pancreatic oxygen saturation after intravenous contrast administration [230]. These effects persisted at 60 min after contrast was given. There is no compelling evidence of this phenomenon in humans, however. A retrospective study at a university hospital found that patients who underwent contrast-enhanced CT had longer hospitalizations than those who did not, despite similar Acute Physiology and Chronic Health Evaluation (APACHE) II scores on admission [231]. A subsequent prospective study randomized patients with severe pancreatitis to CT on the first hospital day with or without bolus intravenous contrast. There was no difference in systemic markers of inflammation, length of hospital stay, or mortality [232]. In practice, a contrast-enhanced CT is usually unnecessary in the first 24 h of hospitalization, but early CT may be performed if it will have an important effect on early management of the individual patient.

Helical CT technique has improved CT sensitivity for detection of bile-duct stones to 85% [233]. Detection of ductal stones using CT is still limited by stone composition; calcium must be present in sufficient amounts to yield a CT density different than that of the surrounding bile or soft tissue. When looking for biliary stones, helical CT should be done without contrast so that a bright rim at the periphery of a stone is not mistaken for contrast enhancement of the bile-duct wall.

Magnetic resonance imaging

Magnetic resonance imaging (MRI) is similar to CT when imaging the acutely inflamed pancreas (Fig. 68.17) [234]. When intravenous gadolinium is given, MRI demonstrates areas of poor pancreatic perfusion similar to those identified with CT. Animal studies have shown no deleterious effect of intravenous gadolinium on the inflamed pancreas. Although MRI is more cumbersome and expensive than CT and more difficult to perform quickly, it does have fewer contraindications (such as pregnancy and contrast allergy), and can detect pancreatic duct disruption. Like EUS, MRI is more accurate than CT for demonstration of solid debris in a pancreatic fluid collection; complex collections often have a homogeneous appearance on CT [235]. However, MRI does not demonstrate pancreatic calcifications.

Secretin-stimulated MRI has a role in the diagnosis of functional pancreatic duct outlet obstruction, and in noninvasive diagnosis of pancreatic duct disruption and leak [236]. Magnetic resonance angiography (MRA) and MRCP are additional techniques that offer noninvasive diagnosis of vascular complications and persistent bile-duct stones in acute pancreatitis. The sensitivity of MRCP exceeds 90% for bile-duct stones, although its yield in patients with normal-caliber ducts and small ampullary stones is not as well defined.

Endoscopic retrograde cholangiopancreatography (ERCP)

There is no role for ERCP in establishing the diagnosis of acute pancreatitis. Most patients with acute pancreatitis have normal pancreatograms, although some may have pancreatic duct strictures or ductal disruptions. However, ERCP is useful in the differential diagnosis of otherwise unexplained, recurrent pancreatitis, especially for diagnosis of mild chronic pancreatitis, pancreas divisum, or sphincter of Oddi disease. It has been used to diagnose and treat persistent bile-duct stones in acute pancreatitis, as discussed in section "Urgent ERCP and EUS in gallstone pancreatitis".

Endosonography

Endoscopic ultrasound (EUS) is useful in some patients with acute pancreatitis. It is a sensitive test for detection of persistent bile-duct or ampullary stones and can be used to identify

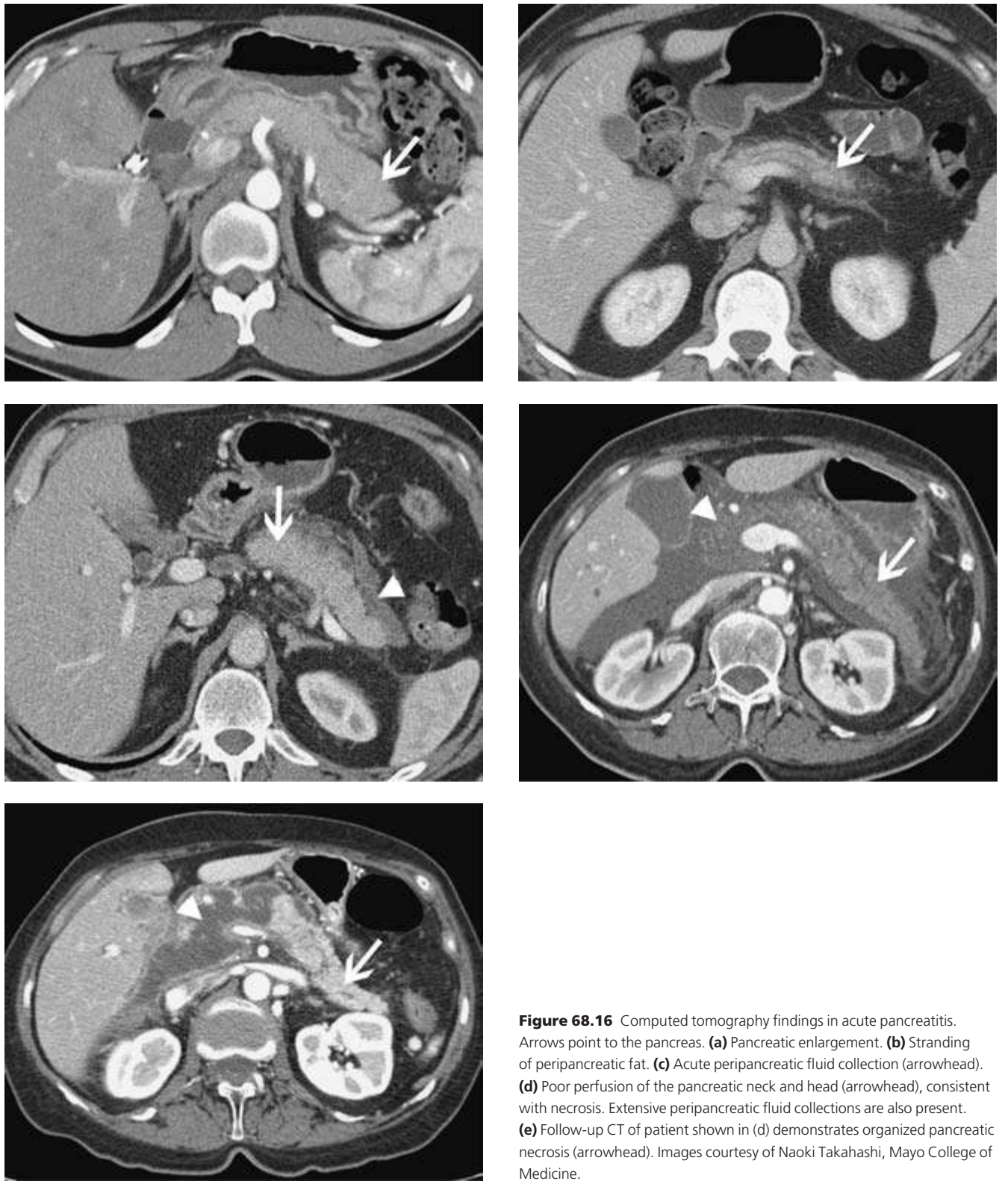
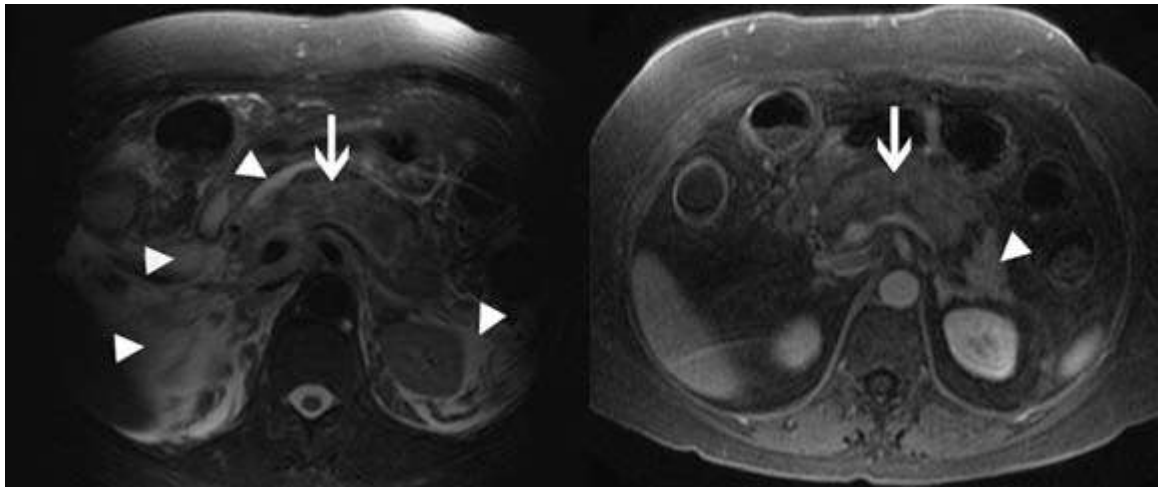


Figure 68.16 Computed tomography findings in acute pancreatitis. Arrows point to the pancreas. **(a)** Pancreatic enlargement. **(b)** Stranding of peripancreatic fat. **(c)** Acute peripancreatic fluid collection (arrowhead). **(d)** Poor perfusion of the pancreatic neck and head (arrowhead), consistent with necrosis. Extensive peripancreatic fluid collections are also present. **(e)** Follow-up CT of patient shown in (d) demonstrates organized pancreatic necrosis (arrowhead). Images courtesy of Naoki Takahashi, Mayo College of Medicine.



(a) (b)

Figure 68.17 Magnetic resonance imaging (MRI) findings in acute pancreatitis. **(a)** T2-weighted image demonstrates enlargement of the pancreatic body with increased signal consistent with edema (arrow), and fluid collections in the peripancreatic and perinephric spaces (arrowheads). **(b)** Post-contrast T1-weighted image shows decreased enhancement of the pancreatic body (arrow) compared with the tail (arrowhead), consistent with necrosis of the body. Images courtesy of Naoki Takahashi, Mayo College of Medicine.

patients likely to benefit from ERCP [237]. Endosonography is also useful for the differential diagnosis of unexplained acute pancreatitis, because it demonstrates biliary sludge and stones missed with other imaging modalities, as well as small pancreatic or ampullary tumors, pancreas divisum, and chronic pancreatitis [214]. Complex material within a pancreatic fluid collection can be demonstrated by EUS, and it can guide endoscopic pseudocyst drainage.

Use of cross-sectional imaging studies in acute pancreatitis

The choice of imaging modality for patients with acute pancreatitis depends on the clinical question that needs to be answered. Imaging studies can establish the diagnosis of pancreatitis, aid in differential diagnosis, provide prognostic information, or assess possible complications.

When the diagnosis of pancreatitis is uncertain both ultrasound and CT may be useful. A patient with rapidly resolving biliary pancreatitis may have near normal serum enzymes but persistent pancreatic edema seen on ultrasound, or peripancreatic inflammatory changes on CT. Computed tomography can show changes of chronic pancreatitis or focal pancreatic masses.

For differential diagnosis, the choice of imaging test depends on the diagnosis suspected. When biliary pancreatitis seems likely, ultrasound is the standard investigation because it detects almost all gallbladder stones. When chronic pancreatitis or an underlying pancreatic malignancy is suspected, CT is more useful. Endoscopic ultrasound may also be warranted to pursue these diagnostic possibilities in the face of negative noninvasive imaging studies. When a metabolic or drug-induced pancreatitis is likely, imaging tests may still be indicated to exclude other common causes such as stones.

In patients with biliary pancreatitis, imaging studies such as EUS or MRCP may usefully identify patients with persistent bile-duct or ampullary stones. Early bile-duct imaging is particularly appropriate when the pancreatitis is severe or laboratory trends suggest an increased risk of a persistent stone.

Contrast-enhanced CT or MRI can identify patients with necrosis, and these patients may benefit from prophylactic antibiotics. However, CT is not necessary in every patient with pancreatitis. Necrosis is uncommon in patients with clinically mild disease who improve rapidly in the hospital. Serial hematocrits and the concentration of C-reactive protein on the second hospital day are useful predictors of the presence or absence of necrosis, as described in the section on complications. When necrosis is suspected or the patient is not improving over the first 48 h of hospitalization, contrast-enhanced CT or MRI is usually worthwhile.

Computed tomography is also valuable in the diagnosis and management of local complications of acute pancreatitis. Jaundice or a marked drop in hematocrit may also be indications for CT. When thrombosis of the splenic or superior mesenteric veins is suspected, MRA or CT may be useful.

Evaluation of severity

Severe pancreatitis is defined as the occurrence of a local or systemic complication of the disease, or death. Definitions of both local and systemic complications of pancreatitis were described at the 1992 Atlanta consensus conference; subsequently multiple organ dysfunction syndrome (MODS) was defined in a broad range of patients with critical illness, and MODS definitions of organ dysfunction have also been used to define severe acute pancreatitis (Table 68.3). There are

Table 68.3 Complications of acute pancreatitis, as defined by the Atlanta consensus conference and the multiple organ dysfunction syndrome (MODS) score

	Atlanta definition[a]	MODS score ≥ 2[b]
Local complications		
Acute fluid collections	Occur early, lack a wall	
Necrosis	Areas of nonviable parenchyma	
Acute pseudocysts	Collection of pancreatic juice Enclosed by a wall	
Pancreatic abscess	Circumscribed collection of pus	
Organ dysfunction		
Shock	Systolic blood pressure < 90 mmHg	Pressure-adjusted heart rate (PAR)[c] > 15
Pulmonary	P_aO_2 ≤ 60 mmHg (room air)	PO_2/F_iO_2 ≤ 225
Renal	Creatinine > 2 mg/dL after rehydration	Creatinine > 2.26 mg/dL
GI	> 500 mL GI blood loss in 24 h	
Hematological	Platelets < 100 000/mm^3; fibrinogen < 1 g/L; fibrin split products > 80 μg/mL	Platelets ≤ 80 000/mm^3
Metabolic	Serum calcium < 7.5 mg/dL	
Neurological		Glasgow Coma Score ≤ 12

a Bradley et al. [9].
b Marshall et al., with hepatic dysfunction excluded as explained in the text [249,272].
c PAR = product of the heart rate multiplied by the ratio of the right atrial (central venous) pressure to the mean arterial pressure.

several important reasons to identify the severity of acute pancreatitis. Because severity correlates with prognosis, such evaluation may allow the clinician to predict the patient's course and anticipate complications or the need for closer monitoring in an intensive care unit. Prognostic information may guide the use of prophylactic antibiotics, urgent bile-duct imaging, and early ERCP. Finally, prognostic markers allow the clinical investigator to measure the success or failure of therapeutic interventions. The tools used to predict severity and prognosis fall into three categories: specific laboratory tests, clinical and physiological assessment, and CT.

Laboratory tests

The standard laboratory tests used to diagnose pancreatitis provide little prognostic information, but other tests appear to separate patients with mild disease from those with severe disease. The first group of tests measures the severity of the inflammatory response. These include the neutrophil elastase and the monokine IL-6 tests. Both are elevated within 24 h and correlate with the severity of pancreatitis. C-reactive protein is induced by IL-6 and is a later marker of severity, distinguishing patients with severe disease when measured at 48 h (Fig. 68.18). Because C-reactive protein is easily and inexpensively measured and appears to be a good prognostic marker, it should be widely used to assess prognosis.

The hematocrit is a simple, readily available laboratory test that provides prognostic information in acute pancreatitis [238]. An elevated hematocrit (> 44%) at the time of hospital admission, or failure of the hematocrit to decrease in the first 24 h of hospitalization, are seen in almost all patients who develop pancreatic necrosis or distant organ failure [238]. Perhaps the most useful benefit of the hematocrit is its ability to exclude severe disease; a normal hematocrit on admission has a negative predictive value for severe pancreatitis of over 95% [239], and may be used to identify patients who do not require CT.

The finding that a rising hematocrit is associated with necrosis in up to 50% of cases has raised the possibility that aggressive fluid resuscitation could prevent pancreatic necrosis. Failure of the hematocrit to normalize has not been linked with disease severity in all studies, however [240], and limited available treatment data do not prove the hypothesis that aggressive hydration prevents necrosis [241].

The TAP (trypsinogen activation peptide) assay is an indirect measure of the amount of active trypsin. From a pathophysiological standpoint, this is an elegant measure of severity: the more trypsin activation, the more pancreatic damage. In one large prospective study, TAP levels discriminated between mild and severe disease [224]. In another prospective study, urinary TAP levels were more accurate than initial hematocrit for prediction of disease severity [242]. Unfortunately, TAP assays are not yet widely available.

Laboratory tests may usefully predict the likelihood of pancreatic necrosis, which is in turn linked to severe disease. A serum C-reactive protein level greater than 150 mg/L is seen in 95% of patients with necrosis by the second hospital day

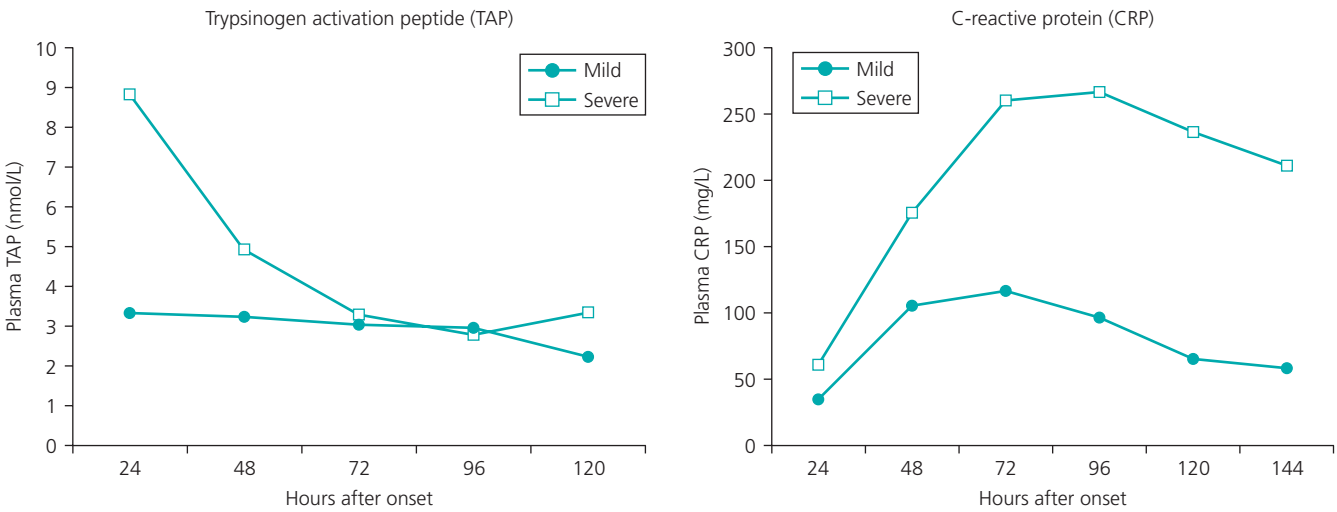


Figure 68.18 Serum markers of severity. Serum values of the trypsinogen activation peptide (TAP) and C-reactive protein (CRP) as a function of the duration and severity of acute pancreatitis. Derived from Kemppainen et al. [224], and Wilson C, Heads A, Shenkin A, Imrie CW. C-reactive protein, antiproteases and complement factors as objective markers of severity in acute pancreatitis. Br J Surg 1989;76:177.

(Fig. 68.18) [243]. When the hematocrit is less than 44% on admission and does not increase in the first 24 h, necrosis has been demonstrated in only 4% [238].

Computed tomography

Both non-contrast CT and contrast-enhanced CT yield useful prognostic information. A prognostic scoring system called the CT Severity Index (Table 68.4) has been shown to correlate with the Ranson criteria for assessing severity [19]. In one study, patients with CT Severity Index scores up to 2 had only 4% morbidity and no mortality, but those with scores from 7 to 10 experienced 92% morbidity and 17% mortality (Fig. 68.19). Contrast-enhanced CT demonstrates the presence and extent of pancreatic necrosis, and is hence an important predictor of complications and death, as discussed in the section on pancreatic necrosis.

Table 68.4 Computed Tomography Severity Index

	Points
Grade of acute pancreatitis	
A: Normal pancreas	0
B: Pancreatic enlargement	1
C: Inflammation confined to pancreas and peripancreatic fat	2
D: One peripancreatic fluid collection	3
E: Two or more fluid collections	4
Degree of necrosis	
No necrosis	0
Necrosis of one-third of pancreas	2
Necrosis of one-half of pancreas	4
Necrosis of more than one-half	6

Computed Tomography Severity Index = grade points + degree of necrosis.
Adapted from Balthazar et al. [19], with permission from the Radiological Society of North America.

Clinical and physiological assessment

The criteria established by Ranson and colleagues are still the most widely applied to the assessment of acute pancreatitis. The Ranson criteria (Table 68.5) distinguish between mild and severe pancreatitis about 80% of the time. As shown in Fig. 68.19, Ranson score correlates with morbidity and mortality [244]. The disadvantage of the Ranson criteria is that they include 11 values that must be monitored over 48 h. Blamey and colleagues developed the Glasgow classification, which can be calculated any time within the first 48 h of hospitalization and measures only eight parameters (Table 68.5) [245]. The simplified Glasgow criteria appear to have prognostic accuracy similar to that of the Ranson criteria. The Glasgow criteria were developed in a population of predominantly biliary pancreatitis, whereas the Ranson criteria were developed in patients with more alcoholic pancreatitis.

Although some prognostic classifications have dropped the base deficit and fluid requirements from the Ranson criteria, others have identified obesity as an independent risk factor. The higher risk in the obese patient may result from respiratory compromise from physical restraints, or from the provision of toxic substrates such as free fatty acids from triglycerides and harmful cytokines from peritoneal fat stores.

The Acute Physiology and Chronic Health Evaluation (APACHE) II has been used to establish the prognosis in patients with pancreatitis [246]. This system uses 14 routinely measured parameters of physiological activity and biochemical function, and generates a numerical score that

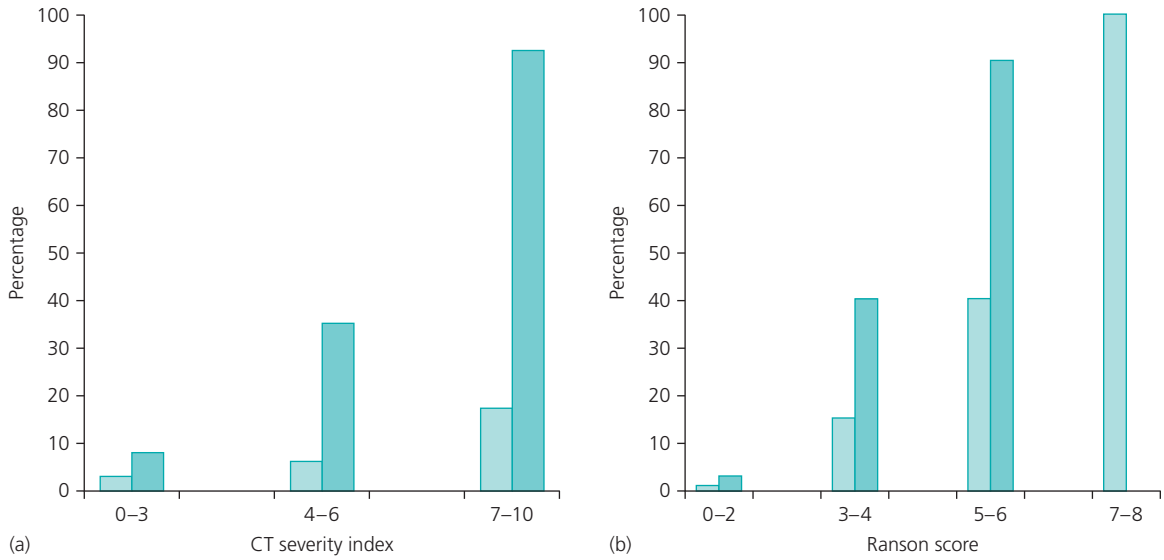


Figure 68.19 Stratification of severity according to Computed Tomography (CT) Severity Index (Table 68.4) and Ranson criteria (Table 68.5). The light bar represents mortality. Morbidity (dark bar) was defined as greater than 7 days in the intensive care unit. Derived from Balthazar et al. [19] and Ranson [244].

Table 68.5 Ranson criteria and simplified Glasgow prognostic scoring criteria

Ranson criteria	Simplified Glasgow criteria
On admission	**Within 48 h**
Age > 55 years	Age > 55 years
WBC > 16 000/mm^3	WBC > 15 000/mm^3
LDH > 350 IU/L	LDH > 600 IU/L
Glucose > 200 mg/dL	Glucose > 180 mg/dL
AST > 250 IU/L	Albumin < 3.2 g/dL
	Calcium < 8 mg/dL
Within 48 h	Arterial Po_2 < 60 mmHg
Hematocrit decrease by > 10%	Urea > 45 mg/dL
Urea nitrogen increase by > 5 mg/dL	
Serum calcium < 8 mg/dL	
Arterial Po_2 < 60 mmHg	
Base deficit > 4 mEq/L	
Estimated fluid sequestration > 6 L	

AST, aspartate aminotransferase; LDH, lactate dehydrogenase; WBC, white blood cells.

depends on the deviation of those parameters from the normal range. The patient's age, organ insufficiency, neurological state, and postoperative state are also weighted. The potential advantage of the APACHE is that it can be calculated instantaneously from routine measurements. The initial value discriminates patients at high risk for complications and death, and trends in the patient's APACHE score over time add to its predictive value. A major shortcoming of this classification is its complexity. It is difficult to apply the APACHE system routinely outside of an intensive care unit.

The presence of the systemic inflammatory response syndrome (SIRS) is an important early predictor of multiple organ dysfunction syndrome (MODS) and mortality from acute pancreatitis, particularly if SIRS persists beyond 48 h. The systemic inflammatory response syndrome is defined by simple clinical data (Table 68.6) [247,248] that are readily available for patients with acute pancreatitis. In one large series, the prevalence of SIRS among patients with acute pancreatitis was 21% on hospital admission, and 18% had persistent SIRS at 48 h. Persistent SIRS was associated with development of MODS. The mortality rate was 0.7% in patients without SIRS, 8% in patients with transient SIRS, and 25% in those with persistent SIRS beyond 48 h [249].

Are these prognostic scoring systems of use to the clinician? A group of experienced pancreatic surgeons compared bedside assessment at admission and after 48 h with the APACHE II, Ranson, and Glasgow scores for predicting the severity of acute pancreatitis [246]. Bedside assessment alone missed over half of the cases that ultimately proved to have a complicated course. Thus, a high score on a standardized scoring system alerts the clinician to the possibility of complications. This in turn should prompt frequent reevaluation of the patient and the adequacy of supportive treatment, perhaps in an intensive care unit.

Table 68.6 Systemic inflammatory response syndrome (SIRS)[a]

Temperature > 38°C or < 36°C
Heart rate > 90 beats/min
Respiratory rate > 20 breaths/min or arterial Pco_2 < 32 mmHg
WBC count > 12 000 or < 4000 cells/mm^3

a SIRS is present if two or more of the four criteria are present [249].

Local complications

Local complications of pancreatitis include fluid collections, retroperitoneal hemorrhage, vascular and splenic complications, and duodenal, biliary, or colonic obstruction. The commonest of these is the development of a pancreatic or peripancreatic collection. A useful classification of pancreatic collections was adopted at a consensus conference on acute pancreatitis held in Atlanta in 1992, and this has since been widely accepted, replacing older terminology such as "phlegmon" [9]. The Atlanta classification distinguishes acute fluid collections, necrosis, pseudocysts, and abscesses. Each of these entities has a different pathology, natural history, and treatment.

Pancreatic fluid collections

Acute fluid collections

Acute fluid collections form in or near the pancreas, and are common among patients with pancreatitis. They occur early in the course of the acute illness, and do not have a defined wall. Fluid collections may be simple and exhibit a uniform CT density that approximates that of water; or they may be complex with multiple CT densities and poorly defined margins (Fig. 68.16). Even those that appear simple on CT often contain locules and inflammatory debris when imaged by MRI or EUS.

Most acute fluid collections resolve spontaneously. The factors determining resolution of an acute collection, versus persistence of the collection and progression to a pseudocyst, are incompletely understood. An underlying pancreatic duct disruption that empties into a fluid collection certainly contributes to their persistence and enlargement in some patients. Nevertheless, prospective studies show that ductal disruptions often resolve with conservative management alone, without surgical or endoscopic intervention [250].

Although some have advocated drainage of acute fluid collections, evidence demonstrating a benefit for such intervention is lacking. Acute collections may be difficult to drain percutaneously, and CT scans often underestimate the complexity of these collections. Unless an acute collection is rapidly expanding or associated with clinical evidence of infection, it should be observed. Infection is an uncommon complication; if suspected, diagnosis is best made by sterile aspiration of the fluid collection under CT guidance, with Gram stain and culture of the aspirate. Infected collections have to be drained and have been successfully treated using percutaneous catheters. A percutaneous catheter should not be placed unless infection is confirmed, as it can serve as a portal for entry of bacteria into a previously sterile collection. Because fluid collections are frequently associated with acute pancreatitis, and most resolve spontaneously, a fluid collection should be defined as a pseudocyst only if it persists for more than 4 weeks.

Necrosis and infected necrosis

Any form of acute pancreatitis may progress to severe disease and extensive pancreatic necrosis. Pancreatic necrosis may result from ischemia or marked inflammation and represents a severe form of disease. Necrosis may affect the pancreas diffusely or focally, and necrotic areas may be separated by segments of viable tissue. Histological exam may show devitalized pancreatic tissue with vessel damage and necrosis of parenchyma and ducts, as well as hemorrhage and fat necrosis.

Pancreatic necrosis is diagnosed clinically with dynamic contrast-enhanced CT, which demonstrates well-marginated zones of nonenhanced pancreatic tissue (Fig. 68.16). Although some nonenhancing parenchyma may be viable, there is generally good correlation between CT, operative findings, and histology in patients with necrosis undergoing surgery. Computed tomography is less useful for diagnosis of peripancreatic fat necrosis.

Necrosis is uncommon in patients with clinically mild disease. Laboratory predictors of necrosis include a serum C-reactive protein level greater than 150 mg/L at 48 h (Fig. 68.18), a Ranson score greater than or equal to 3, or an APACHE II score exceeding 7. Necrosis is associated with other major complications and death from acute pancreatitis. Major complications of necrosis include distant organ failure and infected pancreatic necrosis. Early in the course of necrotizing pancreatitis there may be a marked systemic inflammatory response (SIRS) with increased capillary permeability, a sepsis-like syndrome, and other organ failure most often affecting the lungs. Organ failure is more likely in patients with necrosis of over 50% of the pancreas [251]. Infection of the necrotic pancreatic tissue may develop. Because there is no fibrotic wall or capsule to contain the infection, adjacent inflamed tissues become involved and overwhelming sepsis is likely unless adequate debridement of the infected tissue is achieved.

Infected pancreatic necrosis is a leading risk factor for death from acute pancreatitis. Infection of pancreatic necrosis occurs in 30%–70% of patients not receiving prophylactic antibiotics [252]. This high frequency is in large part due to increased bacterial translocation from the gut into the portal venous system, with seeding of the necrotic tissue. Alterations in mucosal villus architecture and bowel microvasculature occur in animal models of acute pancreatitis, with bacterial translocation from the intestine to the pancreas, liver, and spleen [253]. In patients, the same bacterial species that infect the pancreas can be previously cultured from the colon [254]. The prevention and treatment of infected necrosis is described in greater detail later (see "Antibiotic prophylaxis" below).

Infected necrosis may be heralded by a sudden increase in abdominal tenderness, high fever, marked leukocytosis, and bacteremia. The presentation may also be subtle, particularly in patients receiving prophylactic antibiotics, with persistent or worsening organ failure despite ongoing supportive

care. Negative blood cultures do not exclude the diagnosis. Although infection of pancreatic necrosis was thought to occur mainly after the first 10–14 days of illness, subsequent reports have stressed its occurrence earlier and later in the course of necrotizing pancreatitis.

The main diagnostic modality for infected necrosis is CT-guided fine-needle aspiration of the necrotic pancreatic tissue. Samples should be immediately examined by Gram stain, which has a high utility in this setting. Fungal stains are advisable as well, and cultures are also mandatory. Polymicrobial bacterial gram-negative enteric and anaerobic organisms are most common, but with the widespread use of prophylactic antibiotics bacterial gram-positive and fungal infections are increasingly frequent [255].

Laboratory predictors of infected pancreatic necrosis have been sought. The serum procalcitonin and IL-6 levels may distinguish between sterile and infected necrosis [256–258]. Further clinical experience with these markers of infected necrosis is desirable.

Sterile pancreatic necrosis has a variable clinical course. The necrotic tissue may gradually disappear, leaving residual inflammation and scar. In other cases the necrotic region gradually evolves into a region of organized pancreatic necrosis (Fig. 68.20). In a minority of patients, a persistent sequestrum of sterile pancreatic necrosis is responsible for persistent organ failure despite prolonged supportive care. Drainage of sterile necrosis may be considered in patients with persistent symptoms or organ failure, as described later (see "Drainage of sterile necrosis" below).

The term "organized pancreatic necrosis" has been used to describe a region of necrosis that is at least 4 weeks old and has developed a wall, but still contains necrotic material [259]. The distinction between organized pancreatic necrosis and pseudocyst has importance when nonsurgical drainage is considered. Organized pancreatic necrosis can be drained endoscopically using a transmural gastric or duodenal approach, or percutaneously. Aggressive endoscopic or percutaneous techniques are needed adequately to remove the

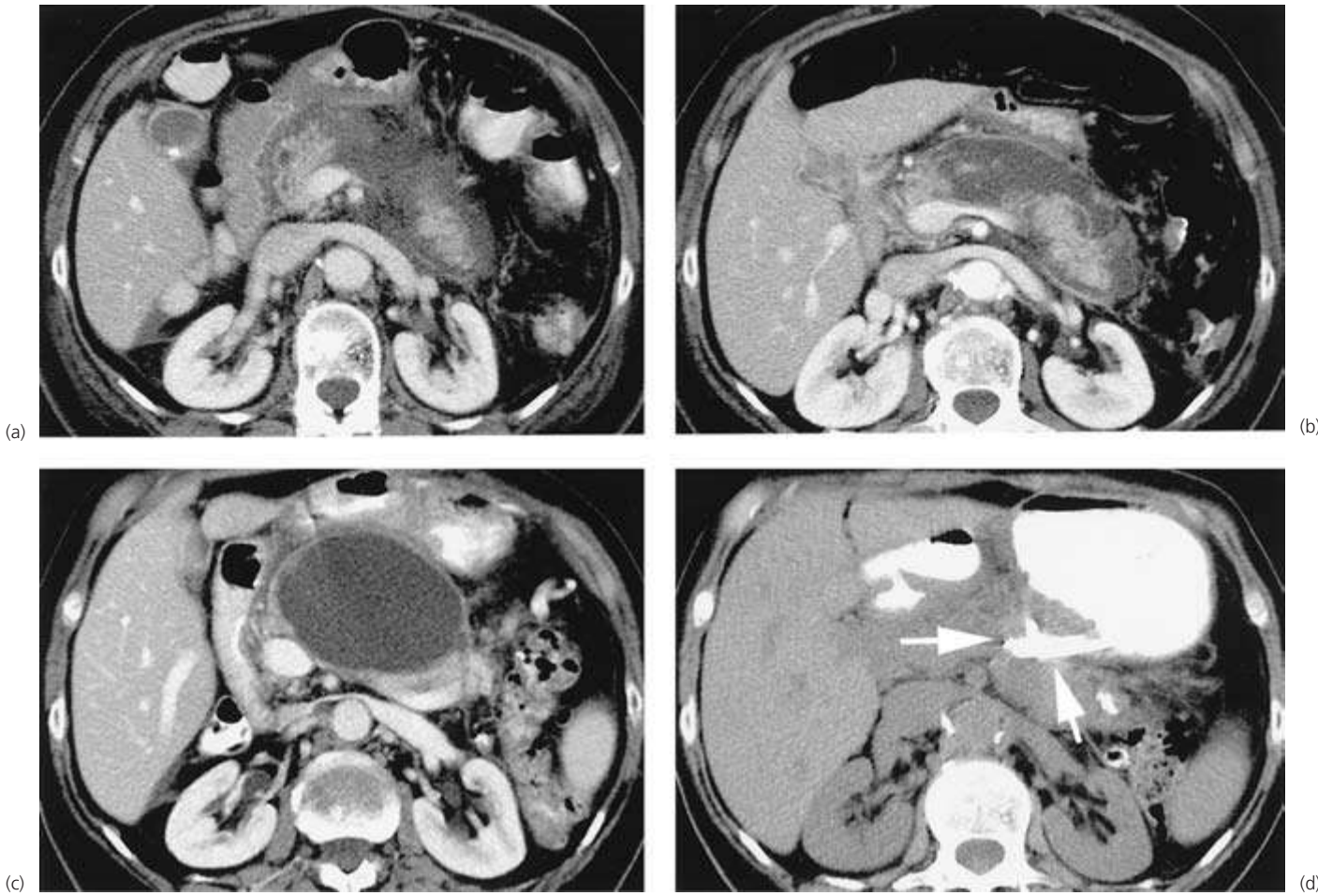


Figure 68.20 Evolution of central necrosis. **(a)** Necrosis of the pancreatic body in a patient with gallstone pancreatitis. **(b)** One week after onset of pancreatitis. **(c)** Seven weeks after onset. **(d)** The collection has resolved after endoscopic drainage with placement of multiple internal stents (arrows).

thick necrotic material, including creation of a large tract to the collection using large-caliber dilating balloons, placement of multiple stents and drains, lavage by percutaneous or nasocystic catheters, and direct endoscopy of the collection by the transmural or percutaneous tract for debridement of necrotic material. Attempts at drainage may otherwise result in a poorly drained, infected collection. The complexity of an organized area of pancreatic necrosis is often underestimated by CT, and both EUS and MRI have been used to identify solid material in these collections.

Necrosis is often accompanied by a disruption of the pancreatic duct. When the disruption occurs in the mid-portion of the pancreas, the necrotic region may gradually expand as pancreatic juice from the viable pancreatic tail drains into it. This so-called "disconnected duct syndrome" usually requires resection of the disconnected tail, sometimes with islet cell autotransplantation, although creation of a durable fistula to the gut has been described using endoscopic techniques.

Pseudocysts

Pseudocysts are collections of pancreatic fluid contained by a wall of fibrosis or granulation tissue. The presence of a well-defined wall distinguishes pseudocysts from acute fluid collections and pancreatic necrosis. An epithelial lining is absent (in contrast to true pancreatic cysts). Formation of a well-defined wall generally requires at least 4 weeks. Pseudocysts usually contain fluid rich in pancreatic enzymes, and often contain particulate matter.

The advent of pancreatic imaging by ultrasonography and CT led to the realization that pancreatic pseudocysts are common and appear in up to 10% of patients with acute pancreatitis [260]. Although they may form most frequently in patients with alcoholic pancreatitis, up to 30% of cases arise in gallstone pancreatitis. The most common presenting symptom of a pseudocyst is upper abdominal pain that is either constant or postprandial. Other symptoms referable to a pseudocyst include early satiety and nausea and vomiting secondary to gastric outlet obstruction. Pseudocysts may also enlarge rapidly, rupture, hemorrhage, leak pancreatic fluid, obstruct the biliary tract, compress the inferior vena cava, erode into surrounding structures, extend into the mediastinum, and become infected.

The risk of complications varies with the size of a pseudocyst and its age. Symptomatic pseudocysts may also be more prone to complications. Early reports based on ultrasound detection of pseudocysts in persistently symptomatic patients found a complication rate of 30%–50% in pseudocysts that persisted for more than 6 weeks, and were the basis for recommendations to drain all persistent pseudocysts. Subsequent studies of asymptomatic pseudocysts detected with liberal use of CT document a low incidence of complications, even in large pseudocysts, and show that most pseudocysts less than 6 cm in diameter resolved over time. One-third of lesions more than 10 cm in diameter also do well with observation alone [260].

Indications for early drainage include infection or progressive enlargement of the pseudocyst. Symptomatic pseudocysts typically require drainage. In the absence of symptoms, pseudocysts can be followed conservatively, although large lesions that fail to shrink with time should probably be drained. Pseudocysts can be drained endoscopically, percutaneously, or surgically. If there is a question about whether the lesion is a true pancreatic cyst or pancreatic cystic neoplasm rather than a pseudocyst, surgical drainage gives the opportunity to obtain a large piece of the cavity's wall for histological exam. This is especially important when a pancreatic "pseudocyst" is found in the absence of a clinical bout of pancreatitis, or in recent pancreatitis of unclear etiology (Fig. 68.21).

The location of a pseudocyst and its relationship to the pancreatic duct can affect the approach taken for cyst drainage. Pancreatography may be helpful in delineating obstruction of the pancreatic duct and ductal communication with a pseudocyst. Those that do not communicate are amenable to either internal or external drainage. Pseudocysts that communicate with the duct, especially when the duct is strictured or disrupted, often resolve slowly when drained externally, and percutaneous drainage may lead to a pancreaticocutaneous fistula. Such pseudocysts are best considered for internal drainage.

Endoscopic therapies are attractive nonsurgical options for pseudocyst drainage in many cases. Endoscopic techniques include transpapillary and transmural drainage into the stomach or duodenum with placement of internal stents. Pseudocysts of the pancreatic head may be best treated with transpapillary stenting, especially if the cyst communicates with the pancreatic duct and there is an associated ductal stricture. Transpapillary stenting is most successful when the stent traverses the ductal disruption [261,262]. A high incidence of major hemorrhage was initially reported with endoscopic transmural drainage of pseudocysts, but use of an over-the-wire Seldinger technique, rather than electrocautery, lowers the risk of major complications such as bleeding or perforation to less than 5% [263]. Endoscopic ultrasound can facilitate pseudocyst drainage, particularly when an obvious bulge is not visible endoscopically or venous collaterals are present [264]. Endoscopic drainage of infected pseudocysts has been described.

Percutaneous drainage is also a well-established method for draining sterile or infected pseudocysts. When technically possible, a transgastric approach is favored, as internal drainage into the stomach through a mature catheter tract can result. External drainage may otherwise result in persistent drainage of pancreatic juice through the external fistula created by the catheter.

Incomplete pseudocyst drainage by either endoscopic or percutaneous means may result in infection of the pseudo-

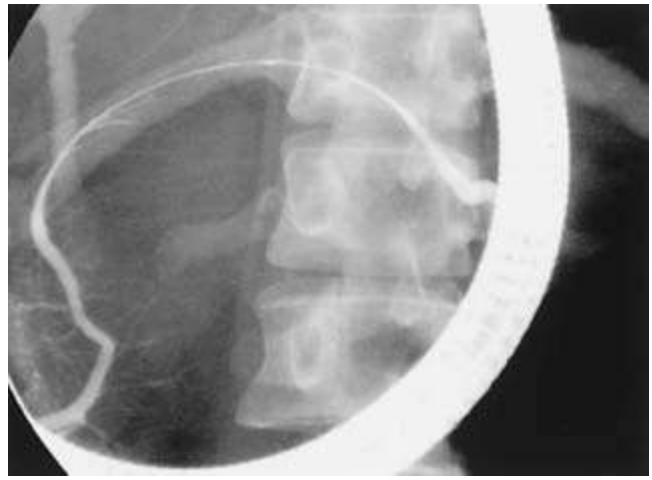
(a)  (b)

Figure 68.21 Cystic neoplasm simulating a pseudocyst. The patient developed epigastric pain without a discrete episode of pancreatitis. **(a)** Computed tomography demonstrates a round fluid collection of the pancreatic body with a well-defined wall. **(b)** Endoscopic retrograde cholangiopancreatography (ERCP) shows bowing of the pancreatic duct around the lesion, without communication. Pathology examination of the resected lesion showed cystadenocarcinoma.

cyst contents. This is particularly common in patients with "organized pancreatic necrosis" mistaken for a simple pseudocyst, as described above. A complex collection should be suspected in patients with necrosis that has evolved into a pseudocyst-like structure on serial CT scans. Prior to drainage, EUS or MRI may be used to assess for solid material in the cavity. If solid matter is present in the collection more aggressive endoscopic or percutaneous techniques are necessary for adequate treatment, such as placement of multiple stents and a nasocystic tube to allow lavage of the pseudocyst cavity.

There are several reasons why the fluid contents of a pseudocyst should be sampled during drainage. First, a high amylase concentration within a cyst probably reflects its persistent communication with the pancreatic duct and bodes a poor outcome for external drainage. Second, symptoms of pseudocyst infection may be indolent and suspected only after bacterial and fungal culture. Third, true cystic lesions of the pancreas, including cystic neoplasms, may be mistaken for pseudocysts. Thus, amylase, viscosity, cytology, and tumor markers (including CEA) should be obtained from cyst aspirates when possible.

Pancreatic abscesses

Pancreatic abscesses are circumscribed intraabdominal collections of pus, usually within or in close proximity to the pancreas. In contrast to infected pancreatic necrosis, a pancreatic abscess contains little or no necrosis and typically presents later in the course of the illness, often more than 4 weeks after the onset of acute pancreatitis. Clinical and laboratory findings of acute pancreatitis have usually resolved before pancreatic abscess is detected. Because the infected collection is walled off and the surrounding tissue is viable, spread of infection to adjacent tissues is uncommon. Endoscopic or percutaneous drainage of pancreatic abscess is usually successful, although a minority of patients will require surgery. Endoscopic transpapillary drainage is also feasible when the abscess cavity communicates with the pancreatic duct [265].

Other local complications

Ascites and fistulae

Several processes may contribute to the formation of ascites in acute pancreatitis. Peritoneal inflammation resulting from chemical peritonitis usually generates a small amount of fluid. Attendant chronic liver disease may contribute varying amounts of ascites. Pancreatic ascites refers to the presence of large amounts of fluid rich in pancreatic secretions; it results from a communication between the pancreatic duct or a pseudocyst and the peritoneal cavity. Pancreatic ascites produces a gradual increase in abdominal girth, and is sometimes associated with subcutaneous fat necrosis. Because the pancreatic enzymes usually remain inactive, peritonitis is uncommon. The diagnosis should be suspected when ascites fails to respond to diuretic therapy and the serum amylase level is persistently elevated. Marked elevations of the ascitic fluid amylase level and ascites protein concentrations above 3 g/dL are typical of pancreatic ascites. The site of ductal disruption can sometimes be identified by CT or MRI, but ERCP is often required [210].

Three forms of treatment have been used to treat massive pancreatic ascites. Endoscopically placed pancreatic stents can be used to bridge the ductal disruption. Total parenteral nutrition together with administration of somatostatin or

octreotide has also been used, with or without paracentesis. A minority of patients will require surgical drainage or pancreatic resection. Endoscopic therapy appears to be more effective than medical therapy [266].

Pancreatic fistulae usually arise after a bout of alcoholic pancreatitis or from pancreatic pseudocysts, but also may form after pancreatic trauma, acute pancreatic duct disruptions, percutaneous catheter placement, or pancreatic surgery. Anterior pancreatic ductal discontinuities can generate ascites, whereas posterior disruptions may result in fistulae that burrow through to the skin or track through the retroperitoneum. Fistulae sometimes extend through the esophageal or aortic hiatus into the chest, producing pleural effusions or mediastinal collections rich in amylase. Internal fistulae may communicate with the colon, gallbladder, and small bowel. Fistulas to vascular structures such as the portal vein have rarely been reported, and are often associated with subcutaneous fat necrosis.

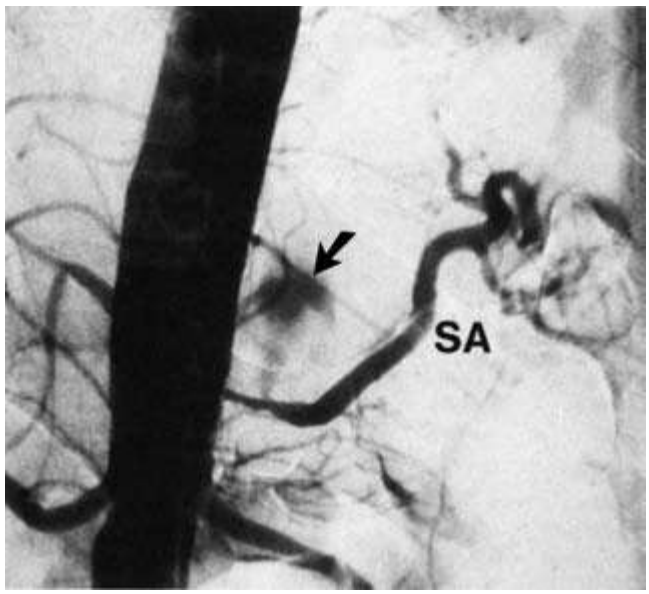
The fluid from a pancreatic fistula has high protein and amylase contents. The anatomy of an external fistula may be revealed by a fistulogram. Although ERCP is the most useful test for identifying an internal fistula, CT scanning may be useful for fistulae that communicate with the thoracic cavity. Spontaneous healing is more common for cutaneous fistulae than internal fistulae. Drainage of fluid collections by thoracentesis or paracentesis may help to heal serosal discontinuities. Reduction of pancreatic secretion by administering somatostatin analogues has been reported to promote the resolution of fistulae, particularly those with cutaneous communications. Stenting of the pancreatic duct or surgical resection, however, are usually required for internal fistulae. Pancreatic stents are most effective when they bridge the site of ductal disruption [261,262].

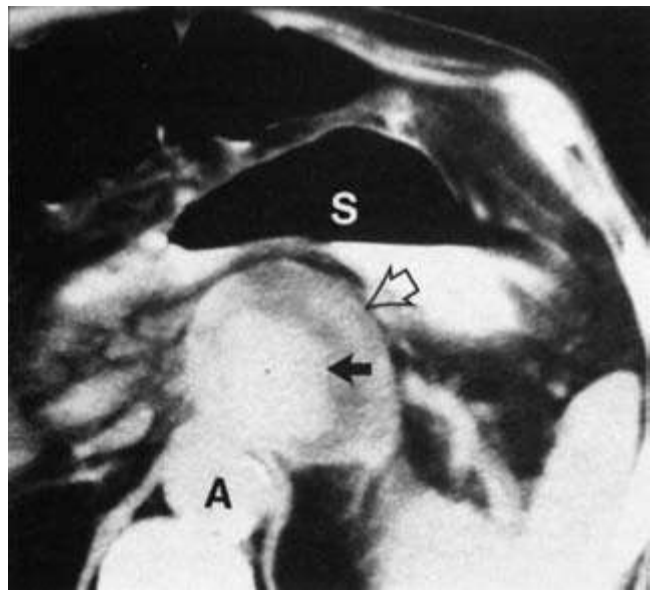
Vascular and splenic complications

Pancreatitis infrequently results in bleeding or thrombosis of peripancreatic vessels. The commonest venous complication of pancreatitis is occlusion of the splenic vein, which is adjacent to the posterior superior margin of the pancreas throughout most of its course. Splenic vein thrombosis results in splenomegaly, gastric varices, and rarely colonic varices. Occlusion of the superior mesenteric vein can also occur, causing mesenteric venous congestion and small bowel edema and ischemia. Severe pancreatic inflammation or a large pseudocyst may thrombose or compress these vessels. When acute thrombosis of these veins is detected thrombolytic therapy is sometimes considered, but there are few data regarding the safety of such therapy and the risk of retroperitoneal hemorrhage in patients with ongoing pancreatitis.

Arterial hemorrhage may also occur as a result of pancreatitis, and is a life-threatening event. This typically occurs when a pseudocyst erodes into a pancreatic artery, transforming the pseudocyst cavity into a pseudoaneurysm whose walls are partially formed by the artery and partially by the pseudocyst (Fig. 68.22). The splenic artery may be involved if the pseudocyst is in the pancreatic body or tail, whereas the gastroduodenal artery or branches of the pancreaticoduodenal arcade are typically affected in the head of the pancreas. If the pseudocyst communicates with the pancreatic duct, hemosuccus pancreaticus (or bleeding into the duodenum

(a)





(b)

Figure 68.22 Pseudoaneurysm complicating acute pancreatitis. **(a)** Angiogram demonstrates pseudoaneurysm arising from the splenic artery (SA) and a jet of contrast leaking from the pseudoaneurysm (arrow). **(b)** Dynamic contrast-enhanced computed tomogram of the same patient demonstrates the pseudoaneurysm (open arrow) with contrast enhancement (black arrow) anterior to the aorta (A) and posterior to the stomach (S). Courtesy of Caroline Taylor, West Haven, CT.

through the pancreatic duct and ampulla) ensues. Diagnosis is made by CT scanning and angiography. The bleeding artery can often be treated by embolization, but sometimes requires surgical ligation and, occasionally, broad surgical resection. If hemorrhage is controlled angiographically, subsequent endoscopic treatment of the pseudocyst has been described [267].

Extension of a pseudocyst, or occasionally inflammation alone, from the pancreas into the spleen may lead to a splenic hematoma and rupture. This complication is most often associated with chronic disease and may present with abdominal pain that is referred to the left shoulder, fever, and an abdominal mass. Ultrasonography or CT suggests the diagnosis. Arteriography may demonstrate splenic artery pseudoaneurysms. Emergency splenectomy is required for splenic rupture, and prophylactic splenectomy has been advocated by some authors even when splenic pseudocysts are asymptomatic [268]. Splenic pseudocysts have been successfully drained endoscopically.

Gastrointestinal obstruction

Severe acute pancreatitis may result in compression of the duodenum, with gastric outlet obstruction. Bile-duct obstruction may also occur. These forms of gastric or biliary obstruction will usually resolve unless there is underlying chronic pancreatitis or pseudocyst.

It is common for pancreatic inflammation to extend to the transverse colon and splenic flexure. Obstruction secondary to spasm and edema may cause the classic colon cutoff sign, with gas present in the right colon but absent distal to the region of obstruction. This finding is seen in 1%–10% of patients with acute disease, and cecostomy is occasionally required to prevent colonic perforation in patients with colonic obstruction and progressive dilation of the proximal colon [269]. Extension of pancreatic inflammation may lead to persistent colonic strictures, fistulas, or even perforation. During surgery for acute pancreatitis, especially when performed for debridement, the colon is particularly susceptible to devitalization and subsequent perforation. Rarely, erosion into colonic blood vessels may result in acute lower gastrointestinal hemorrhage or inferior mesenteric vein thrombosis and colonic infarction.

Systemic complications

Systemic complications of acute pancreatitis may be caused by the direct toxicity of circulating activated pancreatic enzymes, the systemic inflammatory response to pancreatic injury and necrosis, circulating endotoxins arising from bacterial translocation from the gut, or combinations of these factors. Organ failure can result, and is usually manifested as respiratory insufficiency, vascular complications including shock, a sepsis-like syndrome, or disseminated intravascular coagulation, and renal failure. Gastrointestinal bleeding and confusion or coma may also occur, and MODS may develop. Organ failure is seen uncommonly in interstitial pancreatitis, but develops in about 50% of patients with pancreatic necrosis, and is an independent predictor of mortality [248]. Early mortality in acute pancreatitis is often due to multiple organ failure, whereas late mortality is more often related to infection.

Multiple organ dysfunction syndrome (MODS) and systemic inflammatory response syndrome (SIRS)

Organ failure or dysfunction in acute pancreatitis is associated with high levels of circulating tumor necrosis factor and IL-6 [270], and systemic activation of macrophages and the complement system [271]. Respiratory failure is the most common form of organ dysfunction in acute pancreatitis, followed by vascular and renal complications. Consensus criteria for the diagnosis of organ failure or dysfunction were proposed in 1992 by the Atlanta Symposium on Acute Pancreatitis, which defined organ failure as either absent or present in a variety of physiological systems (Table 68.3) [9]. Marshall and colleagues subsequently proposed a scoring schema for "multiple organ dysfunction syndrome" (MODS) that grades the degree of organ dysfunction in six physiological systems. The function of each organ system is scored from 0 to 4, with a score equal to or greater than 2 indicating dysfunction of that organ system [272]. The resulting Marshall score, which has been validated and widely applied to a range of critical illnesses, can range from 0 to 24. Scores greater than 20 have been associated with intensive care unit (ICU) mortality rates of 100% [272]. When applied to acute pancreatitis, investigators have modified the Marshall system, omitting the hepatic dysfunction scale because hyperbilirubinemia in gallstone pancreatitis is more often due to biliary obstruction than hepatic failure. The MODS scoring system used in pancreatitis trials is shown in Table 68.3 [249].

Predictors of MODS include the admission APACHE II score, the serum CRP level at 48 h, the presence of pancreatic necrosis, and the presence of SIRS (Table 68.6) [247–249].

Organ dysfunction is a dynamic process that is reversible in some patients. The time course of organ dysfunction predicts mortality from acute pancreatitis. Those patients whose organ dysfunction resolves in the first week do well, whereas those with deteriorating organ dysfunction have a mortality rate of over 50% [247]. Similarly, SIRS may also be transient or persistent. In one large series, the prevalence of SIRS among patients with acute pancreatitis was 21% on hospital admission, and 18% had persistent SIRS at 48 h. Persistent SIRS was associated with development of MODS and mortality from acute pancreatitis [249].

Respiratory failure

The most common form of organ failure in acute pancreatitis is pulmonary insufficiency due to the adult respiratory

distress syndrome (ARDS). This hypoxemic state is associated with a normal pulmonary venous wedge pressure, decreased pulmonary compliance, and pulmonary capillary injury. Obese patients and those with hypertriglyceridemia are at increased risk for developing this complication. In patients with severe ARDS, respiratory support with positive end-expiratory pressure is required.

Pulmonary function may be further affected by pleural effusions and decreased diaphragmatic movement due to ascites or retroperitoneal inflammation. The contribution of decreased diaphragmatic movement is unclear, but may be a factor in the increased risk of a pulmonary death in those with acute pancreatitis and obesity. Effusions are reported in 4%–17% of patients with acute pancreatitis; up to 50% may be associated with pseudocysts or pancreatic fistula. Most effusions are found on the left side or are bilateral, and most resolve spontaneously [229]. The early appearance of effusion has been associated with more severe disease. Thoracentesis is suggested when needed for diagnosis, to exclude infection, or when the size of the effusion results in respiratory compromise. Marked elevation of the pleural fluid amylase level may suggest an underlying pancreatic fistula, which may respond to antisecretory or endoscopic therapy.

Fat necrosis

Clinically evident fat necrosis in regions distant from the pancreas is seen in less than 1% of patients with acute pancreatitis. Pancreatic enzymes have been detected in areas of fat necrosis and are presumably responsible for initiating this injury. The initial injury may arise from the cleavage of adipocyte plasma membrane glycerosphingolipids by phospholipase A. Adipocyte-associated or circulating lipase activities may then enter the cell and convert triglycerides to monoglycerols and toxic free fatty acids. Several clinical syndromes are associated with fat necrosis. Cutaneous necrosis generates widely disseminated, raised, erythematous, and tender nodules of less than 2 cm in diameter. The process is frequently associated with fever and eosinophilia. The nodules typically resolve in days to weeks; however, some may rupture and heal with hyperpigmentation. Fat necrosis may affect the marrow cavity and lead to vascular damage and bone infarction. The typical bone lesions are frequently painless, osteolytic, develop 3–6 weeks after the onset of pancreatitis, and involve the ends of long bones such as the femur. Synovial pad fat necrosis may be associated with arthritis. The increased amounts of free fatty acids recovered from such joints may initiate this inflammatory process. Fat necrosis may rarely affect the middle ear.

Other systemic complications

Confusion and coma can occur and may be due to cerebral edema. Gastrointestinal hemorrhage may be caused by mucosal erosions and ulcerations, gastric varices, colonic varices, or a pancreatic pseudoaneurysm, as described above (see "Vascular and splenic complications"). Temporary blindness has been reported in pancreatitis and may be attributed to the retinal ischemia induced by leukocyte emboli.

Acute treatment

The treatment of acute pancreatitis is largely supportive. Pancreatitis patients are often in critical need of such care, and appropriate treatment is frequently lifesaving. Aggressive fluid replacement, management of pain and nausea, prompt identification and treatment of complications, nutritional support, and prophylaxis and treatment of infection are important elements of supportive care.

Some treatments have lost favor due to lack of convincing efficacy. These include peritoneal lavage and nasogastric suction. The use of nasogastric suction in the past was based on the unproven concept that removing the acid-dependent stimulation of the pancreas would allow the inflamed organ to rest and promote recovery. However, clinical trials showed no benefit from nasogastric suction for treatment of underlying pancreatitis. Nasogastric suction should be reserved for patients with persistent emesis, and the occasional patient with evidence of gastric or colonic obstruction complicating acute pancreatitis.

Supportive care includes careful observation of the patient. Regular assessment of vital signs and oxygen saturation should be a routine part of care. Decreased urine output, hemodynamic instability, or worsening oxygen saturation should lead to prompt evaluation and treatment, perhaps in an ICU.

Parenteral narcotics are often required for treatment of pain in acute pancreatitis, and meperidine has traditionally been the narcotic of choice because it does not cause pancreatitis or significantly alter sphincter of Oddi function [273]. However, meperidine is a less effective analgesic than many other narcotics, and accumulation of meperidine metabolites may cause paranoia, psychosis, or seizures. Morphine administration is discouraged in acute pancreatitis because it can cause sphincter of Oddi spasm and, rarely, pancreatitis. The effects of other narcotics on the sphincter of Oddi are less well studied, but hydromorphone or other agents may be appropriate in some cases, particularly if meperidine is ineffective, large doses are required, or prolonged use of narcotics is anticipated.

Fluid and electrolyte replacement

The cornerstone of supportive therapy is maintenance of an adequate intravascular volume. The analogy between the injury of severe pancreatitis and a burn is a useful one: pancreatitis often leads to "third spacing" of fluid and intravascular volume depletion. This may in turn worsen pancreatic

perfusion and exacerbate the pancreatitis. Hemoconcentration is associated with the development of pancreatic necrosis. The clinician should regularly reassess intravascular volume and aggressively replace fluids and electrolytes to avoid volume depletion. Often such assessment is made on the basis of urine output and vital signs. An elevated hematocrit or renal insufficiency may be signs of inadequate volume replacement. In severely ill patients, central venous pressure monitoring may be useful. Although dextrans have been used to expand intravascular volume and enhance pancreatic blood flow, their use has not been widely accepted.

Metabolic abnormalities

Hyperglycemia is present in some patients with acute pancreatitis. Tight glycemic control (blood glucose 80–100 mg/dL) has been associated with improved outcomes in critically ill patients [274], mainly due to decreased rates of organ dysfunction and infection. Although specific data are not available, tight glycemic control may be of benefit in severe acute pancreatitis as well. However, limited glucagon and glycogen reserves, especially in alcoholics, diminish the ability of pancreatitis patients to respond to iatrogenic hypoglycemia.

Serum calcium levels may be decreased during acute pancreatitis, and levels below 7.5 mg/dL are associated with a poor prognosis. Factors that account for this decrease are sequestration of calcium by free fatty acids that have been generated by peritoneal fat necrosis, and dilution by intravenous administration of calcium-poor fluids. In addition, hypoalbuminemia may result in low total serum calcium with normal serum ionized calcium. Humoral factors such as parathyroid hormone resistance associated with hypomagnesemia, and hypersecretion of calcitonin and glucagon have been implicated in hypocalcemia, but are unlikely to play a major role. Calcium should be slowly administered to patients with decreased ionized serum calcium.

Hyperlipidemia may lead to pancreatitis, accompany alcoholic pancreatitis, or be a consequence of acute pancreatitis. Therapy should generally not be instituted for hyperlipidemia encountered during acute pancreatitis, but values should be remeasured after recovery. One exception is patients with severe pancreatitis and respiratory failure. In this subgroup, and in the occasional patient with extreme hyperlipidemia, lowering serum triglycerides with plasmapheresis may be useful [275].

Inhibition of enzymes, secretion, and inflammation

Pharmacological therapy could theoretically be used to limit the severity of pancreatitis at several levels: by inhibiting active pancreatic enzymes, inhibiting pancreatic secretion, or interrupting the inflammatory cascade. The first agents to be tested extensively in humans were drugs that inhibit activated pancreatic enzymes. Of these "protease inhibitors" the best studied is gabexate mesilate, which is not available in the United States.

Most studies of gabexate have shown a benefit in acute pancreatitis that did not reach statistical significance. Two metaanalyses evaluating controlled clinical trials of gabexate in acute pancreatitis found that gabexate does not reduce mortality from acute pancreatitis, but does appear to reduce the incidence of complications by about 30% [199,276]. Although a subsequent trial showed decreased early mortality in patients with severe disease, it is unlikely to alter the basic conclusions of the metaanalyses, which drew on studies enrolling over 800 patients with acute pancreatitis [277]. The lack of a more decisive clinical effect is consistent with the fact that activated pancreatic enzymes play their most important role early in the course of pancreatitis, before the patient presents for treatment.

Somatostatin and octreotide, two agents that inhibit pancreatic secretion, have also been extensively studied in clinical trials. When only randomized controlled trials were considered, metaanalysis showed a statistically significant reduction in mortality with somatostatin therapy, and a lesser benefit with octreotide that did not reach statistical significance [278]. Curiously, despite their effect on mortality, neither agent was shown to decrease complication rates. The lack of demonstrated benefit with octreotide therapy may reflect its effects on sphincter of Oddi function and pancreatic blood flow. Although somatostatin appears to have a significant impact on mortality, it is not available in the United States.

Severity of the systemic immune response, as measured by blood levels of circulating proinflammatory cytokines, correlates with the development of local and systemic complications in pancreatitis. The inflammatory response often continues to increase after the patient is hospitalized, suggesting that pharmacological blockade of proinflammatory cytokines soon after hospital admission could ameliorate the consequences of pancreatitis. Although animal studies support this hypothesis, an inhibitor of platelet-activating factor (a proinflammatory cytokine) tested in a large clinical trial did not have a significant effect on complication rates or mortality [279]. Experimental studies suggest that specific cytokines may promote injury in distinct tissues. Therefore, a different cytokine inhibitor or a combination of inhibitors might be needed to achieve benefit.

Enteral and parenteral nutrition

One longstanding tenet of supportive treatment has been to avoid pancreatic stimulation by giving the patient nothing to eat during their acute illness. Patients with mild disease who rapidly improve and resume oral intake require no special form of nutritional support, but those with more protracted disease are generally given nutritional therapy.

Controlled trials of total parenteral nutrition (TPN) have shown little or no benefit for patients awaiting surgery or undergoing chemotherapy. Although there are few controlled data for pancreatitis, some retrospective studies suggest that

pancreatitis patients given early parenteral hyperalimentation are at increased risk for complications such as sepsis and pancreatic infection. Although intravenous lipid infusion does not stimulate pancreatic secretion, it can produce hyperlipidemia that can then trigger further pancreatitis. Because TPN-induced elevations of the triglyceride level have been reported to cause pancreatitis, it seems prudent to monitor triglyceride levels and attempt to keep them below 500 mg/dL in pancreatitis patients receiving TPN [280].

A growing body of evidence suggests that enteral feeding is safe in pancreatitis, and preferable to parenteral nutrition. Oral feeding stimulates pancreatic secretion, but feeding directly into the distal small bowel may result in negligible pancreatic stimulation [281]. Theoretically, enteral feeding could limit the changes in gut mucosal integrity seen in pancreatitis, with a resultant decrease in bacterial translocation, systemic immune response, and pancreatic infection. These considerations have led to controlled trials of enteral vs parenteral nutrition in pancreatitis.

In one randomized study, enteral feeding through a nasojejunal tube was compared with TPN in patients with mild acute pancreatitis mostly attributed to alcohol [282]. Enteral feeding was begun within 48 h of admission and was well tolerated in all cases. There was no significant difference between the two groups in major endpoints, including time to normalization of serum pancreatic enzyme levels, resolution of pain, time to oral feeding, or incidence of infection. Several patients tolerated enteral feeds well despite failing subsequent trials of oral intake, or had recurrent pancreatic symptoms when the tip of the enteric feeding tube fell back into the stomach.

Two other studies have randomized patients with severe disease to TPN or early enteric feeding. In both studies enteric feeding was safe and less costly than TPN, and was associated with improved outcomes as determined by either laboratory markers of inflammation or the incidence of infections and other complications [283]. Patients with clinical evidence of ileus generally tolerated enteric feeding well. An additional study showed no benefit of enteral feeding ascertained by markers of the immune response or intestinal permeability, but tube dislodgement was common in this study, and patients received only 20% of their predicted caloric requirements [284].

Enteral feeding can be recommended in acute pancreatitis, especially when it is unlikely that the patient will quickly resume oral intake. Early initiation of nasojejunal feeding (within 48 h of admission) is safe and may improve the course of the illness in patients with severe disease. The major impediment to this therapy is the practical difficulty of placing a nasojejunal tube. Methods for simple enteric feeding tube placement have been described, including both fluoroscopic techniques and bedside transnasal endoscopy [285]. Isocaloric full-strength formula can be used, beginning at 25 mL/h, and increasing the rate every 4 h until the patient's target rate is met. Feeding is generally successful even in the presence of ileus, but high gastric residuals or symptoms of nausea and abdominal fullness may prompt a decrease in the infusion rate.

Nasogastric tube feeding is a simpler alternative for enteral nutritional support. Two small randomized controlled studies of nasojejunal vs nasogastric feeding in patients with severe acute pancreatitis, many of whom were in an ICU, showed no difference in patient outcomes [286,287]. Because intragastric feeding does stimulate subsequent pancreatic secretion in healthy persons, it is unclear why nasogastric tube feeding would be well tolerated in acute pancreatitis. Patients with severe acute pancreatitis may have a "stunned pancreas" that is less responsive to stimulation.

When the patient is ready for oral feeding tradition holds that they should be started on a low-fat diet, but there is little evidence that this restriction affects recovery. Indeed, lumenal amino acids are also potent stimulants of pancreatic enzyme secretion. Probably more important than fat restriction is the subjective response of the patient to feeding. The most reliable marker for beginning oral feeding is hunger. Steady improvement in pain and tenderness allowing discontinuation of parenteral pain medication is also a useful indicator. Persistently abnormal serum enzymes or CT scans in the absence of symptoms or complications should not dissuade the clinician from feeding a hungry patient. There is no reason to believe that the mechanical consistency of the food is important in refeeding.

A significant minority of patients will have pain with refeeding, especially those with necrotizing pancreatitis. In one series, pretreatment with lanreotide (a somatostatin analogue), 30 mg intramuscularly, markedly decreased the rate of refeeding pain in patients with necrosis compared with historical controls [288]. Prospective controlled study of this strategy is warranted.

Antibiotic prophylaxis

Infection of pancreatic necrosis is a life-threatening complication and a major risk factor for death from pancreatitis [289]. Strategies for the prevention of infected necrosis have been evaluated, including systemic antibiotic prophylaxis and selective decontamination of the gut.

Older studies of antibiotics to prevent pancreatic infection found no benefit. These studies probably enrolled many patients at low risk for infection, and did not select antibiotics based on their ability to penetrate pancreatic tissue. Subsequent investigations demonstrated that penetration of pancreatic tissue by ampicillin and aminoglycosides is poor, whereas imipenem–cilastatin, mezlocillin, third-generation cephalosporins, metronidazole, and quinolones exhibit moderate to excellent penetration [290].

Prospective randomized studies of antibiotics known to penetrate necrotic pancreatic tissue have demonstrated bene-

fits for prophylactic intravenous antibiotic therapy in patients with necrosis. Prophylactic imipenem–cilastatin, cefuroxime, and a combination of ceftazidime, amikacin, and metronidazole have been tested against placebo, and each regimen resulted in a significant reduction in the incidence of infectious complications [291,292]. These studies have been criticized for the absence of physician blinding, frequent crossover between study groups, and frequent use of off-protocol antibiotic regimens. Only the cefuroxime study showed a significant reduction in mortality, but had a high rate of nonpancreatic and staphylococcal infections, and did not demonstrate a significant reduction in the incidence of pancreatic infections. Two metaanalyses of antibiotic prophylaxis concluded that prophylaxis decreases sepsis and mortality in patients with necrosis [293,294]. In one comparative study, pefloxacin was less effective than imipenem–cilastatin for prevention of infectious complications. In a randomized study comparing early (at admission) vs late (> 48 h after admission) initiation of prophylactic antibiotics, there was a statistically significant benefit from early initiation of treatment, with pancreatic infection rates of 13% and 31% in the early and late groups, respectively [295].

The first double-blind randomized controlled trial of prophylactic antibiotics was published in 2004, comparing a 21-day course of intravenous ciprofloxacin and metronidazole with placebo in patients with severe acute pancreatitis. A total of 114 patients were enrolled, the majority of whom had pancreatic necrosis. Patients were switched to open antibiotic treatment if they developed new sepsis or SIRS, new MODS, documented pancreatic or extrapancreatic infection, or an increased serum CRP with clinical suspicion of infection; 28% of the treatment group and 46% of the placebo group were switched to open antibiotic treatment. On an intention-to-treat basis, infected necrosis developed in 12% of the antibiotic group versus 9% of the placebo group. The commonest bacteriological isolates from infected necrosis included enteric organisms and *Staphylococcus epidermidis*. Mortality was similar in both groups (5% and 7%) [296].

These data have renewed the controversy surrounding prophylactic antibiotic administration in severe acute pancreatitis. It is unclear whether antibiotics should be initiated in all patients with severe acute pancreatitis, or only "on demand" in patients with SIRS, MODS, documented infection, or clinically suspected infection [296]. Until additional double-blinded randomized studies are available, antibiotic prophylaxis or "on-demand" use of antibiotics are both reasonable strategies. Patients with necrosis documented by CT or, in the absence of early contrast-enhanced CT, patients with severe pancreatitis (who are at highest risk for necrosis) should be treated. If prophylactic treatment is given, it should generally be started within 48 h of admission and continued for 2–3 weeks, and imipenem–cilastatin (500 mg every 8 h) is probably the most effective agent. Unfortunately, prophylactic antibiotics do not eliminate the risk of infected necrosis. Large series of patients treated with prophylactic antibiotics show an incidence of infected necrosis as high as 32%, with a shift toward gram-positive and fungal infections [255]. Fluconazole penetrates pancreatic tissue [297] but its role in the prophylaxis of pancreatic fungal infections is unproven. Methods for the diagnosis of infected pancreatic necrosis are discussed earlier (see "Complications" above).

Other strategies for prevention of infected pancreatic necrosis

An alternative to systemic antibiotic prophylaxis is "selective decontamination" of the gastrointestinal tract with lumenal antibiotics. The rationale for this approach is the finding that bacterial translocation from the gut is the likely source of infection in pancreatic necrosis [253]. Studies in animal models of severe pancreatitis show a benefit from intralumenal antibiotics and intestinal lavage. One large controlled clinical trial has tested this prophylactic strategy; patients with severe pancreatitis were randomized to receive no antibiotic prophylaxis or colistin sulfate (200 mg), amphotericin (500 mg), and norfloxacin (50 mg) orally every 6 h, and in a rectal enema daily. Intravenous cefotaxime was also given until gram-negative bacteria were eliminated from the mouth and rectum. The incidence of pancreatic infection was halved by selective decontamination, and mortality was significantly decreased as well [254]. The complex study regimen may be difficult to introduce in some practice settings, and further investigation of selective decontamination is needed.

Probiotic administration has been suggested as a potential method of preventing infected necrosis, but data are limited.

Treatment of infected pancreatic necrosis

As discussed earlier in this chapter, the diagnosis of infected pancreatic necrosis should be entertained in patients with systemic signs of infection, or in patients with persistent or worsening organ failure despite supportive care. Diagnosis requires a high level of suspicion. Available noninvasive laboratory and imaging tests do not reliably exclude infection, and CT-guided aspiration of necrotic areas for Gram stain, fungal stain, and cultures are the most important diagnostic modalities.

Anecdotal reports suggest that an occasional patient with infected necrosis will survive with antibiotic treatment alone [298]. However, antibiotics alone are generally inadequate for treatment of documented infected necrosis, and the infected necrotic material should be removed. Prompt surgical debridement has been the standard therapy, and repeated reexploration for further debridement is often necessary. This treatment results in mortality rates of between 20% and 60% [299]. Better surgical outcomes are in part associated

with changes in technique, including open packing of the wound and prolonged postoperative lavage.

Percutaneous drainage of infected necrosis was long thought to be an inadequate therapy, because the solid necrotic material would not drain out through small-caliber percutaneous catheters, and the drainage tube would only serve to introduce other organisms into the necrotic debris. However, reports have demonstrated that percutaneous catheter drainage can be effective [300,301]. In contrast to percutaneous treatment of pseudocysts or pancreatic abscesses, repeated interventions and aggressive techniques are required adequately to remove the infected material. Techniques may include placement of multiple large-bore (28-French) drainage tubes, frequent bedside catheter irrigation, periodic vigorous manual irrigation under fluoroscopic or CT guidance, and endoscopy of the percutaneous catheter tract for removal of debris using jet irrigation, forceps, and snares. Reported mortality rates of 15%–20% from expert centers compare favorably with surgical series. Complications include hemorrhage from retroperitoneal vessels, and patients with persistent pancreatic sepsis requiring surgery had a high mortality rate.

Endoscopic drainage of infected necrosis has also been described, in patients whose infection occurs in the setting of organized pancreatic necrosis (typically at least 2–3 weeks into the course) [259,302]. An aggressive endoscopic approach is necessary, as placement of a single plastic stent into an infected necrotic collection will not result in adequate drainage or allow debridement of the necrotic tissue. In one series multiple collections were drained into the stomach or duodenum under EUS guidance; in addition to endoscopic placement of stents and lavage catheters, cyst-enterostomies were dilated with large-caliber balloons to permit direct endoscopy of the necrosis cavity with endoscopic debridement [303]. Endoscopic debridement may be repeated daily or weekly. Outcomes in small series from expert centers compare favorably with the larger published surgical experience, although success is lower than for endoscopic drainage of pseudocysts [302]. Endoscopists and radiologists undertaking drainage of pancreatic necrosis should be prepared to perform repeated interventions over time as necessary to remove necrotic material and ensure adequate drainage.

Drainage of sterile necrosis

The role of surgery in sterile necrosis has been debated. Although routine surgical debridement of sterile pancreatic necrosis was advocated in the past, the weight of available evidence does not show a benefit from surgery over supportive medical care [304]. Nevertheless, intervention may be warranted in a small subset of patients in whom sterile necrosis is associated with either a deteriorating course despite maximal supportive care, or prolonged organ failure [305]. In such patients the percutaneous and endoscopic techniques described above are often appropriate as well.

Some patients with sterile necrosis require intervention not because of organ failure or an ongoing systemic inflammatory response, but because of recurrent pancreatic symptoms and an enlarging collection with attempts at refeeding. This is particularly common in patients with "central necrosis" (extensive necrosis of the pancreatic body) and is usually associated with a persistent disruption of the pancreatic duct, resulting in an isolated pancreatic tail and ongoing leakage of pancreatic juice from the disruption. Internal drainage of the collection may suffice, but resection of the pancreatic tail is required in many cases [301,306]. Drainage of sterile necrosis may also be required in patients with gastrointestinal obstruction caused by the necrotic collection.

Urgent ERCP and EUS in gallstone pancreatitis

Persistent bile-duct stones are associated with more severe pancreatitis, and both surgeons and endoscopists have investigated the theory that prompt relief of ampullary obstruction by removal of an impacted stone would decrease the severity of gallstone pancreatitis. Although studies of urgent surgery have yielded disappointing results, endoscopic studies have identified subgroups of patients who benefit from urgent ERCP.

Several randomized studies have assessed the value of urgent ERCP in patients with gallstone pancreatitis. The first, performed in the United Kingdom, found that ERCP within 2 days of presentation decreased the complication rate of gallstone pancreatitis [307]. Sphincterotomy was done only if stones were present in the ampulla or bile duct. The benefit was limited to the subset of patients who were predicted to have severe pancreatitis on initial assessment using the modified Glasgow criteria (Table 68.5). These results suggested that urgent ERCP could alter the course of acute gallstone pancreatitis.

A similar study subsequently reported from Hong Kong also found a decreased complication rate when urgent ERCP was performed and sphincterotomy was done for ampullary or ductal stones [144]. This study also showed that benefit was limited to patients predicted on admission to have severe pancreatitis, using the simpler urea–glucose criteria. The Hong Kong study included some patients with other causes of pancreatitis, and probably included more patients with primary pigment stones of the bile duct. In contrast to the British study, the benefit of ERCP appeared to be largely due to prevention of bacterial cholangitis rather than amelioration of pancreatitis.

Both of these studies found a benefit in patients predicted to have severe pancreatitis and showed that ERCP can be safely performed in this setting. It remains unclear if the benefit was the result of a decrease in the severity of pancreatitis or to treatment and prevention of cholangitis. The pancreas was not directly imaged by CT to assess the severity of pancreatitis in these studies. There was no evidence that ERCP could prevent a predicted mild episode of pancreatitis from progressing in severity, and there were no statistically

significant changes in survival. A third large study showed a significant improvement in mortality with early ERCP and sphincterotomy, but this was only published in abstract form [308].

A fourth study with a similar design from Germany showed a trend toward higher mortality and more severe complications in patients undergoing early ERCP [309]. This study excluded patients with a serum bilirubin above 5 mg/dL, probably excluding many patients with persistently impacted stones while including patients with milder disease and nonobstructing ductal stones. Because nonobstructing stones theoretically have little effect on either the development of cholangitis or the severity of pancreatitis, this study probably excluded those patients most likely to benefit from urgent ERCP. Unfortunately, none of these studies reported the incidence of impacted ampullary calculi. Further studies using noninvasive methods to visualize ampullary stones and grade the severity of pancreatic inflammatory changes may shed more light on the benefit achieved by ERCP and sphincterotomy.

When should the clinician consider urgent interventions to diagnose and remove bile-duct stones in patients with acute pancreatitis? The clearest indication is the presence or suspicion of biliary sepsis. Anicteric patients with mild pancreatitis and no signs of cholangitis are unlikely to require urgent ERCP. Jaundiced patients and those with predicted severe pancreatitis, especially those who fail to improve over the first 12–24 h of observation, may benefit from prompt bile-duct evaluation. In these patients, newer and less invasive methods of demonstrating ductal or ampullary stones (such as MRCP, helical CT, and endoscopic ultrasound) will probably be helpful in choosing patients who should undergo urgent ERCP [237]. A randomized prospective study comparing EUS and ERCP showed that urgent EUS could replace diagnostic ERCP in acute biliary pancreatitis, with therapeutic ERCP reserved for those patients with persistent bile-duct stones. Stone detection rates were higher in the EUS group, and there was a trend toward decreased morbidity in the EUS group [310].

Traditionally, cannulation of the pancreatic duct has been avoided when possible in patients with acute biliary pancreatitis undergoing ERCP. Yet some experts have advocated intentional visualization of the pancreatic duct early in the course of severe acute pancreatitis, for identification and endoscopic treatment of pancreatic duct disruptions. However, some disruptions resolve spontaneously [250], and instrumentation may introduce infection into a previously sterile collection. Controlled data evaluating this strategy are needed before it can be generally recommended. Similarly, a strategy of early ERCP and sphincterotomy in all patients with biliary pancreatitis cannot be generally recommended.

Preoperative ERCP in mild gallstone pancreatitis

Most patients with gallstone pancreatitis rapidly improve with supportive management, and by the time of their cholecystectomy only a minority have bile-duct stones. The bile duct should be imaged in such patients, and the choice of imaging technique depends on the likelihood of finding a bile-duct stone, the surgeon's ability to extract bile-duct stones laparoscopically, and the confidence of the biliary endoscopist.

Patients with gallstone pancreatitis whose serum liver tests and pancreatic enzyme levels steadily fall after admission have a low (8%) incidence of bile-duct stones [228], and are best evaluated with intraoperative cholangiography (IOC). Patients in whom any of these chemistries increase in the first 48 h of admission, and patients with a dilated bile duct on ultrasound, have an intermediate (30%) incidence of ductal stones. These patients may also best be evaluated with intraoperative cholangiography, depending on the comfort and skill of the available surgeon and endoscopist. Preoperative ERCP, MRCP, or EUS may also be appropriate in this patient group.

A randomized study compared preoperative ERCP with IOC in patients with mild to moderate biliary pancreatitis. Only patients with risk factors for retained ductal stones (bile-duct dilation, elevation of the serum bilirubin, or persistent hyperamylasemia) were enrolled. Patients with severe pancreatitis and those with cholangitis were excluded. Only 24% of patients in the IOC group had bile-duct stones and required postoperative ERCP. Patient outcomes were similar in the two groups. Hospital stay was longer and treatment costs were higher in the preoperative ERCP group [227].

Direct visualization of a ductal stone on ultrasound, CT, MRI, or EUS establishes a high risk (60% or greater) of a bile-duct stone in gallstone pancreatitis, and such patients, as well as those with persistent jaundice, may reasonably undergo preoperative ERCP.

Sphincterotomy as sole therapy for gallstone pancreatitis

Endoscopic sphincterotomy usually eliminates the common channel between the bile duct and pancreatic duct, which afterwards enter the duodenum at slightly different locations. Sphincterotomy appears to prevent recurrence of gallstone pancreatitis in patients whose gallbladder remains in place. At least 15% of patients treated in this fashion will subsequently develop other complications of gallstones, however, including biliary obstruction, cholangitis, and acute cholecystitis [311]. These complications are commonest within 6 months of sphincterotomy but may occur years later. In addition, a small percentage will develop stenosis of the sphincterotomy site. Cholecystectomy thus remains preferable to sphincterotomy in patients with uncomplicated pancreatitis who are fit for surgery. When the risks of cholecystectomy are high (as in patients with unstable cardiovascular disease or advanced cirrhosis), endoscopic sphincterotomy is a reasonable therapeutic alternative.

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69 Chronic pancreatitis

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The terminology surrounding the classification of inflammatory disease of the pancreas is confusing and is based on both clinical and morphological criteria. Since the 1970s, multiple classifications have been proposed. Differentiation by etiology allows for the prediction of the factors responsible for pancreatitis but gives little information on potential overlapping pathophysiological mechanisms. Clinical and pathological classifications by themselves have prognostic implications, with fulminant or hemorrhagic pancreatitis having a poor prognosis and edematous pancreatitis being associated with a good prognosis; only in a minority of patients is histological information ever obtained, however, and clinical classifications are not helpful pathophysiologically. In 1963, a symposium on pancreatitis was held in Marseilles, France, in which a clinical classification of pancreatitis was proposed, and this classification has since been widely used [1]. According to the Marseilles classification, pancreatitis can be divided into acute and chronic, with both types having relapsing and nonrelapsing varieties. The essential difference between acute and chronic pancreatitis is the presence of permanent and progressive morphological or functional damage in the latter. Both subtypes are further classified into relapsing and nonrelapsing varieties depending on their clinical presentation. In acute pancreatitis, if the primary causes or factors are eliminated, clinical and morphological restitution of the pancreas occurs. In contrast, in the chronic form, anatomic or functional pancreatic damage remains even after the primary causes are eliminated.

The major drawback of the Marseilles classification is the frequent inability to distinguish between acute and chronic forms of the disease based on their clinical presentation. This prompted two subsequent international symposia in 1983 [2] and 1984 [3] to discuss the classification of pancreatitis. Some minor modifications of the original Marseilles classification were proposed. The classification of pancreatitis should be reduced to include only acute and chronic forms because it is frequently difficult to distinguish between recurrent acute pancreatitis and recurrent attacks of chronic pancreatitis, and several years' follow-up may be needed. Accordingly, chronic pancreatitis is defined as an inflammatory disease of the pancreas characterized by persistent and often progressive lesions, whereas the lesions of acute pancreatitis regress when the cause is suppressed or removed. In an addendum, it was recognized that alcohol-induced pancreatitis may present acutely and is not inevitably progressive.

The second symposium at Marseilles [2] also proposed to distinguish obstructive chronic pancreatitis from other forms of chronic pancreatitis. Obstructive chronic pancreatitis is characterized by dilation of the ductal system, diffuse atrophy of the acinar parenchyma, and uniform fibrosis. In contrast to other forms of chronic pancreatitis, intraductal plugs or stones are usually rare or absent, and both structural and functional changes may improve when the obstruction is relieved. Based on morphology, biochemistry, molecular biology, and epidemiology, Sarles [4] reclassified chronic pancreatitis into four groups. The first and largest group, lithogenic pancreatitis (chronic calcifying pancreatitis), consists of five subgroups of stones: hereditary pancreatitis, transparent stones, nutritional pancreatitis, hypercalcemia pancreatitis, and pure calcium stones. The other three groups include obstructive pancreatitis, caused by obstruction of the pancreatic ducts preceding the onset of pancreatitis, with uniform distribution of the lesions caudal to the obstruction; inflammatory pancreatitis, which is characterized by diffuse fibrosis and the destruction of exocrine parenchyma with infiltration of mononuclear cells; and pancreatic fibrosis, characterized by silent, diffuse perilobular fibrosis.

A recent classification of chronic pancreatitis, the TIGAR-O system, incorporates new insights into genetic, environmental, immunological, and pathobiological risk factors associated with chronic pancreatitis [5]. The TIGAR-O etiological

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Table 69.1 TIGAR-O System: etiological factors associated with chronic pancreatitis

Toxic-Metabolic	**Autoimmune**
Alcoholic	Isolated autoimmune chronic pancreatitis
Tobacco smoking	Syndromic autoimmune chronic pancreatitis associated with
Hypercalcemia	– Sjögren syndrome
Hyperlipidemia	– Inflammatory bowel disease
Chronic renal failure	– Primary biliary cirrhosis
Medications – phenacetin abuse	
Toxins – organotin compounds (e.g., dibutyltin dichloride)	
Idiopathic	**Recurrent and severe acute pancreatitis**
Early onset	Postnecrotic (severe acute pancreatitis)
Late onset	Recurrent acute pancreatitis
Tropical	Vascular diseases/ischemia
	Postirradiation
Genetic	**Obstructive**
Hereditary pancreatitis – cationic trypsinogen	Pancreas divisum
CFTR mutations	Sphincter of Oddi disorders (controversial)
SPINK1 mutations	Duct obstruction (e.g., tumor)
	Preampullary duodenal wall cysts
	Posttraumatic pancreatic duct scars

Adapted from Etemad and Whitcomb [5], with permission from Elsevier.

classification consists of six groups (see Table 69.1): Toxic-metabolic, Idiopathic, Genetic, Autoimmune, Recurrent and severe acute pancreatitis, and Obstructive. The relationships among the six groups remain unclear but conceptually, this system categorizes the groups by potential shared pathogenic mechanisms. Significant insight into shared mechanisms of disease has evolved with the identification of similar or different genetic mutations associated with conceptually different categories of chronic pancreatitis discussed in this chapter. As stated by the authors, "Further research is needed to determine the clinical and natural history of chronic pancreatitis developing in the context of various risk factors" [5].

Incidence and prevalence

The prevalence of chronic pancreatitis in autopsy materials ranges from 0.04% to 5% [6,7]. Most epidemiological data are obtained either from retrospective studies [8–10] or by calculations using data given in clinical material [11,12]. The only prospective study on the incidence and prevalence of chronic pancreatitis was performed in Copenhagen, Denmark, in 1978 and 1979 [13]. It showed an incidence of 8.2 new cases per 100 000 inhabitants per year, and a prevalence of 26.4 cases per 100 000 inhabitants. The epidemiological studies clearly demonstrate that there are enormous differences in the incidence rates between different areas. Alcohol consumption is considered to be the major factor in the development of chronic pancreatitis. This may well explain the low incidence (rate) of chronic pancreatitis in Japan, which has traditionally a very low alcohol intake. On the other hand, Switzerland has higher alcohol consumption than Denmark [14], but the incidence of chronic pancreatitis is lower in Switzerland than in Denmark.

Environmental or hereditary factors may influence susceptibility to alcohol-induced pancreatitis. Alternatively, this may be related to regional differences in diagnostic criteria for chronic pancreatitis. Figures for frequency of chronic pancreatitis differ markedly from one center to another; most likely, this does not reflect a real difference in frequency but points strongly to regional differences in patient selection and diagnostic criteria.

Etiology

Alcohol in Western societies (70%–80%) and malnutrition worldwide represent the major etiologies of chronic pancreatitis [15,16]. In addition, metabolic and mechanical disturbances, as well as hereditary disposition, have been implicated (Table 69.2).

Alcohol

Alcohol consumption is the major risk factor for chronic pancreatitis, but probably requires additional anatomic, environmental, or genetic cofactors to induce the disease because less

Table 69.2 Etiology of chronic pancreatitis

Alcohol, 70%
Idiopathic (including tropical), 20%
Other, 10%
Hereditary
Hyperparathyroidism
Hypertriglyceridemia
Autoimmune pancreatitis
Obstruction
Trauma
Pancreatic divisum

than 10% of alcoholics develop pancreatitis [17]. In Western societies, about 70% of cases are caused by alcohol [18]. The incidence of pancreatitis found at autopsy in alcoholics is as high as 45–50 times the rate in nondrinking controls [19]. There is a linear relationship between the logarithm of the risk for development of chronic pancreatitis and the mean daily consumption of alcohol [20]. Furthermore, it has been demonstrated that the risk for abstainers is lower than the risk for people drinking a low quantity of alcohol (i.e., 1–20 g/day). Therefore, it appears that there is no statistical threshold for alcohol toxicity but a continuous spectrum of individual thresholds. This makes it difficult to distinguish between low-threshold, alcohol-induced pancreatitis and idiopathic pancreatitis. The duration of alcohol consumption is also important. For the same daily intake, the risk increases with duration [21], but the type of alcoholic beverages chosen and the pattern of drinking (weekend vs daily users) have no influence on the risk for development of chronic pancreatitis [22]. This concept, however, has been challenged [23].

In general, prolonged alcohol intake is required to produce symptomatic chronic pancreatitis (6–12 years), although the length of time required for histological damage in humans is unknown [24]. Clinically, alcoholic chronic pancreatitis is characterized by an initial period of recurrent acute attacks lasting 5–6 years, followed by the development of chronic pain, exocrine and endocrine insufficiency, or both. A natural history study [25] demonstrated a median time to exocrine insufficiency of 13 years in patients with alcoholic chronic pancreatitis and a median time to endocrine insufficiency of 20 years. Interestingly, not all patients with an acute episode of alcohol-induced pancreatitis progress to clinical chronic pancreatitis. It is generally accepted that at the time of the initial attack of pancreatitis, histological, chronic pancreatitis is already present. Two retrospective studies [26,27] reported that 15%–20% of patients who were followed for up to 30 years after an initial attack of alcoholic chronic pancreatitis did not develop pancreatic calcification or exocrine or endocrine insufficiency. The factors responsible for this variability in disease progression are not clear but do not appear to be caused by differences in avoidance of further alcohol. Patients with progressive disease were more likely to have more severe and more frequent acute clinical attacks of pancreatitis, and these patients frequently developed significant abnormalities affecting the main pancreatic duct.

A variety of studies provide evidence that one or more cofactors are necessary to sufficiently increase the risk (or lower the threshold) for alcohol to induce chronic pancreatitis. Kondo and colleagues [28] reported that the aberrant pancreas might not be susceptible to alcoholic pancreatitis. Anatomic differences in the aberrant pancreas, such as innervation and different ductal drainage, may explain the lack of damage after chronic alcohol ingestion. In addition, dietary factors may play a significant role. Both experimental and epidemiological studies indicate that the risk for alcohol-induced pancreatitis is higher in people with high-fat, high-protein diets. Severe focal lesions of chronic pancreatitis, however, were observed in rats fed higher amounts of fat, which resulted in striking potentiation of alcohol-induced pancreatic injury [29,30]. Observations from these experimental studies were supported by clinical and epidemiological studies. It has been observed in many European countries that alcoholics consuming a high-fat, high-protein diet are predisposed to chronic pancreatitis [6,31–33]. Durbec and Sarles [20] noted that the risk was less with average consumption of fat but was increased by both high (more than 100 g/day) and low (less than 85 g/day) daily fat intakes. Furthermore, the effects of alcohol, protein, and fat consumption appear to be additive. This may explain why chronic pancreatitis is more commonly observed in patients who drink more alcohol and eat more protein and fat than in controls [21]. Observations made in Europe, however, are not confirmed by studies reported from the United States and Australia [34–36]. It appears that whereas a high-fat or high-protein diet may predispose to pancreatitis, such a diet is not a prerequisite for the development of pancreatitis in chronic alcoholics. Differences in the quantity of alcohol and the type of fat consumed by various populations may explain some of these conflicting observations, but recent studies indicate that other factors may also play a role, including concurrent group B Coxsackie virus infection, genetic predisposition, and cigarette smoking.

Group B Coxsackie virus infection is a proposed and underexplored cofactor for alcoholic chronic pancreatitis. It is well known that Coxsackie virus causes necrotizing pancreatitis in humans. Experimental studies show that the virus causes acute pancreatitis in a virus titer-dependent fashion [37,38], which may progress to chronic pancreatitis when there is a lack of tissue repair, a defect associated with preferential activation of M1 macrophages and T helper type 1 cells rather than M2 macrophages and T helper type 2 cells [39]. Further, short-term (5–14 days) and subchronic (> 28 days) administration of oral ethanol potentiates virus-induced pancreatitis and impairs pancreatic regeneration following injury in a dose- and duration-dependent fashion [38]. Alcohol

may modify rather than trigger acute pancreatic injury, possibly by increasing tissue virus infection or replication, impairing tissue healing, and promoting chronic injury. Further research is needed to determine how group B Coxsackie virus infection occurs and increases the risk of clinical alcoholic chronic pancreatitis.

A strong genetic susceptibility factor for alcoholic chronic pancreatitis has not been identified [40], but information about the association between mutations of the serine protease inhibitor, Kazal type 1 (*SPINK1*) gene and alcoholic pancreatitis is conflicting [41–44]. The association of cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations with alcoholic pancreatitis is uncertain; it is absent in several large studies [45–47] and present in 23%–40% of patients in large Italian [48], Spanish [49], and Japanese [50] studies. The latter three studies raise the possibilities that an increased frequency of *CFTR* mutations occurs in patients with idiopathic and alcoholic chronic pancreatitis, that there may be regional genetic differences in patients with alcoholic chronic pancreatitis, and, as reported by Casals and colleagues [49], that different *CFTR* mutational spectra may be present in patients with alcoholic vs idiopathic chronic pancreatitis. The Japanese study noted the rarity of cystic fibrosis in Japan (1 per 350 000 compared with 1 per 2500 in Western countries). In support of these data, Naruse and colleagues [51] showed in a pilot study that *CFTR* dysfunction (indicated by the finger sweat test) was present in 52% of patients with chronic pancreatitis (21 alcoholic and 4 idiopathic) compared with 16% of control subjects. It is not known whether mutations in the *CFTR* gene are associated with alcoholic chronic pancreatitis and whether the simple finger sweat test is clinically useful to determine *CFTR* dysfunction in patients with pancreatitis.

The pathogenic mechanisms of alcohol-induced chronic pancreatitis are uncertain. Results from a prospective clinicopathological study of alcohol-induced recurrent attacks of acute pancreatitis evolving to chronic pancreatitis [52] support the hypothesis of the necrosis–fibrosis sequence, originally proposed in 1946 by Comfort and associates [53]. Chronic alcoholism causes an increase in basal secretion of proteases, amylase, and lipase, and a decrease in trypsin inhibitor in rats [54]. Furthermore, an increased responsiveness of the pancreas to cholecystokinin (CCK) stimulation has been reported in dogs [55] and humans [56].

The augmented responsiveness of the exocrine pancreas may account for the high-protein, low-bicarbonate, and low-volume pancreatic secretory output after chronic alcohol consumption [57]. Theoretically, protein precipitates would be formed, particularly in areas of low flow rate such as the secondary pancreatic ducts. This may explain the frequent involvement of the secondary duct, with relative sparing of the main pancreatic duct, in early stages of chronic pancreatitis.

The discovery of a pancreatic stone protein (lithostatin) found in pancreatic juice and calculi has generated considerable interest because this protein is capable of inhibiting the formation of insoluble calcium salts in a supersaturated milieu [58,59]. Lithostatin is decreased in the pancreatic juice of patients with alcoholic pancreatitis and in some patients with nonalcoholic chronic pancreatitis [60]. Hence, it is conceivable that a deficiency of lithostatin may play an important role in the development of pancreatic calcification [58]. The importance of this protein remains unclear, however, because not all studies have shown a reduction in lithostatin in pancreatic juice from patients with chronic pancreatitis [61].

One report [62] showed that GP2, a protein found on the zymogen membrane that is secreted into the pancreatic duct by exocytosis, is a reproducible integral component of the protein plugs found in patients with noncalcific, chronic pancreatitis. On the other hand, lithostatin was found less frequently and in variable amounts. Interestingly, GP2 has significant homology with the urinary protein uromodulin (Tamm–Horsfall protein), which is involved in the formation of protein precipitates in urine. It has been suggested that GP2 may be involved in the formation of protein plugs in the pancreatic duct in patients with chronic pancreatitis.

In addition to hypersecretion of protein, the viscosity of pancreatic juice is enhanced in chronic pancreatitis because of a higher concentration of hexosamine [63]. There is a correlation between the concentrations of protein, hydrolytic enzymes, hexosamine, and viscosity [63]. Hyperviscosity of pancreatic juice may result in decreased flow and contribute to protein precipitation in chronic pancreatitis. Therefore, it appears that chronic alcoholism results in a number of changes in the pancreatic juice that create an environment that is conducive to the formation of intraductal protein plugs that block small ductules in a random fashion. Ductal blockade produces progressive structural abnormalities in the ducts and acinar tissue. In time, calcium is complexed to protein plugs, initially in small ductules and, eventually, in the main pancreatic duct. Progressive blockade results in injury and destruction of pancreatic tissue [64].

In addition to the perturbation in the content and secretion of pancreatic enzymes, chronic alcohol consumption also causes perturbation in the lysosomal enzymes; acid phosphatase was increased in cell fractions, and cathepsin B was increased in the mitochondrial–lysosomal fraction and the zymogen granule fractions of the pancreas from alcohol-fed rats [65]. Therefore, it is conceivable that alcohol produces pancreatitis by interfering with the intracellular transport and discharge of digestive enzyme, causing colocalization of digestive and lysosomal hydrolases, a condition that is conducive to the initiation of autodigestion. As evidence, in vitro studies showed that alcohol increased the sensitivity for intraacinar trypsinogen activation induced by cerulein hyperstimulation [66]. Furthermore, in vivo experimental acute pancreatitis studies showed that alcohol sensitizes rats to pancreatitis caused by CCK-8 and was associated

with upregulation of proinflammatory cytokines and chemokines [67]. In an experimental model leading to chronic pancreatitis, Deng and colleagues [68] confirmed not only the potentiating effect of alcohol (Lieber–DeCarli protocol) on CCK-8-induced acute pancreatic injury in rats but also that the onset of pancreatic fibrosis was accelerated by both ethanol exposure and the increased severity and duration of the pancreatitis trigger. These findings support the concept that ethanol plays a modifying rather than a triggering role in pancreatitis and that acute injury with persistent inflammation fosters fibrogenesis.

The biochemical mechanisms responsible for the occurrence of fibrosis in chronic pancreatitis are also unclear. Recent evidence suggests that pancreatic stellate cells (PSCs) undergo activation in response to fibrogenic stimuli or tissue injury and play a central role in pancreatic fibrosis and tissue restitution [69–71]. PSCs are perivascular [72] and derived from vitamin A-containing cells that can acquire a myofibroblast phenotype [69,73]. These cells may be activated by transforming growth factor-β1 (TGB-β1), basic fibroblast growth factor and fibrogenic cytokines (e.g., TGF-β1, tumor necrosis factor-α, and interleukins 1 and 6) [69,74]. Furthermore, ethanol and the oxidative metabolite acetaldehyde (and oxidative stress) may directly activate PSCs [74], and the nonoxidative ethanol metabolites, the fatty acid ethyl esters, inhibit degradation of the extracellular matrix [75]. Activated PSCs secrete extracellular matrix proteins leading to tissue restitution or fibrosis depending on whether the duration of signaling by profibrotic growth factors and cytokines is a self-limited process (e.g., following an acute injury) or a perpetual process [71,76].

Intracellular or paracrine signaling pathways may be potential therapeutic targets to inhibit pancreatic fibrogenesis. General strategies directed towards PSCs may include inhibiting/inactivating PSCs, eliminating PSCs by inducing apoptosis [77] – which is an important step in wound healing [78] – or providing PSCs that have a restitutive phenotype with the ability to phagocytose inflammatory cells and reduce inflammation [79]. Major proteins of therapeutic interest as targets are the mitogenic peptides TGF-α and TGF-β, which are involved in cellular proliferation and differentiation. The central fibrogenic role for TGF-β was illustrated by showing that conditional loss of TGF-β signaling in mouse pancreas prevents fibrogenesis [80]. Furthermore, transgenic mice that overexpress TGF-α develop interstitial pancreatic fibrosis. Human studies demonstrated high levels of TGF-α and its messenger RNA in pancreatic tissue from patients with chronic pancreatitis, coupled with high levels of epidermal growth factor receptor [81], the receptor through which TGF-α acts. Another study [82] demonstrated overexpression of acidic and basic fibroblast growth factors in tissue from patients with chronic pancreatitis. Paracrine signaling loops between immune cells and PSCs likely perpetuate inflammation and PSC stimulation, as demonstrated by the finding that PSCs inhibit lymphocyte apoptosis by secreting interleukin-15 and in turn lymphocytes secrete PSC-activating inflammatory cytokines [83]. Although these observations are intriguing, it remains to be determined whether these abnormalities are contributing to the progression of disease or are merely a result of the disease process.

Cigarette smoking

An increasing number of studies indicate that cigarette smoking increases the risk and accelerates the progression of pancreatitis. Specifically, cigarette smoking increases the risk of developing acute [84,85] and chronic [84–88] alcoholic pancreatitis, and idiopathic chronic pancreatitis [85]. Lin and associates [88] reported that the odds ratio (95% confidence intervals) for chronic pancreatitis for smokers compared with nonsmokers, after adjustment for body mass index, education level, and alcohol consumption, was 7.8 (2.2–27.3) for all smokers, and greater risk was observed for those who had smoked for 25 years or more. The risk for chronic pancreatitis significantly increased with increasing cumulative amount of smoking. Other studies showed that cigarette smoking increases the risk for pancreatic calcification of late-onset but not of early-onset idiopathic chronic pancreatitis [89]. Furthermore, smoking is associated with earlier onset of alcoholic chronic pancreatitis [87] and accelerated progression of both alcoholic chronic pancreatitis [86] and late-onset, but not early-onset, idiopathic chronic pancreatitis [90]. An important interaction among calcification, smoking, and alcohol ingestion is that calcifications occurred more frequently in smokers and correlated with the amount and duration of drinking [20,91,92].

Based on these and other studies, the role of smoking in the causation of chronic pancreatitis in the absence of alcoholism is strongly suspected but not conclusively proved. However, it appears that smoking enhances the progression of chronic pancreatitis, especially of calcification. In hereditary pancreatitis, smoking increases the risk for pancreatic cancer and lowers the age at onset by about 20 years [93]. Finally, exposure to cigarette smoking and, probably, alcohol consumption contribute to cardiovascular disease, which is the leading cause of death in patients with alcoholic chronic pancreatitis [25]. Hence, patients with chronic pancreatitis, regardless of the cause, should stop smoking.

Tropical (nutritional) pancreatitis

Tropical chronic pancreatitis, one of the major nutritional forms of chronic pancreatitis, is an important disease among juveniles and young adults in some African and Asian countries (Indonesia, southern India, and tropical Africa) [94,95]. The natural history of tropical chronic pancreatitis is succinctly summarized by Gee Varghese [95] as "recurrent abdominal pain in childhood, diabetes around puberty, and death at the prime of life." Abdominal pain characterizes the onset of tropical pancreatitis. The onset of diabetes typically

occurs a few years after the onset of abdominal pain. Diabetes is characteristically brittle, with marked fluctuations of blood glucose. At this time, abdominal radiographs invariably show diffuse pancreatic calculi. Microscopically, dilation of the ducts, pancreatic lithiasis, chronic inflammatory cell infiltration, and atrophy of the pancreatic parenchyma are seen.

The etiology of tropical pancreatitis is not clearly understood, but appears to be associated with both nutritional and genetic risk factors. In most the common denominator appears to be malnutrition [96]. Evidence suggests that other factors, such as toxic products in certain nutritional components (cassava), may be more important [97]. Cassava is consumed in large quantities by most poor people in many African and Asian countries. It contains 65 mg of toxic glycoside per 100 g. When glycosides react with gastric hydrochloric acid, hydrocyanic acid is liberated. The enzyme rhodanase acts on hydrocyanic acid, leading to thiocyanate production in the presence of adequate amounts of methionine and cysteine. Cyanogens impair a number of enzymes, including superoxide dismutase, an important scavenger of free radicals, which are proposed to cause cell injury [98,99]. Associated nutritional deficiencies, such as deficiencies of zinc, copper, and selenium, which are common in malnutrition, interfere with the detoxification of cyanogens. Thus, the pathogenesis of tropical pancreatitis may be partly explained by micronutrient and antioxidant deficiencies and by unopposed free radical injury secondary to dietary cyanogens.

Investigation of risk factors other than dietary elements is relevant for understanding the pathobiological mechanisms of tropical pancreatitis because the disease also occurs independently of cassava consumption and in people without suspicion of malnutrition. Variable familial clustering of the disease further raised suspicions of a genetic defect. Previous studies showed no association of tropical pancreatitis with gene mutations for *CFTR* [100], *reg1A101*, or *PRSS1* [102,103]. However, multiple concurrent investigations led to the detection of the serine protease inhibitor, Kazal type 1 (*SPINK1*) gene mutation at residue N34S in 20%–55% of patients with tropical pancreatitis [104–109], compared to 10%–14% in early-onset type II (non-insulin-dependent) diabetes mellitus [106,109], and 1.3%–5.6% in control subjects [106–109]. Conceptually, mutations in the *SPINK1* gene impair the normal functioning of trypsin inhibitor, a safety mechanism for preventing pancreatitis by inhibiting activated trypsin within acinar cells. However, unlike in hereditary pancreatitis (discussed below), most clinical studies support the idea that the *SPINK1* missense mutation in codon N34S is a disease modifier rather than a cause of pancreatitis [110] because the prevalence of N34S *SPINK1* gene mutations (~ 2%) is far greater than the prevalence of chronic pancreatitis (0.006%) [110] and the pancreatitis phenotype is no different with either homozygous or heterozygous N34S *SPINK1* gene mutations [107,111]. Hence additive risk arising from multiple rather than single factors is likely responsible for tropical pancreatitis, including dietary, genetic, and, perhaps, other risk factors.

Hereditary pancreatitis

The clinical picture of hereditary pancreatitis was well described in 1952 by Comfort and Steinberg [112]. Since then, many similar cases have been reported, primarily in Caucasians from different areas of the world (United States, Ireland, France, and New Zealand), but also in some Japanese families [113]. It is an autosomal dominant disorder with 80%–96% penetrance and variable expression that begins in childhood [114,115]. The incidence is about equal in both sexes [116]. Between 52% and 81% of patients with hereditary pancreatitis have mutations in the gene for cationic trypsinogen (*PRSS1*) [114,115,117]. LeBodic and colleagues [118] from France, and Whitcomb and associates [119] from the United States, mapped the gene to the long arm of chromosome 7 (7q55). Subsequently, by positional cloning, Whitcomb and colleagues [117,120] identified two genetic mutations in the cationic trypsinogen gene in hereditary pancreatitis – a missense mutation R117H on exon 3117 and a mutation in exon 2 of the gene (N21I) [120] – in relatives without the R117H mutation. It was hypothesized that cleavage at these sites of the trypsinogen molecule is part of a failsafe mechanism to inactivate trypsin within the pancreas. Gene mutations result in failure of cleavage and persistent tryptic activity, causing autodigestion of the pancreas and pancreatitis. These mutations have since been described in families with pancreatitis around the world [121–125]. They represent the causative mutation in about two-thirds of the families with classic hereditary pancreatitis and are the only mutations for which genetic testing is recommended.

In addition to the typical hereditary form described by Comfort and Steinberg, which begins at a young age, familial aggregations of two or three cases, with attacks beginning in the third or fourth decade [126], have been reported. This suggests that there may be different forms of hereditary transmission. Mutations at codons 16 [127], 22 [128], and 23 [123] in the trypsinogen gene, although significantly associated with chronic pancreatitis, have a low penetrance. These mutations may be present in patients with a diagnosis of sporadic idiopathic pancreatitis. However, trypsinogen gene mutations are not the cause of tropical pancreatitis, even if many family members are affected [102].

The clinical picture of hereditary pancreatitis differs little from that of nonhereditary pancreatitis [116] and carries a high risk for pancreatic cancer [129]. Patients frequently experience recurrent attacks of severe upper abdominal pain. Overt diabetes develops 8–10 years after the onset of pain in 20% of cases, and gross steatorrhea in 15%–20%. The diagnosis of hereditary pancreatitis should be suspected if two or more family members have pancreatitis in the absence of alcohol consumption or other causes of chronic pancreatitis. This criterion may be too stringent [113] based

on reports of "sporadic" cases of hereditary pancreatitis [113,123,127,128,130] and because up to 11% of mutation-positive individuals may be silent carriers [115]. Indeed, variances in the application of diagnostic criteria to clinical studies will influence a study's outcome. For example, in a European cohort of 112 hereditary pancreatitis families identified by these "strict" criteria [114], 81% had mutations of R122H, N29I, or A16V, compared to 51% reported previously [115] and 96% had disease penetrance, compared to 80% [115]. Similar findings in the two studies are the life-time pancreatic cancer risk of 44% [114] compared with 40% [115]. Moreover, cigarette smoking accelerates the onset of pancreatic cancer, which occurs 14 years earlier in smokers than innonsmokers [131]. Genotype/phenotype analyses indicate that individuals with the more common R122H mutation presented 4–5 years earlier than persons with a mutation in N29I or with no identifiable mutations [114] and were more frequently hospitalized than the N29I mutation patients [114]. Conversely, patients with N29I mutations were treated at an earlier age with pancreatic resection for pain [114]. Hence, in contrast to a previous report [120], the data are conflicting on whether the R122H mutation increases the risk for more aggressive disease [114,120]. As a result of the high disease penetrance and markedly increased life-time risk of pancreatic cancer, current guidelines favor genetic screening for *PRSS1* gene mutations when hereditary pancreatitis is suspected [132].

Hyperparathyroidism

Calcified chronic pancreatitis occurs in untreated hyperparathyroidism. Surveys suggest that the incidence is decreasing and is no greater than 1%–2% [133]. A likely explanation is that serum calcium is measured routinely in virtually all patients who undergo a medical checkup, and, as a result, hyperparathyroidism does not remain undiscovered and untreated for many years. The pathogenesis of pancreatitis in hyperparathyroidism is presumed to be related to the effect of hypercalcemia. Acute hypercalcemia is a potent stimulus of human pancreatic enzyme secretion [134,135]. Furthermore, chronic hypercalcemia causes a significant increase in pancreatic calcium secretion in patients with hyperparathyroidism and in experimental animals. This results in precipitation of intraductal calcium in pancreatic juices. Moreover, chronic hypercalcemia causes a decrease in the diffusion barrier between the pancreatic interstitial compartment and the ductal system, resulting in excessive diffusion of calcium ions into the pancreatic juices. These events may damage the pancreas and promote the development of calcified chronic pancreatitis.

Hyperlipidemia

As discussed in Chapter 68, it is well known that hyperlipidemia can cause acute pancreatitis. After repeated episodes of acute pancreatitis, hyperlipidemia may, on rare occasions, cause chronic pancreatitis. It is important to consider this unusual cause because effective therapy is available. Glueck and associates [136] highlighted the risk for estrogen replacement in postmenopausal women with underlying hypertriglyceridemia. Twelve women receiving estrogen replacement were studied; 10 of these had fasting triglycerides greater than 1200 mg/dL while receiving estrogens, and six developed pancreatitis. Stopping estrogen replacement therapy and treatment of the underlying hypertriglyceridemia returned triglycerides to normal levels. Fasting triglyceride levels of less than 300 mg/dL pose no risk for estrogen-induced pancreatitis, whereas levels greater than 750 mg/dL are associated with a high risk. Therefore, it is important to measure fasting serum triglycerides before initiating estrogen replacement therapy in postmenopausal women to prevent acute pancreatitis and avoid the potential for chronic pancreatitis.

Autoimmune pancreatitis

Chronic pancreatitis may be caused by autoimmunity [137]. The prevalence of autoimmune pancreatitis is reported as 2%–8% in non-US populations [138,139]. Data from the United States indicate that autoimmune pancreatitis is mistaken for pancreatic cancer and is diagnosed at surgery in 2.5% of cases [138]. The number of publications per year on autoimmune pancreatitis increased exponentially in the last 6 years, reaching nearly 100 publications in 2006. Many of the first case series were reported in the Japanese literature followed by a multitude of publications from other countries leading to identification of different features or subsets of the disease (see Table 69.3) and to the generation of two sets of diagnostic criteria: the Mayo Clinic [140] HISORIt criteria (Histology, Imaging, Serology, Other organ involvement, Response to steroid therapy) and the revised Japanese Pancreas Society criteria [141]. Autoimmune pancreatitis typically affects the elderly (mean age 63–68 years) [140,142],

Table 69.3 Geographic variation in the clinical presentation of autoimmune pancreatitis

Clinical presentation	Mayo experience	Japanese experience
Jaundice (painless)	73%	86%
Abdominal pain (mild)	34%	
Mass	10%	
New-onset diabetes	~ 3%	41%
Steatorrhea	~ 3%	Uncommon
Pancreatitis	~ 3%	
Calcifications	Uncommon, ~ 50% of relapses	Uncommon, 50% of relapses

Data collected from Chari et al. [140] (Mayo Clinic) and from Takayama et al. [139], Kamisawa et al. [142] and Horiuchi et al. [143] (Japanese publications).

men (> 80% predominance) [140,142], and presents as painless jaundice (73%–86%) [139,140,142,143] followed by mild abdominal pain (34%) [140] or the appearance of a pancreatic mass lesion (10%) [140]. Uncommon initial presentations [139,140,142,143] include pancreatitis, steatorrhoea, and calcification. New-onset diabetes mellitus is reported to be rare in the Mayo Clinic series [140] but is common (41%) in the Japanese literature [139,142,143]. The major clinical presentation, obstructive jaundice, is caused by stenosis of the common bile duct, which is otherwise rare in other types of pancreatitis [144]. Steroid therapy is usually effective in alleviating the stenosis of the common bile duct as well as that of the pancreatic duct [145,146], providing a provocative example of a potentially reversible type of chronic pancreatitis.

Not only pancreatic core biopsy but also imaging studies, laboratory data, and, potentially, genetic profiling provide useful information for diagnosing autoimmune pancreatitis. In Japan, computed tomography (CT) and ultrasonography usually show a diffusely enlarged pancreas, with a sausage-like appearance and a capsule-like rim that is of low density on CT [141]. Initially, pancreatic calcification or pseudocyst is seldom observed, but calcifications occur in around 50% of patients who relapse following prednisone therapy [143]. Endoscopic retrograde cholangiopancreatography (ERCP) usually shows segmental or diffuse narrowing of the main pancreatic duct and extrinsic stenosis of the intrapancreatic portion of the common bile duct, resulting in dilation of the proximal biliary tract. The differential diagnosis of diffuse pancreatic enlargement includes malignant lymphoma, plasmacytoma, metastasis, and diffuse infiltrative pancreatic carcinoma. Definitive diagnosis by the Japanese criteria requires diffuse pancreatic enlargement and diffuse or segmental pancreatic duct narrowing plus either abnormal serological tests (described below) or suggestive histopathology [141]. In contrast, based on prospectively collected data from 59 patients at the Mayo Clinic, the HISORIt criteria (see Table 69.4) are less rigid and allow for the diagnosis to be made in one of three ways:

- histology
- imaging and serology
- serology, other organ involvement and response to steroid therapy [140].

Diagnostic histological findings most commonly show fibrotic changes with infiltration of lymphocytes and plasma cells and, sometimes, eosinophils around the pancreatic duct associated with obliterative phlebitis, cholangitis, and edema, labeled as lymphoplasmacytic sclerosing pancreatitis [147]. The lymphocytes are usually CD4+ and human leukocyte antigen (HLA) DR+ T cells [148–150]. The HLA-DR is also expressed on pancreatic duct cells [148,151]. A less common histological variant found in younger patients shows neutrophil predominant lobular inflammation without phlebitis, termed idiopathic duct centric chronic pancreatitis [152]. When pancreatic tissue specimens are not available, laboratory data and clinical history provide useful information that may increase the suspicion of autoimmune pancreatitis. Patients frequently have circulating autoantibodies (e.g., antinuclear antibody, antilactoferrin antibody, anticarbonic anydrase II antibody, rheumatoid factor, anti-smooth muscle antibody) and may have another autoimmune disease (Sjögren disease, primary sclerosing cholangitis, ulcerative colitis, systemic lupus erythematosus, primary biliary cirrhosis) [153]. A recent, inclusive characterization of the disease is that autoimmune pancreatitis is one manifestation of an immunoglobulin G4 (IgG4)-related autoimmune disease [138], because increased serum β-globulin or IgG4 levels are common and IgG4 deposition may be detected in a variety of tissues [138]. Reported IgG4 organ involvement includes sclerosing sialadenitis, sclerosing cholangitis, sclerosing cholecystitis, retroperitoneal fibrosis, joint involvement in patients with rheumatoid arthritis and amyloidosis, gastrointestinal tract mucosal deposition, bone marrow involvement in myelodysplastic syndrome, inflammatory pseudotumors of the spleen, lung and liver, lymphadenopathy, tubulointerstitial nephritis, and interstitial pneumonia [154–164]. A genetic basis of autoimmune pancreatitis is under investigation and indicates that up to 33% of patients have *SPINK1*

Table 69.4 Diagnosis of autoimmune pancreatitis by the Mayo Clinic HISORIt Criteria: one of three combinations of data are diagnostic

	H	I	S	O	Rt
	Histology	**Imaging (CT):**	**Serology elevated serum IgG4**	**Other organ involvement**	**Response to steroids therapy**
Group A: (n = 29)	X				
Group B: (n = 3)		X	X		
Group C: (n = 17)			X or X		X

Modified from Chari et al. [140], with permission from Elsevier.

gene mutations [43], which are associated also with idiopathic and tropical pancreatitis, and some patients have a DRB1*0405-DBQ*0401 haplotype [165]. The large number of epidemiological, histopathological, and genetic studies on autoimmune pancreatitis have provided further understanding of subgroups of patients who were formerly classified as having late-onset but also early-onset idiopathic chronic pancreatitis [166].

Obstruction

Obstruction of the main pancreatic duct by tumors, benign vaterian stenosis [167], scars (e.g., from traumatic pancreatitis), and pseudocysts [168] can lead to a distinct form of chronic pancreatitis known as *obstructive chronic pancreatitis*. This is characterized by acinar atrophy and fibrosis and dilation of the ductal system. In contrast to alcohol-induced chronic pancreatitis, intraductal plugs or stones are very rare in obstructive chronic pancreatitis, and both structural and functional changes may improve when the obstruction is relieved [167]. The regression of fibrotic lesions may be explained by the finding that fibrosis in experimental obstructive pancreatitis consists of fractions of collagen with short half-lives (fibronectin, laminin, collagen III, and procollagen III) rather than the long-lived fraction collagen I [169].

Trauma

Trauma to the abdomen or back may be clinically insignificant and still produce significant pancreatic injury, leading to chronic pancreatitis. The pathogenesis may follow that of the obstructive type, but inflammation and pseudocysts frequently develop. Recognition of trauma as the cause for chronic pancreatitis is important because most cases are associated with severe ductal disruption, which responds poorly to medical treatment, but the results of surgical correction (particularly partial pancreatectomy) have been excellent [170,171].

Idiopathic chronic pancreatitis

The major form of nonalcoholic chronic pancreatitis in North America and Europe is the idiopathic type (10%–40%). Epidemiological and clinical studies suggest that idiopathic chronic pancreatitis may be divided into two distinct groups: a juvenile type, with onset of symptomatic disease at a median age of 18 years; and a senile type, with an incidence peak at 60 years of age. In juvenile chronic pancreatitis, abdominal pain dominates the clinical picture at initial presentation, whereas in senile groups, painless disease is frequent, and most patients present with exocrine insufficiency, diabetes, and pancreatic calcification [172].

Landmark genetic studies indicate that *CFTR* gene mutations are linked to some patients with chronic pancreatitis. Sharer and colleagues [46] searched for 22 *CFTR* mutations in 134 patients who had idiopathic chronic pancreatitis or chronic pancreatitis as the result of alcoholism, hyperparathyroidism, or hypertriglyceridemia. In these patients, *CFTR* mutations were seen at 2.5 times the expected frequency, and the frequency of the 5T allele (which is associated with a reduced proportion of functional *CFTR*) was twice the expected frequency. In a separate study, Cohn and associates [173] found that 10 of 27 patients with idiopathic chronic pancreatitis had at least one of 17 *CFTR* mutations; both alleles were affected in three patients. The 5T allele was present in 5 of 27 patients; this was twice the expected frequency. Hence, it is intriguing that at least a subset of patients who would be classified as having idiopathic chronic pancreatitis has *CFTR* mutations. Subsequent genetic studies show that the true prevalence of *CFTR* mutations is approximately 50% in idiopathic pancreatitis compared with 22% in controls when exhaustive gene identification methods are performed [174]. Genetic studies also provide evidence of mutations other than *CFTR* in early-onset idiopathic chronic pancreatitis; around 50% of patients formerly classed as having early-onset idiopathic chronic pancreatitis have *SPINK1* [41,43,175–177] (see section on tropical pancreatitis) or *CFTR* [46,173,176,178] gene mutations. The functional significance and clinical relevance of these mutations on the pancreas are unclear, but most patients in the above studies did not have classic cystic fibrosis. They all had normal sweat chloride test results and nasal potential difference. Cohn and associates, in a review, stated, "at present, the role of *CFTR* mutation testing in idiopathic chronic pancreatitis is uncertain because no guidelines exist for genetic counseling or altered clinical management of idiopathic chronic pancreatitis based on the results of such testing," [179].

Pancreas divisum

Pancreas divisum is the most common congenital abnormality of the pancreas, comprising in 4%–24% [180]. Fusion of the dorsal and ventral pancreatic ducts is absent or incomplete, and drainage of the major portion of the pancreas occurs through the minor papilla (see Chapter 67). The clinical significance of pancreas divisum is unknown, although considerable controversy surrounds this issue [180].

A central point of contention is whether the incidence of idiopathic pancreatitis, particularly idiopathic recurrent acute pancreatitis, is increased in subjects with pancreas divisum, a question raised almost exclusively by endoscopic studies. Earlier endoscopic studies by Cotton [181], Sahel [182], and Richter [183] and their colleagues indicated that the prevalence of pancreas divisum was increased in idiopathic pancreatitis but this was contradicted by later studies by Burtin [184], Delhaye [185], and Sugawa [186] and their associates. To explain the differences between these two groups of studies, it is possible that the results from the earlier studies [181–183] might be biased by patient selection and that the patients had poorly characterized pancreatic diseases. Authors use different terminology for pancreatitis and likely enter different subsets of patients into their studies.

Finally, patients with pancreas divisum and patients with idiopathic pancreatitis are likely to be referred to centers with exceptional endoscopic skills, and, as a result, the incidence of pancreas divisum may appear to be increased, whereas such an increase is not present in a more random sampling of patients.

The prevalence of pancreas divisum in idiopathic pancreatitis and the general population was clarified by a comprehensive bi-part analysis of data published as a clinical controversy topic [180]. Prevalence data for pancreatitis in pancreas divisum was determined for 28 803 patients from 77 studies – 23 autopsies [187–209], 41 ERCP studies [181–186,209–243], and 13 magnetic resonance cholangiopancreatography (MRCP) studies [235,244–255] – and categorized according to the following terms: no pancreatitis, idiopathic pancreatitis, and acute pancreatitis and chronic pancreatitis when sufficient detail supported this designation [180]. Whereas autopsy and MRCP studies provide overwhelming evidence that the prevalence of pancreas divisum in the general population is around 8%, ERCP studies report a prevalence of approximately 4% for pancreas divisum in patients idiopathic pancreatitis (general population) and about 8% prevalence in patients with idiopathic pancreatitis (see Fig. 69.1). Hence, there does not appear to be an association between pancreas divisum and idiopathic pancreatitis and the increased frequency of pancreas divisum reported in some endoscopic studies is likely attributable to underrecognition of pancreas divisum in the general population or referral bias.

Many patients with idiopathic pancreatitis and pancreas divisum are commonly mislabeled as having idiopathic recurrent acute pancreatitis. The data from Choudari and colleagues [256] indicate that in patients with idiopathic pancreatitis and pancreas divisum, predisposing factors (e.g., *CFTR* gene mutations) other than pancreas divisum are necessary for pancreatitis to develop [46]. It has been hypothesized that the opening of the lesser papilla might be too small to permit the free flow of pancreatic juice into the duodenum, and, as a result, pancreas divisum might cause a form of obstructive pancreatitis. Indeed, the results of an uncontrolled surgical study showed that reduction in pancreatic duct and sphincter pressures following sphincteroplasty predicted a good outcome [257]. These findings should be interpreted cautiously because as was pointed out, "... diagnostic tests for assessing the presence of functional obstruction are not specific (secretin induced duct dilatation detected by ultrasound/MRCP is 'abnormal' in 50% of normal controls

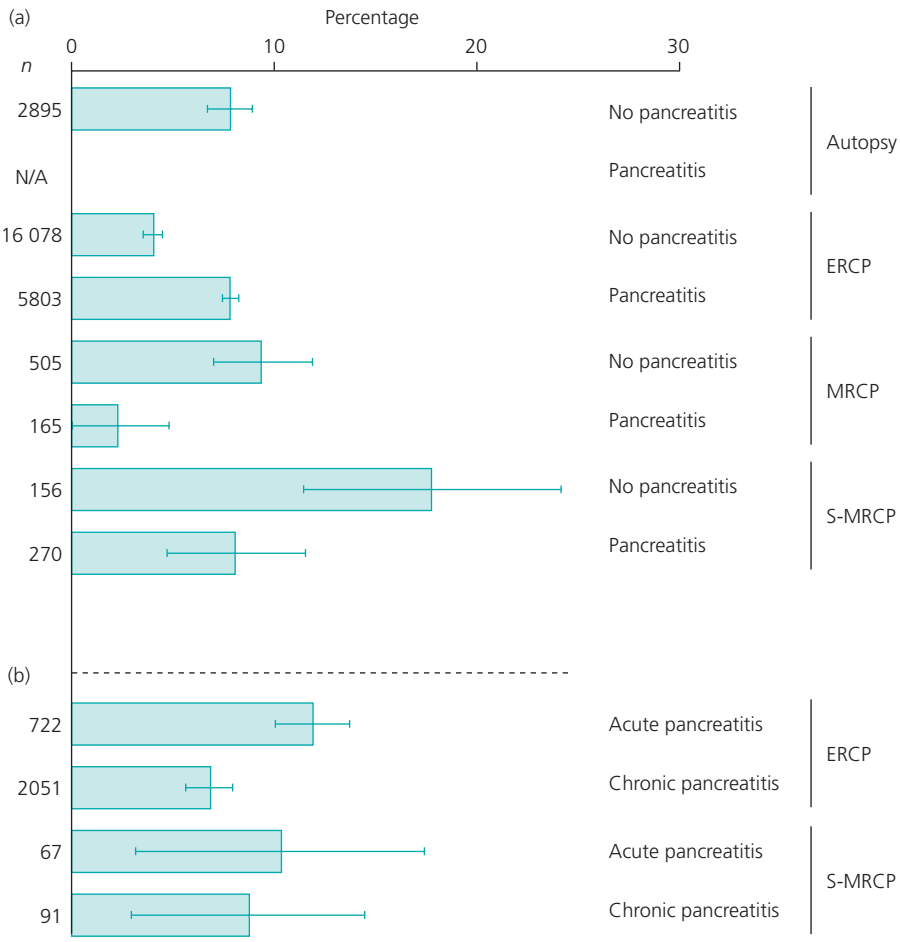

Figure 69.1 Prevalence of pancreas divisum. Bars represent mean pancreas divisum as % total cases (*n*) and error bars are 95% confidence intervals. Panel **3(a)** is the prevalence of pancreas divisum without or with pancreatitis reported in autopsy, endoscopic retrograde cholangiopancreatography (ERCP) and secretin-magnetic resonance cholangiopancreatography (S-MRCP) studies. Panel **3(b)** is the prevalence of pancreas divisum with acute and chronic pancreatitis reported in ERCP and S-MRCP studies. Adapted from Fogel et al. [180], with permission from Lippincott Williams and Wilkins.

[258] and does not differ in patients with or without pancreas divisum [249,250]), or results of standardized tests (sphincter manometry) do not correlate with symptoms or predict response to intervention [180,259]."

Gelrud and colleagues [260] further illustrated that endoscopic therapy has unclear effectiveness for treating pain in those with idiopathic pancreatitis, pancreas divisum and CFTR dysfunction. Gelrud and colleagues [260] substantiated the clinical association of aberrant CFTR function with idiopathic pancreatitis and pancreas divisum and reported that only 2 of 12 patients had resolution of symptoms even though all had 2–5 ERCPs, 10 of the 12 had endotherapy (stent/sphincterotomy), and 8 of the 12 had a cholecystectomy. Although the therapeutic component of this study was not randomized or controlled, the results do not endorse invasive therapy.

Clinical presentation

Pain

The presenting symptom of most patients with chronic pancreatitis is abdominal pain. The pain is usually epigastric, dull rather than sharp, and constant rather than colicky. Although the pain may radiate to both upper quadrants and, occasionally, to the lower quadrants, a characteristic feature is radiation directly through to the back. The pain is partially relieved by sitting with the trunk bent forward or lying prone; the supine posture aggravates the discomfort. The almost instantaneous aggravation of pain by food ingestion is characteristic of chronic pancreatitis or carcinoma of the pancreas, and this symptom should always raise the suspicion of pancreatic disease. Although food ingestion may aggravate the pain of other abdominal conditions (i.e., irritable bowel syndrome), there is usually a much greater interval between the meal and the discomfort than is the case with pancreatic disease. Ingestion of alcohol may also aggravate the pain, although a sizable fraction of patients claim that the pain develops after 12–24 h of abstinence [261].

Pain patterns may differ depending on the type of chronic pancreatitis [25]. At the beginning of disease, pain is present in 75% of patients with alcoholic chronic pancreatitis, 50% of patients with late-onset idiopathic chronic pancreatitis, and nearly all patients with early-onset idiopathic chronic pancreatitis. Pain tends to be less severe in the late-onset idiopathic group.

A prospective longitudinal study of alcoholic chronic pancreatitis identified two major patterns of pain. Ammann and Muellhaupt [262] reported that 44% of the patients had short episodes of pain, type A pain – defined as short episodes of usually less than 10 days' duration, separated by long pain-free intervals of months to years. The patients with mild to moderate chronic pancreatitis rarely had constant pain, so none of these patients underwent surgery for pain relief. By contrast, 56% of patients had episodes of constant daily pain, type B pain – defined as occurring on 2 or more days per week for at least 2 months. Importantly, most patients with constant (type B) pain had local complications such as pancreatic pseudocysts or obstructive cholestasis that were amenable to surgical correction. It is important to emphasize that the above study only evaluated alcoholic chronic pancreatitis patients who were not addicted to narcotics. Therefore, these findings are not applicable to patients who are addicted to narcotics and who have any form of chronic pancreatitis.

To address the limitations of the above study, a long-term natural history study described pain patterns that are common or unique to alcoholic and non-alcoholic chronic pancreatitis [90] based on a mixed cohort of patients. Ammann's group [90] emphasized that the natural history of chronic pancreatitis differs among etiologies. They corroborated and extended the data from a previous study [25], by reporting a "significantly delayed progression of exocrine insufficiency and calcification from onset in IJCP (juvenile or early-onset idiopathic chronic pancreatitis)/HP (hereditary pancreatitis) compared to alcoholic chronic pancreatitis (alcoholic)/ISCP (senile or late-onset idiopathic chronic pancreatitis)." A major finding was that type A pain (see above) was the predominant pain pattern in senile idiopathic chronic pancreatitis and hereditary pancreatitis and was managed without invasive procedures (surgery). Type B pain (see above) occurred in approximately 60% of alcoholic chronic pancreatitis and juvenile idiopathic chronic pancreatitis, was predominantly associated with local complications, and required surgery. These data have important negative implications for invasive therapies in chronic pancreatitis patients without local complications.

Pain in chronic pancreatitis may continue, diminish, or disappear completely. Several reports have suggested that pain may disappear as the severity of pancreatitis increases [25,53,263–265]. According to Ammann [265], pain disappears coincident with the appearance of calcifications, steatorrhea, and diabetes, which occurs 5–8 years after the onset of chronic pancreatitis. With different etiologies for chronic pancreatitis, the disappearance of pain occurs at a similar frequency but over variable time spans; pain decreased/disappeared in early-onset idiopathic chronic pancreatitis, late-onset idiopathic chronic pancreatitis, and alcoholic chronic pancreatitis in 67%, 64%, and 77% of patients over a mean duration of 27 ± 3, 13 ± 2, and 14 ± 1 years, respectively [25]. Chronic pancreatitis is painless or relatively painless (i.e., insufficient pain to require medical evaluation) in about 15% of patients [266]. Idiopathic pancreatitis is more likely to be painless than the alcoholic variety. Most studies report that pain diminishes/disappears over time, and at least one study reported that the frequency of pain relief was similar in those who had or did not have surgery [25,267] so the recommendation for offering invasive therapies to patients with chronic pancreatitis, particularly type A pain,

should be carefully weighed against the natural history of pain in this disease.

Weight loss

Nausea, vomiting, anorexia, and weight loss are common in chronic pancreatitis. The major cause of weight loss is decreased caloric intake owing to fear of aggravation of the abdominal pain, although malabsorption or uncontrolled diabetes may also play a role. Marked weight loss is relatively unusual in other painful abdominal conditions, such as peptic ulcer or irritable bowel syndrome, and the combination of chronic upper abdominal pain and rapid loss of weight should always suggest the possibility of pancreatic disease.

Malabsorption

Diarrhea, steatorrhea, and azotorrhea occur when exocrine secretion of pancreatic enzymes is insufficient to maintain normal digestion. Although most attention has been directed to the malabsorption of fat and protein in this condition, studies using hydrogen breath tests have also demonstrated malabsorption of starch [268].

Malabsorption does not occur until enzyme secretion is reduced to less than 10% of normal [269,270] so diarrhea and steatorrhea occur relatively late in the course of chronic pancreatitis. As a rule, fecal weight is less in pancreatic malabsorption than in other conditions with comparable steatorrhea. This relatively low fecal weight reflects less fecal water, and patients may pass bulky, formed stool, as opposed to the frank watery diarrhea observed in other conditions. The relatively low fecal water in pancreatic insufficiency probably results from the better absorption of some carbohydrates, such as disaccharides, that occurs in conditions with mucosal pathology, such as celiac sprue. Occasionally, the patient observes that gross oil leaks from the rectum or floats as an "oil slick" on the surface of the water in the toilet bowel. If ingestion of mineral oil is excluded, this finding is usually indicative of pancreatic steatorrhea. For a given degree of steatorrhea, the absorption of fat-soluble vitamins (A, D, E, and K) is much better in pancreatic insufficiency than in celiac sprue [271]. Marked deficiency of these vitamins is seldom observed in pancreatic insufficiency, and presumably this reflects the relative unimportance of lipolysis for the absorption of these compounds. The body stores of these vitamins, however, have been shown to be reduced in a large fraction of patients with chronic pancreatitis, although clinical manifestations of the deficiencies are rare [272].

Pancreatic diabetes

Glucose intolerance is common early in the course of chronic pancreatitis. Data are limited but in patients with mild to moderate alcoholic chronic pancreatitis an abnormal oral glucose tolerance test was present in 68% and hyperglycemia was found in 32% [273]. Clinically evident diabetes may be an initial manifestation of asymptomatic chronic pancreatitis [274], an observation known to clinicians, particularly in South Africa and India, but it more commonly occurs relatively late in the disease. Endocrine insufficiency eventually occurs in most patients with chronic pancreatitis. In a follow-up study of 500 patients with chronic pancreatitis, of whom 85% were alcoholic, it was reported that diabetes developed in 83% within 25 years after the clinical onset of chronic pancreatitis, and more than half required insulin treatment [275]. The prevalence of diabetes was similar in patients who underwent surgery and those who received medical treatment. However, for those who had pancreatic surgery, the prevalence of diabetes was significantly higher 5 years after distal pancreatectomy (57%) than after pancreaticoduodenectomy, pancreatic drainage, or cystic drainage (24%–36%). Pancreatic drainage did not prevent the onset of diabetes. Distal pancreatectomy and early onset of pancreatic calcifications were the only independent risk factors for diabetes.

Ketoacidosis and diabetic nephropathy are relatively uncommon in pancreatic diabetes. For a given duration of diabetes, however, retinopathy and neuropathy are thought to occur with a frequency similar to that in idiopathic diabetes mellitus [276].

Other clinical features

Other clinical presentations of chronic pancreatitis include jaundice, secondary to common bile duct compression by the pancreas; ascites or pleural effusion, caused by leak of pancreatic secretions from a ruptured duct or pseudocyst; painful nodules, usually over the lower extremities, resulting from fat necrosis; and, rarely, a polyarthritis of the small joints of the hands. In the United States, at least 70% of chronic pancreatitis is attributed to prolonged, heavy ingestion of ethanol, whereas the bulk of the remaining 30% is idiopathic. Historical information concerning length and duration of ethanol ingestion is therefore critical in all suspected cases of chronic pancreatitis. In contrast, gallstone disease is almost never the cause of chronic pancreatitis. A family history of pancreatitis or chronic abdominal pain of unknown origin may suggest the diagnosis of familial pancreatitis.

Physical examination is usually of limited assistance in the diagnosis of chronic pancreatitis. There is epigastric tenderness during the painful episodes (and sometimes during periods of remission). Tenderness ranges from mild to marked, but involuntary guarding and rebound are unusual. The physical signs characteristically are trivial relative to the intensity of the patient's complaints. Complications of chronic pancreatitis, such as pseudocysts, ascites, or pleural effusions, may be detected on physical examination.

Pathomechanism of symptoms

Abdominal pain and malabsorption are the major symptoms of chronic pancreatitis. The pathophysiological processes

responsible for the development of these symptoms are discussed in the following sections.

Pain

The mechanisms of pain in chronic pancreatitis are unclear. Possible causes include inflammation of the pancreas, increased intrapancreatic pressure, microvascular ischemia, neural inflammation, and extrapancreatic causes, such as common bile duct stenosis, duodenal stenosis, and pseudocysts.

Acute inflammation of the pancreas, during a relapsing attack of chronic pancreatitis or peripancreatic inflammation involving the duodenum and retroperitoneum, may cause pain. This may be mediated by inflammation or fibrosis. Cytokines such as interleukin-6 and interleukin-8 [277] and chemokines such as Mob-1 and tumor necrosis factor-α may be involved in inflammatory and fibrosis pathways. Whether fibrosis by itself produces pain is unclear, but in chronic pancreatitis the link between fibrosis and pain is probably related to pancreatic stellate cells, which are responsible for pancreatic fibrosis [278]. With their contractile potential and their perivascular location, these cells could cause microvascular ischemia and pain [278] when stimulated by growth factors [69].

Clinical and experimental evidence suggests that pain may also be related to increased intraductal pressure secondary to continued pancreatic secretion in the face of ductal obstruction caused by strictures or intraductal stones [279]. The increased pancreatic ductal and parenchymal pressure may also produce a compartment syndrome that induces ischemia and pain. This thesis is supported by experimental studies that showed increased interstitial and perfusion pressures and decreased blood flow in feline chronic pancreatitis [280,281]. These abnormalities were significantly reversed by surgical incision of the gland and draining of the pancreatic duct [281].

Several clinical studies showed that pain relief correlates with the development of pancreatic insufficiency in patients with alcohol-induced chronic pancreatitis [115,116]. Measurements of intraductal pressure have been made in patients without pancreatic disease and in those with ductal abnormalities. Among patients without pancreatic disease, intraductal pressure has been found to be 7 mmHg by direct puncture at surgery in one patient and between 10 and 16 mmHg by ERCP among 33 patients without pancreatic disease [282–285]. By comparison, in 59 patients with a dilated duct, intraductal pressure measured by direct puncture at surgery ranged from 18 to 48 mmHg [285]. In one study [282], all 19 patients with a dilated main pancreatic duct with elevated intraductal pressure had prompt relief of pain after decompression surgery. Pancreatic tissue pressure also appears to be elevated among patients with chronic pancreatitis with a dilated pancreatic duct and those with pseudocyst [279,286]. The pancreatic tissue pressure normalized after decompression of the duct or cyst, and this resulted in pain relief in 12 of the 14 patients. Hence, these observations support the thesis that pain in chronic pancreatitis is related to increased intraductal and pancreatic tissue pressure. This provides the rationale for treatments that may reduce pancreatic secretion, such as octreotide, specific diets, and pancreatic enzyme therapy.

Intrapancreatic neural inflammation is another factor that may play an important role in the genesis of pain in chronic pancreatitis. Morphological studies indicate that pancreatic nerves appear to be larger and more numerous in chronic pancreatitis [287]. The organization of intraneural organelles, such as microtubules, is disrupted. Most significantly, however, there is an alteration in the perineural sheath that ordinarily shields nerves from surrounding connective tissue [287]. The damaged perineurium allows penetration of biologically active materials from the surrounding extracellular matrix, and pain may result from the continual stimulation of the sensory fibers by noxious substances. In this regard, immunohistological studies have shown that the amount of neurotransmitters, such as substance P and calcitonin gene-related peptide (CGRP), are increased in afferent pancreatic nerves in human chronic pancreatitis [288,289]. Immune cell infiltration and growth-associated protein 43 also correlate with pain intensity [290]. There is also evidence that eosinophils are increased in the perineural space among patients with recent alcohol consumption [291]. It is conceivable that degranulation of these eosinophils might be a factor in the generation of pain.

Capsaicin-sensitive afferent sensory neurons are considered a common final pathway in neurogenic pancreatic inflammation, including plasma extravasation, neutrophil infiltration, and parenchymal necrosis [292,293]. Capsaicin-sensitive C fibers release the proinflammatory neuropeptides substance P, CGRP, and neurokinin A294 [295]. These neuropeptides have been shown to promote inflammation and activate the transient receptor potential vanilloid 1 (TRPV1) receptor, which releases substance P and CGRP from afferent neurons and mediates inflammatory pain in many tissues [296].

In chronic pancreatitis, several neuropeptides and downstream receptor signaling are thought to contribute to pain. First, nerve growth factor is overexpressed in the pancreas and substance P and CGRP are overexpressed in the dorsal root ganglion in rodents [297] and humans [298], including enlarged pancreatic nerves in humans [299]. Second, because painful compared with painless human chronic pancreatitis was associated with a 3.5-fold increase in mast cells in pancreatic tissue [300], the same group proposed that mast cell degranulation products such as nerve growth factor may activate or sensitize nociceptive (sensory) neurons by activating proalgesic receptors and channels such as tyrosine kinase A (TrkA) and receptor potential family V receptor (TRPV1). As evidence supporting this possibility, antagonism of TRPV1, CGRP, and substance P receptors may suppress

pain in acute pancreatitis. Further, disruption of "neurogenic signaling," specifically substance P and neurokinin I receptor signaling, inhibits the inflammatory response and reduces the severity of experimental acute pancreatitis [301–303], similar to effects in other tissues [304]. Whether these interventions may have the same effect on inflammation and pain in human chronic pancreatitis is not known.

As a seemingly paradoxical observation, stimulation of other neural pathways may have an antiinflammatory effect in pancreatitis [305]. In an experimental model of acute pancreatitis, disruption of CCK-stimulated, efferent vagal cholinergic, nicotinic receptor signaling (by left unilateral cervical vagotomy or inhibition of nicotinic receptor signaling with mecamylamine) had a proinflammatory effect on disease and increased its overall severity. Conversely, agonism of this pathway with the selective α7 nicotinic receptor agonist GTS-21 had antiinflammatory effects on murine cerulein-induced pancreatitis and reduced its severity. The authors attributed these observations to efferent vagus neurotransmitter release of acetylcholine, interacting with nicotinic α7 acetylcholine receptors present on macrophages and neutrophils, resulting in the suppression of inflammatory mediators.

There is no clear explanation for the findings that capsaicin-sensitive vagal afferent pathways and specific vagal efferent pathways can have pro- and antiinflammatory effects. This question is fertile ground for future research. A study by Buscher and colleagues [306] provides evidence for pancreas-independent neural mechanisms of pain in chronic pancreatitis. According to their study, two mechanisms may contribute to painful chronic pancreatitis:

- persistent nociceptive input from ongoing pancreatic inflammation leads to central desensitization and visceral hyperalgesia
- continuing nociceptive input eventually alters central pain processing so that central pain perception becomes autonomous and independent of peripheral input.

These investigators used quantitative sensory testing to measure pain thresholds to pressure and electrical stimulation at various dermatomes in a small pilot study of 10 chronic pancreatitis patients on opioids. Thresholds were significantly lower in dermatomes associated with the pancreatic region, indicative of hyperalgesia of central origin. This type of pain may be less responsive to peripheral therapies aimed at the pancreas or peripheral nerve block but may be amenable to target disruption of central pain processing centers. This type of research is in its infancy. As one example, investigators are examining repetitive transcranial magnetic stimulation of the secondary somatosensory cortex [307] or secondary somatosensory area [308] to treat chronic visceral pain and transcranial direct current stimulation to treat chronic central pain [309].

Peripancreatic inflammation, involving the duodenum and retroperitoneum, may cause pain. Extension of active inflammation of the pancreatic tissue within the wall of the duodenum may result in extensive fibrosis and stenosis of the descending duodenum [310]. Peripancreatic inflammation may cause stenosis of the distal common bile duct, and this has been noted to be associated with severe abdominal pain. Pain may also result from continued inflammation of the head of the pancreas [310]. It is likely, therefore, that the pain in chronic pancreatitis is multifactorial. Depending on the etiological factors, structure, alteration, severity of the disease, and degree of pancreatic insufficiency, different factors may predominate in the genesis of pain. A study correlating pancreatic pathological condition and symptoms found that intermittent attacks of pain resulted from recurrent tissue necrosis, whereas chronic pain appeared to be secondary to segmental distention behind obstructed ducts [311].

Malabsorption

There is a 10-fold reserve for exocrine pancreatic enzyme secretion. Malabsorption occurs only after the capacity for enzyme secretion is reduced by more than 90% [269] (Fig. 69.2). In chronic pancreatitis secondary to alcoholism, it usually takes 10–20 years for severe pancreatic insufficiency to develop, but lipase secretion decreases more rapidly than secretion of proteolytic enzymes [270] (Fig. 69.3). Hence, steatorrhea is often an earlier and more severe problem than azotorrhea. The importance of colipase in fat malabsorption in adult patients is unknown, although colipase appears to be an important factor in children because steatorrhea and colipase correlate better than lipase and steatorrhoea [312].

Concurrent with the reduction of pancreatic enzyme secretion, there is also decreased bicarbonate secretion in patients with severe chronic pancreatitis. Not infrequently,

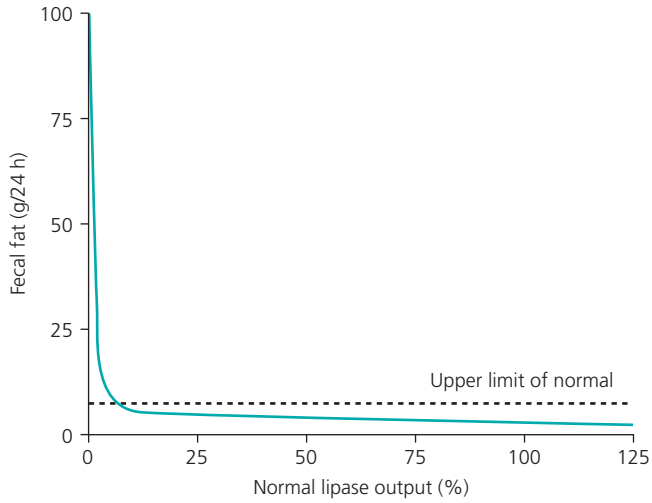

Figure 69.2 Relation between steatorrhea and lipase output. Steatorrhea does not occur until lipase output is reduced below 10% of normal. Adapted from DiMagno et al. [269].

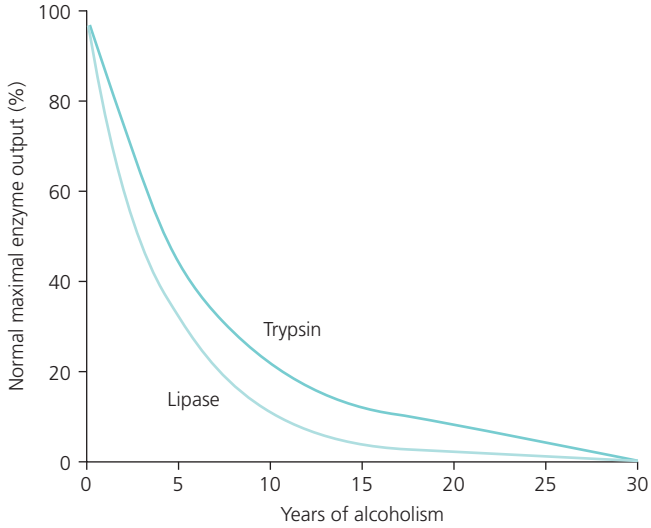

Figure 69.3 Relation between reductions in enzyme output and years of alcoholism in patients with alcoholic pancreatitis. Adapted from DiMagno et al. [270], with permission from Blackwell Publishing.

Table 69.5 Tests for chronic pancreatitis

I. Measurement of pancreatic products in blood
   A. Enzymes
   B. Pancreatic polypeptide
II. Measurement of pancreatic exocrine secretions
   A. Direct measurement
      1. Enzymes
      2. Bicarbonates
   B. Indirect measurement
      1. Bentiromide test
      2. Dual Schilling test
      3. Fecal chymotrypsin concentration
      4. [^{14}C]olein absorption
II. Imaging techniques
   A. Plain film radiography of abdomen
   B. Ultrasonography
   C. Computed tomography
   D. Endoscopic retrograde choliangiopancreatography
   E. Magnetic resonance cholangiopancreatography
   F. Endoscopic ultrasonography

the duodenal pH may fall to 4.0 or less [313,314] 90 minutes after eating. The abnormally low duodenal pH (less than 4.0) reduces lipid digestion by inactivating pancreatic enzymes and precipitating bile acids. These factors are important to consider in the treatment of pancreatic steatorrhea.

Diagnosis

Overview of diagnostic approaches

The diagnosis of chronic pancreatitis is usually suggested by historical information and then confirmed by radiography or laboratory tests. Over the years, an enormous number and variety of tests have been developed for the diagnosis of pancreatitis (Table 69.5). Many of these tests have not been adequately evaluated, and their true sensitivity and specificity are unknown. This section reviews many of the proposed tests for chronic pancreatitis; most are of limited clinical value. An understanding of the rationale underlying the test, however, often provides an insight into the physiological alterations that occur in chronic pancreatitis. The diagnosis of chronic pancreatitis can often be made on the basis of history and relatively simple radiographic tests. Only the rare patient requires extensive and complicated testing so the workup should be tailored to the individual patient.

Laboratory evaluation

Routine laboratory findings

Routine blood studies are not usually helpful in making the diagnosis of chronic pancreatitis.

Leukocytosis may be observed during acute exacerbations. The anemia frequently associated with malabsorption secondary to celiac disease is seldom present in patients with pancreatic malabsorption. Although patients with chronic pancreatitis commonly have an abnormal Schilling test result owing to the inability to digest R protein normally, megaloblastic anemia is rare [315]. Fat-soluble vitamin deficiency states (hypocalcemia, hypoprothrombinemia) are seldom observed in association with the steatorrhea of chronic pancreatitis, in contrast to their frequent occurrence in celiac sprue.

Cholestasis develops to varying degrees from compression of the common duct by a fibrotic process in the pancreas [316]. Alkaline phosphatase elevation in the absence of jaundice or other symptoms is the most common manifestation. Clinical jaundice results from more severe compression. Although liver disease is extremely common in alcoholics, this condition usually does not take the form of relatively pure cholestasis. The finding of cholestatic liver function in patients with pancreatitis requires extensive radiological testing, usually culminating in ERCP, to establish that the biliary obstruction results from chronic pancreatitis.

Specific tests for the diagnosis of chronic pancreatitis

The multiple tests available for the diagnosis of chronic pancreatitis can be separated into chemical measurements of pancreatic function and radiological procedures that provide information on pancreatic structure.

Several factors make it difficult to determine accurately the true sensitivity and specificity of these tests. First, biopsies of the pancreas are not usually obtained in patients with possible chronic pancreatitis, and most series report that patients have not been followed for a period sufficient to establish the

presence or absence of this condition with certainty. In most early or questionable cases, there is no histological gold standard that can be used to determine the accuracy of the more indirect chemical or radiological tests. Second, the sensitivity of the various diagnostic tests depends on the extent of the pancreatic damage. In long-standing disease with marked functional and anatomic alterations, virtually all proposed tests are positive. The sensitivity of the tests falls dramatically in early or mild chronic pancreatitis, however. The apparent sensitivity and specificity of the various tests also depend on the composition of subjects used to establish the normal range. For example, patients with a variety of disease states such as diabetes mellitus [317], celiac sprue [318], and cirrhosis [319] may have subnormal pancreatic secretion of enzymes. If such patients are used to establish the normal range, the lower limits of normal are reduced, and the sensitivity of the test for chronic pancreatitis drops accordingly. If the normal range is established with healthy controls, the specificity of a low secretion is reduced so although claims are made with regard to the sensitivity and specificity of tests for chronic pancreatitis, these claims have to be interpreted in light of the types of patients composing the pancreatitis groups as well as the subjects used to establish the normal range.

Serum pancreatic enzymes

In contrast to attacks of acute pancreatitis, in which the serum level of pancreatic enzymes is almost always elevated, serum enzyme levels may be elevated, normal, or low in chronic pancreatitis. In an acute exacerbation of the chronic process, the levels of these enzymes (amylase, lipase, trypsin) may be elevated; however, their serum concentrations seldom reach the levels observed in acute pancreatitis. A high serum concentration of pancreatic enzymes during a painful episode in a patient with advanced chronic pancreatitis should raise the possibility that some other abdominal process, such as gut perforation or infarction, is responsible for the pain.

Tests of pancreatic exocrine function

Tests of pancreatic exocrine function can be divided into those that directly assess pancreatic exocrine function (e.g., measurements of enzyme or bicarbonate secretion) and those that test some secondary effect of impaired enzyme secretion (e.g., malabsorption of a compound that requires pancreatic digestion for normal absorption).

Direct tests of pancreatic exocrine secretion

The basal secretory rate of the pancreas is highly variable so meaningful direct measurements of pancreatic secretion must be carried out in the stimulated state. Varying doses of secretin or CCK have been used for this purpose as well as a standard meal (the Lundh test). Early studies used simple drainage of the intestine at the ligament of Treitz to obtain pancreatic secretions. This technique does not recover all luminal fluid passing the collection site so studies have used a constant perfusion of a nonabsorbable marker, such as polyethylene glycol, into the duodenal bulb. Measurements of the concentration of this marker passing the distal collection site permit assessment of the rate at which fluid passes this site and make it possible to quantify the rate at which pancreatic secretory components pass this site [269].

A wide variety of pancreatic juice components, including the concentration or output of bicarbonate, amylase, lipase, and trypsin, have been assessed to find the most sensitive and specific indicator of chronic pancreatitis. In general, comparative studies suggest that all of them tend to be equally depressed in chronic pancreatitis and that there is no clear-cut advantage of one measurement over another. Few studies, however, measured pancreatic juice lipase output, which in two studies was less variable or was the earliest and most sensitive indicator of chronic pancreatitis, compared to lipase concentration, other enzyme concentrations/outputs, and bicarbonate concentration or output [320,321]. Critical to these studies was the use of continuous infusion rather than bolus administration of secretagogues. None of these measurements accurately differentiates chronic pancreatitis from carcinoma of the pancreas. Collection of pure pancreatic juice by way of cannulation of the ampulla of Vater during ERCP apparently adds little to the accuracy of the test [322].

Stimulation of pancreatic secretion has been produced over a wide range of doses and dosing schedules using secretin [323], CCK [324], cerulein [325], and bombesin [326] singly or in combination. Although it is difficult to compare the results of different studies, there appears to be no clear-cut advantage of one stimulatory technique over another. The relative value of direct stimulation of the pancreas with secretin-CCK vs indirect stimulation by a Lundh meal is also somewhat controversial, although most comparative studies suggest that direct stimulation is superior [327,328].

For many years, the finding of a subnormal pancreatic secretion of enzymes or bicarbonate in aspirated duodenal contents was considered the most sensitive test for chronic pancreatitis, and, if pancreatic carcinoma could be excluded, a low secretion was assumed to be diagnostic of chronic pancreatitis. Natural porcine secretin has been unavailable because its use for the treatment of autism has not been officially approved. Synthetic porcine secretin has been produced and compared favorably to natural porcine secretin in the diagnosis of chronic pancreatitis [329]. The sensitivity of the CCK or secretin pancreatic function tests was examined in 48 patients with suspected chronic pancreatitis [321]. All patients were evaluated with ultrasound, CT, and endoscopic retrograde pancreatography (ERP). All 10 patients with normal ERP results demonstrated normal function by direct hormonal stimulatory testing, and all 16 patients with advanced changes on ERP had grossly abnormal function testing. In 12 patients with mild to moderate changes on ERP, all demonstrated some abnormality of function, and of 10 patients with

minimal or equivocal changes on ERP, all had some degree of functional impairment. The CT and ultrasound were accurate in advanced chronic pancreatitis (94%–100%) but performed poorly in patients with less advanced changes on ERP (40%–50% accurate in patients with equivocal changes). This study is in agreement with other previous reports, which indicated that direct hormonal stimulation testing might be the most sensitive test (70%–95%) [330–334] for the diagnosis of chronic pancreatitis in all stages. As perhaps the strongest evidence, studies with definite histological evidence of chronic pancreatitis report a sensitivity of 67%–97% and specificity of 78%–100% [269,320,324,335–341]. However, it should be noted that patients with a variety of conditions, including diabetes mellitus [317], hepatic cirrhosis [319], Billroth II gastrectomy [342], and celiac sprue [318] may have diminished pancreatic secretory output with no clinical or radiological evidence of chronic pancreatitis. Another major consideration is that direct hormonal stimulation testing is only available at a few centers; therefore, the search continues for a convenient and readily available diagnostic test.

Indirect tests of pancreatic exocrine secretion

The complexity and patient discomfort involved in the direct measurement of pancreatic secretory capacity have led to a variety of simpler tests that indirectly assess the secretion of pancreatic enzymes. Most of these tests measure the absorption of some compound that first requires digestion by pancreatic enzymes. However, clinically detectable malabsorption of nutrients does not occur until pancreatic enzyme secretion has diminished to less than 10% of normal, so it is axiomatic that indirect tests of pancreatic function will not be able to detect early chronic pancreatitis and will therefore have very poor sensitivity.

The bentiromide test is used rarely and involves ingestion of *N*-benzoyl-L-tyrosyl-*p*-aminobenzoic acid (NBT-PABA), a tripeptide that is digested by chymotrypsin with the release of paraaminobenzoic acid (PABA) [343,344]. Free PABA is absorbed in the small bowel and excreted by the kidney; the quantity excreted in urine is used as a measure of pancreatic exocrine function. The sensitivity of the bentiromide test varies with the extent of damage to the exocrine pancreas, with sensitivity as high as 100% [345] reported with very severe disease and as low as 40%–50% with minor damage [346,347]. The specificity of this test is far from perfect. Reduced PABA excretion has been reported to occur commonly in diabetes mellitus [348], renal insufficiency [349], liver diseases [349], and malabsorption states other than pancreatic insufficiency, including celiac sprue [350], Crohn's disease [351], and postgastrectomy states [349]. The NBT-PABA test has low sensitivity so the major role of this test is not in diagnosis of chronic pancreatitis but rather in determining the extent of the pancreatic insufficiency that is present in patients with known chronic pancreatitis.

The demonstration that vitamin B-12 is malabsorbed in patients with pancreatic insufficiency, owing to their inability to degrade R protein [352], provided the rationale for a test for chronic pancreatitis. In this test, the subject ingests both [^{57}Co]cobalamin bound to intrinsic factor and [^{58}Co]cobalamin bound to R protein, plus excess intrinsic factor and a cobalamin analogue that saturates all empty R protein-binding sites [353]. Theoretically, the patient with pancreatic insufficiency should absorb the cobalamin bound to intrinsic factor much more efficiently than that bound to R protein. All other subjects should absorb both forms of cobalamin equally. Although the initial paper describing this technique reported excellent sensitivity and specificity for chronic pancreatitis, a subsequent study found very poor sensitivity [354].

Measurements of fecal chymotrypsin and elastase are rapid and simple but are inaccurate indicators of mild to moderate chronic pancreatitis. The diagnosis of pancreatic insufficiency by way of measurements of fecal chymotrypsin was first described 40 years ago [180] and a number of subsequent reports have evaluated the sensitivity and specificity of various modifications of the initial technique. The far more cumbersome measurement of total fecal chymotrypsin output in timed fecal collections appears to offer little advantage over the much simpler measurement of chymotrypsin concentration in a random fecal sample [180]. Reports of sensitivity of the test range from 45% [355] to 100% [356], depending on the extent of pancreatic damage in the study population. Specificity ranges from 49% [357] to 90% [358] with false-positive results reported for malabsorptive conditions such as celiac sprue, damage secondary to Crohn's disease, and postgastrectomy states [358,359].

Similar to fecal chymotrypsin, both the monoclonal and polyclonal enzyme-linked immunosorbent antibody fecal elastase-1 tests have unacceptably low sensitivity and specificity for diagnosing exocrine pancreatic insufficiency (see Chapter 139). For this reason, fecal chymotrypsin and elastase measurements are poor indicators of chronic pancreatitis in the clinic and in epidemiological studies [360]. Patients with appreciable chronic pancreatitis malabsorb fat when it is ingested in the form of triglyceride, and a number of indirect tests have been developed to assess such malabsorption. Most of these tests involve the administration of [^{14}C]olein. Hydrolysis of the triglyceride and absorption of [^{14}C]oleate lead to the production and pulmonary excretion of $^{14}CO_2$. Measurement of breath $^{14}CO_2$ excretion can be used as a simple measure of the normality of the hydrolysis of the labeled triglyceride [361]. Unfortunately, the test is negative in a high percentage of patients with early chronic pancreatitis, and false-positive results are observed in many malabsorption states other than chronic pancreatitis [362].

Imaging studies

The demonstration of diffuse, speckled calcification of the pancreas on a plain film of the abdomen is diagnostic of

chronic pancreatitis. Although the sensitivity of this finding is limited (perhaps 30%–40%), a plain film of the abdomen should be the first diagnostic test used when attempting to establish the diagnosis of chronic pancreatitis because a positive finding obviates the need for additional testing.

The development of ultrasound, CT, and ERCP has made it possible to assess, routinely, the gross structure of the pancreas. These tests all have excellent specificity and reasonably good sensitivity. Of major importance is the ability of these imaging procedures to differentiate between carcinoma of the pancreas and chronic pancreatitis. As a result, imaging techniques have largely supplanted tests of pancreatic function in the workup of the patient with possible chronic pancreatitis.

Ultrasound is the simplest and least expensive of the three imaging techniques. Findings on ultrasound that correlate with marked pancreatic changes on ERCP include dilation of the main pancreatic duct to more than 4 mm, large (more than 1 cm) cavities, and calcifications [363]. Findings associated with less severe changes on ERCP include dilation of the duct up to but not exceeding 4 mm, small cavities, reduction in echogenicity or echogenic foci in the parenchyma, and an irregular contour to the gland [363]. When a satisfactory ultrasound examination is obtained, the reported sensitivity of this test for chronic pancreatitis is of the order of 70%, and the specificity is high, roughly 90% [364–367]. The finding of chronic pancreatitis on ultrasound usually requires no additional confirmatory testing.

CT is 10%–20% more sensitive than ultrasound for the diagnosis of chronic pancreatitis [366–368]; these two techniques have roughly comparable specificities. CT is more expensive than ultrasonography and involves exposure to ionizing radiation. Therefore, in the workup for chronic pancreatitis, CT should usually be limited to patients who have negative or unsatisfactory ultrasound examinations. The most common diagnostic findings of chronic pancreatitis on CT include duct dilation, calcifications, and cystic lesions [369–371] (Fig. 69.4). Less common diagnostic findings include enlargement or atrophy of the pancreas and heterogeneous density of the parenchyma. This imaging technique is particularly helpful in the differentiation of chronic pancreatitis from carcinoma of the pancreas. The dilated duct system resulting from carcinoma tends to be smooth or beaded, in contrast to the irregular, calcified ducts that are typical of chronic pancreatitis. The sensitivity of CT for the diagnosis of chronic pancreatitis is in the neighborhood of 80%, whereas the specificity is roughly 90% [366,372,373]. ERCP is commonly considered to be the most sensitive and specific test available for the diagnosis of chronic pancreatitis even though there are few comparisons between ERCP and histopathology [337–339], in contrast to the numerous studies that provide diagnostic accuracy of direct pancreatic function tests by using gold standard histopathology (see Table 139.3 in Chapter 139) [269,320,324,335–341]. Furthermore, ERCP has become the gold standard against which all other tests are evaluated. In the absence of histological confirmation of disease in most patients, it is difficult to speak with complete certainty about the diagnostic accuracy of ERCP (because ERCP is the gold standard). Most studies indicate that the sensitivity and specificity of ERCP are 70%–90% and 80%–100%, respectively [321,373–376].

Figure 69.4 Chronic pancreatitis. Computed tomography scan shows pancreatic atrophy along with multiple intraductal calculi and dilation of the pancreatic duct (arrow).

The most commonly used classification of ERCP changes in chronic pancreatitis was developed at an international symposium. The Cambridge classification of chronic pancreatitis is based on abnormalities seen in the main pancreatic duct and in the side branches [377,378] (Table 69.6). In minimal pancreatitis, the changes are limited to the branches and fine ducts (less than three), which show dilation and irregularity (Fig. 69.5). There is considerable observer variation in the detection of these minor changes, and differentiation of normal from abnormal is not always clear. Minor ductal dilation and intraductal calculi mimicking the changes seen in chronic pancreatitis are observed with aging (age > 60 years) [379], as found in postmortem pancreatograms [380–383], and following acute pancreatitis [376,384]. Indeed, ductal changes occur and commonly persist in 9%–33% of patients after acute pancreatitis [376,384] but likely represent scars rather than a specific sign of chronic pancreatitis as they rarely lead to reduced pancreatic function or definite or prob-

Table 69.6 Cambridge classification of chronic pancreatitis by endoscopic retrograde pancreatography

Grade	Main pancreatic duct	Side branches
Normal	Normal	Normal
Suggestive	Normal	< 3 abnormal
Mild	Normal	≥ 3 abnormal
Moderate	Abnormal	> 3 abnormal
Severe	Abnormal plus at least one of the following: – Large cavity – Duct obstruction – Dilation or irregularity – Intraductal filling defects	

Modified from Axon et al. [377], with permission from the BMJ Publishing Group.

Figure 69.5 Endoscopic retrograde cholangiopancreatography study shows minimal changes consistent with chronic pancreatitis. The main duct is slightly irregular, and the branches are slightly dilated. These findings are not diagnostic.

able evidence of chronic pancreatitis at follow-up [376,384]. Further, 50% of patients with mild ERCP changes may not have histological evidence of chronic pancreatitis [381,385]. Moderate pancreatitis is characterized by abnormalities in at least three or more side branches and the additional finding of dilation, tortuosity, and stenosis of the main pancreatic duct (Fig. 69.6). Advanced pancreatitis has the additional findings of cyst formation, duct obstruction, severe ductal dilation, or irregularity (Fig. 69.7). Pancreatic carcinoma usually can be distinguished from chronic pancreatitis in that there is a single strictured region in malignant obstruction, in contrast to the multiple stenoses, irregular branching ducts, and intraductular calculi observed in chronic pancreatitis.

In general, there is a good correlation between the changes observed on ERCP and measurements of pancreatic secretory

Figure 69.6 Endoscopic retrograde cholangiopancreatography study shows changes of moderate pancreatitis. The main duct is irregular and dilated, and there is a pseudocyst in the tail of the pancreas.

capacity. An occasional patient, however, may have markedly diminished secretory function with seemingly minor changes on ERCP. Patients with minor abnormalities on ERCP often have normal secretory function. For further discussion please see Chapter 139.

It should be noted that pancreatitis occurs in about 5% of patients undergoing ERCP [386]. This risk for pancreatitis increases significantly in female patients with normal serum

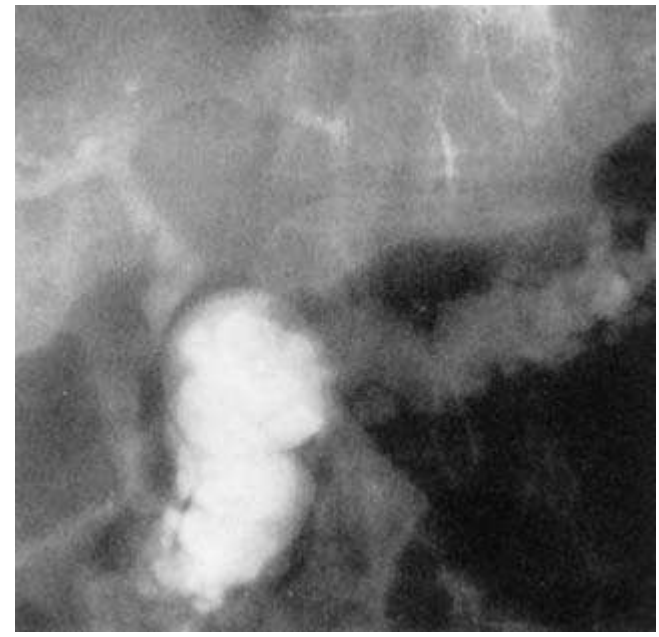

Figure 69.7 Endoscopic retrograde cholangiopancreatography study shows changes of advanced chronic pancreatitis. There are massive cystic dilations of the main duct and its branches.

bilirubin, patients suspected of sphincter of Oddi dysfunction, and those with a previous history of post-ERCP pancreatitis [386]. In fact, patients with the least probability of harboring truly obstructive pathology are at the highest risk for pancreatitis after ERCP, even if the procedure is for diagnosis alone. ERCP is an expensive procedure and has a low but not insignificant rate of associated complications, so this test ordinarily should be reserved for the rare patient with chronic pancreatitis in whom the diagnosis cannot be clearly established using other imaging techniques.

Although chronic pancreatitis and carcinoma of the pancreas usually can be reliably distinguished based on imaging studies, in an occasional patient, this differentiation may be difficult [387]. Attempts to use serum or pancreatic juice tumor markers, such as CA 19-9, to differentiate benign from malignant pancreatic processes have not been impressive [387], and it is usually necessary to make a tissue diagnosis. Although laparotomy with open biopsy was required in the past, percutaneous fine-needle aspiration biopsy makes it possible to obtain a histological diagnosis in a very high percentage of patients with pancreatic carcinoma. Under CT or ultrasonographic guidance, a small-gauge needle is inserted into the pancreatic mass, and aspirated material is examined for the presence of malignant cells. The reported specificity of this test approaches 100%, and the sensitivity is 80%–90% [388].

In recent years, advances in magnetic resonance (MR) imaging have led to the development of MR cholangiopancreatography (MRCP) for the evaluation of the biliary and pancreatic ducts (Fig. 69.8). Similar to ERCP, MRCP allows direct visualization of the pancreatic duct but without radiation exposure or the use of oral or intravenous contrast. Hence, it can be used in patients who are pregnant or have contrast allergies. In severe chronic pancreatitis with marked changes of the main pancreatic duct, agreement between MRCP and ERCP is excellent [389–392]. However, MRCP is relatively insensitive in mild chronic pancreatitis, in which changes are limited to the secondary ducts. Emerging modifications and application of MRCP technology, including secretin-enhanced MRCP and "diffusion-weighted" MR imaging, are under investigation and are reviewed in Chapter 132.

Endoscopic ultrasonography (EUS) is a major advance in imaging the pancreas because it may provide more detailed structural information of the pancreas than routine ultrasonography and CT scanning [393]. EUS features of chronic pancreatitis may be used to establish the diagnosis and evaluate the severity of the disease [394]. These include ductal changes and parenchymal changes, such as echo texture of the gland, calcifications, lobulations, and bands of fibrosis (Figs 69.9 and 69.10; see Table 69.7). For EUS to be considered an accurate test for chronic pancreatitis, there must be good interobserver agreement. To address this issue, EUS was performed on 33 patients with suspected chronic pancreatitis based on typical symptoms as well as on 12 control patients [395]. There was moderately good overall agreement for the final diagnosis of chronic pancreatitis ($\kappa = 0.45$). Agreement was good for individual features of duct dilation ($\kappa = 0.6$) and lobularity ($\kappa = 0.51$) but poor for the other seven features ($\kappa < 0.4$). The presence of stones was considered the most predictive feature of chronic pancreatitis, followed by visible side branches, cysts, lobularity, irregular main pancreatic duct, hyperechoic foci, hyperechoic strands, main pancreatic duct dilation, and main duct hyperechoic margins. Hence, EUS

Figure 69.8 Magnetic resonance cholangiopancreatography shows calcification in the head of the pancreas and obstructions to both the pancreatic duct and the common bile duct. Courtesy of Dr Theesa Rangsitwatana.

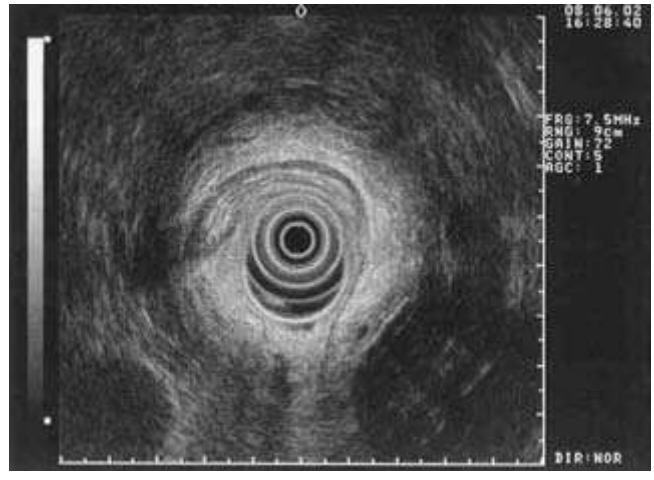

Figure 69.9 Endoscopic ultrasound showing mild chronic pancreatitis. Characteristic features include hyperechoic pancreatic duct margins and irregular contour of the pancreatic duct.

Figure 69.10 Endoscopic ultrasound showing a large pseudocyst (4.4 x 4.6 cm) with a calcified rim from a patient with chronic pancreatic pancreatitis.

Table 69.7 Endoscopic ultrasound features of chronic pancreatitis

Endoscopic ultrasound feature	Implication
Ductal changes	
Duct size > 3 mm	Ductal dilation
Tortuous pancreatic duct	Ductal irregularity
Intraductal echogenic foci	Stones or calcification
Echogenic duct wall	Ductal fibrosis
Side-branch ectasia	Periductal fibrosis
Parenchymal changes	
Inhomogeneous echo pattern	Edema
Reduced echogenic foci (1–3 mm)	Edema
Enhanced echogenic foci	Calcifications
Prominent interlobular septae	Fibrosis
Lobular outer gland margin	Fibrosis, glandular atrophy
Large, echo-poor cavities (> 5 mm)	Pseudocyst

Adapted from Catalano et al. [394], with permission from Elsevier.

appears to be a reliable method for the diagnosis of chronic pancreatitis, with good interobserver agreement among experienced endosonographers. A prospective evaluation of EUS compared with ERP and the secretin stimulation test in the diagnosis of chronic pancreatitis was conducted by Catalano and colleagues [394]. Eighty consecutive patients with recurrent pancreatitis participated in the study. EUS criteria for chronic pancreatitis included mild (one to two features), moderate (three to five features), and severe (more than five features; see Table 69.7). Abnormal EUS, ERP, and secretin test were observed in 63, 36, and 25 patients, respectively. The secretin test had 100% agreement with normal and severe chronic pancreatitis by EUS criteria, but agreement was poor for mild (13%) and moderate (50%) disease. On the other hand, the agreement between ERCP-specific and EUS-specific criteria was excellent for normal (100%), moderate (92%), and severe (100%) chronic pancreatitis and poor for mild (17%) disease. The main source of disagreement in this and other studies involves patients with abnormal results of EUS and normal ERCP or pancreatic function tests. Therefore, it appears that with the exception of mild changes noted on EUS, there is excellent agreement between EUS and ERCP in the diagnosis of chronic pancreatitis. Similar findings were reported in another prospective study to determine the ability of EUS to diagnose, exclude, or establish the severity of chronic pancreatitis found by ERP [396]. In general, histology is the most obvious candidate for a diagnostic gold standard, but few studies have compared EUS with pancreatic histology. In a preliminary study comparing EUS with the histological features of chronic pancreatitis in 34 patients who underwent EUS and later pancreatic surgery (21 with chronic pancreatitis and 13 with pancreatic carcinoma), the sensitivity of EUS for diagnosis of chronic pancreatitis was 78%, specificity was 73%, and the threshold for diagnosis was four or more echo features [397]. However, these findings are only applicable to mostly severe pancreatitis. The specificity of EUS changes in mild or early chronic pancreatitis is unknown but probably very low. Reliance on a few EUS criteria, such as inhomogeneity, hyperechoic strands, and lobularity, probably would give rise to many false diagnoses of chronic pancreatitis. Long-term follow-up of patients with mild EUS changes is needed to determine the usefulness of EUS in diagnosing mild chronic pancreatitis. In a recent study, 130 patients with a history of chronic use of alcohol and recurrent abdominal pain underwent ERCP and EUS [398]. All patients with chronic pancreatitis confirmed by ERCP (70.8%) had ductal or parenchymal changes detected with EUS. Among 38 patients (29.2%) with normal ERCP, 32 (84.2%) presented with morphological features consistent with chronic pancreatitis by EUS. During follow-up (median, 18 months), chronic pancreatitis was confirmed by repeat ERCP in 22 of these 32 patients (68.8%). Hence, it appears that EUS is more sensitive than ERCP in the detection of early chronic pancreatitis in patients with abdominal pain and a history of chronic and continued ingestion of alcohol. More long-term follow-up studies are needed to determine the usefulness of EUS in diagnosing mild nonalcoholic chronic pancreatitis.

Rational use of tests in the diagnosis of chronic pancreatitis

The evaluation should begin with a plain film of the abdomen. The finding of pancreatic calcification is virtually diagnostic of chronic pancreatitis, and no further testing is required. If calcifications are not present, the next test should be ultrasound. Once again, if this test demonstrates findings

indicative of chronic pancreatitis, further procedures are not necessary. If the ultrasound examination is nondiagnostic, CT is indicated only if there is a high degree of clinical suspicion of chronic pancreatitis. Only a small percentage of patients with chronic pancreatitis have negative ultrasound and CT findings. Thus, ERCP should be reserved for those patients in whom the diagnosis of chronic pancreatitis is thought to be likely on clinical grounds or for patients in whom ultrasound, CT, and fine-needle biopsy have not been able to differentiate between carcinoma of the pancreas and chronic pancreatitis. If ultrasound, CT scan, and ERCP are normal, a secretin test may disclose evidence of mild chronic pancreatitis. Unfortunately, the secretin test is not widely available. Studies suggest that EUS may be useful to establish the diagnosis and evaluate the severity of the disease. Additional studies are needed to define the sensitivity of EUS for the diagnosis of early disease. MRCP may be used as a backup alternative if ERCP fails and EUS is unavailable or technically not feasible, such as in patients with a Roux-en-Y gastrojejunostomy.

Complications

Pseudocyst

A pseudocyst is a collection of pancreatic juice, outside the normal boundaries of the ductal system, which is enclosed by a fibrous tissue membrane. It is the most common complication of chronic pancreatitis, occurring in up to 25% of cases in some series [399]. Most chronic pseudocysts occur in patients with chronic alcoholic pancreatitis [400], and they are found more commonly in the body of the pancreas than in the head or tail [400].

Little is known about the mechanism responsible for the initial formation of pancreatic pseudocysts. They probably result from rupture of a pancreatic duct, activation of interstitial pancreatic juices, necrosis of the surrounding parenchyma, escape of the pancreatic juice into the lesser sac, and reaction of local mesothelial cells to wall off the collection with a fibrous membrane. Once initiated, expansion of the embryonic pseudocyst continues until the surrounding tissue pressure equals pancreatic secretory pressure, which is usually elevated in chronic obstructive pancreatitis. Although not conclusively demonstrated, some evidence exists to suggest that pancreatic juice contained within a pseudocyst can exchange with plasma. If so, this may represent a mechanism responsible for resorption of immature pseudocysts and spontaneous resolution of chronic pseudocysts.

Pain is the major symptom of pancreatic pseudocysts. A pseudocyst should be suspected in a patient with stable chronic pancreatitis who experiences worsening of abdominal pain. Abdominal ultrasonography is most frequently used in the diagnosis and management of pseudocysts. It is best used as a serial monitor of pseudocyst size in those patients in whom definitive drainage is delayed. CT has emerged as the single most accurate method of diagnosing pancreatic pseudocysts [401]. In addition to its high accuracy rate, it is capable of providing structural details, such as the size of the common duct or pancreatic duct. This has important bearing on the choice of an operative approach. In the presence of dilation of either or both the common bile duct and pancreatic duct, drainage of the pseudocyst alone is rarely sufficient [402]. Although some surgeons have advocated ERCP for all patients with pseudocysts [403,404], others have not found routine preoperative pancreatography desirable for fear of secondary infection, which may occur in up to 25% of cases [404]. It is generally agreed, however, that patients with suspected concomitant common duct obstruction should undergo ERCP before surgical drainage.

In contrast to acute pseudocysts, chronic pseudocysts, especially those larger than 6 cm, almost never resolve spontaneously [405,406]. On the other hand, they also do not carry the same risk for serious complications as acute pseudocysts. In a study involving 75 patients with chronic pancreatitis and pseudocyst, about half of the patients could be managed conservatively [407]. Only one pseudocyst-related complication occurred in this group, and there was no mortality. According to this study, indications for operative management include persistent abdominal pain, enlargement, or complications of pseudocyst.

Effective treatment of a pseudocyst includes excision and internal or external drainage. Surgical excision provides definitive treatment and is feasible when the cyst is localized to the tail of the pancreas. In most cases, however, internal drainage is the treatment of choice. Pseudocysts that are more than 6 weeks old usually have a mature cyst wall that permits internal drainage, where the cyst is anastomosed to the stomach (cystogastrostomy), duodenum (cystoduodenostomy), or jejunum (cystojejunostomy). The fluid in the pseudocyst drains into the gastrointestinal tract, and the cyst cavity collapses and becomes obliterated, usually within 1 week. There also have been reports of endoscopic drainage for the treatment of pseudocysts in chronic pancreatitis [408,409]. A pseudocyst that indents the stomach or duodenum can be punctured transendoscopically using electrocautery or lasers to create a fistulous tract between the pseudocyst and the stomach or duodenum. Long-term, endoscopic studies report 62%–84% success rates [410–413] compared to surgical success rates of 90%–93% [411,414,415]. In a medium-term follow-up study, Cremer and colleagues [408] reported a success rate of 96% for endoscopic cystoduodenostomy and 100% for endoscopic cystogastrostomy in 33 patients with chronic pancreatitis. No significant complications were observed after successful endoscopic cystoduodenostomy, and the two complications of endoscopic cystogastrostomy were gastric hemorrhage and iatrogenic pseudocyst infection. To decrease the risks for bleeding and

perforation, the use of endosonography-guided drainage of a pancreatic pseudocyst has been reported [416]. Potentially, the precise ultrasonographic definition of the cyst and local anatomy at the time of endoscopic drainage may improve the safety of endoscopic drainage, but the results are too preliminary for adequate evaluation.

External drainage usually is used for patients with infected pseudocysts and for those in whom the wall of the pseudocyst is not mature enough to hold sutures. Drainage is achieved by surgical placement of a large catheter in the pseudocyst, and the fluid drains externally through the abdominal wall. This form of treatment usually is safe but is associated with a higher recurrence rate than internal drainage [417].

CT-guided, percutaneous, transabdominal catheter drainage of chronic pseudocysts has gained popularity and is considered by some a preferred alternative to surgical treatment in the management of pancreatic pseudocysts. Van Sonnenberg and colleagues [418] reported a 90% success rate in the percutaneous drainage of 101 pancreatic pseudocysts. The mean duration of drainage was 20 days. There was no mortality, and the only major complication was superinfection in four cases. Adams and Anderson [419], in a retrospective study, also found that percutaneous catheter drainage was as effective as internal drainage in the management of pseudocysts, although drain tract infection was as high as 48%. In a long-term retrospective study of 42 patients undergoing percutaneous catheter drainage, however, Criado and associates [420] reported that in nine patients, the pseudocysts failed to resolve, and seven recurred after initial resolution. Hence, the effectiveness of percutaneous drainage in the management of pseudocysts remains to be established.

Somatostatin is a potent inhibitor of pancreatic secretion and therefore may be useful in the treatment of pancreatic pseudocyst. Gullo and Barbara [421] reported that 2-week treatment with octreotide, a long-acting somatostatin analogue, resulted in a 42% reduction in size of the pseudocysts in four of seven patients. In addition, octreotide also appears to be effective in decreasing persistent catheter drainage from chronic pseudocyst [422,423]. Therefore, patients with pancreatic pseudocyst resistant to drainage should be offered a course of octreotide before surgery is contemplated.

Pancreatic ascites

The incidence of pancreatic ascites in chronic pancreatitis is usually considered to be less than 1%. It occurs as a consequence of persistent leakage of pancreatic juice from a pseudocyst or the pancreatic duct. Fifteen percent of patients with a pseudocyst may have pancreatic ascites [424]. On the other hand, 60% of patients with pancreatic ascites have pseudocysts [425,426]. Pancreatic ascites occurs typically in alcoholic patients with cirrhosis, who may complain of mild to moderate abdominal pain because of distention. The diagnosis is often erroneously attributed to decompensated cirrhosis; however, paracentesis reveals elevation of pancreatic enzyme levels and a high total protein or albumin level. These findings are diagnostic.

Most patients need surgery. A period of 2–3 weeks of observation and medical treatment is warranted. During this period, the patients are given nothing orally and are placed on parenteral nutrition to improve their nutritional status. Suppression of pancreatic secretion by agents such as octreotide may be tried (100–250 μg subcutaneously every 4 hours). With this regimen, the ascites may resolve spontaneously in up to one-third of patients, and surgery can be avoided. For patients who require an operation, ERCP should be performed to identify the precise site of the leak.

Pancreatic fistula

External pancreatic fistula as a complication of chronic pancreatitis is rare. It occurs most commonly after operative or percutaneous drainage of a pseudocyst. It also may follow pancreatic biopsy or arise from a leaky pancreatic anastomosis. Clinically, pancreatic fistula should be suspected when clear fluid drains from a cutaneous orifice, and the diagnosis is confirmed when the amylase content of the fluid is found to be elevated. Treatment is conservative and consists of fluid and electrolyte replacement, nutritional support, and eradication of infection if present. Somatostatin, which suppresses pancreatic secretion and decreases the volume of fistula drainage, may be used. During the course of therapy, a fistulogram should be obtained when the tract is well formed. If there is ductal obstruction between the site of the leak and the duodenum, surgical repair may be necessary. Most of the fistulae, however, close spontaneously.

Splenic vein thrombosis

Splenic vein thrombosis with extrahepatic (sinistral) portal hypertension is a well-known complication of chronic pancreatitis [427,428]. The splenic vein courses along the posterior surface of the pancreas so thrombosis may occur as a result of peripancreatic inflammation associated with acute attacks or with pseudocyst formation and extension. Rosch and Herfort [429] found occlusion of the splenic vein in about 4% of cases of chronic pancreatitis studied with splenoportography. Almost all patients had some impingement on the splenic vein. Varices involving the esophagus, stomach, duodenum, and colon have been described with resultant hemorrhage. A more recent study [428] involving 484 consecutive patients with chronic pancreatitis reported that splenic vein thrombosis occurred in 7% (34 patients), of whom 35% (12 patients) had gastroesophageal varices. Overall, 18% of them bled either before first surgery or on follow-up. The 22 patients who underwent splenectomy never bled after surgery. These findings support the recommendation that patients who have symptomatic sinistral portal hypertension who are undergoing surgery should have concomitant splenectomy. Splenectomy also should

be strongly considered in patients undergoing surgery for chronic pancreatitis who have varices secondary to splenic vein thrombosis regardless of the presence or absence of a previous history of variceal bleeding.

Treatment

Treatment of chronic pancreatitis is aimed mainly at the control of pain and the correction of malabsorption with adequate pancreatic enzyme replacement. Clinical diabetes occurs relatively late in the disease, and insulin is usually needed for its management.

Control of pain

The management of pain in chronic pancreatitis can be a challenging problem. It is the symptom that most commonly prompts the patient to seek medical attention and is the one that is most difficult to manage. Pain patterns are highly variable, and this may reflect different causative mechanisms. The problem is further exacerbated by a lack of good controlled trials. As highlighted in an American Gastroenterological Association technical review on treatment of pain in chronic pancreatitis [430,431], "a reliable database for making sound judgements and recommendations is lacking in most areas."

Avoidance of alcohol

A long-held clinical aphorism is that avoiding alcohol ingestion decreases the frequency and severity of abdominal pain in chronic alcoholic pancreatitis. Trapnell [385] reported that 75% of his patients with chronic alcoholic pancreatitis experienced pain relief when they stopped drinking. This observation may be explained by the stimulatory effect of alcohol on pancreatic secretion [432]. The relationship between alcohol and pain in chronic pancreatitis, however, is controversial. Bornman and colleagues [432] noted that half of their patients with painful pancreatitis and half of their patients with painless pancreatitis continued to drink. Similar observations were made by Marks and associates [433]. These findings call into question whether continued alcohol ingestion is related to pain. It is conceivable that pancreatic pain is related to exocrine pancreatic secretion. In patients who maintain significant exocrine secretory function, pain may be provoked by alcohol, which acts as a secretagogue. In patients whose exocrine secretion is drastically reduced, alcohol no longer plays a role in the mechanism of pain. Further studies are needed to clarify the role of alcohol in pain production.

Analgesics

Analgesics remain the main method for pain control in chronic pancreatitis. Initially, nonnarcotic analgesics, such as salicylates or acetaminophen, should be used. Preferably, these analgesics should be given before meals to prevent postprandial exacerbation of pain. Drug doses should be individualized, and the lowest effective dose should be used. As the severity of pain increases, the dose strength or frequency of these simpler analgesics should be increased before switching to narcotics. In severe cases, however, opiate analgesics are required, and they should not be withheld for fear of narcotic addiction.

Celiac plexus block

Percutaneous, radiologically or ultrasonographically guided, celiac ganglion alcohol injection has been used for control of pancreatic pain. This technique has been helpful in patients who have severe pancreatic pain secondary to pancreatic cancer [434]. In small, uncontrolled series of patients with chronic pancreatitis and debilitating pain this procedure has produced mixed results. The occasional benefits almost never last for more than a few months [435], and repeated treatment may not be as effective. The risk for procedure-induced transient hypotension, nerve root pain, and focal neuropathic damage, combined with the need for repeated blocking, even if successful, make this an unattractive technique for most patients [435]. An open trial of celiac plexus block using botulinum toxin A instead of alcohol was unsuccessful [436].

The advances in EUS have allowed the performance of celiac plexus block under the guidance of EUS. This has become a relatively simple and short outpatient procedure. Because of the anterior transgastric approach taken during the procedure, it has mostly eliminated the risk for nerve or spinal cord injury. A prospective study with EUS-guided celiac plexus block was performed in 90 patients with painful chronic pancreatitis [437]. A significant improvement in pain score occurred in 55% of patients. Unfortunately, the benefit persisted beyond 24 weeks in only 10%. Hence, although EUS-guided celiac plexus block is safe and well tolerated, it is not very effective for treating pain in chronic pancreatitis because it offers only temporary relief.

Enzyme therapy

Much interest has been generated by a series of observations suggesting that the intraluminal action of pancreatic proteases plays an important role in regulating pancreatic enzyme secretion. The underlying concept of feedback regulation of the pancreas is based primarily on studies in rats that showed that diversion of pancreatic juice from the duodenum stimulates CCK release and pancreatic enzyme secretion [438,439]. On the other hand, intraduodenal administration of trypsin or chymotrypsin inhibits the release of CCK and pancreatic enzyme secretion. The increased plasma CCK levels and pancreatic secretion after diversion of pancreatic juice appears to be mediated by a trypsin-sensitive substance secreted by the proximal small intestine that has been designated CCK-releasing factor [440]. When trypsin is present, this peptide is

cleaved and inactivated. The CCK-releasing factor may act as a mediator of pancreatic enzyme secretion in response to dietary protein intake in rats. Dietary protein in the intestine competes for the trypsin that would otherwise inactivate CCK-releasing factor [441]. The resulting increase of CCK-releasing factor in the intestinal lumen enhances CCK release, stimulating pancreatic enzyme secretion. A number of CCK-releasing peptides have been identified. These include a peptide extracted from porcine intestinal extracts with a structure identical to the diazepam-binding protein [442] and a luminal CCK-releasing peptide isolated from the duodenal secretion of rats [443]. The physiological significance of these releasing peptides is yet to be defined.

The protease-sensitive feedback mechanism was first reported to occur in humans in 1977 [444]. Subsequently, several groups of investigators confirmed that intestinal administration of trypsin or chymotrypsin inhibits pancreatic enzyme secretion [444–448]. Thus, it is conceivable that in patients with chronic pancreatitis, decreased enzyme secretion may result in the hyperstimulation of the pancreas by elevated plasma CCK levels, with resultant pain. Some [449–451], but not all [452,453], studies reported elevated plasma CCK levels in patients with chronic pancreatitis. It has been proposed that effective enzyme replacement therapy should reduce pancreatic stimulation, decrease intraductal pressure, and diminish pain. This hypothesis was tested in several clinical studies. In a double-blind, crossover study, Isaksson and Ihse [454] reported pain relief in 15 of 19 patients with chronic pancreatitis. There was a 30% reduction in pain intensity and a significant decrease in the frequency of pain attacks in patients on active enzymes. Slaff and colleagues [445] studied 20 patients with painful chronic pancreatitis in a double-blind, crossover study. Pain reduction was observed in 9 patients with mild to moderate pancreatic insufficiency, whereas only 2 of 11 with severe insufficiency responded to the treatment with enzyme replacement. Most of the good responders were women with idiopathic chronic pancreatitis, whereas most of the poor responders were men with alcohol-induced chronic pancreatitis. Favorable response was also reported by Ramo and associates [455], who showed significant pain relief after self-administration (ad libitum) of an enzyme preparation, compared with administration of the regular dose in 10 patients with painful chronic pancreatitis. These three double-blind studies [445,454,455] demonstrated good results for enzyme treatment in 73% (36 of 49) of the patients evaluated. A double-blind, 4-week crossover study from Denmark, however, showed that oral administration of two capsules of Pancrease with each meal did not significantly control pain compared with placebo [456]. Another multicenter 2-week controlled study in more than 40 patients using an enteric-coated supplement also showed no benefit [457]. Feedback inhibition of pancreatic enzyme secretion depends on delivery of high concentrations of protease to the duodenal lumen. Enteric-coated preparations preferentially release most of their enzymes distal to the duodenum and may therefore be less effective at suppressing CCK levels and producing pain relief [458]. Finally, a 4-month double-blind, crossover study used an enteric-coated preparation with microspheres that release their contents at a lower pH (pH > 5.0) than most commonly used microspheres (pH > 6.0). Despite a high delivery rate of trypsin into the duodenum, this study showed no benefit in pain scores in patients receiving pancreatic enzymes compared with placebo [459]. However, this study contained a small number of women and evaluated patients with recurrent rather than chronic pain. Long-term trials comparing nonenteric and enteric-coated preparations in patients stratified according to etiology, severity, and pattern of disease should help to clarify these issues. Until then, it seems reasonable to initiate a trial of high-dose, nonenteric-coated oral pancreatic enzymes taken with meals for several weeks in any patient with painful chronic pancreatitis. The best results may be seen in pancreatitis of nonalcoholic etiology, with symptoms of constant, rather than recurrent, pain and only mild to moderate pancreatic insufficiency [460].

Treatment with octreotide

Somatostatin is a naturally occurring hormone that has been shown to inhibit pancreatic secretion [461]. Theoretically, it may also have cytoprotective action and beneficial effects on the reticuloendothelial system in the setting of pancreatic inflammation [462–464]. In addition, clinical studies have shown that somatostatin has antinociceptive activity in human [465,466] and animal [467,468] models of pain, suggesting that this peptide may be helpful for pain control in chronic pancreatitis. Somatostatin also appears to gain its beneficial effects in inflammatory conditions through its inhibition of cytokine release. In a recent study, somatostatin was shown to reduce markedly the secretion of interleukin-8 and interleukin-1 from intestinal epithelial cells [469]. In another study, somatostatin significantly inhibited interleukin-8 and interferon-γ production from activated peritoneal macrophages [470].

Octreotide is a synthetic, long-acting analogue of somatostatin that has been shown to inhibit CCK release and both basally and neurally stimulated pancreatic secretion [471–473]. Octreotide's long duration of action allows subcutaneous dosing and makes it a more practical alternative for the treatment of chronic pancreatitis than the short-acting native somatostatin. The drug is well tolerated, although long-term therapy predisposes to cholesterol gallstones. Unfortunately, controlled trials using octreotide for the pain of chronic pancreatitis are limited. Short 3-day studies, using 100 μg and 150 μg three times a day, showed no benefit [474]. A multicenter randomized 4-week study of 91 patients with advanced chronic pancreatitis and severe pain was performed [475]. Octreotide, at doses ranging from 40 μg to 200 μg three times daily, was compared with placebo. Although results in this

pilot study did not reach statistical significance, 200 μg three times a day of octreotide produced the greatest pain relief (65% vs 35% of patients with placebo), especially in patients with constant as opposed to intermittent pain. Additional studies are needed to clarify the role of octreotide in the management of chronic pancreatitis pain.

A long-acting formulation of octreotide acetate (Sandostatin LAR Depot) was developed for patients requiring long-term octreotide therapy. It consists of octreotide acetate microencapsulated by a biodegradable polymer. Drug release occurs slowly because cleavage of the polymer takes place primarily through tissue fluid hydrolysis. After single intramuscular injection, the serum octreotide reaches plateau concentrations at about day 14 and remains relatively constant during the following 3–4 weeks. This long-acting form of octreotide may provide significant benefit over octreotide because it requires only monthly injection. This should be prospectively evaluated with a double-blind, randomized, multicenter study.

Endoscopic therapy

Endoscopic therapy has been used for control of pain in chronic pancreatitis, with the aim of alleviating obstruction of flow caused by ductal strictures, stones, or papillary stenosis. Ductal strictures are sometimes treated by balloons or dilating catheters, but in most cases, dilation is followed by stent placement across the stricture. Technical success in reported studies ranges from 82% to 100% [476–482] and pain improvement occurs in 55%–100% of patients during a follow-up of 8–39 months. True success rates are difficult to decipher, however, because stent placement frequently was performed with other procedures. Ponchon and colleagues [482] used a standardized protocol to evaluate endoscopic stenting for pain relief in a highly selected subgroup of patients with chronic pancreatitis. Patients with multiple strictures, pancreatic calculi, pancreatic divisum, common duct narrowing, any duodenal impingement, or a pseudocyst larger than 1 cm were excluded. They successfully placed 10-French, multi-side-hole stents after biliary and pancreatic sphincterotomy and balloon dilation of strictures in 28% of 33 patients (85%) with a distal pancreatic duct stricture and upstream dilation. The stents were exchanged at 2-month intervals, for a total stenting duration of 6 months. The report was based on 23 patients observed for at least 1 year after removal of the stent. During the stenting period, 21 patients (91%) had resolution or reduction in pain, and 17 discontinued analgesic medications. Twelve patients (52%) had a persistent beneficial outcome for at least 1 year after stent removal. Disappearance of the stenosis on pancreatography, at stent removal, and 1 year later and reduction in the diameter of the pancreatic duct were significantly associated with relief of pain. Caution, however, should be urged to avoid extrapolating these observations to widespread use of endoscopic therapy, which may expose patients to greater risks than proven benefit. According to guidelines published by the American Society of Gastrointestinal Endoscopy [483], the diameter of the pancreatic duct or resolution of pancreatic duct strictures in response to endoscopic therapy does not correlate with resolution of pain [483]. Furthermore, what data are available is difficult to interpret because "Confounding the literature on pancreatic duct stent therapy are other therapies performed at the same time of stent placement and the tendency of the chronic pancreatitis pain to wax and wane and decrease with time as deterioration of the pancreas function occurs" [483].

Based on reports in the literature, the incidence of occlusion of pancreatic stents appears to be similar to that for biliary stents [484]. Sherman and Lehman [485] reported that 50% of pancreatic stents (5–7 French) were occluded within 8 weeks of placement. Another complication is stent migration, which may be upstream (into the duct) or downstream (into the duodenum). Migration in either direction may be heralded by return of pain or pancreatitis. Johanson and associates [486] reported inward migration in 5.2% of patients and duodenal migration in 7.5%. Modifications in the design of pancreatic stents may reduce the frequency of such occurrences [487]. Long-term studies of stent placement also indicated that morphological changes occurred in most patients. These were usually mild and were reversible in most patients [488,489].

There are two prospective, randomized trials comparing endotherapy and surgery for treatment of painful, obstructive chronic pancreatitis failing conservative therapy [490,491]. Dite and colleagues [490] randomized 72 consecutive patients to surgery (80% resection and 20% drainage) or endotherapy (52% sphincterotomy and stenting or 23% stone removal). Aftert 5 years but not 1 year, patients had greater pain resolution (34% vs 15%) and weight gain (52% vs 27%) in surgical vs endoscopic groups. Drawbacks to this study are that only 50% of the eligible patients underwent random assignment, 50% were lost to follow-up, there was no true control group (no endoscopic or surgical intervention), and the pain pattern was not provided, which prevents characterization of patients' pain according to the clinically important descriptions by Ammann as "type A pain" or "type B pain."

The second randomized controlled trial by Cahen and colleagues [491] randomized 39 patients with severe pain and ductal obstruction to surgery (90% drainage or 10% combination drainage/resection) or endotherapy (sphincterotomy and stenting with or without stone removal), the latter following endoscopic shock-wave lithotripsy in 84% of patients (for stones > 7 mm). At 2 years, surgically drained patients had lower pain scores, greater pain relief (75% vs 32%) and higher quality of life scores ($P = 0.003$) compared with the endoscopic group. This study was particularly robust because few subjects were lost to follow-up (1/39) and there were multiple end points.

The two randomized controlled trials comparing endoscopic and surgical therapy indicate that surgical decompres-

sion has better long-term results than endoscopic techniques for treating pain and improving quality of life in patients with chronic pancreatitis and pancreatic ductal obstruction (strictures, stones, or dilation). Furthermore, both studies showed that endoscopic therapy had no greater effect on pain than the 38% endoscopic placebo response rate reported by others [492]. Surgery may be superior because surgery also reduces pancreatic tissue pressure, an endpoint that may predict the magnitude of pain resolution [493]. In contrast to a common misconception, pain relief was immediate (and not delayed) with surgery but was delayed with endoscopic decompression [491]. Importantly, these studies do not clarify whether surgical and endoscopic therapies differ for patients with type B pain but without pancreatic ductal strictures, stones, or dilation. Hence, for individual patients with painful, obstructive chronic pancreatitis, the decision to offer endoscopic, surgical or conservative treatment should be influenced by recognition that surgery has superior results compared with endoscopy, pain in most patients with chronic pancreatitis decreases over time, pain can recur in surgical groups [431] and in the presence of comorbidities, which may preclude surgical intervention. Finally, patients with type A pain should be managed with conservative medical treatment (no surgery or endotherapy) and those with refractory type B pain should be offered surgical therapy, except in select circumstances, particularly when surgery is contraindicated.

Endoscopic techniques also have been used for the removal of pancreatic stones in chronic pancreatitis. In different series, pancreatic duct stones were removed after endoscopic sphincterotomy in 27%–100% of patients [494,495]. In some cases, stone removal was facilitated by fragmentation with extracorporeal shock-wave lithotripsy, with clinical improvement observed in 50%–80% of cases in which the stones were successfully removed [495]. Inherent in these various therapies is the belief that stones are themselves causing symptoms by intensifying obstruction and that their removal will improve pancreatic flow and thereby reduce symptoms. On the other hand, it is also possible that pancreatic stones form as a result of pancreatic stricture and abnormal pancreatic juice protein and have little to do with pain. None of the studies on endoscopic treatment of chronic pancreatitis is controlled, and no valid comparisons have been made among medical, surgical, and endoscopic treatments. These procedures should therefore be considered experimental and performed only in patients entered into prospective, randomized trials.

In one study, minor papilla endoscopic sphincterotomy was performed in 52 patients with pancreas divisum who had chronic abdominal pain, acute recurrent pancreatitis, or chronic pancreatitis [496]. Among the three groups of patients, only those with acute recurrent pancreatitis benefited from the endoscopic procedure. This suggests that minor papilla sphincterotomy should be avoided in patients with pancreatic divisum and chronic pancreatitis.

Surgical treatment

After all medical measures have failed to relieve pain, surgery should be considered. The type of surgery is selected according to the perceived mechanism for the pain. When the main pancreatic duct is dilated, it is assumed that there is pancreatic duct obstruction and that pancreatic duct hypertension is the cause of the pain. On the other hand, if there is no ductal dilation, pain may result from diffuse parenchymal disease or blockage of small side ducts by stones, protein plugs, or scars. Relief of major duct obstruction should not affect this type of pain. Therefore, the type of surgery should be chosen according to the severity of pain, ductal morphology, and the extent of parenchymal disease. Patients who have ductal dilation have a 70%–80% chance of obtaining pain relief with either a partial resection with pancreaticojejunostomy or lateral pancreaticojejunostomy (modified Puestow procedure). The modified Puestow pancreaticojejunostomy is particularly suitable for patients who have ductal obstruction and dilation. It is a safe and effective operation, with a morbidity rate of less than 5%, mortality rate of less than 2%, and effective pain relief rate in the order of 80% [497,498]. On the other hand, for patients with moderate to severe parenchymal disease and no ductal dilation, partial pancreatic resection should be considered. Ninety-five percent distal resection is recommended for patients with diffuse parenchymal disease [433], whereas local resection of major site of involvement may be sufficient for those with regional parenchymal disease [433]. Overall, 50% of these patients have had satisfactory results.

Near-total pancreatectomy, or Whipple procedure, includes sacrificing portions of normal stomach and duodenum and can result in metabolic and digestive complications. To correct these problems, the original Whipple procedure underwent two subsequent procedural modifications. In 1978, Traverso and Longmire [499] described pylorus-preserving pancreatic duodenectomy, thereby avoiding the digestive complications of partial gastric resection. Several years later, Beger and associates [500] described a more conservative procedure that resected the diseased pancreatic head and preserved all extrapancreatic organs, including the duodenum (Fig. 69.11). A randomized trial comparing these two procedures in patients with painful chronic pancreatitis was performed [501]. There was no postoperative mortality, and morbidity was comparable in both groups (15%–20%). After 6 months of follow-up, patients who underwent duodenum-preserving pancreatic head resection (the Beger procedure) experienced less pain, better preservation of insulin secretion and glucose tolerance, and more stable weight than those who underwent a pylorus-preserving Whipple resection. This suggests clinical superiority of the Beger procedure as compared with the pylorus-preserving Whipple resection. However, the limited sample size and lack of long-term follow-up preclude any definitive conclusions.

In 1987, Frey and Smith [502] described another operation for painful chronic pancreatitis that consisted of local

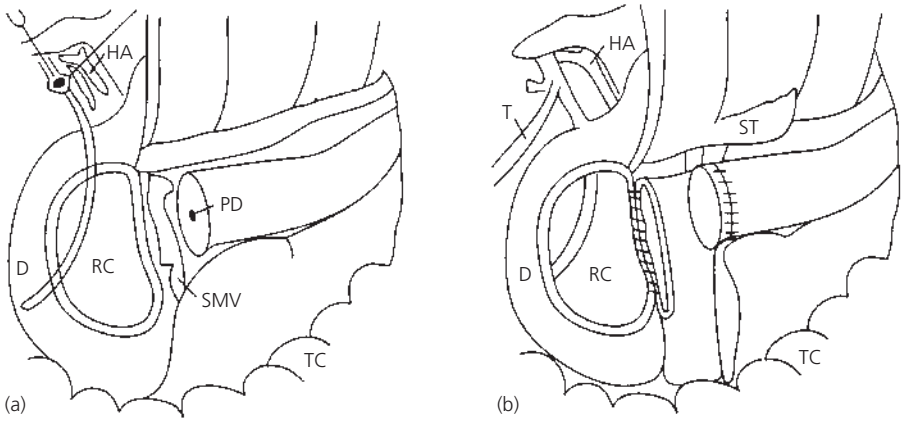

Figure 69.11 Duodenum-preserving resection of the pancreatic head as described by Beger. **(a)** A metal probe is inserted through the common bile duct into the duodenum to prevent inadvertent injury during resection of the pancreatic head. **(b)** Reconstruction includes an end-to-end pancreaticojejunostomy and a side-to-side anastomosis between the jejunum and the rim of the cavity remaining after resection of the pancreatic head. D, duodenum; HA, hepatic artery; PD, pancreatic duct; RC, resection cavity of the pancreatic head; SMV, superior mesenteric vein; ST, stomach; T, tube; TC, transverse colon. From Izbicki et al. [504], with permission from Lippincott Williams and Wilkins.

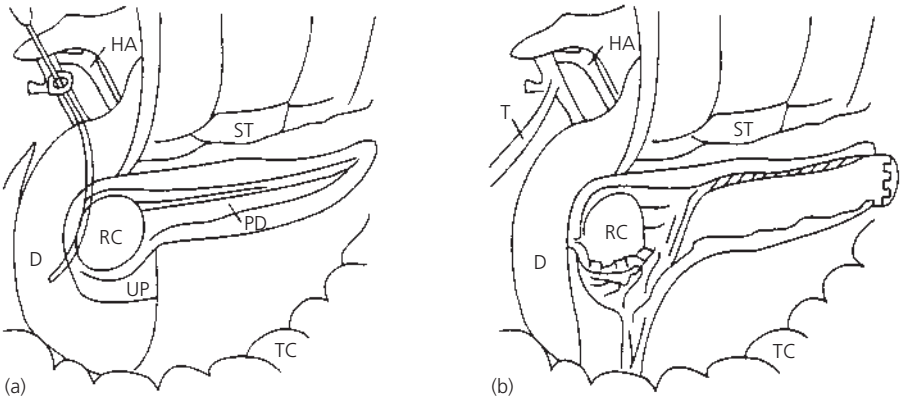

Figure 69.12 **(a)** Duodenum-preserving resection of the pancreatic head as described by Frey. A metal probe is inserted through a choledochotomy into the duodenum before resecting the pancreatic head mass to minimize the risk for bile duct injury. **(b)** Reconstruction consists of a side-to-side pancreaticojejunostomy including the resection cavity of the pancreatic head. D, duodenum; HA, hepatic artery; PD, pancreatic duct; RC, resection cavity of the pancreatic head; ST, stomach; T, tube; TC, transverse colon; UP, uncinate process. From Izbicki et al. [504], with permission from Lippincott Williams and Wilkins.

resection of the pancreatic head combined with longitudinal pancreaticojejunostomy (Fig. 69.12). This procedure was designed to improve decompression of the head of the pancreas, which often was not drained well by conventional pancreaticojejunostomy. Frey and Amikura [503] reported their results in 50 patients with chronic pancreatitis in a 7-year follow-up study. There were no operative deaths, and 22% of patients developed postoperative complications. Pain relief was complete or pain was improved in 87% of patients. Twenty percent of patients developed new-onset postoperative diabetes, and 11% experienced worsening of their diabetes.

The two techniques of duodenum-preserving resection of the pancreatic head (the Beger and Frey procedures) were compared in a prospective study [504]. There was no operative mortality in both groups, and postoperative morbidity was significantly lower in patients who had undergone the Frey procedure, which is technically less demanding. Both groups of patients reported similar postoperative decreases in pain and improved quality of life index scores. Postoperative pancreatic endocrine and exocrine function was also similar in both groups. These results are encouraging, but the mean follow-up of 1.5 years is relatively short. Long-term studies are needed to determine whether either or both procedures will suffer from attrition of the initial good results. It should also be noted that the duodenum-preserving resections are not appropriate for all patients with symptomatic chronic pancreatitis but seem especially useful for patients with predominant involvement of the pancreatic head.

Nealon and Matin [505] evaluated the success rates of different surgical procedures in relieving both the chronic abdominal pain and the recurrent exacerbations associated with chronic pancreatitis. Patients were divided into three groups based on the characteristics of their pain: severe unrelenting pain alone, severe pain with intermittent acute exacerbations, and intermittent acute exacerbations only. Postoperative pain relief was achieved in 83% of patients overall, with 86% relief from lateral pancreaticojejunostomy, 67% from distal pancreatectomy, and 91% from pancreatic head resection. It appears that pancreatic head resection was most effective for the relief of chronic pain (91%), whereas distal pancreatectomy was least effective (67%). On the other hand, lateral pancreaticojejunostomy gave the best relief of acute pain exacerbations (91%), whereas distal pancreatectomy was rather ineffective (33%). Pancreatic head resection was much more effective in reliev-

ing chronic pain (91%) than pain related to acute exacerbations (58%). These observations suggest that stratification of patients according to their pain patterns may be helpful to select the most effective surgical procedure for pain relief.

In patients undergoing a near-total pancreatectomy, islet cell autotransplantation by infusion of islet cell preparations into the portal system may prevent diabetes [506–508]. Islets from the resected pancreas can be isolated by mechanical disruption and collagenase digestion of the dispersed tissue and injected into the portal vein. Using this method, Farney and colleagues [508] reported that 9 of 22 patients with intraportal islet autografts were insulin independent for at least several months after surgery. Wahoff and associates [509] reported long-term insulin independence in 33% of 48 patients who underwent pancreatic resection combined with islet autotransplantation and followed up for 2–10 years. Insulin independence after islet autotransplantation correlated most closely with the number of islets transplanted and therefore relates to the available mass of pancreatic tissue and the degree of pancreatic fibrosis. Of those patients who received fewer than 200 000 islets, only 22% achieved long-term insulin independence. By comparison, nearly 60% of patients who received more than 300 000 islets achieved long-term independence.

With recent advances in islet isolation techniques, there appears to be an overall improvement of the results of islet autotransplantation. Recent studies indicate that about 50% of patients undergoing total or subtotal pancreatectomy can become insulin independent after intraportal autologous islet transplantation [510,511]. In the largest published series of 43 patients undergoing total or near-total pancreatectomy for chronic pancreatitis, insulin independence persisted in 34% of patients 2–10 years after surgery, and no graft failure occurred after 2 years [509]. Patient selection is important to determine the success of islet cell autotransplantation on diabetes after total pancreatectomy for chronic pancreatitis. Patients with end-stage fibrotic glands and those who are narcotic-dependent are unlikely to do well postoperatively [511].

Summary of treatment approach for control of pain

We recommend the following approach to treat the pain of chronic pancreatitis. When a patient first presents with pain or shows a different pain pattern, we determine whether there is an anatomical reason for the occurrence of pain or a change in pain. Pseudocyst, phlegmon, obstruction, and peptic ulcer are ruled out by appropriate tests. Patients are advised to stop all alcohol ingestion and to use salicylate or acetaminophen as needed. We also recommend the use of enzyme (e.g., 8 tablets of Viokase with each meal), especially in patients with non-alcohol-induced chronic pancreatitis. If pain continues, a trial of octreotide may be considered. After all medical measures have failed, a number of selected patients may benefit from pancreatic duct stenting and for others we recommend surgery; some centers, however, may consider celiac plexus block before surgery (Fig. 69.13).

Management of pancreatic insufficiency

At first glance, treating malabsorption secondary to pancreatic insufficiency might appear to be relatively easy. It would seem that simple oral replacement of pancreatic enzymes would be efficacious. Unfortunately, complete correction of steatorrhea is rarely accomplished. This is related to insufficient amounts of enzyme in oral supplements and acid pepsin inactivation of pancreatic enzymes.

The maximal postprandial delivery of pancreatic lipase is about 140 000 IU/h for 4 h after meals [270]. It has been shown that malabsorption does not occur if more than 5% of the normal maximal enzyme output is delivered to the duodenum. To meet this requirement, 28 000 IU of lipase should be delivered during a 4-h postprandial period. Pancreatic supplements are highly variable in enzyme activity, with lipase content ranging from 0 to 8000 IU per tablet [313,512,513]. Therefore, if the clinician uses a commercially available preparation containing 3500 IU per tablet of lipase, then under the best of circumstances, at least 8 tablets or capsules must be taken per meal to abolish malabsorption. Smaller amounts of enzymes may reduce steatorrhea but not abolish it. Therefore, the clinician should choose those commercial enzyme preparations that have been shown to contain higher lipase activity, and ensure that sufficient amounts have been used (Table 69.8).

Another important factor to consider is acid peptic inactivation of pancreatic enzymes. When duodenal samples were examined for lipase and trypsin activity, less than 8% of ingested lipase and less than 22% of ingested trypsin was recovered, regardless of the dosing schedule used (either prandial or hourly dosing). To eliminate steatorrhea in pancreatic insufficiency, a large number of tablets are necessary. Hyperuricosuria [514] and kidney stones have been associated with such high dosing of pancreatic supplements secondary to their high purine content, and therefore, this high dosing schedule may not be clinically justified. The relatively low delivery of lipase into the proximal small bowel compared with the delivery of trypsin with routine treatment schedules may explain the relative impossibility of completely correcting steatorrhea in pancreatic insufficiency as well as the usual correction of azotorrhea.

Preventing acid peptic neutralization of enzyme supplements by coating the capsules with acid-resistant and alkali-sensitive materials or by using antacids has produced mixed results [515,516]. In most studies, there has been little success with oral antacids or enteric coating with regard to clinical improvement or alleviating steatorrhea. Antacids increase gastric secretion, and dilution of enzyme concentrations below critical levels may explain the relative ineffectiveness of antacids [516]. Enteric coating is effective only if

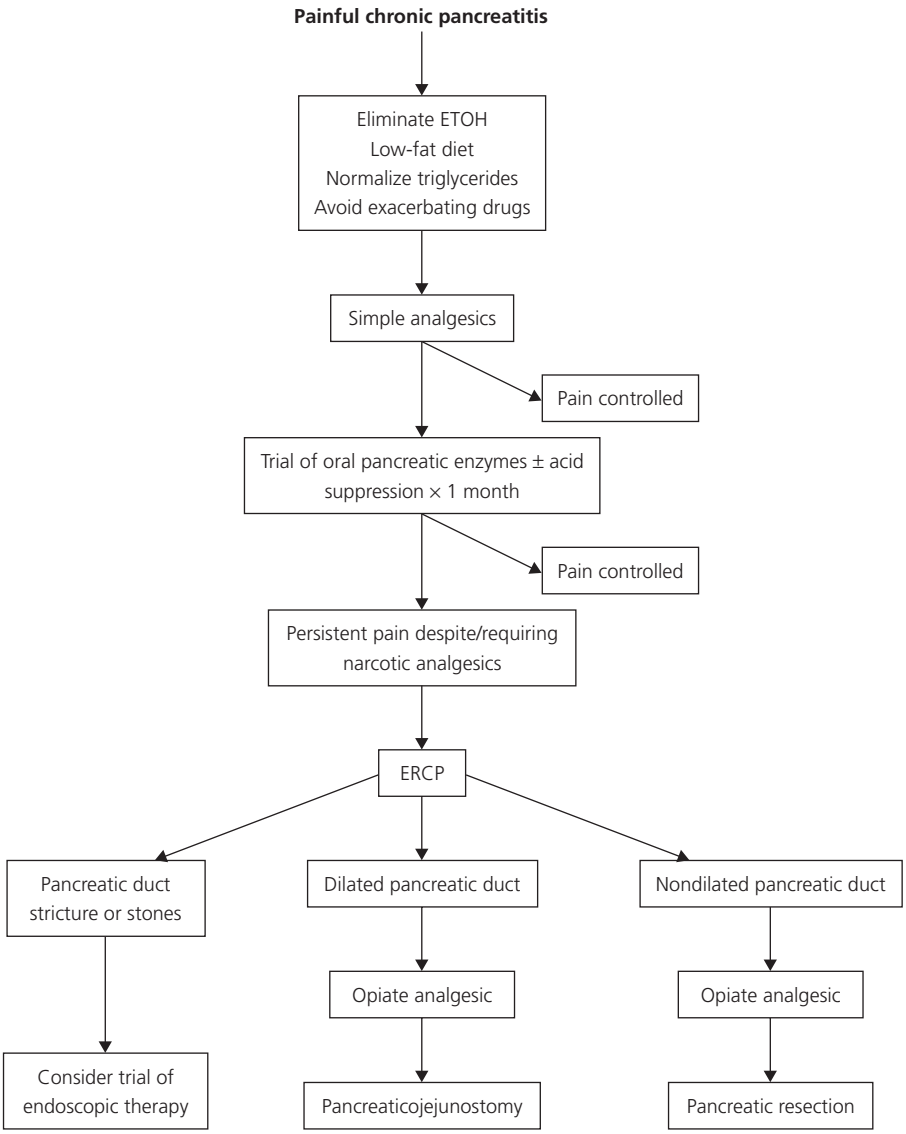

Figure 69.13 Algorithm for management of the patient with painful chronic pancreatitis. ERCP, endoscopic retrograde cholangiopancreatography; ETOH, methyl alcohol.

pancreatic enzymes are delivered into the duodenum with ingested food from the stomach and adequate intraduodenal dissolution occurs. Emptying of large tablets from the stomach may be different from that of typical antral contents, and duodenal and gastric pH have been shown to be low (pH < 4.0) for prolonged periods in many patients with pancreatic insufficiency [314,517], so enteric coating may not be an effective improvement over oral pancreatic supplements alone.

Pancrease (pancreatin coated with a pH-dependent polymer) is another improvement in pancreatic enzyme supplements [518]. This supplement is stable at an acidic pH (pH < 4.0) and dissolves at a pH greater than 5.0 so it is most effective (i.e., better than standard tablets alone) in patients who maintain acidic pH levels in the stomach postprandially. This maneuver delivers pancreatic enzymes to the upper small bowel intact, whereas standard supplements are irreversibly inactivated. In several studies, the mean reduction of steatorrhea with the use of Pancrease is not better than that with Viokase given with meals [516,519]. Normally, in chronic pancreatitis, the gastric pH increases to above 5.0 in the early postprandial period and then decreases to less than 4.0. It is likely that, under these circumstances, pancreatin initially will be liberated from its enteric coat, but its enzyme activity will be irreversibly inactivated when the intragastric pH falls to less than 4.0. Conversely, if gastric and duodenal pH remain low (pH < 4.0) throughout the postprandial period [520], the enteric coat of Pancrease remains intact as it traverses the upper gastrointestinal tract, and pancreatin is liberated en masse only as it reaches the jejunum, where intraluminal pH is greater than 5.0. This may explain the effectiveness of Pancrease in abolishing steatorrhea in some patients who secrete more acid and presumably can maintain an acidified upper small intestine for a prolonged period of time.

Table 69.8 Commercial pancreatic enzyme preparations

Preparation	Type	Content (Units)		
		Lipase	Amylase	Protease
Cotazyme	C	8000	30 000	30 000
Cotazyme-S	ECMS	5000	20 000	20 000
Creon 10	ECMS	10 000	33 200	37 500
Creon 25	ECMS	25 000	74 700	62 500
Ilozyme	UCT	11 000	30 000	30 000
Ku-zyme HP	ECMS	8000	30 000	30 000
Pancrease	ECMS	4000	20 000	25 000
Pancrease MT-4	ECMT	4000	12 000	12 000
Pancrease MT-10	ECMT	10 000	30 000	30 000
Pancrease MT-16	ECMT	16 000	48 000	48 000
Protilase	ECMS	4000	20 000	25 000
Viokase	UCT	8000	30 000	30 000
Viokase	P	16 800	70 000	70 000
Zymase	ECMS	12 000	24 000	24 000

Adapted from Berardi and Dunn-Kucharski [529], with permission from The McGraw-Hill Companies.
C, capsule; ECMS, enteric-coated microspheres encased in a cellulose capsule; ECMT, enteric-coated microtablets encased in a cellulose capsule; P, powder; UCT, uncoated tablet.

Until recently, the major criterion for determining the efficacy of enteric-coated enzyme preparations was the size of the microspheres, which influences the timing of enzyme delivery to the intestine. It has been shown that microspheres with a diameter of about 1.4 mm appear to mix with chyme most thoroughly and are emptied from the stomach at the same rate as food; large spheres remain in the stomach after food has been emptied into the small bowel and are less effective in correcting malabsorption in chronic pancreatitis [521]. Some commercially marketed microspheres (more than 2 mm) of pancreatin empty too slowly to be effective in the digestion of food. This may explain why some enteric-coated preparations are not effective in correcting pancreatic steatorrhea. However, a recent prospective, randomized, double-blind, multicenter, crossover trial in patients with chronic pancreatitis who have a stool fat excretion of 7.5 g/day showed that there was no difference between Creon given as microspheres with a diameter of 1–2 mm and as mini-microspheres with a diameter of 0.7–1.6 mm [522]. The mean stool fat excretion per day was about 16 g in both groups. Reducing microsphere size does not therefore improve the efficacy of the enzyme supplements. An explanation for this observation may be that microspheres aggregate in vivo into larger particles in the presence of free oil [523]. It should be noted that high doses of pancreatic enzyme supplements in children with cystic fibrosis have been associated with recurrent bowel obstruction as a result of fibrosing colonopathy, characterized by dense submucosal fibrosis in the large bowel. In recent years, two cases of fibrosing colonopathy have been reported in adult patients, one with and one without cystic fibrosis [524,525]. The cause of fibrosing colonopathy associated with high doses of pancreatins remains speculative [526].

Histamine H_2 receptor antagonists decrease acid production, pepsin activity, and gastric secretory volume simultaneously and should optimize pancreatic enzyme concentrations [516,527]. Duodenal lipase and trypsin activities are consistently increased with concomitant Viokase and histamine H_2 receptor antagonist treatment. Cimetidine should be considered only in patients who do not respond to standard oral pancreatic supplements because of lifelong increased cost and potential unknown long-term side effects. It has been shown that omeprazole further reduces fecal fat excretion in patients with cystic fibrosis receiving enzyme replacement for pancreatic insufficiency [528]. Therefore, in patients in whom histamine H_2 receptor antagonist fails to abolish steatorrhea, use of omeprazole should be considered.

We recommend the following approach to treat patients with pancreatic insufficiency. It is critical that sufficient amounts of enzyme tablets be given (e.g., 8 tablets of Viokase with each meal) to abolish azotorrhea and significantly reduce steatorrhea. Most patients on this regimen achieve satisfactory nutritional status and become relatively asymptomatic. In some of the symptomatic patients, the number of tablets given prandially can be increased or the amount of dietary fat can be reduced. These measures usually are effective in alleviating symptoms. In occasional patients, the use of Pancrease may be necessary. The addition of a histamine H_2 receptor antagonist or omeprazole should be reserved for those patients who are resistant to these maneuvers and who have documented acidic duodenal pH levels, because of cost considerations as well as potential long-term side effects. If all of these measures are ineffective, documentation of diagnosis (by pancreatic function testing) and exclusion of other contributing causes (celiac sprue, terminal ileal disease, or bacterial overgrowth) must be carried out.

Management of pancreatic diabetes

Clinical diabetes occurs relatively late in the disease and is an independent predictor of mortality of chronic pancreatitis. Ketoacidosis and diabetic neuropathy were relatively uncommon in this form of diabetes. This is probably because in pancreatic diabetes, insulin secretion is not completely lost, and glucagon secretion is reduced. Although some patients may respond to oral hypoglycemics, insulin is needed in most patients. Insulin requirements are usually lower than for most patients with genetic diabetes because insulin receptors usually are not down-regulated and insulin antibodies initially are not present. Overzealous control of blood glucose should be avoided because this may result in life-threatening hypoglycemia.

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E PANCREAS

70 Nonendocrine tumors of the pancreas

James J. Farrell, Howard A. Reber

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Ductal adenocarcinoma of the pancreas

Nonendocrine tumors of the pancreas cover a wide spectrum of pathologies, including solid tumors (adenocarcinoma, acinar cell carcinoma) and cystic tumors (serous cystadenoma, mucinous cystadenoma/adenocarcinoma, intraductal papillary mucinous tumor of the pancreas). However, by far the most common is pancreatic ductal adenocarcinoma, and consequently this will be the focus of this chapter.

It is estimated that 213 000 men and women die of pancreatic cancer worldwide each year. It is the fourth leading cause of cancer death in the United States (33 000 cases annually) and the sixth leading cause in Europe (30 000 cases annually) [1]. Most patients present late, have only a few months to live, and will die from the disease. Worldwide, the 1-year and 5-year survival rates are 25% and 5%, respectively. The mortality rate is nearly identical to the incidence rate. Survival is improved when disease is confined to the pancreas, although this only occurs in 15%–20% of cases.

Potentially beneficial developments continue to occur in the field of pancreatic cancer research. They include a rapid growth in the characterization of the genetic abnormalities associated with the disease, the appearance of new diagnostic techniques that have improved our ability rapidly to diagnose and stage it, and effective new therapeutic modalities, which allow nonsurgical palliation in many patients. Curative surgery is performed with operative mortality rates of only 1%–2%, compared with 10 times that in the 1980s. A number of new operations exist, which hold the promise of more effective treatment with less morbidity. In this chapter, we discuss these advances and present a broad overview of the diagnosis and management of patients with pancreatic neoplasms.

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Epidemiology and risk factors

Although the peak incidence of pancreatic carcinoma occurs in the seventh decade of life, 10% of patients develop pancreatic cancer at or before the age of 50 years [2]. There is a slight male predominance [3]. In general, high rates are found in developed industrialized countries (8.5 per 100 000 males; 5 per 100 000 females); lower rates occur in developing regions of the world (2.4 per 100 000 males; 1.6 per 100 000 females) [4]. The overall incidence of the disease is 30%–40% higher in the African-American population than in Caucasians in the United States (10.2 per 100 000 vs 6.6 per 100 000).

Smoking

The risk factor most strongly linked to pancreatic cancer is cigarette smoking, which may account for 25%–30% of cases [5,6]. It approximately doubles the chance of developing the disease. The relation between number of pack years of smoking and the risk of pancreatic cancer is not as strong as it is with lung (10–15-fold increased risk) or oropharyngeal cancers, but it is generally true that the risk is greater with more cigarettes smoked [7]. The risk of pancreatic cancer rapidly decreases when individuals discontinue cigarette use. The relative risk falls to about 1 only 10–15 years after quitting [8]. The smoking of cigars and other forms of tobacco probably does not increase risk.

Chronic pancreatitis

A case–control study for the United States Veterans Administration compared the occurrence of chronic pancreatitis in

2639 pancreatic cancer patients with a matched control group of 7774 subjects. A history of chronic pancreatitis increases the risk of developing pancreatic cancer anywhere from 2-fold to 15-fold in case–control studies [9–12]. In all types of chronic pancreatitis, the risk of pancreatic cancer is elevated. For example, patients with idiopathic or alcoholic pancreatitis have a 15-fold increased risk of pancreatic cancer [10]. For those with hereditary pancreatitis, the lifetime risk of pancreatic cancer is approximately 30%–40% [11,13]. For those patients with tropical pancreatitis (a type of chronic pancreatitis occurring in persons living in the southern regions of India or Africa), the risk of pancreatic cancer is also high [14]. This suggests that the etiology of the pancreatitis is not a factor, but that the changes associated with chronic inflammation and fibrosis in the pancreas are more important. A large number of epidemiological studies in both the United States and Europe have failed to find any consistent etiological association between alcohol consumption (in nonalcoholic patients) and the development of pancreatic cancer [5,6].

Coffee

In an Italian multicenter study, consumption of more than three cups of coffee per day increased the risk for pancreatic cancer even when the data were controlled for potential confounding factors such as cigarette smoking or alcohol use, and when the analysis was restricted to nonsmoking coffee drinkers [15]. However, a case–control study in Japan and a metaanalysis of data published between 1981 and 1993 did not support the notion that either caffeinated or decaffeinated coffee or tea were risk factors for pancreatic cancer [16].

Aspirin use

The role of aspirin use in preventing pancreatic cancer development is not clear. Whereas several case–control studies report no association between aspirin use or NSAID use or the duration of use and the risk of pancreatic cancer [17–19], other studies have variously shown both an inverse relationship and a positive correlation between aspirin use and pancreatic cancer [20–23].

Diet

In general, a high total energy intake correlates with an increased risk of pancreatic cancer [24,25]. Meats and foods of animal origin increase the risk [26,27]. Protein and fat consumption have been shown to be promoters of pancreatic carcinogenesis in experimental animals [3]. Clinical studies showed a correlation between meat consumption and pancreatic cancer [28,29]. The dietary nitrite content has been linked to pancreatic cancer, possibly through the formation of nitrosamines, which are potent carcinogens [30].

In an experimental model, caloric restriction has reduced the frequency of preneoplastic pancreatic lesions, but similar evidence in humans is not yet available [31]. Products of plant origin and dietary fiber, in particular, appear to be protective [32]. Supplemental intake of antioxidants (α-tocopherol or β-carotene) did not reduce the risk of pancreatic cancer over a follow-up period of 5–8 years [33]. There are conflicting data about the protective role of folic acid in pancreatic cancer [34–36].

Obesity

Prospective cohort studies including 46 648 men (ages 40–75) and 117 041 women (ages 30–55) showed that a body mass index (BMI) of at least 30 kg/m^2 was associated with an increased risk for pancreatic cancer [37]. In addition, total physical activity was shown to be inversely related to pancreatic cancer risk for those with a BMI of 25 or greater [37]. The increased risk associated with obesity is thought to be related to abnormal glucose metabolism, insulin resistance, and hyperinsulinemia commonly seen in obese individuals.

Diabetes mellitus

The diabetes–pancreatic cancer relationship has been confusing because some patients with pancreatic cancer develop diabetes as a manifestation of their underlying pancreatic tumor. The diabetes sometimes appears as an early manifestation of pancreatic cancer, occurring many months before the tumor becomes evident. Of the two types of diabetes, type II is the most frequently observed in adults; it is related to obesity and sedentary lifestyle and is the form linked to pancreatic cancer. In a metaanalysis of 20 studies with data on the duration of diabetes prior to pancreatic cancer, individuals with diabetic histories of 5 or more years had a two-fold elevation in pancreatic cancer risk compared with those without a history of diabetes, or diabetes of less than 5 years duration (relative risk, 95% confidence interval [CI] 1.2–3.2) [38]. A population-based study of over 2000 patients with diabetes suggests that approximately 1% of diabetes subjects over the age of 50 years will be diagnosed with pancreatic cancer within 3 years of first meeting criteria for diabetes [39].

Genetic factors

It is estimated that familial factors and genetic susceptibility play significant roles in the development of around 10% of pancreatic cancer cases [40]. In a prospective study of familial pancreatic cancer (FPC) kindreds, defined as kindreds having at least one pair of first-degree relatives with pancreatic cancer, and sporadic pancreatic cancer (SPC) kindreds, defined as families without such an affected pair, the observed-to-expected rate of pancreatic cancer was significantly elevated in members of FPC kindreds (9.0; 95% CI 4.5–16.1), but not in the SPC kindreds (1.8; 95% CI 0.22–6.4). This risk in FPC kindreds was elevated in individuals with three (32.0; 95% CI 10.2–74.7), two (6.4; CI 1.8–16.4), or one (4.6; CI 0.5–16.4) first-degree relative(s) with pancreatic cancer. Risk was greater in smokers than in nonsmokers [41]. The possibility of genetic anticipation in familial pancreatic cancer with the

presentation of cancer occurring earlier with more recent generations, has been suggested [42].

No single pancreatic cancer gene has been identified, but several inherited syndromes with an excess of pancreatic cancer cases have been described. These syndromes include familial breast cancer (*BRCA2* mutations), familial atypical multiple melanoma syndrome (FAMMM), hereditary pancreatitis, hereditary nonpolyposis colorectal carcinoma (HNPCC), Peutz–Jeghers syndrome, familial adenomatosis polyposis, Gardner syndrome, and von Hippel–Lindau syndrome [11,40,43–49]. It is also possible that polymorphic genes (e.g., single nucleotide polymorphisms) play a role in inherited and sporadic cancer. Because smoking is a strong risk factor for pancreatic cancer, it seems reasonable that the polymorphisms in genes that detoxify carcinogens in tobacco smoke could be important (e.g., cytochrome P450 system, *N*-acetyltransferases, glutathione-*S*-transferases) [50–52].

Pathogenesis and molecular pathology

Pancreatic cancer precursors lesions, also known as pancreatic intraepithelial neoplasia (PanIN), are seen more commonly in patients with pancreatic cancer compared with those who do not have the disease (Fig. 70.1) [53,54]. Pancreatic intraepithelial neoplasia (PanIN) precursor lesions can be graded from PanIN-1A (flat mucinous epithelium) to PanIN-2 (columnar epithelium with nuclear atypia) to PanIN-3 (in situ carcinoma) [54]. This histological- and cytological-based model of pancreatic precursor lesions is supported by molecular analyses (mutational analysis, immunohistochemical analysis, and loss of heterozygosity studies), which have identified most of the molecular changes seen in invasive ductal adenocarcinoma in PanIN lesions, with the prevalence of these genetic alterations increasing with increasing degrees of cytological and architectural atypia in the duct lesions in PanIN.

Oncogenes

Oncogene mutations – through point mutations, amplification, or chromosomal translocation – confer on the pancreatic cancer cell a "gain of function," usually manifested as a constitutively active and unrestrained growth-promoting protein, point mutations in the *KRAS* oncogene (most commonly in codon 12, but also codons 13 or 61), and overexpression of ERBB2 are the earliest genetic alterations occurring in pancreatic ducts with minimal cytological and architectural atypia (PanIN-1A and PanIN-1B) [55–61].

Tumor suppressor genes

Inactivation of tumor suppressor genes that have an important role in pancreatic oncogenesis, such as *p53*, *DPC4* (deleted in pancreatic carcinoma locus 4), and *BRCA2*, occurs in later stage precursor lesions. The *p53* gene product is inactivated by mutation in 50%–75% of infiltrating ductal carcinomas [62–65]. The p53 protein is a nuclear binding protein that regulates the transcription of factors important in cellular processes such as cell-cycle modulation, DNA surveillance and repair, differentiation, and apoptosis. The tumor suppressor gene *SMAD4* (small mothers against decapentaplegic 4; also called *DPC4* – deleted in pancreatic carcinoma 4) is a member of the transforming growth factor-β (TGF-β) family. Its protein product is a cytoplasmic protein that, in response to transforming growth factor binding to its receptor, translocates to the nucleus, binds the Smad-binding element of DNA, and regulates transcription. Inactivation of SMAD4 (DPC4) is present in approximately 55% of invasive pancreatic adenocarcinomas [66].

The *p16* tumor suppressor gene function is affected in virtually all infiltrating ductal adenocarcinomas by homozygous deletion or a combination of loss of heterozygosity (LOH) and point mutations or methylation of the promoter region [67]. The *p16* gene encodes a protein that serves as a cyclin-dependent kinase (inhibitor). Its interaction with cyclin C-complexes effectively arrests cells in the G1 phase.

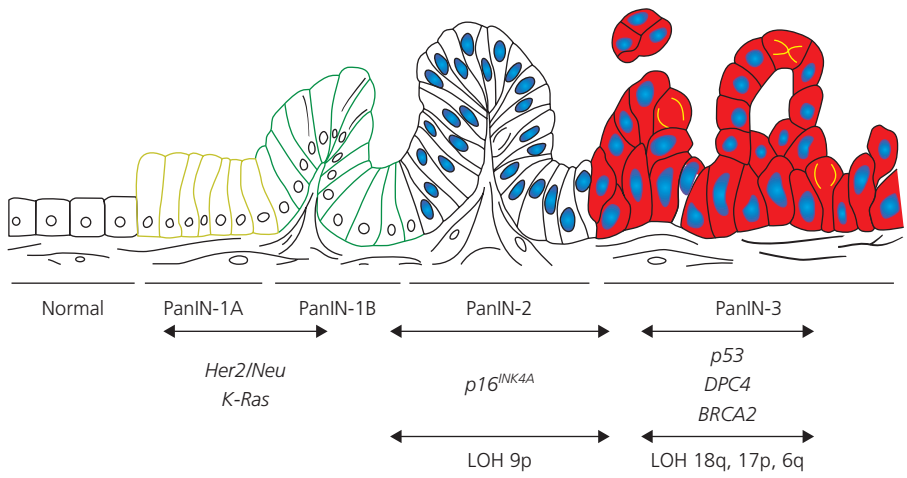

Figure 70.1 Pancreatic carcinogenesis: progression of pancreatic intraepithelial lesions (PanIN) from normal to invasive cancer. LOH, loss of heterozygosity. Adapted from Maitra et al. [62].

Inactivation results in loss of its restrictive action on cell growth.

Cell cycle regulators

Overexpression of cyclin D1 protein, a positive regulator of the cell cycle, has been documented immunohistochemically in 60%–85% of invasive pancreatic adenocarcinomas [68,69]. Cyclin D1 overexpression in pancreatic cancer has been associated with a poor prognosis and decreased survival [68,70], whereas decreased expression of cyclin D1 in pancreatic cancer has been associated with increased chemosensitivity and decreased expression of multiple chemoresistance genes [71].

Epithelial mucins

The apomucins MUC1, MUC2, and MUC5 are frequently overexpressed in epithelial cancers. The principal pancreatic mucin is MUC1, which is expressed in normal pancreatic ducts and acini [72]. The transfection of MUC1-expressing constructs into MUC1-negative tumor cell lines results in increased tumorigenicity and invasiveness in vivo; functional studies have shown MUC1 to be upstream of the oncogenic GRB2–SOS–RAS signaling pathway [73–75]. Expression of MUC1 is commonly (up to 100%) observed in invasive pancreatic adenocarcinomas at both the transcript and protein level. The gastric foveolar mucin, MUC5, is normally expressed in the gastric epithelium but is downregulated in the course of intestinal metaplasia. Unlike MUC1, it is not expressed in normal pancreatic ducts, but like MUC1, it is expressed in the majority of invasive pancreatic ductal carcinomas [62,76].

In addition to these gene mutations, there is also evidence that various growth factor receptors (e.g., epidermal growth factor [EGF] receptor, ERBB2, ERBB3, TGF-β receptors 1–3), growth factors (e.g., EGF, TGF-α TGF-β receptors 1–3, fibroblast growth factors [αFGF and βFGF]), and intercellular and endothelial-leukocyte adhesion molecules (e.g., intercellular adhesion molecule-1, endothelial leukocyte adhesion molecule-1) are upregulated in human pancreatic adenocarcinomas [77,78]. These changes may stimulate further tumor growth and enhance the metastatic behavior of pancreatic cancer cells, thus possibly contributing to shorter postoperative survival following tumor resection [77,79–81]. Vascular endothelial growth factor (VEGF) receptors and ligands promote endothelial cell growth and survival, enhancing angiogenesis. Expression of VEGF occurs in 90% of pancreatic cancers; it correlates with microvessel density and, at moderate–high levels, with reduced survival [82–84].

Developmental signaling and cancer stem cells

The Notch pathway plays an important role in directing decisions about the fate of cells in the developing pancreas and in pancreatic cancer initiation and invasion [85,86]. Upregulation of a number of Notch target genes is seen in preneoplastic lesions and in invasive pancreatic cancer [87]. Hedgehog signaling, which is crucial for the development of the gastrointestinal tract, has a major role in the initiation and growth of pancreatic cancer [88–90]. Cancer stem cells have a great potential for self-renewal and production of differentiated progeny. A $CD44^+CD24^+ESA^+$ phenotype (0.2%–0.8%) isolated from primary pancreatic cancer cells with a 100-fold increased tumorigenic potential has been described [91].

Pathology

Ductal adenocarcinoma and its variants make up more than 90% of all malignant exocrine pancreatic tumors. Others include acinar cell carcinomas, sarcomas, and malignant lymphomas. About two-thirds of ductal adenocarcinomas occur in the head of the gland; the rest occur in the body or tail, or diffusely throughout the pancreas. They are characterized by an intense desmoplastic reaction in which duct-like structures of varying degrees of differentiation are seen. The desmoplastic reaction comprises an extracellular matrix, together with a number of different host cell types, including fibroblasts, small endothelial-lined vessels, residual normal epithelia, and a variety of inflammatory cells, which are both locally derived and recruited from the circulation [92]. The interplay between all these cell types and the pancreatic cancer cells influences new blood vessel formation, invasion, metastasis, and evasion of the host immune system [93]. Because of their proximity to the intrapancreatic portion of the common bile duct, tumors in the head usually produce jaundice because they compress and obstruct the bile duct as they grow. They often obstruct the pancreatic duct as well, and although steatorrhea may result, there may not be any obvious symptoms. Tumors of the head of the pancreas are usually at least 2 cm in diameter when they are first diagnosed. Tumors of the body and tail commonly are larger (5–7 cm) and more advanced when they are discovered because they produce symptoms relatively later than head tumors. The symptoms from tumors in the body and tail are usually caused by malignant infiltration of the retroperitoneal structures and nerves, which produces pain. By the time the diagnosis is made, almost all are unresectable. Nevertheless, there is evidence that resectable body tumors have a similar prognosis to resectable tumors in the head of the gland. Approximately 70%–80% of adenocarcinomas of the head of the pancreas have metastasized to regional lymph nodes by the time they are discovered, which worsens the prognosis but does not preclude cure. These tumors also commonly invade lymphatic channels and perineural spaces. The prognosis is also influenced by the degree of tumor differentiation and by the presence of invasion of the retroperitoneal tissues adjacent to the cancer. Distant metastases (e.g., lung) may occur, but pancreatic cancer typically infiltrates locally into the adjacent structures (e.g., stomach, duodenum, colon,

Table 70.1 Staging of carcinoma of the pancreas

TNM classification	
T0	No evidence of primary tumor
Tis	In situ tumor
Tl	Tumor limited to the pancreas, < 2 cm in greatest dimension
T2	Tumor limited to the pancreas, > 2 cm in greatest dimension
T3	Tumor extends directly into duodenum, bile duct, or peripancreatic tissues
T4	Tumor extends directly into stomach, spleen, colon, or celiac axis vessels
N0	Regional lymph nodes not involved
N1	Regional lymph nodes involved
PN1a	Metastasis in a single regional lymph node
PN1b	Metastases in multiple regional lymph nodes
M0	No distant metastases
M1	Distant metastases present
American Joint Committee on Cancer (AJCC) stage	
IA	T1, N0, M0
IB	T2, N0, M0
II	T3, N0, M0
III	T1, N1, M0; T2, N1, M0; T3, N1, M0
IVA	T4, any N or M
IVB	any T, any N, M1

transverse mesocolon, portal and superior mesenteric veins, superior mesenteric artery). The liver is the most common site of intraabdominal metastasis, and peritoneal seeding of the tumor is also seen. In patients without distant spread, vascular invasion by tumor is the most common reason for unresectability (Table 70.1).

Clinical manifestations

The most common symptoms in patients with cancers in the head of the pancreas are jaundice and abdominal pain, usually with some degree of weight loss. In those with tumors of the pancreatic body, jaundice is unusual. Abdominal pain and back pain are common, and weight loss may be profound.

Pain

The cause of the abdominal and back pain associated with cancer in the head of the pancreas may be gallbladder and bile-duct distention associated with biliary obstruction, pancreatic duct distention associated with pancreatic duct obstruction, and invasion of retroperitoneal or somatic nerves. Abdominal pain associated with pancreatic cancer may also be as a result of acute pancreatitis, which may be the clinical presentation in 5% of pancreatic cancer patients. In the absence of another recognized etiology for an attack of pancreatitis, the possibility of an underlying carcinoma should be considered [94].

Jaundice

Jaundice, accompanied by pain, is the presenting symptom in 80%–90% of patients with cancer of the head of the pancreas. Painless jaundice is uncommon. Rarely, pancreatic malignancy can present with ascending cholangitis.

Weight loss

By the time the diagnosis is made, weight loss of more than 10% of ideal bodyweight is common. There are several possible explanations for such weight loss. The pain associated with the tumor produces anorexia in almost all patients, so decreased food intake is the most common cause. Malabsorption also may occur, especially in patients with pancreatic duct obstruction in the head of the pancreas, as digestive enzyme output from the pancreas may be prevented from entering the duodenum. It is less likely in those with pancreatic duct obstruction from body tumors, because enzymes from the pancreatic head drain normally, and are enough to maintain normal fat and protein absorption. Finally, there is the suggestion that a humoral substance secreted by the tumor increases catabolic activity [95].

Diabetes mellitus

Diabetes sometimes appears as an early manifestation of pancreatic cancer, occurring many months before the tumor becomes evident. Some 5% of patients with pancreatic cancer will have developed diabetes mellitus within the previous 2 years, and recent-onset diabetes may therefore serve as a warning sign [96].

Other clinical features

Light-colored stools and dark urine are characteristic of obstructive jaundice. Other symptoms include pruritus, nausea, vomiting, and weakness. Vomiting may result from duodenal or gastric outlet obstruction caused by tumor invasion. In some cases, when there is no apparent obstruction, it may be caused by abnormal gastric motility for as yet obscure reasons. Less common findings include superficial thrombophlebitis and gastrointestinal bleeding. In advanced stages, gastrointestinal bleeding may occur from direct invasion of the tumor into the duodenum, stomach, or colon.

Physical findings

Physical examination may reveal hepatomegaly, a palpable abdominal mass, or ascites. In the past, a distended palpable gallbladder (Courvoisier sign) was thought to be pathognomonic for bile-duct obstruction caused by a tumor. When gallstones caused the bile-duct obstruction, gallbladder distention was thought to be less likely because chronic inflammation of the gallbladder wall caused scarring and contracture of the viscus. However, the gallbladder is palpable only in about one-half of jaundiced patients with pancreatic cancer,

so the Courvoisier sign is of no practical diagnostic value. In those patients with pruritus, persistent scratching produces excoriation and lichenification of the skin. Rarely, an abdominal bruit can be heard if the tumor compresses the aorta or splenic artery.

Diagnostic investigations

Routine clinical tests

In patients with biliary obstruction, the total serum bilirubin and alkaline phosphatase concentrations are elevated. The total bilirubin level tends to be greater with malignant obstruction than obstruction from benign causes (> 15 vs 5 mg/dL). Serum amylase elevation is seen in only 5% of patients with pancreatic cancer.

Tumor markers

A variety of tumor-associated antigens have been studied as possible markers for pancreatic cancer. The most sensitive (80%) and specific (90%) is cancer antigen (CA) 19-9, but it still falls short of the ideal because it is almost never positive with small tumors (< 1 cm). Values of CA 19-9 also may be abnormal with other cancers (gastric, colorectal cancer) and with some benign conditions (cholangitis). Determination of serum CA 19-9 levels may be useful to provide some assurance that the tumor has been resected in its entirety, to signal the presence of recurrent disease after resection, and to determine the response to adjuvant therapy [97,98].

Rationale of the workup in patients with suspected pancreatic cancer

There are two main goals of the diagnostic workup in patients who are thought to have a pancreatic malignancy. The first is to establish the diagnosis with a high degree of certainty. In patients who are candidates for surgery, this usually does not require histological proof of malignancy. The second is to decide whether the patient should undergo an operation in an effort to either resect or palliate the disease. Because patients with unresectable pancreatic cancer have a median survival of no more than 1 year (Table 70.2), it is particularly important to avoid the morbidity (and cost) of an operation if it will not help [99].

Noninvasive techniques

Transabdominal ultrasound (US) may be useful as a first-line investigation in patients with jaundice to distinguish between intrahepatic and extrahepatic causes. Extrahepatic obstruction from a pancreatic (or any periampullary) cancer would be expected to show dilated intrahepatic and extrahepatic biliary radicles (Fig. 70.2). Transabdominal US can also identify gallstones that may be the cause of obstruction.

Table 70.2 Published survival after pancreaticoduodenectomy with tumor involving surgical margins (R1, R2)

Study	*n*	Survival
Neoptolemos [135]	101	11 months median
Sohn [136]	184	12 months median
Nishimura [137]	70	6 months median
Yeo [139]	58	10 months median
Nitecki [139]	28	6.8% 5-year survival
Willett [140]	37	12 months median

R1, positive margins by microscopy; R2, macroscopic tumor at surgical margin.

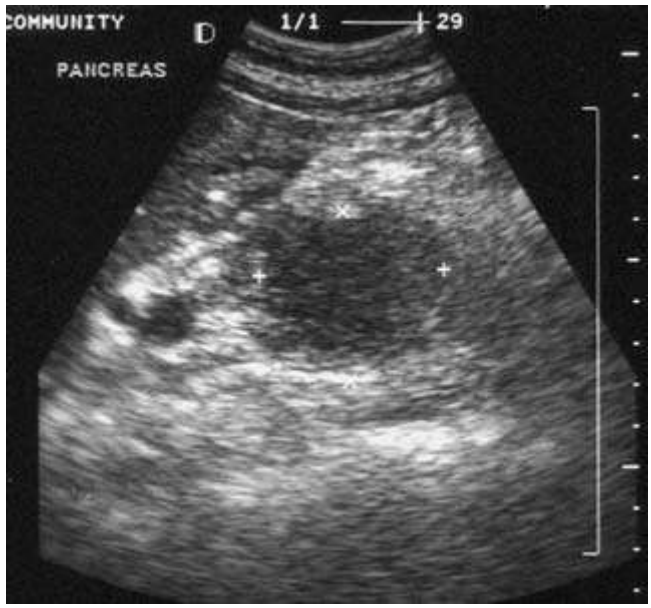

Figure 70.2 Transabdominal ultrasound in a patient showing a hypoechoic mass in the head of the pancreas.

Although transabdominal US provides some information about the relationship of the tumor to surrounding structures, computed tomography (CT) is much better in this regard. Transabdominal US is a relatively inexpensive test, with a sensitivity of 70% and a specificity of more than 90% for the diagnosis of pancreatic cancer [100–102].

Multidetector helical CT with high-quality thin sections is the best overall option for diagnosis, as well as the preoperative staging of pancreatic cancer. It provides information about the nature and site of the lesion (e.g., pancreatic versus other periampullary tumors, hilar bile-duct tumors), its resectability (e.g., presence of hepatic metastases, vascular invasion), and vascular anatomy. It is important to stress that helical CT is the state-of-the-art procedure. When helical CT is compared with the conventional technique, and especially when imaging of both the pancreas and liver is done

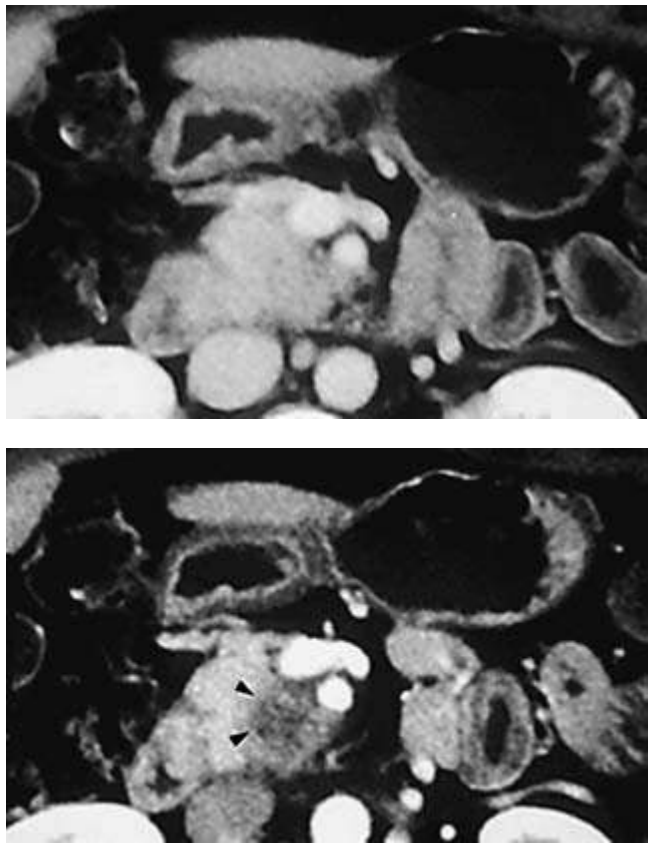

Figure 70.3 **(a)** On a conventional computed tomography (CT) scan, the primary tumor in the uncinate process of the pancreas is not seen. **(b)** The helical (spiral) CT scan performed 4 days later reveals the tumor (arrowheads). Courtesy of David Lu, UCLA Department of Radiology, Los Angeles.

separately according to published protocols, the differences in the sensitivity and specificity of the two approaches are dramatic [103] (Fig. 70.3). Extraordinary detail is provided about vascular anatomy and vascular invasion by the tumor (Fig. 70.4) [99,104]. A CT scan can detect tumors measuring about 2 cm or greater in diameter; these appear as lucent areas because they are less well perfused with blood than the adjacent pancreatic tissue. The technique has a false-positive rate of less than 10%, which is usually caused by focal pancreatitis, or variations in normal pancreatic anatomy that are mistaken for tumor.

Magnetic resonance imaging (MRI) may provide slightly better pancreatic tissue contrast than CT, but spatial resolution is inferior. Magnetic resonance cholangiopancreatography (MRCP) provides detailed ductal images without the risk of ERCP-induced pancreatitis [105]. It was hoped that MRI would be superior to CT in outlining the blood vessels, but such an advantage has not been demonstrated [106]. Consequently, MRI for pancreatic cancer staging is generally limited to instances in which patients cannot receive CT contrast (e.g., allergy to the dye, impaired renal function).

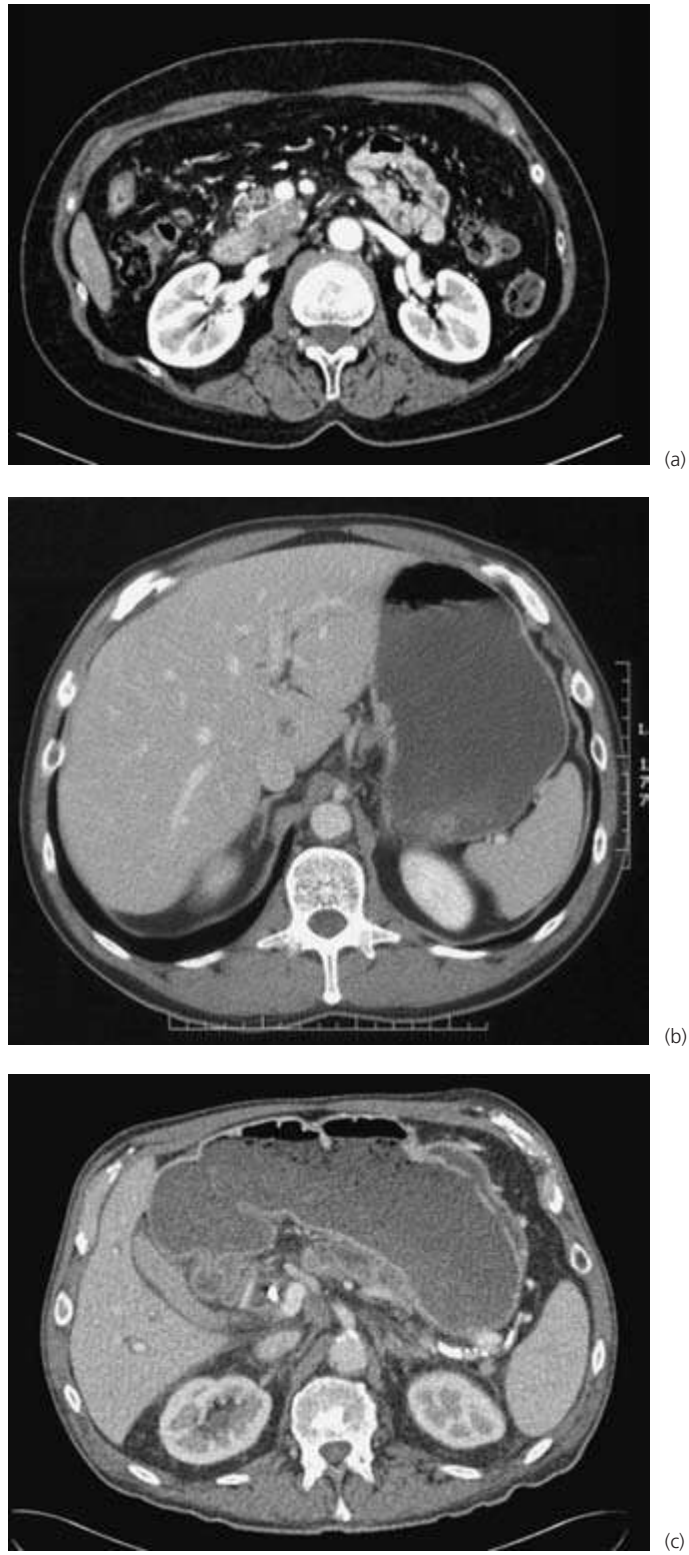

Figure 70.4 Computed tomography evidence of surgical unresectability. **(a)** Pancreatic head mass with superior mesenteric vein involvement. **(b)** Hypointense liver lesion suggestive of metastasis. **(c)** Celiac lymph node metastasis.

The value of positron emission tomography (PET) for the diagnosis of pancreatic malignancy is unclear. According to published studies, PET can identify pancreatic cancer and differentiate it from chronic pancreatitis with a sensitivity of 85%–98% and a specificity of 53%–93% [107,108]. Moreover, PET may be able to clarify diagnoses that are uncertain and to document metastatic disease where CT findings are equivocal [108]. However, it is still unclear how PET will fit into the overall pancreatic cancer staging algorithm [109,110].

Invasive techniques

Endoscopic retrograde cholangiopancreatography (ERCP) has a sensitivity of 95% and a specificity of 85% for the diagnosis of pancreatic cancer [111]. The classic finding that suggests pancreatic cancer is obstruction of both the bile and pancreatic ducts in the head of the pancreas, the so-called double-duct sign. In tumors of the body or tail of the pancreas, only the pancreatic duct is abnormal. At the time of ERCP, brushings for cytology may be obtained from the pancreatic duct, which has sensitivity of about 60% in proving the diagnosis of malignancy. Also at the time that the ERCP is done, a stent can be placed into the obstructed common bile duct; this has an important role in the palliation of patients who do not require surgery.

In spite of its value, diagnostic ERCP is not required in most patients with pancreatic cancer. Its role is confined to palliation of obstructive jaundice, particularly in patients who are not candidates for surgery. If a patient has a history typical for pancreatic cancer (e.g., pain, jaundice, weight loss) with a mass in the head of the pancreas evident on CT scan, then an ERCP is unnecessary for the diagnosis and will add nothing to the workup.

When tumors are small or poorly visualized on CT scan, endoscopic ultrasonography (EUS) provides a minimally invasive, accurate method of defining the extent of the primary tumor–vessel relationships and evaluating surrounding lymph nodes (Fig. 70.5). With surgical pathology as the gold standard, studies have shown that EUS accuracy in tumor staging and estimation of lymph node involvement ranges from 69% to 85% and 54% to 75%, respectively [106,112,113]. Lymph nodes infiltrated by carcinoma usually appear as round, hypoechoic masses more than 1 cm in diameter. Endoscopic ultrasonography can provide information about whether the tumor invades the portal, splenic, or superior mesenteric veins, the celiac axis, or superior mesenteric arteries. Common EUS criteria for vascular involvement include abnormal vessel contour, loss of vessel–parenchymal interface, tumor visible within the vessel lumen, and the presence of dilated peripancreatic venous collaterals [114]. When results are compared with surgical findings, EUS accuracy ranges from 69% to 93% [113–116], and its ability to predict tumor resectability ranges from 72% to 93% [113,116]. Endoscopic ultrasound can detect tumors smaller than those detectable by CT scanning techniques (< 2.0 cm in diameter), with sensitivity and specificity for tumor detection as high as 99% and 100%, respectively [114,117,118].

Figure 70.5 Endoscopic ultrasonogram showing a hypoechoic pancreatic head mass involving the portal vein.

Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) is the method of choice for obtaining a pathological diagnosis of malignancy (Fig. 70.6). Pretreatment confirmation of malignancy is critical for patients with locally advanced or metastatic disease who will be treated nonoperatively. Nearby lymph nodes and liver lesions can also be sampled using EUS-FNA to aid in local staging, with a low complication rate of 1%–2% [114]. However, for patients presenting with a low-density solid mass in the pancreas, who have resectable disease by CT criteria, a histological diagnosis of malignancy is unnecessary. In a patient with a negative CT scan but a history that suggests pancreatic cancer, EUS is useful because it is the most sensitive method for detecting a small tumor that has escaped detection by CT. Likewise, EUS may be useful to estimate the resectability of the tumor because of vascular involvement if the CT scan does not resolve that issue.

Percutaneous transhepatic cholangiography (PTC) is useful in patients with proximal bile-duct obstruction (e.g., from a tumor at the hepatic duct bifurcation) to delineate the bile duct anatomy on the hepatic side of the obstruction. However, MRCP may be the preferred technique in most of these patients. Endoscopic placement of a biliary stent is associated with a lower morbidity and procedure-related mortality rate than the percutaneous transhepatic route, because it minimizes the risk of bile leak and bleeding [119]. If an endoscopic approach is not possible, a combined approach involving the insertion of a transhepatic catheter and guidewire, which can be retrieved by the endoscopist, may be

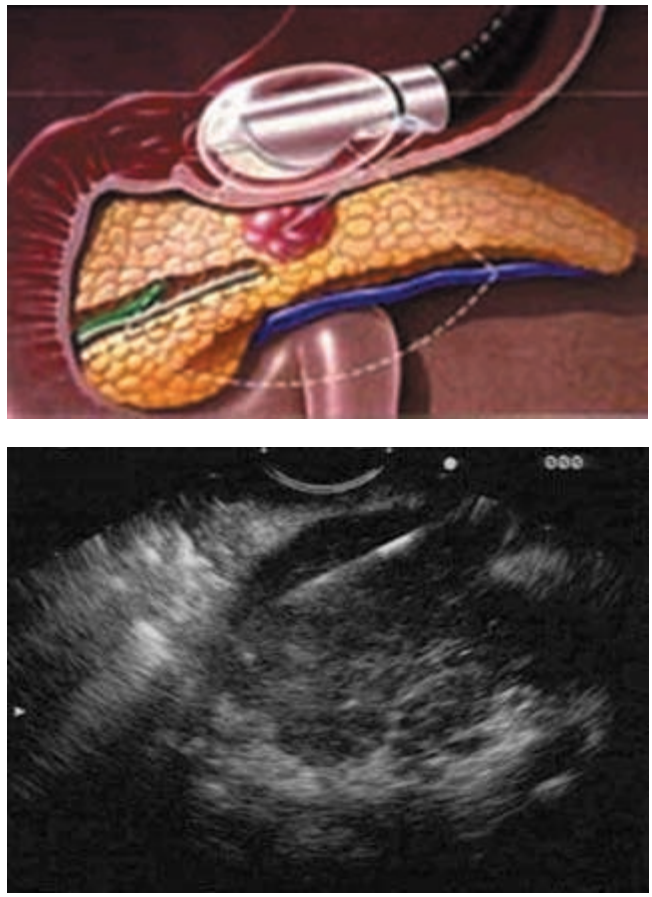

Figure 70.6 **(a)** Endoscopic ultrasound and fine-needle aspiration (FNA), demonstrating a linear echoendoscope biopsying the pancreas transgastrically. **(b)** Endoscopic ultrasound and FNA of a 2.5-cm localized hypoechoic mass in the head of the pancreas. The FNA needle is seen in the top right-hand corner. Cytological analysis of the aspirated specimen revealed adenocarcinoma.

used. This allows successful stent placement in a group of patients for whom endoscopic stenting alone is unsuccessful [120,121].

Computed tomography remains suboptimal for detecting small-volume peritoneal metastases and hepatic metastases measuring less than 1 cm. For this reason, laparoscopy, often combined with laparoscopic ultrasound, may be useful. Staging laparoscopy prior to laparotomy can provide tissue for the diagnosis of metastatic disease, which may avoid laparotomy in some patients [122]. There is debate about whether it should be used in all patients or confined to a selected group. We favor the latter, and often begin with staging laparoscopy when the preoperative CA 19-9 level exceeds 1000 U/mL, when imaging suggests advanced disease that cannot be confirmed by other techniques, or in many patients with cancers in the body or tail of the pancreas.

Tissue diagnosis

Preoperative FNA for cytology can be obtained percutaneously using a fine-gauge needle under CT or US guidance, or endoscopically by endoscopic ultrasound (EUS) or ERCP (Fig. 70.6). Complications include hemorrhage, pancreatitis, pancreatic fistula, and seeding of the needle tract with cancer cells, all of which are uncommon. Fine-needle aspiration should not be done routinely in the workup of patients with suspected pancreatic cancer, but it is indicated in situations where the cytological proof of malignancy will alter management (e.g., in a patient with an unresectable pancreatic tumor). A negative FNA does not rule out the possibility of malignancy, however.

Experienced pancreatic surgeons do not require histological proof of malignancy before proceeding with resection. Resection is performed on the basis of the patient's history, the information from preoperative tests, and the gross findings at operation. With this approach, experienced surgeons err less than 10% of the time (i.e., a resection is performed for what turns out to be benign disease) [123]. This is acceptable because the mortality rate of resection is low (1%–2%), and the majority of patients who undergo resection for benign disease have chronic pancreatitis for which resection is also appropriate.

Treatment: surgery

Preoperative management

As in all patients about to undergo major surgery, it is important to optimize cardiac, pulmonary, and renal function preoperatively. Although these patients have often lost weight, most are still nourished adequately to undergo the operation safely. However, if the serum albumin is less than 3 g/dL, or if surgery must be delayed for more than several weeks after the diagnosis is established, supplemental nutrition is often appropriate.

Preoperative biliary drainage

Because obstructive jaundice can cause defects in hepatic, renal, and immune function, it was hoped that preoperative relief of the jaundice would correct these defects, and decrease postoperative morbidity and mortality rates. Unfortunately, several randomized controlled studies have proven that routine preoperative bile duct stenting does not help [124–133] (Table 70.3). However, if the operation must be delayed several weeks (including in the setting of neoadjuvant therapy), or if cholangitis or pruritus occur, a plastic biliary stent (at least 10 French, if possible) should be placed endoscopically in jaundiced patients. The use of permanent metal biliary stents is discouraged in patients who are candidates for resection. These stents incite a severe inflammatory reaction and eventually may be incorporated into the bile-duct wall. This can complicate or even prevent the resection.

Table 70.3 Studies of preoperative biliary stenting in pancreatic cancer: infectious complications (%)

Study	*n*	Stented	Unstented
Povoski [128]	240	41	25
Sohn [129]	567	32	22
Hochwald [131]	71	66	38
Heslin [132]	74	46	11
Pisters [130]	265	37	11
Hodul [133]	212	28	20

Operative management: resection

The first successful resection for a periampullary cancer was performed as a two-stage operation by the German surgeon Kausch in 1909. In 1935, Whipple performed a similar procedure for an ampullary carcinoma, which by 1942 he had refined into a one-stage resection [134]. That operation has been called a pancreaticoduodenectomy, or Whipple resection, and it is similar to the one performed today (Fig. 70.7). The principal goal of the operation is the removal of all gross and microscopic disease within the pancreas and draining lymph nodes, a so-called margin-negative or R0 resection [135–140]. In the hands of experienced pancreatic surgeons around the world, the operative mortality rate of this procedure is 2% or less.

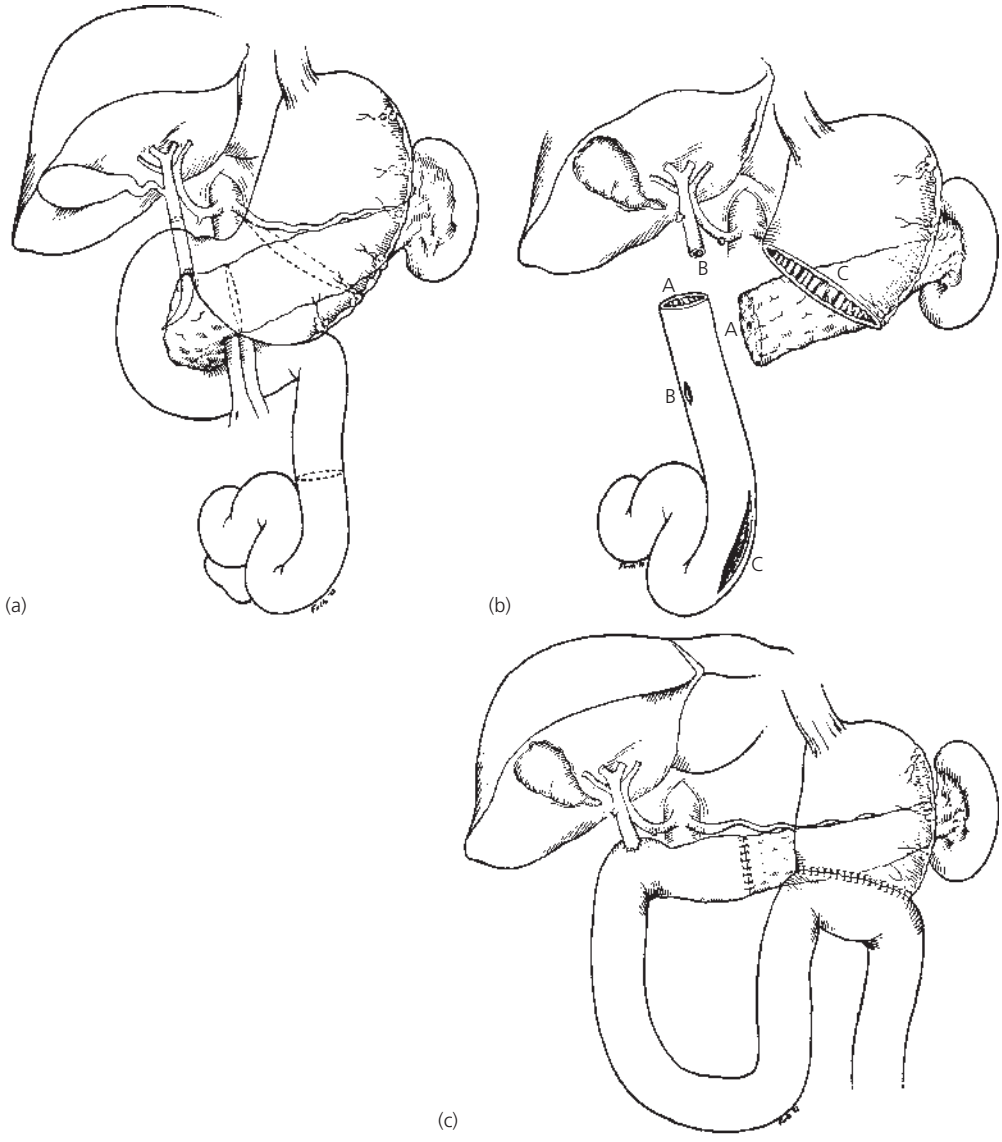

Figure 70.7 The standard Whipple pancreaticoduodenectomy. **(a)** Dashed lines indicate the resection margins in the typical operation for a tumor in the head of the pancreas. **(b)** The specimen has been resected. A, B, and C represent the sites for subsequent anastomoses between the bowel and the pancreas, bile duct, and stomach. **(c)** The completed anastomoses are shown.

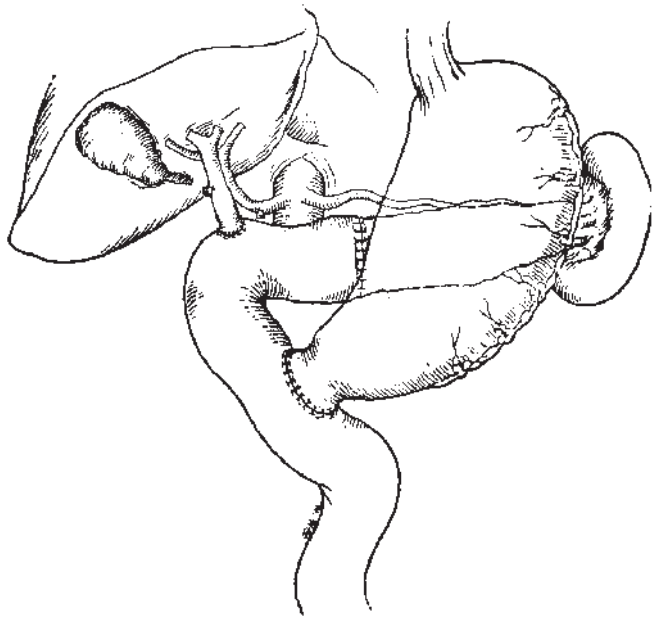

Figure 70.8 The pylorus-preserving modification of the standard Whipple pancreaticoduodenectomy is shown. The entire stomach, the pylorus, and several centimeters of duodenum are retained.

Resectable tumors in the body or tail of the pancreas require a distal pancreatectomy and splenectomy. The head of the pancreas, gallbladder, bile duct, stomach, and duodenum can usually be preserved because the tumor and its lymphatic drainage do not involve these structures. Mortality and morbidity rates are lower than after a Whipple resection. Because of the late presentation of symptoms, most patients with adenocarcinoma of the pancreatic body and tail present with locally advanced disease or distant metastases (or both), which precludes resection [141]. Central pancreatectomy, which may be used to resect benign tumors of the neck and mid-body of the pancreas, should not be used for pancreatic cancer. Nor should laparoscopic pancreatic resections be done for known malignant pancreatic disease.

Modifications of pancreaticoduodenectomy

Pylorus-preserving Whipple resection

Because of concern that the standard Whipple resection is associated with excessive weight loss and nutritional disturbances, many surgeons adopted this modification in which the stomach, pylorus, and first 2–3 cm of duodenum are preserved (Fig. 70.8). The operation was first described in 1944, but it did not become popular until the 1970s, when it was described for patients with chronic pancreatitis; later it was adopted for patients with pancreatic cancer as well [142]. The modified operation does not compromise the chance for cure of the cancer, and is associated with complication, morbidity, and mortality rates similar to the standard operation (Table 70.4) [143–149]. Most surgeons perform the two procedures interchangeably, reserving the standard Whipple for patients with larger, more extensive tumors.

Radical (extended) Whipple resection

Some surgeons have advocated the use of a more radical pancreatic resection to improve cure rates [150]. The standard Whipple (in which peripancreatic and subpyloric lymph nodes are generally removed) is modified by removal of more peripancreatic soft tissue and lymph nodes, often with resection of segments of the superior mesenteric and portal veins, when they appear to be involved by tumor. Although some of the uncontrolled studies reported improved survival, several prospective randomized trials have not [151–153]. Most surgeons in the United States are skeptical that the radical operation cures more patients, and it is rarely done in this country [154,155].

Vascular resection

Tumor involvement of the superior mesenteric or portal vein has generally been considered a contraindication to resection, although it is technically possible to remove limited segments of these vessels and reconnect the two ends. Retrospective data suggest that patients treated by pancreaticoduodenectomy combined with venous resection have a life expectancy similar to those treated with a Whipple resection

Table 70.4 Comparison of outcomes for standard pancreaticoduodenectomy and pylorus-preserving pancreaticoduodenectomy

Study	*n*	Outcomes
Lin [143]	31	Increased delayed gastric emptying in pylorus-preserving pancreaticoduodenectomy ($P = 0.08$)
Seiler [144]	77	Longer operative time in standard pancreaticoduodenctomy No statistically significant difference in the other parameters measured (operative blood loss, surgical morbidity, delayed gastric emptying, mortality, length of hospitalization), disease recurrence, and overall survival
Zerbi [145]	72	No significant differences
Tran [146]	170	No differences in median blood loss, duration of operation, postoperative delayed gastric emptying, positive margins of resection, median disease-free survival, overall survival

Table 70.5 Studies comparing portal vein/superior mesenteric vein resection (VR) with standard pancreaticoduodenectomy (PD)

Study	No. of patients		Results
	VR	Standard PD	
Furhman [157]	23	36	No difference in morbidity or mortality
Leach [158]	31	44	Median survival: 22 vs 20 months ($P = 0.25$)
Bachellier [159]	31	119	Equivalent 2-year survival rates
Nakogohri [160]	33	48	Median survival: 15 months vs 10 months ($P = 0.44$)
Howard [161]	13	23	Median survival: 13 months vs 12 months (P = NS)
Nakano [162]	146	54	Equivalent survival rates between groups
Tseng [163]	110	181	Median survival: 23.4 months vs 26.5 months ($P = 0.17$)

Adapted from Wray et al. [156].

without venous involvement, but that none are cured by the operation. Thus, although such locally advanced disease can be safely removed by extending the resection to include a segment of the vein, this should be viewed as an effort at palliation. However, involvement of the celiac axis, or hepatic or superior mesenteric arteries is an absolute contraindication to resection of the cancer (Table 70.5) [156–164].

Surgical outcomes

Surgical resection offers the only chance for cure. Nevertheless, according to the National Cancer Database, between 1985 and 1995 only 9% of more than 100 000 patients had their pancreatic cancer resected. The 5-year survival rates were between 13% and 17% for resection alone, and 23% for patients who had resection followed by adjuvant therapy. Selected series of patients who have undergone a curative (R0) resection have survival rates of 25%–35% after 5 years; however, up to one-half of the patients who survive 5 years still die from recurrent disease due to micrometastases unrecognized at the time of resection. Thus, adjuvant therapy is widely accepted as an important adjunct to management and the best hope for better long-term results.

Operative mortality and regionalization

Significant improvements in mortality, morbidity, and length of hospital stay have come as a result of improved operative techniques, anesthesia, and perioperative care [165–167]. As a result, mortality rates at most high-volume centers are less than 5%, and numerous centers report rates of under 2%. In specialist centers, resectability rates (the fraction of patients operated on who actually undergo resection) are around 80%, reflecting referral practices and case selection [165,168–170]. The overall 3-year survival is higher for patients treated at high-volume centers (37%) than at medium (29%) and low-volume (26%) centers [171]. Even after adjusting for perioperative deaths and case mix, patients treated at high-volume centers are less likely to experience late mortality [111,172–176]. The more frequently surgeons performed pancreaticoduodenectomy, the lower the in-hospital mortality rate, the length of stay, and hospital charges.

Palliation

Surgical palliation

During exploration, when resection of the primary tumor is not possible, the surgeon must decide whether any palliative procedures should be done. Because most patients with cancer in the head of the pancreas are jaundiced at the time of operation, a bypass to relieve the biliary obstruction is the most common procedure. An anastomosis between the gallbladder and the jejunum (cholecystojejunostomy) or common bile duct (choledochojejunostomy) are both effective bypass techniques (Fig. 70.9) [177,178].

At the time of the initial exploration, it is unusual that patients have duodenal obstruction from the tumor, but up to 15% develop it sometime before they die [178]. Thus, most surgeons perform a prophylactic gastrojejunostomy at the same time that a biliary bypass is done (Fig. 70.10). This is not associated with increased morbidity or mortality rates [179,180]. Occasionally, patients complain of vomiting preoperatively, and no objective evidence can be found for duodenal obstruction. In these patients, a gastrojejunostomy usually does not relieve the symptom, and the patient may still be unable to eat. The problem is probably caused by abnormal gastric motility, perhaps because of tumor infiltration of the nerve plexuses. Although prokinetic agents may help, the symptom is often refractory to all forms of treatment.

Nonsurgical palliation of biliary and duodenal obstruction

Endoscopic stenting of biliary obstruction represents an alternative to surgery in individuals with unresectable pancreatic cancer, which may avoid operation altogether. The

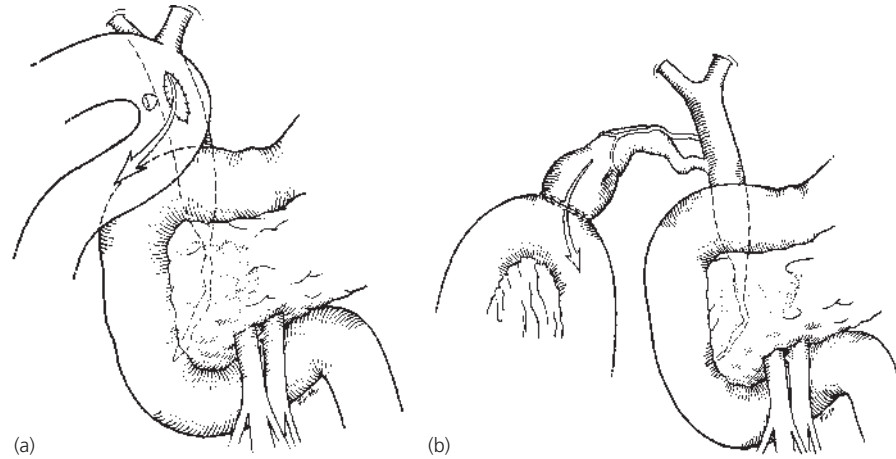

Figure 70.9 (a) Choledochojejunostomy and **(b)** cholecystojejunostomy. Each of these operations effectively relieves biliary obstruction by diverting the bile into the small intestine proximal to the tumor.

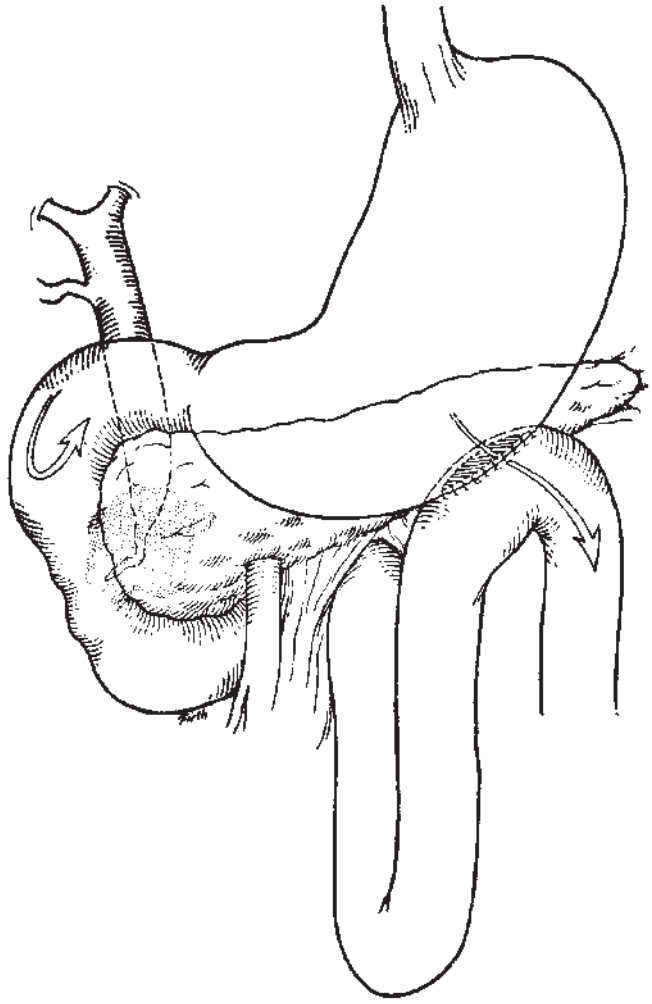

Figure 70.10 Gastrojejunostomy. The stomach can empty into the small intestine directly when the tumor obstructs the duodenum.

advantages and problems associated with both surgical and endoscopic approaches have been defined in several prospective randomized studies that compared the two [181–187]. In general, operative biliary bypass is more expensive, because of charges associated with the surgical procedure itself, and the hospitalization required for recovery. However, surgical bypass is more likely to provide permanent relief of the jaundice than are the endoscopically placed stents. Endoscopic stenting is associated with a lower procedure-related complication rate and mortality when compared with surgery [187]. It seems reasonable to reserve surgery for patients with both good performance status and small tumors, who are likely to survive longer than average, and to place a stent in patient with advanced tumors who are unlikely to survive longer than the usual patency of the stent.

Self-expanding metallic stents are larger (10 mm) than the 10–11-French plastic stents in common use, and they are less likely to become obstructed. As the lumen of this type of stent is so large, biliary drainage is superior to that seen with plastic stents and they rarely block due to debris. However, tumor ingrowth through the mesh of the self-expanding metal stents can occur. The use of thin membranes to cover self-expanding stents may minimize this problem.

To date there has been no randomized comparison between expanding metal stents and biliary bypass surgery. Laparoscopic techniques for both biliary bypass and gastrojejunostomy may decrease the cost and discomfort of the surgical approach, and permit more rapid hospital discharge [188,189]. On occasion, duodenal obstruction can be effectively treated with duodenal stents [190–192].

Malabsorption

Some patients with unresectable pancreatic cancer experience fat malabsorption because the tumor obstructs the pancreatic duct, and insufficient amounts of pancreatic enzymes

enter the duodenum. The result is diarrhea and steatorrhea, which interfere with the ability to gain weight and adversely affect quality of life. About 25% of patients who undergo pancreaticoduodenectomy also may suffer from malabsorption, which requires treatment. Although fat absorption itself cannot be restored to normal, the goal should be to stop the diarrhea, restore adequate nutrition, and maintain body weight. The principles of treatment require that about 30 000–40 000 U of lipase be supplied with each of four to five meals spread throughout the day.

Pain

Conventional treatment of the pain associated with pancreatic cancer includes both opioid and nonopioid analgesics as well as nonsteroidal antiinflammatory drugs, tricyclic antidepressants, and antineoplastic agents [193]. However, pain from advanced pancreatic cancer is often difficult to control, and narcotics may be ineffective. If this occurs, visceral pain may be managed by celiac plexus block, either at the time of surgery or later by a percutaneous or endosonographic approach (Fig. 70.11). Pain is relieved in up to 90% of patients, and significant complications are uncommon [194,195]. Other approaches to pain control in locally advanced or metastatic pancreatic malignancy include pancreatic ductal decompression (endoscopic or surgical), external beam radiation, or chemoradiation [178,196–198].

Figure 70.11 Endoscopic ultrasound-guided celiac nerve block for palliation of pain.

Systemic therapy

Adjuvant therapy

Patients with no detectable evidence of residual disease at the time of resection are likely to harbor microscopic tumor deposits that, if left untreated, will lead to tumor recurrence and death. These patients may benefit from adjuvant therapy after the tumor has been removed surgically. Randomized phase III trials studying the role of chemotherapy (5-fluorouracil, 5-FU) and chemoradiation therapy in the adjuvant setting, suggest that postoperative adjuvant therapy improves survival in patients with resected adenocarcinoma; consequently, this has become the standard of care. Unfortunately, median survival is less than 2 years, and long-term survival is under 20% in most phase III trials. Results from two large randomized trials show that adjuvant systemic chemotherapy increases the 5-year survival from 9%–12% with resection alone to 21%–29% and 23% with either 5-FU and folinic acid or gemcitabine, respectively [199,200]. The role of radiation therapy in this setting has been brought into question by the ESPAC 1 study, which concluded that patients who had radiation alone or in combination with chemotherapy fared worse than those who had chemotherapy alone [135, 199]. However, the mode of radiation therapy delivery, the use of bolus injections of 5-FU, and the lack of quality control for the radiation therapy has raised questions about that conclusion, and the role of radiation remains unsettled. An American Intergroup trial (RTOG 9704) studying adjuvant chemoradiotherapy has reported median and 3-year survival rates. This study used background 5-FU-based chemoradiotherapy together with pre- and post-chemoradiation systemic chemotherapy comprising either 5-FU or gemcitabine. The results show no difference in median survival or 3-year survival across all patients. There was, however, a significant improvement in survival with the gemcitabine-based treatment in patients who had tumors of the pancreatic head [201].

Locally advanced disease

Treatment of locally advanced disease (tumor extension into adjacent major blood vessels or organs, preventing complete surgical resection with negative margins) in the United States has been defined by three randomized trials: two early Gastrointestinal Tumor Study Group (GITSG) trials and an Eastern Cooperative Oncology Group (ECOG) trial [202–204]. The treatment includes continuous infusion 5-FU and gemcitabine in combination with radiation therapy. Based on the results of the available randomized trials, it is unclear if radiation therapy is an essential element of treatment of patients with locally advanced pancreatic cancer.

Metastatic disease

Metastatic pancreatic cancer tends to be a rapidly progressing disease, often accompanied by a constellation of debilitating

symptoms. An important phase III randomized trial compared weekly gemcitabine ($n = 63$) with weekly bolus 5-FU ($n = 63$). Overall survival in patients treated with gemcitabine was significantly improved compared with patients treated with 5-FU (median survival 5.7 vs 4.4 months, respectively; $P = 0.0025$). The 1-year survival rates were 18% for patients treated with gemcitabine vs 2% in patients treated with 5-FU. Clinical benefit response was experienced by 24% of the patients treated with gemcitabine compared with only 5% of the patients treated with 5-FU ($P = 0.0022$) [205]. Most studies have shown no benefit in adding an additional agent to gemcitabine, compared with gemcitabine alone, in the management of metastatic pancreatic cancer. However, a randomized phase III trial has demonstrated statistically significant improved survival in advanced pancreatic cancer by adding erlotinib – an oral HER1 (epidermal growth factor receptor, EGFR) tyrosine kinase inhibitor – to gemcitabine in the management of locally advanced and metastatic pancreatic cancer [206].

Neoadjuvant therapy for locally advanced disease

Neoadjuvant therapy in patients with locally advanced disease may downstage the tumor and allow resection with clean surgical margins. There are several theoretical benefits to this approach where the treatment is given *prior* to surgery. Candidates for neoadjuvant therapy include not only patients with lesions of borderline resectability (e.g., those with CT or EUS evidence of vascular involvement), but also the larger group with radiographically resectable pancreatic cancer. However, for this latter group, no trial has ever shown an improvement in either resectability rate or survival when comparing preoperative with the more common adjuvant therapy. Therefore, neoadjuvant therapy has not been used widely and still must be considered experimental.

Prognosis

Surgical resection provides the only chance for cure, but the median survival after resection is only 18–20 months, and the overall 5-year survival is 20%. However, if the tumor is removed with clear surgical margins and no lymph node metastases are found, the 5-year survival may be as high as 50%. Various prognostic indicators for survival after resection for pancreatic cancer have been evaluated. One of the strongest factors is negative lymph node status. In one center, a median survival of more than 4.5 years was seen in patients with negative nodes, vs 11 months when nodes were involved. Unfortunately, even small tumors have metastasized to nodes in up to 80% of cases. Poorly differentiated tumors are associated with a worse prognosis than well-differentiated ones (10% vs 50% 5-year survival). Patients with smaller tumors (< 2 cm in diameter) tend to have a better prognosis than patients with larger tumors. Another important prognostic factor is whether the resection margins of the resection specimen are involved with tumor.

Less common exocrine pancreatic tumors

Mucinous cystadenoma and cystadenocarcinoma

Mucinous cystic neoplasms are large (often > 5 cm), bulky, unilobular, or multilobulated macrocysts containing mucin (Fig. 70.12). The cysts are lined with mucinous columnar epithelium that forms papillary projections, which may contain varying degrees of dysplasia ranging from benign to borderline to invasive cancer. They are associated with "ovarian stroma," which is pathognomonic of the process. Because mucinous cystadenomas are premalignant, they should be resected in most patients. Ninety percent of these tumors occur in the body/tail region of the pancreas, and the appropriate treatment is distal pancreatectomy. These neoplasms comprise 2%–5% of exocrine tumors and are more common in women (female-to-male ratio 9:1). The peak age of occurrence is 40–60 years [207]. They appear to have a better prognosis than ductal pancreatic cancers; the 5-year survival after a curative resection of a mucinous cystadenocarcinorna is at least 30%.

Serous cystadenoma and cystadenocarcinoma

These lesions are typically large, well-demarcated, multiloculated microcystic tumors. They usually have a characteristic honeycomb appearance, and the individual cysts are 1 cm or less in diameter, which is smaller than the individual cysts in mucinous cystic neoplasms (Fig. 70.13). They account for about 1% of neoplastic pancreatic lesions and usually occur in older adult women in the sixth and seventh decades of life.

Figure 70.12 Computed tomography scan of a patient with a malignant mucinous cystadenocarcinoma, which was resected.

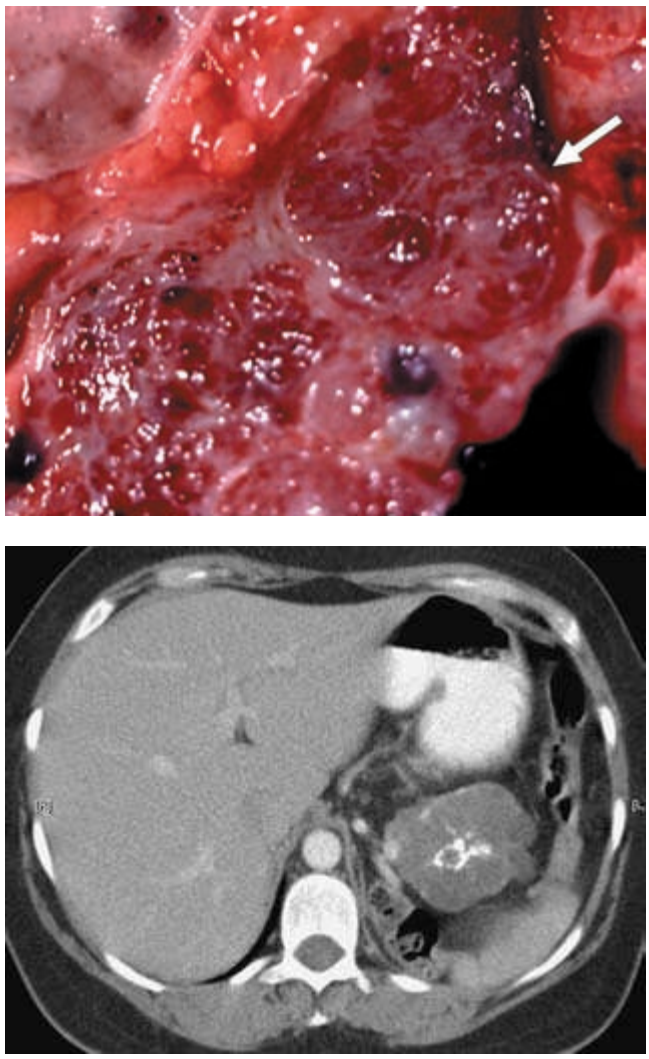

Figure 70.13 **(a)** Surgical resection of a serous cystadenoma (arrow) with the honeycomb appearance. **(b)** Computed tomography scan of patient with benign serous cystadenoma with the characteristic central calcification.

These so-called microcystic adenomas are usually asymptomatic and are found incidentally when a US or CT scan is done for some other reason. One-third of the tumors occur in the head of the pancreas [208,209]. Malignant transformation of serous lesions is rare. Although surgical resection offers a definitive diagnosis, because of the low risk of malignancy, surveillance is also an option for the management of these tumors. These neoplasms, as well as the mucinous cystic tumors previously discussed, should not be confused with pseudocysts, where internal or external drainage procedures are acceptable forms of treatment.

Solid and papillary epithelial neoplasm

Solid and papillary epithelial neoplasms appear to be increasing in frequency [210,211]. They are most common in adolescent girls and young women, and have a characteristic appearance on CT scan. The tumors are often large and may produce vague abdominal discomfort, which leads to their discovery. They have a favorable prognosis, and most patients are cured by resection. Nevertheless, some of these tumors may recur locally and there is one report of liver metastasis. The majority of these tumors occur in the body or tail of the pancreas, where they are treated with distal pancreatectomy. When they occur in the head, pancreaticoduodenectomy is required.

Intraductal papillary mucinous neoplasm

Patients with intraductal papillary mucinous neoplasm (IPMN) often present with repeated episodes of inflammation from underlying chronic pancreatitis [212]. In fact, these patients are likely to present with abdominal pain as the principal complaint. The pancreatitis is caused by obstruction of the pancreatic duct by intraductal tumor growth and inspissated mucus, which these tumors secrete (Fig. 70.14a). A dilated

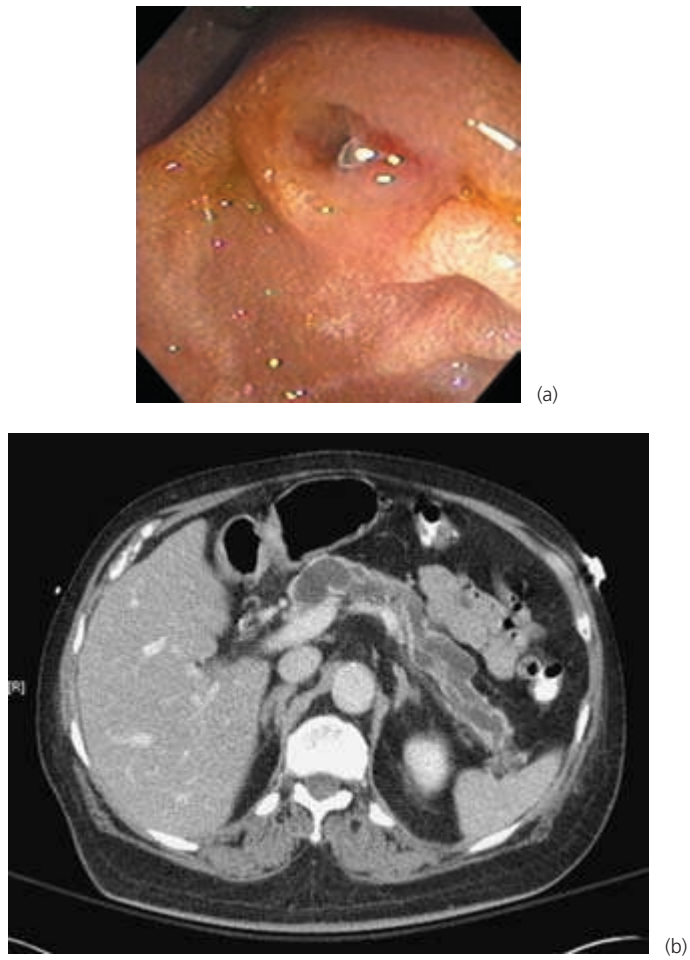

Figure 70.14 Main-duct intraductal papillary mucinous neoplasm (IPMN). **(a)** Endoscopic image of mucin extruding from ampulla in a main-duct IPMN. **(b)** Computed tomography imaging revealing a diffusely dilated main pancreatic duct in main-duct IPMN.

pancreatic duct is revealed by CT scans (Fig. 70.14b). The ductal dilation is confirmed by ERCP, which often shows intraductal mucus appearing as filling detects. A glob of mucus emanating from the gaping orifice of the ampulla of Vater is characteristically seen at the time of ERCP (Fig. 70.14a). Based on the origin of the disease, IPMN is classified into main duct type variant and a branch duct type (Fig. 70.15). Approximately half the lesions show papillary malignant changes, but even these tumors have a better prognosis than the usual ductal adenocarcinoma. A review of a single center's experience with 60 resected IPMNs revealed that these tumors are being recognized more frequently [213]. In this series, 37% of the resected IPMNs were associated with an adjacent invasive adenocarcinoma, and the 5-year survival for those patients undergoing IPMT resection was 57% [213]. Branch-duct-type IPMNs are considered to have less malignant potential compared with main-branch-type. Treatment is required both to remove any premalignant or malignant disease that may be present and also to relieve the episodes of pancreatitis. This may require total pancreatectomy because the entire pancreatic duct can be affected. Because of the lower risk of malignancy, surveillance has been advocated in patients with side branch IPMT [212].

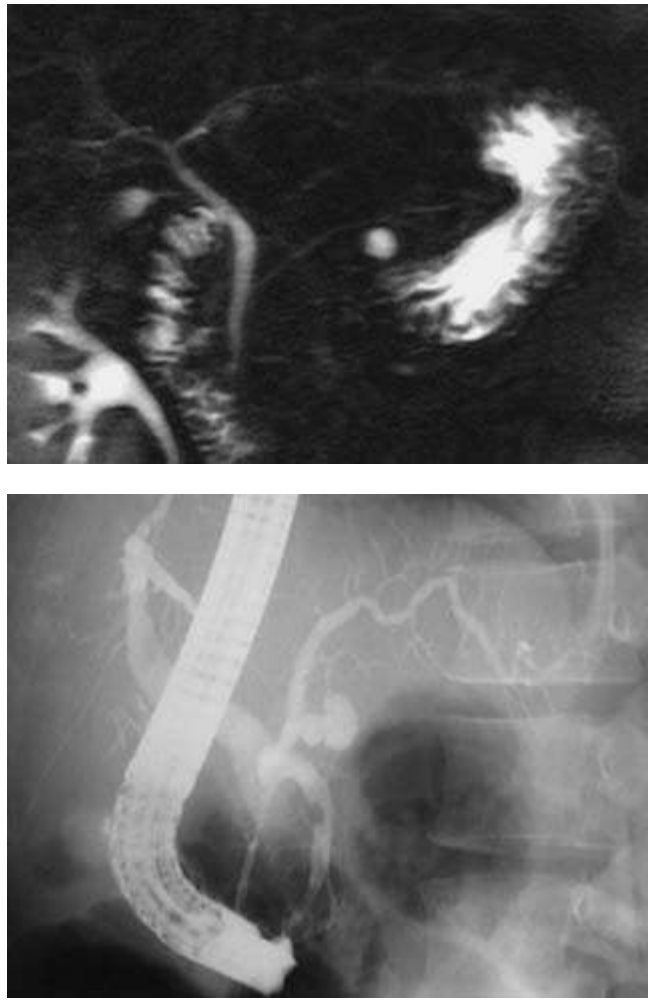

Figure 70.15 Branch-duct intraductal papillary mucinous neoplasm. **(a)** Magnetic resonance cholangiopancreatogram showing a normal pancreatic duct with an associated pancreatic body cyst. **(b)** Endoscopic retrograde cholangiopancreatogram showing opacification of a side-branch cyst.

Acinar cell tumor

Acinar cell carcinomas are uncommon and characterized by an acinar arrangement of cells supported by minimal fibrous stoma [214]. Zymogen granules are present, which may be identified by electron microscopy. Patients may have elevated serum lipase levels and associated nonsuppurative panniculitis of the extremities and bone marrow, and manifest subcutaneous nodules and polyarthritis. These tumors usually occur in adults in their sixth to eighth decades of life [215].

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E PANCREAS

71 Endocrine neoplasms of the pancreas

Robert T. Jensen

Epidemiology, 1875
Pathology and tumor biology, 1876
Clinical features and diagnosis, 1879
Tumor localization, 1890
Treatment, 1897

Endocrine tumors of the pancreas are classified according to the type of clinical syndrome present (Table 71.1). In general they share a number of common features including various aspects of pathology, natural history, considerations in treatment, localization studies, and treatment of metastatic tumors. In each case, except for nonfunctioning pancreatic endocrine tumors (PETs), the principal clinical manifestations of the endocrine tumor are due to the extensive release of hormones by the tumor into the circulation. Each of the various hormones released in the different syndromes occurs naturally and is important in mediating various physiological processes (see Chapter 41). However, in the PET syndromes these hormones are not under normal physiological regulation and are released autonomously by the tumor. Pancreatic endocrine tumors are classified as either functional or nonfunctional (Table 71.1) depending on whether a clinical syndrome caused by an ectopically released hormone is present. Nonfunctional PETs frequently ectopically release hormones and peptides – pancreatic polypeptide (PP), chromogranin, neurotensin, or neuron-specific enolase; however, these cause no distinct clinical syndromes [1–6]. The term "nonfunctional PET" is commonly used and will be retained here to mean any PET not secreting products that cause a distinct syndrome although some actually release peptides and are, strictly speaking, functioning. The term "pancreatic endocrine tumor" (PET) is also retained in this chapter to indicate the tumors listed in Table 71.1, although it is also a misnomer in that a number of these tumors also occur outside the pancreas – i.e., gastrinomas, VIPomas (vasoactive intestinal polypeptide), somatostatinomas, GRFomas (growth hormone-releasing factor), and ACTHomas (adrenocorticotrophic hormone) (Table 71.1). These tumors are also frequently called islet cell tumors; however, it is unproven that they originate from pancreatic islets [7,8]. It is important to realize that although these tumors are usually slow growing in many cases, some are aggressive [2,9,10], therefore effective therapy will require treatment of the autonomous hormone overproduction and treatment directed at the tumor itself. In this chapter the common features of these tumors will be dealt with together and the distinctive features separately. Gastrinoma was discussed in Chapter 4 and will, in general, not be dealt with.

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Epidemiology

In general, PETs are uncommon, having a prevalence of less than 10 per million population [2,11–13]. In some older series insulinomas were reported to be the most common PET, with an incidence in various series of 0.8–0.9 per million population per year, whereas gastrinomas were reported to occur in 0.1–0.4 per million per year [14]. However, in subsequent studies [11,14], gastrinomas were as common as insulinomas (1–3 new cases/year/million population). The remaining symptomatic PETs each occur in fewer than 0.5 per million per year [11,12]. Approximately 400 cases of glucagonoma have been reported worldwide [15–18], 250 cases of VIPomas [19–21], and 201 cases of somatostatinomas [22–26]; the number of cases of GRFomas is unclear. The relative frequency of these tumors is insulinomas, nonfunctional PETs, and gastrinomas occurring approximately with equal frequency, 2–8 times more commonly than VIPomas, and 17–30 times more commonly than glucagonomas [2,11,12]. Somatostatinomas were equal in frequency to glucagonomas in some studies, but less common in others [2,11,12]. In various series PETs had an incidence equal to [12], or half that [27] of carcinoid tumors. In autopsy studies, PETs occur in 0.5%–1.5% of cases [4,9,12]; however, in less than 1 in 1000 cases was a functioning PET thought to occur [9,12]. In surgical studies nonfunctioning PETs are reported to comprise 15%–33% of all PETs removed [1,28–30], and in a large pathology series they accounted for 36% of all PETs [4]. In addition to the established PETs listed in Table 71.1, it has been proposed [31] that PETs secreting calcitonin cause

Table 71.1 Endocrine tumors of the pancreas

Name of tumor	Name of syndrome	Signs or symptoms	Primary location	Hormone causing syndrome
Gastrinoma	Zollinger–Ellison syndrome	Abdominal pain Diarrhea Esophageal symptoms	Pancreas 60% Duodenum 30% Other 10%	Gastrin
Insulinoma	Insulinoma	Hypoglycemic symptoms	Pancreas 99–100%	Insulin
Glucagonoma	Glucagonoma	Rash, anemia Diabetes/glucose intolerance Weight loss Thromboembolic disease	Pancreas 99–100%	Glucagon
VIPoma	Verner–Morrison Pancreatic cholera WDHA	Severe watery diarrhea Hypokalemia	Pancreas 90% Other 10% (neural, adrenal, periganglionic tissue)	VIP
Somatostatinoma syndrome	Somatostatinoma	Diabetes mellitus Cholelithiases Diarrhea Steatorrhea	Pancreas 56% Duodenum/jejunum 44%	Somatostatin
GRFoma	GRFoma	Acromegaly	Pancreas 30% Lung 54% Jejunum 7% Other 13% (adrenal, foregut, retroperitoneum)	GRF
ACTHoma	ACTHoma	Cushing syndrome	Pancreas 4–16% all ectopic Cushing	ACTH
PET causing carcinoid syndrome	–	Diarrhea Flushing	Pancreas 100%	Serotonin, tachykinins, prostaglandins
PET causing hypercalcemia	–	Symptoms due to increased calcium	Pancreas 100%	PTHrP
Nonfunctioning	PPoma Nonfunctional	Weight loss Abdominal mass Occasionally asymptomatic hepatomegaly	Pancreas 100%	None (PP or chromogranin released but no symptoms due to hypersecretion)

ACTH, adrenocorticotrophic hormone; GRF, growth hormone-releasing factor; PET, pancreatic endocrine tumor; PP, pancreatic polypeptide; PTHrP, parathormone-related peptide; VIP, vasoactive intestinal polypeptide; WDHA, watery diarrhea, hypokalemia, achlorhydria.

a distinct syndrome with diarrhea; however, too few have been described to establish them as constituting a distinct syndrome. In addition, rare cases have been described of PETs secreting renin and causing hypertension [32]; secreting erythropoietin to cause erythrocytosis and polycythemia [33]; secreting luteinizing-hormone and causing masculinization [34,35], and ovarian tumors secreting peptide YY (PYY) causing constipation [36–38].

Pathology and tumor biology

Pancreatic endocrine tumors share a number of pathological features [8,39]. These tumors frequently contain ductular structures and produce hormones not normally produced in the adult pancreas. Therefore they are thought by some to originate from an immature stem cell [4]. Because endocrine cells bud off from ductules during ontogenesis of the pancreas and ductular structures are present in these tumors, it has been suggested these cells may originate from ducts. A study in patients with multiple endocrine neoplasia type I (MEN-I), 80%–100% of whom develop PETs, provided evidence that the PETs originate from ducts [40], whereas a second study concluded they developed from islets [41]. The tumors are thought to originate from cells that are part of the diffuse neuroendocrine cell system [8,42]. These cells share cytochemical properties, and these tumors, together with carcinoid tumors, medullary carcinoma of the thyroid,

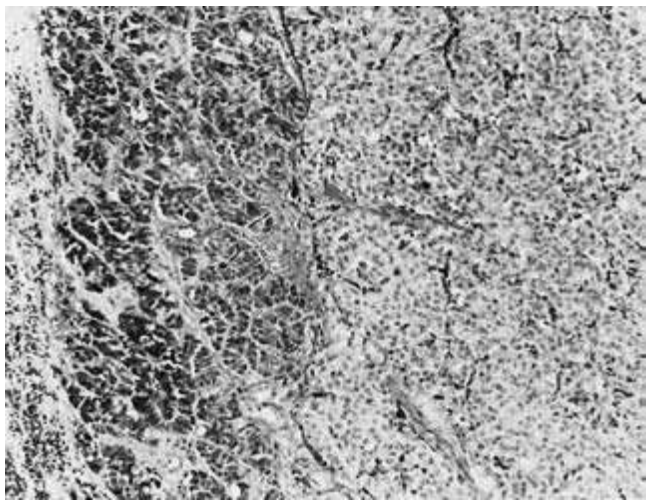

Figure 71.1 Photomicrograph of an insulinoma. This insulinoma (right) demonstrates a typical histological pattern seen with pancreatic endocrine tumors, with monotonous sheets of small, round cells with uniform nuclei and cytoplasm. The tumor is well marginated and interfaces with the normal pancreas (left) by a distinct margin. (H & E stain; original magnification × 100.)

melanomas, and pheochromocytomas, have been called APUDomas (amine precursor uptake and decarboxylation) [2,8,42,43]. The tumors are composed of monotonous sheets of small round cells with uniform nuclei and cytoplasm (Fig. 71.1). Mitotic figures are uncommon. Ultrastructurally, the tumors demonstrate electron-dense granules that contain various peptides, amines, neuron-specific enolase, synaptophysin, and chromogranin A and C [2,8,39,43]. Frequently PETs are multihormonal when examined by immunocytochemical techniques [4,8,14,28,44,45]. In various series over 50% of all PETs contain more than one hormone as determined by immunocytochemistry [14,44–46]. In insulinomas, for example, glucagon has been identified by immunocytochemical methods in 0%–44%, somatostatin in 0%–18%, gastrin in 3%–11%, PP in 18%–39%, and ACTH-like immunoreactivity in 11% [28,43]. Particularly common are PP cells, which have been identified in 22%–39% of insulinomas, 0%–67% of glucagonomas, and 50%–75% of VIPomas [28]. In some cases numerous hormones are released into the circulation whereas in others only one peptide may be detectable [28,44,45]. It is not apparent why patients with a given PET syndrome present only with a syndrome characteristic of hypersecretion of only one of these peptides; although the tumor contains multiple peptides [47].

In addition to producing and frequently releasing multiple gastrointestinal peptides, PETs produce a number of products that are characteristic of neuroendocrine differentiation and are used to identify these tumors [8,39]. These general markers are neuron-specific enolase, chromogranins A, B, and C, synaptophysin, epitope Leu-7, protein gene product (PGP)-9.5, and the alpha or beta subunit of human chorionic gonadotropin (α- or β-HCG) [3,12,48–52]. Chromogranins are acidic glycoproteins that are present in almost all endocrine or neuronal tissue [3]. Chromogranin is released into the plasma in patients with PETs or carcinoid tumors [27,48,53,54], and elevated plasma levels of chromogranin A, B, or C are reported in more than 90% of such patients [3,4,27,48,49,54]. Increased plasma levels of α-HCG and β-HCG also occur in PETs and have been reported by some to be suggestive of malignancy; however, this remains controversial [2,3,14,28,48,55]. Neuron-specific enolase (NSE), the γ-γ dimer of the glycolytic enzyme enolase, occurs in the cytoplasm of most neuroendocrine cells, is present in most PETs as well as carcinoids and other APUDomas [3,42,51], and is frequently released into the plasma and can be used as a tumor marker [48,56]. Synaptophysin is a calcium-binding vesicle membrane glycoprotein that is synthesized independently of the other neuroendocrine proteins [12]. The presence of chromogranin immunoreactivity in the tumor is the most generally used marker to identify a pancreatic tumor as endocrine in origin [8,39].

Because patients with PETs are living longer as a result of increasingly effective therapy, the development of a secondary symptomatic PET syndrome may become more common [44,45]. One study suggests that a secondary symptomatic PET syndrome is common, occurring in 7.5% of patients with a mean follow-up of 19 months [44], whereas another study suggests it is uncommon, occurring in only 2% of patients with gastrinoma followed for a mean duration of 11 years [45]. Because of the frequent presence of multiple peptides in the PET as shown by immunocytochemistry, it has become increasingly difficult, if not impossible in most cases, to determine immunocytochemically which of the hormones found in the tumor are clinically important in that patient [14,43]. Therefore, a functional PET syndrome should only be diagnosed if the clinical symptoms are present and plasma elevations are documented, and not on the basis of immunohistochemical results only.

A WHO classification has been proposed for PETs, as well as carcinoids and other gastrointestinal (GI) neuroendocrine tumors, assigning the tumors to one of three categories (well-differentiated tumor, well-differentiated carcinoma, poorly differentiated carcinomas) based on their size and histology, including measures of proliferation (mitoses, proliferative antigen Ki-67) [8,39]. Furthermore, for the first time a consensus conference has proposed a TNM staging classification of PETs and other foregut neuroendocrine tumors, and also proposed a working formulation for the grading of these tumors based on mitotic index and the Ki-67 index [57]. This classification system is meant to help clinicians in the stratification, treatment, and follow-up of these patients [57]. In general, histological classification of PETs has failed to predict the growth pattern of the tumor or determine whether it is malignant [8,14,43,58]. Furthermore, there is no definite

correlation between the histological pattern and the type of clinical syndrome the tumor is associated with [14,43,59]. Therefore, at present malignancy can only be unequivocally established in those patients who are demonstrated to have metastatic tumor spread either to lymph nodes or the liver, gross invasion or infiltration into adjacent organs, or clear blood vessel invasion [14,43]. Because of this it is not fully established what percentage of PETs are malignant. The benign nature can only be established by long-term follow-up. In general, 5%–10% of insulinomas are reported as malignant, whereas in various series 50%–90% of the other tumors are reported as malignant [2,9,14,18,19,22,25,43,59,60].

The size of the tumor is usually not related to the severity of the hormonally induced symptoms; however, in general, there is a correlation between size and the occurrence of malignancy [9,10,46,57,59,61]. Whereas insulinomas, like gastrinomas, are generally small (< 2 cm), glucagonomas and the other PETs are larger at the time of detection (frequently > 5 cm) [14,18,19,22,25,59,60]. The majority of the primary PETs are solitary and encapsulated when not associated with MEN-I (see below) [8,39]. When metastatic spread occurs it is usually to regional lymph nodes first and later to the liver. Late in the course of the disease, metastases to bone may also occur [10,62].

Pancreatic endocrine tumors may occur either in a nonfamilial form (sporadic) or be associated with an inherited syndrome [7,8,63–66]. Inherited syndromes associated with PETs include MEN1 and three phacomatoses (von Recklinghausen disease, von Hippel–Lindau disease, and tuberous sclerosis) [12,63,65]. Multiple endocrine neoplasia type I (or Wermer syndrome) is an autosomal dominant disorder characterized by tumors or hyperplasia of multiple endocrine organs. Hypercalcemia due to hyperparathyroidism is the most common abnormality (i.e., occurring in 97% of patients) [63,65,67], whereas nonfunctional PETs are the second most frequent abnormality, occurring in 80%–100%. Functional PETs occur in 40%–80% of patients with MEN-I. The incidence of gastrinomas, insulinomas, glucagonomas, and VIPomas in MEN-I is 54%, 21%, 2%–3%, and 1%–3%, respectively [4,68]. Pituitary and adrenal cortical adenomas also occur but are less common [4,67]. The chromosomal defect in MEN-I maps to chromosome 11q13, and a study [69] using positional cloning identified the *MEN1* gene in this region; the gene is proposed to function as a tumor suppressor gene [68]. The *MEN1* gene contains 10 exons and encodes a unique ubiquitously expressed 610-amino acid protein, called menin, a nuclear protein that binds to numerous transcription factors [65]; however, its exact function remains unclear. Studies [70,71] suggest a similar double acquired loss of the *MEN1* gene may be important in sporadic (noninherited) PETs because 32%–38% of these have loss of heterozygosity at the 11q13 locus [65,71]. Furthermore, 27%–39% of sporadic PETs have mutations in the *MEN1* gene, suggesting that some sporadic PETs have a pathogenesis similar to PETs in patients with MEN-I [71]. The exact percentage of patients with PETs who have MEN-I varies in different series and with different tumors, from less than 5% to 25% [14,72]. Multiple endocrine neoplasia type I is present in 20%–25% of patients with gastrinomas [14,73], in 4%–6% with insulinomas [60], in 13%–17% with glucagonomas [17,18], in 6%–11% of patients with VIPomas [19,74], in 7% with somatostatinomas [25], and in 33% with GRFomas [22]. The recognition that the PET can be part of the MEN-I syndrome is important because multiple PETs are frequently present [14]. Furthermore, screening of family members for various features of the syndrome is indicated [14].

Almost all insulinomas either occur in the pancreas (Table 71.1) or are attached to it [60,75–78] although rarely an aberrant insulinoma may be found in carcinoid tumors in the duodenum, ileum, lung, or mesentery [60,75]. Multiple insulinomas occur in only 10%–13% of cases, and in these patients the presence of MEN-I should be suspected [59,60]. Glucagonomas occur almost entirely within the pancreas, usually in the pancreatic body and tail [16–18,59,79,80]. In contrast to insulinomas or gastrinomas, the majority of glucagonomas are usually large (3–10 cm) at the time of diagnosis [16–18,59,79,80]. Almost all VIPomas in adults are pancreatic in location (i.e., > 90%); however, occasionally the VIPoma syndrome is reported in association with a pheochromocytoma or carcinoid tumor of the intestine [19–21]. In children, the VIPoma syndrome is often caused by a ganglioneuroma or ganglioneuroblastoma [21,81,82]. VIPomas are usually large tumors [19,21,80]. Somatostatinomas, similar to gastrinomas, frequently are found in extrapancreatic locations (Table 71.1) [14,22,23,25]. In various reviews [22,25], 47%–56% of somatostatinomas are in the pancreas, usually in the pancreatic head, and 44%–52% are extrapancreatic, usually in the small intestine (duodenum or jejunum). Similar to glucagonomas or VIPomas, somatostatinomas tend to be large (mean size 5 cm) [23,25,83]. Nonfunctioning PETs and PPomas occur in the pancreas, and are usually greater than 5 cm in diameter [1,30]. Other less common functioning PETs such as those associated with acromegaly (GRFomas) [23,84], carcinoid syndrome [85], paraneoplastic hypercalcemia [28,86,87], or Cushing syndrome [28,88–90] are usually large tumors within the pancreas.

In addition to the MEN-I syndrome, there are reports of PETs in patients with von Recklinghausen disease [24,66,91–93], von Hippel–Lindau disease [66,94], and tuberous sclerosis [66]. In a number of patients with von Recklinghausen disease duodenal somatostatinomas are reported [24,25,66,92,93] as well as, rarely, Zollinger–Ellison syndrome [91]. In von Hippel–Lindau syndrome, 10%–17% of the patients have a PET, which is usually nonfunctional, but occasional insulinomas and VIPomas are described [4,66]. Somatostatinomas have also been reported in von Hippel–

Lindau disease [95]. Among patients with tuberous sclerosis, three were reported with insulinomas and one with a nonfunctioning PET [4,66].

For a time the molecular pathogenesis of PETs was largely unknown [7,64,65]. In contrast to most common nonendocrine tumors (e.g., pancreatic, colonic, gastric adenocarcinomas), alterations in neither common tumor suppressor genes (retinoblastoma gene, p53 gene) nor common oncogenes (e.g., *JUN*, *SRC*, *RAS*, *FOS*, *MYC*) are generally present in PETs or the closely related carcinoid tumor [7,63–65], suggesting they are not generally important in their molecular pathogenesis. However, studies suggest alterations in the *MEN1* gene (as discussed above), the *p16/MTS1* tumor suppressor gene, and in the *DPC64/SMAD4* gene; amplification of the *HER-2/neu* (ERBB2) protooncogene; and loss of an unknown tumor suppressor gene on chromosome 1 or 3p, which could also be important [7,9,63–65]. Comparative genomic hybridization and genome-wide allelotyping studies show that both chromosomal losses (especially 1p, 3p, 3q, 6p, and 22q) and gains (especially 7q, 17q, 17p, and 20q) are common events, and that the frequency of these changes differs between PETs and carcinoids, suggesting that they have a different pathogenesis [64,65]. There are mutations in the *MEN1* gene in one-third of sporadic PETs [71], and alterations in the *p16/MTS1* gene in 50%–92% of PETs, suggesting that these are particularly important. Whether the presence of these alterations carries a worse prognosis is unclear.

Clinical features and diagnosis

In each PET syndrome, except with nonfunctioning tumors, early symptoms are caused by the excessive hormone release, whereas late symptoms (e.g., cachexia, pain, bleeding) are primarily due to the metastatic spread of the tumor per se.

Insulinoma

Insulinomas are endocrine tumors that originate in the pancreas, secrete excessive amounts of insulin, and cause a distinct syndrome characterized by symptoms caused by hypoglycemia.

Insulinomas rarely occur in adolescence. The average age at presentation in most series is between 40 and 50 years, with 60% of the insulinomas occurring in females [4,59,60,76,76,77,96].

The clinical manifestations of insulinomas are due to the hypoglycemia secondary to the excess unregulated insulin secretion by the tumor. The symptoms are typically associated with fasting, that is, the attacks characteristically occur before breakfast, when a meal is delayed, or hours postprandially. The majority of patients have symptoms of central nervous system dysfunction secondary to hypoglycemia (neuroglycopenic symptoms), which include headaches, confusion, lightheadedness, visual disturbances, irrational behavior, drowsiness, or even confusion resulting in coma. Patients with insulinomas misdiagnosed as psychiatric or neurological problems have been reported [12]. The majority of patients also have symptoms due to catecholamine excess secondary to the hypoglycemia, such as sweating, tremor, palpitations, and irritability [59]. The average time from onset of symptoms to diagnosis is 3 years, although some studies suggest this to be decreasing [4,97].

The presence of an insulinoma should be suspected in all patients with hypoglycemia, especially patients with a history of fasting hypoglycemia or with a family history of MEN-I. Fasting hypoglycemia can result from a number of different conditions (Table 71.2) [59,76,98–100]. It should be remembered that insulinoma is only one cause of fasting hypoglycemia, and other causes should also be suspected such as inadvertent or deliberate administration of insulin, ingestion of oral hypoglycemia agents, alcoholism with severe liver disease or malnutrition, or other extrapancreatic tumors (Table 71.2). The diagnosis of insulinoma requires the demonstration of hypoglycemia combined with an inappropriate elevation of plasma insulin concentration and ultimately a PET. Insulinomas characteristically cause hypoglycemia during fasting, in contrast to postprandial hypoglycemia, which is seen in a number of conditions such as after gastric bypass surgery for obesity [100–102].

The key to establishing the diagnosis is suspecting the symptoms could be due to hypoglycemia; the Whipple triad [103], described in 1938, was long used to diagnose insulinomas. It consisted of: presence of hypoglycemic symptoms; detection of hypoglycemia (< 50 mg/dL); and relief of symptoms with glucose ingestion. Unfortunately the symptoms are not specific for insulinoma [104]; in a large review of 1085 cases they were present in only 43% of cases of insulinomas [60]. Consequently, a fast with monitoring of plasma insulin, glucose, C-peptide, and proinsulin values is the study usually used to establish the diagnosis of insulinoma [76–78,99]. A 72-hour fast in the hospital (with free access to water) is usually undertaken [59,99]. The above mentioned blood studies should be monitored at regular intervals (2–4 h) and more frequently if the blood sugar level decreases to less than 50 mg/dL. If at any point the patient becomes symptomatic, repeat serum levels of the studies listed above should be obtained before administering glucose. The test is terminated for neuroglycopenic symptoms or for persistent glucose levels of less than 40 mg/dL. Over 90% of insulinomas reported have been detected in this fashion, and 70%–80% of patients will develop hypoglycemia during the first 24 h of fasting, and 90%–98% by 48 h [59,76]. In normal nonobese subjects, serum insulin concentrations decrease to less than 6 μU/mL (i.e., assessed by the standard insulin radioimmunoassay [RIA]) when blood glucose decreases to 40 mg/dL or less, and the ratio of serum insulin to glucose remains less than 0.3 (in mg/dL) [59,98,99]. An insulinoma is considered present if serum insulin concentrations remain

Table 71.2 Causes of fasting hypoglycemia

Category	Cause or agent
Mediated by endogenous insulin or insulin-like factors	Pancreatic islet disease (insulinoma, hyperplasia, nesidioblastosis) Spontaneous autoimmune antiinsulin antibody syndrome Autoantibodies to insulin receptor Noninsulin tumor-associated hypoglycemia
Reduced hepatic glucose output	Deficient gluconeogenesis or glycogen storage Hormonal deficiencies (e.g., adrenal insufficiency) Enzymatic defects (e.g., glucose-6-phosphatase deficiency) Ethanol consumption and poor nutrition Severe liver disease
Drugs or other pharmacological causes	Sulfonylureas or biguanides Insulin administration Ingestion of ackee fruits (hypoglycin) Other drugs (aspirin, pentamidine, haloperidol, quinine)

Modified from Jensen [4]; Boden [59]; Grant [76]; Comi et al. [99].

constant or increase during hypoglycemia. One set of proposed criteria are:

- documented glucose levels near or below 40 mg/dL (2.2 mM)
- concomitant insulin levels ≥ 6 μU/mL (43 pmol/L)
- elevated C-peptide levels (≥ 0.2 nmol/mL in a patient without elevated sulfonylureas in the plasma) [76].

Some add a requirement for a serum proinsulin greater than or equal to 5 pmol/L [77,105] or an insulin:glucose ratio greater than 0.3 [59,99]. In one study all 114 patients with insulinoma operated on between 1980 and 1995 had a positive fast [76], whereas in another review all 132 patients with insulinomas fulfilled the biochemical criteria, but 4 of 132 patients (3%) did not become symptomatic [106]. In the small percentage of patients with a negative test (i.e., serum insulin concentration decreases to < 6 μU/mL with hypoglycemia) but in whom insulinoma is still suspected, additional tests such as various provocative tests may have to be used [28,59,76,98,99,106]. Plasma insulin is being increasingly measured by insulin-specific immunoradiometric assay or immunochemoluminescent assays, which have no cross-reactivity with proinsulin, as do many of the standard insulin RIAs, and thus give lower insulin levels; resulting in 30%–60% of patients with insulinomas having plasma insulin levels below 6 μU/mL [107,108]. Using insulin-, proinsulin-, and C-peptide-specific assays a study involving 33 insulinoma patients and 67 controls [108] demonstrated that the most sensitive criterion for diagnosing insulinoma was the combination of an elevated proinsulin level with a fasting glucose level below 45 mg% [108].

The diagnosis of insulinoma can be difficult because an inappropriately elevated serum insulin concentration associated with hypoglycemia can be observed in a number of different conditions (Table 71.2) [100]. Besides insulinomas, β-cell hyperplasia (or nesidioblastosis), factitious or inadvertent administration of excessive insulin or oral hypoglycemia agents, and autoantibodies against insulin or the insulin receptor can also cause these findings (Table 71.2) [59,99,100]. β-Cell hyperplasia, or nesidioblastosis, consisting of a proliferation of insulin-producing cells, is a leading cause of hyperinsulinism in newborns and has also occasionally been reported in adults [99,109]. Of these varying causes of hypoglycemia and hyperinsulinemia (Table 71.2), surreptitious use of insulin or sulfonylureas is the most difficult to distinguish from an insulinoma [98–100,105,110]. To differentiate these conditions determinations of serum concentrations of proinsulin, C-peptide, antibodies to insulin, and sulfonylurea need to be done [76,98–100,105,110].

Insulin is synthesized as proinsulin, which consists of a single-chain molecule containing both a 21-amino-acid α-chain and a 30-amino-acid β-chain connected by a 33-amino-acid connecting peptide (C-peptide) [111,112]. Insulin and C-peptide are liberated in equimolar amounts, and because proinsulin or its intermediate are also found in granules it is also detected in small quantities in the serum with insulin [99,111,112]. Normal subjects have less than 25% of the serum insulin as proinsulin. Serum proinsulin has been reported to be elevated in at least 80%–90% of patients with insulinoma [98,99,108,112]. Because high proinsulin values are not found in other hypoglycemic disorders (elevated levels can occur in cirrhotics and patients with type 2 diabetes), an elevated value is helpful in the diagnosis of insulinoma. In insulinoma the characteristic findings are either a normal or elevated serum concentration of C-peptide, absence of antibodies to insulin, and lack of detectable sulfonylurea in the blood [76,98,100,108]. In a typical patient with surreptitious use of insulin the C-peptide level is decreased, proinsulin is

normal or decreased, antibodies to insulin may be present, and serum sulfonylurea determinations are negative [98,99]. In patients who surreptitiously use sulfonylureas, the serum concentration of proinsulin is usually normal, antibodies to insulin are not present, and sulfonylurea is detected in the serum or the urine [98,99].

VIPoma

The VIPoma syndrome is due to an endocrine tumor, usually located in the pancreas, that secretes excessive amounts of vasoactive intestinal peptide (VIP), which causes a distinct syndrome characterized by fasting, large-volume diarrhea, hypokalemia, and hypochlorhydria (Table 71.3).

The VIPoma syndrome is also called the Verner–Morrison syndrome because of its original description by these two authors in two patients with profuse watery diarrhea, hypokalemia, and dehydration with a non-β-cell pancreatic islet cell tumor [113]. It has also been termed pancreatic cholera, and the WDHA syndrome (for the watery diarrhea, hypokalemia, and achlorhydria that some patients develop) [20,21,82]. Although early in the presentation the diarrhea may be episodic or intermittent, characteristically the diarrhea is large in volume (> 1 L/day), secretory in nature, and persists during fasting (Table 71.3) [4,20,21,28,114–116]. The diarrhea fluid frequently is described as having the appearance of weak tea. An increased net secretion of sodium and chloride into the small intestine has been demonstrated in these patients [117,118]. The sum of fecal sodium and potassium concentrations multiplied by two will equal the osmolality, thus there is no osmotic gap [21]. In general these patients are reported not to develop steatorrhea [21,116] – in only 16% in one study [21] – nor have abdominal cramping pain, but some studies suggest pain may be more common than reported previously [21,28,74]. Studies assessing malabsorption are usually normal, with a normal fecal fat excretion in 84%, normal D-xylose in 94%, and a normal Schilling test in 87% [21]. The increased fecal loss frequently leads to dehydration (Table 71.3) [21,74]. Hypokalemia is present in most patients (Table 71.3) [19–21,74,114,119,120] and is the result of the large losses of potassium in the diarrhea fluid. Hypochlorhydria rather than achlorhydria is found in 28%–76% of patients with VIPoma syndrome (Table 71.3) [20,74,119]. This has been attributed to the inhibitory effect of VIP on gastric acid secretion [20,28]. Flushing is seen in 13%–28% of patients with the VIPoma syndrome [19,20,74,121] and has been attributed to the vasodilatory effects of VIP (Table 71.3) [20,122]. The fact that prolonged infusion with VIP in humans results in a gradual decrease in the flushing suggests the development of tachyphylaxis [12]. This phenomenon has been used to explain why only a minority of patients develop flushing [20]. Hyperglycemia is noted in 20%–50% of patients with VIPomas [21,74], and it has been attributed [20] to the glycogenolytic effect of VIP on the liver (Table 71.3) [123]. Hypercalcemia also is reported in 25%–76% of patients with VIPoma [20,21,28,74]. At present the mechanism of hypercalcemia in most cases is not established; however, it seems to be related to the tumor itself because it is relieved by complete tumor removal [21]. Only 6%–11% of patients with VIPomas have a history of MEN-I, and only 1%–3% of all MEN-I patients develop VIPomas, and therefore this is rarely the cause of the hypercalcemia [19,21,68,72,74].

Table 71.3 Clinical features of the VIPoma syndrome

Sign/symptom	Frequency (%)
Secretory diarrhea	89–100
Hypokalemia	67–100
Weight loss	33–72
Dehydration	44–95
Hypochlorhydria	34–76
Hyperglycemia	20–50
Hypercalcemia	25–50
Flushing	13–28
Dilated, atonic gallbladder	Unknown

Data from Jensen [4]; Soga and Yakuwa [19]; Matuchansky and Rambaud [21]; Smith et al. [114]; Verner and Morrison [119,136].

Beside VIP a number of substances [20,124], including secretin [12], gastric inhibitory polypeptide [125], pancreatic polypeptide [126], and prostaglandins [127], are reported to cause diarrheogenic secretion in some patients. Controversy arose in early studies about whether or not VIP was the sole cause of the diarrhea in WDHA, because VIP was not found to be elevated in all patients by different investigators, and exogenous VIP infusions for 1 or 2 h did not cause diarrhea in humans [128]. However, in subsequent studies only VIP has been found to be elevated consistently in patients with the WDHA syndrome [20,81,114,120,124,129,130]. Furthermore, continuous infusion of VIP for 10 h in normal subjects to achieve plasma VIP concentrations similar to those seen in patients with WDHA produced profuse watery diarrhea by 6–7 h [129,131]. The diarrhea produced in these patients is similar to that seen in patients with WDHA. The ability of VIP to cause intestinal secretion is consistent with its known mechanism of action. Vasoactive intestinal peptide has been shown to cause net chloride secretion associated with increased short circuit current, to bind to specific receptors on intestinal epithelial cells, and to activate adenylate cyclase and increase cyclic AMP levels in intestinal cells [28,132]. Occasional patients with the WDHA syndrome have normal plasma concentrations of VIP [20]. Another VIP-related peptide, peptide histidine isoleucine (PHI), is reported to be elevated in the plasma of patients with WDHA [21,28,133,134]. Tumor tissue obtained from patients with the WDHA syndrome also demonstrated PHI-like immunoreactivity, which was produced by the same cells as VIP [21,28,133,134].

Furthermore, analysis of a WDHA tumor demonstrated that VIP and PHI sequences are in the same gene [132,134]. Infusions of PHI also caused intestinal secretion although PHI was 32 times less potent than VIP [135]; therefore, although PHI was reported to coexist in 22 of 24 VIPomas [133], because of its low potency it remains to be proven whether PHI is contributing to the diarrheal state [21].

The definite diagnosis of VIPoma requires the establishment of a secretory diarrhea, demonstration of elevated serum concentrations of VIP and identification of a PET. The possibility that VIPoma and not some other cause is responsible for the diarrhea may be suggested by measuring the stool volume [20,21,28,116]. In 70%–80% of patients with VIPomas the stool output is greater than 3 L/day [21,136]; in 100% of 52 patients it was greater than 1 L/day [21]; and when the stool output is less than 700 mL/day the diagnosis of VIPoma is excluded [20]. In addition the diarrhea persists during fasting [20,21,114] and the sum of the concentration of stool sodium and potassium multiplied by two equals the isotonicity [21].

A number of diseases can give chronic secretory diarrheas with similar volumes and need to be differentiated from VIPomas [116]. The diarrheas due to midgut carcinoids in the carcinoid syndrome or medullary thyroid carcinoma are primarily due to motility disturbances and rarely reach the volume typical of VIPoma syndrome [21,116]. However, high-volume fasting diarrhea occurs in patients with Zollinger–Ellison syndrome [14,73,116], diffuse islet-cell hyperplasia [21], patients who surreptitiously use laxatives or diuretics, the pseudopancreatic cholera syndrome [21,81,137], and patients with other disorders such as AIDS [138,139] or, occasionally, sprue [140,141]. The serum gastrin concentration is elevated in patients with Zollinger–Ellison syndrome but not in patients with VIPoma. Furthermore, effective treatment of the gastric hypersecretion stops the diarrhea in patients with Zollinger–Ellison syndrome [14,73,116,142].

To differentiate patients with VIPoma from those with pseudopancreatic cholera syndrome or laxative abuse a reliable VIP radioimmunoassay is required [20,21,81]. The normal fasting plasma VIP concentration measured in a number of laboratories ranges from 0 to 190 pg/mL [20,81,124]. The fasting plasma VIP concentration is best determined at the time when diarrhea is present because between diarrheal periods in an occasional patient with VIPoma, VIP levels may be normal [20]. The mean concentration of plasma VIP in one series of 29 patients with the VIPoma syndrome was 956 pg/mL, with a range of 225–1850 pg/mL [20,28]. In another series [82] of 52 VIPomas the mean value was 702 pg/mL, with a range from 159 to 2530 pg/mL. In several reviews [19,74,114,115], 94%–100% of patients with VIPomas had elevated plasma VIP levels. Normal plasma VIP levels are reported to occur in 12% of cases with a pancreatic tumor and in 82% of cases with pancreatic islet cell hyperplasia [21]. In most studies plasma VIP levels are not elevated in patients with laxative abuse [20,21,81,124,125]. Patients with chronic secretory diarrhea due to chronic laxative abuse can be difficult to detect, but this should be suspected if plasma VIP levels are normal or a laxative screen is positive [143]. Because of the difficulty in diagnosing laxative or diuretic abuse, a laxative screen should be performed in any patient with unexplained chronic diarrhea [144]. Even using a standard laxative screen, laxative abusers may be difficult to detect without sophisticated methods such as using gas-liquid chromatography–mass spectroscopy to assess specimens [145,146].

Elevated plasma VIP levels should not be the sole basis for the diagnosis of a VIPoma [20,147]. Other conditions including prolonged fasting, inflammatory bowel disease, small bowel resection, radiation enteritis, and chronic renal failure can occasionally elevate plasma VIP levels [20,147]. In one study [147] of 193 patients with chronic diarrhea, eight different gastrointestinal hormones (motilin, PP, neurotensin, somatostatin, substance P, VIP, gastrin-releasing peptide, and calcitonin) were assessed, and 45% of the patients had one or more elevated levels; however, no patient had a PET. This study showed that false-positive elevations of plasma levels for those hormones reported to cause diarrhea, including VIP, are not uncommon; therefore, such determinations need to ordered selectively and carefully interpreted in light of other clinical and laboratory findings to establish the correct diagnosis [147].

Glucagonoma

Glucagonomas are endocrine tumors of the pancreas that secrete excessive amounts of glucagon and cause a distinct syndrome characterized by dermatitis, glucose intolerance, weight loss, and anemia (Table 71.4).

The first description of what we know as the glucagonoma syndrome was reported in 1942 [148] in a patient with a skin rash associated with pancreatic cancer. M^{c}Garvan and

Table 71.4 Clinical features of the glucagonoma syndrome

Sign/symptom	Frequency (%)
Dermatitis (migratory necrolytic erythema)	64–90
Hypoaminoacidemia	41–100
Glucose intolerance or diabetes mellitus	38–90
Weight loss	56–96
Anemia	33–85
Diarrhea	14–29
Thromboembolism	12–35
Glossitis, cheilitis	14–40
Psychiatric disturbance	0–17

Data from Jensen [4]; Guillausseau et al. [15]; Wermers et al. [16,170]; Frankton and Bloom [17]; Soga and Yakuwa [18]; Guillausseau and Guillausseau-Scholer [79]; Leichter [153]; Stacpoole [154].

colleagues, in 1966, reported the first well-described case of a patient with the glucagonoma syndrome [4,149]. The patient had elevated immunoreactive glucagon in plasma, diabetes mellitus, a skin rash, and a PET. In 1973, Wilkinson [150] introduced the term "necrolytic migratory erythema" for the skin rash associated with PETs. Mallinson and colleagues in 1974 [151] specifically established the association of glucagonomas with the skin rash when they reported nine cases with the clinical glucagonoma syndrome consisting of dermatitis, diabetes mellitus, unexplained weight loss, hypoaminoacidemia, anemia, and a glucagon-producing tumor of the pancreas.

The peak incidence for the glucagonoma syndrome is in the age range 45–70 years, and the syndrome has not been described in children [15–18,59,79,152]. The characteristic skin rash associated with the glucagonoma syndrome, called necrolytic migratory erythema, is present in the vast majority of cases (Table 71.4) [16–18,79,152–156]. The skin lesion starts as an annular erythema at intertriginous and periorificial sites. It is normally found on the buttock, groin, perineum, and thighs. The erythema subsequently becomes raised and the central parts form superficial bullae. The top of the bulbous lesion frequently detaches, leaving eroded areas that become crusted (Fig. 71.2). Lesions frequently become confluent. The extent and the severity of the lesions may wax and wane [155,156]. Healing in 2 or 3 weeks results in hyperpigmentation (Fig. 71.2). Histologically, early skin lesions demonstrate a superficial spongiosis and necrosis and subcutaneous blister formation [153,154]. The pathogenic mechanism by which the glucagonoma syndrome produces this characteristic skin rash is unclear [155,157]. A number of studies report that the skin rash can be caused by prolonged hyperglucagonemia because it developed in patients who were given glucagon for long periods [158–160]. Glucagon-induced hypoaminoacidemia may be one mechanism because correction of the hypoaminoacidemia has been shown to correct the dermatitis without changing plasma glucagon concentrations [161,162]. The resemblance of the lesions to those seen with zinc deficiency has resulted in trials of zinc therapy, with some responses [17]. However, resolution of the rash has been reported following simple hydration with glucose and saline [163]; therefore, it is not known that either hypoaminoacidemia or zinc deficiency are necessarily causative in all patients. A number of other clinical features are also characteristic of the glucagonoma syndrome (Table 71.4). Angular cheilitis (Fig. 71.2) is a common feature as is nail dystrophy and thinning of the hair [16,17]. Some patients develop a painful glossitis (Table 71.4).

Hypoaminoacidemia occurs in most patients and, as originally pointed out by Mallinson, the levels of amino acids may vary with the intensity of the disease [18,155,164]. The hypoaminoacidemia is thought to be secondary to the hyperglucagonemia because glucagon infusions have been demonstrated to have a controlling influence on amino acid metabolism by altering gluconeogenesis [165]. It has been demonstrated in human subjects that glucagon deficiency caused by either suppression with somatostatin or total pancreatectomy increases plasma amino acid concentrations [28,166,167]. Conversely, the administration of glucagon decreases plasma amino acid concentrations [167]. Metabolic studies [168,169] in patients show an increase in amino acid catabolism, a 15% increase in whole-body lipolysis, and a 15% increase in hepatic glucose production; the energy expenditure, as well as the rate of protein breakdown, were normal.

Diabetes mellitus and glucose intolerance occur frequently in patients with the glucagonoma syndrome (Table 71.4) [18,138,154,155,170]. However, in one study [16], diabetes was present in only 38% at presentation and developed in an additional 38% as the glucagonoma progressed. The relationship of the hyperglucagonemia to the diabetes mellitus or glucose intolerance remains unclear. Glucagon levels in

Figure 71.2 Migratory necrolytic erythema involving the face in a patient with metastatic glucagonoma. These typical skin lesions usually start on the extremities or at intertriginous or periorificial sites. The lesions initially are erythematous and scaly, later become raised and bullous, and finally become crusty, as is evident in this patient. Healing results in hyperpigmentation. Angular cheilitis, a common feature in patients with glucagonoma, is also present in a mild form in this patient. The patient also demonstrates loss of the buccal fat pad and temporal muscle wasting indicative of the generalized wasting these patients characteristically develop.

patients with glucagonomas do not correlate well with the degree of glucose intolerance [153,154,170]. Furthermore, various treatments have resulted in definite changes in plasma glucagon concentrations but have not produced correlated changes in blood glucose. For example, tumor resection and normalization of blood glucose may not result in the correction of the glucose intolerance [79]. The somatostatin analogue, octreotide, may depress plasma glucagon levels, but frequently does not improve the diabetes [12,16,17,171]. In some patients, however, removal of the glucagon-producing tumor has improved glucose tolerance [164]. Whether or not glucagon causes glucose intolerance appears to depend to a large degree on the patient's insulin reserve. If it is intact, hyperglucagonemia is present without glucose intolerance [16,59,170].

Weight loss is a common feature of patients with the glucagonoma syndrome and it may be profound (Table 71.4; Fig. 71.2) [15–18,79,138,153–155]. A number of observations suggest weight loss is a specific feature of the glucagonoma syndrome. Weight loss is prominent in patients with small tumors as well as those with metastatic tumor and is not seen early in other PETs unless malabsorption is present, suggesting the cachexia is an intrinsic feature of the glucagonoma syndrome. The weight loss has been attributed to the catabolic effects of glucagon [169]. However, in one study in which glucagonomas from a pluripotent rat islet tumor were transplanted into normal animals, severe anorexia and adipsia developed. Studies show that bilateral vagotomy had no effect on the development of anorexia, that the satiety peptides, cocaine- and amphetamine-regulated transcript (CART) or leptin, are unlikely to be the mediators, and that the unknown mediator can override the potent appetite-stimulatory effect of neuropeptide Y (NPY) [172,173]. These results suggest that the glucagonoma's anorectic effects are caused by the production of a novel substance by the glucagonoma and not due to glucagon per se because other transplanted glucagonomas producing similar levels of plasma glucagon elevation do not cause anorexia.

Thromboembolic phenomena are more common in patients with the glucagonoma syndrome (Table 71.4) [15–17,79,153,154]. Both deep-vein thrombosis and pulmonary emboli have been reported to occur in a significant number of cases and have been the cause of death in some patients [15,153,154]. Glucagon is not known to affect coagulation parameters, and the pathophysiology of the thromboembolic events in glucagonoma has not been systematically studied. A normochromic, normocytic anemia is also reported to be a frequent finding in patients with glucagonomas (Table 71.4) [16–18,59,79,138,153]. Serum iron, serum vitamin B-12, and serum folate concentrations are usually normal, and the anemia does not respond to therapy with any of these agents. It does, however, respond to resection of the tumor [174–176]. Further evidence that the anemia may be due to glucagon excess is that prolonged therapy with a long-acting glucagon preparation decreased erythropoiesis in rats and mice [176]. Psychiatric disturbances, particularly depression, have been reported in a number of patients with the glucagonoma syndrome; however, the exact frequency or even whether they are more common in patients with glucagonoma has not been established (Table 71.4) [16,17,79,148].

Glucagonoma is usually suspected in patients with chronic unexplained and therapy-resistant dermatitis and elevated erythrocyte sedimentation rates, and is associated with glucose intolerance, thromboembolic phenomena, or the MEN-I syndrome (Table 71.4) [12,17,153–156,170,177]. Necrolytic migratory erythema is the presenting feature in 70% of patients [155,178], occurring from 1 month to 3 years (mean 12 months) before the diagnosis of the pancreatic tumor [156]; in some patients no other extracutaneous symptom is present [179]. Unfortunately, not only is necrolytic migratory erythema frequently misdiagnosed, but a number of other conditions besides glucagonomas can cause this rash, including hepatitis B infections [180], myelodysplastic disorders [181], short bowel syndrome [182], malnutrition [183], cirrhosis, celiac sprue, other malignancies, inflammatory bowel disease, malabsorptive syndromes, and specific nutritional deficiencies [157]. Diagnosis of glucagonoma rests on demonstrating a pathological elevation of the plasma glucagon concentration. In almost all patients with glucagonoma the plasma glucagon concentration is elevated (> 150 pg/mL) [18,153,156,170]. In one study of 23 patients with proven glucagonoma the plasma glucagon concentration had a mean value of 2110 pg/mL (± 1 standard error of the mean [SEM]) whereas the highest value in a patient without glucagonoma was 409 ± 29 pg/mL. In a review of 58 published cases of glucagonoma, plasma glucagon levels exceeded 1000 pg/mL in 90%, were between 500 and 1000 pg/mL in 7%, and were less than 500 pg/mL in 3% [15,79]. In a study of 21 cases from the Mayo Clinic [16], 48% of patients had values greater than 1000 pg/mL, and 86% exceeded 500 pg/mL. It has been suggested that a plasma glucagon concentration of more than 1000 pg/mL is diagnostic of glucagonoma, because other causes of elevated plasma glucagon levels do not produce this degree of elevation [4]. However, in some studies [16,156] plasma glucagon levels below 500 pg/mL have been reported in a subset of patients (< 15%) and therefore a high degree of suspicion for the disease should be maintained, especially in patients with small tumors or MEN-I [17]. Other conditions that are associated with increased plasma glucagon concentrations include renal insufficiency, acute pancreatitis, hyperadrenal corticalism, hepatic insufficiency, severe stress (trauma, exercise, bacteremia, diabetic ketoacidosis), prolonged fasting, familial hyperglucagonemia, or other PETs [17,18,59,79,153,170,177,184,185]. In two studies [18,170], one involving 71 patients with fasting hyperglucagonemia at least twice the upper limit of normal (i.e., > 120) [170], and the other [18] comprising 407 cases of glucagonoma from the literature, only 21% and 57%,

respectively, of the patients included had a clinical glucagonoma syndrome. Therefore, hyperglucagonemia without the glucagonoma syndrome is not necessarily uncommon. In one series [170] the hyperglucagonemia occurred with polyfunctional PETs in 69% of the cases, especially with patients with Zollinger–Ellison syndrome, insulinomas, and carcinoid tumors; in almost all cases the hyperglucagonemia was not associated with the glucagonoma syndrome. In general, in these nonglucagonoma conditions causing hyperglucagonemia, the plasma glucagon is not elevated above 500 pg/mL except in patients with cirrhosis [59,79,153]. For the occasional glucagonoma patient with a fasting plasma glucagon concentration less than 1000 pg/mL that may overlap with other conditions, there is no proven provocative test that will clearly distinguish these conditions [59,79]. Usually, however, these nonglucagonoma conditions can be excluded on clinical grounds. One to three percent of MEN-I patients develop glucagonomas [68], and in various series from 0% to 20% of all patients with glucagonomas had MEN-I [15–17,153,154]; these patients can be difficult to diagnose because they have minimal symptoms early in their disease course [17].

Somatostatinomas

Somatostatinomas are endocrine tumors of the pancreas or intestine that secrete excessive amounts of somatostatin, which cause a distinct syndrome characterized by diabetes mellitus, gallbladder disease, diarrhea, and steatorrhea (Table 71.5).

In 1977 the first two cases of somatostatinomas were independently reported [186,187]. On the basis of these reports and the known actions of somatostatin, it was proposed that the clinical somatostatinoma syndrome consisted of mild diabetes mellitus, gallbladder disease, weight loss, and anemia [4]. Diarrhea, steatorrhea, and hypochlorhydria became additional features as other cases have been described (Table 71.5) [83,188,189].

Table 71.5 Clinical features of the somatostatinoma syndrome

	Frequency (%) in cases of somatostatinoma[a]		
Sign/symptom	**Pancreatic**	**Intestinal**	**Somatostatinoma syndrome[a]**
Diabetes mellitus	90–95	21	95
Gallbladder disease	90–94	43	68
Diarrhea	60–97	10–40	37
Steatorrhea	83	12	47
Hypochlorhydria	86	17	26
Weight loss	30–90	20–45	68

a Somatostatinoma refers to a pancreatic endocrine tumor that shows somatostatin-like immunoreactivity and may occur without (89%) or with (11%) the somatostatinoma syndrome, which is due to somatostatin secretion by the tumor [25,90].
Modified from Jensen [4]; Vinik et al. [22]; Sassolas and Chayvialle [23]; Soga and Yakuwa [25].

There is no general agreement on the definition of a somatostatinoma. In the literature this term is generally used to indicate a PET possessing somatostatin-like immunoreactivity and it may (11%–45%), or may not (55%–89%) be associated with clinical symptoms due to ectopic release of somatostatin [23–25]. In this chapter the term "somatostatinoma syndrome" will be used to indicate a somatostatin-producing PET associated with clinical features due to ectopic somatostatin release (Table 71.5), and the term "somatostatinoma" to include a PET containing somatostatin immunoreactivity.

Somatostatin is a tetradecapeptide originally isolated and purified from ovine hypothalamic tissue [4]. It inhibits the release of almost all other hormones, and has direct effects on a number of gastrointestinal functions, including potent inhibition of basal and pentagastrin- or meal-stimulated acid secretion, cholecystokinin-stimulated pancreatic enzyme secretion, and intestinal absorption of amino acids [190,191]. A number of studies have suggested that somatostatin has a paracrine effect on antral gastrin release. Somatostatin also has marked effects on gut motility and transit time [190,191].

Somatostatinomas primarily occur in the pancreas (41%–75%) or in the upper small intestine (25%–54%) [22,23,25,26,192]. In the pancreas 56%–78% of the somatostatinomas occur in the pancreatic head [22,25,26]. Tumors not in the pancreas occur in the duodenum (43%–96%), ampullary area (48%), jejunum (5%), or cystic duct area [22,25,26]. Most somatostatinomas (> 96%) are solitary and vary from less than 1 cm to 10 cm in diameter, with a mean of 4–5 cm [25]. Pancreatic tumors are larger than duodenal tumors (5 cm vs 2 cm) ($P < 0.0001$) [25], and in one study [22] were found to be more malignant than intestinal tumors (92% vs 6%), but not in another study [25] (i.e., both 50%). With duodenal somatostatinoma the occurrence of lymph node metastases correlates with primary tumor size [193]. Electron microscopic studies show typical D-cell secretory granules in 52%–89% of the tumors [22,25,193].

The mean age of patients with pancreatic somatostatinomas or intestinal somatostatinomas is 45–50 years, with a range of 30–84 years [22,192]. In one series [22], patients with pancreatic somatostatinomas show a 2 to 1 female predominance, whereas in those with intestinal somatostatinoma 60% of the patients are males [22]; however, in another review somatostatinomas occurred with equal frequency in both genders [25].

In assessing the symptoms/signs due to somatostatinomas it is important to differentiate between those due to the PET per se, which are similar to any PET (i.e., pain, icterus, weight loss, hepatomegaly, abdominal tumor), from those that are due to the increased somatostatin secreted by the PET. In one large literature review of 173 cases of somatostatinomas [25], only 18 cases (11%) were associated with the

somatostatinoma syndrome (Table 71.5). In the same study [25], of all patients with somatostatinomas, 93% initially had symptoms or signs, with the most frequent being abdominal pain (40%), weight loss (26%), icterus (23%), abdominal tumor (12%), diarrhea (18%), nausea/vomiting (16%), and hepatomegaly (10%). Diabetes mellitus or glucose intolerance occurs in 63%–90% of patients with somatostatinomas [22,23,83]. However, the diabetes mellitus occurred in 90%–95% of the patients with pancreatic somatostatinomas but in only 21% of those with intestinal somatostatinomas (Table 71.5) [22]. The diabetes is usually mild, and severe hypoglycemia or ketosis, although reported, are uncommon [23,28,189]. The development of diabetes mellitus or glucose intolerance is secondary to the ability of somatostatin to inhibit insulin and glucagon release [22,23] and the replacement of functional islet tissue by tumor [83]. This latter factor may explain why diabetes mellitus is more common in patients with pancreatic than with intestinal somatostatinomas (Table 71.5) [22]. In patients with the somatostatinoma syndrome, 95% had diabetes mellitus (Table 71.5) [25].

Gallbladder disease (cholelithiasis) is reported in 65%–90% of patients with somatostatinomas [22,23,83], and in 90%–94% of patients with pancreatic and 43% of patients with intestinal somatostatinomas (Table 71.5) [22]. Cholelithiasis was present in 68% of patients with the somatostatinoma syndrome (Table 71.5) [25]. The high incidence of gallbladder disease in patients with somatostatinomas and the lack of low incidence in patients with other PETs suggests a causative association between the gallbladder disease and somatostatinoma. This conclusion is supported by studies demonstrating that somatostatin analogues inhibit gallbladder emptying, alter the motility of the sphincter of Oddi, alter hepatic bile secretion, and affect the bile composition [23,190,194]; and by studies of patients with acromegaly or PETs treated long term with octreotide [190,195,196]. Two of the latter studies [190,196] demonstrated that 13%–66% of patients treated long term with octreotide develop gallbladder sludge or gallstones.

Diarrhea and steatorrhea were reported in 36%–90% of patients in various studies [22,23,25,83]. The diarrhea characteristically consists of 3–10 foul-smelling stools per day, and the steatorrhea varies from 20 to 76 g/day [22]. Diarrhea and steatorrhea are much less common in patients with intestinal (10%–40%) rather than pancreatic somatostatinomas (60%–97%) (Table 71.5) [22,25]. In some, but not all cases the severity of the diarrhea and steatorrhea correlates with the size and degree of metastatic spread of the tumor and improves with tumor resection [83]. Somatostatin has been shown to inhibit pancreatic enzyme and fluid secretion as well as gallbladder motility [190]. In addition, somatostatin also inhibits intestinal absorption of lipid, D-xylose, vitamin B-12, and folate [4]. These biological actions of somatostatin may be responsible for the maldigestion, steatorrhea, and diarrhea observed in these patients [83]. The fact that patients with intestinal somatostatinomas have less diarrhea or steatorrhea has been attributed to their lower plasma somatostatin levels or the lack of local effects of the somatostatinoma within the pancreas (Table 71.5) [22]. Diarrhea is present in 37% and steatorrhea in 47% of patients with the somatostatinoma syndrome (Table 71.5) [25].

Hypochlorhydria was found in 33% of patients in one study [83], in 53% of patients in a second study [22], and in 70% in a third study [25]. Hypochlorhydria occurred in 86% of patients with pancreatic and 17% of patients with intestinal somatostatinomas [22], and was present in 26% of patients with the somatostatinoma syndrome (Table 71.5) [25]. Infusion of somatostatin has been shown to inhibit gastric secretion in normal human subjects as well as basal and food-stimulated gastrin release [4]. As with the glucagonoma syndrome, weight loss is common in patients with somatostatinomas [23], being reported in 32%–90% of patients with pancreatic tumors and in 20%–45% of patients with intestinal tumors [25,83] (Table 71.5), with weight loss ranging from 9 to 21 kg. Weight loss was reported in 68% of patients with somatostatinoma syndrome (Table 71.5) [25]. The weight loss may be secondary to the diarrhea and malabsorption. Other less common features reported in patients with somatostatinoma include mild to moderate anemia in 15%–67% of patients with somatostatinomas [25], and in 21% of patients with the somatostatin syndrome [25]. Also, the occurrence of various associated endocrine disorders is reported in patients with somatostatinomas [83]. In one study, four patients were reported to have hypoglycemic episodes with normal or elevated plasma insulin concentrations and were diagnosed as having insulinomas [83].

The diagnosis of somatostatinoma has been applied to numerous PETs in the literature based only on immunohistochemical results; in most cases (55%–89%) this is not associated with the somatostatinoma syndrome due to ectopic somatostatin release [23–25,92,93]. The diagnosis of the somatostatinoma syndrome requires the demonstration of increased plasma concentrations of somatostatin-like immunoreactivity (SLI) in the resected tumor and at least some features of the appropriate clinical syndrome (Table 71.5). The establishment of SLI only in a resected tumor is not sufficient for the diagnosis of the somatostatinoma syndrome. Minimal increases in plasma SLI levels need to be interpreted with caution because they can occur in nonendocrine disorders [23]. Furthermore, whereas plasma SLI levels are frequently elevated with pancreatic tumors, in small intestinal somatostatinomas the SLI levels are frequently normal [23,24]. Frequently SLI is detected as a subpopulation of cells in a variety of PETs, especially nonfunctional tumors [23]. High plasma SLI levels have been reported with endocrine tumors outside the pancreas or intestine, as with small cell lung cancer, bronchial oat cell carcinoma, medullary thyroid carcinoma, pheochromocytomas, and other catecholamine-producing extraabdominal paragangliomas [23]. It is likely in the future that the syndrome will be diagnosed earlier

because of the increased clinical awareness of the somatostatinoma syndrome (Table 71.5) and the greater availability of reliable assays for the determination of SLI in blood. As yet these assays are complicated by the need for extraction of the plasma and are not widely available.

The diagnosis of somatostatinoma at a time when plasma SLI concentrations are only marginally elevated and the tumor has not yet metastasized will require the development of reliable provocative tests. Tolbutamide and arginine have been reported to stimulate plasma SLI increases [83,197]; however, arginine is a well-established stimulant for normal D-cells and thus is unlikely to differentiate normal from supernormal secretion [83]. Furthermore, although tolbutamide stimulates SLI increases in animals [197], it is reported not to cause changes in plasma SLI concentrations in normal human volunteers [198]. Until an adequate provocative test is developed, if somatostatinoma is to be diagnosed earlier, it may be necessary to determine plasma SLI concentrations in all patients with diabetes mellitus without a family history and with any other clinical features of the somatostatinoma syndrome, or in all patients with unexplained diarrhea [188].

In most studies somatostatinomas have been found more or less by accident [83]. Because most somatostatinomas (i.e., 89% [25]) do not produce the somatostatinoma syndrome and the most common presenting symptoms are nonspecific (abdominal pain, weight loss, icterus, diarrhea, nausea/vomiting) [25], the correct diagnosis is rarely suspected prior to the discovery of a PET at laparotomy for cholecystectomy, during imaging studies for nonspecific complaints, or after a biopsy for metastatic liver disease discovered during a workup for nonspecific GI symptoms [22,23,25]. In particular, the presence of psammoma bodies in a duodenal tumor should raise the suspicion that the tumor could be a somatostatinoma [23,25,192,199]. These lesions are found in approximately one-half of duodenal somatostatinomas [23–25], but are uncommonly found (i.e., 2%–5%) in pancreatic somatostatinomas or in other types of duodenal carcinoids or duodenal gastrinomas [23,192,199]. Duodenal somatostatinomas are increasingly associated with von Recklinghausen disease [23,24,92,93,200,201]. Duodenal somatostatinomas in patients with von Recklinghausen disease are similar to sporadic duodenal somatostatinomas in that they are rarely (i.e., 2%) associated with symptoms of the somatostatinoma syndrome, infrequently cause elevated plasma SLI levels, frequently contain psammoma bodies (37%–66%) [24,25], and are less frequently malignant (30% duodenal vs 70% pancreatic) [24,25]. Somatostatinomas are uncommon in MEN-I patients, occurring in 0.65% of patients in one study involving 580 MEN-I patients [68].

GRFomas

GRFomas are endocrine tumors that frequently originate in the pancreas but also occur in other extrapancreatic sites and secrete excessive amounts of growth hormone-releasing factor (GRF), which causes acromegaly. Tumors releasing GRF (GRFomas) were described in 1982 [202,203]. Growth hormone-releasing factor, a 44-amino-acid peptide originally isolated from human PETs causing acromegaly, is structurally similar to VIP [23,202], and GRF-like immunoreactivity (GRF-IR) is present in neurons in the hypothalamus as well as in multiple extrapituitary tissues [204]. The true frequency of this syndrome is still not established but a review in 1997 included 40 cases [84]. In one immunocytochemical study [205] of PETs, four of nine (44%) contained GRF-IR, although only one patient had acromegaly. In another study [206] 6 of 24 (25%) PETs – of which 16 were associated with various clinical syndromes (8 insulinomas, 5 gastrinomas, 2 VIPomas, and 1 Cushing syndrome) – were found to have GRF-IR material but no patient had clinical acromegaly. GRFomas occur in the pancreas in 29%–30% of cases; 47%–70% occur in lung tumors, with the majority in the right lung; 8%–10% occur in the small intestine; and up to 13% are found in other sites including adrenal gland, foregut, and retroperitonium (Table 71.1) [23,84,204,207,208]. The patients were 15–66 years old, with an average age of 38–39 years [84,207,208]. A female predominance (78%) is seen with pancreatic GRFomas, and indeed with all GRFomas (73%) [84,207,208]. The clinical features can be divided into three categories:

- acromegalic features due to ectopically released GRF causing excess growth hormone secretion
- clinical features due to hormones other than GRF coreleased, such as gastrin causing Zollinger–Ellison syndrome, or ACTH causing Cushing syndrome
- local symptoms due to the mass effect of the hormone [23,207,208].

The mean duration from symptom onset to diagnosis is 5–6 years [207,208]. The acromegalic features of GRFomas are indistinguishable from those of classical acromegaly [23,204,207,208]. Patients usually have a large pancreatic tumor (> 6 cm; range 1–25 cm), and in 33%–39% of cases the tumor is metastatic at the time of diagnosis [84,207]. The majority of pancreatic GRFomas occur in the pancreatic tail [84,204,208]. In one series [207] metastatic disease was present in 30% of the patients with pancreatic GRFomas and in two of the three patients with intestinal GRFomas [208]. No relationship between the presence or absence of metastases, tumor size, or plasma GRF level was found [208]. Multiple pancreatic GRFomas occurred in 30% in one series [208], usually in patients with MEN-I [23]. Forty percent of the patients with pancreatic GRFomas had associated Zollinger–Ellison syndrome, Cushing syndrome was present in 40%, and one-third of the patients had associated MEN-I syndrome [28,208–210].

GRFomas are usually suspected in a patient with acromegaly and an elevated growth hormone level with hepatic metastases or with abdominal complaints [23,202,203,207,208].

Pancreatic GRFomas are often associated with a gastrinoma and Zollinger–Ellison syndrome or Cushing syndrome. Therefore, any patient with Cushing syndrome or peptic ulcer symptoms, diarrhea, a pancreatic mass, or with symptoms suggestive of chronic esophageal reflux who presents with acromegalic features should be suspected of having a GRFoma. Furthermore, pancreatic GRFomas have occurred in a number of patients with the MEN-I syndrome [209,210]. Any patient with acromegaly with hyperparathyroidism or a family history of MEN-I should also be suspected of having a GRFoma. The diagnosis should be suspected in any patient with acromegaly without a pituitary adenoma; with acromegaly associated with hyperprolactinemia, which is seen in 70% of GRFomas; with paradoxical growth hormone response to thyroid-stimulating hormone (TSH) or during an oral glucose tolerance test [23,84,207,208]. A GRFoma is a rare cause of acromegaly, occurring in 0 of 177 unselected patients with acromegaly in one study [211]. The diagnosis is confirmed by performing a plasma assay for growth hormone-releasing factor and for growth hormone [23,84,207,208]. Elevated plasma levels of growth hormone (usually > 5 μg/L in men and 10 μg/L in women) and an elevated plasma GRF confirm the diagnosis, whereas GRF levels are normal to low in patients with pituitary acromegaly [204]. One review proposes an empirical diagnostic threshold value for GRFomas of 300 pg/mL for the plasma GRF level [23]. Healthy subjects have GRF levels less than 50–100 pg/mL [84] and patients with pituitary adenomas causing acromegaly have plasma GRF levels of less than 200 pg/mL [23,211]. In addition to elevated levels of growth hormone and GRF, patients with GRFomas also have raised plasma levels of insulin-like growth factor 1 (IGF-1), similar to patients with acromegaly due to a pituitary adenoma [207].

Nonfunctioning pancreatic endocrine tumors

Nonfunctioning PETs (NFPETs) are endocrine tumors that originate in the pancreas and either secrete no peptides or their secreted products do not cause clinical symptoms. Unlike functional PETs, the symptoms that occur are due to the effects of the tumor per se and not to the secreted products [1,22,29,30]. Frequently NFPETs release peptides that cause no clinical symptoms, including chromogranin A (69%–100%), chromogranin B (100%), PP (50%–100%), α-human chorionic gonadotropin (α-HCG; 40%), β-HCG (20%), synaptophysin, neuron-specific enolase, and (in some) neurotensin [1,3,22,47,48,56]. Immunocytochemically these tumors often contain multiple peptides [1,43]. In one study of 30 such tumors [43], 50% had immunoreactivity for insulin, 30% for glucagon, 40%–75% for PP, 13% for somatostatin, and 13% for none of these. Usually NFPETs are solitary, large tumors (72% > 5 cm in one study [212]) and occur primarily in the pancreatic head (ratio of 14:2:3 in the head, body, and tail, respectively, in one study [212]), except in patients with MEN-I, who frequently have multiple NFPETs detected, often less than 2 cm in diameter and histologically invariably have multiple, microscopic tumors [213–215]. Frequently NFPETs are diagnosed after the patient presents with symptoms or signs of metastatic tumor in the liver (cachexia, abdominal pain, hepatomegaly) and a liver biopsy is performed that reveals metastatic endocrine tumor [1,22,29,30,216]. Infusions of PP into animals have been shown to have numerous effects including inhibition of pancreatic secretion, relaxation of the gallbladder, weak inhibition of pentagastrin-stimulated acid secretion, and various stimulating effects on gastrointestinal motility [217]. It is unclear why patients with PETs and high plasma concentrations of PP do not have symptoms cuased by the increased level of PP per se. Typically the patient with a NFPET is 40–60 years of age [1,6,22,29,30,218]. Some series demonstrate a male predominance for NFPETs [29,219], whereas in others there is a female predominance [6,218]. Patients with NFPETs frequently present with abdominal pain (in 36%–56%), jaundice (28%–40%), weight loss (24%–46%), or abdominal masses (8%–40%), and in 16% of the patients tumors were found incidentally at surgery in asymptomatic patients [30,212]. In a large series [6] the NFPET was discovered incidentally by ultrasound in 35% of the patients; these patients were asymptomatic and the tumors were smaller than in patients with NFPETs with symptomatic presentations. In most series [1,5,29,30,216,219], but not all [6,220,221], more than 60% (range 64%–92%) of patients with NFPETs have metastatic disease at the time of diagnosis [1,22,30,212]. The median delay in time from first symptoms to diagnosis varies from 0.5 to 2.7 years [1].

Usually NFPETs are not differentiated from any other malignant tumor of the pancreas prior to histological studies [1,22]. In one series the diagnosis of a NFPET was not made in a single patient prior to surgery [29]. Furthermore, 20% of the patients were asymptomatic and the tumors were found incidentally during other operative procedures [29]. Any patient with a long survival (> 5 years), previously diagnosed as having metastatic pancreatic adenocarcinoma, should be suspected of possibly actually having a NFPET. An elevated plasma level of chromogranin A or B (69%–100%), PP (50%–100%), α-HCG (40%), β-HCG (20%), neuron-specific enolase (31%), or positive somatostatin receptor scintigraphy (SRS) is strong evidence to suggest that a pancreatic mass is a PET [1,3,22,48,56,222]. In 53 patients with adenocarcinoma of the pancreas, none had elevated plasma levels of PP [22]. However, elevated levels of PP do not establish the diagnosis of a PPoma even when a pancreatic mass is present because plasma PP levels are increased in 22%–71% of patients with functional PETs [1,45,223,224] as well as in nonpancreatic carcinoid tumors. In one study [224] plasma PP levels were over 1000 pg/mL in 32% of patients with gastrinomas, in 21% with insulinomas, in 57% with glucagonomas, in 74% with VIPomas, in 33% with somatostatinomas, and in 45% with carcinoid tumors. Furthermore, plasma PP

elevations can occur with renal failure, old age, post-bowel resection, alcohol abuse, certain infections or inflammatory conditions, acute diarrhea, diabetes, pancreatitis, or with eating [224]. To improve the specificity an atropine suppression test has been proposed [224]. In a study of 48 patients with elevated plasma PP levels, atropine (1 mg i.m.) did not suppress the levels in any of 18 patients with PETs, but did suppress it in all patients without PETs. In one study [222] none of 28 pancreatic ductular cancers was positive with SRS whereas 65% of proven PETs were positive. Furthermore, 42% of long-term survivors of pancreatic adenocarcinoma (> 3 years) were positive by SRS, suggesting they were really PETs misdiagnosed [222]. In a study of 61 NFPETs [221], 56 (91%) were well differentiated, 5 (9%) poorly differentiated, and 56% of patients demonstrated metastases. Factors that correlated with malignancy included: the presence of vascular or perineural invasion; a Ki-67 proliferative index greater than 2%; a mitotic rate greater than or equal to 2; tumor size greater than or equal to 4 cm; capsular penetration; nuclear atypia; lack of progesterone receptors; and presence of calcitonin [221]. These factors had prognostic significance for survival [5,221]. As yet there are no data to suggest that NFPETs producing PP or other peptides differ in biological behavior from tumors not producing these peptides [219].

Other tumors

Cushing syndrome associated with a PET (ACTHoma) is usually [88,187,225–227] recognized in patients with another symptomatic PET. The ectopic Cushing syndrome is usually due to release of ACTH by the tumor and occasionally to release of corticotropin-releasing hormone by the tumor [228–230]. In various studies 4%–16% of all cases of ectopic Cushing syndrome are due to PETs secreting ACTH [89]. Cushing syndrome occurs in 19% of patients with MEN-I with Zollinger–Ellison syndrome, and is due to pituitary production of ACTH; the disease in these patients is usually mild [88]. Cushing syndrome occurs in 4%–5% of patients with sporadic Zollinger–Ellison syndrome [10,88,225–227]. In patients with the sporadic form of Zollinger–Ellison syndrome, the symptoms of Cushing syndrome are severe, usually occur in patients with metastatic disease, and the Cushing syndrome is due to ectopic production of ACTH [88,226,227]. In one study [226] each patient with ectopic Cushing syndrome resulting from a PET had metastatic disease in the liver, although in other studies rare patients with nonmetastatic disease with Zollinger–Ellison syndrome and Cushing syndrome due to ACTH release by the gastrinoma are reported [90]. These patients with metastatic disease usually respond poorly to chemotherapy [88]; in one prospective study [10] the development of Cushing syndrome in patients with gastrinomas was an independent predictor of poor survival, with patients surviving for a mean of only 1.7 years after onset of the syndrome [88]. The occurrence of Cushing syndrome only as a clinical manifestation of a PET is uncommon [231,232].

Paraneoplastic hypercalcemia due to a PET releasing parathormone-related peptide (PTH-RP), a PTH-like immunoreactive material, PTH, or an unknown hypercalcemic substance has been reported rarely [86,87,233–235]. In most [86,233], but not all [86,122] cases describing paraneoplastic hypercalcemia with a PET [233] the tumors are large and are metastatic to the liver at the time of diagnosis. In a review [86] of 23 cases of PETs associated with hypercalcemia, 30% occurred in patients with VIPomas. The tumors were generally large (7–18 cm) and in seven of eight patients PTH-RP was demonstrated immunohistochemically [86].

It has been proposed that there is a *neurotensinoma syndrome* associated with PETs [22,28,47,236]. Neurotensin is a 13-amino-acid peptide first extracted from bovine brain and subsequently also isolated from human gastrointestinal tract [237]. Neurotensin has a number of pharmacological effects including causing hypotension, tachycardia, and cyanosis, stimulating pancreatic protein and bicarbonate secretion, affecting intestinal motility, and stimulating jejunal and ileal fluid and electrolyte secretion [237]. The clinical features of the patients described with neurotensinomas with PET include diarrhea with hypokalemia, weight loss, and in some cases diabetes, cyanosis, hypotension, and flushing [22]. In the six cases included in one review [22], one-half were cured by tumor resection and the remainder responded to streptozotocin treatment [22,28,236]. Other authors [45,236] have raised the question of whether a specific neurotensinoma syndrome actually exists. In one study [236] of 180 patients with functional PETs an elevated plasma neurotensin level was found in six patients with VIPomas and the clinical symptoms of those patients did not differ from those without an elevated plasma neurotensin level. In a second study [45], 19% of patients with Zollinger–Ellison syndrome were found to have an elevated plasma neurotensin level and their symptoms did not differ from those without an elevated plasma neurotensin level, suggesting that a distinct neurotensinoma syndrome does not exist.

The *carcinoid syndrome* can be caused by PETs [4,85]. These tumors are characteristically malignant (65%–88%) and large [4,85]. Pancreatic PETs [27] are foregut carcinoids that may lack DOPA decarboxylase, the enzyme that converts 5-hydroxytryptophan to serotonin (5-hydroxytryptamine) [27]; however, 84% of patients with pancreatic PETs associated with the carcinoid syndrome have increased urinary 5-hydroxyindoleacetic acid (5-HIAA) levels, which can be used for the detection of the PETs [4].

A small number of patients have been described with PETs or other abdominal neuroendocrine tumors secreting biologically active peptides, which are associated with various clinical symptoms and thus may be new clinical syndromes, although in almost all cases too few patients have been described to establish this latter point. Rare cases are described of PETs secreting erythropoietin causing polycythemia and erythrocytosis [33]; secreting luteinizing hormone

causing masculinization [34,35]; secreting renin and causing hypertension [32], and ovarian tumors secreting peptide YY (PYY) causing constipation [36–38].

Tumor localization

The techniques used for tumor localization and the rationale for using various localization methods are similar for all PETs. Once the particular type of PET is established by the proper radioimmunoassay and functional studies, tumor localization studies need to be undertaken for a number of reasons. For appropriate management of the patient the tumor's location and extent must be established [238–242]. The only possible long-term cure of these syndromes is by surgical excision of the tumor. To establish whether the tumor is potentially resectable, tumor localization studies are essential. In all cases except insulinoma, the majority, if not all of these tumors, if followed long enough, may be malignant, and therefore if metastatic spread is not present at the time of diagnosis an attempt should be made surgically to cure these patients. Gastrinomas and insulinomas frequently present as small tumors [46,60,78,93,243,244]. Up to 50% of PETs that occur frequently extrapancreatically, such as gastrinomas [14,46,93,243,244], and 10%–20% of PETs that occur almost entirely within the pancreas, such as insulinomas [59], are not found at surgery. Therefore, detailed localization studies may help the surgeon in finding the tumor. Furthermore, except for insulinoma, the other PETs are frequently diagnosed only after metastatic spread to the liver has occurred [5,14,18,19,22,25,59]. To determine whether treatment should be started for the metastatic disease and to assess the results of such treatment, tumor localization is essential [2,5,14,245,246].

Techniques reported to be useful for localizing PETs include:

- abdominal ultrasound [2,14,238,240,247–250]
- computerized axial tomography (CT scan) (Figs 71.3 and 71.4; see also Figs 71.7 and 71.8) [2,4,14,234,238,240,247–253]
- selective abdominal angiography (Figs 71.5 and 71.6) [2,4,14,238,250,252,254,255]
- magnetic resonance imaging (MRI; Fig. 71.7; see also Fig.71.4) [2,238,242,247,248,256]
- radiolabeled somatostatin receptor scanning (SRS) (Fig. 71.8; see also Figs 71.7 and 71.12) [247,257–262]
- endoscopic ultrasound (Fig. 71.9) [241,247,251,263–266]
- selective venous sampling for hormones from portal venous tributaries using a transhepatic approach (i.e., portal venous sampling, PVS) (Fig. 71.10) [14,151,255,267–269]
- hepatic venous sampling for hormones after intraarterial injection of secretin (gastrinomas) or calcium (insulinomas) (Fig. 71.10) [255,269,270]
- intraoperative ultrasonography (Fig. 71.11) [248,271,272].

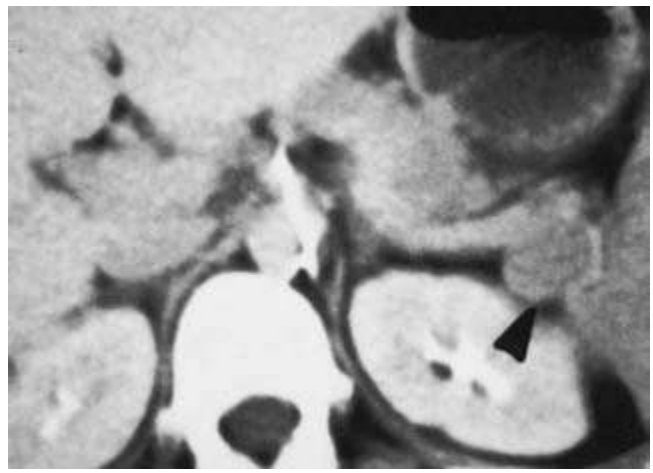

Figure 71.3 Computed tomographic (CT) scan in a patient with an insulinoma in the pancreatic tail, indicated by the arrowhead. The splenic vein courses around the tumor. The CT scan with intravenous contrast will localize 17%–40% of insulinomas. (Courtesy of Dr Jeffrey A. Norton, UCSF, San Francisco.)

Small PETs (< 2 cm) have been the most difficult to localize. The ability of conventional imaging studies (ultrasound, CT scanning, MRI scanning, and selective angiography) to image a PET has been shown to be dependent on the size of the tumor [253,254,256]. For example, using any of the imaging modalities (ultrasound, CT scanning, selective angiography, or MRI) less than 10% of tumors smaller than 1 cm, 30%–40% of tumors 1–3 cm, and 70%–80% of tumors greater than 3 cm were detected [14,273]. Therefore most VIPomas, glucagonomas, and somatostatinomas, which are usually clinically suspected, will only be detected when the tumor is large (frequently > 4 cm). One study demonstrated that small PETs (gastrinomas < 1 cm) also were less frequently detected than large tumors by SRS [258]. Table 71.6 summarizes the results of studies of the ability of the various modalities to localize insulinomas, which are characteristically small (< 2 cm), as are most duodenal gastrinomas at the time of clinical presentation, compared with other PETs [17–20,25,76,223]. The results with insulinomas are presented separately, not only because of their smaller size at presentation, but also because they are found almost exclusively within the pancreas, whereas gastrinomas, VIPomas (in children), and somatostatinomas can frequently be extrapancreatic [18,19,25,60,75,258]. Ultrasound, CT scanning (Fig. 71.3), and MRI (10%–30%) are relatively insensitive, whereas selective abdominal angiography (60%; Figs 71.5 and 71.6), selective portal venous sampling for insulin (80%; Fig. 71.10), intraoperative ultrasound (89%; Fig. 71.11), endoscopic ultrasound (82%; Fig. 71.9), and hepatic venous sampling for insulin after calcium injection (94%; Fig. 71.10) are sensitive methods to localize insulinomas. Insulinomas differ from other PETs in possessing a low density of somatostatin receptors that bind octreotide with high affinity

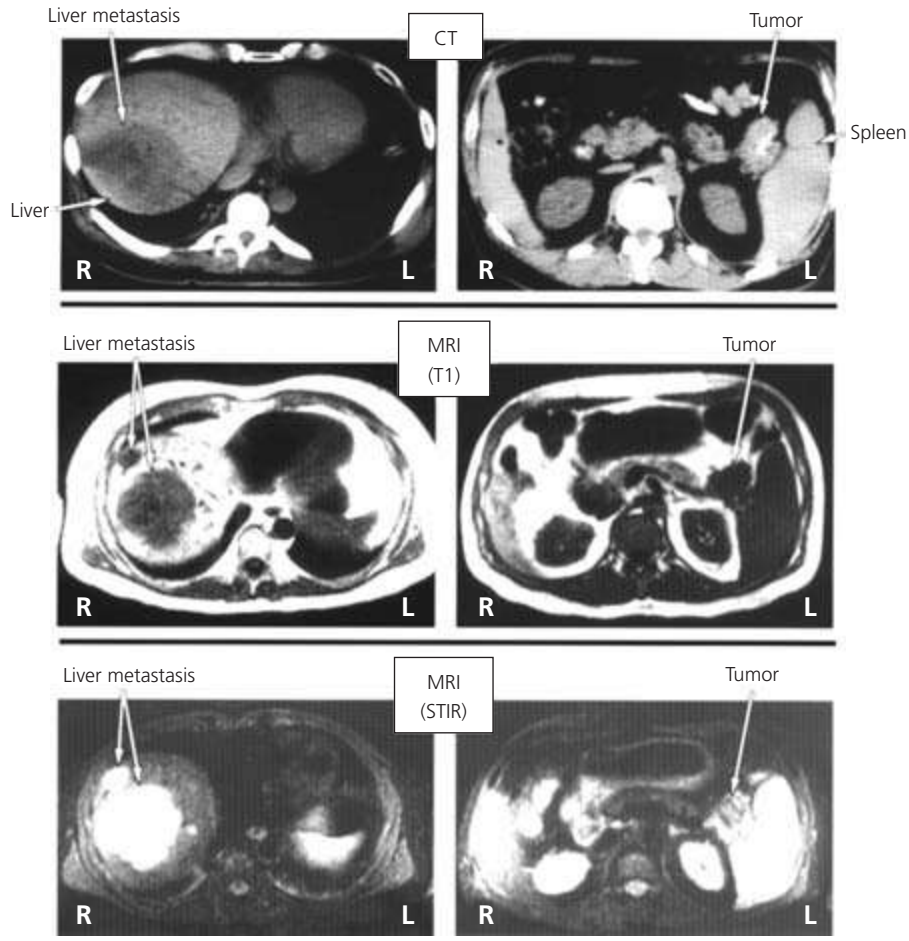

Figure 71.4 Computed tomographic (CT) scan **(top panels)**, magnetic resonance imaging (MRI) T1-weighted image **(middle panels)**, and an STIR (short inversion time inversion recovery) MRI **(bottom panels)** in a patient with a pancreatic endocrine tumor (PET) metastatic to the liver producing parathormone-related peptide causing hypercalcemia. In the left panels the CT scan (top) demonstrates a solitary large metastasis in the right lobe of the liver (arrow), which is not well outlined, whereas on the T1 (middle) and the STIR (bottom) MR images two metastases are clearly seen (arrows) in the right lobe of the liver. In the right panels more caudal views show the primary tumor in the pancreatic tail. The CT scan (top, right) demonstrates clearly the 4-cm pancreatic tail tumor with central calcification, whereas it is less well seen on the MRI T1 (middle) and STIR images (right). This figure demonstrates that metastases from PETs are often much more easily identified on MR images, especially the STIR images, because the tumor has a characteristically bright appearance [14,399]. In contrast the primary tumor is frequently seen better on the CT scan.

(subtypes 2 and 5: sst_2 and sst_5) and therefore SRS is much less sensitive in localizing insulinomas (i.e., 25%, Table 71.6) [259,260]. Results for localization of other PETs except insulinomas are listed in Table 71.6 and compared with the values for insulinomas. These results are primarily based on studies of gastrinomas because the other PETs are uncommon, and frequently the patients with these tumors present with advanced metastatic disease so that routine surgical exploration is not performed. Similar localization data involving large numbers of patients are not available with other PETs within the pancreas. However, because VIPomas, GRFomas, and glucagonomas are almost entirely intrapancreatic it is likely that results similar to those for insulinomas of similar size will be obtained except for the SRS, which will be higher in these tumors. For somatostatinomas, which are frequently extrapancreatic, it is likely that results similar to those obtained with gastrinomas will be found. Ultrasound and MRI are relatively insensitive at localizing noninsulinoma PET primaries (24%–25%); however, CT localizes 50%, angiography finds 68% [14,238,254], selective venous sampling for gastrin reveals 70% [238,267,269], intraoperative ultrasound demonstrates 91% [238,248,271], endoscopic ultrasound finds 70% [238,263], and SRS also localizes 70% [247,257,261,262,274,275]. All PETs are hypervascular and therefore angiography is frequently the most sensitive conventional imaging modality [238,254,255,257]. Figures 71.5 and 71.6 show the typical hypervascular appearance of a primary PET (insulinoma; Fig. 71.5) and hepatic metastases from a malignant insulinoma (Fig. 71.6).

Two newer localization methods that are being increasingly used in patients with PETs are SRS and endoscopic ultrasonography (Table 71.6). Pancreatic endocrine tumors, in addition to a number of other neuroendocrine tumors (carcinoids, medullary thyroid cancer, pituitary adenomas, small cell lung cancer, paragangliomas) and other nonendocrine tumors – lymphomas (Hodgkin disease, non-Hodgkin lymphoma), central nervous system tumors (meningiomas, astrocytomas), breast cancer, prostate cancer – and granulomatous diseases (sarcoidosis, tuberculosis, Wegener granulomatosis), frequently possess high densities of somatostatin receptors, which can be used for localization studies with radiolabeled somatostatin analogues [190,259–261]. For SRS,

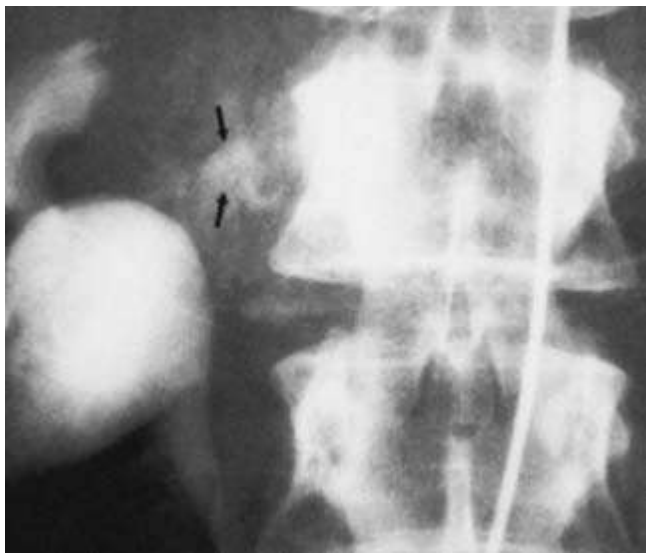

Figure 71.5 Selective angiographic localization of the pancreatic endocrine tumor (PET) in a patient with insulinoma. The insulinoma (1 cm, arrows) is localized in the pancreatic head by a selective celiac artery injection. Insulinomas, as other PETs, are characteristically hypervascular, as demonstrated in the insulinoma in this patient. Because of this hypervascularity even small pancreatic primary lesions are frequently only seen on angiography. Angiography localizes 60% of insulinomas and 68% of the primary tumors in other PET syndromes.

Figure 71.6 Selective common hepatic artery injection in a patient with metastatic insulinoma. The primary insulinoma is demonstrated by the large arrows in the pancreatic head and two hepatic metastases are located in the liver (small arrows). In only 10%–15% of patients with insulinomas are the tumors malignant, whereas in other pancreatic endocrine tumors the majority are malignant. Of the conventional localization methods – ultrasound, computed tomography (CT) scan, magnetic resonance imaging (MRI), angiography – angiography and MRI are the most sensitive modalities for localizing liver metastases (see Table 71.6). Courtesy of Dr Jeffrey A. Norton, UCSF, San Francisco, CA.

in the United States, the radiolabeled octreotide analogue [^{111}In]DTPA-DPhe^{1}octreotide is used [190,260] for tumor localization studies. In vitro autoradiographic studies demonstrate that 100% of gastrinomas, glucagonomas, nonfunctional PETs, 61%–67% of insulinomas, and 88%–96% of carcinoids bind radiolabeled octreotide but 0% of pancreatic adenocarcinomas [257,259,260]. Cloning studies demonstrate that there are five subtypes of somatostatin receptors and that radiolabeled octreotide binds with high affinity to receptor subtypes 2 and 5, with intermediate affinity to subtype 3, and with low affinity to subtypes 1 and 4 [4,190,191]. The overexpression of somatostatin receptors seen on autoradiography in PETs is consistent with the results of SRS in various studies. Tumor is visualized with SRS in 87%–96% of patients with a carcinoid tumor, 73%–100% with a gastrinoma, 46%–61% with an insulinoma, 82%–89% with a nonfunctional PET, 100% with a glucagonoma, and 50%–80% with a VIPoma in two reviews consisting of 477 patients [259,260]. In a comparative study [257] of conventional modalities and SRS in 80 patients with gastrinomas, SRS identified a possible primary tumor in 58% and was superior ($P < 0.001$) to angiography (28%), MRI (30%), CT scan (31%), or ultrasound (9%); moreover, SRS was equal to the combination of all conventional imaging studies combined (48%). Furthermore, SRS [257] alone identified a primary tumor in 20% of patients. Another study [258] confirmed the greater sensitivity of SRS over conventional imaging studies, but demonstrated that SRS can also frequently miss (> 50%) PETs measuring less than 1 cm in diameter. The former study [257] concluded that SRS was the imaging study of choice to localize noninsulinoma PETs. Figure 71.7 demonstrates the increased sensitivity of SRS over the CT scan or MRI in detecting tumor recurrence in a lymph node in a patient with a malignant PET. A number of additional studies have compared the ability of SRS and other imaging studies to localize tumors in some patients with insulinomas [247,251,261] and most patients with other PETs [247,257,276]. In these studies SRS localized an insulinoma in 10% and a gastrinoma in 71%–81% of patients, whereas CT scan, ultrasound, or MRI detected 20% of insulinomas and a primary tumor in 38% of patients with other PETs.

Numerous studies [275,277–281] have examined the ability of SRS to alter clinical management in patients with PETs, which is the most important test of its potential utility. In these studies SRS altered clinical management in a mean of

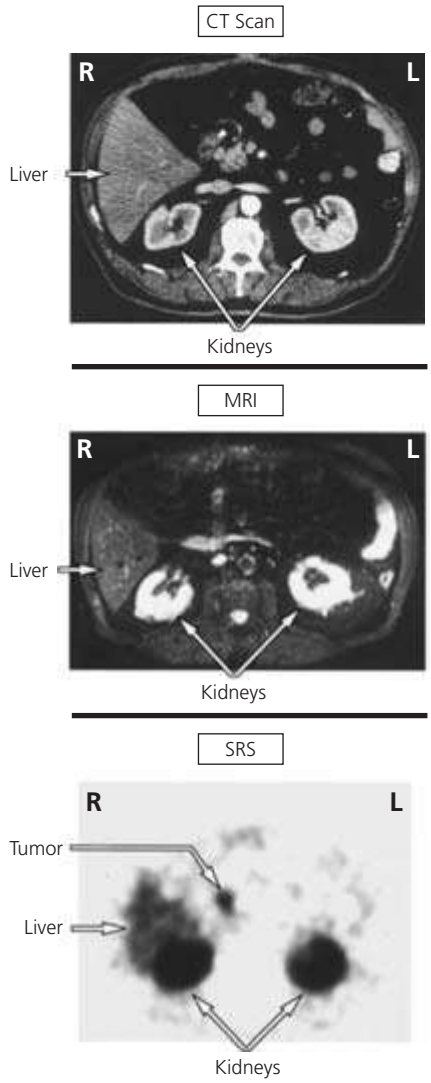

Figure 71.7 Comparison of the ability of computed tomography (CT) scan, magnetic resonance imaging (MRI), and somatostatin receptor scintigraphy (SRS) to localize a pancreatic endocrine tumor (PET). This patient previously had undergone resection of a duodenal PET secreting chromogranin A and gastrin, and underwent reexamination when the serum chromogranin A level increased 1 year postresection. Neither the CT scan **(top)** nor the MRI **(middle)** demonstrated a lesion, whereas the SRS demonstrated a tumor in the pancreatic head area. At surgery a 1.5-cm pancreatic head lymph node containing tumor was found and resected. This study demonstrates the greater sensitivity of SRS for localizing all noninsulinoma PETs [190,257–260] and why it is the initial imaging study of choice in these patients.

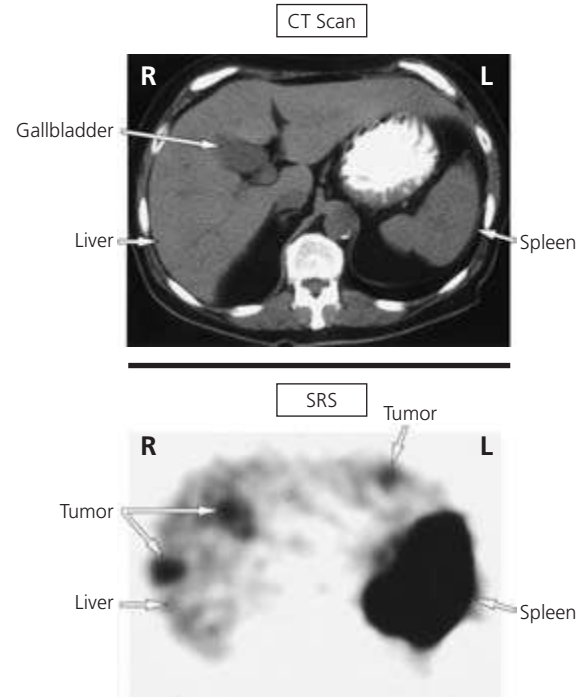

Figure 71.8 Comparison of the ability of computed tomography (CT) and somatostatin receptor scintigraphy (SRS) to localize metastatic pancreatic endocrine tumors (PETs). This patient had right upper quadrant pain with an elevated serum chromogranin level and gastrin level. The CT scan **(top)**, magnetic resonance imaging, ultrasound and angiography were negative; however, the SRS **(bottom)** showed three liver metastases. A percutaneous biopsy demonstrated metastatic PET. This study demonstrates the greater sensitivity of SRS for detecting metastatic disease, both in the liver and at other distal sites, in patients with malignant PETs [4,190,257–260,275,277,282].

30% of the patients (range 12.5%–53%). Management was altered by SRS primarily by clarifying equivocal lesions seen on conventional imaging studies, particularly by identifying liver metastases, a primary tumor, or distant metastases [275,277,278]. One particularly important category of clarifying lesions concerned small hemangiomas of the liver (< 2 cm) [282]. These have a prevalence of 7.3%–15% in autopsy studies, appear indistinguishable from small liver metastases of PETs on MRI or angiography, and cannot be distinguished easily by red blood cell (RBC) scanning or any imaging modality [282]. In one study [282], SRS was positive in 93% of patients with small liver metastases (mean diameter 1.3 cm) of PETs and negative in all small hepatic hemangiomas (mean diameter 1.3 cm). Somatostatin receptor scintigraphy had a significantly better sensitivity, accuracy, and positive and negative predictive value than any other modality for identifying the liver metastases (Fig. 71.8).

Numerous studies demonstrate that endoscopic ultrasound (EUS) is a sensitive method to detect PETs, especially if the PET is intrapancreatic (Fig. 71.9) [241,247,251,264–266,283]. In ten different studies, EUS localized an insulinoma in 57%–92% of patients [241,247,251,263,265,266], and was almost equally sensitive as calcium provocative testing (i.e., 88%) [265], whereas CT scan only localized a tumor in 0%–20%, and SRS in 12%–14%. Similarly, EUS localized a gastrinoma overall in 40%–100% of patients [265], a pancreatic gastrinoma in 75%–100% of cases in four studies

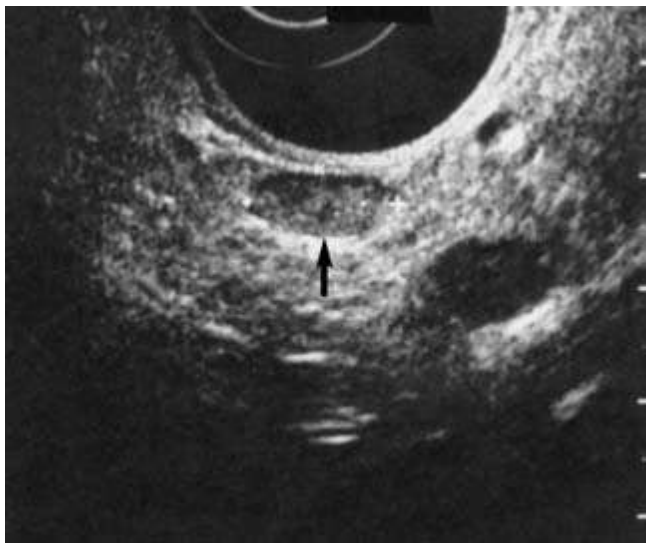

Figure 71.9 Endoscopic ultrasound of a patient with an insulinoma. This image of the head of the pancreas was taken using a 12-MHz probe at the level of the duodenal bulb. It shows a 1.3 × 0.7-cm sharply demarcated, relatively hypoechoic nodule (arrow), which was an insulinoma that was later enucleated at surgery. The splenic vein is seen below the tumor in the 5 o'clock position. The patient was a 29-year-old woman with clinical history and laboratory values diagnostic of insulinoma, but with negative dynamic computed tomography and selective angiography studies. This result shows the sensitivity of endoscopic ultrasound for localizing even small, intrapancreatic endocrine tumors. The author wishes to thank Dr Charles J. Lightdale, Director of Clinical Gastroenterology, Columbia-Presbyterian Medical Center, New York, for providing this picture.

[247,251,264], and a duodenal gastrinoma in 0%–67% in five studies [4,241,247,264]. Therefore, EUS was particularly sensitive for localizing PETs within the pancreas and with insulinomas is more sensitive than SRS, whereas with other PETs in the pancreas it is at least equal in sensitivity to SRS [247,265]. Figure 71.9 shows the ability of endoscopic ultrasound to localize an insulinoma in a patient. To obtain optimum results with EUS requires considerable expertise, whereas SRS can be performed in most radiology departments. However, to obtain optimum results with SRS, SPECT (single photon emission computed tomographic) scanning is needed [259]. Neither EUS nor SRS localizes many small extrapancreatic PETs, especially duodenal gastrinomas [243,258], but SRS has the advantage over EUS of allowing total body assessment at one time; therefore it is especially useful for identifying distant metastases. With both EUS and SRS false-positive results can be obtained; however, the exact rate is not well defined. Somatostatin receptor scintigraphy can be positive in thyroid disorders, breast disease, lymphomas, granulomatous diseases (e.g., sarcoid, tuberculosis), gallbladder filling, wound infections, accessory spleen, various arthritides, and other non-PET neuroendocrine tumors, such as gastric carcinoids [190,260,284,285]. In one prospective study [285], SRS had a false-positive rate of 12% for localizing a gastrinoma; however, when the clinical context was carefully considered, the proportion of cases in which false-positive SRS results altered clinical management was only 3%. There are no comparable studies on the false-positive rate of EUS for small PETs, especially when extrapancreatic.

For metastatic disease to the liver, SRS is more sensitive than conventional imaging studies (Table 71.6) [190,257,275,277,282]. This is shown in Figure 71.8 for a patient presenting with right upper quadrant pain with an elevated serum chromogranin level but normal CT scan and other conventional imaging (MRI, angiography, ultrasound). The SRS demonstrated three liver metastases, which were proven to be metastatic PET. In a comparative study [257] of 24 patients with proven hepatic metastases in patients with gastrinomas, SRS was positive in 92% of the patients, which was significantly better than ultrasound (46%), CT scanning (42%), angiography (62%), and was equal to MRI (71%). The combination of all conventional imaging studies was equal to SRS alone (83% vs 92%, respectively).

Functional localization by venous sampling for hormonal gradients from various portal venous sites or peripheral veins has been used for both insulinomas and other PETs (Fig. 71.10) [14,151,267–269]. This procedure requires considerable expertise and was associated with complications in 20% of the patients in one large study [268]. A simplified method has been developed that has replaced direct venous sampling from portal venous tributaries and does not require transhepatic catheterization. Studies demonstrated that gastrinomas can be functionally localized by measuring hepatic venous samples for gastrin gradients after selective intraarterial injection of secretin, and that this method was more sensitive than PVS, required less expertise, and had lower morbidity [243,286]. A similar procedure has been developed for localizing insulinomas [270,287] using calcium gluconate (0.01–0.025 mEq Ca^{2+}/kg) and is frequently used when the other imaging studies are negative [288,289]. In one comparative study [270] in patients with insulinomas the intraarterial study with selective hepatic venous sampling for insulin levels was positive in 88% of the patients, compared with only 9% for ultrasound, 17% for CT scan, 43% for MRI, 36% for angiography, and 67% for PVS. Calcium infusions may also stimulate the release of hormones from other PETs, so this approach may be useful in these tumors [4]. A typical result is shown in Fig. 71.10 where a much larger increase in insulin concentration in the hepatic vein was seen after the splenic arterial injection; at surgery insulinomas were found in the pancreatic tail in the area supplied by this artery.

Intraoperative ultrasound can be a valuable aid in localizing PETs at the time of surgery (Fig. 71.11) [248,271]. Intraoperative ultrasound is particularly useful for localizing small intrapancreatic PETs, but is less sensitive for extrapancreatic

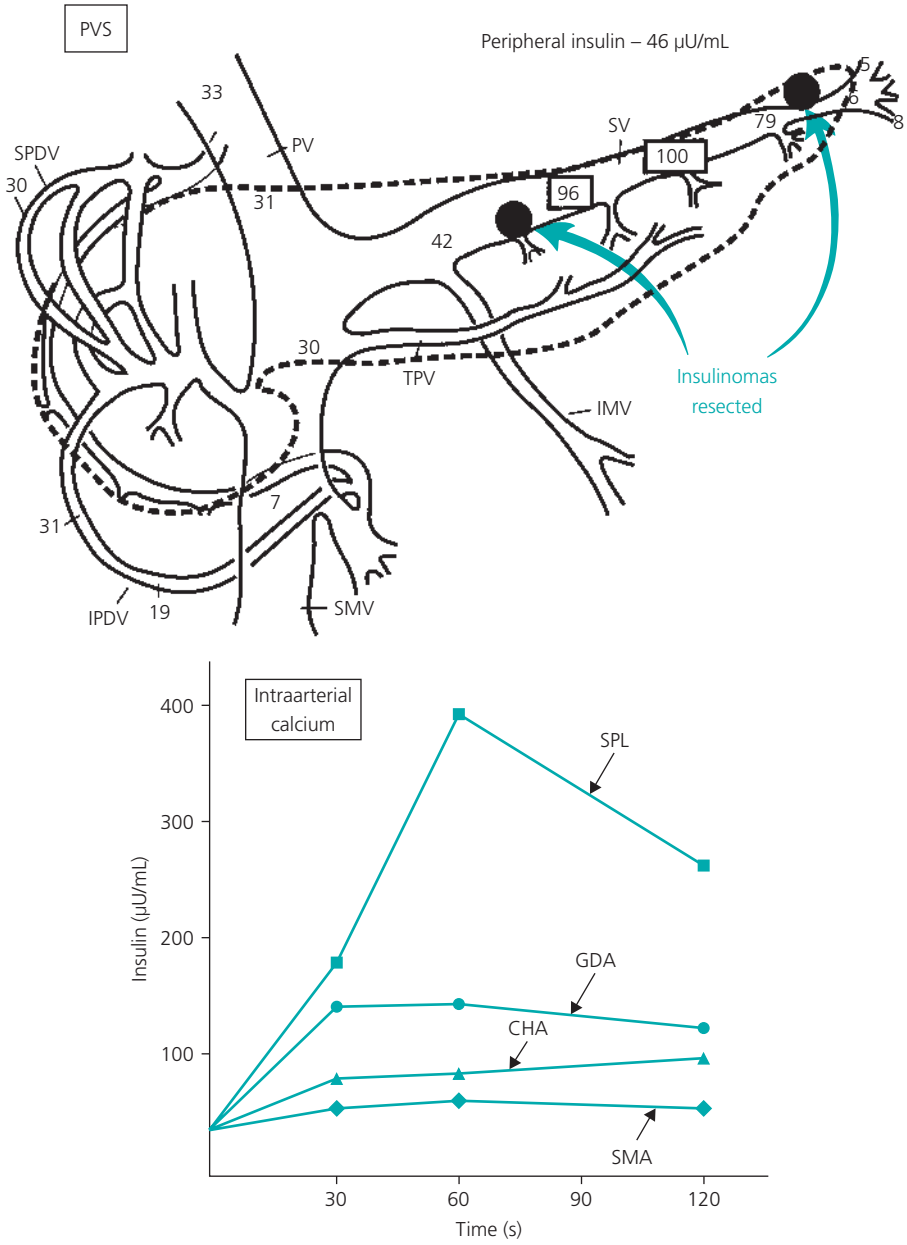

Figure 71.10 Functional location of an insulinoma by selective venous sampling for insulin concentrations in portal venous tributaries (top) or by selective hepatic venous sampling for insulin concentrations after selective intraarterial calcium injections (bottom) in a patient with multiple insulinomas in the setting of multiple endocrine neoplasia type I (MEN-I). **Top panel:** a sampling catheter was passed across the liver and venous samples obtained from the indicated locations; simultaneous peripheral venous samples were obtained and assayed for insulin concentration. The step up to 100 μU/mL is seen in the mid-splenic vein, suggesting a tumor in the pancreatic body or tail. IMV, inferior mesenteric vein; IPDV, inferior pancreaticoduodenal vein; PV, portal vein; SMV, superior mesenteric vein; SPDV, superior pancreaticoduodenal vein; SV, splenic vein. TPV, transverse pancreatic vein; **Bottom panel:** calcium gluconate (0.01 mEq Ca^{2+}/kg) [270] was selectively injected into the indicated arteries and hepatic venous samples obtained prior to injection and at 30 s, 60 s, and 120 s postinjection and analyzed for insulin concentration. There was a marked increase after the splenic artery injection (SPL), whereas after injection into the gastroduodenal artery (GDA), common hepatic artery (CHA), and superior mesenteric artery (SMA) there was a much smaller increase. Two insulinomas were found at surgery (see location by arrows in top panel) in the pancreatic body and tail, which is in the area localized by the PVS sampling as well as the area supplied by the splenic artery. The intraarterial calcium method is more sensitive than portal venous sampling [269,270], requires less expertise, and can be done at the time of angiography thereby avoiding a separate procedure. In some studies [270] the intraarterial calcium is the most sensitive modality for localizing small insulinomas.

tumor [248,271]. Intraoperative ultrasound may also suggest whether the PET is malignant, define the proximity of a PET to the pancreatic duct, and further define the anatomy of a nodule palpated at surgery [248,271]. Intraoperative ultrasound is essential when laparoscopic tumor resection is attempted [290–293]. The use of intraoperative ultrasound at surgery will be discussed further below (see "Surgical treatment").

Bone metastases have been described in patients with advanced PETs [62]. In one study [62] of 115 patients with gastrinomas, bone scanning, MRI of the spine, and SRS were compared for their ability to identify bone metastases. Eight patients (7%) were shown to have bone metastases, and SRS and MRI had the highest sensitivity and specificity [62]. Bone scanning had a low specificity and was less sensitive than SRS or MRI of the spine. Bone metastases occurred in 31% of the patients with liver metastases, were confined to patients with liver metastases, were usually in the axial skeleton initially, and their detection changed management in all cases [62]. Figure 71.12 shows an example of the ability of SRS to image the entire skeleton at once and localize bone metastases in a patient with a malignant PET. In this patient SRS detected more spinal metastases than MRI, demonstrating its high sensitivity.

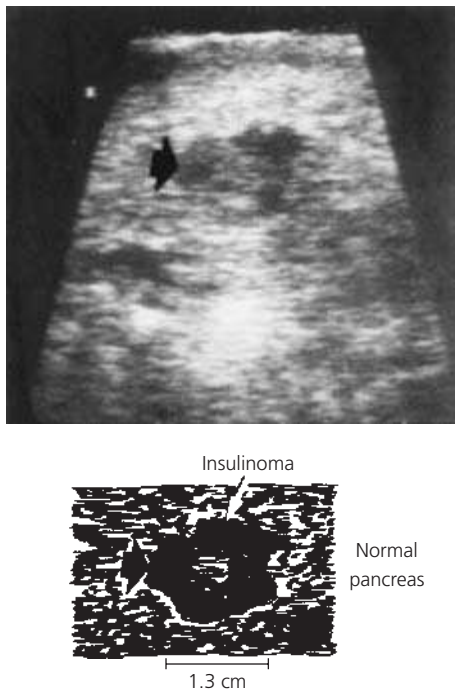

Figure 71.11 Intraoperative ultrasound localization of an insulinoma. **Top:** intraoperative ultrasound image of an insulinoma (arrow). The image was taken with a 10-MHz transducer. **Bottom:** drawing of the ultrasound image with the arrow indicating the tumor. A 1.3-cm, nonpalpable insulinoma was found at surgery in the pancreatic head. Studies [248] demonstrate that intraoperative ultrasound will localize 88% of insulinomas. In this patient the use of intraoperative ultrasound guided the successful enucleation of this tumor, which could not be identified at surgery by any other means. Courtesy of Dr Jeffrey A. Norton, UCSF, San Francisco, CA.

Figure 71.13 summarizes the recommended approach to a patient with a PET that is not an insulinoma. In such a patient both the location of the primary tumor and possible occurrence of liver or distant metastases need to be assessed. Because of its increased sensitivity and ability to image the entire body in one study, SRS is recommended as the initial study with SPECT imaging. If the SRS scan is positive for liver metastases, the extent and number of lobes need to be established to determine whether surgical resection of all imageable tumors is possible, as discussed below under "Treatment". If diffuse metastases are present, a CT- or ultrasound-guided percutaneous liver biopsy should be done (Fig. 71.13). Because it is difficult to evaluate increases in tumor size by SRS, an MRI or CT scan should be performed to evaluate changes in the size of liver metastases over time [245]. If no metastases are present and no primary tumor is seen, endoscopic ultrasound should be performed by an experienced endoscopist. If a primary tumor is still not seen, a selective angiogram should be performed and the use of intraarterial calcium with determination of hepatic venous hormonal gradients considered (Figs 71.10 and 71.13).

Studies from Europe provide evidence that positron emission tomographic scanning using [^{11}C]5-hydroxytryptophan or gallium 68-labeled somatostatin analogues may have greater sensitivity than SRS or other conventional imaging modalities [261,294–296] and therefore be clinically useful in a subset of patients. In a study [294] using a gallium-68-radiolabeled octreotide analogue (^{68}Ga-DOTA-Tyr^3-octreotate) involving 84 patients with gastrointestinal neuroendocrine tumors, positron emission tomography had greater sensitivity (97% vs 55%) and accuracy (96% vs 58%, $P < 0.01$) with equal specificity (92%) to SRS. This technique is not approved for use in the United States.

Table 71.6 Studies assessing the ability of various modalities to localize insulinomas and other pancreatic endocrine tumors

	Mean percentage localized (range)		
		Other pancreatic endocrine tumors	
Localizing modality	**Insulinomas**	**Primary**	**Liver metastases**
Ultrasound	27 (0–64)	24 (6–70)	40 (14–76)
Computed tomography scan	30 (16–61)	50 (33–100)	70 (35–100)
Magnetic resonance imaging	10 (0–25)	25 (21–100)	80 (43–100)
Selective angiography	60 (41–90)	68 (35–100)	69 (33–86)
Selective portal venous hormone sampling	80 (63–96)	71 (46–94)	N/A
Hepatic venous sampling for insulin after intraarterial calcium	94 (88–100)	N/A	N/A
Intraoperative ultrasound	89 (80–100)	91 (80–100)	N/A
Endoscopic ultrasound	82 (57–93)	70 (40–100)	N/A
Radiolabeled somatostatin receptor scintigraphy	50 (12–56)	70 (56–100)	94 (88–100)

Data from Jensen [4,190]; Jensen and Norton [12,28,47,190]; Orbuch et al. [238]; Norton and Jensen [241]; Norton et al. [243]; Zimmer et al. [247]; Norton [248]; London et al. [249]; Rosch et al. [251]; Krenning et al. [259]; Strader et al. [269]; Gibril et al. [474].
N/A, not applicable.

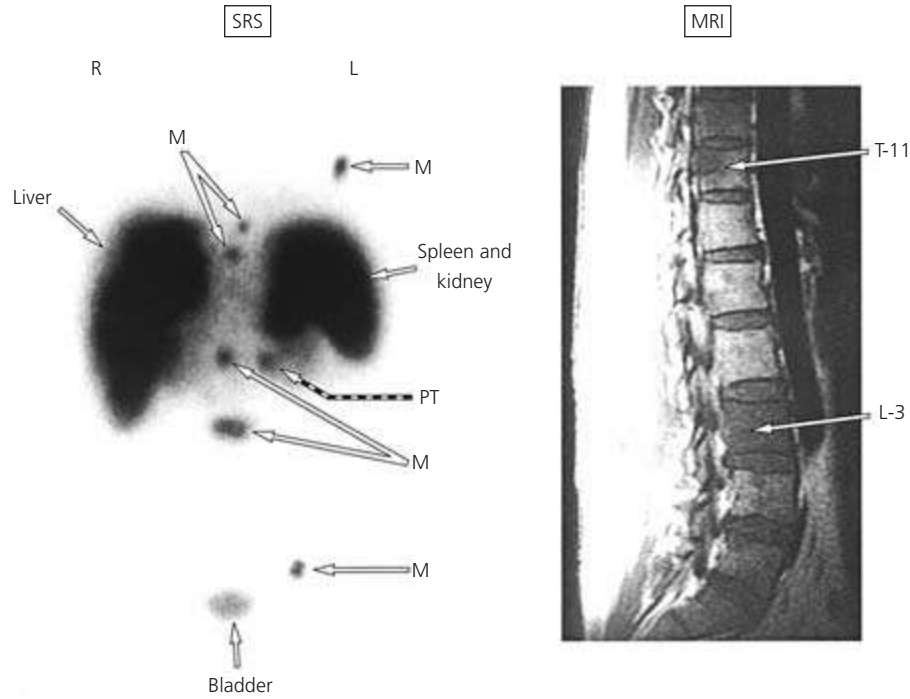

Figure 71.12 Comparison of the ability of somatostatin receptor scintigraphy (SRS) and magnetic resonance imaging (MRI) to localize bone metastases in a patient with a malignant pancreatic endocrine tumor (PET) secreting glucagon and gastrin and located in the pancreatic tail. In this patient with back pain, MRI demonstrated T11 and L3 metastases (**right panel**, arrows) whereas SRS **(left panel)** demonstrated numerous metastases (labeled M) in the thoracic and lumbar spine, ribs, and right pelvis. The broad dotted arrow on the SRS shows the primary tumor (labeled PT). This patient's results show the high sensitivity of SRS for localizing bone metastases and the ability of SRS to allow a complete body scan at one time.

Treatment

General

All patients with functioning PETs have two problems that are interrelated but often have to be dealt with separately. Acutely, the symptoms due to the state of excessive hormone must be controlled, and ultimately the tumor itself must be dealt with. Although excision of the PET would solve both clinical problems, this is frequently not possible because of several possible factors: there is unresectable metastatic disease at time of diagnosis; the primary PET cannot be found at surgery; or curative resection is not achieved. Furthermore, the clinical condition of the patient may have deteriorated because of complications of the hormone excess state necessitating treatment prior to surgery [2].

Even with the increased ability to control the symptoms of the hormone excess state with various medical therapies, a number of factors suggest that all patients with PETs should be considered for surgical resection of the tumor. First, although only 5%–15% of insulinomas are reported as malignant, with the other PETs at least 50% are malignant, and in many reports this percentage of patients or more have metastases at the time of diagnosis [2,297]. For example, in various studies up to 90% of all gastrinomas [14,46,244], up to 90%–100% of nonfunctional PETs [1,5,29,298,299], 82% of glucagonomas [15], and 50%–60% of VIPomas [20,21] were malignant. Furthermore, although in some studies large tumors were more likely to be malignant [9,10,59,61,300–304], metastases occasionally occur with small tumors; therefore, the absence of tumor on the initial imaging studies or small tumor size do not rule out metastatic disease. Although a number of patients with metastatic glucagonomas [59,79], gastrinomas [9,10,14,61], and somatostatinomas [23,305] have been reported to have long-term survivals, detailed studies of PETs, such as gastrinoma, in which there has been effective therapy for the gastric hypersecretion since the late 1980s, suggest that for patients with advanced disease the long-term prognosis is worse than originally thought [9,10,14,79,300,306,307]. Results from studying primarily patients with gastrinomas have provided insights into prognostic factors and determinants of survival in these patients, insights that are likely also important with other less common PETs because of their similar biological behavior, histopathology, growth patterns, and metastatic pattern (Table 71.7) [9,10,46,61,245,300]. These studies demonstrate that various PETs in different patients frequently grow at markedly different rates [9,10,19,61,245,300,308]. In approximately 75% of patients with gastrinomas the tumor demonstrated either no growth or indolent growth, whereas in 25% it demonstrated aggressive growth [10,61]. Furthermore, even in patients with liver metastases, aggressive growth occurred in less than one-half; however, all the deaths occurred in this subgroup of patients [245]. The presence or absence of liver metastases initially, or the development of liver metastases during follow-up, is the single most important prognostic factor (Fig. 71.14) [9,10,19,61,300,303,304,309]. For example, in one study [10]

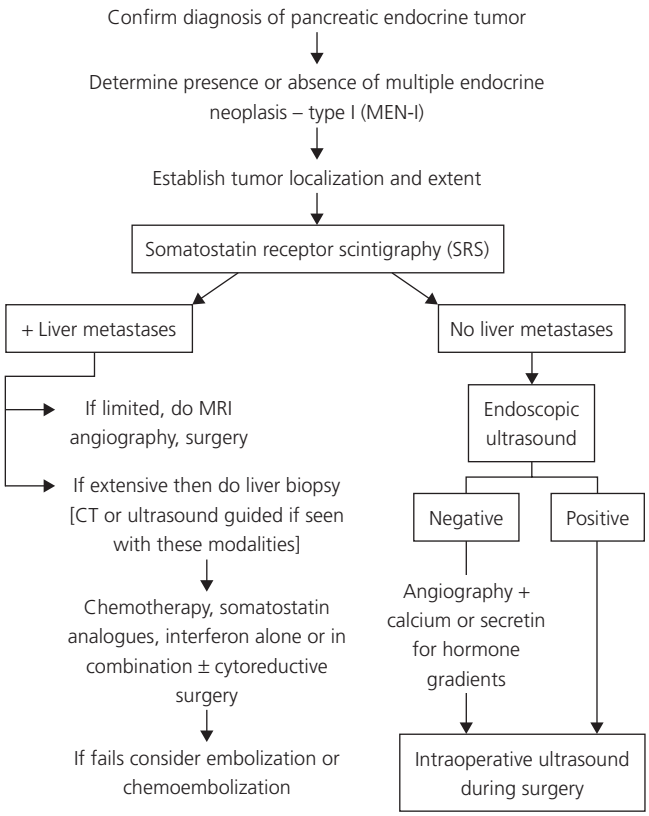

Figure 71.13 Algorithm summarizing the approach to tumor localization in a patient with a pancreatic endocrine tumor that is not an insulinoma. Because of its increased sensitivity for detecting the primary and metastatic disease both in the liver and at distal sites [190,257,260,275,277] and its ability to cover all body areas in one study, somatostatin receptor scintigraphy (SRS) with single photon emission computed tomographic imaging is the initial localization method of choice. SRS will localize more than 90% of patients with liver metastases [4,190,257,260,275,277]. If no liver metastases are seen, endoscopic ultrasound (EUS) is the most sensitive method for localizing the pancreatic primary [263,474]. If the EUS is negative, selective angiography with intraarterial injection of calcium (insulinoma, somatostatinoma, glucagonoma) and sampling of hormonal levels in hepatic venous samples should be done. At surgery intraoperative ultrasound should always be used because it will identify lesions not seen by other methods [248]. Up to 15% of patients with metastatic disease in the liver may have resectable disease and should be considered for surgery [4,246,306,316]. CT, computed tomography; MRI, magnetic resonance imaging.

the 15-year survival in patients with liver metastases that were present during initial assessment was 26%, whereas in patients without liver metastases it was 96%. The extent of liver metastases (one lobe, two lobes, diffuse) (Fig. 71.14), presence of lymph node metastases, bone metastases, a large primary tumor size, primary location (pancreatic worse prognosis than duodenal gastrinoma), various histological features, tumor marker levels (chromogranin A, specific hormone level, such as gastrin), flow cytometric features, and proliferative indices (Ki-67, mitotic index), have all been shown to be

Table 71.7 Prognostic factors in pancreatic endocrine tumors (PETs) for decreased survival

Category	Prognostic factor
Clinical features	Female gender Absence of multiple endocrine neoplasis–type 1 (MEN-I) syndrome
Laboratory features	Increased chromogranin A in some studies Increased gastrin level in gastrinomas Lack of progesterone receptors
Tumor extent/size	Presence of liver metastases Increased extent of liver metastases Presence of lymph node metastases Presence of bone metastases Primary tumor site Primary tumor size (> 2–3 cm = worse prognosis)
Tumor behavior	Growth of liver metastases Incomplete tumor resection Nonfunctional tumor Development of ectopic Cushing syndrome Increased depth of tumor invasion
Histological features	Necrosis High nuclear atypia Poor tumor differentiation High growth indices (high Ki67 index [> 2%], PCNA expression, mitotic rate) Capsular invasion Vascular or perineural invasion Low vessel density
Other	Flow cytometric features (i.e., aneuploidy) *RAS* oncogene or *p53* overexpression

Data from Jensen [4,9,190]; Yu et al. [10]; Weber et al. [61]; La Rosa et al. [221]; Madeira et al. [300]; Hochwald et al. [302]; Panzuto et al. [304]; Metz et al. [310]; Marion-Audibert et al. [475].

important prognostic factors in various studies of PETs (Table 71.7, Fig. 71.14) [2,9,10,57,61,190,221,300,302,304,310]. Studies have shown that survival is directly related to tumor extent. In patients with primary tumors so small that no tumor was found at the time of surgery, the 5- or 10-year survival was 90%–100% [14,79,306]. In patients with complete tumor resection the 5- or 10-year survival was also 90%–100%. But in patients whose tumors were incompletely resected or who developed a recurrence postoperatively, the 5-year survival varied from 14% to 76%, and 10-year survival was 20%. In some studies [311,312], but not others [302,304,313], survival was better with functional than with NFPETs. In patients with unresectable PETs, such as gastrinomas metastatic to the liver, the 5-year survival varied from 20% to 75%, and the 10-year survival in one study was 30% (Fig. 71.14) [14,79,306]. These data suggest that once metastatic disease occurs the prognosis is signific-

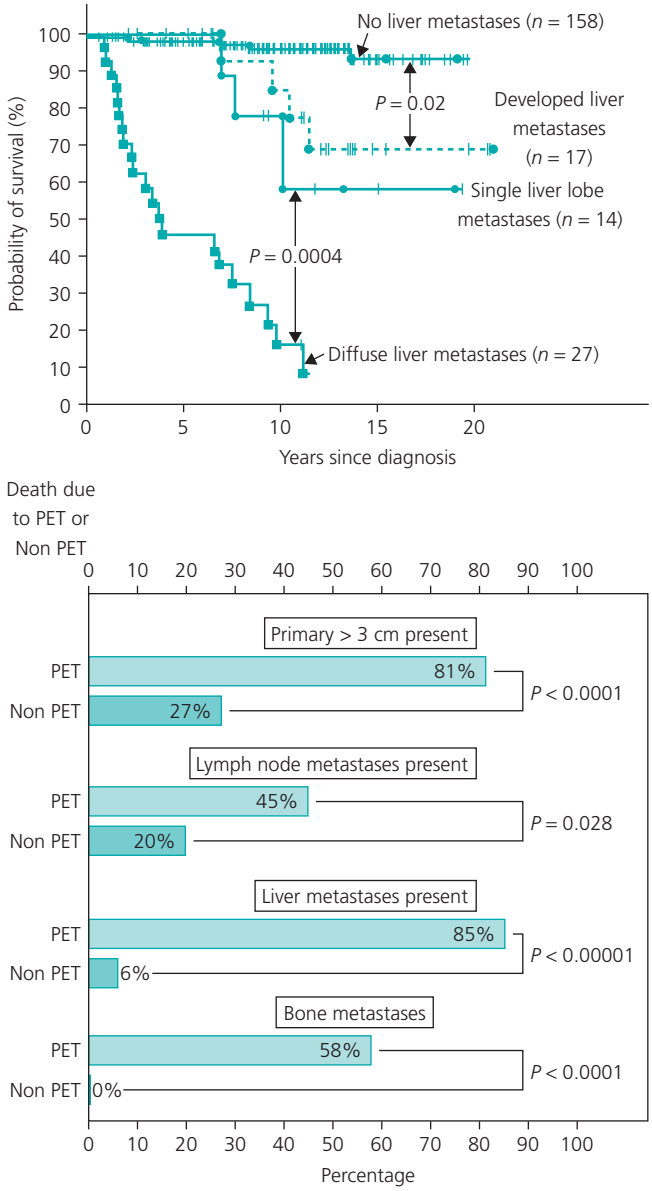

Figure 71.14 Effect of the development or extent of liver metastases from a pancreatic endocrine tumor (PET) **(top panel)**, presence or absence of lymph node or bone metastases, or PET primary size on disease-related survival. Data are from 158 patients and are modified from Yu et al. [10] **(top)** and Jensen [46] **(bottom)**. These results demonstrate that the presence of liver, bone, or lymph node metastases, a large primary PET size, and increased extent of liver metastases are all important predictors of a tumor-related death.

antly worsened. Two important studies [314,315] demonstrate for the first time that resection of the primary PET significantly decreases the rate of development of liver metastases or improves survival (or both). The improvement in survival [315] by surgery was due to resection of the primary PET, because only tumor-related death rates were altered by the surgical resection. Furthermore, because therapy for advanced disease is in many cases only partially effective [2,14,246,304] it is important to attempt to resect the PET while it is still localized. Unlike gastrinoma, the long-term medical therapy of the other various PETs is not well established and it is not known what percentage of patients will be refractory to medical treatment. Therefore, in functional PET syndromes not including gastrinomas, cytoreductive surgery may be helpful because it may improve symptom control [246,316,317]. Lastly, the long-term toxicity of most medical therapies, except those for gastrinomas, is not well established.

Treatment of nonmetastatic disease

Medical treatment

General treatment considerations

The initial management of patients with insulinomas, and the long-term management of those patients with insulinomas who are not surgically cured, is directed at controlling the hypoglycemia. The simplest form of nonsurgical control is dietary management. Frequent small meals may alleviate symptoms [76,99]. In severe cases intravenous glucose combined with various drug therapies may be required. In general, 10% dextrose should be avoided because of the electrolyte imbalances generated by the combination of elevated serum concentrations of glucose and hyperinsulinemia [99]. Some investigators have used slowly absorbed nutrients to maintain normal blood sugars prior to surgery. This may be preferable to diazoxide because of reports of sudden hypotension in some patients receiving diazoxide prior to surgery [318]. However, diazoxide (150–800 mg/day) has been used successfully to manage patients with insulinoma prior to surgery as well as in long-term treatment [76,78,99,319]. Diazoxide is a benzothiadiazide that has a plasma half-life of 28 hs and a peak hyperglycemic effect 12 h after an oral dose or 4 h after an intravenous dose. Its hyperglycemic action is attributed to direct inhibition of insulin release by adrenergic stimulation and by enhancing glycogenolysis [59,76]. The main side effects of diazoxide are sodium retention (47%), gastrointestinal symptoms such as nausea, and occasionally hirsutism [59,76,78,99,319]. Sodium retention can be minimized by administration of diazoxide with a diuretic and restricting sodium intake. The gastrointestinal side effects can be decreased by taking the drug with meals [59,99]. Diazoxide is primarily excreted by the kidneys and is known to displace various protein-bound drugs and increase their free concentrations in the serum [99]. Diazoxide therapy should be initiated with low doses and increased to a maximum of 600–800 mg/day in two or three divided doses as tolerated or until symptoms are controlled. In general approximately 50%–60% of patients will respond [59,76,78,99,319,320]. Patients have been treated for up to 22 years with diazoxide [319], demonstrating that it can be effective in the long term. The calcium channel blocker verapamil [76,78,321,322] and diphenylhydantoin [323] have also been used to control hypoglycemia, with some

success in some patients when diazoxide has failed. The long-acting somatostatin analogue, octreotide (Sandostatin, Novartis), has been shown to be effective in 36% of patients for the acute and long-term control of hypoglycemia in patients with insulinoma [28,121,171,190] and this will be discussed further in the next section.

In the initial management of patients with VIPomas the most important issue is to replace the large volumes of fluid and electrolytes lost, and correct the hypokalemia and acidosis [20,21,114]. This is particularly important because acutely a common cause of death in these patients is renal failure associated with hypokalemia and hypokalemic nephropathy [20]. Formerly, drug therapy included the use of high doses of prednisone (60–100 mg/day) [20], which decreases diarrhea in 40%–50% of patients [20,20], and the use of clonidine [324], angiotensin II [325], norepinephrine (noradrenaline) [325,326], indometacin (indomethacin) [327,328], lithium carbonate [329], phenothiazines [330,331], propranolol [28], metoclopramide [332], lidamidine [333], and loperamide [333], all of which decreased the diarrhea in a small number of patients. These agents primarily enhanced sodium absorption in the proximal small intestine or inhibited intestinal secretion [20]. Subsequent studies demonstrated that the long-acting somatostatin analogue, octreotide controls diarrhea in 87% of all patients with VIPomas [28,114,190], and this has become the drug of choice. It will be considered in detail below (see "Octreotide").

In patients with glucagonoma the rash – migratory necrolytic erythema – may be severe. Some studies suggest that correction of the hypoaminoacidemia with parenteral amino acids and normalization of the plasma amino acid levels will improve the rash [79,161,162]. However, another study noted disappearance of the rash after surgical resection of the glucagonoma but not dietary normalization of the plasma amino acids [163]. Octreotide [121,171,190] improves symptoms in 84% of patients with glucagonomas and its use will be discussed in detail below.

Octreotide

As pointed out earlier, somatostatin has been shown to inhibit the release of a number of hormones in both animals and in human volunteers, and to have direct actions on a number of target organs including inhibition of gastric and pancreatic secretion, intestinal absorption, and gastrointestinal motility [190,191]. Administration of natural somatostatin by continuous infusion to a number of patients with functioning PETs demonstrated symptomatic improvement in patients with VIPomas, insulinomas, gastrinomas, and glucagonomas [28,47,121,190]. However, natural somatostatin has a short half-life ($t_{0.5}$ 2–3 min) [12,190] and therefore has limited therapeutic efficacy because it has to be given by continuous intravenous infusion.

The synthetic octapeptide analogue of somatostatin, octreotide acetate (Sandostatin), has a half-life ($t_{0.5}$ 100 min.) 33 times longer than somatostatin and thus can be administered two to four times a day [121,190,334,335]. In the rat, compared with native somatostatin, it was 70-fold more potent at inhibiting growth hormone release, 3-fold more potent at inhibiting insulin release, 23-fold more potent at inhibiting glucagon release, and 80-fold more potent at inhibiting acid secretion [190,334,335]. Octreotide is usually started at a dose of 50–150 μg b.i.d to t.i.d. subcutaneously given by self-administration, the dose being increased if not effective. Doses of up to 750 μg t.i.d have been used in patients with the carcinoid syndrome and PETs [121,171,190]. There have been reports in small numbers of cases where intermittent subcutaneous administration of octreotide was ineffective or only partially effective and the continuous infusion of octreotide was more effective [190,336]. One long-term study [337] reported on seven patients with various PETs treated for a median period of 20 months (range 13–54 months) in which octreotide was initially effective in all. Worsening of symptoms and rising plasma hormone concentrations occurred over a median period of 5 months after starting therapy; these parameters were initially reversed by increasing the octreotide dose, but after a median of 13 months (range 5–34 months) the symptoms returned and were no longer responsive to higher doses of octreotide.

In one review of 55 patients with insulinoma [190], octreotide improved some symptoms in 31% and plasma insulin concentrations decreased in 41%. However, 65% of the patients received octreotide for less than 1 week because most went to curative surgery. In a second series of 17 patients with insulinoma [338], octreotide controlled symptoms of hypoglycemia in 58%. Octreotide also decreases growth hormone secretion and plasma glucagon levels and thus it may worsen the hypoglycemia. A reduction in hypoglycemia attacks has been noted only occasionally [339]. Octreotide was given for over 1 month in 10 patients [190]. In short-term studies octreotide appears to benefit 36% of patients with insulinoma, but the true percentage of patients with insulinomas who continue to respond to octreotide is not clear [171]. Of the five subtypes of somatostatin receptors, octreotide has high affinity for sst_2 and sst_5, intermediate affinity for sst_3, and low affinity for sst_1 and sst_4 [190,191]. The response rate of insulinomas to octreotide is likely lower than other PETs because insulinomas frequently possess low densities of somatostatin receptors or lack somatostatin receptor subtypes 2 and or 5 (sst_2 and sst_5), which have high affinity for octreotide/lanreotide analogues, whereas 80%–90% of the other PETs possess high densities of high-affinity sst_2 and sst_5 receptors [190,259,340]. This finding may also explain why insulinomas appear to be less responsive than glucagonomas, VIPomas, gastrinomas, or GRFomas to octreotide and are imaged less frequently by SRS [190,259,259,260]. This proposal is supported by a study [338] that correlated sst receptor expression in insulinomas and the functional responses to octreotide administration as

well as the ability of radiolabeled somatostatin analogues to image the tumor. In the 50% of insulinomas not responding to octreotide, sst_2 receptors were not found but sst_5 receptors were present, leading to the conclusion that the presence or absence of sst_2 receptors on the insulinoma partially explains the variable responses of insulinomas to octreotide and to imaging with SRS [338].

Octreotide improved diarrhea in 27 (87%) of 31 patients with VIPomas in one review [190], and plasma VIP concentrations decreased in 87% of the patients, which is similar to the 78%–90% response rate in other reports [19,21,114,121,171,190]. In the same review [190], tumor size was evaluated in 18 patients with VIPomas and a decrease in size occurred in 4 patients. All patients demonstrated a decrease in diarrhea within the first 24 h but in three patients the effect lasted only a few days [121]. Octreotide was given for more than 1 month in 90% of the patients and remained effective for over 6 months in 50% of all patients. However, some patients developed decreased responsiveness to octreotide with time, and the dose had to be increased. In 87% of patients there was a decrease in the VIP plasma level with octreotide treatment although in many cases it did not return to the normal range [190,341,342]. In up to 25% of patients the diarrhea may respond without a decrease in plasma VIP levels [171]. The apparent discrepancy in response and plasma hormone levels may be partially explained by the fact that multiple forms of VIP occur in the plasma and after octreotide treatment only the native peptide may disappear; this may represent a small fraction of the total VIP immunoreactivity and, therefore, no change in plasma VIP is detected [342]. An example of such a patient is shown in Fig. 71.15. This patient [341] had severe secretory diarrhea of 5816 ± 185 g/day with daily losses of 616 mmol of sodium and 348 mmol of potassium; the patient required 3 L/day of fluid with 462 mmol of sodium and 400 mmol of potassium to maintain serum potassium at 3.4 mmol/L and bicarbonate at 15–24 mmol/L (Fig. 71.15). Octreotide 100 µg twice a day was started and stool output fell to 292 ± 55 g/day, with daily fecal loss of 5 mmol sodium and 25 mmol of potassium and rising serum potassium and bicarbonate levels. This VIPoma was secreting VIP, motilin, PP, and neurotensin, which with octreotide treatment decreased the plasma levels by 94% for PP, 76% for VIP, 88% for neurotensin, and 70% for motilin (Fig. 71.15) [341]. This patient's diarrhea was controlled for 3 months, at which time she died of respiratory failure [341]. The cost-effectiveness of long-term octreotide treatment in patients with VIPomas has been studied [343]. It was concluded that long-term octreotide treatment decreases medical costs for a VIPoma patient by 50% [343].

In 33 patients with glucagonoma, octreotide improved the skin rash in 24 patients and reduced plasma glucagon concentrations in 78% of patients [16,17,28,47,79,121,171,174, 190,344]. Weight loss, pain, and diarrhea improved in patients with these symptoms, but octreotide may not improve

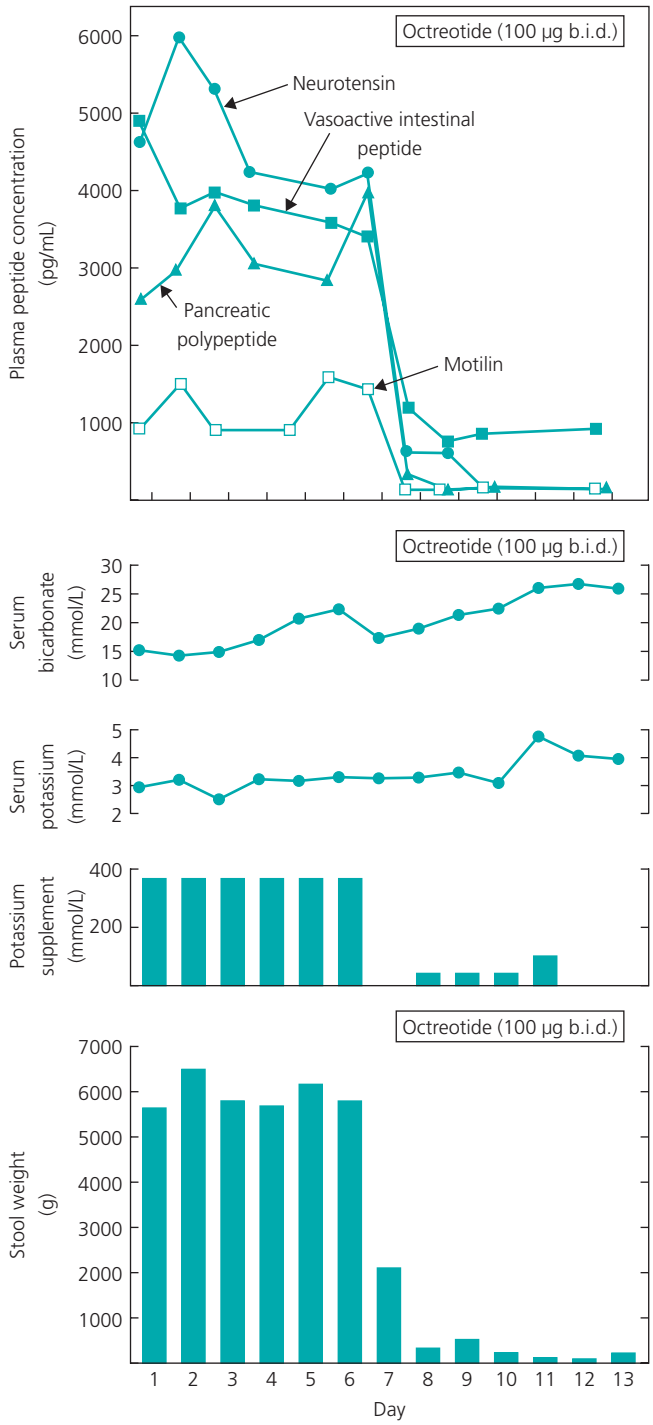

Figure 71.15 Effect of octreotide treatment on hormonal levels, stool weight, serum electrolytes, and potassium replacement in a patient with a metastatic VIPoma. This patient [341,342] had a VIPoma secreting vasoactive intestinal polypeptide (VIP), neurotensin, pancreatic polypeptide (PP), and motilin. Shown are values for the different parameters for 6 days prior to, and 7 days after, receiving octreotide 100 µg b.i.d. subcutaneously. The hormone levels all decreased by more than 70%, serum bicarbonate increased, and potassium intake was decreased by 94%, and stool weight decreased by over 95% (5816 ± 185 g/day to 292 ± 55 g/day). The patient was maintained for 3 months on octreotide, remaining asymptomatic until death of unrelated causes.

the diabetes mellitus [16,79,174]. Octreotide usually does not affect tumor size, decreasing the tumor size in only 8% of cases in one study [16] and in only 4% of cases in the literature [16,190]. Decreases in plasma glucagon concentration occur in 80%–90% of patients but do not always parallel the improvements in the rash [79,121,171]. Octreotide was administered for more than 1 month in 94% of patients and remained effective for over 6 months in 75% of all patients. In one study [16] the duration of treatment ranged from 2 months to 3.5 years.

Bromocriptine, a dopamine agonist, is effective in patients with classical acromegaly; however, it only reduces GRF levels in 25% of patients with GRFomas [84]. Octreotide has become the agent of choice for GRFomas [23,84,190,204,207,209]. Octreotide reduces symptoms of both growth hormone excess and elevated GRF plasma concentrations in patients with GRFomas [23,84,171,190,345]. In most patients reported with GRFomas, octreotide improved symptoms and reduced the GRF levels in all patients [23,84,190,207], although in one study [346] continuous infusion was required. Eight of the nine patients received treatment with octreotide for more than 6 months [171,190,345]. Pituitary shrinkage occurred with octreotide treatment in some studies [207]. In GRFomas, the suppression of growth hormone secretion is mainly due to a suppressive effect at the pituitary level because plasma GRF levels do not become undetectable [84].

A small number of patients with somatostatinomas were treated with octreotide [171,347]. In three of four treated patients plasma somatostatin-like immunoreactivity levels decreased, and in two patients symptoms due to the somatostatinoma syndrome (diarrhea and diabetes) were improved [347].

Long-term treatment with octreotide has a number of unresolved issues. Many patients require increasing doses of octreotide over time, and some patients require high doses [171,190,348]. In a small number of patients when the octreotide was stopped and restarted, the symptoms were controlled at a lower dose of octreotide [171], whereas other patients did not respond. Furthermore, it is unclear why in some cases intermittent subcutaneous (s.c.) injections are not effective, whereas continuous low-dose s.c. infusions are effective. It is also unclear whether the sustained-release preparations (octreotide-LAR, lanreotide-SR, lanreotide-autogel) [349] will be effective in cases where intermittent s.c. octreotide injections prove ineffective. Octreotide, in addition to reducing the ectopic release of hormone, also decreases growth rate in a significant proportion of PETs and decreases tumor size in a small percentage of patients [2,171,190,308]. This will be discussed in a later section (see "Somatostatin analogues") but it is not established that the dose of octreotide is the same for both effects.

Side effects have been recorded in approximately 50% of patients undergoing short-term octreotide treatment, but these have rarely been serious enough to stop therapy [121,171,190]. Most patients only experience some pain or discomfort at the subcutaneous injection site (9%) [121]. The other most common side effects are gastrointestinal, including nausea (15%), vomiting (1%), constipation, crampy abdominal pain, and diarrhea (13%), which may improve with time [171]. Steatorrhea can occur with octreotide [190]: at low doses the steatorrhea is usually mild and transient, but at high doses gross steatorrhea can occur [350]. Dunne and colleagues [121] reported that 13% of patients developed diarrhea, 6% had fatty stools, and 8% experienced loose stools; elevated fecal fat levels were demonstrated in nine patients. Rarely does octreotide have to be stopped because of steatorrhea; and occasionally it may impair glucose tolerance and unmask diabetes mellitus. Occasional postprandial hyperglycemia has been reported [171]. Reactions were graded as mild in 48%, moderate in 38%, and severe in 15% [121]. Most of the side effects were short-lived and did not result in stoppage of octreotide treatment. A number of studies have assessed the safety and adverse effects of octreotide during long-term treatment, especially on gallstone formation and gallbladder function, particularly in patients with acromegaly [190,195,196,351]. In one study [195] of 103 acromegalic patients treated with octreotide (s.c.) for a mean of 30 months, 66% of patients experienced an adverse event in the first year and 46% in the second. Most were related to the GI tract, including diarrhea (58%), abdominal discomfort (44%), loose stools (36%), nausea (30%), flatulence (13%), and constipation (9%). With continued treatment the adverse events usually disappeared, and only 5% of patients discontinued treatment because of adverse events. In a detailed review of octreotide-LAR, 30% of patients developed GI side effects, which were mild in most cases and did not require drug discontinuation [349]. Various studies in acromegalic patients have shown the incidence of gallstones to range from 1% to 80%, with a mean of 29% [4,190,195,349,351]; however, it is reported that only 1% develop symptomatic gallbladder disease [349]. In one study [196] of 33 patients with carcinoid syndrome or PETs treated long-term with octreotide, the overall incidence of cholelithiases or gallbladder sludge was 52%. Seven percent of the patients had symptomatic disease and required an emergency cholecystectomy. The incidence of cholelithiases varied with octreotide dose [196], being 35% in patients on a low dose (150 μg t.i.d.) and 61% with a high dose (500 μg t.i.d.). The incidence of acute cholecystitis was 12% in the low-dose group and 5% in the high-dose group [196]. Eleven percent of patients underwent elective cholecystectomy. Long-term treatment can also alter glucose tolerance significantly in some patients; therefore, glucose levels should be monitored [190].

Long-term treatment with synthetic somatostatin analogues has been greatly simplified by the availability of sustained-released forms [190,349,349,352]. Both a long-acting octreotide formulation (Sandostatin LAR, 30 mg i.m.

every month; available in the United States and Europe) and a slow-release lanreotide (lanreotide SR, 30 mg i.m. every 10–24 days; available in Europe) or sustained-release formulation of lanreotide in gel form (lanreotide autogel) (60, 90, or 120 mg/month i.m.; available in Europe) are being used for the treatment of acromegaly, carcinoid syndrome, and PETs [4,190,349,349,352–354]. Following a 30 mg i.m. injection of octreotide-LAR, a plasma level of 1 mg/mL is achieved and maintained for more than 25 days and therefore this formulation can be administered monthly [349]. The principal side effects of octreotide-LAR are similar to those discussed in the previous paragraph for the shorter-acting formulation, and include pain at the injection site and GI symptoms (usually mild and frequently transient – pain, cramping, diarrhea) [4,349,349,352–356]. Octreotide-LAR and lanreotide SR impair gallbladder emptying [4,194,349,353,354], and octreotide-LAR has been shown to inhibit cholecystokinin (CCK) release [194]. Studies of long-term octreotide treatment have variously reported no increase in the incidence of gallstones (at 12 months), the development of asymptomatic biliary sludge in 15% of patients, and the development of symptomatic gallstones in 1% [4,349,353,354]. Lanreotide SR is reported to cause new gallstones in 10%–26% of patients [352,357]. Other side effects reported with octreotide-LAR or lanreotide autogel with long-term use in patients with acromegaly include hypoglycemia (2%) and hyperglycemia (15%) but they are symptomatic in only a small percentage of patients [4,349,353,354].

Surgical treatment

All patients with PETs should undergo exploratory laparotomy for possible cure [2,243,248,317,358]. Exceptions are patients with unresectable metastatic disease or the occasional patient whose symptoms are well controlled by medical management but who also has a concurrent medical problem that makes the risks of surgery prohibitive. It is particularly important that the surgery is performed only by a surgeon with experience in operating on patients with PETs [14,78,99,244,359]. Even experienced general surgeons and many endocrine surgeons have little experience with these rare tumors and therefore will frequently miss small tumors, perform unnecessary major resections, or fail to perform the appropriate resection to palliate symptoms.

As outlined above (Fig. 71.13), prior to surgery all patients should undergo localization studies and if the tumor localization or extent is still unclear, then functional localization studies (selective hormone sampling of portal venous tributaries) or calcium infusion with hepatic venous sampling (insulinomas, somatostatinomas, VIPomas) should be considered. Even if localization studies are negative, provided metastatic disease or a prohibitive concurrent illness are not present, the patient should undergo exploratory laparotomy [2,5,14,78,80,92,243,244,248,258,317,360].

The surgical approach has been reviewed [78,92,243,252, 317,360], and is guided by the tumor location. At exploration the liver should be carefully explored for evidence of metastatic disease. In the case of PETs that are frequently extrapancreatic in location the entire abdomen, especially the proximal small intestine, should be carefully explored to locate extrapancreatic tumors [243,252]. An extended Kocker maneuver should be performed to allow thorough examination of the pancreatic head area. The entire pancreas must be carefully explored, even if one PET is found, because there may be multiple tumors [99]. A detailed exploration for multiple endocrine tumors is particularly important in those patients with MEN-I [4,14,359,361,362]. Isolated small insulinomas should be removed by enucleation because they are rarely malignant. Isolated noninsulinoma PETs in the pancreatic body or tail should be removed either by enucleation or en bloc by distal pancreatectomy because they may be malignant [243,248,252]. Pancreatic head and proximal pancreatic body tumors require enucleation with careful dissection to avoid damage to the main pancreatic duct and its attendant morbidity. If at surgery an isolated hepatic metastasis is found and can be removed without increased risk, then it should be excised with negative margins [2,252]. Aggressive surgery, such as a proximal cephalic pancreaticoduodenectomy (Whipple operation), is indicated only in selected patients with widespread involvement in the pancreatic head region or with tumors that cannot be enucleated, because it has not been established that the potential benefit outweighs the increased risk of morbidity in most patients [2,14,243,360]. Pneumococcal vaccine (Pneumovax) should be administered preoperatively to lower the risk of postsplenectomy sepsis.

At the time of surgery the use of intraoperative ultrasound may be helpful and is recommended [14,76,248,322,323,360]. Studies show this technique will identify a certain number of PETs that would otherwise not be identified and therefore should be used routinely (Fig. 71.11) [2,14,248,271,363]. In seven studies [248], intraoperative ultrasound localized 89 of 101 (88%) insulinomas. Intraoperative ultrasound changed operative management in 10% of all cases of gastrinomas [271], either by localizing additional tumors or by determining that a tumor was malignant. Intraoperative ultrasound is particularly helpful for intrapancreatic lesions [248]. In one study [271] intraoperative ultrasound localized 96% (22 of 23) of pancreatic gastrinomas, and in a review of four studies [248] it localized 91% of 34 pancreatic gastrinomas. However, it is less sensitive for localizing duodenal tumors, finding only 30% of duodenal gastrinomas in two studies [271]. In 12 consecutive patients with occult insulinomas not visualized by any conventional imaging study, intraoperative ultrasound identified 10 of 11 (91%) of the insulinomas that were correctly identified including five pancreatic head insulinomas not identified by palpation. The use of intraoperative ultrasound not only localizes primary tumors not

detected by other modalities, but also reduces complications like fistulae, pancreatitis, and pseudocysts by allowing better definition of the anatomy prior to a procedure [248].

At the time of surgery in patients with insulinomas there have been occasional reports of hypoglycemia during manipulation of the tumor. Use of the artificial pancreas (Biostator) has documented an increased glucose requirement during resection of the insulinoma [364]. A number of centers document successful tumor removal by demonstrating rebound hyperglycemia. However, a study has shown that rebound hyperglycemia did not occur in 23% patients with successful tumor excision [365], and therefore its use is not recommended.

In most studies, 75%–98% of patients with insulinomas were cured by surgery [59,60,76,78,99,106,250,252,317, 360,363,366]. With glucagonomas there have been a number of reports of successful excision of the tumor [4,12,47,79,80,177,367,368], and in many cases follow-up has been sufficiently long enough to suggest cure. However, at least 50%–95% of the patients with glucagonomas have metastases at the time of diagnosis, with a significant additional percentage at the time of surgery [16,18,79,368]. A number of cases of glucagonomas have shown recurrence during long-term follow-up, despite apparently complete resection of all tumors initially. Therefore, the true long-term cure rate is not established. With VIPomas, somatostatinomas, GRFomas, and PPomas, many patients present with metastatic disease and, although curative resections do occur, the exact percentage of patients cured by surgical excision of the tumor has not been adequately studied [30,74,317].

If the patient is found to have more extensive disease with either local invasion or metastases at time of surgery, or metastatic disease prior to surgery, then the question of debulking or cytoreductive surgery often arises, especially if the patient has a functional PET syndrome [4,246,306,316, 317,360,369,370]. This will be dealt with more extensively in the following section.

Treatment of metastatic disease

Metastatic PETs have traditionally been characterized as slow growing [9,12], albeit based on few data about the rate of growth of untreated metastatic disease, because many patients require antitumor therapy to control symptoms. However, some insights into the natural history of metastatic disease have been gained [9,10,245,307,308]. In one study [245] of 19 patients with proven metastatic gastrinoma in the liver, the tumor demonstrated no growth over a 29-month period in 26% of patients, 32% had slow growth (1%–50% volume increase/month), and 42% had rapid growth (> 50% volume increase/month). These results agree with another study [308], which found that 78% of patients with metastatic neuroendocrine tumor to the liver demonstrated progressive disease over a 6-month period. A third study [10] demonstrated that 50% of patients with gastrinomas die from tumor-related causes, with the extent of metastatic disease and presence of bone metastases being important prognostic factors. These studies demonstrate that in most patients metastatic PETs continue to increase in size, and in approximately 50% the growth is rapid [245]. Survival studies also support these findings [10,245]. In one study [245], 62% of patients with rapidly growing liver metastases died and 0% of the patients with liver metastases showed either no tumor growth or slow growth over a mean follow-up period of 5.5 years. No clinical or laboratory parameter correlated with growth rate; however, the growth rate was highly predictive of death from the tumor [245]. In one large study [367] involving 85 patients with various functional PETs, none of the patients without metastatic disease died (follow-up 3–18 years, median 8 years). However, for the 41 patients with metastatic disease, 66% died from tumor progression, with overall 5- and 10-year survival rates of 54% and 28%, respectively. In patients with metastatic disease not to the liver (primarily lymph nodes) [367], the 5-year survival was significantly better (90%; $P < 0.001$) than those with hepatic metastases at diagnosis (23%). The 5- and 10-year survival rates [367] for patients with malignant insulinomas are 50%–88% and 50%–75%, for gastrinomas 47%–53% and 18%–30%, for glucagonomas 60% and 52%, and for VIPomas 20%–88% and 25%, respectively [18,19,60,74,367]. The natural history of metastatic VIPomas, glucagonomas, somatostatinomas, or insulinomas is not clear because effective therapy for the hormone excess state did not exist in most cases and patients frequently died of complications resulting from the hormone hypersecretion. However, with the availability of the long-acting somatostatin analogue, octreotide, this may change in the future. Excellent long-term survival data are available for patients with gastrinomas, for which effective therapy has existed for over 20 years (Fig. 71.14) [10,14,61,243]. Overall 5- and 10-year survival rates are 74% and 60%, respectively, in six series [14,61], whereas for patients with a tumor completely resected they are 100% and 90% [61]; with a primary tumor so small that no tumor was found at surgery they are 95% and 84% [14,61]; for patients in whom the tumor was resected but who were not cured or had a recurrence they are 75% and 56% [61]; and in patients with unresectable tumors they are 44% and 33%, respectively. These findings are in agreement with studies of patients with PETs without metastases, for whom 5-year survival rates were found to be 80%–90% [303,304,371], with rates of 40%–51% for those with metastases [303,304,371]. These data clearly demonstrate that overall survival correlates with the extent of the disease. Furthermore, two large studies [10,61] of 185 patients demonstrate that the single most important determinant of long-term survival in patients with gastrinoma is the presence of liver metastases. Moreover, of patients with metastatic disease who subsequently died, 50%–70% of the deaths were due to tumor progression [10,14]. There are no data to suggest that the

other metastatic PETs will differ from gastrinoma in their natural history, and in fact the study of survival of patients with other PETs [367] reviewed above suggests a similar natural history. This conclusion is further supported by the fact that similar percentages of patients with the various noninsulinoma PETs have metastatic or malignant disease, suggesting that their malignant potential and growth patterns are similar [20,22,59]. Therefore, most authorities would agree that treatment directed at the metastatic disease is indicated [2,4,5,372–377].

Disagreement occurs over the type, efficacy, and timing of therapy. Chemotherapy [4,14,299,373,375,378–388], tumor cytoreduction by surgical resection or radiofrequency ablation [4,14,246,306,316,370,389–394], hepatic arterial embolization (for functional tumors metastatic to the liver with or without chemotherapy [chemoembolization]) [4,373,374,395–397], interferon [4,398–402], the use of the long-acting somatostatin analogues [4,171,308,401,403–407], liver transplantation [4,408–412], and somatostatin receptor-mediated radiotherapy [4,413,413,414] have all been reported to have positive effects on metastatic PET growth. Each will be considered below.

Chemotherapy

A number of different chemotherapeutic regimens have been evaluated in patients with metastatic PETs [4,14,299,373,375,382,384,388,395,413,413–416]. In most studies, because of the rare occurrence of PETs, chemotherapy for PETs has been considered together as a group or in other cases included in series with metastatic carcinoid tumors [2,4,14,299,382,387]. Although some studies [4,299,382] reported no difference between different types of PETs in their responsiveness to chemotherapeutic agents, it has been pointed out that the sample size of the various tumors included in these studies was too small to establish whether each type of PET responds equally to chemotherapy [14,381]. In fact, some clinical observations suggest there may be significant differences in their responsiveness. For example, in some studies up to 90% of metastatic VIPomas are reported to respond to streptozotocin, frequently with a complete response [20]. In contrast, only 5%–40% of patients with metastatic gastrinomas increasing in size prior to treatment responded to streptozotocin plus 5-fluorouracil with or without doxorubicin, and in no case was there a complete response [4,381]. Furthermore, a high proportion of metastatic glucagonomas are reported to respond to dacarbazine (DTIC), with several complete remissions in some studies [2,4,175,379,417–419], whereas in other PETs DTIC has a low response rate (i.e., 6%) [4].

In almost all studies, streptozotocin is used either alone or in combination with one or both of 5-fluorouracil and doxorubicin [2]. Streptozotocin is a nitrourea antibiotic originally isolated from a streptomycete [420,421]. In early studies it was observed to induce diabetes mellitus [422]. The first report of clinical effectiveness of streptozotocin in a PET was in 1968 [423]. Since then it has been generally used in the treatment of PETs either alone or in combination with other agents [4]. In various series streptozotocin alone is reported to cause objective remissions in 34%–62% of PETs whereas other single-agent therapies have been relatively ineffective, with 5-fluorouracil, etoposide, doxorubicin, carboplatin, methyl-CCNU (1-[2-chloroethyl]-3[4-methylcyclohexyl]-1-nitrosourea), tubercidin, and DTIC giving objective responses in 0%–33% [4,367,373,375,388,395]. Synthesis of chlorozotocin (CZT), a chloroethyl analogue of streptozotocin, was reported in 1975 [423]. Compared with streptozotocin, CZT was reported to have only mild to moderate gastrointestinal toxicity, but myelosuppression was the dose-limiting side effect [4,424], Furthermore, it had the practical advantage of only having to be administered in a single intravenous dose during each course of chemotherapy. In two studies chlorozotocin alone had a response rate of 30%–53%, which is similar to streptozotocin alone [4,382,425]. Streptozotocin with one or both of 5-fluorouracil and doxorubicin has been reported to cause objective responses in 5%–80% of patients, with mean response rate of 43% in the different reports [4,299,379,382,395,426]. The chemotherapeutic agents of choice for metastatic PETs are streptozotocin and doxorubicin in combination [382]. This recommendation is based on an Eastern Cooperative Oncology Group (ECOG) comparative study [382] of streptozotocin and doxorubicin vs streptozotocin plus 5-fluorouracil or CZT in 105 patients with metastatic PETs. Streptozotocin plus doxorubicin had a 69% response rate, which was significantly higher than the 45% response rate achieved with streptozotocin plus 5-fluorouracil, or the 30% response rate with CZT alone. Furthermore, streptozotocin [382] plus doxorubicin had a significant advantage in terms of survival ($P = 0.0004$). A previous prospective study [299] done by the ECOG had also demonstrated the superiority of double-agent therapy over streptozotocin alone, with streptozotocin plus 5-fluorouracil having a higher response rate of 63% compared with 40% with streptozotocin alone in patients with metastatic PETs. In four subsequent prospective studies examining streptozotocin and 5-fluorouracil with or without doxorubicin in patients with liver metastases derived from gastrinomas or PETs, response rates of 5%, 16%, and 40% were reported [4,381,426]. In no study were there any complete responders, and in one study there was no difference in survival between responders and nonresponders [381]. In a study involving 61 patients with malignant PETs [426], the response rate to streptozotocin, 5-fluorouracil, and doxorubicin was 39%; however, none of the 11 patients with gastrinomas responded, suggesting that response rates may differ for different PETs. It is not clear whether this difference in response rates from those previously reported with PETs is due to differences in the tumors themselves, to differences in the extent of metastatic disease, or to differences in assessing tumor response in the different studies.

The use of both streptozotocin and CTZ is associated with significant side effects. In prospective studies 74%–100% of patients experienced nausea/vomiting and 20%–40% developed renal toxicity with increased serum creatinine, proteinuria, or both. The nausea and vomiting are frequently severe but can be controlled in almost every patient with 5-hydroxytryptamine (5-HT) receptor antagonists such as ondansetron [2,427]. In most cases these side effects are reversible, including the early renal effects; however, in the 1992 ECOG study [382], nine patients developed chronic renal failure and seven patients required dialysis. Serum creatinine and urine protein must be assessed prior to each cycle of chemotherapy [299,381].

Various triple-drug combinations with or without streptozotocin have been tried in small numbers of patients, with variable response rates; these include streptozotocin with 5-fluorouracil plus doxorubicin [4,367,381] or with tubercidin, 5-fluorouracil, doxorubicin, and cisplatin, and 5-fluorouracil with DTIC and epirubicin [4,428,429].

Poorly differentiated PETs have a poor prognosis [430]. In one study the combination of etoposide and cisplatin [383] was used in 13 patients with metastatic carcinoid tumors, in 14 patients with metastatic PETs, and in 18 patients with anaplastic tumors. Zero percent of the carcinoid tumors, 14% of the PETs, and 67% of the anaplastic neuroendocrine tumors demonstrated a partial to complete regression [383]. Two subsequent studies [4,431] confirmed the effectiveness of this combination in undifferentiated or poorly differentiated carcinoids or PETs, with response rates of 54% and 42%. It is therefore recommended that platinum-based chemotherapy regimens be used for the treatment of patients with poorly differentiated PETs [373,375,390,432].

Most experts agree that chemotherapy, chemoembolization, hormonal therapy, or interferon are only indicated in patients with extensive metastatic disease (usually to the liver) [2,4,14,246,373,375,376,379,388,396] and are not indicated for treatment of the primary tumor when surgery is not curative. There is no agreement, however, about when to commence chemotherapy, chemoembolization, hormonal therapy, or interferon [2,4,14]. Some groups recommend treatment only when the patient develops symptoms due to the PET [4,299], whereas others recommend it only when the tumor is demonstrated to be increasing in size [2,4,14,381,391].

Other treatments of metastatic disease

Interferon

Because of the toxicity and relatively poor response rates of chemotherapy in patients with extensive metastatic PETs, other approaches have been investigated. Interferon (human leukocyte α-interferon, IFN-α) is reported to be effective in controlling symptoms in a number of patients with PETs and carcinoid tumors [4,376,386,398,400,433,434]. One review [435] analyzed results in 383 patients from 13 studies of various neuroendocrine tumors (carcinoid tumors, PETs) treated with interferon (recombinant IFN-α2a, recombinant IFN-α2b, recombinant IFN-α2c, natural leukocyte IFN-α). A biochemical response (decreased tumor plasma or serum marker) occurred in 44%, there was a decrease in tumor size in 11% [435], and tumor stabilization in 35%. Of the 58 patients with PETs in another review of 322 patients [398], 51% had a biochemical response and in 12% a decrease in tumor size occurred with a mean duration of treatment of 20 months (range 2–96 months) [398]. Patients with VIPomas had a higher response rate (83%, 10 of 12) than those with other PETs [4]. Twenty-eight of the 58 patients had received chemotherapy prior to treatment with interferon, but their biochemical response rate was no different from those without previous chemotherapy (55% vs 44%) [398]. Disease stabilization occurred in 14 of 58 (24%) of patients, with a mean duration of 16 months. A number of studies showed that IFN-α treatment rarely decreases the size of either a malignant PET or carcinoid tumor (0%–12%), but it can inhibit further tumor growth – in 24%–80% of patients with metastatic neuroendocrine tumors [4,398,434,435]. In a review [401] of 274 patients with malignant GI neuroendocrine tumors (NETs: carcinoids, PETs) no patients had a complete response, 11% had a partial tumor response, 70% had tumor stabilization, and 23% had progressive disease with interferon treatment. These studies suggest that IFN-α is minimally effective as a primary tumoricidal agent, but it does have tumoristatic activity in a significant percentage of patients [4,398–401]. Studies of human NETs demonstrate that IFN-α activates the STAT (signal transducer and activator of transcription) cascade, reduces cyclin B levels resulting in inhibition of cyclin-dependent kinase 2 activity, which causes delayed progression from the S to the G2 phase of the cell cycle and inhibition of anchorage-dependent and independent cell growth [402]. Also transactivation of the vascular endothelial growth factor (VEGF) gene is inhibited due to Sp1/Sp3-dependent inhibition of the VEGF promoter resulting in inhibition of angiogenesis [402]. Interferon has also been used in combination with a number of agents such as 5-fluorouracil [4,386], but it is not clear that this enhances its antitumor effect. Interferon is increasingly being used in combination with somatostatin analogues, which will be discussed in the next section [4,400,401,434,436–438].

Somatostatin analogues

A number of studies in animals and in vitro studies in various tumor cell lines have demonstrated that somatostatin analogues can inhibit the growth of various tumors under differing experimental conditions [190,401,406]. The effects of somatostatin analogues are mediated through interaction with five classes of G protein-coupled somatostain receptors, sst_1–sst_5 [191,406]. Somatostatin receptor activation can inhibit growth by blockade of autocrine/paracrine-mediated growth, inhibition of growth factor production, inhibition

of mitogenic signals, induction of apoptosis, and inhibition of angiogenesis [406]. Studies demonstrate that except for insulinomas, more than 90% of PETs possess somatostatin receptors [259,260,401,439–441]. Hormonal therapy with the long-acting somatostatin analogue, octreotide, is reported to cause a decrease in tumor size in 12% of patients with NETs in one review of cases reported up to 1988 [403], and in 0%–8% of patients in various studies [4,308,355,401,404,407,442,443]. However, disease stabilization is reported in 25%–88% of patients with metastatic neuroendocrine tumors treated with somatostatin analogues [4,355,356,401,407,442,443]. The mean duration of stabilization is 18–26 months. Predictors of the antiproliferative response (i.e., stabilization, tumor decrease in a PET progressive prior to treatment) to octreotide have been studied in well-differentiated metastatic PETs [405,443]. In one study [405] a rapid growth rate prior to treatment predicted a poor response rate to somatostatin analogues, whereas in a second study [443] a pancreatic primary (i.e., a PET rather than a malignant carcinoid tumor), no previous tumor surgery, and the presence of distant extrahepatic metastases predicted a poor response. In this latter study responders had a significantly better survival at 3 years than nonresponders (100% vs 53%, $P = 0.004$). These results demonstrate that octreotide alone, similar to interferon, has minimal tumoricidal activity against PETs. However, in a significant number of patients it stabilizes the disease, preventing further progression, but because of a lack of randomized studies it remains unclear whether octreotide causes stabilization for periods of time sufficient to increase survival. The combination of somatostatin (either subcutaneouly or in long-acting forms) with IFN-α is reported to cause disease stabilization in 57%–89% of patients with malignant NETs, some of whom did not previously respond to either agent alone [4]. However, three prospective studies have not shown that the combination of IFN-α and a somatostatin analogue (lanreotide or octreotide) is better than either alone [436–438]. These findings remain controversial and each of these studies has been criticized on various methodological points [434].

Hepatic artery embolization/chemoembolization

Hepatic artery embolization with or without postocclusion chemotherapy (streptozotocin, doxorubicin, 5-fluorouracil, or DTIC) has been used successfully in patients with various PETs [2,4,374,395–397,444–450]. Because the liver derives only 20%–25% of its blood supply from the hepatic artery and 75%–80% from the portal vein, and because most PETs are vascular with an arterial supply, hepatic artery embolization can be used to treat metastatic disease to the liver if the portal vein is patent. In some studies, 55%–100% of patients with metastatic NETs have symptomatic improvement, and 33%–80% have an objective response [4,12,374,397,445,447–449,451]. Chemoembolization with doxorubicin in iodized acid combined with gelatin or sponge particles, or with streptozotocin, 5-FU, cisplatin, or mitomycin C, is reported to improve symptoms in 67%–100% of patients and to decrease tumor size or hormone levels in 33%–100% of patients [4,374,397,446–449,451–453]. The mean duration of objective responses is 6–42 months [4,374,397,447,449–451]. An analysis [448] of 123 patients with metastatic PETs found a reduced response rate in patients with malignant PETs involving more than 75% of the liver and with extrahepatic metastases.

This procedure is not without significant side effects. In the literature [444] the overall mortality for this procedure is less than 3% of cases, but pain develops in 50%–100%, nausea or vomiting in 50%–90%, and fever and leukocytosis are seen in 30%–60% of patients; moreover, 5%–15% are reported to have severe complications including hepatic failure, bleeding, infection, or acute renal failure [4,94,374,396,447,452]. In almost all patients some degree of abdominal pain, nausea, vomiting, and fever occurs, which lasts for up to 10 days [453]. Furthermore, in patients with metastatic PETs and in up to 31% of patients with gastrinoma metastatic to the liver, metastases to bone can occur [62], suggesting that procedures, such as embolization, directed at the liver may be of limited value to control metastatic spread in patients with extensive disease. However, in a patient with a hormonal syndrome that cannot be controlled by other therapies, this procedure may be helpful [2,27,374,397,447].

Hepatic artery embolization has been combined with IFN-α treatment [12,27,454] in patients with metastatic carcinoid tumors, which closely resemble PETs in responsiveness and histology. One year after the treatment, five of seven patients (71%) continued to demonstrate a decrease in the metastatic disease in the liver, whereas with interferon alone ($n = 10$) only 10% demonstrated a decrease. In another study [454] involving 36 patients with metastatic carcinoid tumors, interferon alone resulted in a 24% response rate (decrease in 5-HIAA secretion) and survival rate was 40% after 5 years. With interferon and hepatic artery embolization, in contrast, the response rate was 60% and 5-year survival rate 75%.

Cytoreductive surgery and radiofrequency ablation (RFA)

The systematic removal of all resectable tumor (i.e., cytoreductive or debulking surgery) may prolong the life of some patients with functional PETs [2,4,12,14,30,246,306,316,317,358,370,389,394,455–458]. Systematic removal of all resectable disease has been reported to help control symptoms in patients with malignant insulinomas [4,76,78,317,367], glucagonomas [4,21,59,317,449], VIPomas [4,20,21,80,317], GRFomas [4,23], somatostatinomas [317], and nonfunctional PETs [1,4,5,30] and has led to the suggestion that resection should be performed when possible in cases where extensive tumor is identified at the time of surgery and resection does not entail increased risk. There are no studies in which cytoreductive surgery has been evaluated in a controlled, prospective manner. It is important to distinguish

between the possible benefits of such surgery for patients who are asymptomatic (nonfunctional tumors) or have symptoms well controlled (gastrinomas, effective medical control of other tumors), and for patients in whom symptoms are not controlled. In the former group such surgery is performed to extend survival, although no controlled studies have established this. In the latter case there are numerous examples cited above with each of the symptomatic PETs where cytoreductive surgery improved symptoms. A number of studies [4,316,369,449,455,457,458] and reviews [246,246,394,456] have provided data using this approach. It is important to remember that surgical removal of all visible tumors is only possible in a small proportion (5%–15%) of all patients who have PETs with metastatic disease [4,246,306,370,455,456]. In one study, the tumor was completely excised in 80% of cases with metastatic disease in which surgical excision was initially deemed possible; survival was 79% at 5 years with a mean follow-up of 3 years. In another study [316] 36 extended hepatectomies or hepatic lobectomies and 38 nonanatomical liver resections were performed in patients with advanced disease due to malignant NETs. Perioperative mortality was 2.7%, morbidity 24%, and 4-year survival was 73%, with 90% of patients having symptomatic improvement [316]. In a retrospective comparison of results [449] with cytoreduction or embolization in 120 patients with malignant NETs (33 PETs, 87 carcinoids), the patients treated with cytoreductive surgery had a longer survival with equally effective control of symptoms. In another review [246] of different surgical series, 63 patients were analyzed from five different series of patients with malignant PETs who underwent resection of liver metastases. Operative mortality was 0%–6%, symptom control was achieved in most patients (at least 85%), and 5-year survival was 60%–80%. The authors concluded from this review [246], like the authors of numerous other studies, that cytoreductive surgery should be performed whenever it is possible to resect at least 90% of the malignant PET [4,246,370,449,455,456,458].

However, it is important to remember that this approach is only possible in less than 15% of patients, it has not been systematically evaluated, and it remains unclear whether patients identified with metastatic disease by localization studies prior to surgery and with symptoms well controlled by medical management will have long-term benefit from debulking surgery. In fact a review [389] injects a note of caution about the use of aggressive cytoreductive surgery, pointing out that this surgery can be associated with a high complication rate and that many of these patients survive for a prolonged period of time even with advanced disease. The authors recommend that cytoreductive surgery be performed in patients with metastatic PETs only if the PET is potentially fully resectable; if it is not fully resectable they recommend refraining from performing a partial resection.

Radiofrequency ablation (RFA) is increasingly used for patients with malignant PETs with hepatic metastases, either alone or in combination with other antitumor treatments [115,374,397,459–462]. It can be performed laparoscopically or at the time of surgery, causes tumor destruction by converting radiofrequency waves into heat, and is performed with an RFA catheter with an electrode tip from which current passes into the tissue [459–462]. Both tumor size and the number of lesions are rate limiting (RFA is usually used if there are less than five lesions under 3.5 cm in diameter), although larger tumors have been successfully treated [374,462]. Morbidity is generally low (5%) with occasional cases of abscess formation or hemorrhage [374,459,460]. Response rates of 85%–96% are reported, with responses lasting up to 35 months, including control of symptoms in patients with functional tumors [115,374,459,460]. Although the use of RFA has not been shown to extend survival, its ability to reduce tumor mass and assist in controlling symptoms due to the tumor per se or to uncontrolled hormone secretion makes it an increasingly used procedure for small metastases or limited numbers of metastases, especially in patients with functional malignant tumors [115,374,459–461].

Liver transplantation

Liver transplantation continues to be used used in selected patients with metastatic NETs (PETs and carcinoid tumors) [4,12,408–412,463,464]. The use of hepatic transplantation for many metastatic tumors such as colorectal carcinoma, cholangiocarcinoma, or metastatic sarcomas, has been largely abandoned because of early recurrence. However, because of their more indolent biological behavior of metastatic NETs, patients with these are considered more likely to benefit from liver transplantation [4,412,463,464]. The reports are generally confined to small numbers of patients with limited follow-up [4,408,409,412,463,464]. In a review [463] of reports of liver transplantation in patients with malignant NETs ($n = 103$) (48 PETs, 43 carcinoids), the 5-year survival rate was 45%, and occasional long-term cures are reported [463]. In a series of 15 patients with malignant NETs (10 PETs, 5 carcinoids) treated with liver transplantation, the transplant was found to provide good relief of symptoms and the 5-year survival was 90%. Reviews [412,463] suggest that liver transplantation should be considered in younger patients (< 50 years old) with malignant NETs in whom disease is confined to the liver with an absence of concurrent extensive surgery.

Somatostatin receptor-mediated radiotherapy

The presence of high densities of somatostatin receptors on almost all NETs (PETs and carcinoids) [4,190,259,260], which is widely used to localize these tumors [4,190,259–261,274], is being investigated as a means of possible antitumor treatment using radiolabeled analogues [4,274,413–415,465,466]. Agents reported to have antitumor effects in humans include: [^{111}In]DTPA-octreotide, which emits conversion and auger electrons; and somatostatin analogues, usually coupled by a

DOTA group (1,4,7,10,-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-tetraacetic acid) to yttrium 90, which strongly emits β-particles, or to lutetium 177, which is a beta and gamma emitter [4,274,413–415,465,466]. In 52 patients with malignant NETs (carcinoids, PETs) treated with [^{111}In]DTPA-octreotide in two studies, a complete tumor response, partial tumor response, or stable tumor-related disease occurred in 0%, 0%–8%, and 42%–81% of patients, respectively [415]. In four series involving 229 patients treated with [^{90}Y-DOTA,Tyr^3]octreotide, [^{90}Y-DOTA]lanreotide, or [^{90}Y-DOTA,Tyr^3]octreotate, complete tumor responses, partial responses, and stable disease occurred in 0%–3%, 6%–37%, and 44%–80% of the patients, respectively [413,415]. One series [413,467] reported results from 129 patients with malignant NETs treated with [^{177}Lu-DOTA^0,Tyr^3]octreotate; 2% had a complete tumor response, 32% had a partial tumor response, and 34% developed stable disease.

Generally, somatostatin receptor-mediated radiotherapy has been safe and severe side effects uncommon [413–415,466]. Approximately 30% of treated patients develop acute side effects at the time of the injection or within a few days (i.e., nausea, vomiting, increased tumor pain); these are usually mild and can be controlled by symptomatic treatment. Hematological toxicity occurred in a maximum of 15% in different series, and was generally transient, although 0.3% of treated patients developed a myelodysplastic syndrome [413]. Renal toxicity can also occur, but it can be limited by coadministration of amino acids, especially arginine and lysine, and is reported almost entirely in patients receiving yttrium 90-labeled somatostatin analogues [413,415,466]. The above results suggest this form of treatment holds considerable promise for the treatment of patients with advanced disease. As yet, no controlled study has demonstrated that it can extend life, and it is not a licensed therapy in the United States although it is undergoing evaluation in a number of centers.

Newer antitumor therapies

Studies involving usually small numbers of patients with metastatic progressive GI neuroendocrine tumors (both PETs and carcinoids) have reported the effects of various agents, including:

- angiogenesis inhibitors – endostatin, VEGF-A inhibitor, bevacizumab, sunitinib (SU11248)-tyrosine kinase inhibitor active against VEGFR, c-kit, platelet-derived growth factor receptor (PDGFR); VEGF tyrosine kinase inhibitors, sunitinib, vatalanib, sorafenib
- growth factor inhibitors – VEGF, IGF-1, transforming growth factor-α (TGF-α), PDGF, epidermal growth factor (EGF) – or growth factor receptor inhibitors (imatinib: PDGFR inhibitor; gefitinib: EGFR inhibitor)
- inhibitors of the mammalian target of rapamycin (mTor) (everolimus, temsirolimus)
- the DTIC analogue, temozolomide with thalidomide [388,468–473].

Studies with bevacizumab, everolimus, temozolomide, and thalidomide have shown sufficient promise such that phase III studies, either alone or in combination with other agents, are either planned or likely [468,469].

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72 Hereditary diseases of the pancreas

Jonathan A. Cohn

Cystic fibrosis, 1921
Hereditary pancreatitis, 1930
Rare inherited diseases of the exocrine pancreas, 1933
Hereditary aspects of idiopathic chronic pancreatitis, 1934

The two most common hereditary diseases of the exocrine pancreas in adults are cystic fibrosis (CF) and hereditary pancreatitis (HP). In the United States the prevalence of CF at this time is 30 000, and HP is probably 10–20 times less common. Both conditions are monogenic diseases and show mendelian inheritance. Although most individuals with CF or HP are diagnosed during childhood, nearly all individuals with these conditions survive well into adulthood. Both CF and HP cause potentially serious gastrointestinal problems and often require specialized care from gastroenterologists.

Discoveries made since the late 1980s have largely defined the genetic, molecular, and cellular basis of CF and HP. The main goal of this chapter is to summarize this information and to explain how it has implications for pathogenesis and for clinical practice. The exocrine pancreas is also affected by many less common monogenic diseases and clinical information about these rare conditions will be briefly summarized. Finally, this chapter will review emerging information about how genetic factors influence individual susceptibility to common nonhereditary forms of pancreatic disease.

Cystic fibrosis

Cystic fibrosis (Online Mendelian Inheritance in Man [OMIM] number #219700) is an autosomal recessive disease of the exocrine glands. The main clinical features of CF are recurrent lung infections leading to progressive pulmonary insufficiency, partial or complete pancreatic insufficiency, and abnormal sweat gland function. The first comprehensive description of CF was published in 1938 [1], and the genetic basis of CF was determined in 1989 [2,3].

Cystic fibrosis is the most common life-threatening genetic disease in Caucasians. It occurs in 1 in 3000 live births in the United States. The incidence of CF is similar in many European Caucasian populations, it is lower in African-Americans (1/15 000), and CF is extremely rare in other populations (e.g., 1/350 000 in Japan) [4,5]. Genotypically, CF is caused by two loss-of-function mutations in the gene encoding CFTR, the CF transmembrane conductance regulator protein. Carriers of CF have one loss-of-function *CFTR* mutation plus one normal *CFTR* allele and represent 3%–4% of the general population in the United States.

The epidemiology of CF in the United States has changed markedly since the late 1980s [6]. The median predicted survival was 16.0 years in 1970, 25.5 years in 1985, and 36.5 years in 2005; over 90% of individuals with CF survive into adulthood (past age 18) [7]. This improvement in survival corresponds to a 12-fold increase in the population of CF adults since the late 1970s [8]. Gastrointestinal complications are an increasingly important clinical challenge in this population.

Genetic and molecular basis

In 1989, CF was one of the first human disease genes to be identified by positional cloning [2,3]. The CF gene contains 27 exons, spans approximately 250 kb of DNA on chromosome 7q31.2, and encodes the CFTR protein. The latter is an integral membrane protein of 1480 amino acids that is expressed in many types of epithelial cells. Each CFTR molecule contains 12 transmembrane sequences, which embed the protein in the plasma membrane and form a channel through which anions (chloride or bicarbonate) can passively flow into or out from the cell interior. Each CFTR molecule also contains three cytoplasmic domains, which control ion transport by CFTR.

Identification of the CF gene was crucial for understanding the pathogenesis of CF at the molecular level. Before the CF gene was identified, epithelial cells from many CF-affected tissues were known to exhibit defective cyclic adenosine monophosphate (cAMP)-regulated anion secretion [9,10]. Once the *CFTR* gene was identified, antibodies to CFTR epitopes were developed for studying the biochemical properties,

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distribution, and role of the protein in human tissues and cells [11]. These studies showed that CFTR can be phosphorylated by cAMP-dependent protein kinase [12] and that the protein occurs at sites in many epithelial tissues where CF is known to cause abnormal ion transport [13,14].

The CF gene was also used to produce recombinant CFTR for directly studying the protein's function. When CFTR was purified and inserted into lipid bilayers, the intrinsic anion channel function of CFTR was shown to be stimulated when cAMP-dependent protein kinase phosphorylated regulatory sites on CFTR [15]. In further studies, CFTR was unexpectedly found to function as a regulator of other ion transport proteins including certain sodium channels [16], chloride channels [17], and anion exchangers [18]. Finally, somatic gene transfer was used to show that the CF anion transport defect could be corrected in epithelial cells by expressing functional CFTR protein; when combined with clinical and genetic data, this indicated that CFTR function is necessary and sufficient for correcting the ion transport defect in CF cells and it suggested that CF might eventually be amenable to gene therapy [19,20].

The intrinsic properties of CFTR also help to explain the pathogenesis of organ dysfunction in CF. In most gastrointestinal tissues affected by CF, CFTR occurs at the apical plasma membrane of the cells lining the duct or crypt epithelium [13,14]. At this site, the net flow of anions and water into the duct or crypt lumen is normally subject to tight control by neurohumoral agents acting through cAMP-dependent protein kinase. Depending on the tissue, the predominant secreted anion may be chloride or bicarbonate and the main neurohumoral regulators also are tissue-specific. However, the general pattern is that CFTR functions at the apical plasma membrane of these cells as a cAMP-regulated anion channel and thereby promotes the regulated secretion of fluid (and often also of bicarbonate) into the lumen.

In humans with CF and also in affected tissues of mice lacking CFTR, CFTR function is reduced at the epithelial cell apical plasma membrane and this causes inadequate anion and fluid secretion. In many ductal epithelial tissues (exocrine pancreas, intestine, hepatobiliary tract, and male reproductive tract), this causes plugging or obstruction of the lumen with inspissated secretions, and this can cause injury upstream from the obstructed lumen [13]. In other tissues (e.g., airway), sodium hyperabsorption accompanies anion hyposecretion, and the combined impact of these two defects is to cause an even greater reduction in net fluid secretion into the lumen. At these sites, sodium hyperabsorption in CF is attributed to inadequate regulation of sodium channels due to the loss of CFTR function [16].

CFTR mutations

Over 1500 different *CFTR* mutations have been detected in individuals with CF or related conditions [21]. Annotated information about these mutations is compiled in a database developed by the Cystic Fibrosis Genetic Analysis Consortium and is available online as the Cystic Fibrosis Mutations Database (http://www.genet.sickkids.on.ca/cftr).

Mutations of CFTR have been divided into five categories based on their molecular mechanism [21,22]:

- class I mutations prevent the production of full length CFTR
- class II mutations cause defective intracellular trafficking of newly synthesized CFTR
- class III mutations cause defective regulation of CFTR
- class IV mutations cause abnormal ion channel function
- class V mutations cause partial reductions in the abundance of functional protein.

Class I mutations include stop (nonsense) mutations, frameshift mutations, and some mutations at conserved splice sites. The most common class II and III mutations are small deletions or missense mutations located in one of CFTR's cytoplasmic domains. Class IV mutations are missense mutations in one of the protein's channel-forming transmembrane sequences. Class V mutations are often splice variants causing reduced splicing efficiency or creation of a new splice donor site. Residual CFTR function is usually low in individuals with genotypes consisting of two alleles with class I, II, or III mutations, whereas residual CFTR function tends to be higher in individuals who have a class IV or V mutation [23,24].

One mutation, ΔF508, accounts for most CF-causing alleles [3]. Transcripts of *CFTR* containing ΔF508 produce a mutant protein lacking the phenylalanine normally found at position 508. Although this phenylalanine residue (F508) occurs in a CFTR domain required for ATP-dependent regulation [25,26], the main effect of this mutation is to cause CFTR to misfold, to mislocalize, and to undergo accelerated degradation in CF cell lines [27–29] and in tissues from CF patients [30,31]. Thus, ΔF508 is a class II mutation.

About half of CF patients in the United States are ΔF508 homozygotes, and another 40% are compound heterozygotes who have one ΔF508 allele plus one less common CF-causing allele. Patients who are ΔF508 homozygotes have low residual CFTR function (< 2% of normal) and typically have pancreatic insufficiency. By contrast, pancreatic sufficiency occurs in CF patients who have genotypes causing less severe loss-of-function (e.g., with 5% residual CFTR function) [21,32,33]. The amount of residual CFTR function correlates closely with the clinical features, as summarized in Table 72.1 [34].

This CFTR genotype–phenotype correlation was later extended to genotypes producing higher residual CFTR function (~ 10% of normal). These genotypes often occur in infertile males who have normal pulmonary, pancreatic, and sweat gland function [35–37]. In these cases, infertility is caused by congenital bilateral absence of the vas deferens (CBAVD, OMIM #277180), a condition similar to the cause of male infertility in CF. Although CBAVD patients often have *CFTR* genotypes consisting of two abnormal alleles, they are

Table 72.1 Clinical diagnoses and *CFTR* genotypes correlate with residual function of cystic fibrosis transmembrane conductance regulator (CFTR) protein

	PI-CF	PS-CF	CBAVD, ICP	CF carrier
CFTR mutations	Severe + severe	Severe + milder	Severe + mild–variable	Severe + wild-type
Residual CFTR function[a]	0%–2%	2%–5%	5%–25%	50%
Pancreatic insufficiency	Present[b]	Absent[b]	Absent	Absent
Abnormal sweat test	Present	Present	Variable	Absent
CF lung disease	Present	Present	Absent	Absent
Male infertility	Present	Present	< 30% of cases[c]	Absent
Pancreatitis	Absent	10%–15% of cases[c]	< 30% of cases[c]	Absent

CBAVD, congenital bilateral absence of the vas deferens (male infertility); CF, cystic fibrosis; ICP, idiopathic chronic pancreatitis. PI, pancreatic insufficiency; PS, pancreatic sufficiency.

a Residual CFTR function is expressed as % of normal.

b Present or absent features show mendelian inheritance based on the *CFTR* genotype; these features are present (or absent) in over 95% of individuals (or in under 5%) depending on the *CFTR* genotype.

c These three conditions show complex inheritance (not mendelian); the *CFTR* genotype is predisposing (it increases disease risk markedly) but it is not sufficient to cause the disease.

From Cohn et al. [34].

protected from CF lung disease because one of their mutant alleles causes only partial loss-of-function [36,38,39].

Congenital bilateral absence of the vas deferens can be viewed as a mild, single-organ, nonpulmonary variant of CF. It is associated with *CFTR* genotypes consisting of one severe mutation (CF^{sev}) plus one mild-variable mutation ($CF^{m\text{-}v}$). With CF^{sev} mutations, nearly all CFTR function from that copy of the gene are eliminated; such mutations are generally class I, II, or III mutations. Individuals with CF^{sev}/CF^{sev} genotypes consistently develop CF lung disease [38]. By contrast, $CF^{m\text{-}v}$ mutations are generally class IV or V mutations that reduce CFTR function by 50%–90% and are associated with variable clinical features [38]. Thus, residual CFTR function is roughly 10% of normal in individuals with $CF^{sev}/CF^{m\text{-}v}$ genotypes. In CBAVD, the most common CF^{sev} mutation is ΔF508 (as in CF); the most common $CF^{m\text{-}v}$ mutation in CBAVD is an intron 8 variant consisting of a short polythymidine tract (5T instead of 7T or 9T). The 5T allele reduces the efficiency of splicing at this site, such that about 80% of the resulting CFTR protein molecules are nonfunctional because they lack exon 9 [36,40,41].

Vas deferens disease related to CFTR can show either monogenic (mendelian) or complex (nonmendelian) inheritance depending on the level of residual CFTR function. In individuals with low residual CFTR function (e.g., under 5% of normal), CBAVD shows autosomal recessive mendelian inheritance: CBAVD occurs in nearly every male who has a *CFTR* genotype causing this degree of CFTR malfunction. By contrast, in individuals with higher residual CFTR function (e.g., 5%–20% of normal), the abnormal *CFTR* genotype increases the risk of CBAVD but the genotype is not sufficient to cause the condition; most men who have $CF^{sev}/CF^{m\text{-}v}$ genotypes do not develop CBAVD [42]. Susceptibility to CBAVD is increased in $CF^{sev}/CF^{m\text{-}v}$ genotypes but the effect is not large or consistent enough for the condition to show mendelian inheritance in these cases. Thus, in individuals with $CF^{sev}/CF^{m\text{-}v}$ genotypes, the occurrence of the clinical phenotype (CBAVD) must be influenced by genes other than *CFTR* or by nongenetic factors.

Pancreatic disease related to CFTR also can show either monogenic or complex inheritance depending on the level of residual CFTR function caused by an individual's *CFTR* genotype. If residual CFTR function is low (e.g., 0%–2% of normal), then pancreatic insufficiency occurs consistently along with CF lung disease and other CF manifestations in a condition showing autosomal recessive inheritance for each feature [43]; these individuals do not develop pancreatitis [32]. If residual CFTR function is roughly 5% of normal, then CF lung disease occurs consistently but pancreatic insufficiency is absent; in this group, the pancreatic phenotype is variable in that 10%–15% of these individuals develop recurrent or chronic pancreatitis [32]. Finally, for individuals with even higher levels of residual CFTR function (roughly 10%–20% of normal), CF lung disease is absent but there continues to be an elevated risk of pancreatitis [44]. Thus, CFTR-related pancreatic insufficiency shows monogenic inheritance whereas CFTR-related pancreatitis shows complex inheritance, as summarized in Table 72.1 [34].

Diagnostic criteria

Most CF patients are diagnosed by one year of age. The diagnosis is usually suggested by respiratory symptoms (in 46% of individuals diagnosed in 2000), malnutrition or failure to thrive (29%), steatorrhea or abnormal stools (23%),

meconium ileus or intestinal obstruction (18%), or a family history of CF (15%). Less common presenting features include neonatal screening (8%), nasal polps or sinus disease (4%), rectal prolapse (3%), prenatal diagnoses (3%), electrolyte imbalance related to salt loss (2%), and liver disease (1%). Prenatal and neonatal diagnoses are increasingly common [45].

Two types of criteria must be met for a diagnosis of CF [46]. First, the individual must have clinical evidence consisting of either a positive newborn screening test result or a history of CF in a sibling or at least one phenotypic feature consistent with CF. Many phenotypic features can fulfill this criterion. About half of patients have respiratory symptoms such as persistent sinopulmonary colonization with CF-associated pathogens (e.g., *Pseudomonas aeruginosa*), chronic productive cough, persistently abnormal chest radiographs (e.g., bronchiectasis or lobar atelectasis), airway obstruction, nasal polyps, sinus disease, and digital clubbing. In cases where respiratory symptoms are initially absent, the diagnosis is often supported by gastrointestinal findings such as intestinal obstruction (meconium ileus or distal intestinal obstruction syndrome), rectal prolapse, pancreatic insufficiency, recurrent pancreatitis (especially in adolescents), and focal biliary cirrhosis or multilobular cirrhosis. In other cases, the diagnosis is supported by evidence of protein–calorie malnutrition, malabsorption of fat-soluble vitamins, salt loss syndromes (typically with chronic metabolic alkalosis), or male infertility due to CBAVD.

In addition to meeting one of these clinical criteria, a diagnosis of CF also requires unequivocal functional or genetic evidence of CF. To meet this criterion, the individual needs to have either an abnormal sweat chloride value, or two CF-causing *CFTR* mutations detected by DNA testing, or abnormal CFTR-mediated ion transport detected by measuring nasal potential difference responses. The first option, namely the quantitative pilocarpine iontophoresis sweat test, is usually the most informative laboratory test in adults when CF is suspected. This test uses standardized protocols and is reliably performed at over 100 CF Foundation-accredited care centers throughout the United States [47]. Sweat chloride values below 40 mM are normal, values above 60 mM are abnormal, and intermediate values are borderline; abnormal values should be confirmed by testing on a second day.

If an adult's clinical presentation suggests CF but sweat testing is normal, *CFTR* DNA testing is usually done next. If this identifies two CF-causing *CFTR* mutations, then the DNA testing establishes the diagnosis of CF (assuming that the individual's clinical features also support the diagnosis). It should be emphasized that a mutation is classified as CF-causing only if it consistently causes CF (e.g., in practically all individuals who have the mutation on one allele plus ΔF508 on the other allele). Apart from ΔF508, few *CFTR* mutations are known to consistently cause CF. If DNA testing detects at least one nondiagnostic *CFTR* allele (any of the hundreds of mutations causing an unpredictable phenotype), then the DNA testing is inconclusive. To diagnose CF in these cases, nasal potential difference measurements can be used to test for defective CFTR-mediated ion transport. Reliable testing by this method requires standardized procedures and is performed at a limited number of CF care centers [48].

Common manifestations and complications

Lung disease and abnormal sweat test results occur in over 95% of individuals with CF. For males with CF, the prevalence of infertility due to CBAVD is also over 95%. Most mortality in CF results from lung disease (78%) or complications after lung transplantation (12%) [45].

Table 72.2 compares the frequency of common complications of CF in children and adults. The number of CF adults is increasing rapidly and this predicts that the frequency of certain complications will also increase. Many of these complications result from conditions primarily affecting gastrointestinal organs. One example is diabetes: roughly half of older CF patients have CF-related diabetes as a late complication of their CF pancreatic disease. About half of these individuals require insulin to treat what is typically a brittle form of diabetes. A second example is osteoporosis: this condition also is increasing rapidly in prevalence due to improved survival of adults with CF. Incompletely treated pancreatic insufficiency is one factor promoting osteoporosis in CF individuals. A third example is gastrointestinal cancer. Although cancer occurs rarely in CF patients, the risk of certain gastrointestinal cancers is unexpectedly high. For example, the age-adjusted risk of cancers of the colon, of the small intestine, and of the biliary tract is increased approximately

Table 72.2 Complications occurring during one year in children and adults with cystic fibrosis (% of patients)

Complication	0–18 years	18 + years
Acute GI bleeding	0.2	0.3
Cirrhosis with portal hypertension	0.8	1.6
Gallbladder disease requiring surgery	0.1	0.8
Pancreatitis	0.2	1.4
Peptic ulcer	0.1	0.4
Rectal prolapse	0.5	0.1
Osteoporosis	0.2	2.6
CFRD requiring insulin	2.5	16.2
Massive hemoptysis	0.1	1.8
Nasal polyps requiring surgery	2.8	2.5
Pneumothorax requiring chest tube	0.2	1.4
Cancer (GI cancers explain excess)	0.1	0.3
Depression	1.5	8.6

CFRD, cystic fibrosis-related diabetes; GI, gastrointestinal.
From Cystic Fibrosis Foundation Patient Registry 2000 Annual Data Report [45], with permission from the Cystic Fibrosis Foundation.

20-fold in CF adults [49,50]. This information should be considered when deciding how to evaluate CF patients who have symptoms consistent with these diagnoses. The frequency of these cancers may rise markedly as the number of older CF adults continues to increase.

Pancreatic disease

Pancreatic insufficiency occurs in 85% of CF patients and is usually evident by the age of 5 years [51]. In most cases, pancreatic injury begins in utero with early obstruction of intrapancreatic ductules by inspissated eosinophilic plugs. Fatty infiltration of the exocrine tissue is often prominent, as the pancreas gradually shrinks and becomes fibrotic with cystic changes (Fig. 72.1) [52]. These histopathological changes correlate with findings seen by computed tomography (CT) imaging. Lipomatosis and atrophy are the dominant findings on pancreatic imaging in most CF patients, whereas cystic changes and a grossly normal pancreas are each observed in about one-tenth of cases [53].

Most CF patients with pancreatic insufficiency (PI) have genotypes causing nearly complete loss-of-function for CFTR-mediated ion transport [43]. This applies to all ΔF508 homozygotes and to individuals who have ΔF508 on one *CFTR* allele plus a second mutation causing severe loss-of-function on their second allele. Apart from ΔF508, the G542X, G551D, W1282X, N1303K, and R553X mutations are the most common causes of PI. Affected individuals predictably develop steatorrhea due to severe pancreatic insufficiency, which generally has an early onset and can be anticipated based on the genotype. These individuals need pancreatic enzyme replacement.

Individuals with *CFTR* genotypes producing small amounts of residual CFTR function (about 5% of normal) usually have subclinical defects in pancreatic electrolyte secretion [54]; these individuals are classified as pancreatic sufficient because they do not have steatorrhea [32,54]. In the United States, many of these individuals have genotypes including at least one of the following mutations: G551S, R117H, R347H, P574H, R334W, R352Q, or T338I. Although these and other mutations are associated with pancreatic sufficiency in CF, DNA testing is usually not helpful for predicting pancreatic function in individual cases. First, this group of mutations is only found in a minority of CF individuals who have pancreatic sufficiency. Second, the pancreatic phenotype in individuals with genotypes causing 5% residual CFTR function is less predictable than with genotypes causing nearly complete loss-of-function. One complication is the fact that 10%–15% of pancreatic-sufficient CF patients develop recurrent or chronic pancreatitis [55]. Some of these individuals later develop a secondary form of pancreatic insufficiency (often as young adults) despite having *CFTR* genotypes that would otherwise be associated with pancreatic sufficiency [54]. Although these individuals do not have fat malabsorption as children, they often need pancreatic enzyme supplements as adults.

Pathogenesis

The localization of CFTR protein in normal human pancreas helps to explain the role of CFTR during pancreatic exocrine secretion. The protein occurs in the cuboidal epithelial cells lining smaller intrapancreatic ductules (Fig. 72.2). At this location, CFTR plays a critical role. The pancreatic juice is initially secreted as a concentrated, protein-rich fluid produced by acinar cells (Fig. 72.3). This fluid then flows through narrow pancreatic ductules, which are lined by epithelial cells that secrete bicarbonate into the ductal lumen and thereby modify the composition of the pancreatic juice. Alkalinization and dilution at this site reduces the viscosity of the pancreatic

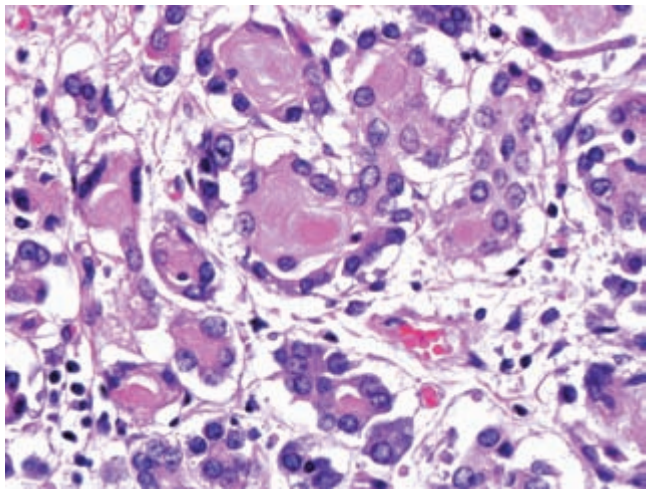

Figure 72.1 Photomicrograph of the pancreas from a patient with pancreatic insufficiency due to cystic fibrosis. Typical changes include prominent fibrosis and fatty infiltration with pancreatic islet preservation. Courtesy of Dr Alan Proia, Duke University Medical Center.

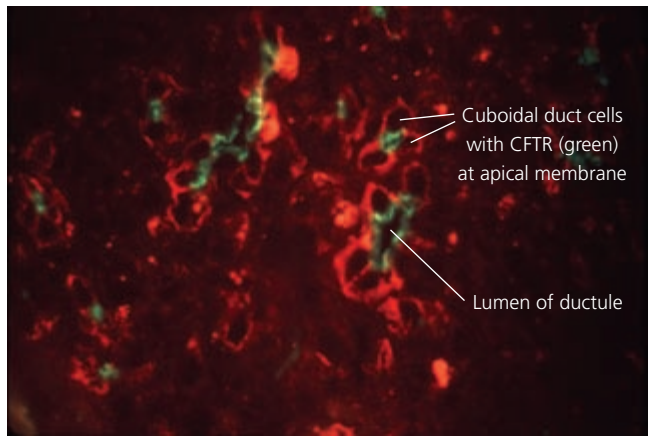

Figure 72.2 Localization of the cystic fibrosis transmembrane conductance regulator protein (CFTR) in normal human pancreas. From Marino et al. [13], with permission from the American Society for Clinical Investigation.

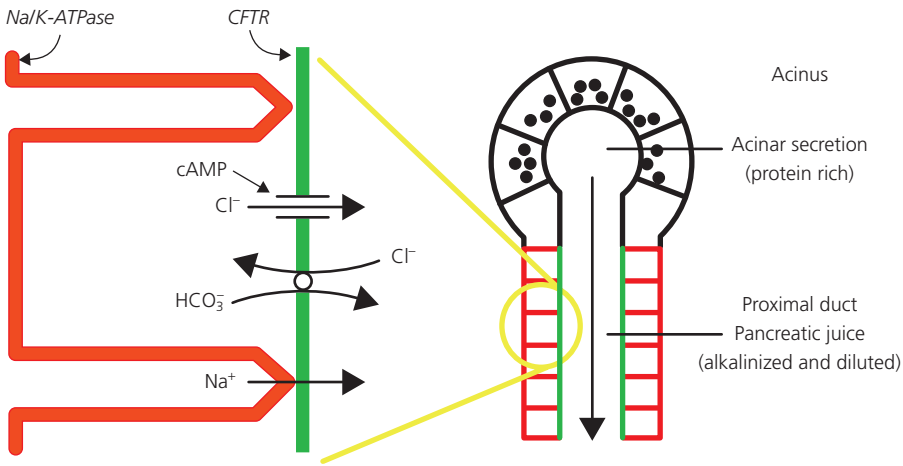

Figure 72.3 A model showing the proposed role of the cystic fibrosis transmembrane conductance regulator (CFTR) protein during pancreatic exocrine secretion. Modified from Marino et al. [13], with permission from the American Society for Clinical Investigation.

juice and promotes the solubility of many protein constituents. Within the pancreatic duct cells, CFTR occurs at the apical plasma membrane where the CFTR protein promotes cAMP-regulated bicarbonate and fluid secretion by functioning as an anion channel and probably also as a regulator of anion exchange [13,18]

Early features of CF pancreatic disease include plugging of the smaller pancreatic ducts [52] and defective bicarbonate secretion [56,57]. These features agree well with what is known about the intrinsic function of CFTR [15] and about the localization of CFTR protein in human pancreas [13]. Taken together, this evidence suggests that CFTR contributes to normal pancreatic secretion by diluting and alkalinizing the pancreatic juice to prevent obstruction of the smaller pancreatic ducts with proteinaceous plugs.

Evaluation

Pancreatic enzyme replacement therapy in CF patients is often started based on symptoms of steatorrhea without formal testing to document malabsorption. This approach is appropriate for most ΔF508 homozygotes and for others with genotypes known consistently to cause severe loss of CFTR function, as long as these individuals will be treated for pancreatic insufficiency. However, when this approach is used with individuals who have less predictive *CFTR* genotypes, it can cause misclassification with respect to pancreatic insufficiency. One report indicates that this type of misclassification is common: when 1215 CF patients were tested for pancreatic insufficiency, roughly 12% had unequivocally normal fecal elastase 1 levels; this agreed well with the known frequency of genotypes causing pancreatic sufficiency in CF. A surprising finding in this study was that most of these pancreas-sufficient CF individuals had been receiving enzyme supplements (67/127 cases, after excluding cases with pancreatitis) until they were tested for research purposes; this study also identified some individuals (24/1074 cases) who needed enzyme supplements but who were previously misclassified as having pancreatic sufficiency [58]. Because CF usually causes profound pancreatic insufficiency, nearly all CF patients in the study had elastase 1 values that unequivocally were either normal (> 200 μg/g stool) or abnormal (< 100 μg/g). These data suggest that many groups of CF patients will benefit from fecal elastase 1 testing. This testing will be especially important for individuals in whom the *CFTR* genotype is not predictive regarding pancreatic insufficiency.

In CF patients, it is also common to assess pancreatic function indirectly by evaluating nutritional status, malabsorption symptoms, and blood levels of fat-soluble vitamins. Secretin testing and quantitative stool fat studies are not widely used in most CF care centers. However, quantitative fat analysis can be helpful when malabsorption is suspected in individuals who have a normal elastase 1 level (> 200 μg/g stool) or who are responding poorly to enzyme supplements. Formal testing for malabsorption also is important for the rare CF patient who has a genotype predicting pancreatic insufficiency but who has clinical findings that suggest pancreatic sufficiency. Finally, pancreatic function can change over time in CF patients who have pancreatitis. Guidelines do not exist for monitoring pancreatic function in this group of CF patients; borderline fecal elastase 1 values may be more common in this group than in others with CF.

Treatment

Pancreatic enzyme supplements are used to treat CF patients with pancreatic insufficiency. For adults, enzyme supplements are usually administered as capsules containing enteric-coated enzymes formulated as microspheres or microtablets. These enzyme products contain multiple pancreatic enzymes and they significantly improve malabsorption of fat and protein in CF children and adults [59,60]. For adults starting on enzyme supplements, the initial dose per meal is typically 500 U lipase per kilogram bodyweight; for snacks, half this dose is used. Traditionally, dose adjustments are guided by

symptoms or other evidence of ongoing malabsorption (e.g., quantitative fecal fat testing). Dose adjustments aim for the minimum effective dose and sometimes require gradual increases in enzyme dose up to a maximum of 2500 U lipase per kilogram bodyweight per meal. Higher enzyme doses are avoided due to the risk of fibrosing colonopathy [61].

Significant but incomplete correction of steatorrhea is expected in most CF patients [59,60]. Persistent steatorrhea can limit nutritional status, and this correlates closely with prognosis in CF [62,63]. Many factors can limit the effectiveness of enzyme therapy in CF patients: one frequent limitation is poor patient adherence to pancreatic enzyme replacement therapy recommendations [64]. Generic or nonproprietary substitution of enzyme products can raise questions about enzyme potency and stability [65]; optimal enzyme delivery to the duodenum depends on when capsules are ingested [66] and can be reduced by gastric dysmotility [67]. Acid suppression is widely used in CF patients and can promote enzyme efficacy by multiple mechanisms [64]. Finally, CF patients develop unrelated conditions causing malabsorption; treatable possibilities should be considered, including celiac disease [68] and giardiasis [69]. More generally, inadequate responses to enzyme replacement in CF may also be due to extrapancreatic consequences of reduced CFTR function. For example, postlipolytic defects contribute to fat malabsorption in CF patients and in murine models of CF [70,71]. Cystic fibrosis has been associated with intestinal pH dysregulation, abnormalities of the unstirred water layer, and abnormalities in bile acid pools; the importance of these and other extrapancreatic events in humans with CF is uncertain [72].

Nutritional aspects

Nutritional status has a major impact on survival in CF. Until the late 1980s, the approach to nutritional support varied widely from one CF care center to another. At that time, many US CF care centers prescribed a carbohydrate-rich, low-fat diet with supplemental medium-chain triglycerides and protein hydrolysates, aiming to minimize steatorrhea symptoms and encourage appetite. By contrast, a limited number of centers (mainly in Canada) prescribed a caloric-dense, high-fat diet that was more palatable but required higher doses of pancreatic enzymes. When clinical outcomes were compared at two such centers in 1988, median survival for CF patients was 9 years longer at the center where the caloric-dense diet was prescribed [62]. Factors other than differences in dietary management did not seem to explain the survival difference and these data led most US CF care centers rapidly to adopt the caloric-dense approach to nutritional support [73]. As anticipated, this change in clinical practice led to a gradual narrowing of the gap in nutritional status between CF patients in Canada and those in the United States over the subsequent decade [74]. Nutritional status is therefore recognized as a powerful predictor of prognosis in CF [63,75], and consensus guidelines strongly recommend using a caloric-dense dietary approach in CF children [76] and adults [8].

In the United States, most CF patients receive their care at CF care centers where nutritional support is emphasized. Trained personnel assist with evaluating nutritional status to assure that malnourished patients are identified promptly and treated aggressively. For adults, nutritional status is mainly evaluated by comparison with ideal bodyweight (IBW) tables or by calculating body mass index (BMI). For IBW, %IBW is calculated based on published data for small-frame individuals [77]. Adults with CF having a %IBW below 90% are classified as having malnutrition and are candidates for more intensive monitoring and nutritional support.

A report indicates that %IBW values underestimate the severity of malnutrition in many individuals, especially children with short stature [78]. For this reason, US CF care centers are modifying their nutritional assessment approach to emphasize BMI as the preferred measurement. Body mass index is calculated as the weight (in kilograms) divided by the square of the height (in meters). For CF adults, when BMI values drop below the normal range of 20–25 kg/m^2, there is a dramatic decrease in lung function (Fig. 72.4) [7]. To prevent this, BMI and weight are followed closely. Small declines in either parameter lead to aggressive and early intervention. Target values for BMI in male and female CF adults are 23 and 22 kg/m^2, respectively. Those CF adults who have BMI values below 19 kg/m^2 are classified as having malnutrition; CF adults who have intermediate BMI values (above 19 but below 22–23 kg/m^2) are treated as having a high risk of developing malnutrition [79].

It is difficult to achieve target %IBW or BMI values in CF patients. Daily caloric intake in CF should be 20%–50%

Figure 72.4 Relationship between lung function and body mass index (BMI) in adults with cystic fibrosis. FEV_1, forced respiratory volume in 1 s. Data from Cystic Fibrosis Foundation Patient Registry 2005 Annual Data Report [7], with permission from the Cystic Fibrosis Foundation.

above that in non-CF individuals [76]. This is based, in part, on the fact that resting energy expenditure is increased at early stages of CF prior to the development of clinically significant lung injury or infection [80,81]; resting energy expenditure is further increased during pulmonary exacerbations due to the impact of infection and airway obstruction on the work of breathing [82,83]. However, caloric intake is often compromised by problems with appetite and absorption.

To maintain adequate caloric intake, CF patients are encouraged to select high-fat foods and snacks because these choices are energy-dense and appetizing. Malabsorption of fat-soluble vitamins is common with pancreatic insufficiency and is managed using supplements specifically formulated for the CF population. Monitoring for vitamin K and vitamin D deficiency is particularly important due to the frequent use of antibiotics and to the increasing prevalence of osteoporosis in older CF patients. Vitamin E deficiency also is common. Appetite stimulants can help some CF patients achieve adequate caloric intake. Small clinical trials have tested megestrol acetate [84,85] and cyproheptadine [86] in CF patients; the available data favor cyproheptadine with respect to effectiveness and safety. Gastroesophageal reflux causes significant symptoms in about half of CF adults; this is a potentially correctable cause of poor appetite and it is treated aggressively. When CF patients develop moderate or severe malnutrition during their clinical course, placement of a gastrostomy or jejunostomy tube should be considered [87,88].

Diabetes

Diabetes occurs in many older pancreatic-insufficient CF adults as a late complication of their CF pancreatic disease. The occurrence of diabetes in CF is signficant because it accelerates the progression of CF lung disease and it shortens survival [63]. In CF children with pancreatic insufficiency, diabetes is rare and pancreatic histopathology characteristically shows sparing of the islets [52]. By contrast, the adult CF pancreas exhibits sclerotic disruption of the islet architecture [89].

Cystic fibrosis-related diabetes differs from both common forms of diabetes (type 1 and type 2) in its clinical features. Diabetics with CF resemble type 1 diabetics (and differ from type 2 diabetics) in having low fasting insulin levels, normal (or high) insulin responsiveness, and a low BMI; however, CF-related diabetes differs from type 1 diabetes with respect to the absence of ketoacidosis, the insidious onset, and the absence of anti-islet antibodies. More importantly, both common forms of diabetes are associated with high glucagon levels, whereas glucagon levels are low in CF-related diabetes; this correlates with an overall loss of islet cells in CF-related diabetes and explains why diabetes tends to be especially brittle in CF patients [90].

Screening for CF-related diabetes depends on annual random blood sugar determinations. If the random blood sugar level is abnormal (> 126 mg/dL), further testing includes measuring the fasting blood glucose or performing an oral glucose tolerance test. Testing for glycohemoglobin is not sensitive enough to reliably identify diabetics in the CF population [90].

The treatment of CF-related diabetes differs from the treatment of other forms of diabetes. In most diabetics, caloric restriction is used for glycemic control, whereas this approach can be dangerous in CF because it is so difficult for CF patients to ingest enough calories to meet their elevated levels of energy expenditure. Fat intake in most diabetics is usually limited to 30% of total calories and sugar intake is strictly restricted; by contrast, a high-fat diet (40% of total calories as fat) with relatively little sugar restriction is advised for diabetics with CF. For most diabetics, salt intake is restricted, whereas this would be contraindicated for many CF patients. Finally, snacks and unstructured meals are discouraged for most diabetics, whereas they are encouraged for most CF patients [8,90].

Intestinal disease

The most common intestinal findings in CF are meconium ileus (MI) and distal intestinal obstruction syndrome (DIOS). Approximately 15%–20% of CF patients present with MI and 4%–20% of CF adults develop DIOS [45,91]. Both conditions result from blockage of the terminal ileum with impacted fecal material. Distal intestinal obstruction syndrome should be distinguished from constipation, a more common problem in CF patients in which intestinal blockage begins in the rectum.

The pathogenesis of MI and DIOS is incompletely understood and probably results mainly from anion hyposecretion caused by the absence of functional CFTR at the apical membrane of enterocytes [14]. The best characterized role of CFTR in enterocytes is to function as the rate-limiting apical chloride channel for cAMP-activated fluid secretion into the colonic crypt lumen [92]. Excessive ion transport by CFTR at this site causes secretory diarrhea; CFTR plays a critical role in this response because the intestinal secretory response to cAMP-dependent agonists (e.g., cholera toxin) is blocked in humans with CF, in transgenic mice lacking functional CFTR, and in animals treated with CFTR inhibitors [93,94]. cAMP-mediated anion secretion has also been linked with CFTR function in the small intestine, although other roles of CFTR may also be important at this site [95–98].

Most CF individuals with MI or DIOS have pancreatic insufficiency [91,99]. In the past, DIOS was attributed to inadequate doses of pancreatic enzyme therapy. However, it has been established that pancreatic insufficiency is associated with the subset of *CFTR* genotypes causing more severe ion transport abnormalities in many CF-affected tissues; it therefore seems likely that the risk of intestinal obstruction is higher in these CF patients because they have lower residual CFTR function in their enterocytes. The risk of MI is highly heritable; concordance rates for this complication in

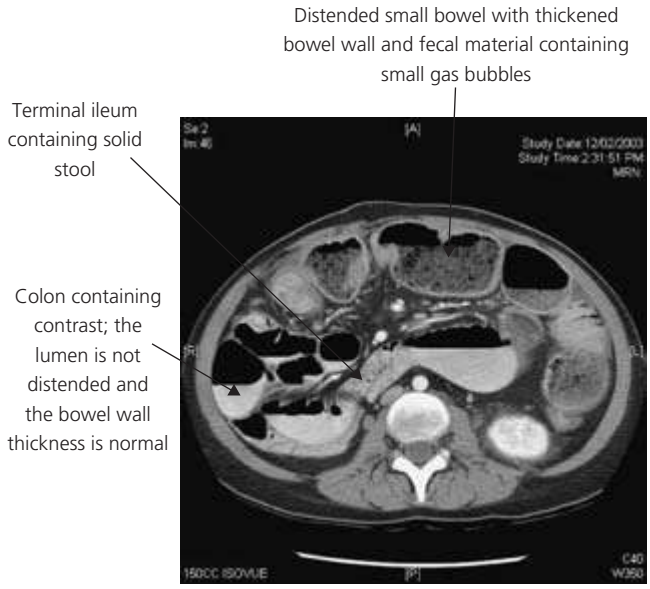

Figure 72.5 Abdominal computed tomography scan of an adult cystic fibrosis patient with distal intestinal obstruction syndrome. The terminal ileum is filled with solid stool. Proximal to this obstruction, the small bowel is distended (note thickened bowel wall and gas bubbles in fecal material). Distal to the transition point, the colon contains contrast and is not distended. Courtesy of Dr Carlos Micames, Duke University Medical Center.

monozygotic twins with CF are higher than in dizygotic twins, and ongoing studies aim to identify the modifier gene(s) responsible for this [99,100]. By contrast, twin studies in individuals with DIOS did not show heritability [99].

MI and DIOS are potentially dangerous complications of CF. Both conditions are characterized by partial or complete bowel obstruction caused by inspissated fecal material in the terminal ileum or cecum. Usually MI occurs within 2 days of birth and does not occur in adults. For DIOS, the mean age when an initial episode occurs is 20 years and recurrence is common [91]. The diagnosis is suggested by abdominal pain with a right lower quadrant mass. Abdominal radiographs usually show impacted stool in the ileum or cecum and this often appears granular or bubbly (Fig. 72.5). Because DIOS can lead to intussusception, volvulus, or ischemia, early recognition and intervention is important. Initial evaluation should include imaging studies to exclude appendicitis (e.g., by CT or sonography). Management includes correction of dehydration and electrolyte abnormalities, nasogastric decompression, and enemas (often using gastrografin with Tween-80) followed by intestinal lavage using polyethylene glycol electrolyte solutions (Golytely). Powdered polyethylene glycol (MiraLax) is effective for constipation in CF and regular use may also reduce the frequency of DIOS episodes. Some patients supplement this by intermittently using Golytely.

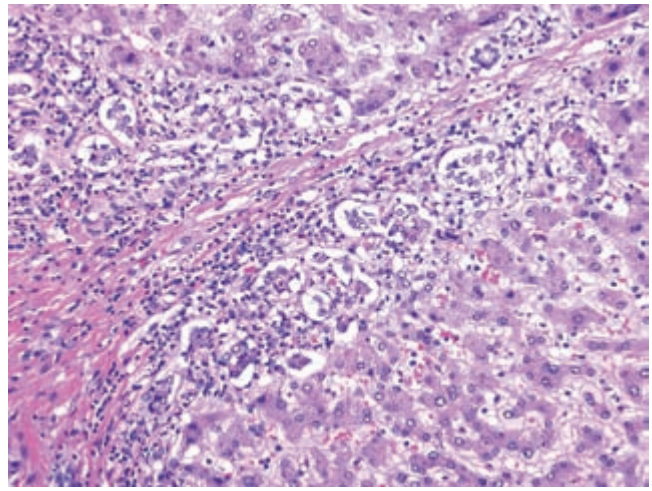

Figure 72.6 Photomicrograph of the liver from a patient with cirrhosis due to cystic fibrosis. Courtesy of Dr Victor Roggli, Duke University Medical Center.

Liver disease

The most important form of liver disease in CF is cirrhosis with portal hypertension (Fig. 72.6). Approximately 2% of CF adults have clinically significant portal hypertension, and liver disease accounts for approximately 2% of mortality in CF [45]. The prevalence of milder forms of liver disease in CF adults is uncertain and cholestasis probably occurs in 20%–30% of individuals [101]. Cholestasis is usually asymptomatic and biochemical markers do not reliably detect progression to focal or multilobular biliary cirrhosis. In one series of closely monitored patients who progressed to cirrhosis, the mean age at onset of cirrhosis was 10 years [102].

Liver histopathology varies widely in individuals with CF. Approximately half of CF patients have periportal steatosis. The most distinctive early feature of CF liver disease is plugging of the intrahepatic bile ducts with eosinophilic concretions [52]. The term *focal biliary cirrhosis* is used to describe a patchy and uneven form of bridging fibrosis often found in CF; the frequency and natural history of bridging fibrosis in CF has not been precisely defined.

The term *multilobular biliary cirrhosis* describes the form of biliary cirrhosis found in more advanced CF liver disease. This condition also tends to be patchy such that one lobe or segment can have normal histopathology whereas another lobe or nearby segment can be cirrhotic. For this reason, there is a high risk of sampling error when using percutaneous or transjugular liver biopsy to evaluate the severity of CF liver disease. Serum markers (e.g., liver function tests) are also unhelpful for monitoring early cirrhosis because they are often abnormal in CF individuals who do not have cirrhosis. Many individuals with multilobular cirrhosis are asymptomatic until developing portal hypertension. The risk of CF liver disease is increased in individuals who have pancreatic insufficiency and in males; some data suggest a further increment in risk in individuals who have had MI [102,103].

The primary defect in CF liver disease resembles that in CF pancreatic disease. The CFTR protein mainly occurs in the apical membrane of the epithelial cells lining the intrahepatic bile ducts. In individuals with CF, reduced CFTR function in the duct cell probably leads to inadequate alkalinization and dilution of the bile as it flows through the ducts, thereby leading to ductal plugging and cholestasis [104]. Ductal obstruction leads to hepatic fibrosis and injury resembling that occurring in other forms of biliary cirrhosis such as primary biliary cirrhosis and primary sclerosing cholangitis. Based largely on the effectiveness of ursodeoxycholic acid for primary biliary cirrhosis [105], ursodeoxycholic acid (10 μg/kg, twice daily) is used at some centers to treat CF patients who have persistently abnormal liver function tests (cholestasis) to try to prevent cirrhosis [106]. In CF, ursodeoxycholic acid can improve clinical and biochemical status [107]. However, this treatment has not been shown to affect mortality or liver fibrogenesis in CF.

The clinical management of portal hypertension and decompensated cirrhosis in CF is similar to that in non-CF individuals. Some patients develop advanced liver disease despite having relatively mild lung disease and these individuals can be favorable candidates for liver transplantation. Liver transplantation was performed on a total of 29 CF patients in the United States during 2004 and 2005; for comparison, lung transplantation was performed on 314 CF patients during the same period [7].

Patients with cystic fibrosis also develop other forms of hepatobiliary liver disease. Approximately half of CF patients have hepatic steatosis. This tends to be periportal and it may partly explain the frequent occurrence of abnormal liver function tests in asymptomatic CF individuals. Inflammation (steatohepatitis) is usually not prominent and it is uncertain whether steatosis plays a significant role in promoting fibrosis in these CF individuals [108]. In the past, profound hepatic steatosis was often found in newly diagnosed patients with severe malnutrition; steatosis in these cases improved with treatment of malnutrition. In other cases, hepatic congestion can occur in the setting of cor pulmonale due to advanced lung disease.

The most common biliary tract finding in CF is the occurrence of a nonfunctional and small gallbladder (microgallbladder) by ultrasonography. This finding occurs in one-fourth of CF individuals and it agrees well with information about the distribution and function of CFTR in the gallbladder. Normally CFTR occurs as an apical protein in the gallbladder epithelium [109]; reduced expression of CFTR at this site correlates with reduced cAMP-dependent anion and fluid secretion [110,111]. Symptomatic gallstones occur in fewer than 1% of CF adults per year [45].

Individuals with CF also develop a sclerosing cholangiopathy. In some patients (fewer than 10%), this leads to bile duct stenosis and the cholangiographic features can resemble primary sclerosing cholangitis. Based on histopathology, the secondary form of sclerosing cholangitis in CF does not exhibit the distinctive features of primary sclerosing cholangitis (e.g., prominent inflammation and concentric rings of periductal fibrosis). The evaluation and management of biliary disease in CF is similar to that in non-CF individuals [112].

Hereditary pancreatitis

Hereditary pancreatitis (HP, OMIM #167800) is an autosomal dominant disease of the exocrine pancreas characterized by recurrent attacks of acute pancreatitis leading to chronic pancreatitis. It was first described in 1952 [113] and the genetic basis for most cases was discovered in 1996 [114].

Families with HP have been identified throughout the United States and Europe, and to date approximately 700 cases have been reported with confirmatory DNA testing [115]. The prevalence of HP is unknown. In regions where there are multiple large HP families, this diagnosis can account for up to 10% of chronic pancreatitis in children evaluated at tertiary referral centers [44]; in other regions, the diagnosis is probably much less common.

Genetics and molecular basis

In 1996, the gene responsible for most cases of HP was mapped to chromosome 7q35 and shown to encode the protein cationic trypsinogen [114]. Cationic trypsinogen is produced by the *PRSS1* gene. It is one of three trypsinogen isozymes and normally represents 60%–70% of the trypsinogen in pancreatic juice. Pancreatic juice also contains anionic trypsinogen produced by the *PRSS2* gene (comprising 30% of trypsinogen) and mesotrypsinogen produced by the *PRSS3* gene (comprising 5%–10%) [116].

Each isozyme is produced as an inactive preproenzyme consisting of a signal peptide, a trypsinogen activation peptide, and a proteolytically active trypsin moiety. The signal peptides each contain 15 amino acids and are removed before trypsinogen is delivered to the zymogen granule. The trypsinogen activation peptides each contain eight amino acids and are removed when trypsinogen is activated, an event normally occurring after exocrine secretion of trypsinogen from the acinar cell. Trypsinogen activation within the acinar cell is ordinarily blocked by a reversible trypsin inhibitor protein located in zymogen granules with trypsinogen. This inhibitor is a 56-amino-acid protein and is encoded by the pancreatic secretory trypsin inhibitor (*PSTI*, also known as *SPINK1*) gene located on chromosome 5q32 [117,118].

Trypsinogen is normally cleaved by enterokinase to produce active trypsin in the duodenal lumen. Once activated, trypsin acts on many proenzyme substrates including other trypsinogen molecules. Because one trypsin molecule can activate many trypsinogen molecules, a rapidly escalating chain reaction can occur if trypsin activation occurs at a site

where trypsinogen is abundant. If such a chain reaction is initiated in the pancreatic parenchyma, this can be dangerous because the resulting protease activity can digest the pancreas and thereby expose additional trypsinogen molecules to produce increasing levels of proteolysis and tissue injury.

Several protective mechanisms normally prevent large amounts of activated trypsin from accumulating in the pancreas. For example, intracellular trypsinogen is normally sequestered in zymogen granules, where it is physically separated from intracellular proteases capable of activating the proenzyme (e.g., in lysosomes). For preventing a potentially catastrophic uncontrolled chain reaction, two protective mechanisms are especially important. First, acinar cells produce the PSTI protein that functions in zymogen granules to reversibly inhibit proteolysis by blocking the active site of trypsin [117]. Second, active trypsin molecules are unstable and become cleaved at a specific site (R122 in trypsin) to produce enzymatically inactive fragments [115].

Autoactivation refers to the ability of trypsin to digest and thereby activate other trypsinogen molecules; autodegradation refers to the ability of one trypsin molecule to digest and inactivate other trypsin molecules. Excessive intrapancreatic trypsin activation with pancreatic injury could result from defects causing enhanced autocatalytic production of active trypsin from trypsinogen (autoactivation) or it could result from defects causing prolonged survival of active trypsin due to reduced autodegradation. Trypsin autodegradation depends sensitively on the trypsin concentration (because autodegradation is a bimolecular reaction) and also on the calcium concentration. At higher calcium levels, calcium binds to trypsin and leads to a conformational change causing R122 to become inaccessible. Thus, high calcium levels can reduce tryptic autodegradation and thereby encourage the accumulation of larger amounts of active trypsin during pancreatitis [115].

The first gene identified as a cause of HP was *PRSS1*. Two mutations have been shown to cause HP as an autosomal dominant disease, R122H and N29I [114,119]. Together, these mutations account for most cases of HP in the United States and Europe [120]. A third *PRSS1* mutation, A16V, has been detected in over a dozen unrelated cases of pancreatitis; this mutation occurs at the signal peptide cleavage site in cationic trypsinogen and it is mainly associated with sporadic cases of chronic pancreatitis in children [115,121]. Dozens of additional *PRSS1* mutations have also been detected in rare or unique families with pancreatitis, but the clinical significance of these mutations is unknown [115].

The impact of the R122H and N29I mutations on cationic trypsinogen was defined by comparing the biochemical properties of recombinant proteins produced with or without disease-causing mutations. When compared with normal (wild-type) trypsinogen, each mutant form of trypsinogen exhibits a biochemical defect, explaining how the mutation causes pancreatitis. The R122H mutation causes trypsinogen to undergo both increased autoactivation and reduced autodegradation, whereas the N29I mutation causes increased autoactivation without affecting autodegradation [122]. Thus, both *PRSS1* mutations change the intrinsic biochemical properties of cationic trypsinogen to shift the balance between trypsin autodegradation and trypsin autoactivation in favor of increased trypsin activation [115].

The discovery that the *PRSS1* R122H mutation causes hereditary pancreatitis was important because it provided new insight into the pathogenesis of pancreatitis [114]. It showed that excessive activation of trypsin is sufficient to cause recurrent attacks of acute pancreatitis in humans. It also showed that recurrent acute pancreatitis can progress to chronic pancreatitis. Although both mechanisms of pathogenesis had been widely discussed before this (e.g., Chiari had suggested that pancreatitis resulted from autodigestion of the gland a century earlier), the clinical, genetic, and molecular characterization of HP and of the *PRSS1* R122H mutation provided compelling evidence that these mechanisms play a crucial role in the pathogenesis of some cases of pancreatitis.

Once *PRSS1* was identified as the gene causing most HP, additional studies of monogenic forms of pancreatitis further explored the link between excessive trypsin activation and pancreatitis. Three findings particularly support this link:

- *PSTI* loss-of-function mutations were identified as the cause of autosomal dominant HP in some families where *PRSS1* mutations are absent [123,124]. The *PSTI* mutations detected in some families (L14R or L14P) produce unstable mutant proteins whereas other cases were caused by deletion of 1336 bp (exon 1) of the *PSTI* gene; either mechanism would produce low levels of functional PSTI protein and thereby predispose to excessive intrapancreatic trypsin activation.
- Some cases of HP were shown to be caused by a large (605 kb) triplication including both *PRSS1* and *PRSS2* [125]. In these cases, high levels of these trypsinogen isozymes would be caused by a gene dosage effect due to increased copy number.
- In murine models of pancreatitis, transgenic over- or under-expression of *PSTI* [126,127]and transgenic expression of the R122H variant of *PRSS1* [128] each caused changes in pancreatic injury consistent with the concept that excessive trypsin activation plays a central role in pancreatitis.

Common manifestations and complications

The initial clinical features of HP are identical to those of non-hereditary forms of pancreatitis. Some individuals have minimal symptoms or only a single attack of acute pancreatitis. The more typical clinical course consists of recurrent attacks of acute pancreatitis leading to chronic pancreatitis over decades. Symptoms usually start in childhood (median age at onset is 13 years) and 90% of patients become symptomatic by age 30. When comparing groups of HP patients who have the R122H vs the N29I *PRSS1* mutations, the R122H

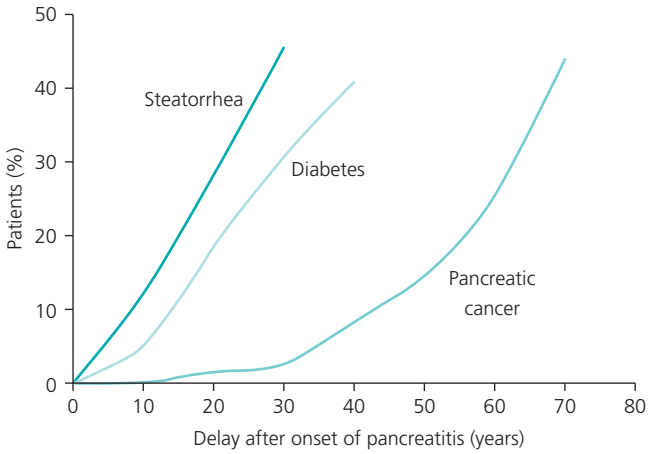

Figure 72.7 Complications of chronic pancreatitis occur gradually in patients with hereditary pancreatitis. Based on data in Howes et al. [120] and Lowenfels et al. [129].

mutation is associated with an earlier age at onset (10 years vs 14 years) [119,120].

Generally, HP patients survive for more than 50 years after the onset of their pancreatitis. Over time, most HP patients gradually develop exocrine and endocrine pancreatic insufficiency. For both complications, there is a usually a delay of decades between the onset of pancreatitis symptoms and the onset of exocrine or endocrine pancreatic failure (Fig. 72.7). The cumulative lifetime risk is over 60% for each complication. When diabetes mellitus occurs in HP patients, the condition tends to be brittle and is distinct from type 1 or type 2 diabetes. Chronic or recurrent abdominal pain can dominate the clinical picture and requires pancreatic resection or a drainage procedure in one-fifth of cases [120].

The most serious complication of HP is pancreatic cancer. Cancer is rare during the first two decades after the onset of HP symptoms. The cumulative risk of pancreatic cancer gradually rises and it exceeds 40% after seven decades [129]. The age-adjusted risk of pancreatic cancer is increased by about 60-fold by HP [130] and is not influenced by mutation status, gender, or maternal vs paternal mode of inheritance [120]. On average, pancreatic cancer occurs 15–20 years earlier in HP patients who smoke cigarettes than in nonsmokers [120,130]; preliminary findings suggest that when HP patients use either tobacco or alcohol, the risk of pancreatic cancer may be increased approximately two-fold [130].

Diagnostic studies

In the pregenomic era, the autosomal dominant nature of HP was emphasized to distinguish this condition from other familial forms of pancreatitis. Dominant inheritance is supported by identifying multiple affected individuals (at least two first-degree or at least three second-degree relatives) in at least two generations. Criteria like this are still used in research studies designed to exclude individuals in whom the inheritance pattern is equivocal or uncertain. However, in clinical practice it is unhelpful to use such stringent criteria because family history information is often incomplete.

With the availability of *PRSS1* mutation testing for clinical use, DNA testing is commonly used to make a new clinical diagnosis of HP. In the United States, *PRSS1* mutation testing is licensed to Ambry Genetics, Irvine, CA. If an individual has a clinical history of recurrent acute pancreatitis (RAP) or chronic pancreatitis (CP) and if *PRSS1* DNA testing detects either of the known HP-causing mutations (R122H or N29I), then this establishes the diagnosis of HP. This can help patients by preventing further testing, which might otherwise be performed to exclude rare causes of pancreatitis. If *PRSS1* DNA testing detects an uncommon mutation, then information about the detected mutation may be available through a database established to collect and distribute clinical and genetic data related to *PRSS1* mutations – the University of Leipzig Database of Genetic Variants in Patients with Chronic Pancreatitis (http://www.uni-leipzig.de/pancreasmutation). Interpretation of *PRSS1* DNA testing can be complicated by the absence of a detected *PRSS1* mutation in many HP families. Patients should understand the limitations and potential risks (e.g., of discrimination) of DNA testing for HP before being asked to provide informed consent for this testing.

In clinical practice, individuals with unexplained RAP or CP are often tested for *PRSS1* mutations regardless of their family history. It is common for individuals to be diagnosed with HP even when their initial family history data seem to be negative for HP [131]. Because the age of onset for HP is much earlier than for other forms of CP or RAP, *PRSS1* DNA testing should particularly be considered in individuals in whom pancreatitis begins at a younger age (e.g., under 20–30 years). For both common HP-causing *PRSS1* mutations (R122H and N29I), the penetrance of HP is approximately 80% [114,119,120]. This predicts that a fraction of HP patients (20%) will have no affected parent in spite of the autosomal dominant inheritance pattern. Outside of research studies, *PRSS1* gene testing should generally not be offered to asymptomatic relatives of HP individuals.

Complications of HP are evaluated using the same approach as in other forms of chronic pancreatitis. Due to the high lifetime risk of pancreatic cancer in HP, there is a particular need to develop screening methods for early cancer detection in individuals with HP. Several approaches to screening have been considered [132], but none has been prospectively tested in this population. In other familial forms of pancreatic cancer, endoscopic ultrasound (EUS) and CT can be useful for screening high-risk individuals [133]. However, these approaches may be less useful for screening individuals with HP due to the pancreatic structural changes commonly found in longstanding HP. A related point is that many abnormalities found by EUS will prove to be benign or low-grade neoplastic lesions. Thus, there is no consensus about the best approach to screening for malignancy in HP [134].

Treatment

Hereditary pancreatitis resembles CP with respect to most aspects of therapy. One general goal is to try to prevent acute attacks of pancreatitis. Patients with HP are often advised to limit their intake of alcohol and fatty foods based on early data associating these exposures with painful attacks in HP [135]. A small pilot study suggested that antioxidants and vitamins may also help prevent acute attacks of pancreatitis [136]. Finally, cigarette use is strongly discouraged based on the impact of smoking on the risk of pancreatic cancer in HP [130].

One distinctive feature of HP is that the lifetime risk of pancreatic cancer is much higher in HP than in other forms of CP. This has implications for the surgical management of HP patients: if an older HP patient is a candidate for pancreatic resection (e.g., for pain control or to remove a suspicious lesion detected by a screening procedure), then total pancreatectomy should be considered [134].

Rare inherited diseases of the exocrine pancreas

Shwachman–Diamond syndrome

Shwachman–Diamond syndrome (SDS; OMIM #260400) is an autosomal recessive condition characterized by pancreatic exocrine insufficiency, bone marrow failure, and skeletal abnormalities [137–139]. At least 250 families have been identified and the frequency of SDS is thought to be approximately 1 in 80 000 [140].

The syndrome is caused by mutations of the *SBDS* gene (Shwachman–Bodian–Diamond), a gene located on chromosome 7 (at 7q11) and predicted to encode a 250-amino-acid protein [141]. This protein occurs in the nucleolus where it apparently participates in RNA processing [142]. Approximately 90% of SDS families have mutations of the *SBDS* gene. Most detected mutations are gene conversions in which part of the *SBDS* gene is exchanged with a closely related but nonfunctional pseudogene located near the *SBDS* gene. The two most common *SBDS* splicing mutations in this condition are 183-184TA→CT and 258+2T→C; half of SDS patients are compound heterozygotes who have one copy of each of these two mutations [141].

Common manifestations

Most SDS patients present with findings related to pancreatic insufficiency such as failure to thrive and steatorrhea. In approximately 90% of cases, this is clinically evident within one year of birth. Pancreatic insufficiency in SDS is caused by defective development of the pancreatic acini. Histopathology shows fatty replacement of the acinar tissue with preservation of the pancreatic ducts and islets. Thus, pancreatic lipomatosis is typical in patients who undergo CT or ultrasound imaging [138]. The histopathological findings in SDS (acinar hypoplasia with ductal preservation) correlate with the pattern of abnormal pancreatic exocrine function observed when SDS patients undergo secretin testing: SDS causes relatively severe impairment of pancreatic enzyme secretion while causing relatively mild effects on bicarbonate secretion [54].

The second characteristic feature of SDS is bone marrow dysfunction. Nearly all SDS patients have persistent or cyclic neutropenia, and approximately half of SDS patients also have anemia or thrombocytopenia [138]. Recurrent bacterial and viral infections occur in most SDS patients and result from multiple mechanisms including neutropenia, defective immune function, and impaired neutrophil chemotaxis [143]. Typical infections include sinusitis, pneumonia, osteomyelitis, urinary tract infection, septicemia, and abscess. Up to one-third of SDS patients eventually develop myelodysplastic syndromes, and roughly half of these individuals develop acute myelogenous leukemia [139].

Growth and skeletal abnormalities also are typical features of SDS. Half of patients develop metaphyseal chondrodysplasia involving the femoral head. A second common finding is shortening or flaring of the ribs with costochondral thickening. Delayed growth is typical and many SDS patients remain below the fifth percentile for height and weight despite treatment of their pancreatic insufficiency.

Clinical evaluation and management

When young children present with steatorrhea or other evidence of pancreatic insufficiency, sweat testing is usually the first diagnostic procedure because CF is much more common than SDS. If sweat testing is normal, then SDS becomes the most likely possibility. In such children, the diagnosis of SDS is often supported by abnormalities in the blood count (e.g., persistent neutropenia). DNA testing is available as a clinical assay and this is the definitive method for confirming a diagnosis of SDS. This test is performed by the Molecular Genetics Laboratory, Hospital for Sick Children, Toronto, Canada. DNA testing is also used to screen siblings of SDS patients. In families where SDS is caused by identified mutations of the *SBDS* gene, DNA testing is sensitive and specific. Rare SDS patients will have no known SBDS mutation when tested; in these cases, the diagnosis of SDS depends on laboratory data showing unequivocal evidence of pancreatic and bone marrow dysfunction.

Pancreatic insufficiency in SDS is treated with pancreatic enzyme replacement therapy starting with enzyme doses similar to those used in CF. This therapy tends to be more effective than in other forms of childhood pancreatic insufficiency (e.g., CF) in which multiple defects contribute to steatorrhea including problems with bicarbonate secretion and postlipolytic absorptive mechanisms. In SDS, pancreatic insufficiency often becomes less severe over time.

The bone marrow disease of SDS requires specialized care by a hematologist. Neutropenic infections can be life-

threatening and are often treated using granulocyte colony-stimulating factor (GCSF) with antibiotics. Due to the high risk of developing myelodysplastic syndromes or acute leukemia, surveillance bone marrow studies are used to try to identify candidates for bone marrow transplantation [139]. The skeletal disease of SDS is usually asymptomatic but occasionally requires orthopedic referral [144].

Other rare diseases of the exocrine pancreas

Johanson–Blizzard syndrome (OMIM #243800) is an autosomal recessive condition consisting of pancreatic exocrine insufficiency with nasal alar hypoplasia or agenesis of the nasal cartilage [145]. This syndrome is also often associated with hypothyroidism, deafness, imperforate anus, scalp defects, and mental retardation [146]. The estimated frequency is 1/250 000 [147]. When detailed family history data can be obtained, consanguinity is often evident [146].

Johanson–Blizzard syndrome is caused by loss-of-function mutations of the *UBR1* gene [146]. This gene encodes a ubiquitin ligase that apparently is required for maintaining normal pancreatic acinar cell function in utero in mice and humans. Johanson–Blizzard syndrome resembles SDS with respect to its effects on pancreatic histopathology and on exocrine function: both conditions cause fatty replacement of the acini with relative sparing of other pancreatic structures and also cause severe impairment of pancreatic enzyme secretion with relative preservation of bicarbonate secretion [148]. However, the two conditions differ in that the development of pancreatic acini seems to be normal in Johanson–Blizzard syndrome whereas it is abnormal in SDS; also, Johanson–Blizzard syndrome does not cause the hematological or skeletal defects seen in SDS [148]. The treatment of pancreatic insufficiency in both conditions is similar.

Pearson syndrome (OMIM #55700) is a rare autosomal dominant condition consisting of refractory sideroblastic anemia and pancreatic insufficiency in young infants [149]. Most cases are caused by large deletions or duplications of mitochondrial DNA [150,151]. The initial clinical presentation is usually dominated by severe macrocytic anemia, with bone marrow studies showing severe hemosiderosis, ring sideroblasts, and prominent vacuolization of erythroid and myeloid cells [152]. Pancreatic findings at autopsy include fibrosis rather than fatty replacement; pancreatic function testing shows abnormalities in both acinar and ductular secretions [149,153].

Pancreatic agenesis (OMIM #260370) is a rare condition causing persistent and severe exocrine and endocrine pancreatic insufficiency in early infancy. Individual cases have been associated with mutations of different genes required for pancreatic development such as *PDX1* and *PTF1A* [154]. By contrast, *agenesis of the dorsal pancreas* (OMIM #167755) can cause diabetes without significant exocrine insufficiency [155]. An unrelated and rare pancreatic cause of steatorrhea is *congenital lipase deficiency* (OMIM #246600) [156].

When exocrine and endocrine pancreatic insufficiencies occur together in older individuals, this can result from maturity-onset diabetes of the young (MODY, type VIII) with exocrine dysfunction (OMIM #609812), an autosomal dominant condition caused by mutations of carboxyl-ester lipase [157]. Early findings in this condition include steatorrhea, reduced serum levels of fat-soluble vitamins and low-density lipoprotein (LDL) cholesterol, and pancreatic fatty infiltration, whereas diabetes occurs as a later finding with a mean age at diagnosis of 34 years. Pancreatic lipomatosis occurs before pancreatic atrophy, fibrosis, and endocrine insufficiency. This condition illustrates how a primary defect affecting an acinar cell protein can produce exocrine insufficiency leading to a monogenic form of diabetes [158].

Hereditary aspects of idiopathic chronic pancreatitis

Idiopathic chronic pancreatitis (ICP) accounts for most nonalcoholic cases of chronic pancreatitis in the United States and Western Europe. The prevalence of chronic pancreatitis is approximately 1.3 per 1000, with ICP accounting for 20%–30% of these cases [159]. Plugging of the smaller pancreatic ducts is a frequent early finding in ICP [160–162]. In this respect, the histopathology of ICP resembles that of CF pancreatic disease [52]. Idiopathic chronic pancreatitis also resembles CF in that it is associated with abnormal sweat tests [163,164]. These considerations were deemed suggestive that some cases of ICP might result from inadequate ductal fluid and bicarbonate secretion and that *CFTR* mutations might thereby predispose to ICP.

This hypothesis was supported by two reports in 1998 [165,166] and confirmed in further studies in which many ICP patients were comprehensively tested for *CFTR* mutations [44,167–170]. By combining the data in these European and US studies the following conclusions emerge. When *CFTR* DNA testing is performed on ICP patients, CF-causing mutations (CF^{sev} using nomenclature explained above under "Cystic fibrosis") are found in approximately 20% of cases. This is roughly five times the frequency of these mutations in the general population and indicates that ICP risk is increased five-fold in CF carriers on average [171]. Roughly 10% of ICP patients are *CFTR* compound heterozygotes who have one CF^{sev} mutation plus one $CF^{m\text{-}v}$ (mild-variable) mutation. These $CF^{sev}/CF^{m\text{-}v}$ genotypes are rare in the general population and they increase the risk of ICP over 50-fold [171].

The inheritance pattern for CFTR-related ICP is complex. In individuals who have $CF^{sev}/CF^{m\text{-}v}$ gentoypes, the risk of ICP is markedly increased. Nonetheless, most $CF^{sev}/CF^{m\text{-}v}$ individuals do not develop ICP. This is analogous to the occurrence of pancreatitis in 10%–15% of individuals who have pancreatic-sufficient CF lung disease [32,55]. In both cases, the *CFTR* genotype is predisposing, but not sufficient,

to cause pancreatitis (see Table 72.1). Thus, genetic or environmental factors other than the *CFTR* genotype must be important in determining whether ICP develops in any particular CF^{sev}/CF^{m-v} individual.

Patients with ICP who have CF^{sev}/CF^{m-v} *CFTR* genotypes differ from healthy individuals and from others with ICP. Residual CFTR function in the CF^{sev}/CF^{m-v} ICP cases is reduced to roughly 5%–25% of normal by the *CFTR* genotype (see Table 72.1), and this is associated with abnormal or borderline sweat chloride values and also with abnormal nasal bioelectric responses in most cases [44,170]. Many of these ICP patients also have extrapancreatic manifestations of reduced CFTR function, including male infertility, chronic bronchitis with CF-associated pathogens (e.g., *Pseudomonas aeruginosa*) and severe sinopulmonary disease [44,171].

Since 1998, many other candidate genes have been tested to explore whether they might influence an individual's risk of developing ICP. So far, two modifier genes for ICP risk have been identified, *PSTI/SPINK1* and *PRSS2*. For *PSTI*, the N34S variant occurs in roughly 2% of healthy individuals and at seven times this frequency in ICP [44,172–175]. N34S occurs with multiple intronic polymorphisms, and indirect evidence suggests that one or more of these polymorphisms is required for the trypsin inhibitor function of *PSTI* to be affected [176]. The proteins produced by the *PSTI* and *CFTR* genes occur at different sites in the pancreas and these genes probably have independent and additive impact on ICP risk [44]. The *PSTI* N34S variant has also been associated with tropical calcific pancreatitis (OMIM #608189) [177,178] and with alcoholic pancreatitis [179,180].

For *PRSS2*, the G191R variant occurs in 3% of healthy individuals and in 1% of individuals with ICP or alcoholic pancreatitis [181]. Thus, individuals with a G191R allele are protected from pancreatitis. This mutation modifies anionic trypsinogen (the *PRSS2* protein) by creating a new cleavage site where the protein is rapidly digested and degraded, to shift the balance between trypsin activation and inactivation in the acinar cell. The *PRSS1* A16V mutation is a second example of a trypsinogen mutation known to influence ICP risk; as discussed above under "Hereditary pancreatitis," this mutation has been detected in many children who have ICP [121].

Genetic testing and ICP

Testing for the *PRSS1* gene should be considered in younger patients who have ICP (see "Hereditary pancreatitis" above). In a mixed population including sporadic and familial cases of pancreatitis, an HP-causing mutation (R122H or N29I) or an ICP-associated mutation (A16V) was detected in 4% of tested individuals [182].

The role of *CFTR* gene testing in ICP patients is not established. Potential benefits of CF gene testing in ICP include helping patients to understand their disease, promoting early detection of extrapancreatic CFTR-related conditions (e.g., CBAVD), identifying CF carriers for genetic counseling, and identifying rare CF patients in whom ICP occurs before the onset of lung disease. The finding of *CFTR* mutations in an ICP patient may improve medical care and patient satisfaction in cases where physicians might otherwise suspect undisclosed alcohol use.

CFTR mutation testing is more likely to be positive in younger ICP patients and in those who have extrapancreatic evidence of reduced CFTR function (e.g., borderline sweat testing). When ICP patients are tested using CF carrier screening assays, false-negative results are common because many ICP-associated alleles are not represented in the mutation panels used in these assays [44,170,182]; this consideration also limits the use of carrier screening assays to test non-Caucasian ICP patients [183]. Comprehensive DNA testing improves assay sensitivity [169,182], but it can cause misunderstandings when it detects an uncharacterized mutation.

Cystic fibrosis gene testing has major limitations in ICP. Although CF gene testing can identify individuals who have a high ICP risk, it does not predict who will develop ICP because pancreatitis never occurs in most individuals with high-risk genotypes (e.g., *CFTR* compound heterozygotes). For individuals already known to have ICP, the results of gene testing do not affect patient care and guidelines do not exist for genetic counseling in this setting.

When CF is suspected in individual ICP cases, early referral to a CF care center is advised to ensure that the diagnostic evaluation is properly performed, interpreted, and explained. Individuals should not undergo DNA testing until they understand the limitations and risks of this testing. When ICP patients do undergo *CFTR* gene testing, the results should be interpreted cautiously [171]. In one-third of cases, comprehensive CF gene testing will detect at least one abnormal *CFTR* allele [182]; in most of these cases, special expertise will be required to interpret the detected genotype and to explain the test result clearly to the patient. DNA testing should not be used to make a diagnosis of CF except in rare cases where the genotype contains two CF-causing mutations [46]. Although many ICP patients have *CFTR* genotypes containing two mutations, it should be emphasized that few ICP patients have genotypes in which both mutations are known consistently to cause CF. When individuals are incorrectly classified as having CF, this misdiagnosis can cause harm in many ways including effects on employability and insurability [184,185]. CFTR gene-testing data can be difficult to interpret in adults who undergo this testing for nonpulmonary indications. Before explaining the results of this testing to individual patients, published consensus guidelines should be considered [46,186].

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73

Gallbladder and biliary tract: anatomy and structural anomalies

Theodore H. Welling, Diane M. Simeone

Embryological development, 1940
Anatomy of the gallbladder, 1940
Anatomy of the extrahepatic biliary ducts, 1944
Congenital variations and malformations, 1948

The extrahepatic biliary tract has a great number of variations, with a wide range of these variations also seen in the vasculature surrounding the extrahepatic biliary tree. Increasing use of diagnostic and interventional radiology as well as therapeutic endoscopy has made it essential for a wide variety of physicians to become familiar with the anatomy of the extrahepatic biliary tract. This review of anatomy and anomalies of the extrahepatic biliary tree will assist surgeons, gastroenterologists, and radiologists in understanding and recognizing the variations they may encounter.

Embryological development

The biliary tract develops from the primitive gastrointestinal tract, in the distal foregut, as a ventral sacculation; it is first apparent at week 5 of gestation when the embryo is 3 mm long (Fig. 73.1a). This sacculation grows and extends into the ventral mesentery, dividing into two buds: the cranial bud develops into the liver and intrahepatic bile ducts and the caudal bud develops into the gallbladder and cystic duct (Fig. 73.1b). The base of the diverticulum ultimately becomes the common bile duct. Another small bud develops from the proximal aspect of the caudal bud and grows inferiorly, ultimately developing into the ventral pancreas (see Fig. 73.1a,b).

The cranial bud divides into two smaller buds that grow upward toward the septum transversum (future diaphragm). These eventually become the right and left lobes of the liver. The caudal bud is carried superiorly by the growth of the cranial bud and stops at the undersurface of the cranial bud to become the gallbladder and cystic duct (Fig. 73.1c). Rarely, continued advancement of the caudal bud too far superiorly will result in an intrahepatic gallbladder, which is almost always embedded within the right lobe of the liver.

As the cranial and caudal buds are advancing, the ventral pancreatic bud rotates through 180 degrees from right to left, allowing it to fuse with the dorsal pancreatic bud to form the complete pancreas. Fusion of the two parts of the pancreas occurs during week 7 of gestation. Because the lower end of the common bile duct is attached to the ventral pancreatic bud, the rotation results in fusion of the junction of the common bile duct and the duodenum on the posteromedial duodenal wall, posterior to the dorsal pancreatic duct (Fig. 73.1d).

The ventral sacculation from which the biliary tract arises is originally composed of a solid cord of endodermal cells and contains no lumen. Beginning at week 7 of gestation, vacuolization begins to occur and a completely open lumen is formed in the gallbladder, cystic duct, hepatic ducts, and common bile duct within 1 week. By the third month of gestation, bile flow is demonstrable through the canalized biliary tract into the duodenum [1].

Anatomy of the gallbladder

Gross anatomy

The gallbladder is a pear-shaped sac that is located along the inferior surface of the liver in line with the division of the anatomic left and right lobes; it lies in a depression known as the gallbladder fossa (Fig. 73.2). The adult gland is typically 7 cm to 10 cm in length and has an average capacity of 30 mL. The gallbladder is intimately attached to the liver by loose connective tissue, which contains small veins and lymphatics that connect between the gallbladder and liver. Occasionally, one or more small accessory ducts from the liver traverse this connective tissue to enter the gallbladder directly. Inadequate ligation of an accessory duct may account for the occasional bile leak that occurs after cholecystectomy.

The remainder of the gallbladder not in direct contact with liver is covered by peritoneum reflected from the liver. The

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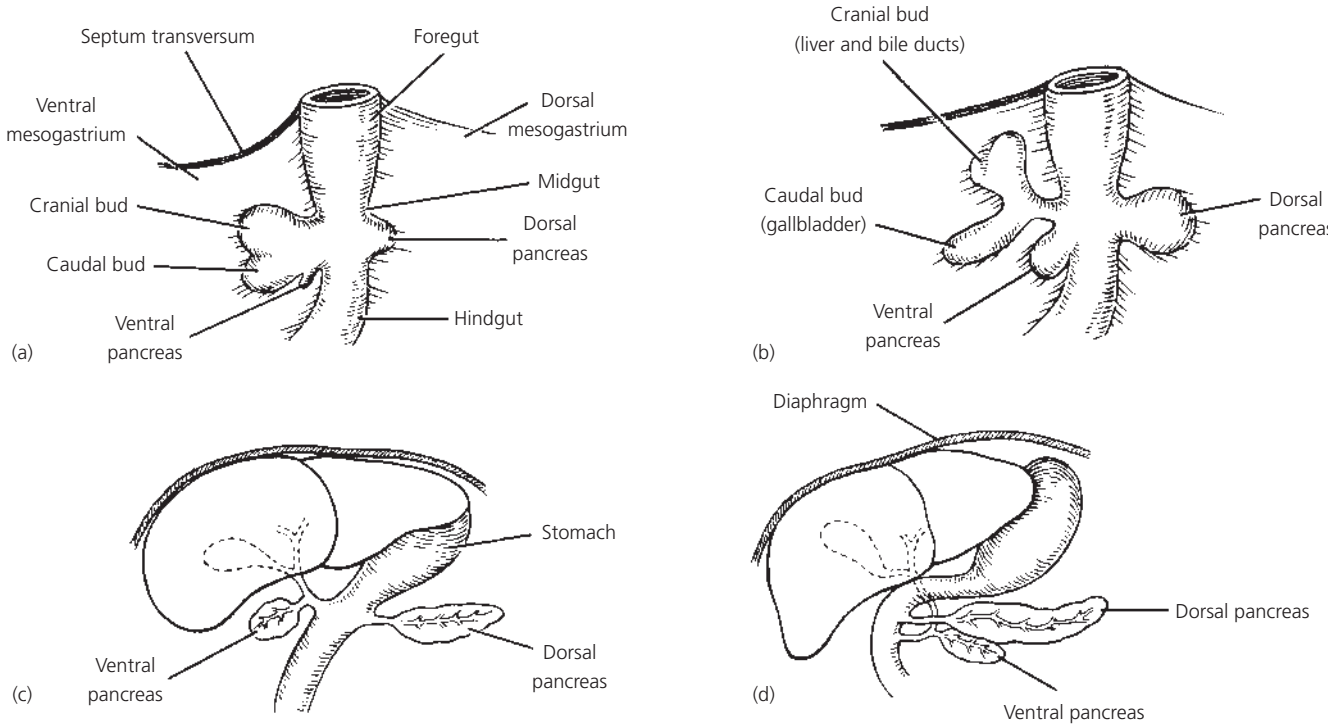

Figure 73.1 Embryology of the developing biliary tract. **(a)** At the 3-mm stage of the embryo, the ventral bud enters the mesogastrium and soon divides into a cranial and a caudal bud. A smaller caudad bud represents the origin of the ventral pancreas. **(b)** As the embryo reaches 5 mm, the cranial bud, which will form the liver and intrahepatic biliary tract, moves toward the septum transversum, pulling the caudal bud, which will form the gallbladder and extrahepatic bile ducts. **(c)** When the embryo reaches 7 mm, the right and left lobe of the liver occupy the position under the septum transversum. The ventral pancreas and the extrahepatic biliary tract are visible. As the ventral pancreas rotates to reach the dorsal pancreas, it pulls the lower end of the common bile duct with it. **(d)** At the 12-mm embryonic stage, the ventral pancreas has rotated, and the anatomic relations between the bile ducts and the gastrointestinal tract have assumed their mature forms.

gallbladder is divided into four anatomical areas: fundus, body, infundibulum, and neck. The fundus is the rounded end of the gallbladder that normally extends 0.5 cm to 1 cm beyond the liver margin and is covered with peritoneum. The fundus rests on the duodenum and hepatic flexure of the colon (see Fig. 73.2) and is in direct contact with the anterior abdominal wall, usually near the lateral border of the right rectus abdominus muscle at approximately the level of the ninth costal cartilage; however, its position may vary. The fundus may occasionally possess a kink, known as a phrygian cap deformity, which has no pathological significance.

The fundus merges imperceptibly into the body, the largest part of the gallbladder, and is the segment that is closely attached to the liver on one side. This intimate contact is responsible for early direct spread of gallbladder carcinoma into the liver. The infundibulum represents a transitional region between the body and neck. It is notable because occasionally a shallow diverticulum may be present on the inferior surface of the infundibulum, referred to as a Hartmann pouch. Gallstones may become impacted within the Hartmann pouch, causing cystic duct obstruction and thus acute cholecystitis.

The neck is a short (usually 5–7 mm) region that tapers into the cystic duct. The neck occupies the deepest part of the gallbladder fossa and lies in the free border of the hepatoduodenal ligament.

Arterial blood supply

The blood supply to the gallbladder is usually from a single cystic artery that arises from the right hepatic artery, traverses the triangle of Calot, and divides into two branches close to the gallbladder wall [2] (Fig. 73.3a). One branch runs along the peritoneal surface of the gallbladder and the other branch runs in the gallbladder fossa between the gallbladder and liver. When the cystic artery arises from the right hepatic artery, its course is often parallel and medial to the cystic duct. Double or accessory cystic arteries have been reported in up to 20% of people. Variations in the site of origin and course of the cystic artery are common. The cystic artery typically arises from the right hepatic artery (95%), but also may arise from the left hepatic artery, the common hepatic artery, an aberrant right hepatic artery that arises from the superior mesenteric artery, the gastroduodenal artery, the superior mesenteric artery, or the celiac axis directly (see Fig. 73.3).

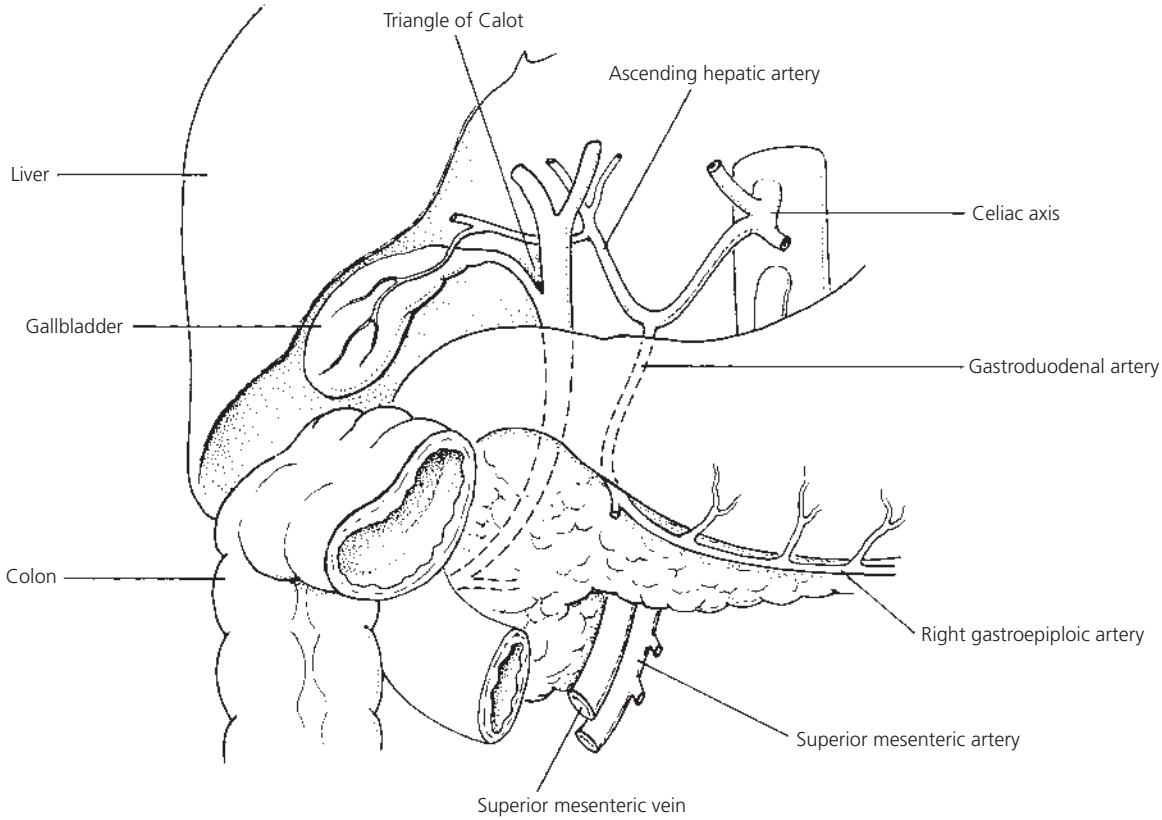

Figure 73.2 Relation of the gallbladder and the extrahepatic biliary tract to the liver, duodenum, colon, and pancreas.

The cystic artery may be short or long, and may pass either behind the hepatic duct (84%) or may cross the hepatic duct anteriorly.

The cystic artery, when it originates from the right hepatic artery, may closely parallel the right hepatic artery for a distance before reaching the gallbladder. In 5%–10% of cases, the cystic artery does not arise from the right hepatic artery until just before it enters the right lobe of the liver, thus creating a short (only a few millimeters) cystic artery. Failure to recognize this may lead to inadvertent ligation of the right hepatic artery during cholecystectomy.

Venous and lymphatic drainage

The gallbladder lacks a major cystic vein but rather drains through a network of small veins. These veins run either from the hepatic surface of the gallbladder directly to the liver or run toward the cystic duct and join venous collaterals from the common bile duct before ultimately draining into the portal vein.

The lymphatic drainage of the gallbladder parallels the venous drainage pattern. Lymph flows directly from the gallbladder to the liver or may drain toward the cystic duct into a single node or group of nodes. From these lymph nodes, lymph may ultimately drain into several nodes along the surface of the portal vein and common bile duct.

Nerve supply

The gallbladder is innervated by branches of both the sympathetic and parasympathetic nervous systems, which pass through the celiac plexus. Preganglionic sympathetic nerves arise from the T8 and T9 levels. Postganglionic sympathetic nerves originate at the celiac plexus and travel along the hepatic artery and portal vein to the gallbladder. Parasympathetic nerves arise from branches of the vagal trunks. Unlike the branches of the posterior vagal trunk, which pass through the celiac plexus, the branches of the anterior vagal trunk reach the gallbladder by way of the gastrohepatic ligament (Fig. 73.4). Visceral pain caused by gallbladder wall distention or inflammation is conducted through afferent sympathetic fibers and is referred to the epigastric, right subcostal, or right scapular regions. The nerve supply to the common bile duct is the same as for the gallbladder.

Although gallbladder contractility is primarily mediated by cholecystokinin (CCK), innervation of the gallbladder also modulates gallbladder contractility. Vagal activity contributes to normal gallbladder tone and enhances the ability of sub-

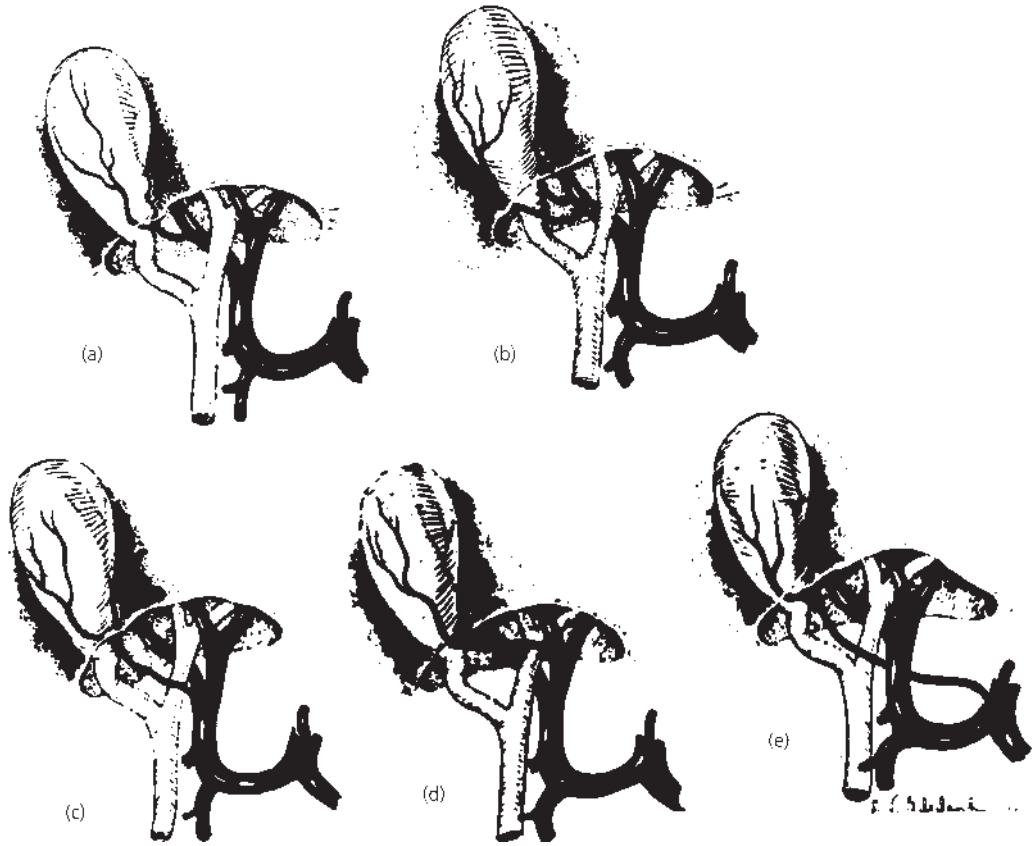

Figure 73.3 Common variations in the origin of the cystic artery. **(a)** It originates most commonly from the right hepatic artery; it traverses the triangle of Calot, and, on reaching the gallbladder, it divides into two main branches. **(b)** Occasionally the two branches come off the right hepatic artery independently. The cystic artery may cross the hepatic duct anteriorly **(c)**, come off the left hepatic artery **(d)**, or, more rarely, come directly from the celiac axis **(e)**. From Lindner [1] with permission from Elsevier.

threshold doses of CCK to promote gallbladder mobility [3]. Sympathetic fibers enhance gallbladder relaxation. In addition to the classic parasympathetic and sympathetic nervous systems, a large number of peptide-containing neurons exist within the gallbladder. The peptides identified thus far by immunocytochemical techniques include CCK [4], vasoactive intestinal polypeptide [4], gastrin-releasing peptide [5], neuropeptide Y [6], pituitary adenylate cyclase-activating polypeptide [7], calcitonin gene-related peptide [7], and substance P [7]. The physiological functions of these peptidergic nerves within the gallbladder remain to be elucidated.

Histology and ultrastructure

The gallbladder has five layers: epithelium, lamina propria, muscularis, perimuscular connective tissue layer, and serosa. The mucosa, lined with a simple columnar epithelium, consists of many folds that increase the efficiency of the gallbladder in concentrating bile. The apical surface of the epithelium is covered with abundant microvilli. The basal surface of the epithelium is in contact with the lamina propria, which is rich in loose connective tissue, elastic fibers, blood vessels, and lymphatics. The muscularis is an arrangement of circular, longitudinal, and oblique fibers without any well-developed layers. Ganglia may be found between the muscle fibers of the muscularis. The subserosa contains loosely arranged collagen and elastic fibers, as well as larger blood vessels and lymphatics. This thick subserosal layer attaches the gallbladder to the liver. Dissection during cholecystectomy usually takes place between the subserosa and serosa, which in normal circumstances is an avascular plane. The serosa is absent from the surface of the gallbladder that is in direct contact with the liver. Mucus is secreted into the gallbladder from tubular alveolar glands found in the mucosa lining the infundibulum and neck of the gallbladder.

At the ultrastructural level, the gallbladder epithelium is well suited for its physiological role of concentrating bile. The columnar epithelial cells have basally located nuclei and apices covered with numerous microvilli that protrude into the gallbladder lumen. Numerous mitochondria are present within the epithelial cells, which facilitate the active process of bile concentration. Epithelial cells are tightly joined together along their lateral membranes by tight junctions, which separate the gallbladder lumen from the intercellular spaces. During active bile concentration, the intercellular

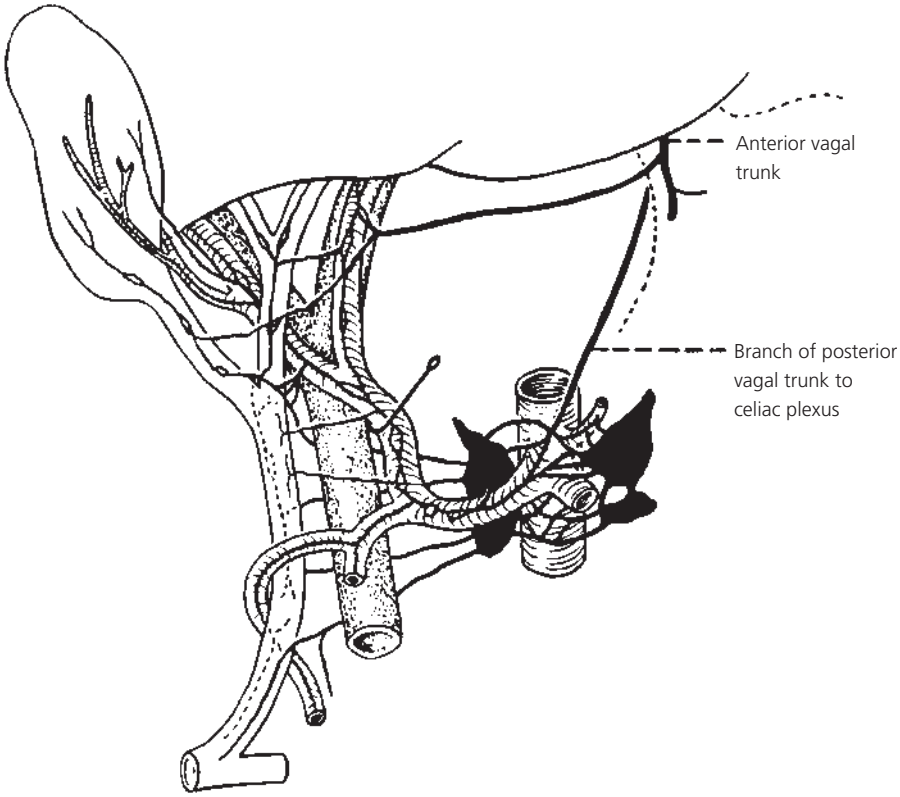

Figure 73.4 Schematic representation of the innervation of the gallbladder and extrahepatic biliary tract. The nerves originate from both vagi and from the celiac axis. They reach the biliary tract, traveling along the walls of the hepatic artery, except for the direct vagal branches of the anterior vagus, which cross through the gastrohepatic ligament.

spaces become distended with water and electrolytes. Water extracted during this process passes into a rich capillary network in the lamina propria.

Anatomy of the extrahepatic biliary ducts

Hepatic ducts

The common hepatic duct is formed by the union of the right and left hepatic ducts. In 95% of cases, this union takes place outside the liver, just below the level of the porta hepatis. In 5% of cases, the left and right hepatic ducts join within the substance of the liver. The lengths of the right and left hepatic ducts vary from 0.5 cm to 2.5 cm in length, with the left hepatic duct usually longer than the right. The ducts may join at a wide or acute angle, depending on their extrahepatic length; however, they usually join about 1 cm below the porta hepatis to form the common hepatic duct. The confluence of the right and left hepatic ducts lies anterior to the portal venous bifurcation, usually overlying the origin of the right branch of the portal vein. An accessory right hepatic duct is present in 10% of people.

Anatomic variations of the hepatic duct confluence most commonly involve the right hepatic duct, with the right anterior and right posterior branches often taking a variable course. The right anterior and posterior branches join to form a common right hepatic duct 60%–65% of the time, whereas the right anterior and posterior duct branches join at the confluence approximately 12% of the time. About 20% of the time, the right anterior duct will join separately onto the common hepatic duct with the right posterior and left hepatic duct joining first to form the common hepatic duct. The common hepatic duct passes downward in the superior portion of the hepatoduodenal ligament and lies anterior to the portal vein and to the right of the hepatic artery (Fig. 73.5). In cases when the cystic duct inserts on the right hepatic duct or at the junction of the union of the right and left hepatic ducts, there is no common hepatic duct. The length of the common hepatic duct varies from 2 cm to 6.5 cm. It joins the cystic duct to form the common bile duct.

The course of the right hepatic artery is variable in relation to the right hepatic duct and common hepatic duct. Usually the right hepatic artery passes posterior to the common hepatic duct near the junction of the right and left hepatic ducts (64%). In 24% of people, the right hepatic artery or the cystic artery may pass anterior to the common hepatic duct. In the remaining patients (12%), the right hepatic artery arises from the superior mesenteric artery and runs parallel and to the right of the common bile duct and posterior to the cystic duct. Because of the inconstant location of the right hepatic artery and its close proximity to the cystic artery, the right hepatic artery may be mistaken for the cystic artery and is particularly vulnerable to injury during biliary tract surgery.

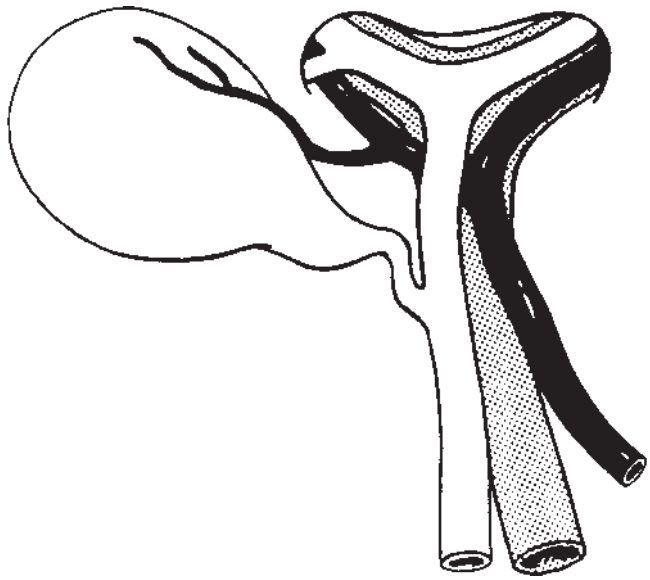

Figure 73.5 Schematic representation of the hilum of the liver. The right anterior and posterior hepatic ducts join to form the right hepatic duct. This duct and the left duct join outside the liver capsule to form the common hepatic duct. The left duct is usually longer and more superficial than the right. The triangle of Calot, with the right hepatic artery and the cystic artery, is also clearly displayed. From Lindner [1] with permission from Elsevier.

The triangle of Calot, originally described in 1891, is bounded superiorly by the hilum of the liver, on the right by the cystic duct, and on the left by the common hepatic duct [8] (see Fig. 73.5). Dissection of the triangle of Calot during cholecystectomy must be carried out with care because many important structures run through this triangle: the right hepatic artery, the cystic artery, 90% of accessory hepatic ducts, and 95% of aberrant right hepatic arteries. In cases when there is a replaced right hepatic artery originating from the superior mesenteric artery, the vessel travels toward the liver in a position posterolateral to the common bile duct and just behind the cystic duct. Here it is vulnerable to injury. Complete dissection of the triangle of Calot, with separation of the base of the gallbladder from the liver bed, allowing identification of the only two structures that should be emanating from the gallbladder, the cystic duct and cystic artery, is critical to avoid injury during cholecystectomy.

Cystic duct

The cystic duct arises from the neck of the gallbladder and joins the common hepatic duct. Its lumen usually measures 1 mm to 3 mm, but it may occasionally be as large as 10 mm, allowing larger gallstones to enter the common bile duct. Whereas the cystic duct typically joins the common hepatic duct directly (70%), the site of the cystic duct junction with the extrahepatic biliary tree may vary from the right hepatic duct down to the level of the ampulla (Figs 73.6 and 73.7). The length of the cystic duct may vary from 0.5 cm to 8 cm.

Figure 73.6 Endoscopic retrograde cholangiopancreatogram demonstrating an anomalous junction of the cystic duct with an accessory right hepatic duct.

The cystic duct may join the right hepatic duct, may run parallel to the common hepatic duct for a distance with connective tissue ensheathing both ducts, and, in some instances, may not enter the common hepatic duct until passing behind the duodenum. Additionally, the cystic duct may spiral around the common hepatic duct, passing either dorsal or ventral to the common hepatic duct before joining it. These anatomic variations may lead to bile duct injury during cholecystectomy, especially if dissection is carried out to clearly define the union of the cystic duct and common bile duct, a practice that is unnecessary during cholecystectomy.

Within the cystic duct, the mucosa forms a series of 5–10 crescent-shaped folds, known as the spiral valves of Heister. They span the length of the cystic duct and project into the cystic duct lumen. These valves serve to prevent excessive distention or collapse of the gallbladder with changes in cystic duct pressure and may function to block the passage of gallstones into the common bile duct. They may make catheterization during intraoperative cholangiogram difficult.

Common bile duct

The union of the cystic duct and common hepatic duct forms the common bile duct. Its length is approximately 7.5 cm, but

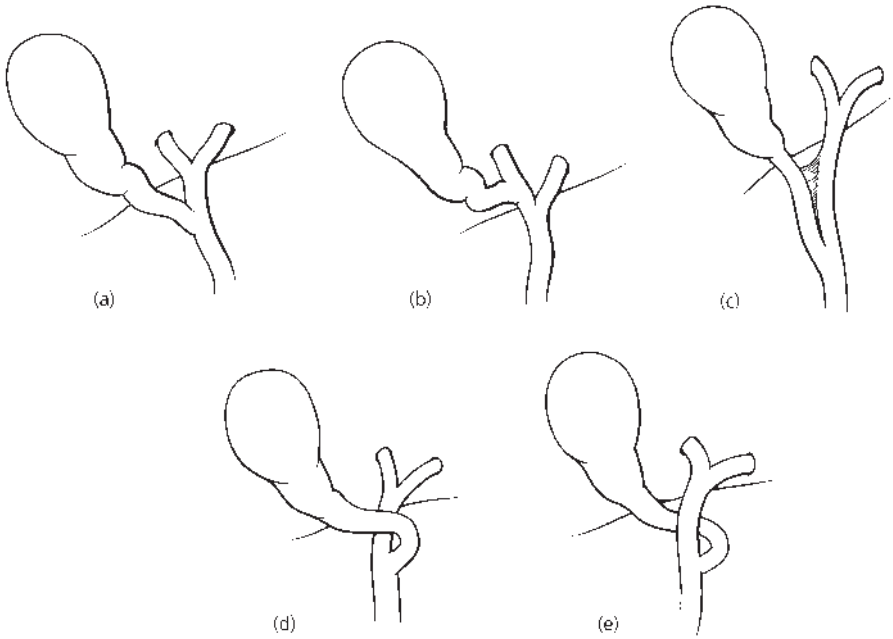

Figure 73.7 Variations in cystic duct anatomy. **(a)** Cystic duct joins common hepatic duct directly (most common). **(b)** Cystic duct joins the right hepatic duct. **(c)** Low junction of cystic duct with common hepatic duct. **(d)** Anterior spiral of cystic duct before joining common hepatic duct. **(e)** Posterior spiral of cystic duct before joining common hepatic duct.

this can vary depending on the relative lengths of the cystic and common hepatic ducts. The mean diameter of the common bile duct is about 6 mm, but it may dilate significantly in the face of distal obstruction. The common bile duct is divided into four anatomic segments: supraduodenal, retroduodenal, pancreatic, and intraduodenal. The supraduodenal segment is usually 2.5 cm in length and is located in the right border of the hepatoduodenal ligament. It lies anterior to the portal vein and to the right of the ascending common hepatic artery. This anatomic relationship allows the surgeon to compress these three structures between an index finger inserted through the foramen of Winslow into the lesser sac and a thumb placed anteriorly across the hepatoduodenal ligament. This maneuver is referred to as the Pringle maneuver and allows rapid occlusion of the blood supply to the liver, which is useful for controlling major liver hemorrhage.

The retroduodenal segment lies posterior to the first part of the duodenum and is about 2.5 cm to 4 cm long. As it runs to the inferior surface of the duodenum, it moves right to left, lying just to the right of the gastroduodenal artery. The retroduodenal common bile duct is loosely attached to the duodenum by a thin layer of areolar tissue. The close relationship of this portion of the common bile duct and the duodenal bulb means that it may occasionally be involved in the inflammatory process of a posterior penetrating duodenal ulcer and may be inadvertently injured during antrectomy.

The pancreatic segment of the common bile duct extends from the lower border of the first part of the duodenum to the posteromedial wall of the second portion of the duodenum where the duct penetrates the duodenal wall. It may be entirely retropancreatic, or it may lie within the substance of the head of the pancreas. Obstruction of this segment of the common bile duct is common with cancers of the pancreatic head, which often initially present with painless jaundice.

The common bile duct then turns 90 degrees to the right to enter the posteromedial wall of the descending duodenum. The final, or intraduodenal, segment of the common bile duct is about 2 cm long and travels obliquely through the duodenal wall with the main pancreatic duct. The common bile duct may join the main pancreatic duct outside the duodenum, or the ducts may form a common channel as they traverse through the duodenal wall, in both of these cases opening through a single ostium on the major ampulla of Vater. This site is typically 7 cm to 10 cm from the pylorus. From the inside of the duodenal lumen, the termination of the ampulla appears as a small, protruding, nipple-like structure marked by a longitudinal duodenal fold. In 29% of cases, a septum persists between the two ducts, and the ducts empty on the papilla as separate ostia.

Ampulla of Vater

Union of the common bile duct and the main pancreatic duct forms the ampulla of Vater. The length of the ampulla is variable, and if there is no junction of the common bile duct and main pancreatic duct, there is no true ampulla of Vater. Rienhoff and Pickrell [9] studied the pancreatic duct system in 250 autopsy specimens. They found an ampulla longer than 2 mm in 46% of cases (range from 3 mm to 14 mm), an ampulla less than 2 mm in 32% of cases, and no junction of the pancreatic and bile ducts in 29% of cases.

The common bile duct narrows significantly as it passes through the wall of the duodenum, and the ampulla narrows before it enters the duodenal lumen. These narrowings

are frequent sites for stones to lodge and cause either biliary or pancreatic obstruction. Additionally, these are potential sites of injury when instrumented during common bile duct exploration.

Sphincter of Oddi

The intraduodenal segment of the common bile duct and the ampulla is surrounded by a sheath of smooth muscle fibers referred to collectively as the sphincter of Oddi. The sphincter of Oddi is a unique group of muscle fibers that arise from the bile duct wall and manometric studies have verified that the sphincter acts independently of the duodenal musculature. The resting pressure of the sphincter of Oddi is approximately 13 mmHg above duodenal pressure [10]. Regulation of bile flow is primarily controlled by the sphincter of Oddi. Relaxation of the sphincter occurs with CCK stimulation and is facilitated by parasympathetic stimulation. Sympathetic stimulation causes increased sphincter tone.

The preampullary portion of the common bile duct is invested in a sheath of circular muscle referred to as the sphincter choledochus (sphincter of Boyden) (Fig. 73.8). The distal main pancreatic duct may have a short sphincter called the sphincter pancreaticus. If present, the sphincter pancreaticus and sphincter choledochus may intertwine in a figure-of-eight manner. The smooth muscle sheath surrounding the ampulla is called the sphincter of the ampulla; if there is no ampulla, the distal sphincter is simply called the sphincter of the papilla. During endoscopic sphincterotomy, the sphincter of Oddi is divided using electrocautery to relieve common bile duct obstruction from a common duct stone.

Arterial blood supply

The arterial blood supply to the extrahepatic bile ducts is segmental; because of the segmental nature of the blood supply, extensive mobilization of the extrahepatic bile ducts may lead to ischemic injury and development of postoperative biliary stricture and should therefore be avoided [11]. The hepatic ducts and the supraduodenal portion of the common bile duct are nourished by small arterial branches from the cystic artery (Fig. 73.9). The retroduodenal portion of the common bile duct is supplied by branches of the retroduodenal and posterior superior pancreaticoduodenal arteries. The

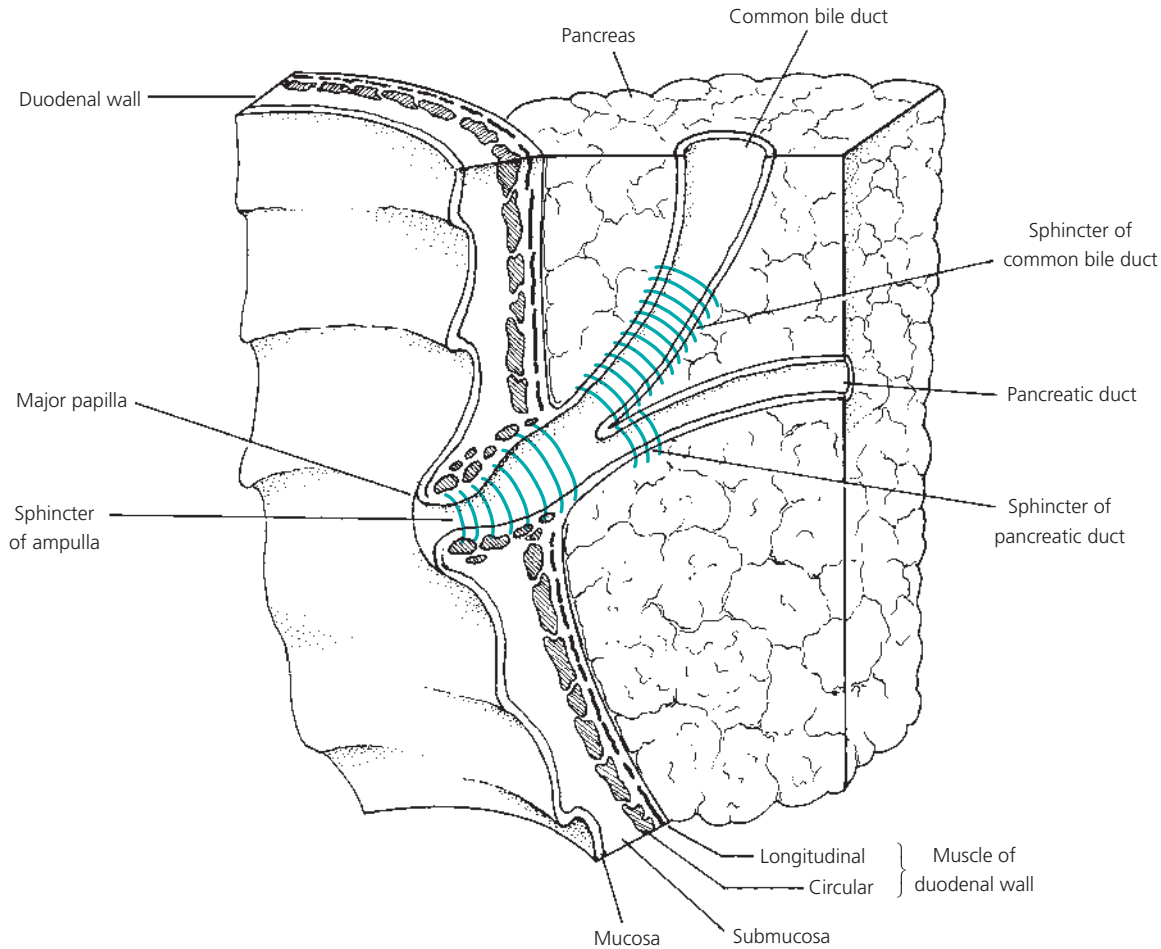

Figure 73.8 The muscular apparatus at the terminal end of the common bile duct. The bile duct is closely associated with the pancreatic duct, and they both enter the medial wall of the duodenum tangentially. Each duct has its own sphincter, which is poorly developed in the pancreatic duct.

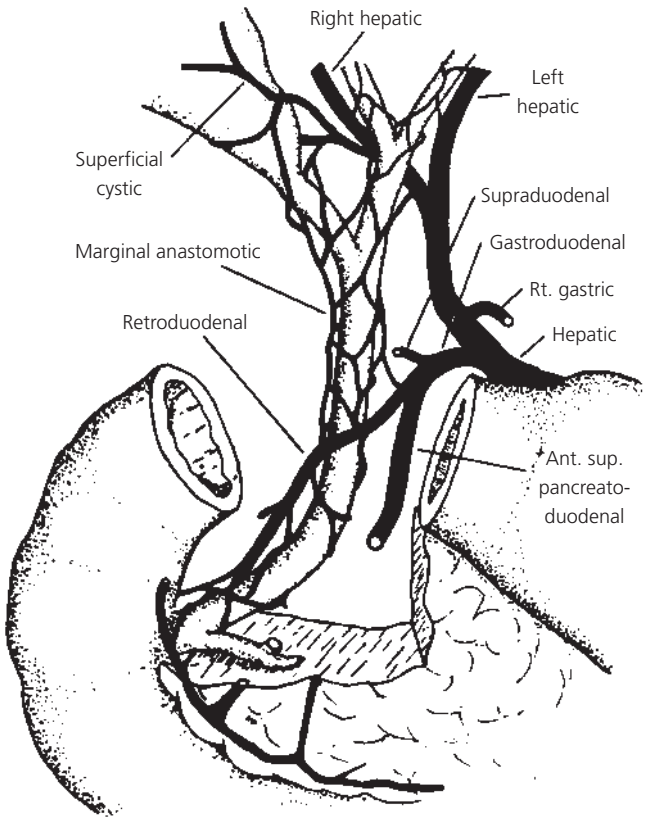

Figure 73.9 The extrahepatic biliary tract is supplied by a rich vascular net of vessels from the hepatic and gastroduodenal arteries. The relationship between the hepatic arteries and the extrahepatic biliary tree is evident.

pancreatic and intraduodenal segments of the common bile duct are supplied by both the anterior and posterior superior pancreaticoduodenal arteries.

Venous and lymphatic drainage

The veins of the hepatic ducts and proximal common bile duct, like those from the gallbladder and cystic duct, enter the liver directly. Veins from the lower portion of the common bile duct drain into the portal vein. The lymphatic drainage from the hepatic ducts and upper common bile duct flows superiorly along the course of the common bile duct to lymph nodes in the porta hepatis. Some lymphatic drainage arising from the inferior portion of the common bile duct may reach the deep pancreatic nodes near the origin of the superior mesenteric artery. All lymphatic drainage ultimately reaches the celiac lymph nodes.

Histology and ultrastructure

The mucosa of the extrahepatic bile ducts contains columnar epithelium surrounded by a layer of connective tissue. The epithelium contains many mucous glands. Muscle fibers in the hepatic ducts and proximal common bile duct are relatively few and discontinuous, and may be arranged in either a longitudinal or a circular direction. As the common bile duct approaches the duodenum, it begins to develop a more substantial muscle layer, which merges into the sphincter of Oddi complex, where distinct bundles of muscle fibers are evident.

Congenital variations and malformations

Gallbladder and cystic duct

Congenital anomalies of the gallbladder may be classified into three different categories: anomalies of number, anomalies of form, and anomalies of position. Duplication of the gallbladder is thought to occur in approximately one of every 4000 human gallbladders [12]. With complete duplication, the gallbladders are separate, and the cystic ducts may join one another before entering the common duct or each cystic duct may enter separately. Partial duplications can result in bilobed gallbladders (Fig. 73.10a), in which the two gallbladder cavities are separate at the fundus but joined at the neck and drain through a common cystic duct. Gallbladders may also be septate (divided by a partial or complete septum) with septa present in either a longitudinal or transverse direction, the latter referred to as an hourglass gallbladder (Fig. 73.10b). Septate gallbladders are thought to be a result of incomplete vacuolization of the solid endodermal cord during development. None of these abnormalities of the gallbladder by themselves have clinical significance unless they are involved in a pathological process.

Lack of development of the gallbladder bud results in agenesis of the gallbladder. Most cases are associated with extrahepatic biliary atresia [13]. In the absence of associated biliary atresia, an intrahepatic gallbladder or left-sided gallbladder must be ruled out before the diagnosis is made.

Abnormal migration of the caudal bud (future gallbladder and cystic duct) of the ventral diverticulum during development may cause an anomalous gallbladder position. If the caudal bud advances beyond the cranial bud (future liver), it may become buried in the liver substance, creating an intrahepatic gallbladder. These are usually identified by ultrasonography before cholecystectomy and may make cholecystectomy slightly more difficult. If the caudal bud lags behind the cranial bud, a floating gallbladder is created. A floating gallbladder is a gallbladder that is completely covered with peritoneum; it is usually suspended from the liver by a mesentery. A floating gallbladder is at a higher risk for torsion. Left-sided gallbladders are rare, but they have been described in the setting of situs inversus (all abdominal viscera are reversed right for left) or in the setting in which the gallbladder alone is transposed [12]. The gallbladder has also been found in other unusual locations such as the falciform ligament [14], the abdominal wall [15], and the retroperitoneum [16]. Regardless of the anomalous position of the gallbladder, it is important to realize that in almost all cases

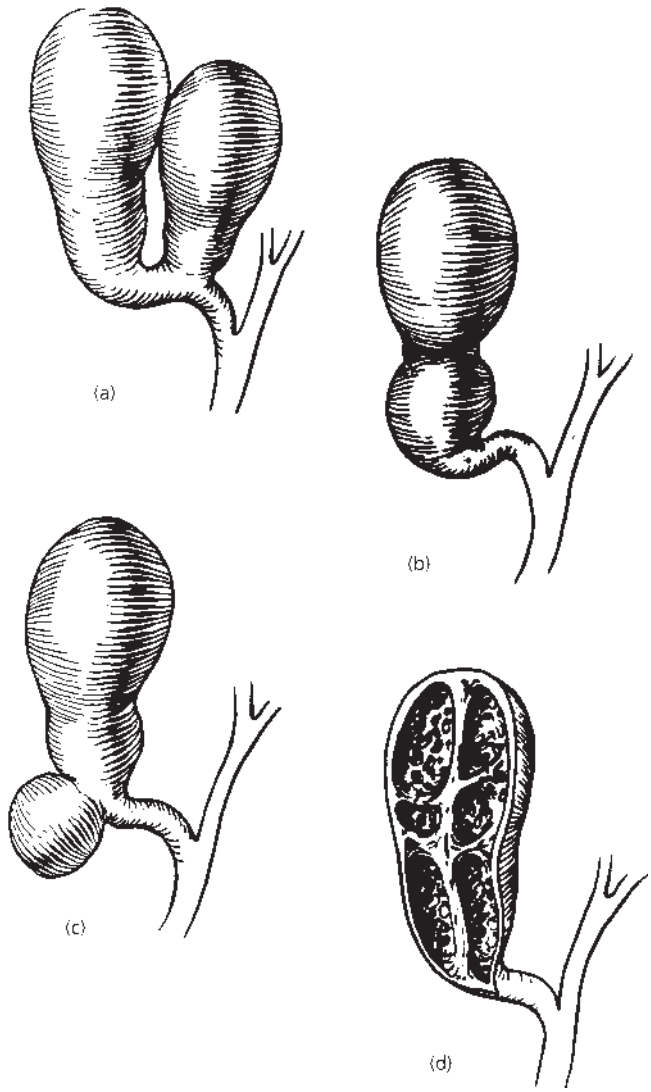

Figure 73.10 Two gallbladders **(a)**, a bilobed gallbladder **(b)**, a diverticulum at the neck **(c)**, and a septated gallbladder **(d)** are all anatomic variations that relate to the embryological development of the biliary tract. From Lindner [1] with permission from Elsevier.

the cystic duct will inevitably join the common bile duct in a relatively normal position.

Abnormal development of the gallbladder bud may also cause anomalies in the cystic duct. Double cystic ducts may drain a single, nonseptate gallbladder and join the biliary system at the common bile duct or right hepatic duct. There may be absence of the cystic duct, and, in this case, the neck of the gallbladder drains directly into the common bile duct. Failure to recognize this anomaly has resulted in excision of a segment of the common bile duct during cholecystectomy in which the common bile duct has been mistakenly identified as the cystic duct. Liberal use of intraoperative cholangiography has been emphasized when intraoperative anatomy is confusing or anomalies are present.

Hepatic ducts and common bile duct

True accessory hepatic ducts result from the development of an extra bud from the biliary anlage during development. Accessory ducts are much more common on the right than the left. They may drain directly into the gallbladder, the right or left hepatic ducts, the cystic duct, or the common bile duct. Accessory hepatic ducts are present in about 10% of individuals. Cystohepatic ducts that drain bile directly from the liver to the gallbladder are rare. Aberrant hepatic ducts, unlike true accessory ducts, result from an extrahepatic course of a duct that is normally contained within the liver parenchyma. Accessory ducts provide a second drainage route from a region of the liver, in contrast to an aberrant duct, which represents the only drainage to a particular region of the liver, but lies in an unusual location. Almost all aberrant hepatic ducts represent the right anterior segmental duct, which joins the right posterior segmental duct outside the liver. This extrahepatic union occurs in about 10% of cases and may result in mistaken identification of the right anterior segment duct as the cystic duct during cholecystectomy, with inadvertent ligation [17]. Both aberrant and true accessory hepatic ducts are at equal risk for injury during cholecystectomy.

Anomalies in the common bile duct do exist but are uncommon. The common bile duct may contain two lumens but appears to be single externally. The common bile duct may be completely duplicated, with one duct draining the right lobe of the liver and one duct draining the left lobe. Duplicated ducts usually empty separately into the duodenum [18,19]. Rarely, the common bile duct may open into the gastrointestinal tract at an ectopic site. The common bile duct has been reported to drain into the stomach [20] and the duodenal bulb [21], as well as the distal duodenum [22,23].

Biliary atresia

Biliary atresia is defined as the obliteration of the extrahepatic or intrahepatic bile ducts, with many different variations described (Fig. 73.11). The incidence of biliary atresia is 1 per 15 000 births. Between 10% and 15% of patients with biliary atresia have associated anomalies of the inferior vena cava (absence), portal vein (preduodenal portal vein), intestine (intestinal malrotation), and spleen (polysplenia) [24]. Most patients have a form of biliary atresia with complete obstruction of the gallbladder and extrahepatic bile ducts, whereas 10%–15% of patients have obstruction of the proximal hepatic ducts with patency of the gallbladder and distal common bile duct. Only 1%–2% of patients have proximal hepatic duct patency.

In biliary atresia, the bile ducts are replaced by dense fibrotic tissue containing evidence of both an acute and chronic inflammatory process [25]. Typically, there is obliteration of the entire extrahepatic biliary tract, including the gallbladder. Extrahepatic biliary atresia frequently extends into the intrahepatic bile ducts, probably as an end result of a

Figure 73.11 The different forms of biliary atresia. Biliary atresia may be partial, affecting the intrahepatic or extrahepatic portions of the biliary tract, or it may be a complete process **(d)**. From Lindner [1] with permission from Elsevier.

destructive process leading to fibrosis and obliteration of the biliary tree, with development of secondary biliary cirrhosis [26]. If left untreated, the typical course is one of progressive hepatic insufficiency, with an average survival of 12–19 months [27].

The pathogenesis of biliary atresia is not known. Existing data seem to contradict the theory that failure of recanalization of the bile ducts during development is the cause. Data accumulated from the surgical experience using portoenterostomy have provided some insight into the disease process. Early establishment of bile drainage, typically before 60 days, can reverse the liver injury and is associated with long-term survival, whereas delay in establishing bile drainage until after 120 days is often associated with progression of cirrhosis and a poor outcome. In addition, infants who develop biliary atresia are rarely jaundiced at birth and frequently have bile-stained meconium; biliary atresia has rarely been demonstrated in autopsy studies of fetuses [28]. These observations suggest that biliary atresia is a dynamic process targeting the extrahepatic bile ducts, with progressive destruction rather than a static process. The best available data suggest that 80% of cases are secondary to infection with a cholangiotropic virus such as cytomegalovirus, reovirus, or rotavirus, followed by an exuberant immune response destructive to the ductal epithelium that may persist even after eradication of the offending agent [29–32].

The clinical presentation of biliary atresia is progressive neonatal jaundice with an onset in the first few weeks of life. Jaundice usually becomes visible in the first 2–4 weeks of life. Biliary atresia must be differentiated from neonatal physiologcial jaundice, as well as from congenital infectious causes, such as cytomegalovirus and rubella, and genetic diseases, including α_1-antitrypsin deficiency.

Technetium 99m-iminoacetic acid (^{99m}Tc-IDA) hepatobiliary scanning is the imaging test of choice for biliary atresia, especially when preceded by a 5-day to 7-day course of phenobarbital to increase bilirubin conjugation and excretion. The sensitivity of the test for biliary atresia is 100%, with a specificity of 94% [33]. An abdominal ultrasound should also be performed as a standard part of the evaluation of the jaundiced infant and is helpful to evaluate the possibility of other sources of biliary obstruction. Liver biopsy is nondiagnostic in up to 25% of patients and is often unnecessary to make a diagnosis.

Mortality associated with medical management of biliary atresia is close to 100% so surgical therapy has become the treatment of choice. The standard operation, hepatic portoenterostomy, termed the Kasai procedure, was first described by Kasai and Suzuki in 1959 [34] following their original observation of residual microscopic bile channels in the fibrous tissue of the porta hepatis in patients with biliary atresia. The Kasai procedure consists of exploratory laparo-

tomy with excision of the occluded extrahepatic biliary system and a Roux-en-Y enteric anastomosis to the transected fibrotic cord at that level of the porta hepatis where microscopic bile ducts have been documented by frozen section. Primary hepatic transplantation is reserved for patients with a delayed diagnosis and advanced cirrhosis at presentation or patients with failed Kasai procedures [35].

Most series report a 40%–60% 5-year survival rate following portoenterostomy [36,37]. In general, one-third have good long-term results with minimal or no liver dysfunction and normal growth and development, one-third do poorly and are dependent on immediate liver transplantation for survival, and one-third have slow, progressive liver dysfunction over a period of months to years and subsequently need liver transplantation. Although the failure rate is fairly high, the fact that one-third of patients will do well and never require transplantation and another subset will experience normal growth for a period of years before potentially requiring transplantation, in addition to the shortage of transplantable organs, has prompted centers to use the Kasai procedure as first-line therapy for biliary atresia [38–40].

Choledochal cyst

See Chapter 76 for detailed discussion.

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F GALLBLADDER AND BILIARY TRACT

74 Gallstones

Cynthia W. Ko, Sum P. Lee

Epidemiology, 1952
Etiology, 1953
Clinical manifestations, 1959
Differential diagnosis, 1961
Clinical course and complications, 1963
Treatment, 1964
Acalculous cholecystitis, 1969
Recurrent pyogenic cholangitis, 1969

Autopsy studies of Egyptian and Chinese mummies have shown that gallbladder stones have existed for more than 35 centuries. Today, over 20 million Americans have gallstones, and more than 700 000 cholecystectomies are performed annually in the United States. Gallstone-related symptoms and complications are among the most common gastroenterological disorders requiring hospitalization, at an estimated annual cost of almost $6.5 billion. This chapter summarizes the epidemiology, pathogenesis, clinical manifestations, and evolving treatment of gallstones.

Epidemiology

This disease is nonfatal and often asymptomatic so the diagnosis of gallstones is not always made before death. Clinicopathological studies suggest that approximately two-thirds of the population with gallstones are asymptomatic [1]. Methods most commonly used to determine gallstone prevalence include autopsy studies, determination of cholecystectomy rates, and ultrasonography. Gallstones are generally classified as cholesterol stones, pigment stones, and mixed stones. In most Western countries, more than 75% of gallstones are the cholesterol type.

Gallstone prevalence increases with age, and gallstones are more prevalent in females of any age group [2]. The prevalence of gallstones varies widely in different countries and among different ethnic groups in the same country. The wide scatter in gallstone prevalence among different nations and ethnic groups is strong evidence that genetic factors are involved in gallstone formation. Those with the highest prevalence of gallstones are the Pima Indians of North America, Chileans, and Caucasians in the United States [1,3,4]. Next are the populations of Sweden, Germany, and Austria, followed by New Zealand, Great Britain, Norway, Ireland, and Greece [5–9]. The Asian populations in Singapore and Thailand have the lowest prevalence of gallstones [10,11]. Animal models have been developed that may help in the identification of genetic factors for gallstones [12–14].

The type of gallstone also varies among cultures. In developed countries, cholesterol gallstones are most common and usually occur in the gallbladder. In Africa and Asia, where the prevalence of gallstones is low, pigment gallstones are most common, and stones also can be found in the bile ducts.

Progressive increases in the prevalence of gallstones support a role for environmental factors in their pathogenesis. Cholecystectomy rates partly reflect the provision of medical services, and the prevalence of gallstones at autopsy may vary even within a country during a given time period. Nevertheless, several studies have shown increases in the prevalence of gallstones over time [8,15–17]. The Westernization of Japan since World War II is one example. Since the late 1940s, the prevalence of gallstones in Tokyo has more than doubled, and there has been a change from pigment to cholesterol gallstones [16]. In Saudi Arabia over a 10-year period, the frequency of cholecystectomy increased sharply, a rise not matched by the increase in population, use of medical services, or number of other surgical procedures. A shift to a more Western diet has been suggested as a possible explanation [18].

Recent studies have shown significant effects of lifestyle and behavioral factors, including obesity, physical activity, diet, and medications, in predisposing to both symptomatic and asymptomatic gallstones. For example, the risk of asymptomatic or symptomatic gallstones increases with increasing body mass index [19,20], with central obesity appearing to be more significant than peripheral obesity [21,22]. Both men and women who have low levels of recreational physical activity are at higher risk for cholecystectomy [23,24]. Diets high in carbohydrates and especially simple sugars, low in vegetable proteins, and low in fiber have also been

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associated with symptomatic gallstones [25–28]. Medications including thiazide diuretics and estrogen replacement therapy may increase the risk of symptomatic gallstones [29,30].

Etiology

Pathogenesis of cholesterol gallstones

The total body pool of cholesterol is supplied by de novo synthesis from acylcoenzyme A (acyl-CoA) and dietary absorption [31,32]. Approximately 20% of biliary cholesterol comes from new hepatic synthesis [33,34]. The remainder originates from a preformed pool within the liver [35,36]. Important contributors into the hepatic pool include hydrolysis of cholesteryl ester stores, dietary sources as chylomicrons, and direct hepatic and extrahepatic synthesis of the lipoproteins high-density lipoprotein (HDL), low-density lipoprotein (LDL), and very-low-density lipoprotein (VLDL). Total hepatic cholesterol is tightly regulated within a narrow range [37,38]. Cholesterol can be eliminated from the body by biliary excretion or by conversion to bile acids (Fig. 74.1). The association of gallstones with serum cholesterol levels is not straightforward; although gallstones may be linked to decreased serum HDL cholesterol and increased serum triglyceride levels, no such association has been found for total serum cholesterol levels [39,40].

Various defective metabolic states can disrupt this regulatory balance, resulting in absolute biliary cholesterol hypersecretion or relative bile acid hyposecretion and predisposing to gallstone formation. Both defects can coexist, as in the case of the Pima Indians [41].

Several mechanisms may be operative in biliary cholesterol hypersecretion [42–46]. Clinical situations associated with excessive secretion of biliary cholesterol include obesity, aging, drug effects, and hormonal therapy [47]. Nonobese patients with gallstones may have higher intracellular levels of total and free cholesterol, suggesting enhanced intracellular cholesterol transport [48]. Table 74.1 summarizes some of these examples and the proposed underlying pathogenesis. Animal studies have shown that there are specific genes that determine susceptibility to cholesterol stone formation in mice [12,14]. Further work has identified the bile salt export pump as a candidate protein for the *Lith1* gene [49]. Other candidate genes associated with gallstones in mice include those for hydroxy-methyl-glutaryl-CoA (HMG-CoA) reductase, canalicular multispecific organic anion transporter (Cmoat), intracellular lipid transporters (Pctp and Fabp6), lipoprotein lipase, and lecithin-cholesterol acyltransferase [14]. The human homologues to these animal genes are still being identified. Recent studies in humans have shown that dysfunction of the MDR3 protein, a phosphatidylcholine transporter found in the biliary canalicular membrane, predisposes to

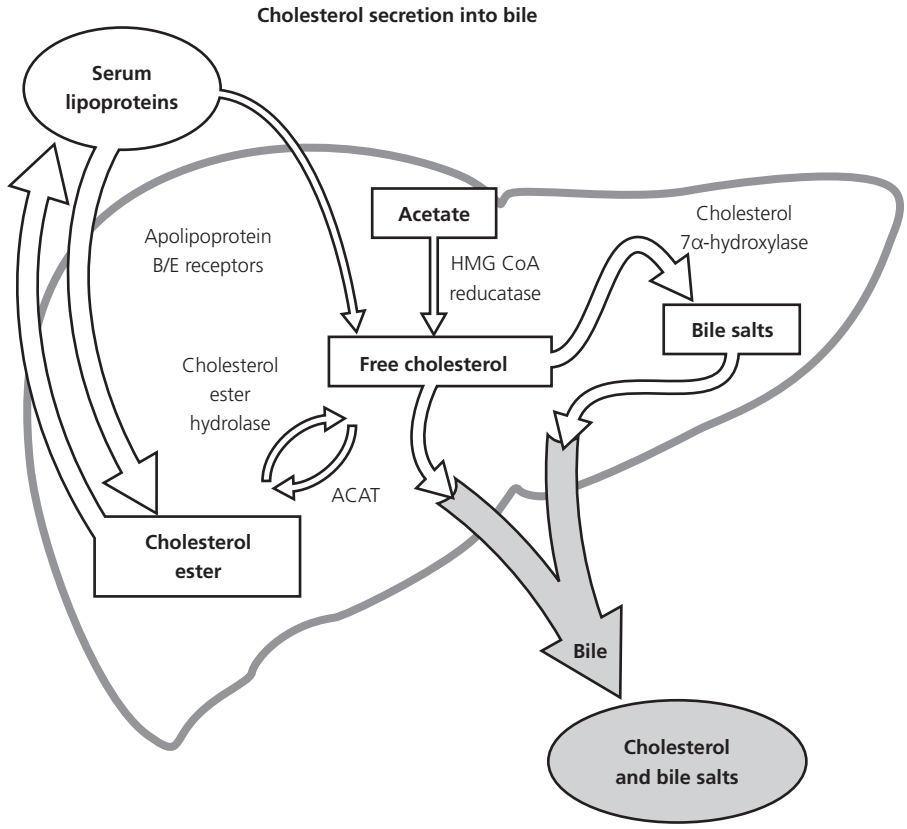

Figure 74.1 Schematic representation of cholesterol and bile acid metabolism in the liver. The liver is the major site for cholesterol synthesis and metabolism in the liver. The free pool of cholesterol in the liver is derived from de novo cholesterol synthesis or uptake from plasma lipoproteins. Cholesterol synthesis from acetate is regulated by hydroxy-methyl-glutaryl-coenzyme A (HMG-CoA) reductase. Cholesterol may be esterified by acyl:cholesterol:lecithin transferase (ACAT) for storage in the cholesterol ester pool in the body. Cholesterol may be eliminated from the body by secretion into bile or by transformation into bile acids through the rate-limiting enzyme 7α-hydroxylase.

Table 74.1 Risk factors associated with cholesterol hypersecretion in bile

Clinical examples	Pathogenesis
Obesity, hyperlipoproteinemia	Increased hydroxymethylglutaryl-coenzyme A activity leading to increased mevalonic acid and cholesterol synthesis
Progesterone, oral contraceptives, clofibrate	Inhibition of hepatic acyl-coenzyme A cholesterol acyltransferase activity leading to decreased conversion of cholesterol to cholesteryl ester stores
Estrogens	Increased lipoprotein receptors B and E leading to increased hepatic cholesterol uptake; inhibition of 12α-hydroxylase leading to decreased synthesis of chenodeoxycholic acid
Selected nonobese Japanese	Increased hepatic intracellular transport of cholesterol
Selected nonobese Caucasians	Reduced activity of 7α-hydroxylase leading to a defect in conversion of cholesterol to bile acids; increased enterohepatic cycling of bile acids, leading to increased biliary deoxycholate levels
Age	Age-related decrease (?) in 7α-hydroxylase activity
Marked weight reduction	Mobilization of tissue cholesterol

formation of intrahepatic and gallbladder cholesterol stones [50–53]. In addition, the *ApoE4* genotype has been linked to an increased prevalence of cholesterol gallstones in humans [54–56]. Apolipoprotein E (ApoE), a lipid transfer protein, is reported to be associated with stones with high cholesterol content [55], faster dissolution when treated with ursodeoxycholic acid, and higher recurrence rates after lithotripsy [56]. ApoE may have a role in the regulation of biliary cholesterol secretion [57]. The role of genetic factors and their interactions with other environmental factors deserves further study in humans.

More recent data have shown a role for serum leptin in regulating biliary cholesterol secretion. Leptin is a hormone produced by adipocytes, and its levels correlate strongly with body mass index. Studies of leptin-deficient and leptin-resistant mice have shown that higher leptin levels may mediate biliary cholesterol saturation and gallbladder motility [58–62], therefore providing a possible molecular link between body mass index, cholesterol supersaturation of bile, and ultimately cholesterol gallstone formation.

Alternatively, relative bile acid hyposecretion may lead to biliary cholesterol supersaturation (Table 74.2) [63–65]. A diminished bile acid pool and secretion rate may result from excessive intestinal losses or decreased production. In nonobese patients with cholesterol gallstones, cholic acid and chenodeoxycholic acid pools are reduced, and deoxycholic acid is often increased in bile [65]. Conversion of cholic acid to deoxycholic acid, possibly in the small intestine or by colonic bacteria, may account for these changes [66,67]. Deoxycholic acid concentrations in bile are increased with prolonged transit time, potentially linking intestinal transit to gallstone formation [67,68]. With an expanded deoxycholate pool in bile, biliary cholesterol secretion rises [69].

Cholesterol solubilization

Free cholesterol is virtually insoluble in aqueous solution. Bile acids, because of their unique amphipathic properties, are able to solubilize cholesterol and phospholipids in mixed micelles [70,71]. Phase equilibrium diagrams can be constructed to characterize cholesterol solubility in a defined

Table 74.2 Risk factors associated with relative bile acid hyposecretion

Clinical examples	Pathogenesis
Ileal disease, bypass, or resection	Impaired bile acid absorption or excessive losses
Cerebrotendinous xanthomatosis	Inherited 26-hydroxylase deficiency leading to incomplete oxidation of the cholesterol side chain and decreased bile acid production
Congenital 12-hydroxylase deficiency	Decreased cholate and deoxycholate synthesis
Selected nonobese Caucasians	Excessive feedback suppression of bile acid synthesis leading to a decreased pool, hepatic return, and secretion
Primary biliary cirrhosis, chronic cholestasis	Decreased bile acid secretion

milieu of various lipid concentrations. Using these data, the cholesterol saturation index can be calculated [72]; this is the ratio of the actual amount of cholesterol in a given bile sample to the maximal cholesterol carrying capacity of that sample determined in vitro. Bile that has a cholesterol saturation index greater than 1 is considered supersaturated. Supersaturated bile that does not form cholesterol crystals is called metastable.

In addition to classic micelle packaging, cholesterol can be solubilized with phospholipid (principally phosphatidylcholine) as unilamellar vesicles ranging in size from 40 nm to 100 nm [73–76]. Vesicles consist of a bilayer of phospholipid interdigitated with cholesterol, without associated bile acids. These vesicles are thought to be assembled within the hepatocyte and then transported to the biliary canaliculus [77]. Vesicles comprise a separate and distinct carriage system that probably represents the major mode of cholesterol transport from the hepatocyte.

Bile acid secretion is the primary driving force behind the biliary secretion of cholesterol and phospholipid [78–79]. As bile acids are actively secreted against a gradient into the canaliculus, they induce the subsequent secretion of cholesterol–phospholipid vesicles into bile. Hydrophobic bile acids are more effective in provoking lipid secretion, although the maximum rate of biliary lipid secretion is similar for all bile acids [80]. Within the canaliculus, the bile acid concentration increases, reaching a micellar concentration that allows conversion to mixed micellar carriers. This interchange continues in a dynamic fashion within the ductules, ducts, and gallbladder [81]. The relative percentage of cholesterol transported in the micellar fraction can vary dramatically, depending on the physiological conditions. For example, at low bile acid secretion rates, as with fasting, the predominant form of cholesterol carriage in hepatic bile is vesicular [73,74]. Conversely, the higher bile acid concentration in the gallbladder favors a shift toward micelle formation (Fig. 74.2 and Fig. 74.3) [73,74].

The cholesterol present in gallstones consists of oxidized species (oxysterols) [82]. The presence of oxysterols also correlates with the presence of bacterial DNA, suggesting that they might be products of biliary cholesterol oxidation as a result of bacterial–leukocyte interactions and generation of reactive oxygen species [83]. The presence of these oxysterols may also affect mucin secretion. Whether these oxysterols contribute to gallstone formation, chronic inflammation or cancer development is unknown.

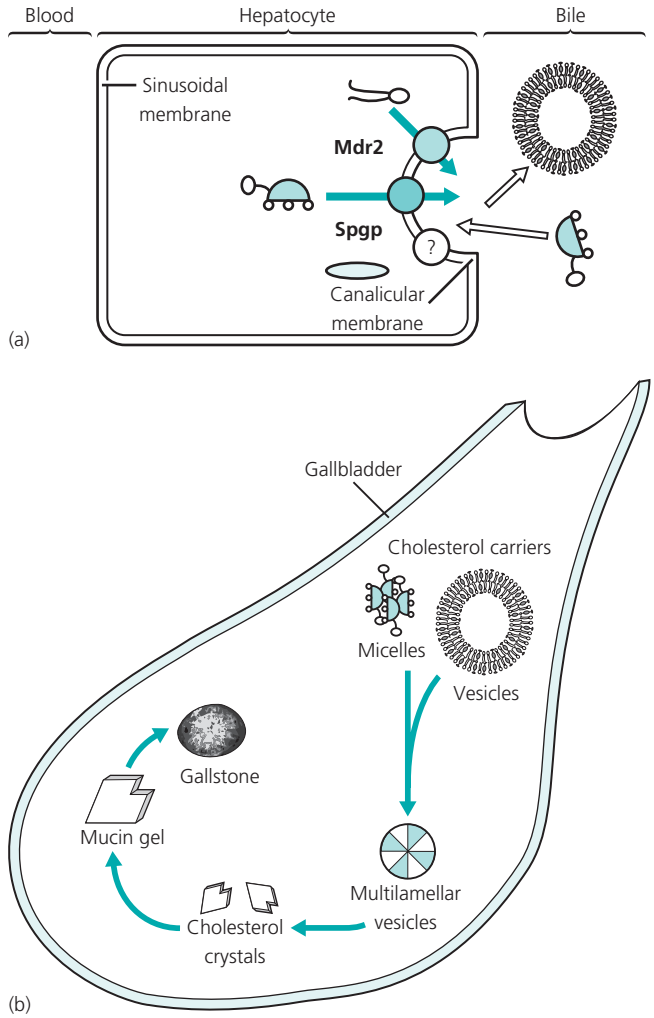

Figure 74.2 Schematic representation of biliary lipid secretion. At the biliary canalicular membrane of the hepatocyte, vesicles of cholesterol and phospholipids are secreted. Bile salts are also secreted by the bile salt export pump **(a)**. Inside the canaliculus, mixed micelles of bile salts, cholesterol, and phospholipids are formed when bile salts reach a critical micellar concentration. As bile is further concentrated in the gallbladder, more vesicles are converted to mixed micelles. Mixed micelles transfer more phospholipid than cholesterol in solubilizing biliary lipids. Vesicles in the gallbladder are relatively depleted in phospholipid and enriched in cholesterol. These vesicles are more unstable and prone to nucleation **(b)**.

Nucleation of cholesterol crystals

An early and indispensable step in cholesterol gallstone formation is nucleation, the initial precipitation of solid cholesterol crystals in saturated bile (Fig. 74.3). Fusion and aggregation of cholesterol-carrying vesicles may be crucial for crystal generation. Under video-enhanced microscopy, crystals appeared to originate from aggregated vesicles [84–86]. Nucleation of crystals results in decreases in vesicular cholesterol without changes in micellar cholesterol [73] so cholesterol in vesicles may be more unstable and prone to precipitate than cholesterol in mixed micelles.

Vesicles in bile are heterogeneous. Gallbladder bile vesicles are likely to participate in nucleation; the vesicles in hepatic bile are much more resistant to nucleation. This discrepant behavior is partly caused by the difference in their relative cholesterol-to-phospholipid contents. Vesicles that have increased ratios of cholesterol to phospholipid aggregate, fuse,

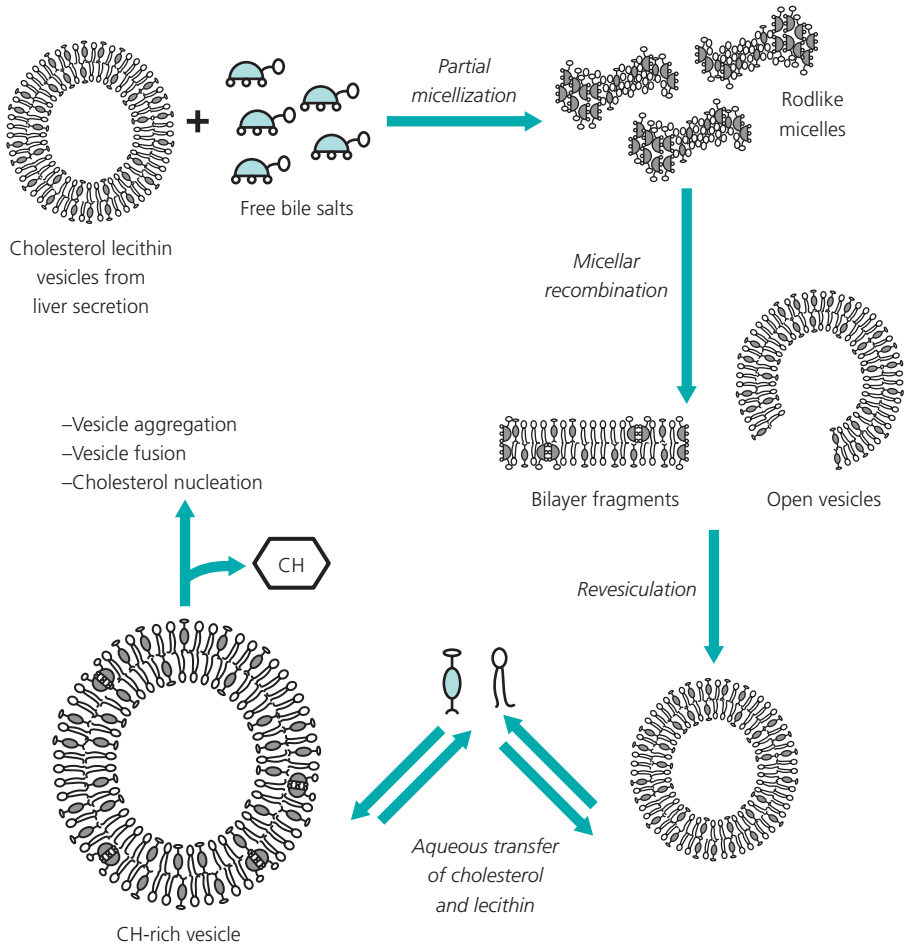

Figure 74.3 Schematic representation of cholesterol nucleation. In bile, cholesterol exists in two soluble forms, vesicles and mixed micelles. These forms are in dynamic equilibrium depending on the total bile salt and lipid concentration. With concentration, vesicles are depleted of phospholipid and enriched in cholesterol, resulting in a tendency for vesicles to aggregate and fuse. Pronucleators may also cause vesicular fusion. The fused vesicles lead to formation of multilamellar liposomes or liquid crystals. Cholesterol separates from the liquid to the solid crystalline form during nucleation. Whether vesicles can nucleate into solid cholesterol crystals is unclear. CH, cholesterol.

and nucleate more readily [86]. The concentrated bile acids within the gallbladder may preferentially remove vesicular phospholipid over cholesterol during the dynamic shift to the micellar phase. The remaining vesicles are relatively cholesterol enriched and nucleation prone. Conversely, hepatic vesicles have lower cholesterol-to-phospholipid contents and are therefore more stable.

Many people secrete supersaturated bile without developing gallstones [87]. Gallbladder bile taken from patients with cholesterol stones nucleates more rapidly than equally saturated bile from controls, suggesting that factors in addition to cholesterol supersaturation must be involved. Considerable efforts have been made to identify specific factors enhancing nucleation. Biliary proteins have generated the most attention, as total biliary protein content is increased in bile with cholesterol crystals compared with samples without crystals [88,89].

Burnstein and colleagues provided the first major evidence for a potent pronucleating agent in bile [90]. Glycoproteins and other proteins or peptides isolated from bile may associate with vesicles, regulate calcium salts, and influence cholesterol precipitation in bile [90–95]. Putative pronucleators include α_1-acid glycoprotein, aminopeptidase N, immunoglobulins G and M, haptoglobin, fibronectin, and α_1-antichymotrypsin [96–103]. However, the role of these proteins in vivo remains controversial [104–106]. In contrast, other biliary protein fractions, such as apolipoproteins AI and AII, a biliary glycoprotein heterodimer, and immunoglobulin A inhibit nucleation during in vitro assays [107–110].

Gallbladder mucin appears to be an important pronucleating factor. Mucin, a high-molecular-weight glycoprotein that is the major organic constituent of gallbladder mucus, consists of a polypeptide core with multiple oligosaccharide side chains. These peptide cores also contain hydrophobic domains that bind pigment, cholesterol, and phospholipid, entrapping vesicles and micelles in their binding sites [111,112]. With this close contact, membrane fusion and mixing can occur with the formation of multilamellar vesicles. Addition of mucin to supersaturated bile greatly accelerates cholesterol crystal nucleation [106,113]. Mucin may become incorporated into the core of the growing gallstone, providing an architectural framework for crystal growth and stone formation [114]. Mucin can therefore act as a pronucleator [115]. Mucus hypersecretion before stone formation appears to be

a universal antecedent in a number of animal models of lithogenesis [116,117]. The stimulus for mucin hypersecretion is not clear, although prostaglandins may play a role. For example, the hypersecretory response can be inhibited by the administration of large doses of oral aspirin in the human gallbladder [118], and aspirin ingestion can completely abolish the subsequent formation of cholesterol crystals and gallstones [119]. However, inhibition of prostaglandin synthesis with indomethacin does not prevent mucin hypersecretion in a prairie dog model [120,121]. Although prostaglandin activity may play a role in the pathogenesis of mucin hypersecretion, the role of other factors such as lithogenic bile or hydrophobic bile salts awaits study [122–124].

Calcium complexes are present in the central matrices of many, if not all, cholesterol stones, where they may serve as potential niduses for cholesterol crystallization [125–128]. Calcium has been shown to bind to mixed micelles, simple micelles, and simple vesicles in vitro, potentially explaining its role in cholesterol crystal nucleation [129]. Calcium may also regulate gallbladder secretion of electrolytes and glycoproteins [130,131]. Nevertheless, various studies have found conflicting data about biliary calcium levels in gallstone patients [132–134].

Role of the gallbladder

The importance of the gallbladder in cholesterol gallstone pathogenesis must be emphasized. Cholecystectomy essentially cures the patient of recurrent de novo cholesterol stone formation. Both gallbladder mucosal function and motility are important. The gallbladder can absorb excess water and electrolytes, concentrating bile [135]. Sodium and chloride ion fluxes are altered in an animal model of lithogenesis [136]. The gallbladder also normally secretes hydrogen ions and mucin.

Alterations of lipid metabolism in gallbladder epithelium that might increase cholesterol content have also been found. HMG-CoA reductase activity, governing cholesterol synthesis, is lower in the gallbladder mucosa than in hepatic tissue, and acyl:cholesterol:lecithin transferase (ACAT) activity, catalyzing the esterification of cholesterol, is several times higher than in the liver [137]. Normal human gallbladders can absorb phospholipids and cholesterol from bile in vitro, resulting in less lithogenic bile [138]. In contrast, gallbladders from patients with cholesterol stones are less able to absorb cholesterol and phospholipids, resulting in potentially more lithogenic bile.

In addition to epithelial cell function, gallbladder motility is of vital importance. Gallbladder stasis is a risk factor for lithogenesis, as mucin gels accumulate with prolonged bile storage. The viscosity of these gels may interfere with mechanical emptying. Increased cholesterol nucleation and crystal growth as well as enzymatic and nonenzymatic hydrolysis of bilirubin conjugates occur with prolonged stasis. The resulting unconjugated bilirubin may precipitate with calcium.

Altered gallbladder contractility before cholesterol stone formation has been shown in a lithogenic animal model [139]. In humans, prolonged total parenteral nutrition (TPN) induces gallbladder hypomotility and profound stasis, predisposing to subsequent biliary sludge and possible stone development [140]. High spinal cord injury is linked to gallbladder stasis and gallstone formation [141,142]. Pregnancy, oral contraceptives, obesity, diabetes mellitus, and octreotide therapy are also associated with impaired emptying and cholelithiasis [143–145].

Biliary sludge

Precipitates in bile have been called by many names – biliary sludge, microlithiasis, and pseudolithiasis. Sludge is best diagnosed by microscopic examination of a fresh sample of gallbladder bile [146]. Sludge also can be seen ultrasonographically as low-amplitude echoes without postacoustic shadow that layer with gravity [147,148] (Fig. 74.4). In certain clinical conditions, sludge may evolve into cholelithiasis.

Biochemically, sludge is composed of calcium bilirubinate granules and cholesterol monohydrate crystals embedded in a mucus gel. Ceftriaxone may precipitate as a calcium salt that appears ultrasonographically as sludge [149]. These calcium precipitates, with cholesterol crystals 50 μm or more in diameter, produce the characteristic ultrasonic echoes in sludge. The deformable mucin gel accounts for its unique layering and flow characteristics [148].

In a prospective study of patients receiving prolonged TPN, 6% of the patients developed sludge during the initial 3 weeks. By 4–6 weeks, 50% of the patients had sludge, and after 6 weeks, its appearance was universal [150]. Stones developed in 43% during follow-up. Significantly, sludge resolved in all

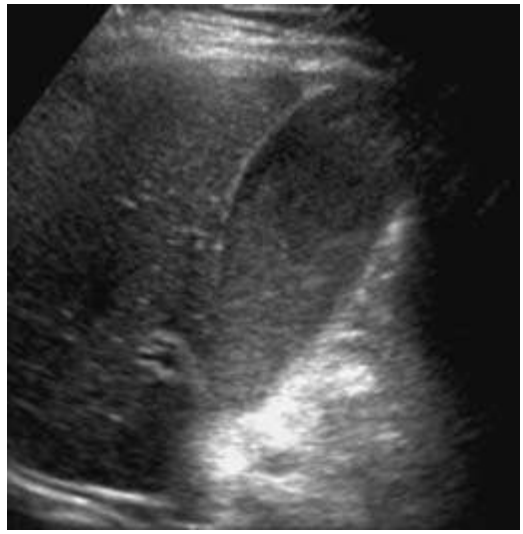

Figure 74.4 Ultrasound scan showing gallbladder sludge. Sludge appears as low-amplitude echoes without postacoustic shadowing that shift with positioning.

the patients within 4 weeks after reinstitution of oral feedings. Although stasis was undoubtedly the major factor in sludge formation, these patients represented a medically ill population with complicating metabolic derangements.

Gallbladder sludge may also appear spontaneously in selected individuals. The clinical outcome of patients who presented with upper abdominal pain and sludge on initial ultrasound examination was examined [148]. In approximately 18%, the sludge resolved over a 2-year period. It had a disappearing and reappearing course in about 60%, and gallstones also developed in 14%. Another retrospective study demonstrated similar findings, with sludge disappearing spontaneously after 2 months in most [151]. Complications including stones or acute acalculous cholecystitis developed in 19.6%. Patients with sludge may represent de novo cholesterol stone formers who have been detected at an early stage. In the absence of distal obstruction, sludge can be associated with abdominal pain (i.e., biliary colic), acute cholecystitis, or pancreatitis and should be regarded as part of the spectrum of gallstone disease [147,148].

In summary, cholesterol lithogenesis begins with altered hepatic secretion of biliary lipids, resulting in cholesterol supersaturation of bile. Gallbladder mucin hypersecretion and stasis develop, perhaps through the action of chemical mediators. As the bile is progressively concentrated, the vesicular fraction becomes relatively cholesterol enriched. These vesicles aggregate and fuse. When the balance of the opposing promoting and inhibitory factors favors nucleation, biliary sludge or microlithiasis forms. The crystals formed are retained within the gallbladder, where they grow and conglomerate with mucin glycoprotein and other constituents such as calcium and bilirubin. Continued stasis and impaired emptying of cholesterol precipitates lead to macroscopic stone formation.

Pathogenesis of pigment gallstones

Chemical composition and clinical associations

Pigment gallstones are subclassified into two types, brown and black, which differ in morphology, pathogenesis, and clinical correlations (Table 74.3). In contrast to cholesterol gallstones, race and ethnicity are not important determinants of pigment gallstones. Black pigment stones can occur in persons with no predisposing factors. In the United States, black pigment gallstones are found in the gallbladder and are not associated with bacterial infection.

Factors associated with the formation of black pigment stones include chronic hemolysis (e.g., sickle cell disease), thalassemia, prosthetic heart valves, advancing age, long-term TPN, and cirrhosis [152–155]. Black pigment stones seldom coexist with cholesterol stones in the same gallbladder. A

Table 74.3 Clinical associations and compositions of black and brown pigment gallstones

Characteristics	Black	Brown
Color	Black	Brown, orange, yellow
Size	0.3 cm to 0.6 cm	0.5 cm to 2 cm
Consistency	Amorphous, powdery, solid	Soft, laminated, muddy
Anatomic location	Gallbladder	Gallbladder and bile ducts
Geography	West and Asia	Mostly Asia
Diet	High-protein diet	Low-protein diet
Disease associations	Hemolysis, cirrhosis, "healthy" persons	Cholangitis, ova, parasites, sutures
Bile cultures	Usually sterile	Infected (*Escherichia coli*, *Bacteroides* sp., ova, parasites)
Recurrent stones	Uncommon	Frequent
Radiological calcification	Diffuse (up to 70% of stones radiopaque)	Usually radiolucent, slightly calcified
Principal components	Pigment polymer, calcium phosphate, calcium carbonate	Calcium bilirubinate, calcium soaps of fatty acids, cholesterol
Etiology	Increased excretion or hydrolysis of conjugated bilirubin	Bacterial hydrolysis of conjugated to unconjugated bilirubin
Bile pigment – total major form	40 (10–90)[a] Insoluble polymer	50 (28–79)[a] Calcium bilirubinate
Calcium	15 (3–40)[a]	5 (3–9)[a]
Calcium salts		
Carbonate	13 (0–65)[a]	None
Phosphate	5 (0–32)[a]	< 1[a]
Palmitate + stearate	1 (0–3)[a]	23 (11–67)[a]
Cholesterol (unesterified)	3 (1–13)[a]	10 (2–28)[a]
Organic matrix (glycoproteins, mucins, carbohydrates)	24 (10–73)[a]	12 (0–30)[a]

a Values are given as a percentage of dry weight of the stone and are means, with ranges in parentheses.
Adapted from Ostrow [327] and Leuschner et al. [328].

calcium-regulatory protein that may mediate calcium precipitation has been isolated from black pigment stones [156].

In Asia, brown pigment stones are often found in the bile ducts and are strongly associated with bacterial infection [157]. There is a high incidence of infection with *Escherichia coli*, with the bacterial presence within stones easily demonstrated [158–160]. Biliary secretory immunoglobulin A is decreased [161]. Infected bile exhibits high bacterial β-glucuronidase activity, often associated with a juxtapapillary duodenal diverticulum [162,163]. Molecular studies have shown that bacterial DNA can be recovered from most gallstones, except for those with very high cholesterol content [164–166], confirming a role for bacteria in pigment gallstone pathogenesis.

Deconjugation of bilirubin

Bilirubin, a tetrapyrrol-like cholesterol, is insoluble in water. After glucuronidation, bilirubin is secreted, mostly as the water-soluble diglucuronide (75%–80%) or monoglucuronide (20%) forms, with a small amount of unconjugated bilirubin (3%). Both the black and brown pigment stones contain calcium-bilirubin salts so the pathogenesis of pigment stones involves the deconjugation and precipitation of bilirubin.

In patients with chronic hemolysis, there is a 10-fold increase in the excretion of conjugated bilirubin in hepatic bile [167]. In an animal model of ileal resection, bilirubin circulates enterohepatically during the early postsurgical period, with increases in biliary levels of this molecule [168]. In patients with Crohn's ileitis or who had undergone ileal resection, concentrations of conjugated and unconjugated bilirubin were increased compared to patients with Crohn's or ulcerative colitis. In these studies, it has been hypothesized that unconjugated bilirubin is absorbed from the colon in the presence of ileal disease. These findings may help explain the increased incidence of pigment stones in patients with ileal disease.

The role of biliary β-glucuronidase in the pathogenesis of pigment gallstones has been controversial. Studies employing molecular genetic techniques have implicated bacterial infection in producing brown pigment stones, with bacterial β-glucuronidase hydrolysis of conjugated bilirubin to form insoluble bilirubin [165,166]. However, this hypothesis does not seem to be relevant to black pigment gallstones [169]. β-Glucuronidase activity in uninfected bile has been demonstrated, suggesting that this enzyme may also originate in the epithelium [170–172]. However, another risk factor, gallbladder stasis, must be present for precipitates of calcium bilirubinate to form. Stasis, similar to the scenario described for cholesterol nucleation, can provide an opportunity for nonenzymatic hydrolysis of bilirubin diglucuronide and its subsequent precipitation [173,174]. Prolonged TPN, with attendant gallbladder stasis, can result in the formation of gallbladder sludge and black pigment stones [150,175].

The gallbladder may contribute to pigment gallstone formation in other ways. Calcium homeostasis is intimately linked to black and brown pigment gallstones, because both kinds contain predominantly calcium bilirubinate. Hepatic bile is supersaturated with calcium carbonate. The gallbladder epithelium can acidify bile, increasing the solubility of calcium carbonate. Impaired acidification of bile in an inflamed gallbladder may contribute to pigment gallstone formation. Gallbladder epithelium secretes a matrix of mucous glycoprotein into bile that binds to bilirubin and other hydrophobic lipids and is contained in black and brown pigment gallstones [111].

In summary, pigment gallstones begin with increased hepatic bilirubin excretion. Gallbladder stasis or bile infection allows the hydrolysis of bilirubin diglucuronide to less soluble forms. Impaired acidification of bile by the gallbladder perturbs calcium and bilirubin solubility, and mucus secreted by the gallbladder epithelium buffers hydrogen ions, enabling calcium carbonate, phosphate, and bilirubin to precipitate. For reasons that are poorly understood, the calcium bilirubinate in brown pigment stones remains in the monomeric form, but undergoes significant cross-linking to become polymeric and crystalline in black pigment stones.

Clinical manifestations

Biliary colic

Approximately one-third of patients with gallstones are symptomatic. Biliary colic as the result of transient obstruction of the cystic duct by sludge or stones is the main complaint in 70%–80% of symptomatic patients. Implicit in the term biliary colic is the fact that the gallbladder mucosa does not show features of acute inflammation. The pain of biliary colic is thought to be caused by functional spasms around an obstructed cystic duct; the pain of acute cholecystitis is caused by gallbladder wall inflammation.

Although it may be variable, biliary pain often has specific characteristics. The pain is episodic and severe, and it is located in the epigastrium or less frequently in the right upper quadrant, left upper quadrant, the precordium, and the lower abdomen [176,177]. The pain may be precipitated by eating a large meal, but often no single food is the lone culprit. Pain may develop without any precipitating events.

Typically, the pain has a sudden onset and rises steeply in intensity over a 15-min interval to a steady plateau lasting as long as 3 h. Resolution of pain is slower. Pain lasting more than 6 h should raise the suspicion of cholecystitis. The pain may radiate to the interscapular region or, rarely, to the right shoulder. Vomiting and diaphoresis are not uncommon. The patient is usually restless and unable to find a comfortable position. Residual tenderness in the upper abdomen may persist after an attack. The interval between attacks is unpredictable and may be weeks, months, or years. True biliary colic should be differentiated from nonspecific dyspepsia.

Flatulence, pyrosis, aerophagia, vague abdominal discomfort, and fatty food intolerance are common complaints of many patients, whether or not they have cholelithiasis [178].

The ability to differentiate true biliary pain from nonspecific abdominal symptoms significantly impacts the success of treating gallstone disease. For example, a cholecystectomy performed for gallstone-induced biliary colic is usually curative, but symptoms often persist if it is performed for patients with nonspecific dyspepsia and cholelithiasis [179,180]. It is worthwhile to clarify prevalent misunderstandings about biliary pain. First, biliary colic is a misnomer, because the pain is steady, not intermittent or fluctuating. Second, the pain is primarily epigastric. It is inappropriate to interpret pain not located in the right upper quadrant as atypical of gallstone symptoms. Third, fat intolerance is not a feature of biliary colic. Fourth, despite the availability of many imaging techniques to demonstrate the presence of gallstones, the diagnosis of biliary colic is ultimately based on clinical judgment.

Acute cholecystitis

The most common cause of acute cholecystitis is obstruction of the cystic duct by gallstones, resulting in acute inflammation of the gallbladder. Approximately 90% of cases are associated with cholelithiasis. Obstruction likely damages the gallbladder mucosa, initiating an inflammatory reaction involving prostaglandins and other chemical mediators [181,182]. Although bacteria are commonly found in the gallbladder bile of patients with acute cholecystitis, this is probably a secondary event [183,184]. Secondary bacterial infection can progress to empyema.

Most patients with acute cholecystitis have had previous attacks of biliary pain. The pain of acute cholecystitis typically lasts longer than 3 h and shifts from the epigastrium to the right upper quadrant with the emergence of localized tenderness and associated vomiting. With time, the intensity of pain may diminish, but tenderness increases. Fever is common, but is rarely over 38.3°C (101°F). The sequence of clinical features represents visceral pain of ductal impaction by stones, proceeding to gallbladder inflammation with parietal pain.

In elderly patients, presenting signs and symptoms may be especially mild. On physical examination, Murphy's sign, an abrupt arrest in inspiration secondary to pain during direct palpation of the right upper quadrant, may be present. In 30% to 40% of patients, a palpable mass may be present. Jaundice is present in 15% of patients with acute cholecystitis, even without choledocholithiasis and obstruction. The pathogenesis may involve edema and compression around the inflamed cystic duct. The differential diagnosis of cholecystitis includes choledocholithiasis, acute cholangitis, and pyogenic or amebic liver abscesses.

Chronic cholecystitis

Patients with chronic cholecystitis usually have gallstones and have had repeated attacks of biliary pain or acute cholecystitis. This results in a thickened and fibrotic gallbladder, which shows evaginated mucosal pouches (Rokitansky–Aschoff sinuses) on histological examination. It is uncommon for the gallbladder to be palpable during an attack of pain. The patient may have fewer symptoms referable to the gallbladder but may present with associated gallstone complications such as recurrent pancreatitis, choledocholithiasis, and cholangitis.

Choledocholithiasis and cholangitis

Small gallstones passing into the common bile duct from the gallbladder may eventually proceed to the duodenum. They may also remain in the common bile duct and give rise to complications. The majority of stones in the common duct have the same composition as those in the gallbladder. However, some are softer and more brown in color because of deposits of calcium bilirubinate and other calcium salts, including fatty acid complexes [153]. Common duct stones are frequently associated with infected bile, which may or may not be clinically apparent.

Choledocholithiasis is one of many causes of obstructive or "surgical" jaundice, which must be differentiated from hepatocellular or "medical" jaundice. Obstruction of the passage of bile results in jaundice and pruritus. The pathogenesis of pruritus is unclear, although retention of bile salts with irritation of sensory nerve endings and retention of endogenous opiate agonists have been proposed as mechanisms. Sometimes pruritus can be the predominant or incapacitating symptom. With biliary obstruction, the feces may be hypocholic or acholic. Light-colored or clay-colored stools are uncommon in bile duct obstruction resulting from gallstones because the obstruction is rarely complete, but are more commonly observed with malignant strictures of the common bile duct.

Unlike with malignant obstruction of the common bile duct, a palpable and nontender gallbladder because of cholelithiasis (Courvoisier law) is uncommon. The biliary obstruction is usually incomplete, and the gallbladder itself is likely to be fibrotic, scarred, and nondistensible secondary to chronic cholecystitis. However, the exceptions to this rule are substantial, with frequent false-positive and false-negative results.

Obstruction of the common bile duct also causes increased intraductal pressure, inhibiting bile flow and causing the reflux of bile from the canaliculus into the sinusoids. The normal pressure in the common bile duct is 10 cmH_2O to 15 cmH_2O. With obstruction, the pressure can rise to above 40 cmH_2O, causing biliary duct dilation [185,186]. Therefore, dilation of the extrahepatic and intrahepatic bile ducts is a valuable sign of common bile duct obstruction and is best seen using ultrasonography or a computed tomography (CT) scan. Clinically, the only manifestations may be mild hepatomegaly or right upper quadrant tenderness.

Prolonged biliary obstruction induces fibrogenesis in the liver, potentially leading to secondary biliary cirrhosis. The

propensity for developing cirrhosis varies with the completeness and duration of obstruction. On average, choledocholithiasis will lead to secondary biliary cirrhosis after about 5 years [187]. Patients with incomplete obstruction more commonly present with portal hypertension, and those with complete obstruction present with hepatic failure [188]. Even if the patient has cirrhosis, every effort should be made to relieve the obstruction, because reversal of portal hypertension and secondary biliary cirrhosis has been reported [189].

A common complication of choledocholithiasis is cholangitis. For bacterial infection to occur, biliary obstruction or bile stasis is always present, although obstruction is not always associated with overt bacterial infection. For example, cholangitis is extremely common in choledocholithiasis, not uncommon in sclerosing cholangitis, and relatively uncommon (10%–15%) in malignant bile duct strictures.

The typical clinical picture, occurring in 70% of cases, consists of biliary pain, jaundice, and chills and rigors (Charcot triad). Pain, usually characteristic of biliary colic, occurs in 90% of patients. Bacteremia with chills and fever occurs in 95%, and clinical jaundice in 80%. Clinical signs are nonspecific, with mild hepatomegaly, tenderness, and occasionally rebound tenderness [190]. For many patients, cholangitis is a short and self-limited illness complicating choledocholithiasis or sclerosing cholangitis. However, shock, multiple liver abscesses, or multiorgan failure may result. Blood cultures are often positive and reflect the organisms infecting the bile ducts. The most commonly found organisms are *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., and enterococci. Coinfection with anaerobes occurs in 15% [191–193].

Acute pancreatitis may be precipitated by the passage of stones or sludge in the common bile duct, and sludge or microscopic stones may account for a significant proportion of cases of idiopathic pancreatitis [146,194].

Differential diagnosis

Clinical signs and symptoms

The clinical symptoms and signs of biliary tract disorders are not highly specific. The patient's history, physical findings, and laboratory data should be carefully examined. A clinical impression of biliary colic should be confirmed by imaging studies. Gallstones are found commonly and may coexist with other disorders so the finding of gallstones does not exclude other diseases contributing to or complicating the patient's clinical picture. Disorders of other visceral organs, including the upper gastrointestinal tract, colon, kidneys, and pancreas, should be excluded by appropriate diagnostic tests. Extraabdominal disorders that may produce a similar clinical picture include angina pectoris, dissecting aortic aneurysm, spinal neuralgia, pleuritis, pericarditis, and uncommon metabolic disorders such as hereditary angioedema (C1 esterase inhibitor deficiency) and acute intermittent porphyria.

In addition to pain, patients with acute cholecystitis usually present with symptoms and signs of local inflammation (e.g., right upper quadrant mass, tenderness) and systemic toxicity (e.g., fever, leukocytosis). The differential diagnosis includes other causes of intraabdominal inflammation or infection. Acute appendicitis can cause a difficult diagnostic problem. The periumbilical pain shifting to the right lower quadrant with an inflamed mass may be confused with biliary colic with an inflamed gallbladder. A gallbladder can be low lying or an appendix can be subhepatic. Fever and leukocytosis are features of both. Ultrasonography or hepatobiliary scintigraphy may be helpful.

It may be difficult to differentiate acute pancreatitis from cholecystitis because the two conditions produce tenderness in an overlapping area. Gallstones may cause acute pancreatitis, and cholecystitis and pancreatitis therefore may coexist. Acute cholecystitis alone can be associated with hyperamylasemia, but pancreatitis often has higher enzyme levels. Biliary scintigraphy and imaging techniques such as ultrasound and CT scans are helpful diagnostically.

Perforated peptic ulcer usually produces more dramatic pain and peritoneal signs. Plain abdominal radiographs or CT scans often show free intraabdominal air. If free air cannot be seen and ulcer perforation is still suspected, an urgent radiographic study with water-soluble contrast should demonstrate the perforation.

Diagnostic studies

Laboratory tests

In uncomplicated biliary colic, the results of hematological and biochemical tests are normal. In acute cholecystitis, leukocytosis with a "left shift" is usually seen, but this does not differentiate it from other intraabdominal infections. Edema and inflammation of the gallbladder can partially obstruct the common bile duct, causing mild elevation of the serum aminotransferases and alkaline phosphatase. Elevation of serum bilirubin can also be associated with the enzyme level changes, especially if the common hepatic duct or common bile duct is involved in the inflammatory reaction. Bilirubin elevation is proportional to the degree of obstruction. Alkaline phosphatase, however, is produced by bile canalicular and ductular cells, and its elevation does not depend on the magnitude or cause of obstruction.

Abdominal pain, fever, and jaundice are often the presenting features of choledocholithiasis. In such cases, the bilirubin level is usually between 2 mg/dL and 10 mg/dL, and the alkaline phosphatase is less than five times normal. If the level of bilirubin is above 15 mg/dL, a neoplastic obstruction should be strongly suspected. If ductal dilatation is documented by ultrasonography or CT scans, a cholangiogram should be obtained to define the cause and level of obstruction.

Radiological studies

Plain abdominal films are rarely useful in uncomplicated biliary colic, because only 13%–17% of gallstones contain sufficient calcium to be radiopaque. In acute cholecystitis, radiographs are obtained to exclude other intraabdominal causes of abdominal pain, fever, and leukocytosis, such as perforated ulcer or intestinal obstruction. Occasionally, when emphysematous cholecystitis is present, intramural gas outlining the gallbladder can be seen, but this is better shown using ultrasonography.

Ultrasonography has high specificity and sensitivity for the diagnosis of biliary sludge or gallstones and should be a routine examination in the evaluation for gallstone disease. Stones appear as high-amplitude echoes with postacoustic shadowing (Fig. 74.5). Biliary sludge appears as low-amplitude echoes without postacoustic shadowing that shift with positioning (Fig. 74.4). Other potential findings include thickening of the gallbladder wall (> 2 mm), intramural gas, and a pericholecystic collection of fluid; these last two suggest active gallbladder inflammation or infection. Dilatation of intrahepatic or extrahepatic bile ducts suggests distal obstruction. However, the sensitivity of ultrasonography for choledocholithiasis is only 40%–50%, and therefore a normal ultrasound does not rule out this diagnosis [195].

Hepatobiliary scintigraphy can confirm or exclude the diagnosis of acute cholecystitis with a high degree of sensitivity and specificity. After a 2-h to 4-h fast, the patient is given an intravenous injection of a technetium 99 m-labeled iminodiacetic acid derivative (^{99m}TC-IDA agent), which is excreted into the bile ducts, and is sequentially imaged under a gamma camera. Several ^{99m}Tc-IDA compounds have been developed and differ with respect to their degree of hepatic uptake and time to reach a peak concentration in bile.

In a normal study, images of the gallbladder, common bile duct, and small bowel appear within 30–45 min. A normal ^{99m}Tc-IDA scan virtually rules out the diagnosis of acute cholecystitis in a patient who presents with abdominal pain. Failure to visualize the gallbladder by 90 min despite adequate views of the liver, common bile duct, and small bowel strongly suggests acute obstructive disease. False positives can result from non-fasting states, prolonged fasting states, or chronic cholecystitis. Delayed repeat scanning after 4 or more hours decreases the false-positive rate and provides sensitivity and specificity of approximately 97% and 90%, respectively. Morphine causes contraction of the sphincter of Oddi, increasing common bile duct pressure and enhancing filling of the cystic duct. Administration of low-dose morphine at 1 h may obviate the need for delayed repeat scans. Hepatobiliary scintigraphy cannot provide anatomic information and cannot directly identify gallstones.

Oral cholecystography is seldom employed as a primary diagnostic test for the detection of gallstones or acute cholecystitis. The study involves the administration of oral contrast tablets such as iopanoic acid the night before. The contrast is absorbed from the small bowel, conjugated and excreted by the liver, and concentrated within the gallbladder. The following morning, abdominal radiographs are obtained. Extrabiliary conditions such as small bowel disease, hepatic dysfunction, or prolonged fasting can all lead to gallbladder nonvisualization [196,197]. Inadequate gallbladder visualization can occur in 15%–50% of patients after a single dose. The chief advantage of oral cholecystography over ultrasound is its ability to assess the patency of the cystic duct and gallbladder function, which may be useful if medical therapy of gallstones is being considered. Moreover, the size, number, and degree of calcification of stones can be accurately determined.

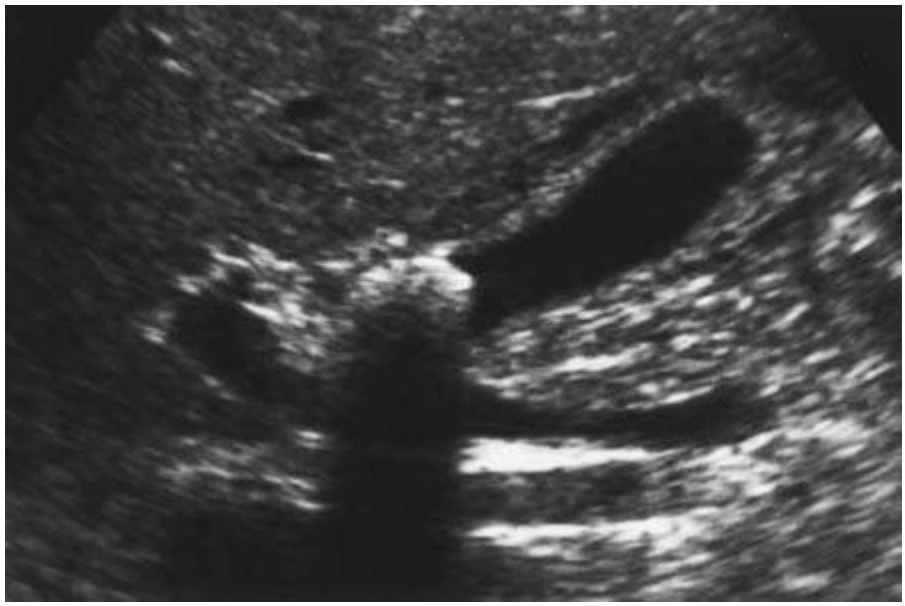

Figure 74.5 Ultrasound scan showing gallstones. The stones generate high-amplitude echoes and are large enough and dense enough to constitute substantial acoustic impedance in the path of the ultrasound beam. Hence, there is a void behind the stone, known as the postacoustic shadow. Some edema of the gallbladder wall is also seen, suggesting acute cholecystitis.

CT can also be used in the detection of cholelithiasis. Although superior to ultrasound in other clinical situations, CT is not as reliable in diagnosing gallbladder stones. However, CT is useful in demonstrating dilated bile ducts and mass lesions and can be considered the test of choice if clinical suspicion of a tumor (e.g., pancreatic cancer) obstructing the common bile duct is strong.

When a more definitive view of the biliary system is needed, cholangiography should be performed by a retrograde endoscopic route (i.e., endoscopic retrograde cholangiopancreatography [ERCP]) or through a percutaneous transhepatic cholangiography (PTC) (Fig. 74.6). The selection of either approach depends on the expertise at a particular institution and on the level and nature of the suspected lesion. In general, ERCP is used to demonstrate the lower limit of an obstruction and has the advantage of the ability to sample tissue. PTC demonstrates the upper limit of an obstructive lesion or a proximal obstruction better. Both tests have a risk of introducing infection in the presence of obstruction.

Other options for less invasive diagnosis of bile duct stones include magnetic resonance cholangiopancreatography (MRCP), helical CT, and endoscopic ultrasound. MRCP has been shown to be over 90% sensitive and specific for the diagnosis of bile duct stones, when compared to ERCP [198–201]. It also has the advantage of being noninvasive and potentially allowing diagnostic imaging without the risks of PTC or ERCP. Helical CT has also been studied, and found to have sensitivity and specificity of 80%–85% [202,203]. The addition of an oral contrast agent for CT cholangiography may provide sensitivities and specificities comparable to MRCP [204]. Endoscopic ultrasound can detect bile duct stones and biliary sludge with high sensitivity, without the morbidity associated with ERCP [205–208]. With the availability of many tests, the choice of diagnostic test should be based on local availability and expertise.

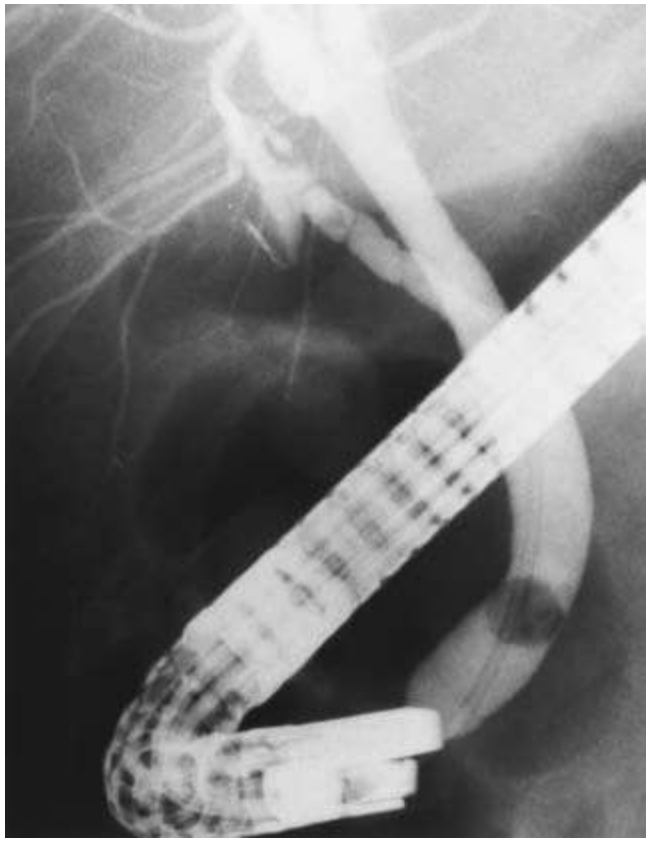

Figure 74.6 Endoscopic retrograde cholangiogram shows a stone within the common bile duct and a small stone or debris in the cystic duct. Courtesy of Dr Scott Schulte.

Clinical course and complications

Natural history of asymptomatic gallstones

The rate with which gallstones develop varies. In patients on TPN or in obese persons with rapid weight loss, the interval can be weeks. In Pima Indians, the progression from cholesterol-supersaturated bile to formation of gallstones takes 5–10 years [209]. When gallstones do form, the risk factors for developing symptoms are unknown. It is estimated that 60%–80% of all gallstones are asymptomatic at a given time [210–212]. However, the rate at which stones give rise to symptoms and complications is relatively small. In contrast, once gallstones start to cause biliary-specific symptoms, the risk of continuing problems is relatively high.

Studies by Wenckert and Robertson [213] and Lund [214] followed a total of 1307 patients with gallstones for up to 20 years and concluded that 50% of patients remained asymptomatic, 30% had biliary colic, and 20% had complications. Some have cited such data as indicating prophylactic cholecystectomy for all patients with gallstones, regardless of the severity of symptoms. However, the patients in these studies, although asymptomatic at the time of entry to the follow-up study, had previously been hospitalized for symptoms related to gallbladder disease.

Gracie and Ransohoff followed a cohort of 123 asymptomatic persons with gallstones that were found by oral cholecystography [215]. These 110 men and 13 women (average age 54 years) were followed for 11–24 years. New-onset biliary colic developed at a rate of 2% per year for the first 5 years, but the cumulative incidence over time plateaued, such that the total incidence was 15% at 10 years and 18% at 15 and 20 years. Three patients (2%) developed complications, all preceded by repeated attacks of biliary colic, and all had uneventful cholecystectomies. No deaths related to gallbladder disease occurred in this cohort.

The GREPCO study has reported follow-up of 161 initially asymptomatic patients with gallstones [216]. Biliary colic developed in 11.9% at 2 years, 16.5% at 4 years, and 25.8% at 10 years. The subjects were also followed for development of complications of gallstones, including cholecystitis, pancreatitis,

and biliary obstruction. After 10 years, 3% of those who were initially asymptomatic developed complications, including one person who developed gallbladder adenocarcinoma.

A 20-year population-based survey of mortality in Pima Indians showed that the overall age- and sex-adjusted death rate was higher in those with a history of gallstones, with a ratio of 1.9 [217]. The death rate attributed to malignancies was 6.6 times higher in those with gallstones. Of the 20 fatal malignancies in patients with gallstones, 11 were cancers of the gastrointestinal tract, with 6 cancers of the gallbladder or bile ducts.

These studies highlight the question of whether a link between gallstones, cholecystectomy, and malignancies exists. A modest increase in the risk of colon cancer, but not of rectal cancer, has been found after cholecystectomy [218–220]. Increased rates of gallbladder carcinoma or carcinoma of the extrahepatic bile ducts in patients with gallstones have been found in an autopsy series and in a case–control study [221,222]. The mechanisms for the increased risk of malignancy have not been clarified.

Natural history of symptomatic gallstones

Once an episode of biliary colic has occurred, there is a high risk of repeated attacks of pain. Cohort studies following symptomatic gallstone patients indicate that 58%–72% of patients have ongoing symptoms and complications [214,223]. More than 90% of complications, such as cholecystitis, cholangitis, and pancreatitis, are preceded by attacks of pain. The most serious complications are gangrene and perforation of the gallbladder, occurring in about 10% of cases of acute cholecystitis.

When the gallbladder perforates, the outcome depends on the anatomic relation with the neighboring structures. The perforation can localize to form an abscess; it can be a free perforation with peritonitis; or it can communicate with another hollow viscus as a fistula. A localized perforation with a pericholecystic abscess is most common. This should be suspected when acute cholecystitis is slow to resolve, especially with a second episode of fever, right-sided abdominal pain, or the appearance of a right upper abdominal mass. Ultrasonography and CT may show the pericholecystic abscess, but this is not dependable. If the degree of clinical suspicion is high, the diagnosis should be confirmed at surgery. This is especially so in elderly patients or patients on long-term steroid treatment in whom fever and the inflammatory response may be minimal.

In 1%–2% of perforations, especially in the fundus of the gallbladder, free perforation occurs and carries a mortality rate of at least 30%. Bile and purulent peritonitis are almost always associated with persistent or progressive abdominal pain. When the diagnosis is suspected, no test can establish the diagnosis without further delaying intervention and causing additional tissue injury. Antibiotics should be given and emergency surgery performed.

When the gallbladder perforates into the adjacent intestine, an attack of acute cholecystitis often subsides as the inflamed organ is decompressed. Cholecystoenteric fistulae are most common in the duodenum, followed by the hepatic flexure of the colon, stomach, and jejunum. If the gallstones are completely discharged and are small enough to pass rectally, an uncomplicated cholecystoenteric fistula results. However, if stones are still present in the gallbladder or in the common bile duct, chronic symptoms may arise.

Bowel obstruction may result when a fistula develops and the gallstone is too large to traverse the intestine (gallstone ileus). The diameter of the stone is usually greater than 2.5 cm and the site of obstruction is commonly in the small intestine, most often at the ileocecal valve. Although patients with gallstone ileus sometimes have a previous history of acute cholecystitis, stones usually erode slowly through the gallbladder, and the symptoms may be minimal. Gallstone ileus should always be considered in older patients with intestinal obstruction [224,225]. Plain abdominal radiographs may show air in the biliary tree or the presence of a calcified gallstone, and contrast studies often reveal the site of communication. Ultrasound scans can detect air in the biliary tree but not the site of the fistula. CT scans are less useful in detecting gallstones and fistulae, although they also may show air in the biliary tree.

Treatment

Because gallstones are common and often asymptomatic, the treatment of gallstones should consider the patient's clinical presentation (Fig. 74.7). For asymptomatic gallstones, before active treatment is instituted, the following questions should be considered. What is the rate at which new-onset symptoms emerge? If symptoms develop, what is the risk of these evolving to complications, and what is the risk of morbidity and mortality from such complications? What is the risk of prophylactic treatment? What are the short-term and long-term costs of prophylactic treatment compared with no treatment or delayed treatment?

The fractional and cumulative risk of developing symptoms is not great (2%–5% per year). Complications generally develop after the emergence of symptoms. Risk–benefit analyses of elective cholecystectomy have shown no advantage to aggressive prophylactic cholecystectomy for patients with asymptomatic gallstones. Once symptoms or complications develop, gallstones should be treated. Options include surgery, dissolution, or fragmentation of symptomatic gallstones (Table 74.4). Cholecystectomy is a safe procedure with a mortality rate of 0.1%–0.2% for either open or laparoscopic procedures [226–228]. The surgical mortality rate of 0.9%–1.8% is higher for patients older than 60 years of age [229,230]. With common bile duct exploration, the mortality rate is approximately two to three times higher.

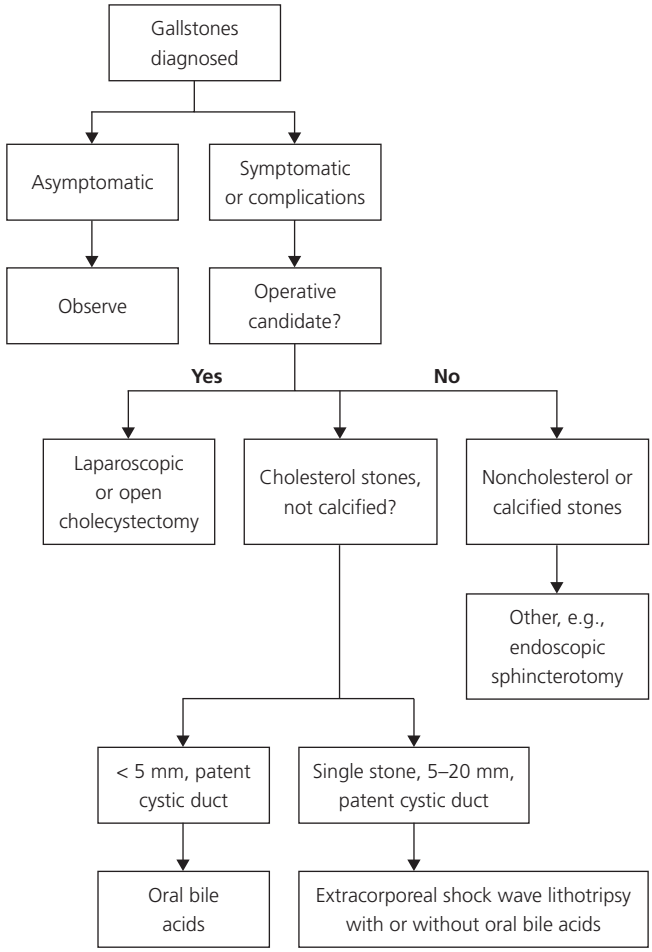

Figure 74.7 Algorithm for management of biliary sludge and gallstones.

Generally, the indications for cholecystectomy should not change, and asymptomatic patients should not undergo surgery despite the widespread availability of laparoscopic cholecystectomy. However, in certain subsets of patients, it may be prudent to make exceptions and proceed with a prophylactic cholecystectomy. These groups include children, patients with porcelain gallbladder, and the morbidly obese [231,232]. A calcified or "porcelain" gallbladder is associated with a higher risk of cancer [233]. In the obese, a cholecystectomy is recommended if the patient undergoes bariatric surgery [234]. Patients who are immunosuppressed on a long-term basis may represent another group, as signs of acute cholecystitis may be "masked." It is also agreed that patients with choledocholithiasis, even if asymptomatic, should have the bile duct stones removed and undergo cholecystectomy. This is reinforced by postmortem studies, which showed that ductal stones contributed significantly to death even in asymptomatic patients [235,236]. Patients with diabetes mellitus are a controversial group. It has been stated that diabetics have a higher risk of complications, morbidity, and mortality from gallstones. However, they may have an increased operative risk with elective and emergency cholecystectomy, largely because of concomitant cardiovascular disease [237,238].

Surgical treatment

Cholecystectomy is the only definitive treatment. Elective cholecystectomy is usually simple, safe, and curative; is indicated for most patients with symptoms; and is the first choice when complications ensue. Nonsurgical modalities of treatment are less favored with the advent of laparoscopic cholecystectomy.

Laparoscopic cholecystectomy

Laparoscopic cholecystectomy was first performed in France in 1987 [239]. The procedure has since become the standard of care for the treatment of symptomatic cholelithiasis and acute or chronic cholecystitis. In fact, the overall rate of cholecystectomy increased rapidly with the introduction of the laparoscopic approach, possibly because of wider patient and physician acceptance of this method of treating

Table 74.4 Therapeutic options for symptomatic gallbladder stones

Therapy	Candidates	Stone clearance (%)	Mortality (%)	Disadvantages
Laparoscopic cholecystectomy	No previous abdominal surgery, normal gallbladder wall	100	< 1	Invasive, requires general anesthesia, possible bile duct injury
Oral bile acids	Patent cystic duct, functioning gallbladder, floating radiolucent stones, < 5 mm in diameter	80–90	0	Delayed stone clearance, possible stone recurrence
Extracorporeal shock-wave lithotripsy with oral bile acids	Patent cystic duct, functioning gallbladder, solitary radiolucent stones up to 20 mm in diameter	70–90	< 0.1	Delayed stone clearance, possible stone recurrence
Contact dissolution with methyl tert-butyl ether	Radiolucent stones, gallbladder attached to liver, patent cystic duct	50–90	Limited experience	Invasive, bile leakage (5%), stone recurrence

gallstones [240]. There is also some evidence that the indications for cholecystectomy have broadened since introduction of the laparoscopic approach [241]. Due in part to the rapid acceptance of the procedure by patients and physicians, there have been few randomized, controlled trials comparing laparoscopic with open cholecystectomy [242,243]. However, publications compiling results for more than 5000 patients have documented its safety and efficacy [244]. Retrospective comparative studies have shown that laparoscopic cholecystectomy produced fewer complications, shorter hospital stays, more rapid returns to normal activities, and minimal use of postoperative analgesia [245–247]. Additionally, overall mortality appears to be lower [248].

The technique involves general anesthesia with endotracheal intubation. A pneumoperitoneum is established, and the laparoscope with video camera attached is inserted through an umbilical incision. Accessory trocars and cannulas are inserted through separate sites. The gallbladder, cystic duct, and cystic artery are identified. An intraoperative cholangiogram can be performed by cannulation of the cystic duct, allowing the detection of common bile duct stones and delineation of the ductal anatomy. The blood supply to the gallbladder is identified and controlled, and then the cystic duct is ligated. The gallbladder is dissected from the underlying liver bed, using electrocautery or laser energy. The gallbladder is removed through the incision site. Stones may need to be mechanically crushed within the gallbladder before removal. Stones spilled into the peritoneal cavity are retrieved [249].

The primary indication for laparoscopic cholecystectomy is identical to that for open cholecystectomy: symptomatic cholelithiasis. Absolute contraindications include an inability to tolerate general anesthesia and uncontrolled coagulopathy. Relative contraindications include scarring or inflammation, precluding access to the gallbladder using the transperitoneal approach, and diffuse peritonitis. As surgeons have accumulated more experience, the technique has been applied to a variety of patients. The procedure has been successfully performed in anticoagulated patients, obese patients, children, and pregnant women prior to the third trimester [250–253].

Examination of the results of large series illustrates the pertinent aspects of the procedure. An early analysis of 1518 laparoscopic cholecystectomies performed at academic and private hospitals was published [254]. In 4.7% of the patients, the procedure was converted to open cholecystectomy, most often because of difficulties in identifying anatomy as the result of inflammation or bile duct injury. Of the 1518 patients in this series, 96.5% had ultrasound examinations before surgery to document cholelithiasis, and preoperative cholangiography was performed in 4.2% of patients. Intraoperative cholangiography was used routinely by some surgeons and selectively or not at all by others. Intraoperative cholangiograms were obtained in 29.3% of patients, with a failure rate of 23.3%.

Complications, including delayed discharge, undesired change in therapy, readmission, biliary injury or leakage, infection, hemorrhage, or conversion to open cholecystectomy, occurred in 5.1% of cases. Superficial wound infection was the most common complication. Injuries to the common bile duct or the hepatic duct occurred in 0.5%. Similar to other groups, the rate of bile duct injury was higher early in a given surgeon's experience with the procedure, illustrating the steep learning curve associated with the procedure. The mean hospital stay was 1.2 days, with a range of 6 h to 30 days. One patient died [228,244].

There is some debate about the appropriate use of intraoperative cholangiography at the time of laparoscopic cholecystectomy. Some argue for routine operative cholangiography to look for unsuspected common bile duct stones and to identify ductal anatomy, anomalies, and injuries. Intraoperative cholangiography is usually safe and successful, and appears to reduce the risk of bile duct injury [255,256]. Others argue for a more selective approach, using preoperative clinical, and ultrasonographic assessment to predict the likelihood of common duct stones. A history of jaundice or pancreatitis, dilated common bile ducts on ultrasound, and elevated levels of bilirubin, aminotransferases or alkaline phosphatase can be used as an indication for preoperative ERCP with possible sphincterotomy or intraoperative cholangiogram [245,257]. Preoperative ERCP and sphincterotomy does not interfere with laparoscopic cholecystectomy or increase the operative complication rate [258]. Statistical models to predict which patients have choledocholithiasis, and therefore might benefit from preoperative ERCP or intraoperative cholangiogram, have been developed [259,260].

If choledocholithiasis is discovered intraoperatively, several options are available. The surgeon may perform a common bile duct exploration with either laparoscopic or open access with transcystic duct retrieval of stones. Bile duct exploration can also be carried out using laparoscopic or endoscopic techniques [261]. In experienced hands, laparoscopic bile duct exploration appears to have high success rates (75%–91%) [262,263]. It also has the potential advantages of using only one procedure for treatment of these patients and avoiding the delay inherent in postoperative ERCP. For those patients in whom the procedure is not successful, postoperative ERCP with sphincterotomy remains an option. The modalities of therapy in this area are evolving rapidly, and the perioperative approach to common bile duct stones should depend on local availability and expertise.

A serious direct complication of laparoscopic cholecystectomy is injury to the common bile or hepatic ducts. This may result from misidentification of the common duct for the cystic duct, resection of part of the common and hepatic ducts, or associated right hepatic arterial injury. The estimated incidence of bile duct injury varies from 0.3%–2.7% [264]. In contrast, biliary tract injuries are estimated to occur in 0.25%–0.5% of open cholecystectomies [265]. A major

risk factor for bile duct injury is the experience of the surgeon. Bile duct injuries appear to be much more common early in a surgeon's experience with the technique. Other risk factors appear to be the presence of aberrant biliary tree anatomy and the presence of local acute or chronic inflammation [264]. Intraoperative cholangiography may reduce the risk of bile duct injuries [256]. Bile leaks may also develop as the result of displacement of the operative clips on the cystic duct.

Bile duct injury leads to two clinical manifestations: bile leakage into the peritoneum, with resulting abdominal pain and bile peritonitis, or biliary obstruction as the result of partial or complete hepatic or common duct obstruction from ductal ligation or stricture. Patients can present 3–7 days after surgery with fever, abdominal pain, anorexia, ileus, ascites, nausea, or jaundice. Patients with late-onset stricture may present months later with jaundice. Biliary scintigraphy using ^{99m}Tc-IDA can be used to diagnose bile leakage [266]. ERCP with stent placement or sphincterotomy can be used both diagnostically and therapeutically. ERCP appears to be the treatment of choice for less severe lesions, such as minor lacerations of the common bile duct [264, 267]. It may also be used to treat bile leakage because of displaced cystic duct clips. Lesions in the proximal biliary tree may be more amenable to percutaneous transhepatic approaches [268]. Surgical repair may be necessary in some patients, such as those with transected common bile ducts [264,268–270]. Intraabdominal bile collections may need to be drained percutaneously.

Certain clinical scenarios remain controversial, such as the management of patients with acute cholecystitis; in particular, the timing and route of cholecystectomy. Although laparoscopic cholecystectomy has been performed successfully in acute cholecystitis, this group of patients has a higher incidence of common bile duct stones, and the procedure may be technically more difficult and last longer [271]. In this setting, intraoperative cholangiograms may be more difficult to obtain and bile duct injuries more common. Randomized trials comparing early surgery (< 3 days) with delayed surgery (4–6 weeks) for acute cholecystitis show no benefit to delaying surgery [272–274]. Patients in the early surgery group had longer operating times, but they had similar rates of conversion to open cholecystectomy and shorter hospital stays. Laparoscopic cholecystectomy appears to provide similar outcomes compared to open cholecystectomy in this setting [275]. Early cholecystectomy for acute cholecystitis, initially using a laparoscopic route, may therefore be appropriate.

Conversion to an open cholecystectomy should be undertaken if the operative field is not adequately visualized or variant anatomy is encountered. Conversion to an open procedure should not be considered a complication. In most series, conversion rates are higher with emergency operations. Reported rates range from 1.5%–15%, with most reporting rates around 5% [228]. With experience, the operative conversion rate appears to fall.

Many studies suggest that the indications for cholecystectomy are broader with the availability of laparoscopy [241]. Cholecystectomy may be performed in some patients whose symptoms are not clearly biliary in origin. In contrast, some patients with symptomatic gallstones may not receive surgery in a timely fashion. Glasgow and colleagues found that 1.3 per 1000 people underwent cholecystectomy for symptomatic gallstone disease in California in 1996 [276]. Of these, 56% had biliary colic, and the remainder had more severe complications such as acute cholecystitis, gallstone pancreatitis, or cholangitis. Patients presenting with biliary colic were more likely to undergo laparoscopic surgery electively, while those with the more severe complications were more likely to require an emergency or open procedure. In many patients, surgery was significantly delayed after the development of initial symptoms. Patients being treated for complicated gallstone disease had significantly longer hospital stay and costs. There is evidence for both underuse and overuse of laparoscopic cholecystectomy. Clarifying the appropriate indications for surgery and ensuring timely recognition and treatment of gallstone symptoms remain as priorities to improve clinical care of these patients.

Endoscopic treatment

Stones may be removed from the common bile duct by means of ERCP with sphincterotomy. Larger stones may require mechanical lithotripsy. In the postoperative period, endoscopic and radiographic techniques may be used to extract stones through a T-tube tract. Stones may be extracted or fragmented with the help of a choledochoscope. In all of these modalities, the gallbladder is left in situ, allowing the possibility of recurrent stone formation. Patients undergoing sphincterotomy alone for treatment of their gallstone disease have a much higher rate of future biliary complications than patients who also undergo cholecystectomy [277,278]. Therefore, cholecystectomy should be considered in all patients who have common bile duct stones even if a sphincterotomy is performed.

Medical dissolution

With the advent of laparoscopic cholecystectomy, medical therapy for gallstone disease has fallen out of favor. These therapies are limited to patients who are not operative candidates.

Oral bile acid litholysis

Two bile acids, chenodeoxycholic acid [3α,7α-dihydroxycholanoic acid (CDCA)] and ursodeoxycholic acid [3α,7β-dihydroxycholanoic acid (UDCA)], have been used for gallstone dissolution [279–284]. These two bile acids induce the secretion of undersaturated hepatic bile and do not increase the absolute amount of bile acids secreted. The

biological effects of administration of CDCA and UDCA include suppression of de novo hepatic cholesterol synthesis by inhibiting HMG-CoA reductase and enhancement of the activity of 7α-hydroxylase, leading to an increase in bile acid synthesis [285,286]. Intestinal absorption or reabsorption of cholesterol is down-regulated [287].

CDCA has more associated side effects and a lower success rate in dissolving gallstones so UDCA, the major bile acid of the Himalayan black bear, is the bile acid of choice for gallstone dissolution. UDCA is a remarkably safe compound. The usual dose is 8 mg/kg per day to 12 mg/kg per day. The proper selection of patients is a major factor affecting the success of bile acid therapy. The selection criteria include gallstones predominantly consisting of cholesterol; pigment stones or calcified stones are excluded. Small stones that are buoyant in an oral cholecystogram examination have a 90% chance of dissolution. Stones should have a large surface area in relation to their mass, and those with a diameter larger than 1.5 cm are not suitable. Patency of the cystic duct should be documented by oral cholecystogram or hepatobiliary scanning.

Extracorporeal shock-wave lithotripsy

Energy generated externally can be directed to fragment gallstones. This method of treatment combines two approaches: oral bile acid therapy and fragmentation of gallstones. Extracorporeal shock-wave lithotripsy (ESWL) requires accurate localization of the gallstones within the gallbladder, which is usually achieved by three-dimensional ultrasonography. The source of energy varies with different methods. Fragmentation of a gallstone into small fragments (< 3 mm) results from successful ESWL. Depending on the type of lithotriptor, one to several sessions of ESWL may be needed before optimal fragmentation can be achieved. The remaining gallstone fragments can pass into the duodenum spontaneously or can be rapidly dissolved by the oral bile acids. UDCA, usually given before and concurrently with ESWL, is continued for several months until the stones have completely disappeared [288].

If patients are properly selected, ESWL is effective and safe [289]. Selection criteria of patients for ESWL are similar to those listed for oral bile acid therapy. Single, small cholesterol stones can be successfully fragmented in more than 90% [290–292]. However, patients with larger or multiple stones have a lower success rate of 34%–71% [293].

About 20% of patients develop biliary colic for several weeks after ESWL (25% of these patients require cholecystectomy), and 1% develop pancreatitis [290,291,293]. Other side effects include local discomfort, petechiae or bruising, transient gallbladder dysmotility, and microscopic hematuria. ESWL is used in the United States mostly for problematic common bile duct stones that are not cleared by ERCP with sphincterotomy [294].

Contact solvents

When gallstones can be accessed by a catheter, solvents can be delivered directly to dissolve the stones. The most commonly used agent is methyl tetrabutyl ether (MTBE). To dissolve gallstones, a catheter is introduced into the gallbladder by a percutaneous transhepatic approach, and MTBE is introduced and withdrawn from the catheter [295,296]. Dissolution can be achieved within a few hours [297]. In well-selected patients in specialized centers, this method is 90% successful and safe. Complications include those caused by the percutaneous puncture procedure and side effects if the MTBE drains into the duodenum (e.g., hemolytic anemia, erosive or hemorrhagic duodenitis, aspiration pneumonia, somnolence). Small cholesterol gallstones without a thick calcified rim are suitable for this therapy. Calcified or pigment gallstones, cirrhosis of the liver with portal hypertension, and coagulopathy are contraindications.

Rational approach to treating symptomatic gallstones

Surgery remains the definitive curative method of gallstone treatment. The advent of laparoscopic cholecystectomy and its widespread acceptance has revolutionized surgical treatment of gallstones. Laparoscopic cholecystectomy is regarded as the treatment of choice for symptomatic gallstones. For patients unable or unwilling to pursue this route, nonsurgical methods such as oral bile acid therapy or lithotripsy are available (Fig. 74.7).

When stones are removed but the gallbladder is left behind, stones may recur. With oral bile acid therapy, the cumulative recurrence rate is about 15% in 2 years and 50% in 10 years [298, 299]. Patients with multiple stones have higher recurrence rates than those who have solitary stones. The long-term recurrence rate after ESWL is also high, 15% at 1 year and 60% at 5.5 years [56]. It is not generally agreed whether long-term, low-dose UDCA can protect against recurrence.

To our knowledge, no formal cost comparison has been made between surgical and nonsurgical treatment. Surgery has a high immediate cost and low long-term cost. Nonsurgical means of treatment may have a low immediate cost but they have high long-term cost because of recurrent or persistent disease. In selecting patients for nonsurgical treatment, a diagnosis of gallstones by ultrasonography is insufficient. The physician must also determine gallstone composition and gallbladder function. Regular monitoring by ultrasonography during nonsurgical treatment may be necessary. These additional tests increase cost.

In approaching a patient with symptomatic gallstones, an elective cholecystectomy remains the gold standard. ERCP may be required to remove common bile duct stones or for management of complications after cholecystectomy. For patients who cannot or will not have surgery, the preferred form of medical treatment is oral bile acid dissolution in appropriate candidates.

Acalculous cholecystitis

Acute cholecystitis can present as an acalculous disorder in 5%–10% [300,301]. This entity is distinct from gallstone-related disease in that it usually occurs in the setting of major surgery, critical illness, trauma, or burn-related injury. The patients tend to be predominantly male and older than 50 years of age. Many of them are on TPN, and bile inspissation or sludge formation may occur. Hypotension and sympathetic vasoconstriction may contribute, predisposing the patient to ischemic injury. Extensive small vessel occlusion has been shown in cholecystectomy specimens from these patients [302]. Another postulated risk factor is mechanical ventilation with positive end-expiratory pressure [300]. The positive end-expiratory pressure may decrease portal perfusion and increase resistance in the common bile duct, with subsequent stasis [303,304]. The pathogenesis of acalculous cholecystitis probably involves some combination of bile stasis, chemical inflammation, and mucosal ischemia. Gallbladder ischemia may be the only predisposing factor found in many cases. Bacterial infection, usually by gram-negative bacteria and anaerobes, appears to be a secondary phenomenon.

In acalculous cholecystitis, complications such as gallbladder gangrene and perforation, emphysematous cholecystitis, or empyema, may develop more rapidly than in calculous cholecystitis [300]. One retrospective study revealed that 70% of patients had gangrene, empyema, or perforation of the gallbladder evident at the time of surgical exploration [305]. This increased complication rate that occurs in the setting of advanced age and other illnesses contributes to the higher mortality rate associated with this disorder. For these reasons, it is important to suspect and pursue an early diagnosis. It should be suspected in patients who are critically ill with signs of sepsis such as fever or leukocytosis, but with no obvious source. Findings on physical examination and laboratory studies are not necessarily helpful. Potential diagnostic modalities include ultrasonography, CT, hepatobiliary scintigraphy, and laparoscopy.

Ultrasonography is usually the initial modality chosen. Criteria for acalculous cholecystitis include a thickened gallbladder wall (> 4 mm), pericholecystic fluid, subserosal edema without ascites, intramural gas, sloughed mucosal membranes, or a sonographic Murphy sign. Reported sensitivity ranges from 67%–92%, with specificity over 90% [306]. Experience with CT is also favorable. Diagnostic criteria are similar to ultrasonography. The sensitivity and specificity of CT for acalculous cholecystitis are over 95% [306]. Another advantage of CT is the ability to look for other intraabdominal pathology. Results with hepatobiliary scintigraphy are variable, with reported sensitivity rates ranging from 68%–91% and frequent false-positive results. Adjunctive use of morphine to enhance gallbladder filling may improve the accuracy of this test [307]. Patients in whom the diagnosis is uncertain or with generalized peritonitis may benefit from diagnostic laparoscopy or exploratory laparotomy. In small series of patients, diagnostic laparoscopy appears to be accurate and safe [308,309]. Some suggest empiric percutaneous cholecystostomy if the diagnosis is suspected but cannot be confirmed with other modalities [310].

Rare causes of acute cholecystitis include specific infections, such as *Salmonella* or *Candida* [311,312]. Cytomegalovirus and cryptosporidia can infect the biliary system and produce cholecystitis and cholangitis in severely immunocompromised patients, such as those with acquired immunodeficiency virus (AIDS) or after bone marrow transplantation [313,134]. In patients with AIDS, infection with microsporidia such as *Enterocytozoon bieneusi*, *Pneumocystis carinii*, and *Isospora belli* have been documented [315]. However, acute calculous cholecystitis may also occur in these patients. No specific symptoms can differentiate these two, and patients may have coexisting gallstones and opportunistic infection. In these patients, acalculous cholecystitis probably represents part of the spectrum of AIDS-related cholangiopathy.

Once a diagnosis of acalculous cholecystitis is made, the gallbladder must be drained or removed. Choosing the treatment modality may be difficult as patients often have severe comorbid conditions. Options include percutaneous or surgical cholecystostomy, surgical cholecystectomy, or endoscopic transpapillary biliary drainage [310,316–318]. Percutaneous cholecystostomy has been gaining favor. Its advantages include the use of local anesthesia and the ability to be performed at the bedside. Also, percutaneous cholecystostomy can be the definitive therapy in acalculous cholecystitis. The overall success rate is 63%–94% with a low complication rate of approximately 10% [319]. Complications include liver laceration, hemorrhage, infection, hemobilia, bile leak, bile peritonitis, and catheter dislodgment. Other disadvantages of cholecystostomy include a limited ability to look for other causes of sepsis and potentially inadequate drainage in cases of pericholecystic abscess, gangrene, or gallbladder perforation. Surgical cholecystostomy or cholecystectomy is the traditional therapy, but patients may be poor operative candidates. Endoscopic transpapillary drainage has been reported in small series and has not gained widespread acceptance [316].

Recurrent pyogenic cholangitis

Recurrent pyogenic cholangitis, also known as Oriental cholangiohepatitis or hepatolithiasis, is a syndrome seen primarily in southeast Asia including Taiwan, China, Japan, and Korea. Patients present clinically with recurrent attacks of abdominal pain, fever, and jaundice. Recurrent pyogenic cholangitis was once among the leading causes of acute abdominal emergencies in Hong Kong [320]. However, the

prevalence of intrahepatic stones is likely decreasing in many parts of Asia [321].

Primary intrahepatic stones are generally calcium bilirubinate or mixed stones. Intrahepatic calcium bilirubinate stones contain less bilirubin and more cholesterol than pigment stones in the extrahepatic bile ducts [322]. The pathogenesis of these stones is complex, and likely involves a combination of bile infection, malnutrition or dietary factors, biliary stasis, and possibly parasitic infestation. It is generally believed that bacterially mediated hydrolysis of bilirubin by β-glucuronidase is crucial to the formation of pigment stones. This leads to the formation of unconjugated bilirubin, which may precipitate as calcium bilirubinate. The β-glucuronidase enzyme often originates from *E. coli* or other bacteria in the biliary tree. There has been a debate about whether bacterial infection precedes or is a sequel of stone formation. However, in at least some patients with bilirubinate stones, bile infection precedes stone formation [162]. β-Glucuronidase can be inhibited by glucarolactone, whose levels are decreased in patients with a low protein and low fat diet [323]. It has therefore been suggested that dietary factors contribute to the formation of intrahepatic stones. The role of infection by parasites such as *Ascaris lumbricoides* or *Clonorchis sinensis* has been controversial. The parasites or their ova have been found as niduses for intrahepatic stones in earlier studies [322]. However, infection with these parasites is widespread, but recurrent pyogenic cholangitis is uncommon outside southeast Asia. Even within endemic countries, there is not a direct correlation between the prevalence of parasitic infection and the occurrence of this syndrome. These lines of evidence suggest that factors in addition to parasites are important for the development of these stones.

Recurrent pyogenic cholangitis frequently presents in patients less than 50 years of age. Men and women are affected equally. It is more common with lower socioeconomic status and malnutrition. Symptoms occur when the stones cause partial or complete biliary obstruction, either with or without associated infection. Many patients will present with epigastric or right upper quadrant pain or discomfort. Acute cholangitis, with fever, right upper quadrant pain, and jaundice, is also common. Physical examination may show right upper quadrant tenderness, hepatomegaly, or an enlarged gallbladder. Laboratory results are generally consistent with the degree of biliary obstruction and infection. In advanced cases, biliary cirrhosis and its sequelae may develop.

Recurrent pyogenic cholangitis is often diagnosed clinically in endemic areas. Delineating the extent and location of stones and strictures is essential to planning further treatment. Abdominal plain films are rarely useful as the stones are usually radiolucent. CT scan may be useful to assess ductal dilation. Percutaneous or endoscopic retrograde cholangiography provides the most accurate information. The left intrahepatic duct is preferentially involved. Frequent findings on cholangiography include stones, biliary strictures, and ductal dilation. The intrahepatic ducts may also appear "pruned" with decreased arborization [324]. Histologically, increased fibrous tissue around the portal tracts may be found with bile ductular proliferation.

Surgery is the primary method of treatment for this syndrome. The role of endoscopic treatment is relatively limited because of the difficulty in treating intrahepatic strictures and stones. Surgery should aim to remove as many stones as possible and to achieve adequate drainage of obstructed ducts [324]. In rare cases, hepatic resection can be undertaken if the stones and strictures are localized. If strictures cannot be managed operatively, many surgeons will create a Roux-en-Y choledochojejunostomy with the end of the loop left in the subcutaneous abdominal wall tissue [325]. This allows easier access for later endoscopic or interventional radiological treatment.

Interventional radiology techniques are also important in the management of recurrent pyogenic cholangitis. If percutaneous transhepatic drainage can be established, cholangioscopy can be used to direct attempts at electrohydraulic or laser lithotripsy, stricture dilation, and stone clearance [326]. Solvents such as MTBE are ineffective, because the stones are not cholesterol stones. Many patients will require a combined surgical and interventional radiological approach to achieve adequate drainage and stone clearance.

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F GALLBLADDER AND BILIARY TRACT

75 Primary sclerosing cholangitis and other cholangiopathies

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Primary sclerosing cholangitis, 1978
Variant primary sclerosing cholangitis syndromes, 1992

Primary sclerosing cholangitis

Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease characterized by inflammation and fibrosis of the intrahepatic and extrahepatic bile ducts. Histological characteristics include the presence of portal tract inflammation, periductal fibrosis, and loss of small bile ducts. Disease progression is almost universal among subjects, resulting in biliary cirrhosis, hepatic failure, and possibly cholangiocarcinoma. Immunological and genetic alterations have been cited as factors in its pathogenesis [1,2]. Understanding the natural history of PSC has become important because of the success of liver transplantation as an effective therapeutic modality.

Epidemiology

With the increasing availability of endoscopic retrograde cholangiopancreatography (ERCP), a growing body of knowledge about the clinicopathological features of PSC has emerged. Fifty to seventy percent of affected individuals are male with an average age at diagnosis of 40 years [3–10]. The male predominance seen in PSC has not been observed in inflammatory bowel disease (IBD) or similar autoimmune liver diseases such as primary biliary cirrhosis (PBC) and autoimmune hepatitis (AIH). However, a significant proportion of female subjects have been reported among select cohorts [8,9]. The suggestion of a reduced survival outcome from PSC in female subjects, however, has yet to be confirmed [9].

The close association between PSC and IBD, however, is well established. Among northern Europeans, 70%–80% of PSC patients have chronic ulcerative colitis (UC) [11–14]. Lower prevalence rates of IBD in PSC, however, have been noted elsewhere [15,16]. These wide variations appear to be related to influences that are not explained by clinical practice. The use of surveillance colonoscopy with mucosal biopsy assessment, while improving the detection of subclinical IBD, is nonetheless required if accurate prevalence estimates for IBD in PSC are to be determined. No meaningful difference in PSC-related survival, however, has been observed among patients with or without IBD [9]. Ulcerative pancolitis or colonic involvement alone by Crohn's disease imparts the greatest risk for PSC [10,11]. Conversely, about 2.4%–4% of patients with UC [10,12–14] and 1.4%–3.4% of patients with Crohn's disease [11,14] either have PSC or develop PSC. From descriptions in the general population there is an estimated prevalence rate of 13–140 cases of PSC per 1 million persons [10]. Population-based epidemiological investigations of PSC have revealed annual incidence and cross-sectional prevalence rates of 0.9–1.3 and 8.5–20 per 100 000 population, respectively [17–19]. However, PSC has been reported at lower rates in geographic areas outside northern Europe and the United States, using less well-defined methods [20,21]. The accuracy of prevalence data is limited by several factors including the failure to identify asymptomatic patients, those with fluctuations in liver biochemical parameters such as serum bilirubin, and those without coexisting IBD. From an estimated prevalence rate of 40–225 cases per 100 000 US population for UC, the prevalence of PSC is estimated to be between 2 and 11 cases per 100 000 persons [10].

A Swedish investigation into the familial occurrence of PSC revealed a prevalence of 0.7%, which represents a 100-fold increased risk of developing PSC in first-degree relatives of patients with PSC. Ongoing investigations into the genetic factors are discussed later in this chapter [22]. An English study found an increased prevalence of PSC in patients with underlying celiac disease compared to the normal population [23]. Whereas a negative relationship between smoking history and UC activity is well established [24], similar investigations

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have also been conducted in PSC. By comparing patients with UC with age-matched and gender-matched controls, a lower prevalence rate of PSC (4.9%) was observed among individuals who smoked compared with nonsmoking controls (26.1%) [25].

Pathogenesis

Progress in understanding the pathophysiology has been slowed by the lack of an animal model for PSC. Several potential animal models exist that exhibit similar immunological and genetic susceptibility to PSC, but none fully represents the human form of the disease. Genetic and immunological alterations as well as nonimmunological features have been cited as factors in the pathogenesis of PSC as well as in disease progression.

Genetics

As previously mentioned, PSC is 100-fold more common in first-degree relatives [22], suggesting genetic inheritance, but it does not follow classic genetic (mendelian) inheritance patterns. To date, no specific autoantigen has been elucidated.

Normal biliary cells express human leukocyte antigen (HLA) class I (A, B, and Cw) but not class II (DR, DQ, and DP) molecules, but class II molecules appear to be aberrantly expressed on the biliary cells of PSC patients [26,27]. An increased prevalence of HLA-A1, HLA-B8, and HLA-DR3 alleles in PSC has been described but remains nonspecific given the occurrence of these alleles in other organ-specific autoimmune diseases [26]. Greater than expected frequencies of the HLA-DRw52a antigen and HLA-DRB3*0101 were initially reported among 100% of patients undergoing liver transplantation at a US center [28]. Subsequent investigations have not supported the DRw52a allele as an important susceptibility marker for disease development [29]. HLA-DRB3*0101 appears to be the most common susceptibility locus in 55%–65% of PSC patients [30] and may be universal in end-stage liver disease from PSC [13]. In Scandinavia, however, the HLA alleles DRB1*0301 and DRB1*1301 have been reported as the primary susceptibility loci in PSC [31]. Of potential significance is the reported association between DRB3*0101 and reduced survival among subjects with PSC [29]. Rapid disease progression has also been suggested with the existence of HLA-DR4 where a protective effect was once previously proposed [32]. A multicenter investigation found the HLA-DR3,DQ2 heterozygous genotype associated with more rapid disease progression in PSC whereas a protective effect was linked to HLA-DQ6 [33]. Overall these genetic associations with PSC are complex and poorly understood.

A number of non-major histocompatibility class candidate genes may also be related to the immune dysregulation in PSC. Serum cytokines including interleukin-1 (IL-1) and IL-10 have been examined as inflammatory response mediators in PSC. No association between IL-1 and IL-10 gene polymorphisms with susceptibility or resistance to PSC has been observed [34,35]. Major histocompatibility class I chain A (MICA) and chain B (MICB) genes have also been studied in PSC. MICA5.1 (90% vs 74%) and MICB24 (58% vs 29%) were significantly increased among PSC patients compared with controls [36]. The frequency of PSC patients carrying all four alleles was also significantly greater than controls (49% vs 18%). Between two independent PSC populations [37], the MICA*008 allele was more common in PSC than in controls (66% vs 48%) whereas the MICA*002 allele had a protective effect from PSC. A chemokine receptor (CCR5) is expressed on T cells and macrophages. A 32-basepair deletion in the chemokine receptor 5 gene (CCR5-Delta32) leads to a nonfunctional receptor. Conflicting evidence exists whether this deletion is associated with PSC. Although one study [38] notes a higher rate of this defect in PSC and a relation to severity of disease, the most evidence suggests that there is no association [39,40]. Matrix metalloproteinases (MMPs) regulate fibrosis production. A common polymorphism in the promoter sequence of stromelysin (MMP-3) results in increased transcription. The influence of a matrix metalloproteinase-3 (MMP-3) functional polymorphism may influence susceptibility and disease progression in PSC as demonstrated in one study [10], but not confirmed in another [41]. Intercellular adhesion molecule-1 (CD54) gene polymorphisms have been implicated in the susceptibility to a range of inflammatory diseases, including IBD, but have not been clearly associated with PSC [42,43].

Immune-mediated

Evidence that PSC may be immune mediated is circumstantial, and largely because of disease associations with other autoimmune processes [44]. Cellular immune abnormalities have been described focusing primarily on the significance of lymphocyte function in PSC. CD4 T-lymphocyte recognition of antigens found on biliary epithelia is hypothesized to initiate the immune-mediated histological damage seen in PSC [30,45]. T-cell reactivity studies have demonstrated the activation of both suppressor and cytotoxic lymphocyte populations with exposure to major histocompatibility antigens in vitro [45]. A recent study using freshly isolated liver-derived T cells from PSC patients showed that cell proliferation and the function of these T lymphocytes are impaired in PSC patients compared with normal controls and other patients with autoimmune liver diseases [46]. The study demonstrated an inability to detect any normal cytotoxic activity among liver-derived T cells and natural killer cells from these patients, which was thought to be a consequence of exposure (maybe even chronic) to high levels of tumor necrosis factor-α (TNF-α) in vivo. The study also showed that these liver-derived lymphocytes from PSC patients showed increased expression of intracellular TNF-α. Interestingly, the addition of anti-TNF antibodies restored the diminished proliferative responses of the T cells in vitro [46]. Bernal and colleagues [47] studied the association of TNF polymorphism with PSC and concluded

that genetic susceptibility to PSC may be determined by polymorphism within the TNF genes, particularly with the TNF2 allele. The increased numbers of hepatic B-cell lymphocytes reported among PSC patients with advanced histological disease are not found with earlier stages of disease, suggesting that cellular infiltration may be a secondary phenomenon related to chronicity [48]. In a Swedish study, anti-biliary epithelial cell antibodies were demonstrated in 63% of PSC patients; in 90% of these patients the autoantibodies reacted only with cytokine-stimulated target cells. Interestingly, the antibody-positive PSC patients had either the HLA-DRB1*0301 or HLA-DR2 allele (see below) compared with those without ($P = 0.007$) [49]. These studies suggest a coordinated inflammatory response that implicates both the cellular and humoral arms of the immune response in the pathogenesis of PSC. Several potential animal models exist that exhibit similar immunological and genetic susceptibility to PSC, but none fully represents the human form of the disease.

A number of clinical investigations have also confirmed the presence of numerous humoral immune markers in the sera of patients with PSC. Non-organ-specific markers such as immunoglobulins G and M, antinuclear antibody, and anti-smooth muscle antibody are often found at low but detectable titers [2,50]. Their relevance in the pathogenesis of PSC may occur among patients displaying clinical features of AIH and PSC. Elevated levels of circulating immune complex molecules have also been reported in up to 80% of PSC patients and other HLA-B8-DR3-associated immune-mediated diseases [50,51]. Selected cross-reactive antibody species among patients with UC and PSC are not seen in patients with PSC alone, suggesting a shared mechanism for immune-mediated damage [52]. Perinuclear antineutrophil cytoplasmic antibody (pANCA) has been reported in 26%–85% of patients with PSC and in 70% of UC patients without PSC [53–55]. These are "atypical" pANCAs and are distinct from the pANCA of microscopic polyangiitis, with the target antigen possibly a neutrophil nuclear envelope protein. In fact, because it is not a cytoplasmic protein, the term peripheral antineutrophilic nuclear antibody (pANNA) has been suggested [56]. The antigenic and subsequent potential pathogenic role of pANCA, however, is unknown [57]. Seropositivity for pANCA in PSC has been reported with extensive involvement of the biliary tree, hepatic dysfunction, and severe cholestasis; titers tend to remain unchanged after liver transplantation [58]. Antibodies to *Saccharomyces cerevisiae* have been observed in PSC, but they appear to be of limited clinical value [59]. Little progress has been made in elucidating the immunological mechanisms of this disease.

Nonimmune-mediated

The primary target of immune-mediated injury in PSC is the biliary epithelium. Increased expression of class II major histocompatibility complex molecules on biliary epithelial cells in PSC in support of their role as antigen-presenting cells remains controversial.

The strong association between PSC and IBD suggests that an infectious etiology may be present in specific individuals [60]. Alterations in the mucosa of diseased bowel facilitating portal bacteremia may be responsible. Increased levels of TNF have been found in animal models linked to portal bacteremia and the resulting activated hepatic Kupffer cells [61]. Clinical evidence in humans, however, is noted for the relative lack of significant colonic inflammation in both patients with PSC and patients after total proctocolectomy for refractory UC [62]. Eksteen and colleagues have shown that T cells activated in the gut are recruited to the liver by aberrant expression of gut-specific chemokines, and that these lymphocytes can persist for years potentially explaining the delayed onset of PSC despite colectomy in some patients [63]. Bjornsson et al. were unable to demonstrate a role for small bowel bacterial overgrowth and increased intestinal permeability in the pathogenesis of PSC [64]. As with primary biliary cirrhosis, the role of *Helicobacter* sp. infection in developing PSC has been examined. The significance of an increased rate of *Helicobacter* sp. DNA in patients with PSC (especially those with UC) compared to those with non-cholestatic liver disease requires further study [65]. An increased rate of α-hemolytic streptococci from explanted liver tissue [66] and bile [67] has been demonstrated in PSC. Among PSC patients with or without previous ERCP, α-hemolytic streptococci were the most frequent isolates despite similar positive culture rates. The impact of α-hemolytic streptococci on etiopathogenesis or disease progression in PSC remains unknown. Infections with viruses such as cytomegalovirus among immunocompromised hosts can result in PSC-like changes [68].

Hepatic histological features of cystic fibrosis can resemble PSC, but gene analysis (*CFTR* gene) did not demonstrate higher gene carriage rates in patients with PSC [69]. Immunodeficiency syndromes have been identified with cholangiographic changes suggestive of PSC including, familial immunodeficiency syndrome, X-linked inmunodeficiency, angioimmunoblastic lymphadenopathy, acquired immunodeficiency syndrome (AIDS) without known explanation [70].

Bile acid metabolite hepatotoxicity has been observed in animal models after chenodeoxycholic acid breakdown to lithocholic acid by bacterial 7-α-dehydroxylation in the colon. No major bile acid abnormalities have been identified among human subjects with PSC [71]. A possible role of biliary lipotoxicity, in the sense of deranged and consequently toxic bile composition, has been suggested as a trigger for the development of cholangitis, but it requires further investigation [72]. Biliary tree injury resulting from hepatic artery chemoembolization with fluorodeoxyuridine, 5-fluorouracil, and mitomycin can yield cholangiographic features compatible with PSC [73]. Direct toxicity or ischemia to bile ducts is hypothesized.

Table 75.1 Symptoms and signs at initial presentation in primary sclerosing cholangitis

Finding	Prevalence (%)
Symptom	
Fatigue	75
Pruritus	70
Jaundice	65
Weight loss	40
Fever	35
Asymptomatic	10
Sign	
Hepatomegaly	55
Jaundice	50
Splenomegaly	30
Hyperpigmentation	25
Xanthelasma	4

Clinical manifestations

Asymptomatic elevations in serum liver biochemistries or the development of symptoms such as fatigue (75%) and pruritus (70%) are often observed with the diagnosis of PSC (Table 75.1). Jaundice (65%) and weight loss (40%) are less common and should raise the suspicion of advanced disease or a complication related to PSC [1,2,4,8]. The average duration of symptoms prior to diagnosis is 2 years. Fever and abdominal pain in a patient with PSC is highly suggestive of bacterial cholangitis, which can manifest at initial presentation [74]. A previous history of IBD and elevated serum liver biochemistries often prompts investigations to exclude PSC. Among patients with refractory UC requiring total proctocolectomy, it has been shown that PSC may still develop among these individuals [62].

Asymptomatic primary sclerosing cholangitis

Whereas symptomatic PSC is commonly associated with jaundice or pruritus, an increasing number of asymptomatic cases are being identified. Asymptomatic patients have represented between 15% and 25% of most reported observational cohorts examined [4–6,9,19,75]. Recent reports, however, have observed prevalence rates of greater than 40% for asymptomatic PSC [8,19,76].

Progression of liver disease can also occur among asymptomatic patients but existing data are conflicting. Over a 6-year period, the evidence for advancing liver disease was noted among 34 of 45 (76%) asymptomatic subjects with a 31% rate of liver transplantation or death [75]. Minimal histological abnormalities (stage I–II) on liver biopsy with no cases of cirrhosis were found in the majority of subjects at study entry. Estimated median survival for asymptomatic patients without liver failure was 65%–75% at 7–10 years compared with 96% for comparable age-matched and sex-matched healthy individuals [19,75]. Helzberg and colleagues [4] reported that 11 of 13 asymptomatic individuals remained symptom free for a mean duration of 37 months, resulting in an overall survival rate of 75% at 9 years. Independently, no significant decompensation over a 4.4-year period among 27 asymptomatic subjects has also been described [3].

Despite a silent or subclinical presentation at diagnosis, there is evidence to support that asymptomatic PSC becomes symptomatic. Fatigue, pruritus, jaundice, or abdominal discomfort develops in 60% of patients [4–6,8]. The similar frequencies in clinical deterioration among asymptomatic and symptomatic patients, however, emphasize the lack of predictive ability associated with subjective complaints at diagnosis [4,6]. Differences in criteria for defining the asymptomatic state may explain the discrepancy in published results. Fatigue and weight loss have been reported in up to 30% and 24% of asymptomatic subjects, respectively. In addition, advanced histological features have been demonstrated in 30% to 43% of subjects considered asymptomatic at diagnosis [3,75].

Symptomatic primary sclerosing cholangitis

The majority of subjects in reported investigations have been described as symptomatic based on findings at diagnosis [3–9]. Helzberg and colleagues [4] first reported an 18% mortality rate among patients with PSC over a mean follow-up period of 56 months. All deaths occurred in symptomatic individuals. A similar mortality rate of 14% over 51 months has been observed with a reduced median survival for symptomatic individuals as well (112 months compared with 144 for the entire cohort) [8]. Two larger series have provided evidence to suggest a more aggressive disease course for symptomatic PSC. Among 174 patients with PSC who were followed at the Mayo Clinic [5], a 41% combined rate for liver failure, cholangiocarcinoma, and need for transplantation was observed over 6.25 years of follow-up. Median survival for the entire cohort was 11.9 years. For symptomatic patients with PSC, the median survival was reduced to between 8 and 9 years. Similar results were reported from Kings College [6], where a median survival of 12 years was observed among 126 subjects over a 6.9-year period.

Biochemical features

Serum hepatic biochemical parameters usually reflect a cholestatic profile. Alkaline phosphatase values often predominate with elevations up to three times the upper limit of normal in nearly 95% of patients [1–9]. Serum alanine aminotransferase and aspartate aminotransferase levels can also be found between two and three times higher than the upper limit of normal [3,4,8,9,74]. Serum total bilirubin is normal in 60% of individuals [1,2,74]. Levels of total bilirubin, which often fluctuate in PSC, are of concern for

advanced disease when persistent elevations occur. No significant differences in serum liver biochemistry profiles have been seen among asymptomatic and symptomatic individuals at diagnosis [75]. Normal serum albumin measurements and prothrombin times, which reflect hepatic synthetic function, are found in the majority of patients at diagnosis [3–9].

Serological features

Serum immunoglobulin levels and autoantibodies are associated with PSC. Elevations in immunoglobulin G and immunoglobulin M in up to 40% and 60% of individuals, respectively, are observed [2,7]. Antinuclear antibody and smooth muscle antibody (SMA) can also be detected but are at lower titers compared to those found in AIH [2,7,77]. Antimitochondrial antibodies (AMA) are exceedingly rare in PSC [5,77]. Atypical pANCAs (see Immune-mediated section) are detected in the serum of PSC patients at rates between 30% and 80% [53,54] but they lack specificity because they are also observed in PBC, AIH, and UC [54,78]. Angulo and colleagues [79] have observed a greater rate of seropositivity for antinuclear antibody, anticardiolipin, antithyroperoxidase, ANCA, and rheumatoid factor among patients with PSC compared to control subjects. Ninety-seven percent of PSC patients were found to have one or more autoantibodies whereas 81% were found with three or more. The presence or absence of IBD was not significantly associated with autoantibody history.

Figure 75.1 Cholangiographic features in primary sclerosing cholangitis. Multifocal strictures with intervening saccular dilation ("beads-on-a-string" appearance) of both intrahepatic and extrahepatic bile ducts are shown.

Radiographic features

Cholangiography

The diagnosis of PSC is made by cholangiography. Both ERCP and percutaneous transhepatic cholangiography (PTC) have been employed in a nonoperative setting, but they are invasive. Magnetic resonance cholangiopancreatography (MRCP) is increasingly being used for diagnostic purposes and ERCP and PTC are saved for difficult diagnoses or therapeutic uses. PSC commonly affects the entire biliary tree in a heterogeneous manner. Intrahepatic duct involvement is almost universal among patients with PSC [80]. Only 15%–20% of patients will have hilar duct involvement with sparing of the remaining extrahepatic duct. Segmental fibrosis of both intrahepatic and extrahepatic ducts with subsequent saccular dilation of normal intervening areas results in the characteristic "beads-on-a-string" appearance seen with cholangiography in PSC (Fig. 75.1). Bile duct strictures are of varying lengths but can be up to several centimeters. The serrated appearance of bile duct walls and occasional diverticula are also identified in extrahepatic portions of the biliary tree in 25% of patients [81].

The initial use of MRCP for suspected PSC has been increasingly recognized [82,83]. Among 23 of 73 patients identified with PSC [82], an overall diagnostic accuracy of 90% for MRCP compared to 97% for invasive cholangiography was reported. Comparable diagnostic accuracy has been demonstrated for MRCP compared with ERCP [84] but some still argue that better diagnostic accuracy for extrahepatic strictures is obtained with ERCP [85]. Advantages for MRCP over invasive cholangiography include the visualization of bile ducts proximal to obstructed areas and hepatic parenchymal assessment for features of cirrhosis and portal hypertension or lobar atrophy. The inclusion of subjects with advanced PSC and overestimation of stricture length by MRCP have been observed as potential biases for both investigations.

Cross-sectional imaging

Computed tomography (CT) [86–88] and magnetic resonance imaging (MRI) [88–90] have been used in the evaluation of PSC. Radiological features of PSC detected by cross-sectional imaging include segmental dilation of intrahepatic bile ducts and a paucity of increasing dilation toward the hilum [86]. Gallstones have been identified in up to 25% of patients with PSC and asymmetric thickening of the gallbladder wall independent of cholelithiasis may also be observed [87]. Marked hypertrophy of the right and caudate hepatic lobes and simultaneous atrophy in the remaining liver are often seen in cirrhotic-stage PSC [88]. As observed for other etiologies of cirrhosis, the presence of confluent hepatic fibrosis and resulting regenerative nodule formation may

be confused for hepatocellular carcinoma. With MRI, the presence of abnormal T2-weighted signal intensity from periportal areas is common and correlates with fibrosis [89]. Enlarged areas (greater than 1.5 cm) of abnormal T2 signal within portal areas are considered suspicious for cholangiocarcinoma [90].

New diagnostic procedures being investigated include the use of dynamic three-dimensional biliary scintigraphy (technetium 99 m hepatobiliary iminodiacetic acid single-photon emission CT [HIDA SPECT]) for differentiating between parenchymal and ductal contributions to deranged liver function [91]. Duodenal endoscopic ultrasound may be able to provide additional diagnostic proof for extrahepatic disease, but its clinical utility is uncertain [92]. Although limited by lack of availability, transpapillary cholangioscopy appears to increase the ability to distinguish between malignant and benign dominant bile duct stenoses in patients with PSC [93]. In addition, cholangioscopy detects stones missed by cholangiography in more than 30% of PSC patients referred for dominant strictures or stones [94].

Histological features

Liver biopsy is required for staging disease severity and determining prognosis in PSC. Periductal fibrosis with inflammation, bile duct proliferation alternating with obliteration, and ductopenia constitute the main histological findings [95]. Fibroobliterative cholangiopathy, which is considered the most diagnostic finding for PSC on liver biopsy, is present in only 10% of patients [96]. However, similar findings may occur in varying stages of other conditions such as PBC, AIH, or chronic extrahepatic bile duct obstruction. The histological abnormalities in PSC are classified into four stages. Stage I (portal stage) is associated with a nonspecific inflammation confined to the limiting plate, which surrounds the portal tract; fibrosis is usually not seen at this stage. Stage II (periportal stage) is associated with inflammation extending beyond the limiting plate with expansion of the portal tracts; fibrosis is seen involving the portal and periportal areas. Stage III (septal stage) is characterized by septal or bridging fibrosis spanning the portal tracts in conjunction with bridging necrosis (Fig. 75.2). Stage IV, or cirrhosis, is when complete septal fibrosis in association with evidence for nodular regeneration is present [95].

Differential diagnosis

Table 75.2 lists the differential diagnoses for PSC. The identification of serum hepatic biochemical elevations consistent with cholestasis in a male patient with concurrent IBD is strongly suggestive of PSC. Excluding structural causes for biliary obstruction, including choledocholithiasis, by ultrasonography or cross-sectional imaging is also required. Cholangiography by direct or imaging methods, which reveals the typical features of PSC, is considered the gold standard for diagnosis. Although liver biopsy is not mandatory to confirm the diagnosis of PSC, its performance is helpful for staging and excluding other causes of chronic cholestasis.

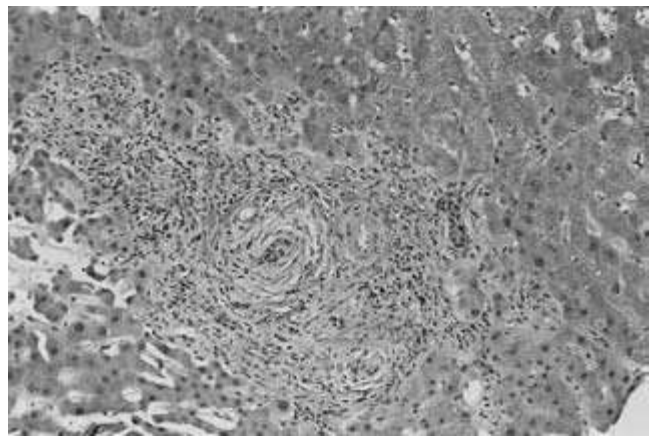

Figure 75.2 Histological features of primary sclerosing cholangitis. Portal tract expansion with chronic nonspecific inflammation, edema, and periportal fibrosis consistent with stage II disease is shown.

Table 75.2 Differential diagnosis of primary sclerosing cholangitis

Extrahepatic biliary tract obstruction
Choledocholithiasis
Strictures
Malignancy
Primary biliary cirrhosis
Autoimmune hepatitis
Autoimmune pancreatitis
Pyogenic hepatic abscesses
Fungal cholangitis
Acquired immunodeficiency syndrome cholangiopathy
Choledochal cyst
Cystic fibrosis
Cirrhosis
Intrahepatic hepatocellular carcinoma
Cholangitis glandularis proliferans
Eosinophilic cholangitis

PBC more commonly affects women than men (with a 9 : 1 ratio) and is rarely associated with IBD. The diagnosis is confirmed by elevated serum titers of AMA. Early histological involvement is characterized by lymphoplasmacytic portal infiltration or granulomatous inflammation ("florid duct lesion") [97]. AMA-negative PBC or autoimmune cholangitis is clinically similar to PSC in the absence of cholangiography. With normal biliary tree visualization, a liver biopsy is required to distinguish this condition from small-duct PSC where involvement with ductular fibrosis is more prominent [98].

AIH is distinguished from PSC by

- predominant elevations in serum aminotransferase levels compared to alkaline phosphatase and bilirubin
- the presence of elevated serum antinuclear antibody or anti-smooth muscle antibody titers

• extensive interface or lobular lymphoplasmacytic inflammation
• normal cholangiography [99].

In children, the initial presentation of PSC often resembles AIH so cholangiography is necessary for accurate diagnosis [100]. Overlapping syndromes between AIH and PSC among pediatric and adult populations have been recognized in 2%–20% of patients based on various definitions [77,101,102]. Cholangiography in these patients, however, must demonstrate features of PSC before consideration of an overlap state is made. There may be potential benefit from corticosteroid therapy for selected individuals.

Autoimmune pancreatitis (AIP) is a disease entity that frequently involves the bile ducts (causing a sclerosing cholangitis, i.e., AIP-SC) creating similar radiographic and histological features to PSC. AIP-SC generally causes stenosis of the distal ducts, but both intrahepatic and extrahepatic changes have been described in up to 50% of patients. Liver biopsy specimens of AIP-SC were indistinguishable the biopsy specimens of patients with PSC. However, AIP is responsive to steroids, whereas PSC is not, which portends a better overall prognosis [103,104].

The development of pyogenic hepatic abscesses with invasion of adjacent bile ducts can result in irregularities that suggest PSC [105]. Frequent attacks of ascending cholangitis in natives of southeast Asia are consistent with a diagnosis of recurrent pyogenic cholangitis (oriental cholangiohepatitis). Cholangiography will show dilation of intrahepatic and extrahepatic ducts, decreased arborization with tapering of peripheral ducts ("arrowhead sign"), and straightening of the intrahepatic ducts from periductal fibrosis [106]. Systemic fungal infection may colonize or invade the biliary tree resulting in the cholangiographic features of PSC [107]. AIDS cholangiopathy presents with striking elevations in serum alkaline phosphatase with or without symptoms of jaundice and abdominal pain. Extrahepatic bile duct dilation and papillary stenosis are most commonly observed. Thickened bile duct walls rather than saccular dilations in AIDS cholangiopathy distinguish this entity from PSC [108].

Choledochal cyst involvement of intrahepatic bile ducts can also resemble PSC. Ultrasonography usually shows marked areas of ductal dilation, and cholangiography is almost invariably diagnostic [109]. Cystic fibrosis, which involves intrahepatic bile duct cystic dilation, can produce a cholangiogram typical of PSC [110].

Diffuse abnormalities of the biliary tree without discrete extrahepatic stricturing may also be observed with conditions such as cirrhosis and intrahepatic hepatocellular carcinoma. In cirrhosis, the presence of distal duct tapering is noted when cholangiography is performed. Cross-sectional imaging and liver biopsy (if indicated) will confirm the presence of cirrhosis or hepatic neoplasia when suspected [111].

Cholangitis glandularis proliferans [112] – a rare disorder similar to adenomyomatosis of the gallbladder – and eosinophilic cholangitis [113] can present with nonstricturing intrahepatic duct dilation resembling sclerosing cholangitis.

Table 75.3 Selected extrahepatic autoimmune diseases associated with primary sclerosing cholangitis

Disease	Prevalence (%)
Ulcerative colitis	70–80
Crohn's colitis	5–8
Pancreatitis	10–25
Diabetes mellitus	5–15
Autoimmune thyroid disease	3–5

Associated conditions

An increased prevalence for concurrent autoimmune disorders has been recognized in PSC but remains less common than in PBC (Table 75.3). UC remains the most commonly associated autoimmune disorder in PSC (70%–80%) [2,5–8,10–14] with colonic involvement from Crohn's disease occurring in 5%–8% of patients with PSC [11,14]. Other diseases include pancreatitis (10%–25%) [114,115], diabetes mellitus (5%–15%) [116], and autoimmune thyroid disease (3%–5%) [116]. Rare associations include Sicca syndrome [115], immune thrombocytopenic purpura [117], mediastinal or retroperitoneal fibrosis [118,119], pseudotumor of the orbit [119], Peyronie disease [120], rheumatoid arthritis [121], ankylosing spondylitis [122], Hodgkin disease [123], membranous nephropathy [124], glomerulonephritis [125], autoimmune hemolytic anemia [126], celiac disease [127,128], histiocytosis X [129], hypereosinophilia [113], focal nodular hyperplasia [130], and Down syndrome [131].

Disease-related complications

Fatigue

The frequency of reported fatigue by individuals affected by PSC is between 60% and 70% [89,74]. Its level of intensity appears to be independent of histological and cholangiographic disease severity [1–8]. Reported fatigue has been associated with reductions in health-related quality of life in PSC [132]. Knowledge of the exact mechanism responsible for fatigue in PSC remains unknown.

Pruritus

In PSC, the prevalence of pruritus ranges from 40% to 60% in reported observational cohort studies [1–8]. Proposed mechanisms of action have included bile salt retention with skin deposition [133] and the accumulation of endogenous opioid ligands and receptors within the central nervous system [134]. As with fatigue, the severity of pruritus in PSC appears to be independent of histological stage, although extrahepatic stricturing can influence symptom intensity [1–8].

A number of medical therapies have been evaluated for symptom relief from pruritus. Initial therapy with antihistamines is often associated with residual sedation and only limited success [97]. Cholestyramine (a bile-acid-binding resin) may decrease the intensity of pruritus but rarely provides complete symptom resolution [135]. Among subjects with intact gallbladders, the use of 4 g before or after breakfast is thought to maximize bile acid sequestration resulting in symptom alleviation. Divided doses over 24 h in subjects postcholecystectomy are more effective than once or twice daily dosing. Administration of cholestyramine several hours apart from other medications is recommended to prevent its elimination without gastrointestinal absorption.

Hepatic enzyme inducers including phenobarbital [136] and rifampin (rifampicin) [137–139] have been associated with improvements in bile acid flow experimentally and used in the treatment of pruritus. Excessive sedation at higher doses has limited the clinical utility of phenobarbital [136]. Rifampin at dosages of 300–600 mg/day is associated with a rapid onset of action and symptom relief in PSC [137]. Although well-tolerated, this medication is also associated with cholestatic liver injury and bone marrow aplasia in up to 10% of patients [138]. Ursodeoxycholic acid (UDCA) at dosages of 13–15 mg/kg/day has not been shown to be effective in the treatment of pruritus [139]. Other therapeutic options used for pruritus in PSC including oral naltrexone [140], activated charcoal hemoperfusion [141], and plasmapheresis [142] have been sporadically applied with good results. Among patients with intractable pruritus that is refractory to medical therapy, liver transplantation is the only existing therapeutic option [97].

Metabolic bone disease

Metabolic bone disease in PSC is related to osteopenia rather than osteomalacia (defective bone mineralization) among North American patients [143]. Decreased bone formation (osteoporosis) [144] and some degree of increased resorption [145] are felt to be important mechanisms in the pathogenesis of bone disease. Calcium and vitamin D metabolism is usually abnormal among anicteric patients with PSC [146]. The recognition of improved bone mineral density approximately 6 months after liver transplantation also suggests the presence of an unidentified substance creating osteopenia [147]. Approximately 50% of patients with PSC have osteopenia (i.e., a Z score less than − 1.0 by lumbar spine bone mineral densitometry) whereas osteoporosis (i.e., a Z score less than − 2.5) is less common in PSC than in PBC (10% compared with 35%) [148,149]. Reasons for this difference include a greater proportion of male patients with PSC with higher baseline bone mass and a slower rate of bone loss compared to women. Only 23% of patients with advanced cholestatic liver disease have normal bone density at the time of transplantation [150]. Up to 50% of liver transplant recipients have bone mineral densities below the fracture threshold. Risk factors for osteoporosis in PSC are low body mass index, older age, postmenopausal status, muscle wasting, high alkaline phosphatase, low serum albumin, and a long duration of IBD [148–150], but bone density is not clearly related to the severity of the hepatic disease [151].

Ensuring adequate intake of calcium (1000–1200 mg daily) with weight-bearing activity is recommended as an initial treatment for all individuals. Measurement of serum vitamin D levels is also an essential part of the preventative management in PSC. The presence of vitamin D deficiency is variable among patients with metabolic bone disease. Oral replacement therapy is indicated if measured serum levels are reduced compared to local normal values. The 25-hydroxylation of vitamin D is intact among PSC patients; therefore, the use of vitamin D rather than 1,25-dihydroxyvitamin D or 25-hydroxyvitamin D can be used. Dosage is generally between 25 000 and 50 000 IU two to three times per week. The efficacy of bisphosphonate therapy in PSC remains unknown. No benefit from calcitonin was observed compared with placebo among PSC patients after liver transplantation [147].

Steatorrhea

Impairment in bile acid delivery resulting in insufficient critical micellar concentrations in the small intestine is the most common cause of steatorrhea with advanced hepatic disease caused by PSC [1]. The coexistence of celiac disease with PSC has been reported in an increasing number of PSC patients [127,128]. Exocrine pancreatic insufficiency has also been observed as a cause of steatorrhea related to overall glandular dysfunction, which may be associated with chronic pancreatitis [114,115]. Determining the exact cause is important because specific therapeutic options can be employed for symptom relief. In patients with decreased bile acid concentrations, the oral replacement of medium-chain triglycerides for long-chain compounds is usually of benefit. Adherence to a gluten-free diet and pancreatic enzyme replacement therapy are recommended for patients affected by celiac disease and pancreatic insufficiency, respectively.

Micronutrient deficiency

Malabsorption of fat-soluble vitamins is common with advanced hepatic disease caused by PSC. Intrahepatic cholestasis and impaired bile acid delivery to the small intestine are putative causes. Vitamin A deficiency observed in 20%–50% of patients is often clinically asymptomatic. When symptomatic, the presence of night blindness is observed and should be corrected by oral replacement therapy with 25 000–50 000 IU two to three times a week. As discussed previously, vitamin D deficiency can occur and is the next most common fat-soluble vitamin deficiency in 15%–50% of patients [146].

Vitamin E deficiency is a rare occurrence in PSC. When present, it may be associated with ataxia from neuropathic

involvement of the posterior vertebral columns of the spinal cord. Oral replacement therapy is indicated in asymptomatic patients [152].

Vitamin K deficiency is most commonly related to severe cholestasis, cholestyramine treatment for pruritus, or significant hepatic dysfunction from end-stage PSC. The initiation of water-soluble oral vitamin K at 5 mg daily with repeat measurement of prothrombin time is effective in determining the extent of malabsorption. If correction of prothrombin time is encountered, daily oral doses between 5 mg/day and 10 mg/day may be initiated.

Biliary calculi

Biliary calculi have been reported in up to 40% of patients with PSC [1,2,74,87]. Cholelithiasis is common and often requires cholecystectomy when symptomatic biliary colic occurs in nonadvanced hepatic disease [87]. Choledocholithiasis is reported at frequencies between 5% and 15% and more commonly among symptomatic individuals with biliary strictures. The most calculi are pigmented in nature and involve both central and peripheral bile ducts [153]. The development of symptoms, including intractable abdominal pain or recurrent bacterial cholangitis, has been successfully treated by endoscopic [154,155] or percutaneous [156] methods to provide biliary decompression and stone extraction. Biliary surgery, including stone extraction and bilioenteric anastomosis [156,157], may be required only in patients that are refractory to endoscopic and percutaneous approaches.

Dominant stricture

Dominant strictures or stenoses occur in 15%–20% of patients with PSC [158]. Clinical manifestations include a sudden increase in serum alkaline phosphatase or bilirubin in the asymptomatic patient, progressive jaundice, and bacterial cholangitis [1,2,74]. Cholangiography is required to determine the nature and extent of existing strictures [158]. Balloon dilation of identified stenoses by endoscopic [154] or radiological [158] approaches often provides significant clinical improvement. For patients with an incomplete response to balloon dilation alone, the use of endoscopic stents for 3–6 months to prevent recurrent stricture formation has been advocated [159,160]. Reports of infectious complications, however, have raised questions about the efficacy of this approach [161]. Cytological brushings with pinch biopsies of all dominant strictures is required to help exclude the presence of cholangiocarcinoma [162]. Cholangioscopic evaluation may be better able to predict benign vs malignant dominant strictures, but access to this procedure is limited [163].

Bacterial cholangitis

Bacterial cholangitis in PSC is most commonly associated with a previous history of biliary tract surgery [74], bile duct calculi [153], or ductal obstruction from a dominant stricture [158]. Once suspected, empiric broad-spectrum intravenous antibiotic therapy should be initiated. Cholangiography (with ERCP if possible) should then be considered to exclude biliary obstruction and to provide biliary decompression. The use of prophylactic antibiotic therapy (oral fluoroquinolone, trimethoprim-sulfamethoxazole) especially in the setting of short-term biliary stent placement may reduce the risk for recurrent cholangitis. Recurrent episodes of bacterial cholangitis in PSC can be an indication for liver transplantation [74].

Cholangiocarcinoma

A measurable proportion of subjects with PSC will develop cholangiocarcinoma as a complication of disease. Prevalence rates appear to vary between 7% and 13% in most reported series [3,5–9,164]. Between 30% and 45% of individuals with PSC will be diagnosed with cholangiocarcinoma at the time of liver transplantation [165–171] or autopsy [3,172,173]. PSC may be simultaneously present in 30%–50% of patients of diagnosed cholangiocarcinoma. The anatomic distribution of cholangiocarcinoma in PSC primarily involves the hilum (75% of patients), intrahepatic ducts (16%), and gallbladder (8%) [174].

Risk factors for cholangiocarcinoma in PSC include inflammatory bowel disease, chronic liver disease, cirrhosis, alcohol use, smoking, human immunodeficiency virus (HIV) infection, and diabetes [175]. No association with the stage of hepatic disease in PSC is observed, with coexisting cirrhosis affecting only 20% of patients with cholangiocarcinoma [174,176]. Advanced age and longer duration of IBD [173] have also been cited. Previous or current cigarette smoking has been suggested to be associated with cholangiocarcinoma in some but not all studies [177]. The presence of colorectal dysplasia/carcinoma in patients with PSC and UC has been noted as a possible risk factor for cholangiocarcinoma [172].

The presence of oncogenic mutations, inactivation of tumor suppressor genes, and apoptosis have been postulated as mechanisms for the development of cholangiocarcinoma in PSC. Mutations in K-*ras* found in the biliary secretions of PSC patients have been associated with cholangiocarcinoma [179,180] but this remains a nonspecific finding. Aberrations of the p53 tumor suppressor gene occur in 30%–80% of patients with PSC whose disease is complicated by cholangiocarcinoma [181] but are often seen as a late finding. Chromosomal loss of 9p21 and p16 tumor suppressor gene inactivation have also been reported [182]. The recognition of biliary dysplasia as a precursor to cholangiocarcinoma has been inconsistently suggested [183,184]. A detailed review of this topic is given in Chapter 77.

The diagnosis of cholangiocarcinoma in PSC remains challenging despite recent technological advances. Using the Mayo PSC model among 48 subjects undergoing liver transplantation, the incidence of biliary tract malignancy was significantly associated with model risk scores greater than 4.4 independent of clinical status [185]. Subsequent investigations to confirm this observation have not been performed.

Marked bile duct dilation in association with a polypoid mass-like structure larger than 1 cm in diameter on cholangiography has been reported in association with cholangiocarcinoma. The distinction between benign and malignant biliary strictures from less prominent findings, however, remains difficult. Comparisons between ultrasound, CT, and MRI have demonstrated an inadequate ability to distinguish between PSC patients with and without cholangiocarcinoma [186]. Emerging techniques such as positron emission testing that show initial promise will require further examination [187].

Serum tumor markers including carbohydrate antigen 19-9 (CA 19-9) and carcinoembryonic antigen (CEA) have been employed for the early detection and diagnosis of cholangiocarcinoma. CA 19-9 levels over 100 U were found to have a sensitivity of 52%–89% and specificity of 76%–86% for detecting cholangiocarcinoma in PSC [188,189]. The combination of both markers (CA 19-9 + CEA × 40) exceeding values of 400 U was reported to have an 86% diagnostic accuracy despite a sensitivity of only 67% [189]. Similar results using this formula were reported in an independent study [190]. The prospective evaluation of four tumor markers (CA 19-9, CEA, CA 50, and CA 242) among 75 patients over 3 years failed to achieve high test performance characteristics for predicting cholangiocarcinoma [178].

The performance of endoscopic biopsy and cytological brushing for the diagnosis of cholangiocarcinoma is noted for good specificity (90%–100%) but poor sensitivity (4%–20%) [162,191]. False-positivity with cellular atypia from chronic inflammation may occur [191]. Detection of DNA aneuploidy by flow cytometry has been reported in 80% of individuals with cholangiocarcinoma compared with only 12% of patients with benign strictures [192]. Digital imaging analysis and fluorescence in situ hybridization are new techniques to detect malignancy in cytological specimens that are otherwise negative for malignant cells [193]. These techniques in addition to cytological examination improve the overall sensitivity to 35%–60% while maintaining the high specificity.

Therapeutic options for cholangiocarcinoma in the setting of PSC are limited. For extrahepatic biliary resection, operative complication and mortality rates exceed 30% and 6%, respectively, in the absence of bile duct cancer [194]. Highly selected patients, however, may derive benefits from surgical resection [195]. Curative attempts with liver transplantation to date have resulted in poor outcomes from residual tumor at surgical margins and local hepatic lymph node involvement [167,170]. The presence of cholangiocarcinoma in situ of less than 1 cm found incidentally at liver transplantation has been associated with similar long-term survival rates as individuals with PSC alone in two studies [169,196] but long-term prognosis remains poor in another study [197].

To improve outcomes in liver transplantation, the implementation of preoperative radiation and chemotherapy in 65 highly selected patients with unresectable cholangiocarcinoma and PSC yielded a 76% 5-year survival after liver transplant at the Mayo Clinic [198]. This protocol appears to provide an acceptable option for patients fitting the strict criteria for inclusion. Use of these protocols should be limited to centers with experience and prospective research involvement.

Portal hypertension

Progressive PSC can result in the typical complications of portal hypertension with or without the development of cirrhosis. These complications include ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, esophagogastric variceal bleeding, portal hypertensive gastropathy, hepatorenal syndrome, and hepatopulmonary syndrome. Patients with a platelet count below 200/mm^3, an albumin level less than 40 g/L, and a bilirubin level above 20 μ/L (1.2 mg/dL) were shown to have an increased likelihood of varices so screening those patients has been suggested [199]. A unique complication is peristomal variceal bleeding which occurs among subjects with IBD who have undergone proctocolectomy with ileostomy formation [200]. Newer surgical techniques including ileal pouch–anal anastomosis at the time of proctocolectomy are indicated among patients in whom PSC has been identified to prevent this complication [201]. Pharmacological (β-blocker) and endoscopic (sclerotherapy) therapies are without long-term efficacy [200]. Transjugular intrahepatic portosystemic shunt placement has been shown to be effective in treating uncontrolled bleeding and preventing further episodes [202].

Colonic dysplasia/carcinoma

The association between an increased risk of colonic dysplasia/carcinoma and PSC in the setting of UC is interesting but remains controversial. Decreased bile acid secretion and increases in secondary bile acid exposure of the colonic mucosa are considered potential mechanisms [203]. In addition, a greater proportion of right-sided colon neoplasia has been observed in UC patients with PSC compared with patients with UC alone [204–206]. Absolute cumulative risks for colorectal dysplasia/carcinoma of 5% (at 10 years), 31% (at 20 years), and 50% (at 25 years) have been observed in patients with PSC and UC compared with risks of 2%, 5%, and 10%, respectively for patients with UC alone [172]. Other studies, however, have failed to confirm a significant risk from PSC in developing colorectal dysplasia/carcinoma [207,208]. A metaanalysis of 11 studies confirmed a significantly increased risk of colorectal neoplasia in patients with UC with and without PSC [209]. The cumulative risk of developing colorectal carcinoma is further increased post-transplantation in PSC patients. In patients with an intact colon and IBD at the time of transplant, the risk was 14% after 5 years and 17% after 10 years [210]. Patients who have had pancolitis for longer than 10 years are at highest risk. Given the potential association between bile acid exposure and colorectal neoplasia, the use of UDCA was strongly

associated with a decreased risk for colonic dysplasia among 59 PSC patients undergoing colonoscopic surveillance for UC. This association remained after adjustment for duration and severity of PSC. A younger age of IBD onset, however, was also associated with an increased dysplasia risk [211]. These findings were confirmed in a controlled trial [212]. Surveillance colonoscopy with biopsy is recommended in patients with PSC and UC, especially after liver transplantation among patients with UC in the absence of proctocolectomy [166,213–215].

Pregnancy

Women with PSC tend to be of childbearing age. A study involving 13 pregnancies in 10 patients with PSC (4 with cirrhosis) showed that in patients with PSC, pregnancy does not seem to have a negative effect on the disease process, nor did mothers or babies show any ill effects from the PSC [216].

Disease-modifying therapies

Liver transplantation is the only effective treatment for end-stage hepatic disease resulting from PSC. As a result of the variable nature of disease progression, the development of randomized clinical trials for the assessment of therapies has been difficult. As a result, no effective medical therapy has been identified for the treatment of PSC (Table 75.4).

Medical therapy

D-Penicillamine

An increase in hepatic copper found among patients with chronic cholestatic liver disease including PSC provided the rationale for examining D-penicillamine. Among 70 patients randomized to active drug or placebo, there was no reported benefit in terms of biochemical improvement, histological stabilization, or survival free of liver transplantation after 3 years of follow-up. Major toxicities including renal disease resulted in drug discontinuation in 20% of subjects [217].

Colchicine

A randomized controlled trial of 84 subjects with PSC (44 given colchicine 1 g/day, 40 given placebo) revealed no differences in biochemical measures, histological results, or survival between groups after 36 months of follow-up [218]. Combination therapy with prednisolone and colchicine has been associated only with initial biochemical improvement after 24 months of treatment [219].

Corticosteroids

The report of beneficial results from corticosteroid use has only been demonstrated in uncontrolled trials. Patients with early-stage histological disease may have symptomatic and biochemical improvement [220]. The use of oral budesonide,

Table 75.4 Medical therapies evaluated in primary sclerosing cholangitis

Medication	Primary action	Therapeutic benefit
Controlled trials		
D-Penicillamine	Cupuretic	None
Colchicine	Antifibrotic	None
Cyclosporine	Immunosuppressive	None
Methotrexate	Immunosuppressive	Biochemical, histological (?)
UDCA	Immunomodulatory	Biochemical, histological (?)
Uncontrolled trials		
Corticosteroids	Immunosuppressive	Clinical, biochemical, histological
Azathioprine	Immunosuppressive	None
Mycophenolate mofetil	Immunosuppressive	None
Tacrolimus	Immunosuppressive	Biochemical
Pentoxifylline	TNF inhibition	None
Etanercept	TNF inhibitor	None (? Effect on pruritus)
Nicotine	Immunomodulatory	None
Silymarin	Antifibrotic	None
Budesonide	Immunosuppressive	Biochemical but osteopenia
Combination therapy	**Compared with**	
UDCA + methotrexate	UDCA monotherapy	Similar to UDCA alone
Corticosteroids + colchicine	Historical controls	Biochemical
UDCA + mycophenolate mofetil	UDCA monotherapy	Similar to UDCA alone
UDCA + metronidazole	UDCA monotherapy	Biochemical, clinical

TNF, tumor necrosis factor; UDCA, ursodeoxycholic acid.

a systemic corticosteroid with significant first-pass hepatic metabolism and reduced toxicity, in 21 patients with PSC was associated with improved hepatic biochemistry results at the expense of worsening osteopenia in a pilot investigation [221]. The use of prednisone or budesonide in combination with UDCA was not associated with significant clinical or biochemical improvement in a double-blinded, randomized controlled trial [222]. A combination of prednisone, azathioprine, and UDCA in 15 patients was noted for significant biochemical and histological improvements. These results were also observed among individuals with partial biochemical responses to UDCA [223]. Case reports detailing beneficial effects from topical corticosteroid therapy administered endoscopically through a nasobiliary drain were complicated by an increased rate of bacterial cholangitis [224,225]. If significant improvement occurs with corticosteroids, the patient is more likely to have had secondary sclerosing cholangitis as a result of autoimmune pancreatitis, because this is highly responsive to steroid treatment [226].

Azathioprine

No randomized controlled trial for azathioprine monotherapy has been reported. Open-label pilot studies are noted for their mixed results for efficacy using biochemical parameter responses [227,228].

Cyclosporine

A single randomized controlled trial of cyclosporine (ciclosporin [rINN]) among 34 patients [229] was noted for the absence of significant symptomatic, biochemical, or histological improvement (but no deterioration throughout the 2-year follow-up). Toxicity from cyclosporine, including hypertension and renal insufficiency, was minimal. Tacrolimus (FK506), a calcineurin-based inhibitor with pharmacological properties similar to cyclosporine, has been associated with significant improvements in biochemical profiles among all 10 subjects in an open-label pilot trial. Mean serum aminotransferase levels among these patients, however, were up to fivefold greater than normal values, which is not seen in patients with typical PSC. No effect on histology was observed based on limited follow-up [230].

Mycophenolate mofetil

No clinical benefit has been demonstrated when mycophenolate mofetil is used either alone or in combination with ursodiol (13–15 mg/kg) [231,232]. Both trials included patients with fairly advanced disease, so the effect of the drug earlier in the disease process is unknown.

Methotrexate

Data from a double-blind, randomized controlled trial comparing oral pulse methotrexate (15 mg/week) with placebo among 24 patients [233] is noted only for reductions in serum alkaline phosphatase. No improvements in total bilirubin histology, or survival were observed. Early-stage histological disease was associated with possible improvements in both symptoms and biochemical parameters [234]. Combination therapy with methotrexate and UDCA in 19 patients was associated with toxicity (from methotrexate) without biochemical improvement when compared to UDCA alone in 9 patients [235].

Ursodeoxycholic acid

The mechanism of action attributed to ursodeoxycholic acid (UDCA) in the treatment of chronic cholestatic liver disease is multifaceted. In addition to suppressing cytotoxic endogenous bile acid secretion [236], there is evidence to suggest that UDCA is associated with membrane stabilization [237] and reduced aberrant HLA type I expression on hepatocytes [238]. Mitochondrial dysfunction induced by hydrophobic bile acids, decreased cytokine production, and inhibition of apoptosis have also been identified in association with UDCA [239].

Early investigations suggested a beneficial effect from UDCA on PSC [240,241]. Three randomized, controlled trials comparing UDCA with placebo, however, did not show significant improvements in histology or survival despite a change in biochemical parameters [242–424]. Two recent investigations of high-dose UDCA (25–30 mg/kg/day) reported additional improvements in biochemical [245,246], cholangiographic, and histological parameters [246] when compared to standard doses. A recent study confirmed a lack of effect with intermediate dose UDCA (17–23 mg/kg/day) [247]. Results from placebo-controlled studies examining high-dose UDCA in PSC continue are awaited.

Novel therapies

Pilot investigations of oral nicotine [248] and pirfenidone [249] have failed to demonstrate improvements in serum hepatic biochemistries or Mayo PSC risk score. Despite properties of anti-TNF attenuation, the use of oral pentoxifylline was not associated with clinical or biochemical improvement among 20 patients with PSC [250]. A pilot study of subcutaneous etanercept (25 mg biweekly) showed no biochemical or radiographic benefit, but did have an effect in reducing pruritus in two of five patients [251]. The nucleoside analogue cladibrine has been associated with progression of histological fibrosis despite improvements in periportal inflammation among four patients with PSC after 24 months of follow-up [252]. A placebo controlled trial of metronidazole (600–800 mg/day) in addition to UDCA (15 mg/kg/day) suggested biochemical benefit to combination therapy as well as a trend toward radiographic improvement [253].

Endoscopic therapy

Endoscopic therapy in PSC has been successful in alleviating cholestasis aggrevated by mechanical biliary obstruction. Controversy remains, however, regarding the safety and efficacy of ERCP based on the limited numbers of patients reported and

variety of endoscopic methods utilized. Among PSC patients undergoing diagnostic ERCP, a 2% complication rate has been reported compared to 14% among patients with jaundice or cholangitis [254]. With advances in technology and endoscopic development, a number of options for therapeutic intervention are possible including endoscopic sphincterotomy, hydrostatic balloon dilation, nasobiliary tube drainage, and biliary stent placement. The current use of endoscopic dilation with sphincterotomy or stenting is associated with clinical response rates from 70% to 90% [156,255].

Among 53 patients requiring ERCP for the evaluation of biliary obstruction [155], a technical performance success rate of 88% was observed with 15 (28%) subjects experiencing procedure-related complications. Twenty-eight patients (56%) reported symptomatic improvement following ERCP for a median of 31 months. No change in cholangiographic findings, however, was seen in over 50% of cases. In the largest series reported to date of 106 patients with PSC [256], dominant stenoses were observed in 43 instances over a median follow-up of 5 years. The majority of these stenoses were treated using balloon dilation. Complications included pancreatitis (5.2%), cholangitis (3.3%), and bile duct perforation (0.5%). Survival after balloon dilation of the first recognized stenosis was significantly better when compared to predicted survival using the Mayo multicenter prognostic model.

Nasobiliary tube placement for drainage and saline irrigation has been employed to prevent recurrent biliary obstruction [224,225]. Among 12 asymptomatic patients with high-grade strictures treated initially by hydrostatic balloon dilation followed by nasobiliary tube irrigation [257], an improvement in clinical, biochemical, and cholangiographic features was documented in 75% of cases over 23 months. One patient had no change in clinical status from unresponsive disease. Progressive disease in three subjects required treatment by liver transplantation. Further use of nasobiliary tube irrigation among symptomatic patients has not been reported.

A retrospective examination of 25 symptomatic patients treated by biliary stent placement is noted for an endoscopic success rate of 84% [159]. Clinical and biochemical improvement in all cases was noted after 6 months. Additional procedures for stent exchange were associated with a complication rate of 14% over a 29-month period. During follow-up, only 12 patients (57%) remained asymptomatic with normal hepatic biochemistries. The use of short-term stenting (mean 11 days) of dominant strictures among 32 patients has been associated with an 83% improvement rate from symptomatic cholestasis (fatigue, pruritus) and biochemical improvement over 4 years [160]. Reintervention rates at 1 year (80%) and 3 years (60%) were observed. An investigation advocating the use of aggressive stricture dilation or stenting combined with UDCA therapy in PSC was based on results in an uncontrolled trial setting [258].

Biliary reconstructive surgery

Among precirrhotic individuals with extrahepatic biliary strictures refractory to endoscopic/percutaneous therapy, the use of intraoperative resection has been performed successfully [194,259–261]. Common sites of involvement in this setting include the common bile duct and perihilar regions. Early reports involving 17 of 22 patients undergoing choledochoenteric anastomosis was noted for good to excellent results in 13 patients (76%) [259]. Transhepatic stenting was employed in six patients. An overall survival rate of 82% at 52 months following surgery was observed. Among 31 patients undergoing extrahepatic bile duct resection and long-term stenting [260,261], excellent results among 26 precirrhotic individuals were reported. A 20% survival rate over 5 years, however, was noted in patients with cirrhosis.

Comparisons between operative and nonoperative therapies have been reported involving 104 patients treated surgically ($n = 54$) or using endoscopic balloon dilation ($n = 35$) and percutaneous stenting ($n = 19$) [194]. In this retrospective investigation, no differences in procedure-related morbidity and mortality were recorded. Improvements in serum bilirubin up to 3 years following resection were significantly better compared to those in nonoperative therapies. Among 40 patients with cirrhosis at surgical resection, overall 5-year survival rates of 85% (including liver transplantation in 21 patients) were noted. Transplant-free survival, however, was 78% at 5 years among subjects without decompensated cirrhosis undergoing resection. A 59% overall and 46% transplant-free 5-year survival, respectively, was observed among medically treated individuals. Cholangiocarcinoma in 9% of medically treated patients was reported in contrast to no cases among surgical patients.

Concerns regarding operative bile duct resection complicating the performance of liver transplantation have been raised [165,167,170,171]. The association between previous bile duct surgery and poor outcomes including decreased survival, however, has not been conclusively determined [166–169]. Among selected individuals with PSC, a history of previous surgery has been associated with longer operative procedure times [165,166,194] greater intraoperative blood loss [165–167], and increased risks for biliary complications after transplantation [165,166,168].

Liver transplantation

Among patients with end-stage liver disease as a result of PSC, the current treatment of choice is liver transplantation. PSC is the fourth most common indication for liver transplantation in the United States, accounting for 5%–10% of all procedures. Indications for liver transplantation are similar to other hepatic diseases including complications from portal hypertension (ascites, variceal bleeding, encephalopathy). Significant impairments in health-related quality of life from fatigue, pruritus, and painful osseous fractures may also be considerations for liver transplantation. Unusual

Table 75.5 Patient survival (%) following liver transplantation for primary sclerosing cholangitis

Site	No. of patients	1-year	2-year	5-year	Ref.
UCSF	37	97	92	88	Polter et al. [135]
UCLA	127	90	86	85	Prince et al. [138]
UNOS	2178	91	–	83	Narumi et al. [220]
Sweden	47	86	82	–	Burgert et al. [221]
Mayo Clinic	150	94	92	86	Angula et al. [166]

UCSF, University of California at San Francisco; UCLA, University of California at Los Angeles; UNOS, United Network for Organ Sharing.

Table 75.6 Etiology of biliary strictures or cholangiopathy after liver transplantation

Early (< 6 months)	Late (> 6 months)
Hepatic artery thrombosis	Chronic rejection (intrahepatic)
Preservation injury (warm/cold ischemia)	Biliary tract infection (including bacterial, cytomegaloviral)
ABO blood group incompatibility	Recurrent primary sclerosing cholangitis
Anastomotic stricture	Cholangiocarcinoma
Bile leak	Cholelithiasis

circumstances prompting referral for liver transplantation in patients with PSC include recurrent cholangitis despite medical therapy, severe extrahepatic biliary obstruction among individuals with advanced hepatic disease precluding operative repair, and uncontrolled peristomal variceal bleeding. Reports from single centers performing liver transplantation in PSC are noted for patient survival rates between 90% and 97% at 1 year and 83% and 88% at 5 years (Table 75.5). Improvements in operative technique and immunosuppression have greatly contributed to improvements in patient survival. Long-term graft survival, however, has been affected by a higher incidence of rejection (acute cellular and chronic ductopenic) and hepatic artery thrombosis, in addition to recurrence of PSC [262].

Among patients with coexistent UC undergoing liver transplantation, it has been recognized that gastrointestinal symptoms may improve with the initiation of immunosuppressive therapy. Exacerbations of UC, however, are reported in up to 30%–50% of patients with some individuals requiring proctocolectomy for disease control [263,264]. An increased prevalence of pouchitis among PSC patients after liver transplantation compared to UC patients transplanted for other hepatic disease states has also been reported [265]. Patients with intact colons appear to be at increased risk for colorectal neoplasia after liver transplantation [166,213–215]. The risk has been shown to be 14% 5 years posttransplant and 17% 10 years posttransplant [210]. A duration of IBD greater 10 years is the highest risk. The cumulative risk for colorectal cancer among individuals transplanted for PSC at the Mayo Clinic [213] was calculated at 1% per year. The cumulative incidence for colonic dysplasia, however, was increased at 15% over 5 years and 21% at 8 years.

Recurrent PSC is thought to occur by 5 years in 9%–20% of patients with underlying PSC who are transplanted [266–269]. A combination of cholangiographic findings of intrahepatic or extrahepatic strictures and beading along with histological findings including biliary fibrosis/cirrhosis, fibroobliterative lesions, and cholangitis with or without ductopenia are required for accurate diagnosis of recurrent PSC [269]. Risk factors for recurrent disease have not been clearly delineated. A metaanalysis of 18 published studies showed that underlying IBD was not a risk factor for recurrence [266]. Chronic rejection is difficult to discern from recurrent PSC and it is not clear that the two entities are mutually exclusive [267]. Kugelmas and colleagues [270] have suggested that patients who received maintenance corticosteroids or orthoclone (OKT3) posttransplant appeared to be at higher risk of recurrent PSC but recurrence was not related to other immunosuppressants. PSC is a risk factor for cholangiocarcinoma but the risk in recurrent PSC, although reported, is unknown [271]. The diagnosis of recurrent PSC requires the exclusion of known causes of stricturing including ABO incompatibility, chronic ductopenic rejection, early and late hepatic artery thrombosis, prolonged cold ischemic time, and infectious causes (Table 75.6). Differentiating recurrent intrahepatic (small duct) PSC from chronic ductopenic rejection is fraught with difficulty and requires experienced pathologists and supporting clinical and radiographic evidence. Differentiating recurrent disease from other biliary complications is dependent on the clinical picture and the timing and location of the biliary strictures. See Liver transplantation section within Post-transplantation cholangiopathies section for further transplant-related issues.

Natural history and prognosis

The majority of published investigations support the contention that PSC is a progressive disease that can ultimately lead to liver failure or death. A median survival of 9–12 years in the absence of liver transplantation appears to be independent of geographic and environmental influences [3–6,9,10]. Subsets of patients with PSC, however, appear to follow different patterns of disease progression. Patients can be categorized as small duct vs large duct disease, symptomatic vs asymptomatic or with or without coexistent IBD. Symptomatic patients may do poorly compared with asymptomatic patients, but this may also reflect lead-time bias. Patients with small duct disease may have less rapidly progressive disease [272,273]. This significant variation in natural history means

that the use of mathematical prognostic models has allowed for improvements in estimating risks for disease progression and PSC-related survival independent of liver transplantation.

Subject age, serum total bilirubin, hemoglobin, a history of inflammatory bowel disease, and histological stage on liver biopsy were observed as independent predictors of death among PSC patients at the Mayo Clinic [5]. A similar prognostic index including subject age, hepatomegaly, splenomegaly, elevated serum alkaline phosphatase, and advanced histological stage has also been reported [6]. To address variations in independent populations, a multicenter prognostic model was developed using 426 patients from five centers [274]. Subject age, serum bilirubin, splenomegaly, and histological stage emerged as independent predictors of death in PSC. A revised version of the original Mayo PSC model has eliminated histological stage in favor of variceal bleeding history, avoiding the need for invasive liver biopsy for estimating prognosis [275].

Examination of the Child–Turcotte–Pugh (CTP) classification as a prognostic model in PSC has yielded mixed results [276,277]. Significant differences in 7-year survival have been demonstrated between CTP class A (89.8%), class B (68%), and class C (24.9%) patients with PSC [276]. Subject age and CTP classification were independent predictors of mortality. A subsequent investigation of 147 patients [277], however, was noted for limitations in predicting survival in CTP class B and C patients. CTP classification was not observed as an independent predictor of death in this cohort in contrast to the Mayo PSC risk score. Once a patient develops decompensated disease the model for end-stage liver disease (MELD) score more accurately predicts survival and is used when listing for liver transplantation [278].

Attempts at defining the optimal timing for liver transplantation in PSC have examined the application of existing prognostic models. Among 216 patients with PSC who underwent liver transplantation between 1981 and 1990 [170], a dramatic survival benefit was observed compared to predicted survival using the Mayo PSC model (73% vs 28% at 5 years, $P < 0.001$). Long-term survival between subjects grouped by risk score, however, was similar. The occurrence of cholangiocarcinoma, however, was associated with a significant decline in survival independent of pretransplant Mayo risk score. The presence of ascites, previous upper abdominal surgery, elevated serum creatinine, IBD, and a history of biliary tree malignancy have also been described as predictors of reduced posttransplant survival [279].

Additional investigations surrounding outcomes after liver transplantation in PSC have focused on outcomes such as health-related quality of life and resource utilization [280–282]. Among 436 patients with cholestatic liver disease including PSC ($n = 208$), the independent predictors of postoperative morbidity were subject age, renal failure (defined as serum creatinine > 2 mg/dL), Child–Pugh classification, and United Network for Organ Sharing (UNOS) status [280]. Increased resource utilization based on hospital charges has been associated with a Karnofsky score greater than 40, poor nutritional status, and renal failure [281]. A subsequent multicenter investigation, however, demonstrated that the CTP classification is a significantly better predictor of clinical and economic outcomes than the Mayo risk score [282].

Variant primary sclerosing cholangitis syndromes

Small duct primary sclerosing cholangitis

A subset of patients with typical features of PSC may have intrahepatic and extrahepatic bile ducts that appear normal by cholangiography [272,273,283–285]. The diagnosis of small duct PSC is based on

- serum biochemical profile consistent with cholestatic liver disease
- the presence of inflammatory bowel disease
- normal cholangiogram
- appropriate findings on liver histology.

Characteristic features described by liver histology assessment include mild portal mononuclear inflammation, ductular proliferation, and nonsuppurative fibrous obliterative cholangitis [284]. Fibrous-obliterative cholangitis, while considered pathognomic for PSC, occurs in only 10%–40% of cases [95]. Granulomatous cholangitis (more commonly seen in primary biliary cirrhosis) is seen in 8% of cases [1]. Disease progression is characterized by periportal fibrosis, ductopenia, and eventual biliary cirrhosis [95] or progression to large duct involvement. Previously termed "pericholangitis," the prevalence of small duct PSC is estimated to be between 6% and 35% among adults with UC [272,283,284]. As many as 33% of patients with large duct PSC are identified from normal cholangiograms during the course of their disease despite histological evidence for PSC. Isolated small duct involvement at diagnosis with eventual recognition of large duct PSC occurs in 12%–40% of cases [272,273,283,285]. Although uncommon, the progression to end-stage liver disease requiring hepatic transplantation has been associated with small duct PSC [170,272,285]. Specific reference to small duct PSC in terms of medical therapy has not been widespread given its rare occurrence. The natural history of small duct PSC appears to portend a better prognosis than large duct disease [272,273,285]. Cholangiocarcinoma has rarely been observed among patients with small duct PSC [272,283,285].

Autoimmune hepatitis–primary sclerosing cholangitis overlap syndrome

Patients with PSC and clinical features reminiscent of AIH are classified as having an "overlap syndrome" between the two conditions [286–289]. Greater than expected serum aminotransferase elevations (5–10 times) and increased titers of

serum autoantibodies, including antinuclear antibody, anti-smooth muscle antibody, and pANCA, are observed in most cases [287–289]. The presence of typical biliary stricturing and dilation seen with PSC is often accompanied by increased lymphoplasmacytic portal or periportal infiltrates on liver biopsy consistent with AIH. In PSC, the presence of overlap with AIH was previously thought to range between 35% and 54% using the original International Autoimmune Hepatitis Group (IAHG) scoring system criteria (11–12) [286]. Using the revised IAHG scoring system, a decrease in prevalence of AIH–PSC overlap between 1.4% and 11% has been reported [287,288]. In contrast, van Buuren and colleagues [289] observed similar prevalence rates of AIH–PSC overlap based on either conventional diagnostic criteria (23%) or the revised IAHG scoring system (19.5%). Empiric immunosuppressive therapy for potential benefit among patients with AIH–PSC overlap has been recommended [286,290].

Idiopathic adulthood ductopenia

Idiopathic adulthood ductopenia is a syndrome described by:

- the presence of biochemical parameters consistent with cholestasis
- the presence of histological evidence of ductopenia involving more than 50% of the bile ducts
- the presence of no evidence for the diagnoses of PSC and IBD
- the exclusion of other known etiologies for cholestasis including PBC and drug-induced hepatotoxicity [291].

Variation in the extent of bile duct loss likely reflects a spectrum in natural history [284]. Other diagnostic possibilities include an unidentified chronic viral cholangitis, small-duct PSC in the absence of IBD after colonoscopic investigation, or advanced AMA-negative PBC with resulting ductopenia [291].

Among 29 reported cases of idiopathic adulthood ductopenia [284,291] there is a median age of 27 years (range 15–67 years) at diagnosis. Male to female gender distribution is estimated at 2 : 1. Jaundice and pruritus are seen at initial presentation in 33% of cases. Disease progression reported among 10 patients occurred over a median duration of 4 years (range 5 months to 15 years). Seven of 23 (30%) patients with follow-up longer than 6.5 years died from liver disease while 4 patients required liver transplantation. Corticosteroid or UDCA therapy was associated with clinical improvement in 3 of 4 cases. Among subjects with features similar to idiopathic adulthood ductopenia despite more than 50% bile duct loss [292], a benign course was reported in 3 of 24 individuals using sequential liver biopsies over 12 years. Four of 5 patients treated with UDCA had normalization of serum liver biochemical parameters.

Other cholangiopathies

AIDS cholangiopathy

Epidemiology

Biliary tract disease in AIDS (AIDS cholangiopathy) was first described soon after the identification of HIV [293]. AIDS cholangiopathy describes a syndrome that resembles sclerosing cholangitis associated with papillary stenosis [294,295]. Pathogens involved with the development of AIDS cholangiopathy do not appear to affect patients who are immunocompromised for other reasons. The mean age of affected patients is reported to be 36.5 years with a preexisting diagnosis of AIDS from 1 to 2 years before symptomatic biliary tract involvement [295,296]. With the advent of highly active antiretroviral therapy (HAART), this entity has become rare and generally only occurs when the CD4 T-cell counts drop below 50 per mm^3. Previously, this diagnosis was known to be associated with significant mortality within 1 year of infection [296]. Since the introduction of HAART in general, as well as specific therapy for cholangiopathy, not only has the incidence of the disease declined, but the survival after developing cholangiopathy has improved to 80% at 24 months from the diagnosis [297].

Pathogenesis

Infection of the biliary or duodenal epithelium is the primary cause for ductal abnormalities in AIDS cholangiopathy. Among pathogens identified, *Cryptosporidium* is the most common and is often found without coexistent infection [294–297]. Among 82 HIV-infected patients exposed to *Cryptosporidium* in a water-borne outbreak [298], the development of abdominal pain associated with biliary tract disease occurred in 24 cases, which were confirmed by cholangiography in only 40%. Simultaneous bouts of new-onset diarrhea as a result of *Cryptosporidium* were also reported [299]. Concurrent active HIV replication and associated *Cryptosprodium parvum* infection act synergistically to increase cholangiocyte apoptosis and thus jointly contribute to AIDS-related cholangiopathies [300]. If undetected by blood or stool cultures, the next most common organism is *Microsporidium* [301,302]. Other recognized infections include cytomegalovirus (CMV) [303], *Enterocytozoon bieneusi* [304], *Septata intestinalis* [305,306], *Mycobacterium avium-intracellulare* [307], *Isospora belli* [308], and possibly HIV infection itself [309]. Hepatic CMV inclusions have been observed in 5%–44% of patients at autopsy. An estimated 33% of patients with AIDS and CMV have cholestatic liver disease, and as many as one-third of these individuals have overt bile duct abnormalities [303]. Ultimately, as many as 50% of patients with AIDS cholangiopathy have no identifiable opportunistic infection [295,301,303,305]. Malignancy such as lymphoma [309] and Kaposi sarcoma [309] has been associated with cholangitis but it rarely presents as a cholangiopathy. Histological findings in AIDS cholangiopathy observed on bile duct and liver biopsy include moderate to severe inflammation and fibrosis [310].

Clinical manifestations

The most common presentation is epigastric or right upper quadrant abdominal pain (64%–88%) with fever (20%–65%).

Jaundice, however, is distinctly uncommon. Diarrhea is also present in most cases, related to small intestinal involvement with the characteristic pathogens. Superimposed bacterial cholangitis is a rare complication [295,296,311]. Serum liver biochemistries are noted for elevations in alkaline phosphatase more than three or five times the upper limit of normal in over 75% of cases. Milder increases in serum aminotransferases (less than threefold) are also noted. Normal biochemical parameters have also been observed in 20% of instances with cholangiographic abnormalities [295,296,301,312]. Ultrasonography or CT imaging can detect biliary duct dilation (intrahepatic or extrahepatic) in most instances [313–315]. Cholangiography is required for the diagnosis of AIDS cholangiopathy. Left-sided biliary duct system involvement is observed with an increase in intrahepatic duct involvement with saccular dilations, debris, and pruning. Irregular structuring of the entire common bile duct with wall thickening in the absence of dilation is common [295,311,314]. The most common characteristic biliary tree manifestation is papillary stenosis with intrahepatic sclerosing cholangitis, which is found in 50% of cases. Other variations include intrahepatic and extrahepatic sclerosing cholangitis without papillary stenosis, papillary stenosis alone, intrahepatic sclerosing cholangitis alone, or long (> 1 cm) extrahepatic strictures [295,311,312,316,317]. Among individuals with severe pain and associated cholangitis, the identification of papillary stenosis is almost universal [295,312,318]. The presence of isolated common bile duct strictures should raise the suspicion for primary lymphoma [309] or pancreatic disease [311].

Differential diagnosis

The differential diagnosis includes choledocholithiasis, acalculous cholecystitis, periampullary tumors including pancreatic and bile duct cancer, and PSC. Choledocolithiasis will also produce intrahepatic duct dilation above the level of obstruction where biliary duct dilation is distinctly uncommon in AIDS cholangiopathy. Bile duct wall thickening appears most often. The presence of elongated stricturing with intrahepatic and peripheral bile duct dilation is more often observed with periampullary carcinoma but can resemble findings when papillary stenosis alone is present. Direct visualization and biopsy of the ampulla are required to exclude carcinoma. Global duct narrowing, diffuse stricturing, and intrahepatic stone formation characterize PSC. The finding of concomitant inflammatory bowel disease also suggests PSC even in subjects with AIDS.

Treatment

The presence of opportunistic infections and a very low CD4 count is a marker of advanced disease, and the emphasis should be on the HIV disorder, not on the AIDS cholangiopathy. Therapy of both is directed (and outcomes improved) by elevation of the CD4 count and depression of viral loads, which can only be obtained by HAART [319]. Additional treatment can be directed at identified pathogens as well as biliary tree abnormalities. Antimicrobial therapy against *Cryptosporidium* sp. with trimethoprim-sulfamethoxazole is indicated, especially among patients with coexistent diarrheal symptoms [299]. Effective treatment against *Microsporidium* sp. remains elusive [302]. Ganciclovir for CMV [303,316] and multidrug regimens against *Mycobacterium avium-intracellulare* [307] should also be provided. Albendazole for *Septata intestinalis* infection has been shown to result in symptom improvement when present [305]. Successful therapy for malignancy-related cholangiopathy with reversal of biliary tract abnormalities has been reported with B-cell non-Hodgkin lymphoma in anecdotal instances [309]. There is no indication for prophylactic treatment in patients unaffected by AIDS cholangiopathy.

Symptomatic patients with a high degree of suspicion for biliary tract disease require cholangiography by ERCP [294,295,311]. For patients with papillary stenosis alone, endoscopic sphincterotomy has been associated with improvement in abdominal symptoms, but not survival [296,316,318,319]. Often, there is little to no change in serum hepatic biochemical parameters in most cases [311]. A continued rise in serum alkaline phosphatase levels, however, suggests the presence of intrahepatic duct involvement as well [296,311]. The performance of balloon dilation or stent placement in cases of papillary stenosis is without dramatic benefit over sphincterotomy [311]. UDCA therapy for symptomatic disease is not highly efficacious [320]. A diagnosis of acalculous cholecystitis requires cholecystectomy [321].

Posttransplantation cholangiopathies

Bone marrow transplantation

Epidemiology

The most frequent etiologies of cholangiopathy within the first 80 days of bone marrow transplantation include acute graft-versus-host disease (GVHD), sinusoidal obstruction syndrome (SOS), cholangitis lenta, and drug-induced cholestasis [322,323]. Chronic GVHD is the most common cause beyond 80 days [323]. Risk factors for developing cholestatic-mediated injury after bone marrow transplantation have been identified. The use of conditioning therapies before the transplant, including combinations of irradiation or immunosuppressive agents, has most often been associated with hepatic venoocclusive disease, which is referred to as SOS [324–326]. Allogeneic donor stem cell recipients are considered to be at increased risk for acute and chronic GVHD [323]. Related sibling donors with identical HLA typing impart the lowest risk of acute or chronic GVHD among all donor source types [327]. The use of immunosuppressive agents for acute GVHD prophylaxis is also associated with an increased risk for cholestatic injury [322,328]. Antimicrobial prophylaxis against viral and fungal infections in the posttransplant period [322,329] while reducing the risk for

hepatobiliary infections may also contribute associated toxicities resulting in liver injury.

Pathogenesis

A number of pathophysiological mechanisms have been implicated in bone marrow transplant-related cholangiopathies. In cholangitis lenta, the release of bacterial cell wall components [330] as well as proinflammatory cytokines including IL-1, IL-6, and TNF-α results in bile flow impairment [331]. Biliary epithelial cells, zone 1 hepatocytes, and periductular glands have been recognized as intrahepatic targets for donor-derived lymphoid cells in acute GVHD [332]. Cell death mediated by apoptosis has also been observed but exact mechanisms for this process are not well understood [333]. Abnormalities in cellular and humoral immunity have been implicated in chronic GVHD [322]. Impaired bile salt and bilirubin transport is associated with hepatotoxicity from cyclosporine [334]. Bile canaliculus injury, hepatocyte necrosis, and elevated serum hepatic biochemistries are observed with supratherapeutic blood levels of cyclosporine [322,335]. Intrahepatic thromboses of terminal venules and central veins are observed in conjunction with clinical manifestations of SOS [324].

Clinical manifestations

Acute GVHD occurs among recipients of allogenic bone marrow and stem cell infusions. The development of a skin rash, evidence for gastrointestinal dysfunction (including nausea and diarrhea), and elevated serum hepatic enzymes are typical manifestations. Hepatic GVHD may also occur independently of skin and gut manifestations but is uncommon. Average time to onset is approximately 15–21 days after the bone marrow transplant. A delay in time to onset for acute GVHD has been reported with the use of immunosuppressive prophylaxis and nonHLA mismatched grafts. Increases in serum aminotransferase and alkaline phosphatase levels (2- to 10-fold each) with hyperbilirubinemia are characteristic. Decompensated liver disease (ascites, coagulopathy) is uncommon in the early stages of acute GVHD. A rapid increase in hyperbilirubinemia may suggest concomitant sepsis, cholangitis lenta, or SOS [322]. Liver biopsy performance is often required in this setting for diagnosis. Histological findings of acute GVHD include bile duct epithelial cell necrosis and apoptosis, mild to moderate hepatocyte necrosis, and cholestasis. Ductopenia has been reported in severe cases [336]. A recent study showed that a high level of lobular inflammation and a low level of hepatocyte ballooning were independent favorable prognostic factors for patients with GVHD [337]. The probability of developing chronic GVHD from acute GVHD is estimated at 50% or more. Progressive hepatic GVHD has been reported as an independent predictor of mortality after bone marrow transplantation. Clinical evidence for portal hypertension is often noted in the absence of histological cirrhosis when present [322].

Cholangitis lenta is associated with fever and mild hyperbilirubinemia in the early posttransplant phase and with the use of immunosuppressive treatment for acute GVHD. The time interval for preceding infection may be days to weeks. Elevations in serum alkaline phosphatase may be observed but isolated hyperbilirubinemia is the most common biochemical finding. Total bilirubin levels can reach values, as high as 15 mg/dL, but hepatic synthetic function is generally intact. Liver histology is noted for cholestasis within pericentral canaliculi and periportal areas in the absence of significant portal inflammation from immunosuppression [322,330]. The use of prophylactic antibiotics and antifungal medications has reduced the incidence of infections and sepsis and so of cholangitis lenta.

Drug-induced cholestasis can occur by idiosyncratic (cyclosporine) [338] or dose-dependent (methotrexate) [322] mechanisms. Among 38 patients receiving cyclosporine at 3 mg/kg/day [339], an increase in serum bilirubin levels was reported compared to patients given 1.5 mg/kg/day despite serum trough levels of cyclosporine in the therapeutic range (< 400 ng/ml) in the high dose group. In dosages up to 20 mg/kg/day, a majority of patients treated for acute leukemia developed jaundice and hyperbilirubinemia, which resolved after drug cessation in 2–5 days [340]. Azathioprine [341] and fluconazole [342] may be associated with hepatocyte necrosis yet more commonly result in asymptomatic serum aminotransferase elevations. Antibiotics including amoxicillin–clavulanic acid [343], flucloxacillin [344], and trimethoprim-sulfamethoxazole [345] have been reported in association with cholestatic hepatitis and chronic liver disease in rare instances. Ceftriaxone has been implicated in the pathogenesis of cholelithiasis [346]. Cholestasis is also observed after chronic total parenteral nutrition. Elevated serum liver biochemistries including total bilirubin, serum aminotransferases, and alkaline phosphatase can be attributed to total parenteral nutrition. Association between prolonged total parenteral nutrition and liver failure, however, remains rare. Reversal of liver biochemical abnormalities often occurs after discontinuation of total parenteral nutrition and resumption of oral intake. Other associated complications in the presence or absence of chronic total parenteral nutrition administration include biliary sludge, cholelithiasis, and acalculous cholecystitis [347].

Chronic GVHD of the liver has been associated with the rapid onset of bile duct destruction or subacute development of intrahepatic cholestasis. It may occur de novo or result from previous acute GVHD. Chronic GVHD generally occurs after 100 days post-bone-marrow-transplant. Hepatic involvement occurs in up to 80% of cases. Biochemical features typically include progressive elevations in serum alkaline phosphatase (as high as 15 times normal) and hyperbilirubinemia. Rapid onset chronic GVHD may present with elevated serum aminotransferases during tapering of immunosuppressive medications [322,323]. Histological features on liver biopsy include ductopenia, lymphoplasmacytic portal

inflammation, periportal fibrosis, and cholestasis. In select cases, a paucity of bile ducts may be observed (vanishing bile duct syndrome) [322,336].

The development of jaundice, hepatomegaly, and weight gain over 5% of ideal body weight is consistent with a diagnosis of SOS. The average time of onset after bone marrow transplant is between 15 and 30 days. Persistent jaundice after an episode of acute GVHD can also be from SOS in the setting of fluid retention. Biochemical features include isolated hyperbilirubinemia as the most common presentation [322,324,348]. Ultrasonography is commonly negative for biliary tract obstruction or hepatic vein thrombosis. Most patients with SOS have mild involvement and eventual spontaneous resolution. Progressive SOS, to include the hepatic venules (and thus venoocclusive disease) is associated with liver failure in select cases [348].

Treatment

Prevention of acute GVHD is the most important aspect of treatment because eventual multiple organ involvement is often fatal [349,350]. Conventional strategies have included combination pharmacological therapy with corticosteroids [322,326,351], cyclosporine [322,326], methotrexate [322,326,328], tacrolimus [328], or antilymphocyte antibodies [328]. Depletion of donor T lymphocytes by elution or monoclonal antibodies is associated with reductions in the incidence and severity of acute GVHD [352]. Reports of Epstein–Barr virus-associated lymphoproliferative disorder from depletion/elution techniques, however, have also been noted [353]. Systemic corticosteroid therapy (prednisone at 2 mg/kg/day) is the treatment of choice in acute GVHD with hepatic involvement [349]. Treatment failures (defined as persistent skin and gut symptoms) are managed with increasing corticosteroid dosages (4–8 mg/kg/day) [350] and the addition of tacrolimus [351] or antithymocyte globulin [352]. Empiric treatment of cholestasis-associated pruritus with UDCA may be of benefit in selected cases [354].

No specific therapy exists for cholangitis lenta. Treatment of the underlying infection often results in a reduction in hepatic biochemical elevations and improvement in bile flow [330].

Discontinuation of potential offending agents is the initial step in treating drug-induced cholestasis. Improvements in cholestasis and serum hepatic biochemistries occur within 5–7 days of cyclosporine discontinuation [322,340]. Dose reduction of cyclosporine or substitution with other anti-T-lymphocyte agents is recommended in the presence of acute GVHD to minimize worsening bile duct injury [322].

The use of immunosuppressive therapy including prednisone and cyclosporine is required for the treatment of chronic GVHD. Salvage therapy options include tacrolimus, thalidomide, or sirolimus [322,323]. Empiric UDCA to modulate symptoms from intrahepatic cholestasis may result in serum hepatic biochemical improvement [354].

Mild to moderate cases of SOS are treated with supportive measures. Diuretic therapy may be useful in patents with symptomatic fluid overload [322,324,348]. For severe cases of SOS, the use of thrombolytic therapy has been shown to improve hyperbilirubinemia in up to 30% of patients. The risk of significant hemorrhage among patients with preexisting thrombocytopenia including a fatality rate approaching 15% is associated with thrombolytic therapy [348,355]. Defibrotide, which has anti-thrombotic and anti-fibrinolytic properties, has been shown to improve survival in severe cases to 35%, with these patients showing complete resolution of the sinusoidal obstruction, with no adverse bleeding events [356]. The addition of antithrombin III infusion to high-dose defibrotide resulted in a complete response to therapy and 100-day survival in 93% of children with varying severity of SOS. This same study showed that the use of preemptive antithrombin III infusion did not prevent SOS development [357]. Transjugular intrahepatic portosystemic shunt placement to improve sinusoidal portal hypertension has been associated with an increased mortality in patients with severe SOS [358].

Liver transplantation

Cholangiopathies associated with liver transplantation can involve either intrahepatic or extrahepatic bile ducts with different entities tending to occur early (< 6 months) or late (> 6 months) in the posttransplant course (Table 75.6). The two most common forms of surgical biliary anastomoses performed in liver transplantation are choledochocholedochostomy (C-C) and choledochojejunostomy (C-J). Factors involved in choosing one type of anastomosis over another include underlying hepatic disease and size match between recipient and donor organ.

Epidemiology

Intrahepatic cholangiopathies after liver transplantation primarily involve the biliary epithelium. Cold ischemic injury and, possibly more importantly, reperfusion injury occur in bile duct epithelial cells [359]. Mild cases resolve spontaneously, but more severe cases may have residual damage (intrahepatic or extrahepatic) or result in primary nonfunction [360]. Blood-group-related antigens (ABH) are expressed on the epithelial cells of large bile ducts, and periductular hepatocytes, thus transplantation of ABO mismatch livers results in complications such as biliary stricturing, hepatic artery thrombosis, and cellular rejection [361]. Chronic "ductopenic" rejection occurs in 2%–5% of transplant patients and generally presents within the 6 weeks to 12 months after transplantation, but has been noted as early as 2–3 weeks postoperation. It is characterized by progressive loss of intrahepatic interlobular and septal bile ducts (ductopenia) with lipid-laden vasculopathy; more than 50% of the portal triads must be involved for it to be termed "ductopenic" [362]. As discussed previously, recurrent PSC

occurs in up to 20% of cases where strict criteria are applied to exclude other potential causes of intrahepatic biliary disease [267–269].

Extrahepatic cholangiopathies following liver transplantation include anastomotic strictures, hilar strictures, ischaemic-type bile duct stricturing, bile leaks, choledocholithiasis, and sphincter of Oddi dysfunction. The overall incidence of biliary complications ranges from 9% to 40% and complications usually develop 2–6 months after liver transplantation. Anastomotic strictures occur in 3%–7% of cases and may be early or late [363,364]. Hilar strictures after liver transplantation are associated with local ischaemia or hepatic artery thrombosis. Prolonged cold ischemia time and ABO incompatibility have also been reported as risk factors [365]. Bile leaks occur in up to 25% of transplant recipients [363,364]. Sites of injury include the biliary anastomosis, T-tube insertion site, reduced size parenchymal graft cut edge, or unrecognized collateral bile duct [363]. T-tube removal in association with C-C anastomosis is reported to comprise over 50% of all bile leaks [363,364]. The finding of choledocholithiasis after transplantation is generally associated with a biliary stricture [363]. Sphincter of Oddi dysfunction has been recognized after C-C reconstruction. Cholangiography is noted for a dilated common bile duct tapering to its origin [363,364].

Clinical manifestations

Patients with intrahepatic or extrahepatic cholangiopathies after liver transplantation often present with asymptomatic elevations in serum alkaline phosphatase or total bilirubin. Mild elevations in serum aminotransferases can occur as well. Alternatively, the presence of fever or right upper quadrant abdominal pain may be consistent with bacterial cholangitis [363,364]. Jaundice and pruritus are uncommon but can be associated with chronic ductopenic rejection [362]. Imaging studies with ultrasound or CT scanning are sensitive for the detection of intrahepatic duct dilation. Examination of hepatic arterial blood flow by Doppler ultrasonography is mandatory in the setting of biliary stricture to exclude thrombosis or stenosis [365]. If a T-tube is present, cholangiography is performed to determine the location and extent of biliary strictures when suspected [363,364]. ERCP has developed into the initial method of cholangiography in transplant patients for diagnostic and therapeutic interventions [366]. The use of PTC is indicated when distal bile duct dilation is not significant or evidence for a proximal biliary obstruction above the bifurcation is observed by imaging studies, as well as cases that cannot be accessed by ERCP (long Roux limbs etc) [363,367]. Histological examination of the allograft by liver biopsy is warranted if cholangiography is unremarkable.

Treatment

Ensuring adequate immunosuppression in the presence of chronic ductopenic rejection is mandatory. Conversion from cyclosporine to tacrolimus (FK506) is often recommended as resulting bile flow impairment reduces effective gastrointestinal absorption of cyclosporine. Treatment options for recurrent PSC deal primarily with stricture dilation with or without long-term biliary tube stenting [269]. Hepatic retransplantation in this population is the only available definitive solution if allograft failure ensues [269,367].

Surgical conversion to a Roux-en-Y choledocojejunostomy for biliary strictures associated with the C-C anastomosis was previously considered the treatment of choice after liver transplantation [363,364,368]. Recent advances in endoscopic and radiological techniques have demonstrated satisfactory results from balloon dilation or stenting. Balloon dilation of anastomotic strictures is successful in most cases. The use of stents significantly reduces the recurrence of stricturing [369]. Endoscopic management also appears more successful in the early strictures (< 6 months) as compared with late strictures (> 6 months) [370]. Patients who fail to respond after these nonoperative measures may then be considered candidates for biliary anastomosis reconstruction. Focal areas of stricturing in association with indwelling T-tubes, often related to postoperative edema, usually resolve with observation [363].

Hepatic artery thrombosis is described in 3%–9% of transplants, with another 3% of patients experiencing nonthrombotic hepatic artery complications, such as hepatic artery stenosis, redundancy, and pseudoaneurysm [371]. As the main blood supply to the biliary system stems from the hepatic artery, later complications tend to be biliary, including bile duct stricturing with subsequent cholangitis, liver biloma, or abscess or biliary necrosis with bile leak. Either the early or late presentation can progress to severe liver dysfunction and failure, however, severe graft dysfunction is observed more frequently in patients with early hepatic artery thrombosis. Retransplantation rates of 50%–80% and mortality rates of up to 50%–70% have been described in patients with hepatic artery thrombosis [372,373]. The correction of aberrant hepatic artery flow (by angioplasty or surgical revision if required) may be beneficial to prevent further bile duct injury and diffuse stricturing in the early postoperative period.

Hilar strictures are difficult to manage by operative intervention based on previous manipulation of the porta hepatis. Interventions with balloon dilation and percutaneous indwelling stent placement are often required. Problems with recurrent biliary obstruction from debris and mucosal sloughing (especially when ischemic cholangiopathy is present) require frequent tube manipulations and changes [368]. The effect of bilateral endoscopically placed stents (polyurethane and expandable metal) compared to percutaneous drainage is unknown.

Diffuse biliary stricturing can initially be managed with nonoperative methods including balloon dilation followed by stenting of dominant strictures [363,368]. Similar to hilar

strictures, the occurrence of stent occlusion has limited the long-term success of this strategy [368]. No benefit with UDCA or prophylactic antibiotic therapy for the prevention of biliary stricturing has been shown. Hepatic retransplantation in eligible candidates is often required for definitive therapy [367].

The management of choledocholithiasis is similar to non-liver-transplantation scenarios with the use of balloon dilation and stone removal by ERCP or PTC. Endoscopic sphincterotomy may or may not be required [363,366]. Sphincter of Oddi dysfunction, when confirmed using accepted criteria, is also treated by endoscopic sphincterotomy despite the absence of abnormal manometric findings [363].

Iatrogenic cholangiopathies

Operative bile duct injury

Epidemiology

Operative bile duct injury is most commonly associated with cholecystectomy (90% of instances). The incidence of bile duct injury with open cholecystectomy is estimated to be between 0.1%–0.2%, which includes minor duct (of Luschka) injuries [374]. Initial reports of bile duct injury from laparoscopic cholecystectomy between 1% and 2% have been reduced to 0.4%–0.6% [375]. Risk factors for bile duct injury include limited operative experience, acute or chronic inflammation involving the biliary tree and gallbladder fossa, and aberrant anatomy [376].

A classification system for bile duct injuries has been proposed by Strasberg and colleagues [377]. Type A injuries affect the minor ducts without the loss of biliary tree continuity, including the cystic duct and gallbladder fossa. Type B and C injuries involve aberrant right hepatic duct injuries. Occlusion of the cystic duct in this scenario is termed a type B injury whereas complete cystic duct transection is termed a type C lesion. Type D injuries involve incomplete lacerations of the primary bile ducts (common bile, common hepatic, left and right hepatic). Evolution of these lesions to type E injuries can occur. Type E injuries include five variations with lesions located between the common hepatic duct and above the bifurcation. Type A, D, and E bile duct injuries are most often reported.

Clinical manifestations

The timing and extent of injury often determines clinical presentation. Bile duct injuries are often not recognized at the time of surgery. The median time to symptoms is between 5 and 7 days but may be weeks. Fever, abdominal pain, or wound drainage can be observed. Jaundice may represent distal common bile duct occlusion or transection. A small proportion of patients will be asymptomatic at presentation. Imaging studies such as nuclear scintigraphy (HIDA scan) can document the existence of bile leakage in 90%–95% of cases. Identification and localization of bile duct injuries by cholangiography is required before repair [375,376]. Late complications including biloma formation and biliary stricture may occur [377].

Treatment

Endoscopic or percutaneous methods have been successfully employed to treat bile duct injuries. These measures are effective in the treatment of active bile leaks as well as benign postoperative strictures. Endoscopic sphincterotomy with or without nasobiliary tube or stent placement are equally effective for bile leak closure. Removal of stents in 3–4 months with repeat cholangiography to document bile leak closure is recommended. A disconnection between distal and proximal bile duct systems requires PTC for diagnosis and therapy [378].

Injuries noted at surgery require conversion to an open procedure with immediate repair. Techniques include suturing of linear lacerations, end-to-end anastomosis, and Roux-en-Y biliary reconstruction. Damage involving the common hepatic duct bifurcation is treated with biliary–enteric reconstructive techniques. Facilitation of surgical repair can be provided by preoperative stent placement. Continued stent placement after operative repair up to 3–6 months is advised [375,377]. Long-term results from surgical bile duct repair are good and are associated with the nature and extent of injury, the type of surgical reconstruction performed, and the level of experience at the treatment center [375,377].

Secondary sclerosing cholangitis

The development of cholangiographic changes similar to PSC has been reported as a secondary phenomenon resulting from complications of therapeutic interventions. The best-known causes of secondary sclerosing cholangitis are related to traumatic ischemic injuries to the vascular and subsequent biliary system [366] and hepatic artery infusion with floxuridine (FUDR) [73]. Prevalence rates for FUDR-associated secondary sclerosing cholangitis remain unchanged between 17% and 56%. Involvement of the common hepatic duct bifurcation with sparing of the common bile duct is characteristic. The arterial supply of extrahepatic bile ducts is derived primarily from the gastroduodenal arcade, which is often excluded from hepatic artery infusion. Ischaemia rather than direct FUDR toxicity to biliary epithelium appears to be the underlying mechanism of action [73]. Whereas injury appears to be contained within the biliary system, liver failure associated with FUDR-induced secondary sclerosing cholangitis is related to coexistent obstructive vasculopathy [73]. The injection of 20% formaldehyde with sodium chloride or ethanol for the treatment of hydatid disease has also been associated with secondary sclerosing cholangitis [379]. Direct sclerosant-type effects on biliary epithelia are the proposed mechanism of action.

Rare entities that have been described as etiologies of secondary sclerosing cholangitis include hepatic inflammatory pseudotumor, portal biliopathy (collective bile duct and gallbladder wall anomalies as a result of extrinsic compression

seen in patients with portal hypertension) and eosinophilic cholangitis [380].

Biliary stricture from chronic pancreatitis

Epidemiology

Biliary strictures from pancreatic disease occur based on anatomic proximity between the distal common bile duct and posterior pancreas. The presence of fixed structures including pancreatic cysts and masses are associated with extrinsic common bile duct compression. Biliary stenosis associated with the inflammation of acute and chronic pancreatitis is usually transient. Chronic biliary obstruction is noted among 4%–10% of patients with alcoholic pancreatitis. Biliary stricture following operative therapy for chronic pancreatitis is observed between 60% and 70% of cases [380,382].

Clinical manifestations

In chronic pancreatitis, there are no specific clinical features that suggest the presence of biliary obstruction. Serum hepatic biochemistries are noted for elevations in alkaline phosphatase without similar degrees of hyperbilirubinemia. Mild aminotransferase (78%) and amylase (47%) elevations can also occur. Among patients with postoperative bile duct strictures as many as 80% of cases are identified within the first postoperative year. Late manifestations may occur years after initial surgery [380,382]. Among 987 patients with benign postoperative biliary strictures followed over 25 years, the most common symptoms at presentation were fever (64%), abdominal pain (49%), and jaundice (44%). Elevations in serum alkaline phosphatase and bilirubin occurred in one-half of cases [382].

Diagnosis

Ultrasonography and cross-sectional imaging techniques are useful for documenting biliary dilation or biloma formation in select cases. Cholangiography (ERCP or PTC) can define both location and extent of stricture involvement [380,382]. A smooth, long stricture of the distal common bile duct between 2 and 4 cm is most characteristic. Hepatic histology can also identify biliary obstruction in 80% of cases involving chronic alcoholic pancreatitis with cirrhosis present in 29% of patients [380].

Course and complications

Complications associated with biliary stricture from chronic pancreatitis include cholangitis, choledocholithiasis, and secondary biliary cirrhosis. As many as 20% of reported patients have developed biliary cirrhosis or liver failure after biliary stricture formation from operative injury [380].

Treatment

In cases of biliary stricturing from chronic pancreatitis, the optimal management has yet to be defined. Surgical decompression is employed for high-grade obstruction complicated by jaundice or cholangitis [382]. Endoscopic approaches with stricture dilation and stenting have been performed with good results and minimal morbidity [383,384]. Symptomatic patients appear to benefit more from stenting with multiple stents compared to single stents [384]. Among patients with abnormal serum hepatic biochemistries and no symptoms, the pursuit of decompression is controversial. Liver biopsy features of progressive biliary fibrosis favor surgical decompression.

Biliary fistulae

Spontaneous enteric biliary fistula

Epidemiology

Prevalence rates between 0.5% and 5% after biliary tract operations have been reported. Gallstones are responsible for 90% of cases followed by peptic ulcer disease (6%) and trauma (4%). Cholecystoduodenal (75%), cholecystocolonic (15%), and cholecystogastric (6%) fistulae are the most frequent sites of tract formation. Cholecystoduodenal fistulae from gallstones almost exclusively involve the periampullary area. Fistulae may be obstructive or nonobstructive. The female to male ratio for both types is between 2 : 1 and 3 : 1. Gallbladder carcinoma has been associated with enteric biliary fistulae in 15% of cases [385–387].

Clinical manifestations

Nonobstructive fistulae are often diagnosed as incidental findings at cholecystectomy or cholangiography. Consequences of these fistulae include ascending cholangitis, weight loss, and bleeding. Obstructive fistulae present with symptoms that are reminiscent of bowel obstruction, which may be complete or partial [386]. Gallstone ileus can present with acute small bowel obstruction from gallstones of diameters greater than 25 mm. The ileocecal area is the most common site of obstruction (75%) followed by the jejunum (20%). Abdominal radiograph findings of pneumobilia are observed in only 30% of enteric biliary fistulae. Biliary reflux of oral contrast is more suggestive of biliary fistula [386].

Treatment

The treatment of choice when possible is operative. For gallstone ileus, the performance of cholecystectomy, fistula excision, and operative cholangiography to exclude choledocholithiasis is recommended. Mortality rates approaching 20% in previous reports have been reduced below 5% [385].

Operative biliary fistula

Most commonly observed in association with bile duct injury after cholecystectomy. The development of a cutaneous or internal bilioperitoneal fistula occurs in 33% of postoperative cases. Hepatobiliary scintigraphy (i.e., HIDA scan) can detect bile leaks and fistulae with greater than 90% sensitivity. Cholangiography may ultimately be required for diagnosis

confirmation. Endoscopic therapy with nasobiliary tube placement or stents have been successful in selected cases. Treatment failures require surgery or interventional radiology [387,388].

Bronchobiliary fistula

Overall prevalence rates are unknown but range between 4% and 10.5% among patients with hepatic and subphrenic abscesses, respectively. Other etiologies responsible for bronchobiliary formation include blunt abdominal trauma, bile duct stenosis, choledocholithiasis, and congenital causes. Clinical manifestations include bilioptysis, jaundice, and cholangitis. Diagnosis has been made by cholangiography and biliary scintigraphy. The therapy of choice is surgical [389].

Miscellaneous biliary fistula

Vascular fistulae with the biliary tree may occur and include both arterial and venous systems. Causes include trauma, infection, or iatrogenic factors (operative, transjugular intrahepatic portosystemic shunt). Hemobilia is the most common sign at presentation. Biliobiliary fistulae involving the gallbladder, cystic duct, or secondary biliary radicle with the main biliary tree can also occur. Compression by stone impaction in the cystic duct or gallbladder neck (Mirizzi syndrome) may result in erosion through the common bile duct resulting in fistula formation [390].

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76 Cystic diseases of the liver and biliary tract

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Introduction, 2009
Cystic diseases of the biliary tract, 2009
Polycystic liver disease, 2017

Introduction

Cystic dilations occur throughout the liver and biliary system. They most commonly affect the common bile duct (CBD) (i.e., choledochal cyst), but cystic dilations involving the intrahepatic ducts alone or in combination with other cystic abnormalities of the extrahepatic ducts are increasingly recognized. The terminology and classification are tremendously confused. The term choledochal cyst has commonly referred to all cystic abnormalities of the biliary tree; however, this term is best restricted to only cystic abnormalities of the CBD. Despite the many variations of these cystic abnormalities, some underlying features unify them. Biliary cysts are distinct from polycystic liver disease (PCLD), which is a rare hepatobiliary fibropolycystic disorder that is characterized by the progressive development of fluid-filled biliary epithelial cysts in the liver. This chapter reviews current understanding of cystic diseases of the biliary tract and PCLD.

Cystic diseases of the biliary tract

Epidemiology

Biliary cysts are rare, with the highest incidence in Japan, where one-half to two-thirds of all reported cases have originated [1–3]. The Japanese incidence (1 in 13 000 births) is significantly higher than in the West (1 in 150 000 births) [3,4]. The incidence is much higher among women, with a female to male ratio of 3 : 1 or 4 : 1. Between 40% and 60% of patients are diagnosed before 10 years of age, 52%–76% before 20 years of age, and 83%–90% before 30 years of age [1–3,5]. This is mainly a disease of children and young adults, although more recent series indicate that the incidence in adults is much higher than previously thought [6].

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Classification

Choledochal cysts were first described by Vater and Ezler in 1723. Alonzo-Lej and colleagues classified cysts into three types [7]. Later, Todani and associates proposed the most complete and practical classification that recognizes five types of bile ducts cysts (Fig. 76.1) [8].

- Type I cysts include choledochal cysts in a narrow sense; segmental choledochal dilations; and diffuse or cylindrical dilations. Between 75% and 85% of cases are type I.
- Type II or diverticulum cysts are found anywhere in the extrahepatic ducts and make up 2%–3% of reported cases.
- Type III or choledochocele cysts, also rare, represent 1.4%–5.6% of reported cases. These are cystic dilations involving only the intraduodenal portions of the CBD. In 1989, Sarris and Tsang reviewed 48 cases in the English-language literature and proposed a further anatomic classification of choledochoceles [9]. In type A, the ampulla opens into the choledochocele, which communicates with the duodenum through another small opening. Type A choledochoceles can be further subclassified into A1, in which the pancreatic duct and CBD share a common opening into the cyst (33% of cases); A2, in which the openings are distinct (4% of cases); and A3, in which the choledochocele is small and entirely intramural (25% of cases). In type B, the ampulla opens directly into the duodenum, with the choledochocele communicating only with the distal CBD (21% of cases). This scheme is useful because of important therapeutic implications.
- Type IV cysts are also subclassified. In type IVA, multiple cysts in the intrahepatic and extrahepatic ducts account for 10%–15% of reported cases [3,10]. In type IVB, multiple cysts occur only in the extrahepatic duct and are much less common.
- Type V intrahepatic bile duct cysts (single or multiple) are also rare. They probably represent Caroli disease as originally described.

Serena and colleagues proposed a type VI biliary cyst, which is cystic dilation of the cystic duct with otherwise normal intrahepatic and extrahepatic ductal systems [11]. Three cases of type VI cysts had been described in the literature up to 1999 [12].

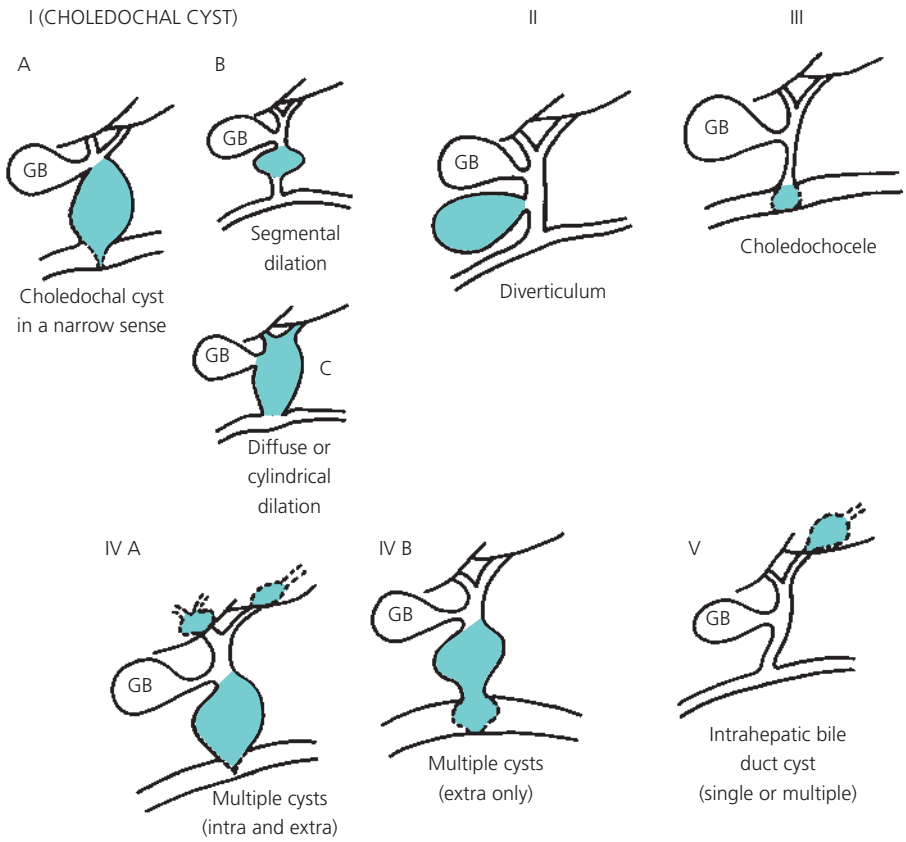


Figure 76.1 Todani's classification of biliary cysts based on location. Hatched areas represent cystic dilations.

Forme fruste choledochal cyst is a variant of choledochal cyst that has little to no cystic dilation but is associated with anomalous pancreaticobiliary ductal union (APBDU) [13]. In one series, 17 of 281 choledochal cysts were of this variant [13].

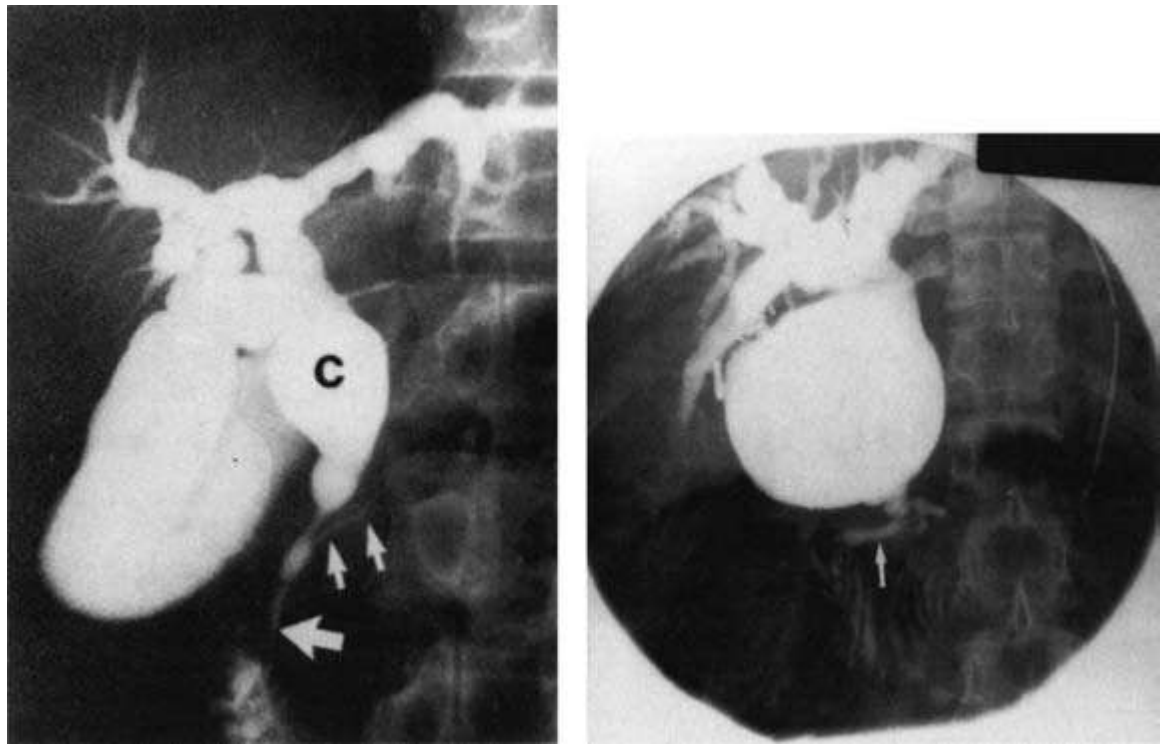
Anatomically based classification is of great practical importance in planning surgical management. Other classifications emphasize clinical presentation rather than ductal anatomy.

Pathogenesis

The cause of biliary cysts has been disputed. The major debate concerns whether cysts are congenital or acquired. Genetic factors may also be involved because the disease is more common in Japanese and familial occurrence has been reported. However, a study of a pair of monozygotic twins showed discordance for both anomalous pancreaticobiliary junction and biliary cyst [14], which is not compatible with single gene control.

Many early investigators favored the congenital malformation theory [15]. Several observations support this. First, biliary cysts appear in all age groups, and antenatal diagnosis has been reported as early as 15 weeks' gestation, making a congenital origin likely. There are also many associated anomalies of the biliary system, such as double common bile duct, double gallbladder, multiseptate gallbladder, biliary atresia, congenital hepatic fibrosis, and annular pancreas [16]. In addition, many cysts fail to shrink after decompressive surgery, suggesting inherently abnormal ductal wall development [2].

Of the theories proposing acquired cysts that of Babbitt and associates has received the most attention [17]. They proposed that choledochal cysts develop because of an anomalous arrangement of the distal pancreaticobiliary tree. In all seven of their patients with choledochal cysts, they found the CBD entered the pancreatic duct at a right angle at an abnormally long distance (> 2 cm) from the ampulla of Vater (Fig. 76.2). This abnormal anatomy impaired normal sphincteric function at the pancreaticobiliary junction. Because the maximal secretory pressure of the pancreas is 30 cmH_2O to 50 cmH_2O and that of the liver is 25 cmH_2O to 30 cmH_2O, pancreatic juice flows into the bile duct in the absence of a sphincter mechanism. This anomaly is associated with recurrent cholangitis, edema, fibrosis, obstruction of the bile ducts, and carcinoma. These complications are believed to result from chronic reflux of pancreatic juice and subsequent activation in bile of various pancreatic proteinases and lipases. In support of this are the high levels of amylase that are frequently found in the cyst fluid and the demonstration that pancreatic juice in the CBDs of animals with an anomalous arrangement can cause cystic dilation of the CBD [5,18]. Although amylase has been found in the cyst fluid, this enzyme probably does not play a pathogenic role. Amylase is not detectable in the duodenal fluid of newborns and infants,



(a) (b)

Figure 76.2 Choledochal cyst–anomalous ductal relationship. **(a)** Cystic enlargement of the common bile duct (C) with associated dilation of the common hepatic and intrahepatic ducts. The abnormal junction of pancreatic (small arrows) and common bile ducts and long common channel (large arrow) is displayed. **(b)** A large cyst involves the common hepatic and common bile ducts accompanied by intrahepatic ductal dilation. A long common channel (arrow) results from the abnormally high junction with the pancreatic duct.

but trypsin activity is the same in newborns and older children [19]. Another substance that is markedly increased in the bile of patients with APBDU (most also had biliary cyst) is lysophosphatidylcholine [20]. This was the result of increased hydrolysis of phosphatidylcholine, a normal component of bile, by increased activity of phospholipase A_2. Lysophosphatidylcholine is cytotoxic so it may play a role in the pathogenesis of biliary cysts by adversely affecting an already weakened bile duct wall and in the later development of malignant degeneration [20]. However, this theory does not explain the cause of intrahepatic cysts because of their distance from the anomalous union [21].

APBDU occurs in 65%–80% of patients with choledochal cysts [15]. APBDU is not unique to patients with biliary cysts, it is also associated with biliary malignancies (especially gallbladder carcinoma) and abnormal pancreatic ducts [22]. In a large study of 1325 patients who were examined with direct cholangiography for symptoms suggestive of hepatobiliary and pancreatic disease, 29 patients had the anomaly (2.2%), of which 21 had associated choledochal cyst. Surprisingly the frequencies of gallbladder carcinoma in patients with the anomaly were 50% and 5% in the absence or presence of associated choledochal cyst, respectively. None of the patients with gallbladder carcinoma had gallstones. The overall frequency of carcinoma of the biliary tract was 14% in patients with choledochal cyst and 50% in those without. This striking difference led the authors to recommend prophylactic cholecystectomy in patients with an APBDU [23]. Similar findings and recommendations have been reported by Sugiyama and Atomi [24].

Many investigators emphasized that cystic dilation results from a combination of two factors: segmental weakness of the duct wall and distal obstruction [25]. The weakness of the wall and the distal obstruction may be congenital or acquired. Several animal experiments support the importance of distal obstruction [2,26]. Ligation of the distal CBD in adult rabbits, rats, and dogs gives rise to diffuse dilation of the intrahepatic and extrahepatic ducts. This also occurs in neonatal lambs [26]. After CBD ligation, all six lambs in the study developed choledochal cysts, and all four adult sheep developed isolated dilation of the gallbladder. However, whether these animal experiments are applicable to humans remain unclear because not all choledochal cysts have demonstrable distal obstruction.

The infantile form of choledochal cysts appears in some cases to result from an obliterative process similar to that producing biliary atresia. Twenty-four (13%) of 188 patients with biliary cysts were also found to have biliary atresia [27]. However, well-documented cases of acquired biliary cysts in adults have appeared, including seven attributed to recurrent

attacks of pancreatitis [28]. More recent studies suggest that many other factors may also be involved, including abnormal sphincter of Oddi pressure [29], viral infection, and genetic mutations [30–32]. Tyler and colleagues found reovirus in 13 of 23 tissues from patients with extrahepatic biliary atresia and 7 of 9 tissues from patients with choledochal cysts, prevalences that were much higher than in patients with other hepatobiliary diseases (21%) or in autopsy cases (12%) [30]. This is intriguing because reoviruses can produce a disease in weanling mice with similar pathological features to human choledochal cysts [30]. However, the number of patients with choledochal cysts examined was small and causality remains to be established. Two cases of choledochal cysts in association with rare genetic syndromes have been described [31,32]. One was in association with familial adenomatous polyposis syndrome [31] and the other was in association with Simpson–Golabi–Behmel syndrome [32]. These investigators raised the possibility that mutations in their respective genes may have contributed to the development of the choledochal cyst.

Two recent studies advanced our understanding of the pathogenesis of Caroli syndrome (type V biliary cysts in association with congenital hepatic fibrosis – see Clinical presentations). First is the identification of impaired protein N-glycosylation because of phosphomannose isomerase deficiency as a cause of congenital hepatic fibrosis, a disorder of ductal plate malformation often associated with type V cysts [33,34]. These patients often present with protein-losing enteropathy, which responds to D-mannose treatment [34]. Whether the hepatobiliary abnormalities will improve is unknown. These recent findings also suggest that normal protein glycosylation is important in the formation of ductal plates. Another advance is the development of a novel animal model with features of autosomal recessive polycystic kidney disease, multiple intrahepatic bile duct dilations, and congenital hepatic fibrosis [35]. These animals exhibit increased proliferative activity and decreased apoptotic activity in the biliary epithelial cells during development compared to controls, suggesting that the remodeling defect in immature bile ducts associated with the imbalance of cell kinetics play a role in the development of the hepatobiliary abnormalities [35]. One study in rats with autosomal recessive polycystic kidney disease suggested that overexpression of the mitogen-activated protein kinase signaling pathway may be involved in biliary dysgenesis because interference with this pathway demonstrated a decrease in the proliferation activity of biliary epithelial cells [36].

It is clear that many factors are important in the pathogenesis of biliary cysts, which encompass a wide spectrum of lesions.

Clinical presentations

Type I or choledochal cysts

Patients with choledochal cysts present with two typical clinical constellations, which are determined mostly by the patient's age at presentation [2]. In infancy, jaundice with or without acholic stools is the most common finding, occurring in as many as 80% of patients [2]. The presentation is often indistinguishable from that of biliary atresia, which is sometimes associated with choledochal cysts [27]. Pain may or may not be a factor, but vomiting and failure to thrive have been reported in as many as 50% of patients [2]. Hepatomegaly is often found, and 30%–60% of patients have a palpable abdominal mass [2]. The mass is usually in the right hypochondrium, soft, elastic, round, mobile laterally, and may follow the movements of the diaphragm [8]. The classic clinical triad of pain, jaundice, and a palpable abdominal mass has been found only in 11%–63% of large series [2]. Frequently, biliary cirrhosis and portal hypertension complicate the management [27].

The noninfantile form (in patients older than 2 years) covers a wide age range, with the oldest patient reported being in the eighth decade. Asymptomatic patients have been described [37]. Chronic and intermittent pain appears to be the most common presenting symptom, reported in 50%–96% of patients [38]. Intermittent jaundice and recurrent cholangitis are also common, reported in 34%–55%. Abdominal mass is much less common; it occurs in about 10%–20% of patients. The classic triad of pain, jaundice, and a palpable mass has been reported in 3%–13% of patients [38]. Cirrhosis and portal hypertension are less often encountered. Pancreatitis may be a presenting symptom in up to 33% of the patients in the noninfantile form, possibly as a result of immature pancreatic exocrine function in infants less than 1 year old [39]. However, whether or not there is actual pancreatic inflammation is debatable [40]. When these patients present with painful attacks, they often show slight elevation of serum bilirubin levels and an increase in the degree of the choledochal dilation, possibly caused by biliary obstruction. The terms fictitious pancreatitis and pseudopancreatitis have been applied to these patients [40].

The other presentation that has been reported only in patients older than 10 years of age is that of carcinoma associated with choledochal cysts [41].

Type II or diverticulum cysts

More than 50 cases of type II or diverticulum cysts have been reported in the world literature, representing 2%–3% of large series [39,42]. Presenting symptoms generally reflect the compression of nearby structures by the cyst.

Type III or choledochocele cysts

About 125 cases of choledochocele have been reported [39,43–45]. More than 73% of reported patients were older than 20 years of age, unlike type I cysts, which are found in 60% of the patients with biliary cysts younger than 10 years of age [44]. There is also less female preponderance, with a female to male ratio of 1.4 : 1 [44]. Common presenting symptoms include intermittent episodes of abdominal pain

and obstructive jaundice. An abdominal mass is not found. Pancreatitis is much more common in choledochocele than in any other type of biliary cyst and is reported in 30%–70% of these patients, presumably caused by reflux of bile or obstruction [45]. Stones in the cele or the CBD are also much more common, reported in 25%–35% of these patients, in contrast to only 8% in type I cysts [39,45]. Rarely, intestinal (duodenojejunal) intussusception may be the presenting feature of choledochocele [43].

Type IV and V cysts or Caroli disease

Caroli and colleagues originally described this disease as a congenital malformation of the intrahepatic bile ducts, characterized by segmental cystic dilation of the intrahepatic ducts; increased incidence of biliary lithiasis, cholangitis, and liver abscesses; absence of cirrhosis and portal hypertension; and association of renal tubular ectasia or similar renal cystic disease [46].

After the first report, Caroli recognized that two distinct disease entities are associated with intrahepatic duct cysts: the simple type, which was his original description, and the periportal fibrosis type [47]. The simple type is associated with medullary sponge kidney in 60%–80% of cases. The periportal fibrosis type is also known as Caroli syndrome. In addition to intrahepatic cystic dilation, congenital hepatic fibrosis, cirrhosis, portal hypertension, and esophageal varices are frequently seen. Hepatic function is usually well preserved in the periportal fibrosis type; the major clinical manifestations are recurrent cholangitis, liver abscess formation, and portal hypertension. It is often associated with the renal abnormalities of autosomal recessive polycystic kidney disease [33].

It is apparent that Caroli disease, including the simple and periportal fibrosis types, represents only part of the spectrum of cystic disease of the intrahepatic ducts. In an excellent review by Barros and associates of 46 well-documented cases with hepatic histopathology and biliary tree studies available, only 13% of patients had the simple type [48]. Thirty-five percent had the periportal fibrosis type, 22% had intrahepatic and extrahepatic cystic dilation (i.e., type IVA of Todani's classification), and 30% had all three abnormalities: cystic dilation of the intrahepatic and extrahepatic bile ducts, and congenital hepatic fibrosis.

Congenital hepatic fibrosis refers to a unique congenital liver histology characterized by bland portal fibrosis, hyperproliferation of interlobular bile ducts within the portal areas with variable shapes and sizes of bile ducts, and preservation of normal lobular architecture [48]. The prevalence of biliary cysts in patients with congenital hepatic fibrosis has probably been underestimated, because most patients did not have cholangiographic studies performed.

The aforementioned forms of intrahepatic biliary cystic disease should be differentiated from PCLD (see Polycystic liver disease) because of significant dissimilarities. PCLD is an autosomal dominant genetic defect, but the manner of inheritance of Caroli disease remains poorly defined [33]. Polycystic liver cysts are large, contain serous fluid, and do not communicate with the biliary tree. Caroli disease cysts communicate with the biliary tree. Portal hypertension is rare in PCLD. The prognosis for PCLD patients is determined by the effect of cystic disease on the kidneys and not the liver [49]. The incidence of malignant transformation in the intrahepatic parenchymal cysts of PCLD is 1.3%, compared with 7% in Caroli disease [50].

Caroli disease is rare, and males and females are equally involved [50]. Symptoms usually appear in early adult life, with recurrent episodes of fever, chills, and abdominal pain as the result of cholangitis. More than 80% of patients present before the age of 30 years. Biliary lithiasis is found in 34% of these patients, which may predispose to recurrent cholangitis [48]. Occasionally, the disease is diagnosed later in life, with the sequelae of portal hypertension, most commonly bleeding esophageal varices [47,50].

Diagnosis

Diagnosis of biliary cysts requires a high index of suspicion. Biliary cyst should be included in the differential diagnosis of recurrent abdominal pain, pancreatitis, obstructive jaundice, or cholangitis. Most patients do not present with the classic triad of pain, jaundice, and abdominal mass. Biochemical tests only reflect the degree of biliary obstruction and infection. Correct diagnosis depends on the use of imaging studies.

Ultrasonography is the best screening method for type I, II, IV, and V, but not type III, biliary cysts [1,2,51]. Ultrasonography has allowed the antenatal diagnosis of choledochal cyst in at least 17 cases [52]. When a choledochal cyst is suspected on prenatal ultrasonography, repeat ultrasonography is indicated immediately after birth so that expeditious treatment can be provided. The major drawback to ultrasonography is that it provides little anatomical or functional information. Furthermore, choledochal cysts can be confused with other cystic lesions, such as hepatic cysts, pancreatic pseudocysts, enteric duplication cysts, hepatic artery aneurysms, and hydatid cysts [53].

Hepatobiliary scintigraphy has been advocated as a sensitive, noninvasive tool for the diagnosis of choledochal cyst. The characteristic findings are biliary tract dilation and tracer retention after 24 h [54]. It provides information about excretory patterns and has therefore been recommended by many for postoperative patient follow-up. This technique is useful for all except type III cysts, because it may fail to demonstrate tracer accumulation in a small choledochocele [51,54].

Ultrasonography and scintigraphy are complementary and provide a sound basis for preoperative diagnosis in patients younger than 2 years of age. For older patients, computed tomography (CT) has been advocated by some to be superior to ultrasound [54,55]. Percutaneous transhepatic cholangiography (PTC) and endoscopic retrograde cholangiopancreatography

(ERCP) have been regarded as providing the best detailed examinations. PTC is especially useful in defining complex anatomic cysts of the intrahepatic ducts, but ERCP provides the best visualization of the distal portion of the biliary tree, especially the relation of the biliary and pancreatic ducts [2]. This notion has been challenged in recent years by improvements made in magnetic resonance imaging [56,57]. Several studies showed that magnetic resonance cholangiopancreatography (MRCP) is as good as ERCP in the evaluation of biliary cysts, especially in adults [56,57]. However, MRCP can overlook small choledochoceles, may be inadequate for visualizing the terminal CBD or APBDU, and have lower concordance with ERCP for type I and type IVA cysts [58]. Nevertheless, because MRCP is noninvasive and is not operator-dependent, it is likely to replace ERCP and PTC as the imaging study of choice in patients with biliary cysts.

ERCP remains useful in the diagnosis of choledochoceles. The typical features are the "clubbed" appearance of the distal CBD and a round, cyst-like, contrast-filled structure in the terminal CBD, which often protrudes into the duodenal lumen. Emptying of contrast material is often delayed. Choledochoceles are easily differentiated from duodenal diverticula and duodenal duplication cysts by filling during cholangiography but not during upper gastrointestinal contrast studies (Fig. 76.3). Duodenal diverticula fill on upper gastrointestinal series but not on cholangiography. Duplication cysts do not fill with either method [59].

Endoscopic ultrasound has also been used as an additional modality with which to visualize extrahepatic biliary cysts and provide detailed images of the cyst wall and APBDU [60].

The definitive diagnosis of biliary cyst and its classification is secured by intraoperative cholangiography at the time of operative intervention. Delineation of the precise anatomy is critical in preventing injury, especially to the pancreatic duct because of the high frequency of the anomalous union, and in providing the best operative management possible [2].

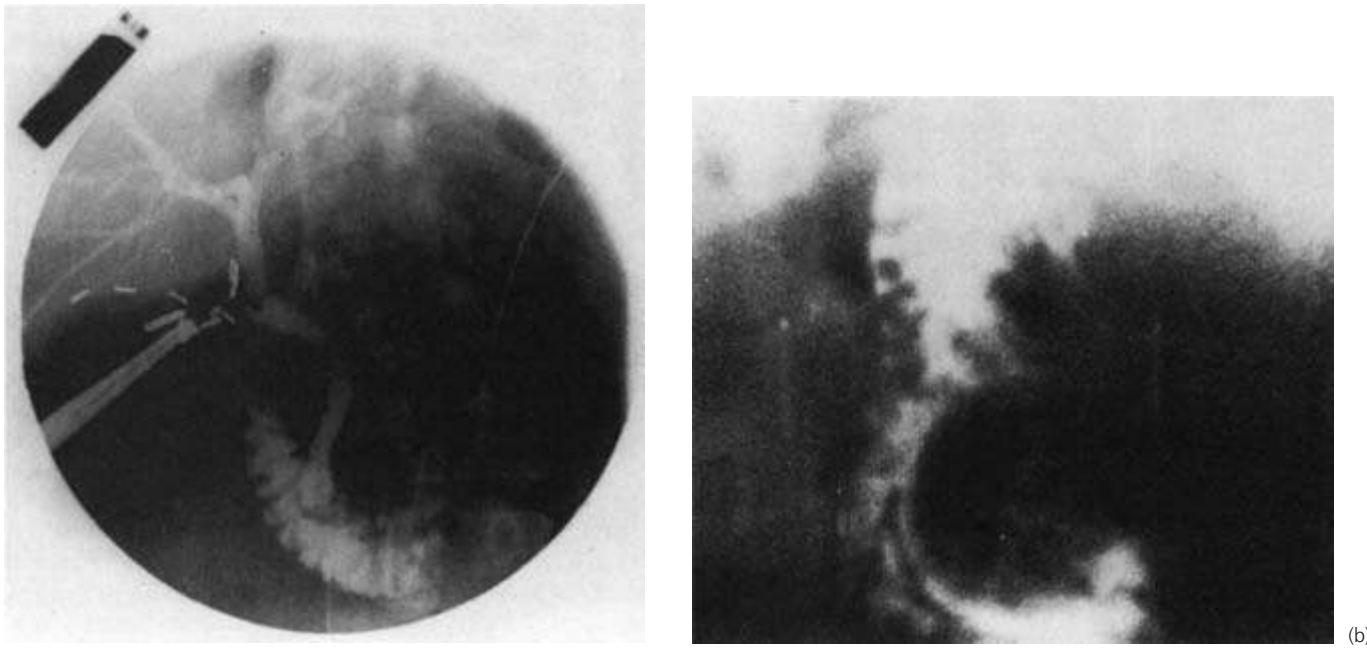
Pathology

The wall of the cyst is usually thickened because of productive fibrosis and inflammation. Histologically, dense connective tissue, fibrocollagenous and sometimes with smooth muscle and elastic elements, is seen. There is no epithelial lining, but islets of cylindrical or columnar epithelium may be preserved. A case of biliary papillomatosis arising in a choledochal cyst wall was reported [61]. Duodenal mucosa covers choledochoceles externally but the internal surface of choledochoceles is variably lined by duodenal mucosa (63% of cases), bile duct mucosa (21% of cases), or unclassified glandular epithelium (13%) [62].

Liver biopsy is abnormal in 60% of patients with biliary cysts, demonstrating biliary cirrhosis, portal fibrosis, or evidence of biliary atresia. Other abnormal findings include liver abscesses, especially in cases of Caroli disease and sludge or stones in the CBD. In newborn patients with biliary cysts, the histology is usually normal or shows mild bile duct proliferation consistent with biliary obstruction [15].

Treatment

Biliary cyst treatment is surgical with primary cyst excision as the procedure of choice [8]. Internal drainage procedures,



(a) (b)

Figure 76.3 Choledochocele type III cyst. **(a)** Cholangiography reveals a club-shaped enlargement of the distal common bile duct bulging into the duodenum. **(b)** Upper gastrointestinal series reveals a smoothly rounded filling defect in the second portion of the duodenum.

such as choledochoduodenostomy and Roux-en-Y choledochojejunostomy, are not recommended because of the high morbidity rates, the need for reoperation, and the risk of malignant degeneration [2,27]. Morbidity rate can be up to 88% with internal drainage procedures, compared with 2%–8% with cyst excision [27]. In large series, 13%–70% of patients have required reoperations after internal drainage, but a 0%–10% reoperation rate was reported after cyst excision [27,63]. Most importantly, the risk of malignant transformation is not diminished after an internal drainage procedure (see Clinical course and complications). Patients who develop biliary carcinoma after the excision of choledochal cyst are extremely rare; only three had been reported up to 1984 [64]. The fundamental reason for poor outcome after internal drainage is that diseased tissue remains and is used for biliary reconstruction. The cyst wall is inherently inflamed and fibrous, precluding normal healing. Biliary stasis persists and predisposes to recurrent cholangitis, and stone and stricture formation.

The aforementioned techniques are applicable for choledochal cysts (i.e., type I and IV cysts involving the CBD). Type II cysts should be excised. Treatment for type III cysts depends on the anatomy (see Fig. 76.1). Sarris and Tsang recommend excision of the duodenal luminal portion of the cyst, leaving the medial portion containing the ampulla intact as the preferred treatment in most cases of type IIIA cysts (i.e., A1 and A2) [9]. Transduodenal sphincteroplasty and ERCP with papillotomy have been advocated as treatment for type IIIA3 cysts. Type IIIB cysts should be treated by excision and sphincteroplasty. The malignant potential of choledochocele appears to be very low [44,45]. However, Schimple and colleagues pointed out that carcinoma has only been reported in choledochoceles that are lined by biliary or undifferentiated epithelium internally (up to 20% in some reports). They advocate complete excision of the choledochocele by separation and reinsertion of the CBD and pancreatic duct into the duodenal wall if the choledochocele is lined by biliary or undifferentiated epithelium, whereas sphincteroplasty should be reserved only for those lined by duodenal epithelium [65].

Intrahepatic cyst (i.e., type IVA, V) treatment depends on the degree of involvement. When segmental cystic disease is confined to one lobe (more often the left lobe), then lobectomy is usually curative [50,66]. If both lobes are involved, some have advocated establishing a permanent-access hepaticojejunostomy after partial hepatectomy to allow easy biliary tree access when necessary [66]. If extrahepatic cysts coexist with intrahepatic cysts (type IVA), they should be excised as previously described. Chronic antibiotic therapy in multilobar Caroli disease may have some benefit. Ultimately, if attacks of cholangitis are frequent and quality of life is poor, hepatic transplantation may be a therapeutic option. A summary of the recommended surgical treatment is given in Table 76.1.

In a long-term study of children with forme fruste choledochal cysts who underwent extrahepatic duct excision followed by Roux-en-Y hepaticojejunostomy, there were significant bile duct changes, including inflammatory cell infiltration, mucosal ulceration, and fibrosis, despite minimal cystic dilation [13]. These pathological changes are similar

Table 76.1 Suggested treatment for biliary cysts

Type of biliary cyst	Suggested treatment
Type IA	Cyst excision with Roux-en-Y hepaticojejunostomy or jejunal
Type IB	Interposition hepaticoduodenostomy
Type IC	Choledochojejunostomy or sphincteroplasty if excision is impossible
Type II	Excision if possible
Type IIIA1	Excision
Type IIIA2	Excision
Type IIIA3[a]	Sphincteroplasty or endoscopic retrograde cholangiopanctreatography with papillotomy
Type IIIB	Excision and sphincteroplasty
Type IVA	Extrahepatic cyst excision with biliary reconstruction as in IA and IB. Lobectomy if intrahepatic cyst is confined to one lobe or permanent access hepatocojejunostomy if intrahepatic cysts are diffuse Transplantation in selected cases
Type IVB	Cyst excision as in IA, IB
Type V	Hepatic resection for localized disease or permanent access hepaticojejunostomy for diffuse disease Transplantation may be an option

a Complete excision of the choledochocele by separation and reinsertion of the common bile duct and pancreatic duct into the duodenal wall if the choledochocele is lined by biliary or undifferentiated epithelium.

to those found in choledochal cysts and may place these children at high risk of malignancy. Shimotakahara and colleagues recommended extrahepatic duct excision followed by Roux-en-Y hepaticojejunostomy for all children with forme fruste choledochal cysts. However, the management of this variant in adults is still controversial [13].

Laparoscopic cyst resections and external anastomosis of choledochojejunostomy for Type I, II, and IV cysts have been reported [67,68].

Cholecystectomy should be performed at the time of cyst excision because continuous free bile flow minimizes the risk of reflux and cholangitis and because the gallbladder has shown clinical and histological evidence of cholecystitis in patients in whom secondary operations have been necessary. Furthermore, the gallbladder is predisposed to malignant change in patients with biliary cysts [69].

If the definitive surgical procedure is successful, the prognosis is generally excellent. Reversal of cirrhosis has even been reported [70].

Clinical course and complications

Complications of biliary cysts include recurrent cholangitis before treatment and after internal drainage procedures, stone formation, stenosis and stricture, pancreatitis, biliary cirrhosis, portal hypertension as the result of cirrhosis or portal vein thrombosis, liver abscess, especially in Caroli disease, and cyst rupture [9,27,71]. Gallstones may be present in approximately 25% of patients [51]. Stones can be trapped within the choledochal cysts, causing cholestasis, cholangitis, and cholecystitis [51]. Pregnancy can precipitate or aggravate symptoms of choledochal cyst, specifically cyst rupture. Twenty-six cases have been reported to rupture during pregnancy or labor [72]. Pregnant women with symptomatic choledochal cyst should avoid labor by having cesarean sections as soon as the fetus is mature.

After surgical intervention of choledochal cysts, early complications include bile leak and cholangitis [73]. Late complications depend on the type of surgery, but often include cholangitis or pancreatitis [73]. Regardless of the type of surgical reconstruction, the incidence of postoperative cholangitis is approximately 20% [74]. After Roux-en-Y hepaticojejunostomy, patients may develop diarrhea, fat malabsorption, and malnutrition.

The most feared complication is malignancy [9,75]. Carcinoma afflicts predominantly adults. The youngest patient found to have carcinoma during initial choledochal cyst surgery was 10 years of age [75]. The mean age for detecting carcinoma after enteric drainage was 35 years, with a mean interval of 10 years after the enteric drainage procedure [75]. The mean age for detecting carcinoma at the initial surgery for biliary cysts was 50 years; this is two decades earlier than the mean age for bile duct carcinoma in the general population [75]. Even though the overall incidence of biliary carcinoma in association with biliary cysts is 2.5%, it is actually 14%–18% in adult patients (thos older than 20 years) and is 50% by 50 years [2,41,75]. In contrast, the overall incidence of biliary carcinoma is 0.012%–0.48% in the general population [76]. The female to male ratio is 2.5 : 1, slightly less than that seen in choledochal cyst.

Carcinomas occur not only in the cyst wall; the remainder of the hepatobiliary and pancreatic tree can be affected. Of 154 cases of carcinoma in cases of biliary cysts, only 58% were actually in the cyst wall, 40% were gallbladder, 1.3% were pancreatic, and 0.6% were intrahepatic carcinomas [75].

The pathogenesis of malignant change in biliary cysts is unknown. Stones are not thought to be important because they are found in only 13.8% of patients with malignant change in the biliary cyst (Fig. 76.4) [75]. Adenocarcinoma is the most common type, constituting 70%–84% of all types. Other histological types are squamous (4%–9%) and undifferentiated or anaplastic (7%–21%), and one case of small cell carcinoma was reported [75]. Numerous studies have found that APBDU is a risk factor for biliary tract carcinoma

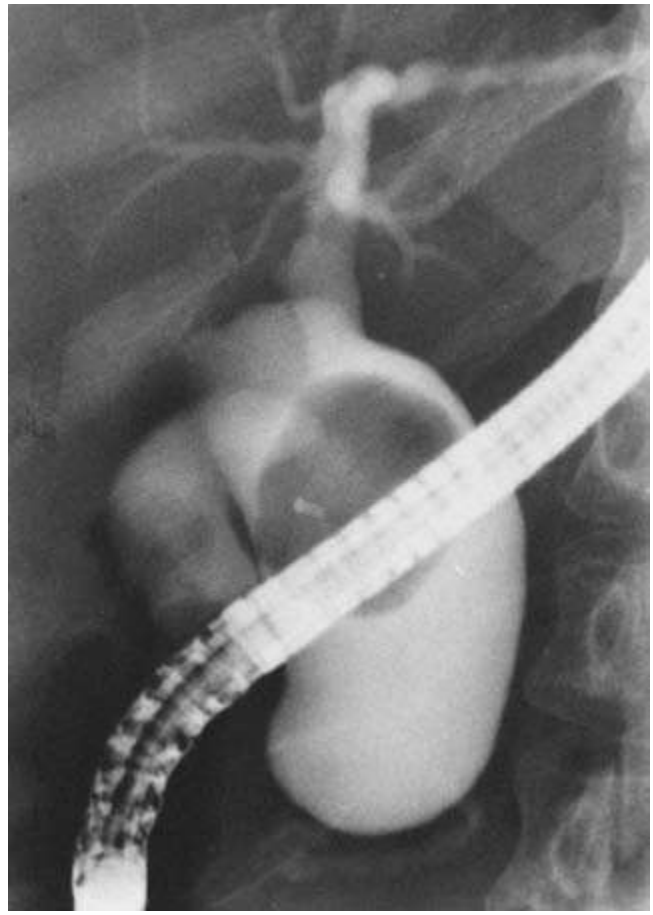


Figure 76.4 Choledochal cyst type IA. Endoscopic retrograde cholangiogram shows cystic dilation of the common bile duct with a very large filling defect as the result of a calculus. Stones are uncommon complications of biliary cysts, only reported in 13.8% of cases. Courtesy of Dr Hartley Cohen, USC School of Medicine.

[78]. This may involve mutational events involving Deleted in Pancreatic Cancer, locus 4 (*DPC-4*), *K-ras*, and *p53* genes [78,79].

Isolated intrahepatic cysts (i.e., Caroli disease) are also associated with a higher incidence of carcinoma. Overall, 7% of patients with Caroli disease were found to have cholangiocarcinoma, with no female predominance [80]. The average age at the time of diagnosis of carcinoma was 51 years [80].

MRCP may be a good diagnostic modality in the surveillance of cholangiocarcinoma, both intraluminal and periductal, but a surveillance protocol has not yet been established [58].

The prognosis of carcinoma related to biliary cysts is dismal. Almost all patients die soon after diagnosis [75,80]. One preventive therapy is total cyst excision during the initial operation. However, cyst excision does not completely eliminate the future risk of carcinoma, because the rest of the pancreaticobiliary tree appears to also be predisposed to malignant change.

Polycystic liver disease

Epidemiology

The prevalence of PCLD in autopsy studies is 0.05%–0.13% [81,82]. The most common form of PCLD coexists with autosomal dominant polycystic kidney disease (ADPKD); however, PCLD can occur without renal involvement [81,83]. Regardless of the etiology of the PCLD, the natural history is similar [84]. Gabow and colleagues observed that the hepatic cysts lag behind renal disease in ADPKD patients [85]. The hepatic cysts are rarely detected before puberty, but approximately 80% of the patients with renal cysts have liver cysts by the fifth decade of life. The liver cysts are more prevalent and prominent in women, and increase dramatically in number and size through the childbearing years [85]. A similar female preponderance is observed in isolated PCLD [84].

Pathogenesis

PCLD that coexists with ADPKD is associated with mutations of the *PKD1* (polycystic kidney disease 1) or *PKD2* [85–88] genes. Liver cysts are believed to arise from progressive dilation of abnormal biliary ducts, particularly the small intrahepatic ducts, which have lost their connection with the biliary tree [89]. The majority of the patients with ADPKD have *PKD1* gene mutations, accounting for 85%–90% of cases, whereas the remaining patients have mutations in the *PKD2* gene [90]. Interestingly, patients with mutations in *PKD2* have later onset of disease [91]. The *PKD1* gene, which encodes an integral membrane protein, polycystin 1, has been localized to the short arm of chromosome 16p13.3-p13.12 [92]. The *PKD2* gene encodes an integral membrane protein, polycystin 2, which has been linked to chromosome 4q21–q23 [93]. Although some studies have suggested that the type and position of mutations may predict phenotypic renal and extrarenal disease, no studies have analyzed the effects on hepatic cystic disease [84]. Studies in animal models demonstrate that autosomal polycystic disease may require a second somatic mutation to lead to cyst formation [94]. It is believed that loss of function of polycystin 1 and 2 leads to clonal expansion of mutated cells. Liver cysts arise from intrahepatic bile duct epithelial cells and detach from the intrahepatic bile duct as they expand and compress the surrounding parenchyma. Histological evaluation of cysts shows variable numbers of biliary microhamartomas, known as von Meyenburg complexes, which are characterized by clusters of dilated bile ducts lined by a layer of cuboidal cells surrounded by a fibrous stoma [95]. Everson and colleagues have suggested that the secretion of fluid within the cyst is triggered by hormonal signaling, primarily secretin [96].

Autosomal dominant PCLD is an entity that is distinct from ADPKD [81]. Reynolds and colleagues localized the mutation to chromosome 19p13.2–13.1 [97]. It has been further linked to a mutation in the protein kinase C substrate *80K-H* gene, which encodes a protein called hepatocystin [98–101]. So far, nine different mutations in hepatocystin have been found in patients with PCLD. All of the mutations lead to premature chain termination. Hepatocystin is a protein kinase substrate that functions as a β-subunit of endoplasmic reticulum glucosidase II [98–101]. Truncation of hepatocystin leads to failure of interaction of hepatocystin and the α-subunits, causing decreased levels of both subunits. This may compromise the processing of N-linked oligosaccharide chains of newly synthesized glycoproteins.

Clinical presentation

Most patients with PCLD are asymptomatic [84]. The cysts range in size from less than 1 mm to more than 10 cm and they may be diffuse or involve only one lobe [102]. The most common presenting symptom is abdominal pain, with or without distention [103,104]. Other symptoms, such as dyspnea and restricted mobility, are related to mass effect from massive hepatomegaly [105]. Complications of PCLD include hepatic cyst hemorrhage, rupture, or infection [105]. Rarely, patients can develop advanced liver disease with portal hypertension [105]. PCLD has been associated with other medical conditions including mitral valve prolapse, diverticulosis, inguinal hernias, and cerebral aneurysms [106].

Diagnosis

Most patients have preserved hepatic function; some have slightly elevated levels of serum alkaline phosphatase and total bilirubin and lower levels of total cholesterol and triglycerides [107]. Conventional radiological imaging, such as abdominal ultrasound and CT scans, is excellent in the detection of hepatic cysts (Fig. 76.5), and frequently, hepatic cysts are found incidentally on routine imaging [105]. Earlier studies using CT quantification have shown that hepatic parenchymal volume is preserved in PCLD, even in patients

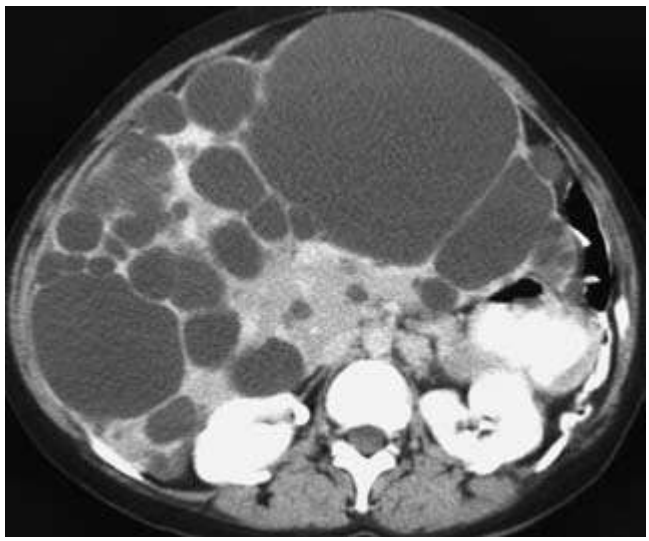


Figure 76.5 Polycystic liver disease. Small and large cysts fill the liver of a patient without polycystic kidneys.

with massive cysts [108]. MRCP can also be a useful modality for better visualizing intrahepatic ductal dilations and distinguishing PCLD from other lesions such as biliary hamartomas and Caroli disease [109].

Treatment

There is no effective medical therapy for PCLD [84]. Interestingly, an observation by Sherstha and colleagues that hepatic cystic disease worsened under the influence of pregnancy or exogenous female steroids but that renal cystic disease did not suggested that women with PCLD should avoid estrogen replacement [110].

A number of techniques are available for the treatment of symptomatic hepatic cysts. Most patients with PCLD have too many cysts or cysts of insufficient size to undergo ultrasound or CT-guided aspiration or sclerosis with ethanol [84]. Bistritz and colleagues reported the success rate of obliterating symptomatic cysts as 50%–67%, but others have reported success rates as high as 70%–90% [105,111,112]. Surgical fenestration of hepatic cysts, involving the deroofing of as many cysts as possible starting superficially and then proceeding more deeply, can be performed through open laparotomy or by laparoscopy. The optimal candidates for laparoscopic fenestration include those with a limited number (< 10) of large (> 10 cm) cysts, rather than those with diffuse involvement and smaller cysts [113,114]. Recent series, however, have reported that laparoscopic fenestration can be associated with perioperative morbidity as high as 50%, and 50% of the patients develop recurrence of cysts, requiring multiple procedures [115–117]. The most common complications following fenestration include hemorrhage, particularly from puncture of the right hepatic vein, and injury to the biliary system, which is distorted in PCLD [113]. Intraoperative ultrasound has been utilized to assess the location and position of the cysts and biliary and vascular structures [118]. Series of patients who underwent fenestration combined with partial hepatic resection demonstrated that nearly all patients experienced long-term symptom relief; however, this procedure was associated with significant perioperative morbidity, including ascites and pleural effusions [83,119].

Patients with severely symptomatic PCLD and significant comorbid conditions or diffuse liver involvement by small cysts may benefit from orthotopic liver transplantation [120]. Everson and colleagues reported that patients with PCLD who undergo liver transplant or combined liver–kidney transplant have relatively good outcomes. Based on data from the US Scientific Registry of Transplant Recipients from 1987 to 2003, survival rates for 1, 3, and 5 years for isolated hepatic transplantation were 78.1%, 71.7%, and 68.7%, respectively. Survival rates for 1, 3, and 5 years for combined liver–kidney transplantation were 79.5%, 75.5%, and 75.5%, respectively [84]. There has also been growing experience with living-donor liver transplantation for severely ill patients with PCLD [121].

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F GALLBLADDER AND BILIARY TRACT

77 Tumors of the biliary tract

Christopher J. Smolock, Jeffrey A. Drebin

Cholangiocarcinoma, 2022
Benign bile duct tumors, 2033
Carcinoma of the gallbladder, 2033
Benign tumors of the gallbladder, 2038

Cholangiocarcinoma

Cholangiocarcinoma is cancer arising from the biliary epithelium. It may arise from intrahepatic or extrahepatic bile ducts. Sporadic cholangiocarcinoma is relatively uncommon, but cholangiocarcinoma has a high incidence in the presence of certain benign conditions. Information on cholangiocarcinoma is mostly derived from small case series gathered over many years and therefore tends to be descriptive. Consequently, claims for the best form of investigation or treatment must be interpreted cautiously.

Classifications

There are several suggested classifications. Clinically, intrahepatic and extrahepatic cholangiocarcinomas are distinct entities (Table 77.1) but they are linked by cell of origin and etiological factors. Intrahepatic cholangiocarcinoma arises from small ducts or ductules and presents as an intrahepatic mass. The differential diagnosis of such a mass includes other intrahepatic tumors such as hepatomas or metastatic lesions. Extrahepatic cholangiocarcinoma arises from large ducts and usually presents as biliary tract obstruction. The extrahepatic tumors may be further subdivided into upper duct tumors (also called perihilar, hilar, or Klatskin tumors) and lower duct tumors [1]. The division into upper and lower duct cholangiocarcinomas is based on staging and treatment considerations. Lower duct tumors are similar to other periampullary tumors, such as adenocarcinomas of the head of the pancreas, in terms of diagnosis and treatment.

Extrahepatic cholangiocarcinoma has been classified, for staging purposes, by a classification introduced and modified by Bismuth and colleagues [2] into four types based on the level of ductal involvement (Fig. 77.1). Cholangiocarcinomas are also classified by the TNM (tumor/node/metastases) system [3], which groups intrahepatic cholangiocarcinomas with other intrahepatic malignancies and considers extrahepatic cholangiocarcinomas as a separate group (Table 77.2). A potentially useful modification of the staging system for intrahepatic cholangiocarcinomas has been proposed [4].

Incidence, etiology, and pathogenesis

The true incidence of bile duct carcinoma is difficult to determine because reports of incidence and survival statistics from these cancers are often included with liver and gallbladder cancers or pancreatic carcinomas. Recent reports suggest that rates of intrahepatic cholangiocarcinoma may be increasing in Great Britain [5] and the United States [6]. The incidence peaks at about 65 years of age and the tumor is more common in men than women.

Sporadic cholangiocarcinoma is uncommon. The tumor usually arises in association with an underlying condition. Underlying conditions are of two types: those that have a high frequency in localized populations, such as parasitic infestation with *Opisthorchis viverrini* in northeast Thailand, and those that have a much lower frequency but a more global distribution, such as primary sclerosing cholangitis (PSC) (Table 77.3). In Thailand, liver fluke-associated cholangiocarcinoma is a leading cause of death [7,8], whereas in the United States, cholangiocarcinoma accounts for less than 1% of malignancies. The diverse predisposing causes of cholangiocarcinoma in many cases share a common pathophysiology – chronic inflammation of the biliary tract.

Opisthorchis species-related and *Chlonorchis* species-related cholangiocarcinoma are caused by eating raw fish [7,8] containing the larval stage of flukes. Eating raw fish is more common among men in the affected populations so liver fluke-associated cholangiocarcinoma is more common in the male population. Infestation is associated with increased nitrate levels in body fluids, which is reversible on treatment with praziquantel [9]. There is evidence from human [9] and animal [10] studies that *N*-nitroso compounds may be the

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Table 77.1 Classification of cholangiocarcinoma

Name	Synonyms
Intrahepatic	Small duct, intraparenchymal, peripheral[a]
Extrahepatic – upper duct	Hilar cholangicarcinoma, perihilar cholangiocarcinoma, Klatskin tumor, proximal[a] bile duct cancer
Extrahepatic – lower duct	Distal[a] bile duct cancer

Note: The classification is the authors'. Intrahepatic and extrahepatic are the main divisions in the classification per the TNM (tumor/node/metastasis) system. Extrahepatic tumors are divided into two rather than three anatomic zones following Nakeeb et al. [1].
a These terms are undesirable, particularly "proximal" and "distal." Distal means away from the center of the body and proximal means near. Note that in pancreatic terminology, distal refers to the part of the duct farthest away from the termination in the duodenum, whereas in biliary terminology, the opposite is true. The terms are properly applied to limbs, and their use in biliary–pancreatic terminology should be abandoned.

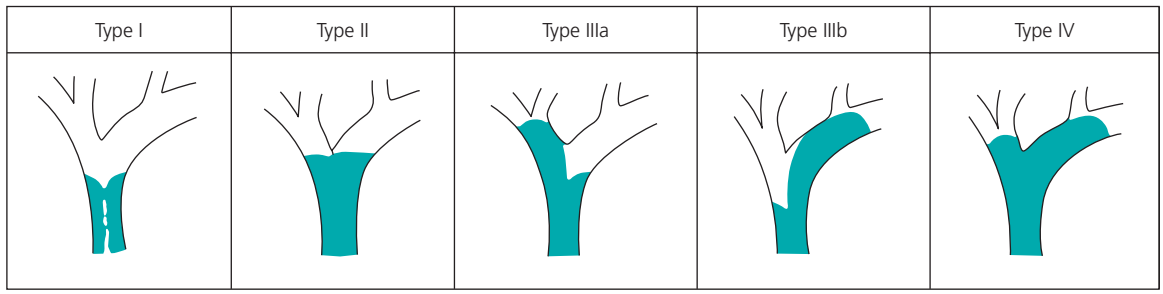


Figure 77.1 Bismuth–Corlette classification of hilar cholangiocarcinomas. Shading indicates level of ductal involvement.

Table 77.2 Tumor/node/metastasis (TNM) classification for cholangiocarcinoma and gallbladder carcinoma

A. Definition of TNM for intrahepatic cholangiocarcinoma

Primary tumor (T)			
T1	Solitary tumor 2 cm or less in greatest dimension without vascular invasion		
T2	Solitary tumor 2 cm or less in greatest dimension with vascular invasion; or multiple tumors limited to one lobe, none more than 2 cm in greatest dimension without vascular invasion; or a solitary tumor more than 2 cm in greatest dimension without vascular invasion		
T3	Solitary tumor more than 2 cm in greatest dimension with vascular invasion; or multiple tumors limited to one lobe, none more than 2 cm in greatest dimension, with vascular invasion; or multiple tumors limited to one lobe, any more than 2 cm in greatest dimension, with or without vascular invasion		
T4	Multiple tumors in more than one lobe, or tumor(s) involving a major branch of the portal or hepatic vein(s)		
Regional lymph nodes (N)			
N0	No regional lymph node metastasis		
N1	Regional lymph node metastasis		
Distant metastasis (M)[a]			
M0	No distant metastasis		
M1	Distant metastasis		
Stage grouping			
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1	N1	M0
	T2	N1	M0
	T3	N0	M0
	T3	N1	M0
Stage IVA	T4	Any N	M0
Stage IVB	Any	T	M1

Table 77.2 (*cont'd*)

B. Definition of TNM for extrahepatic cholangiocarcinoma

Primary tumor (TN)			
Tis	Carcinoma in situ		
T1	Tumor invades the mucosa or muscle layer		
T1a	Tumor invades the mucosa		
T1b	Tumor invades the muscle layer		
T2	Tumor invades the perimuscular connective tissue		
T3	Tumor invades adjacent structures: liver, pancreas, duodenum, gallbladder, colon, stomach		
Regional lymph nodes (N)			
N1	Metastasis in the cystic duct, pericholedochal or hilar lymph nodes (i.e., in the hepatoduodenal ligament)		
N2	Metastasis in the peripancreatic (head only), periduodenal, periportal, celiac, superior mesenteric, or posterior pancreaticoduodenal lymph nodes		
Stage grouping			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1	N1	M0
	T1	N2	M0
	T2	N1	M0
	T2	N2	M0
Stage IVA	T3	Any N	M0
Stage IVB	Any T	Any N	M1

C. Definition of TNM for gallbladder cancer

Primary tumor (T)			
Tis	Carcinoma in situ		
T1	Tumor invades mucosa or muscle layer		
T1a	Tumor invades the mucosa		
T1b	Tumor invades the muscle layer		
T2	Tumor invades the perimuscular connective tissue; no extension beyond the serosa or into the liver		
T3	Tumor perforates the serosa (visceral peritoneum) or directly invades into one adjacent organ, or both (extension 2 cm or less into the liver)		
T4	Tumor extends more than 2 cm into the liver or into two or more adjacent organs (stomach, duodenum, colon, pancreas, omentum, extrahepatic bile ducts, any involvement of liver)		
Regional lymph nodes (N)			
N1	Metastasis in cystic duct, pericholedochal, or hilar lymph nodes (i.e., in the hepatoduodenal ligament)		
N2	Metastasis in peripancreatic (head only), periduodenal, periportal, celiac, or superior mesenteric lymph nodes		
Stage grouping			
Stage 0	Tis	N0	M0
Stage I	T1	N0	M0
Stage II	T2	N0	M0
Stage III	T1	N1	M0
	T2	N1	M0
	T3	N0	M0
	T3	N1	M0
Stage IVA	T4	N0	M0
	T4	N1	M0
Stage IVB	Any T	N2	M0
	Any T	Any N	M1

a This part of the classification is the same for the other two tumors listed in this table.

Table 77.3 Predisposing causes of cholangiocarcinoma

Predisposing causes with a high incidence in localized areas of the world
Infestation with *Opisthorchis viverrin* in northeast Thailand
Infestation with *Chlonorchis sinensis* in China
Intrahepatic stones
Predisposing causes with a low but more global incidence
Primary sclerosing cholangitis
Choledochal cysts
Thorotrast[a]
Ulcerative colitis

a A number of other agents have been claimed to be associated with cholangiocarcinoma, but the evidence is weak.

carcinogens involved. Liver fluke infestation is also associated with development of intrahepatic stones, which themselves are associated with an increased risk for cholangiocarcinoma [11]. Cholangiocarcinoma may develop even after stone removal, if bile duct fibrosis induced by the stone disease leads to stasis and infection [11].

Cholangiocarcinoma occurs in about 10% of patients with PSC [12] with associated biliary dysplasia a suggested precursor [13]. Also, cholangiocarcinoma occurs 10 times more frequently in patients with chronic ulcerative colitis than in the general population [14]. PSC and ulcerative colitis often coexist; in such patients, as might be expected, the incidence of cholangiocarcinoma is even higher [15]. Up to 80% of patients with PSC have inflammatory bowel disease [16]. Cholangiocarcinoma also occurs in about 10% of patients with untreated choledochal cyst disease [16,17]. Malignancy of the bile ducts and gallbladder in patients with choledochal cysts may be related to the presence of an anomalously high pancreatic duct–bile duct junction (see Carcinoma of the gallbladder). Thorotrast-related cholangiocarcinoma, resulting from exposure to high levels of α-particle radiation, is largely a disease of the past [18]. The agent has not been used since 1940.

Molecular genetics

Cancer results from the accumulation of multiple genetic abnormalities in a single cell [19]. This process of multistep carcinogenesis involves genes that control cell cycle progression and apoptotic cell death. Many of the specific genetic events responsible for neoplastic transformation in colon and pancreatic malignancies have been identified [19,20]. Relatively less is known about the events responsible for neoplastic transformation in the biliary tract.

The *erbB2* oncogene (also called the *HER2* oncogene or the *neu* oncogene) encodes a cell surface protein that interacts with several distinct cellular receptors, including the receptor for epidermal growth factor [21,22]. Overexpression of *erbB2* has been identified in tumors of the breast, ovary, stomach, pancreas, lung, and prostate. Several studies have examined expression of *erbB2* in cholangiocarcinomas [23–25]. Although an early report suggested that expression of *erbB2* was present in less than 10% of cholangiocarcinomas [23], studies from several other laboratories have identified *erbB2* expression in 67%–73% of cholangiocarcinomas [24,25]. Overexpression of the epidermal growth factor receptor as well as of cyclooxygenase-2 has also been identified in some cholangiocarcinomas [26,27]. Immunoreactivity for cyclooxygenase-2 is also increased in the surrounding hepatocytes of livers in patients with PSC and hepatolithiasis compared with those of normal adult livers [27]. There are reports that combined targeting of *erbB2* and cyclooxygenase-2 in cholangiocarcinoma cells results in a synergistic effect on suppression of cell growth as well as induction of caspase-3 activation [27,28].

The *ras* genes constitute a distinct family of oncogenes that transduce proliferative signals from cellular receptors to the cell interior [29]. One member of this family, termed K-*ras*, has been shown to be mutated in a significant fraction of colon, pancreas, and lung carcinomas. Several studies have demonstrated K-*ras* expression in cholangiocarcinomas [26,30–32]. Point mutations involving this gene may occur in more than half of all cholangiocarcinomas [31,32].

Several other genes, including the *myc* gene [33] and the *p53* gene [34], have been studied in cholangiocarcinomas. These genes encode proteins that reside in the cell nucleus and control transcription of growth regulatory genes. Expression of the *myc* gene appears to be an almost universal feature of cholangiocarcinomas [31], whereas *p53* expression, suggesting the presence of a mutant *p53* gene, has been identified in 50%–80% of cholangiocarcinomas [34–37].

The studies cited here have just begun to characterize the genetic events responsible for the development of cholangiocarcinoma. It is interesting that the genes that have been linked to cholangiocarcinoma so far, particularly *erbB2*, K-*ras* and *p53*, also appear to be involved in a high percentage of pancreatic carcinomas [28]. It is possible that this reflects exposure to common carcinogens in the biliary and pancreatic epithelium.

Pathology

Malignant tumors of the bile ducts are almost exclusively epithelial tumors (carcinomas). Tompkins and associates [38] reported that the distribution of extrahepatic bile duct cancers is 49% in the upper third, 25% in the middle third, and 19% in the lower third of the duct; 7% of these cancers were diffuse. The Johns Hopkins group reported a distribution of 6% intrahepatic; 67% extrahepatic, upper duct ("perihilar"); and 27% extrahepatic, lower duct ("distal") [1]. It must be stressed that all such figures arising from surgical case series are heavily influenced by referral patterns and may not be representative of the disease generally.

Grossly, extrahepatic bile duct cancers can be divided into three types: polypoid or nodular masses, sclerosing, and diffusely infiltrating. Some cancers may present as only a thickening of the bile duct wall, which appears to be involved in dense fibrous scar. Polypoid or papillary cancers have the best prognosis.

Histological variants of adenocarcinomas of the bile duct include well-differentiated, pleomorphic, giant cell, adenosquamous, oat cell, and colloid carcinoma. Bile duct cancers have a tendency to spread along the bile duct. The longitudinal extent of cancers along the bile duct is often difficult to determine by gross examination. Shimada and colleagues [39] performed a histological examination on 29 patients after resection and found that the mean distance of microscopic invasion beyond the gross margin was 16.8 mm toward the liver and 6.5 mm toward the duodenum. A retrospective review of 253 cases by Ebata and colleagues [40] recommended a 10-mm ductal resection margin in both directions for eradication of invasive carcinoma cells. However, a 20-mm margin was recommended to eliminate noninvasive carcinoma cells [40].

Histological diagnosis of malignant bile duct cancers may be difficult at times. Well-differentiated cancers with little invasion are difficult to differentiate from the bile duct involved in scar formation or PSC. Cancers arising in the upper duct tend to be better differentiated with more sclerotic stroma that contains chronic inflammatory cells [41]. Perineural invasion (i.e., malignant cells within nerve sheaths), a prominent feature of bile duct cancers, is not present in PSC and is the single most important diagnostic criterion of malignancy. Other problems in differential diagnosis occur with cancers of the lower bile duct that involve the ampulla, duodenum, and pancreas. All of these sites may give rise to malignant tumors with similar characteristics so it may be difficult to determine the site of origin conclusively. An important point is that bile duct cancers, compared to pancreatic adenocarcinomas, are less likely to metastasize early. As a result, surgical resection of bile duct tumors is associated with a somewhat more favorable outcome.

Hilar tumors often arise in one of the main bile ducts and produce asymptomatic obstruction of that duct before progressing to obstruct the other main duct, which leads to jaundice. Unilateral obstruction results in atrophy of the affected side of the liver and hypertrophy of the other side. This phenomenon will also occur if there is unilateral portal vein occlusion. The clinical importance of atrophy has been emphasized by Blumgart and Stain [42]. Atrophy may result in axial rotation of structures in the hepatoduodenal ligament. For instance, with atrophy of the left hemiliver and hypertrophy of the right, the structures rotate in a clockwise direction when viewed from below, which hides the hepatic artery, rotates the bile duct to the left, and exposes the portal vein on the right side of the hepatoduodenal ligament in the usual position of the bile duct. The effect of atrophy must be taken into account when adding liver resection to extrahepatic duct resections for cholangiocarcinoma [43].

Cholangiocarcinomas must also be differentiated from benign inflammatory tumors [44], also called *hepatic inflammatory pseudotumors* and *benign fibrosing disease* [45]. These inflammatory masses mimic extrahepatic and intrahepatic cholangiocarcinomas but consist of chronic inflammatory cells and fibrosis. Benign inflammatory tumors appear to occur most frequently in extrahepatic upper ducts but also occur intrahepatically [46], and less commonly in lower ducts. In a recent series of patients undergoing resection for hilar strictures, 15% were benign tumors by histopathology [45]. Failure to appreciate this diagnosis may lead to inappropriate therapies, such as long-term stenting or hepatic resection.

Bile duct cancers usually spread by direct extension to involve adjacent organs and tissues. Upper duct cancers may directly invade the liver. Cancers in the common bile duct or cystic duct may directly invade the gallbladder. Regional lymph node metastases are common, liver metastases distant from the primary occur frequently, but distant metastases are unusual.

It may be difficult to distinguish between a liver metastasis of colonic or pulmonary origin and an intrahepatic cholangiocarcinoma. Grossly, intrahepatic cholangiocarcinomas may be well or poorly demarcated, single or multiple. However, mucin production, fibrosis between acini of tumor tissue, and a more overtly glandular pattern are the main characteristics that differentiate cholangiocarcinoma from hepatoma. Immunohistochemical staining for specific cytokeratins may also be helpful. Also unlike hepatomas, some cholangiocarcinomas stain positively for cancer antigen 19-9 (CA 19-9) or carcinoembryonic antigen (CEA). CA 19-9 may be an especially helpful test in interpreting biliary brush cytology in PSC [47].

Clinical presentation

Extrahepatic upper duct and extrahepatic lower duct tumors cannot usually be separated on the basis of presenting symptoms. Most patients present with painless jaundice and its clinical constellation of dark urine, light stool, and pruritus. Nonspecific gastrointestinal symptoms such as anorexia and nausea, as well as mild weight loss and fatigue, are not unusual. Mild abdominal pain without jaundice may be the sole presenting symptom in upper duct cholangiocarcinoma; many patients with upper duct cholangiocarcinoma have had recent cholecystectomy, presumably because it was believed that gallstones were the source of these symptoms. Cholangitis is uncommon unless the biliary tree is instrumented. On examination, patients are usually jaundiced and, in the case of tumors arising below the insertion of the cystic duct, the gallbladder may be palpable.

Laboratory tests suggest extrahepatic biliary obstruction. Characteristically, the bilirubin rises over several weeks to a level of 20 mg/dL or greater if the jaundice is unrelieved and

the direct reacting fraction accounts for more than 50% of the total bilirubin. Marked elevation of serum alkaline phosphatase and γ-glutamyltransferase levels and mild elevation of aminotransferase levels are also usual.

Cholangiocarcinoma in patients with PSC is often associated with deterioration in clinical status and liver function tests. However, these signs are unreliable and late markers of cholangiocarcinoma. Cholangiocarcinoma may be present without any change in traditional liver function tests [48]. Furthermore, screening of patients at risk, such as those with PSC, using computed tomography (CT) scanning, ultrasound, and even endoscopic retrograde cholangiopancreatography (ERCP) is typically ineffective [49]. However, with the continuing advances in CT technology, there is a recent report that suggests multiphasic helical CT is improving the ability to distinguish malignant from benign common bile duct strictures [50].

CA 19-9 is emerging as a useful diagnostic marker. The Mayo group has reported that a cutoff of 100 IU was 89% sensitive and 86% specific for cholangiocarcinoma in PSC [51]. The King's College group recommend the use of an index that incorporates both CEA and CA 19-9 values and that has attained similar sensitivities for cholangiocarcinoma in PSC [50]. More recently, Levy and colleagues reported that only advanced cases of cholangiocarcinomas were detected by serum CA 19-9 in PSC patients [52]. Bile CEA levels are reportedly elevated in cholangiocarcinoma, but not in benign diseases, other than intrahepatic stones [53].

Intrahepatic cholangiocarcinomas characteristically present with nonspecific right upper quadrant pain. Other symptoms include weight loss and nausea. An intrahepatic cholangiocarcinoma may occasionally be critically situated in the central area of the liver and, as a result, may cause jaundice owing to obstruction of the main ducts by external pressure.

Diagnosis and staging

Extrahepatic, lower duct tumors

The most common cause of painless jaundice or jaundice with mild pain in the cancer age group is pancreatic carcinoma. Consequently, both lower and upper duct cholangiocarcinomas are often initially suspected to be pancreatic carcinoma. The question of which is the best initial investigation of painless jaundice is controversial. Ultrasound, ERCP, and CT scan have all been advocated. Ultrasound has the advantages of low cost and lack of invasiveness and it is the best test for detection of choledocholithiasis and cholecystolithiasis. In young patients with any pain or in older patients with pain that has characteristics of biliary colic, ultrasound is probably still the best first examination. Conversely, in the typical patient with painless jaundice, CT scan may be the best initial investigation. This is because in most instances, when a periampullary mass is detected without evidence of metastatic spread, CT scan is the only investigation needed before proceeding to surgery or staging laparoscopy with ultrasound. When no mass is present on CT scan, a diagnostic ERCP is performed to determine the site and type of biliary obstruction. Magnetic resonance cholangiopancreatography (MRCP) is another imaging modality in the diagnostic repertoire. A recent report lists comparable sensitivities, specificities, and accuracies between ERCP and MRCP in the differentiation of malignant and benign causes of biliary stricture [54].

Staging laparoscopy with ultrasound

Preoperative staging of the tumor is also of great importance. Ideally, staging locates and sizes all tumors in the body. The immediate goal of staging for the surgeon is to determine whether the tumor has spread beyond the limits of operative resection; this may be because of distant metastases or the local extent of the tumor. For staging purposes in lower duct cholangiocarcinoma, *local extent* refers to the macroscopic limits of the tumor in relation to the portal vein and superior mesenteric artery. The value of staging laparoscopy with ultrasound in hepatobiliary–pancreatic malignancies has become evident. The importance of laparoscopy in staging hepatobiliary–pancreatic malignancy was demonstrated by Warshaw's group [55], by John and associates [56], and by Weber and colleagues [57]. Additional staging information, proving that resection for cure was not possible, was obtained by Warshaw's technique in 22 of 50 patients with hepatobiliary–pancreatic malignancies who had been completely evaluated by conventional studies [58]. Of the remaining 28 patients, 26 had disease that was resectable for cure at the time of surgery. Staging laparoscopy permits evaluation of the peritoneal surfaces without laparotomy, and subsequent biopsy if metastatic implants are found. Laparoscopic ultrasound can detect vascular and distant nodal invasion, criteria which generally make the tumor inoperable [58] (Fig. 77.2). Others have found endoscopic ultrasonography [59] useful in determining the extent of tumor involvement and intraductal ultrasound during ERCP [60] valuable in distinguishing malignant from benign strictures. Fine-needle aspiration may also be performed during endoscopic ultrasonography as a diagnostic test when other tests are inconclusive [61]. The role of positron emission tomographic scanning in staging cholangiocarcinoma is still under investigation and its reliability is questionable [62–64].

Extrahepatic upper duct tumors

Altemeier and colleagues [65] and Klatskin [66] wrote classic papers on perihilar cholangiocarcinoma in an era when the biliary tree was inaccessible to preoperative imaging. Consequently, both stressed the difficulty of diagnosing this tumor. Today, there are many imaging techniques that can display the tumor or its effects on adjacent vessels. Examples include transabdominal ultrasound, CT scan, CT with angioportography, magnetic resonance imaging (MRI), percutaneous transhepatic cholangiography (PTC), ERCP, positron emission

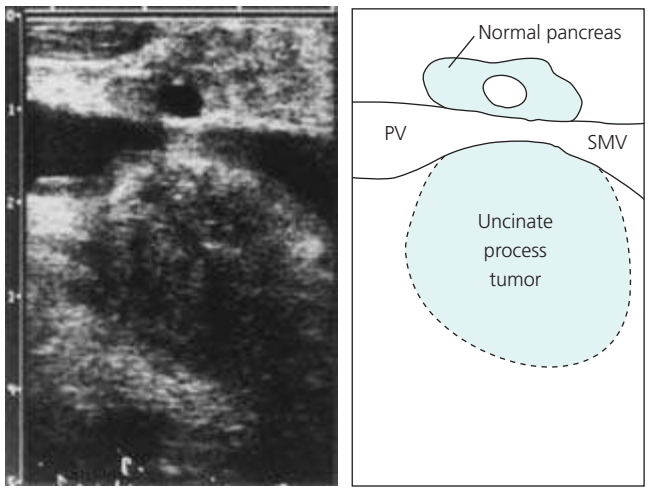


Figure 77.2 High-resolution laparoscopic ultrasound image demonstrating a 2.5-cm to 3-cm inhomogeneous hypoechoic mass in the uncinate process consistent with a pancreatic cancer. The tumor abuts the superior mesenteric vein (SMV) and portal vein (PV) posteriorly behind the neck of the pancreas, which itself appears to be normal. No evidence of actual vascular invasion is seen. At laparotomy, this adenocarcinoma was resected. From Callery et al. [58].

tomography, angiography, endoscopy with ultrasound, and laparoscopy with laparoscopic ultrasound. Diagnosis is usually based on history and imaging characteristics. Any patient who has a perihilar stricture, without evidence of ductal disease elsewhere in the biliary tree suggestive of PSC, and who has not had previous biliary surgery that might have resulted in stricture, is considered to have upper duct cholangiocarcinoma. Inflammatory pseudotumor is not ruled out by these considerations and a confident diagnosis depends on pathological examination of tissue obtained at surgery or cytological brushings obtained by ERCP or PTC. In one report covering 74 patients with biliary and pancreatic strictures, the sensitivity and specificity of brush cytology were 56% and 100% and the positive predictive value was 100% [67]. Therefore, brush cytology is diagnostic and useful when positive but of little value when negative. Perihilar tumors are less accessible than lower duct or pancreatic neoplasms so it is unlikely that a sensitivity of 50% is being achieved in these tumors. Mucobilia on ERCP is an uncommon finding but is highly suggestive of a papillary cholangiocarcinoma, which may be intrahepatic or extrahepatic [68]. Fine-needle aspiration is also useful if the mass can be seen on ultrasound examination or on CT scan. Another technique that we have used with success is to direct the biopsy needle at a stent placed through the site of duct narrowing.

Staging is also of great importance in upper duct tumors. For staging purposes, *local extent* means the macroscopic limits of the tumor in relation to the biliary tree, especially the upper level of the stricture. It also refers to whether and to what degree invasion of adjacent vessels has occurred. The upper level of the stricture provides the information for the Bismuth stage. Blumgart and Stain [42] have described the indicators of unresectable bile duct cancers: bilateral intrahepatic bile duct spread, involvement of the main trunk of the portal vein, involvement of both branches of the portal vein or bilateral involvement of the hepatic artery and portal vein, or a combination of vascular involvement on one side of the liver with extensive bile duct involvement on the other. With vascular replacement, it may be possible to resect some cholangiocarcinomas that were previously considered unresectable.

The local extent of the disease along the biliary tree has usually been determined by direct cholangiography – either ERCP or PTC (Fig. 77.3). It is the upper margin of disease that is of most interest in these tumors so PTC has been generally favored over ERCP. However, ERCP has progressed to the point that a complete picture of the biliary tree can often be obtained even when the obstruction is nearly complete. MRCP is a newer, less invasive method of evaluation of the biliary extent of perihilar cholangiocarcinoma. Although promising, it has not been determined whether it will routinely provide the detail regarding disease extent obtained by direct cholangiography (Fig. 77.4).

Vascular invasion has been assessed by angiography, color-flow Doppler ultrasound, and MRI. Angiography is the gold standard for assessing vascular involvement but is also the most invasive technique. Our preference is for color-flow Doppler ultrasound because the tumor and its extravascular extent, as well as its effect on vessels, are seen in real time and multiple detailed views of the tumor and its relationships may be obtained. Vascular deviation without narrowing is not evidence of unresectability. Color-flow Doppler ultrasound is dependent on the skill of the ultrasonographer and on the available equipment. It has been claimed that Doppler ultrasound is effective in evaluating portal vein involvement (Fig. 77.5) but less so in examining the hepatic artery [69].

Additional information on local extent of disease in or around the biliary tree can be obtained by laparoscopy with laparoscopic ultrasound. However, cytology from peritoneal washings obtained during laparoscopy has not been shown to be predictive of occult peritoneal disease in patients with hilar cholangiocarcinoma [70,71].

Laparoscopic ultrasound permits close contact between the ultrasound probe and the tumor [57,58]. The best position for the probe is often the anterior surface of the liver, looking through a 2-cm to 4-cm thickness of liver into the porta hepatis. Using this method, we have been able to identify tumor extending along the intrahepatic ducts above the level that was evident by the upper level of the biliary stricture identified on direct cholangiography. Vascular encasement and lymph node involvement may be detected (Fig. 77.6). Bilateral biliary involvement to the point that all four sectional ducts [57] are involved precludes curative resection.

Several factors preclude dogmatic statements regarding selection and order of investigation in extrahepatic upper

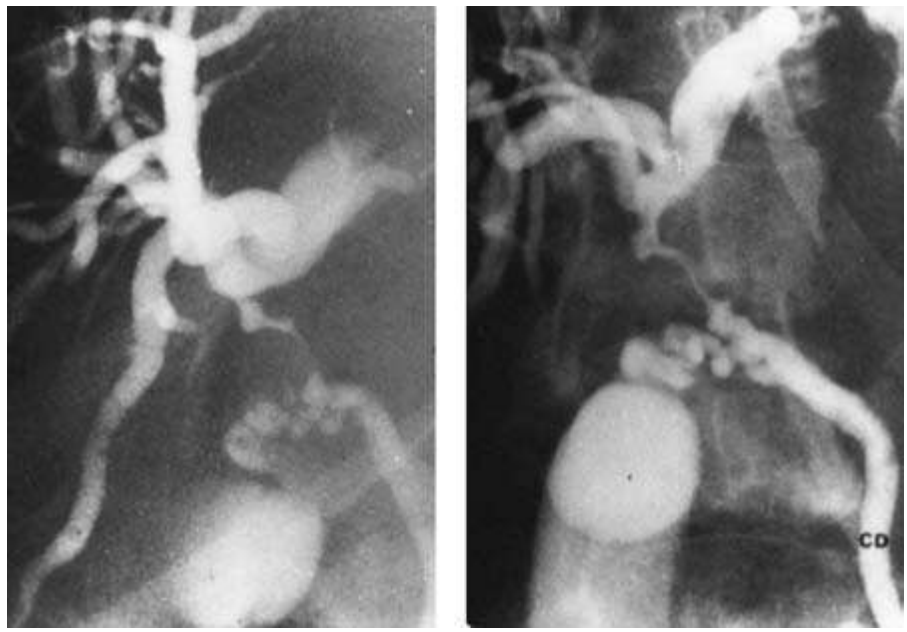


Figure 77.3 Cholangiogram shows a Klatskin tumor. The intrahepatic bile ducts are dilated and the size of the common bile duct (CD) is normal.

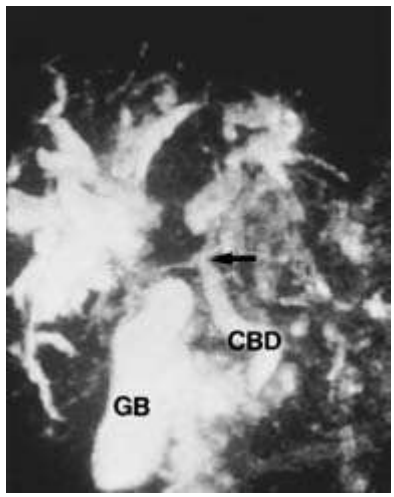
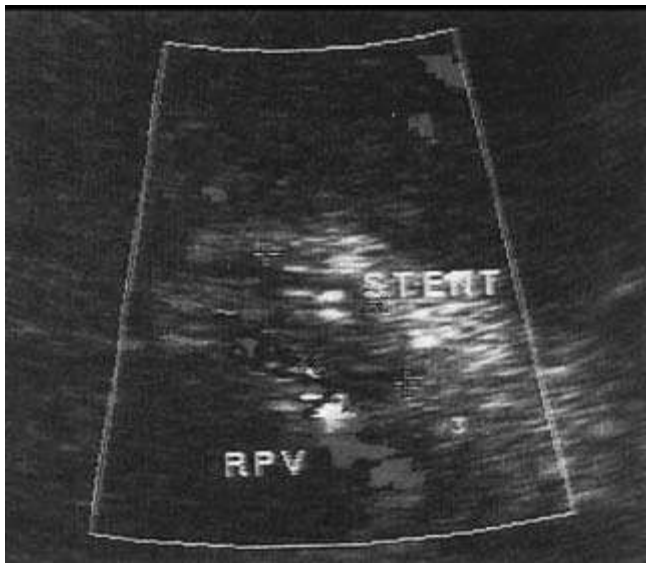


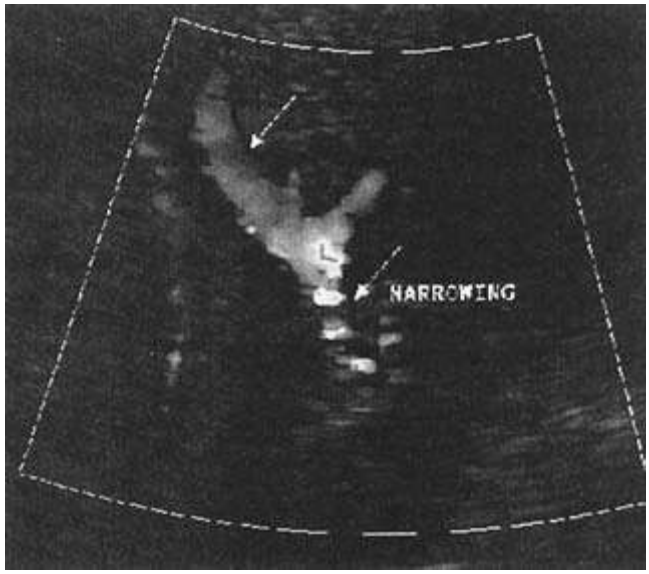
Figure 77.4 Magnetic resonance cholangiogram showing extrahepatic and intrahepatic biliary tract. Narrowing of bile duct produced by a hilar tumor is shown (arrow). CBD, common bile duct; GB, gallbladder. Courtesy of Dr Joseph T. Ferrucci, Department of Radiology, Boston University School of Medicine.

duct cholangiocarcinoma. There are no good comparative studies examining this issue; there are so many potentially valuable investigations, and further developments in imaging are occurring so rapidly, that it is unlikely that good data will be realized. Equipment and expertise vary from center to center; patients are often referred with several studies already completed; at presentation upper duct cholangiocarcinoma is often not distinguishable from lower duct cholangiocarcinoma or other malignancies causing complete obstruction of the lower duct. Consequently, initial investigation cannot be directed specifically to the perihilar region. The important considerations are that a combination of investigations must be used to achieve the goals of staging – evaluating the local extent of disease and whether it is metastatic – and that clinicians have a variety of algorithms to choose from to achieve staging. Our approach is given below.

Summary of our present algorithm for staging in extrahepatic cholangiocarcinoma

In the typical case of painless jaundice, a CT scan is obtained first. If there is a periampullary mass, the next step is staging laparoscopy with ultrasound unless therapeutic decompression of the biliary tree by ERCP or percutaneous stenting is indicated (see later). If no mass is seen, ERCP with brushings is performed when ductal dilation extends into the lower bile duct or when there is no ductal dilation. Either ERCP or PTC with brushings is performed when only the intrahepatic ducts are dilated, suggesting an upper ductal lesion. In the case of lower duct lesions, detection of a single stricture by ERCP is taken as evidence of a periampullary cancer, possibly cholangiocarcinoma, and the next investigation is staging laparoscopy with ultrasound. In the case of upper duct strictures compatible with hilar cholangiocarcinoma, the next step is to evaluate vascular involvement by color-flow Doppler ultrasound or MRI; fine-needle aspiration may be performed at the time of ultrasound. Staging laparoscopy with ultrasound then follows for those patients whose disease is still potentially resectable. The utility of staging laparoscopy in the operative assessment of extrahepatic cholangiocarcinomas has been validated in a large series from the Memorial Sloan Kettering Cancer Center [71].





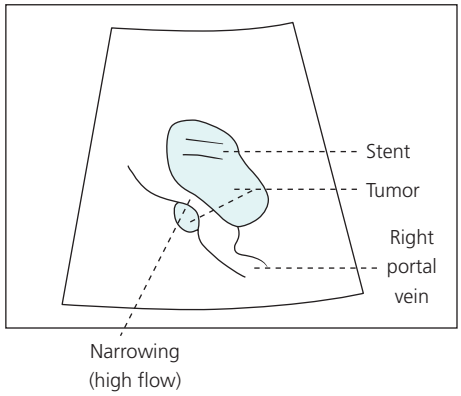


Figure 77.5 Flow Doppler ultrasonograms showing encasement of right portal vein (RPV) by tumor. A stent that had been placed through the tumor can also be seen. Note that in the area of vein encasement higher-velocity flows occur in the compressed area, compared with low-velocity flows in the non-compressed areas.

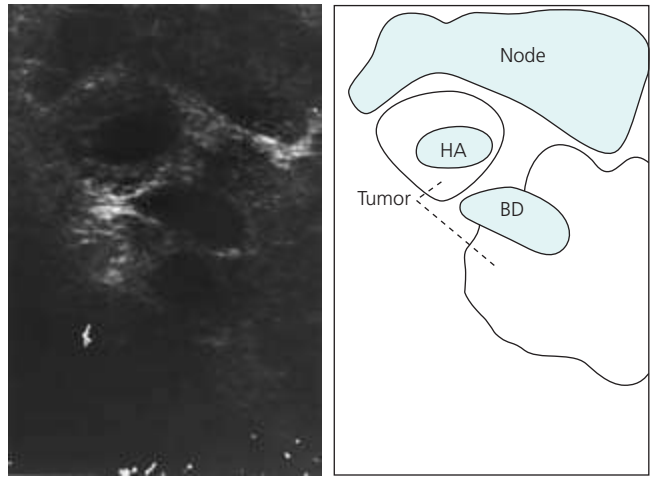


Figure 77.6 Laparoscopic ultrasonogram showing encasement of the hepatic artery (HA) and bile duct (BD). An enlarged lymph node that contains tumor is also seen.

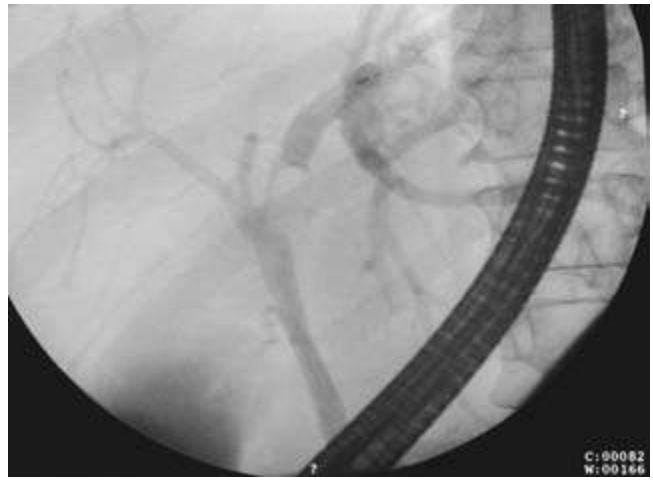


Figure 77.7 Endoscopic retrograde cholangiogram showing a bullet-shaped filling defect in the left hepatic duct. At surgery, this was found to be a tumor embolus from an intrahepatic cholangiocarcinoma. This patient presented with pancreatitis, presumably caused by such an embolus.

Intrahepatic tumors

Intrahepatic cholangiocarcinoma is in the differential diagnosis of the intrahepatic mass. Except for events such as mucobilia or tumor embolizing into the extrahepatic bile ducts, an event that may cause pain, jaundice, or even pancreatitis [72] (Fig. 77.7), intrahepatic cholangiocarcinoma does not produce highly characteristic findings. On axial imaging, the tumor may be single or multiple. Neither CT nor MRI appears superior in characterizing these rare lesions but there may be a diagnostic benefit from using both modalities, with or without ultrasound, because small nodules missed with one test

were often seen with another [73,74]. Fluorodeoxyglucose positron emission tomography scan is not sensitive in the detection of intrahepatic cholangiocarcinoma [74]. On CT scan, the lesion is usually irregular or unencapsulated and of low attenuation with only mild enhancement seen with contrast [73]. The tumors are low attenuation on CT angioportography [75]. On T1-weighted MRI the intrahepatic cholangiocarcinoma is usually of low intensity but on T2-weighted images they are of high intensity [74,76]. Local vascular invasion is often seen by CT, MRI [76] or angiography [77], and ductal dilation is often present peripheral to the tumor. Centripetal filling after gadolinium administration has been noted [76]. Liver biopsy is useful when focal nodular hyperplasia is in the differential diagnosis on the basis of imaging studies or if the tumor is inoperable because of metastatic spread or extent in the liver. Otherwise, in potentially resectable cases, the next investigation after axial imaging by either CT or MRI is staging laparoscopy with ultrasound, followed by surgical exploration and resection as for other hepatic masses [62,63].

Preoperative decompression of the biliary tract in jaundiced patients

Three randomized studies have shown that jaundiced patients do not benefit from preoperative decompression of the biliary tree [78–80]. However, these studies were performed in an era in which the most common surgical procedure performed after preoperative decompression was palliative surgical bypass of the biliary tree – a relatively short procedure associated with limited morbidity. No study has examined the potential benefit of preoperative decompression when the following operative procedure is either pancreaticoduodenectomy or liver resection. One study found that operative morbidity was greater in patients with hilar cholangiocarcinoma who had an aspartate aminotransferase level of more than 90 IU than in patients with lower levels [81]. Until definitive studies are available, preoperative decompression is still an option to be considered. In our opinion, it should be performed if hemihepatectomy for cholangiocarcinoma is planned in a jaundiced patient and may be performed if a pancreaticoduodenectomy is to be performed in a patient with longstanding or severe jaundice. Other preoperative preparations include correction of vitamin K deficiency and bowel preparation.

Treatment

Lower duct tumors

Lower duct cholangiocarcinoma is one of the four types of periampullary tumors treated by pancreaticoduodenectomy, the others being pancreatic, ampullary, and duodenal cancers. The extent of the procedure is the same as that used for pancreatic carcinoma and will not be described in detail in this chapter. The procedure has reached a "near-zero" mortality rate in expert hands at tertiary-care centers specializing in hepatobiliary–pancreatic surgery [82,83]. Often, the tissue of origin of the tumor, whether pancreatic, ampullary, or bile duct in origin, is uncertain until after the specimen has been examined pathologically and even then, doubt can remain. The main complication of this procedure is a fistula from the pancreatic–jejunal anastomosis, which occurs in 5%–10% of patients [82]. Biliary fistulae occur in about 2% of patients. Today, patients rarely die from these complications because of improvements in diagnostic and interventional radiology, intensive care, and treatment of infection [82]. Resection of part of the stomach is no longer required for lower duct cholangiocarcinoma, although there is little or no difference in short-term outcome or quality of life [84] between the pylorus-preserving and standard types of pancreaticoduodenectomy [85]. Many patients require pancreatic enzyme replacement after this procedure, but few become diabetic.

Palliative treatment of unresectable lower duct tumors is by stenting and a combination of chemotherapy and radiation. The only randomized trial of surgical bypass versus endoscopic intubation favored the latter [86]. When unresectability is determined at open exploration, a double bypass is performed to decompress the biliary tree and bypass the duodenum, which may become obstructed. Palliative resection of periampullary tumors may be unavoidable because the full extent of the tumor may not be detectable until the procedure is nearing completion. However, compared with upper duct tumors, this is an uncommon problem. Palliative resections for visceral cancers is an accepted procedure, for example, for colonic and gastric cancers, but has been avoided for periampullary tumors because of the risk of the procedure. However, an argument has been made in favor of palliative resection because the procedure can be performed with relatively little risk [87].

Upper duct tumors

The goals of surgical resection are to remove the tumor with negative resection margins. This may be accomplished with or without an associated hepatic resection depending on the extent of the tumor. In some cases, it will be known preoperatively or by intraoperative staging with ultrasound that a liver resection will be required because the disease will have spread into the liver on one side. At other times, the need for a liver resection becomes apparent only intraoperatively, after exploration, or after the extrahepatic bile ducts are resected and microscopic disease is discovered on one side at the highest possible point of extrahepatic resection. Unfortunately, it is also at this point in the operation when discovery of bilateral microscopic involvement makes it clear that a curative resection has not been accomplished and is not possible. The same considerations apply to the discovery of microscopic involvement of vessels. Operative management is further complicated by the fact that frozen-section diagnosis of the presence or absence of tumor at the resection margin may be difficult in these fibrotic cancers.

A description of the operative technique is beyond the purposes of this text; however, the principal steps in the operation are as follows. The bile duct is divided at the level of the pancreas and a negative margin is confirmed by frozen section. The bile duct and gallbladder are dissected off and away from the portal vein and hepatic arteries, leaving the latter structures bare; that is, all nodes and soft tissue are taken with the specimen. The bile ducts and surrounding tissue are freed to above the bifurcation where the tumor is situated until macroscopically normal ductal tissue is encountered in the right and left bile ducts. These ducts are divided and frozen sections are obtained. Coring out of liver tissue in the porta may permit excision to the level of the first branches of the right and left bile ducts. If the tumor has grown too far up one side to perform an extrahepatic resection, the bile duct is divided on the side to be retained, and the liver, bile ducts, and vascular structures are removed on the other side. Reconstruction is by a Roux-en-Y limb approximately 60 cm in length to avoid reflux of intestinal contents into the biliary tree. Cholangiocarcinoma commonly invades caudate bile ducts, and caudate resection is routinely performed by some surgeons for upper duct cholangiocarcinoma. This area must be carefully evaluated at surgery and caudate resection must be performed if there is any question of caudate invasion.

The important trends in surgery for upper duct cholangiocarcinoma are that a higher percentage of upper duct tumors are being resected [88], largely owing to the more common use of liver resection [89], vascular resection and reconstruction are becoming more common, and surgical morbidity and mortality are decreasing. Mortality has remained at 5% or less for more than a decade [90–93]. Liver resection combined with hilar duct resection is performed for more advanced disease so comparisons between local resection and local resection plus liver resection for outcomes, such as percentage of procedures obtaining negative margins and survival, are meaningless. The results of surgical resection depend highly on whether negative resection margins are achieved [84,93–98]. There are few 5-year survivors when resection margins are positive [84,93,98,99]. Other negative prognostic variables are tumor stage [97], nodal disease [73,97], tumor grade [54,99], bilirubin concentration [54,99], and absence of mucobilia [73]. When negative margins are obtained, the 5-year survival rate varies from 11% to 43% in recent series [1,84,93,97–99]. Again, it must be stressed that these results are in small numbers of highly selected patients. The median survival of patients with tumor-free margin is 3–3.4 years, compared with a median survival of 1–1.2 years for patients with disease at the resection margins.

Palliation of unresectable upper duct tumors may be by percutaneous [100] or endoscopic intubation or by surgical bypass. There have been no randomized trials. Metallic stents have extended the functional life of stents. Plastic stents typically clog at 3–4 months, whereas metal stents last 8–10 months or even longer [101–104]. Surgical treatment is by biliary enteric bypass, usually employing the duct to segment III, the so-called Bismuth–Corlette procedure [105]. Bypass to right-sided ducts is more difficult and less successful. Surgical implantation of large-bore tubes through the tumor has been used in the past but is now rarely employed [106,107]. Both surgical bypass and internal stenting have their proponents [91,99,108]. Stenting has the advantage of initial low morbidity and the disadvantages of occasional failure to clear jaundice, bouts of cholangitis, and the need for repeated procedures to deal with stent obstruction. With improvement in techniques of stenting, one may expect resolution of jaundice in 80% of patients, with a 10% major morbidity rate, a 30-day mortality rate of about 5%, and a median survival of 8 months [109]. Surgical bypass requires a major open surgical procedure and has corresponding early morbidity [91] but the complete clearance of jaundice is usual, and cholangitis is uncommon. There are many nonrandomized comparisons of these techniques, but all are contaminated by patient selection and consequently are of little value in determining which technique is superior. For instance, greater success in clearing jaundice by surgical bypass may partly reflect an earlier stage of disease in these patients. Patient selection is probably appropriate in this disease. Younger, healthier patients, in whom bypass to segment III will decompress 40%–50% of the liver, probably should undergo surgical decompression; whereas older patients, especially those with intercurrent disease or in whom decompression will provide biliary drainage from less than 40% of the liver mass, should be treated by nonsurgical means [110].

Radiation therapy has been used alone or in combination with other techniques. Its value is unclear because most series are small and no randomized comparisons exist. External-beam irradiation was successful in clearing jaundice in 10 of 11 patients in a report where no other decompressive measures were used [111]. Brachytherapy has been applied through percutaneous tubes, with a median survival of 23 months [112]. The combination of surgery and radiotherapy was reported to provide a median survival of 14 months in unresectable or recurrent disease [113]. Another series reported no benefit of radiation [114].

Intrahepatic tumors

The principles of treatment are the same as for other malignant intrahepatic hepatic lesions [115]. The tumor must be resected with a margin of normal tissue to obtain microscopically free resection margins (a 1-cm tumor-free resection margin is the goal) yet leave enough normally functioning liver tissue behind for the patient to have adequate liver function in the postoperative period. The size of the resection may vary from a single segment or less to resection of three of the four hepatic sections [116]. Like upper duct tumors, results depend on whether negative resection margins are achieved. There are few case series that separate these uncommon tumors from other cholangiocarcinomas. One recent series

obtained a 5-year survival rate of 44% [1] in 18 patients, whereas another reported a 32% 2-year survival rate in 19 patients [117].

Liver transplantation has been performed for intrahepatic and upper duct cholangiocarcinoma. Initially the results were disappointing. Goldstein and colleagues reported that only 3 of 14 patients (21%) lived for more than 28 months after the procedure [118], whereas in a study by Shimoda and associates 25 patients reported a 3-year survival of 32% [119]. However, two reports by Heimbach and colleagues at the Mayo Clinic show a series of 28 patients with localized, regional lymph-node-negative, hilar cholangiocarcinoma undergoing neoadjuvant therapy and liver transplantation having an actuarial patient survival of 82% at 5 years [120,121]. Liver transplantation for neoplasms must still be evaluated with the knowledge that many patients with end-stage chronic liver disease are dying while on a waiting list for liver transplantation and the comparative survival rate in this group of patients would be expected to be about 90%.

Benign bile duct tumors

Benign tumors are uncommon. Most information comes from case reports. The clinical presentation is similar to that of choledocholithiasis: pain, jaundice, and less commonly, cholangitis or pancreatitis. Adenomas are the most common benign tumors of the bile ducts. They may be single, multiple, tubular, papillary, or mixed. Multicentricity has been reported but is uncommon. Cystadenoma and granular cell tumors are rare benign tumors of the extrahepatic bile ducts. Cystadenomas are similar to and may coexist with cystadenomas of the liver and pancreas [122]. Granular cell tumors are unusual, occur most commonly in women of African heritage, and may be associated with granular cell tumors at other sites [123,124]. The malignant potential of these lesions is unknown. Reported benign mesenchymal tumors include fibromas, lipomas, leiomyomas, and neurilemomas.

Papillomatosis is a rare condition in which multiple papillary lesions involving a large amount of the bile duct mucosa may be encountered. Malignant transformation or progression to invasive carcinoma has been reported so papillomatosis of the bile duct mucosa should be considered a premalignant lesion [125,126]. This lesion should be treated by complete excision if possible; if not, regrowth is common.

Carcinoma of the gallbladder

Carcinoma of the gallbladder is the fifth most common malignancy of the gastrointestinal tract [127]. As with studies on cholangiocarcinoma, studies of gallbladder cancer tend to be small case series covering many years, and claims for the best form of investigation or treatment must be cautiously interpreted. Cancers arising from the gallbladder mucosa appear to behave in a fashion similar to other adenocarcinomas of the gastrointestinal tract; premalignant to invasive malignant changes can be found, metastatic spread occurs by lymphatic and vascular routes, diagnosis is often delayed, and survival is related to stage. Interestingly, at the population level, mortality is also inversely related to cholecystectomy rates [128].

Incidence

The highest incidence of gallbladder cancer occurs in Chileans and Bolivians [129]. Gallbladder cancer is the fourth leading cause of all cancer deaths in Chile; mortality from gallbladder cancer (5.2%) in Chile is the highest in the world [130]. Other commonly affected population groups include Native Americans, Hispanic American women, Latin American women, Japanese women in Japan, African Zimbabwean women, European immigrant women in Israel, and northern Europeans [131,132]. In contrast to the general population of the United States, in whom the incidence is low at about 3 per 100 000, gallbladder cancer is the most common gastrointestinal malignancy in Native Americans. Low rates of gallbladder cancer occur among Nigerians, New Zealand Maoris, and Chinese natives and immigrants [131].

Cancer of the gallbladder in the United States is a disease of elderly people, with the greatest incidence occurring after 65 years of age. It is three times more common in women than in men, and it is more common in whites than in African Americans. Genetic differences are exceedingly important. In North America, the highest incidence of gallbladder cancer occurs among Native Americans and Mexican Americans. In North and South America, gallbladder cancer is related to Native American rather than Spanish heritage because the incidence of gallbladder cancer in Spain, Cuba, and Puerto Rico is low. In Mexico, *mestizos* (people of mixed ancestry) have the highest incidence, whereas in Bolivia, there appears to be a difference based on tribal origin [133].

Etiology

Etiological factors that appear to be, or have been claimed to be, important for the development of gallbladder cancer include genetic characteristics, gallstone disease, bile composition, calcification of the gallbladder wall, anomalous junction of the biliary and pancreatic ducts, congenital biliary cysts, some infections [134], environmental carcinogens, and drugs. Geographic differences may reflect racial and genetic differences, although cultural differences possibly play a role. A progression from dysplasia to early cancer to metastatic cancer has been observed [135], the progression from dysplasia to cancer taking about 15 years.

Gallstone disease is associated with gallbladder cancer, but the mechanism is unknown. Most reports of the relationship between gallstones and gallbladder cancer have come from operative or autopsy studies; however, a prospective cohort

study of patients with gallstones found that gallstones did increase the risk for gallbladder cancer, but the actual incidence was very low (9 per 10 000 per person-years), and the absolute number of cases of gallbladder cancer (5 of 2583 people) in this population was low [136]. The incidence of gallbladder cancer in patients with cholecystolithiasis ranges between 0.5% and 3% [137]. Cholecystolithiasis is present in 70%–90% of patients with gallbladder cancer. Duration of gallstones, patient age, size of gallstones [138], and possible carcinogenic effects of gallstones, such as from the chemical composition [139] or from bacteria within the stones, may be important [140], although in certain studies, patients with cancers did not have larger stones, nor did their stones have higher cholesterol content [136].

Duration of cholecystolithiasis appears to be a crucial factor for the development of cancer. Patients who have had cholecystolithiasis for longer than 40 years have a significantly higher incidence of gallbladder cancer than those who have had gallstones for a shorter time. Glenn and Hays [141] suggested that 1% of patients older than 65 years of age with gallstones would develop gallbladder cancer. This more likely reflects the duration of stone disease than an effect of age. However, the possibility of cancer is not an indication for cholecystectomy in a patient with asymptomatic stones. In many countries, gallbladder cancer is unknown, which is probably related to the rate of cholecystectomies performed for asymptomatic gallstones or early in the course of patients with symptomatic stones.

Little is known about the pathophysiology of gallbladder cancer in patients with cholecystolithiasis. Chronic irritation of the gallbladder mucosa by stones, followed by repeated episodes of epithelial repair over a period of years, may lead to malignant transformation. The chemical composition of stones or bile may be related to development of gallbladder cancer. Indeed, because bile from high endemic areas is more mutagenic than that from low endemic areas [142], there is probably some factor excreted by the liver into bile that contributes to malignant transformation. Cholesterol stones are the most common type associated with gallbladder cancer. Bile acids can act as cocarcinogens, and there is a structural similarity to the known carcinogen, methylcholanthrene. In the most comprehensive analysis yet, Strom and colleagues [136] performed a case–control study in Mexico and Bolivia that compared patients with gallbladder cancer, patients with gallstones, and patients with no biliary tract disease. Their prior hypotheses, that patients with tumor would have increased levels of lithocholate, was not confirmed, but bile glycoursodeoxycholate levels were unexpectedly elevated in the patients with cancer. This paper must be read to appreciate the difficulty of performing and interpreting such a combined epidemiological and biochemical study. For instance, the authors were unsure at the end of their study whether their unexpected findings in bile acid composition were a cause or effect of the tumor. There are no conclusive data linking bile composition of bile acids to carcinoma of the gallbladder and because bile is the main route of excretion of dietary or chemical metabolites with molecular weight of more than 500, there are a large number of potentially carcinogenic compounds.

A number of recent studies from Japan have linked anomalous pancreatic–biliary duct junction (APBJ) to gallbladder and extrahepatic bile duct cancer [143–145]. There is a much higher incidence of biliary tract cancer in patients with APBJ. About 2% of Japanese patients examined had APBJ; about 75% of these cases were associated with a choledochal cyst and the rest had normal-caliber bile ducts. In those associated with choledochal cyst, cancer may arise in the extrahepatic duct or gallbladder, whereas in those without ductal dilation, the cancer seems almost always to occur in the gallbladder [143,144]. Cancer of intrahepatic ducts does not appear to be associated with APBJ. Patients with APBJ get cancer at a younger age than patients with sporadic gallbladder cancer [144]. Pediatric patients with APBJ frequently have epithelial hyperplasia of the gallbladder [146,147]. The nonmalignant areas of the gallbladder of patients with APBJ-associated gallbladder cancer show increased hyperplastic changes in the gallbladder mucosa compared with patients with sporadic gallbladder cancer [148,149]. In a cat model, side-to-side biliary–pancreatic anastomosis produced hyperplastic changes in the gallbladder within 6 months [150].

Another factor that has been associated with gallbladder cancer is partial or complete calcification of the gallbladder wall (porcelain gallbladder). Although an incidence of coexistent gallbladder malignancy of up to 25% has been claimed in patients with porcelain gallbladder, recent data suggest that this is less common [151]. Others have questioned whether porcelain gallbladder is linked to gallbladder carcinoma at all [152]. *Salmonella typhi* carriage; exposure to toxic environmental factors in the automotive, rubber, textile, and metal industries [38,135,153]; previous gastric operations [154]; and elevated body mass index [138] have been associated with gallbladder cancer.

Molecular genetics

There are relatively few studies examining the molecular events in carcinoma of the gallbladder. As in cholangiocarcinomas, the *erbB2* oncoprotein is expressed in a significant fraction of gallbladder cancers [155]. Furthermore, transgenic mice that express *erbB2* in the gallbladder epithelium develop gallbladder carcinomas [156]. Data regarding the expression of *ras* and *myc* genes in gallbladder cancer are conflicting [155,157,158] but it appears that the expression of these genes is substantially less than in cholangiocarcinomas. Similarly, point mutations involving the K-*ras* gene appear to be less common in gallbladder cancer than in cholangiocarcinomas [158]. Several studies have identified *p53* expression in gallbladder cancers; about two-thirds of

such tumors express *p53* [37,159]. Such overexpression is not seen in adenomas.

Pathology

As noted, there is a progression from dysplasia to carcinoma in situ to invasive cancer in the gallbladder epithelium that appears to take about 15 years [135]. Premalignant lesions are associated with gallbladder cancer, and it is thought that chronic inflammation may play a role in the development of premalignant lesions [41,160]. Two types of metaplasia, intestinal and squamous, have been found in patients with gallbladder cancer. The relation of intestinal metaplasia to subsequent development of gallbladder cancer has not been determined. Squamous metaplasia, squamous epithelium replacing normal gallbladder epithelium, is a rare premalignant lesion that has been found with squamous cell cancer of the gallbladder. Cholecystitis follicularis, a rare type of inflammation, has been reported in a few cases of gallbladder cancer, but its premalignant potential is unclear [41].

The classification of malignant lesions of the gallbladder is shown in Table 77.4. Sixty percent of cancers of the gallbladder are found in the fundus, 30% in the body, and 10% in the neck. They may be isolated or involve the entire gallbladder through intramural spread, analogous to linitis plastica of the stomach.

Most malignant neoplasms of the gallbladder are adenocarcinomas, which behave in a manner similar to adenocarcinomas arising from other epithelial tissues. They originate as mucosal lesions, and as growth progresses, they invade the wall of the gallbladder. The anatomy of the gallbladder wall partly explains the advanced stage of disease at which most patients with gallbladder cancer present. The lack of a well-defined muscularis leads to early entry of invasive gallbladder cancer into the perimuscular connective tissue. Lymphatic, neural, and hematogenous invasion occurs earlier with gallbladder cancer than with cancers of the gut.

Table 77.4 Malignant neoplasms of the gallbladder

Malignant epithelial tumors
Adenocarcinoma
Squamous cell carcinoma
Oat cell carcinoma
Others
Malignant mesenchymal tumors
Embryonal rhabdomyosarcoma
Leiomyosarcoma
Malignant fibrous histiocytoma
Miscellaneous
Carcinosarcoma
Carcinoid
Lymphoma
Melanoma
Others

Gallbladder cancer frequently extends beyond the gallbladder to involve the liver and the extrahepatic biliary tree. Most patients with liver involvement have direct extension of the disease; fewer than 10% of patients have liver metastasis without full-thickness invasion of the gallbladder wall. The routes of lymphatic drainage of the gallbladder have been studied in an attempt to rationalize aggressive surgery for cancer of the gallbladder. Uesaka and colleagues [161] injected a carbon particle suspension into the primary drainage nodes – the cystic and pericholedochal nodes. The main route of drainage was on the right side of the bile duct to the superior retropancreatic node and from there directly to the paraaortic nodes or indirectly to the paraaortic nodes through the posterior pancreatic nodes. Drainage to the left side of the bile duct to the celiac nodes and drainage upward to the hilar nodes and into segment IV of the liver were much less common modes of drainage. Lymph node metastasis to the cystic, pericholedochal, peripancreatic, and celiac nodes occurs early; more than 50% of patients are found to have lymph node metastases at the time of diagnosis. Direct invasion of the duodenum and the colon also occurs. Intraperitoneal spread, Krukenberg tumors (i.e., ovarian metastases), and hematogenous dissemination occur.

A uniform staging system for gallbladder cancer has not been widely applied. Nevin and colleagues [162] developed the first clinically useful staging system. The TNM system, described by the American Joint Committee on Cancer, is the preferred staging system [3]. Survival of patients with gallbladder cancer is related to stage and histological type of cancer. Lymph node involvement is rare in stage T1 tumors [162,163]; that is, lymph node involvement almost never occurs until the muscularis has been penetrated. After that, lymph node involvement is common, occurring in about 50% of stage II patients and in 70%–80% of patients in stage III and IV [162,163]. There is a close correlation between lymph node involvement and prognosis [164–166]. Most long-term survivors are patients with well-differentiated tumors that were minimally invasive. These are usually found incidentally at or immediately after cholecystectomy (Fig. 77.8).

Clinical presentation

Signs and symptoms of gallbladder cancer are nonspecific. This explains the delay in diagnosis in most patients with gallbladder cancer. In TNM stages I and II, symptoms often mimic those of cholelithiasis and cholecystitis [167]. Indeed, in the early stages, the cause of symptoms may be the associated cholelithiasis. Pain is the most common initial complaint; it may be dull and aching, colicky, sharp, constant, or intermittent, and it may or may not radiate to the back. Other symptoms include nausea, vomiting, and anorexia. In later TNM stages, weight loss, jaundice, hepatomegaly, a

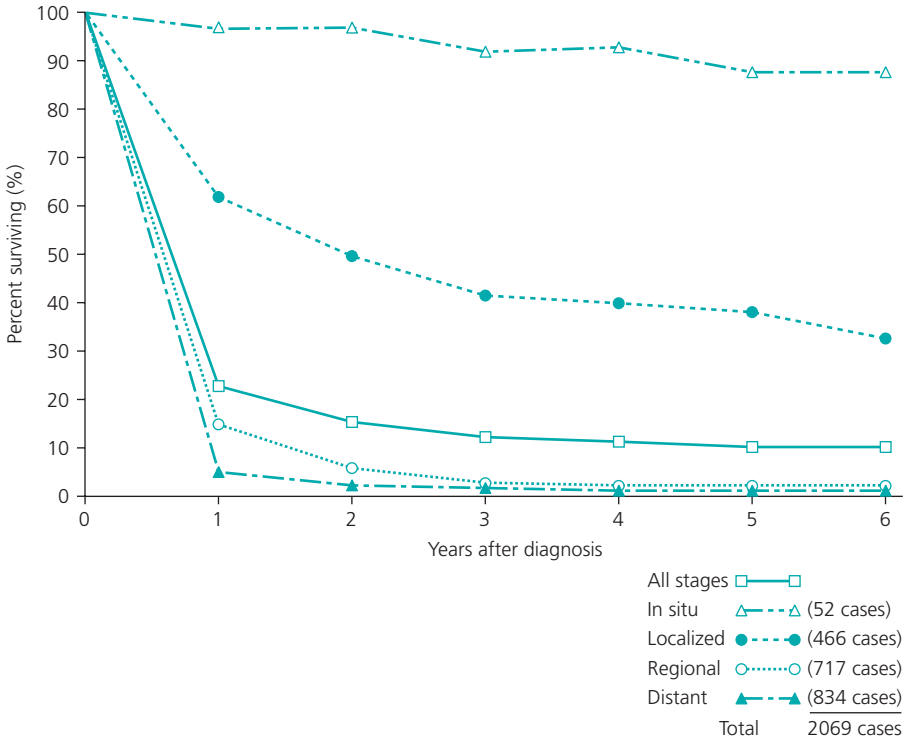


Figure 77.8 Gallbladder cancer survival rates by stage in all races and genders from 1973 to 1981. From Albores-Saavedra & Henson [41], with permission from the American Registry of Pathology.

palpable mass, or ascites may develop. Jaundice develops in 30%–60% of patients and is a poor prognostic sign because it is caused by extension of the tumor beyond the gallbladder, with obstruction of the extrahepatic bile ducts; 85% of patients with jaundice have unresectable tumors [137]. The patients may also have obstruction of the duodenum or colon. Malignant cholecystoenteric fistula may be the first indication of gallbladder cancer.

Diagnosis and staging

In most cases, the diagnosis is not made preoperatively [137]; this is true even in recent series [168]. Laboratory findings are not diagnostic but are related to abnormalities associated with bile duct obstruction. Patients frequently have increased alkaline phosphatase and bilirubin levels. Blood levels of CEA or CA 19-9, another tumor marker, may also be elevated. These findings are not diagnostic because they may have other causes.

Diagnostic techniques include oral cholecystography, ultrasonography, CT scanning, and possibly MRI. The most widely employed diagnostic study for biliary tract disease is ultrasound. Findings on ultrasonography that are suggestive, but not diagnostic, of gallbladder cancer include thickening of the wall, a mass projecting into the lumen, calcification of the gallbladder, multiple masses or a fixed mass in the gallbladder, a mass extending into the liver, or an extracholecystic mass. In a comparison of patients with unsuspected gallbladder cancer and patients with benign gallbladder disease, it was found that solitary stone, displaced stone, intralumenal mass, and invasive mass were more commonly associated with cancer [169]. A report of mucosal thickening should also be viewed with suspicion. CT scanning may help in the diagnosis but is of limited help in staging the disease except in estimating the extent of hepatic invasion [170] (Fig. 77.9).

Gallbladder polyps may be malignant but are rarely so when less than 1 cm in diameter. In a recent series of 103 patients with gallbladder polyps, no polyp was malignant if less than 1 cm in diameter, but 23% of polyps larger than

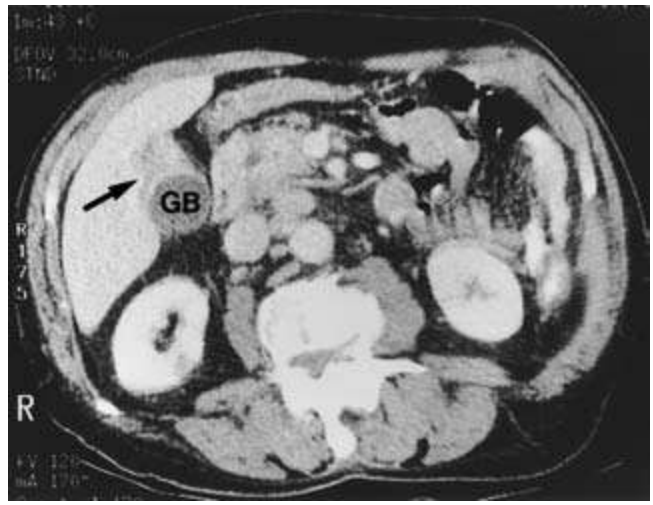


Figure 77.9 Computed tomography scan of patient with carcinoma of the gallbladder (GB) showing invasion of hepatic parenchyma (arrow).

1 cm were malignant [171]. In another series, 88% of polyps larger than 1 cm in diameter were malignant, and polyps larger than 1.8 cm were more likely to contain a more advanced stage of cancer [172]. Fine-needle aspiration appears to be an accurate way of distinguishing between polyps caused by cholesterolosis and neoplastic polyps [173], especially when the polyps are larger than 1 cm in diameter [174]; however, it is much less accurate in determining whether a neoplastic polyp is an adenoma or carcinoma [174]. Duplex Doppler ultrasound imaging of blood flow within a neoplastic polyp may also be useful in distinguishing these lesions from cholesterol polyps.

Many of the staging considerations are similar to those discussed under cholangiocarcinoma. Staging laparoscopy with ultrasound appears to have many of the same advantages for staging of gallbladder cancer that it has for other hepatobiliary malignancies [56,58,175].

Treatment

Both treatment and prognosis of gallbladder cancer depend on stage of disease [162]. Unfortunately, most cancers are diagnosed when symptomatic, and at that time, the stage of disease is usually TNM stage III or IV [165,176]. In most patients with disease that is in advanced stages, their disease is not resectable so the stage distribution at the time of open surgery has been more heavily weighted to later stages than the stage distribution of patients actually resected for cure. For instance, in a German series of 81 operated patients, the TNM stage distribution (I to IV, respectively) was 7%, 12%, 15%, and 66% [165] whereas the stage distribution of 106 radically resected cases in a Japanese series was 14%, 43%, 24%, and 19% [163]. It is to be hoped that staging laparoscopy with ultrasound will alleviate this disparity and reduce the number of patients undergoing open exploration without resection. Of the 106 patients in the Japanese series, curative resection was achieved in 74. Most of the noncurative resections were in patients with stage IVb cancer [163]. In a recent American series containing 149 patients, 58 cancers were explored, and of those, 23 were resected for cure [165]. As previously noted, these figures may not be representative of the broad population of patients contracting the disease.

There are no established principles of treatment because no definite trials establishing these exist. Most studies of treatment regimens are small, not randomized and retrospective [177]. At best, there are rational guidelines based on the facts that T1 lesions have rarely metastasized to the lymph nodes but T2 to T4 lesions have usually done so [162,163] and that radical surgery achieving negative tumor margins has resulted in acceptable results in more advanced disease [178]. There are several levels of resection. In increasing order of magnitude they are

- extraserosal cholecystectomy or cholecystectomy with wedge resection of the liver
- extraserosal cholecystectomy combined with portal lymph node dissection and sometimes extrahepatic bile duct resection
- liver resection with cholecystectomy and lymph node dissection and sometimes extrahepatic bile duct resection
- liver resection with cholecystectomy and lymph node dissection combined with pancreaticoduodenectomy (the most radical).

Beginning with the earliest stages, the recommendation for treatment may be summarized as follows. When gallbladder cancer is suspected, an open procedure should be performed. If there is no evidence of spread outside the gallbladder, an extraserosal cholecystectomy should be performed. An extraserosal cholecystectomy excises the gallbladder on a deeper plane than a standard cholecystectomy, so that the gallbladder and all connective tissue down to actual liver tissue are removed. This can be performed laparoscopically; however, it is our opinion that this should not be attempted because gallbladder perforation and bile spillage are more common in the laparoscopic technique. The negative consequences of tumor implantation or incomplete excision far outweigh any benefit of minimally invasive surgery. The excised specimen should be inked. If resection margins are negative and if the tumor has not penetrated the muscularis (T1 lesion), the procedure is considered to be complete. If margins are positive or if the muscularis has been penetrated, then resection of segments 4b and 5 and a lymph node dissection are performed. If the cystic duct resection margin is positive, then, in addition, the extrahepatic bile duct is excised to clear margins [179].

When there is obvious penetration to the serosal layer on the deep surface of the gallbladder, found with intraoperative ultrasound or palpation, then resection of the gallbladder with segments 4b and 5 and a lymph node dissection with or without extrahepatic bile duct resection is the recommended procedure. Sometimes, because of the local depth of penetration into the liver, a more extensive liver resection is required [180]. Stated otherwise, a cholecystectomy is usually sufficient for T1 lesions and usually results in cure. For T2 to T4 lesions, the extent of resection depends on the route of direct extension, whether into the liver or bile duct; a lymph node dissection is always performed.

Results in recent case series [163,165,178,181,182] appear to show marked improvement over classic reports. Mortality is infrequent, even for major resections, but there is a complication rate of about 25%, including minor complications. Exact comparisons among series are not possible because different operations have often been carried out for the same disease stage. For long-term survival, a curative resection with negative margins must be obtained. If negative resection margins are achieved, 5-year survival rates according to stage are about as follows: stage I, 90%; stage II, 80%; stage III, 40%; and stage IV, 15%. Prognosis in stage IV appears to be best when the tumor is placed in this category because of local hepatic invasion, rather than because of lymph node

involvement [165]. Lymph node involvement is an ominous sign [165], although 5-year survivors have been reported in some series [163,182]. Based on the pattern of lymph node drainage, a pancreaticoduodenectomy has been performed for T2 to T4 disease [183]. However, the results have still been poor in patients with advanced stage disease.

Palliation of gallbladder cancer mainly requires treatment of obstructive jaundice. The considerations are the same as for cholangiocarcinoma, except that percutaneous or endoscopic means of decompression are preferable in this rapidly progressive state. Chemotherapy is experimental and of no proven benefit [184]. Although some gallbladder cancers may be radiosensitive [185], radiation therapy provides little survival benefit because of the extent of tumor at the time of diagnosis. Palliation of jaundice has been reported, and intraoperative irradiation may be beneficial [186,187]. Although these types of treatments should be considered, their role has not been defined.

Incidental gallbladder cancer at laparoscopic cholecystectomy

Gallbladder cancer may be an incidental finding at laparoscopic cholecystectomy, as it was at open cholecystectomy in the past; the incidence ranges from 0.3% to 1%. The difference with laparoscopy is that port-site implantation may occur [188,189]. Port-site implantation may simply be the result of contact between the malignancy and the tissues at the perimeter of the port site at the time of gallbladder extraction. It is reported to occur in 17% of cases [190]. However, positive-pressure pneumoperitoneum appears to be another factor [191]. Preoperative suspicion should be raised by the ultrasonographic findings listed previously. Extraction of the gallbladder in a plastic bag is prudent when these signs exist or when there is evidence of gallbladder wall thickening at surgery. Every gallbladder should be inspected at the time of extraction and questionable areas should be resected for biopsy.

If a cancer is discovered, it should be treated at that time using the principles stated previously. The tissue surrounding the trocar ports should also be excised as part of the treatment because seeding may have occurred. Early reoperation is the approach of choice when the tumor is discovered on later pathological examination, when surgery at the time of the initial procedure could not proceed because of uncertainty of diagnosis or degree of penetration of the tumor, or when the surgeon believes that the proposed procedure must be discussed with the patient. Stage I gallbladder carcinomas that have clear resection margins probably require no further surgical treatment. Overall, the widespread adoption of laparoscopic cholecystectomy did not worsen the survival of patients with gallbladder cancer and patients with serendipitously treated gallbladder cancers did not have a worse survival after laparoscopic manipulation than after a standard open cholecystectomy [192].

Benign tumors of the gallbladder

Benign tumors of the gallbladder are uncommon and infrequently symptomatic. Benign epithelial tumors include adenomas and mixed tumors. Adenomas may be tubular, papillary, or mixed. One-third are multiple, and fewer than 50% are associated with stones. Benign tumors may be symptomatic, causing biliary colic, presumably as a result of shedding of tumor, which temporarily obstructs the cystic duct. Treatment is laparoscopic cholecystectomy when the mass is symptomatic and less than 1 cm in diameter. Cholecystectomy is performed for either symptomatic or asymptomatic tumors when the mass is larger than 1 cm in diameter because of the possibility of malignancy and for the same reason the procedure is performed as open surgery. Fine-needle aspiration may permit the diagnosis of cholesterolosis, in which case surgery is necessary only if symptomatic and laparoscopic surgery may be performed.

Other problems with which benign tumors of the gallbladder are confused are much more common than benign tumors of the gallbladder. These include cholesterolosis and adenomyomatosis. The former is a disease of unknown etiology in which cholesterol accumulates focally, segmentally, or diffusely in the gallbladder. Focal cholesterolosis may present as a polyp and be confused with a tumor. Adenomyomatosis goes by a variety of other names as well. It is a condition in which there is nonneoplastic ingrowth of gallbladder glands into the wall of the gallbladder and even penetration of the wall. The cause is unknown. It may be focal or diffuse. When focal, the site is usually in the fundus, producing the characteristic fundal adenomyoma. When diffuse, it produces wall thickening. Both types may be confused with malignancy. There are characteristic changes on ultrasound and contrast cholangiography, which help differentiate the process from cancer. Benign mesenchymal tumors are rare and include leiomyoma, hemangioma, and lipoma.

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F GALLBLADDER AND BILIARY TRACT

78 Sphincter of Oddi dysfunction (postcholecystectomy syndrome)

John O. Clarke, Anthony N. Kalloo

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Introduction

The sphincter of Oddi (SO) was first described by a 23-year-old Italian medical student named Ruggero Oddi in the late 19th century (1887) after extensive research on the physiology of dogs and detailed histological examination of many species, including humans. Following this, Oddi was promoted to the Chair of Physiology at the University of Genoa but then sank into a world of depression, narcotic abuse, and homeopathic medicine before dying anonymously and mysteriously in Tunisia in 1913 [1]. Like Oddi himself, the sphincter he described has remained an item of intrigue, mystery, and controversy. A recent editorial referred to sphincter of Oddi dysfunction (SOD) as the "most misunderstood and abused diagnosis in gastroenterology" [2]. This chapter aims to clarify (as much as possible) what is known concerning nomenclature, diagnosis, and treatment of SOD.

Definitions

SOD refers to a benign, noncalculous obstruction to bile or pancreatic exocrine secretions through the SO [3]. This may be secondary to a fixed structural lesion, referred to as SO stenosis, or secondary to a motility disorder or spasm, with resultant SO dyskinesia. Other terms used to describe SOD include papillary stenosis, sclerosing papillitis, biliary spasm, biliary dyskinesia, and postcholecystectomy syndrome. It is often difficult (or impossible) to completely distinguish between stenosis and dyskinesia so the term SOD is used to incorporate both groups of patients.

Anatomy

The SO is a complex muscular sheath consisting of circular and longitudinal muscle fibers that surround the distal common bile duct, the main pancreatic duct of Wirsung, and the common channel (ampulla of Vater) before emptying into the second portion of the duodenum. It is divided anatomically into four distinct components:

- the biliary sphincter (*sphincter choledochus*), which consists of circular muscle fibers surrounding the distal common bile duct proximal to its junction with the pancreatic duct
- the pancreatic sphincter (*sphincter pancreaticus*), which consists of the short annular muscle surrounding the distal intraduodenal segment of the main pancreatic duct (of note, this is located in only 30% of the population)
- *sphincter ampullae*, which consists of a thick sheath of longitudinal muscle fibers overlying thinner circular muscle surrounding the ampulla of Vater; and
- *fasciculi longitudinales*, which consists of short muscle fibers connecting the distal end of the joined ducts [4,5].

The intramural length of the SO is 7–22 mm; the common distal segment (which may not exist in 15% of individuals) is 1–9 mm, and the extramural length of the sphincter is about 5 mm [6]. The high-pressure zone is typically 4–10 mm in length. The embryological, anatomical, and physiological features of this musculature are separate from that of the surrounding duodenal musculature (Fig. 78.1).

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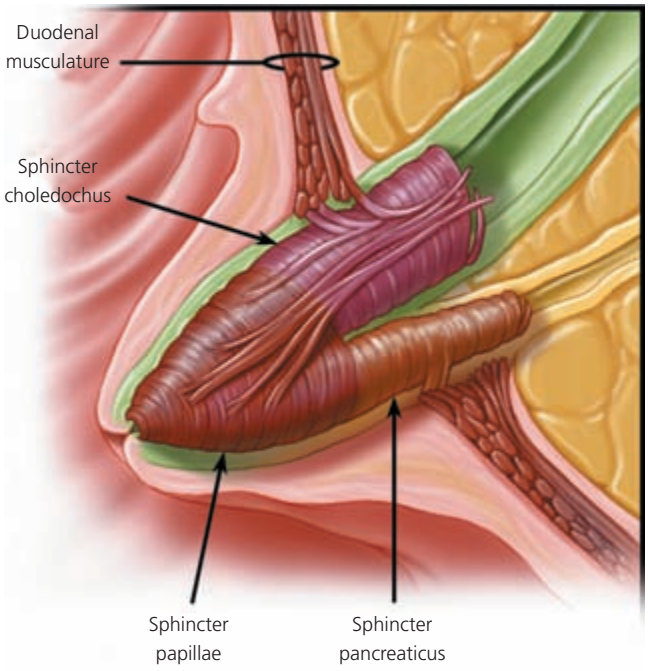

Figure 78.1 Anatomy of sphincter of Oddi.

Physiology

The exact role of the SO remains unclear. Some investigators propose an active role of the SO in propelling bile into the duodenum whereas others suggest that the SO functions only as a regulated resistor of bile flow. The available evidence suggests a role for the SO in the following three areas:

- regulating bile flow and maintaining a low intraductal pressure
- diverting the hepatic bile into the gallbladder during the interdigestive period
- preventing reflux of duodenal contents into the biliary tree [4].

Two types of motor activity have been detailed in association with SO: baseline tonic pressure (approximately 5–15 mmHg above the common bile duct pressure and 10–33 mmHg above duodenal pressure), and superimposed phasic contractions (occurring every 8–15 s and lasting 4–5 s) [7,8].

Sphincter regulation is under both neural and hormonal control although the exact mechanisms of that control are not clearly elucidated. Three regulatory systems have been described in relation to SO activity:

- synchrony with gastric and duodenal motor activity during the interdigestive period (reflex regulation)
- hormonal control, with cholecystokinin (CCK) in particular believed to play a major role
- neural control primarily through cholinergic parasympathetic fibers.

The maximal contractive activity of the SO occurs during phase III of the migrating motor complex [9–11]. Both intraduodenal acidity and food content have been shown to affect SO activity. In some animals, gastric distention has been shown to cause an increase in SO activity; however, in humans that does not appear to be the case [12]. CCK reduces the baseline pressure and decreases the phasic contractile activity of the SO in humans; however, in other species (opossum and prairie dog), CCK increases the phasic activity of the SO without affecting the baseline pressure [4]. In general, the following hormones are believed to have a stimulatory effect on SO motility: gastrin, secretin, motilin, bombesin, galanin, neuropeptide Y, peptide YY, pancreatic polypeptide, and substance P; whereas the following hormones are believed to be inhibitory: cholecystokinin, glucagon, secretin, calcitonin gene-related peptide, nitric oxide, and cerulin [4]. In addition, numerous pharmacological agents appear to have varied effects on SO motility. Morphine, fentanyl, pentazocin, ethyl alcohol, bethanechol, and physostigmine have stimulatory function; atropine, nitroglycerin, nifedipine, amyl nitrate, and barbiturates are inhibitory. The regulation of SO motility is complex and not completely understood [4].

Diagnosis and classification

The diagnosis of SOD rests on the presence of typical clinical symptoms in the setting of associated radiographic and laboratory abnormalities.

Milwaukee Biliary Group Classification

Several classification schemes have been proposed; however, the classic ("Milwaukee Biliary Group Classification") criteria proposed by Hogan and Geenen in 1988 remain the best studied [13]. Under this system, three groups of patients with pain suggestive of biliary etiology are recognized. These patients are divided as follows:

- type I patients fulfill the following three criteria:
  - pain associated with abnormal serum aminotransferases (alanine aminotransferase and aspartate aminotransferase more than twice the normal value on at least two occasions)
  - a common bile duct dilated more than 10 mm on ultrasound or 12 mm on endoscopic retrograde cholangiopancreatography (ERCP)
  - delayed drainage of contrast from the common bile duct after more than 45 min in the supine position
- type II patients have typical biliary-type pain and, in addition, fulfill one or two of the above criteria
- type III patients have typical biliary-type pain but fulfill none of the above criteria [13].

When manometry is considered to be the gold standard, the predictability of SOD varies among these three groups with the following values: type I, 65%– 95%; type II, 50%–63%; type III, 12%– 28% [14]. Given the logistical difficulties

in assessing delayed drainage times, this criterion is often excluded in clinical practice and research studies, resulting in the modified Hogan–Geenen classification scheme [15]:

In addition, three classification groups have also been developed for pancreatic SOD. These are as follows:

- type I patients fulfill the following two criteria:
  - abnormal pancreatic enzymes on two occasions
  - pancreatic duct dilated more than 6 mm
  - delayed drainage of pancreatic duct after ERCP by more than 8 min
- type II patients present with typical pancreatic-type pain and, in addition, fulfill one or two of the above criteria
- type III patients present with typical pancreatic pain but do not fulfill any of the above objective criteria [13,16].

Rome III criteria

In 2006, the Rome III Committee established three gallbladder and biliary sphincter disorders; referred to as functional gallbladder disorder, functional biliary SO disorder and functional pancreatic SO disorder. The hallmark of all three disorders is episodic pain located in the epigastrium or right upper quadrant and all of the following:

- episodes lasting 30 min or longer
- recurrent symptoms occurring at different intervals (not daily)
- the pain builds up to a steady level
- the pain is moderate to severe enough to interrupt the patient's daily activities or lead to an emergency department visit
- the pain is not relieved by bowel movements
- the pain is not relieved by postural change
- the pain is not relieved by antacids
- exclusion of other structural disease that would explain the symptoms.

Supportive criteria are: associated nausea and vomiting, radiation of pain to the back or right subscapular region, and awakening from sleep in the middle of the night.

Functional gallbladder disorder was defined as the above symptoms in the context of an intact gallbladder and normal liver enzymes, conjugated bilirubin, and amylase/lipase. Functional biliary SO disorder was defined as typical pain (meeting all the features detailed above) in conjunction with normal amylase and lipase measurements. Elevated serum aminotransferases, alkaline phosphatase, or conjugated bilirubin temporally related to at least two pain episodes was considered to be a supportive criterion. Functional pancreatic SO disorder was defined as typical pain in conjunction with elevated amylase/lipase [17].

Clinical presentation and demographics

SOD is estimated to affect approximately 13% of patients with unexplained right upper quadrant pain following cholecystectomy and 0.9% of all individuals undergoing cholecystectomy [18,19]. Although SOD may occur in patients of any age, the classical presentation is typically a middle-aged woman [20].

In terms of clinical presentation, abdominal pain is the sine qua non of this disorder. The pain is typically located in the epigastrium or right upper quadrant, lasts for from 30 min to several hours, and may be disabling. In some patients the pain is continuous with intermittent exacerbations. It may radiate to the back or shoulder. Associated nausea and vomiting may be present. The pain may begin following a cholecystectomy; however, this is not required and patients may also present with biliary-type pain that is not relieved with cholecystectomy. Jaundice, fevers, and chills are not typically observed and should prompt consideration of an alternative diagnosis [21]. Physical examination is characterized by the absence of objective abnormalities with the most common finding being nonspecific abdominal tenderness on palpation.

Following the initial history and physical examination, initial diagnostic evaluation should focus on etiologies typical of pancreatobiliary symptomatology. Initial evaluation should include standard liver chemistries, serum amylase or lipase, and abdominal ultrasonography or computed tomography (CT) scan. If possible, laboratory evaluation should be obtained during bouts of pain. Mild elevations in aminotransferases (less than twice the upper limits of normal) are frequently observed in SOD; however, significantly greater abnormalities are more suggestive of other etiologies, such as tumors, stones, and parenchymal liver disease. CT scans can often be normal in SOD, although mild ductal dilation is not uncommonly observed and is supportive of a diagnosis of SOD. More common gastrointestinal disorders such as gastroesophageal reflux and peptic ulcer disease should also be evaluated and treated concurrently. In the presence of typical biliary-type pain and the absence of improvement with treatment of more common disorders, further work-up for possible SOD is warranted.

Diagnosis using noninvasive imaging

Sphincter of Oddi manometry (SOM) is considered to be the gold standard for diagnosis of SOM and stratification of therapy. However, this procedure is not without risk and most series suggest an increased rate of post-ERCP pancreatitis in this patient population with values ranging from 17% to 31% [22,23]. Given this, most centers would recommend that the diagnostic evaluation begin with noninvasive imaging unless the clinical, laboratory, and radiographic evidence clearly suggests SOD. This point is particularly emphasized in the Rome III guidelines published in 2006 [17]. After initial laboratory analysis and ductal imaging, consideration should be given to the tests listed below.

Morphine–prostigmin provocation test (Nardi test)

The Nardi test represents historical precedent more than established clinical care. Morphine has been shown to cause SO contraction (measured manometrically). Prostigmin (neostigmine) is a cholinergic secretory stimulant. Given together, morphine (10 mg) and prostigmin (1 mg) should theoretically induce increased biliary and pancreatic flow in tandem with SO contraction. A positive test has been defined as reproduction of the patient's typical pain in tandem with a fourfold increase in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, amylase, or lipase levels [24,25]. However, the clinical utility of this test is questioned given that specificity and sensitivity have been poor when the Nardi test has been compared to results of biliary manometry or outcome after endoscopic sphincterotomy. This test has been largely replaced by the subsequent studies listed later in this chapter [26].

Secretory imaging studies

Ductal diameter is felt to be a significant predictor of SOD and is listed as one of the classic "Milwaukee" criteria. Provocatory imaging tests have been postulated as a noninvasive means of increasing specificity for the diagnosis of SOD. The theory behind this is as follows: following a lipid-laden meal or CCK injection, the gallbladder contracts, hepatocyte-induced biliary flow increases, and the SO relaxes; similarly, after a lipid-laden meal or secretin administration, pancreatic exocrine output increases and the SO relaxes. Therefore, if there is SOD, increased ductal dilation may be more preferentially seen after increased secretory output. In normal subjects, the bile duct diameter should remain constant or decrease with stimulation. Normative values are not clearly established but most studies define an increase of more than 1 mm to be abnormal and suggestive of possible SOD [27,28]. Studies have included patients with and without intact gallbladders. Provocatory ductal diameter has been measured using several different imaging modalities: transcutaneous ultrasound, endoscopic ultrasound, and magnetic resonance cholangiopancreatography (MRCP).

Transcutaneous ultrasound has been studied in several small studies with variable correlation when compared with SOM [29–33]. Endoscopic ultrasound with secretin stimulation has also been compared to ERCP with SOM with results suggesting "excellent negative and positive predictive values but poor overall sensitivity" [34]. Dynamic MRCP with secretin administration has been examined for noninvasive diagnosis [27,35–38]. Initial results have been encouraging with concordance between dynamic MRCP and ERCP reported to be 86% in one study; however, long-term data with larger patient populations and multiple centers are needed before this test can enter the clinical arena as anything other than a supportive research tool. Given these factors, dynamic imaging with transcutaneous ultrasound, endoscopic ultrasound, and MRCP are possibly useful clinical adjuncts to diagnosis but data do not support their use as an exclusive means of diagnosis.

Hepatobiliary scintigraphy

Quantitative hepatobiliary scintigraphy assesses bile flow through the biliary tract using radionucleotide imaging (Fig. 78.2). Functional or structural obstruction results in impaired radionucleotide flow and can support a diagnosis of SOD. However, the precise criteria used to define an abnormal study remain controversial with commonly measured variables including: the time taken to the maximal count over the biliary tree (T_{MAX}), the time taken for 50% of the tracer to accumulate over the biliary tree ($T_{1/2}$), the duodenal appearance time (DAT), the hilum-to-duodenum transit time (HDTT), and prolonged excretion of tracer from the biliary tree. Among these measurements, the most common criteria for diagnosis consist of a DAT longer than 20 min and an HDTT greater than 10 min [39–48]. Results of clinical studies have been mixed; some studies have reported sensitivities and specificities of 100% [49] while others have reported poor concordance between scintigraphic results and subsequent manometric studies [48]. Although scintigraphy has enormous appeal as an adjunct to diagnosis, its status in the management of SOD remains unclear.

ERCP with SOM

ERCP with SOM is the gold standard for diagnosis of SOD and the only means to directly measure SO motor activity [44]; however, because of the technical challenges and risk of complications inherent to this procedure, the decision to perform ERCP with manometry is not made lightly. In general, ERCP with SOM should not be considered as a pure diagnostic test unless the severity of the symptoms is such that sphincterotomy would be performed if the results of manometry were abnormal.

Technique

SOM is typically performed at the same time as ERCP. A side-viewing duodenoscope is inserted and advanced to the papilla at which time retrograde cannulation of the sphincter is achieved with a pressure-transducing manometry catheter. All medications that inhibit (anticholinergics, nitrates, calcium-channel blockers, glucagon) or stimulate (narcotics, cholinergics) the sphincter should be avoided for 8–12 h before manometry. Benzodiazepines are not thought to affect SO pressure in humans and are felt to be acceptable for sedation. Likewise, current evidence suggests that meperidine does not affect basal sphincter pressure (although it does affect phasic contraction) if used at a dose less than or equal to 1 mg per kg body weight [50]. Given that basal sphincter pressure is typically the only parameter used to guide SOD therapy, meperidine has been used frequently for conscious

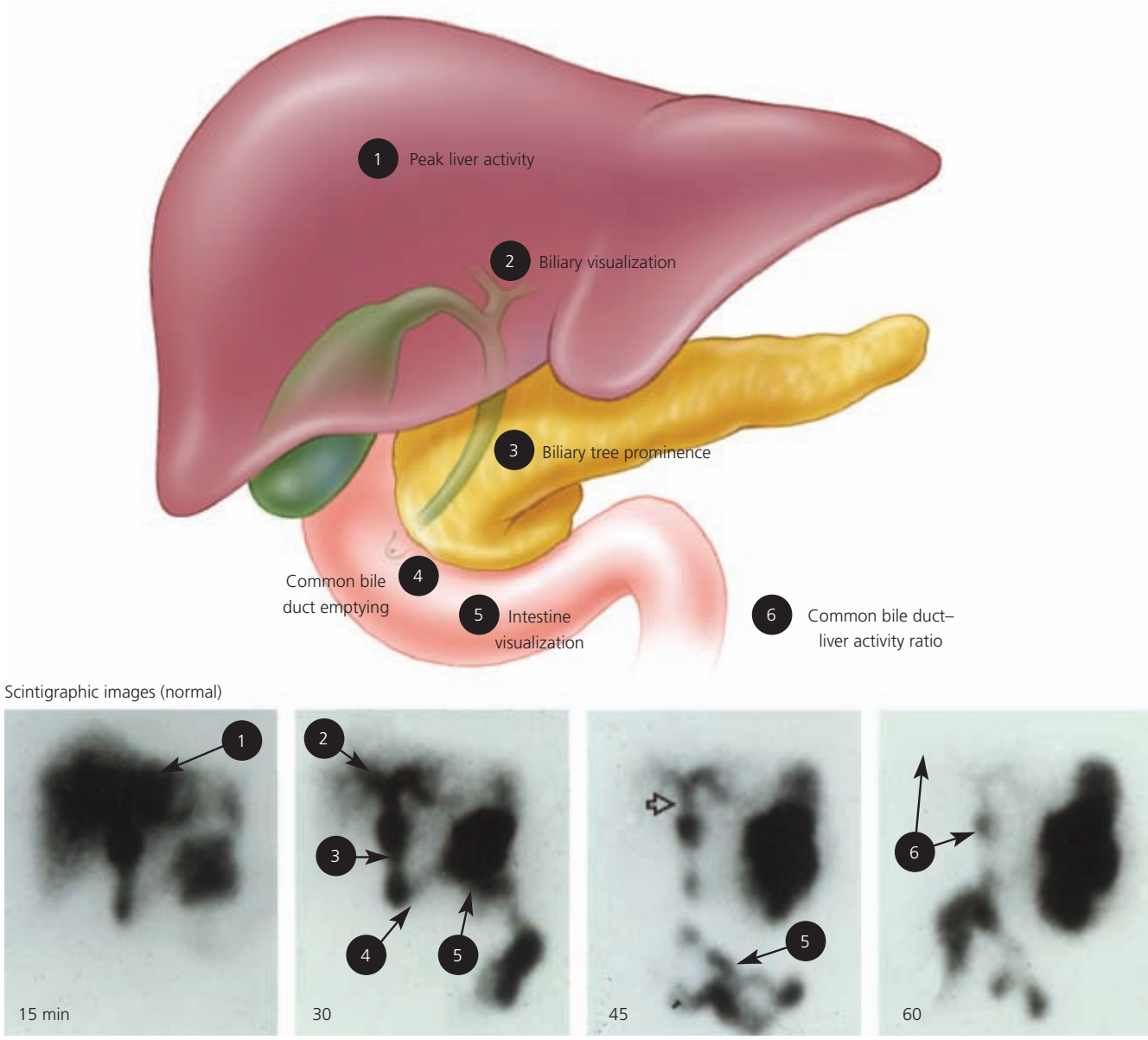

Figure 78.2 Technique of biliary scintigraphy for diagnosis of sphincter of Oddi dysfunction.

sedation at the dosing detailed above. Propofol has also been used for SOM. Although the data showing that propofol does not affect basal sphincter pressure in vivo are limited [51], there are in vitro data showing that propofol has less manometric effect than meperidine [52] – making it "standard of care" for SOM at our institution. If glucagon is needed for cannulation, at least an 8–10 min waiting period is required to allow the basal sphincter pressure to return to baseline before the manometry.

A 5-French, triple-lumen manometry catheter should be used because most standard parameters have been based on this catheter size. Aspiration catheters in which one port is sacrificed to permit end-hole and side-hole aspiration of intraductal fluid and perfusate are recommended because of the decreased risk of associated pancreatitis [23]. A minimally compliant pneumohydraulic capillary infusion system is used to perfuse the catheters. The usual rate of perfusion is 0.25 ml/channel; however, lower rates are used at some centers. Typically, a lower rate will convey an accurate basal sphincter pressure; however, the phasic wave information may not be as accurate. Distilled water is generally the perfusate of choice because saline has been known to crystallize in the capillary tubing and requires frequent flushing.

Selective cannulation of the common bile duct and pancreatic duct is required for accurate manometry. It is possible to distinguish reliably between the two ducts by gently aspirating: yellow-colored fluid signifies entry into the common bile duct; clear liquid signifies entry into the pancreatic duct. On achieving selective cannulation, the next step is to obtain a cholangiogram and pancreatogram before performing SOM

because this may provide additional information obviating the need for manometry. It has been demonstrated that obtaining a cholangiogram before manometry does not significantly alter the manometry results [53]. However, no such data are available for pancreatography.

Following selective cannulation, the manometry catheter is withdrawn across the sphincter at 1-mm to 2-mm intervals by a standard station pull-through technique. If possible, manometry should be studied in both the pancreatic duct and common bile duct because there are data that suggest that abnormal pressure may be detected on only one side in approximately 35%– 64% of patients [15,54–58]. According to at least one report, the manometric abnormality is more likely to be in the pancreatic sphincter in patients with recurrent pancreatitis and is more likely to involve the biliary sphincter in patients with biliary-type pain and elevated liver function tests [55] (Fig. 78.3).

Before performing manometry, it is necessary to either "zero" the catheter in the duodenum or to record continuous intraduodenal pressure with a separate manometry catheter attached to the outside of the endoscope. After this baseline has been established, at least two separate station pull-throughs should be obtained from both sphincters – although, for practical purposes it is often possible to use one pull-through if these results are felt to be of good manometric quality. The highest basal pressure that is sustained for 30 s should be identified. At that point, the four lowest amplitude points in that zone are identified. The mean of these four recordings is labeled as the basal sphincter pressure for that particular lead. The average of the basal sphincter pressures for all interpretable leads is then calculated as the final basal sphincter pressure. Once the basal sphincter pressure is calculated, the amplitude of phasic wave contractions is measured. This consists of the pressure increase from the basal pressure to the peak of the contraction wave. Four sample waves are taken from each lead with the mean pressure representing the phasic amplitude. The duration of phasic waves and frequency per minute can also be computed. However, most authorities rely solely on the basal sphincter pressure for diagnosis of sphincter of Oddi dysfunction as this measurement is more stable over time [16], has stronger interobserver reliability [59], is more reproducible with repeat testing [60], and is associated with response to therapy [16,61]. In addition, there is some evidence to suggest that basal sphincter pressure correlates with biliary pressure (which is markedly easier to calculate); however, this evidence needs to be substantiated in larger trials before it can enter into the realm of standard clinical care [62].

Data establishing normal values for SOM are limited. Perhaps the best data come from Guelrud and associates [63], who evaluated 50 asymptomatic patients for the purposes of measuring intraductal pressure, basal sphincter pressure, and phasic wave parameters. Manometry was then repeated on 10 subjects to establish reproducibility. However, because of the risks inherent with SOM, this study is unlikely to be repeated in a larger sample. At the present time, most authorities use a value of 35 mmHg or 40 mmHg as the upper limit of normal for mean basal sphincter pressure. As stated above, most authorities rely solely on basal sphincter pressure for the diagnosis of SOD.

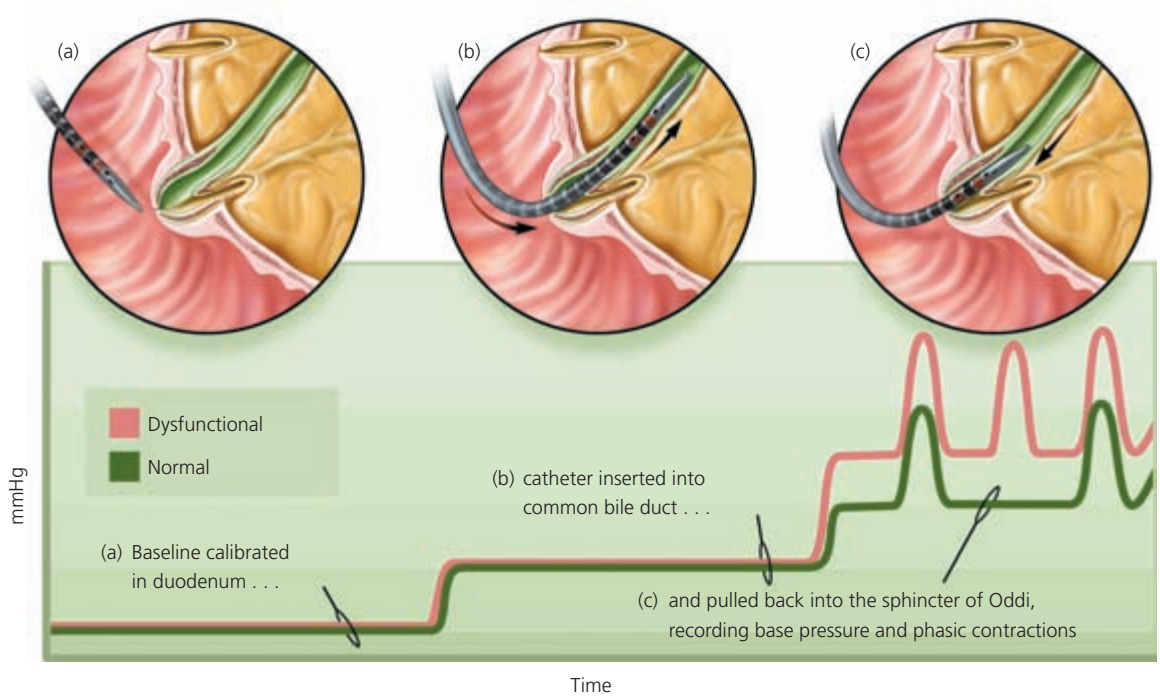

Figure 78.3 Technique of biliary manometry.

Therapy

The goal of therapy in SOD is to improve the impaired flow of biliary or pancreatic secretions into the duodenum. This can be accomplished by pharmacological, endoscopic, and surgical means. The success of therapy rests on the strength of clinical diagnosis. The various treatment options available will be discussed below.

Pharmacological therapy

Medical therapy for SOD has received little focused attention. Given that SOD is felt to result from increased sphincter pressure, theoretical consideration has been given to muscle-relaxing agents such as calcium-channel blockers and nitrates. Nifedipine has been shown to reduce basal SO pressure in volunteers and patients with SOD [64,65]. In a single small placebo-controlled crossover trial, nifedipine was associated with statistically significant decreases in the severity and frequency of pain along with emergency room visits and narcotic usage [66]. However, in vivo studies in the opossum model have shown that nifedipine has greater effects on blood pressure than SO pressure at low doses [67]. A subsequent study showed that nifedipine use was statistically associated with vascular side effects and was no more efficacious than placebo [68].

Nitrates have been shown to decrease SO tone in animal models and in humans [69]. A potential role for nitrates as therapy for SOD was suggested by one case report in which recurrent pain was relieved with nitrate administration; in this particular case, pain resolution was associated with a decrease in both basal and phasic SO activity [70]. To date, there have been no controlled trials of nitrate therapy for SOD.

Acupuncture

Intriguing data from a single pilot study suggests that electroacupuncture may be of therapeutic benefit in relaxing the SO. With selective stimulation of GB34 (*Yanglingquan*), a specific electroacupuncture site located on the lateral surface of the right leg below the head of the fibula, known in Oriental medicine to be a specific acupoint that affects the hepatobiliary system, it was possible to significantly decrease the SO basal pressure as well as the amplitude, frequency, and duration of phasic wave contractions. Moreover, this decrease correlated with an increase in CCK plasma levels. These changes were noted to be partially reversible on discontinuation of acupuncture. However, the study only examined SO pressure for 10 min after the acupoint stimulation, raising questions as to the long-term benefit and clinical relevance of these findings [71]. No long-term studies using acupuncture as therapy for SOD have been published.

Surgery

Surgical therapy for SOD is possible and was historically the mainstay of therapy. The traditional surgical approach includes a transduodenal biliary sphincteroplasty with a transampullary septoplasty (pancreatic septoplasty). There are two theoretical benefits to this approach over endoscopic therapy. First, surgery may reduce the chance of recurrent stenosis due to scarring. Second, a surgical approach may be more effective at relieving pancreatic duct stenosis because of the endoscopic difficulty of severing the transampullary septum without risking duodenal perforation [72,73]. Reported data suggest that between 50% and 60% of patients with biliary-type pain or recurrent pancreatitis treated with surgical sphincterotomy experienced clinical benefit [74–82]. However, there has been some suggestion that patients with pancreatitis may do worse than patients with recurrent biliary-type pain [79].

Despite these benefits, surgical therapy has largely been replaced by endoscopic therapy for numerous reasons: similar clinical outcomes, decreased morbidity, decreased mortality, cosmetic benefits, and increasing endoscopic expertise. Surgical therapy has been reserved for patients with restenosis following endoscopic sphincterotomy and when endoscopic evaluation is not technically feasible [44]. Nevertheless, in many centers, operative therapy continues to be the standard of care because of local expertise [14,83].

Endoscopy

Endoscopic sphincterotomy is the standard of care for patients with sphincter of Oddi dysfunction. Sphincterotomy has been extensively evaluated for different subsets of patients based on the Milwaukee classification and sphincter subset (biliary versus pancreatic). These categories will be addressed below.

Biliary sphincter of Oddi dysfunction

Consensus opinion holds that endoscopic sphincterotomy without sphincter of Oddi manometry is the standard of care for type I SOD. This argument is based on the supposition that type I SOD implies a fixed stenosis in the sphincter; hence explaining the ductal dilatation, prolonged drainage, and abnormal laboratory parameters in the context of symptomatology. In addition, because of the possibility of small tumors in the intrapapillary region leading to a fixed stenotic defect, sphincterotomy is recommended to ensure adequate visualization and suitable biopsy specimens. Nevertheless, this opinion is based on relatively scant clinical evidence and there are no randomized or controlled trials specifically evaluating therapy for type I SOD. In a small retrospective study evaluating 31 patients with type I SOD treated with sphincterotomy, Thatcher and colleagues noted clinical improvement in 87% of patients at a 3-month interval [84]. Boender and colleagues evaluated empiric endoscopic sphincterotomy without manometry in 11 patients with type I SOD and found clinical improvement in 100% of patients at a 2-year follow-up [85]. Rolny and associates described 17 patients with classic type I SOD who underwent manometry followed

by sphincterotomy (although 2 of the 17 patients underwent surgical rather than endoscopic sphincterotomy) [86]. Although all had significant clinical improvement, manometry was normal in 35%. Finally, Cicala and colleagues prospectively evaluated 30 patients with type I or type II SOD using manometry and scintigraphy [47]. Of the 8 patients with type I SOD, 6 accepted endoscopic sphincterotomy and all were clinically improved at follow-up of at least 10 months. Interestingly, when performed in the setting of type I SOD, manometry is normal in a sizeable percentage of cases (ranging from 13% to 65%) and does not appear to correlate with clinical outcome to sphincterotomy [86–89]. Therefore, at the present time, the available evidence for type I SOD indicates that sphincterotomy should be performed empirically because manometry data do not appear to impact clinical outcome and may even falsely exclude patients who would otherwise benefit clinically from therapy.

The data for optimal management in biliary type II SOD patients are not as clear. Theoretically, whereas the etiology for type I SOD patients can be viewed as a fixed stenotic process, type II SOD patients likely represent more of a transient, dyskinetic picture with less convincing evidence (based on radiographic and laboratory data) for SO obstruction. Three randomized controlled trials have been published on type II SOM management (although one only in abstract form). In a landmark study by Geenen and colleagues [16], 47 patients with type II SOD were randomized to sham sphincterotomy or endoscopic sphincterotomy. At 1-year follow-up, clinical improvement was noted in 65% of patients who underwent sphincterotomy compared with only 30% of patients who had undergone a sham procedure. An abnormal basal sphincter pressure was found to be the strongest predictor of benefit and when patients were stratified based on a basal pressure cutoff of 40 mmHg, 91% of patients with abnormal basal pressures experienced clinical improvement with sphincterotomy as opposed to only 25% with normal sphincter pressures. After a cross-over period, 17 of 18 patients with elevated pressures at study entry treated with sphincterotomy were clinically improved after a follow-up of 4 years. Toouli and associates [61] evaluated clinical outcomes to sphincterotomy versus sham sphincterotomy in 81 patients with primarily type II SOD. Sphincterotomy resulted in clinical improvement at 2 years in 11 of 13 (85%) patients with elevated basal sphincter pressures as opposed to only 5 of 13 (38%) patients who underwent a sham procedure; sphincter hypertension was the only statistically significant predictor of improvement. In an abstract, Sherman and colleagues evaluated 23 patients with manometrically documented type II SOD [90]; their data show clinical improvement in 5 of 6 (83%) patients treated with endoscopic sphincterotomy and 8 of 10 (80%) patients treated with surgical sphincteroplasty as opposed to only 1 of 7 (14%) patients treated with sham sphincterotomy. However, data from smaller non-randomized clinical series have been less definitive for patients with type II SOD with several studies showing less impressive results to manometrically guided sphincterotomy [91,92], no significant correlation between manometry and clinical outcome [84,93], or clinical improvement to empiric sphincterotomy without manometry [85,94,95]. Given the discordance in data, there is considerable debate as to whether patients with type II SOD should undergo manometrically guided therapy versus empiric sphincterotomy [96]. It is hoped that additional long-term prospective studies will further address this issue.

Type III SOD by definition consists of pain in the absence of abnormal objective measurements. It is unclear if this truly represents dysfunction of the sphincter itself as opposed to generalized intestinal dysmotility, visceral hyperalgesia, somatization disorders, or other functional abnormalities. Data in this subset are scarce [97]; however, available data support clinical improvement in approximately 30%–60% of patients with manometrically documented sphincter hypertension [90,91,98]. In addition, the complication rates appear to be significant in this population and more prospective information is needed to help guide management in this category.

Recurrent pancreatitis

The differential diagnosis of acute recurrent pancreatitis is broad and includes gallstones, microlithiasis, subclinical chronic pancreatitis, alcohol, structural lesions, tumor, drugs, pancreas divisum, and hypertriglyceridemia. Once these entities have been appropriately excluded, the possibility of SOD becomes significantly more likely. Studies by Coyle and colleagues [99], Sherman and cooleagues [100], and Geenen and Nash [101] demonstrated SOD in 31%, 33%, and 31% of cases of idiopathic acute recurrent pancreatitis, respectively. Toouli and associates identified manometric abnormalities in 25 of 28 patients with idiopathic acute recurrent pancreatitis after an extensive evaluation which was otherwise unrevealing [102].

Data regarding sphincter ablation for pancreatic SOD are largely limited to retrospective case series. However, these data indicate a response rate to sphincterotomy of approximately 50%–80%. Guelrud and colleagues evaluated 16 patients with type II pancreatic SOD [103]. After biliary endoscopic sphincterotomy and 6-month follow-up, 9 of the 16 patients (56%) were asymptomatic. Stone and colleagues evaluated 30 patients treated with sphincter ablation (endoscopic or surgical) for idiopathic acute pancreatitis [104]. In patients with documented sphincter hypertension, clinical improvement was seen in 15 of 18 patients (83%) whereas improvement was only seen in 4 of 12 patients (33%) without documented manometric abnormalities. The groups of Catalano [105] and Sherman [100] reported clinical improvement in 70% and 75% of patients with acute idiopathic pancreatitis and documented sphincteric hypertension treated with sphincter ablation, respectively. Guelrud and colleagues

reported clinical improvement in only 5 of 18 patients (28%) with documented type II pancreatic SOD treated with biliary sphincterotomy alone whereas dual sphincterotomy of both the biliary and pancreatic sphincter (either in one session or two) resulted in improvement in 86% and 77% of patients, respectively [106]. Wehrman and associates reported improvement in 8 of 9 patients (89%) treated with dual sphincterotomy for idiopathic recurrent pancreatitis and documented sphincter hypertension [98]. Coyle and colleagues reported improvement in 20 of 21 patients at 6-month follow-up [99]. However, the studies mentioned above have all been small case-series (most of which were published only in abstract form) and there are no randomized controlled trials evaluating pancreatic SOD. Based on the information above, the current standard of care is to perform manometry and subsequent sphincterotomy if the manometric values are abnormal. However, further studies are needed to guide therapy in this complicated area.

Chronic pancreatitis

Data evaluating pancreatic duct pressure and SOM in patients with chronic pancreatitis have been conflicting. Sphincter pressure has been reported to be normal [107,108], abnormal [54,109], or abnormal only in response to dynamic testing [110]. Moreover, there are data indicating that pancreatic juice viscosity may have a more direct impact on pancreatic duct pressure than sphincteric pressure [110,111]. The relationship between SOD and chronic pancreatitis was explored by Tarnasky and associates [112]. One hundred and four patients with presumptive biliary SOD (of whom 68 had confirmed sphincteric hypertension) underwent evaluation with endoscopic ultrasonography, pancreatography, and secretin stimulation intraductal function testing. Chronic pancreatitis was demonstrated in 29% of patients with confirmed sphincter hypertension as opposed to only 7% of those without SOD. At the present time, it is unclear if SOD directly leads to chronic pancreatitis or if changes associated with subtle chronic pancreatitis may induce alteration of the sphincteric pressure. It is hoped that this relationship will be clarified in subsequent studies. There is no clearly defined role for SOM in the setting of chronic pancreatitis.

Stenting, balloon dilatation, and botulinum toxin injection

Balloon dilatation of the SO was postulated to be of benefit in treating SOD under the supposition that it would be less invasive than sphincterotomy and may preserve anatomical function. However, the complication rate with this procedure was shown to be unacceptably high and there is no role for balloon dilatation in the management of sphincter of Oddi dysfunction [113]. Likewise, biliary stent placement has been evaluated for symptom relief in this disorder with similarly unacceptable complication rates [114]. Neither balloon dilatation nor stent placement (as an alternative to sphincterotomy) can be supported for the treatment of SOD.

Botulinum toxin (Botox) is a potent inhibitor of acetylcholine release that has been studied extensively in various motility disorders in the gastrointestinal tract including achalasia and gastroparesis. In a pilot study, botulinum toxin injection into the SO resulted in a significant decrease in basal sphincter pressure and improved biliary flow [115]. In a subsequent study, response to botulinum toxin in type III SOD was used as a variable to predict response to sphincterotomy [116]. In this study, 22 patients with manometrically documented type III SOD underwent botulinum toxin injection into the intraduodenal sphincter segment. Of the patients with a clinical response to botulinum toxin injection, 11 of the 12 patients later benefited from endoscopic sphincterotomy. Of the 10 patients who did not experience clinical improvement with botulinum toxin injection, only 2 of the 10 had clinical improvement with subsequent sphincterotomy. Botulinum has also been explored as a means to decrease post-ERCP pancreatitis following sphincterotomy. In a randomized controlled study of 26 patients who underwent SOM with sphincterotomy, postsphincterotomy injection of botulinum toxin resulted in a trend towards decreased postERCP pancreatitis [117]. However, this did not reach clinical significance and the rate of post-ERCP pancreatitis in this study appears to be higher than generally reported, raising questions as to the applicability of these results. More clinical data are needed before botulinum toxin can enter into the armamentarium of treatment for SOM and its use remains experimental within the context of clinical trials.

Complications

Pancreatitis is the most common major complication following SOM with reported rates as high as 31% with use of a standard perfusion catheter [22,23,118]. These numbers emphasize the risk associated with manometry and they have limited the widespread application of this diagnostic modality. Several methods have been proposed to decrease the incidence of postmanometry pancreatitis, including the use of an aspiration catheter, gravity drainage of the pancreatic duct after manometry, decrease of the perfusion rate to 0.05–0.10 ml/lumen/min, limitation of pancreatic duct manometry time to less than 2 min, and use of a nonperfused manometry system [119–121]. Moreover, if manometry is abnormal and sphincterotomy is performed, routine temporary stenting of the pancreatic duct has been shown to decrease resultant pancreatitis [122]. As has been detailed above, botulinum toxin has also been studied in this context. Using an aspiration catheter (allowing aspiration of the perfused fluid from end and side holes while simultaneously recording pressure from the two remaining side-ports), Sherman and associates were able to decrease the risk of manometry-induced pancreatitis from 31% to 4% in a series of 76 patients [23]. Using many of the above modifications

and careful patient selection criteria, more recent quotations of post-ERCP pancreatitis for patients with SOD undergoing manometry and sphincterotomy have been in the realm of 8% [123].

Approaches to treatment failure

There are several potential explanations for an apparent failure for adequate biliary sphincterotomy in a patient with well-documented SOD. First, the pain may not be of pancreatobiliary etiology. There are increasing data linking type III SOD with altered gastrointestinal motility, functional bowel disorders, hyperalgesia, somatization, depression, and anxiety as compared to control subjects [124–128]. Second, the biliary sphincterotomy may have either been inadequate or the site may have restenosed. Data on the incidence of restenosis are extremely limited; however, the one study to examine this issue reported a rate of 4.7% over a follow-up period of 7 years [129]. In these cases, the standard of care is to repeat sphincter ablation; however, there are no data available concerning the symptoms of these patients compared to control patients without restenosis.

A third possibility is pancreatic sphincter hypertension in cases where a dual sphincterotomy was not performed. Eversman and associates reported abnormal pancreatic sphincter pressure in 90% of patients with pancreatitis or persistent pain after biliary sphincterotomy [130]. Likewise, Soffer and Johlin reported abnormal pancreatic sphincter pressure in 25 of 26 patients with SOD who failed to respond to biliary sphincterotomy [131]. Finally, Elton and colleagues performed pancreatic sphincterotomy on 43 patients with type I or type II SOD who failed to respond to biliary sphincterotomy alone [132]. Following this procedure, 72% of the patients were asymptomatic. These studies have led many experts to recommend dual sphincterotomies rather than biliary sphincterotomy alone. Research into this issue is ongoing.

A fourth possibility is that certain patients with presumed SOD may in fact have subtle hitherto-undiagnosed chronic pancreatitis. Chronic pancreatitis is clearly associated with SOD and this may either cloud the diagnosis of SOD or explain an apparent lack of response to seemingly appropriate therapy. Tarnasky and colleagues demonstrated the presence of chronic pancreatitis in 20 of 69 patients with confirmed sphincter hypertension [112]. Moreover, patients with chronic pancreatitis have been shown to have a significantly decreased clinical response compared to control patients with SOD and to have no evidence of chronic pancreatitis. This was illustrated by Okolo and associates in a retrospective case series in which a significantly higher rate of recurrent pain was seen in patients with SOD and chronic pancreatitis (42%) when compared with patients with SOD but no evidence of chronic pancreatitis (27%) [133].

A final possibility is the presence of an obstructing lesion not previously identified such as a tumor, stricture, or stone either leading to an initial misdiagnosis or developing since the initial work-up. Given this possibility, a reevaluation of the sphincter with contrast is always recommended in this scenario.

Controversies

Although it has been approximately 30 years since the advent of biliary manometry [134] there is still much that remains unknown with regards to the appropriate diagnosis, treatment, natural history, and outcome of SOD. Whereas consensus is reasonably clear with regard to type I SOD, considerable debate exists regarding the optimal management of the remaining categories. Should patients with type II SOD undergo mandatory manometry before sphincterotomy? Is there a way to better define patients with type III SOD to lessen the risk of pancreatitis in this population and optimize which patients will benefit from sphincterotomy? When therapy is offered, should the initial therapy of choice be a dual sphincterotomy, biliary sphincterotomy, or selective sphincterotomy depending on the nature of the presenting symptoms? Given that manometry is abnormal in approximately one-third of patients with type I SOD, how reliable is manometry using a single time-point in the remaining, less objectively defined categories? Is there a way to increase the diagnostic yield of manometry either at a single temporal interval or through the development of a safe ambulatory technique to optimize the diagnostic yield? Is there a way to better predict which patients will respond to invasive therapy before subjecting those patients to the risks of manometry and sphincterotomy? How reliable are noninvasive diagnostic studies and is there a way to optimize these modalities? Should evaluation for this disorder even be considered in patients with an intact gallbladder?

These questions need to be addressed through randomized controlled studies. With this information, it is hoped that a universal standard of care can be developed to better guide management of this difficult disorder.

Conclusion

SOD is a complicated disorder that is challenging to diagnose and treat. At the present time, although numerous questions remain, the following general conclusions can be offered. Patients should be stratified according to the Milwaukee criteria after more common causes of recurrent postprandial abdominal discomfort have been eliminated. Patients with type I SOD should be offered sphincterotomy. There is debate regarding the necessity of manometry in type II SOD patients but we believe that manometry should be offered before therapy given the results of randomized controlled trials. Manometry is mandatory before sphincterotomy for patients with type III SOD. Given the high rate of post-ERCP

pancreatitis in this population, all efforts should be made to exclude other diagnoses before considering SOM and the procedure should only be performed by a practitioner who is skilled in the technical aspects of this disorder.

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G LIVER

79 Liver: anatomy, microscopic structure, and cell types

Gary C. Kanel

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The liver is a unique organ with a wide range of both structural and physiological functions. Understanding the arrangements of all the individual components of the liver on both gross and light microscopic examination assisted by electron microscopic and immunoenzymatic features allows a better understanding of liver histopathology and pathophysiology. Study of the embryological evolution of the hepatic and hepatobiliary elements also assists in understanding many of the key concepts in developmental abnormalities seen in a wide variety of pediatric and adult liver diseases. This chapter outlines the gross and microscopic characteristics of the liver and briefly addresses liver function, with an introductory presentation of embryological development.

Embryology

The hepatic primordium anlage first appears toward the end of the third week of gestation and is seen as a hollow midline outgrowth stalk (hepatic diverticulum) of the endodermal epithelium at the distal aspect of the foregut (future duodenal loop) [1–7]. By the fourth week, the diverticulum enlarges by way of proliferation of the endodermal cell strands (hepatoblasts) and projects cranially into the mesoderm of the septum transversum (the mesodermal plate between the pericardial cavity and the yolk sac stalk), eventually giving rise to the hepatic parenchyma and intrahepatic duct structures (Fig. 79.1). The cephalic end ultimately develops into the right and left hepatic lobes. The stalk between the diverticulum and foregut narrows, forming the extrahepatic biliary system and gallbladder. The proliferating endodermal cells initially form solid cords, which then anastomose and form isolated vesicles and cribriform tubules with centrally located lumenal structures (biliary canaliculi). The cords eventually become confluent and form channels that subdivide the cords by small capillaries, which co-mingle with the developing vitelline and umbilical veins. These channels ultimately form the hepatic sinusoids. The individual hepatoblasts are progenitor cells that develop into the mature hepatocyte, with those cells immediately adjacent to the portal mesenchyme becoming the ductal plates [8]. The rapid growth rate of the hepatic cords allows sheets several cells thick, termed *muralium multiplex*, to develop; these persist until birth, after which the cell sheets narrow to two cells (*muralium duplex*) and eventually evolve into a one cell thick trabecular cord (*muralium simplex*) by 5 years of age [9,10].

The mesoderm of the septum transversum initially surrounds the liver and is in continuity with the lesser curvature of the stomach, duodenum, and ventral body wall. The mesoderm becomes stretched between the liver and lesser curvature, forming the lesser omentum, and between the diaphragm and ventral abdominal wall, forming the falciform, coronary, and triangular ligaments. A portion also develops into the hepatic (Glisson) capsule. The developing hepatic artery and vagus nerve branches follow the mesoderm along and adjacent to the portal vein. The mesoderm on the liver surface is also in continuity with the peritoneum, whereas that portion in contact with the future diaphragm remains uncovered (*bare area*). The mesoderm is the main focus for the development of hematopoiesis, which begins at about 6 weeks and becomes most active during the sixth and seventh months, then rapidly regresses owing to developing bone marrow activity. Hematopoiesis is responsible for the prominent liver size in the fetus (up to 10% body weight by week 10, with the liver occupying most of the abdominal cavity) but regresses at birth so that only a few islands of hematopoietic cells are noted. The liver then weighs about 5% of body weight. Hematopoiesis is usually absent by 4 weeks of age. Initially, the enlarged fetal liver has symmetric right

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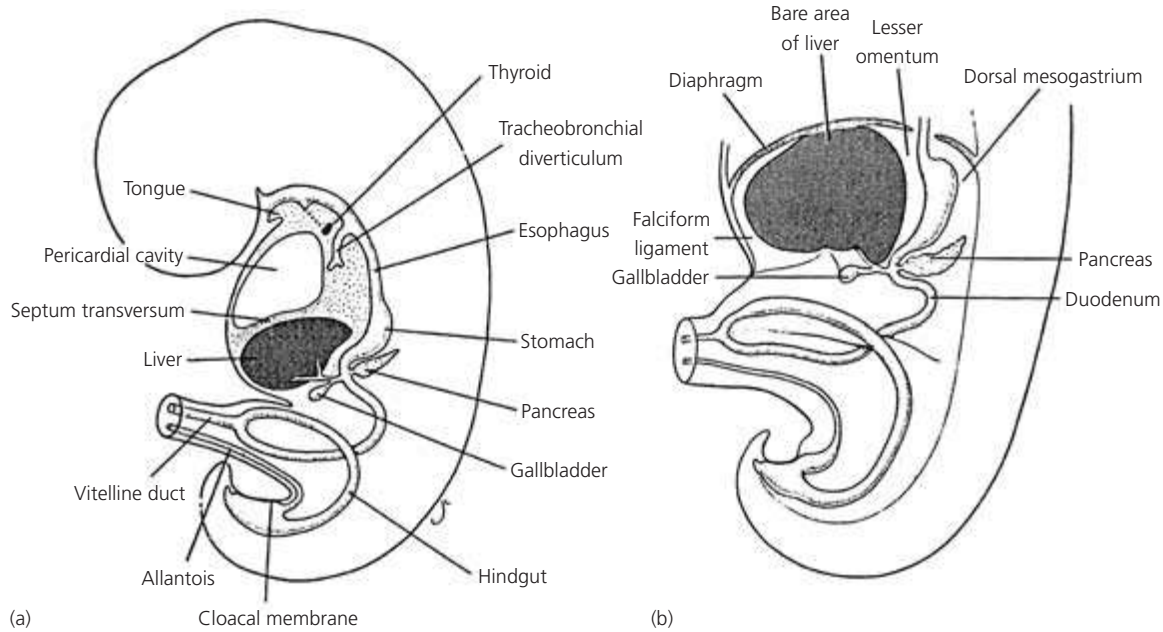

Figure 79.1 Developing embryo. **(a)** The drawing represents a 9-mm embryo, estimated at about 36 days' gestation. The liver is derived from the hepatic diverticulum (midline outgrowth stalk of the distal foregut) and projects cranially into the septum transversum and caudally into the abdominal wall. **(b)** The drawing represents an embryo that is slightly older, with the falciform ligament noted between the hepatic parenchyma and the anterior abdominal wall. From Langman [5] with permission from Lippincott, Williams & Wilkins.

and left lobes; with time, however, the growth rate diminishes but affects the left lobe more than the right, causing the size difference seen at birth. The caudate and quadrate lobes develop as subdivisions of the right lobe.

The vascular network is originally derived from the development of both the vitelline and umbilical veins and occurs at the same time as proliferation of the hepatoblasts. The cords and vessels anastomose, forming the hepatic sinusoids. The sinusoidal plexus initially receives blood through the vitelline vein and is drained into the sinus venosus, but by the fifth week the right and left umbilical veins also supply blood to the sinusoidal plexus. By the fifth week, most of the major vessels are identified and include the right and left umbilical vein, the transverse portal sinus, and the ductus venosus, which shunts blood directly from the umbilical vein into the inferior vena cava. At this time, the left umbilical vein becomes the main source of blood flow, with the right umbilical vein becoming atrophic by the fifth week. The portal vein develops from the vitelline vein and then subdivides into the right and left branches. The hepatic and portal vein branches divide the parenchyma into lobules and acini. The left umbilical vein blood flow has three major branching routes: through the sinusoidal plexus of the left half of the liver; through the sinusoidal plexus of the right half of the liver by retrograde flow from connections with the left branch of the portal vein; and through the ductus venosus. At birth, a sphincter mechanism closes the ductus venosus, causing cessation of blood flow through the umbilical vein, with the liver now receiving blood from the left branch of the portal vein. The resultant closed segment of the umbilical vein then becomes the umbilicus, and the fibrotic ductus venosus becomes the ligamentum venosum.

The biliary apparatus develops from membranous infolding occurring between the junctional complexes between individual hepatoblasts and appears initially as intercellular spaces with no distinct wall. The biliary canaliculi are first seen at week 6, with bile synthesis occurring by week 9 and bile secretion by week 12. The hepatoblasts immediately adjacent to the mesenchyme of the portal tracts form the ductal plate, which is two-layered; by 3 months, a lumen is seen within the ductal plate, with formation of double-layered tubular structures. Invasion of these cells into the portal mesenchyme occurs, forming an anastomosing network of portal duct structures. These ducts are not fully developed at term [11], after which remodeling forms the true interlobular bile ducts that assume their major role as a link between the canalicular biliary apparatus and the extrahepatic biliary tree. This biliary network is fed by a hypervascular complex of arterioles and capillaries formed from the peribiliary plexus. The immature structures at birth may persist for up to 1 month. The extrahepatic biliary tree develops from the stalk of the original hepatic outgrowth.

Individual cell functions become apparent at different but early times in embryological development [9,12,13]. α-Fetoprotein, which is found in high amounts at birth, initially is present by 1 month gestation. Glycogen may be seen

by 2 months, with glycogen synthesis becoming most apparent by 3 months; at birth, the amount of glycogen rapidly diminishes owing to rapid and active glycogenolysis. Fatty change within the hepatocyte parallels that of glycogenesis. Hemosiderin is usually visible in early stages and becomes most marked as intrahepatic hematopoiesis decreases; it then gradually decreases but may still be seen at birth in the periportal hepatocytes. The perisinusoidal cells and Kupffer cells appear by 3 months' gestation.

Gross anatomy

The liver takes up the majority of the right upper abdominal cavity and extends from the right lateral aspect of the abdomen 15–20 cm transversely toward the xiphoid [1,2,14–16]. The weight of the adult liver varies from 1200 to 1800 g, dependent on overall body size, and constitutes about 1.8%–3.1% of total body weight; however, at birth, the liver is larger compared with adjacent thoracic and abdominal viscera and constitutes about 5%–6% of body weight [1,17,18]. Anatomically, the liver has four lobes: right, left, caudate, and quadrate. The right lobe accounts for half to two-thirds of total liver volume; however, functionally, the right and left lobes are of about equal size and are divided by a line extending from the inferior vena cava superiorly to the middle of the gallbladder fossa inferiorly. A total of eight functional segments are present, each demarcated by the vascular and biliary drainage [1,2,9,14,19–21] (Fig. 79.2): the lateral (segments VI and VII) and medial (segments V and VIII) divisions of the right lobe, the medial (segment IV) and lateral (segments II and III) divisions of the left lobe, and the caudate lobe (segment I), the latter a watershed area of the right and left lobe vasculature.

The hepatoduodenal ligament connects the liver to the superior aspect of the duodenum and supports the hilar vessels and duct structures. The transverse fissure separates anteriorly the right lobe from the caudate lobe, whereas the umbilical fissure is located to the left of the quadrate lobe, which itself is bordered on the right by the gallbladder. The peritoneal layers forming the falciform ligament, which extends between the liver and the anterior abdominal wall, separate to form the superior layer of the coronary ligament and the left triangular ligament. The ligamentum teres is located along the lower edge of the falciform ligament and contains the obliterated umbilical vein remnant. The total surface area of the liver is structured by direct continuity with the surrounding abdominal organs, ligaments, and fascia.

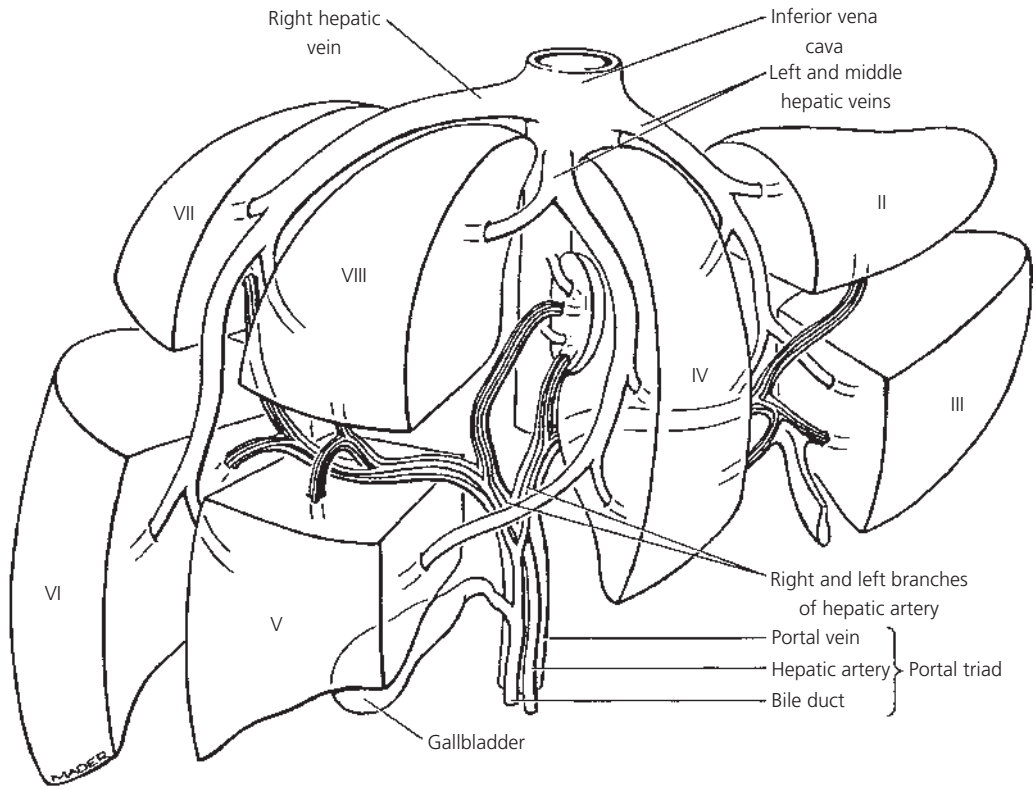

Figure 79.2 Anatomical and functional subdivisions. The liver is divided into eight functional anatomical segments, each having its own vascular flow and biliary drainage. These include the right lateral (segments VI and VII) and medial (segments V and VIII) divisions, the left medial (segment IV) and lateral (segments II and III) divisions, and the caudate lobe (segment I). From Moore KL, Dalley AF II. Clinically Oriented Anatomy, 4th edn. Philadelphia: Lippincott, Williams & Wilkins, 1999.

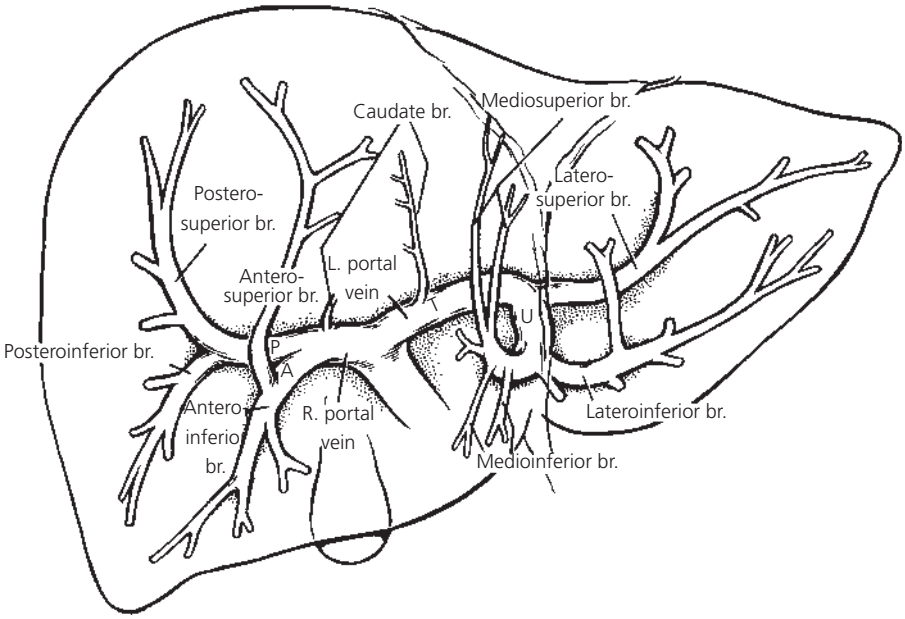

Figure 79.3 Intrahepatic branches of the portal vein. A, anterior segment; br., branch; L., left; P, posterior segment; R., right; T, pars transversus; U, embryonic ductus venosus. From Skandalakis LJ, Colborn GL, Gray SW, et al. Surgical anatomy of the liver and extrahepatic biliary tract. In: Nyhus LM, Baker RJ (eds). Mastery of Surgery, 2nd edn. Boston: Little, Brown, 1992.

The portal vein is the main route of vascular drainage of the gastrointestinal tract and is formed by merger of the superior mesenteric and splenic veins. It also receives blood from the coronary and cystic veins. The portal vein is located along the hepatoduodenal ligament posterior to the hepatic artery and common bile duct and ends at the porta hepatis at the main lobar fissure, dividing into the right and left main branches. The right branch divides early into anterior and posterior segments, whereas the left branch divides into two segments: the pars transversus, which extends to the left in the porta hepatis, and the pars umbilicus, which descends into the umbilical fossa in line with the left segmental fissure. The caudate lobe veins arise from both the right and left main portal vein branches (Fig. 79.3).

The hepatic vein is composed of three major tributaries (right, middle, left), each having intrahepatic branches. The middle and left hepatic veins often converge to form a single outflow vessel before draining into the inferior vena cava, whereas the right hepatic vein opens through a separate ostium. The caudate lobe drains directly into the inferior vena cava.

The hepatic artery is a branch of the celiac artery and ascends along the hepatoduodenal ligament and eventually divides into the right and left main branches. The right hepatic artery is usually seen behind the common hepatic duct after giving rise to the cystic artery, and eventually divides into the anterior and posterior segmental branches. The left hepatic artery passes obliquely upward and to the left in the porta hepatis, eventually dividing into the medial and lateral segmental branches. The quadrate lobe is fed by a middle hepatic artery branch, whereas the caudate lobe is fed by both right and left hepatic artery branches.

The biliary system originally arises from the bile canaliculi and can be grossly demonstrated in the larger interlobular branches. The biliary drainage of the right lobe is derived from anterior and posterior segmental branches that merge to form the right hepatic duct. Lateral and medial segmental branches merge to form the left hepatic duct that drains the left lobe. The caudate lobe is drained from three duct branches directly into the right and left hepatic ducts (Fig. 79.4). The smaller ducts do not have a distinct wall, but the larger septal branches demonstrate a thin wall secondary to periductal collagen fibers and can easily be demonstrated on gross inspection. The bile duct structures are directly fed by the hepatic artery and its branches, which parallel the ducts as they course through the various hepatic segments.

The lymphatic channels provide both sympathetic and parasympathetic innervation and are divided into deep and superficial branches. The deep lymphatic branch parallels the portal and hepatic vein branches, whereas the superficial lymphatic structures arise from the capsule of Glisson and drain through the adjacent falciform ligament, diaphragm, esophagus, and hilar lymph nodes [1,2,22].

The nerve supply parallels the main hepatic artery and portal vein and is divided into parasympathetic and sympathetic fibers. The former are preganglionic and derived from the vagus nerve, whereas the latter are postganglionic and receive their nerve supply from T7 through T10. The nerve supply enters the hepatic hilum through both anterior and posterior routes and feeds the arteries and bile ducts through sympathetic innervation. The nerve fibers branch through the main portal tracts, with smaller unmyelinated branches feeding the periportal hepatocytes. In addition, many of the nerve fibers terminate on endothelial cells lining the smallest

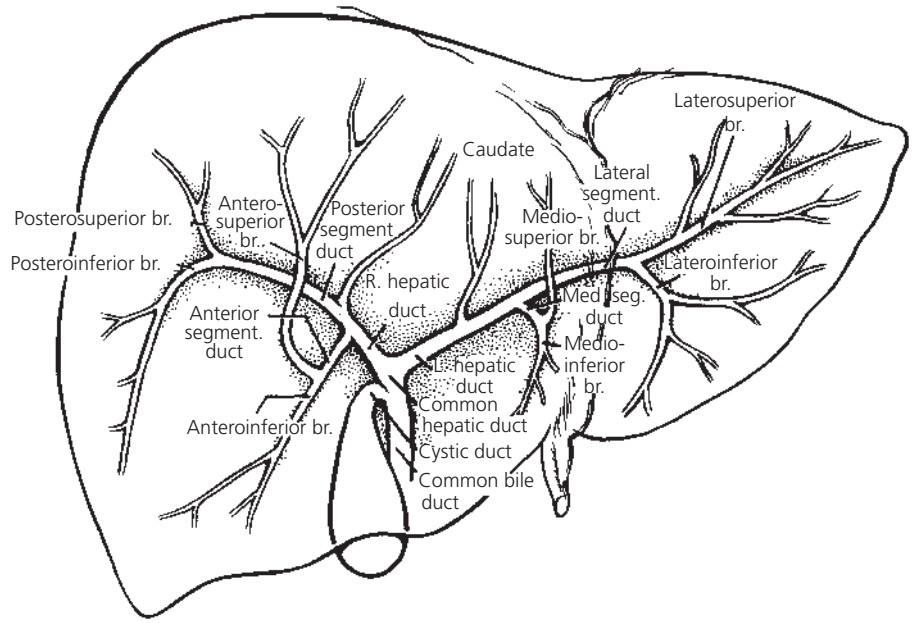

Figure 79.4 Branching segments of the intrahepatic bile ducts. br., branch; L., left; R., right. From Skandalakis LJ, Colborn GL, Gray SW, et al. Surgical anatomy of the liver and extrahepatic biliary tract. In: Nyhus LM, Baker RJ (eds). Mastery of Surgery, 2nd edn. Boston: Little, Brown, 1992.

arterioles and along Kupffer cells, stellate (fat-storing) cells, and hepatocytes [23].

Microanatomy

Normal histology

Architecture

The basic microanatomical structure of the liver can best be appreciated on a three-dimensional drawing of the portal tracts, parenchyma, and vascular flow (Fig. 79.5). This architectural arrangement is evenly spaced throughout all lobes of the liver and is best assessed under low- and medium-power microscopy [24,25]. Although the portal inflammatory and cellular components and the size of the liver cell plates vary, dependent on the developmental stage of the liver, the architectural framework can be seen in fetal liver as well. Although the architecture may at times be difficult to assess when acute liver injury is present, on recovery the arrangements universally return to normal; on the other hand, the distortion of the architecture seen in advanced chronic liver disease is for the most part irreversible.

Portal tracts

The portal tracts (Fig. 79.6) are often referred to as *portal triads*; this term is misleading in that more than three components are present. The interlobular bile ducts number from one to two per portal structure, although in infants the ducts appear early on to be slightly less frequent. The ducts are usually seen immediately adjacent to the hepatic arterioles, which are responsible for their blood supply. The hepatic arterioles are usually present singly. The portal venules similarly are a single structure, although transverse cuts of the portal tracts in biopsy material can mistakenly appear as if two or more ducts and vessels are present. True increase in portal venules and lymphatics are characteristic of portal hypertension and are usually seen in conjunction with portal fibrosis or cirrhosis. The fibrous tissue that supports the major portal components varies in amount, depending on the distance of the portal tract from the hepatic hilum, and can mistakenly be described as *fibrotic* when the biopsy specimen is taken toward the hilum. The infiltrating cellular inflammatory components within the fibrous tissue consist of scattered lymphocytes, which are scanty but often present to some degree even in normal liver.

Parenchyma

In adults, the hepatic lobules constitute about 80% of the total hepatic volume and are composed predominantly of liver cell cords one cell thick made up of polyhedral hepatocytes. The adjacent sinusoids are lined by both endothelial cells and Kupffer cells. The perisinusoidal space is located between the endothelial cells and hepatocytes. Stellate cells and collagen fibers are also present along the perisinusoidal space, but on routine hematoxylin and eosin staining of normal liver tissue, these structures are usually inconspicuous. The sinusoids drain from the portal venule and hepatic arterioles into the terminal hepatic venules (Fig. 79.7).

Parenchymal cellular components

Hepatocyte

The hepatocyte comprises about two-thirds of the total number of cells within the liver and about four-fifths of total liver volume [1–3,26–28]. Hepatocytes generally measure

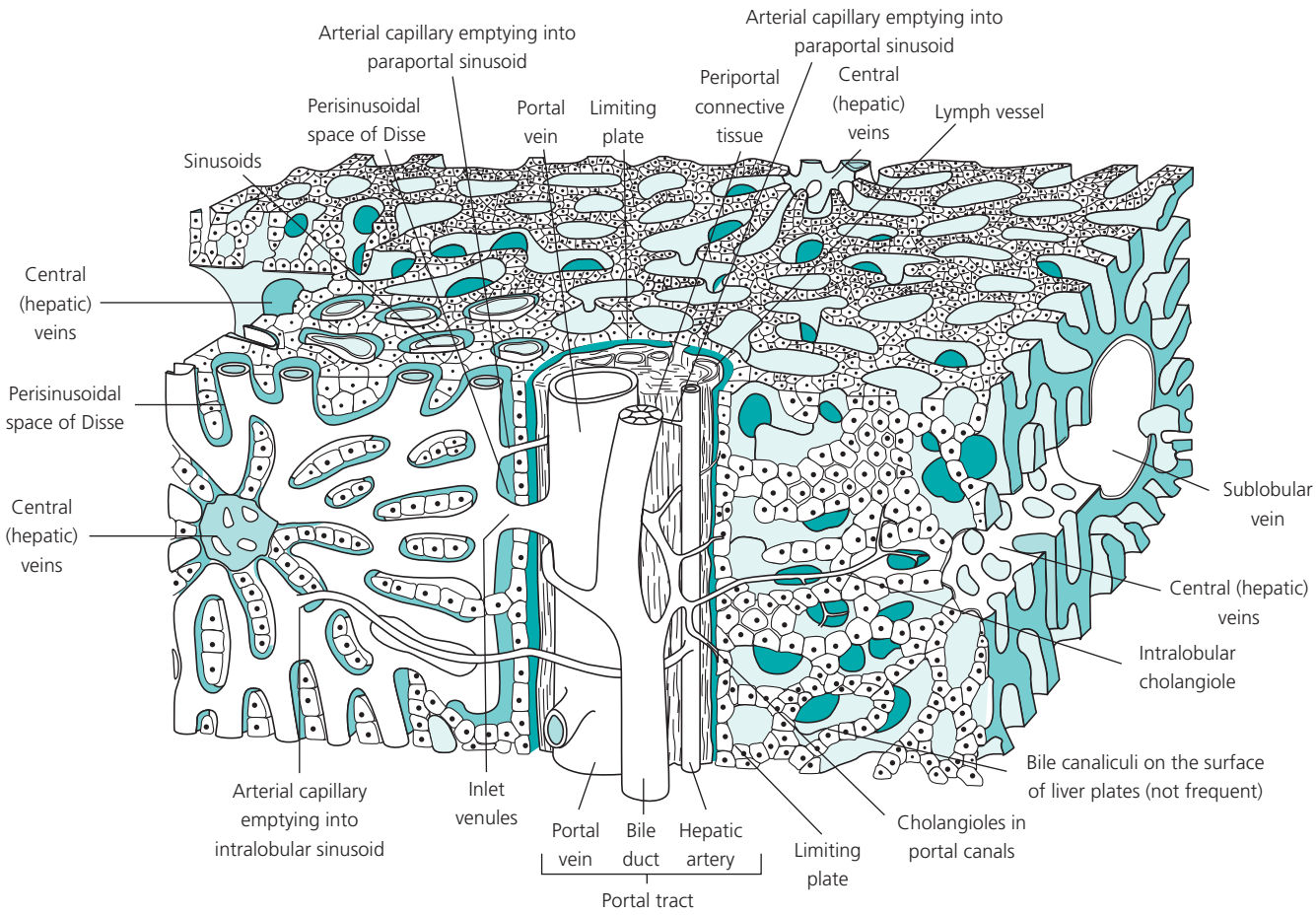

Figure 79.5 Structure of the normal liver. From Sherlock & Dooley [2], with permission from Blackwell Publishing.

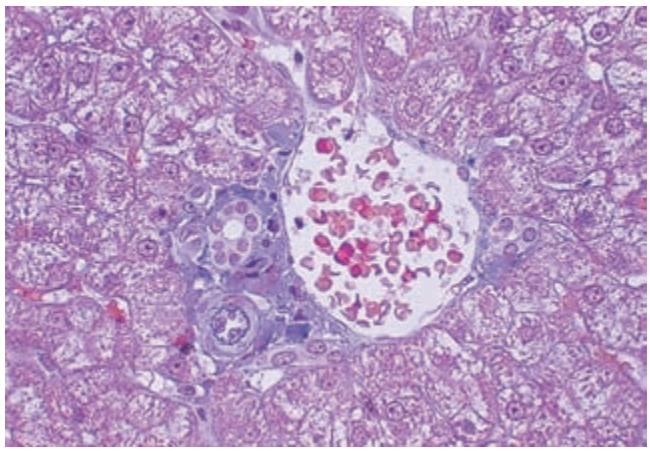

Figure 79.6 Portal tract (Masson trichrome). The major components include the hepatic arteriole, portal venule (large vessel), and bile ductule (cuboidal epithelium). There is a normal amount of collagen seen in this portal tract.

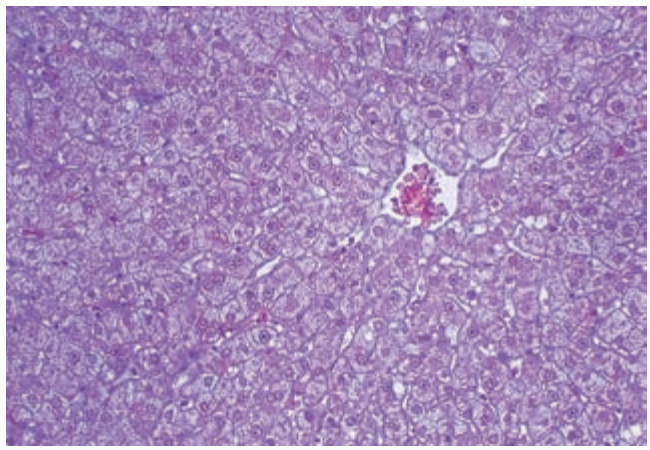

Figure 79.7 Parenchyma. The liver cell plates are one cell thick and are composed of polyhedral hepatocytes with centrally placed nuclei. These cells are bordered by sinusoids lined by both Kupffer and endothelial cells. The sinusoids drain directly into the terminal hepatic (central) venules.

25–40 μm in diameter, depending on their zonal location and patient age, and are polyhedral and multifaceted. The cells in the adult are arranged in cords that are one cell thick and have three distinct cell boundaries: sinusoidal, lateral (intercellular), and canalicular membranes [9,26]. The sinusoidal or basolateral surface area constitutes about 60%–70% of the total surface area. Microvilli measuring about 0.5 μm in length can be seen along the surface extending into

the perisinusoidal spaces. The lateral membranes lie between adjacent hepatocytes and constitute about 15% of the surface area. They are divided into a number of junctional subunits [1,26]. Gap junctions represent two adjacent membranes with a distinct 2- to 4-μm gap, whereby communication between cells for transport of metabolites occurs by way of projected particles (*bobbins*) directed from each cellular membrane. No microvilli are seen. Desmosomes are attached to the intermediate filaments and play a role in the resilience of the cell membrane. Intermediate junctions are attached to various cytokeratin filaments that also aid in cellular resilience. Tight junctions are zones of contacted membranes and represent a permeability barrier to macromolecules [29].

The canalicular membranes constitute about 15% of membrane surface area, lie toward the center of the intercellular junction, and represent the origin of biliary drainage (Figs 79.8 and 79.9). They are lined by microvilli and measure in diameter from 0.5 μm in the perivenular zone (zone 3 of Rappaport) to 2.5 μm in the periportal zone (zone 1).

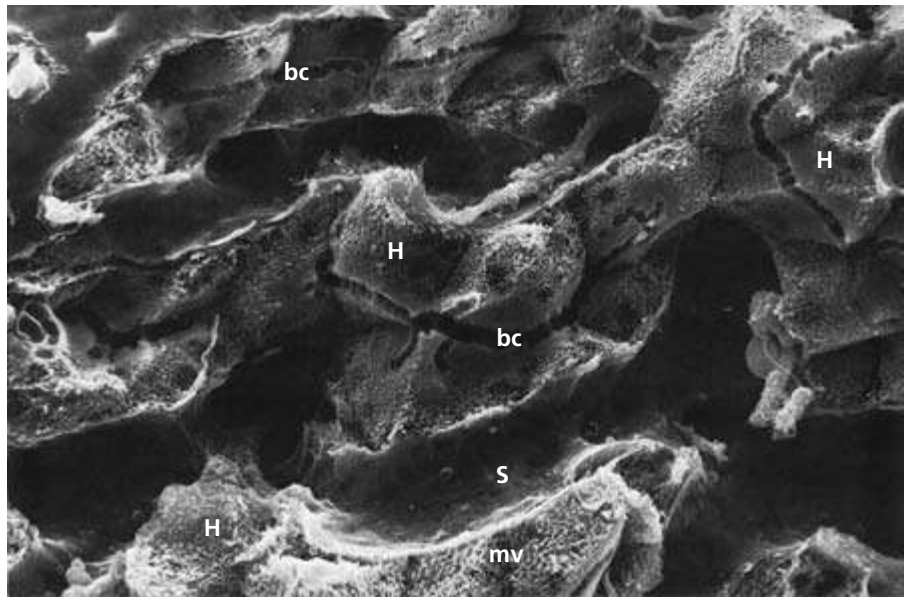

Figure 79.8 Biliary canaliculi. Using scanning electron microscopy, the liver cell plates and adjacent sinusoids (S) are seen with the bile canalicular (bc) network located between the adjacent hepatocytes (H). Microvilli (mv) are also present. From Phillips et al. [26].

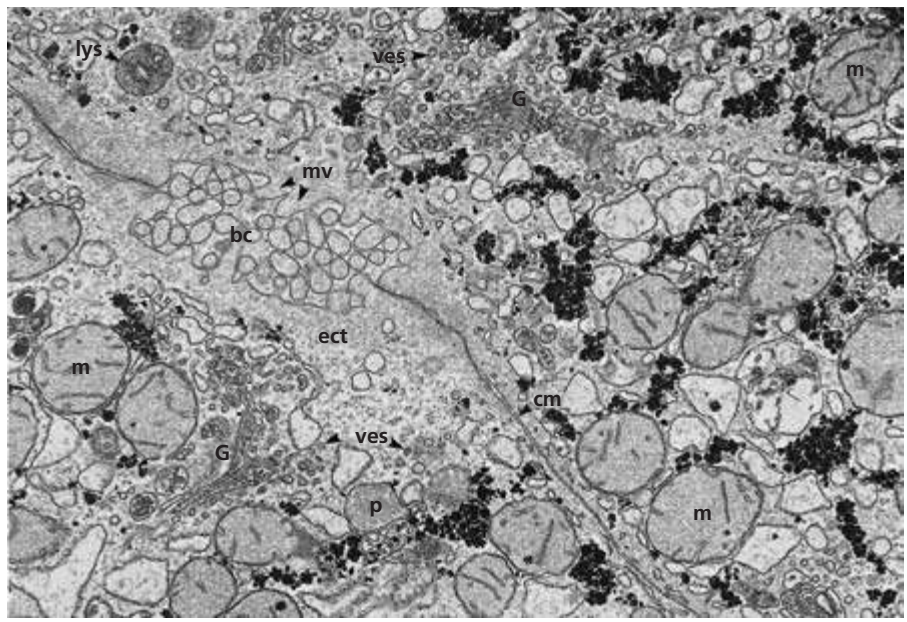

Figure 79.9 Biliary pole. Using electron microscopy, the bile canaliculi (bc) can be seen to the left of the field, surrounded by microvilli (mv) and pericanalicular ectoplasm (ect). Golgi complexes (G), vesicles (ves), mitochrondria (m), peroxisomes (p), and lysosomes (lys) can also be seen in the adjacent liver cell cytoplasm. cm, liver cell membrane. From Phillips et al. [26].

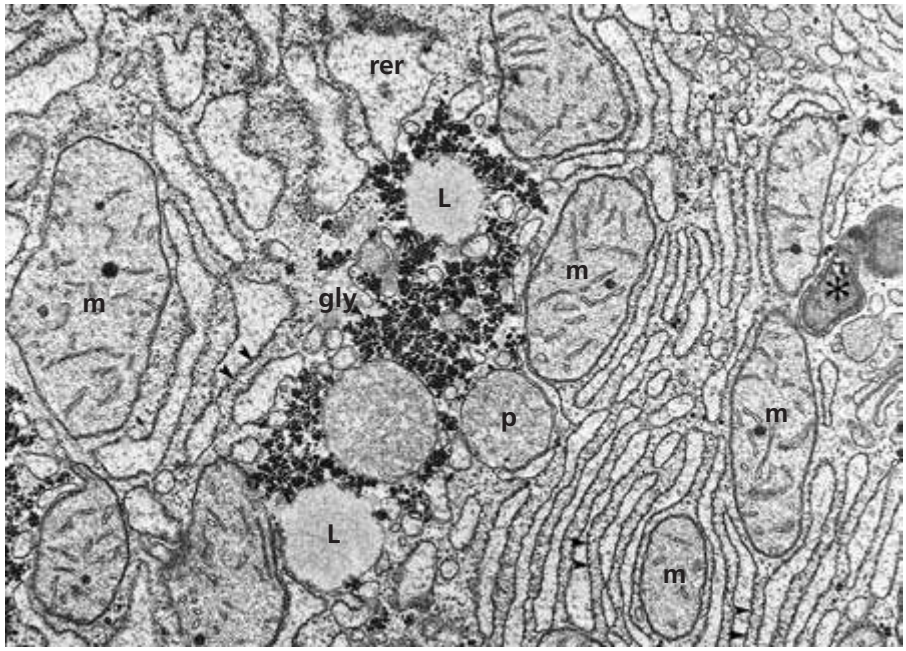

Figure 79.10 Liver cell cytoplasm. Using electron microscopy, the cytoplasm is composed of rough endoplasmic reticulum (rer), mitochondria (m), glycogen (gly), lipid droplets (L), peroxisomes (p), and secondary lysosomes (*). From Phillips et al. [26].

Nucleus

The liver cell nucleus is centrally located within the hepatocyte, measures about 10 µm in diameter, and contains clumped chromatin and nucleoli [26,30]. The nuclear membrane is composed of two envelopes separated by a narrow zone. These membranes have numerous apertures or pores that provide cytoplasm–nuclear communication. The outer membrane is often lined by ribosomes, which are in direct communication with adjacent rough endoplasmic reticulum fibers. The intranuclear chromatin fibers are composed of heterochromatin, which is round to irregular dense metabolically inactive granules, and euchromatin, which actively transcribes RNA [26]. Anywhere from one to six nucleoli can be seen, their number and size in direct proportion to their degree of activity (ribosomal RNA function). The hepatocyte usually contains one nucleus, although bilobed forms may also be seen that are more often identified in the elderly population. Mitotic figures are infrequent but may be prominent in instances of active liver cell regeneration (e.g., resolving acute viral hepatitis). Various nuclear inclusions may be identified [26,31,32] and include glycogen (frequently seen in diabetic patients), lipid, and cytoplasmic invaginations (pseudoinclusions, or nuclear membrane irregularities).

Cytoplasm

The cytoplasm constitutes about 90% of the volume of the hepatocyte and contains numerous functionally important organelles (Fig. 79.10). The cytoplasm and intracellular components vary in size, ultrastructure, and function depending on the zonal location of the hepatocytes (see Functional components: liver cell heterogeneity; see also Tables 79.1 and 79.2). The superstructure is maintained by the cytoskeleton

Table 79.1 Liver cell structural zonal variations

Zone 3 (perivenular)
Mitochondria: round; less numerous and smaller; fewer inner membranes
Peroxisomes prominent
Lysosomes numerous
Bile canaliculi with fewer microvilli, smaller in diameter
Surface area of smooth endoplasmic reticulum larger
Sinusoids form parallel vessels that open into terminal hepatic venules, are wider (30 µm), fewer in number
Endothelial cells more numerous but smaller; increase in number of fenestrations and porosity
Larger nuclear volumes
Increase in number of microbodies
Slight increase in stellate (Ito) cells[a]
Predominant collagen types I, III, VI
Zone 1 (periportal)
Mitochondria: oval and oblong; larger diameter, volume and number; greater cristae area
Rough endoplasmic reticulum more abundant
Bile canaliculi with numerous microvilli, larger in diameter
Kupffer cells more numerous
Sinusoids form interconnecting polygonal network, are smaller (6 µm), more tortuous, more numerous in number
Abundant Golgi-rich volume
Endothelial cells larger; endothelial fenestrations larger but less numerous
Numerous large granular lymphocytes (pit cells)
Predominant collagen types IV, V

a Zone distribution varies considerably depending on the nutritional state.

Table 79.2 Liver cell functional zonal variations

Zone 3 (perivenular)
Glycolysis
Glycogen synthesis from glucose
Lipogenesis
Removal of ammonia from blood by glutamine
Detoxification, biotransformation of the majority of drugs and toxins (P450 enzymes)[a]
Ketogenesis
Bile acid synthesis
Bile salt-independent fraction of bile formation; bile acid uptake (sodium independent)
Glucuronidation
Mixed function oxidase
Increase in Kupffer cell phagocytic activity
Zone 1 (periportal)
Gluconeogenesis
Glycogen synthesis from lactate
β-Oxidation of fatty acids
Amino acid catabolism
Urea synthesis
Cholesterol synthesis
Bile acid secretion
Bile salt-dependent fraction of bile formation; bile acid uptake (sodium dependent)

a Note that certain drugs and toxins (e.g., allyl formate, phosphorus) are metabolized and may cause liver cell injury in zone 1 because of different pathophysiological mechanisms.

of the hepatocyte and includes three major subdivisions: microfilaments, microtubules, and intermediate filaments [9,26,33–35].

The mitochondria are one of the most prominent intracellular organelles [9,26], averaging up to 2200 per hepatocyte. They have numerous critical functions that include oxidative phosphorylation and fatty acid oxidation and contain components essential for the urea and citric acid cycles. They are larger in the perivenular zone but more numerous in the periportal zone and generally maintain a constant volume within each hepatocyte. They are oval to oblong in shape, and measure 0.4–3.5 μm in diameter. They may be divided into outer and inner membranes separated by a gap.

The endoplasmic reticulum [26,36] is composed of a convoluted network of cisternae, saccules, tubules, and vesicles that are distributed throughout the liver cell cytoplasm and constitute about 15% of cytoplasmic volume. It is divided into two components, the rough endoplasmic reticulum and smooth endoplasmic reticulum. The rough endoplasmic reticulum is seen predominantly surrounding the nucleus and biliary channels, whereas the smooth endoplasmic reticulum forms a meshwork of small tubules that are devoid of ribosomes and often communicate with the Golgi apparatus.

The Golgi apparatus [9,26,36,37] is composed of highly polarized, parallel, flattened, dilated saccules or vesicles, is about 1 μm in diameter, and is most often present adjacent to the liver cell nucleus and biliary canaliculi. Anywhere from 40 to 60 Golgi can be seen within the cytoplasm. Golgi function includes production of secretory vacuoles for transportation and excretion of synthesized proteins into the sinusoids, bile secretion, incorporation of carbohydrates into proteins, and membrane synthesis and repair.

Lysosomes [9,26,38,39] appear as electron-dense, pleomorphic, single-membrane-bound vesicles containing enzymes such as acid phosphatase, esterases, proteases, and lipases; they are most frequently identified adjacent to the canalicular membrane. They are divided into primary and secondary lysosomes, which are actively involved in phagocytosis and eventual degradation of various cellular components.

Peroxisomes are single-membrane intracellular organelles, also termed *microbodies*, that vary in size from 0.2 to 1.3 μm [9,26,40,41]. They are round to oval, exhibit a finely granular homogeneous matrix, contain various oxidases and catalases, and primarily function in the oxidation and degradation of numerous substrates with formation of hydrogen peroxides. They are also involved in oxidation of long-chain fatty acids.

There are various intracellular components readily seen by light microscopy that contribute to the function and appearance of the liver cell [24,25]. Lipochrome pigment appears as fine to coarse brown granules and is derived from increase in lysosomal activity and intracellular condensation of various cellular remnants. Two other intracellular pigments that can resemble lipochrome include bile and hemosiderin. Bile is present as clumped green to green-yellow globules that stain positively with the Hall stain for bilirubin. Usually, the presence of intracellular bile is also accompanied by intracanalicular bile. Hemosiderin pigment is coarsely granular and golden brown, is best highlighted by the Perl iron stain, and represents red blood cell degradation remnants. Intracellular lipids appear as clear, distinct, rounded vacuoles and are composed predominantly of neutral triglycerides. The size of the fat droplets varies, with the extremely small variants (termed *microvesicular* or *foamy*) often difficult to assess on routine light microscopy unless thin 1-μm sections are prepared or fat stains (Oil red O) on frozen sections are performed. Intracellular glycogen is distributed throughout the cytoplasm but is more frequently seen adjacent to the smooth endoplasmic reticulum. Glycogen is easily demonstrated by periodic acid–Schiff (PAS) stain on frozen-section material. Nuclear glycogen, when abundant, gives the nucleus a clear appearance and may be seen in a number of liver diseases, including diabetes and Wilson disease.

Kupffer cell

Kupffer cells are sinusoid lining cells that function as tissue macrophages. They represent more than 75% of fixed macrophages throughout the body and take up about one-third of

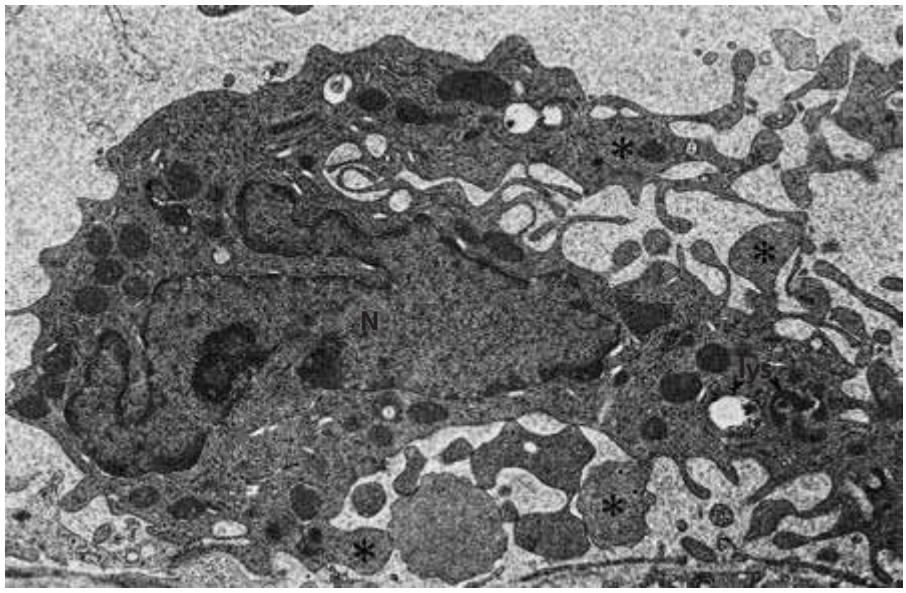

Figure 79.11 Kupffer cell. Using electron microscopy, the Kupffer cell exhibits a cleft nucleus (N), pseudopodia (*), and numerous lysosomes (lys). From Phillips et al. [26].

the hepatic sinusoidal cell volume [9,26,42–46]. Although originally derived from the circulation, they eventually rest along the sinusoidal borders and maintain the ability to divide. Kupffer cells have oval to elongated nuclei, abundant pyramidal stellate cytoplasm that contains numerous lysosomes, and measure up to 9 μm in length (Fig. 79.11). They overlie but do not form junctional complexes with the smaller endothelial cells. However, Kupffer cells may be identified in gaps between adjacent endothelial cells, with cytoplasmic processes extending through endothelial fenestrations. Their primary functions relate to phagocytosis and eventual clearance of particulate material, clearance of endotoxins and degenerating cellular components, synthesis and catabolism of lipids, clearance of senescent erythrocytes, sequestration of antigens, and clearance of immune complexes.

Endothelial cell

The endothelial cells are flattened elongated sinusoidal cells that range in size from 50 to 80 nm and represent almost half of the sinusoidal cell volume [26,42,45,47–50] (Fig. 79.12). Numerous cytoplasmic projections and clustered fenestrae or gaps that range in size from 0.1 to 0.2 μm are present (Fig. 79.13). These fenestrae function as a filtration barrier. The individual cells are loosely attached. A main function of the endothelial cell involves filtering of various macromolecules from the sinusoidal blood by receptormediated endocytosis, enabling substances such as glycoproteins and polysaccharides direct contact with the hepatocyte, but excluding and protecting the liver cell from numerous larger cellular components and various soluble compounds by receptor-mediated endocytosis (ligand binding to coated pits on the cell surface), pinocytosis (trapping of extracellular fluid droplets), and phagocytosis (plasma membrane encircling and internalizing particles) [51].

Stellate (perisinusoidal) cell

Also termed *fat-storing cells* and *Ito cells*, the stellate cells are located within the perisinusoidal liver cell recesses along the space of Disse [26,42,45,52–56] (Fig. 79.14) and constitute about one-fifth of the sinusoidal cell volume. These cells

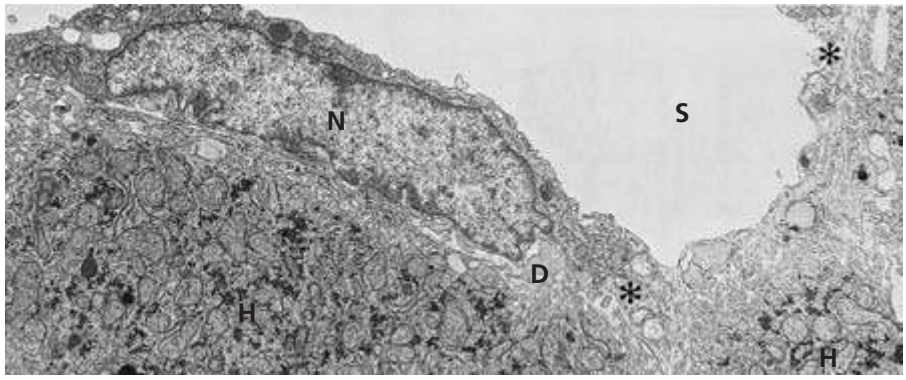

Figure 79.12 Endothelial cell. Electron microscopy of a sinusoid (S) shows an endothelial cell with an elongated nucleus (N) and scanty cytoplasm (*). The space of Disse (D) and adjacent cytoplasm of two hepatocytes (H) are noted. From Phillips et al. [26].

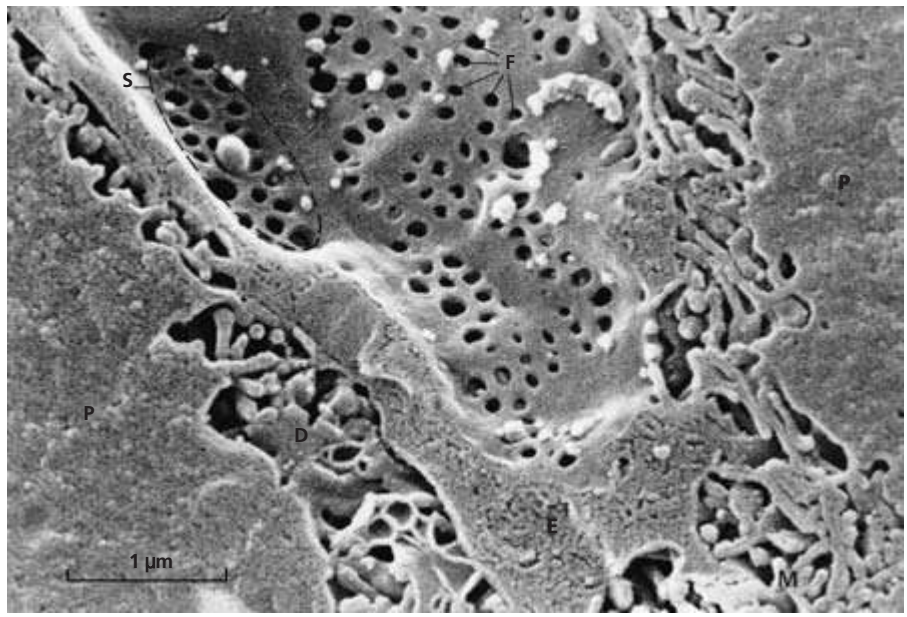

Figure 79.13 Scanning electron micrograph of the hepatic sinusoid. Numerous fenestrae (F) grouped into sieve plates (S) are demononstrated. D, space of Disse; E, endothelial cell; M, microvilli; P, parenchymal cell. From Sherlock & Dooley [2], with permission from Blackwell Publishing.

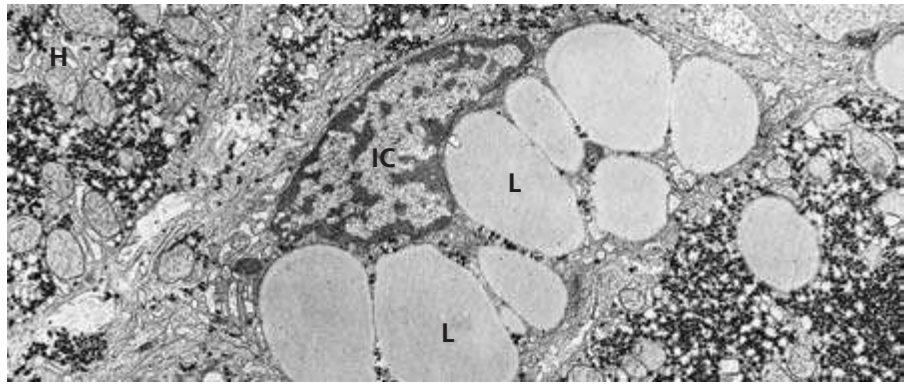

Figure 79.14 Stellate (perisinusoidal) cell. These cells, also termed Ito cells (IC), are seen by electron microscopy along the perisinusoidal recesses and characteristically demonstrate cytoplasmic lipid droplets (L) containing vitamin A. Adjacent hepatocytes (H) are noted. From Phillips et al. [26].

range in diameter from 2 to 10 µm and contain small star-shaped nuclei without prominent nucleoli. The cytoplasm often contains variably sized lipid droplets having a high concentration of vitamin A (retinoyl palmitate) that can easily be demonstrated on frozen sections as intensely green, rapidly fading fluorescence when excited at a wavelength of 328 nm [51]. Besides being the major source of vitamin A storage, the cells synthesize extracellular matrix by way of cytokine activation and resultant transformation to myofibroblasts in response to liver injury, with enhancement of protein and collagen synthesis [57,58].

Pit cell

Also termed *liver-associated lymphocytes* and *large granular lymphocytes*, pit cells are nonparenchymal T cells distributed within the sinusoidal lumen in loose contact with the endothelial or Kupffer cells. They can occasionally be seen within portal tracts [50,51,59–62]. These cells function as natural lymphocyte-activated killer (NK) cells, and contain multivesicular body-related dense granules and rod-cored vesicles [63]. They are often called pit cells because of the resemblance of these cytoplasmic granules to grape seeds [1,64]. These cells are often seen in direct contact with the endothelium in response to various immunological mechanisms and can be targeted in viral hepatitis, acute posttransplantation cellular rejection, and various primary and metastatic neoplastic processes [9,51].

Stroma (extracellular matrix)

The stroma constitutes about 10% of liver volume and overall supports the basic hepatic architectural arrangement, produces intercellular cohesion and communication, and effects cellular differentiation. The capsule of Glisson, composed of dense hypocellular collagen, encapsulates the liver and extends at the hilum into the hepatic parenchyma, forming the tensile structure of the portal tracts. Extension within the sinusoids into the space of Disse as reticulin fibers maintains the intralobular framework. Five basic types of

collagen are seen, with types I and III representing more than 95% of total collagen. Type I represents mature collagen strands, whereas type III represents new collagen (reticulin fibers). Type IV is present in the basal lamina (membrane) around small vascular structures and ducts and represents about 1% of total hepatic collagen. The noncollagenous proteins are numerous matrix glycoproteins and include laminin, the major glycoprotein component within the basement membranes; fibronectin, synthesized by perisinusoidal cells and responsible for collagen adhesion; and elastin, which stabilizes blood vessel walls. Collagen deposition is often triggered by activation of stellate cells in certain instances of liver cell necrosis and impaired or delayed regeneration of hepatocytes.

Biliary network

The main function of the biliary tract is to transport bile synthesized in the hepatocyte into the gastrointestinal tract by way of the extrahepatic biliary network. In addition, the transport proteins synthesized by biliary epithelium and located within the microvilli aid in both the secretion of bicarbonate-rich fluid and the reabsorption of various fluids and solutes that generally enhance bile flow [65]. The biliary tract can be divided into its structural components [66], the smallest of which are the biliary canaliculi. These are located along the intercellular spaces between hepatocytes, range in diameter from 0.5 to 1 μm, and are lined by microvilli. The canaliculi have numerous anastomotic connections and may undergo contractions secondary to actin, myosin, and tropomyosin [67], enabling and enhancing forward bile flow.

The canaliculi that enter the portal tracts are called the terminal ductules, periportal cholangioles, or canals of Hering. These duct structures are derived from hepatocytes located at the limiting plate and provide communication with the interlobular bile ducts. The ducts at this point develop a basement membrane and have both liver cell and ductal ultrastructural and histochemical features. Although in the normal liver these ducts are usually inconspicuous on routine light microscopy, their prominent proliferation (duct transformation, duct metaplasia) is most apparent in instances of severe liver cell necrosis that extends to and involves the periportal hepatocytes.

The interlobular bile ducts range in diameter from 15 to 20 μm, are located within the smaller portal structures, and are lined by a single layer of cuboidal cells with discrete round nuclei, usually inconspicuous nucleoli, and scanty eosinophilic cytoplasm. The duct cytoplasm contains sparse as well as relatively small mitochondria, free ribosomes, scanty endoplasmic reticulum, and abundant intermediate cytokeratin filaments (cytokeratins 7, 19) [3]. The lumenal surface contains numerous pinocytotic vacuoles. Adjoining duct epithelium shows complex interdigitations. The lumenal surface also contains microvilli. A basement membrane is apparent and easily demonstrated on PAS stain. Although the smaller ducts have no apparent wall, the larger interlobular ducts, which measure up to 100 μm in diameter, develop a small periductal fibrous sheath. Anastomoses of the smaller interlobular ducts is frequent. The main blood supply is the smaller branches of the hepatic artery and the peribiliary plexus, which run in parallel with the duct structures. These ducts express class I major histocompatibility antigens, with cytokine-mediated class II expression in instances of liver allograft rejection and certain chronic biliary tract diseases that attack ducts, such as primary biliary cirrhosis.

The larger interlobar and septal ducts measure more than 100 μm in diameter, have a fibrous wall, and are lined by a single layer of cuboidal to columnar epithelium with nuclei located toward the basement membrane. Some degree of periductal fibrous tissue is common but should not be confused with the distinct periductal concentric collagenosis seen in instances of long-term bile duct obstruction.

The interlobar and septal ducts lead into the segmental ducts that measure up to 800 μm in diameter, which eventually form the major hilar ducts that measure up to 1.5 mm in diameter. The hilar ducts ultimately branch into the main right and left hepatic ducts. The hilar ducts are lined by columnar mucus-secreting epithelium, have a distinct fibromuscular wall, and are associated with both intramural and extramural seromucinous peribiliary glands, which communicate with the bile duct lumen. The same type of peribiliary glands are also demonstrated around the intrahepatic large bile ducts [68].

Vascular network

The major blood vessels that supply the liver are the portal vein and hepatic artery. The portal vein sequentially develops interlobar, segmental, interlobular veins and preterminal branches, with the terminal portal venules measuring about 20–30 μm in diameter and seen in the smaller triangular portal tracts. The vascular inflow from the portal veins is controlled by various sphincters. The hepatic artery branches accompany the portal vein and divide within the smaller portal tracts into two segments: the periportal plexus, which branches around the portal vein and drains into the sinusoids; and the peribiliary plexus, which provides blood supply to the accompanying interlobular bile ducts by way of small capillaries that are layered around the ducts. Various connections are seen between the small arterioles and the sinusoids that are most prominent in the periportal zone (zone 1 of Rappaport). The arterial flow depends on both sphincters and contractile mechanisms that define the degree of arterial versus portal venous blood flow, with the arterial flow varying inversely with the portal venous circulation.

There are a number of organizational approaches in assessing the structure as well as function of the liver lobule [69–71]. On the basis of vascular injection studies, Rappaport and colleagues [72] described the concept of the *liver acinus*, with a stratified order of simple acini, complex acini, and acinar

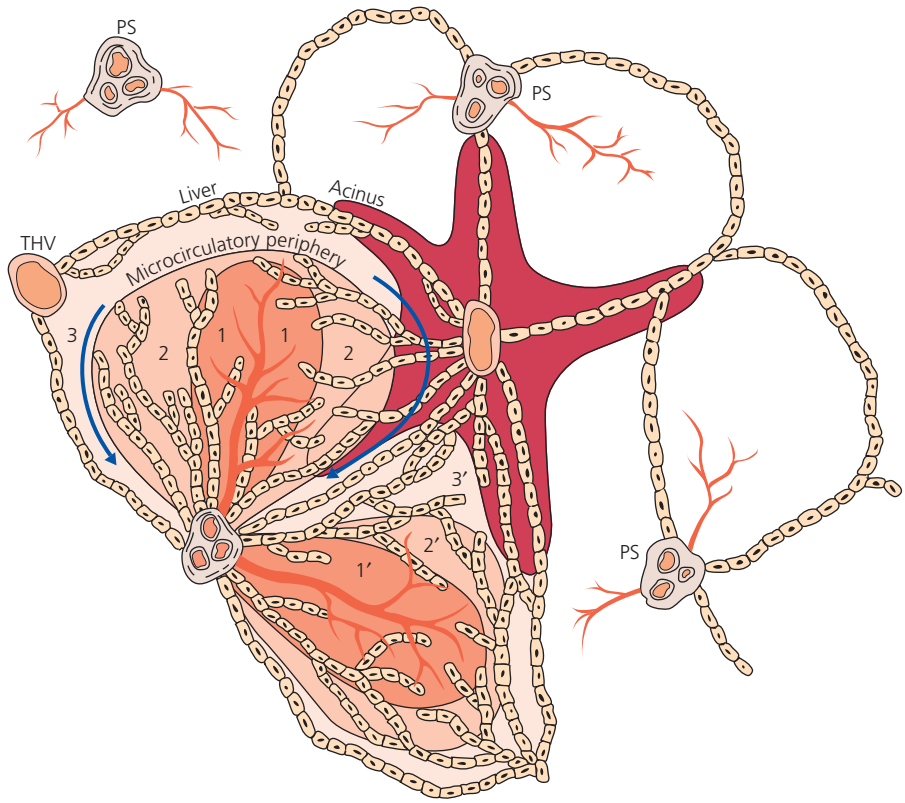

Figure 79.15 Hepatic acinus. The simple liver acinus demonstrates the three hepatic zones and their relationship to the microcirculatory blood supply. PS, portal structures; THV, terminal hepatic venule. From Sherlock & Dooley [2], with permission from Blackwell Publishing.

agglomerates. This approach is a most useful tool in assessing lobular microstructure. The simple acinus is the smallest functional parenchymal unit and centers around the preterminal portal venules, hepatic arteriole, and terminal bile ductules (Fig. 79.15). The acinus is divided into three zones (zones of Rappaport): periportal (zone 1), which includes the limiting plate; midzone (zone 2); and perivenular (zone 3), with the terminal hepatic venule at its outer lateral margin. There is no discrete demarcation separating these zones from one another. The watershed areas occur at the periphery of the acini where blood can be derived from the smallest arteriolar branches and portal venules from adjacent acini. Biliary drainage runs parallel to the vascular sinusoidal circulation. The complex acinus is derived from three adjacent simple acini fed by a preterminal portal vein and arterial branches. A small aggregate or sleeve of liver cells (acinuli) directly surrounds the preterminal branch. The acinar agglomerate is composed of about four complex acini and is fed by a portal venous branch measuring 300–1200 μm in diameter. The concept of modular microarchitectural arrangement of the hepatic parenchyma has also been introduced using a metabolic marker (alkaline phosphatase activity and its presence within endothelial cells) instead of morphology alone, and demonstrated both primary and secondary modules. The primary modules are composed of portal tracts with vascular septa drained by a common central venular network, this approach emphasizing the three-dimensional rather than two-dimensional arrangement of the liver [73].

The space of Disse lies between the hepatocyte and the endothelial cells, measures 0.2–1.0 μm in width, and forms a space that is not appreciated on routine light microscopy of biopsy material but which can sometimes be seen in autopsy livers secondary to liver cell shrinkage from autolysis. Numerous microvilli can be seen projecting from the liver cell membrane into the space of Disse. The discontinuity of the adjacent endothelial cells enables plasma to have easy access to the liver cell membranes. The space of Disse contains reticulin fibers, which can best be appreciated on special reticulin stains and which are easily seen on electron microscopy. The stellate or perisinusoidal cells (Ito cells) also protrude into this space. The sinusoids eventually drain into the terminal hepatic venules, which have no fibrous wall. These vessels then drain into the terminal hepatic and sublobular intercalated veins, and then exit the liver from the three main hepatic vein branches into the inferior vena cava.

Most hepatic lymph is derived from the space of Disse, whereas about 10% comes from capillary leakage from the peribiliary plexus. Its main function is to drain excess proteinaceous fluid from the interstitial hepatic spaces [1,74]. The hepatic lymph drainage within the space of Disse travels into the smallest lymphatic vessels within the portal tracts by way of *endothelial massaging* by circulating erythrocytes

and leukocytes within the sinusoids. The terminal branches form plexuses lined by endothelial cells and are accentuated around the hepatic arterioles in the smaller portal structures, although lymphatics are also seen adjacent to portal veins and bile duct tributaries in larger portal tracts. Small branches can also be seen along the hepatic venous outflow vessels. A lymphatic plexus is also identified within the capsule of Glisson and communicates with the intrahepatic lymphatics through anastomotic channels. Most lymphatics leave the liver at the porta hepatis, although lymphatic drainage is prominent through the capsule of Glisson in instances when the hepatic venous drainage is impaired (e.g., acute and chronic hepatic venous outflow obstruction, cirrhosis). The larger lymphatic channels have an identifiable wall and are valved.

Neural network

The nerve fibers are composed of both parasympathetic and sympathetic branches and release neurotransmitters from intrasinusoidal fibers that contribute to modulation of liver cell function. Small nerve segments can be seen within the larger portal tracts, but smaller unmyelinated fibers can be discerned by way of electron microscopy and immunohistochemical studies within the space of Disse.

Functional components: liver cell heterogeneity

The hepatocytes within the various parenchymal zones have many different specialized physiological functions that often parallel the light microscopy, ultrastructural features, and enzyme distribution patterns [1–3,23,75–81] (Tables 79.1 and 79.2). This functional heterogeneity applies not only to liver cells but also to the sinusoidal and perisinusoidal spaces, Kupffer and endothelial cells, the extracellular matrix, and even bile duct epithelial cells [82–84]. These functions are manifestations of:

- nutrient and hormonal gradients delivered to the various zones
- sinusoidal vascular perfusion and oxygen concentration gradients
- availability of innumerable substrates and cofactors
- expression of various enzyme activities through gene expression and local (zonal) genetic variations.

As a consequence, functional and morphological zonal liver cell changes and injury are end-stage manifestations of modifications, alterations, and impairment of many of these critical and complex parameters.

Stem cells

The bile duct epithelial cells harbor a subdivision of stem cells that are located near the canals of Hering [3,85]. The importance of these cells is that they have the potential to differentiate along either liver or biliary cell lines and have a critical potential in liver cell regeneration and repair [86,87]. Although the hepatocyte has been shown in several animal models to have more than adequate ability to repopulate the liver in the transplant setting [88] and shows striking regenerative activity after partial hepatectomy for tumor or in partial livers after living-related transplantation, the ability of stem cells to regenerate in instances where viable hepatocytes are not present (i.e., fulminant hepatitis with massive hepatic necrosis) shows their critical potential. The morphology in these cases, for example, shows that at the border of the portal tracts and parenchyma there is a striking proliferation of bile ductular-type cells that potentially develop from these stem cells. These cells have also been shown by immunological techniques to have parathyroid hormone-type activity that can further influence liver cell differentiation and cell division [3]. Progenitor cells isolated from normal adult liver have also been shown to express mesenchymal stem cell markers and have the ability to differentiate into mature hepatocytes when cultured in the presence of hepatocyte and fibroblast growth factors [89]. In addition, hepatic stellate cells, which normally play an important role in hepatic fibrogenesis, also have precursor potential in the developement of both endothelial cells and hepatocytes [90].

Studies have also suggested that some of these stem cells in the liver have the potential to differentiate into hematopoietic precursors [91]. Furthermore, stem cells located within the bone marrow have shown an ability to mobilize and migrate into the liver [92], with differentiation into hepatocytes and duct epithelial cells [93–96], supporting the view that these stem cells have multipotential abilities; however, it is not certain that the bone marrow-derived cells play a significant physiological function in replacing epithelial cells in the setting of hepatic injury [93].

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G LIVER

80 Acute viral hepatitis

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Five viral agents have been associated with acute hepatitis. These viruses were denominated A, B, C, D, and E in order of their discovery. They are a genetically diverse group of viruses linked by their tropism for the liver, their primary site of replication. They all cause an acute hepatitis, which can range in clinical severity from an asymptomatic illness to fulminant hepatitis. Hepatitis B, C, and D viruses have the propensity to progress to chronic hepatitis. These hepatotrophic viruses account for 90%–95% of all cases of acute viral hepatitis in the United States [1]. Data from the Centers for Disease Control and Prevention (CDC) Sentinel Counties Study of acute viral hepatitis indicated that hepatitis A virus (HAV) was the cause of almost half the reported cases, hepatitis B virus (HBV) caused a third, hepatitis C virus (HCV) caused 15%, and the remaining cases were caused by non-A, non-C viruses [2]. In the United States, acute viral hepatitis has a low case fatality rate, 1%–2%, but is associated with substantial morbidity. Death from end-stage liver disease, a long-term consequence of acute viral hepatitis, ranks in the top 10 of all causes of mortality in the United States.

Other viruses from the families Arenaviridae (e.g., Lassa fever virus), Bunyaviridae (e.g., Rift Valley fever and Hanta viruses), Flaviviridae (e.g., yellow fever and dengue viruses), Filoviridae (e.g., Marburg and Ebola viruses), and Herpesviridae may cause hepatitis as part of a systemic illness but are not considered primary hepatotrophic viruses.

Epidemiology

Overview

The hepatotrophic viruses are found worldwide, and there are distinct geographic regions where each virus is highly endemic. The global distribution of each virus is closely linked to its mode of transmission. Enterically transmitted viruses, HAV and hepatitis E virus (HEV), are more common in regions of the world that lack proper sanitation and have limited access to fresh water. In contrast, the parenterally transmitted viruses, HBV, HCV, and hepatitis D virus (HDV), predominate in areas of the world where social or customary practices facilitate their transmission, explaining the high rate of HCV infection in injection drug users in the United States and HDV in the South American Indians of the Amazon basin. Despite the institution of public health measures to educate the general public on transmission risks, the number of cases of acute viral hepatitis has not declined substantially in recent years. This may be partly because risk factors for acute viral hepatitis have not been identified in 30%–40% of cases [1]. If elimination of infection is the ultimate goal, further studies on the various modes of transmission are necessary.

Hepatitis A virus

Prevalence

HAV infection occurs worldwide. The prevalence of antibody to hepatitis A (anti-HAV) correlates inversely with the socioeconomic status and the prevailing standard of hygiene of a region. In highly endemic areas, the prevalence of anti-HAV is almost universal by 5 years of age, whereas in industrialized nations, it is generally less than 5% for all age groups sampled. This finding has important public-health implications. Outbreaks of HAV infection are more common in low-prevalence areas because of a susceptible population and they are rare in endemic areas because of natural herd immunity. This explains the cyclic epidemics observed every 10–15 years in areas of intermediate prevalence, such as the United States. Additionally, a paradoxical increase in the number of cases of clinical hepatitis is observed in low- and intermediate-prevalence areas owing to a greater number of adult infections.

About one-third of the US population has serological evidence of past HAV infection. Prevalence was found to increase

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with age, being born outside the US, and being of Mexican American ethnicity, according to data obtained from the Third National Health and Nutrition Examination Survey (NHANES-III, 1988–1994) [3]. The relationship with age can be explained by infections that occurred during childhood when sanitary conditions were poorer than present-day standards and by travel to endemic regions by adults. The age-adjusted prevalence of anti-HAV was highest in Mexican Americans (70%) and lowest in non-Hispanic whites (23%). This difference is probably related to socioeconomic factors rather than racial susceptibility to infection. The incidence of acute HAV infection in the United States has remained fairly constant over the past several decades. However, since the introduction of vaccination, there has been a dramatic decline in the incidence of HAV infection. Between the periods 1990–1997 (when there was no vaccination) and 2003 (vaccination) the rate of hepatitis A declined by 76% from a low of 9.1 cases per 100 000 (in 1991) to 2.6 cases per 100 000 persons [4]. Greater declines were observed in high-risk states that adopted vaccination strategies. Rates declined by 88% to 2.5 per 100 000 persons in states with vaccination programs compared to a 53% decrease elsewhere to 2.7 per 100 000 persons [4].

Transmission

HAV is transmitted primarily by the fecal–oral route through person-to-person contact or through ingestion of contaminated food or water. Viral titers are highest in stool and can reach 10^8 virions/mL [5]. This is because HAV lacks a lipid envelope and is readily secreted by hepatocytes into bile and the intestinal tract [6]. The virus is detected in stool during the incubation period, and the viral titer peaks 2 weeks before the onset of symptoms [7]. Therefore, the highest period of infectivity is before the onset of symptoms. In most cases, viremia becomes undetectable after the peak of the alanine aminotransferase (ALT) level [8]. However, experimental transmission studies performed in human volunteers and chimpanzees revealed that viremia may persist for long periods during the convalescent phase of the illness [7–9]. This suggests that blood obtained during the convalescent phase may be potentially infectious. Furthermore, the virus could be demonstrated in convalescent stool by reverse transcription polymerase chain reaction (PCR) and may be a source of infection in sporadic cases.

Viral titers in serum are about twofold less than those in stool; therefore, parenteral spread is not a significant route of transmission. Transmission has been reported occasionally in blood transfusion recipients, patients who require clotting factors, and injection drug users who share needles [10–12].

HAV is shed in the saliva during an acute illness. However, transmission has not been associated with saliva, semen, or urine [5]. Sexual transmission of hepatitis is reported in both heterosexuals and homosexuals. Outbreaks in homosexual men seem to be related to oral–anal contact during the sexual encounter [13,14]. Nosocomial spread from undiagnosed cases has been reported [15].

In the United States, the highest rates of spread are reported in household contacts, in the intimate contacts of an index case, and in child day-care centers (person-to-person spread) [16]. Data from the Viral Hepatitis Surveillance Program at the CDC in 1995 showed that contact with another person with hepatitis A was overall the most frequently identified risk factor. Attendees or employees of day-care centers accounted for 10%–15% of cases. However, nearly 50% of cases in both 1994 and 1995 reported no known risk factor [16]. It has been suggested that transmission from an asymptomatic infant or child may account for most of the adult cases of HAV infection [17]. Outbreaks of HAV have been associated with ingestion of shellfish [18,19], raw onions [20,21], salads [22], and frozen strawberries [23], but these account for a small number of cases overall.

Hepatitis B virus

Prevalence

Hepatitis B occurs worldwide; the areas of highest prevalence are China, southeast Asia, subSaharan Africa, and Alaska. Almost 50% of the world's population live in these regions, where the prevalence of hepatitis B surface antigen (HBsAg), a serological marker of chronic infection, can exceed 8% [24]. Another 40% of the world's population lives in areas with intermediate prevalence, 3%–5%, such as Japan and India. In low-prevalence areas, such as the United States, Western Europe, and Australia, the rate of HBsAg positivity is 0.1%–2%. In endemic areas, most infections occur in children, whereas in areas of low seroprevalence, most infections occur in adults.

The prevalence of HBsAg was found to increase with age in the US population and is highest in people aged 20–29 years, probably reflecting transmission through injection drug use or sexual exposure [25]. Certain US populations, such as Eskimos, have higher rates of HBsAg positivity [26]. The incidence of acute HBV infection has declined dramatically in the United States, primarily as a result of the use of the hepatitis B vaccine in high-risk groups, behavioral changes in response to the human immunodeficiency virus (HIV) epidemic, and federal regulations introduced to limit nosocomial spread.

Transmission

HBV is transmitted primarily by the percutaneous and sexual routes including exposure of the mucous membrane to bodily fluids. Not surprisingly, the highest titers of virus are seen in serum and blood; intermediate titers are seen in semen, vaginal fluid, and saliva; and lowest titers are observed in tears, urine, feces, and breast milk. The infectivity of other body fluids is 100-fold to 1000-fold less compared with blood.

Transfusion of blood products used to be the major route of transmission before the introduction of blood screening in 1985. Studies of multitransfused patients with hemophilia

before 1985 indicated that 40%–50% had markers of previous or active HBV infection [27]. Transfusion-related HBV infection has been virtually eliminated by screening of blood donors.

The transmission of HBV varies in endemic and nonendemic regions. In the United States and Western Europe, areas of low endemicity, injection drug use, and sexual exposure to an acute case or chronic carrier are the major modes of transmission. Data from the CDC Viral Hepatitis Surveillance Program showed that sexual exposure was the predominant risk factor in the United States; 26% of cases reported sexual contact with a person known to have HBV. Injection drug use was the next most common risk factor (13%). The risk for sexual transmission was directly related to number of lifetime sexual partners, use of paid sex, and previous history of sexually transmitted diseases. The risk for transmission appears to be greater from men to women.

In contrast, in endemic regions, perinatal spread (vertical transmission) and person-to-person spread (horizontal transmission) are more common modes of spread. Perinatal spread is responsible for the high rate of infection seen in China and southeast Asia [28]. Perinatal spread is more efficient if the mother is positive for HBe antigen (HBeAg). Fetal exposure to blood during passage through the birth canal is thought to be the cause of infection rather than transplacental spread. In subSaharan Africa, another highly endemic area, the major route of spread is horizontal transmission, occurring in young children between the ages of 4 and 6 years, presumably through close physical contact or intrafamilial spread. The reason for the lower rate of vertical transmission in the African population may be the low prevalence of HBeAg in the serum of females of childbearing potential [29].

Health-care workers were at major risk for exposure in the 1980s; however, widespread use of the hepatitis B vaccine has reduced the incidence of acute hepatitis markedly, from 9% in 1985 to 0.8% in 1994/1995 [30]. Nosocomial infection continues to remain a source of infection, and transmission has been reported from physician to patient, from contaminated multiuse vials and medical instruments, in hemodialysis units, and through organ transplantation, especially in the case of antibody to hepatitis B core antigen (anti-HBcAg)-positive donors after orthotopic liver transplantation (OLT).

Hepatitis C virus

Prevalence

HCV infection occurs worldwide, and it is estimated that 3% of the world's population is infected based on blood donor studies [31,32]. The geographic prevalence of antibody to HCV (anti-HCV) is fairly consistent throughout the world, ranging from 0.5% to 2%. However, the prevalence of HCV is disproportionately high in Egypt and Japan. In Egypt, this has been explained by the use of mass parenteral therapy for schistosomiasis with reusable glass syringes during the period between 1961 and 1985 and in Japan it is explained by the use of blood transfusions during World War II [33–35].

Data from the NHANES-IV study found a prevalence of anti-HCV of 1.6% in the general US population [36]. Prevalence was highest in injection drug users and in African Americans. Overall, the rate of anti-HCV was twice as high in African Americans as in whites. Groups with higher prevalence than the general population included patients with hemophilia, injection drug users, patients receiving hemodialysis, and people attending sexually transmitted disease clinics. Injection drug users are at the highest risk for acute HCV infection. Since 1989, the incidence of acute HCV infection has been on the decline, primarily as a result of screening of the blood supply and a decrease in injection drug use.

Transmission

HCV is transmitted by percutaneous, sexual, and perinatal routes. The highest viral titers are seen in blood and serum. Before the introduction of blood-screening procedures (anti-HBc and ALT levels were used as surrogate markers for non-A, non-B hepatitis beginning in 1985 until they were replaced by anti-HCV testing in 1991), transfusion of blood or blood products was a major source of infection, accounting for up to 20% of cases of infection. HCV is efficiently transmitted by blood. Between 60% and 95% of patients with hemophilia who received clotting factor before 1985 tested positive for anti-HCV [37,38]. With the introduction of screening measures, the risk for acquiring hepatitis C through blood transfusion in the United States is estimated to be 1 in 400 000 [39]. Transmission of HCV through the blood supply still occurs in countries where testing for HCV is not routinely performed. Injection drug use is a highly efficient means of transmission and is the major route of transmission of HCV in the United States. The prevalence of anti-HCV in active injection drug users who share needles is 70% after 1 year and more than 90% after 8 years. Prevalence of anti-HCV in this group has been shown to be associated with duration of injection drug use and sharing needles with a long-time drug user. Intranasal cocaine use has been suggested as a mode of transmission [40]. A cross-sectional study of volunteer blood donors in the United States reported that a history of intranasal cocaine use was independently associated with anti-HCV positivity [40].

HCV can be transmitted sexually but not efficiently. Epidemiological case–control and cross-sectional studies report independent associations between HCV infection and exposure to an infected sex partner, increasing number of sex partners, failure to use a condom, and sexual activities involving trauma [41,42]. The presence of other sexually transmitted diseases (e.g., herpes and syphilis) has been shown to facilitate the transmission of HCV. The rate of sexual transmission from an infected case to a partner is low in monogamous relationships, 1.5%, but increases with

duration of the partnership and averages 3%–5% [43,44]. The risk of transmission is higher in those with multiple sexual partners [45]. The rate of sexual transmission is the same for heterosexual and homosexual relationships. Overall, sexual transmission accounts for a relatively small percentage of acute cases, less than 10%.

Perinatal transmission of HCV occurs but the rate is low and is significantly lower compared with HBV. The rate of transmission is estimated to be 3%–5% for mothers not infected with HIV-1 and increases to 12%–14% in HIV-1-positive women [46,47]. Risk factors for perinatal transmission include high maternal viral load, HIV/HCV coinfection, and vaginal delivery [47–49]. It was suggested that cesarean section may be associated with a lower rate of perinatal transmission [50]. Transmission of HCV through breastfeeding has not been reported [51]. Mother-to-child transmission occurs but is uncommon [47,52].

Nosocomial transmission of HCV occurs but is not a major cause of new infections [53,54]. Transmission through contaminated multidose vials, through endoscopes, from physician to patient, from unsafe therapeutic injections, organ transplantation, and through surgical procedures was identified as potential sources of infection [55–58]. Needle-stick injury is a risk factor for health-care workers (see later).

Hepatitis D virus

Prevalence

HDV, the infectious agent of delta hepatitis, is found throughout the world. Its prevalence mimics that of HBsAg because it is dependent on HBV for its life cycle [59]. It is estimated that 5% of HBsAg carriers are coinfected with HDV [60]. However, there are areas where there is discordance between the prevalence of HDV and HBV, such as in China, where the rate of HBV infection is high but that of HDV is low. The reason for this is unknown. HDV is endemic in the southern Mediterranean, Amazon Basin, and tropical and subtropical Africa. Prevalence of HDV is low in the United States [61]. Recent studies indicate that the seroprevalence of HDV in southern Europe is declining. Studies in Italy confirmed a decrease in incidence of acute cases from 3.1 per 100 000 population in 1987 to 1.2 per 100 000 population in 1992 [62,63]. This decline was attributed to the introduction of hepatitis B vaccination programs and to a decrease in injection drug use [62].

Transmission

HDV is transmitted in a similar fashion to HBV. The single most important factor influencing transmission of HDV is the HBsAg status of an individual. HDV cannot be transmitted in the absence of HBsAg. Exposure to blood and blood products used to be the major route of transmission, but this has been virtually eliminated by the screening of blood for HBV [59]. In developed countries, injection drug use and sexual transmission are the major routes of spread. The mode of spread in populations from Africa and the Amazon Basin is not well understood, but tribal practices that expose individuals to blood or sexual exposure may play a role. Perinatal transmission is rare.

Hepatitis E virus

Prevalence

HEV is the major causative agent of enteric non-A, non-B hepatitis and the primary cause of sporadic hepatitis in the developing world [64,65]. HEV is found worldwide, and the highest prevalence is found in areas of the world where there is inadequate sanitation. It is endemic in southeast and central Asia. Epidemics of HEV infection, usually involving many thousands of people, occur in endemic areas. In endemic areas, the prevalence of antibody appears to increase with age; about 5% of children younger than 10 years old have antibody to HEV, but 10%–40% of adults are seropositive [66,67]. This suggests that HEV infection does not occur frequently in children in endemic areas. This discrepancy may be in part the result of the sensitivity of the test used for serological testing. The prevalence of anti-HEV in the United States is low.

Transmission

HEV is transmitted by the fecal–oral route. Highest attack rates are seen in individuals aged 15–40 years [67]. In endemic areas, consumption of contaminated water accounts for most new cases of HEV. Person-to-person spread is rare, and secondary attack rates appear to be distinctly uncommon, occurring in 0.7%–2.2% of household contacts of hepatitis E, compared with rates of 50%–75% in susceptible household contacts of hepatitis A [67]. There is no evidence for parenteral or sexual transmission. Vertical transmission is known to occur but accounts for only a small number of cases [68]. HEV is responsible for sporadic outbreaks of hepatitis. However, the reservoir of HEV during sporadic outbreaks is unclear. Recent data based on the detection of HEV in the feces of domestic swine in Nepal and the United States and anti-HEV in the sera of pigs, cattle, sheep, and rodents suggest that HEV may be a zoonosis [69–71]. Cases of HEV infection in the United States are rare and usually associated with a history of travel to an endemic area. Infection has been reported without a history of travel, lending support to the zoonosis theory.

Virology

The five known hepatitis viruses, A to E, belong to completely different virus families. Although all of them can cause acute hepatitis, only HBV, HCV, and HDV infection can lead to chronic infection. The virological characteristics of each virus are summarized in Table 80.1 and are described in detail in this section.

Table 80.1 Human hepatitis viruses

Virus	Genome	Genome size (kb)	Envelope	Classification Family	Genus
HAV	RNA positive sense, single-stranded, linear	7.5	–	Picornaviridae	Hepatovirus
HBV	DNA partially double-stranded, circular	3.2	+	Hepadnaviridae	
HCV	RNA positive sense, single-stranded, linear	9.6	+	Flaviviridae	Hepacivirus
HDV	RNA positive sense, single-stranded, linear	1.7	+	Unclassified (viroid)	Deltavirus
HEV	RNA positive sense, single-stranded, linear	7.5	–	Unclassified	Togavirus-like and alphavirus-like

Hepatitis A virus

HAV is classified in the *Hepatovirus* genus in the Picornaviridae family. It has one serotype but multiple genotypes. The virus is nonenveloped and stable to both acid and heat treatment. The HAV has a linear, positive-sense, single-stranded RNA genome of about 7.5 kilobases (kb). The genome has a protein covalently attached to its 5′ end and a poly A tail at the 3′ end [72]. The genome is organized similarly to other picornaviruses in that it codes for a single polyprotein of about 2235 amino acids [73] (Fig. 80.1). The polyprotein is divided into three main functional domains from the 5′ end, P1, P2, and P3. Individual proteins are cleaved from the functional domains, with P1 encoding the viral capsid proteins, whereas P2 and P3 encode the nonstructural proteins. The 5′ untranslated region (UTR) is the most conserved segment of the genome [74] and contains an internal ribosomal entry site that functions to initiate protein synthesis from two in-frame AUG codons [75]. Mutations in this region appear to enhance the adaptation of virus growth in cell culture but do not appear to affect in vivo virulence [76].

Cleavage within the P1, P2, and P3 domains of the polyprotein gives rise to the individual viral proteins. Four capsid proteins, designated 1A to 1D, are encoded in P1, with the P2 and P3 regions encoding proteins 2A to 2C and 3A to 3D, respectively. The 3B protein covalently attaches to the 5′ terminus of the genome and serves as the primer for replication [72]. The functions of 2A, 2B, and 3A are unclear. The 3C is probably a serine proteinase [77] involved in cleavage of the polyprotein. 3D is the putative viral RNA polymerase [78].

The replication process of HAV has been inferred from studies of other picornaviruses [79]. Entry of the virus into the host is mediated by a cell surface receptor, which has been proposed to be a mucin-like class 1 integral membrane glycoprotein [80]. Viral entry is followed by uncoating and initiation of viral protein synthesis [81]. Viral RNA synthesis proceeds from negative to plus strand and occurs in the cytoplasm. Viral assembly follows a sequence similar to that of picornaviruses in a cellular membrane compartment [82].

Hepatitis B virus

HBV has been classified into seven genotypes, A to G, based on sequence divergence [83,84]. Three types of viral particles are visualized in infectious sera using electron microscopy. Two of the viral particles are smaller spherical structures with a diameter of 20 nm and filaments of variable length with a width of 20 nm, both of which vastly outnumber the Dane

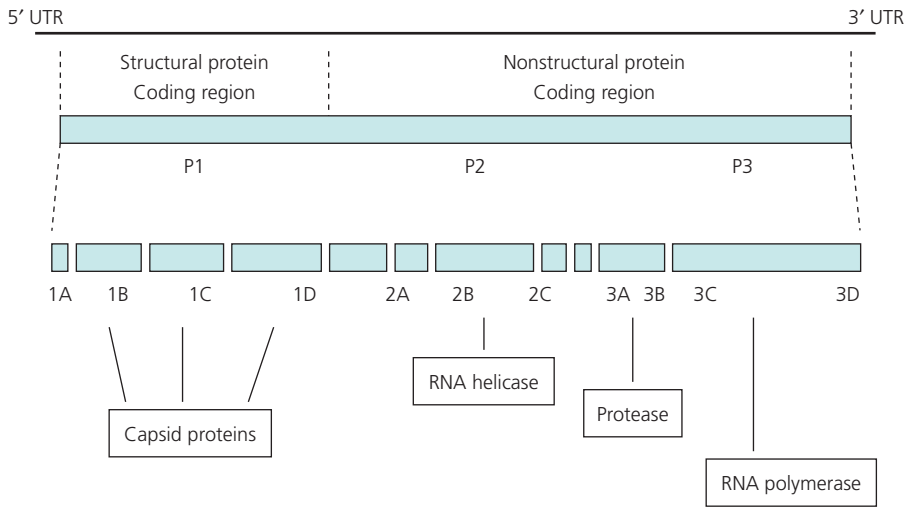

Figure 80.1 Structure of the hepatitis A virus and genetic organization. The *top line* represents the hepatitis A virus RNA genome. Translation of the genome yields the polyprotein; the *lower diagram* illustrates the three regions of the polyprotein and the individual protein products. UTR, untranslated region.

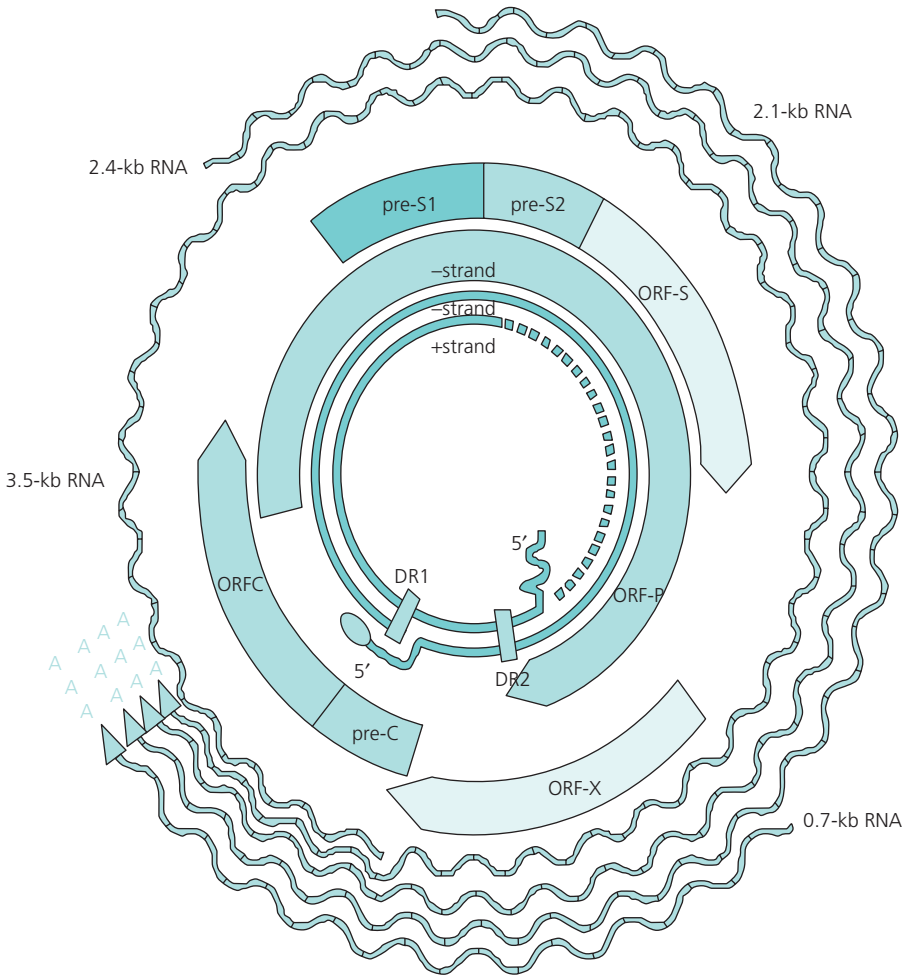

Figure 80.2 Genome structure and organization of hepatitis B virus. The hepatitis B virus open reading frames (ORFs): pre-C and C (precore and core proteins), P (polymerase protein), pre-S1, pre-S2, and S (L, M, and S surface envelope proteins), and X protein are shown. The viral genome structure is composed of the full length (–)-DNA strand and variable length (+)-DNA strand (*solid* followed by *dashed line*). The pol protein is covalently attached to the 5′ of the (–) strand and a capped oligoribonucleotide (*angulated line*) to the (+) strand. Direct repeats (DRs) 1 and 2 (*small rectangular boxes*) are shown on the genome. The *outer lines* represent the four transcripts, all terminating at a common polyadenylation site.

particles in infectious serum [85]. The spheres and filaments are composed of HBsAg and host-derived lipids and are noninfectious because they lack a nucleic acid [86]. The infectious HBV virion (the Dane particle) has a 42-nm spherical, double-shelled structure, consisting of a lipid envelope containing HBsAg that surrounds an inner nucleocapsid. The HBcAg forms a complex with viral-encoded polymerase and viral DNA genome to form the nucleocapsid [87]. The genome of HBV is a partially double-stranded circular DNA of about 3.2 kb pairs. The viral polymerase is covalently attached to the 5′ end of the minus strand [88].

The viral genome encodes four overlapping open reading frames (ORFs) (Fig. 80.2). The S ORF encodes the viral surface envelope proteins, the HBsAg, and comprises the pre-S1, pre-S2, and S regions. The core gene consists of precore and core regions. Multiple in-frame translation initiation codons are a feature of the *S* and *C* genes, which give rise to functionally distinct proteins. HBeAg and the viral nucleocapsid HBcAg are translated from initiation at the precore and core regions, respectively (Fig. 80.3). The core protein has the intrinsic property to self-assemble into a capsid-like structure and contains a highly basic cluster of amino acids at its C terminus with RNA-binding activity [89]. The precore ORF codes for a signal peptide that directs the translation product to the endoplasmic reticulum, where the protein is further processed to form the secreted HBeAg. The role that HBeAg serves is still unknown because mutations inactivating the precore region do not appear to affect viral replication [90]. One speculative role for HBeAg is as an immune tolerogen, whose function is to promote persistent infection [91]. The polymerase (pol) is a large protein (about 800 amino acids) and is functionally divided into three domains: the terminal protein domain (TP), which is involved in encapsidation and initiation of minus-strand synthesis; the reverse transcriptase domain, which catalyzes genome synthesis; and the RNase H domain, which degrades pregenomic RNA and facilitates replication. The HBX is a 16.5-kDa protein and has been attributed with multiple functions, including signal transduction, direct transcriptional activation, DNA repair, and inhibition of protein degradation [92–94]. The mechanism of this activity and the biological function of HBX in the viral life cycle remain largely unknown. However, it is well

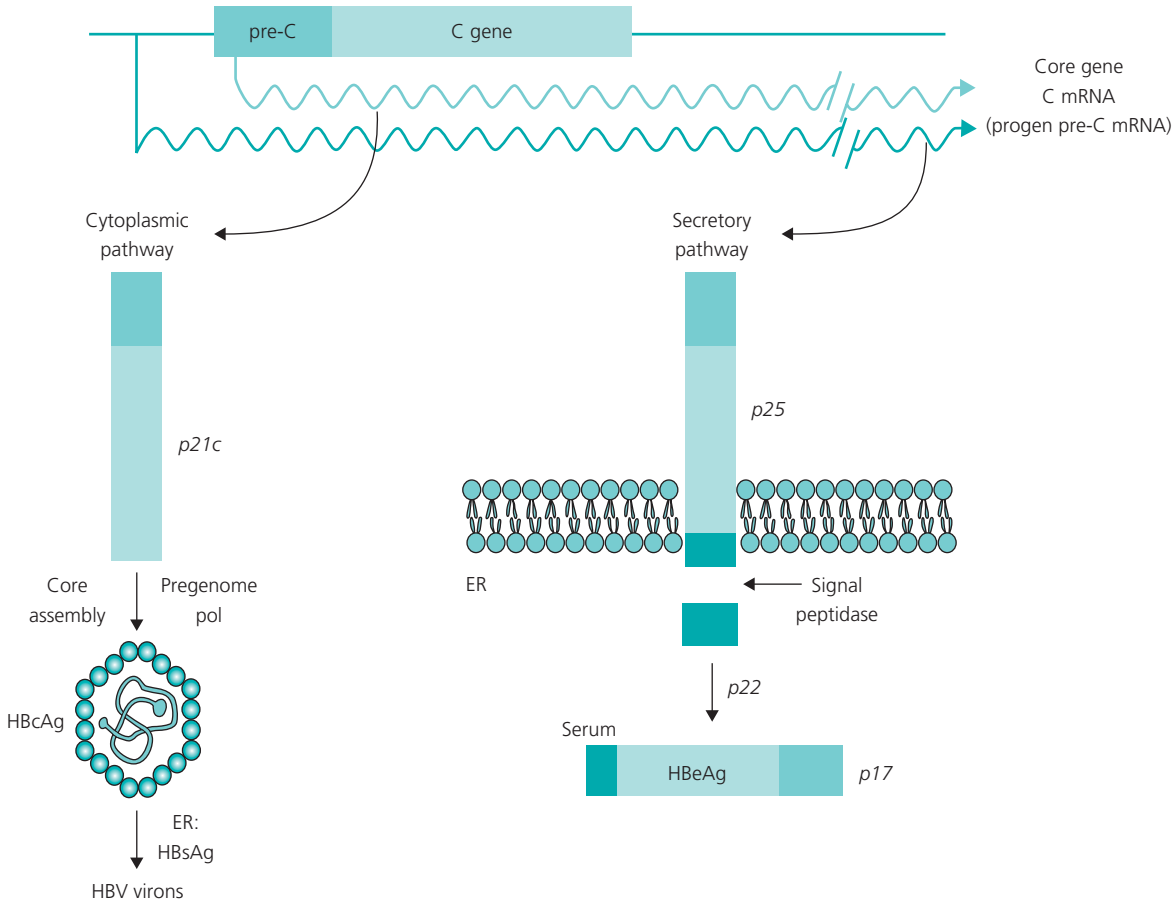

Figure 80.3 Two distinct processing pathways of *HBV* core gene. Two species of core RNAs are transcribed from the core gene. One starts before the pre-C and the other in the middle of the pre-C region. The former codes for the pre-C/C protein and is directed to the secretory pathway through the signal peptide sequence in the pre-C. After further proteolytic cleavage in the endoplasmic reticulum (ER), hepatitis Be antigen (HBeAg) is formed and secreted. The latter RNA codes for the core protein, which is involved in the nucleocapsid assembly.

established that HBX plays an essential role in HBV infection in vivo [95,96] and may contribute to the oncogenic potential of HBV [97].

Other functionally important elements within the HBV genome include two direct repeats (DR1 and DR2) in the 5′ ends of the minus and plus strands, which are required for strand-specific synthesis during replication [98]. Two enhancer elements, designated as En I and En II, confer liver-specific expression of viral gene products [99]. A glucocorticoid-responsive sequence within the S domain [100], a polyadenylation signal within the core gene [101], and a posttranscriptional regulatory element overlapping En I and part of HBX ORF have been described [102].

The HBV replication pathway has been studied in great detail and is summarized in Fig. 80.4. The initial phase of HBV infection involves the attachment of mature virions onto host cell membranes, likely involving the preS domain of the surface protein [103]. Various cellular factors have been proposed to be the viral receptors, but only the carboxypeptidase D has been shown to play an essential role in viral entry for the duck HBV [104]. Mechanisms of viral disassembly and intracellular transport of viral genome into the nucleus are not well understood and probably involve modification of nucleocapsid core protein [105]. After entry of viral genome into the nucleus, the single-stranded gap region in the viral genome is repaired by the viral pol protein, and the viral DNA is circularized to the covalently closed circular form [105]. This form of HBV DNA serves as the template for transcription of several genomic and subgenomic RNAs. Structurally, the transcripts are unspliced, polyadenylated, and possess a 5′ cap structure. The 3.5-kb genomic transcripts consist of two species with different 5′ ends: the pregenomic and the precore RNAs. The pregenomic RNA serves as the template for reverse transcription and the messenger RNA as template for core and polymerase; the precore RNA directs the translation of the precore gene product [106]. The polymerase is initiated at the pol start codon of the pregenomic RNA, probably as a result of a ribosomal scanning mechanism [107]. The large HBsAg (LHBsAg) protein is translated from the 2.4-kb subgenomic RNA, the middle (M) and small HBsAg (SHBsAg) proteins from the 2.1-kb RNA, and the HBX protein from the 0.7-kb RNA.

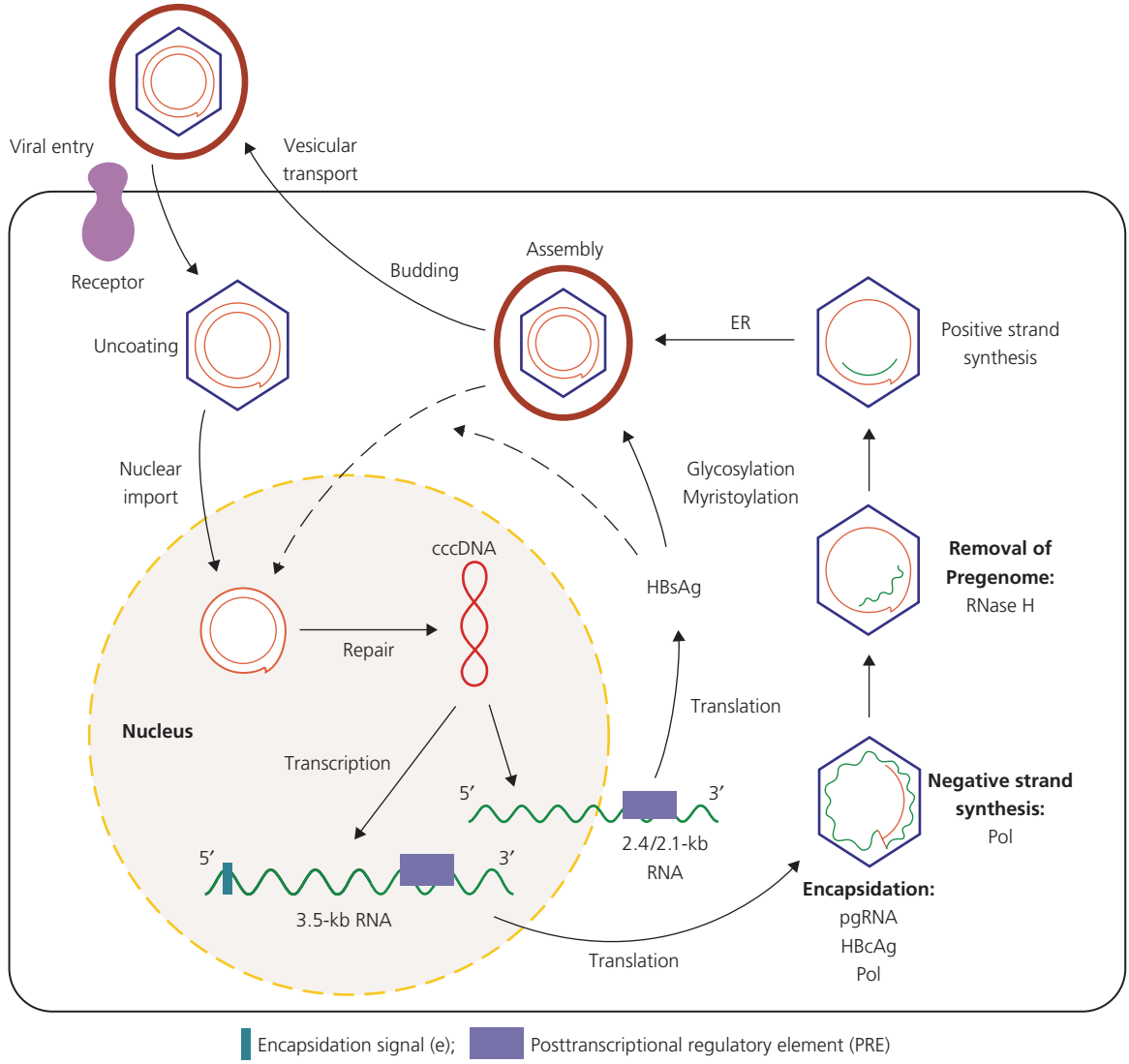

Figure 80.4 Replication of hepatitis B virus. Infectious virions probably attach to hepatocytes through the pre-S1 domain of the L protein. On entering, the nucleocapsid is delivered to the nucleus, and the viral genome is repaired to the covalently closed circular form (cccDNA). Viral transcripts are translated in the cytoplasm, and the core and polymerase proteins interact with the genomic length RNA to form the nucleocapsids. Reverse transcription occurs, and the mature virions are assembled in the endoplasmic reticulum (ER), where they acquire the surface proteins. The virion is then secreted through vesicular transport. The encapsidation signal (dark box) and posttranscriptional regulatory element (light box) on the *HBV* transcripts are shown as *rectangular boxes*.

The S protein accounts for most *S* gene products, with the L protein constituting 1%–2% and the M protein 5%–15%. Each surface protein has a glycosylation site in the S domain. Additional modifications of the M and L proteins occur at the pre-S2 domain with an N-linked oligosaccharide and a myristic acid at the N-terminal glycine residue of the pre-S1 domain [108]. The distribution of the three envelope glycoproteins varies among the types of viral particles, with little M and L protein in the 20-nm particles but relatively more L protein in the Dane particles.

Assembly of HBV begins with encapsidation of the genome. The packaging signal is a *cis*-acting element referred to as epsilon, which contains a stem-loop structure [109]. The terminal protein of the pol interacts with the epsilon and in concert with the core protein forms the nucleocapsid [110]. After encapsidation, the pol mediates the reverse transcription of the pregenomic RNA to minus-strand DNA and subsequent positive-strand synthesis. The circular form of the DNA is completed through several complicated steps of strand transfer [111]. The nucleocapsid then interacts with the envelope proteins in the endoplasmic reticulum to assemble into mature virions.

Hepatitis C virus

HCV is an enveloped virus and has been classified into six genotypes, 1–6. The virus circulates predominantly in complex

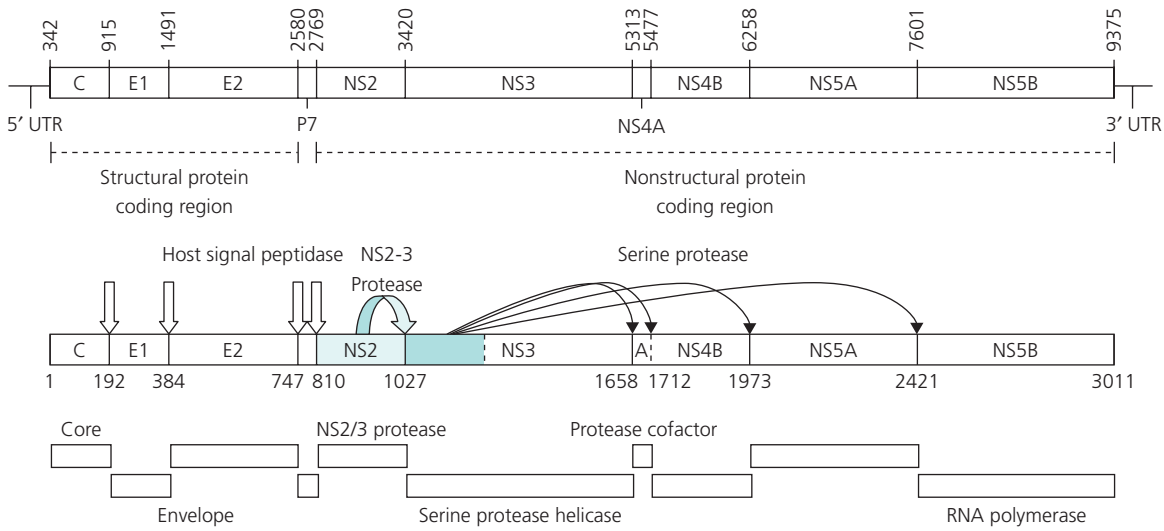

Figure 80.5 Genome organization of hepatitis C virus (HCV). The 5′ and 3′ untranslated regions (UTR) flanking a polyprotein open reading frame are shown at the *top*. Numbering refers to nucleotide positions of genes, based on the sequence of an *HCV* genotype 1a infectious clone. The middle panel shows HCV polyprotein processing with cleavage sites of host signal peptidase (*open arrows*), NS2-3 protease (*gray arrow*), and NS3 serine protease (*thin arrows*). Numbering denotes amino acid position upstream of cleavage sites. The processed HCV proteins are shown at the bottom.

with immunoglobulins and lipoproteins [112,113]. It is a member of the Flaviviridae family and has a positive-sense, single-stranded RNA genome about 9.6 kb in length with a single large ORF and highly conserved UTRs at the 5′ and 3′ ends [114–116] (Fig. 80.5). The 5′ UTR contains an internal ribosomal entry site that mediates translation. The 3′ UTR has a variable length with a 5′ to 3′ sequence followed by a polypyrimidine tract and finally by a highly conserved 98-nucleotide sequence [116]. The 3′ UTR is also predicted to have extensive secondary structures that probably play a role in viral replication. In the large ORF, the structural genes for core and two envelope glycoproteins, E1 and E2, are located at the N-terminal end and the nonstructural genes for NS2, NS3, NS4A, NS4B, NS5A, and NS5B are at the C-terminal end.

The HCV polyprotein of about 3000 amino acids is processed co- and posttranslationally by cellular and viral proteases to produce the individual gene products [117,118]. Cellular proteases in the endoplasmic reticulum catalyze the cleavage of the structural proteins, whereas viral encoded proteases cleave the nonstructural proteins [119].

The highly conserved core protein is the putative viral nucleocapsid and encompasses the first 191 amino acids of the polyprotein. The E1 and E2 are envelope glycoproteins with C-terminal hydrophobic transmembrane domains [120]. The 27 N-terminal amino acids of E2 have been designated the hypervariable region 1, owing to significant amino acid variations among all isolates [121]. Studies have suggested that sequence variations of the hypervariable region 1 result from immune pressure as a mechanism to evade host immune response.

The NS2 region encodes a metalloproteinase that requires cell membrane and N-terminal NS3 for efficient function [122]. The NS2-3 protease mediates autocatalytic cleavage between the NS2 and NS3 (Fig. 80.5). The NS3 region encodes a multifunctional protein with an N-terminal serine protease and a C-terminal RNA helicase and nucleotide triphosphatase (NTPase) [123]. The NS3 protease, distinct from NS2-3 protease activity, is involved in processing the downstream polyprotein [124]. The NS4A interacts with and acts as a cofactor for the NS3 protease [125]. The crystal structure of NS3 protease has a trypsin-like structure but becomes more chymotrypsin-like when cocrystallized with NS4A [126]. NS4B is thought to function in the formation of replicative complex in a membranous compartment [127]. The NS5A may play a role in sensitivity to interferon [128]. The NS5B is the RNA-dependent RNA polymerase that mediates viral replication. Similar to replication of other members of the family Flaviviridae, HCV replicates in the cytoplasm, presumably in a membrane-associated compartment (Fig. 80.6). The NS5B recognizes a structure in the 3′ UTR and initiates minus-strand RNA synthesis in a primer-independent template-specific process [129]. The synthesis of plus strand is presumably carried out by NS5B in a similar manner. HCV replication in vivo probably occurs in the context of multiple viral nonstructural proteins. The process of viral assembly is poorly understood and probably requires the interactions among viral genome, core, and E1 and E2 proteins.

Hepatitis D virus

HDV requires coinfection with HBV for replication [130]. Delta antigen is the inner ribonucleoprotein component of a subviral particle that is enveloped by HBsAg. HDV has many features in common with viroids, the subviral agents of plants [131]. The ribonucleoprotein complex consists

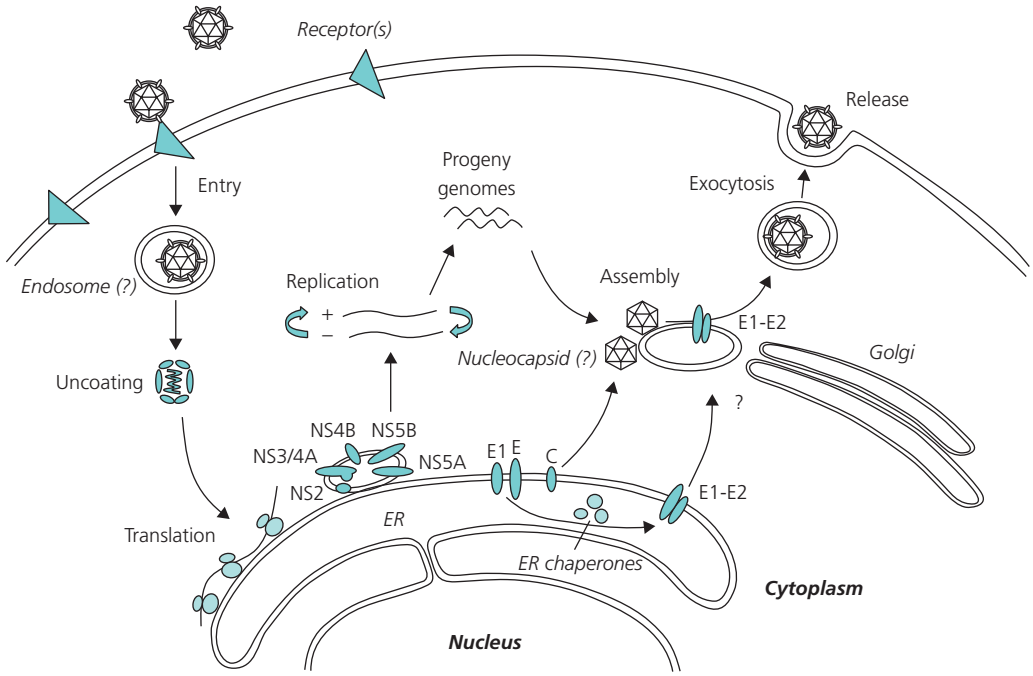

Figure 80.6 Replication of hepatitis C virus (HCV). The virion attaches and enters the susceptible cell through pathways that are not yet completely defined. The viral genome is then directed to a membranous component in the perinuclear endoplasmic reticulum (ER) region and serves as template for HCV protein synthesis. The nonstructural proteins form a replication complex with the genomic RNA and direct RNA replication (to negative and then plus strands). The structural proteins, which are retained in the endoplasmic reticulum, interact with the progeny genomes and assemble into virions. The virions are then secreted through an unknown exocytotic pathway, probably not passing through the Golgi compartment.

of small (SHDAg) and large (LHDAg) delta antigens and a single-stranded circular RNA genome 1.7 kb in length that has extensive self-complementation to form a rodlike structure (Fig. 80.7). HDV attachment to the host cell is mediated by HBsAg [132]. The HDV genome uses host RNA polymerase II to carry out RNA-directed RNA synthesis that is dependent on the small delta antigen [133,134]. Both genomic and antigenomic RNAs possess ribozyme activities that catalyze RNA self-cleavage and self-ligation. Similar to plant viroids, transcription and replication are integrated into a single process using a double rolling-circle mechanism [131]. After entry into cells, the HDV genome serves as a template for replication, resulting in the production of multimeric antigenomes. Nascent antigenomes, through their intrinsic ribozyme activities, form circular monomeric RNAs that, in turn, serve as templates for the production of HDV genomes. Alternatively, the elongating product can be cleaved and released as polyadenylated messenger RNAs, which then direct delta antigen synthesis [135].

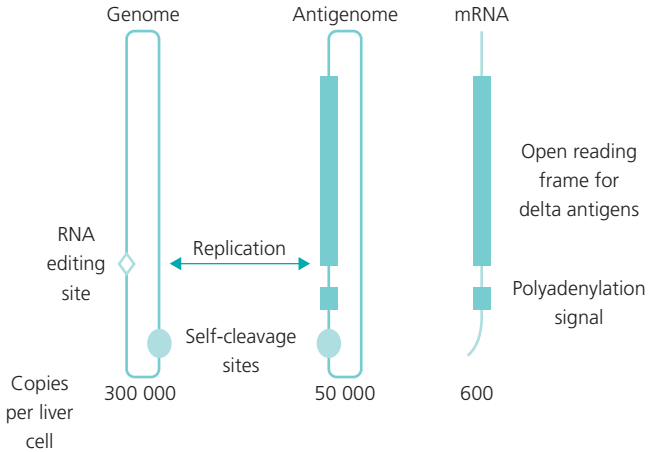

Figure 80.7 Genomic organization of hepatitis D virus (HDV). The RNA genome has a rod-like structure and contains an RNA editing and a self-cleavage site (*circle*). The antigenome is synthesized from the genomic RNA and is the template for HDV messenger RNA encoding the delta antigens. The antigenome also serves as the template for genome synthesis. The estimated copy numbers of the RNA species in the infected liver are shown *below*.

Translation of HDV messenger RNA yields the small and large delta antigens, which differ by the addition of 19 amino acids in the C-terminal end of LHDAg [136]. The small and large delta antigens have distinct functions during HDV replication. The additional C-terminal amino acids of the LHDAg contain an isoprenylation signal that appears to be important for interaction with HBsAg during viral assembly [137]. The RNA editing process results in two mRNA species coding for the two forms of HDAg and involves the double-stranded RNA-activated adenosine deaminase [138]. This RNA editing activity results in the C-terminal extension of the LHDAg [139].

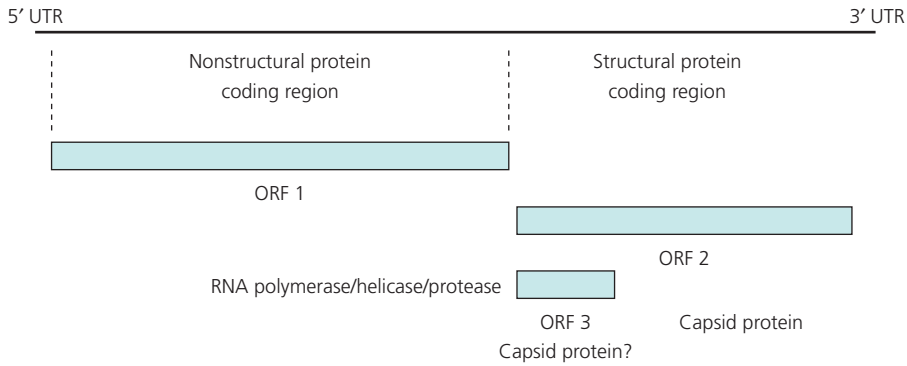

Figure 80.8 Genomic organization of hepatitis E virus. The open reading frames (ORF) are flanked by 5′ and 3′ noncoding regions and a 3′ polyadenylation. Within ORF1: MT (methyl transferase), X and Y (unknown functions), Pro (protease), Hel (helicase), H (proline-rich hinge region), Pol (RNA-dependent RNA polymerase). ORF2 codes for the capsid, and ORF3 codes for a protein of unknown function.

HDV assembly begins with the association of the delta antigens with the newly synthesized genome to yield a ribonucleoprotein complex [140]. The ribonucleoprotein complex is transported from nucleus to cytoplasm, presumably mediated by the nucleocytoplasmic shuttling function of delta antigens. The LHDAg of the ribonucleoprotein interacts with HBsAg to facilitate assembly. Large delta antigen is required for particle assembly, whereas small delta antigen is copackaged but not required for particle formation [141].

Hepatitis E virus

HEV is a nonenveloped virus and is unclassified but it is similar to togavirus and alphavirus. The viral genome is a single-stranded, positive-sense RNA genome of about 7.5 kb. It is polyadenylated and has a capped 5′ end [142]. The genome is organized into three overlapping ORFs flanked by noncoding regions (Fig. 80.8). ORF1 appears to encode the nonstructural gene products, ORF2 encodes the capsid protein, and ORF3 encodes a protein with possible nucleocapsid function [143]. Based on sequence analysis and comparison with other viral genomes, ORF1 contains several functional domains: methyl transferase, papain-like cysteine protease, helicase, and an RNA-dependent RNA polymerase [144]. ORF2 begins 38 nucleotides downstream from the 3′ terminus of ORF1 and contains arginine-rich regions believed to be involved in genome encapsidation. ORF3 overlaps one 3′ nucleotide of ORF1 and extends into ORF2.

Replication of HEV has not been characterized because of a lack of similarity to other viruses and efficient cell culture systems. The mechanisms of viral attachment, entry, and uncoating are unknown. The released genome likely uses the host translation machinery to synthesize viral gene products. Processing of the ORF1 polyprotein is carried out by either cellular proteases or a viral papain-like proteinase encoded by ORF1. The viral encoded polymerase mediates synthesis of plus- and minus-strand genomes as well as subgenomic RNA species, which serve as templates for the translation of viral antigens. The mechanism of viral assembly is unknown but probably involves the capsid function of ORF2 and ORF3.

Pathogenesis

Acute infection with hepatitis viruses is associated with a spectrum of liver diseases and systemic manifestations, ranging from asymptomatic infection, through self-limited hepatitis, to fulminant hepatitis. In addition, three of the viruses (B, C, and D) are associated with persistent infection and chronic hepatitis, the clinical manifestations of which are variable. The outcome, extent, and severity of acute viral hepatitis depend on a variety of viral and host factors. Although host responses play a major role in viral clearance and disease pathogenesis, the outcome of infection is often predicated on a variety of viral adaptive mechanisms. The pathogenesis of acute viral hepatitis involves a complicated set of virus–host interactions and is likely a result of diverse host immunological responses to the viral infection. In this section, the pathogenesis of acute viral hepatitis is discussed in the general context of viral infection and host responses and with respect to relevant issues specific to each of the viruses. Among the hepatitis viruses, the pathogenetic mechanisms have been much better studied in HBV and HCV than in the other viruses; therefore, we focus more on HBV and HCV. Furthermore, the pathogenesis of acute hepatitis is often inferred from the various studies during chronic infection (for B, C, and D), and we attempt to highlight these issues during the course of discussion in this section.

Primary infection

After gaining entry into a susceptible host, the hepatitis viruses are carried by the blood to the liver, which is the major site of viral tropism. For HAV and HEV, the viruses enter through the gastrointestinal epithelium, and the uptake mechanisms remain largely unknown. It is possible that a round of local replication may occur at the site of entry before the virus is further spread to the liver through the portal circulation. HBV, HDV, and HCV have all been reported to infect nonhepatocytes so the existence of an intermediate compartment of replication before infection of the liver has also been proposed but remains controversial [145,146].

Many initial events of viral infection have been characterized in the animal models for each virus. HBV and probably HCV replicate in a noncytopathic manner without causing injury to hepatocytes, whereas the cytopathicity of other viruses is unknown.

Immune responses

During primary infection, the initial pathway of antiviral immune response is largely unknown. Initial viral infection is associated with activation of innate immunity in the liver. Recognition of infected hepatocytes by resident natural killer or natural killer T cells leads to the activation of these cells and the induction of antiviral cytokines, including interferons [147,148]. This phase of innate immunity leads to the initial control of viral replication. This antiviral response is likely associated with a noncytopathic mechanism so little or no hepatocellular injury is evident. The innate immunity also plays a critical role in the activation of the adaptive immunity, including humoral and cellular responses. Induction of humoral immune response with the production of neutralizing antibodies prevents viral spread and leads to subsequent elimination of circulating viruses [149]. This mechanism is particularly relevant for HAV and HBV infections because of the high level of viral replication and viremia. Neutralizing antibodies against the capsid (for HAV and HEV) or envelope (for HBV) can be induced and lead to viral clearance and protection from subsequent exposure [150–152]. This constitutes the basis for the successful formulation of HAV (probably HEV as well) and HBV vaccines based on the capsid and envelope proteins, respectively. In HAV, HBV, HDV, and HEV, the immunoglobulin M (IgM) class of antibodies is detected first and serves as a useful marker for acute infection. The IgA class of antibodies has been detected in HAV and HEV infections, but the implication of its presence in viral clearance and protective immunity remains unknown [150]. On the other hand, neutralizing antibodies probably play little or no role in viral clearance during acute HCV infection [153]. A clearly defined anti-HCV IgM response has not been identified, and the antibody response [154], especially against the envelope proteins, tends to occur much later than in other hepatitis viral infections [155].

For HBV, the antibody response to the envelope proteins is a T-cell-dependent process [156]. A minority of patients infected with HBV fail to produce antienvelope antibodies, therefore contributing to the establishment of persistent infection. This deficiency resides at the level of T-cell nonresponsiveness, probably as a result of tolerance or anergy induction toward the HBV proteins [157]. The molecular mechanisms underlying this deficiency, however, are largely undefined. For HAV infection, whether the relapsing or prolonged phase is secondary to transient failure of the humoral immune response is unknown. On the other hand, antiviral antibodies may play a role in viral pathogenesis. Cell surface forms of viral antigens on infected hepatocytes could be potential targets for antibody-directed cell cytotoxicity [158,159]. However, this mechanism has not been shown to be operative in hepatocellular injury associated with any of the viral infections. In contrast, immune complex diseases with complement fixation, including glomerulonephritis, vasculitis, and cryoglobulinemia, which are major extrahepatic manifestations of HBV and HCV infections, can result from an abnormally activated antibody response to viral antigens.

The other limb of the immune response, the cell-mediated immunity, is critical for the long-term control of viral infections, including the hepatitis viruses. Although this aspect has only been studied in great detail for HBV and HCV, it is not unreasonable to suspect that cell-mediated immunity may play a role in viral clearance and pathogenesis for HAV, HDV, and HEV. Much of the information for hepatitis B has been gleaned from studies in mouse models, chimpanzees, and infected humans [157,160]. Nonprimate animal models that are susceptible to hepadnaviral infection (woodchucks, ground squirrels, ducks) have also proved useful in this avenue of research. In acute HBV infection, individuals can mount a vigorous, multispecific, and polyclonal cellular immune response to HBV. In contrast, chronically infected patients have a weak or barely detectable anti-HBV response [157]. This is true for both CD4 and CD8 responses. During acute hepatitis B, a vigorous human leukocyte antigen (HLA) class II-restricted, CD4+, helper T-cell response to multiple epitopes of HBc/eAg predominates in virtually all patients. Such a response to HBV envelope antigens, however, is much less evident. The class II-restricted, CD4+ T cells specific for nucleocapsid play an important immunoregulatory role in the control of viral infection [157,161]. By helping B cells produce neutralizing antienvelope antibodies and activating HBV-specific cytotoxic T lymphocytes (CTL), this CD4+ helper T-cell population may direct the initial antiviral response. During chronic HBV infection, the once strong nucleocapsid-specific CD4+ T-cell response becomes barely detectable, presumably as the result of a switch to T-cell exhaustion or nonresponsiveness. During exacerbation or successful treatment of chronic hepatitis B, these nucleocapsid-specific T-cell responses could reemerge [162,163].

In most viral infections, the activation of virus-specific CD8+ CTLs is critical for viral clearance. These cells can detect and eliminate or cure virus-infected cells through recognition of viral peptides in the context of HLA class I molecules. Using short synthetic peptides that mimic processed viral antigens and cells that are genetically engineered to express viral proteins, CTL responses to HBV in humans have been studied extensively [157]. These studies indicate that patients acutely infected with HBV develop a strong, polyclonal, HLA class I-restricted CTL response that is directed against multiple epitopes in all viral proteins. Similar to the class II-restricted CD4 responses in a minority of infected people, the class I-restricted CTL responses disappear and are barely detectable during the chronic phase of HBV infection [161,164].

Studies of immune responses in HCV revealed that both CD4 and CD8 responses can be detected against various viral proteins during acute infection, but the vigor and character of these responses appear to be directly correlated with the outcome of the viral infection [153]. A vigorous and multispecific CD4 and CD8 response is associated with viral clearance in both human and experimental chimpanzee infections [165,166]. On the other hand, antibodies against the envelope proteins, presumably containing neutralizing activities, arise much later in the infection and appear not to play a major role in viral clearance [167]. During acute HCV infection, those who progress to chronic infection appear to exhibit weaker cell-mediated immunity than those who recover. Several recent studies have pointed to a defect in dendritic cells in antigen presentation, presumably as a result of infection of these cells by HCV [168]. However, other studies did not confirm any defects in dendritic cell functions. Other mechanisms, such as the immunomodulatory effects of HCV core protein and the interferon inhibitory property of the NS5a protein, may also contribute to an overall weak immunity to this viral infection [169,170].

In chronic HBV and HCV infections, the detection of virus-specific CD4 and CD8 cells, albeit with low frequency and weak activities, in the peripheral blood and liver of chronically infected individuals suggests a pathogenic relationship between the indolent cellular immune response and necroinflammatory liver disease associated with chronic hepatitis [157]. Therefore, the cell-mediated immunity is a double-edged sword: vigorous response leads to viral clearance, whereas ineffective response results in chronic hepatocellular injury.

For HBV infection, the molecular and cellular mechanisms of viral clearance and hepatocellular injury have been elucidated in animal models, including both transgenic mice and chimpanzees [157,160] (Fig. 80.9). Adoptive transfer of murine CD8+, class I-restricted HBsAg-specific CTLs into syngeneic transgenic mice expressing HBV envelope protein in the liver leads to acute hepatitis [171,172]. The CTLs first target the liver through interaction between the HBV-specific T-cell receptors and the antigen-presenting HLA class I molecules on the hepatocytes and cause scattered apoptosis of hepatocytes. By secreting cytokines, including interferons, the CTLs recruit a variety of antigen-nonspecific inflammatory cells into the liver, resulting in more extensive necroinflammatory injury of the liver that resembles acute hepatitis B in humans. The predominant infiltrating effector cells are the macrophages, which probably mediate most of the hepatocellular

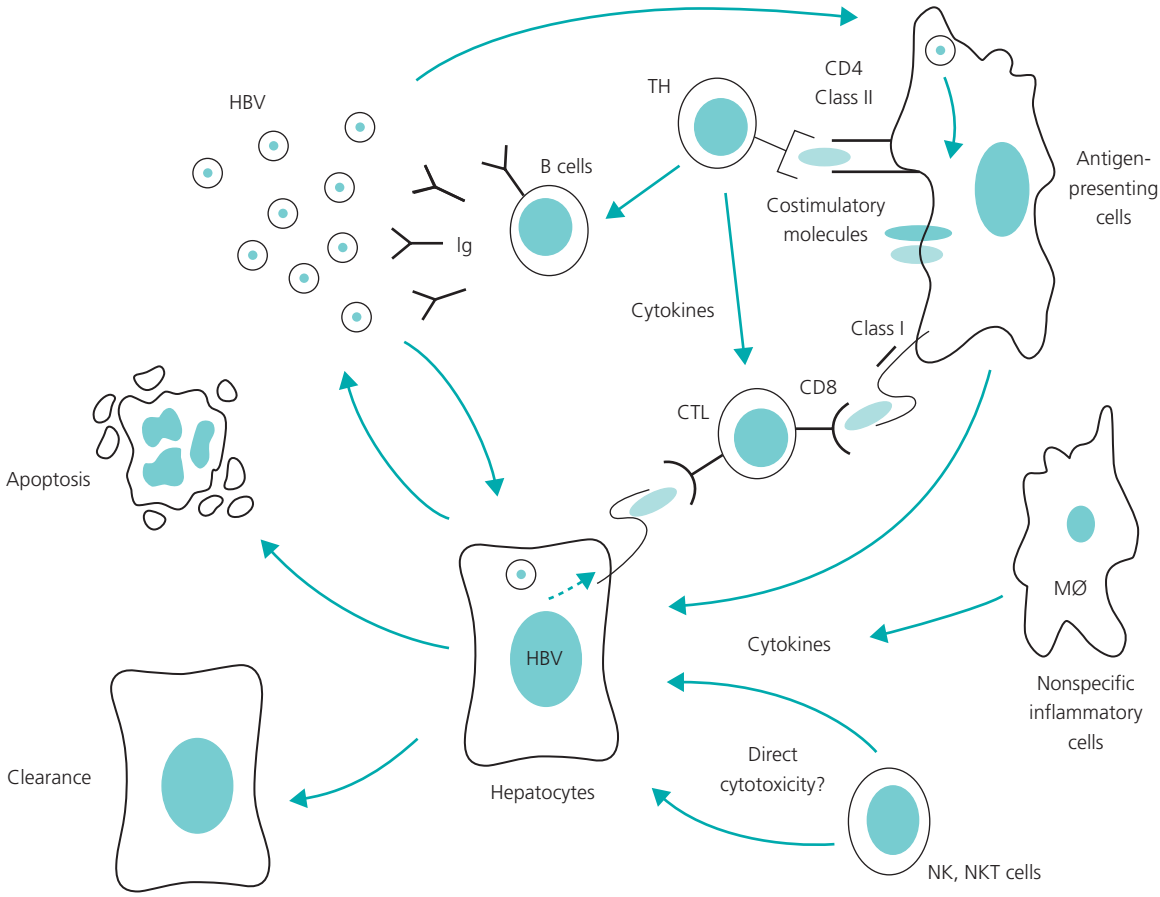

Figure 80.9 The immunopathogenesis of hepatitis B. See text for details. CTL, cytotoxic T lymphocyte; HBV, hepatitis B virus; NK, natural killer cell; NKT, natural killer T cell; TH, thymocyte.

injury in this model [173]. The CTLs, although not primarily responsible for most of the hepatocellular injury, initiate the cascade of immunological events leading to hepatitis in this model. A major consequence of these immunological events is the marked inhibition of hepatocellular *HBV* gene expression and viral replication [174]. Although the CTLs may contribute to a proportion of inhibition by direct cytolysis of *HBV*-expressing hepatocytes, a noncytolytic mechanism likely plays a substantial role in this effect [174]. Interferon-α and tumor necrosis factor-α produced by activated CTLs and other effector cells are the key cytokines involved in this mechanism [174]. A recent study in chimpanzees [160] also provided support for the noncytopathic mechanism as the major antiviral mechanism responsible for viral clearance during acute hepatitis. The resident natural killer or natural killer T cells, as discussed previously, provide the first line of defense; the CTLs, together with other inflammatory cells and production of antiviral cytokines, likely constitute the final assault on the virus. The CTL response appears to be long-lived because patients recovered from acute HBV infection harbor HBV-specific CTLs many years after viral clearance [164]. The CTLs have been postulated to persist as a result of continuous stimulation by low-level viral replication and in the meantime keep the infection in check. It is not clear how prevalent is this occult HBV infection, but low-level HBV virus can be frequently detected in patients with serological evidence of recovery [175–177]. Furthermore, active HBV infection can be reactivated in these patients on immunosuppressive therapy, and their serum or organs are capable of transmitting HBV infection [178–180].

In HCV infection, long-term persistence of virus-specific T cells has also been demonstrated despite evidence of recovery [181]. However, low-level viral infection is probably not the reason for this persistence. It is possible that these virus-specific memory T cells have unusual longevity in vivo and can be rapidly activated on viral antigen stimulation years after infection. Whether these virus-specific T cells can offer protective immunity to subsequent viral infection is unknown. The observation that chimpanzees can be reinfected despite recovery from previous infection [182,183] suggests that protective immunity may not persist after viral clearance. On the other hand, partial protective immunity has been demonstrated in both humans and chimpanzees after repeated exposure to HCV [184–186].

The cellular immune responses to HAV, HDV, and HEV are largely unknown. Although HAV-specific CD8 CTLs have been detected in liver biopsies during acute hepatitis A, their roles in viral clearance and pathogenesis remains undefined [187,188].

Clinical manifestations

General

Symptoms related to acute viral hepatitis are variable and nonspecific. It is impossible to distinguish one form of viral hepatitis from the other based solely on clinical features. The clinical course of hepatitis varies widely from an asymptomatic phase recognized only by elevation in aminotransferase levels to fulminant hepatitis with frank jaundice and hepatic coma [189–191]. About 20% of cases of acute hepatitis present with jaundice. There is a variable incubation period for each virus, ranging from 1 week to 6 months. The initial presentation is dependent on age at exposure, integrity of the host immune system, presence or absence of underlying liver disease, and the level of viral inoculum. Overall mortality is low. An overview of the clinical features of each of the five hepatotrophic viruses is given in Table 80.2.

Table 80.2 Clinical overview of the five viral hepatidides

Virus	A	B	C	D	E
Source	Stool	Blood	Blood	Blood	Stool
Transmission	Enteric	Percutaneous/ permucosal	Percutaneous/ permucosal	Percutaneous/ permucosal	Enteric
Epidemics	Yes	No	No	Yes	Yes
Incubation period (days)	15–45, mean 25	30–180, mean 75	15–160, mean 50	30–180, mean 35	14–60, mean 40
Acute hepatitis	Yes	Yes	Yes	Yes	Yes
Acute infections (× 1000)/years, USA	125–200	140–320	35–180	6–13	?
Fulminant hepatitis	Yes	Yes	Yes	Yes	Yes
Fulminant deaths/year, USA	100	150	?	35	?
Risk for chronic hepatitis	No	Yes	Yes	Yes	No
Risk for hepatocellular cancer	No	Yes	Yes	Yes	No
Prevention	Pre/postexposure immunization	Pre/postexposure immunization	Blood donor screening; risk behavior modification	Pre/postexposure immunization; risk behavior modification	Safe drinking water

Before the onset of symptoms, there is a prodromal phase that can last from 1 day to 2 weeks and averages 5–7 days, during which there are nonspecific constitutional symptoms. By far the most common symptom reported is loss of appetite. Patients report a distinct aversion for food that may worsen as the day progresses [192]. They may also lose the taste for tobacco and alcohol. Fatigue and weakness are common symptoms reported by 90% of patients and may be severe enough to confine them to bed. Before the onset of jaundice, two-thirds to three-quarters of patients complain of low-grade fever and flu-like symptoms. Mild headache is reported by 50%–70% of patients. Vomiting and nausea are experienced by some patients but are not predominant features of acute viral hepatitis, and their continued presence should alert one to consider alternative diagnoses. Diarrhea is not a common feature of acute hepatitis and occurs in less than 25% of cases of HAV infection. Patients often complain of a dull ache under the right rib cage or even frank pain. Closer to the onset of jaundice, the urine may begin to darken.

The prodromal phase is followed by the icteric phase. The onset of jaundice usually coincides with the peak of the serum ALT. The duration of jaundice is variable, ranging from 4 days to several months, but averages 2–3 weeks. Patients initially notice a yellow discoloration of the mucous membranes and sclerae, followed later by a sallow discoloration of the skin. The urine becomes intensely dark owing to the release of conjugated bilirubin from necrosed hepatocytes, and stools become pale as the result of an inability of the hepatocytes to conjugate bilirubin to its secretory form. During this phase, half of patients may experience itching, which is usually mild and transient. Weight loss of 2–10 kg is not uncommon. A minority of patients experience depression.

During the convalescent phase, most symptoms resolve. Jaundice disappears, with a return of normal skin, urine, and stool color. The return of appetite and gain in weight are favorable signs indicating recovery. Fatigue may persist for up to 2–6 months.

The physical examination during acute viral hepatitis may be normal, but almost all patients have some degree of hepatic tenderness. Most patients have mild hepatomegaly (12–14 cm), and the liver edge should feel soft and smooth. On occasion, it may feel firm to the examining hand, but it should never feel hard or nodular. If these findings are elicited, underlying cirrhosis should be suspected and an acute on chronic hepatitis should be considered. Splenomegaly and spider angiomas are features not commonly observed with acute hepatitis, but they may occur rarely. Jaundice is present in icteric cases, involving the mucous membranes, sclerae, and skin. The total bilirubin level usually has to be greater than 2.5 mg/dL before jaundice is clinically evident.

Extrahepatic manifestations

Extrahepatic manifestations may occur in patients with acute viral hepatitis and result from direct infection of another organ or as an immune-mediated phenomenon. A serum-sickness-like syndrome may be present in 5%–15% of patients with acute HBV infection and in fewer patients with HCV infection. It presents as a low-grade fever, rash, arthralgias, and angioneurotic edema [193,194]. The rash is usually urticarial but may be maculopapular. The arthralgias tend to involve the large joints, such as the elbows, wrists, knees, and ankles. Symptoms are thought to be immune complex mediated and resolve with onset of hepatitis.

Polyarteritis nodosa is well described in association with HBV infection. In one series, polyarteritis nodosa occurred in almost one-third of newly diagnosed cases of HBV [195]. Polyarteritis nodosa is thought to result from the deposition of antigen–antibody complexes in the intima of the vessel wall; HBsAg is the suspected antigen [196,197]. Symptoms include arthralgias, fever, abdominal pain, renal disease, mononeuritis, and skin rash. Polyarteritis nodosa occurs more often with chronic HBV infection [198]. The mortality rate ranges from 30% to 50%.

Essential mixed cryoglobulinemia (EMC) is a systemic vasculitis characterized by palpable purpura, arthralgias, and weakness. It has been linked to hepatitis A, B, and C based on the high prevalence of liver disease in patients with EMC and on the detection of viral antigens, antibody, and virus in the cryoprecipitate and the vascular lesions [199–201]. The association of EMC and viral hepatitis is strongest with HCV infection [202]. The prevalence of HCV in EMC ranges from 80% to 98% [202,203]. Cryoglobulins are serum proteins that precipitate at temperatures below 37°C [204,205]. They usually consist of polyclonal IgG complexed with monoclonal IgM with cross-reactivity against rheumatoid factor [206]. The prevalence of cryoglobulins in unselected patients with hepatitis C is unknown. Studies from referral centers report a prevalence ranging from 29% to 54% [199,207]. Most cases of HCV-related EMC are associated with chronic infection and do not correlate with viremia or genotype but appear to be related to the duration of infection and to more advanced stages of liver disease.

Renal disease is often seen in patients with acute viral hepatitis, and specific pathology is best characterized for HBV and HCV. Manifestations of renal involvement range from mild proteinuria and abnormal urinary sediment, including red blood cell and hyaline casts, to frank glomerulonephritis with renal failure. The renal lesion is thought to result from the deposition of immune complexes in the glomerular basement membrane. HBsAg, HBeAg, and HCV antigen have been demonstrated in the immune complexes from the glomerular basement membrane [208–210]. The most common renal lesions seen in HBV infection were membranous [211] and membranoproliferative glomerulonephritis [212]. Membranous glomerulonephritis occurs mainly in children from endemic regions. The natural history of membranous glomerulonephritis is not well defined [213]. Nephrotic syndrome and proteinuria are the most common presentations.

Children are more likely to be asymptomatic and to have spontaneous resolution of the nephrotic syndrome [211]. Progressive renal failure usually develops in adults. Most patients with glomerulonephritis have evidence of active viral replication, but the severity of the renal disease does not correlate with the activity of the liver disease or the viral level in serum. Membranoproliferative glomerulonephritis is the most common renal lesion seen in HCV infection, usually in association with EMC [214]. In the presence of cryoglobulins, 98%–100% of patients with membranoproliferative glomerulonephritis have detectable anti-HCV. In the absence of cryoglobulinemia, patients with chronic HCV infection may have a variety of renal lesions suggestive of primary glomerular disease including nephrotic syndrome and nonnephrotic proteinuria.

Papular acrodermatitis (Gianotti syndrome) has been described in children with acute hepatitis B [215,216]. The syndrome is most commonly seen in young children aged 1–6 years. It is characterized by erythematous papules on the arms, legs, and face with truncal sparing and lymphadenopathy. The mucous membranes are spared. It is not related to the infecting subtype of HBV. It usually runs a benign course, improving with the onset of hepatitis.

Other organ systems affected during viral hepatitis, although rarely, include the heart and gastrointestinal and nervous systems. Cases of myocarditis, pericarditis and bradycardia, and pericardial and pleural effusions have been described. Pancreatitis is commonly reported, especially in children [217] and may be the result of direct infection of the gland in the case of HAV infection. Neurological manifestations have included encephalitis, meningoencephalitis, mononeuritis multiplex, transverse myelitis, and Guillain–Barré syndrome [218–220]. A variety of hematological disorders may follow viral hepatitis, including pure red cell aplasia, pancytopenia, aplastic anemia, and hemolytic anemia.

Diagnosis and differential diagnosis

The diagnosis of acute hepatitis is based on the constellation of clinical, biochemical, histological, and serological findings. The CDC case definition of acute viral hepatitis is an illness with a discrete date of onset, accompanied by jaundice or elevated serum aminotransferase levels more than 2.5 times the upper limit of normal. The clinical symptoms associated with viral hepatitis were described in the previous section and are nonspecific for etiology. As the clinical features are similar for all the hepatotrophic viruses, specific diagnosis depends on the results of serological testing. The sensitivity of current serological assays should allow the etiology of acute viral hepatitis to be established in almost all cases by the time patients become symptomatic. In instances in which there may be a late or weak antibody response, sensitive RNA and DNA assays may be helpful in the diagnosis.

Serology

In the setting of a known exposure and appropriate symptoms with elevated serum aminotransferases, acute hepatitis A is diagnosed by the detection of IgM antibody to HAV (anti-HAV IgM) and the absence of other viral markers in serum [221,222]. Anti-HAV IgM is usually present in serum 5–10 days into the incubation period and remains detectable for up to 6 months after infection, when IgG-type antibody rises to a high titer and persists for life [223] (Fig. 80.10). Commercial assays for total anti-HAV measure both IgG and IgM, and thus a positive test result is not helpful in distinguishing acute from chronic infection. A past history of HAV infection is diagnosed by the detection of anti-HAV IgG. HAV RNA can also be detected in serum and stool during the incubation phase but this is primarily a research tool and is not used for diagnostic purposes. These molecular techniques are particularly helpful in characterizing the epidemiology of HAV infection and in identifying sources of outbreaks.

A number of viral antigens and their respective antibodies can be detected in serum after infection with HBV, and their interpretation is often the source of great confusion to physicians and patients alike. HBsAg and HBeAg are the first two viral proteins detected in serum [152] (Fig. 80.11). HBsAg may be detected as early as 1–2 weeks or as late as 11–12 weeks after infection, and its persistence is a marker of chronicity. HBeAg is considered a marker of HBV replication and infectivity. It is more important for assessing risk for transmission in chronic HBV infection [224]. HBcAg in serum is usually too scarce to detect but it can be visualized by immunostaining of liver tissue. A cytoplasmic distribution of HBcAg in hepatocytes was shown to correlate with disease activity.

Antibodies to three of the viral proteins (HBsAg, HBeAg, and HBcAg) can be detected in convalescent sera. Anti-HBc IgM class is the first to appear, followed by antibody to HBeAg (anti-HBe) and finally antibody to HBsAg (anti-HBs),

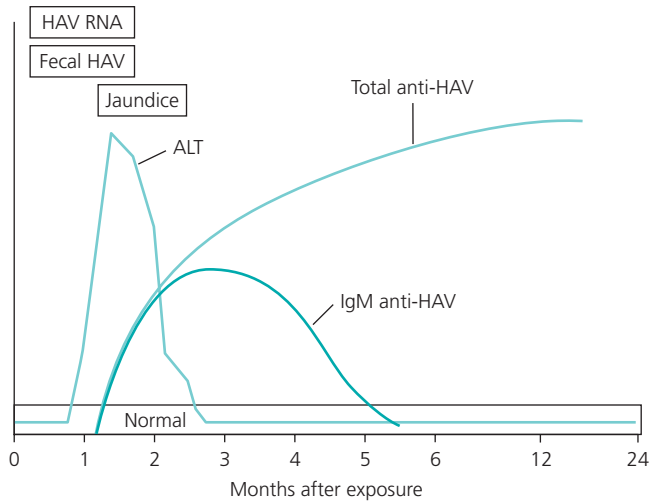

Figure 80.10 Typical serological course of acute hepatitis A virus (HAV) infection. ALT, alanine aminotransferase; IgM, immunoglobin M.

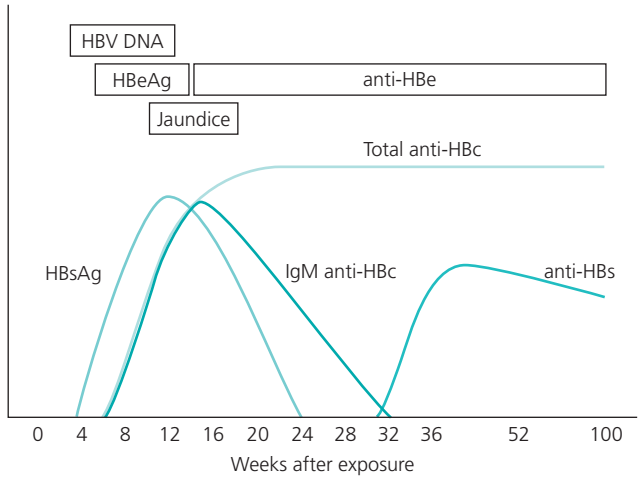

Figure 80.11 Typical serological course of acute hepatitis B virus (HBV) infection. HBeAg, hepatitis Be antigen; HBsAg, hepatitis surface antigen; IgM, immunoglobulin M.

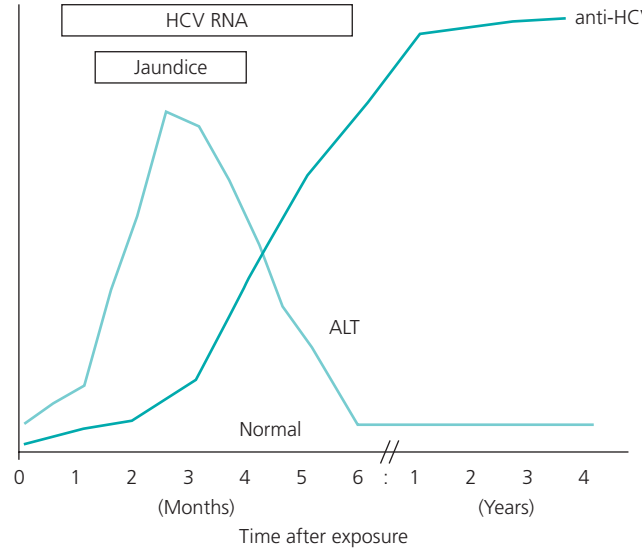

Figure 80.12 Typical serological course of acute hepatitis C virus (HCV) infection. ALT, alanine aminotransferase.

which is the marker of recovery. Anti-HBc IgG type persists after recovery and is a useful marker for prevalence studies of past exposure to HBV [225]. It should not be used to diagnose acute or chronic infection. HBsAg and anti-HBc IgM are the most frequent markers present during the symptomatic phase of the illness [152,226]. Rarely during acute HBV infection, HBsAg is rapidly cleared from serum, and anti-HBc IgM may be the only marker detectable; this particular scenario is referred to as the "window" period. This situation may also be seen during the convalescent phase of the illness when HBsAg and aminotransferase levels are declining and anti-HBs is not yet detectable. Given the sensitivity of current assays for HBsAg, this window period is rarely observed in clinical practice. Diagnosis of acute hepatitis B rests on the demonstration of anti-HBV IgM and HBsAg and negative tests for anti-HAV and anti-HCV. HBV DNA is present during the incubation and symptomatic phases of infection. Like HBeAg, it correlates with disease activity and infectivity. Current PCR-based assays have a cutoff of 100 virions/mL blood, but routine testing for HBV DNA is not recommended unless the patient is immunocompromised [227]. Persistently high levels of HBV DNA following resolution of hepatitis may be indicative of a failure to control the infection and risk for chronic infection.

Acute hepatitis C infection is difficult to recognize because fewer than 20% of cases present with jaundice [228]. Anti-HCV can be detected as early as 4 weeks after acute infection and in the majority of cases remains detectable lifelong [229,230] (Fig. 80.12). The diagnosis of acute hepatitis C infection should be based on a history of exposure, absence of previous liver disease, negative tests for other hepatitis viruses, and a positive test for anti-HCV and HCV RNA [231]. HCV RNA is detectable within 2 weeks of established infection and is also used to confirm acute infection [232]. However, routine HCV RNA testing for screening is not advocated because there is no standardization of the test. It is recommended in the investigation of immunocompromised patients who may have a low or absent antibody response.

Acute delta hepatitis may occur in two settings: simultaneously with HBV (coinfection) or after infection of a chronic hepatitis B carrier (superinfection). The serological pattern differs in each case. During coinfection, markers of HBV are usually detected first, followed later by markers for HDV. The presence of anti-HBc IgM is an important finding of HDV coinfection and a discriminating marker for distinguishing HDV coinfection from superinfection. Early in the course of coinfection, HDAg is not detectable unless the hepatitis is severe, but within 1–2 weeks, antibody to hepatitis D (anti-HDV) IgM should be detectable (Fig. 80.13). Anti-HDV IgG is usually delayed for several weeks after the onset and in some cases is present only transiently during the convalescent phase. The late and poor antibody response in acute delta coinfection makes the diagnosis difficult. It is advisable to perform repeat testing for anti-HDV IgM to confirm the HDV coinfection. HDV RNA can be detected early in the course of coinfection, but commercial assays are not available. The diagnosis of acute delta coinfection depends on the detection of anti-HDV IgM, HBsAg, and anti-HBc IgM.

Acute delta superinfection occurs in the setting of chronic hepatitis B. HDAg and HDV RNA can be detected early in the course of infection by experimental assays. In contrast to HDV coinfection, anti-HDV IgM and IgG are both present early during the symptomatic phase of acute delta superinfection (Fig. 80.14). Anti-HBc IgM is usually absent or present in low titers. Diagnosis of acute delta superinfection thus rests on the detection of anti-HDV and HBsAg and absence of anti-HBc IgM.

Diagnostic tests for antibody to HEV (anti-HEV) are available although none are approved by the US Food and Drug

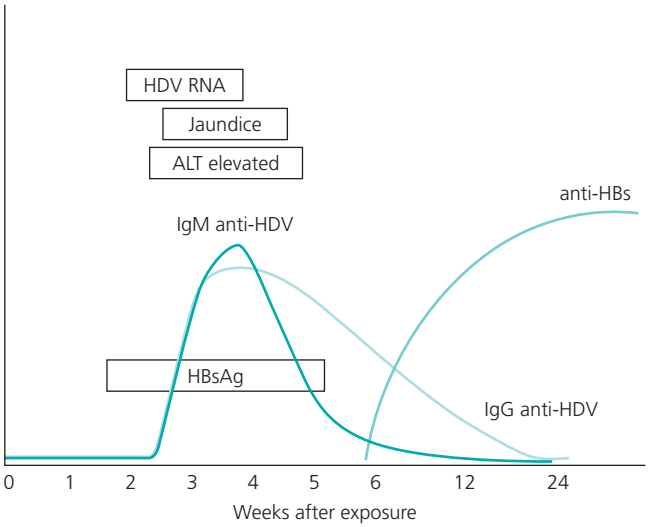

Figure 80.13 Typical serological course of acute hepatitis D virus (HDV) coinfection. ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; IgM, immunoglobulin M.

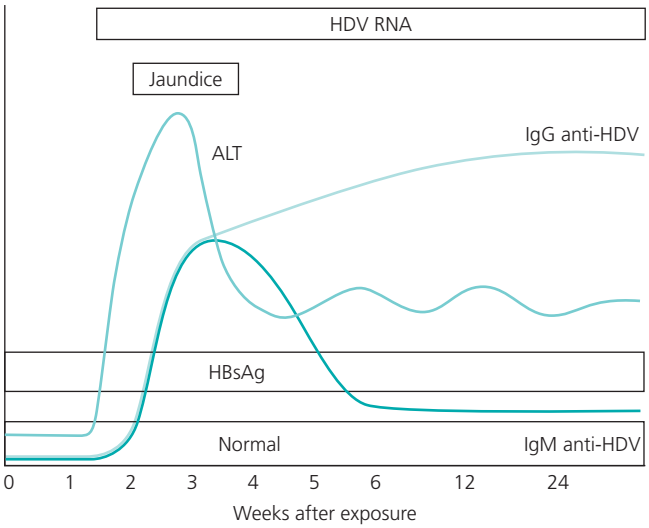

Figure 80.14 Typical serological course of acute hepatitis D virus (HDV) superinfection. ALT, alanine aminotransferase; HBsAg, hepatitis B surface antigen; IgG, immunoglobulin G; IgM, immunoglobulin M.

Administration. These tests have performed well in detecting anti-HEV in sera that are known to be positive. However, when used in panels of blood donor sera, these tests provide discordant results making them unreliable for clinical use [233]. In outbreak settings caused by HEV, anti-HEV IgM is detectable in more than 90% of patients' sera during the symptomatic phase and remains detectable for a period of 2 months after the onset of illness (Fig. 80.15). Anti-HEV IgG persists after recovery and appears to be protective against reinfection, at least in the short-term. In the appropriate setting, diagnosis of acute hepatitis E is confirmed by the demonstration of anti-HEV IgM.

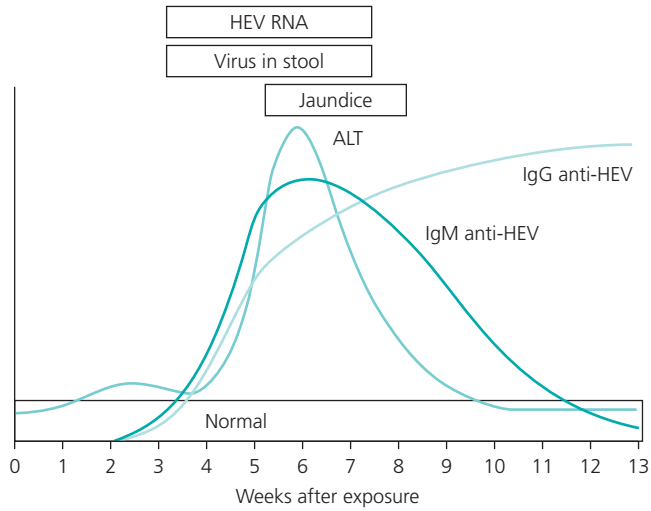

Figure 80.15 Typical serological course of acute hepatitis E virus (HEV) infection. ALT, alanine aminotransferase; IgG, immunoglobulin G; IgM, immunoglobulin M.

Laboratory features

The laboratory hallmark of acute viral hepatitis is an elevation in the serum aminotransferase levels [234,235]. The serum ALT level is more specific for hepatocyte necrosis and is usually higher than the serum aspartate aminotransferase level. During an acute hepatitis, levels range from 10 times the upper limit of normal to more than 20 times the upper limit of normal and they peak with the onset of jaundice. The height of the ALT or aspartate aminotransferase elevation usually correlates with the degree of hepatocyte destruction but not with clinical outcome. The serum alkaline phosphatase level, a marker of biliary epithelial cell damage, may rise to two to three times the upper limit of normal but in most cases remains in the normal range. The conjugated and unconjugated serum bilirubin fractions are usually elevated. The level is dependent on the extent of hepatocyte damage but typically ranges from 85 to 340 µmol/L (5–20 mg/dL). In fulminant cases, laboratory markers of hepatic function become markedly abnormal: the prothrombin time prolongs, serum albumin falls, and higher bilirubin levels are seen. These are ominous signs that indicate impending hepatic failure and should prompt early patient referral to a liver transplantation center. During the acute phase of the illness, leukopenia with neutropenia and lymphopenia may be observed. Rarely, aplastic anemia may complicate acute viral hepatitis.

Histology

A liver biopsy can distinguish acute from chronic hepatitis and viral from nonviral causes but is rarely necessary to establish the diagnosis. Regardless of the etiology, acute viral hepatitis has certain characteristic findings.

There is an acute inflammatory response involving the entire liver [236,237]. Hepatocyte damage is greatest near the

terminal hepatic venules (zone 3). Here, the classic histological features of acute hepatitis are observed. The hepatocytes appear swollen (ballooning change). Ballooned hepatocytes have a granular, pale-staining cytoplasm with enlarged nuclei and are occasionally multinucleated. Numerous perivenular hepatocytes undergo acidophil change as a result of apoptosis. Their cell cytoplasm becomes densely stained and irregular in shape with pyknotic nuclei. These cells are thought to be the precursor of the acidophil body (Councilman body). The acidophil body is not specific for viral hepatitis but is a characteristic feature when present in abundance. The remaining hepatocytes show regenerative hyperplasia. The combination of cell swelling, cell shrinkage, apoptotic body formation, and cell loss gives rise to disruption of the liver cell plate pattern or lobular disarray [238]. When only a few hepatocytes are involved, the damage is designated *focal necrosis*. When groups of adjacent hepatocytes are involved, it is termed *confluent necrosis*.

Inflammatory cells are present within the hepatic lobule, among the damaged cells. Lymphocytes and macrophages are the major cell types. Plasma cells, neutrophils, and eosinophils are present to varying extent. Kupffer cell hyperplasia is recognizable. The mononuclear cell infiltrate also involves the portal tract. The inflammatory infiltrate may spill out of the portal tract, but this should not be confused with piecemeal or interface necrosis, a feature of chronic viral hepatitis. Surprisingly, despite the extensive destruction of liver tissue, the reticulin framework of the liver remains remarkably preserved. Cholestasis may or may not be present. Bile ductular proliferation may be seen but is not a prominent feature. Mild fatty change is seen more frequently with HCV infection. Liver fibrosis is generally absent but may be seen if the biopsy is performed late in the course of the illness.

In some cases, the confluent necrosis is severe enough to involve the entire central zone of the acinus. The necrotic cells are replaced by the condensed reticulin framework and inflammatory cells, predominantly macrophages forming central-to-portal bridging necrosis. This entity may be the precursor to fulminant hepatitis, and in one series, its presence was associated with a poorer outcome [239]. In extreme cases, there may be involvement of all zones of the acinus, referred to as *massive necrosis* with total loss of hepatocytes. The parenchyma is replaced by the collapsed reticulin framework and inflammatory cells. This is a manifestation of severe hepatitis seen in the clinical setting of fulminant hepatitis and coma.

The histological changes associated with acute viral hepatitis persist for several weeks after onset so a nearly normal liver biopsy 2–3 weeks after an episode of jaundice effectively rules out acute hepatitis. Late biopsy findings include a decrease in the degenerative changes, macrophages that stain intensely with periodic acid-Schiff stain, and numerous mitotic figures.

Staining the liver tissue with antibodies for hepatitis B surface and core antigens in the case of hepatitis B and delta antigen for delta hepatitis can help with the identification of the etiological agent of the hepatitis. Staining for hepatitis C antigen in liver tissue is not reliable [240,241].

Differential diagnosis

An array of diseases may mimic acute viral hepatitis and should always be considered in the differential diagnosis while awaiting the results of serological testing. As with any acute medical illnesses, more rapid diagnosis permits the prompt institution of specific therapy that may prevent an adverse outcome. The differential diagnosis of acute viral hepatitis is summarized in Table 80.3.

The prevalence of drug-induced liver disease is probably underestimated in clinical practice. Drug-induced liver disease should be considered in every case of acute hepatitis until the cause is firmly established. A wide variety of over-the-counter drugs are associated with hepatic toxicity, which may result in a clinical picture indistinguishable from acute viral hepatitis. Therefore, it is mandatory to obtain a detailed drug history from every patient who presents with acute hepatitis. Clinical clues to the etiology of the acute hepatitis are the timing of the rise in serum aminotransferase in relation to the onset of jaundice and presence of a serum eosinophilia. In drug-induced hepatitis, the jaundice tends to lag behind the rise in aminotransferases, whereas in viral hepatitis, the serum aminotransferase level tends to peak with the onset of jaundice. The concomitant finding of a serum eosinophilia may be suggestive of a drug-induced hepatitis.

Autoimmune hepatitis may present as an acute hepatitis in up to 30% of cases [242,243]. The typical presentation is that of a young woman with a high titer of antinuclear antibody and smooth muscle antibody or anti-LKM (liver/kidney/microsome antibody) and other clinical evidence of autoimmune disorders such as arthralgias and autoimmune hemolytic anemia. The globulin level may not be elevated during an acute presentation. Testing for the HLA markers A1, B8, and DR3 may provide substantiating evidence for confirming the diagnosis of autoimmune hepatitis.

Wilson disease is "the great imitator" and has diverse presentations, including that of acute hepatitis [244]. The diagnosis is difficult to confirm during an acute presentation and requires a high index of suspicion. It is important to establish the diagnosis early because of an extremely high mortality rate. In a recent series of fulminant hepatitis, no patients with Wilson disease referred to a transplantation center survived despite undergoing OLT [245]. The usual diagnostic criteria may be normal during an acute presentation. Ceruloplasmin level may be normal because of widespread release from necrosed hepatocytes, and urinary copper excretion may be low owing to altered renal clearance. Diagnostic clues may be the young age of the patient, a low alkaline phosphatase level, and the presence of a hemolytic anemia.

The liver may be an innocent bystander in infections that have multisystem involvement. Yellow fever, malaria, and Q

Table 80.3 Acute viral hepatitis: differential diagnosis, diagnostic testing and specific therapy

Condition	Diagnostic clue or test	Specific treatment
Drug toxicity, e.g., isoniazid, acetaminophen, phenelzine, halothane	Complete drug history including over-the-counter and complementary alternative medications. Acetaminophen level > 10 mg/dL	Withdraw offending agent. *N*-Acetylcysteine for acetaminophen toxicity[a]
Autoimmune hepatitis	ANA, SMA	Prednisone 60 mg/day
Wilson disease	Serum ceruloplasmin, 24-h urinary copper	D-Penicillamine (if diagnosed early)
Ischemic liver injury	Clinical evidence of hypotension	Correct hypotension
Toxic injury, e.g., *Amanita phalloides* poisoning	History of mushroom ingestion	None
Infections (non-A–E hepatitis), e.g., Q fever, yellow fever, malaria	Culture and serology, history of travel to endemic area	Treat specific infection
Choledocholithiasis	Ultrasound, cholestatic laboratory features	Antibiotics and drainage
Special hosts		
Bone marrow transplant recipients		
Acute graft-versus-host disease	Liver biopsy	Prednisone
Venoocclusive disease	Liver biopsy	None
Adenovirus, CMV, HSV, EBV	Serology and culture	Acyclovir for HSV and EBV; gangcyclovir for CMV; cidofivir for adenovirus
Pregnancy		
Acute fatty liver	Third trimester	Delivery
HELLP syndrome	Evidence of preeclampsia, thrombocytopenia, hemolysis	Delivery

a *N*-Acetylcysteine is administered orally in a loading dose of 140 mg/kg followed by 70 mg/kg every 4 h for 15–20 doses.
ANA, antinuclear antibody; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HELLP, *h*emolysis, elevated *l*iver enzymes, and *l*ow *p*latelets; HSV, herpes simplex virus; SMA, smooth muscle antibody.

fever may involve the liver and cause a hepatitis-like picture. Epstein–Barr virus (EBV), cytomegalovirus (CMV), herpesviruses, and adenoviruses may cause severe hepatitis in immunocompromised patients and in patients at the extremes of age. These agents should be suspected when routine serological testing is negative in an immunocompromised patient or when a history of recent travel to an endemic area is obtained from the patient.

Acute cardiovascular failure with hypotension from pump failure or sepsis can result in ischemic injury to the liver, so-called shock liver. In this setting, there can be massive hepatocyte destruction with serum aminotransferase levels more than 20 times the upper limit of normal, mimicking the presentation of an acute viral hepatitis. Bilirubin levels usually are only mildly elevated despite the degree of aminotransferase elevation. The history of hypotension and aminotransferase levels that rapidly normalize should establish the diagnosis.

Given the vast number of metabolic functions carried out by the hepatocyte, it is surprising that toxic injury is not seen more commonly. Mushroom poisoning from ingestion of *Amanita phalloides* and exposure to carbon tetrachloride are the best examples of toxic injury leading to markedly elevated aminotransferase levels. The diagnosis depends on a careful medical history indicating ingestion or exposure.

Rarely, biliary tract obstruction (choledocholithiasis or acute cholecystitis) can be confused with acute hepatitis [246]. Usually, other clinical features are present to suggest the diagnosis, such as an elevated serum alkaline phosphatase level and more intense right upper quadrant pain.

Finally, in special populations, such as bone marrow transplant recipients, unique diseases should be considered during the investigation of an acute rise in serum aminotransferase levels. These include acute graft-versus-host disease, which typically occurs within the first 28 days after bone marrow transplantation, and venoocclusive disease, which occurs within the first 2–3 weeks [247–249]. In pregnancy, acute fatty liver and the HELLP syndrome (*h*emolysis, *e*levated *l*iver enzymes, and *l*ow *p*latelets) should be considered [250,251].

Course and complications

Course

Most cases of acute hepatitis are uncomplicated and resolve without sequelae. This is the expected outcome after infection with HAV and HEV in 99% of instances. Between 1% and 2% of cases are complicated by a fulminant course associated with a high mortality rate. Infection with HBV, HCV, and HDV may progress to a chronic hepatitis in 10%–90% of cases depending on the infecting virus, age at exposure, and immune status of the host. Chronic hepatitis is defined as persistent elevation in the aminotransferase levels 6 months

after the onset of infection and detection of specific serological or virological markers. It is best assessed by liver biopsy. Occasionally, acute hepatitis may have a prolonged course over 4 months or even up to 1 year, the best example of which is the prolonged course of HAV infection. This variant presentation needs to be differentiated from chronic hepatitis, and the most appropriate means of doing so is watchful waiting until the aminotransferase levels return to normal.

The prolonged form of HAV infection is characterized by pruritus, fever, diarrhea, and weight loss with serum bilirubin levels greater than 10 mg/dL and a clinical course lasting a minimum of 12 weeks [252,253]. It is seen more commonly in males and older individuals. Importantly, all patients recover without lasting sequelae. Some patients with this variant of hepatitis A may benefit from corticosteroid therapy [253].

In addition to the prolonged variant of HAV infection, a relapsing form is seen in up to 20% of HAV cases, distinguishable from the protracted form by its biphasic presentation [254]. Patients initially present with an acute hepatitis followed by apparent recovery only to have a recrudescence of hepatitis 5–10 weeks later. The second phase tends to be associated with higher bilirubin levels and to have a more prolonged course compared with the first phase [255]. Steroid therapy during the early phase of the illness and alcohol consumption have been identified as potential risk factors for relapsing HAV infection [254].

Despite these complications, complete resolution is the rule. Serological evidence of HAV IgM in serum during recrudescence of the illness and detection of HAV in stool during relapse are helpful tests for excluding other cause of hepatitis. The pathophysiology of these atypical presentations is unknown. A recent study in children reported an association between the protracted form of hepatitis A and HLA-DRB1*1301, a marker for pediatric autoimmune hepatitis [256]. Case reports have noted that acute hepatitis A infection may trigger the onset of autoimmune hepatitis in adults [257,258].

Other atypical courses of viral hepatitis have been described, which sometimes lead to diagnostic confusion. A biphasic pattern of hepatitis may also be observed after hepatitis D coinfection, although cholestasis is not a feature. The first peak is related to HBV infection and the second to HDV infection. On occasion, HBsAg-negative delta hepatitis is encountered during acute infection as the result of suppression of HBV replication by HDV [259]. This presentation can be perplexing for the clinician, and repeat testing for anti-HBc IgM and markers of HDV infection is recommended in suspected cases. Flares of chronic hepatitis B or reactivation are well recognized in association with chemotherapy or immunosuppression. It is important to distinguish reactivation from acute infection or nonviral causes of hepatitis because the management is different. The diagnosis of reactivation can be tricky. In most cases, the clinical picture is recognizable by elevated aminotransferase levels with detectable HBsAg and absent anti-HBV IgM in serum. However, anti-HBV IgM may be detectable in 25% of cases of reactivation, leading to confusion with acute hepatitis B. The only way to resolve this dilemma is to obtain a history from the patient of previous HBV infection or to perform a liver biopsy, which should demonstrate changes of both acute and chronic hepatitis in the case of reactivation.

Complications

Fulminant hepatitis is a complication of acute hepatitis characterized by a rapid progression to hepatic failure with associated hepatic encephalopathy. Only 1%–2% of all cases of acute viral hepatitis result in fulminant hepatitis. Fulminant hepatitis has been reported with all of the hepatotrophic viruses [260–263]. It has an extremely high mortality rate; fortunately survival has improved with improved intensive care units and with the widespread availability of OLT [264].

Risk factors for fulminant hepatitis A include older age at infection and underlying chronic hepatitis C [265,266]. The risk of fulminant hepatitis A with underlying chronic hepatitis C has not been substantiated by other studies [267,268].

Epidemics of fulminant hepatitis B have been reported with variant strains of the virus. The precore mutant, which is defective in HBeAg synthesis, has been reported to cause outbreaks of fulminant hepatitis [269–272]. However, other studies did not report a causal relationship of this mutation with fulminant hepatitis B [273–275]. This mutation was also found to arise de novo in patients with chronic HBV infection who did not develop fulminant hepatitis B, suggesting that other factors probably play a role in this multifactorial process [276]. Fulminant hepatitis B has also been reported with emergence of the antiviral drug-resistant virus, for example the rtM204V/I variant under lamivudine therapy [277,278].

Fulminant hepatitis may occur more frequently with HDV coinfection [279,280]. In addition, periodic epidemics of a severe fulminant form of delta hepatitis have been described in the Amazon Basin, referred to locally as LaBrea hepatitis [281]. This finding may be related to the particular genotype of HDV, genotype 3 [282].

Fulminant hepatitis E occurs at surprisingly high rates of 15%–25% in pregnant women during the third trimester [283,284]. The reason for this is unknown. Survival is poor.

A hepatitis-associated aplastic anemia and pure red cell aplasia may complicate acute hepatitis and is associated with a high mortality rate [285–287]. It appears to be more common in the Far East and in adolescent boys and young men [288].

Chronic hepatitis is a complication seen only with HBV, HCV, and HDV. Rates of chronic infection depend on immune status of the host and age at time of infection and vary from 10% to 90%. The causes of chronicity are unknown but undoubtedly involve a failure of the cellular immune response.

Other long-term complications of acute viral hepatitis include hepatocellular carcinoma, especially in the case of HBV and HCV infection, and lymphoma associated with HCV infection.

Prevention and immunoprophylaxis

Hepatitis A virus

Infection with HAV can be prevented by three strategies: active immunization through vaccination, which has the advantage of lifelong immunity; passive immunization through the administration of antibody made from pooled human sera processed by cold ethanol fractionation; or a combination of the two strategies.

Passive immunization

Immunoglobulin provides protection against HAV infection by the passive transfer of antibody. It can be used to provide immunity before and after exposure. The recommended dose is 0.02 mL/kg of immunoglobulin administered intramuscularly for both preexposure and postexposure prophylaxis. If used for preexposure prophylaxis, a dose of 0.02 mL/kg provides protection for less than 3 months. A higher dose of 0.06 mL/kg will provide protection for about 5 months. For postexposure prophylaxis, administering immunoglobulin within 2 weeks of the exposure is more than 85% effective [289]. Later administration is ineffective in preventing infection but will attenuate the course of disease [290]. Serious adverse events are rare after administration of immunoglobulin, but anaphylaxis has been reported after repeat administration to patients with known IgA deficiency [291]. It is safe to administer during pregnancy or breastfeeding and can be coadministered with inactivated vaccines, oral polio vaccine, and yellow fever vaccine [292]. Immunoglobulin can interfere with the immune response to other live attenuated vaccines, and these vaccines should be postponed for about 2 weeks [292].

Active immunization

Two commercially inactivated hepatitis A vaccines are available in the United States: HAVRIX and VAQTA. Both vaccines are highly immunogenic in adults, adolescents, and children, with 94%–100% achieving protective levels of antibody 1 month after a single dose of vaccine [293]. The lower limit of antibody required to prevent HAV infection is unknown, but in vitro studies indicate that levels of less than 20 mIU/mL can be neutralizing [293,294].

The efficacy of both vaccines was demonstrated in clinical trials. HAVRIX was shown in a large double-blind randomized controlled trial to have an efficacy of 94% after two doses 1 month apart [295]. Similarly, VAQTA was evaluated in a double-blind, placebo-controlled, randomized trial and had an efficacy of 100% after one dose of vaccine [296]. The vaccine has not been evaluated in the postexposure setting. A small randomized trial showed that hepatitis A vaccine was 79% efficacious in preventing HAV IgM positivity after household exposure to hepatitis A compared with no treatment [297]. Hepatitis A vaccine has not been directly compared to immunoglobulin in postexposure prophylaxis.

Table 80.4 People for whom hepatitis A vaccine is recommended

People traveling to or working in countries that have high or intermediate endemicity of infection
Men who have sex with men
Illegal drug users
People who have occupational risk factors for infection
People who have clotting factor disorders

In studies, the vaccine appears to confer long-term protection against infection, with follow-up ranging from 6 to 7 years [298,299]. Hepatitis A vaccination provides preexposure protection against HAV infection, and it is recommended for people who are at increased risk and for anyone wishing to obtain immunity. Individuals who are at increased risk for hepatitis A infection and who should be routinely vaccinated are summarized in Table 80.4. Both vaccines appear to be safe. In Europe, Asia, and the United States, about 8 million doses of hepatitis A vaccine have been administered, and serious events, anaphylaxis, Guillain–Barré syndrome, brachial plexus neuropathy, transverse myelitis, multiple sclerosis, encephalopathy, and erythema multiforme, although rare, have been reported [295].

The hepatitis A vaccine can be safely coadministered with the hepatitis B vaccine without affecting the immunogenicity of either vaccine or increasing the frequency of side effects [300]. Safety has not been evaluated in pregnancy, but because the vaccine is inactivated, the risk to the fetus is expected to be low. Similarly, no special precautions are required when vaccinating immunocompromised patients [301]. The vaccine is safe and effective when administered to patients with chronic liver disease [302,303]. Response to HAV vaccination in HIV-positive men is variable but appears to be reduced in those with low CD4 counts compared to HIV-negative men. Rates of anti-HAV range from 47% to 88% in HIV-positive individuals [304]. Antibody titers were also noted to be lower in those with HIV infection [305]. Prevaccination serological testing for susceptibility is not recommended because of the additional costs and because vaccination poses no risk to a person with a history of hepatitis A infection. Postvaccination testing is not recommended because of the high rate of response. A combined hepatitis A and B vaccine (HEPAB, Twinrix) is commercially available and appears to be safe and effective [306].

Hepatitis B virus

The goals of vaccination against hepatitis B are similar to those for hepatitis A: to prevent infection in susceptible individuals and to prevent spread of infection and chronic liver disease. Additionally, vaccination against hepatitis B can prevent infection with delta virus and prevent hepatocellular carcinoma related to HBV infection. HBV infection can be prevented by passive or active immunization or a combination of the two.

Passive immunization

Hepatitis B immunoglobulin (HBIG) is prepared from plasma that contains high-titer anti-HBs. In the United States, HBIG has an antibody titer of more than 100 000 IU/mL by radioimmunoassay. HBIG is used in conjunction with hepatitis B vaccination for postexposure prophylaxis to prevent transmission of HBV from an HBsAg-positive mother to her child or alone to prevent recurrence of HBV infection after OLT.

Active immunization

Two yeast-derived recombinant hepatitis B vaccines are available in the United States. A plasma-derived vaccine that was licensed for use in the United States in 1982 is no longer available. The vaccine is part of the routine immunization program in many countries and is effective in reducing the incidence of HBV infection in endemic areas of the world.

The recommended schedule is a series of three intramuscular doses of hepatitis B vaccine into the deltoid muscle of adults and anterolateral thigh of neonates and infants. The recommended dosing schedule induces an antibody response (anti-HBs titer higher than 10 mIU/mL) in more than 90% of adults and more than 95% of children and adolescents [307,308]. Increasing the interval between the first and second doses of HBV vaccination has little effect on immunogenicity or final antibody titer. The third dose confers optimal protection. Longer intervals between the second and third doses result in higher final titers of anti-HBs [309]. Administration of hepatitis B vaccine does not interfere with the antibody response to other vaccines [310]. The immune response is comparable to a standard course of vaccination if the product of one manufacturer is used with that of another. Hemodialysis patients usually require a higher dose or a greater number of vaccinations to achieve an adequate immune response [311,312]. Response rates among HIV-positive individuals were reported to be lower when compared to HIV-negative individuals; rates ranged from 33% to 56% and were related to CD4 count [304]. In one study, doubling the dose of HBV vaccine increased the response rate from 34% to 47% but this strategy was only effective in those with CD4 counts greater than 300 cells/mm^3 [313]. This probably also applies to immunocompromised individuals but has not been confirmed.

Clinical trials in the United States and other countries have shown that the hepatitis B vaccine is 80%–95% effective in preventing HBV infection in susceptible people [308,314–316]. If a protective antibody response is elicited, then the vaccine is 100% effective. Factors associated with nonresponse are a genetic predisposition, immunosuppression and certain chronic illnesses, age over 30 years, HIV infection, obesity, alcoholism, and male sex [317]. Studies suggest that even patients who fail to develop an adequate immune response to the vaccine (anti-HBs titer less than 10 mIU/mL) are probably protected against infection. Likewise, individuals who lose detectable antibody are probably protected because of lasting immunological memory. The vaccine appears to afford long-term protection. Studies in which the plasma-derived vaccine was used indicate that 13%–60% of patients may lose antibody after 9 years of follow-up [318–320]. A recent study from Taiwan reported a decrease in anti-HBs titers to below 10 mIU/ml in 29% of children vaccinated with the plasma-derived vaccine 15 years earlier [321]. Surprisingly anti-HBc was detected in 33% of these children but HBsAg, the marker of chronic infection, was found in only one child. In a similar long-term follow-up study from Alaska, 1578 native Alaskans vaccinated with three doses of the plasma-derived vaccine were tested after 15 years or longer [322]. The geometric mean concentration of anti-HBs decreased from 822 IU/L (measured 6 months after the third dose) to 27 IU/l after 15 years. Sixteen asymptomatic breakthroughs and eight possible breakthroughs were observed. Factors associated with higher titers of anti-HBs after 15 years included high initial serum anti-HBs level, male sex and vaccination at an older age [322].

The efficacy of the hepatitis B vaccine to prevent transmission of HBV was shown in several studies after the introduction of vaccine programs in endemic regions. Before vaccination, the carrier rate of HBV infection in Taiwan was estimated to be 15%–20%. A program of universal vaccination was initiated in 1984. This program was highly effective in reducing the rate of chronic HBV infection. Over the next decade, the rate of chronic HBV infection fell from 10.5% to 1.7% in children aged 6 years, and the annual incidence of hepatocellular carcinoma fell from 0.7 per 100 000 to 0.36 per 100 000 [323–325]. These findings were confirmed by other studies from other endemic regions [326].

Vaccination is not contraindicated in pregnant women or in those who are breast-feeding. Postvaccination testing is not recommended after routine vaccination of children or adolescents. Testing for immunity is advised only for people whose subsequent management depends on their immune status, such as infants born to HBsAg-positive mothers, dialysis patients and staff, and patients with HIV infection [320]. Booster doses are not recommended for vaccinated individuals, nor is routine serological testing recommended to determine immune status, unless a person was exposed to

the virus. Revaccination of nonresponders to a primary course of vaccine should be considered because 15%–25% develop an adequate antibody response after one dose and 30%–50% after three doses of vaccine [318]. For patients undergoing hemodialysis, vaccine-induced immunity may wane, and patients should probably undergo testing for antibody levels annually and receive a booster dose if anti-HBs levels fall below 10 mIU/mL [320].

Pain and fever are the most common side effects associated with HBV vaccination; however, in placebo-controlled trials, these side effects occurred no more frequently than with placebo [307,308,314,316]. Over 1 billion doses have been administered worldwide, and serious adverse events occur rarely [24]. A possible association between the plasma-derived vaccine and Guillain–Barré syndrome and demyelinating diseases has been reported [327]. Two recent studies failed to confirm such an association between hepatitis B vaccine and multiple sclerosis [328,329]. In addition, there are large differences in the geographic prevalence of HBV and the incidence of multiple sclerosis. Furthermore, analysis of postmarketing studies in the United States showed no increase in multiple sclerosis after hepatitis B vaccination, and reanalysis of the French data by the Viral Hepatitis Prevention Board and World Health Organization concluded that there was no association between hepatitis B vaccination and multiple sclerosis [330].

Variant viruses, with "escape mutations" in the immunodominant "a" determinant region of the HBsAg gene after vaccination, have been described. These variants have been reported worldwide. Therefore, a theoretical concern of hepatitis B vaccination is the replacement of wild-type virus by the variant strain to which vaccinated individuals in the community will be susceptible. A report from the Taiwan vaccination program described an increased prevalence of vaccine escape variants from 8% to 28% over a 10-year period, lending credence to this concern [331]. However, this finding has not been confirmed in other studies [332,333]. Furthermore, chimpanzees vaccinated with commercial hepatitis B vaccines were protected from infection after challenge with an infectious strain of the variant virus [334].

Hepatitis B vaccination is recommended for the prevention of perinatal HBV infection, for infants born to HBsAg-positive mothers, and as part of the universal vaccination of all preschool-aged children [320]. Individuals who are at high risk for acquiring HBV infection should receive hepatitis B vaccination and are listed in Table 80.5. The recommendations for use of the hepatitis B vaccine in the postexposure setting are outlined in Table 80.6. These recommendations have been updated. The major changes were to improve prevention of perinatal and early childhood HBV transmission and to improve vaccine coverage of children and adolescents who were not primarily vaccinated. Readers are referred to the following reference for a complete list of all the updated recommendations [335].

Table 80.5 People for whom hepatitis B vaccine is recommended

People with occupational risk
Clients and staff of institutions for the developmentally disabled
Hemodialysis patients
Recipients of clotting factor concentrates
Household contacts and sex partners of HBV carriers
Adoptees from countries where HBV infection is endemic
International travelers
Injection drug users
Sexually active men and women
Inmates of long-term correctional facilities

HBV, hepatitis B virus.

Table 80.6 Recommendations for postexposure immunoprophylaxis for hepatitis B

Exposure	Recommendation
Perinatal	Vaccination + HBIG
Sexual (acute case)	HBIG +/– vaccination
Sexual (chronic carrier)	Vaccination
Household (acute case)	None
Household (acute case, known exposure)	HBIG +/– vaccination
Household contact (chronic carrier)	Vaccination
Inadvertent percutaneous/permucosal	Vaccination +/– HBIG

HBIG, hepatitis B immunoglobulin.

Hepatitis C virus

No effective vaccine is available to prevent infection with HCV. This is primarily the result of a lack of neutralizing antibody, the high mutation rate of the viral genome, existence of viral quasispecies, and a lack of knowledge about protective immunity. Protection against infection after challenge with a homologous strain has been demonstrated in chimpanzees using hyperimmune serum against region 1 of the E2 protein but not against heterologous strains [336–338]. Studies in chimpanzees using a DNA vaccine encoding the E2 protein failed to protect against infection with a homologous strain of virus but may have attenuated the course of infection and prevented chronicity [339]. The recent discovery of natural immunity to HCV and the results of several vaccine studies in chimpanzees hold promise for the development of an HCV vaccine. Current strategies have been more successful in preventing acute infection from progressing to chronic infection rather than preventing acute infection. However, a recent study reported that a recombinant DNA vaccine encoding the nonstructural region of HCV in an adenovirus vector was able to elicit effective immunity against heterologous virus challenge in chimpanzees [340]. Four of five chimpanzees inoculated with 100 chimpanzee 50% infec-

tious doses (CID_{50}) were protected against acute hepatitis after infection with a heterologous virus which differed from the vaccine sequence by more than 13% at the amino acid level compared to none of five control chimpanzees inoculated with a control adenovirus and plasmid DNA vector encoding the HIV gag antigen [340].

Studies in the late 1970s showed that use of immunoglobulin failed to prevent non-A, non-B hepatitis (HCV) [341,342]. Intravenous infusions of immunoglobulin may attenuate the severity of non-A, non-B hepatitis (HCV) [343,344]. It was demonstrated that neutralizing antibodies to HCV are present in immunoglobulins derived from anti-HCV positive plasma [345]. Enrichment of such plasma containing broadly reactive neutralizing antibodies may provide a strategy for preventing HCV infection. Until further studies are carried out, use of immunoglobulin as postexposure prophylaxis for HCV infection cannot be recommended.

Hepatitis D virus

The dependence of HDV replication on HBV means that the same preventative and postexposure measures used to prevent HBV infection can be employed to prevent hepatitis D coinfection. Risk behavior modification is the only current measure to prevent HDV superinfection.

Hepatitis E virus

A recombinant vaccine for HEV is being evaluated in Nepal. Immunoglobulin has not been evaluated for postexposure prophylaxis.

Needlestick injury

Transmission of bloodborne pathogens through needlestick injury is an important issue for health-care workers. It is a significant contributor to job-related injuries and emotional stress for health-care workers. Each year, about 600 000–800 000 needlestick injuries occur in the United States. Infection with HBV, HCV, or HIV constitutes the major risk for the health-care worker after needlestick injury.

The risk for HBV infection is primarily related to the degree of contact with blood in the work environment and to the HBeAg status of the source. In studies of nosocomial transmission of HBV through needlestick injury, the risk for clinical hepatitis if the source was positive for both HBsAg and HBeAg was 22%–31%; the risk for serological evidence of HBV infection was 37%–62%. In contrast, the risk for developing clinical hepatitis from a contaminated needle from an HBsAg-positive, HBeAg-negative individual was 1%–6%, and the risk for HBV seroconversion was 23%–37% [346]. Although percutaneous exposure is an efficient mode of transmission of HBV, it accounts for a relatively small number of nosocomial infections because of increased hepatitis B vaccine use in health-care workers.

HCV is not transmitted efficiently by needlestick injury. The rate of seroconversion to anti-HCV after needlestick injury averages 1.8% per injury and ranges from 0% to 7% [347,348]. About 2.4% of new cases of HCV infection are the result of needlestick injury. A deep injury, use of a hollow-bore needle, and visible blood on the needle have been identified as risk factors for transmission of HCV [347].

Exposure prevention is the primary strategy to reduce HBV and HCV transmission by needlestick injury. Health-care workers should be educated concerning the risk for and prevention of bloodborne infections, including use of personal protective equipment and vaccination against HBV. Health-care workers were required to adopt universal precautions under regulations that were introduced in 1987 [349]. Despite these recommendations, needlestick injuries continue to occur. Regulations to prevent needlestick injury should be enforced in the workplace.

General management after a needlestick injury involves washing the wound with soap and water for a minimum of 15 min. Postexposure management should include prompt reporting, evaluation, counseling, treatment as necessary, and follow-up. The person whose blood was the source of exposure should be evaluated for evidence of HBV, HCV, and HIV infection. No special precautions are necessary to prevent secondary transmission during the follow-up period. However, exposed people should refrain from donating blood, plasma, organs, tissue, and semen. The exposed person does not need to modify sexual practices or to avoid becoming pregnant. Employers should follow all federal and state requirements for recording and reporting occupational injuries and exposures [350,351].

Specific recommendations for postexposure management are outlined by the US Public Health Service [352]. Any unvaccinated person who sustains a needlestick injury should receive the hepatitis B vaccine series. The recommendations for management of exposure based on HBsAg status of the source and vaccine status of the exposed individual are shown in Table 80.7. Both HBIG and hepatitis B vaccine can be administered simultaneously.

No specific therapy is available for prevention of HCV infection after needlestick injury. Recommendations for postexposure management are intended to achieve early identification of chronic cases and, if present, to refer to a specialist for treatment options. The source of exposure should be tested to determine anti-HCV status. Baseline anti-HCV and ALT testing should be performed on the exposed individual and repeated in 4–6 months. Testing should be extended to 12 months for HIV–HCV coinfected sources. If desired, HCV RNA testing can be performed after 4–6 weeks. All positive tests should be confirmed by testing for HCV RNA. Immunoglobulin administration is not recommended because of the absence of neutralizing antibody and the failure of immunoglobulin to prevent transmission of HCV [341,344]. Antiviral agents are not recommended for post-exposure management. Counseling should be provided to exposed people.

Table 80.7 Recommendations for postexposure prophylaxis for health-care workers following exposure to hepatitis B virus

Vaccination and antibody status of exposed health-care worker	Source and treatment		
	HBsAg-positive	**HBsAg-negative**	**Unknown/Not available for testing**
Unvaccinated	HBIG[a] × 1 and initiate hepatitis B vaccine series	Initiate hepatitis B vaccine series	Initiate hepatitis B vaccine series
Previously vaccinated			
Known responder	No treatment	No treatment	No treatment
Known nonresponder	HBIG × 1 and initiate hepatitis B vaccine series or HBIG × 2	No treatment	No treatment
Antibody response unknown	Test exposed person for anti-HBs If titer > 10 mIU/mL, no treatment is necessary If inadequate, administer HBIG × 1 and vaccine booster	No treatment	Test exposed person for anti-HBs If titer > 10 mIU/mL, no treatment is necessary If inadequate, administer vaccine booster and recheck titer in 1–2 weeks

a Dose of HBIG is 0.06 mL/kg intramuscularly.
HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

Treatment

Treatment of acute viral hepatitis is largely supportive and directed to ensuring adequate nutrition and hydration and to monitoring patients for the development of fulminant hepatitis. Drug therapy is rarely indicated for acute viral hepatitis A and E because 90%–95% of patients are expected to recover without sequelae. Therapy of acute hepatitis B has not been well studied; however, given the high recovery rate of 90%–95% in adult cases, therapy is not recommended. Based on the high response rate to interferon therapy, it may be reasonable to treat patients with clearly documented acute HCV [353].

General recommendations

Most patients with acute hepatitis can be managed outside the hospital setting as long as they can maintain adequate hydration and intake of calories. The old recommendation of bed rest is no longer advocated unless the patient experiences severe fatigue [354]. No specific dietary measure or supplement has been shown to be effective. Protein should be restricted only in patients exhibiting clinical signs of hepatic encephalopathy. During the convalescent phase, a high-protein diet may be necessary to aid in recovery. Alcohol should be avoided, and use of medications should be kept to a minimum. Drugs that are metabolized by the liver, such as benzodiazepines, should be avoided or the dose reduced if they are absolutely essential. Patients should be assessed weekly during the early phase of the illness and followed through recovery. They should be monitored for symptoms and signs of encephalopthy, such as increased somnolence, drowsiness, and asterixis and, if any of these are present, the patient should be assessed with serial trail tests. Monitoring of the serum prothrombin time is a useful marker for assessing hepatic decompensation and for deciding when to refer to a liver transplantation unit. The monitoring of serum aminotransferase levels is not helpful for assessing hepatic function because levels may return to normal in fulminant hepatic failure after massive destruction of hepatocytes. Antiemetics can be used to improve symptoms of nausea. Patients who exhibit clinical features of fulminant hepatitis should be rapidly referred to a liver transplantation unit for management. OLT may be a lifesaving procedure for patients who decompensate after acute hepatitis (see Chapter XX on fulminant hepatitis). It is not necessary to isolate patients with acute hepatitis. People caring for patients with acute HAV and HEV infection should wash their hands with soap and water. Close contacts of people with acute HBV infection should receive hepatitis B vaccine.

Specific therapy

The rationale for antiviral therapy in acute viral hepatitis is to prevent fulminant hepatitis, to ameliorate symptoms, and to prevent progression to chronic hepatitis. The indications for antiviral therapy in acute hepatitis B have not been clearly delineated. Interferon-α was compared with placebo in retrospective fashion for the treatment of acute hepatitis B. A higher rate of clearance of HBsAg and HBeAg and loss of HBV DNA were observed in the group that received interferon-α, 80% vs 54%. Therapy was well tolerated, but the concern remains that interferon therapy may worsen the hepatitis. To date, there have been no randomized, prospective trials evaluating interferon therapy for acute hepatitis B and use of this agent should be on a case-by-case basis using sound clinical judgment.

Lamivudine, a nucleoside analogue, was shown to be effective in the treatment of chronic HBV infection and may hold promise as a therapeutic agent for acute fulminant infection. This is based on the benefit determined from a few case reports in the setting of subfulminant or fulminant hepatitis B, and reports of success in treating reactivation of hepatitis B after chemotherapy and acute hepatitis B after OLT [355–358]. In one uncontrolled study 15 patients with clinically severe acute hepatitis B were treated with lamivudine 100 mg daily [359]. Lamivudine resulted in a marked improvement in clinical status in 13 patients within a mean period of 2 weeks. Encephalopathy and coagulopathy improved rapidly within 1 week and all patients were HBV-DNA-negative by PCR-based assay by 2 weeks [359]. Two patients required OLT, of whom one died. A report from Germany looking at lamivudine use before OLT in patients presenting with severe acute or fulminant hepatitis B showed similar findings with 14 of 17 patients surviving, without the need for liver transplantation, all of whom cleared HBsAg within 6 months [360]. Two patients required OLT and one patient died. Based on these uncontrolled studies it might be appropriate to consider lamivudine therapy for cases of acute hepatitis B presenting with a fulminant picture [360]. Lamivudine cannot be recommended for all cases of acute hepatitis B until more data are obtained. Studies on therapy of acute delta hepatitis are not available. Until further data are available, specific antiviral therapy is not recommended for acute HBV or HDV infection. Lamivudine should be used to prevent reactivation in patients receiving chemotherapy or high-dose immunosuppressive therapy [361,362].

Therapy for acute hepatitis C is controversial. The rationale for treatment of acute hepatitis C includes a high rate of chronicity ranging from 50% to 85%, and the relatively low response rate of 54%–56% when treatment is initiated in the chronic phase [363–365]. However, the decision to institute therapy early must be weighed against spontaneous viral clearance and the risks of therapy. Small published studies indicate that treatment of acute hepatitis C using interferon monotherapy reduces the severity of the acute hepatitis and reduces the rate of chronic infection [366–369]. The sustained loss of HCV RNA ranged from 39% to 64%, compared with 0%–20% in untreated controls. A metaanalysis of 12 controlled trials, which included 414 patients, assessed the efficacy of interferon vs no therapy for posttransfusion or sporadic acute hepatitis C [370]. The overall rate of chronicity in untreated subjects was high, ranging from 65% to 75%. Using a sustained virological response (SVR), defined as the outcome of undetectable HCV RNA 6 months after stopping therapy, interferon therapy significantly increased the SVR with a risk difference of 49% in comparison to no treatment [370]. Delaying treatment by 8–12 weeks after the onset of disease did not compromise the SVR rate. An uncontrolled study of interferon-α2b 5 MU daily for 4 weeks followed by 5 MU thrice weekly for 20 weeks was associated with a SVR in 43 of 44 patients (98%) when treatment was initiated within a mean of 89 days from the onset of infection, suggesting that early therapy was highly effective at clearing virus [353]. The argument against early treatment of acute hepatitis C is based on the fact that a substantial proportion of acute cases, 25%–30%, resolve spontaneously and that treatment during the acute phase is problematic. Two studies from Germany highlight these issues. In one study, 24 of 46 patients (52%) presenting with symptomatic, acute hepatitis C spontaneously cleared virus usually within 12 weeks after the onset of symptoms [371]. All asymptomatic patients ($n = 9$) progressed to chronic hepatitis C. It was noteworthy that delaying the start of treatment beyond 3 months did not markedly impact on SVR with a reported rate of 80% [371]. The second study treated 89 patients with acute hepatitis C using pegylated interferon-α2b 1.5 μg/kg/week for 24 weeks [372]. The SVR rate was only 71%, much lower than other studies had previously reported. Adherence to therapy was suboptimal because only 73% of patients received 80% of the dose for 80% of the prescribed duration. Psychiatric side effects were common, 27%, and one patient committed suicide [372].

Delaying therapy beyond 1 year appears to be detrimental based on the results of a Japanese study that compared early treatment within 8 weeks of onset of acute hepatitis vs delaying therapy by 1 year [373]. Delaying therapy decreased the SVR rate from 87% to 53% [373]. However, it remains to be proved whether antiviral therapy initiated during the acute stage is superior to treatment started shortly after the infection becomes chronic. Additionally, the best regimen is yet to be determined. Based on available data, a reasonable approach would be to monitor patients for a period of 8–12 weeks after the onset of hepatitis to identify those patients who will clear virus spontaneously. If patients fail to clear virus, treatment should be discussed with the patient and probably should not be delayed beyond 24 weeks after presentation. Although the optimal therapy has not been determined, it is reasonable to use monotherapy either with standard interferon 5 MU daily for 4 weeks followed by thrice weekly for 20 weeks, or pegylated interferon for 24 weeks. Long-term efficacy is not available from any of the published series.

Other viruses

Other nonhepatotrophic viruses can cause hepatitis as part of a generalized systemic infection. Members of the Herpesviridae family are covered here because of their worldwide prevalence and their special role in bone marrow transplant recipients, patients receiving chemotherapy, and post-OLT patients. The identification of other putative hepatotrophic viruses and their role in acute hepatitis are briefly discussed.

Herpes simplex virus types 1 and 2

Infection with herpes simplex virus (HSV) occurs worldwide and affects people of all ages [374]. The clinical manifestations of primary disease depend on the age and immune status of the individual. Primary infection in children tends to be a mild and subclinical illness in almost all cases. Severe infections occur more frequently in infants and immunocompromised patients. HSV-1 classically causes infection above the waist and HSV-2 below the waist. The clinical manifestations of primary disease include vesicular and ulcerative gingivostomatitis and anogenital ulceration [374]. Hepatitis caused by HSV-1 and HSV-2 infections tends to occur in the setting of generalized primary infection or reactivation. HSV hepatitis is not a major cause of non-A to non-E hepatitis [375]. Rates of fulminant hepatitis and mortality are high in infants, patients receiving chemotherapy, bone marrow transplant recipients, and women during the third trimester of pregnancy [376–378]. HSV superinfection in patients with chronic liver disease has been reported to cause fulminant hepatitis [379].

Fever and abdominal pain are the major symptoms of hepatic involvement, and patients frequently appear ill. The characteristic mucocutaneous lesions usually seen in primary infection are present in less than one-third of cases; therefore, a high index of suspicion is required to make the diagnosis. Herpes simplex should be considered in cases of fulminant hepatitis presenting in immunocompromised and sexually active individuals. Hepatomegaly may be present on clinical examination. There is often discordance between the aminotransferase levels and serum bilirubin level, with marked elevations in aminotransferases but minimal jaundice. There may be an associated leukopenia, thrombocytopenia, and coagulopathy. Liver biopsy shows nonspecific hepatocellular necrosis and a mild, scattered inflammatory infiltrate. Multinucleated hepatocytes and nuclear inclusion bodies are specific for the disease.

The diagnosis depends on positive viral cultures from body fluids and PCR detection of viral DNA from serum. Serology is not helpful because the prevalence of antibody is high in the general population. A fourfold rise in IgG titer between acute and convalescent titers is diagnostic but is not practical in acutely ill patients. Immunostaining or PCR analysis of liver tissue is rapid and specific for the diagnosis. When the diagnosis is suspected in acutely ill patients, liver biopsy for culture and immunostaining is recommended for rapid diagnosis [380]. The recently developed real-time PCR may lead to diagnosis in 3 h [381].

There have been no clinical trials evaluating therapy for acute HSV hepatitis. Acyclovir, 5–10 mg/kg, is the treatment of choice based on its success in numerous case reports [382]. Therapy should be initiated promptly while awaiting diagnostic tests because of the high mortality rate. Successful resolution has been reported with vidaribine, and it may be used as an alternative agent to acyclovir. OLT may be a lifesaving procedure [383].

Epstein–Barr virus

EBV occurs worldwide. Most cases in childhood are asymptomatic, whereas in adults, the typical symptoms of infectious mononucleosis are present: fever, malaise, headache, and cervical and axillary lymphadenopathy. The virus is lymphotrophic, and after initial infection and replication in epithelial cells of the pharynx, infects B lymphocytes. The liver is frequently involved in primary infection, but in contrast to HSV- 1 and HSV-2 infections, the hepatitis is usually mild and subclinical. Severe hepatitis has been described in immunosuppressed individuals but is uncommon. Most severe cases are the result of reactivation of latent infection.

The clinical features of EBV-related hepatitis in adolescents and adults include fever, malaise, pharyngitis, abdominal pain, and mild adenopathy. The aminotransferase level is mildly elevated and peaks during the second to fourth week of infection [384]. No pattern is specific for EBV. Occasionally, the serum alkaline phosphatase level may be markedly elevated, more than 15 times the upper limit of normal. A dissociation between the serum alkaline phosphatase and bilirubin, with an elevated alkaline phosphatase and normal serum bilirubin, has been observed. Jaundice is present in about 10% of cases and is usually mild (bilirubin less than 5 mg/dL). The coexistence of a hemolytic anemia may lead to higher than usual bilirubin levels. Most cases resolve in a few weeks, but aminotransferase levels may remain elevated for several months. Splenomegaly is frequently detected in primary infection (50%) and hepatosplenomegaly in 25% of cases. In children, a common presentation is fever, hepatomegaly, and disseminated intravascular coagulation. EBV hepatitis has been described after OLT but is infrequent and not a major cause of graft loss [385]. It may be associated with use of antilymphocyte preparations in the OLT setting. Chronic EBV hepatitis has not been reported. Cases of fulminant hepatitis occur but are rare [386].

For practical reasons, the virus is not cultured in diagnostic laboratories, and primary infection is diagnosed by demonstration of nonspecific heterophile antibody using Paul–Bunnell or slide agglutination reactions (monospot test). Heterophile antibodies may only be detected after the second week of infection and may be present for only a short time. This test has been replaced by the monospot test, which is specific and sensitive for EBV. If more specific and sensitive tests are required, immunofluorescent tests for the detection of specific antibodies to EBV proteins are available. Antiviral capsid antigen IgM and IgG antibodies are usually present at the time of onset of clinical symptoms. EBV DNA can be detected in blood but is primarily a research tool. The liver biopsy shows a mononuclear cell infiltrate involving the portal area and sinusoids with minimal hepatocyte necrosis and Kupffer cell proliferation. The liver biopsy findings may be nonspecific. To aid histological diagnosis, liver tissue can be stained for EBV nuclear antigen 1 or probed for EBV-encoded small nuclear RNA by in situ hybridization [387,388].

No controlled trials have been performed to assess treatment of EBV hepatitis because of its mild nature. Successful outcome in severe cases has been reported with acyclovir. Based on these case reports, acyclovir should be tried in severe cases of EBV hepatitis. OLT can be lifesaving when medical management fails. In the post-OLT setting, EBV hepatitis should be managed with reduction of immunosuppressive medication and judicious use of intravenous immunoglobulin [389].

Cytomegalovirus

CMV infection occurs worldwide. In cross-sectional studies, more than 50% of the population exhibited past evidence of infection with CMV [390]. Most infections are asymptomatic, especially in children. Routes of transmission are intrauterine, perinatal, and sexual through contact with blood, genital fluid, or saliva.

In infants, CMV infection can cause a neonatal hepatitis syndrome characterized by jaundice, hepatomegaly, hemolytic anemia, and thrombocytopenic purpura with mild elevations in aminotransferase level. The diagnosis may sometimes be confused with extrahepatic biliary atresia, and indeed CMV infection has been proposed as a causative agent [391]. Most cases resolve without long-term sequelae.

Primary infection in adults presents with symptoms similar to those of infectious mononucleosis. The liver is commonly involved in primary infection, but the hepatitis tends to be mild; jaundice is rarely present [392]. Patients at risk for severe hepatitis include neonates, bone marrow and liver transplant recipients, and those receiving chemotherapy. In severe cases, the level of aminotransferases may mimic acute hepatitis. Distinguishing features are prolonged fever and atypical mononuclear cells on peripheral blood smears. Fibrosing cholestatic hepatitis has been reported to occur with CMV infection [393].

The liver biopsy usually shows multinucleated giant cells in neonatal hepatitis. Intranuclear inclusions are observed in hepatocytes, Kupffer cells, and biliary epithelial cells. Hepatic lobular microabscesses are reported in liver transplant recipients [394].

The presence of atypical mononuclear cells on peripheral blood smears may suggest the diagnosis. More definitive diagnosis rests on recovering the virus from infected body fluids. The shell vial assay, which uses a combination of virus culture and immunological detection of immediate to early gene expression, is the test of choice. IgM is sensitive for the detection of CMV macroglobulins but does not reliably indicate acute infection. The presence of IgG antibody is not specific for acute infection. A fourfold rise in IgG antibody between acute and convalescent sera is diagnostic but is seen in less than 50% of cases. Liver tissue can be stained with monoclonal antibody against CMV early and late antigen, and PCR techniques can yield a faster diagnosis than routine culture [395,396].

There are no controlled trials of therapy for CMV hepatitis. There are several case reports citing the benefits of gancyclovir for severe hepatitis. Patients with normal immunity generally have a mild course and do not require treatment. Treatment should be reserved for immunocompromised patients because of associated toxicity.

Recently discovered viruses

Studies indicate that in 10% of cases of transfusion-related hepatitis, 50% of cases of fulminant hepatitis, and 20% of community hepatitis, no cause can be identified [397]. This has led to the search for other hepatotrophic viruses. Using molecular techniques, three candidate viruses have been discovered. Hepatitis G virus (HGV) or GB virus C (GBV-C), basically the same virus, was the first described, TT virus (TTV) (named after the initials of the patient from whom it was discovered) was the second, and SEN virus (SENV) the third.

Hepatitis G virus

HGV is a member of the Flaviviridae family and is distantly related to HCV. Based on blood donor studies, the virus appears to have worldwide prevalence, with a rate of viremia of 1%–2%. It can be transmitted by blood transfusion, and other modes of transmission, such as the percutaneous route, may be possible. It appears to have a high rate of persistence (91%). Initial studies suggested that this was a causative agent of non-A to non-E hepatitis, but this has not been substantiated in other studies [398,399]. Rates of HGV infection appear to be similar in transfusion recipients who developed hepatitis and in those in whom hepatitis was not seen [398]. Additionally, most cases were not associated with a rise in aminotransferases. HGV may not even be hepatotrophic because inoculation studies in a chimpanzee failed to demonstrate liver disease. Based on these studies, it has been concluded that HGV is not a cause of acute or chronic hepatitis.

TT virus

TTV was initially discovered in 1997 from the serum of a patient with non-A to non-G hepatitis [400]. TTV is a single-stranded DNA virus belonging to the Circovirus family. Studies of multitransfused individuals and blood donors suggest a worldwide prevalence, with rates of viremia ranging from 5% to 90% [400–402]. Initial studies from Japan indicated that the virus was related to hepatitis in three of five patients after blood transfusion, suggesting that it was the cause for non-A to non-E hepatitis [400]. However, a study of blood donors and prospectively followed transfusion recipients with and without hepatitis indicated a similar rate of newly acquired TTV infection in patients with (23%) and without (22%) hepatitis [402]. Transmission using serum and stool from two infants with acute TTV infection was observed in chimpanzees, but clinical and histological evidence of hepatitis was not demonstrated [403]. These data support the conclusion that TTV is not a hepatotrophic virus.

SEN virus

SENV is the most recently identified candidate virus as a potential cause for non-A to non-E hepatitis. SENV is a single-stranded DNA virus probably belonging to the Circovirus family and distantly related to TTV. Five strains have been identified (A, B, H, D, and E), but only strains SENV-D and SENV-H had low enough rates in donor populations and high rates in transfusion-related hepatitis to qualify as potential agents of acute hepatitis. Preliminary data supporting SENV as an infectious hepatitis agent included a high prevalence of SENV in injection drug users and multitransfused patients, and the prevalence of SENV was high in cases of non-A to non-E hepatitis but low in control populations [404]. In a prospective study of transfusion-related hepatitis, there was a strong association between SENV with transfusion-associated non-A to non-E hepatitis (92%), compared with transfused controls who did not develop hepatitis (24%) [405]. Persistent viremia was demonstrated for up to 12 years, and chronic elevation of aminotransferases was observed, but chronic hepatitis was not documented by liver biopsy. However, most patients with SENV infection did not have hepatitis, and replication within the liver has not been established [405]. These data appear to indicate that SENV-D and SENV-H may have a causative role in non-A to non-E hepatitis.

Summary

In summary, HGV and TTV are not causative agents of non-A to non-E hepatitis. They can be transmitted by blood transfusion, but it is debatable whether they cause liver disease after infection or are hepatotrophic at all. It is possible that they may cause liver disease in certain settings, as in immunocompromised patients infected with CMV and EBV, but this remains to be proved. They do not appear to worsen underlying viral or nonviral liver disease. SENV, another transfusion-transmitted virus, may lead to persistent infection, but more definitive studies are awaited before it can be added to the hepatitis alphabet.

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G LIVER

81 Chronic hepatitis B viral infection

Robert G. Gish, Stephen Locarnini

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There are estimated to be over 400 million carriers of hepatitis B virus (HBV) worldwide [1]. The prevalence of chronic HBV varies depending on the geographical region. In the Western world, HBV is usually an adult-acquired disease with a prevalence ranging from 0.2% to 1% of the population, and is responsible for less than 10% of chronic liver disease. In Asia, Africa, and the Middle East, chronic hepatitis B (CH-B) is acquired at birth or early in life, has a prevalence of 5%–20%, and is among the leading causes of death in those regions. At least 20%–30% of those carriers will die of complications of chronic liver disease, including cirrhosis and hepatocellular carcinoma (HCC) [2,3]. The World Health Organization (WHO) places CH-B in the top 10 causes of death worldwide [4]. The estimated viral burden in the United States is at least 1.25–2 million people, with the disease concentrated in ethnic subgroups and populations with high-risk behavior [5]. With statistics based on immigration over the last 10 years and the known prevalence in those populations, the US prevalence could be as high as 2 million [6–10]. The costs of managing CH-B in the United States exceeds 350 million dollars per year [11]. Although the United States is considered an area of low prevalence for hepatitis B infection, the incidence of new cases, the prevalence of chronic infection, and the burden of acute and chronic disease maintains hepatitis B among the important communicable diseases.

The incidence of acute HBV disease fell markedly (by 67%) in the United States and Canada between 1990 and 2002 [12], compared with a marked increase during the 1980s [13]. In the 1980s, the estimated number of new (acute) cases of hepatitis B disease in the United States was more than 430 000 per year; by 1997 the estimate was only 185 000 new cases per year [14]; and in the mid-2000s, the estimate was just 78 000 cases per year [15]. These acute infections lead to 27 000–42 000 chronic infections, 17 000 hospitalizations, and ultimately 4000–5500 deaths per year from cirrhosis and HCC, as well as to the morbidity and cost of liver transplantation in about 350 patients per year [16–18].

The risk of developing chronic HBV infection after acute exposure ranges from 1% to 5% for adults, and is greater than 90% for infants born to infected mothers who are positive for hepatitis B e antigen (HBeAg), a secreted protein that is normally detectable in the bloodstream when wild-type hepatitis B virus is actively replicating. Persistent and stable HBV DNA levels detected over the first 1–4 weeks following acute hepatitis B disease indicate a strong likelihood of not clearing HBV infection; in such cases, consideration should be given to intervention with antiviral therapy [19].

Understanding of the prevalence and pattern of HBV infection, which patients are at risk, the clinical presentation, serological (Table 81.1) and molecular tests, as well as the current standards of monitoring and treatment are all essential parts of practice for clinical physicians. Advances in molecular biology and virology as well as developments in antiviral research have led to a much greater understanding of this virus, allowing implementation of new therapies and more appropriate and targeted testing, the combination of which results in improved patient outcomes. Sharing of knowledge between investigators and clinicians worldwide will continue to advance our understanding of this global public health problem.

The serious consequences of end-stage liver disease and HCC confront patients and physicians throughout the world. The combined risk of liver cancer and cirrhosis with its inherent complications of death or transplant for patients with CH-B is 25%–30% [20–24]. Communicating the high rate of severe consequences of this disease is essential to encourage patients to participate in monitoring and follow-up. Testing patients at risk for HBV infection, interpreting the serological

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Table 81.1 Significance of a positive test for hepatitis B virus

Positive test parameter	Clinical significance
HBsAg	Infected and infectious
Anti-HBs	Immune (in the absence of HBV DNA)
Anti-HBc	Exposed
IgM	Recent or acute infection
IgG	Historical exposure (with possible current infection)
	or false positive
	or low-level carrier
HBeAg	High replication
Anti-HBe	May indicate low replication or presence of precore/BCP mutant
HBV DNA qualitative	Presence of HBV DNA, sensitive
HBV DNA quantitative	Presence of HBV DNA and level of replication
Anti-HD	Recent or current infection with delta hepatitis
Delta antigen/HDV RNA	Replication of delta hepatitis

BCP, basal core promoter; HBc, hepatitis B core (antigen); HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HDV, hepatitis delta virus; IgG, immunoglobulin G; IgM, immunoglobulin M.

tests available, disseminating patient education, and implementing regular follow-up are all important steps in the management of infected patients. Vaccination, the key step in eliminating this global scourge (Fig. 81.1), must be applied to all infants [25], household contacts of HBV-infected individuals, health-care workers, and adolescents and individuals who are sexually active with more than one sexual partner or use nonprescribed injectable drugs [26–28]. Vaccination of all newborns has been calculated to be cost-effective in developed countries [29,30], and may become cost-effective

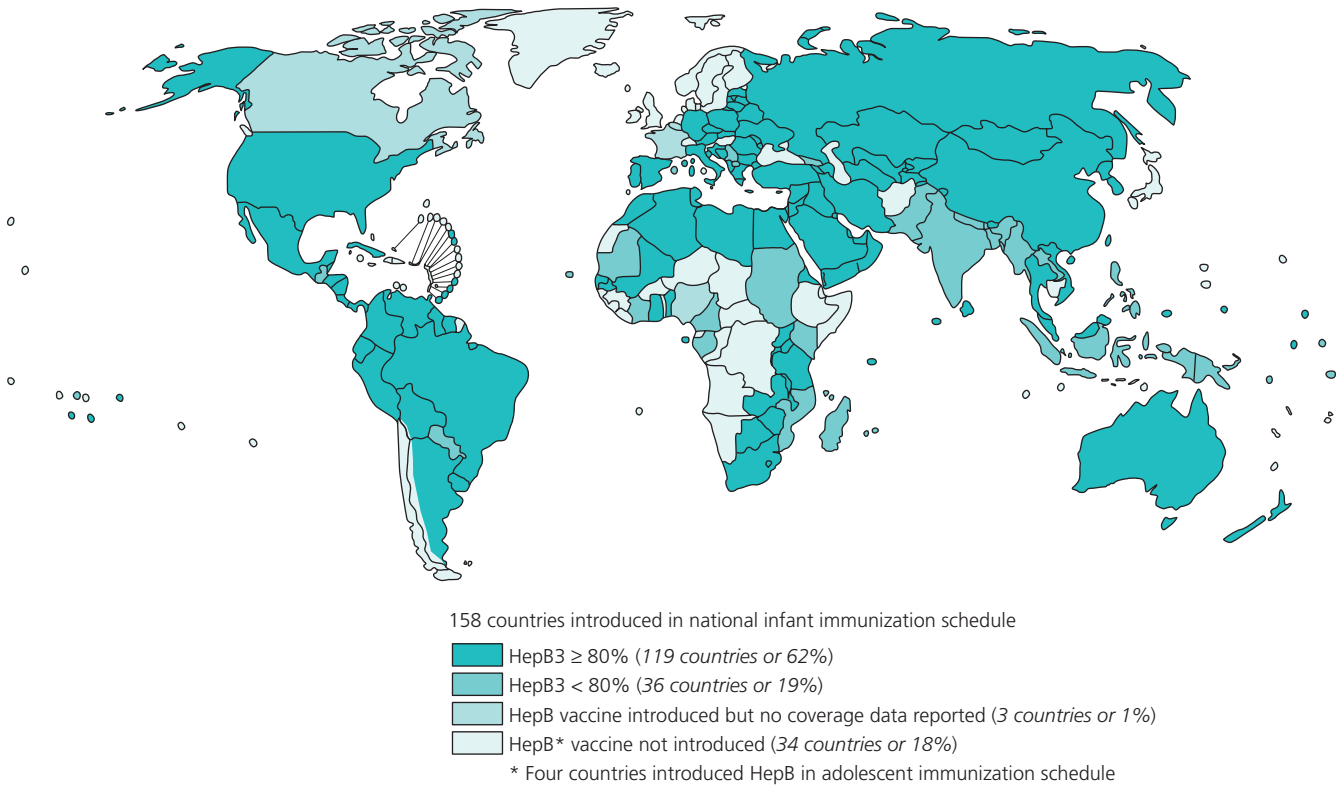

Figure 81.1 Global status of hepatitis B immunization policy. Modified from World Health Organization website, www.who.int/immunization_monitoring/diseases/HepB_map_schedule.JPG

in developing countries depending on the cost of vaccine and methods of administration. Regularly evaluating patients and counseling them about the monitoring process is important because of the ongoing revolution in antiviral therapy and its potential benefits for patients who are infected with HBV [31].

Terminology and testing for HBV have confounded many students of medicine since the discovery of the hepatitis B surface antigen (HBsAg) [29,30,32,33] because of confusing and misleading nomenclature. Inconsistent and inaccurate terms such as the oxymoron "healthy carrier" must be removed from our terminology as well as discouraging the use of the term "silent carrier." These outdated terms, often used to reassure patients or physicians, do not represent the true clinical outcome for any patient group and cannot be confidently applied to any individual patient.

The liver biopsy has a declining role in evaluating patients with CH-B and is not required for all patients. Fortunately, the outpatient liver biopsy is safe, with a risk of serious complications being less than 1:2000, and the risk of death being less than 1:12 000 [34,35]. The liver biopsy determines the *stage* of disease (fibrosis) and the extent or level of inflammation (*grade*), and evaluates for other important and treatable diseases such as fatty liver or iron overload (see Chapter 97). The liver biopsy allows the patient and physician to decide on the necessity and urgency of treatment, the presence of or risk of progression to cirrhosis, and the need and frequency of liver cancer screening as well as monitoring of viral replication and liver panels. In the future, serum tests for fibrosis and percutaneous sound-wave devices that test elasticity may further obviate the need for liver biopsy. Most importantly, levels of HBV DNA aid in the determination of a patient's risk for liver cancer, cirrhosis, and death, and are eliminating the need for liver biopsy in many patients [36–39] (Figs 81.2 and 81.3).

From the first use of the oral antiviral medication lamivudine, to inhibit HBV replication directly, increasing rates of resistance to medications have been observed over time.

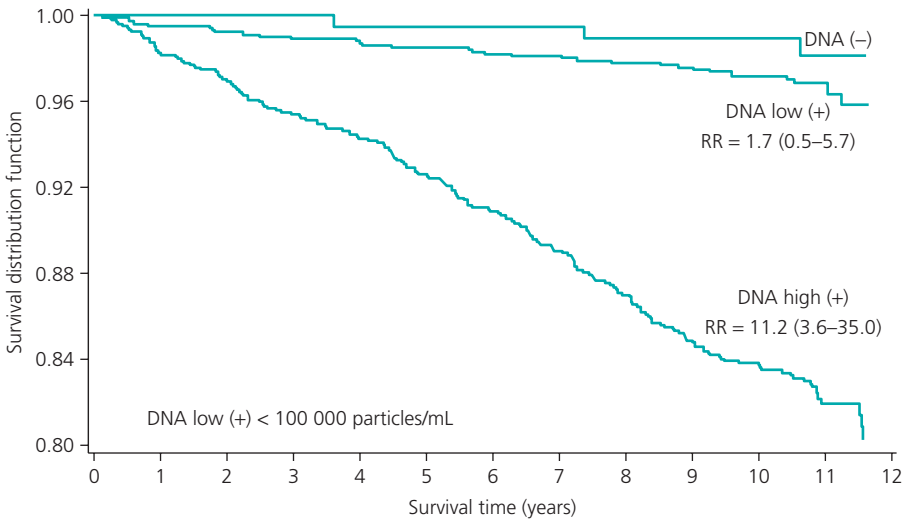

Figure 81.2 HCC mortality curves by viral load category at study entry. From Chen G, Lin W, Shen F, et al. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. Am J Gastroenterol 2006;101:1797.

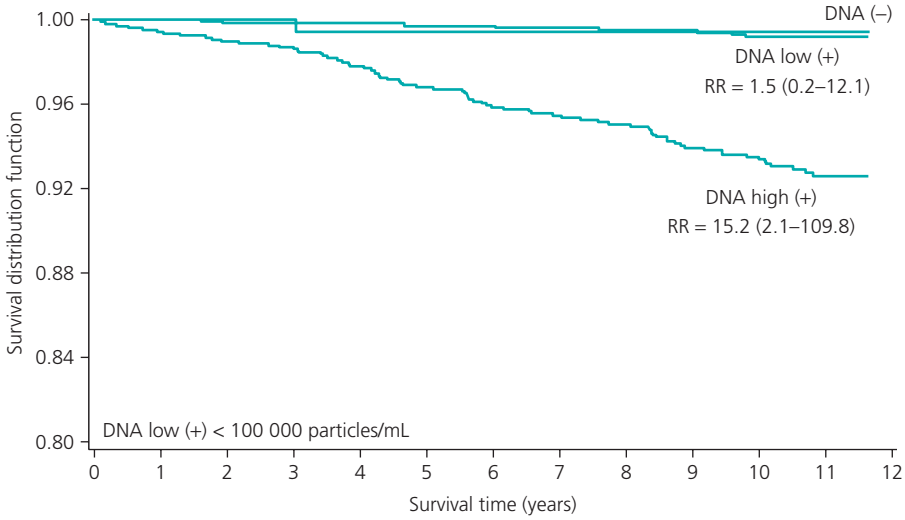

Figure 81.3 Chronic liver disease mortality curves by viral load category at study entry. From Chen G, Lin W, Shen F, et al. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. Am J Gastroenterol 2006;101:1797.

The clinical significance of these medication-resistant viruses will be discussed below. Fortunately, with the advent of telbivudine, adefovir, and entecavir, there has been a continued decline in the rate of resistance. This has encouraged practitioners toward earlier intervention for patients with CHB. Of greatest importance are those patients with advanced liver disease and impending liver failure because they require immediate treatment with the most effective available therapy. The most rapid suppression of virus occurs with entecavir and telbivudine, and the lowest rate of resistance with entecavir. Combination therapy with a nucleoside (e.g., lamivudine, telbivudine, or entecavir) and nucleotide (e.g., adefovir or tenofovir) is important if there is preexisting resistance to antiviral medications.

Chronic hepatitis B

Chronic hepatitis B disease is defined as hepatic necroinflammation resulting from the persistent presence of HBV infection. These patients are positive both for HBsAg and for HBV DNA, with serum levels of HBV greater than 2000–20 000 IU/mL or the presence of hepatitis B core antigen (HBcAg) staining in the liver. Liver enzyme levels are either persistently elevated or intermittently elevated over periods of 6 months or longer. The WHO case definition of CH-B requires the detection in serum of HBsAg on two occasions over a 6-month period. Liver biopsy is an important component in defining the severity of liver disease in these patients. Abnormal liver enzyme levels are defined as an alanine aminotransferase (ALT) of more than 17–20 IU/mL in women and 25–30 IU/mL in men when individuals have a normal body mass index [40–42].

The hepatitis B e antigen (HBeAg) is a secreted protein that is normally detectable in the bloodstream when wild-type virus is actively replicating; however, mutant strains of HBV can be easily selected out that replicate without producing HBeAg. Thus, patients with CH-B can be divided into two subgroups, HBeAg-positive (wild-type) and negative (basal core promoter and precore mutants), which will be discussed further under "Hepatitis B virus variants and mutants" below. Patients with normal ALT levels but high levels of HBV DNA and necroinflammation on liver biopsy are also included in the group with CH-B because of the similarity in natural history [20,22–24]. "Flares" of hepatitis B occur in some patients and are defined as intermittent elevations of serum aminotransferase levels to more than twice baseline and 10 times the upper limit of normal (ULN) [43]. These events occur in 10%–15% of patients each year and may be asymptomatic and undetected.

There are four phases of CH-B infection, although not all patients go through all phases. These are the immunotolerant phase, the immunoelimination phase (also referred to as immune clearance or immune active), the nonreplicative phase (also referred to as inactive carrier state), and the reactivation phase.

Immunotolerant phase

Individuals who are HBeAg-positive with high levels of virus in their blood (HBV DNA greater than 20 000 IU/mL) but persistently normal liver enzymes are termed "immunotolerant" and typically have normal liver histology. These individuals are typically in their second or third decades of life, develop elevated liver enzymes later in life, and can go on to cirrhosis or can undergo spontaneous seroconversion of either their HBeAg and subsequently HBsAg to the respective antibody, although this is a rare event. If HBV DNA levels remain elevated and ALT levels rise above the upper limits of normal as defined in the previous section in this chapter, consideration of a liver biopsy should be discussed [44], as well as the possibility of initiating therapy. This phase is generally seen in patients with perinatally acquired infection; it is rarely observed in those with childhood- or adult-acquired infection.

Immunoelimination phase

Individuals in the immunoelimination phase are HBeAg-positive with initially high serum levels of HBV DNA and elevated ALT with moderate or severe necroinflammation on liver biopsy. In patients with perinatally acquired HBV, the transition from the immunotolerant phase to the immunoelimination phase commonly takes place during the second to fourth decades. Most patients with childhood- or adult-acquired infection are in this immunoelimination phase at presentation. During this phase, the immune response to HBV is more active, with resulting reductions in serum HBV DNA levels. In some patients, immune control of HBV will result in substantial drops in HBV DNA with HBeAg seroconversion.

Nonreplicative phase

Individuals in the nonreplicative phase (also referred to as the inactive carrier state) are identified by the presence of HBsAg in serum, no detectable HBeAg, low levels of HBV DNA (< 2000–20 000 IU/mL), and persistently normal ALT levels for periods of longer than 6 months. A liver biopsy can be performed in such patients, but is not a usual practice, unless the ALT becomes elevated or serum HBV DNA remains persistently positive [44]. The liver disease activity index is typically less than 3 on a 1–22 scale (see Chapter 97 on liver biopsy and histology scoring systems). The clinical course of patients in this phase will be affected by the level of liver damage prior to HBeAg seroconversion, and the durability of the phase. These patients are at risk for relapse to active liver disease and replication of virus as well as HBV DNA integration into the hepatocyte nuclear DNA with the attendant risk of developing HCC later in life. They should be monitored at least every 6 months with serum tests for HBV replication, serology, liver enzymes, and synthetic tests.

Reactivation phase

Individuals in the reactivation phase are HBeAg-negative and anti-HBe-positive. Reactivation of hepatitis B disease occurs when a person is in the nonreplicative phase and develops an elevation of liver enzyme levels and a significant rise in HBV DNA levels (≥ 1.0 log IU/mL). Levels of ALT may be persistently elevated and stable, elevated but fluctuating, or only intermittently elevated. Levels of HBV DNA can also vary from high to undetectable, making serial tests necessary to identify accurately patients in the reactivation phase. The HBeAg can become positive in some patients. The liver biopsy typically has a hepatic activity index (HAI) score of 4 or greater [45]. Reactivation can occur naturally or with the use of immunosuppressive therapy. Individuals in this phase tend to be older and to have more advanced liver disease with a higher likelihood of adverse clinical outcomes.

Resolved infection

Resolved hepatitis B infection is defined by serological tests showing negative for both HBsAg and HBV DNA in a person with a history of acute or chronic HBV infection and currently normal levels of liver enzymes. These patients may remain anti-HBc positive for 5–10 years or longer and are at risk of transmitting disease on rare occasions (such as the donation of solid organ tissue). With supersensitive testing, HBV DNA may be found in the blood in a substantial number of patients. In addition, those patients who appear to have resolved HBV infection may develop active liver disease if they are treated with immunosuppressive medications such as prednisone, methotrexate, or chemotherapy. The standard is to treat these patients during chemotherapy with a nucleoside or nucleotide analogue, as discussed below.

Coinfection definition

Coinfection with hepatitis delta virus (HDV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) is defined as the presence of active viral replication with one or more of the above defined viruses determined by molecular assays, or in the case of HIV, HIV-positivity confirmed by the standard enzyme-linked immunosorbent assay (ELISA)/Western blot tests. These infectious problems will be discussed in detail below.

Serological and molecular diagnosis

As a general principle of laboratory-based investigation for a virological cause of chronic liver disease, testing should include all the major etiological agents (HBV, HBV plus HDV, and HCV) [46]. These are common infections in immigrants, and in high-risk groups such as injecting drug-users, the multiply transfused, and those who are sexually active with multiple partners. The principal screening assay for acute hepatitis B (AH-B) and CH-B, as well as for the screening of blood and organ donors, is detection of the HBsAg in serum [47], although molecular testing with more advanced nucleic acid tests such as polymerase chain reaction (PCR) and transcription-mediated amplification (TMA) are being instituted in the United States and considered worldwide.

The typical serological profiles found in active or past HBV infection are shown in Table 81.1. Hepatitis B core-specific IgG antibodies (IgG anti-HBc) are almost always present in the serum of patients with CH-B and may play a role in fetal and neonatal immunological responses to HBV where there is mother-to-child transmission (MCT). These antigens and antibodies cross the placenta in utero and thus the newly infected neonate has high titers of maternal IgG anti-HBc at birth (indicating a strong T-helper cell type 2, or Th2, response), partly contributing to its "tolerized" state to the HBV. These antibodies are not neutralizing and do not appear to influence viral replication. The detection of IgM anti-HBc is generally diagnostic of acute HBV infection [48]. Most patients with underlying inflammatory activity in the liver will show evidence of viral infection with one or more of the available tests. Typically, HBV DNA is detected and measurable by molecular techniques [49,50]. Although historically levels of HBV DNA have been measured in terms of copies per unit volume (i.e., per mL), the WHO has recommended that laboratories convert to international units (IU/mL) (Table 81.2). Relative levels of HBV DNA often correlate inversely with the degree of necroinflammatory activity in the liver, reflecting attempts by the host's immune response to control and eliminate the virus. The serum level of HBV DNA can be used for prognosis [36–39] (Figs 81.2 and 81.3).

Prevalence

The WHO has identified regions of high, intermediate, and low endemicity. High levels of endemicity (positive HBsAg

Table 81.2 Viral load testing: conversion to international units

Assay	Conversion factor
Digene Hybrid-Capture II (Digene Corp.)	No CE (European Conformity) mark
Versant HBV DNA 3.0 (bDNA) (Bayer Diagnostics)	1 IU/mL = 5.2 copies/mL
Cobas Amplicor HBV monitor (Roche Molecular Systems)	1 IU/mL = 5.6 copies/mL
Cobas Taqman 48 HBV (Roche Molecular Systems)	1 IU/mL = 5.8 copies/mL

Conversion factors given by manufacturers.

with ≥ 8% of population infected) affect more than 45% of the world's population; intermediate rates of infection (2–7%) affect another 43% of the population; and low prevalence (< 2%) occurs in the final 12%. As assessed by a combination of serological assays, 5% of the US population has evidence of present active or past infection with HBV. About 1 out of 200 people in the United States is infected with HBV, and some ethnic subgroups have prevalence rates of 7%–20%, which closely approximate rates in their country of origin.

Transmission

Modes of transmission vary depending on the country of origin; ethnic background; local, traditional, and Western medicine practices; and, most importantly in developed countries, risk behavior. Understanding of risk factors and intervention aimed at each of the major routes of transmission is the key for preventing HBV transmission. The combination of education, prevention strategies, and vaccination could lead to eradication of the disease [51], a focus of the US Centers for Disease Control and Prevention (CDC). In populations that acquired HBV as adults, heterosexual contact (42%), men having sex with men (15%), and injecting drug use (21%) represent the most common modes of acute transmission and may represent the most common causes of chronic disease.

Perinatal transmission is considered to be the leading cause of HBV spread worldwide. However, other modes of transmission involving iatrogenic routes and the application of folk medicine in young populations are important [52]. Even taking these newly identified risks into account, perinatal transmission probably accounts for more than 60% of all childhood-acquired disease.

Nosocomial/occupational exposure and transmission is also an important issue. Health-care workers exposed to patients with CH-B and patients exposed to chronically infected medical practitioners are both at risk [53,54]. Renal dialysis patients are, historically, considered a high-risk group for acquiring or transmitting HBV because many have had extensive blood exposure in the dialysis setting [53–55]. Some US states legally require practitioners to remove themselves from practice if they perceive that they pose a risk (in this case an infectious risk) to their patients. Health-care workers who are exposed or potentially exposed to needlestick injuries or blood splash exposure are also at risk of acquiring HBV [56]. The most important steps are preventive vaccination before risk exposure, immediate testing for immunity to hepatitis B if exposure takes place, and application of hepatitis B immune globulin (HBIG) and HBV vaccine if a nonimmune health-care worker is exposed to blood or contaminated sharps [53,54,57]. Hepatitis B vaccination is strongly advised for all individuals who work in a health-care setting that may expose them to blood or body fluids. Elimination in developing countries of the practice of reusing needles may lead to a marked reduction in childhood, adolescent, and adult-acquired HBV infections [58].

Household transmission has been clearly identified as a means of acquiring HBV [16]. The route of exposure is not clear but HBV is a DNA virus that can exist outside the body for prolonged periods on fomites. Recommendations state that the sharing of household utensils that may carry blood, such as toothbrushes and razor blades, must be avoided. The transmission of HBV by means of common kitchen items and food sharing has not been described. All household members living with an individual who is positive for HBsAg or known to have CH-B should be tested for evidence of HBV infection and vaccinated if not immune. Studies have even suggested that bed bugs can be a source of HBV transmission [59,60].

Hepatitis B is also a sexually transmitted disease [16,61,62]. Individuals attending clinics treating sexually transmitted diseases commonly have evidence of exposure to HBV as indicated by the presence of anti-HBs and anti-HBc; in a subset of these patients, there will also be HBsAg in serum. Many of these patients have asymptomatic infection although some patients will present with acute HBV with jaundice and elevated liver tests following a high-risk sexual exposure.

Blood transfusion is an unlikely source of HBV in developed countries where effective screening of blood takes place. Screening of all blood units for HBV as well as predonation screening for risk behavior should become a standard practice throughout the world. The risk of acquiring HBV through a blood transfusion in the United States is less than 1 in 400 000 units. The best test to screen blood for HBV infection is determination of HBV DNA by nucleic acid testing (NAT), also termed "molecular testing," coupled with improved HBsAg and anti-HBc assays [63–66]. The risk from blood products arises because some patients donate blood before the onset of an immune response to HBV infection or appearance of clinical evidence of hepatitis. Nucleic acid testing looks set to make the blood supply even safer [64]. A number of developing countries do not screen blood or blood products for HBV, posing a major risk to patients who may undergo blood product transfusion in those countries.

Approximately 20%–35% of individuals with adult-acquired HBV infection in the United States have no known risk factor or easily identifiable risk factor [16]. Possible transmission through sexual contact, medical or dental treatment, injection drug use (IDU), sharing household utensils, or blood exposure in an occupational setting must all be considered.

Coinfection: risk groups

In patients who are infected with HBV, coinfection with HCV [67] or HIV exists in certain risk groups, especially those with adult high-risk behavior. All patients with HBV infection must be tested for HCV infection; if a history of risky sexual

exposure or IDU is discovered, HIV infection testing is also necessary. Infection with HIV clearly influences the response to HBV vaccine and treatment as well as risk of progression. Hepatitis delta virus coinfection is also an important medical issue because this RNA virus, when superimposed on HBV, results in a higher rate of chronic disease, cirrhosis, and HCC. Infection with HDV is specific also to populations in certain regions, such as Amerindians in South America, as well as high-risk populations in the Mediterranean area and the Pacific islands.

Prevention

Vaccination is the only medical intervention to date that can markedly change the incidence and prevalence of this disease. The list of HBV vaccine products available worldwide is extensive and individuals should review local product availability in their own country. All products appear to be similar in terms of immune response and level of anti-HBs titer after a series of vaccinations. Worldwide vaccination for HBV has finally come of age, with most countries utilizing or planning a broad-based HBV vaccine program (Fig. 81.1) [17,68–70]. Vaccination has already resulted in a marked decline in childhood prevalence of HBV disease in Taiwan, and more importantly has yielded a major fall in HCC even in this young age group [71–73]. This is the ultimate in cancer treatment: prevention through vaccination. The following groups of people should all be vaccinated against HBV infection:

- individuals who are exposed to blood or blood products
- those who plan to travel to developing countries
- people who live in a household with or have intimate contact with a chronically infected individual
- those who work in a health-care setting with patient contact
- children born to HBsAg-positive mothers
- patients with chronic liver disease.

Safe sexual practices, needle and syringe exchange programs or nonreusable needles, thorough blood screening, and medical instrument sterilization are all essential components of a prevention program.

For children born to HBV-infected mothers, prevention of perinatal and early horizontal transmission with injection of HBIG combined with vaccine, or vaccine alone, is recommended or considered the standard of care in most parts of the world [74]. Because of the expense, HBIG administration to the infant at the time of birth (in combination with vaccination) is only available for use in countries with higher per capita health-care expenditures. Without HBIG, vaccination appears to protect more than 80%–90% of infants when administered at birth; it must be followed by two additional doses at 1 and 6 months. Intramuscular injection of HBIG (0.5 mL) combined with HBV vaccine has an even higher efficacy, protecting more than 98% of infants. Some special populations have a low or exceedingly low response to conventional vaccines, which has led to the development of more immunologically active vaccines or adjuvants to promote an increased response rate. Examples of such populations include older patients, organ transplant patients, renal dialysis patients, and immunosuppressed populations, including patients coinfected with HIV. Hepatitis A vaccination of patients with CH-B is emerging as the standard of care in many countries because of the high mortality rate for acute hepatitis A virus (HAV) superinfection, as demonstrated in outbreaks such as the Shanghai epidemic [75].

Postvaccination testing is only required for those individuals who need to know their HBV immune status for their medical management or for potential occupational exposure risks. Revaccination results in an antibody response in more than 30% of all persons who do not respond to a primary vaccination series [76]. Postvaccination anti-HBs levels remain above the value regarded as protective (10 IU/mL) in about 50% of patients for up to 15 years. It is also clear that patients who do not have measurable antibodies can mount an amnestic response and be protected from HBV infection [77–79]. There is no evidence that HBV vaccine is associated with any form of arthritis or neurological sequelae [80–82]. Vaccination has no role in the treatment of the patient with CHB.

Based on the epidemiological picture described above, the populations to screen for evidence of HBV infection and to vaccinate if not infected with HBV are:

- patients who are born to parents from endemic regions of the world
- patients with a history of high-risk sexual activity or more than 50 lifetime sexual partners
- men who have sex with men (MSM)
- any person with a history of injecting drug use
- individuals who have or have had sexually transmitted diseases
- household contacts or sexual contacts of individuals who are positive for HBsAg
- persons who have had medical care or injections in a developing country
- any person with acutely or chronically elevated liver enzymes
- individuals with HIV or HCV infection.

Children are a special subgroup of patients who need to be considered at risk of chronic infection if they are born to a mother who is HBsAg-positive and do not receive HBV vaccination (with or without HBIG) at birth. Also, all children who originate from orphanages in developing countries should be tested for HBsAg. Moreover, all immigrants from developing countries where there is a significant prevalence of HBV need to be screened for HBsAg, as well. Both pediatric and adult travelers to developing countries are at risk for HBV infection and need to be tested if evidence of liver disease is present.

Hepatitis B virus variants and mutants

Although HBV is a DNA virus, replication is through an RNA intermediate requiring an active viral reverse transcriptase/polymerase enzyme. The polymerase (reverse transcriptase) of HBV lacks a conventional proofreading function as is found in other higher-order DNA polymerases. Therefore, HBV exhibits a mutation rate more than 10-fold higher than other DNA viruses, more closely resembling retroviruses such as HIV. The prevalence or number of nucleotide substitutions found in the viral genome varies depending on the duration and stage of liver disease. The natural evolutionary rate for the HBV genome in CH-B is approximately $1.4–3.2 \times 10^{-5}$ substitutions per site per year. In the liver transplant setting, this increases by up to 100-fold as a result of increased viral replication that results in an average increase of 1–2 $\log_{10}$ IU/mL in HBV viral load. This higher substitution rate may be a result of the immunosuppression regimen associated with transplantation, especially the use of steroids, and the positive enhancing effect on the HBV glucocorticoid response element (GRE) located in the viral genome. Mutant viruses are defined as subpopulations of HBV that emerge when there is either immune or medication selection pressure that allows particular populations or quasispecies to emerge.

Mutants affecting HBeAg production

The pre-C/C open reading frame (composite ORF) encodes the core protein (p21) and the precore (p25) protein/HBeAg (p14–17). The core protein is the major polypeptide of the nucleocapsid and is expressed as the HBcAg. The C ORF is preceded by a short, upstream in-phase ORF known as the precore region from which the soluble HBeAg is synthesized. Both HBcAg and HBeAg proteins are targets for immune-mediated viral clearance mechanisms. The two major groups of mutations that affect HBeAg synthesis are precore region mutations (nucleotide G1896A), which block translation, and mutations in the basal core promoter (BCP), which reduce transcription, usually at nucleotides 1762 and 1764, all resulting in diminished production of HBeAg, usually as a consequence of increased host immune pressure. Precore mutations frequently occur temporally related to core gene mutations/deletions. Patients with precore mutations typically have about 1–2 log lower levels of serum HBV DNA compared with those patients with "wild-type" HBeAg-positive CH-B. Mutations in the BCP are likely to increase viral replication and enhance disease activity, especially in genotype C HBV-infected patients [83].

X gene mutants

The X protein is a nonstructural protein, encoded by the X gene, that can transactivate a variety of cellular and viral promoters by activating signaling pathways [84]. X has been reported to interact with a number of nuclear proteins involved in cell-cycle regulation, DNA repair, and transcription. Persistently high levels of the HBx expression have been associated with the development of liver tumors in transgenic mice. The X protein can transform nontumorigenic cell lines in vitro.

Pre-S/S gene mutants

The S envelope gene contains three in-frame start codons that divide the gene into the three surface proteins of HBV: The pre-S1 region, the pre-S2 region, and the S region. Three corresponding mRNAs serve for the synthesis of these proteins. The pre-S1 promoter (SPI) is located upstream of the S gene, whereas transcription of the pre-S2 and S mRNAs is directed by the S promoter (SPII), which is located within the pre-S1 region. Surface mutations have obvious clinical relevance in the prevention of HBV because of their impact on prophylactic vaccination as well as laboratory-based diagnosis [29,85,86]. Several large HBV vaccination programs in endemic regions have revealed a 2%–3% incidence of vaccine escape mutants resulting from alterations in the HBsAg protein. Generally, the amino acid substitution Gly145Arg of the S protein makes this epitope unlikely to bind to anti-HBs generated to wild-type HBsAg. The "a" determinant of the HBsAg is a peptide sequence located between amino acids 99 and 170 of the S protein, which represents the major immune target of anti-HBs. This antibody reactivity is directed mainly against the second loop of this determinant, comprising amino acids 139–147. In patients infected with HBV exhibiting surface mutations affecting the "a" determinant, the mutant HBsAg may not be detectable by commonly used HBsAg assays, especially if based on monoclonal antibody "capture" reagents. As a result, these mutants do represent a potential public health problem because patients harboring HBV with these surface mutants remain infectious but do not exhibit readily detectable HBsAg and hence may escape diagnosis [14,87–90]. Viral HBsAg escape mutants have been described in the liver transplant setting where hepatitis B immune globulin exerts pressure on the virus. Subsequent changes in HBsAg have been documented with resultant fulminant hepatic failure in a few patients.

Polymerase gene mutations

The polymerase ORF is the longest within the HBV genome, covering almost 80% of the whole genome and overlapping the other three ORFs. The HBV polymerase protein mediates encapsidation of the pregenomic RNA into the core particle and synthesizes the HBV DNA genome therein. It has four enzymatic activities: DNA synthesis priming activity; RNA-dependent reverse transcriptase (RT) activity; DNA-dependent DNA polymerase activity; and RNaseH (RNA degradation) activity. Hepatitis B virus quasispecies with mutations in the

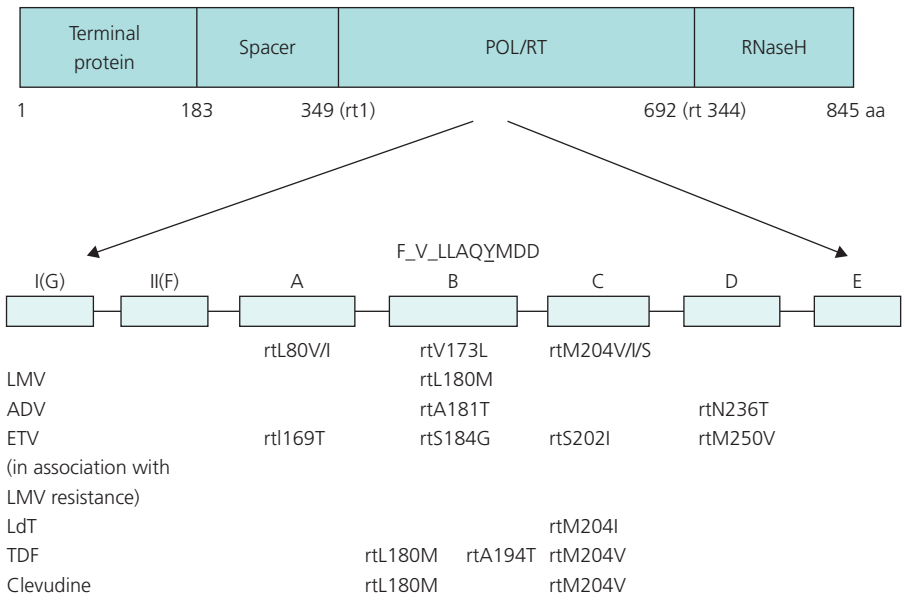

Figure 81.4 Mutation locations associated with antivirals. Adapted from Locarnini S. Molecular virology of hepatitis B virus. Semin Liver Dis 2004;24(Suppl. 1):3.

polymerase gene have been detected in patients undergoing oral antiviral therapy. The locations of the major mutations associated with the antivirals lamivudine, adefovir, entecavir, telbivudine, and tenofovir have been determined (Fig. 81.4). These mutations significantly decrease the in vitro and in vivo sensitivity of the polymerase to nucleoside/nucleotide analogues [91].

Genotypes and serotypes

Hepatitis B virus has a number of genetic variants described as serotypes (adw, ayw, and adr) and genotypes (A, B, C, D, E, F, G, and H). Our understanding of the clinical significance of HBV genotypes is evolving (Table 81.3). For example, genotyping can be used to determine who is the best candidate for interferon therapy (genotype A), who may be at higher risk for liver cancer (genotype C), and who may have an increased risk of resistance to medications (genotype D for adefovir resistance). Genotype assessment and nucleic acid sequence analysis may allow epidemiologists to track the transmission and movement of HBV strains through the world or regions of the world, to establish other epidemiological behavior of HBV infection, including the investigation of common source outbreaks [92], and to establish clinical behaviors specific to each subgroup.

Genotyping is accomplished by sequencing the HBV genome, using a line probe assay (capture hybridization), or by using serologically based antibody testing. Routine genotype testing before initiation of antiviral therapy has been advised [44].

Depending on the predominant HBV genotype in the particular geographical setting, patients infected with genotypes other than A (such as B, C, D, and E) often present to the liver clinic seropositive for anti-HBe rather than HBeAg.

Table 81.3 Hepatitis B virus genotypes and associations

Genotype	Associations
Ae	Spontaneous sAg seroconversion Interferon response
B	Spontaneous eAg seroconversions Better response to lamivudine
C	Increased rate of HCC Increased rate of perinatal transmission
D	High rate of adefovir resistance
E	
F	Unstable HBeAg seroconversions High HCC rate Fulminant hepatic failure
G	Unable to secrete HBeAg
H	

eAg, e antigen; HBeAg, hepatitis B e antigen; HCC, hepatocellular carcinoma; sAg, surface antigen.

These patients are infected with the precore G1896 stop codon precore mutation ("precore mutant") or basal core promoter mutants A1762T+G1764A. Genotype A HBV-infected patients still tend to present to the clinic seropositive for HBeAg.

Clinical manifestations and laboratory tests

Clinical manifestations of chronic HBV infection range from no signs or symptoms of disease (the majority of patients) to the warning signs of end-stage liver disease [93]. The clinical

manifestations of end-stage liver disease are the same as those seen in patients with other causes of liver failure. Some patients with HBV develop extrahepatic manifestations of infection including periarteritis nodosa and leukocytoclastic vasculitis with the increased likelihood of major damage of target organs (including peripheral nerves, kidneys, brain, and gut) caused by immune complex deposition [94,95]. Examination of the creatinine level in each patient with HBV infection and assessment for proteinuria is important. All of the medications used for the treatment of chronic HBV need to be dose adjusted if there is a decrement in renal function. Peripheral skin rashes can be assessed by punch biopsy to look for signs of vasculitis. Some patients will need a visceral and renal angiogram to identify angiographic signs of vasculitis in those organs due to periarteritis nodosa.

Cirrhosis may manifest early and subtly with changes in sensorium, memory loss, and day–night reversal. The signs of sequential liver synthetic test result abnormalities (including low albumin, elevated bilirubin, prolonged international normalized ratio [INR], low cholesterol, and high ammonia) and progression to marked signs of portal hypertension are not peculiar to HBV infection (see Chapters 90 and 91). The presence of portal hypertension is manifested by splenomegaly, ascites, varices, thrombocytopenia, leukopenia, and coagulopathy, as seen in patients with cirrhosis of other causes. Acute flares of disease in patients with HBV with cirrhosis are more common than in conditions such as HCV infection or autoimmune diseases in which the inflammatory process is often "burned out."

Laboratory tests are an integral part of evaluating patients with both AH-B and CH-B. It is important to differentiate liver enzymes from liver function tests, although these two terms are often confused or, unfortunately, combined. Liver enzymes are markers of liver inflammation and do not reflect liver function. The ALT, aspartate aminotransferase (AST), alkaline phosphatase, and γ-glutamyltransferase (GGT) enzyme determinations are useful markers for liver inflammatory activity only. Normal ranges for ALT are being fully reassessed and practitioners need to examine closely how their local laboratory sets a "normal" range and whether this is truly a "healthy" ALT. Normal "healthy" ALT is estimated to be < 19 IU/mL for women and < 30 IU/mL for men [44]. These tests, if showing elevated values, should lead a clinician to a full laboratory evaluation for HBV infection as well as investigating other causes of liver disease such as fatty liver, herbal medications, and alcohol-induced liver disease [56,96], over-the-counter and prescription medications, HCV infection, hemochromatosis, autoimmune disease, and Wilson disease. An elevated liver enzyme level in a patient who is HBsAg-positive provides a compelling indication for liver biopsy or consideration of treatment without a biopsy.

Liver function tests commonly include cholesterol, albumin, INR, bilirubin, and ammonia. When the results of liver function tests become abnormal, there is usually severe liver injury present, which could indicate end-stage liver disease or a flare of hepatitis superimposed on chronic liver disease. A flare, or marked rise, in liver enzyme levels, and a decline in liver function co-occur with an increased immune response directed at the HBV-infected hepatocyte. Such a flare of disease activity can also represent superimposed infection such as HCV or HDV, worsening HIV disease (if HIV infection is present), and potential progression to end-stage liver disease. When results of liver synthetic tests become abnormal in a patient with CH-B, a clinician should strongly consider consulting with a transplant center if the patient has no contraindications for transplantation. There should also be a strong consideration of immediate initiation of antiviral therapy with a nucleoside/nucleotide analogue. In the setting of liver synthetic abnormalities, interferon is relatively contraindicated, unless used by expert hands, typically in a tertiary referral center.

Laboratory surrogates to assess for the severity of liver disease include signs of hypersplenism, such as a low platelet or low white blood cell (WBC) count. Platelet counts below 100 000–150 000/mL should elicit strong suspicion of advanced fibrosis. An elevated α-fetoprotein (AFP) is usually associated with inflammation and ongoing liver injury; however, AFP is a poor parameter for the presence or absence of hepatocellular carcinoma and is no longer a first-line test for HCC screening [97]. The degree of AFP elevation informs about the risk of HCC in the future and occasionally is associated with the presence of hepatocellular carcinoma. A high ferritin and iron saturation could arouse suspicion of hemochromatosis although abnormalities in biochemical markers of iron overload are also common in patients with marked fibrosis or cirrhosis. A "reversed" AST/ALT ratio, where the AST is higher than the ALT, also supports advanced fibrosis or cirrhosis.

Patients with CH-B may have a moderately reduced quality of life compared with those without persistent HBV infection. Patients with end-stage liver disease will have a markedly reduced quality of life, although this is true for all patients with advanced liver disease and is not specific to HBV infection. Patients who undergo treatment with interferon often have a reduced quality of life during treatment but most tolerate full doses and a full course of treatment. If the patient clears HBV infection, his or her quality of life may improve after treatment to regain a normal level; hence, treatment may be not only cost-effective but also cost-saving [98,99].

Hepatocellular carcinoma is a serious risk for all individuals who are persistently HBsAg-positive (see Chapter 93). The estimated lifetime risk for HCC in patients who are chronically HBsAg-positive is estimated to be about 20%. This risk is markedly increased in patients with lifelong HBV infection, cirrhosis, active liver disease as demonstrated by biopsy, elevated liver enzyme levels, elevated HBV DNA level (Fig. 81.2), a history of alcohol abuse, chronic infection lasting more

than 30–50 years, dietary exposure to aflatoxins, a family history of HCC, an elevated AFP, or coinfection with HCV, HIV, or HDV [100–103]. Issues such as prevention of HCC by vaccine are covered in other sections, and prevention of HCC by use of interferon and antiviral therapies will be discussed under "Treatment" below. Patients with CH-B need to be informed of the risk of HCC and should be considered for HCC screening, depending on resources and available treatments. The initiation and frequency of screening depend on the relative risk of HCC in special groups of patients with HBV and, most importantly, in patients with cirrhosis.

Differential diagnosis

All patients with elevated levels of liver enzymes (see discussion above for what are considered normal and elevated liver enzymes) need to be screened for fatty liver, medication toxicity, elevated iron (blood and tissue concentrations), HBV and HCV infection, and alcohol abuse. The differential diagnosis of chronic HBV infection includes considering acute HBV infection, which usually can be differentiated by the presence of anti-HBc IgM (Table 81.1). Patients with elevated liver test results and detectable presence of anti-HCV in serum can be evaluated by the use of molecular testing. If HBV DNA is not measurable and HCV RNA is found to be elevated, HCV is considered the dominant disease and therapy should focus on the HCV infection. Autoimmune disease rarely occurs concomitantly with HBV disease and is also rarely caused by HBV. One must rule out biliary obstruction if the GGT or alkaline phosphatase concentration is higher than the AST or ALT serum levels. A baseline ultrasound is considered standard of care in all patients with elevated levels of liver enzymes to rule out other diseases, assess for fatty liver, assess spleen size, evaluate for varices, and look for space-occupying lesions as well as to reassure the patient about the absence of liver cancer. Examination of the liver biopsy for plasma cells and testing for antinuclear antibodies and anti-smooth muscle antibody is important, especially if serum globulin levels are greater than 4 g/dL. Patients with elevated levels of liver enzymes who are HBsAg-positive with adult-acquired liver disease need to be assessed for HDV infection by antibody testing, at a minimum [104–107].

Course and complications

The clinical course of CH-B infection is variable and affected by multiple factors, including those related to the patient (including age, gender, immune capacity and response to the virus, genetics, coinfection with other viruses, and habits such as alcohol consumption or smoking) and the virus itself (replication level, genotypes, and molecular variants). Each subgroup of patients with HBV infection has a different clinical course. The individual with persistently positive HBsAg, elevated HBV DNA level, and consistently elevated liver enzymes is the patient with the greatest risk of progression to cirrhosis and the subsequent complications. The next highest risk of progression is in the patient who presents with flares of liver enzymes and reactivation of liver disease as well as HBV DNA levels greater than 10^4 IU/mL. Each cycle of liver enzyme elevation purportedly is due to immune activation, which results in attempts to clear HBV infection. These are often ineffective. Spontaneous seroconversion from HBeAg to anti-HBe occurs at a rate of 1%–10% per annum in patients with CH-B, but seroconversion from HBsAg to anti-HBs, with clearance of HBV from the liver, is uncommon (at or less than 0.5%–1% per year) [108]. The rate of HBsAg seroconversion is higher in patients with more active liver disease (as measured by liver enzymes) than in those with persistently normal liver enzymes, but is in general less than 15% over any 3-year interval and occurs more commonly in genotype A [21,22]. During these immune "attacks" or flares there is ongoing hepatocellular damage and, commonly, progressive fibrosis. Thus, in general, the serum level of HBV DNA is the most important factor for determining risk of progression, but the degree of liver enzyme elevation and the number of flares often correlate over time with the severity of liver injury. Blood testing for progressive liver disease and screening for HCC should also take place more frequently in those patients with established cirrhosis compared with patients with mild or no fibrosis.

The patient with CH-B who has a persistently normal level of liver enzymes (< 19–30 IU/mL) and HBV DNA levels less than 200 IU/mL has a much lower risk for progressive liver disease and cancer, although the overall risk for HCC is still significant. Blood testing at least every 3–6 months is important because annual testing may be insufficient to document a stable patient or to define inactive or reactivated disease. For such patients, the frequency of screening with blood tests should be at least biannually; a more thorough evaluation with testing every 3 months may be justified.

During regular laboratory testing of liver enzymes, quantitative HBV DNA, anti-HBe, and HBeAg testing can establish if a precore or a BCP mutant virus has emerged in the patient. These viral mutants can be suggested by the presence of moderate (HBV DNA > 2000 IU/mL) to high HBV DNA serum levels in the absence of HBeAg. The larger laboratories offer HBV DNA sequencing services to determine the presence of HBV mutants of clinical significance. One widely available commercial test, produced by Innogenetics NV (Ghent, Belgium), is the INNOLiPA assay, which can confirm the presence of the precore and core promoter mutations. Patients with the precore or BCP mutant probably have a higher risk of developing liver cancer and progression to liver disease. In rare circumstances, patients who appear to clear HBsAg but do not develop anti-HBs may have developed surface mutant viruses.

Coinfection

HBV coinfection with hepatitis D (delta virus)

The patient with dual HBV and HDV infection has a typical serological profile of HBsAg and anti-HBe positivity (rarely HBeAg positivity) as well as IgG anti-hepatitis delta (anti-HD) seropositivity and HDV RNA positivity (Table 81.1). Specific immunoassays are available for detection of both IgM- (for both acute and active infection) and IgG-specific anti-HD (seen in chronic disease as well as resolved infection). Serum assays for detecting HDAg and HDV RNA are restricted and can typically only be requested from central reference laboratories or the larger commercial specialist laboratories. Hepatitis delta infection is easy to miss if not considered for all individuals who are persistently positive for HBsAg, or patients with acute liver disease, and testing should include HDV RNA and anti-HD IgG/IgM as well as the standard tests for HBV infection. These tests should be repeated in suspected cases if the first test is negative and aggressive liver disease continues.

In the United States, HDV infection occurs predominantly amongst IDUs in large cities and those who engage in high-risk sexual activity [109]. Ethnic populations who may carry HBV from the Mediterranean region, the Pacific islands, Greenland, the Middle East, or the Orinoco river valley in South America need to be tested for HDV infection as well. Hepatitis delta virus infection can lead to more severe liver disease (fulminant hepatitis) in those patients who acquire HDV and HBV at the same time compared with those patients who acquire acute infection with HBV alone (see chapter on acute hepatitis) [104–107], but often the two diseases are clinically indistinguishable. If they survive, patients with acute coinfection with HBV and HDV have a high rate of clearance of both viruses. When new HDV infection is superimposed on chronic HBV infection, the liver disease commonly accelerates and cirrhosis often develops in a much shorter time. Compared with patients with chronic HBV monoinfection, patients with HDV and HBV coinfection may also be at greater risk for liver cancer, probably because cirrhosis develops at a higher rate [110].

Infection with HDV can be treated with interferon although results are disappointing, with patients rarely clearing HDV and HBV in the long term. Therapy requires at least 1 year of treatment. Therapy with a nucleoside analogue, such as lamivudine, does not suppress HDV infection, probably because it has minimal effect on the HBV covalently closed circular (ccc) DNA levels, the main transcriptional template of HBV, from which HBsAg is synthesized and which HDV then uses to complete its replication. Liver transplantation for patients with acute HDV infection will often be followed by milder liver disease if HBV and HDV infections persist. However, HBIG therapy to prevent graft infection with HBV can also prevent HDV infection of the new graft [111]. Patients with HDV infection typically have low levels of HBV DNA as a result of the suppressive effect of HDV on HBV viral replication. The mechanism for this is unknown. HBeAg and anti-HBe status is variable (Table 81.1).

The manner in which HDV causes liver injury remains controversial. Histological observations of microvesicular steatosis and cytoplasmic eosinophilia in the absence of a mononuclear cell or lymphocytic infiltrate in the liver biopsy specimen of patients with acute HDV infection are consistent with a direct cytotoxic effect of HDV. In vitro molecular studies have supported this view, with experimental evidence that the smaller form of HDAg (S-HDAg) expression in cells results in the production of pyknotic nuclei and shrunken eosinophilic cytoplasm as well as reduced cellular nucleic acid synthesis. By contrast, evidence supporting an immune-mediated cytotoxic pathway is sparse and a direct link between HDV-associated disease expression and the immune response is lacking.

Suppression of markers of HBV replication during HDV superinfection has been observed in humans as well as in the woodchuck and chimpanzee animal models. In over 90% of hepatitis B patients superinfected with HDV, seroconversion occurs from an HBeAg-positive to an HBeAg-negative/anti-HBe-positive state with low or absent serum HBV DNA levels. Serum HBV-specific DNA polymerase activity and intrahepatic HBcAg expression can no longer be detected in these patients. On rare occasions, HDV superinfection has been associated with termination of the HBsAg carrier state and seroconversion to anti-HBs. The mechanism for this interference has not been conclusively established.

HBV coinfection with HIV

Coinfection of HIV with HBV results in more aggressive liver disease with faster progression to cirrhosis and liver failure in approximately one-third of patients, although other patients have minimally active liver disease with little or no evidence of progressive liver disease [112–114]. Why certain patients have progressive liver disease in this setting and others do not is unclear and is not correlated with any specific clinical factor or laboratory test, although the emergence of lamivudine-resistant HBV has been implicated [112]. The discrepancies may be explained by an interaction between the host immune system, T-cell types present (Th1 vs Th2), host factors that predict fibrosis, and levels of HBV and HIV viral replication.

Treatment with lamivudine may slow the progression of liver disease but is problematic from the perspective of increased resistance compared with patients who are not coinfected [115]. Current guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents recommend that if therapy for either HBV or HIV infection, or both, is indicated in the coinfected individual then a fully suppressive antiretroviral regimen should be initiated in order to prevent development of antiretroviral resistance [116]. This

is recommended for the HIV/HBV-coinfected regardless of CD4 count. These guidelines list the combination of tenofovir with either emtricitabine or lamivudine as the preferred nucleoside analogue backbone, as these medications also suppress HBV, along with the addition of the other antiretroviral agents required to create an effective anti-HIV regimen.

It is very important to avoid HBV treatment of an HIV-infected patient with an agent known to have activity against HIV, such as entecavir, unless that patient is on a fully HIV-suppressive highly active antiretroviral therapy (HAART) regimen. Using a single agent known to have activity against both viruses would lead to rapid selection of medication-resistant HIV strains, as this would be equivalent to antiretroviral monotherapy. Because of the high level of cross-resistance with many antiretrovirals and the need for effective nucleoside analogues as the backbone for most HIV treatment regimens, this could leave the patient with few options for durable HIV suppression. It is known that lamivudine, emtricitabine, and entecavir all select for the development of the M184V mutation. The medications currently used in the treatment of HBV and/or HIV that are known to have activity against both viruses include adefovir (Hepsera), emtricitabine (Emtriva, also in the combination antiretroviral medications Truvada and Atripla), entecavir (Baraclude), lamivudine (Epivir-HBV and Epivir, also in the combination antiretroviral medications Combivir, Trizivir, and Epzicom), and tenofovir (Viread; also in the combination antiretrovial medications Truvada and Atripla). In light of the delayed recognition that entecavir has activity against HIV, a problem that resulted in some HIV/HBV-coinfected patients being treated with monotherapy [117], experts have pointed to the need for very careful evaluation of possible anti-HIV activity for all future hepatitis B medications proposed for use in coinfected patients.

It is also important that coinfected patients never be treated with less than two agents with anti-HBV activity in order to slow development of HBV resistance and limit the risk of a clinically significant hepatitis flare in the setting of HAART-induced immune reconstitution [118]. The risk of hepatitis flare with discontinuation of HBV therapy also mandates that in those who need a change in their HAART regimen, medications with HBV activity should not be discontinued without the substitution of other agents that can effectively suppress HBV, unless there has been HBeAg seroconversion followed by an adequate course of consolidation treatment [118].

In cases when HIV treatment is not an option or is not desirable, it is very important that HBV treatment choices be limited to only those medications that do not have clinically relevant dual activity. Of the antiviral agents currently approved for the treatment of HBV, only telbivudine (Tyzeka, Sebivo) has no known activity against HIV. However, current guidelines recommend against its use as monotherapy in HIV/HBV-coinfected patients due to the risk of selection of the M204I mutation in the YMDD motif [118]. Adefovir, which has minimal HIV activity at the approved HBV dose of 10 mg per day, could be used with telbivudine. However, the safest alternative is to use only pegylated interferon therapy as it does not lead to development of medication-resistant HIV or HBV.

As well as the issue of accelerated liver disease progression due to the effect(s) of HIV on HBV replication, other challenges include the immune reconstitution inflammatory syndrome that occurs when an effective HAART regimen restores immune function and increases the capacity to mount an inflammatory response. Another concern is direct hepatotoxicity from the multiple agents that comprise the HAART regimens in common use with HIV disease. However, an analysis of 5272 HIV-infected patients (30% female, 39% intravenous drug users, and nearly 48% HCV/HBV coinfected) with 18 259 person-years of follow-up found that HAART did not significantly increase the risk of liver enzyme elevations but HBV or HCV coinfection did increase risk [119].

HBV coinfection with HCV

Coinfection with HCV and HBV results in increased risk of both cirrhosis and liver cancer. Patients often have one dominant viral disease. Treatment focused on the dominant virus, as revealed by blood tests of viral replication, is probably the best management step [120–125].

Hepatitis A disease

Acute HAV infection sometimes complicates chronic HBV infection. Patients infected with HBV and having an acute HAV infection are at increased risk of developing severe liver disease and death [126]. Vaccination against HAV is advocated for all patients with chronic HBV infection [126].

Treatment

Definitions of response to treatment

Definitions of response to antiviral therapy are important when evaluating patients who are about to undergo antiviral therapy or for previously treated patients who appear to need subsequent treatment. Indications of response are defined as follows:

- biochemical – a decrease in levels of liver enzymes into the normal range
- virological – suppression of viral replication to less than 20 000 IU/mL (potentially useful with interferon therapy) or to a level undetectable by PCR (the goal of therapy with all nucleoside or nucleotide analogues)
- serological – HBeAg seroconversion to anti-HBe as well as HBsAg seroconversion
- histological – improvement in liver biopsy scores, with histological activity index improving by at least 2 points and no worsening in fibrosis.

These responses can be *initial* (occuring during therapy), *end of therapy* (ETR), or *sustained*. A sustained biochemical response is commonly defined as normal levels of liver enzymes at the end of therapy that are maintained for at least 6–12 months following cessation of therapy. A partial biochemical response means a marked fall (> 50%) in liver enzyme levels to the near-normal range, reductions that are typically associated with improvement in histology.

In the past, the virological response was defined as either a reduction of HBV DNA to less than 20 000 IU/mL when pretreatment levels were greater than 20 000 IU/mL, or a greater than 1–2 log reduction from baseline. With the advent of more powerful agents and the concept that patients with any level of viral replication have a higher risk of liver cancer and death due to liver failure, the ability to control HBV replication and bring viral DNA down to undetectable levels (defined as less than 60 IU/mL, or 300 copies/mL – the limits of the PCR COBAS Amplicor assay, the most common assay used in licensing trials) is emerging as the goal of antiviral therapy. If viral load reduction is less than 1 log from baseline every 3 months, and HBV DNA is still detectable by 24 weeks for nucleoside analogues and by 48 weeks for nucleotide analogues, it is recommended that there be a change in therapy.

The timing of a change or addition of therapy depends on the risk of resistance, which is highest with lamivudine and lowest with entecavir. Choices include switching to the most potent agent available or adding medications to create effective long-term combination therapy. The reduction in serum HBV DNA viral levels can correlate with HBeAg loss (either naturally or if treatment is given). Conversion from anti-HBe negative to positive would complete what is termed triple seroconversion, although the rate of HBeAg seroconversion is higher with interferon therapy than with any of the nucleoside or nucleotide analogues after 1 year of treatment. Virological responses can also be termed initial, ETR, or sustained if there is loss of viral replication for 6–12 months after the end of treatment. The terminology "HBeAg seroconversion" is used when the HBeAg is lost (negative) in the long term and anti-HBe is gained, with HBV DNA levels less than 2000 IU/mL and normal ALT levels.

Histological improvement is defined using the histology activity index, the Ishak score, the Metavir score, or one of the modifications. There is no international consensus about which scoring system is best although the US Food and Drugs Administration (FDA) is utilizing a combination of the modified Knodell and Ishak scores. A significant improvement is based on a decrease in the necroinflammation score by two points and no worsening of fibrosis because the fibrosis score typically lags behind changes in inflammation in the liver.

Complete response means normalization of liver enzymes, serological response, virological response, and loss of HBsAg. This complete response is further defined as (before HBsAg seroconversion takes place): HBeAg negativity, anti-HBe positivity, and HBV DNA negativity by the most sensitive molecular test available. Interferon responder patients may still have low levels of HBV DNA but HBeAg seroconversion and normal ALT levels. The HBV DNA tests we use can typically measure down to 60 IU/mL (Table 81.2). Hence, this is synonymous with resolved (HBsAg seroconversion) or controlled (HBeAg seroconversion or long-term DNA negativity) HBV infection. These states do not require the presence of anti-HBs and clinically do not require a follow-up liver biopsy. The use of more advanced molecular testing, such as TMA, which has a sensitivity of 15 IU/mL, and real-time TaqMan/Artus PCR, which has a sensitivity of 50 IU/mL, is improving the quality and reliability of quantitative HBV DNA testing.

Another important perspective on response to HBV treatment is the question of whether HBV infection is ever "cured." As discussed above, the HBV transcriptional template exists in the nucleus of the infected cell as a population of cccDNA molecules organized into a viral minichromosome. These molecules of HBV cccDNA have a long intracellular half-life (Fig. 81.5). There are no clearly identified antiviral agents that inhibit HBV cccDNA generation or processing although clevudine, adefovir, and interferon seem to at least reduce intracellular cccDNA levels.

Medication resistance testing

As discussed in depth below for individual agents, the issue of medication resistance greatly affects the likelihood of success with many of the available treatments. With continued use, the virus is likely to develop resistance to not only an antiviral agent currently being used, but also possibly to future agents where there is cross-resistance. This has been found to be a particular problem with the use of lamivudine, which leads to cross-resistance with many other agents. It has been proposed that lessons learned with HIV resistance assays might translate into more effective management of CH-B. The use of both genotypic and phenotypic assays to assess antiretroviral resistance in HIV-infected patients has been shown significantly to improve the outcome of therapy. Anti-HBV medication resistance testing may become equally useful for making therapy choices. Assays specific for HBV that identify mutations associated with resistance to certain medications are commercially available, although their use is not yet as widespread as that of assays used in HIV treatment. So far there are no commercially available HBV-specific phenotypic assays.

Several consensus groups have released guidelines for the use of antiretroviral resistance testing in HIV-infected patients. No such guidelines exist for HBV medication resistance testing, so its use is at the discretion of individual physicians. It is generally thought that the use of the available assays to identify mutations associated with resistance to specific medications could be important for effective clinical

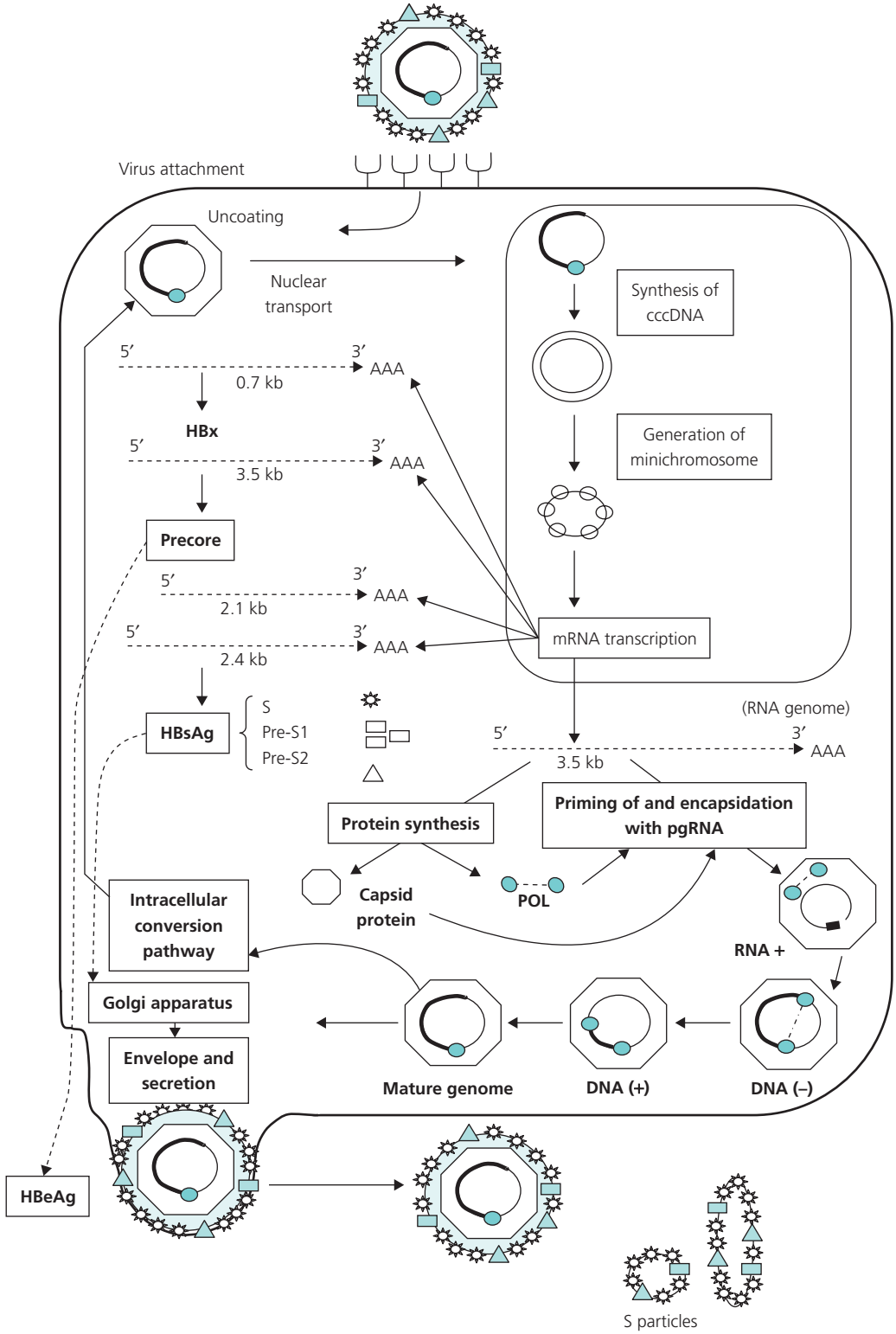

Figure 81.5 Life cycle of covalently closed circular DNA (cccDNA). HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBx, hepatitis B x antigen; pgRNA, pregenomic RNA; POL, "polymerase" (reverse transcriptase); S, S envelope protein.

management. One limitation is that resistance testing may not be accurate for medications that the patient is not currently taking because in that setting there will be no selective pressure to increase the population of the medication-resistant viruses. Thus, although present in minority populations, these resistant viruses may not be identified on initial HBV DNA Pol sequencing (sometimes referred to as "resistance genotyping" but best termed sequencing or using INNOLiPA for resistance). Since these resistant viral populations are present as a minor group, they may not be detected by nucleic acid testing assays. The most common assays are the sequencing assays that can measure down to viral populations of 20% or line probe assays that can measure down or detect viral variants as low as 5% of a given total population. Newer resistance tests that can identify mutations present in only 0.01% of the viral population are in development and should be available soon.

Therapeutic agents

Algorithms for the treatment of CH-B are updated regularly. Clinicians should be aware of the most recent guidelines [44,118], as well as any updates published on the available therapeutic agents, to make the best treatment decisions. Below is information on the individual agents approved for HBV treatment, as well as investigational agents in development.

Interferons

The term interferon is used to describe a large family of naturally occurring peptides that are included within an even larger group of molecules termed cytokines, which modulate immune function. This family of proteins is often involved in the host's control or elimination of acute and chronic viral infections. Interferon directly decreases viral replication by interference with DNA and RNA replication at the genome level. Interferon also indirectly modulates viral replication and viral protein synthesis by activating other immune pathways and modulating the level of other cytokines. The JAK/STAT pathway, responsible for triggering increased synthesis of antiviral signals and enzymes, such as 2′,5′-oligoadenylate synthetase and protein kinase, results in effective antiviral mechanisms within the cell, blocking viral replication at the translational (PKR, protein kinase R) and transcriptional (RNase L) levels. By enhancing major histocompatibility antigen (MHC) expression, interferon increases the identification of cells infected with HBV and leads to both cytolytic and noncytolytic viral clearance. Activation of natural killer (NK) cells and dendritic cells are other key steps in viral clearance.

At least 20 natural forms of interferon exist in humans including α2-interferon (IFN-α2; produced in lymphocytes), IFN-β (produced in fibroblasts), IFN-γ (produced in T cells), and IFN-ω. A number of these interferons (IFN-α2a and IFN-α2b) have been isolated and shown to have direct antiviral effects on HBV replication in humans [127]. Interferon is the only medication that has been proven to reduce and eliminate HBV infection, as shown by a high frequency of HBsAg seroconversion in chronically infected patients given interferon in randomized controlled trials [128,129]. Interferon therapy is not associated with medication resistance. Clearance of cccDNA is a key step in the control of CH-B, and interferon treatment is correlated with cccDNA suppression and clearance. Both patients with HBeAg-positive and HBeAg-negative CH-B benefit from treatment [129,130], demonstrated by improved outcomes in randomized controlled clinical trials (Fig. 81.6).

Over time, there is a cumulative increase in the number of patients who are HBV DNA negative, HBeAg negative, and anti-HBe positive with HBsAg seroconversion. These virological endpoints have been directly linked with a decrease in the number of patients with decompensated liver disease and HCC. Prednisone has been studied in clinical trials and practice and appears to be beneficial only for certain rare subgroups of patients such as those with modest viral replication and near-normal levels of liver enzymes, and is therefore not used in clinical practice [131].

Only an "ideal" subset of patients responds to interferon and clears HBsAg. These are patients with the higher levels of serum liver enzymes that signal a more active, ongoing, and effective immune response; lower serum levels of HBV DNA; and genotype A. The presence of elevated levels of liver enzymes and the presence of hepatic inflammation identify these "ideal" patients, who seem primed in their immune response to clear chronic HBV infection. Levels of HBV DNA also need to be in a mid to low range (< 20 000 IU/mL). Interferons such as IFN-α2b (Intron A; Schering-Plough, Kenilworth, NJ) and pegylated interferon alpha-2a (PEGASYS; Roche Pharmaceuticals, Nutley, NJ), have been approved for the treatment of CH-B in many countries worldwide, including for the treatment of adults and children in the United States. Treatment with IFN-α2b results in HBeAg clearance in 30%–50% of selected patients and HBsAg clearance in up to 15% of patients with long-term follow-up [132].

Results of several large randomized studies of pegylated IFN-α in patients with CH-B have been published [129,130,133,134]. These studies compared the safety and efficacy of pegylated IFN-α2a or IFN-α2b administered either alone or in combination with lamivudine. Janssen and colleagues [134] studied 307 patients with HBeAg-positive CH-B who were given pegylated IFN-α2b, at doses of 100 μg/week for 32 weeks, followed by 50 μg/week for 20 weeks, either with placebo or in combination with lamivudine (100 mg/day). There was no lamivudine monotherapy arm in this study. Patients were followed for an additional 26 weeks of treatment. At 52 weeks, loss of HBeAg was achieved in 29% of the patients treated with pegylated IFN-α2b alone and in 44% of the patients treated with pegylated IFN-α2b

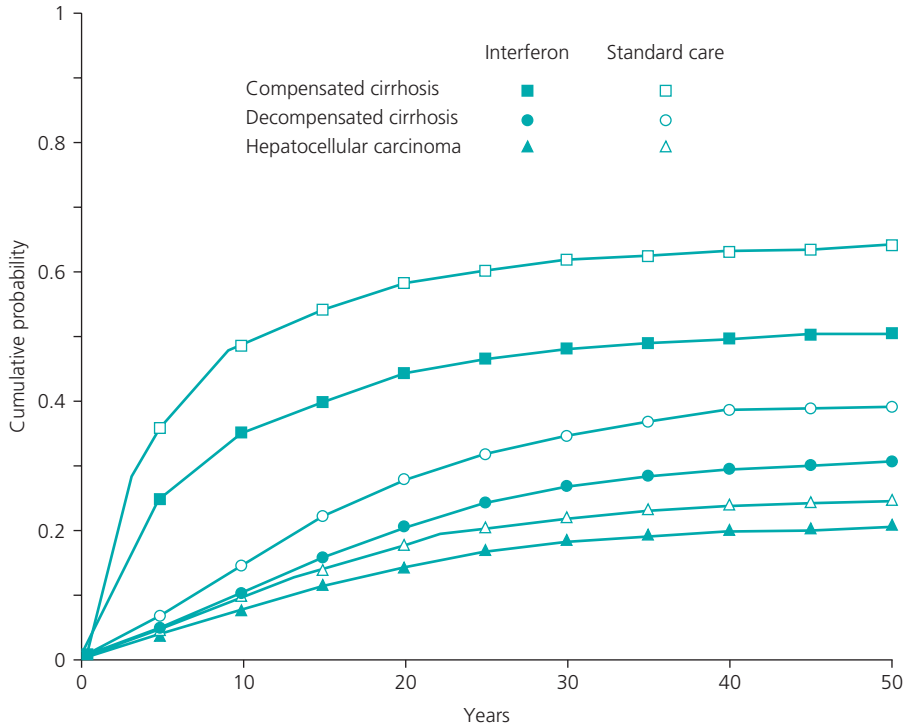

Figure 81.6 Outcomes of interferon treatment for hepatitis B virus infection. From Wong et al. [98], with permission from the American College of Physicians.

in combination with lamivudine ($P = 0.01$). However, at the end of follow-up, the proportions of patients in the two groups with loss of HBeAg were comparable: 36% (49/136 patients) in the pegylated IFN-α2b monotherapy group and 35% (46/130 patients) in the combination therapy group ($P = 0.91$). Clearance of HBeAg at the end of treatment but relapse during follow-up occurred more frequently among the combination therapy group (44%; 57 patients) than among the monotherapy group (29%; 40 patients) ($P = 0.01$). For response defined as suppression of serum HBV DNA or improvement in ALT concentration, there was no difference in response rate between the treatment groups. Response rates as defined by loss of HBeAg varied by HBV genotype ($P = 0.01$): rates were 47% for genotype A (42 patients), 44% for genotype B (10 patients), 28% for genotype C (11 patients), and 25% for genotype D (26 patients).

The ideal dose of the pegylated interferons varies according to the manufacturer (Schering-Plough vs Roche) but typically it is the hepatitis C dose, given once per week subcutaneously for 12 months. Interferon causes significant and various side effects that are well described. It is hoped that they will be reduced with newer forms of interferon or with lower doses used in combination with other forms of less toxic therapy, such as nucleoside or nucleotide analogue antivirals. Some examples of interferon side effects (which are often dose-related) are bone marrow suppression, psychiatric disturbances, alopecia, flu-like syndromes, nausea, weight loss, increased risk of bacterial infections, and hyperglycemia. The immunomodulator thymosin-α1 (Zadaxin, Sciclone Pharmaceuticals, San Mateo, CA), has been shown to be associated with HBeAg seroconversion and triple seroconversion when used as a single agent, but it may be more useful in combination with interferon. It is approved in the United States for treatment of liver cancer.

Nucleoside and nucleotide analogue antivirals

Modified nucleosides such as adenosine arabinoside (ara-A), ganciclovir, famciclovir, and other nucleoside analogues have been studied extensively over the last few decades. Adenosine arabinoside was the initial nucleoside analogue studied for the treatment of HBV infection. Treatment with ara-A resulted in a decrease in levels of HBV DNA in the serum but had significant untoward side effects including, in some patients, an irreversible peripheral neuropathy. Toxicity and weak antiviral effects were the major factors behind the failure of ara-A, ganciclovir, and similar medications. Fialuridine (FIAU), a fluorinated nucleoside with more potency than ara-A, was studied in the early 1990s and in humans was found to have major mitochondrial toxicity that resulted in pancreatitis, acidosis, liver failure, and death. Lobucavir was also studied in humans, but clinical trials were halted when this agent was associated with gynecological cancers in animals. These complications support close short- and long-term scrutiny of these powerful medications that can disrupt host cellular homeostasis and DNA replication. There are four nucleoside analogues approved for the treat-

ment of CH-B in the United States: lamivudine, adefovir dipivoxil, entecavir, and telbivudine.

Lamivudine

Lamivudine (3-thiacytidine, 3TC; Epivir-HBV, Zeffix, GlaxoSmithKline, Philadelphia, PA) was the first FDA-approved oral medication with proven efficacy against HBV, as shown by HBeAg seroconversion and triple seroconversion compared with placebo. Lamivudine is approved throughout the world for the treatment of CH-B. Data to date concerning efficacy include a rate of 17% HBeAg seroconversion at 1 year of therapy with lamivudine (100 mg daily) [28,135–137] and HBeAg seroconversion rates of up to 40%–45% by year 4–5 of therapy. Importantly, in the first trial to compare a nucleoside with placebo in a prospective study, lamivudine (100 mg daily) decreased the rate of liver decompensation events and even decreased the rate of liver cancer [138]. Unfortunately, resistance to lamivudine is common: after 1 year of treatment (150 mg daily) the frequency is greater than 20%; and by 5 years the frequency of resistance rises to 70%–100% [139]. This resistance can be associated with elevations in levels of liver enzymes; flares with decompensation and death or transplant have also been reported. Liaw and colleagues reported that patients infected with lamivudine-resistant HBV had an increasing rate of liver decompensation over the period of their study, approaching the rate of the events in the placebo group [138]. More importantly, resistance to lamivudine affects the sensitivity to the other nucleoside analogues and can affect the response to the nucleotide analogues. For these reasons, lamivudine is considered second-line therapy. Resistance to this agent is considered in more detail under "Lamivudine resistance" below.

Adefovir

Adefovir (adefovir dipivoxil; Hepsera, Gilead Sciences, Redwood City, CA) is a nucleotide analogue of adenine that has a high binding affinity for the HBV polymerase. Adefovir is FDA approved for the treatment of HBeAg-negative and HBeAg-positive CH-B as well as the treatment of lamivudine-resistant hepatitis B. Compared with placebo, adefovir caused reductions in HBV DNA and ALT in patients with the HBV precore and BCP mutants, as well as in those with wild-type virus. All patients were treated with adefovir at 10 mg/day for a minimum of 1 year in the two main placebo-controlled trials. The primary endpoint of treatment was histological improvement, which was achieved in 67% of patients on adefovir and 19% of patients on placebo. The data in the HBeAg-positive patients demonstrated histological improvement in 53% of the treatment group versus 25% of the placebo group [140]. Longer-term data have shown increasing improvement in the majority of adefovir-treated HBeAg-negative patients [141]. After 192 or 240 weeks of treatment, over 83% of patients had improvement in necroinflammation. Ishak fibrosis scores improved compared with baseline in 35%, 55%, and 71% of patients after 48, 192, and 240 weeks, respectively. At 240 weeks, serum HBV DNA levels were less than 1000 copies/mL in 67% of patients, with normalization of ALT in 69% of patients.

Renal injury due to adefovir and the related compound tenofovir is uncommon at the approved/licensed therapeutic doses, occurring at a rate of about 1%–2% per year in the first 2 years, and only rising to 3% after 5 years of adefovir monotherapy [141]. Monitoring of creatinine should be carried out routinely, either as per the product insert, in 6-monthly cycles, or with any suspected changes in renal status or co-administration of medications that may affect renal status.

Cumulative data with both HBeAg-positive and HBeAg-negative populations continue to show improved seroconversion with each additional year of use, and stable long-term suppression (up to 90%) off therapy in those patients with HBeAg seroconversion. Duration of therapy after seroconversion may affect the durability of seroconversion. In one study, of those patients who seroconverted from HBeAg-positive to anti-HBe while receiving adefovir (10 mg daily), seroconversion was maintained in 91% over a median off-treatment follow-up of 3 years [142]. The 9% of patients who reverted to HBeAg-positive status within 16 weeks off therapy had a shorter duration of postseroconversion adefovir treatment. Interestingly, with initial treatment response assessment, there is no difference in terms of viral suppression among the various HBV genotypes.

Resistance to adefovir is low in the first few years of monotherapy (0%, 3%, and 11% in HBeAg-negative patients at 1, 2, and 3 years, respectively [143]) but rises to 29% after 240 weeks in HBeAg-negative patients [141] and 42% after 235 weeks in HBeAg-positive patients [144]. However, the cumulative probability of mutations combined with virological resistance was 20% after 240 weeks in HBeAg-negative patients. Resistance to adefovir is associated with the rtN236T + rtA181T/V mutations (rt, reverse transcriptase).

In patients with preexisting lamivudine resistance who are given adefovir monotherapy, adefovir resistance develops much more rapidly. After 48 weeks of adefovir treatment, adefovir resistance has been seen in 18% of lamivudine-resistant patients compared with 0% in treatment-naive patients [145]. At 2 years, the cumulative incidence of resistance seen with lamivudine-resistant patients treated with adefovir monotherapy was 25.4% [146].

Much better results are seen when adefovir is added to lamivudine therapy (add-on combination). In one study of patients with lamivudine resistance and virological and clinical breakthroughs with lamivudine monotherapy, patients who were switched to adefovir monotherapy were compared with those given adefovir in addition to continuing lamivudine therapy [147]. At 12 months, there were no significant differences between the two groups of patients, with undetectable serum HBV DNA seen in 71% and ALT normalization in 90% of all patients. However, at a median treatment

duration of 40 months, these responses were maintained in all patients with adefovir added to lamivudine, whereas adefovir resistance and resulting virological and biochemical breakthroughs occurred at 15–18 months from start of treatment in 21% of patients on adefovir monotherapy.

The timing of an adefovir addition to lamivudine therapy in patients showing lamivudine resistance appears to be important. In one study that compared the results of adding adefovir on the development of "genotypic resistance only" (defined as 3–6 $\log_{10}$ copies/mL of HBV-DNA and normal ALT levels) with the results of adding adefovir on the development of "phenotypic resistance" (defined as > 6 $\log_{10}$ copies/mL of HBV DNA and high ALT levels), HBV DNA was undetectable by month 3 in all patients with only genotypic resistance compared with only 46% of patients with phenotypic resistance [148]. At 2 years, the rates of virological response were 100% in those with only genotypic resistance when adefovir was added compared with 78% in those with phenotypic resistance when the addition was made. Overall, combination therapy with lamivudine and adefovir, either in naive patients or in those patients with lamivudine resistance, does appear to be of benefit, with the rate of resistance greatly reduced if the two medications are started concomitantly (de novo combination), although there is no additional HBV DNA suppression.

Tenofovir is also a choice, with limited case series data supporting its use in this setting. The best option to reduce medication resistance is not to use agents with a significant or overlapping resistance profile. In patients with adefovir resistance (HBV Pol: rtA181T/V + rtN236T), entecavir is the best option although there are limited data showing that tenofovir can also result in additional viral suppression.

Entecavir

Entecavir (Baraclude, Bristol-Myers Squibb, Princeton, NJ) is a novel oral deoxyguanosine analogue that has demonstrated potent activity against HBV when used in doses of 0.5 mg daily for treatment-naive patients and 1.0 mg daily for lamivudine-experienced patients [149–151]. The potency of entecavir is derived from its efficient cellular phosphorylation, high intracellular concentrations, and competitive inhibition of HBV polymerase at three levels: priming, positive-strand synthesis, and negative strand synthesis [152,153].

In nucleoside-naive HBeAg-positive patients at 48 weeks, entecavir (0.5 mg daily) was superior to lamivudine (100 mg daily) for histological improvement (72% of entecavir-treated patients vs 62% of lamivudine-treated patients), reduction to undetectable serum levels of HBV DNA (67% for entecavir vs 36% for lamivudine), reduction in mean HBV DNA level from baseline (−6.9 $\log_{10}$ copies/mL vs −5.4 $\log_{10}$ copies/mL), ALT normalization (68% for entecavir vs 60% for lamivudine), and HBeAg seroconversion (21% for entecavir vs 18% for lamivudine) [150]. A second study compared the safety and efficacy of entecavir (0.5 mg daily) and lamivudine (100 mg daily) in 638 nucleoside-naive patients with HBeAg-negative chronic hepatitis B [154]. Clinical trial endpoints found that at 48 weeks entecavir was superior to lamivudine for histological improvement (70% of entecavir-treated patients vs 61% of lamivudine-treated patients), reduction to undetectable serum levels of HBV DNA (90% for entecavir vs 72% for lamivudine), reduction in mean HBV DNA level from baseline (−5.0 $\log_{10}$ copies/mL vs −4.5 $\log_{10}$ copies/mL), and ALT normalization (78% for entecavir vs 71% for lamivudine). Improvement in fibrosis, as measured by the Ishak fibrosis score, was similar among both treatment groups (36% for entecavir vs 38% for lamivudine). The 2- and 3-year data for both of these populations continue to show improvement in liver enzymes, increases in the number of patients with serum HBV at undetectable levels, and, in the HBeAg-positive patients, higher rates of HBeAg seroconversion [155,156].

The safety and efficacy of entecavir and lamivudine were also evaluated in lamivudine-refractory patients with HBeAg-positive chronic hepatitis B [157]. Entecavir (1.0 mg daily) was superior to lamivudine in terms of histological improvement (55% of entecavir-treated patients vs 28% of lamivudine-treated patients); achievement of the composite endpoint of HBV DNA less than 0.7 Meq/mL by branched chain DNA (bDNA) and ALT less than 1.25 × ULN (55% for entecavir vs 4% for lamivudine); HBV DNA less than 60 IU/mL through week 48 (21% for entecavir vs 1% for lamivudine); mean reduction in HBV DNA at week 48 (−5.1 $\log_{10}$ copies/mL for entecavir vs −0.5 $\log_{10}$ copies/mL for lamivudine;); and serum ALT less than or equal to 1 × ULN at week 48 (61% for entecavir vs 15% for lamivudine). HBeAg seroconversion at week 48 was similar for both groups (8% for entecavir and 3% for lamivudine).

The safety profiles of entecavir and lamivudine were comparable with respect to the incidence of serious adverse events and of total adverse events. Fewer patients in the entecavir group than in the lamivudine group discontinued because of adverse events. In addition, fewer ALT elevation "flares" occurred among entecavir patients than among lamivudine-treated patients, during both on-treatment and off-treatment periods. In lamivudine-naive patients, resistance to entecavir is approximately 1% after 3 years of treatment. However, in lamivudine-experienced patients, it is over 30% after 3 years.

Telbivudine

Telbivudine (Tyzeka, Sebivo, L-deoxythymidine, LdT; Idenix Pharmaceuticals, Cambridge, MA) is FDA-approved in the United States for treatment of CH-B in adults. Telbivudine (600 mg) has demonstrated superiority to lamivudine (100 mg) at both 1 year [158] and 2 years [159] in terms of HBeAg seroconversions and HBV DNA undetectability for HBeAg-positive disease, and HBV DNA undetectability for HBeAg-negative disease. Resistance to telbivudine is

common after 1–2 years of monotherapy [159]. It is an L-nucleoside that is completely cross-resistant with lamivudine.

Investigational compounds

Compounds approved for other indications or in phase III trials for the treatment of hepatitis B include the following.

Nucleoside or nucleotide antivirals

- Emtricitabine (FTC, Emtriva; Gilead Pharmaceuticals, Foster City, CA); in phase III trials for hepatitis B; approved in the United States for treatment of HIV.
- Clevudine (L-FMAU; Pharmasset, Princeton, NJ); in phase III trials for hepatitis B.
- Tenofovir (Viread; Gilead Sciences, Foster City, CA); in phase III trials for hepatitis B; approved in the United States for treatment of HIV.
- Tenofovir combined with FTC in a single tablet (Truvada; Gilead Sciences, Foster City, CA); in trials for treatment of CH-B and HIV/HBV coinfection; approved in the United States for treatment of HIV.

Nonnucleoside antivirals

- HepeX-B (XTL-001; XTL Biopharm, Cambridge, MA); human monoclonal antibodies; in phase II/III trials for hepatitis B; has orphan drug approval in the United States for liver cancer.

Noninterferon immune enhancers

- Thymosin-α1 (Zadaxin, SciClone, San Mateo, CA); immune stimulator that has orphan drug approval in United States for liver cancer.

Future developments

Future medications that are in development to treat CH-B include:

- interferons fused to albumin
- nucleoside and nucleotide analogue antivirals, including amdoxovir (DAPD; RFS Pharma, Tucker, GA), ANA 380 (LB80380; Anadys Pharmaceuticals, San Diego, CA), pradefovir (Remofovir; Valeant Pharmaceuticals; Costa Mesa, CA; licensed from Metabasis, San Diego, CA), racivir (RCV; Pharmasset; Princeton, NJ), valtorcitabine (LdC; Idenix Pharmaceuticals, Cambridge, MA), and fluoroguanosine (FLG; Medivir AB, Huddinge, Sweden)
- small interfering RNAs (siRNAs) and expressed interfering RNAs (eiRNAs), including HepX (Nucleonics, Horsham, PA)
- therapeutic vaccines, including Hi-8 HBV (Oxxon, Oxford, United Kingdom), HepaVaxx B (Virexx, Edmonton, Alberta, Canada), and HBV core antigen vaccine (Emergent BioSolutions, Rockville, MD).

Treatment might also involve DNA vaccines that provide ongoing internal immune suppression through production of HBV-derived proteins [59,160]. Liposomes may eventually be used to deliver vaccines, as with those originally developed for hepatitis B by means of intramuscular injections.

Genotypic resistance of hepatitis B virus to nucleoside analogues

As discussed previously, the emergence of HBV mutants that show resistance to nucleoside analogues as a result of genetic mutation has been observed. The discussion that follows concerns important amino acid changes in the HBV POL sequence that have been detected in clinical isolates and shown to confer or modify resistance to lamivudine, adefovir, entecavir, and other nucleoside/nucleotide analogues. A standardized numbering system for the HBV POL was proposed by Stuyver et al. [161], and this consensus is widely accepted.

Lamivudine resistance

Initial clinical trials established that treatment of chronic HBV infection with lamivudine was safe and well tolerated, and caused rapid and substantial decreases in viremia. However, decreases in viremia were not stable and rebounded in the majority of cases during treatment or after treatment was stopped [162–165]. As long-term trials progressed, it became apparent that rebounds could occur during treatment, the majority of which have been attributed to the development of medication resistance. This resistance was observed at a greater frequency and at an earlier time point in the transplant setting [166–168] compared with that seen with immunocompetent patients [169–172]. Lamivudine-resistant populations of HBV were also detected in patients who were coinfected with HBV and HIV and who were receiving lamivudine as part of their HAART [173–175]. Although varying frequencies of resistance and different lengths of time preceding the detection of resistance have been reported, the incidence of resistance in both immunocompetent patients and immunosuppressed patients clearly correlates with treatment duration. During the first year of lamivudine therapy, resistance developed in 14%–32% of cases studied [176–178], a frequency that increased to greater than 50% after 2 years [171] and to 70%–100% by 5 years. Lamivudine is universally accepted as second-line therapy due to this resistance issue with its concomitant risk of flare, decompensation, liver disease progression, and death [44].

The site in the HBV reverse transcriptase or "polymerase" most commonly associated with lamivudine resistance is the YMDD motif in the C domain (rtM204V/I). These substitutions changed the conserved YMDD tetrapeptide in motif C to YIDD or YVDD; this is analogous to the base substitutions causing the amino acid substitution M184V in the HIV reverse transcriptase, which likewise confers lamivudine resistance [179]. Mutations that alter the YMDD motif appear to have multiple effects on the biological properties of the polymerase but, in general, they seem to increase fidelity and reduce the ability to process the viral genomic code. Three-dimensional models of the HBV polymerase predict that substitution of

either isoleucine or valine – both of which are smaller than methionine – would increase the size of the dNTP binding pocket and decrease binding affinity for lamivudine triphosphate [180,181].

Numerous different mutational patterns appear to be responsible for the lamivudine resistance, but the majority of cases of HBV resistance to lamivudine affect the rtM204 codon (Fig. 81.4). One pattern is associated with two amino acid substitutions (rtL180M + rtM204V) whereas a second pattern causes a single substitution (rtM204I) [166,168]. A variety of other mutations, which cluster mainly in the polymerase A (rtL80V/I), B (rtV173L and rtL180M) and C motifs, have also been described in association with rtM204V/I [182,183]. In a small number of cases, lamivudine resistance can be caused by selection of the rtA181T/V (B domain) change, which is cross-resistant with adefovir and partially cross-resistant with tenofovir, the two available nucleotide analogues.

Nucleotide changes, which produce the rtM204V/I polymerase mutations, also alter codons in the overlapping surface antigen ORF and may lead to HBV isolates with reduced anti-HBs binding, which may have public health implications for hepatitis B vaccinse or immune escape. The nucleotide change responsible for the rtM204V change also results in an isoleucine to methionine substitution at residue 195 (SI195M) of HBsAg. Several nucleotide substitutions can cause the rtM204I change in the polymerase. Depending on the specific nucleotide substitution, there are three possible alterations to the surface antigen:

- a tryptophan to serine change at position 196 (SW196S)
- a tryptophan to leucine change at position 196 (SW196L) or
- the introduction of a stop codon, which would truncate the surface antigen.

It is unlikely that these surface antigen changes affect medication resistance, but they may perhaps affect antigenicity and subsequent binding of anti-HBs, either natural antibody or through therapeutic administration of HBIG directed at the "a" determinant. Mutations that generate the rtL80V/I or rtL180M are silent for the surface antigen ORF; however, the rtV173L does result in sE164D in HBsAg, which is within the neutralization domain of the envelope and results in reduced anti-HBs binding.

Other mutations that alter the amino acid sequence of HBV POL have emerged under selection pressure of lamivudine in vivo. Although their functional consequences remain largely unknown, two possibilities exist: changes may enhance medication resistance or replication efficiency (or both). Presumably, increases in fidelity and processivity (respectively) are major contributors to each process.

Adefovir and tenofovir resistance

Resistance to adefovir dipivoxil is initially low in the treatment-naive patient given adefovir monotherapy, but it rises over time. It may not be seen in the first year, but then increases to approximately 3% after 2 years and 11% after 3 years [143]. At week 192, the cumulative probability of adefovir resistance is 15%–18% [184,185]. After 240 weeks, it rises to 29% in HBeAg-negative disease [141] and 42% in HBeAg-positive disease [144]. Adefovir resistance is conferred by substitution of threonine for asparagine at codon 236 (rtN236T), which is located in the D domain of the polymerase, a change that does not significantly affect sensitivity to lamivudine. However, another mutation selected in cases of adefovir failure, rtA181T/V, which is located in the B domain of the enzyme, does affect lamivudine sensitivity. The mechanism of rtN236T-induced resistance has been shown to be indirect perturbation of the triphosphate binding site, whereas indirect steric hindrance accounts for resistance caused by rtA181T/V [186]. More importantly, there are data showing the cumulative incidence of adefovir resistance in patients who are lamivudine resistant to be 6.4% at 12 months, but substantially higher (25.4%) at 24 months [146].

Resistance to tenofovir has been proposed in patients coinfected with HIV and HBV who are receiving both lamivudine and tenofovir as part of HAART [187]. The profile of tenofovir resistance includes rtA194T + rtL180M + rtM204V, and the backbone of lamivudine resistance (rtL180M + rtM204V) is required in order for the rtA194T to be associated with medication resistance.

Entecavir resistance

Resistance to entecavir in treatment-naive patients is uncommon in the first 2 years and around 1% by year 3. However, in lamivudine-experienced patients, entecavir resistance emerges quickly and reaches over 30% by year 3. Entecavir resistance requires three mutations in total: rtL180M + rtM204V plus one other mutation of rtT184, rtS202, or rtM250. The initial evidence from phase II and III studies indicated that the accumulation of additional mutations in preexisting lamivudine-resistant HBV polymerase was required for expression of resistance to entecavir [157,188]. Thus, at least three point mutations in most, and up to four or five point mutations in some, are necessary before a rise in HBV DNA levels will occur during treatment with entecavir. Three-year resistance data on entecavir reveal a cumulative rate of medication resistance of 1.1% in lamivudine-naive patients, but in patients with preexisting 180 and 204 mutations, the rate of resistance is over 30% [156], supporting the contention that single-medication therapy for patients with these resistance mutations is not ideal and should be supplanted by combination therapy. The data from the phase II dose-ranging study in lamivudine-refractory patients [151] and the phase III lamivudine refractory study [157] were the basis for the dosage recommendations of 1.0 mg/day for patients with previous exposure to lamivudine who show evidence of lamivudine resistance. The phase III lamivudine refractory study showed a 5.1 $\log_{10}$ decrease in HBV DNA, with 20% of patients becoming HBV DNA negative by PCR, a

rate comparable with or better than that seen with adefovir monotherapy in separate trials. The pattern of resistance to entecavir includes: rtI169T (B domain), rt184G (B domain), rtS202I (C domain), and rtM250V (E domain) in various combinations of rtL180M + rtM204V [188].

Telbivudine resistance

Telbivudine is the β-L-isomer of thymidine. After 24 weeks of therapy, it results in a 4–6 log drop in HBV DNA levels with a daily oral dose of 600 mg. The cumulative rates of resistance at 2 years have been reported as 21.6% for HBeAg-positive patients and 8.6% for HBeAg-negative patients when patients with a 1 log rise from nadir were sequenced for viral resistance mutations [159]. Week 24 viral load has been shown to predict telbivudine resistance at year 2, with minimal resistance seen when viral load is undetectable at week 24 (2% for HBeAg-negative; 4% for HBeAg-positive) but high-level resistance at year 2 in those with elevated viral loads at 24 weeks [159]. These findings would support an algorithm that proposes to change or add a second agent at 6 months if a patient is not HBV DNA negative at that point. Resistance results from the selection of the rtM204I mutation indicate cross-resistance with lamivudine; this substitution has been found in all telbivudine patients with confirmed virological breakthrough. In some breakthrough patients, secondary mutations have been identified, including rtA181T/V; however, these mutations are of uncertain significance, with no breakthrough seen to date with these mutations alone.

Clevudine resistance

Clevudine (L-FMAU) is a pyrimidine nucleoside analogue that has been found to be effective against HBV, causing a 4–5 log decrease in viremia. As with telbivudine, the main resistance selected is the "lamivudine resistance" profile, including rtL180M + rtM204V.

Antiviral cross-resistance of HBV variants

Clinically, a variety of medication-resistant HBV mutants have emerged under the selective pressures of lamivudine, adefovir, and entecavir. Rational design of effective chemotherapeutic strategies for the future will require determination of the sensitivities of these mutants to both existing and novel nucleoside and nucleotide analogues, as they become available. This will be necessary both to provide effective treatment to patients who are already infected with medication-resistant HBV and to prevent, or at least minimize, the possibility of resistance arising in treatment-naive individuals.

Adefovir dipivoxil can inhibit the replication of most strains of lamivudine-resistant HBV (rtM204V/I but not A181T/V). However, as discussed above, the most successful use of adefovir in lamivudine-resistant patients is when it is added to lamivudine rather than substituted as monotherapy, and used at the first appearance of resistance mutations, well before virological failure occurs.

Combination therapy to counter resistance

There is no FDA-approved combination therapy for treating HBV, much less for treating lamivudine-refractory patients. However, there is an emerging body of data suggesting that combination therapy can decrease antiviral resistance, the single factor that is likely to be of the greatest importance for maintaining effective long-term therapy, and that such combination therapy is likely to be more effective in treatment-naive patients rather than when used to add or replace an antiviral agent after resistance develops [189]. Clinical experts are proposing using adefovir with lamivudine from the outset, or using tenofovir with lamivudine, emtricitabine, or entecavir [44], with ever more substantial data to support such a creative approach.

Management of special populations

Alcohol use in patients chronically infected with HBV increases the risk for progression to cirrhosis, results in more rapidly progressive liver disease, and also increases the risk of liver cancer [29,190]. Patients with chronic liver disease should not drink alcohol. Patients with documented chronic HBV infection should be educated about the risks of alcohol use and advised not to drink any alcohol because the threshold for complications of alcohol use is unknown.

Dialysis patients need to be tested for evidence of HBV infection. Those without evidence of immunity need to be vaccinated with a double dose of vaccine, often repeatedly, until anti-HBs seroconversion occurs. Patients who are chronically infected have historically been isolated from other patients in dialysis centers, but advances in the sterile techniques used, the exchange of equipment in dialysis centers, and other improvements in therapy no longer mandate complete separation of such patients.

Treating chronic HBV infection in dialysis patients should focus on the presence or absence of progressive liver disease as demonstrated by biopsy because liver enzymes have little value in managing such patients. Interferon probably has reduced efficacy in dialysis patients because of their relative state of immunosuppression. Adefovir and entecavir can also be used, but dose adjustments must be made to take into account the dominant renal clearance of these medications and potential renal toxicity of adefovir.

The management of HBV infection after liver transplant is complex [29]. The most important step is to prevent graft infection or reinfection; this can start before liver transplant by screening donors for anti-HBc. Recipients who are HBsAg-positive must be screened for HBV DNA replication. Suppressing HBV replication to low levels using nucleoside or nucleotide analogues before the patient enters the operating room is a key step. Ideally, patients should not undergo liver transplantation if they are HBV DNA positive in their serum. Perioperative and postoperative HBIG therapy is used

to bind circulating virus and prevent graft infection [191], and results in improved survival and a much lower rate of HBV graft infection, thereby preventing aggressive liver disease. Lamivudine maintains viral replication at a low level, and when used in combination with HBIG results in a risk of less than 5%–20% for graft infection in compliant patients [192]. Newer nucleoside and nucleotide analogues may also be used in a similar clinical setting. The use of two oral agents may obviate the need for the costly use of HBIG. The emergence of mutant viruses that are resistant to lamivudine is a major problem in the treatment of HBV disease after liver transplant, with over 30%–40% of patients developing resistance, followed by more aggressive liver disease [193]. Combination therapy with adefovir and lamivudine pretransplantation to achieve HBV DNA PCR negativity, followed by maintenance of such combinations with HBIG posttransplant, significantly reduces the risk of severe viral recurrence. The role of entecavir, tenofovir, and combinations of these agents is evolving. It is hoped that in the near future we may be able to eliminate HBIG therapy.

Other solid organ transplant recipients, including bone marrow, heart, and kidney recipients, are at risk for reactivation of HBV disease after organ transplant [194–198]. An expert in hepatitis B management must evaluate these patients before they undergo transplantation. If active HBV replication is present, avoiding or delaying organ transplantation is advised unless successful suppression of HBV disease can be achieved. Some transplant centers will not perform organ transplants on patients who are HBsAg positive. Another clinical possibility is the transmission of HBV from the organ donor to the recipient. In such cases, the risk of transmission is nearly 100% if the donor is HBsAg positive. Donation is rarely performed in this circumstance. A further risk is the presence of anti-HBc in the donor. This poses a small (< 3%) risk of HBV transmission for organ transplant recipients other than liver, but a significant risk to liver transplant recipients, ranging up to 70% [199,200]. Finally, recipients who are anti-HBc positive can reactivate "latent" HBV disease when immunosuppression is initiated [201]. During any chemotherapy or application of immunosuppression, these patients should be treated prophylactically with an oral agent or monitored with liver tests monthly. After solid organ transplant, any patient who develops acute liver disease needs to be assessed by HBsAg testing. Conversely, bone marrow transplants have resulted in HBsAg clearance by adoptive immunity from the donor's immune cells [202].

In summary, chronic HBV infection continues to pose a serious health-care problem worldwide. Emerging molecular, clinical, and treatment information will markedly enhance our ability to manage patients with CH-B and to prevent liver cancer, death, and liver transplantation. Hopefully, in the near future, with the advent of universal infant vaccination combined with treatment of all patients with CH-B, we can reduce the impact of this deadly disease and remove HBV from the top 10 list of causes of death worldwide.

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Epidemiology

Background

Chronic hepatitis C virus (HCV) infection has reached pandemic proportions, with an estimated 170 million individuals infected worldwide, which represents an overall prevalence rate of 2% [1–5]. There is a marked geographic and temporal variation in the incidence and prevalence of HCV infection [2,3]. In the United States, the prevalence of antibody to HCV (anti-HCV) in an updated survey was 1.6%, with 1.3% or 3.2 million persons actively infected based on detection of HCV RNA [1]. Data from population-based studies demonstrate that chronic HCV infection, the leading cause of chronic liver disease, is the reason for approximately 40% of liver transplantations, and causes an estimated 8000–10 000 deaths annually in the United States [2].

Incidence

Chronic hepatitis C is the most common chronic bloodborne infectious disease in the United States, even though the number of new infections per year has declined since 1989 by more than 80% to 36 000 cases in 1996 [5]. The incidence of HCV infection has declined yet further since 1996, with the Centers for Disease Control and Prevention (CDC) estimating that there were 26 000 new cases in 2004 [6]. Based on surveillance data, it appears that the incidence of HCV infection rose during the 1960s and 1970s, remained stable in the 1980s, and has subsequently declined. New infections were particularly common among young Hispanic males between 20 and 39 years of age [1,2,5]. Injection drug use is the most common risk factor associated with the transmission of HCV infection and is responsible for as many as 38% of newly diagnosed cases [5].

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Prevalence

The worldwide prevalence rates of chronic HCV infection vary from as low as 0.4% in the Western European countries to as high as 22% in Egypt and other parts of Africa [2,7–12]. Approximately 85% of patients develop chronic liver disease after acute HCV infection. Therefore, the past incidence of acute HCV infection is a key predictor of the current prevalence rate of chronic hepatitis C [4]. The Third National Health and Nutrition Examination Survey (NHANES III), conducted from 1988 to 1994, provided data on the past incidence of acute HCV infection using national seroprevalence and age-specific incidence data from the sentinel counties surveillance program [13]. The overall national prevalence of anti-HCV was 1.8%, which corresponds to an estimated 3.9 million individuals infected with HCV. Approximately 65% of these individuals were 30–49 years of age. Serum HCV RNA was detectable in 74% of cases, which corresponds to an estimated 2.7 million actively infected individuals nationwide. Of these chronically infected individuals, 74% were infected with genotype 1 (57% with genotype 1a, and 17% with genotype 1b). The most recent updated estimate of prevalence from NHANES data collected from 1999 to 2002 is that 1.6%, or 4.1 million, have anti-HCV, of which 79.9% are actively infected with detectable serum HCV RNA [1]. In this updated survey, the peak in age-specific prevalence moved from persons aged 30–39 years to those aged 40–49 years. The prevalence rate was higher in non-Hispanic blacks than the other two ethnic groups analyzed, i.e., non-Hispanic whites or Mexican Americans. Injection drug use was the strongest risk factor, but 20 or more lifetime sexual partners and blood transfusion before 1992 were also risk factors for HCV infection.

Demographic characteristics

Infection with HCV has no boundaries and affects individuals from all walks of life, ranging from children to the elderly. The highest incidence of acute hepatitis C is found among individuals between 20 and 59 years of age, with male patients outnumbering female patients [1,2,5]. African Americans

and whites have a similar incidence of acute HCV infection, whereas individuals with Hispanic background have the highest incidence rate. In the general population, the highest prevalence rates of HCV infection are found among individuals between the ages of 30 and 49 years of age, with males predominantly affected [1,2,4]. In contrast to the rate of acute HCV infection, African Americans have a substantially higher prevalence rate of chronic HCV infection when compared with whites. A study conducted to estimate seroprevalence rates of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and HCV among individuals with severe mental illness in the United States showed a 20% prevalence rate of HCV infection, which is 11 times the overall estimated prevalence rate for the HCV infection in the general population [14]. Higher rates of HCV infection were also noted in patient groups with HIV and HBV infection. Individuals with asymptomatic HCV infection are frequently undiagnosed and fail to receive appropriate treatment to eradicate viremia and retard disease progression; they may, therefore, be a source of infection to others [14].

Infection with HCV may differ in the pediatric age group (as compared with adults) with respect to transmission, natural history, and response to treatment. The prevalence of chronic HCV infection in children ranges from 0.05% to 0.4% [15]. The major mode of acquisition has shifted from parenteral transmission to maternal–infant transmission. However, the actual rate of vertical transmission is low, ranging from 4% to 10% [2].

Transmission patterns

Based on age-specific prevalence data, at least three distinct transmission patterns of HCV infection have been established. First, in developed nations such as the United States and Australia, chronic HCV infection mainly affects individuals between the ages of 30 and 49 years, indicating that the risk for HCV infection was at its peak in the recent past (10–30 years ago) and primarily affected young adults [1,2]. Second, in nations such as Japan and Italy, HCV infection mainly affects older individuals, reflecting that the risk for HCV infection peaked in the distant past. Finally, Egypt is a classic example of an ongoing risk for acquiring HCV, i.e., high rates of infection are observed in all age groups. In countries with the first pattern, injection drug use is the most common risk factor associated with chronic HCV infection, whereas in those with the second or third patterns, unsafe injections and contaminated equipment used in health-care facilities were primarily responsible for HCV transmission [1,2]. The contribution of different risk factors predicts the variability in the transmission patterns of HCV infection between various regions and countries.

Modes of transmission

In the United States, the relative importance of the two most common exposures associated with transmission of HCV, blood transfusion and injection drug use, have undergone a substantial change [2,16]. Blood transfusion, which accounted for a significant proportion of HCV infection acquired more than 10 years ago, has been almost completely eliminated as a risk factor for HCV infection because of improved screening of blood and organ or tissue donors. Since 1990, the risk for transfusion-transmitted HCV infection has been almost nonexistent, as demonstrated by the inability of the CDC sentinel counties' viral hepatitis surveillance system to report any transfusion-associated cases of acute hepatitis C [6]. Therefore, the risk of transfusion-related transmission of HCV is negligible. In contrast, injection drug use has consistently been associated with a significant proportion of chronic HCV infections and accounts for 50%–60% of HCV transmission in the United States [1,2,5]. Other known exposures (occupational, hemodialysis, household, perinatal) together account for about 10% of HCV transmission. In up to 10% of individuals with chronic HCV infection, no recognized source of infection can be identified, and most individuals in this category come from a low socioeconomic status. Case–control studies have found no association of increased HCV transmission with military service [16].

The variation in HCV prevalence rates in a certain population is determined by the mode of transmission [2,16]. The highest prevalence rate of HCV infection is associated with high-dose or recurring direct percutaneous blood exposure, which occurred in injection drug users, hemophiliacs who received clotting factor concentrates produced before 1987, and transfusion recipients of HCV-positive blood or blood products before 1992 [2,12,16,17]. Moderate prevalence rates of HCV infection are associated with smaller direct percutaneous exposures, such as experienced by individuals on long-term hemodialysis. Lower prevalence rates are associated with percutaneous or mucosal exposures through high-risk sexual behavior or with low-dose percutaneous exposures experienced by health-care workers. The lowest prevalence rate of HCV infection is found among individuals who lack high-risk characteristics, as exemplified by the screened volunteer blood donor population.

Injection drug use

In the early 1990s, the decrease in the incidence of HCV infection was associated with a sharp fall in HCV infection among injection drug users [4,5]. Up to 65% of injection drug users have detectable anti-HCV within 1 year of starting this high-risk behavior [2,17]. Despite the high proportion of new infections caused by injection drug use, for reasons that are unclear, the dramatic decline in incidence of acute hepatitis C since 1989 correlates with a decrease in cases among injection drug users [2,4,5]. Several differences in risk factors associated with HCV prevalence rates were observed in two populations of young injection drug users from New York City, suggesting that indirect transmission of HCV may occur [17]. Among subjects aged 17–59 years, the strongest factors

independently associated with HCV infection were injection drug use and high-risk sexual behavior. Independent drug-related risk factors included frequent injections, heavy crack smoking, injecting in a shooting gallery, and syringe-mediated sharing. Sharing of drug preparation equipment has been implicated as a possible route of HCV transmission [2,5]. Intranasal cocaine use can also lead to transmission of HCV infection [18]. Other factors independently associated with HCV infection included poverty, having had 12 or fewer years of education, having been divorced or separated, and residence in an urban area as compared with the suburbs [19,20].

Blood transfusion

The current risk of acute infection with any pathogen associated with transfusion has decreased to less than 5% [4]. The exact risk of transfusion-related HCV infection is 0.6% per patient and 0.03% per unit of blood transfused [21–24]. Among donors whose blood units passed all the screening tests, the risks of donating blood during an infectious window period were estimated as follows: for HIV, 1 in 493 000; for HTLV, 1 in 641 000; for HCV, 1 in 103 000; and for HBV, 1 in 63 000 [23]. The incidence of posttransfusion HCV infection has decreased appreciably since the implementation of donor screening using surrogate markers initially and then specific tests for anti-HCV [2,4,5,21,25–27]. In 1984, the sharp decline in the incidence of transfusion-associated hepatitis was noted when donors were screened with alanine aminotransferase (ALT) and anti-HBc. In 1990, anti-HCV testing was added to the screening protocol and resulted in a further decrease in the incidence of transfusion-related hepatitis. Before 1984, transfusion was the most common route of HCV infection; in particular, patients receiving multiple transfusions had the highest risk [2,5]. In hemophiliacs, unheated and dry heat-treated clotting factor concentrates carried a high risk of transmitting HCV infection [28]. In comparison, clotting factor concentrates inactivated by vapor heating had a significantly lower (close to zero) risk of transmitting HCV infection [28,29].

Chronic hemodialysis

The transmission of HCV infection is common among hemodialysis patients. Transmission of infection in this setting has been related to the number of blood transfusions, the duration of hemodialysis, male gender, African American race, diabetes, HBV infection, previous renal transplant, alcohol or substance abuse, and nosocomial transmission of HCV in the dialysis unit [30–34]. Studies have failed to determine the most likely mode of transmission in dialysis units; therefore, emphasis is placed on strict adherence to blood collecting and handling using universal safety precautions, careful attention to hygiene in the dialysis units, and sterilization of dialysis machines to minimize the risk of HCV transmission [30,31,34,35]. Nosocomial transmission of HCV not related to blood transfusions has been reported between patients dialyzed on the same shift and not sharing the same dialysis machine [36]. In dialysis units in Italy and France, the incidence and prevalence rates of HCV infection are 1%–2% and 20%–60%, respectively [37], and the mean prevalence of HCV infection in the analysis of dialysis units from three continents was 13.5%, ranging from 2.6% to 22.9% [30].

Occupational

The reported prevalence rate of HCV infection in health-care workers such as orthopedic and oral surgeons is 1%–2%, which is comparable to the prevalence rate in the general population [38–43]. However, some surveys have demonstrated seroprevalence rates ranging between 0.6% and 4.5% in health-care workers, exceeding the seroprevalence rate of HCV infection in blood donors at the same facility by up to 4.5 times [43–45]. This observation has been supported by other studies that have demonstrated significantly higher prevalence rates of HCV in dentists and oral surgeons as compared with controls [46]. There are no reports documenting transmission of HCV through mucus membranes. However, accidental needlestick exposure and blood splashes to the conjunctiva have been associated with transmission of HCV infection [42,47,48]. The incidence of HCV transmission by accidental needlestick exposure varies from 0% to 10% and has been associated with the viral load in the inoculum, depth of inoculation, and size of needle [5,49–51]. The risk may be higher with needles that have hollow channels compared with non-hollow sharps [50].

Nosocomial transmission of HCV infection has been suspected, and health-care workers have been reported to transmit HCV infection to their patients [36,52]. HCV was detected in the biopsy suction channel after an endoscopic procedure in 27% of patients with chronic HCV infection, and HCV RNA was detected on the biopsy forceps in 6% of cases [53,54]. A thorough disinfection procedure appears to be effective in eliminating the risk of HCV transmission. Percutaneous transmission of HCV as the result of lack of sterile practices and reuse of contaminated supplies in traditional medicine, folk medicine, tattooing, body piercing, commercial barbering, circumcision, and scarification has been documented [55–57]. On the other hand, there are case–control studies that show no evidence of increased HCV transmission associated with exposures resulting from medical, surgical or dental procedures, tattooing, acupuncture, ear piercing, or foreign travel [16].

Sexual transmission

It is estimated that up to 20% of patients with chronic hepatitis C and no identifiable risk factor may have acquired HCV sexually [2,58,59]. HCV RNA has been detected in the semen of as many as one-third of HCV viremic men [58]. Although seminal viral loads are low, semen could be infectious and play a role in the spread of HCV infection. The risk of acquiring

HCV rises in men who have sex with men and in bisexual men [60,61], in heterosexuals with multiple sexual partners [60], with the number of sexual contacts [62], with the presence of other sexually transmitted diseases such as syphilis and HIV [63], in partners of injection drug users [10], in prostitutes and their contacts [64], with failure to use condoms [60,61], with sexual activities involving trauma [60,61,64], and with divorce and separation [3]. Patients with HCV and HIV coinfection have a fivefold greater risk of transmitting HCV to their partners [65]. Male-to-female transmission of HCV is higher than that of female-to-male [66,67]. The risk of sexual transmission of HCV is significantly lower than HBV, HIV, and other sexually transmitted diseases [61].

Vertical transmission

The rate of mother-to-child transmission of HCV is low, ranging from 4% to 10% [2,68–71]. However, based on the prevalence of HCV infection in pregnant woman in the Western world (0.09%–0.25%), a reasonable number of pediatric HCV infections may occur [71]. One prospective trial showed that the transmission rate of mother-to-infant HCV infection is 7.8% in anti-HCV-positive mothers [72]. It has been demonstrated that high maternal viral load and HCV and HIV coinfection are significant risk factors for vertical transmission of HCV, although a threshold HCV RNA has not been identified [2]. Review of the data suggests that the mode of delivery (vaginal versus cesarean) does not affect the risk of transmission of HCV. Most infected infants do not test positive for HCV RNA during the first month after birth, suggesting that infection occurs at the time of delivery rather than in utero. The placenta acts as a barrier and prevents free, direct communication between maternal and fetal blood [73–75]. This barrier is broken at birth, resulting in free communication between the maternal and fetal blood [76]. Elective cesarean delivery is associated with the lowest amount of microtransfusion from mother to fetus [76], suggesting that patients with high HCV load and other risk factors for vertical transmission might benefit from elective cesarean section [66,77].

Molecular virology and pathogenesis

Molecular organization and characteristics

HCV RNA was isolated in 1989 using a random-primed complementary DNA library established from the plasma of patients with suspected non-A, non-B hepatitis infection [78–80]. Using electron microscopy, HCV has been visualized in chimpanzee hepatocytes and human T and B cells [81]. HCV genome is a 9.6-kilobase (kb), single-stranded RNA molecule. The genomic size and molecular organization suggest close association with flaviviruses and pestiviruses [78]. Data from filtration studies indicate that the HCV ranges in size from 30 to 60 nm in diameter [82]. The virus is coated with a bilayer lipid envelope [83]. The crucial structural constituent of the HCV genome is the core protein, which is found at the N-terminal of the polyprotein. The core protein is strictly conserved in HCV genotypes. The coating glycoproteins that form the HCV envelope are referred to as E1 and E2 [84]. The E2 glycoprotein has two hypervariable regions (HVR), HVR1 and HVR2. These hypervariable segments provide HCV with the basic mechanisms for evading the host immune defenses and establishing chronic infectivity [85–88]. Anti-HVR1 antibodies provide protection against HCV infection, supporting the fact that this region harbors a crucial signal for a neutralization factor. HVR1 activates helper T-cell responses during HCV infection. It was noted that the frequency of anti-HVR1 T-cell responses was more pronounced in patients who recovered after interferon (IFN) therapy as compared with individuals who did not respond [89,90]. The HCV must bind to a receptor on the cell membrane to facilitate entry into the cell. It has been shown that E2 envelope proteins have the ability to bind cell membranes [91]. The entry into the cell and exposure to the intracellular acidic environment causes irreversible structural changes in envelope proteins coating the HCV genome [92]. This is followed by release of genomic RNA triggering protein translation. Furthermore, multiple independent adaptive mutations cluster in the HCV nonstructural protein NS5A and confer increased replicative ability in vitro [93].

Genotypes and quasispecies

The open reading frame of HCV RNA demonstrates polymorphism and encodes a polyprotein precursor consisting of approximately 3000 amino acids, varying among different genotypes. HCV is classified into four hierarchical levels, which include genotypes, subtypes/subgenotypes, isolates, and quasispecies [94–98]. HCV appears to exist simultaneously within an individual as a series of related, but immunologically distinct, variants called quasispecies. The existence of these quasispecies may provide a mechanism for the virus to escape the host immune response. The HCV genotype, however, is stable and does not change over the course of infection in an individual. The evolutionary dynamics of the HCV quasispecies during the acute phase of a hepatitis C infection predict whether the infection will resolve or persist [99]. Individuals infected with HCV can develop superinfection with other HCV genotypes leading to eradication or suppression of the primary genotype. Simultaneous infection with multiple HCV genotypes may result in concomitant infection or eventual dominance by one genotype [100]. The genotype distribution showed no relation to the severity of the liver disease, or to the duration of the disease [101].

Pathophysiology and immunopathogenesis

The mechanistic pathways involved in the pathogenesis of chronic HCV infection include direct HCV-induced cyto-

pathic insult to hepatocytes or injury resulting from specific or nonspecific immune responses [102,103]. It has been demonstrated that cytotoxic T cells and cytokines produced by both CD4+ (T helper) and cytotoxic T cells may trigger the inflammatory response in the hepatocyte. The T helper type 2 immunity, as well as the weak HCV-specific T-cell response, is associated with viral persistence [102–104]. The production of interleukin-12 and nitric oxide is critical for the induction of T helper type 1 and innate immunity [104]. Patients infected with HCV mount a humoral immune response to epitopes of HVR1. However, HCV infection may be difficult to eradicate because of the rapidly evolving nature of HVR1, which results in the generation of variants that are not recognized by preexisting antibodies [105]. Treatment-induced control of viremia is associated with the development of HCV-specific T-cell responses with enhanced IFN-γ and low interleukin-10 production [106]. The greater efficacy of combination therapy using IFN-α plus ribavirin may be related to its ability to suppress HCV-specific interleukin-10 production [106]. The resolution of HCV infection is not followed by protective immunity against reinfection with homologous or heterologous strains [107].

Histopathology

Chronic hepatitis C does not have characteristic pathognomic features and demonstrates wide variation of pathological findings [108]. The mechanism underlying hepatocyte and bile duct injury in HCV infection results from the host immune response to the virus and direct viral cytopathogenicity. Acute hepatitis C is histologically indistinguishable from acute viral hepatitis with other causes [108,109]. HCV infection can present histologically as a triad of lymphoid aggregates in portal tracts, epithelial damage of small bile ducts, and microvesicular and macrovesicular steatosis [108]. Antibodies directed against HCV antigens allow the identification of viral proteins by immunohistochemistry using monoclonal or polyclonal antibodies, particularly when frozen tissues are employed [109]. Detection of HCV RNA sequences in fixed liver tissues by in situ hybridization appears to be a more sensitive method than immunohistochemical staining [108,109]. Immunostaining should be performed to rule out other chronic liver diseases.

A number of numerical scoring systems have been used to grade the amount of inflammation and stage and degree of fibrosis in liver biopsy specimens from patients with chronic hepatitis C. The histology activity index (HAI) of Knodell was the original scoring system and has been used in many clinical investigations [110]. With the HAI system, biopsies are graded into four categories: periportal necrosis, intralobular necrosis, portal inflammation, and fibrosis, with inflammatory activity on a scale from 0 to 18 and fibrosis on a scale from 0 to 4 [110]. The HAI was more recently modified by Ishak and colleagues [111] and by the METAVIR system [112], and all of these systems are in current use. The numerical designations used for staging the degree of fibrosis are shown in Table 82.1 [110–113]. These scoring systems provide definitive endpoints for statistical analysis of serial changes in liver histology and have replaced the older descriptive terms, chronic active hepatitis, chronic persistent hepatitis, and chronic lobular hepatitis.

Table 82.1 Scoring of hepatic fibrosis

Fibrosis	HAI [110][a]	METAVIR [112][a]	Ishak [111][a]
None	0	0	0
Portal (some)	1	1	1
Portal (most)	1	1	2
Bridging (few)	3	2	3
Bridging (many)	3	3	4
Cirrhosis (incomplete)	4	4	5
Cirrhosis (established)	4	4	6

a Scoring system and reference citation.

Screening and diagnosis

Screening for HCV infection

Primary-care physicians should routinely screen new patients for a history of risk factors associated with HCV infection. During the history and physical examination, findings suggestive of chronic liver disease include encephalopathy, ascites, edema, spider angiomas, palmar erythema, a firm liver edge, and splenomegaly. However, the majority of patients with chronic HCV infection is asymptomatic or has nonspecific constitutional symptoms, such as fatigue and decreased energy levels. Elevated serum ALT levels can predict the prevalence of HCV infection in some populations [114–116]. In summary, a test for anti-HCV should be performed if an elevated ALT level is found, if there is a positive history of risk factors for HCV infection, or if physical findings suggest the presence of chronic liver disease. A sensitive polymerase chain reaction (PCR) test for HCV RNA is warranted in patients who test positive for anti-HCV, particularly those with normal ALT levels or no HCV risk factors, to confirm HCV infection and rule out a false-positive test or recovery from past HCV infection. The latter patients have a detectable anti-HCV but negative serum HCV RNA, and their ALT levels are normal.

Chronic HCV infection may be a multisystem disease, and patients should be screened for cryoglobulinemia by checking serum cryoglobulins and serum creatinine, and performing a urinalysis. More than 50% of patients with chronic HCV infection have cryoglobulinemia, but only 1%–5% experience related symptoms. Additional baseline laboratory tests that are commonly obtained following the initial diagnosis of

chronic hepatitis C include a standard liver panel, complete blood cell count, prothrombin time, tests for coinfection with HBV or HIV, test for immunoglobulin G antibody to hepatitis A virus (anti-HAV) to determine if immunity is present or if vaccination is recommended, antinuclear antibody to exclude coexistent autoimmune hepatitis, α-fetoprotein level, and abdominal ultrasound. If treatment is being considered, a quantitative rather than qualitative serum HCV RNA test and HCV genotype are also obtained. In acute hepatic injury, prothrombin time and, to a lesser extent, total bilirubin are the best indicators of disease severity. Although ALT is useful for detecting acute and chronic hepatic injury, it is not related to the severity of acute hepatic injury and is only weakly related to the severity of chronic hepatic injury [117].

Diagnosis of HCV infection

The discovery of HCV in 1989 led to the development of diagnostic serological and virological tests [118–125]. The diagnostic tests used to detect HCV infection can be divided into two main classes:

- serological tests/assays that detect anti-HCV
- molecular tests/assays that detect, quantify, and characterize HCV RNA [119–121].

Serological assays have been subdivided into screening tests for anti-HCV, such as the enzyme immunoassay (EIA), and supplemental tests, such as the recombinant immunoblot assay (RIBA). Three generations of anti-HCV tests have been developed, and each generation has provided higher sensitivity for detecting anti-HCV. Third-generation anti-HCV tests (EIA-3 and RIBA-3) contain antigens from the HCV core, nonstructural 3, nonstructural 4, and nonstructural 5 genes. The EIA-1 contained a single HCV recombinant antigen derived from a nonstructural gene. The specificity of EIA-1 was 30%–50% in low-risk populations, such as blood donors, and the development of detectable antibodies after acute infection required several months [119]. The use of additional antigens in the EIA-2 and EIA-3 tests has increased the sensitivity and shortened the time to detection after acute infection to a median of 7–8 weeks, but false-positive measurements of anti-HCV by EIA still occur in low-risk populations. EIA-3 did not detect more HCV-infected individuals in a donor population that previously tested negative in EIA-2, but it did detect HCV antibodies earlier in some patients with acute HCV infection. EIA-2 and EIA-3 are significantly more sensitive than RIBA-2 and RIBA-3 [122]. Supplemental anti-HCV tests are designed to resolve false-positive testing by EIA, and are appropriately used in low-prevalence settings in which false-positive anti-HCV tests remain a problem [121,122]. In practice, confirmation of HCV infection is performed by the detection of HCV RNA by PCR or the branched chain DNA (bDNA) assay [118–121]. Transcription-mediated amplification is superior to PCR in identifying patients with sustained HCV RNA clearance [124,125]. The EIA-3 has an estimated sensitivity of 98.9% and specificity of 100% in patients with chronic HCV infection, whereas the sensitivity of RIBA-3 is 78.8% in the hemodialysis population [122,123]. These data provide evidence for the good sensitivity and specificity of EIA-3, particularly in high-risk patient groups, and confirms their use for screening in these populations.

Detection of serum HCV RNA provides evidence of active HCV infection and is useful as a confirmatory test [118–125]. Optimal HCV RNA assays using PCR at present have a sensitivity of less than 100 copies/mL. There are several methods for assessing HCV RNA levels, i.e., various PCR assays, the bDNA assay, and transcription-mediated amplification [118–121,124,125]. Quantitative PCR is the most sensitive test for quantifying hepatitis C viral load, whereas the bDNA test appears to be the most specific method. However, it must be recognized that these molecular tests have their own limitations, which include inadequate dynamic range, the high variability of PCR assays, and the poor sensitivity of the bDNA test [118–125]. Commonly used serum HCV RNA qualitative and quantitative second-generation PCR assays are Amplicor HCV v2.0 and Amplicor HCV Monitor v2.0, respectively. Second-generation assays are one log more sensitive and are genotype independent, compared with their respective first-generation tests (10^2 copies/mL versus 10^3 copies/mL for qualitative tests; 10^3 copies/mL versus 10^4 copies/mL for quantitative tests) [118–121].

An anti-HCV-positive result in a low-risk setting, such as blood donation, should be confirmed with an analytical antibody test. Subsequently, a serum HCV RNA test should be performed on donors with a positive or indeterminate confirmatory test result. On the other hand, an anti-HCV-positive test result in high-risk populations is likely to be a true positive. A quantitative HCV RNA test and genotyping should be performed if therapy is being considered [118,119].

According to data from the CDC, 200 000–700 000 new cases of acute viral hepatitis occurred in the United States each year during the 1980s and 1990s, but this rate has been declining in recent years for all three viruses [1–6]. In 2004, there were an estimated 56 000 new cases of hepatitis A virus (HAV) infection, 60 000 new HBV infections, and 26 000 new HCV infections [6]. From these annual cases, there are about 100 deaths per year from fulminant hepatitis A and 150 deaths from fulminant hepatitis B; hepatitis C has rarely been implicated as a cause of acute liver failure. Although the death rate from acute viral hepatitis is low, many adult patients with HBV infection (5%) and HCV infection (85%) become chronically infected, and a significant percentage of these patients ultimately develop cirrhosis or hepatocellular carcinoma (HCC) over two or more decades. Since 75% of acute viral hepatitis results from infection with either HAV or HBV, initial laboratory investigation should include serological tests to exclude HAV or HBV infection. If these studies are negative, then further testing should be performed to rule out acute HCV infection. Serum HCV RNA is detectable 1–2 weeks

after the onset of infection, whereas antibodies to HCV are not detected until 8–10 weeks after infection with HCV. In clinically stable patients, it may be plausible to wait and check antibodies to HCV.

Natural history

Acute HCV infection

Acute infection with HCV is usually asymptomatic and therefore is not diagnosed and is underestimated. Acute HCV infection is the third most common cause of acute hepatitis associated with jaundice. The most common cause of acute hepatitis in the United States is acute hepatitis A, which accounts for 49% of all cases [27,126]. This is followed by acute hepatitis B (35% of cases) and acute HCV infection (16% of cases) [27]. As many as 43% of cases of acute hepatitis C are associated with intravenous drug use, 17% with sexual exposure to an infected individual or with high-risk sexual behavior, and less than 10% with other risk factors also known to transmit HCV infection [27,127]. No known risk factors can be identified in 31% of patients with acute HCV infection [27].

The incubation period from infection to onset of symptoms can range from 2 to 12 weeks, with an average of 6–7 weeks [128,129]. Serum HCV RNA is detectable within 7–21 days after an acute infection [129]. Serum HCV RNA, in titers of 10^5–10^7 copies/mL, can be detected at the onset of jaundice. Aminotransferase levels are elevated between 14 and 28 days after infection, and anti-HCV becomes detectable approximately 60 days (varies from 20 to 150 days) after the onset of infection [129].

Preicteric period

A wide constellation of clinical features including low-grade fever, decreased energy level, fatigue, nausea, vomiting, right-sided abdominal pain, myalgias, and arthralgia may develop in 15%–20% of patients. This clinical syndrome occurs within 2–12 weeks of acute HCV infection and also lasts for 2–12 weeks. Systemic symptoms may be followed by jaundice. In rare circumstances, patients can develop rash, hives, and arthralgias that are most likely secondary to a serum sickness syndrome precipitated by immune complexes [128,129]. The majority of patients with acute hepatitis C, however, remain asymptomatic.

Icteric period

The nonspecific symptoms that develop in the preicteric phase usually persist during the period of jaundice. Infrequently, jaundice can be a presenting symptom of acute HCV infection and typically lasts for 1–2 weeks. Serum HCV RNA usually becomes detectable with the onset of jaundice. Fulminant liver failure following HCV infection has been reported but is a rare occurrence [27,129].

Resolution versus chronicity

Following the acute onset, HCV infection is self-limiting and resolves after 3–6 months in 15%–30% of cases, with the return of ALT levels to normal and the disappearance of serum HCV RNA, although anti-HCV remains detectable. During convalescence patients report resolution of jaundice, improved appetite, weight gain, and a feeling of well-being. However, nonspecific clinical features may persist for a few months in a small minority of patients who recover from acute HCV infection. A higher rate of HCV eradication is noted in patients with clinically symptomatic acute HCV infection compared with those who are asymptomatic. Therefore, the outcome of HCV infection is dependent on the virus–host immunological interactions in the first few weeks of infection. Patients who recover from acute HCV infection usually do so within the first 12 weeks of infection. Nearly 10% of patients who develop an anti-HCV response will eventually lose the nonneutralizing antibody and evidence of past acute infection [130]. Some patients may not develop an anti-HCV response with short-duration, acute hepatitis C; it is difficult to estimate the proportion of patients with this response. Spontaneous clearance and resolution of HCV infection are rare after 6 months of persistent infection [131]. Up to 75%–85% of adults and 55% of children will develop chronic HCV infection [128,129,132–135].

Chronic HCV infection

Chronic HCV infection develops in as many as 85% of patients after acute infection [136]. The majority of patients with chronic infection have asymptomatic elevation of aminotransferase levels and lack physical findings suggestive of liver disease [137,138]. Fatigue is the most common symptom reported by patients with chronic HCV infection [137]. The correlation of symptoms with histological severity of liver disease is poor in patients with chronic hepatitis C; patients may have fatigue associated with either mild or severe disease. Vague and intermittent right upper quadrant pain is the second most frequent symptom noted in patients with chronic HCV infection. Other less common nonspecific symptoms include low-grade fevers, nausea, vomiting, myalgias, and arthralgia. Up to 15%–20% of patients with chronic HCV infection have psychological or psychosocial problems, which complicate the initial assessment and later decisions regarding treatment. One-third of patients with chronic HCV infection have normal ALT levels, and 25% have ALT levels that are elevated to less than twice the upper limits of normal [136,139]. ALT elevations are persistent in 26% of cases and intermittent in 40%–70% of cases, with resolution to normal range in 17% of patients on long-term follow-up [136,139,140]. Self-limited, intermittent ALT flares have been noted and may be associated with an increase in serum HCV RNA, but not with viral eradication [141]. A 10-fold rise in ALT levels has been associated with severe piecemeal

necrosis [136,137,142]. The titer of serum HCV RNA is not associated with histological severity.

HCV-related cirrhosis

Several studies have confirmed that chronic HCV infection demonstrates minimal clinical progression in the initial two decades after infection [2,138]. However, chronic hepatitis C leads to cirrhosis in at least 20%–25% of immunocompetent patients within 20 years of the onset of infection [138,143]. The median duration of chronic HCV infection required for the development of cirrhosis can vary from 13 years in men who use alcohol and are older than 40 years of age at the time of infection to as long as 42 years in women who avoid alcohol and are less than 40 years of age at the time of infection [143]. The overall risk of cirrhosis is less than 10% over a 10-year period in patients with mild chronic hepatitis, 44% in those with moderate hepatitis, and 100% in those with severe hepatitis with bridging fibrosis [144].

Risk factors associated with rapid progression to HCV-related cirrhosis

Cirrhosis and end-stage liver disease may develop more rapidly under certain circumstances [135]. Factors influencing the rate of progression of fibrosis include gender (male sex), genetic background (Japanese at higher risk compared with Americans), age at exposure (higher risk of cirrhosis with age greater than 40 years at time of HCV infection), duration of HCV infection (longer), route of transmission (blood transfusion greater risk than injection drug use), and coexisting conditions including alcoholic liver disease (daily alcohol consumption of 50 g or more), chronic tobacco use, HIV coinfection, and HBV coinfection [144–155]. Up to 20% of patients with chronic hepatitis B have detectable anti-HCV [151,152]. Alternatively, 2%–10% of patients with anti-HCV demonstrate markers of HBV infection. Coinfection with HBV and HCV results in more severe liver disease compared to patients with isolated HBV or HCV infection.

Clinical presentation of HCV-related cirrhosis

The mean age of patients presenting with HCV-related cirrhosis is 55 years compared with mean ages of 42 years in patients presenting with chronic HCV infection without cirrhosis and 66 years in patients with HCC [146]. Other studies have also confirmed a mean difference of at least 10 years between the diagnosis of cirrhosis and the detection of HCC in patients with chronic HCV infection [153–156]. HCV-related cirrhosis affects men more commonly than women, with a ratio ranging from 1.3 : 1 to 3.2 : 1 [139,157].

The diagnosis of HCV-related compensated cirrhosis is often made incidentally during screening of blood donors or at the time of routine laboratory testing [139]. A wide spectrum of nonspecific symptoms can be noted in patients with compensated and decompensated cirrhosis, including fatigue in 75%, abdominal pain in 24%, and anorexia in 13% [136]. Systemic symptoms are more severe in patients with decompensated cirrhosis, and the annual risk of decompensation is 3.9% [139]. The clinical presentation can be dramatic after hepatic decompensation and manifests with ascites in 48%, variceal bleeding in 22%, hepatic encephalopathy in 8%, jaundice in 6%, or a combination of complications in 17% of patients [139]. Other clinical features noted with hepatic decompensation include lower extremity edema, easy bruising, pruritus, muscle wasting, muscle cramps, and sexual dysfunction. Patients with HCV-related compensated cirrhosis have a mean survival rate of 80% at 10 years, compared with a mean survival of 50% at 5 years once decompensation occurs [139].

Physical findings suggestive of cirrhosis include a palpable firm liver edge in 79%, hepatomegaly in 50%–78%, splenomegaly in 25%–37%, and cutaneous stigmata of advanced liver disease in 31% [139]. The majority of patients with HCV-related cirrhosis have mild to moderate elevation of aminotransferase levels, with less than 5% of patients having ALT levels 10-fold higher than the upper limit of normal [139]. The association between ALT levels and histological severity of chronic HCV is poor [142,158]. Coagulopathy, hypoalbuminemia, hyperbilirubinemia, and thrombocytopenia are associated with a decrease in survival rate at 10 years [139]. The annual risk of HCC in patients with chronic hepatitis C with cirrhosis is 1.4%–3.3% in the United States and Europe, and as high as 6.9% in Japan and Italy [159]. The mean length of time from HCV infection to the development of HCC is 29 years [136,139].

Clinical manifestations and complications

Chronic HCV infection can be associated with extrahepatic organ involvement or lead to complications primarily involving the hepatic parenchyma. Most of these manifestations are directly associated with HCV infection; however, HCC nearly always develops in the setting of HCV-related cirrhosis. For other complications associated with HCV-related cirrhosis, see section on HCV-related cirrhosis.

Extrahepatic clinical manifestations

The prevalence of extrahepatic clinical manifestations is relatively low but can be associated with significant morbidity and even mortality. The precise pathogenesis of these extrahepatic manifestations has not been determined, although the majority represents the clinical consequences of an immune-mediated response [160–163].

Autoantibodies

There is a high prevalence of a variety of autoantibodies, usually in low titers, associated with chronic HCV infection. Antinuclear antibody, antismooth muscle antibody, and antithyroid antibody are detectable in 40%–65% of patients with

chronic HCV infection [164,165]. The clinical significance of most of these autoantibodies is negligible, except for liver–kidney–microsomal type 1 antibodies (anti-LKM1) [165,166]. Patients with anti-LKM1 can be differentiated into two groups, anti-HCV-positive and anti-HCV-negative [167,168]. Type 2 autoimmune hepatitis with high anti-LKM1 titers and negative anti-HCV primarily affects young women with moderate to marked elevation of ALT. These patients are responsive to immunosuppressive therapy and are human leukocyte antigen-DR3-positive. In contrast, patients with chronic HCV infection and a positive anti-HCV associated with a positive anti-LKM1 have distinctive features, such as an equal distribution between men and women, mildly elevated ALT levels, low anti-LKM1 titers, and responsiveness to IFN therapy [169]. Finally, patients with classic type 1 autoimmune hepatitis can have a false-positive anti-HCV, which can be confirmed with the finding of a negative serum HCV RNA [170,171].

Renal disorders

A wide array of renal disorders can occur in association with chronic HCV infection, and renal complications may be the presenting feature of chronic hepatitis C [172–175]. Patients may present with vasculitis secondary to cryoglobulinemia, proteinuria, microscopic hematuria, acute renal failure, or nephrotic syndrome [172]. The pathogenesis of HCV-related renal disorders generally remains undefined; however, deposition of circulating immune complexes in the subendothelial space and mesangium in the glomeruli plays a central mechanistic role. Uremic patients with chronic HCV infection who are undergoing regular dialysis frequently develop immunological abnormalities. The most common HCV-related renal disorder is type I membranoproliferative cryoglobulinemic glomerulonephritis with a chronic course interspersed by acute recurrent episodes. The membranoproliferative cryoglobulinemic glomerulonephritis is usually associated with type II cryoglobulinemia with immunoglobulin M rheumatoid factor, polyclonal immunoglobulin G anti-HCV antibodies, and HCV RNA immunoglobulin M as the principal constituents in cryoprecipitate. The treatment of renal disorders associated with chronic HCV infection is discussed in the section on treatment [176–181].

Endocrine disorders

Infection with HCV may contribute to the development of diabetes mellitus. Both disorders are common; therefore, cross-sectional studies were performed to confirm their close association [182–184]. Patients with chronic HCV infection have an increased prevalence of diabetes mellitus type II. HCV-related cirrhosis can induce insulin resistance and predispose patients to diabetes mellitus. However, the increased prevalence of diabetes is independent of cirrhosis [183]. Further investigation is needed because the pathogenesis may be multifactorial and unique to HCV. In the United States, diabetes mellitus type II occurs more often in individuals with chronic HCV infection who are older than 40 years of age [184].

Autoimmune thyroiditis can be associated with HCV-related liver disease [218,219]. Antithyroid antibodies are found in 5%–13% of patients with chronic HCV infection, predominantly affecting older women [185,186]. Autoimmune thyroiditis is manifested as hypothyroidism in 3%–6% of patients with chronic HCV infection. Interferon-related thyroid disease is more frequently noted in patients with preexisting thyroid antibodies. However, IFN can induce the formation of antithyroid antibodies. Discontinuation of IFN therapy is usually accompanied by resolution of the thyroid disorder. Salivary and lacrimal gland involvement is common in HCV-infected individuals, but HCV antigens are not detectable in affected glands [187]. Testicular atrophy and sexual dysfunction can be associated with HCV-related cirrhosis.

Rheumatological disorders

Rheumatological disorders associated with chronic HCV infection are common and include mixed cryoglobulinemia, vasculitis, sicca symptoms, myalgia, arthritis, and fibromyalgia [187–190]. There is a wide spectrum of clinical manifestations that can be noted and are not characteristic of HCV infection. However, a characteristic joint syndrome is associated with the presence of mixed cryoglobulinemia and characterized by intermittent monoarticular or oligoarticular, nondestructive arthritis affecting large and medium-size joints. Treatment of patients with HCV-associated arthritis is limited and remains controversial.

Cutaneous and mucosal lesions

There are a number of dermatological disorders associated with chronic hepatitis C, with variable responses to IFN therapy [191]. The prevalence of anti-HCV varies from 4%–65% in patients with cutaneous lichen planus or oral lichen planus [191,193]. These estimates were obtained from populations with a high overall prevalence of HCV infection, such as the southern Europe and Japan [193]. Other lesions noted in association with HCV infection include porphyria cutanea tarda, necrotizing cutaneous vasculitis, and Mooren corneal ulcer [188,191]. The high prevalence of HCV infection in patients with porphyria cutanea tarda is based on epidemiological data [188]. The precise role of HCV in the pathogenesis of porphyria cutanea tarda is unknown.

Cardiac dysfunction

Recent studies have demonstrated an association between chronic hepatitis C and hypertrophic cardiomyopathy or dilated cardiomyopathy [194]. In a collaborative research project of the Committees for the Study of Idiopathic Cardiomyopathy, the prevalence of anti-HCV was 11% in patients with hypertrophic cardiomyopathy and 6% with those with dilated cardiomyopathy, both of which were significantly

higher than the prevalence rate of HCV infection in volunteer blood donors in Japan [194]. Various other cardiac abnormalities were found, of which arrhythmias were the most frequent.

Miscellaneous extrahepatic manifestations

Many systemic disorders can be associated with chronic HCV infection including polyarteritis nodosa-type systemic vasculitis [195], colonic vasculitis [196], idiopathic thrombocytopenic purpura [188], celiac sprue [197], Sjögren syndrome [188], polymyositis [198], and multiple myeloma [199]. Systemic sclerosis has been reported in patients with chronic HCV infection, manifested by severe Raynaud phenomenon, progressive skin thickening, painful fingertip ulcers, dysphagia, and Sjögren syndrome [200]. The prevalence of chronic HCV infection in patients with systemic lupus erythematosus is greater than the prevalence of HCV in blood donors from the same population [201]. Patients with coexistent systemic lupus erythematosus and HCV infection tend to manifest a lower frequency of cutaneous systemic lupus erythematosus lesions and anti-double-stranded DNA antibodies, and a higher rate of hepatic involvement, hypocomplementemia, and cryoglobulinemia [201].

Lymphoproliferative diseases

The role of HCV infection in the etiology and pathogenesis of lymphoproliferative diseases is supported by several studies reporting high seroprevalence of HCV in patients with some types of B-cell non-Hodgkin lymphoma [202–206]. HCV demonstrates a high affinity for lymphoid tissue. HCV is associated with type II mixed cryoglobulinemia, a benign monoclonal lymphoproliferative disorder that can transform to overt B-cell non-Hodgkin lymphoma.

Primary hepatic lymphoma

The etiological association between HCV and primary hepatic lymphoma is unclear [202,206]. Numerous reports of primary hepatic lymphoma of B-cell origin in association with HCV infection have been published, and the cell types are typically immunocytoma, marginal zone lymphoma, and diffuse large cell lymphoma. Only a few cases of T-cell lymphoma have been described [206].

Hepatocellular carcinoma

The incidence of HCC in the United States has more than doubled over the past 20 years from 1.4 cases per 100 000 in 1975 to 1977 to 3.0 per 100 000 in 1996 to 1998, primarily related to the impact of HCV infection acquired during the 1960s and 1970s [159,207–209]. The increase affected most age groups above 40 years, with the greatest increase in the group aged between 45 and 49 years [208]. By contrast, the incidence of HCC is as high as 120 cases per 100 000 in China, the Far East, and sub-Saharan Africa [159,210,211]. The occurrence of HCC in association with HCV-related liver disease shows marked geographic variation [159,209–211]. The rate of detectable anti-HCV in patients with HCC can vary from 60%–70% in Spain and Italy, 50% in Japan, and 27% in the United States to less than 10% in Hong Kong [159]. Approximately 20%–25% patients with chronic HCV infection will develop cirrhosis within 20 years from the onset of infection, and HCC develops at a rate of 1.5%–9% per year in patients with chronic hepatitis C and cirrhosis [139,212]. Therefore, there is a 5% risk of developing HCC in patients with HCV infection over a course of 20 years. The risk factors associated with the development of HCC in the setting of HCV infection include age over 60 years, male gender, alcohol use, coinfection with HBV, porphyria cutanea tarda, increased hepatic iron content, and severity of cirrhosis [209,212–214]. The incidence of HCV-related HCC is steadily rising, and HCV accounts for nearly half of all cases of HCC in the United States [208–210].

Ultrasound of the abdomen and measurement of serum α-fetoprotein are reasonable tests in a screening strategy for early detection of HCC in patients with chronic HCV infection and cirrhosis [214]. There is no consensus on the frequency with which these tests should be performed, but most experts recommend an interval of every 6–12 months. Screening is recommended only for patients with known or suspected cirrhosis, because the risk of HCC is low in patients who do not have cirrhosis.

Cholangiocarcinoma

There is also a somewhat higher incidence of cholangiocarcinoma in patients with HCV-related cirrhosis, and cholangiocarcinoma is also associated with poor prognosis [215,216].

Antiviral therapy

Peginterferon plus ribavirin combination therapy is the current standard of care for treatment-naive patients with chronic hepatitis C [217]. The 2002 NIH Consensus Development Conference and recent guidelines recommend that all patients with chronic hepatitis C should be considered as potential candidates for therapy [217–220]. Patients with HCV-related compensated cirrhosis can be cautiously treated, whereas patients with decompensated cirrhosis should be referred to a liver transplant center for consideration for liver transplantation, which is the treatment of choice when liver failure complicates chronic HCV infection.

Interferon-based antiviral therapy

Treatment is recommended by clinical guidelines on the management of chronic hepatitis C in adults who are at the greatest risk for progression to cirrhosis [149,217–220]. These patients have persistently elevated ALT levels for more than 6 months, detectable serum HCV RNA, portal or bridging fibrosis with at least a moderate degrees of inflammation

Table 82.2 Contraindications for interferon and ribavirin therapy

Hematological
Hemoglobin < 12 g/dL in women and < 13 g/dL in men
White blood cell count < 1500/mm^3
Platelet count < 100 000/mm^3
Hepatic
Normal aminotransferase levels
Decompensated cirrhosis
Cardiovascular
Severe coronary artery disease
Unstable angina
Endocrine
Poorly controlled diabetes mellitus
Autoimmune diseases
Untreated thyroid disease
Neuropsychiatric
Seizure disorder
Severe psychiatric illness
Obstetric
Pregnant or unable to practice contraception

and necrosis on liver biopsy, and no evidence of serious coexisting conditions or contraindications to antiviral therapy. Patients who are treated in clinical trials are typically required to have elevated ALT levels of more than 1.5 times the upper limit of normal, clinically compensated liver disease, and no other significant medical or psychiatric illness that contraindicates therapy (Table 82.2).

Virological and biochemical response

Responses to antiviral therapy of chronic HCV infection may be defined on the basis of the biochemical, virological, and histological outcomes. The persistence of a biochemical (normal ALT) or virological (undetectable HCV RNA) response for 6 months or more after cessation of therapy is the operational definition of a *sustained response* [221]. Several studies, including a mean 4-year follow-up of 80 patients and a 10-year follow-up of five patients who all had an initial posttreatment 6-month sustained virological response (SVR), showed long-term sustained response rates of 95%–100% [222–224]. In addition, there was improvement of liver histology, including evidence of regression of hepatic fibrosis in noncirrhotic patients [223]. These studies suggest that some patients may indeed be "cured" of chronic HCV infection and that a benefit on mortality should be expected if cirrhosis, with its risk of HCC, could be prevented. A posttreatment elevated ALT level and detectable HCV RNA that had been normal and undetectable, respectively, during treatment define *relapse* after a course of therapy. *Nonresponse* to antiviral therapy is defined as persistently elevated ALT levels or detectable serum HCV RNA, and a variant of nonresponse called *breakthrough* is characterized by an initial decrease of ALT levels into the normal range or the disappearance of detectable HCV RNA with subsequent elevation of ALT or the presence of detectable HCV RNA while still on treatment [224]. An early virological response (EVR) is defined as a drop in the HCV RNA level of 2-$\log_{10}$ or more compared to baseline [226,227]. Patients with undetectable levels of HCV RNA at 12 weeks are considered to have a complete EVR. Patients with an EVR who demonstrate a drop in HCV RNA greater than 2-$\log_{10}$, but who continue to demonstrate low-level viremia (partial EVR), should be treated for 24 weeks with continuation of therapy beyond 24 weeks in only those who become HCV RNA negative by week 24 of therapy. Analyses of data from two registration trials demonstrated that the EVR was a reliable predictor of subsequent SVR with 97% and 100% positive predictive value in two studies [226,227]. Failure to clear HCV RNA after 12 weeks of combination therapy predicts the lack of an SVR and has been used as a stopping rule for antiviral therapy. However, histological improvement may occur with longer maintenance therapy, and so continuation of therapy may be beneficial in selected cases on an experimental basis.

Aims of antiviral therapy

The primary goal of antiviral therapy in patients with chronic hepatitis C is long-term viral eradication as determined by undetectable HCV RNA in serum and liver [217–220]. Secondary objectives of treatment include normalization of ALT levels and improvement in histology (reduced inflammation and fibrosis) on liver biopsy.

Selective role of liver biopsy

A liver biopsy is the gold standard to assess the severity of underlying liver disease. However, liver biopsy is not mandatory before treatment and is best used selectively [228,229]. Liver biopsy can help to distinguish those patients that are most likely to benefit from therapy, i.e., those with moderate histological disease, from those who may be less likely to benefit, i.e., those with mild disease and no or minimal fibrosis or those with advanced disease and cirrhosis. A decision to perform a liver biopsy can be influenced by many factors, such as patient preferences, presence of relative contraindications, and clinical suspicion of coexistent liver diseases suggested by laboratory abnormalities like high-titer antinuclear antibody with hypergammaglobulinemia or abnormal iron studies. Liver biopsy is costly, invasive, painful, requires prolonged monitoring, and is associated with sampling error [230,231]. Apprehension and anxiety is frequently noted in patients before a liver biopsy is performed. A percutaneous liver biopsy is a blind procedure, which can cause pain in 30%, severe complications in 0.3%, and death in 0.03% of patients [230,231]. In addition, liver biopsy incurs both direct

health-care-related and indirect work-loss-related costs. Based on a cost-effectiveness analysis, it has been recommended that the most suitable strategy in the management of chronic HCV infection is to initiate therapy in all patients and avoid a liver biopsy [232]. Considering these arguments, there is a trend to initiate therapy without performing a liver biopsy. The role of liver biopsy might be thought of as selective rather than mandatory in identifying candidates for antiviral therapy, e.g., for the patient with genotype 1 infection who prefers to defer therapy if mild disease with no or minimal fibrosis is present [217–220]. On the other hand, patients with genotype 2 or 3 infection, with expected high rates of an SVR, may not need a pretreatment liver biopsy.

A number of studies have been performed in patients with chronic HCV infection to investigate the utility of laboratory tests, such as the aspartate aminotransferase to ALT ratio or the platelet count, to establish the severity of liver disease, but none of the proposed predictors are as sensitive and specific as a liver biopsy [233,234]. In one study, a more complex formula correctly classified 46% of patients with or without significant fibrosis into these two respective categories [235]. This formula required three assays that were not routinely performed in patients with chronic HCV infection (α_2-microglobulin, haptoglobin, and apolipoprotein A-1), in addition to three routine tests (γ-globulin, γ-glutamyltransferase, and total bilirubin), but commercial application of these and similar tests are available through referral laboratories. The greatest use of these tests is to confirm suspected mild or advanced fibrosis; the sensitivity and specificity of these tests is poor in patients with intermediate stages of fibrosis [233,234].

The future treatment of chronic hepatitis C may include antiviral drugs and also antifibrotic agents, which might necessitate the performance of a liver biopsy or the identification of reliable serum markers of fibrosis to establish the endpoints of therapy. A wide variety of serum fibrosis markers have been studied to predict the stage of fibrosis, of HCV-related liver disease [233,234,236]. The serum markers that have been studied include procollagen type III N-terminal peptide (PIIINP), 7S fragment of type IV collagen (PIVNP), hyaluronan (HA), matrix metalloproteinase 1 (MMP-1), MMP-2, and tissue inhibitor of metalloproteinases 1 (TIMP-1). The Ishak fibrosis scores of treatment-naive patients, including 205 white and 194 African Americans, with HCV genotype 1 enrolled in the Viral Resistance to Antiviral Therapy of Chronic Hepatitis C study (Virahep-C) were modeled using simple and multiple logistic regression analysis [237]. The model was then validated in an independent cohort of 461 previously untreated patients with HCV. The value of the area under the receiver operating characteristic curve (AUROC) of the Virahep-C model (0.837) was significantly better than in other published models. The AUROC of the Virahep-C model was 0.851 in the validation population. In summary, a model consisting of widely available clinical and laboratory features predicted severe hepatic fibrosis equally well in white and African American patients with HCV genotype 1 and was superior to other published models [237]. The excellent performance of the Virahep-C model in an external validation cohort suggests that the findings are replicable and potentially generalizable.

Interferon-induced viral and immune kinetics

The potency of IFN is determined by measuring antiviral activity in biological assays relative to a number of international reference standards. Further insight into IFN-induced viral kinetics and host immune modulation has been provided by recent studies [238–240]. It has been demonstrated that multitarget, potent CD4+ proliferative T-cell responses are maintained long-term after eradication of HCV infection compared to the focused and weak responses in patients with persistent HCV infection [240]. On the other hand, the HCV-specific CD8+ T-cell response is quantitatively weaker in both responders and nonresponders.

Treatment of naive patients

The four types of IFN-α that have been evaluated in a large number of patients with chronic HCV infection include IFN-α2b [241], IFN-α2a [242], IFN-αn1 (lymphoblastoid interferon) [243], and IFN-αcon-1 (consensus interferon [CIFN]) [244]. In the United States, IFN-α2b, IFN-α2a, IFN-αcon-1, combination IFN-α-2b with ribavirin, peginterferon alfa-2a plus ribavirin and peginterferon alfa-2b plus ribavirin are licensed by the Food and Drug Administration (FDA) for the treatment of chronic HCV infection. The treatment of choice for chronic hepatitis C approved by the FDA is peginterferon alfa-2a or peginterferon alfa-2b plus ribavirin for 24 or 48 weeks.

The FDA originally licensed interferon in 1991 for the treatment of chronic hepatitis C, and the initial strategy of treating patients for 24 weeks yielded 10%–15% sustained response rates. It was later found that 48–72 weeks of therapy increased the response rates up to 20%–25% [221]. Interferon and ribavirin combination antiviral therapy became the standard treatment for chronic HCV infection in 1998 [217–220]. Ribavirin in doses of 1000 mg to 1200 mg daily in two divided doses combined with IFN-α2b in a dose of 3 million units (MU) three times a week for 24 weeks was found to significantly improve the biochemical and virological sustained response rates compared with IFN alone in an early pilot experiment [245]. Phase III American and European trials comparing standard IFN monotherapy with combination IFN-α2b plus ribavirin therapy showed a significant improvement in response rates for all measures of efficacy, including virology, ALT levels, and histology in treatment-naive patients with chronic HCV infection [246,247]. Serum HCV RNA was eradicated in 31%–35% of patients after 24 weeks of combination therapy and in 38%–43% of patients with 48 weeks of combination therapy as compared to 13%–19% of patients given interferon monotherapy. These studies also showed

important differences in the outcome based on genotype, i.e., approximately 30% of patients with genotype 1 versus approximately 65% with genotypes 2 or 3 had an SVR. In addition, patients with genotype 1 had increased rates of SVR with 48 versus 24 weeks of therapy, whereas patients with other than genotype 1 achieved no additional benefit after 24 weeks of combination therapy. These studies indicate that combination therapy should be administered for 48 weeks to patients with genotype 1 but for only 24 weeks to those with genotypes 2 and 3 to achieve maximal SVR rates. Multiple factors, in addition to genotype, are known to predict the likelihood of successful therapy with ribavirin and IFN and may influence the decision of whether or not to administer a course of therapy.

Polyethylene glycol (PEG) is a large, inactive water-soluble polymer that can attach to IFN with a covalent bond [248]. The process of pegylation results in reduced clearance of the pegylated molecule and up to a 10-fold increase in the drug half-life, which allows once a week administration [248]. Peginterferon has improved efficacy because a steady serum level is maintained, but there may be a variable loss in IFN activity determined by the size of the PEG molecule and the site of pegylation [239,248]. The two peginterferons that have been studied include the long branched 40-kDa peginterferon alfa-2a (Pegasys, Roche), which is metabolized by the liver, and the short linear 12-kDa peginterferon alfa-2b (PEG-Intron, Schering), which is excreted by the kidneys [249,250]. Pilot studies demonstrated that the safety profile of peginterferon administered in ascending subcutaneous doses to healthy subjects was comparable to that of standard interferon [249–251]. Peginterferon monotherapy was more efficacious than standard interferon monotherapy in a dose-dependent fashion [249–251]. In one clinical trial, 531 IFN-naive patients with chronic HCV infection were treated for 48 weeks with peginterferon alfa-2a, 180 μg once weekly, and showed an SVR rate of 39% at 24 weeks following discontinuation of therapy, in comparison to a 19% SVR rate with standard IFN-α2a three times a week [249]. In another study, 1219 IFN-naive patients with chronic HCV infection were treated with peginterferon alfa-2b weekly using a weight-based dosing of 1.0 μg/kg or 1.5 μg/kg weekly and with IFN-α2b three times a week in the control group for 48 weeks [250]. The SVR rates were 25% for 1.0 μg/kg peginterferon alfa-2b once weekly, 23% for 1.5 μg/kg peginterferon alfa-2b once weekly, and 12% for IFN-α2b thrice weekly. Therefore, monotherapy with either peginterferon has twice the efficacy of standard IFN monotherapy in terms of SVR rates. The response rates were reduced in patients with genotype 1 and higher viral loads. Both peginterferons were tolerated as well as the standard interferons, with discontinuation rates ranging from 6% to 11% in all treatment groups. Peginterferon alfa-2b was approved and licensed by the FDA in 2001 and peginterferon alfa-2a in 2002. There are no clinical trials comparing the efficacy of the two peginterferons face to face; however, based on available data it is expected that any differences between the two drugs are not significant.

The combination of peginterferon with ribavirin has improved the efficacy of antiviral therapy of chronic HCV infection even further [226,227]. Peginterferon alfa-2b at a weekly dose of 1.5 μg/kg plus ribavirin, 800 mg per day, showed a significantly higher response rate when compared with standard IFN plus ribavirin combination in a large phase III trial [226]. The overall SVR rate was 54% (42%, genotype 1; 82%, genotype 2 or 3). The group treated with peginterferon plus ribavirin combination reported higher frequencies of fever, nausea, and injection site reaction with erythema compared to the control group. The rate at which the drugs were discontinued was similar in the two arms of the study. The dose of ribavirin may not have been ideal, with higher doses achieving yet better results. Two large, phase III studies of peginterferon alfa-2a plus ribavirin reported an SVR rate of 56%–63% (46%–52%, genotype 1; 76%–84%, genotype 2 or 3) [227,252]. These results are probably not significantly different from the results using peginterferon alfa-2b, making these two drugs interchangeable. Therefore, based on these efficacy data, the use of peginterferon monotherapy is only appropriate if there are contraindications to ribavirin use, such as significant baseline anemia or advanced chronic renal failure. Based on data from these trials, peginterferon and ribavirin combination therapy is the standard of care in treating patients with chronic HCV infection [226,227,252]. Table 82.3 compares the SVR rates reported with peginterferon plus ribavirin therapy in the key pivotal trials by genotype and viral load for genotype 1. The treatment duration and ribavirin dose registration study established that 48 weeks of therapy and 1000 or 1200 mg of ribavirin (based on body weights of less than or more than 75 kg, respectively) was needed for an optimal response in patients with genotype 1, whereas 24 weeks of therapy using 800 mg of ribavirin was sufficient therapy for patients with genotypes 2 and 3 [252].

Table 82.3 Sustained virological response with peginterferon and ribavirin treatment in pivotal trials

Treatment [ref.]	Overall	GT 1	GT 1 (HVL)	GT 2 or 3
Peginterferon alfa-2a plus ribavirin [227]	56%	46%	41%	76%
Peginterferon alfa-2a plus ribavirin [252]	63%	52%	47%	84%
Peginterferon alfa-2b plus ribavirin [226]	54%	42%	30%	82%
Weight-based RBV[a]	61%	48%	37%	88%

a Weight-based RBV, > 10.6 mg/kg ribavirin by secondary analysis of the study of Manns et al. [226].
GT, genotype; HVL, high viral load (> 800 000 IU/mL); RBV, ribavirin.

Viral, host and treatment-related variables associated with lower SVR rate include genotype 1, high baseline viral load, advanced fibrosis (bridging fibrosis and cirrhosis), obesity, African American race, older age, contraindications to treatment, previous relapse or nonresponse to treatment, and ribavirin dose [253,254]. Patients with HCV genotype 1 and high viral load (> 600 000–800 000 IU/mL) demonstrated SVR rates of 30%–47% [226,227,252]. Recent data have demonstrated that measurement of HCV RNA at 4 weeks may allow for the individualization of the duration of treatment. Patients with undetectable HCV RNA at week 4 of therapy are characterized as having a rapid virological response (RVR) [255]. A recent retrospective review of treatment duration and ribavirin dose registration [252] showed that 24% of patients with HCV genotype 1 in the 24-week peginterferon alfa-2a plus ribavirin treatment group developed a RVR that was associated with an 89% probability of an SVR [255]. These data are promising but they need to be reproduced in a prospective, controlled trial before treatment recommendations can be modified. Therefore, all patients with HCV genotype 1 should be treated for at least 48 weeks if they develop an EVR at 12 weeks of therapy. On the other hand, data have demonstrated a significantly improved SVR rate of 29% with extended therapy of 72 weeks (versus 17% with the standard 48 weeks) in patients with HCV genotype 1 who develop a partial EVR at week 12 of therapy (> 2-$\log_{10}$ drop but still have detectable HCV RNA) but subsequently become HCV RNA negative at 24–weeks [256]. In particular, patients with low level viremia [< 6000 IU/mL] at 12 weeks benefited from 72 weeks of treatment, whereas patients with undetectable HCV RNA at weeks 4 and 12 had no added benefit from the extended 72 weeks of therapy [256]. Similarly, in a recent prospective clinical trial, 510 treatment-naive patients were treated with peginterferon alfa-2a (180 μg/week) plus ribavirin (800 mg/day) and treated for 48 or 72 weeks. Patients with detectable HCV RNA levels (HCV RNA < 50 IU/mL) at week 4 were randomized to complete 48 or 72 weeks of treatment [257]. The end-of-treatment response rate (61%) was similar in both groups, but the SVR rate was 32% with 48 weeks of therapy (compared with 45% in the 72-week treatment group). In genotype 1-infected patients randomized to 48 weeks or 72 weeks of treatment, SVR rates were 28% and 44%, respectively – a significant difference. The incidence of adverse events was similar in both study arms. Treatment discontinuation was significantly more frequent in the 72-week group (36%) than in the 48-week group (18%). Therefore, the extended 72 weeks (versus the standard 48 weeks) of therapy with peginterferon plus ribavirin significantly increases the rate of SVR in patients with detectable viremia at week 4 of treatment [256,257].

Results from three clinical trials performed in an attempt to curtail the treatment duration for patients with HCV genotypes 2 and 3 to 12, 14, or 16 weeks (rather than 24 weeks) suggested that patients who achieve an RVR with peginterferon alfa-2a or -2b plus ribavirin can discontinue treatment early (12, 14, or 16 weeks) and gain no added benefit by continuing therapy for usual 24 weeks [258–260]. Unfortunately, these data have been marred by flawed study designs, small number of patients enrolled, and unconventional doses of peginterferon and ribavirin. The results of the largest prospective study in patients with HCV genotypes 2 and 3 (n = 1469, the Accelerate trial) demonstrated that 24 weeks of peginterferon alfa-2a 180 μg/week plus ribavirin 800 mg/day was significantly superior to 16 weeks (76% versus 65%) [261]. The lower SVR rate in the 16-week group was associated with a higher relapse rate. Further subanalysis of the Accelerate trial showed that patients with an RVR and low viral load (up to 400 000 IU/mL) might be suitable candidates for shorter therapy (16 weeks). Prospective studies must be performed to reproduce this observation and before modifying treatment recommendations.

Patients with compensated cirrhosis are suitable candidates for treatment, but the SVR rates are lower and there is a small risk of hepatic decompensation induced by therapy [262,263]. Patients with hepatic decompensation tolerate treatment poorly and serious adverse effects, including death, have been reported [263]. However, treatment guidelines have recommended that patients with mild hepatic decompensation can be treated cautiously [262]. A low accelerating dosage regimen of peginterferon and ribavirin achieved an SVR rate of 20%–30% (13% in genotype 1, and 50% in genotypes 2 and 3) in the setting of hepatic decompensation [263]. Ideally, patients with hepatic decompensation should initially be listed for liver transplantation, followed by consideration for cautious antiviral therapy in patients with mild hepatic decompensation. For patients with hepatic decompensation who are poor candidates for liver transplantation, treatment should be individualized by weighing the risks of therapy versus the benefits.

Several studies have demonstrated that hepatic fibrosis improved on serial liver biopsies following interferon-based therapy, especially in patients who developed an SVR [264]. A significant reduction in the rate of hepatic fibrosis was noted in a set of data pooled from 3010 treatment-naive patients with chronic hepatitis C who underwent pretreatment and posttreatment liver biopsies in four randomized clinical trials using 10 different IFN-based regimens [264]. Although, the best results were noted with a peginterferon plus ribavirin combination, all regimens significantly reduced the fibrosis progression rates in comparison to rates before treatment. In particular, advanced fibrosis was reversed in 49% of patients with baseline cirrhosis. Independent predictors of posttreatment minimal fibrosis included age less than 40 years, body mass index less than 27 kg/m^2, HCV RNA level less than 3.5 million copies/mL, SVR, baseline fibrosis stage, and no or minimal baseline activity [264]. Antiviral therapy for chronic hepatitis C is also associated with a decrease in the incidence of HCC. The Inhibition of Hepatocarcinogenesis by Inter-

Table 82.4 Favorable impact of antiviral therapy on the natural history of chronic hepatitis C

Long-term virological response after SVR: **> 95%**
Clinical relevance of detection of small amounts of HCV RNA uncertain
Fibrosis progression rate: **slowed**
Evidence of reversal of fibrosis, including early cirrhosis
Incidence of hepatocellular carcinoma: **reduced**
Especially among patients with SVR
Life expectancy and survival: **improved**

HCV, hepatitis C virus; SVR, sustained virus response.

feron Therapy (IHIT) Study Group in Japan has followed a cohort of more than 3000 Japanese patients with chronic hepatitis C and demonstrated that IFN therapy reduces the risk of HCC by 50% [265]. The risk of HCC was reduced to one-fifth in biochemical and virological responders versus treatment-naive patients. Recent data with long-term follow-up have shown reduced overall death rate and reduced liver-related deaths [266,267]. Thus, evolving data indicate the natural history of chronic hepatitis C is being favorably influenced by antiviral therapy (Table 82.4).

Treatment of variant clinical manifestations

Patients with chronic hepatitis C included in the following section were either excluded from or insufficiently studied in the registration trials. Therefore, treatment recommendations in these subgroups are limited by lack of data and may not be FDA approved.

Acute hepatitis C

Although interferon-based antiviral therapy has not been approved for acute hepatitis C, several clinical trials have shown promising results [368,269]. A study of 44 patients with acute hepatitis C treated with IFN-α2b 5 MU daily for 4 weeks and then three times a week for another 20 weeks showed a remarkable 98% SVR rate 6 months after completion of therapy, i.e., prevention of chronic hepatitis C [268]. In a recent study, patients treated with peginterferon alfa-2b (1.5 μg/kg/week) for 24 weeks (treatment was initiated after a median of 76 days after infection, range 14–150 days) demonstrated an SVR rate of 89% [269]. An ALT level of more than 500 U/L before therapy was the only factor associated with a higher response rate. The adverse effects with peginterferon alfa-2b were significant in this patient population and led to a high number of dropouts [269]. The optimal regimen for acute hepatitis C remains undefined and further studies are needed. Unfortunately, up to 75% patients with acute hepatitis C will develop chronic infection without therapy. Therefore, 24-week treatment with peginterferon at standard doses plus ribavirin at 800 mg/day appears prudent [220]. The SVR rates are promising (89%–98%) if treatment is continued for 24 weeks and initiated within 6 months after the onset of infection [220,268,269].

Children with HCV infection

Chronic hepatitis C infection is usually asymptomatic in children, but significant liver disease may occur [270]. Interferon-based treatment of children with chronic hepatitis C is not yet approved. Preliminary data suggest that an IFN plus ribavirin combination is effective, safe, and well tolerated in children with chronic hepatitis C [270,271]. Prospective controlled trials are ongoing to study the role of combination therapy in children with chronic hepatitis C. It may be prudent to delay therapy in children with mild, asymptomatic chronic hepatitis C and minimize quality of life issues including performance in school.

Normal ALT levels

As many as 25% of patients with chronic HCV infection have persistently normal ALT levels [272]. Most patients with persistently normal ALT levels have mild, nonprogressive histological damage [27,272–274]. However, mildly abnormal liver histology may be encountered in these patients, and the reported rate of cirrhosis varies from 0.3% to as high as 10% in patients with normal ALT levels [275,276]. In these patients, advanced fibrosis was associated with a coexistent significant amount of alcohol consumption [276].

In summary, the degree of ALT elevation does not consistently predict the severity of histological damage and response to therapy. Therefore, treatment in patients with chronic hepatitis C who have normal ALT levels should be individualized [217–220]. This patient population should not be universally excluded from antiviral therapy [272–275]. Patients with persistently normal ALT or histologically mild chronic hepatitis C can be treated if they decide to pursue antiviral therapy, particularly those with debilitating symptoms, unresolving acute hepatitis C, and genotypes 2 or 3.

Mixed cryoglobulinemia and glomerulonephritis

Mixed cryoglobulinemia is characterized by precipitation of cold-insoluble immunoglobulin complexes or cryoglobulins in the serum of 40%–50% of patients with chronic hepatitis C. Cryoglobulin production is triggered by chronic viremia, generation of rheumatoid factor, and stimulation of B cells by antigen–immunoglobulin complexes. In 10% of patients with HCV-related cryoglobulinemia various clinical manifestations, including vasculitis, glomerulonephritis, arthritis, or neuropathies, may develop. Cryoglobulinemia is associated with progressive liver damage and rapid posttransplant HCV recurrence. Monotherapy with IFN has been shown to be beneficial in the treatment of essential mixed cryoglobulinemia and glomerulonephritis associated with HCV infection [177–179,277]. Long-term therapy is usually required to prevent relapse of the underlying disorder [177–179]. In the absence of significant renal impairment, combination therapy with IFN-α and ribavirin provides much better efficacy

and should be the treatment of choice [177,277]. Cryoglobulinemia resolves and its clinical manifestations improve following viral clearance. Other forms of therapy used in the management of HCV-induced cryoglobulinemic glomerulonephritis include cytotoxic therapy and immunosuppressive therapy [180–182]. Cyclophosphamide and corticosteroids, often in combination with plasma exchange, have been used with some success [179–181]. Cyclophosphamide and corticosteroids are effective in providing symptom relief, but may stimulate viral replication. Prolonged use of immunomodulators should be limited to severe forms of vasculitis, aggressive glomerulonephritis, and following failure of antiviral therapy. In patients with severe debilitating illness or acute exacerbation, plasmapheresis may provide rapid but temporary relief. Rituximab, an anti-CD20 monoclonal antibody, has been noted to have clinical efficacy in lupus nephritis, membranous idiopathic nephropathy, and nonrenal manifestations of type II mixed cryoglobulinemia [182]. Preliminary data demonstrate that rituximab therapy is effective and safe in cryoglobulinemia-related glomerulonephritis in the setting of chronic hepatitis C [182].

Autoimmune liver disease

Interferon-based therapy can precipitate a flare-up of coexistent otherwise quiescent autoimmune chronic hepatitis. It is recommended that patients with coexisting chronic autoimmune hepatitis and HCV infection should initially be treated with immunosuppressive therapy to prevent acute exacerbation of autoimmune liver disease [278,279].

Immunocompromised status

The efficacy of IFN plus ribavirin treatment of HCV infection following liver transplantation, in end-stage renal disease, or following renal transplantation, and HIV coinfection is less than optimal [280–286]. End-stage liver disease is the leading cause of death in hospitalized HIV-seropositive patients [287]. An increasing number of patients with either HCV or HIV are being identified as coinfected with both viruses because of the similar routes of transmission. Up to 30% of all patients infected with HIV are also infected with HCV, whereas 10% of those infected with HCV may have HIV coinfection. It has been shown that patients coinfected with HCV and HIV progress more rapidly (up to threefold) than HCV-positive patients without HIV [283,288]. There is a plethora of data indicating that both HCV RNA levels and survival are directly influenced by HIV infection. These results suggest that HIV infection-induced CD4 depletion is independently associated with severity of liver fibrosis in chronic HCV infection [288,289]. In addition, HCV-related hepatitis may occur with initiation of antiretroviral therapy, which is presumably related to an immune restoration syndrome.

It is recommended that patients with HCV and HIV coinfection should undergo preemptive therapy against HCV infection irrespective of ALT levels and histological findings. If chronic hepatitis C is detected early in the course of HIV infection before the initiation of antiretroviral therapy is necessary, treatment of the hepatitis C is advised. However, if there is severe immune deficiency (CD4 count < 200 cells/mm^3), the CD4 count should be improved with antiretroviral therapy and the patient must have been on a stable regimen for at least 3 months before beginning treatment for their chronic hepatitis C. Three pivotal studies have demonstrated that HCV–HIV coinfection can be successfully treated with a peginterferon plus ribavirin combination, with SVR rates about half of those with HCV monoinfection but ranging from 14% to 29% for genotype 1, and 44%–73% for genotypes non-1 [284–286] (Table 82.5). The regimen approved by the FDA for treatment of HCV–HIV coinfection is peginterferon alfa-2a 180 μg/week plus ribavirin 400 mg twice a day for 48 weeks regardless of HCV genotype [284]. Studies with HCV monoinfection have indicated that patients infected with genotype 1 benefit from higher ribavirin dosages (1000 or 1200 mg/day), with an increase in SVR rate. Therefore, ribavirin may be given at dosages as high as 1200 mg daily, depending on the body weight. Peginterferon plus ribavirin does not affect HIV RNA levels, but CD4 counts can decline

Table 82.5 SVR with peginterferon and ribavirin therapy in patients with hepatitis C virus–human immunodeficiency virus coinfection

Trial [ref.]	Treatment	No.	Overall	GT 1	GT 2,3
APRICOT [284]	PEG alfa-2a + RBV 400 mg b.i.d.	868	40%	29%	62%
ACTG [285]	PEG alfa-2a + RBV 600–1000 mg/day	133	27%	14%	73%
RIBAVIC [286]	PEG alfa-2b + RBV 400 mg b.i.d.	412	27%	17%	44%

b.i.d., twice a day; GT, genotype; PEG alfa-2a, peginterferon alfa-2a at 180 μg/week; PEG alfa-2b, peginterferon alfa-2b at 1.5 μg/kg/week; RBV, ribavirin.

during therapy with a return to baseline following discontinuation. Unfortunately, the peginterferon plus ribavirin combination results in a higher rate of adverse effects and subsequent treatment discontinuation in coinfected patients.

At least two of the protease inhibitors used for the treatment of HIV infection, ritonavir and indinavir, can cause serious liver toxicity [290]. Ritonavir leads to late liver toxicity, normally as the result of triglyceride abnormalities. Indinavir, on the other hand, usually causes only a Gilbert-like syndrome, although in some cases it may cause a more significant toxicity. The protease inhibitors, saquinavir and nelfinavir, have only minimal liver toxicity. The use of highly active antiretroviral therapy has prolonged the healthy lifespan of patients infected with HIV, and deaths among individuals with AIDS have declined for the first time in 1996 with the institution of this therapeutic approach [283,290].

African Americans

A recent clinical trial confirmed the findings of two earlier studies that African Americans compared with white Americans with chronic hepatitis C, genotype 1 are less likely to respond to IFN-based antiviral therapy [291–293]. In the most recent multicenter clinical trial, 196 African Americans and 205 white Americans who were treatment-naive patients with HCV genotype 1 infection were treated with peginterferon alfa-2a (180 μg/week) and ribavirin (1000–1200 mg/day) for up to 48 weeks, and the primary endpoint was SVR [291]. Baseline features were comparable, including HCV RNA levels and histological severity, but African Americans had significantly higher body weights, a higher prevalence of diabetes and hypertension, and lower ALT levels. The SVR rate was 28% in African Americans and 52% in white Americans [291]. In multiple regression analyses, the white Americans had a higher SVR rate than the African Americans. Other factors independently associated with higher SVR included female sex, lower baseline HCV RNA level, less hepatic fibrosis, and more peginterferon taken. Racial differences in viral responses were evident as early as treatment week 4. African Americans demonstrated a higher frequency of breakthrough viremia than white Americans (13% versus 6%), but relapse rates were comparable (32% versus 25%). The proportions of patients with serious adverse events and dose modifications and discontinuations were similar among African Americans and white Americans. In summary, African Americans with chronic hepatitis C genotype 1 have lower rates of SVR to peginterferon and ribavirin than white Americans. These differences are not explained by disease characteristics, baseline viral levels, or amount of medication taken and may be explained by racial differences in global transcriptional response to interferon [294].

Adverse effects of interferon and ribavirin

Common side effects associated with ribavirin and IFN treatment, most of which are mild to moderate but may necessitate discontinuation of therapy in 10%–15% of patients, are listed in Table 82.6 [295–297]. The major side effect of ribavirin is a dose-dependent hemolytic anemia, which is reversible and usually stabilizes after 5–6 weeks of treatment. If severe anemia develops, treatment must be discontinued. The off-label use of growth factors (epoetin-α and granulocyte colony-stimulating factor) and antidepressants has come into common use. Patients must be informed that the use of growth factors and antidepressants is not approved by the FDA during antiviral therapy for chronic hepatitis C [295,297]. There are several contraindications to combination therapy with IFN and ribavirin (Table 82.2). Patients with preexisting anemia usually cannot tolerate the degree of hemolysis that occurs with ribavirin therapy and it can be dangerous. Patients with significant cardiovascular disease are particularly at risk should severe anemia develop during therapy. Patients with chronic hepatitis C and premorbid conditions preventing the use of ribavirin, particularly anemia, can be treated with peginterferon monotherapy [295].

Table 82.6 Adverse effects of interferon and ribavirin therapy

Nonspecific	Flu-like symptoms, including headache, fatigue, rigors, and fever
Cutaneous	Injection site reaction, pruritus, rash, dry skin, and hair loss
Respiratory	Cough and dyspnea
Gastrointestinal	Anorexia, nausea, vomiting, and dyspepsia
Neuropsychiatric	Depression, insomnia, anxiety, and irritability

Retreatment of relapsers and nonresponders

A selective approach to retreatment is prudent. Patients with favorable predictors for retreatment (genotypes 2 or 3) and at higher risk for progressive liver damage (stage 2 or greater fibrosis) should be considered for retreatment [298–301]. The primary goal of retreatment is eradication of HCV RNA and an SVR. In patients who fail to clear HCV RNA, secondary goals include, retarding or reversing hepatic inflammation and fibrosis, reducing the risk of HCC, preventing hepatic decompensation, and avoiding liver transplantation. Retreatment response rates have been highly variable, depending in large part on the response to the initial course of the treatment (nonresponse versus relapse). Relapsers demonstrate a significantly higher overall SVR rate to retreatment than nonresponders. The failure to respond to initial interferon-based antiviral therapy may be associated with a suboptimal therapeutic regimen, host variables, disease characteristics,

and viral factors. Retreatment of patients who were previously nonresponders to IFN monotherapy with subsequent peginterferon plus ribavirin achieved overall SVR rates between 16% and 28% [299–301]. Previous nonresponders to IFN plus ribavirin achieved overall SVR rates of 6%–15% following peginterferon plus ribavirin retreatment with higher response rates in patients with favorable predictors. Previous relapsers to IFN monotherapy were noted to have a 47% SVR to retreatment with IFN plus ribavirin, whereas relapsers to IFN plus ribavirin showed an overall SVR rate of 32%–50% with peginterferon plus ribavirin retreatment. Clinical trials testing retreatment strategies for treatment failures to peginterferon plus ribavirin are ongoing and include daily IFN-αcon-1 plus ribavirin, higher doses of peginterferon plus standard doses of ribavirin, extended therapy with peginterferon plus ribavirin and peginterferon maintenance monotherapy [302–304]. Low-dose maintenance peginterferon monotherapy with the goal of prevention of disease progression rather than viral eradication is an option for HCV treatment failures with advanced disease with increasing donor shortage for liver transplantation [303,304].

Novel investigational treatment strategies and agents

The efficacy, adverse effects profile and treatment duration of approved anti-HCV therapy is not ideal and is suboptimal. Therefore, new treatment strategies are being tested and developed with a goal to optimize the current regimens (avoid dose reduction and reduce the risk of adverse effects), the use of additional agents working through novel mechanisms (improve efficacy, reduce adverse effects, and shorten treatment duration), and antifibrotic strategies (retard progression, reverse damage, reduce the risk of hepatocellular carcinoma, and prevent hepatic decompensation). Several novel antiviral compounds are being studied in preclinical and clinical trials [305]. The therapies include those directed against hepatitis C targets (polymerase and protease inhibitors, antisense nucleotides), immunomodulators (histamine and thymosin), silymarin, vaccines, alternative IFNs and IFN delivery systems, ribavirin prodrugs, and L isomers. Clinical trials with two promising protease inhibitors, VX-950 (Vertex Pharmaceuticals) and SCH 503034 (Schering-Plough) are under way. Data from pilot studies showed at least a 2-$\log_{10}$ decline in viral load in every patient treated with VX-950 and SCH 503034 demonstrated a dose-dependent decline in viral load, with a mean reduction of 2.06 $\log_{10}$ from baseline [306–308]. No new drugs are expected to gain FDA approval for the next 3–5 years [305]. Furthermore, IFN will maintain its important status in future multidrug regimen.

Preventive and safety measures

Patient should be informed that, although the risk of transmitting HCV infection by sexual contact is low (3%–5%), HCV is potentially transmissible. Patients should be careful about blood exposure of any type to partners and family contacts. Open wounds must be covered, and razors or toothbrushes should not be shared. Although sexual or intrafamilial HCV transmission is rare, testing sexual partners or other family members if there is a concern regarding infection usually provides reassurance. Patients with chronic HCV infection should be counseled to avoid excessive consumption of alcohol, which can accelerate the progression of hepatitis C when used in moderate or large amounts. It has been recommended that the less alcohol consumed the better, and that complete abstinence is ideal [217–220]. Finally, it is recommended that hepatitis A and B vaccine be given to patients with chronic HCV infection who are not already immune because acute hepatitis A or B superimposed on chronic HCV infection may be more severe [309]. The safety and efficacy of these vaccines have been demonstrated in patients with mild to moderate chronic HCV infection [310], and a more convenient combined hepatitis A and B vaccine was licensed in 2001. The inability to culture the virus and its genetic heterogeneity are obstacles to developing a vaccine against HCV infection [311,312]. Investigational vaccination candidates, including recombinant proteins, peptides, virus-like particles, naked DNA, and recombinant viruses, are being explored.

Economic implications

Current estimates of medical and work-loss costs of HCV-related acute and chronic liver disease are in excess of $600 million annually based on unpublished CDC data. In 1995, the outcomes of hospitalizations were analyzed, focusing primarily on inpatient mortality and health-care cost [313]. It was estimated that HCV-related liver disease resulted in 26 700 hospitalizations and 2600 deaths in acute, nonfederal hospitals in the United States. The cumulative cost for these hospitalizations was $514 million. The cost of a 48-week treatment with peginterferon and ribavirin ranges from $30 000 to $40 000 [220]. The use of erythropoietic growth factors for patients with anemia occurring during HCV combination therapy appears to be cost-effective [314].

Liver transplantation

In the United States, about 8000 patients die each year from complications of HCV-related cirrhosis [2,16]. Chronic hepatitis C with decompensated cirrhosis, alone or in combination with alcoholic liver disease, is the most common indication for liver transplantation in the United States and Europe [27]. Based on statistics obtained from the United Network for Organ Sharing (UNOS), 23% of patients who underwent liver transplantation between 1994 and 1998 carried the diagnosis of HCV infection [315]. There has been a dramatic increase in the number of patients with HCV infection undergoing liver transplantation, and HCV accounts

for up to 50% of liver transplants both in the United States and Europe [316,317].

Candidacy for liver transplantation

Patients who are being considered for liver transplantation must meet one of the following minimal listing criteria:

- Child–Pugh class B or C (Child–Turcotte–Pugh [CTP] score of 7 or greater) in the setting of cirrhosis
- history of gastrointestinal bleeding caused by varices, or a single episode of spontaneous bacterial peritonitis irrespective of CTP score
- small unifocal or multifocal HCC (one lesion < 5 cm in diameter or three lesions < 3 cm involving the same lobe of the liver) with no portal vein invasion or metastases [318,319].

Patients who fulfill the UNOS specified listing criteria for liver transplantation have an estimated 1-year survival from chronic liver disease of less than 90%, which is the likely outcome with liver transplantation at major institutions [319]. In patients with compensated HCV-related cirrhosis, the 4-year to 5-year survival rate is 91% and the estimated risk of decompensation is 18%–20% [139]. Liver transplantation is the treatment of choice for chronic hepatitis C with decompensated cirrhosis, but HCV reinfection poses challenging management issues that may arise either early or late after transplantation [320].

Posttransplantation recurrent infection

Chronic hepatitis C with cirrhosis or HCC is the leading indication for liver transplantation in the United States [321–323]. Of the approximate 6000 liver transplantations performed annually, 45% or 2500 are for hepatitis C. Reinfection with HCV is virtually universal at the time of transplantation, and the rate of fibrosis is accelerated such that the median time to recurrent cirrhosis is less than a decade. Three patterns of recurrence are recognized: evolution over time to chronic hepatitis and cirrhosis (most common), an indolent course of reinfection over at least the first decade, and cholestatic hepatitis with early graft failure (infrequent) [321]. There are a number of conflicting reports in the literature regarding risk factors for poor outcomes after liver transplantation for chronic hepatitis C, but the factors most often cited include high pretransplant viral load (> 1 million IU/mL), high posttransplant viral load (> 10 million IU/mL), genotype 1b, early recurrence within months of transplant, older donor age (> 40–50 years of age), cytomegalovirus infection, prolonged cold and warm ischemia times, nonwhite race, female gender, and treatment of acute rejection [321–323] (Table 82.7). Use of corticosteroid boluses and antilymphocyte therapies are most consistently implicated as detrimental, but the impact of other agents, such as cyclosporine (ciclosporin [rINN]) versus tacrolimus, or the use of mycophenolate mofetil remains controversial. There were some preliminary reports that live donor transplant recipients were at greater risk of recurrence compared with deceased donor recipients, but these observations have not been confirmed and remain uncertain [321–323].

Table 82.7 Risk factors for severe recurrence of hepatitis C virus (HCV) posttransplantation

Host
Human leukocyte antigen matching
Age
Nonwhites
Cytomegalovirus viral coinfection
Absence of pretransplant coinfection with HBV
Presence of immunoglobulin M anti-HCV core antibodies after transplantation
Viral
Genotype 1b
Pretransplant HCV quasispecies
High (> 1×10^6 Eq/mL) pretransplant HCV RNA levels
High serum or liver, early posttransplantation HCV RNA viral load
Allograft
Prolonged rewarming time during allograft implantation
Year of transplant (recent year associated with rapid rate of progression)
Early posttransplant histological injury
Episodes of rejection
Drugs
High level of immunosuppression
Mycophenolate use
OKT3 use
Methylprednisone boluses
High cumulative steroid dose

Natural history of recurrent hepatitis C – rationale for treatment

If HCV infection is not eradicated before transplantation, reinfection occurs in essentially 100% of patients [321–323]. The rate of disease progression is variable, but approximately 25% will develop recurrent cirrhosis within 5 years of transplantation. In follow-up studies of 5 or more years, the outcomes for HCV-positive transplant recipients are worse than those for HCV-negative recipients, with a significant reduction in both patient and graft survival. The goals of HCV management in the transplant setting are primarily to prevent graft loss through recurrent disease. The treatment strategies differ in terms of the timing of the intervention:

- prophylactic therapy, starting at time of transplantation
- preemptive therapy, starting in the early posttransplant period
- treatment of established, often progressive, histological disease.

The latter approach has been the most commonly used. However, there is a lack of well-controlled studies of antiviral therapy for recurrent hepatitis C after liver transplantation to provide solid evidence-based medicine to support this approach. On the other hand, this strategy appears reasonable pending further data.

Prophylactic therapy

HBIG has been an effective means of preventing HBV reinfection following liver transplantation. A similar approach has been examined for HCV. There are indirect data from a cohort study of HBV–HCV coinfected patients that received HBIG (before screening of HCV donors) and had lower rates of recurrent HCV than those who did not receive HBIG [322,323]. Experiments in chimpanzees exposed to HCV and then treated with hepatitis C immunoglobulin (HCIG) found that antibody therapy could prevent or attenuate HCV infection. These studies provide the rationale for use of HCV antibodies to prevent HCV reinfection following transplantation. The studies to date suggest that HCV reinfection is not prevented by HCIG but higher dose therapy may modify the natural history of early posttransplant infection (lower ALT levels and less necroinflammation) [323]. Antibody therapy appears to be well tolerated so this form of therapy may be useful in the early posttransplant period as a bridge to other forms of therapy.

Preemptive antiviral therapy

Preemptive treatment of patients with chronic hepatitis C after liver transplantation before the development of histological recurrent disease makes sense. Studies have shown that preemptive IFN monotherapy can lead to the eradication of HCV infection posttransplantation, but tolerability of therapy has been a limiting factor. In a randomized study of 124 patients transplanted for HCV, only 51 (41%) were candidates for preemptive antiviral therapy by the eighth week posttransplant [324]. Patients with low pretransplant MELD and CTP scores and those who were live donor recipients were most suitable for early initiation of therapy. An SVR was achieved in only 18% receiving standard IFN or peginterferon plus ribavirin compared with 5% treated with monotherapy. In another randomized trial involving 60 patients treated with peginterferon alfa-2a monotherapy, only 8% achieved SVR. Clinical trials using preemptive IFN monotherapy, although generally positive, show only a modest benefit, with SVR rates from 5% to 39% [324–327]. Rates of response appear higher with combination therapy than with IFN monotherapy [326,328,329]. Both response rates and tolerability of therapy vary from study to study. Sicker patients are generally less able to tolerate early initiation of posttransplant antiviral therapy, and live donor liver transplant recipients may be good candidates for treatment.

At the present time, preemptive therapy cannot be recommended universally. If our ability to identify persons at risk for rapidly progressive disease improves, preemptive therapy may be useful in these patients as targeted therapy.

Treatment of established HCV disease

Study protocols for the treatment of established recurrent HCV disease have generally initiated treatment between 6 and 24 months after transplantation. The advantages of initiating treatment when recurrent disease is clinically or histologically apparent rather than as preemptive treatment include lower doses of immunosuppression (may enhance response to antiviral therapy), improved clinical status to better tolerate treatment, lower risk of acute rejection, and cost savings because only those with progressive disease are treated. Potential disadvantages of the "wait and treat recurrent disease" approach include higher viral load and more fibrosis, which may reduce the likelihood of response.

Most of the studies evaluating antiviral therapy for established recurrent HCV disease have been uncontrolled and of small sample size, and with variable IFN/ribavirin doses and treatment duration [330]. Reported SVR rates in IFN monotherapy studies, including peginterferon monotherapy, are more than 20%. Combination of standard IFN and ribavirin is associated with slightly higher rates of response (SVR rates 20%–30%) and the limited data available for peginterferon plus ribavirin report SVR rates of 30%–45%. Combination therapy with peginterferon plus ribavirin is therefore the treatment of choice.

Only two controlled studies have been reported, one with a combination of IFN and ribavirin and the other with peginterferon monotherapy [325,331]. Combination IFN and ribavirin resulted in SVR in 21% of treated patients compared with 0% of untreated controls. The response rates were lower with peginterferon monotherapy (12%) but still superior to untreated patients (0% response). Histological improvements were reported in both responders and nonresponders. Despite titrated regimens using low initial doses of peginterferon and ribavirin, dose reductions and discontinuations were frequent.

The optimal duration of treatment for recurrent HCV disease is unknown. In an immunosuppressed population of liver transplant recipients, longer treatment periods may be necessary to enhance SVR rates. Prolonged low-dose maintenance IFN is being evaluated in several clinical trials for its histological and clinical benefits in patients who fail to clear HCV after combination IFN and ribavirin therapy. This strategy needs to be evaluated in the transplant setting.

In summary, the treatment of recurrent HCV disease is focused primarily on the treatment of recurrent histological disease with hepatic fibrosis of stage 2 or beyond. Although large-scale and controlled studies are lacking, use of peginterferon plus ribavirin would appear to be the treatment of choice. Strategies to improve tolerability are needed because this would be predicted to increase response rates. Fortunately, the use of IFN or peginterferon has only rarely been

associated with precipitation of acute cellular rejection or chronic ductopenic rejection. Finally, this is a patient population for whom new antiviral agents with improved efficacy and tolerability are urgently needed.

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83 Drug-induced liver disease

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Epidemiology

Drug-induced hepatotoxicity is a frequent cause of acute liver injury of exceptional severity, comprising more than 50% of all cases of acute liver failure (ALF) in the United States [1,2] (Fig. 83.1). Hepatotoxicity has been described for a large number of drugs [3], although the number of cases is low given the number of prescriptions written.

Different agents cause liver injury in different ways at different rates. Most reactions are directed against hepatocytes but biliary injury, as well as combined hepatocyte/biliary injury or damage to specific organelles (e.g., mitochondria), produce the different disease patterns observed. While some agents such as isoniazid cause liver injury in as many as 1 in 100 people and fatality in 1 in 10 000, other agents result in liver damage in only 1 in 50 000 or may never cause liver injury.

Few data on the epidemiology of drug-induced liver disease are available. A population-based study from France performed between 1997 and 2000 demonstrated an annual incidence of ~ 13.9 per 100 000 with significantly more cases found in people over 50 years of age [4]. Cases with a fatal outcome are more limited and most studies are subject to underreporting [5]. In developing parts of the world, drug-induced liver disease is much less common and related to very few drugs [6]. However, probably only a small fraction of actual cases are reported, and a true estimate of the incidence of drug-induced liver disease may be impossible to obtain. This is, in part, linked to the difficulty in establishing the diagnosis, as well as to inadequate reporting systems.

The exact number of drug-induced liver injuries per year in the United States is unknown, but the severity of many of these cases and the tragedy involved in a presumed preventable injury makes it imperative that all sensible precautions be taken to avoid such incidents.

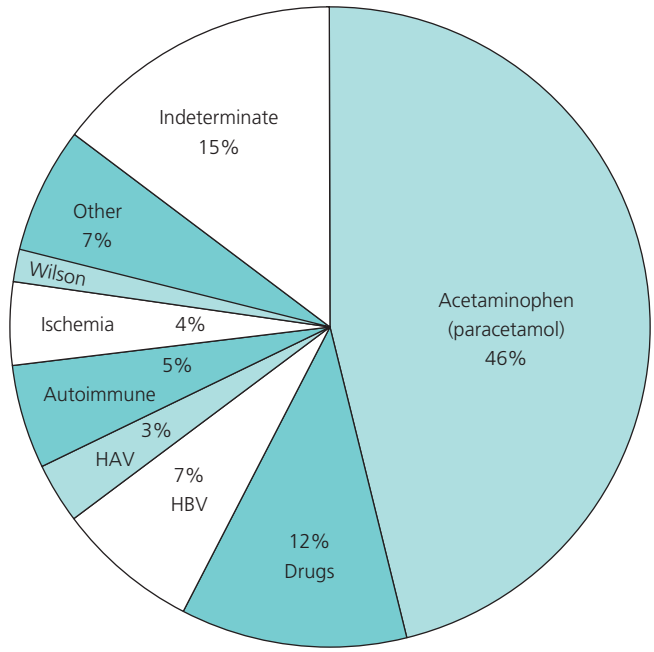

Figure 83.1 Number of cases of acute liver failure among 1033 patients grouped according to etiology in a multicenter study between 1998 and 2006 at 23 sites around the United States participating in the Acute Liver Failure Study Group. Acetaminophen and idiosyncratic drug reactions were the presumed cause in 594 (58%) cases of acute liver failure in this series.

Drug metabolism and mechanisms of hepatotoxicity

The liver, situated between the absorptive surface of the gastrointestinal tract and the target of drug effects throughout the body, is central to the metabolism of foreign substances.

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Since hepatocyte metabolism is required for virtually every drug, it is remarkable how seldom injury to liver cells occurs. Most drugs and xenobiotics cross the intestinal brush border because they are lipophilic. *Biotransformation* is the process whereby lipophilic therapeutic agents are rendered more hydrophilic by the hepatocyte, resulting in excretion of the drug in urine or bile. In most instances, biotransformation changes a nonpolar compound to a polar compound through several steps. Foremost is an oxidative pathway (e.g., hydroxylation) mediated by the cytochromes P450 (CYPs) [7]. This is typically followed by esterification to form sulfates and glucuronides, which results in the addition of highly polar groups to the hydroxyl group. These two enzymatic steps are referred to as phase I (P450 oxidation) and phase II (esterification). Other important metabolic pathways involve glutathione S-transferase, acetylating enzymes, and alcohol dehydrogenase, but the principal metabolic pathways for most pharmacological agents involve P450 and subsequent esterification.

The exact details of the pathogenesis of liver injury remain unclear for most drugs. A single drug may cause its toxic effects in several ways. An oversimplified approach suggests that high-energy unstable metabolites of the parent drug, the result of P450 activation, bind to cell proteins or DNA and disrupt cell function. Perhaps the best example is acetaminophen. Although used universally for nonnarcotic pain relief, acetaminophen taken in large quantities causes profound centrilobular necrosis [8]. The metabolic pathway for acetaminophen involves both phase I and phase II reactions, glutathione detoxification, and the formation of reactive intermediates (Fig. 83.2). Glucuronidation and sulfation occur as the initial detoxifying step because the parent compound contains a hydroxyl group. Since glucuronidation and sulfation capacity greatly exceeds daily needs, even patients with far-advanced liver disease continue to have adequate glucuronidation capacity, which explains why no obvious enhancement of toxicity is observed in patients with cirrhosis who are taking acetaminophen [9].

Enzyme polymorphism

The rarity of drug toxicity begs the question of how an infrequent event (1 in 10 000) occurs. Genetically variant CYP isoenzymes, such as are observed with the metabolism of debrisoquine, partially explain the observed individual variation in responses to drugs. Debrisoquine is an antihypertensive drug marketed in Europe that is hydroxylated by CYP2D6, an isoform that is totally lacking in 5% of healthy individuals, greatly prolonging the half-life of the parent compound in affected individuals [10]. Fast and slow acetylator patterns affect whole races, and have been implicated in isoniazid metabolism, which includes an acetylation step [11]. Genetic variants, which occur relatively frequently, cannot explain the formation of a toxic intermediate in only a rare individual. While there might be other metabolic variant P450

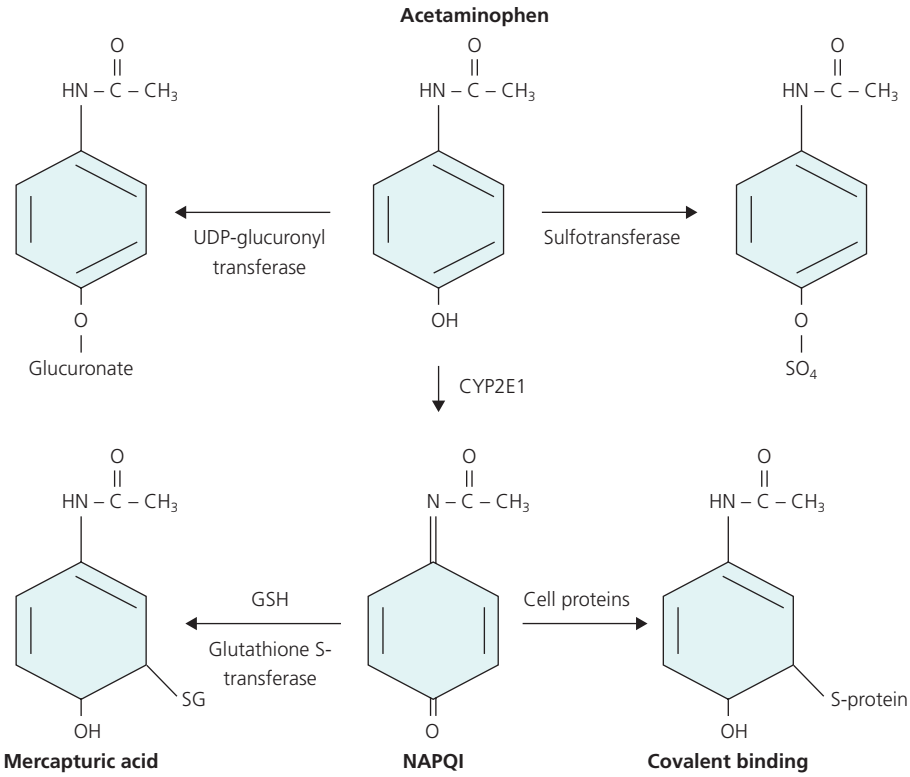

Figure 83.2 Metabolic pathway for acetaminophen. CYP2E1, cytochrome P2E1; NAPQI, *N*-acetyl-p-benzoquinoneimine.

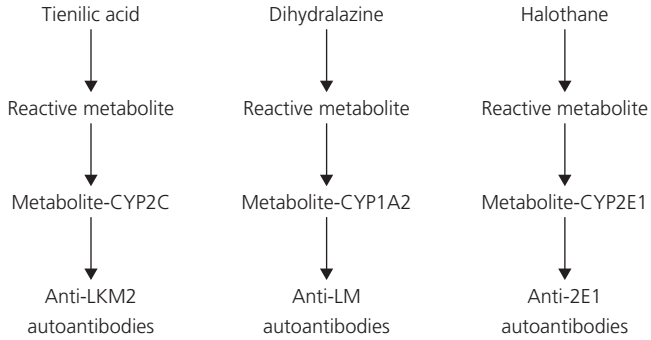

Figure 83.3 Modification of host P450 enzymes renders them immunogenic. Autoantibodies can be detected, which recognize the enzyme–metabolite adducts and the enzyme itself. CYP1A2, cytochrome P1A2; Adapted from Robin et al. [12].

species that are even rarer, little evidence for these has been found in affected patients. Other explanations are necessary.

Most drugs are small organic compounds that are unlikely to evoke an immune response. While some toxic drug reactions are associated with an obvious allergic response, most are not. Nevertheless, immune mechanisms not associated with systemic allergic immunoglobulin E (IgE) reactions or skin hypersensitivity might be involved. Studies suggest that the products of CYP metabolism, the highly reactive intermediates formed within the microsomes, covalently bind to the enzyme itself to form a drug–hapten adduct that disables the enzyme and injures the cell [12]. Haptenization then evokes an immune response directed against the newly formed antigen or neoantigen. P450s have been shown to traffic to the plasma membrane allowing the P450–drug adduct to become the target of a subsequent cytolytic attack (Fig. 83.3). Whether these adducts or smaller peptides that are processed and presented by way of the major histocompatibility complex class I and class II schemes are the targets remains unclear. Still, the association of neoantigens, autoantibodies, and hepatotoxic drugs implicates an immunological mechanism.

Whether the drug causes significant cell necrosis or not, the P450–drug adducts can evoke the immune response. Any subsequent P450–drug adduct present on the hepatocyte surface would evoke a further response. Responses may be antibody-mediated or occur from direct cytolytic attack by primed T cells [12,13] (Fig. 83.4).

Such a combined toxic/immunological mechanism is involved in the liver injury caused by halothane. Halothane was a widely used fluorinated hydrocarbon anesthetic that causes severe, often fatal, liver injury after multiple exposures [13]. Other fluorinated hydrocarbons also occasionally produce the same response [14–16]. While halothane has never been withdrawn, its use has been limited by the advent of safer agents. Both direct cytotoxicity and immune-mediated toxicity are observed in keeping with the clinical observations that severe halothane toxicity occurs with repeat exposures. Still, evidence of injury can usually be identified

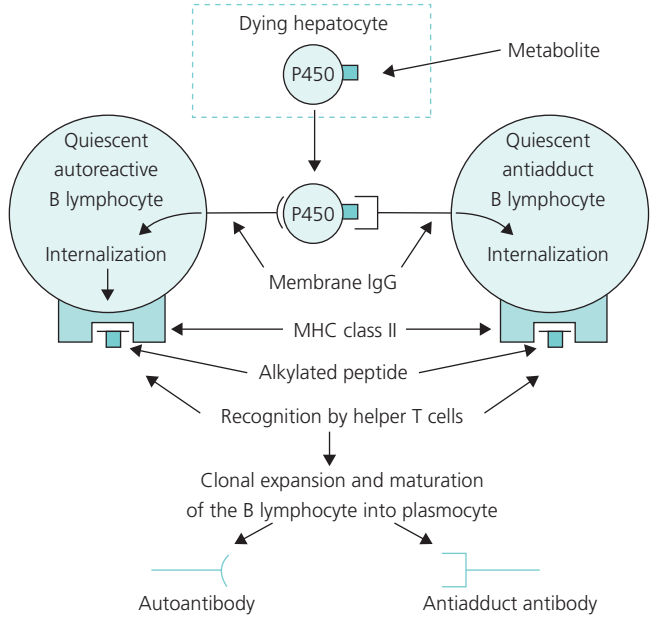

Figure 83.4 Possible models of liver injury due to drugs. Direct toxicity may play a role but immune mechanisms are also operative. In this model, rechallenge or continuing to receive the same drug would simply elicit a preformed cytolytic response. IgG, immunoglobulin G; MHC, major histocompatibility complex. From Robin et al. [12], with permission from Elsevier.

within a week of the first exposure. As befits an immune reaction, the interval to toxicity is shortened and the damage is more severe with each successive exposure.

Specific genetically determined components of the immune response might be important. For example, the binding of peptides for antigen presentation depends on human leukocyte antigen (HLA) configurations that are genetically determined. This variation among individuals is thought to underlie the diverse responses observed in patients encountering the hepatitis B virus. The highly variable severity of reactions observed depends on the fit of antigen peptides in the HLA groove. A specific HLA haplotype has been associated with amoxicillin–clavulanate-induced hepatitis, being found in 57.1% of patients compared with 11.7% of controls [17]. Polymorphisms have also been identified for the interleukin-10 (IL-10) promoter and for tumor necrosis factor-α (TNF-α). These variations in immune responsiveness could modulate the severity of the responses observed. For example, different IL-10 promoter phenotypes are recognized. A C-to-A substitution at position 627 is linked to severe asthma and, by inhibiting IL-10 secretion, an up-regulation of T helper type 2 immune reactions. This same phenotype has been linked to hepatitis C-related liver injury and to the severity of alcoholic liver injury [18,19]. Variant TNF-α phenotypic expression has been implicated in determining the severity of drug reactions related to acetaminophen [20]. A multistep, immune-based mechanism would best explain both the rarity of idiosyncratic reactions, and their severity,

Table 83.1 Factors influencing the metabolic fate of drugs

Age: drug reactions appear to impact the elderly more often, P450s vary [127]. Adults more susceptible than children in some scenarios (acetaminophen, halothane, isoniazid) and less susceptible in others (e.g., aspirin, valproic acid)
Gender: females are more prone to drug reactions statistically, mechanism unknown
Size/weight: effects on women relate to intrinsic gender differences but also to size
Pregnancy: effects of drugs in pregnancy have been poorly studied
Preexisting liver disease: hepatic disease may *protect* against idiosyncratic reactions and may enhance toxicity in dose-dependent hepatotoxins (such as acetaminophen) [131]
Renal disease: slowed disappearance of parent compound yields higher concentrations and affects P450 [132]. Tetracycline toxicity is enhanced in renal disease
Certain foods: grapefruit has an unknown substance that interferes with metabolism [133–135]
Concomitant drugs: drug–drug interactions are common causes of adverse effects (e.g., valproate and chlorpromazine together lead to enhanced cholestasis).
Genetic factors: enzyme polymorphisms (e.g., enhanced phenytoin liver disease in patients with defective epoxide hydrolase activity), human leukocyte antigen phenotypes (nitrofurantoin susceptibility).

as well as the findings of mild, nonprogressive liver injury in some patients – those with "protective" phenotypes. Recent studies suggest that, for specific agents, genetic markers can be identified that associate with the toxic reaction. Such pharmacogenomic observations may bear fruit, particularly for high-risk drugs [21]. While an immunological explanation for many reactions is plausible, the exact mechanism to account for most drug reactions remains obscure. Both cell necrosis and apoptosis have been recognized, and sinusoidal lining cells as well as Kupffer cells are part of the process [22].

There should be little doubt that the metabolic fate of any compound is a complex process. There are other important environmental and host variables outlined in Table 83.1. Often multiple factors are at play simultaneously, including drug interactions, either induction or competition. Common inducing agents include ethanol, phenobarbital, and phenytoin, but cigarette smoke is also a potent inducer of certain P450 species. Induction or substrate competition for available enzyme may not result in hepatotoxicity but strongly impacts plasma drug levels. For example, the effect of ketoconazole on enhancing cyclosporin levels is the result of induction [23], whereas competitive inhibition by ketoconazole increases serum levels of astemizole (Hismanal) with resulting torsades de pointes [24].

Other mechanisms

In drug-induced cholestasis, disruption of specific transport proteins, or processes in hepatocytes or cholangiocytes may be the event that results in cholestasis. Bile salt transport from plasma into the hepatocytes is provided for by two basolateral (sinusoidal) transport systems: the sodium-taurocholate cotransporter, and the organic-anion transporting polypeptide, whereas several canalicular export pumps have been identified [25]. Estrogen may cause multiple canalicular membrane transport changes [26], affecting, among others, the canalicular bile salt pump [27].

Uncoupling or inhibition of mitochondrial respiration may in some instances lead to microvesicular steatosis [28]. Mitochondrial β-oxidation of fatty acids is impaired and this may decrease the cellular energy supply leading to severe liver dysfunction. The mitochondrial β-oxidation may be affected either directly or by impairment of mitochondrial respiration [29].

Drug-induced liver injury may be modulated and enhanced by inflammatory mediators that may trigger hepatocyte apoptosis [30]. Hepatocyte apoptosis is complex and may be controlled by the intracellular energy status [31,32] and by the redox state of the cell [33]. How the specific drugs involved in hepatotoxicity affect hepatocyte apoptosis remains to be studied.

As noted above, new techniques of pharmacogenomics may be helpful in predicting an individual's risk of hepatotoxicity for a given drug based on the discovery of genetic susceptibility profiles associated with liver injury [34].

Classification of hepatotoxic agents

Two main categories of drugs can produce liver disease. One group consists of intrinsic (predictable) drugs, whereas a second group consists of idiosyncratic (unpredictable) drugs. Unfortunately, the vast majority of drugs involved in liver disease belong to the idiosyncratic, and so unpredictable, group.

Intrinsic (dose-dependent) agents

Hepatotoxins of this group produce liver disease in most patients in a dose-related fashion if toxic amounts of the drug are ingested. Furthermore, similar lesions can often be found in animal models [35]. Hepatotoxicity may be caused by the drug itself or, most frequently, by toxic effects of its metabolites. Table 83.2 provides a list of known dose-dependent drugs.

Until recently, occupational hepatotoxic agents, such as carbon tetrachloride [36] and yellow phosphorus [37], were frequently encountered. Since 1980, however, these agents have rarely been seen. Instead, acetaminophen (paracetamol) has emerged as the most dominant intrinsic hepatotoxic drug. Taken in small doses (4 g or less per day) acetaminophen is an extremely safe drug. However, its therapeutic index is low because as little as 10–12 g may cause extensive hepatic necrosis [38,39].

In acetaminophen metabolism, the phase II reactions predominate, with only a small fraction of acetaminophen meta-

Table 83.2 Drugs/toxins in which a dose–response effect is observed

Drug	Response
Acetaminophen	Total dose, single versus multiple time points
Amiodarone	Total dose over time
Bromfenac	Toxicity only occurs after extended use
Cocaine	Dose-related vascular collapse
Cyclophosphamide	Dose-related, worse with previous ALT elevations
Cyclosporine	Cholestasis with toxic blood levels, CYP3A phenotype
Methotrexate	Aminotransferase/fibrosis; single dose/total dose
Niacin	Large doses yield vascular collapse
Oral contraceptives	Prolonged usage yields hepatic adenomas
Tetracycline	Total dose, renal dysfunction
Toxins (yellow phosphorus, carbon tetrachloride, amanita toxin, bacterial toxins)	Total dose

ALT, alanine aminotransferase; CYP3A, cytochrome P3A.

bolized by CYP450, until the quantity of acetaminophen exceeds phase II capacity, at which point significant amounts of a toxic intermediate, *N*-acetyl-p-benzoquinoneimine (NAPQI), are formed, primarily through CYP2E1 (see Fig. 83.2) [40,41]. NAPQI binds covalently to cell macromolecules disrupting mitochondrial and nuclear function [35]. Nitrotyrosine residues can be detected by immunohistochemistry as evidence of oxidative stress in the livers of patients (or experimental animals) demonstrating toxicity [42]. These residues are formed by the rapid reaction of superoxide and nitric oxide (formed by the Kupffer cells) to form peroxynitrite, unless covalent bonding of NAPQI is prevented by its conjugation (via glutathione-S-transferase) to form mercapturic acid, a harmless water-soluble product excreted by the kidney [43]. Depletion of glutathione lowers this last defense against the formation of NAPQI-related intracellular adducts. Starvation and chronic alcohol intake by depleting glutathione enhance toxic injury [44–48], whereas *N*-acetylcysteine, by replenishing glutathione, protects against acetaminophen-induced injury [49]. This direct toxic reaction occurs predictably in all individuals and is not an allergic reaction. The final step leading to cell death remains unclear but may involve an increase in levels of cytosolic calcium altering the cytoskeleton and membrane integrity, leading to "blebbing" of the cell membrane and loss of its integrity [50]. Dose-related necrosis (lysis) of hepatocytes occurs but apoptotic pathways are also implicated. A finding that peroxisomal proliferator activation prevents the liver injury associated with acetaminophen links this liver damage to apoptosis but does not preclude a combined necrosis/apoptosis effect [51,52].

Acetaminophen overdose is the most common cause of acute liver failure with hepatic encephalopathy in several Western countries including the United Kingdom and the United States [39,53,54]. Although the prognosis for acetaminophen-induced acute liver failure is relatively good, with a spontaneous survival (i.e., survival without liver transplantation) of approximately 57% [1]. It is still the leading cause of fatal acute liver failure in the United States [2].

Except for acetaminophen-induced liver disease, intrinsic drug cases are rare.

Idiosyncratic reactions

While acetaminophen is a dose-related toxin, most drug reactions are idiosyncratic, occurring from 1 in 1000 to 1 in 50 000 patients. The etymology of "idiosyncratic" from the Greek loosely translated is "the unique composite of the self" – the particular features of a given individual. This places the emphasis appropriately on the patient's characteristics rather than on the drug itself. Idiosyncratic reactions are not the result of the drug itself because almost everyone can tolerate them, but of something unique about the patient who ingests them and has a toxic reaction. Theories abound to explain these reactions. Any theory of pathogenesis must "explain" the features shown in Table 83.3. The proportion of drug-induced liver disease varies greatly among drug classes as evidence of class effect. Nonsteroidal agents, antibiotics, and anticonvulsants are highly associated with drug-induced liver disease, whereas hormones, antihypertensive drugs, digoxin, and antiarrhythmic drugs are rarely associated. Idiosyncratic reactions occur in small numbers such that some drugs continue to be used when usefulness or uniqueness makes the risk acceptable. Isoniazid is such a drug, virtually the only drug implicated in developing countries, where drug-induced liver injury is otherwise unheard of [55]. Some 15%–20% of individuals receiving isoniazid as a single agent for tuberculosis prophylaxis may develop increased transaminases, but these usually stabilize or improve, so that less

Table 83.3 Idiosyncratic drug reactions

1. Occur rarely
2. A pattern consistent for each drug
3. When similar drugs exhibit similar features, called "class effects"
4. Individual drugs in a class still vary considerably
5. Reactions occur at varying time intervals after first ingestion
6. Reactions vary in severity, but typically severe and fatal if drug continued
7. Mild injury can sometimes disappear with continued use
8. Rarity of most reactions suggests possibility of multiple hits
9. Rechallenge is often met with greater severity, shorter latency

than 1% may develop severe hepatic necrosis [56]. More recently, a lower estimate of severe liver injury of 1 in 1000 has been given for isoniazid from a large tuberculosis public-health clinic [57], because 11 of 11 141 patients developed isoniazid-induced liver disease. This is still a high rate of injury compared to other idiosyncratic drug reactions but the usefulness of the drug has precluded its withdrawal.

Aside from isoniazid, nonsteroidal analgesics may be the most commonly associated drug class capable of inducing idiosyncratic reactions [58]. The newer cyclooxygenase-2 (COX-2) inhibitors have been implicated [59,60]. It is important to recognize that certain classes, principally antibiotics and nonsteroidal antiinflammatory drugs (NSAIDs), are known to be associated with toxic injury whereas for others an association is much less likely.

Adaptation

It has been recognized that many drugs that cause rare idiosyncratic reactions are associated with more frequent aminotransferase elevations that are limited and resolve even if administration of the suspected agent continues [61]. This was known to be true for isoniazid but is recognized as a common feature of the cholesterol-lowering "statin" drugs. How this nascent liver injury is aborted or modulated by the host remains unclear. An interesting correlate is that acetaminophen appears to cause significant aminotransferase elevations in up to 40% of users within the first 2 weeks, if they take the recommended maximum of 4 g/day [62].

Types of drug reactions, clinical pictures

While most liver injury involves direct hepatocyte necrosis/apoptosis, some drugs primarily injure the bile ducts or canaliculi, causing cholestasis without significant hepatocyte damage. Others affect sinusoidal cells or present a particular pattern of liver injury affecting multiple cell types (mixed type). In a rough way, drug reactions can be grouped as hepatocellular, cholestatic or mixed, but these are only general terms and do not apply to all circumstances. An additional way to categorize drug reactions emphasizes the histological changes involved as well as the cell type (Table 83.4).

Table 83.4 Types of drug reactions, with examples

Hepatocellular: isoniazid, trazodone, diclofenac, nefazodone, venlafaxine, lovastatin
Cholestatic: chlorpromazine, estrogen, erythromycin
Mixed: amoxicillin/clavulanate, carbamazepine, herbs, cyclosporin, methimazole
Immunoallergic: halothane, phenytoin, sulfamethoxazole
Granulomatous: allopurinol, diltiazem, nitrofurantoin, quinidine, sulfa drugs
Steatohepatitis: amiodarone, perhexiline maleate, tamoxifen
Autoimmune: nitrofurantoin, methyldopa, lovastatin
Fibrosis: methotrexate, vitamin A excess
Vascular collapse: nicotinic acid, cocaine, ecstasy
Venoocclusive disease: busulfan, herbal medicines

A distinction between the various types of liver injury can be made based on the liver tests for alanine aminotransferase (ALT) and alkaline phosphatase (AP) [63]. The liver injury is considered *hepatocellular* when ALT alone is more than twice the upper limit of normal (ULN) range, or when the ratio (ALT/ULN) : (AP/ULN) is greater than or equal to 5. The liver injury is termed *cholestatic* if AP alone is more than twice the ULN, whereas the term *mixed* designates a situation in which both ALT and AP are more than twice the ULN and the ALT/ULN : AP/ULN ratio is between 2 and 5.

Hepatocellular reactions

Hepatocellular reactions are the most common type of drug-induced liver disease, constituting up to 90% of cases [64]. They are characterized by a hepatocellular pattern of serum liver tests, as defined above. Many drugs have been implicated in hepatocellular type drug-induced liver disease (Table 83.5). Usually, improvement occurs quickly after discontinuation of the drug (1–2 months), and only a few patients develop fulminant, acute liver failure with hepatic encephalopathy [65].

Histological findings include necrosis and cellular infiltration. Necrosis may be zonal (e.g., acetaminophen-induced or

Table 83.5 Agents that may cause liver injury of hepatocellular type

Acetaminophen
Carbon tetrachloride
Cloxacillin
Diclofenac
Halothane
Isoniazid
Lovastatin
Nefazodone
Trazodone
Venlafaxine

CCl_4-induced) or diffuse (e.g., halothane-induced), and the inflammatory response consists of lymphocytes or eosinophils. Massive necrosis may cause acute liver failure and death – the exact quantity of remaining hepatocytes necessary to support life has not been established [8].

Acetaminophen has been the best-understood example of direct hepatocyte toxicity. Liver injury occurs predictably after intentional suicidal overdose [54], and during the unintentional "therapeutic misadventure," in which acetaminophen used in therapeutic or excessive doses for pain relief leads to severe liver injury [44,66]. Enhanced toxicity occurs as a result of the enzyme induction and glutathione depletion by alcohol as well as of fasting as outlined previously [45]. Acetaminophen toxicity is the most common form of acute liver failure observed in the United States [2]. In a medical record review from an urban county hospital in the United States, 71 patients admitted in a 39-month period had actual or *potential* hepatotoxicity due to acetaminophen [53]. Those who ingested acetaminophen accidentally, without suicidal intent, fared worse because they presented for treatment late, and did not realize that they had done anything harmful. This group was more likely to develop severe liver injury and to die from the episode. By contrast, those with suicidal intent took larger doses, presented to a hospital earlier, and received *N*-acetylcysteine, an effective antidote. One-fifth of the suicidal cases had severe injury and the potential for a fatal outcome should not be underestimated. Key features in the accidental group were excessive chronic alcohol intake (usually more than six drinks daily), and the use of acetaminophen for a specific pain problem, but generally exceeding the package recommendations. The extremely elevated aminotransferase values (often greater than 6000 IU/L and sometimes as high as 30 000 IU/L) observed in suicidal and accidental acetaminophen ingestion help to distinguish these cases from viral hepatitis or other drug injury [53]. *N*-Acetylcysteine should be given by nasogastric tube on admission, and for the ensuing 72 h, to provide glutathione substrate. In Europe, intravenous *N*-acetylcysteine is the standard treatment [39]. Expected survival is greater than 80%, although transplantation is occasionally indicated. The incidence of acetaminophen poisoning varies widely throughout the world, but it is becoming more frequent and widespread as indicated by a report from Taiwan [67]. Acetaminophen poisoning is an increasing cause of ALF in children [68–70]. As in adults, two forms are seen: teenagers may overdose with suicidal intent but more ominous are instances of inappropriate overdosing of acetaminophen in small infants because of the parents' ignorance of the high risks involved.

The US Acute Liver Failure Study Group, a national registry established in 1998, reviewed the experience with 275 patients who developed ALF defined as altered mentation and coagulopathy, as a result of acetaminophen toxicity [54,71]. The majority (79%) were women, although the gender breakdown for other etiological categories of ALF is similar, and accidental overdose comprised 48% of patients, compared to 44% who were considered suicides; for 8% the type of overdose could not be determined. More than one-third of patients ingested more than one acetaminophen-containing compound simultaneously. Two-thirds of the unintentional overdose patients were taking a narcotic-containing acetaminophen compound. Both these observations suggest that patients are frequently unaware of the potential for toxicity or even of what is contained in the preparations they are using.

One advance in our understanding that may be useful clinically is the recognition that the reactive metabolite of acetaminophen bound to cell proteins can be measured reliably in plasma from patients with acetaminophen toxicity. This can be of value in cases where no history of overdose is given [72]. Acetaminophen adducts bound to the sulfhydryl group of cell proteins are found in serum in a pattern similar to aminotransferase levels, long after the parent acetaminophen compound has been metabolized. The presence of adducts is a sensitive and specific biomarker test for liver injury due to acetaminophen and it can identify adult as well as pediatric patients who were not previously suspected of being the result of acetaminophen overdose.

Cholestatic reactions

Cholestatic reactions have been described for a number of drugs, some of which are listed in Table 83.6. Cholestasis is best defined as a failure of bile to reach the duodenum [73], and common symptoms are jaundice and pruritus. *Pure cholestasis* with no signs of hepatocellular necrosis is almost exclusively seen in patients taking oral contraceptives, anabolic steroids, or sex hormone antagonists such as tamoxifen [74].

Table 83.6 Drugs involved in cholestatic drug reactions

Pure cholestasis	Anabolic steroids
	Tamoxifen
	Estrogens
Cholestatic hepatitis	Allopurinol
	Amoxicillin/clavulanate
	Azathioprine
	Barbiturates
	Captopril
	Carbamazepine
	Chaparral (herbal)
	Chlorpromazine
	Clindamycin
	Erythromycin
	Flucloxacillin
	Gold salts
	Phenytoin
	Ticlopidine
	Trimethoprim-sulfamethoxazole

Acute *cholestatic hepatitis* is histologically characterized by cholestasis (dilated canaliculi, brown granules in the cytoplasm of hepatocytes) and some degree of liver cell necrosis as well as bile duct injury and inflammatory infiltration with polymorphonuclear leukocytes. Drugs related to this type of reaction include carbamazepine [75], trimethoprim-sulfamethoxazole [76], captopril [77], and ticlopidine [78].

Generally, drug-induced cholestasis needs a longer time to resolve than the hepatocellular type of drug reactions [3]. In some instances progressive destruction of segments of the intrahepatic biliary tree may occur, the so-called *vanishing bile duct syndrome* [79] that occurs after a protracted course (more than 6 months) of drug-induced cholestasis. The result is a state of chronic cholestasis, resembling primary biliary cirrhosis [80]. Approximately 30 drugs have so far been implicated in the vanishing bile duct syndrome, including chlorpromazine [81] and ajmaline [82].

A sclerosing cholangitis-like syndrome with jaundice caused by intrahepatic and extrahepatic strictures of the bile ducts is sometimes observed in patients receiving intra-arterial floxuridine chemotherapy for hepatic metastases of colorectal cancer [83].

Immunoallergic reactions

Drugs may be associated with reactions that are definitely allergic in nature. Hypersensitivity reactions such as fever, eosinophilia, or rash are common. Halothane induces fever, eosinophilia, and antimitochondrial antibodies [13]. Halothane was formerly widely used as an inhalation anesthetic. However, it has been implicated in a high number of very severe cases of liver disease [84]. Halothane causes a hepatocellular injury as evidenced by findings of necrosis – ranging from spotty necrosis to bridging hepatic necrosis and multilobular necrosis – in liver biopsies [85].

Phenytoin (Dilantin) induces the simultaneous onset of fever, rash, lymphadenopathy, or eosinophilia [86]. The mechanisms responsible for the combined allergic and hepatotoxic reaction are unknown, but the slow resolution of the illness suggests that the allergen remains on the hepatocyte surface for weeks or months. With phenytoin, a mononucleosis-like picture may also be seen and is frequently confused with a viral illness or streptococcal pharyngitis [87]. When the offending agent is not discontinued promptly, despite signs of developing hepatitis, a severe Stevens–Johnson drug eruption and prolonged fever may result [88]. As with any therapeutic agent, rapid recognition of the presence of a toxic drug reaction and immediate discontinuation of the compound are the keys to limiting hepatic damage. It is important to remember that features of an allergic reaction may not be obvious. Even in the absence of systemic signs of allergy, eosinophilia or granulomas may be present on liver biopsy.

Sulindac is an NSAID that causes immunoallergic liver disease with a cholestatic pattern of injury in two-thirds of liver injury cases [89]. Sulindac may also cause hepatocellular liver injury and is more frequently implicated in drug-induced liver disease than other NSAIDs, such as ibuprofen.

Steatohepatitis

Steatosis in the liver can be present either in a microvesicular or in a macrovesicular pattern. Macrovesicular steatosis is the most common form and is histologically characterized by hepatocytes containing a single vacuole of fat filling up the hepatocyte and displacing the nucleus to the cell's periphery [28]. Macrovesicular steatosis is typically caused by alcohol, diabetes, or obesity. Sometimes drugs such as corticosteroids or methotrexate may cause these hepatic changes [90].

In microvesicular steatosis, hepatocytes contain numerous small fat vesicles, not displacing the nucleus [28]. These lesions are associated with disruption of mitochondrial DNA with resulting anaerobic metabolism leading to lactic acidosis in the most severe cases. Not uncommonly, macrovesicular and microvesicular lesions are observed concomitantly and in these cases the microvesicular lesions should be given more attention because they are associated with a more severe prognosis [29]. Hepatocellular necrosis may also be present. Acute fatty liver of pregnancy [91] and Reye syndrome [92] are two examples of severe liver diseases caused by microvesicular steatosis.

Drugs involved in microvesicular steatosis include valproate [93], tetracycline [94], fialuridine [95] (Table 83.7). Use of aspirin in children has been associated with Reye syndrome [96] and the incidence of Reye syndrome has decreased since warnings concerning aspirin use in children were issued [97]. The exact causal relationship between aspirin and microvesicular steatosis remains to be studied [92]. A relatively new situation is that of considering the impact of drugs such as the cholesterol-lowering "statins" in the setting of known fatty liver disease caused by the metabolic syndrome. Although these agents may be associated with elevations in aminotransferase levels, they appear to be remarkably safe and can be used without monitoring liver enzyme levels in patients with or without fatty liver disease [98].

Other drug reactions

There are several other types of drug reactions involving the liver that are of lesser importance in terms of number and severity. These include granulomatous reactions, fibrosis,

Table 83.7 Agents that may cause microvesicular steatosis of the liver

Aspirin
Ethionine
Fialuridine
Nucleoside analogues
Valproic acid
Tetracycline (intravenous administration)
Troglitazone

ischemic injury, and chronic autoimmune liver injury (see Table 83.4). The type of reaction observed can be helpful in determining the probable agent because most drugs have a specific injury profile.

A pattern of venoocclusive disease with obliteration of small intrahepatic veins, sinusoidal congestion, and necrosis is frequently observed in patients receiving chemotherapy (e.g., busulfan) following bone marrow transplant [99–101]. Symptoms include rapidly accumulating ascites, painful hepatomegaly, and jaundice occurring shortly after the conditioning regimen has been instituted. Rarely, herbal medicines may cause venoocclusive disease [102].

Treatment

Prompt discontinuation of a suspected drug is mandatory. General supportive therapy may be necessary according to the state of the patient, ranging from none through intravenous fluid replacement to the intensive monitoring and treatment of patients with acute liver failure with hepatic encephalopathy [63,103,104]. Liver transplantation is performed in more than 50% of patients with idiosyncratic drug-induced acute liver failure because spontaneous survival in this setting is less than 20% [1].

Criteria for causal assessment of drug-induced liver injury

How do we decide whether a certain drug is responsible for the liver disease encountered in a certain patient? It is important to understand that we are always trying to assess a possible association from the *past*, unlike most other scoring models that try to predict the *future* [105]. A standardized reporting form developed by an international panel working in France provides a worthwhile causality assessment scoring system [61], called the RUCAM (*R*oussel *U*claf *c*ausality *a*ssessment *m*ethod) (Table 83.8). These guidelines outline the steps that an experienced clinician uses to assess the likelihood of drug reactions [106]. Causality assessment methods typically include temporal relationship, course after cessation of drug, risk factors, concomitant drugs, a search for nondrug causes (viral hepatitis), previous information concerning the drug, and response to rechallenge, which is usually not available. Validation of the RUCAM scoring system suggests that a RUCAM score could be classified as *highly probable* (RUCAM score > 8), *probable* (score 6–8), *possible* (score 3–5), *unlikely* (score 1–2), or *excluded* (score ≤ 0) [107].

A newer model called the clinical diagnostic scale (CDS) was developed in Portugal [108] and tested in the United Kingdom [109]. Features from the CDS include most of the features from the RUCAM plus extrahepatic manifestations such as rash, fever, arthralgia, cytopenia, and eosinophilia [108]. The British study suggests using a CDS score of 9 or more as a strong indicator of drug-induced liver disease [109]. However, a recent Spanish comparison study in 215 patients suggested that the RUCAM system is more accurate than the CDS model [110].

Lessons from drugs withdrawn from the market

Two drugs withdrawn because of severe and fatal liver injury were bromfenac and troglitazone. There are important lessons to be learned from each one, illustrating the difficult issues surrounding drug-induced liver injury. NSAIDs as a class have been associated with considerable hepatotoxicity [111,112]. Bromfenac was an NSAID marketed as Duract, and introduced in 1997 as a short-term analgesic for orthopedic pain. Aminotransferase elevations had been noted during clinical testing of bromfenac, but no instances of severe toxicity had been reported. However, increased aminotransferases were associated with longer use, although the total number of patients undergoing long-term treatment was small. No instances of ALF were observed, but fewer than 1000 patients were studied. There were concerns that long-term use might be hazardous so approval was given with the limitation that the drug should only be used for intervals of 10 days or less. Once released, bromfenac was associated with more than 50 cases of severe liver injury and the drug was withdrawn in June 1998. All patients in whom toxicity was observed had been taking the drug for more than 8 weeks [113,114]. With hindsight, a drug that effectively relieved pain would not be prescribed or used reliably only for a 10-day period. The risk to benefit ratio must be an important consideration in the approval process for any new drug – bromfenac offered little that was new and on that basis should have met an even stricter test for safety.

Troglitazone (Rezulin) was approved by the US Food and Drug Administration (FDA) in January 1997, the first of a new class of compounds, the thiazolidinediones. As an agonist of the nuclear regulatory factor peroxisomal proliferator activator receptor-γ (PPAR-γ), troglitazone reduces insulin resistance and increases insulin-stimulated glucose disposal, resulting in improved glycemic control for patients with type II diabetes. In clinical trials, reversible elevations of aminotransferase levels were observed, occasionally reaching more than eight times the ULN but, again, no examples of ALF. However, once the drug was approved, several reports of severe and fatal liver injury appeared [115–118]. Troglitazone has a variable time frame for demonstrating toxicity, from a few days to several months [119]. The pathogenesis is not understood. Unlike bromfenac, troglitazone was not immediately removed. As a new agent, its benefits were initially thought to outweigh the risks. The drug was recalled in the United Kingdom in 1999. The renewed warning in the package

Table 83.8 Scoring system for assessing causality for hepatocellular types of reactions: a positive score of 6 or more represents a strong causal relationship

Temporal relationship of start of drug to start of illness	
Initial treatment 5–90 days; subsequent treatment course: 1–15 days	+2
Initial treatment < 5 or > 90 days; subsequent treatment course: > 15 days	+1
From cessation of drug: ≤ 15 days[a]	+1
Course	
ALT decreases ≥ 50% from peak within 8 days	+3
ALT decreases ≥ 50% from peak within 30 days	+2
If the drug is continued, inconclusive	0
Risk factors	
Alcohol[b]	+1
No alcohol[b]	0
Age ≥ 55 years	+1
Age < 55 years	0
Concomitant drug	
Concomitant drug with suggestive time of onset	–1
Concomitant drug known hepatotoxin with suggestive time of onset	–2
Concomitant drug with further evidence of involvement (e.g., rechallenge)	–3
Nondrug causes: six are primary: hepatitis A, B, or C, biliary obstruction, alcoholism (AST ≥ 2 × ALT), recent hypotension (heart disease).	
Secondary group: underlying other disease; possible CMV, EBV or HSV infection	
In this category, all causes ruled out	+2
Four or five causes ruled out	+1
Fewer than four causes ruled out	–2
Nondrug cause highly probable	–3
Previous information on hepatotoxicity of the drug in question	
Package insert mention	+2
Published case reports but not in package label	+1
Reaction unknown	0
Rechallenge	
Positive (ALT doubles with drug alone)[c]	+2
Compatible (ALT doubles, compounding features)[c]	+1
Negative (increase in ALT but ≤ 2 × ULN)[c]	–2
Not done	0

For cholestatic reactions:
a ≤ 30 days
b alcohol or pregnancy
c read alkaline phosphatase (or total bilirubin) instead of ALT.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HSV, herpes simplex virus; ULN, upper limit of normal.
Adapted from Danan and Benichou [106] and Benichou et al. [107].

insert (in a black box) mandated monthly monitoring during the first 6 months of use. However, the number (> 90) and severity (68 fatal, 10 transplanted) of these cases, as well as doubts over the efficacy of monitoring, led to drug withdrawal in 2000, 3 years after its approval. A factor in the FDA decision was the arrival of two new PPAR-γ agonists, rosiglitazone (Avandia) and pioglitazone (Actos), approved, respectively, in May and July 1999. Although of the same class, these agents do not seem to have the same degree of toxicity. So far, neither drug has shown the 1 : 50 000 incidence of severe hepatotoxicity seen with troglitazone. Although there may be intrinsic differences in the drugs, more careful screening of patients by physicians, monitoring of ALT levels, and early discontinuation of the drug in moderately severe cases may play a part in limiting the severe toxicity associated with this class of drugs [119]. Two drugs found to have significant toxicity were ximelagatran (Exanta) and telithromycin (Ketek). The first was licensed in Europe as a thrombin inhibitor that would work in a similar way to warfarin. Unfortunately, aminotransferase elevations were observed in 7.9% of patients in preapproval trials and examples of toxicity were observed as long as several weeks after

the drug had been discontinued – approval was denied in the United States and the sponsor withdrew the drug from the European market [120]. Ketek was approved in the United States and Europe in 2004 as the first in a new class of antibiotics, the ketolides; they have activity against penicillin-resistant and erythromycin-resistant pneumococci. After more than 50 reports of serious liver injury, the drug has been given more severe restrictions on its use and is to be used only as a "second-tier" drug [121,122].

The drug approval process

How can new drugs get through the drug approval process and still lead to so many deaths that they are ultimately withdrawn? In brief, drug development is divided into three stages: initial research and development, clinical testing for new drug application (NDA), and the postmarketing experience.

Research and development

The initial stage of drug development includes drug discovery and initial testing for efficacy or toxicity in animals or in vitro model systems. Most new compounds fail to make it through this stage, either because of toxicity or lack of efficacy. Compounds may be "discovered" in several ways: synthesized to resemble previous compounds, discovered in the field by purification of naturally occurring peptides (e.g., cyclosporin A), or generated by computer modeling. A compound shown to have a desirable effect in vitro or in vivo then undergoes extensive preclinical testing in a variety of animals using doses up to 50 times that predicted to be useful in humans to ascertain the types of toxicity that might be expected. While metabolic pathways differ in some specific aspects, the similarities between lower mammals and humans are notable. Animals are euthanized after short-term experiments and all their organs are examined; those dying during experiments undergo necropsy to determine the cause of death. Long-term exposure studies are performed looking for carcinogenicity or other delayed effects. Preclinical testing, which may take 5–6 years to complete, is still a crude technique and no substitute for clinical trials in humans. The use of massive dosing in animals may in part compensate for metabolic differences between species, but human trials are ultimately needed. Toxicogenomics offers some promise of early identification of "toxicity" gene expression profiles (i.e., signatures).

Clinical trials

In phase I testing, progressively larger doses of the test medication are given to well-paid healthy volunteers. Routine monitoring includes vital signs, electrocardiogram, assessment of reported side effects, and blood measurements including serum aspartate aminotransferase/ALT, amylase, and creatine phosphokinase. In phase II testing, patients are exposed for the first time and the emphasis shifts from safety alone to safety *and* efficacy. Depending on the intended use of the medication and the prevalence of the disease to be treated, from 500 to 5000 study patients may test the medication for periods of up to a year. In early phase II trials, a progressive dosing scheme identifies the maximal dose that is effective and still safe. If a given dose is effective, it is then determined if there are any short- or long-term side effects. Several NSAIDs have made it to phase II trials only to be withdrawn because of frequent aminotransferase level increases. However, this is where safety concerns regarding idiosyncratic reactions founder. It is easy to pick up common reactions but hard to pick up the truly rare drug injury during the preapproval process. A "signal" of aminotransferase elevations is often associated with more severe toxicity as can be observed with isoniazid and possibly ximelagatran, but most aminotransferase elevations do not lead to severe injury.

Dr Hyman Zimmerman, long recognized as the founder of the study of drug-induced liver injury, stated that if a drug causes enough liver injury to lead to jaundice, even rarely, then 10% of affected patients will develop acute liver failure ("Hy's rule") [90]. Put another way, any drug that in phase II or III testing demonstrates not only aminotransferase elevations, but increases in bilirubin or jaundice, will likely lead to ALF when larger numbers of patients are exposed. This sounds like an imprecise "rule" but it has served well over the years, and there does not seem to be anything better.

How certain can we be that clinical studies identify instances of liver injury? First, all studies are conducted according to previously established guidelines of good clinical practice. In each clinical study, a detailed assessment of liver biochemical parameters is part of every company's NDA filing. Data supporting the safety of the drug include placebo-controlled trials where the incidence of abnormalities must be shown to be similar to that observed in the placebo group. Aminotransferases exceeding three times the ULN generally require discontinuation of the drug. Increased aminotransferase levels *without* bilirubin elevations may not lead to discontinuation during a phase III trial, but frequent or more severe aminotransferase increases (more than 8 times the ULN) or accompanying increases in bilirubin will likely bring a new drug trial to a halt. If any case of ALF occurs, the trial is discontinued, as was the case with fialuridine.

The FDA approves approximately 50 new drugs each year. The approval process takes between 6 months and a year, once the NDA is filed. Approval brings with it instant widespread, intense marketing efforts and the necessity for all US pharmacies to stock the drug. As noted previously, the number of prescriptions frequently rises rapidly into the millions, within a year or less. This fact explains why some drugs only demonstrate problems once they receive FDA approval. Idiosyncratic events occurring in only 1 in 50 000 patients are not going to be recognized in a study of 4500 patients. The "rule of threes" applies: to reliably identify a single case of liver injury due to a drug with 95% confidence, there must

be three times the number of patients studied as the incidence of the drug reaction. In other words, a 1 in 1500 reaction requires 4500 patients to reliably detect a single case; a 1 in 50 000 reaction would require 150 000 patients. No clinical trial will reliably pick up rare drug reactions. Approval by the FDA provides a wider experience than the limited exposure of the carefully controlled clinical trial. It should not be surprising, therefore, that drug reactions are observed in the postmarketing period and not before. However, postapproval drug recall still takes time to evolve while the drug continues to be prescribed despite the recognition of adverse events.

Postmarketing surveillance

The greatly increased number of patients receiving a new drug ensures that untoward or unusual drug effects will be observed. In addition to increased numbers, a wider range of patients than the defined clinical trial population is exposed. For example, most studies do not include patients with renal failure, heart failure, HIV/AIDS, pregnant women, the elderly, or children. Any of these groups may show enhanced toxicity. Even the best-randomized controlled clinical trial is not a "real-life" experience. The difficulty is in identifying these drug reactions quickly and accurately, once the product is released. During clinical trials and the after market period, pharmaceutical companies must report serious adverse events to the FDA within 24 h. A serious adverse event is any unexpected medical occurrence that at any dose results in death, is life threatening, requires hospitalization, or results in permanent disability or a birth defect. Pharmaceutical manufacturers maintain a safety monitoring force that gathers reports, assesses likelihood of the reaction being attributed to their product, and issues a report to the FDA and to clinical investigators if there is still an ongoing trial. However, there is bound to be a bias toward any new product, just as there is bias built in to the design of clinical trials [123].

There are several additional shortcomings to the effectiveness of postmarketing surveillance. First, the reporting system is passive. Physicians and pharmacists are under no obligation to report adverse events. The FDA introduced the Medwatch program to improve surveillance, asking physicians and pharmacists to report on a standardized form all drug reactions they observe. However, it is estimated that less than 10% of severe adverse drug reactions are reported to the company or the FDA. Reasons for underreporting include: failure to recognize "hepatitis" as being due to a drug, concern about malpractice implications, reluctance to get involved, complacency ("too busy"), and so forth. Reports received from pharmacists or drug representatives (or physicians for that matter) seldom contain full clinical information. Privacy issues may preclude further inquiries and raise concern regarding possible legal implications. For all these reasons, a passive reporting system is inadequate. Nevertheless, the main source of information is the Medwatch system, plus case reports [124].

Hepatotoxicity in the patient with chronic liver disease

Hepatologists are frequently asked "Is the patient with liver disease more susceptible to liver injury?" Intuitively, this makes sense, until we realize that hepatotoxic reactions represent the culmination of hepatic enzyme activity. If liver function is impaired, one might predict diminished activity of certain enzyme systems. Patients with liver disease do not appear to be at increased risk for hepatic injury compared with their counterparts without underlying liver problems. Dr Zimmerman put it best [90]: "A stubborn [misconception] has been the view that patients with preexisting hepatic disease are more likely than others to suffer hepatic injury on exposure to drugs that cause liver damage. There is virtually no evidence for this view." What do we know of the liver function of patients with cirrhosis? Many enzyme systems are well preserved even in advanced disease. For example, patients with terminal alcoholic hepatitis still are able to conjugate most of their bilirubin. Therefore, enzyme activity in many instances exceeds the daily requirement, so that even severe liver injury would not be expected to lead to an adverse drug reaction. In general, phase I reactions may be diminished but this is not uniformly so. In severe liver disease the activity of CYP2C19 is greatly decreased whereas that of CYP2D6 is intact [125]. In nonalcoholic steatohepatitis, the enzyme CYP2E1 is increased, particularly in the centrilobular region, so that acetaminophen toxicity should be enhanced in patients with nonalcoholic steatohepatitis [126]. So far, this has not been appreciated clinically; further support in an animal model of nonalcoholic steatohepatitis has been presented [127].

Drug metabolism in patients with cirrhosis can be reduced by as much as 50%. Whether the cells in a patient with cirrhosis are sick or simply reduced in number but functioning normally is not clear. Neither answer is exactly correct. It appears that the physiological changes seen with fibrosis along the hepatic sinusoids results in a widening of the barrier between the bloodstream and the hepatocyte. In support of this, patients with cirrhosis with comparably diminished metabolism of acetaminophen and theophylline normalize theophylline disposal, but not acetaminophen, with oxygen supplementation [128]. The metabolism of theophylline uses CYP1A1 and CYP1A2, which requires oxygen as substrate, unlike acetaminophen conjugation (phase II). The limitation to metabolism is the barrier to oxygen absorption. These studies support the "intact hepatocyte/sick membrane" hypothesis. In summary, dosage adjustments may need to be made in patients with cirrhosis, but these individuals do not appear to have an abnormally sensitive hepatic metabolic system, just less reserve if a hepatotoxic insult were to occur [128,129].

In general, patients with liver disease suffer more renal than hepatic insults. They are particularly prone to nephrotoxicity

as the result of the altered renal circulation of the cirrhotic patient. Nephrotoxicity of aminoglycosides, radiocontrast, and prostaglandin inhibitors such as indomethacin are a frequent problem for patients with cirrhosis, but doses of antibiotics, antipsychotics, and so forth, are seldom adjusted, although any medication with sedating effects may be a problem if metabolism is slowed.

Avoiding further liver injury in the patient with preexisting liver disease is a difficult task. Antituberculous therapy cannot be withheld from patients just because they have alcoholic cirrhosis. In these instances, frequent monitoring appears to be helpful, but the value of this monitoring has not been proven in controlled trials, is seldom adhered to, and can prove expensive. Despite surveillance using liver enzyme levels, acute liver failure has developed in patients treated with isoniazid. In many instances, the presence of preexisting liver disease is subclinical, (e.g., in patients with nonalcoholic steatohepatitis). Whether the diabetic population is more at risk for troglitazone hepatotoxicity because of their diabetic fatty liver is still debated. Nevertheless, a healthy regard for the possibility of increased hepatotoxic reactions in patients with preexisting liver disease, and the use of periodic surveillance during treatment, should allow the maximum chance to avoid prescribing drugs that are harmful.

Clinician's guide to handling new drugs

The best advice in prescribing new pharmaceutical agents is not to prescribe them. It is wise to defer embracing new drugs during their first year of introduction, particularly if they demonstrate no unique advantages over accepted formulations. Marketing hype exceeds real-life experience with any new agent. Physicians must strive to instill in their patients a healthy level of alertness with regard to drug-induced liver injury, particularly for agents with known hepatotoxicity. Physicians and pharmaceutical companies should strike a careful balance between alerting patients to the potential for severe reactions without frightening them so that they avoid needed medications. Monitoring aminotransferase levels is suggested for known hepatotoxins such as isoniazid or diclofenac on a monthly basis but is unlikely to be cost-effective when an adverse reaction occurs in only one in 50 000 patients. Since many drug reactions develop within days, monitoring provides no guarantee [130]. Most fatal drug reactions could be prevented if the offending agent were withdrawn immediately, at the first sign of illness. The patient most likely to be harmed is the one who believes in the complete safety of drugs, does not realize that drug-induced injury is possible, or is encouraged to be compliant when signs of toxicity are beginning.

New drugs should be prescribed with caution, keeping an eye out for case reports. Some of the newer agents implicated in acute liver necrosis are listed in Table 83.9. The diagnosis of drug-induced liver injury necessitates determining the precise timing of the drug ingestion, making a careful record of all drugs ingested, and being particularly suspicious of known hepatotoxic agents begun within 3 months of the onset of illness. After withdrawal of the offending agent, improvement should be rapid, within days. Cautious re-challenge may be made *only* if the toxicity observed was highly questionable and if no other drug is available for a serious problem. If jaundice, coagulopathy, or any degree of encephalopathy is present initially, then hospitalization is required because drug reactions worsen quickly, and fatal outcomes are common.

Table 83.9 Current list of newer drugs reported to cause severe toxicity

Atorvastatin [136]
Agent orange [137]
Carbamazepine [74]
Celecoxib [59]
Clarithromycin [138]
Coumarin compounds [139]
Cyproterone acetate [140]
Fosinopril [141]
Fluconazole [142]
Fluoxetine [143]
Indinavir [144]
Levofloxacin [145]
Losartan [146]
Mesalazine [147]
Metformin [148]
Nefazodone [149]
Norfloxacin [150,151]
Paroxetine [152]
Pemoline [153]
Pravastatin [154]
Ranitidine [155]
Risperidone [156,157]
Telithromycin [121]
Terbinafine [158]
Ticlopidine [77]
Tolcapone [159,160]
Trazodone [161]
Trovafloxacin [162,163]
Valproate [164,165]
Venlafaxine [166]
And don't forget herbs! [102]

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G LIVER

84 Autoimmune hepatitis

E. Jenny Heathcote

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When first described [1,2], autoimmune hepatitis (AIH) was thought to be a potentially fatal form of chronic hepatitis that predominantly affected young women. It is now recognized that this disease may affect all age groups [3,4] and women are affected four times as often as men. All racial groups are at risk of AIH. Its prevalence in Europe is about 1.9 per 100 000 [5]. Spontaneous remission may occur but subsequent relapse is usual; only rarely is the disease curable. Although this disease can be fatal if severe and left untreated [6–8], earlier recognition of the manifestations of AIH has shown that in some instances the disease may be asymptomatic and its course perhaps more benign than was originally thought.

Pathogenesis

There are many features of AIH which suggest the disease is immune-mediated. Certain human leukocyte antigen (HLA) class II alleles are associated with both disease susceptibility and treatment responsiveness [9]. In individuals from North America and Europe the class II HLA-DRB*0301 and HLA-DRB*0401 alleles are more common in individuals with type I AIH. In South America and Japan the HLA pattern associated with AIH is different. A common antigenic peptide which recognizes a positively charged amino acid at positions 67–72 on the DRB allele may be the link [10]. The immune response is complex, involving several promoters such as costimulatory signals for T-cell activation, adhesion between immune synapses, cytokine production, and T-cell receptor configuration. Genetic polymorphisms of one or more of these promoters may be relevant to the pathogenesis of AIH [11].

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AIH may be associated with low serum complement levels and a null allotype at the C4A or C4B gene location (a silent *C4AQ + 0* gene is identified in such patients) [12]. This C4A gene deletion is associated with both relapse on therapy and increased mortality [13].

Immunoglobulin levels are elevated twofold to threefold in active AIH, indicating marked B-cell activation, the cause being multifactorial [14,15]. Several reports imply that the onset of AIH may be precipitated by an acute viral infection in genetically susceptible individuals [16–19]. The induction of AIH in a few patients being treated with interferon for chronic hepatitis C has also been described [20]. Certain drugs (e.g., minocycline) may also induce an AIH-like disease [21–23].

The nonorgan-specific, nonspecies-specific antibodies typically associated with AIH are antinuclear antibodies (ANAs) and smooth muscle (specifically anti-actin) antibodies (SMAs). The detection of these antibodies in up to one-third of individuals with other liver diseases not thought to have an autoimmune basis [24] makes it unlikely that they have any pathogenic role on their own. Another autoantibody, anti-soluble liver antigen (anti-SLA), is described in 11% of patients with type 1 AIH. This antibody is identical to liver/pancreas antibody [25,26]. Anti-SLA may also be detected in 14%–30% of patients given a diagnosis of cryptogenic cirrhosis. This latter observation supports the concept that some patients with a diagnosis of cryptogenic cirrhosis may have an autoimmune basis for their liver disease [27].

Another distinct form of AIH (type 2) has been most often described in Europeans but does occur in North America [28]. In these individuals an antibody against liver and kidney microsomes (anti-LKM1) (specifically cytochrome P450-2D6) can be detected; serum generally tests negative for ANA and SMA. It remains unknown as to whether this antibody has any pathogenic significance. If the immunofluorescence slides are not read carefully, this antibody could be misinterpreted as

Table 84.1 Comparison of the clinical and immunological features of types 1 and 2 autoimmune chronic active hepatitis

	Type 1 (anti-actin)	Type 2 (anti-LKM)	Anti-SLA
Age at presentation (years)	10–25 and 45–70	< 15	Mean 37
Associated disorders	10%	17%	58%
Immunoglobulins			
γ-Globulins (g/L)	37 ± 11	23 ± 8	mean 32.2; range 1.8–5.2
IgG (g/L)	37 ± 16	25 ± 10.4	
IgA (g/L)	3.7 ± 1.3	1.8 ± 0.9	
IgM (g/L)	1.7 ± 1.1	2.4 ± 1.5	
Autoantibodies			
Anti-SMA (%)	100	0	74
ANA (%)	33	2	29
AMA (%)	2	0	14
Progression to cirrhosis after 3 years (%)	43	82	75

AMA, antimitochondrial antibodies; ANA, antinuclear antibodies; LKM, liver and kidney microsomes; SLA, soluble liver antigen; SMA, smooth muscle antibody.

From Johnson PJ, McFarlane IG, Eddleston AL. The natural course and heterogeneity of autoimmune-type chronic active hepatitis. Semin Liver Dis 1991;11:189, with permission from Thieme.

being an anti-mitochondrial antibody (AMA) [29]. Sometimes anti-LKM1 can be detected in the serum of persons with chronic hepatitis C [30].

There are many other autoantibodies described in association with AIH. Asialoglycoprotein receptor antibodies can be detected in 90% of patients with all forms of AIH. This antibody is directed at the receptor for $CD4^+$ T cells [31]. Antineutrophil cytoplasmic antibodies were initially believed to be a hallmark for primary sclerosing cholangitis (PSC) but subsequent case series indicate that these antibodies are not specific and are also common in AIH [32].

A rare condition known as autoimmune polyglandular syndrome type 1 (APS-1) is associated with AIH in 10%–20% of patients. APS-1 is associated with a homozygous mutation in an autoimmune regulator (*AIRE*) gene, which encodes a nuclear transcription factor. A survey of individuals with a variety of autoimmune liver disease did not indicate that this same mutation was present in individuals with AIH [33].

Some patients thought to have AIH are found to test positive for only anti-SLA, and have been categorized as having type 3 AIH. However, more than likely as the symptoms are so similar, these rare patients can be categorized as having type 1 AIH. AIH type 1 is the form most frequently encountered in North America; type 2 appears to be more common in Europe. The latter more commonly affects young persons, generally presents acutely, and is more frequently associated with cirrhosis.

The HLA patterns in type 1 and type 2 AIH are also distinct but this may be partially explained by the very different geographic distribution of these two forms of AIH. The HLA pattern that appears to confer resistance to AIH type 1 confers susceptibility to AIH type 2 (i.e., DRB1*07 and DRB1*15 and DQB1*06) [34], suggesting that the genetic susceptibilities to these two forms of AIH are quite separate. Type 1 and type 2 AIH can often be distinguished clinically by the natural history of the disease, the laboratory parameters for each type, and the different responses to treatment [35] (Table 84.1).

Clinical manifestations

Presenting symptoms

At first presentation the diagnosis of AIH may be missed because the symptoms of a mild, self-limiting, acute hepatitis are very nonspecific [36]. Most often AIH is diagnosed in individuals found to have markedly abnormal liver biochemical tests when they present with jaundice. Occasionally, presentation may be as an acute [37] and sometimes fulminant hepatitis [38]. Careful history-taking may reveal past nonspecific but relevant symptoms such as episodes of malaise, nausea, and arthralgias. There may be a family history of other autoimmune diseases. Sometimes the disease may present de novo with hepatic decompensation (e.g., ascites, variceal hemorrhage). On occasion a complication of AIH (e.g., secondary amenorrhea or delayed menarche) may be the only complaint leading to a visit to the physician. AIH is sometimes entirely asymptomatic, diagnosed as the result of blood tests performed as part of a routine physical examination or as part of the workup for another autoimmune disease.

As with most chronic liver diseases, fatigue is the most common symptom; jaundice is a presenting symptom in more than half. Other gastrointestinal complaints include

anorexia, diarrhea, sometimes pruritus, and occasionally right upper quadrant discomfort. Associated autoimmune diseases include thyroiditis, inflammatory bowel disease, and rheumatoid arthritis. In children there is a very strong link between AIH and sclerosing cholangitis [39,40]; up to 50% are found to have both diseases simultaneously at presentation [40]. This dual presentation is much less frequently observed in adults.

Physical examination

Generally, hepatomegaly is present and jaundice is frequent but there may be no abnormal physical findings noted, particularly if cirrhosis is absent. The spleen may be palpable (50%) and spider nevi may be present but they do not necessarily indicate cirrhosis. Hepatic decompensation is unusual but occasionally patients with *burned-out* disease may present with ascites (20%), hepatic encephalopathy (14%), or bleeding varices (8%). The liver is generally small by this time [36].

Laboratory investigations

The alteration in liver enzymes in AIH is typically hepatocellular. Elevation in serum aminotransferase levels may be quite marked, greater than 1000 IU/L in some cases. A conjugated hyperbilirubinemia is common. Further evidence of liver dysfunction may be present, such as a coagulopathy and hypoalbuminemia. The blood film may reveal a pattern typical of hypersplenism (low total white cell count, platelet count, or hemoglobin), even at presentation in those with already established cirrhosis.

Infection with hepatitis A, hepatitis B, and hepatitis C viruses must be ruled out through serological testing. Other viruses may affect the liver as part of a systemic infection, for example Epstein–Barr virus, adenovirus, parvovirus, and cytomegalovirus (in the immunocompromised individual), and these should be sought if an infectious etiology is considered likely.

If viral hepatitis is excluded, testing for serum ANAs and SMAs should be requested: the immunofluorescence technique is sufficient. Anti-LKM1 and ANA/SMA are nearly always mutually exclusive, and thus anti-LKM1 should be requested once serum tests negative for ANA and SMA. If there is concern that anti-LKM1 may have been misread, then an enzyme-linked immunosorbent assay (ELISA) can be used to confirm the identity of AMA. It is probably not clinically helpful to screen for any other autoantibodies. Only when ANA or SMA are associated with at least a twofold increase in IgG should a diagnosis of type 1 AIH be seriously considered. These two antibodies, generally in low titer, are detected in up to one-third of individuals with other forms of chronic liver disease but the hypergammaglobulinemia is much greater in type 1 AIH. In type 2 AIH, the hypergammaglobulinemia may not be as profound, and even low IgA levels may be observed [34].

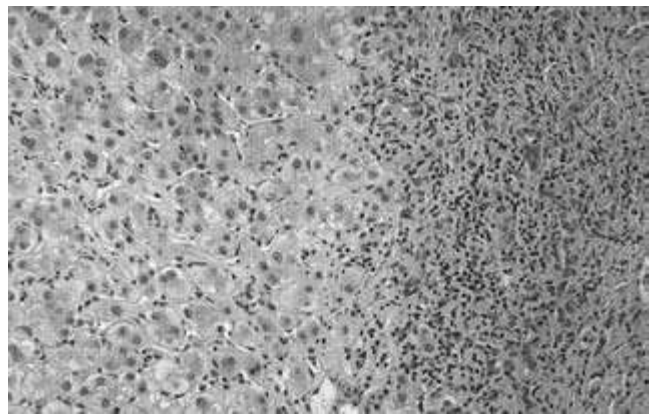

Figure 84.1 Typical histological picture of autoimmune hepatitis.

Liver histology

To confirm a diagnosis of AIH liver histology is essential even though the pattern of disease is not entirely specific. Both viral hepatitis and drug-induced hepatitis may be histologically indistinguishable from AIH. If there is no evidence of hepatic decompensation, liver biopsy can be performed via the percutaneous route, otherwise the transjugular route is necessary. The liver biopsy should be reviewed by an experienced hepatopathologist. The portal tracts and hepatic lobules need to be examined in minute detail looking for evidence of an interface hepatitis at the junction of the portal region and the liver lobule and a lobular hepatitis caused by infiltration with lymphoplasmacytic chronic inflammatory cells (Fig. 84.1). The intrahepatic bile ducts generally appear normal. If there are cholangitic features, then strong suspicion of an associated cholangitis or a misdiagnosis should be entertained. Copper retention within hepatocytes is one of the hallmarks of Wilson disease but may also be seen to a lesser degree in individuals with AIH with or without cholangitic features. Staining for α_1-antitrypsin globules with the periodic acid–Schiff stain (found then to be diastase-resistant) should be negative and there should be no excess iron noted. In those who present with severe disease, large areas of bridging necrosis may be noted. Even when the clinical presentation is acute, it is common for patients to have evidence of chronic liver disease, possibly even established cirrhosis. The composite histological picture is 81% specific and the positive predictive value is 68% [41] when tissue from individuals with AIH is compared with tisssue from those with viral hepatitis and cryptogenic cirrhosis.

Differential diagnosis

There are other liver diseases that may present in a similar fashion to AIH and therefore need to be routinely excluded before a confident diagnosis of AIH can be made. Measure-

ment of serum ceruloplasmin level is essential in those individuals who present when young (< 40 years). If the levels are reduced, measurement of 24-h urinary copper and slit-lamp examination of the eyes for Kayser–Fleischer rings are necessary so as to exclude Wilson disease. Falsely low values for ceruloplasmin may be seen in patients with severe hepatic decompensation from any cause. Falsely high urinary copper may result from severe cholestasis but rarely to the degree (10–20-fold elevated) associated with Wilson disease. There are so many genetic mutations associated with this disease that no one mutation can be used as a specific hallmark.

There are several drugs that may cause the clinical, biochemical, serological, and histological features typical of AIH. The drugs most often associated with typical features of type 1 AIH are minocycline [23] and nitrofurantoin [22]. The picture of type 2 AIH may be mimicked by tienilic acid. Withdrawal of the drug may not be instantly associated with biochemical improvement, although resolution should occur over a matter of months not years.

Fortunately, there are now sensitive and specific tests to identify viral hepatitis. Nevertheless, hepatitis C may mimic both type 1 and type 2 AIH [42]. In individuals from areas where the prevalence of viral hepatitis is high it is possible for chronic viral hepatitis and AIH to coexist.

Cryptogenic cirrhosis is the diagnosis given to patients in whom no obvious etiology can be identified. Czaja and colleagues [43] compared 12 such cases with 94 cases of clear-cut AIH and showed that there was no statistical difference between the two diseases with respect to gender, age, pattern of histological activity at presentation, or frequency of concurrent autoimmune disorders. However, total gammaglobulin and IgG concentrations were significantly lower in those with a diagnosis of cryptogenic cirrhosis. Patients with cryptogenic cirrhosis had no ANA, SMA, anti-LKM1, AMA, thyroglobulin, or microsomal thyroid antibodies in their serum. However, HLA class I and II patterns were similar between the groups, suggesting that the etiology in these individuals was likely autoimmune. All patients were treated with corticosteroid therapy and improved. Other authors have described a similar population [44]. However, so-called cryptogenic cirrhosis may be caused by other silent diseases (e.g., nonalcoholic steatohepatitis) [45] and it is probably unwise to treat "cryptogenic" cirrhosis with immunosuppressive therapy unless there are some autoimmune features present.

Autoimmune hepatitis/primary sclerosing cholangitis overlap syndromes

Particularly in children, overlapping features of AIH and PSC are common [39,40]. It is unclear whether the cholangitis visible on radiological delineation of the biliary tree in children with otherwise obvious AIH is the same cholangitis that may be diagnosed in adults. Both are frequently associated with chronic inflammatory bowel disease. There may be no distinguishing features between individuals with AIH and those with AIH plus PSC (i.e., serum alkaline phosphatase/γ-glutamyltransferase and immunoglobulin values may be elevated in both). The therapeutic response cannot distinguish between the two. Thus a high index of suspicion is needed. Data suggest that in children it is probably appropriate to examine the biliary tree via endoscopic retrograde cholangiopancreatography (ERCP) or magnetic resonance imaging (MRI), even if the primary diagnosis is AIH [40]. The cholangitis may affect only the intrahepatic bile ducts, although in most individuals progression to extrahepatic disease occurs later in life.

When Boberg and colleagues [46] applied the first AIH scoring system to 114 adults given a primary diagnosis of PSC, they found that two individuals scored *definite* and eight *probable* for AIH. Subsequently the application of the modified AIH scoring system [47] has reduced the number of patients with radiologically proven PSC who score *probable* for AIH. Two publications indicate that the rate of PSC/AIH overlap ranges from 4% [48] to 8% [49] of patients with PSC. In the latter study the patient population was younger, so it may be reporting the pediatric PSC/AIH overlap described by Gregorio and colleagues [40].

The AIH scoring system can be employed to determine the strength of evidence that an individual has AIH (Table 84.2). Factors considered important (by a panel of experts) when making the diagnosis have been weighted either positively or negatively. These include gender, alkaline phosphatase (ALP)/aspartate aminotransferase (AST) (or alanine aminotransferase [ALT]) ratio, elevation of IgG, titer of ANA, SMA or LKM1, AMA status, viral serology, drug history, average daily alcohol intake, liver histology (interface hepatitis, predominant lymphoplasmacytic infiltrate, rosetting of liver cells, biliary changes), and other autoimmune diseases. Additional measures can include seropositivity of other autoantibodies, HLA-DR3 or HLA-DR4, and response to immunosuppressive therapy [47].

Natural history of autoimmune hepatitis

Now that immunosuppressive treatment is the standard of care for symptomatic AIH, the natural history of this disease is rarely observed. Examination of the data from subjects who received placebo in the randomized controlled trials of corticosteroids with or without azathioprine reported in the early 1970s indicate that AIH is a rapidly progressive disease, with a 5- and 10-year survival rate of only 50% and 10%, respectively [4–6] (Fig. 84.3). Death is generally due to liver failure often complicated by sepsis. Hepatocellular carcinoma is rare [50]. However, it is important to note that 30 years ago, when these trials were conducted, most patients presented to their physician with hepatic decompensation and thus the

Table 84.2 Summary of revised score sheet for autoimmune hepatitis

	Score
Gender: female	+ 2
Biochemistry (ALP/AST ratio)[a]	
< 1.5	+ 2
1.5–3	0
> 3	– 2
Total globulin or IgG (fold elevation)	
> 2	+ 3
1.5–2	+ 2
1–1.5	+ 1
< 1	0
Autoantibody (ANA, SMA, LKM1) titer (IF)	
> 1 : 80	+ 3
1 : 80	+ 2
1 : 40	+ 1
< 1 : 40	0
Antimitochondrial antibody (AMA)	
AMA positive	– 4
Viral hepatitis markers	
IgM HAVAb, HBsAg, IgM HBcAb	Positive – 3
Anti-HCV and HCV RNA, ?CMV/EBV	Negative + 3
Hepatotoxic drug history (current)	Positive – 4 Negative + 1
Average alcohol intake (current)	
< 25 g/day	+ 2
> 60 g/day	– 2
Liver histology	
Interface hepatitis	+ 3
Mostly lymphoplasmacytic infiltrate	+ 1
Rosetting of liver cells	+ 1
None of the above	– 5
Biliary changes	– 3
Other changes	– 3
Other autoimmune diseases in patients or first-degree relatives	+ 2
Optional additional parameters	
Seropositivity of other defined autoantibodies (pANCA, anti-LC1, anti-SLA, anti-ASGPR, anti-LP, anti-sulfatide)	+ 2
HLA-DR3 or -DR4	+ 1
Response to therapy	
Complete	+ 2
Relapse	+ 3
Nonresponse (cholangiography required)	

a ALP (IU, upper limit of normal)/AST (IU, upper limit of normal).
Interpretation of aggregate scores: (1) pretreatment – > 15, definite AIH, 10–15, probable AIH; (2) post treatment – > 17, definite AIH; 12–17, probable AIH.
Ab, antibody; ALP, alkaline phosphatase; ANA, antinuclear antibodies; ASGPR, asialoglycoprotein receptor; AST, aspartate aminotransferase; CMV, cytomegalovirus; EBV, Epstein–Barr virus; HAV, hepatitis A virus; HBc, hepatitis B core (antigen); HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HLA, human leukocyte antigen; IF, immunofluorescence; LKM, liver and kidney microsomes; pANCA, perinuclear antineutrophil cytoplasmic antibody; SLA, soluble liver antigen; SMA, smooth muscle antibody.
From Alvarez et al. [47], with permission from Elsevier.

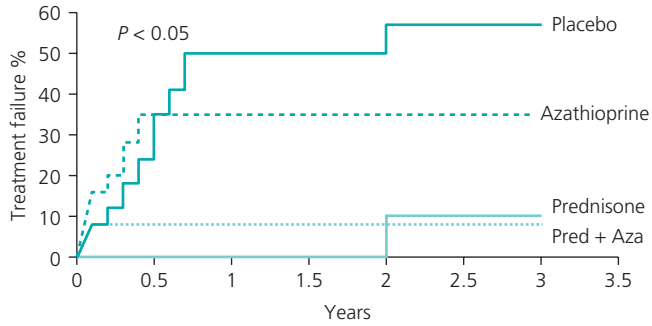

Figure 84.2 Response to immunosuppressive therapy. From Soloway et al. [8], with permission from Elsevier.

majority had well-established cirrhosis at baseline. With the introduction of routine laboratory checks of liver biochemistry and a heightened awareness of liver disease in general, it is likely that the proportion of individuals diagnosed with less advanced disease is increasing. Sometimes AIH may be diagnosed in the absence of any symptoms. There are no published data on the natural history of asymptomatic AIH.

Treatment, which is successful in more than 80% of affected individuals, has allowed most patients with AIH to lead a normal life, with a life expectancy similar to age- and gender-matched control populations (i.e., 98% at 10 years) [51]. This study also indicated that the 10-year survival in AIH is not dependent on baseline liver histology (Fig. 84.3). For the most part, young women with this disease, if treated appropriately, may have successful pregnancies (corticosteroids or azathioprine should not be stopped during pregnancy). If flareups do occur, they are nearly always in the postpartum period [52].

Unfortunately, most of the complications of AIH are due to side effects from long-term immunosuppressive therapy needed to control liver disease. It is essential to monitor systemic blood pressure, bone mineral density, and blood or urine glucose. Regular eye examinations looking for cataracts should also be performed.

Patients with AIH may develop other autoimmune diseases during follow-up (e.g., thyroiditis and rheumatoid arthritis). Thus patients given a diagnosis of AIH need to recognize their need for lifelong follow-up. The complications of liver failure are as for all liver diseases, namely ascites, hepatic encephalopathy, and variceal hemorrhage. It must be determined if liver failure is due to a flareup of disease, in which case medical therapy is appropriate, or due to burned-out end-stage disease, in which case liver transplantation is probably the best option.

Treatment

For details see Table 84.3. The first therapeutic trials in patients with AIH conducted in the late 1960s and early 1970s

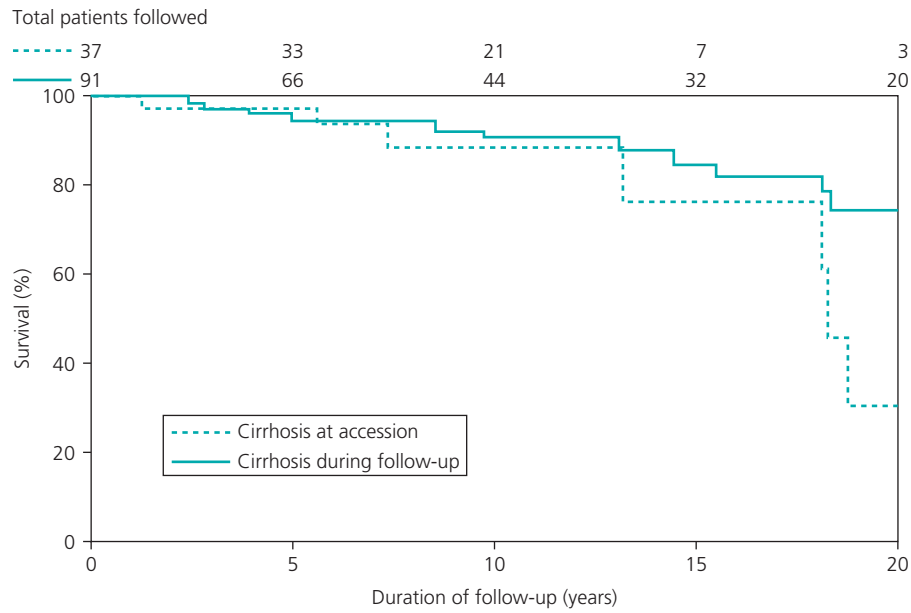

Figure 84.3 Survival of patients with type 1 autoimmune hepatitis: comparison of survival, using death and orthotopic liver transplantation as end points, from development of cirrhosis at accession versus follow-up. From Roberts et al. [51], with permission from Elsevier.

Table 84.3 Suggested therapeutic regimen: for autoimmune hepatitis

Initial therapy Prednisone 30–40 mg/day monotherapy or prednisone 30 mg/day and azathioprine 50–100 mg/day combined therapy
Dose reduction Slowly reduce prednisone (2.5–5 mg every 1–3 months) if ALT in normal range
Maintenance Minimum-dose prednisone or azathioprine to maintain ALT in normal range
Stop therapy If ALT and IgG normal for 1–2 years; liver biopsy indicates inactivity
Retreatment of relapse Reintroduce therapy as for initial treatment
Failure to respond To above, use either high-dose prednisone 40–60 mg/day or another immunosuppressant, or consider liver transplant if appropriate

ALT, alanine aminotransferase.

indicated a highly significant improvement in survival following therapy with corticosteroids [4–6]. The studies from the Mayo Clinic indicated that treatment with high-dose corticosteroid (60 mg daily initially with 20 mg daily as maintenance) and lower-dose corticosteroid combined with azathioprine (30 mg prednisone daily initially, reducing the dose to 10 mg daily as maintenance plus 50 mg daily of azathioprine) were equally effective. Monotherapy with azathioprine 100 mg daily was no better than placebo [7,8]. Several lessons were learned from these trials. First, high-dose corticosteroids are associated with the rapid onset of severe side effects (osteoporosis, diabetes, systemic hypertension, and cataracts) and thus it is wise to treat symptomatic patients from the start with a lower dose of steroids (20–30 mg daily) combined with the steroid-sparing agent azathioprine. As azathioprine takes at least 6 weeks to take effect, it makes sense to start this agent when steroid treatment is initiated. The dose of steroid can be tapered once serum aminotransferase levels have fallen to less than a twofold elevation. Tapering by 2.5–5 mg every 1–3 months with careful monitoring of aminotransferase levels is appropriate. Most patients have a rapid symptomatic and somewhat slower biochemical response within the first few weeks of therapy. There are some patients whose serum aminotransferase levels do not return to normal for many months. A fall in the level of IgG is also a good indicator of response. Liver histology does not improve until the biochemical tests have been normal for at least 6 months. About 20% respond poorly, with 13% achieving only a partial response and only 7% who do not respond to treatment at all [53]. Poor response to immunosuppressive therapy is more common in patients whose liver biopsy shows areas of submassive necrosis [54]. Patients with cirrhosis, even if decompensated, generally respond well to therapy. Therefore even patients being considered for liver transplantation, if they have active disease, should be given immunosuppressive therapy without delay. No randomized controlled trials of therapy in patients who have asymptomatic disease have been conducted. An unsuccessful attempt was made, but patients refused to be included, put off by the long list of side effects attributed to corticosteroids (Gregory P, personal communication, 1997).

Once the serum aminotransferase levels have returned to near-normal values it may be possible to wean the patient off corticosteroids altogether (this was not done in the early trials) and maintain patients on azathioprine alone at a dose of 1–2 mg/kg daily [55,56]. Such a strategy reduces the deleterious consequences of long-term corticosteroid therapy. The white blood cell count needs to be regularly checked because of the potentially toxic effects of azathioprine on the bone marrow. Some patients cannot tolerate azathioprine, mainly because of its gastrointestinal side effects. Alternate-day immunosuppressive therapy is ineffective in AIH.

It is generally advised that immunosuppressive therapy be continued for up to 2 years after the liver biochemical tests have normalized before cessation of immunosuppressive therapy is contemplated. This timing is arbitrary; however it is likely that at least 1 year of therapy is essential. Some would advocate that prior to stopping treatment another liver biopsy should be performed to confirm that the hepatic inflammation has disappeared. A reduction in fibrosis may be observed [57,58] but unfortunately half of those patients not cirrhotic at presentation will progress to cirrhosis despite ongoing immunosuppressive therapy and an apparent biochemical response [59].

In those rare patients with AIH in whom standard therapy with a combination of prednisone and azathioprine fails, other immunosuppressive agents have been tried. Alternatives to classic treatment are also needed for children who may experience devastating growth retardation due to the use of high-dose corticosteroids. However, carefully monitoring corticosteroid therapy is probably still the best treatment in children, although cyclosporine has been used with benefit in children [60] both with type 1 and type 2 AIH [61,62]. A newer agent used to prevent transplant rejection, mycophenolate mofetil, may be preferable as it has less nephrotoxicity, but it often causes diarrhea [63]. Methotrexate has been reported to be of benefit, as has cyclophosphamide in refractory type 1 AIH [64,65].

Budesonide is a corticosteroid that has a 90% first-pass effect in the liver, but which nevertheless appears to have a beneficial effect at this site, causing aminotransferase levels to normalize in AIH [66]. Unfortunately, these encouraging results were not confirmed in another study [67]; in addition steroid side effects were observed in this latter study likely because intrahepatic or extrahepatic portosystemic shunting was present, thus obviating the beneficial properties of budesonide.

Once it is believed that the disease is in long-term remission (sustained normalization of serum aminotransferase and IgG levels, with histological evidence on liver biopsy of quiescent disease), immunosuppressive therapy may be stopped. Unfortunately, the majority (80%) of individuals with AIH who are weaned off treatment will relapse [68], generally within the first few months after treatment cessation but sometimes not until several months or even years later. Reinstitution of immunosuppressive therapy is generally successful.

It is now quite unusual for patients who have been treated for AIH to progress to end-stage liver disease. Risk factors for disease progression include an HLA-DR4-negative phenotype and the presence of anti-LKM1 antibodies as well as massive necrosis on first biopsy and low albumin and high prothrombin time at presentation [69]. However, when liver transplant is necessary, it is extremely successful (5-year survival > 90%), although a pattern of recurrent liver disease similar to AIH may recur in the transplanted liver in up to 40% [70,71]. In most cases this responds to increased immunosuppressive therapy. For this reason attempts at withdrawal of antirejection therapy in those who receive a liver transplant for previous AIH are not recommended.

In addition to specific therapy for liver disease, individuals with AIH may require additional supportive therapy. All should be encouraged to lead an entirely normal life but certain precautions need to be taken, as is the case for anyone on long-term immunosuppressive therapy. In addition the complications of long-term corticosteroid therapy should be anticipated and prevented if possible. Those intending foreign travel should receive appropriate advice from travel medicine specialists, particularly with regard to appropriate vaccinations and how to avoid certain parasitic infections (e.g., *Strongyloides*). All patients on immunosuppressive treatment should learn to recognize any infection early in its course and seek medical attention rapidly.

In young women who are successful in becoming pregnant it is important to check for the presence of esophageal varices in those who are cirrhotic, as nonselective β-blocker therapy may well be required during pregnancy. Advice to the obstetrician to avoid a prolonged second stage of labor is also important. It is quite safe to maintain corticosteroid or azathioprine therapy during pregnancy; sometimes requirements for immune suppression may lessen during the pregnancy. Flareups, if they occur, are most often observed following delivery, although they may occur during pregnancy [52].

Baseline measurement of bone mineral density is wise. Adequate intake of calcium and vitamin D should be recommended to all individuals with AIH given corticosteroids. Preventive bisphosphonate therapy has been shown to be of value.

Summary

Although AIH is one of the less common causes of a chronic hepatitis, it is essential that the diagnosis not be missed, as therapy is so successful, even curative in up to 20%. Once in remission most patients can be maintained in good health on azathioprine monotherapy with no untoward side effects. Treatment of AIH with immunosuppressive agents does not always prevent progression to cirrhosis, but it does significantly reduce the risk of dying of liver failure.

Key points

- Two types of AIH, distinguished by autoantibody status
- Presentation may be with a silent, or a symptomatic or even fulminant hepatitis
- Differential diagnosis: drug-induced hepatitis, Wilson disease, viral hepatitis
- Immunosuppressive therapy markedly improves survival but is curative in only 20% of patients.

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Introduction

Primary biliary cirrhosis (PBC) is a chronic liver disease that is defined by its clinical presentation, histological features, and serological findings. The underlying abnormality in PBC is a slowly progressive, non-purulent inflammatory destruction of the biliary epithelial cells lining the small to medium-sized interlobular ducts [1]. The larger biliary ducts are spared. The pathophysiology of bile duct destruction is poorly understood, but the process is felt to be autoimmune in nature. Genetic and environmental factors appear to be important in disease susceptibility and progression. Autoantibodies are a characteristic feature of the disease; over 90% of patients with PBC have antimitochondrial antibodies (AMA) and about 50% have antinuclear antibodies [2] (Table 85.1). Loss of normal biliary drainage eventually leads to a clinical picture of chronic hepatic cholestasis and its potential complications.

Pathogenesis

Antimitochondrial antibodies

PBC is presumed to be an autoimmune disease, although the evidence to support that hypothesis is largely inferential. Clinically, PBC shares features with other autoimmune diseases such as predominance in middle-aged females and frequent coincidence with other autoimmune disorders. In addition, virtually 100% of patients with PBC have circulating autoantibodies, often to mitochondrial and nuclear antigens.

In 1987, Gershwin and colleagues identified the primary structure of the major target antigen of the PBC AMAs [3]. This autoantigen was identified as the human mitochondrial enzyme, dihydrolipoamide acetyl transferase, also referred to as the E2 component of the pyruvate dehydrogenase complex (PDC-E2). PDC belongs to a family of multienzyme complexes involved in cellular respiration called the 2-oxo-acid dehydrogenase complexes. Although PDC-E2 is the major autoantigen in AMAs, patients with PBC may have antibodies to any of the 2-oxo-acid dehydrogenase complexes, including branched chain 2-oxo-acid dehydrogenase complex (BCOADC) and 2-oxoglutarate dehydrogenase complex (2-OGDC), or other components of the PDC complex (E1, E3, X) [4] (Table 85.1).

The role of these autoantibodies in the pathogenesis of bile duct destruction has been the subject of considerable debate. Clinically, AMAs appear 6–10 years before other clinical signs of the disease, and thus may be present at the initiation of the disease process. AMA titer, however, does not correlate with disease severity, and patients lacking AMA have a clinical presentation and course that are similar to those found in patients with AMA. Experimental models have thus far failed to demonstrate that generation of AMAs leads to chronic destruction of bile ducts in PBC. Rats, guinea pigs, rabbits, and rhesus monkeys have all been immunized with the recombinant form of PDC-E2. Antibodies to PDC-E2 were readily induced, but none of the animals developed abnormal liver tests or abnormal histology after 8 months of follow-up [5].

PDC-E2 is a mitochondrial enzyme that is expressed in the intracellular compartment of all aerobic cells and on the surface of biliary and salivary duct epithelial cells in PBC [6]. The events leading to the expression of PDC-E2 on bile duct cells in PBC are still not understood. One hypothesis is that cholangiocytes are first injured by another process, allowing release of intracellular antigens, which are then displayed on the cell surface. Biliary epithelial cells differ from other types of cells in that they do not add glutathione to the lysine-lipoyl domain of PDC-E2 during apoptosis, and so apoptotic cholangiocytes may expose unmodified PDC-E2 to the immune system [7]. Another hypothesis is that mitochondrial antigens are carried to the surface complexed with immunoglobulin A (IgA) during the normal route of IgA excretion into bile. Alternatively, the abnormal surface

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Table 85.1 Prevalence of autoantibodies in primary biliary cirrhosis

Autoantibody	Prevalence by immunofluorescence %	Prevalence by immunodiffusion, ELISA, or immunoblottting %
Antimitochondrial	85–95	
Anti-PDC-E2[a]		95–97
Anti-PDC-E1		41–66
Anti-PDC-E3		38
Anti-PDC-X		95–97
Anti-OGDC-E2		39–88
Anti-BCOADC-E2		53–55
Antinuclear	25–70	50–81
nuclear rim/peripheral/peri-nuclear	26	
Anti-gp210 (nuclear pore complex)[a]		10–30
Anti-lamin B (nuclear membrane)[a]		1
homogeneous/diffuse	10	
Anti-histone		81
Anti-DS DNA		10
centromere	10–24	
speckled	10–20	
multiple nuclear dot	4–17	
Anti-sp100/anti-PML[a]		5–40
coarse nuclear speckled/nucleolar		
Anti-Smith (Sm) (small RNP)		24
Anti-Scl-70 (DNA-topoisomerase 1)		24
fine nuclear speckled		
Anti-Ro (SS-A) (small RNP)		25–30
Anti-La (SS-B) (small RNP)		21
negative by immunofluorescence		
Anti-SS DNA		71
Antismooth muscle	20	
Anti-actin		13–52
Antiliver membrane	42	
Anti-asialoglycoprotein receptor		15–23
Anticarbonic anhydrase		8–45
ANCA	16–26	
Anti-platelet		40

a Autoantibodies relatively specific for primary biliary cirrhosis.
ANCA, antineutrophil cytoplasmic antibody; BCOADC, branched chain 2-oxo-acid dehydrogenase complex; DS, double-stranded; 2-OGDC, 2-oxoglutarate dehydrogenase complex; PDC, pyruvate dehydrogenase complex; RNP, ribonucleoprotein; SS, single-stranded.

staining could result from surface expression of an abnormal or partial-length PDC-E2, or another molecule that is cross-reactive with antibodies generated against PDC-E2.

The mechanism underlying loss of self-tolerance to PDC-E2 in PBC remains the missing key to understanding the pathogenesis of PBC. Reactivity to mitochondrial antigens might develop as a result of molecular mimicry, a process in which the immune system erroneously recognizes a self-protein as foreign because of similarity to another previously encountered foreign protein. PDC enzymes are well conserved across species. The immunogenic lipoylated domain of PDC-E2 has a particularly high degree of homology with proteins from *Novosphingobium aromaticivorans*, a gram-negative bacterium that is ubiquitous in soil. Sera from almost all patients with PBC react strongly to these bacterial proteins [8]. The organism itself is identified by DNA in the stool of approximately 25% of patients and controls. The rough mutant of *Escherichia coli* expresses a PDC epitope that is also recognized by PBC AMAs [9], and this bacterium has been found in the urine and feces of patients with PBC more frequently than in the urine and feces of liver disease controls [10,11]. Patients with a history of previous urinary tract infections have a slightly higher risk of PBC (Table 85.2) as compared with controls.

Table 85.2 Risk factors for primary biliary cirrhosis (PBC)

Risk factor	Odds ratio
PBC in an identical twin	57 100
Nagasaki bomb survivor	32.5
PBC in another first-degree relative	10.7
Sjögren syndrome in a first-degree relative	5.8
Systemic lupus erythematosus in a first-degree relative	2.2
Urinary tract infection	1.5–2.4
Smoking	1.6–1.8
Hair dye use	1.5–1.8
Hormone replacement therapy	1.6

Exposure to chemicals, toxins, or xenobiotics (natural substances that are foreign to the body) has also been proposed to be responsible for altering native mitochondrial proteins, rendering them immunogenic. Synthetically altering the inner lipoyl moiety of PDC-E2 to mimic changes that might occur after the interaction of PDC-E2 with halogenated xenobiotics in the environment produces a compound with greater reactivity to PBC serum than the native PDC-E2 itself [12]. Animals immunized with these halogenated compounds develop AMAs [13]. However, these AMAs do not persist, as they do in humans with PBC [14].

Environmental risk factors

Epidemiological data also support a possible connection between chemical exposure and PBC. For example, there is an increased prevalence of PBC in survivors of the Nagasaki atomic bomb explosion [15]. Case–control epidemiological studies of PBC have identified links between PBC and smoking, use of hair dye, and use of hormone replacement therapy (Table 85.2) [16,17]. Although the quantity of cigarettes smoked was not related to the development of PBC, smoking more than 10 pack-years has been associated with more advanced fibrosis, irrespective of age or alcohol history [18]. Similarly, the geographic distribution of patients with PBC listed for liver transplant in New York City clusters in neighborhoods that are close to toxic waste sites, whereas cases of primary sclerosing cholangitis patients awaiting transplant and early-stage disease PBC do not cluster in these same areas [19]. It is important to note, however, that even though case–control studies may show clustering of PBC, a population-based cohort from the United Kingdom did not find evidence of geographic clustering.

Genetics

While weak associations with environmental exposures do exist, a positive family history of autoimmune disease (systemic lupus erythemoatosus, Sjögren disease, or PBC) confers a much stronger risk of developing PBC, emphasizing the clear role of genetic susceptibility in acquiring this disease (Table 85.2). The prevalence of PBC in family members of patients with PBC is approximately 4% (range 1%–7%) [20,21]. Of first-degree relatives with PBC, mother–daughter pairs are the most common. The concordance rate for PBC in monozygotic twins is approximately 66%, which is one of the highest reported concordance rates for any autoimmune disease [22,23]. The prevalence of AMA in family members of probands with PBC is also increased [24]. Exactly which genes are responsible for the familial clustering of PBC remains under investigation. Various human leukocyte antigen (HLA) gene alleles and single nucleotide polymorphisms (SNPs) in immunomodulatory genes have been linked to disease susceptibility, but the lack of a reproducible genetic association in the majority of patients suggests that PBC, like diabetes, is a complex multigenic disease. The most frequently observed HLA association across studies is a weak link with HLA-DR8. In North America and Europe, 11%–36% of white females with PBC carry the HLA-DR8 antigen, as opposed to 4%–9% of control subjects. In Japan, where HLA-DR8 is present in 23% of the normal population, HLA-DR8 is found in up to 79% of patients with PBC. Two independent studies identified a SNP (A49G) in cytotoxic T lymphocyte-associated antigen 4 (a molecule that regulates T-cell activation), which confers susceptibility to PBC [25,26]. The *BSMI* polymorphism of the vitamin D receptor is also associated with PBC. Although the relationship of this SNP to bone disease in PBC is not clear, polymorphisms in the vitamin D receptor are known to affect the immunomodulatory activity of vitamin D. Genes on the X chromosome are of particular interest because of the female preponderance of PBC, as well as the large number of immunoregulatory genes located on the X chromosome. There is a higher frequency of X chromosome monosomy in the peripheral leukocytes of patients with PBC as compared to their frequency in healthy controls, similar to what has been described in scleroderma and other autoimmune diseases [27]. Whether increased X chromosome monosomy results from the disease process (e.g., oligoclonal expansion) or contributes to it (e.g., selective loss of inhibitory genes) is still not known.

Immune-mediated destruction of biliary epithelial cells

Once the autoimmune process is initiated, bile ducts are destroyed in a highly specific manner. There is a growing body of evidence that supports the hypothesis that bile duct destruction occurs through T-cell-mediated mechanisms in PBC. This is not surprising considering the abundance of T lymphocytes in PBC portal tract infiltrates [28].

Both $CD4^+$ and $CD8^+$ T cells have been implicated in the pathogenesis of PBC. In vitro, cytotoxic $CD8^+$ cells are the major lymphocyte subset that binds to cholangiocytes [29]. The frequency of PDC-E2-specific $CD8^+$ cells is 10-fold higher

in the liver than the blood of patients with PBC [30]. Aberrant staining for HLA class II and PDC-E2 on biliary epithelium, however, suggests that $CD4^+$ T-cell recognition of antigens on cholangiocytes may be important. The frequency of the precursors of PDC-E2-reactive $CD4^+$ T cells in the liver and lymph nodes is 100-fold higher than in peripheral blood [31]. About 55% of patients with PBC also have circulating T cells reactive to the purified E2/X component of PDC, compared with only 1%–13% of controls [31,32]. Although bile duct cells express HLA class I and II antigens, and mitochondrial antigens on their surface, the ability of cholangiocytes to present antigens to T cells could not be demonstrated in vitro [29].

Natural killer T cells, which recognize CD1d-presented glycosphingolipids from gram-negative bacteria that lack lipopolysaccharide, are also enriched in the liver of patients with PBC [33]. Following stimulation, natural killer T cells produce large amounts of interleukin-4, which drives the differentiation and growth of T helper type 2 cells. Natural killer T cells regulate autoimmune responses and help fight bacterial infections in the liver. Regulatory T cells, which are important in regulating autoimmune responses, are decreased in liver and blood of patients with PBC [34].

The cytokine profile of the liver in PBC is mixed, but favors a predominant T helper type 1 response. The type 1 cytokine interferon-γ is detected at higher levels in the livers of patients with PBC than in control livers from patients with autoimmune hepatitis or in normal liver tissue from transplant donors [35,36]. Moreover, cells expressing interferon-γ messenger RNA in the liver in PBC are clustered around damaged bile ducts [36]. Patients with PBC are also more likely to have an interleukin-1β gene allele associated with high interleukin-1β production [37].

Biliary cell death ultimately occurs through a combination of apoptosis and necrosis. Fas antigen is present on biliary epithelium, and biliary epithelial cells from patients with PBC have been observed to undergo apoptosis more frequently than control biliary epithelial cells [38]. Death of biliary epithelial cells continues throughout all stages of PBC [39].

Alternative recent hypotheses regarding the pathogenesis of PBC include a graft-versus-host disease related to fetal microchimerism, or an immune response to either endogenous retroviruses or chlamydia pneumonia. Evidence for these events, however, is no more frequent in patients with PBC than in matched controls [40–42].

Clinical manifestations

Clinical presentation

The diagnosis of PBC is suspected when a patient presents with evidence of chronic cholestasis. In the early stages, this may be an asymptomatic elevation of serum alkaline phosphatase levels or γ-glutamyltransferase. Over time, fatigue or clinical signs of chronic cholestasis such as pruritus or hypercholesterolemia may develop. Jaundice occurs late in the disease process and is a poor prognostic sign. Early reports of patients with PBC described primarily patients with jaundice and advanced disease [43,44]. Over time, clinical case series have included a decreasing percentage of jaundiced patients and an increasing number of asymptomatic patients [45]. This phenomenon is attributed to increasing physician awareness of the disease as well as widespread use of serum alkaline phosphatase and specific autoantibody tests to help establish an early diagnosis. With earlier diagnosis, a long presymptomatic stage of PBC has become well recognized. In one study of 45 patients with PBC identified during this asymptomatic stage, 84% remained asymptomatic 5 years after the diagnosis had been originally suspected [45]. Patients with PBC may experience symptoms from associated conditions, such as sicca complex, thyroid disease, or arthritis before symptoms of the liver disease itself [46]. Eventually, however, many patients will experience the insidious onset of fatigue or pruritus [47].

Signs/symptoms

Fatigue is the most common complaint of patients with PBC. It is present in 50%–80% of patients and significantly impacts quality of life. Its etiology remains obscure, and its severity may be disproportionate to other disease manifestations [47]. Randomized controlled trials of ondansetron, a serotonin $5\text{-}HT_3$ receptor antagonist [48], as well as antioxidants [49] have failed to demonstrate an improvement in symptoms. Lifestyle changes, such as adequate rest and regular exercise, may offer some benefit [50]. Concomitant depression, anxiety, or sleep disturbance is not uncommon, and these comorbidities may benefit from pharmacological therapy. However, treatment with the antidepressant, fluvoxamine, did not improve fatigue in one randomized controlled trial [51].

The generalized pruritus of PBC is characterized by periodic exacerbations and improvements. Pruritus is often more severe in the evenings. Dry skin and decreased ultraviolet exposure during the winter months or intense heat and sweating during hot weather may intensify symptoms. Pruritus is more common in women than men [52], and pregnancy or exogenous estrogens may precipitate or worsen the itching. The etiology of the pruritus is still unknown. The serendipitous discovery that bile acid sequestrants improve symptoms has led to the hypothesis that elevated bile acid levels are responsible. However, a poor correlation between serum or skin levels of bile acids and symptoms argues against the idea of bile acids being a direct pruritogen [52]. Bile salts may act as important intermediaries in the generation of pruritus. A centrally mediated mechanism of pruritus, potentially mediated by endogenous opioids, was proposed by Bergasa, who was able to elicit scratching in monkeys by injecting PBC plasma into their brains [53]. Endogenous opioid levels are increased in cholestatic patients, and symptoms of pruritus

are relieved by administration of opioid receptor antagonists. Interestingly, this intervention is often accompanied by symptoms of narcotic withdrawal [52].

Right upper quadrant discomfort is present in about 17% of patients with PBC [54]. It may be present in the absence of hepatomegaly, and it has no correlation with levels of alkaline phosphatase, bilirubin, or aminotransferases, or with histological stage. It is typically non-specific in character, is not progressive in nature, and, in fact, often disappears spontaneously.

Xanthomas and hyperpigmentation may develop in hyperlipidemic patients with PBC [47]. Xanthomas are most often seen around the eyes (xanthelasma), but may also develop over tendons and in palmar–digital creases. Hyperpigmentation, which results from increased melanin deposition, is most often found on the trunk and arms. Skin may also darken in areas that are repetitively scratched, which may result in a butterfly pattern of sparing in the middle of the back. Both xanthomas and hyperpigmentation are more often seen in patients with prolonged cholestasis but can also be seen before cirrhosis is found on biopsy [55].

Serological findings

The laboratory abnormalities seen in PBC reflect its cholestatic and immunological nature. Elevated alkaline phosphatase and γ-glutamyltransferase are the predominant features, although aminotransferases may also be moderately elevated (usually < 500 units/L). Hyperbilirubinemia ensues in later stages of the disease. Prothrombin times may be prolonged because of either malabsorption of vitamin K or deteriorating hepatic synthetic function. Hypercholesterolemia often occurs as a consequence of reduced biliary excretion of sterols and impaired feedback regulation of retained bile acids [56]. In early stages of the disease, it primarily consists of high-density lipoprotein particles. Mild eosinophilia may also be seen in early stages of the disease. Elevated serum and hepatic copper levels also develop as a consequence of chronic cholestasis. Although impressive copper deposition can be seen in the liver, there is no evidence that this contributes to disease progression [57]. As with other etiologies of portal hypertension and cirrhosis, decreased albumin and platelet counts reflect the underlying hepatic synthetic function and hypersplenism, respectively. Elevated erythrocyte sedimentation rates are not uncommon, but are not useful in following clinical status.

Almost 100% of patients with PBC have circulating autoantibodies (Table 85.1). The most sensitive and specific autoantibodies are the antimitochondrial antibodies, present in 90%–95% of patients with PBC and rarely found in other conditions [58]. Antinuclear antibodies are present in 25%–70% of patients with PBC, and the immunofluorescence pattern is typically speckled, homogeneous, nuclear dot, centromere, or rim-like. The target antigens of PBC antinuclear antibodies are predominantly nuclear pore complex, centromeric, and occasionally lamin proteins. Antinuclear dot (anti-sp100, anti-PML, anti-SUMO) or antinuclear pore complex (anti-gp210, anti-lamin B receptor) are relatively specific markers for PBC [59,60], although anti-sp100 can also be found in 10% of patients with systemic lupus erythematosus [61] or hepatitis C [62]. Unlike AMAs, the presence of anti-sp100 and anti-PML antibodies has been linked to faster disease progression [63]. Anticentromere antibodies are specifically associated with patients who have limited scleroderma, also called CREST syndrome [64], a subpopulation that may be more prone to portal hypertensive complications rather than jaundice and liver failure [65–67]. It is not uncommon to find other autoantibodies that are usually associated with other connective tissue diseases, but these autoantibodies are usually an isolated finding that is not accompanied by other clinical features of the connective tissue disease. Hyperglobulinemia is not as prominent as in autoimmune hepatitis, but selective elevations of the IgM fraction are found in 80%–95% of patients with PBC [2]. Levels of IgM may also be increased in up to 20% of patients with common bile duct obstruction and 70% of patients with alcoholic cirrhosis, but in these diseases, IgM elevations are usually accompanied by similar elevations in IgA or IgG [67].

A positive serum test for AMA is characteristic of patients with PBC. When present in higher titers (at least 1 : 80), it is the most specific laboratory abnormality of this disease. Using indirect immunofluorescence, approximately 90%–97% of patients will be AMA-positive [58]. AMAs detected by immunofluorescence are rarely associated with diseases other than PBC, but have been reported in association with syphilis, myocarditis, tuberculosis, collagen vascular disease, and iproniazid therapy [69]. Low titers (1 : 40 or below) of AMAs have also been reported in individuals with other liver diseases such as autoimmune hepatitis [70]. Low titers may also be found in PBC, and these are sometimes transient [71]. Therefore, repeat testing is warranted when the clinician suspects a diagnosis of PBC but the AMA test is initially negative. AMAs that are identified in seemingly healthy individuals may represent subclinical PBC and deserve to be followed up. Most of these asymptomatic individuals will eventually develop an elevated alkaline phosphatase, histological findings compatible with PBC, and fatigue or pruritus [72].

Histology

The hallmark histological finding of PBC is focal and segmental nonsuppurative cholangitis. Various authors have developed histological staging systems that group the histological abnormalities into four distinct stages [73,74] (Fig. 85.1). Stage I, as described by Ludwig and colleagues [73], is characterized by lymphocytes infiltrating the portal tracts. In stage II of Ludwig and colleagues, the degree of inflammation is greater, extending beyond the limiting plate. In all systems, Stage III is defined by the presence of bridging fibrosis, and the presence of cirrhosis represents Stage IV.

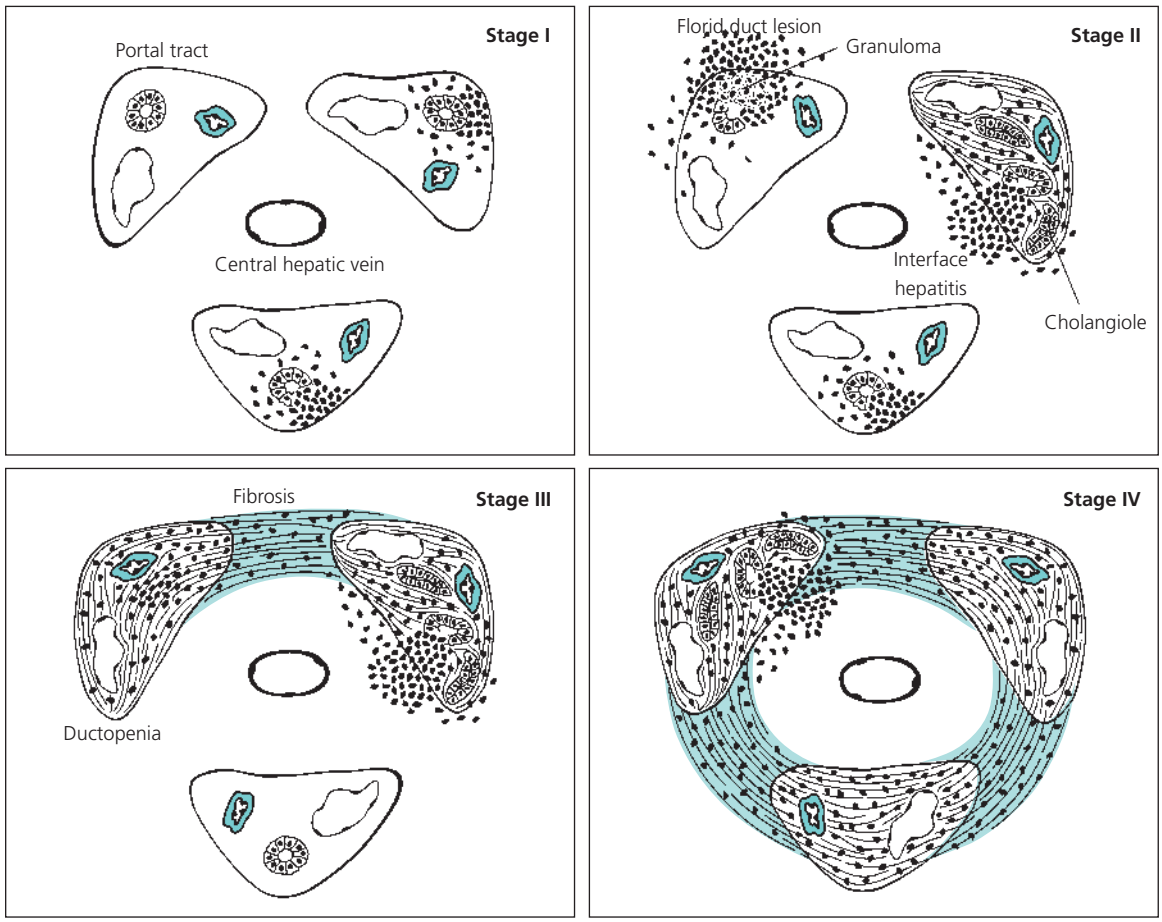

Figure 85.1 Histological progression of primary biliary cirrhosis is categorized into four stages. Characteristic features of each stage are illustrated here, but the lesions are often patchy in nature. Stage I: mononuclear portal tract infiltrates, sometimes involving the bile duct. Stage II: intense mononuclear portal tract infiltrate with interface hepatitis, a florid duct lesion (granulomatous involvement of bile duct), and pseudoductular (cholangiolar) proliferation. Stage III: bridging fibrosis, mononuclear portal tract infiltrates with interface hepatitis, pseudoductular proliferation, and ductopenia. Stage IV: cirrhosis, mononuclear portal tract infiltrates with interface hepatitis, pseudoductular proliferation, and ductopenia.

In earlier stages, bile ducts may show evidence of damage, but are still present in adequate numbers. The most specific (although not pathognomonic) histological finding is the "florid duct lesion," in which the bile duct is surrounded by an intense lymphocytic or granulomatous infiltrate, and the basal integrity of the bile duct has been breached by individual lymphocytes. Granulomas in close proximity to the bile duct are also highly suggestive of PBC. Because of the patchy nature of bile duct involvement in PBC, many portal tracts remain normal or display a nonspecific pattern of mononuclear cell infiltration. The mononuclear infiltrate of PBC consists primarily of T cells, but plasma cells and eosinophils may be seen. The presence of a large number of polymorphonuclear cells or edema surrounding the bile duct, however, should prompt consideration of extrahepatic obstruction. Interface hepatitis (piecemeal necrosis) and foci of lobular inflammation may be present in PBC so the presence of bile duct involvement is an important factor in distinguishing PBC from other forms of chronic inflammatory liver diseases such as autoimmune hepatitis. Bile staining and cholate stasis with feathery degeneration of hepatocytes is evident in some patients, and Mallory hyaline may be found in the affected hepatocytes. Bile ductular proliferation (cholangioles or pseudoducts) is common, particularly along the periphery of the portal tract and is used as a defining characteristic of stage II PBC in the Scheuer staging system [74]. Scattered neutrophils are often found around the proliferating ducts. As the disease advances, ductopenia becomes evident. It is essential to have an adequate number of portal tracts on the biopsy to evaluate the presence of ductopenia. A minimum sample of four portal tracts (with all four portal tracts containing arterioles but no ducts), but ideally 20 portal tracts (with at least 10 portal tracts containing arterioles but no ducts), is needed to make a diagnosis of ductopenia

[75]. Rhodamine and orcein stains for copper and copper-binding protein may be positive in the liver of patients with PBC. The intensity of staining correlates with disease severity, and copper retention may reach levels comparable to that seen in Wilson disease [76].

Epidemiology

The prevalence of primary biliary cirrhosis in the United States and Europe is estimated to be 3.5 per 100 000 individuals (range 0.7–24) with a yearly incidence of 0.9 per 100 000 [20,77]. Some of the highest prevalences have been reported in England and Sweden, where interested researchers have conducted careful epidemiological studies. PBC has been infrequently reported in native Africans [78] and Asians [79]. However, a higher incidence has been reported in persons from India and south Asia who have migrated to western countries [80]. In addition, the prevalence in Japan (5.4 per 100 000) appears to be comparable to that in many European countries [81]. Over the last two decades, the reported prevalence of PBC in North America and Europe has been increasing more rapidly than for any other autoimmune disease [77]. This increase began shortly after the discovery of the AMA and the widespread use of automated chemistry panels, suggesting that greater physician awareness and easier access to diagnostic tests is leading to an increase in the number of patients who are identified at an earlier, frequently asymptomatic phase. Most patients are still identified during middle age, with a median age of presentation between 40 and 55 years.

Women are affected with PBC much more frequently than are men. The reported female : male ratio ranges from 3 : 1 to 22 : 1, with most studies reporting about 12 : 1 [20]. The reason for such a strong female predilection is not known. One hypothesis is that estrogens play an important role in the development or clinical expression of PBC, as has been noted for other autoimmune diseases. The fact that PBC has not yet been reported in a prepubertal female also supports this hypothesis. Of note, whereas many autoimmune diseases improve during pregnancy, the symptoms of PBC may worsen as the result of an increase in cholestasis. While most features of PBC in men are similar to those in women, men have been found to have less pruritus and fewer other autoimmune-associated conditions [50].

Extrahepatic conditions reported to be associated with PBC

PBC is frequently accompanied by other extrahepatic conditions, many of which are also felt to be autoimmune in nature. Up to 84% of patients with PBC manifest features of other autoimmune diseases (Table 85.3).

The most common symptomatic comorbid condition by far is Sjögren syndrome, which occurs in 30%–58% of patients with PBC [2,44,82]. Xerostomia and xerophthalmia of sicca syndrome are the usual manifestations. Vasculitis, renal, pulmonary, or neurological involvement is rarely a clinically significant problem. Evidence of renal tubular acidosis is also present in one-third of patients with PBC, but this association appears to be independent of Sjögren syndrome [83].

Table 85.3 Extrahepatic diseases associated with primary biliary cirrhosis

Extrahepatic disease	Prevalence (%)
Sjögren syndrome	30–58
Gallstones	30–50
Decreased pulmonary diffusion capacity	40–50
Renal tubular acidosis	20–33
Osteoporosis	15–40
Bacteriuria	11–35
Arthropathy	4–38
Rheumatoid arthritis	3–26
Hypothyroidism	11–32
Raynaud phenomenon	7–14
CREST syndrome[a]	3–6
Autoimmune thyroiditis	3–6
Autoimmune anemias	1–2
Psoriasis	1–13
Lichen planus	0.5–6
Ulcerative colitis	0.5–1

a CREST, syndrome of calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias.

The CREST syndrome (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, and telangiectasias – also called "limited scleroderma") is present in about 5% of patients with PBC [2,44,82,84]. The most common features seen are Raynaud phenomena and telangiectasias. Isolated Raynaud syndrome occurs in an additional 7%–14%. Esophageal dysmotility usually presents as gastroesophageal reflux disease or symptomatic hiatal hernia, but frank dysphagia may be present and can be worsened by concomitant xerostomia from Sjögren. Progressive systemic sclerosis, with involvement of other internal organs such as kidney and heart, is actually rare in patients with PBC.

About 10%–20% of patients with PBC complain of arthralgias [2,82,85], which may have a variety of causes. Because PBC often occurs in older individuals, osteoarthritis is the most common reason for joint pains. Rheumatoid arthritis is, however, more common in patients with PBC than in the general population. One study found that 53% of patients with PBC had circulating rheumatoid factor, although only 7% met the established criteria for the diagnosis of rheumatoid arthritis [85]. Radiological surveys of patients with PBC demonstrate that erosive arthritis is common and may not always be accompanied by a positive rheumatoid factor. Hypercholesterolemic arthropathy and hypertrophic osteoarthropathy are also potential reasons for joint disease in this population [86].

Thyroid disease is another frequent comorbid condition, present in about 20% of patients with PBC [2,44,52,82]. Usually this manifests as hypothyroidism, but Hashimoto thyroiditis is also common. Fatigue is a frequent symptom of PBC. However, hypothyroidism should always be excluded before attributing the fatigue to PBC itself.

Osteoporosis is observed in up to 40% of patients with PBC who are referred for transplant [87]. The severity of the bone disease correlates with the severity of the liver disease, and therefore patients with stage IV disease should be screened with bone densitometry at regular intervals. In an unselected group of stable patients with PBC, osteopenia (defined as a T-score of −1.5 to −2.5) was present in 45% and osteoporosis (defined as a T-score less than −2.5) was present in 15% [88]. Osteoporosis in these patients is the result of both increased resorption and decreased formation of bone [89]. Vitamin D deficiency as the result of malabsorption in later stages of disease may also lead to osteomalacia.

Decreased pulmonary diffusion capacity is common in PBC, but it is usually asymptomatic. It is disputed as to whether this finding is related to Sjögren syndrome, CREST syndrome, or the presence of cirrhosis [90]. Symptomatic pulmonary disease is present with a low frequency (< 1%) and has been reported to be the result of a variety of conditions, including sarcoidosis, interstitial pulmonary fibrosis, fibrosing alveolitis, bronchiolitis obstructing obliterizing pneumonia, primary pulmonary hypertension, and hepatopulmonary syndrome [91–93].

Urinary tract infections are frequent, recurrent, and often asymptomatic in female patients with PBC. Up to 61% of female patients report previous urinary tract infections. Upon random screening, about 20% of female patients with PBC will have bacteriuria, as compared with about 7% of age-matched female patients with other liver diseases, 5% of healthy age-matched females, and 20% of age-matched females with cirrhosis [94]. At least half of patients with PBC with bacteriuria are asymptomatic. When followed over time, an additional 10% will develop bacteriuria over a 3-month interval, and 65% will develop pyuria over a 12-month interval. Recurrent infections are often with a different organism, and treatment of asymptomatic infections does not decrease the recurrence.

Despite the frequent occurrence of circulating immune complexes, immune complex disease has only occasionally been reported to occur in PBC. Systemic lupus erythematosus [95], temporal arteritis [96], and other vasculitis syndromes can be found in patients with PBC, but are not particularly increased in frequency. Several reports of membranous glomerular nephritis and leukocytoclastic vasculitis indicate that IgM complexes can be found in the kidney and skin, suggesting that the elevated IgM levels may play a role [97]. In addition, more than a dozen cases of polymyositis with PBC appear in the literature, many of which are in Asian patients [98].

Numerous case reports testify that celiac sprue can occur in patients with PBC. A single study from Ireland found that 11% of patients with PBC had antiendomysial antibodies, which were often accompanied by villous atrophy [99]. However, three larger studies examining the prevalence of antiendomysial antibodies in unselected patients with PBC failed to find a valid association [100].

Gallstones are found by radiological imaging in 30%–50% of patients with PBC, particularly in later stages of the disease when pigment stones are more likely to be formed [101]. Their presence often leads to confusion and delays in diagnosis of PBC, when the patient presents with cholestatic liver test abnormalities, right upper quadrant discomfort, and gallstones, and a presumptive diagnosis of extrahepatic biliary disease is made.

The incidence of extrahepatic cancer in PBC has been studied because of numerous case reports of cancers found in patients with PBC, as well as reports from the 1980s that the relative risk of breast cancer was increased 4.4-fold compared with the risk in other individuals. More recent population studies, however, have failed to find an increased risk of extrahepatic malignancies in PBC [101,102].

The clinical course of associated conditions is not altered by treatment of PBC [82]. The clinician must maintain a high index of suspicion for those conditions that are commonly encountered in patients with PBC so that these diseases may be recognized and managed appropriately.

Differential diagnosis

The differential diagnosis of a patient who presents with chronic cholestatic liver tests may initially include extrahepatic obstruction, or intrahepatic cholestatic liver diseases, as well as granulomatous and infiltrative diseases of the liver.

Extrahepatic obstruction, from gallstones, tumors, or sclerosing diseases of the bile ducts, should first be excluded with good sonographic imaging or cholangiography. Serological testing for AMA will help to differentiate PBC from other intrahepatic cholestatic liver diseases. In patients who are AMA-negative, cholangiography is usually necessary. Liver biopsy in patients who are suspected of having PBC, can confirm the suspected diagnosis, stage the patient, and rule out other etiologies of liver disease. In patients who are AMA-negative, liver biopsy is essential to demonstrate a nonsuppurative cholangiopathy and to exclude other disorders [47].

A variety of diseases may resemble PBC clinically or histologically, but they do not lead to AMAs. Patients who have had prolonged extrahepatic obstruction may also develop a secondary biliary cirrhosis which histologically resembles primary biliary cirrhosis. A rare variant of primary sclerosing cholangitis affects only the small intrahepatic ducts [104]. However, these biliary lesions are characterized by an exuberant fibrotic reaction ("onion skinning") and less lymphocytic

infiltration. Sarcoidosis or idiopathic granulomatous hepatitis may mimic PBC with similar biochemical liver tests, right upper quadrant discomfort, hepatomegaly, and hepatic granulomas. Typically, the granulomas of sarcoidosis are larger, well-formed granulomas found in the liver parenchyma rather than involving the bile ducts [105]. It is possible to have both conditions concurrently; there are several well-documented cases of patients with AMA-positive PBC who also have multi-organ involvement with sarcoidosis [91]. A rare form of chronic hepatitis C that is cholestatic has also been reported [106], and cirrhotic patients with hepatitis C may present with fatigue and pruritus. Infection with the hepatitis C virus is so prevalent that viral infection with hepatitis C should be excluded in all patients with suspected PBC. It is also important to recognize that a variety of drugs have been implicated as causes of nonsuppurative cholangitis and ductopenia, which in some cases may progress to biliary cirrhosis [107]. Other rare causes of intrahepatic cholestasis, such as lymphoma and idiopathic ductopenia, are best excluded by examining liver tissue.

Differentiating PBC from autoimmune hepatitis can be difficult because of overlapping histological and serological features. A published scoring system by the International Autoimmune Hepatitis Group can be used to facilitate the distinction and is particularly helpful when the AMA test is negative [108]. Antinuclear antibodies are common in both diseases and their titer does not help to distinguish between autoimmune hepatitis and PBC. Rarely, some patients will meet diagnostic criteria for both autoimmune hepatitis and PBC, or will develop autoimmune hepatitis while being treated for PBC, so called "overlap syndrome."

It is clear that a small number of patients have a clinical and histological picture similar to that of PBC, except they have atypical antibody profiles, including the absence of AMAs, higher prevalence of antinuclear antibodies (70%–100%), and lower (although still elevated) total IgM levels [109]. This syndrome has been called "autoimmune cholangitis" or "AMA-negative PBC." It is unclear whether the pathogenesis of this condition is distinct from that of AMA-positive PBC, or whether the serological differences between the two syndromes merely reflect the individual diversity of the humoral immune response. Large series of patients with autoimmune cholangitis have not been extensively examined because the frequency of this disorder is so low that few centers see more than a handful of cases. Interpretation of studies is further complicated by the lack of uniform diagnostic criteria. The limited studies that have been conducted suggest that the natural history and response to treatment of this disorder is similar to that of AMA-positive PBC [110]. Similar immunological disturbances, including the presence of PDC-E2-reactive material on the surface of bile ducts and oligoclonality of T-cell populations, have been noted in both conditions [111,112]. In fact, there is reluctance among many experts to embrace the hypothesis that this syndrome represents a distinct entity from the syndrome of AMA-positive PBC.

Course and complications

Clinical course

PBC may exist for relatively long periods of time in an asymptomatic state [47,113]. Indeed, data from natural history studies suggest that a fraction of asymptomatic patients will never develop symptoms during their natural lifespan [113]. However, approximately 66%–83% of untreated asymptomatic individuals become symptomatic (usually pruritus or fatigue) within 10 years, and only 5% remain asymptomatic after 20 years [114]. Most individuals with established disease eventually progress to cirrhosis. In two studies, the mean time of progression from stage 1 or 2 disease to cirrhosis in untreated patients was 4–6 years [115,116]. Survival is shortened in patients with PBC compared with healthy controls. In a recent large, geographically based cohort of mostly untreated individuals, the median survival was 9 years, although only 42% of deaths were liver-related [114]. Clinical progression is foretold by worsening of specific biochemical parameters, particularly serum bilirubin levels. The mean survival of patients with bilirubin values over 2 mg/dL is 4 years, and for those with bilirubin values over 6 mg/dL, mean survival drops to 2 years [115]. Several mathematical models exist that incorporate bilirubin and other parameters into formulas that are clinically useful to predict mortality and judge timing for transplant referral of individual patients [114,116,117]. The most extensively validated and commonly used survival model is the Mayo Risk Score [116]. The Mayo Risk score calculates an *R*-score based on the following formula (adapted from original to use international normalized ratio [INR], personal communication with Dr E.R. Dickson and Dr Terry Therneau, Mayo Clinic): $R = 0.871 \log_e(\text{bilirubin mg/dL}) + [-2.53 \log_e(\text{albumin gm/dL})] + 0.039 \text{ age (years)} + 0.881 \log_e(\text{INR}) + 5.92 + 0.859$ (edema scores of 0, 0.5, or 1.0 for none, mild, or moderate edema, respectively). The computed *R*-score is then used to predict the fraction of patients expected to survive for: 1 year = $0.970^{(R-5.07)}$, 2 years = $0.941^{(R-5.07)}$, 3 years = $0.883^{(R-5.07)}$, 4 years = $0.833^{(R-5.07)}$, 5 years = $0.774^{(R-5.07)}$, 6 years = $0.721^{(R-5.07)}$, or 7 years = $0.651^{(R-5.07)}$. Notably, in all prognostic models, serum bilirubin values play a prominent role in predicting survival. Serum albumin, age, and time are found to be independent predictors of survival in some but not all prognostic models. The Mayo Risk score has been demonstrated to be useful in judging the appropriate time for transplantation. However, in some populations, it has been found to overestimate mortality [118]. An updated version of the model, which evaluates interval changes in the patient's condition to estimate short-term survival, is less likely to overestimate long-range mortality in patients with early disease [119].

Complications

Once cirrhosis is established, the clinical complications of portal hypertension and deteriorating liver function are similar to those of patients with cirrhosis from other causes. In patients with PBC, presinusoidal fibrosis alone may be sufficient to cause portal hypertension and esophageal varices and associated bleeding complications may precede the development of stage 4 fibrosis [120]. The risk of hepatocellular carcinoma in patients with PBC is also increased compared with healthy individuals [103]. This risk is almost exclusively limited to cirrhotics, and is higher for males. In general, the reported risk of hepatocellular carcinoma in PBC is significantly less than the reported risk of hepatocellular carcinoma in other liver diseases such as hereditary hemochromatosis, hepatitis B, or chronic hepatitis C. One possible exception is the subgroup of males with advanced PBC in whom incidences as high as 20% have been reported [103].

Pruritus is a common, and often vexing, symptom of patients with PBC. Its severity often fluctuates and is not correlated with disease progression. However, in the terminal stages of the disease, pruritus often disappears. Its etiology is unknown, but elevated bile acid levels and endogenous opioid levels have been implicated [121].

Decreased osteoblastic and increased osteoclastic activities contribute to accelerated osteoporosis in patients with primary biliary cirrhosis [89]. The risk increases with disease severity, and regular screening of bone density in cirrhotic patients facilitates treatment. Preserving bone mass is particularly important for patients who ultimately undergo liver transplantation, because bone mass decreases further in the immediate posttransplant period of heavy immunosuppression.

Like osteoporosis, deficiencies of the fat-soluble vitamins are common in patients with PBC, and correlate with disease severity. For this reason, asymptomatic patients with cirrhosis should be screened. Poor bile salt excretion is the primary reason for deficiencies, which may be made worse by treatment with bile acid sequestrants or antibiotics. Vitamin A is frequently the first fat-soluble vitamin to become deficient, and patients may not complain of night blindness. Vitamin D deficiency may cause osteomalacia, and vitamin K deficiency may result in prolonged coagulation times. Vitamin E serum levels may be falsely elevated in patients with hyperlipidemia. A ratio of serum vitamin E to total lipid that is less than 0.8 mg/g is considered deficient.

Treatment

Therapy of PBC

Therapy with ursodeoxycholic acid (UDCA), at a dosage of 13–15 mg/kg/day has become the mainstay of therapy for PBC (Fig. 85.2). The medication may be administered in two or three divided doses, or as a single evening dose. The potential mechanisms of UDCA's beneficial effect on PBC are multiple [122]. UDCA is a bile acid that is less toxic to hepatocytes than other more hydrophobic bile acids. It is normally present in human bile at low concentrations (1%), but when ingested at a daily dosage of 13–15 mg/kg, it replaces about 40% of the bile acid pool. It promotes insertion of bile salt export protein on the canalicular membrane, increasing intracellular and canalicular transport of hydrophobic bile acids, which decreases the toxic effects of bile on the liver during cholestasis. UDCA stabilizes hepatocyte membranes, decreases rates of apoptosis of biliary epithelial cells, and has antioxidant properties such as the ability to inhibit nitric oxide synthetase. UDCA has been found to decrease the expression of adhesion molecules (lymphocyte function-associated antigens I and II, and intercellular adhesion molecule 1), to decrease HLA class I and II expression in the liver, and to decrease T-cell reactivity and cytokine production.

The ability of UDCA to impact the natural history of PBC is still controversial, and the lack of sufficiently long placebo-controlled trials precludes a definitive statement on its effect on survival. However, comparison of the *actual survival* of patients treated with UDCA with *expected survival* calculated from validated models, indicates that UDCA delays progression to cirrhosis in patients with early-stage disease. Five large, randomized, double-blind controlled trials have demonstrated the beneficial effects of UDCA therapy [123–127]. Collectively, these studies randomized 890 individuals to either UDCA or placebo and followed them for an average of 2 years before switching the placebo patients to UDCA. In all trials, UDCA produced a rapid and impressive improvement in serum liver tests, primarily alkaline phosphatase, bilirubin, aminotranferases, and IgM levels. Biochemical improvement was usually noted within the first 1 or 2 months of therapy and improvements continued, with a maximal response seen by about 6 months. Effects on symptoms were unimpressive, although a small number of patients reported an improvement in itching. Four of the five trials reported beneficial effects on histology, primarily improvement in inflammatory features and less ductopenia in the treated groups. No effect on the development of cirrhosis or portal hypertension, or death/transplantation could be detected at 2 years in any of the individual trials. A combined analysis of patients from three of the trials, which included patients that were switched from placebo to UDCA after 2 years, reported an improvement in transplant-free survival after 4–6 years in patients originally randomized to UDCA [128]. The conclusions from this combined study, however, have been disputed by two subsequent larger metaanalyses. One traditional metaanalysis of 11 randomized trials, including 1272 patients, failed to show any difference between UDCA and placebo in the incidence of death or transplantation, or development of liver disease complications such as ascites or gastrointestinal bleeding [129]. The second metaanalysis was a Cochrane Hepato-Biliary Group systematic review, examining 16 randomized trials including 1422 patients. It also

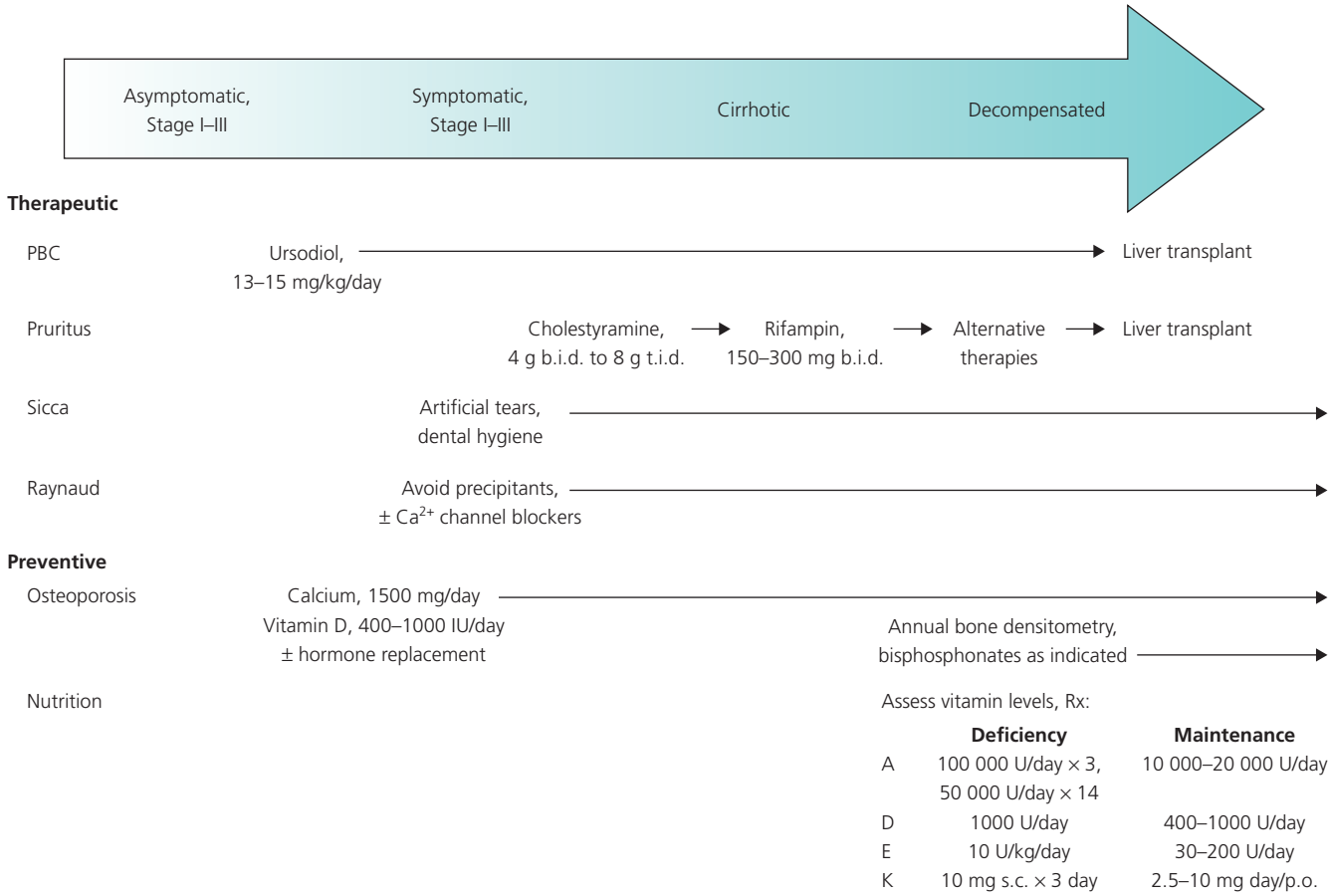

Figure 85.2 Management of primary biliary cirrhosis (PBC) and associated conditions; adapted from Heathcote [173]. Complications of portal hypertension (not shown) are managed in the same manner as in other forms of cirrhosis.

failed to show a difference in mortality or liver transplantation, worsening of histological stage, or development of varices with UDCA treatment. The number of patients developing ascites, however, was lower in persons taking UDCA [130]. The difficulty in clearly demonstrating survival benefits of UDCA is not surprising considering the slowly progressive course of PBC and the relatively short duration of the placebo phase in most randomized controlled trials. A subsequent randomized controlled study which lasted 12 years was still unable to show a mortality benefit of UDCA in PBC [131]. In addition, the potential benefit of UDCA may be greatest when it is started early in the disease course, making it even more difficult to demonstrate survival effects in clinical trials. Follow-up studies of patients treated with UDCA for prolonged periods (10–20 years) demonstrate that patients started on UDCA during stage 1 or 2 have survival rates similar to the general population, but patients started on UDCA in stage 3 or 4 have increased mortality. In fact, survival of patients with late-stage PBC treated with UDCA was no better than predicted survival without any treatment [132]. Progression to cirrhosis appears to be slower in individuals taking UDCA. The incidence of cirrhosis in 183 patients treated with UDCA and followed for 10 years from stages 1, 2 and 3 was 17%, 27%, and 76%, respectively [133]. Importantly, UDCA was extremely well tolerated and safe in all trials. Therefore, because of its excellent safety profile, proven ability to improve markers of cholestasis, and the suggestion of long-term benefits, UDCA is recommended as treatment for all stages of PBC.

The prevailing viewpoint is that bile duct destruction is immune-mediated so immunosuppressive and antiinflammatory drugs have historically been selected as potential medical treatments for PBC. Unfortunately, the results of clinical trials have been disappointing or inconclusive for almost all of them. In striking contrast to UDCA therapy, the major problems encountered in the use of immunosuppressive therapy have been their serious side effects, which have an important cumulative impact in the therapy of a slowly progressive, lifelong disease.

Chlorambucil was evaluated in a controlled trial of 24 patients followed for 4 years [134]. Modest improvements in bilirubin levels, IgM levels, and inflammatory scores were seen in the group given chlorambucil. However, bone marrow depression, requiring cessation of treatment in 17% of

patients, was a significant problem. It was felt that the potentially dangerous side effects of chlorambucil, combined with its modest effects on the disease, made it an impractical therapeutic choice.

D-Penicillamine has been tested in three large controlled clinical trials on 472 patients with long-term follow up [135–137]. Between 22% and 46% of patients experienced serious side effects necessitating discontinuation of the drug. Despite its antiinflammatory, immunosuppressive, antifibrotic, and copper-chelating properties, D-penicillamine was found to have no therapeutic benefit in PBC.

Prednisolone was evaluated in a controlled trial of 36 patients followed for 3 years [138]. There were significant improvements in alkaline phosphatase, aminotransferase, and immunoglobulin levels. Improvements in fatigue, pruritus, and bilirubin levels were transient, and there was a trend toward an improvement in liver histology. These modest benefits were outweighed by the deleterious side effects of prednisolone, especially with respect to accelerated loss of bone density. Budesonide, a corticosteroid with a high first-pass hepatic metabolism, has been tested as a second agent with UDCA [139–141] and found to significantly improve liver histology, liver enzymes, and immunoglobulin levels. However, systemic effects, including bone loss, did occur, particularly in patients with advanced disease.

Cyclosporine (ciclosporin [rINN]) was evaluated in a multicenter, controlled trial in which 349 patients were followed for up to 6 years [142]. Significant improvements in bilirubin, alkaline phosphatase, albumin, aminotransferases, and pruritus were seen, but liver histology was not affected. Survival (time to death or transplant) was modestly prolonged in the cyclosporine group, but complications such as hypertension and renal toxicity were severe enough to discourage cyclosporine from further consideration.

Azathioprine has been tested in two controlled clinical trials [143,144] on a total of 293 patients followed for 2–3 years. Both trials failed to see a survival difference between those treated with azathioprine and those treated with placebo. However, both trials also included a large percentage of jaundiced patients with advanced disease. In the European trial, when patients with higher bilirubin levels were excluded from the analysis, a slight survival advantage was seen in the azathioprine group. Azathioprine was well tolerated by patients with PBC in both studies. Nevertheless, after these trials, azathioprine was largely abandoned as a therapy for PBC.

Colchicine has both antiinflammatory and antifibrotic properties, and so has been seriously considered as a potential therapy for PBC. In some controlled trials [145–147] serum levels of bilirubin, albumin, alkaline phosphatase, cholesterol, and aminotransferases were modestly improved, but symptoms and histological progression were not affected. In one trial [148], a trend toward decrease in liver mortality was seen at 4 years. However, in a Cochrane Database analysis of 10 trials involving 631 patients, no significant differences were detected between colchicine and placebo/no intervention regarding mortality or liver transplantation, liver complications, liver biochemical variables, liver histology, or adverse events [149]. In fact, the authors were unable to exclude a risk of increased mortality from colchicine.

Methotrexate has been reported to improve liver enzymes and histology in selected patients with PBC who do not respond fully to UDCA [150]. However, a randomized double-blind, controlled trial of 265 PBC subjects found that adding methotrexate to UDCA had no impact on survival, histology, or development of portal hypertensive complications after 7 years [151].

Mycophenolate mofetil has been tested as a conjunctive therapy with UDCA in a pilot study of 25 patients who did not achieve biochemical remission on UDCA alone. There were only marginal improvements in alkaline phosphatase and aspartate aminotransferase, and the authors concluded that there was no clinical benefit [152].

At present, UDCA is the only approved recommended therapy for PBC. While it is hoped that UDCA therapy will delay liver disease progression and prolong survival, it is clear that it does not obviate the need for liver transplantation. Patients with complicated cirrhosis or poor estimated survival (Mayo Risk score ≥ 7, model for end-stage liver disease > 15) should be considered for transplant referral.

Transplantation for PBC

In general, patients with PBC have excellent posttransplant outcomes, with a 75% 5-year survival and with 95% of survivors returning to work. AMAs frequently persist after transplant, although often at lower titers than pretransplant. Histological recurrence of PBC in the transplanted liver occurs in up to 32% of recipients [153]. Fortunately, because of the slow progression of recurrent disease, it is not a significant clinical problem. Tacrolimus-based immunosuppressant regimens may be associated with earlier and more frequent recurrence compared with cyclosporine-based regimens. Out of 485 patients transplanted for PBC, the median time to recurrence was 62 months in those receiving tacrolimus, as compared with 123 months in those receiving cyclosporine [154].

Management of complications

Managing the complications of cholestasis comprises a considerable portion of the clinician's treatment of the patient with PBC (Fig. 85.2).

Pruritus

Bile acid sequestrants, such as cholestyramine and colestipol, given at 4–16 g/day in divided doses, improve pruritus in 80%–85% of patients with cholestatic pruritus within 4–11 days. They are the first line of medical treatment for cholestatic pruritus because of their efficacy and safety profile [47].

Unfortunately, compliance with these drugs is sometimes impaired by dislike of the powdered drug delivery system or difficulty in timing other medication doses so that the sequestrant does not inhibit drug absorption (manufacturers recommend that all other medicines be taken at least 1 h before or 4 h after taking the sequestrant). The sequestrants are not absorbed so side effects are limited to the gastrointestinal tract. Rifampin has also been demonstrated to relieve cholestatic itching in small controlled trials [155,156]. The mechanism is unknown but is thought to be mediated through enhancement of the mixed function oxidase system leading to an increase in the rate of bile acid metabolism and excretion or competition for hepatic bile acid uptake. The latter may also lead to an increase in serum bilirubin levels in some patients, which may make monitoring of disease progression difficult. Rare cases of severe hepatotoxicity have been reported. At a total dosage of 300–600 mg/day given in divided doses, the mean time to relief is 2 days, and it is also effective long term. It has been demonstrated by one group to be superior to phenobarbitol therapy [157]. Phenobarbitol, however, is also more effective than placebo. The mechanism of action is likely related to hepatic enzyme induction or sedation. The drug dosage (15–180 mg/day) is titrated to balance efficacy and sedation, but usually much lower dosages are required than for the treatment of epilepsy. Antihistamines are often used to treat itching in patients with PBC, but no controlled trials have demonstrated their efficacy. In fact, there is a lack of evidence that cholestatic pruritus is histamine-mediated. However, anecdotal success in patients with mild itching may be attributable to their sedating properties. Naloxone, naltrexone, and nalmefene, opioid receptor blockers, have been demonstrated to be superior to placebo in alleviating cholestatic pruritus [158]. These drugs can sometimes elicit an opioid withdrawal syndrome of nausea, pallor, colicky abdominal pain, anorexia, and hypertension. The reaction is usually temporary and subsides over 2–3 days of continued treatment. Breakthrough of pruritus and unmasking of chronic pain disorders have also been reported with prolonged therapy. Serotonin may be another important neurotransmitter in the perception of pruritus. A recent pilot study of sertraline, a selective serotonin reuptake inhibitor, at a dosage of 75–100 mg/day demonstrated a beneficial effect on pruritus [159]. However, these findings await confirmation in larger studies. Initial studies of ondansetron, a serotonin $5HT_3$ receptor blocker, showed promise as a new therapeutic agent [160], but a subsequent double-blind, randomized controlled trial showed no benefit [161]. Plasmapheresis and filtration using the molecular adsorbent recirculating system has been used as a desperate measure to control difficult pruritus of cholestasis. In a small number of patients, transient success has been reported lasting anywhere from 24 h to several months. Phototherapy has also been anecdotally successful in ameliorating cholestatic pruritus, but its effectiveness has never been adequately tested in clinical trials. UDCA is associated with little to no relief of pruritus.

Osteoporosis

Accelerated osteoporosis can be a devastating complication of chronic cholestasis, and careful monitoring and preventative treatment are the best therapy. Calcium supplementation (1500 mg/day for postmenopausal females and 1000 mg/day for men and premenopausal females) is well tolerated and widely used as a preventative measure. Estrogen replacement for postmenopausal females is also well tolerated. Estrogens inhibit canalicular transport of bilirubin and have the potential to worsen cholestasis. However, the majority of patients with PBC can benefit from transdermal or low-dose oral estrogen replacement without experiencing a clinically significant increase in cholestasis [162]. Additional therapy is indicated if the bone density decreases into the osteoporotic range (T-score > 2.5 SD) Raloxifene, a selective estrogen receptor modulator that reduces fracture rates in noncholestatic, postmenopausal women, has been tested in a pilot study of nine patients with PBC with promising improvements in bone density [163]. Larger studies in cholestatic patients are required. Alendronate, a bisphosphonate, increases bone density in patients with PBC with few or no side effects [47]. The theoretical risk of inducing variceal hemorrhage has not been reported, although few subjects with varices have actually been treated. Sodium fluoride has also been demonstrated in one trial to increase bone density in patients with PBC [47]. However, less overall experience and increased side effects (severe gastritis) make this drug a less attractive choice compared to alendronate.

Vitamin deficiencies

Deficiencies in the fat-soluble vitamins should be remedied. There is the potential for toxicity from overdoses so levels of vitamins A and D should be monitored in patients receiving supplements.

Hyperlipidemias

Serum cholesterol levels are typically elevated in patients with PBC, even in early stages of the disease. However, two retrospective studies indicate that this does not translate into increased mortality from cardiovascular events [164,165]. Potential reasons for this include:

- onset of disease during middle age rather than childhood resulting in less time for plaque formation
- a favorable high-density lipoprotein : total cholesterol ratio during early stages of the disease
- a favorable lipoprotein profile including the presence of lipoprotein X and low lipoprotein A levels.

Therefore, the decision to intervene medically should be based on factors such as unfavorable lipoprotein profiles, significant personal or family cardiac history, or the presence of painful xanthomas. Patients with PBC are often resistant

to dietary management of hypercholesterolemia, and they may respond paradoxically to clofibrate therapy [166]. Almost all controlled trials of UDCA have shown significant decreases in serum cholesterol levels. Case series have reported efficacy with hydroxymethylglutaryl-coenzyme A reductase inhibitors [167,168] or low-density lipoprotein apheresis [169], but no large randomized clinical trials have been published.

Sicca syndrome

Many patients with PBC have sicca syndrome and are troubled by xerophthalmia and xerostomia. Carboxymethylcellulose lubricants may be used to relieve irritation of the cornea and help prevent ulceration. More severe cases may respond to ocular cyclosporine emulsion, hydroxypropyl cellulose inserts, or punctual occlusion. Xerostomia may be relieved with adequate access to fluids, use of sugar-free hard candies, and prescription moisturizers when necessary. It is important to have regular evaluation by a dental professional because decreased saliva secretion leads to decreased clearance of food particles and increased dental caries.

Treatment of variant forms of PBC

In autoimmune cholangitis, UDCA is effective at improving liver biochemistries, and 3-year clinical outcome is similar to that of AMA-positive PBC [110]. Steroid therapy may modestly improve liver biochemistries in autoimmune cholangitis, as it does in AMA-positive PBC [138], but steroids have been associated with accelerated bone density loss and other undesirable side effects in cholestatic patients. UDCA, therefore, appears to be better tolerated as a long-term therapy than corticosteroids.

Whether patients with overlap syndrome (autoimmune hepatitis + PBC) should be treated with UDCA alone or with UDCA in combination with steroids or azathioprine is debatable. Small, retrospective analyses of cohorts with overlap syndrome demonstrate that patients who do not respond well to an initial trial of UDCA may respond to corticosteroids [170]. However, studies differ as to whether the 2-year outcome is different in dual therapy from UDCA monotherapy [171,172]. No randomized trials have been performed. Thus, clinical judgment must be used to determine the predominant syndrome, balance the risk of medication side effects with possible therapeutic benefit, and initiate therapy accordingly.

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G LIVER

86 Hemochromatosis

Jacob Alexander, Kris V. Kowdley

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The classic triad of diabetes, bronze pigmentation of the skin, and cirrhosis was first described by Trousseau in 1865, and was given the name hemochromatosis by von Recklinghausen in 1889. The seminal study by Sheldon in 1935 identified the role of iron in the pathogenesis of this disease and its hereditary nature. The linkage to human leukocyte antigen (HLA) A3 and localization of the gene to the short arm of chromosome 6 was subsequently established by Simon's group. Feder and colleagues identified the *HFE* gene and the homozygous C282Y mutation in probands with phenotypic hemochromatosis in 1996 using positional cloning [1]. With a prevalence of 1 : 200 to 1 : 250 for homozygosity and 1 : 8 to 1 : 12 for heterozygosity, the C282Y mutation of the *HFE* gene is one of the commonest genetic mutations among persons of northern European descent [2]. Other forms of hereditary hemochromatosis are the result of mutations in other genes regulating iron transport, although these other types are rare in comparison to *HFE*-associated hemochromatosis [3]. The pathophysiology, clinical features, and treatment of hemochromatosis are reviewed in this chapter.

Iron homeostasis

The normal daily turnover of iron is approximately 20 mg. Intestinal absorption mediated by villous enterocytes in the duodenum accounts for only 10% of the turnover; because there is no regulated mechanism for the excretion of iron, regulation of intestinal absorption is crucial for the maintenance of iron homeostasis [4,5]. An incompletely defined signaling pathway between the liver, erythrocytes, macrophages, and enterocytes regulates the intestinal absorption of iron (Fig. 86.1).

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Intestinal absorption

Dietary iron is present either in inorganic form (in vegetables and cereals) or as heme iron (predominantly from hemoglobin and myoglobin in animal proteins). The mechanism for heme absorption has not been elucidated as clearly as that for inorganic iron [5].

Absorption of inorganic iron

Intestinal absorption of inorganic iron involves:

- the reduction of iron from the ferric (Fe^{3+}) to the ferrous (Fe^{2+}) state
- uptake across the mucosal surface of the enterocyte
- transport across the cell to the basolateral aspect
- release into the circulation bound to transferrin [6].

Duodenal ferric reductase (Dcytb) present on the apical plasma membrane of the enterocyte reduces the highly insoluble, and therefore nonabsorbable, ferric (Fe^{3+}) iron to ferrous (Fe^{2+}) iron [7] which is then transported across the apical membrane by divalent metal transporter 1 (DMT1) into the enterocyte [8,9]. Iron is oxidized back to the ferric state by hephaestin, a ferroxidase with homology to ceruloplasmin, and is then exported from the enterocyte to the blood by ferroportin and transferrin receptor (TfR1) located on the basolateral aspect of the enterocyte [7,10].

Iron not exported into the circulation is stored within the enterocyte bound to ferritin and is excreted in the feces by sloughing of senescent enterocytes [6]. Apart from menstrual blood loss, sloughing of senescent enterocytes is the only physiological means of significant iron excretion from the body [11].

Absorption of heme

Heme is transported intact into the intestinal epithelial cells where the iron is released from the heme by the microsomal heme oxygenase system. The only heme transporter identified in the enterocytes is the heme carrier protein 1 (HCP1) [12]. However, the relatively small amount of heme transported by HCP1 suggests that other proteins or cofactors are

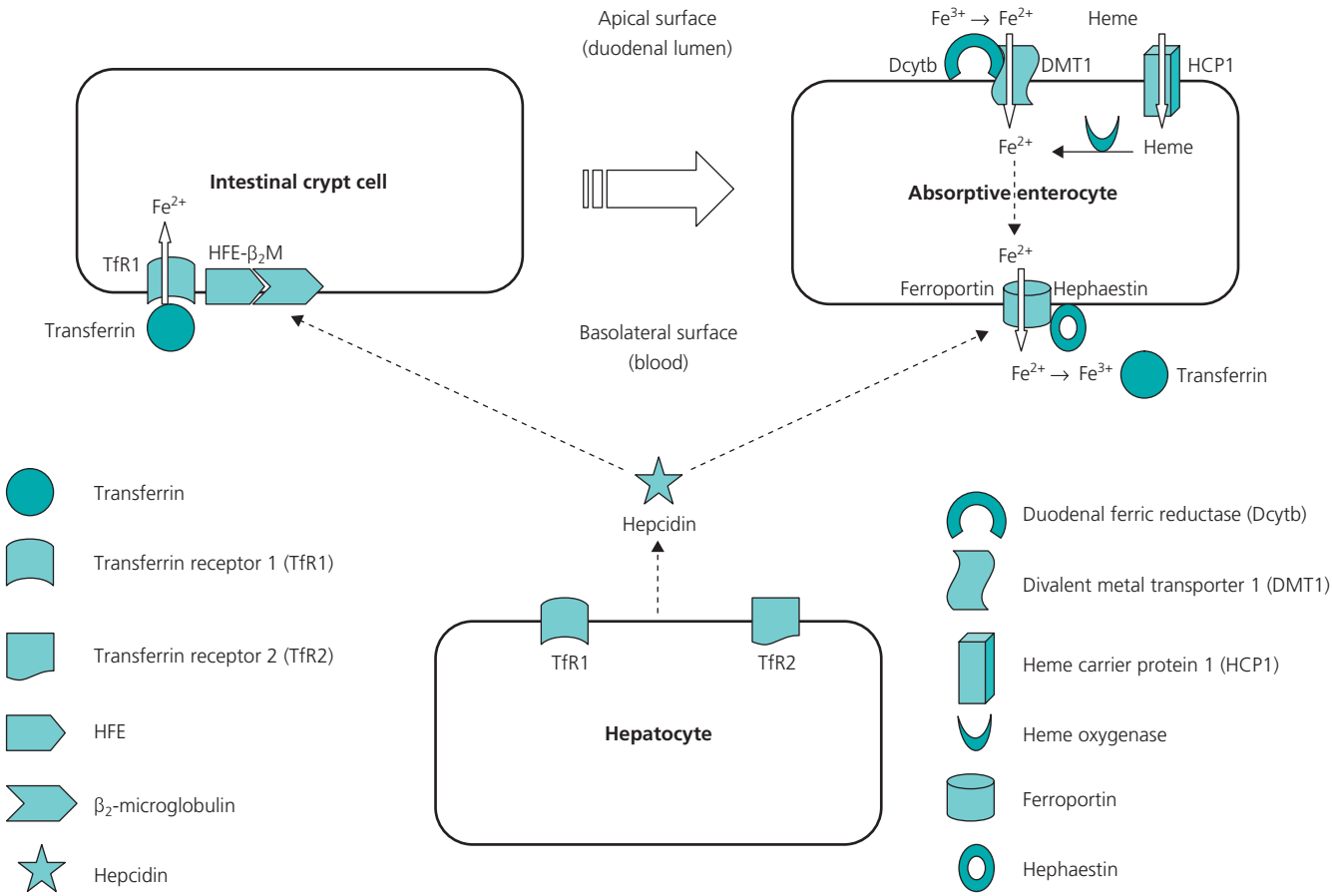

Figure 86.1 Model for control of iron absorption.

also involved in intestinal heme transport [13]. Two other heme transporters, the receptor for feline leukemia virus C (FLVCR) and breast cancer resistance protein (Bcrp) are found in hematopoietic cells, but have not been found to have a role in intestinal iron transport [14,15].

Iron in the circulation

In blood, iron is bound to the plasma protein transferrin, which prevents precipitation of iron in plasma, attenuates the toxicity associated with its redox activity, and facilitates the delivery of iron to cells by binding to TfR1 [16]. Normally, serum transferrin is only about 30% saturated with iron, leaving the remainder readily available for binding free iron.

Iron in the cells

Cellular uptake of transferrin-bound iron is mediated by TfR1, which is expressed on cell surfaces and binds to iron-loaded transferrin to form transferrin–TfR1 complexes. These are then internalized into endosomes where iron is released from transferrin [17]. Export of iron from cells is mediated by the proteins ferroportin [18] and ceruloplasmin [19].

Most of the transferrin-bound iron in the circulation enters hematopoietic cells and ultimately reaches macrophages when senescent red blood cells are phagocytosed [20]. Hepatocytes take up iron by way of TfR1, transferrin receptor 2 (TfR2), and possibly other non-transferrin mediated mechanisms [16].

Genes and proteins related to hemochromatosis

HFE gene and HFE protein

HFE, a 343-amino-acid protein, is a nonclassical major histocompatibility complex (MHC) class I molecule structurally similar to other MHC class I proteins [2]. It is encoded by the *HFE* (previously known as *HLA-H*) gene located on chromosome 6p21.3 and is ubiquitously expressed [2]. In the small intestine, HFE is predominantly expressed in cells in deep crypts, with little or no expression in the villi [21]. The precise role of HFE in iron metabolism is not clear, but its interactions with β_2-microglobulin and TfR1 are considered to be important in the regulation of intestinal iron absorption.

The C282Y mutation, the most common *HFE* mutation associated with hemochromatosis, disrupts a disulfide bridge

in the α3 domain of HFE protein, resulting in a change in the three-dimensional structure of the HFE protein leading to impaired binding with the β_2-microglobulin [22]. Failure of binding with β_2-microglobulin results in defective Golgi processing, accelerated intracellular degradation, and lack of cell-surface presentation of the HFE protein [23].

The nature of the interaction between HFE and TfR1 is less clearly understood. It has been postulated that the interaction of HFE with TfR1 facilitates the entry of iron into crypt cells, and that the HFE mutation impairs this effect, leading to intracellular iron deficiency in the crypts and secondary up-regulation of DMT1 and ferroportin, resulting in increased iron absorption [24]. This model supports the crypt migration hypothesis, which proposes that the iron-deficient crypt cells develop into mature villous cells programmed to absorb excess iron through increased activity of DMT1 and ferroportin 1 (FPN1) [4]. However, this model does not account for the effect of hepcidin, a peptide secreted from the liver that has been found to influence multiple steps in iron metabolism as discussed below. Moreover, this model does not explain the observation that HFE lowers intracellular iron levels independently of its interaction with the TfR1 [24,25].

HAMP gene and hepcidin

The *HAMP* gene located on chromosome 19q13.1 codes for hepcidin, a 25-amino-acid peptide that is secreted from the liver and excreted in urine [26]. Basic amino acids in hepcidin confer on it antimicrobial properties, and four disulfide bridges make it structurally similar to the defensin family of antimicrobial peptides [27]. Hepatic expression of hepcidin is increased in response to infection, inflammation, and iron overload; and decreased in response to anemia and hypoxia. Hepcidin inhibits iron absorption in the small intestine, iron transport across the placenta, and iron release from macrophages [26]. With its effects on multiple steps in iron metabolism, hepcidin is increasingly regarded as a primary regulator of iron homeostasis.

Hepcidin binds to ferroportin, resulting in internalization and lysosomal degradation of ferroportin, so inhibiting iron efflux from cells [28]. However, the mechanism of feedback regulation of hepatic hepcidin expression in response to iron status is not clearly elucidated. Recent observations suggest that proteins of the transforming growth factor-β superfamily, such as transforming growth factor-β and bone morphogenetic proteins, mediate the hepatic expression of *HAMP* by way of a signal transduction pathway involving a class of proteins named SMAD (small mothers against decapentaplegic) [29,30]. It has been proposed that HFE, TfR2, and hemojuvelin (HJV) mediate the upregulation of *HAMP* gene transcription in response to the body iron status and that the impaired hepatic secretion of hepcidin resulting from mutations in either the *HAMP* gene itself or in *HFE*, *TFR2*, or *HJV* is the pathophysiological basis of increased intestinal absorption of iron in various forms of hemochromatosis [31].

HJV gene and hemojuvelin

Hemojuvelin is a 426-amino-acid protein coded by the *HJV* (previously named *HFE2*) gene located on chromosome 1q21 [32]. Like *HAMP*, *HJV* is expressed primarily in the liver, heart, and skeletal muscle [32]; mutations in either gene are associated with a phenotype of juvenile hemochromatosis [33]. The role of hemojuvelin in iron homeostasis has not yet been elucidated, but there is some evidence that it regulates the expression of hepcidin in response to changes in iron concentration [34]. Recent data suggest that the up-regulation of hepcidin expression by hemojuvelin is mediated by bone morphogenetic proteins [35].

TfR2 gene and transferrin receptor 2 (TfR2)

TfR2 is an 801-amino-acid protein encoded by the *TfR2* gene located on chromosome 7q22 [36]. TfR2 is structurally homologous to TfR1, and functions as a receptor for transferrin [36]. However, TfR2 is different from TfR1 in that its expression is mostly confined to the liver [36] and it is not regulated by cellular iron status [37,38]. Initial observations also suggested that unlike TfR1, TfR2 does not interact with HFE in vitro [39]. However, it was demonstrated that TfR2 does interact with HFE in cells, that mutations in *TfR2* and *HFE* do not interfere with this interaction, and that TfR2 competes with TfR1 for binding to HFE [40]. Based on these observations, the interaction between TfR2 and HFE has been proposed to be the molecular basis of a systemic iron sensor in hepatocytes. According to this model, transferrin-bound iron competes with HFE for binding to TfR1. In the presence of elevated transferrin-bound iron, HFE dissociates from TfR1 and binds to TfR2, leading to the formation of a signal transduction effector complex, which subsequently up-regulates the synthesis of hepcidin [40].

FPN1 gene and ferroportin

Ferroportin, a 571-amino-acid protein encoded by the *FPN1* (also known as *SLC40A1*) gene located on chromosome 2q32 [41], is the major iron export protein in enterocytes, macrophages, hepatocytes, and placental cells [18]. Impairment of the iron export function has been postulated to cause retention of iron within cells, especially in reticuloendothelial cells, which have a high iron flux, resulting in tissue iron accumulation (manifested as high serum ferritin) and decreased availability of iron for circulating transferrin (manifested as low serum transferrin–iron saturation; TS, given by serum iron divided by total iron-binding capacity) [42]. Increased intestinal absorption consequent to marginal anemia secondary to impaired delivery of iron to hematopoietic cells also contributes to iron loading in this condition [42]. A subset of patients with *FPN1* gene mutations has a phenotype similar to other types of hemochromatosis, with elevated TS, and parenchymal, rather than reticuloendothelial distribution of iron deposition [43,44]. This phenotype is postulated

to be the result of resistance of ferroportin to negative regulation by hepcidin [45].

Mutations associated with hereditary hemochromatosis

HFE gene mutations

Type 1 hemochromatosis, the most common form of hemochromatosis, is associated with mutations in the *HFE* gene and is inherited as an autosomal recessive trait. The three mutations most commonly associated with hemochromatosis are C282Y, H63D, and S65C. The overwhelming majority of cases with phenotypic *HFE*-associated hemochromatosis are accounted for by the homozygous C282Y mutation. A relatively small proportion of cases with phenotypic hemochromatosis are compound heterozygotes for the C282Y and H63D mutations. The clinical significance of compound heterozygozity of S65C with C282Y or H63D is not clear and is likely to be minimal; similarly, the H63D homozygozity may result in elevated serum iron markers but has not been associated with significantly increased body iron stores [46,47].

The C282Y mutation is primarily found in persons of northwestern European ancestry [2]. The prevalence of the C282Y homozygozity among white patients with phenotypic hemochromatosis varies from 60% in Italy, 70%–90% in France, and 80%–90% in the United States to more than 90% in Ireland and Australia [2]. It has been proposed that the C282Y mutation originated in Celtic or Nordic people, and the current distribution of the mutation reflects the proportion of Nordic or Celtic ancestry of these populations [48]. The H63D mutation has a more widespread distribution, with the allele frequency being > 5% in countries in the Mediterranean region, parts of the Middle East, and the Indian subcontinent [2,49].

The high prevalence of *HFE* mutations in different populations across the globe has prompted the hypothesis that *HFE* mutations may have conferred some selective survival advantage to carriers, possibly through protection from iron deficiency or infectious diseases [49]. In addition, the similar epidemiology of celiac disease and hemochromatosis has led to the hypothesis that *HFE* mutations may have compensated for deficient iron absorption in populations at risk for celiac disease [50].

Other mutations associated with hemochromatosis

Mutations in the *HJV* gene account for the majority of cases of type 2 hemochromatosis, whereas mutations in the *HAMP* gene are much less common [3,32]. Mutations in the *TfR2* gene responsible for type 3 hemochromatosis are extremely rare and are found mostly in southern Europeans and Japanese individuals [3].

Pathophysiology of iron-induced tissue injury

Oxidative stress generated by iron-stimulated free radical production is postulated to be the major mechanism of iron-mediated tissue injury. Proteins such as transferrin and ferritin, which bind free iron; enzymes such as superoxide dismutase, catalase, and glutathione peroxidases, which convert the reactive oxygen species to nontoxic by-products; and antioxidants such as glutathione, α-tocopherol, and β-carotene minimize the toxicity from iron under physiological conditions. However, these defense mechanisms are overwhelmed in conditions of iron overload, leading to free radical-mediated damage of cellular macromolecules. Oxidative stress is also thought to cause hepatic stellate cell activation, leading to fibrogenesis, and ultimately cirrhosis. In addition, iron-mediated immune dysfunction, DNA damage, and stimulation of cellular proliferation are considered to be responsible for hepatocarcinogenesis [51,52].

Clinical manifestations of iron overload

A comparative overview of types of hemochromatosis is given in Table 86.1 [11] and the various clinical manifestations are summarized in Table 86.2 [51].

Type 1 – classic hemochromatosis

The natural history of patients with *HFE*-associated hemochromatosis may progress through three stages with increasing iron accumulation over time. In the initial stage, body iron stores are normal or only mildly to moderately increased (total body iron stores less than 5 g) and there is no end-organ damage. In the second stage, there is moderate iron loading (body iron stores of 10–20 g) and patients may exhibit nonspecific symptoms such as fatigue, lassitude, or arthralgias or arthropathy, particularly involving the metacarpophalangeal joints. In the advanced stage (body iron stores of 20–40 g), cirrhosis is frequently present, often along with diabetes mellitus and hypogonadotropic hypogonadism.

The manifestations in patients identified on the basis of biochemical or genetic markers differ quantitatively from those diagnosed on the basis of classical clinical presentation [53]. The proportion of patients diagnosed with severe end-organ damage appears to have been decreasing over time, possibly because of increased awareness of the disease and earlier diagnosis [54,55]. Most patients (> 75%) with type I hemochromatosis are identified before the development of symptoms; cirrhosis, diabetes, *or* skin pigmentation is present in only a minority (< 25%) at the time of diagnosis and presence of all the three components of the classic triad is rare [56].

Table 86.1 Comparative overview of types of hemochromatosis

	***HFE*-related hemochromatosis**	**Juvenile hemochromatosis**		***TfR2*-related hemochromatosis**	**Ferroportin-related hemochromatosis**
Type	Type 1	Type 2, subtype A	Type 2, subtype B	Type 3	Type 4
Implicated gene and its chromosomal location	*HFE* 6p21.3	*HJV* 1q21	*HAMP* 19q13.1	*TfR2* 7q22	*FPN1* 2q32
Gene product	HFE	Hemojuvelin	Hepcidin	Transferrin receptor 2	Ferroportin
Pattern of inheritance	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal recessive	Autosomal dominant
Distribution of iron accumulation	Parenchymal	Parenchymal	Parenchymal	Parenchymal	Reticuloendothelial
Potential for organ damage	Intermediate	High	High	Intermediate	Low
Age of onset of symptomatic organ disease	4th or 5th decade	2nd or 3rd decade	2nd or 3rd decade	4th or 5th decade	4th or 5th decade
Response to phlebotomy	Excellent: decrease in serum ferritin parallels TS; low risk of anemia	Excellent: decrease in serum ferritin parallels TS; low risk of anemia	Excellent: decrease in serum ferritin parallels TS; low risk of anemia	Excellent: decrease in serum ferritin parallels TS; low risk of anemia	Fair: rapid decrease in TS with persistently high serum ferritin; high risk of anemia

TS, transferrin–iron saturatim. Adapted from Pietrangelo [11], with permission from the Massachusetts Medical Society.

The variable penetrance of the C282Y homozygozity resulting from environmental or genetic factors could also contribute to the clinical heterogeneity of hemochromatosis [53]. Male gender, a diet rich in iron, supplementation of iron or vitamin C, ingestion of alcohol in excess, and hepatitis C infection facilitate iron accumulation [57–60]. Conversely, the presence of chronic blood loss and a diet poor in iron could be protective. Iron loss associated with menstruation, pregnancy, and childbirth keeps penetrance lower in women. Celiac disease, which has similar epidemiology and is associated with HLA DQ2 located in close proximity to the *HFE* locus, has also been found to decrease the penetrance of the C282Y mutation [61]. Polymorphisms or mutations of other genes involved in iron homeostasis have also been hypothesized to modulate the penetrance of C282Y mutation [53].

The risk of decompensated cirrhosis, hepatocellular carcinoma, diabetes mellitus, and cardiomyopathy is 10-fold to 119-fold higher in patients with hemochromatosis than in an age- and sex-matched population without hemochromatosis [62]. Hepatocellular carcinoma is reported to develop in 7.5%–36% of patients with hemochromatosis, and is implicated in up to 45% of the deaths, with other complications of cirrhosis contributing another 20% [62–64]. Among patients with hemochromatosis undergoing liver transplantation, 27% has been found to have primary liver cancer (hepatocellular carcinoma or cholangiocarcinoma), out of which 70% are unsuspected before the time of surgery [64].

The risk factors for hepatocellular carcinoma in hemochromatosis are presence of cirrhosis, increased amount and duration of iron excess, advanced age, alcoholism, tobacco smoking, and concomitant infection with hepatitis C or hepatitis B [65–69]. Only a limited number of cases of hepatocellular carcinoma have been reported in patients with hemochromatosis but without cirrhosis [70]. *HFE* mutations have also been reported to be associated with extrahepatic cancers such as esophageal, gastric, colorectal, and hematological malignancies and melanoma [71–74], but this association has been questioned by others [75–77].

Type 2 – juvenile hemochromatosis

As in type 1 hemochromatosis, hypogonadism, cardiac disease, cirrhosis, diabetes, arthropathies, and skin pigmentation are seen in type 2 hemochromatosis. However, in type 2 hemochromatosis, iron overload is observed from an earlier age, the phenotypic expression is similar in men and women and extrahepatic involvement, especially endocrine and cardiac, are more severe than in type 1 hemochromatosis [78,79].

Table 86.2 Clinical features of hereditary hemochromatosis

Symptoms
Nonspecific, systemic symptoms
Weakness, fatigue, lethargy, apathy
Specific, organ-related symptoms
Abdominal pain secondary to hepatomegaly
Arthralgia
Amenorrhea
Loss of libido, impotence
Congestive heart failure, arrhythmias
Signs
Asymptomatic
Hepatomegaly
Symptomatic
Liver
Hepatomegaly
Cutaneous stigmata of chronic liver disease
Portal hypertension (splenomegaly, ascites, encephalopathy)
Joints
Arthritis
Heart
Dilated cardiomyopathy
Skin
Increased pigmentation
Endocrine
Testicular atrophy
Hypogonadism
Hypothyroidism

Adapted from Harrison and Bacon [51], with permission from the European Association for the Study of the Liver.

The most common age of presentation is early second decade of life, and hypogonadism, the most common manifestation, develops in nearly all patients [78,80]. Heart failure or cardiac arrhythmias, reported to occur in 69%, dominate the clinical course of the disease and are the most common causes of death, which is common before the age of 30 years [80,81].

Type 3 – *TfR2*-related hemochromatosis

Type 3 hemochromatosis is phenotypically similar to type 1 hemochromatosis with the exception that the severity of iron overload and the age of clinical presentation are intermediate between type 1 hemochromatosis and type 2 hemochromatosis [82].

Type 4 – ferroportin-related iron overload

In contrast to other types of hemochromatosis where elevation of serum transferrin iron saturation precedes elevation of serum ferritin, type 4 hemochromatosis is characterized by elevated serum ferritin from the first decade of life, with elevation in TS developing in the third to fourth decades [83,84]. Another distinct feature of type 4 hemochromatosis is mild anemia, which may be aggravated on phlebotomy [83]. Hepatic involvement is characterized by iron deposition predominantly in reticuloendothelial cells with varying degrees of sinusoidal and portal fibrosis, which rarely progress to cirrhosis [42].

Other iron overload states

In hematological diseases like sideroblastic anemia, hemolytic anemias, and myelodysplastic syndromes, iron overload is the result of increased intestinal absorption secondary to increased demand for iron in the bone marrow consequent to ineffective erythropoiesis [85]. Blood transfusions could contribute to iron overload in these conditions.

Iron overload is frequently observed in chronic liver diseases, particularly in alcoholic liver disease, in the absence of mutations associated with hemochromatosis [86–89]. Stainable liver iron has been found in 31%, increased liver iron concentration in 19%, and liver iron concentration in the hemochromatosis range in 7% of patients with chronic liver disease [90]. Consumption of any amount of alcohol has been shown to decrease the risk of iron deficiency, and consumption of more than two alcoholic drinks per day increases the risk of iron overload [91]. The potential mechanisms of iron overload in association with alcohol intake include increased ingestion of iron (African iron overload), increased intestinal absorption, up-regulation of hepatic transferrin receptors, down-regulation of hepatic hepcidin expression [92], secondary anemia as the result of hemolysis or hypersplenism, ineffective erythropoiesis, hypoxemia as the result of intrapulmonary shunts or ventilation/perfusion mismatch, and spontaneous or surgically constructed portosystemic shunts [93]. Nonalcoholic steatohepatitis has also been reported to be associated with iron overload in up to 58% of patients [94]. The prevalence of *HFE* mutations is higher in these patients, and increased hepatic iron concentration has been found in some studies to be associated with increased fibrosis [95,96]. Chronic hepatitis C is also associated with iron overload, and iron overload in this condition has been found to be linked to more advanced hepatic fibrosis, hepatocellular carcinoma, and reduced response to interferon monotherapy [59,97].

African iron overload has historically been attributed solely to excessive intake of iron derived from traditional beer brewed in iron drums and cans, but has been found to be associated with ferroportin mutations and other inherited and environmental factors such as *HFE* C282Y, heritable anemias, alcohol consumption, and chronic viral hepatitis [98,99].

Neonatal hemochromatosis is a rare syndrome of perinatal hepatic failure associated with increased tissue iron deposits in liver, pancreas, heart, and endocrine glands, with sparing of macrophages. It leads to death in utero or in the early neonatal period [100,101].

Hereditary aceruloplasminemia is a rare autosomal recessive disorder characterized by complete lack of ceruloplasmin ferroxidase activity caused by mutations in the ceruloplasmin gene, resulting in impaired export of iron from cells [102,103]. This disease presents in adulthood with hepatic iron overload, diabetes, peripheral retinal degeneration, dystonia, dysarthria, and dementia. Laboratory findings include mild microcytic anemia, low TS, elevated serum ferritin, magnetic resonance imaging findings suggestive of iron accumulation in the liver and basal ganglia, thalamus, and dentate nucleus of brain, and complete absence of serum ceruloplasmin ferroxidase activity [102,103].

Hereditary atransferrinemia is a rare disorder characterized by iron overload and severe hypochromic anemia, where delivery of iron to the marrow is impaired because of deficiency of transferrin [104].

Diagnosis

Appropriate and early diagnosis of hemochromatosis requires knowledge of the protean manifestations of iron overload, the frequent absence of the classic symptoms of "bronze diabetes," and the vague and nonspecific nature of the symptoms. As both screening tests and confirmatory tests are widely available, the most important factor in making the diagnosis of hemochromatosis is the awareness of clinicians about the possibility of iron overload in at-risk individuals [105,106]. Hemochromatosis should be suspected in all adult patients (especially those in middle age), presenting with fatigue, malaise, arthralgia, hepatomegaly, or elevated serum aminotransferase levels. Children or adolescents with hypogonadotropic hypogonadism or unexplained heart failure should be evaluated for possible juvenile hemochromatosis. A suggested algorithm for the evaluation of suspected hemochromatosis is shown in Fig. 86.2 [107].

Biochemical tests of iron overload

The first tests to be done in those with suspicious symptoms, signs, or liver test abnormalities should be TS and serum ferritin [105]. Together, these tests have a negative predictive value of 97% [108]. No further evaluation is required for hemochromatosis if both these tests are normal [105].

The TS should be the initial screening test because it is the earliest phenotypic abnormality in hemochromatosis [56]. The recommended threshold TS for further evaluation of hemochromatosis is 45%, which has a reported sensitivity ranging from 64% to 98% for detection of C282Y homozygozity [105,109,110]. However, TS may be increased as the result of excessive alcohol consumption [91], inflammatory conditions [105], and other causes of chronic liver disease [88,89] or iron overload. The unsaturated iron-binding capacity, the inverse of the TS, which has similar performance characteristics but is less expensive to perform because

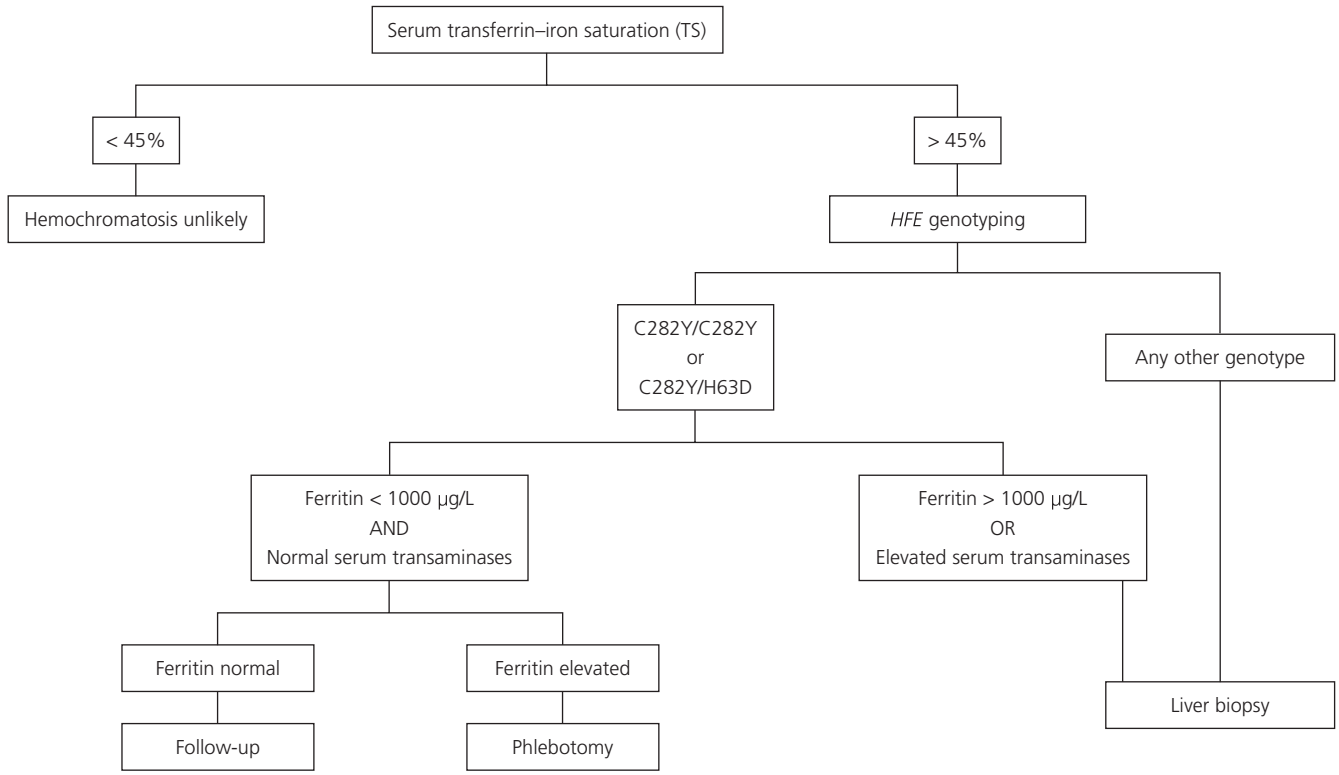

Figure 86.2 Algorithm for management of hemochromatosis. From Alexander and Kowdley [107], with permission from the Annals of Hepatology.

it is a one-step test, could be an alternative to TS for large-scale screening or as part of an automated battery of tests [89].

As TS in fasting samples could be as much as 50% lower than in random blood samples, the test should ideally be performed when patients are fasting and have not taken vitamins or iron supplements for 24 h [111]. The American Association for the Study of Liver Diseases Practice Guideline recommends a random sample for the initial screening test, and a fasting sample for confirmation if the initial value is more than 45% [105]. Serum ferritin is usually measured with the repeat fasting sample, but it can be elevated in inflammatory disorders such as rheumatoid arthritis and in various neoplastic diseases such as lymphoma; it can also be elevated in up to 50% of patients with chronic liver disease in the absence of hemochromatosis [56,91]. Serum ferritin also increases with age, and is higher among men [112]. Therefore, an elevated serum ferritin is much more likely to be associated with iron overload in the presence of an elevated serum TS than if the TS is normal; a notable exception being ferroportin disease, as discussed previously.

Genotyping

Patients with elevated serum TS and ferritin should undergo genotyping for detection of the *HFE* mutations C282Y and H63D [105]. C282Y homozygotes and C282Y/H63D compound heterozygotes do not require any further evaluation for diagnosis of hemochromatosis. Since the majority of white subjects with phenotypic hemochromatosis have one of these two mutation patterns, *HFE* genotyping is sufficient to establish the diagnosis in the vast majority of cases. Liver biopsy is no longer necessary for diagnostic purposes alone among patients with *HFE*-associated hemochromatosis but may be useful to determine the presence of fibrosis, as discussed below. Gene testing for mutations in other iron-related genes is not available.

Liver biopsy

The following are the potential roles of liver biopsy in hemochromatosis.

- Liver biopsy can be used to confirm the diagnosis of hemochromatosis if genotyping is not available or does not reveal C282Y homozygozity or C282Y/H63D compound heterozygozity. Findings diagnostic of hemochromatosis (except type 4 hemochromatosis) include [113]
  - grade 4 stainable iron in hepatocytes, with periportal distribution, and sparing of Kupffer cells
  - hepatic iron concentration (HIC) greater than 80 μmol (4500 mg)/g dry weight
  - hepatic iron index (HII; which is given by [HIC in μmol/g]/age in years) greater than 1.9
- Liver biopsy is useful to exclude coexistent or alternative causes of liver disease
- In patients with a confirmed diagnosis of hemochromatosis, liver biopsy is needed to determine the presence or absence of cirrhosis, which is the single most important adverse prognostic factor [66].

Therefore, liver biopsy is recommended in patients with an increased likelihood of cirrhosis. Elevated serum aminotransferases and a serum ferritin greater than 1000 μg/L at the time of diagnosis are independent predictors of cirrhosis [114] and the combination of absence of hepatomegaly, normal serum aspartate aminotransferase levels, and serum ferritin greater than 1000 μg/L is predictive of absence of severe fibrosis [115]. Therefore, liver biopsy is required in C282Y homozygotes and C282Y/H63D heterozygotes only if serum ferritin is over 1000 μg/L, or if serum transaminases are elevated (Fig. 86.2).

Diagnosis of juvenile hemochromatosis

The diagnosis of juvenile hemochromatosis is usually made in the right clinical context: presentation below the age of 30 years (at least in one affected member of the family) with iron overload in the absence of both causal HFE mutations and causes of secondary iron overload [78].

Screening

Several large-scale screening studies have suggested that a large proportion of C282Y homozygotes have elevated TS and serum ferritin, particularly among males [55,116–118]. Among white subjects, various studies have reported a population prevalence of 0.4%–0.5% for C282Y homozygozity, with 75%–86% of males and 40%–100% of females having elevated TS, and 71%–88% of males and 33%–57% of females having elevated serum ferritin [57,116,119]. Despite the high prevalence and the high penetrance as manifested by the proportion of individuals with elevated TS and serum ferritin, morbidity associated with C282Y homozygozity has been argued to be relatively low [54,120]. However, hepatic fibrosis and cirrhosis have been documented in 6%–25% of C282Y homozygotes indicating that a substantial number of individuals with the mutation are at risk of developing long-term complications [116,117,120]. Population surveillance for hemochromatosis using *HFE* gene testing is not recommended because of these conflicting data about the potential benefits of identifying asymptomatic individuals and concerns about possible negative social, legal, and insurance consequences for the identified individual with homozygous *HFE* mutations [111]. The systematic review by the US Preventive Services Task Force evaluated these risks and benefits and concluded that data are inadequate to allow a decision on the benefit of genotypic screening of general population for hemochromatosis [119]. Genotypic screening with mutational analysis is also expensive when applied to the general population and is recommended only in first-degree relatives of confirmed cases of hemochromatosis and in individuals with elevated serum iron markers (in addition

to those with unexplained liver function abnormalities or symptoms as mentioned under the heading "Diagnosis") [106,121,122].

A recent systematic review by the American College of Physicians observed that the association between phenotypic markers of iron overload such as serum ferritin and TS and the development of iron-related complications is inconsistent [123]. However, phenotypic screening with TS has been found to be a cost-effective strategy for screening, even if as few as 20% of the identified patients develop symptomatic disease [121,122]. Therefore, phenotypic screening with TS may be appropriate for use in individuals as part of routine health maintenance [106]. Furthermore, the availability of an inexpensive, safe and effective treatment in the form of phlebotomy and the fact that at least some of the patients with elevated TS would develop the complications of iron overload support population screening with TS [124,125]. Age between 18 and 30 years has been suggested as the ideal time for such phenotypic screening because it has to be done after TS is elevated, but before significant organ damage has occurred [126].

Treatment and prognosis

Phlebotomy

Phlebotomy is the safest, most effective, and most economical therapeutic modality for iron depletion, and should be initiated in all individuals with significantly increased body iron stores based on serum ferritin or hepatic iron content [11].

One unit of blood (containing approximately 250 mg iron) should be removed once or twice a week until the serum ferritin level is below 50 ng/mL. This might take up to 2 or 3 years. Once the serum ferritin reaches the target level, the goal of phlebotomy treatment is to maintain the serum ferritin below 50 ng/mL. The required frequency of maintenance phlebotomy is highly variable, usually ranging from once in 3–4 months to once every month, reflecting the individual variability in iron absorption [106].

Certain clinical features, such as malaise, fatigue, skin pigmentation, insulin requirements (in patients with diabetes), and abdominal pain, improve with phlebotomy, whereas arthropathy, hypogonadism, and cirrhosis generally do not respond [106]. Life expectancy in patients with hemochromatosis is normal if phlebotomy is initiated in the precirrhotic stage; however, once cirrhosis has developed, life expectancy is shortened and there remains an increased risk of hepatocellular carcinoma, even with iron depletion. Vitamin C should be avoided by patients undergoing phlebotomy [106].

Liver transplantation

Liver transplantation should be considered in patients with hepatic decompensation or hepatocellular carcinoma [106]; however, survival after liver transplantation is decreased in hemochromatosis compared with other chronic liver diseases [64,127–130]. Decreased survival in hemochromatosis has been attributed to increased serious infections, especially fungal infections, which are more common in patients with hepatic iron overload, and are considered to be secondary to the immunological effects of iron overload [131,132]. However, a recent study of a large cohort of patients in the National Hemochromatosis Transplant Registry found that patients with *HFE*-associated hemochromatosis have even worse posttransplant outcomes than those with hepatic iron overload because of other causes, suggesting that factors other than iron overload might also have a role in the adverse outcome [127].

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G LIVER

87 Metabolic diseases of the liver

Ronald J. Sokol

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Inborn errors of metabolism commonly involve the liver. In these disorders, mutations in genes that regulate a single enzyme within an important metabolic pathway or that code for essential transport proteins result in significant blocks in the metabolism or transport of the substrate. Either a deficiency of an important product of the enzyme or the accumulation of toxic precursors leads to the disease phenotype. In many hepatic metabolic diseases, environmental exposure to a specific substrate (galactose, fructose, copper, protein load) is an essential cofactor that modifies the time of onset and severity of the disease phenotype. As new genetic causes of many diseases are identified, many prior cryptogenic acute and chronic liver diseases have been attributed to specific metabolic defects, and there are certainly many more to be discovered. It has become apparent that modifier genes most certainly play a major role in determining the clinical phenotype, severity of disease, and onset of illness for many metabolic and genetic liver diseases that were previously thought to be caused by single gene mutations.

Metabolic liver disease may present in a variety of clinical phenotypes, including the appearance of acute hepatitis, bacterial sepsis, metabolic coma, mimicking ingestion of a toxin or drug overdose, or as end-stage liver disease with portal hypertension. A high index of suspicion is necessary in order to perform the correct metabolic testing and establish the diagnosis of a metabolic disorder. Clinical features that suggest a metabolic liver disease include hepatic steatosis, acute fulminant liver failure, presence of neuromuscular or cardiac findings, hypoglycemia, hyperammonemia, organic acidemia, hepatic synthetic functional failure, mildly elevated aminotransferases, episodes of recurrent vomiting or severe gastroesophageal reflux, developmental delays, failure to thrive, chronic diarrhea, cataracts, abnormalities of skin or hair, and a family history of similarly affected infants or children, or of consanguinity. Most metabolic liver diseases are inherited in an autosomal recessive manner; however, several segregate into autosomal dominant and X-linked inheritance patterns. Diseases with maternal inheritance (mitochondrial DNA diseases) also commonly involve the liver.

Childhood metabolic liver diseases present clinically in three patterns. The neonatal cholestasis syndrome (Table 87.1) is a common presentation that requires rapid diagnostic evaluation so that effective therapy can be instituted to avert permanent injury to the liver or other organs. A second presentation is that of chronic liver disease with progressive hepatic fibrosis, frequently with accompanying steatosis, culminating in cirrhosis and portal hypertension. Finally, a *metabolic syndrome* may include hypoglycemia, metabolic acidosis, encephalopathy, variable ketonemia or lactic acidosis, or hyperammonemia. A complete categorization of all metabolic liver diseases affecting children and adults is beyond the scope of this chapter. The reader is referred to recent textbooks on liver disease in children for extensive review of this topic [1]. This chapter focuses on six major areas of metabolic disease: Wilson disease, α_1-antitrypsin deficiency, Reye syndrome, porphyria, a group of important metabolic liver diseases presenting in early childhood, and mitochondrial hepatopathies.

Wilson disease

Clinical features

Mutations in the *ATP7B* gene cause Wilson disease, a progressive accumulation of copper in the liver accompanied by copper deposition in other organs that generate its clinical features. Clinical symptoms are rarely apparent prior to 4–5 years of age [2]. Asymptomatic elevation of aminotransferases occurs in the first decade of life in most patients: the youngest reported patient presenting with hepatic clinical symptoms was 4 years old [3]. After the storage capacity of the liver is exceeded, circulating non-ceruloplasmin-bound copper levels increase, distributing the excess copper to other target organs that produce the clinical manifestations of

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Table 87.1 Metabolic and genetic causes of neonatal cholestasis

Disease	Inborn error	Hepatic pathology	Diagnostic studies
Galactosemia	Galactose 1-phosphate uridyltransferase	Cholestasis, steatosis, necrosis, pseudoacini, fibrosis	Galactose 1-phosphate uridyltransferase assay of red blood cells
Fructose intolerance	Fructose 1-phosphate aldolase	Steatosis, necrosis, pseudoacini, fibrosis	Liver fructose 1-phosphate aldolase assay or leukocyte DNA analysis
Tyrosinemia	Fumarylacetoacetase	Necrosis, steatosis, pseudoacini, portal fibrosis	Urinary succinylacetone, funarylacetoacetase assay of red blood cells
Cystic fibrosis	Cystic fibrosis transmembrane regulator gene mutations	Cholestasis, neoductular proliferation, excess bile duct mucus, portal fibrosis	Sweat test and leukocyte molecular analysis
Hypopituitarism	Deficient production of pituitary hormones	Cholestasis, giant cells	Thyroxine, TSH, cortisol levels
α_1-Antitrypsin deficiency	Abnormal α_1-antitrypsin molecule (PiZZ or SZ phenotype)	Giant cells, cholestasis, steatosis, neoductular proliferation, fibrosis, PAS- and diastase-resistant cytoplasmic granules	Serum α_1-antitrypsin phenotype or genotype
Gaucher disease	β-Glucosidase	Cholestasis, cytoplasmic inclusions in Kupffer cells (foam cells)	β-Glucosidase assay in leukocytes
Niemann–Pick disease	Lysosomal sphingomyelinase	Cholestasis, cytoplasmic inclusions in Kupffer cells	Sphingomyelinase assay of leukocytes or liver, molecular analysis
Glycogen storage disease type IV	Branching enzyme	Fibrosis, cirrhosis, PAS- and diastase-resistant cytoplasmic inclusions	Brancher enzyme analysis of leukocytes or liver, molecular analysis
Neonatal hemochromatosis	Unknown	Giant cells, portal fibrosis, hemosiderosis, cirrhosis	Histology, iron stains
Peroxisomal disorders (e.g., Zellweger syndrome)	Deficient peroxisomal enzymes or assembly	Cholestasis, necrosis, fibrosis, cirrhosis, hemosiderosis	Plasma very long-chain fatty acids, qualitative bile acids, plasmalogen, pipecolic acid, liver electron microscopy
Abnormalities in bile acid metabolism	Several enzyme deficiencies defined	Cholestasis, necrosis, giant cells	Urine, serum, duodenal fluid analyzed for bile acids by fast atom bombardment–mass spectroscopy, molecular analysis
Progressive familial intrahepatic cholestasis (PFIC)	*FIC-1*, *BSEP*, and *MDR3* gene mutations for PFIC 1, 2 and 3, respectively	Cholestasis, necrosis, giant cells, fibrosis	Histology, family history, normal cholesterol, low or normal γ-glutamyltransferase (types 1 and 2), molecular analysis
Alagille syndrome (syndromic paucity of interlobular bile ducts)	*Jagged 1* gene mutations	Cholestasis, paucity of interlobular bile ducts, increased copper levels	Three or more clinical features, liver histology, molecular analysis

BSEP, bile salt export pump; FIC1, familial intrahepatic cholestasis 1; MDR3, multiple drug resistance 3; PAS, periodic acid–Schiff; TSH, thyroid-stimulating hormone.

Wilson disease. This redistribution of copper deposition is best illustrated by the organ involvement at presentation. Of those diagnosed before 10 years of age, the majority (80%) present with hepatic symptomatology with only 17% showing neuropsychiatric manifestations. In contrast, between 10 and 18 years of age approximately 50% present with either hepatic or neuropsychiatric symptoms, and after 18 years of age 25% present with hepatic and 75% neuropsychiatric symptoms [4]. After hepatic saturation with copper, copper may also accumulate in the ocular cornea, leading to the characteristic Kayser–Fleischer (KF) ring, a greenish-brown pigment at the periphery of the cornea on its posterior surface in the membrane of Descemet [5]. Less commonly, a *sunflower cataract* is caused by deposits of copper in the anterior and posterior lens capsule. Other features include a variety of renal disturbances including proteinuria, glucosuria,

phosphaturia, generalized aminoaciduria, microscopic hematuria, and nephrolithiasis [6]. Acute renal failure is a common feature of the fulminant presentation of Wilson disease. Intravascular hemolysis is present in approximately 15% of patients. Cardiac involvement includes echocardiographic abnormalities in up to one-third of adults [7], as well as arrhythmias, orthostatic hypotension, and histological changes in the heart [7]. Skeletal manifestations include bone demineralization, rickets, spontaneous fractures, osteochondritis desiccans, chondromalacia patellae, and degenerative arthritis [8]. Skin pigmentation may be increased, particularly on the anterior aspect of the lower legs [9]. Endocrine abnormalities have also been reported [10,11].

The liver is both the site of the Wilson disease biochemical defect in copper metabolism and the initial target of copper toxicity. Clinical presentations include acute hepatitis, fulminant hepatic failure, chronic active hepatitis, and cirrhosis. Occasionally, elevated aminotransferase levels are detected in asymptomatic patients for whom a chemistry panel is drawn, or in siblings of a patient with Wilson disease. Any patient under 50–60 years of age with chronically elevated aminotransferase levels and no clear etiology should have Wilson disease excluded by appropriate studies.

Acute hepatitis is the presentation in approximately 25% of patients with Wilson disease, with typical findings that mimic the features of infectious hepatitis. Laboratory tests show typically elevated aminotransferases, normal serum albumin and prothrombin time, with conjugated hyperbilirubinemia. If serological testing for viral hepatitis is negative, Wilson disease needs to be considered. A mild hemolytic anemia or a depressed serum uric acid level (due to renal tubular losses) may be key features to alert the clinician to this diagnosis. If not identified, patients generally recover from this presentation and the diagnosis may be missed.

Fulminant hepatic failure is a dramatic presentation of Wilson disease. It typically occurs in the adolescent or young adult who develops an acute icteric hepatitis that rapidly evolves to liver failure within days, with extreme jaundice, fatigue, hepatic coma, severe coagulopathy, ascites, renal failure, and invariably death, if liver transplantation is not performed. A Coombs-negative hemolytic anemia is caused by the rapid release of massive amounts of copper from the necrosing liver, and is the single most important diagnostic feature suggesting Wilson disease in this clinical setting. Acute renal failure rapidly ensues usually requiring dialysis. Interestingly, serum aminotransferase levels are not as high as in other patients with acute liver failure, alkaline phosphatase is generally very low, and serum aspartate aminotransferase (AST) is significantly more elevated than alanine aminotransferase (ALT) [12]. Serum copper levels are paradoxically elevated above normal in this presentation of Wilson disease, because of the massive release of copper from the liver. Because KF rings are present in no more than half of patients with this presentation [4], their absence does not exclude the presence of Wilson disease. This presentation is not amenable to medical therapy, so prompt referral of patients with this presentation to a liver transplantation center is essential in order to save the patient's life. A similar presentation of acute fulminant liver failure and death has been described in Wilson disease patients who had been successfully treated with copper chelation therapy, but who unadvisedly discontinued therapy or became noncompliant for as little as 8 months.

Approximately 30% of patients have a presentation of chronic hepatitis, generally occurring during adolescence or young adulthood. Cirrhosis is common at the time of diagnosis. Typical findings of malaise, anorexia, fatigue, abdominal pain, nausea, and weight loss may precede the onset of jaundice. Amenorrhea, delayed onset of puberty, edema, polyarthralgia, ascites, and clubbing may also indicate the chronic nature of this hepatitis. Physical examination invariably reveals hepatomegaly and usually splenomegaly. Liver histology mimics autoimmune or chronic viral hepatitis, and thus a high suspicion for Wilson disease is essential. It is important to note that histochemical stains for copper-associated proteins in the liver may be negative, and should never be used as the diagnostic criteria for copper overload. KF rings are present only 50% of the time. Thus, it is imperative that biochemical screening for Wilson disease be undertaken in all patients younger than 50–60 years who present with chronic hepatitis and no identifiable cause. The presence of steatosis is common in Wilson disease and can lead to confusion with alcoholic hepatitis and nonalcoholic fatty liver disease.

Cirrhosis and its complications are relatively common features of advanced Wilson disease. Virtually all adults with the neuropsychiatric presentation of Wilson disease have underlying compensated cirrhosis. Other than the findings of a mild hemolytic anemia, a KF ring, positive family history, or neuropsychiatric symptoms, there is little to clinically distinguish this cause of cirrhosis from others.

Cholelithiasis is relatively common in adolescents and young adults with Wilson disease because of ongoing hemolysis and the presence of cirrhosis. Interestingly, hepatocellular carcinoma has been quite unusual in patients with Wilson disease, even those with advanced cirrhosis.

Neuropsychiatric presentation

Neurological or psychiatric symptoms are the first indication of illness in approximately 40–50% of patients with Wilson disease, having been recorded in children as young as 6 years and in adults as old as 72 years of age [13]. Primary involvement is the motor system with extrapyramidal or cerebellar dysfunction [14,15]. Symptoms segregate into pseudoparkinsonism, pseudosclerosis, and dyskinesia [4], each of which correlates with specific magnetic resonance imaging (MRI) lesions in the brain [16]. Extrapyramidal symptoms include facial grimaces, drooling, a fixed grin, dysphasia, and

contractures of the jaw and extremities. Features of pseudoparkinsonism include bradykinesia, cognitive impairment, cogwheel rigidity, and an organic mood syndrome. The pseudosclerosis presentation is characterized by ataxia, tremor, and reduced functional capacity. Because cognitive function is preserved, frustration and depression are common. The neurological basis for these abnormalities is dysfunction of the basal ganglia and cerebellum, although corticospinal and corticobulbar pathways are also affected.

It is well appreciated that a primary psychiatric presentation may include organic dementia, neurotic behavior, bipolar disorder, schizophrenic psychosis, or antisocial behavior. In children and adolescents, a major change in affect or personality may impair socialization skills and school performance. Psychiatrists must measure plasma ceruloplasmin and search for a KF ring in all young patients with psychiatric disorders. KF rings are present in over 90% of patients with the neuropsychiatric presentation of Wilson disease.

Histology of the liver

Early histological findings in the liver of asymptomatic children with Wilson disease include periportal, glycogen-filled, swollen nuclei [17] and microvesicular and macrovesicular steatosis [18]. Macrovesicular steatosis may be confused with alcoholic liver disease or nonalcoholic fatty liver disease. A chronic hepatitis appearance may predominate, characterized by portal tract mononuclear cell infiltrates, interface hepatitis, Mallory bodies, and varying amounts of periportal fibrosis. Eventually, mixed micronodular and macronodular cirrhosis develops. In the fulminant hepatitis presentation, liver histology is characterized by microvesicular fat, coagulative cell necrosis, pigment-laden Kupffer cells, collapse of stroma, and dropout of hepatocytes, with underlying well-established cirrhosis. Histochemical stains for copper or copper-associated proteins may be negative in Wilson disease despite massive accumulation of copper in the liver, and may be positive in other causes of cholestasis and chronic hepatitis. There is no correlation between histochemical staining of copper and quantitative copper measurements of liver biopsy samples in Wilson disease. Therefore, these histochemical stains should *not* be used to establish or exclude this diagnosis.

Electron microscopy may be helpful in Wilson disease because of characteristic ultrastructural lesions. In the early stage of Wilson disease, hepatocellular mitochondria are pleomorphic and large, and show widened intracristal spaces, increased matrix density, large granules, and sometimes crystalline, vacuolated or dense inclusions in the mitochondrial matrix. There is usually microvesicular steatosis present at the same time. These mitochondrial changes diminish with progression of the lesion toward cirrhosis.

Genetics

The *ATP7B* gene, located on the long arm of chromosome 13, was identified in 1993 as the gene mutated in Wilson disease [19–22]. By 2007, over 260 mutations in this gene had been identified from patients with Wilson disease, most being small deletions or missense mutations [23,24]. In addition, over 40 non-disease-causing allelic variations have been identified. There are no predominant mutations, although specific mutations appear to be more common among certain ethnic groups [23]. Thus, the most common mutation (His1069Glu) may be present in as many as 40% of cases in specific ethnic populations. Genotyping of suspected patients is now offered in a number of clinical and research laboratories and may be helpful in questionable cases (see http://www.genetests.org). Haplotype analysis (microsatellite markers or single nucleotide polymorphisms) may be particularly helpful in evaluating relatives of a known case in which markers are informative [25].

Pathogenesis and biochemistry

The *ATP7B* gene is highly expressed in the liver and kidney, with lower expression in the lung, placenta and other tissues. The encoded protein is a P-type cation-transporting ATPase, and includes domains for copper-binding sites and ATP binding. ATP7B is located in the trans-Golgi compartment of the hepatocyte and is essential for the transport of copper into vesicles bound for lysosomes and targeted to the bile canaliculus. ATP7B also makes copper available for synthesis of ceruloplasmin. In Wilson disease, *ATP7B* mutations cause impairment of both copper secretion into bile and copper incorporation into ceruloplasmin, resulting in toxic levels of copper within the hepatocyte. Ceruloplasmin is the primary carrier by which copper is secreted from the liver into the systemic circulation. Ceruloplasmin functions to mobilize iron from tissues by oxidizing ferrous iron for transfer into transferrin. Although ceruloplasmin levels in plasma are very low in Wilson disease, the ceruloplasmin gene is normal.

More than 80% of copper absorbed from the diet is normally excreted in bile. Therefore, copper accumulates in the liver in any condition that causes cholestasis; however it is primarily lysosomal-bound in these conditions and may not necessarily be hepatotoxic. In Wilson disease, the copper appears to be free to enter other compartments, such as the mitochondria and nucleus. Although the precise intracellular target for the toxic action of copper is not known, results have suggested that mitochondrial oxidative injury initiated by copper may be one of the primary pathogenetic events in Wilson disease [26–28]. Several studies have also demonstrated that hepatocyte apoptosis may be the primary mode of cell death in copper toxicity [29,30].

Diagnosis

The diagnostic criteria for Wilson disease vary with the clinical presentation [31]. In patients presenting with neurological or psychiatric symptoms, the absence of a KF ring in combination with a normal ceruloplasmin level virtually exclude the diagnosis of Wilson disease. However, in a series

Table 87.2 Diagnostic tests for Wilson disease

Diagnostic test	Diagnostic values	Causes of false positive	Causes of false negative
Serum ceruloplasmin	< 20 mg/dL	Kwashiorkor, nutritional copper deficiency, protein-losing state, fulminant hepatitis, hepatic failure, hereditary hypoceruloplasminemia or aceruloplasminemia, Wilson disease heterozygote, Menkes syndrome, normal neonate	Acute inflammation (hepatitis), malignancy, pregnancy or estrogen therapy in Wilson disease (5% of patients), immunoassays of apo-ceruloplasmin
Hepatic copper concentration	> 250 μg/g dry weight	Primary biliary cirrhosis, Indian childhood cirrhosis, chronic cholestatic liver disease, primary sclerosing cholangitis, Alagille syndrome, liver tumors, newborn liver	Copper chelation therapy in Wilson disease
24-Hour urine copper excretion	> 100 μg/24 h	Copper chelation therapy, chronic active hepatitis, chronic cholestatic liver diseases, primary sclerosing cholangitis, hepatic failure, nephrotic syndrome	Copper chelation therapy in Wilson disease
Presence of Kayser–Fleischer rings	Present	Chronic cholestatic liver diseases, primary biliary cirrhosis, neonatal cholestasis	Early Wilson disease
Incorporation of ^{64}Cu into ceruloplasmin	Low	Ceruloplasmin < 20 mg/dL, Wilson disease heterozygote	Pregnancy, estrogens, inflammation or malignancy in Wilson disease

Adapted from Scheinberg & Sternlieb [2], with permission.

of 20 patients with neurological symptoms, two did not have KF rings [32]. In the presence of "normal" neurological or psychiatric function, the absence of a KF ring does not exclude Wilson disease, as it is present in only 50% of these patients. The common biochemical tests used for diagnosis of Wilson disease are plasma ceruloplasmin, hepatic copper concentration, and 24-h urine copper excretion (basal and after penicillamine challenge) (Table 87.2). For patients presenting with liver dysfunction, ceruloplasmin is generally less than 20 mg/dL, 24-h urine copper is above 100 μg, and hepatic copper concentration is greater than 250 μg/g dry weight. Other conditions that can give rise to increased hepatic copper concentration (Table 87.2) must be excluded with appropriate testing. Two studies [33,34] demonstrated improved discrimination between Wilson disease and other liver disorders when 24-h urine copper was measured after a penicillamine challenge (500 mg given orally immediately before and repeated 12 h into the urine collection). Values above 1575 μg of copper in this 24-h urine collection indicated Wilson disease. Quantitative hepatic copper determination is essential, with normal copper content below 50 μg/g dry weight [35] but virtually always above 250 μg/g dry weight in Wilson disease [2], if not considerably higher. It should be stressed that liver biopsy samples must be obtained in copper-free stainless steel needles to prevent contamination.

Unfortunately, the assays used to analyze serum for ceruloplasmin have changed over recent years from chemical to immunological techniques. Apo-ceruloplasmin is recognized by these assays, resulting in higher values than those previously measured by oxidase techniques [31]. Therefore, a ceruloplasmin level between 20 and 30 mg/dL cannot be used on its own to definitively exclude Wilson disease. If there is a high suspicion of Wilson disease, the 24-h urine copper excretion, slit-lamp examination, and perhaps liver biopsy or genetic testing should be performed despite a low normal level of ceruloplasmin. If a sibling has Wilson disease, genetic diagnosis or microsatellite (haplotype) analysis is helpful for diagnosing Wilson disease. Incorporation of copper 64 (^{64}Cu) into ceruloplasmin is no longer an available test. Finally, genotyping of *ATP7B* (see http://www.genetests.org for available laboratories) may be useful if diagnosis cannot be definitely established using biochemical and clinical criteria [36].

In patients with fulminant hepatic failure, specimens to establish the diagnosis of Wilson disease may be difficult to obtain. Standard liver biopsy may be contraindicated because of coagulopathy, and the patient may be in acute renal failure so collection of a 24-h urine specimen for copper analysis may not be possible. However, the diagnostic features of a severe hemolytic anemia, extremely high serum bilirubin, acute oliguric renal failure, low serum alkaline phosphatase, elevated serum copper values, and inappropriately mild elevations of aminotransferase levels are very suggestive of this diagnosis. If transjugular liver biopsy is performed, quantitative copper should be obtained. Because these patients will either succumb to their illness or undergo a liver transplant, the explanted or autopsied liver should be evaluated for histology and for copper content to confirm this diagnosis.

Treatment

Wilson disease is uniformly fatal without effective therapy. Therefore the hallmark of medical management is early

diagnosis followed by reduction (or chelation) of the accumulated burden of copper and prevention of further retention of copper [31]. For symptomatic patients, most authorities recommend copper chelating agents and a low-copper diet. For maintenance therapy, copper chelators or oral zinc therapy may be effective.

When the diagnosis of Wilson disease is established in a symptomatic patient, treatment should begin with either triethylenetetramine dihydrochloride (trientine) or penicillamine [31]. In recent years it has become evident that trientine is better tolerated in most patients than penicillamine. The dose of trientine in adults and adolescents is approximately 1–2 g daily in three divided doses 1 h prior to meals. The dose of penicillamine is also 1–2 g per day in three divided doses, given at least 30 min before or 2 h after meals [2]. In young children, the dose for each of these drugs is approximately 20 mg/kg daily rounded to the nearest multiple of 250 mg given in divided doses over 24 h. After stabilization, the dose of trientine or penicillamine can be divided into two daily doses, and reduced to approximately 1 g/day in adults. Penicillamine may have an antipyridoxine effect [2], and thus all patients receiving this medication should also receive pyridoxine 25 mg daily. It is recommended that penicillamine or trientine be started at one-fourth to half the desired dose and increased gradually over 1–2 weeks to assure tolerance of the medication. Within the initial month of therapy the patient should be monitored for signs of acute allergic reactions or other toxicities. Thus, a complete blood count, urinalysis, and renal and liver tests should be obtained weekly for the first month. If the patient responds appropriately, monitoring is then performed each 1–3 months for the first year, and then every 6–12 months thereafter. Some experts recommend following 24-h urine excretion of copper to monitor chelation therapy, obtained several times in the first 12 months and then yearly thereafter. Several hundred milligrams to several grams of copper should initially be excreted over 24 h, but after several years of chelation as little as 0–300 μg of copper should be excreted daily [2,51]. If urinary copper excretion is very low or very high, it suggests poor compliance with therapy. If KF rings had initially been present, then serial ophthalmological examinations annually should be performed to be certain that these lesions disappear with time. The failure of disappearance again suggests poor compliance. Many authorities recommend lifelong chelation therapy with trientine or penicillamine in patients who respond well to therapy and show no sign of toxicity rather than converting to zinc monotherapy (see below). Sudden discontinuation of chelation therapy can precipitate fulminant liver failure within months, which is usually fatal or requires liver transplantation [37]. Recent reports suggest that the combination of trientine and zinc therapy may offer benefit in patients with decompensated liver disease [38].

It has been demonstrated that up to 30% of patients [39] will have some worsening of neurological symptoms when treated with penicillamine. It has been proposed that the cause of this neurological worsening is a transient increase in blood copper and then brain copper exposure as the penicillamine mobilizes hepatic copper stores [40,41] or that penicillamine may form a complex of intracellular copper that is more toxic [41]. Neurological exacerbation has also been reported following initiation of treatment with trientine [42], thiomolybdates [43], and zinc [44]; however the frequency seems to be lower than with penicillamine. For these reasons, Brewer and colleagues [45] have proposed the use of ammonium tetrathiomolybdate for initial therapy in neurologically affected patients, although it remains an investigational compound. Ammonium tetrathiomolybdate complexes with dietary copper, thus preventing its absorption, and when absorbed forms complexes with copper and albumin in blood that prevent cellular uptake and decrease intracellular copper stores. Concerns have been raised regarding bone marrow suppression and other toxicities of ammonium tetrathiomolybdate, and this drug is under continuing investigation. A study has suggested improved preservation of neurological function in newly diagnosed patients treated with ammonium tetrathiomolybdate compared with trientine [46].

Penicillamine may produce both allergic and toxic effects in up to 20% of patients [31] Trientine is associated with a lower frequency of side effects, and is therefore recommended by many authorities as a safer initial chelator for Wilson disease. Side effects of penicillamine include fever, skin rash, lymphadenopathy, pancytopenia, proteinuria, nephrotic syndrome, drug-associated systemic lupus erythematosus, Goodpasture syndrome, optic neuritis, and bone marrow suppression. Long-term therapy with penicillamine may cause a dermatopathy [47,48].

Patients who present with fulminant hepatic failure caused by Wilson disease rarely, if ever, survive unless orthotopic liver transplantation is performed. These patients require critical care therapy in a liver transplantation center fully equipped to use hemodialysis, plasmapheresis, and other means of sustaining the patient until liver transplantation can be performed.

The use of oral zinc therapy has been proposed as maintenance or adjunctive [49] therapy for Wilson disease. Zinc inhibits intestinal absorption of copper by increasing metallothionein binding of copper in intestinal epithelia, which are sloughed in the gut. Some authorities recommend starting zinc acetate at 50 mg elemental zinc three times daily [50] or zinc sulfate 150–220 mg three times daily [50]. The zinc must be given between meals, and compliance may limit the effectiveness of this therapy. Zinc therapy is generally not used as initial therapy of symptomatic patients because of its slower onset of action; however, it has been substituted for copper chelators as chronic maintenance therapy with excellent results [31,51]. It may also play a role in presymp-

tomatic patients or siblings (especially young children) who are diagnosed with Wilson disease. Iron status needs to be monitored on patients undergoing chronic zinc therapy as it may interfere with iron absorption.

Finally, the use of antioxidants as adjunctive therapy in Wilson disease has not been fully explored. Because copper induces oxidative damage to the hepatocyte [27,52] and because α-tocopherol levels may be low during copper overload [27,52], it has been proposed that α-tocopherol might be an adjunctive therapy in symptomatic patients with Wilson disease [36].

The role of orthotopic liver transplantation is well established in patients with fulminant liver failure caused by Wilson disease [31]. In addition, patients with end-stage liver disease caused by Wilsonian cirrhosis should be considered for liver transplantation, as would other patients with cirrhosis. Long-term survival is excellent following liver transplantation and the liver lesion does not recur following transplant because of the presence of ATP7B in the donor liver. Neurological features generally improve following liver transplantation, although transplantation is not recommended for treating the isolated neurological presentation of Wilson disease.

Reye syndrome

In the 1960s and 1970s, reports of children developing an apparently new disease called encephalopathy with fatty liver appeared in Australia, the USA, and Europe. Mortality was 40–70%, and the disease tended to occur in epidemics, linked to influenza B, varicella infection, and other viral infections. The incidence of Reye syndrome peaked in the early 1980s, rapidly falling during the late 1980s and 1990s following the restriction of aspirin use in children [53]. The decline in incidence was also associated with the discovery and identification of numerous inherited metabolic defects of fatty acid transport and mitochondrial fatty acid oxidation, which harbor similar clinical features to Reye syndrome. Therefore, it is not completely clear whether the near disappearance of Reye syndrome was the result of reduction of aspirin use or the reclassification of many patients into newly discovered inborn errors of metabolism, or both. Many of these fatty acid oxidation defects are now detected by expanded newborn metabolic screening, so clinical presentation at older ages will become rarer in the next decade.

Clinical features

Reye syndrome is characterized by a prodromal febrile illness, usually a viral respiratory illness, followed by the abrupt onset of protracted vomiting after it appears that the child is in a recovery phase from the viral illness. Although cases have most often been linked to epidemics of influenza A and B and varicella infections, Reye syndrome has been reported in associated with almost every viral illness. The vomiting occurs within 3–7 days of the onset of the viral illness, and leads to dehydration and hyperpnea. Patients then rapidly pass through graded stages of encephalopathy, progressing to seizures, cerebral edema, coma, and possibly death. In over 90% of cases in the USA, a history of aspirin use for the treatment of the initial viral illness is obtained. Physical examination reveals an anicteric state with mild to moderate hepatomegaly, neurological dysfunction, overly brisk deep tendon reflexes, and varying degrees of dehydration. Biochemical testing reveals elevated AST and ALT (several hundred to several thousand units per liter), a normal serum bilirubin and albumin, elevated prothrombin time and international normalized ratio (INR), hyperammonemia, and hypoglycemia, particularly in young children. The Centers for Disease Control case definition of Reye syndrome includes the presence of acute noninflammatory encephalopathy with a normal cerebrospinal fluid cell count and protein; liver involvement characterized by microvesicular steatosis, AST/ALT elevation of threefold or greater, or hyperammonemia; and no other known explanation for the patient's illness. In recent years various metabolic disorders that induce fatty liver have been identified in patients presenting in this manner. During epidemics, patients with Reye syndrome were generally school age to teenage children who were previously healthy; however, a subgroup of children presented within the first few years of life, frequently with underlying failure to thrive and hypoglycemia in the presentation. Interestingly, respiratory alkalosis was present, lactic acidosis was rare, but dicarboxylic organic aciduria was common.

Hepatic histology

Reye syndrome causes extensive hepatic panlobular microvesicular steatosis, characterized by foaminess of hepatocytes with centrally placed nuclei. The fat deposition may be subtle and require special fat stains to identify microvesicular steatosis. Cholestasis is absent, and inflammation is unusual. Electron microscopic examination of the liver shows striking mitochondrial morphological abnormalities. Variable degrees of mitochondrial swelling, pleomorphism, loss of dense bodies, and matrix edema are present, correlating with severity of the disease. Striking microvesicular steatosis is present early in the course, progressing to macrovesicular steatosis during the recovery phase. Similar findings have been reported in fatty acid oxidation disorders and mitochondrial respiratory chain deficiencies. Hepatocytes do not undergo necrosis, but rather suffer from temporary metabolic (mitochondrial) failure. The central nervous system shows marked cerebral edema, particularly in fatal cases, with changes in neuronal mitochondria resembling those in the liver. Kidney lesions also show lipidosis and mitochondrial abnormalities. Thus, Reye syndrome is believed to be caused by generalized mitochondrial dysfunction and was one of the first well-described mitochondrial hepatopathies.

Genetics

Reye syndrome occurs in children generally aged between 4 and 12 years, with only very rare cases being reported in adults. Occasional cases of recurrent Reye syndrome were reported in very young children, now believed to have been caused by fatty acid oxidation defects and other metabolic disorders that were not identified at the time. Familial cases of Reye syndrome have also been reported, most likely caused by autosomal recessively inherited inborn errors of metabolism.

Pathogenesis and biochemistry

Generalized acute mitochondrial dysfunction underlies the biochemical and clinical abnormalities observed in patients with Reye syndrome [54]. The etiology of the mitochondrial lesions is most likely a combination of viral-induced cytokine-mediated cellular injury and the effect of aspirin on mitochondrial oxidative phosphorylation in perhaps genetically predisposed individuals. Because Reye syndrome has been reported in patients with collagen vascular disorders receiving large doses of aspirin who did not have apparent viral infections, isolated cases may have been caused purely by the effects of aspirin. The similar clinical presentation of fatty acid oxidation defects suggests that many cases of Reye syndrome in the past were undiagnosed defects in this important pathway for cellular energy metabolism, perhaps exacerbated by aspirin ingestion or the effects of viral infection.

The epidemiological association between aspirin intake and Reye syndrome was established in the USA in the late 1970s [55,56]. Subsequently, in 1982, the United States Surgeon General advised against the use of salicylates in children with febrile illnesses, and warning labels were placed on all aspirin products. A dramatic reduction in the use of aspirin in children followed over the next several years, with a coincident decline in the number of reported cases of Reye syndrome. However, in other parts in the world, such as Australia and Asian countries, aspirin use was not associated with Reye syndrome, yet there was dramatic reduction in the number of cases. Thus it has been proposed [57,58] that the discovery of new metabolic diseases (e.g., fatty acid oxidation defects) and closer examination of cases resulted in a clinical reclassification of cases that previously would have been labeled as Reye syndrome. Others claim that the effects of salicylates on mitochondrial oxidative phosphorylation may have brought to clinical recognition cases of subclinical metabolic liver disease [59].

Diagnosis

The diagnosis of Reye syndrome is made clinically, based on a prodromal viral illness, intake of aspirin products, and acute onset of vomiting with progressive encephalopathy and cerebral edema [60]. Hyperammonemia, elevated AST and ALT, prolongation of prothrombin time, and normal or only slightly elevated bilirubin are characteristic. A normal cerebrospinal fluid excludes infectious or other causes of similar illnesses. Toxin ingestion needs to be excluded, as do fatty acid oxidation defects, respiratory chain deficiencies, organic acidopathies, adrenal insufficiency, urea cycle enzymopathies, intracranial lesions such as brain tumors, and nonaccidental trauma. The finding of microvesicular steatosis in a non-inflamed liver with characteristic mitochondrial changes on electron microscopy is consistent with the diagnosis, but may be seen in other inborn errors of metabolism as well [61].

Treatment

Management of Reye syndrome requires early diagnosis and a high index of suspicion for the disease. Initial treatment includes controlled rehydration for the vomiting, and administration of 6–8 mg/kg per min of concentrated solutions of dextrose plus electrolytes to maintain blood glucose above 100 mg/dL. Excessive sedation is avoided and the treatment of hyperammonemia varies among institutions. Intracranial pressure monitoring is necessary for patients who reach the encephalopathy stage of delirium and combativeness, with treatment (intravenous mannitol, pentobarbital coma, hypothermia) aimed at carefully reducing intracranial pressure to maintain cerebral perfusion pressure above 45–50 mmHg [62]. Complications such as pancreatitis, acute renal failure, sepsis, pulmonary hemorrhage or cerebral hemorrhage increase the risk of mortality. Unfortunately, most contemporary cases of Reye syndrome present in stage 3–4 coma, and do not have acceptable central nervous system outcome, despite survival. It should be pointed out that the metabolic liver failure in Reye syndrome undergoes spontaneous reversal (consistent with the effects of a toxin or metabolic disorder) during the first 2–3 weeks of illness, except for continued hepatic steatosis.

α_1-Antitrypsin deficiency

α_1-Antitrypsin deficiency affects 1 in 600 to 1 in 2000 live births. The PiZZ homozygous phenotype (and the rarer PiSZ phenotype) causes an autosomal recessive disorder in which there is defective folding and secretion of α_1-antitrypsin from the liver, resulting in serum levels of only 10–20% of normal. The mutant α_1-antitrypsin Z protein is trapped in the endoplasmic reticulum of the hepatocyte, causing overt liver injury in 10–20% of affected individuals [63]. The protein belongs to the family of circulating serine protease inhibitors called *serpins*, and is an approximately 55-kDa secreted glycoprotein that inhibits neutrophil proteases and elastases. In recent years, other functions of α_1-antitrypsin have been identified that may be of clinical importance [64]. For reasons that are not clear, only 10–15% of PiZZ-affected individuals develop liver abnormalities during the first 20 years of life. In adults, emphysema is common in affected homozygotes, particularly in association with chronic exposure to cigarette smoke and other pollutants. α_1-Antitrypsin deficiency is the most common genetic cause of liver disease in children

that leads to cirrhosis, and hence is the leading genetic indication for orthotopic liver transplantation.

Clinical features

Persistent neonatal cholestatic jaundice is the most common childhood presentation of α_1-antitrypsin deficiency. Occasional infants present with acholic stools, direct hyperbilirubinemia, and high serum levels of γ-glutamyltransferase, mimicking the clinical appearance of biliary atresia. Affected infants tend to have been small for gestational age at birth. Serum aminotransferase levels, alkaline phosphatase, and γ-glutamyltransferase may all be elevated. Hepatomegaly with a firm liver and splenomegaly are variable on physical examination. Occasionally patients present with a secondary vitamin K deficiency coagulopathy causing bleeding in the first few months of life. Other affected infants and young children will present abruptly with symptoms of portal hypertension, such as esophageal variceal bleeding or hypersplenism. A very small number of patients present in acute fulminant liver failure during infancy. In adults, α_1-antitrypsin deficiency may cause chronic hepatitis, cirrhosis, portal hypertension, or present as hepatocellular carcinoma [65]. There is also a slight increase in the risk of cryptogenic cirrhosis in adults who are PiMZ heterozygotes.

Sveger [66] has conducted a long-term prospective study examining α_1-antitrypsin phenotype in 200 000 newborn infants in Sweden. Of these 200 000 infants, 127 PiZZ infants were detected, 14 of whom had cholestatic jaundice in infancy, nine of whom had severe liver disease. Approximately 50% of the PiZZ individuals had abnormal aminotransferase levels without any other indication of liver involvement. Through childhood, more than 85% of the 127 patients had normalization and persistently normal aminotransferase levels with no evidence of liver disease or liver dysfunction. This prospective study in Swedish children suggests that only 5–10% of all PiZZ children will develop significant liver disease. However, it is well appreciated that subclinical hepatic fibrosis and even cirrhosis can occur in this disease without elevated liver blood tests.

Several other alleles of α_1-antitrypsin have been associated with liver disease. Children with the less common phenotype PiSZ appear to develop liver disease in a similar manner to PiZZ-affected children. PiSS-affected children do not develop liver disease. Other rare forms of α_1-antitrypsin deficiency have occasionally been associated with liver disease [67,68].

It is rare for emphysema or any significant pulmonary involvement to affect children with α_1-antitrypsin deficiency. In one study there were no significant differences between pulmonary function of PiZZ children and that of an age-matched control group between 13 and 17 years of age [69]. In adults over the age of 25 years, approximately 60–70% with α_1-antitrypsin deficiency may develop variable degrees of emphysema, generally in the fourth or fifth decade. Thus it is recommended that α_1-antitrypsin phenotype or genotype be evaluated in all adults with chronic obstructive pulmonary disease.

Hepatic histology

Liver histology in PiZZ homozygotes ranges from a benign-appearing, almost normal liver to one of extensive cirrhosis. The classic hallmarks of this deficiency are the periodic acid–Schiff (PAS)-positive, diastase-resistant globules that appear in periportal hepatocytes. These globules represent retained α_1-antitrypsin material in the endoplasmic reticulum of hepatocytes. Globules may not be easily recognized in PiZZ infants until up to 6 months of age [70], and thus initial liver biopsy in an affected infant may not reveal these globules. In the neonate, a giant-cell hepatitis with multinucleated giant cells, lobular disarray, cellular and canalicular cholestasis, and bile duct proliferation is commonly observed. Occasionally, the extent of bile duct proliferation and bile duct plugging suggests the diagnosis of biliary atresia. As the child becomes older, a more benign appearance of bridging hepatic fibrosis with minimal or no giant-cell transformation is observed. The liver can also manifest the appearance of a chronic hepatitis, and show increased iron deposition. Approximately 10–30% of affected infants with cholestatic jaundice develop a mixed macronodular and micronodular cirrhosis. Bile ducts are generally normal; however, in 5–10% of children who present with neonatal cholestasis, paucity of interlobular bile ducts will develop, causing chronic cholestatic disease.

Genetics

α_1-Antitrypsin is encoded by a 12.2-kb gene located on human chromosome 14q31–32.2 [71,72]. Structural variants of the α_1-antitrypsin protein are classified by the protease inhibitor phenotype system defined by agarose electrophoresis or isoelectric focusing of plasma [72]. A letter is assigned to variants according to the position of migration of α_1-antitrypsin in these gel systems. The more common normal variant migrates in an intermediate isoelectric point, and has been designated M. The most severe deficiency form migrates to a high isoelectric point, and has been designated Z. More than 100 allelic variants of α_1-antitrypsin have been reported, all inherited in an autosomal recessive fashion. With some variants there is no α_1-antitrypsin detectable in serum, the so-called null variants. Not all variants are associated with clinical disease. Emphysema and liver disease have been associated with the PiSZ, PiZZ, M_{malton} and M_{duarte} variants. The heterozygote state does not confer disease susceptibility, except for the small increased risk that the PiMZ heterozygote state poses for cryptogenic cirrhosis in adults. Studies have also suggested that the PiMZ state may predispose to more severe liver disease in a variety of hepatic disorders [73].

Pathogenesis and biochemistry

The pathogenesis of liver injury in α_1-antitrypsin deficiency has not been completely defined. It is clear that the

accumulation of mutant α_1-antitrypsin protein in the endoplasmic reticulum of liver cells is involved in its pathogenesis. In fact, transgenic mice carrying the mutant Z allele of the human α_1-antitrypsin gene develop PAS-positive, diastase-resistant globules in the liver as well as liver injury [74,75], despite possessing the normal mouse α_1-antitrypsin gene. It is difficult therefore to understand why only 10–15% of patients develop significant disease, since all PiZZ-affected individuals have globules present in their liver. Perlmutter and colleagues have demonstrated delay of degradation of mutant α_1-antitrypsin Z protein in fibroblasts from PiZZ individuals with liver disease compared to those without liver disease [76], providing evidence that other factors affecting the fate of mutant α_1-antitrypsin molecules may play a role in determining genetic susceptibility to liver disease. It has also been suggested that another normal cellular mechanism for disposing of endoplasmic reticulum-accumulated proteins, autophagy, may be genetically defective in those patients who develop liver disease [77]. This important role for a modifier gene may help explain the 10–15% incidence of significant liver disease in this and other single-gene mutation metabolic disorders [78].

Diagnosis

Diagnosis is established by isoelectric focusing or agarose electrophoresis of a patient's serum α_1-antitrypsin, or by genotyping for the Z allele. Generally, with the PiZZ phenotype, total serum levels of α_1-antitrypsin are found to be 10–20% of normal values. Establishing the exact phenotype or genotype assists in determining the risk to the patient for liver disease. For example, the PiSS phenotype elicits no risk for liver disease, whereas PiSZ and PiZZ do pose this risk. All infants with neonatal cholestatic jaundice require α_1-antitrypsin testing, inasmuch as it may be very difficult to clinically or histologically identify the disease at this time. Infants with α_1-antitrypsin deficiency may mimic the presentation of biliary atresia, including acholic stools, failure of biliary excretion on nuclear scintigraphy, and liver biopsy findings that are similar. It is said that α_1-antitrypsin concentrations may increase during the acute-phase response; however, serum concentrations virtually never exceed 50–60 mg/dL in patients with the PiZZ phenotype, allowing the use of serum concentrations as a screen for deficiency. The finding of PAS-positive, diastase-resistant globules on liver biopsy should never in itself be used to either confirm or exclude the diagnosis of α_1-antitrypsin deficiency, as some patients with the MZ phenotype have been reported to harbor these inclusions on liver biopsy.

Treatment

There is no specific therapy for α_1-antitrypsin deficiency-associated liver disease; however, several measures are taken in infants with the cholestatic presentation. Fat-soluble (vitamins A, D, E and K) supplementation and an infant formula containing medium-chain triglyceride oil are administered until the cholestasis resolves. In addition, ursodeoxycholic acid is frequently prescribed to increase bile flow and reduce liver injury from cholestasis, as in other neonatal cholestatic diseases. Although early studies suggested that breast-feeding might be beneficial [79], subsequent data from the Swedish Nationwide Screening Study did not support this contention [80]. Approximately 10–30% of infants diagnosed with α_1-antitrypsin deficiency who have neonatal cholestasis progress within the first 5 years to cirrhosis and end-stage liver disease, requiring liver transplantation sometime during childhood. The remaining 70–90% of patient either show complete recovery from liver injury or have variable degrees of chronic elevated aminotransferases and progressive hepatic fibrosis.

Complications of portal hypertension are treated as they are in other childhood liver diseases. Orthotopic liver transplantation is required for children with end-stage liver disease, in whom hepatic synthetic functional failure or severe complications do not respond to standard therapy.

Patients with emphysema caused by α_1-antitrypsin deficiency are now treated with replacement therapy with purified or recombinant plasma α_1-antitrypsin, either by intratracheal aerosol administration or by intravenous administration [81]. Improvement in serum concentrations of α_1-antitrypsin without significant side effects results from these therapies. One nonrandomized trial suggested that there was slower decline in forced expiratory volume in 1 s (FEV1) in patients on replacement therapy [82]. Lung transplantation has been performed in a number of patients with severe emphysema. Actuarial survival is approximately 50% at 5 years [83]. Clinical trials with 4-phenylbutyrate, a chemical chaperone used to potentially increase α_1-antitrypsin secretion from the hepatocyte, failed to show any clinical benefit yet with considerable side effects [84]. α_1-Antitrypsin deficiency is certainly a disease that could benefit from successful gene replacement therapy and investigation in this arena will continue.

Finally, siblings of all affected patients with the PiZZ phenotype should be screened by isoelectric focusing, agarose gel electrophoresis, or genotyping. If identified as a homozygote, strict avoidance of cigarette smoke and other air pollutants may help prevent the onset of emphysema later in life.

Porphyria

Eight metabolic disorders called the porphyrias are caused by inherited deficiencies or inhibition of the enzymes involved in the heme biosynthesis pathway. Each of these disorders is characterized by the accumulation of distinct substrates or precursors of heme that provide characteristic biochemical footprints in blood, urine, and feces, allowing differentiation of the specific type of porphyria. The accumulated compounds, rather than impaired synthesis of heme, are responsible for

Table 87.3 Clinical features of human porphyrias

Disease	Affected enzyme	Mode of inheritance	Site of expression	Urine porphyrins	Fecal porphyrins	Clinical manifestations
Acute porphyrias						
ALA dehydratase deficiency	ALA dehydratase	Recessive	Liver	ALA		Neuropsychiatric
Acute intermittent porphyria	PBG deaminase	Dominant	Liver	ALA, PBG		Neuropsychiatric
Hereditary coproporphyria	Coproporphyrinogen oxidase	Dominant	Liver	ALA, PBG, coproporphyrin	Coproporphyrin	Neuropsychiatric, cutaneous
Variegate porphyria	Protoporphyrinogen oxidase	Dominant	Liver	ALA, PBG coproporphyrin	Protoporphyrin, coproporphyrin	Neuropsychiatric, cutaneous
Cutaneous porphyrias						
Porphyria cutanea tarda	Uroporphyrinogen decarboxylase	Dominant or acquired	Liver ± RBCs	Uroporphyrin, 7-carboxylporphyrin	Isocoproporphyrin	Cutaneous
Hepatoerythropoietic porphyria	Uroporphyrinogen decarboxylase	Recessive	Liver, RBCs	Uroporphyrin, 7-carboxylporphyrin	Isocoproporphyrin	Cutaneous
Congenital erythropoietic porphyria	Uroporphyrinogen III synthase	Recessive	RBCs	Uroporphyrin I, coproporphyrin I	Coproporphyrin I	Cutaneous, hemolytic anemia
Protoporphyria	Ferrochelatase	Dominant or recessive	RBCs and liver		Protoporphyrin, coproporphyrin	Cutaneous, liver disease

ALA, aminolevulinic acid; PBG, porphobilinogen; RBC, red blood cell.
Adapted from Freese [219], with permission from Lippincott Williams Wilkins.

the cutaneous, hepatic, neuropsychiatric, and hematological manifestations of the porphyrias. Three of the porphyrias are inherited in autosomal recessive fashion, and five by autosomal dominant inheritance. Most patients present during or after puberty; however, onset in infancy or early childhood has been reported on occasion [85–91]. Five of the porphyrias are expressed exclusively in the liver, two in both liver and erythroid cells, and one type has only erythroid expression [85–88]. Porphyrias are generally classified into the acute and cutaneous porphyrias based on their clinical presentation. In the acute porphyrias, one or both of the porphyrin precursors, aminolevulinic acid (ALA) and porphobilinogen (PBG), is increased. In the cutaneous porphyrias, porphyrins rather than ALA accumulate. Diagnostic studies and clinical features of the major porphyrias are outlined in Table 87.3.

Acute porphyrias

Four hepatic porphyrias present with acute attacks of potentially life-threatening neurological dysfunction. These attacks generally begin around puberty, and may diminish after the fifth decade of life. The most common symptoms include abdominal pain, constipation, and vomiting, presumably caused by autonomic nervous system dysfunction of the gastrointestinal tract. A peripheral neuropathy may develop into flaccid quadriplegia with respiratory compromise in severe cases. Psychiatric symptoms may include depression, psychosis, and hysterical behavior [85–87,92–98]. The development of neurological symptoms is related to blood levels of ALA, although the exact mechanism by which ALA functions as a neurotoxin has not been determined. Drugs and toxins that increase cytochrome P450 activity may precipitate acute attacks; relative iron deficiency and cyclical progesterone excretion in menstruating females may be responsible for the higher frequency of acute attacks in women compared to men.

Hepatic abnormalities in the acute porphyrias are characterized by mild AST and ALT elevations during acute attacks. Histology of the liver typically shows steatosis and increased iron deposition. Intramitochondrial crystalline inclusions have also been described [92,93,99–103]. A disturbing high incidence of hepatocellular carcinoma has been described in patients with inducible porphyrias [104].

Acute intermittent porphyria

The most common form of acute porphyria is acute intermittent porphyria (AIP). Porphobilinogen deaminase (also known as hydroxymethylbilane synthase) is the defective enzyme in this autosomal dominant disease. The prevalence is approximately 1 in 1000 to 1 in 2000 [92,105]. Diagnosis is established by the determination of large amounts of ALA and PBG in 24-h urine collections during acute attacks, with

PBG excretion exceeding ALA. A characteristic of AIP is a black or red color of a patient's urine after exposure to light or air. In asymptomatic patients, urinary PBG and ALA excretion may be normal. A diagnosis is then established by enzyme analysis in liver tissue or red blood cell lysates, or identification of disease-causing mutations in leukocyte DNA [93,106]. There have been more than 240 mutations identified in porphobilinogen deaminase.

Mild attacks of AIP are treated by careful management of fluids and electrolytes and intravenous carbohydrate loading, avoidance of drugs that may precipitate symptoms, and observation. Pain is controlled by narcotic analgesics. Restlessness and insomnia may be treated with benzodiazepines. Severe neurological symptoms are treated with intravenous hematin, which inhibits hepatic ALA synthetase activity, yielding lower circulating levels of ALA [107,108]. The standard dose is 3–4 mg of heme in the form of lyophilized hematin (Ovation Pharmaceuticals). Neuropathy may require months or years to show complete recovery. Liver transplantation has been used in very unusual circumstances for severe unremitting symptoms [109]. The risk of hepatocellular carcinoma is increased, frequently unassociated with elevated serum α-fetoprotein. Annual hepatic imaging has been recommended to screen for hepatocellular carcinoma.

Other acute porphyrias

Hereditary coproporphyria (HCP), variegate porphyria (VP), and ALA dehydratase deficiency comprise the other acute porphyrias. HCP and VP are inherited in an autosomal dominant fashion. Urinary and fecal coproporphyrin III excretion is elevated in these two disorders, with fecal protoporphyrin being present in VP. Cutaneous manifestations predominate over neurological symptoms in these two dominant conditions. The accumulation of porphyrins in the skin is responsible for the cutaneous lesions. Neurological symptoms can resemble those of AIP. ALA dehydratase deficiency is a very rare autosomal recessive disorder, yielding exclusive excretion of ALA without PBG in the urine. The disease is characterized by severe acute neuropsychiatric porphyria episodes that are poorly responsive to medical treatment [110]. Treatment of neurological attacks in all these conditions follows the protocol for the treatment of AIP attacks.

Cutaneous porphyrias

Four of the porphyrias are characterized by cutaneous lesions without neurological or psychiatric involvement, although significant hepatic involvement may be observed. These diseases include porphyria cutanea tarda (PCT), hepatoerythropoietic porphyria (HEP), congenital erythropoietic porphyria (CEP), and protoporphyria. Two types of skin photosensitivity develop in the cutaneous porphyrias. Skin lesions appear to be the result of photoexcitation of porphyrins located in the upper levels of the epidermis or walls of dermal blood vessels, with resulting reactive oxygen species generation leading to tissue injury by a variety of proposed mechanisms [111–116]. Patients with PCT, HEP, and CEP have increased skin fragility in light-exposed areas, manifested by the formation of bullous lesions, secondary scarring, and hypertrichosis. Patients with protoporphyria show immediate photosensitivity with itching, burning, and erythema soon after light exposure. Although they do not develop bullae, chronic skin changes evolve into leathery hyperkeratotic skin in light-exposed areas.

Porphyria cutanea tarda

PCT is the most common form of porphyria in North America and Europe, inherited as an autosomal dominant disorder or acquired during chronic hepatitis C and other infectious diseases. PCT may also be associated with toxins, particularly halogenated hydrocarbons. The genetic form of PCT is caused by deficiency of uroporphyrinogen decarboxylase. Urinary and fecal uroporphyrins are elevated (Table 87.3) but PBG levels are normal. Most patients with PCT present in adulthood, with photosensitivity skin reactions, scarring, infection, and lichenification of the skin. Because many individuals with the identical enzyme deficiency remain asymptomatic, others have sought coexisting abnormalities that must be present in order to develop overt disease. These include iron overload, a mutated allele in the hereditary hemochromatosis (*HFE*) gene, the presence of hepatitis C viral infection, ethanol ingestion, or Alagille syndrome. Ingestion of ethanol, toxins, and estrogens may also be involved in the development of sporadic cases of PCT.

Liver involvement is a frequent finding in PCT, even in those without chronic hepatitis C or ethanol abuse, manifested by hepatomegaly, focal inflammation, granuloma formation, and hepatic hemosiderosis. Progression to cirrhosis is not uncommon [117–119]. It remains controversial whether there is an increased risk for hepatocellular carcinoma in patients with PCT [120,121].

Therapy for PCT includes phlebotomy or chelation therapy to reduce the iron burden. Chloroquine has been used to form complexes with uroporphyrin to enhance removal of these deposits from skin, especially if there is a contraindication to phlebotomy. These treatments may be combined in patients with significant liver disease, although their efficacy is still unclear [87,90,117,122]. It has been recommended that treatment of concurrent hepatitis C infection should be delayed until the PCT has been treated and is in remission.

Erythropoietic protoporphyria

Erythropoietic protoporphyria is one of the most common forms of porphyria in North America, caused by a defect in ferrochelatase. The enzyme can be measured in liver and red blood cells, being less than 50% of normal in almost all cases. Affected patients have elevated levels of erythrocyte, plasma, and fecal protoporphyrin [123–125]. The primary clinical

manifestation of protoporphyria is photosensitivity, which generally appears in childhood as burning, pruritus, erythema, and edema in light-exposed areas. Scarring and lichenification of the skin develop with time. Avoidance of light, use of sunscreens, and oral administration of carotenoids appear to be the most efficacious treatments for the skin lesions. Approximately 20% of patients may have minor abnormalities of liver function; however, 5–10% of affected patients develop significant hepatic involvement, with reports in children as young as 10 years of age. Jaundice is generally the first indication of hepatic dysfunction, although rapid progression of decompensated cirrhosis may lead to liver failure within several years of clinical presentation [126–130]. The liver is black in color, enlarged, and exhibits fibrosis and macronodular cirrhosis. Dark brown pigment representing protoporphyrin deposits are found in hepatocytes, Kupffer cells, and small biliary ducts. Insoluble protoporphyrin appears to plug small bile ducts, interrupting bile flow, and may initiate oxidative injury to hepatocytes. It is unclear why only 5–10% of patients develop progressive liver disease, but other gene modifiers or environmental factors must be involved. Other manifestations of protoporphyria include hemolysis, neurological dysfunction, and gallstones.

Treatment of individuals with liver disease is aimed at reduction of protoporphyrin levels in the liver. Iron supplementation and blood transfusions have been used in the past to diminish erythropoiesis, thereby reducing protoporphyrin production [131]. Plasmapheresis or hematin therapy has similarly been shown to decrease plasma protoporphyrin in several patients [132]. Interestingly, administration of chenodeoxycholic acid has been demonstrated to increase protoporphyrin excretion into bile [87–90,122]. Cholestyramine and activated charcoal both bind protoporphyrin, interrupting the enterohepatic circulation and increasing fecal excretion [93,133]. These modalities do decrease circulating levels of protoporphyrin, but have not been shown to conclusively affect the course of liver disease. Although orthotopic liver transplantation has been performed in several patients with end-stage liver disease, protoporphyrin production continues in the bone marrow, depositing protoporphyrins in the newly transplanted allograft [134,135]. In addition, skin lesions and neurological symptoms may continue, complicating the postoperative course. Thus, bone marrow transplant, either alone or following liver transplant, has been suggested in patients with severe disease [136,137].

Congenital erythropoietic porphyria

CEP is an autosomal recessive disorder caused by deficiency of uroporphyrinogen III cosynthase. It is quite rare, with fewer than 100 patients reported. The major manifestations are photosensitivity, hypertrichosis, and hemolysis. Liver disease is present and may progress to cirrhosis, with iron overload playing a significant role [87,90,122,138]. Treatment includes limiting light exposure, sunscreens, and β-carotene. Hematin or blood transfusions to decrease erythropoiesis have also been attempted in patients with liver disease [139,140]. Bone marrow and cord blood transplantation have been effective in several severely affected children [141,142].

Metabolic diseases of the neonate and of childhood

Hepatic involvement may be the presenting symptom of a variety of metabolic disorders that occur primarily or exclusively in infancy and childhood. Because effective treatments have been developed for many of these diseases, patients are now surviving into adulthood. Disorders of amino acid, carbohydrate, lipid, ammonia and mitochondrial metabolism may all have hepatic involvement as the primary clinical manifestation. It is beyond the scope of this chapter to describe in detail all childhood metabolic liver diseases; the reader is referred to recent textbooks for further details [1]. This chapter describes several of the most common genetic metabolic liver diseases of infancy and early childhood.

Hereditary tyrosinemia

Hereditary tyrosinemia is caused by deficiency of the enzyme fumarylacetoacetate hydrolase (FAH), the terminal enzyme in the pathway of phenylalanine and tyrosine degradation. Many mutations of the *FAH* gene have been identified, with unique mutations being common in specific ethnic groups, although there are no definite genotype–phenotype correlations in severity of the disease. Thus undefined environmental or other genetic factors play a role in determining the clinical severity of tyrosinemia. A number of metabolites of tyrosine accumulate proximal to the FAH block, including tyrosine and succinylacetone. Succinylacetone and succinylacetoacetate inhibit enzymes such as porphobilinogen synthase, which causes the build-up of ALA and symptoms similar to that of AIP [143,144]. It is unclear how accumulated toxic compounds initiate liver injury in tyrosinemia.

Tyrosinemia may present in infancy as acute hepatic failure, neonatal cholestasis, rickets, failure to thrive, or as compensated or decompensated cirrhosis later in childhood [145]. There is also a very high incidence of hepatocellular carcinoma in untreated affected children. Episodes of acute peripheral neuropathy may lead to pain, respiratory depression, and even death [146]. Children who present during infancy have decreased survival compared to those with a later presentation in childhood [147]. Cardiomyopathy has been described in 30% of newly diagnosed patients, with inteventricular septal hypertrophy being the commonest finding, although this lesion resolved during treatment in almost all patients [148].

Liver histology is characterized by macrovesicular steatosis, pseudoacinar formation of hepatocyte rosettes, hemosiderosis,

and varying degrees of hepatocyte necrosis and apoptosis. A fine diffuse fibrosis develops, eventually progressing to micronodular cirrhosis. Regenerative nodules are frequently present, and frank hepatocellular carcinoma may occur in patients as early as 2 years of age. The incidence of tyrosinemia is approximately 1 in 100 000; however, there are pockets of high frequency secondary to a founder effect, such as in the Saguenay-Lac Saint Jean region of Quebec, Canada, where the incidence is 1 in 1800 live births [149].

The diagnosis of hereditary tyrosinemia is suggested by features of metabolic liver disease in infants or older children, including diminished hepatic synthetic function (hypoglycemia, coagulopathy, hypoalbuminemia) in the face of only mildly elevated aminotransferases and cirrhosis. Bilirubin may be elevated or normal, and hypophosphatemic rickets may be present. Renal tubular dysfunction causes glycosuria, proteinuria, aminoaciduria, and hyperphosphaturia. Serum α-fetoprotein levels are usually extremely high for age. The diagnosis of tyrosinemia is established by the finding of succinylacetone in the urine. In some research laboratories, FAH activity can be measured in red blood cells. Genetic diagnosis is generally not necessary; however, in Quebec, 100% of patients have a single mutation of the *FAH* gene [150]. Hypertyrosinemia is a nonspecific finding and is not used to establish this diagnosis. Neonatal screening on newborn blood spots dried on filter paper is being carried out in high incidence regions, such as Quebec.

Treatment of hereditary tyrosinemia involves a diet restricted in phenylalanine and tyrosine, although this does not prevent the liver disease but may have beneficial effects on kidneys. Orthotopic liver transplantation reverses the hepatic metabolic abnormalities and reduces the risk for hepatocellular carcinoma, prevents neurological disease, and is usually associated with stabilization of kidney disease. Liver transplant has been considered the preferred option for children with tyrosinemia over the age of 1–2 years. However, the metabolic inhibitor 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC, also called nitisinone) has been shown to be effective in tyrosinemia diagnosed early in infancy [151]. This compound inhibits 4-hydroxyphenylpyruvate dioxygenase, the second enzyme involved in tyrosine degradation which, when inhibited, prevents the production of succinylacetone. Therefore, toxic precursors do not accumulate, liver disease improves dramatically, patients exhibit reduction of α-fetoprotein levels, liver synthetic function improves, and renal tubular damage may resolve. Hundreds of patients have been treated with NTBC. Neurological crises and liver failure are prevented if treatment is initiated within the first month of life. In Quebec, no hepatic nodules have developed in infants treated in this manner, although initiation of therapy at 2–8 months of age has permitted the development of nodules in several patients [151]. The major concern has been that mice homozygous for the *FAH* gene mutation and which develop tyrosinemia respond to NTBC but still develop hepatocellular carcinoma [152]. Whether treatment with NTBC will reduce the high risk for development of hepatocellular carcinoma in children with tyrosinemia over the long term is unknown. In addition the long-term safety of this compound has not been demonstrated. The current recommendation is for continued surveillance for hepatic masses and ocular lesions in children under long-term NTBC therapy. Neurological crises are treated with intravenous dextrose, pain control, NTBC, or hematin.

Galactosemia

Classic galactosemia, an autosomal recessive disorder, results from mutations in the gene for galactose 1-phosphate uridyltransferase (GALT), leading to deficiency of the enzyme [153]. Patients develop chronic cholestatic liver disease, failure to thrive, mental retardation, and cataracts in classic galactosemia. Two other conditions result in elevated plasma galactose concentrations, although without observable liver disease. These include uridine diphosphate 4-epimerase deficiency and galactokinase deficiency, the latter causing only juvenile cataracts. Classic galactosemia is a disease in which toxicity of the liver results from ingested dietary galactose and its failure to be converted to glucose. Most cases are now detected by newborn screening programs, such that it is an unusual patient who presents with neonatal liver disease because treating infants detected by newborn screening prevents liver injury.

The classic presentation of galactosemia is that of vomiting, diarrhea, and failure to thrive following the institution of lactose-containing infant feedings, either breast milk or lactose-containing infant formula [154]. Within weeks to months of birth, hepatomegaly, cholestatic jaundice, and cataracts develop in the affected infant. If left completely untreated, mental retardation develops as well as cirrhosis of the liver. Another classic presentation is that of *Escherichia coli* urinary tract infections and bacteremia, sometimes with a fatal course. Affected females may show ovarian failure due to in utero injury from the galactose toxic products. Laboratory findings include elevated serum aminotransferase levels, prolonged prothrombin time, conjugated hyperbilirubinemia, and hypoalbuminemia. Hemolytic anemia occurs frequently. Renal tubular dysfunction is characterized by generalized aminoaciduria in combination with proteinuria and galactosuria [154].

Pathogenesis

Galactose 1-phosphate accumulates in classic galactosemia, causing injury to the affected organs [153]. Galactose may also be reduced to galactitol, which causes cataracts when it accumulates in the lens of the eye. It appears that galactose 1-phosphate or galactosamine accumulation are responsible for the renal, ovarian, hepatic, and brain abnormalities. Galactose 1-phosphate may also interfere with phosphoglucomutase, an enzyme which allows the release of glucose from glycogen.

Liver biopsy reveals macrovesicular steatosis in the first months of life, with pseudoacinar transformation of hepatocytes, although giant-cell transformation is generally absent. Bile duct proliferation is accompanied by portal fibrosis and the development of cirrhosis over the first several months of life if untreated. The diagnosis of galactosemia is suggested by the detection of reducing substances in the urine with a negative glucose oxidase paper test on the urine. Since vomiting or poor intake of lactose-containing formula or breast milk may limit galactose excretion in the urine, false-negative results of urine reducing substance tests may occur in galactosemia. More specific diagnosis is based on measurements of erythrocyte GALT. If an infant with galactosemia has received a red blood cell transfusion, the red cell enzyme level may be raised into the normal range and the diagnosis missed. Newborn screening programs have been established across the USA and in many areas of the world [154]. Measurement of GALT activity or galactose compounds is the analysis performed on newborn blood spots. There are other causes of elevated galactose in newborn blood spots (e.g., congenital portosystemic shunts), so further evaluation is needed.

Treatment

Treatment of galactosemia is based on the complete elimination of dietary galactose. Infant lactose- and galactose-free formulas have been marketed around the world and are instituted as soon as a diagnosis is made. As the child becomes older, a complete lactose- and galactose-free diet must be maintained [155,156]. It has been suggested that endogenous production of some galactose may be the mechanism that underlies some of the delayed toxicity present in children maintained on a lactose- and galactose-free diet [157]. Follow-up studies have revealed that some patients treated from infancy still suffer poor growth, learning disabilities, and primary ovarian failure in females [157,158].

Hereditary fructose intolerance

Hereditary fructose intolerance is an autosomal recessive disorder caused by deficiency of fructose 1-phosphate aldolase B enzyme activity in the liver. Symptoms occur when fructose is added to the infant's diet, usually in the form of fruit juices or infant formulas that contain sucrose [159,160]. Infants develop vomiting, irritability, hypoglycemia, and even convulsions following the ingestion of fructose. If fructose is not removed from the diet, hepatomegaly, failure to thrive, chronic diarrhea, conjugated hyperbilirubinemia, and metabolic dysfunction ensue, including proximal renal tubular abnormalities [161]. Older children may have a self-aversion to sweets, knowing that these tend to make the child feel ill. Growth delay, the consequences of repeated episodes of hypoglycemia on brain development, and metabolic acidosis eventually complicate the clinical picture.

Liver histology is characterized by diffuse macrovesicular steatosis, scattered hepatocellular necrosis, bile duct proliferation, and periportal fibrosis. Cirrhosis has also been described with complications of portal hypertension. The toxic moiety leading to liver injury is accumulated fructose 1-phosphate. Both hypoglycemia and ATP depletion in hepatocytes is caused by the accumulation of this compound [161].

Diagnosis of hereditary fructose intolerance is suggested by the onset of hypoglycemia, a profound hypophosphatemia, and hyperuricemia following fructose ingestion with detection of fructose in the urine by chromatography. Several common mutations in the gene for this enzyme allow genetic diagnosis in most cases [162]. The incidence of the disease is approximately 1 in 20 000 newborns [163,164]. Because the enzyme is also present in jejunum, it has been suggested that jejunal biopsies can be used for enzyme analysis to establish the diagnosis. Genetic testing is available in clinical and research laboratories [165].

Treatment of hereditary fructose intolerance is based on the complete and lifelong elimination of fructose, sucrose, and sorbitol-containing foods, juices, medications or other compounds from the diet [159,166–168]. Low levels of chronic fructose ingestion may lead to growth failure [166] and persistent hepatic steatohepatitis.

Glycogen storage diseases

Excessive accumulation of glycogen or abnormal synthesis of glycogen in the liver or muscle tissue is the underlying basis for a group of diseases called the glycogen storage diseases (GSD). Seven of the glycogen storage diseases primarily involve the liver, causing a variety of hepatic manifestations. Types Ia, Ib, Ic, and III lead to hypoglycemia and characteristic metabolic derangements; types III, IV, and IX are associated with hepatomegaly and periportal fibrosis; and types VI and IX are associated with benign hepatomegaly. Understanding the underlying genetic enzymatic defects in each of these disorders has produced a better understanding of the pathophysiology and the development of treatment strategies (Table 87.4).

GSD type I

There are three forms of GSD type I, involving the glucose 6-phosphatase enzyme system. Glucose 6-phosphatase catalyzes the last step in the enzymatic degradation of glycogen with release of glucose into the blood. Failure of the proper function of glucose 6-phosphatase leads to profound hypoglycemia during the fasting state. GSD type Ia is caused by deficiency of the glucose 6-phosphatase enzyme. GSD type Ib involves abnormalities in the translocase responsible for movement of glucose 6-phosphate across microsomal membranes to the site of action of glucose 6-phosphatase. GSD type Ic is caused by deficiency of the phosphate–pyrophosphate translocase [169].

GSD type Ia

GSD type Ia (von Gierke disease) is characterized by recurrent episodes of profound and severe hypoglycemia, lactic

Table 87.4 Hepatic glycogen storage diseases (GSD)

GSD type	Enzyme defect	Hepatic symptoms	Liver lesions	Laboratory tests
Ia	Glucose 6-phosphatase	Hypoglycemia, hepatomegaly, lactic acidosis, growth failure	Glycogen accumulation, macrovesicular steatosis	AST/ALT elevations, hyperlipidemia, lactic acidosis, hyperuricemia,
Ib	T1 translocase	Hypoglycemia, hepatomegaly, lactic acidosis, neutropenia and infections, growth failure	Glycogen accumulation, macrovesicular steatosis	AST/ALT elevations, hyperlipidemia, lactic acidosis, hyperuricemia, neutropenia
III	Amylo-1,6-glucosidase (debrancher enzyme)	Hypoglycemia, lactic acidosis, growth failure, variable fibrosis and portal hypertension, hypotonia, cardiomyopathy	Glycogen accumulation, portal fibrosis	AST/ALT elevations, variable hyperlipidemia, lactic acidosis, hyperuricemia
IV	Amylo-1,4→1,6-transglucosidase (brancher enzyme)	Jaundice, hepatosplenomegaly, liver failure, portal hypertension, hypotonia	Glycogen accumulation, cirrhosis, PAS-positive storage material	AST/ALT elevations, coagulopathy, conjugated hyperbilirubinemia, hypoglycemia, hyopalbuminemia
VI	Liver phosphorylase	Hepatomegaly	Glycogen accumulation	AST/ALT elevations
IX	Liver phosphorylase b kinase	Hepatomegaly, rarely portal hypertension	Glycogen accumulation, variable fibrosis	AST/ALT elevations

ALT, alanine aminotransferase; AST, aspartate aminotransferase; PAS, periodic acid–Schiff.

acidemia, hyperuricemia, hypertriglyceridemia, and hypercholesterolemia with accompanying massive hepatomegaly, short stature, and immaturity. The hypoglycemic events frequently start during infancy, when the interval between feedings becomes prolonged beyond 2–3 h, or when the child starts sleeping for longer at night. The untreated child suffers from growth failure and short stature, extreme hepatomegaly without splenomegaly, bleeding episodes caused by platelet dysfunction, renomegaly, and a cherubic appearance secondary to the deposition of "baby fat" in the face and trunk. The abdomen is protuberant, and arms and legs are frequently thin. Impaired glucose production through glycogenolysis leads to overproduction of glucose 6-phosphate, which is shunted into pathways for synthesis of lactate, triglycerides, cholesterol, and uric acid [170]. As a result, glucagon levels are high and insulin levels low. Patients suffer from growth failure if untreated or poorly treated, and eventually develop multiple hepatic adenomas that may evolve into hepatocellular carcinoma. As glucose 6-phosphatase is also expressed in the kidney, enlarged kidneys are common, and nephrocalcinosis or glomerulosclerosis may also develop over time [171]. Hyperuricemia may contribute to chronic renal disease as well as to gouty arthritis [172]. Progressive hepatic fibrosis and portal hypertension virtually never occur in this disorder, although hepatomegaly is extremely prominent. The diagnosis is established by demonstrating elevated hepatic glycogen content and low activity of glucose 6-phosphatase on frozen liver tissue.

Liver histology shows markedly enlarged hepatocytes with pale cytoplasm, causing a mosaic histological appearance. Macrovesicular steatosis is frequent and there is an accumulation of glycogen in both cytoplasm and nucleus under electron microscopy. There is no hepatic fibrosis, cirrhosis, or chronic inflammation.

Treatment of GSD type Ia is directed at providing a continuous source of exogenous glucose to prevent hypoglycemia and the resultant hormonal and metabolic changes [173]. Frequent daytime high-starch meals along with uncooked cornstarch supplements are provided. Cornstarch is given in doses of 1.5–3 g/kg every 3–6 h as needed. In order to maintain blood sugar at night, constant drip feedings of a high glucose-containing elemental formula or glucose solutions are provided through either a nasogastric tube or a gastrostomy tube. The goal is to maintain blood sugar of at least 70 mg/dL. As children become older, many opt to discontinue constant drip feedings at night and ingest uncooked cornstarch at bedtime, and awaken every 4 h at night to drink a dose of cornstarch. Either of these techniques can effectively maintain blood sugar, reverse metabolic abnormalities, and provide adequate growth [174–180]. However, blood sugars need to be monitored periodically and care taken to prevent malfunction of nocturnal enteral pumps. It should be pointed out that hypoglycemia is better tolerated when the children are hyperlipidemic and acidotic than when they are adequately treated with cornstarch or constant drip feedings. However, once the children are well controlled with these modalities, they may react severely to episodes of hypoglycemia, with neurological depression, convulsions, and even death. For patients who cannot be controlled with these therapeutic modalities and for patients with multiple hepatic

adenomas, liver transplantation has been advocated [181]. However, the vast majority of patients can maintain a high quality of life with their native liver.

GSD type Ib

GSD type Ib presents in the same manner as GSD type Ia, although patients also have neutrophil dysfunction and frequently are neutropenic. Therefore they have a propensity toward recurrent bacterial infections, inflammatory bowel disease-like lesions, and oral ulcerations [182–186]. The diagnosis of GSD type Ib is suggested by the finding of neutropenia in a patient who appears to have all the other classic features of GSD type Ia (hypoglycemia on fasting, lactic acidosis, elevated AST and ALT, hypertriglyceridemia, hyperuric acidemia, hepatomegaly, growth failure). This disorder is confirmed by measurements of liver glucose 6-phosphatase activity. In GSD type Ib, which involves the glucose 6-phosphate T1 translocase enzyme, fresh liver homogenate will yield defective activity, whereas frozen liver homogenate will have normal glucose 6-phosphate activity. The reason for this is that freezing disrupts the microsomal enzyme barrier, allowing access of the substrate to glucose 6-phosphatase, thereby bypassing the need for the translocase enzyme. In fresh homogenate tissue, the microsomes are intact, thus necessitating the requirement for the translocase enzyme. This disease is treated similarly to GSD type Ia, plus injections of granulocyte colony-stimulating factor (G-CSF) to elevate neutrophil counts. Inflammatory bowel lesions generally respond to G-CSF therapy but may require other traditional medications for inflammatory bowel disease.

GSD type III

GSD type III is due to deficiency of debrancher enzyme [170], also known as amylo-1,6-glucosidase. This enzyme degrades the α-D-1,6 branch-points of the glycogen molecule after hepatic phosphorylase has hydrolyzed the outer branches. Patients with GSD type III have either liver and muscle involvement (type IIIa) or liver involvement alone (type IIIb), the latter being the minority of patients. Patients may present in identical manner to GSD type Ia, with hepatomegaly, hypoglycemia, hyperlipidemia, hyperuricemia, growth retardation, and similar biochemical features. However, blood lactate and uric acid levels may be normal in GSD type III, with moderate elevations of serum AST and ALT. Patients have the potential to develop progressive portal fibrosis, leading to cirrhosis and complications of portal hypertension [187] as well as hepatocellular carcinoma [188]. Myopathic weakness may worsen as the patient ages into adulthood. Cardiomyopathy with ventricular hypertrophy may also occur in GSD type III patients.

GSD type III is inherited in an autosomal recessive manner, the human gene being an 85-kb gene composed of 35 exons located on chromosome 1p21. Mutations in this gene have been described in both types IIIa and IIIb GSD. Molecular and tissue enzymology diagnoses are possible [189]. Liver histology in GSD type III resembles that found in type Ia, although there is less steatosis and periportal fibrosis progressing to cirrhosis may be present [190]. Treatment of GSD type III depends on the symptomatology. In patients with hypoglycemia, acidosis, and hypertriglyceridemia, treatment is similar to that in GSD type Ia. However, many patients with GSD type III can be managed on a high-starch diet, without the need for cornstarch therapy. There is one report of a high-protein diet and continuous nocturnal infusion of a high-protein formula improving muscle strength in a patient with myopathy. There is no other effective treatment for the progressive myopathy or cardiomyopathy.

GSD type IV

GSD type IV (Andersen disease, amylopectinosis) is caused by an autosomal recessive deficiency of the glycogen brancher enzyme, amylo-1,4→1,6-transglucosidase, present in leukocytes and in liver. The disease is extremely heterogeneous in terms of tissue involvement, age of onset, and clinical manifestations. An abnormal amylopectin-like glycogen is formed in the liver that contains few branch-points, which appears to be toxic to hepatocytes [191]. The human gene coding for this enzyme is on chromosome 3p12 and consists of 16 exons [192]. There appears to be a phenotype–genotype correlation that may explain clinical variability [193]. Patients may present with three clinical scenarios:

- progressive liver injury and cirrhosis, leading to liver failure and death during the first 5 years of life
- chronic liver disease without progressive fibrosis
- abnormal neuromuscular development with hypotonia, muscle atrophy, absent deep tendon reflexes.

Cardiomyopathy may also develop because of deposition of amylopectin in myocardial cells. Adults with milder symptoms, but with similar muscle histology have been described [193]. Children with early-onset liver failure present with failure to thrive, hepatosplenomegaly, cholestatic jaundice, and occasionally hypotonia. Complications of cirrhosis such as ascites and coagulopathy prevail and death usually occurs by 2–4 years of age [194]. Diagnosis is established by finding the characteristic abnormal structure of glycogen on electron microscopy in muscle culture fibroblasts or liver, or through molecular testing [192]. Verification of deficient branching enzyme activity may also be determined in muscle, leukocytes, cultured fibroblasts, or liver. Liver histology is characterized by micronodular cirrhosis and hepatocytes that appear to contain excessive glycogen, which is PAS-positive and diastase-resistant [195]. Electron microscopy shows large irregular areas of glycogen fibrils, and finely granular material [195]. Liver transplantation appears to be the only effective therapy for children with progressive liver failure and cirrhosis [196–198]. Although many patients have done well following liver transplantation without cardiac or

muscle involvement, a single report suggests that cardiac disease may be a posttransplantation complication [199].

GSD types VI and IX

GSD types VI and IX are caused by genetic deficiencies of the phosphorylase system of enzymes that stimulate degradation of hepatic glycogen. Deficiency of hepatic phosphorylase causes GSD type VI, of which there are many subtypes. Patients generally present with asymptomatic hepatomegaly, elevated aminotransferases, variably with hypoglycemia or hyperlipidemia. Growth retardation occasionally occurs, but many children are found to be normal in stature. Lactic acidosis is rare and the prognosis is generally very good. Hepatomegaly improves as the patient reaches adulthood, and growth also is generally normal by adulthood. Liver biopsy findings include glycogen-filled hepatocytes, but generally the absence of inflammation, cell injury, and fibrosis.

GSD type IX is caused by phosphorylase b kinase deficiency. This disorder is characterized by asymptomatic hepatomegaly, mild elevation of aminotransferases, glycogen-filled hepatocytes on liver biopsy, and variable degrees of hepatic fibrosis. Slow progression to cirrhosis has been reported in several cases with the development of portal hypertension. Diagnosis is established through enzyme analysis of liver and erythrocytes [200,201] or by molecular analysis [202]. Phosphorylase b kinase deficiency (GSD type IX) is more frequent than true phosphorylase deficiency. Rarely, patients with GSD type IX develop biochemical features of GSD type I or type III. Treatment is generally not necessary for GSD type IX.

Mitochondrial hepatopathies

Hepatic involvement is a common feature of disorders affecting mitochondrial oxidative phosphorylation. Many of the disorders of the mitochondrial respiratory chain may present as neonatal acute liver failure, hepatic steatohepatitis, cholestasis or cirrhosis with chronic liver failure of insidious onset. Mitochondrial hepatopathies occur primarily in early childhood, although secondary disorders present at any age. Diagnosis is based on biochemical testing, enzymology, and genetic testing. Treatments for mitochondrial support are inadequate at present.

Classification of mitochondrial hepatopathies

Sokol and Treem [203] have developed a classification scheme for mitochondrial hepatopathies (Table 87.5) that divides these disorders into primary disorders, in which the mitochondrial defect is the primary cause of the liver disorder, and secondary disorders, in which a secondary mitochondrial insult is caused by a genetic defect affecting nonmitochondrial proteins (e.g., Wilson disease) or by an acquired injury to mitochondria (e.g., exogenous toxin or drug). Leonard and Schapira [204] further divided primary mitochondrial diseases into those caused by mutations in mitochondrial DNA (mtDNA) genes, called class 1a, and those caused by mutations in nuclear genes that encode proteins or cofactors which affect mitochondrial biogenesis, repair or structural proteins (e.g., respiratory chain complex subunit), called class 1b. This section focuses on four of the primary mitochondrial hepatopathies.

Table 87.5 Classification of primary mitochondrial hepatopathies

Electron transport (respiratory chain) defects
Neonatal liver failure
Complex I deficiency
Complex IV deficiency (*SCO1* mutations)
Complex III deficiency (*BCS1L* mutations)
Multiple complex deficiencies
Mitochondrial DNA depletion syndrome (*DGUOK*, *MPV17*, *POLG* mutations)
Delayed-onset liver failure: Alpers–Huttenlocher syndrome (*POLG* mutations)
Pearson marrow–pancreas syndrome (mtDNA deletion)
Mitochondrial neuro-gastrointestinal encephalomyopathy (*TP* mutations)
Chronic diarrhea (villous atrophy) with hepatic involvement (complex III deficiency)
Navajo neurohepatopathy (mtDNA depletion; *MPV17* mutations)
Fatty acid oxidation and transport defects
Long-chain hydroxyacyl-CoA dehydrogenase deficiency
Acute fatty liver of pregnancy (LCHAD enzyme mutations)
Carnitine palmitoyltransferase I and II deficiencies
Carnitine-acylcarnitine translocase deficiency
Fatty acid transport defects
Electron-transfer flavoprotein (ETF) and ETF-dehydrogenase deficiencies
Disorders of mitochondrial translation process
Urea cycle enzyme deficiencies
Phosphoenolpyruvate carboxykinase (PEPCK) deficiency (mitochondrial)

CoA, coenzyme A; LCHAD, long chain hydroxyacyl-CoA dehydrogenase; mt, mitochondrial.
Adapted from Lee & Sokol [209], with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.

Clincal features

Neonatal liver failure

Mitochondrial hepatopathies involving the respiratory chain frequently present as acute liver failure with onset within the first weeks to months of life. Symptoms include lethargy, hypotonia, vomiting, poor suck, and seizures [205,206]. It is difficult to differentiate the symptoms of liver failure from those of neuromuscular involvement in some infants. In other infants, a viral infection or some other undefined inciting

event triggers hepatic deterioration in an otherwise normal-appearing infant. Key biochemical features are a markedly elevated plasma lactate concentration (2.5–10 mmol/L), an elevated molar ratio of plasma lactate to pyruvate (> 20 and frequently > 30), and a raised β-hydroxybutyrate and arterial ketone body ratio of β-hydroxybutyrate to acetoacetate (> 2.0). Respiratory chain complex analysis of liver or muscle generally shows low activity of complex I, III, or IV. Molecular analysis may indicate mutations in the nuclear *SCO1* gene (associated with complex IV deficiency) [207] and *BCS1* gene (associated with complex III deficiency) [208]. Liver failure generally progresses to death within weeks to months, although occasional infants have recovered or have undergone successful liver transplantation if involvement was limited to the liver.

mtDNA depletion syndrome

The mtDNA pool is maintained either by import of cytosolic deoxyribonucleoside triphosphates (dNTPs) through dedicated transporters or by salvaging deoxynucleosides within the mitochondria. The mitochondrial deoxynucleoside salvage pathway is regulated by nuclear-encoded enzymes, including deoxyguanosine kinase (DGUOK) and thymidine kinase-2 (TK2). Imbalance of this mitochondrial dNTP pool has been proposed as responsible for a group of diseases in which there is inadequate synthesis of mtDNA relative to nuclear DNA, called the mitochondrial DNA depletion syndromes (MDS) [209]. In these disorders, there is insufficient synthesis of respiratory chain complexes I, III, IV, and V. There are two forms: the hepatocerebral and myopathic forms of MDS. Patients with the hepatocerebral form present within the first weeks to months of life with hepatomegaly, progressive liver failure, and generally death a few months later [210]. Presenting symptoms include vomiting, severe gastroesophageal reflux, failure to thrive, or developmental delay. Lactic acidosis, hypoglycemia, moderately raised serum ALT and AST, coagulopathy, and elevated total and conjugated bilirubin are common. Liver biopsy shows macrovesicular and microvesicular steatosis, cellular and canalicular cholestasis, variable degrees of periportal fibrosis depending on the age of the patient, and iron deposition in hepatocytes and sinusoidal cells [211]. There is considerable overlap between the clinical and laboratory features of patients with MDS and those with the neonatal liver failure form of respiratory chain disease. Mutations in several genes have been identified in patients with MDS: *DGUOK*, *POLG*, and *MPV17* in the hepatocerebral form and *TK2* in the myopathic form [209].

Alpers–Huttenlocher syndrome (delayed-onset liver disease)

Typically, the onset of symptoms occurs between 2 months and 8 years of life and is characterized by hepatomegaly, jaundice, progressive coagulopathy, and hypoglycemia. In most of these children liver failure is preceded by the development of hypotonia, feeding difficulties, intractable vomiting or symptoms of gastroesophageal reflux or failure to thrive, and ataxia followed by the onset of refractory partial motor epilepsy or multifocal myoclonus [212]. Multiple anticonvulsants are generally used to control the seizures; the addition of valproic acid may further impair respiratory chain enzyme activity and precipitate liver failure. Diagnostic criteria include refractory mixed-type seizures that include a focal component, psychomotor regression that is often episodic and triggered by intercurrent infections, and hepatopathy with or without acute liver failure [213]. Mutations in the *POLG* gene have been shown to be common in patients with Alpers–Huttenlocher syndrome [214].

Navajo neurohepatopathy

Navajo neurohepatopathy (NNH) is a unique sensorimotor neuropathy with progressive liver disease confined soley to full-blooded children or young adults of the Native American Navajo tribe. This disorder presents with muscular weakness, hypotonia, areflexia, virtual complete loss of sensation in the extremities leading to mutilation of fingers and toes, trigeminal neuropathy leading to corneal ulceration, poor growth, short stature, and serious systemic infections [215]. Liver involvement is characterized by Reye syndrome-like episodes, cholestasis, and cirrhosis or liver failure in infancy or childhood. Cerebral MRI of the brain demonstrates progressive white matter lesions, and peripheral nerve biopsies show severe loss of myelinated fibers [216]. Three clinical presentations have been described: an infantile form, with jaundice and poor growth progressing to liver failure and death within the first 2 years of life, with or without neurological findings; a childhood form presenting between 1 and 5 years of age with rapid development of liver failure; and the classical form in which progressive neurological findings dominate although liver dysfunction is present in all patients. Liver biopsy demonstrates macrovesicular and microvesicular steatosis, pseudoacinar formation of hepatocytes, multinucleated giant cells, cholestasis, periportal inflammation, and portal fibrosis or micronodular cirrhosis [215]. The liver involvement is progressive, with liver failure developing within months to years in most patients. There is no effective treatment for affected children. In 2006, sequencing of the *MPV17* nuclear gene, which has been shown to cause MDS, in six NNH patients from five families demonstrated a single homozygous disease-causing R50Q mutation in exon 2 of all patients [217].

Diagnosis

Diagnosis of a respiratory chain disorder is typically established by enzymatic analysis for respiratory chain activity of an affected tissue. These studies can be performed on frozen samples of small biopsies of liver, muscle, kidney, myocardium, or other tissue since they do not require the isolation

of mitochondrial fractions and can be carried out on tissue homogenates, lymphocytes, or cultured skin fibroblasts. Molecular diagnosis is becoming more useful as more nuclear genes are identified that cause specific respiratory chain and MDS disorders. For nuclear genes, whole blood for isolation of genomic DNA will usually suffice for genotyping. For mtDNA mutations or deletions, which are less common causes of mitochondrial hepatopathies (with the one exception of Pearson marrow–pancreas syndrome) affected tissues must be analyzed. Molecular analyses are now available at a number of clinical and research laboratories (see http://www.genetests.org).

Treatment

There is no effective therapy for most patients with respiratory chain disorders, including those with liver failure and more slowly progressive liver disease. A variety of mitochondrial substrates, cofactors, antioxidants, and other compounds have been suggested, although it is not clear that any currently available medical therapy significantly alters the course of severe disease [209]; some patients have experienced improvement of neuromuscular symptoms. Liver transplantation can be successfully performed in patients with no clinical, biochemical or imaging evidence of extrahepatic involvement, although progressive systemic symptoms may develop following liver transplantation [209,218]. Liver transplantation does not halt the progression of neurological, muscular, cardiac or other involvement, and is thus contraindicated in patients with significant signs of other organ involvement.

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G LIVER

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Alcoholic liver diseases

Suthat Liangpunsakul, David W. Crabb

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Epidemiology

In the United States, about 15–20 million people suffer from alcoholism. According to a 1990 survey by the US Department of Health and Human Services, alcohol is the third leading cause of preventable mortality, behind cigarette smoking and obesity, with about 100 000 deaths annually and an annual economic burden of more than $100 billion in the United States [1,2]. Surveys have demonstrated that alcohol-induced liver injury accounted for around 40% of all deaths from chronic liver disease in the United States between 1990 and 1998 [3].

Alcohol abuse can be found in all age groups. However, the age group at highest risk for hospitalization as the result of alcohol-related liver diseases (ALD) is that between 45 and 64 years, with a prevalence of 94.8 per 10 000 [4–6]. In elderly hospitalized patients, the prevalence of alcohol-related diagnoses ranges from 48.2 to 65.1 per 10 000 [4–6]. Of note, the overall prevalence decreases with increasing age, but the prevalence always remains higher for men than for women. There were notable ethnic differences in the prevalence of ALD and mortality [7]. The highest and lowest alcohol-related cirrhosis mortality rates between 1991 and 1997 were white Hispanic and black Hispanic, respectively. However, black nonHispanic women had the highest alcohol cirrhosis mortality rate among all women [7]. Alcohol intake is best quantified in terms of grams of ethanol per day. Each common unit of drinking contains about 10–15 g of ethanol: 1-ounce or shot of distilled spirits (30 mL), a 12-oz bottle of beer (355 mL), or a 4-ounce glass of wine (118 mL). There is no difference in the risk of ALD associated with the use of any of these forms of alcohol. Drinking alcohol at times other than at meals, which includes binge drinking, was associated with increased risk of liver injury [8]. There is general agreement that the risk of ALD increases with increasing alcohol consumption. The amount of ethanol that can be safely drunk is estimated to be up to 20 g (two drinks) per day for men and up to 10 g (one drink) for women, and in fact this amount of alcohol consumption is associated with reduced risk of myocardial infarction [9]. These levels of drinking are referred to as "moderate drinking." Women have a higher risk of developing cirrhosis than men do even at the lower daily alcohol intake and lower cumulative exposure to alcohol [10–12]. In men, the risk of alcoholic cirrhosis increases with daily alcohol consumption greater than 60–80 g/day; women have a lower threshold and are considered to be at risk with daily alcohol intake greater than 20 g/day. Additionally, there is a more rapid development of liver disease in women who abuse alcohol compared with men. The peak incidence of ALD is in the 40- to 55-year age range in men, but occurs a decade earlier in women. Of note, a larger percentage of women progress to more advanced liver disease [13,14]. The male to female ratio of alcoholism is about 10 to 1, but that for ALD is about 3 to 1. This strongly supports the clinical impression of an increased susceptibility of women to ALD, and emphasizes the need to help women control abusive drinking at a younger age. In the past, ALD was attributed to malnutrition (hence the term "Laennec nutritional cirrhosis"). However, alcoholic cirrhosis can occur in well-nourished persons. To date, it remains an unsolved question why only a subset of alcoholics develops serious liver injury (no more than 35% of heavy drinkers develop alcoholic hepatitis and only 20% develop cirrhosis).

Metabolism of ethanol and genetic predisposition to alcoholism and ALD

In humans, more than 90% of ingested alcohol is eliminated through metabolic degradation in the liver, and 2%–10% is

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eliminated in urine and breath. Ethanol is first metabolized into acetaldehyde through several enzymatic and nonenzymatic mechanisms [15–17]; which include the alcohol dehydrogenase (ADH) pathway in the cytosol; the cytochrome P4502E1 (CYP2E1) pathway in the smooth endoplasmic reticulum, and the catalase pathway in peroxisomes (Fig. 88.1). Acetaldehyde is converted by aldehyde dehydrogenases (ALDH) to acetate, which is released from the liver and metabolized by the heart and muscle [17]. The rate of ethanol metabolism by ADH and ALDH may be critical in determining its toxicity because the intermediates of this pathway are themselves potentially toxic. The maximal activities of ADH and ALDH in the liver are similar, so that each enzyme contributes to the overall control of the rate of alcohol oxidation.

Alcohol dehydrogenase (ADH)

ADH is a cytosolic, zinc-dependent dimeric enzyme using the oxidized form of nicotinamide adenine dinucleotide (NAD^+) as a cofactor [18]. It is responsible for the bulk of hepatic alcohol oxidation [19]. Human ADHs are categorized into five classes on the basis of their structural and kinetic characteristics [17]. However, under physiological conditions, the main isoenzymes involved in human ethanol metabolism are ADHs from classes I, II, and IV. Class I contains α, β, and γ isoenzymes. These enzymes have a low Michaelis constant (K_m < 5 mM) for ethanol and they are abundant in the liver, and therefore play a major role in ethanol metabolism. Class I ADH comprises three genes, *ADH1*, *ADH2*, and *ADH3*, making up gene clusters located on chromosome 4q21–23, spanning about 80 kilobases [20]. These loci have been renamed *ADH1A*,

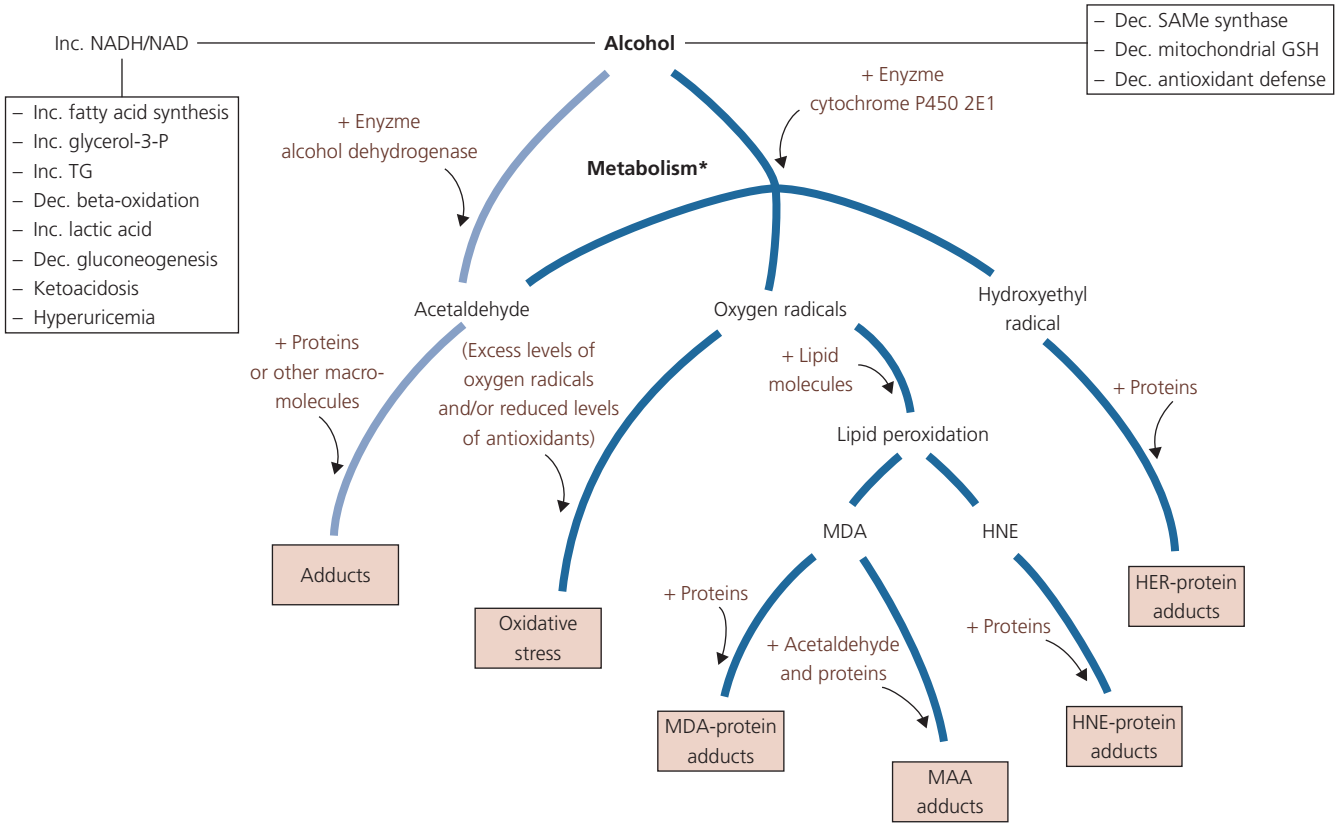

Figure 88.1 Pathway of alcohol metabolism and its effect on metabolism of hepatocytes. The major alcohol-metabolizing enzymes are alcohol dehydrogenase (ADH) and cytochrome P450 2E1 (CYP2E1). Acetaldehyde is then oxidized by aldehyde dehydrogenase (ALDH). The ADH and ALDH reactions generate free reduced nicotinamide adenine dinucleotide (NADH). The increase in the ratio of NADH to NAD^+ (the oxidized form of NAD) modifies many metabolic pathways, which leads to increased fatty acid synthesis and decreased fatty acid oxidation. Acetaldehyde can react with other proteins in the cell to generate hybrid molecules known as adducts. CYP2E1 also generates acetaldehyde, as well as highly reactive oxygen-containing molecules called oxygen radicals, including the hydroxyethyl radical (HER) molecule. Elevated levels of oxygen radicals can generate oxidative stress, which through various mechanisms leads to cell damage. Oxygen radicals can also interact with fat molecules (lipids) in the cell in a process known as lipid peroxidation, resulting in reactive molecules such as malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). Both of these can react with proteins to form MDA–protein and HNE–protein adducts. MDA can also combine with acetaldehyde and protein to form mixed MDA–acetaldehyde-protein adducts (MAA). Other effects of alcohol include depletion of antioxidants such as *S*-adenosylmethionine (SAMe) and glutathione (GSH). Other abbreviations: Dec, decrease; Inc, increase; TG, triglyceride. From Tuma & Casey [100] and http://www.niaaa.nih.gov/Resources/GraphicsGallery/Liver/metabolismp286.htm

Table 88.1 Properties of polymorphic forms of human alcohol dehydrogenases (ADH)

Gene locus	Subunit	K_m (ethanol)	Population
*ADH2*1*	β1	0.05	White, African Americans
*ADH2*2*	β2	0.9	Asians
*ADH2*3*	β3	34	African Americans
*ADH3*1*	γ1	1	All groups
*ADH3*2*	γ2	0.63	White
*ADH3*3*	γ3		Native Americans

From Crabb et al. [17], with permission from Cambridge University Press.

ADH1B, and *ADH1C*; however, this chapter will use the older nomenclature. Among these genes, polymorphisms with physiological significance exist in the *ADH2* and *ADH3* loci. These polymorphic variants differ in their efficiency for ethanol oxidation. People of different racial backgrounds inherit different sets of ADH isoenzymes. African Americans who carry a copy of the *ADH2*3* gene, encoding an enzyme with high maximum velocity, have a somewhat faster rate of alcohol metabolism [21]. The kinetic properties and population distributions of these allelic enzymes are shown in Table 88.1.

Class II ADH (π-ADH) was first found in human liver; it has a higher K_m for ethanol and is less sensitive to pyrazole inhibition than class I enzymes [22]. Class II ADH plays a less important role in liver ethanol metabolism than class I. The class IV enzyme (μ-ADH, for the *m*ucosal isozyme or σ-ADH, for the *s*tomach isoenzyme) has been purified from the stomach and esophagus. This ADH form is involved in the first-pass metabolism of ethanol in the stomach [23]. The ADH reaction rate is controlled by the amount of ADH, the acetaldehyde level, and the ratio in the cytosol of free reduced NAD to oxidized NAD ($NADH/NAD^+$) [24]. The amount of ADH in the liver is not induced by chronic drinking; however, with fasting, protein malnutrition, and liver disease, ADH activity and the ethanol elimination rate are decreased [25]. The properties of the enzymes in each of the ADH classes are summarized in Table 88.2.

Microsomal ethanol oxidizing system

Aside from the ADH pathway, ethanol can be metabolized by a microsomal ethanol oxidizing system (MEOS) involving mostly the cytochrome P450 2E1 (CYP2E1) [26]. Two other cytochromes, CYP1A2 and CYP3A4, also contribute to the MEOS, although to a lesser extent [16,27–29]. CYP2E1 is associated with NADPH-cytochrome P450 reductase in the microsomal membrane, and reduces molecular oxygen to water as ethanol is oxidized to acetaldehyde [26]. It has a low ethanol catalytic efficiency relative to ADH and is therefore responsible for only a small part of total ethanol metabolism [16]. CYP2E1 is inducible by chronic drinking [28,29], especially in the perivenular zone [30], and it may contribute to the increased rates of alcohol elimination in heavy drinkers. CYP2E1 can also be induced in fasting and diabetes and by a diet high in fat [31]. It has a high K_m for ethanol and may assume a greater role in ethanol metabolism at high blood alcohol levels. CYP2E1 is unusually "leaky" and generates reactive oxygen species, including hydroxyl radicals ($OH\cdot^-$), superoxide anions ($O_2\cdot^-$), hydrogen peroxide (H_2O_2), and hydroxyethyl radicals [31]. Thus, CYP2E1 is a major source of oxidative stress in the hepatocytes. Of importance, this P450 enzyme also has capacity to activate many xenobiotics to toxic metabolites, most notably acetaminophen [32].

Table 88.2 Properties of alcohol dehydrogenases (ADH) in man

Gene locus	New nomenclature	Subunit type	K_m (ethanol)	Tissue distribution
Class I				
ADH1	*ADH1A*	α	4	Liver
ADH2	*ADH1B*	β	0.05–34.0	Liver, lung
ADH3	*ADH1C*	γ	0.6–1.0	Liver, stomach
Class II				
ADH4	*ADH2*	π	34	Liver, cornea
Class III				
ADH5	*ADH3*	χ	1000	Most tissues
Class IV				
ADH7	*ADH4*	σ, μ	20	Stomach, esophagus
Class V				
ADH6	*ADH5*		30	Liver, stomach

From Crabb et al. [17], with permission from Cambridge University Press.

Catalase

The enzyme catalase has also been shown to oxidize ethanol to acetaldehyde within the peroxisomes. This process is hydrogen peroxide-dependent. However, under normal physiological conditions, it plays only a minor role in ethanol metabolism [16,29,33].

Aldehyde dehydrogenase (ALDH)

The second step of ethanol metabolism is mediated by ALDH. It converts acetaldehyde to acetate, which is released from the liver and metabolized by heart and muscle [34]. Like ADH, ALDH enzymes use NAD^+ as a cofactor, but are expressed in a wider range of tissues than the ADH isoenzymes. In humans, there are multiple forms of ALDH that are divided into nine major families [35]. However, only some of these isoforms are significantly involved in acetaldehyde metabolism, whereas the others metabolize a variety of substrates. To date, a significant role in acetaldehyde oxidation has been identified only for cytosolic ALDH1 and mitochondrial ALDH2 [17,36]. Mitochondrial ALDH2 plays the central role in human acetaldehyde metabolism because of its submicromolar K_m for acetaldehyde [17]. ALDH2 is inhibited by disulfiram (Antabuse). Consumption of alcohol while taking disulfiram or a number of other drugs (metronidazole, and some sulfonylureas and cephalosporins) results in the accumulation of acetaldehyde, producing vasodilation, facial flushing, tachycardia, nausea, and vomiting [37]. Similar to the class I *ADH* gene, the *ALDH2* gene is polymorphic and the variants demonstrate the vital role of ALDH2 in ethanol oxidation. The normal allele is termed *ALDH2*1*. The allele *ALDH2*2* has been thoroughly studied because it causes inactivation of the enzyme leading to acetaldehyde accumulation [38]. The *ALDH2*2* allele contains a G→A substitution resulting in the replacement of glutamate with lysine at position 487 in ALDH2 [38,39]. *ALDH2*2* homozygotes have essentially no ALDH2 activity, whereas heterozygotes have markedly reduced but still detectable activity [40]. *ALDH2*2* is found almost exclusively in populations of Asian origin [41] and approximately 45% of Japanese have the ALDH2-deficient phenotype [42]. Therefore, flushing as the result of acetaldehyde accumulation after alcohol ingestion is commonly seen in the Asian population. The properties and tissue distribution of human ALDHs are summarized in Table 88.3.

Nonoxidative ethanol metabolism

In addition to the main oxidative pathways described above, ethanol is also metabolized, although to a minor extent, by a nonoxidative pathway to form fatty acid ethyl esters [43–45]. This reaction is catalyzed by fatty acid ethyl ester synthase and may be of importance in organs lacking ethanol oxidative metabolism, such as the heart and pancreas. Fatty acid ethyl esters disturb membrane function, and may thus contribute to alcohol-induced organ dysfunction [46].

Table 88.3 Properties and tissue distribution of human aldehyde dehydrogenase (ALDH)

Gene locus	K_m (acetaldehyde)	Tissue distribution
Class I		
ALDH1	30 μM	Many tissues, liver
Class II		
ALDH2	≤ 1 μM	Low levels in most tissues Liver > kidney > muscle > heart
Class III		
ALDH3	11 mM	Stomach, liver, cornea

From Crabb et al. [17], with permission from Cambridge University Press.

Genetic predisposition to alcoholism and ALD

The strongest genetic associations identified to date are those between risks of alcoholism and genes encoding alcohol-metabolizing enzymes. The *ADH2*2* (*ADH1B*2*) allele has been shown to reduce the occurrence of alcohol abuse and alcoholism in Asians and Jewish populations in which this allele has a relatively high prevalence [17]. Several studies have reported a higher frequency of *ADH2*2* in nonalcoholics and in moderate drinkers relative to heavy drinkers [47–49]. Since *ADH2*2* codes for a high activity enzyme (β2-ADH), it is postulated that this allele protects against alcoholism because of the unpleasant symptoms associated with acetaldehyde accumulation after alcohol drinking [50]. However, individuals with this genotype do not have increased steady state venous blood acetaldehyde concentration after alcohol drinking, despite a somewhat higher rate of ethanol elimination [51,52]. It is possible that these individuals have increases in arterial, and therefore brain, acetaldehyde after drinking.

The *ALDH2*2* allele also provides strong protection against alcoholism, especially in *ALDH2*2* homozygotes, presumably because of the severity of their flushing [49,53]. Several studies demonstrated a reduced frequency of the *ALDH2*2* allele in alcoholics compared with nonalcoholics [54–56]. Individuals with the *ALDH2*2* allele have a 10-fold reduction in the risk of alcohol dependence [54]; they also drink less alcohol and have a lower prevalence of binge drinking [57,58].

The evidence for a genetic risk for ALD is less strong than that for the risk for alcoholism. The largest study, the US Veterans Administration Twin Panel Study, showed a higher concordance for cirrhosis in monozygotic twins than in dizygotic twins, indicating a genetic component to the risk [17,59]. Reanalysis of the database supported this conclusion, but found that most, but not all, of the genetic liability for cirrhosis was the result of shared risk for alcoholism [60]. This study is limited by lack of information on the prevalence of hepatitis C in the cohort. The effect of ADH and ALDH

variants on the risk of ALD could be complex [61]. High activity of ADH variants and low activity of *ALDH2*2* decrease alcoholism risk, but if individuals with these isoenzymes persist in drinking, hepatic injury might result from high intrahepatic concentrations of acetaldehyde. Indeed, one study has demonstrated an increased risk of ALD among *ADH2*2* heterozygotes or homozygotes [62]. Another study suggested that *ALDH2*2* heterozygotes who drink heavily develop alcoholic hepatitis at a lower cumulative alcohol intake than those with active ALDH2 [63]. To date, none of the ADH and ALDH polymorphisms has been clearly identified in replicate studies as predisposing to the development of ALD [17].

Pathogenesis of alcoholic liver disease

Although the ultimate control of ALD will require the prevention of alcohol abuse, better understanding of the mechanisms of alcohol-induced liver injury may lead to treatments of fatty liver, alcoholic hepatitis, and prevention or delay of occurrence of cirrhosis.

Role of redox state

Fatty liver, the accumulation of triglyceride droplets in the liver, is the most common and earliest response of the liver to alcohol in heavy alcohol use [64]. Synthesis of fatty acids and triglyceride in excess of the capacity to oxidize it or export it in very-low-density lipoprotein particles results in hepatic steatosis [65]. The effect of ethanol was initially attributed to increased levels of cytosolic and mitochondrial NADH, generated from the ADH and ALDH reactions [66]. Increasing intracellular NADH levels inhibit the oxidation of fatty acids (Fig. 88.1). Increased levels of L-glycerol-3-phosphate and fatty acids, as well as induction of a number of enzymes involved in de novo fatty acid synthesis, result in accelerated synthesis of triglycerides. Existence of this redox mechanism was supported by in vitro studies. Cell lines engineered to express ADH metabolized alcohol and generated a reduced cytosolic redox potential, reflected by an increase in the lactate: pyruvate ratio in the medium. Coincident with this, the cells accumulated large amounts of triglyceride and free fatty acids [67].

The redox hypothesis of alcoholic fatty liver has been challenged by studies in which antioxidants prevented the development of fatty liver in animals receiving large single doses or chronic feeding of alcohol [68]. In addition, with chronic alcohol feeding of baboons, the redox shift in the liver becomes less severe, but the fat accumulation persists. This suggests that over time, the redox state is no longer the primary cause of alcoholic-induced liver injury [69]. Several additional mechanisms for alcohol-induced steatosis have been investigated over the past two decades, discussed in detail below.

Role of oxidative stress

There are two principal ways by which chronic alcohol use can induce oxidative stress, the CYP2E1 pathway and the mitochondrial electron transfer system [29] (Fig. 88.1). In both cases, increased rates of single electron transfers (at the sites of flavin coenzymes in the cytochrome catalytic cycle or the electron transfer system) facilitate the transfer of electrons to oxygen. CYP2E1 is induced by chronic alcohol use and is "leaky", in that electrons transferred to it from CYP450 reductase can be transferred to molecular oxygen in the absence of substrate, forming superoxide [70]. The preferential expression of CYP2E1 in the central zone of the liver corresponds with the prominence of alcohol-induced injury to this zone [29]. An end result of oxidative stress is lipid peroxidation, a chain reaction of lipid radical formation that follows the removal of an electron from polyunsaturated fatty acid (PUFA). The lipid free radical undergoes addition of oxygen to form a lipid hydroperoxide, and ultimately decomposition to form reactive aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) [71].

The most convincing data indicating that oxidative stress contributes to ALD come from studies using the intragastric infusion model. In these studies, ALD was associated with enhanced lipid peroxidation, protein modification, formation of 1-hydroxyethyl radical, and lipid radicals, and decreases in hepatic antioxidants, particularly glutathione [72–74]. Moreover, changes in the animals' diets that helped promote or reduce oxidative stress led to corresponding changes in the extent of liver injury. For example, when PUFA were replaced with saturated fats or medium-chain triglycerides, lipid peroxidation as well as alcohol-induced liver injury was reduced [75]. Studies in the *in vitro* system also demonstrated the important role of oxidative stress in the pathogenesis of ALD. Exposure of CYP2E1-expressing HepG2 cells to high concentrations of ethanol produced morphological changes and DNA fragmentation, activation of caspases 1 and 3, and apoptosis [76]. These changes were not observed in control HepG2 cells, and could be prevented by an inhibitor of CYP2E1, by antioxidant compounds, or by the inhibition of the caspases [77,78], and were exacerbated by the presence of PUFA. Taken together, these findings indicate that alcohol-induced oxidative stress is a pivotal factor in the development of ALD.

Role of diet and nutrition

It is important to emphasize that ethanol itself, rather than a nutritional deficiency, can cause ALD. Baboons chronically given large amounts of ethanol, despite a nutritionally complete diet, developed fatty liver and significant fibrosis [79]. Rats fed by constant intragastric infusion of a nutritionally adequate liquid diet containing ethanol comprising 50% of total calories developed fatty liver, necrosis, and fibrosis. Of importance, there are interactions between diet and alcohol in the development of liver injury. The most obvious is that

severe liver injury in the rat model required a high content of PUFA in the diet [80] and hepatic steatosis is in fact prevented by substituting saturated fat or medium-chain triglycerides for the PUFA [81]. This has been suggested to be the result of increased susceptibility of PUFA to lipid peroxidation. It is interesting that there appears to be an inverse relationship between dietary saturated fat and risk of ALD [82] and such a protective effect is postulated to result from increased secretion of adiponectin by adipocytes [83]. Administration of ethanol in a liquid diet that is very low in carbohydrate also causes liver inflammation and severe fatty liver in rats [84]. Lastly, obesity has been found to be a risk factor for the development of cirrhosis in humans [85]. Obesity doubles the risk of steatosis among heavy drinkers [86]. Dietary folate deficiency may also promote steatosis (see below).

Role of ethanol effects on transcription factors and metabolic regulators

Recent studies have shown that alcohol-induced fatty liver is associated with alterations in two transcription factors, peroxisome proliferator-activated receptor-α (PPAR-α) and sterol regulatory element binding protein 1 (SREBP-1), and the regulatory enzyme adenosine monophosphate-dependent protein kinase (AMPK) [87–89]. PPAR-α is a member of the nuclear hormone receptor superfamily, which, when dimerized with retinoid X receptors, controls transcription of a set of genes containing peroxisome proliferator response elements involved in transport and oxidation of free fatty acids [90]. SREBP-1 belongs to a family of basic helix–loop–helix leucine zipper transcription factors and regulates the enzymes responsible for cholesterol, fatty acid, and triglyceride synthesis in liver and other tissues. In liver, SREBP-1c is involved in fatty acid synthesis, whereas SREBP-2 plays a major role in the regulation of cholesterol synthesis [91,92].

Ethanol inhibits PPAR-α function in cultured cells and in ethanol-fed mice [88]. Ethanol feeding decreased PPAR-α–retinoid X receptor α binding to its consensus sequence, and the levels of messenger RNAs for PPAR-regulated genes such as long-chain and medium-chain acyl coenzyme A (acyl-CoA) dehydrogenases. Treatment of the mice with the PPAR-α agonist WY14643 increased the levels of PPAR-α and reversed fatty liver despite continued feeding of ethanol.

The role of SREBPs in alcoholic fatty liver was suggested by the battery of lipogenic enzymes induced by ethanol feeding. Ethanol treatment significantly increased SREBP activity in hepatoma cells. In vivo, a substantial increase in the amount of mature SREBP-1 protein was found in the livers of ethanol-fed mice, and the levels of messenger RNA for several SREBP-1 target hepatic lipogenic genes including fatty acid synthase, steroyl-CoA desaturase, malic enzyme, adenosine triphosphate citrate lyase, and acetyl-CoA carboxylase (ACC) [89].

AMPK is emerging as a "metabolic master switch" regulating pathways of hepatic triglyceride and cholesterol synthesis [93]. It phosphorylates and inhibits the enzymes involved in lipid metabolism, such as 3-hydroxy-3-methyl glutaryl-CoA reductase and ACC. ACC is generally regarded as the rate-limiting enzyme in fatty acid synthesis [93]. It has been firmly established that SREBP-1 can be both transcriptionally and posttranscriptionally regulated by AMPK [89,94,95]. Chronic ethanol feeding inhibited hepatic AMPK activity and enhanced the activity of its target protein ACC [96]. Activators of AMPK, e.g., metformin or aminoimidazole-4-carboxamide ribonucleotide, partially blocked the ethanol-mediated induction of SREBP-dependent promoter activity in hepatoma cells [87]. These findings suggest that AMPK may play a role in regulating the effects of ethanol on hepatic SREBP-1 activation and fatty acid metabolism. Potential mechanisms underlying the effect of alcohol on these transcription factors are summarized in Fig. 88.2.

Role of protein adduct formation

Protein adducts are covalent linkages formed between the amino acid side chains (typically lysine) of liver proteins and a low-molecular-weight reactive chemical such as acetaldehyde, hydroxyethyl radical, and lipid peroxide-derived aldehydes, such as 4-HNE and MDA (Fig. 88.1) [97]. There is much evidence that adducts are generated in vivo during ethanol metabolism, and that these adducts elicit an immune response [98]. Antibodies directed against acetaldehyde, MDA, HNE, mixed MDA–acetaldehyde protein adducts, and hydroxyethyl-radical-derived adducts are found in animals and humans that chronically consume alcohol [98–100]. In fact, hepatitis can be induced in guinea pigs by feeding alcohol to animals previously immunized with acetaldehyde adducts [101].

Acetaldehyde adducts form early in ALD and primarily in the perivenous region of the liver, which is also the primary region affected by alcohol-induced liver injury [100]. Moreover, in advanced liver disease they are found in the same areas that show evidence of inflammation and fibrosis [98,102]. Several mechanisms by which adducts could contribute to liver damage besides the formation of neoantigens are known. Aldehydes can interfere with the functions of proteins with critical lysine residues [103], such as lysine-dependent enzymes, calmodulin, and the cytoskeletal protein tubulin. Acetaldehyde–tubulin adducts appear to be especially relevant to alcohol-induced liver injury [104]. Impaired microtubule function could account for defects in protein secretion and other protein transport pathways that result from chronic alcohol consumption [104], and might contribute to endoplasmic reticulum stress (see below).

Role of cytokines, lipopolysaccharide, and the Kupffer cell

The Kupffer cell serves as the primary effector cell of the innate immune response within the liver [105], and plays a key role in the early pathogenesis of alcohol-induced liver

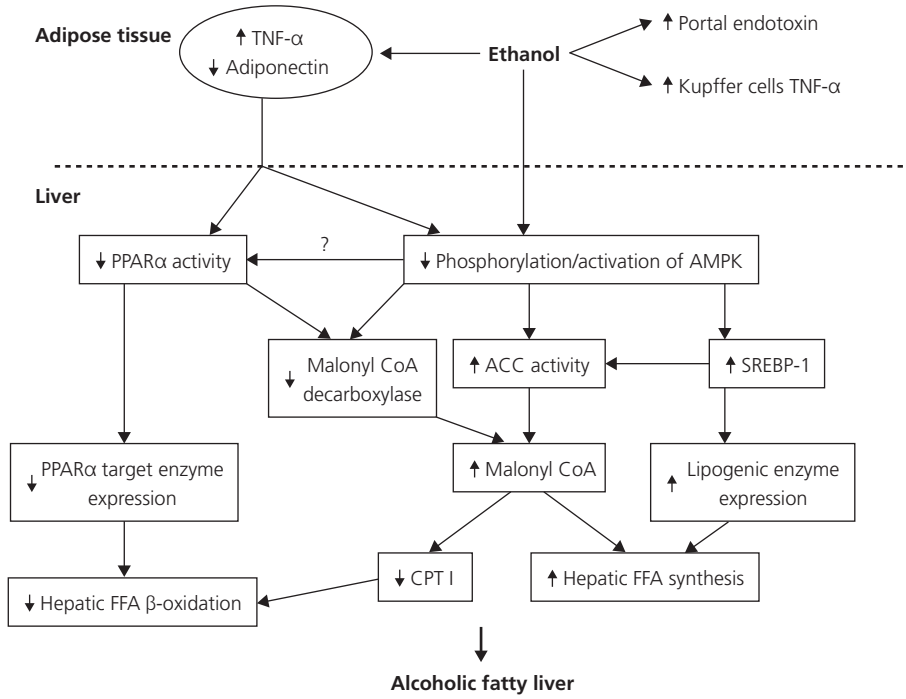

Figure 88.2 Potential mechanisms underlying the effect of alcohol on transcription factors, tumor necrosis factor-α (TNF-α), and adiponectin. Ethanol may influence the activity of peroxisome proliferator-activated receptor-α (PPAR-α), sterol regulatory element binding protein 1 (SREBP-1), and adenosine monophosphate-dependent protein kinase (AMPK) directly or by way of effects on adiponectin and TNF-α. These effects in turn activate lipogenic pathways, inhibit fatty acid oxidation pathways, and increase the concentration of malonyl-coenzyme A (CoA). Malonyl-CoA inhibits the entry of free fatty acid (FFA) into the mitochondria and blocks fatty acid oxidation in the mitochondrion. ACC, acetyl-CoA carboxylase; CPT I, carnitine palmitoyl transferase. From You & Crabb [323], with permission from the American Physiological Society.

injury [106–108]. Inactivation of Kupffer cells by gadolinium chloride ameliorates the ALD seen with chronic ethanol administration using the Tsukamoto–French model [109]. Endotoxin, or lipopolysaccharide (LPS), is derived from the cell wall of gram-negative bacteria (Fig. 88.3). There are three possible mechanisms involved in alcohol-induced endotoxemia:

- dysfunctional Kupffer cells with reduced ability to clear endotoxin
- bacterial overgrowth in the gut leading to excessive generation of endotoxin
- disruption of intestinal barrier function and increased permeability to endotoxin and bacteria [110].

Alcohol feeding increases endotoxin levels in the portal vein and induces the expression of the LPS binding protein and its receptor present on the Kupffer cell. CD14 (the Kupffer cell endotoxin receptor) plays an important role in ALD. Endotoxin binds CD14 in association with Toll-like receptor 4 (TLR4) to initiate intracellular signaling [107]. It has been shown that mice deficient in CD14 or TLR4 are resistant to ethanol-induced liver injury [108].

Kupffer cells that are stimulated by LPS produce inflammatory mediators, among them tumor necrosis factor-α (TNF-α), which is a cause of hepatocyte apoptosis; transforming growth factor-β1 (TGF-β1), the most important stimulus for collagen synthesis by stellate cells; interleukin-8 (IL-8), which attracts neutrophils to the liver (plasma IL-8 levels are markedly increased in patients with alcoholic hepatitis on admission, decreasing with recovery); IL-1 and IL-6, which elicit an acute-phase response; eicosanoids, which are implicated in the increased rate of hepatocyte oxygen consumption during ethanol metabolism; other chemokines that stimulate the migration of leukocytes into the liver; and platelet-derived growth factor, a major stimulator of hepatic stellate cell proliferation [111]. Kupffer cells are also capable of producing large amounts of potentially damaging free radicals, including superoxide, through the NADPH oxidase system. TNF-α appears to play a particularly important role in ALD [112]. TNF-α signals through two distinct cell surface receptors (TNFR1 and TNFR2). Multiple experimental approaches have revealed that TNFR1 initiates the majority of the biological activities of TNF-α in the liver [113]. Knockout mice lacking TNFR1 [114] and rats given antibody against TNF-α [115] are protected against liver injury in the Tsukamoto–French model.

Role of methionine cycle and folate metabolism

Chronic ethanol feeding of rodents impairs their ability to synthesize methionine, elevates homocysteine levels, reduces the synthesis of *S*-adenosylmethionine (SAM), and increases the levels of *S*-adenosylhomocysteine (SAH). This effect is explained by ethanol inhibiting methionine synthase (which converts homocysteine to methionine in conjunction with N5-methyl-tetrahydrofolate and vitamin B-12 [116,117]). McClain and colleagues demonstrated that a decrease in the SAM to SAH ratio was associated with significant increases in hepatic TNF-α levels, caspase-8 activity, and apoptotic rates [117]. SAH also sensitized hepatocytes to caspase-mediated apoptosis [118]. Folate deficiency commonly occurs in alcoholics and compounds the hyperhomocysteinemia [119].

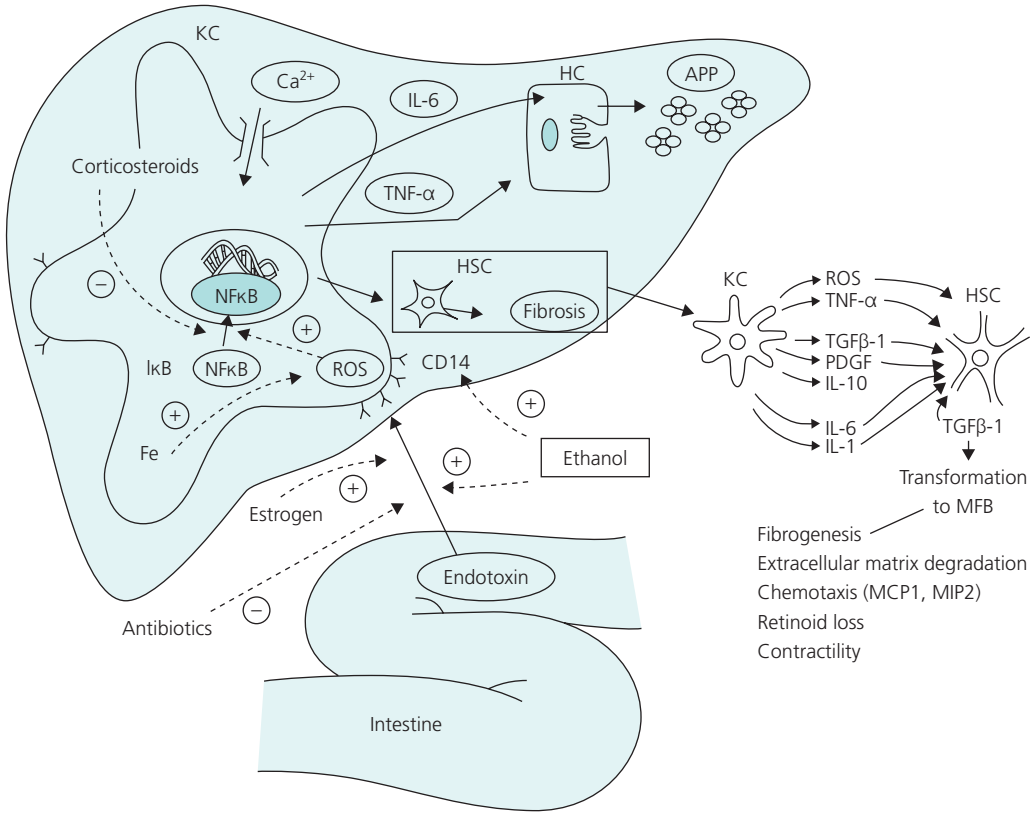

Figure 88.3 Ethanol consumption increases the absorption of lipopolysaccharide (LPS, endotoxin) from the gut. LPS in turn stimulates the endotoxin receptors (CD14) on Kupffer cells (KC). This action is more pronounced in females because of the effect of estrogen. Binding of endotoxin to the CD14 leads to activation of nuclear factor-κB (NF-κB); which then activates numerous inflammation-related genes, notably tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and transforming growth factor-β1 (TGF-β1). These cytokines have effects on the hepatocyte (HC), such as synthesis of acute-phase reactant protein (APP) and generation of reactive oxygen species (ROS). Their effects on hepatic stellate cells (HSC) lead to retinoid loss, and changes in extracellular matrix and collagen synthesis. Other abbreviations: IκB, inhibitor of NF-κB; Fe, iron; MFB, myofibroblast; MCP, monocyte chemotactic protein; MIP, macrophage inflammatory protein.

Micropigs fed ethanol on the background of a folate-deficient diet developed steatosis much earlier than those fed ethanol with a folate-supplemented diet [120], and also induced CYP2E1 and increased the levels of SREBP-1c [121]. Taken together, these data suggest that both folate deficiency and abnormal hepatic methionine metabolism may play an important role in the pathogenesis of ALD.

Role of endoplasmic reticulum stress

Recent work has implicated hyperhomocysteinemia in the pathogenesis of ALD through a mechanism involving endoplasmic reticulum stress [122,123]. The current hypothesis is that homocysteinylation of proteins causes their accumulation or malfolding in the endoplasmic reticulum, leading to a set of responses referred to as the endoplasmic reticulum stress response [124]. These include alterations of glucose-regulated proteins 78 and 94, SREBP, and caspase 12 [124] resulting in steatosis and apoptosis. Indeed, several recent studies have shown that alcohol may modulate both apoptotic and fat synthetic gene expression through homocysteine-induced endoplasmic reticulum stress [121,125] and correction of hyperhomocysteinemia by betaine reduced hepatic steatosis and the endoplasmic reticulum stress response [123,125].

Roles of adipokines

Adiponectin is a hormone secreted by adipocytes. Full-length adiponectin undergoes proteolytic processing, and a small amount of globular adiponectin circulates in plasma. Two adiponectin receptors, AdipoR1 and AdipoR2, have been identified [126]. AdipoR1 is a high-affinity receptor for globular adiponectin and a low-affinity receptor for the full-length ligand, whereas AdipoR2 is an intermediate-affinity receptor for both forms of adiponectin. AdipoR1 is abundantly expressed in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver [126]. Decreased levels of circulating adiponectin are associated with obesity, insulin resistance, type 2 diabetes, and atherosclerosis, and administration of adiponectin diminished the abnormalities

associated with the metabolic syndrome [127]. The effect of adiponectin is largely mediated by an increase in fatty acid oxidation associated with activation of AMPK and PPAR-α pathways downstream of the adiponectin receptors, both in vitro and in vivo [94]. Chronic consumption of a high-fat, ethanol-containing diet significantly decreased circulating concentrations of adiponectin by 30%–40% after 3–4 weeks [128]. Delivery of recombinant adiponectin into these mice dramatically alleviated hepatomegaly and hepatic steatosis. Whereas chronic ethanol feeding significantly decreased the rate of hepatic fatty acid oxidation, adiponectin treatment restored it [128]. It is possible that reduction of hepatic fatty acid oxidation during ethanol consumption results from the suppression of adiponectin (Fig. 88.2). The inhibitory effect of alcohol on adiponectin is mediated through alcohol's ability to activate TNF-α [128], suggesting the importance of interactions between cytokines and adipokines in the pathogenesis of ALD.

Role of microsomal triglyceride transfer protein, c-Met, and hepatocyte growth factor

Export of triglyceride from the hepatocyte requires the synthesis of apolipoprotein B and microsomal triglyceride transfer protein (MTP) [129,130]. Ethanol feeding has been shown to down-regulate the transcription of the MTP gene [131] and decrease its activity [132]. Hepatocyte growth factor can reverse alcohol-induced steatosis by inducing expression of the genes for both MTP and apolipoprotein B [133]. Hepatocyte growth factor acts by way of a receptor encoded by the protooncogene c-Met [134]. Of note, a recent study showed that pioglitazone, a PPAR-γ agonist, can also activate c-Met, increase hepatic apolipoprotein B expression, and thereby attenuate ethanol-induced hepatic steatosis [135].

Role of apoptosis

Numerous factors related to alcohol metabolism can induce apoptosis of hepatocytes. It may result from oxidative stress induced by ethanol, reducing glutathione levels [136], and increased levels of TNF-α [137] and TGF-β [111]. Hepatocyte apoptosis is regulated by a system consisting of Fas and Fas ligand [138,139]. Fas (CD95) is a receptor found on hepatocytes, and it can interact with the Fas ligand present on the surface of certain T cells. These receptor/ligand proteins are evolutionarily related to the TNF/TNFR proteins. The interaction between the Fas ligand and soluble Fas triggers activation of apoptosis-inducing caspases and release of cytochrome c from the mitochrondria [139]. Apoptosis is a rapid process so routine hematoxylin & eosin staining for apoptotic bodies underestimates the extent of apoptosis; so, the TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) assay and immunohistological methods are needed to evaluate apoptosis. Apoptosis is widespread in alcoholic hepatitis, and the apoptotic index is correlated with Maddrey discriminant function (see below) [140] and histological severity [141] in patients with alcoholic hepatitis.

Role of cytokines in activation of hepatic stellate cells

The final elements in alcoholic liver injury are activation of the hepatic stellate cells (HSC) and production of collagen and other matrix proteins [142]. This fibrogenic process requires the interaction of Kupffer cell-derived cytokines with the HSC (Fig. 88.3). One of the earliest events is the activation of nuclear factor-κB (NF-κB) in the HSC [143]. Products of ethanol metabolism and lipid peroxidation such as acetaldehyde, MDA, MDA–acetaldehyde adducts, and 4-HNE can also induce HSC to express collagen genes [142]. The γ-isoform of PPAR may participate in the control of HSC differentiation. Expression of this receptor falls during HSC activation, and treatment of the cells with PPAR-γ ligands retards the activation of the cells [144,145].

Once NF-κB is activated, the HSC express receptors for and can be stimulated by platelet-derived growth factor [146]. They also can be stimulated to secrete collagen by TGF-β1 and IL-1 [142]. This activation may become irreversible, leading to progressive fibrosis and cirrhosis. The irreversible activation may be driven by the changes the cells make in the extracellular matrix, such as deposition of type I collagen, production of TGF-β1 by the HSC themselves, and depletion of retinyl ester stores in the HSC [142]. HSC themselves can be activated directly by endotoxin through the CD14/TLR4 pathway [147]. Of note, these factors may lead to progressive collagen deposition even if the patient stops drinking.

Gender differences in the response to alcohol

There are important gender differences in the pathogenesis of ALD. At any given level of alcohol consumption, women have a higher likelihood of developing ALD than men [14]. This phenomenon is not fully understood; however, one study showed that the levels of gastric ADH were lower in women than in men, which led to higher blood alcohol concentrations in women who consumed the same amount of alcohol [148,149].

Female rats are more susceptible to ALD than male rats. Kupffer cells from female rats fed ethanol had a significantly greater response to LPS than Kupffer cells from male rats [150]. Moreover, female rats produced more TNF-α in response to chronic ethanol than males, suggesting that Kupffer cells in females are more active following ethanol exposure [150]. CD14 expression and NF-κB activation are also higher in Kupffer cells isolated from female mice. Estrogen might play role in ALD in females by modulating the oxidative stress of ethanol metabolism [151]. In summary, many mechanisms may conspire to make female alcoholics more prone to the development of alcoholic hepatitis and cirrhosis.

Clinical manifestations

ALD represents a spectrum of clinical illness and pathological change in individuals with acute and chronic alcohol consumption. Patients may have minimal abnormalities from steatosis or may develop more severe signs and symptoms of liver disease associated with the inflammation seen in alcoholic hepatitis or cirrhosis. Since alcohol abuse or dependence may be present in all stages of ALD, physicians should take a thorough history concerning alcohol use and standard screening questionnaires should be performed on all patients [152]. Denial is a major component of alcohol abuse, and alcoholics typically underestimate their consumption or deny that they have a problem with alcohol [153]. Several questionnaires have been developed and validated to screen for or establish the diagnosis of alcoholism. The CAGE questionnaire is the most commonly used for screening because it is fast and easy to remember [154]. The CAGE questionnaire consists of a battery of four questions:

- Have you ever felt the need to **C**ut down on drinking?
- Have you ever felt **A**nnoyed by criticism of your drinking?
- Have you ever felt **G**uilty about drinking?
- Have you ever had an **E**ye opener (early morning drinking)?

As a screening tool, the CAGE questionnaire does not discern between current and past drinking patterns. Another tool, the Alcohol Use Disorder Identification Test (AUDIT), was developed to improve the sensitivity of identifying heavy drinking and active alcohol use [155]. When using the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) criteria for an alcohol-use disorder as the gold standard, the AUDIT performed best at a cutoff score of 9 (sensitivity, 0.76; specificity, 0.79), and CAGE at a cutoff score of 1 (sensitivity, 0.69; specificity, 0.63). The AUDIT performed better overall than the CAGE test [156].

Clinical diagnosis of the three major stages of ALD is difficult because these stages may occur in any combination in the same patient, emphasizing the importance of liver biopsy. A study from Italy showed that there was no clear-cut association between typical histological features of ALD and the amount of alcohol consumption [64]. However, for purposes of instruction, it is useful to divide ALD into its three major types, alcoholic fatty liver, alcoholic hepatitis, and cirrhosis, and to discuss the clinical, laboratory, and pathological features of each. The role of physical examination to diagnose ALD is limited and the findings may vary from normal to evidence of hepatic decompensation or cirrhosis. A number of less common variants are listed in Table 88.4 and are discussed within the following sections.

Table 88.4 Less common pathological features of alcoholic liver disease

Alcoholic fatty liver with perivenular fibrosis
Alcoholic foamy degeneration (alcoholic microvesicular steatosis)
Sclerosing hyaline necrosis
Alcoholic cirrhosis with chronic hepatitis[a]
Cholestasis
Hepatic iron overload
Lipogranuloma
Massive hepatic necrosis (due to acetaminophen ingestion)
Focal fatty liver or focal fat sparing

a A significant proportion of these patients likely have concomitant hepatitis B or C virus infection.

Alcoholic fatty liver

Consumption of ethanol in sufficient quantities (in the range of 120–150 g/day for 2–3 weeks) will result in fatty liver and a classic study showed that abstinence for 4 weeks leads to resolution [157]. Stigmata of alcoholism (e.g., Dupuytren contractures, testicular atrophy, palmar erythema, spider angiomata, and gynecomastia) may also be present. Evidence of hypogonadism and feminization are evident in alcoholic males before the onset of liver disease, resulting from the toxic effects of ethanol on Leydig cells, leading to decreased testosterone production; impairment of hypothalamic–pituitary function resulting in low levels of luteinizing hormone; and induction of aromatase in adipose tissue and increased conversion of androgens to estrogens [158].

Alcoholic fatty liver is rarely diagnosed clinically because most patients are asymptomatic and do not seek medical attention. However, some cases of biopsy-proven alcoholic fatty liver are associated with constitutional symptoms of weakness, cachexia, fever, anorexia, nausea, vomiting, jaundice, hepatic tenderness, splenomegaly, or ascites. Most likely, such cases represent a combination of fatty liver and alcoholic hepatitis, because the classic lesions of alcoholic hepatitis are difficult to find in livers with panlobular steatosis, or may be missed because of sampling error. Laboratory abnormalities in alcoholic fatty liver are generally mild. Bilirubin is elevated in 25% of cases, but is usually less than 5 mg/dL. As in all stages of ALD, serum aspartate aminotransferase (AST) and alanine aminotransferases (ALT) are usually less than 300 IU/l (with the exception of foamy degeneration, see below). The AST is usually higher than serum ALT (with the AST : ALT ratio being at least 2 in 80% of cases). Hepatic deficiency of pyridoxal-6-phosphate, a cofactor necessary for ALT enzymatic activity, is thought to cause the smaller ALT elevation in these patients [159]. Alkaline phosphatase is usually no more than 300 IU/dL. The ratio of γ-glutamyltransferase to alkaline phosphatase activity can be more than 5, as a result of the disproportionate induction of γ-glutamyltransferase by alcohol abuse. This ratio is more specific than sensitive provided that the patient is not on drugs that also induce γ-glutamyltransferase (e.g., anticonvulsants). In alcoholic fatty liver, serum albumin and globulin levels are expected to be normal in the absence of coincidental medical

conditions, including malnutrition. Microscopically, there is great variation in the number of cells affected, with a tendency for fat accumulation in the perivenous and midzones of the liver lobule. Occasionally, hepatocytes rupture to form a fatty cyst, and then a lipogranuloma. Generally, there is sparse cell necrosis or inflammation. Intrahepatic cholestasis and mild cholangiolitis may be seen in the absence of extrahepatic biliary obstruction [160].

Another clinical variant of ALD is alcoholic foamy degeneration [161]. It is characterized by a marked, transient elevation of serum aminotransferases, alkaline phosphatase, serum lipids, and bilirubin levels. Liver biopsies demonstrate an intact lobular architecture, and perivenular hepatocytes show the presence of megamitochondria, extensive microvesicular fat accumulation without nuclear displacement, and cytoplasmic bile pigment deposition. Foci of hepatocyte dropout without a significant parenchymal infiltration with neutrophils are seen in these patients, more consistent with a degenerative than an inflammatory process [161]. All patients recover rapidly after abstaining from alcohol. Alcoholic foamy degeneration has been noted in about 2% of a series of over 300 patients in Spain [162]. There are yet other variants of fatty liver diagnosed radiographically as focal fatty liver and focal fat sparing. As these names imply, the former is the result of focal accumulation of fat while the rest of the liver is not steatotic. The latter indicates the reverse. These lesions may be confused with mass lesions on ultrasound or abdominal computed tomography (CT) scan. In the past, these conditions could be diagnosed by liver spleen scan [163,164]. With today's technology, magnetic resonance imaging will provide definitive diagnosis of these conditions and will distinguish them from mass lesions.

Alcoholic hepatitis

Alcoholic hepatitis is the most florid manifestation of ALD and is associated with a high mortality [165]. Classically, alcoholic hepatitis presents with fever, jaundice, hepatomegaly, and signs of decompensated liver disease, such as ascites and hepatic encephalopathy. Fever is common and it is important to exclude other potential sources of infection, including spontaneous bacterial peritonitis, pneumonia, and urinary tract infection. Once set in motion, alcoholic hepatitis seems to progress despite stopping drinking. Physical examination usually reveals obvious signs of protein-calorie malnutrition [166]. Stigmata of chronic liver disease should increase the suspicion of underlying cirrhosis. On laboratory examination, pancytopenia is not uncommon and it is secondary to the toxic effects of ethanol on the bone marrow, impaired pyridoxine metabolism, folic acid deficiency, iron deficiency from blood loss, or hypersplenism. Patients might present with leukocytosis, and it is probably driven by increased levels of IL-8 generated in the liver or concurrent infections [167,168]. Serum AST and ALT are elevated in all cases and the AST : ALT ratio is usually greater than 2. Alkaline phosphatase is elevated in 80% of cases. High values of serum AST and ALT (> 300 IU/L) should raise the suspicion of alternative diagnoses, such as coincidental viral hepatitis, hypoxic liver injury (in which the AST : ALT ratio is also increased), acetaminophen overdose, or sclerosing hyaline necrosis (see below). Bilirubin is usually increased, whereas albumin is usually decreased and the prothrombin time is often prolonged. Electrolyte abnormalities are frequent in alcoholic hepatitis, and include hyponatremia, hyperchloremia as the result of renal tubular acidosis, hypokalemia, and hypophosphatemia from poor dietary intake, vomiting, or diarrhea, hypomagnesemia from increased urinary loss, and respiratory alkalosis from hepatic encephalopathy.

The common histological findings in alcoholic hepatitis are ballooning degeneration, focal hepatocyte necrosis, and neutrophilic infiltration [160]. The latter two are considered absolute criteria for the diagnosis of alcoholic hepatitis. More than 30% of patients develop Mallory bodies, an aggregation of perinuclear, eosinophilic, amorphous material. Although highly suggestive of ALD, Mallory bodies are also seen in many other liver diseases (Table 88.5). Other pathological findings in alcoholic hepatitis include steatosis and fibrosis or cirrhosis [160]. These pathological findings may be indistinguishable from features seen in nonalcoholic steatohepatitis. Clinically, patients with alcoholic hepatitis are much sicker than those with nonalcoholic steatohepatitis. In a few cases of alcoholic hepatitis, there is *sclerosing hyaline necrosis*, which is characterized by severe, irregular, confluent, centrilobular necrosis with obliteration of the central vein by collagen [169]. Venoocclusive lesions have also been described and these pathological findings are associated with recalcitrant ascites, variceal bleeding, and a high mortality rate [170].

Alcoholic cirrhosis

Patients with alcoholic cirrhosis have similar clinical features to those with other types of cirrhosis. Cirrhosis may be suspected in patients with stigmata of chronic liver disease on physical examination or laboratory abnormalities suggestive of synthetic dysfunction. Of note, alcoholic cirrhosis may be asymptomatic in 10%–20% of patients. The liver test abnormalities in alcoholic cirrhosis are less pronounced than those in alcoholic hepatitis. In fact, in compensated cirrhosis, many of the liver tests are close to normal. Laboratory abnormalities may include mild increases in aminotransferases and alkaline phosphatase, low serum albumin, polyclonal elevation of serum globulins (greater than 4 g/dL), thrombocytopenia, and anemia. Interestingly, thrombocytopenia is an excellent predictor for the presence of large varices and therefore cirrhosis [171].

The pathological end-stage of ALD is characterized by fibrous bands connecting portal triads with central veins, and by regenerative nodules. The nodules are typically small (1–3 mm) and uniform in size [160]. Ethanol suppresses hepatic regeneration [172]; so with prolonged abstinence,

Table 88.5 Differential diagnosis of liver diseases exhibiting Mallory hyaline

Alcoholic cirrhosis (especially with continued drinking)[a]
Steatohepatitis
Alcoholic steatohepatitis[a]
Nonalcoholic steatohepatitis[a]
Chronic hepatitis C
Parenteral nutrition-induced liver disease
Drug-induced liver disease (e.g., griseofulvin, nifedipine, nicardipine, didanosine, diethylstilbestrol, tamoxifen, tetracycline, valproic acid, vitamin A, diltiazem, glucocorticoids, methotrexate, or amiodarone)
Chronic cholestatic disorders
Primary biliary cirrhosis[a]
Primary sclerosing cholangitis
Chronic biliary obstruction
Extrahepatic biliary atresia
Copper overload conditions
Wilson disease[a]
Indian childhood cirrhosis[a]
Congenital hepatic fibrosis
Copper-associated liver disease of childhood
Hepatocellular neoplasms/masses
Hepatocellular carcinoma
Hepatic adenoma
Dysplastic hepatocellular nodule
Focal nodular hyperplasia
Adenomatous hyperplasia
Weber–Christian disease
Inherited diseases of metabolism
Abetalipoproteinemia
α_1-Antitrypsin deficiency
Glycogen storage disease, type Ia[a]
Postinfantile giant cell hepatitis
Juvenile Parkinson disease

a Indicates that Mallory bodies are found occasionally to commonly; in the other conditions, they are rare.

nodular regeneration becomes more vigorous and may result in mixed micro/macronodular or macronodular cirrhosis. Superimposed fatty change and alcoholic hepatitis are often seen with cirrhosis. There may also be increased stainable iron in biopsy samples. Iron overload in alcoholic cirrhosis can be differentiated from hereditary hemochromatosis by genetic analysis for *HFE* mutations or by quantitative iron analysis. In hereditary hemochromatosis, the hepatic iron index (hepatic iron content expressed as μmol/g dry weight divided by the patient's age) in the cirrhotic liver is more than 1.9 whereas that in the cirrhotic liver of alcoholics is less than 1.9 [173]. In some patients with alcoholic cirrhosis, there is portal inflammation with piecemeal necrosis. This feature may reflect an autoimmune component of ALD, unsuspected Wilson disease (particularly in young patients), or, most commonly, coexistent chronic viral hepatitis – particularly hepatitis C virus (HCV) infection [174].

Role of liver biopsy

Liver biopsy provides valuable information by evaluating for alternative etiologies of liver disease, providing prognostic information by the severity of liver pathology, and confirming the diagnosis of alcoholic hepatitis; however, it is controversial whether a liver biopsy should be performed in patients with suspected ALD. A liver biopsy may be needed if the diagnosis is in doubt or other alternative diagnoses are suspected. However, a percutaneous liver biopsy in such patients is often contraindicated because of the underlying coagulopathy, thrombocytopenia, and ascites. In such cases, transjugular liver biopsies may be necessary [175].

Differential diagnosis

There are numerous pitfalls in the diagnosis of ALD, among them failing to consider ALD in patients not conforming to the alcoholic stereotype, and assuming that abnormal liver tests in an alcoholic patient are the result of ALD. It should be noted that in some populations, more than 50% of patients with alcohol abuse who develop chronic liver disease have concurrent viral hepatitis, especially HCV [176]. As previously mentioned, alcoholism should be considered in any patient with liver disease, and a careful drinking history should be obtained from the patient and a reliable third party, such as a family member.

Alcoholic fatty liver

As outlined in Table 88.6, fatty liver can result from many processes. The differential diagnosis of massive hepatomegaly includes fatty liver, right heart failure, constrictive pericarditis, Budd–Chiari syndrome, infiltrative processes (e.g., amyloidosis, myeloproliferative disorders, reticuloendotheliosis, and lipid storage diseases), and neoplasms. A helical CT scan with dual (arterial/venous) phases is the diagnostic modality of choice to evaluate the cause of hepatomegaly because it is more sensitive in detecting focal hepatic masses than a CT scan performed only in the venous phase, an ultrasonogram, or a sulfur colloid scan [177].

Alcoholic hepatitis

The clinical presentation of patients with alcoholic hepatitis may mimic the presentation of patients with nonalcoholic steatohepatitis or cholangitis from choledocholithiasis. Generally, patients with alcoholic steatohepatitis are much sicker than those with nonalcoholic steatohepatitis and they tend to have higher AST : ALT ratios [165]. Imaging of the liver and biliary tree may be necessary to exclude choledocholithiasis. Depending on the experience of the ultrasonographer, the sensitivity of abdominal ultrasound to detect

Table 88.6 Potential causes of fatty liver

Macrovesicular fat
Alcoholic steatohepatitis
Nonalcoholic steatohepatitis
Hepatitis C
Toxins and drugs: methotrexate, halogenated hydrocarbons, niacin, HIV protease inhibitors, glucocorticoids, heavy alcohol use
Nutritional disorders: obesity, diabetes, choline deficiency, systemic carnitine deficiency, celiac disease, kwashiorkor, parenteral nutrition
Lipodystrophy
Wilson disease
Abetalipoproteinemia
Inflammatory bowel disease
Weber–Christian disease
Q fever
Microvesicular fat[a]
Reye syndrome
Parenteral alimentation
Yellow fever
Heat stroke
Toxins and drugs: valproic acid, intravenous tetracycline, toxic shock syndromes, salicylate overdosage in children, Jamaican vomiting disease, FIAU, nucleoside reverse transcriptase inhibitors, mycotoxins
Metabolic diseases: cholesterol ester storage disease, galactosemia, Wolman disease, long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency, medium-chain acyl-CoA deficiency
Complications of pregnancy: acute fatty liver of pregnancy, eclampsia, HELLP syndrome

a Microvesicular fatty liver is usually the result of mitochondrial damage and defects in lipid oxidation pathways; however, there is significant overlap with many disorders occasionally producing microvesicular fatty liver.

CoA, coenzyme A; FIAU, fialuridine; HELLP, hemolysis, elevated liver enzymes, and low platelet syndrome; HIV, human immunodeficiency virus.

choledocholithiasis is around 50%–90%. A CT scan of the abdomen has similar sensitivity to ultrasound to detect common bile duct stones [178–180]. Magnetic resonance cholangiopancreatography and endoscopic ultrasound are much better diagnostic tests to detect common duct stones with sensitivities of 90% and 95%, respectively [180]. However, these tests might not be readily available in most centers. The gold standard test to detect choledocholithiasis is endoscopic retrograde cholangiopancreatography, which (compared to other tests) has the advantage of permitting intervention if a common bile duct stone is present. However, endoscopic retrograde cholangiopancreatography is invasive and may cause complications such as pancreatitis.

Alcoholic cirrhosis

Alcoholic cirrhosis is usually micronodular but may sometimes be macronodular. The differential diagnosis of alcoholic cirrhosis includes posthepatitic cirrhosis (whether as the result of chronic viral infection, drugs, metabolic disorders, or autoimmune hepatitis), the cirrhotic stage of primary or secondary biliary cirrhosis, Wilson disease, hemochromatosis, and cirrhosis caused by nonalcoholic steatohepatitis. The degree of fatty infiltration of the liver may decrease as cirrhosis develops [181]. Alcoholic cirrhosis with features of chronic hepatitis may reflect superimposed hepatitis B or C or an autoimmune process incited by alcoholic injury [176]. Disease processes that mimic cirrhosis (e.g., constrictive pericarditis, Budd–Chiari syndrome, venoocclusive disease, idiopathic portal hypertension, portal vein thrombosis, and myeloid metaplasia) should be considered.

Special considerations

HCV and ALD

Alcohol abuse and HCV infection coexist with chronic liver disease in many patients. The prevalence of HCV markers in alcoholic patients without liver disease ranges from 0% to 14%; in all alcoholic patients the prevalence ranges from 11% to 35%; and in alcoholic patients with chronic liver disease the prevalence ranges from 18% to as much as 51% [182]. There is no clear-cut explanation for the increased prevalence of hepatitis C infection in alcoholics. Some reports suggested that the increased prevalence of anti-HCV in alcoholics is primarily the result of a past history of intravenous drug use [183,184]. Several studies have shown that alcohol accelerates the progression of liver injury in patients with HCV, leading to cirrhosis and hepatocellular carcinoma [185]. Histological activity and fibrosis increase with the amount of alcohol use. One study from France showed that in patients with HCV who drank more than 50 g of alcohol per day, there was a 30% increased rate of fibrosis compared with those who drank less [186]. The effect of alcohol consumption on the progression of HCV is also seen in patients who drank even moderate amounts of alcohol, as low as 30–50 g/day in men and 20–50 g/day in women [187]. Another possibility for the progression of fibrosis in patients with HCV and ALD is the concurrent use of illicit drugs, especially marijuana, in alcoholics. The cannabinoids present in marijuana exert biological effects through cannabinoid receptors CB1 and CB2, which regulate progression of experimental liver fibrosis. In a recent population-based study, daily cannabis use was found to be an independent predictor of fibrosis progression rate when compared to nonusers or occasional users (odds ratio 3.6; 1.5–7.5). Furthermore, severe fibrosis was also predicted by daily cannabis use (odds ratio 2.5; 1.1–5.6; $P = 0.034$), independently of Metavir activity grade, excessive alcohol intake, age at liver biopsy, and underlying steatosis [188]. The results of these studies highlight that there is no known safe drinking level for patients with HCV infection, and that patients with HCV

infection should be advised to refrain from drinking alcohol and from illicit drug use.

The mechanisms of advanced liver injury caused by coexisting ALD and HCV remain poorly understood. Hepatitis C core protein can inhibit MTP, leading to hepatic steatosis [189]. Alterations in the immune system secondary to alcohol use have also been reported. One study in mice showed that ethanol-fed mice have inhibition of T helper and cytotoxic T-lymphocyte responses to HCV core protein, and so have reduced production of the cytokines needed to eradicate the virus. Additionally, in the presence of HCV, alcohol consumption causes an enhanced inflammatory response in the liver. So the immunosuppressive effect of alcohol might contribute to both the lack of clearance of HCV and the viral persistence, which results in an increased nonspecific inflammatory response [185,190,191]. Oxidative stress might also play a role in the progression of fibrosis. Both ethanol consumption [29] and HCV core protein [192] cause oxidative damage by triggering the release of mitochondrial reactive oxygen species, leading to increased rates of lipid peroxidation. A recent study showed a synergistic effect of alcohol and HCV on the production of several cytokines such as TGF-β and TNF-α [193].

Alcohol also exerts effects on HCV quasispecies generation. HCV quasispecies complexity was increased, leading to viral escape from the immune response in alcoholic patients [194]. There are conflicting reports about the effects of alcohol on serum HCV RNA. A recent metaanalysis of 14 studies failed to show an association between alcohol use and HCV viral load [195].

Alcohol consumption has been shown to decrease the response to interferon in patients with HCV. Tabone and colleagues studied the effect of interferon treatment in patients with hepatitis C whose ALT levels remained twice the upper limit of normal despite 6 months of complete alcohol abstinence [196]. The sustained virological response was 33% in non-drinkers, 20% in mild drinkers, and only 9% in those who drank heavily. Alcohol use also has an impact on treatment response and it is associated with treatment failure in patients with hepatitis C undergoing treatment with pegylated interferon and ribavirin [197]. These findings suggest that patients should be abstinent from alcohol before the initiation of hepatitis C therapy.

Hepatic iron stores and ALD

Iron is a vital element in cellular electron transport and is involved in heme-containing enzyme function. However, excess iron facilitates the generation of reactive oxygen species and catalyzes the formation of hydroxyl radicals from hydrogen peroxide [198]. Increased hepatic iron stores and serum markers of iron overload have been observed in alcoholics [199,200]. The mechanism of the increase in iron accumulation is not known, although increased hepatocyte iron uptake secondary to upregulation of the transferrin receptor has been found in animal models and in humans [201].

The degree of iron accumulation in ALD is usually mild. However, approximately 4% of patients may have hepatic iron stores twice the upper limit of normal [198,202]. The confusion as to whether hepatic iron overload is a form of ALD or hereditary hemochromatosis has been resolved by the use of the hepatic iron index and by the availability of *HFE* gene analysis [173,198]. The former is valuable in the distinction of ALD with iron overload from hereditary hemochromatosis by emphasizing that the index is greater than 1.9 μmol/g dry weight of liver per year of life in individuals with hereditary hemochromatosis [203], but not ALD. Moreover, with the availability of *HFE* gene analysis, the distinction of hereditary hemochromatosis from other liver diseases with iron overload has become further defined [198]. Patients with ALD and a high hepatic iron index are usually homozygotes for the C282Y *HFE* mutation [204]. Patients with hereditary hemochromatosis who are heavy alcohol drinkers (> 60 g/day) have a higher prevalence of cirrhosis at lower levels of hepatic iron and a reduced long-term survival, despite adequate phlebotomy therapy, which is indicative of the synergy between the hepatotoxic effect of alcohol and iron [205].

Clinical course and prognosis of alcoholic liver disease

Alcoholic fatty liver

Historically, patients with evidence of only hepatic steatosis on biopsy were thought to have a benign prognosis. However, in 1995 Teli and colleagues demonstrated that 18% of patients with simple hepatic steatosis progressed to fibrosis or cirrhosis in 10 years [206,207]. Severity of steatosis, female sex, and the presence of giant mitochondria were independent predictors of disease progression [206]. In animals, fatty livers are highly susceptible to toxicity from endotoxin [208], and it is well known that steatotic donor livers increase the risk of graft nonfunction after liver transplantation [209,210], therefore, fatty liver is not a benign condition as previously thought.

Alcoholic steatohepatitis

Alcoholic steatohepatitis occurs less frequently than alcoholic steatosis but carries a worse prognosis. It has been estimated that 10%–30% of heavy drinkers will develop alcoholic steatohepatitis in their lifetime [152,211]. Factors influencing the progression of disease in patients with steatohepatitis include continued alcohol consumption, female gender, age, and high body mass index [211,212]. As many as 56% of patients with steatohepatitis who continue to drink heavily will progress to cirrhosis and improvement in histology is found in 27% of patients who achieved abstinence [212].

Alcoholic hepatitis is perhaps the most severe manifestation of ALD and is associated with a high mortality. Identification of those patients at greatest risk of death is vital not only for decisions relating to individual management but also for the design of clinical studies assessing possible treatments for this condition. Several clinical scoring systems have been derived to predict the clinical outcome of patients with alcoholic hepatitis. These scores include the Child–Turcotte–Pugh score (CTP) [213], which is used commonly to stage the severity of cirrhosis, the combined clinical and laboratory index of the University of Toronto (CCLI) [214], the Maddrey discriminant function [215], the MELD (model for end stage liver disease) scores [216], and most recently the Glasgow alcoholic hepatitis score (GAHS) [165]. The admission CTP score is independently associated with mortality; however, this scoring system is not sensitive to predict the mortality during the hospital stay [217] The CCLI permits a linear estimate of acute mortality in persons with alcoholic hepatitis. Its major disadvantages are the large number of variables that must be scored and the complexity of the calculation itself [214]. The discriminant function (DF) score was derived in clinical trials of patients with alcoholic hepatitis and has been widely applied clinically. The score is based on prothrombin time and bilirubin, and it is calculated as follows: (4.6 × prothrombin time prolongation) + total serum bilirubin in mg/dL. In combination with the presence of encephalopathy, a DF score greater than 32 is highly correlated with a more than 50% short-term (30-day) mortality rate. Although DF is a continuous scoring system, its interpretation (using the cutoff of 32) has converted it into a categorical method of classification [207]. Therefore, this scoring system suffers from the problem that once patients' scores have exceeded the cutoff value, they cannot be further characterized [207]. The model for end-stage liver disease (MELD) is a recently developed scoring system that has been validated as an independent predictor of short-term survival in patients who are on the liver transplantation list. In a study of 34 patients, there was a 45% 30-day survival in patients with a MELD score greater than 11, whereas those with a score of 11 or less had a survival of 96% [216]. Two recent studies showed that the MELD score is useful for predicting 3-month mortality of patients with severe alcoholic hepatitis who are not treated with corticosteroids [217,218]. Its accuracy was comparable to that of DF in predicting 3-month mortality of patients with severe alcoholic hepatitis [218,219]. Using the optimal cutoff point (21 for MELD and 37 for DF), MELD had a sensitivity of 75% and a specificity of 75% and DF had a sensitivity of 88% and a specificity of 65%. Another study confirmed that DF and MELD had similar accuracy in predicting survival of patients with alcoholic hepatitis [217,219].

The GAHS was developed and validated in 2005 [165]. The GAHS was derived from five variables independently associated with outcome: age, and (based on day 1 results) serum bilirubin, blood urea nitrogen, and (based on day 6–9 results), serum bilirubin, prothrombin time, and peripheral white blood cell count (Table 88.7). Values obtained ranged from 5 to 12 and there was a marked rise in mortality when a score greater than 9 was attained. Although this cutoff value had less sensitivity to predict mortality when compared with the DF score, a GAHS of at least 9 on day 1 had an overall accuracy of 81% when predicting 28-day outcome. In contrast, the DF had an overall accuracy of 49%. Similar results were found using information at 6–9 days to predict 84-day mortality [165].

Table 88.7 The Glasgow Alcoholic Hepatitis Score

	Score		
	1	**2**	**3**
Age (years)	< 50	≥ 50	
White blood cell count (× 10^9/mL)	< 15	≥ 15	
Blood urea nitrogen (mmol/L)	< 5	≥ 5	
Prothrombin time ratio (INR)	< 1.5	1.5–2.0	> 2.0
Bilirubin (μmol/L)	< 125	125–250	> 250

From Forrest et al. [165], with permission from the BMJ Publishing Group Ltd.

Alcoholic cirrhosis

As previously mentioned, the amount of ethanol that can be safely drunk (moderate drinking) is estimated to be up to 20 g (two drinks) per day for men and up to 10 g (one drink) for women. Approximately 15%–20% of patients who chronically drink alcohol will progress to cirrhosis in their lifetime [211]. Abstinence from alcohol is essential in treatment because it has been shown to abrogate the progression of disease and need for liver transplantation [220]. The prognosis is poor in patients who continue to drink. One study reported 80% mortality within 7 months in those with decompensated alcoholic cirrhosis who continued to drink [211,221]. Overall 5-year survival rates range from 20% to 50% [222,223].

Complications of alcoholism and alcoholic liver disease

Metabolic disorders

One of the metabolic complications in alcoholic patients is alcoholic ketoacidosis [224]. Alcoholics who are fasting while drinking heavily exhibit similar metabolic alterations to an insulin-deficient state; i.e., high serum free fatty acids, low insulin, and high glucagon and catecholamine levels. Patients with alcoholic ketoacidosis typically present with an otherwise unexplained high anion gap metabolic acidosis, ketonemia (specifically, elevated β-hydroxybutyrate, as the result of the redox shift that accompanies alcohol metabolism), and a variable plasma glucose concentration that may be low, normal, or modestly elevated. Of note, lactic acidosis can also

occur in these patients [225]. Other electrolyte disorders, including hyponatremia, hypokalemia, hypophosphatemia, hypocalcemia, and hypomagnesemia, are common [226].

Another unique metabolic complication in alcoholic patients is hypoglycemia. The high NADH concentration occurring during alcohol metabolism promotes the formation of reduced metabolites, which in turn inhibit gluconeogenesis, resulting in fasting hypoglycemia. Treatment for these patients includes adequate hydration and administration of glucose. Generally, this syndrome is rapidly reversible and has a low mortality [226].

Lastly, the interaction between induced activity of CYP2E1 in alcoholics and acetaminophen is clinically important [227]. Although therapeutic doses of acetaminophen are harmless when consumed by alcohol-naive individuals, in alcoholics such doses can produce serious liver disease with extremely high aminotransferase levels, massive perivenular hepatic necrosis, pancreatitis, and renal failure. Heavy drinkers should be warned to avoid acetaminophen, and in particular to read medication labels of over-the-counter medications to be sure that they do not inadvertently consume more than 2 g/day. On the other hand, small doses of acetaminophen are safer in patients with cirrhosis than nonsteroidal antiinflammatory drugs, which have adverse effects on platelet and renal function.

Infections

Alcoholics are more prone to infections than other patients with liver disease as a result of malnutrition, impaired phagocyte function, and other immune defects [228]. Bacteremia and spontaneous bacterial peritonitis are common in patients with cirrhosis. Other infections include pneumonia and lung abscess, bacterial meningitis, and urinary tract infections [229]. Patients with cirrhosis are also at increased risk of sepsis from opportunistic fungal infections, *Listeria monocytogenes*, or *Vibrio* spp., especially *V. vulnificus*, when they consume raw seafood such as oysters [230] or have skin wounds that are exposed to brackish water. Patients with possible infections should have a chest X-ray, urinalysis, surveillance blood cultures, and perhaps lumbar puncture. A sample of ascites, if present, should be analyzed for the serum–ascites albumin gradient, the cell count, and the differential, and cultured in blood culture bottles. An absolute neutrophil count over 250/μL indicates infection [231]. Direct inoculation of ascitic fluid into blood culture bottles substantially improves the chances of culturing an organism because the concentration of bacteria in the fluid is low [232].

Another important issue is the concomitant infections occurring during or after variceal bleeding in patients with alcoholic cirrhosis. Bacterial infections occur in up to 20% of patients who are hospitalized with gastrointestinal bleeding [233]. Multiple studies and a recent metaanalysis demonstrated that prophylactic antibiotics reduced the overall infectious complications and mortality rate in patients with cirrhosis hospitalized for bleeding [233–236]. Antibiotics may also reduce the risk of recurrent variceal bleeding in hospitalized patients [237].

Altered neurological status

It is difficult to evaluate neurological status in sick and intoxicated alcoholic patients. Some neurological disorders are predominantly the result of inadequate nutrition (e.g., thiamine deficiency), but others appear to involve the neurotoxicity of ethanol. It is essential to exclude intracranial pathology, such as chronic or acute subdural hematoma from head trauma, stroke, or meningitis. Other metabolic or toxic causes should be fully sought as the potential causes for altered mental status. These include hypoglycemia, hyponatremia, and hepatic encephalopathy [238]. In most patients with impaired consciousness, a noncontrasted head CT should be performed and it is highly desirable before performing lumbar puncture, unless the clinical suspicion of meningitis is high, in which case lumbar puncture should be performed promptly [238]. The requirement for thiamine is increased by carbohydrate so alcoholic patients should be given thiamine promptly on admission. Supplementation of other water-soluble vitamins, especially pyridoxine and folate, is advisable.

Hepatic encephalopathy may be difficult to diagnose [239]. Patients with hepatic encephalopathy usually have advanced chronic liver disease and have many of the physical and laboratory stigmata associated with severe hepatic dysfunction. Generally, hepatic encephalopathy is reversible and a precipitating cause (e.g., gastrointestinal bleeding; electrolyte and acid-base imbalance; high protein intake; severe constipation; noncompliance with medications; drug use, including sedatives; and infection), rather than worsening of hepatocellular function, can be identified in the majority of patients.

Renal dysfunction

Renal insufficiency commonly complicates the clinical course of patients with alcoholic cirrhosis. There are several causes of renal failure in such patients, such as volume depletion from diuretics or poor oral intake, shock from sepsis or bleeding, exposure to nephrotoxic drugs, or other intrinsic renal disease. However, renal failure in these patients normally occurs in the absence of these precipitating factors. This type of renal dysfunction is known as the hepatorenal syndrome [240]. There are no specific clinical findings in hepatorenal syndrome, although most patients have features of advanced liver disease. Renal failure in hepatorenal syndrome is associated with oliguria, benign urinary sediment, sodium retention (urinary Na^+ more than 10 mEq/L), dilutional hyponatremia, and low fractional excretion of sodium [240]. As a result of the lack of specific diagnostic tests to differentiate between hepatorenal syndrome and other causes of renal insufficiency, the diagnosis is based on the guidelines proposed by the International Ascites Club [241]. The therapy for hepatorenal syndrome is beyond the scope of this review.

Treatment

Treatment for alcoholism

Abstinence is important to prevent further liver injury and it appears to benefit patients at every stage of the disease [242]; therefore, establishing long-lasting sobriety should be the highest priority for these patients. The success of therapies for alcohol abuse varies from 30% to 90% and the results are improved when the abuse is detected at an early stage and the patient has social support from family, peers, and employers [243,244]. Medications to facilitate abstinence, such as naltrexone and acamprosate, both singly and together, have been shown to be effective in some patients [245]. A 6-month, randomized, double-blind, placebo-controlled trial attempted to determine the efficacy and tolerability of a long-acting intramuscular formulation of naltrexone for the treatment of alcoholism. Compared with placebo, 380 mg long-acting naltrexone resulted in a 25% decrease in the event rate of heavy drinking days and 190 mg resulted in a 17% decrease [246]. Generally, treatment of alcohol disorders through the use of combinations of pharmacological and behavioral modalities may be more effective than single-modality approaches. A large multicenter clinical trial sponsored by the National Institute on Alcohol Abuse and Alcoholism, Combining Medications and Behavioral Interventions (COMBINE), showed that patients receiving medical management with naltrexone, combined behavioral intervention, or both, fared better on drinking outcomes. Acamprosate was not efficacious in this trial. Medical management of alcohol dependence with naltrexone, if implemented with behavioural intervention, could greatly expand patient access to effective treatment [247].

Current methods for monitoring alcohol consumption in patients who are under treatment are not satisfactory. Serum carbohydrate-deficient transferrin has been approved as a tool to identify possible chronic heavy alcohol consumption. This form of transferrin lacks the normal number of sialic acid moieties as a result of the effects of ethanol on its post-translational modification in the Golgi apparatus, and on its rate of removal from the plasma. The sensitivity and specificity of this test for significant alcohol intake are 60%–70%, and 80%–90%, respectively [248,249], and a rise in its concentration is suggestive of relapse [250]. Lower sensitivity (< 50%) has been reported [251], therefore, the usefulness of this test to detect heavy alcohol use is unclear.

Other modifiable factors that might interfere with the progression of liver disease are cigarette smoking, obesity, and caffeine consumption. Several epidemiological studies showed that fibrosis worsens more rapidly in patients with ALD who smoke, presumably secondary to the effect of oxidative stress from smoking [252,253]. Obesity is associated with the development of steatosis and high body mass index has been shown to be an independent risk factor of ALD [212]. Several epidemiological studies have found an inverse association of coffee consumption and the levels of liver enzymes [254–257]. Interestingly, coffee drinking was found to be a protective factor against alcoholic and nonalcoholic liver cirrhosis in case–control studies [252,258] and against alcoholic cirrhosis in several prospective studies [253,259]. Such a protective effect is believed to be from the antioxidant property of caffeine [254]. Therefore, lifestyle modification is important in the treatment of ALD.

Treatment for alcoholic hepatitis

Achieving sobriety is paramount in the treatment of alcoholic hepatitis. Many therapeutic agents have been used for the treatment of alcoholic hepatitis. Specific medical therapies are discussed below.

Corticosteroids

Corticosteroids suppress the immune system activated by enhanced generation of neoantigens, such as MDA and acetaldehyde protein adducts, cytochrome P4502E1 and 3A4, and liver membrane antigen [242]. It also inhibits the production of TNF-α, activation of HSC, and possibly fibrogenesis. At least 12 randomized, controlled clinical trials have assessed the effectiveness of corticosteroids in patients with alcoholic hepatitis. Several studies found no benefit [260–266], whereas others reported improved survival [215,267–270]. Subsequent metaanalyses also produced conflicting results [271]. Two suggested a beneficial role of corticosteroids [272,273], whereas one questioned their efficacy [274]. The American College of Gastroenterology recommended glucocorticoid treatment (prednisolone, 40 mg daily for 4 weeks followed by a taper) in patients with severe alcoholic hepatitis (DF > 32) with hepatic encephalopathy [152]. The therapy should not be initiated in patients with gastrointestinal bleeding, active infection, renal failure, or acute pancreatitis. A major disadvantage to corticosteroids is therefore the fact that they cannot be used by many patients with alcoholic hepatitis. It has been estimated that seven patients need to be treated with corticosteroids to prevent one death [271].

Patients with alcoholic hepatitis are invariably malnourished and the severity of protein-calorie malnutrition has been shown to closely correlate with mortality rate [166]. The underlying mechanisms responsible are unknown, although it has been speculated that protein-calorie malnutrition worsens mortality in patients with alcoholic hepatitis by effects on the immune system, by impairing protein synthesis, which is essential for hepatic regeneration [271,275], and by aggravating depletion of glutathione. A recent randomized controlled trial compared the therapeutic efficacies of corticosteroid therapy (40 mg/day) vs enteral nutrition (2000 kcal/day) with outcomes assessed at 28 days (the end of therapy) and 1 year later or at death. Both therapies showed similar initial outcomes, however, fewer long-term infections were found in those treated with enteral nutrition [276]. This important study suggests that aggressive nutritional

support is as effective as treatment with prednisone. Another small pilot study suggests that enteral nutrition in combination with a short course of steroids might be a good therapeutic strategy for severe alcoholic hepatitis; however, this treatment strategy deserves investigation in a randomized controlled trial [277].

Anabolic steroids

There are several reasons to use anabolic steroids in patients with alcoholic hepatitis. These medications were shown to improve nutritional status in patients with wasting syndrome [278] and they accelerated the disappearance of steatosis and promoted the synthesis of coagulation factors [207]. In animal models, these medications can prevent chemically induced liver injury and promote liver regeneration [207,279]. Several randomized trials have tested the benefit of using anabolic steroids, oxandrolone or testosterone, in patients with alcoholic hepatitis. In a multicenter, randomized, placebo-controlled study, 263 patients with moderate and severe alcoholic hepatitis were randomized to one of three treatments: prednisolone, oxandrolone, or placebo. During the first 30 days, mortality in the groups receiving steroid therapy was not significantly different from mortality in the placebo group. Although neither steroid improved short-term survival, oxandrolone therapy was associated with a beneficial effect on long-term survival, especially in patients with moderate alcoholic hepatitis (as estimated by bilirubin and prothrombin time). As most patients with alcoholic hepatitis were malnourished, a combination therapy with nutritional treatment and oxandrolone was conducted in a veteran population. Oxandrolone appeared to be most beneficial in patients with moderate malnutrition, particularly in those who received sufficient calories during the study [280]. This study supports the notion that nutritional support in patients with alcoholic hepatitis is essential and combined medical and nutritional therapies may be more efficacious than agents used singly. A metaanalysis on the efficacy of anabolic–androgenic steroids for alcoholic liver disease was performed in a Cochrane review. This systematic review could not demonstrate any significant beneficial effects of anabolic–androgenic steroids on any clinically important outcomes of patients with alcoholic liver disease [281].

Propylthiouracil

Propylthiouracil (PTU) has been used to treat alcoholic hepatitis, based on the prediction that it would decrease the hypermetabolic state induced by alcohol and protect perivenous cells from hypoxic injury [282,283]. It also inhibits neutrophil myeloperoxidase and may thereby reduce oxidative stress from hypochlorous acid released by neutrophils [284]. There have been several trials of PTU in patients with alcoholic hepatitis. In one study, PTU improved symptoms and laboratory tests [285]; however, in another series of more severely ill patients, PTU was not effective [286]. A Cochrane database systematic review published a metaanalysis by combining the results of six randomized clinical trials to determine the effectiveness of PTU in alcoholic hepatitis [287]. The analysis of 710 patients demonstrated that there were no significant effects of PTU vs placebo on all-cause mortality (relative risk [RR] 0.93, 95% confidence interval [CI] 0.66–1.30), liver-related mortality (RR 0.80, 95% CI 0.50–1.29), complications of the liver disease, or liver histology. There is therefore no evidence for using PTU for ALD outside randomized clinical trials [287].

Anti-TNF therapy

As previously mentioned, TNF-α appears to play a particularly important role in ALD. Several recent studies have focused on the use of medications that exert their function on the activities of TNF-α. Pentoxifylline, a phosphodiesterase inhibitor, has been shown to decrease the transcription of the TNF-α promoter and the production of TNF-α, and to inhibit the activation of NF-κB [288,289]. A prospective, randomized, double-blind study using pentoxifylline was performed in 101 patients with severe alcoholic hepatitis (DF > 32) [290]. The study showed that the patients who received 400 mg pentoxifylline three times daily for 4 weeks had a 40% reduction in mortality compared to the placebo group (receiving vitamin B-12). Based on the difference in mortality between the pentoxifylline and placebo groups, the number needed to treat to prevent one death was about 5. The reduction in 4-week mortality was mainly attributed to the concomitant decrease in the incidence of hepatorenal syndrome by 65%–70%. The mechanism whereby pentoxifylline decreased the development of the hepatorenal syndrome is unclear [290].

Based on the result of this study and animal data, infliximab, a chimeric mouse–human anti-TNF monoclonal antibody, has been tested in patients with severe alcoholic hepatitis. The first trial evaluated tolerance and effectiveness of infliximab combined with steroids in reducing the 2-month mortality rate among patients with severe alcoholic hepatitis, defined by a DF greater than 32 [291]. Patients were randomly assigned to group A, receiving intravenous infusions of infliximab (10 mg/kg) at weeks 0, 2, and 4; or group B, receiving placebo. All patients received prednisolone (40 mg/day) for 28 days. Patients received standard nutritional support as well as prophylactic antibiotic therapy. After randomization of 36 patients, the study was terminated by the data safety committee because of a substantially higher death rate in the infliximab group (39% compared with 18%) [291]. However, a problem with this study was that the dose of infliximab was high, particularly in combination with prednisone, which may further impair both humoral and cell-mediated immune responses and increase the risk of infection. Another trial was an open label study that included 12 patients with biopsy-proven alcoholic hepatitis [292]. All patients received a single infusion of infliximab,

5 mg/kg body weight, in the first 5 days along with supportive care. Two patients died during hospitalization from sepsis whereas 10 remained alive for a median of 15 months (range 12–20 months). Another study has focused on the use of etanercept, a dimeric fusion protein comprising the extracellular ligand-binding portion of the human TNF receptor linked to the Fc portion of human immunoglobulin G. Etanercept (32 mg/m^2 body surface area, up to 50 mg intravenously on day 1, and followed by 25 mg administered subcutaneously on day 4, day 8, and day 12) was given to 13 patients with moderate or severe alcoholic hepatitis as assessed by a DF value greater than 15 or the presence of spontaneous hepatic encephalopathy [293]. The 30-day survival rate of patients receiving etanercept was 92%. Adverse events that were encountered included infection, hepatorenal syndrome, and gastrointestinal bleeding; these required premature discontinuation of etanercept in 23% of patients [293]. Based on the results of these studies, a larger randomized control trial is warranted to determine the efficacy and safety of anti-TNF therapy in the treatment of alcoholic hepatitis.

S-Adenosyl-L-methionine

Abnormal methionine metabolism occurs in animals fed ethanol and in patients with end-stage cirrhosis as a result of abnormalities in the enzymes of methionine and glutathione metabolism. Patients with alcoholic hepatitis have decreased hepatic methionine, *S*-adenosyl-L-methionine (SAMe), and glutathione levels [294]; however, randomized controlled trials using SAMe in patients with alcoholic hepatitis are limited because most studies were performed in patients with alcoholic cirrhosis. A Cochrane systematic review failed to demonstrate any significant effect of SAMe on mortality, liver-related mortality, mortality or liver transplantation, and liver complications of patients with ALD (see below).

Colchicine

Colchicine is an antiinflammatory and antifibrotic medication [295]. A few studies have been conducted to determine the therapeutic efficacy of colchicines in alcoholic hepatitis; however, none showed a therapeutic benefit [296,297]. A recent metaanalysis by the Cochrane group showed no significant effects of colchicine on mortality (RR 1.00, 95% CI 0.87–1.16) and liver-related mortality (RR 1.08, 95% CI 0.88–1.33) in patients with ALD [298].

Vitamin E

A small study was performed to determine the effect of vitamin E administration on clinical and laboratory parameters of liver function and on markers of fibrogenesis in patients with mild to moderate alcoholic hepatitis [299]. Twenty-five patients received 1000 IU vitamin E per day, while 26 patients received placebo for 3 months. Vitamin E did not result in significantly greater decreases in serum aminotransferases and bilirubin or in greater increases in serum albumin compared with placebo. Four patients in the treatment group and five in the placebo group died during the 1-year study. This small study showed that vitamin E treatment has no beneficial effects on tests of liver function in patients with mild to moderate alcoholic hepatitis [299].

Liver transplantation

Liver transplantation for patients with alcoholic hepatitis is not recommended according to the current guidelines [300]. In an era of organ shortage, use of liver transplants in patients with severe alcoholic hepatitis, who by definition have been actively drinking recently (less than 6-month sobriety), may negatively affect the public attitude on transplantation and organ donation [219].

Treatment for alcoholic cirrhosis

Several medical interventions have been studied in the setting of alcoholic cirrhosis.

Colchicine

Colchicine (1 mg/day) was studied in a randomized, placebo-controlled trial of 100 patients with cirrhosis over a 14-year period [301]. Of these, 45 patients had alcoholic cirrhosis. Seventy-three patients were in class A, 26 were in class B, and one was in class C. A survival benefit was seen in the treated patients at both 5 years (75% vs 34%) and 10 years (56% vs 20%) years. The VA Cooperative study group reported another randomized, placebo-controlled trial of colchicine in the treatment of alcoholic cirrhosis [302]. The aim of this study was to compare the efficacy and safety of long-term colchicine, compared with placebo. Five hundred forty-nine patients with advanced (Pugh B or C) alcoholic cirrhosis were randomized to receive either colchicine 0.6 mg twice per day (274 patients) or placebo (275 patients) and the treatment lasted for from 2 to 6 years. In an intention-to-treat analysis, all-cause mortality was similar in the colchicine-treated (49%) and placebo-treated (45%) patients. Mortality attributed to liver disease was 32% in patients given colchicine and 28% in patients given placebo. There were several differences between this study and the previous one [301] that reported improved survival with colchicine treatment. This recent study was larger (549 patients compared with 100 patients), excluded patients with early (Class A) cirrhosis, enrolled only patients with alcoholic cirrhosis, and treated for a shorter duration (maximum 6 years compared with maximum 14 years, respectively). A Cochrane systematic review failed to demonstrate any significant benefit in survival outcome in patients with alcoholic cirrhosis treated with colchicine [298].

Propylthiouracil

The rationale of using PTU in the treatment of ALD was previously mentioned. Orrego and colleagues observed, in a larger cohort, that the administration of PTU reduced the

mortality due to ALD [303]. In this randomized clinical trial of 310 patients, a significant improvement in survival was seen in patients treated for a maximum of 2 years with PTU (300 mg/day) compared to those who received placebo: 13% vs 25% overall and 25% vs 55% in severely ill patients. This randomized placebo-controlled clinical trial was remarkable for the care taken to monitor alcohol consumption by the study participants, thereby controlling for the greatest confounding factor complicating the interpretation of investigations of treatment for ALD [303,304]. As mentioned previously, a Cochrane metaanalysis review failed to find evidence to support the use of PTU in ALD [287].

Phosphatidylcholine

Phosphatidylcholine, purified from polyunsaturated lecithin, was reported to oppose alcohol-induced fibrosis in baboons by decreasing the activation of stellate cells to transitional cells, and possibly also by stimulating collagenase activity [305]. A large study of phosphatidylcholine supplementation in 789 veterans with ALD was conducted. Treatment was maintained for 2 years; however, no histological benefit was observed in the treatment group when compared to placebo [306].

S-adenosylmethionine

Abnormal methionine metabolism occurs in animals fed ethanol and in patients with end-stage cirrhosis, which is the result of abnormalities in the enzymes of methionine and glutathione metabolism. Patients with alcoholic liver disease have decreased hepatic methionine, SAMe, and glutathione levels [116]. Mato and colleagues published a randomized, placebo-controlled clinical trial, using SAMe in 62 patients with alcoholic cirrhosis [307]. During the 2-year follow-up period, there was no difference in overall mortality in treated patients when compared to controls. However, when patients in Child class C were excluded from the analysis, the overall mortality/liver transplantation rate was significantly greater in the group given placebo than in the group given SAMe (29% compared with 12%) [307]. SAMe was also shown to improve 2-year survival. However, a recent Cochrane systematic review that included eight placebo-controlled trials found no significant benefit of using SAMe in patients with ALD [308].

Silymarin (milk thistle)

Several studies have shown the antioxidant property of silymarin, the active ingredient in milk thistle [309]. The results of clinical trials using silymarin in ALD are inconclusive. In a double-blind, prospective, randomized clinical trial involving 170 patients with cirrhosis (91 with alcoholic cirrhosis), 87 patients were treated with 140 mg silymarin three times daily and 83 patients received placebo. The mean follow-up period was 41 months. Fifty eighty percent of silymarin-treated patients, compared with 39% of placebo-treated patients, survived for 4 years or longer. In another study, 200 patients with histologically proven alcoholic cirrhosis were randomized to receive either silymarin (150 mg three times per day) or placebo [310]. There was no survival benefit noted during the 2-year study period. A recent Cochrane systematic review concluded that when compared to placebo, silymarin had no significant effect on overall mortality (RR 0.78, 95% CI 0.53–1.15), complications of liver disease (RR 0.95, 95% CI 0.83–1.09), or liver histology [311].

Liver transplantation

Guidelines for liver transplant evaluation have been established by the American Association for the Study of Liver Diseases [300]. Indications for orthotopic liver transplantation in patients with end-stage liver disease from alcohol are no different than those for other causes of end-stage liver disease. Abstinence is the only effective treatment for most patients and many patients with apparently far-advanced ALD who completely abstain can recover to the degree that transplantation is not required [300]; therefore, a period of abstinence before providing transplantation for patients with ALD seems to be prudent. More than 85% of transplantation programs in the United States require 6 months of abstinence and careful evaluation by professional counselors to directly address the addiction to alcohol before transplantation [300,312]. Of note, it has been shown that pre-orthotopic liver transplantation abstinence of less than 6 months is an important predictor of post-orthotopic liver transplantation recidivism [313]. If a patient has a drinking relapse while on the transplant waiting list, 97% of transplant centers in the United States would remove the patient from the list for at least 3–6 months and 15% would remove them permanently [312].

The outcome after liver transplantation for alcoholic liver disease is comparable to that of patients transplanted for other conditions. One-year and 5-year patient survival was 80%–85% and 70%–82%, respectively [314]. Five-year graft survival was 60%–70% [314,315]. Patients with alcoholism and hepatitis C have been shown to have slightly lower graft and patient survival [316]. The alcohol relapse rates vary considerably from center to center. Most studies have shown a recidivism rate between 20% and 30% by 2 years after orthotopic liver transplantation [317]. However, graft loss as a consequence of abusive drinking after transplantation is uncommon [300,318,319].

One important issue in patients who underwent liver transplantation for ALD is the higher rate of late deaths as a result of lung and oropharyngeal cancer [320,321]. This is likely because alcohol and tobacco use commonly cooccur, with at least 90% of those with an alcohol problem also using tobacco [322]. A study from Pittsburgh found that transplant recipients who had ALD resume smoking early posttransplantation and quickly become tobacco-dependent [322]. There are no recommendations for the posttransplant sur-

veillance for such malignancies. However, health-care providers should be aware of these conditions, especially in those who continue to smoke after transplantation.

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G LIVER

89

Nonalcoholic fatty liver disease

Arun J. Sanyal

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Nonalcoholic fatty liver disease (NAFLD) represents a spectrum of changes in liver histology characterized by predominantly macrovesicular steatosis [1]. By definition, these changes are not caused by the consumption of alcohol. In North America the most common cause of persistent abnormalities in liver enzyme tests is NAFLD [2]; its prevalence appears to be increasing and is related to the widespread problem of obesity. It is also increasingly recognized as a cause of end-stage liver disease and a potential risk factor for hepatocellular cancer [3,4]. The histological features, pathogenesis, clinical evaluation, and management of NAFLD are discussed below.

Histological definition

There are two broad histological patterns of NAFLD:
- hepatic steatosis or fatty liver
- steatohepatitis.

Contrary to its literal meaning, steatohepatitis does not simply represent the presence of steatosis and inflammation. Steatohepatitis is defined by the presence of hepatic steatosis along with evidence of cell injury, that is, cytological ballooning or Mallory hyaline along with varying degrees of inflammation and pericellular fibrosis (Fig. 89.1) [5]. These changes are typically most marked in the centrilobular regions. The inflammation associated with steatohepatitis is usually modest and mainly lobular in distribution. Although some reports have suggested that the inflammation is mainly neutrophilic in nature [6], others have described a mixed inflammatory infiltrate [7]. A dense lymphocytic infiltrate in the portal areas is not typical of NAFLD, and an alternate cause of such an infiltrate should be sought when it is present. Portal fibrosis may be associated with NAFLD, particularly in pediatric subjects and in those who are morbidly obese [8,9].

Grading and staging

The grade of steatohepatitis reflects the amount of cell injury and necroinflammatory activity that is present. The stage of the disease reflects the amount of fibrosis or how far an individual has progressed towards cirrhosis. The ideal system for grading and staging of NAFLD remains to be defined. An optimal system should be easy to use, highly reproducible, relate to long-term outcomes, show sensitivity to change, and be free of ceiling and floor effects. Thus, the distribution of grade and stage should be such that a large proportion of subjects should not be at the lowest (floor effect) or highest grade or stage (ceiling effect). Unfortunately such a system remains to be designed.

A fundamental requirement of any scoring system is a high degree of reproducibility. Younossi et al. examined 19 individual parameters grouped under steatosis, hepatocyte injury, inflammation, and fibrosis in a blinded manner by four pathologists and twice by two pathologists [10]. Substantial concordance was observed with respect to assessment of extent of steatosis, sinusoidal location of steatosis, perivenular fibrosis, stage of fibrosis, ballooning degeneration and glycogen nuclei. There was, however, considerable interobserver variation in the assessment of inflammation. All of the abovementioned observations have been corroborated by the National Institutes of Health (NIH) NASH clinical research network [11].

Several systems of grading and staging of NAFLD have been proposed. However, only two systems have been validated to any degree. A landmark study by Brunt et al. examined 12 separate histological parameters [12]; these included hepatic macrovesicular steatosis, hepatocellular ballooning, intraacinar inflammation, portal tract inflammation, Mallory hyaline, acidophil bodies, glycogen nuclei, lipogranulomas,

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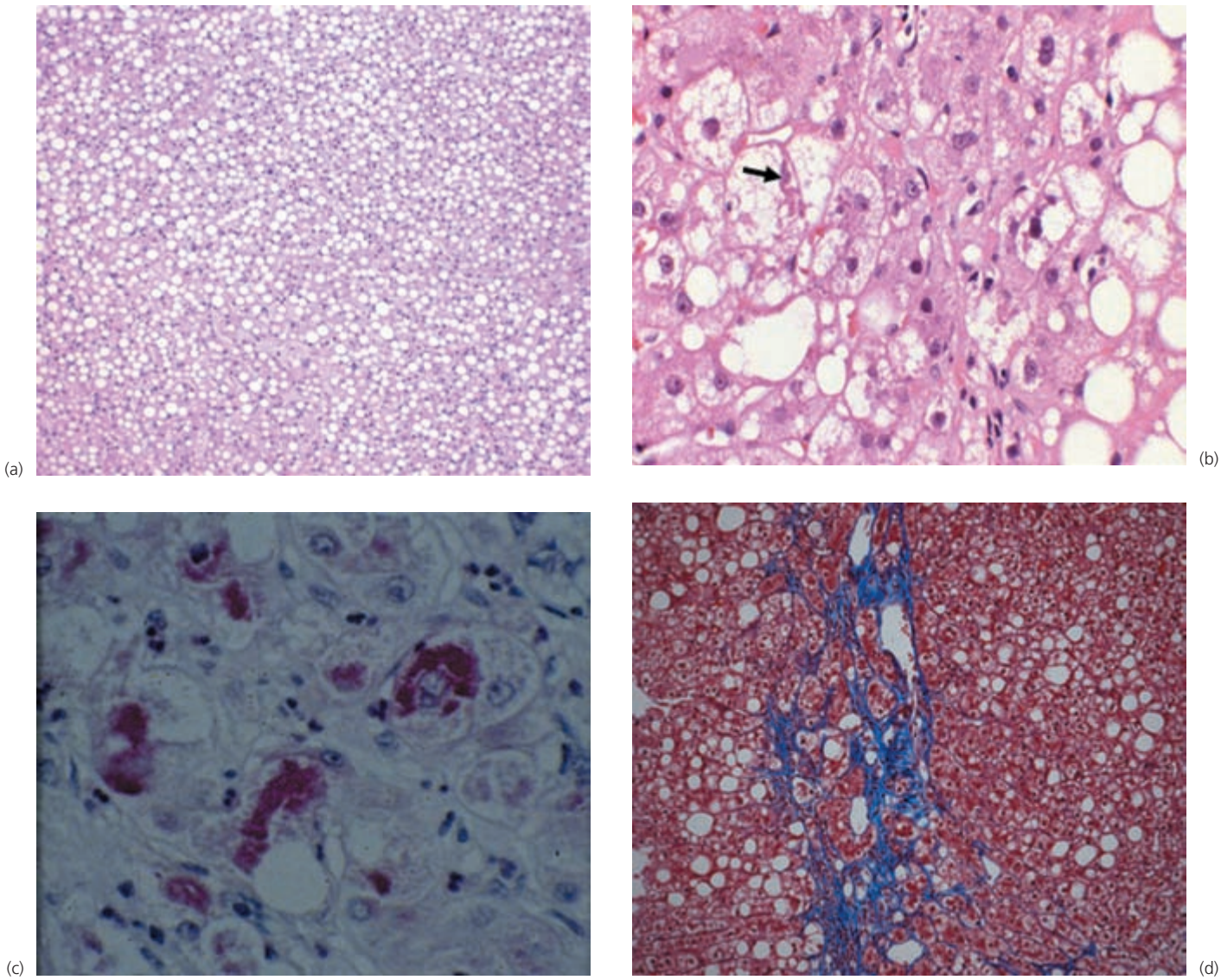


Figure 89.1 The hepatic histology of nonalcoholic steatohepatitis. **(a)** Macrovesicular hepatic steatosis (hematoxylin–eosin stain). **(b)** Cytological ballooning, with Mallory hyaline within a ballooned cell (see arrow). **(c)** Mallory body stained with antibodies to ubiquitin. **(d)** A Masson trichrome stain demonstrates pericellular fibrosis, which is mainly centrilobular in location.

hepatocellular iron, perisinusoidal fibrosis, portal fibrosis, and bridging fibrosis. Based on these parameters, a three-grade and four-stage system of classifying NAFLD was developed. The necroinflammatory grade correlated with the alanine aminotransferase (ALT) activity.

Subsequently, using the Brunt classification as a starting point, the NIH NASH clinical research network has developed a histological scoring system for NAFLD that has been validated [11]. Although 14 separate parameters were evaluated, four were scored semiquantitatively. These include steatosis (0–3), cytological ballooning (0–2), inflammation (0–2), and fibrosis (0–4). A NASH activity score was then developed that included the unweighted scores for steatosis, inflammation, and fibrosis (Table 89.1). The NASH activity score correlated well with the presence of steatohepatitis during a blinded validation process and was typically associated with a score greater than or equal to 5. Those with a score of 3 or less were usually not found to have steatohepatitis, whereas a score of 4 was associated with some divergence of opinion (Fig. 89.2). Importantly, this system divides stage 1 into several subsets (Table 89.1) thereby improving the sensitivity to change in the earlier stages of the disease. This system is expected to be extremely valuable as a research tool for the design and analysis of clinical trials in this area. However, its role in routine clinical practice remains to be established.

A major limitation of any histological scoring system is sampling variability. In one study, two biopsies were obtained

Table 89.1 Grading and staging of nonalcoholic fatty liver disease

Parameter	Description	Score
1. Macrovesicular steatosis	< 5%	0
	5–33%	1
	34–66%	2
	> 66%	3
2. Cytological ballooning	None	0
	Few cells	1
	Many cells	2
3. Inflammation	None	0
	< 2 foci per 200 ×	1
	2–4 foci per 200 ×	2
	> 4 foci per 200 ×	3
4. Fibrosis	None	0
	Perisinusoidal centrilobular mild	1a
	Perisinusoidal centrilobular moderate	1b
	Periportal	1c
	Pericentral and periportal	2
	Bridging	3
5. Mallory hyaline	None to rare	0
	Many	1

Based on Kleiner et al. [11].
The NASH (nonalcoholic steatohepatitis) activity score is based on scores for steatosis, cytological ballooning, and inflammation.

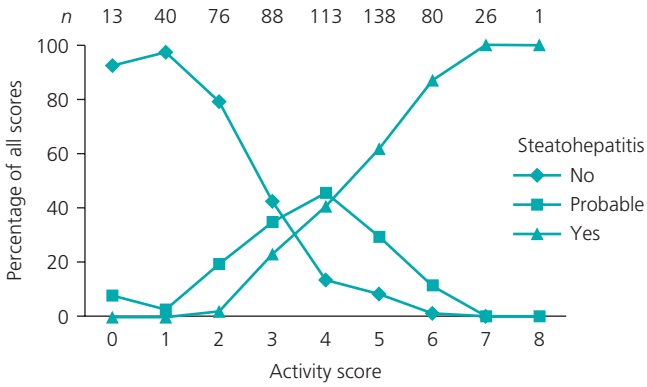


Figure 89.2 The relationship between the NASH activity score and the probability of having steatohepatitis. Steatohepatitis was diagnosed to be present, absent, or probable based on an overall gestalt by a group of experienced pathologists. The same biopsies were also independently scored to determine the NASH activity score by the same pathologists, who remained blinded to their evaluation of steatohepatitis. A score greater than 4 was considered to indicate a high probability of steatohepatitis. From Kleiner et al. [11], with permission from John Wiley & Sons, Inc.

from the same site at the same time [13]. Approximately 20% of subjects had at least a one-stage variability between biopsies, whereas 12% had a two-stage variation. Similar data have been obtained from studies where biopsies from the left and right lobes have been compared [14]. Although a 4-cm core is ideal [15], it is imperative to obtain at least a 2-cm-long core with a 15 or 16 gauge needle to evaluate a biopsy for grading or staging of any liver disease.

Epidemiology and associated conditions

(Table 89.2)

It is established that NAFLD represents the hepatic manifestation of the metabolic syndrome [16]. The metabolic syndrome is characterized by obesity, hypertension, diabetes,

Table 89.2 Conditions associated with nonalcoholic fatty liver disease (NAFLD)

Metabolic syndrome
Drugs, e.g., amiodarone, tamoxifen, HAART
Abetalipoproteinemia
Lipodystrophy
Weber–Christian syndrome
Jejunoileal bypass surgery
Total parenteral nutrition

HAART, highly active antiretroviral therapy.

Table 89.3 Diagnostic criteria for the metabolic syndrome

Abdominal obesity (waist circumference > 102 cm for men and > 88 cm for women)
Triglycerides > 150 mg/dL
HDL < 40 mg/dL for men or < 50 mg/dL for women
BP ≥ 130/85 mmHg
Fasting blood glucose ≥ 110 mg/dL

Based on Adult Treatment Panel (ATP) III criteria [18].
BP, blood pressure; HDL, high-density lipoprotein.

hypertriglyceridemia, and low levels of high-density lipoprotein (HDL) cholesterol (Table 89.3) [17]. It is estimated that about 47 million individuals in the United States are afflicted with the metabolic syndrome, and over 80% of such subjects have NAFLD [18,19]. The corollary is that over 90% of subjects with NAFLD have some features of the metabolic syndrome [20].

The common thread that connects the disorders that define the metabolic syndrome is the presence of underlying insulin resistance [21]. Although several systems for defining the metabolic syndrome have been proposed [17,22,23], these fail to capture with optimal sensitivity the presence of the underlying insulin resistance [24]. Indeed, many individuals in the general population have biochemical evidence of insulin resistance even in the absence of features of the metabolic syndrome [25,26]. It may be argued that the development of the metabolic syndrome is analogous to the development of end-organ disease. Thus, although it is important to identify those with end-organ disease for treatment, it may be even more important to identify those at risk, that is, those with insulin resistance, before the metabolic syndrome develops. This would appear to be a rational approach given the impact of insulin resistance-related disorders to overall morbidity and mortality in the general population [27].

Several approaches have been taken to define the prevalence of NAFLD in the general population. On the one hand, hospital-based studies are flawed because of ascertainment bias. On the other hand, population-based studies have mainly relied on noninvasive imaging studies, such as sonography, to define the presence of NAFLD [28–30]. Unfortunately, sonography has poor specificity for the diagnosis of NAFLD [31]. Magnetic resonance imaging (MRI) has been used to define and quantify the amount of hepatic steatosis [32–34]. This technique has been validated and used to define the prevalence of hepatic steatosis in the general population. Based on this technique, it is estimated that about 33% of the US population has hepatic steatosis [35]. In Hispanic subjects, the prevalence reaches 45%, whereas African Americans have a lower prevalence of NAFLD (24%). Approximately 70%–80% of affected subjects have a normal ALT level [36]. These data are in keeping with the observation that 7.9% of the US population have persistently elevated ALT values and that most individuals with such elevations have underlying NAFLD [37].

In an autopsy series where liver histology was used to define the presence of a fatty liver [38], hepatic steatosis was found in approximately 2.7% of lean individuals and 18.5% of obese individuals. Similar data have been reported from autopsies of air-crash victims [39]. A relationship between the body mass index and the presence of a fatty liver has also been established in otherwise apparently healthy individuals being considered as donors for live-donor liver transplantation [40].

Regardless of the methodology used, several aspects of the epidemiology of NAFLD are consistently observed. Fatty liver and NASH occur in all age groups, including children [41,42]. Whereas studies published prior to 1990 [1,6,43] emphasized that NASH occurred mostly in women (53%–85% of all patients), later studies [7,44] have shown that NASH occurs with equal frequency in males (~ 50%). The prevalence of NAFLD is directly related to the body mass index (BMI), with over 80% of subjects with a BMI greater than 35 having steatosis [16,45,46]. The waist circumference may even be a better predictor of underlying insulin resistance and NAFLD than the BMI [47].

An important confounder is the impact of social consumption of alcohol on the development and categorization of subjects with NAFLD. The precise levels of alcohol consumption that separate those with alcoholic liver disease from NAFLD have not been clearly defined. It is certainly feasible that subjects with risk factors for NAFLD may be at higher risk of hepatic steatosis if they consume alcohol in any quantity. Indeed, it has been found that obese individuals who regularly consume alcohol have the highest prevalence of hepatic steatosis [48].

The complexity of NAFLD means that the phenotype of this condition is highly likely to reflect complex interactions between environmental and lifestyle-related factors with the underlying genetic composition. Obesity and diabetes are often clustered within families [49,50]. The causes of such familial clustering include both genetic and environmental factors [51]. In one study [52], eight families with a total of 18 members with NAFLD including NASH with cirrhosis were described, but no clear-cut pattern of inheritance of risk for NAFLD was identified.

Numerous other conditions have also been associated with NAFLD (Table 89.2). These include abetalipoproteinemia and some rare syndromes characterized by severe insulin resistance, such as lipoatrophic diabetes and Mauriac syndrome [53–55]. The liver disease in both abetalipoproteinemia and lipodystrophy can progress to advanced fibrosis and cirrhosis [53,56]. There is also an association between NAFLD and the use of several drugs, including amiodarone, tamoxifen, and diltiazem [57–59]. The mechanisms involved

in the development of steatosis are somewhat variable across these conditions and they should ideally be considered separately from fatty liver disease, which is associated with the metabolic syndrome.

Hepatic steatosis is also related to the presence of underlying hepatitis C infection or human immunodeficiency virus (HIV) infection [60–63]. Hepatic steatosis is a risk factor for increased hepatic fibrosis in subjects with hepatitis C [64] and is associated with the presence of underlying metabolic syndrome for most subjects with genotype 1 infection [65]. It is also associated with a higher risk of failure to respond to antiviral therapy [66]. The development of steatosis in those with HIV infection has been linked to the use of highly active antiretroviral therapy (HAART), the presence of lipodystrophy, dyslipidemia, and insulin resistance [67,68]. A detailed description of these conditions is beyond the scope of this chapter and the reader is referred to reviews for more information on the subject [63,69].

Pathogenesis

There are two fundamental lesions in NAFLD: hepatic steatosis and steatohepatitis. It has traditionally been considered that if the liver was able to adapt to the development of steatosis it manifested the phenotype of fatty liver alone whereas if these adaptive responses were inadequate it would lead to steatohepatitis. This was the basis for the two-hit hypothesis, in which the development of a fatty liver was the first hit and an additional pathophysiological abnormality was required to cause steatohepatitis [70]. Over time, the concepts have evolved somewhat from these original ideas and focus on NAFLD as an expression of disordered energy homeostasis. To appreciate how disordered energy homeostasis leads to steatosis and steatohepatitis, one must first understand the normal physiology of energy homeostasis.

Normal energy homeostasis

Adipose tissue plays a critical role in energy homeostasis. It serves as an "energy bank" where excess energy is stored and then released to meet the energy needs of the body. Oxidation of free fatty acids (FFA) yields more energy than proteins or carbohydrates, and triglycerides are therefore the most efficient "currency" for energy homeostasis. During periods of caloric excess, the extra "energy" is stored as triglyceride. This normally causes increased expression of leptin, which acts centrally to decrease food intake. When diet-derived calories are not available or are inadequate to meet the "energy" requirements of the body, adipose tissue triglycerides undergo lipolysis releasing FFA and glycerol into the circulation, from where they can be taken up by the liver. This is regulated by the adipocytokine profile at a given adipose tissue location (Table 89.4) and by several hormones. Insulin is a key systemic regulator of adipocyte function and promotes lipogenesis while inhibiting lipolysis. Its actions are balanced by counterregulatory hormones such as growth hormone and glucagon. In addition to endocrine, paracrine, and autocrine regulation of adipose tissue, β_3-adrenergic neurons innervating adipose tissue add another layer of regulatory influence on the balance between lipogenesis and lipolysis [71].

Regulatory mechanisms of metabolic pathways in adipose tissue and liver

Adipocytokines

Adipocytes produce numerous cytokines that have metabolic functions. These include tumor necrosis factor-α (TNF-α), adiponectin, interleukin-6 (IL-6), resistin, and plasminogen activator inhibitor-1 (PAI-1) [72,73]. Of these TNF-α, IL-6, and PAI-1 have been implicated in promoting inflammation and the atherothrombotic predilection in obesity and the metabolic syndrome [74]. By contrast, a low adiponectin level has been associated with insulin resistance and NAFLD [75].

Tumor necrosis factor-α is a proinflammatory cytokine that is secreted by adipose tissue and macrophages [76]. It interferes with insulin signaling through the activation of intracellular signaling pathways such as those involving the Jun N-terminal kinase and inhibitor κ-B kinase-β [77]. In addition, it inhibits adiponectin, which, as described above, has many important actions with respect to insulin sensitivity and lipid metabolism [78]. Thus, high levels of TNF-α seen in the setting of low adiponectin contribute to the development of disordered lipid metabolism and severe insulin resistance [79]. An association has also been reported between NAFLD and promoter polymorphisms in the TNF receptor as well as increased expression of TNF in both adipose tissue and in the liver [80,81].

Adiponectin is another adipocytokine that bears similarity to complement [82]. It increases fatty acid β-oxidation in muscle and liver [72]. It also improves hepatic insulin sensitivity resulting in improved insulin-mediated suppression of hepatic glucose output [83] It acts upstream of AMP kinase, an allosteric inhibitor of acyl CoA carboxylase, which catalyzes the rate-limiting step for lipogenesis [84,85]. AMP kinase also promotes fatty acid oxidation in the mitochondria. By decreasing lipogenesis and increasing FFA oxidation, adiponectin decreases hepatic steatosis. Low levels of adiponectin have been associated with NAFLD [86,87]. In murine models of NAFLD, adiponectin improves insulin sensitivity and hepatic steatosis [88].

Hormones

The ability of the body to respond to different substrate concentrations and varying energy requirements depends on a closely integrated regulation of carbohydrate and lipid metabolism (Fig. 89.3). Insulin promotes glucose uptake and utilization by glycogen synthesis and glycolysis, respectively. It also promotes lipogenesis, and under conditions of long-term

Table 89.4 Adipocytokines and their known effects on inflammation and insulin actions

Adipocytokine	Effect on inflammation	Effect on insulin actions
TNF-α	↑ ICAM-1, VCAM-1, MCP-1 ↑ E selectin ↑ EC apoptosis ↑ NF-κB	↓ Insulin signaling ↑ Lipolysis
Leptin	↑ NO and ET-1 ↑ EC and VSMC proliferation ↑ Macrophage survival/proliferation ↑ Angiogenesis	↑ Glucose transport ↑ Sympathetic tone Corrects insulin resistance in lipodystrophy
IL-6	↑ ICAM-1, VCAM-1, MCP-1 ↑ Acute-phase reactants	↑ Insulin resistance ↑ Preadipocyte differentiation
ANG II	↑ ICAM-1, VCAM-1, MCP-1 ↓ NO and ↑NF-κB ↑ Fibrosis	–
Resistin	↑ ET-1 release ↑ ICAM-1, VCAM-1, MCP-1 ↓ TRAF-3	↓ Glucose clearance in muscle ↑ Hepatic insulin resistance
PAI-1	↓ Fibrinolysis	?
Adipsin	Complement activation	?
Adiponectin	↓ ICAM-1, VCAM-1, selectins ↓ NF-κB	↑ Lipid oxidation ↑ Insulin sensitivity
Visfatin	↑ Susceptibility to endotoxin-mediated EC injury in lungs	↑ Insulin signaling

ANG II, angiotensin II; EC, ; ET-1, endothelin-1; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; MCP-1, monocyte chemotactic protein-1; NF-κB, nuclear factor-kappa B; NO, nitric oxide; PAI-1, plasminogen activator inhibitor-1; TNF-α, tumor necrosis factor-α; TRAF-3, TNF receptor-associated factor-3; VCAM-1, vascular cell adhesion molecule-1; VSMC, vascular smooth muscle cells. Adapted from Rinella & Sanyal [136].

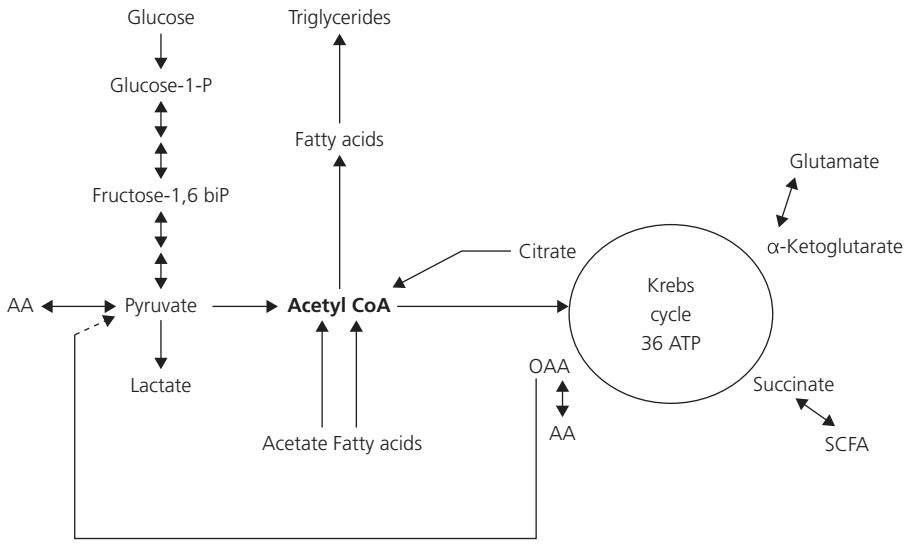


Figure 89.3 An integrated view of intermediary metabolism. Glycolysis leads to pyruvate formation. Under anaerobic conditions, lactate formation is used for ATP generation (Embden–Meyerhof pathway). Under aerobic conditions, pyruvate enters the Krebs cycle, which yields 36 ATP. During periods of caloric excess, extra calories are shunted into acetyl CoA, which is used to form triglycerides and cholesterol. During periods of starvation, oxaloacetate (OAA) can lead to phospho*enol*pyruvate and reverse synthesis of glucose (gluconeogenesis). Under conditions of severe starvation, amino acids (AA) can be transaminated to yield metabolic intermediates that can be used in the Krebs cycle. Short-chain fatty acids (SCFA) can also be converted to succinate, an intermediate in the Krebs cycle.

energy excess, glucose is converted to triglycerides for storage. These are important mechanisms for the postprandial control of metabolism and the utilization of the calories that are consumed. In the interdigestive phase, the glucose supply for tissues that are critically dependent on it is provided by glycogenolysis and gluconeogenesis. Similarly, during periods of food deprivation or during periods of increased energy requirements, for example, growth, wound healing, or inflammation, there is an increase in the mobilization of FFAs from adipose stores and their utilization as a source of fuel for energy-requiring processes. This is accomplished by increased levels of counterregulatory hormones that antagonize the effects of insulin, notably glucagon.

The immediate effects of insulin and its counterregulatory hormones are mediated by alterations in signal transduction cascades following binding of the hormones to their receptors. These signaling pathways alter the activity of regulatory kinases (e.g., protein kinase A), which in turn affect the activity of specific enzymes in various metabolic pathways. In the long term, these hormones regulate the metabolic pathways by transcriptional up- or down-regulation of key enzymes.

Transcription factors that mediate the effects of cytokines and hormones

Several transcription factors play an important role in metabolic homeostasis and mediate the effects of insulin and its counterregulatory hormones (Table 89.5). The sterol regulatory element binding protein (SREBP) group of basic helix-loop-helix/leucine zipper transcription factors are important transcriptional regulators of lipogenesis. They are synthesized as inactive precursors bound to the endoplasmic reticulum [89]. Their activation requires their cleavage and transport to the Golgi apparatus, where they undergo further cleavage thereby releasing the N-terminal region of the protein, which translocates to the nucleus and binds to sterol response elements in the promoters of target genes. Three isoforms of SREBP are known: SREBP-1a, SREBP-1c, and SREBP-2. Of these, SREBP-1c plays a major role in mediating insulin-directed lipogenic activity. It increases de novo lipogenesis (DNL) by transcriptionally increasing the expression of several key lipogenic enzymes (Fig. 89.4). It also increases expression of acetyl CoA synthetase, which increases production of acetyl CoA, the starting point for lipogenesis [90]. The SREBP-1c isoform further contributes to a positive triglyceride balance by inhibiting expression of the microsomal transfer protein resulting in decreased formation of very-low-density lipoproteins (VLDL) [91].

The cellular abundance of SREBP is affected by dietary nutrients, metabolites, hormones, and cytokines. Whereas glucose, saturated fats, and sterols increase SREBP abundance, polyunsaturated fats decrease SREBP and thus decrease lipogenesis [92,93]. Insulin mediates its signature "lipogenic activation" profile through liver X receptor (LXR)-mediated activation of SREBP-1c [94,95]. Several hormones and cytokines, such as leptin, also affect SREBP transactivation

Table 89.5 Transcription factors involved in fatty acid and cholesterol synthesis

Transcription factor	Target genes	Location	Function
SREBP-1c	ACC, FAS, ACS, ATP citrate lyase	1. Liver 2. Adipose tissue	↑ Lipogenesis
SREBP-2	HMG CoA reductase, LDL receptor	1. Liver 2. Constitutive	↑ Cholesterol synthesis
ChREBP	Pyruvate kinase, FAS, ACC	1. Liver 2. Adipose tissue	↑ Lipogenesis (in carbohydrate excess states)
PPAR-α	ACS, FATP1, CPT1, ABCA 1	1. Liver 2. Muscles	1. ↑ Catabolism 2. ↑ Cholesterol efflux
PPAR-γ	LPL, FATP, ACS	1. Adipose tissue 2. Intestines	1. ↑ TG synthesis/storage 2. ↑ Adipocyte differentiation
LXR	CYP7A1, ABCG1, ABCA1, SREBP1c, LPL, CETP, apoE	1. Liver 2. Intestines 3. Adipose tissue	1. FA synthesis, BA synthesis 2. XOL/PL absorption and synthesis 3. Lipoprotein metabolism

ABCA, ATP-binding cassette genes; ACC, acyl CoA carboxylase; ACS, acyl CoA synthetase; apoE, apolipoprotein E; BA, bile acids; CETP, cholesterol ester transporter protein; ChREBP, carbohydrate responsive element-binding protein; CPT1, carnitine palmitoyl transferase-1; CYP, cytochrome P450; FA, fatty acid; FAS, fatty acid synthetase; FATP-1, fatty acid transporter protein-1; HMG CoA, hydroxymethylglutaryl CoA; LDL, low-density lipoprotein; LPL, lipoprotein lipase; LXR, liver X receptor; PPAR, peroxisome proliferation-activated receptor; SREBP, sterol regulatory element binding protein; TG, triglyceride; XOL/PL, cholesterol and phospholipids.
From Choudhury & Sanyal [288], with permission from Frontiers in Bioscience.

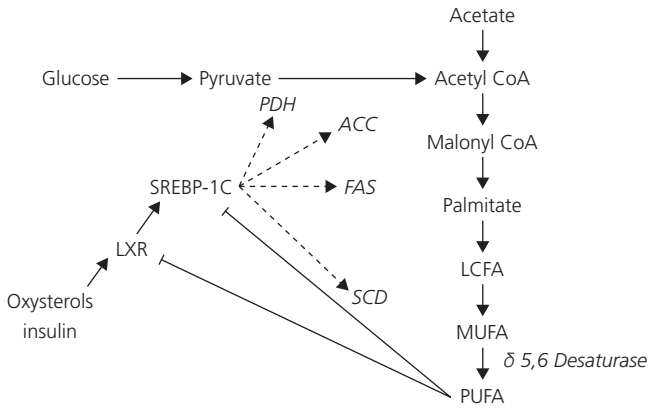


Figure 89.4 Regulation of lipid (fatty acid) synthesis by the sterol regulatory element binding protein-1c (SREBP-1c). The maturation of SREBP-1c is enhanced by liver X receptor (LXR). Insulin promotes SREBP-1c activity by means of LXR. Whereas saturated fats enhance SREBP-1c activity, polyunsaturated fatty acids inhibit SREBP-1c activity. ACC, acyl CoA carboxylase; FAS, fatty acid synthase; LCFA, long-chain fatty acids; MUFA, monounsaturated fatty acids; PDH, pyruvate dehydrogenase; PUFA, polyunsaturated fatty acids; SCD, stearoyl-CoA desaturase.

through phosphorylation by various mitogen-activated protein kinases (MAPKs) [96].

Liver X receptor is another important transcriptional regulator of metabolism. It binds to its response elements (LXRE) in DNA promoters of its target genes along with its heteromeric partner retinoid X receptor (RXR) [97]. On ligand binding, corepressor proteins are inactivated and coactivation proteins are recruited resulting in gene transcription [98]. Liver X receptor-α is present in liver and adipose tissue and is activated by oxysterols and insulin. Liver X receptor increases lipogenesis by increasing SREBP-1c transcription (Fig. 89.4) [94,99]. It also promotes cholesterol utilization by increasing primary bile acid synthesis [100]. Liver X receptor activates ABCA1 (ATP-binding cassette A1) transporters and promotes cholesterol efflux [101,102].

The carbohydrate response element binding protein (CHREBP) is another important transcriptional regulator of metabolism. Under periods of carbohydrate overload, it promotes the conversion of carbohydrates to lipids by the transcriptional increase in expression of the enzymes involved in lipogenesis [103,104]. It is activated by glucose and along with its heteromeric partner, MAX-like protein X (MLX), binds to carbohydrate response elements of the pyruvate kinase, acetyl CoA carboxylase, and fatty acid synthetase genes, thereby increasing their transcription [105,106]. Carbohydrate feeding affects energy balance and lipid homeostasis by increasing carbohydrate oxidation and lipogenesis as a result of increased SREBP and CHREBP activity.

The peroxisome proliferation-activated receptors (PPARs) also play a major role as metabolic regulators. Three isoforms have been identified: PPAR-α, -β/δ, and -γ. The PPAR-α isoform is expressed in organs where fatty acid oxidation is an important means of energy utilization, such as liver, heart, kidney, whereas PPAR-γ is preferentially expressed in adipose tissue and the intestine [107]. Long-chain fatty acids and oxidized phospholipids activate PPAR-α, which then increases FFA uptake, activation to acyl CoA esters, and mitochondrial FFA β-oxidation [108,109]. Agonists of PPAR-α increase FA utilization and limit the availability of FFA for lipogenesis [109,110]. In the small intestine, PPAR-β/δ increases expression of fatty acid binding protein (FABP) and may play a role in the intestinal adaptation to changes in dietary lipid content [111]. The PPAR-γ isoform promotes adipocyte differentiation and lipoprotein lipase activity thereby making triglyceride-derived FA available for uptake. It also induces fatty acid transport protein and acyl CoA synthetase, thus increasing adipose tissue lipogenesis, and induces expression of adiponectin, which is an important insulin-sensitizing cytokine [112]. The synergy between PPARs and LXRs means that they increase each other's expression [113]. The PPAR-γ isoform also antagonizes NF-κB, and thus has antiinflammatory effects [114,115].

Integration of systemic and local regulatory mechanisms of intermediary metabolism

From a physiological perspective, the formation of acetyl CoA (Fig. 89.3) is a key step that connects the glycolytic pathway to the Krebs cycle as well as to the lipogenic pathway. Although these pathways exist in all cells, there is organ-specific differentiation of the various limbs of these pathways. The rapid uptake of glucose by striated muscle and liver allows clearance of the postprandial glucose load. This is stored mainly as glycogen in the short term and as triglyceride within adipocytes, muscle, and liver in the long term.

During periods of caloric abundance, excess carbohydrate-derived energy can be diverted through acetyl CoA to lipid synthesis by the lipogenesis pathway under the control of insulin and several transcription factors, as noted above (e.g., LXR and CHREBP). Similarly, excess dietary fat can be stored as triglycerides. Acetyl CoA may also be derived from FFA oxidation and serve as a substrate for FFA synthesis again if required.

Yet another source of acetyl CoA is citrate, through the action of citrate lyase, another insulin-sensitive enzyme. This pathway assumes importance when the Krebs cycle slows down because of an altered NADPH:NAD ratio and citrate accumulates in the cell [116,117]. Also, when energy is required, proteins may be deaminated to ketoacids or to intermediates in the Krebs cycle, thereby providing a source of acetyl CoA and energy. This pathway is particularly important for the response to starvation or severe caloric deficiency. Free fatty acids derived from lipolysis may be oxidized to release acetyl coA, which can be used for the Krebs cycle. But FFAs interfere with hepatic insulin signaling and are associated with an increase in local and systemic lipolytic cytokines, such as TNF-α [118–121].

There is increasing evidence that FFAs themselves may play an important role as integrators of lipid and carbohydrate metabolism. As the major metabolic product of adipocytes, they have both direct effects on intermediary metabolism and also specifically affect insulin signal transduction pathways [122,123]. In particular, FFAs derived from visceral adipose tissue impair insulin signaling and promote gluconeogenesis [124,125]. Thus, insulin regulates lipolysis and thus the availability of FFAs, whereas FFAs regulate the function of insulin.

Insulin resistance and its effects on energy homeostasis

Several lines of evidence have implicated insulin resistance in the pathogenesis of NAFLD. The original clinical descriptions of NAFLD noted an association between obesity and diabetes, the two conditions best known to be associated with insulin resistance (IR) [1]. Since then, several large epidemiological studies have found a direct correlation between body mass index (BMI) and the probability of having a fatty liver as defined by hepatic sonography [126,127]. Two studies have examined the relationship between NASH and the presence of the metabolic syndrome. In the first study [128], patients with NASH were found to have lower insulin sensitivity and higher insulin secretion rates compared with age and gender-matched normal controls. In the second study [20], IR, as measured by the homeostatic model (IR_{HOMA}), was present in 65/66 subjects whereas the metabolic syndrome was only present in 55/66 subjects. Insulin resistance was present in lean as well as obese individuals. Importantly, the metabolic syndrome was present in 75% of patients with NASH compared with only 8.3% of those age- and gender-matched subjects with hepatitis C, indicating that the association between NASH and IR is highly specific for the condition. Finally, the presence of IR has been confirmed using a euglycemic, hyperinsulinemic clamp in nondiabetic, precirrhotic individuals with fatty liver as well as NASH [21].

The development of IR is multifactorial and involves both genetic and environmental factors. Inherited disorders of IR are extremely rare and include leprechaunism, Rabson–Mendenhal syndrome, and type A insulin resistance syndrome [129,130]. The genetics of IR and the phenotypic variations that may be observed as a consequence of isolated gene defects have been studied in detail using single-gene-knockout mouse models. Although these models are excellent tools for understanding the specific mechanisms of IR, they do not reflect the genomic alterations associated with the actual disease process in the body. This difficulty arises from the polygenic nature of IR and the polymorphism of different genes involved in the process of pancreatic insulin secretion and their action on specific target organs.

Studies have shed light on the early events in the genesis of insulin resistance [131,132]. With increasing adipose mass, especially visceral adipose mass, the adipose tissue is infiltrated by macrophages (Fig. 89.5). Relative hypoxia with release of

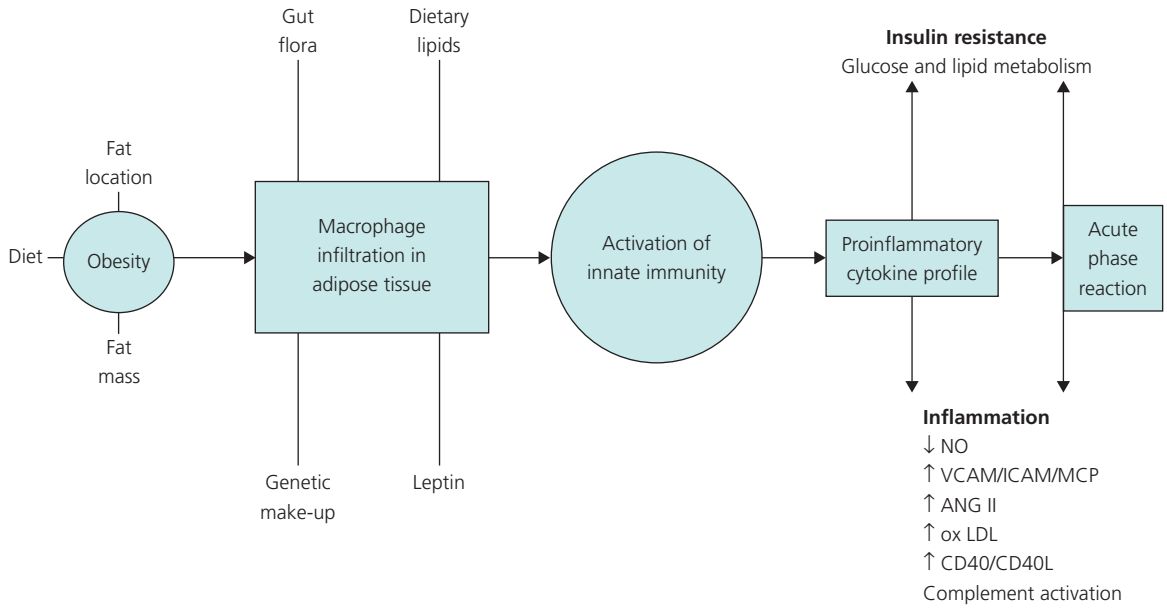


Figure 89.5 Early pathophysiological events in the development of insulin resistance. As fat mass increases, a variety of factors including relative ischemia, dietary components, cytokines (e.g., leptin), and gut flora lead to chemotactic-induced migration of macrophages into the adipose tissue. This is most prominent in visceral adipose tissue. The cell–cell interactions between macrophages and adipocytes lead to activation of the macrophages (part of the innate immune system) and production of proinflammatory cytokines. These have metabolic effects that promote insulin resistance, and they also promote inflammation. These cytokines further stimulate the liver to produce additional cytokines, which are part of the acute-phase reaction, which often accompanies the insulin-resistant state. ANG II, angiotensin II; ICAM, intercellular adhesion molecule; MCP, monocyte chemotactic protein-1; NO, nitric oxide; ox LDL, oxidized low-density lipoprotein; VCAM, vascular cell adhesion molecule.

hypoxia-inducible factor, specific dietary constituents, hormones (e.g., leptin), and gut flora-derived products have all been implicated in this process [133,134]. The interactions between these activated macrophages and adipocytes cause release of adipokines with a net proinflammatory profile, which produce a chronic inflammatory state within this tissue [135]. They also have metabolic effects that cause insulin resistance. These cytokines further affect the liver and lead to an acute-phase reaction. The net effect is a systemic proinflammatory and fibrogenic state along with metabolic insulin resistance [136].

Metabolic consequences of insulin resistance

Insulin resistance is defined by the ability of insulin to clear glucose from blood [25]. This is also how insulin sensitivity is defined, and the two terms essentially reflect two sides of the same phenomenon. It is also important to remember that the sensitivity to insulin is measured on a continuous rather than a categorical scale. Thus, insulin sensitivity and resistance each vary along a continuous scale and there is no threshold cut-off value that marks the onset of insulin resistance in a given individual.

The fundamental physiological abnormality in the insulin-resistant state is an impairment in the insulin-mediated suppression of lipolysis [21,25]. Visceral adipose tissue is less sensitive to insulin-mediated suppression of lipolysis than subcutaneous adipose tissue [137]. It is believed that this is the result of increased expression of 11β-hydroxysteroid dehydrogenase, the rate-limiting enzyme for steroid synthesis. Thus, increased FFA release from visceral adipose tissue affects hepatocytes, where the FFAs interfere with insulin signaling and cause increased gluconeogenesis and impaired glucose utilization. In striated muscle, FFAs impair glucose uptake by impairment of insulin signaling and decreased translocation of the GLUT-4 transporter to the cell surface [138,139]. Increased FFA levels and glucose load consequent to impaired clearance results in pancreatic compensation and hyperinsulinemia. Thus, the insulin level for any given plasma glucose concentration is elevated; this is the biochemical hallmark of the insulin resistant state. The role of FFAs as a potential integrator of carbohydrate and lipid metabolism has also been referred to as the single gateway hypothesis of metabolic regulation [140].

It is possible that different organs and metabolic pathways may have differential sensitivity to insulin. Thus, although IR exists almost universally in subjects with NAFLD, it is important to delineate the specific metabolic pathways that are affected. Thus, whereas there is considerable resistance to the effects of insulin on lipolysis, its effects on lipogenesis are maintained and the rates of lipogenesis are increased [141,142]. This may be due to the effects of hyperinsulinemia on SREBP and lipogenic enzyme expression and activity.

Insulin resistance and the pathogenesis of hepatic steatosis

A characteristic feature of NAFLD is the accumulation of triglycerides in the liver [16]. Triglycerides accumulate in the liver because of an imbalance between production and turnover. They are formed by the esterification of a glycerol moiety with three fatty acid molecules.

Triglyceride synthesis in the liver and the genesis of hepatic steatosis

The net pool of free fatty acids (FFAs) available for triglyceride synthesis depends on the balance between formation and utilization of fatty acids. There are two major sources of FFAs in the liver:

- FFAs that are taken up from circulation
- FFAs that are formed in the hepatocyte itself.

The availability of FFAs for triglyceride synthesis also depends on their utilization for phospholipid synthesis and for oxidation.

There are two main sources of FFAs in the circulation, diet and peripheral lipolysis. There are only limited data on the dietary intake of subjects with NAFLD and its contribution to circulating FFA levels. A high level of saturated fat, high concentration of refined sugars, and a low fiber content in the diet have all been associated with the development of insulin resistance [143–145]. There are, however, no data in subjects with NAFLD comparing them with those with obesity but without NAFLD to see if there are any specific excesses or deficiencies in their diet. In one study [146], increased fat intake correlated with increased steatosis whereas increased carbohydrate intake correlated with the presence of inflammation in the liver.

Insulin resistance is associated with increased peripheral lipolysis and higher levels of fasting FFA concentrations [147]. Compared with age, gender, and race-matched controls, NAFLD is associated with higher fasting concentrations of FFAs [21]. Moreover, fatty liver and steatohepatitis are associated with progressive resistance to insulin-mediated suppression of lipolysis as determined by the rates of appearance of glycerol during a euglycemic hyperinsulinemic clamp [21]. Studies are ongoing to define the nature and relative quantities of FFAs in circulation in subjects with NAFLD.

The hepatic pool of FFAs is also derived from FFAs that are synthesized de novo in the liver. This pathway, also known as the de novo lipogenesis (DNL) pathway, involves the synthesis of FFA from acetyl CoA [148]. Under physiological circumstances, DNL contributes to only 5%–8% of triglycerides synthesized in the liver [149]. However, the physiological relevance of DNL is not well understood. The substrates for DNL include acetate, carbohydrate-derived acetyl CoA, and acetyl CoA derived from lipid oxidation. The relative contributions of these sources to DNL under varying conditions are not known. Nonalcoholic fatty liver disease is associated

with increased DNL [150]. As noted above, the enzymes of DNL are under the control of SREBP-1c, which is an insulin-sensitive transcription factor [151]. Hyperinsulinemia is also associated with NAFLD [21]. It appears that despite the resistance of lipolysis in peripheral adipose tissue to the effects of insulin, its effects on DNL in the liver are maintained in NAFLD. It is likely that this is facilitated by low adiponectin levels, which would decrease AMP kinase activity and thus disinhibit acyl CoA carboxylase, the rate-limiting enzyme in DNL [84]. Low levels of polyunsaturated fatty acids (PUFAs) in the liver may also promote maturation of SREBP-1c from the endoplasmic reticulum–Golgi system [152,153]. This forms the rationale for the use of PUFAs in NAFLD. De novo lipogenesis is also increased by the intake of fructose, especially high levels of corn syrup fructose, a common additive to many foods and beverages [154]. It bypasses the regulated steps in carbohydrate metabolism and forms glycerol-3-phosphate and acetyl CoA, both of which can serve as precursors for DNL. The national consumption of total fructose has been increasing and correlates well with the increasing prevalence of obesity in the United States [154].

It is well known that acetyl CoA can be used either for the formation of malonyl CoA or for mevalonate. The former leads to FFA synthesis and is under the control of SREBP-1c, whereas the latter leads to cholesterol synthesis and is regulated by SREBP-2 [155,156]. The two pathways are known to be coordinately regulated. If so, up-regulation of DNL should be accompanied by increased cholesterol synthesis. However, the principal features of the dyslipidemia associated with the metabolic syndrome and NAFLD are hypertriglyceridemia and a low concentration of HDL cholesterol. Liver X receptor senses the intracellular levels of oxysterols and modulates lipid synthesis in the liver [157–159]. The potential role of LXR in the dyslipidemia associated with NAFLD and the increase in DNL remains to be defined. Also, the potential role of farnisoid X receptor (FXR), which determines bile acid transport into bile, and its interactions with LXR in defining the dyslipidemia associated with the metabolic syndrome and NAFLD, are under active investigation. Liver X receptor affects the ATP-binding cassette lipid transporter (ABCA1) and modulates the export of cholesterol to HDL [160]. Also, steroyl CoA desaturase, a SREBP-1c-sensitive enzyme that catalyzes the formation of monounsaturated fatty acids, destabilizes the ABCA1 function [161]. These are likely mechanisms that cause the low HDL-cholesterol levels in NAFLD.

The availability of FFAs for triglyceride synthesis is also affected by their utilization for oxidation and for phospholipid synthesis. Subjects with NAFLD do not have systemic defects in the incorporation of FFA into phospholipids [21]. Phosphatidylcholine serves as a reservoir for PUFAs in the cell and, theoretically, lowering phospholipase A_2 ($iPLA_2$) should reduce levels of PUFAs and increase SREBP-1c activity. The potential role of such a mechanism in NAFLD remains to be explored.

Finally, the availability of FFAs for triglyceride synthesis is also affected by their utilization for oxidation. Free fatty acids can undergo β-oxidation in the mitochondria or peroxisomes [162]. They may also be oxidized by the microsomal enzyme or cytochrome P450 system [163]. Compared with matched controls, NAFLD is associated with increased β-hydroxybutyrate levels and higher lipid oxidation rates [21]. Also, during a euglycemic clamp, normal individuals suppress β-hydroxybutyrate levels, indicating that mitochondrial fatty acid oxidation is normally suppressed by insulin. However, fatty liver and NASH are associated with an impairment in insulin-mediated suppression of serum concentrations of β-hydroxybutyrate, confirming that these conditions are associated with resistance to insulin effects on fatty acid oxidation. These data are compatible with the known effects of increased FFA delivery on mitochondrial fatty acid oxidation. At a molecular level, FFAs may directly interfere with insulin signaling and inhibit insulin-mediated suppression of ketogenesis [164,165]. Also, increased activity of the suppressor of cytokine signaling-1 (SOCS-1) has been associated with impairment of insulin signaling pathways [166,167]. Although there are no human data on peroxisomal fatty acid oxidation in those with NASH, peroxisomal proliferation has been reported in those with NAFLD [168].

Triglyceride export from the liver and the genesis of hepatic steatosis

There are no accurate estimates of quantitative flux rates for the amounts of triglyceride synthesized from DNL vs reesterification of circulating FFA vs transport from the liver in subjects with NAFLD. Triglycerides are packaged in VLDL in the liver by a process requiring the microsomal transfer protein (MTP) and apolipoprotein B100. Several rare syndromes of NAFLD are associated with defects in the MTP or in VLDL assembly [169,170]. The incorporation of labeled leucine into apoB-100 is decreased in subjects with NAFLD [171]. It is, however, unclear if this is a disease-specific effect or if it is a cause or effect of the NAFLD. In summary, hepatic steatosis results from either or both increased synthesis and decreased export from the liver. Insulin resistance contributes to hepatic steatosis in multiple ways, by increasing levels of FFA for reesterification, increasing the rate of DNL, and providing the substrates for DNL.

In summary, the overall integration of metabolic pathways are closely regulated to maintain homeostasis (Fig. 89.8). An imbalance in hepatic lipid metabolism (synthesis and import vs metabolic utilization and export) causes fat accumulation in hepatocytes. Imbalances in these processes, which are controlled by transcription factors such as SREBP, result from dysregulated transcriptional control, which is, in turn, influenced by the abnormal endocrine and paracrine milieu associated with insulin resistance. All of these layers of complexity are further modulated by many factors such as genetic makeup, diet, and lifestyle. Metabolism is a complex biological

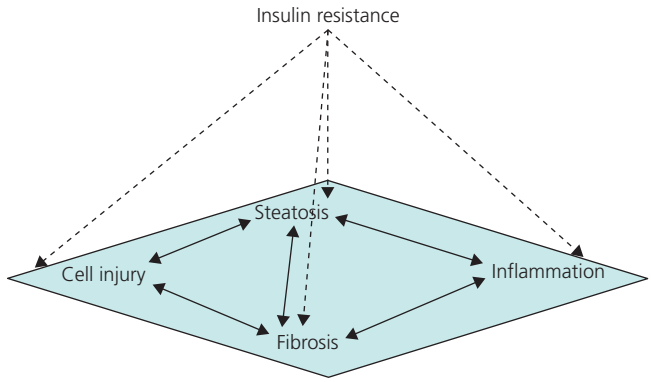


Figure 89.6 Schematic illustrating the pathogenesis of nonalcoholic steatohepatitis (NASH). Insulin resistance promotes steatosis, cell injury, inflammation, and fibrosis. Each of these factors further promotes each other, eventually causing cell injury, inflammation, and fibrosis – the hallmarks that distinguish steatohepatitis from steatosis alone.

state, and this explains why simple paradigms focused on a single mechanism fail in one or more clinical scenarios.

Pathogenesis of steatohepatitis

It was originally proposed that the development of NASH required two hits: insulin resistance that produced hepatic steatosis, and an additional hit that led to development of steatohepatitis. Since the 1990s, it has become apparent that the situation is more complicated (Fig. 89.6). Insulin resistance has been shown to promote directly or indirectly hepatic steatosis, hepatocyte injury, inflammation, and fibrosis. Moreover, the pathways involved in each of these processes affect the pathogenesis of all of the other endpoints that produce the phenotype of steatohepatitis.

Role of oxidative stress

It is generally believed that oxidative stress plays an important role in the pathogenesis of steatohepatitis. Animal models of NAFLD have demostrated the presence of oxidative stress [172]. In human studies, NAFLD has been associated with increased lipid peroxides, a marker of oxidative stress [21]. Evidence of increased lipid peroxides is seen in both those with hepatic steatosis alone as well as those with steatohepatitis. However, subjects with NAFL have lower levels of lipid peroxides compared with those with NASH [21].

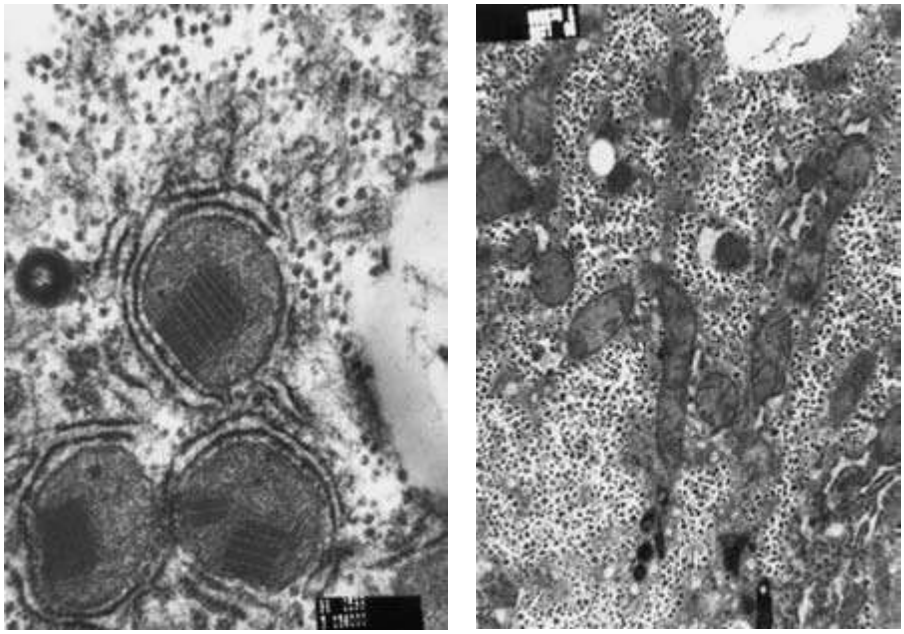
Oxidative stress results from increased production of prooxidants – reactive oxygen species (ROS) or reactive nitrogen species (RNS) – or from decreased antioxidant defenses. There are several potential sources of ROS in the liver (Table 89.6). These include mitochondria, peroxisomes, the microsomal oxidative system, and iron overload. The mitochondria of hepatocytes in liver afflicted by NASH are markedly abnormal; they become ballooned and lose cristae (Fig. 89.7) [21,173]. This is accompanied by formation of paracrystalline intramitochondrial inclusions. These changes are relatively specific for NASH and are not commonly seen in NAFL. Within each hepatocyte some 10%–40% of mitochondria demonstrate these abnormalities.

The precise composition of the paracrystalline inclusions is not known. Similarly, nothing is known about the mechanisms by which mitochondrial morphology become abnormal. However, such inclusions are commonly seen in other cell types in diseases associated with mitochondrial DNA mutations [174]. Mitochondrial DNA encodes several components of the electron transport chain in the mitochondria. It has been shown that there is a generalized decrease in the activity of the hepatic electron transport chain enzymes in subjects with NASH [175]. Decreased electron transport chain activity in the face of continued fatty acid β-oxidation due to increased fatty acid delivery to the liver and intrahepatic mitochondrial insulin resistance should cause an uncoupling of oxidation and phosphorylation [176,177]. Uncoupled oxidation–phosphorylation causes ROS formation and enhances oxidative stress. This concept is further supported by evidence of decreased ATP formation in the liver of subjects with NASH [178]. It is possible that minor degrees of ROS formation from increased fatty acid oxidation cause oxidative damage to mitochondrial DNA, which becomes depleted over time causing the electron transport chain activity to decline, thereby further worsening oxidative stress. This remains to be verified experimentally. It is also not known whether mitochondria regenerate if insulin resistance is corrected.

Another source of oxidative stress is the cytochrome P450 system. This system, particularly CYP2E1, is overexpressed in subjects with NAFLD [179,180]. Using chlorzoxazone as a substrate for this enzyme, it has been shown that the activity of this enzyme is also increased in subjects with NASH [181]. This member of the cytochrome P450 system can be induced by the insulin-resistant state as well as by FFAs [182,183]. Impairment of insulin signaling-induced CYP2E1 overexpression in NASH is most likely secondary to posttranslational enzyme stabilization, although more direct transcriptional control also may have a role in this process [184]. It is believed that overexpression of this enzyme is associated with futile cycling of the enzyme, which generates ROS. Induction of CYP2E1 may be expected to be associated with expansion of the endoplasmic reticulum [183]. There are no studies evaluating the presence or absence of an expansion of

Table 89.6 Potential sources of oxidative stress in nonalcoholic fatty liver disease (NAFLD)

Mitochondria
Microsomal ethanol oxidizing system
Peroxisomes
Iron overload
Decreased antioxidant defenses



NASH Fatty liver

Figure 89.7 Mitochondrial morphology in nonalcoholic steatohepatitis (NASH). Whereas hepatocyte mitochondria in fatty liver are generally normal **(right panel)**, the mitochondria in NASH are often ballooned with loss of cristae and development of paracrystalline inclusions **(left panel)**. From Sanyal et al. [21], with permission from Elsevier.

the endoplasmic reticulum in subjects with NASH. Also, CYP2E1 induction has been described in subjects with the metabolic syndrome even in the absence of a fatty liver [185]. Finally, there is induction of CYP2E1 even in subjects with NAFL, and additional mechanisms must be required to produce steatohepatitis.

Peroxisomes also oxidize long-chain fatty acids but do not generate ATP. As a consequence, peroxisomal fatty acid oxidation causes ROS formation. It has been shown that peroxisomes are more numerous in subjects with alcoholic as well as nonalcoholic fatty livers [168]. There are almost no data on the function of peroxisomes in subjects with NASH. Iron overload has been associated with increased oxidative stress [186]. Although some studies have shown an association between NASH and iron overload [187], others have failed to find such an association [188,189].

The impact of oxidative stress in the liver depends on the balance between prooxidant and antioxidant activity. Little is known of the status of antioxidant pathways in the liver of subjects with NASH. A single study showed decreased paraoxonase-1 activity in the metabolic syndrome [190]. It has also been suggested that promoter polymorphisms in several Mn-superoxide dismutase may affect the progression of NASH [191].

Oxidative stress produces its biological effects through several mechanisms. These include direct lipid peroxidation of membranes of organelles, oxidative changes in DNA, mitochondrial DNA depletion, and changes in the expression of redox-sensitive genes [192]. These changes in gene expression have far-reaching effects on a multitude of cellular functions and can lead to adaptive changes or cell death. Readers are referred to several excellent reviews for in-depth discussion of this subject [192–195].

Mechanisms of cell injury and death

The phenotype of steatohepatitis is characterized by the presence of cytological ballooning, Mallory hyaline, apoptosis, inflammation, and pericellular fibrosis [196]. The pathogenesis of cytological ballooning is not known. A priori, one would expect a defect in the maintenance of normal cell volume regulation because an increase in cell volume is the hallmark of cytological ballooning. Cell volume is regulated by a number of complex processes including osmotic receptors and the intracellular actin network, which tethers the cell membranes [197]. In a fibroblast cell line, insulin has been shown to produce reorganization of the actin network [198]. The potential role of hyperinsulinemia and dysregulation of insulin signaling in the liver in the genesis of cytological ballooning remains to be defined. It is also unclear why occasional cells undergo cytological ballooning whereas other adjacent cells do not.

It has been shown that Mallory bodies are composed of heat-shock proteins that are ubiquitylated but fail to undergo proteosomal degradation [199]. Heat-shock proteins are a family of proteins that are produced in response to a variety of cellular stresses, including oxidative stress – a direct function of insulin resistance and uncoupling of oxidation and phosphorylation in mitochondria. It is likely that these proteins are induced in response to the oxidative stress found in subjects with either NAFL or NASH. However, in subjects with NASH, there may be defects in protein trafficking to the proteosomes or in proteosomal function itself that lead

to accumulation of heat-shock proteins within intermediate filaments. The nature and mechanisms involved in defective protein trafficking and proteosomal function in NASH remain unknown. Of note, Mallory bodies are almost always seen within ballooned hepatocytes. The mechanistic relationship between altered cytological ballooning and the development of Mallory hyaline also remains unknown.

Another characteristic feature of NASH is extensive apoptotic activity [200]. Several mechanisms have been invoked to explain the proapoptotic state in NASH. Triglyceride accumulation itself increases apoptosis by the interaction of unoxidized palmitoyl CoA with serine to form dihydrosphingosine, a precursor of ceramide [201]. Ceramide is a potent inducer of apoptosis through an inducible nitric oxide synthetase (iNOS)-mediated pathway that requires the transcription factor NF-κB [120]. It has also been shown that increased FFA delivery to hepatocytes, secondary to increased peripheral lipolysis in the insulin-resistant state, may also induce apoptosis [121]. Free fatty acids produce a translocation of BCL2-associated X protein (BAX) to lysosomes, where it causes releases of cathepsin. Cathepsin, acting by means of NF-κB, induces expression of TNF-α and activation of TNF-receptor-associated death pathways.

Interest has surrounded the potential role of endoplasmic reticulum stress (ER stress) in the development of cell injury in NASH. The ER is the principal site for synthetic activity within cells. Such activity requires not only appropriate synthesis but also local mechanisms to ensure that the proteins are correctly folded because this is essential for their recognition by the appropriate receptors and trafficking to their final destination. Under conditions where there is increased protein or lipid synthetic activity in the ER, depletion of ATP, depletion of calcium, or altered glucose homeostasis, the regulatory function of the ER in maintaining normal synthetic function is disrupted. This leads to activation of an intracellular program called the unfolded protein response (UPR) [202].

Activation of the UPR initially leads to an adaptive response in which protein synthesis decreases and allows restoration of normal ER function. Simultaneously, proteosomal degradation of unfolded proteins is increased – ER-associated degradation (ERAD). However, if the initiating factors are not corrected, alarm pathways are activated; these include activation of a number of stress kinases, which eventually result in activation of C/EBP homologous protein (CHOP), a potent apoptosis-inducing factor. Although animal studies suggest that ER stress is induced in fatty liver, there are no human data to confirm that ER stress plays a role in the genesis of cell injury in NASH.

Mechanisms of inflammation

Nonalcoholic steatohepatitis is characterized by a scattered, predominantly lobular, mixed inflammatory infiltrate. The precise mechanisms of inflammation in NASH have not been defined. It is generally presumed that the products of cell death and the release of inflammatory cytokines within the liver cause changes in blood flow and endothelial function that promote inflammation.

At a cellular level, activation of proinflammatory pathways is associated with activation of the stress kinase JNK (c-JUN N-terminal kinase) (Fig. 89.8). This enzyme can be activated by ER stress, oxidative stress, and inflammatory cytokines, for example, TNF-α [203–205]. It may also be activated by TNF-independent pathways [206]. Activation of JNK has numerous effects: it activates other kinases, which results in activation of the transcription factors NF-κB and activator protein-1 (AP-1) [207], which leads to transcriptional induction of inflammatory cytokines (e.g. TNF-α) [208,209]. These transcription factors also directly affect cellular metabolic activity [210]. Activated JNK can also produce serine phosphorylation of the insulin receptor substrate and further worsen insulin resistance and the propensity to develop steatosis [211]. Activation of JNK can also be modulated by the suppressor of cytokine signaling (SOCS) [212]. It must be noted that these data have been obtained almost entirely in animal models and their activation in NASH remains to be demonstrated.

Another important factor is the role of the innate immune system in the promotion and progression of inflammation in the liver in subjects with NASH (Fig. 89.5). An early event in the development of insulin resistance is the infiltration

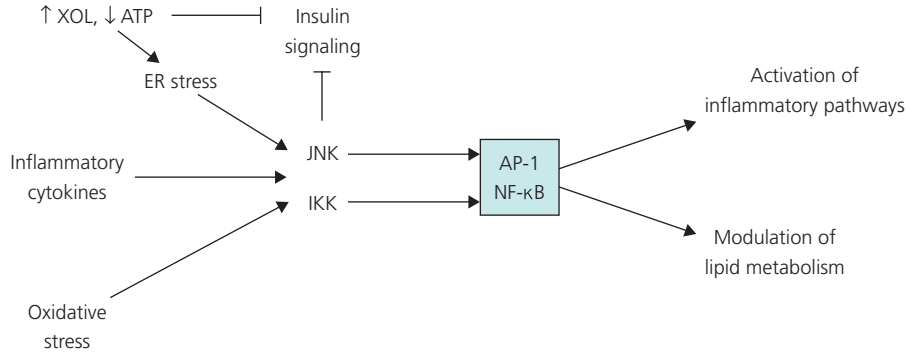


Figure 89.8 Cellular pathways involved in steatohepatitis. Endoplasmic reticulum stress (ER stress), inflammatory cytokines, and oxidative stress all lead to activation of c-JUN terminal kinase (JNK) and IκB kinase (IKK) resulting in activation of the transcription factors AP-1 and NF-κB. These increase expression of genes regulating proinflammatory cytokines and also further impair metabolic homeostasis and cause both glucose intolerance and hepatic steatosis. XOL, cholesterol.

of adipose tissue, especially visceral adipose tissue, by macrophages [132]. Relative tissue hypoxemia and induction of hypoxia-inducible factor-1 along with several dietary constituents have been implicated in this process [213,214]. These macrophages produce a number of proinflammatory cytokines, which further induce production of additional cytokines from adjacent adipocytes [74,215]. These cytokines antagonize insulin-mediated suppression of lipolysis and also produce a proinflammatory milieu within the adipose tissue [73]. They also enter the circulation and induce an acute-phase reaction by the liver. Interleukin-6 plays a key role in this process [114,216]. These cytokines and the secondary cytokines produced in the liver alter endothelial function and promote vascular dysfunction, and leukocyte adherence and migration. The role of the innate immune system in the initiation and maintenance of inflammation within the liver is under active investigation.

Mechanisms of fibrosis

Pericellular fibrosis in the hepatic sinusoids is a histological hallmark of NASH [217]. This results from activation of quiescent hepatic stellate cells, which then produce a collagenous matrix. Over time, the fibrosis extends to form central-to-central and central-to-portal bridges, which then evolve to cirrhosis. Several factors have been implicated in the activation of the stellate cells. These include a variety of cytokines, of which transforming growth factor-β (TGF-β) is the best known [218]. Both hyperinsulinemia and increased leptin levels increase collagen synthesis by activated stellate cells [219,220]. It is also conceivable that if stellate cell apoptosis is inhibited, the total fibrotic process may be enhanced. Some studies have focused on the role of N-cadherin, tissue inhibitor of metalloproteinase (TIMP), and endocannabinoids in this process [221,222]. The mechanisms involved in activation and perpetuation of the fibrotic processes is an area of active investigation. Importantly, following improvement of insulin resistance, hepatic fibrosis can regress [223,224].

Progression of NASH to cirrhosis

The progression of NASH to cirrhosis reflects a balance between cell injury vs repair and regeneration (Fig. 89.9). On the one hand, cell injury results from both the metabolic disturbances associated with insulin resistance and the inflammatory state of the liver. The latter is associated with the presence of proinflammatory cytokines (e.g., TNF-α) and products of inflammation (e.g., leukotrienes). On the other hand, tissue repair involves both repair of cells that have sublethal injury as well as the removal and replacement of dead hepatocytes with either new hepatocytes derived from stem cells or by scar tissue. Our understanding of the mechanisms of hepatic tissue repair and pathological wound healing in disease states is limited. Specifically, the mechanisms by which hepatic architecture is disrupted with progression to cirrhosis remain poorly understood.

Clinical features

Symptoms and signs

Most subjects with NAFLD are asymptomatic (Table 89.7). In such cases, the diagnosis is often made when the person is discovered to have either abnormal liver enzymes or features of a fatty liver on an imaging study performed for unrelated reasons. Others are diagnosed because the liver looked unusual during abdominal surgery, and yet others are diagnosed because of persistent hepatomegaly. It is, however, important to remember that only a small minority of subjects with NAFLD have been diagnosed and the great majority of afflicted individuals remain undiagnosed.

When symptoms do occur, the most common ones to bring NAFLD patients to clinical attention include malaise, fatigue, and right upper quadrant discomfort. The discomfort is often described as a nondescript vague discomfort rather than pain. Such symptoms antedate the diagnosis of NAFLD in one-third of patients [225].

Fatigue is often present in patients with NAFLD and is frequently the most debilitating aspect of the condition for the individual subject. In our experience, fatigue is the most common symptom in this patient population. Several clinical variations of the fatigue syndrome can be recognized. Most commonly, subjects complain of chronic malaise and a sensation of severe lethargy and sleepiness during the day. This is associated with disturbed sleep patterns, and patients often wake up feeling tired. Many subjects have associated sleep disorders and, on direct questioning, will acknowledge that they snore. The spouses of affected individuals often report that the patient is restless at night. It has been recognized that the insulin resistance syndrome is not simply a function of diet and lifestyle but is also markedly affected by abnormal sleep patterns [226,227]. Thus, abnormal sleep leads to insulin resistance whereas the insulin resistance syndrome is associated with abnormal sleep patterns [228,229]. It has been our experience that some of these subjects have underlying sleep-apnea syndrome, and correction of this condition produces a dramatic improvement in energy levels. This type of fatigue does not correlate with the grade or stage of steatohepatitis. The relationship of other sleep disorder-related abnormalities and insulin resistance remains poorly defined.

Other subjects wake up relatively rested but tire easily. This pattern is more often associated with more advanced disease. Some patients have mixed patterns of fatigue that are often difficult to evaluate and manage. Approximately 25% of subjects with NAFLD also carry a diagnosis of chronic fatigue syndrome [230]. Some patients complain of aching soreness in their muscles along with fatigue, and about 20% of all subjects suffer from a chronic pain disorder [231]. At our institution, 27% of subjects diagnosed with NAFLD were taking analgesics, including narcotics (7%), at the time of diagnosis [230].

Table 89.7 Common symptoms and signs of 400 subjects with nonalcoholic fatty liver disease (NAFLD)

Feature	NAFL (*N* = 75)	NASH (*N* = 325)
Symptoms and signs (%)		
Asymptomatic	60	55
Fatigue	30	45
Pruritus	2	4
RUQ discomfort	30	32
Edema	4	5
Hepatomegaly	22	28
Stigmata of chronic liver disease	8	10
Obesity	65	60
Diabetes	45	50
Hypertension	60	65
Dyslipidemia	65	69
Lab abnormalities (ULN) (mean ± SD)		
AST (< 75 IU/L)	65 ± 55	76 ± 66
ALT (< 75 IU/L)	135 ± 70	148 ± 78
Alkaline phosphatase (< 110 IU/L)	132 ± 21	128 ± 34
Total bilirubin (< 2 mg/dL)	0.8 ± 0.3	0.7 ± 0.7
Albumin (3.5–4.5 g/dL)	3.8 ± 0.4	3.7 ± 1
Prothrombin time (< 11 s)	10.9 ± 0.6	11.1 ± 0.6
Proportion of cirrhotics	0	16

ALT, alanine aminotransferase; AST, aspartate aminotransferase; NAFL, nonalcoholic fatty liver; NASH, nonalcoholic steatohepatitis; RUQ, right upper quadrant; ULN, upper limit of normal.
From Ramesh & Sanyal [231], with permission from Elsevier. Data from the NAFLD clinic at Virginia Commonwealth University.

Obesity is the most common physical finding, and is present from in 50%–90% of subjects [16]. About two-thirds of subjects with NAFLD also have other features of the metabolic syndrome. Hepatomegaly is the most common liver-related physical finding in subjects with NAFLD [7]. A minority of subjects have stigmata of chronic liver disease, such as spider angiomas or palmar erythema. Jaundice and features of portal hypertension, such as ascites and variceal hemorrhage, are seen in a small minority of subjects who present with advanced liver disease [232].

Other common associated conditions

The association of NAFLD with obesity, diabetes, hypertension, and hypertriglyceridemia is well known. I have performed a detailed inventory of associated symptoms and conditions in a cohort of 61 subjects with NAFLD [230]. In addition to confirming the classic associations with NAFLD, it was found that 26% of subjects had various allergies and 25% suffered from chronic depression, whereas 10% carried a diagnosis of an anxiety disorder and over 50% of subjects were taking a psychotropic drug at the time of diagnosis. On detailed questioning, the onset of weight gain often corresponded with a life event that is also associated with depression or anxiety, for example, divorce, accident, or child birth. In some of these cases, the onset of weight gain was temporally related to the development of the behavioral disorder and the use of an antidepressant drug. Given the propensity of several antidepressant drugs to cause weight gain, the potential role of such drugs in the weight gain and subsequent development of insulin resistance is an important and as yet unexplored question.

Whether depression and anxiety disorders are unrelated or related to NAFLD remains to be evaluated. Also, the prevalence of NAFLD in the general population of subjects with chronic pain or fatigue is unknown. One should, however, be aware that NAFLD may also be present in subjects with chronic fatigue, fibromyalgia, and depression.

Studies have highlighted the importance of a fatty liver as an independent risk factor for a variety of endpoints related to the metabolic syndrome. In a 5-year follow-up study of 633 subjects, subjects with abnormal liver enzymes had a 2.2–2.8-fold increased risk of developing the full-blown metabolic syndrome and outperformed the C-reactive protein [233]. Similarly, other studies have shown that those with abnormal liver enzymes have a higher risk of developing hypertension and diabetes [234,235]. The presence of NAFLD (either NAFL or NASH) is also independently associated with endothelial dysfunction, abnormal vascular reactivity, increased carotid intimal thickness, and the number of plaques in the carotids [236,237]. These data indicate that although having a fatty liver may not have many clinical consequences for the liver, it can have multiple deleterious effects on health in general.

Insulin resistance and the metabolic syndrome, which are present, respectively, in over 75% and 90% of subjects with NAFLD [20], are associated with numerous abnormalities of the coagulation cascade. The metabolic syndrome is associated with increased concentrations of circulating tissue factor (TF) and factor VII thereby creating a prothrombotic state [238]. The severity of insulin resistance, that is, hyperinsulinemia and adipose tissue mass, both determine the levels of TF and factor VII [239–241]. Factor VII and TF form a complex that activates factor X, which, together with factor V, converts prothrombin to thrombin. This step is further amplified by activation of factor IX by the TF–factor VII complex, as well as by thrombin itself along with recruitment of cofactors V and VIII. Thrombin converts fibrinogen to fibrin thereby forming a clot. The metabolic syndrome has also been associated with high levels of factor VIII and fibrinogen, further promoting the procoagulant state in this condition [242].

The tendency to form clots is counteracted by inhibitors of the clotting pathways, and by fibrinolysis, which is initiated by plasminogen activators. The impact of insulin resistance on inhibitors of coagulation, such as protein c, protein s,

and TF pathway inhibitor, has not been fully defined. Plasminogen activator inhibitor-1 (PAI-1) is the principal physiological inhibitor of fibrinolysis. Insulin resistance is associated with increased PAI-1 levels resulting from increased production and release by adipose tissue and adipose-tissue-associated stromal cells [243]. Expression of PAI-1 is increased by TNF-α, TGF-β, insulin, hyperglycemia, increased FFAs, and triglycerides [243–245].

In summary, the presence in a patient of risk factors for NAFLD, right upper quadrant discomfort, hepatomegaly, or elevated liver enzymes should trigger an evaluation for NAFLD. Other subjects with persistently elevated liver enzymes (e.g., ALT) who do not have viral hepatitis, hemochromatosis, or other common liver diseases, such as Wilson disease, should also be evaluated for NAFLD. It is also important to remember that there are no symptoms or signs to distinguish between those with NAFL and those with NASH.

Laboratory abnormalities

Approximately 7.9% of the US population have persistently abnormal liver enzymes with negative tests for viral hepatitis and other common causes of liver diseases [37]. The presence of abnormal liver enzymes is related to the BMI and other risk factors associated with NAFLD. Thus, the majority of these subjects have NAFLD. It is also important to note that a large number of subjects with NAFLD have persistently normal liver enzymes, and the entire histological spectrum of NAFLD can be seen in such individuals.

The most common liver enzyme abnormality is an elevated serum concentration of ALT. The degree of elevation is usually modest and when the values exceed 300 IU/L, alternate causes of liver disease should be carefully sought. The aspartate aminotransferase (AST) level is elevated to a lesser degree, and the AST:ALT ratio is usually less than 1. In some individuals, an elevated serum level of alkaline phosphatase is the only abnormality. When elevated, it rarely exceeds twice the upper limit of normal.

The hepatic synthetic functions are not altered until the disease is far advanced. The serum albumin and prothrombin time become abnormal before the bilirubin level becomes elevated. In diabetic subjects, isolated hypoalbuminemia can result from diabetic nephropathy. Hematological parameters are usually normal unless cirrhosis and portal hypertension lead to hypersplenism. About 30%–50% of subjects with NAFLD have elevated blood glucose levels, and about 60% of patients with NAFLD also have associated hypertriglyceridemia or a low HDL cholesterol level [20,231]. An elevated ferritin level is also often seen in subjects with NAFLD [7,187]. It is, however, not associated with iron overload in most cases and usually reflects an acute-phase response [7].

From a practical point of view, the laboratory evaluation of a subject with suspected NAFLD involves:

- exclusion of alternate causes of liver enzyme abnormalities
- documentation of hepatic steatosis
- making the distinction between hepatic steatosis and steatohepatitis
- evaluating the stage of the disease
- evaluating the presence and severity of insulin resistance and other complications of the metabolic syndrome (Table 89.8).

In a given subject, each of these must be carefully considered and a decision made about how aggressively the answer to each is to be sought. A liver biopsy remains the gold standard for diagnosing steatohepatitis and for the staging of the liver disease unless clinically evident cirrhosis is present.

Natural history (Fig. 89.9)

There are only limited data concerning the natural history of NAFLD. Most studies are cross-sectional in nature and have focused on highly selected patient populations. In such studies, the majority of subjects have NAFL. It is generally believed that NAFL rarely progresses to more advanced liver disease [44]. However, there are several reported cases of development of steatohepatitis in subjects with NAFL, particularly after rapid weight loss following bariatric surgery [45]. This occurs more frequently after jejunoileal bypass and only rarely after proximal gastric bypass or vertical banded gastroplasty [246,247].

The long-term natural history of subjects with NAFLD is affected by the presence of underlying metabolic syndrome, and these patients are at risk from morbidity related to diabetes and cardiovascular disease. One study has found increased cardiovascular mortality in subjects with cirrhosis due to NASH compared with those with hepatitis C [248]. Subjects with the metabolic syndrome are also at risk for

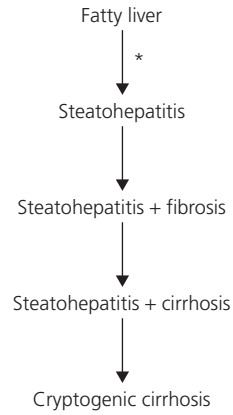


Figure 89.9 The natural history of nonalcoholic fatty liver disease (NAFLD). *It is debated whether steatosis progresses to steatohepatitis. The frequency with which this occurs, especially in the absence of severe weight loss such as that seen after bariatric surgery, remains debated. About 20% of subjects with nonalcoholic steatohepatitis (NASH) develop increasing fibrosis and eventually cirrhosis. Following development of cirrhosis, the hepatic steatosis and the other features of NASH may disappear leading to the presentation of cryptogenic cirrhosis.

Table 89.8 Evaluation of a subject with nonalcoholic fatty liver disease (NAFLD)

1. Exclusion of alternate causes of abnormal liver enzymes
2. Evaluation of presence of hepatic steatosis:
   a: sonography[a]
   b: CT scan
   c: MRI
3. Assessment of presence of steatohepatitis:
   a: liver biopsy only definitive method of making assessment
4. Assessment of stage of disease:
   a: This is sometimes clinically evident (cirrhosis) but usually requires a biopsy. Subjects may be selected on basis of markers of advanced fibrosis: increasing age, persistently abnormal ALT, elevation of AST greater than ALT, presence of other end organ diseases associated with the metabolic syndrome, e.g., diabetes and increasing body mass index and waist-to-hip ratio
5. Assessment of presence of other features of metabolic syndrome:
   a: diabetes – fasting blood sugar
   b: hypertension
   c: dyslipidemia – fasting lipid profile
   d: body mass index and waist circumference
6. Evaluation of other diseases commonly associated with NAFLD:
   a: obesity
   b: sleep apnea
   c: coronary artery disease: monitor Framingham risk score
7. Measurement of degree of insulin resistance:
   IR_{HOMA} (homeostatic model): fasting insulin and glucose[b]

a Sonography is the most commonly used method.
b A fasting insulin level serves just as well.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CT, computed tomography; IR_{HOMA}, homeostasis model assessment of insulin resistance; MRI, magnetic resonance imaging.

development of gallstones. Although gallstones are well known to occur in subjects with obesity, the specific prevalence of gallstones in subjects with NAFLD is unknown.

The natural history of NAFLD was evaluated by chart review of subjects diagnosed in the last 20 years in Olmstead county [249]. The overall survival of subjects with NAFLD was lower than that of a matched population in Minnesota. Higher mortality was associated with age (hazard per decade 2.2; 95% confidence interval [CI] 1.7–2.7), impaired blood glucose (hazard ratio 2.6; 95% CI 1.3–5.2), and cirrhosis (hazard ratio 3.1, 95% CI 1.2–7.8). Liver disease was the third leading cause of death in this population, whereas it was the 13th cause of death in Minnesota. These data confirm previous studies in which the presence of cirrhosis and steatohepatitis were associated with increased mortality [250].

There are only limited data about the progression of NASH to cirrhosis. Most studies are retrospective and methodologically flawed. However, based on the limited data available, the risk of progression to cirrhosis is estimated to be about 20% [251]. In a study of 103 subjects who had multiple liver biopsies over a median duration of 3.2 years, 37% of subjects showed progression of fibrosis whereas 29% showed regression [252]. It is, however, unclear whether these findings reflect sampling variability. It has been reported that a biopsy length of about 4 cm is required to obviate such variability [15]; this length is rarely obtained in routine clinical practice. In cross-sectional studies, increasing age, diabetes, and BMI have been associated with advanced fibrosis [188]. In other studies, a combination of hypertension, abnormal ALT levels, and insulin resistance as measured by the homeostatic model have been associated with a higher probability of having advanced fibrosis in severely obese individuals [46]. Evaluation of the existing literature suggests that older subjects with additional features of the metabolic syndrome, such as diabetes and hypertension, are more likely to have advanced disease.

Once cirrhosis develops, the natural history of NASH is characterized by an approximately 4% annual rate of decompensation [248]. In a 10-year follow-up of 150 subjects with cirrhosis due to NASH, the rate of decompensation was found to be slightly but significantly lower compared with a matched population of subjects with hepatitis C and cirrhosis. These difference were mainly due to lower rates of development of ascites and synthetic failure (Fig. 89.10). Although some studies have suggested that hepatocellular cancer occurs frequently in NASH, they have not evaluated the population at risk, that is, the denominator [253,254]. One study found that 10/149 subjects with cirrhosis due to NASH developed hepatocellular cancer over a 10-year period compared with 25/147 subjects with hepatitis C (Fig. 89.11) [248].

Another aspect of NASH is that hepatic steatosis and other features of steatohepatitis decrease after cirrhosis develops [232]. In some cases, these features disappear completely

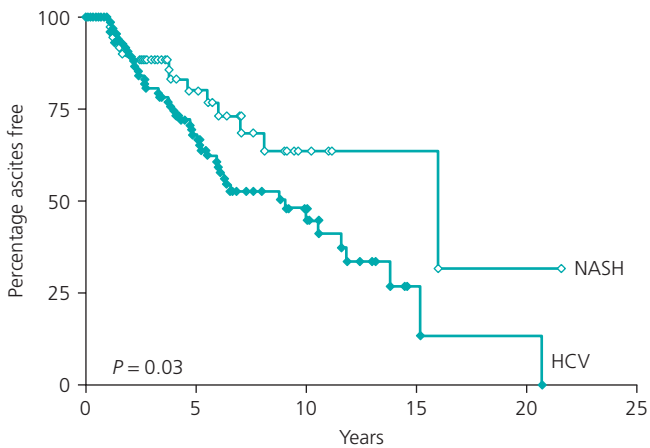


Figure 89.10 The natural history of cirrhosis due to nonalcoholic steatohepatitis (NASH): ascites. The probability of developing ascites and decompensation in those with cirrhosis caused by NASH is less than that seen in subjects with cirrhosis due to hepatitis C. From Sanyal et al. [248], with permission from John Wiley & Sons, Inc.

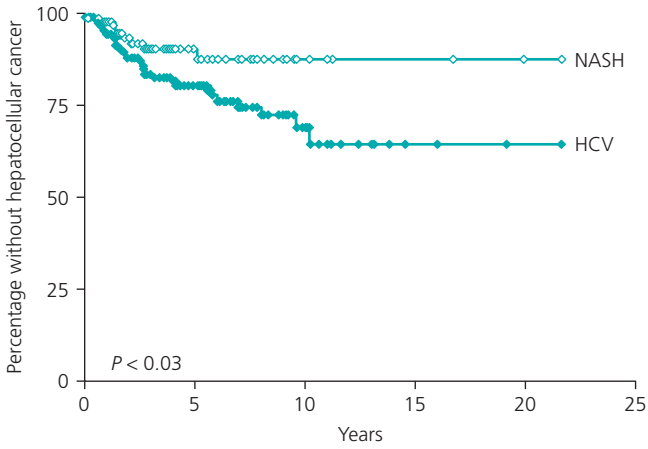


Figure 89.11 The natural history of cirrhosis due to nonalcoholic steatohepatitis (NASH): hepatocellular cancer. Subjects with cirrhosis due to NASH can develop hepatocellular cancer but the cancer risk appears to be somewhat lower than that seen in those with hepatitis C (HCV). From Sanyal et al. [248], with permission from John Wiley & Sons, Inc.

and such cases present as cryptogenic cirrhosis. In fact, most subjects with cryptogenic cirrhosis have underlying fatty liver disease. About 7%–14% of subjects referred for liver transplantation have cryptogenic cirrhosis [3,255]. Hepatic steatosis develops universally in subjects with cryptogenic cirrhosis after liver transplantation compared with 20% of those with hepatitis B, primary biliary cirrhosis, and alcoholic liver disease [256]. Steatohepatitis develops at a later time and most such subjects have recurrence of disease between 2 and 5 years after transplant. However, graft function is maintained over the first 5–10 years posttransplant and there is no evidence of an increased rate of graft loss compared with subjects undergoing transplant for other indications.

Diagnostic approach

A complete evaluation of a patient with NAFLD includes confirmation of the diagnosis, assessment of the etiology, and delineation of the grade and stage of the disease. The diagnosis of NAFLD requires two steps:

- to establish the presence of fatty liver disease
- to establish the "nonalcoholic" nature of the condition.

Also, several other forms of chronic liver disease are extremely prevalent in the general population, such as hepatitis C, and these can often coexist with NAFLD [65]. It is therefore imperative that the initial evaluation includes appropriate laboratory studies to exclude other common causes of liver disease.

The gold standard for the diagnosis of fatty liver disease is a liver biopsy. However, given the invasive nature, costs, and risks of a liver biopsy, there is considerable interest in trying to make the diagnosis using noninvasive methods.

Figure 89.12 An ultrasound picture of hepatic steatosis. The parenchyma is hyperechogenic and brighter than the adjacent kidney. Also, the edges of the blood vessels are blurred. From Ramesh & Sanyal [231], with permission from Elsevier.

Noninvasive diagnostic methods

Two-dimensional sonography, computerized tomography (CT), and magnetic resonance imaging (MRI) are the most-studied methods that are also available at most medical centers. The sonographic features of NAFLD include increased hepatic parenchymal echotexture and vascular blurring [257,258]) (Fig. 89.12). However, these features are also seen in any form of chronic liver disease and, although sensitive (85%–95%) they are nonspecific (positive predictive value 62%) [6]. Also, the ability to detect fatty liver by sonography drops off markedly once the degree of hepatic steatosis decreases to 30% or less [31].

Imaging of the liver by CT provides a more specific method for the noninvasive diagnosis of NAFLD (Fig. 89.13). Hepatic steatosis decreases the CT attenuation of the liver. When the hepatic parenchymal attenuation is 10 or more Hounsfield units lower than the spleen on a non-contrast-enhanced scan, a diagnosis of hepatic steatosis can be made. When intravenous contrast is administered, the hepatic enhancement lags behind the spleen and the liver-to-spleen attenuation differential exceeds 20 Hounsfield units [259]. Although these features allow hepatic steatosis to be defined with a 76% positive predictive value [31], they do not permit distinction between fatty liver and steatohepatitis. Also, the diagnostic sensitivity of the test depends on the severity of the steatosis, and falls off when the steatosis is mild. Computed tomography imaging also does not provide any information about the stage of fibrosis in the liver unless features of portal hypertension are present. This only occurs in the presence of cirrhosis of the liver. Finally, it is worth remembering that CT scans are substantially more expensive than sonography.

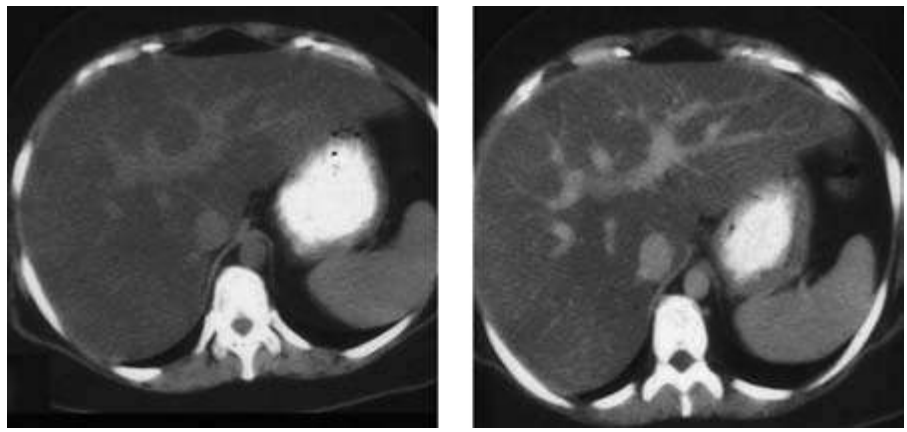


Figure 89.13 Computed tomography (CT) images of hepatic steatosis. In non-contrast-enhanced scans **(left panel)**, the liver attenuation is decreased resulting in the blood vessels standing out. Also, the liver is darker compared with the spleen. When intravenous contrast is given **(right panel)**, the liver attenuation increases but to a lesser degree than the spleen. When the difference exceeds 20 Hounsfield units, hepatic steatosis can be diagnosed with relative specificity. From Ramesh & Sanyal [231], with permission from Elsevier.

Magnetic resonance imaging is even more sensitive than a CT scan for the assessment of hepatic steatosis. Techniques of MRI have been refined to provide a highly reproducible and validated measure of hepatic triglyceride content [34]. Unfortunately, MRI also has many of the same limitations as CT imaging noted above. It is even more expensive than a CT scan and is not universally available. In clinical practice, a hepatic sonogram is the most commonly used modality to evaluate the presence of steatosis. However, it is important to note that none of these methods can diagnose steatohepatitis or accurately assess the stage of the disease.

Role of a liver biopsy

A liver biopsy is the gold standard for the diagnosis of NAFLD, separating those with a fatty liver alone from those with steatohepatitis and evaluation of the stage of hepatic fibrosis. Unfortunately, it is invasive, uncomfortable, expensive, and occasionally causes severe morbidity and mortality. There is also no established, specific pharmacological treatment for NASH at this time. Establishing a diagnosis of NASH vs NAFL does not therefore lead to specific treatment interventions in routine clinical practice. The invasive nature of a liver biopsy coupled with the lack of management options have led to a reluctance on the part of many physicians to perform a liver biopsy in cases of suspected NAFLD.

In order for the practicing clinician to make intelligent decisions regarding who to biopsy and when to biopsy, he or she must consider the information to be obtained and how this will affect the individual undergoing the biopsy. It is important to remember that, in subjects with persistently abnormal ALT values and negative viral serologies, approximately 20%–30% of subjects have an alternate diagnosis [260,261]. The probability of having NAFLD is likely to be higher if other features of the metabolic syndrome are also present in such individuals. The clinical corollary is that individuals without risk factors for NAFLD are more likely to have an alternate cause for their abnormal liver enzymes. A treating physician who is interested in identifying such cases may consider selecting such individuals for liver biopsy to establish the diagnosis. In other cases, the need for a biopsy simply to make a diagnosis of NAFLD should be individualized. For example, demonstration of isolated hepatic steatosis may allow an individual to obtain life insurance.

The second unique piece of information obtained from a liver biopsy is the distinction between hepatic steatosis and steatohepatitis. It is generally believed that steatohepatitis can progress to cirrhosis in 15%–20% of individuals [262]. There are numerous clinical centers engaged in phase II and phase III clinical trials with a variety of agents at this time. I therefore recommend that the physician discusses with the patient the possibility that they have NASH, with its attendant risks of progression, and ask the patient if they would like to be referred to a center to participate in a clinical trial. In those who are interested in pursuing this approach, a liver biopsy may be considered. Also, vertical banded gastroplasty has been shown to improve NASH [263,264]. In cases where NASH is found on a liver biopsy, this option may be considered if the BMI exceeds 35.

A liver biopsy is also the best way to assess the degree of fibrosis in the liver. Therefore, if the physician is concerned about the possibility of having advanced fibrosis, a liver biopsy is the only way to answer the question. In some circumstances, the presence of bridging fibrosis may direct management. For example, in a morbidly obese subject who is likely to be turned down for liver transplant listing because of their obesity, the presence of bridging fibrosis may lead the treating physician to consider aggressive weight loss measures such as pharmacological or surgical interventions. The risk of having advanced fibrosis (i.e., bridging fibrosis or cirrhosis) increases with advancing age, elevated BMI, and the presence of diabetes [188]. These criteria may be used to identify those who are most likely to benefit from a biopsy.

Treatment

There is no clearly established treatment of NAFLD. The focus of management remains the treatment of the risk factors for

NASH, namely obesity and insulin resistance. Dietary modification and lifestyle changes are the principal methods used for weight management. Although several drugs have been used for either weight control or treatment of insulin resistance, they remain experimental.

Weight management

The goal of weight management is to bring the patient's weight as close as possible to the ideal bodyweight. However, it is not essential for the weight to be normalized to improve the metabolic status of overweight or obese individuals. Even modest weight loss can be accompanied by substantial improvement in insulin resistance [265]. There have been a large number of studies of various dietary and lifestyle interventions to improve bodyweight in subjects with obesity. The results are somewhat heterogeneous but still allow certain conclusions to be made.

First, most diets work in the short term. However, long-term weight loss with diet alone is difficult to accomplish. Similarly, lifestyle interventions often appear to be effective in the short term but are difficult to sustain. Combined approaches using intense diet and exercise interventions have also had limited effect because of the high recidivism rate after discontinuation of the studies. The data underscore the need for diet and lifestyle changes that are palatable to the patient, and easy to accomplish and maintain in the context of the socioeconomic, family, and cultural milieu in which a given patient lives. This further indicates that it is unlikely that a single magic solution will work for all subjects. Using a transtheoretical model to analyze the behavioral status of patients with NASH, a preliminary report indicates that most subjects are in the precontemplation phase [230]. This indicates that most subjects with NASH do not perceive that they have a weight-related problem; these data provide a clue about why most patients with NASH do not achieve long-term benefits from lifestyle interventions.

It is my practice to educate the patient as much as possible before they embark on a diet and exercise program. Also, in those with long-standing diabetes or cardiovascular risk factors, it is prudent to perform a basic cardiac, vascular, ophthalmic, and neurological evaluation before starting an exercise program. In subjects who are overweight or obese, the National Heart, Lung and Blood Institute (NHLBI) guidelines for weight management are the best evidence-based treatment guidelines [266]. It is generally recommended that the diet should be planned to achieve a daily caloric deficit of 2–4 MJ (500–1000 cal). Also, an increase in everyday activities should be encouraged. For diabetic individuals, the American Diabetes Association guidelines for diet should be followed [267]. For subjects with a BMI greater than 30, or with a BMI greater than 27 plus other comorbidities (e.g., sleep apnea), pharmacological weight management with orlistat or sibutramine may be considered [266]. The latter is a serotonin reuptake antagonist and is contraindicated in subjects with heart disease. Orlistat causes fat malabsorption; a nonrandomized trial suggested that this agent could produce a beneficial effect on NASH [268].

Table 89.9 Drugs used for the treatment of nonalcoholic steatohepatitis (NASH)

Insulin sensitizers
Metformin
Pioglitazone
Rosiglitazone
Antioxidants
Vitamin E
Silymarin
Vitamin C
Hepatoprotective agents
Betaine
S-Adenosylmethionine
Ursodeoxycholic acid
Hypolipidemic drugs
Fibrates (e.g., gemfibrozil)
Statins
Anti-TNF regimens
Pentoxifylline
Adiponectin
Angiotensin receptor blockers
Losartan

TNF, tumor necrosis factor.

Both proximal gastric bypass and vertical banded gastroplasty have been shown to be safe in subjects with NASH. In the first few months following these procedures, the steatosis decreases whereas the inflammation may worsen [45]. However, in the long term, the hepatic steatosis, cell injury, and fibrosis all regress once the weight stabilizes following these operations [45,269]. In subjects with a BMI greater than 35, these operations are viable treatment options provided the operative risks are within the acceptable range.

Pharmacological treatment (Table 89.9)

There are two classes of drugs that have been shown to correct insulin resistance:

- biguanides (e.g., metformin)
- thiazolidinediones (e.g., rosiglitazone and pioglitazone).

The former work by mechanisms that are undefined. The roles of all of the pharmacological agents discussed below are experimental.

Metformin

In an animal model of steatohepatitis, metformin treatment improved hepatic steatosis and inflammation. However, in

human studies, although the ALT improves and liver size decreases, metformin has not been consistently found to improve liver histology [270]. In an open-label randomized trial, metformin (2 g/day given orally) improved ALT more often than vitamin E (800 IU/day) (odds ratio 3.1, $P < 0.001$) [271]. However, only limited histological data are available from this study. The role of metformin remains experimental and a large-scale clinical trial is underway to define the utility of metformin for NASH.

Thiazolidinediones

This class of drugs act through the peroxisome proliferator-activated receptor-γ and improve insulin sensitivity [223,224,272–274]. A small nonrandomized trial of troglitazone demonstrated an improvement in ALT in 9/10 subjects and improvement in inflammatory scores in five subjects. Troglitazone has also been shown to be beneficial in those with NAFLD associated with lipodystrophy. Three pilot trials supported the use of thiazolidinediones for NASH [223,224,274], and are the basis for a large ongoing NIH-sponsored clinical trial. Caution should be exercised when interpreting these data because of the small number of subjects. The potential risk for hepatotoxicity with these drugs warrants caution when interpreting the data, and their use should be limited to clinical trials.

Lipid-lowering agents

In one controlled trial, clofibrate had no beneficial effects on liver functions or hepatic histology, whereas in another small controlled trial, gemfibrozil improved liver chemistry [275]. However, no histological data are available from the latter study. Thus, in general, such agents are not used for the treatment of NASH.

Vitamin E

In one published series of 11 pediatric patients with NASH who received vitamin E (DL-α-tocopherol) 400–1200 IU, the ALT improved [276]. However, no follow-up liver histology was available from this study, thus preventing us from knowing whether this was a cosmetic effect or whether the hepatic histology was indeed improved. In a study undertaken by I and my colleagues, we found that 400 IU of DL-α-tocopherol improved hepatic steatosis significantly but only produced a trend toward improvement in other histological parameters [223]. In a single pilot study [277], a combination of vitamins E + C (1000 IU/day vitamin E + 1000 mg/day of vitamin C) improved hepatic fibrosis but did not affect inflammation.

Ursodeoxycholate (UDCA)

Ursodeoxycholate is a hydrophilic bile acid with hepatoprotective properties. In one study, UDCA produced improvement in liver enzymes and a decrease in hepatic steatosis [278]. The long-term benefits of UDCA and its optimal dose remain to be established. A randomized clinical trial was unable to establish the benefits of UDCA for NASH [279].

Taurine

Taurine is believed to function as a lipotropic factor and improve mobilization of hepatic fat. In a single uncontrolled series, 10 children treated with taurine supplements orally had radiological resolution of their fatty liver [280]. Radiological improvement was accompanied by a decrease in glycine/taurine-conjugated bile acids.

Betaine

Betaine is a precursor of *S*-adenosylmethionine, a hepatoprotective factor. In one study, 10 subjects received betaine anhydrous for 12 months in two daily divided doses. Seven of the ten subjects completed 1 year of treatment. In this group, an improvement in aminotransferase activity as well as liver histology was noted [281]. Similar data were reported in another study, in which betaine was administered along with diethanolamine glucoronate and nicotinamide ascorbate [282]. These findings require confirmation in large prospective trials.

Pentoxifylline

This agent antagonizes TNF-α and is available for long-term oral use. In two small pilot studies [283,284], the ALT improved following several months of treatment at a dose of 400 mg three times a day. However, histological data are not available. Also, although the drug was well tolerated in one study, 9/20 subjects in the other study dropped out due to side effects, especially nausea. Several trials are underway to confirm these initial findings.

Losartan

Angiotensin II has been implicated in hepatic stellate cell activation and matrix production [285]. A small pilot study of an angiotensin receptor blocker, losartan, found an improvement in ALT [286]. These data also require validation in large-scale studies before any recommendations can be made.

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90 Ascites and its complications

Guadalupe Garcia-Tsao

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Ascites is the accumulation of fluid in the peritoneal cavity. In the Western world, cirrhosis is the main cause of ascites, accounting for more than 75% of the cases (Table 90.1). Other less common causes of ascites are peritoneal malignancy, which accounts for ~ 12%, cardiac failure in 5%, and peritoneal tuberculosis in only 2% of the cases [1], although the latter is still an important cause of ascites in many developing countries. Approximately 5% of patients will have more than one cause of ascites, such as cirrhosis and heart failure, cirrhosis and tuberculous peritonitis, or cirrhosis and malignancy [2].

In patients with cirrhosis, ascites is one of the complications that mark the transition from a compensated to a decompensated stage [3]. Initially, ascites is "uncomplicated," that is, it responds well to diuretics and is not infected. As cirrhosis progresses and the mechanisms that lead to ascites formation worsen, ascites ceases to respond to diuretics (refractory ascites). Bacteria may infect ascites, an entity known as spontaneous bacterial peritonitis (SBP), which occurs mainly in hospitalized patients with severe liver disease. With further progression of cirrhosis, the patient with ascites may develop hyponatremia and functional renal failure (hepatorenal syndrome). The hemodynamic alterations that lead to ascites and refractory ascites are the same as those that lead to hyponatremia and hepatorenal syndrome, differing only in the degree of abnormality, with the latter complications denoting a more deranged circulatory status.

The approach to a patient with ascites depends on the setting surrounding its presentation. In a patient with new-onset ascites, the priority is to determine the etiology of ascites, because this will determine its management. In a patient with ascites caused by cirrhosis, management depends on the phase that the patient has reached, ranging from uncomplicated ascites to hepatorenal syndrome.

Table 90.1 Etiology of ascites and classification by serum–ascites albumin gradient (SAAG) and ascites protein level

	SAAG	Ascites protein
Main etiological factors of ascites		
Cirrhosis or alcoholic hepatitis	High	Low
Congestive heart failure	High	High
Peritoneal malignancy	Low	High
Peritoneal tuberculosis	Low	High
Other etiologies of cirrhosis (account for < 2% of all cases)		
Massive hepatic metastases	High	Low
Nodular regenerative hyperplasia	High	Low
Fulminant liver failure	High	Low?
Budd–Chiari syndrome (late)	High	Low
Budd–Chiari syndrome (early)	High	High
Constrictive pericarditis	High	High
Venoocclusive disease	High	High
Myxedema	High	High
Nephrogenous (dialysis) ascites	High	High
Mixed ascites (cirrhosis + peritoneal malignancy)	High	Variable
Pancreatic ascites	Low	High
Serositis (connective tissue disease)	Low	High
Chlamydial/gonococcal	Low	High
Biliary	Low	High?
Ovarian hyperstimulation syndrome	Low?	High
Nephrotic syndrome	Low	Low

Those assessments followed by a question mark are theoretical and have not been confirmed by data in the literature.

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Suspected ascites

History and physical examination are important in determining the presence and etiology of ascites, but they are not definitive.

The most frequent symptoms associated with the development of ascites are increased abdominal girth, described as tightness of the belt or garments around the waist, associated with weight gain. However, sometimes weight gain is masked by concomitant loss of muscle mass as a result of cirrhosis or malignancy. The rapid onset of symptoms in a matter of weeks helps to distinguish ascites from obesity, which develops over a period of months to years.

Physical examination is relatively insensitive for detecting ascitic fluid, particularly when the amount is small or the patient is obese. When present in small to moderate amounts (> 1500 mL), ascites can be identified on examination by bulging flanks, flank dullness, and shifting dullness. Shifting dullness is the most sensitive finding in the clinical diagnosis of ascites (compared with abdominal distention, bulging flanks and fluid wave) and its absence can rule out ascites with over 90% accuracy [4]. The presence of an umbilical hernia or diastasis recti suggests that the patient has had tense ascites.

Imaging investigations

The initial, least invasive, and most cost-effective method to confirm the presence of ascites is *abdominal ultrasonography*. It can detect amounts as small as 100 mL and is considered the gold standard for the diagnosis of ascites [5]. Abdominal ultrasound is also useful in locating the optimal site to perform a paracentesis, particularly in patients with a small amount of ascites or in those with loculated ascites. Additionally, ultrasound can be accompanied by Doppler examination of the hepatic venous system – an important initial test to rule out the presence of hepatic vein obstruction, a frequently overlooked cause of ascites [6].

Abdominal *computed tomography (CT) scan* is also highly sensitive and specific in diagnosing the presence of intra-abdominal fluid; however, its cost precludes it as a first-line diagnostic method to confirm the presence of ascites. Abdominal CT scan is more sensitive than ultrasonography in determining the presence of cirrhosis or malignancy. Finding a small nodular liver, splenomegaly, and collaterals on CT scan establishes the diagnosis of cirrhosis.

New-onset ascites

In a patient with new ascites, the priority is to establish the cause of ascites because this will determine its management.

Initial evaluation

The history and physical examination are important in guiding the evaluation of ascites. Several aspects of the physical examination should receive special attention. The presence of spider angiomas, palmar erythema, and muscle wasting in addition to jaundice or signs of portal hypertension, such as splenomegaly and abdominal wall collaterals, suggests cirrhosis. Finding a palpable (or ballotable) left lobe of the liver (in the epigastric area) is almost pathognomonic of cirrhosis. Distended neck veins, gallop rhythm (S3), and uniform hepatomegaly with hepatojugular reflux or peripheral edema suggests congestive heart failure. The presence of a small nodule at the umbilicus (Sister Joseph Mary nodule) or an enlarged supraclavicular lymph node (Virchow node) suggests malignant ascites. Large veins with an upward flow localized in the back suggest obstruction of the inferior vena cava.

Diagnostic paracentesis

The simplest and most inexpensive way to orient the diagnostic workup of a patient with new-onset ascites is through the analysis of ascitic fluid. A diagnostic paracentesis should be the first test performed in a patient with new-onset ascites (see Fig. 90.1). It is a safe procedure with a low incidence of serious complications, the most serious being the development of large hematomas, which occurs in 0.2%–0.9% of cases [7,8]. Coagulopathy is not a contraindication to performing a diagnostic paracentesis particularly if it is performed with a small-gauge needle [7,8]. Care should always be taken to avoid surgical scars, abdominal wall collaterals, and the area of the inferior hypogastric artery, which lies midway between anterior superior iliac spine and pubic tubercle. If there are doubts about the presence of ascites or if no fluid is obtained in the first attempt, paracentesis should be performed under sonographic guidance.

In a diagnostic paracentesis, 20–50 mL of ascitic fluid is obtained. Tests that should be performed on the fluid are shown in Table 90.2, and their use in the differential diagnosis of ascites is detailed below.

Appearance of ascitic fluid

Uncomplicated "normal" ascitic fluid is transparent, straw colored to slightly yellow. In patients with marked jaundice, the fluid may have a deeper yellow color. Fluids with a leukocyte count greater than 5000/mm^3 appear cloudy, and those with a leukocyte count exceeding 50 000/mm^3 appear frankly purulent and indicate the presence of a gross intra-abdominal infection (secondary peritonitis) or an abscess [1].

Purulent ascites may be confused with the milky fluid associated with a high fat content (chylous ascites). Lack of odor and a triglyceride level greater than 200 mg/dL establishes the diagnosis of chylous ascites. An easier way to confirm the presence of chylous ascites is to refrigerate a tube with ascites – in case of chylous ascites, a creamy layer will separate at the top.

Fluids with a red blood cell count exceeding 10 000/mm^3 will appear pink, whereas frank bloody fluid is usually associated with a red blood cell count greater than 20 000/mm^3. In

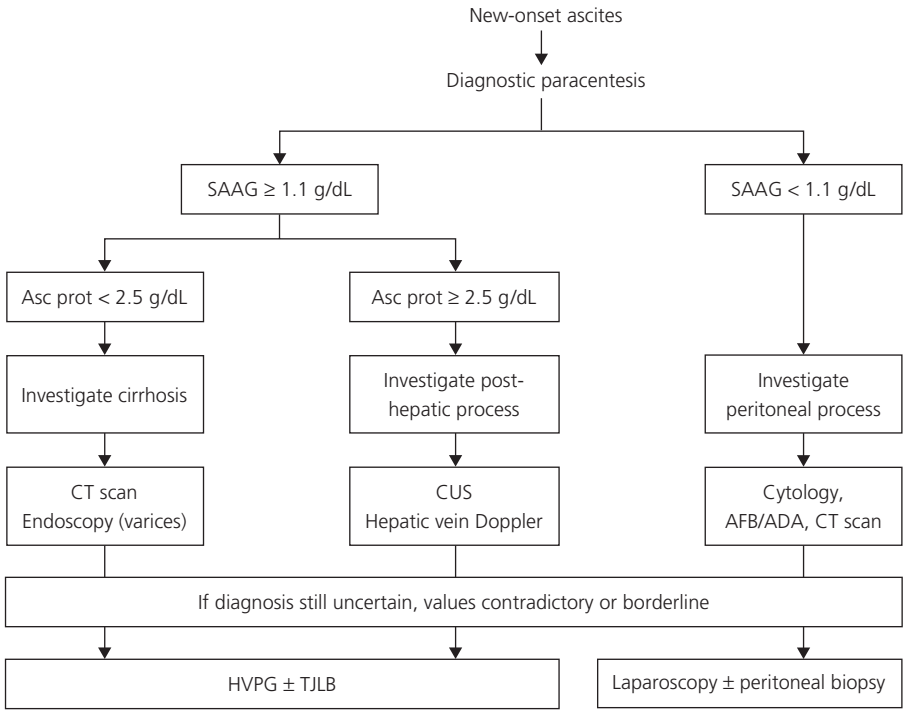

Figure 90.1 Approach to the patient with new-onset ascites. ADA, adenosine deaminase; AFB, acid-fast bacilli; Asc prot, ascites total protein levels; CT, computed tomography; CUS, cardiac echosonography; HVPG, hepatic venous pressure gradient; SAAG, serum–ascites albumin gradient; TJLB, transjugular liver biopsy.

Table 90.2 Tests performed in diagnostic paracentesis

Routine analysis of ascitic fluid
Gross appearance
Total protein
Albumin (with simultaneous estimation of serum albumin) so that the ascites–serum albumin gradient can be calculated by subtracting the ascitic fluid value from the serum value
White blood cell count and differential
Bacteriological cultures
Focused analysis of ascitic fluid
Cytology (to exclude malignant ascites)
Amylase (if pancreatic ascites is suspected)
Acid-fast bacilli smear and culture and adenosine deaminase determination (if peritoneal tuberculosis is suspected)
Glucose and lactic dehydrogenase (if secondary peritonitis is suspected in a patient with ascites PMN > 250/mm^3)
Triglycerides (if the fluid has a milky appearance, i.e., chylous ascites)
Red blood cell count (if the fluid is bloody)

PMN, polymorphonuclear leukocytes.

the presence of bloody fluid, the question will be whether it is the result of a traumatic tap or whether it is due to hemoperitoneum. Blood in the fluid resulting from a traumatic tap will clot, whereas fluid from a hemoperitoneum will not clot because blood has already clotted (intraperitoneally) and the clot has lysed. A tap performed immediately in the opposite flank will also clarify the situation. Hemoperitoneum suggests malignant ascites.

A tea-colored fluid can be seen in pancreatic ascites and the result of breakdown of ascitic fluid red cells. This phenomenon is more pronounced in hemorrhagic pancreatitis, resulting in a black fluid. Bile-stained fluid is green and can be seen with gallbladder or intestinal perforation.

Serum–ascites albumin gradient and ascites total protein levels

These two inexpensive tests taken *together* are the most useful in determining the etiology of ascites and thereby in further directing the workup of patients with ascites (see Table 90.1).

The *serum–ascites albumin gradient (SAAG)* is obtained by subtracting the concentration of ascites albumin from that of serum albumin (ideally determined from specimens obtained on the same day) and is a reflection of hepatic sinusoidal pressure [9,10]. The SAAG concept is based on Starling forces. When sinusoidal hypertension (hydrostatic pressure) is the cause of ascites, the colloidosmotic gradient (exerted mainly by serum and ascites albumin) has to increase to counterbalance the high hydrostatic pressure that is driving fluid into the peritoneal cavity. SAAG correlates well with the hepatic venous pressure gradient ($r = 0.72$), a measure of hepatic sinusoidal pressure [9]. The cutoff value that best distinguishes patients in whom ascites is caused by sinusoidal hypertension vs ascites caused by peritoneal malignancy is a SAAG of 1.1 g/dL, with a SAAG greater than 1.1 g/dL indicating ascites resulting from sinusoidal hypertension (e.g.,

cirrhosis, heart failure) [11]. Interestingly, the SAAG value of 1.1 g/dL roughly corresponds to a hepatic venous pressure gradient (HVPG, a measure of sinusoidal pressure) of 11–12 mmHg [9], which is the threshold pressure necessary for the development of ascites in cirrhosis (see below). The accuracy of SAAG is reduced if samples are not obtained simultaneously, if serum albumin levels are low or if ascites is chylous (falsely high SAAG). Serum hyperglobulinemia (> 5 g/dL) leads to a high ascitic fluid globulin concentration and can narrow the albumin gradient by contributing to the oncotic forces. To correct the SAAG in the setting of a high serum globulin level, the uncorrected SAAG should be multiplied by (0.16) × (serum globulin [in g/dL] + 2.5) [12].

The *ascites total protein content* at different cutoff values (2.5–3.0 g/dL) has been traditionally used to divide ascitic fluid into "exudates" secondary to peritoneal processes (malignancy, tuberculosis) or "transudates" in case of ascites secondary to sinusoidal portal hypertension. It is increased in peritoneal processes because of leakage of high-protein mesenteric lymph from obstructed lymphatics or from an inflamed peritoneal surface [13]. However, exudative ascites is also present in ascites secondary to heart failure [2], in which the mechanism is an increased hepatic sinusoidal pressure. In this situation, the process that leads to sinusoidal hypertension is posthepatic and therefore the hepatic sinusoids are normal. Normal hepatic sinusoids are uniquely permeable ("leaky") and hepatic congestion secondary to hepatic vein or inferior vena cava obstruction or cardiac failure has been shown to lead to profuse outpouring of protein-rich lymph into the peritoneal cavity [14]. In cirrhosis, there is deposition of fibrous tissue in the sinusoids ("capillarization of the sinusoid") that renders the sinusoid less leaky to macromolecules, and this leads to an abnormally low protein content of liver lymph [15,16]. Therefore, in addition to identifying peritoneal causes of ascites (high protein), the ascites total protein enables differentiation of intrahepatic (e.g., cirrhosis) causes of ascites (low protein) from posthepatic causes (e.g., heart failure, Budd–Chiari) of ascites (high protein), in both of which the SAAG is elevated [2]. This differential is extremely important because constrictive pericarditis is one of the few curable causes of ascites. Also, the distinction between cardiac or hepatic origin of ascites is especially important in alcoholic patients, who can have ascites secondary to alcoholic cardiomyopathy or from cirrhosis, with significant management implications.

In addition to being useful in determining the etiology of ascites, total protein levels in ascites are useful to determine susceptibility of developing bacterial infection [17]; cirrhotic patients with an ascites protein concentration below 1.0 g/dL have a higher risk of developing infection (spontaneous bacterial peritonitis). Contrary to what occurs with pleural fluid, ascites does not become an "exudate" with infection [18]; however, protein levels greater than 1.0 g/dL have been described as being suggestive of secondary (surgical) peritonitis.

All causes of ascites and their known/expected SAAG and total protein levels are shown in Table 90.1. The diagnostic workup for the three main causes of ascites is outlined in Fig. 90.1. In cases in which the etiology of ascites is still undetermined because of contradictory or borderline results of SAAG or ascites total protein, the ultimate test will be the determination of the HVPG.

Hepatic venous pressure gradient measurement

The hepatic venous pressure gradient (HVPG) is an indirect measure of portal pressure and a direct measure of hepatic sinusoidal pressure. The concept is the same as the measurement of the pulmonary wedged pressure. The HVPG is obtained by introducing a catheter in the right femoral vein or the internal jugular vein and advancing a balloon catheter into the right main hepatic vein. Pressures are recorded with the balloon inflated (wedged hepatic venous pressure, or WHVP) and deflated (free hepatic venous pressure, or FHVP). The WHVP is the direct measurement of sinusoidal pressure but it is usually corrected by subtracting the FHVP (a measure of systemic pressure that acts as an internal zero) thereby yielding the HVPG. Normal WHVP is 5–7 mmHg, normal FHVP is 1–2 mmHg, and normal HVPG is 3–6 mmHg. Portal hypertension is defined as an HVPG greater than 6 mmHg; however, the development of ascites requires that a threshold portal pressure gradient of 12 mmHg is exceeded [19,20]. Importantly, patients in whom the HVPG is reduced below 12 mmHg, or at least 20% from baseline values, show a decrease in the development of ascites [21].

Therefore, and as shown in Table 90.3, in cirrhotic ascites the HVPG will be elevated at the expense of an elevated WHVP and a normal FHVP, and the levels are usually greater than or equal to 12 mmHg. In cases of cardiac ascites, both the WHVP and the FHVP will be elevated (reflecting elevated systemic pressures) and therefore the HVPG will be normal [22]. In cases of peritoneal ascites (i.e., malignancy or tuberculosis), all hepatic venous pressure measurements (WHVP, FHVP, and HVPG) will be normal, unless the patient has coexisting cirrhosis or heart failure. When performed properly, HVPG measurements are reproducible and safe [23]; in

Table 90.3 Differential of ascites based on hepatic venous pressure gradient (HVPG) measurements

Cause of ascites	WHVP	FHVP	HVPG
Cirrhosis	Increased	Normal	Increased
Cardiac ascites	Increased	Increased	Normal
Peritoneal malignancy or TB	Normal	Normal	Normal

FHVP, free hepatic venous pressure; TB, tuberculosis; WHVP, wedged hepatic venous pressure.

fact, hepatic vein catheterization for measurement of hepatic vein pressures enables the performance, in the same procedure, of a transjugular liver biopsy, which will further define the etiology of ascites [24].

Evaluation of infection

Tests to investigate infection should be performed if the ascites is purulent or in any patient with cirrhotic ascites with symptoms or signs suggestive of spontaneous bacterial peritonitis (SBP) or who is admitted to the hospital [25]. A low ascites protein content (< 1.0 or 1.5 g/dL), present in most patients with cirrhosis, is the most important predictor of ascites infection [17]. SBP is the infection of ascitic fluid in the absence of an obvious local source, and it is the most common bacterial infection in hospitalized patients with cirrhosis. Importantly, the development of SBP is not associated with a significant change in ascites protein levels [18].

Total and differential cell counts

A predominance of neutrophils or polymorphonuclear leukocytes (PMN) in the ascitic fluid indicates the presence of an acute intraabdominal inflammatory process. Processes such as cholecystitis, appendicitis or diverticulitis can lead to ascites with a high neutrophil count, but in the absence of cirrhosis, the amount of fluid is generally minimal.

The diagnosis of SBP, that is, the infection of cirrhotic ascites, is established with an ascites PMN count greater than 250/mm^3 [25]. In patients with bloody ascites (i.e., ascites RBC count > 10 000/mm^3), 1 PMN per 250 RBC should be subtracted to adjust for the presence of blood in ascites.

Ascites PMN cell counts are performed manually and this may take several hours and not be available outside regular work hours. Although the use of reactive strips for leukocyte esterase has been proposed as an alternative to manual counting [26], several studies (mostly including small numbers of patients with SBP) show sensitivities that range widely between 50% and 100% [27]; therefore these strips cannot be recommended until larger studies are performed and the method is standardized.

A predominance of mononuclear cells indicates a chronic process and would suggest the presence of tuberculous peritonitis.

Smears and bacteriological culture

Sample collection at the bedside inoculating blood culture bottles (both aerobic and anaerobic) with 10 mL of ascitic fluid is more likely to yield an infecting organism compared with samples that are centrifuged and plated [28]. Although ascites culture is negative in approximately 40% of patients with clinical manifestations suggestive of SBP and increased ascites PMN [25], it is important to isolate an infecting microorganism, as this will further guide management [29]. Gram stain is positive in less than one-third of cases of SBP.

Ascites glucose and lactate dehydrogenase levels

In contrast to pleural fluid, ascites glucose and lactate dehydrogenase (LDH) levels are of little value in determining the cause of ascites or in determining the presence of infection. The ascites/serum LDH ratio is approximately 0.4–0.5 in uncomplicated cirrhotic ascites and increases in SBP, presumably because of the release of LDH from PMNs [18]; however, its diagnostic accuracy is low. It has been proposed that a ratio greater than 1.0 (i.e., if the ascites LDH is higher than serum LDH) and an ascites glucose below 50 mg/dL would suggest the presence of secondary bacterial peritonitis, although the specificity of these criteria is low [30] and they have not been validated prospectively.

Ascites lactate and pH

These tests were thought to be useful in the rapid diagnosis of SBP. However, several studies have demonstrated that they are no more useful than the ascites PMN determination [31].

Evaluation of malignancy

This should be undertaken in patients with a compatible clinical presentation. It is important to distinguish malignant ascites secondary to an infiltrated peritoneum (peritoneal carcinomatosis) from ascites secondary to massive liver metastases or "pseudocirrhosis" secondary to profound desmoplastic response to infiltrating breast cancer [32] or secondary to nodular regenerative hyperplasia related to chemotherapy [33]. In patients with cancer and ascites, peritoneal carcinomatosis is the cause of ascites in two-thirds of the patients [34].

Cytology

Cytological examination of ascites should be performed in all patients with suspected malignancy, particularly in those with low SAAG and high ascites protein, findings that suggest the presence of peritoneal carcinomatosis (as opposed to hepatic malignancy or pseudocirrhosis). In peritoneal carcinomatosis, SAAG is low, total protein high, and the diagnostic yield of cytology is as high as 97% (but could be as low as 42% [35]), whereas in patients with massive liver metastases, SAAG is high, ascites protein is low, and cytology is often negative [34].

It has been suggested that flow cytometry may be a useful adjunct to cytology in the diagnosis of peritoneal carcinomatosis, particularly when related to lymphoma or pancreatic cancer [35,36]; however, in a patient with suspected malignancy, low SAAG, high ascites protein, and negative results on cytological examination, the test of choice is probably a peritoneoscopy with peritoneal biopsies.

Tumor markers in ascites

Although CA-125 levels may be considered a sensitive tumor marker in patients with ovarian cancer, high serum CA-125 levels are elevated in *any* patient with ascites regardless of etio-

logy. In fact, serum and ascites CA-125 levels were comparable among patients with cirrhotic ascites, patients with malignant ascites, and those with "benign" ascites [37]. Awareness of this association can prevent unnecessary workup and cost.

Other ascites markers such as carcinoembryonic antigen, fibronectin, and cholesterol have been found to be nonspecific and unhelpful in the diagnosis of malignant ascites, mostly because in the studies analyzing such markers patients were not stratified by the type of malignant ascites [38].

Evaluation of peritoneal tuberculosis

Although the incidence of peritoneal tuberculosis (PTB) appears to be rising in the United States, it remains an uncommon cause of ascites although it commonly occurs in patients with cirrhosis [39]. This makes its identification more problematic. In a noncirrhotic patient, ascites secondary to peritoneal tuberculosis is associated with a low SAAG and a high ascites protein. In a cirrhotic patient, findings associated with cirrhosis will predominate, that is, high SAAG and low protein. A diagnostic algorithm of patients with suspected PTB has been proposed [40].

Acid-fast bacilli culture and smear

Although isolating acid-fast bacilli (AFB) in ascites is the gold standard in the diagnosis of PTB, less than 40% of patients with PTB have positive ascitic fluid cultures, and Ziehl–Neelsen staining of the ascitic fluid for mycobacterial detection is positive in only about 3% of cases with proven PTB [40]. Additionally, conventional culture media require 4–8 weeks to detect AFB, which can lead to a delay in the initiation of specific therapy. Newer methods of identifying mycobacterial isolates include liquid chromatography and DNA probes, which reduce the turnaround time, and rapid amplification-based tests like polymerase chain reaction (PCR), which can yield results in days. However, their diagnostic value requires further analysis.

Ultimately, laparoscopy is the best test to diagnose PTB, either by visualization alone (typical findings) or in combination with biopsy, culture, and histological examination. This test should be considered at an early stage whenever PTB is suspected.

Adenosine deaminase ascitic fluid activity

With difficulties in the bacteriological diagnosis of PTB, there has been an ongoing search for alternative rapid and noninvasive tests, with adenosine deaminase (ADA) activity in the ascitic fluid being the most promising.

Adenosine deaminase is an enzyme related to proliferation and differentiation of lymphocytes, and establishing its activity appears to be a fast and discriminating test for diagnosing PTB, as shown by a metaanalysis of 12 studies (some performed in countries with a higher prevalence of PTB) including 264 patients, of which 50 (18.9%) had peritoneal tuberculosis. ADA levels showed high sensitivity (100%) and specificity (97%) using cutoff values from 36 to 40 IU/L, with an optimal cutoff point of 39 IU/L [41]. Although a retrospective study suggested that ADA activity is much less sensitive in detecting PTB in the setting of cirrhosis [39], other systematic reviews have not confirmed this contention [42]. Given its diagnostic accuracy and easy availability, performance of this test is recommended in suspected PTB.

Evaluation of pancreatic ascites

Pancreatic ascites is caused by extravasation of pancreatic fluid from the pancreatic ductal system, either from a leaking pseudocyst or from a ruptured pancreatic duct. Therefore, this fluid has high amylase levels (much higher than serum amylase) and a protein content greater than 2.5 g/dL. Because pancreatic ascites is not secondary to sinusoidal hypertension, the SAAG will be lower than 1.1 g/dL. In a relatively large series, only about two-thirds of the patients experienced abdominal pain [43], and it has been suggested that pancreatic ascites occurs more often than reported and a workup for it should be done even in the face of unconvincing radiographic evidence [44]. Of note, nonpancreatic tumors (e.g., ovarian) may cause an increase in the serum and ascitic fluid amylase, which may be incorrectly diagnosed as pancreatic ascites. However, isoenzyme analysis of this amylase will show that it is salivary isoamylase [45].

Evaluation of chylous ascites

Chylous ascites is an uncommon form of ascites resulting from an accumulation of peritoneal fluid rich in triglycerides, due to the presence of lymph in the abdominal cavity. A triglyceride level greater than 200 mg/dL is diagnostic of chylous ascites.

It occurs because of disruption of the lymphatic system resulting from obstruction (malignant infiltration or inflammatory process) or traumatic injury (surgery, trauma) with leakage of chylomicron-rich fluid into the peritoneum. Altered hemodynamics leading to increased caval (constrictive pericarditis) and hepatic venous pressures (cirrhosis) can also lead to an increased formation of hepatic duct lymph.

The most common cause of nontraumatic chylous ascites in adults is abdominal malignancy, with non-Hodgkin lymphomas accounting for at least one-third to half of these malignancies [46,47]. In addition to lymphomas other tumors that cause chyloperitoneum may arise from the ovary, colon, kidney, prostate, pancreas, and stomach. The most common inflammatory causes of chylous ascites are abdominal or pelvic radiation, pancreatitis, and tuberculosis. Surgical procedures that involve extensive retroperitoneal dissection are most commonly associated with postoperative chylous ascites, including the distal splenorenal shunt. Blunt trauma, stab wounds, and gunshot wounds can also lead to traumatic chylous ascites.

Cirrhotic ascites has chylous characteristics in only 0.5–1.3% of cases. Spontaneous transformation of previously clear ascites appears to be associated with a poor prognosis.

In contrast, the appearance of chylous ascites de novo in a cirrhotic patient appears to indicate a more favorable outcome [48].

Given the multiple etiologies of chylous ascites, workup of the patient is directed by clinical and ascitic fluid findings as described for nonchylous ascites. The only caveat is that the SAAG may be falsely elevated in chylous ascites. Additional tests to determine the presence of lymphatic disruption include lymphangiography and lymphoscintigraphy.

Cirrhosis and ascites

To understand the natural history of the patient with cirrhosis and ascites and its management at each stage it is important to understand the pathophysiology of ascites.

Pathogenesis and natural history of cirrhotic ascites

In cirrhosis, ascites results from two main pathogenic mechanisms: sinusoidal hypertension and sodium retention (Fig. 90.2). Ascitic fluid leaks into the peritoneal space as a result of sinusoidal hypertension, which in turn results from hepatic venous outflow block secondary to regenerative nodules, fibrosis, and active vasoconstriction. As mentioned previously, there is a minimal sinusoidal pressure necessary for ascites formation [19,20]. This would be a self-limited process but for the continuous replenishment of the intravascular volume through sodium and water retention. Sodium retention results from splanchnic and peripheral vasodilation resulting in a decrease in effective arterial blood volume, and activation of neurohumoral systems (angiotensin, renin, aldosterone) that leads to renal sodium retention [49]. As liver disease worsens, vasodilation also worsens leading to the nonosmotic release of antidiuretic hormone, which then leads to water retention and hyponatremia [50]. In the final stage of cirrhosis, vasodilation is extreme and in addition to marked sodium and water retention (refractory ascites with or without hyponatremia) there is renal vasoconstriction, which leads to a decrease in glomerular filtration rate and renal failure (hepatorenal syndrome, HRS).

Therefore, a patient with cirrhosis goes through a sequence of diuretic-responsive ascites, followed by refractory ascites, and then hyponatremia and HRS [51], with each stage reflecting a more deranged circulatory state. Overall, most cirrhotic patients with ascites have "uncomplicated" ascites, that is, ascites that is not infected, is not associated with renal dysfunction, and responds to diuretic therapy [52]. However, the prevalence of uncomplicated ascites is lower in certain settings (e.g., transplant centers, in hospital).

Therapies for ascites

Different therapies for ascites and their site of action are depicted in Fig. 90.3. Except for transplant, none of the different treatments of ascites has resulted in significant improvements in survival. However, treating ascites is important, not only because it improves the quality of life of the cirrhotic patient but also because SBP, a lethal complication of cirrhosis, does not occur in the absence of ascites.

Liver transplant

The development of ascites in a patient with cirrhosis denotes a poor prognosis, with a median survival of about 1.5 years, compared with a median survival of more than 12 years in patients who remain compensated [3]. In the presence of

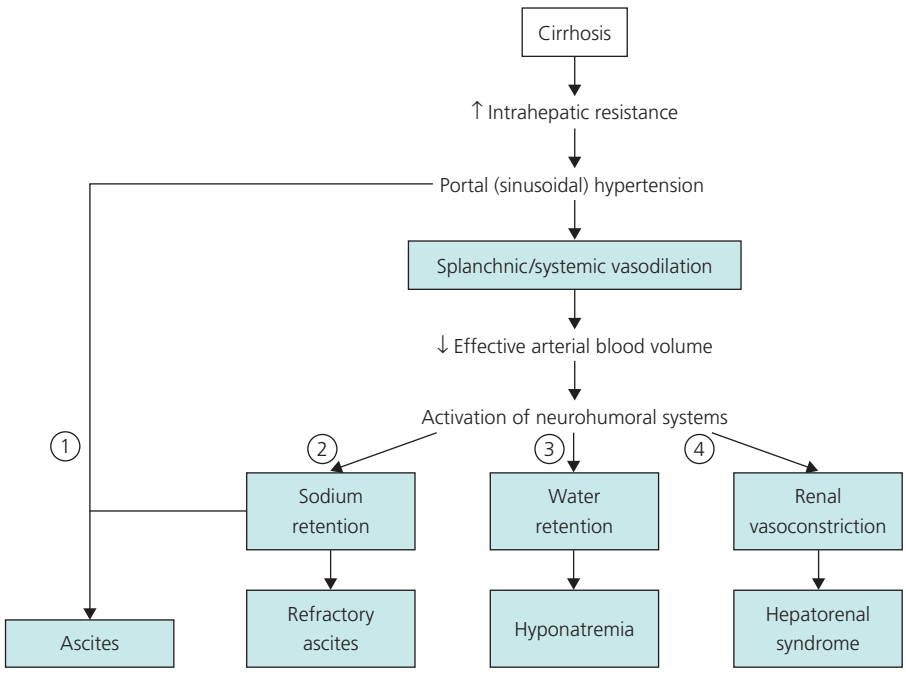

Figure 90.2 Common pathogenesis of ascites, hyponatremia and hepatorenal syndrome. Ascites (1) results from increased sinusoidal pressure and sodium retention. Sinusoidal pressure increases as a result of increased intrahepatic resistance. Sodium retention results from splanchnic and systemic vasodilation that leads to decreased effective arterial blood volume and subsequent upregulation of sodium-retaining hormones. With progression of cirrhosis and portal hypertension, vasodilation is more pronounced, leading to further activation of the renin–angiotensin–aldosterone and sympathetic nervous systems. The resulting increase in sodium and water retention can lead to refractory ascites (2) and hyponatremia (3), respectively, while the resulting increase in vasoconstrictors can lead to renal vasoconstriction and hepatorenal syndrome (4).

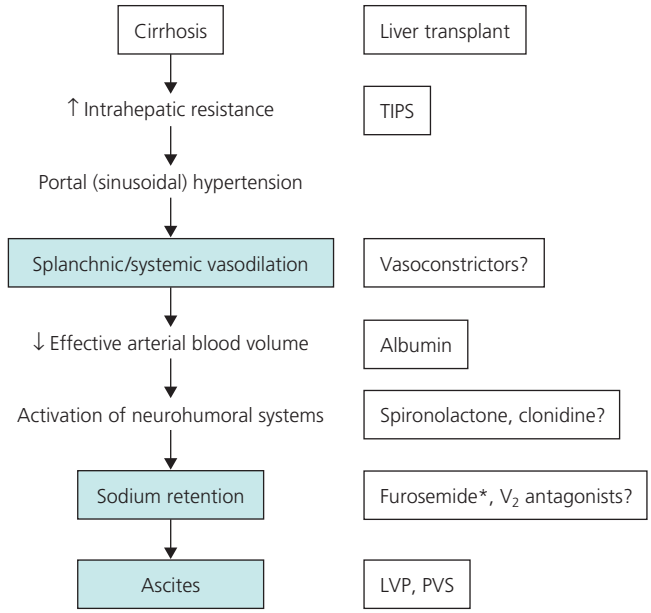

Figure 90.3 Site of action of different therapies for ascites. *Furosemide should only be used in conjunction with spironolactone. LVP, large-volume paracentesis; PVS, peritoneovenous shunt; TIPS, transjugular intrahepatic portosystemic shunt; V_2, arginine vasopressin type 2 receptor.

ascites, mortality in cirrhosis is about 20% per year [53]. Patients with cirrhosis who develop ascites should therefore be evaluated for liver transplantation because this constitutes the ultimate treatment for ascites and its complications.

Sodium restriction and diuretics

Sodium restriction is recommended in all cirrhotic patients with ascites. Restriction to approximately 90 mEq/day (i.e., 2 g sodium/day = 5.2 g of dietary salt/day) is the recommended goal [52]. Further restriction of sodium is unrealistic and difficult to achieve. Nonpalatability of a salt-restricted diet may lead to inadequate food intake. In these cases, liberalizing sodium restriction and adding or increasing diuretics is preferable to compromising the already compromised nutrition of the cirrhotic patient with ascites.

Although loop diuretics such as furosemide are the most potent natriuretics, randomized controlled trials have demonstrated that spironolactone is significantly more effective than furosemide alone in the treatment of cirrhotic ascites [54,55]. When furosemide is used alone, sodium not reabsorbed in the loop of Henle is taken up at the distal and collecting tubules as a result of the hyperaldosteronism present in most cirrhotic patients with ascites. Therefore, spironolactone, which acts by blocking aldosterone, is the diuretic of choice in the treatment of ascites. Furosemide can be used together with spironolactone but should not be used alone.

There is no evidence that other diuretics such as metolazone, thiazides, or torasemide offer an advantage over spironolactone and furosemide.

In contrast to the treatment of acute heart failure, where the response to therapy needs to be fast and an intravenous (i.v.) route is preferable, elimination of cirrhotic ascites should be slow (only about 500 mL/day can be eliminated) and an oral route is preferred. Nonsteroidal antiinflammatory drugs or aspirin blunt the natriuretic effect of diuretics and should be avoided in cirrhotic patients with ascites [56,57]. Although selective cyclooxygenase-2 (COX-2) inhibitors have not been shown to impair natriuresis or to induce renal dysfunction in cirrhotic rats [58], preliminary data in patients indicate that celecoxib may be related to a decrease in renal function [59] and therefore COX-2 inhibitor use should also be avoided until more clinical data become available.

Complications of diuretic therapy

Common complications are renal impairment caused by intravascular volume depletion (25%), hyponatremia (28%), and hepatic encephalopathy (26%) [60–62]. Spironolactone is often associated with adverse events, mainly painful gynecomastia, related to its antiandrogenic activity. Potassium canrenoate, one of the major metabolites of spironolactone, has a comparable diuretic effect and a lower antiandrogenic activity and could be used in cases in which gynecomastia and mastalgia are side effects of spironolactone therapy. However, this drug is not available in the United States. Amiloride, another potassium-sparing diuretic, does not produce gynecomastia and is recommended in patients with intolerable painful gynecomastia, but it has significantly less natriuretic effect than spironolactone [63]. Amiloride is used at an initial dose of 20 mg/day and can be increased to 60 mg/day. In patients in whom the natriuretic response with amiloride is suboptimal it may be worthwhile to attempt retreatment with spironolactone.

Large-volume paracentesis

Several randomized studies have demonstrated that large-volume paracentesis (LVP) associated with i.v. albumin is as effective as standard therapy with diuretics in patients with uncomplicated ascites but with a significantly faster resolution and the same or a lower rate of complications [60,61]. However, LVP is a local therapy that does not act on the mechanisms of ascites formation (Fig. 90.3) and ascites recurrence is the rule. Additionally, the procedure is more costly and requires more resources than the administration of diuretics.

The standard therapy for refractory ascites is LVP + albumin. Although initially the recommendation was to perform daily 5-L paracenteses until the disappearance of ascites, it was subsequently determined that total paracentesis (i.e., removal of all ascites in a single procedure accompanied by the concomitant infusion of 6–8 g albumin per liter of ascites removed) was as safe as repeated partial paracenteses [64].

The frequency of LVP is determined by the rate of ascites reaccumulation and, ultimately, on the need to relieve the

patient's discomfort. The rate of ascites reaccumulation depends largely on the patient's compliance with salt restriction and diuretics and the degree of sodium retention. A randomized controlled study showed that the administration of diuretics after LVP is associated with longer time to recurrence of ascites without any differences in complications [65]. Notably, in this study the urinary sodium of patients in the diuretic group in whom ascites recurred by day 30 was significantly lower at 3 days and 2 weeks (8 and 15 mEq/L, respectively) compared with that of patients in whom ascites had not recurred by day 30 (109 and 67 mEq/L, respectively). This suggests that although sodium restriction and diuretics should be used after LVP, in patients with a urinary sodium less than 30 mEq/L diuretics are not useful and should be discontinued, particularly if associated with complications [52].

Complications of large-volume paracentesis

Procedure-related complications consist mainly of bleeding and ascites leakage. Major *bleeding* occurs rarely but may be lethal [66] and has been related to rupture of mesenteric varices rather than as a result of coagulopathy. In fact, in a series of over 1000 LVPs there was no significant bleeding, not even in patients with marked thrombocytopenia or prolongation in the prothrombin time [67]. Renal dysfunction, more than coagulopathy, appears to be associated with a higher risk of post-LVP hemorrhage [8]. LVP should probably be avoided in patients with renal failure and clinically evident severe coagulopathy. Care should be taken to avoid abdominal wall collaterals and to avoid the area of the inferior hypogastric artery, which lies midway between anterior superior iliac spine and pubic tubercle. Technically, the midline below the umbilicus is often recommended as a site for paracentesis because of its presumed avascularity. However, a laparoscopic study in 20 patients with cirrhosis demonstrated that, in patients with portal hypertension, this area is commonly vascular [68].

Leakage of ascitic fluid is rare and occurs when extraction of ascites is incomplete. Therefore, this complication can be resolved by completing the LVP preferably in a site remote from the leaking puncture site. Another complication of paracentesis that is rare but should be recognized is the development of sudden *scrotal edema* that results from subcutaneous tracking of peritoneal fluid into the scrotum. This can be resolved by elevation of the scrotum [69].

One of the main complications of LVP is the development of *postparacentesis circulatory dysfunction* (PCD), defined as a significant increase in plasma renin activity (PRA) 6 days after LVP. Development of PCD is associated with a faster recurrence of ascites, development of renal dysfunction, and a higher mortality [70,71]. Two factors are independent predictors of the development of PCD: the amount of ascites removed and the type of volume expander used in association with LVP [70]; the lowest rates are observed when less than 5 L is removed or when albumin is used as a plasma volume expander (rate around 16%) [70,72–74]. The presence (or absence) of peripheral edema is not a predictor of the development of PCD. Albumin should be administered at a dose of 6–8 g of albumin i.v. per liter of ascites removed. For LVP less than 5 L, a synthetic plasma expander (Haemaccel, Dextran-70) or even saline solution can be used instead of albumin, and it has been suggested that no plasma expansion may be necessary in this setting [52,70,74]. The pathogenesis of PCD appears to be a worsening of the vasodilatory state of the cirrhotic patient with a consequent further decrease in effective arterial blood volume and marked activation of neurohumoral systems that lead to further sodium retention, renal vasoconstriction, renal dysfunction, and death [71]. Therefore LVP should not be performed in the setting of conditions that have been associated with a worsening in the vasodilatory state of cirrhosis, such as SBP.

Regarding the possibility of infection in outpatients undergoing serial LVP, three studies have shown that SBP is not a significant complication in this setting [75–77].

Transjugular intrahepatic portosystemic shunt

Transjugular intrahepatic portosystemic shunt (TIPS) is a nonsurgical, percutaneous procedure performed by an interventional radiologist by which a portosystemic shunt is established inside the liver parenchyma by connecting, through a metal stent, a main portal branch with a large hepatic vein. By acting as a side-to-side portocaval shunt, it relieves sinusoidal pressure, the main driving force in the formation of ascites. Although TIPS placement is associated early on with a worsening in systemic hemodynamics (increase in cardiac output, further decrease in systemic vascular resistance) and a worsening of liver synthetic function, these changes are no longer present 3 months after TIPS insertion [78]. Additionally, despite deterioration of the hyperdynamic circulatory state and of liver synthetic function, urinary sodium excretion increases significantly as soon as 7 days after TIPS placement and correlates closely with a decrease in plasma renin activity (PRA) and aldosterone levels [78–84]. In addition to sinusoidal decompression, TIPS placement has the advantage of, at least transiently, increasing the effective arterial blood volume by transferring blood volume from the splanchnic to the systemic circulation.

Five prospective randomized trials have compared TIPS (using uncovered stents) with LVP in the treatment of refractory ascites [81,85–88]. Metaanalysis of these trials demonstrates that an uncovered TIPS stent is more effective at preventing ascites from recurring; however, it is associated with an increased occurrence of hepatic encephalopathy without differences in survival [89]. However, when the trial that included Child C patients [81] is excluded, metaanalysis showed a tendency for an improved survival with TIPS [89]. In these trials, the rate of post-TIPS shunt morbidity was an important issue that increased its cost [86]. However, new polytetra-fluoroethylene (PTFE)-covered stents are associated

with improved TIPS patency, and a decrease in the number of clinical relapses and reinterventions, without increasing the risk of encephalopathy [90]. In fact, a case–control study suggested that patients undergoing TIPS with covered stents have higher 2-year survival rates when compared with patients with uncovered TIPS [91]. This benefit needs to be prospectively evaluated in patients with refractory ascites. Until then, the consensus recommendation is to consider TIPS placement when the frequency of LVP is greater than two to three times per month [52].

Complications of transjugular intrahepatic portosystemic shunt

The procedure-related complication rate is around 9%, with the most common being intraperitoneal hemorrhage [92]. Other important complications are heart failure and hemolysis, which may develop in 10%–15% of patients. As mentioned above, long-term complications of TIPS are new onset or worsening of hepatic encephalopathy and shunt dysfunction. Each occurs in about one-third of the patients, with a mean follow-up of about 1 year [78]; the incidence for both complications appears to be lower with covered TIPS stents. Resolution of ascites post-TIPS has been associated with the development of abdominal wall hernia incarceration [93].

Contraindications to transjugular intrahepatic portosystemic shunt

TIPS can precipitate death secondary to liver failure. The strongest predictor of survival post-TIPS placement for patients with refractory ascites is the serum bilirubin concentration [78,89], and a cutoff level of 3 mg/dL has been proposed [94]. Consensus recommendations also suggest that an age greater than 70 years, preexisting cardiac dysfunction, and a Child–Turcotte–Pugh (CTP) score greater than 11 should be considered contraindications to TIPS placement in the setting of refractory ascites [52]. Serum bilirubin may be the decisive factor in reaching the CTP cutoff score of 11 because patients with refractory ascites have, by definition, the highest score for ascites (i.e., tense ascites) and most have the highest score for albumin [78]. TIPS is not recommended in patients with heart failure (ejection fraction < 55%), firstly because with high right-sided pressures there may not be an adequate pressure gradient between the portal and the systemic venous systems for TIPS to function, and secondly because TIPS placement in this setting may precipitate exacerbation of heart failure. Patients with alcoholic cirrhosis who are drinking alcohol may improve with alcohol abstinence and therefore TIPS should be delayed in these patients.

Peritoneovenous shunt

The peritoneovenous (PVS) shunt is a therapy that combines removal of intraperitoneal fluid and replenishment of the intravascular volume. It consists of a silicone tube system and a one-way valve that transfers ascites from the peritoneal cavity to the internal jugular vein (i.e., the systemic circulation). The increase in fluid return to the cardiopulmonary circulation can lead sequentially to decreased activity of sodium-retaining and vasoconstrictive mechanisms (such as the renin–angiotensin–aldosterone system), a marked rise in urinary sodium excretion, and a modest elevation in glomerular filtration rate.

In randomized controlled trials of refractory ascites PVS has been shown to be more effective than diuretics [95] (a predictable result) and as effective as LVP + albumin [96,97]. In these studies, no differences in survival have been observed among therapies. However, as a result of its high obstruction rate, PVS requires frequent admissions for shunt revision or for the management of other more serious complications. The use of PVS has been progressively abandoned over the years because LVP + albumin is a simpler procedure that can be performed in the outpatient setting and because of the advent of TIPS. In a small randomized trial comparing PVS with uncovered TIPS, control of ascites was achieved sooner after PVS, but longer-term (3-year) efficacy favored TIPS, with numerous interventions required to assist patency with both shunts. This study suggests that PVS is a reasonable option for a short (< 1-year) period, particularly because the mean patency of the shunt is around 6 months [95,96]. In fact, a case series of 36 patients listed for liver transplantation who had PVS placed for refractory ascites and who were followed for a mean of 9 months, reported adequate palliation (i.e., no need for further LVP until transplant) in 83% with a lower occurrence of renal failure (compared with historical controls) [98]. Although PVS might complicate liver transplant surgery given its ability to produce peritoneal adhesions, none of the 16 patients who were transplanted in this series had intraoperative complications secondary to PVS [98].

Traditionally, placement of PVS has been performed by surgeons under general anaesthesia and employing venous cutdown. But there is increasing experience of these shunts being placed by radiologists in the interventional suite, with successful revision in case of dysfunction in the majority of patients [99].

Therefore, placement of PVS should clearly be considered in patients who are not TIPS candidates, and it can also be considered (based on local surgeon preference) for patients on the transplant list.

Complications of peritoneovenous shunt

The major problem with PVS is the relatively high rate of complications, although in randomized controlled trials PVS has a similar rate of complications as LVP + albumin [96,97]. The complications related to PVS include disseminated intravascular coagulation (due to introduction of endotoxins and other procoagulants from ascites into the systemic circulation), infection of the shunt (which can can lead to bacteremia and requires shunt removal), variceal bleeding or heart

failure resulting from volume expansion and a concurrent rise in systemic and portal venous pressure, and small bowel obstruction from peritoneal adherences [100]. The transoperative drainage of ascites can minimize the complications related to massive ascites infusion, such as disseminated intravascular coagulation and increased systemic and portal pressures [101].

Therapies under investigation for the treatment of ascites

Sympathetic system antagonists

Clonidine is a centrally acting α_2-agonist with antiadrenergic activity in cirrhosis. In a small randomized pilot study, the administration of clonidine, at a dose of 0.075 mg orally (p.o.) twice a day, plus spironolactone to patients with refractory ascites increased natriuresis and significantly decreased plasma norepinephrine (noradrenaline) and aldosterone levels and plasma renin activity. In a mean follow-up of 10.5 months and compared with patients who were treated with LVP + albumin ($n = 10$), patients who received clonidine plus spironolactone ($n = 10$) had fewer readmissions for ascites, a longer time to ascites reaccumulation, and decreased spironolactone requirements [102].

A single-center randomized double-blind placebo-controlled trial from the same group of investigators evaluated the efficacy of clonidine in patients with uncomplicated ascites and an activated sympathetic nervous system (as defined by serum norepinephrine levels > 300 pg/mL). Patients randomized to clonidine ($n = 32$) had a significantly lower number of readmissions for ascites and a longer time to readmission, and lower requirements for LVP, spironolactone, and furosemide compared with patients randomized to placebo ($n = 32$), with a lower rate of hyperkalemia and renal impairment [103]. These promising results require further investigation before clonidine can be widely recommended.

V_2 receptor antagonists

Selective inhibition of arginine vasopressin type 2 receptors (V_2) was considered important only in the treatment of dilutional hyponatremia in cirrhosis. However, data from experimental animals show that V_2 receptor antagonists also have a natriuretic effect [104–106]. In fact, a phase II study of satavaptan, a V_2 receptor antagonist, in 148 patients with cirrhosis and ascites without hyponatremia demonstrated a dose-related increase in urine volume and a dose-related decrease in body weight without changes in serum creatinine [107]. Furthermore, in a randomized double-blind placebo-controlled study in 151 patients treated with LVP plus spironolactone, satavaptan was associated with a longer time to recurrence of ascites requiring LVP and a significantly lower number of paracenteses [108].

Therefore, V_2 receptor antagonists could be useful adjuvants to diuretics in the treatment of ascites (refractory or nonrefractory ascites) by preventing diuretic-induced hyponatremia and by a synergistic natriuresis. However, further analysis is required.

Vasoconstrictors

Because vasodilation is one of the main mechanisms in the formation of cirrhotic ascites, a rational therapeutic approach is the use of vasoconstrictors. There is limited experience with these agents. A pilot study shows that a 7-day course of midodrine (at a dose of 10 mg p.o. three times a day) was associated with significant increases in urinary sodium, mean arterial pressure, and creatinine clearance and with significant decreases in plasma renin activity and plasma aldosterone levels [109]. Its effect as an adjuvant to diuretics in the treatment of ascites remains to be determined.

Two small randomized studies have compared terlipressin with albumin in the prevention of postparacentesis circulatory dysfunction [110,111]. Both studies showed that the postparacentesis circulatory dysfunction (i.e., plasma renin activity before and 4–6 days after LVP) did not differ between the study groups; however, given the small numbers of patients included in these studies, results are not conclusive.

Cirrhosis and uncomplicated ascites

The approach to the patient with cirrhosis and uncomplicated ascites is depicted in Fig. 90.4.

In contrast to the treatment of heart failure, where achieving a negative sodium and water balance demands a certain urgency given the risk/presence of pulmonary edema, therapy of cirrhotic ascites is not an emergency because the risk of death is not implicit unless the fluid is infected. Therefore, most patients with uncomplicated ascites should treated in a stepwise, unhurried fashion, and treatment should only be initiated in a "stable" cirrhotic patient, that is, one in whom complications such as gastrointestinal hemorrhage, bacterial infection, or renal dysfunction are absent or have resolved. One exception is the patient with tense ascites who experiences not only abdominal discomfort but also respiratory distress. In these patients, a single large-volume paracentesis should be performed prior to or concomitant with starting diuretic therapy.

Because renal dysfunction secondary to diuretics results from a reduction in effective arterial blood volume, diuretics should not be initiated in patients with concomitant complications of cirrhosis known to be associated with a decreased effective arterial blood volume, such as variceal hemorrhage and SBP.

Treatment aimed at achieving a negative sodium balance (i.e., sodium restriction and diuretics) still constitutes the mainstay of therapy in patients with uncomplicated ascites, given its general applicability, low cost, and ease of administration. However, in hospitalized patients with moderate/tense ascites in whom other complications have been resolved,

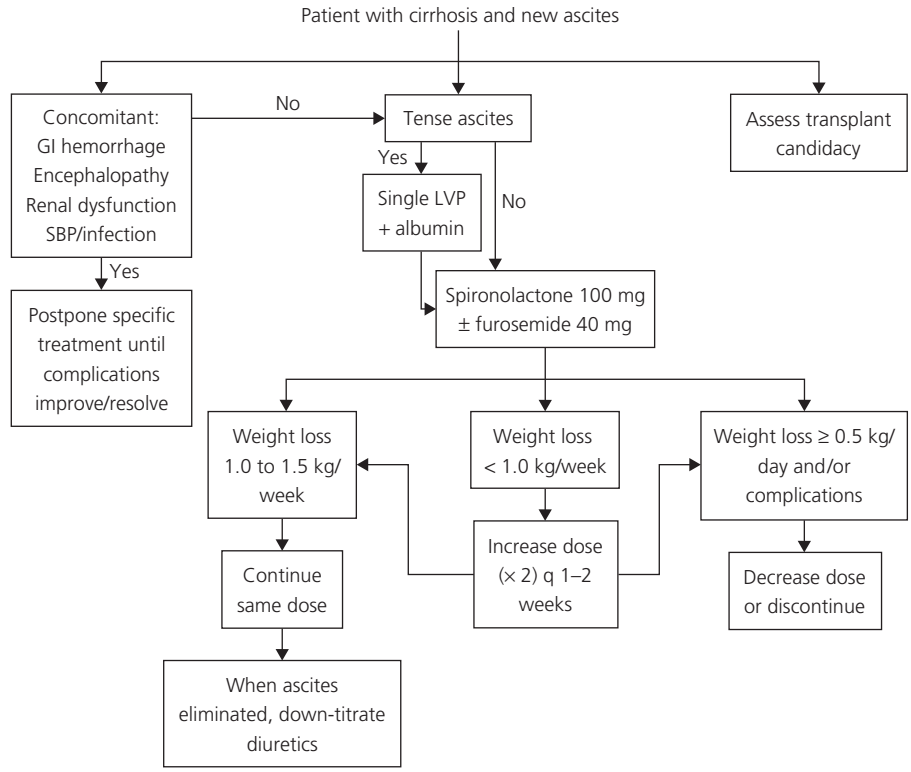

Figure 90.4 Approach to the patient with cirrhosis and uncomplicated ascites. GI, gastrointestinal; LVP, large-volume paracentesis; SBP, spontaneous peritonitis.

it is reasonable to initiate therapy with total paracentesis as this will accelerate discharge from the hospital.

Diuretic therapy can be initiated with spironolactone alone (initial dose of 100 mg p.o. daily) or with spironolactone (100 mg p.o. daily) plus furosemide (40 mg p.o. daily). Both schedules are equally effective; however, dose adjustments are needed more frequently in patients in whom treatment is initiated with combination therapy because of a more rapid mobilization of fluid with consequent increases in blood urea nitrogen or decreases in serum sodium [54,112]. In an inpatient setting, where renal function can be monitored more frequently, combination therapy can be used. However, in the outpatient setting it is preferable to initiate therapy with spironolactone alone at a daily dose of 100 mg p.o. Because spironolactone takes several days to act it should be administered in a single daily dose. If weight loss is not optimal (weight loss < 1 kg in the first week and < 2 kg/week in subsequent weeks) the dose should be increased in a stepwise manner with increases of 100 mg every 1–2 weeks to a maximum of 400 mg p.o., q.i.d [52]). If hyperkalemia develops before the maximal dose is reached, furosemide should be added at an initial single daily dose of 40 mg, increased in a stepwise fashion in increments of 40 mg to a maximum of 160 mg/day.

Before considering that ascites is refractory to diuretics, it is necessary to ascertain whether the patient has adhered to the prescribed sodium-restricted diet and has restrained from using nonsteroidal antiinflammatory drugs. Nonadherence to dietary sodium restriction or diuretics should be suspected if patients fail to lose weight despite an adequate 24-h urine sodium excretion (> 50 mEq/L or greater than daily sodium intake). In a patient in whom ascites disappears, one can attempt to downtitrate the dose of diuretics and maintain it at the minimal effective dose so that complications can be minimized.

In patients who develop renal dysfunction (elevation in creatinine > 50% to a creatinine > 1.5 mg/dL) or encephalopathy, diuretics should be temporarily discontinued and restarted at a lower dose when creatinine returns to baseline. Patients who develop hyponatremia (serum sodium < 130 mEq/L) while on diuretics should be managed with fluid restriction and a decrease in the dose of diuretics. The role of V_2 receptor antagonists in this setting is promising [106,113]. To minimize the rate of complications, patients without edema should not lose more than 0.5 kg/day, whereas in patients with edema a weight loss of 1 kg/day is allowable.

There are insufficient data to support the use of long-term antibiotic prophylaxis to prevent SBP and other bacterial infections in patients with uncomplicated ascites who are not hospitalized with gastrointestinal hemorrhage or who have not had a previous episode of SBP, even in those with an ascites protein concentration < 1 g/dL [27]. Limiting the use of prophylactic antibiotics is important given the increased rate of infections with quinolone-resistant and trimethoprim–sulfamethoxazole (cotrimoxazole)-resistant

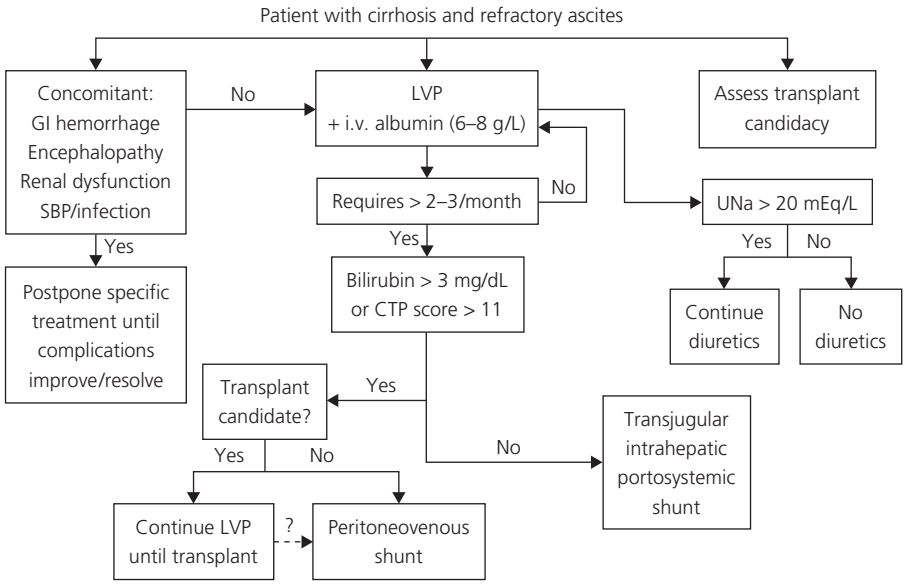

Figure 90.5 Approach to the patient with cirrhosis and refractory ascites. CTP, Child–Turcotte–Pugh; GI, gastrointestinal; i.v., intravenous; LVP, large-volume paracentesis; SBP, spontaneous bacterial peritonitis; UNa, urine sodium concentration.

organisms observed in patients on long-term norfloxacin prophylaxis [114,115].

Cirrhosis and refractory ascites

The approach to the patient with cirrhosis and refractory ascites is depicted in Fig. 90.5.

Refractory ascites assumes either diuretic-resistant ascites (ascites that is not eliminated even with maximal diuretic therapy) or diuretic-intractable ascites (ascites that is not eliminated because maximal doses of diuretics cannot be reached given the development of complications) [116]. The majority of patients with refractory ascites have the diuretic-intractable type [51]. Vasodilation is more pronounced in patients with refractory ascites (Fig. 90.2), thereby leading to a further decrease in effective arterial blood volume and further activation of the renin–angiotensin–aldosterone and sympathetic nervous systems. Further alteration in hemodynamics results in a greater susceptibility to develop hyponatremia or renal dysfunction in these patients. In fact, hepatorenal syndrome (HRS) type 2, in which renal dysfunction is characterized by a moderate and steady or slowly progressive renal failure (serum creatinine < 2.5 mg/dL), is almost exclusively seen in patients with refractory ascites [116]. Patients with type 2 HRS are predisposed to develop type 1 HRS following infections or other precipitating events.

The median survival of patients with type 2 HRS (4–6 months) is worse than that of patients with nonazotemic cirrhosis with ascites [117]. Therefore, a patient with refractory ascites who has not yet been evaluated for liver transplant candidacy should be evaluated without delay.

First-line therapy for patients with refractory ascites is serial LVP, adding albumin if more than 5 L is removed at once. In patients in whom 5 L or less is being removed, a plasma volume expander can be used. To increase the time between paracenteses, patients should continue on maximally tolerated diuretic dose provided that the urinary sodium is greater than 30 mEq/L. Otherwise, diuretics can be discontinued [52].

Routine cell count and culture analysis are not warranted in asymptomatic cirrhotic patients undergoing outpatient paracenteses for refractory ascites because studies have shown a low prevalence (0%–3.5%) of SBP in this setting [75–77]. Data from a randomized double-blind placebo-controlled trial suggest, however, that in patients with a low concentration of ascites protein (< 1 g/dL) who also have evidence of deranged circulatory status (creatinine > 1.2 mg/dL or blood urea nitrogen (BUN) > 25 mg/dL or a serum sodium ≤ 130 mEq/L) or severe liver disease (CTP score ≥ 9 and a serum bilirubin concentration ≥ 3 mg/dL) primary prophylaxis with norfloxacin at a daily dose of 400 mg p.o. is associated with a significantly lower incidence of first SBP and a reduced 3-month mortality [118]. These criteria will probably be met by most patients with refractory ascites.

In patients who require LVP more than two to three times per month or in whom ascites is loculated and cannot be entirely removed with a single LVP, evaluation for TIPS placement should be undertaken. In general, TIPS should not be performed in patients with serum bilirubin greater than 3 mg/dL, a CTP score greater than 11, age over 70 years, or evidence of heart failure [52,78] because these factors are associated with a poorer survival and a poorer shunt function. Although studies of TIPS for refractory ascites were performed using uncovered stents, covered stents should be used because of the lower rate of shunt dysfunction and potential benefits regarding development of encephalopathy and survival [90].

Although the goal of TIPS is to reduce the portosystemic pressure gradient to less than 12 mmHg (the threshold for the formation of ascites), too low a reduction (e.g., to levels

< 5 mmHg) carries a higher risk of post-TIPS portosystemic encephalopathy [119] and should therefore be avoided.

Most cases of post-TIPS encephalopathy respond to standard therapy, and only about 3%–10% develop recurrent, severe encephalopathy that can be treated with stent reduction [120], with preliminary evidence suggesting that covered reduction stents are better than uncovered reduction stents [121]. In a randomized controlled trial, prophylactic therapy with lactitol or rifaximin for 1 month after TIPS placement was ineffective in preventing portosystemic encephalopathy [119], and prophylaxis is therefore not recommended.

Because TIPS placement improves but does not normalize sodium excretion [82], peripheral edema may continue to be a problem and the use of diuretics after the procedure is advisable. Diuretic therapy immediately after the procedure also has the theoretical advantage of improving the pressure gradient across the shunt by reducing central pressure more than portal pressure.

Serial assessment of shunt patency is not necessary after TIPS unless there is recurrence of ascites, a clinically obvious event indicative of stent dysfunction.

In patients requiring frequent LVP and who are not TIPS candidates, a peritoneovenous shunt (PVS) should be considered. Placement of a PVS may hinder future placement of TIPS. The intraabdominal limb of the PVS is associated with the formation of peritoneal adhesions and may complicate transplant surgery; therefore it has also been considered a contraindication in patients who are candidates for transplant. However, a case series of pretransplant patients showed that PVS is not associated with surgical complications [98].

Cirrhosis and hepatic hydrothorax

Pleural effusion (hepatic hydrothorax) develops in approximately 5%–10% of patients with cirrhosis, most probably as the result of the transdiaphragmatic movement of fluid from the peritoneum to the pleural space through diaphragmatic defects [122,123]. The negative intrathoracic pressure favors the movement of fluid across these defects, and patients usually have minimal or mild ascites. In fact, hepatic hydrothorax may be present in patients without detectable ascites [124]. Pleural effusion is right-sided in 85%, left-sided in 13%, and bilateral in 2% of the cases [125].

Although large amounts of ascites can accumulate in the peritoneal cavity before resulting in significant patient discomfort, the accumulation of smaller amounts of fluid (1–2 L) in the pleural space results in severe shortness of breath and hypoxemia. A diagnostic radionuclide ascites scan is useful in the diagnosis of hepatic hydrothorax, particularly in cases where ascites is absent [126]. It consists of the injection of technetium-99m-labeled sulfur colloid [127] or macroaggregated serum albumin [126] into the peritoneal cavity followed by chest imaging every 15–30 min. Transdiaphragmatic movement of ascites into the pleural space is demonstrated generally within 2 h of intraperitoneal injection of the radiotracer.

Hepatic hydrothorax should be treated in the same manner as cirrhotic ascites, that is, the mainstay of therapy is sodium restriction and diuretics. Before determining that hydrothorax is refractory, a trial of in-hospital diuretic therapy should be attempted. In patients with refractory hepatic hydrothorax other therapeutic options such as repeated thoracenteses, TIPS, or pleurodesis should be considered. Regarding thoracentesis, and given that no more than 2 L is removed at a time because of the risk of reexpansion pulmonary edema, the procedure may need to be repeated frequently. When thoracentesis is required every 2–3 weeks, alternative strategies such as TIPS should be considered. Uncontrolled studies of TIPS have shown resolution of the pleural effusion or a decrease in the need for thoracentesis in 67% of patients; however, mortality is high, particularly in nonresponders to TIPS [128]. In patients who have not responded to TIPS, an option is video-assisted thoracoscopy to repair diaphragmatic defects and to perform pleurodesis. However, information is available on only a small number of patients, and the procedure is associated with significant morbidity and mortality [123]. Placement of a chest tube should be avoided in patients with hepatic hydrothorax because it has been associated with multiple complications, mainly volume and electrolyte disturbances.

Spontaneous bacterial peritonitis

Spontaneous bacterial peritonitis (SBP) is the most common type of bacterial infection in hospitalized cirrhotic patients, occurring in about 9% of the patients and accounting for about 25% of all infections [115].

SBP is an infection of ascitic fluid that occurs in the absence of perforation of a hollow viscus or of an intraabdominal inflammatory focus, such as an abscess, acute pancreatitis, or cholecystitis.

Bacterial translocation – the migration of bacteria from the intestinal lumen to mesenteric lymph nodes and other extraintestinal sites – is the main mechanism implicated in the pathogenesis of SBP. Impaired local and systemic immune defenses and intestinal bacterial overgrowth promote bacterial translocation and, together with shunting of blood away from the hepatic Kupffer cells through portosystemic collaterals, allow a transient bacteremia to become more prolonged, subsequently colonizing ascitic fluid. Bacterial translocation and the development of SBP in cirrhosis worsen the hyperdynamic circulatory state by a cytokine-mediated aggravation of vasodilation [129–131]. By worsening vasodilation,

SBP can lead to renal dysfunction and the hepatorenal syndrome [132].

SBP occurs in patients with reduced ascites defense mechanisms, such as low ascites complement levels. Because ascites complement levels correlate with ascites protein levels, it follows that the most important predictor in the development of SBP is a low concentration of ascites protein (< 1 g/dL) [17,133].

When first described, the mortality of SBP exceeded 90%; however, with early recognition of the disease and prompt and appropriate antibiotic therapy, mortality has been reduced to around 20%–30% [134], a much lower but still significant mortality.

The approach to the patient with cirrhosis and SBP is depicted in Fig. 90.6. Early recognition of SBP involves the performance of a diagnostic paracentesis (for total and differential cell counts and bacteriological culture) in any patient in whom SBP is suspected. Suspicion of SBP should be raised in patients with symptoms or signs directly due to SBP, such as fever, abdominal pain or tenderness, and leukocytosis, but should also be raised in the presence of unexplained encephalopathy, jaundice, or worsening renal failure – complications of sepsis. Simultaneous blood and urine cultures and a chest radiograph should also be obtained because any bacterial infection in cirrhosis can lead to similar manifestations. In patients with hepatic hydrothorax in whom an infection is suspected and SBP has been ruled out, a diagnostic thoracentesis should be performed to rule out spontaneous bacterial empyema, an entity akin to SBP that may occur in the absence of ascites or SBP and that should be managed as described below for SBP [135].

Isolation of an infecting organism is definitive in establishing the diagnosis of SBP. The most common organisms isolated in SBP are gram-negative bacteria, and the infections are mostly monomicrobial [31,115]. However, despite the use of sensitive culture methods that include inoculation of ascites into blood culture bottles, ascites cultures are negative in up to 60% of patients with clinical manifestations compatible with SBP and increased ascites PMN counts [115].

Therefore, the diagnosis of SBP is established with an ascites PMN count greater than 250/mm^3, and treatment should be initiated before obtaining bacteriological culture results [25]. If the PMN count is below 250/mm^3, culture results must be awaited. If ascites culture is positive (bacterascites), the ascites cell count should be repeated and, if the repeat PMN count is greater than 250/mm^3, SBP has developed and should be treated. However, if the PMN count remains below 250/mm^3, the results of a second bacteriological culture should be awaited. In most cases, the repeat culture will be negative but if it continues to be positive for the same organism it is assumed that bacteria are being seeded into ascites from an extraabdominal source and, although it is not causing peritonitis, bacterascites should then be treated [25].

The most effective (~ 90% resolution rate) and safe antibiotics are intravenous cefotaxime (2 g i.v. every 12 h) or other third-generation cephalosporins, such as ceftriaxone (1–2 g i.v. every 24 h) or ceftazidime (1 g i.v. every 12–24 h) [136–139]. The combination of amoxicillin/clavulanic acid (1 g/0.2 g i.v. every 8 h) has been shown to be as effective and safe as cefotaxime in the treatment of SBP [140]. Patients who develop SBP on prophylactic quinolones have been shown to

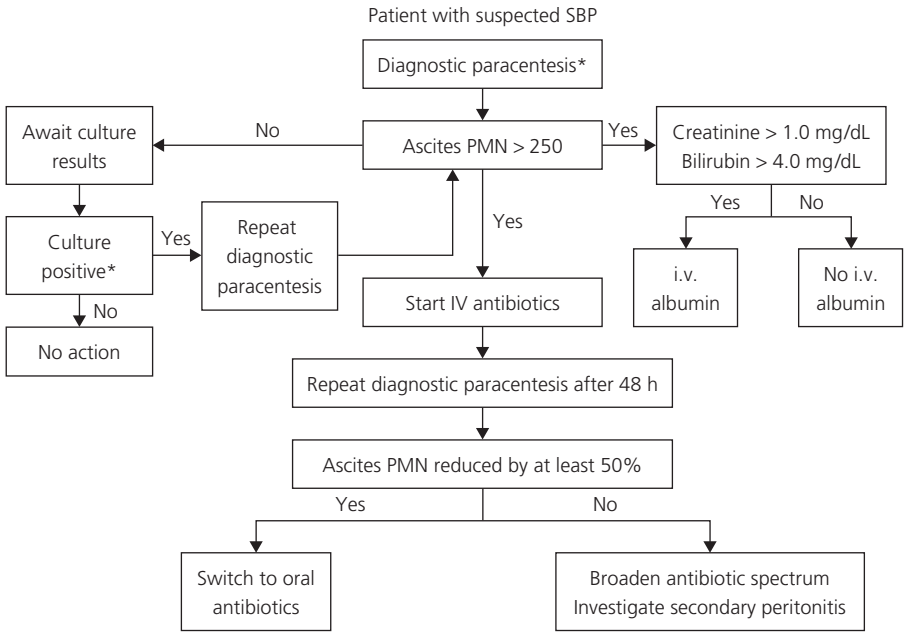

Figure 90.6 Approach to the patient with suspected spontaneous bacterial peritonitis (SBP). *A diagnostic paracentesis should be performed in any patient with symptoms or signs suggestive of SBP, any patient with unexplained renal dysfunction or encephalopathy, and in any hospitalized patient with cirrhosis and ascites. i.v., intravenous; PMN, polymorphonuclear leukocytes.

respond as well to cefotaxime as patients not on prophylaxis [141]. The antibiotics recommended above have been associated with few side effects and no renal toxicity. By contrast, aminoglycosides should be avoided in patients with cirrhosis because of their increased propensity to develop aminoglycoside-induced nephrotoxicity [142,143].

Renal dysfunction is the main cause of death in SBP and both can be prevented by the use of intravenous albumin [144]. Because renal dysfunction and death in SBP occur almost exclusively in patients with a baseline concentration of serum bilirubin greater than 4 mg/dL and a serum creatinine level exceeding 1 mg/dL [144,145], the prophylactic use of albumin in SBP can probably be restricted to this subgroup of high-risk patients [146]. The dose of albumin used is arbitrary: 1.5 g/kg of body weight during the first 6 h, followed by 1 g/kg on day 3, with a maximum of 100 g/day. There is a subgroup of patients with SBP – those with community-acquired infection, no encephalopathy, and normal renal function – that have a 100% cure rate and 100% survival with antibiotic therapy alone [147]. Albumin would not be indicated in these patients [146].

A control paracentesis performed 48 h after starting therapy is recommended to assess the response to therapy and the need to modify antibiotic therapy (depending on the isolation of a causative organism) or to initiate investigations to rule out secondary peritonitis [25]. If clinical improvement is obvious, this control paracentesis may not be necessary. Failure of initial therapy, defined as a decrease in PMN of less than 25% from baseline, occurs in up to 23% of the cases and is associated with a poor prognosis. In the presence of either clinical or cytological improvement, intravenous antibiotics can be switched to oral antibiotics after 2 days of therapy. This strategy has been shown to be as effective as continuous intravenous antibiotic therapy in two trials that used ciprofloxacin (first intravenous and then oral) [148,149]. Although a growing number of infections involving quinolone-resistant organisms may limit the use of quinolones as first-line antibiotic therapy in SBP [115], switching from intravenous cephalosporins to oral quinolones is a common practice recommended in complicated urinary tract infection in adults [150].

Total duration of antibiotic therapy should be for a minimum of 5 days. However, in prospective trials in which ascites PMN counts were carefully monitored, the median time to SBP resolution (i.e., a decrease in PMN count below 250/mm^3) was 8 days and therefore this duration of therapy is probably more recommendable [151].

In patients who survive an episode of SBP, the one-year cumulative SBP recurrence rate is high, at about 70%. Recurrence, particularly secondary to gram-negative organisms, is significantly and markedly lower with the use of norfloxacin at a dose of 400 mg p.o. per day [152,153]. It is therefore essential that patients surviving an episode of SBP are started on antibiotic prophylaxis to prevent recurrence. The use of weekly quinolones is not recommended because it has been shown to be less effective in preventing SBP recurrence and is associated with a higher rate of development of quinolone-resistant organisms [153]. Prophylaxis should be continuous until disappearance of ascites (i.e., patients with alcoholic hepatitis), death, or transplant.

Cirrhosis and acute renal failure

Acute renal failure occurs in 14%–25% of hospitalized patients with cirrhosis [143,154,155]. There are three types of acute renal failure: prerenal failure, which results from renal hypoperfusion without a glomerular or tubular lesion; intrinsic renal failure, which results from tubular necrosis (ischemic or toxic), glomerulonephritis, or interstitial nephritis; and post-renal failure, which results from urinary tract obstruction.

In hospitalized patients with cirrhosis, the most common cause of acute renal failure is prerenal (accounting for 60%–80% of the cases) [154,156,157], followed by acute tubular necrosis (20%–40%) [154,156,157], with postrenal causes accounting for less than 1% of the cases [156,157].

Prerenal failure results from any factor that will further decrease the already decreased effective arterial blood volume of the patient with cirrhosis. Therefore prerenal failure can result from factors that cause hypovolemia, such as gastrointestinal hemorrhage, overdiuresis, or diarrhea; factors that worsen vasodilation, such as sepsis, use of vasodilators, and the postparacentesis circulatory dysfunction; and factors that cause renal vasoconstriction, such as nonsteroidal anti-inflammatory drugs or intravenous contrast agents. These factors account for up to 80% of the causes of prerenal failure [154]. The persistence of acute renal failure despite exclusion or reversal of the above-mentioned precipitant factors indicates the presence of type 1 hepatorenal syndrome (HRS), which accounts for only about 20% of cases of acute renal failure in cirrhosis [156]. HRS type 1 is a type of prerenal failure because it results from hemodynamic abnormalities (extreme vasodilation) leading to renal vasoconstriction [49] (Fig. 90.2). The other type of HRS, type 2, although also resulting from vasodilation, is a chronic form of renal failure associated with refractory ascites and is not considered in the differential diagnosis of acute renal failure in cirrhosis.

In patients with cirrhosis, acute tubular necrosis (ATN) is mainly caused by an ischemic insult to the renal tubules, mostly as a result of a hypotensive event, although the use of aminoglycosides (which cause renal failure by direct toxicity to renal tubules) was found to be the most important predictor of renal failure in cirrhosis in a study performed in US veterans [143]. Studies are needed on the natural course, diagnosis, and treatment (e.g., hemodialysis) of acute tubular necrosis in patients with cirrhosis.

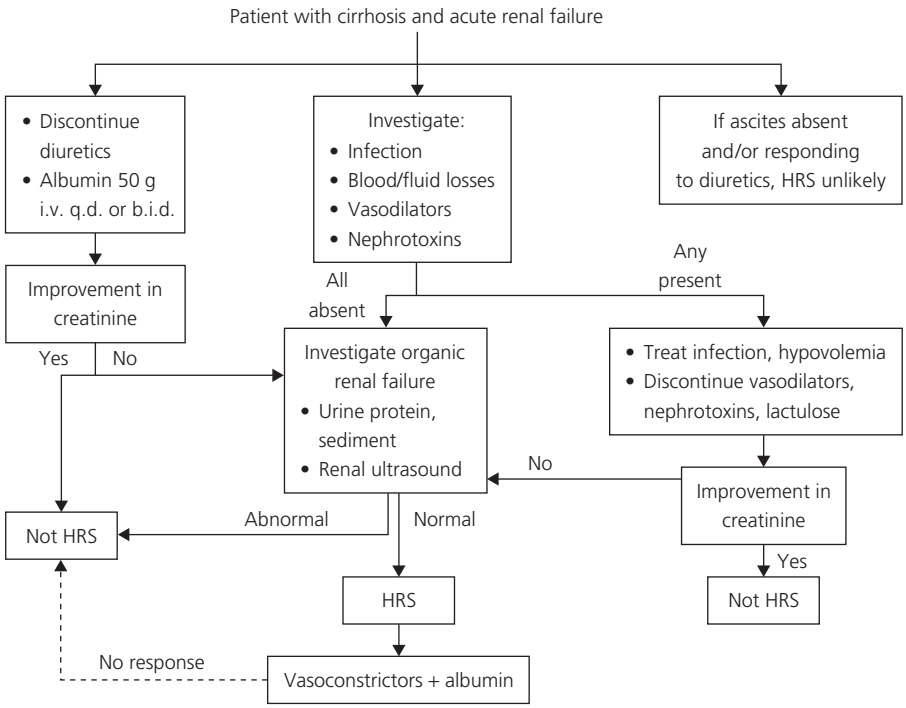

Figure 90.7 Approach to the patient with cirrhosis and acute renal failure. b.i.d., twice a day; HRS, hepatorenal syndrome; i.v., intravenous; q.d., once a day.

The approach to a patient with cirrhosis and acute renal failure is depicted in Fig. 90.7 [132,158].

In a patient with cirrhosis who presents with an acute deterioration in renal function as evidenced by a doubling of serum creatinine to more than 2.5 mg/dL within a 2-week period [116], the main issue is to rule out HRS as this entity involves a totally separate therapeutic approach (see below). Indirect estimates of glomerular filtration rate such as the Modification of Diet in Renal Disease (MDRD) equation have not yet been evaluated in the assessment of renal failure in patients with cirrhosis.

Urine indices (urine osmolality, urinary sodium concentration, and fractional excretion of sodium) may help to distinguish prerenal failure (including HRS) from acute tubular necrosis. The tubular ability to reabsorb sodium and to concentrate urine is preserved in prerenal azotemia and is impaired in acute tubular necrosis. Therefore, patients with prerenal failure have low urinary sodium concentrations (< 20 mEq/L) and elevated urine osmolality (> 500 mOsm/kg) whereas patients with tubular necrosis have high urinary sodium concentrations (> 40 mEq/L) and a urine osmolality below 350 mOsm/kg. However, the urinary sodium concentration may be low early in the course of certain processes that lead to tubular necrosis, such as sepsis, and some cases of HRS with elevated urinary sodium concentrations have been reported [158].

If the patient with acute renal failure does not have ascites, the diagnosis of HRS is highly unlikely, because HRS is the result of maximal derangement in the hemodynamics that lead to ascites formation (Fig. 90.2) [49].

A first step in the diagnostic workup is to discontinue diuretics and to expand the intravascular volume with albumin at a dose of 50 g i.v. This dose can be repeated in 12 h if the creatinine has not normalized. A reduction in creatinine will rule out HRS.

At the same time, investigations to rule out the precipitants of renal dysfunction should be undertaken, specifically diagnostic paracentesis (to rule out SBP), blood and urine bacteriological cultures and chest radiograph to rule out a bacterial infections other than SBP, and stool investigations to rule out gastrointestinal hemorrhage. If any of these precipitants is identified, the patients should be treated accordingly.

Once precipitants of renal dysfunction are excluded or treated and if creatinine does not improve or continues to worsen despite diuretic discontinuation and continued volume expansion the differential diagnosis is between intrinsic renal failure, postrenal failure, or HRS. To rule out intrinsic renal failure, urinary sediment and urine protein should be analyzed. To rule out postrenal failure, a renal ultrasound should be obtained. To ensure that volume has been adequately expanded, it is reasonable to perform central venous pressure measurements at this point.

If ATN or other organic or postobstructive causes of renal failure are ruled out, the patient has HRS type 1. The differentiation between acute tubular necrosis and HRS is the most difficult, and it has been suggested that the response to vasoconstrictors plus albumin (see below) may be used to establish this differential [158].

Hepatorenal syndrome

HRS is a potentially reversible functional renal failure that occurs in patients with cirrhosis, ascites, and liver failure and in patients with acute liver failure and alcoholic hepatitis [132]. HRS is secondary to renal vasoconstriction that results from markedly decreased effective arterial blood volume and the resultant activation of renal vasoconstrictive systems. Decreased effective blood volume is in turn the result of intense vasodilation (Fig. 90.2) and, as postulated, of a relatively low cardiac output [132]. Because HRS represents the extreme of the spectrum of abnormalities that lead to sodium and water retention, ascites unresponsive to diuretics is universal, and dilutional hyponatremia is almost always present (Fig. 90.2).

HRS is divided into two types based on clinical characteristics and prognosis. Type 1 HRS is characterized by rapidly progressive renal failure defined by doubling of the serum creatinine concentrations to a level greater than 2.5 mg/dL in less than 2 weeks. It may appear spontaneously, but often develops after a precipitating event, particularly SBP. Its prognosis is poor, with a median survival of 2–4 weeks [159,160]. Type 2 HRS is a moderate renal failure (serum creatinine 1.5–2.5 mg/dL), with a steady or slowly progressive course, and is associated with refractory ascites. Survival of patients with type 2 HRS is shorter than that of nonazotemic cirrhotic patients with ascites but better than that of patients with type 1 HRS (median survival about 6 months) [132,160]. Type 2 HRS will not be considered in this section.

Patients with ascites have 1- and 5-year probabilities of developing HRS of around 20% and 40% respectively [159]. The likelihood of developing HRS is highest in patients with more marked sodium and water retention and more marked activation of vasoconstrictive systems (renin–angiotensin and sympathetic nervous system) [159], indicative of marked vasodilation (Fig. 90.2). A low cardiac output and high plasma renin activity were found to be independent predictors of HRS in patients with cirrhosis and uncomplicated ascites [161].

As mentioned previously, HRS is diagnosed by exclusion of other known causes of acute renal failure (Fig. 90.8).

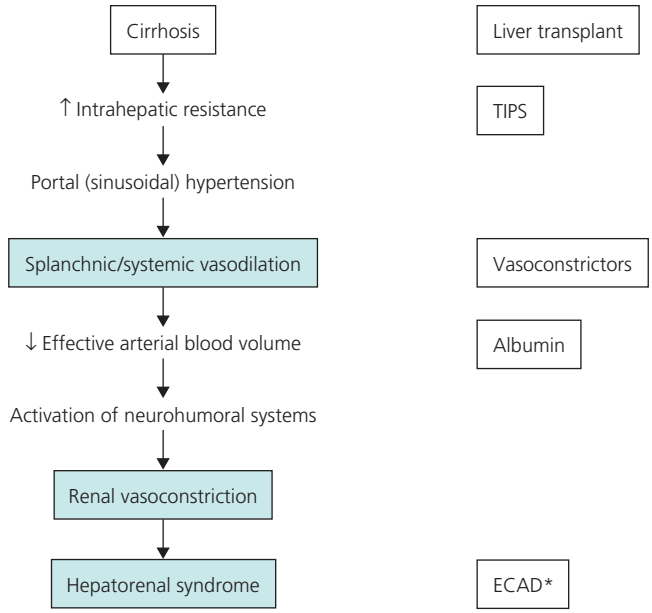

Figure 90.8 Site of action of different therapies for hepatorenal syndrome. *ECAD (extracorporeal albumin dialysis) is an experimental therapy that seems to improve hepatorenal syndrome; it probably acts by decreasing the amount of circulating vasodilators. TIPS, transjugular intrahepatic portosystemic shunt.

Therapies for hepatorenal syndrome

Different therapies for HRS and their site of action are depicted in Fig. 90.8. Except for transplant, none of the different treatments of HRS has resulted in significant improvements in survival. However, treating HRS is important because patients who respond to therapy have a better survival and because the presence of renal disease has an impact on post-liver transplant outcomes [162].

Liver transplant

The first and only definitive therapy for HRS is liver transplantation because it is the only therapy that will provide long-term survival. With the implementation of the Model for End-stage Liver Disease (MELD) score in the allocation of organs in the United States, priority for transplant is given to patients with a high creatinine [163]. In a study aimed at assessing prognostic factors in HRS, MELD score and the type of HRS (type 1 or 2) were independent predictors of death. Whereas all patients with type 1 HRS had a high MELD score (> 20) and had a poor outcome (median survival 1 month), survival of patients with type 2 HRS was longer and depended on their MELD score [160]. This suggests that the development of HRS type 1 per se should indicate a high priority for transplant, independent of MELD.

Patients with HRS who are transplanted have more complications and a higher in-hospital mortality rate than those without HRS [164–167]. Therefore, HRS should be treated before transplantation in an attempt to improve renal function. In fact, the outcome of liver transplantation in patients with HRS treated with vasopressin analogues prior to transplant is similar to that of patients transplanted without HRS [168]. HRS alone should not be a reason for combined kidney and liver transplant; however, this is being considered in patients with HRS who become dialysis-dependent and show no recovery after 8 weeks of dialysis (the usual recovery time for acute renal failure) [162]. The need for dialysis should theoretically be prevented by the early treatment of HRS with the "bridging" therapies outlined below.

Vasoconstrictors plus albumin

The rationale behind this approach is to improve the deranged circulatory function by ameliorating vasodilation thereby improving effective arterial blood volume and decreasing renal vasoconstriction. Albumin, an intravascular volume expander, has been used to increase effective blood volume. However, albumin dialysis (see below) is associated with an increase in blood pressure attributable to the ability of albumin to bind vasodilators. It is therefore conceivable that an improvement of renal function in patients with HRS treated with vasoconstrictors and albumin is the result of the additive effects of both agents in producing peripheral vasoconstriction.

Data about the use of vasoconstrictors in HRS have been mostly derived from uncontrolled studies. The vasopressin analogue ornipressin was the first vasoconstrictor to be used, and although it was found to be effective, its use was abandoned because of a high rate of ischemic side effects [169,170]. Terlipressin, another vasopressin analogue, has been the most widely used vasoconstrictor agent in type 1 HRS and is associated with a low incidence of side effects [156,171–175]. The combination of midodrine plus octreotide has been effective in two small uncontrolled studies [176,177]. It is uncertain whether the effectiveness of this therapy is the result of midodrine alone (an α-adrenergic agonist) or the combination, because octreotide alone has been shown to have no effect in HRS when compared with placebo [178] or with a vasopressin infusion [179]. The combination of intravenous noradrenaline (another α-adrenergic agonist) plus albumin was effective and safe in a small uncontrolled study [180]; however, the need for medication infusion in an intensive care setting limits its applicability.

In most of these studies, albumin was associated with the vasoconstrictor, and one of them showed that treatment with terlipressin and albumin was associated with a significant decrease in the serum creatinine level and an increase in arterial pressure, changes that were not observed in a nonconcurrent group of patients treated with terlipressin alone [181].

In total, 11 uncontrolled studies have been published using vasoconstrictors and including 215 patients with HRS type 1. Reversal of HRS (decrease in creatinine below 1.5 mg/dL) was observed in 64% of the cases and, interestingly, HRS recurred in only a minority (23%) of these patients once therapy was discontinued. One-month survival was 39%, with a better survival in patients who responded to vasoconstrictors [156,179]. Terlipressin may induce ischemic side effects and arrhythmias requiring drug discontinuation.

A small, nonblinded, placebo-controlled study suggested that terlipressin (compared with placebo) was associated with an improvement in HRS (5/12 vs 0/12) and in survival (5/12 vs 0/12) [182]. However, a large multicenter, randomized, placebo-controlled double-blind trial of terlipressin vs placebo in the treatment of type 1 HRS showed that, although reversal of HRS was significantly better in patients in the terlipressin group (19/56, or 34%, in the terlipressin group vs 7/56, or 13%, in the placebo group), survival was identical in both treatment groups (27/56, or 48%, survival at 60 days for both groups) and, notably, HRS reversal rate (34%) was much lower than that demonstrated in uncontrolled studies [183]. The study also confirms that survival is significantly better in patients who responded to terlipressin.

Transjugular intrahepatic portosystemic shunt

Only three small prospective but uncontrolled studies have assessed the role of TIPS in HRS type 1, using 6, 14, and 5 patients (who had responded to octreotide/midodrine), respectively [177,184,185]. All studies showed a decrease in serum creatinine in most patients and a decrease in plasma renin activity. The rate of decrease in creatinine in most patients was slower than that obtained using terlipressin plus albumin. Recurrence of HRS was rare as long as the shunt remained patent but portosystemic encephalopathy was a frequent complication. Post-TIPS resolution of HRS type 1 appeared to improve survival. Long-term success was demonstrated in the study that explored sequential treatment with vasoconstrictors and albumin followed by TIPS [177]. Notably, a great majority of patients included in these three studies had alcoholic cirrhosis, many with active alcoholism, and therefore the improvement observed could have resulted from improvement in an acute-on-chronic process. Also, all three studies excluded patients with a CTP score equal to or greater than 12.

Given the paucity of data, TIPS could be used as a treatment of type 1 HRS only in the setting of prospective randomized trials.

Extracorporeal albumin dialysis

In a small, randomized study, the molecular adsorbent recirculating system, a modified dialysis method using an albumin-containing dialysate (eventually combined with intermittent venovenous hemofiltration), was shown to improve 30-day survival in eight patients with HRS type 1 compared with five patients treated with intermittent venovenous hemofiltration alone [186]. Because extracorporeal albumin dialysis (ECAD) incorporates a standard dialysis machine or a continuous venovenous hemofiltration monitor and glomerular filtration rate was not measured, the decrease in serum creatinine observed in most patients could be related to the dialysis process. However, clear beneficial effects on systemic hemodynamics and on hepatic encephalopathy were observed. ECAD is still considered an experimental therapy and its use in patients with type 1 HRS cannot be recommended outside prospective pathophysiological or therapeutic investigations.

Suspected hepatorenal syndrome

Patients with suspected type 1 HRS should be managed as inpatients for diagnostic investigation and treatment. Vital

signs, urine output, and blood chemistry should be closely monitored. Diuretics should continue to be withheld in these patients to prevent further decreases in effective arterial blood volume. Saline solution should not be administered as it will lead to significant increases in ascites and edema because sodium retention is severe in these patients. Because most patients have dilutional hyponatraemia, fluids (both oral and intravenous) should be restricted to approximately 1000 mL/day. Early identification of bacterial infections and treatment with broad-spectrum antibiotics is fundamental, because severe infections are common and contribute to death in these patients. The patient should be evaluated for liver transplantation and, if already on a transplant list, his or her status should be updated.

Specific treatment with vasoconstrictors plus albumin should be initiated as soon as the diagnosis is suspected. The best evidence supports the use of terlipressin, which should be started at a dose 0.5–1 mg i.v. every 4–6 h. If there is no early response (> 25% decrease in creatinine levels after 2 days), the dose can be doubled every 2 days up to a maximum of 12 mg/day (i.e., 2 mg i.v. every 4 h). Treatment can be stopped if serum creatinine does not decrease by at least 50% after 7 days at the highest dose, or if there is no reduction in creatinine after the first 3 days. In patients with early response, treatment should be extended until reversal of HRS (decrease in creatinine below 1.5 mg/dL) or for a maximum of 14 days [132].

A more rational method for adjusting the dose of vasoconstrictors is by monitoring mean arterial blood pressure (an indirect indicator of vasodilation). This method has been used for adjusting the dose of midodrine plus octreotide [176], an alternative to terlipressin in places such as the United States where terlipressin is not available. In this study, the doses of octreotide and midodrine were titrated to obtain an increase in mean arterial pressure of at least 15 mmHg; midodrine was administered orally at an initial dose of 7.5 mg t.i.d and, if necessary, increased to 12.5 mg t.i.d; octreotide was administered subcutaneously at an initial dose of 100 μg t.i.d and, if necessary, increased to 200 μg t.i.d [176]. In another study, different doses of midodrine (2.5 mg p.o., t.i.d) and octreotide (i.v. infusion of 25 μg/h after an initial bolus of 25 μg) were used [177].

Other alternatives to terlipressin are vasopressin or noradrenaline. Noradrenaline is used as a continuous intravenous infusion at an initial dose of 0.5 mg/h, adjusted to achieve an increase in mean arterial pressure of at least 10 mmHg or an increase in 4-h urine output to more than 200 mL. If these goals are not reached, the dose is increased every 4 h in steps of 0.5 mg/h, up to the maximum dose of 3 mg/h [180]. Vasopressin is also used as a continuous intravenous infusion, starting at a low dose of 0.01 U/min and titrating up to a dose of 0.5 U/min, depending on changes in mean arterial pressure and urine output and the presence of ischemic side effects. In the study that assessed vasopressin for HRS, the mean vasopressin dose in the group of responders was 0.23 ± 0.19 U/min [179].

Albumin should be administered with the vasoconstrictor. The maintenance dose, once the diagnosis of HRS is established and vasoconstrictors are initiated, is 20–40 g/day. Albumin may be discontinued if the serum albumin concentration exceeds 45 g/L and should be withdrawn in case of pulmonary edema. Because this complication is uncommon, catheterization to monitor central venous pressure is not mandatory, but careful physical and radiological monitoring of cardiopulmonary function is recommended [132].

In early responders, treatment with vasoconstrictors and albumin should be maintained until normalization of serum creatinine (< 1.5 mg/dL). Once reversal of HRS is obtained (creatinine < 1.5 mg/dL), the dose of vasoconstrictors can be titrated down until discontinuation.

Renal replacement therapy should be considered in patients who develop pulmonary edema, severe hyperkalemia, or metabolic acidosis not responding to medical therapy.

Prophylaxis of hepatorenal syndrome

Three randomized controlled studies suggest that HRS can be prevented in three specific clinical settings: SBP, ascites with low protein associated with severe liver and/or renal dysfunction, and alcoholic hepatitis.

In a non-placebo-controlled study in patients with SBP, the intravenous administration of albumin (1.5 g/kg at the diagnosis of the infection and 1 g/kg 48 h later) together with antibiotics decreased the risk of HRS by 66% compared with treatment using antibiotics alone (10% vs 33%) and improved hospital survival [144]. The beneficial effect of albumin is probably related to its capacity to improve effective arterial blood volume, as shown by a decrease in plasma renin activity, and is mainly observed in high-risk patients (i.e., those with a serum creatinine > 1.0 mg/dL or a serum bilirubin > 4 mg/dL) [145].

In a placebo-controlled study, the daily administration of norfloxacin (400 mg p.o.) in patients with low ascites protein (< 1.5 g/dL) and either renal or severe liver dysfunction was associated with a lower probability of developing HRS than placebo-treated patients [118].

In a double-blind, placebo-controlled study in patients with alcoholic hepatitis, the administration of pentoxifylline (400 mg t.i.d) was associated with significantly lower rates of HRS (8% vs 35%) and mortality (24% vs 46%) compared with patients randomized to placebo [187]. The beneficial effect of pentoxifylline could be related to its capacity to inhibit production of tumor necrosis factor and the resultant vasodilation; however, the study failed to show significant changes in plasma levels of tumor necrosis factor and did not report on hemodynamics.

In these three studies, the relation between prevention of HRS and improved survival is further proof that renal failure is an important determinant of death in patients with decompensated cirrhosis [3].

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G LIVER

91 Central nervous system and pulmonary complications of end-stage liver disease

Javier Vaquero, Andres T. Blei, Roger F. Butterworth

Hepatic encephalopathy, 2327
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Portopulmonary hypertension, 2342

End-stage liver disease and advanced portal hypertension result in blood from the splanchnic circulation accessing the systemic circulation without being adequately detoxified by the liver. In addition, the injured liver may be a source of abnormal mediators or fail to produce essential metabolites. The consequence of these alterations is the exposure of peripheral organs to an altered composition of blood. Hepatic encephalopathy, hepatopulmonary syndrome, and portopulmonary hypertension are the result of the exposure of brain and lungs to this altered milieu. Together with variceal bleeding, ascites, and hepatorenal syndrome they are the major manifestations of advanced liver disease and can cause significant deterioration of quality of life, morbidity, and mortality. Medical therapies often attenuate the symptoms or delay the progression of these complications, but liver transplantation is considered the only definitive cure.

Hepatic encephalopathy

Definition and classification

Hepatic encephalopathy (HE) is a serious neuropsychiatric complication of both acute and chronic liver failure, with a significant impact on quality of life. Results of a working group were published in 2002 and they include a definition of HE and a new system of classification (Table 91.1). HE is defined as a spectrum of neuropsychiatric abnormalities seen in patients with liver dysfunction after exclusion of other known brain diseases [1]. This multiaxial definition of HE defines both the type of hepatic dysfunction and the characteristics of the neurological manifestations. Three types of hepatic abnormalities are defined, namely:

Table 91.1 Classification of hepatic encephalopathy (HE)

Type **A**	Encephalopathy associated with **a**cute liver failure
Type **B**	Encephalopathy associated with portosystemic **b**ypass and no intrinsic hepatocellular disease
Type **C**	Encephalopathy associated with **c**irrhosis and portal hypertension or portosystemic shunts
	1 Episodic HE
	a Precipitated
	b Spontaneous
	c Recurrent
	2 Persistent HE
	a Mild
	b Severe
	c Treatment-dependent
	3 Minimal HE

- Type A: HE associated with **a**cute liver failure
- Type B: HE associated with portosystemic **b**ypass with no intrinsic hepatocellular disease
- Type C: HE associated with **c**irrhosis and portal hypertension or portosystemic shunts.

In the case of chronic liver disease, the terms *episodic HE* and *persistent HE* were coined and the term *minimal HE* was invoked to replace *subclinical encephalopathy*. It is anticipated that this new system of classification of HE will help to dispel the confusion frequently present in classical textbook definitions and to facilitate recruitment of patients into multicenter clinical trials.

Diagnosis

Neuropsychiatric symptoms characteristic of HE include shortened attention span, sleep abnormalities, and motor incoordination progressing through lethargy to stupor and

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coma. Psychiatric symptoms, particularly anxiety and depression, are not uncommon. Type B HE is, in practice, uncommon. In type C HE, symptoms take an undulating course progressing relatively slowly. In type A HE associated with acute liver failure, the neurological disorder may progress from altered mental status to coma within days, seizures may occur, and mortality rates are high. In these patients, death is frequently the result of brain herniation caused by brain edema and intracranial hypertension.

In patients with an episode or worsening of HE, it is essential to identify potential precipitating factors and to initiate corresponding treatment immediately. Common precipitating factors are gastrointestinal bleeding, hypovolemia, hyponatremia, hypokalemia, hypoxia, hypoglycaemia, infection, and the use of sedative drugs [2].

Diagnosis of HE in chronic liver failure is generally based on clinical and laboratory criteria following exclusion of other causes of neuropsychiatric dysfunction (Table 91.2). Differential diagnosis may require the use of computed tomography (CT) scans to eliminate other causes of encephalopathy such as traumatic brain injury, tumors, or edema.

Grading of HE in clinical practice is generally performed using the West Haven criteria (Table 91.3), which assess mental status on a scale of I to IV based on levels of consciousness, intellectual function, and personality changes [3]. Asterixis or flapping tremor (common in patients in stage II and III) refers to the presence of rapid involuntary flexion and extension movements of the wrist when the patient is told to maintain them actively in dorsiflexion with the fingers extended. Flapping tremor may also be observed during tongue protrusion or pedal dorsiflexion. Patients in stage IV of the West Haven classification may be better assessed using the Glasgow coma scale (Table 91.4).

Psychometric testing is commonly used to quantify the degree of impairment of neurological function, particularly in mild or minimal HE. Tests most commonly used include the Number Connection Test (NCT) and measurement of reaction times to visual or auditory stimuli. The Psychometric Hepatic Encephalopathy Score (PHES) consists of a series of psychometric tests including NCT-A, NCT-B, line tracing test, digit symbol test, and the serial dotting test. This battery of tests examines such entities as visual perception, construction, and visuospatial orientation in addition to motor speed, accuracy, attention, and memory function [4].

Electroencephalographic (EEG) changes in HE start with a bilateral synchronous decrease in wave frequency, increase in amplitude, and loss of normal α rhythm. Computer-assisted spectral EEG analysis facilitates measurement of mean dominant EEG frequency and decreases interoperator variability. Other more sophisticated tests used for grading of HE in chronic liver failure include the use of event-related evoked responses (typically the P300 peak following auditory stimuli) [5] and measurement of critical flicker frequency [6].

Table 91.2 Differential diagnosis of hepatic encephalopathy

Metabolic encephalopathies
Hypoglycemia
Electrolyte imbalance
Hypoxia
Carbon dioxide narcosis
Azotemia
Ketoacidosis
Toxic encephalopathies
Alcohol
Acute intoxication
Withdrawal syndrome
Wernicke–Korsakoff syndrome
Psychoactive drugs
Salicylates
Heavy metals
Intracranial lesions
Subarachnoid, subdural, or intracerebral hemorrhage
Cerebral infarction
Cerebral tumor
Cerebral abscess
Meningitis
Encephalitis
Epilepsy or postseizure encephalopathy
Neuropsychiatric disorders

Adapted from Riordan and Williams [238], with permission from the Massachusetts Medical Society.

Table 91.3 West Haven criteria for clinical grading of hepatic encephalopathy

Stage 0	No abnormality detected
Stage I	Trivial lack of awareness, euphoria, or anxiety Shortened attention span Impairment of addition or subtraction
Stage II	Lethargy Disorientation for time Obvious personality change Inappropriate behavior
Stage III	Somnolence to semistupor, but responsive to stimuli Confusion Gross disorientation Bizarre behavior
Stage IV	Coma Tests of mental state not possible

Neuropathology and neuroimaging

Neuropathology

The principal neuropathological finding in type C HE is altered astrocyte morphology. The term *Alzheimer type II astrocyte* is

Table 91.4 Glasgow coma scale

Criteria	Points awarded
Eyes open	
Spontaneous (eyes open does not imply awareness)	4
To speech (any speech, not necessarily a command)	3
To pain (should not use supraorbital pressure for pain stimulus)	2
Never	1
Best verbal response	
Oriented (to time, person, place)	5
Confused speech (disoriented)	4
Inappropriate (swearing, yelling)	3
Incomprehensible sounds (moaning, groaning)	2
None	1
Best motor response	
Obeys commands	6
Localizes pain (deliberate or purposeful movement)	5
Withdrawal (moves away from stimulus)	4
Abnormal flexion (decortication)	3
Extension (decerebration)	2
None (flaccidity)	1

used to describe the characteristic morphological features manifested by astrocytes in type C HE, which consist of large, pale (watery-looking) nuclei, margination of the chromatin, and prominent nucleoli. The nuclei take on a variety of shapes from round (in cerebral cortex) to irregular or lobulated forms (in basal ganglia), and in many cases occur in pairs or triplets suggestive of hyperplasia [7]. Intranuclear glycogen inclusions are also evident in these cells. The number of cells that manifest the Alzheimer type II phenotype is significantly correlated with the severity of encephalopathy [8,9].

Mixed glial-neuronal cultures exposed to sera from HE patients develop morphological changes characteristic of Alzheimer type II astrocytes [10]. Although rare, neuronal cell death and severe neuronal dysfunction have been documented in several clinical entities in chronic liver failure, which include acquired (non-Wilsonian) hepatocerebral degeneration, postshunt myelopathy, cerebellar degeneration, and extrapyramidal disorders (parkinsonism) [11].

Neuroimaging

Bilateral signal hyperintensities have been described in globus pallidus on T1-weighted magnetic resonance imaging (MRI) (see Fig. 91.2a; see also "Manganese" below), and T2-weighed Fast-FLAIR MRI reveals white matter lucencies along the corticospinal tract in cirrhotic patients with overt HE [12]. Both of these MRI alterations resolve following liver transplantation.

Studies using positron emission tomography (PET) and $^{13}NH_3$ have been used to investigate brain ammonia metabolism in cirrhotic patients. In one study, patients with mild HE presented a significant increase in the cerebral metabolic rate for ammonia, accompanied by an increase of the blood–brain barrier permeability to ammonia, which suggested an increased ease of ammonia for entering the brain in patients with HE [13]. This could explain the hypersensitivity of cirrhotic patients to ammoniagenic conditions such as protein loading, gastrointestinal bleeding, and constipation, and the occurrence of HE in patients with near normal arterial ammonia levels. Results of a subsequent $^{13}NH_3$ PET study, however, suggested that increased cerebral trapping of ammonia in cirrhotic patients with HE was primarily the result of increased circulating ammonia rather than altered blood–brain ammonia kinetics [14].

Pathophysiology

Bloodborne toxins

Ammonia

There is evidence for a pathogenetic link between hyperammonemia and the phenomenon of Alzheimer type II astrocytosis, because the latter phenotype has been described in hyperammonemic syndromes associated with congenital urea cycle enzyme defects, in experimental animals with urease-induced hyperammonemia, and in primary cultures of rat cortical astrocytes exposed to ammonia [9].

A network of organs is involved in ammonia homeostasis (Fig. 91.1) and there is a convincing body of evidence to suggest that hyperammonemia in liver failure results from altered interorgan trafficking of ammonia [15]. In chronic liver failure, hyperammonemia results primarily from reduced hepatic

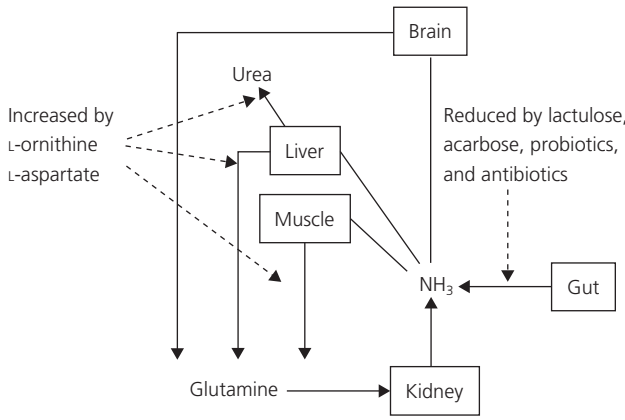


Figure 91.1 Interorgan trafficking of ammonia. Ammonia (NH_3) produced by the gut and kidney is, under normal physiological conditions, removed primarily by the liver (as urea and glutamine) and to a lesser extent by muscle and brain (as glutamine). In liver failure, liver urea and glutamine production is severely impaired, and muscle (not brain) becomes the organ principally responsible for removal of circulating ammonia. Ammonia production in the gut is lowered by treatment with lactulose, acarbose, probiotics, and antibiotics. Hepatic ammonia removal as urea and glutamine together with muscle ammonia removal (as glutamine) are stimulated with L-ornithine L-aspartate.

urea synthesis rather than increased intestinal ammonia production [16]. Urea is synthesized in the liver from ammonia by the urea cycle in periportal hepatocytes, whereas perivenous hepatocytes also transform ammonia into glutamine by glutamine synthetase. Cirrhotic patients have intra- and extrahepatic portosystemic shunts that account for a large portion of portal blood flow. This, in addition to the loss of hepatocytes and impaired residual hepatocyte function, results in decreased ammonia removal by the liver. Increased renal ammonia synthesis reported in liver failure is offset by increased ammonia excretion into the urine [16].

In contrast to the liver, skeletal muscle and brain are devoid of an effective urea cycle and rely only on glutamine synthesis for ammonia removal. In liver failure, muscle becomes the major route for ammonia detoxification [17], because chronic hyperammonemia results in increased glutamine synthetase (GS) activity because of a posttranslational increase of GS [18]. In contrast to skeletal muscle, brain does not adapt its ammonia removal capacity by induction of GS. On the contrary, GS activities are consistently decreased in brains of animals with experimental chronic liver failure [18] and in autopsied brain tissue from cirrhotic patients who died in hepatic coma [19]. This decreased GS activity in brain may be explained by increased GS protein tyrosine nitration, as demonstrated in both in vitro and in vivo models of hyperammonemia and liver failure [20]. Moreover, NMR spectroscopic studies in liver-impaired animals [21] have been unable to demonstrate significant increases of de novo glutamine synthesis in the brain, suggesting that the increased brain glutamine reported in liver failure results primarily from decreased glutamine release from astrocytes or decreased glutamine degradation by glutaminase.

Manganese

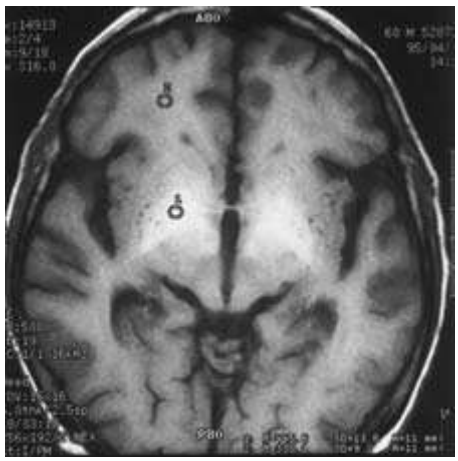
Evidence suggests that manganese deposition is the cause of the bilateral T1-weighed hyperintensities of basal ganglia observed in the MRI of cirrhotic patients (Fig. 91.2). Manganese, which is normally eliminated by the hepatobiliary route, is increased in the blood of cirrhotic patients, who manifest pallidal signal hyperintensities on MRI. Similar MRI pallidal signals probably caused by manganese deposition have been reported in other cholestatic conditions such as Alagille syndrome [22], intrahepatic bile duct paucity, and in patients during total parenteral nutrition [23]. Direct measurements using neutron activation analysis reveal up to sevenfold increases in manganese content of dissected globus pallidus obtained postmortem from cirrhotic patients who died in hepatic coma (Fig. 91.2b) [24]. Experimental animals with surgical portacaval shunts also manifest selective accumulation of manganese in pallidus globus and in other basal ganglia structures [25].

Cerebral blood flow and energy metabolism

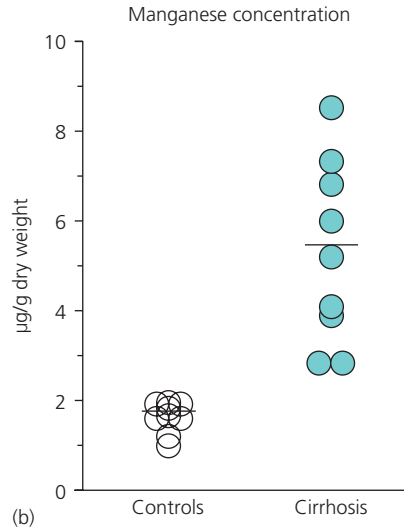
Alterations of brain glucose utilization, of cerebral blood flow, and of brain glucose metabolic pathways have consistently been reported in HE resulting from chronic liver failure. PET studies using ^{18}F-deoxyglucose revealed significant decreases in glucose utilization localized to the anterior cingulate cortex in cirrhotic patients with mild HE [26], and the extent of such decreases correlated with the reduced performance in attention-demanding tasks such as NCT and digit symbol psychometric tests. Anterior cingulate cortex is known to be implicated in the control of the anterior attention system responsible for monitoring the selection of responses to visual stimuli.

Measurement of cerebral blood flow using ^{15}[O]H_2O-PET reveals a redistribution of flow from cortical regions such as the anterior cingulate to subcortical structures such as the thalamus [27].

Precipitation of severe encephalopathy and coma in experimental animals with chronic liver failure following the administration of ammonium salts results in severe alterations of



(a)



(b)

Figure 91.2 **(a)** Typical bilateral signal hyperintensities in globus pallidus on T1-weighted magnetic resonance imaging of a 47-year-old cirrhotic patient with minimal hepatic encephalopathy. Signal hyperintensities resolved following liver transplantation. **(b)** Increased pallidal manganese concentrations in autopsied brain tissue from cirrhotic patients who died in hepatic coma. Data from Pomier-Layrargues et al. [24].

brain glucose metabolism leading to accumulation of lactate [28]. Increased brain lactate concentrations are consistent with impaired oxidation of pyruvate in brain in liver failure. Increased brain and cerebrospinal fluid lactate concentrations have consistently been reported in HE patients [29,30].

Neurotransmitters

Neurotransmission defects in HE have been ascribed to direct neurotoxic effects of the ammonium ion (NH_4^+) on inhibitory (GABA[γ-aminobutyric acid]ergic) and excitatory (glutamatergic) neurotransmission, to the accumulation of neuroactive metabolites of tryptophan, to altered glutamatergic synaptic regulation, and to activation of "peripheral-type" benzodiazepine receptors resulting in the synthesis of neuroactive steroids with potent GABA agonist properties.

Glutamate

In a report by Schmidt et al. [31], the exposure to blood obtained from cirrhotic patients with varying degrees of HE resulted in a dose-dependent inhibition of the uptake of D-aspartate (a nonmetabolizable glutamate analogue) in rat hippocampal slices, and the relative potency of the inhibition correlated with blood ammonia concentrations. Other studies demonstrate ammonia-induced inhibition of high-affinity glutamate uptake into rat synaptosomal preparations [32] and cultured rat astrocytes [33]. Alterations of several subclasses of glutamate binding sites expressed by both neurons and astrocytes have been reported in experimental models of chronic liver failure [34,35].

Serotonin (5-hydroxytryptamine, 5-HT)

Tryptophan is increased in the cerebrospinal fluid (CSF) of patients with hepatic coma [36], and its blood–brain barrier transport is enhanced in dogs with portacaval anastomosis [37]. Because tryptophan hydroxylation (the rate-limiting step in brain 5-HT synthesis) is not saturated at normal blood and brain tryptophan concentrations, increased availability of tryptophan has the potential to result in increased 5-HT synthesis in brain. Supporting an increased synthesis and turnover of 5-HT in the brain in HE, the concentration of 5-hydroxyindoleacetic acid (a metabolite of 5-HT) is increased in the CSF of cirrhotic patients in hepatic coma [36], and in autopsied brain tissue from both HE patients [38] and portacaval-shunted rats [39]. Measurement using an in vivo decarboxylase inhibition assay also revealed increased 5-HT turnover [40] in the brains of portacaval-shunted animals. Increased brain 5-HT turnover correlates with the degrees of portosystemic shunting and hyperammonemia in rats with variable portal vein stenosis [41], an animal model of minimal HE. Other evidence for altered 5-HT metabolism and function in human HE include the report of increased activity and mRNA of monoamine oxidase A (MAO-A; the enzyme responsible for 5-HT breakdown in human brain) [42] and of increased concentration of 5-hydroxyindoleacetic acid in brain tissue from patients who died in hepatic coma [38]. Increased densities of postsynaptic 5-HT_2 receptors were also described in this material [43]. Together, these findings suggest a serotonin synaptic deficit in HE. 5-HT_2 plays a key role in the regulation of sleep, and altered serotoninergic neurotransmission is present in a range of psychiatric disorders in humans. Changes in cerebral 5-HT_2 turnover in HE, therefore, could underlie some early signs and symptoms, such as altered sleep patterns and depression.

γ-Aminobutyric acid

Throughout the 1980s a great deal of attention was focused on the theory that HE was the result of alterations of the GABA neurotransmitter system in brain. Abnormalities of the brain GABA system were initially reported in animal models of acute liver failure [44]. In animal models of chronic liver failure, however, no alterations of this system have been reported, whether reflected by GABA content, its related enzymes, or receptor sites [45–48]. Autopsied brain tissue from cirrhotic patients who died in hepatic coma also contains normal activities of GABA-related enzymes and unchanged densities and affinities of GABA binding sites [19,49,50].

In the central nervous system, benzodiazepine binding sites form part of the GABA-A receptor complex. Stimulation of the benzodiazepine binding site "facilitates" the action of GABA on the functionally linked GABA-A site of the complex, and results in increased chloride channel opening leading to hyperpolarization and inhibition. Administration of the benzodiazepine antagonist flumazenil has been noted to ameliorate the neurological status in cirrhotic patients with HE [51,52]. It was suggested that the beneficial action of flumazenil in HE was the result either of inhibition of increased densities of benzodiazepine binding sites or of inhibition of the action of an "endogenous" ligand at these sites. Subsequent investigations revealed no alterations of densities or affinities of binding sites in either experimental or human HE [50], leaving open the possibility that the beneficial effects of flumazenil were the result of the blockage of "endogenous" benzodiazepines [53]. Initial reports demonstrated that CSF and sera from patients with advanced HE contained significant amounts of "benzodiazepine-like" substances, among which were isolated two known benzodiazepines that are positive allosteric modulators of GABA neurotransmission (diazepam and its *NN*-desmethyl metabolite) [54]. Unfortunately, the interest generated by these reports was tempered by the fact that these benzodiazepines were in most cases pharmaceutical in origin. Moreover, the concentrations of benzodiazepines reported in blood, CSF, and brain extracts of HE patients were well below levels associated with their sedative actions.

An alternative theory to explain increased GABAergic neurotransmission in HE has emerged. Two distinct types of benzodiazepine receptors are expressed in brain. The first type, mentioned in the previous paragraph, forms part of the GABA–benzodiazepine receptor complex and is situated on

the postsynaptic neuronal membrane. The second type is the "peripheral-type" benzodiazepine receptor (PTBR), so-called because of its initial discovery in peripheral tissues but subsequently identified on the outer mitochondrial membrane of astrocytes. Increased densities of PTBRs have been reported in autopsied brain tissue from cirrhotic patients who died in hepatic coma [55], and increased PTBR binding sites and mRNA have been reported in the brain of portacaval-shunted rats [56,57]. Endogenous ligands for the PTBR include the neuropeptide diazepam binding inhibitor and its processing peptide octadecaneuropeptide (ODN) [58]. Portacaval anastomosis in the rat results in increased ODN-immunolabeling of astrocytes and other nonneuronal elements in several brain regions [59]. Exposure to PTBR ligands results in proliferation and swelling of mitochondria in cultured glioma cells [60] and, because mitochondrial proliferation and swelling are observed in astrocytes in liver failure, PTBR activation could play a role in the pathogenesis of this astrocytic response.

Activation of PTBRs in brain in liver failure results in increased synthesis of a novel class of compounds known as neurosteroids, many of which (e.g., allopregnanolone) are potent GABA-A receptor agonists and consequently potent inhibitory agents. Allopregnanolone concentrations are increased up to sevenfold in autopsied brain tissue from cirrhotic patients who died in hepatic coma [61].

Histamine

Histamine is involved in a wide range of physiological functions including sleep, arousal, and circadian rhythmicity. In rats, portosystemic shunting results in increased hypothalamic concentrations of both histamine and its principal metabolite, tele-methylhistamine, and in increased hypothalamic release of histamine [62], suggesting increased histaminergic activity. Increased brain histamine could result from increased brain availability of the precursor amino acid L-histidine. Postsynaptic histamine H_1 receptors are implicated in the entrainment of circadian rhythms to the light–dark cycle in mammals [63], and their activation suppresses deep slow-wave sleep [64]. Upregulation of histamine H_1 receptors has been reported in cerebral cortex of patients with HE and of portacaval-shunted rats. Therefore, it is plausible that increased histaminergic activity through H_1 receptors contributes to the disorganization of the normal sleep cycle and decreased total duration of slow-wave sleep that are characteristically observed in cirrhotic patients with HE [65]. Similar changes in sleep patterns and diurnal rhythms have been reported in portacaval-shunted rats [66]. Histamine H_1 receptor blockade, known to affect sleep quality in humans, significantly improved circadian rhythmicity in these animals [66].

Dopamine

Studies in autopsied brain tissue from cirrhotic patients who died in hepatic coma reveal several-fold increases in the dopamine metabolite homovanillic acid [38]. Similar findings, consistent with increased dopamine metabolism or turnover, were reported in the brains of rats following portacaval anastomosis [67]. A possible explanation for these findings could relate to the increased activities of the monoamine metabolizing enzyme MAO-A [42]. Densities of postsynaptic dopamine (D_2) receptors are significantly reduced in brain tissue obtained at autopsy of cirrhotic patients who died in hepatic coma [68], and in living patients with mild HE studied by PET using [^{11}C]methylspiperone [69].

Opioids

Cirrhotic patients are particularly sensitive to morphine, and portacaval shunting is known to increase pain sensitivity, which involves the endogenous opioid neurotransmitter system. Increased circulating levels of endogenous opioid [Met]enkephalin have been reported in patients with primary biliary cirrhosis [70], and brain levels of β-endorphin are increased in experimental chronic liver failure [71]. Portacaval anastomosis in the rat results in region-selective increases of μ and δ opioid receptor sites in brain [72], a finding that has been linked to increased ethanol consumption in these animals [73].

Oxidative/nitrosative stress

Portacaval anastomosis in the rat results in increased expression of neuronal nitric oxide synthase (nNOS) in brain [74]. NOS activities are also increased by ammonia-induced stimulation of L-arginine uptake into neuronal preparations both in vitro and in vivo. Exposure of cultured astrocytes to millimolar concentrations of ammonia results in oxidative stress and protein tyrosine nitration [20], a process dependent on increases in the inducible isoform of NOS (iNOS). Astrocytic protein tyrosine nitration was also described in the brains of rats following portacaval anastomosis [20], confirming the presence of nitrosative stress under these conditions.

Inflammation

It is becoming increasingly evident that ammonia and manganese neurotoxicity are not the only pathophysiological processes with the potential to affect adversely cerebral function in liver failure. In particular, there is evidence that infection and inflammation also play a significant role.

The severity of HE in acute liver failure is significantly influenced by the so-called systemic inflammatory response syndrome (SIRS) [75], which is a response to the action of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) or the interleukins IL-1β and IL-6. Infection and inflammation are also common in cirrhotic patients, in whom increased circulating levels of TNF-α and IL-6 have been described [76]. Shawcross et al. studied 10 patients with cirrhosis who had evidence of infection and SIRS but no clinically evident HE [77]. In these patients, exogenously induced hyperammonemia was associated with significant

deterioration in neuropsychological testing during the inflammatory state but not after its resolution, indicating that SIRS may modulate the effects of ammonia on brain function.

Therapy

Therapy for HE continues to be a challenge. The reasons for this include a lack of standardization of assessment of mental status, the hesitation by some ethics boards to sanction placebo controlled trials, confounding issues such as the presence of precipitating factors (infection, hyponatremia, unidentified neuroactive drugs, and others), and difficulties related to the effective blinding of patients and clinical investigators to the treatment regimen. A schematic approach to the management of HE is shown in Fig. 91.3.

Nutritional support

Severe restriction of dietary protein is no longer recommended as a means of preventing HE in cirrhotic patients [78]. Long-term nitrogen restriction is potentially harmful, and a positive nitrogen balance is essential to promote liver

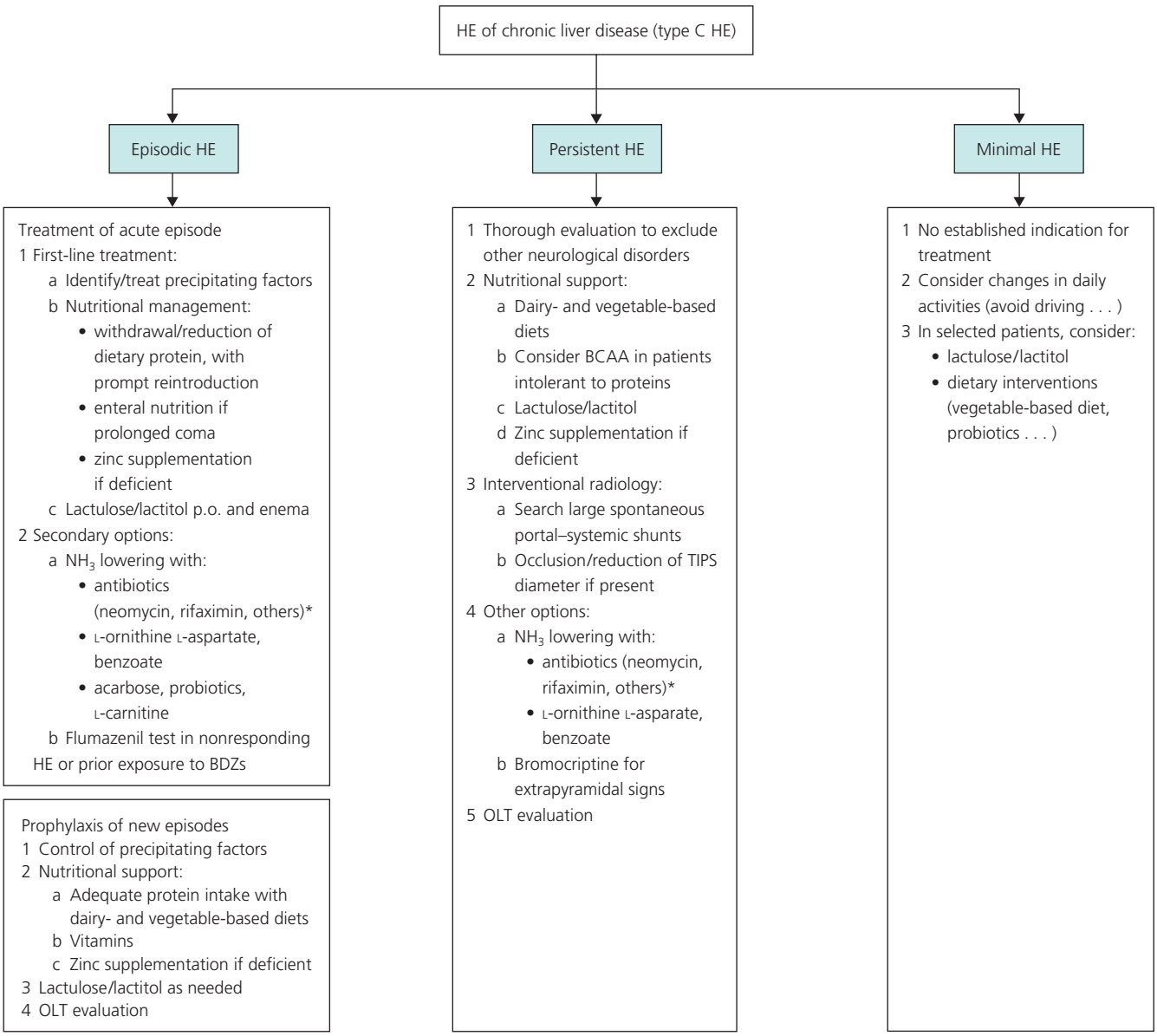


Figure 91.3 General management of hepatic encephalopathy (HE) of chronic liver disease (type C HE). No therapy has conclusively been shown to improve HE compared with placebo in randomized clinical trials. A majority of patients with HE, however, are effectively managed by following the present orientative scheme, based on published guidelines [2,238]. The management of the recurrent type of episodic HE would be similar to that of persistent HE. *Neomycin for chronic use requires periodic monitoring to avoid toxicity (renal, otological). BCAA, branched-chain amino acids; BDZ, benzodiazepine; OLT, orthotopic liver transplant; p.o., orally; TIPS, transjugular intraheptic portosystemic shunt.

regeneration and to increase the capacity for ammonia removal by skeletal muscle. Protein intake in the 1–2 g/kg/day range is generally recommended to maintain an adequate nitrogen balance [2]. Oral supplements of branched-chain amino acids have been suggested to offer significant improvement in severely protein-intolerant patients with chronic HE [79].

Ammonia-lowering strategies (Fig. 91.1)

Nonabsorbable disaccharides, such as lactulose and lactitol, are routinely used to decrease ammonia production in the gut despite a paucity of adequate controlled clinical trials. In the case of lactulose, the ammonia-lowering effect involves increased fecal nitrogen excretion by facilitation of the incorporation of ammonia into bacteria as well as a cathartic effect. In the colon, lactulose and lactitol are catabolized, respectively, to lactic and acetic acids, leading to a lowering of colonic pH to around 5.0. This reduction in pH favors the formation of the less absorbable NH_4^+ from NH_3 thus effectively trapping ammonia in the colon. The dose of lactulose (45–90 g/day) is titrated to achieve 2–3 soft stools per day. Major side effects include abdominal cramps, flatulence, and diarrhea. Antibiotics such as neomycin are also traditionally used to lower blood ammonia by inhibiting bacterial ammonia production. However, neomycin therapy is associated with significant side effects and is increasingly being replaced by alternative antibiotics such as rifaximin.

Significant lowering of blood ammonia and concomitant improvement in neuropsychiatric status in cirrhotic patients with grade I–II HE has been demonstrated following administration of the novel hypoglycemic agent acarbose [80]. One suggested mechanism of action of acarbose involves inhibition of proteolytic flora responsible for gut ammonia production. Along these same lines, there is renewed interest (after a 40-year hiatus) in the area of lowering gut ammonia production by oral supplementation of probiotics [81]. The objective of probiotics is to increase the intestinal proportion of urease-negative bacteria thereby decreasing gut ammonia production. Lactic acid-producing probiotics have the advantage of reducing gut ammonia absorption. Synbiotic preparations consist of both lactic acid-producing probiotics and fermentable fiber. Controlled clinical trials with these agents reveal significant improvement of neurological status and lowering of venous ammonia [81].

An alternative means of lowering blood ammonia in chronic liver failure involves the stimulation of ammonia fixation. Ammonia is normally removed by urea formation (periportal hepatocytes) and by glutamine formation (perivenous hepatocytes, muscle and brain) (Fig. 91.1). Several strategies are aimed at stimulating residual urea or glutamine synthesis. One of the most successful, L-ornithine L-aspartate, has been shown to lower blood ammonia and concomitantly improve neuropsychiatric status in cirrhotic patients in controlled clinical trials in Europe and Asia [82]. Studies in experimental chronic liver failure reveal that this beneficial effect is primarily due to the stimulation of urea and glutamine formation in the liver and in the stimulation of glutamine formation by skeletal muscle [83]. In experimental acute liver failure, the ammonia-lowering effect of L-ornithine L-aspartate appears to be due predominantly to the stimulation of muscle glutamine synthetase. Successful lowering of blood ammonia concentrations in cirrhotic patients has also been accomplished using sodium benzoate, an agent that combines with glycine to form hippurate. A controlled clinical trial with 5 g sodium benzoate twice daily in patients with acute HE found it to be as effective as lactulose but 25 times less expensive [84].

Studies in both experimental animal models of HE [85] and in cirrhotic patients [86] demonstrate that L-carnitine is effective in lowering blood ammonia and improving neurological status. The mechanism of action probably involves improvements in mitochondrial metabolism.

Neuropharmacology

As the precise pathophysiological mechanisms and alterations in neurotransmitter systems responsible for the pathogenesis of HE become more clearly defined, novel pharmacological approaches are starting to emerge.

Agents acting on the GABA-receptor complex

A number of controlled clinical trials have evaluated the efficacy of the benzodiazepine receptor antagonist flumazenil in HE patients [2]. In a subset of HE patients, improvements following flumazenil are spectacular [51]. However, enthusiasm for this approach has been tempered by the possible confounding effects of prior exposure to pharmaceutical benzodiazepines (used as sedatives or as part of an endoscopic workup) and by the poor correlation between the clinical response and blood levels of benzodiazepines in these patients. Adding to these difficulties is the short half-life and lack of an oral formulation for flumazenil.

Benzodiazepine partial inverse agonists display weak negative intrinsic activity and act as mild GABA-A receptor antagonists [87]. One such agent, Ro15-4513, ameliorates the symptoms of HE in rats with thioacetamide-induced [88] and ischemic [89] acute liver failure (ALF), and similar results were obtained with a second benzodiazepine partial inverse agonist, sarmazenil. A major mechanism responsible for the beneficial effect of these agents appears to involve the attenuation of the effects of neurosteroids such as allopregnanolone at the GABA-A receptor complex [90]. Surprisingly, no studies have so far been undertaken to test the efficacy of benzodiazepine partial inverse agonists in patients with acute or chronic liver failure.

Dopamine receptor agonists

Both L-DOPA (the amino acid precursor of dopamine) and the dopamine receptor agonist bromocriptine have been

used in clinical trials in patients with portosystemic encephalopathy [2]. Although results were not encouraging in terms of overall cognitive improvement, it is possible that these agents have a beneficial effect on motor performance.

Histamine H_1 receptor antagonists

Following up on the demonstration of a beneficial effect of histamine H_1 antagonists on neurological function in an experimental animal model of HE [66], results of a controlled clinical trial revealed improvements in sleep quality in cirrhotic patients with minimal HE following administration of the H_1 blocker hydroxyzine [91].

Liver assist devices

In chronic liver disease, the role of liver assist devices to treat HE is likely to be relevant only to a small proportion of patients, because of the complexity, associated adverse effects, and discontinuous use of these devices, in addition to the frequent resolution of HE episodes with standard medical therapy. These devices have been used mainly in patients who had advanced chronic liver disease with superimposed acute injury (so-called acute-on-chronic liver failure) [92]. Dialysis against albumin, which allows the removal of water-soluble and non-water-soluble toxins bound to albumin in the blood, has received most attention. In one study of patients with acute-on-chronic liver failure, treatment with albumin dialysis was associated with an improvement of HE, which was not observed in those treated only with standard medical therapy [93]. In a preliminary report of 70 patients with advanced cirrhosis and severe HE, those randomized to albumin dialysis presented significant improvements of HE compared with those receiving standard medical therapy alone [94]. The use of liver assist devices for treating HE, however, is still experimental.

Liver transplantation

The appearance of HE in a patient with cirrhosis is a poor prognostic factor and should prompt evaluation of the patient for liver transplantation [95]. Elective liver transplantation should not be performed during an acute episode of HE; it is preferable to wait until the episode and its precipitating factors are corrected.

Liver transplantation corrects the majority of alterations related to HE, including neuropsychiatric symptoms and altered neuropsychometric tests [96,97], alterations of electroencephalogram [96], alterations of regional cerebral blood flow [98] and regional brain glucose metabolism [99], cerebral osmolyte disturbances [100], basal ganglia hyperintensities [100], and the decrease of the magnetization transfer ratio in the brain [100]. The posttransplant normalization of the cerebral osmolyte disturbances correlates well with the correction of the neuropsychological alterations [100].

Despite the reversibility of HE, neurological complications are common (~ 30%) following liver transplantation, especially within the first weeks [101,102]. In one study, the presence of HE pretransplant was an independent risk factor for developing these complications [101], which include central pontine myelinolysis, cerebrovascular disease, anoxia, seizures, and infections [101,102]. Neurotoxicity from immunosuppressive medications is also common in the early postoperative period [103], but it usually responds to the reduction or discontinuation of the drug. Liver transplantation candidates may also have neurological impairment related to the etiology of liver disease, such as frontal lobe and cerebellar atrophy in alcohol consumption [104], or cognitive and ^{1}H-NMR spectroscopic alterations associated with hepatitis C virus infection [105]. The posttransplant evolution of these preexisting conditions has not been completely characterized.

Hepatopulmonary syndrome

The association between liver disease and pulmonary dysfunction has been known for more than 100 years, since Fluckiger described in 1884 the case of a woman with chronic liver disease presenting cyanosis and digital clubbing [106]. Subsequently, similar cases were reported, and a wide range of structural alterations in the pulmonary vascular tree of patients with liver disease were described [107]. The term *hepatopulmonary syndrome* (HPS) was coined in 1977 by Kennedy and Knudson as an analogy to the *hepatorenal syndrome*, recognizing the vascular nature of the altered gas exchange in these patients [108].

Today, HPS distinguishes a subgroup of patients with liver disease or portal hypertension who present alterations of arterial oxygenation caused by the development of intrapulmonary vascular dilations (IPVDs). The term IPVD reflects the variety of pulmonary vascular alterations leading to altered gas exchange in HPS, from diffuse peripheral dilations of pulmonary capillaries to true anatomical arteriovenous shunts.

Definition

HPS is defined by the three following criteria, all of which must be present to establish the diagnosis [109,110]:

- presence of chronic liver disease or portal hypertension
- alteration of arterial oxygenation, usually defined as a widened age-corrected alveolar–arterial oxygen gradient on room air with or without arterial hypoxemia
- evidence of IPVDs.

In early definitions, the presence of other cardiopulmonary causes of hypoxemia was incompatible with the diagnosis of HPS. However, it is generally accepted that HPS can be diagnosed in patients with coexisting intrinsic cardiopulmonary disease [110]. Increased experience and the ability to detect specifically the presence and degree of IPVDs has made it possible to distinguish HPS in such a setting.

Epidemiology

Most patients with HPS have underlying liver cirrhosis with variable degrees of portal hypertension. A prevalence of HPS of approximately 10%–20% has been reported in cirrhotic patients evaluated for liver transplantation [111–115] and in less selective populations [116–120]. HPS is not associated with any specific etiology of liver disease, and there is no clear relation between HPS and the degree of hepatic dysfunction [110]. Therefore, HPS should be suspected independently of the stage of liver disease.

HPS has also been diagnosed in noncirrhotic portal hypertension (extrahepatic portal vein thrombosis, Budd–Chiari syndrome, and others) and in liver diseases where portal hypertension is not a significant feature, such as in acute and chronic viral hepatitis without cirrhosis [110]. In a series of critically ill patients with diverse cardiopulmonary diseases, criteria of HPS were fulfilled by almost 50% of those who developed hypoxic hepatitis compared with 0% of patients without liver disease [121]. Structural pulmonary vascular changes characteristic of HPS have also been described in patients with fulminant hepatic failure [122]. These reports underscore that both portal hypertension and liver dysfunction (acute or chronic) are important factors in the pathogenesis of HPS.

Natural history

The natural history of HPS has not been completely elucidated. Approximately 10%–20% of cirrhotic patients present evidence of IPVDs without alterations of arterial oxygenation [112,116–118], not fulfilling the diagnostic criteria of HPS. This situation may represent an initial phase of HPS, but its evolution is unknown.

Once established, the diagnosis of HPS has important implications for the patient with liver disease. Although spontaneous improvement of HPS has been reported [123], progressive deterioration of arterial oxygenation occurs in most cases even in the setting of stable liver disease [124,125]. In patients with HPS awaiting liver transplantation, a mean P_aO_2 decline of 5.2 mmHg per year has been estimated [125]. Diagnosis of HPS is also associated with increased mortality [115,125]. In a prospective study of 111 patients with cirrhosis, the median survival time was 10.6 months in those with HPS compared with 40.8 months in those without HPS ($P < 0.05$), with the survival disadvantage for HPS being independent of the severity of liver disease [115]. In patients who do not undergo liver transplantation, the 5-year survival is significantly diminished in those who have HPS (20% vs 32%–63% in those without HPS) [115,125]. The severity of HPS also influences prognosis, with a $P_aO_2 < 60$ mmHg being correlated with worse survival [115,125]. The causes of mortality, including respiratory failure, are similar in patients with and without HPS and are mainly liver- or portal hypertension-related events [115,125].

Pathophysiology

Pathology

Alterations in the pulmonary vascular tree, generally within a normal lung parenchyma, are the distinctive pathological feature of HPS. In 1956, Hoffbauer and Rydell first described numerous abnormal vascular channels connecting the pulmonary arteries and veins in a patient with cirrhosis and cyanosis [126]. In subsequent years, a range of pulmonary vascular abnormalities were described in necropsy studies of patients with hypoxemia and liver disease. A diffuse dilation of pulmonary vessels mainly at the precapillary and capillary levels was the most conspicuous finding [127–129]. Other common alterations included pleural and subpleural spider nevi [127,129], thickening of walls of pulmonary capillaries and venules by collagen [128], discrete or multiple anatomical pulmonary arteriovenous communications [127,130], and communications between pulmonary veins with portal [131] or systemic veins [132]. All these alterations allow venous or mixed venous blood to pass directly to the pulmonary veins without being fully oxygenated.

Mechanisms of altered arterial oxygenation

Early studies suggested that the cause of hypoxemia in patients with cirrhosis was a decreased affinity of hemoglobin for oxygen, but subsequent research has shown that it was not a sufficient explanation and pointed instead to pulmonary causes of hypoxemia [133]. Among these, hypoventilation can be dismissed because hyperventilation is commonly found in HPS. The other theoretical pulmonary causes of hypoxemia, namely ventilation/perfusion mismatch, diffusion impairment, and vascular shunt, may all play a role in HPS (Fig. 91.4) [133]. Determination of which mechanism is more relevant in individual patients may have therapeutic implications.

Ventilation/perfusion mismatch is the most common cause of hypoxemia in clinical medicine. In normal conditions, the relation between ventilation and perfusion is maintained constant in each lung unit by the modulation of tone in pulmonary arterioles. Thus, arterioles that perfuse poorly ventilated alveoli undergo vasoconstriction and divert blood to better ventilated areas where it can be adequately oxygenated. The presence of abnormally elevated perfusion due to IPVDs results in areas with a low ventilation/perfusion ratio, or "physiological" shunts, which is considered the predominant mechanism of hypoxemia in mild-to-moderate HPS [134]. Characteristically, hypoxemia due to alterations of ventilation/perfusion mismatch has an excellent response ($P_aO_2 > 500$ mmHg) to breathing 100% oxygen (Fig. 91.4). In addition, an impaired pulmonary vasoconstrictive response to hypoxia is present in many patients with cirrhosis [135,136], and it has been associated with more advanced hypoxemia, pulmonary and systemic vasodilation, and ventilation/perfusion mismatch [135]. The extent to which a blunted hypoxic pulmonary vasoconstriction contributes to

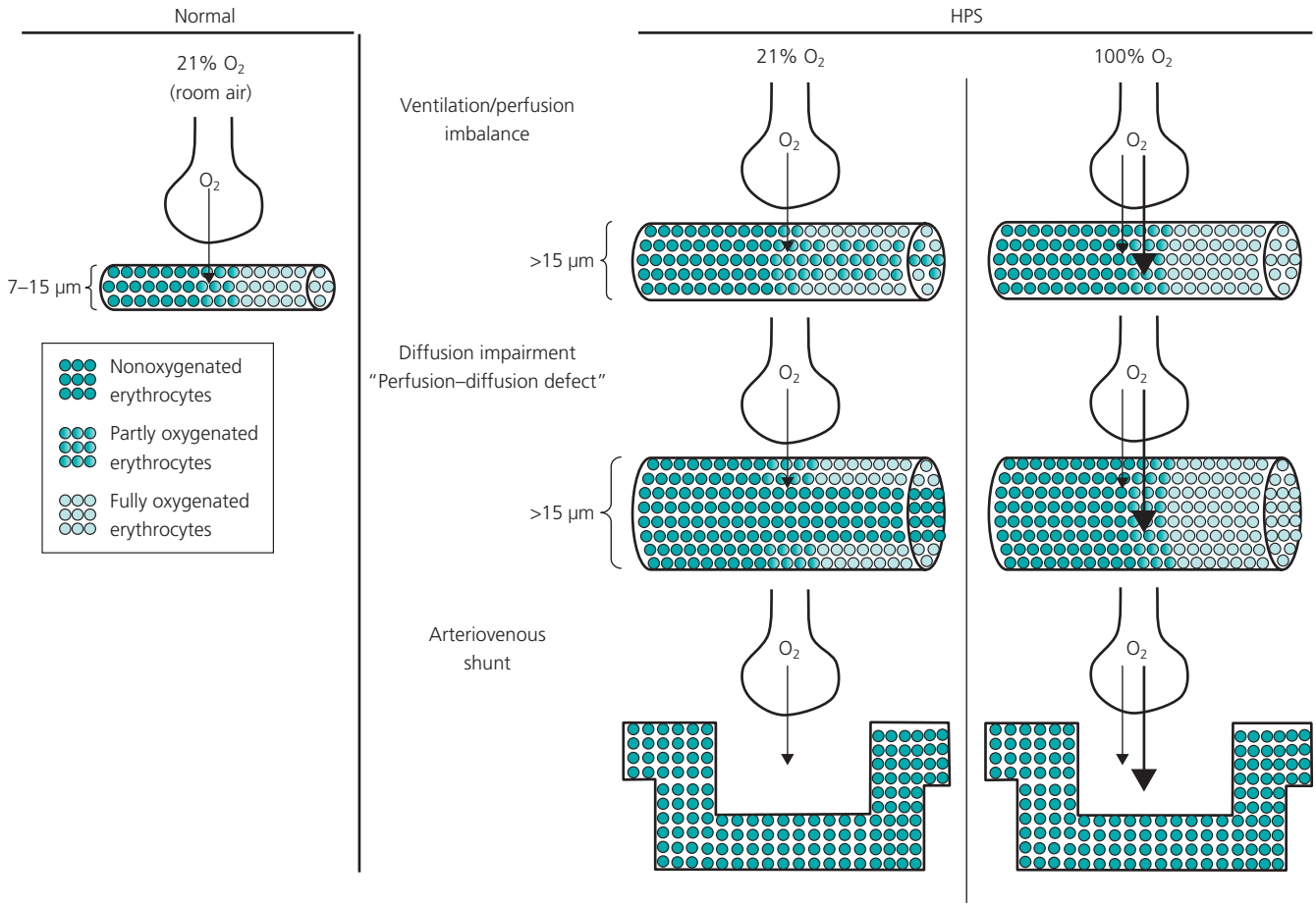


Figure 91.4 Schematic representation of the major mechanisms of hypoxemia in hepatopulmonary syndrome (HPS) and response to breathing 100% oxygen.

ventilation/perfusion mismatch in patients with HPS is, however, controversial [136].

Diffusion impairment is common in patients with liver disease, with up to 55% of candidates for liver transplantation [111,118,137–139] and almost 100% of patients with HPS [111,124,140] presenting an abnormal diffusing capacity for carbon monoxide. A so-called diffusion-perfusion defect has been suggested in HPS [141], where the oxygen pressure within the alveoli would not be sufficient to oxygenate the erythrocytes that travel in the central portion of pulmonary precapillaries and capillaries, because of their abnormal dilation (Fig. 91.4). Based on this hypothesis, the excellent response to 100% oxygen in HPS could be explained by the increased oxygen pressure enhancing the oxygenation of those central erythrocytes. Interestingly, impaired diffusion persists after liver transplantation in many patients despite the reversibility of the hypoxemia and the IPVDs [142], suggesting that the impairment of diffusion is multifactorial.

True anatomical arteriovenous shunts may be present in patients with HPS. Large arteriovenous fistulae between the pulmonary artery and veins are occasionally detected in pulmonary angiography [143]. These arteriovenous communications separate blood from the respiratory interface and, therefore, breathing 100% oxygen does not improve arterial oxygenation (Fig. 91.4). Vascular plexuses between bronchial arteries and veins are common in the pleural surface of patients with cirrhosis, and may be recognized on visual inspection at autopsy as spider nevi [127]. The contribution of portopulmonary anastomoses to the hypoxemia in HPS is likely to be small, because portal venous blood is relatively rich in oxygen (~ 50 mmHg) and the flow through these vessels is small [133].

Importantly, *extrapulmonary factors* also influence gas exchange in these patients. In particular, increased cardiac output may impair arterial oxygenation by decreasing the transit time of erythrocytes through the dilated alveolar capillaries [144].

Mechanisms of pulmonary vasodilation

Pulmonary vascular alterations similar to those of HPS occur in rare cases of congenital heart malformations with anomalous drainage of hepatic veins [145]. Such alterations are

reversible after surgical correction, indicating that factors produced or cleared by the liver strongly influence the pulmonary vascular structure. Mechanisms involved in the development of HPS include altered regulation of pulmonary vascular tone and processes of vascular remodeling and angiogenesis [146]. The cellular and molecular factors implicated have been investigated in humans and in an animal model of HPS, the rat with common bile duct ligation (CBDL) [147,148]. CBDL rats develop severe cholestatic liver disease, hyperdynamic circulation, and, starting 2 weeks after CBDL, progressive arterial oxygenation abnormalities and IPVDs [147,148]. HPS does not develop in other animal models of portal hypertension or liver disease [149].

Nitric oxide (NO), a vasodilator molecule that stimulates guanylyl cyclase in vascular smooth muscle, may be a major mediator of the pulmonary vascular abnormalities of HPS [110,150,151]. The development of HPS, however, is not a mere consequence of the increased NO in the systemic circulation that occurs in portal hypertension. The levels of exhaled NO, which represent local production in the lungs, are increased in patients with HPS compared with cirrhotic patients without HPS or healthy controls, and correlate with the alterations of arterial oxygenation [117]. Transient improvements of arterial oxygenation have been reported in patients with HPS after the inhibition of guanylyl cyclase (using methylene blue) [152,153] or nitric oxide synthases (NOS) (using $N_{(G)}$-nitro-L-arginine methyl ester [L-NAME]), a nonselective inhibitor [154]. L-NAME also improves arterial oxygenation [155] and prevents the development of IPVDs and HPS in CBDL rats [156]. In this animal model, the expression of the endothelial isoform of NOS (eNOS) increases progressively in the pulmonary vascular endothelium from the second week after CBDL in parallel with the IPVDs and the altered gas exchange [157–159], suggesting that it is a major source of increased NO production. An important but transient increase of the inducible isoform of NOS (iNOS) can also be detected later in time, localized in accumulated pulmonary intravascular monocyte/macrophages [158–160]. Alterations of the endothelin-1 system, a major regulator of vascular tone, may determine the altered pulmonary expression of NOS and NO production in CBDL rats [110,150]. In particular, the combination of increased circulating levels of endothelin-1 [149,159] and increased expression of endothelin type B (ETB) receptors in pulmonary vascular endothelium [159] appears to be unique to CBDL rats. Signaling of endothelin-1 through ETB receptors mediates vasodilation by stimulating endothelial NOS activity and NO production. Inhibition of ETB receptors in CBDL rats reduces the expression of both endothelial NOS and ETB receptors, and ameliorates HPS [159]. Rats with prehepatic portal hypertension, which have increased pulmonary expression of the ETB receptor but normal circulating endothelin-1, also develop increased pulmonary expression of endothelial NOS, altered oxygenation and IPVDs when they are infused with low doses of endothelin-1 [149]. Whether these mechanisms are relevant to human HPS, however, is unknown.

Carbon monoxide is another molecule that can induce vasodilation by stimulating guanylyl cyclase. Patients with cirrhosis and HPS present higher arterial levels of carboxyhemoglobin than those without HPS, and the levels moderately correlate with gas exchange abnormalities [114]. In CBDL rats, the expression of heme oxygenase 1, an enzyme that produces carbon monoxide from heme, is induced in liver tissue and pulmonary macrophages, and inhibition of heme oxygenase activity normalizes carboxyhemoglobin levels, attenuates gasexchange abnormalities and IPVDs, and restores hypoxic pulmonary vasoconstriction [158,161]. These findings, therefore, suggest that carbon monoxide production by heme oxygenase contributes to the pulmonary vascular alterations of HPS.

Other factors may influence the development of HPS. In CBDL rats, the presence of bacterial translocation is associated with higher levels of circulating TNF-α and more severe HPS [162]. Treatment with the antibiotic norfloxacin selectively decreases gram-negative bacterial translocation and attenuates gas-exchange abnormalities and IPVDs in these rats [160]. Pentoxifylline, a nonspecific inhibitor of TNF-α, also prevents the development of HPS in CBDL rats [163]. In rats with prehepatic portal hypertension that receive an intravenous (i.v.) infusion of endothelin-1, the development of HPS is associated with an increase of circulating TNF-α [149]. Overall, these studies suggest that inflammation and bacterial translocation, which are frequent in patients with portal hypertension, may play a role in the development of HPS.

Diagnosis

Clinical manifestations

The diagnosis of HPS requires a high index of suspicion. Symptoms related to HPS may be erroneously attributed to progression of the underlying liver disease, which in most cases had been diagnosed years before, or to other conditions common in these patients, such as anemia, malnutrition, ascites, or intrinsic lung disease. This may delay the diagnosis of HPS, and thus it is common to find significant hypoxemia when the diagnosis is finally established.

There are no symptoms or physical signs pathognomonic of HPS. Many patients with HPS may be asymptomatic. The most common complaint (57%–78% of patients) is the insidious onset of dyspnea on exertion and subsequently at rest, but dyspnea is also frequent in cirrhotic patients without HPS (25%–46%) [111,112,164]. Platypnea (worsening of dyspnea in the standing position) is more specific for HPS, but it is present in only a few patients [111]. Spider angiomas, cyanosis, and finger clubbing are signs associated with HPS. Spider nevi are found in the majority of patients with HPS (> 80%) [111,112,165], and have been associated with more

severe liver disease, prominent hyperdynamic circulation, and worse arterial oxygenation [112,135]. However, spider nevi can also be found in 40%–70% of cirrhotic patients without HPS [111,112]. Cyanosis and finger clubbing, by contrast, are highly specific for advanced HPS, reflecting the impact of chronic hypoxemia. Their sensitivity for HPS, however, is low as they are absent in many patients [111,164].

Arterial blood gases

Demonstration of altered arterial oxygenation is required for establishing the diagnosis of HPS. Several thresholds and parameters of arterial oxygenation have been used for defining HPS, partly explaining the variable prevalence reported [112]. In general, HPS can be diagnosed if the age-corrected alveolar–arterial oxygen pressure gradient is higher than normal (age-corrected upper limit of normal = 10 + [0.26 × age – 0.43]) with or without hypoxemia (P_aO_2 < 70 mmHg) [110]. The use of the alveolar–arterial oxygen gradient increases the sensitivity, as it includes in its calculation a correction for the value of P_aCO_2. This gradient is known to increase with age and, therefore, an age correction is necessary to avoid HPS overdiagnosis. The ERS Task Force on Pulmonary-Hepatic Vascular Disorders has staged the severity of HPS based on the P_aO_2 value on room air into mild (≥ 80 mmHg), moderate (≥ 60 and < 80 mmHg), severe (≥ 50 and < 60 mmHg), and very severe (< 50 mmHg; < 300 mmHg if on 100% oxygen), a classification that has prognostic significance [109].

Orthodeoxia (worsening of arterial oxygenation in the standing position) is relatively common in patients with HPS [124], but it can also be encountered in other medical conditions. In patients with HPS, orthodeoxia has been defined as a fall in P_aO_2 ≥ 5% or ≥ 4 mmHg from supine, and it is caused by a gravity-induced heterogeneous redistribution of blood flow to lung bases that increases intrapulmonary shunt and ventilation/perfusion imbalance [165]. To maximize the chance of revealing HPS, the determination of arterial blood gases should be done in the sitting or standing position.

Analysis of arterial blood gases while breathing 100% oxygen may add valuable information, and also allows the quantification of the "physiological shunt." Many patients with HPS present an excellent response to 100% oxygen (P_aO_2 > 500 mmHg), indicating that ventilation/perfusion imbalance or diffusion impairment are the principal mechanisms of hypoxemia. A poor response to 100% oxygen (P_aO_2 < 300 mmHg) indicates the presence of perfusion without ventilation, and suggests (especially if P_aO_2 < 150 mmHg) the presence of anatomical arteriovenous shunts.

Chest radiography and lung function tests

Chest radiography and lung function tests must be done to detect other cardiopulmonary conditions that may cause hypoxemia (see Table 91.5). These conditions may be related to liver disease, such as a hepatic hydrothorax, or may be secondary to intrinsic cardiopulmonary diseases. Importantly, one-third of patients with HPS have coexisting chronic pulmonary conditions, such as chronic obstructive pulmonary disease or bronchial asthma [166,167]. If liver transplantation is being considered it is crucial to evaluate the extent to which these conditions are responsible for the hypoxemia.

The chest radiograph of patients with HPS may reveal bilateral interstitial and pulmonary vascular markings in lower lobes that correspond to the presence of IPVDs [124], but this finding is not specific.

In lung function tests, the majority of patients with HPS present mild to moderate alterations of the diffusing capacity of carbon monoxide [111,124,140], but this is also frequent in patients without HPS [111,118,137–139]. A restrictive pattern can be found in patients who have ascites, pleural effusions, or muscular wasting. In patients with coexisting intrinsic pulmonary diseases, lung function tests will present the corresponding patterns.

Assessment of intrapulmonary vascular dilations

The diagnosis of HPS requires the documenting of the presence of intrapulmonary vascular dilations (IPVDs).

Transthoracic contrast-enhanced echocardiography

Transthoracic contrast-enhanced echocardiography (TT-CEE) is the preferred screening method, because it is noninvasive, highly sensitive for IPVDs, and allows the simultaneous detection of cardiac anomalies and the estimation of right ventricular systolic pressure. Approximately 20% of patients with cirrhosis and a positive TT-CEE for IPVDs, however, do not present alterations of arterial oxygenation [112,116–118]. TT-CEE is usually performed by injecting 10 mL of agitated saline i.v., which produces echogenic microbubbles 24–180 μm in diameter [119]. The microbubbles can be detected in right heart chambers within seconds, they are then trapped in lung capillaries (7–15 μm in diameter) and, in normal conditions, no contrast is observed in left heart chambers. If they are detected in left heart chambers after three heartbeats of their detection in the right chambers, it indicates intrapulmonary shunting. If detected before three heartbeats, it is indicative of intracardiac communication, such as a patent foramen ovale. Different contrast agents, such as indocyanine green solution, may have slightly different sensitivity for IPVDs [119]. TT-CEE cannot quantify the degree of shunt or determine the type of intrapulmonary shunt present (microdilation of fine vessels vs arteriovenous fistula). Transesophageal CEE (TE-CEE) may provide more anatomical information by visualizing specific pulmonary veins, and it is also more sensitive for detecting IPVDs, especially when image quality is bad with the transthoracic approach [118]. However, TE-CEE is more invasive and expensive than TT-CEE, and therefore its main use is in patients with a high suspicion of HPS and negative TT-CEE [118].

Radionuclide lung perfusion scanning

Evidence of right-to-left shunting can be obtained by the intravenous injection of technetium-labeled macroaggregated albumin particles (^{99m}Tc-MAA, 20–90 μm in diameter) combined with whole-body scintigraphy. In normal subjects, ^{99m}Tc-MAA particles are trapped in lung capillaries, and less than 6% of the isotopic activity can be detected in brain, kidneys, spleen, or liver. When the ^{99m}Tc-MAA activity in extrapulmonary tissues (usually calculated from the activity in the brain) is higher than 6% of the total, it indicates the presence of a right-to-left shunt. The ^{99m}Tc-MAA technique permits quantification of the shunt fraction, allowing a better evaluation of the contribution of HPS to the hypoxemia in patients with coexisting cardiopulmonary diseases [140,167]. The calculated ^{99m}Tc-MAA shunt fraction is strongly correlated with arterial oxygenation parameters in patients with HPS [140]. Compared with TT-CEE, the main disadvantages of ^{99m}Tc-MAA are that it may be negative in patients with less severe disease (P_aO_2 > 60 mmHg) [140], and that it cannot distinguish between intracardiac or intrapulmonary shunt, evaluate cardiac disease, or estimate the pulmonary arterial pressure.

Pulmonary angiography

Pulmonary angiography is an invasive, nonsensitive test for the detection of IPVDs. Low pulmonary arterial pressure and low pulmonary vascular resistance are common findings in HPS. Two different angiographic patterns of IPVDs have been described. Type I (diffuse) is characterized by a diffuse dilation of small peripheral branches of the pulmonary artery, mainly in lower lobes. The less common type II (focal) refers to the presence of discrete pulmonary arteriovenous communications [124]. The main indication for pulmonary angiography is patients with a poor response to 100% oxygen (P_aO_2 < 150 mmHg), who may have pulmonary arteriovenous communications amenable to embolization [168]. In patients with a good response to 100% oxygen, the likelihood of finding discrete arteriovenous communications is minimal [167].

High-resolution chest computed tomography (CT)

High-resolution chest CT is useful to diagnose coexisting intrinsic cardiopulmonary disease. When evaluated with this technique, patients with HPS present greater peripheral pulmonary artery diameters and pulmonary artery/bronchus ratios compared with healthy controls and normoxemic patients with cirrhosis [169,170]. The degree of dilation is correlated to some extent with the severity of gas exchange abnormalities [169]. The reliability of this technique for diagnosing IPVDs in HPS, however, requires further study.

Screening

All candidates for liver transplantation should be screened for HPS. Detection of HPS allows a better evaluation of the prognosis and the risk of perioperative complications in individual patients. Guidelines also support the expedited transplantation of patients with severe HPS (P_aO_2 < 60 mmHg on room air) by providing additional points for the Model for End-stage Liver Disease (MELD) [171], because of the unacceptable posttransplant mortality if P_aO_2 falls below 50 mmHg [172]. Under these conditions, screening for HPS in liver transplant candidates is cost-effective, especially with pulse oximetry, and may improve survival [173]. In patients who are not candidates for liver transplantation, general screening for HPS is not recommended, but the diagnostic evaluation of those presenting dyspnea, finger clubbing, or cyanosis should include consideration of HPS.

A general diagnostic approach is shown in Fig. 91.5. If pulse oximetry is used, arterial blood gases should be obtained if oxygen saturation is ≤ 97%. Lower thresholds could miss the diagnosis in many patients because pulse oximetry overestimates arterial oxygenation [113]. Most diagnostic strategies recommend the use of TT-CEE for the detection of IPVDs, reserving the use of ^{99m}Tc-MAA lung perfusion scanning to evaluate those patients with positive TT-CEE and coexisting cardiopulmonary disease [109,110].

Therapy

Oxygen therapy

Mortality in HPS increases with the severity of hypoxemia and is mainly due to liver-related complications [115,125], suggesting that hypoxemia may accelerate the progression of liver disease. Experimental studies suggest that hepatocellular hypoxia enhances fibrogenesis [174]. For these reasons, oxygen therapy is used in patients with significant hypoxemia. Improvement of liver function after long-term oxygen therapy has been reported in two patients with HPS [175]. Despite the rationality behind its use, no study has assessed the effects of oxygen supplementation on the evolution of HPS or liver disease.

Pharmacological therapy

There are no effective pharmacological agents for treating HPS. Somatostatin analogues, indometacin (indomethacin), almitrine bismesylate (a pulmonary vasoconstrictor), inhaled NO, and norfloxacin, have all been tested in small numbers of patients showing minimal (or no) beneficial effects [110,164]. Inhibitors of the NO–guanylyl cyclase pathway (L-NAME, methylene blue) transiently improved hypoxemia, intrapulmonary shunt fraction, and systemic hemodynamics in some studies [152–154], but no effects or even worsening of hypoxemia have also been reported [176,177]. Of note, improvement of arterial oxygenation has been noted in a total of 16 of 33 patients (49%) with HPS treated with garlic powder [178–182]. Despite some promising observations, no clear conclusions can be made regarding the efficacy or safety of any of these agents, because they were all tested in noncontrolled, small series of patients.

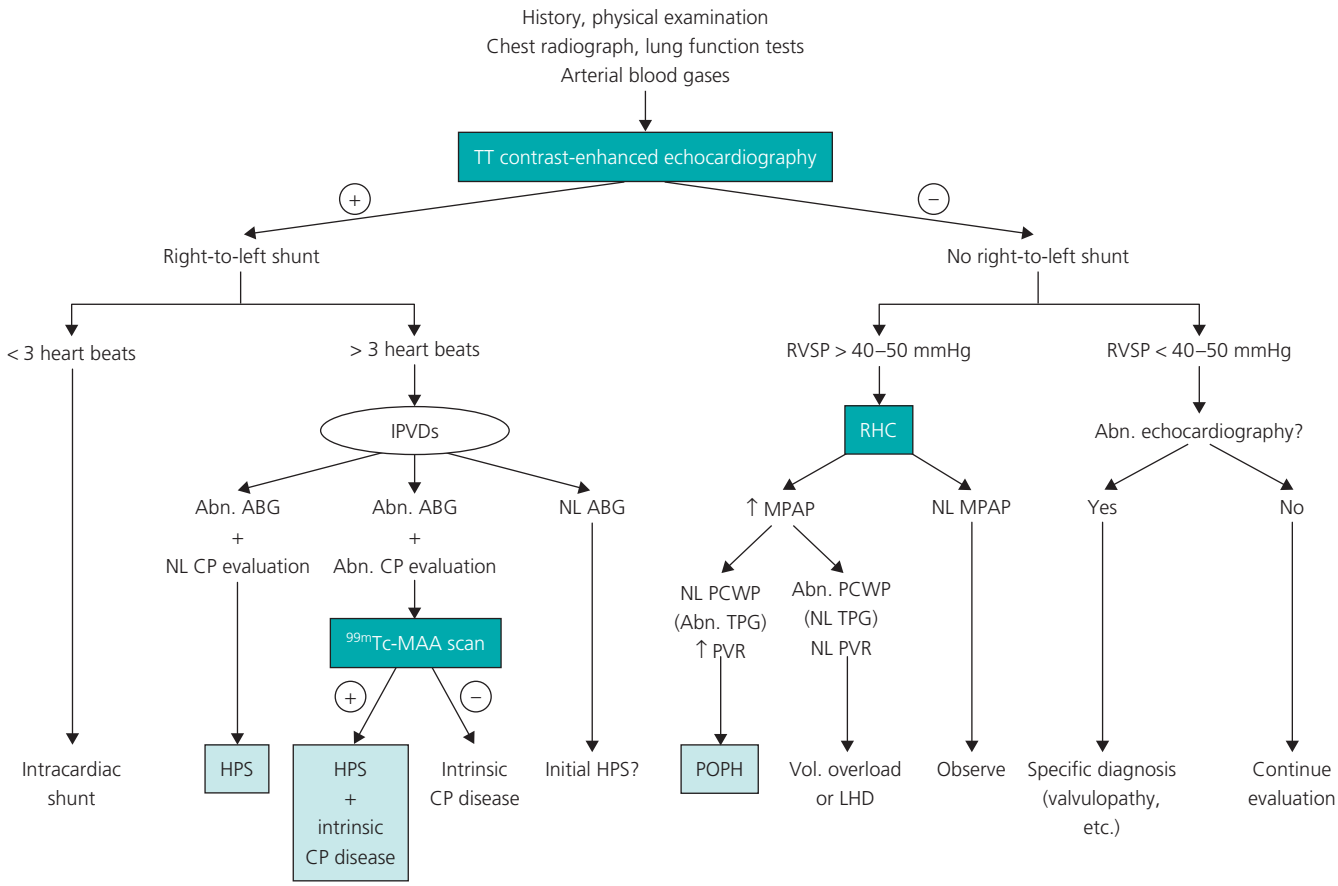


Figure 91.5 General diagnostic approach for hepatopulmonary syndrome (HPS) and portopulmonary hypertension (POPH). Patients with liver disease or portal hypertension presenting with respiratory symptoms or specific signs should be evaluated. All patients being evaluated for major surgical procedures (including liver transplantation) should undergo screening for HPS and POPH, even if asymptomatic. ABG, arterial blood gases; Abn., abnormal; CP, cardiopulmonary; IPVD, intrapulmonary vascular dilation; LHD, left heart dysfunction; MPAP, mean pulmonary arterial pressure; NL, normal; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RHC, right heart catheterization; RVSP, right ventricular systolic pressure; ^{99m}Tc-MAA, technetium-labeled macroaggregated albumin; TPG, transpulmonary gradient; TT, transthoracic; Vol., volume.

Interventional radiology

Pulmonary angiography with coil embolotherapy may improve oxygenation in selected patients with HPS and discrete pulmonary arteriovenous fistulae [168].

Transjugular intrahepatic portosystemic shunts (TIPS) could theoretically improve HPS by relieving portal hypertension, but reports of both improvement and no improvement of HPS have appeared [110]. Short follow-up times and coexistence of hepatic hydrothorax limit the interpretation of the case reports. In general, significant IPVDs persist in most patients after TIPS, suggesting that oxygenation is improved because of blood flow redistribution to regions with normal ventilation/perfusion ratios rather than a true reversal of HPS [164]. Consequently, TIPS should not be considered a therapy for HPS until more evidence of its efficacy and safety is provided [109].

Liver transplantation

Liver transplantation is the only effective therapy for HPS, based on the resolution of hypoxemia and intrapulmonary shunt noted in the majority of reported cases [125,133,183]. In some patients with stable liver disease, severe HPS may constitute the indication per se for liver transplantation. The reversibility of HPS after liver transplantation may be slow (> 1 year), probably reflecting the presence of important structural alterations in the pulmonary vasculature [146]. In general, patients with HPS who undergo liver transplantation have better survival than those who do not, but the effect of comorbid conditions in the latter cannot be dismissed [125]. In patients undergoing liver transplantation, mortality tends to be higher in those with HPS compared with those without HPS [125,172], and it increases with the severity of HPS. A $P_aO_2 \leq 50$ mmHg and a ^{99m}Tc-MAA shunt

fraction ≥ 20% are strong predictors of postoperative mortality [172]. The most common posttransplant complications in patients with HPS include infections and respiratory failure with persistent or worsening hypoxemia [172], but more specific complications of HPS may occur, such as cerebral embolic hemorrhages [184] or the development of portopulmonary hypertension [185]. Despite the beneficial influence of liver transplantation, the criteria used for selecting or prioritizing patients with HPS in the waiting list still require refinement to optimize outcomes and organ allocation [171].

Portopulmonary hypertension

In the early 1950s, Mantz and Craige first described cor pulmonale in a patient with portal vein thrombosis, portal hypertension, and portosystemic shunt [186]. Today, portopulmonary hypertension (POPH) refers to a pathological obstructive process in the pulmonary vasculature of patients who have portal hypertension, resulting in increased resistance to blood flow and pulmonary arterial hypertension. In common with HPS, POPH is also a complication of portal hypertension. In contrast to HPS, however, POPH is characterized by pulmonary vasoconstriction rather than vasodilation.

Definition

POPH is defined as pulmonary arterial hypertension (PAH) associated with portal hypertension, with or without underlying liver disease [109,187]. POPH is not considered a "secondary" form of PAH, but is classified in the same group as idiopathic PAH, familial PAH, and PAH associated with diverse conditions (collagen vascular disease and others) [187].

The following traditional criteria must be fulfilled to diagnose POPH:

- presence of portal hypertension with or without cirrhosis
- mean pulmonary arterial pressure (MPAP) > 25 mmHg (3.33 kPa) at rest
- pulmonary capillary wedge pressure (PCW) < 15 mmHg (2.0 kPa)
- pulmonary vascular resistance (PVR) > 240 dyn·s·cm^5 (3.0 mmHg/L/min).

Performance of right heart catheterization with complete hemodynamic study is, therefore, required to diagnose POPH correctly.

Epidemiology

By definition, the diagnosis of POPH is made in patients with portal hypertension. Most patients have underlying cirrhosis, but POPH can also develop in patients with noncirrhotic portal hypertension, such as nodular regenerative hyperplasia or portal vein obstruction [188]. The diagnosis of portal hypertension usually precedes by more than 4 years that of POPH [189]. POHP may occur at any age, including childhood, but the diagnosis is most common in the fifth decade, compared with the fourth decade in idiopathic PAH [188].

POPH is a rare complication of portal hypertension, but it appears to be more common than reported in early studies. In 1983, a series of 17 901 unselected autopsies noted changes characteristic of idiopathic PAH more commonly in patients with cirrhosis than in the whole series (0.73% vs 0.13%) [190]. In a cohort of 507 patients with portal hypertension (of cirrhotic origin in 85%) undergoing hemodynamic studies, the prevalence of POPH was 2% [189]. In two studies in candidates for liver transplantation, the prevalence of POPH was more than double these rates (5%–6%) [191,192], maybe reflecting a longer duration of portal hypertension. No clear correlations exist, however, between the severity of POPH and the etiology or severity of liver disease, degree of portal hypertension, or hyperdynamic circulation [188,189,192]. In patients with refractory ascites evaluated for TIPS placement, an unusually high prevalence of POPH of 16% has been reported [193].

Natural history

The diagnosis of POPH carries a dreadful prognosis [194]. No cases of spontaneous resolution of POPH have been reported. A median survival as low as 6 months was noted in the preliver transplantation era [195]. In subsequent series, the overall 3- to 5-year probability of survival (irrespective of the medical management, including liver transplantation) was approximately 30%–50% [196–198]. The natural history of POPH is not well defined, however, and predicting survival in individual patients is a difficult task [189].

Complications of liver disease and those directly related to POPH (right-sided heart failure, sudden death) account for a similar proportion of deaths in patients with POPH. A low cardiac index is a strong predictor of mortality in these patients, particularly for death resulting from cardiopulmonary complications [189,197]. Increased plasma levels of brain natriuretic peptide reflect right ventricular stress and have been associated with poor prognosis in idiopathic PAH [199]. Limited data suggest that vasodilator therapy and liver transplantation influence the course of POPH, but the extent to which these interventions may reverse the condition or improve survival is unknown.

Pathophysiology

Pathology

The pathology of POPH is indistinguishable from that observed in idiopathic PAH, and is characterized by vasoconstriction and structural changes in the pulmonary arterial tree that result in the obstruction of pulmonary blood flow [190,200]. The main changes affect small pulmonary arteries and arterioles, and consist of hypertrophy of medial smooth muscle, with proliferation or fibrosis of endothelium and adventitia. Thrombosis with recanalization may develop in situ in these vessels. The plexiform lesion, characteristic of idiopathic PAH,

can also be observed in POPH. It consists of small branches of pulmonary arteries dilated close to their origin that present thinning of medial smooth muscle, and an intralumenal plexus of endothelial cells and narrow slit-like channels.

Pathogenesis

Knowledge of the pathogenesis of POPH is limited, due to the rarity of the condition and the lack of adequate animal models. The mechanistic connection between portal and pulmonary hypertension is not completely understood, but some pathogenetic factors postulated for idiopathic PAH may be enhanced in patients with portal hypertension [201]. Two major features of portal hypertension may affect the pulmonary circulation [187,188]. First, the hyperdynamic circulation with increased cardiac output increases shear stress in the pulmonary vasculature and may lead to altered endothelial expression of genes involved in regulation of vascular tone and angiogenesis. Second, portosystemic shunting and defective liver metabolism expose the pulmonary vasculature to blood that has not been processed by the liver, which presumably causes an imbalance between vasodilator/vasoconstrictor and antiproliferative/growth-promoting mediators in the pulmonary circulation. This may include the elevation of levels of potent vasoconstrictors involved in the pathogenesis of idiopathic PAH, such as endothelin-1 (through ETA receptors) and 5-HT_2, and others such as prostaglandin $F_{2\alpha}$, thromboxane B_2 or angiotensin I. By contrast, the expression of prostacyclin synthase, the enzyme that produces the vasodilator prostacyclin, is decreased in small pulmonary arteries of patients with POPH [202]. Portosystemic shunting of bacterial products translocated from the intestines may lead to macrophage sequestration and secretion of cytokines in the pulmonary vasculature, which may contribute to the pathological process. The coagulopathy of patients with cirrhosis may also influence the development of in situ thrombosis in the affected vessels, causing further resistance to blood flow.

Many patients with portal hypertension are likely to present the above factors, but it is unclear why only a small subset of them develops POPH. An autoimmune origin of POHP has been postulated in some patients based on a high prevalence of autoimmune antibodies [197], but this finding is not conclusive. A genetic predisposition may also be a factor, but genetic variations linked to idiopathic PAH affecting bone morphogenetic protein receptor II, activin-like kinase type-1 or the 5-HT transporter have not been confirmed in patients with POPH [109,187].

Diagnosis

Clinical manifestations

Patients with POPH usually have a previous diagnosis of portal hypertension. Many patients may be asymptomatic [189], underscoring the need for screening patients with liver disease who will undergo surgical procedures, particularly liver transplantation. The diagnosis of POPH may be delayed more than 1 year after the onset of symptoms [189], with dyspnea on exertion being the most common complaint [195]. Patients can also present with progressive fatigue and orthopnea. The presence of chest discomfort and syncope indicates advanced disease. In addition to signs of liver disease and portal hypertension, patients with POPH will present signs derived from progressive right-sided heart dysfunction, which include jugular venous ingurgitation, cardiac auscultation with increase of the P_2 pulmonic valve component and tricuspid regurgitation murmur, right ventricular heave, and edema of lower extremities.

The differential diagnosis of dyspnea in liver disease is broad (Table 91.5), and reaching a diagnosis of POPH may require an extensive diagnostic workup [187,188]. Arterial blood gases in patients with POPH usually reveal an increase of the alveolar–arterial oxygen gradient, hypocapnia, and, less frequently, a mild degree of hypoxemia, even in the

Table 91.5 Differential diagnosis of cardiopulmonary dysfunction in liver disease

Intrinsic cardiopulmonary conditions
Obstructive lung diseases
Chronic obstructive pulmonary disease
Asthma
Others, including emphysema
Diseases of lung parenchyma
Pneumonia
Interstitial lung disease
Others, including atelectasis
Pulmonary vascular diseases
Pulmonary embolism
Secondary pulmonary hypertension (due to heart/lung disease or others)
Disorders of the heart and circulatory system
Congestive heart failure
Valvular heart disease
Cardiomyopathy
Others (including arrhythmias, coronary artery disease)
Conditions related to liver disease and portal hypertension
Associated with specific liver diseases
α_1-Antitrypsin deficiency: emphysema
Primary biliary cirrhosis: fibrosing alveolitis, pulmonary granulomas
Sarcoidosis: lung restrictive disease, nonnecrotizing granulomas, cardiomyopathy
Hemochromatosis: cardiomyopathy, arrhythmias
Cystic fibrosis: bronchiectasis, pneumonia
General complications of liver disease and portal hypertension
Ascites
Hepatic hydrothorax
Muscular wasting
Pulmonary vascular abnormalities
Hepatopulmonary syndrome
Portopulmonary hypertension

setting of moderate-to-severe POPH [203]. In moderate-advanced POPH, a chest radiograph may show a prominent main pulmonary artery and cardiomegaly without any parenchymal alterations. An electrocardiogram indicating right atrial enlargement, right ventricular hypertrophy, or right bundle branch block should increase the suspicion of POPH. Lung function tests are important to exclude significant air flow obstruction and other lung diseases, and frequently reveal a reduced diffusing capacity for carbon monoxide in patients with POPH. Ventilation/perfusion lung scanning may show a "mosaic" pattern in patients with POPH; segmental defects would instead suggest pulmonary embolism. Compared with the pulmonary angiographic pattern of idiopathic PAH, POPH presents a lesser degree of sparse arborization and tapering of peripheral arteries, and a dilation instead of narrowing of proximal pulmonary arteries [204].

Transthoracic echocardiography

Transthoracic Doppler echocardiography is the most important noninvasive test for screening POPH (Fig. 91.5). It may exclude valvulopathies and other cardiac causes of elevated MPAP, and also screens for HPS. Most patients with POPH (~ 80%–90%) present a tricuspid regurgitant jet, which allows estimation of the right ventricular systolic pressure (RVSP). The echocardiographic findings reflect the cardiac consequences of progressive resistance to pulmonary blood flow, including pulmonic valve insufficiency, right atrial dilation, right ventricular hypertrophy and dilation, interventricular septal wall thickening, and paradoxical septal motion.

The estimated RVSP is a useful screening parameter for POPH, but there is no consensus regarding which value should be used [205]. Most studies use estimated RVSP cutoff values of 40–50 mmHg, which have a sensitivity of almost 100% for detecting POPH [206]. The correlation between the RVSP values estimated by echocardiography and those directly measured during right heart catheterization is weak [192,207]. Furthermore, echocardiography cannot evaluate PVR. For these reasons, approximately 30%–40% of patients satisfying the previous estimated RVSP thresholds will have normal PVR measured during right heart catheterization and they will not be diagnosed with POPH [191,192]. Another echocardiographic parameter proposed for screening POPH is a pulmonary acceleration time > 100 ms [208], which may have better diagnostic accuracy and can be used in patients in whom RVSP cannot be estimated [192]. In addition to screening, transthoracic echocardiography is useful for following up patients with a diagnosis of POPH.

Hemodynamic assessment

Diagnosing POPH requires the performance of right heart catheterization (Fig. 91.5). This test allows the direct measurement of MPAP, PCWP, and cardiac output, and the calculation of PVR and systemic vascular resistance (SVR). An increase of MPAP alone is not diagnostic of POPH in patients with portal hypertension. Hemodynamic parameters distinguish three origins of increased MPAP in these patients [109,206]:

1 *Hyperdynamic circulatory state*. This hemodynamic pattern is the most common in patients with liver disease. It is characterized by a significant increase of cardiac output and decreased SVR, with a normal PCWP and normal or low PVR. MPAP may be mildly elevated as a result of increased cardiac output.

2 *Increased pulmonary venous volume*. In this case, MPAP is mildly or moderately elevated in the setting of an increased PCWP, reflecting the presence of volume overload or left ventricular dysfunction. PVP is normal or mildly elevated.

3 *Pulmonary vascular obstruction*. This pattern must be present to diagnose POPH. It is distinguished by the significant increase of PVR, leading to elevations of MPAP that may be severe. PCWP is normal or mildly decreased. In contrast to idiopathic PAH, cardiac output is usually increased in patients with POPH. A decrease of cardiac output in POPH indicates advanced right-sided heart failure, and is a sign of poor prognosis [189,197].

Hyperdynamic circulation and volume overload are common in patients with liver disease. According to traditional criteria, the diagnosis of POPH would be missed in these patients if PCWP is greater than 15 mmHg. To avoid this situation, use of the transpulmonary pressure gradient (TPG = MPAP – PCWP) has been proposed [109]. A TGP > 12 mmHg indicates obstruction to flow, independent of the value of PCWP [192].

The value of MPAP obtained during right heart catheterization is used to stage the severity of POPH into mild (> 25 and < 35 mmHg), moderate (≥ 35 and < 45 mmHg) and severe (≥ 45 mmHg) grades, which provides important prognostic information [109].

If POPH is diagnosed, an acute vasodilator testing should be performed during right heart catheterization, generally using epoprostenol i.v. or inhaled NO. A vasodilatory response is significant when MPAP and PVR decrease by more than 20% from baseline values without a decrease of cardiac output [109]. This test reflects to what extent the hypertensive state is caused by reversible vasoconstriction, helping to characterize the severity of the disease and the therapeutic expectations. In a small study of 14 patients with moderate-to-severe POPH, 6 (43%) presented a significant vasodilatory response to i.v. epoprostenol [209]. In these patients, epoprostenol i.v. induces greater increases of cardiac output than inhaled NO [210], and has been suggested to detect a higher proportion of responders [109].

Treatment

General medical treatment

The general management of patients with POPH consists in avoiding circumstances that may worsen portal hypertension, pulmonary vasoconstriction, or stress to the heart. Surgery

should be indicated with caution in patients with POPH, even in those with mild disease, and it would require special anesthetic care [211]. TIPS is not recommended in patients with POPH, because the increase in cardiac output may worsen pulmonary hypertension and precipitate right-sided heart failure. Patients with POPH and hypoxemia may benefit from oxygen therapy to avoid hypoxic pulmonary vasoconstriction. Diuretics (furosemide, spirolonolactone) may be used in patients with volume overload. Withdrawal of β-blocker treatment in patients with POPH has been associated with improvements in exercise capacity and pulmonary hemodynamics [212]. Alternative methods for preventing variceal bleeding, therefore, may be desirable. Oral anticoagulation and calcium channel blockers, medications used for idiopathic PAH, are not recommended in patients with portal hypertension because they may increase the risk or severity of bleeding and the hepatic venous pressure gradient, respectively [213].

Specific vasodilator therapy

Specific vasodilator therapy improves symptoms, pulmonary hemodynamics, and survival in large clinical studies of idiopathic PAH [201]. In patients with POPH, however, the experience is limited to case reports and small, uncontrolled studies, impeding the establishment of conclusive guidelines. In general, this therapy is not offered to asymptomatic patients with mild POPH (MPAP < 35 mmHg), who should undergo only periodic follow-up with transthoracic echocardiography. Patients with moderate-to-severe POPH (MPAP > 35 mmHg) and a significant vasodilatory response may benefit from this therapy, especially if combined with liver transplantation. The duration of the effect of vasodilator therapy and its impact on POPH survival, however, are unknown.

Prostacyclin analogues

Epoprostenol, a potent pulmonary vasodilator with antiproliferative and anti-platelet aggregating properties, is the best-studied agent. Because of the short half-life (3–5 min) of epoprostenol, treatment requires long-term catheterization of a central vein for continuous i.v. administration. In series totalling more than 45 cases, both the acute and long-term administration of epoprostenol significantly decreased MPAP and PVR and increased cardiac output in patients with POPH [209,214–217]. Common side effects include headache, flushing, diarrhea, nausea, muscle pain, and hypotension [201]. Development of splenomegaly with leukothrombocytopenia was reported in four patients [218]. The potential complications arising from long-term venous access, such as infection or thrombosis, are the major limitations for this therapy. Moreover, accidental interruption of the perfusion may cause life-threatening sudden pulmonary vasoconstriction. More stable prostacyclin analogues, such as iloprost and treprostinil (approved for idiopathic PAH by the US Food and Drug Administration [201]), and a variety of routes of administration (inhalation, oral, subcutaneous, i.v.) have been investigated. The experience with these agents in POPH, however, is still limited to anecdotal reports [219,220].

Endothelin receptor antagonists

Bosentan is a dual ETA and ETB receptor antagonist that can be administered orally. A dose-dependent increase of liver enzymes, however, occurred in approximately 11% of patients treated with this agent in clinical trials of idiopathic PAH [201]. In preliminary reports comprising a total of 13 patients with POPH and well-compensated cirrhosis, treatment with low-to-medium doses of bosentan for more than 1 year resulted in improvement of exercise capacity and pulmonary hemodynamics without notable adverse effects, except systemic arterial hypotension in one patient [221–223].

Phosphodiesterase inhibitors

Sildenafil is an oral medication also approved for idiopathic PAH. Sildenafil inhibits the degradation of NO by phosphodiesterase-5, promoting vasodilation. Preliminary reports have communicated variable improvements of exercise capacity or pulmonary hemodynamics in a total of 17 patients with advanced cirrhosis and severe POPH treated with sildenafil alone or in combination [224–227]. The administration of sildenafil, however, may exacerbate portal hypertension and hyperdynamic circulation [228], and variceal bleeding during treatment with sildenafil has been reported [229].

Other agents

It has been reported that MPAP and PVR were decreased by isosorbide-5-mononitrate [230] in one patient, and by inhaled NO in five out of six patients with POPH [231].

Liver transplantation

It is unclear how liver transplantation influences the natural history of POPH. Normalization, improvement, no change, and worsening of POPH have all been reported in patients undergoing liver transplantation [109,205]. In favorable cases, POPH may take months to years to resolve. De novo development of POPH, transition from HPS to POPH, and recurrence of POPH in cases of graft failure have also been noted after liver transplantation [187]. For these reasons, in contrast to HPS, POPH is not an indication for liver transplantation.

Impact of POPH on liver transplantation outcomes

The presence of POPH is associated with increased morbidity and mortality in patients undergoing liver transplantation. A transplant hospitalization mortality of 36% has been reported in these patients, with 40% of the deaths occurring intraoperatively [232]. Screening of POPH in liver transplant candidates and adequate staging by right heart catheterization are critical for assessing the risk of complications [233]. Despite adequate screening, a few patients may be diagnosed in the operating room [191,232]. The rate of mortality in

patients with POPH undergoing liver transplantation increases from 0% if MPAP < 35 mmHg or TPG < 15 mmHg, to 50% if MPAP is between 35 and 50 mmHg and PVR ≥ 250 dyn·s·cm^5 (3.125 mmHg/L/min), to 100% if MPAP ≥ 50 mmHg [233]. Based on this and other studies, a MPAP ≥ 50 mmHg is usually considered an absolute contraindication for liver transplantation [109]. Successful liver transplantation of these patients, however, has been occasionally reported [234]. Eligible candidates with mild POPH (MPAP < 35 mmHg) could benefit from expedited liver transplantation before POPH progresses, but the provision of additional MELD points is controversial [205].

The risk of complications in patients with POPH undergoing liver transplantation is maximal during the induction of anesthesia, before and after graft reperfusion, and in the immediate postoperative period [235]. Increases of MPAP during reperfusion of the graft may cause right-sided heart failure [109], the main cause of intraoperative and perioperative mortality in these patients [232]. Dobutamine stress echocardiography with volume challenge may improve the prediction of this complication [236]. Circumstances that impair heart function, such as hypoxia, hypothermia, hyperkalemia, metabolic acidosis, or hypercarbia, should be prevented. Anesthesia with isofluorane and intraoperative use of vasodilators may help to control increases of MPAP in these patients [109,235]. Combined liver, heart, and lung transplantation has been reported in highly selected patients but remains experimental [237].

Specific vasodilator therapy for rescuing candidates for liver transplantation

Patients with moderate-to-severe POPH (MPAP > 35 mmHg) who do not have contraindications for liver transplantation are the most likely to benefit from vasodilator therapy. Using this approach, up to 75% of them may achieve reductions of MPAP below 35 mmHg and PVR below 400 dyn·s·cm^5 (5.0 mmHg/L/min) and can be successfully bridged to liver transplantation [187]. Preliminary data suggest that this combination provides a survival advantage to these patients [205,217]. Vasodilator therapy should continue during and after liver transplantation, but withdrawal has been safely achieved within 1 year in many patients [209,216,217]. Despite the evidence supporting the use of vasodilator therapy, a prospective collection of data from 1996 to 2001 in 10 liver transplant centers of the United States showed that vasodilator therapy is not commonly offered to these patients [232].

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Introduction

In the fourth decade since the inception of human orthotopic liver transplantation [1], this procedure has become a highly successful treatment for patients with life-threatening, advanced liver disease, and is performed in 137 centers across the United States [2]. According to data from the Organ Procurement and Transplantation Network for all adult liver transplants performed in the United States from 1996 to 2005, patient survival following deceased donor liver transplantation was 93% at 3 months, 87% at 1 year, and 73% at 5 years (Fig. 92.1) [2]. The applicability of liver transplantation as a therapy is, however, limited by a critical donor organ shortage [3]. The total number of transplants performed yearly has increased steadily over the last 10 years, reaching 6441 procedures in 2005. This rise is mainly attributable to deceased donor liver transplantation, the number of which increased by 30% since 2001 after rising more slowly over the previous decade (Fig. 92.2). The size of the waiting list for a liver transplant has increased continuously, with a peak in 2001 at 14 897 patients (Fig. 92.3) [3]. The interruption of this rise in 2002 coincided with the implementation of the allocation system based on the Model for End-stage Liver Disease and Pediatric End-stage Liver Disease (MELD/PELD). A total of 12 822 candidates were actively listed for liver transplantation in 2005, compared to 12 650 in 2003. Although the death rate among patients on the waiting list has declined steadily over the past decade (174.3 deaths per 1000 patient-years at risk in 1996 compared with 125.5 deaths per 1000 patient-years at risk in 2005), since 1999, there have been approximately 2000 deaths per year among recipients on the waiting list [3]. The continued disparity between cadaveric donor organ supply and recipient demand has undoubtedly contributed to these deaths, and remains a major challenge in this field and continues to motivate the need for improving strategies for optimizing donor organ availability and organ allocation, and developing expertise in the management of patients with severe acute and end-stage liver disease.

This chapter reviews the aspects of surgical technique and donor organ sources and organ preservation, the indications and process of evaluation for liver transplantation, disease-specific issues, outcomes, and the management of posttransplant complications and immunosuppression.

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Surgical technique, sources of donor organs, and organ preservation

General considerations

Matching an appropriate donor organ with a liver transplant recipient requires compatibility of the ABO blood type but not the human leukocyte antigen (HLA) tissue type. The conventional recipient operation involves orthotopic transplantation of the donor organ (i.e., in the normal anatomical location). The procedure is conventionally divided into three phases: the hepatectomy phase (removal of the recipient native liver), the anhepatic phase, and the reperfusion phase (circulation of blood through the newly transplanted liver). The order of anastomoses usually commences with the venous revascularization of the donor organ (systemic and portal venous anastomoses) followed by arterial reconstruction, followed finally by biliary reconstruction. Numerous variations exist in the technique of implanting the donor organ, based on surgical preference, anatomical variations, and considerations imposed by recipient disease etiology [4]. Strategies for systemic venous reconstruction involve either caval replacement, which is applicable only in the setting of deceased donor liver transplantation, or the "piggyback" technique applicable for either deceased or living donor transplantation. When the cava is replaced, the suprahepatic and infrahepatic vena cava is clamped and the retrohepatic caval segment of the recipient is replaced with that of the

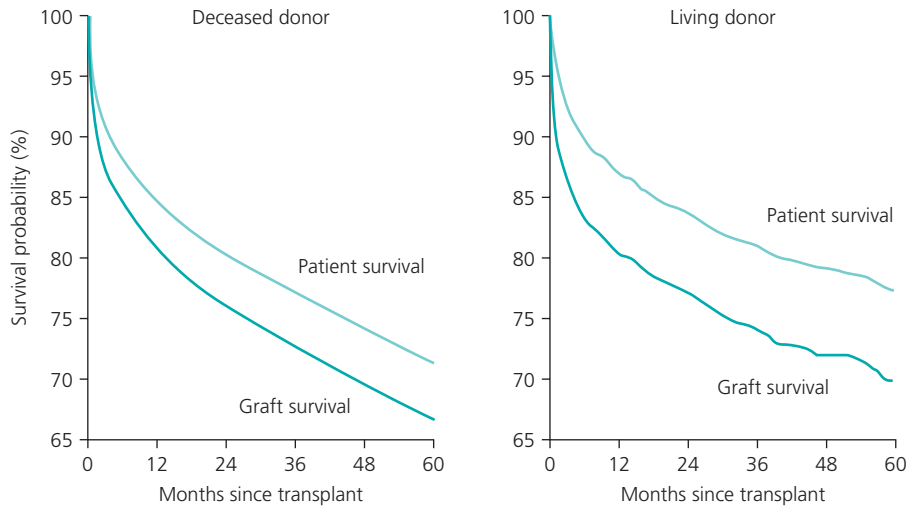

Figure 92.1 Unadjusted patient and graft survival for all deceased donor liver transplantation (DDLT) and living donor liver transplantation (LDLT) recipients In the United States between 1996 and 2005. Patient survival following DDLT was 93% at 3 months, 87% at 1 year, 79% at 3 years, and 73% at 5 years. The corresponding patient survival was slightly better for recipients of LDLT (96%, 92%, 83%, and 77%, respectively). A similar pattern is evident when analyzing graft survival. From the Scientific Registry of Transplant Recipients [352].

donor. Two systemic venous anastomoses are then performed to reestablish caval continuity. When the piggyback technique is used, additional dissection is performed to divide the small hepatic veins that enter the cava directly up to the main right, middle, and left hepatic veins to preserve the recipient's vena cava in situ. Then, the hepatic veins can be clamped with or without partial caval clamping and the anastomosis to the donor liver's suprahepatic cava or right hepatic vein (in the case of a right lobe living donor liver transplantation) is performed. Avoidance of full clamping of the inferior vena cava may decrease the risk of cardiovascular stress, hemodynamic instability, and venous hypertension of the kidneys during the anhepatic phase. Hepatic arterial reconstruction is commonly achieved with an end-to-end anastomosis of the donor celiac axis (with or without a Carrel patch of aorta) for deceased donor livers or the right hepatic artery for living donor livers to the recipient common, proper, or right hepatic artery. Vascular grafts extending from the recipient aorta are occasionally necessary if inflow at the level of the hepatic artery or the celiac axis is inadequate, most frequently as a result of vascular dissection. Typically, deceased donor iliac artery or recipient saphenous, gonadal, or jugular vein is used as the conduit. The usual approach to biliary reconstruction employs a duct-to-duct anastomosis (choledochocholedochostomy for deceased donor transplants or hepaticocholedochostomy for living donor transplants). Common exceptions include pediatric transplants, multiple donor ducts (as is frequent in living donor transplants), disease involving the recipient duct (primary sclerosing cholangitis, biliary atresia), and a large discrepancy in size between the recipient and donor ducts. In these cases, biliary reconstruction is performed with a Roux-en-Y limb of jejunum. To reduce the risk of hemodynamic instability and minimize congestion of the kidneys or bowel during the anhepatic phase, some centers employ venovenous bypass of the systemic circulation with or without the portal circulation [4].

The biggest challenge facing liver transplantation today is the insufficient supply of organs to meet the increasing demand. In response, liver transplant surgeons have aggressively expanded the frontiers of utilization for deceased donor organs and even traversed into the realm of living donors, both of which are discussed in greater detail below.

Deceased donor liver transplantation

The first incremental advance towards maximal use of deceased donor livers emerged in the early 1990s as an outgrowth of technical innovations spurred by the high mortality rate of pediatric liver transplant candidates. The relative shortage of size-matched organs for infants and toddlers considered together with the segmental anatomy of the liver inspired the development of reduced-size liver transplantation, the transplantation of portions of a liver appropriate for the age and size of the intended recipient [5,6]. The maturation of this technique led to the next innovation, split-liver transplantation whereby a single deceased donor liver was divided into two smaller, transplantable grafts [7–9]. Today, this technique is frequently applied to the transplantation of one adult and one pediatric recipient and occasionally applied to the transplantation of two adults [10,11].

In addition to incremental technical innovations, which have increased the number of available donor organs, there has been gradual expansion of the criteria for donor organs to be considered acceptable for transplantation. It has long been known that young, healthy people dying of trauma provide the ideal organs. Such ideal organs have already become the exception rather than the rule. Whole livers from donors between 18 and 40 years of age who have suffered brain death from trauma account for approximately one-third of transplants performed today [12]. Characteristics that exert the greatest negative impact on graft function and survival are increased donor age, split or partial liver transplantation, and donation after cardiac death. Compared to livers from donors younger than 40 years, livers from donors

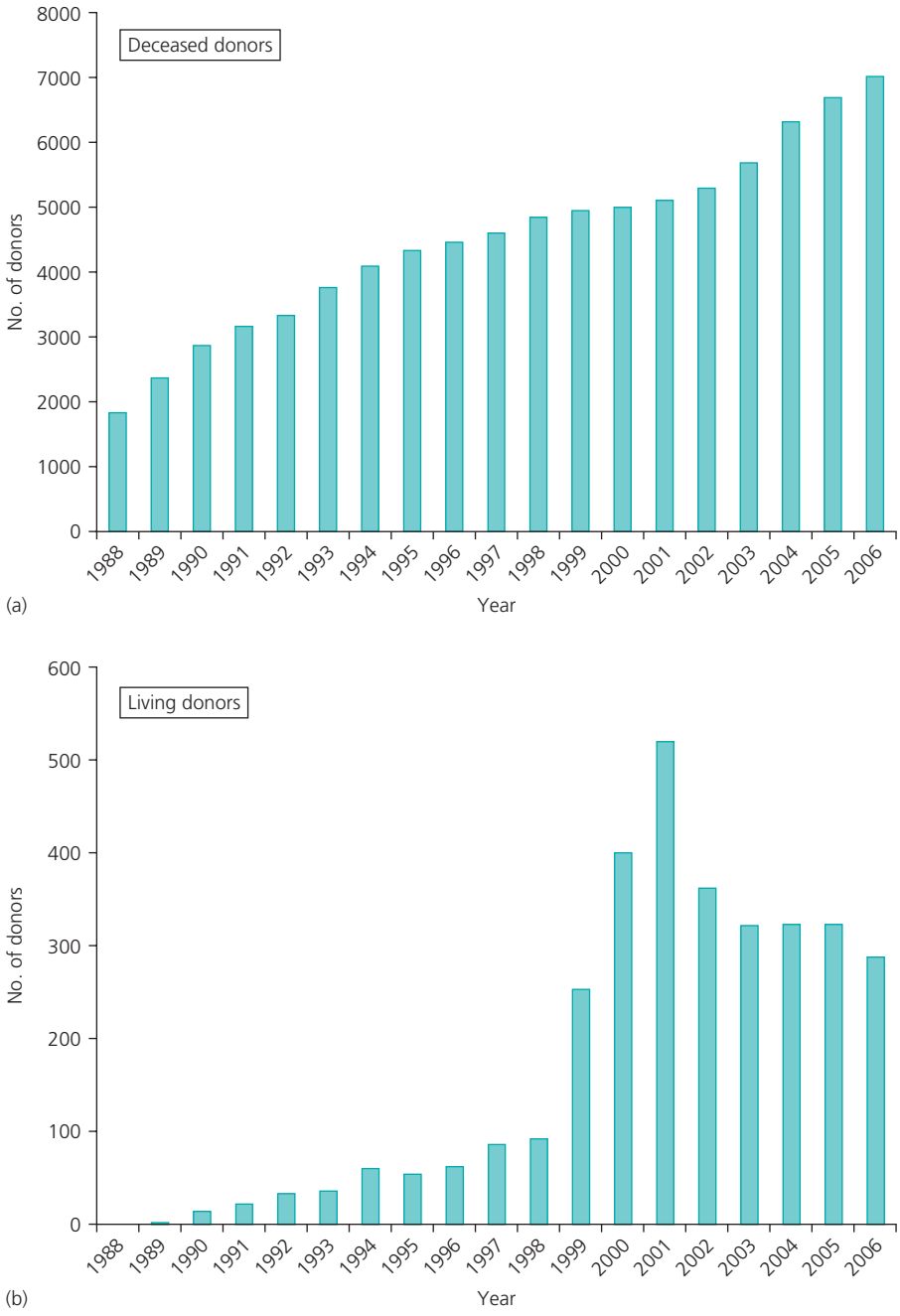

Figure 92.2 Donors recovered in the United States by year between 1988 and 2006. **(a)** Deceased donors; **(b)** living donors. Data from the Organ Procurement and Transplantation Network (OPTN).

aged between 60 and 69 years have a 49% increased risk of graft failure (95% confidence interval: 32%–68% increased risk) whereas livers from donors older than 70 years have an 88% increased risk of graft failure (95% confidence interval: 60%–121% increased risk) [12]. Interestingly, the deleterious effect of increased donor age on transplant outcomes is not uniformly exerted but is differentially exerted on recipients based on disease etiology, affecting those with hepatitis C the most and those with hepatitis B the least [13]. Split/partial livers and livers donated after cardiac death are both associated with approximately a 50% increased risk for graft loss compared to whole livers and livers donated after brain death, respectively [12]. The number of split liver transplants increased over 10-fold during the late 1990s (from 8 in 1995 to 95 in 2000) but this rate of increase has since leveled out. The requirements for relatively ideal donors, a high level of technical expertise, and both a pediatric and an adult recipient suggest that the volume will remain relatively stable. In contrast, the steeply increasing trend of liver utilization from donation after cardiac death is anticipated to continue [8]. The Institute of Medicine has concluded that donation after cardiac death is an ethically proper approach to recovering

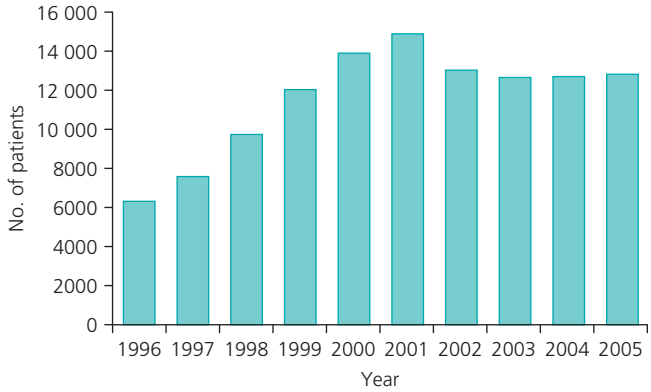

Figure 92.3 Number of patients on the active liver waiting list, 1996–2005. From the Scientific Registry of Transplant Recipients [352].

organs from a deceased patient for the purpose of transplantation [14]. Donation after cardiac death can occur when family member(s) or surrogate decides to withdraw life support (typically by extubation) from someone who has suffered devastating neurological injury but does not meet the criteria for brain death. After determination of death according to circulatory criteria, organ donation proceeds as determined by the wishes of the family member(s) or surrogate [14,15]. The time period between withdrawal of life support and circulatory death engenders a variable amount of warm ischemic injury, which is compounded by additional warm ischemic injury during the time period between circulatory death and initiation of organ preservation. This injury, which does not occur with donation after brain death, is largely responsible for the increased risk of short- and long-term graft failure associated with liver transplantation using organs donated after cardiac death [12,14,15].

Organ preservation

A major advance in the preservation of donor organ viability and extension of the geographic distance over which organs can be procured and transported was the development of the University of Wisconsin (UW) cold-storage solution with which the donor organ is flushed and cooled prior to removal and transportation [16]. The UW solution has extended the cold ischemia time for liver preservation for up to 24 h, although it is uncommon for organs that have been preserved for longer than 16 h to be used on a routine basis. Key principles of the UW solution are a high K^+ and Mg^{2+} and low Na^+ and Ca^{2+}. This solution maintains intracellular ion concentrations, using adenosine as a substrate for adenosine triphosphate synthesis, hydroxyethyl starch as an oncotic solute to reduce interstitial edema, and allopurinol and glutathione as antioxidants.

An alternative preservation solution has been developed called Histidine-Thym-K (HTK) solution. This is a crystalloid solution with much lower viscosity than UW solution that, in theory, may result in improved perfusion through the hepatic microcirculation and thereby improve biliary tract preservation. Several centers have reported their experience using this new preservation solution and shown no significant functional advantage in patient or graft survival or biliary tract complications over traditional solutions [17,18].

Living donor liver transplantation

Living donor liver transplantation represents incremental technical innovations that have expanded the organ pool. Like the split or partial deceased donor transplantation discussed above, living donor transplantation was an outgrowth of reduced liver transplantation. The strategy was first developed in the early 1990s for pediatric candidates whereby typically, the left lateral segment (Couinaud segments 2 and 3) or the left lobe was procured from a blood-type-compatible parent. The strategy was then adapted to adult candidates in the late 1990s using the right lobe, which typically represents approximately 60% of the entire liver mass [7,8,19] (Fig. 92.4).

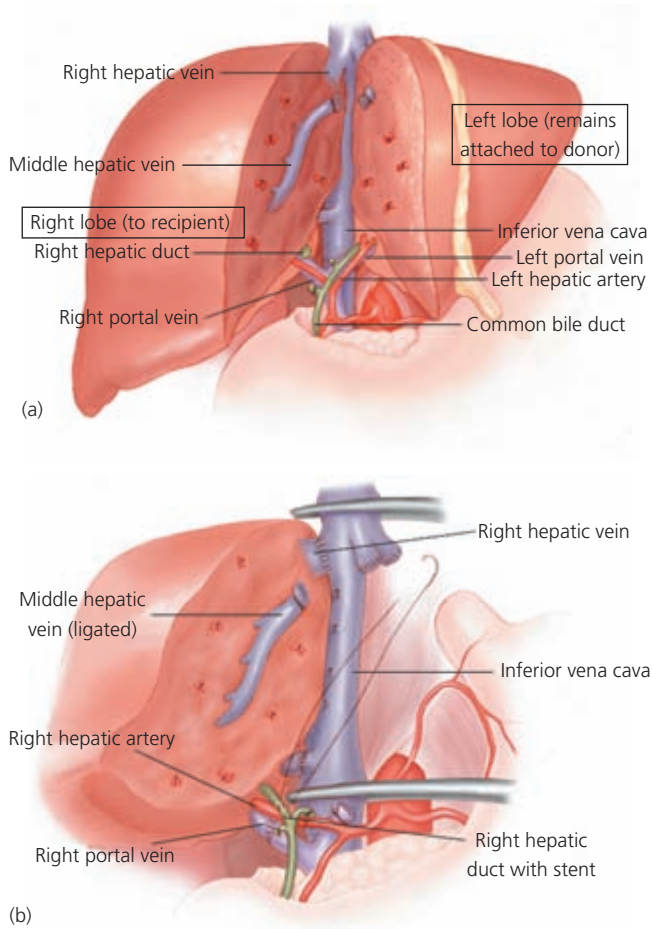

Figure 92.4 Illustration of technique of living donor liver transplantation. **(a)** Right donor hepatic lobectomy; **(b)** right lobe implantation into recipient. Courtesy of John P. Roberts, MD, Department of Surgery, University of California, San Francisco, CA.

In both the donor and the recipient, there is rapid regeneration of the liver. While the recipient appears to achieve the anticipated liver mass within 6–8 weeks, the donor may not regenerate fully even after a full year [19–21].

From the candidate's perspective, the primary advantage of living-related liver transplantation is the assurance of a transplant opportunity at an elective time point in the course of progressive liver disease. The primary disadvantage of living-related liver transplantation is the increased rate of vascular and biliary complications. Hepatic artery thrombosis, portal vein thrombosis, and especially biliary leaks and strictures occur at significantly increased frequency compared to deceased donor liver transplantation [22–27]. From the donor's perspective, there is the risk of not only morbidity but also mortality with donor hepatectomy [28–31]. It is generally acknowledged that the risk of left lateral segmentectomy is less than that of right hepatectomy, although the full and accurate extent of risk is undetermined because both the numerator and the denominator are not precisely known [20,32,33]. To date, the surgical mortality risk is estimated at 0.1% for left lateral segment donation and may approach 0.5% for right lobe donation [32]. There have been several position papers presenting the ethics and surgical techniques of adult-to-adult, living donor liver transplantation and practice guidelines have been established at both national and international consensus conferences [8,32,33].

Indications for liver transplantation

Prognostic models for liver failure and non-disease-specific complications

Liver transplantation should be considered for patients with cirrhosis when one or more signs of hepatic decompensation develop. In a study from Barcelona [34], the probability of decompensation was 58% at 10 years after the diagnosis of cirrhosis. Ascites was the most common manifestation of hepatic decompensation, followed by jaundice, hepatic encephalopathy, and variceal bleeding. The median survival with compensated cirrhosis was 8.9 years, compared to only 1.6 years for decompensated cirrhosis [34].

The Child–Turcotte–Pugh (CTP) scoring system [35] has been used for several decades to determine prognosis for patients with advanced chronic liver failure from a wide spectrum of liver diseases [36]. This scoring system is based on five variables:

- stage of encephalopathy
- severity of ascites
- serum bilirubin level
- serum albumin level
- international normalized ratio (INR) for prothrombin time.

A CTP score of 7 was determined to be the minimum criterion for listing for cadaveric liver transplantation in the past [37], but this requirement is no longer necessary. This system was also previously employed by the United Network for Organ Sharing (UNOS) to determine priority for organ allocation [38], but it lacks objectivity in assessing the severity of encephalopathy and ascites. In 2002, this system was replaced by the MELD system for determining the priority for receiving a cadaveric organ among liver transplant candidates.

Model for End-stage Liver Disease (MELD)

The MELD prognostic score was developed using a formula based on serum creatinine, serum bilirubin, and INR for prothrombin time to predict short-term mortality for patients with end-stage chronic liver disease (Table 92.1). This model was based on multivariate analysis of readily and objectively measurable variables that was originally developed to assess the short-term prognosis of patients with cirrhosis undergoing the transjugular intrahepatic portosystemic shunt (TIPS) procedure [39]. The MELD scoring system was subsequently shown to be also useful in grading the risk of death at 1 week, 3 months, and 1 year for patients with end-stage chronic liver disease (Table 92.1) [40]. The MELD score was implemented by UNOS in 2002 as a disease severity index to determine priority for organ allocation [41], replacing the previous system based on the CTP score and other specific clinical criteria [38].

Using the MELD, the patient with the highest score (range 6–40) – reflecting the greatest risk for short-term mortality – will receive a liver transplant first, and the length of waiting time will be a minimal factor in determining organ allocation [41]. It has been shown in one study that the survival advantage of liver transplantation increases with increasing MELD score, and is only evident when the recipient MELD score exceeds 15 at the time of liver transplantation [42]. In this

Table 92.1 The Model for End-stage Liver Disease (MELD) scoring system determining disease severity and priority for organ allocation: 3-month mortality acccording to the MELD score[a]

	MELD score				
	≤ 9	10–19	20–29	30–39	≥ 40
Hospitalized patients with cirrhosis	4% (6/148)	27% (28/103)	76% (16/21)	83% (5/6)	100% (4/4)
Outpatients with cirrhosis	2% (5/213)	6% (14/248)	50% (15/30)	–	–

a Data from Wiesner et al. [41]
MELD risk score[b] = 10 × (0.957 × log e [creatinine mg/dL] + 0.378 × log e [bilirubin mg/dL] + 1.120 × log e [INR]) + 6.43.
Current calculation of a MELD score uses a minimum value for the creatinine of 1.0 mg/dL, bilirubin of 1.0 mg/dL, and INR of 1.0.
A maximum value of 4.0 mg/dL applies to creatinine. The scores are rounded to the nearest whole number with a score range of 6 (minimum) to 40 (maximum).
The MELD calculator is available from the Internet: http://www.thedrugmonitor.com/meld.html

study, patients with a lower MELD score, below 15, have a greater likelihood of being alive on the waiting list without liver transplantation than surviving the first year after liver transplantation [42]. Since 2005, UNOS policy also requires that donor livers be allocated to local candidates with MELD scores less than 15 only if there are no regional candidates with MELD scores of 15 or more. Patients with fulminant liver failure, who are at greatest risk for short-term mortality, are classified in a separate category and listed at the highest priority (status 1).

Ascites and related complications

Approximately 50% of patients with cirrhotic ascites succumb in 2 years [43]. Up to 10% of patients with cirrhotic ascites are refractory to standard diuretic therapy. Treatment options for refractory ascites include serial therapeutic paracentesis, peritoneal venous shunt, and TIPS [44]. Survival for patients with refractory ascites is only about 25%–50% at 1 year regardless of the type of treatment they have received [44–48]. Ascites may be associated with spontaneous bacterial peritonitis and hepatorenal syndrome, both of which further shorten survival. The 1-year probability of survival was reported in one study to be 66% for patients with cirrhosis and ascites without spontaneous bacterial peritonitis, compared to only 38% for patients with this complication [49]. In one-third of patients with spontaneous bacterial peritonitis, renal impairment develops despite treatment of the infection [50,51]. The use of intravenous albumin has been shown to be of benefit in improving renal impairment in patients with spontaneous bacterial peritonitis [51]. The use of midodrine and subcutaneous octreotide may lead to improvement of hepatorenal syndrome in patients with end-stage liver disease [44,52].

Portal hypertensive bleeding

Bleeding from esophageal or gastric varices carries a high 30-day mortality ranging from 30% to 50%, and the risk of recurrent variceal bleeding is about 60%–70% within 2 years of the index hemorrhage [53]. Better understanding of the basic pathophysiology of portal hypertension and bleeding has led, in addition to endoscopic therapy, to important advances in pharmacological therapy including beta-blockade for primary and secondary prophylaxis of variceal bleeding, and somatostatin for the initial control of hemorrhage [53].

A major advance in the treatment of variceal bleeding is the use of TIPS, which has a more than 90% technical success rate in experienced hands [54,55]. Compared to endoscopic treatments, TIPS confers a lower rate of rebleeding but no survival advantage [53,56,57]. The current accepted indications for TIPS include emergency (rescue or salvage) therapy for uncontrolled, acute portal hypertensive bleeding and prevention of recurrent portal hypertensive bleeding for which endoscopic or pharmacological therapy has either failed or cannot be applied [57]. Severe hyperbilirubinemia occurring after the creation of TIPS is a sign of rapid hepatic decompensation, associated with a particularly ominous short-term prognosis [58]. Encephalopathy develops in about 30% of patients after TIPS and is usually reversible with medical therapy [53].

A major problem with TIPS is the high incidence of shunt stenosis or occlusion, estimated to be 30% over the first year and 50% at 2 years [59]. TIPS has not been shown in a study from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) Liver Transplant Database to have any significant impact on liver transplantation in terms of total operative time, blood product requirements, survival or length of hospital stay [60]. Consequently, preoperative portal decompression solely for facilitating liver transplantation is not an acceptable indication for TIPS. Occasionally, TIPS also creates technical difficulties for liver transplantation secondary to stent migration or extension into the extrahepatic portal or hepatic vein [57].

Portosystemic shunt surgery provides more definitive control of bleeding compared to sclerotherapy [53,61,62] or TIPS [63] in patients with adequate hepatic function to tolerate surgery, but does not improve survival and is also associated with an increased incidence of encephalopathy. Mesocaval or splenorenal shunts are the preferred surgical approaches in consideration for future liver transplantation because the hepatic hilum is avoided.

Hepatic encephalopathy

Hepatic encephalopathy is a complex neuropsychiatric syndrome that may be present in 50%–70% of all patients with cirrhosis, including those with subtle abnormalities only demonstrable by psychometric testing [64]. Hepatic encephalopathy is aggravated by portosystemic shunting by way of collaterals secondary to portal hypertension, and is also associated with surgical portosystemic venous shunting and the creation of TIPS [53]. Nonabsorbable disaccharides such as lactulose, and antibiotics including neomycin and rifaximin with activities against urease-producing bacteria are effective in reversing hepatic encephalopathy. The efficacy of lactulose and neomycin in the treatment of hepatic encephalopathy appears to be equal. Rifaximin, a nonabsorbed oral antibiotic used in the treatment of traveler's diarrhea, provides an effective, safe alternative for the treatment of hepatic encephalopathy [65]. Symptomatic hepatic encephalopathy is an indication for consideration of liver transplantation. Uncommonly, some patients with hepatic encephalopathy have progressive, debilitating neurological or neuropsychiatric syndromes such as dementia, spastic paraparesis, cerebellar degeneration, and extrapyramidal movement disorders, associated with structural abnormalities of the central nervous system [64]. If very advanced, neurological injury may not be reversible with liver transplantation, although one report has suggested that there may be gradual improvement after liver transplantation [66].

Quality-of-life issues

Encephalopathy, ascites, and debilitating fatigue are some of the common manifestations of end-stage liver disease that markedly impair a patient's quality of life. Even subclinical hepatic encephalopathy, determined only by psychometric analysis, can cause significant impairment of daily functioning [67,68]. Liver transplantation improves quality of life, and facilitates a return to gainful employment in over half of patients [69–71]. However, improving the quality of life of a patient should not be the sole indication for liver transplantation in the absence of advanced liver disease evidenced in a MELD score of 15 and CTP score of 7.

Disease-specific issues and outcome after liver transplantation

More than 60 distinct diseases have been treated with liver transplantation [72]. Hepatitis-C-related cirrhosis is the most common indication for liver transplantation in the United States, accounting for close to 50% of all liver transplants. The guidelines for liver transplantation, special considerations, the controversial aspects of certain disease indications, and posttransplant outcome for specific types of liver disease are selectively reviewed (Table 92.2). Many of the less common types of liver disease will not be discussed in detail in this Chapter.

Table 92.2 Summary of disease-specific guidelines for liver transplantation

Liver disease	Guidelines for liver transplantation and pretransplant issues	Special considerations
Alcoholic liver disease	• Abstinence ≥ 6 months • Favorable assessment by substance-abuse professional • Acceptance by center evaluation committee • Appropriate medical indications for liver transplantation	• Lack of reliable predictors for recidivism • Reduced long-term graft survival if return to abusive alcohol consumption
Hepatitis B virus	Suppression of HBV DNA before liver transplantation with nucleoside/nucleotide analogues reduces risk for posttransplant recurrence and may improve liver function	• HBIG prophylaxis effective but expensive • Combination of HBIG and lamivudine or other antivirals now standard prophylaxis • Potential role of low-dose HBIG or short-term HBIG + antiviral in low-risk patients
Hepatitis C virus	Suppression of HCV RNA with antiviral therapy before liver transplantation in selected patients (LADR)	• High rates of recurrence despite favorable medium-term survival • Suboptimal response and poor tolerance to interferon and ribaviron after transplant • Adverse impact of recurrent HCV on graft survival ≥ 5 years
Hepatocellular carcinoma	Milan criteria for liver transplantation: • single lesion ≤ 5 cm • fewer than three lesions each ≤ 3 cm	• Improved recurrence-free survival using the Milan criteria • Impact of MELD organ allocation on waiting time and dropout • Potential role for expansion of Milan criteria and down-staging
Hemochromatosis	Cardiac dysfunction due to iron overload should preclude liver transplantation	• Reduced survival after liver transplantation due to cardiac and infectious complications • Early diagnosis and phlebotomy
Wilson disease	List status 1 for acute Wilson disease with fulminant liver failure	Neuropsychiatric manifestations may not reverse after liver transplantation
PBC/PSC		• The groups with the best survival after liver transplantation • Increased risk of cholangiocarcinoma in PSC • Recurrent PBC and PSC in about 20%, generally mild disease
Fulminant hepatic failure	• List for liver transplantation (status 1) when stage 2 encephalopathy • King's College and other prognostic criteria to help predict need for liver transplantation	• Cerebral edema and sepsis as major causes of death • ICP monitoring for advanced stage encephalopathy • Sustained cerebral perfusion pressure (MAP – ICP) < 40 mmHg precludes liver transplantation

HBIG, hepatitis B immunoglobulin; HBV, hepatitis B virus; HCV, hepatitis C virus; ICP, intracranial pressure; MAP, mean arterial pressure; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

Alcoholic liver disease

Once considered a highly controversial indication for liver transplantation on both medical [73] and ethical [74] grounds, alcoholic liver disease is second only to hepatitis C as the leading indication for liver transplantation, accounting for up to 20% of all cases [75]. Early reports have suggested that survival at 5 years following liver transplantation for alcoholic liver disease is at least as good as for most other indications [75,76]. The obvious concern, however, is the possibility of returning to alcohol consumption after liver transplantation. Long-term follow-up data have emerged showing a significant adverse impact of alcohol recidivism on long-term patient survival at 10 years after liver transplantation [77,78], especially among patients who returned to abusive drinking [77]. There are insufficient data to fully understand the impact of modest alcohol consumption after liver transplantation, and the extent and pattern of graft injury. Nevertheless, the patient transplanted for alcoholic liver disease who drinks again creates a highly emotional and demoralizing issue and is often perceived as a treatment failure, even though the absolute alcohol intake may be low and graft function normal. The reported incidence of recidivism following liver transplantation has typically been about 20%, but the definition of recidivism varies widely in these studies [75,79]. In a recent prospective observational study using structured interviews and a standardized Alcohol Abuse and Alcoholism quantity–frequency questionnaire [80], 42% of patients transplanted for alcoholic liver disease admitted to using alcohol at least once following liver transplantation, and over 20% had returned to abusive drinking. Better insight into patient selection, closer monitoring, and ongoing counseling for these patients after liver transplantation are therefore needed.

There is a strong consensus based on a survey of liver transplant centers and a National Institutes of Health workshop to require an established period of abstinence, typically 6 months, before approval for listing for liver transplantation [81,82]. The required period of abstinence is useful because abstinence alone can often lead to sustained clinical improvement, thereby obviating the need for liver transplantation. Although some studies have reported a significant correlation between a short duration of alcohol abstinence of less than 6 months and the risk of recidivism [77,83], the 6-month rule for alcohol abstinence as a predictor of long-term sobriety remains controversial [84], and should be used in conjunction with other psychosocial and psychiatric parameters in the patient selection process [85]. In most transplant centers, the current criteria for liver transplantation in patients with alcoholic liver disease include:

- alcohol abstinence of at least 6 months
- favorable assessment by a substance abuse professional
- approval by the transplant center evaluation committee
- fulfillment of the medical criteria to be listed for liver transplantation.

Hepatitis B

In the late 1980s and early 1990s, liver transplantation for patients with cirrhosis as a result of chronic hepatitis B infection was considered a relative contraindication because of the high rates of hepatitis B recurrence and graft failure. The most aggressive form of hepatitis B recurrence, known as fibrosing cholestatic hepatitis, often leads to death within weeks of onset of graft reinfection [86]. Subsequently, the use of high-dose intravenous hepatitis B immunoglobulin (HBIG) after liver transplantation to maintain hepatitis B surface antibody titer above 100 IU/L has significantly reduced the incidence of hepatitis B recurrence and improved survival [87–90]. Patient and graft survival in patients undergoing liver transplantation for hepatitis B are comparable to that in patients transplanted for other nonmalignant liver disease [91,92]. The benefit of HBIG appears to be greatest among patients with negative hepatitis B DNA and hepatitis B e-antigen before liver transplantation [87]. Despite excellent graft and patient survival, hepatitis B recurrence still occurs in 6%–52% of patients who receive intravenous HBIG prophylaxis after liver transplantation [93]. Graft reinfection despite HBIG prophylaxis may be caused by inadequate neutralization because of overwhelming amounts of hepatitis B virus replication or breakthrough infection by hepatitis B surface antigen protein escape mutants [94,95]. Another concern of intravenous HBIG is its high cost, estimated to be $30 000–$50 000 in the United States during the first year of therapy after liver transplantation.

Another major advance in the treatment of hepatitis B infection in the past decade is the development of oral nucleoside and nucleotide analogues. Four of these antiviral agents, lamivudine, adefovir, entecavir, and telbivudine, have been approved by the Food and Drug Administration for the treatment of hepatitis B virus infection in the United States. In contrast to interferon-α, these antiviral agents appear to be safe and well tolerated in patients with advanced decompensated cirrhosis caused by chronic hepatitis B [96–99]. The primary goal of treatment is to suppress virus replication to achieve undetectable hepatitis B DNA before liver transplantation, thereby lowering the risk for posttransplant hepatitis B recurrence [91]. In some patients with decompensated cirrhosis due to hepatitis B virus infection, treatment with lamivudine has been shown to improve or stabilize liver function to the extent of delaying or even preventing liver transplantation [73–76]. An important concern of long-term use of lamivudine beyond 6–9 months, regardless of the clinical setting, is the high rate of emergence of lamivudine-resistant mutant virus (YMDD mutant). Newer agents such as adefovir and entecavir with a lower incidence of drug recurrence are preferable to lamivudine in the treatment of hepatitis-B-related decompensated cirrhosis. Although data are limited, combination therapy with agents such as lamivudine plus adefovir may be the best approach in patients with decompensated cirrhosis to maintain sustained virological

suppression and minimize the risk for drug resistance, which can precipitate acute deterioration of liver function. Combination therapy is particularly prudent in patients with a history of lamivudine resistance [91,100].

Currently, the combination of HBIG plus lamivudine or another nucleoside/nucleotide analogue is the most commonly used prophylactic regimen for patients infected with hepatitis B virus [91]. The use of lamivudine alone without HBIG is associated with a high reinfection rate of 22%–50% at 3 years after liver transplantation as a result of the selection of resistant mutants [101–103]. In low-risk patients with undetectable hepatitis B DNA at the time of liver transplantation, HBIG can be safely discontinued after short-term use (usually 12 months) following liver transplantation, with continued long-term antiviral therapy [104]. High-risk patients, including those with a high level of hepatitis B DNA without antiviral therapy or those with virological breakthrough or persistent viremia despite antiviral therapy, would likely require indefinite HBIG and maintenance antiviral therapy [91].

The use of a lower dosage of intramuscular HBIG in combination with lamivudine has also been evaluated as a cost-saving strategy in small pilot studies, with promising results in terms of the risk for hepatitis B recurrence [105,106]. Recently, the Australia and New Zealand group reported impressive results using a low dosage (400–800 IU/month) of intramuscular HBIG plus lamivudine in 147 patients with hepatitis B infection after liver transplantation [107]. The 5-year patient survival rate was 88%, and the actuarial risk of hepatitis B recurrence was only 4% at 5 years. The baseline hepatitis B DNA level was associated with hepatitis B recurrence. This strategy of low-dose HBIG given intramuscularly plus lamivudine appears to be highly effective and cost saving and should be further validated. More studies are also needed to evaluate the efficacy of newer antiviral agents in combination with HBIG as well as a combination of two antiviral agents in the prophylaxis of hepatitis B virus infection after liver transplantation.

Active immunization using standard or high-dose conventional hepatitis B vaccines to replace HBIG has been investigated as a potential strategy to prevent graft reinfection [108,109]. An earlier report showed encouraging results in selected patients at low risk of hepatitis B recurrence (negative hepatitis B DNA at the time of liver transplantation) who had been given HBIG prophylaxis for at least 18 months before receiving a series of three double doses of conventional recombinant hepatitis B vaccine. Fourteen of 17 patients (82%) developed a protective hepatitis B surface antibody titer of more than 10 IU/cm^3 [85]. However, subsequent studies reported low efficacy of this strategy with a response rate of less than 10% [91,109]. Consequently, active immunization should not be used in posttransplant patients as a means of preventing recurrent hepatitis B infection outside clinical trials.

Hepatitis C

Hepatitis C is the leading indication for liver transplantation in the United States and in Europe. The major problem concerning these transplant recipients is the high rate of graft reinfection with hepatitis C virus which occurs almost universally in these recipients, with histological evidence of hepatitis developing in about 40%–60% of patients at a median of 4–6 months after liver transplantation [91,110,111]. It is also increasingly evident that the course of posttransplant recurrent hepatitis C is more rapid when compared with that before liver transplantation. Between 10% and 40% of patients with recurrent hepatitis C virus infection progress to cirrhosis within the first 5 years after liver transplantation, and the median rate of fibrosis is estimated to be between 0.3 and 0.6 stages per year [110–113]. Some studies have reported that graft survival at or beyond 5 years following liver transplantation is reduced compared to patients without hepatitis C infection [114]. A severe form of recurrence, resembling the syndrome of fibrosing cholestatic hepatitis in patients infected with hepatitis B virus, has also been reported with recurrent hepatitis C [115]. Factors predictive of more severe recurrent disease and worse outcome after liver transplantation have not been fully elucidated, but those most consistently linked with more severe recurrent disease include older donor age [113,116–118], and treatment of acute rejection with OKT3 (Muromonab-CD3) or corticosteroid boluses [110,119–123]. Cytomegalovirus (CMV) infection has also been associated with more severe recurrent hepatitis C infection [124,125]. Other viral factors, including a high pretransplant hepatitis C RNA titer (at least 10^6 Eq/mL) [126] and genotype 1b [88,127], have also been suggested to correlate with more severe recurrent infection. Other studies, however, have not supported this association [128]. The type of calcineurin inhibitors in the immunosuppression regimen (cyclosporine [ciclosporin rINN] vs tacrolimus) does not appear to have any significant impact on the incidence or severity of hepatitis C recurrence [129]. The impact of mycophenolate mofetil on hepatitis C recurrence is also unclear. Additional studies are needed to further evaluate the impact of different immunosuppressive regimens as well as the use of new immunosuppressive agents on the course of recurrent hepatitis C.

The most common approach to the management of recurrent hepatitis C virus infection is to initiate antiviral therapy when there is histological evidence of recurrent disease [91,110,130]. Considerable variation exists among liver transplant centers in terms of the threshold for the disease severity according to the stage of fibrosis before treatment is initiated, in part because of the suboptimal treatment response rate and the significant side effects of treatment. The recommendation from an International Liver Transplantation Society Expert Panel Consensus Conference is to initiate treatment for patients with stage II fibrosis [112]. Combination therapy with pegylated interferon-α and ribavirin is the standard

treatment for hepatitis C in both the transplant and the nontransplant populations. A number of studies using standard interferon-α or pegylated interferon-α plus ribavirin for recurrent hepatitis C after liver transplantation, including two randomized controlled trials [131,132], have shown a sustained virological response rate of 12%–35% after about 48 weeks of therapy [131–136]. Poor patient tolerance and the effects of immunosuppression are important factors contributing to the suboptimal response in this population. Factors contributing to poor patient tolerance include renal insufficiency, anemia, and thrombocytopenia commonly observed in these patients especially in the early posttransplant period. The dosage of ribavirin used in these patients is typically less than that used in the nontransplant setting, in large part because of the anemia and reduced glomerular filtration rate associated with either cyclosporine or tacrolimus. Most patients require the support of growth factors, including erythropoietin and granulocyte colony-stimulating factor, during treatment.

Another approach that has been evaluated in several studies is preemptive therapy initiated between 2 and 8 weeks following liver transplantation [132,137,138]. The sustained response rate does not appear to be clearly better than that achieved with the administration of treatment later in the course when histological recurrence is established [130,137,138]. Furthermore, factors contributing to poor patient tolerance and the need for dose reductions, which include pancytopenia, anemia, and renal insufficiency, are often encountered with higher frequency and severity in the early postoperative period. Consequently, most experts do not recommend preemptive therapy [91,130]. Although interferon-α treatment has been suggested to cause acute rejection in the liver graft, the incidence and risk of this association have not been clearly defined [139]. In randomized controlled studies of interferon treatment compared with no treatment for recurrent hepatitis C [131,132], the incidence of acute rejection did not appear to be significantly higher in the treated group than in the control group.

The magnitude of the problem with recurrent hepatitis C infection following liver transplantation has led to a number of strategies being investigated in the hope of reducing the risk of graft reinfection. The University of Colorado group reported the use of a low, accelerating dose regimen (LADR) of interferon and ribavirin for patients with mildly decompensated cirrhosis due to hepatitis C awaiting liver transplantation in an attempt to achieve virological clearance before transplant [140]. The overall sustained virological response rate was 22%, and was much higher in patients with genotype 2 or 3 than in patients with genotype 1 (60% vs 11%, respectively). Sustained virological response to treatment before liver transplantation also correlated with absence of recurrent hepatitis C after transplantation. This strategy should only be considered in patients with mildly decompensated cirrhosis (Child's A and B) who are listed and ready for liver transplantation in the event of hepatic decompensation after treatment. A high rate of serious adverse events with this combination therapy has been reported in those patients infected with hepatitis C virus who have more advanced liver failure and are awaiting liver transplantation [141]. A randomized controlled study from the Adult-to-Adult Living Donor Liver Transplant Consortium (A2ALL) in the United States is in progress to determine the benefit of pegylated interferon and ribavirin therapy for patients with hepatitis C genotype 1 infection who are awaiting liver transplantation.

With the success of HBIG in the prophylaxis of hepatitis B virus infection after liver transplantation, several groups have examined the safety and efficacy of hepatitis C immunoglobulin (HCIG) to prevent hepatitis C reinfection [91]. However, a randomized controlled study did not show a significant benefit of HCIG in reducing the hepatitis C RNA level when compared to no treatment [142].

Transplantation of a hepatitis C-positive donor liver into a recipient with hepatitis C cirrhosis does not appear to result in a worse outcome in the initial years after liver transplantation [143], and may prove to be an important strategy for expanding the donor pool for liver transplantation. Whether patients infected with hepatitis C virus have worse results after living donor transplantation than after deceased donor liver transplantation remains highly controversial. In an analysis of the UNOS database comparing 279 living donor transplant recipients with 3955 deceased donor transplant recipients, the short-term graft survivals at 1 and 2 years appeared to be equal in the two groups [144]. According to the A2ALL study [145], the overall 3-year graft survival, but not patient survival, was significantly lower for live donor transplantation compared with cadaveric liver transplantation. However, the difference in graft survival between the two groups was limited to the first 20 live donor transplantation operations at each center, suggesting that limited experience and technical complications during the learning phase of live donor transplantation might have a significant contribution to the unfavorable outcome.

Hepatocellular carcinoma

Liver transplantation for hepatocellular carcinoma (HCC) in unselected patients, including those with extensive and bulky tumors, was associated with dismal outcome, primarily because of aggressive tumor recurrence [146]. The observation that small incidental tumors have no adverse impact on posttransplant survival compared to no tumors has shaped the development of restrictive tumor criteria to limit the risks for tumor recurrence after liver transplantation [146]. In the seminal study by Mazzaferro and colleagues from Milan, Italy [147], 35 patients who met the specific criteria of a solitary tumor not exceeding 5 cm or no more than three tumor nodules, none greater than 3 cm (Milan criteria) had excellent overall and recurrence-free survival rates of 85% and 92%, respectively, at 4 years after liver transplantation.

These criteria have been subsequently validated in a number of studies, showing overall 5-year patient survival rates to be consistently 70% or better [148]. The Milan criteria [147] have been incorporated into the UNOS modified staging classification for HCC [149] as T1 (one lesion 1.9 cm) and T2 (one lesion 2–5 cm, or two or three lesions all 3 cm). The adoption of the Milan criteria as selection guidelines for liver transplantation in the United States was largely responsible for the steady improvement in posttransplant survival in recent years according to UNOS data [150]. Currently, under the MELD system of organ allocation, HCC accounts for about 10% of all transplants in the United States [3], and this number roughly equals that for cholestatic liver disease (Fig. 92.5). Factors predicting recurrence of HCC after OLT have been evaluated in many published reports. The most consistently shown factors correlating with HCC recurrence include tumor stage based on size and number [147,151–153], vascular invasion [153–157], poorly differentiated histological grade [154–156], and high preoperative α-fetoprotein [152,153,155,157].

Despite excellent survival after liver transplantation for selected patients with HCC, tumor growth in the face of increased waiting time for liver transplantation in the United States presents a considerable challenge, as eventually, acceptable criteria for liver transplantation may be exceeded. As a result of the high dropout rate related to tumor growth in patients on the waiting list [158], the current MELD system for organ allocation is adjusted to give higher priority to patients with HCC who are at increased risk for exclusion based on tumor characteristics [159]. This system is designed to reduce the waiting time, and so the rate of dropout from the waiting list. When the HCC-adjusted MELD system of organ allocation was first implemented in 2002, there was an initial overestimation of the rate of dropout from the waiting list. Consequently, the adjusted MELD scores for these patients have been downgraded several times since then. Following the latest modification, only patients with T2 HCC are eligible for priority listing starting with a MELD score of 22. These patients are also eligible for upgrade of the priority score for every 3 months on the waiting list [149]. Patient with T1 HCC (single tumor mass < 2 cm in diameter) are no longer eligible for priority listing for liver transplantation because these patients are at low risk of dropout from the waiting list [158], and there is also a high rate of false radiological diagnosis with no tumor in the liver explant in patients with presumed T1 HCC undergoing liver transplantation [160].

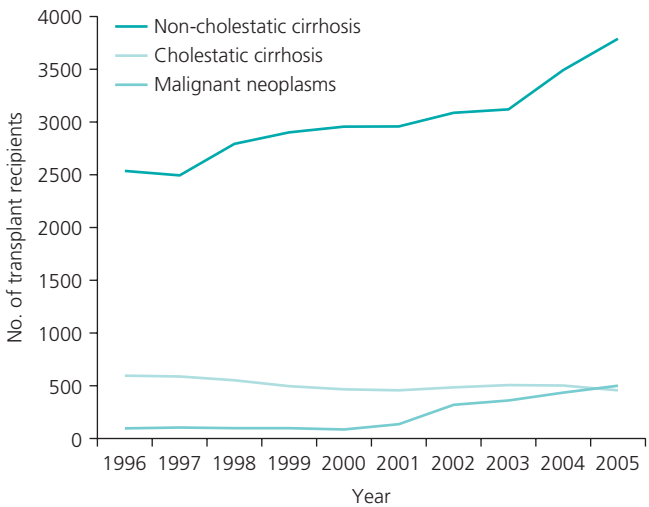

Figure 92.5 Liver transplantation in the United States according to major primary diagnosis. Noncholestatic cirrhosis includes viral hepatitis C and B, alcoholic and cryptogenic cirrhosis. Cholestatic liver disease/cirrhosis includes primary biliary cirrhosis and primary sclerosing cholangitis (but not biliary atresia). Malignant neoplasms are comprised mainly of hepatocellular carcinoma. Data from the Scientific Registry of Transplant Recipients [352].

To control tumor growth while waiting for a suitable organ, many liver transplant centers routinely use locoregional therapies, including chemoembolization, percutaneous ethanol injection, and radiofrequency ablation, as "bridging" procedures to liver transplantation [146]. However, the benefits of these procedures in reducing the rate of dropout from the waiting list and preserving the chance for successful liver transplantation, or to improve outcome following liver transplantation remain unproven [146,161,162]. Based on cost-effectiveness analysis, these treatments are recommended to slow tumor growth when the waiting time for liver transplantation is expected to be at least 6 months [146,162].

The ideas of expanding the conventional HCC criteria for liver transplantation and down-staging larger tumors to meet conventional criteria take center stage in a widespread debate [163–165]. The proposed University of California, San Francisco (UCSF) criteria represent a modest expansion of the tumor size limits beyond the Milan criteria (one lesion 6.5 cm, or two or three lesions all 4.5 cm with total tumor diameter 8 cm) [151]. Several groups have validated the UCSF criteria based on tumor pathology [152,166,167], but the ability of these criteria to serve as selection guidelines based on preoperative imaging has been questioned [167]. A prospective study is in progress to validate the UCSF criteria based on pre-operative imaging. Preliminary data from our institution have also shown that successful down-staging of large tumors by locoregional treatments can be achieved in the majority of highly selected patients, in association with excellent posttransplant outcome [164,168]. Data from longer follow-up with a larger cohort are needed to confirm these encouraging results.

Whether the results of living donor transplantation are the same as those with deceased donor liver transplantation for patients with HCC remains unclear. While studies from Asia reported similar outcome with living donor transplantation for patients with HCC meeting Milan or UCSF criteria [166,169], a retrospective study from the A2ALL group sug-

gested worse outcome following living donor transplantation when compared to deceased liver transplantation [170]. The inclusion of a high proportion of patients with advanced HCC beyond Milan criteria may be an important contributing factor to the high HCC recurrence rate in the group receiving living donor transplants. Concerns have also been raised that the short waiting time with living donor transplantation or under the MELD system would include patients with less favorable tumor biopsy results, who would have been selected out by the traditionally longer waiting time for liver transplantation [171]. To address this concern, a recent study showed no significant correlation between waiting time and the risk of HCC recurrence or survival following liver transplantation [171].

Hemochromatosis

Patients with hereditary hemochromatosis are at increased risk for the development of cirrhosis, liver cancer, cardiomyopathy, and diabetes [149]. Most cases of hereditary hemochromatosis have been linked to mutation in the *HFE* gene, including the homozygous C282Y mutation and the heterozygous C282Y/H63D mutation. Studies from the past 10 years have consistently demonstrated a less favorable outcome following liver transplantation for patients with hemochromatosis [173–178]. According to the National Hemochromatosis Transplant Registry involving 260 patients with end-stage liver disease and hepatic iron overload enrolled from 12 liver transplant centers [178], *HFE* mutation with homozygosity for the C282Y was identified in 14 patients (7.2%). Their 1- and 5-year survivals following liver transplantation were only 64% and 34%, respectively, which were significantly worse than in patients heterozygous for the C282Y/H63D mutation, as well as wild-type patients. Non-*HFE*-associated patients with hepatic iron overload also had significantly worse survival when compared with the overall population undergoing liver transplantation. Cardiac and infectious complications accounted for about half of the deaths in patients with hepatic iron overload following liver transplantation [178]. A high incidence of hepatic malignancies (27%) was also observed at the time of liver transplantation in one study [174]. The diagnosis of hemochromatosis was often not made until after liver transplantation [173,175,178], a factor that might have contributed to the poor outcome. Collectively, these studies suggest that hereditary hemochromatosis should be considered a controversial indication for liver transplantation. Once the diagnosis of hemochromatosis is confirmed, early initiation of phlebotomy may diminish the likelihood of posttransplant cardiac complications. Thorough cardiac evaluations with right heart catheterization and possibly endomyocardial biopsy are recommended before liver transplantation [173,175]. Cardiac dysfunction as the result of excessive iron infiltration should preclude liver transplantation in view of the high perioperative and late postoperative cardiac mortality [173,176,178].

Wilson disease

The hallmark of Wilson disease, a relatively rare, inherited autosomal recessive disorder of copper metabolism, is its highly variable clinical presentation [179,180]. The diagnosis of Wilson disease is suggested by a young age at presentation and the presence of low serum ceruloplasmin, neuropsychiatric symptoms, and Kayser–Fleischer rings on slit-lamp opthalmological examination, although atypical clinical presentations and the absence of these classic signs and are also common [179,180]. Wilson disease may present as fulminant hepatic failure with a high mortality when managed with medical treatment alone [181,182]. Such patients can be listed as UNOS status 1 for liver transplantation [183]. The presence of a Coomb negative hemolytic anemia and a serum alkaline phosphatase to serum bilirubin ratio of less than 2 is suggestive, but not diagnostic, of fulminant Wilson disease [175]. Nazer and colleagues have developed a useful prognostic score (based on serum bilirubin, serum aminotransferase levels, and prothrombin time), which stratifies patients with Wilson disease according to the risk of death without liver transplantation [184]. Recently, additional prognostic models have been tested for fulminant Wilson disease in adults and children showing good discriminatory power for outcome and need for liver transplantation in these patients. They include a MELD score of 30 [183], as well as a revised King's College Prognostic Index (based on aspartate aminostransferase, albumin, bilirubin, INR for prothrombin time, and white cell count) [185,186]. The neurological and psychiatric manifestations of Wilson disease improve in most but not all patients after liver transplantation [175,182,187].

Non-alcoholic fatty liver disease (NAFLD)

Nonalcoholic fatty liver disease (NAFLD) has been considered the hepatic manifestation of a metabolic syndrome related to insulin resistance, with central obesity as a major component [188,189]. NAFLD includes a spectrum of histological lesions ranging from benign steatosis to nonalcoholic steatohepatitis [188,189], the latter being increasingly recognized as a significant cause of cirrhosis [188–191]. Many patients with cryptogenic cirrhosis appear to have NAFLD as the etiology [190]. According to UNOS data [2,189], the proportion of patients with NAFLD undergoing liver transplantation has increased 35-fold from less than 0.1% in the period 1995–2000 to 3.5% in 2005. During the same time interval, the proportion of patients with cryptogenic cirrhosis undergoing liver transplantation decreased from 9.6% to 6.6%. It seems likely that more cases previously referred to as cryptogenic cirrhosis are recognized as resulting from NAFLD [189].

The adverse impact of obesity in association with NAFLD and other liver disease on morbidity and mortality following liver transplantation has been increasingly recognized. According to an analysis of UNOS data [192], patients who were

severely obese (with a body mass index [BMI] of 35) or morbidly obese (BMI 40) had a significantly greater risk of primary graft nonfunction and a higher 5-year mortality compared to patients who were not obese (BMI < 25). At the authors' institution, patients who are morbidly obese are required to lose weight to achieve a BMI of 40 before they are considered as potential candidates for liver transplantation.

Several reports have suggested that NAFLD may recur after liver transplantation [189,191,193]. This phenomenon has also been described in patients with cryptogenic cirrhosis following liver transplantation, with the presumption that NAFLD is the unrecognized underlying cause of their liver disease before liver transplantation [194,195]. The course of recurrent NAFLD may not be benign. In one study, 9 of 15 patients had recurrence of NAFLD after liver transplantation, and 2 of these patients had rapid progression to cirrhosis or liver failure requiring retransplantation [191]. Additional studies are needed to evaluate the long-term outcome of liver transplantation for NAFLD in a larger group of patients. Recurrent NAFLD after liver transplantation highlights the importance of host-dependent factors, as opposed to liver-dependent factors, in the pathogenesis of the disease. De novo development of NAFLD after liver transplantation in patients with other diagnoses and no risk factors for NAFLD has also been reported [196].

Other metabolic liver diseases

A number of other diseases in the category of inborn errors of metabolism have been successfully treated with liver transplantation [49,175]. The indication for liver transplantation is end-stage cirrhosis caused by some of these diseases, including α_1-antitrypsin deficiency [197], glycogen storage disease (types I, III, and IV) [198], cystic fibrosis [199], erythropoietic protoporphyria [200], and tyrosinemia [201]. At the other end of the spectrum, liver transplantation has been performed solely for the correction of nonhepatic manifestations of certain metabolic diseases in which the genetic defect is expressed in the liver in the absence of significant hepatic dysfunction. Examples include Crigler–Najjar syndrome [202], primary hyperoxaluria [203], familial amyloid polyneuropathy [204], and severe familial hypercholesterolemia [205].

Autoimmune hepatitis

Progression of autoimmune hepatitis to cirrhosis and liver failure requiring liver transplantation is usually seen in patients refractory to conventional immunosuppressive therapy and those who present late in the course of disease without previous diagnostic confirmation or appropriate therapy [206]. Acute, fulminant presentation of severe autoimmune hepatitis refractory to corticosteroid therapy has also been described, for whom status I listing for liver transplantation may be appropriate. Although the outcome of liver transplantation for autoimmune hepatitis is generally excellent, it is increasingly recognized that there is a risk for disease recurrence [207–210]. Bearing in mind a significant limitation with respect to the small number of patients in many single-center reports, and the differences in the definition of recurrent disease based on histology, autoantibodies, liver biochemical profiles, or a combination of these factors, the recurrence rate is estimated to be between 20% and 30% [207–210]. The diagnostic criteria for recurrent autoimmune hepatitis have not been well established. The utility of monitoring autoantibodies after liver transplantation is unclear, and most studies have found that these markers persist to some extent in all patients transplanted for autoimmune hepatitis. Published data on titer or persistence of the autoantibodies are insufficient for predicting recurrence or disease activity after liver transplantation [209]. Histological features of recurrent autoimmune hepatitis may be difficult to differentiate from those of acute cellular rejection. Risk factors for recurrent disease in some studies have included suboptimal immunosuppression, and HLA haplotypes DR3 and DR4 [208]. The use of tacrolimus vs cyclosporine as primary immunosuppression has not been shown to have a significant impact on the risk of disease recurrence [207]. Recurrent autoimmune hepatitis rarely develops in the first year after liver transplantation, presumably because of the higher level of immunosuppression used during this period [209,210]. The course of recurrent autoimmune hepatitis is typically indolent, and the histological features for recurrent disease rapidly resolve in most cases when adequate immunosuppression is restored [209]. One study, however, reported graft failure requiring retransplantation in 6 of 24 patients with recurrent autoimmune hepatitis and more than 1-year of follow-up [210].

Cholestatic liver disease

Patients with cholestatic liver disease, chiefly primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), have traditionally been considered the best candidates for liver transplantation based on superior survival statistics [211,212]. The Mayo clinic risk scores for PBC [213] and PSC [214] have been widely used as prognostic models for predicting survival without therapeutic intervention. The MELD scoring system serves the role of identifying patients with PBC and PSC in need of liver transplantation and prioritizing them on the waiting list. Patients with cholestatic liver disease commonly present with pruritus, which can be severe and refractory to medical therapy [215], and metabolic bone disease with osteopenia predisposing to fractures [216]. No therapy other than liver transplantation has been shown to increase bone mineralization in patients with cholestatic liver disease [216,217]. Although bone loss continues in the first 3–6 months after liver transplantation, bone density then returns to pretransplant levels at 1 year, and may be restored to close to normal within 2–3 years after liver transplantation [217]. Liver transplantation is also effective for xanthomatous neuropathy, an unusual complication of cho-

lestatic liver disease. Biliary atresia is the most common indication for pediatric liver transplantation.

Primary biliary cirrhosis

PBC is characterized by progressive destructions of small intrahepatic bile ducts, leading to eventual cirrhosis and liver failure [218]. PBC is one of the five most common indications for liver transplantation in the United States, associated with superior patient survival of about 85% at 5 years after liver transplantation [212]. Ursodeoxycholic acid (UDCA) improves the biochemical profiles, especially the bilirubin level, in patients with PBC [218]. Some concerns have been raised that using the Mayo risk score, and possibly also the MELD score, in UDCA-treated patients may underestimate their mortality [219]. Although existing data are still inconclusive, UDCA appears to benefit survival in PBC and delay the need for liver transplantation [218,220].

It is widely accepted that PBC does recur in the allograft, with an estimated incidence of about 10%–20% after liver transplantation [207,221,222]. The diagnosis of recurrent PBC can only be made on histological criteria, which may be difficult to differentiate from other causes of bile duct damage in the allograft such as acute or chronic rejection and biliary complications. In a study from the Mayo Clinic using strict histological criteria for disease recurrence defined as the presence of a florid duct lesion or destructive lymphocytic cholangitis within a dense portal infiltrate, the incidence of recurrent PBC was reported to be 17% [221]. A large study from the United Kingdom involving 400 patients also reported a similar rate of recurrence [222]. Serum antimitochondrial antibody titer has not been shown to predict histological recurrence of PBC [221]. The risk of recurrence also does not appear to be different between tacrolimus and cyclosporine as primary immunosuppression [207]. To date, disease recurrence has had little impact on the quality of life or graft survival, and very few patients develop advanced stages of fibrosis or require retransplantation [207,221,222]. Whether UDCA alters the rate of progression of recurrent PBC has not been adequately studied.

Primary sclerosing cholangitis

PSC typically involves both the intrahepatic and extrahepatic bile ducts; with segmental biliary strictures, beading, and dilations. No medical therapy, including high dosages of UDCA, has clearly been proven to be effective in improving the natural history of PSC [223,224]. One of the indications for liver transplantation in PSC is recurrent bacterial cholangitis, which is often associated with bile duct stones or obstructing strictures. Endoscopic stenting or balloon dilation of strictures may benefit some patients, although controlled data are limited. With the extensive scarring of the diseased common bile duct, anastomosis to a Roux-en-Y limb of jejunum is the standard surgical approach at liver transplantation. Patients with PSC are also at risk for developing cholangiocarcinoma, with an estimated incidence of up to 20%–30% at the time of liver transplantation [225–227]. Establishing the diagnosis of cholangiocarcinoma in patients with PSC is often difficult. Neither brush cytology nor serum tumor markers including CA 19-9 are reliable diagnostic tools [228,229]. Fluorescence in situ hybridization from bile duct brushings and aspirates has been shown to improve the sensitivity over routine cytology in detecting cholangiocarcinoma in PSC [229]. Preliminary data on dynamic positron emission tomography scanning for detecting small cholangiocarcinoma in patients with PSC appears to be promising [230]. Survival after liver transplantation is reduced when occult cholangiocarcinoma is present [227,231]. About 70% of patients with PSC also have inflammatory bowel disease, and are at risk for developing colorectal cancer. There have been several reports of colorectal cancer diagnosed after liver transplantation in patients with PSC and ulcerative colitis [232,233]. Continued surveillance by yearly colonoscopy after liver transplantation to detect dysplasia is recommended.

The reported incidence of recurrent PSC after liver transplantation has ranged from 10% to 40% [234,235]. Distinguishing cholangiographic features of recurrent disease from some other conditions, such as ischemic strictures related to prolonged cold ischemia time, ABO incompatibility, and hepatic artery thrombosis can be difficult. In one study from the Mayo Clinic [235], nonanastomotic strictures of intrahepatic and extrahepatic bile ducts developing more than 90 days after liver transplantation defined cholangiographic recurrence, whereas fibroobliterative lesions and fibrous cholangitis were consistent with histological recurrence. Exclusion criteria included hepatic artery thrombosis, chronic ductopenic rejection, donor-recipient ABO incompatibility, and nonanastomotic strictures before day 90 posttransplant. The incidence of recurrence was 18% among 120 patients with PSC receiving liver transplantation, compared with 1% of a control group of 415 patients transplanted for other indications [235]. Recurrent PSC does not appear to impact adversely on patient or graft survival over the intermediate term [208].

Fulminant hepatic failure

Fulminant hepatic failure is traditionally defined as the development of hepatic encephalopathy within 8 weeks after the de novo onset of jaundice [236]. Subacute or subfulminant hepatic failure is observed in a small subgroup of patients in which encephalopathy develops later, up to 26 weeks after the onset of illness [237]. According to recent data from the US Acute Liver Failure Study Group, the predominant cause of fulminant hepatic failure in the United States is acetaminophen-induced hepatic injury [238]. Idiopathic liver failure possibly from an unidentified virus, other types of known viral hepatitis (hepatitis A, B, D, and E, but not hepatitis C) and idiosyncratic drug-induced liver injury comprise the next most common group [238], although differences exist between Europe, the United States, and

Eastern countries [239]. Patients with fulminant hepatic failure typically have no preexisting liver disease, but there are well-recognized examples of hitherto asymptomatic chronic liver disease, such as autoimmune hepatitis and Wilson disease, presenting with fulminant hepatic failure [240]. Acetaminophen-induced acute liver failure may be the result of intentional overdose or a so-called "therapeutic misadventure" [241]. Early diagnosis and administration of *N*-acetylcysteine as soon as possible are of paramount importance in reducing the risk of fulminant liver failure associated with acetaminophen. *N*-Acetylcysteine is proven to be effective when given in the first 24 h after acetaminophen overdose, but may still be of value 48 h or longer after ingestion [240]. Measurement of acetaminophen levels is of limited value in the setting of acute liver failure, and a low level should not dissuade one from treating the patient with *N*-acetylcysteine. A recent report has shown that measurement of serum acetaminophen–protein adducts improves the diagnostic yield of acetaminophen-induced liver failure [242].

Currently, about 6% of liver transplants in the United States are performed for patients with fulminant liver failure [2]. With improved supportive care for these critically ill patients, about 40% of patients with fulminant hepatic failure survive without liver transplantation [240]. Recent reports showed a high rate of spontaneous recovery (62%–68%) without liver transplantation in patients with acetaminophen-induced fulminant liver failure [238,241], but the chance of survival without liver transplantation was 25% or less in cases of drug reaction and indeterminate cause [238]. A variety of medical therapies, including charcoal hemoperfusion, hemodialysis, plasmapheresis, prostaglandin analogues, and corticosteroids have failed to improve outcome in controlled clinical trials involving patients with fulminant liver failure. The well-validated King's College prognostic criteria identify the subgroup of patients at highest risk of dying who should undergo liver transplantation as soon as possible [243] (Table 92.3). A coagulation factor V level of less than 20% (Clichy criteria) also appears to have significant discriminatory prognostic value in non-acetaminophen-induced fulminant hepatic failure [244,245]. A low serum phosphate as a marker for hepatic regeneration has been reported to predict a greater chance of recovery without liver transplantation in patients with acetaminophen-induced acute liver failure [246]. All these prognostic models are, however, imperfect in predicting the likelihood of spontaneous recovery vs the need for liver transplantation. Consequently, the decision of whether or not to proceed with liver transplantation remains a challenge in the management of these patients.

Early referral of patients with fulminant liver failure and also of patients with severe acute liver failure, even in the absence of altered mental status, to a liver transplant center is of paramount importance. Patients with fulminant liver failure should be listed for liver transplantation with the highest priority (UNOS status 1) as soon as they develop clinically evident hepatic encephalopathy [183]. Rapid deterioration and death, most commonly from multiorgan failure, cerebral edema, and sepsis, can occur within days after the development of stage 3 or 4 encephalopathy [239,240]. Intracranial pressure monitoring is commonly instituted in patients with grade 4 hepatic encephalopathy, but this procedure carries a significant risk for intracranial hemorrhage [247,248] and an alternative monitoring approach using the lumbar subarachnoid space is being assessed (Fix, OK, Pitts, LH, Davern, TJ and Bass, NM, unpublished results). At the authors' institution, patients deteriorating beyond stage 3 hepatic encephalopathy are electively intubated and ventilated with adequate sedation both to protect the airway and to prevent surges in intracranial pressure related to psychomotor agitation. Sustained elevation of intracranial pressure with cerebral perfusion pressure (mean arterial pressure – intracranial pressure) below 40 mmHg for at least 2 h despite treatment with mannitol and pentobarbital is associated with a very poor prognosis secondary to irreversible neurological damage and precludes liver transplantation [247]. In addition to mannitol and pentobarbital [240], hypothermia has also been shown to be of benefit in lowering the intracranial

Table 92.3 King's College prognostic criteria for fulminant hepatic failure

Prognostic factors	Predicted mortality rate (%)
Acetominophen-induced fulminant liver failure[a]	
pH < 7.3	95
Grade 3 or 4 encephalopathy, Prothrombin time > 35 s Serum creatinine > 3.5 mg/dL	67
Nonacetaminophen-induced fulminant liver failure[b]	
Prothrombin time > 35 s	100
Age < 10 or > 40 years	96
Bilirubin > 17.5 mg/dL	93
Jaundice for > 7 days before encephalopathy	97
Unfavorable etiology	90
idiosyncratic drug reactions	
Wilson disease	
non-A, non-B hepatitis	
halothane hepatitis	

a Liver transplantation is indicated for pH < 7.3 irrespective of the grade of encephalopathy; or prothrombin time > 35 s and creatinine > 3.5 mg/dL with grade 3 or 4 encephalopathy.
b Liver transplantation is indicated for prothrombin time > 35 s or any three of the remaining variables irrespective of the grade of encephalopathy.
Data from O'Grady [243]. The prothrombin time is converted to equivalent values in the United States.

pressure in these patients [249]. Faulty monitor function must be carefully excluded because false readings may result in erroneous decisions with respect to the patient's candidacy for liver transplantation [247]. The usual protocol for patients in hepatic coma with intracranial monitoring is to maintain cerebral perfusion pressure between 50 and 60 mmHg and intracranial pressure below 20 mmHg both before and during liver transplantation to ensure full neurological recovery after surgery [240,247].

The 1-year survival following liver transplantation for fulminant hepatic failure approaches 90% in the United States [212], perhaps reflecting advances in the care of critically ill patients and improved patient selection. Liver transplantation for fulminant hepatic failure is, however, limited by the severe shortage of cadaveric organ donors and by the very brief time available to obtain suitable organs. There has been considerable interest in the use of extracorporeal liver-support devices to provide temporary liver support, and serve as a "bridge" to liver transplantation. Results of some early studies were, however, disappointing in that no survival benefit could be demonstrated among patients so treated compared with those receiving supportive care only before liver transplantation [240], thus dampening the initial enthusiasm regarding this technology. A more recent multicenter trial did report improved short-term survival for a subgroup of patients with fulminant liver failure treated with a porcine hepatocyte-based bioartificial liver [250]. The application of extracorporeal liver support needs further research and validation [240]. Auxiliary orthotopic liver transplantation [251] is a special procedure in which part of the native liver is left in situ, and the reduced-size graft provides temporary support until subsequent regeneration of the native liver. Thereafter, the auxiliary graft is either surgically removed or allowed to atrophy following withdrawal of immunosuppression. Although auxiliary liver transplantation has the potential advantage of not subjecting patients to lifelong immunosuppression with its attendant risks, technical difficulties have prevented its widespread use for fulminant hepatic failure.

Evaluation for liver transplantation

Although the approach to the evaluation of patients for liver transplantation may vary according to the individual liver transplantation center, the principles are generally similar (Fig. 92.6). The evaluation process typically involves a multidisciplinary team including hepatologists, transplant surgeons, transplant nurse coordinators, social workers, and individuals with expertise in substance-abuse issues. The use of other consultants varies according to individual transplant centers and patient needs. The main roles of the transplant hepatologist are:

- to confirm that the patient has appropriate medical indications for liver transplantation
- to implement a plan for the management of the complications of liver disease
- to evaluate disease-specific issues that may potentially impact on outcome after liver transplantation
- to assess other comorbid conditions and possible contraindications to liver transplantation.

Surgical risks and technical considerations for liver transplantation need to be carefully evaluated, primarily by the transplant surgeon. Extensive portal and mesenteric venous thrombosis, previous abdominal surgery near the hepatic hilum, and severe obesity can make the transplant operation difficult, if not impossible. In some patients with a TIPS, extension or migration of the stent into the extrahepatic portal vein, the hepatic vein, or vena cava may compromise the success of liver transplantation [57]. Patients should be fully informed of the risks for liver transplant surgery, the possibility and likelihood of recurrent disease after liver transplantation, and the potential alternatives to liver transplantation. For instance, some patients with well-compensated or mildly decompensated cirrhosis may benefit from portal decompressive surgery for refractory variceal bleeding. Surgical resection for HCC may be an alternative to liver transplantation in some patients with well-compensated cirrhosis, a single lesion less than 5 cm, and no portal hypertension. Psychosocial assessment is an integral part of the liver transplant evaluation process to address substance-abuse issues, the risk for recidivism, compliance, and adequacy of social support. In addition to a thorough history and physical examination, an abdominal imaging study (ultrasonography with Doppler, computed tomography, or magnetic resonance imaging) to exclude intrahepatic or extrahepatic tumors and to evaluate hepatic vessel patency, and laboratory tests are part of the routine evaluation process.

Cardiac evaluation before liver transplantation is intended to exclude coronary artery disease, heart-valve disease, and cardiac failure from various etiologies, the extent of which may also depend on the cardiac risk factors present in the individual patient. Special attention should be given to the cardiac evaluation of patients with alcoholic liver disease and hemochromatosis because they are at increased risk for cardiomyopathy, whereas patients with diabetes, metabolic syndrome, and NAFLD are at higher risk for coronary vascular disease. A chest radiograph and arterial blood gas analysis are routinely performed in most transplant centers. Patients with α_1-antitrypsin deficiency, chronic tobacco use, or known or suspected intrinsic lung diseases should undergo full pulmonary function testing. It is also important to identify two pathophysiologically distinct pulmonary syndromes that are uncommon but clinically important among patients with cirrhosis. These are hepatopulmonary syndrome and portopulmonary hypertension [252]. Hepatopulmonary syndrome is characterized by arterial hypoxemia ($P_aO_2 < 70$ mmHg or alveolar–arterial gradient > 20 mmHg) and pulmonary capillary bed dilation supported by contrast echocardiography and

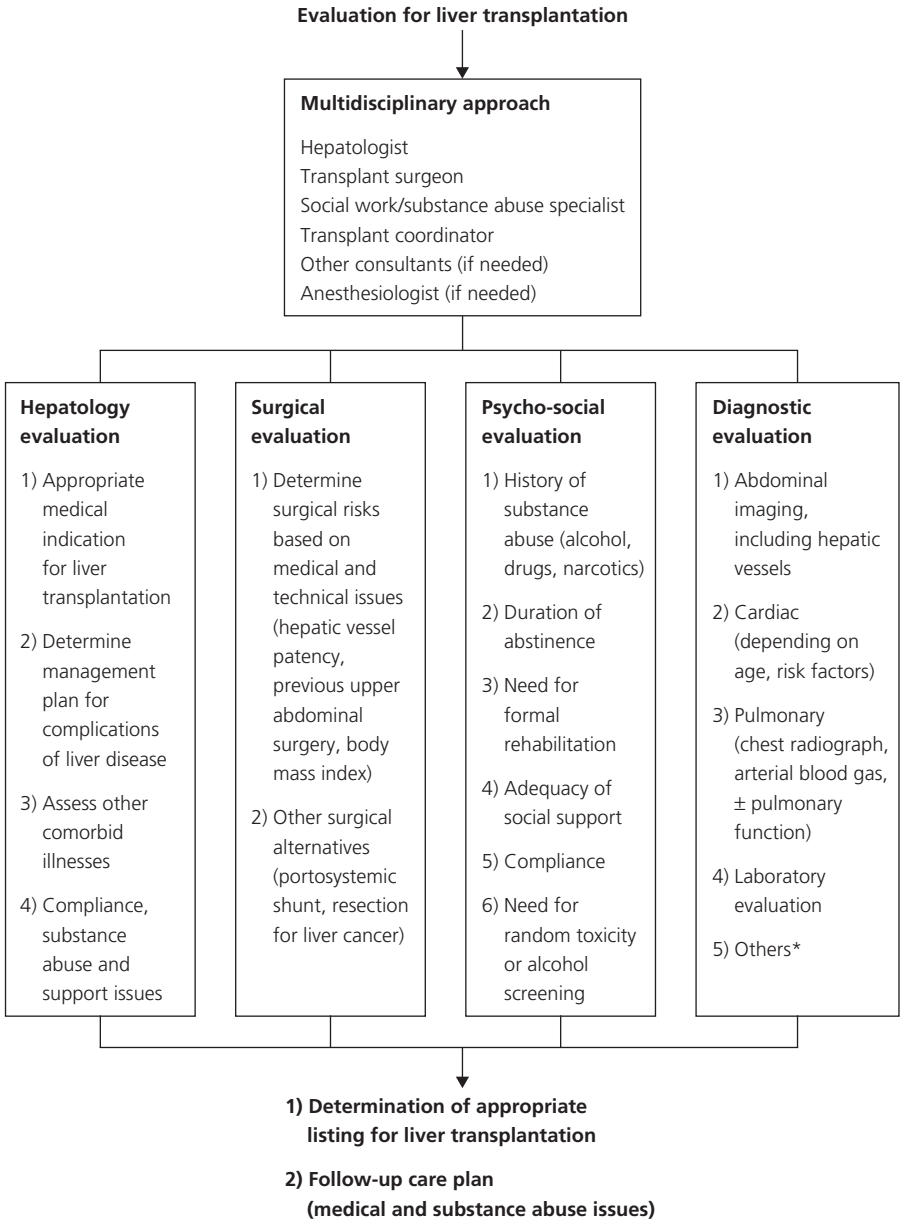

Figure 92.6 Principles in the evaluation of potential candidates for liver transplantation.

technetium macroaggregated albumin lung scanning [252]. Hepatopulmonary syndrome is reversible after liver transplantation, but a high perioperative mortality of about 30% has been reported in patients with a baseline P_aO_2 of 50 mmHg undergoing liver transplantation [253,254]. Portopulmonary hypertension is defined as a mean pulmonary arterial pressure (PAP) greater than 25 mmHg and pulmonary capillary wedge pressure of less than 15 mmHg [252,255]. The perioperative mortality associated with liver transplantation was reported to be 50% among patients with moderate portopulmonary hypertension (mean PAP between 35 and 50 mmHg) and 100% for those with severe portopulmonary hypertension (mean PAP 50 mmHg) [255]. Right heart catheterization is indicated to confirm the diagnosis when the estimated mean PAP is 35 mmHg by echocardiography [256]. Patients with mean PAP 35 mmHg and elevated pulmonary vascular resistance (> 3.0 Woods Units) should be considered for intravenous epoprostenol (prostacyclin) or other treatment protocols [252] in an attempt to decrease the PAP before liver transplantation [257,258].

Contraindications to liver transplantation

The commonly cited absolute and relative contraindications to liver transplantation, which also reflect the authors' opinion rather than a true consensus, are listed in Table 92.4. The absolute contraindications include severe comorbid medical illness such as cardiac and pulmonary diseases that are not reversible and adversely impact on the patient's short-term life

Table 92.4 Contraindications to liver transplantation

Absolute contraindications:
Severe, irreversible comorbid medical illnesses that adversely impact short-term life expectancy
Severe, untreated pulmonary hypertension (mean PAP ≥ 50 mmHg)
Extrahepatic malignancy (excluding some skin cancers)
Extensive hepatocellular carcinoma or with macrovascular or lymph node invasion
Uncontrolled systemic sepsis
Extensive portal vein and mesenteric vein thrombosis
Active alcohol or drug abuse
Noncompliance
Unacceptable risks for recidivism from drugs or alcohol
Severe, uncontrolled psychiatric disease
Relative contraindications:
Moderate pulmonary hypertension (mean PAP between 35 and 50 mmHg)[a]
Severe hepatopulmonary syndrome with P_aO_2 of ≤ 50 mmHg
Severe obesity (Body Mass Index ≥ 40)
Poor social support
Advanced age (≥ 70 years)
Cholangiocarcinoma[a]
HIV/AIDS[a]

a Liver transplantation has been performed in some transplant centers under an experimental treatment protocol.
AIDS, acquired immune deficiency syndrome; HIV, human immunodeficiency virus; PAP, pulmonary arterial pressure.

expectancy, and extrahepatic malignancies excluding certain skin cancers. Patients with advanced HCC and cholangiocarcinoma are generally excluded from liver transplantation, although some centers have performed liver transplantation on these patients under certain experimental treatment protocols. One example is the Mayo clinic protocol for cholangiocarcinoma using external-beam radiation therapy and neoadjuvant chemotherapy before liver transplantation in a highly selected group of patients, showing excellent 5-year survival of 82% [259]. This protocol may be more widely adopted by other transplant centers in the future.

As already described, patients with severe portopulmonary hypertension should not undergo liver transplantation because of an unacceptably high perioperative mortality. At the authors' institution and some other transplant centers, selected patients with moderate or severe pulmonary hypertension are placed on intravenous epoprostenol (prostacyclin) in an attempt to decrease the PAP before liver transplantation [257]. Systemic infections must be adequately treated before liver transplantation. Psychiatric and psychosocial contraindications include active substance abuse, high recidivism risk, noncompliance, and poorly controlled psychiatric illness. Poor social support is also a relative contraindication to liver transplantation. Technical contraindications include extensive thrombosis involving both the portal and mesenteric veins. Severe obesity, defined as a BMI of 35, was shown in a recent analysis of UNOS data to be associated with significantly worse survival after liver transplantation [192]. It is therefore important for these patients to lose weight before liver transplantation. At the authors' institution, patients who are morbidly obese are required to lose weight to achieve a BMI of 40 before they are accepted for liver transplantation.

Individuals infected with the human immunodeficiency virus (HIV) survive longer with current advances in highly active antiretroviral therapy (HAART). Whether they should be considered for liver transplantation remains a highly controversial issue. In the past few years, data on liver transplantation for HIV-infected patients have been reported by several centers in the United States (UCSF, Pittsburgh, Miami) and Europe, although the total number of patients in these studies is still relatively small [260]. In a small series from King's College, London, three of five patients with HIV and hepatitis C coinfection who received transplants died of aggressive recurrent hepatitis C infection between 6 and 25 months after the transplantation [261]. Another study from Barcelona, Spain including nine patients coinfected with HIV and hepatitis C showed no significant difference in 3-year actuarial patient survival compared to a control group [262]. In the largest series reported to date by the group from University of Pittsburgh [263], the 5-year posttransplant survival of 27 patients coinfected with HIV and hepatitis C was only 33%, compared with 72% for the HIV-negative, hepatitis C-positive controls. Recurrent hepatitis C, including three cases of fibrosing cholestatic hepatitis C infection, was a major cause of graft loss and mortality in this study [263]. Further studies with larger numbers of patients are needed to fully address the impact of hepatitis C on posttransplant outcome in this population. At present, liver transplantation for HIV-infected individuals should be restricted to centers with well-defined protocols for patient selection, managing HAART therapy and drug interactions, and treatment for recurrent disease.

Posttransplant management

Complications

Vascular complications

Hepatic artery thrombosis occurs in 2%–4% of all adult whole deceased donor liver transplants [264,265]. The incidence is approximately doubled after partial or split deceased donor liver transplants or living donor liver transplants [25,26,266,267]. Although risk factors for hepatic artery thrombosis include small hepatic arterial size, complex anatomy, the need for extension grafts, prolonged ischemic time, and hypercoagulable states have been identified [266], typically, the specific cause of individual cases is unknown

[268]. The presentation of hepatic artery thrombosis can be highly variable, ranging from clinical silence to frank hepatic necrosis. Diagnosis is usually suggested by Doppler examination of the hepatic vessels and confirmed by arteriography. If hepatic artery thrombosis is identified early after liver transplantation, operative exploration with thrombectomy may succeed in reestablishing arterial flow [268]. Since the blood supply of the biliary tree comes exclusively from the hepatic artery and ischemia frequently results in diffuse biliary strictures, often associated with episodic cholangitis, and less frequently, hepatic abscesses, retransplantation is often necessary [266]. Late hepatic artery thrombosis is more frequently occult and can have a more variable course because collateral blood supply may have developed. Management is dictated by the clinical scenario.

The second most common vascular complication is portal vein thrombosis, which can manifest solely as liver test abnormalities or can manifest with ascites, encephalopathy, or variceal bleeding [266]. Risk factors for portal vein thrombosis include jump graft reconstruction during transplantation, presence of portosystemic surgical shunts, and hypercoaguable states. Balloon dilation and stenting may be effective in some cases of portal vein stenosis, which abrogates the need for surgical revision or retransplantation [269]. Thrombosis or stenosis of the hepatic vein or the inferior vena cava with resultant outflow obstruction is generally uncommon. This complication however has a higher incidence in recipients with recurrent Budd–Chiari syndrome following liver transplantation [270], recipients of partial or split liver transplants either from a deceased or living donor [271], and livers implanted using the piggyback technique [272,273].

Biliary complications

Choledochocholedochostomy (end-to-end anastomosis) with or without a T-tube is the most frequently used biliary anastomosis in adult deceased donor liver transplantation. The alternative, a Roux-en-Y choledochojejunostomy, is used primarily in patients with preexisting bile duct abnormalities such as PSC and biliary atresia or in whom technical difficulty is encountered in fashioning a duct-to-duct anastomosis. In adult living donor liver transplantation, hepaticocholedochostomy is possible, typically when the graft has only a single duct. However, many right lobe grafts have more than a single bile duct, necessitating the use of Roux-en-Y hepaticojejunostomies. The use of T-tubes for duct-to-duct anastomoses or stents for choledochojejunostomy or hepaticojejunostomy is dictated by center practice. While these devices may have some therapeutic effect (prevention of leak or stricture), these devices are also inserted to ensure diagnostic access to the biliary tree [274].

The overall incidence of biliary complications after deceased donor whole liver transplantation ranges between 10% and 15% [275]. In a series of almost 1800 patients reported from the University of Pittsburgh [276], 80% of biliary complications were identified within the first 6 months after liver transplantation, and one-third were diagnosed in the first month. Bile leaks and biliary strictures account for 80% of biliary complications [275–277]. Many biliary complications, particularly those that occur early after transplantation, can be managed endoscopically, in the case of duct-to-duct anastomoses, or with interventional radiology, in patients with choledochojejunostomy or hepaticojejunostomy anastomoses [275,278,279]. Leaks occur most commonly at the anastomotic site, T-tube exit site, or the cut edge of reduced-size liver grafts. A bile leak results in a biloma or bile peritonitis, frequently complicated by superinfection. Nonoperative management with biliary stent insertion and percutaneous drainage is successful in most cases [278]. Surgical revision is typically reserved for failure of nonoperative strategies [278]. Biliary strictures occur most often at the anastomosis, presumably secondary to edema early after transplantation and local ischemia leading to scarring late after transplantation. Endoscopic biliary stenting with balloon dilation is generally successful in resolving early strictures and less successful in resolving late strictures [275]. Occasionally, diffuse stricturing of not only the extrahepatic but also the intrahepatic biliary tree develops. Although these are more frequent with allografts from ABO-incompatible or "donation after cardiac death" donors, or in the setting of insufficient hepatic arterial flow from thrombosis or stenosis, often, no definite etiology can be identified [280].

Biliary complications occur much more frequently after living donor liver transplantation, with an estimated incidence of 30%–40%. Graft preparation necessitates hilar dissection, which can inadvertently disrupt the biliary microcirculation. Moreover, the frequent occurrence of more than one hepatic duct also predisposes to complications. Similar to deceased donor transplantation, the majority of biliary complications after living donor transplantation are managed nonoperatively with stenting and biloma drainage, as needed, for leaks and stenting and dilation for strictures. The approach – endoscopic or transhepatic – is dictated by whether the anastomosis was to a duct or to a Roux-en-Y limb.

In summary, biliary complications remain fairly frequent, particularly after transplants from living donors or using donations after cardiac death. Although the overall impact of biliary complications on mortality is small because of improvements in diagnostic and therapeutic techniques strategies [278], they result in significant morbidity, including graft loss in 1%–3% of transplant recipients [278].

Recurrent disease in the allograft

Disease-specific recurrence following liver transplantation has already been reviewed. Disease recurrence is an important issue, particularly in light of the fact that retransplantation is associated with significantly worse survival compared to primary transplantation [281] and further depletes the limited donor pool. In general, recurrent autoimmune hep-

atitis and cholestatic liver disease have a minor impact on graft survival [208], whereas recurrent viral hepatitis has been a highly important cause of graft failure within the first 5 years following liver transplantation [266]. The use of HBIG and the addition of a nucleoside or nucleotide analogue have diminished the adverse impact of hepatitis B recurrence on graft survival [87–90,96–99]. The magnitude of the adverse impact of recurrent hepatitis C on graft survival, on the other hand, has become increasingly evident in the last few years. The problem related to liver transplantation for hepatitis C infection will likely worsen as many patients with hepatitis C infection are at risk for cirrhosis, liver failure, and HCC as the disease advances to the third and fourth decade following acute exposure of the chronically infected population (see Disease-specific issues and outcome after liver transplantation).

It is clear that all HCV recipients who are viremic at the time of transplant will have recurrent disease of variable and unpredictable severity. Currently, the most potent risk factor for aggressive recurrent disease is increased donor age. Interferon plus ribavirin therapy remains the only possible treatment for hepatitis C, but this treatment unfortunately is characterized by poor tolerability and low efficacy, particularly for unfavorable genotypes. In contrast, liver transplant candidates who are not viremic at the time of transplantation appear to be protected against recurrent disease. Currently, there are trials exploring the feasibility of pretransplant treatment, particularly for candidates who are less severely ill, such as those poised for living donor liver transplantation [140,141]. Despite improved outcome in recent years for liver transplantation among patients with HCC, many patients still succumb to recurrent HCC, which tends to be highly aggressive in immunosuppressed, posttransplant patients and is rarely curable by surgical resection or retransplantation. Better understanding of the mechanisms and predictors of tumor recurrence, refinements of the selection criteria for liver transplantation, and modification of organ allocation policies to reduce waiting time and the pretransplant risk of tumor dissemination may further improve the outcome for HCC.

The course of recurrent NAFLD has not been well characterized because of the small number of patients studied. It appears that the course is relatively benign, although some cases progress to cirrhosis and graft failure. The development of NAFLD following liver transplantation may occur not only in patients with this original diagnosis, but also in patients with different original diagnoses. Obesity and rapid weight gain in the posttransplant period may be important risk factors in these patients. Finally, alcoholic liver disease may recur with heavy alcohol intake in the posttransplant period. Surreptitious alcohol consumption should be considered in the diagnostic evaluation of unexplained graft dysfunction, especially among those with a history of heavy alcohol consumption before liver transplantation.

Medical complications of immunosuppression

Direct adverse effects of immunosuppressants

Clearly, an advantage of current immunosuppression strategies is the possibility to "mix and match" a variety of immunosuppressants, each with a distinctive toxicity profile, to minimize overall side effects. Most side effects occur in a dose-related manner and are amenable to management by dose reduction rather than by discontinuation. Some of the well-known direct adverse effects of the primary immunosuppressive agents are listed in Table 92.5. Corticosteroids are associated with a multitude of well-known side effects including hypertension, dyslipidemia, hyperglycemia/diabetes, osteoporosis, cataracts, weight gain, Cushingoid appearance, and mood alterations. Calcineurin inhibitors, tacrolimus, and cyclosporine share common side effects of nephrotoxicity, neurotoxicity, hypertension, hyperlipidemia, hyperkalemia, impaired glucose metabolism, and gastrointestinal disturbances. Neurotoxicity, decreased glucose tolerance, and gastrointestinal adverse effects are more common with tacrolimus whereas hypertension and hyperlipidemia are more common with cyclosporine. Hyperkalemia is usually managed with fludrocortisone [282]. Hair loss is specifically associated with tacrolimus whereas hirsutism and gingival hyperplasia are specifically associated with cyclosporine [283]. It is important to recognize that a long list of drugs can interfere with the metabolism of the calcineurin inhibitors (Table 92.6). Most interactions occur as a result of either competitive inhibition or induction of metabolism at the level of cytochrome P450 3A4. Increased drug levels can lead to toxicities whereas decreased drug levels can precipitate rejection. The primary adverse effect of immunosuppressants such as azathioprine, mycophenolate mofetil, and rapamycin is bone marrow suppression. Gastrointestinal toxicity, including a hemorrhagic gastritis, is also associated with mycophenolate mofetil. Prominent side effects of rapamycin include hyperlipidemia, ulceration of the oral mucosa, thrombocytopenia, anemia, and leukopenia. Less common but distinctive associations include peripheral edema and interstitial pneumonitis.

Among the most important complications of immunosuppression is the increased incidence of infections and malignancies, as well as renal dysfunction, cardiovascular abnormalities, and metabolic derangements. These are discussed in greater detail below.

Infectious complications

Over 50% of liver transplant recipients experience one or more episodes of infection in the postoperative period [284]. Historically, infectious complications account for most cases of death in the first year after liver transplantation [284]. The timetable of infection in all forms of solid-organ transplantation is most easily organized into three periods [285]: the first month, 1–6 months, and more than 6 months after transplantation. During the first posttransplant, month, conventional bacterial and fungal nosocomial infections predominate.

Table 92.5 Side effects of immunosuppressive drugs

Corticosteroids	Azathioprine	Mycophenolate mofetil	Rapamycin
Infection	Leukopenia	Leukopenia	Leukopenia
Poor wound healing	Thrombocytopenia	Thrombocytopenia	Thrombocytopenia
Osteonecrosis	Hepatotoxicity	Gastrointestinal	Hyperlipidemia
Cataracts	Gastrointestinal	nausea/vomiting	Interstitial pneumonitis
Diabetes	Pancreatitis	abdominal pain	
Cushingoid habitus	Cough	ulceration/gastritis	
Peptic ulcer	Arthralgia	Pancreatitis	
Hypertension	Retinopathy	Arthralgia	
Obesity	Hypersensitivity		
CNS symptoms			
Growth retardation			
	Cyclosporine	**Tacrolimus**	
Nephrotoxicity	++	++	
CNS toxicity	++	++	
headaches			
tremor			
paresthesia			
confusion			
nightmares			
seizure			
Hypertension	++	+	
Glucose intolerance	++	++	
Hyperkalemia	+	++	
Hypomagnesemia	++	++	
Hyperuricemia	+	+	
Gastrointestinal	++	++	
diarrhea			
nausea/vomiting			
abdominal pain			
anorexia			
Hirsutism	++	–	
Alopecia	–	+	
Gingival hyperplasia	+	–	
Hyperlipidemia	++	+	

–, not reported; +, occasional; ++, common.
CNS, central nervous system.

Opportunistic infections with various viral or fungal infectious agents occur most commonly between 1 and 6 months. Beyond 6 months after transplantation, as the degree of immunosuppression decreases, community-acquired infections or recurrent/persistent infections predominate.

With regard to specific infections and organisms, the typical bacterial infections in the early postoperative period are wound infections, indwelling catheter-based infections, and pneumonia with *Staphylococcus* and *Enterococcus* species as the most common pathogens [284]. Vancomycin-resistant *Enterococcus* has emerged as an important nosocomial pathogen in liver transplant recipients [286]. After the first month, viruses (such as CMV, herpes virus, Epstein–Barr virus), fungi, *Nocardia*, *Listeria*, tuberculosis, and *Pneumocystis carinii* pneumonia are important concerns [285]. Fungal infections usually present 1–6 months after liver transplantation. *Candida albicans* is the most common fungal pathogen, although there have been steep increases in the frequency of non-albicans *Candida* infections [287,288]. Invasive aspergillosis carries a high mortality, and its incidence among liver transplant recipients may be rising [289]. Other mycoses, such as cryptococcosis, coccidioidomycosis, and histoplasmosis, are rare except in cases of exposure in specific geographic endemic areas [284,285]. Oral nystatin, clotrimazole, or weekly fluconazole (Table 92.6) are the most frequently used agents for fungal prophylaxis [290]. *Pneumocystis carinii* pneumonia is

Table 92.6 Potential drug interactions with cyclosporine and tacrolimus (partial list)

Potential inhibitors of metabolism resulting in increased levels[a]
Macrolide antibiotics
erythromycin
azithromycin
clarithromycin
Antifungal agents
ketoconazole
fluconazole
itraconazole
Calcium-channel blockers
verapamil
diltiazem
nicardipine
Cimetidine
Doxycycline
Danazol
Potential inducers of metabolism resulting in decreased levels[a]
Anti-seizure medications
phenobarbital
phenytoin
carbamazepine
Antituberculosis medications
isoniazid
rifampin

a Most interactions occur as a result of either competitive inhibition or induction of metabolism at the level of cytochrome P450 3A4.

very rare in liver transplant recipients because of highly effective long-term prophylaxis with trimethoprim–sulfamethoxazole [291].

Historically, CMV has represented one of the most important infectious complications after organ transplantation, causing mortality, morbidity, and heavy resource utilization [292,293]. However, the availability of oral antiviral agents and their use for prophylaxis [294–300] has reduced the incidence and severity of this complication. Distinction should be made between CMV "infection", which refers simply to the detection of the virus in tissue or body fluids, and CMV "disease", defined as CMV infection accompanied by symptomatic clinical manifestations [293]. Patients at highest risk for CMV infection and disease are those who are CMV-seronegative at the time of transplant and those treated with potent immunosuppression regimens [301]. Previously, the incidence of CMV disease was reported to be as high as 15%–40% but the overall incidence has decreased to below 15% [298,299]. The differential susceptibility of high-risk vs low-risk patients still persists. Historically, CMV disease occurred most commonly early after transplantation, within the first 3 months. The widespread use of prophylaxis has delayed the time of onset with CMV disease frequently occurring after cessation of prophylaxis [298,299]. Patients typically present with a "CMV syndrome" characterized by fever, malaise, arthralgia, myalgia, and hematological abnormalities such as leukopenia and thrombocytopenia. Clinical manifestations in liver transplant recipients can include fever, hepatitis, pneumonia, gastroenteritis, and chorioretinitis in descending order of frequency [301].

There are many available strategies to diagnose CMV infection. Histopathology depends on the identification of classic tissue intranuclear inclusion bodies (Cowdry type A). Conventional CMV culture from tissue or blood is slow and relatively insensitive. Its replacement, the shell vial assay, which uses a monoclonal antibody against the early CMV antigen, has improved sensitivity and has a rapid turnaround of less than 24 h. Several newer diagnostic assays are available, including:

- CMV antigenemia assay, which uses monoclonal antibodies to detect the viral pp65 antigen expressed on blood leukocytes
- polymerase chain reaction
- hybrid capture CMV DNA assay [293,302].

While these tests are highly sensitive and specific for detecting CMV infection, the specificity for predicting CMV disease is lower, in the 30% to 60% range, depending on the clinical manifestation [293,302]. The CMV antigenemia assay is the predominant diagnostic test used at the authors' institution because of the rapid turnaround time compared to the polymerase chain reaction assay.

Today, standard treatment of CMV disease is intravenous ganciclovir or oral valganciclovir for 14–21 days at the high treatment dosage, followed by 3–6 months at the low prophylaxis dosage. There is no evidence from controlled trials in solid organ transplantation that the addition of CMV immunoglobulin increases treatment efficacy [293]. Clinical response to intravenous ganciclovir is seen in the majority of patients. Of the 20% of patients with recurrent CMV infections, about two-thirds respond to a second course of ganciclovir treatment [303]. There is no consensus regarding the best regimen for CMV prophylaxis after liver transplantation. At the authors' institution, oral ganciclovir or valganciclovir is given to all liver transplant recipients for CMV prophylaxis. Reports have suggested no clear difference in efficacy between oral ganciclovir and valganciclovir for CMV prophylaxis in spite of the fact that the latter has higher oral bioavailability [296–298].

Posttransplant malignancies

De novo malignancy developing after transplantation has been a well-known complication of liver and other solid-organ transplantation [304,305]. The most common is skin cancer, followed by lymphoma/posttransplant lymphoproliferative disease (PTLD), cancer of the lip, Kaposi sarcoma, vulval and perineal cancers, hepatobiliary cancers, and sarcoma [305]. PTLD develops in about 2% of liver transplant

recipients in the first 5 years after transplantation under both cyclosporine and tacrolimus immunosuppression regimens [282,305]. PTLD most often occurs within the first year after liver transplantation, and involves the liver allograft in one-third of cases. The usual histology is B-cell-derived, large cell non-Hodgkin lymphoma. The association between PTLD and Epstein–Barr virus infection has been well documented [306]. Management of PTLD can be considered in a hierarchical fashion, beginning with withdrawal of immunosuppression and administration of antiviral agents such as ganciclovir/valganciclovir or interferon, through anti-B-cell agents such as rituximab, and culminating in conventional chemotherapy.

Renal, cardiovascular, and metabolic complications

Renal insufficiency is recognized as a major cause of long-term morbidity and mortality for all nonkidney solid organ recipients in general and for liver transplant recipients in particular. Previously, the cumulative incidence of end-stage renal failure necessitating hemodialysis or kidney transplantation was reported to be 5% at 5 years and 10% after 10 years [307,308]. However, the reported incidence of advanced chronic kidney disease appears to be much higher with 15% of liver transplant recipients having a glomerular filtration rate of below 30 within 5 years after transplantation [309]. The steepest decline in renal function is observed during the first 12–18 months after transplantation. Direct toxicity of the calcineurin inhibitors is believed to be a major contributing factor to posttransplant renal insufficiency [282,307,308]. The other prime risk factor is pretransplant renal dysfunction that, in the era of organ allocation according to the MELD score, is increasingly prevalent [310,311]. Pretransplant renal dysfunction can be acute, secondary to hepatorenal syndrome or acute tubular necrosis or chronic, most frequently secondary to various types of glomerulonephritis [307]. Although many instances of acute pretransplant renal dysfunction are, in theory, completely reversible shortly after transplantation, it is recognized that dysfunction lasting for several weeks before transplantation may not completely reverse [311–314]. The increasing use of induction immunosuppression [315] with a concomitant delay in the introduction of calcineurin inhibitors undoubtedly reflects an attempt to optimize renal recovery in the early posttransplant timeframe. Moreover, the steadily increasing use of mycophenolate mofetil as part of a triple immunosuppression regimen [315] may also facilitate relative sparing of toxicity from calcineurin inhibitors, although the safety and efficacy of such an approach has not been demonstrated by prospective randomized studies. Several single center studies have examined the safety and efficacy of mycophenolate mofetil and rapamycin as rescue therapy for liver transplant recipients who develop renal insufficiency several years after transplant. Results of reducing or discontinuing calcineurin inhibitors with concomitant initiation of mycophenolate mofetil and rapamycin have been mixed. From the safety perspective, some studies have reported acceptably low rates of acute or chronic rejection whereas others have observed high rates of acute or chronic rejection, some leading to graft loss. From the efficacy perspective, some studies have suggested benefit with improvement in renal function whereas others have suggested no benefit, surmising that renal dysfunction had become irreversible and therefore unaffected by late alterations in immunosuppression [316–318].

Hypertension is common after liver transplantation and may be related to renal insufficiency or immunosuppressive drugs. The incidence of hypertension appears to be lower with tacrolimus than cyclosporine [282]. The first-line agents for hypertension after liver transplantation have been β-blockers, both selective and nonselective (metoprolol, atenolol, and labetolol), and the dihydropyridine calcium-channel blockers (nifedipine, amlodipine, isradipine, felodipine, and nicardipine) [307]. With the exception of nicardipine, this group of calcium-channel blockers has no significant interactions with the calcineurin inhibitors, in contrast to that observed with the other groups of calcium-channel blockers such as verapamil or diltiazem (see Table 92.6). Second-line antihypertensive agents include β-adrenergic and α_1-adrenergic agents, loop diuretics, and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers [307,308]. The use of the α-adrenergic agents such as clonidine and doxazosin is limited by their central nervous system toxicity, particularly drowsiness. It is best to avoid the angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in the first several months after transplantation when calcineurin inhibitors are being introduced and maintained at high levels because they may aggravate renal dysfunction and hyperkalemia [307]. About one-third of patients achieve effective control of their hypertension with a single agent, and another one-third require the addition of a second agent [307].

Obesity, diabetes, and hyperlipidemia occur commonly after liver transplantation, either singly or in combination [319,320]. In an analysis of about 800 adults from the NIDDK Liver Transplant Database, approximately 20% of nonobese liver transplant recipients became obese over the 2 years after liver transplantation [321]. Diabetes is present in about 5%–15% of patients with advanced cirrhosis awaiting liver transplantation [319]. These patients are at higher risk for posttransplant diabetes than those without diabetes before liver transplantation. Some patients develop short-term diabetes early after transplantation because of high-dose corticosteroids but this resolves once the corticosteroids are tapered. For those who remain diabetic, their management is not substantially different from management in the nontransplant setting, although the use of various oral agents that reduce insulin resistance, particularly those with potential hepatotoxicity, requires careful monitoring. Conflicting results have been reported with respect to the impact

of diabetes on medium- to long-term survival [319]. One study revealed a fourfold higher mortality among diabetic patients [322], whereas another study showed a higher survival rate in diabetics than nondiabetics [323]. A more consistent finding, however, is a higher incidence of bacterial and fungal infections after liver transplantation among diabetic patients compared to nondiabetic patients [319]. The intriguing observation from a few studies that the prevalence of diabetes after liver transplantation is higher among patients with hepatitis C infection and that diabetes is an independent risk factor for progressive recurrent hepatitis C needs to be further investigated [324–326].

Hyperlipidemia often occurs concurrently with obesity or diabetes mellitus. Experience has shown that hydroxymethylglutaryl-coenzyme A reductase inhibitors are both safe and efficacious for liver transplant recipients [327]. Other frequently used agents are, however, problematic. Cholestyramine interferes with gastrointestinal absorption of calcineurin inhibitors so its administration must be separated from that of calcineurin inhibitors. Niacin has multiple side effects and can be hepatotoxic.

Despite the increase in cardiac risk index for many of these patients after liver transplantation, long-term follow-up data regarding the risks and incidence for cardiovascular events and mortality following liver transplantation are only beginning to emerge. It is important to remember that liver transplant recipients are a highly selected population, one that has likely undergone extensive cardiovascular assessment as part of the pretransplant evaluation process. Therefore, in theory, they should have a lower prevalence of cardiovascular disease and a lower rate of cardiovascular events than age-matched and gender-matched controls. One study found that among patients who survived longer than 5 years after liver transplantation, the prevalence of cardiac disease was no higher than that in the nontransplant population in the United States [328]. Two other studies, on the other hand, reported cardiovascular events as the cause of 14%–24% of deaths beyond 1 year after liver transplantation [324,330]. Clearly, more studies with longer follow-up are needed. At present, it is important to try to aggressively correct potentially reversible cardiovascular risk factors following liver transplantation.

Immunosuppression

Induction agents

The historical aim of induction agents, typically an antibody preparation given for a short, predetermined course, was to rapidly and potently suppress the immune system around the time of transplantation. The primary short-term side effects associated with administration of these agents include a cytokine-release syndrome typically resulting in severe flu-like symptoms such as fevers, chills, headaches, myalgias, and diarrhea but sometimes involving hypotension or bronchospasm. The primary long-term side effects are those associated with "over-immunosuppression", an increased incidence of infections and malignancy, especially PTLD. While many or most programs used induction in the early era of liver transplantation when immunosuppressants were limited in both number and efficacy, the emergence of newer and more potent agents nearly eliminated all use of induction in the mid- to late 1990s [331–333]. However, there has been resurgence in the use of antibodies but with somewhat different aims – to facilitate minimization or elimination of a maintenance agent (most commonly steroids) or to delay the introduction of calcineurin inhibitors (most commonly in the setting of renal dysfunction) [334–339]. Studies are underway to explore the safety and efficacy of these strategies.

Antibody induction agents can be polyclonal (antilymphocyte globulin and equine or rabbit antithymocyte globulin) or monoclonal (Muromonab-CD3, which is also known as OKT3, the newer anti-interleukin-2 receptor antagonists, daclizumab and basiliximab, or alemtuzumab). Polyclonal preparations are γ-globulins isolated from animals that have been injected with human lymphocytes or thymocytes. Monoclonal preparations have specificities against the pan T-cell CD3 receptor (OKT3), the CD52 receptor (alemtuzumab), or the interleukin-2 receptor (basiliximab and daclizumab). Today, the primary polyclonal and monoclonal agents used in the United States are rabbit thymoglobulin and the anti-interleukin-2 receptor antibody preparations, respectively [333].

Maintenance agents

The typical maintenance immunosuppressive regimen consists of prednisone and a calcineurin inhibitor, with or without an antiproliferative agent. The immunological mechanisms of action for these and other immunosuppressive drugs used today are described briefly below and in greater detail elsewhere [340,341]. Although the specifics of maintenance immunosuppression differ according to the preference of individual transplant centers, the general strategy is to reduce the number and dosage of immunosuppressants with time, as long as there has been clinical stability. Center-specific protocols can specify different strategies based on the pretransplant diagnosis of liver disease, prescribing lower levels of maintenance immunosuppression for recipients with viral hepatitis and higher levels of maintenance immunosuppression for patients with autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, and possibly, fulminant hepatic failure.

Corticosteroids

Corticosteroids are nonspecific antiinflammatory agents that inhibit cytokine production and thereby prevent T-cell recruitment and activation. Typically, corticosteroids are started intraoperatively and given in large but rapidly tapering doses, reaching the maintenance dose of 5 mg/day within several weeks after transplantation. Frequently, corticosteroid

doses are then more gradually reduced such that the drug is completely discontinued sometime during the first posttransplant year. As evidenced by the decreasing percentage of recipients on corticosteroids at discharge from the transplant admission, there is a trend towards minimizing corticosteroids, giving them only in conjunction with induction immunosuppression or avoiding corticosteroids altogether [333,338,339,342].

Calcineurin inhibitors – cyclosporine and tacrolimus

Calcineurin inhibitors, first cyclosporine and then tacrolimus, have been the mainstay of immunosuppression for liver transplantation for nearly three decades [340,341]. Both drugs bind to cytosolic proteins termed immunophilins to form a complex that inhibits calcineurin, a critical enzyme in the production of interleukin-2 by T cells. Interleukin-2 has a major role in orchestrating the complex immune response through the recruitment and activation of not only T cells but also B-cell and natural killer cell populations and through induction of other cytokines.

The first available formulation of cyclosporine, Sandimmune, was notable for erratic bioavailability because its absorption was highly dependent on food intake, bile flow, and gastrointestinal mobility. The newer, microemulsion formulation products, Neoral and Gengraf, have a much more consistent bioavailability profile. Typically, calcineurin inhibitors are usually initiated immediately after liver transplantation. However, for patients with renal dysfunction, these agents, which have known nephrotoxicity, are often delayed, to optimize the recovery of renal function. Calcineurin inhibitors are typically continued indefinitely, albeit at lower doses. Discontinuation in the long-term is typically motivated by significant toxicity, most commonly, deteriorating renal function.

Azathioprine

Azathioprine is an antimetabolite, available since the late 1960s. As a derivative of mercaptopurine, it antagonizes purine metabolism thereby inhibiting DNA synthesis. Although it was frequently used as a maintenance immunosuppressant into the 1990s, it has been largely replaced by mycophenolic acid, which acts through a similar mechanism but has higher potency and lower toxicity.

Mycophenolic acid

Mycophenolic acid inhibits inosine monophosphate dehydrogenase, an enzyme that is essential for de novo purine synthesis, and thereby blocks DNA replication with some specificity in T and B lymphocytes, which are unable to use alternative salvage pathways. Currently, the drug is available as mycophenolate mofetil (MMF), a semisynthetic prodrug which is immediately released, or Myfortic, which is released in a delayed manner to ameliorate gastrointestinal side effects. Since the drug is inactivated by glucuronidation in the liver and then eliminated by the kidney, liver dysfunction results in higher exposure.

The use of mycophenolic acid, typically as a third maintenance agent in conjunction with corticosteroids and calcineurin inhibitors, has increased steeply during the past decade [333]. Now, more than 50% of all transplant recipients in the United States are discharged after transplantation on mycophenolic acid, although it is commonly discontinued sometime during the first posttransplant year when the risk of acute rejection has decreased substantially.

Rapamycin

Rapamycin is a macrolide antibiotic that blocks signal transduction of a multitude of growth factor receptors, inhibits ribosomal protein synthesis, and ultimately blocks cell cycle progression and proliferation. Since it is a potent immunosuppressant that lacks nephrotoxicity, there was great enthusiasm for exploring its appropriate use in not only kidney transplants but also liver transplantation. Early studies in liver transplantation, however, suggested an increased incidence of hepatic artery thrombosis, leading the United States Food and Drug Administration to issue the following black box warning: "The safety and efficacy of Rapamune (sirolimus) as immunosuppressive therapy have not been established in liver transplant patients, and therefore, such use is not recommended." Although later studies have to some degree allayed this fear, use of rapamycin early after transplantation as a maintenance immunosuppressant remains limited in the United States [333].

Acute and chronic rejection

Acute cellular rejection occurs in 30%–60% of all liver transplant recipients within the first year after transplantation with the majority of episodes within the first 4–6 weeks after transplantation [282,283,340,341,343]. It is generally thought to be a reaction to mismatches in class I and II major histocompatibility complex antigens [344]. Historically, in the study from the NIDDK Liver Transplant Database (343], acute rejection was not shown to exert an overall detrimental impact on patient and graft survival. Only the subgroup of patients with a severe episode of rejection defined by histological criteria was at higher risk for death or retransplantation [343]. These findings were in contrast to those in renal transplant recipients, in whom even a single acute rejection episode was associated with a significantly reduced graft survival. In the past decade, advances in immunosuppressive drugs have resulted in a progressive decline in the incidence of acute rejection episodes after liver transplantation [266]. A primary immunosuppressive regimen of a calcineurin inhibitor, mycophenolate mofetil, and prednisone is associated with a rejection rate of only about 30% [266]. Acute rejection is most commonly seen within the first 6 weeks, especially between days 4 and 14 after liver transplantation [282,283,340,341,343].

The incidence of acute rejection generally decreases with time after liver transplantation. The diagnosis is suggested by nonspecific elevations in the liver enzymes. Fever, leukocytosis, and eosinophilia may be present, but are also nonspecific. The other diagnostic considerations for elevated liver enzymes in the early postoperative period include "preservation/reperfusion" injury, hepatic artery thrombosis, and biliary complications. Histologically, acute cellular rejection is characterized by the triad of portal mixed cellular inflammation with predominantly lymphocytes, bile ductular injury, and subendothelial inflammation of the portal or terminal hepatic venules (endotheliitis) [345]. At least two of the three features are required for the diagnosis [345]. Eosinophils are commonly present in the mixed cellular infiltrate although this is a nonspecific feature.

The initial treatment for acute cellular rejection is usually high-dose methylprednisolone at 1000 mg/day for 2 days. In some centers, this intravenous treatment is followed by a tapering schedule of oral prednisone from over several weeks back to maintenance doses. Some centers, however, use only the intravenous bolus or the oral recycle. Steroid-resistant rejection occurs in 10%–20% of patients treated with cyclosporine, but the incidence is reported to be less than 10% with a tacrolimus-based immunosuppressive regimen [283]. Previously, Muromonab-CD3 (also known as OKT3), a murine monoclonal antibody to the CD3 receptor on T lymphocytes, was commonly used for the treatment of steroid-resistant acute cellular rejection. Muromonab-CD3 use is almost always accompanied by a cytokine-release syndrome, which can include fever, chills, bronchospasm, and, rarely pulmonary edema within the first hour after administration. This resolves within 4–6 h. Patients are given methylprednisolone, antipyretics, and antihistamines as a premedication regimen about 1 h before receiving muromonab-CD3 to relieve some of these symptoms. Aseptic meningitis is associated with muromonab-CD3 in 3%–5% of cases. Rabbit thymoglobulin, a polyclonal preparation generated against human thymocytes, has become the preferred agent, mostly because administration is associated with a much milder side effect profile. The use of either is associated with an increased incidence of serious infections, particularly CMV. Hyperacute rejection is a rare and devastating event mediated by preformed antibodies that bind to allogeneic epitopes on donor endothelial cells, leading to rapid graft failure within hours to days after liver transplantation [344]. It is most commonly due to ABO incompatibility.

Historically, chronic ductopenic rejection [346] was seen in about 10% of all liver transplant recipients but its incidence has decreased substantially with the availability of more potent immunosuppressants [347,348]. It is characterized by progressive loss of interlobular bile ducts and an obliterative arteriopathy [346]. Chronic rejection usually develops after multiple episodes of acute rejection, but may be seen after an unresolved episode of acute rejection or indolently over months to years after liver transplantation without a definite rejection episode. Chronic rejection is generally much more difficult to reverse than acute rejection [346,349,350,351] but again, the availability of potent immunosuppressants has improved treatment efficacy. Irreversible chronic rejection that results in diffuse stricturing of bile ducts in the allograft and cholestasis may require retransplantation.

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Hepatocellular carcinoma

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Epidemiology of hepatocellular carcinoma

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third most frequent cause of cancer death worldwide, with an estimated 626 000 new cases per year [1]. Estimates from the International Agency for Research on Cancer for the year 2002 show worldwide age-specific incidence ratios of 15.8 cases per 100 000 per year in men and 5.8 cases per 100 000 per year in women [1]. As a result of the low overall cure rate for HCC, the worldwide age-specific mortality ratios are nearly identical to the incidence ratios at 14.9 deaths per 100 000 per year in men and 5.7 deaths per 100 000 per year in women. There are strong etiological associations with chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, alcoholic cirrhosis, other causes of chronic liver disease, and dietary aflatoxin exposure. The strong associations with diseases and risk factors that have distinct geographic distributions and different frequencies within different ethnic groups produce a preferential distribution of cases of HCC to certain geographic regions and specific at-risk populations.

International variation in incidence of hepatocellular carcinoma

There is considerable international variation in rates of HCC. By region, the highest incidence is in eastern Asia, with age specific ratios of 36.9 per 100 000 in men and 13.4 per 100 000 in women. China, with the largest population in the region, had an estimated 346 000 cases of HCC in 2002, representing 55% of the total number of cases worldwide. Other regions of high incidence are subSaharan Africa, southeastern Asia, and southern Europe. The lowest incidence rates are found in northern Europe; North, Central and South America; south central Asia; and Australia/New Zealand. The regions of highest incidence in Asia and Africa have a high prevalence of chronic HBV infection as well as high-level exposure to dietary aflatoxins. These combined risk factors play a synergistic role in the development of HCC. Other combinations of risk factors that are important in the epidemiology of HCC include coinfection with HBV and HCV and alcohol use in individuals with chronic hepatitis from viral or other causes. Cigarette smoking and long-term use of oral contraceptives also appear to be independent risk factors for HCC [2]. The risk of development of HCC increases with age. The age distribution of cases of HCC also varies in different populations. A significant proportion of HCC occurs in young and middle-aged individuals at the peak of their productive lives. This results in a high public health and economic impact, particularly in the countries with the highest incidence rates. There is a 15- to 40-year lag time between development of chronic hepatitis or cirrhosis and diagnosis of HCC. This lag time is shorter in persons with active chronic hepatitis C, therefore the increasing prevalence of chronic hepatitis C has led to a decrease in the age of onset of HCC in many populations. For HBV-induced HCC, the age at acquisition of HBV infection is important because individuals who become infected in the perinatal period or in early childhood are more likely to develop chronic hepatitis or become chronic carriers, with an attendant increased risk of development of cirrhosis and HCC [3,4].

Gender variation in incidence of hepatocellular carcinoma

There is a significant effect of gender on the risk of development of HCC. The worldwide male to female incidence ratio is 2.7 : 1. In general, the male : female ratio is highest in the regions with the highest incidences of HCC. Higher levels of

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androgen signaling may be associated with the increased risk of HBV-related HCC in men [5].

Epidemiology of hepatocellular carcinoma in North America

In the United States, there has been a well-documented increase in the number of cases of HCC over the past 20 years. Analysis of data from the Surveillance, Epidemiology, and End Results (SEER) database of the National Cancer Institute showed a 71% increase in incidence of histologically proven HCC from 1.4 per 100 000 population for the period from 1976 to 1980 to 2.4 per 100 000 for the period from 1991 to 1995 [6]. In particular, the incidence of HCC increased significantly among younger persons, aged 40–60 years old, during the period from 1991 to 1995 compared with earlier periods. Between 1981–1985 and 1991–1995, there was a 41% increase in the overall mortality rate from HCC. There is also an effect of gender on the incidence of HCC in the United States, with an incidence in men that is three times the incidence among women. There is a striking effect of race on the incidence of HCC in the United States. For the period from 1991 to 1995, Hispanics, Asians, Pacific Islanders, and Native Americans had the highest age-adjusted incidence of HCC, 7.4 per 100 000. For African-American men, the incidence was 6.1 per 100 000 per year, whereas for white men, it was 2.8 per 100 000. The incidence of HCC is highest among recent Asian immigrants to the United States, with age-standardized rates of 23.9 per 100 000 in Korean men and 5.5 per 100 000 in Korean women. These immigrant populations have a high prevalence of chronic HBV infection. Subsequent US-born generations typically have lower rates of chronic HBV infection and a lower risk for HCC [6]. In general, individuals of lower social class in urban US communities have a higher risk of HCC. This may be related to the inverse relationship of social class with prevalence of HBV infection and cigarette smoking.

Analysis of hospitalization rates for HCC in a population of US veterans showed that the recent increase in incidence of HCC was primarily the result of chronic HCV infection. Between 1993–1995 and 1996–1998, age-adjusted hospitalization rates for HCC associated with HCV increased threefold, from 2.3 per 100 000 in 1993–1995 to 7.0 per 100 000 in 1996–1998. During the same period, the age-adjusted hospitalization rates for HCC associated with HBV, alcoholic cirrhosis, and in persons without known risk factors remained relatively stable [7,8].

A number of recent studies have shown that diabetes is associated with development of HCC, with a relative risk of approximately 2.5 [9–11]. Diabetes exerts a synergistic effect with heavy alcohol use and also with viral hepatitis in increasing the risk of HCC in both African-Americans and whites in the United States [12]. Obesity, which is a risk factor for both diabetes and nonalcoholic steatohepatitis, is also associated with an increased risk of HCC [13]. Given the increasing rates of obesity and diabetes in the United States, these risk factors are likely to be increasingly important in the epidemiology of HCC during the next few decades.

Despite improvements in early diagnosis and management of HCC, survival of patients with HCC in the United States has not improved significantly over the past 20 years. A recent analysis of data from the SEER database showed that the overall 1-year relative survival rate increased from 14% in 1977–1981 to 23% in 1992–1996 [14]. However, 5-year relative survival rates did not change as much, increasing from 2% to 6%. Median survival after diagnosis increased only slightly, from 0.57 years in 1977–1981 to 0.64 years in 1992–1996. There were no significant influences of gender or ethnicity on survival. Most patients with HCC have advanced disease at the time of diagnosis. In the period from 1987 to 1991, only 0.8% of patients with HCC underwent radical surgery with curative intent; these patients had a significantly better survival than patients with untreated advanced disease, with a 1-year relative survival of 60% and a 5-year relative survival of 40%. In the same period from 1987 to 1991, 7.4% of patients underwent palliative surgery; these patients had a 1-year survival of 50% and a 5-year survival of 20%. The proportion of patients undergoing radical surgery in the later period from 1991 to 1996 was unchanged from the earlier period at 0.8%. These data suggested that screening programs aimed at identifying patients with HCC early enough to allow radical surgery were not widely practiced, were ineffective, or were not coupled with widespread availability of the specialized surgical skills needed for optimal radical surgery. The proportion of patients with HCC able to undergo radical surgery is probably also limited by the recognition in recent years that surgical outcomes in patients with cirrhosis and clinically significant portal hypertension are very poor [15]. There is an urgent need to develop methods of identifying the individuals who are at highest risk of developing HCC, to determine the optimal and most cost-effective screening methods to allow early diagnosis of HCC, and to provide access to specialized multidisciplinary management of HCC. In the 1992–1999 time period, there was a substantial increase in the proportion of individuals with HCC who received radical, potentially curative treatment to 13% of cases in the age group over 65 years of age, perhaps reflecting the results of efforts to enhance screening of at-risk individuals [16]. With substantial evidence accumulating that liver transplantation can effectively treat both early HCC and also replace the surrounding cirrhotic liver, there have been additional priority points awarded to patients with early HCC who are candidates for liver transplantation; consequently, the percentage of patients with HCC who are candidates for surgical resection or liver transplantation has increased further. These improvements in the management of HCC, particularly the increasing availability of liver transplantation for patients with early disease, are likely to result in further improvements in survival.

Etiology and risk factors for hepatocellular carcinoma

Chronic hepatitis B

There are 350 million chronic HBV carriers worldwide. The prevalence of hepatitis B surface antigen positivity ranges from high (> 8%) in Africa, Asia, and the Western Pacific, through intermediate (2%–7%) in Southern and Eastern Europe, to low (< 2%) in Western Europe, North America, and Australia. Fifty million new cases of HBV infection occur each year, of these, 5%–10% of adults and up to 90% of infants become chronically infected. In the United States, at least 250 000 persons contract hepatitis B each year. The predominant mode of viral transmission in a population varies with the prevalence of HBV infection. In low-prevalence countries, sexual contact among high-risk adolescents and adults is the predominant mode of transmission. In highly endemic countries, most HBV infections are contracted perinatally or in early childhood. Between one-fourth and one-third of individuals with chronic hepatitis B develop progressive liver disease. Cirrhosis develops in 0.1%–2% of patients with chronic hepatitis B each year, depending on the duration of HBV replication, the severity of disease, and the presence of coexisting infections or alcohol use. In individuals who develop cirrhosis, the risk of progression to HCC varies from 2% to 10% per year [17]. Both host and viral factors influence the natural history of chronic hepatitis B. Certain HBV strains predominate in HCC, suggesting that particular genotypes are more effective at initiating or promoting hepatocarcinogenesis [18]. Individuals with high serum HBV DNA levels are at substantially higher risk of developing cirrhosis and HCC than those with undetectable levels [19,20]. Vaccination of infants and at risk individuals decreases the prevalence of chronic HBV infection and the incidence of HCC [21]. However, there is no conclusive evidence that treatment of patients with high HBV DNA levels with antiviral agents decreases the risk of HCC.

Chronic hepatitis C

An estimated 170 million people are infected with HCV worldwide, and in some countries HCV has overtaken HBV as the major cause of HCC. HCV is an RNA virus that causes acute and chronic hepatitis. It is contracted through parenteral exposure to infected material. Those at highest risk for acquiring hepatitis C are recipients of contaminated blood transfusions, injection-drug users, people who snort cocaine with shared straws, and health-care workers at risk for needlestick and other exposures. Although the incidence of acute HCV infection has fallen dramatically in the United States during the past two decades, the prevalence of infection remains high (approximately 2.7 million Americans) because chronic hepatitis C develops in about 75% of those infected. Both acute and chronic hepatitis C are asymptomatic in most patients. Chronic hepatitis C is a slowly progressive disease that eventually results in severe morbidity in 20%–30% of infected persons. Virological features of HCV such as genotype and viral load are associated with progression and response to therapy [3]. There is a marked difference in the geographic distribution of HCV genotypes, with types 1, 2, and 3a being most frequently found in Western countries. Persistent hepatitis, with high average annual alanine aminotransferase levels, is a significant predictor of the development of HCC [22]. Individuals with active hepatitis, those with coinfections with HBV or human immunodeficiency virus (HIV) and persons with other causes of chronic liver disease such as alcoholic liver disease are more likely to develop liver-related complications. Persons with combined hepatitis B surface antigen positivity and chronic HCV infection are at particular risk of developing HCC [23]. HCV-positive individuals who are anti-hepatitis B core antigen-positive also have a higher prevalence of cirrhosis, lower HCV RNA levels, and an impaired ability to respond to interferon treatment [24].

HIV and HCV have shared routes of transmission so persons infected with HIV are at risk for coinfection with HCV. With the advent of highly active antiretroviral therapy, there has been decreased morbidity and increased survival of patients with HIV infection. As a result, HIV–HCV coinfected patients are living longer and are at increased long-term risk for hepatic cirrhosis, end-stage liver disease, and HCC [25].

After surgical resection of HCC, the presence of active hepatitis and hepatitis C viremia are risk factors for tumor recurrence. Prior treatment of hepatitis C with interferon is protective against the initial development of HCC or its recurrence after surgical resection. The protective effect of interferon is most marked in patients who respond to interferon treatment by clearing the virus and achieving a sustained virological response; there is no evidence of benefit in patients who do not clear the virus. In spite of the decreased risk of HCC after sustained virological response, the risk is still significant and continued surveillance for the early detection of HCC is warranted [26–32].

Dietary aflatoxin exposure

Dietary exposure to fungal aflatoxin is an important risk factor for development of HCC. Aflatoxin (derived from A*spergillus* fla*vus* toxin) is a mycotoxin produced by two fungal species, *Aspergillus flavus* and *Aspergillus parasiticus*. *Aspergillus flavus* is widespread in nature, occurring in soil and decaying vegetation, hay, and grains, particularly in conditions of high moisture content and high temperature. There are at least 13 different types of aflatoxin, with aflatoxin B_1 considered the most toxic. Epidemiological studies have indicated a relationship between aflatoxin intake and incidence of liver cancer in several countries [33,34].

Aflatoxins are metabolized in the liver by the cytochrome p450 and glutathione *S*-transferase enzyme systems. Aflatoxin

B_1 is a procarcinogen that is converted in the liver to the mutagenic metabolite aflatoxin B_1-8,9-exo-epoxide by hepatic microsomal cytochrome p450. Glutathione *S*-transferases and other phase II enzymes subsequently detoxify this DNA-reactive metabolite [35]. Interindividual differences in the biotransformation of dietary aflatoxins result in differing susceptibility to aflatoxin-induced carcinogenesis. The glutathione *S*-transferase M1 (*GSTM1*) gene exhibits genetic polymorphism, with a proportion of normal individuals having a homozygous deletion of the gene. Individuals living in regions where dietary aflatoxin levels are high who have the *GSTM1* null genotype are at increased risk of developing HCC [36]. The presence of additional high-risk genotypes in aflatoxin-metabolizing or DNA repair genes confers an even higher risk of development of HCC [37]. Aflatoxin exposure predisposes to mutations in the gene for p53, particularly a G to T transversion at codon 249, an event that contributes to the pathogenesis of HCC [38]. In many countries, particularly in tropical regions, grains, peanuts, beans, and other foods are harvested and stored under conditions that allow fungal growth and accumulation of high levels of aflatoxin. Measures are being instituted in many countries to decrease contamination of foodstuffs with aflatoxin. There are also efforts to develop drugs that enhance aflatoxin detoxification and excretion [39].

The carcinogenic potential of dietary aflatoxins is significantly increased by the presence of concomitant chronic HBV infection. In one study, persons negative for HBV had 0.01 cases of HCC per 100 000 persons per year per ng of aflatoxin B_1 ingested/kg body weight per day. In contrast, HBV-positive persons had 0.3 cases per year per 100 000 persons, a 30-fold higher risk than in the absence of hepatitis B surface antigen in the serum. Individuals with chronic HCV infection are also probably at increased risk from dietary aflatoxin [40].

Cirrhosis from alcohol and other chronic liver diseases

Cirrhosis from any cause, including chronic alcohol abuse, increases the risk of HCC. The HCC risk is higher for individuals with chronic hepatitis and ongoing hepatocellular injury. Therefore, individuals who stop drinking significantly reduce their risk of HCC. Similarly, persons with hereditary hemochromatosis and cirrhosis who are iron depleted by phlebotomy have a reduced risk of HCC. Certain causes of cirrhosis, such as autoimmune hepatitis, carry a relatively low risk of HCC [41].

Familial and genetic influences on risk of hepatocellular carcinoma

Particularly in areas of the world with a high incidence of HCC, a family history is a significant risk factor for HCC development [42]. The biological basis for this phenomenon is unknown. Epidemiological studies have suggested either the presence of a recessive allele that contributes to risk of HCC or a familial predisposition to a prolonged HBV replication phase [43,44]. Recent evidence from Taiwan has identified a potential susceptibility locus for familial predisposition to HBV-induced HCCs at chromosome 4q25, adjacent to the *LEF1* gene, which encodes a transcriptional effector of the Wnt signaling pathway [45]. In findings that may be related to the inherent ability to clear or suppress HBV infections, initial results suggest that HBV-infected individuals who carry the low-activity genotypes of major T helper type 1 cytokines or the high-activity genotypes of major T helper type 2 cytokines may be at particularly high risk for development of HCC [46]. Genetic polymorphisms of the carcinogen-metabolizing enzymes cytochrome P450 (*CYP*), *GSTM1*, and *N*-acetyltransferase (*NAT2*) as well as polymorphisms in the gene for p53 may also contribute to familial risk of HCC [47].

Factors associated with decreased risk of hepatocellular carcinoma

A number of recent epidemiological studies have identified factors that are associated with a decreased risk of progression of chronic liver disease or development of HCC. These factors include a high serum retinol level and high coffee consumption [48–53]. These findings may have future relevance for efforts at chemoprevention of HCC.

Pathogenesis of hepatocellular carcinoma

Oncogene activation and tumor suppressor inactivation play important roles in carcinogenesis. Oncogene activation occurs through transcriptional activation, gene amplification, or development of activating mutations of genes involved in cellular growth control. Tumor suppressor inactivation can occur through genomic deletions, translocations, or mutations or through epigenetic modifications such as promoter methylation. Normal human somatic cells have a finite life span in vivo and undergo senescence after a predictable number of cell divisions. Cellular senescence is triggered by the activation of two interdependent mechanisms. One mechanism leads to the induction of cell cycle arrest as a result of activation of two tumor suppressor genes, *p53* and *pRb*. The second mechanism is activated by a critical shortening of chromosomal ends as a result of the progressive shortening of the telomeres with each cell division. When both cell growth regulatory mechanisms are inactivated, unchecked cell division and progression to cancer occur. Cells that are turning over at higher than normal rates because of local growth factor up-regulation or chronic cell injury and regeneration, have a higher likelihood of developing genomic alterations that lead to oncogene activation or tumor suppressor inactivation [54]. Environmental exposure to carcinogens can also cause oncogene activation or tumor suppressor inactivation (Fig. 93.1). A number of approaches

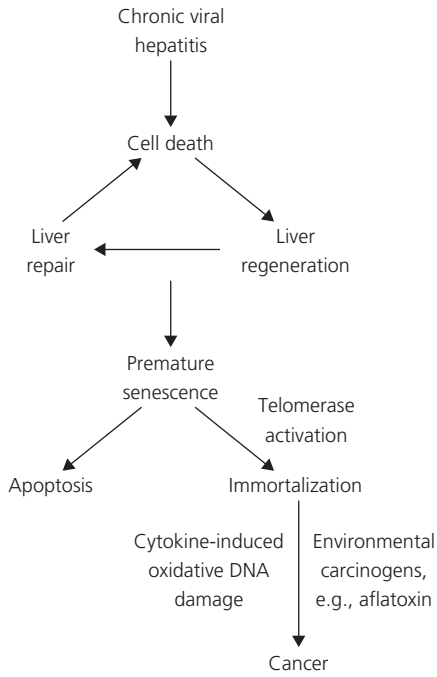


Figure 93.1 The role of chronic hepatitis with recurrent liver injury, regeneration, and repair in the development of hepatocellular carcinoma. Premature senescence of liver cells occurs by apoptosis. Cells undergoing telomerase activation escape apoptosis. The resulting immortalized cells are susceptible to the influence of oxidative and environmental carcinogen-induced DNA damage, leading to the eventual development of transformed neoplastic hepatocytes.

have been used to elucidate the molecular mechanisms of liver carcinogenesis. These include:

- the use of chemical tumor initiators and promoters in animal models
- studies of growth factors and their signaling pathways
- transgenic mouse models overexpressing cytokines, growth factors, or oncogenes
- studies of immune-mediated mechanisms of hepatocellular injury
- analysis of the molecular genetic changes that occur in HCCs, including studies of chromosomal allelic imbalance, comparative genomic hybridization, restriction landmark genomic scanning, and gene expression analysis using complementary DNA microarrays
- studies of the molecular consequences of HBV integration and the interaction of the protein products of the HBV and HCV with host cell processes.

These studies have contributed to our growing appreciation of the multiplicity of mechanisms and pathways that may contribute to the carcinogenic process in toxin-affected, chronically inflamed, or otherwise injured liver tissue. In cirrhotic livers, macroregenerative nodules with foci of hepatocyte dysplasia have been identified as preneoplastic lesions of HCCs. Histologically, dysplastic lesions are classified as small cell or large cell lesions or as foci of adenomatous hyperplasia; available evidence suggests that small cell dysplasia and adenomatous hyperplasia are the predominant preneoplastic lesions [55]. Unfortunately, we are still far from a unified, comprehensive understanding of liver carcinogenesis. This is in part because HCC is initiated in multiple genetic and environmental contexts and almost certainly emerges as a consequence of multiple possible pathways. The lack of a comprehensive view of the pathogenesis of HCC has also prevented the development of effective, targeted, preventive, or therapeutic interventions that are elegant and also simple enough to be applicable to the vast majority of patients with this disease. Recent analyses using genome-wide approaches and novel animal models have led to a better understanding of the major pathways involved in liver carcinogenesis and allowed initial attempts at subclassifying the apparently molecularly heterogeneous HCCs into distinct molecular and prognostic subtypes [56–60].

While the generally accepted paradigm of carcinogenesis is that malignant transformation occurs through the stepwise accumulation of genetic and epigenetic alterations that lead to the progressive acquisition of the cancer phenotypes, recent studies have led to the concept that a minimum number of molecular alterations leads to the acquisition of the key cancer phenotype, unconstrained cell proliferation. This "cancer platform" concept posits that growth-promoting oncogenic pathways generally also activate pathways of cellular senescence or apoptosis that limit their growth effects, producing a natural homeostasis of tissue mass. When activation of oncogenic events is coupled with inactivation of this "oncogene-induced apoptosis", unconstrained proliferation results. The other cancer phenotypes are then acquired secondarily through the interaction of the surrounding stroma with the growing mass of tumor cells [61,62]. The major regulator of oncogene-induced apoptosis appears to be the tumor suppressor p53, however, p53 action is accomplished through a network of regulatory molecules, which when disrupted individually or in combination can mimic the effects of p53, hence the observation that cancers are molecularly heterogeneous and there are multiple genes that contribute to the development of cancer. However, this new paradigm also suggests that targeting the key pathways that regulated unconstrained proliferation may have profound effects on tumor growth.

Another major advance in our understanding of cancer has come from the recognition that the primary events leading to unconstrained cell proliferation may occur in a small population of cancer stem cells from which the main bulk of a malignant tumor is derived through partial differentiation [63]. Consequently, to effectively induce tumor regression, therapeutic measures will have to target the key molecules and pathways that regulate unconstrained proliferation of the stem cell compartment. Experiments using mice in which *myc* oncogene expression is regulated by oral administration

of doxycycline have elegantly demonstrated the ability to induce HCC or to cause its regression through modulation of *myc* expression in a small stem cell compartment [64,65].

These studies hold great promise for allowing careful characterization of tumors and personalization of targeted therapies in the future.

Tumor suppressor genes

Tumor suppressor genes are normal cellular genes that, when homozygously inactivated, can contribute to tumor development. Tumor suppressor genes generally operate in a recessive manner, requiring loss of both copies for tumorigenesis, in contrast to oncogenes, which can exert their effects in a dominant fashion. Loss of heterozygosity (LOH) is loss of one allele in a tumor cell from a chromosomal region for which the individual's normal cells are heterozygous. LOH is detected using polymorphic DNA markers that can distinguish between the two alleles. If one allele of a tumor suppressor gene is inactivated by mutation, then deletion of the second allele, detected by LOH, is associated with loss of expression of the gene. Promoter hypermethylation also leads to decreased expression of tumor suppressor genes. DNA hypermethylation on CpG islands and DNA hypomethylation on pericentromeric satellite regions are early events during hepatocarcinogenesis. CpG island hypermethylation leads to decreased tumor suppressor gene expression, while pericentromeric hypomethylation results in chromosomal instability and deletions [66].

Known or putative tumor suppressor genes shown to be mutated or down-regulated in HCCs include *p53* [67], *RB* [68], *M6P/IGF2R* [69], *BRCA2* [70], *DLC1* [71,72], *p16* [73], *Smad2* [74], *Smad4* [74], *axin1* [75], *axin2* [76], *Parkin* [77], *SULF1* [78], Kruppel-like factor 6 [79–82], the IκB kinase (IKK) subunit *NEMO/IKKγ* [83], *Prickle1* [84], vitamin D binding protein, fibrinogen γ-chain, fibrinogen-like protein 1, metallothionein 1G, α_2-plasmin inhibitor [85], *DDX3* [86], and *TIP30* [87]. In human HCCs, LOH has been reported in multiple chromosomal regions, including 1p, 1q, 2q, 4q, 5q, 6q, 8p, 8q, 9q, 10q, 11p, 12p, 13q, 14q, 16q, and 17p [88,89]. A number of these regions contain known tumor suppressor genes, such as at 17p13.1 (*p53*), 6q26–27 (*M6P/IGF2R*), 8p21.3–22 (*DLC1*), and 13q12–q32 (*Rb* and *BRCA2*). For a few of these chromosomal loci, clinicopathological associations have been demonstrated, such as the association of LOH on chromosome 1p with early stage HCC [90] and that of LOH on chromosome 16q with progression of HCC [91]. In addition, patients with LOH at multiple regions have more advanced stage disease, less well-differentiated tumors, higher serum α-fetoprotein levels, and a worse prognosis [92]. Chronic HBV infection and integration of the HBV genome are also associated with high rates of genomic instability [93]. Comparative genomic hybridization (CGH) allows the identification of gains and losses of DNA sequences across the entire tumor genome. Analysis of HCC tumors and cell lines by CGH has revealed genomic DNA copy number gains on chromosomal arms 1p, 1q, 2q, 3q, 6p, 7p, 7q, 8q, 10p, 11q, 12q, 17q, 20p, and 20q. Recurrent losses have been found on 1p, 3p, 3q, 4p, 4q, 5q, 6q, 8p, 9p, 10q, 11q, 13q, 14q, 15q, 16q, 17p, 18q, 21q, and on the Y chromosome [94–101]. Significantly, a majority of the overrepresented regions harbor known protooncogenes, and half of the underrepresented regions coincide with sites of known or putative tumor suppressors [96]. Notably, comparison of CGH analysis of HCCs that are the result of HBV infection, as compared to HCC with non-viral etiology, has a lower frequency of loss at 4q, 16q, and 17p in nonviral HCC samples, suggesting that these abnormalities are associated with HBV infection [102]. CGH analyses also reveal a similar pattern of chromosomal alterations in HBV-induced and HCV-induced HCCs. Thus a subset of alterations may preferentially contribute to virus-induced carcinogenesis. In general, LOH and CGH studies have shown variable rates of chromosomal instability. Patient populations with low rates of virus-induced HCCs have lower levels of chromosomal instability than populations with high rates of virus-induced HCCs. Despite this variability, there is remarkable consistency in the locations of chromosomal instability, suggesting that particular gene targets are affected by genomic instability in HCC.

Investigation of gene mutations in the tumor suppressor gene *p53* in HCC has been particularly revealing because HCCs from patients with high dietary exposure to fungal aflatoxin B_1 have a high frequency of point mutations at the third position of codon 249 of the *p53* gene, resulting in a G : C to T : A transversion [103]. The frequency of this mutation in HCC increases in proportion with the level of dietary exposure to aflatoxins. A G : C to T : A transversion at the second position of codon 249 is often found in patients with chronic HBV or HCV infection. The occurrence of this mutation correlates with oxyradical exposure. Both of these mutations lead to decreased binding of p53 to its nuclear DNA targets [104]. Mutations in *p53* have been demonstrated in nonneoplastic liver cells in subjects from communities with high dietary aflatoxin B_1 exposure, suggesting that the mutations are early events in neoplastic transformation (Fig. 93.2). However, *p53* mutations can also occur late in tumor progression. The hepatitis B virus HBx gene product has also been shown to interact with p53 and to strongly inhibit p53 sequence-specific binding, leading to inhibition of p53-mediated apoptosis. Abnormalities in the *Rb* gene have been noted in association with *p53* mutations in advanced HCCs, particularly in poorly differentiated tumors, and *p53* and *Rb* mutations may have an additive effect in liver carcinogenesis [105]. HCCs from different populations show different frequencies of *p53* mutations. In Alaskan Indians, *p53* mutations are rare or absent, suggesting that a difference in environmental or host factors leads to a different mechanism of hepatocarcinogenesis [106]. In a conditional mouse model of HCC, loss of *p53* has been shown to be required for the

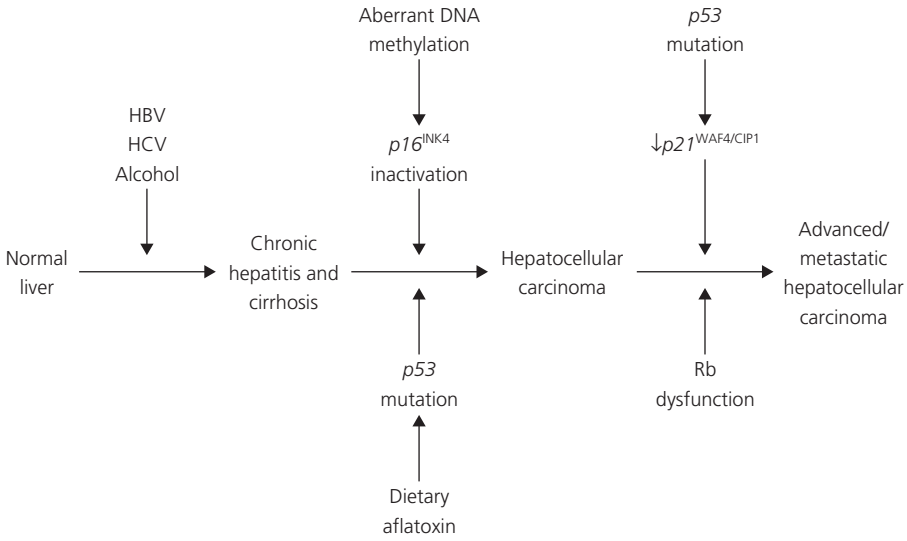


Figure 93.2 Major molecular pathways of hepatocarcinogenesis. Hypermethylation of the p16 gene promoter is one of the earliest changes in development of hepatocellular carcinoma. In regions with high dietary aflatoxin exposure, the development of p53 mutations also occurs early. In areas with low aflatoxin exposure, p53 and pRb pathway disruption occurs late in hepatocarcinogenesis.

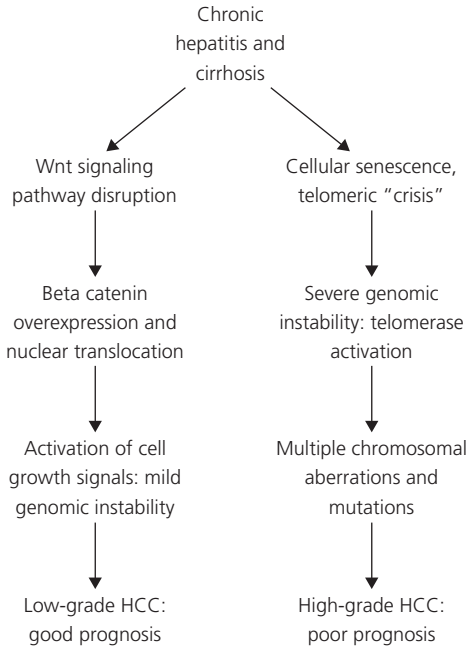


Figure 93.3 Two distinct pathways of liver carcinogenesis. Disruption of the Wnt signaling pathway occurs in approximately 25% of hepatocellular carcinomas. β-Catenin mutations prevent degradation of β-catenin and lead to accumulation of nuclear β-catenin. β-Catenin cooperates with the Tcf transcription factor to activate cellular growth control genes. Tumors with β-catenin mutations tend to have lower rates of genomic instability and to be lower grade tumors with a better prognosis.

maintenance of aggressive HCCs [107]. In this model, reactivation of endogenous p53 induced cellular senescence and an enhanced innate immune response that produced complete tumor regression. These studies underline the importance of p53 in restricting tumorigenesis. Molecular genetic studies show that HCC appear to fall into two groups, with either low or high levels of chromosomal changes [108]. In general, the group of tumors with low level chromosomal instability is also more likely to have disruption of the Wnt signaling pathway, with β-catenin mutations, low-grade histology, and a better long-term prognosis (Fig. 93.3). In contrast, tumors with high-level chromosomal instability are more likely to have *p53* mutations.

Promoter hypermethylation is an important cause of loss of expression of tumor suppressor genes in hepatocarcinogenesis. Genes inactivated by this mechanism include *p16* [109], *SOCS* [110], *TSLC1* [111], E-cadherin [112], *RASSF1A* [113], *NORE1B* [114], Fas-associated phosphatase-1 [115], and spleen tyrosine kinase [116]. In particular, *p16* inactivation by hypermethylation appears to be one of the earliest genetic events in hepatocarcinogenesis (Fig. 93.3) [117].

Oncogenes

Protooncogenes are cellular genes involved in the control of cell growth. Mutation, overexpression, or amplification of these genes leads to oncogenic activity that contributes to neoplastic transformation. Oncogenes known to be overexpressed in HCC include c-*fos* and c-*myc* [118], c-*erbB2* [119], c-*met* [120], β-catenin [121], gankyrin [122], and pituitary tumor-transforming gene-1/survivin [123,124]. Overexpression of c-*myc* is frequently a consequence of amplification of the c-*myc* locus in HCC [94]. Activation of the c-*myc* and c-*fos* oncogenes also occurs as a consequence of the transactivating function of the hepatitis B virus x protein (HBx) and the C-terminal truncated middle hepatitis B surface protein (MHBst) [125]. Oncogene activation may also occur through promoter hypomethylation [126], through pathways mediated by reactive oxygen species, or through protein kinase C or mitogen-activated protein kinase pathways. Processes mediated by reactive oxygen species or lipid peroxidation are also important in the pathogenesis of chronic liver injury induced by alcohol, genetic hemochromatosis, and α_1-antitrypsin deficiency.

Growth factors and growth factor signaling pathways

A number of different growth factors are involved in hepatocarcinogenesis. Insulin and insulin-like growth factors (IGFs) 1 and 2 promote hepatocyte growth. IGF-2 is frequently overexpressed in HCCs. In addition, insulin receptor substrate 1 (IRS-1), a cellular mediator of insulin-like growth factor signaling, is frequently overexpressed in HCCs [127]. Signaling events downstream of IRS-1 lead to activation of the mitogen-activated protein kinase cascade and to cell proliferation through up-regulation of cellular growth genes and inhibition of apoptosis [128]. Transforming growth factor-α (TGF-α) and the structurally related epidermal growth factor are another potent class of hepatocellular growth factors. TGF-α is overexpressed in the liver of patients with chronic hepatitis. As TGF-α levels are often increased in HCCs, it is likely that it contributes to cellular proliferation in cancer, and may be a factor in tumor initiation or progression in patients with chronic hepatitis. In a transgenic mouse model, TGF-α dramatically enhances c-*myc*-induced hepatocarcinogenesis by promoting the proliferation and survival of HCC cells [129]. The TGF-α/c-*myc* transgenic mouse model also shows hyperphosphorylation of the retinoblastoma protein pRb, which results in inactivation of pRb and release of E2F transcriptional activity, resulting in cell cycle progression and enhanced mitosis [130]. TGF-β_1 inhibits cell proliferation and promotes cellular differentiation, fibrogenesis, and apoptosis. Increased TGF-β_1 may create an environment in which selection of hepatocyte clones resistant to TGF-β_1-induced apoptosis occurs. The *Smad* genes are involved in TGF-β signaling and mutations in the *Smad2* and *Smad4* genes have been described in HCC [74]. Hepatocyte growth factor, the ligand for the c-*met* oncogene, activates the Akt/phosphatidylinositol-3 kinase pathway to suppress Fas-mediated cell death in human HCCs [131]. High molecular mass forms of basic fibroblast growth factor have also been shown to have a mitogenic effect on hepatocytes [132]. Recent studies suggest that the Ras, Jak/STAT, and Akt pathways are activated in the majority of HCCs [133]. As previously mentioned, there is substantial evidence for a significant role of Wnt pathway activation in hepatocarcinogenesis. Emerging evidence suggests that Wnt pathway regulation in HCC is complex, with differential effects of mutated β-catenin and Axin1 in different subclasses of HCCs [59,134]. There is also evidence for involvement of the hedgehog signaling pathway in hepatocarcinogenesis [135]. Clearly, multiple growth-promoting molecules are involved in hepatocellular carcinogenesis.

Telomere length and telomerase activity

Telomeres are specialized protein–DNA structures at the ends of chromosomes that contain long stretches of TTAGGG hexameric repeats. Telomeres prevent degradation of chromosome ends and end-to-end fusion with other chromosomes. Aging of somatic cells is associated with reduction in telomere length as a result of the inability of traditional DNA polymerases to completely replicate the end of the chromosomal DNA. In contrast, germ-line and neoplastic cells express telomerase, an enzyme that restores telomere length. There is progressive shortening of telomeres during progression from chronic hepatitis to cirrhosis and eventually to HCC [136]. This occurs as a consequence of the multiple cycles of cell injury, death, and regeneration that occur in injured liver, leading to premature hepatocellular senescence. Telomere shortening beyond a critical length leads to a proliferative block referred to as telomeric crisis, which is characterized by chromosomal instability, end-to-end fusions, and cell death. Stabilization of the telomeric DNA through either telomerase activation or the activation of an alternative mechanism of telomere maintenance is essential if the cells are to survive and proliferate indefinitely (Fig. 93.1) [137]. There is cross-talk between the oncogene and telomerase pathways, as c-*myc* has been shown to activate the telomerase gene promoter [138]; p53 dysfunction also cooperates with telomere-induced chromosomal instability in hepatocarcinogenesis [54]. Hepatocarcinogenesis is characterized by the evolution of clones of hepatocytes with increased telomerase expression and an immortalized phenotype [139]. Therefore, almost all HCCs show reactivation of telomerase activity [140].

Microsatellite instability

Microsatellite instability (MSI) is defined as a change of any length as the result of either insertion or deletion of repeating units in a DNA microsatellite within tumor genomic DNA when compared to normal tissue. This form of genomic instability is associated with defective DNA mismatch repair in tumors, which is important in the pathogenesis of the hereditary nonpolyposis colon cancer (HNPCC) syndrome and associated malignancies [141]. To date, six mismatch repair genes have been identified in humans: *hMLH1*, *hMSH2*, *hPMS1*, *hPMS2*, *hMSH3*, and *hMSH6*. Colorectal cancers may be characterized as having high-frequency MSI (MSI-H) if more than 30%–40% of more than five microsatellite loci analyzed show instability, low-frequency MSI (MSI-L) if less than 30%–40% of more than five loci analyzed show instability, or as being microsatellite stable (MSS) if 0% of loci show instability. Of sporadic colorectal cancers, 70% are MSS, 15% are MSI-L, and 15% are MSI-H. MSS or MSI-L colorectal cancers do not have an associated defective mismatch repair phenotype or the clinicopathological features of HNPCC tumors; instead they behave in the same manner as sporadic colon cancers. Clear criteria have not been defined for MSI in noncolonic tumors. To date, a number of studies have demonstrated either no instability or a relatively low frequency of low-level MSI in HCC [142,143]. As is the case with other noncolonic tumors, it is unclear whether this finding is of significance in the etiology or pathogenesis of HCCs or is instead simply a consequence of the generalized genomic instability found in cancer. It has

been suggested that cumulative low-level MSI at multiple loci leads to tumor progression. Further investigation is needed to resolve this potentially important question.

Chromosomal fragile sites

Chromosomal fragile sites are specific genetic loci which are susceptible to forming gaps, breaks, and rearrangements in metaphase chromosomes of cells cultured under conditions that inhibit DNA replication, such as treatment with the DNA α-polymerase inhibitor, aphidicolin. Fragile sites are grouped into "common" or "rare" classes based on their frequency of occurrence and the culture conditions required for their expression. So far, 89 common and 28 rare chromosomal fragile sites have been identified. Common fragile sites are present in all individuals. The most frequently observed common chromosomal fragile sites occur at 3p14.2 (*FRA3B*), 16q23 (*FRA16D*), 6q26 (*FRA6E*), 7q32 (*FRA7H*), and Xp22 (*FRAXB*) [144]. Common fragile sites span a distance of 250–1500 kilobases and display characteristics of unstable, highly recombinogenic DNA in vitro. In particular, they are preferred sites for sister chromatid exchanges, chromosomal deletions and rearrangements, integration of viral sequences and transfected plasmid DNA, and initiation of bridge breakage–fusion cycles, which lead to gene amplification. As a result of these characteristics and the frequent coincidence of fragile sites with chromosomal breakpoints in malignant cells, it has been hypothesized that fragile sites are involved in carcinogenesis [145]. The most convincing evidence of the potential significance of the fragile sites in carcinogenesis is the location of *FRA3B*, the most highly inducible common fragile site, at chromosome 3p14.2. This chromosomal region is frequently deleted in lung cancer, renal cell carcinoma, and pancreatic cancer, and is also the location of the fragile histidine triad (*FHIT*) gene [146]. The *FHIT* gene has been proposed to be a tumor suppressor and has been identified as a preferential target in HCC [147]. Additional fragile site genes, including the *FRA16D* gene *WWOX* (16q23) and the *FRA6E* gene *Parkin* (6q26), which are both located in regions at which there is increased LOH in HCC, encode proteins that have been shown to have tumor suppressor functions in HCC [77,148,149].

Angiogenesis

New vessel formation is required for the continual growth of the tumor and provides a gateway for cells to escape the confines of the primary tumor. HCCs are usually hypervascular and show evidence of active angiogenesis. Arteriolar density in the tumors correlates with angiographic vascularity and also with the proliferating cell nuclear antigen labeling index [150]. It has been shown that IGF-2 production is stimulated by hypoxia, and that IGF-2 in turn activates expression of vascular endothelial growth factor (VEGF), an important regulator of tumor angiogenesis [151]. IGF-2 has also been shown to directly stimulate angiogenesis. The hypoxia inducible factor (HIF-1) also plays a role in angiogenesis in HCC [152]. HIF-1 stimulates angiogenesis by activating transcription of the gene encoding VEGF. In parallel with the increase in angiogenic stimuli, it has been shown that the expression of collagen XVIII, the precursor of the antiangiogenic molecule endostatin, is decreased in larger and more vascular HCCs [153]. Antiangiogenic agents have been shown to be effective against HCC in mouse models, and initial results from clinical trials of antiangiogenic agents in human HCC suggest that they are of benefit [154–156].

Hepatitis B virus in the pathogenesis of hepatocellular carcinoma

Multiple mechanisms appear to contribute to HBV-induced hepatocarcinogenesis. Although persistent hepatitis with regenerative hepatocellular turnover and cirrhosis is presumed to play a significant role in HBV-induced carcinogenesis, a significant percentage of HBV-related HCCs occur in the absence of cirrhosis, suggesting a specific role for HBV in the development of these cancers. HBV integrates into the host genome in almost all patients with chronic hepatitis. Viral integration almost invariably precedes the development of HCC. HBV DNA appears to integrate into random sites in the human genome, however, with recent advances in molecular genetics, there is emerging evidence that, as with other viruses, viral integration preferentially targets specific genes or common chromosomal fragile sites [149,157,158]. Integrated HBV is thought to contribute to the pathogenesis of HCC through a number of potential mechanisms:

- by local genomic effects as a result of production of genomic instability and focal deletions and translocations
- by interruption of genes, leading to overexpression, suppression, or production of novel fusion transcripts
- by the transactivating effects of viral proteins, particularly the X and preS gene products.

Viral integration has been shown to result in deletions, translocations, and gene amplification at the site of integration [159]. In a number of cases, HBV integration has been shown to occur adjacent to or within genes involved in cell growth or apoptosis. Genes found at HBV integration sites include the retinoic acid receptor β-gene (*RARB*) [160], the cyclin A gene [161], and the sarco/endoplasmic reticulum calcium ATPase (*SERCA*) gene [162]. In the case of the *RARB* and cyclin A integrations, expression of a fusion transcript results in oncogenic effects [163,164]. Integration of HBV into the *SERCA* gene leads to accumulation of chimeric HBx/SERCA-1 proteins that induce apoptosis. HBx protein inhibits the function of the tumor suppressor p53 and activates cytoplasmic mitogenic signaling cascades, including the mitogen-activated protein kinase and the Janus family tyrosine kinase (JAK)/signal transducer and activators of transcription (STAT) pathways. HBx also interacts with transcription factors in the nucleus to transactivate multiple genes involved in cellular growth control, including the nuclear factor-κB (NF-κB),

activator protein 1 (AP-1), and serum response element (SRE) pathways [165]. The HBx protein has been shown to play a role in fibrogenesis. HBx interacts with Smad4 and stabilizes the complex of Smad4 with components of the basic transcriptional machinery, leading to enhanced transcriptional activity in response to TGF-β. In addition, confocal microscopy studies suggest that HBx facilitates and potentiates the nuclear translocation of Smads, further enhancing TGF-β signaling. This suggests that HBx-enhanced Smad-mediated signaling may contribute to HBV-associated liver fibrosis [166]. The hepatitis B surface and core genes and proteins may also play significant roles in hepatocarcinogenesis. It has been shown that a truncated C-terminal variant of the HBV S protein directly interacts with DNA and transactivates several cellular and viral promoters, including the c-*jun* and c-*fos* promoters [167]. Hepatocellular carcinoma has also been associated with HBV strains that have deletions or insertions in the HBV core promoter region, suggesting a role for changes in core protein expression in persistence of HBV infection, HBV integration, and carcinogenesis [168].

Hepatitis C virus in the pathogenesis of hepatocellular carcinoma

In patients with chronic HCV viremia, persistent liver damage with abnormal alanine aminotransferase levels plays an important role in the development of HCC. This may be the result of repeated cycles of liver cell injury and regeneration, resulting in premature cellular senescence and the development of genomic aberrations. However, in addition, it has been shown that individuals with persistent liver damage develop a population of HCV quasispecies which changes over time, whereas individuals with persistently normal aminotransferases have a stable infection with a single species of HCV [169]. Even within the same patient, tumor samples show greater variability in the core region of HCV isolates than is found in adjacent benign samples [170]. This phenomenon may be important in hepatocarcinogenesis, by allowing for selection of more oncogenic variants of the HCV core protein. In a transgenic mouse model, HCV proteins suppress Fas-mediated hepatocellular apoptosis, and may use this mechanism to maintain the persistence of HCV infection [171]. Of the seven HCV proteins, core protein and nonstructural (NS) proteins NS2, NS3, NS4A, NS4B, NS5A, and NS5B, the core protein is the most potent cellular signaling activator, causing activation of the NF-κB, AP-1, and SRE pathways and the mitogen-activated protein kinase/extracellular-regulated kinase cascade [172]. Activation of these cascades leads to cell proliferation. The HCV core protein has been shown to interact with the 14-3-3 protein, leading to activation of Raf-1 kinase [173]. The core protein also up-regulates Wnt-1 expression and stimulates hepatocyte growth [174]. The NS4B protein activates the NF-κB pathway, and the NS5A protein has been shown to repress the interferon-induced protein kinase, PKR. The envelope protein E2 is transcribed from the most variable region of the HCV genome, and is thought to allow the virus to escape from neutralizing antibodies, in addition, E2 also inhibits PKR and contributes to interferon resistance of HCV [175].

Surveillance for hepatocellular carcinoma

Surveillance in a population assumes that:

- the disease has an asymptomatic stage but if left alone will produce significant morbidity or mortality
- a high-risk population can be identified
- surveillance tests are inexpensive, noninvasive, and accurate
- clearly defined recall procedures exist
- effective therapy is available (Table 93.1).

For HCC almost all of these criteria can be met in principle. Patients with cirrhosis from alcohol abuse, viral hepatitis, hemochromatosis, α_1-antitrypsin deficiency, and primary biliary cirrhosis all have an increased incidence of HCC [176–180]. Asians and Africans who are chronic HBV carriers are also at significant risk for the development of HCC [181]. In contrast, HCC is unusual in Wilson disease,

Table 93.1 Criteria for instituting population surveillance

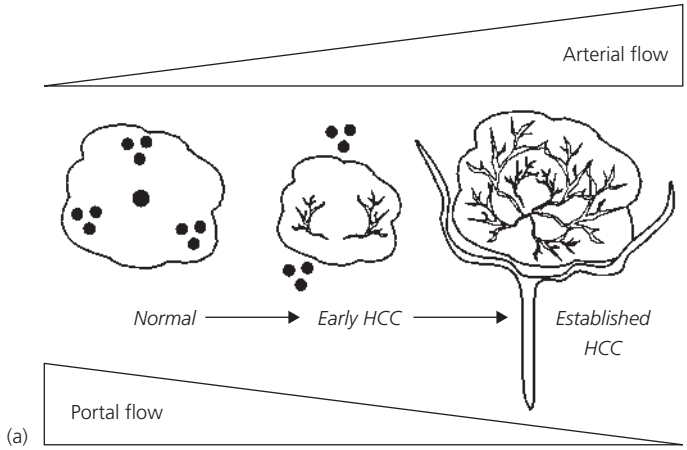
Criteria	Criteria met for HCC
Asymptomatic stage	Yes
Late stages are incurable and cause mortality	Yes
High-risk population	Yes, cirrhosis
Surveillance testing is inexpensive and noninvasive	Yes, ultrasonography
Established recall procedures	Yes
Effective therapy	Liver transplantation, surgical resection, ablative procedures; TACE[a]

a These therapies can only be applied on a selective basis, and the nontransplant therapies are fraught with recurrent disease.
HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

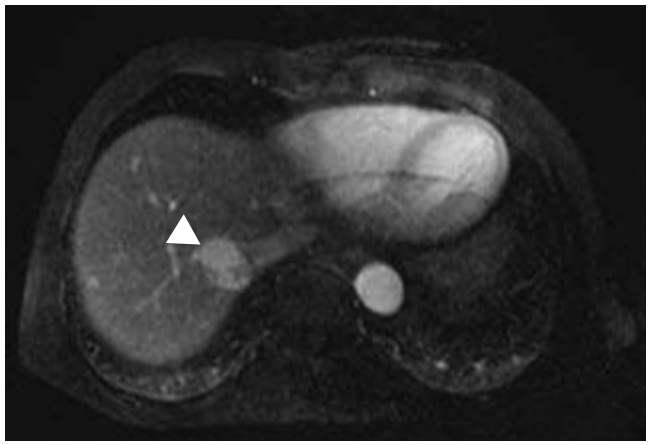
autoimmune hepatitis, and primary sclerosing cholangitis [182–184]. In high-risk cirrhotic patients the incidence of HCC is 2%–6%, which is more than the calculated incidence of 1.5% that is thought to be the cutoff point needed for surveillance to be cost-effective [185]. Current guidelines recommend surveillance for all patients with cirrhosis of any etiology as well as for patients with chronic hepatitis B who are Africans over 20 years of age, Asian men of at least 40 years of age, Asian women of at least 50 years of age, or who have a family history of HCC [186]. Ultrasonography is relatively inexpensive, noninvasive, and accurate in the diagnosis of early stage HCC, and, therefore, it is recommended that ultrasonography be used to screen for HCC at 6-month intervals in patients with cirrhosis. This radiographic technique has a sensitivity of 70% and a specificity of 90% for identifying HCC in an asymptomatic population [187]. The 6-month interval time schedule comes from data on the doubling time of HCCs [188,189]. The utility of serum α-fetoprotein determinations in screening for HCC is diminished by the low sensitivity of the test for early-stage tumors, which are most amenable to potentially curative therapy, and recent guidelines discourage its use except in settings where expertise in liver ultrasound is not available or in overweight patients in whom ultrasound is less sensitive [186,190]; however, an elevated serum α-fetoprotein does identify patients at increased risk for developing HCC [191]. Current recommendations for recall procedures (the definition of an abnormal result and the procedure for investigating abnormal results) are based on studies of the size, growth, and imaging characteristics of early HCCs. Although cirrhotic nodules can be as large as 2 cm, most nodules less than 1 cm in size are not HCCs. Therefore, new nodules in a cirrhotic liver that are less than 1 cm in size should be followed with repeat ultrasound at 3- to 4-month intervals. If the nodules remain stable in size over 18–24 months, it is reasonable to presume that they are benign and resume standard surveillance every 6 months. Nodules 1 cm or greater in size should be evaluated further using cross-sectional imaging of the liver (see Diagnosis of hepatocellular carcinoma). Recent population-based studies of surveillance for HCC confirm that it is associated with the detection of disease at an earlier stage and enhanced survival [192].

Differential diagnosis, diagnosis, and staging of hepatocellular carcinoma

This section will focus on the differential diagnosis of liver mass lesions in the presence of cirrhosis. The major problem in cirrhosis is differentiating HCCs from macroregenerative nodules (MRNs), which are large nodules that form in the liver as part of the cirrhotic process. The presence of large MRNs is itself a risk factor for the development of HCC, and therefore, the distinction between MRNs and HCC is difficult. Indeed, nomenclature suggests that instead of MRN the term dysplastic nodule should be used [193]. The imaging hallmark of HCC is increased vascularity of the lesion as compared to the surrounding tissue in the arterial phase and "washout" of contrast from the lesion so that it is less enhancing than the surrounding nonneoplastic tissue in the portal venous and late venous phases. These characteristics have a 98% specificity for the diagnosis of HCC [194]. For lesions 1–2 cm in size, arterial enhancement and venous "washout" using two cross-sectional imaging modalities is considered necessary for a noninvasive diagnosis of HCC; for lesions greater than 2 cm in size both these characteristics found on one cross-sectional imaging modality are sufficient for noninvasive diagnosis (Fig. 93.4) [195,196]. Also, if the lesion is accompanied by an α-fetoprotein measurement greater than 400 ng/mL, the diagnosis of HCC is likely [196]. If these strict criteria are adopted, imaging misdiagnosis is unlikely. In a robust study, the diagnosis of HCC was established preoperatively without biopsy with an accuracy of 99.6%, sensitivity of 100%, specificity of 98.9%, and positive and negative predictive values of 99.3% and 100%, respectively [197]. In the absence of these noninvasive criteria for the diagnosis of HCC (Table 93.2), histological or cytological verification of the malignancy should be obtained. However, the risk of tumor seeding has been estimated to be 0.76%–5.1% [198–200], and a biopsy should only be performed if it will change therapy. Recent experience with the use of needle introducer sheaths, through which a cutting needle is passed into the tumor, suggests that they reduce the risk of tumor seeding [201]. Also, the false-negative rate (negative biopsy report in a patient with cancer) is approximately 10%. In many instances, expert clinicians may elect to proceed with surgery or an ablative therapy based on the presence of a clear-cut mass lesion in a cirrhotic liver because it is difficult to interpret a negative biopsy. The detection of small (< 1 cm) hypoechoic or hyperechoic nodules during ultrasonography of the liver should raise the suspicion of HCC. Hypoechoic nodules are consistent with neoplastic growth of tissue whereas hyperechoic nodules suggest the presence of steatosis within an early cancer. It is well established that many small HCCs contain fat, which is subsequently lost as the cancer progresses [202]. Therefore, both hypoechoic and hyperechoic lesions must be considered malignant in a cirrhotic liver until further characterized. However, pathological studies have shown that only approximately half of such nodules less than 1 cm in size are neoplastic. These small lesions, unfortunately, are too small to assess their vascularity by imaging studies or to reliably obtain tissue for pathological examination. It is recommended that these lesions be followed with serial imaging studies at 3- to 4-month intervals. Growth of the lesion or the development of contrast enhancement on cross-sectional imaging studies would signify the development of HCC. Ultrasound and spiral computed tomography (CT) or magnetic resonance imaging (MRI)



(a)



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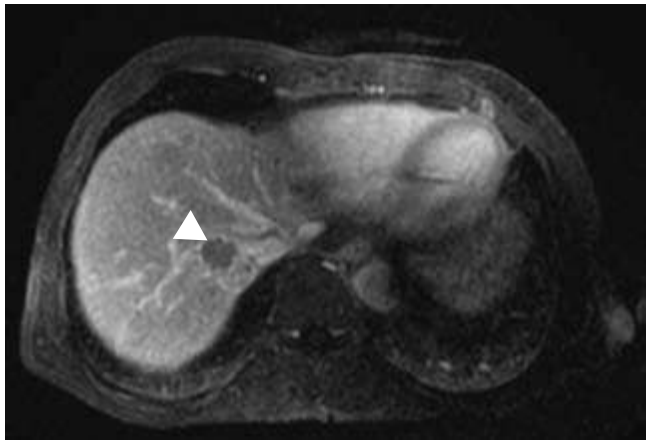

(c)

Figure 93.4 **(a)** Schematic representation of arterialization during hepatocellular carcinoma (HCC) development. **(b)** A contrast magnetic resonance imaging scan in the arterial phase showing enhancement of an early HCC. This pathological feature of HCC allows more accurate detection of malignant nodules. The technique of transarterial chemoembolization also relies on the preferential arterial vascularization of HCC rather than the normal hepatic parenchyma. **(c)** Delayed image showing venous phase washout of the small HCC shown in **(b)**. This phenomenon is accounted for by the later flow of contrast-carrying portal venous blood into the benign liver parenchyma, after the arterial flow has emptied out of the vascular HCC. Washout is highly specific for HCC in a cirrhotic liver.

Table 93.2 Noninvasive radiographic criteria for the diagnosis of hepatocellular carcinoma

Lesions 1–2 cm in size:	
Radiographic criteria	two coincident imaging studies demonstrating arterial hypervascularization and portal venous washout
Lesions > 2 cm in size:	
Radiographic criteria	one imaging study demonstrating arterial hypervascularization and portal venous washout

scans are the standard imaging modalities for the follow-up of these early and diagnostically challenging lesions.

Natural history of hepatocellular carcinoma

An understanding of the natural history of HCC is necessary for appropriate interpretation of the results of published treatment trials. The natural history of HCC is dependent on the severity of the underlying cirrhosis, tumor characteristics (i.e., size, growth rate, multicentricity, presence or absence of vascular invasion, pathological grade, and metastases), symptoms as manifest by the performance status of the patient, comorbid diseases, and the efficacy of treatment interventions.

Table 93.3 Prognostic stage classification systems for hepatocellular carcinoma

Classification	Parameters
BCLC	performance status constitutional symptoms vascular invasion extrahepatic spread
CLIP	Child–Turcott–Pugh stage tumor size or nodularity vascular invasion α-fetoprotein ≥ 400 mg/mL
GETCHC	Karnofsky index < 80% bilirubin ≥ 3 mg/dL α-fetoprotein > 35 mg/mL alkaline phosphatase > 2 × ULN vascular invasion
Okuda	ascites tumor size > 50% of liver albumin < 3 g/L bilirubin > 3 mg/dL

BCLC, Barcelona Clinic Liver Cancer; CLIP, Cancer of the Liver Italian Program; GETCHC, Group d'Etude et de Traitement du Carcinoma Hepatocellulaire; ULN, upper limit of normal.

Several prognostic classifications for HCC have been developed (Table 93.3). All of these are imperfect, in part because they do not take into account specific information on the tumor (e.g., information on the genetic changes or specific mechanisms involved in pathogenesis of the tumor), are based on information at the time of the initial diagnosis and, therefore, do not incorporate changes in prognostic variables over time, and are based on retrospective analysis. The first was developed by Kunio Okuda and colleagues and is simply known as the Okuda classification [203]. This classification takes into account hepatic factors (bilirubin, albumin, and ascites) and a single tumor factor (extent of liver replacement > 50% or < 50%). Given its relatively gross assessment of tumoral factors and absence of patient health parameters, this classification is seldom used in clinical assessment. The other three prognostic classifications take into account more refined assessments. The Barcelona Clinic Liver Cancer (BCLC) prognostic assessment takes into account the patient performance status, presence or absence of constitutional symptoms, vascular invasion, and extrahepatic spread [204]. The Cancer of the Liver Italian Program (CLIP) uses the Child–Turcotte–Pugh Score, tumor size and multicentricity, vascular invasion, and the serum α-fetoprotein to establish a prognosis [205,206]. The Group d'Etude et de Traitement du Carcinoma Hepatocellulaire (GETCHC) takes into account the Karnofsky index, serum bilirubin, α-fetoprotein, serum alkaline phosphatase, and vascular invasion [207]. It is clear from an assessment of these models that vascular invasion, poor performance status, and an elevated α-fetoprotein with tumor multicentricity are uniform poor prognostic parameters. The BCLC staging system has been externally validated by two groups and is the staging system of choice, having been endorsed by two professional societies (Table 93.4; Fig. 93.5) [208,209]. This classification system also predicts which patients will do well for extended periods of time. For example, using data from patients randomized to no treatment in well-designed randomized control trials, the BCLC group found that patients with unicentric disease, no symptoms, and no evidence for vascular invasion nor extrahepatic spread had a 50% 3-year survival [210]. This subgroup of patients with HCC are frequently those selected for locoregional therapies (see below) and the survival in the treatment groups is frequently no better than this untreated group of patients. This information on the natural history of HCC needs to be kept in mind when counseling patients and helping them select treatment options.

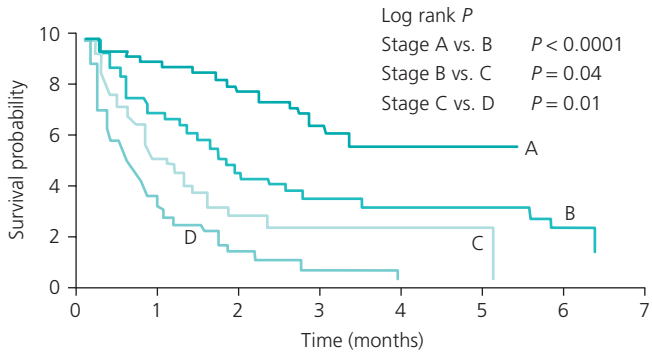

Figure 93.5 Staging of hepatocellular carcinoma. Survival as a function of Barcelona Clinic Liver Cancer stage. Modified from Marrero et al. [208].

Table 93.4 Barcelona Clinic Liver Cancer (BCLC) staging system

Stage	Tumor features	Liver features
Stage 0 (very early HCC)		
PST 0	Single < 2 cm	Child–Pugh A
Stage A (early HCC)		
PST 0	Single tumor	Child–Pugh A–B
PST 0	Three tumors, all < 3 cm	Child–Pugh A–B
Stage B (intermediate HCC)		
PST 0	Multinodular	Child–Pugh A–B
Stage C (advanced HCC)		
PST 1–2	Vascular invasion or extrahepatic spread	Child–Pugh A–B
Stage D (end-stage HCC)		
PST 3–4	Any	Child–Pugh C

HCC, hepatocellular carcinoma; PST, performance status.

Table 93.5 Clinical manifestations of symptomatic hepatocellular carcinoma

Presentation	Clinical syndrome
Tumor	malaise, anorexia, weight loss, night sweats
Hepatic decompensation	jaundice, ascites, variceal bleeding
Acute abdominal pain	tumor bleeding, capsular extension
Pyrexia	fever and leukocytosis
Metastases	brain, lung, or bone metastases
Cholestasis	large bile duct obstruction or diffuse infiltrating cancer
Paraneoplastic	↑ red blood cell mass, ↑ Ca^{2+}, ↓ glucose, diarrhea

Clinical manifestations of hepatocellular carcinoma

With better institution of surveillance strategies, most HCC will be identified at an asymptomatic stage. However, many patients continue to present with advanced HCC. The clinical manifestations of advanced HCC are protean and include decompensation of cirrhosis, tumoral symptoms, acute abdominal catastrophe due to intratumoral or peritumoral hemorrhage, cholestasis, fever of unclear etiology, paraneoplastic phenomena, and the metastatic presentation (Table 93.5). HCC may result in decompensation of cirrhosis by vascular invasion of the major portal vein or its large branches. This event worsens portal hypertension and frequently results in the development of ascites and refractory variceal bleeding. Infiltrating cancers in the liver also increase portal hypertension at the level of the sinusoids promoting ascites formation and variceal bleeding. Also, the diffuse infiltrating cancers further worsen hepatic function resulting in hyperbilirubinemia, coagulopathy, and encephalopathy. Even small cancers may be associated with hepatic decompensation either because advanced cirrhosis is frequently associated with HCC or because the small cancers have effects on the immune system or hepatic function in general. For example, approximately 20% of patients hospitalized with spontaneous bacterial peritonitis will have HCC [211]. As a result of this association, all hospitalized cirrhotic patients should have appropriate imaging studies to look for HCC.

Tumoral and acute presentations of HCC are less common than the presentation of hepatic decompensation. The tumoral presentations include anorexia, weight loss, general malaise, and painful hepatomegaly. The acute presentation is associated with spontaneous rupture of the cancer into the liver parenchyma, into the peritoneum, or extension through the capsule. Mortality is high with hemoperitoneum because of exsanguination. Selective acute arterial embolization of the bleeding artery is the preferred treatment for this complication. Patients with pain from tumor extension through the capsule likely have pain from this rupture event. The pain is usually acute, limited in time (< 24 h) and is usually assumed to be biliary colic. The screening ultrasound of the liver often unexpectedly identifies a liver mass when the patient seeks medical attention.

Cholestasis may result from tumor-associated extrinsic compression of the large bile ducts, tumor invasion of the large bile ducts, or miliary metastasis throughout the liver. Tumor invasion of the bile ducts can be associated with hemobilia and gastrointestinal bleeding [212]. The miliary pattern of metastases is usually associated with disproportionate high serum aminotransferases in addition to hyperbilirubinemia and jaundice.

Fever of unknown etiology is an unusual presentation of HCC [213]. Interestingly, the febrile episodes may be discrete episodes and associated with leukocytosis. Tumors need not be large to generate this symptom complex. Given the frequent difficulty in diagnosing HCC by a single imaging study, multiple imaging studies may be necessary to make the diagnosis of HCC in this context. In contrast to fever, night sweats are quite common in advanced HCC and portend a poor prognosis.

Paraneoplastic phenomena associated with HCC are rare but include hypercalcemia, erythrocytosis, hypoglycemia, thrombophlebitis migrans, arterial hypertension, and the syndrome of watery diarrhea, hypokalemia, and achlorhydria. The hypercalcemia is the result of production of a parathyroid hormone-like molecule, the erythrocytosis is due to erythropoietin generation by the tumor, and hypoglycemia is mediated by insulin-like growth factors [214]. Arterial hypertension is uncommon in advanced cirrhosis and its presence, therefore, should prompt imaging studies for HCC. In addition to the rare syndrome of watery diarrhea, hypokalemia, and achlorhydria, diarrhea without electrolyte abnormalities is relatively common in advanced HCC [214]. In children with hepatoblastomas, isosexual precocity may be a paraneoplastic syndrome [214]. In adults, feminization and gynecomastia are also associated with HCC, but these syndromes are often present in cirrhosis without HCC and are, therefore, difficult to attribute to HCC. Cutaneous manifestations of HCC are equally rare and include dermatomyositis, pemphigus foliaceous, and Leser–Trelat sign. The latter is the sudden onset of seborrheic dermatitis and freckles with a rapid increase in the number of the lesions accompanied by pruritus. Pityriasis rotunda (round or oval hyperpigmented scaly lesions) may be associated with HCC in black South Africans [214].

Metastatic complications of HCC are not dissimilar from the metastatic complications of other neoplastic diseases. However, HCC does have some unique metastatic presentations. One metastatic complication of HCC is dyspnea as the result of tumor pulmonary emboli, and another is headache associated with pituitary metastases [215]. Common sites for HCC metastases include the diaphragm with associated pleural effusions, bone metastases with pain, and regional lymph node metastases, which may be associated with abdominal

discomfort. In general, abdominal pain in patients with HCC is a poor prognostic symptom and usually indicates advanced disease.

Staging for selection of therapy

The staging procedure depends in part on the intensity of the selected treatment. A chest radiograph should be performed for all patients to exclude pulmonary metastases; at least two imaging studies should be carried out to stage intrahepatic disease and exclude vascular invasion (ultrasound, contrast CT, contrast MRI), and many centers will perform a CT scan of the chest and a bone scan if liver transplantation is being contemplated. It is important to note that abdominal lymphadenopathy is common in cirrhosis, especially the perihilar lymph nodes, and even more generalized lymphadenopathy occurs in hepatitis C. One therefore has to be very cautious in attributing lymphadenopathy to metastatic disease. In suspicious cases, the authors have found endoscopic ultrasound with fine-needle aspiration of hypoechoic lymph nodes useful for detecting metastatic involvement of lymph nodes. Finally, the dilemma of what to do with a small HCC remote from a portal vein thrombosis remains problematic. If tumor thrombosis is present, radical or ablative therapies are not effective; if the thrombus is bland, then these therapies may be contemplated. Many experienced centers use color-flow Doppler and ultrasound-guided fine-needle aspiration of the thrombus to make a decision [216–218]. The appearance of an arterial signal on the color-flow Doppler is most indicative of tumor thrombus as these lesions are frequently arterialized despite their residence in a vein. A negative color-flow Doppler along with a negative fine-needle aspiration would be strong evidence in favor of a bland thrombus.

Locoregional therapies

Locoregional approaches include surgical and percutaneous ablative techniques. The outcome of these therapies for HCC is dependent on several readily identifiable prognostic factors. Poor prognostic factors identified in numerous studies include unicentric tumors larger than 5 cm, multicentric disease with more than three lesions, especially if any of the lesions is larger than 3 cm in size, satellite lesions adjacent to the main tumor (a marker for tumor invasion and therefore metastatic potential), and vascular invasion [204]. In particular, invasion of the main portal vein or one of its segmental branches, a striking feature of HCC, is a relative contraindication to radical therapy.

Surgical approaches

Surgical resection is commonly accepted as the initial treatment of choice for localized HCC worldwide. Despite the acceptance of surgery for the treatment of HCC, there are no randomized controlled trials demonstrating its efficacy. This approach is limited in its application because few patients are suitable for partial hepatectomy because of tumor size, vascular invasion, multicentricity, the presence of portal hypertension, or overall hepatic functional reserve. Intrahepatic recurrence of HCC is common among patients who undergo apparently curative resection because the neoplastic potential of the nonresected liver remains unchanged. Indeed, the 5-year recurrence rate is 52% either as the result of de novo tumor recurrence or the progression of previously unrecognized intrahepatic metastases [15]. Hepatic decompensation is high in patients with any elevation of the serum bilirubin or evidence of portal hypertension (Fig. 93.6) [219]. A wedged hepatic venous pressure gradient above 10 mmHg is considered to be a contraindication for resection. However, few liver centers routinely perform this test and, instead, many surgeons use a platelet count of less than 100 000/fL (indicative of congestive splenomegaly) or the presence of varices on endoscopy or cross-sectional imaging studies of the abdomen (indicative of a wedge hepatic vein pressure gradient of at least 12 mmHg) as contraindications to surgery. Operation-related mortality is also high, especially in patients with limited hepatic functional reserve. Partial hepatectomy should therefore only be considered for patients without cirrhosis or those with mild liver disease (Child's A cirrhosis), normal portal pressure, and normal bilirubin [204]. A Model for End-Stage Liver Disease (MELD) score of 9 or greater has been shown to predict a significantly worse outcome after surgical resection for HCC [220]. The best outcomes are observed in patients with unicentric disease, no vascular invasion, tumors smaller than 5 cm, and relatively inactive liver disease. Given these selection criteria, it is estimated that only about 5% of patients with cirrhosis and HCC are candidates for surgical resection [221]. In this highly selected patient population, the best reported outcomes are 50% at 3 years [204]. This outcome is not that much different from the natural history of untreated, asymptomatic,

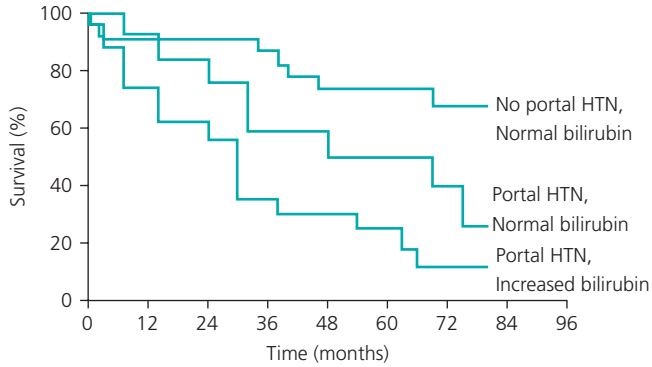

Figure 93.6 Hepatic decompensation leads to a worse outcome after surgical resection for hepatocellular carcinoma in patients with clinically significant portal hypertension and an increased serum bilirubin. Modified from Llovet et al. [15].

small, unicentric disease without vascular invasion or that obtained with ablative techniques (see above). Many hepatologists seldom advocate resection for HCC except in ideal candidates for these reasons. However, if appropriate adjuvant therapies or secondary chemopreventive agents could be developed then surgery would be a more attractive option. Along these lines, polyprenoic acid has been shown in a single study to decrease recurrence rates following surgical resection of HCC [222]. If these studies can be confirmed, the use of this agent should enhance outcomes following surgery or ablative therapies.

Orthotopic liver transplantation (OLT) for patients with HCC offers several advantages over partial hepatectomy. It can be employed for patients with all stages of liver disease and the cancer, the neoplastic potential of the underlying liver disease, and the liver disease itself are all therapeutically addressed at the same time. Indeed, numerous studies have demonstrated that in patients with unicentric disease smaller than 5 cm or with multicentric disease of up to three lesions with each lesion 3 cm or less, the results with liver transplantation are superb. For example, a study of 120 cirrhotic patients with HCC showed that transplanted patients with a maximum of two small (< 3 cm) nodules had improved survival without recurrence (83% at 3 years) compared to patients treated with partial hepatectomy (18% at 3 years) for tumors of the same size [223]. One large series found that HCC less than 5 cm in diameter or the presence of three or fewer tumors all less than or equal to 3 cm in diameter was associated with overall survival of 85% and disease-free survival of 92% at 4 years [224]. Subsequently, transplant centers throughout Europe and the United States have adopted these criteria in HCC patients for OLT. In the United States, patients with HCC are assigned additional MELD points with the goal of matching their dropout rate from tumor progression to that of patients with advanced liver disease who die while on the waiting list. Initial limited studies based on post-transplant pathological examination of explanted livers suggested that unicentric tumors with a capsule and without vascular invasion of up to 6.5 cm or multicentric tumors with up to three lesions, each no larger than 4.5 cm in size, having a total diameter of up to 8 cm, may do equally well with OLT; these are commonly referred to as the UCSF (University of California at San Francisco) criteria [225]. In addition, data from Germany suggest that very well-differentiated large tumors have acceptable outcomes following OLT [226]. Consequently, it has been suggested that the current guidelines of one lesion less than 5 cm or three lesions all less than 3 cm in diameter are restrictive criteria developed to assure the best outcomes, and should be reevaluated as more information becomes available. However, a recent study suggests that when applied before liver transplantation, the additional patients with disease stage beyond the Milan criteria who meet the UCSF criteria have a worse outcome; the 5-year survival was 46% in the patients meeting UCSF criteria but beyond Milan criteria compared to a survival of 60% in those meeting the Milan criteria [227]. In future, molecular staging techniques may allow optimal selection of the candidates who are most likely to have a recurrence-free survival after transplant. The most important limitation of OLT is an increasing organ shortage, which is problematic for several reasons. OLT for HCC removes livers from the common donor pool and may thus aggravate the situation for patients with end-stage cirrhosis. In addition, a long waiting period for a donor organ often allows a small tumor to become large or even metastatic. Indeed, the drop-out rate for patients initially meeting the criteria for OLT may be as high as 25% during 6 months of waiting time in the absence of therapy [15]. However, these patients were selected on the criteria that all lesions had to be identified by two imaging modalities, resulting in an understaging bias. In contrast, most transplant centers in the United States have an overstaging bias, which is to take the maximum number of lesions identified on any given study to be the actual number of tumors. Nonetheless, given the long waiting times for OLT, most centers prefer to treat the lesions before OLT to prevent tumor growth and dissemination. Ablative approaches, chemoembolization, or radioembolization are usually employed [228]. None of these therapies has been subjected to randomized controlled trials in combination with OLT and, therefore, the optimal approach remains to be ascertained.

In summary, tumor criteria identifying patients with HCC who are most likely to benefit from surgical approaches (resection and transplantation) have been established. Many experts feel that patients meeting criteria for surgical approaches should be offered this option as definitive therapy. In patients who are candidates for both resection and transplantation, the best outcomes are obtained with transplantation because of the high recurrence rate following liver resection [15]. However, if effective secondary chemoprevention therapies can be established, surgical resection may become preferred as it avoids the risks and need for long-term immunosuppression. In patients with HCV infection, this decision must be balanced against the risk of recurrent HCV and its sequelae. For patients who are not candidates for surgical approaches, locoregional forms of treatment are reasonable. These options include percutaneous alcohol injection, radiofrequency ablation, transcatheter arterial chemoembolization, and transcatheter arterial radioembolization and will be discussed in the following sections.

Percutaneous alcohol injection

Percutaneous alcohol injection (PEI) has become a widely accepted form of therapy for small, localized HCC. The advantage of PEI over other local ablative minimally invasive therapies is its relatively simple technique and low cost. PEI can be safely performed in patients who are not candidates for resection or OLT, even in the presence of advanced cirrhosis [229]. It is generally agreed that HCCs less than 3 cm

in size and with fewer than three nodules are suitable for PEI. The destruction of tumor cells by injection of absolute alcohol is probably the result of a combination of cellular dehydration, coagulative necrosis, and vascular thrombosis followed by tissue ischemia.

For PEI, sterile ethanol (95%) is slowly instilled throughout the tumor nodule under ultrasound or CT guidance. Since it is important to achieve uniform distribution of the ethanol, the injecting needle is usually placed at the far end of the lesion and then withdrawn in a stepwise fashion during the injection. For small lesions, a single treatment is often sufficient while larger lesions may require several treatments over a number of days. The extent of necrosis achieved is dependent on tumor size. Small nodules less than 3 cm in diameter are usually completely destroyed, larger tumors are only partially destroyed [230,231]. Patients should therefore be followed up regularly with spiral CT scans. PEI can be repeated for local recurrences and new lesions within the liver. PEI is associated with a complication rate of 1.7% and a 0.1% mortality rate. Patients may experience abdominal pain, which is often the result of leakage of ethanol into the peritoneal cavity and can be prevented by leaving the needle inside the lesion for 30 seconds or more after injection. A low-grade fever may be present for 1–3 days after the procedure because of tumor necrosis. Contraindications to PEI include massive ascites, coagulopathy, and obstructive jaundice, which increase the risk of postprocedural bleeding and bile peritonitis.

Long-term survival is as high as 70% at 3 years in patients with cirrhosis undergoing PEI for single HCCs less than 3 cm in diameter [230,231]. In the largest series published, Livraghi and colleagues reported survival rates in 746 cirrhotic patients with HCC treated with PEI [229]. In patients with a single tumor < 5 cm in diameter ($n = 462$), the 3- and 5-year survival rates, respectively, were 79% and 47% for patients with Child A stage disease, 63% and 29% for patients with Child B stage disease, and only 12% and 0% for patients with Child C disease. In patients with Child A cirrhosis, survival was much lower in those with multiple HCCs (35% at 5 years), single HCCs larger than 5 cm (30% at 5 years), and advanced HCCs (0% at 5 years). Unfortunately, there have been no prospective randomized controlled trials comparing PEI to surgical resection or transplantation for early-stage HCC. In a large retrospective study from Japan in which patients were matched for severity of the liver disease and stage of tumor, there was no significant difference in survival between patients treated with PEI and those undergoing surgical resection [232]. Long-term survival after PEI may therefore be equivalent to survival after surgical resection in a selected subgroup of patients with small tumors and mild cirrhosis. Similar to the results after surgical resection, tumor recurrence occurs in 50% of patients following PEI [233]. In summary, PEI is a safe, simple, and inexpensive treatment for patients with HCC. Although relatively few reports of long-term results are available, PEI seems to be an effective method for patients with single, small tumors. For this subgroup of patients, PEI might be equal to surgical resection with fewer complications and less procedure-related morbidity and mortality. However, more controlled studies comparing PEI to other treatment modalities are needed to better define its role in the management of HCC. Treatment of larger lesions is also possible, but is less likely to completely ablate the tumor and is therefore associated with higher rates of relapse and decreased survival.

Radiofrequency ablation

Radiofrequency ablation (RFA) is emerging as a preferred therapeutic method in the management of HCC. The first papers reporting ultrasound-guided RFA of hepatic tissue were published in 1990 and suggested that RFA could prove useful to create focal coagulative necrosis of hepatic tumors while sparing normal liver tissue [234]. Significant advances in equipment design have led to increasing success in the use of this method in the management of HCC. RFA destroys tissue by thermal energy generated with an alternating electric current generator that operates in the radiofrequency range from 200 to 1200 kHz. Like PEI, RFA is usually performed by a percutaneous approach in the outpatient department. After local anesthesia, a needle electrode is introduced through the skin under sonographic guidance and advanced into the center of the tumor. Treatment is guided by impedance measurements between components of the needle electrode. The goal of each procedure is to ablate the tumor and a small margin of normal tissue around the tumor. Depending on the equipment used, this is can be achieved in one or two short sessions (< 15 min) for lesions up to 5 cm. Large blood vessels act as a heat sink so RFA is not effective for lesions adjacent to large veins in the liver. It is also more technically difficult to treat lesions in segment 8 (lesions high in the dome of the liver) with RFA than with PEI. Spiral contrast enhanced CT scanning is usually used to assess tumor necrosis after RFA because ultrasound is usually not sensitive enough for this purpose. RFA can be repeated for residual tumor found on follow-up or for new tumor nodules that develop in the liver.

As experience with RFA has accumulated, the initially promising results have been largely confirmed. In 1995, Rossi and colleagues reported initial results from 24 patients with one or more tumor nodules smaller than 3 cm who were treated with intent to cure [235,236]. Complete tumor necrosis was achieved in all but two patients after one session; both patients successfully received additional thermal ablations. About half the patients had recurrence of the tumor during follow-up, which could be retreated in 70% of these patients. The survival rate was 95% the first year, 84% the second year, 67% the third year, and 45% the fourth and fifth years. Livraghi and colleagues used a large cohort of patients ($n = 112$) with small HCCs, less than 3 cm in diameter,

to compare the effectiveness of RFA with that of PEI [237]. Complete tumor necrosis was achieved in 90% of tumors with RFA in an average of only 1.2 sessions per tumor and in 80% of tumors with PEI in 4.8 sessions. However, in contrast to other series, four minor and one major complication were reported in patients treated with RFA; in contrast, no complications occurred in patients treated with PEI. One study suggests that RFA may be associated with a high rate of needle track seeding (12.5%) because of the large needle used in this approach, especially for high-grade and surface lesions [238].

Studies comparing PEI and RFA have shown superiority in outcome for patients treated with RFA [239]. More importantly, a randomized controlled trial comparing RFA with surgical resection yielded similar outcomes. Consequently, RFA is rapidly becoming a preferred approach for treatment of unicentric HCC less than 3 cm in diameter [240].

Transcatheter arterial chemoembolization

Transcatheter arterial chemoembolization (TACE) is a technique that uses angiography to selectively embolize the arterial supply to an HCC. A cytotoxic agent such as doxorubicin or cisplatin is mixed with lipiodol, gelfoam, or Ivalon particles to form a suspension that is then injected into the feeding artery of the tumor using an angiographic technique by way of the common femoral artery. This results in both selective ischemic and chemotherapeutic effects on the HCC. As a result of the dual blood supply of the liver, TACE causes minimal damage to normal liver parenchyma, because 80%–100% of the blood-flow to liver tumors, but only 20%–30% of the flow to normal liver, is supplied by the hepatic artery. After the procedure the suspension remains in the tumor, where the cytotoxic agent is slowly released to exert its effect. Although lipiodol serves a dual role in TACE by carrying the chemotoxic agent to the tumor and also embolizing the vasculature, it can remain in the lesion for prolonged periods of time and preclude adequate radiographic assessment of tumor necrosis by contrast-enhanced CT scanning. The relative contribution of the chemotherapeutic agents to treatment of HCCs compared to the simple ischemic effect of occluding the tumor vasculature has never been established.

Risks of TACE include contrast allergy, contrast-induced nephropathy, and bleeding or pseudoaneurysm formation at the catheter site. Most patients develop a postembolization syndrome with fever and right upper quadrant pain and are therefore hospitalized. Liver function tests can be elevated for up to 2 weeks. Major complications include hepatic abscess, which is most common in patients with a preexisting biliary–enteric anastomosis, hepatic artery dissection or thrombosis, and ischemic cholecystitis. The morbidity rate of TACE was 5.1% and treatment-related mortality rate was 4.1% in one retrospective study [241]. Advanced Child C stage cirrhosis is a relative contraindication for TACE because of the high risk of potentially fatal complications such as liver failure. Other contraindications include hepatic encephalopathy, biliary obstruction, portal vein occlusion, a surgical portosystemic shunt, a transjugular intrahepatic portosystemic shunt device, bilirubin greater than 5 mg/dL, international normalized ratio greater than 1.7, or creatinine more than 1.8.

The efficacy of TACE is determined by the size of the lesion. One study reported a survival rate of 100% at 3 years for tumors less than 2 cm in diameter, whereas 3-year survival was 0% for tumors greater than 5 cm [242]. TACE also avoids the risk of needle-track seeding so transplant centers often use TACE as a bridge to transplantation for HCC patients. It prevents progression of the lesions and potential micrometastases while the patient waits for a donor organ. In a pilot study at our institution, none of the transplant recipients pretreated with TACE developed recurrent HCC during a mean follow-up of more than 2 years [243]. More recent results confirm that patients who have a complete or partial response to pretransplant TACE, as assessed by CT, have substantially better 5-year survival rates (complete response, 85.7%, partial response 66.2%) than those with no response or with tumor progression (19.3%) [244]. The value of TACE as a palliative procedure for patients with intermediate stage HCC has been established in the past few years by the results of two randomized controlled trials that both confirmed increases in survival of patients receiving TACE when compared to untreated controls [245,246]. An analysis of the available randomized trials also confirmed the superiority of TACE [247].

Transcatheter arterial radioembolization

Transcatheter arterial radioembolization (TARE) is similar to TACE in that radiolabeled particles are infused into the hepatic artery and preferentially flow into the highly vascular HCCs, with relative sparing of the nontumorous liver, which is primarily supplied by the portal venous circulation. TARE is available in two variants, as yttrium-90-impregnated glass microspheres (TheraSpheres, MDS Nordion, Ottowa, ON) and as resin-based microspheres (Sir-Spheres, SIRtex Medical Limited, Wilmington, MA) [248,249]. There has been more published experience with TheraSpheres. Significant differences between TheraSphere administration and TACE include the fact that the Thera-Sphere microspheres have high specific activity and consequently a relatively small mass of beads is infused into the tumor vasculature. The beads therefore do not occlude the vasculature, as occurs with TACE, and TheraSphere treatment does not cause the tumor lysis syndrome that is typically induced by TACE [250]. TARE using TheraSpheres can therefore be administered to patients with HCC and portal vein thrombosis, which is typically a contraindication for the use of TACE. Since there is no tumor lysis syndrome and the emission of β-particles by yttrium-90 is limited to 1–2 mm from the microspheres, the treatment also has the advantage of being

administered on an outpatient basis. There are risks of radiation injury to the lungs or the adjacent gastrointestinal tract supplied by hepatic artery branches. Therefore, a planning angiogram is performed before the TheraSphere procedure in which any hepatic artery branches that supply the stomach or duodenum are occluded. During the planning angiogram a small dose of technetium-99-labeled macroaggregated albumin is injected into the hepatic artery distribution followed by a lung scan to quantify the percentage of infused radiation that is shunted to the lungs. A high lung shunt would lead to potential radiation lung injury from the radiolabeled microspheres and is a contraindication to therapy. The TheraSphere protocol has been shown in uncontrolled series to be effective for down-staging patients with intermediate stage HCC to resection, RFA, and as a bridge to liver transplantation. [251]. Additional studies, particularly randomized controlled comparisons of TARE with TACE, are needed to determine the optimal strategy for patients who are eligible for treatment using these modalities.

Systemic therapy

Four forms of systemic therapy have been applied to the treatment of advanced HCC without substantial efficacy, including cytotoxic therapy, immunotherapy, hormonal therapy, and gene therapy. Studies using new treatments that target molecular pathways involved in cancer growth and angiogenesis are being reported. With improvements in our understanding of the molecular pathogenesis of HCC, these new, targeted therapies hold promise for providing substantial improvements in the treatment of advanced HCC, adjuvant treatment of intermediate stage HCC in combination with surgery or locoregional therapies, secondary chemoprevention after surgery for cure, and primary chemoprevention in high-risk individuals.

Cytotoxic chemotherapy

HCC is extremely refractory to current available cytotoxic therapies. Moreover, the presence of cirrhosis with impaired clearance of drugs metabolized by the liver increases drug toxicity. Portal hypertension with leukocytopenia and thrombocytopenia also exacerbate drug-induced bone marrow toxicity. The overall response rate is < 20% for systemic chemotherapy given intravenously using either single or combined agents [252]. Intraarterial therapy also has not been shown to impact disease progression or survival [253]. There is therefore no current proven chemotherapeutic regimen for HCC.

Hormonal therapy

The presence of androgen receptors in HCC, the sexual dimorphism of the liver, and the male predominance of HCC led to the use of antiandrogen therapy for this cancer. Unfortunately, studies were negative and this approach was abandoned [254]. The presence of estrogen receptors in the liver also led to the use of tamoxifen (an antiestrogen) for the treatment of this disease. Initial studies suggested a survival benefit [255]. However, several randomized controlled trials and a metaanalysis of these trials have shown no survival benefit. Finally, HCCs express somatostatin 2 receptors, and administration of somatostatin appeared to improve the survival of patients with advanced HCC cancer in a pilot study [256]; however, subsequent randomized studies have shown no significant survival benefit [257–259].

Immunotherapy

Two forms of immunotherapy have been applied in HCC, using systemic therapy with interferon or employing lymphokine-activated natural killer cells. Although interferon was superior to no treatment or adriamycin in one study, this result was not reproduced in a second study [260]. Further, the administration of interferon in doses required for antitumoral effects was associated with a high rate of adverse side effects necessitating discontinuation of therapy. Lymphokine-activated killer cell therapy is associated with significant toxicity, is expensive, and difficult to implement [261]. Results with this approach have also been modest and, therefore, it has not been adopted as a therapeutic option.

Gene therapy

Gene therapy is rational for HCC because a transient transfection (e.g., liposome-mediated transfer of plasmid DNA constructs) or transduction (e.g., administration of a virus expressing the desired construct) may be sufficient to destroy the tumor cells. It also may be possible to effect selective tumor targeting using the α-fetoprotein promoter or angiography. In addition, bystander effects have been observed with all gene therapy strategies, indicating that not all cells have to be transduced. However, these goals have yet to be realized in the clinical context. Several trials, including those attempting to transfect HCC cells with wild-type p53, have evolved to human studies, but the results have been disappointing so far. Nonetheless, it is premature to abandon this approach and further developments are likely.

Targeted molecular therapies

Advances in our understanding of the molecular pathogenesis of epithelial cancers in general and HCC in particular have led to the recognition of specific molecular pathways that are deranged in cancer and that provide opportunities for targeted intervention. Growth-signaling pathways including the receptor tyrosine kinase signaling pathways and their downstream Raf kinase, mitogen-activated protein kinase, phosphatidylinositol-3/Akt kinase pathways, proangiogenic pathways activated by VEGF and mediated by the VEGF receptors, the Wnt signaling pathway, pathways leading to inactivation of multiple tumor suppressors, including the

methylation and histone deacetylase and the proteasomal protein degradation pathways, all represent rational targets for anticancer drug development. A phase II study of the Raf kinase and VEGF receptor kinase inhibitor sorafenib showed modest efficacy in HCC [154]. Subsequently, the first large phase III study of a receptor tyrosine kinase inhibitor in HCC, using sorafenib, was terminated early because of a presumed significant positive effect on patient survival. Sorafenib may become the treatment of choice for advanced HCC. The major toxicities are fatigue, a hand–foot skin rash, acne, and diarrhea. The study was conducted in patients with Child–Pugh class A disease, largely with BCLC stage C disease. The toxicity profile and efficacy in patients at Child–Pugh class B and C is unknown. This early positive result gives hope for additional future gains in the use of targeted agents for systemic therapy and prevention of HCC.

Summary

The selection of an appropriate treatment strategy for patients with HCC depends on careful tumor staging and assessment of the underlying liver disease (Fig. 93.7). All patients with localized HCC (involvement of one single lobe, no vascular invasion or extrahepatic disease) should be evaluated for the potentially curative therapy options partial hepatectomy or OLT. Candidates for partial hepatectomy must have no liver disease or Child stage A cirrhosis, normal portal pressure, and normal serum bilirubin. For patients not meeting these criteria, OLT should be considered if there is a solitary lesion less than 5 cm in size or three or fewer lesions less than 3 cm in size. Local ablative therapies such as PEI, RFA, TACE, and TARE offer palliation for patients with contraindications for surgical approaches. PEI and RFA are minimally invasive and can be used on an outpatient basis, usually for tumor nodules smaller than 3 cm. When used for small tumors, the survival rates can be similar to those achieved by partial hepatectomy. TACE may be used as an interim treatment for patients waiting for OLT or patients with BCLC stage 3 HCC. Given the paucity of efficacy data, there are no proven systemic chemotherapy regimens, immunotherapy approaches, or hormonal therapies that can be recommended. Early results suggest that we may be on the threshold of new advances in treatment of HCC using targeted therapeutics, especially sorafenib.

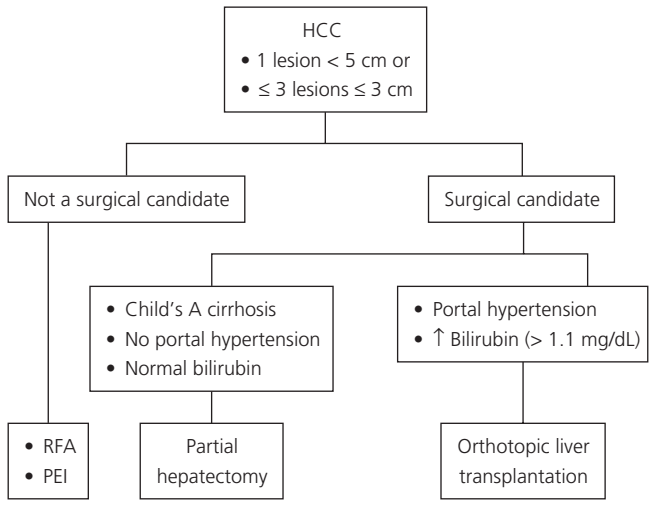

Figure 93.7 Management algorithm for curative treatment of hepatocellular carcinoma.

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G LIVER

94 Liver abscess

David S. Raiford

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Amebic versus pyogenic abscess

This chapter describes the clinical presentation, imaging characteristics, laboratory findings, natural history, and response to treatment of hepatic abscess. Although there is considerable overlap in these features between amebic and pyogenic abscesses, the differences in epidemiology, associated conditions, treatment, and prognosis underscore the need for the physician to distinguish these entities. Effective management depends critically on prompt and correct definition of the abscess type.

Epidemiology

There are important differences in the epidemiology of amebic abscess, as it presents in the United States, and pyogenic abscess. Intestinal amebiasis is a necessary prelude to hepatic amebic abscess so patients with amebic abscess typically have emigrated from or traveled to areas where intestinal amebiasis is prevalent [1,2]. Ethnicity of the local patient population is important. For example, in a large series from Los Angeles, 92% of 457 patients with amebic liver abscess had Hispanic surnames, compared with 37% of the local patient population [3]. In contrast, ethnicity of patients with pyogenic abscess did not differ from that of the general hospital population. Another difference identified between amebic and pyogenic liver abscess in this series was a striking male predominance (84%) in those with amebic abscess, whereas the gender distribution in those with pyogenic abscess was about equal. The median age of patients with amebic abscess (28 years) was significantly lower than that of those with pyogenic abscess (44 years) [3].

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Since the classic review by Ochsner and colleagues [4] in 1938, and after the introduction of antibiotics in the 1940s, the frequency of pyogenic liver abscess associated with intra-abdominal infection has diminished [5,6]. Although pylephlebitis (mostly due to appendicitis) accounted for 43% of cases of pyogenic liver abscess in the first decades of the 20th century [4], a series of patients from 1973 to 1993 indicated that in only 7% of cases was pyogenic liver abscess believed to be the result of portal bacteremia [7]. During the past few decades, biliary obstruction, both benign and malignant, has emerged as the most common etiology of pyogenic liver abscess, accounting for 50%–60% of cases in several recent series [7–9]. This trend undoubtedly reflects improvements in early diagnosis and management of extrabiliary abdominal infections attributable to modern imaging modalities and use of antibiotics. Over time the incidence of cryptogenic hepatic abscess has remained stable at ~20% of cases [4,7,10]. Several series suggest that diabetes mellitus may predispose to pyogenic liver abscess because 15%–25% of cases occur in patients with diabetes [3,7,8,11]. Finally, since the 1980s, it has become apparent that recipients of liver transplant allografts have an elevated risk for pyogenic liver abscess [12].

Clinical manifestations

Fever and right upper quadrant pain are the principal symptoms of hepatic abscess, both amebic and pyogenic. Fever is evident in virtually all patients. Although spiking fever and chills favor pyogenic abscess, these may be seen with amebic abscess. Pain is reported by 75%–90% of patients, is usually constant, is of variable intensity, and may exhibit pleuritic features with radiation to the right shoulder if diaphragmatic involvement is present [3,13–15]. Most patients have symptoms for less than 2 weeks before seeking medical care. Nonspecific symptoms, such as weakness, anorexia, nausea, and weight loss, are common. About one-third of patients with either type of liver abscess report diarrhea, and one-fourth have a nonproductive cough.

On physical examination, sequential measurements of body temperature should be made to detect fever. Hepatic enlargement and tenderness are typical, but not invariably present. Jaundice is rare in amebic abscess and, when present, should suggest biliary tract obstruction with pyogenic infection or underlying chronic liver disease. Percussion dullness, diminished breath sounds, or other chest findings at the right lung base are evident in 20%–30% of patients and suggest involvement of the superior portion of the right hepatic lobe. Occasionally signs of weight loss, dehydration, and anemia are evident [3,7,14,16].

Diagnosis and differential diagnosis

Imaging modalities

The clinical constellation of fever, right upper quadrant discomfort, and hepatic enlargement with tenderness should prompt an imaging study early in the diagnostic assessment. Depending on the age of the patient and level of clinical suspicion for cholelithiasis or biliary obstruction, either ultrasonography or computed tomography (CT) will be performed. These techniques facilitate discrimination of liver abscess from cholecystitis, bile duct obstruction, or pancreatitis. Radionuclide liver–spleen scanning after administration of a technetium 99m–sulfur colloid has been supplanted by ultrasonography and CT because the latter two are more sensitive for small lesions (less than 3 cm) and offer better precision in localizing lesions that may require percutaneous aspiration or drainage. Of note, lesions near the dome of the right hepatic lobe may be difficult to see by ultrasonography. Typically, on ultrasound abscesses appear as hypoechoic lesions, sometimes with internal echoes [17,18] (Fig. 94.1). CT scanning identifies abscesses as low-density lesions (Fig. 94.2), often with peripheral enhancement after intravenous administration of contrast, and may provide better definition of extrahepatic pathology associated with pyogenic abscess (e.g., appendiceal or diverticular abscess) [19]. Although both modalities are sensitive for detecting abscesses and biliary obstruction, neither can distinguish reliably between amebic and pyogenic abscesses [20–22]. Although less widely used, magnetic resonance imaging (MRI) also has high sensitivity for detection of hepatic abscess. Characteristically, liver abscesses are hypointense on T1-weighted images and hyperintense on T2-weighted images, and wall enhancement soon after gadolinium infusion is typical [23]. Both CT and MRI provide multiplanar imaging so these modalities may be particularly valuable when ultrasound findings are ambiguous or when coronal or sagittal images will help guide

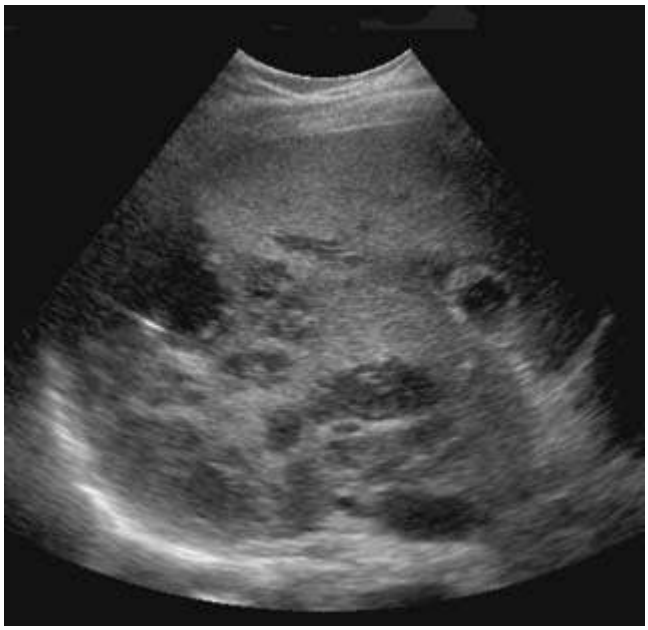

Figure 94.1 Sonogram of the right hepatic lobe showing multiple hypoechoic pyogenic abscesses. This image is from a 54-year-old man who developed multiple hepatic abscesses associated with sigmoid diverticulitis. Blood cultures obtained before antibiotic administration yielded *Bacteroides thetaiotaomicron*. Gram stain of abscess material obtained after antimicrobial therapy was initiated showed gram-positive cocci, gram-negative rods, and gram-positive rods. He was treated initially by percutaneous drainage and intravenous ceftriaxone and metronidazole. After clinical improvement, he was discharged home and completed uneventfully a several week course of oral ciprofloxacin and metronidazole.

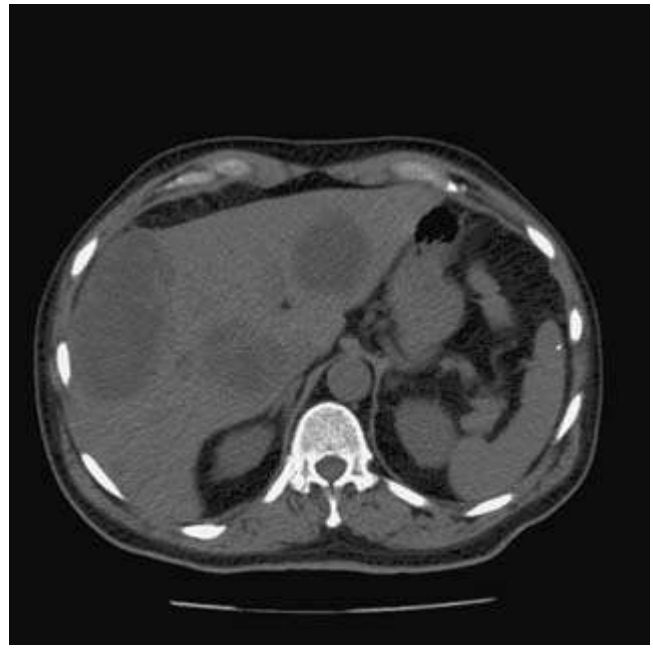

Figure 94.2 Computed tomography image showing numerous low-density lesions in both hepatic lobes in a 65-year-old man with colon carcinoma. *Streptococcus* (γ-hemolytic) was cultured from fluid obtained by abscess aspiration. His liver lesions were treated by aspiration without drain placement and by administration of levofloxacin and metronidazole. He improved and subsequently tolerated resection of his colonic lesion.

management of a lesion in the superior portion of a hepatic lobe [24]. Most abscesses, both amebic and pyogenic, occur in the right hepatic lobe. The presence of multiple abscesses strongly suggests pyogenic infection, as does identification of concomitant biliary tract obstruction. Chest radiographs in patients with an abscess adjacent to the diaphragm may show elevation of the right diaphragm, subpulmonic effusion, and right lower lobe atelectasis or infiltrate. It is well to remember that hepatic tumors may present with necrosis and secondary infection [25], mimicking a primary abscess.

Laboratory testing

Commonly employed blood tests are of limited utility in reaching a specific diagnosis of hepatic abscess. This being said, certain abnormalities are typically present. Leukocytosis (more than 10 000/mm^3) is present in more than 90% of patients. Mild anemia is common, with hemoglobin levels of less than 12 g/dL seen in two-thirds of patients. Mild to moderate elevation of serum alkaline phosphatase activity is typical but of little discriminating value in the absence of information from imaging studies [3,7,26].

Detection of antiamebic antibodies is of primary importance in diagnosis of amebic liver abscess. Although serological tests may rarely be negative very early in infection, more than 90% of patients with hepatic abscess develop antibodies to *Entamoeba histolytica* in high titer. A positive serological response to *E. histolytica* indicates tissue invasion by the parasite and not simply intestinal colonization. Both indirect hemagglutination and enzyme-linked immunosorbent assays are available [2,27]. Importantly, seropositivity does not distinguish current from prior disease. Persistence of antiamebic antibodies after resolution of amebic abscess (usually in diminishing titer) may lead to confusion in the differential diagnosis of a subsequent liver lesion, especially in areas in which infection is endemic [1,2,28–30].

A key element in diagnosis and treatment of pyogenic liver abscess is identification of the organisms in the abscess. Blood cultures are positive for bacteria in about 50% of patients so at least two separate specimens of blood should be taken for culture before administration of antibiotic agents. Most pyogenic abscesses are caused by enteric gram-negative aerobic rods, streptococci, and anaerobes. Although blood cultures typically identify a single organism, cultures from abscess aspiration are frequently polymicrobial [3,32,33]. Needle aspiration of an abscess is the best and most direct method to distinguish amebic from pyogenic abscess. Material from an amebic abscess is brown-red in color and typically is not particularly malodorous. A pyogenic abscess yields material that is creamy, tan-green in color, and often putrid, reflecting anaerobic infection. Gram stains of amebic abscess contents show neutrophils but no bacteria, unless secondary infection is present. Smears of pyogenic abscess contents usually identify at least one bacterial form. Meticulous handling of aspirated material to avoid exposure to air enhances the recovery and identification of anaerobic species. Reliable and complete identification of infectious agents ensures proper selection of an antibiotic treatment regimen [33].

Course and complications

Amebic abscess

Without treatment, amebic liver abscess tends to enlarge and ultimately extend through the diaphragm or rupture into the peritoneal cavity. Pleuropulmonary involvement is the most common complication [13,34–36]. On extension through the diaphragm, amebic empyema or lung involvement with development of hepatobronchial fistula may occur. Rupture into the right pleural cavity is less frequent than into the lung, probably because of fusion of the diaphragmatic and visceral pleura induced by inflammation. Erosion into a bronchus may be followed by expectoration of amebic pus and by amelioration of symptoms. This mechanism of abscess drainage affords an opportunity for spontaneous resolution. Less frequently seen is rupture into the pericardium, typically a complication of amebic abscess within the left hepatic lobe. This may be accompanied by retrosternal pain, a pericardial friction rub, and findings of cardiac tamponade, such as jugular vein distention with pulsus paradoxus. Historically, rupture into the pericardium has carried a high mortality [13,34].

When an amebic abscess ruptures abruptly into the peritoneum, diffuse abdominal pain and signs of peritonitis occur. Less frequently encountered is a contained abscess adjacent to the site of hepatic rupture. In neither instance is surgery required because cure typically results from antimicrobial therapy alone [13,35]. Other rare intraabdominal complications include rupture into bowel and hemobilia through formation of an arteriobiliary fistula.

Pyogenic abscess

The mortality rate associated with untreated pyogenic liver abscess approaches 100% [4,8,10]. As with amebic abscess, complications of pyogenic liver abscess include rupture and extension into the surrounding tissues, with pleuropulmonary involvement most common. In contrast to amebic peritonitis, should rupture into the peritoneum occur from a pyogenic liver abscess, mortality is very high without surgical management. Abscess-associated thrombosis within the portal vein (Fig. 94.3) or a hepatic vein is associated with anaerobic infections [5,6] and may lead to residual portal hypertension or the Budd–Chiari syndrome after otherwise successful treatment of the abscess. Patients with large or multiple pyogenic abscesses are at increased risk for developing infections at remote body sites as a result of bacteremia. Patients with polymicrobial abscesses have a higher mortality rate than do those with monomicrobial infections. In one series, all 14 patients who had two or more organisms isolated

Figure 94.3 Computed tomography image showing filling defects within the intrahepatic portal vein, scattered hepatic hypodense lesions, and free fluid within the abdominal cavity. These findings are compatible with septic pyelothrombophlebitis. This image is from a 44-year-old man who presented with sigmoid diverticulitis and pericolonic abscess. He was treated by segmental colectomy and his liver abscesses resolved without aspiration after prolonged antibiotic therapy. He was treated postoperatively with heparin, then coumadin. After 3 months of anticoagulation therapy, his portal vein thrombus resolved and there were no residual imaging abnormalities.

from blood cultures died [8]. Mortality appears to be higher in patients with pyogenic abscesses of biliary origin, probably because these patients tend to have multiple abscesses. Even with prompt diagnosis and treatment, the mortality rate from pyogenic liver abscess remains high, ranging from 10% to 30% [3,8].

Treatment

Liver abscess should be considered in any patient with fever, leukocytosis, pain in the right upper quadrant, and tenderness over the liver or right lower chest wall. Depending on the age of the patient and the nature of the discomfort and associated symptoms elicited by history, either ultrasonography or CT should be obtained. Either modality will detect reliably one or more hepatic lesions, with imaging characteristics suggesting abscess. Should jaundice or evidence for biliary obstruction be discovered, consideration of cholangiography and biliary decompression is appropriate. Regardless of whether amebic or pyogenic abscess is believed more likely, at least two sets of blood cultures should be obtained before administration of antimicrobial agents.

Amebic abscess

If the patient is a young man who has emigrated from or traveled to an area where amebiasis is endemic, who has a single lesion on an imaging study, and who does not appear toxemic, then amebic abscess is the more likely diagnosis. Stool specimens should be examined for amebic cysts and trophozoites and serum should be tested for *E. histolytica* antibodies. Treatment in adult patients should then be initiated with metronidazole, 750 mg given orally three times daily for 10 days [1,2,33]. An alternative regimen is chloroquine phosphate, 1000 mg (600 mg chloroquine base) given orally daily for 2 days, and then 500 mg (300 mg chloroquine base) given orally daily for 2–3 weeks [1,2,37]. Clinical improvement is expected within 3 days. After treatment with either of these tissue amebicides, therapy with a lumenal amebicide is appropriate to eradicate amebas within the bowel because these may cause relapsing or persisting infection. Iodoquinol, 650 mg given orally three times daily for 20 days, is the drug of choice for this purpose [37]; paromomycin is a satisfactory alternative [2,33]. Aspiration of the abscess is indicated if there is no clinical improvement within several days, if the diagnosis is in doubt, or if the abscess is large or located such that rupture is believed likely. Amebic pleural effusions are treated by aspiration and one of the amebicidal regimens. Amebic pericarditis is treated by pericardiocentesis and by aspiration of the (usually left lobe) hepatic abscess in addition to amebicidal antibiotics. Surgery is not indicated for uncomplicated amebic liver abscess but may be appropriate in some instances for rupture or, rarely, for failure of medical therapy alone [38]. Ultrasound-guided percutaneous drainage is effective and should obviate the need for surgical management in most cases [39]. In uncomplicated amebic liver abscess, the prognosis is good, with a mortality rate of about 1% [13]. Extension into the chest or pericardium increases mortality significantly, to 6% or 25%–30%, respectively [13,35].

Pyogenic abscess

If the patient is older, lacks a history suggesting risk for amebiasis, or has multiple lesions or bile duct obstruction on an imaging study, pyogenic abscess is more likely. After blood cultures have been obtained, treatment with a broad-spectrum antibiotic regimen is appropriate. The mixed nature of many pyogenic infections dictates that the initial empiric regimen should provide effective coverage against aerobic enteric bacilli, microaerophilic streptococci, and enteric anaerobes. Suitable regimens include ampicillin–sulbactam, piperacillin–tazobactam, cefoxitin, imipenem, or a third-generation cephalosporin plus metronidazole [33]. Previous instrumentation or obstruction of a patient's biliary tree and local patterns of bacterial antibiotic resistance should prompt broader coverage

or antibiotic selection to strengthen coverage against specific pathogens such as *Enterococcus* and *Pseudomonas* species [7]. In most instances, diagnostic aspiration for bacterial culture is appropriate. Ideally, this should occur before antibiotics are administered, but treatment should not be delayed injudiciously if prompt aspiration is not feasible. Although many choose to leave a drain in place after aspiration, several series have indicated that aspiration without catheter drainage (in concert with antibiotic therapy) is curative in most instances [3,7,9,16,40–47]. Antibiotic therapy alone should be employed only for those with multiple small abscesses not amenable to aspiration and for those with a contraindication to aspiration and drainage (e.g., coagulopathy) [48]. Surgical drainage, either open or laparoscopic [49], is appropriate for patients who fail to improve with percutaneous drainage and antibiotic therapy and for those who have an extrahepatic or biliary source of hepatic abscess in need of surgical treatment. It is important to note that resolution of abscess cavities (as determined by sequential imaging studies) typically lags behind clinical improvement in effectively treated patients. Thus, clinical judgment and not simply persistence of an abscess cavity should guide decision-making regarding the need for recurrent aspiration or operative drainage. Surgical intervention may ultimately be necessary in up to one-third of patients with pyogenic abscess [50,51]. Although percutaneous drainage and current antibiotic regimens have reduced mortality from pyogenic liver abscess significantly, it remains high (20%–40%) in patients with significant comorbidity, multiple abscesses, malignant biliary obstruction, and inadequate drainage [7,8].

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G LIVER

95 Vascular diseases of the liver

Laurie D. DeLeve

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Systemic circulatory disease

Ischemic hepatitis

Ischemic hepatitis or "shock liver" may occur as the result of reduction in systemic blood pressure with or without hypoxemia. Ischemic hepatitis often occurs in patients with underlying heart disease [1–3] and it has been suggested that right-sided heart failure with hepatic venous congestion predisposes to ischemic hepatitis because of systemic hypotension [3]. Decreased cardiac output may have cardiac causes, such as myocardial infarction, severe congestive heart failure, cardiac arrhythmias, severe cardiomyopathy, cardiogenic shock, constrictive pericarditis, or pericardial tamponade. Systemic causes of hypotension include septic shock or hypovolemia due to massive hemorrhage, extensive burns, or extravasation of fluid into the peritoneum, e.g., in acute pancreatitis.

The diagnosis is made on clinical grounds when there is systemic hypotension and severe and rapidly reversible elevation of serum aminotransferases in the absence of other causes of acute hepatitis. In addition to these features, a marked, rapid elevation of lactate dehydrogenase [4] with a low ratio of serum aminotransferase to lactate dehydrogenase is helpful in distinguishing ischemic hepatitis from acute viral hepatitis [5]. Serum aminotransferases are most commonly 20 or more times the upper limit of normal and an elevation less than 20 suggests the need for further confirmation of the diagnosis [6]. Concomitant renal hypoperfusion with elevation in creatinine and blood urea nitrogen are also usually present. The classic histological feature is centrilobular necrosis.

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Congestive hepatopathy

Chronic passive congestion of the liver in congestive heart failure causes atrophy of hepatocytes in zone 3, sinusoidal congestion, and enlargement of sinusoidal endothelial fenestration. It has been suggested that the mechanisms leading to hepatocyte atrophy are pressure on the hepatocytes and perisinusoidal edema, which impairs the diffusion of oxygen and nutrients to hepatocytes [7].

Causes of congestive hepatopathy include chronic right-sided heart failure as the result of cardiomyopathy or valvular heart disease, constrictive pericarditis, and pulmonary hypertension. Clinical findings include a dull ache in the right upper quadrant, hepatomegaly, increased jugular venous pressure, and hepatojugular reflux. Liver tests are usually normal. Congestion will cause sinusoidal dilation in the centrilobular sinusoids with preservation of normal architecture in the periportal areas. Histologically this pattern is referred to as a "nutmeg" appearance. Centrilobular fibrosis may occur with long-standing disease, but frank cardiac cirrhosis is rare.

Treatment of both ischemic hepatitis and congestive hepatopathy is directed towards the underlying cardiopulmonary disease.

Hypoxia

Cases of presumed ischemic hepatitis may sometimes be the result of hypoxia rather than of decreased perfusion, although more commonly this will be the result of the combination of left-sided heart failure with chronic respiratory failure than the latter in isolation [8]. Primary pulmonary causes of hypoxemia leading to hepatocellular necrosis may include massive pulmonary embolus, end-stage chronic obstructive lung disease, or restrictive lung disease.

Budd–Chiari syndrome

Budd–Chiari syndrome (BCS) is the eponym for hepatic venous outflow tract obstruction, independent of the level or

mechanism of obstruction [9,10]. BCS is considered primary when the blockage is intrinsic to the vein and secondary when the obstruction or compression originates external to the vein. In the West, a pure hepatic vein obstruction is more common. In non-Western countries obstruction of the hepatic portion of the inferior vena cava (IVC) is more common than hepatic vein obstruction.

Membranous obstruction of the hepatic portion of the IVC is frequently seen in patients in countries such as South Africa, India, Japan, Nepal, and China, whereas in the West it is uncommon. The original theory was that the membrane was congenital, but current thinking is that it is a consequence of thrombus organization [11,12]. One of the initiating factors may be an infectious process. Patients with prothrombotic disorders also have a predisposition to obstruction of the hepatic portion of the IVC, but it is unknown why that particular section of the IVC is more vulnerable to thrombosis. Behçet disease is more likely to cause obstruction in the hepatic portion of the IVC than in the hepatic veins [13,14]. The symptoms of hepatic vein obstruction and obstruction of the hepatic portion of the IVC are the same.

Clinical features

Presenting signs and symptoms in patients with overt disease include fever, abdominal pain and tenderness, ascites, lower extremity edema, gastrointestinal bleeding, and hepatic encephalopathy, but any of these symptoms may be absent in symptomatic cases [15–17]. In obstruction at the level of the IVC, patients may develop distended subcutaneous veins across the abdomen, chest, and back, which form part of the collateral circulation; this finding is uncommon, but specific for IVC block. The presentation of BCS may be acute, acute-on-chronic, subacute, or chronic, although the last two presentations are more common. Severity of symptoms will depend on the extent of the involvement of the hepatic veins, the time-course over which the obstruction develops and the duration of untreated disease. Slower development of the obstruction or occlusion allows the formation of collaterals, which alleviates sinusoidal congestion. There is a wide spectrum of disease presentation. Concomitant portal vein obstruction is common in patients with severe forms of the disease [15,18–20]. Patients with an acute presentation are more likely to present with fulminant hepatic failure with coagulopathy, encephalopathy, and hepatorenal syndrome. Patients with a chronic presentation may present with chronic liver disease with (near-)normal aminotransferases, low albumin, and a prolonged prothrombin time. If there is extensive formation of collaterals or involvement of a single hepatic vein, the patient may have preservation of normal liver function and no ascites. BCS may be asymptomatic in up to 20% of cases [21]. The most common presentation is a history of vague complaints for less than 6 months and onset of ascites.

Serum aminotransferases and alkaline phosphatase may be normal or elevated. Serum albumin, serum bilirubin, and prothrombin can be normal or abnormal, and in some patients are markedly abnormal, although frank jaundice is relatively uncommon. The serum–ascites albumin gradient will be greater than 1.1 g/dL. An ascitic fluid protein concentration of greater than 3 g/dL is consistent with BCS, but may also occur with cardiac or pericardial disease.

The caudate lobe drains through the right inferior hepatic vein into the IVC, which develops thromboses less frequently, so that flow through the caudate lobe may become an important route of blood flow when there is occlusion of two or all three main hepatic veins. This increased flow leads to caudate lobe hypertrophy in about half of patients with BCS. Significant caudate lobe hypertrophy may compress the IVC behind it, complicating decompression by portacaval shunts (which are of course below the level of compression of the IVC).

Risk factors

Primary BCS is the result of an intrinsic venous process and the risk factors are those that lead to thrombosis or phlebitis. The major risk factors (Table 95.1) are chronic myeloproliferative diseases and prothrombotic coagulation disorders. Around 25%–30% of patients will have multiple concurrent risk factors [22,23]. Myeloproliferative disorders are the leading causal factor in 50% of patients [22,24–26]. The chronic myeloproliferative disorder most closely associated with hepatic vein thrombosis is polycythemia vera, whereas essential thrombocythemia is a less common cause. In a large series, 10% of patients with polycythemia vera, and 13% of patients with essential thrombocythemia, developed hepatic vein thrombosis [27]. None of the 232 patients with chronic myelogenous leukemia in this series developed thrombosis of the major abdominal vessels. The increased risk for hepatic vein thrombosis is linked to both overt and latent myeloproliferative disease.

In a recent series, one or more prothrombotic risk factors was identified in 87% of patients with BCS [22]. Two recent case series have identified factor V Leiden as an important risk factor [22,23], but these studies differed as to whether protein C deficiency was a risk factor. The prevalence of antithrombin deficiency, protein S deficiency, the G20210A prothrombin gene mutation, and the methylene-tetrahydrofolate reductase gene mutation in patients with hepatic vein thrombosis is similar to that expected for a healthy control population. Once BCS causes chronic liver disease, diagnosis of protein C deficiency, protein S deficiency, or antithrombin deficiency may be misleading because synthesis of these coagulation factors decreases as liver function declines; studies of family members may be needed to rule out these clotting disorders as a risk factor. Antiphospholipid antibodies are a risk factor for BCS and may be primary or related to one of the various connective tissue diseases. Oral contraceptives, pregnancy, and the puerperium are associated with an increased risk of BCS, but there will most commonly be a

Table 95.1 Risk factors for Budd–Chiari syndrome

Primary Budd–Chiari syndrome	Secondary Budd–Chiari syndrome
Myeloproliferative diseases	Hepatocellular carcinoma[a]
Polycythemia vera	Renal cell carcinoma[a]
Essential thrombocythemia	Adrenal carcinoma[a]
Prothrombotic coagulation disorders	Primary hepatic hemangiosarcoma
Factor V Leiden mutation	Epitheloid hemangioendothelioma
Protein C deficiency	Sarcoma of the inferior vena cava[a]
Antiphospholipid antibodies	Right atrial myxoma[a]
Prothrombin G20210A mutation	Hydatid cysts[a]
Oral contraceptives, pregnancy, puerperium	Amebic or pyogenic abscess[a]
Rare causes	Sarcoidosis
Behçet disease[a]	Trauma[a]
Paroxysmal nocturnal hemoglobinuria	Aspergillosis
Hypereosinophilic syndrome	Abdominal surgery
Granulomatis venulitis	
Ulcerative colitis	

a More commonly associated with membranous obstruction of the hepatic portion of the inferior vena cava than with obstruction at the level of the hepatic vein.

concurrent risk factor. Uncommon risk factors for BCS include paroxysmal nocturnal hemoglobinuria [28], postoperative complications, and Behçet disease. Although Behçet disease may cause hepatic vein thrombosis, it is more likely to cause obstruction of the IVC [13,14].

Procoagulants and anticoagulants circulate systemically throughout the vasculature. If the hemostatic balance of coagulation factors was the only determinant of thrombosis, then any change in the balance should have an impact on all vascular beds. As noted in the preceding paragraph, some of the prothrombotic disorders do and others do not cause hepatic vein thrombosis. Predisposition to thrombosis in a particular vascular bed is a characteristic feature of prothrombotic disorders. This selectivity of thrombosis for discrete segments of the vasculature may be the result of endothelial cell-dependent procoagulant and anticoagulant activities [29].

Secondary BCS is the result of a process originating outside the vein that compresses or invades the vein. The major causes are cancers and infections (Table 95.1). Some of the causes listed in Table 95.1 may cause obstruction at the level of the hepatic veins, but they are more commonly associated with obstruction of the hepatic portion of the IVC. Uncommon causes of secondary BCS are trauma with formation of an intrahepatic hematoma, granulomata caused by sarcoidosis, and aspergillosis invading hepatic veins [30].

Diagnosis

Imaging with Doppler ultrasound can make the diagnosis, although in some centers dual-phase computed tomography (CT) or magnetic resonance (MR) angiography may be needed to confirm the diagnosis (Table 95.2). Imaging may demonstrate obstruction or absence of flow at the level of the major hepatic veins or IVC; intrahepatic, capsular, or systemic col-

Table 95.2 Sequential steps in diagnostic imaging and therapy of Budd–Chiari syndrome

Sequential steps	Diagnostic imaging	Therapy
1	Doppler ultrasound	Anticoagulation
2	Magnetic resonance imaging	Angioplasty and stenting
3	Venography and transvenous biopsy	TIPS (or surgical shunt)
4		Liver transplantation

Recommendations from a workshop organized by the European Group for the Study of Hepatic Vascular Diseases. From Janssen et al. [10], with permission from Elsevier.
TIPS, transjugular intrahepatic portosystemic shunt.

lateral formation; hepatic parenchyma with variegated appearance and nodularity; caudate lobe enlargement; ascites; or splenomegaly. If normal hepatic veins with phasic flow are detected, the diagnosis of BCS is excluded. In difficult cases angiography may be required to establish the diagnosis and to precisely localize the obstruction.

Ultrasound is superior to MR imaging (MRI) for the detection of intrahepatic collaterals [31]. MRI may be more accurate than venography for the characterization of solid endolumenal material [31]. In the older literature, CT did not provide a reliable assessment of the hepatic veins [31], but the accuracy of current CT technology has not yet been reported.

The value of liver biopsy for establishing prognosis or determining management is greatly limited by significant sample variation. Liver biopsy is, however, the only method to establish a diagnosis of BCS of the small intrahepatic veins. Liver biopsy findings in acute BCS include centrilobular and sometimes midlobular sinusoidal congestion, acute hemorrhage, and hepatocyte ischemia or drop out. Chronic outflow obstruction leads to hepatocyte dropout and bridging fibrosis between central veins with sparing of the portal tracts. Thrombi in the hepatic veins may have multiple layers, consistent with ongoing or recurrent thrombosis. Portal vein thrombosis occurs secondarily in some of the small branches of the portal vein and in the main portal vein in about 25% of cases. Areas of the liver subject to the most severe outflow obstruction demonstrate sinusoidal fibrosis. Benign regenerative nodules are common.

In addition to the diagnosis of BCS itself, the diagnostic work-up needs to uncover the predisposing factor(s). Frequently there are two or more causes, one of which may be a myeloproliferative disorder [22,23]. In this population, portal hypertension with hypersplenism and with gastrointestinal bleeding leading to iron deficiency anemia will obscure the peripheral blood changes of the myeloproliferative disorder in most patients [25], resulting in occult myeloproliferative disease. No single test is diagnostic in all patients with occult myeloproliferative disease [32–34]. Diagnosis may be made by demonstrating the 1849GmT point mutation (V617F) of the tyrosine kinase *JAK2* gene in myeloid cells [32–35], by demonstration of clusters of dystrophic megakaryocytes on bone marrow biopsy [36], or by endogenous erythroid colony formation in culture of bone marrow cells or circulating myeloid progenitor cells [25,37,38].

Management of primary Budd–Chiari syndrome

The sequential steps for management of BCS recommended by consensus statement reports in 2002 [10] and 2005 [9] are:

- anticoagulation, treatment of predisposing conditions, and prophylaxis for or treatment of complications of portal hypertension
- an active search for and treatment of short-length venous stenoses amenable to angioplasty or stenting
- transjugular intrahepatic portosystemic shunt (TIPS), if angioplasty or stenting is not possible or is unsuccessful
- if TIPS is unsuccessful, evaluate for liver transplantation (Table 95.2).

Treatment of condition predisposing to thrombosis

Myeloproliferative diseases should be treated, although therapeutic end points are not well defined. Phlebotomy alone will increase the risk of thrombosis in patients with polycythemia vera, whereas one nonrandomized trial has suggested a reduced risk of thrombosis in patients receiving phlebotomy plus hydroxyurea [39,40]. Hydroxyurea reduces the risk of thrombosis in patients with essential thrombocythemia [41].

Oral contraceptives are contraindicated. There are reports of successful pregnancies in patients on anticoagulation during pregnancy. Most other predisposing conditions for primary BCS will require anticoagulation.

If the predisposing condition is not curable, indefinite anticoagulation should be considered. Indefinite anticoagulation may improve survival for patients with a good prognosis, but may not for individuals with an intermediate or poor prognosis [15].

There are insufficient data to determine the benefit of thrombolytic therapy. The largest case series suggests that the best results may be obtained with local infusion of thrombolytic therapy rather than systemic treatment, after recent thrombosis and following partial recanalization by mechanical means [42]. However, these findings are based on a small retrospective study.

Angioplasty and stenting

Angioplasty, with or without stenting, should be considered in patients with focal or segmental obstruction. Patency may be better maintained in patients who remain on anticoagulants for at least 6 months [43]. Recurrent obstruction is more common after primary angioplasty than after primary stenting for both hepatic vein and IVC obstruction. Reobstruction may be less common after IVC stenting than after hepatic vein stenting [43].

Portosystemic shunting

Increased sinusoidal pressure and decreased liver perfusion in BCS lead to parenchymal necrosis, fibrosis, cirrhosis, and portal hypertension. Sinusoidal decompression performed before significant fibrosis and loss of liver function may stabilize disease progression. Other indications for decompression include refractory ascites and uncontrollable gastrointestinal hemorrhage. Options for decompression of obstruction in the hepatic vein include TIPS and surgical shunt. The goal of TIPS or of a surgical shunt is to create hepatofugal flow through the portal vein.

The use of TIPS for BCS has been increasing. Polytetrafluoroethylene-covered stents have a lower rate of dysfunction

than uncovered stents [44]. Bleeding complications of TIPS may be higher for BCS than for other chronic liver diseases, but the incidence of encephalopathy may be lower.

The availability of TIPS has greatly reduced the need for surgical shunts. There may not be any improvement in survival with a shunt unless patency of the shunt is maintained [45]. The choice of surgical shunt will often depend on the preference of the surgeon. If there is significant compression of the IVC by caudate lobe hypertrophy or substantial obstruction of the IVC by thrombosis or a web, pressure in the retrohepatic IVC may be higher than in the portal vein. Under these conditions shunts proximal to the obstructed IVC (portacaval or mesocaval shunt) may fail and a mesoatrial shunt should be considered. Side-to-side portacaval shunts, with direct anastomosis of the side of the portal vein to the side of the vena cava, have the highest patency rate but may make future liver transplantation more difficult: a portacaval shunt will require extensive dissection around the porta hepatis and reversal of the shunt is a major undertaking. Mesocaval shunts are technically less demanding and may facilitate surgery at the time of liver transplantation, but when performed with a synthetic graft they have a higher thrombosis rate than a side-to-side portacaval shunt.

Liver transplantation

Liver transplantation is the treatment of choice for acute BCS with fulminant hepatic failure and for end-stage liver disease after a chronic course. In more recent series of patients transplanted for BCS, survival rates have been much improved over earlier series. Three-year survival of 78%–88% and 5-year survival of 75% have been reported [46,47]. The improved survival is likely to be the result of earlier treatment after onset of symptoms and the recognition of the need for lifelong anticoagulation in most patients. Liver transplantation may cure one of the underlying predispositions, such as factor V Leiden, protein C deficiency, or antithrombin deficiency, but a thorough diagnostic work-up may reveal the presence of two or more remaining prothrombotic risk factors that will still require anticoagulation.

Portal vein thrombosis

After a review of the common risk factors, this section will separately review the acute and chronic phases of portal vein thrombosis.

Risk factors

A local precipitating factor can be identified in 30% of patients, whereas a systemic risk factor is present in 70%–80% [22,23,32] (Table 95.3).

The most common local precipitating factors include hepatoma, pancreatic cancer, and cirrhosis. Other local factors include: any abdominal malignancy; inflammatory processes in the abdomen such as pancreatitis, diverticulitis, appendicitis, cholecystitis, inflammatory bowel disease, or cytomegalovirus hepatitis that lead to a pylephlebitis; and abdominal trauma or surgery. Portal vein thrombosis in patients with malignancy may be the result of local tumor invasion or of a hypercoagulable state. Pancreatitis may cause portal vein thrombosis either through extrinsic compression of the portal vein by a pseudocyst or through pylephebitis secondary to acute pancreatitis. Cirrhosis may predispose to portal vein thrombosis because of sluggish blood flow through the liver in intrahepatic portal hypertension, but cirrhosis-related hepatocellular carcinoma may also manifest as portal vein thrombosis.

Table 95.3 Risk factors for portal vein thrombosis

Systemic risk factors	Local risk factors
Myeloproliferative diseases	*Abdominal malignancies*
Polycythemia vera	Hepatoma
Essential thrombocythemia	Pancreatic cancer
Inherited clotting disorders	Cirrhosis
G20210A prothrombin	*Inflammatory/infectious processes*
Antithrombin deficiency	Pancreatitis
Protein C deficiency	Diverticulitis
Protein S deficiency	Cholecystitis
	Appendicitis
	Cytomegalovirus hepatitis
	Inflammatory bowel disease
	Abdominal trauma and surgery

The major systemic risk factors are myeloproliferative diseases and inherited clotting disorders. About one-third of patients with portal vein thrombosis will have a primary myeloproliferative disease, notably polycythemia vera and essential thrombocythemia. As noted earlier in the chapter on the section for BCS, in most patients the myeloproliferative disease will be occult, i.e., not manifested in peripheral blood smears, because of hypersplenism and iron deficiency anemia. No one diagnostic test is sufficient to diagnose all cases of occult myeloproliferative disease. Diagnosis may be made by the presence of dystrophic megakaryocytes on bone marrow biopsy [36], endogenous erythroid colony formation in cultures of bone marrow or circulating progenitors [25], or the presence of the 1849GmT point mutation (V617F) of the tyrosine kinase gene *JAK2* in myeloid cells [32,34,48,49].

The inherited clotting disorders that predispose to portal vein thrombosis are the prothrombin gene mutation (factor II G20210), antithrombin deficiency, protein C deficiency, and protein S deficiency [22,23,32]. Inherited protein C, protein S, and antithrombin deficiency need to be distinguished from acquired deficiency because of impaired hepatic synthesis in liver disease. Factor V Leiden and hyperhomocysteinemia do not predispose to thrombosis of the portal vein. It is unclear why prothrombotic disorders predispose to

clotting in particular regions of the vascular bed. Endothelial cells in various organs and even within organs show tremendous heterogeneity [50]. The selectivity of a prothrombotic disorder to predispose to clotting in a particular vascular bed may be related to specific procoagulant and anticoagulant activity of the endothelial cells in that particular segment of the vasculature [29].

Analogous to BCS, most patients will have two or more risk factors for portal vein thrombosis.

Acute portal vein thrombosis

Clinical presentation

The most common presenting symptom of acute portal vein thrombosis is abdominal or lower back pain. When there is partial occlusion of the vein, symptoms are milder, whereas the intestinal congestion of a complete obstruction leads to severe, continuous colicky pain. The patient may develop an ileus with abdominal distention, but without other features of intestinal obstruction. Other symptoms include nonbloody diarrhea and a persistent fever. Laboratory tests may reveal a transient increase in serum aminotransferases, but liver function is otherwise preserved. Acute portal vein thrombosis is reversible with recanalization or development of a cavernoma.

Propagation of the thrombus into the mesenteric venous arches may lead to intestinal ischemia or even infarction. In the presence of mesenteric vein thrombosis, patients may develop severe pain lasting longer than 5–7 days, bloody diarrhea, and ascites. With progression to intestinal infarction patients may develop renal insufficiency, acidosis, and respiratory dysfunction. Without timely surgical intervention, transmural intestinal infarction will lead to perforation, peritonitis, sepsis, and death.

In a patient with acute portal vein thrombosis with high fever, chills, and right upper quadrant pain, a pylephlebitis is likely. The patient should be evaluated for an abdominal infection as the precipitating event. In this setting, liver abscesses are common and blood cultures are usually positive for enteric organisms.

Diagnosis

CT scan before contrast injection can demonstrate hyperattenuating material in the lumen of the vessel and CT after contrast injection demonstrates the lack of lumenal enhancement, increased arterial and decreased portal phase enhancement of the liver [51,52]. If CT is not readily available, Doppler ultrasound can demonstrate hyperechoic material in the portal vein, distention of the vein, and lack of flow. Either CT or MR angiography can be used to demonstrate propagation of the thrombus into the mesenteric circulation.

Management

The three treatment goals in acute portal vein thrombosis are to recanalize the obstructed vessel, to diagnose and treat the predisposing factor, and, if there are indicators of infection, to treat the infection.

Recanalization rarely occurs spontaneously, but partial or complete recanalization may occur in 40% and 50%, respectively, of patients on anticoagulation [53]. Therapy can be initiated with low-molecular-weight heparin and then switched to oral anticoagulation. Anticoagulation should be given for at least 3 months. Long-term anticoagulation should be considered for patients with a predisposing risk for clotting that cannot be cured, with extension of the thrombus into the mesenteric circulation, or in whom the mesenteric thrombus does not recanalize. The current literature suggests that recanalization rates with thrombolytic therapy are similar to those with anticoagulation alone, but with a much higher incidence of complications. A work-up should be initiated to find the source of infection in patients with fever or leukocytosis, but even if the source of infection cannot be documented antibiotics should be considered in these patients. Intestinal infarction requires emergent laparotomy to resect necrotic bowel.

Prompt institution of anticoagulation will significantly improve the prognosis. Outcome is dependent on recanalization of the portal and mesenteric veins and prevention of intestinal infarction. Recanalization of the portal vein and at least one of its branches will prevent portal hypertension. Intestinal infarction will be prevented if extension of the thrombus into the mesenteric circulation can be prevented or if the mesenteric vein is recanalized. Rarely, patients with intestinal ischemia may develop intestinal strictures and present with small bowel obstruction at a later time point. With prompt diagnosis and treatment before intestinal infarction, the outcome is good. Overall mortality is around 10% and this is mainly the result of postoperative complications or the predisposing condition [54].

Chronic portal vein thrombosis

Clinical presentation

In the developed nations, portal vein thrombosis is increasingly being recognized during the acute phase [53]. The most common presenting features of chronic portal vein thrombosis are splenomegaly or variceal hemorrhage due to portal hypertension. Patients with cirrhosis who develop a chronic portal vein thrombosis may present with worsening ascites. Recanalization of the portal vein clot leads to formation of small tortuous collateral veins, so-called cavernomatous transformation, which is the hallmark feature of chronic portal vein thrombosis. Initially patients will develop extensive collaterals throughout the upper gastrointestinal tract, portal hypertensive gastropathy, and splenomegaly. Some patients will have a tender enlarged liver. Blood flow to the liver from the hepatic artery and from perihepatic varices is usually adequate to maintain normal liver function. However, ascites or encephalopathy may be transiently present after a gastrointestinal bleed, during sepsis, or with unrelated renal

failure. With complete obstruction of the portal vein, enlarged biliary veins can compress large bile ducts, leading to portal cholangiopathy. About 10% of patients have hepatopulmonary syndrome [55].

In the absence of cirrhosis, liver tests are usually normal. Prothrombin time may be moderately prolonged. Patients who develop portal cholangiopathy may have cholestasis.

Diagnosis

The diagnostic feature of chronic portal vein thrombosis is cavernomatous transformation. Ultrasound, CT, or MRI will demonstrate the presence of a cluster of small irregular collateral vessels and the absence of the main portal vein and its main branches. Doppler ultrasound, CT or MRI before and after contrast can detect both the size and the patency of the portal vein. Hepatic arteries are usually enlarged and the caudate lobe may be enlarged.

Management

The most common presenting symptom of portal vein thrombosis is variceal hemorrhage. Acute variceal bleeding may be managed endoscopically. Prevention of recurrent variceal bleeding can be addressed with endoscopic band ligation or β-blockers. Patients with cavernomatous transformation who do not present with variceal hemorrhage should be screened for varices. The two options for intractable variceal hemorrhage are shunt surgery and TIPS. Shunt surgery in this population carries an increased risk of thrombosis of the shunt and should be performed with larger veins [56]. TIPS may be considered for intractable bleeding, but is not technically feasible in most patients [57].

Given the risk of variceal hemorrhage on one hand and extension of thrombosis on the other, the question arises as to the risk–benefit ratio of anticoagulation. Three retrospective studies have found no increase or a decreased incidence of gastrointestinal hemorrhage with anticoagulation [58–60] with a significantly decreased risk of recurrent thrombosis [60]. In 2005, a consensus panel recommended that anticoagulation should only be considered in patients with a permanent prothrombotic condition [9]. Long-term anticoagulation should be considered for patients with chronic portal vein thrombosis without cirrhosis, who have no contraindication for anticoagulation, who have received adequate treatment or prophylaxis for varices, and who either have a permanent prothrombotic condition or who have extension of the thrombus into the mesenteric circulation [61].

Patients with jaundice or biliary symptoms due to portal cholangiopathy may be treated by endoscopic retrograde cholangiopancreatography and stent placement after extraction of any stones. Half of these patients will not have recurrent symptoms when the stent is removed [62]. Recurrence will require a repeat procedure. If a surgical approach is deemed necessary, biliary decompression should be coupled with portal decompression.

Outcome is largely related to complications from portal cholangiopathy and transformation of a myeloproliferative disease to leukemia or myelofibrosis. In a 5-year follow-up study, fewer than 5% of patients died from either gastrointestinal hemorrhage or intestinal infarction. The prognosis may be worse in patients with extension into the mesenteric circulation [59].

Portal vein thrombosis in cirrhosis

Much of the preceding applies to portal vein thrombosis in cirrhotics, but a few special considerations apply to this population.

The risk of portal vein thrombosis increases with the severity of cirrhosis. The most common risk factor in cirrhosis is hepatocellular carcinoma. Evaluation of inherited deficiency of antithrombin, protein C, and protein S is complicated by acquired deficiency because of decreased hepatic synthetic function and evaluation of family members may be needed to make the diagnosis.

Splenectomy and surgical portosytemic shunting markedly increase the risk of portal vein thrombosis in this population. It is unclear whether treatment of varices precipitates the development of portal vein thrombosis or whether severe portal hypertension that leads to varices also predisposes to portal vein thrombosis.

Long-term anticoagulation should be considered for patients with compensated cirrhosis, a permanent prothrombotic risk factor and adequate treatment or prophylaxis for gastrointestinal hemorrhage [61]. In patients with decompensated cirrhosis with portal vein thrombosis, anticoagulation should only be instituted in a transplant center [61].

Sinusoidal obstruction syndrome (hepatic venoocclusive disease)

In 1920 and again in 1945, papers were published describing a novel liver disease [63,64]. The most striking histological feature was occlusion of the central veins and it was subsequently named hepatic venoocclusive disease [65]. Clinicopathological studies have demonstrated that the central veins are not occluded in up to 45% of patients [66,67] and experimental studies have shown that the disease process originates in the sinusoids [68,69]. Based on these findings, investigators in this field have proposed renaming this disorder sinusoidal obstruction syndrome (SOS) [70].

Pathogenesis

In contrast to most liver diseases, SOS presents with features of portal hypertension, followed subsequently by evidence of parenchymal dysfunction. This is indicative of the primary circulatory nature of the disease. Experimental studies indicate that drugs that cause SOS target the sinusoidal endothelial cell [68,71–73]. Sinusoidal endothelial cells become

rounded and gaps form within and between these cells [68]. This permits blood to enter into the space of Disse and dissect off the sinusoidal lining cells, which then embolize downstream and block sinusoidal flow [69]. Interventions that preserve the integrity of the sinusoidal endothelial cell lining completely prevent the development of SOS [74,75], confirming the sinusoidal origin of the disease.

Risk factors

The original descriptions of SOS were the result of ingestion of herbal teas ("bush tea disease") or foodstuffs contaminated with pyrrolizidine alkaloids, particularly in protein-malnourished individuals. In many parts of the world, ingestion of pyrrolizidine alkaloids is still the major cause of SOS. In North America and Western Europe the most frequent cause of SOS is myeloablative conditioning in preparation for hematopoietic stem cell transplantation (bone marrow transplantation), either high-dose combination chemotherapy or chemotherapy plus irradiation [67,76–78]. SOS may occur after long-term immunosuppression with azathioprine or treatment with 6-thioguanine [79–84]. It is also seen after treatment with chemotherapeutic agents unrelated to stem cell transplantation and has been associated with drugs such as gemtuzumab ozogamicin, actinomycin D, dacarbazine, cytosine arabinoside, 6-thioguanine, and urethane [85–89]. Treatment of Wilms tumors with actinomycin D carries a significant risk for SOS and this risk is greater in right-sided Wilms tumors or when actinomycin D is combined with abdominal irradiation [90,91]. The risk of gemtuzumab ozogamicin is particularly high when the drug is given in close temporal proximity to myeloablative conditioning therapy [88,92]. In a small series of patients, an interval of less than 3.5 months between gemtuzumab ozogamicin and myeloablative conditioning lead to a 90% incidence of SOS [92].

Clinical presentation

The classic features of SOS are hyperbilirubinemia, right upper quadrant pain of liver origin or hepatomegaly, and weight gain. The course of disease is protracted in SOS as a result of chronic exposure to pyrrolizidine alkaloids, whereas SOS in the setting of stem cell transplantation evolves more rapidly. In patients treated with cyclophosphamide-containing conditioning regimens, onset is within 10–20 days of initiation of treatment [77]. In other conditioning regimens onset of SOS may occur later than for cyclophosphamide-containing regimens [93].

Diagnosis

A tentative diagnosis can be made based on the presenting features in the setting of hematopoietic stem cell transplantation with a conditioning regimen that is hepatotoxic, but the importance of ruling out other conditions cannot be overemphasized. The differential diagnosis in this population includes (hyper)acute graft-versus-host disease; cholestatic jaundice caused by sepsis; fluid-overload caused by renal failure, congestive heart failure, or vigorous fluid resuscitation; medication-induced cholestasis; and liver involvement by viral or fungal infections seen during immunosuppression. More than one of these conditions may occur simultaneously in this population so that it can be difficult to determine which disease is the predominant cause of a particular sign or symptom. Up to 10%–20% of patients with liver dysfunction in the first 10–20 days after stem cell transplantation cannot be diagnosed with certainty based on clinical grounds [94,95].

Two groups of investigators developed clinical criteria used to diagnose SOS after hematopoietic stem cell transplantation that were intended for clinical research purposes (Table 95.4). However, application of these criteria requires careful exclusion of other causes. According to the criteria published by the Seattle investigators, two of three findings must occur within 20 days after transplantation: bilirubin greater than 2 mg/dL, tender hepatomegaly, and weight gain greater than 2% as the result of fluid accumulation. These criteria were developed for SOS caused by conditioning regimens that contain cyclophosphamide and the temporal criterion may not apply to regimens without cyclophosphamide. The Baltimore criteria require bilirubin to be greater than 2 mg/dL and two of three other findings: (usually painful) hepatomegaly, greater than 5% weight gain, and ascites.

Imaging studies can confirm hepatomegaly and ascites and can exclude biliary obstruction and tumor invasion into the hepatic parenchyma or vasculature, but there is not a single imaging feature that by itself is diagnostic. Imaging features that are highly suggestive of SOS include reversal of portal

Table 95.4 Criteria for diagnosis of sinusoidal obstruction syndrome

Seattle criteria [127]	Baltimore criteria [78]
Diagnosis requires two of three criteria within 20 days of transplantation: • bilirubin > 2 mg/dL • hepatomegaly or pain of liver origin • > 2% weight gain due to fluid retention	Hyperbilirubinemia (> 2 mg/dL) plus at least two of the following three findings: • (tender) hepatomegaly • > 5% weight gain • ascites

venous flow, gallbladder wall edema, attenuation of hepatic venous flow, and, perhaps, increased resistive index of hepatic artery flow.

The main differential diagnosis that might warrant a liver biopsy is (hyper)acute graft-versus-host disease. In a moderately or severely ill patient in whom the diagnosis is uncertain, a transvenous liver biopsy with hepatic venous pressure gradient using the occlusive balloon technique may help to distinguish SOS from graft-versus-host disease. In the setting of stem cell transplantation, a hepatic venous pressure gradient greater than 10 mmHg has a specificity of more than 90% and positive predictive value greater than 85% [96].

Prevention

Prevention is largely limited to identification of those at high risk for SOS, who may then be limited to less hepatotoxic alternatives. The major risk factors are hepatitis C, hepatic fibrosis, previous exposure to a myeloablative regimen, and previous history of SOS. Cirrhosis is an absolute contraindication to a myeloablative regimen.

Nonmyeloablative regimens are used to treat malignancy through a graft-versus-tumor effect. These regimens do not appear to have a risk for SOS and reduce immediate transplant-related mortality, but may have more late complications than myeloablative regimens [97,98]. For patients at significant risk for SOS, non-myeloablative regimens seem to be the best approach. Even with nonmyeloablative regimens, patients with cirrhosis have an increased risk of death from liver decompensation [98]. Lower doses of total body irradiation [99] and pharmacokinetic monitoring followed by targeted dosing of drugs such as cyclophosphamide [100,101] and busulfan [102–105] may also lower the risk of SOS. Adjustment of busulfan dosing based on plasma concentrations has been shown in some studies to be beneficial, but this could not be confirmed in a number of studies (see review [106]). The value of therapeutic monitoring of busulfan may depend on other factors, such as the age of the patient, the underlying disease, and the other drugs in the conditioning regimen [106]. Investigators from Seattle have shown benefit from metabolism-based dosing of cyclophosphamide, but it remains to be seen whether this will become more widely available and whether it will indeed reduce the risk of fatal SOS. Other approaches to avoiding SOS caused by myeloablative regimens include reversing the order of drugs in the busulfan–cyclophosphamide regimen (i.e., cyclophosphamide first) [107] or avoiding cyclophosphamide-containing regimens altogether.

Prophylactic treatment has been tried with heparin, low-molecular-weight heparin, prostaglandin E, and pentoxifylline, but none of these compounds have been shown to reduce the incidence of fatal SOS (see review [70]). The largest randomized controlled trial found no benefit for ursodeoxycholic acid [108], but a recent metaanalysis reported that ursodeoxycholic acid was beneficial in SOS [109]. The likely explanation for the apparent benefit is that SOS commonly occurs in conjunction with cholestatic liver disease, which may improve with ursodeoxycholic acid.

Treatment

The majority of patients with SOS recover spontaneously. Patients with severe SOS are deeply jaundiced, but commonly lack some of the features of liver failure, such as hyperammonemia, cerebral edema, and prolonged international normalized ratio. Death is usually the result of multiorgan failure with renal and pulmonary failure. Supportive care requires maintenance of fluid and electrolyte balance. Extravascular fluid overload is treated with diuretics in conjunction with paracentesis, hemodialysis, and hemofiltration when indicated for respiratory impairment due to ascites.

Defibrotide is an experimental compound that has been tried for a variety of vascular diseases. It is a single-stranded polydeoxyribonucleotide with antithrombotic, antiischemic, and thrombolytic effects and that reduces leukocyte accumulation. Uncontrolled clinical trials in moderate to severe SOS have been promising, but randomized controlled trials have not yet been performed.

TIPS decompresses portal pressure and relieves ascites, but does not improve the outcome of SOS [110–112]. Liver transplantation may be considered in patients who develop SOS after hematopoietic stem cell transplantation for a benign disease or whose underlying malignancy is expected to have a good outcome (e.g., chronic myelogenous leukemia in chronic phase). However, liver transplantation is not indicated when the risk of recurrent malignancy is high.

Prognosis

Diagnostic criteria for SOS and fatal SOS vary between transplantation units. These differences may account for the wide range in published case fatality rates [77,78,113–115]. Two large studies with cyclophosphamide-containing regimens reported a 70% recovery rate from SOS [77,113] and a study of SOS due to other alkylating agents reported an 84% recovery rate [93]. For patients who develop SOS due to cyclophosphamide-containing regimens, outcome can be predicted based on bilirubin level and weight gain using published graphs [116].

Adaptation to heterogeneous perfusion

The current consensus is that there are a variety of pathological lesions and clinical manifestations that are the result of uneven perfusion of the liver [117–120]. According to this hypothesis, these lesions (Table 95.5) are a single entity with a common pathogenesis, but a range of presentations. These lesions include nodular regenerative hyperplasia, partial nodular transformation, incomplete septal cirrhosis, and idiopathic noncirrhotic intrahepatic portal hypertension.

Table 95.5 Lesions due to heterogeneous perfusion of the liver

Liver lesions	Morphology [118]
Nodular regenerative hyperplasia	Monoacinar nodules composed of hyperplastic hepatocytes diffusely distributed throughout the liver, no surrounding fibrous septa
Partial nodular transformation	Multiple nodules that are several centimeters in diameter, composed of hyperplastic hepatocytes, encompassing several portal tracts, located in perihilar region
Incomplete septal cirrhosis	Large, diffusely distributed nodules, bordered by incomplete slender fibrotic septa; septa extend from periportal or perivenular fibrosis; spacing between portal tracts and between portal tracts and central veins is abnormal
Idiopathic non-cirrhotic intrahepatic portal hypertension	No nodular lesions; portal tracts are fibrotic, thin fibrotic septa may be present, if present then bridging fibrosis is subcapsular; sinusoidal dilation is common

More than one of these lesions may be found in some patients, supporting the concept that these lesions are a spectrum of responses with a shared etiology. By definition, these lesions occur in the absence of cirrhosis or chronic liver diseases that might lead to cirrhosis.

The concept of liver lesions caused by heterogeneous perfusion was first described for nodular regenerative hyperplasia [120]. The hypothesis is that impaired perfusion in some of areas of the liver leads to atrophy with apoptotic or atrophic hepatocytes and reactive hyperplasia in adjacent areas with preserved blood flow.

Risk factors

Underlying predispositions include collagen vascular diseases, clotting disorders, myelo- and lymphoproliferative diseases, some immunological disorders, and a variety of drugs and toxins (Table 95.6). Although the diseases and predisposing factors vary greatly, a shared element is the ability to potentially impair the circulation at the venous or sinusoidal level. Inflammation of the hepatic artery in collagen vascular diseases or immune complex diseases may lead to inflammatory destruction of adjacent portal veins [121,122]. Prothrombotic disorders may cause thrombosis in either the venous or sinusoidal circulation [117]. Azathioprine and myeloablative conditioning therapy for stem cell transplantation may impair the sinusoidal circulation by damage to sinusoidal endothelial cells [72,73].

Table 95.6 Conditions leading to heterogeneous perfusion of the liver

Collagen vascular diseases
- Rheumatoid arthritis
- Scleroderma
- Systemic lupus erythematosus
- Polyarteritis nodosa
- Glomerulonephritis

Hematological diseases
- Polycythemia vera
- Essential thrombocythemia
- Agnogenic myeloid metaplasia
- Chronic myeloid leukemia
- Hodgkin disease
- Non-Hodgkin lymphoma
- Multiple myeloma

Immunological disorders
- Cryoglobulinemia
- Antiphospholipid syndrome
- Myasthenia gravis

Drugs and toxins
- Anabolic steroids
- Azathioprine
- Myeloablative conditioning regimens for stem cell transplantation
- Oral contraceptives
- Oxaliplatin
- Thoratrast
- Toxic oil syndrome
- 6-Thioguanine

Clinical presentation

The clinical presentation can vary from asymptomatic disease diagnosed at autopsy to decompensated liver disease. Nodular regenerative hyperplasia is an uncommon clinical diagnosis. Large autopsy series have found a prevalence of nodular regenerative hyperplasia of around 2.5% of the population [119,120], demonstrating that most cases of nodular regenerative hyperplasia are asymptomatic. The most common symptoms of overt disease are bleeding varices and splenomegaly as the result of portal hypertension. Liver function is usually preserved, so that hypoalbuminemia and ascites are uncommon. Decompensated liver disease or frank liver failure may be precipitated by severe gastrointestinal hemorrhage or by another concurrent disease. The most common liver test abnormalities are mild elevations of alkaline phosphatase, aspartate aminotransferase, or alanine aminotransferase. Morphology of lesions attributed to heterogeneous perfusion of the liver is described in Table 95.5.

Treatment

In the vast majority of patients, no treatment is needed for the liver disorder itself, although the underlying predisposing condition or precipitating factor may require therapy. Manifestations of portal hypertension are treated along conventional lines.

Peliosis hepatis

Peliosis hepatis is a rare abnormality characterized by blood-filled cystic lesions in the hepatic parenchyma distributed irregularly throughout the liver. The peliotic cavities range in

Table 95.7 Conditions associated with peliosis hepatis

AIDS/*Bartonella* infection
Tuberculosis
Myeloproliferative diseases
Leukemia
Lymphoma
Multiple myeloma
Macroglobulinemia
Anabolic steroids
Oral contraceptives
Azathioprine
Arsenic
Thoratrast
Vinyl chloride
6-Thioguanine

size from less than 1 mm to several centimeters. Although most common in the liver, peliotic lesions may also occur in the spleen, abdominal lymph nodes and bone marrow.

Risk factors

Risk factors for peliosis hepatis include drugs, AIDS, tuberculosis, lymphoma, multiple myeloma, leukemia, and myeloproliferative disorders (Table 95.7). Historically, peliosis was found at autopsy in patients with chronic wasting illnesses, in particular tuberculosis and cancer. In patients with acquired immunodeficiency syndrome (AIDS), infection with *Bartonella henselae* or *Bartonella quintana* may cause peliosis as well as bacillary angiomatosis.

The initial change in peliosis hepatis seems to be sinusoidal dilation, followed by formation of cavities without sinusoidal endothelial cells [123,124]. Eventually the endothelial lining may be restored in parts of the peliotic cavities. Studies in AIDS patients most clearly support the concept that peliosis is initiated by damage to sinusoidal endothelial cells. In AIDS patients, *Bartonella* bacilli can be detected by electron microscopy in sinusoidal endothelial cells of peliotic lesions [125] and there is disruption of the sinusoidal endothelial cell lining [123].

Peliosis caused by some drugs (azathioprine, 6-thioguanine, oxaliplatin, urethane) may also be the result of direct damage to sinusoidal endothelial cells. These drugs have been linked to other disorders that are thought to be the result of damage to sinusoidal endothelial cells and in some cases up to all four lesions (i.e., peliosis, SOS, nodular regenerative hyperplasia, and sinusoidal dilation) have been described within the same liver. Azathioprine and oxaliplatin have been associated with all four lesions; 6-thioguanine has been linked to peliosis, nodular regenerative hyperplasia, and SOS; and urethane has been associated with peliosis and SOS. Other drugs (glucocorticoids, methotrexate, anabolic steroids, vitamin A, oral contraceptives) cause peliosis through an as yet undefined mechanism.

One might speculate that, analogous to AIDS, infection with *Bartonella* sp. may play a role in some of the other risk factors that cause immunosuppression, such as tuberculosis, cancer, malnutrition, and glucocorticoid therapy. However, *Bartonella* spp. have not been documented in peliosis in these disorders.

Clinical presentation

Peliosis hepatis is usually a silent disease. There are reports of portal hypertension, ascites, cholestasis, and liver failure. Rupture of a lesion may lead to either a hepatic hematoma or an intraperitoneal hemorrhage that may progress rapidly to shock and death. If the precipitating cause of peliosis is withdrawn or resolves, there is regression of the lesions. Diagnosis may be made by Doppler ultrasound, CT, or MRI [50,126].

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96 Experimental therapies: hepatocyte transplantation, gene therapy, and liver assist devices

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Introduction

Hepatocyte transplantation, liver-directed gene therapy, and artificial liver assist devices are being developed in a parallel but interdependent manner. For example, hepatocyte transplantation can be used as a vehicle for liver-directed gene therapy. On the other hand, gene transfer can be used to expand hepatocytes in culture for transplantation and abrogation of the immune response. This article will provide a short overview of advances, as well as the remaining hurdles in these areas.

Hepatocyte transplantation

Orthotopic liver transplantation has significantly improved the prognosis for patients with acute or end-stage liver failure, or potentially lethal inherited metabolic disorders. Because of the expense, complexity, and associated morbidity and mortality of this procedure, hepatocyte transplantation is being explored as an alternative. Hepatocyte transplantation is performed by percutaneous or transjugular injection into the portal vein, spleen, or peritoneal cavity so this procedure is less invasive than liver transplantation. As the host liver remains intact, the loss of graft function should not worsen liver function. While intact livers must be transplanted shortly after procurement, isolated liver cells may be cryopreserved for later use [1]. The success of hepatocyte transplantation in experimental animals has encouraged clinical trials of this procedure at several institutions. Potential indications for hepatocyte transplantation include acute or chronic liver failure and inherited metabolic disorders. Hepatocytes could be used as a vehicle for ex vivo gene therapy of inherited metabolic disorders. Hepatocyte transplantation is also being considered for rescuing patients from radiation-induced liver damage following radiotherapy for liver tumors.

Treatment of liver-based metabolic diseases

Hepatocyte transplantation has been shown to correct several liver-based metabolic disorders in animals. The Gunn rat, an animal model of Crigler–Najjar syndrome type 1, lacks bilirubin-uridine diphosphate-glucuronosyltransferase (UGT1A1) activity and consequently, exhibits toxic levels of hyperbilirubinemia 1 [2]. Gunn rats and Nagase genetically analbuminemic (NAR) rats have been studied extensively in hepatocyte transplantation experiments. Transplantation of normal donor hepatocytes has ameliorated the metabolic abnormality in both animal models [3]. In the absence of cirrhosis, hepatocytes injected into the splenic pulp or infused into the portal vein engraft in the liver, where they integrate into the hepatic cords and function throughout life [4,5]. Hepatocyte transplantation has resulted in partial correction of metabolic disorders in low-density lipoprotein receptor-deficient [6] Watanabe heritable hyperlipidemic rabbits (an animal model of familial hypercholesterolemia), the Long–Evans Cinnamon rat (an animal model for Wilson disease) [7], the *mdr2* mouse (a model of progressive familial intrahepatic cholestasis type 3) [8], the *spf-ash* mouse (a model of ornithine transcarbamylase deficiency) [9], the $Agxt^{-/-}$ mouse (a model for primary hyperoxaluria-1) [10], and the hyperuricemic Dalmatian dog [11].

Although many biologically active liver proteins are present in excess, the expectation that liver-based metabolic deficiencies could be corrected by the transplantation of a very

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small number of hepatocytes did not materialize. Repeated hepatocyte infusions can improve the response to transplantation [12], but more dramatic results have been obtained by progressive repopulation of the host liver by engrafted hepatocytes.

Massive repopulation of the liver

Studies in fumarylacetoacetate hydrolase (FAH) mutant mice (a model of hereditary tyrosinemia type I) showed that transplanted normal hepatocytes can spontaneously repopulate the liver of animals, in which hepatocytes are dying because of a metabolic disorder [13]. A small number of hepatocytes from normal mice, engrafted into the livers of FAH mutant mice, almost completely replace the native liver cells [13]. However, the transplant recipients remain susceptible to the development of hepatocellular carcinomas, despite extensive repopulation of their livers with normal hepatocytes. Therefore, risk of cancer or recurrent disease should be an important consideration in the clinical application of hepatocyte transplantation for a given disease. A small number of other metabolic diseases that provide a competitive repopulation advantage to donor cells include Wilson disease and α_1-antitrypsin deficiency. Unfortunately, in many cases, the liver disease is so advanced at the time of diagnosis that the structural damage to the host liver poses a hurdle to successful treatment by hepatocyte transplantation.

For most metabolic diseases, where host hepatocytes do not die spontaneously, strategies are being developed to inhibit host hepatocyte regeneration, while providing a proliferative stimulus to the transplanted cells by partial hepatectomy, induction of Fas-mediated apoptosis of host hepatocytes, or administering pharmacological doses of thyroid hormone [14,15]. A plant alkaloid, retrorsine blocks the hepatocyte cell cycle and has been used for this purpose [16]. As retrorsine is potentially carcinogenic, X-irradiation of the liver has been utilized as part of the preparative regimen [17,18]. Transplantation of normal hepatocytes from congeneic donor rats into Gunn rats that had undergone partial hepatectomy and hepatic irradiation resulted in complete normalization of serum bilirubin levels. Functional correction was also shown in a mouse model of primary hyperoxaluria type 1, where hepatic irradiation was combined with expression of hepatocyte growth factor by gene transfer, in lieu of partial hepatectomy [10]. It is possible that with detailed analysis in large animals, including nonhuman primates, preparative hepatic irradiation could find clinical application as a preparative regimen to expand donor hepatocytes after transplantation in patients with liver-based metabolic disorders.

Hepatocyte transplantation in patients with inherited metabolic disorders

Patients with ornithine transcarbamylase deficiency [19,20], α_1-antitrypsin deficiency [21], glycogen storage disease type 1a [22], infantile Refsum disease [23], factor VII deficiency [24], bile salt export protein deficiency (PFIC2) [25], and Crigler–Najjar syndrome type 1 [26] have been transplanted with allogeneic hepatocytes. Partial improvement in the metabolic syndrome has been documented in several of these diseases. In a patient with glycogen storage disease type 1a, hepatocyte transplantation resulted in improved glycemic control, and in the child with infantile Refsum disease, serum dihydroxycoprostanoic acid levels remained improved for more than a year after transplantation.

Unequivocal evidence of function of transplanted human hepatocytes was obtained in a 10-year-old patient with Crigler–Najjar syndrome type I (UGT1A1 deficiency) [26,27]. After transplantation, the serum bilirubin level fell by 50%, hepatic bilirubin glucuronidating activity increased from pretransplantation levels that were essentially unmeasurable to approximately 5% of normal and bilirubin glucuronides were excreted in bile. Allograft rejection was prevented with standard tacrolimus therapy. The procedure was not associated with any significant complications. Continued function of the engrafted cells was documented for nearly 3 years after transplantation. After this, the patient underwent partial liver organ transplantation because of continued requirement for phototherapy and slowly rising bilirubin levels [25].

Hepatocyte transplantation for patients with acute liver failure

Conceptually, hepatocyte transplantation should be particularly suitable for treating acute liver failure, because the liver remains architecturally normal and retains considerable restorative potential. Hepatocyte transplantation can rescue rodents with acute liver failure induced by hepatotoxins, liver ischemia, or 90% hepatectomy [28,29] and prevent the development of intracranial hypertension in pigs with acute, ischemic liver failure [30]. However, the outcome of hepatocyte transplantation in patients with acute liver failure has been disappointing, except, when used as a bridge to liver transplantation.

In the first clinical study, seven patients with fulminant liver failure were injected with human fetal hepatocytes into the peritoneal cavity. Some of the recipients recovered from encephalopathy [31]; the survival of patients with grade III hepatic encephalopathy was superior to that observed in the untransplanted control group. Since long-term survival of hepatocytes following direct injection into the peritoneum has not been shown, investigators in the United States have used other sites. Ethical concerns have led to the use of adult hepatocytes rather than fetal cells. In one study, seven potential transplant candidates received hepatocyte infusion through the splenic artery, five of these survived to undergo orthotopic liver transplantation [32]. In other studies, patients who were not candidates for organ transplantation were transplanted with hepatocytes by infusion into the portal vein for engraftment in the liver [33,34]. Engrafted hepatocytes were found in the spleens and livers of some of

the transplant recipients, and there are anecdotal reports of improvement in ammonia, prothrombin time, encephalopathy, cerebral perfusion pressure, and cardiovascular stability. Complications have been few, but include transient hemodynamic instability during intraportal hepatocyte infusion, sepsis, and embolization of hepatocytes into the pulmonary circulation [35]. Portal hypertension as a result of transplantation through the portal vein has been generally transient. Although the results suggested that the transplanted hepatocytes provided some benefit, unequivocal evidence for engraftment and function of the transplanted cells has not been obtained. Part of the problem could be that relatively small numbers of hepatocytes were transplanted in many of the patients.

In contrast to the situation in animal models of acute liver failure, liver regeneration is significantly inhibited in patients with acute liver failure [36]. Since injections of bone marrow cells and hepatocyte lysates have also been shown to affect animal survival, hepatocyte transplantation in the animal models might have helped by enhancing the regeneration of the host liver, rather than through functional engrafted cells. Based on studies with a mouse model of acute liver failure that more closely parallels the clinical situation [37], it is likely that multiple hepatocyte infusions over time may be required for improved patient survival.

Treatment of chronic liver failure

Studies in experimental animals

Because of the difficulty in generating animal models of stable and irreversible liver cirrhosis causing hepatic failure, most studies on evaluating hepatocyte transplantation for chronic liver failure have been performed in other animal models of hepatic encephalopathy. Intrasplenic transplantation of hepatocytes in rats with end-to-side portacaval shunt resulted in improvement of behavioral score, partial correction of the amino acid imbalance [38], and prevention of hepatic coma induced by ammonium chloride administration [39]. The engrafted hepatocytes proliferated in the spleen, organizing into structures resembling hepatic chords.

Hepatocyte transplantation has been shown to improve many of the physiological abnormalities associated with decompensated liver failure in rats with end-stage liver cirrhosis, induced by chronic administration of phenobarbital and carbon tetrachloride. Hepatocyte transplantation also prolonged the survival of these animals [40]. Transplantation of hepatocytes into cirrhotic livers results in a severe and prolonged increase in portal pressure [41]. While some transplanted hepatocytes migrate into cirrhotic nodules following intraportal infusion, it is unlikely that enough cells can engraft in the cirrhotic liver to significantly affect liver function. Moreover, it is unclear whether the transplanted cells could function normally within the deranged structure of the cirrhotic nodules. For these reasons, in the studies on cirrhotic rats, hepatocytes were engrafted in the spleen.

Clinical experience

The initial clinical trial was performed on 10 Japanese patients with liver cirrhosis to test the hypothesis that the functional failure of hepatocytes within the cirrhotic liver is the result of the deranged architecture of the organ. Between 10 million and 600 million hepatocytes harvested from their own left lateral liver segments were injected directly into the spleen, or infused into the splenic artery or the portal vein. In one patient, ascites and encephalopathy eventually resolved and engrafted hepatocytes were detected in the spleen by technetium-99m(Sn)-*N*-pyridoxyl-5-methyltryptophan radioisotope uptake 11 months after transplantation [42]. Subsequently, in the United States, infusion of up to 10^{10} allogeneic donor hepatocytes into the splenic artery in patients with decompensated chronic liver disease was shown to be well tolerated and associated with improvement in encephalopathy, hepatic protein synthesis, and renal function [35]. Nuclear scanning demonstrated the presence of engrafted hepatocytes in the spleen.

If extrahepatic hepatocyte transplantation proves to be effective in improving liver function, it could be performed in combination with transjugular intrahepatic portosystemic shunt to relieve portal hypertension. However, the risk for hepatocellular carcinoma would persist, and routine surveillance would still be needed. Unfortunately, the improvement of liver function following hepatocyte transplantation in patients with chronic liver failure has been relatively modest, compared with that observed in animal models. The discrepancy may have resulted from the fact that hepatocytes were transplanted by injection into the splenic pulp in rats, whereas they were infused into the splenic artery of the patients [35]. In fact, hepatocytes infused in the arterial bed of rats are lost rapidly [43]. Consistent with this, in patients, only a few hepatocytes were found in the spleen after infusion into the splenic artery.

Ongoing issues

Technically, the number of hepatocytes that can be transplanted at any one time is limited. Using present techniques, less than 30% of transplanted cells engraft in the liver. A better understanding of the factors that allow hepatocyte engraftment might reduce the need for repeated cell infusions and multiple donors. The most important issue limiting the application of hepatocyte transplantation is the availability of donor livers, which is reduced by competition for use in whole organ transplantation. The ability to cryopreserve and bank hepatocytes might allow the pooling of cells from multiple donors, increasing cell availability for transplantation. Unfortunately, cryopreserved liver cells have not yet been shown to engraft as well as fresh hepatocytes [44] and the viability of human hepatocyte cryopreserved by current methods is variable.

Despite some progress in growing primary hepatocytes in culture [45], only hepatocytes that have been immortalized

by gene transfer are capable of long-term growth and correcting metabolic abnormalities in liver failure after transplantation [39,46]. An alternative solution for the scarcity of human donor cells is the transplantation of xenogeneic hepatocytes [47]. Studies in both rodents and primates indicate that xenogeneic hepatocytes are not susceptible to the immunological processes that lead to hyperacute rejection of vascularized whole organ xenografts [48]. However, xenograft survival in humans has been of such short duration that whether organs or tissues from another species will function adequately to replace human organ function remains uncertain [49]. It is also a concern that xenogeneic liver cells could transfer infectious agents to the recipient and, potentially, beyond [50]. On the other hand, it is possible, that transplanted nonhuman hepatocytes could be resistant to recurrent infection by human-specific viruses, such as hepatitis C virus.

Finally, it is difficult to detect early rejection of the transplanted hepatocytes and new, noninvasive approaches to the detection of donor hepatocytes, such as magnetic resonance-based methods, are needed [51].

Liver-directed gene therapy

Goals of liver-directed gene therapy

Targets of liver-directed gene therapy include both inherited metabolic disorders and acquired conditions, such as infectious and neoplastic diseases, cirrhosis of the liver, and immune rejection of transplants. Liver-directed gene therapy could be used for diverse purposes. Gene products that are missing in inherited disorders could be replaced by transferring transcription units expressing those proteins. In other cases, certain genes could be overexpressed for specific therapeutic benefits, such as treatment of cirrhosis by expression of metalloproteases. The large size of the liver and its capability to secrete proteins into the plasma could be exploited to generate extrahepatic proteins, such as coagulation factors or hormones. Proteins that are normally expressed at extrahepatic sites could be expressed in the liver for specific purposes. For example, the catalytic subunit of the apolipoprotein B messenger RNA (mRNA) editing enzyme (APOBEC-1), which is expressed normally in the intestinal epithelial cells, could be expressed ectopically in the liver to switch the hepatic apolipoprotein production from apolipoprotein B-100 to apolipoprotein B-48, thereby reducing the generation of low-density lipoproteins [52]. Similarly, PDX, a homeobox protein that is responsible for pancreatic differentiation, could be expressed in the liver, to switch the hepatocyte phenotype to an insulin-generating phenotype [53]. Gene transfer to the liver could be used to generate pharmacological gene products, such as vaccines, single chain antibodies, dominant negative proteins, immunomodulatory substances, and pro-apoptotic or antiapoptotic proteins. On the other hand, nucleic acids may be used to inhibit the expression of deleterious proteins, such as viral proteins or mutant α_1-antitrypsin. This could be accomplished by transferring synthetic antisense RNAs, ribozymes or small interfering RNAs. Genes could be transferred to express antisense RNAs [54], ribozymes, short hairpin RNAs (shRNAs), micro RNAs (miRNAs), or dominant negative proteins [55]. Finally, technologies are being developed to correct mutations within endogenous genes in intact organisms. This could be accomplished by targeted replacement of the defective gene [56], or by site-directed correction of a target genomic sequence [57]. These newer strategies permit the repaired gene to remain under the control of the endogenous promoter, whereby physiological regulation is retained. Table 96.1 is a partial list of inherited and acquired disease targets for liver-directed gene therapy.

Table 96.1 Some hepatic disorders that are targeted for gene therapy

I. Inherited liver disorders
Crigler–Najjar syndrome type I
Familial hypercholesterolemia and other lipid metabolic disorders
Maple syrup urine disease
Progressive familial intrahepatic cholestasis
Phenylketonuria
Tyrosinemia
Mucopolysaccharidosis VII
α_1-Antitrypsin deficiency
Ornithine transcarbamylase deficiency
Wilson disease
Glycogen storage diseases, e.g., von Gierke disease and Pompe disease
II. Inherited systemic disorders
Hemophilia A and B
Oxalosis
III. Acquired disorders
Infectious diseases, e.g., hepatitis B and C
Malignant neoplasms: hepatomas, cholangiocarcinomas, metastatic tumors
Extrahepatic tumors (inhibition of neovascularization)
Cirrhosis of the liver
Allograft or xenograft rejection

Gene therapy for cancer

Like other therapeutic approaches, the target is to eliminate the tumor cells while minimizing the damage to normal cells. Gene transfer could be used to kill the tumor cells or inhibit their growth, reduce the vascular supply to tumors, generate immune response against tumor cells, or augment the effect of chemotherapy and radiotherapy. Enhancement of apoptosis of the tumor cells could be promoted by expressing *p53*, a sentinel gene of the cell cycle [58]. Tumor killing is attempted by transferring "suicide genes," such as the herpes simplex virus thymidine kinase (HSV-TK), which converts a prodrug,

ganciclovir, to its active phosphate derivative [59], or cytosine deaminase and purine nucleoside phosphorylase (which converts fludarabine to a diffusible toxic metabolite) [60]. The activated toxin, such as ganciclovir phosphate, may diffuse to neighboring cells, killing them by "bystander effect". Toxicity to normal cells could be reduced by tumor-specific delivery through tagging the DNA to a monoclonal antibody, such as that directed against AF-20, a 180-kDa, tumor-specific cell surface glycoprotein, expressed in hepatoma cell lines [61], and by using tumor-specific promoters (e.g., α-fetoprotein or carcinoembryonic antigen) to drive the expression of transgene. In an alternative approach, E1B-mutant adenoviruses that are capable of replicating in tumor cells deficient in functional p53, have been used in an attempt to kill tumor cells [62], although the replication of these mutant adenoviruses may not necessarily depend on the absence of p53.

Direct killing of the tumor cells by topical expression of toxic gene products does not eliminate those at metastatic sites. As tumor growth requires neovascularization, inhibition of new blood vessel formation by angiostatin or endostatin may retard the growth of both primary and metastatic tumors [63,64].

Permanent elimination of the tumor cells may require a host immune response against tumor-specific "neoantigens" that are not expressed by normal cells. Specific tumor antigens, such as those from melanoma cells, may permit DNA-based tumor vaccination. The host immune response against the neoantigens may be feeble because of inadequate presentation of the antigens by antigen-presenting cells. Genetic manipulation of antigen-presenting cells may augment the host immune response against tumor cells. Secretion of cytokines, such as transforming growth factor-β or interleukin-10, by large tumors may suppress the immune response [65]. "Debulking" the tumor by surgery, radiofrequency ablation, radiotherapy, chemotherapy, or gene therapy may enhance the immune response. Currently, gene therapy for cancer has the greatest potential as an adjunct to chemotherapy or radiotherapy. Irradiation enhances the number of tumor cells transducible by recombinant viruses. Transgene expression could be driven by promoters that are stimulated by irradiation [66]. On the other hand, inhibition of the expression in the tumor cells of radioprotective proteins, such as ATM (*a*taxia *t*elangiectasia *m*utated), could increase their radiosensitivity.

Gene therapy for infectious diseases

Nucleic-acid-based approaches are being explored for both prophylaxis and treatment of hepatic viral infections. DNA vaccination [67] eliminates the possibility of the vaccines being contaminated by microbes or other organic material. The injected DNA itself serves as an adjuvant, enhancing the immune response. For viral antigens that are presented poorly, the antigenic peptides may be expressed directly in antigen-presenting cells by gene transfer. Gene transfer may also be used to express cytokines that promote the antigen-presenting cell proliferation and maturation of T cells to helper T cells. In a different approach, termed intracellular vaccination, single chain antibodies or antibody fragments may be expressed within the cells to make them resistant to viral infections [68].

Gene therapy can also be used to interfere with the virus life cycle by introducing synthetic antisense RNAs, ribozymes, or DNA ribonucleases (see below). Ribozymes, antisense RNAs, shRNAs, miRNAs, or dominant negative proteins [69] can also be expressed within the target cells by gene transfer.

Gene therapy for inherited disorders

As many inherited disorders are caused by the abnormality of a single gene, the effect of gene therapy can be evaluated directly and precisely in these conditions. For this reason, although the inherited disorders are much less common than acquired diseases, single gene abnormalities continue to be important targets of liver-directed gene therapy. Table 96.1 contains a partial list of inherited disorders that are targets of gene therapy.

Methods of gene transfer to the liver

Genes may be delivered to the liver by isolated hepatocytes, termed ex vivo gene therapy, or by delivery of the gene into the intact liver. Hepatocyte transplantation-based methods have been discussed above. Here we will consider methods of delivering genes to the intact organism, using replication-deficient recombinant viruses or nonviral vectors. For gene transfer to hepatocytes, important considerations include the attainable infectious titer, ability of the virus to infect nondividing cells, integration into the host genome, repeatability of administration, and safety of the vector system. Frequently used methods of gene transfer to the liver are listed in Table 96.2. Some commonly used recombinant viral vectors are described below.

Vectors based on recombinant viruses

Retrovirus-based vectors

The complementary DNA (cDNA) generated from the RNA genome of retroviruses is integrated into the host genome and transmitted to the progeny of the transduced cells [70]. Oncoretroviruses, particularly, Moloney murine leukemia virus, are used commonly for generating recombinant vectors (Figs 96.1 and 96.2). The viral genes are replaced by target transgenes, but the packaging signal (ψ) is retained [71]. The viral proteins are provided in *trans* by "packaging cell lines," generating a replication-deficient recombinant virus. The range of species that recombinant retroviruses can infect is determined by the envelope protein. The host range can be expanded by engineering the viral envelope to contain proteins, such as the G protein of the vesicular stomatitis virus. Inside the cell, the RNA genome is reverse-transcribed into double-stranded DNA, which is transported to the nucleus

Table 96.2 Characteristics of liver-directed gene therapy methods

Method	Integration and persistence	In vivo gene transfer efficiency to liver	Liver-specificity	Immunological and other issues
Recombinant viral vectors:				
Murine leukemia-based retroviruses	Integration is required	Requires mitosis Low efficiency in quiescent cell types, e.g., hepatocytes	None	Non-immunogenic Difficult to obtain very high titers Envelope can be pseudotyped with other proteins to increase stability and broaden host range
Lentivirus-based retroviruses	Integration is required	Can infect non-dividing cells, but cell cycling may be needed Low to intermediate efficiency for liver	None	Non-immunogenic Difficult to obtain very high titers Envelope can be pseudotyped with other proteins to increase stability and broaden host range
Adeno-associated virus	Can exist in both episomal and integrated forms	Can infect quiescent and dividing cells Low to intermediate efficiency for liver	None	Causes humoral immune response Can be grown at high titers Site-specificity of integration of the wild-type virus is lost in the absence of *rep* Can undergo lytic cycle in the presence of helper proteins Limited packaging space
Simian virus 40	Can exist in both episomal and integrated forms	Infects both quiescent and dividing cells High efficiency	None	No significant immune response Can be grown at high titers Limited packaging space
Adenovirus	Episomal Can persist for several months in the absence of host immune response	Efficiently infects both dividing and non-dividing cells Very high efficiency for liver	Liver targeted	Evokes both humoral and cell-mediated host immune response Viral gene deletion reduces primary immunogenicity, but does not permit repeated injection Host tolerization permits repeated gene transfer
Hybrid viruses	Combines advantages of different viruses	Efficiency of adenoviral vectors is combined with persistent nature of other viruses	Liver targeted	Adenoviral proteins provided in *trans* may evoke immune response Site-specific integration or episomal replication may be possible
Non-viral vectors:				
Injection of naked DNA or RNA	Transient	Intermediate efficiency	Liver targeted	Probably nonimmunogenic
Receptor or liposome-mediated plasmid delivery	Transient	Low efficiency	Liver targeted	Probably nonimmunogenic
Oligonucleotides designed to form DNA triplex or to convert single nucleotides	Permanent	Low efficiency	Liver targeted	Nonimmunogenic

and is integrated into the host genome. This process requires mitosis of the host cell; this is infrequent in hepatocytes of the adult liver. To overcome this obstacle, immunoretroviruses, particularly lentiviruses, which form preintegration complexes that can be translocated into nondividing nuclei, are being explored for liver-directed gene therapy [72].

Recombinant adenovirus

Adenoviruses are large, linear, double-stranded DNA viruses. Adenoviruses types 5 and 2 are commonly used for preparing gene transfer vectors. Recombinant adenoviruses can be generated at high titers, can infect both dividing and quiescent cells, and on intravenous administration in rodents,

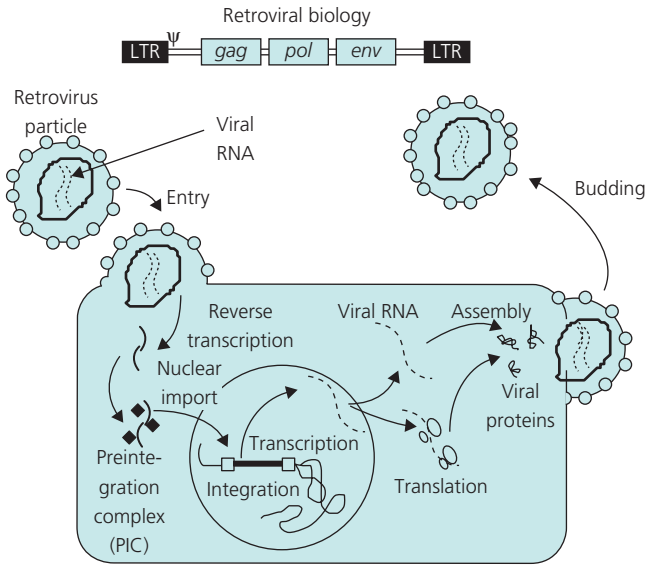

Figure 96.1 Life cycle of wild-type Moloney murine leukemia virus. The RNA genome of the virus consists of the retroviral genes, *gag*, *pol*, and *env*, flanked on the 5′ and 3′ ends by the "long terminal repeats" (LTRs), consisting of a promoter, enhancers, and polyadenylation signals. Sequences upstream to the 5′ LTR contain the packaging signal (ψ) that is needed for packaging of the viral RNA genome into viral particles. After entry into the infected cell, the RNA genome undergoes reverse transcription and the DNA transcript is incorporated into a preintegration complex (PIC), which is imported into the nucleus. Dissolution of the nuclear envelope, which occurs during mitosis, may be needed for this step. The proviral DNA is then integrated into the host genome. The entire unspliced transcript provides the new viral genome, which is packaged with the viral proteins expressed from the integrated proviral DNA. The assembled virus buds out and is ready to infect another cell.

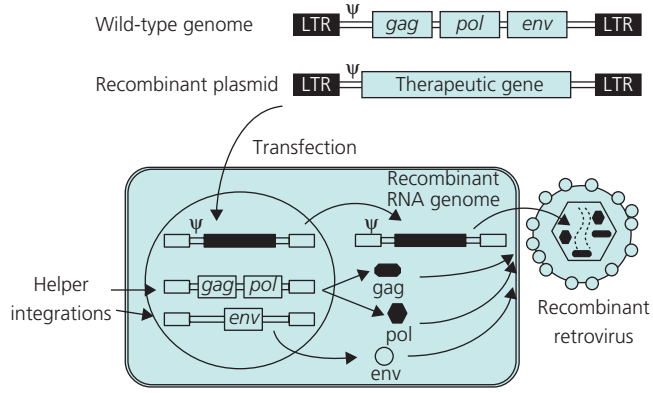

Figure 96.2 "Domestication" of the Moloney murine leukemia virus. The packaging cell is stably transfected with multiple DNA segments expressing different viral proteins (gag, pol, and env). A DNA version of the viral genome is cloned into a plasmid. All viral genes are replaced by target transgene, but the packaging signal (ψ) is left intact. The recombinant plasmid is transfected into the packaging cell, which provides the viral proteins in *trans*. The viral RNA genome can be assembled with the viral proteins because of the presence of the packaging signal, ψ. The assembled recombinant virus buds out of the packaging cell into the culture medium. The recombinant virus can infect other cells but is replication-incompetent because of the lack of the viral genes.

localize preferentially to the liver [73]. The adenoviral receptor, coxsackievirus and adenovirus receptor (CAR), is highly concentrated in rodent liver, but less so in the human liver. It is therefore unclear whether the high gene transfer efficiency observed in the rodent liver will be replicated in human liver.

Recombinant adenoviral vectors are produced by inserting the transgene into the early region-1 (*E1*) gene, which encodes transcription factors required for the expression of adenoviral genes. The recombinant virus is generated in packaging cells that provide the *E1* gene products in *trans*. All adenoviral genes may be deleted to increase the "stuffing space" and to reduce immunogenicity by preventing any possibility of expression of viral proteins [74]. These "gene-deleted" or "gutless" vectors require helper adenoviruses to provide the structural proteins in *trans*.

Strong immunogenicity of the viral antigens limits the clinical application of adenovectors. Many human subjects have preexisting immunity against adenoviruses from natural infection. In addition, there can be innate immune response after administration of adenovectors in humans. Neutralizing antibodies generated after the first injection block gene transfer following subsequent administrations of adenovector. Cytotoxic lymphocytes against adenoviral antigens attack the adenovirally infected host cells, causing liver damage and rapid loss of the transgene after secondary gene transfer [75]. Despite the deletion of all viral genes, the helper-dependent, gutless adenovectors retain their immunogenicity by virtue of the capsid proteins in the input virus [76], thereby frustrating the attempts at repeated gene transfer. Adenovectors are being generated from nonhuman strains of adenoviruses that are antigenically different from human adenoviruses, so that vectors based on different adenoviral strains can be used serially to express a transgene.

Development of an effective immune response requires costimulatory interaction between antigen-presenting cells and cytotoxic lymphocytes through the B7–CD28 and CD40–CD40 ligand interactions. Although inhibition of the B7–CD28 costimulation by injecting cytotoxic T-lymphocyte antigen 4–immunoglobulin (CTLA4–Ig) alone does not permit repeated administration of adenovectors [77], coexpression of CTLA4–Ig along with the target transgene permits multiple administration of the recombinant adenovirus [78]. Alternatively, host tolerance toward adenoviral proteins can be induced by injecting recombinant adenoviruses in utero or in newborn rats [73], inoculating adenoviral proteins into the thymus of young adult rats [79], or orally administering small doses of adenoviral proteins [80]. However, safety concerns

persist regarding tolerization of humans to adenoviral antigens, because wild-type adenoviruses are pathogenic to humans.

Herpes simplex virus 1

Herpes simplex virus type 1 is a 150-kilobase (kb) double-stranded DNA virus with a broad host range [81] and the ability to infect nondividing cells. However, long-term gene expression in the liver has not been achieved with the available herpes simplex virus vectors.

Recombinant baculovirus

The *Autographa californica* nuclear polyhedrosis virus is usually employed for generating recombinant proteins in insect cells [82]. Interestingly, among mammalian cells, hepatocytes can be infected by baculovirus vectors and the transgene expression can be driven by mammalian promoters. Hybrid viruses, consisting partly of baculoviral sequences, are being evaluated for *in vivo* application.

Recombinant adeno-associated virus

Adeno-associated virus (AAV) is a human parvovirus with a single-stranded DNA genome encapsidated in an icosahedral shell ~ 25 nm in diameter. The genome is approximately 5 kb long and contains two open reading frames. The reading frame on the left encodes replication proteins whereas the right-hand open reading frame encodes capsid proteins. AAV is a dependent parvovirus that relies on coinfection by adenovirus or herpes viruses or genotoxic stimuli, e.g., ultraviolet light or irradiation [83] for a productive, lytic infection. In the absence of helper viruses, wild-type AAV integrates preferentially on the q13.4-ter arm of human chromosome 19 [84] and remains latent in the host genome. Of the capsid proteins, VP1, VP2, and VP3, the key structural component VP3 constitutes nearly 80%–90% of the virion shell. VP1 contains a phospholipase A2 (PLA2) domain required for infectivity. Recombinant AAV vectors require the 145-base-pair (bp) terminal repeats of the AAV genome whereas all other viral factors can be provided in *trans* for production (Fig. 96.3). Following the initial development of an infectious AAV type 2 (AAV-2) clone in 1982 [85], rAAV-2 vectors have gained popularity in gene therapy applications, and have been tested in preclinical studies and clinical trials for a variety of diseases [86]. Binding of AAV-2 to a heparin sulfate proteoglycan at the cell surface is required for its internalization. The 145-bp inverted terminal repeats are needed for integration into the host genome. Following introduction into cells, recombinant AAV vectors persist mainly as episomes. The viral Rep protein, which directs the site specificity of AAV integration into chromosome 19, is absent in the recombinant vectors, which, therefore, lose the ability to integrate and remain in the nucleus as episomes [87].

Initial studies of intramuscular administration of AAV-2-based vectors for delivering coagulation factor IX in patients with hemophilia B resulted in low levels of protein production [88]. Infusion of higher doses of the recombinant virus into the portal vein of factor IX-deficient dogs resulted in the appearance of 5% of normal levels of factor IX activity in plasma [89]. The efficiency of AAV-mediated cancer gene therapy could be augmented when used in combination with conventional tumor therapies, such as irradiation or chemotherapy [90]. Later studies showed that various AAV serotypes have distinct tissue tropism, depending on their capsids. To date, 11 different serotypes, AAV-1 to AAV-11, and over 100 variants have been isolated from human and nonhuman tissues [91]. Of these 11 serotypes, AAV serotypes 1–9 are being developed as gene therapy vectors. As the AAV-2 genome is well-characterized, it has been cross-packaged with capsids of other serotypes to increase the efficiency of gene transfer. AAV-2/AAV-8 hybrids are particularly efficient for transferring genes into hepatocytes, although the pancreas, heart, and skeletal muscles are additional targets of this hybrid vector.

While recombinant AAV vectors persist for up to a year as episomes in hepatocytes, they are eventually lost because of their episomal nature. AAV vectors cause humoral and cellular immune responses, which are hurdles to readministration of a single vector. However, it may be possible to use vectors of alternative capsid serotypes for repeat administration. Attempts are being made to generate AAV vectors with the desired characteristics by mutagenesis of the capsid genes, based on known structural characteristics. An alternative approach is to use random DNA recombination, which leads to viral diversity in nature. Using DNA shuffling techniques, the process of evolution can be accelerated to generate vectors

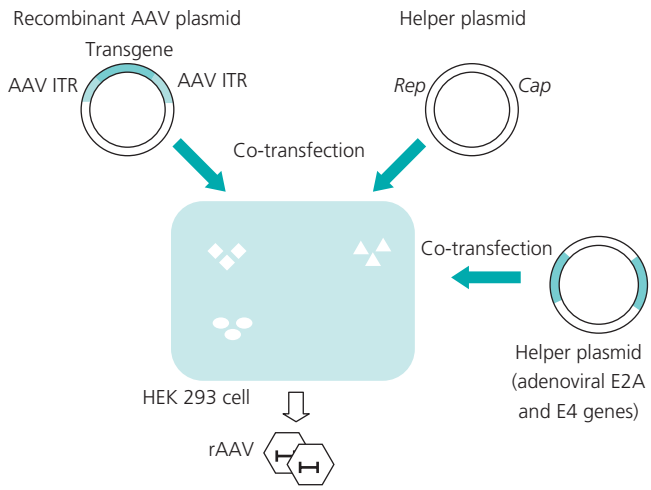

Figure 96.3 Generation of recombinant adeno-associated viruses (AAV). To generate AAV vectors, two plasmids, one containing the transgene, flanked by the inverted terminal repeats (ITRs) and the other encoding rep, cap, and adenoviral proteins, are cotransfected into 293 cells, which provide adenoviral E1A in *trans*. The recombinant virus is "rescued" by infection with a helper adenovirus (*E1A*-deleted) or, more commonly, by transfecting a plasmid that expresses adenoviral proteins.

with certain desired attributes, such as the ability to avoid recognition by antibodies and cytotoxic lymphocytes, or to transfer genes into specific tissues with high efficiency [92].

Despite several advantages of recombinant AAV vectors, the small stuffing space offered by the small virus limits the size of cDNA that can be accommodated within these vectors. To overcome this problem, the split AAV vector technology has been used to expand the packaging capacity of AAV through head-to-tail dimerization. Essentially, two recombinant vectors are generated. A 5′ vector includes the enhancer/promoter cassette and a half-intron carrying a splice donor site. A complementing 3′ vector contains another half-intron carrying a splice acceptor site linked with the cDNA encoding the target gene and a polyadenylation signal. Following coinfection into cells (e.g., 293 cells), genomes of the 5′ and 3′ vectors dimerize together, thereby expressing the target gene [93].

Simian virus 40-based vectors

Simian virus 40 (SV40) is a non-enveloped virus of the papova family with a 5.2-kb double-stranded DNA genome. The large (Tag) and small (tag) T antigens are transcription factors required for the expression of the viral structural genes, VP1, VP2, and VP3. The recombinant vector is generated by replacing the *Tag* genes of the viral genome by the target transgene. Various lengths of the structural genes can also be deleted to make additional stuffing space. The recombinant viral genome is transfected into a helper cell line (e.g., COS cells that provide Tag in *trans*). Absence of the *Tag* gene makes the recombinant virus replication deficient and markedly reduces its immunogenicity. Up to 4.7 kb of exogenous DNA can be inserted into SV40 vectors, which can be generated at 10^9 infectious units (IU)/mL and concentrated to 10^{12} IU/mL [94,95]. Recombinant SV40 integrates into the genome of both dividing and nondividing cells. The range of target cells is broad and includes hepatocytes and bone marrow cells [94,95].

Nonviral vectors

Liposomes, lipoplexes, and polyplexes

Non-viral vectors comprise:

- lipid-based delivery systems, e.g., lipid microcapsules (liposomes) or lipid-nucleic acid complexes (“lipoplex”)
- polycations, e.g., poly-L-lysine (PLL), polyethylenimine (PEI), polyglucosamines, lipopolyamines, and cationic peptides
- polycation–lipid hybrids (“Lipopolyplex”).

DNAs are usually compacted with polycations before lipid encapsulation into liposomes or formation of complexes with lipids. Lipopolyplex particles are smaller than the liposomes or lipoplexes and provide greater protection of the nucleic acids from degradation by nucleases. Cationic lipid transfecting agents (e.g., lipofectamine, 1,2-dioleolyl-3-trimethylammonium-propane), and cationic polymers, (e.g., PLL and PEI), provide relatively effective transfection [96], but are inefficient in the presence of plasma and too toxic for use in vivo. To target the gene delivery to hepatocytes, the gene transfer vehicles have been modified to include ligands that are endocytosed by hepatocyte-specific receptors, such as, the asialoglycoprotein receptor (ASGPr). Galactose-terminated peptides (e.g., asialoorosomucoid) or galactose can be conjugated to PLL or PEI for hepatocyte-specific gene delivery [97]. Similarly, liposomes composed of galactocerebrosides serve as ligands for the ASGPr by way of the galactose residues [98]. Incorporation of nuclear localization signal peptide motifs on plasmid constructs enhances their translocation to the nucleus [99].

Nucleic acid transfection generally has a transient effect in vivo; this may be sufficient when using synthetic antisense RNAs or ribosymes. A long-term effect is possible if the DNA integration can be enhanced by some mechanism, e.g., using the transposon–transposase system, or if the transfected nucleic acid triggers a permanent change, e.g., site-directed gene repair. Some specific applications of nonviral gene transfer methods are discussed below.

Ribozymes, antisense RNA, and DNA ribonucleases

Ribozymes are RNA enzymes that hybridize to complementary RNA sequences and catalyze endoribonucleolytic cleavage, causing rapid degradation of the RNA molecule [100]. For hairpin ribozymes the presence of a guanosine residue immediately downstream to the cleavage site is essential, but the effect is enhanced by a GUC sequence. Hammerhead ribozymes require only a UA, UC, or UU dinucleotide for cleavage. Ribozymes can be synthesized and packaged for cellular uptake, or expressed in cells from transfected DNA.

Hammerhead [100] and hairpin [101] ribozymes have been shown to inhibit the production of hepatitis B and hepatitis C viruses in cells. Antisense oligonucleotides targeted to a 5′-untranslated region of hepatitis C virus were transferred to a differentiated human hepatoma cell line HuH7 using ASGPr–polylysine complexes, resulting in specific inhibition of the hepatitis C vrus-directed protein synthesis in the cells [101]. Multiple sites within the hepatitis C virus RNA genome may be targeted simultaneously using a series of ribozymes expressed from a single vector, thereby inhibiting the emergence of drug-resistant mutants [102]. Hepatitis B virus, a partially double-stranded DNA virus, replicates through a pregenomic RNA intermediate, which could also be a target for ribozyme-based RNA cleavage [100].

DNA ribonucleases are synthetic single-stranded DNAs comprising a 15-nucleotide catalytic domain, flanked by two RNA-binding domains, that catalyze RNA cleavage with a greater efficiency than ribozymes [103]. DNA ribonucleases against the hepatitis C viral genome can specifically cleave the viral RNA. DNA ribonucleases designed to cleave hepatitis B virus RNA transcript substantially inhibit viral gene expression [104].

Small inhibitory RNAs, short hairpin RNAs, and micro RNAs

The introduction of single-stranded siRNAs and shRNAs that express siRNAs within cells has ushered an exciting era in down-regulation of gene expression as a therapeutic strategy in liver diseases. Synthetic single-stranded siRNAs can be transfected into cells to inhibit the translation of specific mRNAs and to destabilize the mRNA. However, for in vivo gene therapy, it is more convenient to use vectors to express shRNAs that are processed in the cell into siRNAs [105]. The most vigorously investigated targets are hepatitis B and hepatitis C viruses [106], although other disease targets are also being explored. Plasmid or viral vectors are used to express shRNAs, usually from polymerase III promoters (e.g., U6 or H1). However, the use of polymerase II promoters may also be feasible [107]. A recent advance in gene silencing has involved modification of the stem sequence of miR-30 miRNA hairpins to achieve gene knockdown [108]. Modified miR-30 can achieve more effective knockdown than shRNA-based methods and can be expressed readily from polymerase II promoters. A number of natural miRNA hairpins have been found to exist in clusters of multiple identical or different copies [105], suggesting that polycistronic transcripts might naturally enhance the efficiency of target gene repression or result in linked multigene repression. Simple multihairpin designs have been shown to exhibit improved knockdown efficiency compared with single hairpin constructs [108]. Gene knockdown technologies using shRNAs and miRNAs are likely to progress to clinical trials [106].

Homologous recombination

Site-specific recombination of DNA could be a precise means of repairing mutated or damaged DNA. Several proteins, including homologues of yeast recombination proteins rad51 or rad52, are important in the homologous recombination in higher eukaryotes [109]. Although used extensively in cultured cells, the process is inefficient and has rarely been successful in vivo [110]. As homologous recombination is cell cycle-regulated, it occurs extremely infrequently in quiescent cells, such as hepatocytes [111].

Triplex DNA

This method is based on the formation of a triple-DNA by the binding of an exogenous single-stranded nucleic acid to the major groove of a homopurine region of the double-stranded genomic DNA [112]. The polypurine regions must be guanine rich and 12–14 nucleotides in length for adequate triplex formation to occur. Conventionally, cross-linking agents, such as psoralen or other mutagens, are covalently attached to the triplex-forming oligonucleotide [112]. After intercalation of the psoralen at the target 5′-ApT-3′ site, ultraviolet irradiation causes cross-linking of the thymines in the two strands. This substrate is then repaired by endogenous cellular DNA repair mechanisms, producing the characteristic T : A to A : T transversions. Single-strand DNAs with cross-linking agents can also be used to promote insertions and deletions at target through the excision repair pathway [113]. Target sequence constraints limit the application of this method in vivo. Bifunctional oligonucleotides comprising regions that form triple-helical structures as well as conventional Watson and Crick base pairs have partly overcome this hurdle in cultured cells [114].

Single nucleotide modification

A method that uses the cells' mismatch repair machinery to correct single base mutations has been introduced. A synthetic oligonucleotide is generated that is complimentary to the targeted genomic DNA, except at a single mismatched base. The majority of the successful studies have utilized a DNA/RNA chimera. The RNA component, consisting of 2′-*O*-methyl-RNA residues, is included to increase the strength of hybridization with the targeted DNA sequence. The mismatch between the oligonucleotide and the genomic DNA triggers the mismatch repair system of the cell, so that the genomic mutation is permanently corrected. In *Escherichia coli* strains that are deficient in specific repair proteins [115], this approach was shown to require RecA and MutS, proteins involved in DNA pairing and mismatch repair, respectively, suggesting the involvement of both the homologous recombination and mismatch repair pathways. Both recombination and mismatch repair pathways are evolutionarily conserved. Extracts of human hepatoma cells (HuH-7) containing the mismatch repair protein hMSH2 can complement MutS-deficient *E. coli* in converting the mutant aminoglycoside resistance gene. Wild-type p53 may inhibit the initial pairing step in this repair process by inhibiting the pairing activity of RecA and its human homologue Rad51 [116].

In intact rats, RNA–DNA chimeric oligonucleotides were delivered to the liver by receptor-mediated endocytosis, using lactosylated PEI or galactocerebroside-containing liposomes. In initial studies, a Ser365Arg conversion of the rat factor IX gene was achieved with significant frequency [117]. Subsequently, the guanosine base deletion in the bilirubin-uridine diphosphate-glucuronosyltransferase (*UGT1A1*) gene in jaundiced Gunn rats was repaired at a frequency that was sufficient to express detectable enzyme activity in the liver and reduce serum bilirubin levels significantly [118]. Nucleotide insertion was verified by differential hybridization, Southern blot analysis, and Western blot analysis. Tagalakis and colleagues demonstrated a high level of conversion of the gene expressing human apolipoprotein E-2 in a transgenic mouse to apolipoprotein E-3 after intraperitoneal injection of an RNA–DNA chimeric oligonucleotide [119]. In addition to hepatocytes, the RNA–DNA chimera has been used successfully for site-directed gene conversion of the apolipoprotein A-2 gene in human lymphocytes [119], the nonsense

mutation of carbonic anhydrase II in nude mouse primary kidney tubular cells [120], and the missense mutation in the tyrosinase gene in albino mouse melanocytes [121].

Transposon-based gene integration

Transposable genetic elements (transposons) are ubiquitous in eukaryotic genomes and are capable of moving from one chromosomal location to another by a "cut-and-paste" mechanism. The Emmons laboratory characterized five transposable elements from fish that were similar in structure to the Tc1 transposon of the nematode *Caenorhabditis elegans* [122]. These transposons of the *Tc1/mariner* superfamily consist of short inverted repeats that flank an open reading frame, encoding a transposase. Ivics and associates generated a synthetic transposable element from defective copies of an ancestral Tc1-like fish transposon. This transposon was termed *Sleeping Beauty*, because it had remained dormant for millions of years during evolution and became functional only after correcting mutations that had accumulated over many generations [123]. It is a 1.6-kb element, flanked by 250-bp terminal inverted repeats. DNA sequences, flanked by the terminal inverted repeats, can efficiently integrate into mammalian cellular genome, provided the transposase is expressed in the same cell. The integration occurs at TA dinucleotide sites, which are duplicated on insertion of the transposable element [123]. The coding region of human factor IX has been inserted into the genome of hepatocytes of factor IX-deficient hemophilic mice by cotransfection of one plasmid containing the factor IX transcription unit flanked by the inverted repeats and another plasmid expressing the *Sleeping Beauty* transposase [124].

Extracorporeal liver assist devices

The fact that some patients with acute liver failure recover spontaneously led to the idea that patient survival could be improved by providing metabolic support until the liver had had time to regenerate. During the 1960s and early 1970s, ex vivo and in situ cross-circulation with animal and human livers was shown to improve clinical and biochemical parameters in animal models of liver failure and in uncontrolled human trials [125]. However, without the option of transplantation, there was no evidence of increased survival. In the 1980s the life-saving effects of liver transplantation in fulminant hepatic failure were shown, resulting in expanding demand for a finite number of donor organs, increasing the difficulty of obtaining suitable organs in a timely manner. The concept of temporary liver support as a "bridge" to successful liver transplantation emerged. Since coagulopathy, renal failure, encephalopathy, and cerebral edema negatively affect the prognosis after liver transplantation, survival could improve if these complications could be held at bay while waiting for a suitable organ. Liver assist devices have been considered also for use in acute exacerbation of chronic liver failure to alleviate encephalopathy following gastrointestinal bleeding or to prevent hepatorenal syndrome, and for intractable pruritus caused by cholestatic liver disease. Many of these patients are not transplant candidates so the potential for liver assist devices to improve survival is limited.

Many liver support systems are in the development stage but only a few have been tested in clinical series. Although the survival benefit of these devices in patients with acute liver failure is yet to be shown unequivocally in controlled, randomized trials, much progress has been made in the bioengineering of artificial liver support systems. Hepatic support systems comprise three basic groups:

- cell-free liver assist devices
- entirely biological systems, such as extracorporeal liver perfusion
- hybrid bioartificial devices, which include both nonbiological and biological components.

A brief discussion of these devices follows.

Cell-free liver assist devices

To substitute for the detoxification and excretion functions, hemodialysis [126], exchange transfusion [127], and hemoperfusion [128] through charcoal or resins have been attempted during the past 40 years, without consistent beneficial outcomes. Currently, two such systems are available for clinical use, the molecular absorbent recirculating system (MARS) and the Prometheus system. A third, the BioLogic-DT plasma filter system, has been extensively evaluated in patients but is no longer available [129]. In the last of these, the patient plasma comes into contact with a powdered charcoal suspension [128]. Many putative toxins that are thought to contribute to the complications of hepatic failure bind to the charcoal in vitro. Clinical trials have suggested improvement in patients with decompensated chronic liver disease, but studies in acute hepatic failure did not show any benefit over standard clinical management [130].

Of the two systems available for use in patients, there is most experience with MARS (Fig. 96.4). MARS is a cell-free system in which an albumin-enriched dialysate is passed through an anion exchange resin column and a charcoal column, and recirculated through the dialysis unit. This system effectively reduces plasma bilirubin and bile acid concentrations in patients with cholestasis. As standard dialysis is included in the circuitry, some water-soluble substances are also removed [131]. Use in several hundred patients has suggested that treatment with this system may improve encephalopathy scores and the hemodynamic status, thereby increasing urine output in hepatorenal syndrome reducing ascites [129]. Significant improvement in cases of acute-on-chronic decompensation was documented in controlled trials [129]. Patients with fulminant hepatic failure have been bridged to transplantation and cases of primary graft hypofunction have been supported until recovery with the

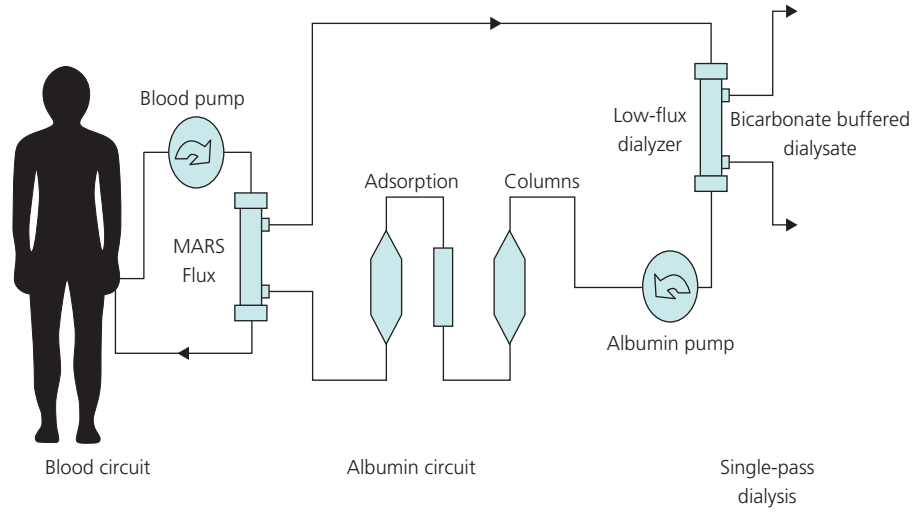

Figure 96.4 The MARS (molecular absorbent recirculating system) unit, a cell-free liver assist device. An albumin-enriched dialysate is passed through a series of absorption columns, including an anion-exchange resin column and a charcoal column, and recirculated through the dialysis unit.

MARS system [132,133]. MARS has also been used in cases of intractable pruritus due to cholestatic liver disease with clear improvement in itching and in some cases, but not all, a durable response, eliminating or reducing symptoms for weeks [134]. Based on a similar principle to MARS there have been case reports of proprietary single-pass albumin dialysis (SPAD) systems where the albumin-enriched dialysate is discarded rather than "scrubbed" and recycled [135].

The Prometheus system relies on fractionated plasma separation (filter cut-off 250 kDa) and passage of the patients' plasma proteins (mostly albumin) through two adsorption columns of neutral resin adsorber followed by an anion exchange resin [136,137]. The cleansed plasma is then returned to the primary dialysis circuit. The blood then passes through a high-flux dialyzer before being returned to the patient. Similar to previous systems, Prometheus has been demonstrated to remove putative toxins, both protein-bound and water-soluble, that accumulate in hepatic failure [138,139]. In comparative studies with MARS, Prometheus showed higher efficiency for removal of urea and bilirubin and similar efficiency for bile acid clearance [140,141]; however, MARS improved the hyperdynamic circulatory changes seen in some patients whereas Prometheus did not [142]. Safety profiles were comparable between the two systems.

Although these results are encouraging, the cell-free plasma detoxification systems cannot substitute for the metabolic and synthetic functions of the liver. Therefore, other liver support systems including liver tissues or liver-derived cells are being developed.

Extracorporeal liver perfusion

Otto and colleagues in 1958 demonstrated that allogeneic extracorporeal liver perfusion could reduce plasma ammonia in hyperammonemic dogs [143]. Eiseman and colleagues [144] were the first to apply this concept in patients using both human and xenogeneic livers. By 1994, 87 cases treated with extracorporeal perfusion using porcine liver or human cadaver livers had been reported [145]. At the University of Nebraska, 14 patients received extracorporeal liver perfusion using eight human and eight pig livers. Of the 10 patients who were considered suitable for transplantation, nine were "bridged" to liver transplantation [146]. In one study, livers from transgenic pigs expressing human CD55 and CD59 to limit hyperacute rejection were used for the management of two patients with fulminant hepatic failure [147]. Although controlled trials of extracorporeal liver perfusion have not been carried out, the complete recovery of three patients and the prolonged survival of some patients suggest improvement over conventional treatment. However, even the best results using extracorporeal liver perfusion are not equal to that of liver transplantation. Membranes, filters, and tubings included in the circuitry may activate leukocytes, leading to cytokine release. Since immunosuppression is avoided to reduce the risk of sepsis, humoral and cellular rejection can occur. Finally, perfusion of the extracorporeal organ with systemic, rather than the portal blood may reduce hepatic function. Logistic hurdles to extracorporeal perfusion include the limited availability of human donor organs and the difficulty of procuring porcine livers, unless an institution maintains its own colony. Concerns over safety and efficacy of porcine organs persist.

Hybrid bioartificial liver support devices

Standardized machinery, including bioreactors containing metabolically active hepatocytes, has been in development over the last 20 years. Initially, Matsumara used a device containing liver cell suspension to treat one patient [148], but it is recognized that hepatocytes function more effectively when anchored to a surface. In a clinical trial on patients with acute liver failure, perfusion of patient plasma over porcine hepatocytes attached to Biosilon microcarriers, 37 of the 59 patients recovered, but only 25% of those with

grades 3 and 4 encephalopathy survived [149]. The hepatocytes lost function after a few hours of application. Other systems that use hepatocytes cultured onto stacked flat surfaces [150] or three-dimensional structures [151] have been designed but not yet tested in clinical studies. The largest clinical experience is with systems consisting of a large number of cellulose-acetate hollow fibers packaged into plastic cartridges. The intralumenal and the extralumenal compartments can be perfused independently. In all devices undergoing clinical trial, the intralumenal compartment is used for perfusion of blood or plasma, and the hepatocytes are inoculated into the extralumenal compartment, although Nyberg and colleagues have designed an experimental system in which gel-entrapped hepatocytes are packed within the intralumenal compartment [152].

Sussman and colleagues employed C3A cells, a human hepatoblastoma-derived cell line, grown to confluence in the extralumenal compartment of hollow fiber cartridges in their Extracorporeal Liver-Assist Device (ELAD) [153]. Whole blood from patients was perfused through the intralumenal compartment. Although initial uncontrolled observations in 11 patients with acute hepatic necrosis were encouraging, a pilot control trial demonstrated no difference in outcome compared to a control group receiving conventional supportive therapy [154]. In this study, the survival rate was surprisingly high in both the treatment (78%) and the control (75%) groups, probably because of the inclusion of patients with early stages of acute liver failure.

Demetriou and associates used cryopreserved primary pig hepatocytes, attached to collagen-coated microcarriers in their device, the Bioartificial Liver (BAL) also known as HepatAssist (Fig. 96.5) [155]. In this device, hepatocytes are introduced into the extralumenal compartment of hollow fiber cartridges and the intralumenal compartment is perfused with patient plasma that has been previously circulated through a charcoal column to remove toxins in the "hepatic failure" plasma. Each cartridge functions for 6 h, but the course may be repeated several times. In 2004 the results of a randomized multicentered controlled trial using this device in acute liver failure were published [156]. A total of 171 patients (86 controls and 85 BAL) were enrolled at 11 US and nine European centers. All patient had fulminant or subfulminant hepatic failure (FHF/SHF) or primary nonfunction (PNF) following transplantation and all had advanced encephalopathy, grades III or IV. Thirty-day survival was not significantly different between the treated and control groups but using a time-dependent proportional hazard model to control for the effect of liver transplantation, it was shown that the mortality of FHF/SHF (excluding the PNF patients) was almost halved for the BAL treatment group ($P = 0.048$).

A third hybrid hollow fiber system, Modular Extracorporeal Liver Support (MELS), uses woven polyether-sulphone capillaries that exit from the bioreactor in discrete bundles, so that different bundles can serve separate functions such as plasma flow and oxygenation [157]. The three-dimensional structure is claimed to improve the function of the cultured hepatocytes. CellModule bioreactors containing primary porcine hepatocytes were used to treat eight patients with liver failure, all of whom were carried through to successful transplantation [158]. Primary human hepatocytes obtained from cadaver donor organs were used, leading to encouraging results in 12 patients with acute failure [159]. Several other hybrid hepatic support systems have been used in preliminary human studies of hepatic failure [160–163].

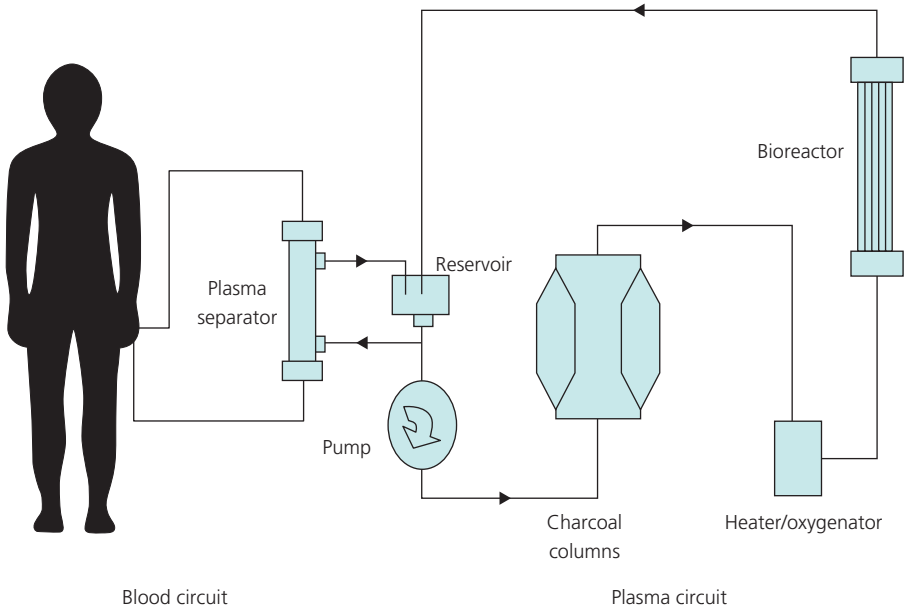

Figure 96.5 The HepatAssist device, example of a hybrid liver assist device. Plasma is separated from blood cells and passed through a charcoal column being perfused through the intralumenal compartment of a hollow fiber bioreactor. The extralumenal space of the bioreactor is packed with cryopreserved primary pig hepatocytes, attached to collagen-coated microcarriers.

Hurdles that need to be overcome for the development of extracorporeal liver support

Currently, porcine livers are the main source of the biological component of artificial liver devices. Limitations of using xenogeneic sources include hyperacute or delayed immune response to the cells or their products, and concerns regarding the presence of infectious organisms, such as the porcine endogenous retrovirus. The use of human hepatocytes would be logical, but is limited by donor organ shortage. Therefore, more progress is needed for generating human hepatocytes that can be expanded in vitro. The potential sources, including embryonic stem cells, hepatocyte progenitors, fetal hepatocytes, and genetically engineered immortalized hepatocytes, are being explored actively.

Preclinical evaluation of extracorporeal liver assist devices has been limited by the lack of animal models of fulminant hepatic failure that fully represent the clinical course of acute liver failure in patients. The relatively low incidence of acute liver failure makes it difficult to perform a detailed clinical evaluation of a hepatic support system at any one center, on the other hand, multicenter trials add to the variables to be considered in the trial design. The wide spectra of etiology, severity at presentation, and rapidity of progression of acute liver failure complicate the standardization of entry criteria and randomization. The level of encephalopathy in patients is difficult to quantify. Since liver transplantation is known to improve survival fulminant hepatic failure, the extracorporeal hepatic support may be applied only until a donor organ becomes available, making the data analysis difficult. However, even if the present generation of devices does not prove sufficiently efficacious, the experience gained will undoubtedly help in the future development of an effective extracorporeal liver support.

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G LIVER

97 Liver biopsy and histopathological diagnosis

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This chapter describes the morphological changes of various disease processes that are relevant in attempting to interpret a needle biopsy of the liver. Attention is drawn to the changes that are most important in analyzing a liver biopsy to arrive at a diagnosis and differential diagnoses. Sections in other chapters deal with more detailed analysis of these disease processes.

The first step in liver biopsy interpretation is recognition of the lobular architecture differentiating normal from fibrosis, cirrhosis, and noncirrhotic nodular regeneration. Systematic analysis of the hepatic lobule starts with the examination of the portal tract, followed by terminal hepatic veins, and the assessment of the parenchyma and the sinusoidal spaces. Based on this analysis, the following differentiations are made:

- acute versus chronic
- parenchymal versus biliary disease
- possible etiological factors
- if neoplastic, benign versus malignant, and primary versus metastatic.

Technique of liver biopsy

Obtaining liver tissue for histological examination has been useful for diagnostic and prognostic purposes since the 19th century [1]. Several approaches can be taken, using a number of available biopsy needles, each with specific advantages and shortcomings.

The most common method of liver biopsy is transcutaneous (transthoracic or subcostal) with or without ultrasound or computed tomography (CT) guidance [2,3]. Occasionally, the clinical circumstance prevents the transcutaneous route and alternative methods should be considered. The most common of these situations are summarized in Table 97.1. Alternatively, patients with coagulopathy can be biopsied transcutaneously after infusion of platelets and fresh frozen plasma [3,4]. Patients with inherited bleeding disorders such as hemophiliacs can be biopsied transcutaneously, provided they are infused with the appropriate coagulation factors and are followed by experienced hematologists during the peri-biopsy period [5].

Table 97.1 Reasons for avoiding the transcutaneous liver biopsy route

Clinical situation	*n*	Percentage of patients
Ascites	37	9
Abnormal coagulation	213	52
Ascites + coagulopathy	81	20
End-stage renal disease	18	4
Obesity	20	5

From Smith et al. [4].

The transjugular route appears superior in yield and safer than percutaneous biopsy in patients with renal disease undergoing hemodialysis [6].

Needle types

Needles used for liver sampling rely either on suction (Menghini, Klatskin, and Jamshidi) or cutting technique (Tru-Cut and Vim Silverman) [3]. The latter technique is applied with a spring-load mechanism (Quick-core, Cook Medical, Bloomington, IN; BioPince, Angiotech Medical, Denmark; or INRAD, SE Kentwood, MI) that allows a specific length of tissue, usually 15–20 mm, to be retrieved with a minimum of needle manipulation in the liver parenchyma [7]. In cirrhotic

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Table 97.2 Advantages and disadvantages of different biopsy needles

Biopsy needle	Size (gauge)	Advantages	Disadvantages
Jamshidi	17	Long cores with minimal needle insertion	Fragmented tissue with increased fibrosis
Tru-Cut	16	Rare fragmentation, large core	17–20 mm maximum length in one pass, less tissue than other needles
Inrad, BioPince	16 or 18	Rare fragmentation, large core, adjustable throw up to 34 mm length with one pass	Long throw, more procedural pain

From deMan et al. [23].

livers, cutting techniques result in less fragmented specimens than the suction technique (Table 97.2). Most percutaneous liver biopsies result in the retrieval of sufficient liver tissue for diagnosis (see below) after a single pass, but more than one pass is required in 4%–12% of patients [2,8]. Although in our institution, the intercostal approach has been preferred, the subcostal approach with an 18G needle has been shown to result in adequate samples with similar frequency of adverse events [9].

Imaging for liver biopsy

The use of real-time imaging (ultrasound being more cost-effective than CT) or ultrasound X-marking of the entry site prior to biopsy has significantly decreased the percentage of patients who experience pain requiring analgesia (50%–35%) [2,3,8] as well as the chance of hospitalization [2]. A decrease in mortality has not been shown [2,8]. but the sample size required to show a difference would be exceedingly large.

Surgical approaches

Laparoscopic liver biopsy may be indicated in patients with ascites as hemostasis is carried out after liver sampling by the surgeon. The technique has the advantage of offering visual inspection of the liver surface and biopsy of focal lesions [10]. One study suggested that the diagnosis of cirrhosis was increased with minilaparoscopic biopsy, owing to the visual diagnosis of a nodular liver surface [10].

Percutaneous approach

Liver biopsy is usually performed with an unsedated patient given a local anesthetic injection at the site of needle entry [11]. In our institution, oral or sublingual temazepam is administered at the patient's request. Some physicians administer intravenous midazolam prior to biopsy. The patient is marked by ultrasound, either at the bedside or in the imaging department, where a mark is made in deep expiration. We ask the technician to indicate the angle of entry to avoid large vessels and gallbladder. After the procedure approximately 50% of patients have some epigastric or right shoulder pain [11]. Oral or intravenous analgesia is then administered. A history of intravenous drug abuse (IVDA) has been associated with pain medication use [11]. Typically 98%–99% of patients can be discharged after a 3-h observation. Some radiology departments reimage the patient after the biopsy and if no hematoma is noted and the patient has no or minimal symptoms, discharge is carried out after 1 h [12].

Adequacy of specimen

Failure to obtain tissue occurs in 1%–3% of biopsies [11]. In addition, several studies have addressed the issue of the appropriate biopsy length for an accurate diagnosis. Typically a biopsy length of less than 10 mm and with less than six portal tracts is considered inadequate, although this depends on the indication [1]. For example, it may be possible to diagnose acute cellular rejection with a small biopsy but not to stage fibrosis accurately. At the other end of the spectrum a length greater than 25 mm and including at least 11 portal areas is usually considered adequate [1]. Little has been written about the needle gauge; a 14G needle is likely to retrieve a thicker specimen with conceivably a better assessment of fibrosis stage. But a larger needle is also more likely to be associated with more complications.

Complications

The estimated percentage of patients that need admission after a liver biopsy is approximately 2%. The most common causes are severe pain (2%), hemoperitoneum (0.04%), hemobilia (0.01%), and sepsis (0.01%) [13,14]. Hemo-, pneumo-, or hydrothorax was reported in 0.07% of cases [13]. Perforations of other organs such as gallbladder, lung, colon, kidney, or adrenal gland have all been described [2,8,13]. Pancreatic tissue has been observed together with liver tissue.

Biopsy of malignant liver masses is associated with a small but significant probability of seeding the track with neoplastic cells: the percentage varied from 1% in hepatocellular cancer (HCC) to 19% with colorectal metastasis [15,16]. The vast majority of complications occur within the first 3 h after biopsy, so a 3–6-h monitoring period is usually carried out, with a friend or family member to take charge of the patient for 24 h [14]. A study describing 629 high-risk patients,

including HIV infection [17], reported an unusually high (0.5%) mortality rate after liver biopsy. The finding of delayed bleeding (> 24 h) in 70% of patients who bled was also unusually high. Death has been reported in 1 per 1000–10 000 cases, and basically is always due to massive hemoperitoneum, and most often associated with cirrhosis or malignancy [13,18]. The risk of bleeding appears to increase with the number of passes, associated severe systemic disease including infections, and the use of heparin or corticosteroids [17]. Typically only two passes are customarily allowed [18]. There is little difference in the complication rates that occur with trainees versus attending physicians [11,14,18,]. However, the former tend to retrieve less satisfactory specimens compared with the latter [18]. In the radiological literature, experience with ultrasound-guided percutaneous liver biopsy was not a factor regarding specimen adequacy or complications [19]. There are some differences in specimen adequacy and complications with suction, cutting, or spring-load techniques [2,8]. The impact of a lower needle gauge (14G vs 16 or 18G) or the use of "plugging" devices on the risk of complications is unsettled [3].

Alternatives to liver biopsy

Two main approaches have been taken to evaluate fibrosis (and inflammation) in the hepatic parenchyma. The first is by modeling of blood tests that estimate fibrosis; some are easily obtained (AST/platelets ratio [APRI], Forns, Fib-4, cirrhosis discriminant score [CDS]) [20]. Others are proprietary and cost about $200 per test. These tests have a far better predictive value for lower fibrosis levels and, depending on the cutoff used, may only save liver biopsy in 20%–25% patients with hepatitis C [21]. Further validation needs to be obtained in other types of liver disease. The other approach is to estimate fibrosis by assessing hepatic stiffness using an ultrasound wave. The results are integrated with a Fibroscan (Echosens, Paris) machine. A study noted a negative predictive value of 99% for cirrhosis when the liver stiffness was less than 9.2 kPa in patients with hepatitis B and C and alcoholic and nonalcoholic liver diseases [22]. Unfortunately, prediction of earlier stages was not reported. Thus it appears that with a few exceptions, liver biopsy will remain the most accurate, although not perfect, way to assess necrosis, inflammation, and fibrosis of the liver.

Processing of liver biopsy tissues

Fixation and processing techniques vary from laboratory to laboratory; however, the most commonly used fixative is 10% buffered formalin. For crisp nuclear details and rapid fixation, we use B5 solution containing 6 g of mercuric chloride and 2.074 g of hydrated sodium acetate dissolved in distilled water and mixed with 10 mL of 40% formaldehyde (pH 5.8) at time of use. Embedding in plastic resins such as Araldite, compared with paraffin embedding, improves the ability to obtain thin (2–3 μm) sections with greater cytological details, such as cellular mitochondria or microvesicular fat [24]. Besides regular hematoxylin and eosin (H & E) stains, the most useful routine stains are Masson trichrome for collagen (Fig. 97.1), periodic acid–Schiff (PAS) for glucose, diastase-digested PAS for glycoprotein (i.e., α_1-antitrypsin) (Fig. 97.2), iron stain (Fig. 97.3), Shikata orcein stain for hepatitis B surface antigen (HBsAg) (Fig. 97.4), and copper-binding proteins (Fig. 97.5) and reticulin stain for identification of cord sinusoidal structures. Other special stains that are helpful in diagnosis are those for acid-fast and fungal organisms

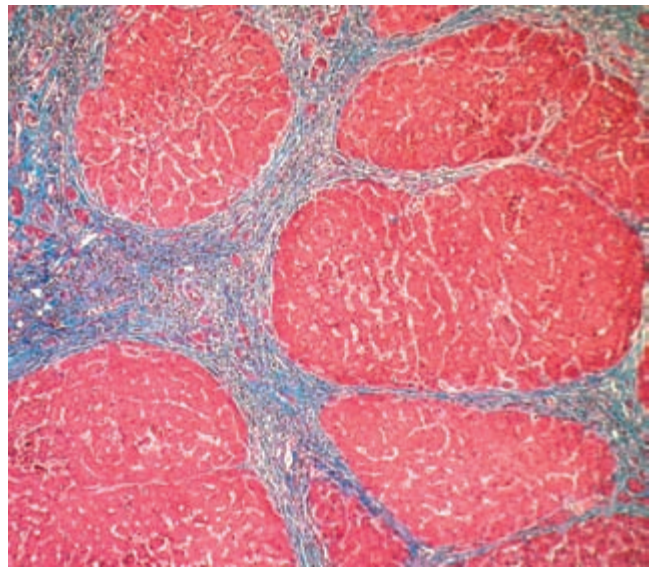

Figure 97.1 Cirrhosis of liver with fibrous septa and regenerative nodules. (Masson stain; original magnification × 40.)

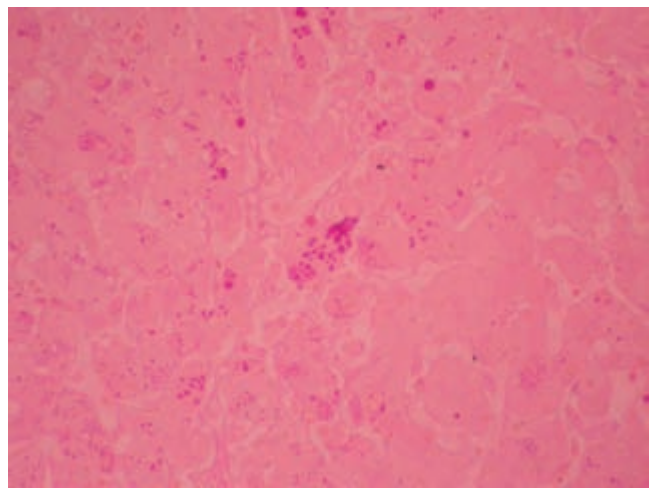

Figure 97.2 α_1-Antitrypsin globules in the periportal hepatocytes – diastase-resistant, PAS-positive. (Di-PAS stain; original magnification × 200.)

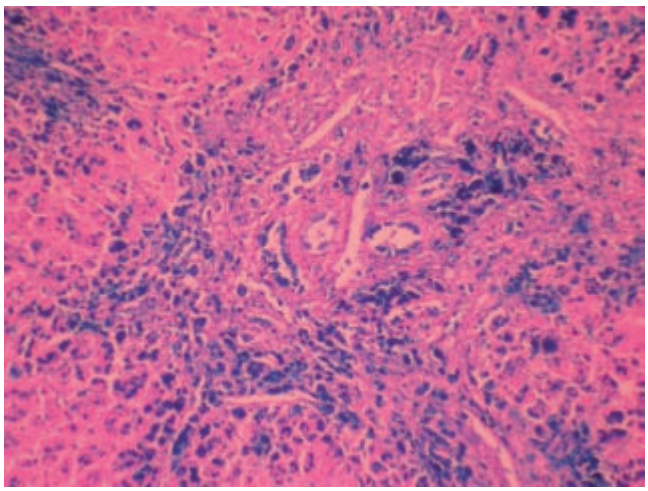

Figure 97.3 Perls iron stain demonstrating bright-blue granules in hepatocytes and duct epithelial cells in hemochromatosis. (Original magnification × 100.)

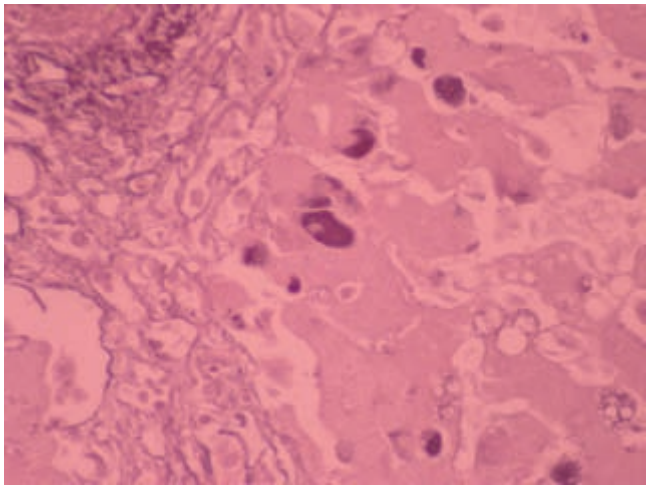

Figure 97.4 Shikata stain demonstrating the presence of HBsAg in the hepatocytes in chronic hepatitis B virus. (Original magnification × 200.)

in cases of granulomas; phosphotungstic acid–hematoxylin for fibrin in disseminated intravascular coagulation or necrosis in cases of Q fever; rubeanic acid for copper; and immunoperoxidase stains for viral proteins, α_1-antitrypsin, α-fetoprotein, factor VIII, and others.

Special requirements

Frozen sections of fresh or fixed tissue are needed to demonstrate fat with oil red "O" stain in cases of Reye syndrome, fatty liver of pregnancy, and fat-soluble vitamin A in Ito cells in hypervitaminosis A. For quantitative analysis of hepatic copper or iron, a part of the tissue needs to be saved either fixed or fresh frozen prior to processing for paraffin embedding. Electron microscopic examination is not a routine necessity and is used for special studies.

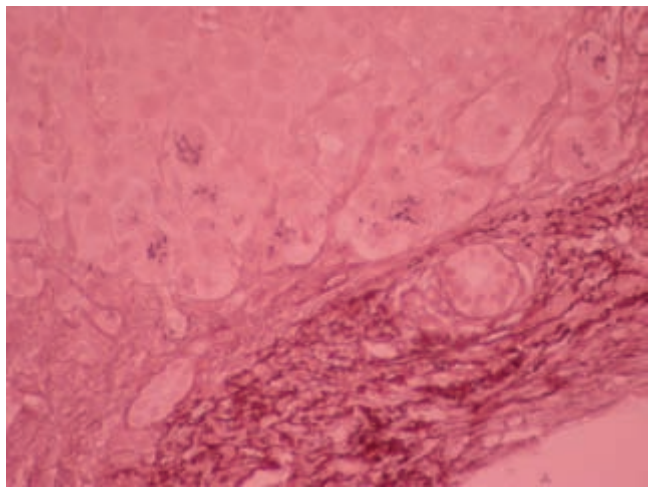

Figure 97.5 Shikata stain demonstrating dark-black granules of copper-binding protein in periseptal hepatocytes in Wilson disease. (Original magnification × 200.)

Systematic approach to the review of liver biopsies

The biopsy section should be reviewed on a low-power field to screen all fragments present in the section. At this time, an evaluation of the overall architecture is made. Regularly placed portal tracts and hepatic venules with their proper relationship constitute normal architecture. The portal areas can be expanded with fibrosis. When there are fibrous septa from portal to portal or to perivenular areas with formation of regenerative nodules, a diagnosis of cirrhosis is made (Fig. 97.1). In nodular regenerative hyperplasia, the parenchyma exhibits regenerative nodules compressing the adjacent hepatic cords without fibrous tissue around them (Fig. 97.6).

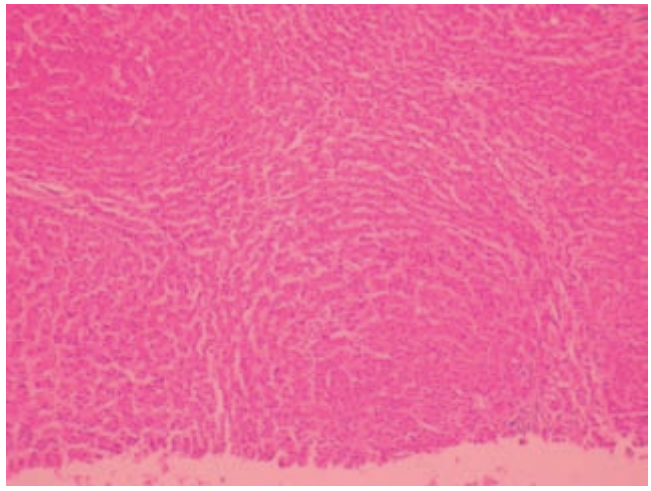

Figure 97.6 Nodular regenerative hyperplasia demonstrating regeneration of parenchyma compressing the surrounding parenchyma without fibrous septa formation. (H & E stain; original magnification × 40.)

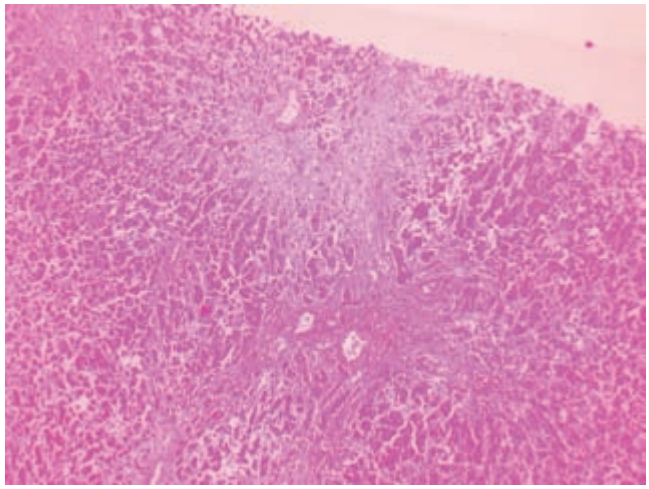

Figure 97.7 Submassive hepatic necrosis with collapsed perivenular reticulum network. (H & E stain; original magnification × 40.)

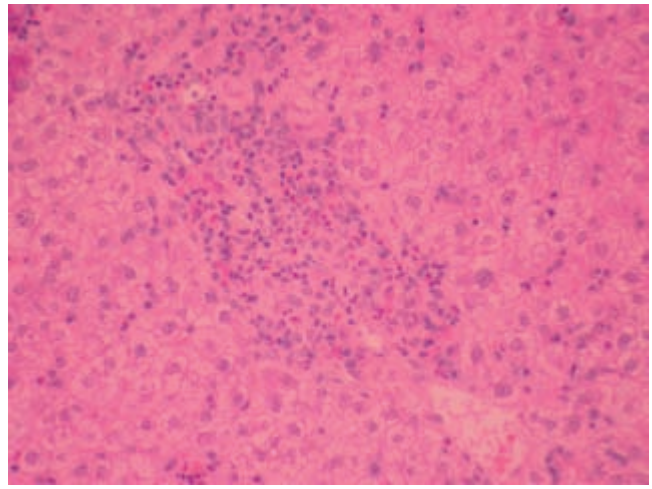

Figure 97.9 Portal area with prominent eosinophils among the inflammatory infiltrates in a case of Dilantin-induced hepatotoxicity. (H & E stain; original magnification × 200.)

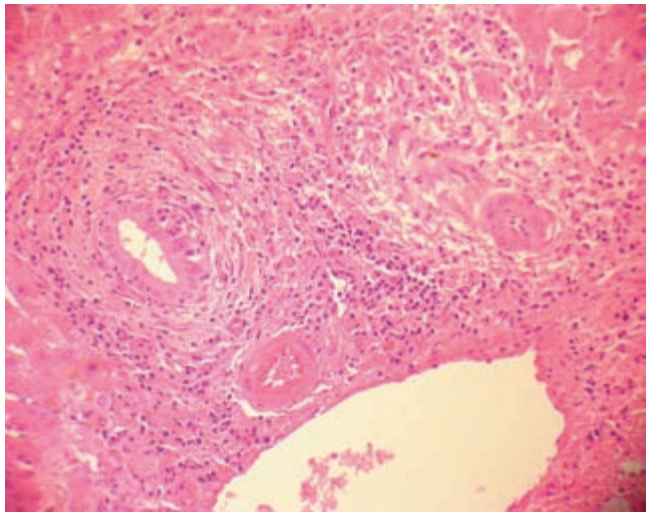

Figure 97.8 Portal area with prominent neutrophils in close proximity to the dilated interlobular bile duct in acute cholangitis. (H & E stain; original magnification × 100.)

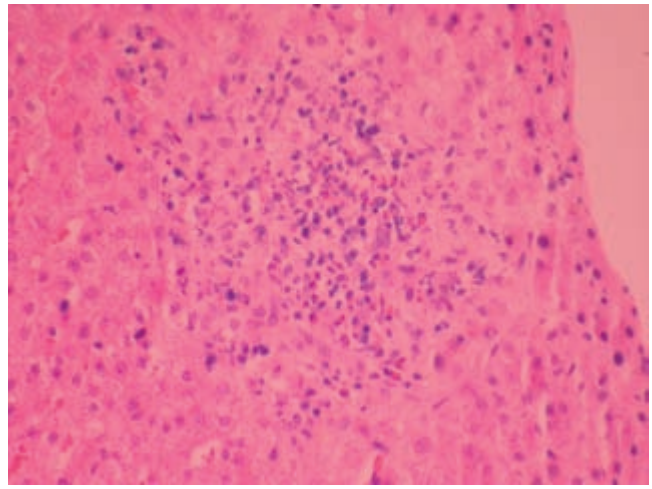

Figure 97.10 Portal area with increased number of eosinophils in a case of early rejection of orthotopic liver transplantation. (H & E stain; original magnification × 200.)

Collapsed reticulin fibers can be differentiated from true fibrosis by reticulin and trichrome stains. When there is confluent or submassive liver necrosis, there is loss of hepatocytes from hepatic cords resulting in a collapsed or compressed reticulum network. If this occurs in the perivenular region, it can mimic fibrosis (Fig. 97.7). Immediately following the overall assessment of the hepatic architecture, special attention is paid to each of the elements of the hepatic lobule.

Portal areas

Enlargement of the portal tracts can be due to either cellular infiltration or fibrosis. Cellular infiltrate can be of inflammatory reactive changes, such as in a variety of viral, drug-induced, or autoimmune types of hepatitis, or neoplastic infiltrate, such as lymphomas. Inflammatory infiltrates are further categorized as acute with neutrophils, usually seen in acute cholangitis with orientation around the dilated ducts due to biliary obstruction (Fig. 97.8) or diseases such as oriental cholangiohepatitis. Predominantly eosinophilic infiltrates, not oriented to the ducts, can be seen in allergic drug reactions (Fig. 97.9), parasitic diseases, and acute rejection following liver transplantation (Fig. 97.10). Among the inflammatory infiltrates of mononuclear type, mainly plasma cells are seen in autoimmune chronic hepatitis (Fig. 97.11), mixed mononuclear cells are seen in viral-induced chronic hepatitis, and cells oriented around the bile ducts are seen in primary

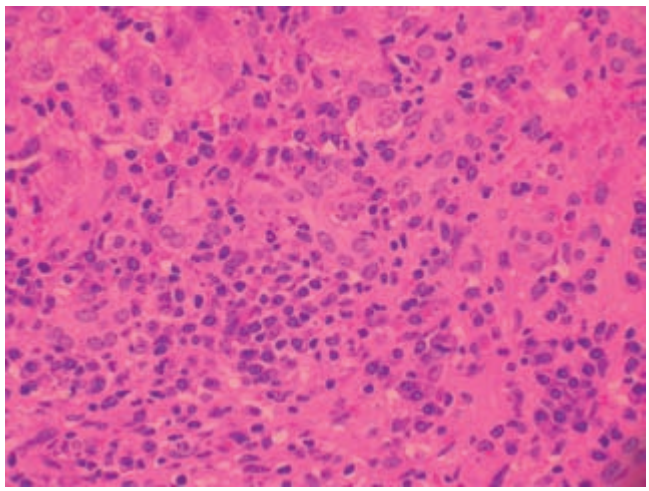

Figure 97.11 Prominent plasma cells among the infiltrates in the portal tract of autoimmune chronic hepatitis. (H & E stain; original magnification × 400.)

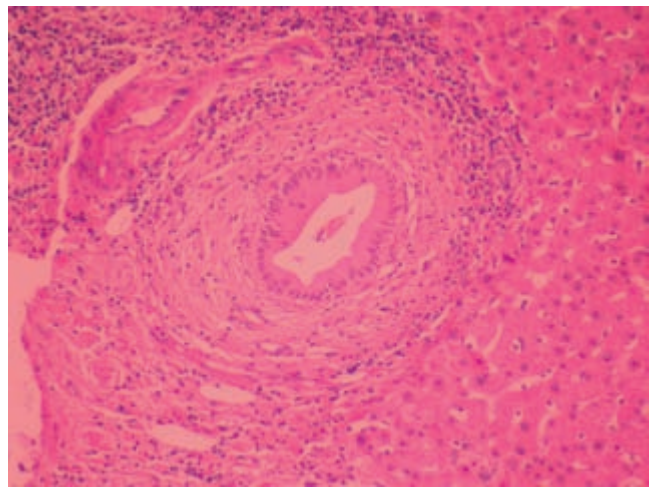

Figure 97.13 Lamellar periductal fibrosis in chronic bile duct obstruction. (H & E stain; original magnification × 100.)

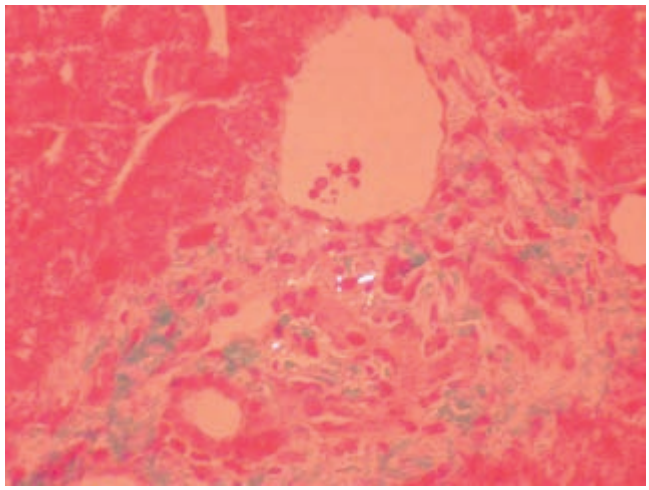

Figure 97.12 A portal area under polarizing light to demonstrate polarizable crystals in an intravenous drug user. (H & E stain; original magnification × 200.)

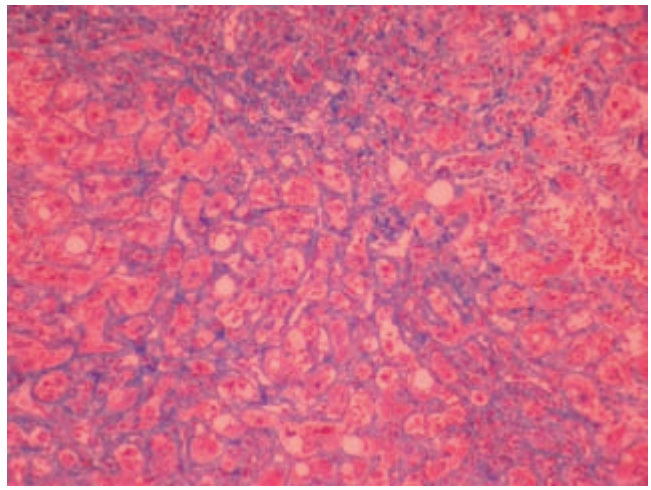

Figure 97.14 Arachnoid portal fibrosis with periportal extension of collagen in chronic alcoholic liver disease. (Masson trichrome stain; original magnification × 100.)

biliary cirrhosis. Portal tracts may contain granulomas due to various etiologies, as well as hypertrophic macrophages with phagocytosed pigments, such as iron or foreign material. Polarizable talc crystals are often seen in intravenous drug abusers (Fig. 97.12). The pattern of portal fibrosis varies with the type of disease. For example, in chronic biliary tract diseases the fibrosis is often periductal, concentric, and lamellar (Fig. 97.13) in nature, whereas in chronic alcoholic liver disease it is arachnoid with irregular extension into the parenchyma (Fig. 97.14). Bile ducts are involved in acute as well as chronic disease processes. In acute cholangitis, usually due to mechanical obstruction, there is dilation of the duct with periductal edema and polymorphonuclear leukocytes infiltrating the duct epithelium and within the duct lumina (Fig. 97.8). In chronic biliary obstruction there is bile duct proliferation and biliary fibrosis with periductal orientation (Fig. 97.15). Periductal concentric fibrosis is also seen in sclerosing cholangitis (Fig. 97.16). There is a chronic inflammatory reaction involving the duct epithelium in the early stages of primary biliary cirrhosis (PBC) (Fig. 97.17) and, in the late stage, the ducts are absent. Similar changes are seen in chronic rejection of a liver transplant. Anomalous duct changes include Meyenburg complexes (Fig. 97.18) and cystic disease such as Caroli disease. Paucity of bile ducts is seen in intrahepatic biliary atresia, whereas biliary fibrosis and bile duct proliferation are seen in extrahepatic biliary

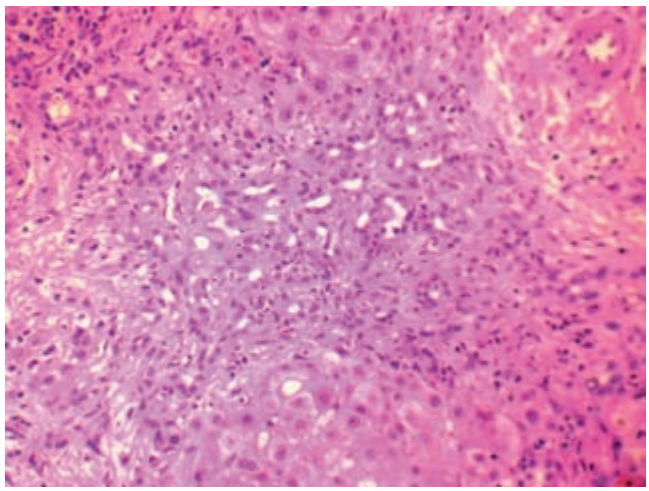

Figure 97.15 Portal area with marked cholangiolar proliferation in mechanical duct obstruction. (H & E stain; original magnification × 100.)

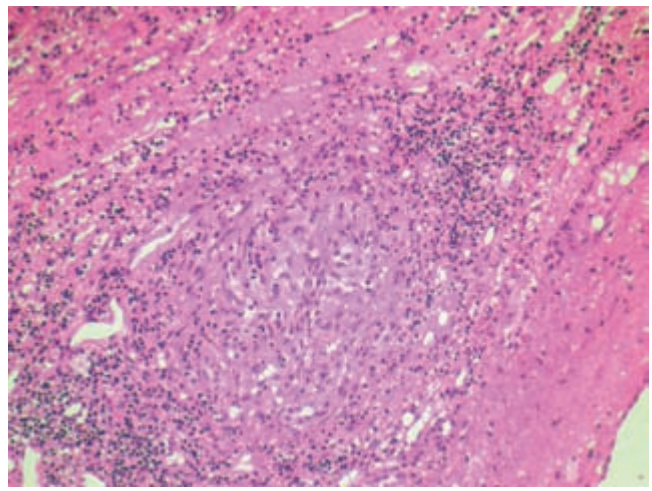

Figure 97.17 Primary biliary cirrhosis with granuloma. (Original magnification × 200.)

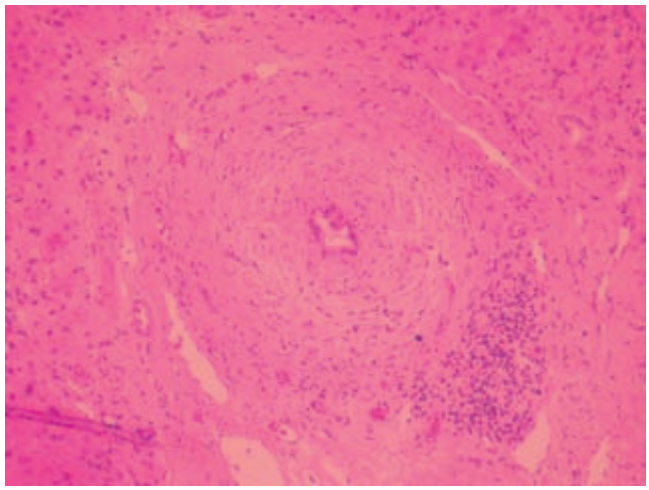

Figure 97.16 Primary sclerosing cholangitis with evidence of periductal fibrosis and chronic inflammatory infiltrate. (H & E stain; original magnification × 100.)

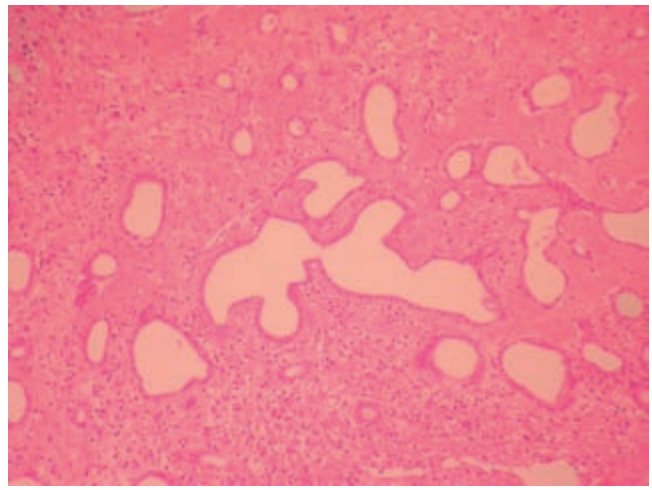

Figure 97.18 A few dilated duct structures with abnormal epithelium surrounded by loose collagen representing Meyenburg complex. (H & E stain; original magnification × 100.)

atresia (Fig. 97.19). Portal veins are thin-walled vascular structures, and are increased in number and dilated in portal hypertension (Fig. 97.20). There is inflammation and thrombosis of portal veins in pyelophlebitis. Primary or metastatic tumors can be seen in the portal venous structures. The hepatic artery can reveal hyalinization in diabetics. Polyarteritis nodosa and giant cell arteritis can produce a typical inflammatory reaction involving the arterial wall (Fig. 97.21). There is arterial hypoplasia in congenital hepatic fibrosis. In Osler–Weber–Rendu syndrome there is an increase in abnormal vascular channels suggestive of arteriovenous shunting (Fig. 97.22). There is deposition of amyloid on the arterial wall in systemic amyloidosis.

Terminal hepatic venules

Terminal hepatic venules are thin, endothelial-lined vascular spaces without collagen deposition. There is perivenular fibrosis in most types of alcoholic liver disease (ALD) (Fig. 97.23). Endophlebitis with inflammatory reaction of terminal hepatic venules is seen in acute rejection of liver transplants (Fig. 97.24). In Budd–Chiari syndrome, there is obliteration of the terminal hepatic veins along with hemorrhagic necrosis of the perivenular hepatocytes and dilation of sinusoids (Fig. 97.25).

Hepatic parenchyma

The hepatic parenchyma should be evaluated with orientation to the three zones of Rappaport. Zone 3, or the perivenular

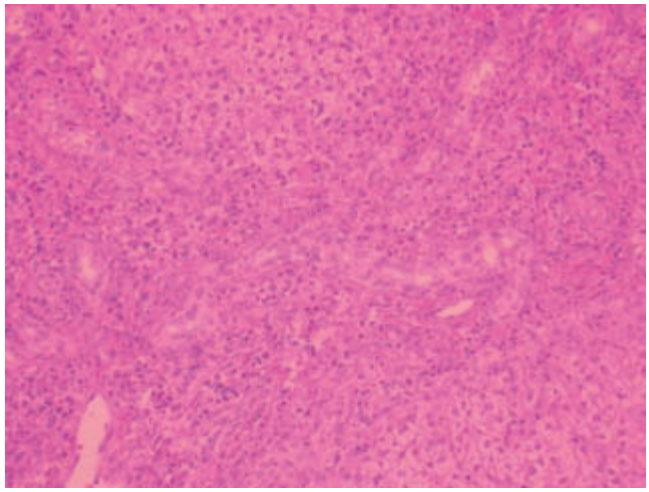

Figure 97.19 Biliary fibrosis and ductular proliferation in a 3-month-old infant with extrahepatic biliary atresia. (H & E stain; original magnification × 100.)

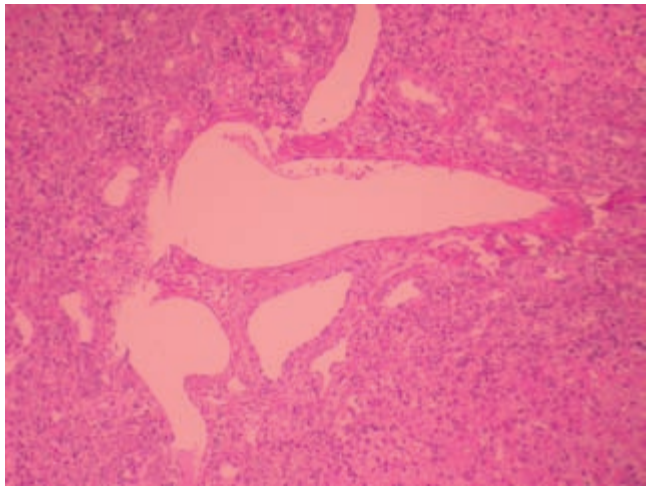

Figure 97.20 Increased number of thin-walled vascular structures representing portal venous radicles reflective of portal hypertension. (H & E stain; original magnification × 100.)

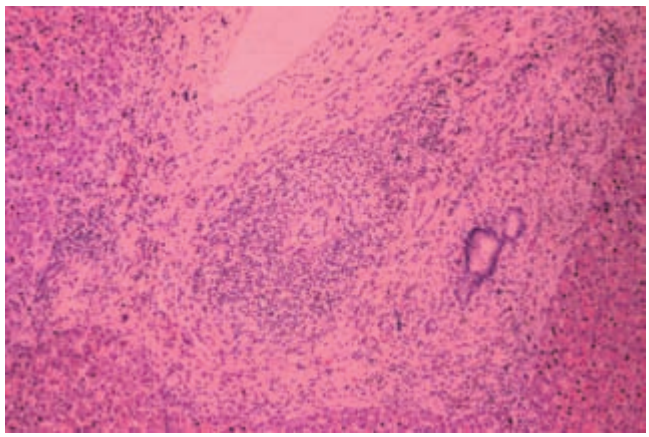

Figure 97.21 Severe necrotizing inflammatory reaction around hepatic arteriole in polyarteritis nodosa. (H & E stain; original magnification × 100.)

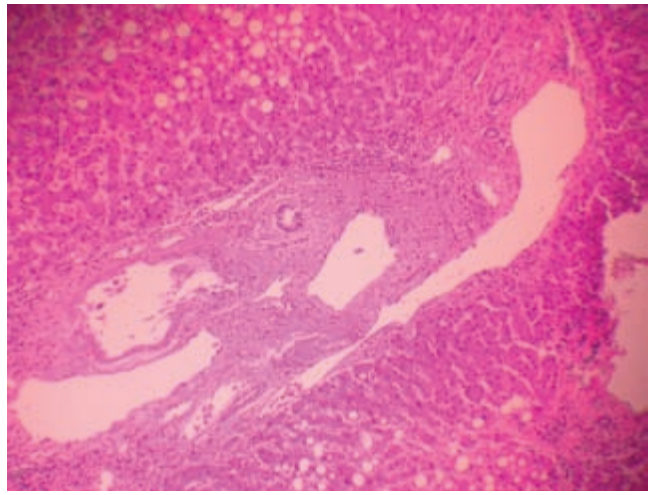

Figure 97.22 Increased number of abnormal vascular structures in a portal tract in Osler–Weber–Rendu syndrome. (H & E stain; original magnification × 100.)

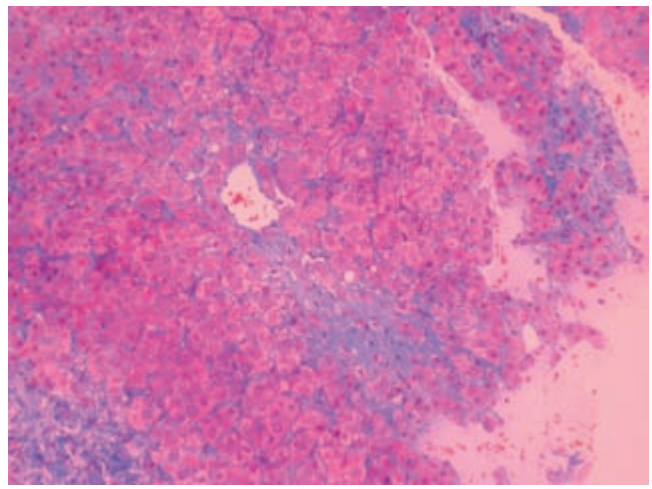

Figure 97.23 Marked perivenular fibrosis in alcoholic liver disease. (Masson trichrome stain; original magnification × 100.)

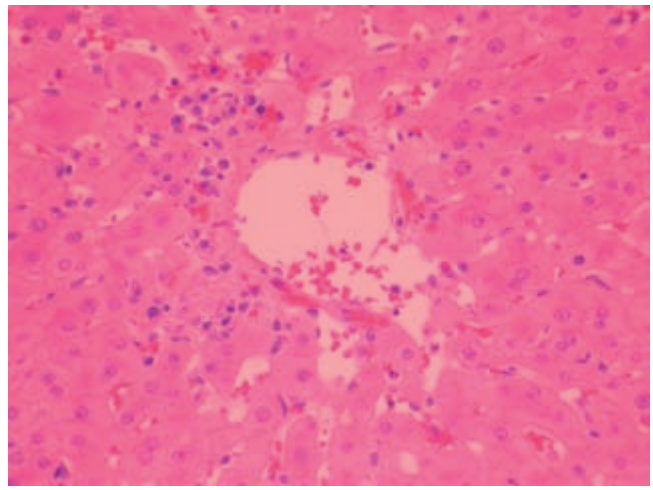

Figure 97.24 Endothelialitis showing inflammatory changes of a terminal hepatic venule in acute rejection of orthotopic liver transplantation. (H & E stain; original magnification × 200.)

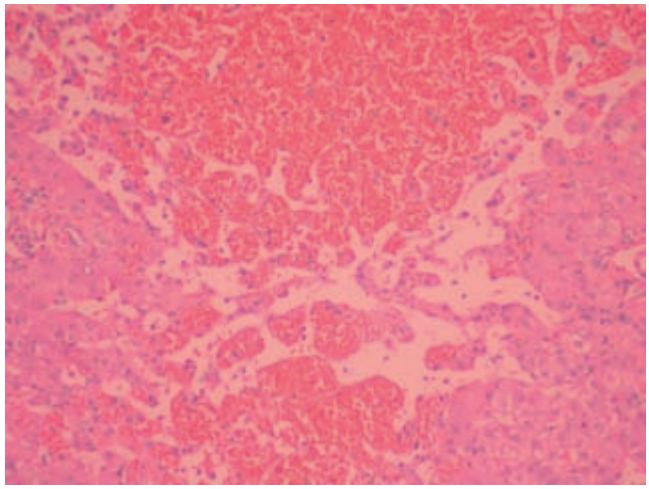

Figure 97.25 Budd–Chiari syndrome with perivenular hemorrhage, necrosis, and sinusoidal dilation. (H & E stain; original magnification × 200.)

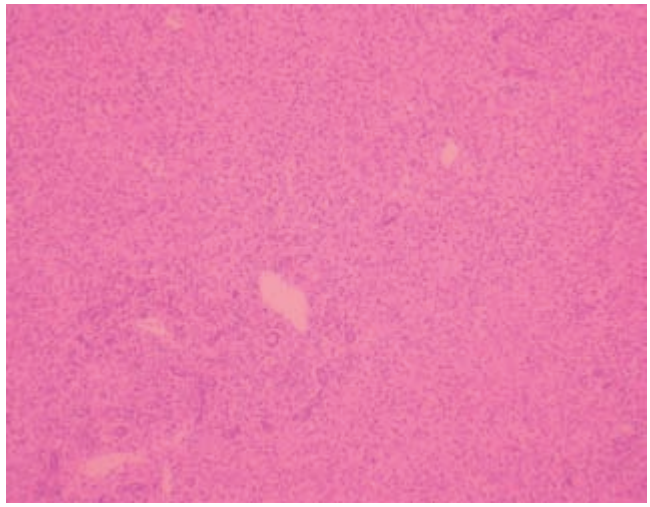

Figure 97.27 Massive hepatic necrosis involving the entire parenchyma with islands of portal tracts remaining. (H & E stain; original magnification × 40.)

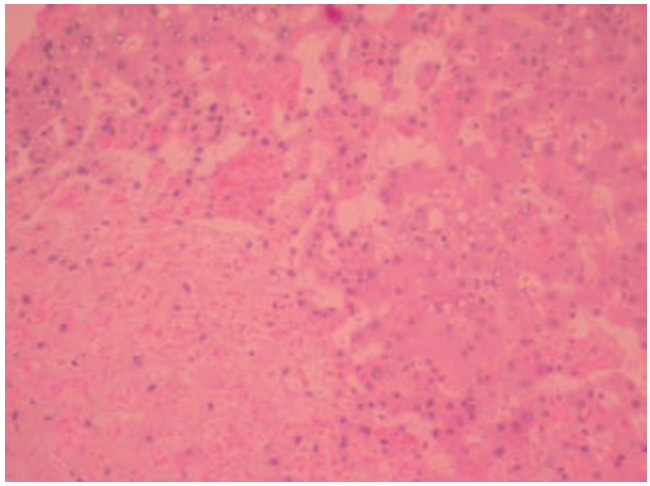

Figure 97.26 Confluent necrosis in the perivenular zone due to anoxia. (H & E stain; original magnification × 200.)

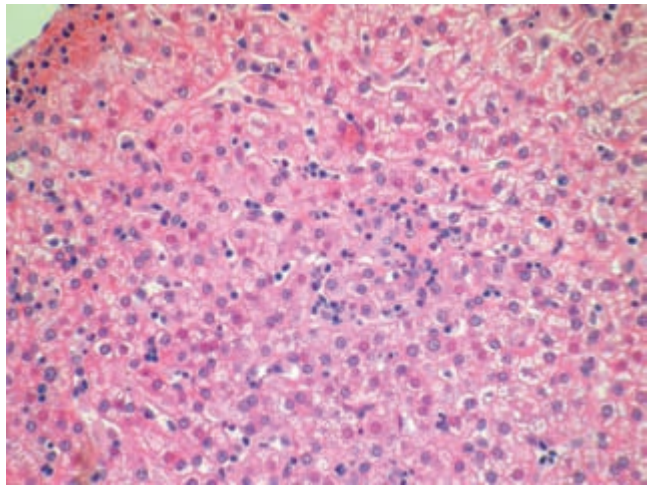

Figure 97.28 Punched-out granulomatous necrosis of the parenchyma in mononucleosis due to Epstein–Barr virus. (H & E stain; original magnification × 200.)

zone, is most susceptible to anoxic changes and most drug-induced liver necrosis occurs here. Acute alcoholic injury, as well as acute viral hepatitis, reveals a predominance of zone 3 involvement. Periportal cell necrosis can be seen in some cases of acute type A viral hepatitis, in ferrous sulfate toxicity, and in toxemia of pregnancy. The term *spotty necrosis* is used when there are small scattered foci of hepatocytolysis replaced by a small group of hyperplastic Kupffer cells. Confluent necrosis occurs when a larger number of liver cells, such as an entire zone, is necrotic (Fig. 97.26). Submassive and massive necrosis are more severe forms. In submassive necrosis, entire lobules are involved with sparing of a few periportal hepatocytes, whereas in massive necrosis no viable hepatocytes are present (Fig. 97.27). The term granulomatous necrosis is used when the area of necrosis is well circumscribed and comprises a compact arrangement of macrophages, lymphocytes, and plasma cells (Fig. 97.28). This differs from true granulomas in lacking epithelioid cells and multinucleated giant cells. Coagulative necrosis denotes eosinophilic change of hepatocytes with loss of nuclei and maintenance of reticulin without inflammatory infiltration. This is seen in anoxia (Fig. 97.26) or toxic necrosis due to halothane or acetaminophen (Figs 97.29 and 97.30).

Parenchymal inclusions

The most common pigment seen in the liver cells is lipochrome or lipofuscin (wear-and-tear pigment) prominent in zone 3. Hemosiderin pigment is usually seen in periportal

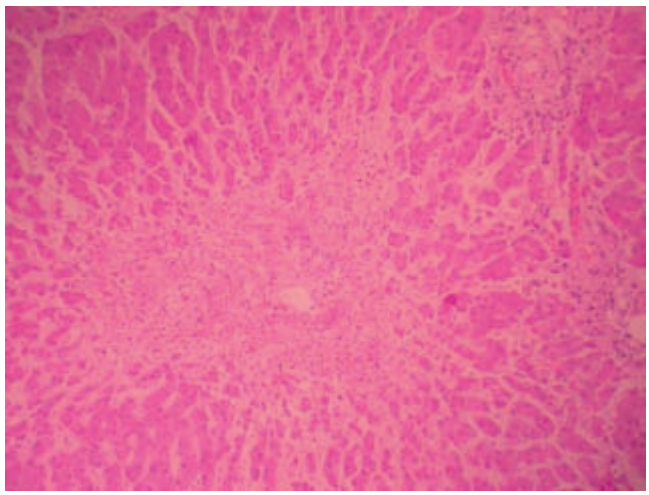

Figure 97.29 Acetaminophen toxicity resulting in perivenular coagulative necrosis without hepatocyte swelling. (H & E stain; original magnification × 100.)

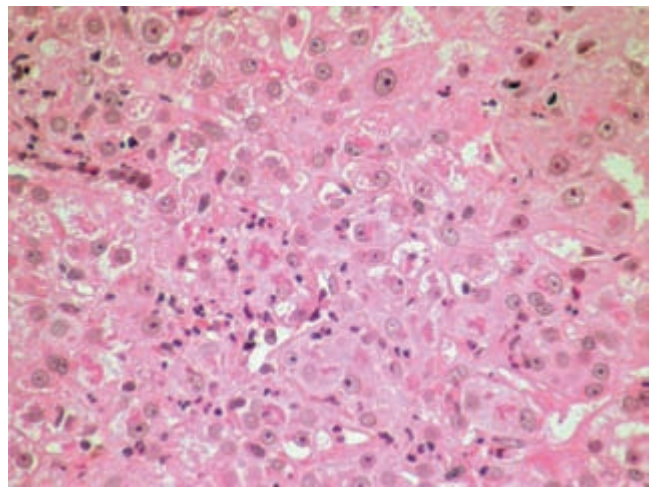

Figure 97.31 Perivenular hepatocytes containing Mallory hyaline with neutrophilic reaction around them. (H & E stain; original magnification × 200.)

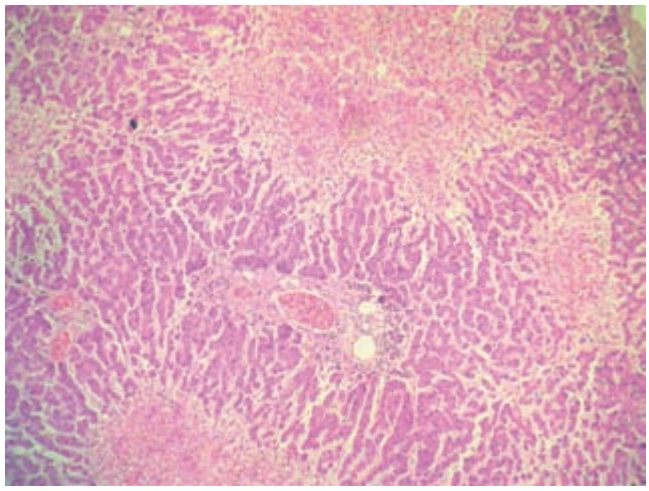

Figure 97.30 Halothane-induced perivenular and midzonal coagulative necrosis. (H & E stain; original magnification × 100.)

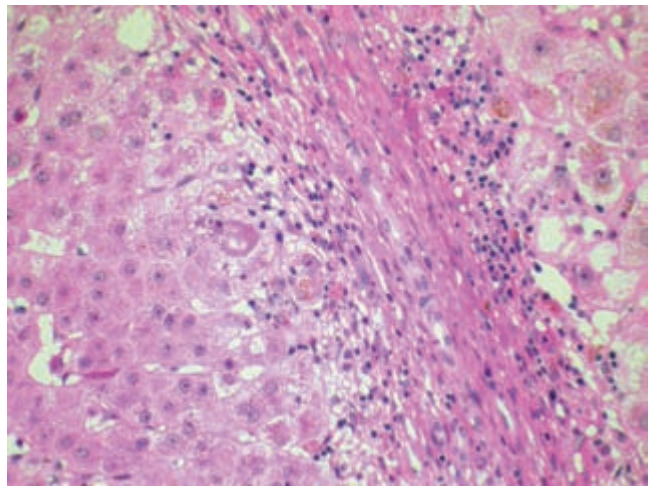

Figure 97.32 A periportal hepatocyte containing Mallory hyaline in primary biliary cirrhosis. (H & E stain; original magnification × 200.)

hepatocytes (Fig. 97.3). Bile can be seen in the hepatocytes and Kupffer cells but often it is associated with canalicular bile plugs. Mallory hyalin is seen as eosinophilic clumpy, ropy material in hepatocytes that are hydropic. It represents aggregates of intermediate filaments. In acute ALD, hyaline necrosis is associated with neutrophilic infiltrates usually in perivenular areas (Fig. 97.31). Mallory bodies can be seen in periportal hepatocytes in chronic biliary diseases such as primary biliary cirrhosis or chronic obstruction (Fig. 97.32). Another eosinophilic inclusion body that is often spherical but of variable size is the α_1-antitrypsin globule, which is seen in heterozygous or homozygous deficiency of α_1-antitrypsin resulting in accumulation of the protein in hepatocytes. This is also seen in periportal hepatocytes. These globules are PAS-positive with resistance to diastase digestion (Fig. 97.2). In some cases of ALD, giant mitochondria that are spherical or needle-shaped can be seen in hepatocytes (Fig. 97.33). Among the viral inclusions, ground-glass hepatocytes with excessive HBsAg in chronic hepatitis B virus (HBV) infection, nuclear and cytoplasmic inclusions of cytomegalovirus (CMV), and nuclear inclusion of herpes simplex are important.

Cord pattern

Hepatocytes are arranged in a radial fashion alternating with the sinusoidal blood spaces. In cases of diffuse cell swelling, this pattern is altered. When there is uniform cell enlarge-

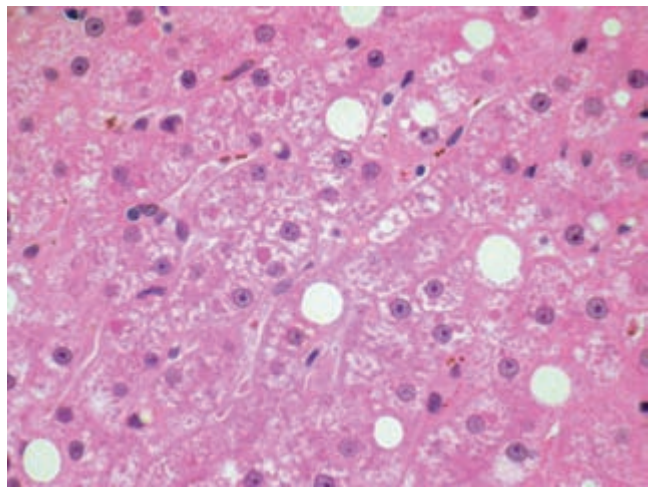

Figure 97.33 Hepatocytes containing spherical megamitochondria in alcoholic liver disease. (H & E stain; original magnification × 200.)

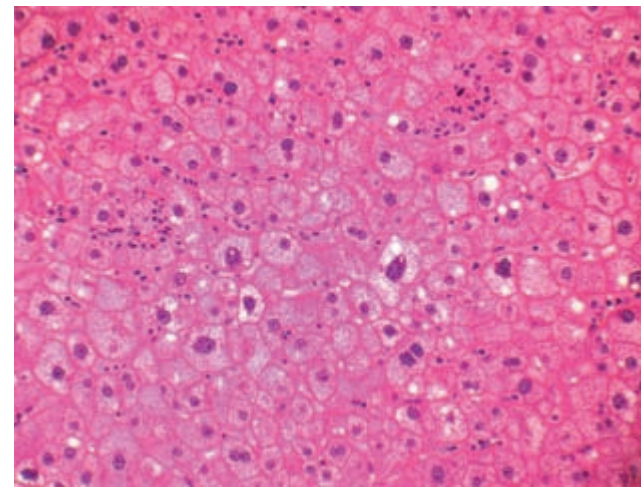

Figure 97.35 Focal dysplastic change consisting of enlarged cells with large nuclei in chronic hepatitis B. (H & E stain; original magnification × 200.)

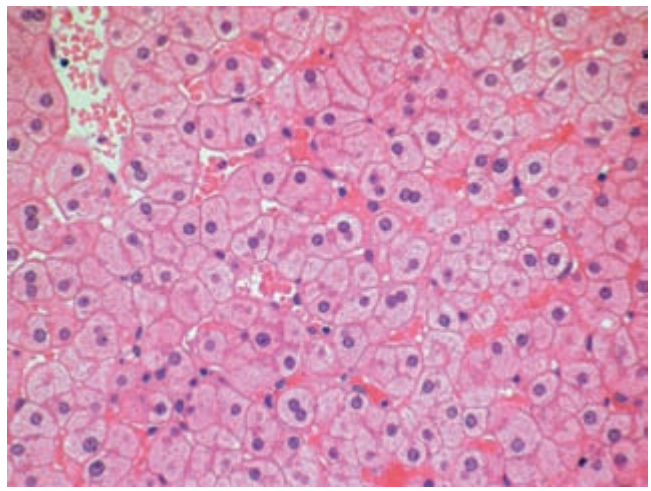

Figure 97.34 Focal regeneration with cobblestone arrangement of hepatocytes in chronic hepatitis. (H & E stain; original magnification × 200.)

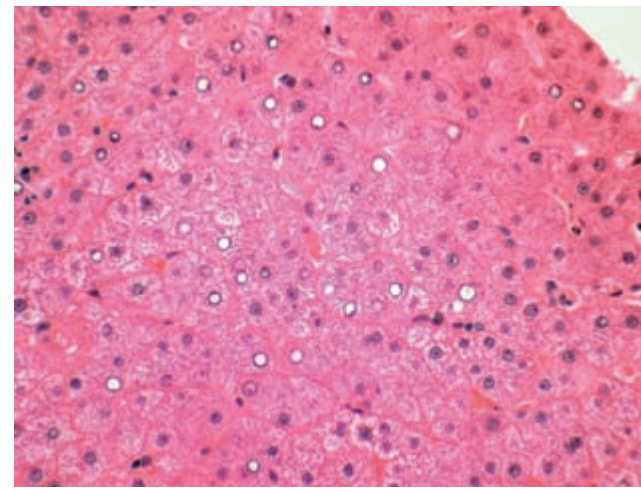

Figure 97.36 Hepatocytes with glycogen vacuolated nuclei. (H & E stain; original magnification × 200.)

ment with crisp nuclei and the cells are arranged in a cobblestone formation, it indicates focal regenerative activity (Fig. 97.34). A diffuse cobblestone pattern is seen in persistent viral hepatitis.

Nuclear changes

Nuclear changes of hepatocytes include dysplasia, defined as enlargement of a group of cells with a high nuclear to cytoplasmic ratio (Fig. 97.35). Nuclear polyploidy means irregularity and enlargement of individual cell nuclei. Nuclear membrane invagination and glycogen vacuolization are other frequent changes (Fig. 97.36). Syncytial change represents multinucleated liver cells, often seen with neonatal hepatitis (Fig. 97.37).

Sinusoidal spaces and lining cells

In chronic passive congestion and outflow obstruction, the sinusoids in zone 3 are dilated along with atrophy of hepatocytes (Fig. 97.38). In left-sided heart failure, red cells traverse the space of Disse and enter the hepatic trabecula (Fig. 97.39). Focal sinusoidal dilation can be seen in space-occupying lesions in the adjacent parenchyma. Collagen deposition in the space of Disse eventually leads to narrowing and occlusion of sinusoidal spaces (Fig. 97.40). This is commonly seen in ALD, hypervitaminosis A, chronic outflow obstruction including constrictive pericarditis, and inferior vena cava web lesions, as well as in methotrexate toxicity. In amyloidosis there is deposition of amyloid either as a reticular type or globular amyloid in the space of Disse (Figs 97.41 and 97.42).

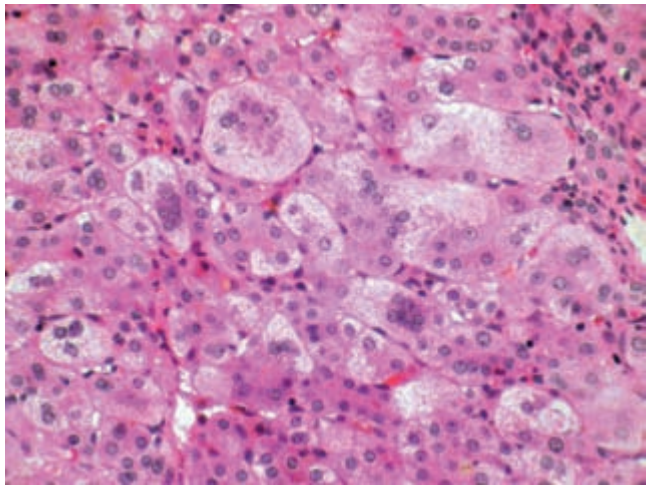

Figure 97.37 Syncytial hepatocytes in neonatal hepatitis. (H & E stain; original magnification × 200.)

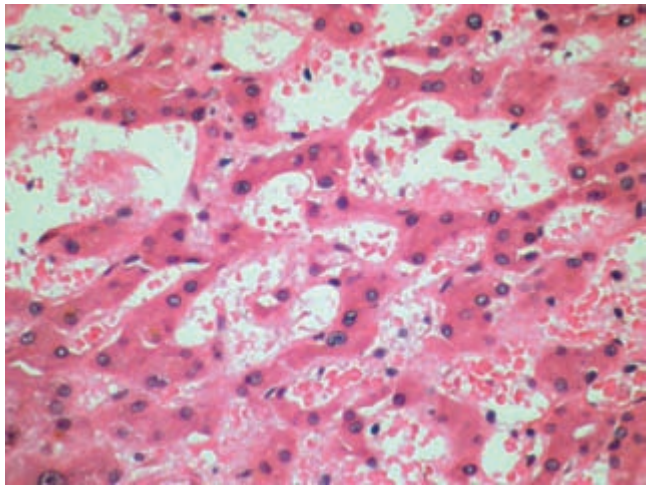

Figure 97.38 Chronic passive congestion causing perivenular sinusoidal dilation and atrophic hepatic cords. (Masson trichrome stain; original magnification × 400.)

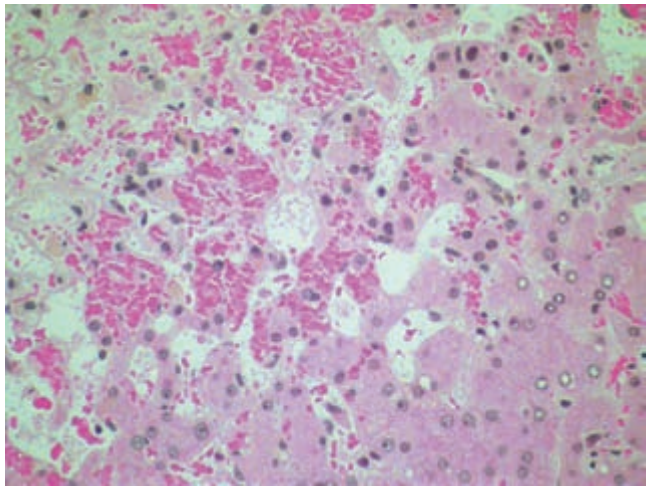

Figure 97.39 Perivenular hepatic parenchyma with dilated sinusoids and the presence of red blood cells within the hepatic cords in left-sided heart failure. (H & E stain; original magnification × 100.)

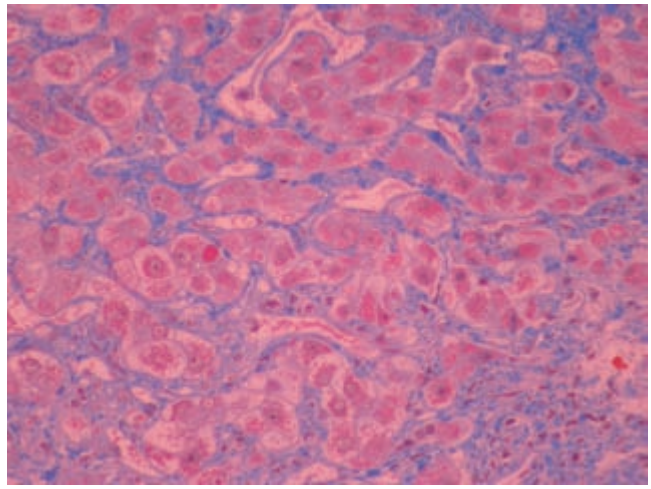

Figure 97.40 Collagen fibers along the sinusoids in the space of Disse in alcoholic liver disease. (Masson stain; original magnification × 200.)

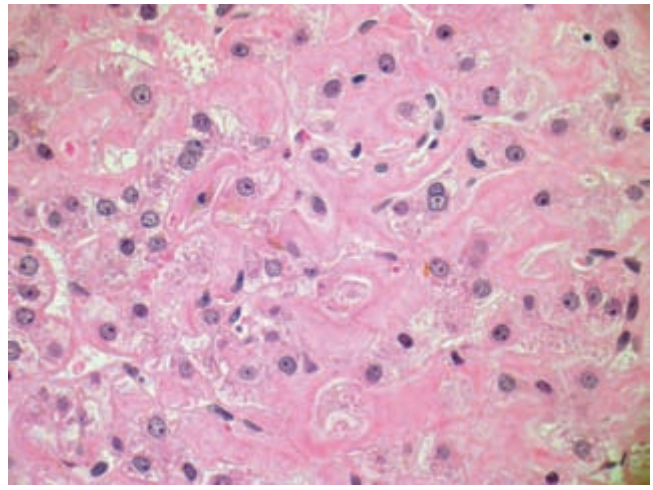

Figure 97.41 Reticular amyloid deposition in the space of Disse. (H & E stain; original magnification × 200.)

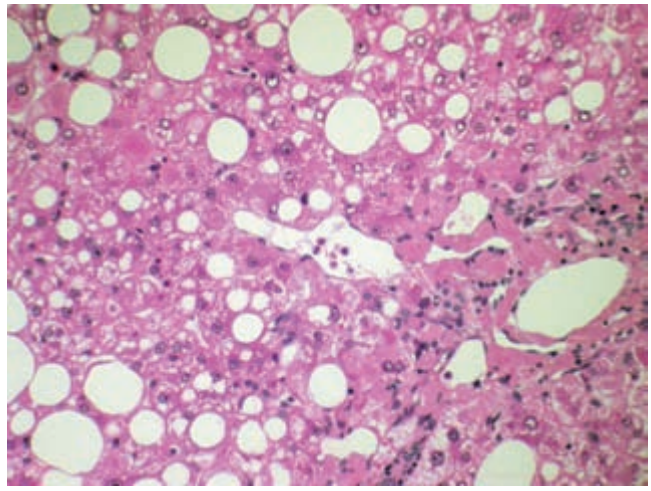

Figure 97.42 Globular amyloid deposition. (H & E stain; original magnification × 400.)

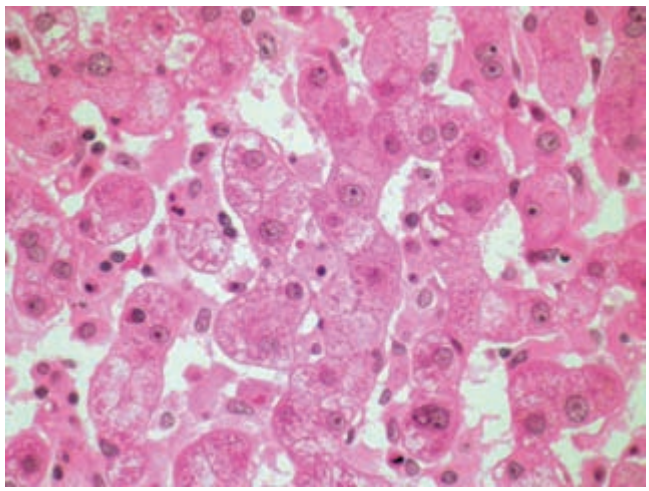

Figure 97.43 Hypertrophic Kupffer cells in salmonellosis. (H & E stain; original magnification × 200.)

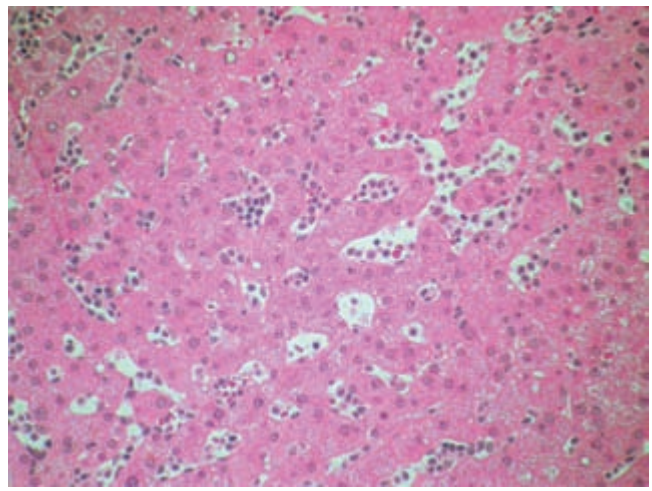

Figure 97.45 Leukemic cells in the sinusoidal blood space in a case of lymphocytic leukemia. (H & E stain; original magnification × 200.)

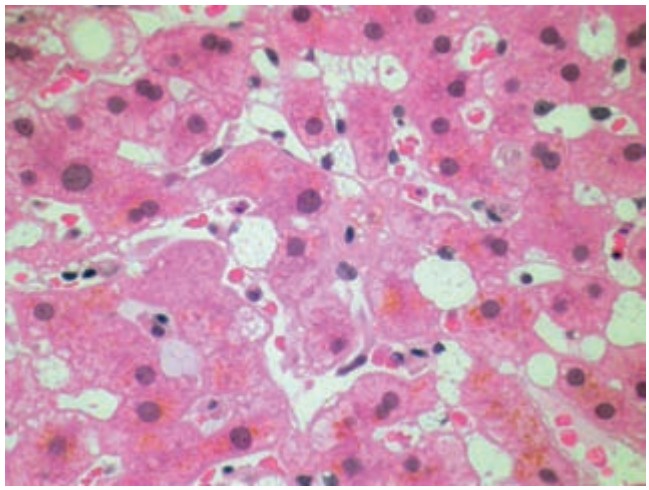

Figure 97.44 Ito cells with foamy fatty cytoplasm along the sinusoidal surface in hypervitaminosis A. (H & E stain; original magnification × 400.)

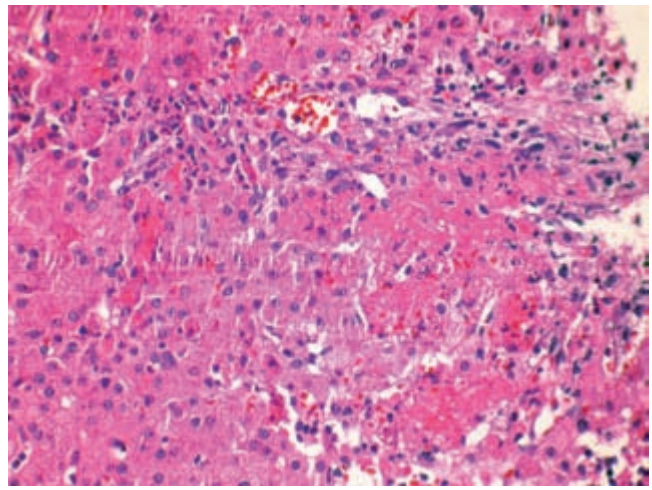

Figure 97.46 Periportal sinusoidal space filled with fibrin thrombi in toxemia of pregnancy. (H & E stain; original magnification × 100.)

Hypertrophy of Kupffer cells is seen in many inflammatory diseases of the liver, but is most striking in salmonellosis (Fig. 97.43). Stellate cells or Ito cells are located in the space of Disse; they are the storage cells of retinoids (vitamin A metabolites). Through a process of activation, these cells become myofibroblasts causing sinusoidal fibrosis (Fig. 97.44). Peripheral circulating cells are visualized in sinusoidal blood spaces. Atypical cells of mononucleosis, leukemic cells, and cells of hairy cell leukemia can be seen (Fig. 97.45). Fibrin thrombi are seen packed within the sinusoids in disseminated intravascular coagulation and in toxemia of pregnancy (Fig. 97.46). Sickled red blood cells are often seen in small clumps within the sinusoidals in sickle-cell anemia (Fig. 97.47).

Cholestasis

Bile stasis can be within the lobules in dilated biliary canaliculi or in periportal cholangioles or bile ducts. Cholestasis in zone 3 is often associated with biliary obstruction or drug-induced injury (Fig. 97.48), whereas periportal (zone 1) bile stasis is seen in late stages of primary biliary cirrhosis. Simple cholestasis with no associated morphological changes of bile ducts is seen in conditions such as benign recurrent intrahepatic cholestasis, benign postoperative cholestasis, pregnancy, and administration of drugs such as estrogen or anabolic steroids, as well as in bacterial sepsis.

Fat

Two major types of fatty changes can occur in hepatocytes:

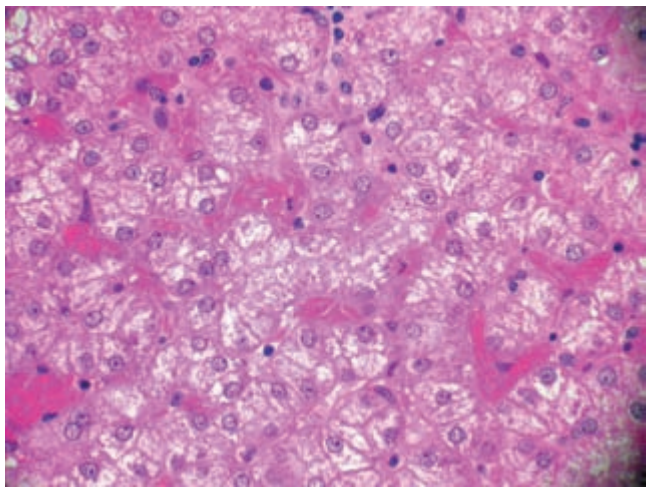

Figure 97.47 Clumps of sickled red blood cells packed in the sinusoidal spaces. (H & E stain; original magnification × 200.)

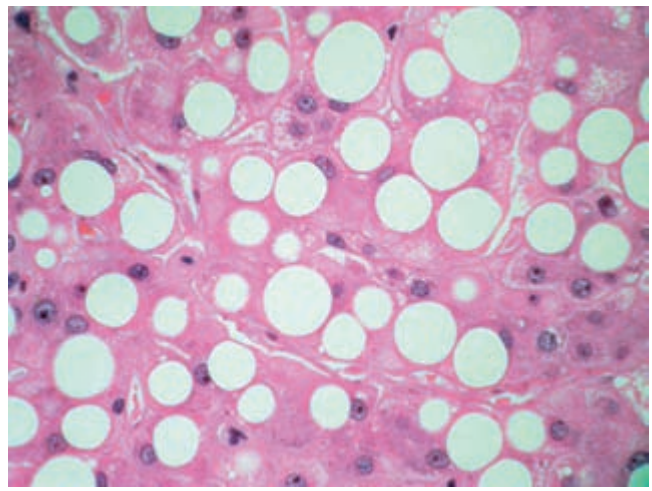

Figure 97.49 Macrovesicular fatty change of hepatocytes in alcoholic liver disease. (H & E stain; original magnification × 200.)

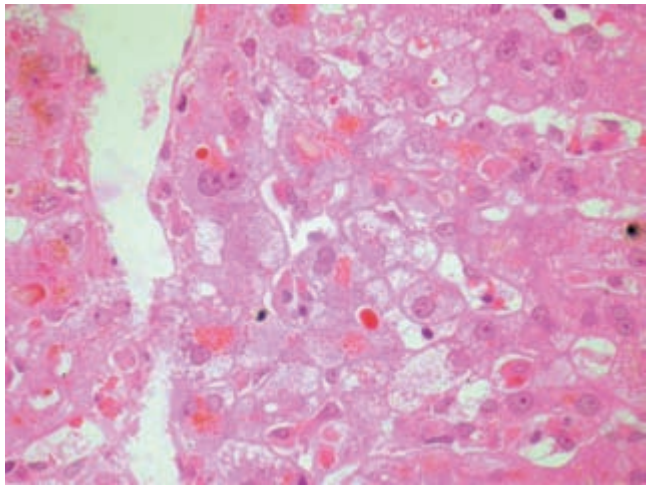

Figure 97.48 Cholestasis in dilated canaliculi in zone 3 in chlorpromazine-induced liver disease. (H & E stain; original magnification × 200.)

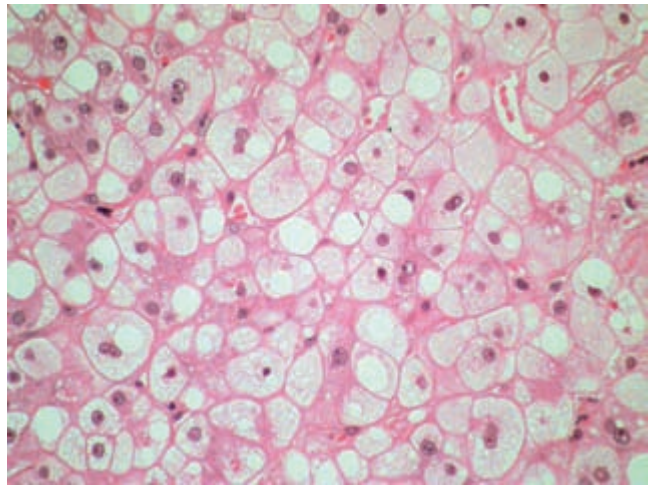

Figure 97.50 Diffusely enlarged hepatocytes with foamy fatty change in acute alcoholic liver disease. (H & E stain; original magnification × 100.)

macrovesicular and microvesicular types. Macrovesicular fat droplets appear as a large vacuole dislocating the nucleus and the cytoplasmic material to the periphery of the cell. Macrovesicular fatty change involving zone 3 is often seen in ALD (Fig. 97.49) and also in diabetes mellitus and in obesity. Steroid-induced fatty change involves all zones. Microvesicular fat, composed of small droplets of fat, sometimes appears as foamy change of the cytoplasm, without displacement of the hepatic nucleus. This is seen in alcoholic foamy degeneration, fatty liver of pregnancy, tetracycline toxicity, Reye syndrome, and in valproic acid toxicity (Fig. 97.50).

Terminal hepatic venules and venous outflow

The most common pathological change of terminal hepatic venules is perivenular and obliterative sclerosis seen in alcohol-induced liver injury (Fig. 97.23). Similar fibrosis can also be seen in Budd–Chiari syndrome and other causes of outflow obstruction, such as constrictive pericarditis, inferior vena caval obstruction, or web lesions. Endotheliolitis or inflammatory cell infiltration of terminal hepatic venules is a feature of acute rejection of liver transplants (Fig. 97.24).

Space-occupying lesions of the liver

Primary tumors: benign and malignant

These can be neoplastic lesions, cysts, or abscesses. A CT- or ultrasound-guided liver biopsy can result in a sample that is diagnostic of the lesion present. Cysts and abscesses are usually aspirated. Among the benign solid mass lesions, focal

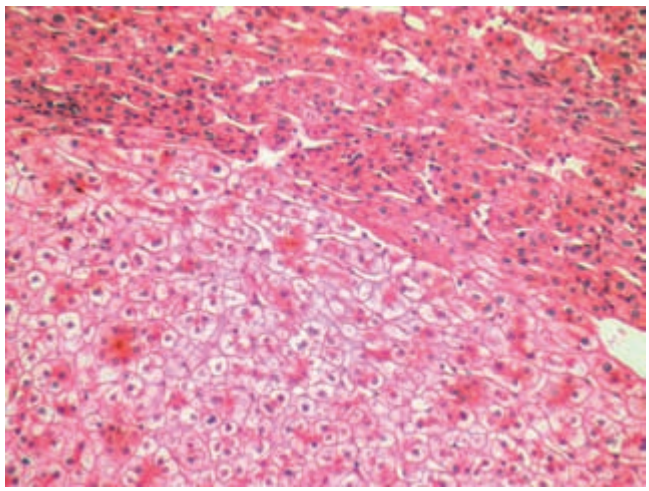

Figure 97.51 Liver cell adenoma with clear cells and the adjacent normal parenchyma. (H & E stain; original magnification × 100.)

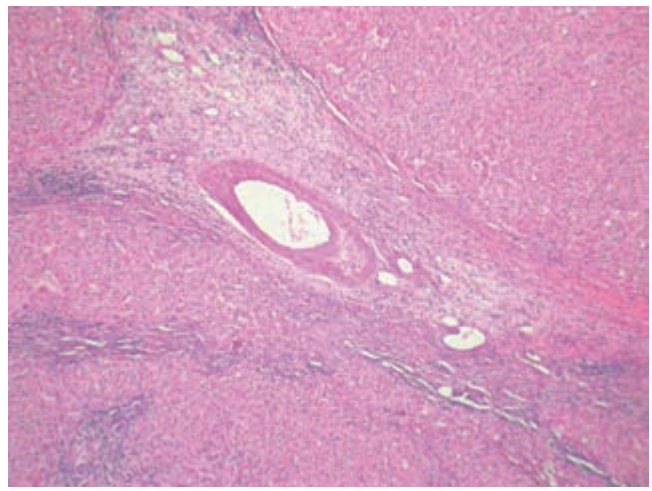

Figure 97.53 Focal nodular hyperplasia (FNH) with central stellate scar. (H & E stain; original magnification × 40.)

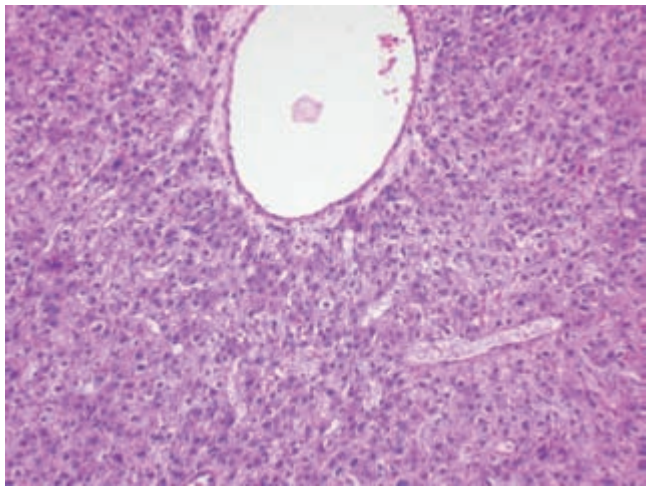

Figure 97.52 Liver cell adenoma with thick-walled vessels and lack of portal tracts. (H & E stain; original magnification × 100.)

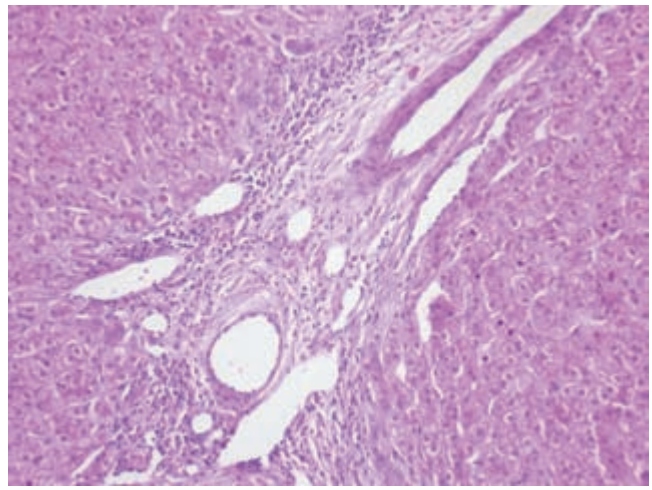

Figure 97.54 Focal nodular hyperplasia (FNH) with the scar exhibiting lack of bile ducts and presence of vascular structures. Liver cells are uniform and regenerative in appearance. (H & E stain; original magnification × 100.)

nodular hyperplasia is one of the most common and must be differentiated from liver cell adenoma [25,26]. Liver cell adenoma does not have a capsule but compresses adjacent parenchyma (Fig. 97.51). The tumor cells are benign with normal nuclear-to-cytoplasmic ratios, and with lack of portal tracts and terminal veins in the lesions. There are prominent, thick-walled arteries present along the edge of the tumor (Fig. 97.52). Focal nodular hyperplasia has a typical central stellate scar with loose vascularized fibrous tissue containing atypical ductal elements along the periphery of the scar (Fig. 97.53). This lesion also lacks normal portal areas and terminal hepatic veins (Fig. 97.54). Among the primary malignant neoplasms of the liver, hepatocellular carcinoma (HCC) is the most common form [27]. Other forms include cholangiocarcinoma and angiosarcoma. The tumor cells of the majority of typical HCCs have a trabecular pattern with increased numbers of cell thicknesses forming finger-like projections into vascular spaces. These trabeculae are lined by endothelial cells and surrounded by vascular spaces (Fig. 97.55). The tumor can have focal acinar changes with secretory material in the lumina. Sclerosing hepatic carcinoma is a variant with a dense collagenized stroma (Fig. 97.56). The fibrolamellar variant has lamellar strands of collagen separating thin cords of large eosinophilic neoplastic hepatocytes [28] (Fig. 97.57). Cholangiocarcinomas are composed of neoplastic ductal elements surrounded by dense fibrous stroma [27] (Fig. 97.58). The tumor is clearly demarcated from the surrounding liver.

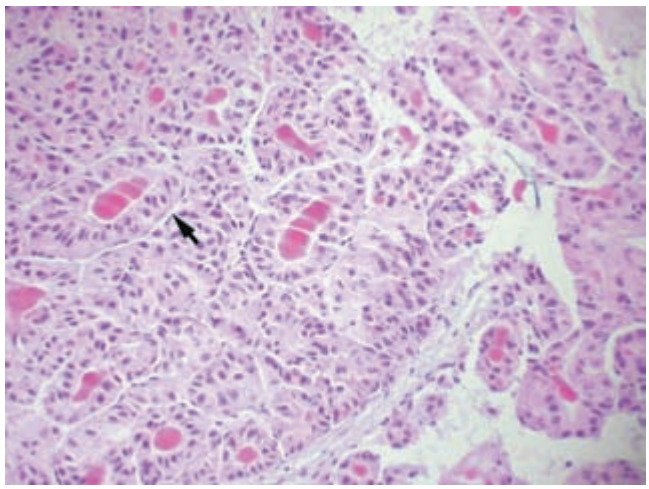

Figure 97.55 Well-differentiated trabecular hepatocellular carcinoma with endothelial lining. (H & E stain; original magnification × 100.)

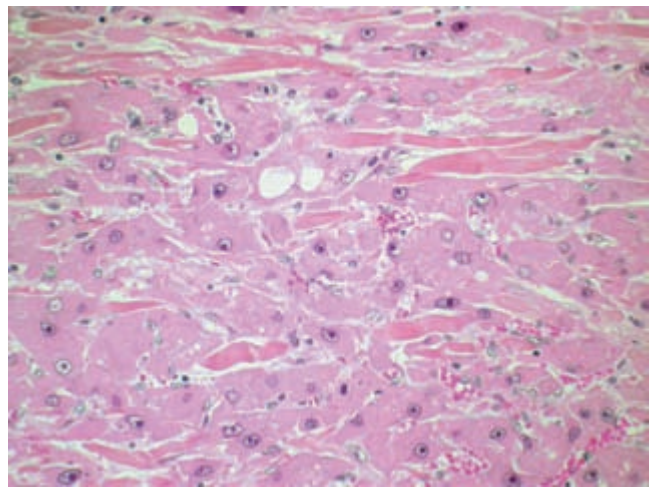

Figure 97.57 Eosinophilic neoplastic hepatocytes with lamellar fibrous stroma in fibrolamellar hepatocellular carcinoma. (H & E stain; original magnification × 100.)

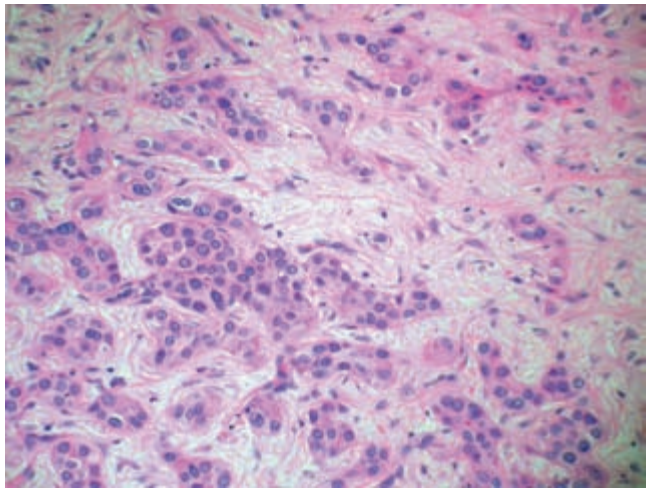

Figure 97.56 Sclerosing hepatic carcinoma with dense fibrous stroma. (H & E stain; original magnification × 100.)

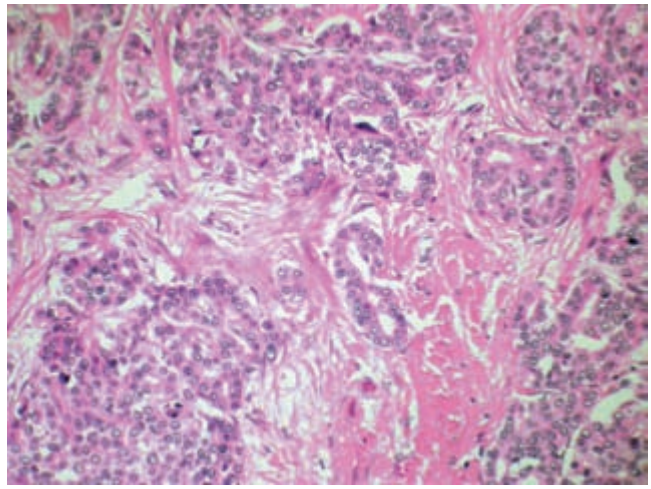

Figure 97.58 Neoplastic ductal structures with fibrous stroma in cholangiocarcinoma. (H & E stain; original magnification × 100.)

Nodular regenerative hyperplasia (NRH)

This is a rare form of hyperplasia of liver cells with formation of nodules without fibrous septa [29]. Microscopically, the hyperplastic nodules are better identified on low power exhibiting compression by the surrounding parenchyma (Fig. 97.6). A reticulum stain outlines these nodules, which can vary from small and microscopic to large, up to 23 cm. Patients with NRH may have evidence of portal hypertension.

Metastatic tumors

Metastatic tumors in the liver can be easily diagnosed from an ultrasound-guided needle biopsy. The tumor cells usually grow into the adjacent sinusoids, compressing and separating the hepatic trabeculae (Fig. 97.59). Examination of the junction between the tumor and nontumorous tissue is useful in differentiating HCC from metastatic tumors. In HCC, the tumor cells merge into the hepatic cords where there is a transition from normal to neoplastic cells (Fig. 97.60). In metastatic tumors, as mentioned earlier, the tumor grows into the sinusoidal spaces.

Granulomas

Granulomas are identified in the liver at a rate of 25% of all liver biopsies in most of the large centers [30]. The most common cause of epithelioid granulomas in the liver is sarcoidosis (Fig. 97.61). Among infectious agents causing granulomas in the liver, the most common are *Mycobacterium tuberculosis, Mycobacterium avium intracellulare*, Q fever, and brucellosis

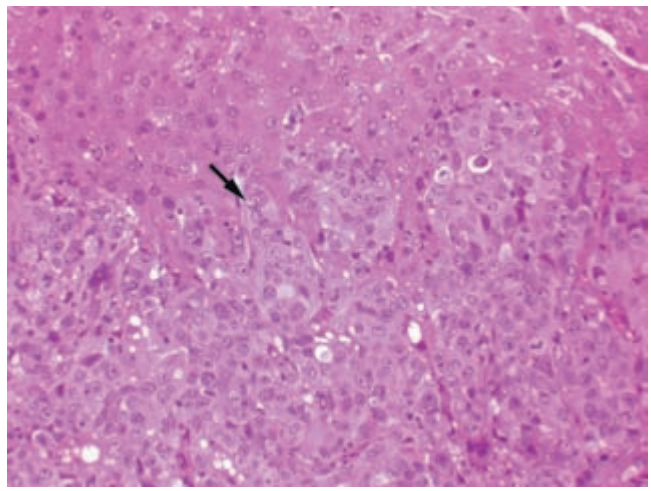

Figure 97.59 Metastatic, poorly differentiated adenocarcinoma infiltrating into the sinusoids. (H & E stain; original magnification × 200.)

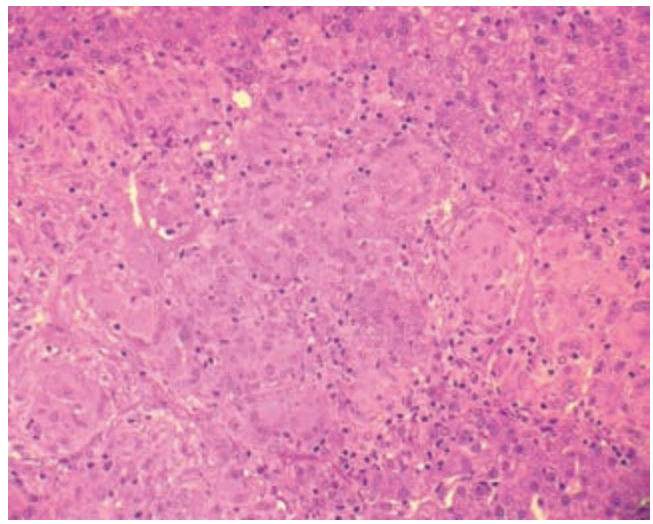

Figure 97.61 Partially segmented, exuberant epithelioid granuloma of sarcoidosis. (H & E stain; original magnification × 100.)

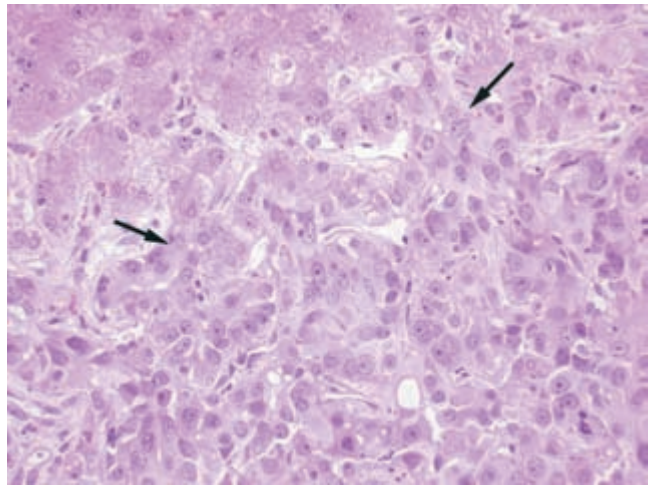

Figure 97.60 Junction of tumor and nontumor liver in hepatocellular carcinoma. The tumor cells grow into the hepatic cords (arrows). (H & E stain; original magnification × 100.)

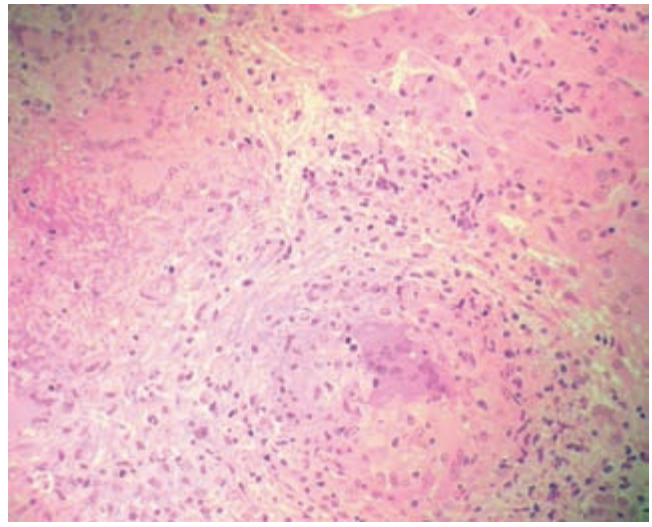

Figure 97.62 Epithelioid granuloma with Langhans giant cells in *Mycobacterium tuberculosis* infection of liver. (H & E stain; original magnification × 100.)

(Figs 97.62–97.64). Rare fungal infections such as blastomycosis and coccidioidomycosis are the next most common causes. Parasitic infections such as schistosomiasis with hepatic granulomas are common in some endemic areas of the world. Epithelioid granulomas are well circumscribed and comprise multinucleated giant cells, epithelioid cells, and lymphocytes along the periphery. Granulomatous lesions, which are not true granulomas, are small punched-out areas of hepatic necrosis with aggregates of macrophages and lymphocytes without epithelioid cell formation. These lesions are seen in viral infections (e.g., CMV, Epstein–Barr virus [EBV]), rickettsial infections (e.g., Q fever), and in liver injury caused by drugs such as phenytoin, allopurinol, phenylbutazone, and sulfonamides. Some of these drugs can also elicit an epithelioid reaction with true granuloma formation (Fig. 97.65). Hepatic granulomas can also be associated with Hodgkin disease, post-jejunoileal bypass, and PBC (Fig. 97.17). Some unique features useful in the differential diagnosis of hepatic granulomas include:

- a central vacuolated space with a fibrin ring in granulomas [31] of Q fever representing the so-called "donut" lesions (Fig. 97.64)
- demonstrations of acid-fast and fungal organisms using special stains
- the presence of eosinophils in granulomas associated with drug reactions, parasitic diseases, and Hodgkin disease

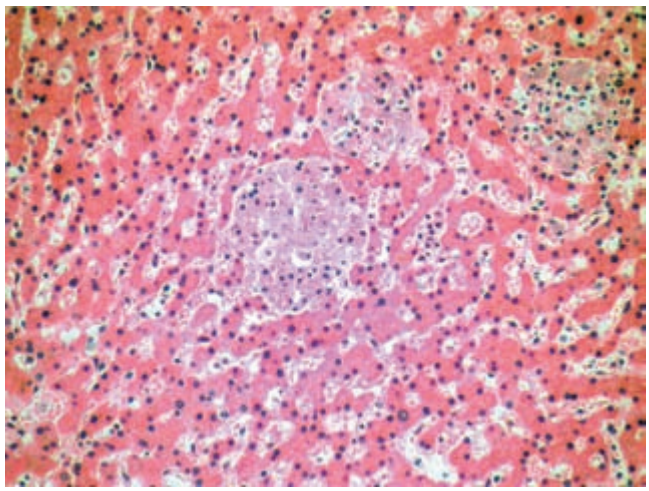

Figure 97.63 Well-circumscribed clusters of large foamy histiocytes in *Mycobacterium avium intracellulare* infection of the liver. These cells contain abundant acid-fast organisms on special stain (not shown). (H & E stain; original magnification × 100.)

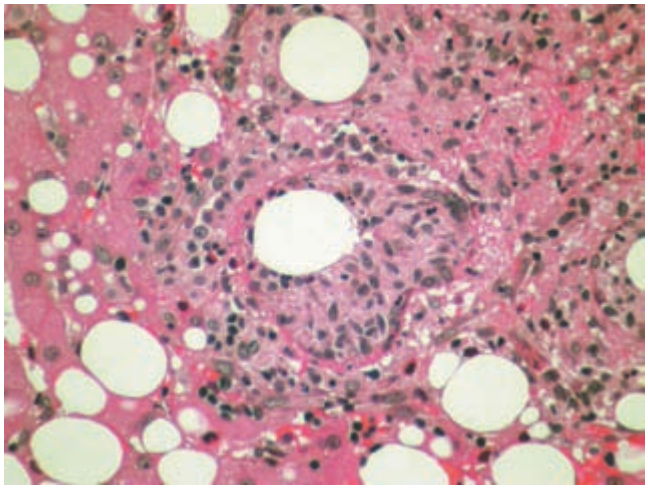

Figure 97.64 Granulomatous lesion with central vacuolization surrounded by a fibrin ring in Q fever. (H & E stain; original magnification × 200.)

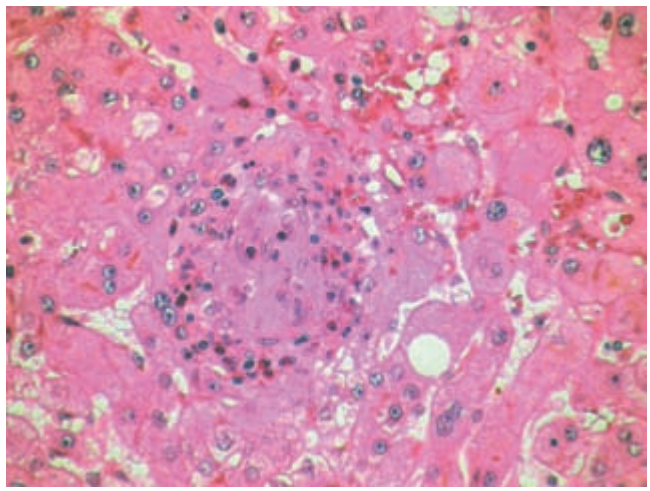

Figure 97.65 Small, well-circumscribed granuloma in sulfonamide-induced hepatic necrosis. (H & E stain; original magnification × 100.)

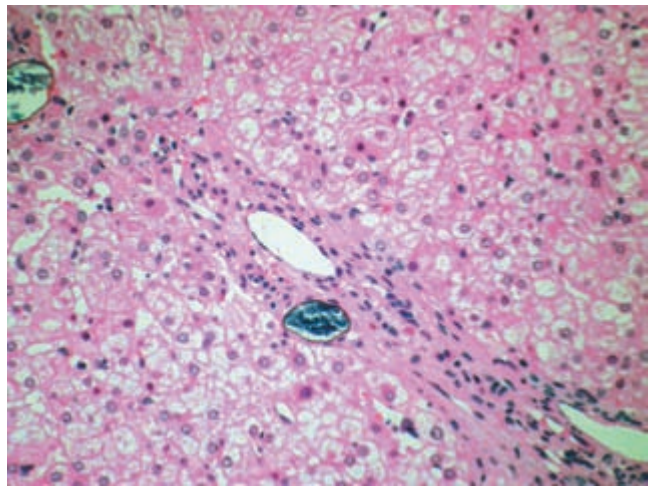

Figure 97.66 Remnants of ova of schistosomal organisms in a fibrous portal area. (H & E stain; original magnification × 200.)

- the presence of remnants of ova in schistosomiasis (Fig. 97.66)
- caseation necrosis, rarely occurring in tuberculous granulomas (Fig. 97.62).

Differential diagnosis of cirrhosis

Based on its morphological features, cirrhosis of the liver can be classified as alcoholic, nonalcoholic, biliary, and cardiac cirrhosis. Based on the size of the regenerative nodules, a broad distinction is made between micronodular (< 3-mm nodules) and macronodular (> 3-mm nodules) cirrhosis. A typical alcoholic cirrhosis is a micronodular type diagnosed by the presence of dense, broad, fibrous scars with small parenchymal nodules exhibiting sinusoidal collagenosis (Fig. 97.1). Fat, alcoholic hyaline, and other acute changes due to alcohol can be superimposed. Mild chronic inflammatory reaction of a nonspecific nature can be present in the fibrous septa without periseptal or parenchymal activity. Cirrhosis due to hemochromatosis, also a micronodular cirrhosis, is characterized by the presence of hemosiderin deposition in the parenchymal cells as well as in the fibrous septa and in bile duct epithelia. There is a striking lack of inflammatory process in this type of cirrhosis. Nonalcoholic cirrhosis, usually of macronodular type, is a broad category where the cirrhosis is the end result of chronic hepatitis of various etiologies. Fibrous septa are relatively thin and vascularized and reveal prominent chronic inflammatory infiltration. The inflammation extends into the periseptal parenchyma, which exhibits a varying degree of necroinflammatory activity. In biliary cirrhosis, the fibrous septa reveal a character-

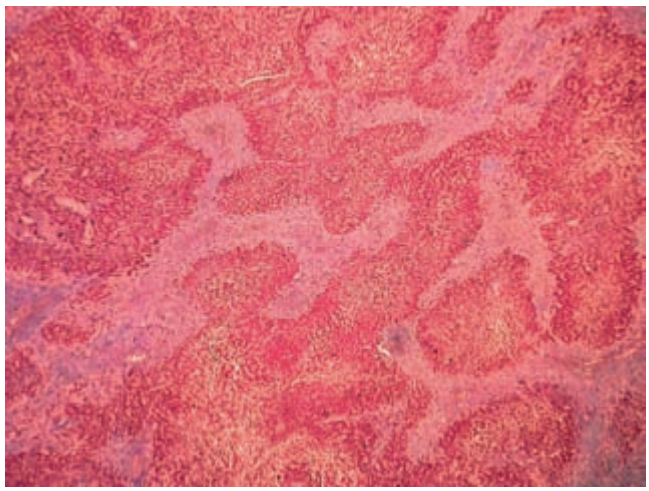

Figure 97.67 Jigsaw-puzzle appearance of biliary cirrhosis. (Masson stain; original magnification × 40.)

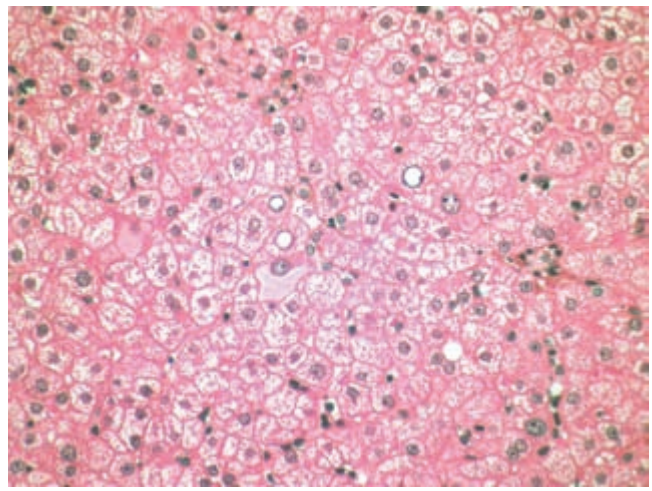

Figure 97.68 Scattered ground-glass cells in chronic hepatitis B. (H & E stain; original magnification × 100.)

istic lamellar pattern and extend from portal to portal areas leaving islands of parenchyma. This is best described as a jigsaw puzzle-like appearance (Fig. 97.67). Cardiac cirrhosis, in contrast, has a reverse lobular pattern, as the perivenular fibrous areas form septa leaving the portal areas relatively uninvolved. Following a morphological identification of the type of cirrhosis, an etiological diagnosis is attempted. The morphology of alcoholic cirrhosis can also be seen in post-jejunoileal bypass liver disease. Among the etiologies of nonalcoholic cirrhosis, chronic HBV infection can be diagnosed by the presence of ground-glass cells (Fig. 97.68) and significant dysplastic changes of the hepatocytes (Fig. 97.35). Autoimmune hepatitis can be suspected as the etiology if there is a predominance of plasma cells among the portal and parenchymal infiltrates (Fig. 97.11). In addition, areas of collapse may exist even in the cirrhotic stage in these cases. Wilson disease can be suggested if there is evidence of increased hepatic copper and copper-binding protein (Fig. 97.5). In cases of biliary cirrhosis, absence of bile duct elements with the presence of lymphoid infiltrates in the septa is suggestive of primary biliary cirrhosis, whereas evidence of bile duct proliferation with or without acute cholangitis is suggestive of biliary obstruction leading to cirrhosis. Cirrhosis in infants and children due to α_1-antitrypsin deficiency, intra- or extrahepatic biliary atresia, and in cystic fibrosis involving the liver also appears biliary in nature. Clinical, laboratory, and radiological data are needed to arrive at a more accurate etiological diagnosis of cirrhosis in most cases.

Specific liver diseases

Necroinflammatory diseases

Acute viral hepatitis

Regardless of the etiological agent, typical perivenular hepatocytolysis, extreme hydropic swelling of the hepatocytes, marked Kupffer cell hyperplasia, and mononuclear exudative reaction in the parenchyma as well as the portal tracts are found in cases of acute viral hepatitis [32] (Figs 97.69 and 97.70). Numerous acidophilic bodies are also present. The acidophilic bodies represent individual hepatocytes undergoing necrosis with cytoplasmic condensation and nuclear pyknosis. Cholestasis may or may not be present. The hepatocytes in zone 1 usually reveal uniform hydropic change suggestive of regeneration. Portal tracts are expanded with inflammation without fibrosis (Fig. 97.70). Some of the following specific changes are associated with different viral agents. Acute type A hepatitis can exhibit a periportal accentuation of the necrosis [33]. Enteric (hepatitis E virus, HEV) acute hepatitis tends to have prominent acinar changes with hepatocytes arranged around dilated biliary canaliculi resembling rosettes throughout the lobules [34] (Fig. 97.71). In acute hepatitis due to delta (hepatitis D virus, HDV) agent, the presence of hepatitis D antigen (HDAg) can be demonstrated by immunoperoxidase. When the necrosis in acute viral hepatitis (AVH) is severe, it involves the entire zone 3 and is called confluent necrosis. When the necrosis involves zones 2 and 3 and part of zone 1 with hepatocytes remaining viable in the periportal zones, it is classified as submassive necrosis. Massive necrosis is the term used when the necrosis involves all zones without any viable hepatocytes.

Chronic viral hepatitis

Terminology

Chronic hepatitis is the universal terminology used for all liver biopsies obtained from patients whose virological markers persist for more than 6 months in the presence of abnormal liver enzymes. Terms such as persistent hepatitis, chronic active hepatitis, chronic lobular hepatitis, and so forth are no longer used by pathologists. The diagnosis of

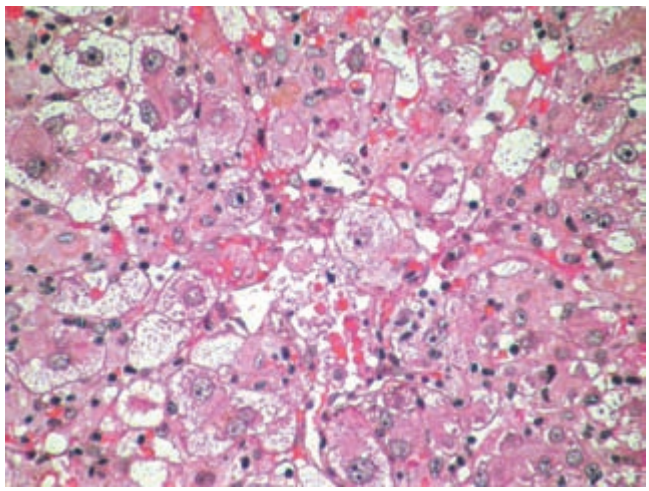

Figure 97.69 Perivenular zone in acute viral hepatitis demonstrating hydropic hepatocytes, hepatocytolysis, inflammatory exudate, and rare acidophilic bodies. (H & E stain; original magnification × 200.)

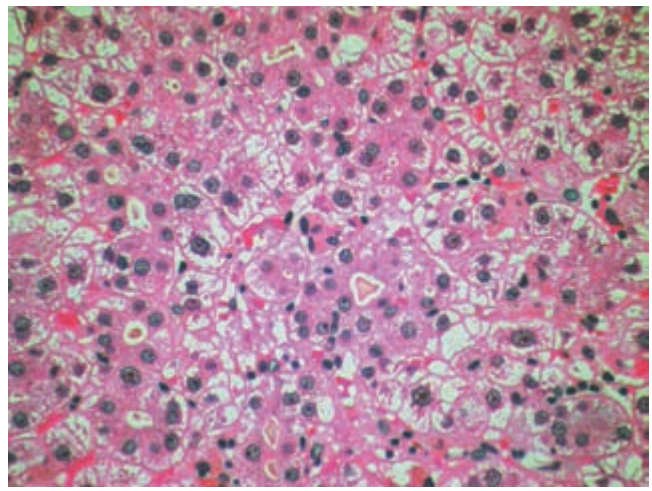

Figure 97.71 Prominent acinar transformation of hepatocytes in enterically transmitted acute hepatitis, type E. (H & E stain; original magnification × 200.)

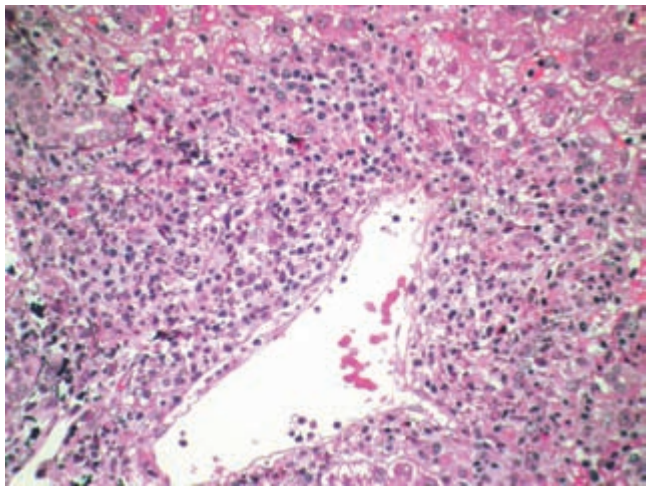

Figure 97.70 Portal area in acute viral hepatitis with mononuclear infiltration extending to the periportal regions. (H & E stain; original magnification × 200.)

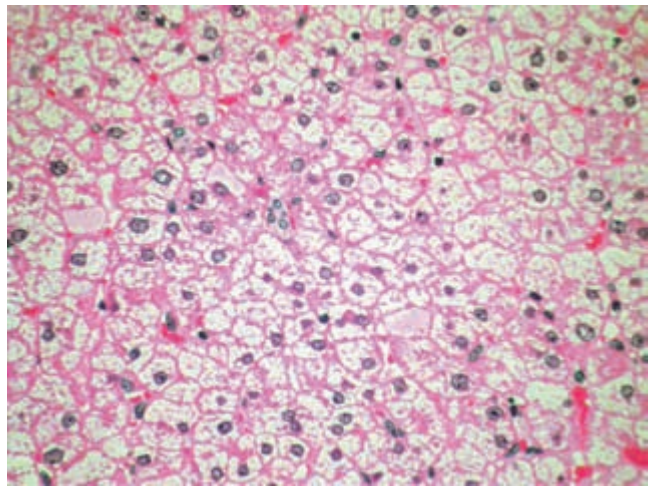

Figure 97.72 Uniform cobblestone appearance of parenchyma in chronic hepatitis, type B. A few ground-glass cells are seen. (H & E stain; original magnification × 200.)

"chronic hepatitis" is further elaborated by adding the name of the causative virus (i.e., type B, type C, or type D) and by adding the assessment of necroinflammation and fibrosis by a semiquantitative method, using grades (0–4) and stages (1–4) as proposed by Ludwig [35]. Detailed quantitation assessments have included many scoring systems; the most commonly used are those devised by Knodell and colleagues and Ishak and colleagues, and the Metavir scoring system [36–38]. *Knodell scoring* has a total of 18 for necroinflammation including lobular, periportal, and portal inflammation, and a total of 4 for fibrosis (0–4). The *Ishak scoring system* has more details for necroinflammation (0–18) and expanded scores for fibrosis (0–6). *Metavir scoring* is simpler with a combination score for inflammation including periportal and lobular activity (A0–A3) and a fibrosis score on a scale of F0–F4.

The most common morphological features of chronic viral hepatitis are portal fibrosis, portal and periportal mononuclear inflammation, and necroinflammatory changes within the parenchyma with an irregular distribution between one lobule and another. There is also regenerative activity in an irregular distribution (Figs 97.72 and 97.73). Lymphocytes and Kupffer cells are seen cuffing around the target hepatocytes (Fig. 97.74). The degree of necrosis depends on the disease activity – more severe in exacerbations or reactivation and less so in quiescent stages. Fibrosis progresses to cirrhosis and the periseptal inflammatory activity continues with

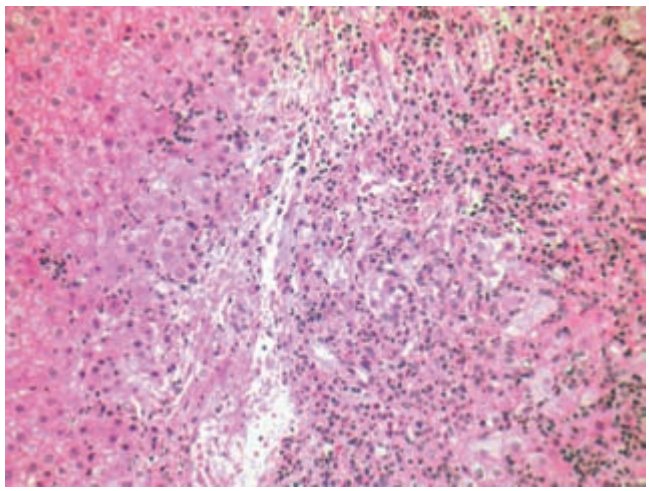

Figure 97.73 Portal areas with fibrosis and mononuclear inflammation extending to the parenchyma exhibiting piecemeal necrosis in chronic hepatitis. (H & E stain; original magnification × 100.)

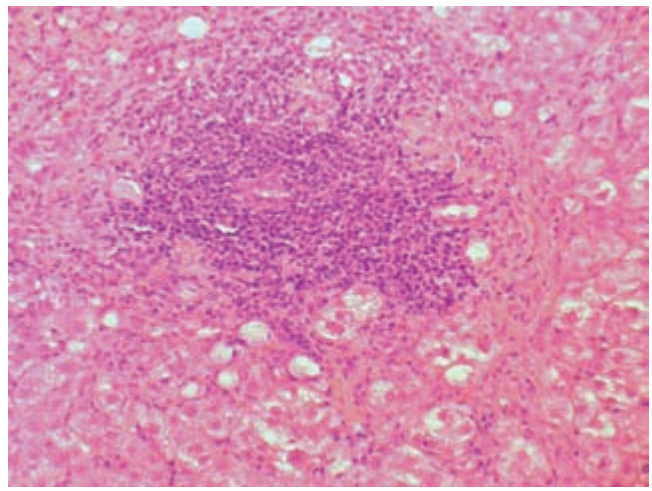

Figure 97.75 Chronic hepatitis C showing portal fibrosis and inflammation, macrovesicular fat, and inflammation in the adjacent parenchyma. (H & E stain; original magnification × 100.)

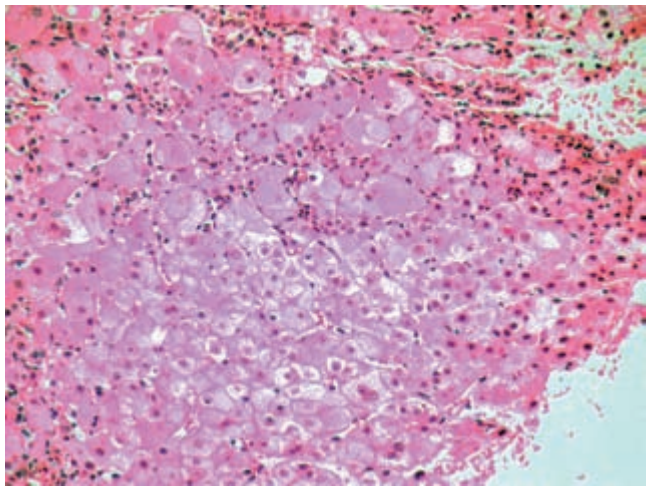

Figure 97.74 Inflammatory cells are seen cuffing around the hepatocytes in chronic hepatitis. (H & E stain; original magnification × 100.)

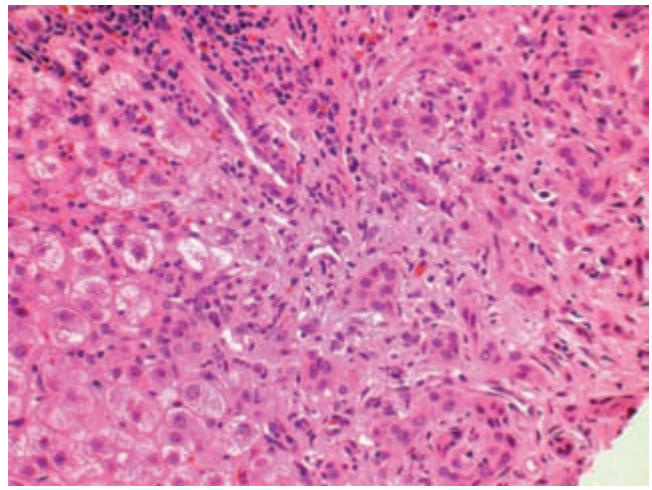

Figure 97.76 Atypical bile ducts in a portal tract of chronic hepatitis C. (H & E stain; original magnification × 200.)

cirrhosis. The term piecemeal necrosis is used to describe the periportal extension of mononuclear inflammation in association with hepatocytolysis involving the limiting plate. In chronic hepatitis B there is significant nuclear dysplasia (Fig. 97.35). In cases of chronic hepatitis C the following unique features are seen in most cases: macrovesicular fatty change, mild sinusoidal collagenosis in the periportal areas, prominent lymphoid reaction of portal areas, and moderate to marked atypia of the bile duct epithelium [39] (Figs 97.75 and 97.76). In cases of chronic hepatitis B immunoperoxidase can be used to stain for the viral proteins such as hepatitis B core antigen (HBcAg), HBsAg, and hepatitis B e antigen (HBeAg) [40]. The HBsAg protein is also demonstrable using Shikata orcein stain or Victoria blue (Fig. 97.4). In chronic hepatitis D virus infections HDAg is demonstrable by immunoperoxidase techniques [41] (Fig. 97.77).

Autoimmune etiology

In addition to the features of chronic hepatitis already described, the changes unique to autoimmune chronic hepatitis (the term *lupoid hepatitis* is used by some, but should be discouraged as it is not related to systemic lupus erythematosus) are:

- the presence of significant numbers of plasma cells among the portal and the parenchymal infiltrates (Fig. 97.11)
- evidence of collapse and striking degree of periportal as well as perivenular necrosis
- trapped hepatocytes in the fibrous portal or septal areas [42].

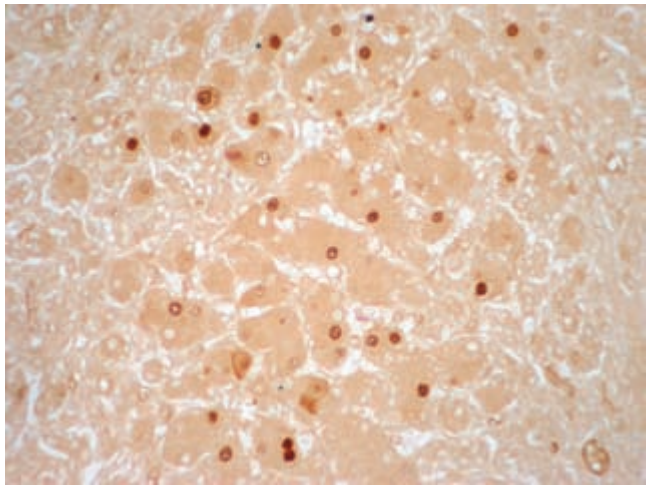

Figure 97.77 Immunoperoxidase stain demonstrating hepatitis D antigen in the nuclei of hepatocytes in chronic hepatitis D. (Original magnification × 100.)

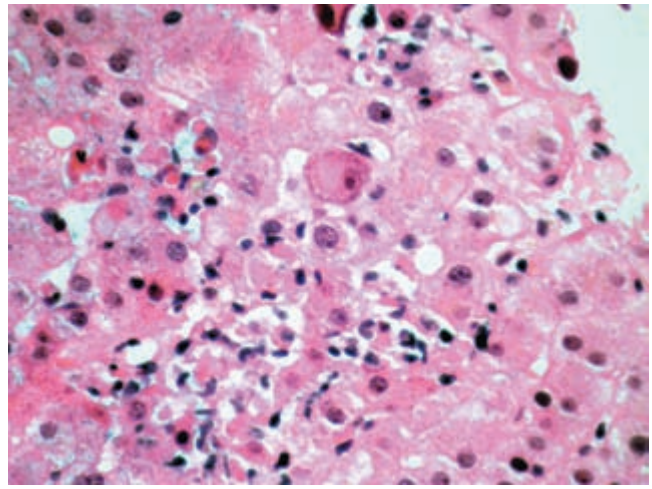

Figure 97.79 Intranuclear and cytoplasmic inclusions of cytomegalovirus (CMV) in a hepatocyte surrounded by polymorphonuclear leukocytes in an orthotopic liver transplant infected with CMV. (H & E stain; original magnification × 200.)

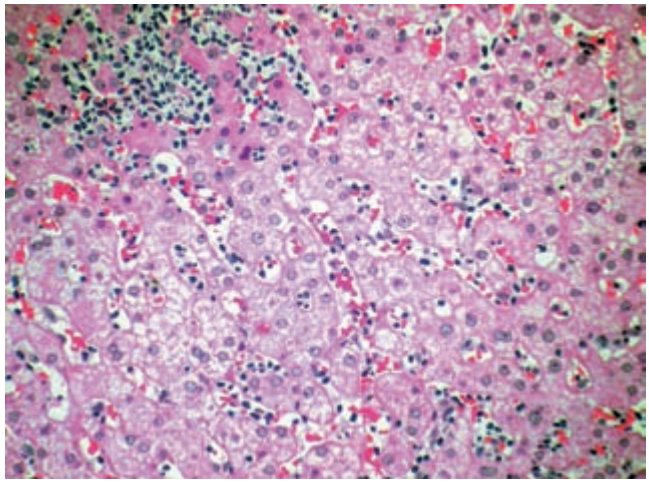

Figure 97.78 Striking sinusoidal lymphocytosis of atypical type in Epstein–Barr virus-induced mononucleosis. (H & E stain; original magnification × 200.)

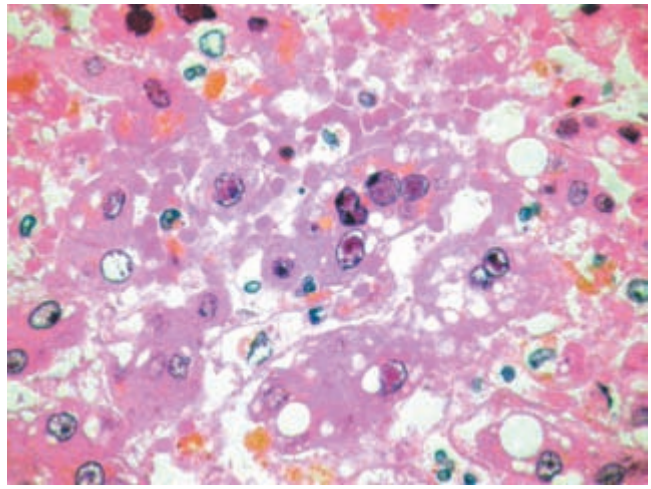

Figure 97.80 Intranuclear inclusions of Cowdry type A of herpes simplex seen in hepatocytes. (H & E stain; original magnification × 200.)

Necroinflammatory changes are minimal in patients on steroid therapy with good response.

Other viral infections

A mononucleosis pattern of hepatitis can be seen in hepatitis due to EBV, CMV, and some cases of hepatitis C virus (HCV) [43]. In this form, the hepatocytes are not swollen and the cord pattern is maintained. There is striking sinusoidal lymphocytosis, many of these cells being atypical in nature (Fig. 97.78). There is portal infiltration by the same type of cells. In addition, there is multifocal punched-out hepatocytolysis (Fig. 97.28) without confluent or zonal necrosis. Rarely, epithelioid granuloma can be seen. In immunocompromised individuals, the CMV infection results in an abundance of virally infected cells with typical nuclear inclusion bodies in the bile duct epithelium or reticuloendothelial cells. Following an orthotopic liver transplantation, CMV infection causes typical microabscess formation with the polymorphonuclear leukocytes surrounding the cells with viral inclusion bodies [44,45] (Fig. 97.79). Viral antigen in CMV hepatitis in these cases is demonstrable using immunochemical methods. Herpes simplex infection of the liver is rarely encountered in liver biopsies, because the prothrombin time is too prolonged for a biopsy in these patients. However, the typical changes consist of large, discrete, irregular zones of coagulative necrosis of parenchymal cells with the presence of eosinophilic intranuclear inclusions in the viable hepatocytes at the margins of necrosis [46] (Fig. 97.80).

Alcoholic liver disease

Changes in liver biopsies due to alcohol can be broadly divided into acute cellular damage and its characteristic features, and chronic, slowly progressive changes with evidence of chronic liver disease.

Acute changes

The most common form of acute damage is seen as fatty change of a macrovesicular type with or without cholestasis. The patients present with large fatty change with or without jaundice. Needle biopsy reveals large vacuoles of macrovesicular fat involving the perivenular hepatocytes (Fig. 97.49). Occasionally, the entire lobule is uniformly involved. Cholestasis and sinusoidal fibrosis may be seen. Acute foamy fatty change (FFC) represents a variant of acute alcoholic hepatitis [47]. The perivenular hepatocytes are enlarged with foamy or microvesicular fat (Fig. 97.50). The nucleus is central in location and the cells may exhibit abnormal giant mitochondria visible as eosinophilic round bodies (cherry bodies) (Fig. 97.33). Sinusoidal collagen deposition can be prominent in the perivenular zones. Foamy fatty change can also be seen in association with the classic acute sclerosing hyaline necrosis (ASHN) in patients with alcoholic hepatitis [48]. The morphological hallmarks of ASHN are:

- hydropic hepatocytes with cytoplasmic Mallory hyaline bodies
- neutrophil reaction around these cells with hyaline necrosis (Fig. 97.31)
- sclerosis of the terminal hepatic venules with sinusoidal collagenosis.

Other microscopic features, such as fatty change, cholestasis, and portal fibrosis with arachnoid extension, can also be part of this morphology. Other uncommon biopsy findings of acute ALD include lytic necrosis of liver cells, in which the hepatocytes are extremely hydropic with lysis and fusion of adjacent cells, and acute portal reaction, in which there is prominent neutrophilic reaction within the portal tract without any orientation to the bile ducts. These portal tracts often have classic arachnoid fibrosis.

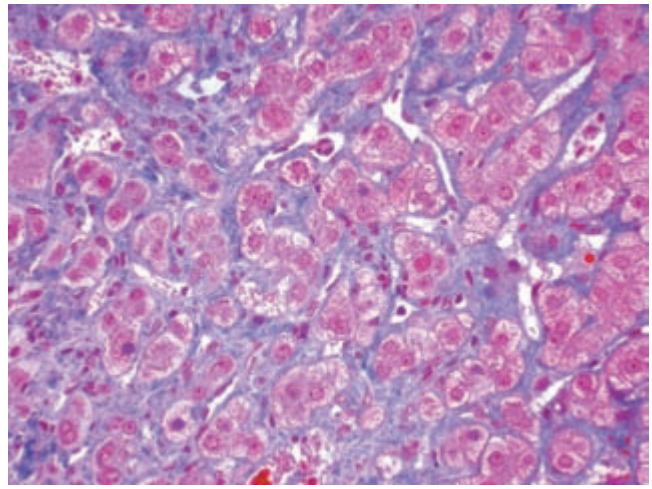

Figure 97.81 Diffuse interstitial fibrosis in chronic alcoholic liver disease. (Masson stain; original magnification × 100.)

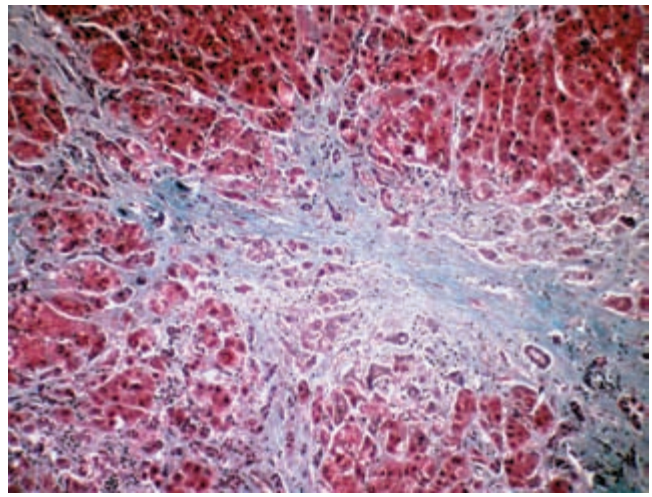

Figure 97.82 Marked perivenular fibrous scarring with mild portal fibrosis and lack of regenerative nodules in progressive perivenular fibrosis of alcoholic etiology. (Masson stain; original magnification × 100.)

Chronic alcohol-induced liver damage

This primarily manifests as fibrosis. Three forms of progressive fibrosis are seen in the liver [49]:

- portal and perivenular fibrosis leading to fibrous septa formation, resulting in cirrhosis with small regenerative nodules (Fig. 97.1)
- diffuse interstitial fibrosis with collagen extending throughout the lobule encircling individual hepatocytes and without parenchymal regeneration or septal formation (Fig. 97.81)
- progressive perivenular fibrosis with dense scar-like collagen involving zone 3 with relatively minimal portal fibrosis (Fig. 97.82).

In the early stages of development of cirrhosis the fibrous septa appear thin, with larger pseudolobules or parenchymal nodules. There is evidence of prominent intranodular sinusoidal fibrosis. As the disease progresses, the septa become denser and wider, and the nodules become subdivided into smaller nodules, with continued sinusoidal fibrosis in the periseptal regions. In advanced stages, there is extensive scarring with islands of parenchymal nodules scattered within. Masson trichrome and PAS stains clearly outline this ratio of fibrous tissue to the parenchyma. Following an established state of cirrhosis, if there is prolonged abstinence from alcohol, the regenerative parenchymal nodules enlarge, pushing the fibrous septa centrifugally with a remarkable reduction in the amount of collagen both within the parenchyma and the septa. At this inactive stage, it is difficult to identify

alcohol as the initial agent responsible for the cirrhosis. Any of the acute cellular changes described previously can occur superimposed on a cirrhotic or fibrotic liver. The distribution of Mallory hyaline, neutrophilic infiltrate, or fatty change may be irregular and focal, not uniform. When there are additional complications, such as anoxia due to variceal bleeding or general anesthesia, typical coagulative necrosis can be seen within the central zone of the cirrhotic nodules.

Differential diagnosis

Nonalcoholic steatohepatitis (NASH) or nonalcoholic fatty liver disease (discussed in the next section) are unrelated to alcohol or any other recognized cause of liver disease, but most often present as progressive liver disease with histological features resembling alcoholic liver disease [50,51]. Identical morphological changes of ASHN can be seen in Indian childhood cirrhosis, liver disease secondary to jejunoileal bypass surgery for morbid obesity, and drug-induced reactions in patients receiving amiodarone [52]. Mallory bodies without accompanying neutrophilic reaction can be seen in a number of conditions such as Wilson disease, primary biliary cirrhosis, chronic biliary obstruction, and in livers bearing HCC (in either the tumor or nontumor cells). Sinusoidal collagen deposition typical for ALD can be seen in a moderate degree in chronic HCV hepatitis, hypervitaminosis A, and methotrexate toxicity. A much more localized perivenular fibrosis can be seen in venous outflow obstruction such as Budd–Chiari syndrome. Studies of serological testing for HCV have identified a high prevalence (> 50%) of chronic HCV infection among those with ALD. In many of these patients, the liver biopsy reveals features of both ALD and chronic hepatitis of HCV type. There is an increased degree of chronic inflammatory infiltrate in the portal tracts or fibrous septa with spillover to the periseptal parenchyma.

Nonalcoholic fatty liver disease (NAFLD)

This covers the spectrum from fatty liver to nonalcoholic steatohepatitis (NASH) and to end-stage cirrhosis. Although these are not necessarily sequential, they represent increasing gravity of the disease process. Morphological features of NAFLD (Figs 97.83 and 97.84) include steatosis (both macrovesicular and microvesicular), ballooning change of hepatocytes and perisinusoidal fibrosis predominantly in zone 3 in adults and in zone 1 in children, and lobular inflammation including mononuclear cells and neutrophils. A finding of Mallory hyaline (MH) within the hepatocytes with ballooning change, neutrophilic infiltrates, megamitochrondria, and pericentral fibrosis are all features of NASH as well as ASH (alcoholic steatohepatitis). However, MH is usually more abundant in ASH. Several scoring systems have been described to assess the severity and stage of NAFLD. These include microvesicular and macrovesicular steatosis, perivenular and periportal sinusoidal fibrosis with or withoutnecroinflammation, and Mallory bodies. Degree of necroinflammation and

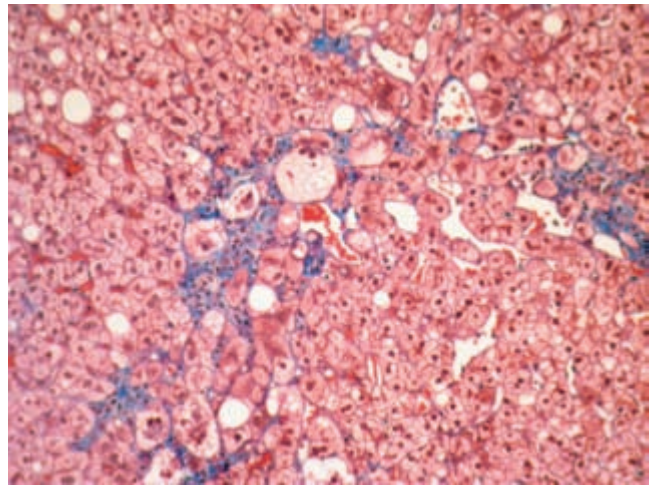

Figure 97.83 Perisinusoidal collagen in nonalcoholic steatohepatitis (NASH). (Masson trichrome stain; original magnification × 100.)

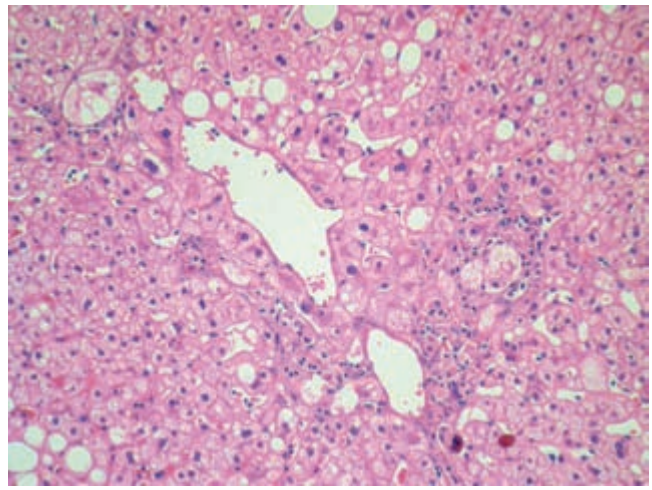

Figure 97.84 Hepatocytes with ballooning change and Mallory bodies in nonalcoholic steatohepatitis (NASH). (H & E stain; original magnification × 100.)

ballooning changes (scale of 1–3) as well as fibrosis (1–4) have been graded by a system developed by Brunt and colleagues [51]. Subsequently, a NASH clinical research network group has proposed a scoring system called the NAFLD activity score, or NAS [53]. This scores the sum of steatosis (scale of 0–3), lobular inflammation (0–2), and ballooning of hepatocytes (0–2). A score greater than 5 is diagnostic of NASH, whereas a score of less than 3 excludes NASH. The fibrosis stage is on scale of 0–4, with 1 = perisinusoidal or periportal; 2 = perisinusoidal and periportal; 3 = bridging fibrosis; and 4 = cirrhosis. Although other features such as MH and megamitochrondria are often seen in NASH, these features are associated with hepatocyte ballooning change, which is a component of the NAS system and therefore these features are not independently added to the scoring system.

Biliary diseases

Mechanical duct obstruction

Mechanical obstruction of the bile duct is often diagnosed without a liver biopsy. When the various procedures, such as ultrasound, endoscopic retrograde cholangiopancreatography (ERCP), and others, are inconclusive, a liver biopsy is performed. The changes can be divided into parenchymal and portal. Although the predominance of one type of change over the other varies from patient to patient, the earliest change might be parenchymal cholestasis in the perivenular hepatocytes. This is followed by canalicular dilation with bile plug formation. The hepatocytes become hydropic with a reticular rarefaction of the cytoplasm termed "feathery degeneration." The portal tracts reveal edema with loose fibrous tissue around dilated bile ducts. Portal inflammatory reaction includes polymorphonuclear leukocytes (PMNs) and mononuclear cells. An orientation of PMN infiltration around the bile duct, and within the duct lumen, strongly suggests mechanical duct obstruction (Fig. 97.8). Prolonged biliary obstruction results in marked cholangiolar proliferation as well as periductal fibrosis (Figs 97.13 and 97.15). Long-standing obstructions can result in disappearance of the ducts resembling PBC. During the acute stage, drug-induced cholangitic and cholestatic reaction, acute viral hepatitis of cholestatic type, impaired regeneration syndrome, changes due to parenteral hyperalimentation, and septic shock should be considered in the differential diagnosis. Biliary fibrosis and cirrhosis follow if there is continued duct obstruction (Fig. 97.67).

Primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC)

The diagnosis of PBC based on a needle liver biopsy might be difficult if adequate portal tracts are not present in the specimen. In addition to the classic destructive duct lesions with marked lymphoplasmacytic infiltrates, the presence of granulomas, absence of ducts in small portal tracts, hydropic changes of periportal hepatocytes containing Mallory hyaline and copperbinding protein (Shikata-positive granules), and periportal cholestasis are all helpful morphological features in diagnosing PBC [54]. A staging system of 1–4 has been used by some; briefly, the histological hallmarks of each of these stages are as follows:

- Stage 1: destructive changes of interlobular bile ducts with or without granulomas
- Stage 2: proliferation of atypical ductules mostly along the periportal areas
- Stage 3: absence of interlobular bile ducts in most of the portal tracts and portal-to-portal bridging fibrosis
- Stage 4: cirrhosis.

However, in many patients the biopsies reveal changes overlapping between different stages [55].

Diagnosis of PSC is much more difficult in needle biopsies of liver. A wide range of pathological changes from minimal portal fibrosis and inflammatory reaction, changes of biliary obstruction secondary to extrahepatic sclerosing cholangitis, to typical marked concentric periductal fibrosis with chronic inflammation can be seen [56] (Fig. 97.16). Radiological studies are necessary for definitive diagnosis.

Drug-induced liver diseases

Liver biopsy interpretation should always consider the possibility of drug-induced changes as a differential diagnosis [57]. A careful history of drug intake should be considered prior to final morphological diagnosis.

Parenchymal changes

Parenchymal changes resembling acute viral hepatitis are seen in both isoniazid- and methyldopa-induced hepatic damage (Fig. 97.85). Changes resembling mononucleosis pattern are seen in liver disease induced by diphenylhydantoin, paraaminosalicylic acid, and, occasionally, sulfonamides (Fig. 97.86).

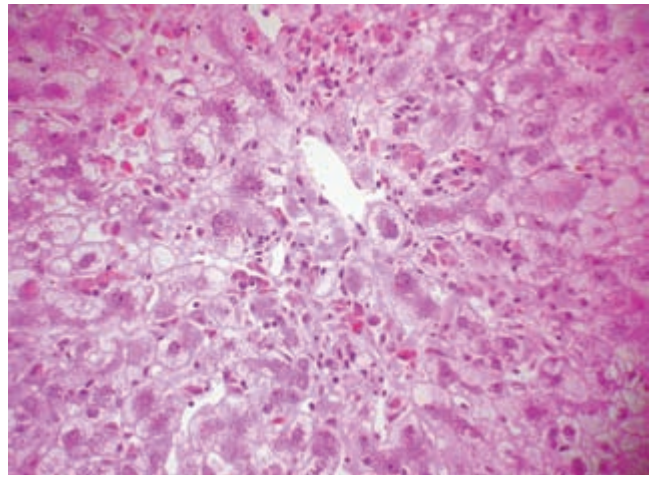

Figure 97.85 Hepatitis-like activity resembling acute viral hepatitis in Aldomet-induced hepatotoxicity. (H & E stain; original magnification × 200.)

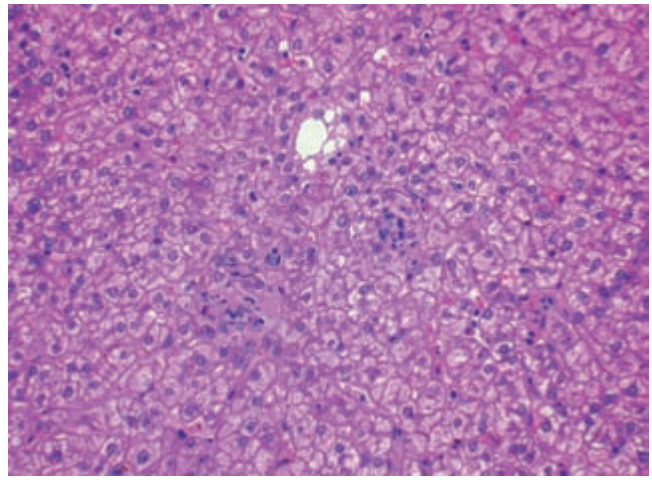

Figure 97.86 Dilantin-induced hepatic changes resembling mononucleosis. (H & E stain; original magnification × 100.)

Coagulative necrosis without much inflammatory reaction is seen in acetaminophen- and halothane-induced damage (Figs 97.29 and 97.30). The necrosis is typically in perivenular and mid-zonal locations. Periportal coagulative necrosis is seen in ferrous sulfate poisoning. Fatty change of a microvesicular type is caused by tetracycline and valproic acid. This is accentuated in zone 3, resembling alcoholic FFC. Macrovesicular fatty change is most commonly seen with corticosteroid and methotrexate use. Carbon tetrachloride and tritetrachlorethylene toxicity results in fatty change as well as liver cell necrosis without inflammatory changes. Hepatocyte necrosis with Mallory body formation can be seen with amiodarone, perhexilene maleate, and with synthetic estrogen preparations used for prostatic carcinoma. Parenchymal cholestasis in zone 3 without inflammation or necrosis can be seen with the use of anabolic steroids and with oral contraceptives. Cholestasis with mild hepatocellular necrosis is associated with total parenteral hyperalimentation, antibiotics such as erythromycin, penicillin, sulfonamides, nitrofurantoin, oral hypoglycemic agents, tranquilizers such as chlorpromazine (Fig. 97.48), antihypertensive agents, captopril, gold therapy, and some antineoplastic agents. Some of these drugs, such as sulfonamides, cause granulomatous necrosis in addition to cholestasis. Rare cases of allopurinol-induced submassive hepatic necrosis have been reported. Morphological patterns of CAH can result from hepatotoxicity from nitrofurantoin, methyldopa, oxyphenisatin, dantrolene, papaverine, and, occasionally, isoniazid. A number of drugs can elicit granulomatous reactions in the liver. Phenylbutazone and sulfonamides cause granulomas resembling sarcoidosis, either portal or parenchymal. Allopurinol, Dilantin, quinidine, nitrofurantoin, methyldopa, Pronestyl, and, rarely, isoniazid can cause a granulomatous reaction, consisting of punched-out areas of hepatocytolysis replaced by hyperplastic Kupffer cells, lymphocytes, and other inflammatory cells in a circumscribed fashion resembling a granuloma. Among the drugs causing hepatocyte inclusions, a ground-glass appearance can be caused by cyanamide, and proliferation of smooth endoplasmic reticulin (SER), resulting in a similar appearance, by phenobarbital, chlorpromazine, and Dilantin [58].

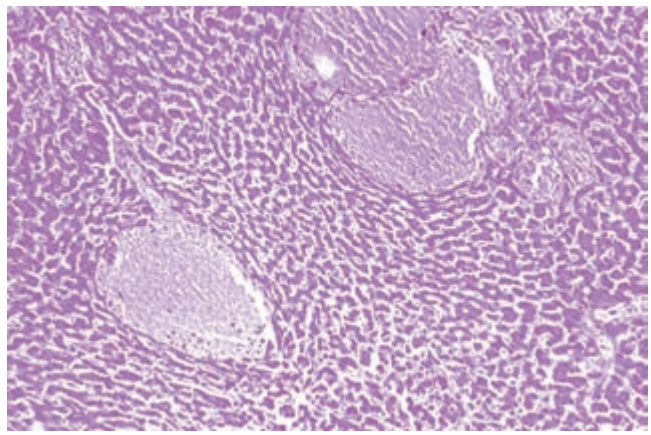

Figure 97.87 Peliosis hepatis with blood-filled spaces without endothelial lining. (H & E stain; original magnification × 100.)

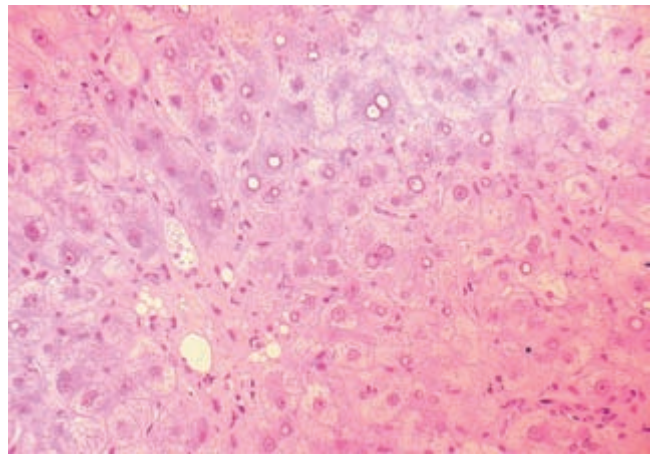

Figure 97.88 Sinusoidal fibrosis and nuclear dysplastic changes in methotrexate toxicity. (H & E stain; original magnification × 400.)

Portal tract changes

Oral hypoglycemic agents and tranquilizers mentioned previously, as well as allopurinol, can cause variable degrees of portal inflammation and bile duct epithelial abnormalities, resembling mechanical duct obstruction or PBC. Dilantin toxicity results in a mononucleosis pattern along with a portal inflammatory reaction with a predominance of eosinophils (Fig. 97.9). Allopurinol causes portal eosinophilia with a cholangitic reaction.

Vascular changes

These include sinusoidal dilation following administration of oral contraceptive agents or anabolic steroids, and in azathroprine therapy, and peliosis hepatis seen in anabolic steroid and methyl testosterone treatment (Fig. 97.87). Venous outflow occlusion with its hepatic morphological changes can be seen as follows: perivenular sclerosis with sinusoidal collagenosis resembling alcohol is associated with hypervitaminosis A, methotrexate (Fig. 97.88), azathioprine, and certain antineoplastic drugs, such as mitomycin C. Venoocclusive disease with coagulative necrosis and severe congestion in zone 3 is seen in toxicity due to bush tea containing pyrrolizidine alkaloids, as well as toxicity resulting from 6-thioguanine and azathioprine. Hepatic vein thrombosis leading to Budd–Chiari syndrome has been associated with prolonged use of oral contraceptives (Fig. 97.25).

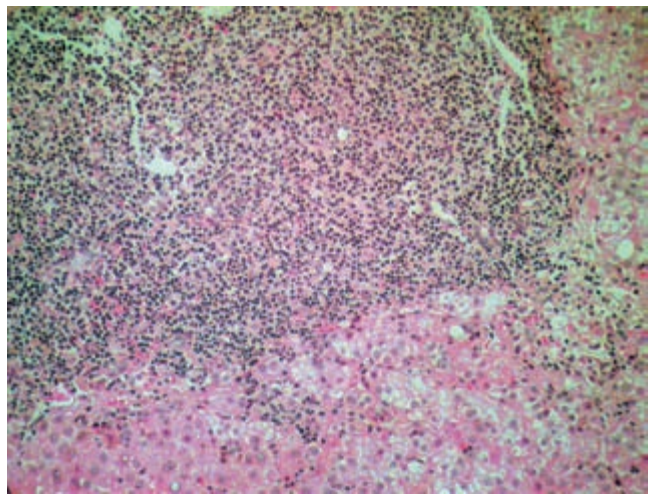

Figure 97.89 Portal infiltrate in non-Hodgkin lymphoma. (H & E stain; original magnification × 100.)

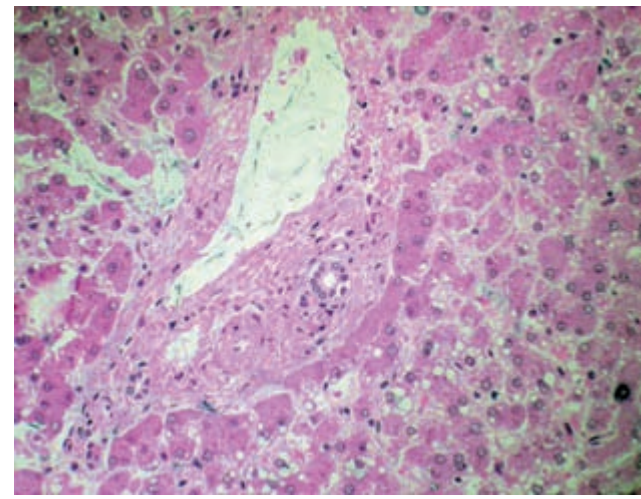

Figure 97.91 Portal area with lymphopenia in a patient with AIDS. (H & E stain; original magnification × 200.)

Liver biopsy changes in systemic diseases

Hematopoietic system

Lymphomas with hepatic involvement reveal monomorphic portal infiltrates of neoplastic lymphocytes (Fig. 97.89). Bile ducts and parenchymal cells are not involved. However, in Hodgkin disease, the portal infiltrate, including atypical or typical Reed–Sternberg cells, seems to produce destructive lesions of bile ducts (Fig. 97.90). In addition, epithelioid granulomas of a nonneoplastic nature are seen in both portal and lobular zones. Cholestasis can also be a prominent feature in Hodgkin disease. Liver biopsies in leukemias reveal immature neoplastic white cells in the sinusoidal spaces as well as in the portal zones (Fig. 97.45). Hairy-cell leukemia exhibits typical leukemic cells in the sinusoidal spaces as well as infiltrating the parenchyma. Sickle-cell disease can be diagnosed by identifying clumped red cells as well as sickled cells in the sinusoidal spaces (Fig. 97.47). Disseminated intravascular coagulation manifests as fibrin thrombi within the sinusoidal spaces.

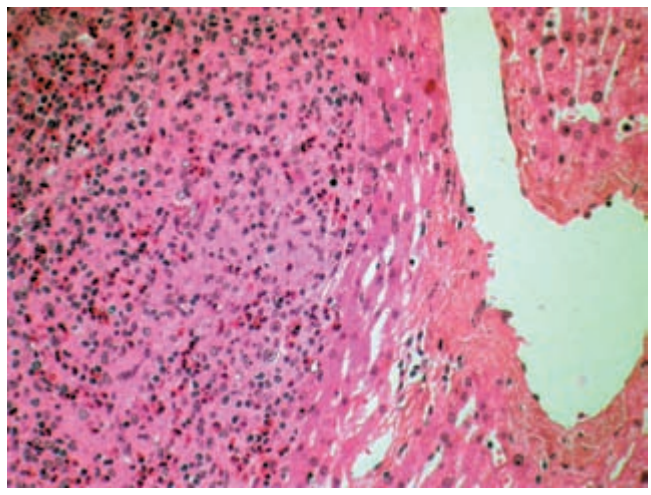

Figure 97.90 Portal infiltrate in Hodgkin lymphoma with an atypical Reed–Sternberg cell. (H & E stain; original magnification × 200.)

Acquired immunodeficiency syndrome (AIDS)

Liver biopsies in patients with AIDS should be examined for evidence of opportunistic infections commonly seen in these patients. In general, the portal areas are relatively lymphopenic, in spite of the inflammatory processes in the liver [59] (Fig. 97.91). *Mycobacterium avium–intracellulare* (MAI) infection in these patients typically results in numerous clusters of bloated Kupffer cells, appearing foamy and containing numerous acid-fast stainable organisms (Fig. 97.63). No true granulomas are seen. Similarly, fungal infections such as cryptococcosis reveal a lack of granuloma formation in the presence of organisms identified by special stains and cultures. Viral inclusion bodies of CMV and herpes simplex involving either the reticuloendothelial cells or duct epithelium cells, or parenchymal cells can be seen. Cryptosporidiosis involving the biliary system can be identified by examining the lumenal surface of the duct epithelial cells using special stain such as Giemsa (Fig. 97.92). Sclerosing cholangitis identified in patients with AIDS has been described as a sequela of CMV and cryptosporidiosis of the biliary system [60]. In patients with Kaposi sarcoma elsewhere in the body, liver biopsy can also exhibit the presence of the tumor [61] (Fig. 97.93). Because these patients are on a number of drugs on a long-term basis, hepatotoxic changes due to the drugs should also be considered in the differential diagnosis.

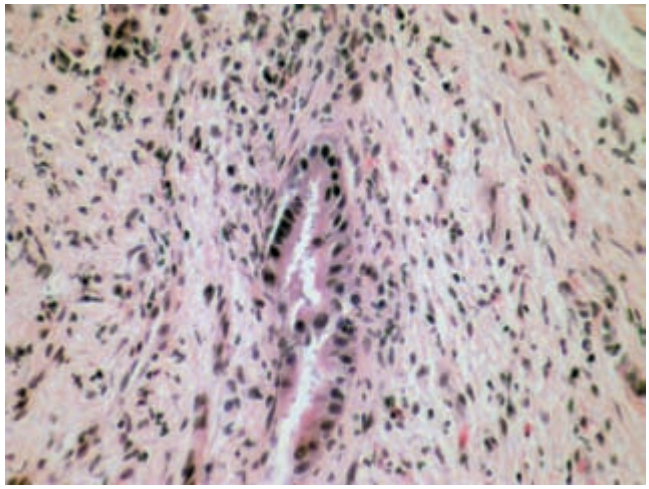

Figure 97.92 Bile duct epithelium along the lumenal surface demonstrates the presence of cryptosporidiosis, which are 3–4-μm size. (H & E stain; original magnification × 400.)

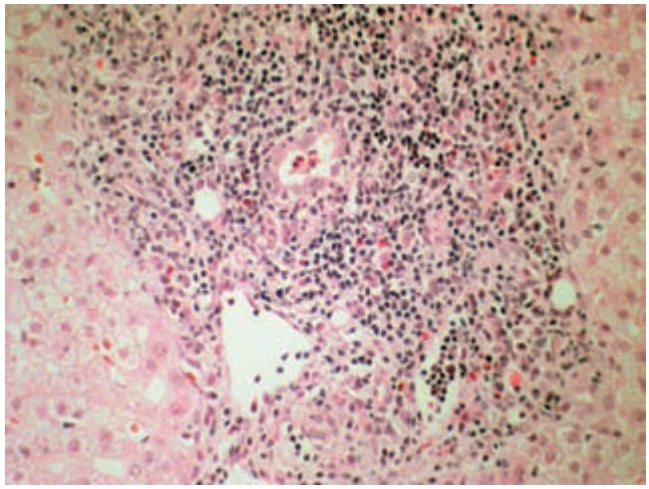

Figure 97.94 Portal inflammatory infiltrate, changes of interlobular bile ducts, and endothelialitis of portal vein radicles in acute rejection. (H & E stain; original magnification × 200.)

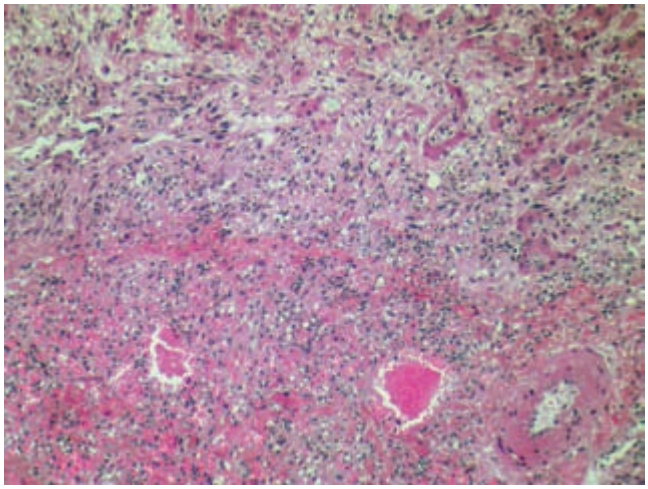

Figure 97.93 Kaposi sarcoma involving the liver. (H & E stain; original magnification × 100.)

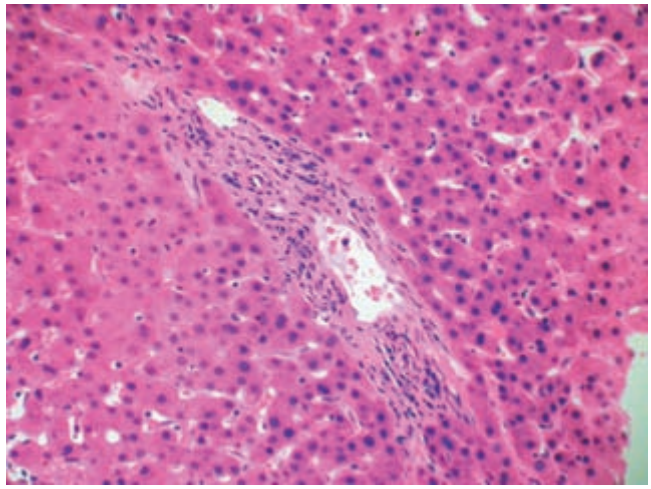

Figure 97.95 Portal inflammatory infiltrate with loss of bile ducts in chronic rejection. (H & E stain; original magnification × 200.)

Pathology of transplantation

Allograft rejection

Acute rejection is a common event seen in varying degrees in 70% of allografts. The histological characteristics, as described by Snover and colleagues [62], include portal inflammatory infiltration, inflammation and destructive changes of interlobular bile ducts, and endothelialitis involving hepatic and portal venous structures (Fig. 97.94). Portal inflammation consists of activated lymphocytes or "immunoblasts," neutrophils, and eosinophils. These cells are seen infiltrating the epithelial cells of interlobular bile ducts. In addition, the duct epithelium reveals pyknosis of the nuclei, hydropic changes, and disruption of basement membrane. Endothelialitis is exhibited as lymphocytes attached to the venous endothelium and infiltrating through the subendothelium. The hallmark of chronic rejection consists of progressive destruction and eventual loss of bile ducts [63] (Fig. 97.95). In addition, there is perivenular cholestasis and multifocal hepatocytolysis. There is also evidence of obliterative vascular lesions involving hepatic arteries and arterioles. Chronic rejection is less common than acute rejection and occurs in about 4% of graft recipients.

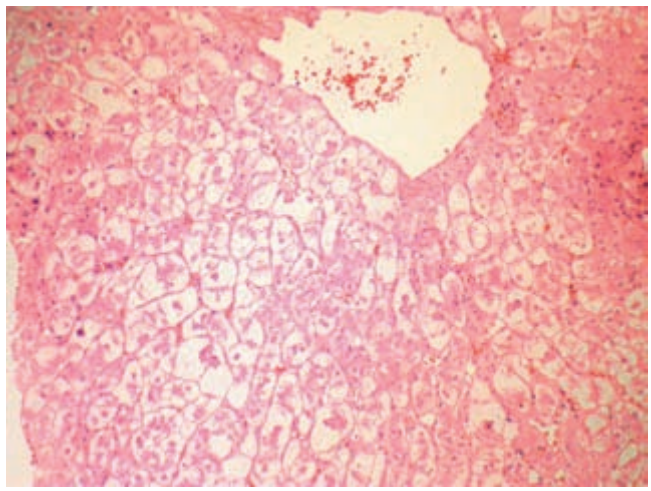

Figure 97.96 Marked ballooning of the hepatocytes in acinar zone 3 representing harvest injury. (H & E stain; original magnification × 100.)

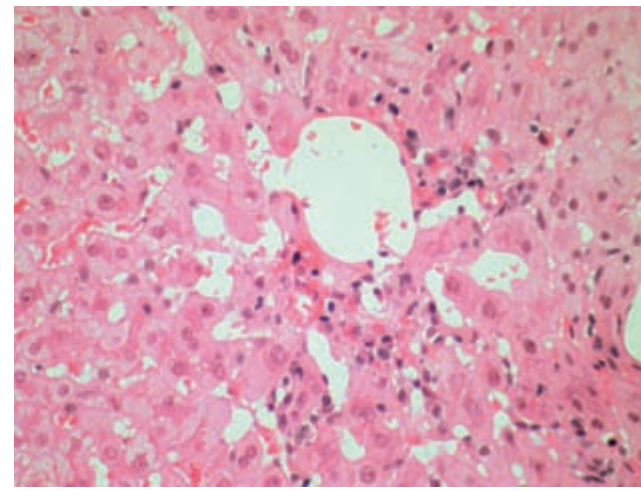

Figure 97.97 Perivenular zone with necroinflammatory changes in recurrent acute hepatitis B infection of allograft. (H & E stain; original magnification × 200.)

Harvest or preservation injury

In the majority (54%) of orthotopic hepatic grafts, there is marked ballooning of the hepatocytes in acinar zone 3 (Fig. 97.96). This usually occurs in the first 2 weeks and in some patients as early as 48 h. This is often associated with cholestasis and high serum alanine aminotransferase (ALT) levels. Ischemia is considered one of the pathogenetic factors. Ballooning without associated necrosis has a better prognosis and is not a sign of graft rejection. Among the other features included under preservation injury are microvesicular fatty change and mild acidophilic degeneration of the hepatocytes.

Cytomegalovirus hepatitis

Cytomegalovirus hepatitis in allografts is often associated with parenchymal microabscesses, seen as small aggregates of neutrophils in the hepatic lobule. Infected cells have large eosinophilic intranuclear inclusions. Immunoperoxidase stains demonstrate these inclusions clearly.

Recurrent hepatitis B in allograft

Liver allograft recipients who had chronic hepatitis B with cirrhosis invariably develop recurrence of hepatitis B infection of the graft. The recurrence usually develops after more than 3 months but can occur earlier in association with an accelerated course. The recurrent HBV infection is similar to that seen in non-liver-graft patients. The acute process consists of lobular disarray and ballooning hepatocytes with necroinflammatory changes (Fig. 97.97). Portal areas reveal inflammatory hyperplasia without any evidence of bile duct epithelial changes or vascular endothelial damage. At this early stage, HBcAg is demonstrable in the hepatocytes by immunoperoxidase stains. Although the acute events can be self-limited, viral infection is never cleared and invariably leads to chronic infection, with progressive increase in the expression of viral antigens in the liver. A unique variant of recurrent hepatitis in the allograft is termed *fibrosing cholestatic hepatitis* [64]. The characteristic pathological changes include periportal fibrosis with thin strands of pericellular collagen, cholestasis, and ballooning of hepatocytes with apoptotic necrosis. There is an abundance of HBsAg and HBcAg demonstrable in hepatocytes. Lymphocytic infiltrates are minimal. This morphology has been associated with a poor outcome. Chronic hepatitis and cirrhosis are the eventual outcomes.

Recurrent hepatitis C

Reinfection of allografts is universal but the disease progression varies widely. Acute changes can be seen as early as 80–90 days posttransplant. They include sinusoidal lymphocytosis, acidophilic bodies, and lobular disarray. Portal lymphoid aggregates, progressive fibrosis, and changes similar to chronic HCV in the non-transplant setting are seen in later stages. A small percentage (2%–10%) of patients with recurrent HCV develop severe fibrosing cholestatic hepatitis similar to that seen in recurrent HBV [65].

Graft-versus-host disease

Graft-versus-host disease is seen in association with bone marrow transplantation and less often with other solid organ transplantation [66]. In the acute stage, there is evidence of hepatocellular necrosis and endothelialitis. In chronic stages, there is extensive bile duct damage and the interlobular bile duct epithelium shows cytoplasmic vacuolization, nuclear hyperchromasia, and atypia. Destructive bile duct changes resemble PBC.

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98

Abdominal cavity: anatomy, structural anomalies, and hernias

Sareh Parangi, Richard A. Hodin

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Embryology of the abdominal cavity

Abdominal cavity

Knowledge of the embryology of the abdominal contents is imperative to understanding the pathophysiology of the various congenital and structural anomalies of the abdominal cavity. The abdominal or peritoneal cavity is formed from a large intraembryonic coelomic cavity during the fourth week of embryonic development. The cells of the somatic mesoderm lining the intraembryonic coelomic cavity become mesothelial and form the parietal layer of the serous membranes lining the outside of the peritoneal, pleural, and pericardial cavities. In a similar manner, the cells of the splanchnic mesoderm layer form the visceral layer of the serous membranes covering the abdominal organs, lungs, and heart [1]. During the fifth to seventh weeks of development, the pleuroperitoneal folds fuse with the septum transversum, and the mesentery of the esophagus thus separates the thoracic cavity from the abdominal cavity. In the cephalic and caudal parts of the embryo, the primitive gut (an endodermal-lined cavity) forms a blind-ending tube, the foregut and the hindgut, respectively. The middle part, the midgut, remains temporarily connected to the yolk sac by means of the vitelline duct or yolk stalk. The upper gastrointestinal tract develops from the foregut and includes the esophagus, stomach, duodenum, liver, gallbladder, and pancreas. The midgut develops into the small bowel and the right and transverse colons. Development of the hindgut forms the left colon, sigmoid, rectum, and the upper part of the anal canal.

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Development of the foregut, midgut, and hindgut

During embryonic development, the foregut rotates 90° clockwise around its longitudinal axis, causing the left side of the stomach to face anteriorly and its right side posteriorly (Fig. 98.1). The foregut blood supply is the celiac artery.

The development of the midgut is characterized by rapid elongation of the gut and its mesentery, resulting in the formation of the primary intestinal loop. Over its entire length, the midgut is supplied by the superior mesenteric artery. As a result of this rapid elongation and the simultaneous expansion of the liver, the abdominal cavity of the fetus becomes too small to contain all the intestinal loops. These loops enter the extraembryonic coelomic cavity in the umbilical cord, forming a physiological umbilical herniation during the sixth week of development (Fig. 98.2). Coincident with this rapid growth, the midgut rotates 270° counterclockwise around the axis of the superior mesenteric artery. At about the third month, the herniated intestinal loops return to the abdominal cavity. The proximal portion of the jejunum is the first part to reenter, and it comes to lie on the left side, whereas the primitive cecum is the last to reenter and descends into the right iliac fossa.

The hindgut gives rise to colon and rectum past the distal third of the transverse colon as well as the upper part of the anal canal. The junction of the endodermal and ectodermal parts of the anal canal is formed by the pectinate line, which is found just below the anal columns. The endoderm of the hindgut also gives rise to the internal lining of the bladder and urethra. The hindgut blood supply is the inferior mesenteric artery.

Mesenteries of the abdominal cavity

During the development of the peritoneal cavity, the splanchnic mesoderm covers the developing gut. Eventually, most of this ventral mesentery is resorbed, except for a small portion between the liver and the stomach that persists as the

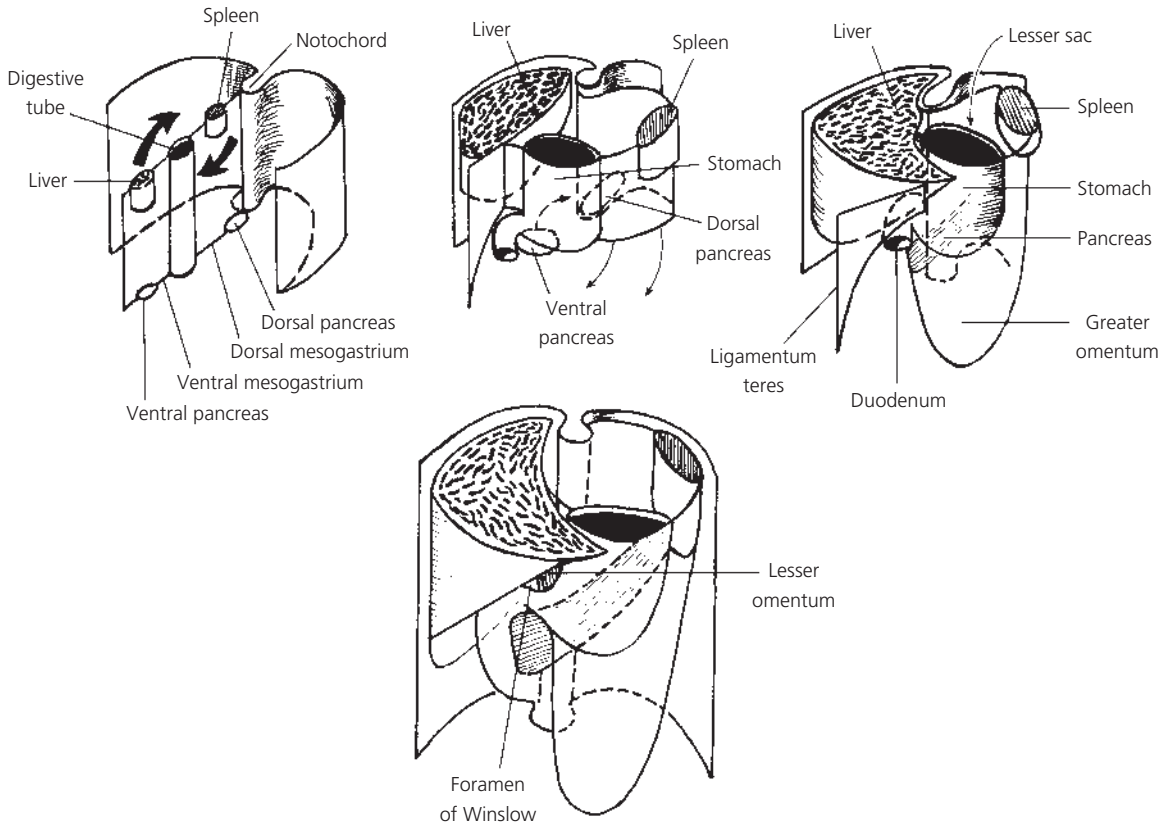


Figure 98.1 Embryonic development of the foregut. The organs of the upper gastrointestinal tract rotate 90° clockwise (when viewed from above) in a transverse plane. Originally, ventral structures like the liver and ventral pancreas are situated to the right, and posterior organs such as the spleen and dorsal pancreas and the dorsal aspect of the future stomach are positioned to the left side of the abdominal cavity. The dorsal mesogastrium elongates and folds to the left to form the greater omentum.

gastrohepatic ligament (or lesser omentum) (see Fig. 98.1), and the ligaments of the liver, i.e., the coronary, falciform, and teres. The dorsal mesogastrium remains and changes in position and size; the spleen is positioned to the left, the pancreas is situated posteriorly, and the space within the mesogastrial fold becomes the lesser sac. The dorsal mesogastrium enlarges, forming the greater omentum, which drapes anteriorly. As the midgut elongates during development, the dorsal mesodermic attachment elongates as well and forms the mesentery. The right colon adheres to the posterolateral abdominal wall and becomes retroperitoneal; the rest of the midgut is intraperitoneal.

Anatomy of the abdominal cavity

The abdominal cavity in the adult is defined by the diaphragm superiorly, the abdominal walls laterally, and the pelvis inferiorly. The peritoneal covering is the endodermal investment of the surface of the organs of the abdominal cavity. The pancreas, parts of the duodenum, right and left colon, and the rectum, being retroperitoneal, are only covered anteriorly by peritoneum. The organs are supported by thickened bands of peritoneum: the gastrohepatic, gastrosplenic, splenorenal, and splenocolic ligaments. The liver is suspended from the diaphragm also by the falciform and coronary ligaments and is joined to the abdominal wall by the ligamentum teres that contains the remnant of the umbilical vein.

The abdominal cavity has three compartments: lesser sac, supramesocolic, and inframesocolic. The lesser sac is limited by the stomach and gastrocolic ligaments anteriorly; by the spleen, splenorenal, and gastrosplenic ligaments on the left side; and by the pancreas and duodenum posteriorly (see Fig. 98.1). The lesser sac communicates with the rest of the abdominal cavity through the foramen of Winslow. The boundaries of the foramen are the liver superiorly, hepatic pedicle anteriorly, duodenum and pancreas inferiorly, and the retroperitoneum posteriorly.

The supramesocolic compartment lies cephalad to the transverse mesocolon and contains the duodenum, pancreas, liver, spleen, and lesser sac. The inframesocolic compartment contains the small intestine, colon, and suspensory ligaments of the spleen and liver. The area between the colon and the inferior margin of the liver is called the Morison pouch and

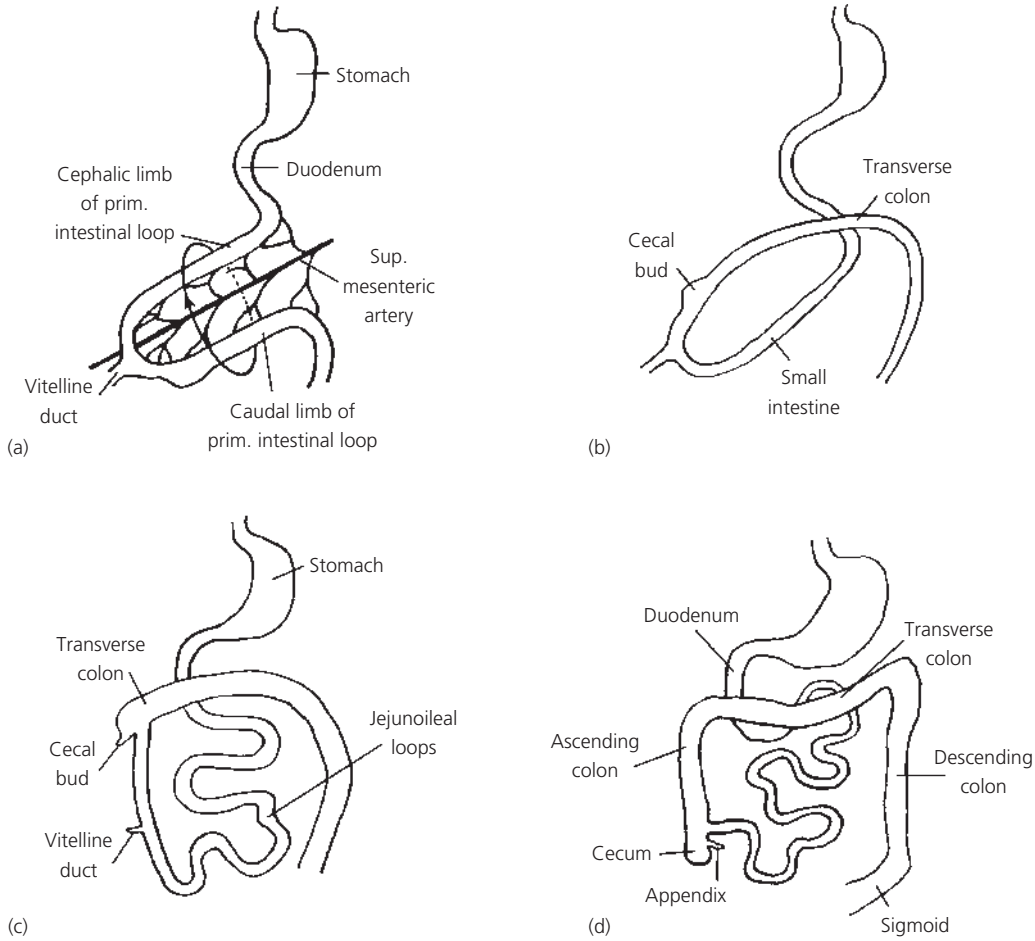


Figure 98.2 Embryonic development of the midgut. **(a)** Schematic drawing of the primary intestinal loop before rotation (lateral view). The superior mesenteric artery forms the axis of the loop, and the arrow indicates the counterclockwise rotation around this axis. **(b)** Similar views as in **(a)** showing the primary intestinal loop after 180° counterclockwise rotation. The transverse colon is shown as passing in front of the duodenum. **(c)** Anterior view of the intestinal loop after the 270° counterclockwise rotation. Note the cecal bud in the right upper quadrant. **(d)** Similar views as in **(a)** with the intestinal loops in the final position. Cecum and appendix are now in the right lower quadrant. Adapted from Sadler [1].

acts as a potential space for fluid accumulation. In addition, fluid can accumulate along the pouches created by the right and left parietocolic peritoneal coverings.

The inferior limit of the peritoneum covers the pelvic urogenital organs and the inguinal region. Most inferiorly is the pouch of Douglas, a peritoneally covered pouch between the rectum and uterus in females and the rectum and bladder in males.

The anatomy of the inguinal region (the groin) is complex and is discussed in the section Groin hernias.

Congenital anomalies

In the embryo, the abdominal cavity is too small to accommodate the intestines, and a physiological herniation occurs into the umbilical cord with return of the intestines to the abdominal cavity by the 10th week. *Omphalocele* (Fig. 98.3a) is failure in the growth of the coelomic walls with a large herniation of the abdominal viscera into the base of the umbilical cord. The herniated viscera are covered by a sac composed of amnion. The incidence of omphalocele is estimated to be 1 in 6000 to 1 in 10 000 live births. *Gastroschisis* (Fig. 98.3b) is a similar anomaly, but the defect in the abdominal wall is located lateral to the umbilicus and is caused by failure of closure of the abdominal wall. No sac is present to cover the herniated intestines, and the size of the defect is often smaller. In gastroschisis, the intestines can be inflamed and thickened, and the mesentery may be foreshortened. The incidence of gastroschisis is 1 in 3200 to 1 in 10 000 births, with some male predominance; associated congenital anomalies include incomplete rotation and fixation of the midgut in 40% of cases.

(a) (b)

Figure 98.3 (a) Omphalocele with large herniation of the abdominal viscera. Note the amnionic sac covering the viscera. **(b)** Gastroschisis with defect lateral to the umbilicus. Note that the herniated intestines are engorged and discolored and that no sac is present.

At least 50% of infants with omphalocele have associated anomalies of the skeleton, gastrointestinal tract, or nervous or genitourinary system [2]. Ultrasound can detect these defects prenatally, and vaginal delivery is safe for all newborns except those with the biggest omphaloceles, which may contain liver [3]. Both conditions are surgical emergencies of the newborn. The airway should be managed first, and a nasogastric sump tube should be placed to prevent intestinal distention. If present, the sac is painted with antiseptic solutions, and the herniated contents are kept moist and covered with warm, moistened, saline-soaked gauze and plastic to prevent dehydration and desiccation. Alternatively, the entire lower torso can be covered by a plastic intestinal bag. Once stable, an operation is needed to reduce the herniated viscera and perform a fascial closure. If necessary, the viscera are wrapped in a sheet of silicone (Silastic chimney) that is sutured to the abdominal wall, allowing time for growth of the abdominal wall and eventual reduction of the herniation.

Remnants of the omphalomesenteric (vitelline) duct may present as abnormalities related to the abdominal wall or remain asymptomatic for long periods. An *umbilical sinus* results from the continued presence of the umbilical end of the duct and can result in persistent drainage and infection at the umbilicus. Persistence of the entire duct is heralded by passage of enteric contents from the umbilicus, and excisional treatment avoids complications of intussusception or volvulus. Cystic remnants should be excised if seen at operation or if presenting as an infection at the umbilicus. *Meckel diverticulum* results when the intestinal end of the vitelline duct persists. This is a true diverticulum, composed of all layers of the intestinal wall.

Congenital diaphragmatic hernias

The diaphragm appears in the third week of development as a septum transversum that separates the thorax from the abdomen. The diaphragm is derived from the following structures: the septum transversum, which forms the tendinous part of the diaphragm; the pleuroperitoneal membranes; the muscular components from the lateral and dorsal body walls; and the mesentery of the esophagus, which forms the crura of the diaphragm (Fig. 98.4a). A diaphragmatic hernia is one of the more common malformations of the newborn (1 in 2200) and is most frequently caused by failure of the pleuroperitoneal membranes to separate completely the peritoneal and pleural cavities posteriorly [4].

Posterolateral hernia (hernia of Bochdalek) is usually large and on the left side; the intestinal loops, stomach, spleen, and part of the liver may enter the thoracic cavity (Fig. 98.4). The presence of abdominal viscera in the chest results in compression of the heart and hypoplasia of the lung. Animal models have shown that long-term compression of the lung leads to abnormal development of the lung and the pulmonary vascular bed [5]. The neonate presents with acute respiratory distress; the heart, mediastinum, and lungs are displaced to the right, and the abdomen may be scaphoid. Respiratory sounds are absent on the affected side, and heart sounds may be audible in the right chest. The diagnosis is easily made with a chest radiograph showing one or more air–fluid levels in the left thorax, displacement of the mediastinum, and loss of the sharp diaphragmatic line separating the thorax and abdomen (Fig. 98.4b). Radiological studies with water-soluble contrast show the intraabdominal contents in the left chest. Lung hypoplasia results in acute respiratory distress and is the main cause of death in these patients. Bowel obstruction, strangulation, and other gastrointestinal malformations can result in other complications. Mortality depends on the age of the patient, associated malformations, and most importantly the degree of lung hypoplasia. Mortality rates are as high as 50% in those infants requiring emergency operations during the first week of life but decrease to 10% in infants with a higher degree of lung maturity. Extracorporeal membrane oxygenation is

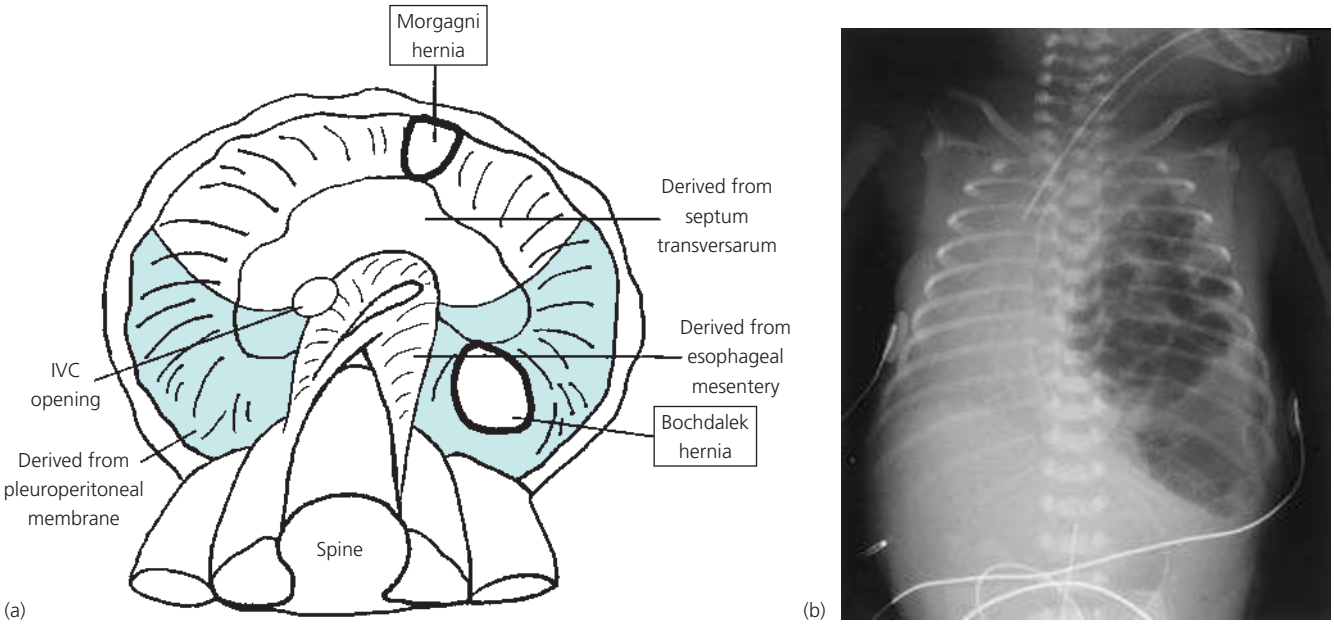


Figure 98.4 Development of the diaphragm and congenital diaphragmatic hernia. **(a)** Abdominal surface of the diaphragm and the derivation of the components during development. The pleuroperitoneal membranes, the septum transversum, and the esophageal mesentery form the diaphragm. A Bochdalek hernia forms when there is a posterolateral defect. Morgagni hernias are less common and are present anteriorly. **(b)** Chest radiograph of a child with a congenital posterolateral (Bochdalek) diaphragmatic hernia on the left. The mediastinum is displaced to the right by the intestinal loops present in the left chest.

used in some instances to support the maturing lungs before and after repair of the hernia [6].

Occasionally, a small part of the muscular fibers of the diaphragm fails to develop, and a hernia may remain undiscovered until later in life. Such a defect is seen in the anterior portion of the diaphragm and is known as a *parasternal hernia* (hernia of Morgagni; see Fig. 98.4a). These hernias are usually small and can contain stomach, omentum, colon, or small intestine with a peritoneal covering.

Umbilical hernias of childhood

Herniation of intraabdominal viscera through the umbilicus is an *umbilical hernia*. A congenital defect in the umbilical ring during development of the abdominal wall results in this hernia. Congenital umbilical hernias are more common in black and male infants, most not reaching maximal size until the infant is 1 month of age. The younger the neonate, the more common this congenital deformity, reaching 20% in premature infants. Predisposing conditions include Down syndrome, gargoylism, amaurotic familial idiocy, cretinism, Beckwith–Wiedemann syndrome, and diseases that increase intraabdominal pressure. This type of hernia has a high propensity to reduce and heal spontaneously, so that repair is generally not indicated in children younger than 3 years of age [7]. Large or symptomatic hernias, especially with incarceration or strangulation, require immediate attention.

Hernias of the abdominal cavity in adults

> Hernia is a protrusion of any viscus from its proper cavity (Sir Astley Cooper, 1804).

Hernias are composed of a herniated viscus, the hernia sac, and the opening of the hernia (the hernial ring) (Fig. 98.5). The hernial sac is the internal wall of the hernia lined by peritoneum. An external hernia comprises a viscus abnormally located outside the abdomen; an internal hernia is when the viscus is abnormally located within the intraabdominal space. Any viscus can become part of a hernia, but most commonly a segment of intestine is partially or completely herniated.

A *Richter hernia* occurs when only a portion of the wall of the herniated bowel is included in the hernia (Fig. 98.5). Such a hernia is unique in that it can strangulate without causing complete small bowel obstruction. Herniation of two adjacent loops through the same hernial opening is called a *Maydl hernia* (Fig. 98.5). This kind of hernia is dangerous because the closed loop may perforate, leading to peritonitis. A *sliding hernia* is when a partially retroperitoneal structure (such as colon) is part of the wall of the hernial sac (Fig. 98.5). The symptoms of hernias depend on a number of factors, such as the exact viscus that is herniating, the location of the herniated bowel (i.e., jejunum or ileum), and whether there is a full or partial strangulation. When omentum alone is strangulated within a hernia, obstruction of the gut does not occur.

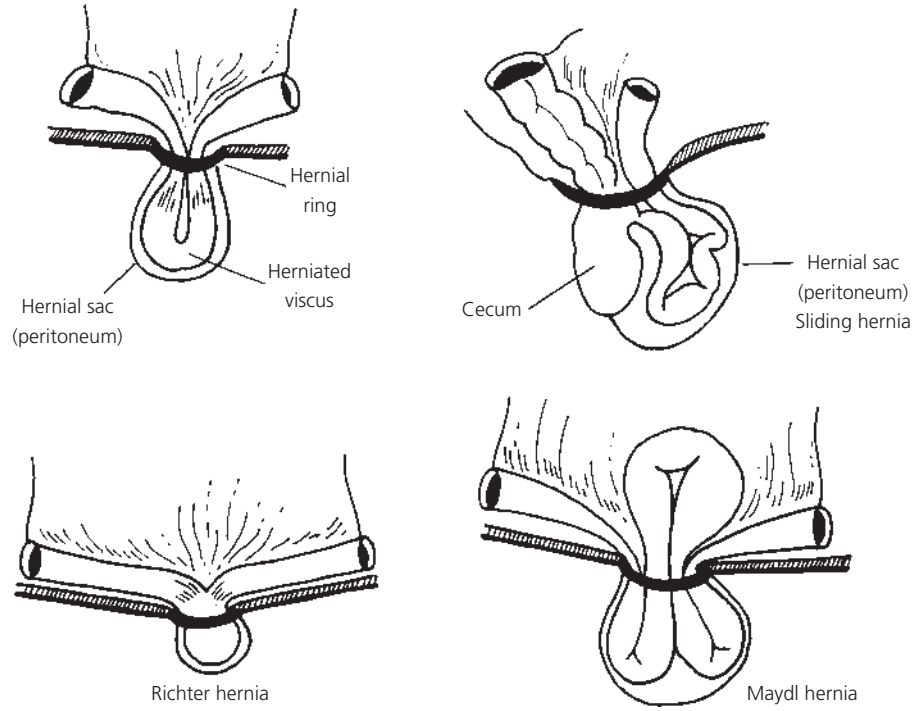


Figure 98.5 Different types of intestinal hernias.

A *reducible hernia* occurs when the hernial sac contents return to the abdominal cavity spontaneously or with external manipulation. The major complications of hernias are incarceration, bowel obstruction, and bowel strangulation. A complication is more likely to occur if the hernial opening is small and in hernias in which the bowel has access to the sac. Bowel incarceration greatly increases the chance that strangulation will occur. An *incarcerated hernia* occurs when the hernial contents cannot be dislodged from the sac. Incarceration occurs when there are adhesions from the hernial contents or around the opening of the hernia, making reduction impossible, or when there is edema or contortion of the hernial contents such that hernial contents once entered into the sac cannot exit. A hernia, reducible or incarcerated, becomes a *strangulated hernia* when the blood supply to the herniated viscus is compromised.

Epidemiology

Hernias are common occurrences; 5% of people have a spontaneous abdominal hernia during their lifetime. Many people also develop secondary hernias, most frequently at the site of a previous incision or injury to the abdominal wall. Hernia is the most common cause of intestinal obstruction worldwide, and in the USA it is the third most common cause. In surgical practice, 10%–15% of all procedures are related to hernias. Of all hernias, 90% are incisional, umbilical, or inguinal hernias. Groin hernias account for 85% of all hernias (Table 98.1). Indirect inguinal hernias are the most common, followed by direct inguinal hernias. Incisional hernias develop in 3%–5% of all abdominal operations and represent 7% of abdominal wall hernias. Internal hernias are uncommon and have been seen in 0.5% of autopsies.

There is a male to female prevalence in inguinal hernias of about 7 : 1, whereas there is a female dominance in femoral, umbilical, and incisional hernias of about 2 : 1. Although femoral hernias are seen more commonly in females, the most common hernia in women still remains an indirect inguinal hernia.

Table 98.1 Epidemiology of groin hernias[a]

Type	Occurrence (%)
Inguinal	80
Indirect	60
Direct	30
Both	10
Femoral	5
Inguinal and femoral	2
With a sliding component	12
Sigmoid	8
Cecum	4
Maydl hernia	2

a Groin hernias account for 85% of all hernias.
Adapted from Ponka JL. Hernias of the Abdominal Wall. Philadelphia: WB Saunders, 1980; Nyhus LM, Condon RE (eds). Hernia. Philadelphia: JB Lippincott, 1978.

Pathophysiology

Our understanding of the pathophysiology of inguinal hernias has changed over the years. In the past, indirect hernias were attributed at least in part to a patent processus vaginalis, which is present in many infants and up to 25% of young children. However, more recent studies have shown the inguinal ring and any patent processus vaginalis to close with a valve-like effect because of tension on the abdominal wall musculature when there is increased intraabdominal pressure [8]. In cases of indirect inguinal hernia, this valve-like effect may become ineffective, allowing a viscus to enter the patent processus vaginalis [9]. Chronic injury in the form of traumatic overstretching of muscles and aponeurotic structures undoubtedly contributes to hernia formation, although rigorous scientific studies have not been performed. Clinicians often see the onset of herniation related to such activities as extreme athletics or conditions associated with increased intraabdominal pressure such as pregnancy, chronic cough, chronic obstructive pulmonary disease, obstructive uropathy, massive ascites, or obstipation from colorectal cancer.

In cases of incisional hernias, infection or tension during the wound healing or scar remodeling phase can lead to weakened musculoaponeurotic tissue, which in turn leads to herniation at a later date. Some studies have shown altered collagen formation and breakdown in tissue samples from the inguinal floor of patients with hernias [10].

Diagnostic considerations

Most abdominal wall hernias are detected using history and physical examination. The patient needs to be questioned regarding the presence of a bulge, conditions that make the bulge appear and disappear, and any associated pain or gastrointestinal symptoms. History of any previous hernias, abdominal wall incisions, or a family history of any connective tissue disorder such as Marfan syndrome should be sought. A review of systems to include any of the above-mentioned causes of intraabdominal pressure should be obtained because failure to recognize these factors will increase the chance for recurrence if repair is undertaken. A complete physical examination of the abdomen and rectum should be done, including a prostate examination and testing of the stool for occult blood. All scars should be noted. The examination is best initiated with the patient in the standing position because gravity tends to pull the intraabdominal contents into hernias. If no bulge is obvious, a Valsalva maneuver is used to increase intraabdominal pressure. If a bulge is seen, a gentle attempt to reduce the hernia can be made with the patient standing or in the recumbent position. Examination of the area in the recumbent position often allows better palpation of the hernial defect and opening.

Radiographic studies are rarely needed but may be useful in cases in which the abdominal wall fat layer prevents accurate diagnosis. Ultrasound is rarely helpful, but in some cases it can delineate the abdominal wall musculature and note the presence of air or peristalsing bowel. Computed tomography (CT) is good at detecting abdominal and pelvic defects; use of the Valsalva maneuver may sometimes be helpful during imaging [9,11].

Specific types of hernia

Epigastric hernias

Epigastric hernias occur in the midline of the abdominal wall between the umbilicus and the xiphoid in 5% of the population (males more than females). Most epigastric hernias are asymptomatic. An area of congenital weakness in the linea alba allows protrusion of bowel, omentum, or just preperitoneal fat. Symptoms range from a small painless nodule below the xiphoid to epigastric pain with exertion or rarely small bowel obstruction. Diagnosis is easy if the hernia is large but can be difficult if there is a small hernia in an obese patient. Tenderness while palpating the linea alba with elevation of the head can sometimes be produced in small hernial protrusions. These hernias should be surgically repaired. About 20% of patients have multiple hernial defects in other locations along the linea alba [12] and this should be taken into consideration at the time of repair.

Umbilical hernias

Umbilical hernias in adults often occur in obese people, multiparous women, and patients with ascites. The diagnosis is usually self-evident with a protuberant mass at the umbilicus. Incarceration of omentum or small bowel is common, and up to 30% of cases present with strangulation of bowel. Of patients with ascites and end-stage liver disease, 40% have umbilical hernias. In this setting, ulceration and perforation of the hernia can lead to peritonitis with a high mortality rate [13]. Discoloration or ulceration of overlying skin heralds imminent rupture. Umbilical hernias in adults generally need surgical repair. Small defects can be repaired primarily if there is no tension; defects larger than 3 cm should be repaired with prosthetic mesh or by using some other method to ensure tension-free repair (e.g., release of the lateral fascial attachments). Patients with ascites should have their ascites controlled before repair, otherwise the repair will likely fail. In some cases of refractory ascites, portacaval shunt or transjugular intrahepatic portacaval shunt allows successful surgical herniorrhaphy after the ascites has disappeared. Alternatively, concomitant peritoneovenous shunt and herniorrhaphy should be considered to prevent breakdown of the repair [14]. Liver transplantation is another alternative in the appropriate candidate.

Groin hernias

Hernias of the groin are the most common of all hernias. The inguinal anatomy is defined superiorly by the arch of the aponeurosis of the transversus abdominis muscle and inferolaterally by the upper ramus of the pubis and the psoas muscle. The inguinal canal is about 4 cm in length and is located

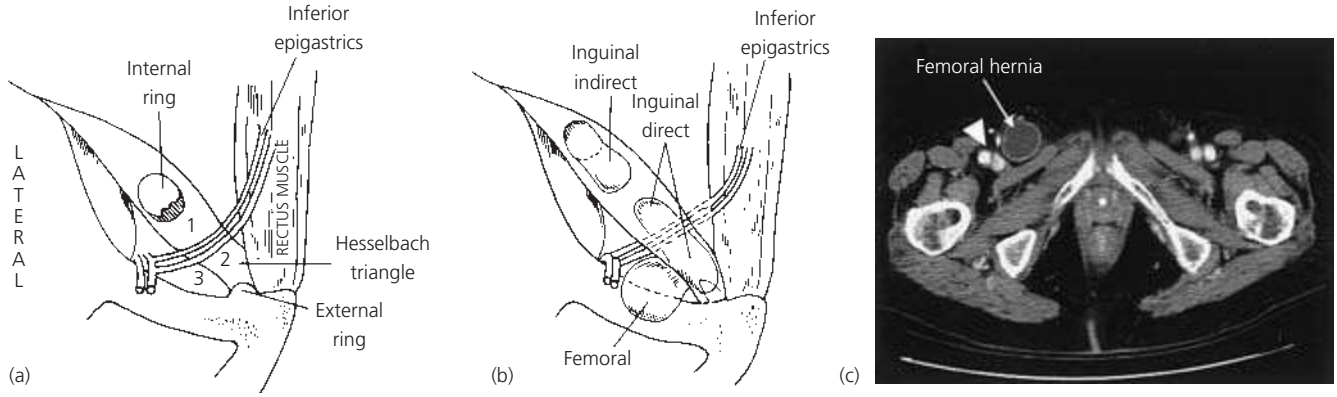


Figure 98.6 The right inguinal region. **(a)** Schematic anatomy showing internal and external rings and Hesselbach triangle. Spaces where hernias occur include Hesselbach triangle (2), the inguinal canal (1, 2), and the femoral space (3). **(b)** The most common groin hernias are direct and indirect hernias above the inguinal ligament and femoral hernias below the inguinal ligament. **(c)** Computed tomography of an incarcerated femoral hernia on the right. Note that a loop of small intestine is present medial to the femoral vessels; the vessels are marked by a white arrowhead.

just above the inguinal ligament between the internal and external rings (Fig. 98.6). The inguinal canal contains the spermatic cord in males (vas deferens, spermatic artery and vein, and cremasteric muscle) and the round ligament in females, in addition to the ilioinguinal nerve. The superior entrance to the inguinal canal is the internal ring (the deep inguinal ring) and is an outpouching of the transversalis fascia lateral to the inferior epigastric vessels. The external ring is a slit-like opening between the diagonal fibers of the aponeurosis of the external oblique muscle (Fig. 98.6a). The anterior wall of the inguinal canal is composed mainly by the aponeurosis of the external oblique. The superior wall is formed by the arching fibers of the internal oblique and transverse abdominal muscles, otherwise called the conjoined tendon. The inferior wall is formed by the inguinal ligament (the folded aponeurosis of the external oblique) that forms a shallow ledge, and more medially by the lacunar ligament along the pectineal line of the pubis. The inguinal ligament is the landmark between the abdomen and thigh. It is fixed medially to the pubis and laterally to the anterosuperior spine of the ilium and fascia of the thigh. The pectineal ligament (Cooper ligament) lies between the pubic tubercle and the femoral vein. The posterior wall (floor) is formed mainly by transversalis fascia; medially, this wall is reinforced by formation of the conjoint tendon (inguinal falx), the merging of the internal oblique and transverse abdominal aponeuroses. The floor or the posterior wall has no intrinsic strength, and as the fibers of the transversalis fascia deteriorate, a direct hernia results [15]. The Hesselbach triangle is bounded by the inguinal ligament inferiorly, the inferior epigastric vessels laterally, and the lateral border of the rectus medially (Fig. 98.6a). A weakness in the floor of this triangle (the transversalis fascia) results in a direct inguinal hernia. Hernias protruding lateral to the Hesselbach triangle are termed *indirect hernias* (Fig. 98.6b). Inguinal hernias include direct and indirect hernias found above the inguinal ligament, and femoral hernias are found below the inguinal ligament in the femoral canal. The inferior epigastric artery serves as an important defining anatomical landmark. Indirect hernias originate lateral to this artery and protrude into the inguinal canal along the spermatic cord. Direct hernias are located medial to the inferior epigastric artery and come through a weakened inguinal floor composed of the transversalis aponeurosis and fascia (Fig. 98.6). A *pantaloon hernia* is composed of both direct and indirect components. *Sliding hernias* have one wall composed of colon or bladder. A special type of indirect inguinal hernia containing a Meckel diverticulum is *Littré hernia*.

The main symptom of groin hernia is a bulge in the inguinal area that appears intermittently or with increased intraabdominal pressure. Pain is usually mild; local pain or colicky abdominal pain is suggestive of incarceration or strangulation. Palpation should be performed in the standing and recumbent position and using Valsalva maneuver to sustain increased intraabdominal pressure. Both sides of the groin and scrotum should be examined. To perform a digital examination, the index finger is introduced into the inguinal canal through the external inguinal ring by invaginating the skin in the area (Fig. 98.7). Using the index finger, the external inguinal ring is first palpated, and with a small further push the internal ring can be palpated. Using this simple technique, an indirect hernia can be felt exiting the internal ring at the fingertip, and a direct hernia can be felt more medial to the finger. In femoral hernias the inguinal canal has no hernia, but a mass is felt below the inguinal ligament in the medial thigh near the saphenofemoral junction, medial to the femoral vein. Obesity makes the examination difficult, especially in detection of femoral hernias, and radiological studies may be helpful [16] (Fig. 98.6c).

The differential diagnosis of an inguinal bulge includes lipoma, tumors, lymphadenopathy, abscess, cysts, endometrial

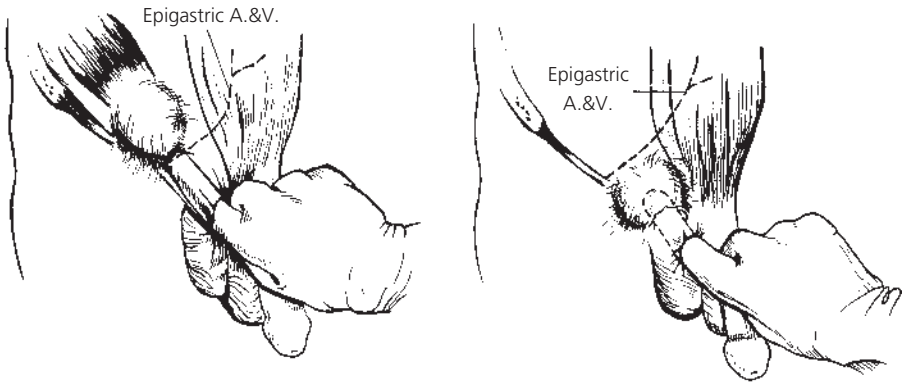


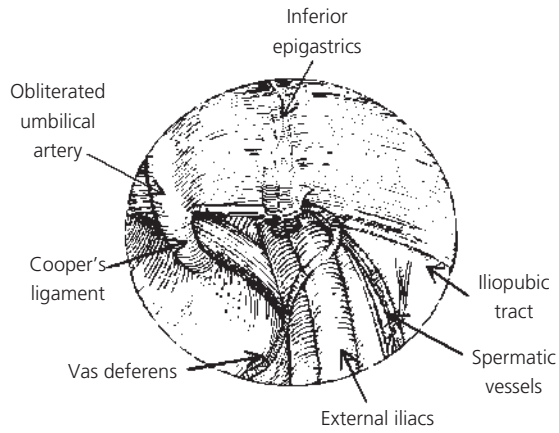
Figure 98.7 Digital examination of inguinal hernias. **(a)** Indirect inguinal hernias are palpated in the inguinal canal at the fingertip. **(b)** Direct inguinal hernias are palpated on the sides of the examiner's finger.

deposits, testicular torsion in an undescended testicle, hydrocele, varicocele, pseudoaneurysm, and saphenous varix. Strangulated hernia can present with signs and symptoms of small bowel obstruction with the addition of a painful, tender, or tense swelling in the groin. Without an operation, it is difficult to determine whether bowel loops are strangulated; therefore, all painful tense hernias should be considered strangulated and repaired imediately. Because both strangulated inguinal and femoral hernias require urgent operations, differentiating them is of intellectual but not practical interest [17].

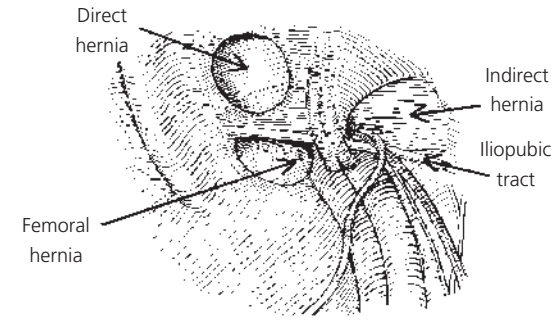
Surgical treatment is generally recommended for most inguinal and femoral hernias, because of the chance of strangulation (5% in indirect hernias and up to 30% in femoral hernias). Watchful waiting may be a reasonable option in men whose inguinal hernias are minimally symptomatic. In one randomized study of 336 patients assigned to watchful waiting over the subsequent 4 years, two required surgery due to strangulation or small bowel obstruction caused by the inguinal hernia; and 23% of patients assigned randomly to watchful waiting chose to have their hernias repaired in the 2-year period of the study, most commonly due to increase in pain [18]. Elective repair can be undertaken as outpatient surgery using general anesthesia, spinal anesthesia, intravenous sedation, or local anesthesia alone. Repair of the hernia requires delineation of anatomy, identification of the hernia sac, and reduction of sac contents. Indirect hernia sacs are ligated at the level of the internal inguinal ring; direct hernia sacs are pushed below the repair site. Suitable native tissue can be used for repair. The modified Bassini repair apposes the shelving edge of the inguinal ligament to the conjoint tendon (inguinal falx), the merging of the internal oblique and transverse abdominal aponeuroses. The Shouldice repair is similar but uses an overlapping technique with two rows of sutures. The McVay repair involves opening the inguinal floor and approximates the conjoint tendon and transversalis fascia to the pectineal ligament (Cooper ligament); this repair is suitable for femoral hernias. Relaxing incisions of the anterior rectus sheath are used to reduce tension on repairs using native tissue.

Polypropylene mesh repair is now widely used as standard repair for all direct and larger indirect hernias. The Lichtenstein repair involves overlay of the mesh along the inguinal floor underneath the spermatic cord; the mesh is sutured to the shelving edge of the inguinal ligament, the pubis medially, and the internal oblique fascia superiorly. A slit in the mesh allows passage of the spermatic cord [19]. Recurrence rates are thought to be somewhat lowered by the use of mesh, but a higher risk for infection must be taken into consideration [20].

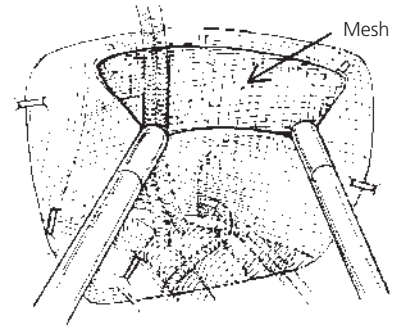
The laparoscopic approach to repair of hernias has been popularized. A transabdominal or preperitoneal approach can be taken, and knowledge of anatomical considerations is key [21] (Fig. 98.8a). The transabdominal, preperitoneal, and totally extraperitoneal repairs are the most commonly used laparoscopic approaches [22]. After reducing the hernia contents, the repair is performed by securing a sheet of polypropylene mesh over the internal aspect of the inguinal floor and internal ring area using surgical clips or staples (Fig. 98.8b,c). Proponents of laparoscopic inguinal hernia repair cite advantages such as improved cosmesis, less postoperative discomfort, earlier return to work, and easier repair of bilateral hernias. The laparoscopic approach also has disadvantages. Complications related to laparoscopy, such as intestinal or vascular injury during trocar insertion, potential for adhesions, the need for general anesthesia, and increased cost, are potential downsides of laparoscopic repair. Prospective randomized trials have been performed showing advantages of laparoscopic repair for one or more of the above-cited parameters [23–25]. Larger randomized studies [26] have shown some benefit in terms of less pain and more rapid return to work, but the same authors have shown more recurrences and higher costs in the laparoscopic repair groups [27]. Overall, many surgeons still feel the potential risks associated with laparoscopy and the increased cost outweigh the advantages and do not routinely recommend laparoscopic hernia repair. The laparoscopic approach has been recommended by some surgeons as ideal for repair of recurrences, for bilateral hernias, and for patients who need to return to a very active lifestyle rapidly [20,28].



(a) Laparascopic hernia anatomy



(b) Types of inguinal hernias



(c) Laparoscopic hernia repair (TAPP)

Figure 98.8 Laparoscopic repair of inguinal hernias. **(a)** Anatomy of the inguinal region as seen through the laparoscope using the transaabdominal preperitoneal (TAPP) approach. **(b)** Spaces through which hernias commonly occur as seen through the laparoscope. (**c**) After reduction of the hernia, the polypropylene mesh is secured over the inguinal floor using surgical clips or staples.

Pelvic hernias

The intestine can herniate through the pelvic floor (weakness, perhaps, from multiparity or previous trauma) in areas such as the obturator foramen, the greater or lesser sciatic foramen, or the perineal muscle. The most common of these hernias is the *obturator hernia*. The obturator foramen is covered by a membrane except for one small area, and herniation occurs when the intestine (usually ileum) enters the foramen through this small defect and into the obturator region of the thigh (Fig. 98.9). This hernia is seen more commonly in females (male to female ratio of 1 : 6), on the right side, and in the sixth decade of life. Bilateral hernias can be seen, albeit rarely [29]. Most obturator herniations present with acute intestinal obstruction with no previous signs or symptoms. Occasionally, dysesthesias occur in the medial thigh because of compression of the obturator nerve. The Howship–Romberg sign (pain in the medial thigh radiating to the knee or hip exacerbated with extension, adduction, or medial rotation of the thigh) is present in 50% of the patients. A tender mass may be palpated near the obturator canal on rectal or vaginal examination. Radiological studies may show a gas shadow in the region of the obturator foramen; a small loop of intestine in this region can be also seen on CT [29]. Surgical treatment using a transabdominal approach is indicated in all cases [30,31].

Sciatic hernias are extremely rare and can present with a slowly enlarging mass in the gluteal fold area. A CT scan is often needed for diagnosis, and treatment is surgical using a transabdominal or combined transabdominal and gluteal approach. *Perineal hernias* occur through the pelvic floor musculature, and symptoms can vary but are usually related to a protruding mass in the perineal area, worst with standing. These hernias can be seen after removal of the rectum in abdominoperineal resection for rectal carcinoma. Repair is undertaken using a transabdominal approach; polypropylene mesh may be needed.

Lumbar hernias

The posterior abdominal wall has two naturally weak areas. The superior triangle of Grynfeltt–Lesshaft, also called the lumbocostoabdominal triangle, is the most common site for lumbar herniation. This area is bordered by the 12th rib, the posterior border of the internal oblique, and the anterior border of the sacrospinalis. The inferior triangle of Petit or lumboiliac abdominal triangle is limited by the iliac crest, the anterior border of the latissimus dorsi, and the posterior border of the external oblique muscle.

Lumbar hernias are seen most commonly in men and are congenital (20%), acquired (50%), or due to trauma (25%), for example rib fractures or removals, iliac crest fractures, or

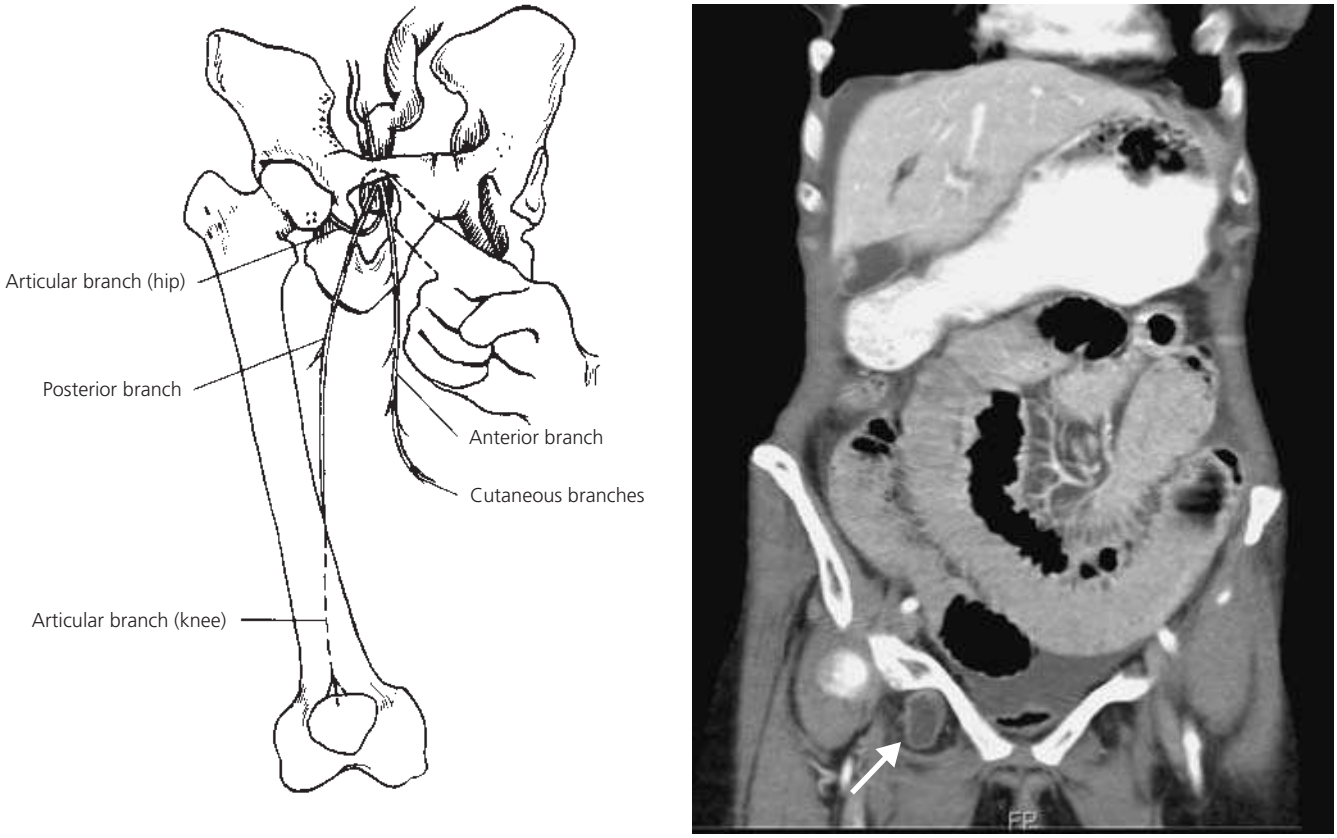


Figure 98.9 Rectal or vaginal palpation of the obturator foramen may confirm the existence of an obturator hernia. Adapted from Nyhus LM, Condon RE (eds). Hernia. Philadelphia: JB Lippincott, 1978. **(b)** Computed tomography of an obturator hernia; white arrow points to small intestine trapped in the hernia.

removal of a segment of iliac crest for bone grafting [12]. These hernias are usually left-sided. Symptoms usually include a tender mass or pain in the lumbar area or radiating to the pelvis. Lumbar hernias should be surgically fixed when first noted because they tend to enlarge, and larger hernias are more difficult to repair.

Spigelian hernias

A Spigelian hernia is rare, occurring through the linea semilunaris, lateral to the rectus abdominis, with protrusion through the external oblique fascia. Spigelian hernias occur in elderly people, are often small and difficult to diagnose, and always present below the arcuate line of Douglas. Symptoms include local pain or discomfort worsened by increased intraabdominal pressure. Diagnosis can be made with ultrasound or CT if physical examination is difficult because of obesity or if differentiation is needed from abdominal wall tumor [32]. Surgical repair is needed because of the risk for incarceration; the results are excellent.

Diaphragmatic hernias

Acquired diaphragmatic hernias are the result of blunt or penetrating trauma. Most hernias occur on the left, and herniated viscera can include stomach, spleen, colon, or the left lobe of the liver. Herniation and traction on the mesentery can lead to nausea, vomiting, diaphoresis, and general abdominal discomfort. Respiratory symptoms occur if there is lung or mediastinal compression. Some patients present years after a traumatic injury [33].

The diagnosis is based on physical examination with dullness to percussion along the left chest; chest radiography shows abdominal viscera or a nasogastric tube curled in the thorax (Fig. 98.10). CT can be useful in visualizing defects with protruding viscera [34]. If the index of suspicion is high for a diaphragmatic injury, normal radiological studies should not deter laparoscopy or surgical exploration of the diaphragm [35]. All diaphragmatic hernias should be fixed on diagnosis to avoid strangulation or acute respiratory distress. Chronic herniations may be best approached transthoracically to allow release of intrathoracic adhesions, although most can be repaired transabdominally.

Internal hernias

An internal hernia is a protrusion of any intraperitoneal viscus into a compartment within the abdominal cavity. There is no hernia sac, and frequently the herniated viscus is

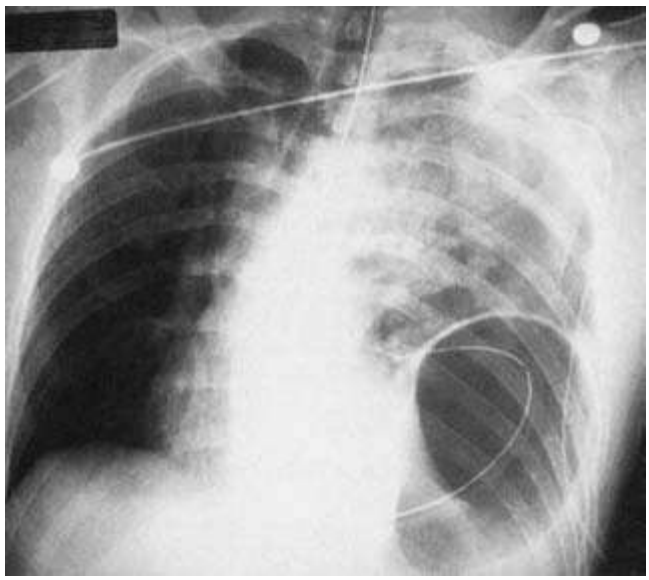


Figure 98.10 Traumatic hernia of the diaphragm on the left. The diaphragmatic line has disappeared and the gastric fundus is in the left thoracic space. The contour of the nasogastric tube should be noted in the left chest.

entering a known anatomical space or foramen; some hernias occur in surgically created or congenital defects. In one review, 0.2%–0.9% of cadavers had incidentally noted internal hernias, most commonly paraduodenal (53%), followed by pericecal (13%) and the foramen of Winslow (8%), and supravesical and intersigmoid hernias (6%–7% each) [36].

Most symptoms are intermittent, and patients often present with small bowel obstruction or signs and symptoms of strangulation. Radiological studies are not always helpful, and the differential diagnosis must include foreign bodies, intestinal volvulus, adhesions, and tumors [37,38]. Surgical treatment is needed and includes reduction of the herniated viscus and repair of the defect.

Paraduodenal hernias

The left paraduodenal fossa of Landzert is a peritoneal pocket located lateral to the fourth portion of the duodenum and posterior to the inferior mesenteric vein. A left paraduodenal hernia (75%) results from dorsal malrotation of the midgut resulting in a hernial opening with the inferior mesenteric artery covering the anterior margin. A right paraduodenal hernia (25%) is formed by incomplete rotation of the midgut such that the small intestine is in a sac covered anteriorly by the mesocolon and the superior mesenteric artery. The cecum remains in the right upper quadrant and is attached to the right abdominal wall by thickened parietocolic peritoneal bands of tissue called Ladd bands.

Signs and symptoms are elusive and can include any signs associated with chronically incarcerated hernias. Contrast radiography shows stasis and delay in transit with large portions of small bowel trapped in one anatomical position. Surgical correction is necessary, and damage to the small bowel vasculature should be carefully avoided.

Hernias of the foramen of Winslow

Herniation of a mobile cecum and distal ileum can occur into a larger than usual foramen of Winslow. Signs and symptoms are vague but can include crampy epigastric pain relieved with flexion of the torso. Radiographic studies show a gas-filled loop of bowel behind the stomach and pushing the stomach anteriorly and to the left. Surgery with reduction and cecopexy may be needed.

Pericecal hernias and intersigmoid hernias

The distal ileum can pass through a defect in the cecal mesentery occupying the right paracolic gutter. Most present with right lower quadrant pain with or without small bowel obstruction. Intersigmoid hernias are composed of herniated small bowel present in the peritoneal pocket between the two loops of sigmoid. These hernias are usually picked up incidentally at operation or during a radiological study such as barium enema or CT [38].

Transmesenteric and transomental hernias

About 5%–15% of internal hernias occur through defects in the mesentery or the omentum. Transmesenteric hernias occur most commonly in children, along the mesentery of the jejunum. Prenatal ischemic events may be the causative agent because the area of defect is often associated with atretic segments of intestine. Symptoms of small bowel obstruction may develop, and surgery is required at diagnosis.

Iatrogenic hernias

Iatrogenic hernias result from alteration of intraabdominal anatomy, most often at surgery. Internal herniation can be related to surgical defects created by prior operations, e.g., in the paracolic or paraileal spaces adjacent to end stomas, or in a retroanastomotic location. Of iatrogenic hernias, 75% manifest in the first postoperative year.

Retroanastomotic hernias

A retroanastomotic hernia occurs through any defect in the mesenteric space left open after completion of an intestinal anastomosis. Hernias can occur after a Billroth II anastomosis in the space between a gastrojejunostomy and the posterior abdominal wall. Most patients present with intestinal obstruction and require immediate surgical correction. The presentation will generally be indistinguishable from other causes of intestinal obstruction, so that an accurate preoperative diagnosis is rarely made. Surgical repair of the defect should be performed along with resection of any nonviable bowel. If discovered incidentally at the time of laparotomy for other reasons, such defects should be repaired to avoid future problems.

Given the rapidly increasing number of patients undergoing gastric bypass operations, herniation and volvulus associated with the new anatomy created by Roux-en-Y gastric bypasses deserves special mention. These hernias used to be quite rare and rarely reported, but now appear to be a major cuase of morbidity and mortality after open or laparoscopic gastric bypass. Retrocolic/retrogastric herniation of most of the small intestine has been reported due to internal herniation at the mesenteric defect of the jejunojejunostomy after both laparoscopic and open gastric bypass surgery [39]. This unusual but not rare complication is potentially life-threatening due to possible ischemic necrosis of most of the small bowel as well as perforation of the gastric remnant, which is almost uniformly fatal. Internal hernias appear to be more common after laparoscopic Roux-en-Y gastric bypasses, possibly due to the relative lack of postoperative adhesions. Patients with retrocolic placement of the Roux limb appear to be at highest risk of herniation [40]. Patients often present with intermittent abdominal pain, small bowel obstruction and normal radiological studies, including contrast studies and CT scans, but can also present with signs and symptoms of an intraabdominal catastrophe. One retrospective analysis of 2000 consecutive patients who underwent laparoscopic Roux-en-Y gastric bypass found 63 patients with such internal hernias requiring operative intervention, an incidence of 3.1%. The site of internal herniation included 44 in the mesocolon, 14 in the jejunal mesentery, and five in the space of Petersen. Of these patients, 20% had normal small bowel series or CT scans [41]. In one study of laparoscopic gastric bypass procedures, the overall incidence of internal hernias was 6.0% of patients with retrocolic procedures and 3.3% of patients with antecolic procedures. Most were in the Petersen defect (55.9%) and at the enteroenterostomy site (35.3%), while 23% of patients with internal hernia were diagnosed in the early postoperative period (2–58 days) and 77% were diagnosed at a later date, up to 3 years after the original surgery [42]. In another study, volvulus and ischemia requiring resection of small intestine was present in over 50% of patients with transmesenteric hernias, and absence of the *whirl sign* and other radiological signs of bowel ischemia did not exclude volvulus or ischemia [43]. Diagnosis of internal herniation in this patient population remains extremely challenging given the relative lack of experience with this situation and because of extreme obesity leading to difficulty with reliability of both physical examination and laboratory findings. A high index of suspicion, diligent patient evaluation with special attention to symptoms, early abdominal CT scanning, communication with the surgical team involved with the patient's gastric bypass (if different from current physician team), and urgent operative intervention are necessary to achieve a successful outcome. All defects must be closed to minimize the risk of hernia, whether antecolic or retrocolic [44].

Incisional hernias

Incisions of the abdominal wall result in future herniation 2%–5% of the time. Multiple factors are responsible for a postoperative incisional hernia, including defective suture material, undue tension on the sutured fascia, obesity, previous incisions, infections, seromas or hematomas, malnutrition, and smoking [45]. Different incisions have different rates of future herniation. Midline incisions have the highest rates; paramedian and transverse incisions have lower rates of herniation. Incisional hernias can range in size from 1 cm to large hernias containing nearly all the intestines. With increasing popularity of laparoscopic surgery, attention needs to be paid to all trocar site defects larger than 0.5 cm to avoid future herniation.

The diagnosis of incisional hernia is usually evident by history as well as physical examination. The hernial ring is often palpable with the edges of the muscle retracted laterally. Incisional hernias often incarcerate but tend not to strangulate. Operative repair should be undertaken in most patients. For many years, the repair of incisional hernia was associated with a high recurrence rate. Most recurrences are thought to be due to infection, insufficient dissection and exposure of other defects, or closure under excess tension. Tension should be avoided at the time of repair, and prosthetic mesh is often needed. The introduction of synthetic prosthetic materials has provided the opportunity to perform a tension-free repair, thereby reducing the rate of recurrence [45]. In addition, musculoaponeurotic flaps are used along with relaxing incisions in cases in which prosthetic material is best avoided. Natural tissue substitutes, such as fascia lata or pig small intestinal submucosa, are also being used to repair incisional hernias [46].

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99

Intraabdominal abscesses and fistulae

Paul Knechtges, Ellen M. Zimmermann

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Intraabdominal abscesses and fistulae are complications that arise from a variety of abdominal and pelvic processes. They should be approached as specific clinical entities, with close attention to the underlying etiology. In the past, most abscesses and fistulae required open surgical therapy. However, the modern therapeutic approach includes sophisticated diagnostic imaging, percutaneous or endoscopic aspiration and drainage, and medical therapy, leaving fewer cases requiring aggressive surgery. A thorough understanding of the pathogenesis, diagnostic options, and therapeutic strategies will lead to more efficient and effective care of complex cases.

Epidemiology

The most common cause of intraabdominal abscesses are complications of abdominal operations, penetrating trauma, perforations of the gastrointestinal tract, diseases of the pancreas or biliary system, and infections of the male and female genitourinary tracts [1]. Half to three-fourths of intraabdominal abscesses arise in the postoperative setting with most resulting from a documented anastomotic leak [2]. The causes of nonsurgical intraabdominal abscesses include diverticulitis, appendicitis, inflammatory bowel disease, pancreatitis, perforated peptic ulcers, and perforated visceral carcinomas. The incidence of intraabdominal abscesses appears to be decreasing, but the overall mortality remains high at 10%–30% [3,4]. Intraabdominal abscesses are being increasingly seen in elderly or immunocompromised patients where their detection and management may be difficult [1].

A gastrointestinal fistula is defined as an abnormal communication between the gastrointestinal tract and another epithelialized surface such as the skin or an adjacent loop of bowel. Gastrointestinal fistulae can be further subdivided into congenital and acquired [5]. Congenital gastrointestinal fistulae are the result of embryological processes (e.g., tracheoesophageal fistula) and are most commonly encountered in the neonatal period and childhood. These are not considered further in this chapter. Acquired fistulae can be classified by their anatomical origin and end point, and are either internal (e.g., enteroenterofistula) or external (e.g., enterocutaneous). Functionally, they are separated into low output (< 500 mL/day) or high output (> 500 mL/day). Most occur in the postoperative setting due to inadvertent organ injury, failure of an anastamosis, or in proximity to a drain, tube or other foreign body. Posttraumatic fistulae can be due to direct injury or be associated with an open abdominal wound. Fistulae form in the setting of inflamed tissue as in Crohn's disease, pancreatitis, or post irradiation. Finally, malignant or necrotic tissue can form fistulae, particularly if they are adjacent to bowel or the abdominal wall [2,6]. Acquired fistulae in adults are often either the cause or the consequence of intraabdominal abscesses and therefore are considered together.

Pathophysiology

Intraabdominal abscesses

Intraabdominal abscesses develop as a result of localization of diffuse peritonitis, in which case they may reside in dependent regions of the peritoneum such as the pelvis, perihepatic spaces or the paracolic gutters, or as a result of localized abscess around an area of trauma, surgery, or adjacent to a diseased organ. An intraabdominal abscess is a well-defined collection of inflammatory cells and bacteria, either viable or nonviable, that is walled off from the rest of the peritoneal cavity by inflammatory adhesions, omentum, abdominal viscera, or the gut [6]. Intraabdominal abscesses typically arise when previously sterile visceral organs or abdominal spaces are seeded by bacteria. The majority of visceral organ abscesses result from hematogenous seeding, whereas the majority of

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abscesses in the peritoneal space result from perforation of an abdominal viscus or dehiscence of a surgical anastamosis [2,6]. Abscesses in the retroperitoneal space can result from both perforation of the gastrointestinal tract and lymphatic or hematogenous spread. The location of abscesses varies by series depending on the etiologies. In one large series, 20% were perihepatic, 44% were in the right lower quadrant, 14% in the left lower quadrant, and 14% in the pelvis [7].

When pyogenic bacteria enter a previously sterile anatomical space, they trigger an acute inflammatory reaction. Numerous leukocytes are drawn to the infection to contain and eradicate the invading bacteria. This leads to the formation of an abscess cavity consisting of a localized region of edema, neutrophils, and necrotic tissue [6]. An abscess cavity typically consists of three distinct histological layers. The center of an abscess consists of necrotic leukocytes and the necrotic cells of the infected tissue. This necrotic center is surrounded by a second layer of preserved neutrophils. Outside this region, there is a third layer characterized by vascular dilatation and fibroblastic reaction. It is this zone of vascular dilatation that gives a mature abscess its characteristic *ring enhancement* during contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI) [8]. The central devascularized area of necrotic cells and bacteria has no blood flow and is difficult to reach with antibiotics. Abscesses often require percutanous or surgical drainage in addition to antibiotic treatment.

Intraabdominal abscesses are typically polymcrobial. Anaerobes can be found in 60%–70% of cases. In one series, *Bacteroides fragilis* was the most common pathogen, with anaerobic cocci and clostridia found in 50%. Other common bacteria include *Escherichia coli*, *Klebsiella/Enterobacter* group, *Proteus* spp., *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and enterococci [1,9–11].

Gastrointestinal fistulae

The pathophysiology of gastrointestinal fistulae is incompletely understood. In some settings it is likely that disruption of the normal tissue planes creates a path of least resistance that then perpetuates effluent from a lumenal organ. The effluent itself may inhibit normal healing of the tract. The tissue planes could be disrupted by a foreign body (e.g., gastrostomy tube), inflammation (e.g., pancreatitis), infection, surgery, or tumor. In some conditions such as Crohn's disease, downstream intestinal strictures create a high-pressure region facilitating the fistula [2]. However, only approximately 50% of Crohn's fistulae in the terminal ileum are associated with a downstream stricture, and perianal fistulae are not usually associated with significant rectal or anal stenosis [12–14]. The molecular mechanisms underlying fistula formation are poorly understood, although in Crohn's disease and postirradiation fistulae, matrix metalloproteinases play a local role in matrix degradation [15,16]. Infection likely contributes in many settings as fistula formation is commonly associated with an abscess or local tissue infection. Local ischemia may play a role especially in the postoperative setting. Finally, patient factors including nutritional status and concomitant medications contribute to fistula formation and delay spontaneous closure [17,18].

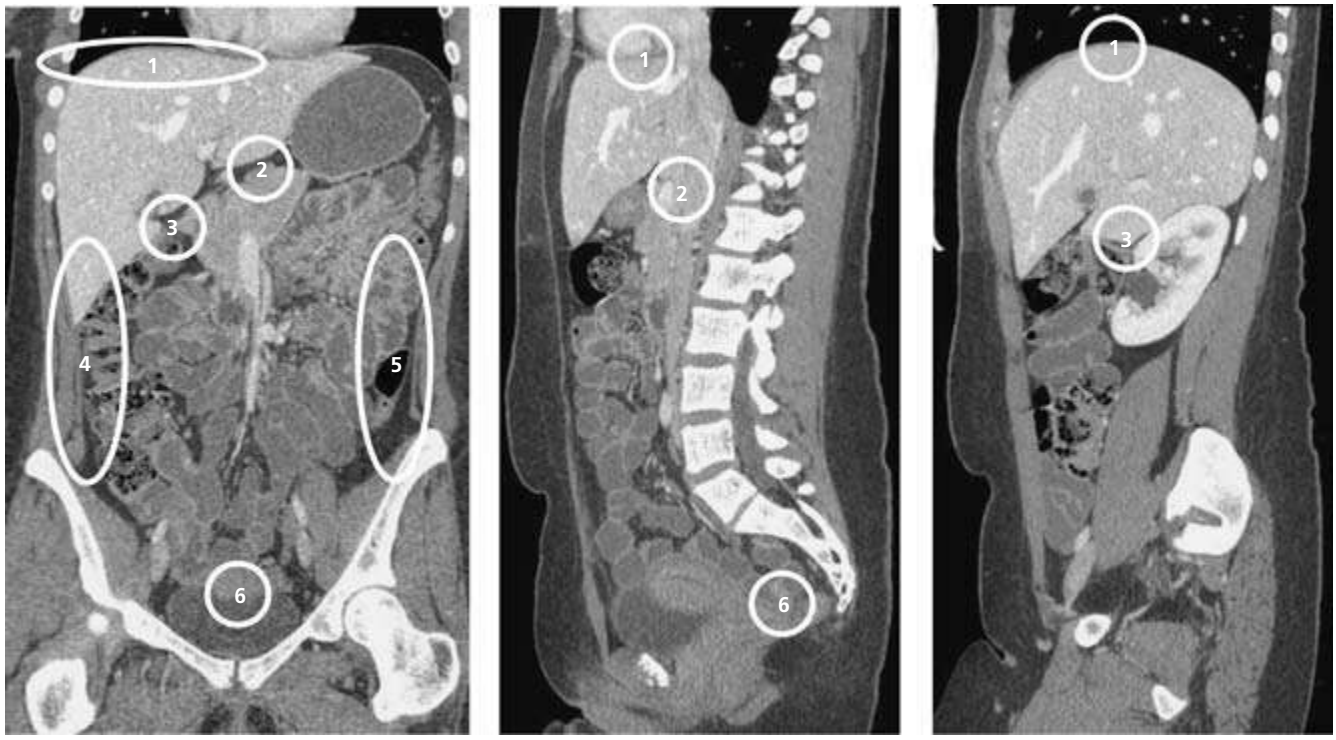
Anatomical considerations

The peritoneal and retroperitoneal compartments consist of a series of communicating compartmentalized potential spaces that determine the site and clinical symptoms associated with gastrointestinal abscesses and fistulae (Fig. 99.1). The potential space between the visceral and parietal peritoneum defines the peritoneal space. The organs within the peritoneal cavity are connected and supported by ligaments including omenta and mesenteries [8]. The ligaments define the potential spaces in the peritoneum.

Key structures limit or localize infection in the abdomen. The greater omentum joins the greater curvature of the stomach to the colon and then extends downward anterior to the small bowel. The small bowel mesentery is a broad fan-shaped fold of peritoneum that connects the jejunum and ileum to the posterior abdominal wall. It originates at the duodenal jejunal flexure (ligament of Treitz) in the left upper quadrant and extends diagonally to the ileocecal junction in the left lower quadrant. The transverse mesocolon is the mesentery that extends from the ventral surface of the pancreas to the transverse colon.

The region near the liver has two major potential spaces: the perihepatic space and the lesser sac (omental bursa). These two spaces communicate via the epiploic foramen (foramen of Winslow). The perihepatic space extends superiorly to the junction of the bare area of the liver and the diaphragm and is the site of subphrenic abscesses. The posterior subhepatic space projects cephalad into the recess between the liver and the right kidney. This recess constitutes the hepatorenal fossa (pouch of Morrison). The hepatorenal fossa is the most dependent portion of the subhepatic space when the patient is supine and consequently is a common site for the accumulation of intraperitoneal fluid collections. Intraperitoneal inflammation can cause the lesser sac to seal off from the remainder of the peritoneal space. As a result, abscesses in the main cavity rarely enter the lesser sac and abscesses in the lesser sac usually do not spread to the rest of the peritoneal space [2,8].

Inferior to the small bowel mesentery the potential space extends down into the pelvis as the pouch of Douglas (also called the rectouterine space in women and the rectovesical space in men). The pouch of Douglas is the most dependent portion of the peritoneal cavity in both the erect and supine positions, and is a common site of abscess formation after bowel perforation. The right and left pericolic gutters border the ascending and descending colon, respectively. These form important tracts for infected fluid between the lower and upper abdomen. Finally, the retroperitoneum is a potential



(a) (b) (c)

Figure 99.1 Peritoneal compartments in which abscesses commonly form. **(a)** Coronally reconstructed CT of the abdomen and pelvis. **(b)** Midline sagittally reconstructed CT of the abdomen and pelvis. **(c)** Right para-midline sagittally reconstructed CT of the abdomen and pelvis. 1, Subphrenic space; 2, lesser sac; 3, subhepatic space (pouch of Morison); 4, right paracolic gutter; 5, left paracolic gutter; 6, rectouterine space in female patient (rectovesical space in males).

space that is also separated into regions defined by fascia. The anterior pararenal space contains most of the pancreas and the retroperitoneal portions of the duodenum, ascending colon, and descending colon. Soft tissue of the retroperitoneal space facilitates the development of fistulae.

Clinical manifestations

Intraabdominal abscesses

Classically, patients with intraabdominal abscesses have fever, abdominal pain, anorexia and malaise. The symptom complex can vary widely based on the underlying etiology, location of the abscess in the abdomen, and patient factors. The fever is usually intermittent and spiking, and may be associated with intermittent bacteremia. Abdominal pain may be localized to the region of the abscess. An abscess that is associated with the parietal peritoneum may produce localized pain, tenderness, and a palpable mass. Abscesses within the mesentery or between bowel loops may produce diffuse, poorly localized visceral pain, and a mass may be more difficult to appreciate. Subphrenic abscesses can cause shoulder pain, tachypnea, cough, and hiccups. Pelvic abscesses may cause tenesmus, diarrhea, or urinary retention. Lumbar or ileopsoas muscle pain may be present. Retroperitoneal abscesses cause fewer symptoms than an intraperitoneal process. Patients with intraabdominal abscesses usually appear ill, and fever, tachycardia and tachypnea are common. Vascular collapse and a classic picture of septic shock may be present, but is rare. Abdominal examination may or may not localize the region of the abscess. Peritoneal signs may be present but are not common. Abdominal distention and decreased bowel sounds suggest an ileus. Pelvic abscesses can often be palpated during rectal or vaginal examination. A pleural effusion may be associated with a subphrenic abscess. Involvement of the psoas muscle may cause pain on flexion of the hip (psoas sign).

Patients may have minimal signs and symptoms of an intraabdominal abscess. History and physical examination can be more difficult in obtunded patients, and may underestimate pathology in elderly patients or patients on antibiotics, narcotics, or steroids. Intraabdominal abscesses may be difficult to distinguish from the underlying disease process in patients with Crohn's disease or diverticulitis for example. Finally, an intraabdominal abscess in a postoperative patient may be difficult to detect. Therefore, a high index of suspicion needs

to be maintained when evaluating patients with diseases or clinical settings where the development of intraabdominal abscesses occurs commonly.

Laboratory examination usually demonstrates leukocytosis with a classic left shift. Bacteremia may be present and should be sought with at least three blood cultures. Urine examination may reveal white or red blood cells due to irritation of the ureters or bladder. Serum bilirubin may be elevated if sepsis is present. Amylase may be elevated if a lesser sac abscess is present. Chest radiography will often show a pleural effusion on the side of the subphrenic abscess. An elevated diaphragm and atelectasis of the adjacent lung may be present. Plain abdominal radiographs may reveal air–fluid levels, gas bubbles localized outside the intestinal tract, or if the abscess is large, displacement of bowel loops.

Gastrointestinal fistulae

Clinical manifestations of gastrointestinal fistulae vary widely depending on the underlying etiology and the organs in communication. Internal enteroenterofistulae may be relatively asymptomatic and without physiological consequences if they span a short segment of bowel. Fistulae to the skin from the stomach or duodenum can result in fluid and electrolyte losses, hypochloremic hypokalemic metabolic alkalosis, and skin breakdown. Loss of pancreatic secretions from pancreatic fistulae can result in metabolic acidosis and maldigestion of nutrients. The presence of activated digestive enzymes in fistulae effluent can cause significant skin breakdown.

Sepsis can accompany fistula development as the fistula may rupture into the peritoneal cavity or cause an abscess at a distant site. Alternatively, a fistula can present as a draining surgical wound or a localized abscess that then continues to drain. Pain can accompany fistula formation, or pain may decrease as the fistula itself decreases pressure in an abscess or in a bowel segment upstream of a stricture as occurs in Crohn's disease [1,2,17,18].

Diagnostic studies

Over the last several decades, CT has become the imaging method of choice for evaluating intraabdominal abscesses [19–21]. Other modalities such as ultrasound, MRI, and nuclear imaging can play an important role in complex cases, in specific clinical indications (e.g., ultrasound for tuboovarian abscess), or when there are relative contraindications to CT (e.g., pregnancy).

Radiography and fluoroscopy

Plain film radiography has low sensitivity and specificity for detecting an intraabdominal abscess. However, several nonspecific findings suggest the presence of an abscess, including a mass effect on the bowel or adjacent organs, a mottled extralumenal gas collection, and loss of fat/soft tissue interfaces [8,19]. Occasionally, fluoroscopy can be used to direct injection of sterile water-soluble contrast into a drain that has been previously placed into an abscess cavity. This is done in order to determine the size of the residual abscess cavity and therefore the continued need for the drain. Overpressurization of an abscess cavity during injection can result in rigors and even sepsis, so patients are often premedicated with antibiotics prior to this procedure. Drain injections should be reserved for special circumstances such as identifying a potential fistulous communication between the abscess cavity and an abdominal organ.

Ultrasound

The physics of sound transmission severely limit ultrasound's ability to detect intraabdominal abscesses. When sound strikes an interface between soft tissue and gas, nearly all of the sound is reflected [22] and therefore ultrasound cannot "see" gas collections. Consequently, ultrasound cannot be used to find abscesses among multiple air-filled loops of bowel [8]. Further, ultrasound can not easily distinguish a gas-containing abscess from an air-filled bowel loop. Finally, the lack of commercially available contrast agents for ultrasound often makes it nearly impossible to differentiate an abscess cavity from a postoperative seroma or hematoma.

Despite these limitations, ultrasound is the primary imaging modality in specific situations such as the evaluation of tuboovarian abscess [23]. Transabdominal ultrasound is able to visualize the pelvic structures because of the excellent sonographic window provided by a fluid-filled urinary bladder. Transvaginal scanning allows the operator to bring the ultrasound probe into close apposition with the pelvic structures, often avoiding interference from gas-filled loops of bowel. Ultrasound can also be used to guide real-time imaging for needle aspiration and catheter drainage, but this should generally only be performed after a diagnostic CT has excluded additional abscesses and better defined the location of adjacent bowel [8] (Fig. 99.2).

Nuclear medicine

Over the past few decades, nuclear medicine/radionuclide image has been largely supplanted by anatomical imaging modalities, and has evolved a complementary role as a problem-solving tool [24]. Three major radionuclide markers are utilized in the imaging of infectious and inflammatory disease processes: gallium 67 citrate, technetium 99m-labeled leukocytes, and indium 111-labeled leukocytes. Of these three agents, ^{111}In-labeled leukocytes are the most appropriate for the localization of intraperitoneal abscesses because the label does not demonstrate physiological uptake in normal bowel. While spatial resolution is limited, it can provide important physiological data about the infectious or inflammatory status of fluid collections and soft tissue stranding seen on CT. This information can be valuable for differentiating postoperative changes from infection. In addition, leukocyte imaging

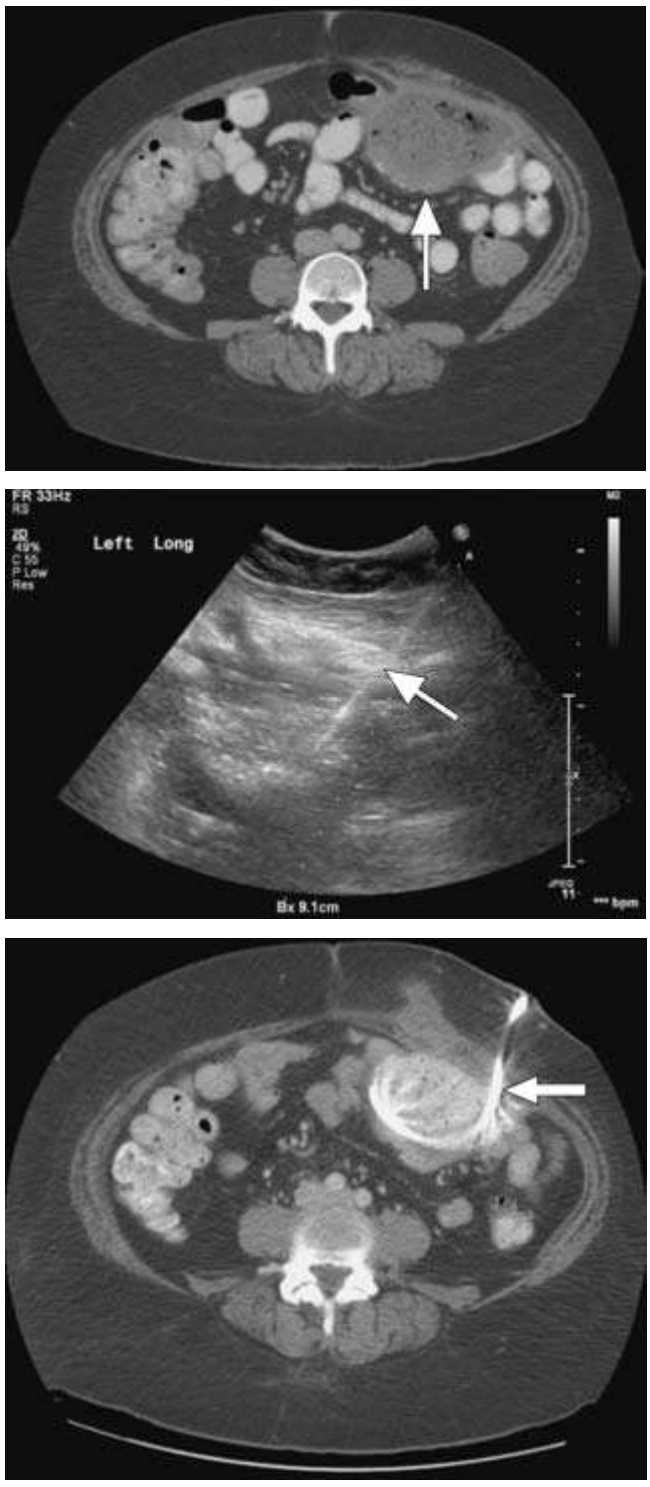


Figure 99.2 **(a)** A patient with a prior history of diverticulitis presents with a rim-enhancing fluid collection containing mottled gas in the left lower quadrant consistent with an abscess (arrow). **(b)** Under ultrasound guidance, a needle (arrow) was introduced into the abscess and brown turbid fluid was aspirated. A drainage catheter was subsequently placed. **(c)** Follow-up CT scan reveals the drainage catheter in the abscess cavity. There is now oral contrast in the abscess cavity consistent with internal fistulization with adjacent bowel.

has proven superior to anatomical imaging for determining if prostheses (e.g., vascular prosthetics, orthopedic hardware) have been seeded by bacteria from an intraabdominal process [24].

Magnetic resonance imaging

Technical issues limit routine application of MRI in the abdomen, but this may change with rapid advances in magnetic resonance technology. Magnetic resonance sequences are relatively slow, especially when compared with the speed of modern CT. Motion artifact results in "ghosting," an imaging phenomenon that can blur images [25,26]. Respiratory motion can often be overcome by breath-holding and respiratory gaiting, although this is difficult in ill patients. The random motion produced by peristalsis also significantly hinders attempts to identify abscesses among bowel loops [25]. The air–fluid–fat interface, as well as surgical clips and metallic objects, cause further artifacts [26]. Many limitations of traditional MRI are being overcome by modern techniques such as magnetic resonance enterography and magnetic resonance colonography, although these techniques have limited availability [27,28].

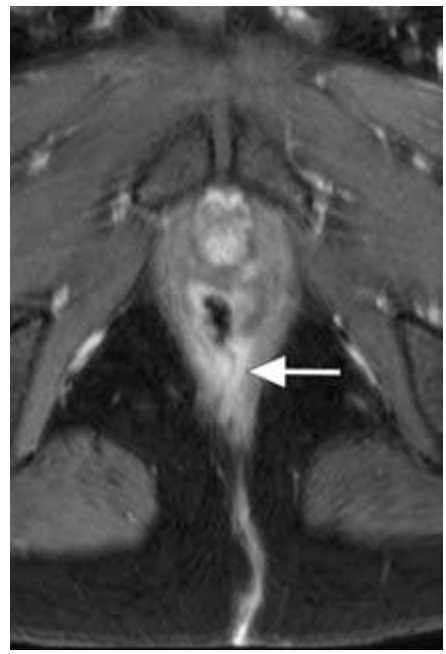
MRI serves an important problem-solving role for distinguishing small hepatic abscesses from cysts and cavernous hemangiomas that are too small to characterize by CT [29]. In addition, MRI provides exquisite detail of the retroperitoneal and pelvic structures and is a superb imaging modality for perianal abscesses and fistulae [30,31] (Fig. 99.3).

Computed tomography

CT has an accuracy of over 90% in detecting intraabdominal abscesses [19,20,32]. Several findings on CT are highly suggestive of an intraabdominal abscess including loculation, ring enhancement, and gas (Fig. 99.4). The very low attenuation mottled gas collections seen on CT can either result from a gas-producing infection or from gas that has escaped from an adjacent gastrointestinal perforation. In a solid organ with no recent operative intervention, the presence of a gas-containing fluid collection is virtually pathognomonic of an abscess [33].

Ideally, CT of the abdomen and pelvis should be performed with intravenous and oral contrast when evaluating for abscess. The use of iodinated intravenous contrast can help differentiate an inflamed rim-enhancing abscess from a sterile loculated fluid collection such as a seroma. Unfortunately, due to renal failure or allergy many patients cannot receive intravenous contrast. While the lack of intravenous contrast can reduce the accuracy of CT for abscess evaluation, it should not preclude the performance of a CT scan for this indication.

The administration of an appropriate positive oral contrast is important for identifying and characterizing abscess cavities adjacent to loops of bowel. Positive oral contrast is defined as any agent that is higher in attenuation than water, i.e., the traditional barium and iodine-based agents. Without any oral



(a)

(b)

Figure 99.3 **(a)** Axial MR image (post-contrast spoiled gradient echo with fat saturation) demonstrates a trans-sphincteric fistula-in-ano (arrow) originating at the posterior anal mucosa and traversing the internal and external anal sphincters before emerging in the perianal skin. **(b)** Diagrammatic representation shows the fistula in black traversing the internal and external anal sphincters. Adapted from Knechtges et al. [37].

Figure 99.4 Right lower quadrant abscess in a patient with perforated appendicitis. This multiloculated rim-enhancing fluid collection contains tiny gas bubbles (arrowhead) and enhancing septae (arrow).

contrast or with some of the newer water-density contrast agents, it can be very difficult to differentiate fluid in the bowel lumen from extralumenal fluid collections like abscesses. In addition, the presence or absence of spillage of oral contrast into the abdominal cavity can help differentiate a bowel perforation from an abscess. Therefore, whenever possible, positive oral contrast should be administered to allow adequate opacification of the entire bowel.

Imaging fistulae

Diagnostic imaging plays an important role in the triage and treatment of patients with gastrointestinal fistulae. Gastrointestinal fistulae may be associated with chronic disease processes, or a more acutely life-threatening processes (e.g., intraabdominal abscess and dehiscence of surgical anastamoses). Accordingly, the imaging workup can be divided into the acute and chronic phases. Acutely ill patients or patients with peritoneal signs are the most likely to benefit from plain film radiography, because it is a rapidly available test that is quite sensitive for free air as in an uncontained bowel perforation. Contrast-enhanced CT may be utilized as a first-line agent to evaluate acutely ill patients, because it can rapidly assess the entire abdomen for associated conditions like intraabdominal abscesses and perforations. In addition, newer multislice CT scanners are often able to define larger fistulous and sinus tracts without any additional imaging.

In subacutely and chronically ill patients, more specialized and time-consuming examinations (e.g., MRI, fistulography, small bowel follow-through) can provide greater anatomical detail regarding the presence and extent of smaller fistulae. These tests are more specifically tailored to evaluate fistulae and can provide valuable information to guide medical and surgical intervention focused specifically on fistula treatment.

A hybrid approach combining cross-sectional imaging studies (e.g., CT and MRI) with more traditional studies (e.g., fistulography and contrast fluoroscopy) can provide complementary information. Such a hybrid/multimodality approach to fistula imaging is becoming more common as imaging technology advances [8] (Fig. 99.5).

Fistulography

Fistulography has traditionally been a first-line test for imaging of cutaneous gastrointestinal fistulae. This is a relatively

Figure 99.5 **(a)** Inflamed terminal ileum (arrow) demonstrating marked wall thickening in a patient with Crohn's disease. There is an enhancing gas-containing tract (arrowhead) between the terminal ileum and the sigmoid colon which was suspicious of an enterocolic fistula. **(b)** A small bowel follow-through was performed which demonstrated that the tract seen on CT was a blind-ending sinus tract (arrowhead). The small bowel follow-through was able to detect subtle ileocecal fistulae (small arrows) that were not visible on the conventional CT of the abdomen and pelvis. The terminal ileum is markedly narrowed secondary to Crohn's involvement (large arrow).

simple test that involves placing a catheter into the fistulous opening and gently instilling water-soluble contrast [34]. The contrast instillation is typically observed under fluoroscopy in order to define the fistulous tract and communications with any organs or abscess cavities. Because of the risk of pressurizing an abscess cavity or the biliary tree and possibly inducing sepsis, some institutions elect to premedicate patients with broad-spectrum antibiotics prior to fistulography. Fistulography has proven relatively inaccurate and unreliable in perianal fistulae [35,36], and has been replaced by MRI, and to a lesser extent endoanal ultrasound, for the evaluation of perianal Crohn's disease and fistula-in-ano [30,31,37].

Therapy

Intraabdominal abscesses

Drainage is the mainstay of therapy for intraabdominal abscesses. Advances in imaging have allowed detailed localization and description of the abscess, and often clues as to the underlying etiology. Abscesses can usually be distinguished from normal structures such as the bowel lumen or sterile collection of fluid or blood by contrast agents and by comparison of different imaging techniques. Aspiration of the contents is critical to confirming the diagnosis of an abscess and providing guidance as to the choice of antibiotic regimen. CT- or ultrasound-guided drainage is being used increasingly in appropriate clinical settings with good results. Requirements for optimal percutaneous catheter drainage include:

- an abscess that can be approached adequately via a safe percutaneous route
- an abscess that is unilocular
- an abscess that is not vascular and the patient has no coagulopathy
- joint radiological, surgical, and medical evaluation with adequate back-up for any complications
- the possibility of dependent drainage for the percutaneously placed drain [1,8].

When these criteria are met, percutaneous drainage is successful in 80%–90% [38,39]. Attempts at drainage of loculated, poorly organized, multiple or extensive collections are less successful. The complications in most series range from 5% to 15% and include septicemia, hemorrhage, peritoneal spillage, and fistula formation [38,39]. Percutaneous drainage may be associated with less morbidity and mortality than surgical treatment [1,40].

Antimicrobial therapy should be started as soon as appropriate samples are collected; typically this occurs before drainage. Initial antibiotic therapy is directed at the anaerobes, especially *B. fragilis* and Enterobacteriaceae. The antibiotic regimen should be adjusted based on in vitro testing of the organisms isolated from the blood or purulent material. Repeated cultures of blood and fluid collections may be necessary during prolonged illnesses, as changes in the pathogenic organisms can occur during therapy. Small abscesses can occasionally be treated with antibiotics alone without drainage [1,2].

Supportive care for ill patients with intraabdominal abscesses is important and includes attention to fluids and electrolytes, gastrointestinal drainage with a nasogastric tube for patients with ileus or obstruction and, when necessary, blood transfusion and respiratory support.

An operative approach to an intraabdominal abscess should be considered if there is bowel perforation as in a postsurgical anastamotic leak or traumatic perforation [2]. In addition, laparotomy may be indicated if the purulent collection is large or complex, or if there is suspicion of necrotic or severely compromised organ, such as necrotic bowel. Finally, decompressive laparotomy may rarely be indicated in extreme cases of acute increase in intraabdominal pressure, which itself can lead to multiorgan failure and shock [2].

Gastrointestinal fistulae

The treatment of gastrointestinal fistulae depends on the anatomy, the underlying etiology, and the physiological consequences [17,18,41]. The treatment approach is based on

Table 99.1 Factors influencing spontaneous closure of gastrointestinal fistulae

Favorable	Unfavorable
Tract < 2 cm	Tract > 2 cm
Intestinal continuity	Diseased adjacent bowel
Absence of obstruction	Distal obstruction
Postoperative/anastamotic	Malignancy, Crohn's disease, radiation
Low fistula output (< 500 mL/day)	High fistula output (> 500 mL/day)
No sepsis	Sepsis
Serum albumin > 2.5 g/dL	Serum albumin < 2.5 g/dL
Well-nourished patient	Malnourished patient
Short duration	Chronic duration
Origin: pancreaticobiliary, colon	Origin: gastric, small bowel

careful imaging to define an underlying organ leak or infection. External fistulae with their origins in the stomach, pancreas, small bowel, or colon are the most common. General treatment principles include adequate external drainage, control of infection, and intensive nutritional support and replacement of nutrient and electrolyte losses. In the case of traumatic or postsurgical fistulae, 4–8 weeks of intensive nutritional and medical therapy should be continued to allow spontaneous closure [2]. Factors affecting spontaneous closure of fistulae are listed in Table 99.1. When surgery is undertaken general principles include resection of the fistula, serosal patching or internal drainage of the fistula, correction of any associated pathology, and provision of adequate wound coverage [2].

Management of nutrition is highly important to successful closure of the fistula, as wound healing mechanisms are necessary for closure of fistulae to occur [17,18,41]. The issue of enteral versus parenteral feeding is an ongoing discussion. Based on the critical care literature, the enteral route should be employed when possible as it is more effective in improving nutritional state, with beneficial effects on wound healing and possible favorable effects on gut mucosal integrity [42–45]. Further, enteral nutrition avoids metabolic, infectious, and other safety concerns associated with total parenteral nutrition (TPN) [46]. Historical data on gastrointestinal fistulae clearly demonstrate improved outcomes in the TPN era [47–49]. However, randomized trials have not been performed, and the exact effects of TPN are difficult to determine from these studies. The concept that bowel rest is beneficial is based on a 30%–50% decrease in gastrointestinal secretions in patients on TPN and receiving nothing by mouth [50]. However, while bowel rest will improve fluid and electrolyte management, data supporting spontaneous fistula closure or other significant outcomes are largely lacking [17]. TPN is generally reserved for patients with high-output fistulae (> 500 mL/day), when the patient is unable to tolerate enteral nutrition, or when there is distal obstruction. If oral feeding is used, it is recommended that calorie counts and nutritional assessment be performed to ensure that nutritional needs are being met. Enteral feeding bypassing or directly through the fistula has been reported [51].

High-output fistulae are less likely to close spontaneously than low-output fistulae. This observation underlies the practice of decreasing fistula output, although the evidence that decreasing output improves spontaneous closure is less convincing [17]. It is common to increase the salt and glucose content of oral fluids similar to the management of short bowel syndrome. Proton pump inhibitors and slowed bowel transit using antimotility agents are used. Somatostatin, or its longer half-life analogue octreotide, is widely used to decrease gastrointestinal secretions, although the data supporting its use are mixed. A number of controlled trials of somatostatin or octreotide have compared these agents to standard treatment alone [17,52]. Most trials showed a reduction in the time to fistula closure, although only one study showed a significant improvement in the spontaneous closure rate. Somatostatin may be more effective than octreotide, but the difficulty with constant infusion compared with subcutaneous administration favors octreotide.

Specific considerations

Appendiceal abscesses

A periappendiceal abscess or phlegmon is present in 2%–4% of patients presenting with typical signs and symptoms of acute appendicitis; the incidence is higher if symptoms have been present for several days [53,54] (see Fig. 99.4). Immediate appendectomy is technically difficult and associated with high morbidity. Imaging should be performed to confirm the diagnosis and percutaneous drainage should be considered. Antibiotic coverage for bowel flora should accompany percutaneous drainage [55,56]. After primary nonsurgical treatment, the recurrence rate is less than 10%, and is often associated with an appendicolith. Whether interval appendectomy should be performed after primary nonsurgical

management of an appendiceal abscess is less clear. There is an approximately 2% risk of missed serious diagnosis of cancer or Crohn's disease with a nonsurgical approach. A recent metaanalysis favors a nonsurgical approach without interval appendectomy, but stressed the importance of follow-up with colonoscopy or imaging especially for patients over age 40 [57].

Diverticular abscesses

Complicated diverticulitis occurs when there is an abscess or phlegmom, fistula, stricture, bowel obstruction, or peritonitis and occurs in approximately 25% of patients with acute diverticulitis. In patients with a diverticular abscess but without peritonitis, the size of the abscess is an important determinant of outcome. Abscesses less than 4 cm can be treated effectively with bowel rest and broad-spectrum antibiotics [58,59]. Several recent studies of percutaneous drainage demonstrate that, for larger abscesses, percutaneous drainage with antibiotics improves the patient's clinical course [58,60–64] (see Fig. 99.2). Most patients with complicated diverticulitis require hospitalization and may require repeat imaging to confirm improvement in the radiographic findings. This is particularly true in immunocompromised patients including elderly patients or patients on steroids who may have a paucity of symptoms or clinical findings. A more thorough discussion of recurrence rates and operative management can be found in Chapter 61.

Crohn's-related intraabdominal abscesses and fistulae

Abscesses occur in up to 20% of patients with Crohn's disease [12,14]. They can arise from any involved region of the gut, with the terminal ileum being the most common. They commonly arise postoperatively at the site of an anastamosis. Approximately 50% arise proximal to a narrowed area, resulting in a relative high-pressure region. An abscess is formed if the infection is walled off by inflamed tissue and mesentery. If there is an adjacent bowel loop or an adherent scar, an enteroenterofistula or an enterocutaneous fistula may form. Enterovesical or enterovaginal fistula form in some patients. Fistulae and abscesses in the perianal region present particular clinical and diagnostic considerations that are described in Chapters 57 and 66.

Abdominal abscesses are best identified with CT using intravenous and positive oral contrast. Management of intraabdominal abscesses in Crohn's disease is complicated by the underlying pathology and the immunosuppressive medications used to treat serious Crohn's disease [12]. Percutaneous drainage and antibiotics may successfully treat the immediate infection, but half of patients treated nonoperatively will ultimately require surgery [65]. The use of potent immunosuppression in the setting of an abscess creates a difficult clinical dilemma, especially inpatients presenting with an abscess and intestinal obstruction or near obstruction. Generally, parenteral steroids or anti-tumor necrosis factor (TNF) therapy are deferred until the abscess is drained and antibiotics are ongoing. It is anticipated, but not proven, that treatment with disease-modifying therapy earlier in the course of the disease will prevent complications such as strictures, abscesses, and fistulae and reduce the occurrence of these difficult situations.

Data on medical therapy for Crohn's fistulae are derived from studies that include predominantly patients with perianal disease, with fewer patients in each series having internal or other fistulae. Corticosteroids are not effective in closing fistulae. Antibiotics are indicated if there is infection present, and leads to healing in some patients, but is not definitive therapy for most. Controlled trials of azathioprine and 6-mercaptopurine demonstrate efficacy in fistula closure and improved symptoms [66,67]. Other agents such as tacrolimus also show efficacy [68]. The most robust data on fistula closure of all types suggests the use of anti-TNF therapy, where closure rates approach 60% [14,69,70]; most fistulae reopen when the therapy is stopped. A combined approach of medical (commonly anti-TNF therapy) and limited surgical therapy is commonly used and often successful [66,67,71]. Medical therapy for fistulae is considered in Chapter 57 in more detail.

Gastric fistulae

Most cases of gastric fistulae are postsurgical. In past decades, surgery for ulcer disease was the most common cause, with ischemia and anastamosic tension considered the major factors. Gastric fistulae are more commonly the result of long-term gastrostomy tube placement. Fistulae in this setting are related to the duration of tube placement and nutritional status of the patient [72]. The rate of gastrocutaneous fistulae following operations for nonmalignant conditions such as ulcer disease, reflux, and obesity is 0.5%–3.9% [2]. With the increasing rate of surgical therapy for obesity, it is anticipated that obesity surgery will be a more common cause of gastrocutaneous fistulae in the future. Spontaneous gastrocutaneous fistulae are uncommon, but may result from ulcer disease, ischemia, radiation, and cancer. The effluent from a gastrocutaneous fistula can be high volume, and result in hypochloremic hypokalemic metabolic alkalosis and skin breakdown. These symptoms may be improved with high-dose proton pump inhibitors, and good skin care. Treatment is generally surgical, but endoscopic approaches have been utilized [73,74].

Pancreatic abscesses and fistulae

A pancreatic abscess is a devastating complication of severe necrotizing pancreatitis. It is typically considered when a patient with severe acute pancreatitis has CT findings suggesting pancreatic necrosis. Development of a pancreatic abscess occurs late in the course of acute pancreatitis, occurring in the second or third week in 40%–70% of patients

[75]. Persistent fever, elevated white blood cell count, sepsis, bacteremia, or failure to demonstrate improvement raises the index of suspicion for a pancreatic abscess. Data including a recent metaanalysis favor prophylactic antibiotics to reduce the mortality and morbidity of pancreatic necrosis [75]. As with abscesses in other abdominal sites, percutaneous aspiration to document infection and refine antibiotic therapy is recommended [76,77]. Surgical therapy for pancreatic abscess is associated with high mortality, and therefore minimally invasive laparoscopic, radiographic or endoscopic techniques for debridement and drainage have been tried [78,79]. Surgical therapy remains the standard, but patient factors and local experience and expertise may determine the exact approach. Necrotizing pancreatitis and pancreatic abscess are discussed in more detail in Chapter 68.

A pancreatic fistula develops as a complication of severe acute pancreatitis or trauma or as a result of surgery to the pancreas. Endoscopic retrograde cholangiopancreatography is used to determine pancreatic duct anatomy and establish continuity; a pancreatic duct stent can improve drainage in certain settings. CT can also help to delineate anatomy and rule out abscess or other pathology that could affect drainage. Pancreatic fistulae may be high volume and may have significant metabolic consequences, with bicarbonate loss resulting in acidosis and malabsorption occurring due to loss of digestive enzymes. Duodenal ulcers can occur due to diversion of the alkaline pancreatic secretions from the duodenum. Fluid and electrolyte management, proton pump inhibitor therapy, and pancreatic enzyme replacement are used. Octreotide is effective in decreasing fistula volume and may improve spontaneous closure rates [17,52]. Nonoperative therapy is generally successful and only 10%–30% of patients require surgery for fistula closure [2].

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H ABDOMINAL CAVITY

100 Diseases of the peritoneum, retroperitoneum, mesentery, and omentum

Shawn D. Larson, B. Mark Evers

Peritoneum, 2505
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Peritoneum

Embryology, anatomy, and physiology

The peritoneum is the mesothelial lining of the peritoneal cavity and its contained viscera. Embryologically, it develops from the primitive celom, which is formed by a splitting of the lateral mesoderm into somatic and splanchnic layers [1]. The parietal peritoneum, derived from the somatic layer, lines the abdominal cavity, diaphragm, and pelvis. The visceral peritoneum, from the splanchnic layer, covers the intraperitoneal organs and forms the mesenteries by which they are suspended.

The peritoneum and mesentery are supplied mainly by the splanchnic blood vessels and, to a lesser extent, by branches of the lower intercostal, lumbar, and iliac arteries. Differences arise in the innervation of the visceral and parietal peritoneum, which lead to different perceptions of painful stimuli. The visceral peritoneum receives afferent innervation from the autonomic nervous system and responds primarily to traction and pressure; painful stimuli are perceived as a poorly localized, dull pain. In contrast, the parietal peritoneum is innervated by both somatic and visceral afferent nerves; noxious stimuli are perceived as a localized, sharp pain with rebound tenderness.

The peritoneum, with a total surface area of approximately 2 m^2, serves as a bidirectional dialysis membrane through which the passage of both large- and small-molecular-weight solutes is accomplished predominantly by simple passive diffusion [2,3]. Factors that can alter absorption include intraabdominal pressure, temperature, pH, increased portal pressure, lymphatic blockade, and scarring of the peritoneum [4,5]. The peritoneum contains a complex multileveled defense system that provides an innate immune system to protect against inflammatory processes [6,7]. This system consists mainly of mechanical clearance of the peritoneal cavity; sequestration mechanisms, such as fibrin trapping of bacteria activation of complement, particularly C3a and C5a, which stimulate neutrophil chemotaxis; and the bacteria-killing actions of polymorphonuclear neutrophils and macrophages. The disease processes of the peritoneum discussed in this section include inflammation of the peritoneum (i.e., peritonitis), primary mesothelioma, and pseudomyxoma peritonei.

Peritonitis

Peritonitis involves a local or generalized inflammation of the parietal and visceral peritoneum. With the development of broader-spectrum antibiotics, newer anesthetic techniques, and modern preoperative and postoperative monitoring in the intensive care unit, the mortality from peritonitis has been greatly reduced; however, despite these advances, peritonitis remains a formidable challenge confronting physicians.

Peritonitis can be classified as either *primary* or *secondary*. Primary peritonitis is less common and involves a spontaneous infection of preexisting ascites in the absence of any obvious intraabdominal source [8,9]. The spectrum of bacteria causing this syndrome and the patient population primarily affected has changed. Spontaneous bacterial peritonitis is more common in adults than in children and shows no differential gender incidence. The group most commonly affected, formerly children with nephrosis, is adults with cirrhosis. Whereas gram-positive organisms formerly caused most of these infections, gram-negative enteric bacteria account for 60%–80% of organisms in ascitic fluid cultures, with *Escherichia coli* and *Klebsiella pneumoniae* the most common species [10]. Primary, or spontaneous, peritonitis is discussed in Chapter 90.

Secondary peritonitis is caused by diseases of or injury to the intraabdominal organs causing a mechanical break in the gastrointestinal tract [11]. Acute suppurative peritonitis,

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granulomatous peritonitis, and chemical (aseptic) peritonitis belong to this category and are discussed in this section.

Acute suppurative peritonitis

Etiology

Spillage of intestinal contents into the peritoneal cavity as a result of primary intraabdominal disease (e.g., perforated peptic ulcer, appendicitis, diverticulitis, perforated carcinoma), penetrating trauma, or iatrogenic perforation after instrumentation or radiological procedures is the usual cause of acute suppurative peritonitis [12,13].

Clinical manifestations

Abdominal pain, which may be sudden in onset if associated with a perforated viscus, is the predominant symptom [14]. Characteristically, patients with peritonitis lie supine with knees flexed and with frequent and limited intercostal respirations. Examination of the abdomen of a patient with generalized peritonitis reveals distention and bowel sounds that are either hypoactive or absent. Of the various physical findings of peritonitis, tenderness and muscle guarding are the most important. Tenderness is often maximal over the organ in which the process originated; however, as the inflammation spreads, tenderness may be present over the entire extent of the peritoneum. Rebound tenderness, defined as the sudden and severe pain produced with the release of the hand after deep abdominal palpation, is often described as a useful adjunct in the diagnosis of the acute abdomen. This test, however, elicits no more information than that obtained from a careful and gentle examination of the abdomen and, furthermore, may cause unexpected and undue pain to the patient. Symptoms associated with acute suppurative peritonitis include anorexia, nausea that is often accompanied by vomiting, fever (38°C–40°C), and signs of hypovolemia (e.g., tachycardia, dry mucous membranes, and hypotension). Typical diagnostic findings in patients with acute peritonitis include a leukocytosis, often with an increased number of bands in the differential count. Radiographic findings are usually nonspecific, with evidence of a paralytic ileus of both the small bowel and colon in some patients. Free air under the diaphragm may be seen in an upright chest radiograph if a ruptured viscus is the cause (Fig. 100.1). Although conventional plain radiographs remain as a first-line investigation, extraluminal air is identified in only 50%–70% of cases [15]. Computed tomography (CT) is recognized to be more sensitive for the detection of free air with reported sensitivities of 70%–100% [15]. The improved sensitivity of CT allows for the detection of free air that would not normally be detected with conventional plain films. Furthermore, indirect findings of bowel perforation such as phlegmon, abscess, or free peritoneal fluid can easily be detected with CT imaging [16]. Generally, the diagnosis of acute suppurative peritonitis is based on clinical manifestations and a thorough physical examination; laboratory and radiological procedures serve mainly in a confirmatory role.

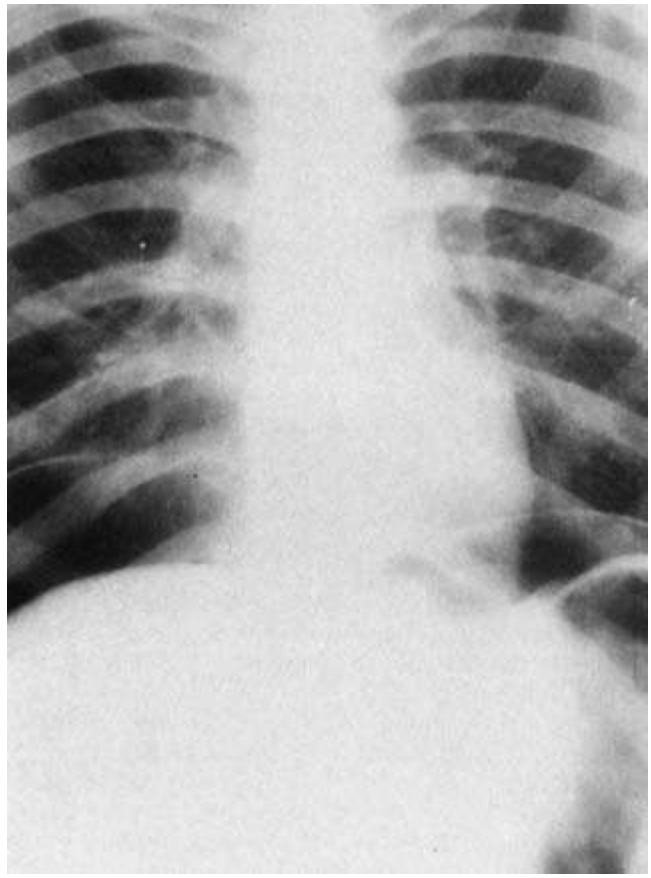

Figure 100.1 Upright chest radiograph demonstrates free air under both diaphragms after perforation of a duodenal ulcer. Courtesy of Charles J. Fagan, MD, Galveston, TX.

Clinical course and complications

Usually, suppurative peritonitis has an abrupt onset and a relatively short course with a rapid progression. Mortality results from fluid shifts and systemic endotoxin, which may cause hypovolemia and septic shock. Early diagnosis with prompt surgical intervention and aggressive preoperative and postoperative management is essential to reduce the morbidity and mortality from multiple organ system failure resulting from untreated peritonitis [17–20].

Treatment

The hallmarks of treatment include resuscitation with intravenous fluids to restore normal physiology, broad-spectrum antibiotics, and operative management to control the source of peritoneal contamination and irrigate the peritoneal cavity (Table 100.1).

Resuscitation involves aggressive administration of isotonic crystalloid fluids (e.g., Ringer's lactate) to correct hypovolemia

Table 100.1 Treatment of acute suppurative peritonitis

Restore normal physiology
Fluid resuscitation
Correct electrolyte imbalances
Supplemental O_2
Monitoring (e.g., vital signs, urine output, CVP, PCWP)
Broad-spectrum antibiotics
Operative intervention
Control the source of infection
Irrigate peritoneal cavity
Planned reoperations if source of infection not controlled
Investigational adjuvant therapies
Immunostimulation (e.g., administration of biological response modifiers, vaccination)
Immunorepletion (e.g., administration of antiendotoxin antibody)
Immunoabrogation (e.g., cytokine antagonism, inhibition of prostaglandin synthesis)

CVP, central venous pressure; PCWP, pulmonary capillary wedge pressure.

and electrolyte imbalances. Careful monitoring of the response to rapid fluid resuscitation is necessary, particularly if large fluid volumes are required or the patient has impaired cardiac reserve. Monitoring usually entails frequent assessment of vital signs, hourly measurement of urine output, and frequent determination of central venous pressure or, preferably, placement of a Swan–Ganz catheter for pulmonary capillary wedge pressure and pulmonary artery pressure determinations in an intensive care unit. Oxygen may be administered to overcome the mild hypoxemia that is commonly present, and nasogastric intubation is required to decompress the stomach.

Antibiotics play an important, albeit adjuvant, role in the management of patients with peritonitis. To be effective in the treatment of peritonitis, antibiotic therapy must be initiated before and continued during and after surgical therapy. In addition, broad-spectrum antimicrobial therapy should be directed toward both aerobic and anaerobic pathogens. The combination of an aminoglycoside (e.g., gentamicin or tobramycin) with clindamycin has long been considered the "gold standard" in the treatment of intraabdominal infections. However, concerns about nephrotoxicity have prompted investigators to evaluate the use of single agents (e.g., third-generation cephalosporins, broad-spectrum penicillins with β-lactamase inhibitors and carbapenems) in the treatment of intraabdominal infections. A number of single and combination drug choices have been recommended, all with comparable efficacy, by the Surgical Infection Society for the treatment of intraabdominal infections [21,22]. A more recent Cochrane review demonstrated no difference in morbidity and mortality when single agents were compared to combination regimens; however, the combination of fluoroquinolones/antianaerobes and cephalosporins/β-lactamase inhibitors did appear to be statistically more effective [23].

Expeditious surgical intervention remains the mainstay of treatment [24]. After an adequate period to ensure resuscitation, laparotomy should be performed to irrigate the peritoneal cavity copiously and to repair the rupture. Aerobic and anaerobic cultures should be obtained to ensure appropriate antibiotic coverage. If the source of infection is not eliminated, the debridement is incomplete, or definitive abdominal closure cannot be accomplished, staged laparotomies may be required for redebridement, removal of abdominal packs, and repeat irrigation until the source of infection is controlled and abdominal closure is obtained. In this situation, the placement of a large abdominal zipper can greatly facilitate abdominal opening and closing. Other procedures that have been described, but found not to be beneficial, include continuous postoperative irrigation with Tenckhoff catheters and radical peritoneal debridement. Recently, laparoscopy has been used as both a diagnostic and a therapeutic modality in selected patients with peritonitis [25–27]; the future role and possible advantages of laparoscopy compared with a formal laparotomy remain to be defined.

The clinical syndrome of sepsis results from a systemic host response to infection that, in turn, triggers the cytokine cascade. A number of cytokines have been identified and are generally classified as either proinflammatory (e.g., tumor necrosis factor-α [TNF-α] and interleukin-1 [IL-1]) or anti-inflammatory (e.g., IL-10 and IL-1 receptor antagonist). It is generally accepted that a careful balance exists between proinflammatory and antiinflammatory cytokines; a disruption in this balance leads to an exaggerated immune response resulting in multiple organ system failure and possibly death. Therefore, novel agents directed at immunomodulation offer an attractive area of research and may offer new therapies in the treatment of sepsis. One such approach has focused on neutralizing the proinflammatory cytokine TNF-α using monoclonal anti-TNF antibodies. Three large clinical trials (i.e, the North American Sepsis Trials [NORASEPT I and II] and the International Sepsis Trial [INTERSEPT]) were conducted to test the efficacy of anti-TNF therapy in the treatment of severe sepsis [28–30]. These trials generated some conflicting results, but overall, anti-TNF therapy was noted to have possible survival benefits in a select subgroup of patients (e.g., patients in septic shock) when administered early (< 24 h from the onset of symptoms). Other approaches to immunomodulation are under investigation. One such approach is the use of interferon-γ, granulocyte–macrophage colony-stimulating factor and intravenous immunoglobulins to promote monocyte activity [31]. These have demonstrated encouraging results in small studies; however, large randomized trials are needed to test the clinical efficacy of these modalities in sepsis. Finally, anticoagulant therapies, such

as tissue-factor-pathway inhibitors and activated protein C, have emerged in the treatment of sepsis [31]. While these therapies may not be regarded as true immunomodulators, research has suggested that the coagulation cascade interacts with various inflammatory pathways. A recent large, multicenter placebo-controlled trial has demonstrated significantly increased survival following infusion of recombinant human activated protein C [32].

Granulomatous peritonitis

Etiology

Granulomatous peritonitis is a disease process characterized by peritoneal inflammation that is associated with formation of granulomas and an increased incidence of adhesions. The disease most commonly associated with granulomatous peritonitis is tuberculosis [33,34]. Other, less common, causes include fungal (e.g., *Candida*, *Histoplasma*), amebic, and parasitic infections [35–37]. Iatrogenic causes of granulomatous peritonitis also occur, usually related to the presence of glove lubricants (e.g., talc, cornstarch) or cellulose fibers from gauze, surgical drapes, or gowns within the peritoneum [38].

Tuberculous peritonitis

Epidemiology

After years of a decreasing incidence of tuberculosis in the United States, there has been a general reemergence of this disease secondary to an increase in cases of acquired immunodeficiency syndrome (AIDS) and an increase in the number of immigrants [39–44]. Cases of tuberculosis have decreased in the United States since 1984 but the number of new cases worldwide has increase approximately 20% [45]. The recent appearance of multidrug-resistant strains of tuberculosis is particularly disturbing [44,46,47]. In addition to patients with AIDS, other groups at high-risk include poorly nourished, debilitated patients, patients requiring ambulatory peritoneal dialysis, and those with cirrhosis, diabetes, or malignancy [48,49].

Etiology

Tuberculous peritonitis is a form of abdominal tuberculosis that can involve the omentum, intestinal tract, liver, spleen, or female genital tract in addition to the parietal and visceral peritoneum. It accounts for approximately 11%–16% of extrapulmonary cases [50]. The overall incidence of abdominal tuberculosis in the United States is about 0.5%–1% of all cases of tuberculosis [33,34]. Tuberculous peritonitis is usually associated with a primary focus of tuberculosis elsewhere. This primary focus is usually the lung; however, only about one-third of cases have clinical or radiographic evidence of pulmonary tuberculosis. The pathological organism, *Mycobacterium tuberculosis*, can gain entry to the peritoneal cavity by one of three mechanisms: transmurally from diseased bowel, from tuberculous salpingitis, or, more commonly, by hematogenous spread from a pulmonary focus [33,34].

Clinical manifestations

Generally, the onset is insidious, with more than 70% of patients having had symptoms for more than 4 months before definitive diagnosis. The most common symptoms are constitutional and include fever, anorexia, weakness, malaise, and weight loss. Abdominal distention caused either by ascites or by partial obstruction may be present. On examination, the abdomen is diffusely tender in most patients; however, the classic doughy abdomen is rarely found. Tuberculous peritonitis should be suspected in high-risk or immunocompromised patients with ascites, fever, unexplained generalized symptoms, and diffuse abdominal pain or tenderness [33,34].

Differential diagnosis and diagnostic studies

Routine laboratory and radiographic studies are rarely diagnostic. A normal leukocyte count is present in most patients, and anemia is found only occasionally. Tuberculin skin tests are usually positive in patients with tuberculous peritonitis; however, a negative result is of no help in excluding the disease. Chest radiographs are abnormal in 80% of patients and include findings of pleural effusions or pulmonary infiltrates. Radiographs of the abdomen are seldom of benefit; however, a CT scan may be useful in identifying thickened bowel and ascites.

Examination of the peritoneal fluid may prove useful (see Chapter 90). In patients with tuberculous peritonitis, the protein content characteristically exceeds 3.0 g/dL, and glucose concentration is below 30 mg/dL in more than 80%. Most patients have a leukocyte count higher than 250 cells/mm^3 and a relative lymphocytic pleocytosis. Acid-fast stains are rarely positive unless at least 1 L of ascitic fluid is obtained, concentrated by centrifugation, and then examined. To compound the diagnostic problem, bacterial cultures of *M. tuberculosis* often require 4–6 weeks of incubation. For these reasons, much attention has been given to the development of a rapid diagnostic test for tuberculosis. Promising new approaches include adenosine deaminase, polymerase chain reaction, ligase chain reaction, and enzyme-linked immunosuorbent assays [48,51]. Although these tests hold great potential, a number of issues surround their routine use including cost, availability, and low predictive value.

The diagnosis of tuberculous peritonitis is often suggested by findings at laparoscopy or laparotomy. With reported sensitivities of 100% and low complication rates, laparoscopy with biopsy is considered the gold standard in diagnosing peritoneal tuberculosis [33,52]. Tuberculous peritonitis is characterized by stalactite-like fibrinous masses from the parietal peritoneum; in addition, the peritoneum may be studded with small granulomas.

The differential diagnosis of tuberculous peritonitis is variable, depending on the acuteness of the symptoms. In patients with a prolonged history, tuberculous peritonitis most commonly is confused with Crohn's disease or carcinoma. In patients presenting acutely, the differential diagnosis must

include such entities as acute appendicitis, cholecystitis, perforated ulcer, and salpingitis.

In summary, the diagnosis of tuberculous peritonitis is often difficult to make and requires an initial suspicion based on the clinical presentation (i.e., fever, anorexia, and abdominal pain) in combination with findings of a nonexudative ascites. It is crucial that a suspected diagnosis be confirmed by open or laparoscopy-assisted biopsy before committing the patient to a long-term treatment regimen with multiagent therapy.

Clinical course and therapy

Before the advent of chemotherapy, the mortality from tuberculous peritonitis was as high as 60%; the disease is, for the most part, readily curable with the available agents. Current recommendations for tuberculous peritonitis follow the principles of treatment for pulmonary tuberculosis. Treatment consists of a regimen of isoniazid, rifampin, pyrazinamide, and ethambutol for 6 months. Therapy should be prolonged for patients who exhibit a slow response to treatment. Although not proven experimentally, corticosteroids for 2–3 months may be beneficial in preventing the formation of dense fibrous adhesions, which could lead to bowel obstruction. The most worrying feature of the resurgence of tuberculosis has been the recent outbreaks of multidrug-resistant isolates that often fail to respond to isoniazid and rifampicin, the two cornerstone antituberculosis drugs [39–44,46,53]. Treatment of patients with multidrug-resistant tuberculosis should ideally be managed by physicians with expertise in drug-resistant disease.

Chemical (aseptic) peritonitis

Etiology

Peritoneal inflammation can result from spillage of irritant materials that are initially sterile; with time, secondary bacterial contamination occurs with signs and symptoms similar to those of acute suppurative peritonitis. Most of the substances that cause a chemical peritonitis are capable of acting as adjuvants to promote the growth of bacterial contaminants. These agents include bile, usually resulting from open or laparoscopic biliary tract operations without adequate external drainage, from inadvertent injury to the biliary tract, or from external trauma [54–56]; urine, from intraperitoneal bladder rupture [57]; and chyle, which occurs secondary to injuries of large lymphatic vessels during operations on retroperitoneal organs [58]. In addition, spillage of barium sulfate, secondary to perforation of the gastrointestinal tract from diagnostic procedures, results in a severe peritoneal irritation, and, in combination with accompanying enteric bacteria, poses a lethal threat to the patient [59,60].

Treatment

The same principles that apply to the management of acute suppurative peritonitis also apply to the treatment of chemical peritonitis. Adequate intravenous fluids to replace the peritoneal fluid sequestration and antibiotics are followed by laparotomy to irrigate the abdomen and control the source of the peritoneal contamination.

Peritonitis as a complication of chronic peritoneal dialysis

Etiology

Continuous ambulatory peritoneal dialysis (CAPD) has gained increasing acceptance as an efficacious and cost-effective alternative to chronic hemodialysis in patients with end-stage renal disease. It is estimated that, in the United States alone, about one-fourth of the more than 100 000 dialysis patients use CAPD. Despite its obvious advantages, the most common complication of CAPD is infectious peritonitis resulting from bacterial contamination of the peritoneal cavity [61]. Even with better patient education regarding sterile techniques and proper cleaning around the catheter site, peritonitis in this group of patients is the major source of morbidity and the largest single cause of patient failure on CAPD. Although the incidence of peritonitis varies with the institution, peritonitis as a complication of CAPD averages 1.1–1.3 episodes per patient-year of treatment [61,62].

Epidemiology

The most frequent portals of entry for bacteria in CAPD patients include the exit site of the dialysis catheter and the tunnel through which the catheter traverses the abdominal wall. Infection usually occurs as a result of a break in aseptic technique with secondary contamination. Other possibilities for contamination include hematogenous or lymphatic dissemination from a septic focus originating from the patient. In contrast to other types of peritonitis, CAPD-related peritonitis usually is caused by a single organism, in most cases by gram-positive cocci (*Staphylococcus epidermidis*, *Staphylococcus aureus*, streptococci, and enterococci) [61–65]. Gram-negative bacteria account for about 25% of all cases of CAPD peritonitis, with the most common being *E. coli* and *Pseudomonas aeruginosa*. The presence of anaerobic organisms or a mixed flora should raise suspicion of an intestinal perforation or other intraabdominal disease as the cause of peritonitis. Fungal peritonitis is relatively uncommon but remains the most serious type of infective peritonitis in CAPD patients because of treatment difficulties [66]. At least 20 different fungal species have been cultured; however, *Candida albicans* and *Candida tropicalis* remain the most common.

Clinical manifestations

The severity of symptoms is dependent on the infecting organism; however, the signs and symptoms in most patients with CAPD peritonitis are clinically less severe than those in patients with suppurative peritonitis. Some cases may even be asymptomatic and detected only by the presence of a cloudy effluent. The predominant symptom found in

patients is a diffuse abdominal pain, usually associated with a lowgrade fever and leukocytosis. Other symptoms may include hyperhydration, diarrhea, hypotension, and pressure pain above the catheter tunnel. Characteristically, a turbid dialysis effluent is found in nearly all cases of CAPD peritonitis.

Diagnosis

The diagnosis of CAPD peritonitis is based on both clinical and laboratory findings [61,65,67]. The clinical diagnosis should be based on the presence of two of three criteria: abdominal pain or tenderness, a turbid dialysate containing more than 100 neutrophils/mm^3, and a positive culture from the peritoneal fluid. Even if the patient's history and examination are consistent with the diagnosis of peritonitis, it is essential that the dialysate be evaluated to confirm the diagnosis and to identify the causative organisms. In some centers, the use of bacteria-concentrating techniques yielded positive cultures in more than 90% of cases.

Therapy and clinical course

Of patients with CAPD peritonitis, 70%–80% can be treated successfully on an outpatient basis without hospitalization or interruption of dialysis [61,63–66,68–70]. CAPD peritonitis is treated most effectively by intraperitoneal administration of antibiotics. The initial choice of antibiotics should be broad enough to cover the most common pathogens causing CAPD peritonitis and then should be changed according to susceptibility testing. Recently, the International Society for Peritoneal Dialysis (ISPD) has altered its recommendations for empirical therapy of peritonitis. Current recommendations include a first-generation cephalosporin for gram-positive organisms, a third-generation cephalosporin for gram-negative organisms, and a combination of first- and third-generation cephalosporins when no organism is identified [65]. Aminoglycosides may be substituted for treatment of gram-negative organisms in patients with residual urine output of < 100 cm^3/day. Furthermore, ISPD no longer recommends the routine use of vancomycin; however, this recommendation remains controversial. In addition to antibiotics, heparin also is added to the dialysis bag to reduce fibrin formation and, thereby, the incidence of postinfective adhesions. Indications for catheter removal include persistence of peritonitis after 4–5 days of treatment and the presence of fungal or tuberculous peritonitis, fecal peritonitis, or severe skin infection at the catheter site.

Peritonitis in the immunocompromised host

Etiology and diagnosis

The AIDS epidemic and the increasing use of immunosuppressive therapy for cancer, autoimmune disease, and multiple organ transplantation procedures have combined to increase greatly the incidence of opportunistic infections of the gastrointestinal tract [53,71]. The differential diagnosis of abdominal pain in AIDS is broad and involves both AIDS- and non-AIDS-related disorders (see Chapter 104) [71–73]. Perforation of the small bowel or colon secondary to cytomegalovirus enteritis is a frequent cause of peritonitis in patients with AIDS [74,75]. Other organisms reported to cause peritonitis in these patients include *Mycobacterium avium-intracellulare*, *M. tuberculosis*, *Cryptococcus neoformans*, *Strongyloides* spp., and *Leishmania* spp. [71–74,76–78].

Clinical manifestations and treatment

Immunocompromised patients with peritonitis usually present with severe abdominal pain as the predominant symptom, like patients with suppurative peritonitis. Principles of management include emergent laparotomy and resection of the involved portion of bowel, intravenous fluid resuscitation, and broad-spectrum antibiotics.

Primary mesothelioma

Incidence and etiology

Primary mesotheliomas, arising from mesenchymal and epithelial components, are uncommon. The reported incidence in the United States is only 2.2 cases per million population; however, studies suggest that the incidence is increasing throughout the world and especially in industrialized countries [79–83]. Only 20%–40% of mesothelioma occurs in the peritoneum, with most of these tumors originating from the pleura. Malignant mesothelioma is linked to exposure to asbestos, and there is a strong association with occupations that require workers to handle or work in proximity to asbestos, such as those in the textile, insulation, building, demolition, and shipyard industries [81,82,84–86]. There is a latency period of 20–40 years from significant asbestos exposure to development of mesothelioma; two-thirds of the patients are diagnosed at 45–64 years of age, and there appears to be a male predominance. Crocidolite is considered the most oncogenic type of asbestos, whereas the oncogenic potential of chrysotile is still debated. Although asbestos is the predominant cause of this disease, other etiological agents may be associated with primary mesothelioma, including genetic factors, use of the angiographic contrast material Thorotrast (thorium dioxide), radiation exposure, and recurrent peritonitis.

Clinical manifestations

The clinical presentation is nonspecific, with the predominant complaint being abdominal pain present in the epigastrium or right upper quadrant. Other symptoms include nausea and vomiting, malaise, fever, weight loss, diarrhea, and anemia. Ascites is the most common physical finding and occurs in 90% of patients. Peritoneal mesotheliomas can produce and secrete a variety of ectopic hormones, including antidiuretic hormone, growth hormone, and insulin-like factors, which can produce associated paraneoplastic syndromes with symptoms of hypoglycemia, hyponatremia, thrombocytosis, and increased production of fibrin-degradation products

[79–82,86]. Interestingly, peritoneal mesothelioma has been described in association with other synchronous neoplasms such as colorectal cancer.

Differential diagnosis and diagnostic studies

Malignant mesothelioma is a difficult malignancy to diagnose early [79–82,86,87]. Mesotheliomas are generally firm, white tumors that present as individual nodules studding the peritoneal surface and tend to spread over the surface of the intraabdominal organs, eventually encasing the viscera. Ultrasound and CT studies can suggest the diagnosis, demonstrate the extent of tumor, and also aid in directed biopsy of suspicious lesions; however, in most cases, the diagnosis is confirmed after diagnostic laparoscopy or laparotomy.

Microscopic and immunohistochemical studies are available to aid in the diagnosis [88,89]. Immunohistochemistry is useful for two reasons:

- to determine whether the mesothelial cells present are benign or malignant
- to distinguish mesothelioma from an adenocarcinoma.

The typical analysis of a suspected mesothelioma should include electron microscopy, periodic acid–Schiff (PAS) stain, Alcian blue stain to detect hyaluronic acid, and an anticarcinoembryonic antigen (anti-CEA) antibody stain. Ultrastructural features on electron microscopy include long, slender microvilli and diffuse cytoplasmic or perinuclear distribution of tonofilaments. Mesotheliomas lack intracellular mucin on PAS stains, should produce negative results on anti-CEA antibody stain, and often stain positively for hyaluronic acid. In contrast, adenocarcinomas often stain positively for mucin and CEA and do not contain hyaluronic acid. Well-characterized monoclonal and polyclonal antibodies hold great promise for improving the diagnostic accuracy [90]. Recent studies indicate that staining with calretinin, a member of the family of calcium-binding proteins, and cytokeratin 5/6 appear to be the most sensitive and specific positive members for distinguishing between malignant epithelial mesotheliomas (positive staining) and metastatic adenocarcinomas (negative staining) [89]. Among the antibodies that are considered to be negative members for mesothelioma, MOC-31, CEA, and BG-8 seem to be the best diagnostic discriminators. Other useful negative markers include B72.3, Leu-M1, Ber-EP4, and thrombomodulin. Immunostaining for N-cadherin and E-cadherin appears to be promising in the diagnosis of mesothelioma. Another useful marker is epithelial membrane antigen, which has been shown to be of value in distinguishing between benign and malignant mesotheliomas. Additional molecular studies that may serve as useful adjuncts to the diagnosis include the identification of high levels of the platelet-derived growth factor (PDGF) in malignant mesotheliomas [90]. Another promising area of research involves gene expression microarray analysis [91]. Microarray data may help to identify single genes or gene expression patterns that will aid in diagnosis and predicting prognosis.

Less common primary peritoneal neoplasms that should be included in the differential diagnosis include benign papillary mesotheliomas, benign fibrous mesothelioma, adenomatoid tumors, and multicystic peritoneal mesothelioma [81,82].

Genetic factors

With the advances in molecular techniques and the ability to identify genetic abnormalities in various tumors, studies have focused specifically on potential genetic abnormalities in mesotheliomas [79,80,84,85,91–93]. The accumulation of numerous chromosomal deletions in a number of mesotheliomas suggests a multistep process of tumorigenesis, characterized by the loss or inactivation of various tumor suppressor genes. Genetic abnormalities that have been identified include frequent deletions of specific sites within chromosome arms 1p, 3p, 6q, 9p, 13q, 15q, and 22q [92,93]. Loss or mutation of the $p16^{Ink4a}$ tumor suppressor gene at 9p21 has been noted with high frequency (~ 85%) and may contribute to the pathogenesis of these tumors. In addition, another recurrent theme of genetic abnormalities in mesotheliomas is mutation or allelic loss of neurofibromatosis type 2 (*NF2*), a tumor suppressor gene, at 22q12. Recent studies have identified the presence and expression of simian virus 40 (SV40) in a number of mesotheliomas [94,95]. SV40 large T antigen has been shown to inactivate the tumor suppressor gene products Rb and p53, suggesting the possibility that asbestos and SV40 could act as cocarcinogens in mesothelioma. Furthermore, circulating antibodies to SV40 antigens have been described, raising the possibility that the immune system may be modulated to treat patients with mesothelioma. Finally, expression of growth factors, including PDGF-A and PDGF-B, insulin-like growth factors I and II, and transforming growth factors α and β, has been identified in these tumors. A possible paracrine/autocrine role for PDGF has been postulated because the PDGF receptor is expressed in a number of mesotheliomas. Continued studies are required to determine whether these genetic abnormalities play a role in the pathogenesis and progression of the mesothelioma tumors and may serve as potential targets for novel treatment strategies.

Treatment and prognosis

Therapy for mesothelioma has proved disappointing, mainly because of the advanced stage of the disease at diagnosis and the local aggressiveness of the tumor [96,97]. Rarely can surgery be performed for a curative resection; rather, laparotomy serves to establish a tissue diagnosis and to perform palliative procedures such as bypassing obstructive lesions or tumor debulking. Chemotherapy offers little improvement in overall patient survival. Doxorubicin, alone or in combination with other antineoplastic agents, achieves the best, albeit a minimal, response. Studies suggest that surgical cytoreduction and multimodality therapy, especially with paclitaxel and cisplatin chemotherapy, may confer a survival

advantage. In addition, there is emerging evidence of improved patient survival using hyperthermic intraperitoneal infusion of the chemotherapeutic agent in patients with primary peritoneal mesotheliomas [98–100]. Radiation therapy has been used in combination with intracavitary instillation of various chemotherapeutic agents or with systemic chemotherapy but with only minimal success.

Future directions for the management of mesothelioma include the use of IL-2, interferon-γ, TNF-α, monoclonal antibodies, and cancer vaccines, which may prove beneficial when used in combination with other therapies. The results of recent trials suggest potential benefits of chemoimmunotherapy for the treatment of mesothelioma. Novel treatment strategies under investigation include the introduction of the herpes simplex virus thymidine kinase gene into the mesothelioma so that growth is inhibited after administering ganciclovir and the transfection of antisense oligonucleotides to the growth factors PDGF-A or -B [79,80,101,102]. Multiple obstacles, including transfection efficiency and proper vector construction, must be overcome before these types of gene therapy protocols will be clinically beneficial. The prognosis for mesothelioma of the peritoneum is poor. Death usually occurs within 12–14 months of the diagnosis from progressive gastrointestinal tract obstruction and debility.

Pseudomyxoma peritonei

Incidence and etiology

Pseudomyxoma peritonei is a rare condition manifested by diffuse, gelatinous implants of the peritoneal cavity and omentum arising from mucinous neoplasms of either the appendix or ovary. The reported incidence of pseudomyxoma peritonei is approximately 2 in 10 000 laparotomies. The vast majority of female patients with pseudomyxoma peritonei have disease in both the appendix and ovary; therefore, there is no general agreement regarding the tissue of origin. Recent applications of immunocytochemistry and genetic analysis by polymerase chain reaction strongly suggest that most cases are the result of metastases from appendiceal neoplasms [103–105].

Clinical features and diagnosis

Women between 45 and 55 years of age make up about 75% of patients with pseudomyxoma peritonei. This is usually an unexpected diagnosis made at laparotomy for a preoperative diagnosis of appendicitis or ovarian tumor. Patients can present with an increasing abdominal girth secondary to mucinous ascites or intestinal obstruction, abdominal pain, or a mass in the abdomen [105–107].

Histologically, pseudomyxoma peritonei is characterized by a benign appearance with simple columnar epithelium containing mucin-filled vacuoles [107,108]. Certain features, noted on sonography or CT scan, are characteristic for this disease. Sonographic findings include multiple intraperitoneal multilocular cysts and ascitic septation. A characteristic CT finding of pseudomyxoma peritonei is "scalloping" of the hepatic and bowel margins secondary to extrinsic compression by ascitic spaces containing gelatinous material (Fig. 100.2). The role of magnetic resonance imaging (MRI) in diagnosing pseudomyxoma remains to be defined. T2-weighted images accentuate differences between mucinous and fluid ascites; however, a major disadvantage of MRI is the added expense compared with CT scan.

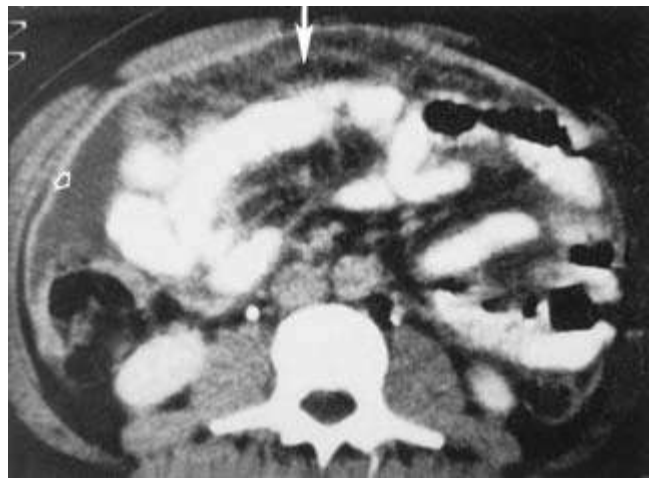

Figure 100.2 Computed tomography scan demonstrates large gelatinous masses (arrows) in a patient with pseudomyxoma peritonei. Courtesy of Charles J. Fagan, MD, Galveston, TX.

Treatment and prognosis

Pseudomyxoma peritonei is a low-grade malignancy that rarely metastasizes or invades contiguous viscera. The treatment of pseudomyxoma peritonei is primarily surgical, with aggressive debulking of all intraabdominal tumor and omentectomy [109]. In addition, because of the usual involvement of both the appendix and the ovaries, it is recommended that appendectomy and bilateral oophorectomy be performed in all patients. Recurrence occurs in about 75% of patients; repeat laparotomies are indicated for recurrent disease. Recently, new strategies in the treatment of this disease have included a chemotherapy regimen with surgical treatment [81,110–112]. These reports suggest a survival advantage using early postoperative chemotherapy (intraperitoneal or systemic) combined with cytoreductive surgical treatment compared with repeated surgical procedures in the absence of chemotherapy. Radiation treatment, mucolytic agents, and phototherapy have been described but appear to be of little benefit [113]. Long-term survival is about 54% at 5 years, with death occurring usually as a result of extensive peritoneal disease and intestinal obstruction.

Retroperitoneum

The retroperitoneum is the space behind the abdominal cavity extending from the posterior peritoneum and the mesenteries to the spinal column and the psoas, quadratus lumborum, and transversus abdominis muscles. It extends superiorly from the diaphragm and inferiorly to the levator muscles of the pelvis. The retroperitoneum is divided into the anterior compartment, which contains the pancreas, duodenal loop, and the ascending and descending colon, and the posterior compartment, which contains the perinephric space with the kidneys, ureters, and adrenal glands and an extensive network of vessels, lymphatics, and neural structures. The space also contains fatty and areolar tissue and connective tissue.

Modern diagnostic techniques, such as ultrasonography, CT scan, MRI, and arteriography, have greatly enhanced our ability to diagnose and treat pathological processes in the retroperitoneum; however, this region remains elusive. Early clinical manifestations are nonspecific so pathological processes become relatively advanced before producing symptoms. Retroperitoneal disease processes often present as a mass. (The differential diagnosis of retroperitoneal masses and other disease processes is shown in Table 100.2.) This section specifically discusses retroperitoneal hemorrhage and infections, idiopathic retroperitoneal fibrosis, and primary neoplasms of the retroperitoneal space. In addition to these clinical entities, fluid collections consisting of lymph, gastrointestinal secretions (e.g., duodenal, pancreatic), bile, or urine also may collect in this space, usually secondary to iatrogenic or traumatic injuries [114,115]. A CT scan or ultrasound may be a useful diagnostic imaging study; percutaneous drainage with fluid analysis may aid in further establishing the diagnosis and, in certain instances, may be useful in the treatment.

Table 100.2 Differential diagnosis of retroperitoneal masses and disease processes

Fluid collections
Lymph
Duodenal succus
Pancreatic juice
Bile
Urine
Hematoma
Spontaneous hemorrhage
Traumatic hemorrhage
Fat necrosis
Acute necrotizing pancreatitis
Abscess
Primary
Secondary: extension from abdomen, kidney, vertebra
Neoplasm
Benign
Cyst
Benign soft tissue tumor
Malignant
Sarcoma
Germ cell-derived neoplasm
Lymphoma
Lymph node metastases
Aortic aneurysm
Retroperitoneal fibrosis

Retroperitoneal hemorrhage

The most common etiology for retroperitoneal bleeding and hematoma is secondary to traumatic injuries associated with pelvic or vertebral fractures, avulsion of the vascular pedicle of the kidney, or penetrating injuries [115–118]. Other causes for hemorrhage may be secondary to anticoagulation therapy, acute pancreatitis, spontaneous hemorrhage into an adrenal gland or retroperitoneal tumor, ruptured aortic aneurysm, and ruptured ureteral or ovarian veins during pregnancy. In addition, massive retroperitoneal hemorrhage has been associated with femoral vein catheterization and liposuction [119–121].

If hemorrhage occurs quickly and in significant amounts, signs of hypovolemic shock may be present. Signs of retroperitoneal hemorrhage that may occur late include pain felt most in the abdomen and back and ecchymosis of the flank (Grey Turner sign). Laboratory examination may demonstrate a low hematocrit. Evaluation of the retroperitoneal hemorrhage may include plain films of the abdomen, which can show obliteration of the psoas shadow. A CT scan may be useful in the stable patient to evaluate the possible etiology and extent of the hemorrhage (Fig. 100.3).

Treatment of retroperitoneal hemorrhage is secondary to the specific etiology. For example, retroperitoneal hemorrhage

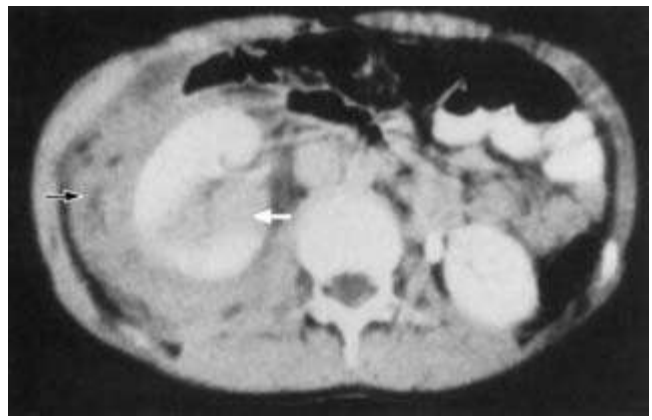

Figure 100.3 Computed tomography scan demonstrating a large retroperitoneal hematoma (open arrow) due to blunt injury to the kidney (closed arrow). Courtesy of Melvyn H. Schreiber, MD, Galveston, TX.

secondary to a pelvic fracture may be treated by fracture stabilization or arteriography and embolization of the bleeding vessel. Vascular injuries or a ruptured abdominal aortic aneurysm require emergent surgical intervention and control of the ongoing hemorrhage.

Retroperitoneal infections

Abscesses in the retroperitoneal space are normally secondary to diseases of surrounding abdominal organs, the urinary tract, or the vertebral column [112,122–126]. Most commonly, the abscesses result from perforated diverticular disease or colonic cancer, appendicitis, acute pancreatitis, penetrating duodenal ulcer, or biliary tract disease. A psoas abscess may occur secondary to intraabdominal or vertebral infections; extension from Crohn's disease, appendicitis, colon diverticulitis, and cancer are the most frequent causes. Primary bacterial infections in the retroperitoneal space can occur but are rare. Except for the Gerota fascia, there are no anatomical boundaries to the retroperitoneal space; therefore, abscesses can be large, extend bilaterally, and present late in their course. As a result, retroperitoneal abscesses may be associated with higher mortality.

Clinical manifestations include fever, chills, anorexia, and weakness; pain in the lower back and abdomen, thigh, or flank is present in about 60% of the patients. A palpable mass may be present on physical examination. Leukocytosis is often noted as the most common laboratory abnormality. The CT scan is the most useful diagnostic test to establish the diagnosis (Fig. 100.4); CT-guided percutaneous needle aspiration of the abscess may allow for bacteriological examination so that an appropriate antibiotic treatment regimen can be chosen based on the specific organisms and sensitivities. Depending on the etiology, percutaneous drainage may be the effective treatment for some of these collections; surgical drainage is required secondary to inadequate fluid evacuation. Coliform organisms and *S. aureus* are the most common organisms encountered. Other organisms can occur rarely and include *M. tuberculosis*, which most commonly effects the urinary tract or spine; opportunistic fungi (e.g., *Candida*, *Aspergillus*, *Cryptococcus* species) may spread to the retroperitoneum, particularly in patients who are immunocompromised. Actinomycosis or retroperitoneal infections with *Nocardia* organisms can be the result of secondary extension from a primary focus. For example, actinomycosis may be secondary to perforation of the appendix or colon and nocardiosis may be associated with a pulmonary infection.

Figure 100.4 Computed tomography scan demonstrating a retroperitoneal infection and abscess (arrow) with air bubbles present, originating from infection of the left kidney. Courtesy of Melvyn H. Schreiber, MD, Galveston, TX.

Retroperitoneal fibrosis

Etiology and epidemiology

Retroperitoneal fibrosis is an uncommon disease process characterized by progressive nonspecific inflammation and fibrosis of connective and adipose tissue in the retroperitoneal space [127–131]. This process originates just below the level of the aortic bifurcation near the sacral promontory and then spreads throughout the retroperitoneum with extension bilaterally along the aorta and inferior vena cava. Symptoms are the result of gradual compression of the tubular structures in the retroperitoneal space, particularly the ureter.

Retroperitoneal fibrosis was first described by the French urologist Albarran in 1905 [127]. The initial description of retroperitoneal fibrosis in the English literature is credited to Ormond in 1948 [128]. In fact, idiopathic retroperitoneal fibrosis is often referred to as Ormond disease. Retroperitoneal fibrosis is relatively uncommon, with an instance of 1 in 200 000. There appears to be an overall male predominance of 2 or 3 to 1, with presentation usually in the fifth and sixth decades of life. The etiology of this disease is, for the most part, not known. A number of factors have been postulated to contribute to this disease process, including extravasation of urine, surgery, and nonspecific gastrointestinal inflammatory diseases, including Crohn's disease; however, most cases of retroperitoneal fibrosis are considered idiopathic (Table 100.3). Various medications have been associated with retroperitoneal fibrosis as a side effect. Methysergide, an antiserotonin drug, is the best-described agent in which the occurrence of retroperitoneal fibrosis has been clearly linked [132]. Other drugs implicated in this fibrotic process include ergotamine, pergolide, hydralazine, methyldopa, and, β-adrenergic blocking agents such as metoprolol [133]. An immune etiology for retroperitoneal fibrosis has been suggested, particularly because an association with other immune-mediated connective tissue disorders such as systemic lupus erythematosus, Hashimoto thyroiditis, Raynaud disease, systemic vasculitis, and ankylosing spondylitis has been demonstrated in certain cases [134,135]. In addition, amelioration of symptoms can occur with corticosteroid treatment. In some patients, retroperi-

Table 100.3 Etiologies of retroperitoneal fibrosis

Primary
Idiopathic
In conjunction with other fibrotic processes (e.g., mediastinal fibrosis, mesenteric fibrosis, sclerosing cholangitis)
Drug-associated (e.g., methysergide, ergotamine, hydralazine, and β-adrenergic drugs)
Paraneoplastic (e.g., sarcoma, Hodgkin disease, carcinoid tumor)
Secondary
Radiotherapy
Retroperitoneal infection
Retroperitoneal fluid collection or hematoma
Inflammatory abdominal aortic aneurysm

toneal fibrosis can occur secondary to severe retroperitoneal infection (e.g., pancreatitis) or inflammatory aortic aneurysms and in response to neoplasms, most commonly Hodgkin lymphoma, sarcomas, carcinoid tumors, and certain adenocarcinomas [136].

Pathology

Retroperitoneal fibrosis appears grossly as a woody, white, fibrous plaque, which, as it progresses, surrounds the ureters and vascular structures in the retroperitoneum [129–131]. Early microscopic features include active chronic inflammation occurring with abundant lymphocytes, plasma cells, and macrophages interspersed with fibroblasts and collagen bundles [129,130]. As the disease progresses, the tissues become relatively acellular with scattered calcifications, an abundant collagen deposition, and fibrous scarring.

Clinical manifestations

The symptoms of retroperitoneal fibrosis are generally nonspecific, the most common symptom being a dull, constant pain localized to the back or flank [127–131]. This pain may radiate to the lower abdomen, groin, or anteromedial aspect of the thigh. Other symptoms include anorexia, nausea, diarrhea, fever, and weight loss; patients may present with hypertension. Laboratory abnormalities may include a leukocytosis, an elevated erythrocytic sedimentation rate, and elevated alkaline phosphatase levels. As the disease progresses, late complications of retroperitoneal fibrosis occur as the result of encasement and compression of the tubular retroperitoneal structures, most commonly involving the ureters, and eventually leading to azotemia, renal insufficiency, and possible renal failure. Lower extremity edema may occur from compression of lymphatics and venous obstruction [137]. The aorta can be involved, albeit uncommonly, with symptoms consistent with arterial insufficiency (e.g., claudication, rest pain). Rarely, this fibrotic process may involve the duodenum, the common bile duct, or colon, resulting in obstruction [129,130,138–140].

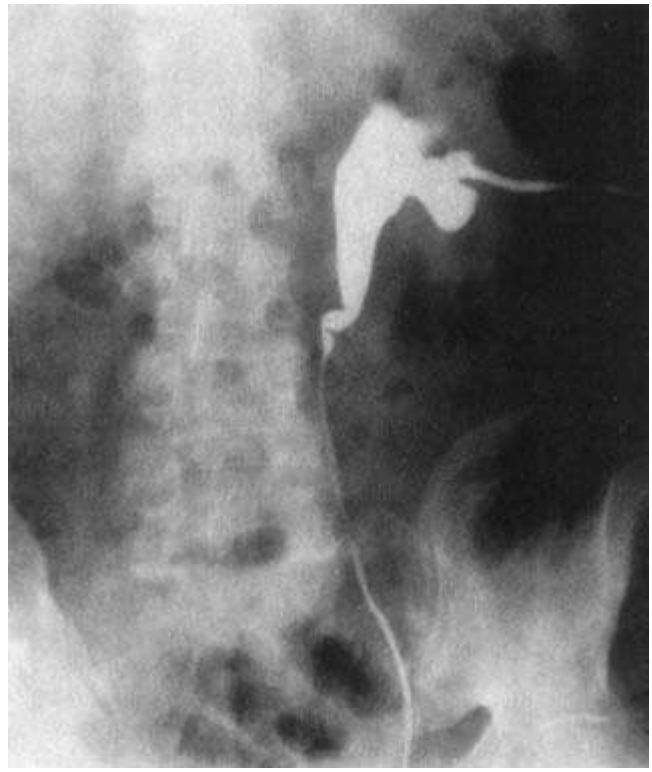

Figure 100.5 Pyelogram of the left kidney through a nephrostomy tube demonstrating marked hydronephrosis, narrowing of the ureter at the L4 level, and medial displacement of the ureter. Courtesy of Melvyn H. Schreiber, MD, Galveston, TX.

Differential diagnosis and diagnostic studies

The differential diagnosis of retroperitoneal fibrosis includes retroperitoneal hemorrhage, primary neoplasms, and metastasis to the retroperitoneum. Imaging studies useful in establishing the diagnosis of retroperitoneal fibrosis include an intravenous pyelogram, in which a triad of classic findings have been described: delayed excretion of contrast material with hydronephrosis, narrowing of the ureters at approximately the L4–5 level, and a medial displacement of the ureters (Fig. 100.5) [129,130]. In fact, this medial deviation of the ureters was considered a pathognomonic sign of retroperitoneal fibrosis, in contrast to retroperitoneal neoplasms in which deviation of the ureters normally occurs laterally. Recent studies, however, indicate that medial deviation is not a constant finding in patients with retroperitoneal fibrosis. The CT scan has emerged as the procedure of choice for the diagnosis and follow-up of patients with retroperitoneal fibrosis [130,141]. Findings include a periaortic soft tissue mass of variable thickness that envelops the retroperitoneal structures, with obliteration of the fat plane between the mass and adjacent psoas musculature. Increasingly, MRI has been used to assess retroperitoneal fibrosis, particularly using T2-weighted images to delineate the stage of the disease more clearly (Fig. 100.6) [140,142–144]. The

Figure 100.6 Axial T2-weighted magnetic resonance scan demonstrating a periaortic soft tissue mass (arrow) that obliterates the fat plane in a patient with retroperitoneal fibrosis. Courtesy of Greg Chalchub, MD, Galveston, TX.

advantages of MRI compared with CT are a more exact anatomical definition because of multiplanar capability and the avoidance of nephrotoxic contrast agents.

Treatment

Goals of treatment include establishing the diagnosis, relieving the obstruction (usually involving the urinary tract), and preventing progression of the disease. Medications associated with retroperitoneal fibrosis should be discontinued. Surgical management is directed at ureterolysis for ureteral obstruction with intraperitoneal transposition of the ureters. Other maneuvers described to prevent recurrence of the ureteral obstruction include wrapping the ureters in omental fat or interposing retroperitoneal fat between the ureters and the fibrosis [145,146]. The use of laparoscopic ureterolysis in selected cases has been described with good results in short-term follow-up [147]. Symptoms caused by venous obstruction are best treated nonoperatively, with elevation and elastic support of the leg eventually resulting in the development of collateral circulation. The medical management of retroperitoneal fibrosis includes corticosteroids, which are effective in the early, active inflammatory stage. Other immunosuppressive drugs, such as azathioprine and cyclophosphamide, also have been used with varying success [148,149]. Recently, two prospective observational trials have demonstrated remission of retroperitoneal fibrosis with steroid treatment [150,151]. In one trial, remission was achieved in 11 of 12 patients with prednisolone alone whereas, in the second trial, seven patients were successfully treated with a combination of prednisolone and mycophenolate–mofetil. Recent studies suggest that tamoxifen, a nonsteroidal antiestrogen agent, may be effective in regression of the fibrotic process and amelioration of symptoms secondary to increased synthesis and secretion of transforming growth factor-β, an inhibitory growth factor [152]. In patients with idiopathic retroperitoneal fibrosis without renal compromise and effective ureterolysis, the prognosis is excellent with long-term success rates exceeding 90% [129].

Retroperitoneal neoplasms

Etiology and pathology

Tumors in the retroperitoneal space arise from mesodermal, neuroectodermal, or embryonic remnants, including fat, connective tissue, fascia, muscle, nervous tissue, lymph vessels, and lymph nodes. Malignant tumors are about four times more prevalent than benign neoplasms, with the most common neoplasms being malignant lymphomas or liposarcomas [153,154]. Other malignant neoplasms in this area include other types of sarcomas, such as liposarcoma and leiomyosarcoma [155–157]. Of the benign tumors, the most frequent are lipomas. Most patients presenting with primary retroperitoneal tumors are in the fifth or sixth decades of life, although about 15% of these tumors are found in children aged younger than 10 years. In addition to primary tumors of retroperitoneal structures, the retroperitoneum is also a common site for metastasis, primarily lymphatic metastasis from neoplasms of abdominal organs.

Clinical manifestations

Retroperitoneal tumors can grow to a large size before becoming symptomatic [153,154]. Early symptoms are normally vague or lacking and may include fatigue, weakness, weight loss, abdominal discomfort, and a sense of fullness or abdominal heaviness. Nausea and vomiting and a change in bowel habits may occur with intestinal compression. Swelling of the lower extremities may occur secondary to obstruction of lymphatics or venous return. Larger tumors can cause pain radiating to both thighs as a result of involvement of the lumbar and sacral nerve roots. Rarely, patients may present with hypoglycemia. The most common physical finding is the presence of a nontender abdominal mass.

Differential diagnosis and diagnostic studies

Primary retroperitoneal tumors should be differentiated from other masses in this region, including renal lesions, pancreatic cysts and tumors, malignancies of the gastrointestinal tract, ovarian tumors, and cysts of the omentum and mesentery. The CT scan is a useful imaging modality to identify the mass, determine its size and origin, and assess its relationship, proximity, and invasion of surrounding structures (Fig. 100.7) [158]. In addition, CT-guided needle biopsy can often provide a histological diagnosis preoperatively. MRI has become increasingly important as a diagnostic tool for retroperitoneal neoplasms [144,159,160]; it provides a better degree of definition between the mass, surrounding muscle groups, and vascular structures compared with CT (Fig. 100.8). Before surgery, angiography provides important information about the vasculature supplying the neoplasm. The main

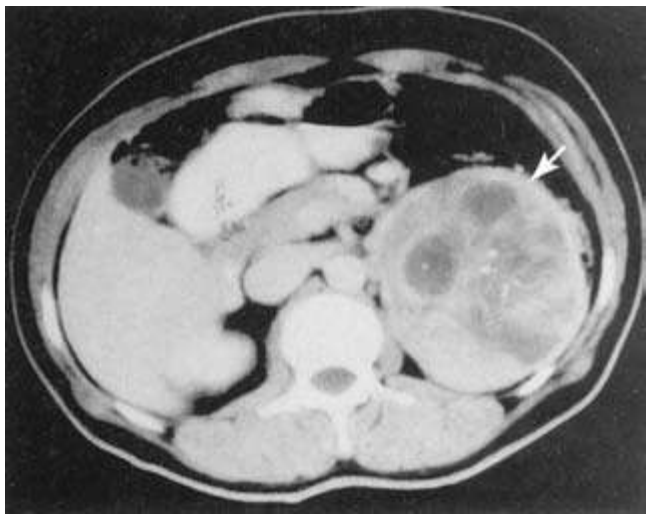

Figure 100.7 Computed tomography scan of a large retroperitoneal leiomyosarcoma (arrow). Courtesy of Melvyn H. Schreiber, MD, Galveston, TX.

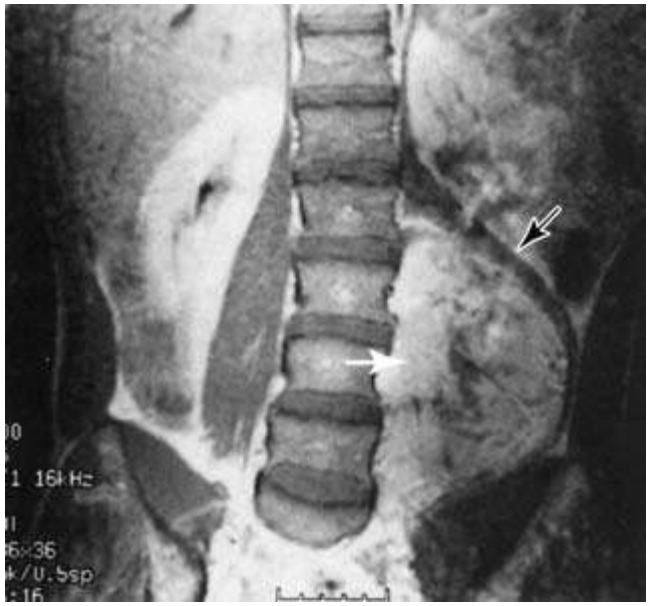

Figure 100.8 Coronal T1-weighted magnetic resonance scan after administration of the contrast medium gadolinium shows a large retroperitoneal sarcoma (black arrow) with displacement of the left psoas muscle laterally (white arrow). Courtesy of Greg Chalchub, MD, Galveston, TX.

value of barium studies is to delineate primary intestinal malignancies from retroperitoneal tumors.

Treatment

Surgical excision offers the greatest prospect for cure and remains the best option for treatment of these retroperitoneal malignancies [161,162]. Some retroperitoneal tumors are benign and are cured by complete excision. Treatment of malignant tumors involves surgical resection or radiation or a combination of the two modalities. With the exception of lymphomas, chemotherapy appears to offer little to the multimodal management of these tumors. It is important to remember that even though the overall prognosis for malignant retroperitoneal tumors is relatively low, en bloc resection of a primary malignancy can achieve 5-year survival rates of more than 60%. Furthermore, debulking procedures can provide symptomatic relief and may offer a better response rate to radiation therapy in the postoperative period in patients with tumors that cannot be excised because of size or invasion of surrounding structures. Malignant tumors have a recurrence rate in the range of 30%–50% over a 3-year period.

Mesentery and omentum

During fetal development, the splanchnic mesoderm forms the mesentery covering the developing gut [1]. The greater omentum, which extends from the greater curvature of the stomach caudally to fuse with the transverse colon and mesocolon, develops from the dorsal mesogastrium. The omentum and mesentery are rich in lymphatics and blood vessels and provide a major source of macrophages and lymphocytes to aid in the removal of foreign material or infection in the abdominal cavity. The small bowel mesentery contains the lymphatic and vascular network responsible for transporting all of the nutrients absorbed from the small bowel. Primary diseases of the mesentery and omentum are relatively uncommon. This section will describe some of the diseases that primarily or prominently involve the omentum, mesentery, or both mesenchymal structures (Table 100.4).

Mesenteric panniculitis and retractile mesenteritis

Incidence and etiology

Mesenteric panniculitis (also called primary liposclerosis, lipogranuloma, isolated lipodystrophy, and mesenteric Weber–Christian disease) is a nonspecific, rare inflammatory process involving the adipose tissue of the mesentery [163,164]. This process includes a spectrum of diseases, from inflammatory to fibrotic lesions, which have been called mesenteric panniculitis and retractile mesenteritis, respectively. Possible etiologies include trauma, autoimmunity, infection, ischemia, previous abdominal surgery, or abdominal malignancy, but in the majority of cases, the precise etiology is not known.

Pathology

Gross pathological findings include a diffusely thickened and rubbery mesentery, usually as a solid mass in the root of the mesentery or as several adherent masses. Microscopic findings include excessive growth of normal fat, with subsequent degeneration, fat necrosis, and xanthogranulomatous inflammation with lipid-laden macrophages, infiltration with

Table 100.4 Classification of mesenteric and omental diseases

Mesenteric diseases	Omental diseases
Primary mesenteric inflammatory diseases	**Mass lesions**
Mesenteric panniculitis	Primary tumors and cysts
Retractile mesenteritis	Metastatic disease
	Vascular lesions damaging blood supply
Mesenteric cysts	Torsion
Embryonic and developmental cysts	Primary
Traumatic or acquired cysts	Secondary: hernia, adhesion, tumor
Neoplastic cysts	Infarction
Infective and degenerative cysts	Primary
	Secondary: torsion, incarceration in hernia
Mesenteric tumors	
Benign tumors	
Lipoma	
Hemangioma	
Leiomyoma	
Ganglioneuroma	
Malignant tumors	
Leiomyosarcoma	
Liposarcoma	
Rhabdomyosarcoma	
Metastatic disease	
Mesenteric fibromatosis	

histiocytes, lymphocytes, and occasional foreign body giant cells, leading to fibrotic scarring and calcification [163].

Clinical manifestations

Patients with mesenteric panniculitis generally present in middle or late adulthood (average age 53 years). There is a slight male predominance. When symptomatic, patients may present with cramping abdominal pain, which may be local or generalized; weight loss; nausea and vomiting; and low-grade fever. Patients may occasionally present with findings consistent with an acute abdomen. Approximately 60% of patients present with a palpable abdominal mass noted by physical examination; the remainder of patients present with an incidental mass noted at examination or laparotomy [163,164]. The differential diagnosis includes a wide range of mesenteric pathologies such as mesenteric lymphoma, fat necrosis, primary or secondary mesenteric tumors, and mesenteric fibromatosis. In certain instances, an abdominal aortic aneurysm may be considered because of the transmission of aortic pulsations to the anterior abdominal wall [165].

This diagnosis can be suspected by various radiographic studies. Plain radiographic findings include displacement of intestinal segments and extrinsic compression of the bowel. Findings by CT scan include fat density masses arising from the mesentery, surrounding mesenteric vessels, displacing bowel loops without evidence of invasion, a left-sided orientation, a hypodense fatty halo surrounding nodules and vessels, and a hyperattenuated stripe [166] (Fig. 100.9). Ultrasonography has also been successfully used as a diagnostic tool in these patients [167].

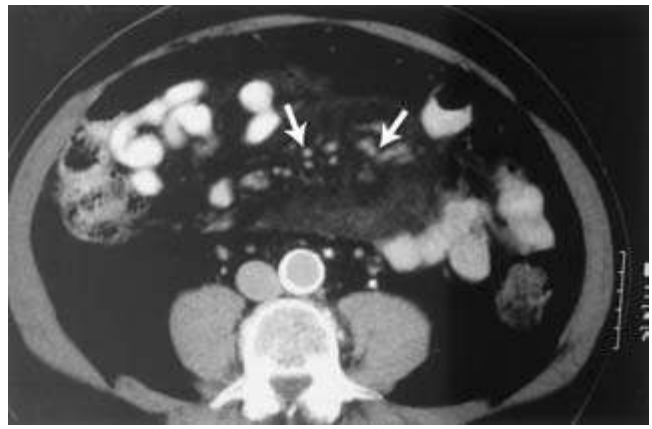

Figure 100.9 Computed tomography scan of mesenteric panniculitis demonstrating a fat-density mass surrounding mesenteric vessels with a characteristic "fatty halo" surrounding the vessels (arrows). Courtesy of Luis B. Morettin, MD, Galveston, TX.

Over time, some cases of mesenteric panniculitis progress to retractile mesenteritis characterized by a thickened mesentery that is fibrotic and retracted. There may also be mesenteric pseudocysts from fat necrosis. Rarely, there is involvement of the colonic mesentery, and fewer than 5% of patients have retroperitoneal involvement. Symptoms include continuous abdominal pain, fever, and weight loss. Some patients develop small bowel obstruction, mesenteric thrombosis, and lymphatic obstruction with ascites, steatorrhea, or protein-losing enteropathy.

Treatment and prognosis

Patients with mesenteric panniculitis should undergo biopsy of the lesion to rule out malignancy. This may be accomplished laparoscopically or by an open procedure. Resection of the entire mass is usually not possible and should not be attempted. In patients with symptomatic progressive sclerosing or retractile mesenteritis, dramatic responses have been reported after treatment with progesterone, prednisone and azathioprine or with cyclophosphamide [168–170]. Experience with these immunosuppressive agents is too small to determine their efficacy in this usually self-limiting disease. Surgical diversion or bypass is indicated for symptomatic relief in cases where extrinsic bowel obstruction because of mesenteric inflammation and scarring has occurred. The prognosis for mesenteric panniculitis is usually good but depends on the underlying disease processes.

Mesenteric fibromatosis

Incidence and etiology

Fibromatous proliferation of the mesentery, called mesenteric fibromatosis or mesenteric desmoid, is a rare and noninflammatory condition [171]. These mesenteric lesions can infiltrate the surrounding structures and, similar to desmoid tumors in other locations, tend to recur locally, although they have never been reported to metastasize. The majority of the reported cases have been associated with familial adenomatous polyposis syndromes (in particular, Gardner syndrome). Mesenteric fibromatosis affects about 3% of patients with familial adenomatous polyposis and 14%–29% of patients with Gardner syndrome [172,173]. This process has also been reported after abdominal surgery, trauma, prolonged estrogen intake, and pregnancy [171]. In addition, it may occur as a primary condition (primary mesenteric fibromatosis) in the absence of predisposing factors.

Clinical manifestations

Patients with mesenteric fibromatosis may present with an asymptomatic abdominal mass on physical examination or with a mesenteric mass at laparotomy. Symptoms of intestinal obstruction are the most common complaint. Aggressive mesenteric desmoids can become infiltrative and can involve mesenteric vessels, leading to intestinal infarction and perforation. The radiographic features of mesenteric fibrosis are generally nonspecific. The desmoid tumor appears as a solid mass by ultrasound. CT scan shows a nonenhancing mass with soft tissue density. At laparotomy, desmoids are found to involve the mesentery of the small bowel or transverse mesocolon, and the most common site is the base of the small bowel mesentery [174,175]. These lesions can be difficult to differentiate from low-grade sarcoma and can lead to misdiagnosis at operation and extensive small bowel resection.

Treatment and prognosis

In the past, the treatment of choice for patients with mesenteric desmoids has been wide local excision, but, unlike extraabdominal desmoids, no clear evidence exists showing a benefit from negative margins on the rate of recurrence of mesenteric fibromatosis. Furthermore, the severity and frequency of complications, recurrence rates as high as 85%, and relatively good long-term survival in unresectable patients lead some to reserve resection for patients suffering from complications secondary to desmoid tumors (e.g., obstruction, perforation, or fistula formation) [176,177].

There are anecdotal reports of successful treatment with prostaglandin inhibitors (i.e., sulindac or indomethacin) and antiestrogens (e.g., tamoxifen) alone and in combination [178,179]. Cytotoxic chemotherapy with dactinomycin, vincristine, and cyclophosphamide, and radiotherapy have been utilized, individually or in combination, with variable success [180–182].

Cysts and tumors of the mesentery and omentum

Mesenteric and omental cysts

Incidence and etiology

Cysts of the mesentery and omentum are uncommon lesions, with an incidence of about 1 case for every 140 000 general hospital admissions and about 1 per 20 000 pediatric hospital admissions [183,184]. These cysts represent benign proliferation of ectopic lymphatics lacking communication with the normal lymphatic system [185]. Although they are most commonly found in the small bowel mesentery, they can occur anywhere along the gastrointestinal tract. Some authors differentiate between mesenteric/omental cysts and cystic lymphangiomas.

Clinical manifestations

Cystic lymphangiomas characteristically are found in children (mean age, 6–10 years) and are frequently large and almost always symptomatic, usually presenting with abdominal distention, pain, or vomiting, and occasionally with an acute abdomen mimicking appendicitis [184]. Lymphangiomas are most often located in the small bowel mesentery but occasionally may be found in the mesocolon, the omentum, or, more rarely, the retroperitoneum. They may be single or multiple.

In contrast to cystic lymphangiomas, the mean age of presentation of patients with nonlymphangiomatous mesenteric

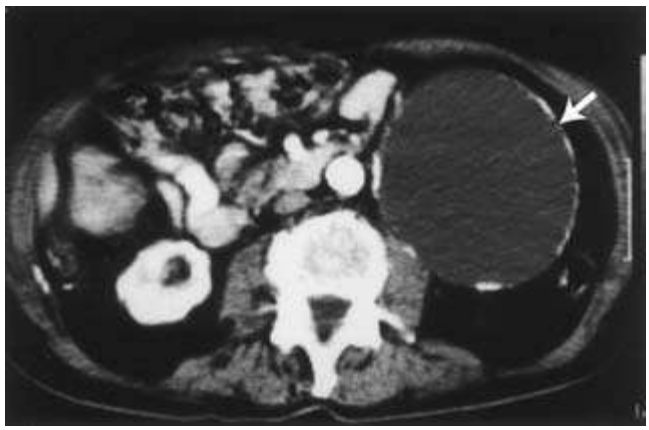

Figure 100.10 Computed tomography scan of a large lymphatic cyst arising from the mesentery (arrow). Courtesy of Luis B. Morettin, MD, Galveston, TX.

cysts is 44 years [186]. These cysts include nonpancreatic pseudocysts, enteric cysts, and mesothelial cysts. As many as one-third of the nonlymphangiomatous cysts may be located in the omentum, and only 25% are symptomatic.

Diagnosis and treatment

The identification of an intraabdominal cystic mass can be obtained by radiographic studies. Plain radiographs often demonstrate a mass displacing bowel gas and may demonstrate proximal dilation of the bowel. Ultrasonography, CT, and MRI may demonstrate the multilocular or unilocular nature of the cysts, which may have homogeneous or nonhomogeneous contents (Fig. 100.10). Of the three imaging modalities, ultrasonography probably yields the most information for the least expense.

Surgical resection represents the definitive diagnosis and treatment for these lesions. Percutaneous aspiration and biopsy are not warranted because they are not likely to provide a definitive diagnosis and may lead to infection or rupture of the cyst. Total excision of the cystic lymphangioma requires resection of adjacent attached abdominal organs (usually segments of small bowel) in a majority of cases, but most other types of mesenteric cysts can simply be excised [185,186]. Rarely, the location of the cyst precludes complete resection and requires drainage or marsupialization. However, this is usually associated with a high recurrence rate [187,188]. Recent reports have begun to emerge demonstrating that mesenteric and omental cysts can be effectively treated using a laparoscopic approach [189–191].

Solid tumors of the omentum and mesentery

Incidence and etiology

Primary tumors of the omentum and mesentery are exceedingly rare and may arise from any of the cellular elements that constitute these tissues [192]. By far the most common types of tumors that involve the omentum and mesentery are malignant tumors that arise from intraabdominal viscera, such as the ovaries, stomach, colon, or pancreas, and secondarily involve the omentum or mesentery through metastasis or direct invasive extension.

Clinical manifestations

Patients may present with a constellation of nonspecific or vague complaints; however, the most consistent presenting feature of omental and mesenteric tumors is a palpable abdominal mass that is often movable, often associated with pain, and sometimes associated with ascites or vomiting [192,193]. Plain abdominal radiographs and gastrointestinal contrast studies can demonstrate intestinal displacement and are useful in excluding the intestine as the origin of the tumor. A CT scan usually identifies the location of the mass within the mesentery or omentum and can exclude other organs as the origin of the mass; However, CT is nonspecific with regard to the diagnosis [158].

Treatment

The treatment of primary omental and mesenteric tumors is surgical excision, if possible. The malignant tumors of the mesentery and omentum spread by local invasion and by peritoneal implantation and kill by involvement of vital abdominal organs. Surgery always should be considered in patients with symptoms of bowel obstruction or ischemia.

Metastatic tumors of the mesentery are more common than primary mesenteric tumors and are usually the result of enlarged lymphomatous or carcinomatous lymph nodes. In addition, malignant midgut carcinoid tumors often metastasize or spread directly to the mesentery and exhibit pronounced fibrosis causing mesenteric shortening, angulation, and fixation of the bowel, which can result in bowel obstruction and intestinal infarction [34,194,195]. Surgical palliation of patients with metastatic carcinoid tumors is warranted because of the indolent behavior of these tumors.

Mesenchymal tumors (e.g., leiomyomas or gastrointestinal stromal tumors) of the gut wall are also treated by surgical resection. The gastrointestinal stromal tumors may express gain-of-function mutations of the *c-kit* protooncogene, which encodes a tyrosine kinase receptor. If the stromal tumor stains positive for *c-kit*, the tumor appears to be responsive to the inhibitor imatinib (Gleevec). Recently, a large Phase III trial was completed which demonstrated significant overall survival when compared to conventional chemotherapy [196].

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I MISCELLANEOUS

101 Nutritional supplementation

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Nutritional assessment

Most methods used to evaluate nutritional status are aimed at identifying specific nutrient deficiencies or protein–energy malnutrition. The diagnoses and treatments of specific nutrient deficiencies are usually straightforward. For example, potassium deficiency can be identified by measuring the serum potassium concentration and can be corrected by oral or intravenous potassium supplementation. In contrast, the diagnosis of protein–energy malnutrition is more complicated. Commonly used indicators of nutritional status have been validated by linking nutritional indicators with clinical outcome; patients with abnormal indicators have a worse outcome than patients with normal indicators. However, all indicators are influenced by illness or injury, so it is difficult to separate the contribution of malnutrition from that of the illness itself. Therefore, if nutritional status is linked to the severity of disease, patients who are "malnourished" may have a poor outcome simply because they are sicker than patients who are "well nourished." Moreover, nutritional therapy may not improve outcome unless malnutrition is an independent contributor to adverse medical events.

Nutritional assessment techniques

Specific nutrient deficiency

A careful history and physical examination, routine blood tests, and selected laboratory tests based on the history and physical examination findings can be used to diagnose specific macronutrient, mineral, vitamin, and trace mineral deficiencies (Tables 101.1–101.3) [1].

Protein–energy malnutrition

Ideally, nutritional assessment is used to identify those patients who will benefit from nutritional support. However, the indicators used to assess protein–energy malnutrition have been validated by determining their relationship to clinical outcome rather than to the response of patients to nutritional therapy.

Body mass and composition

In general, unintentional weight loss is associated with an adverse clinical outcome [2]. However, the assessment of changes in body weight can be confounded by errors in recall and changes in body water. Anthropometry, the measurement of triceps and subscapular skinfold thickness, provides an index of body fat and muscle mass, which is compared with the values in standard tables. Although the interpretation of these data may be limited by differences in age and ethnicity between patients and the standard population, interrater variability, and hydration, markedly abnormal values (below the 5th percentile) usually predict a poor clinical outcome. Body muscle mass can also be assessed in the clinical setting by measuring the urinary creatinine excretion [3]. Patient values can be compared with those in tables indicating an expected amount of creatinine excretion in relationship to height in subjects consuming a meat-free diet [1]. The body mass index (BMI), which is obtained by dividing weight (kilograms) by the square of the height (meters squared), can help identify patients at increased risk for medical complications (Table 101.4).

Serum protein concentrations

The concentrations of several plasma proteins, particularly albumin, correlate with clinical outcome: a low plasma albumin concentration is associated with an increased incidence of

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Table 101.1 Some physical signs of nutritional deficiency

	Finding	Deficiency
Hair	Thin, sparse	Protein, zinc, biotin
	Flag sign (transverse depigmentation)	Protein, copper
	Easy pluckability	Protein
Nails	Spoon-shaped (koilonychia)	Iron
	No luster, transverse ridging	Protein–energy
Skin	Dry, scaling (xerosis)	Vitamin A, zinc
	Seborrheic dermatitis	Essential fatty acids (EFAs), zinc, pyridoxine, biotin
	Flaky paint dermatosis	Protein
	Follicular hyperkeratosis	Vitamin A, vitamin C, EFAs
	Nasolabial seborrhea	Niacin (B-1), pyridoxine (B-6), riboflavin (B-2)
	Petechiae, purpura	Vitamin C, vitamin K, vitamin A
	Pigmentation, desquamation	Niacin (pellagra)
	Pallor	Folate, iron, cobalamin, copper, biotin
Eyes	Angular palpebritis	Riboflavin
	Blepharitis	B vitamins
	Corneal vascularization	Riboflavin
	Dull, dry conjunctivae	Vitamin A
	Bitot spots	Vitamin A
	Keratomalacia	Vitamin A
	Fundal capillary microaneurysms	Vitamin C
	Ophthalmoplegia	Thiamin (Wernicke encephalopathy)
Mouth	Angular stomatitis	B vitamins, iron, protein
	Cheilosis	Riboflavin, niacin, pyridoxine, protein
	Atrophic lingual papillae	Niacin, iron, riboflavin, folate, cobalamin
	Glossitis (scarlet, raw)	Niacin, pyridoxine, riboflavin, folate, cobalamin
	Decreased sense of taste and smell	Vitamin A, ?zinc
	Swollen, bleeding gums	Vitamin C
Glands	Parotid enlargement	Protein
	"Sicca" syndrome	Ascorbic acid
	Thyroid enlargement	Iodine
Heart	Enlargement, tachycardia, high-output failure	Thiamin (wet beriberi)
	Small heart, decreased output	Protein–energy
	Cardiomyopathy	Selenium
	Cardiac arrhythmias	Magnesium, potassium
Extremities	Edema	Protein, thiamin
	Muscle weakness	Protein–energy, selenium
	Bone and joint tenderness (child)	Vitamin C, vitamin A
	Osteopenia, bone pain	Vitamin D, calcium, phosphorus, vitamin C
Neurological	Confabulation, disorientation	Thiamin (Korsakoff psychosis)
	Decreased positional and vibratory senses, ataxia	Cobalamin, thiamin
	Decreased tendon reflexes	Thiamin
	Weakness, paresthesia	Cobalamin, pyridoxine, thiamin
	Mental disorders	Cobalamin, niacin, thiamin, magnesium
Other	Delayed wound healing	Vitamin C, protein, ?zinc, ?EFAs
	Hypogonadism, delayed puberty	Zinc
	Glucose intolerance	Chromium

Table 101.2 Clinical laboratory tests for detection of vitamin deficiency

Vitamin	Test	Source	Reference range (units)[a] Marginal	Deficient	Usefulness
B-1	Transketolase ratio	RBC	1.16–1.24	> 1.25	+ When severe
	Thiamin	Serum		< 12.7 (nmol/L)	Direct measure
	Thiamin	Urine		< 27 (μg/g creatinine)	
B-2	GSH-reductase ratio	Serum	1.20–1.40	> 1.40	Body stores
	Riboflavin	Urine	27–79	< 27 (μg/g creatinine)	Recent intake
B-6	AST activity ratio	RBC	1.70–1.85	> 1.85	Body stores
	Pyridoxal 5-PO_4	Plasma	20–30	< 20 (nmol/L)	Stores, sensitive
	4-Pyridoxic acid	Urine		< 3.0 (μmol/day)	Recent intake
	Total vitamin B-6	Urine		< 0.5 (μmol/day)	Recent intake
Niacin	*N*-methylnicotinamide	Urine	0.5–2.5	< 0.5 (mg/g creatinine)	Recent intake
	2-Pyridone	Urine	2.0–3.9	< 2.0 (mg/g creatinine)	Recent intake
Folate	Folic acid	Plasma	3.0–5.9	< 3.0 (ng/mL)	Stores + intake
	Folic acid	RBC	140–159	< 140 (ng/mL)	Body stores
Folate or B-12	Homocysteine	Plasma	12–15	> 15 (μmol/L)	Function
B-12	Cobalamin	Serum	150–200	< 150 (pg/mL)	Body stores
	Methylmalonic acid	Serum		> 376 (nmol/L)	Function
	Holotranscobalamin II	Serum	40–60	> 60 (pg/mL)	Function
C	Ascorbic acid	Serum	11–23	< 11 (μmol/L)	Recent intake
	Ascorbic acid	WBC	10–20	< 10 (μg/10^8 cells)	Stores
A	Retinol	Plasma	10–19	< 10 (μg/dL)	Stores + intake
	Retinol-binding protein	Plasma		< 50 (mg/L)	Function
D	25-OH vitamin D	Serum	12–25	< 12 (nmol/L)	Body stores
	1,25-dihydroxyvitamin D	Serum	48–65	< 48 (pmol/L)	Function
E	α-Tocopherol	Serum	5.0–7.0	< 5 (μg/mL)	Body stores
	α-Tocopherol/total lipid	Serum	0.8–1.0	< 0.8	Preferred
	H_2O_2 hemolysis	RBC	10–20	> 20 (%)	Function
K	Prothrombin time	Plasma	1.5–2.0	2.0 (s over control)	Function
	Phylloquinone			< 0.35 (nmol/L)	Recent intake

a Precise values may vary between laboratories.
AST, aspartate aminotransferase; GSH, reduced glutathione; RBC, red blood cells; WBC, white blood cells.
Adapted from Alpers et al. [1].

medical complications [4,5]. Illness or injury, however, rather than malnutrition per se, are responsible for hypoalbuminemia in sick patients [6]. Inflammation and injury decrease albumin synthesis, increase albumin degradation, and increase albumin transcapillary losses. Specific gastrointestinal, renal, and cardiac diseases can increase albumin losses through the gut or kidney. Wounds, burns, and peritonitis cause losses from surface tissues. Nutritional support itself does not usually alter serum albumin levels [7]. Serum concentrations of transferrin, retinol-binding protein, and thyroxine-binding prealbumin are also affected by inflammation, so that they are unreliable as a measure of protein–energy malnutrition.

Immune system function

The total circulating lymphocyte count and delayed cutaneous hypersensitivity to skin test antigens have been used as clinical indicators of malnutrition. Although depression of circulating lymphocytes (< 200/mm^3) and anergy assessed by delayed cutaneous hypersensitivity are associated with a poor clinical outcome, they are not specific for nutritional deficiency and are affected by specific diseases, injury, and certain drugs.

Subjective global assessment

The subjective global assessment involves a focused history and physical examination to determine the adequacy of

Table 101.3 Laboratory detection of micronutrient mineral deficiency

Nutrient	Test	Source	Reference range (units)[a]	Usefulness
Iron	Iron	Serum	50–200 (μg/dL)	Poor measure of body stores
	Total iron-binding capacity	Serum	245–400 (μg/dL)	
	Iron-binding saturation		15–50 (%)	Insensitive for iron status
	Transferrin	Serum	200–400 (μg/dL)	Preferred over TIBC if available
	Ferritin	Serum	12–300 (ng/mL)	Measures body stores: high specificity when stores low, poor sensitivity
Zinc	Zinc	Plasma	20–130 (μg/dL)	Poor specificity for body stores
	Zinc tolerance test	Plasma	> Twofold increase over baseline at 2 h	For malabsorption
Copper	Copper	Serum	55–175 (μg/dL)	Insensitive for body stores
	Ceruloplasmin	Plasma	10–60 (mg/dL)	Independent of body stores
Selenium	Selenium	Serum	100–340 (ng/mL)	Insensitive for body stores
	Glutathione peroxidase	Plasma	455–800 (U/L)	More sensitive for body stores

a May vary in different laboratories.
TIBC, total iron-binding capacity.
Adapted from Alpers et al. [1].

Table 101.4 Body mass index (BMI) as a measure of associated disease risk

Weight category	BMI (kg/m^2)	Risk
Extremely underweight	< 14.0	Extremely high
Underweight	14.1–18.4	Increased in smokers, chronic illness
Normal	18.5–24.9	Normal
Overweight	25–29.9	Increased
Obesity		
Class I	30.0–34.9	High
Class II	35.0–39.9	Very high
Class III	≥ 40.0	Extremely high

Adapted from National Institute of Diabetes and Digestive and Kidney Diseases. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults – the evidence report. Obes Res 1998;6:S53.

nutrient intake and the possible effect of malnutrition on body composition and organ function (Fig. 101.1) [8,9]. In addition, it includes an analysis of the patient's future ability to ingest adequate nutrients. The findings of the history and physical examination are subjectively weighted to rank patients as well nourished, moderately malnourished, or severely malnourished. Patients who are judged to be severely malnourished are at high risk for medical complications and adverse outcome [8,10].

There is no "gold standard" for determining nutritional status. All current approaches are influenced by the severity of illness and have been validated by assessing clinical outcome rather than by nutrition-specific parameters. Therefore, current nutritional assessment techniques identify sick patients. Although it is often assumed that "malnourished" patients will benefit from nutritional support, this assumption has not been documented in prospective randomized clinical trials [11].

Choosing the route for nutritional support

The decision to initiate nonvolitional nutritional support is complex and involves a clinical assessment of the patient's nutrient intake, body fat and protein mass, protein and energy requirements, severity of illness, and anticipated duration of inadequate volitional intake. Once it has been decided that nonvolitional nutritional support is indicated, the route of nutritional support must be considered. The usual course is to choose the oral or enteral route when this approach can be used effectively and safely. There are several reasons for this choice. First, enteral nutrition may provide nutrients that are not available from parenteral nutrition. The best example of this principle is the provision of short-chain fatty acids to the colonic mucosa through the bacterial degradation of fiber or unabsorbed carbohydrate. The provision of this important colonic energy source may maintain colonic function [12]. Second, it is felt that lumenal nutrients may prevent atrophy in the small intestinal mucosa. Atrophy occurs in rats fed by

A History

1 Weight change

Overall loss in past 6 months: amount = _____ kg

Change in past week: _____ increase

_____ no change

_____ decrease

2 Dietary intake change (relative to normal)

_____ No change

_____ Change: duration = _____ weeks

type: _____ suboptimal solid diet _____ full liquid diet

_____ hypocaloric liquids _____ starvation

3 Gastrointestinal symptoms (that persisted > 2 weeks)

_____ none _____ anorexia _____ nausea _____ vomiting _____diarrhea

4 Functional capacity

_____ No dysfunction (e.g., full capacity)

_____ Dysfunction: duration = _____ weeks

_____ working suboptimally

_____ ambulatory

_____ bedridden

5 Disease and its relation to nutritional requirements

Primary diagnosis (specify) _____

Metabolic demand (stress) _____ none _____ low _____ moderate _____ high

B Physical (for each trait specify: 0 = normal, 1+ = mild, 2+ = moderate, 3+ = severe)

_____ loss of subcutaneous fat (triceps, chest)

_____ muscle wasting (quadriceps, deltoids, temporals)

_____ ankle edema, sacral edema

_____ ascites

_____ tongue or skin lesions suggesting nutrient deficiency

C SGA rating (select one)

_____ A = Well nourished (minimal or no restriction of food intake or absorption, minimal change in function, weight stable or increasing)

_____ B = Moderately malnourished (food restriction, some function changes, little or no change in body mass)

_____ C = Severely malnourished (definitely decreased intake, function, and body mass)

Figure 101.1 Subjective global assessment (SGA) of nutritional status. Adapted from Klein et al. [11].

parenteral nutrition, but the importance of this phenomenon in humans is still uncertain [13]. Finally, enteral feeding may be safer than parenteral nutrition [14–16].

If oral or enteral feeding is chosen, intestinal motility and the absorptive surface must be adequate. Other disorders that interfere with enteral feeding include severe diarrheal illnesses, extensive inflammatory bowel disease, acute gastrointestinal bleeding, conditions that will require abdominal surgery in the near future, obstructing small bowel or colonic lesions, and acute inflammation of the pancreas or biliary tract. Guidelines have been developed that provide useful and practical information for the clinician [17,18]. The guidelines for use in specific disorders, such as AIDS, are practical modifications of the generalized guidelines, with special emphasis on the problems of feeding patients with that disorder [19]. However, the clinician should not rely on a set pattern of guidelines for decisions regarding nutritional support. The published guidelines should be used as resource material to aid in the clinical judgment required for each patient.

Enteral nutrition

The availability of so many different types of nutritionally complete diets and defined formulas makes it possible for many hospitalized patients to receive adequate nutrient intake by oral or enteral tube feedings.

Whole-food diets include a regular hospital diet and diets modified in either consistency (e.g., clear-liquid, full-liquid, pureed, and soft diets) or nutrient content (e.g., low-residue, low-fat, low-sodium, low-protein, and high-fiber diets). In many cases, nutrient intake can be increased by encouraging the ingestion of ordinary food and by avoiding missed meals and unpalatable diets. Many patients eat more if they are given assistance at mealtimes and provided with desired foods, and if some food is supplied by relatives and friends is allowed.

Defined formulas are commercially made products with a known "defined" nutrient composition (Table 101.5). These formulas can be divided into five categories: feeding modules,

Table 101.5 Classification of commercially available enteral nutrition products

Modular type	Indication	Examples
Intact protein	Standard	Boost, Ensure, Isosource, Nutren 1.0, Osmolite, Peptinex 1.0, Peptinex 1.5, Peptinex DT, Resource
	Volume-restricted	Boost Plus, Ensure Plus, Isosource 1.5, Jevity 1.5, Nutren 1.5, Nutren 2.0, Resource 2.0
	High-protein	Boost High Protein, Isosource HN, Nutren Replete, Osmolite 1, Osmolite 1.5, Promote
	Disease-specific	Boost Diabetic, Diabetisource AC, Ensure Plus, Glucerna, Juven, Nepro, Novasource Pulmonary, Novasource Renal, Nutren Glytrol, Nutren Pulmonary, NutriHep, Oxepa, Pulmocare, Renalcal, Suplena
	Immunonutrition	Crucial, Impact, Impact Advanced Recovery, Impact Glutamine, Impact with Fiber, Perative, Pivot 1.5, TraumaCal
	Fiber supplement	Ensure Fiber, Fibersource, Jevity 1, Nutren 1.0 Fiber, Nutren ProBalance, Nutren Replete Fiber, Promote With Fiber, Resource Benefiber
	Milk-based	Carnation Instant, Carnation Instant No Sugar Added, Resource Instant Breakfast, Resource Milkshake
Elemental	Digestion-impaired	Crucial, Peptamen, Vivonex TEN, Vital HN, Optimental, Peptinex, FAA free amino acid diet
Protein-limited	Protein supplement	Additions (526 kcal/100 g), Resource Beneprotein (357 kcal/100 g)
CHO-limited	CHO supplement	Resource Benecalorie (800 kcal/100 g), Polycose liquid (10 kcal/5 mL), Polycose powder (380 kcal/100 g)
Fat-limited	Fat supplement	MCT oil (830 kcal/100 g)

Note: caloric density ranges from 1.0 to 1.5 kcal/mL for the intact protein and elemental products, except for the volume-restricted products, in which the caloric density is 1.5–2.0 kcal/mL and is often identified in the product name by number.
CHO, carbohydrate.

monomeric formulas, oligomeric formulas, polymeric formulas, and disease-specific formulas. Feeding modules consist of single nutrients, such as protein (intact protein, hydrolyzed protein, or crystalline amino acids), carbohydrate (glucose polymers), or fat (long-chain or medium-chain triglycerides), that can be used to supplement a specific diet or be part of a modular enteral system composed of several nutrient modules.

Monomeric (elemental) formulas contain nitrogen in the form of free amino acids and usually only small amounts of fat (< 5% of total calories). Therefore, these formulas can be useful in patients who require an extremely low-fat diet. However, monomeric formulas are hyperosmolar, unpalatable, and more expensive than polymeric formulas, and in the absence of pancreatic insufficiency, they are absorbed no better than oligomeric or polymeric formulas [20].

Oligomeric (semielemental) formulas contain hydrolyzed protein in the form of small peptides and sometimes free amino acids. Fat represents 5%–40% of energy content as either long-chain triglycerides or a combination of long-chain and medium-chain triglycerides. Data from several studies suggest that oligomeric diets can reduce diarrhea and other gastrointestinal side effects in critically ill patients [21] and in patients receiving abdominal irradiation and chemotherapy [22]. However, the purported clinical benefits of oligomeric formulas require confirmation in large prospective clinical trials before precise recommendations regarding their use can be made.

Polymeric formulas contain nitrogen in the form of whole proteins. These formulas can be categorized into three groups: blenderized food formulas, milk-based formulas, and lactose-free formulas. Milk-based formulas usually contain milk as a source of protein and fat and tend to be more palatable than other defined diets. Although milk-based formulas can be problematic for lactose-intolerant patients, they are often tolerated when infused continuously to decrease the load of lactose delivered to the intestine at any one time. Lactose-free formulas are the most commonly used polymeric formulas in hospitalized patients, and many modifications in protein and fat content are available. Disease-specific formulas have been designed for patients who have specific illnesses, including hepatic insufficiency, renal insufficiency, pulmonary insufficiency, diabetes, and severe metabolic stress. In general, the clinical superiority of these formulas over less expensive, standard polymeric enteral formulas remains controversial. However, branched-chain amino acid-enriched formulas may permit greater nitrogen intake in selected patients with chronic protein-intolerant hepatic encephalopathy (see "Nutritional support in the hospitalized patient with gastrointestinal disease"), and renal failure formulas contain much smaller amounts of sodium, potassium, phosphorus, and magnesium than standard formulas.

Over the last 10 years more than 500 articles have been published addressing the benefits and risks of immunonutrition. Immunonutrition formulas are supplemented with one or a combination of immune stimulants, such as arginine, glutamine, omega-3 fatty acids, and nucleotides (Table 101.5). A metaanalysis of randomized clinical trials comparing immunonutrition to standard products concluded there were no significant effects on mortality, infectious complications,

length of stay, or time on the ventilator in critically ill patients [23]. A higher mortality rate has been observed in septic patients receiving immunonutrition when compared with similar patients receiving parenteral nutrition [24]. However, a significant reduction in infectious complications and length of stay was observed in elective surgery patients. Interpreting the data on immunonutrition is hampered by differing study populations, inconsistent use of blinding, the use of different control feeds, and small sample sizes.

A positive caloric balance is necessary to optimize and sometimes to achieve positive nitrogen balance, and to incorporate amino acids into new protein. To achieve this goal, the ingestion of about 25–35 kcal/g of protein (105–146 kJ/g) (~ 150–200 kcal/g [630–835 kJ/g] of nitrogen) is required. This ratio is based on the derivation of 10%–15% of caloric needs during catabolism from protein degradation.

Oral rehydration therapy

Patients with protracted vomiting or diarrhea can lose excessive amounts of fluid and electrolytes. Oral rehydration therapy enhances sodium and water absorption by stimulating sodium/glucose cotransport in the small intestine. Oral rehydration therapy has been demonstrated to have considerable clinical benefits in patients with severe gastrointestinal fluid and mineral losses, such as those with cholera-induced diarrhea, high-output ostomies, and short bowel syndrome [25–31].

A variety of oral rehydration solutions are available (Table 101.6). The World Health Organization developed a solution based on the results of studies in patients with cholera [30]. The glucose component of an oral rehydration solution should be provided at concentrations between 70 and 150 mmol/L. Higher concentrations of glucose can be deleterious because the increase in osmolality may reduce water absorption. Making the solution hypotonic by replacing glucose with rice solids or other polymeric forms can decrease stool output compared with isotonic solutions [26–29]. In patients with short bowel syndrome ending in a jejunostomy, net sodium and fluid balance is related to jejunal length. Patients with less than 100 cm of jejunum are usually net secretors, whereas those with more than 100 cm of jejunum are usually net absorbers [31]. The use of oral rehydration therapy can markedly decrease ostomy output and convert some patients from net secretors to net absorbers. In these patients, the consumption of electrolyte replacement drinks containing 60 mmol or less of sodium per liter causes a negative sodium and fluid balance, whereas the balance becomes positive after the ingestion of solutions containing 90 mEq/L and is maximal at concentrations of 120 to 160 mEq/L [32,33]. Sport drinks and commercially made defined liquid formula diets do not contain an adequate amount of sodium for maximal fluid absorption in patients with severe short bowel syndrome and a jejunostomy.

Tube feeding

General principles

Enteral tube feeding is useful in patients who have a functional gastrointestinal tract but who cannot or will not ingest adequate nutrients. Many approaches to tube feeding are possible (e.g., nasogastric, nasoduodenal, nasojejunal, gastrostomy, jejunostomy, pharyngostomy, esophagostomy tubes) and depend on physician experience, clinical prognosis, anticipated duration of feeding, patency and motility of the gut, risk for aspiration of gastric contents, and patient preference.

Short-term feeding (< 6 weeks) can be achieved by the placement of a soft, small-bore nasogastric or nasoenteric feeding tube. These tubes are made of silicone or polyurethane and can be left in place without the tissue irritation or pressure

Table 101.6 Characteristics of selected oral rehydration solutions

Product	Na (mEq/L)	K (mEq/L)	Cl (mEq/L)	Citrate (mEq/L)	kcal/L	CHO (g/L)	mOsmol/kg
Equalyte	78	22	68	30	100	30	305
CeraLyte 70	70	20	98	30	165	40	235
CeraLyte 50	50	20	40	30	240	60	240
Pedialyte	45	20	35	30	100	20	300
Gatorade	20	3	N/A	N/A	210	45	330
WHO[a]	90	20	80	30	80	20	200
Washington University[b]	105	0	100	10	85	20	250

a WHO (World Health Organization) formula: mix 3/4 tsp (teaspoon) sodium chloride, 1/2 tsp sodium citrate, 1/4 tsp potassium chloride, and 4 tsp glucose (dextrose) in 1 L (4 1/4 cups) of distilled water.

b Washington University formula: mix 3/4 tsp sodium chloride, 1/2 tsp sodium citrate, and 3 tbsp (tablespoons) + 1 tsp Polycose powder in 1 L (4 1/4 cups) of distilled water.

CHO, carbohydrate.

Adapted from Klein S. Nutritional therapy. In: Ahya S, Flood K, Paranjothi S (eds). The Washington Manual of Medical Therapeutics, 30th edn. Philadelphia: Lippincott Williams & Wilkins, 2000:27.

necrosis caused by larger polyvinyl chloride tubes. Many patients are able to eat with the tube in place, which permits the use of tube feeding to supplement oral intake. Nasogastric feeding is usually the most appropriate route, but orogastric feeding can be used in cases of nasal injury or gross nasal deformity. Nasoduodenal or nasojejunal feeding is useful in patients with gastroparesis. In addition, by taking advantage of the barrier function of the pyloric and gastroesophageal sphincters, nasoenteric feeding may decrease the incidence of aspiration in high-risk patients. However, the prevention of aspiration by feeding beyond the pylorus has not been proved in clinical trials.

Most patients who require long-term tube feeding (> 6 weeks) should have a gastrostomy or jejunostomy tube placed. These tubes can be placed endoscopically, radiologically, or surgically, depending on the clinical situation and local expertise. Percutaneous endoscopic gastrostomy can be performed within 30 min and is successfully completed in more than 90% of attempts [34]. Gastrostomy tubes can be placed percutaneously without endoscopy by inserting the catheter directly into the stomach by a peel-away sheath introduced over a previously placed J-wire guide [35]. This approach is particularly useful in patients with an obstructing lesion of the esophagus or hypopharynx that prevents passage of an endoscope or a gastrostomy tube bumper. A summation of data from several large series suggests that percutaneous endoscopic gastrostomy tube placement is associated with mortality in 0.5%, major complications (peristomal leakage with peritonitis, necrotizing fasciitis of the anterior abdominal wall, gastric hemorrhage) in 1%, and minor complications (minor wound infections, stomal leaks, tube extrusion or migration, aspiration, gastrocolic fistula, ileus, fever) in 8% of patients [36]. Jejunal tube placement can be achieved by threading a tube through an existing gastrostomy or by percutaneous endoscopic jejunostomy in patients with previous partial or total gastrectomy [37–41].

Surgical gastrostomy is more expensive than percutaneous endoscopic gastrostomy unless the tube is placed during an operation performed for another reason. A jejunostomy tube may also be placed at the time of abdominal surgery and consists of a subserosal tunnel or needle catheter jejunostomy. Gastrostomy and jejunostomy tubes can also be placed by using laparoscopic techniques.

Administration of tube feeding

Patients with feeding tubes in their stomach can often tolerate intermittent gravity feedings, in which the total amount of daily formula is divided into four to six equal portions that are infused by gravity over 30–60 min. The patient's upper body should be elevated by more than 30° during and for at least 1 h after feeding. Intermittent feedings are useful for patients who cannot be positioned with the head of the bed continuously elevated or who require greater freedom from feeding. However, patients who experience nausea and early satiety with bolus gravity feedings may require continuous infusion at a slower rate. Patients who have gastroparesis often tolerate gastric tube feedings when they are started at a slow rate (e.g., 10 mL/h) and advanced by small increments (e.g., 10 mL/h every 8–12 h). However, patients with severe gastroparesis require passage of the feeding tube tip past the ligament of Treitz. Continuous infusion should always be used when feeding directly into the duodenum or jejunum to avoid distention, abdominal pain, and dumping syndrome.

Complications

Mechanical, metabolic, and gastrointestinal complications can occur in patients receiving tube feedings. Although the placement of nasogastric tubes is usually safe, tubes can be misplaced, particularly in unconscious patients. Intubation of the tracheobronchial tree has been reported in up to 15% of patients, and intracranial placement can occur in patients with skull fractures. Feeding tubes can cause nasopharyngeal erosions, pharyngitis, sinusitis, otitis media, pneumothorax, and gastrointestinal tract perforation.

Small-diameter nasogastric tubes (e.g., 8F or 9F) are usually used to lessen nasopharyngeal irritation. These small tubes often become occluded by inspissated feedings or pulverized medications given through the tube. Frequent flushing of the tube with 30–60 mL of water and avoiding the administration of pill fragments whenever possible help maintain patency. Many techniques for unclogging tubes have been published and include the gentle infusion of small quantities of carbonated beverage, pancreatic enzymes, and 95% ethyl alcohol into the tube. In addition, commercially made products can be obtained that either dissolve or mechanically remove the obstruction. Metabolic complications, such as hypokalemia, hyponatremia, hypophosphatemia, and hyperglycemia, can occur [40]. Patients should be monitored for metabolic abnormalities, particularly severely malnourished patients during the initial refeeding period.

Gastrointestinal side effects of tube feedings include nausea and vomiting, pulmonary aspiration, abdominal pain, and diarrhea. Diarrhea is the most common complication and occurs in 30%–50% of critically ill patients receiving tube feedings. Diarrhea is often caused by antibiotic use [41] and the use of liquid medications and elixirs that contain nonabsorbable carbohydrates, such as sorbitol [42]. Patients with normal gastrointestinal tract function can tolerate larger volumes of tube feedings. In one study, tube feeding did not cause diarrhea in healthy subjects until it was given at rates exceeding 200 mL/h [43]. Several case reports have also documented an association between small bowel necrosis and jejunal feedings in critically ill patients [44]. Most of these patients were receiving pressors to maintain an adequate blood pressure, suggesting that the increased oxygen requirements associated with feeding can cause ischemia in patients whose intestinal blood flow is unable to increase.

Parenteral nutrition

In some patients, enteral feedings are either contraindicated or cannot be provided in sufficient quantities to meet nutritional requirements. In these cases, parenteral nutritional support can be a valuable adjunctive and sometimes lifesaving therapy. Certainly, patients who are unable to ingest "adequate" nutrients for a "prolonged" period of time require nutritional therapy to prevent the adverse effects of malnutrition. However, the precise definitions of "adequate" and "prolonged" are not clear and are likely to differ from patient to patient depending on the amount of body energy stores and lean body mass, the presence of preexisting medical illnesses, and the level of metabolic stress. The use of parenteral nutrition is often recommended if enteral intake has been, or is anticipated to be, inadequate for 5–10 days [45]. However, carefully performed, well-designed, prospective randomized clinical trials to support the efficacy of this approach for many clinical situations are few in number [46]. Therefore, the use of parenteral nutrition requires a careful integration of data from pertinent clinical trials, clinical expertise in the illness or injury being treated, a reasonable estimate of the anticipated duration of inadequate food intake, clinical expertise in nutritional therapy, and input from patients and their families.

Central parenteral nutrition

General principles

The ability to use a central vein to supply nutrient requirements for growth and maintenance was realized more than 30 years ago when catheters were inserted into the superior vena cava of beagle dogs to provide the sole source of nutrients for 72–256 days [47]. Today, the use of central parenteral nutrition (CPN), also known as *total parenteral nutrition* (TPN), is commonplace. Although percutaneous infraclavicular subclavian vein catheterization with advancement of the catheter tip to the junction of the superior vena cava and right atrium is the most commonly used technique for catheter placement, many other approaches have been successfully performed when the subclavian vein is not accessible, including internal jugular, basilic, saphenous, and femoral veins, and even thoracotomy with direct insertion into the right azygos vein [48] or the right atrial appendage [49]. In addition, peripherally inserted central venous catheters can be used to provide CPN. In this approach, insertion by a physician is not required, and the technique cannot cause pneumothorax or traumatic injury to arteries or nerves, but it is associated with an increased risk for thrombosis if the catheter tip is placed proximal to the superior vena cava [50].

Parenteral nutrient solutions provide all the basic nutrient requirements, including fluids, proteins, carbohydrates, fats, minerals, trace elements, and vitamins (Table 101.7). The lipid component can be piggybacked to the primary nutrient mixture, or a total nutrient admixture can be prepared, which reduces handling costs and potential breaks in sterility. However, total nutrient admixtures prevent visible inspection for particulate matter. Invisible calcium and phosphorus precipitates have caused fatal pulmonary emboli in patients receiving parenteral nutrition [51]. Therefore, pharmacy standards regarding physicochemical compatibility should be closely followed in all patients receiving CPN, and in-line filters should be used with all parenteral nutrient solutions. Even a careful inspection of clear nutrient solutions cannot detect small microprecipitates, which can obstruct small pulmonary capillaries [52].

The specific formulation prescribed for a patient depends on the patient's estimated nutrient requirements and ability to tolerate specific nutrients without adverse effects. The patient's protein, energy, and fluid requirements are the most important initial considerations in designing the appropriate parenteral formulation. Protein requirements are met by infusing standard solutions composed of crystalline amino acids containing 40%–50% essential and 50%–60% nonessential amino acids, usually with little or no glutamine, glutamate, aspartate, asparagine, tyrosine, and cysteine. Most hospitalized patients require between 1.0 and 1.5 g of protein per kilogram body weight per day. Some amino acid solutions have been modified for specific disease states, such as those enriched in branched-chain amino acids (advocated for patients with hepatic encephalopathy) and those containing 67%–100% essential amino acids (advocated for patients with renal insufficiency). However, the clinical superiority of these formulations over standard amino acid solutions has not been well documented in clinical trials [53,54].

The major sources of energy are glucose and lipid. However, infused amino acids are also oxidized and should be included in the estimate of energy provided as part of the parenteral formulation. Glucose (dextrose) is usually the predominant energy source in CPN formulations and is the required fuel for erythrocytes, white blood cells, bone marrow, and renal medulla (~ 40 g/day) and the preferred fuel for the brain (~ 120 g/day). Each gram of hydrated dextrose provides 3.4 kcal (14.2 kJ) and is readily oxidized unless excessive amounts are infused. In stable postoperative patients, increasing the rate of glucose infusion up to, but not more than, 7 mg/kg/min increases the rate of glucose oxidation [55,56]. Lipid emulsions contain soybean oil or a combination of soybean and safflower oil triglycerides, egg yolk phospholipids as an emulsifying agent, and glycerin to maintain isosmolarity. These emulsions provide energy and are a source of essential fatty acids, linoleic and linolenic acids. Lipid calories are as effective as additional glucose calories in conserving body nitrogen economy and supporting protein metabolism once absolute tissue requirements for glucose have been met [57,58]. The optimal percentage of calories that should be infused as fat is not known. At least 5% of total calories should be given as lipids to prevent essential

Table 101.7 Delivery of nutrients during total (central) parenteral nutrition

Nutrient	Formulation for delivery	Frequency, suggested dose
Water	Diluent in base solution	Daily, 2–3 L
Amino acids	3.5%–10% solutions of crystalline amino acids in base solution or 3:1 mixtures	Daily, 12%–24% of total kcal
Carbohydrate	20% dextrose in base solution or 3:1 mixtures	Daily, 55%–65% of total kcal
Phospholipid	10%–30% soybean/safflower oil emulsion in 3:1 mixtures (1.1–3.0 kcal/mL)	Daily to weekly, 20%–25% of total kcal
Essential fatty acids	3:1 mixtures i.v., corn oil orally (5–15 mL), or topical skin application	Daily to weekly, ~ 11 g/day (1%–2% of kcal/day)
Vitamins A, D, E, C, B complex	Multivitamin preparation in base solution	Daily or q.o.d., 10 mL
Vitamin B-12	Injection s.c. (1 mg/mL) or oral tablet	Monthly (1 mg s.c.) or daily (100 µg/day orally)
Vitamin K	Aqueous colloidal solution	Weekly, 10 mg s.c.
Sodium	Amino acid solutions and NaCl/acetate added	Daily, 60–120 mEq
Potassium	Amino acid solutions and KCl added	Daily, 30–80 mEq
Chloride	Amino acid solutions and Na/KCl added	Daily, 80–140 mEq
Calcium	Calcium gluconate added to base solution	Daily, 4.6–9.2 mEq
Magnesium	Amino acid solutions and $MgSO_4$	Daily, 8.1–24.3 mEq
Phosphorus	Na/K phosphate added to diluted base solution	Daily, 12–24 mmol
Iron	Iron dextran added separately to maintain stores	Monthly, titrated to needs
Trace minerals	Trace mineral solutions	Daily, 1 mL

Note: Each component of total parenteral nutrition can be ordered separately, according to patient need. The amino acid concentration is chosen according to the need for nitrogen. Lipid is not needed beyond the requirement for essential fatty acids but is often provided daily to restrict volume.
i.v., intravenously; q.o.d., every other day; s.c., subcutaneously.

fatty acid deficiency. The rate of lipid emulsion infusion should probably not exceed 0.7 kcal/kg/h because most complications associated with lipid emulsion infusions have been reported when more than 1.0 kcal/kg/h (0.11 g/kg/h) was provided [59]. Lipid emulsion infusion, containing ω-6 polyunsaturated fatty acids (PUFAs), can cause pulmonary dysfunction [60], hepatic phospholipidosis [61], impaired immune system function [62], pancreatitis [63], decreased platelet aggregation [64], fat overload syndrome [65], and hypersensitivity reactions [66–68]. Therefore, olive oil-based lipid emulsions and medium- and long-chain triglyceride lipid emulsions are being developed [69,70]. Together, carbohydrate and lipid should provide 25–35 kcal/g of protein to optimize incorporation of amino acids into new protein.

Complications

Mechanical, infectious, metabolic, and gastrointestinal complications are associated with the use of CPN. Administering CPN under the guidance of experienced teams decreases the incidence of most complications [71]. Percutaneous insertion of a central venous catheter can damage local structures and cause pneumothorax, brachial plexus injury, subclavian and carotid artery puncture, hemothorax, thoracic duct injury, and chylothorax. In addition, the catheter can be sheared off, become occluded, or cause subclavian vein or superior vena cava thrombosis [72–74].

Catheter-related sepsis is the most common serious complication in patients receiving CPN; even when meticulous care is provided, catheter-related sepsis occurs in up to 4% of hospitalized patients [75]. Multiple-lumen central catheters that are used for CPN are associated with an increased incidence of infections compared with single-lumen catheters [75]. Catheter-related sepsis should be considered in all patients in whom a fever or leukocytosis develops while they are receiving CPN. The catheter tunnel and exit site should be carefully inspected, and any exit site drainage should be cultured. Blood cultures should be obtained from the central line and a peripheral vein. The presence of subcutaneous infection along the catheter tunnel or clinical toxicity (e.g., hypotension) without another known source of infection is

an indication for immediate catheter removal. If no evidence of tunnel infection or clinical toxicity is found, the catheter can remain in place, and empirical antibiotic therapy should be started through the central venous catheter. Blood cultures should be obtained again in 48–72 h to ensure clearance of bacteremia. The catheter should be removed from patients who have blood cultures that are positive for fungus or *Staphylococcus aureus,* polymicrobial infection, persistent or recurrent catheter-related bacteremia, or persistent fever without another suspected source of infection [76].

The metabolic complications observed in patients receiving parenteral nutrition are usually caused by inappropriate nutrient administration resulting in nutrient excesses or deficiencies or both. Parenteral nutrition has a propensity to cause hyperglycemia in hospitalized patients. Since 2001, there exists good evidence that even minor elevations in blood glucose (BG) levels in intensive are unit (ICU) patients lead to increased mortality when compared with true normoglycemia (BG 80–110 mg/dL) [77,78]. In the less stressed patient a BG goal of less than 150 mg/dL may be more appropriate as there have been no clinical trials published in this patient group. Blood glucose should be kept below a concentration of 120 mg/dL in pregnant patients to avoid the complications of gestational diabetes and large-for-gestational-age births. Metabolic bone disease, including osteopenia and osteomalacia, has been observed in patients receiving long-term (> 3 months) CPN [79].

Several hepatobiliary abnormalities have been observed in patients receiving CPN, and these are usually more severe and more frequent in infants than in adults [80]. Hepatic abnormalities include alterations in liver biochemistries, steatosis, steatohepatitis, lipidosis and phospholipidosis, cholestasis, fibrosis, and cirrhosis. Most hepatic abnormalities occur early and are transient, but in a small subset of patients receiving long-term (> 16 weeks) CPN, progressive and more serious liver disease develops [81]. Biliary abnormalities include acalculous cholecystitis, gallbladder sludge, and cholelithiasis, and these usually occur in patients receiving relatively prolonged (> 4 weeks) courses of CPN. Acalculous cholecystitis has been reported in about 5% [82], cholelithiasis in about 30%, and gallbladder sludge in up to 100% [83] of patients receiving prolonged CPN. The pathogenesis of acalculous cholecystitis is unclear. Gallbladder stasis is an important contributing factor in the pathogenesis of gallbladder sludge and stones.

Maintaining regular gallbladder contractions by either enteral feedings or cholecystokinin injections prevents gallbladder sludge and gallstone formation [84,85].

Peripheral parenteral nutrition

Peripheral parenteral nutrition (PPN) consists of a mixture of nutrients containing a final concentration of 5%–10% dextrose, 2%–5% amino acids, electrolytes, and vitamins and minerals. This crystalloid solution is mixed with a 10% or 20% lipid emulsion as an all-in-one admixture, or the lipid emulsion can be piggybacked to the distal port of the intravenous infusion line. To ensure that adequate calories can be given in a reasonable volume, the lipid component usually provides approximately half of the total calories.

The use of a peripheral vein to provide nutritional support avoids many of the mechanical and infectious complications of CPN but is associated with a high risk for thrombophlebitis, which occurs in up to 94% of patients [86]. However, the use of PPN has become possible in many patients since the development of safe isotonic lipid emulsions and a better understanding of the causes of PPN-induced thrombophlebitis.

Several factors are important in the pathogenesis of thrombophlebitis in patients receiving PPN [87,88]:

- the osmolality, pH, and lipid content of the PPN solution, and the presence of particulate matter
- the diameter, length, and composition of the catheter
- the duration, rate, and volume of the infusion
- the diameter and anatomical position of the vein
- the insertion technique.

Adherence to the following principles can increase the life of a single infusion site to more than 10 days in many patients:

- provide at least 50% of total energy as a lipid emulsion, add 500–1000 U of heparin and 5 mg of hydrocortisone per liter of solution, and add sodium hydroxide if needed to achieve a pH of 7.4
- insert a fine-bore 22- or 23-gauge polyvinyl pyrrolidine-coated polyurethane catheter in as large a vein as possible in the proximal forearm with use of a sterile technique
- place a 5-mg glyceryl trinitrate ointment patch over the infusion site
- infuse the PPN solution with a volumetric pump and keep the total infused volume below 3500 mL
- filter the solution with an in-line 1.2-μm filter.

Home parenteral nutrition

The ability to provide parenteral nutrition at home was first demonstrated in 1970 [89]. In 1992, approximately 40 000 patients (120 per million residents) received home parenteral nutrition (HPN) in the United States [90], a tenfold higher rate than that in Europe (1–12 per million residents) [90–92]. Patients with benign diseases have a much better outcome than those with terminal illnesses. Patients with benign gastrointestinal disease and receiving HPN have a better outcome and decreased HPN days spent in the hospital than those with terminal illnesses. In addition, it has been reported that patients with benign disease and receiving HPN had a 5% 1-year mortality compared with 80% of their counterparts with terminal illnesses [93].

HPN is usually given through an implantable subcutaneous port or a catheter inserted in the subclavian vein and tunneled subcutaneously to exit on the anterior chest. Nutrient formulations are infused for 8 to 12 h overnight to

allow patients to be active during the day. Intravenous lipids may not be necessary for patients who eat and are able to absorb adequate amounts of fat, whereas others may receive 20%–30% of their calories as a lipid emulsion. The development of multichamber bags with an extended shelf life allows the delivery of more CPN bags to home patients at one time [94]. The most serious common complication is catheter-related sepsis, most often caused by *S. aureus* or *Staphylococcus epidermidis*. However, a 5-year review of HTPN patients demonstrated a 50% decline in the incidence of catheter-related sepsis from 1997 to 2001 [95]. Nonetheless, venous thrombosis, chronic liver disease, cholelithiasis, and metabolic bone disease are all associated with the prolonged use of HPN.

Nutritional support in the hospitalized patient with gastrointestinal disease

The major purpose of nutritional therapy is to prevent or correct specific nutrient deficiencies and to prevent the adverse effects of protein–energy malnutrition. Nutritional support has also been proposed as a primary therapy for patients with certain gastrointestinal diseases, such as inflammatory bowel disease and hepatic encephalopathy. In this section, we review the available data evaluating the clinical efficacy of nutritional support in patients with gastrointestinal diseases. Whenever possible, only the data from prospective randomized clinical trials are considered because this approach is the most reliable technique for evaluating the usefulness of therapy.

Short bowel syndrome

Patients who have undergone massive intestinal resection often require nutritional support for optimal function and survival. The length of the remaining jejunum, the presence of the ileocecal valve, and whether the colon is intact are critical determinants of the need for nutritional support [96]. In general, patients with a jejunostomy and less than 90 cm of jejunum, or those with a colon in continuity but less than 50 cm of jejunum, are likely to require parenteral nutrition. Providing nutrients with continuous nighttime tube feedings [97] or fluid and electrolytes with oral rehydration therapy [32] (see "Oral rehydration therapy" above) can sometimes decrease or eliminate the need for parenteral therapy.

Inflammatory bowel disease

Enteral nutrition

Three metaanalyses of published prospective randomized clinical trials concluded that enteral nutrition is not as effective as corticosteroids. In addition, no benefit of elemental over nonelemental formulas was noted [98–100]. The true clinical efficacy of enteral nutritional therapy has not been evaluated because no study has compared patients receiving dietary therapy with an untreated control group. However, the overall remission rate reported with enteral nutrition therapy is about 60% [98,99], which is higher than the range of 20%–40% reported for placebo-treated patients with moderate disease [101,102].

Parenteral nutrition

Data from several prospective randomized clinical trials found that "bowel rest" is not necessary to achieve clinical remission in patients with active Crohn's disease; patients who received TPN plus bowel rest fared no better than those receiving TPN plus oral/enteral feeding [103,104]. A beneficial effect of TPN in patients with Crohn's disease or ulcerative colitis has not been demonstrated in prospective randomized clinical trials. No significant differences in clinical response rates were found in hospitalized patients with severe disease exacerbations who were randomized to receive TPN and steroid therapy or an oral/enteral diet and steroid therapy [105–107]. Nonetheless, TPN may be useful in patients with severe inflammatory bowel disease who cannot eat because of prolonged ileus or who might benefit from bowel rest because of severe diarrhea or anticipated surgery.

Specific nutrient therapy

Fish oil supplementation may produce a modest decrease in the activity of ulcerative colitis [108,109], may help to lower the steroid dose, and may prevent early but not late relapse [110], but it is not effective by itself. Two prospective randomized clinical trials of maintaining clinical remission in patients with Crohn's disease have shown both success [111] and failure [112]. Short-chain fatty acids have not been shown to be effective for rectal disease in ulcerative colitis [113].

Gastrointestinal fistulae

No prospective randomized clinical trials have evaluated the efficacy of TPN, but "bowel rest" may improve the clinical outcome of selected patients with fistulae. Before the use of TPN, mortality in patients with gastrointestinal fistulae was caused by malnutrition, fluid losses, and peritonitis. A retrospective analysis of patients with small bowel fistulae showed an improved outcome for those who received nutritional support [114]. Anastomotic fistulae at sites of recent resection in Crohn's disease may close permanently with TPN and bowel rest, although octreotide therapy may also be necessary for success in some patients [115]. However, fistula closure is not well maintained after an oral diet is reinstituted in patients with active disease at the origin of the fistula, or with postfistula obstruction.

Growth failure in children

Total parenteral nutrition or oral/enteral supplemental feedings can initiate catch-up growth, resulting in marked increases in height and weight, in children with inflammatory bowel disease and growth failure [116–119]. In fact, even intermittent enteral feeding can improve growth [120,121].

Acute pancreatitis

Most patients with acute pancreatitis are unable to eat because oral feeding can cause abdominal pain and increase serum amylase and lipase concentrations. Nonetheless, nutritional support is not useful in patients with mild or moderate pancreatitis because of the rapid resolution of symptoms and ability to begin oral feeding within several days. In addition, no benefit of parenteral nutrition compared with enteral nutrition has been demonstrated in patients with mild or moderate pancreatitis [122], and in one study, the use of TPN was associated with a higher prevalence of catheter-related sepsis and insulin requirements [123]. However, nutritional support may be necessary in about 10%–20% of patients with pancreatitis who have severe and complicated disease when a prolonged period of starvation is anticipated.

It has been shown that it is possible to provide enteral feeding to patients who have mild to moderate [122] or severe [124–126] pancreatitis, without causing an exacerbation of symptoms, when feeding is delivered distal to the ligament of Treitz into the upper jejunum. Moreover, in prospective randomized clinical trials that compared enteral with parenteral nutritional therapy in patients with severe pancreatitis, those who were randomized to receive enteral nutrition showed a greater reduction in acute-phase-response proteins, a greater improvement in disease severity scores, and fewer medical complications than those given parenteral nutrition [124]. These findings led to the recommendation by an international consensus conference to provide nutrition support by jejunostomy feedings rather than CPN in patients with severe acute pancreatitis [127]. However, it is not known whether enteral feeding was beneficial or whether parenteral feeding was harmful because no study included an unfed control group.

Providing a portion of total calories as lipid can help prevent hyperglycemia in glucose-intolerant patients with pancreatitis. However, it is important to ensure adequate triglyceride clearance because hypertriglyceridemia induced by infusion of fat emulsions can cause a relapse of pancreatitis [63].

Liver disease

Alcoholic hepatitis

One prospective randomized clinical trial compared PPN with an anabolic steroid, oxandrolone, or the combination for 21 days and found no change in mortality but improved Child–Pugh scores in those receiving PPN plus oxandrolone [128,129]. In another study, nutritional therapy improved liver function and reduced early mortality (1- and 6-month) in a subgroup with moderate "malnutrition" or liver disease [130]. No improvement was noted in the group with severe disease. It is clear that these markers correlate with the severity of liver disease and with clinical outcome [131].

Alcoholic cirrhosis

Several prospective randomized clinical trials showed that enteral nutrition in patients hospitalized for complications of cirrhosis led to an improved clinical status [132–134]. A single study showed that enteral feeding of a branched-chain amino acid formula reduced mortality [135]. However, the ad lib diet group ingested only about half the calories of the enterally fed group, and the control group had an unusually high mortality rate (47%).

Hepatic encephalopathy

In a metaanalysis of nine prospective randomized clinical trials evaluating the use of TPN enriched with branched-chain amino acids in patients with acute encephalopathy [53], recovery from encephalopathy was improved during short-term (7–14 days) treatment. However, the variability in mortality rates was so great that no conclusion could be reached regarding that outcome measure. No benefit of branched-chain amino acid therapy was detected in the one study that compared a group that received branched-chain amino acid-enriched TPN with a control group that received standard amino acids. Studies of the use of enteral solutions containing branched-chain amino acids in patients with chronic encephalopathy have yielded conflicting results. However, the studies with the largest number of patients showed clinical benefits in protein-intolerant patients [136].

Micronutrient deficiency and treatment

Tissues that proliferate rapidly (skin, oral and gastrointestinal mucosa, hair, bone marrow) or in which metabolic rates are high (muscle, gastrointestinal mucosa) are at greatest risk for signs of deficiency at an early stage. Clues regarding deficiency come from a history of reduced intake or increased losses (or of conditions that make those situations more likely), physical examination findings, and results of laboratory tests that ideally determine body stores of the nutrient. The signs and symptoms of nutritional deficiency in adult patients are listed in Table 101.1. The laboratory values for the major vitamins and minerals are listed in Tables 101.2 and 101.3. A more detailed discussion of these nutrients and the assessment of their status can be found in general references [1,137,138].

Sodium (Na^+)

Deficiency

Sodium depletion usually presents with symptoms of dehydration because of concomitant water loss. Severe depletion presents with nausea, vomiting, exhaustion, cramps, seizures, and ultimately cardiovascular collapse. On the physical examination, mucosal xerosis, sunken eyeballs, and mental confusion can be present, but none of these are specific findings. In older patients, other explanations are often present for all these signs [139]. Orthostatic hypotension is often used to estimate the intravascular volume and is defined as a

systolic blood pressure below 20 mmHg or a diastolic blood pressure below 10 mmHg within 3 min after standing, with or without a rise in the heart rate of 5 to 10 beats/min [140]. The signs of total body sodium excess are weight gain and edema. The presence of either of these would not reflect sodium deficiency. If sodium deficiency is the result of increased gastrointestinal or cutaneous losses, the urine osmolality is increased and the urine sodium concentration is less than 10 mEq/L. If sodium deficiency is the result of diuretic therapy, the urine osmolality is decreased and the urine sodium concentration is more than 10 mEq/L.

Assessment

The serum sodium concentration primarily reflects water, not sodium, balance. Thus, any serum concentration is possible when deficiency is present, depending on the relative deficits of water and sodium. If hyponatremia is present but the cause is not apparent, the serum osmolality should be measured. In hospitalized patients, hyponatremia is most often the result of dilution by excess free water, not depletion of sodium. The free water excess is usually caused by cardiac, hepatic, or renal insufficiency or by overly enthusiastic fluid administration and is accompanied by edema and weight gain. When normovolemia is present, the hyponatremia may be caused either by inappropriate secretion of antidiuretic hormone or by displacement of sodium osmoles with elevated glucose or urea as osmoles.

Treatment

When signs of volume depletion are present, at least a 10% reduction in extracellular fluid volume has occurred [141]. The sodium deficit can be estimated as follows: Na deficit (mEq) = ([Na desired] − [Na observed]) × 0.6 × weight (kg). Sodium is usually replaced with NaCl (1 g = 17 mEq Na), either orally, enterally, or parenterally. Symptomatic hyponatremia (e.g., seizures, lethargy) is treated with intravenous 0.9% NaCl or occasionally with hypertonic (3% or 5%) NaCl. Sodium infusion should be designed to increase the serum concentration by 1 mEq/h until it reaches 120 mEq/L. Too rapid restoration has been associated with permanent neurological deficits resulting from central pontine myelinolysis. When sodium depletion is accompanied by hypernatremia or normonatremia, the appropriate amount of water should be supplied along with the sodium to achieve or maintain a normal serum sodium concentration. Chronic losses from diarrhea, vomiting, or ileostomy should be treated in adults with an oral rehydration solution that provides a carbohydrate source along with adequate sodium, so that Na/solute-coupled cotransport can occur and maximize the rate of sodium repletion. These solutions are discussed in the section "Enteral nutrition" above. Sodium toxicity can result from excessive administration of NaCl. Symptoms of toxicity include vomiting, diarrhea, peripheral vascular collapse, respiratory depression, and death.

Potassium (K^+)

Deficiency

Hypokalemia (< 3.5 mEq/L) rarely is caused by decreased intake but can result from shifts between the extra- and intracellular compartments of the body occurring while the total body potassium remains normal. This shift can be caused by alkalosis and the administration of glucose or insulin. Alternatively, decreased body stores result from increased renal excretion or intestinal losses, with subsequent shift of potassium from extra- to intracellular compartments. Loss of gastric contents by vomiting produces hypovolemia and metabolic acidosis, leading to increased renal K^+ loss. Increased K^+ loss from diarrhea produces hypovolemia, but with metabolic acidosis, yet can still produce hypokalemia from Na^+–K^+ exchange in the colon. Other causes of renal losses include mineralocorticoid excess, some antibiotics, and, rarely, renal tubular acidosis. In pure deficiency states, each decrease of 1 mEq/L corresponds to a loss of 200–300 mEq of body potassium. The loss of 5%–10% of body stores is usually tolerated without symptoms, but when serum levels fall below 3.0 mEq/L, weakness, paresthesias, confusion, cramps, myalgias, and cardiovascular abnormalities can occur [142].

Assessment

The normal serum level of potassium is 3.5–4.5 mEq/L and reflects both body stores and the availability of glucose as an energy source. Red blood cell hemolysis or extreme leukocytosis without hemolysis can result in spurious hyperkalemia. Total body potassium deficit cannot be estimated accurately especially by urinary K^+([K^+]u), as that merely reflects the renal response to circulating volume, pH, or plasma K^+ ([K^+]p) (Table 101.8). The transtubular K^+ concentration gradient (TTKG) is a simple calculation that can provide a semiquantitative measure of K^+ secretion in the cortical collecting duct (CCD) [143]: [K^+]ccd = [K^+]u/(Osm_u/Osm_p), the urine–plasma osmolality ratio. If the urine osmolality exceeds that of plasma, then TTKG = [K^+]ccd/[K^+]p, and it can be used to assist in the differential diagnosis of hypokalemia. TTKG is correlated with plasma aldosterone levels in hypokalemic patients.

Treatment

Treatment should be provided if symptoms are attributed to the hypokalemia, or if other conditions, such as chronic liver disease, are present that would exacerbate the hypokalemia. Alkalosis tends to lower the serum potassium concentration, whereas acidosis raises it. Thus, alkalosis can be treated first before the addition of potassium salts, but the same should not be done when acidosis is present. Oral replacement should be used if possible because this allows the serum potassium concentration to rise slowly in equilibrium with the intracellular compartment. Either food or supplement (Table 101.9) sources should be used. Foods rich in K^+ (> 400 mg/serving) include cantaloupe, potato, dried fruits, avocado, skim milk, banana, fruit juices, and many fruits. The administration of

Table 101.8 Expected values for tests that are helpful in the differential diagnosis of hypo- or hyperkalemia

Test	Normal	Hypokalemia	Hyperkalemia
24-h K^+ excretion	60–80 mmol/day 6–8 mmol/mmol creatinine	< 10 mmol/day < 1–1.5 mmol/mmol creatinine	> 150 mmol/day > 10–15 mmol/mmol creatinine
Urinary Na^+/K^+	2.3–2.7	GI loss 2.5–2.6 Diuretics 2.6	Aldosteronism 1.4
Spot urine K^+	Variable due to K^+ secretion and water reabsorption	Diarrhea < 20 Renal > 20	Variable
TTKG	2–5	Diarrhea < 2 Diuretics 5–10 Vomiting 2–5	Aldosteronism > 10

GI, gastrointestinal; TTKG, transtubular potassium concentration gradient.

Table 101.9 Oral potassium products comparison chart

Dosage forms	Potassium salt(s)	Brand names	Potassium content	Comments
Liquid	Potassium chloride	Various	10, 15, 20, 30, 40 mEq/15 mL	Rapid absorption, low frequency of gastrointestinal ulceration, unpleasant taste
	Mixture of potassium acetate/bicarbonate/citrate	Trikates, Tri-K	45 mEq/15 mL	Preferred forms in patients with delayed gastrointestinal transit time, avoid nonchloride salts in metabolic alkalosis
Powder	Potassium gluconate Potassium chloride Mixture of potassium bicarbonate/citrate	Kaon Various Various	20 mEq/15 mL 15, 20, 25 mEq/packet 25, 50 mEq/packet	Must be dissolved in water before use, avoid nonchloride salts in metabolic alkalosis
Effervescent tablets	Potassium chloride	Various	25, 50 mEq/tablet	
Sustained-release capsules, tablets	Potassium chloride	Various	8, 10, 20 mEq/capsule	Wax matrix or polymer-coated crystals in gelatin capsule, bioequivalent to liquid forms, avoid in patients with delayed gastrointestinal transit time, poorly effective in short bowel syndrome
Salt substitutes	Potassium chloride	Adolph's, Morton's, Nu-Salt, No Salt, various	Powder 50–70 mEq/tsp	Inexpensive dietary source, prescribe specific amount to avoid hyperkalemia; contraindicated in oliguria, severe renal disease

Adapted from Knoben JE, Anderson PO. Handbook of Clinical Drug Data, 7th edn. Hamilton, IL: Drug Intelligence Publications, 1993:663; Olin BR (ed.) Facts and Comparisons. St. Louis: Facts and Comparisons, 1992:16; and Stanaszek WF, Romankiewicz JA. Current approaches to management of potassium deficiency. Drug Int Pharm 1985;19:176.

20–40 mEq at a time up to four times a day is safe. The chloride salt is preferred when renal or gastric losses account for the hypokalemia. When hypokalemia is secondary to diarrhea, one of the alkalinizing salts (e.g., citrate, acetate, bicarbonate) is preferred. The slow-release, matrix, and microencapsulated forms are better tolerated than liquid preparations. The slow-release forms have reduced the risk of small bowel ulcers and strictures but may not be useful for patients with short bowel syndrome. For intravenous therapy, the infusion rate should not exceed 20 mEq/h unless cardiac monitoring is included. The chloride salt is usually best for intravenous therapy, except when phosphate is used for treating the associated hypophosphatemia of diabetic ketoacidosis. In the presence of acidosis, potassium acetate is the best choice. Hyperkalemia should be avoided during therapy because levels above 8.0 mEq/L carry a severe risk

for cardiac dysfunction, including bradycardia, asystole, and ventricular fibrillation. Skeletal muscle weakness, areflexia or hyperreflexia, paralysis, and fatigue may also occur at these high levels.

Calcium

Deficiency

The conditions that cause hypocalcemia requiring calcium supplementation include vitamin D deficiency, failure of vitamin D synthesis or action, hypoparathyroidism, hypomagnesemia, severe acute pancreatitis, malabsorption syndromes, neoplasms with osteoblastic metastases, and medications, including aminoglycosides, cisplatin, calcitonin, furosemide, mithramycin, phosphates, and anticonvulsants [144]. Acute manifestations include tetany, altered myocardial function and arrhythmias, hyperreflexia, paresthesias, seizures, mental status changes, and choreoathetotic movements. The electrocardiogram shows prolonged Q–T intervals with normal QRS complexes. Chronic deficiency may not be associated with hypocalcemia. With a total body content of 1000 g and a net daily loss of 100 mg/day, a 20% decrease in body stores (which could be easily detected) would take 2000 days, or about 6 years, to develop. Osteopenia usually presents with fractures or bone pain. However, patients identified as being at high risk for the development of osteopenia should begin treatment well before this occurs. Risk factors include cigarette smoking, family history of osteoporosis, sedentary lifestyle, long-term glucocorticoid therapy, long-term anticonvulsant therapy, early surgical or natural menopause (< 50 years), and testicular failure (secondary to drug therapy or disease).

Assessment

Recent intake or absorption is assessed by 24-h urinary calcium [137]. At levels of absorption above 2 mg/kg (corresponding to an intake of 6 mg/kg), urinary calcium equals absorbed calcium. When absorption is low, urinary calcium levels do not decline proportionally, unlike the levels of sodium, potassium, magnesium, and phosphorus, all of which are better absorbed with better-regulated urinary excretion. Urinary calcium is used primarily to determine that absorption is low and to follow the patient after oral replacement is begun, to ensure that absorption becomes normal and that hypercalciuria does not ensue. Urinary calcium excretion of more than 300 mg/day is an indication to reduce the dose of calcium (or vitamin D). Serum calcium is tightly regulated and only falls below normal when severe deficiency occurs. Pseudohypocalcemia can be caused by hypoalbuminemia and by gadolinium administration, a treatement that precipitates calcium [145]. Body stores of calcium are assessed by bone densitometry, with many centers using dualenergy x-ray absorptiometry. The accuracy of this method is equal to that of quantitative computed tomography and is sensitive to changes of 9%–12% in bone mass [146]. Results must be compared with those of age- and gender-matched controls and are useful for diagnosis and following the response to therapy. Bone densitometry should be used to screen all patients at high risk for the development of osteopenia and to guide treatment decisions for selected postmenopausal women, but it is not recommended for routine screening. Serum calcium and alkaline phosphatase levels do not become abnormal until total body calcium depletion is severe.

Treatment

Treatment of acute symptomatic hypocalcemia is required immediately to prevent laryngospasm, seizures, and cardiac arrhythmias. A total of 200–300 mg of elemental calcium should be given intravenously over 5 min as 1 g of calcium chloride or 2–3 g of calcium gluconate (10 mL of 10% calcium gluconate = 1 g of calcium). This should be followed by an infusion of calcium of 0.5–2.0 mg/kg/h [144,147]. The serum calcium should normalize in 6–12 h while the vital signs and electrocardiographic activity are monitored. Patients with chronic hypocalcemia or osteopenia on the basis of poor intake or absorption should be treated with oral supplements of up to 2.5–3.0 g/day given in divided doses. The best dietary source of calcium is milk, and this can be used if clinically tolerated. Otherwise, a number of oral calcium salts are available that vary in elemental calcium content (Table 101.10) [148]. Calcium carbonate is used most often because of the high percentage of elemental calcium, but it is rather insoluble. If the urinary calcium does not increase with calcium carbonate, another, more soluble salt (e.g., calcium gluconate) may be tried, with or without the addition of vitamin D. People who take calcium supplements may be at risk for ingesting lead, although the amount of lead in bone meal has dropped as a consequence of a decrease in the levels of environmental lead from gasoline and paint. When tested by the Natural Resources Defense Council, many of the 20 major brands of calcium supplements marketed in California contained enough lead to increase the average intake of 5–10 µg by 50% [149]. The supplements tested that were lowest in lead content (< 0.5 µg per dose) were Children's Mylanta, Posture-D High Potency Calcium with Vitamin D, and Tums 500 Calcium Supplement.

When malabsorption is present, the doses of vitamin D used are large (25 000–50 000 IU given from once a week to daily). Alternatively, the more soluble forms of vitamin D, especially 1,25-dihydroxyvitamin D, may be effective. Treatment programs must be individualized and monitored closely. Thiazides can decrease the urinary loss of calcium (thereby rendering the urinary determination less meaningful) but also increase the urinary loss of magnesium. Thus, their use in patients with osteopenia must be carefully considered. Supplemental calcium may predispose some individuals to kidney stones but decreases the risk for oxalate stones in patients with short bowel syndrome. The develop-

Table 101.10 Selected oral calcium supplements

Salt in mineral supplements	Percentage of elemental calcium	Tablet size (mg)	Amount of calcium per tablet (mg)
Calcium carbonate	40		
Caltrate + D (200 IU)		1500	600
Os-Cal 500 + D (200 IU)		1250	500
Tums Ultra		1000	400
Viactin (100 IU D)		1250	500
Calcium phosphate	38		
Posture + D		1600	600
Calcium acetate	25	1000	250
Calcium citrate	21		
Citracal + D (200 IU)		1200	315
Calcium citrate 1000			250
Calcium lactate	13	650	85
Calcium gluconate	9	500	45
Calcium glubionate	6.5	1800/5 mL	115/5 mL

Foods	Serving	Amount/serving (mg)
Skim milk/yogurt	1 cup	302
Cheese, hard	1 oz	~ 250
Cheese, soft	1 oz	~ 175
Tofu	1/2 cup	~ 250
Broccoli, boiled	1/2 cup	36
Kale, cooked	1/2 cup	47

D, vitamin D; 1 cup = 8 fl oz (240 mL); 1 oz = 28 g.

ment of calcium toxicity in patients with malabsorption is rare but can occur in patients with osteopenia and normal absorption, especially if they are also receiving supplemental vitamin D. Hypercalcemia can cause anorexia, nausea, constipation, polyuria, fatigue, weakness, confusion, lethargy, decreased deep tendon reflexes, shortened Q–T and prolonged P–R interval and QRS complex duration, and cardiac arrest [147].

Magnesium

Deficiency

Symptomatic magnesium deficiency is usually the result of decreased absorption or increased losses in the urine or from the gastrointestinal tract, especially in patients with extensive small bowel resection [150]. Excessive urinary loss, usually caused by decreased tubular reabsorption, occurs in a variety of circumstances [151]. Medications that promote renal loss include diuretics, aminoglycosides, cyclosporine (ciclosporin), amphotericin B, cisplatin, and digitalis [152]. Increased renal losses also occur in hypercalcemia, volume expansion, tubular dysfunction, acute and chronic alcoholism, diabetes mellitus, hyperparathyroidism, and hypophosphatemia. Magnesium deficiency may occur in type 2 diabetes mellitus and precede the diabetes. Added magnesium may improve diabetic control if the serum levels are low initially [153]. Hypomagnesemia caused by a shift of magnesium from the extracellular to the intracellular compartment may result from feeding, treatment of diabetic ketoacidosis, parathyroidectomy, acute pancreatitis, and correction of acidosis in renal failure. Hypomagnesemia is often associated with and can be a cause of hypocalcemia, hypokalemia, and hypophosphatemia [153]. Hypomagnesemia can present as tremor, myoclonic jerks, ataxia, tetany, ventricular dysrhythmias, tachycardia, hypotension, cardiac arrest, apathy, depression, delirium, hallucinations, and psychosis [154]. The signs and symptoms of hypomagnesemia are often a consequence of the hypocalcemia resulting from the same condition (e.g., malabsorption) or of the decreased parathyroid hormone secretion that occurs when hypomagnesemia is severe.

Assessment

In the absence of excess fecal or renal losses, urinary excretion reflects intake and absorption of magnesium [138]. When the serum magnesium is low in patients with short bowel syndrome, the urinary magnesium excretion may

also be low and perhaps more sensitive than the serum magnesium [155]. Serum magnesium concentrations do not correlate well with total body stores, but it is reasonable to assume that depletion is present when the serum concentration is less than 1.0 mg/dL [152]. Because magnesium is primarily an intracellular ion, the best indicator of depletion may be a measurement of the content in white cells, although only a few studies have compared this value with those in other tissue pools [138]. A load test for magnesium depletion has been described, but its usefulness has not been satisfactorily documented.

Treatment

Moderate to severe deficiency should be treated parenterally, especially if tetany or ventricular arrhythmias are present. An intravenous loading dose of 4 to 8 mmol should be given first, followed by 25 mmol/day thereafter until the plasma magnesium is above 0.4 mmol/L [156]. If hypocalcemia or hypokalemia is present, magnesium should be replaced first. Repletion is best achieved with slow, continuous infusions to avoid rapid increases in urinary excretion. Replacement should be monitored closely with serial serum concentrations and evaluation of deep tendon reflexes. Hypermagnesemia (> 3.0 mg/dL) should be avoided because vasodilation, nausea, vomiting, drowsiness, lethargy, hypotension, bradycardia, depressed deep tendon reflexes, and a prolonged P–R interval can develop.

Patients with mild deficiency or ongoing magnesium losses should receive foods rich in magnesium or an oral supplement. A variety of magnesium salts are available, but many of them, especially the oxide and hydroxide, are virtually insoluble (Table 101.11). Because magnesium is poorly absorbed, it is best to give it in frequent small doses. Potassium-sparing diuretics such as spironolactone decrease urinary magnesium losses and may be useful, especially in patients who are also hypokalemic. However, some patients will require parenteral supplements. Outpatients unresponsive to oral therapy can be treated with intramuscular injections of 2.0 mL of 50% magnesium sulfate solution (8.1 mEq) in each buttock at intervals as needed.

Table 101.11 Magnesium products comparison chart

Product	Dosage forms[a]	Magnesium content (mEq/g)[b]	Comments
Magnesium carbonate	Tablet, 250 mg	23.7	Poorly soluble, low absorption
Magnesium, chelated	Tablet, 500 mg	8.2	Amino acid chelate; sodium-free, oral use only
Magnesium chloride	Solution, 200 mg/mL	9.8	Used i.v. or orally as a 5% solution; alternative to parenteral $MgSO_4$
	Enteric-coated tablet, 535 mg		Sold as Slo-Mag
Magnesium citrate	Solution, 60 mg/mL	4.4	Oral use only
Magnesium gluconate	Tablet, 500 mg, 1000 mg/5 mL	4.8	Very soluble, well absorbed
Magnesium hydroxide	Suspension, 40–80 mg/mL	34.0	Readily available in combination antacid formulations; start with 5-mL suspension or 1 tablet, increase as tolerated to four times daily; may require gastric acid to be absorbed; inexpensive
	Tablet, 600 mg		
Magnesium lactate	Sustained-release tablet, 700 mg	9.8	Sold as Mag-Tab SR
Magnesium oxide	Capsule, 140 mg Tablet, 250 mg, 400 mg, 420 mg, 500 mg	49.6	Poorly soluble; net absorption low, especially in malabsorption
Magnesium sulfate	Solution, 10%, 12.5%, 50%	8.1	Use i.v., i.m., or p.o.

a Magnesium products exhibit variable absorption; increase dose incrementally until no further rise in serum magnesium occurs or until diarrhea ensues. Oral magnesium alleviates diarrhea in some patients with malabsorption.
b 1 mEq = 12 mg = 0.5 mmol Mg.
i.m., intramuscularly; i.v., intravenously; p.o., orally.
Adapted from Knoben JE, Anderson PO. Handbook of Clinical Drug Data, 7th edn. Hamilton, IL: Drug Intelligence Publications, 1993:657; Olin BR (ed.) Facts and Comparisons. St. Louis: Facts and Comparisons, 1992;14a; and Fink EB. Magnesium deficiency: etiology and clinical spectrum. Acta Med Scand Suppl 1981;647:125.

Phosphorus

Deficiency

Most patients in whom clinical evidence of hypophosphatemia develops have an underlying wasting condition [157,158], such as malabsorption, malnutrition of any cause, cancer, or chronic alcoholism. Causes of hypophosphatemia include increased urinary losses, shifts of serum phosphorus into the intracellular compartment, and reduced absorption. Absorption is decreased with the use of phosphate-binding antacids, vitamin D deficiency, and parathyroid hormone deficiency. Renal loss is increased in metabolic alkalosis, diuretic therapy, renal tubular dysfunction, osmotic diuresis, hyperparathyroidism, and long-term corticosteroid therapy [156]. When carbohydrate is administered or when phosphate-rich lean tissue is restored and insufficient phosphorus is available, serum levels can fall [156]. Signs and symptoms of hypophosphatemia include hemolytic anemia (serum phosphorus concentration < 0.5 mg/dL), rhabdomyolysis (< 1.0 mg/dL), and other complications that occur with lesser degrees of depletion, including platelet dysfunction, metabolic acidosis, peripheral neuropathy, central nervous system dysfunction, metabolic encephalopathy, and cardiac failure. Most cases of hypophosphatemia appear to be associated with intercompartmental shifts of phosphorus rather than with decreases in the total body pool, although this distinction is not easy to make.

Assessment

The normal serum phosphorus concentration is 2.5–4.5 mg/dL. Levels lower than 1.0 mg/dL usually cause symptoms [156,157].

Treatment

If hypophosphatemia is the result of therapy with phosphate-binding medications (antacids, sucralfate), stopping the medication is sufficient. If oral or enteral intake is possible, supplementation for other causes of a serum phosphate level below 2.5 mg/dL can be provided with milk (1000 mg or 32 mmol/quart (0.9 L) of skim milk) or with other oral preparations in doses of 2–3 g/day (e.g., Neutra-Phos K or Uro-KP Neutral with 250 mg or 8.0 mmol per capsule or dose of powder). Most of the oral preparations, however, cause diarrhea (secondary to lactose intolerance in the case of milk and the phosphate content in the others), which may limit their use in patients with malabsorption. Intravenous supplementation should be limited to those who cannot take oral preparations or who have severe hypophosphatemia (< 1.0 mg/dL). The exact amount needed to restore serum phosphorus cannot be predicted because severe hypophosphatemia may occur with normal body stores of phosphorus. The dose of intravenous phosphate can be adjusted to serum phosphorus levels and to body weight (Table 101.12). When hypocalcemia or renal failure is present, great caution should be used in administering phosphate by any route, but especially intravenously. Too rapid administration can cause calcium phosphate precipitation in renal tubules and other tissues.

Table 101.12 Intravenous phosphorus repletion protocol according to body weight

	Single phosphorus dose (mmol)		
	Body weight (kg)		
Serum [P] (mg/dL)	40–60	61–80	81–120
< 1.0	30	40	50
1.0–1.7	20	30	40
1.8–2.2	10	15	20

A single dose restores serum [P] to normal within 24 h in 76% of patients.
From Taylor BE, Huey WY, Buchman TG, et al. Treatment of hypophosphatemia using a protocol based on patient weight and serum phosphorus levels in a surgical intensive care unit. J Am Coll Surg 2004;198:198.

Iron

Deficiency [158,159]

For clinicians, the sequelae of the last stage of iron deficiency, when stores are depleted and anemia occurs, are equated with iron deficiency. However, the role of delivering oxygen to the tissues is dependent not only on hemoglobin concentration but also on the ability of iron-containing proteins in mitochondria to process the oxygen. Such changes may account for the symptoms more prevalent in children (i.e., anorexia, decreased resistance to infection, and reversible protein-losing enteropathy) and for other features not easily explained by anemia, such as angular stomatitis, atrophic lingual papillae, koilonychia, and behavioral or cognitive changes [160]. Cognitive changes have been correlated with low hemoglobin levels in dieting adults, although it is not clear whether the iron was causative [161]. When anemia develops, weakness and pallor predominate. Iron supplements can decrease fatigue in women who are not anemic (mean ferritin concentration 10.5 μg/L), showing that iron deficiency can be recognized and treated preclinically [162].

Assessment

The hemoglobin and hematocrit are the initial and best screening tests [163], but the results should be interpreted with caution. The patient's age and gender have to be considered in assessing the iron status of any individual. Moreover, hemoglobin concentrations can be altered by dehydration or overhydration, chronic inflammation or infection, protein–calorie malnutrition, cobalamin or folate deficiency, hemoglobinopathies, and pregnancy. Thus, hemoglobin is at best an indirect index of the iron status. The ferritin levels in serum are directly proportional to marrow iron stores and

inversely proportional to transferrin levels, with each milligram per liter representing 10 mg of storage iron. Low levels of serum ferritin (< 15 ng/mL) in the presence of anemia nearly always reflect decreased iron stores, and values up to 35 ng/mL are suggestive [1]. However, ferritin levels can be elevated in cases of acute and chronic inflammation, cobalamin or folate deficiency, leukemia or lymphoma or other tumors, alcohol intake, and hyperthyroidism. Thus, values between 15 and 100 ng/mL can be seen in patients with iron deficiency. Serum iron levels alone are not a good measure of the iron status because they do not reflect a stable body pool. About 35 mg is turned over in the plasma each day, and levels can change rapidly in response to acute inflammation. In iron deficiency, serum transferrin levels rise whereas iron levels fall, so that the percentage of saturation falls below 15%. When chronic inflammation, infection, or liver disease is present, serum transferrin levels fall. Transferrin synthesis is downregulated by iron status at the level of translation, so that transferrin levels are elevated in iron deficiency. Thus, a low percentage of saturation in the presence of low transferrin levels does not reflect iron deficiency but rather the anemia of chronic inflammation. Table 101.13 outlines the predictive value of the usual tests in iron deficiency compared with anemia of chronic inflammation [163].

How to proceed when ferritin values are above 35 ng/mL is not clear, and transferrin receptors have been suggested as a possible aid [164]. The amount of uptake of transferrin into cells is regulated by alterations in the number of soluble transferrin receptors (sTfR) on the surface of cells and is most dependent on marrow erythropoiesis activity [164], although intracellular concentration of iron is also important. The highest concentration of transferrin receptors is on cells with the greatest need for iron, such as reticulocytes. A truncated form of the receptor is found in human plasma bound to transferrin, and its content is elevated in iron deficiency, even if mild and of recent onset. The levels of transferrin receptor are not affected by inflammation or liver disease, because cytokines and other factors suppress erythropoiesis through inhibition of erythropoietin production. Thus, the levels of transferrin receptor can be used to distinguish iron deficiency from the anemia of chronic disease. Normal sTfR levels average 5.0 ± 1.0 mg/L, but the lack of an international standard makes comparison of laboratory values difficult. Receptor levels are also increased when erythropoiesis is stimulated by hemolysis or ineffective erythropoiesis.

Table 101.13 Serum tests that differentiate anemia of chronic inflammation (ACI) from iron deficiency anemia (FeD)

Serum test	ACI	FeD	Both
Iron	↓	↓	↓
Transferrin	↓ or NL	↑	↓
Transferrin saturation	↓	↓	↓
Ferritin	↑	↓	↓ to NL
Soluble transferrin receptor (sTfR)	↓	↑	NL to ↑
sTfR/log ferritin	< 1	> 2	> 2
Cytokine levels	↑	NL	↑

NL, normal.
Adapted from Weiss & Goodnough [163].

Treatment

Oral iron is available in a wide variety of preparations as a single nutrient or in combination with other vitamins and minerals. The amount used is not important so long as it is adequate to replenish body stores, with an assumption of 20% absorption at the start of treatment for iron deficiency. The approximate percentages of the preparations as elemental iron are as follows: sulfate anhydrous, 30%; sulfate $7H_2O$ hydrated, 20%; fumarate, 33%; and gluconate, 11.6%. The dose should be calculated based on the elemental iron content. A standard dosing schedule is ferrous sulfate (65 mg of elemental iron) two to three times a day between meals. Side effects include nausea, indigestion, diarrhea, and abdominal cramping and can limit the amount that can be ingested. Side effects may be less common when slow-release forms are used, but the iron may be made less available by delaying release to beyond the duodenum, the site of maximal absorption. The total iron deficit can be determined roughly by calculating the amount needed to replenish hemoglobin, with an additional gram for repleting tissue stores: iron deficit (mg) in males = body weight (lb) × (15 – hemoglobin [g/dL]) + 1000. A hemoglobin value of 12 or 13 g/dL can be used for females, 14 or 15 g/dL for males. If 10% absorption throughout therapy is assumed, the total dose required can be calculated. Once the daily tolerable supplement is determined for a patient, the length of treatment necessary to replete the stores can be calculated. Oral preparations should be continued for 1–3 months after the hemoglobin level is restored to allow for restoration of other tissue stores. Parenteral iron is used only when oral iron is not tolerated or effective. Iron dextran (50 mg/mL) is available for intramuscular or intravenous use. The total dose required to replete stores is based on the patient's weight and hemoglobin level:

$$\text{Total iron (mg)} = 0.3 \times \text{body weight (lb)} \times 100 - \frac{\text{hemoglobin (g/dL)} \times 100}{14.8}$$

The intravenous route is safe when the iron is diluted in normal saline solution and administered over 2–3 h [165] (Table 101.14). A test dose of 25 mg of iron dextran should be given initially, and the daily dose should not exceed 100 mg if one wishes to minimize the risk for side effects. A test dose is not required for the other parenteral iron preparations. However, medium-dose (100–400 mg) or high-dose (500–

Table 101.14 Parenteral iron therapy

Parameter	Iron dextran	Sodium ferric gluconate	Iron sucrose
Product	INFeD (100 mg/2 mL) DexFerrum (100 mg/2 mL) Desferal (500 mg/vial)	Ferrlecit (62.5 mg/5 mL)	Venofer (100 mg/5 mL)
Test dose	Required	Not required	Not required
Recommended dose	100 mg/day to repletion	125 mg/dialysis session × 8 doses May repeat p.r.n. for ferritin < 100 ng/mL and Tf saturation < 26%	100 mg 1–3 times/week × 10 doses – repeat p.r.n.
Maximal dose	May give to full replacement	10 mL (25 mg)	5 mL (100 mg)
Method	Diluted in 250 mL–1 L over 4–6 h	Undiluted 12.5 mg/min Diluted in 100 mL NS over 60 min	Undiluted up to 20 mg/min, or diluted in 100 mL NS over > 15 min
TPN compatible	Nonlipid solutions	Not studied	Not studied
Limitations	Stop oral iron Do not exceed calculated dose	↓ Dose by 50% for ferritin > 100 ng/mL, or Tf saturation > 35% Hold dose for ferritin > 500 ng/mL or Tf saturation > 50%	Same as Fe gluconate

NS, normal saline; p.r.n., as required; Tf, transferrin; TPN, total parenteral nutrition.
Adapted from Kumpf [165] and Aronoff [167].

1000 mg up to full replacement) regimens of iron dextran can be used safely and more conveniently, provided adequate precautions are taken and the patient is observed during the infusion [165]. Anaphylaxis is extremely rare when iron dextran is given in this way. However, the patient should be closely supervised and parenteral iron given with caution to anyone with asthma or allergy. Epinephrine (adrenaline) should be available for acute hypersensitivity reactions, and antihistaminics can be given for symptoms of urticaria, rashes, sweating, dizziness, headache, nausea, and fever. Ferric gluconate is the preparation preferred by some experts [166]. All parenteral iron preparations are fairly safe, with the incidence of serious adverse events ranging from 0.4% to 2.5% [167]. Most patients with Crohn's disease who require parenteral iron will respond, but erythropoietin can enhance the response [168].

Zinc

Deficiency

Zinc deficiency is difficult to identify, because plasma zinc is an insensitive predictor of zinc status; thus, deficiency is probably underdiagnosed. Overt deficiency in otherwise healthy individuals is not reported in the United States, but the growth of children can be limited by inadequate dietary zinc [169]. Patients with gastrointestinal disease (Crohn's disease, short bowel syndrome), cystic fibrosis, pancreatic insufficiency, or chronic diarrhea from any cause are at increased risk for zinc deficiency because of increased losses in the stool and decreased intake [170]. Diarrheal stool contains about 17 mEq of zinc per liter, in excess of the recommended dietary allowance (RDA). Other conditions predisposing to zinc deficiency include cirrhosis, sickle cell anemia, pregnancy, pica, and penicillamine treatment [171]. Clinical manifestations include growth retardation (in children), skin lesions on the face and limbs (which can vary from moist and pustular to seborrheic and acneiform), alopecia, diarrhea, apathy, night blindness, and possibly poor wound healing and loss of taste. However, the single double-blinded study of the effect of zinc supplements on taste and smell dysfunction did not support a role for zinc [171].

Assessment

None of the available methods reliably reflects either recent intake or absorption or body stores. The fasting plasma concentration is most often used, but it correlates poorly with total body zinc except when the patient is severely deficient. Most zinc in blood is in red cells, so that even small amounts of hemolysis can alter plasma zinc. Most plasma zinc is bound tightly to α_2-macroglobulins or loosely to albumin. Thus, hypoproteinemia can lower zinc levels. The plasma level may be falsely low because it can be decreased by many other factors, such as stress, infection, polypharmacy (displacing

zinc from albumin), and corticosteroids [1,138]. Levels below 50 μg/mL are often associated with some symptoms, and patients with levels below 30 μg/mL nearly always have some manifestation of deficiency. Metallothionein is a zinc/copper-binding protein that is reduced in zinc deficiency, and its red cell content has been low when zinc plasma levels were normal, perhaps reflecting a functional zinc pool [172]. At present, however, if zinc deficiency is suspected, it is best diagnosed by the symptomatic response to zinc therapy.

Treatment

Zinc is available as a single nutrient and as a component of many multivitamin and mineral preparations. Zinc supplementation should be provided to persons at risk for the development of deficiency, as well as to those with symptomatic deficiency [173]. Zinc sulfate (67 mg or 220 mg) contains 15 or 50 mg of elemental zinc. Thus, unlike a single dose of other minerals, a single dose of zinc exceeds the RDA, and oral therapy is usually sufficient although absorption is only about 20%. Parenteral zinc therapy is also available as the sulfate or chloride. Zinc is safe to use; the minimum oral toxic dose is 500 mg/day [1]. Long-term ingestion of high doses (450 g/day) can induce copper deficiency [174]. Zinc supplements have reduced the incidence, prevalence, duration, and severity of diarrhea and pneumonia in children from developing nations, presumably because the availability of dietary zinc in such regions is limited [175]. Because of its effect in diarrhea of children, zinc has been added to some oral rehydration solutions. Zinc does not appear to help in treatment of the common cold [176]. Supplemental zinc decreases measures of iron status in patients with low iron reserves [177]. Although zinc is not usually considered a toxic metal, a familial syndrome of hyperzincemia is associated with recurrent infections, hepatosplenomegaly, inflammatory skin lesions, arthritis, and anemia [178].

Copper

Deficiency

Copper deficiency in adults is rare. It can occur in patients receiving TPN without copper supplementation and in patients treated with chelating agents, such as D-penicillamine, with or without large doses of zinc [179]. Premature infants, malnourished or malabsorbing patients, and persons with Menkes syndrome, an inherited defect in copper absorption, are at higher risk for the development of deficiency [180]. Neutropenia, anemia, diarrhea, and scurvy-like bone changes can occur, but detection of early deficiency can be difficult [181]. A clinical myelopathy similar to subacute combined degeneration is being increasingly reported with severe copper deficiency [182]. Copper supplementation should also be considered for patients with increased gastrointestinal fluid losses, especially from chronic biliary tract fistulae, because biliary secretion is the major excretory route for copper [179].

Assessment

Laboratory assessment of copper status is difficult because serum concentrations do not correlate well with tissue levels. Because 80% of copper is bound to ceruloplasmin (each milligram binds 3.3 μg of copper) and the rest to other proteins, the level does not correlate with intake [183]. However, tissue copper levels are relatively stable and change slowly. Thus, when deficiency occurs, the plasma level often falls earlier than the tissue levels. Normal levels are increased by estrogens or oral contraceptives, pregnancy, and acute and chronic infections. Levels are decreased in nephrosis, Wilson disease, and any cause of protein malnutrition. Free copper levels are more instructive than total levels and can be estimated by calculating bound copper (bound copper = ceruloplasmin [μg/dL] × 3) and subtracting it from the total. Values below 25 μg/dL are normal [184]. This calculation is more sensitive in detecting elevated copper levels (as in Wilson disease) than in detecting copper deficiency.

Treatment

Oral supplementation can be provided by the copper sulfate in multivitamin and mineral preparations, which contain 0.4 mg of elemental copper per milligram of copper sulfate. The daily dose of 2–3 mg is adequate to treat deficiency in adults. The suggested maintenance intravenous dose is 0.3–1.5 mg/day, but the dose should be reduced to 0.15 mg/day for patients with cholestasis [185]. To treat deficiency, 2 to 3 mg/day has been given intravenously, repeated thrice weekly until the ceruloplasmin level normalizes, usually in about 10 days. There is a large margin of safety in using copper supplements, but acute oral ingestion of more than 15 mg of elemental copper produces nausea, vomiting, diarrhea, abdominal cramps, and mucosal ulceration. At larger doses, hemolysis, gastrointestinal bleeding, azotemia, and jaundice can occur. The treatment of toxicity includes gastric lavage and 1 g of D-penicillamine per day [186].

Thiamin (vitamin B-1)

Deficiency

Deficiency of thiamin may be caused by decreased intake or increased tissue utilization, or by a combination of factors. Thus, the usual clinical settings include pregnancy, chronic alcoholism, malabsorption syndromes, chronic nausea and vomiting, prolonged febrile illnesses, and chronic renal dialysis. The total requirement is usually more than 1 mg/day in adults, and the total stores are only about 30 mg, so deficiency can develop fairly rapidly. In parts of the world where polished rice is the staple cereal in the diet, beriberi (weakness, paresthesias, high-output cardiac failure) is seen. In the United States, where food thiamin is abundant, the deficiency syndrome is often seen in conjunction with deficiency of other B vitamins. Symptoms and signs include peripheral neuropathy, limb weakness, cerebellar dysfunction, subacute necrotizing encephalomyelopathy, and Wernicke encephalopathy

(apathy, confusion, ataxia, photophobia, nystagmus, paralysis of upward gaze). Signs of deficiency may worsen if glucose is given without thiamin. If lactic acidosis is also present, signs of heart failure may develop [187]. Thiamin deficiency should be considered in the differential diagnosis of lactic acidosis.

Assessment

Recent intake or absorption, but not body stores, is correlated with urinary excretion. The amount excreted is greater during childhood, when growth is rapid, so the result (micrograms of thiamin per gram of creatinine) must be compared with the values in age-matched controls. Body stores are measured either directly in blood or serum by high-performance liquid chromatography or by the erythrocyte transketolase assay, which measures a thiamin-dependent enzyme activity. The activity coefficient of the enzyme is determined by the activity in the presence and absence of added thiamin. In deficiency states, more stimulation occurs, and the activity coefficient is above 1.25 [138].

Treatment

Thiamin hydrochloride is available as a single nutrient in oral and parenteral forms and as a constituent of almost all multivitamin preparations. Adults with mild deficiency should receive 10–20 mg intramuscularly or 25–50 mg orally twice daily for 1 week, followed by an oral maintenance dose of 2–5 mg/day. Benfotiamine (*S*-benzoylthiamine-*O*-monophosphate) is a lipid-soluble thiamin analogue that achieves plasma levels five times that of comparable doses of thiamin [188]. There is no clinical correlation with these higher plasma levels. Critically ill patients, especially those with central nervous system manifestations of deficiency, should receive doses of 50–100 mg twice daily intravenously for 3 days, followed by oral supplementation of 5–30 mg/day until a normal diet is resumed. The thiamin should be given before any carbohydrate is administered to avoid enhancing the deficiency. Because thiamin deficiency is often associated with deficiencies of other B vitamins, multiple vitamins should be given. Rare responses to parenteral thiamin include feelings of warmth, tingling, pruritus, nausea, sweating, and anaphylactic reaction [189]. It has been suggested that thiamin improves energy levels during exercise and in the elderly, and cognition in Alzheimer disease, but the few studies performed do not support these conclusions [190].

Riboflavin (vitamin B-2)

Deficiency

Riboflavin deficiency usually occurs along with deficiencies of other B vitamins, especially in patients with malabsorption and chronic alcoholism. Early symptoms relate to oral or eye lesions. Angular stomatitis (maceration and fissuring of the mucocutaneous junction at the angles of the mouth), cheilosis (inflammation of the lips), glossitis, geographic tongue, seborrhea-like dermatitis, pruritus, photophobia, corneal vascularization, and visual impairment may occur. The differential diagnosis of the lip lesions includes poorly fitting dentures with malocclusion, allergy (e.g., lipstick, toothpaste), and iron deficiency anemia. Other vitamin B deficiencies can cause the tongue lesions. Some of these symptoms occur in vitamin B-6 deficiency because the oxidase necessary to produce functional vitamin B-6 is riboflavin-dependent [191].

Assessment

Urinary excretion correlates well with intake because the vitamin is excreted unmetabolized. With fasting or prolonged bed rest, urinary excretion can be falsely elevated. Levels below 40 μg/g of creatinine signify extremely low intake in an adult. Body stores of riboflavin are assessed by measuring the riboflavin-dependent enzyme erythrocyte glutathione reductase in the blood. An activity coefficient above 1.2 (stimulated/control activity) signifies deficiency [138].

Treatment

Deficiency can be treated with 5–10 mg/day orally, along with other vitamins [192]. When malabsorption is present, a prophylactic dose of 3 mg/day is recommended, usually as part of a multivitamin preparation. Riboflavin may cause a yellow-orange appearance to the urine but is remarkably nontoxic.

Niacin (vitamin B-3)

Deficiency

Because of the niacin supplementation of grains and breads, the classical deficiency syndrome of pellagra (dermatitis, dementia, diarrhea) is rare in the United States. Deficiency is seen most often in patients on a diet of meat, cornmeal, and molasses, with chronic alcoholism, malabsorption syndromes, or carcinoid syndrome, in which a large amount of tryptophan (niacin precursor) is converted instead to serotonin. Pellagra rarely occurs in the carcinoid syndrome, and when it does it can be overcome with oral niacin. Hartnup disease is associated with a defect in tryptophan absorption and can also predispose to deficiency [193]. Isoniazid therapy can lead to deficiency because hydrazines form adducts with pyridoxal phosphate. The last enzyme in the conversion of tryptophan to nicotinic acid, kynureninase, requires pyridoxal, thereby linking the deficiency of these two vitamins. Moreover, isoniazid resembles nicotinic acid and acts as an inhibitor. In niacin deficiency, the dermatitis occurs over exposed areas and is scaly. Twenty percent of patients get dyssebacea, referring to plugs of inspissated sebum projecting from deleted orifices of sebaceous glands, appearing first along the alae nasi, spreading over the nose, and then the forehead, lips, and chin. It is not inflammatory, and is unrelated to sunlight, unlike the dermatitis, and can precede the dermatitis by weeks. Painful tongue and angular stomatitis are seen but may be caused in part by accompanying riboflavin deficiency. Diarrhea is probably caused by the direct

effect of niacin deficiency on epithelial cell function. Central nervous system dysfunction includes irritability, headache, insomnia, psychosis, hallucinations, and seizures.

Assessment

The urine content of the niacin metabolite *N*-methylnicotinamide reflects intake and absorption and is less than 0.5 mg/g of creatinine in adults with deficient intake. No test is available for body stores of niacin, although the results of one study in young men suggested that an erythrocyte ratio of nicotinamide adenine dinucleotide (NAD) to nicotinamide adenine dinucleotide phosphate (NADP) below 1.0 might reflect deficiency [138].

Treatment

Isolated niacin deficiency is unusual in the United States, so treatment with other B vitamins often accompanies niacin replacement. Depending on their severity, the symptoms of pellagra respond to oral doses of 100–500 mg of niacin per day [193]. Mental, gastrointestinal, and oral symptoms clear rapidly, but the resolution of skin lesions may require weeks to months. Large doses of nicotinic acid (1–3 g/day) used to treat hypercholesterolemia can cause flushing, pruritus, burning sensations, nausea, vomiting, heartburn, diarrhea, dizziness, and tachycardia. Hyperglycemia and increased serum aminotransferase and bilirubin levels can occur at doses as low as 750 mg of nicotinic acid per day [194].

Pyridoxine (vitamin B-6)

Deficiency

Because vitamin B-6 is abundant in foods and is produced by colonic bacteria, dietary deficiency is rare. It is most often reported as a result of treatment with pyridoxine antagonists, especially isoniazid, hydralazine, oral contraceptives, dopamine, and D-penicillamine. Rarely, deficiency occurs in chronic alcoholism or malabsorption, usually in association with deficiencies of other B vitamins. The clinical manifestations include peripheral neuropathy, seborrheic dermatitis around the eyes and nasolabial folds, and oral lesions (angular stomatitis, cheilosis, glossitis), similar to those seen in riboflavin and niacin deficiencies. Seizures and sideroblastic anemia may also occur [195]. Hyperhomocystinuria has been identified as an independent risk factor for vascular disease and may be caused in part by a deficiency of cystathionine synthase, the vitamin B-6-dependent enzyme that catalyzes the conversion of homocysteine to cystathionine. Twelve large-scale randomized studies have examined the effect of B-vitamin supplementation on cardiovascular risk, and shown that homocysteine levels can be normalized, but any effect on clinical outcomes is still uncertain [196]. It is premature to recommend the use of these supplements to alter atherogenesis in this subset of patients with hyperhomocystinemia [197]. Low vitamin B-6 levels have been associated with an increased risk for first venous thrombosis in a single study [198], so it is not inconceivable that vitamin supplementation might carry some risk on its own.

Assessment

Recent intake is reflected by the urinary excretion of metabolites. A low intake correlates with excretion of less than 500 μg of 4-pyridoxic acid per day. Body stores are measured by the erythrocyte aminotransferase index, with a determination of enzyme activity in the presence and absence of pyridoxal phosphate. Deficiency is correlated with an erythrocyte aspartate aminotransferase (E-AST) index above 2.2. Alternatively, pyridoxal phosphate can be measured in whole blood (50–120 nmol/L) or plasma (> 30 nmol/L), although the levels associated with deficiency have not been precisely determined [138].

Treatment

Pyridoxal phosphate is available in both oral and parenteral forms. Suspected deficiency should be treated with 50–150 mg/day orally, especially when neuropathy is present. A multiple vitamin preparation containing 2–5 mg of pyridoxine should be added to provide the other B vitamins. Some experts suggest such a supplement for all patients on isoniazid and similar inhibitors, whereas others suggest supplements only for those patients at high risk for the development of neuropathy. Pyridoxine is used in large doses for isoniazid overdose, *Gyromitra* (monomethylhydrazine) mushroom poisoning, and hydrazine overexposure [199]. When pyridoxal phosphate is ingested in large amounts (2–6 g/day), a peripheral neuropathy may develop [200]. Pyridoxine antagonizes levodopa by stimulating the decarboxylation of dopa to dopamine. Therefore, patients taking levodopa should limit their pyridoxine intake to less than 5 mg/day [201].

Folate

Deficiency

Folate deficiency is associated with poor socioeconomic and dietary conditions, especially with chronic alcoholism (usually > 80 g/day) and malabsorption syndromes. Alcohol can increase urinary folate excretion [202]. Deficiency also results from competition for absorption (sulfasalazine), long-term anticonvulsant therapy (usually years), increased utilization (pregnancy, hemolytic anemia, leukemia, chronic myelofibrosis), and inhibition of dihydrofolate reductase (the enzyme that converts folate to the active coenzyme) by methotrexate, trimethoprim, and pyrimethamine [203]. A cerebral folate deficiency syndrome can be produced by folate receptor antibodies in infants, older children, and occasionally in adults, resulting from blocked folate transport into the brain [204]. Serum and erythrocyte folate levels and serum homocysteine are normal, but 5-methyltetrahydrofolate concentration in the cerebrospinal fluid is low and accounts for the developmental and neurological abnormal-

ities. If folate stores are normal initially, symptoms will appear in about 4 months. The primary manifestation of classical folate deficiency is macrocytic anemia, often accompanied by thrombocytopenia and leukopenia. Other symptoms may include glossitis, diarrhea, fatigue, and possibly (but uncommonly) neurological signs.

Two other significant forms of folate deficiency occur in the absence of classical evidence of deficiency. Women consuming too little folic acid during early pregnancy are at increased risk for delivering children with neural tube defects. The Dietary Reference Intake Committee has confirmed the recommendation of the US Department of Health and Human Services that all women capable of becoming pregnant ingest 400 μg of synthetic folic acid daily [203]. Hyperhomocystinemia can be caused by folate deficiency if it is not accompanied by elevated methylmalonic acid levels. High levels of homocysteine are correlated with an increased risk for myocardial infarction in some studies, in about 10% of patients [197]. Although the folate levels are normal, the hyperhomocystinemia can be corrected by supplemental folate, but it is not clear that the risk for heart disease is altered. The epidemiological data implicating homocysteine as a factor in cardiovascular risk are strong, but data from some prospective interventional studies have been less consistent [197,205]. Although a low folate status is a strong determinant of elevated total homocysteine levels, the folate status was not associated with homocysteine levels [143], nor with an increased risk for coronary atherosclerosis [206]. These data are consistent with overrating of homocysteine as a risk factor for heart disease. Similarly, the importance of homocysteine and folate levels as risk factors for stroke is probably small [207].

Assessment

Because folate deficiency develops during decreased intake or absorption, the first laboratory abnormality to develop is a decrease in the serum folate (2–6 ng/mL), which is sensitive to changes in intake [138]. Values below 2 ng/mL are usually associated with megaloblastic anemia and decreased tissue reserves. Because about 50% of serum folate is bound to albumin, hypoalbuminemia can produce falsely low serum folate levels. Body stores of folate can be measured by red cell folate more accurately than by serum folate. Both red and white cells contain much more folate than serum does, so hemolysis or a leukemoid reaction can falsely elevate the serum folate. When both serum folate (< 2 ng/mL) and red cell folate (< 140 ng/mL) are low, folate deficiency is the cause. When cobalamin deficiency is present, folate may not be well utilized in 15%–25% of cases, so serum folate rises and red cell folate falls. Multilobed polymorphonuclear cells and macrocytic anemia develop after tissue folate levels fall [138]. Serum homocysteine levels above 30 μmol/L are consistent with folate deficiency if the methylmalonic acid levels are normal.

Treatment

Oral folate supplements are available in tablets up to 1 mg, but the unreduced pteroylglutamic acid is the form used. Most of this form is excreted unchanged or after degradation, so the dose of this supplement cannot be compared with the true folate requirements. Folinic acid (5-formyltetrahydrofolate) is needed for treating the cerebral folate syndrome. The parenteral route is used only if oral therapy is not possible or malabsorption is severe. A reticulocyte response is seen in 3–5 days, and a peak response occurs in 5–10 days. The 400-μg dose found in multivitamin preparations is usually adequate as maintenance therapy for patients with malabsorption or alcoholism. For patients on sulfasalazine or with hemolytic anemia, a dose of 1 mg/day is suggested. Folate can lower plasma homocysteine, but the data do not yet support a preventive role for folate in cardiovascular disease, stroke, or other chronic diseases [205–208]. Betaine (trimethylglycine) is a methyl donor, approved by the US Food and Drug Administration (FDA) for the treatment of homocystinuria, that does lower plasma homocysteine, but not more so than folate [209]. Foltx (folic acid 2.5 mg, cyanocobalamin 1 mg, and pyridoxine 25 mg) is an over-the-counter preparation promoted for hyperhomocysteinemia, but its benefit compared with existing preparations is unknown. Folate is remarkably nontoxic. Large doses (> 100 times the RDA) can precipitate convulsions in patients treated with phenytoin [210]. Giving folate to a patient with cobalamin deficiency corrects the anemia but does not prevent irreversible neurological damage. The tolerable upper intake level (UL) for folate of 1000 μg/day has been challenged because all but eight cases of masking neurological progression (not just worsening anemia) in vitamin B-12 deficiency occurred in patients taking more than 5 mg of folate per day [211].

Cobalamin (vitamin B-12)

Deficiency

A dietary deficiency of cobalamin occurs only in lacto-ovo-vegetarians who do not consume any food of animal origin. The most common causes in adults are gastric lesions (especially the atrophic gastritis associated with pernicious anemia or gastrectomy), lesions or resection of the terminal ileum (especially in Crohn's disease), and bacterial overgrowth. Gastric pathology accounts for well over half the cases. An increasingly common cause of low serum cobalamin levels is AIDS. The significance of the low levels is not always clear, but some patients manifest subtle alterations in mental and cognitive abilities [212]. Reversible chorea and dystonia have been reported as a result of cobalamin deficiency [213]. Deficiency also occurs rarely in patients with severe chronic pancreatitis, congenital deficiency of the carrier protein transcobalamin II (TCII), or an inability to utilize food-bound cobalamin in the absence of other gastric pathology [214]. Symptoms are insidious and develop over 2–3 years, sooner if malabsorption is the cause. A sore

tongue, paresthesias, anorexia, loss of taste, diarrhea, dyspepsia, hair loss, impotence, irritability, and psychiatric illness (e.g., depression) can be present. Numbness and tingling, especially in the lower extremities, can progress to loss of vibratory sensation, loss of coordination, muscle weakness, and atrophy, and memory disturbances can develop [215]. Macrocytic anemia with megaloblastic bone marrow and multilobed nuclei in the polymorphonuclear leukocytes is frequently, but not always, present. The macrocytic anemia must be differentiated from chronic liver disease and from hypothyroidism. Many patients, especially those who are elderly, present with neurological symptoms and signs in the absence of anemia assessment.

Assessment

The diagnosis of cobalamin deficiency is usually suspected on the basis of hematological findings [216]. The serum concentration of cobalamin usually correlates with body stores. Unlike the folate concentration, the cobalamin concentration in red cells is no higher than that in serum, so hemolysis is not a cause of falsely elevated values. In humans, only a small proportion of cobalamin is carried on TCII, the protein that delivers the vitamin to the tissues. Thus, there can be a stage at which the serum level is normal (cobalamin is bound to haptocorrin) but tissue levels are low. Falsely high levels can be seen in leukocytosis (release of haptocorrin, increasing the total serum binding capacity) and in acute liver disease (release of body stores). Conversely, serum levels can be low when body stores are normal. Protein deficiency can lower serum levels without affecting tissue delivery if the TCII levels are normal. Levels are low in pregnant women because of dilution and redistribution of binding proteins. Serum cobalamin levels below 150 pg/mL (110 pmol/mL) are always associated with deficiency if causes of falsely low levels are not present (folate or protein deficiency, pregnancy). Levels between 150 and 200 pg/mL should be considered suspect and should lead to further testing with methylmalonic acid and homocysteine levels, correlation with abnormal hematological or neurological findings, or both. A subtle presentation of cobalamin deficiency is especially likely in elderly patients [217]. In doubtful cases, a therapeutic trial with cobalamin is safe, and reversal of the abnormal findings is diagnostic.

Biochemical tests for cobalamin deficiency can clarify cases in which the serum cobalamin levels combined with the clinical picture are not definitive. The workup should also include a careful neurological examination. The serum can be assayed for metabolites that increase when the function of the two cobalamin-dependent enzymes is impaired: methylmalonyl-CoA mutase (methylmalonic acid increased) and methionine synthase (homocysteine increased). Folate deficiency leads to an increase in homocysteine levels (> 20 µmol/L) but not in methylmalonic acid levels, whereas cobalamin deficiency increases methylmalonic acid (> 390 nmol/L) as well [218]. Methylmalonic acid levels can be elevated in the absence of cobalamin deficiency when renal insufficiency is present. Because TCII is the protein that delivers cobalamin to tissues but accounts for less than 10% of serum cobalamin binding, low holo-TCII concentrations have been reported as the earliest sign of negative cobalamin balance [89]. This test is not used routinely. The selection of the various tests for cobalamin deficiency depends on the stage of deficiency suspected (Table 101.15). Early sensory neuropathy can be linked to cobalamin deficiency by evidence of myelopathy obtained by spiral magnetic resonance imaging (MRI) [219].

Because abnormal serum measurements of cobalamin metabolism precede the late manifestations of tissue damage, it is recommended that populations at risk are screened.

Table 101.15 Laboratory tests in sequential stages of cobalamin deficiency

	Stage of cobalamin deficiency				
Parameter	**None**	**I**	**II**	**III**	**IV**
Cbl balance	Normal	Negative balance of stores	Depletion (early)	Tissue damage (late)	
Serum holo-TCII	> 60 pg/mL	Low	Low	Low	Low
Serum Cbl	> 201 pg/mL	> 201 pg/mL	> 201 pg/mL	Low	Low
Serum MMA	< 376 nmol/L	< 376 nmol/L	< 376 nmol/L	High	High
Serum Hcy	< 15 µmol/L	< 15 µmol/L	< 15 µmol/L	High	High
RBC folate	> 160 pg/mL	> 160 pg/mL	> 160 pg/mL	> 140 pg/mL	> 100 pg/mL
Neurological symptoms	None	None	None	Sometimes	Frequent
MCV	Normal	Normal	Normal	Normal	High
Hemoglobin	Normal	Normal	Normal	Normal	Low

Cbl, cobalamin; Hcy, homocysteine; MCV, mean corpuscular volume; MMA, methylmalonic acid; RBC, red blood cell; TCII, transcobalamin II.
Adapted from Herbert VD. Round Table Series 66. London: Royal Society of Medicine Press, 1999.

These include strict vegetarians, persons older than 65 years of age with decreased food intake, and patients with any of the following: unexplained neurological or psychiatric symptoms or anemia, long-term use of proton pump inhibitors, autoimmune diseases, AIDS, previous gastric surgery, Crohn's disease of the ileum, and malabsorption from any cause. Screening should be done with serum holo-TCII measurements if available, either alone or in conjunction with serum cobalamin. If serum cobalamin alone is used and values below 350 pg/mL are obtained, metabolite assays should be performed if cobalamin deficiency is suspected clinically. The combination of a serum cobalamin value below 350 pmol/mL and an elevated methylmalonic acid concentration has a specificity of 98% for cobalamin deficiency [220].

Intake or absorption should be assessed only when clinically indicated. If gastric atrophy is demonstrated by biopsy, or if gastrectomy is known from the history or the radiographic findings, it is not usually necessary to confirm the absence of intrinsic factor by a Schilling test. Urinary excretion of less than 5% labeled cobalamin is diagnostic of malabsorption, whereas excretion of more than 10% labeled cobalamin is normal, but many results are indeterminate (5%–10%) [1,138]. This lack of sensitivity is also a factor in deciding whether to proceed with the test. The two major causes of a falsely normal Schilling test result are an erroneous value (intertest variability can be as high as 30%–50%) and malabsorption of food cobalamin, presumably because of an inability of gastric proteases to liberate the vitamin. The Schilling test must be performed with labeled food cobalamin (usually in liver or scrambled eggs) to make the diagnosis because the standard Schilling test uses free cobalamin. However, the commercially available versions of the test have not been uniformly standardized.

Treatment

If cobalamin deficiency is caused simply by decreased intake without malabsorption, 3–6 μg of oral cobalamin per day will suffice. However, if malabsorption is present, either because of lack of intrinsic factor or of intestinal disease, then losses of up to 10 μg/day must be allowed for. Deficiency is treated with 100 to 1000 μg/day for 5–10 days, followed by at least 300 μg/month, given by subcutaneous injection, indefinitely. Alternatively, oral or sublingual cobalamin can be given with equal efficacy (2 or 1 mg/day, respectively) to take advantage of the inefficient (~ 1%) absorption of the vitamin in the absence of intrinsic factor [221]. Nasal spray and nasal gel formulations of cyanocobalamin (Nascobal) are available, in the recommended dose of 500 μg once per week [222]. Although normal cobalamin levels can be restored by larger doses, no studies have documented equal efficacy with the much more expensive nasal preparation. Increased well-being is noted within 24 h of initiating treatment, painful glossitis improves in 48 h, and reticulocytosis begins in 5 to 7 days. Serum folate falls rapidly. The reversal of neurological signs may take 6 months or more. No significant toxicity has been reported with therapeutic doses of oral or parenteral cobalamin.

Ascorbic acid (vitamin C)

Deficiency

Scurvy develops in 2–3 months if the diet is deficient in ascorbic acid. It occurs in the United States only rarely, usually in cases of chronic alcoholism, malabsorption, or food faddism. Early symptoms are weakness, lassitude, irritability, aching joints and muscles, and weight loss. Later, perifollicular hyperkeratotic papules appear on the buttocks, thighs, and legs, followed by petechiae on the lower legs [223]. In advanced deficiency, the gums become swollen, red, and spongy and hemorrhaging occurs, especially from the gums and in the skin and muscles. Anemia is common. No deficiency syndrome other than scurvy has been reported.

Assessment

Plasma ascorbate levels reflect recent intake. Thus, a low serum concentration precedes clinical scurvy [138]. Low levels do not necessarily denote scurvy, however. Levels can be falsely low in patients with chronic inflammatory diseases, in cigarette smokers, after severe emotional stress, and in women taking oral contraceptives. Levels below 0.2 mg/dL (11 μmol/L) denote deficient intake or absorption; that level is reached in 3–5 months. Leukocyte ascorbate concentration is better correlated with body stores (< 150 μmol/L represents a high risk for deficiency), but the assay is technically difficult and requires large blood samples. Because the diagnosis of scurvy should be made quickly, plasma ascorbate is the initial test of choice.

Treatment

Scurvy may respond to as little as 10 mg of vitamin C per day, but doses between 60–100 mg/day and 250 mg four times daily are recommended to replenish body stores. Large doses of ascorbic acid (2–6 g/day) have been reported to cause diarrhea, promote the formation of renal oxalate stones, increase the excretion of basic drugs by acidifying the urine (e.g., tricyclic antidepressants), decrease the excretion of acidic drugs (e.g., aspirin), and interfere with many laboratory tests, including the fecal tests for occult blood that depend on oxidation of the substrate for a positive reaction. However, the reports of clinically adverse effects with supplemental vitamin C have largely not been substantiated in normal healthy populations [224]. However, despite small benefits regarding the duration and severity of rhinovirus infection in experimental settings, there is no evidence that vitamin C reduces the incidence or severity of the common cold in the general population [225]. Epidemiological studies have noted an inverse relationship between serum levels and coronary artery disease, hypertension, cataracts, and carcinomas, but no evidence for benefit from intervention has been found

[226]. It is not indicated to recommend supplemental vitamin C for these conditions.

Biotin

A dietary deficiency of biotin is rare [203,227], but it can be caused by excessive consumption of raw egg white, which contains avidin, a biotin-binding glycoprotein. The serum assay is available, but is complex, involving high-performance liquid chromatography combined with avidin binding. The effects of deficiency include anorexia, nausea, dermatitis, alopecia, mental depression, and organic aciduria. Symptoms disappear with doses of 0.15 to 0.3 mg parenterally, or with 0.2–10 mg/day orally for a few days. No adverse effects of such treatment have been reported.

Pantothenic acid

Definite clinical deficiency has not been reported in humans, probably because the vitamin is so abundant in foods [203]. The "burning feet" syndrome seen in malnourished patients may respond to pantothenic acid, but it is unclear whether this represents a specific deficiency. If deficiency is suspected, 10 mg of calcium pantothenate can be used.

Vitamin A [228,229]

Deficiency

Vitamin A deficiency is usually the result of decreased intake or of fat malabsorption. Contributing factors include impaired conversion of carotenoids to vitamin A (in mucosal disease), decreased storage capacity (in liver disease), decreased levels of serum transport proteins (in liver disease or protein malnutrition), and increased urinary losses (in cancer, tuberculosis, or urinary tract infections) [228].

Inadequate intake is rare in the United States, but the number of new cases of corneal disease caused by vitamin A deficiency worldwide approaches 1 million each year [229]. It takes about 2 years to deplete hepatic stores. Symptoms of deficiency are night blindness, xerophthalmia, follicular hyperkeratosis, altered taste and smell, increased cerebrospinal fluid pressure, and increased infections [230]. When zinc deficiency is also present, the effect on visual adaptation may be magnified.

Assessment

The intake of both carotene and vitamin A is reflected in the serum levels. Decreased intake is reflected in serum vitamin A levels between 10 and 20 μg/dL [138]. A level below 10 μg/dL indicates deficiency. Low carotene levels are meaningful only if carotene is being ingested in the diet because there are no body stores of carotene. In severe liver disease and chronic infection, serum vitamin A levels may fall because retinol-binding protein is not produced in normal amounts. However, carotene levels tend to rise because less carotene is converted to vitamin A in these conditions. Vitamin A can be elevated with normal carotene levels in patients ingesting excess amounts of vitamin A or in patients on chronic hemodialysis because of a decreased conversion of retinol to retinoic acid. Elevated carotene levels with normal retinol are seen in persons ingesting excess amounts of carotene and also in patients with anorexia nervosa, hypothyroidism, hyperlipidemia, or the hypercholesterolemia of diabetes [138].

Treatment

Vitamin A is provided in the form of free retinol. Deficiency states respond to daily oral doses of the vitamin of between 5000 and 50 000 IU. Intramuscular delivery can also be used [1]. Because of their antioxidant properties, carotenoids have been implicated in many chronic diseases often linked with vitamins E and C, the other vitamin antioxidants [223]. However, no convincing evidence is yet available to support supplementation with any antioxidant vitamins, carotenoids, or preformed vitamin A to prevent malignancies or cataracts. Some data suggest that vitamin A may help children with infections to grow [231]. Doses of vitamin A suggested for patients with severe malabsorption are 50 000 IU/day for young infants (0–6 months), 100 000 IU for older infants (6–12 months), and 200 000 IU for children over 1 year of age. Doses of vitamin A above the RDA are contraindicated in pregnant women because of the potential for teratogenicity. The minimum daily dose that is toxic to patients without malabsorption is 25 000–50 000 IU. Toxicity is correlated with serum levels of vitamin A above 1000 μg/dL. Symptoms of chronic toxicity include dry mouth and mucous membranes, skeletal pain, increased cerebrospinal fluid pressure, alopecia, anorexia, irritability, hepatic dysfunction, exophthalmos, and hypercalcemia. Carotene cannot be converted to retinol fast enough for even large doses to produce retinol toxicity.

Vitamin D (cholecalciferol)

Deficiency

Vitamin D deficiency secondary to inadequate intake is rare in the United States, in part because milk products are fortified with the vitamin. Even persons who do not consume dairy products usually obtain adequate sunlight exposure to prevent vitamin D deficiency. However, in elderly persons with little exposure to sunlight, decreased vitamin D intake or synthesis along with inadequate calcium intake may contribute to a high incidence of bone fractures [232]. Deficiency should be considered in any patient with steatorrhea (malabsorption of dietary vitamin and polar metabolites by the enterohepatic circulation) or severe liver disease (decreased 25-hydroxylase activity) or kidney disease (lack of 1α-hydroxylase), as well as in patients with Crohn's disease or previous ileal resection [233]. Vitamin D deficiency causes hypocalcemia and hypophosphatemia, which result in increased parathyroid hormone secretion and bone demineralization. In time, this sequence leads to osteomalacia in adults and rickets in children and can be an unrecognized

factor in acute hip fracture [234]. Tetany results from severe hypocalcemia, and muscle weakness is correlated with hypophosphatemia and depletion of muscle phosphate. Patients with risk factors for the development of osteopenia (cigarette smoking, family history, sedentary lifestyle, long-term glucocorticoid therapy, long-term anticonvulsant therapy, early menopause, testicular failure) should be followed even more carefully if they are at risk for vitamin D deficiency. Long-term alcohol abuse is also an overlooked cause of osteoporosis in men [235]. Risk factors for metabolic bone disease in patients with liver disease include female gender, Caucasian race, estrogen deficiency, low–normal BMI, corticosteroid use, and tobacco abuse [236].

Assessment

The 25-hydroxyvitamin D serum level is low when body stores, intake, or endogenous production of the vitamin are low [237]. Thus, this parameter is satisfactory for assessing vitamin D status with respect to deficiency and toxicity. However, the assay is variable: the competitive protein binding assay gives results some 20%–30% higher than the radioimmunoassay (RIA) [166]. Levels of 32 μg/L (80 nmol/L) have been advocated as the upper range of normal, based on the ability to restore plasma parathyroid hormone (PTH) concentrations to normal [238,239]. The production of 25-hydroxyvitamin D is not closely regulated and rises or falls as its substrate is made available. The concentration in serum is 5–10 times that in other tissues except for adipose tissue. It is bound in serum to a binding protein that is normally only 5% saturated, and the half-life is long (24 h). Thus, recent exposure to sunlight or increased oral intake are reflected in the serum level. Levels are low in dietary deficiency, decreased absorption, lack of sunlight, prematurity, and severe liver disease; low levels are also associated with drugs that alter the metabolism (e.g., anticonvulsants). High levels are seen in growing children, conditions in which parathyroid hormone levels are elevated, sarcoidosis, and some forms of idiopathic hypercalciuria.

Patients at risk for deficiency should be followed with periodic measurements of the serum 25-hydroxyvitamin D level, as well as bone density and 24-h urinary calcium measurements, to assess the status of calcium stores and intake (see "Calcium" above). 1,25-Dihydroxyvitamin D can also be measured in the serum. The production of this isoform is closely regulated, but by extracellular ionized calcium not by vitamin D stores, unless the latter are extremely low. The level of this vitamin correlates with vitamin D function more than with stores. Serum 1,25-dihydroxyvitamin D levels are low in profound vitamin D deficiency, chronic renal disease, hypoparathyroidism, vitamin D-resistant rickets type I, and osteolytic conditions not caused by increased PTH levels (cancer, hyperthyroidism). Primary hyperparathyroidism, vitamin D-resistant rickets type II, and pregnancy are conditions in which levels are elevated. Hypervitaminosis D elevates 25-hydroxyvitamin D levels markedly, but 1,25-dihydroxyvitamin D levels are little affected. Table 101.16 provides suggested guidelines for evaluating the vitamin D status.

Treatment

Oral vitamin D does not maintain the vitamin D status as well as endogenous vitamin D; the latter is released more constantly from the skin and is not converted so rapidly to other isomers by the liver, to which virtually all orally administered vitamin D is exposed following absorption. Nonetheless, oral supplements are needed sometimes, especially in patients with fat malabsorption, uremia, long-term corticosteroid use, and possibly postmenopausal osteoporosis, although the proper place of vitamin D supplementation in the management of osteoporosis is still uncertain. Adults with vitamin D deficiency should receive 0.1–0.2 mg (4000–8000 IU) of cholecalciferol daily by mouth. Much larger doses should be used if malabsorption is present, although the exact dose needed may vary widely. Theoretically, the more polar forms, 25-hydroxyvitamin D (calciferol) and 1,25-dihydroxyvitamin D (calcitriol), should be useful in patients with malabsorption [240,241], but the absorptive advantage is probably not sufficient to justify the great increase in cost. Analogues of vitamin D are available for use in chronic renal failure, being designed to decrease the hypercalcemic effect while restoring bone mass [242]. Paricalcitol

Table 101.16 Suggested guidelines for evaluating vitamin D status

Test	Deficient	Low	Acceptable	High
25-Hydroxyvitamin D				
(nmol/L)	≤ 30	< 60	≥ 80	> 200
(ng/mL)	≤ 12	< 24	≥ 32	> 80
1,25-Dihydroxyvitamin D				
(pmol/L)			48–100	
(pg/mL)		20–42		
24-h urinary calcium (mg/kg)	< 2		> 2	
Bone density (SD below mean)	> 2.5	> 2	1–1.5	

(19-nor-1α-25-dihydroxyvitamin D-2) also improves survival from cardiovascular and infectious causes of death. The efficacy of treatment should be assessed by periodic measurement of the 25-hydroxyvitamin D level, with the dose adjusted to keep the serum level within the normal range. In patients with malabsorption, one should determine that the function of vitamin D has also been restored – that is, that calcium absorption has been normalized – by bringing the 24-hour urinary calcium into the normal range. The risk for toxicity in patients with malabsorption is extremely slight. In other patients, hypercalcemia and hypercalciuria may develop. The estimated minimal toxic daily oral dose for healthy adults is 0.125 mg (5000 IU) [1,232]. Nausea, anorexia, itching, polyuria, abdominal pain, constipation, bone pain, metallic taste, and dehydration may be present early, followed by other manifestations of hypercalcemia. Vitamin D toxicity is reversible if renal damage is not severe.

Vitamin E

Deficiency

Deficiency of vitamin E is uncommon in humans. Persons at risk include newborns and premature infants, patients with fat malabsorption or biliary obstruction, and food faddists. Vitamin E does not cross the placenta well and is poorly absorbed in newborns. Severe fat malabsorption in cystic fibrosis, abetalipoproteinemia, short bowel syndrome, biliary obstruction, and excessive mineral oil ingestion can lead to deficiency [243]. Unsteady gait, tremor, weakness, ophthalmoplegia, pigmentary retinopathy, and proprioceptive impairment have been noted in adult malabsorptive syndromes, along with red cell hemolysis. A similar progressive neurological syndrome has been reported in children with cholestatic liver disease [244].

Assessment

Deficiency can be documented by a low serum α-tocopherol concentration (< 11.6 μmol/L or < 5.0 μg/mL) [89]. Because the vitamin is carried in serum by lipoproteins, the plasma concentration of vitamin E is low in any hypolipidemic state. Therefore, the ratio of α-tocopherol to total lipid (triglyceride + cholesterol) is a more accurate indicator of vitamin E status than is the vitamin level alone [245]. Ratios of more than 0.8 mg of α-tocopherol per gram of total lipid or more than 0.22 mg of α-tocopherol per gram of cholesterol are normal. Ethane and pentane are generated through the peroxidation of *n*-3 and *n*-6 fatty acids, respectively. Breath ethane has been used to evaluate the vitamin E status and follow therapy in children because the level rises with deficiency [246].

Treatment

Large oral doses of D-α-tocopherol acetate, up to 600 mg (800–900 IU) per day, are needed, especially in patients with malabsorption. A water-soluble form of the vitamin (D-α-tocopherol-PEG-1000 succinate) is available over the counter as Liqui-E [247]. This compound forms micelles when given in doses above 25 mg/kg/day and may assist in the absorption of other fat-soluble vitamins. The results of the use of vitamin E to treat chronic diseases have been mixed, but overall are negative for heart disease, either alone or in combination with other antioxidants [248]. The American Heart Association consensus statement does not recommend the routine use of vitamin E [249]. Data for protection by vitamin E in prostate and other cancers is still inconclusive [250]. No consistent ill effects are seen with up to 3200 IU (2112 mg) per day in healthy persons. At doses from 100 to 1100 mg/day, oxidation of vitamin K to its active form can be inhibited, and high-dose vitamin E may be problematic in patients with bleeding disorders. However, the use of 800–1200 IU/day had no effect on the prothrombin time of patients on warfarin therapy [190].

Vitamin K

Deficiency

Nutritional deficiency of vitamin K is uncommon in adults because of the large amount in foods and because bacteria synthesize the vitamin in the intestinal lumen. Deficiency is most commonly the result of fat malabsorption, often compounded by poor intake and diminished liver function or decreased bile excretion [251]. Inhibition of bacterial biosynthesis by broad-spectrum antibiotics can be important. Deficiency is manifested by easy bruising and clotting abnormalities.

Assessment

The most commonly used clinically applicable test for vitamin K deficiency is the one-stage prothrombin time. The clotting factors tested (II, V, VII, and IX) are all responsive to vitamin K except for factor V. Factor VII has the shortest half-life and is the usual rate-limiting factor. The prothrombin time does not test vitamin K stores and is abnormal when deficiency is present, when the synthesis of clotting factors is impaired by liver disease, or when factors are consumed in intravascular coagulation. Thus, the test is nonspecific. Response of the prothrombin time to 5–10 mg of parenteral vitamin K for 2 to 3 days confirms the presence of a deficiency state. Plasma phylloquinone reflects recent intake, but is not a good measure of body stores. Determination of the urinary α-carboxyglutamic acid (Gla) level may be even more sensitive because it tests a function of vitamin K, and the level can be low when blood coagulation is normal [252]. Plasma levels of Gla proteins may be useful in detecting deficiency of vitamin K but have not been widely used. These proteins include undercarboxylated prothrombin (PIVKA-II) and carboxylated/undercarboxylated osteocalcin [8].

Treatment

Long-term therapy with doses titrated to the prothrombin time (~ 2 mg daily or every other day) should be restricted to patients with malabsorption. Patients on anticoagulant

therapy should eat a well-balanced diet with consistent intake of vitamin K, avoiding fluctuations of high-content foods (egg yolk, vegetable oils, green leafy vegetables). For anticoagulant overdose, 1–2.5 mg oral phytomenadione (vitamin K-1) will decrease the international normalized ratio (INR) from 5–9 to 1–5 in 24–48 h [253]. For an INR greater than 10, a 5-mg initial dose of vitamin K-1 is recommended. The vitamin is available for subcutaneous or intramuscular injection at 2 or 10 mg/mL. Vitamin K is not a component of any multivitamin preparation and must be prescribed individually.

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I MISCELLANEOUS

102 Obesity

Louis A. Chaptini, Steven R. Peikin

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Obesity is defined as an excessive amount of body fat arising from an excess energy intake that exceeds energy expenditure. Accurate measurement of body fat requires expensive and sophisticated techniques not readily available to physicians. The body mass index (BMI) correlates closely with the mass of adipose tissue [1] and therefore constitutes an acceptable estimate of body fat. The BMI represents an index of the relationship between height and weight (calculated as weight in kg divided by height in m^2 or as weight in pounds multiplied by 704 divided by height in inches squared). It is used as a clinical tool to identify overweight (BMI between 25 and 29.9 kg/m^2) and obese (BMI > 30 kg/m^2) patients. This BMI classification is primarily based on several epidemiological studies that assessed the relationship between BMI and mortality and found that men and women with a BMI greater than 30 kg/m^2 are at increased risk of health hazards and mortality [2] (Table 102.1).

According to the most recent National Health and Nutrition Examination Survey (NHANES 2003–2004), the prevalence of obesity among adults in the United States was 32.9% and the prevalence of obesity and overweight was 66.2% (Table 102.2). The number of obese adults has doubled from 1980 to 2000 and the number of morbidly obese (BMI > 40) has quadrupled. More than 300 000 deaths per year are attributed to obesity [3] and poor diet and physical inactivity may soon overtake tobacco as a leading cause of death in the United States [4]. Furthermore, because of the substantial rise in the prevalence of obesity and its life-threatening complications, we may experience a potential decline in life expectancy in the United States in the 21st century [5].

Obesity has particular relevance to gastroenterologists because of the associated causality with different gastrointestinal diseases. Gastroenterologists should be knowledgeable in the treatment of obesity (dietary interventions and pharmacological treatment) and in the treatment of patients presenting with complications after bariatric surgery. This chapter reviews the complex mechanisms that regulate food intake and body weight, comorbidities in particular those involving gastrointestinal and hepatic complications associated with obesity and the clinical approach to the patient with obesity.

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Regulation of energy storage, intake, and expenditure

Concept of energy homeostasis

Fat is the primary form of energy storage. According to the first law of thermodynamics, the amount of stored energy is equal to the difference between energy intake and energy expenditure. This law applies to any biological system, including the human body. Weight gain results from an imbalance in homeostatic mechanisms that, in normal conditions, maintain the difference between intake and expenditure close to zero. A very small imbalance over a long period of time can have a large cumulative effect. To keep a perfect balance between energy intake and expenditure, homeostatic mechanisms rely on neural signals that emanate from adipose tissue, endocrine, neurological, and gastrointestinal systems and are integrated by the central nervous system (CNS) [6,7] (Fig. 102.1). The CNS subsequently sends signals to multiple organs in the periphery to control energy intake and expenditure and maintain energy homeostasis over long periods of time.

Gastrointestinal hormones and food intake

The gastrointestinal neuroendocrine system plays a substantial role in regulating food intake and maintaining energy homeostasis. Specialized enteroendocrine cells contain chemoreceptors that are sensitive to nutrients present in the chyme as it progresses through the gastrointestinal tract [8]. In response to different classes of nutrients, the enteroendocrine

Table 102.1 Classification of weight by BMI, waist circumference, and associated disease risk

BMI (kg/m^2)	Classification	Disease risk[a] Men ≤ 102 cm (≤ 40″) Women ≤ 88 cm (≤ 35″)	Disease risk[a] Men > 102 cm (> 40″) Women > 88 cm (> 35″)
< 18.5	Underweight	–	–
18.5–24.9	Normal	–	–
25.0–29.9	Overweight	Increased	High
30.0–34.9	Obese class I	High	Very high
35.0–39.9	Obese class II	Very high	Very high
≥ 40	Obese class III	Extremely high	Extremely high

a Disease risk for type 2 diabetes, hypertension, and cardiovascular disease, relative to normal weight and waist circumference.
BMI, body mass index.
Adapted from World Health Organization [117].

Table 102.2 Age-adjusted prevalence of overweight and obesity among US adults, age 20–74 years

	NHANES II (1976–1980) (*n* = 11 207)	NHANES III (1988–1994) (*n* = 14 468)	NHANES (1999–2000) (*n* = 3603)	NHANES (2001–2002) (*n* = 3916)	NHANES (2003–2004) (*n* = 3756)
Percentage of overweight or obese (BMI ≥ 25.0)	47.1	55.9	64.5	65.7	66.2
Percentage of obese (BMI ≥ 30.0)	15.0	23.2	30.9	31.3	32.9

BMI, body mass index; NHANES, National Health and Nutrition Examination Survey.
From Centers for Disease Control and Prevention website accessed on 01/07/2008: www.cdc.gov/nchs/products/pubs/pubd/hestats/overweight/overwght_adult_03.htm

cells secrete peptides that act either as hormones by entering the bloodstream or in a paracrine fashion by diffusing through the extracellular fluid to adjacent cells. Most of these peptides stimulate satiety with the exception of ghrelin, which has an orexigenic effect.

Cholecystokinin

Cholecystokinin (CCK) is the prototypical satiety hormone, produced by I cells in the duodenum and jejunum. It is secreted in response to the presence of nutrients within the gut lumen, specifically fat and protein [9]. The satiating effect of CCK is mediated by the vagus nerve. The activated vagus in turn stimulates cells in the brainstem (nucleus tractus solitarius) leading to signals to other areas of the brain (paraventricular nucleus and ventromedial hypothalamus) that cause the individual to stop eating [10]. CCK also has endocrine effects on the gastrointestinal system and the CNS, which may be important in stimulating satiety. These include contraction of the pylorus, inhibition of gastric emptying and possibly direct binding to CCK receptors in the area postrema at the base of the fourth ventricle [11].

Peptide tyrosine tyrosine, pancreatic polypeptide and neuropeptide Y

Peptide tyrosine tyrosine (PYY), pancreatic polypeptide (PP), and neuropeptide Y (NPY) are all members of the pancreatic polypeptide family. NPY, a potent short-term stimulus of appetite, is released from NPY neurons in the arcuate nucleus (Fig. 102.1). PYY is secreted by L cells in the distal ileum and colon and is present in two forms, PYY(1–36) and PYY(3–36). PYY release from the gastrointestinal tract is proportional to the caloric density of the ingested nutrients, primarily lipids and carbohydrates [12]. When administered peripherally, PYY(3–36) has been shown to reduce food intake in rodents, primates, and in normal weight and obese humans [13,14]. PP is secreted from specific pancreatic islet cells in response to a meal. Several reports have shown a reduction in food intake after peripheral administration of PP [15,16] (Fig. 102.1).

Glucagon-like peptide-1 and oxyntomodulin

Glucagon-like peptide-1 (GLP-1) is derived from the post-translational modification of proglucagon in the enteroendocrine L cells in the ileum and colon. The secretion of GLP-1

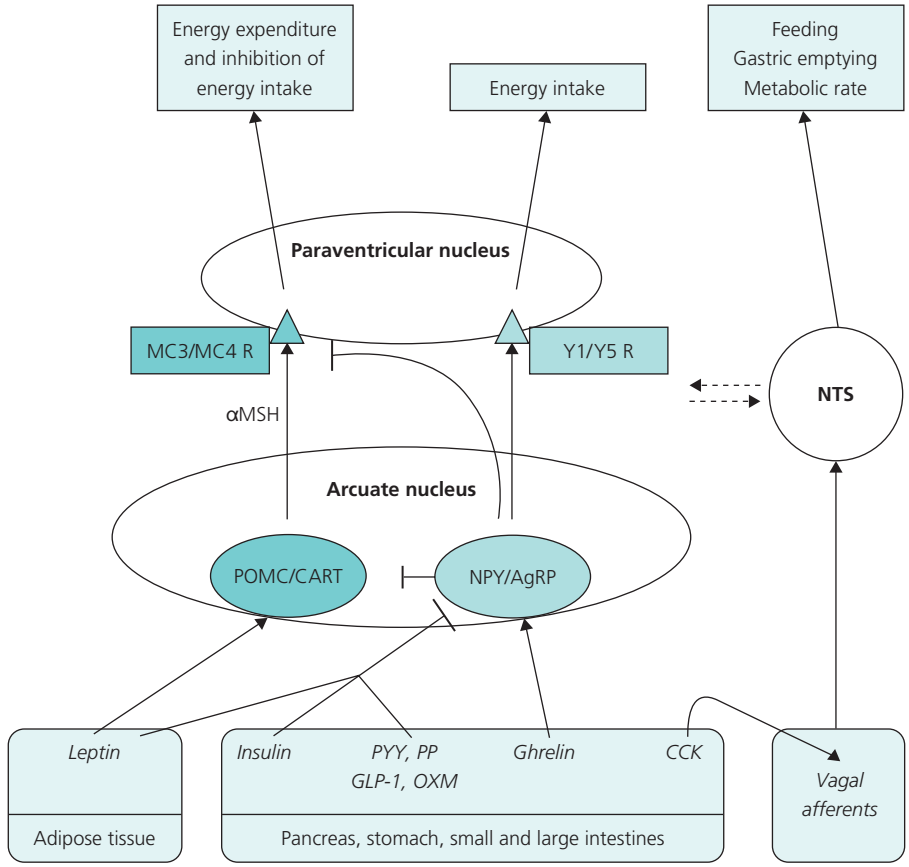

Figure 102.1 Pathways of regulation of food intake. Representation of potential action of gut peptides on the hypothalamus. Primary neurons in the arcuate nucleus contain multiple peptide neuromodulators. Appetite-inhibiting neurons (dark purple) contain proopiomelanocortin (POMC) peptides such as α-melanocyte-stimulating hormone (αMSH), which acts on melanocortin receptors (MC3 and MC4) and cocaine- and amphetamine-stimulated transcript peptide (CART), whose receptor is unknown. Appetite-stimulating neurons in the arcuate nucleus (light purple) contain neuropeptide Y (NPY), which acts on Y receptors (Y1 and Y5), and agouti-related peptide (AgRP), which is an antagonist of MC3/4 receptor activity. Integration of peripheral signals within the brain involves interplay between the hypothalamus and hindbrain structures, including the nucleus tractus solitarius (NTS), which receives vagal afferent inputs. Inputs from the cortex, amygdala, and brainstem nuclei are integrated as well, with resultant effects on meal size and frequency, gut handling of ingested food, and energy expenditure. →, direct stimulatory; ⊣, direct inhibitory; PYY, peptide tyrosine tyrosine; PP, pancreatic polypeptide; GLP-1, glucagon-like peptide-1; OXM, oxyntomodulin; CCK, cholecystokinin. Adapted from Badman & Flier [7], with permission from the American Association for the Advancement of Science.

results from direct nutrient stimulation in the distal small intestine as well as indirect neurohumoral stimulation in proximal regions of the small intestine [17]. A major action of GLP-1 is to help stimulate insulin secretion during a meal. GLP-1 also has an anorectic effect in healthy [18], diabetic [19], and obese [20] human subjects. The peripheral administration of GLP-1 is thought to elicit satiety by its inhibitory effects on gastrointestinal transit and reduced gastric emptying [21].

Oxyntomodulin is another peptide derived from proglucagon's posttranslational processing in L cells. It is also an anorectic peptide and reduces caloric intake in humans. Part of its anorectic effect may result from suppression of plasma ghrelin levels [22].

Amylin

Amylin is a peptide secreted by pancreatic B cells in parallel with insulin secretion during meals. The peptide inhibits gastric emptying and gastric acid secretion. Several reports showed a decrease in meal size after systemic administration of amylin [23,24]. Pramlintide, an amylin analogue, has been shown to induce weight loss in diabetic patients [25].

Leptin

Leptin is an adipocyte-derived factor that plays a dominant role in the central regulation of food intake and energy balance by signaling adipose energy reserve to the brain [26]. Leptin is secreted by subcutaneous adipose tissue in response to fat storage or overfeeding. Leptin is also produced in extra-adipose sites such as placenta, skeletal muscle, and stomach. Up to 25% of circulating leptin derives from fundic glands in the stomach [27]. Leptin has the ability to reduce food intake and body weight [28] by suppressing the expression of NPY, a potent appetite stimulatory peptide, and increasing the expression of α-melanocyte-stimulating hormone, which acts to decrease appetite (Fig. 102.1). In contrast, leptin deficiency reduces energy expenditure [7]. The effects of gastric leptin are probably similar to the adipocyte-derived leptin [28]. Although total leptin deficiency is responsible for the obesity seen in the *Ob/Ob* mouse [7], the majority of obese humans actually have elevated leptin levels [29], suggesting an end-organ decreased response to the peptide.

Ghrelin

Unlike the satiety hormones discussed so far, ghrelin is an orexigenic or "appetite" hormone that stimulates hunger [30].

It is primarily synthesized and secreted from the fundic area in the stomach but can also be produced by cells in the duodenum, ileum, cecum, and colon. Plasma levels of ghrelin rise during fasting, peak (to double the baseline concentration) immediately before meals and fall rapidly after a meal, suggesting a role as a meal initiator [31]. The role of ghrelin in obesity in not clear because, unlike what one would expect, ghrelin levels tend to be low in obese humans, only increasing after dietary weight loss [32].

History and physical examination

History taking should be the first step in the evaluation of the obese patient. A review of the patient's lifestyle including eating habits and physical activity may provide helpful information as to why the patient is obese. Eating disorders are a relative contraindication for the treatment of obesity and require the intervention of a specialist in the area. Binge eating is reported more frequently in women with higher BMI [33]. Information about the age at onset of obesity, recent weight changes, previous attempts of weight loss with details of the modalities followed, and family history of obesity is important. A search for drugs that can promote weight gain such as steroid hormones (glucocorticoids, progesterone), neurotropic and psychotropic medications (lithium, antidepressants, phenothiazines), and antidiabetic medications (sulfonylureas, insulin, thiazolidinedione) should be carried out and if possible alternatives should be offered. A review of systems should look for symptoms of secondary causes of obesity such as hypothyroidism, Cushing syndrome and polycystic ovarian syndrome. Attention should be directed to medical conditions associated with obesity like diabetes, hyperlipidemia, hypertension, coronary artery disease, and osteoarthritis. The physician should be aware of symptoms and signs of sleep apnea (loud snoring, daytime fatigue, and morning headaches) and the patient should be referred accordingly. From the gastroenterologist's perspective, a search for symptoms related to gastrointestinal complications of obesity should be undertaken. Those can range from conditions such as gallstones and gastroesophageal reflux disease (GERD) to more serious cases involving cirrhosis or malignancies.

Physical examination should start with the measurement of the height and weight, and calculation of BMI to categorize the risk and severity of obesity. Clinical judgment is necessary in assessing a muscular patient because the BMI may overestimate the degree of fatness. The excess body fat in obese persons may be distributed in an android or gynecoid pattern. Measurement of the patient's waist circumference is important to assess the abdominal distribution of adipose tissue, which by itself is associated with an increased risk of medical complications [34]. Women and men with a waist circumference greater than 88 cm (35 inches) and 102 cm (40 inches), respectively are considered at increased risk for diabetes and other complications of obesity independent of their BMI. Blood pressure should be checked with an appropriately sized cuff. Other key features in the clinical assessment include looking for signs of secondary causes of obesity and conditions that are caused by obesity. Particular attention should be given to the examination of the thyroid gland and the search for signs of hypothyroidism and Cushing syndrome. Hyperinsulinemia, which is typically associated with obesity, can be manifested by acanthosis nigricans around the neck and axilla. Laboratory evaluation should screen for complications of obesity and associated conditions such as nonalcoholic steatohepatitis, type 2 diabetes, and hyperlipidemia that are not necessarily detected on physical examination. Thyroid-stimulating hormone level should be measured to rule out hypothyroidism.

Gastrointestinal complications of obesity

Esophageal disease

The prevalence of GERD and GERD-related complications, particularly erosive esophagitis, Barrett esophagus, and esophageal adenocarcinoma, has been increasing over the past 20 years [35]. Several reports link this increase to the obesity epidemic. The association between reflux symptoms and obesity has been examined in several cross-sectional studies. Most of these studies showed a significant positive association [36–38] with an increased risk ranging from 1.5- to 3-fold in obese subjects, whereas other studies showed no significant link [39,40]. The association between obesity and erosive esophagitis was examined in multiple case–control, cross-sectional, and cohort studies [41–44]: most reports showed a positive association with adjusted odds ratios ranging from 1 to 2.1 in overweight and 1.6 to 14.6 in obese patients.

The incidence of esophageal adenocarcinoma has increased in parallel with that of obesity in the past 15 years, which prompted the performance of several studies looking at an association between them [44–46]. Estimates from pooled studies showed a 2.1-fold increase in the overall risk of esophageal adenocarcinoma in patients with a BMI greater than 25 compared to subjects with a normal BMI [44].

Gallbladder disease

Obesity is an important risk factor for gallbladder disease. Data from the Nurses Health Study demonstrated that the risk for symptomatic gallstones increases linearly with BMI. A twofold and a sevenfold increase in risk for symptomatic gallstones was noted in women with BMI greater than 30 and BMI greater than 45, respectively [47]. Several other epidemiological studies have found the same positive association between BMI and the risk of gallstones [48,49]. The mechanism of gallstone formation in obese individuals involves an increase in cholesterol saturation index and a decreased

response to cholecystokinin [50]. The risk of gallbladder cancer increases with gallstones, hence obesity increases this risk [51].

Pancreatic disease

The increased risk of pancreatitis in obesity is thought to be explained by the increased risk of cholelithiasis. Acute pancreatitis is more severe in overweight and obese subjects [52]. There is an increase in the incidence of infectious complications as well as in the length of hospitalization in obese individuals.

Obesity has also been associated with pancreatic cancer but it has been difficult to determine whether this association is direct or secondary to the association of obesity to diabetes, the latter being an established risk factor for pan-creatic cancer. A metaanalysis estimated that obese subjects have a 20% increase in the risk of developing pancreatic cancer when compared to people with a normal body weight [53].

Liver disease

Liver damage can be increased when liver disease occurs in the setting of obesity. This has been shown in chronic hepatitis C, alcoholic liver disease, and toxin-induced liver disease [54,55]. The most common liver abnormality associated with obesity is nonalcoholic fatty liver disease (NAFLD). Several studies have demonstrated a strong link between obesity and NAFLD. There is an increase in prevalence of NAFLD with BMI increase in both genders and in all races. Furthermore, liver biopsies from morbidly obese individuals performed at the time of bariatric surgeries demonstrated NAFLD in over 80% of cases [56]. NAFLD is a spectrum of conditions that range from steatosis at one end to cirrhosis at the most serious end of the spectrum. Nonalcoholic steatohepatitis (NASH) is an intermediate stage in this spectrum, characterized by hepatocyte injury and death accompanied by infiltration of acute and chronic inflammatory cells. NAFLD is the hepatic consequence of insulin resistance and the associated metabolic syndrome that leads to a relative overproduction of proinflammatory mediators [57]. It has also been suggested that hyperleptinemia plays a role in the occurrence of fibrosis by activating hepatic stellate cells (the source of hepatic collagen) [58]. NAFLD is usually diagnosed when patients are referred for further evaluation of abnormal liver enzymes or when fatty infiltration is detected on an abdominal imaging study performed for other reasons. Serum aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl transferase are usually increased less than fourfold and do not correlate with the severity of the condition. The main target in the treatment of NAFLD is the improvement of insulin sensitivity, which can be achieved by dietary modifications and by pharmacological treatment. A gradual weight loss of 10% or more of body weight can correct abnormal liver chemistries and decrease liver size, fat content, and features of steatohepatitis but the impact on the long-term outcome and the progression of the disease remains unknown. Several small clinical trials looking at the efficacy of pharmacological agents such as insulin-sensitizing agents (metformin, troglitazone), lipid-lowering agents (gemfibrozil, atorvastatin), antioxidants (vitamin E), tumor necrosis factor-α-inhibiting agents, and ursodeoxycholic acid showed conflicting results and their impact on the natural progression of the disease remains unknown [59].

Colon disease

Several cohort and case–control studies have looked at the association between obesity and colorectal cancer and a positive association was noted especially in men, with an increased risk of about 1.5- to 3-fold [44,60]. The association was found to be weaker in women, especially older and postmenopausal women [61]. The risk of colon polyps is also increased about twofold in obese individuals [44,62] and appears to be higher in men than in women.

Irritable bowel syndrome

Gastrointestinal motility is regulated by some of the gut peptides involved in feeding behaviors. Hence, the association between irritable bowel syndrome (IBS) and obesity is worth studying. Few studies have examined this association: one demonstrated a significant increase in some IBS symptoms (constipation, diarrhea, straining, and flatus) in obese patients and another showed a threefold increase in the risk of IBS with obesity [63]. Further studies are needed to confirm this association.

Systemic complications of obesity

Since the 1990s, the obesity epidemic has generated a rapid increase in the metabolic syndrome. The metabolic syndrome is defined as the constellation of insulin resistance (manifested by type 2 diabetes or glucose intolerance), visceral adiposity (resulting in abdominal obesity and increased waist circumference), hypertension, and dyslipidemia [64]. One of the direct consequences of metabolic syndrome and obesity is an increase in the prevalence of diabetes, hypertension, and dyslipidemia leading to a two- to threefold increase in cardiovascular disease [64,65] (Fig. 102.2).

Treatment of obesity

Treatment guidelines

Determination of BMI and waist circumference and the search for comorbid conditions (such as cardiovascular risk factors) help in assessing the degree of overweight and the overall risk status. The next step is to assess how motivated the patient is to lose weight and their degree of understanding of obesity as a disease, its causes, its consequences, and

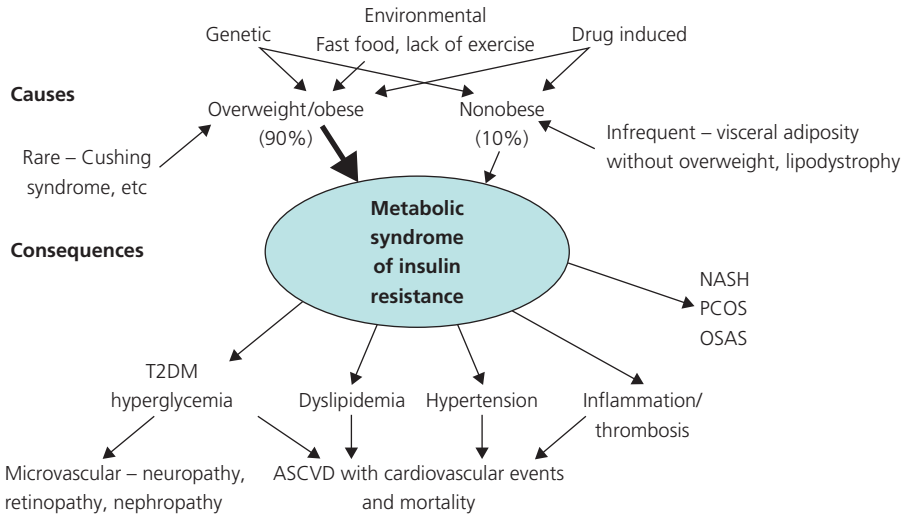

Figure 102.2 Metabolic syndrome. NASH, nonalcoholic steatohepatitis; PCOS, polycystic ovary syndrome; OSAS, obstructive sleep apnea syndrome; ASCVD, atherosclerotic cardiovascular disease; T2DM, type 2 diabetes mellitus. From Richardson & Vinik [64], with permission from Elsevier.

Table 102.3 Steps in the assessment and treatment of the overweight and obese patient

Calculate body mass index and measure waist circumference
Look for causes of weight gain, including medications
Assess and treat comorbidities
Assess patient motivation to lose weight and discuss any previous unsuccessful attempt to lose weight:
If motivated, set reasonable weight loss goal and discuss the options
If not motivated, urge weight maintenance and manage comorbidities
Develop a treatment plan based on Table 102.4

the treatment options, and to discuss any previous unsuccessful treatments and the reasons for failure of those treatments [66] (Table 102.3).

The initial goal of the treatment is a 10% reduction of body weight from baseline. A reasonable time line to achieve this reduction is 6 months of therapy. Further weight loss can be attempted later if indicated [66]. Depending on the degree of overweight and the overall risk status of the patient, different modalities are available to the clinician to achieve the weight loss target. These include lifestyle modifications (dietary interventions and physical activity), pharmacotherapy, and surgical interventions (Table 102.4). Efforts to maintain the

Table 102.4 Treatment options for the overweight and obese patient

	BMI				
Treatment	**25–26.9**	**27–29.9**	**30–34.9**	**35–39.9**	**≥ 40**
Diet, exercise, behavior therapy	+ (with comorbidities) ± (without comorbidities)[a]	+ (with comorbidities) ± (without comorbidities)[a]	+	+	+
Pharmacotherapy	–	– (without comorbidities) + (with comorbidities)	+	+	+
Surgery	–	–	–	– (without comorbidities) + (with comorbidities)	+

a While weight loss is not necessarily recommended for those with a BMI of 25 to 29.9 kg/m^2 unless they have two or more comorbidities [66], motivated subjects should be offered lifestyle therapy.
BMI, body mass index.
From NIH [66].

achieved weight loss should be put in place through a weight maintenance program involving dietary and behavioral therapy along with physical activity.

Lifestyle modifications

Dietary interventions

To induce weight loss, a calorie deficit must be achieved. Balanced diets should contain a minimum of 1000–1200 kcal per day and provide adequate amounts of all essential nutrients. An average loss of 500 kcal per day should produce a weight loss of about 450 g (1 lb) weekly. There is evidence that weight loss of 5%–15% of initial weight is enough to reduce the risks of dyslipidemia, diabetes, and hypertension associated with obesity [67].

The role of dietary fat in the development of obesity has been studied extensively. Almost all dietary guidelines recommend a reduction in the daily intake of fat to 30% of energy intake or less regardless of weight status. On the other hand, despite a decrease in consumption of fat in the past 30 years, the rate of obesity has risen to an all-time high [68]. This shows that reducing the percentage of fat alone will not produce weight loss unless combined with a reduction of total calories. Furthermore, a metaanalysis of six clinical trials (comparing low-fat to other types of weight-reducing diets) showed that low-fat diets were no better or worse than other weight-reducing diets for achieving sustained weight loss [69]. Despite the controversial role of low-fat diet in the treatment of obesity, the National Institutes of Health (NIH) recommend a low-calorie diet consistent with a step I or II diet (depending on the patient's risk status) in which fat constitutes 30% or less [66].

The popularity of low-carbohydrate diets (Atkins, Sugar Busters, Zone, South Beach) has been increasing since the late 1990s. These diets result in a rapid initial weight loss explained by sodium diuresis secondary to ketosis caused by glycogen mobilization in the presence of carbohydrate restriction.

Very-low-calorie diets are diets that provide 500–800 kcal per day. Despite an impressive short-term success, these diets provide no greater weight loss at 1 year than do low-calorie diets (1000–1500 kcal per day) [70].

Portion-controlled, vitamin/mineral-fortified meal replacements have been increasingly used as part of low-calorie diets. These diets using meal replacements are marketed now by several manufacturers and used as components in several large-scale clinical trials. A metaanalysis of trials utilizing these diets showed significant weight loss (7%–8% of body weight) at 3 months and 1 year [71].

Several epidemiological studies looked at the magnitude of weight loss with the different types of diets discussed above. A recent randomized trial comparing four different diets [very-low-carbohydrate (Atkins); macronutrient balance controlling glycemic load (Zone); calorie restriction (Weight Watchers); very-low-fat (Ornish)] with a design intended to simulate their use in a real-world setting [72]. A trend toward higher dropout rates was noted after 1 year with the very low-carbohydrate and very low-fat diets than with the more moderate diets. Average weight loss at 1 year was approximately 4–7 kg (in those who completed the study), and was similar among the four diets. In the intention-to-treat analysis, mean weight loss was similar for all four diets and was more modest (2.1–3.3 kg). Dietary adherence rates were low for all diets and were more important predictors of weight loss. This study illustrates the difficulty of achieving significant weight loss with dietary therapy in a real-world setting.

Physical activity

Physical activity is a key component of any long-term weight loss therapy and weight maintenance program. Physical activity modestly contributes to weight loss in overweight and obese adults. It may also decrease abdominal fat and it increases cardiorespiratory fitness [66]. The combination of a low-calorie diet and increased physical activity produces greater weight loss than diet alone or physical activity alone. Adults should accumulate at least 30 minutes of moderate intensity physical activity on most days of the week.

Behavior therapy

The role of behavior therapy is to alter the eating and activity habits of an obese patient and overcome barriers to compliance with dietary therapy and increased activity [66]. It can be achieved either on an individual basis or in group settings (the latter being less expensive). Behavior therapy can produce a weight loss in the range of 10% of baseline weight over 4 months to 1 year [66,73] but most patients return to baseline weights in the absence of continued intervention. Specific behavioral strategies include self-monitoring, stress management, stimulus control, positive reinforcement, and social support. Self-monitoring consists of recording dietary habits (types, amounts, caloric values, nutrient composition) and physical activity habits (frequency, intensity). Stress management through coping strategies, meditation, and relaxation techniques can defuse situations leading to overeating. Stimulus control includes avoiding stimuli that may encourage eating and situations associated with little amount of exercise. Positive reinforcement through the use of rewards for specific actions encourages behavior change. Social support (family members, friends) can assist in maintaining motivation and providing positive reinforcement [66].

Pharmacotherapy

Antiobesity drugs are indicated as adjuncts to diet and physical activity for patients with BMI of 30 or above in the absence of obesity-related risk factors or diseases, and for patients with BMI of at least 27 in the presence of such conditions [66]. Usually drugs are used after nonpharmacological treatment has been attempted for 6 months with unsatisfactory results of weight loss (less than 450 g per month).

Table 102.5 Medications for the treatment of obesity

Medication	Typical dosing	Classification	Common adverse effects
Medications approved by the FDA specifically for weight loss indication			
Phentermine	15–37.5 mg/day	Adrenergic agent	Tachycardia, hypertension
Sibutramine	10–15 mg/day	Serotonergic/adrenergic	Hypertension, tachycardia
Orlistat	120 mg three times/day	Lipase inhibitor	Malabsorption, steatorrhea
Medications approved by the FDA for other indications but shown to have weight loss effect			
Bupropion	150–300 mg/day	Depression	Anticholinergic; agitation
Metformin	500–1000 mg/day	Type 2 diabetes	Hepatic oxidative injury
Topiramate	50–100 mg/day	Seizure disorder	Cognitive impairment
Zonisamide	400–600 mg/day	Seizure disorder	Cognitive impairment

FDA, Food and Drug Administration.
Adapted from Kaplan [81], with permission from Elsevier.

Pharmacotherapy for obesity underwent a gigantic turn in the 1990s when Weintraub et al. showed that the combination phentermine-fenfluramine (Phen-fen) induces a sustained weight loss for more than 3 years [74]. Fenfluramine was subsequently withdrawn from the market because of an association with valvular heart disease and pulmonary hypertension. Sibutramine and orlistat are the two medications currently approved in the United States for long-term use. Several others, including phentermine, are approved for short-term use but are often used off-label for longer periods of time. Studies are underway regarding new promising antiobesity agents. Other drugs approved for indications other than obesity have been shown to induce some weight loss (Table 102.5).

Sibutramine

Sibutramine (Meridia) is a serotonin and norepinephrine reuptake inhibitor and a weak dopamine reuptake inhibitor. Increasing the availability of these neurotransmitters in the CNS results in appetite suppression. Placebo-controlled trials showed 5%–8% weight reduction after 6 months of sibutramine treatment compared with 2%–4% weight loss in subjects receiving placebo [75,76]. Of note, most of these trials include dietary or exercise interventions for subjects in both the treatment and placebo groups, which likely explains the weight loss in the placebo group. In one study, continued treatment for up to 2 years was associated with some weight regain (approximately 50% of the initial weight loss); however, around 25% of subjects who continued to take sibutramine maintained their reduced weight for the entire 2 years of observation [77]. Side effects of sibutramine include hypertension and tachycardia, therefore routine clinical monitoring of vital signs is recommended. Other less dangerous adverse effects include headache, insomnia, dry mouth, and constipation.

Orlistat

Orlistat (Xenical) alters fat metabolism by inhibiting pancreatic lipase. As a result, ingested fat is not completely digested. Taken with a meal, orlistat can inhibit the absorption of up to 30% of ingested fat [78]. Clinical trials demonstrated that orlistat treatment in conjunction with nutritional counseling results in weight loss of 8%–10% at 1 year, compared with 6% with placebo [79,80]. Extension of the treatment with 2 years results in weight regain of approximately one-third of the initial weight loss (compared with regain of two-thirds with placebo) [79,80]. The use of orlistat is associated with gastrointestinal side effects that include flatulence, steatorrhea, increased stool frequency, fecal incontinence and oily rectal discharge [81]. Malabsorption can be associated with a deficiency in the fat-soluble vitamins A, D, E, and K. Patients on orlistat should receive a daily supplement containing these vitamins given at least 2 h before or after each dose of orlistat [81]. Furthermore, vitamin D levels should be measured before and periodically during orlistat therapy [81]. Few cases of orlistat-induced severe hepatotoxiciy and hepatic failure have been reported [82,83].

Phentermine

Phentermine is an adrenergic reuptake inhibitor that works as an appetite suppressant. It is approved for use over 12 weeks or less. Because phentermine is an adrenergic agonist, it can be associated with tachycardia and hypertension. A weight loss of 1.8 kg (4 lb) over 4 weeks is considered an acceptable therapeutic response [81]. Other noradrenergic agents approved by the Food and Drug Administration (FDA) for short-term use in the treatment of obesity include diethylpropion, phendimetrazine, and benzphetamine [84]. Studies using this class of medications demonstrated up to 10-kg weight loss when compared with placebo [84].

Rimonabant

Rimonabant (Acomplia) is a cannabinoid receptor antagonist. Animal studies have shown that the cannabinoid-1 receptor plays a role in appetite and body weight regulation [85]. Rimonabant has been studied as a potential treatment for both obesity and smoking cessation. In a randomized trial comparing rimonabant to placebo, subjects who took 20 mg rimonabant experienced an average weight loss of 6.6 kg whereas subjects who took placebo lost on average 1.8 kg. Furthermore, improvements in waist circumference, high-density lipoprotein, triglycerides, and insulin resistance were noted with rimonabant [86]. Two other recent randomized controlled trials showed similar effects of rimonabant on weight loss and associated metabolic risk factors [87,88]. In one of the trials, weight regain was noted in subjects who were switched to placebo after 1 year while those who continued on 20 mg rimonabant experienced sustained weight loss at 2 years [88]. Side effects of rimonabant include depression, nausea, headache, dizziness, and diarrhea.

Drugs approved for other indications

Bupropion, a psychotropic agent used in the treatment of depression, is associated with a modest weight loss in the range of 4%–5% when compared with placebo [89]. Similarly, the antidiabetic agent metformin has been associated with weight loss in the range of 4% of the initial body weight over 1–2 years [81]. Topiramate and zonisamide, both anticonvulsant drugs, have been shown to induce some weight loss (up to 6% of the initial body weight with zonisamide) [81].

Over-the-counter products

Over-the-counter dietary supplements are widely used by individuals attempting to lose weight. Such supplements include ephedra (no longer available in the United States to treat obesity), green tea, chromium, chitosan, ginseng, and guar gum. The use of these agents should be discouraged because of the lack of evidence of their efficacy and safety [90].

Surgical therapy

Bariatric surgery is indicated for carefully selected patients with clinically severe obesity (BMI ≥ 40 or BMI ≥ 35 with comorbid conditions) when less invasive methods of weight loss have failed [66]. To be considered effective, a bariatric procedure should result in a sustained excess weight loss of more than 50% and resolution of comorbid conditions. Bariatric surgeries can be divided into two varieties, malabsorptive and restrictive, depending on the mechanism by which weight loss is induced.

Malabsorptive procedures include jejunoileal bypass, biliopancreatic diversion, and biliopancreatic diversion with duodenal switch. These procedures induce weight loss by shortening the length of functional small bowel segment. Profound weight loss can be achieved with these malabsorptive procedures but significant metabolic abnormalities occur, including protein calorie malnutrition and various macronutrient deficiencies [91]. As a result of these dangerous consequences, malabsorptive procedures have been replaced by the restrictive procedures: Roux-en-Y gastric bypass (RYGB), vertical banded gastroplasty (VBG), and laparoscopic adjustable gastric banding (LAGB). RYGB is primarily restrictive but has also some malabsorptive characteristics.

RYGB is accomplished by creating a small, 15–20 mL, gastric pouch and a Roux-en-Y gastrojejunostomy (Fig. 102.3a), hence restricting food intake and at the same time inducing some malabsorption by bypassing the distal stomach, the duodenum, and a variable length of jejunum. RYGB is the most common bariatric procedure performed in the United States. More weight loss is achieved with this procedure than with VBG [92,93]. On average, 50%–75% of excess weight loss is achieved after 1–2 years [94]. Complications of the gastric bypass procedure include stomal stenosis, marginal ulcers, staple line disruption, dilatation of the bypassed stomach, internal hernias, dumping syndrome, and malabsorption of nutrients. RYGB can be performed laparoscopically and results in similar sustained weight loss compared to the open approach [91]. Advantages of the laparoscopic approach include a lower incidence of incisional hernia and wound infection, faster recovery and shorter hospital stay [94,95].

VBG is a purely restrictive procedure that involves creating a gastric pouch by stapling the gastric fundus and placing a circumferential band around the pouch. VBG was the most commonly performed procedure before the advent of gastric bypass (Fig. 102.3b). The average excess weight loss at 3–5 years is around 30%–50% [96]. These more modest long-term results compared with RYGB, along with the significant adverse events associated with VBG (GERD, vomiting, solid food intolerance), explain the decline of popularity of this procedure. Postoperative complications requiring intervention include staple line disruption, stomal stenosis, band erosion, band disruption, and pouch dilatation.

LAGB is another purely restrictive procedure that involves placing a tight, adjustable prosthetic band around the proximal stomach (Fig. 102.3c). The prosthetic band is connected to an infusion port that is placed in the subcutaneous tissue and is accessible with a needle. Injection of saline into the port leads to increased restriction. Advantages of LAGB include technical ease of placement, lack of anastomosis, adjustability (deflation of the port), and reversibility (band removal). Because of its simplicity and lower rate of complications, this procedure has largely replaced VBG, especially in Europe and Australia. Despite initial disappointing data in the United States [97], subsequent studies showed excess weight loss similar to the Australian and European experience, in the range of 45%–75% [98–101].

Patients undergoing bariatric surgery should be followed by a multidisciplinary team including medical, behavioral, and nutritional experts. Gastroenterologists should assess the patient preoperatively and manage any chronic gastrointestinal

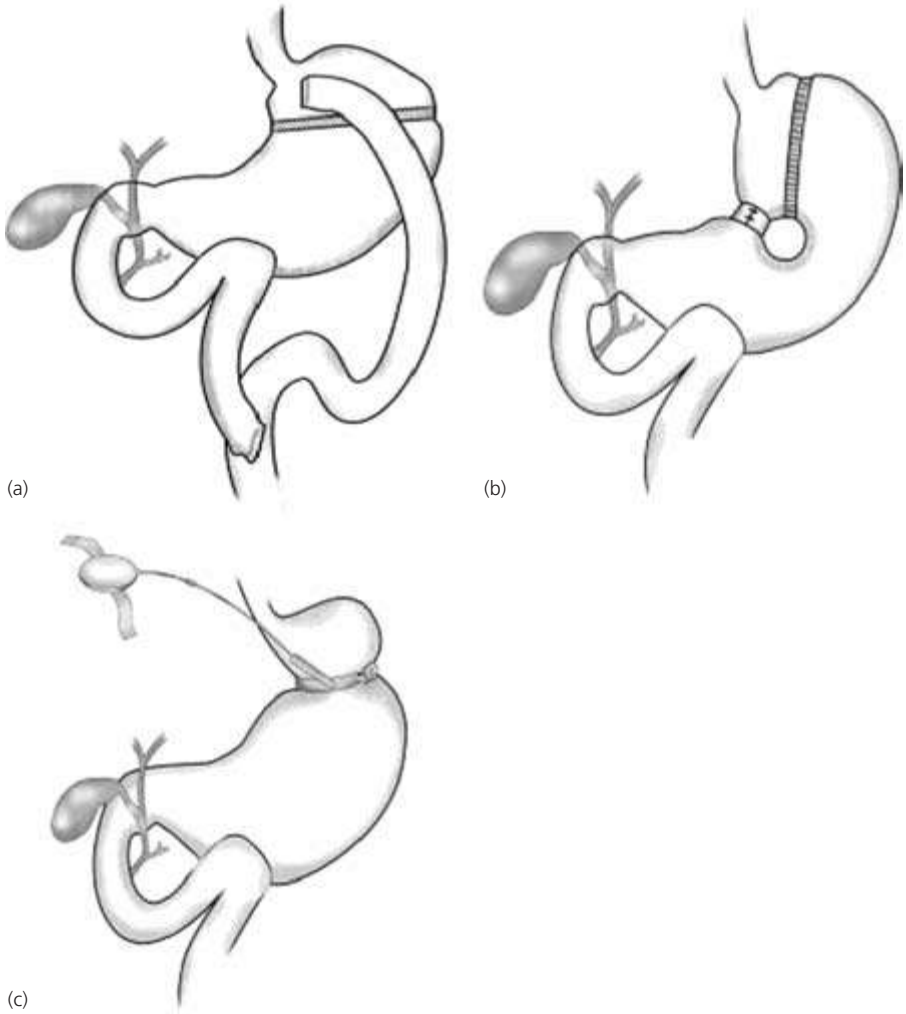

Figure 102.3 Types of bariatric surgeries: **(a)** Roux-en-Y gastric bypass; **(b)** vertical banded gastroplasty; **(c)** laparoscopic adjustable gastric banding. Redrawn from Brolin [118], with permission from the American Medical Association.

disorders that may complicate the postoperative care. For example, attention should be paid to detecting any gastric, pancreatic, or biliary diseases preoperatively because the alteration of the anatomy with some of the performed surgeries makes parts of the gastrointestinal tract (distal stomach, biliary tree) inaccessible to endoscopic and radiological procedures. Patients should be screened for iron deficiency anemia before surgery and gastrointestinal blood loss should be excluded to avoid confusion after surgery. Fatty liver disease is present in most morbidly obese patients requiring surgery and 3%–7% of these patients have cirrhosis [102]. Portal hypertension and the presence of varices (esophageal and/or gastric) are contraindications to gastric operations and should be excluded before surgery [103].

Gastroenterologists also have a role in the management and follow-up of the bariatric surgery patients postoperatively. Complications of surgery usually present as nausea, vomiting, pain, bleeding, diarrhea, and constipation. Immediately after the surgery, these symptoms are probably directly associated with the operation and are often managed by the surgical team. Later on, the gastroenterologist may get involved to deal with complications indirectly related to the surgery, such as anastomotic ulceration, band erosion, stricture, and fistula, which may require endoscopic therapy. Depending on the type of operation, some nutritional complications may occur. Iron, vitamin B-12, and calcium deficiencies can be seen with gastric bypass.

Endoscopic therapy

Minimally invasive endoscopic treatment for obesity is an attractive option, especially in patients who do not respond to lifestyle modifications and pharmacotherapy but are not candidates for bariatric surgery. Various intragastric space-occupying devices have been evaluated for short- and long-term use. In 1985 the FDA approved the Garren–Edwards gastric bubble [104], an air-filled bubble floating in the stomach that is inserted through the esophagus using a gastric tube. Because of a lack of significant weight loss in sham-controlled studies and significant complications such as gastric erosions, gastric ulcers, Mallory–Weiss tear, and esophageal

lacerations, this device was abandoned and is no longer commercially available. Several subsequent modifications to the device failed to show improvement in efficacy. More recently, the Bioenterics intragastric balloon (BioEnterics Corp., Carpinteria, CA, USA) has been widely used internationally but not yet in the United States. It consists of a soft, saline-filled balloon placed endoscopically to partially fill the stomach mimicking an intragastric bezoar. Mean excess weight loss with Bioenterics intragastric balloon is reported to be 38%–48% after 4–6 months [105]; however, only a minority of patients has sustained weight reduction after balloon removal [106]. In a recent sham-controlled study, the difference in mean weight loss between patients with Bioenterics intragastric balloons and controls was not significant (10.4% and 9%, respectively) at 3 months [107]. Complications are less frequent with the Bioenterics intragastric balloon than with earlier balloons. They include intolerance to the balloon, vomiting, abdominal pain, esophagitis, and gastric ulcers and erosions [104].

Future endoscopic approaches will aim to duplicate bariatric surgical procedures. Ongoing animal work is directed at attempting gastric partitioning (the equivalent of VBG). Preclinical work is also being undertaken to refine an endoscopic gastrojejunostomy technique [104].

Complications of weight loss

During rapid weight loss, the risk for cholesterol gallstone formation increases [108]. Patients with higher initial BMI and greater absolute rate of weight loss seem to have the highest risk of gallstone formation. The mechanism of this increase in gallstone formation involves an increase in the cholesterol saturation index and in the crystallization promoting proteins (mucin) and a decrease in gallbladder motility with low-calorie low-fat diet [109–111]. Once an individual has lost weight and maintained weight loss, the risk of gallstone formation decreases. Some surgeons recommend the performance of cholecystectomy at the time of gastric bypass surgery because of the high incidence of gallbladder disease in the seriously obese and to avoid postoperative complications as a result of gallstones [112]. More recently, the use of ursodeoxycholic acid has been shown to reduce the risk of gallstone formation in the first 6 months after bariatric surgery [113]. Another complication of rapid and large weight loss is the occurrence of liver disease. Two weight loss studies, one involving patients undergoing gastroplasty and the other involving patients on a very low-calorie diet, showed a decrease in the prevalence of steatosis but an increase in the prevalence of steatohepatitis with the fall of BMI [114,115]. The risk of liver damage may be related to the rapidity of weight loss, which determines the rate of increased delivery to the liver of free fatty acids from lipolysis of adipose triglycerides [116].

Conclusions

Obesity has become a major health problem in most industrialized nations. It has particular relevance to gastroenterologists because of their involvement in managing the associated gastrointestinal disorders, their role in the evaluation and treatment of obese patients before and after bariatric surgery, and their potential future role in the endoscopic therapy for obesity. By becoming actively involved in the management of obesity, gastroenterologists can provide a valuable clinical service to their patients.

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I MISCELLANEOUS

103 Eating disorders

Henry C. Lin, Eric E. Stone

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Each year an estimated 5 million US citizens are affected by an eating disorder [1]. The fourth edition of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) classifies eating disorders into those of childhood and those of adolescence and adulthood [2]. The former category includes pica, rumination disorder, and feeding disorder of infancy or early childhood. The latter includes anorexia nervosa (AN), bulimia nervosa (BN), and "eating disorder – not otherwise specified." This chapter will focus on the two prominent eating disorders of adolescence and adulthood, namely AN and BN. Because patients with eating disorders "won't eat," their unwillingness to resume "normal" eating is usually considered evidence of an underlying psychological disorder. The coexistence of obsessiveness, an altered body image, hyperactivity, anxiety, and depression further support this viewpoint. Accordingly, patients with an eating disorder are commonly referred to a psychiatrist for treatment. There is, however, a growing body of evidence that many of the features of eating disorders may be maladaptive physiological responses to caloric restriction. In this chapter, we will review AN and BN, emphasizing this biological perspective.

Epidemiology

About 0.5%–1% of adolescent and young adult females in the United States have a diagnosis of AN, with a lifetime incidence as high as 3.7% [2]. Bulimia nervosa is diagnosed slightly more often, with a reported prevalence of 1.5% [3]. Eating disorders are more common in females, by a ratio of 10–20:1 ratio [4]. Nearly 75% of individuals manifest features of their eating disorder while in adolescence [5], with the average age of onset for AN being 15 years [6]. Increasingly, late-onset cases are being recognized [7].

A dramatic rise in the incidence of eating disorders during the 20th century was reported for the United States, Western Europe, and Japan [8–10]. Some have questioned this conclusion after a critical review of epidemiological data [11,12]. Although long assumed to be more prevalent in industrialized than nonindustrialized societies [1], cases of AN are being increasingly reported in Nigeria, leading some to claim that its prevalence may be as common in African populations as in Western countries [13,14]. Rieger et al. argue against the validity of two often-cited assumptions regarding the weight concerns of anorexics, namely, that these concerns are specifically a contemporary, Western manifestation of the condition or that the increasingly recognized prevalence of AN in non-Western societies is solely attributed to their adopting Western ideals of thinness [15].

Although eating disorders can be found in all ethnic and socioeconomic groups in the United States [16–18], the majority of cases are diagnosed in middle- to upper-middle-class families [5]. They are more common in Caucasians (up to 95% in some series of patients) and Asians [4]. The increasing incidence of eating disorders is said to parallel a significant increase in dieting by adolescent girls and young women. Other risk factors for developing an eating disorder have been proposed and include a family history of an eating disorder, history of past sexual abuse, previous substance abuse, and type 1 diabetes mellitus [19].

Recognizing eating disorders

Anorexia nervosa

Cases of AN have been described in medical writings dating back 300 years [20]. Increasing attention was given to AN

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in the 1900s, with various etiologies for the condition proposed during that time [21]. Three distinct components were proposed by Hilde Bruch in 1962 as the principal hallmarks of the disease [22]:
- a distorted body image
- alteration in perception of signals of hunger and satiety
- an overwhelming feeling of ineffectiveness.

She also described a preoccupation with food and eating in anorexics [20]. In the absence of an underlying organic disease, she proposed that AN was a mental disorder [22]. This is the model that is used today as the classic approach to understanding AN.

The overarching abnormality that characterizes AN is an unwillingness to maintain an appropriate bodyweight. The diagnosis is driven by clinical criteria, which have been expanded and incorporated within DSM-IV. The four diagnostic criteria for AN under DSM-IV include:
- bodyweight < 85% of expected
- intense fear of weight gain
- inaccurate perception of own body size or weight
- amenorrhea in postmenarcheal females [8] (Table 103.1).

Bulimia nervosa (BN)

This condition was first described as a clinical entity in 1979 [23]. In addition to excessive concern about weight or body shape, which is similar to AN, patients diagnosed with BN also have recurrent episodes of binge-eating followed by inappropriate compensatory activities, such as vomiting. Four diagnostic criteria for BN are listed in DSM-IV:
- recurrent binge eating (at least twice per week for 3 months)
- recurrent purging, excessive exercise, or fasting (at least twice per week for 3 months)
- excessive concern about bodyweight or body shape
- the absence of anorexia nervosa (Table 103.1).

Although a common component, vomiting is not mandatory to the diagnosis. Other compensatory activities that may be employed in BN include abuse of laxatives, diuretics, or enemas, or excessive exercise or fasting. Because fasting behavior can be encountered in BN, just as binge–purge behavior can be seen in AN, the phenotypic expression of these two conditions can exhibit considerable overlap.

Table 103.1 Diagnostic criteria for anorexia nervosa and bulimia nervosa

A. Anorexia nervosa
1. Inability to maintain a minimally appropriate bodyweight, as indicated by bodyweight < 85% of expected for age
2. Intense fear of weight gain
3. Disturbance in the perception of one's own body shape, size, and weight
4. Amenorrhea lasting for three consecutive months in females who are postmenarche

B. Bulimia nervosa
1. Recurrent episodes of binge eating as defined by eating an excessively large amount of food within a discrete period of time, with these episodes often associated with individuals feeling a lack of control over their eating
2. Recurrent, inappropriate, compensatory behaviors to avoid weight gain (e.g., self-induced vomiting, misuse of laxatives, diuretics, enemas, or other medications, or excessive exercise)
3. Binging and purging behaviors occur at least twice a week for 3 months
4. Body shape and weight unduly influence one's self-evaluation

Adapted from American Psychiatric Association [8], with permission.

Could anorexia nervosa and bulimia nervosa share an underlying pathophysiology?

Bulimics and anorexics are different in one conspicuous way: bulimics are normal weight, whereas anorexics are underweight. In addition, cycles of binge eating and subsequent compensation, such as cathartic-induced purging and vomiting, are necessary for the diagnosis of BN. However, could this separation by phenotype be an artificial one? In her original description of AN, Bruch included binging and purging behavior as a part of the condition [22], and Russell presented bulimia as a special form of anorexia [23]. Could there be a common etiology?

Sodersten et al. suggest that AN and BN represent a continuum of the same underlying process [24]. Furthermore, there is a newer diagnostic category of "eating disorder – not otherwise specified" [8], which suggests a difficulty in drawing a clear distinction between AN and BN in some individuals. The significant overlap between AN and BN is further highlighted in DSM-IV by its attempts to subclassify eating disorders. Anorexia nervosa can be divided into a restricting type and a binge-eating/purging type [8]. Similarly, BN can be classified as either a purging type or nonpurging type. This chapter will discuss the different eating disorders as different clinical manifestations of the same disease, falling within a spectrum of phenotypes for the same underlying pathophysiological process. We will use the term anorexia nervosa (AN) broadly to cover the range of eating disorders described in DSM-IV.

Three cardinal features of eating disorders

Distorted body image

Ever since Bruch described a distorted body image as a core feature in AN [22] there has been debate about the specific nature of this disturbance [25]. Generally, two components

have been described as making up this body-image dysfunction, namely a perceptual body size distortion and a cognitive evaluation dissatisfaction [25]. A perceptual distortion is manifested as an abnormality in accurately gauging one's body size. A cognitive evaluation dissatisfaction involves a negative attitude towards one's size, that is, body dissatisfaction. Although a distorted body image has long been associated with eating disorders, there are data that argue against this feature being specific to individuals with eating disorders. One study reported greater body uneasiness and inappropriate attitudes towards eating in participants of recreational sports, such as body-building, dancing, and gymnastics, which emphasize muscularity and thinness [26]. In some individuals, might a distorted attitude of body image take root in the fact that a specific body shape may be a required component of their recreational endeavors or occupations? Other studies have reported increased body dissatisfaction in female dieters who do not have an eating disorder [27–29], particularly when hungry [30] and after consumption of a high-caloric drink, such as a milkshake [31,32]. These data support the view that body dissatisfaction is not unique to patients with an eating disorder.

Alteration in processing of signals of hunger and satiety

Patients with eating disorders have reported low levels of hunger and heightened levels of fullness [33]. Although these findings suggest an alteration in how the brain processes signals of hunger and satiety as a contributing cause of an eating disorder, experiments in obese subjects suggest another possible explanation [34]. Specifically, after a 3-week, near-fasting diet of only 840 kJ/day (200 kcal/day), hunger scores in these obese patients decreased to nearly zero, which argues that prolonged food deprivation leads to physiological loss of the desire to eat [35]. In states of malnutrition, delayed gastric emptying may account for early satiety [36], raising the possibility that loss of hunger and greater satiation may not be unique to patients with an eating disorder.

A period of diminished food intake often precedes binge eating [37]. Observations from prisoners of war after World War II have provided some insight into feelings of satiety during the resumption of eating after prolonged starvation. When refeeding of the prisoners was undertaken, they demonstrated lower levels of satiety and dramatically increased rates of food intake, with this increased drive to eat lasting for several weeks [38]. Thus, resumption of caloric intake after long-term food deprivation may be associated with a diminished satiation and binge-eating in some instances [37].

Feelings of ineffectiveness and other psychological changes

Bruch described a paralyzing feeling of "ineffectiveness" in her anorexic patients, creating a sense of helplessness, passivity, and fear of losing control [39]. Many other psychological disturbances have been associated with AN, including irritability, depression, phobias, inertia, magical thinking, difficulty with concentration, and insomnia [40]. The coexistence of depression or dysthymia has been reported in 50%–75% of patients [41]. In one series, about two-thirds of patients with an eating disorder had one or more lifetime anxiety disorders, with obsessive-compulsive disorder being the most common, affecting ~ 40% of anorexics [42]. An increased risk of substance abuse and personality disorders has also been described [2]. Because anxiety disorders, often with features of obsessive-compulsive features, have long been recognized as a common feature of AN, some assert that these are different phenotypic expressions of related mental illnesses and that there may be an evolution from one phenotype to another. Sodersten et al. questioned the hypothesis that an underlying genotype would manifest as an obsessive-compulsive disorder (OCD) during childhood, with a phenotypic progression to AN during adolescence, and then ultimately to an anxiety disorder later in life, by citing the higher incidence of OCD in boys, which contrasts with a higher incidence of AN in girls [43]. In general, increased rates of psychological distress, including feelings of ineffectiveness, have been described in dieters, demonstrating that these feelings are not unique to patients with an eating disorder [44,45].

Natural history of eating disorders

There are no formal definitions regarding what constitutes recovery from an eating disorder [46], which creates some ambiguity when comparing data on long-term outcomes. The goals of treatment are generally said to be two-fold: weight gain and normalization of eating patterns. It is reported that about half of individuals with AN will recover from their illness, 30% will demonstrate residual symptoms (which may fluctuate over time), and 10% will demonstrate more severe, persistent, and unrelenting symptoms [47]. The remaining 10% will eventually die from complications of AN, giving an overall mortality rate of 0.56% per year [48]. Others have reported an even lower rate of sustained remission, of only 33% [49]. During one long-term follow-up study lasting 21 years, 16% of patients died of disease complications [50].

Regarding bulimic activity, studies suggest that patterns of aberrant eating habits usually wax and wane over the course of many months to years. Similar to AN, 50% of bulimics will eventually recover from their illness. Twenty to thirty percent suffer continued, persistent symptoms. An additional 30% who had achieved remission experience a relapse [51]. Mortality in eating disorders is most commonly associated with complications of severe malnutrition.

Biology of response to caloric restriction

A fluctuating food supply challenges the homeostasis of all animals. As such, semistarvation is a key stressor. What is the expected response to this stress? How does this response compare with the changes seen in AN? Similarities between the biology of malnutrition in underfed, otherwise normal individuals and those with eating disorders have been described [52]. The body's two main physiological imperatives necessary for survival during starvation are provision of a continual supply of energy to the brain, in the form of glucose, and conserving the body's protein stores [53–55]. The physiological changes described in starvation associated with AN are analogous to those changes seen in many other states of malnutrition and starvation.

Energy expenditure

The resting energy expenditure (REE) of the body is decreased as lean body mass is reduced during the starvation of AN. This may, at least partly, be influenced by diminished secretion of thyroid hormones (T_3 and T_4). The muscle unit in an anorexic patient also expends less energy than normal muscle, with a consequent decreased efficiency in work performed [56]. Following refeeding, undernourished muscle demonstrates a "superefficiency." The total energy expenditure (TEE) is similar in anorexics and controls, and for this to be the case there must be an increase in activity-related energy expenditure to offset a reduced REE [57]. The decreased REE and improved economy of muscle function allows anorexics to maintain an increased level of activity with relative conservation of energy.

Using the body's stored calories during inadequate caloric intake

A regular supply of nutrients is normally required to ensure access to all three forms of energy for the body: fat, carbohydrates, and protein. During periods when the body's energy needs are not being met by oral intake, stored fat, protein, and glycogen are mobilized to make up this shortcoming. As starvation progresses, the body switches from primarily using glucose, derived from amino acids by gluconeogenesis, to using ketone bodies, derived from fatty acids [58]. The fat stores harbor a much greater potential to meet the body's energy needs than glycogen or protein. Most tissues can utilize fatty acids and ketone bodies, except nervous tissue, erythrocytes, and the immune system, which still need glucose. The plasma ketone levels rise dramatically with advancing starvation. When plasma levels reach a certain threshold, the brain can begin using ketone bodies as an alternative energy source [59]. Likewise, with advanced starvation, muscle tissue adapts so it can utilize ketone bodies and free fatty acids for energy, which induces a perpetual low-insulin state. The body strives to conserve its protein stores. Early in the process of starvation, 10 g N/day is lost from protein stores, falling to 3 g N/day once the body shifts to the utilization of ketones and fatty acids as the primary energy source [60].

Searching for calories in the face of starvation

Throughout history, food has been inconsistently available to humans, with periods of famine and starvation that vary in length. During famine, the need for survival will reinforce behavior that is conducive to this end. Increased physical activity, increased focus and preoccupation with food, and the motivational drive from "obsessive" thinking and thoughts of desperation would all serve the individual well, from an evolutionary standpoint, in the pursuit of food.

Hyperactivity

Keys published one of the sentinel works examining the effects of starvation in humans [61]. This study enrolled 36 male conscientious objectors to significant caloric deprivation (~ 40% of normal intake) for an average of 168 days. These individuals were normal weight at baseline and young (range 20–33 years), and lost 24% of their baseline bodyweight down to an average body mass index (BMI) of 17.5. The effects of prolonged semistarvation were recorded in detail. During their caloric restriction, the subjects were reported to have increased levels of hyperactivity.

Similar behavior has been described in eating disorders. Remarkably, despite weight loss and food deprivation, patients with eating disorders often exhibit exaggerated levels of physical activity [62]. This is a phenomenon that is seen in experimental animals as a response to food deprivation [60]. The rat model has been the best studied, in which the imposition of food restriction on rats leads to increased activity [62]. When an exercise wheel is available, rats deprived of food will use the wheel to the point of markedly accelerating weight loss. From an evolutionary perspective, this would be an advantageous response in the face of a food shortage.

Impulsivity and obsession

An evolutionary adaptation to resource scarcity is a heightened sensitivity to risk. As a self-protective mechanism, impulsive behavior may convey an advantage, as the appropriate adaptation to scarcity of food [63]. In his 1950 study, Keys observed markedly altered behavior in his semistarved subjects, including hyperirritability, impulsive behavior, and violent ideations, which emerged with progressing weight loss [43,61]. He also reported depression in many of the malnourished subjects, and during their refeeding there was a linear relationship between improved nutritional status and improved symptoms of depression. Sodersten et al. explain

that an "obsession" with food would make sense during a period of significant food deprivation [43]. With ongoing starvation, a preoccupation with food would prompt the individual to devote more time to searching for food. Therefore, might the obsessive thinking associated with an eating disorder represent an understandable adaptation to food deprivation, rather than indicating a true psychiatric illness [43]?

The presence of anxiety in those with eating disorders is also well described. A fear of becoming fat is listed as a key diagnostic criterion in DSM-IV [8]. Can the anxiety in AN be explained as a correlate of a fear of becoming fat? Sodersten et al. propose that the cognitive recognition of an "obsession" with food, which arises with extreme fasting, may transform into a fear of acting on this obsession, in which case obesity becomes the undesirable outcome to avoid [43]. The desire to avoid an aversive outcome (i.e., becoming fat) can become reinforced by the preoccupation with food seen in eating disorders, and this persistent desire becomes anxiety-provoking.

Mechanisms explaining hyperactivity and anxiety in anorexia nervosa

Role of low blood glucose

The stress of restricted-feeding regimens on rats and the resulting hyperactivity has received significant attention since it was first reported in 1954 [64]. In this original description, the rats demonstrated abnormal behaviors similar to obsessive-compulsive behaviors witnessed in semistarved humans with AN. In these experiments, gastric ulcers were seen as another sign of stress. Takeda et al. subjected rats to 7 days of a food-restricted diet while allowing for continued access to either water or a glucose solution of varying concentration [65]. Prevention of hypoglycemia with glucose consumption inhibited many of the stress-induced outcomes in the rats, suggesting that hypoglycemia is one of the driving forces for the abnormal behavioral and physical responses in this rat model.

"Obsessive" behavior and OCD have long been recognized in individuals with AN [41,66]. Diminished food intake, weight loss, and hyperactivity are often elements of OCD, as they are in AN. Elevated cerebrospinal fluid (CSF) levels of corticotropin-releasing hormone (CRH) and vasopressin are found in patients with AN and patients with OCD, suggesting a common disturbance [67,68]. Based on positron emission tomography (PET) scans showing alterations in glucose metabolism in the brains of individuals with OCD [69], abnormally low serum glucose levels may play a role in the pathogenesis of OCD.

Role of hypoleptinemia

As the neuroendocrinology of the body's adaptation to starvation has become better understood, the role of leptin in this process has been a source of tremendous interest. Leptin was discovered in 1994 as a secretory product of adipose tissue. The original studies showed that leptin-deficient mice were subject to overeating and obesity [68], so leptin was assumed to mediate a negative feedback to prevent overeating. Subsequent studies in humans failed to confirm leptin deficiency to be a cause of human obesity [70]. Our understanding of leptin is evolving, and it is known to be an important mediator of some biological changes of starvation [70–72].

Serum leptin levels are generally higher in obese individuals [70]. By contrast, starvation is associated with low levels of leptin. Leptin levels drop dramatically after only 2–3 days of restricted nutritional intake [72,73]. Low leptin levels act as a signal to the hypothalamus of a reduced-energy state [71,74]. As the control center of many hormonal pathways, the hypothalamus directs a shift from energy use to energy conservation. Levels of thyroid and reproductive hormones fall. Studies of healthy men who fasted for as little as 2–5 days reported reduced serum luteinizing hormone (LH) and testosterone concentrations [75,76]. The role of leptin in hormonal feedback control was demonstrated when exogenously administered recombinant leptin (r-metHuLeptin) prevented the fall in LH and testosterone levels seen with fasting [72]. In contrast to the fall in sex hormone levels, levels of stress hormones rise as part of an attempt to mobilize energy stores. Likewise, growth hormone (GH) levels rise, but without a corresponding rise in insulin-like growth factor-1 (IGF-1). During starvation, GH induces lipolysis, which allows utilization of the body's alternative energy reserves. Because IGF-1 levels remain low, the body's energy-consuming, growth-related pathways are not stimulated [74]. As a whole, the body is diverting energy to pathways most critical for survival.

Intense exercise is associated with amenorrhea in many female athletes [77]. The combination of strenuous physical activity and inadequate nutritional intake creates a relative energy deficiency. This energy imbalance results in a hypothalamic amenorrhea. Leptin has been implicated as a regulatory agent in this process. These athletes have a low-leptin state, similar to the semistarved state of AN, which results in lower levels of sex hormones, thyroid hormones, and IGF-1, but increased secretion of cortisol and GH [78–80].

In addition to leptin, adipose tissue secretes a number of other biologically active proteins, often referred to as adipocytokines. Adiponectin is one such protein that is receiving increasing attention; it is involved in regulating energy homeostasis and glucose and lipid metabolism [81]. Adiponectin levels are low in obese individuals [82], and weight reduction increases the serum concentration of adiponectin in obese rats and humans [83,84]; therefore, its plasma level rises with a decreasing BMI. Several studies have reported elevated plasma adiponectin in AN [85,86], but at least one

other study has described lower levels of adiponectin in AN and BN compared with normal-weight controls, regardless of BMI [81]. However, these levels were restored after weight gain in the anorexic group, suggesting that feeding behavior may also influence adiponectin levels in eating disorders. A complex interplay of low leptin and high adiponectin exists in AN, mediating insulin sensitivity, neuroendocrine abnormalities, and osteopenia [87].

Leptin secretion may increase with the resumption of eating after a prolonged fast [88]. Such increased secretion limits food intake and may arrest weight gain as a means of averting obesity, which would be the undesirable outcome of an overcorrection in caloric intake after a period of starvation. It is suggested that by this mechanism leptin might be a factor in the relapse of anorexia in some patients [88]. Several studies have also identified a role for hypoleptinemia in hyperactivity and impulsivity in rats and humans [89,90]. This is corroborated by animal data showing that intracerebroventricular administration of leptin suppresses the hyperactivity associated with the semistarvation state in rats [91].

Role of elevated neuropeptide Y

The body strives for homeostasis with regard to its weight by either stimulating or suppressing the drive to eat through changes in the signals from the gut and energy stores [92]. Anorexia nervosa does not function in this way. It is a condition of starvation, yet anorexics are not intrinsically stimulated to eat. Some have suggested that the actions of many orexigenic (stimulating food intake) and anorexigenic peptides are more complex than initially appreciated and may be partially dependent on the physiological context [43].

One of the best recognized orexigenic peptides is neuropeptide Y (NPY). High leptin levels reduce the production of NPY by the hypothalamus, which would be the expected result for the hyperleptinemia of obesity [93]. Yet, this does not lead to less eating for many of these individuals. The classic theory of bodyweight regulation asserts that external signals are processed by hypothalamic centers, which then adjust food intake. Reduced leptin levels induce an upregulation of orexigenic peptides, including NPY, which should increase nutritional intake [94–96]. In AN, however, these changes do not stimulate eating [93,97].

Insights from animal data suggest a different biological action of NPY that may explain why higher levels of an orexigenic peptide may not be associated with stimulation of food intake. Food deprivation increases the level of NPY mRNA in the hypothalamic arcuate nucleus of female rats. The most striking characteristic of these rats was that they engaged in progressively more running as food deprivation continued, and those provided with an exercise wheel lost more weight than animals without access to an exercise wheel [98]. Administration of a NPY Y1 receptor antagonist to rats blunted the effect that food deprivation had on stimulating wheel running, demonstrating the role of NPY in the hyperactivity response [99]. Correspondingly, increased physical activity was reproduced with intracerebroventricular administration of NPY to these rats without stimulating increased food intake. Interestingly, in the situation of food-deprived rats that did not have access to an exercise wheel, but did have continual access to food, exogenous NPY did stimulate food intake [98]. These results are consistent with the suggestion of Morley et al. in 1987 that NPY may have a stimulatory effect on behavior [100]. These findings in experimental animals, whereby NPY (an orexigenic peptide) paradoxically decreases food intake in the setting of stimulating physical activity, fit into a model of a complex interplay of behavioral, physiological, and biochemical adaptations to starvation in humans [101].

Neuropeptide Y has also been implicated in food-anticipatory activities, including hoarding behavior [102–105]. Evolutionarily, such anticipatory behavior has been explained as a logical precursor to the impending search for food. Anorexics have been reported as engaging in similar anticipatory behavior [106].

Explanation for female predominance

Is there an explanation as to why women are so much more susceptible to developing an eating disorder than men? Differences in energy homeostasis and utilization of substrates between the genders have been claimed [107]. Studies have also asserted that there is a difference between men's and women's abilities to withstand starvation [61,107,108]. Sodersten et al. reported such a difference in a cohort of 30 (17 women and 13 men) high-school-aged subjects. The quantity of food consumed by each gender was measured at baseline and then after skipping two successive meals. After this short-term fast, the amount consumed by the male subjects on resuming eating was markedly greater than their baseline, whereas the women actually ate less food after this short-term fast [24]. The authors felt this might suggest an inherent difficulty for females to compensate for a period of fasting by increasing their food intake. Therefore, dieting may be more risky for females than males. Dieting may start an individual down the road of reduced caloric intake leading to physiological changes of starvation. Once this progression begins, it may be particularly difficult for females to reverse this process.

Medical complications of eating disorders

Potentially serious medical complications may result directly from the malnutrition associated with food deprivation, and indirectly from metabolic derangements following purging

Table 103.2 Laboratory abnormalities seen in eating disorders

Anorexia nervosa
Hypokalemia, hyponatremia, metabolic alkalosis
Metabolic acidosis
Hypoglycemia
Mildly increased creatinine
Anemia, thrombocytopenia
Mild leukopenia
Low erythrocyte sedimentation rate
Mildly increased hepatic aminotransferases
Mildly increased serum proteins
Hypercholesterolemia
Low alkaline phosphatase
Decreased TSH, T_3, and T_4
Decreased FSH, LH, and prolactin
Elevated cortisol
Low magnesium, phosphorus, and zinc levels
Carotenemia
Bulimia nervosa
Hypokalemia, hyponatremia, hypochloremic alkalosis
Metabolic acidosis
Hypoglycemia
Azotemia
Elevated cortisol
Increased prolactin
Low magnesium

FSH, follicle-stimulating hormone; LH, luteinizing hormone; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid-stimulating hormone.

Table 103.3 Medical complications of eating disorders

ANOREXIA NERVOSA
Neurological
Depression
Sleep disorders
Decreased attention and concentration
Diminished learning and problem-solving
Decreased short-term memory
Labile moods
Cardiovascular
Cardiac arrhythmias
Sudden death
Bradycardia
Hypotension
Mitral valve prolapse
Left ventricular dysfunction
Pulmonary
Aspiration pneumonia
Ventilatory failure
Gastrointestinal
Impaired gastric emptying/early satiety
Postprandial bloating
Constipation
Decreased motility
Hypogeusia
Abdominal pain
Pancreatitis
Peptic ulcer disease
Superior mesenteric artery syndrome
Renal
Prerenal azotemia
Mesangial sclerosis
Nephrolithiasis
Endocrine
Amenorrhea
Decreased libido
Osteopenia and pathological fractures
Impaired functioning of the hypothalamic–pituitary–adrenal axis
Diabetes insipidus
Hypothermia
Increased growth hormone accompanying low levels of insulin-like growth factor (IGF-1)
Decreased insulin production
Metabolic
Decreased metabolic rate
Increased β-hydroxybutyrate
Immunological
Decreased cell-mediated immunity
Granulocyte dysfunction
Decreased serum complement
Altered cytokine levels

behaviors common to these patients. Although still controversial, most investigators in this area support the idea of an increased risk of premature death in people with eating disorders, with a mortality odds ratio (OR) of 10.5 (95% confidence interval [CI], 5.5–15.5) [109]. The medical complications of eating disorders are generally identical to those reported in healthy individuals placed on a semistarvation diet, with changes affecting every organ system, including the skin, digestive, cardiovascular, respiratory, skeletal, renal, immune, and endocrine systems [110,111] (Tables 103.2 and 103.3).

Gastrointestinal complications

Hypogeusia (decreased taste acuity), delayed gastric emptying, loss of digestive capacity, and constipation are the most common complications of the digestive tract in anorexics and bulimics [112–114]. A reduced sensitivity to sweet, salty, sour, and bitter flavors was demonstrated in anorexics [115]. Individuals who specifically employed vomiting as their primary means of purging, also demonstrated a similar loss of sensation on the tongue [116], perhaps related to the frequent exposure of their mouths to the acidity of the gastric contents. In addition, low serum levels of zinc, copper, and

Table 103.3 (*cont'd*)

Musculoskeletal
Decreased muscle mass and weakness
Proximal myopathy

Dermatological
Lanugo hair
Brittle nails
Pruritus
Acrocyanosis

BULIMIA NERVOSA
Neurological
Seizures (diet pill toxicity)
Neuromyopathy (ipecac toxicity)

Cardiovascular
Arrhythmias
Hypotension
Mitral valve prolapse
Palpitations

Pulmonary
Aspiration pneumonia
Pneumomediastinum

Gastrointestinal
Constipation
Cathartic colon
Dysphagia
Esophagitis
Esophageal ulcer, stricture
Mallory–Weiss tear
Abdominal pain/dyspepsia
Gastroparesis
Pancreatitis
Gastric rupture

Renal
Pseudo-Bartter syndrome
Hematuria
Proteinuria
Azotemia

Endocrine
Amenorrhea or irregular menses
Absence of follicular development
Luteal phase dysfunction
Increased rates of birth complications and miscarriages

Oral/dental
Caries
Perimolysis – lingual and occlusal surfaces
Cheilosis
Pharyngeal soreness
Sialadenosis
Salivary hyperamylasemia

Dermatological
Russell sign (i.e., calluses over fingers)

triiodothyronine (T_3 hormone), which many anorexics demonstrate, have been implicated as influencing taste [115]. Often, there is also a loss of dental enamel [117,118], with an increased rate of caries formation and increased sensitivity to hot, cold, and acidic foods [119,120].

Numerous studies have documented a delay in gastric emptying in AN [121–124]. A lack of food in the stomach may cause the delay [125]. Gastric smooth muscle atrophy may result from protein malnutrition [126]. Other proposed mechanisms include the presence of gastric dysrhythmias [124] and a reflexive inhibition of gastric emptying by the rectal distention of constipation [127]. The data are somewhat conflicting as to whether weight gain improves the delay in gastric emptying: some studies demonstrated improvement after weight gain [128–130] whereas others did not [124,125]. Delayed gastric emptying is associated with symptoms of bloating, early satiety, and abdominal distention [125,128,130]. These symptoms may prove to be an impediment to refeeding and weight gain, as eating may increase physical discomfort and exacerbate bloating and distention, reinforcing a perception of feeling "fat" [113]. A similar association of delayed gastric emptying with BN has also been reported in some studies [131,132], but not in others [125,133,134]. Geliebter et al. report that bulimics require a larger volume of gastric balloon distention to produce a feeling of fullness [131]. Proposed explanations for this include abnormalities in gastric relaxation and diminished release of the anorexigenic signal cholecystokinin (CCK) [135–137].

Constipation is also a common problem encountered in AN, with diminished oral intake contributing to this. Anorexics frequently complain also of bloating and abdominal distention [113,127]. Whole-gut transit times were found to be delayed in AN and BN compared with controls [127], and an association with abnormalities in anal canal resting pressure and rectal sensation have also been suggested [138]. The delayed colonic transit times improved with refeeding.

Cardiovascular complications

Potentially the most serious cardiac complication in AN is the prolongation of the Q–T interval, a marker for certain arrhythmias and sudden cardiac death [139]. Several studies have linked AN with a prolonged Q–T interval [140–143] and an increased risk of ventricular arrhythmias [142]. Bradycardia and hypotension are two additional abnormalities associated with AN. One series showed that 100% of anorexics had a sinus bradycardia with a heart rate of less than 60 beats/min compared with controls [144]. Several causative mechanisms for this bradycardia have been proposed, including vagal hyperactivity of starvation [143,145,146] and reduced secretion of T_3 hormone [143]; electrolyte abnormalities, a decrease in myocardial glycogen stores, and myocardial cell atrophy may be additional aggravating factors [141].

Endocrine complications

The hormonal changes that are observed in AN are best understood as an adaptation to malnutrition and starvation rather than a manifestation of a primary endocrinopathy [147]. Normal hypothalamic functioning is impaired in the setting of a significant energy deficit. The role of leptin in this process has already been described (see "Role of hypoleptinemia" above). Whereas amenorrhea is the consequence of this hypotropic hypogonadism in women, males manifest a diminished libido [148]. Teleologically, the body is attempting to divert energy from the reproduction pathway to other processes more crucial for survival. The hypothalamic dysfunction of AN also disrupts thyroid hormone secretion, with the resulting hypothyroidism contributing to lower body temperature, lethargy, dry skin, constipation, and bradycardia.

Feeding is a chief regulator of the daily rhythms of the hypothalamic–pituitary–adrenal (HPA) axis, which mediates secretion of plasma corticosteroids [52]. Persistently elevated serum cortisol levels are reported in anorexics, with an abolition of the normal daily circadian rhythm observed in healthy individuals. Similarly, an elevated 24-hour urinary cortisol has been described in bulimics [149]. There is blunting of the CRH-induced response of adrenocorticotrophic hormone (ACTH) and cortisol in AN and BN [149–151]. Because bulimics are normal in weight, these changes may be independent of BMI. Yet, many of the changes do largely reverse with increased nutritional intake. As is seen in AN, CRH hypersecretion is also described in depression and is suggested as a common pathogenic mechanism to explain some of the clinical similarities of these conditions [150].

Anorexia nervosa is also associated with reduced bone mineral density in adolescent and adult females [152–154]. Although the degree of estrogen deficiency may be similar in AN and other causes of hypothalamic amenorrhea, anorexics have a much greater reduction in bone density, which observation argues for other contributing factors [155], such as malnutrition, excessive exercise, hypercortisolemia, acquired GH resistance, and low levels of IGF-1 [151,154]. Secretion of GH by the pituitary is nutritionally regulated and stimulates hepatic secretion of IGF-1, which functions as a bone trophic factor. However, IGF-1 levels remain low in AN, despite elevated levels of GH [151].

Treatment

Basic approach to treatment

A multidisciplinary team, including an internist, psychiatrist, dietician, and also, in some cases, a gastroenterologist, endocrinologist, and psychotherapist, is advocated as the optimal approach in managing eating disorders [156]. Intervention and counseling by the primary physician alone are adequate in only a few cases involving younger patients with a short duration of symptoms. Most patients, particularly those with more serious and long-standing symptoms, benefit from referral to a psychiatrist who is knowledgeable about eating disorders [149]. Indications for mandatory psychiatric referral include a failure to respond to current treatment with clinical deterioration, significant underlying depression with suicidal behavior, or serious family issues.

It is reported that 50% of patients with AN can be successfully treated in the outpatient setting [157]. Patients with a higher probability of successful outpatient treatment include those who are younger, have a shorter duration of disease, and have cooperative families. There are no randomized controlled trials (RCTs) comparing inpatient with outpatient treatment to guide this decision [158]. A structured, inpatient program is recommended for those who are 25%–30% below a healthy bodyweight [159].

The general physician's primary role is nutritional management, which includes following serial weights and labs, educating, counseling, and recognizing the need for referral [149]. Initial treatment for AN should focus on medical stabilization and weight restoration [156]. Adequate nutritional replacement can be achieved by the oral route in most; only in rare instances is nasogastric feeding or hyperalimentation needed. As a rule, caloric supplementation is progressively increased to achieve weight gain. In severely malnourished individuals, the clinician must be cautious with the rate of nutrition reintroduction to avoid the electrolyte abnormalities and other complications of the refeeding syndrome.

A multivitamin preparation with vitamin D and supplemental calcium should be part of the nutrition plan. Exogenous estrogen and progesterone have not demonstrated any meaningful improvement over placebo in bone density [160]; these agents should not be given to adolescents who are still growing. Excessive exercise should be discouraged [161]. A crucial component of a nutritional rehabilitation program is to correct the patient's distorted views towards eating and dieting [149].

Psychiatric approach: treatment directed at psychological change

The association of eating disorders with other psychiatric diagnoses is well described, such as anxiety and AN, or depression and BN. Many of the tools and treatments traditionally used for these psychiatric conditions have been tried for eating disorders, particularly in the setting of individuals with coexisting psychiatric diagnoses [162]. Selective serotonin reuptake inhibitors (SSRIs) are commonly used in the treatment of both AN and BN. Randomized controlled trials of fluoxetine have not established clear efficacy with improved clinical outcomes for AN [163–168]. Severely malnourished individuals have diminished synaptic release of serotonin (5-hydroxytryptamine; 5-HT), which may account for a decreased effect of the SSRIs in AN [156]. As a general rule, the American Psychiatric Association does not endorse

the use of SSRIs in the treatment of significantly underweight patients with AN [169]. Published reviews on the pharmacological management of AN acknowledge that no medication to date has demonstrated a significant impact on weight gain or the psychological features of AN [170,171]. However, although evidence about their efficacy in AN is lacking, SSRIs are well tolerated and are frequently employed as therapy directed at depressive and obsessive-compulsive symptoms [156]. For BN there is better evidence that high-dose fluoxetine decreases binging and purging and may reduce relapse at 1 year [172].

The efficacy of other psychotropic drugs in eating disorders has been examined. For example, amitriptyline, cyproheptadine, and clomipramine did not demonstrate consistent clinical improvement in AN [173–175]. However, limited data suggest that the tricyclic antidepressants (TCAs) may reduce vomiting episodes in BN [172]. The TCAs are associated with constipation, hypotension, and certain cardiac arrhythmias, side effects that are particular concerns in AN. Furthermore, based on anecdotal experience, antianxiety and "atypical" antipsychotic medications have been tried in some patients with AN [176].

Many forms of behavioral interventions have been tried for eating disorders, including cognitive, dynamic, family, individual, and group therapy. One study suggested that cognitive behavioral therapy (CBT) reduced the rate of relapse and led to improved outcomes in those who had already achieved inpatient weight gain [177]. The efficacy of CBT in the acute setting of still-underweight anorexics is less clear [171]; yet, in general, CBT is assumed to be helpful in AN [178]. For BN there is relatively better evidence that CBT should be the primary treatment [172]. Family therapy has not been shown to be effective in adolescents suffering from AN [179], but may be beneficial in younger patients with a shorter duration of symptoms [180,181]. Overall, the research on psychotherapy in AN is limited [182], and the combination of pharmacological and behavior therapy has not been thoroughly studied [171].

The course of AN is often protracted, with suboptimal long-term outcomes in many [47,183]. The framework on which most treatment is based is that an eating disorder is an inherent psychiatric abnormality. In many instances, this approach has resulted in only marginal improvements. Perhaps developing a new framework to understand eating disorders will open new avenues, down which future treatment may be directed.

Biological approach: treatment directed at the response to starvation

Anorexia nervosa may primarily represent a perturbation in the biological response to caloric restriction, which is an alternative framework for trying to understand the diagnosis. The cascade of events in this process is as follows. Voluntary caloric restriction is undertaken, perhaps prompted by societal influences, or the subject's occupation or athletic pursuits. As a pronounced energy and nutritional deficit develops, hypoleptinemia ensues, influencing the body's hypothalamus-directed hormonal axes [74]. Levels of NPY rise in response to hypoleptinemia, driving hyperactivity [98]. Hypoglycemia also may contribute to impulsive behavior. Furthermore, the individual is driven to continue the pattern of decreased food intake and hyperactivity by the activation of the dopaminergic reward pathway in the brain [184]. It is hypothesized that these behaviors become entrenched within the individual, as a perceived "reward" leads to conditioning of these behaviors. In patients experiencing the acute physiological consequences of malnutrition, it may not be surprising that a traditional approach of medication and psychotherapy is often only minimally effective in changing behavior [185,186].

Can a biological framework for understanding AN lead to more effective treatments? Anorexics often demonstrate significantly increased levels of exercise and participation in sports, even predating their diagnosis [187]. Increased physical activity may be a crucial component in the pathogenesis and progression of AN. The euphoria associated with physical activity is well described and, thus, serves as a positive driver to the perpetuation of the eating disorder [188]. Hyperactivity seems then to be a key component of AN, because increased physical activity results in a greater metabolic demand, and this alone hampers efforts to successfully gain weight. In the rat model of AN, providing access to an exercise wheel while instituting food restriction, was key for beginning the cycle of uncontrollable weight loss [98]. In the rat given NPY, stimulated eating (the expected effect of an orexigenic peptide) was suppressed when the animal had access to an exercise wheel but was unmasked when the wheel was removed [98]. Thus, the two main physiological risk factors for AN and BN may be dieting and hyperactivity [188]. Although treatment recommendations for anorexics often include discouraging overly excessive exercise, might treatment outcomes in AN be more successful if strict attention is given to limiting increased physical activity, so as to alter the physiological response to NPY?

Intriguing preliminary research exists in which investigators utilized a biological framework for understanding AN to devise an innovative, multifaceted treatment program [186,189]. Their approach used a computerized device to help patients relearn how to eat and how to recognize satiety. Patients' activity was strictly limited. They were also treated with external warmth, which some studies suggest further reduces activity and anxiety in the individual [91,186,190]. A randomized trial from 2002 reported that 75% of patients treated in this manner were able to achieve a normal BMI over an average of 14 months of follow-up [186]. Even more remarkably, only 10% of enrollees demonstrated relapse after 5 years. The authors felt that these results support the contention that a conceptual shift to a more biologically

based treatment program, including strict attention to controlling physical activity, is needed to improve clinical outcomes in treating eating disorders.

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I MISCELLANEOUS

104 Complications of AIDS and other immunodeficiency states

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The gastrointestinal tract plays a fundamental role in the pathogenesis of the acquired immunodeficiency syndrome (AIDS). Consequently, it is not surprising that gastrointestinal complications are common in patients infected with human immunodeficiency virus-1 (HIV-1), the causative agent of AIDS (Table 104.1). Infectious processes, particularly in resource-limited settings, comprise the majority of these complications and are responsible for relentless morbidity and, in some cases, mortality [1,2]. This chapter focuses on the intestinal infections and key gastrointestinal syndromes associated with AIDS and other common immunodeficiency states.

Role of the gastrointestinal mucosa in HIV-1 pathogenesis

All HIV-1 infections, excluding those acquired parenterally, are transmitted across a mucosal surface [3]. In addition to their role in HIV-1 transmission, mucosal surfaces participate in many key events in HIV-1 pathogenesis. First, mucosal cells are probably involved in selecting from the pool of inoculated viruses the phenotypically and genotypically restricted HIV-1 that is transmitted in acute infections [4–6]. Second, mucosal (lamina propria) lymphocytes are the initial target cell for HIV-1 infection [7–9]. Third, HIV-1 replication occurs in the mucosa [10–13], a microenvironment containing an abundance of cytokines capable of enhancing HIV-1 replication in infected cells [14]. Fourth, the subsequent decline in the number of circulating $CD4^+$ T lymphocytes, a fundamental characteristic of AIDS, begins in the intestinal mucosa [15–19]. Finally, local and systemic reduction in $CD4^+$ T cells, and related cellular and humoral perturbations, predispose the gastrointestinal tract mucosa to an array of opportunistic infections and neoplasms. Consequently, the crucial role of mucosal immunity in HIV-1 infection has gained prominent consideration in vaccine strategies designed to prevent HIV-1 transmission [20,21].

Diarrheal diseases

HIV-1-related diarrhea may first occur during the syndrome of primary HIV-1 infection 1–4 weeks after virus inoculation [22–25]. This mononucleosis-like syndrome, characterized by fever, sore throat, rash, lymphadenopathy, fatigue, weight loss, and myalgias, occurs in 30%–90% of persons acutely infected with HIV-1 and lasts for 1–3 weeks. Acute, self-limited diarrhea occurs in approximately one-third of persons with the syndrome [24]. Less frequent gastrointestinal manifestations of acute HIV-1 infection include thrush and ulcerations of the oral cavity, esophagus, and rectum, as well as enteropathy and weight loss. Resolution of the syndrome coincides with seroconversion and decline in the level of circulating virus [24].

Chronic diarrhea, defined as two or more loose or watery stools per day for at least 1 month, is common in patients with clinically apparent AIDS. Such patients have lower numbers of circulating $CD4^+$ T lymphocytes and a higher incidence of extraintestinal opportunistic infections than patients without diarrhea, indicating a causal relationship between the immunosuppression and the diarrhea. When an enteric pathogen is not detected, the diarrhea has been termed "AIDS enteropathy" [25,26]. The ability of the HIV-1 transactivating factor protein (Tat) to induce epithelial cell ion secretion without direct infection of the cells suggests a potential mechanism for diarrhea associated with the AIDS

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Table 104.1 Major gastrointestinal complications of AIDS

Diarrhea:	Acute HIV-1 syndrome, infectious enteritis/colitis, drug-induced and AIDS enteropathy
Biliary disorders:	Acalculous cholecystitis, sclerosing cholangitis and papillary stenosis
Pancreatitis:	Drugs (pentamidine and dideoxyinosine), infections
Gastrointestinal bleeding:	Upper tract: infectious esophagitis, Kaposi sarcoma and lymphoma Lower tract: infectious colitis, idiopathic colonic ulcers, non-Hodgkin lymphoma and Kaposi sarcoma
Neoplasms:	Non-Hodgkin lymphoma and Kaposi sarcoma
Anorectal disease:	Anus: condylomata, infection, fistula, fissure and squamous cell carcinoma Rectum: nonspecific proctitis, infectious proctitis and perirectal abscess

AIDS, acquired immune deficiency syndrome; HIV-1, human immunodeficiency virus type 1.

enteropathy [27]. However, among HIV-1-infected persons with diarrhea, vigorous microbiological evaluation frequently reveals an identifiable pathogen [28–32]. Important causes of diarrhea in HIV-1-infected persons include opportunistic and nonopportunistic infections and, during the last decade, antiretroviral therapy as well. Diarrhea in HIV-1-infected persons is associated with wasting and decreased survival in infants as well as adults [33,34]. In addition to the effect of diarrheal illness on the individual, the resultant loss of the ability to work and the high cost of providing health care are major drains on the financial resources of families and ultimately society in developing as well as developed countries.

Diagnostic evaluation

A vigorous diagnostic evaluation in HIV-1-infected patients with severe chronic diarrhea is usually justified, because pathogen-specific therapy can reduce the volume and frequency of the diarrhea [28,29]. Increased awareness of the infectious processes involving the gastrointestinal tract (Table 104.2) and improved diagnostic techniques have led to the identification of enteric pathogens in 44%–85% of patients with AIDS who have diarrhea [28–32]. A gastrointestinal pathogen is more frequently identified in symptomatic patients who also have weight loss and fewer than 100 $CD4^+$ T cells/mm^3 than in those patients without weight loss and with more than 100 $CD4^+$ T cells/mm^3, indicating that patients with more severe illness and greater immunosuppression are more likely to have an identifiable enteric pathogen [30,35–37]. Two or more enteric pathogens may coexist in approximately 25% of patients with AIDS who have diarrhea [38].

Step 1

Evaluation begins with a thorough history, including a review of the patient's drugs. In North America and Europe, antimicrobial agents and antiretroviral drugs are among the most common causes of gastrointestinal symptoms and diarrhea.

Table 104.2 Sites of infections in the gastrointestinal tract in HIV-1-infected persons

Organ	Pathogens
Esophagus	*Candida albicans*, herpes simplex virus, cytomegalovirus
Stomach	Cytomegalovirus, *Mycobacterium avium* complex
Biliary tract	Cytomegalovirus, *Cryptosporidium*, microsporidia, *Isospora belli*
Gallbladder	Cytomegalovirus, *Crytosporidium*, microsporidia, *Isospora belli*
Pancreas	Cytomegalovirus, *Mycobacterium avium* complex, herpes simplex virus
Small intestine	*Cryptosporidium*, microsporidia, *Isospora belli*, *Cyclospora*, *Mycobacterium avium* complex, *Salmonella* spp., *Campylobacter jejuni*, cytomegalovirus
Colon	Cytomegalovirus, *Mycobacterium avium* complex, *Shigella* sp., *Clostridium difficile*, *Campylobacter jejuni*, *Histoplasma capsulatum*, adenovirus, enteroaggregative *Escherichia coli*
Rectum	Herpes simplex virus, lymphogranuloma venereum, papillomavirus

HIV-1, human immunodeficiency virus type 1.

In addition, the increased use of antimicrobial agents in HIV-1-infected patients may be associated with increased rates of *Clostridium difficile*-associated diarrhea [39,40]. In the largest retrospective review of bacterial diarrhea in HIV-1-infected persons, *C. difficile* has become the most commonly identified bacterial pathogen in the United States [32]. The geographic region(s) in which a patient has resided or traveled is important because some AIDS-defining pathogens are endemic or more common in certain geographic regions, e.g., *Histoplasma capsulatum* in the Mississippi valley [41], *Cryptosporidium parvum* and *Isospora belli* in developing countries [42,43], and rotavirus in Australia [44,45] and Peru [31].

Step 2

After the history and physical examination, up to three stool specimens are cultured for *Salmonella* spp., *Shigella* spp., and *Campylobacter jejuni*, which are among the most common bacterial diarrheal pathogens identified in persons with and without HIV-1 infection [31,32,46,47]. *Aeromonas*, *Yersinia*, and *Escherichia coli* 0157:H7 are not more common among patients with AIDS, so stool samples are not routinely cultured for these bacteria. Each specimen is also examined for ova and parasites using saline, iodine, trichrome, and acid-fast preparations of direct or concentrated samples of stool. Antigen detection assays for *Giardia lamblia* and *Cryptosporidium* are increasingly available in clinical laboratories. Stool is assayed for *C. difficile* toxin, but because approximately 10% of patients without AIDS but with *C. difficile* infection are toxin-negative, stool is cultured for *C. difficile* when the bacterium is suspected despite a negative toxin assay [48]. The identification of enteric pathogens by stool examination alone in up to 55% of HIV-1-infected patients with diarrhea [29,49] underscores the importance and cost effectiveness of stool examination as the initial diagnostic test [50]. When fever is present, blood cultures are obtained for *Salmonella* spp., *Shigella* spp., and *C. jejuni* because bacteremia may accompany these infections at increased rates in HIV-1-infected persons.

Step 3

When stool examination does not reveal a potential pathogen or when therapy for the identified pathogen(s) does not improve symptoms, we perform esophagogastroduodenoscopy (EGD) and colonoscopy to inspect the mucosa and obtain biopsies for histopathological and microbiological evaluation. Small bowel fluid from HIV-1-infected patients rarely contains parasites [51] or excessive levels of bacteria ($> 10^4$/mL) [52], so we do not aspirate fluid from the small intestine to diagnose parasitic or bacterial overgrowth. Among HIV-1-infected patients with diarrhea and pathogen-negative stool studies, a diagnosis was obtained in 26% of patients by EGD [51], in 50% by EGD plus sigmoidoscopy [26], and in 44% by EGD plus colonoscopy [36]. The diagnostic yield of an endoscopic procedure is significantly greater for patients with $CD4^+$ T-cell counts below 500 cells/mm^3 [30], especially counts lower than 100 cells/mm^3 [36], and weight loss greater than 10 kg [53]. The diagnosis of cytomegalovirus (CMV) colitis, microsporidiosis, *Mycobacterium avium* complex, *Histoplasma capsulatum*, and adenovirus is made most frequently in such patients, who typically have advanced disease. Endoscopic procedures are an important adjunct to the diagnostic evaluation of unexplained chronic diarrhea in HIV-1 disease, and the diagnostic yield is greater for patients with more profound immunosuppression and severe diarrhea.

Step 4

When Steps 1–3 do not yield a detectable pathogen or when therapy is ineffective, glutaraldehyde-fixed tissue specimens obtained during Step 3 can be evaluated by electron microscopy for microsporidia (duodenal biopsies), and adenovirus and enteroaggregative bacteria (colon biopsies). The cost required to perform electron microscopy should be weighed against the low prevalence of these pathogens.

Approach to therapy

During the diagnostic evaluation of diarrhea, many patients without febrile, inflammatory, or bloody diarrhea benefit from supportive therapy with fluids, electrolytes, and antimotility agents. However, because the array of opportunistic and nonopportunistic pathogens that cause diarrhea in HIV-1-infected patients is extensive (Table 104.3), and drug-related side effects and drug interactions are common, specific antimicrobial therapy is preferable to empiric therapy [54]. In this regard, many microorganisms are resistant to, or develop resistance to, antimicrobial agents, necessitating close monitoring with in vitro drug susceptibility testing. Importantly, diarrhea and the AIDS cholangiopathy are often multifactorial in etiology, and, therefore, treatment may be only partially beneficial. Nevertheless, long-term suppressive therapy is often necessary for infections that reactivate or recur after treatment. Although specific antimicrobial therapy and supportive care can enhance the quality of life and duration of survival in many patients, highly active antiretroviral therapy (HAART) has obviated the need for antimicrobial therapy for some pathogens, as described next.

Highly active antiretroviral therapy

A major advance in the therapeutic management of HIV-1 infection has been the development of HAART, which combines reverse transcriptase inhibitors (RTI) and protease inhibitors (PI) and, more recently, fusion and integrase inhibitors, and HIV coreceptor antagonists (Table 104.4). RTIs include nucleoside analogues (NRTIs), such as zidovudine, and non-nucleoside analogues (NNRTIs), which inhibit the ability of HIV-1 RNA to encode viral DNA. The PIs block the cleavage of HIV-1 Gag-Pol polyprotein precursor into structural core proteins and enzymes (reverse transcriptase, integrase, and protease), causing the production of abnormal

Table 104.3 Opportunistic and nonopportunistic pathogens in the gastrointestinal tract in HIV-1 infection

	Opportunistic	Nonopportunistic
Bacteria	*Salmonella* sp. *Shigella flexneri* *Campylobacter jejuni* *Mycobacterium avium* complex Enteroaggregative *Escherichia coli*	*Clostridium difficile*
Protozoa	*Cryptosporidium parvum* *Enterocytozoon bieneusi* *Septata intestinalis* *Cyclospora cayetanensis* *Isospora belli*	*Giardia lamblia* *Entamoeba histolytica*
Viruses	Cytomegalovirus Herpes simplex virus Adenovirus Picobirnavirus/Astrovirus[b]	Rotavirus[a] Norwalk virus
Fungi	*Histoplasma capsulatum* *Candida albicans*	

a Nonopportunistic in United States and Africa, but more common in HIV-1-infected persons in Australia and Europe.
b More common in patients infected with HIV-1 with diarrhea; pathogenesis under investigation.
HIV-1, human immunodeficiency virus type 1.

viral proteins and noninfectious virions. HAART regimens typically include three or more agents, usually two NRTIs and one PI, along with one or more of the newer agents for patients with drug-resistant infections, to diminish or ablate viral replication, prevent the development of resistance, and enhance the recovery of immunological competence [55–59]. Consequently, HAART has dramatically reduced the rate of death [60] and the incidence of many systemic and gastrointestinal opportunistic infections, especially cryptosporidiosis [61,62], microsporidiosis [62], and enteric CMV disease [63], as well as oral candidiasis [64], in patients with HIV-1 infection, probably as the result of the inhibition of HIV-1 replication and the resultant recovery of immune function. Coinciding with the increased use of HAART, *C. difficile*-associated diarrhea [32] and medication-associated diarrhea [65] have emerged as leading causes of diarrhea in HIV-1-infected persons. The recovery of immune function takes several months so rates of opportunistic infections decline most prominently after 2 months of antiretroviral therapy. Prophylactic antimicrobial therapy may be discontinued in most patients on HAART when the number of $CD4^+$ lymphocytes has increased to 200 cells/mm^3 and remained at this level for more than 12–24 weeks [66–68].

A spectrum of toxicities is associated with antiretroviral drugs, but many antiretroviral agents have prominent side effects: nausea, vomiting, and diarrhea (Table 104.4). These toxicities occur more frequently among patients with advanced HIV-1 disease ($CD4^+$ T cells < 200 cells/mm^3) [3]. The PIs such as nelfinavir are associated with higher rates of diarrhea than are more recent agents, such as lopinavir/ritonavir [69]. Pancreatitis and hepatic toxicity also may occur with some agents. Many of the toxic effects of antiretroviral medications are dose-dependent, and dose reduction may allow the continuation of therapy. However, because optimal antiretroviral activity is usually dose-dependent, particularly for PIs, changing antiretroviral agents may be preferable to lowering doses. Drug resistance may develop so virological status should be monitored. The management of overlapping toxicities, side effects and complications associated with anitretroviral agents requires in-depth knowledge of the agents and close patient monitoring.

Bacterial infections

Salmonella spp., *Shigella* spp., and *Campylobacter jejuni*

The gram-negative bacteria, both *S. enteritidis* (particularly in the United States) and *S. typhimurium* (particularly in Africa) [70–72], *Shigella flexneri* [73–75] (although *Shigella sonnei* is more comon in the general population), and *C. jejuni* [76,77], cause a similar clinical illness in HIV-1-infected persons. This syndrome may be characterized by chronic or recurrent diarrhea, abdominal cramps, and fever. During symptomatic infection, the stool frequently contains fecal leukocytes and either gross or microscopic blood, although noninflammatory diarrhea may also be present. Each of these bacterial infections occurs more often, causes more prolonged or recurrent diarrhea, and is more commonly associated with bacteremia and antibiotic resistance in HIV-1-infected persons than in seronegative persons. The *C. jejuni* may cause visible mucosal inflammation. *Campylobacter* sp. other than *C. jejuni* have been detected in HIV-1-infected patients with diarrhea [78], but a causative role for these species in the diarrhea awaits confirmation.

Diagnosis

These bacteria are diagnosed by culture of the organism in a stool specimen. The blood should also be cultured when diarrhea is accompanied by fever.

Treatment

The drug of choice for infection with *Salmonella* spp. is ceftriaxone or ciprofloxacin, for *Shigella* spp. ciprofloxacin is used, and for *C. jejuni* erythromycin or ciprofloxacin is used (Table 104.5). Because recurrence is common and drug resistance may develop, repeat culture of stool and blood (when bacteremia occurs) and drug sensitivity testing are used to monitor the therapeutic response, particularly when symptoms persist.

Table 104.4 Gastrointestinal complications of antiretroviral therapy and potential interactions with other medications used in gastrointestinal medicine

Type of medication	Medication	Trade name	Primary gastrointestinal toxicity[a]	Gastrointestine-associated drug interactions
Antiretroviral				
• Nucleoside analogue RTI (NRTI)	Zidovudine (AZT, ZDV)	Retrovir	Anorexia; nausea (4%–26%); vomiting (3%–8%); rare hepatitis, steatosis, lactic acidosis	Activity may be inhibited by ribavirin
	Didanosine (ddI)	Videx; Videx EC	Indigestion; diarrhea (16%); pancreatitis (4%–8%); ↑ALT/AST (6%–20%); rare steatosis and lactic acidosis	Potential increased rate of pancreatitis with pentamidine, azathioprine; buffers associated with ↓ absorption of itraconazole, ketoconazole, dapsone; tetracycline, ciprofloxacin; ddI levels ↑ with ranitidine, ganciclovir
	Zalcitabine (ddC)	HIVID	Stomatitis (self-limited) (2%–17%); pancreatitis (0.5%–9%)	Few; possible ↑ neuropathy with metronidazole, disulfuram
	Stavudine (d4T)	Zerit	Diarrhea (33%), nausea/vomiting (26%), abdominal pain (23%); ↑ALT/AST (65%); significant GI symptoms and hepatic abnormalities in 4%–10%; rare lactic acidosis and pancreatitis	Few; possible ↑ neuropathy with ethanol; ribavirin may increase activity (in vitro data)
	Lamivudine (3TC)	Epivir	Limited; mild and transient diarrhea, nausea, abdominal pain; rare pancreatitis, steatosis, and lactic acidosis	Few
	Abacavir (ABC)	Ziagen	Nausea (45%), diarrhea (25%), vomiting (15%) and abdominal pain (15%) may decrease over weeks of therapy; hypersensitivity reaction with fever ± rash, malaise, nausea, vomiting, ↑ALT/AST in 2%–5% within 1 month	None reported
	Emtricitabine (FTC)	Emtriva	Minimal toxicity; occasional nausea; diarrhea and nausea; rare pancreatitis, steatotis, and lactic acidosis	
	Tenofovir (TDF)	Viread	Well tolerated; occasional nausea, vomiting, diarrhea, flatulence, abdominal distention, anorexia	
• Nucleotide analogue RTI	Adefovir dipivoxil	(Preveon)	Usually mild nausea, diarrhea, occasional vomiting (1%–8%); ↑ALT/AST (4%); (renal toxicity 38%)	None reported
Combinations	ZDV/3TC	Combivir	See individual agents	
	ZDV/3TC/ABC	Trizivir	See individual agents	
	3TC/ABC	Epzicom	See individual agents	
	FTC/TDF	Truvada	See individual agents	
• Non-nucleoside RTI (NNRTI)	Nevirapine (NVP)	Viramune	Nausea, vomiting; ↑ALT/AST (1%; lower if begin with 2 weeks at lower dose); isolated ↑GGT common; (rash most common, ≈ 10%)	
	Delavirdine (DLV)	Rescriptor	Nausea (7%); diarrhea (4%); ↑ALT/AST (≤ 5%); (rash most common; usually transient)	Level ↓ with rifampin, rifabutin (P-450 system) and antacids (↑ gastric pH); level ↑ with clarithromycin, rifabutin, cisapride
	Efavirenz (EFV)	Sustiva	Most common: transient rash and CNS symptoms of headache, dizziness, impaired concentration; some nausea, vomiting, diarrhea, ↑ALT/AST	Avoid cisapride; should not be taken within 2 h of antacids; not recommended with clarithromycin; (interacts with other PI)
	Etravirine (TMC125)	New agent; not released	Initial GI symptoms not greater than control regimens	
	EFV/FTC/TDF	Atripla	See individual agents	

• Protease inhibitors (PI)[b] (most PI metabolized by liver)	Saquinivir (SQV)	Invirase	Nausea, diarrhea, abdominal pain, dyspepsia (5%–10%)	Cisapride contraindicated; SQV level ↑ with ketoconazole, clarithromycin; SQV level ↓ with rifampin, rifabutin; clarithromycin level ↑ with SQV; (interacts with other PI)
	Ritonavir (RTV)	Norvir	Nausea, diarrhea, vomiting, anorexia, abdominal pain (20%–40%), esp. in first few weeks of therapy; Taste perversion (10%); Increased triglycerides (60%, but > 1500 mg/dL in 2%–8%); ↑ALT/AST (10%–15%), esp. with NRTI; fewer complications when used only at lower doses for boosting levels of other PIs	Cisapride contraindicated; caution with dronabinol, ondansetron, cimetidine, promethazine, corticosteroids; RTV level ↑ with ketoconazole, itraconazole; RTV level ↓ with rifampin; clarithromycin, erythromycin, rifampin, rifabutin levels increased with RTV; (interacts with other PI)
	Indinavir (IDV)	Crixivan	Nausea, vomiting, diarrhea, abdominal pain (4%–15%); Increased indirect bilirubin (10%); (nephrolithiasis 5%–10%)	Cisapride contraindicated; IDV level ↓ with rifabutin; IDV level ↑ with ketoconazole; rifabutin level ↑ with IDV; (interacts with other PI)
	Nelfinavir (NLF)	Viracept	Diarrhea (usually mild) (2%–19%)	Cisapride and rifabutin contraindicated; NLF levels ↓ with rifampin; rifabutin levels ↑ with NLF; (interacts with other PI)
	Amprenavir	Agenerase	Mild diarrhea, nausea, vomiting (7%–33%); fatigue; taste disorder; (rash 18%);	Cisapride and rifampin contraindicated; Amprenavir levels ↓ with rifabutin; rifabutin levels ↑ with amprenavir; (interacts with other PI)
	Fosamprenavir (FPV)	Lexiva	Nausea, diarrhea, vomiting; mouth numbness; ↑ALT/AST; (rash common 19%)	As above; cytochrome P450 and CYP3A4 inhibitor[b]
	Lopinavir-Ritonavir (LPV/RTV)	Kaletra	Diarrhea (10%–20%); nausea, vomiting, stomach pain; ↑ALT/AST (8%)	Similar to other PI
	Atazanavir (ATV)	Reyataz	Occasional GI symptoms and ↑ALT/AST; elevated indirect bilirubin/jaundice (7%–9% but subclinical)	As above; cytochrome P450 and CYP3A4 inhibitor[b]
	Tipranavir (with ritonavir)	Aptivus	Nausea, vomiting, diarrhea, fatigue, and headache (rates similar to those with comparator PIs); ↑ALT/AST (> 10%) and occ. fatal hepatic failure	Avoid drugs that are metabolized by or induce CYP34A
	Darunavir (DRV/RTV) (with ritonavir)		Nausea, vomiting, diarrhea, fatigue, and headache (rates similar to those with comparator PIs); ↑ALT/AST (2%–3%)	Avoid drugs that are metabolized by or induce CYP34A
• Fusion inhibitors	Enfuvirtide	T20; Fuzeon	Decreased appetite; ↑ALT/AST; (local injection and systemic hypersensitivity reactions)	Does not affect cytochrome P450
• Other	Interleukin-2	Proleukin	Diarrhea, abdominal pain, stomatitis (7%); nausea; vomiting; isolated ↑ bilirubin (8%); ↑ALT/AST; acalculous cholecystitis; constitutional flu-like symptoms (fever, chills, muscle/joint pain) (45%)	
	Hydroxyurea	Hydrea	Nausea (12%); ↑ALT/AST (2%); stomatitis (8%); diarrhea; occasional anorexia, vomiting, diarrhea, constipation (myelosuppression; rash)	
	New classes: – entry inhibitors (CCR coreceptor)	Maraviroc	Limited to date	
	– integrase inhibitors	Raltegravir	Limited to date	
	– maturation inhibitors			

a Rates may vary among studies.

b As with other inhibitors of cytochrome p450 system, specifically CYP 3A4, protease inhibitors should not be given with cisapride (Propulsid), astemizole (Hismanal), terfenadine (Seldane), midazolam (Versed), or triazolam (Halcion), or ergot derivatives.

Adapted from data reviewed in refs [48–52]. Useful sources for drug interactions are: (1) www.hiv-druginteractions.org/ and (2) http://clinicaloptions.com/HIV.aspx. For information about HIV/AIDS: (1) http://www.aids-etc.org; (2) http://aidsinfo.nih.gov; (3) http://hivinsite.ucsf.edu/InSite.jsp?page=KB; (4) http://www.hopkins-aids.edu/; (5) http://www.thebody.com/index.html.

↑ALT/AST = alanine/aspartate aminotransferase elevation > 2.5-fold to 5-fold above normal values; CNS, central nervous system; GGT= γ-glutamyl transfersase; GI, gastrointestinal; RTI, reverse transcriptase inhibitors.

Table 104.5 Therapy for intestinal infections in HIV-1-infected adults

Pathogen	Treatment	Alternative treatment
Protozoal:		
Cryptosporidium	Nitazoxamide 500 mg b.i.d. × 14 days	Paromomycin 500 mg q.i.d./1000 mg b.i.d. × 14 days
Microsporidium sp.	Albendazole 400 mg b.i.d. × 4 weeks (*Septata intestinalis*)	Fumagillin 60 mg/day (*Enterocytozoon bieneusi*); Nitazoxanide 1000 mg b.i.d.
Isospora belli[a]	Trimethoprim-sulfamethoxazole 1 DS[b] q.i.d. × 10 days	Pyrimethamine 25 mg + leucovorin acid 5–10 mg/day × 10 days Ciprofloxacin 500 mg b.i.d. × 10 days, then b.i.d. × 3 weeks Maintenance: 1 TMP-SMX q.d. or 3 ×/week
Giardia lamblia	Metronidazole 250 mg t.i.d. × 5 days	Tinidazole 2 g single dose Furazolidone 100 mg q.i.d. × 7–10 days
Cyclospora	Trimethoprim-sulfamethoxazole 1 DS q.i.d. × 10 days	
Viral:		
Cytomegalovirus[a,c,d]	Ganciclovir 5 mg/kg i.v. q12h × 3–4 weeks Valganciclovir 900 mg b.i.d. with food × 3–4 weeks	Foscarnet 60 mg/kg i.v. q8h or 90 mg i.v. q12h × 2–3 weeks
Herpes simplex[a,c,d]	Acyclovir 200–800 mg 5/day × 7–14 days	Foscarnet 40 mg/kg i.v. q8h × 3 weeks
Adenovirus	NA[e]	
Rotavirus[f]	NA	
Astrovirus	NA	
Picobirnavirus	NA	
Bacterial:		
Salmonella sp.[a,d]	Ceftriaxone 1–2 g q.d. × 7 days Ciprofloxacin 500 mg b.i.d. × 7 days	Amoxicillin 1 g t.i.d. × 7–14 days Trimethoprim-sulfamethoxazole 1 DS b.i.d. × 10–14 days
Shigella flexneri[a,f]	Ciprofloxacin 500 mg b.i.d. × 3–7 days Ampicillin 500 mg q.i.d. × 5 days	Trimethoprim-sulfamethoxazole 1 DS b.i.d. × 3–14 days Ceftriazone 1–2 g q.d. × 7 days
Campylobacter jejuni[a,d]	Erythromycin 500 mg q.i.d. × 7 days Ciprofloxacin 500 mg b.i.d. × 7 days	Tetracycline 500 mg q.i.d. × 7 days
Mycobacterium avium complex[a,d]	Clarithromycin 500–1000 mg b.i.d. Rifabutin 300 mg p.d., and Ethambutol 15–25 mg/kg q.d.	Azithromycin 500 mg q.d.
prophylaxis:	Clarithromycin 500 mg q.d. or Azithromycin 500 mg q.d.	Ribabutin 300 mg q.d.
Clostridium difficile Bacteriosis[f]	Metronidazole 500 mg p.o. t.i.d. × 7–10 days Ciprofloxacin 500 mg b.i.d. × 4 weeks	Vancomycin 125 mg p.o. q.i.d. × 10–14 days
Fungal:		
Histoplasma capsulatum[a]	Amphotericin B 0.7 mg/kg i.v., q.d. × 3–14 weeks (or liposomal AmB 3 mg/kg/day i.v.) (maintenance phase up to 12 weeks)	Itraconazole 200 mg b.i.d. × 4–8 weeks[g]

All treatments are per os unless otherwise indicated. Treatment of all diarrheal syndromes should include supportive care, particularly repletion of fluids and electrolytes. Use of loperamide, lomotil (diphenoxylate-atropine) or paregoric an option with noninflammatory untreatable chronic infections, such as adenovirus, astrovirus, picornavirus, and calicivirus. Use of effective antiretroviral therapy is important initial and supportive therapy for many opportunistic infections, including *Cryptosporidium*, *Microsporidium*, herpes simplex virus, *M. avium* complex, etc.

a Chronic suppression at reduced dosage may be necessary.

b Double-strength tablet.

c Reduce dosage for decreased creatinine clearance.

d Resistance may develop; susceptibility testing necessary.

e Not available.

f Pathogenicity in human immunodeficiency virus type 1 infection under investigation.

g Also maintenance regimen.

b.i.d., twice a day; DS, double-strength; i.v., intravenous; p.o., orally; q8h, 8-hourly; q12h, 12-hourly; q.d., each day; q.i.d., four times a day; t.i.d., three times a day.

Mycobacterium avium complex

Mycobacterium avium complex (MAC) comprises two acid-fast, obligate, intracellular bacteria: *M. avium* and *M. intracellulare*. The gastrointestinal tract is the likely portal of entry for MAC, and exposure is common in all persons, as the organisms are ubiquitous in the environment. In immunocompetent hosts, MAC is not an enteric pathogen, but immunosuppressed HIV-1-infected patients ($CD4^+$ T cells < 100 cells/mm^3) are highly susceptible to infection. Macrophages are an important reservoir for the bacteria, and during HIV-1 infection macrophages phagocytose MAC, but appear unable to kill them. The organism up-regulates the macrophage HIV-1 production in dual-infected cells [79] and alters macrophage function [80].

MAC is the most common cause of systemic bacterial infection in HIV-1-infected persons in the United States. MAC is much less common in Africa and Haiti. End organ involvement and bacteremia usually do not occur until the number of circulating $CD4^+$ T cells declines to below 100 cells/mm^3. Gastrointestinal tract disease, which is usually a manifestation of disseminated infection, may be associated with diarrhea, abdominal pain, malabsorption, weight loss, and fever, with or without night sweats [81,82]. Concomitant hepatomegaly or splenomegaly with anemia as the result of MAC infection is often present. The numerous mycobacteria typically present in the gastrointestinal tract mucosa suggest that MAC contributes to the associated gastrointestinal symptoms. Endoscopic abnormalities are nonspecific and include erythema, friability, erosions, and fine white nodules. Occasionally, an inflammatory mass, which can be mistaken for a tumor, is the major gastrointestinal manifestation. The small intestine is reported to be involved more often than the colon [81], but this may reflect endoscopic access. Histological examination shows diffuse infiltration of the lamina propria by macrophages filled with bacilli. Granulomas are usually absent or poorly developed, possibly because of the impaired chemotactic activity of monocytes in patients with AIDS [83].

Diagnosis

The diagnosis of MAC is based on the identification of typical acid-fast organisms in stool or gastrointestinal biopsy specimens (fixed and touch preparations) [81,82]. Culture of gastrointestinal tissue may increase the positivity rate compared to acid-fast staining alone [84]. Isolation and speciation are achieved by culture of organisms from stool and biopsy specimens. Although the incidence of both pulmonary and extrapulmonary *Mycobacterium tuberculosis* infections is increased among HIV-1-infected persons, intestinal involvement is uncommon.

Treatment

Although initial trials of anti-tuberculosis agents proved disappointing, improved results with newer multi-drug regimens provide reason for cautious optimism in treating MAC [85]. Combination regimens have produced more sustained benefit, but such regimens are expensive, have adverse effects, and are often difficult to tolerate. The current recommended three-drug therapy is two anti-mycobacterial agents, including a macrolide azole agent and rifabutin, and a third agent, such as ethambutol [86]. For prophylactic therapy when the $CD4^+$ T-cell number falls below 50 cells/mm^3, clarithromycin or clarithromycin plus rifabutin are effective; MAC disease occurred in 9% and 7% of patients treated with clarithromycin alone or with combination therapy, respectively [87]. For patients on HAART who respond with an increase in the number of $CD4^+$ lymphocytes to more than 100 cells/mm^3 for more than 3–6 months with sustained suppression of HIV-1 plasma RNA for the same period, prophylactic therapy may be discontinued.

Clostridium difficile

The gram-positive bacterium *C. difficile* is an important pathogen in the differential diagnosis of diarrhea in persons with HIV-1 infection. During the first decade of HAART, *C. difficile* emerged in the United States as the most common bacterial cause of diarrhea among HIV-1-infected persons [32]. Risk factors for *C. difficile*-associated diarrhea in HIV-1-infected persons include recent antimicrobial therapy (particularly clindamycin and pyramethamine) and hospitalization [88], risk factors similar to those in immunocompetent persons without HIV-1 infection [89]. Failure to generate specific antibodies to *C. difficile* toxins, important mechanisms of defense, and resolution of infection, may be compromised during HIV-1 infection [90] and predispose severely immunocompromised persons with HIV-1 infection to *C. difficile*-associated diarrhea. Importantly, the rate for *C. difficile*-associated diarrhea is significantly higher for persons with more advanced HIV-1 infection, particularly persons with clinical AIDS [32]. Although asymptomatic carriage occurs, *C. difficile* infection in HIV-1-infected persons is commonly associated with profound watery diarrhea, abdominal pain and tenderness, fever, leukocytosis, and the presence of fecal leukocytes and occult blood. Infection with *C. difficile* is among the most common bacterial causes of diarrhea in HIV-1-infected persons in the United States [32,91] but the severity of the clinical illness and the response to therapy appear to be similar to those of seronegative subjects [92,93]. Thus, *C. difficile* is a frequent, but nonopportunistic, pathogen associated with diarrheal illness in HIV-1-infected patients.

Diagnosis

The diagnosis of *C. difficile*-associated diarrhea or colitis is based on the presence of diarrhea during antimicrobial use and the detection of either *C. difficile* toxin or organisms in stool or endoscopically confirmed pseudomembranous colitis. The potential usefulness of stool culture is underscored by the higher rates of positive stool cultures (90%–97%)

compared with positive toxin assays (70%–73%) in HIV-1-seronegative subjects with *C. difficile* infection [48,94], although asymptomatic carriage is well-described. Newer toxin assays show increased sensitivity for toxins A and B and are capable of detecting toxin A variant strains of *C. difficile* [95].

Treatment

Metronidazole and vancomycin are highly effective for the treatment of *C. difficile* infection in HIV-1-infected persons (Table 104.5).

Enteroaggregative bacteria

Intestinal infection with enteroaggregative *E. coli* has been reported in HIV-1-infected persons in the United States and Africa [96,97]. The bacterium adheres to enterocytes predominantly in the colon and ileum, where focal areas of epithelial cell injury (effacement, displaced microvilli, necrosis) are associated with attached bacteria. Accumulation of inflammatory cells in the epithelium or lamina propria is minimal or absent. Enteroaggregative *E. coli* have been identified more frequently in stool from HIV-1-infected adults with diarrhea than in stool from asymptomatic seropositive subjects [98]; the mechanism of the diarrhea is not known. Therapeutic eradication of the bacteria with ciprofloxacin has been associated with restoration or improvement of diarrheal symptoms [99].

Protozoan infections

Cryptosporidium parvum

Cryptosporidium parvum is a unicellular, spore-forming coccidian protozoan taxonomically related to *Isospora*, *Toxoplasma*, *Sarcocystis*, and *Cylospora* [100]. Early in the AIDS epidemic, cryptosporidia were present in approximately 20% of AIDS patients with diarrhea in the United States [28,29,101] but in as many as 55% of AIDS patients in developing countries such as Zaire and Haiti [35]. Persistent infection occurs predominantly in HIV-1-infected patients with more than 200 cells/mm^3 [102,103]. A relatively common cause of self-limited mild diarrhea in immunocompetent persons [101,104,105], cryptosporidiosis-associated disease may be characterized by chronic, debilitating diarrhea in patients with AIDS. The diarrhea can be voluminous, nonbloody, and watery; abdominal cramps, anorexia, malaise, and weight loss are also prominent symptoms. Asymptomatic carriage may occur [106], but symptoms of dehydration and wasting are more common. Malabsorption of both fat and carbohydrate is associated with cryptosporidiosis. Rarely, *Cryptosporidium* may spread to the biliary tract, leading to biliary tract obstruction (see below). The parasite has been identified throughout the gastrointestinal tract, developing within a parasitophorous vacuole beneath the epithelial cell membrane but outside the cytoplasm. Despite its ability to cause severe disease, the spectrum of *Cryptosporidium*-associated symptoms is broad, may vary over time, and is strongly influenced by the level of immunosuppression [107].

Diagnosis

Infection with *Cryptosporidium* is easily identified in stool microscopically with a modified acid-fast stain. Concentration of stool by zinc sulfate or sucrose flotation enhances the detection of rare or infrequent oocysts during intermittent shedding. A single stool examination was sufficient to diagnose 96% of cases in one retrospective study [108]. When *Cryptosporidium* is suspected despite negative stool examination, hematoxylin & eosin-stained tissue specimens obtained by endoscopy can reveal the parasite. Biopsy of the terminal ileum has a higher sensitivity (91%) than that of the duodenum (53%) for the detection of the parasite [109]. Sensitive and specific antigen detection assays for stool are also commercially available.

Treatment

Despite anecdotal reports of successful treatment of cryptosporidiosis in HIV-1-infected persons with spiramycin, bovine colostrum, transfer factor, somatostatin, and zidovudine, therapy with these agents has not been consistently effective, and controlled clinical trials have not yet been performed. Paromomycin, a nonabsorbable aminoglycoside, was shown to be no more effective than placebo for HIV-1-associated cryptosporidiosis [109]. Nitazoxamide, which has induced parasitological cure and complete resolution of the diarrheal syndrome in a high proportion of patients [110,111], is a reasonable first-line agent for HIV-1-infected patients with cryptosporidiosis. Somatostatin may reduce the frequency and volume of *Cryptosporidium*-associated diarrhea, but therapy is complicated by the requirement that the drug be given chronically several times daily and either intravenously or subcutaneously. As noted above, HAART appears to decrease the incidence, severity, and duration of *Cryptosporidium* infections [61,112].

Microsporidia

Microsporidia are spore-forming, obligate intracellular protozoa that infect the small intestine (*Enterocytozoon bieneusi*), small intestine with dissemination (*Septata intestinalis*), liver (*Encephalitozoon cuniculi*), and cornea (*Encephalitozoon hellem*). The parasite has distinctive ultrastructural features; many organisms at different stages of development can be identified in the same enterocyte, causing cytopathic effect [113] (Fig. 104.1). *Enterocytozoon bieneusi* infects only enterocytes in the small intestine. *Septata intestinalis*, which is ultrastructurally distinct from *E. bieneusi*, also infects epithelial cells but can penetrate into the lamina propria, infect macrophages and disseminate to other organs such as the kidney [114–116]. Microsporidia were initially identified exclusively in HIV-1-infected persons, but the parasite has been diagnosed in seronegative liver and renal transplant recipients [117,118]

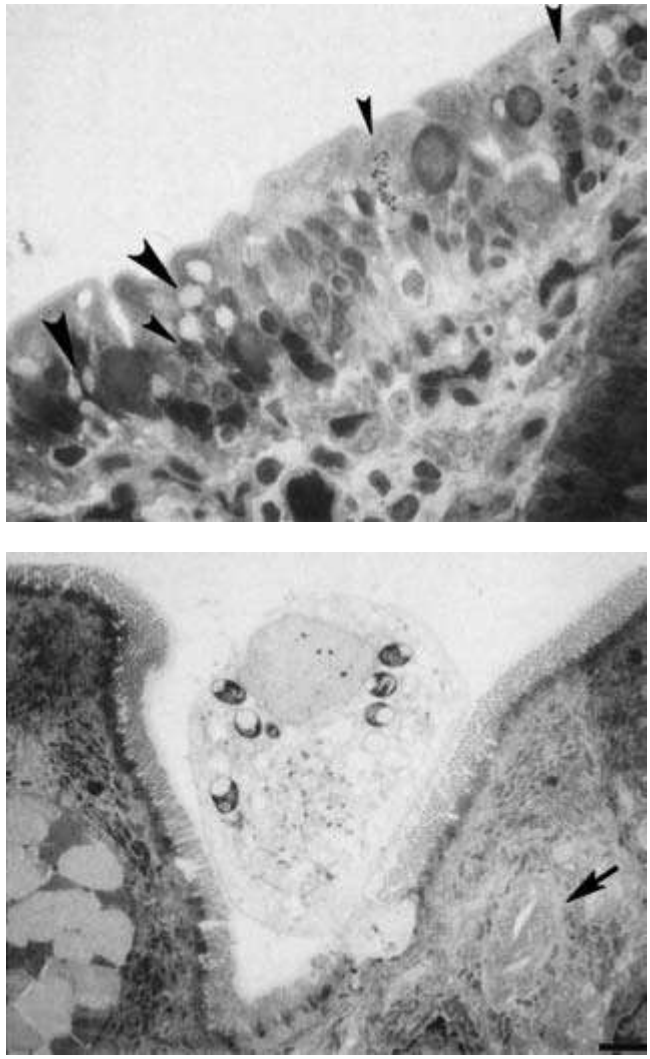


Figure 104.1 **(a)** Densely stained microsporidial spores are detected in the cytoplasm of several epithelial cells by light microscopic examination of an intestinal biopsy from a patient with microsporidiosis (semithin plastic section; methylene blue-azure II and basic fuchsin stain, original magnification × 630). **(b)** Electron micrograph shows a necrotic intestinal enterocyte containing six microsporidial spores in the final stage of being sloughed into the lumen (original magnification × 10 000). Courtesy of Dr Jan M. Orenstein.

and infrequently in immunocompetent persons [119]. In resource-limited settings in South America, intestinal microsporidiosis is associated with poor sanitary conditions as well as low (< 400) $CD4^+$ cells/mm^3 [120].

Infection with the parasite is associated with chronic, watery, nonbloody diarrhea of variable severity, frequently with substantial fluid and weight loss. Microsporidiosis has been reported to cause 40%–50% of the cases of unexplained diarrhea among patients with HIV-1 infection [121], but microsporidia infection is not invariably accompanied by diarrhea [122]. *Enterocytozoon bieneusi* is equally common in HIV-1-infected patients with and without diarrhea. The highest density of *E. bieneusi* is located in the jejunum [123], where the parasite infects enterocytes and may cause villus atrophy. The organism has been associated with AIDS-related biliary tract disease, including acalculous cholecystitis, cholecystitis with cholelithiasis, and cholangitis, presumably by ascending the proximal small intestine to infect the biliary tract, as discussed below.

Diagnosis

The diagnosis of microsporidiosis is based on the identification of the parasite in small intestinal tissue or fluid. Biopsies should be taken at the most distal site, where the parasite burden is the highest. Because of their small size, poor staining qualities, and minimal associated inflammation, microsporidia were formerly diagnosed exclusively by transmission electron microscopy. Microsporidia can be identified by light microscopy in semi-thin plastic sections of small intestinal biopsies stained with methylene blue-azure II-basic fuchsin, touch preparations of mucosa stained with Giemsa, and stool specimens stained with a small modified trichrome (chromotrope 2R) stain. One light microscopic technique appears to obviate the need for electron microscopy in over 50% of cases, and a combination of any two techniques obviates the need in more than 75% of cases [38]. A rapid fluorescence technique based on Uvitex 2B binding to chitin, a component of the spore wall, is reportedly effective for detecting microsporidia [121]. However, because Uvitex 2B binds to any chitin-containing microorganism, morphological evaluation may be necessary to confirm the diagnosis. A polymerase chain reaction assay using primers for the ribosomal RNA gene of *E. bieneusi* and *S. intestinalis* to identify microsporidia in tissue specimens is not widely available [124].

Treatment

Curative therapy for microsporidiosis is not available. Albendazole, a benzimidazole derivative related to mebendazole, induces improvement in or resolution of symptoms in patients with microsporidia infection (Table 104.5) and appears to be more effective against *S. intestinalis* than *E. bieneusi* [116,125]. Patients on HAART have shown both improvement and resolution of diarrheal symptoms due to microsporidia infection. Nitozoxanide is another therapy with potential efficacy for these infections.

Isospora belli

Isospora belli is an obligate, intracellular coccidian parasite that resembles Cryptosporidium in its life cycle and small intestinal habitat. In the early years of the AIDS epidemic, *I. belli* was identified in fewer than 3% of AIDS patients in the United States but in up to 15% of patients in developing nations such as Haiti and Zambia [43,126]. Isosporosis is typically a chronic illness characterized by profuse, nonbloody,

watery diarrhea that may be indistinguishable from the diarrheal illness associated with cryptosporidia and microsporidia infections. Weight loss of at least 10% may occur during the months before diagnosis [43,127]. Nausea and abdominal cramps also typically accompany the illness; fever and vomiting are less frequent. In Haitian patients, dehydration requiring hospitalization has been reported in nearly 50% of patients [43]. Steatorrhea and eosinophilia may also be present. Histology usually shows inflammation associated with some degree of villus atrophy; in contrast to *Cryptosporidium*, *I. belli* may invade the mucosa. Although concentrated in the small intestine, *I. belli* has been identified throughout the gastrointestinal tract and has been associated with biliary disease, specifically acalculous cholecystitis. Rarely, the organism disseminates to extraintestinal sites, including mesenteric and tracheobronchial lymph nodes.

Diagnosis

The diagnosis of *I. belli* is established by the identification of the typical highly refractile, spherical oocysts (containing two sporoblasts) in stool using the modified Kinyoun acid-fast stain. Stool concentration may be necessary to detect infrequent or rare oocysts.

Treatment

The most effective therapeutic agent for *I. belli* is trimethoprim-sulfamethoxazole (Table 104.5). Because infection recurs in as many as 50% of cases following treatment, prolonged or repeat therapy with this agent may be required to suppress clinical illness.

Cyclospora cayetanensis

Cyclospora are obligate, intracellular coccidian parasites with morphological and staining characteristics of a cyanobacterium-like organism [128,129]. The spherical cyst-like organisms measure 8–10 μm in diameter and on modified acid-fast staining resemble *Cryptosporidium*. The *Cyclospora* oocyst has two sporocysts, each containing two sporozoites, distinguishing the parasite from the larger *I. belli* [128]. Both sexual and asexual forms occur in small intestinal epithelial cells, suggesting that the organism can complete its life cycle in a single host. The organism has been identified in persons with diarrhea from Latin America (primarily Peru), the Caribbean, Southeast Asia, Eastern Europe, and the United States. In immunocompetent persons, the diarrhea is typically abrupt in onset, watery, and prolonged (lasting a mean of 6 weeks) but self-limited. The histopathology of the small intestine in patients with *Cyclospora*-associated diarrhea shows acute and chronic inflammation, surface epithelial disarray, and varying degrees of villous atrophy and crypt hyperplasia. *Cyclospora* infection in persons with AIDS is associated with prolonged or relapsing diarrhea and weight loss as great as 10% [129,130]. Diffuse, cramping abdominal pain accompanies the diarrhea in over half of patients and fever in a third. *Cyclospora* outbreaks have been associated with contaminated drinking water and imported raspberries [131,132].

Diagnosis

Modified Kinyoun stain is used to identify *Cyclospora* oocysts in a concentrated fresh stool sample; oocysts appear as 8–9-μm, acid-fast, granular spheres.

Treatment

Trimethoprim-sulfamethoxazole appears to be effective therapy for *Cyclospora* infection [129] (Table 104.5).

Giardia lamblia and *Entamoeba* species

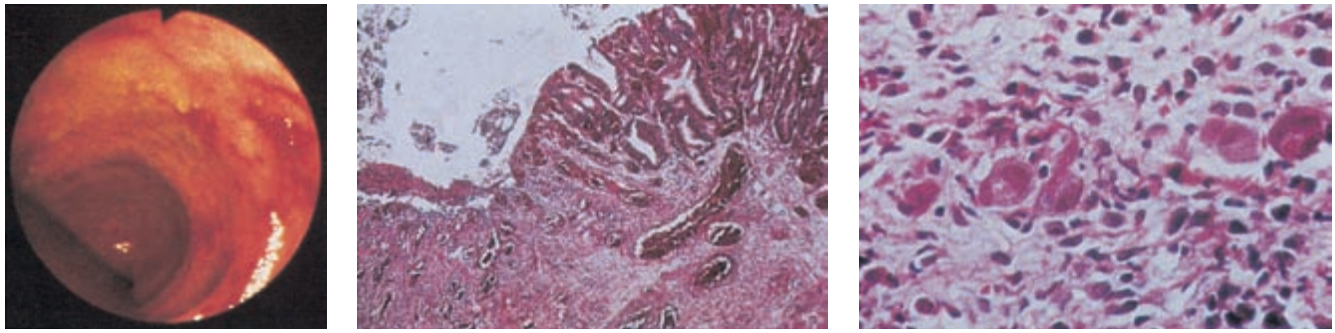
In immunosuppressed HIV-1-infected patients, *Giardia lamblia* does not cause more frequent or severe infection than in seronegative persons and responds appropriately to antimicrobial therapy with metronidazole [133]. A retrospective case review showing that a small proportion of HIV-1-infected persons exposed to *Entamoeba histolytica* develop *E. histolytica* colitis or liver abscess warrants prospective study [134].

Viral infections

Cytomegalovirus

CMV is a double-stranded DNA virus in the herpesvirus group. After initial infection, which in immunocompetent persons is usually asymptomatic, the virus enters a latent, nonproductive phase in circulating mononuclear cells [135]. Consequently, serious organ disease due to CMV infection is extremely rare in immunocompetent persons, with only 34 such cases having been reported by 1996 [136]. When the host becomes immunosuppressed, virus is reactivated, leading to replication and cell lysis. Replication occurs in the nuclei of permissive cells, and virus accumulates in nuclear and cytoplasmic inclusions, producing cell enlargement and the characteristic nuclear and cytoplasmic inclusions. In the immunosuppressed host, CMV has been detected in intestinal mononuclear, epithelial, endothelial, and smooth muscle cells [137]. The local inflammatory response appears to involve CMV-induced release of proinflammatory cytokines [138,139].

The clinical manifestations of CMV disease in the gastrointestinal tract include esophagitis, gastritis, small intestinal enteritis, and, less frequently, acalculous cholecystitis, papillary stenosis, sclerosing cholangitis, pancreatitis, and appendicitis. Colitis appears to be the most common manifestation of enteric CMV disease [46,140]; among AIDS patients with colitis or enteritis, CMV has been identified in biopsies in 45% of patients [46]. CMV colitis is characterized by diarrhea, fever, and weight loss. Abdominal pain and hematochezia are also frequently present and help distinguish CMV colitis from the protozoan diarrheal illnesses described above. The colon appears to be particularly susceptible to



(a) (b) (c)

Figure 104.2 (a) Endoscopic visualization of mucosal ulceration and inflammation in a patient with cytomegalovirus colitis. Light microscopic view of a colon biopsy from the same patient shows ulceration and hemorrhage **(b)** and infiltration by inflammatory cells **(c)**, many containing cytomegalic inclusions. (**b**,**c**, hematoxylin & eosin: **b**, original magnification × 30; **c**, original magnification × 62). From Smith et al. [138], with permission from the American Society for Clinical Investigation.

progression to ischemic necrosis and perforation during CMV infection. Regardless of whether diarrhea is present, CMV-associated disease of the biliary tract (usually without icterus or pruritus) should be included in the differential diagnosis of abdominal pain, nausea, and vomiting in patients with AIDS. Endoscopic findings that suggest CMV-induced disease range from localized hyperemia through hemorrhagic erythema to superficial or deep ulceration (Fig. 104.2).

Diagnosis

CMV should be suspected when endoscopic or colonoscopic visualization show discrete, often hemorrhagic, erosions or ulcerations with normal intervening mucosa in an HIV-1-infected patient. Colitis may be patchy in approximately 40% of cases and involve only the right colon or cecum in 18% [140], underscoring the importance of colonoscopy in the diagnostic evaluation of colitis in an HIV-1-infected patient. The diagnosis of CMV gastrointestinal disease is established by the histopathological identification of large (cytomegalic) mononuclear, endothelial, or epithelial cells containing intranuclear or cytoplasmic inclusions with surrounding inflammation [141] and confirmed by immunocytochemical staining, *in situ* hybridization, or DNA amplification.

Treatment

Ganciclovir and foscarnet are the drugs of choice for the treatment of CMV (Table 104.5). Because these agents are virustatic and not virucidal, recurrent CMV disease is common after the cessation of therapy. Ciclovovir is a new nucleoside analogue with potent prolonged *in vivo* activity against CMV in patients with CMV retinitis, but the agent has not been studied in patients with gastrointestinal CMV disease. The availability of valganciclovir, an oral form of ganciclovir with substantial bioavailability (≈ 50%–60%), has been of substantial value in controlling CMV disease in a range of immunocompromised patients, both for primary and maintenance therapy.

Herpes simplex virus

Herpes simplex virus (HSV) is also a double-stranded DNA virus in the herpesvirus group. Latent in sensory nerve ganglia, HSV reactivation during immunosuppression leads to spread of the virus along sensory nerve pathways to mucocutaneous sites where the virus next spreads among epithelial cells by lysis, releasing virions that infect and lyze adjacent cells. Reactivation increases HIV-1 levels in plasma. Gastrointestinal HSV disease is confined to the perianal region, distal rectum, and esophagus. The perianal lesions range from small, unrecognized mucosal ulcers or fissures to chronic, deeply ulcerated necrotic lesions that cause localized pain but not diarrhea [142]. Involvement of the rectum (proctitis) is often associated with perianal disease. Proctitis manifests as severe anorectal pain, tenesmus, constipation, inguinal lymphadenopathy, and, less often, difficulty with urination and sacral paresthesias. Such symptoms may also be associated with proctitis in seronegative homosexual men. Diarrhea is not associated with typical proctitis, although the mucopurulent discharge may be misinterpreted as diarrhea. Proctocolitis, which occurs when the proctitis extends proximally into the distal sigmoid colon, can cause mild diarrhea associated with hematochezia, but the predominant symptoms are generally those of the proctitis. Overall, HSV proctocolitis is a rare cause of diarrhea in HIV-1-infected persons [132].

Diagnosis

Anoscopy and sigmoidoscopy are required to diagnose HSV proctitis and proctocolitis, respectively. Typical lesions begin as small vesicles, these progress to erosions that coalesce

into diffuse ulcers. Diagnosis is predicated on the cytological identification of intranuclear (Cowdry type A) inclusions in cells within the lesion and is confirmed by virus isolation.

Treatment

Acyclovir and related agents are the drugs of choice (Table 104.5) for HSV infection. Recurrent HSV is common so long-term suppressive therapy may be necessary. Despite increased use of suppressive acyclovir therapy, increased acyclovir-resistant HSV (type 2) has not been reported [143].

Adenovirus

Adenovirus is a double-stranded non-enveloped DNA virus that is well recognized as a cause of morbidity and mortality in immunocompromised persons such as recipients of stem cell and organ transplants [144]. In HIV-1-infected patients, adenovirus has been isolated from various body sites and may induce hepatic necrosis, but intestinal involvement is rare. In the United States, adenovirus excretion is equally common in HIV-1-infected patients with and without diarrhea [45]. In contrast, 23% of Australian HIV-1-infected patients with diarrhea reportedly excrete adenovirus compared to 5.4% without diarrhea [44]. Adenovirus has been identified with transmission electron microscopy and culture in inflamed colonic tissue of patients with AIDS with chronic, watery, nonbloody, nonmucoid diarrhea [145]. Weight loss was also a prominent symptom. Endoscopically, the colonic mucosa showed areas of discrete, often raised, erythematous lesions that were several millimeters in diameter. Light microscopy showed chronic inflammation surrounding epithelial cells containing large, amphophilic intranuclear, but not cytoplasmic, inclusions. Adenovirus appeared to infect only epithelial cells, especially goblet cells, sparing lamina propria cells, which are frequent targets of CMV. At the ultrastructural level, adenovirus was associated with degeneration, death, and focal necrosis of infected epithelial cells, some of which had been extruded into the lumen (Fig. 104.3). Although adenovirus can cause pathogenic changes in the colonic mucosa, a causal relationship between adenovirus and diarrhea has not been established. An array of antiviral agents has been used in immunocompromised patients with adenovirus infection, but only treatment with cidofovir, a cytosine analogue with nephrotoxicity, has been associated with a significant reduction in adenovirus-associated mortality [144].

Astrovirus, picobirnavirus, and rotavirus

Prospective evaluation of chronic diarrhea in HIV-1-infected persons indicated an association between the presence of astrovirus and picobirnavirus and diarrhea in HIV-1-infected persons [146], but further epidemiological and clinical studies are needed to define the role of these viruses in HIV-1 disease. Rotavirus is not associated with diarrhea in HIV-1-infected patients in the United States [29,45,146] or Africa

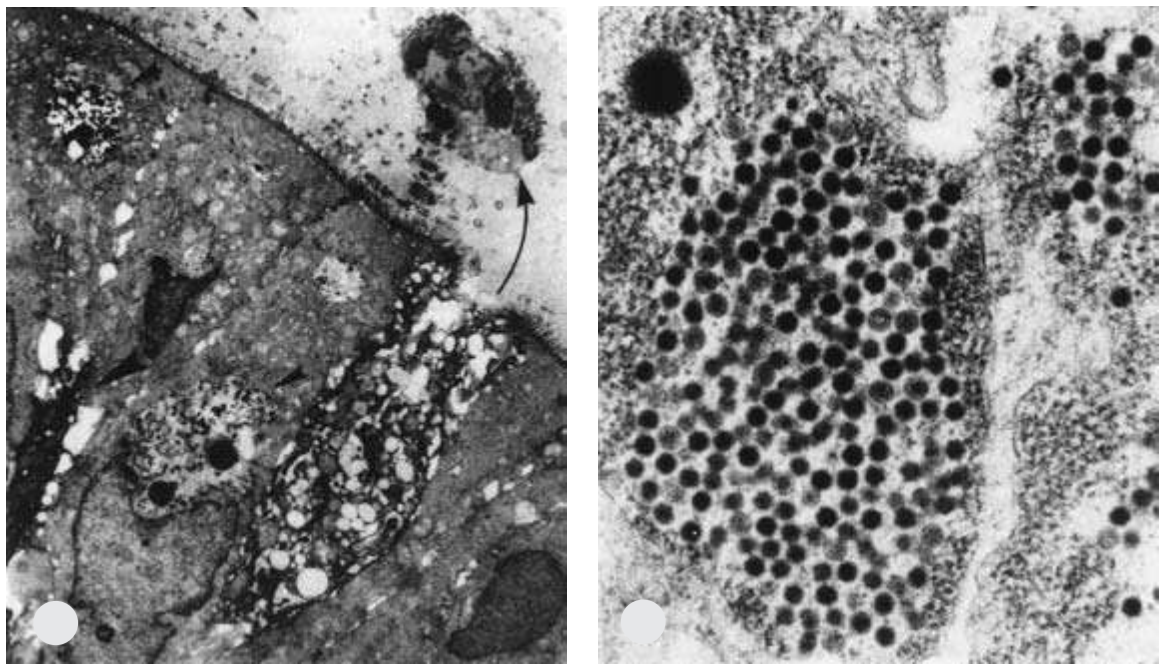


Figure 104.3 **(a)** Transmission electron micrograph of a colonic biopsy specimen from a patient with adenovirus colitis shows a nucleus from a necrotic enterocyte extruded into the lumen (arrow). Adjacent cells have foci of disrupted cytoplasm containing secondary lysosomes (original magnification × 34 000). **(b)** Higher magnification of a portion of the extruded nucleus in **(a)** shows numerous hexagonal virions characteristic of adenovirus (original magnification × 49 000). From Janoff et al. [145], with permission from Elsevier.

(Zaire) [147,148], but it is the predominant virus detected in the stools of Australian HIV-1-infected homosexual men with diarrhea [44] and is common in HIV-1-infected adults with diarrhea in Peru [31]. These findings suggest geographic variation in the prevalence of rotavirus-associated diarrhea in HIV-1-infected persons, but additional studies are needed.

Fungal infections

Histoplasma capsulatum

Histoplasma capsulatum is a dimorphic fungus that exists as a mycelial form with septate branching hyphae that contain readily airborne and infectious spores and a yeast form present in tissue macrophages. The organism is endemic in the central region of the United States and some tropical, subtropical areas, including Central and South America, where bird excrement provides microfoci of infection.

Disseminated *H. capsulatum* is the most common of the endemic mycoses in HIV-1-infected persons with $CD4^+$ T-cell counts less than 100 cells/mm^3 who reside in endemic areas [149–151]. Gastrointestinal involvement generally reflects disseminated disease, which is primarily the result of reactivation of quiescent infection. Among persons with disseminated *H. capsulatum*, gastrointestinal involvement has been detected in approximately 70% of cases by evaluation of biopsy specimens, but gastrointestinal symptoms are present in only about 10% of patients [152]. Symptoms include diarrhea, weight loss, fever, and abdominal pain, which often accompany respiratory symptoms. *Histoplasma* skin lesions are uncommon in patients in the United States but occur in up to 85% of patients in Brazil, possibly as the result of genetic differences between the *H. capsulatum* strains in the United States and those in Latin America [153]. Most patients with gastrointestinal manifestations of *H. capsulatum* have colonic involvement. Colonoscopy in such patients may show inflammation, ulcerations, or fungating mass lesions.

Diagnosis

The histological examination of Giemsa-stained sections shows small yeast-like cells within macrophages or histiocytes. When infection is intense, the organism is not difficult to identify. However, the diagnosis of histoplasmosis is established by culture. Serological detection of complement fixing antibodies to *H. capsulatum* mycelial antigens provides supportive evidence for infection.

Treatment

Amphotericin B is the drug of choice for disseminated histoplasmosis (Table 104.5). The recommended initial course of therapy in HIV-1-infected persons is amphotericin B 0.5–0.7 mg/kg intravenously daily for 4–8 weeks, or longer, depending on the clinical response. Long-term therapy with amphotericin B (50–80 mg intravenously every 2 weeks) is also highly effective in suppressing relapses after initial treatment [150]. Itraconazole may also be effective for milder disease and chronic suppressive therapy. The role of caspofungin acetate, a fungicidal echinocandin, is under evaluation.

Biliary disorders

HIV-1-infected persons and patients with AIDS may develop cholecystitis and cholangitis as the result of cholelithiasis and the same gram-negative bacteria that cause biliary tract disease in seronegative persons. In addition, HIV-1-infected persons are susceptible to a spectrum of biliary tract disorders related to opportunistic pathogens (Tables 104.2 and 104.3) and lymphoma, referred to collectively as the AIDS cholangiopathy. The disorders associated with infection include acalculous cholecystitis, biliary tract obstruction due to stenosis of the distal common bile duct, and narrowing and irregularity of the intrahepatic and extrahepatic bile ducts, associated most commonly with *Cryptosporidium* or CMV in the gallbladder and bile duct papillary stenosis and sclerosing cholangitis [154,155]. Other opportunistic pathogens implicated etiologically in biliary tract disease include *Enterocytozoon bieneusi*, *Septata intestinalis*, *Isospora belli*, and *Pneumocystis carinii* [156–158]. Endoscopic retrograde cholangiography is the most effective diagnostic tool for evaluating the extent of the disease and excluding other processes, such as cholelithiasis. Sphincterotomy is an effective therapeutic modality for relieving the obstruction and pain. Rarely, HIV-1-associated non-Hodgkin lymphoma may cause sclerosing cholangitis, resulting in a similar clinical syndrome. The long-term prognosis of HIV-1-infected patients with hepatobiliary cholangiopathy is similar to that of patients without the syndrome.

Pancreatitis

Pancreatitis is an important gastrointestinal complication in patients with HIV-1 infection. Autopsy studies have identified an array of opportunistic pathogens, including viruses (CMV and HSV), fungi (*Cryptococcus neoformans*), bacteria (*Mycobacterium*) and protozoa (*Toxoplasma gondii*), as well as neoplasms (non-Hodgkin lymphoma and Kaposi sarcoma), in the pancreas [159]. Although CMV and tuberculosis may cause clinical pancreatic disease [160,161], most infectious and neoplastic processes involving the pancreas in HIV-1-infected persons are subclinical. In contrast, drug-induced pancreatic disease is a common cause of clinical pancreatitis among persons with HIV-1 infection [162]. The two most common drugs implicated in HIV-1-associated pancreatitis are pentamidine and dideoxyinosine. Pentamidine-induced disease, which may occur with either inhaled or parenterally administered drug, causes mild to severe, and even fatal, pancreatitis. Dysregulation of glucose metabolism is common in this setting, although pentamidine itself may

cause dysregulation in the absence of clinical pancreatitis [163]. Dideoxyinosine therapy may induce pancreatitis in up to 10% of patients [164,165]; occasionally, dideoxyinosine-induced pancreatitis is fatal. Low-level hyperamylasemia (serum amylase level less than three times the upper limit of normal) may occur in patients with AIDS despite the absence of underlying pancreatic disease [166,167]. After antiretroviral drug-induced pancreatitis has been excluded, the presentation, evaluation, and management of acute pancreatitis in HIV-1-infected persons are similar to that in seronegative persons.

Gastrointestinal bleeding

Upper gastrointestinal bleeding is uncommon in HIV-1-infected persons, who are as likely to bleed from HIV-1-related etiologies as non-HIV-1 causes. The most common HIV-1-related causes are viral esophagitis, gastroduodenal lymphoma, and MAC disease of the proximal small intestine [168,169]. Regarding viral esophagitis, HSV is the most common cause of bleeding. Unlike HIV-associated CMV colitis, CMV esophagitis is rarely associated with significant bleeding [170,171].

Similar to the low prevalence of bleeding in the upper gastrointestinal tract, intestinal and colonic bleeding in HIV-1-infected persons is uncommon. Although the differential for lower gastrointestinal tract bleeding includes traditional causes, opportunistic infections are, in our experience, the most common etiology for bleeding from the lower gastrointestinal tract in persons with HIV-1 infection. In contrast to the esophagus, where HSV is the most common infection associated with bleeding, lower tract bleeding is more frequently caused by CMV [172]. Typically, bleeding is slow and associated with abdominal pain and diarrhea; bleeding from CMV colitis is rarely severe, but may be associated with intestinal perforation [173]. Other HIV-1-related causes of lower gastrointestinal tract bleeding include non-Hodgkin lymphoma, unusual infections, such as *Histoplasma capsulatum*; and idiopathic ulcerations. The approach to diagnosis and management of gastrointestinal bleeding in HIV-1-infected patients is similar to that in seronegative persons.

Gastrointestinal neoplasms

Kaposi sarcoma and non-Hodgkin lymphoma are the most common gastrointestinal neoplasms associated with HIV-1 infection. Recognized formerly as a rare neoplasm of the skin in older men of Jewish or Eastern European origin, Kaposi sarcoma emerged in the 1980s as one of the most common opportunistic processes heralding the AIDS epidemic. Although the incidence of Kaposi sarcoma has declined dramatically in recent years [174], the tumor remains an important gastrointestinal malignancy in HIV-1-infected, immunosuppressed, homosexual and bisexual men; Kaposi sarcoma occurs rarely in those who acquire HIV-1 intravenously or through heterosexual contact. Human herpesvirus 8 is etiologically linked to the development of Kaposi sarcoma [175–178]. Histologically, the tumor is characterized by the proliferation of spindle-shaped endothelial cells in a stroma of proliferating abnormal vessels, fibroblasts, and infiltrating leukocytes. The local presence of angiogenic growth factors, human herpesvirus 8, and impaired immunosurveillance mechanisms appear to predispose the gastrointestinal tract to Kaposi sarcoma formation.

Endoscopically, Kaposi sarcoma begins as a small, hyperpigmented plaque or macule, progressing to a violaceous nodule and eventually a tumor-like mass. In the early stage of the AIDS epidemic, visible tumor was identified in the gastrointestinal tract in 40%–50% of patients with AIDS [179,180]. Although the tumor may be more common in the upper tract, Kaposi sarcoma occurs throughout the gastrointestinal tract. Oropharyngeal Kaposi sarcoma is highly suggestive of upper tract involvement. The vast majority of persons with enteric Kaposi sarcoma are asymptomatic; consequently, therapy for gastrointestinal lesions is rarely necessary. Interferon-α and doxorubicin treatment have been associated with tumor regression [181,182]. Unusually large tumors that cause bleeding, obstruction, or perforation can be treated emergently with radiation therapy. Antiretroviral therapy likely has a positive impact on the incidence and progression of this tumor.

Among HIV-1-infected patients with non-Hodgkin lymphoma, the most frequently involved gastrointestinal sites are the colon (46%), ileum (39%), and stomach (23%) [183]. Symptoms associated with gastrointestinal lymphoma include abdominal pain, weight loss, obstruction, and bleeding [183–185]. The presence of latent and replicating Epstein–Barr virus DNA in the lymphomas of patients with AIDS suggests that lymphoproliferative lesions in the gastrointestinal tract could represent the outgrowth of Epstein–Barr virus-transformed or modulated mucosal B cells [186]. Chemotherapy, surgery, radiotherapy, or combination therapy have not substantially improved the survival of HIV-1-infected persons with gastrointestinal lymphoma.

Anorectal disease

Anal complications associated with HIV-1 disease include perianal condylomata, infection, fissure, fistula, and squamous cell carcinoma. These complications occur predominantly in HIV-1-infected homosexual and bisexual men. Homosexual men, particularly those who are HIV-1-positive, have a high incidence of anal infection with human papillomavirus (HPV) [187], which has been implicated as a risk factor for anal condylomata and anal squamous cell carcinoma

[188]. The link between HPV, particularly types 16 and 18, and anal carcinoma may be through the ability of the virus to induce local dysplasia in a high proportion (15%) of immunosuppressed, HIV-1-infected homosexual men [189]. The role of cytological screening for HPV and local HPV-related lesions is under investigation and is recommended by some authorities. Although quadrivalent vaccine against papillomavirus has been shown to reduce the incidence of HPV-associated anogenital diseases in young women [190], the vaccine has not yet been evaluated for efficacy in men, particularly those with HIV-1 infection. Perianal infection is commonly caused by HSV, but involvement of the rectal mucosa is rare (see section on herpes simplex virus). Rectal complications of HIV-1 infection include nonspecific proctitis (rectal inflammation in the absence of an identifiable pathogen [191]) and infectious proctitis due to *Chlamydia trachomatis*, *Treponema pallidum*, *Neisseria gonorrhoeae*, or HSV [192]. Perirectal abscess in HIV-1-infected persons is often idiopathic [193] or may be associated with HPV [194,195] or CMV [196] infection.

The initial presentation of anorectal disease includes dyschezia (pain on defecation), bleeding, drainage from the anal canal, palpable mass, and sepsis syndrome (local pain and fever). Dyschezia usually results from ulcerative lesions in the anal canal caused by infection, abscess, fissure, hemorrhoids, or neoplasm. A palpable mass suggests neoplasm. When the mass is tender and accompanied by fever, perirectal abscess is the most likely differential diagnosis. Tenesmus, urgency, and frequent small-volume stools suggest proctitis. Perianal drainage is usually caused by a fistulous tract or, less commonly, severe proctitis. Careful inspection, palpation of the perianal area and anal canal, and visualization of the rectum are mandatory components of the anorectal evaluation. Biopsy is performed for microbiological evaluation and histological examination, and computerized tomography is helpful for staging neoplasms and evaluating perirectal abscess. Symptomatic treatment, pathogen-specific antimicrobial therapy, and surgery in selected patients [197] are effective for most nonmalignant anorectal lesions. Anorectal neoplasms are treated with radiation therapy, chemotherapy, and, when localized, surgical resection [198].

Other immunodeficiency states

Hematopoietic stem cell transplant recipients

Gastrointestinal disease is an important cause of morbidity and mortality in recipients of allogeneic hemopoietic stem cell or solid organ transplants. Hematopoietic stem cell transplantation, in which recipients receive donor bone marrow or peripheral blood stem cells, is performed to cure hematological malignancies and certain nonmalignant disorders of blood cells. Along with donor progenitor cells, $CD4^+$ and $CD8^+$ αβ T cells are transferred into the recipient. Although the αβ T cells reconstitute T-cell immunity and eradicate malignant cells in the recipient, they also attack recipient nonself antigens in host tissues in a process known as graft-versus-host disease [199]. Stem cell transplantation is further complicated by the need for immunosuppressive therapy, which impairs immune reconstitution and increases the risk of infection. Consequently, Recipients of stem cell transplantation experience more frequent and more severe gastrointestinal disease than solid organ (kidney, liver, heart, lung) recipients, mainly as the result of graft-versus-host disease and more profound neutropenia and cell-mediated immune deficiency.

Acute graft-versus-host disease occurs usually within 3–4 weeks after transplantation, and often involves the skin, liver, lung, and intestine. Intestinal disease presents as severe watery diarrhea that may be accompanied by anorexia, nausea, vomiting, abdominal pain, and gastrointestinal bleeding. [200,201]. The latter may involve the esophagus, stomach, and small intestine and is usually caused by CMV infection, graft-versus-host disease itself, and vascular ectasias (stomach) [202]. When rash and jaundice are also present, graft-versus-host disease is likely. However, because the gastrointestinal symptoms are nonspecific, the diagnosis of acute graft-versus-host disease is confirmed by the presence of single-cell apoptosis of the epithelium, crypt distortion, and abscesses with minimal or no inflammation [203,204] (Fig. 104.4) in endoscopically obtained biopsy specimens. Chronic graft-versus-host disease, which is less common than acute graft-versus-host disease, typically occurs 3–12 months after marrow transplantation. The major gastrointestinal manifestations of chronic graft-versus-host disease include mucositis of the oral cavity, dysphagia as the result of esophageal desquamation and stricture, and small bowel dysmotility associated with bacterial overgrowth [205–207].

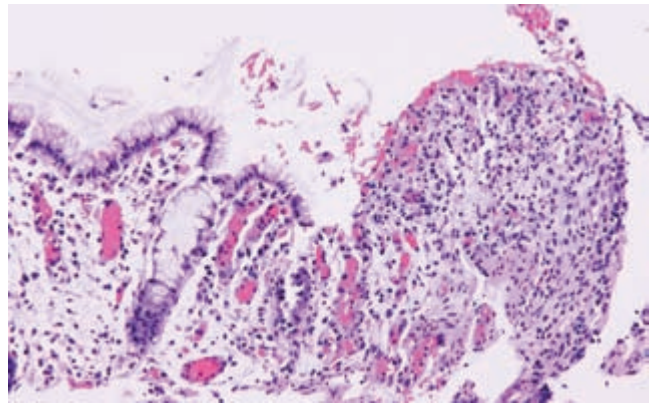


Figure 104.4 Intestinal biopsy from a patient with graft-versus-host disease shows frequent epithelial cell apoptosis in the basal zone of the crypts, crypt degeneration and loss, edema, and ulceration (× 20). Courtesy of Dr N. Jhala.

Solid organ transplant recipients

In contrast to recipients of hemopoietic stem cell transplants, the gastrointestinal complications of solid organ recipients are mainly infections. Among lung transplant recipients, for example, 51% developed operative and nonoperative gastrointestinal complications [208]. The nonoperative complications included esophagitis, gastritis, colitis, enteritis, peptic ulcer disease, gastrointestinal bleeding, cholecystitis, diverticulitis, and Ogilvie syndrome; 65% of these processes were caused by infections, excluding cholecystitis and diverticulitis.

The frequency and severity of infections diagnosed in transplant recipients varies considerably, depending on the factors listed in Table 104.6. As discussed above, stem cell transplant recipients have more frequent and more severe enteric infections than solid organ transplant recipients. The amount of time elapsed since the transplant operation also has an important influence on the development of gastrointestinal complications [208–210]. During the first month after transplantation, marrow recipients are vulnerable to fungal and bacterial infections as a consequence of cytoreductive therapy, whereas during the same period organ recipients are more susceptible to the complications of surgery and surgery-related procedures. One month after marrow transplantation, acute graft-versus-host disease should be in the differential diagnosis of diarrhea. Between 1 and 6 months after transplantation, both stem cell and solid organ recipients are highly susceptible to gastrointestinal infections caused by viruses, including CMV, adenovirus, HSV, varicella zoster virus, and rotavirus (children); fungi, such as *Candida* spp. and *Histoplasma capsulatum* [211,212]; bacteria, particularly *Clostridium difficile*; and, less commonly, parasites, such as *Cryptosporidium* [213–215]. CMV infection is an important complication of transplantation because it affects up to 25% of organ transplant recipients [216–218], causes major gastrointestinal morbidity in such patients [219], and is associated with a marked increase in mortality among transplant recipients [220]. The presence of graft-versus-host disease in hemopoietic stem cell transplant recipients also significantly increases the risk of CMV disease, and, conversely, CMV infection increases the risk of graft-versus-host disease [221]. After 6 months, stem cell and organ transplant recipients may develop gastrointestinal manifestations of chronic graft-versus-host disease. Susceptibility to an array of enteric pathogens also increases after 6 months (Table 104.7). In addition, recipients of bone marrow or organ transplants are susceptible to the gastrointestinal complications of chemotherapy, radiation therapy, and typhlitis.

Table 104.6 Factors that influence the development and management of gastrointestinal infections in transplant recipients

Type of transplantation
Hematopoietic stem cell vs solid organ
Time since transplantation
< 1 month: fungal and bacterial infections
~ 1 month: acute graft-versus-host disease
1–6 months: viruses, especially cytomegalovirus
> 6 months: chronic graft-versus-host disease; enteric pathogens; chemotherapy and radiation therapy complications
Prevalence of potential pathogens in the environment
Prevalence of latent infections in the donor and host before transplantation
Especially cytomegalovirus
Level of immunosuppression
Profound suppression/hematopoietic stem cell recipients
Type of immunosuppressant (less with cyclosporine [ciclosporin rINN])
Availability of diagnostic tests
Transplant center vs community hospital
Rigor of diagnostic evaluation for enteric pathogens
Transplant center vs community hospital

Pathogens endemic to certain regions may be more common in transplant recipients. For example, *Strongyloides stercoralis*, which is more prevalent in the southeastern part of the United States, has been reported in renal transplant recipients in that region [222,223] and in Brazil. Similarly, *Histoplasma capsulatum* infections are more common in transplant recipients in the midwestern and southeastern part of the United States. Although amebiasis and malaria are common in immunocompetent persons in the tropics, *Entamoeba histolytica* and *Plasmodium falciparum* infections appear not to be more common in organ (renal) transplant recipients in the same region [224]. In addition, the prevalence of latent infections in the donor and host before transplantation strongly influences the development of infection by the same pathogen after transplantation. For example, among bone marrow and liver transplant recipients, the highest rate of symptomatic CMV infection occurs when the donor is seropositive and the recipient is initially seronegative and the lowest rate when both the donor and recipient are seronegative [225–227]. The relative risk for death 1 year after transplantation is also affected by the presence of latent CMV infection. One year after liver transplantation, the highest relative risk (4.56-fold) for death occurred when the donor was CMV-seropositive and the recipient was CMV-seronegative [220]. In addition, the more profound the immunosuppression, as in bone marrow transplant recipients, the more severe the infections. The rate of infectious complications is also affected by the type of immunosuppressive regimen. Compared with recipients of heart transplants, who received conventional immunosuppressive therapy, recipients who received cyclosporine (ciclosporin rINN) had lower rates of complications and morbidity due to CMV and HSV [217].

Table 104.7 Gastrointestinal pathogens in immunocompromised subjects[a]

Site	Viral	Parasitic	Fungal	Bacterial
Oral cavity	Herpes simplex virus Epstein–Barr virus		*Candida* sp.	
Esophagus	Herpes simplex virus Cytomegalovirus		*Candida* sp. *Histoplasma capsulatum*	*Mycobacterium tuberculosis* *Pseudomonas aeruginosa*[b] *Lactobacillus acidophilus*[b] *Streptococcus* sp.[b] *Staphylococcus* sp.[b] *Bacillus* sp.[b]
Small intestine	Cytomegalovirus Rotavirus Adenovirus Coxsackievirus Astrovirus Calicivirus Picobirna/parvovirus	*Giardia lamblia* *Cryptosporidium parvum* *Strongyloides stercoralis* Microsporidia[b], *Cyclospora*[c]	*Aspergillus* sp. *Histoplasma capsulatum*	*Salmonella* sp. *Campylobacter* sp. *Aeromonas* sp.
Colon	Cytomegalovirus Herpes simplex virus[c]	*Entamoeba histolytica*	*Histoplasma capsulatum* *Aspergillus* sp.	*Clostridium difficile* *Campylobacter* sp. *Shigella* spp.
Liver	Cytomegalovirus Herpes simplex virus		*Candida* sp.	*Mycobacterium* sp.

a Excluding persons infected with human immunodeficiency virus type 1.
b Rare.
c Proctitis.

Finally, the availability of diagnostic tests and the rigor with which patients are evaluated for enteric pathogens have strong influences on the detection of gastrointestinal pathogens in immunocompromised patients.

Neutropenia

A reduction in the absolute number of circulating neutrophils to less than 1000 cells/mm^3 is associated with increased susceptibility to infections. When the level of neutrophils declines to less than 500 cells/mm^3, normal host defense against endogenous gastrointestinal flora is substantially impaired. Neutropenic patients are far more likely to present with fever and sepsis from gram-negative bacteria and fungemia than with primary gastrointestinal syndromes. Importantly, the intestinal flora is the source of most bacteremias in neutropenic patients [228,229], and approximately 15% of such patients have polymicrobial infections [230]. Data suggest that the risk for the development of bacteremia in neutropenic patients colonized with potential gram-negative bacterial pathogens is up to 200-fold greater than the risk in noncolonized patients; for this reason, some clinicians advocate surveillance cultures of mucosal and other surfaces [228]. However, the clinical responses to the presence of such colonization are not well characterized nor have the beneficial consequences been validated.

Three gastrointestinal syndromes may complicate neutropenia: oral infections, anorectal infections, and enterocolitis. The oral manifestations of neutropenia include gingivitis, periodontitis, and oral ulcers. These complications occur most commonly when the neutrophil count declines below 500 cells/mm^3 [231–234]. Anorectal disease is an important and life-threatening complication of neutropenia and is discussed above (see section on anorectoal disease).

Necrotizing enterocolitis typhlitis, also referred to as neutropenic enterocolitis and the ileocecal syndrome, is an infrequent complication of chemotherapy-induced neutropenia in patients with leukemia or lymphoma [235,236]. The etiology of necrotizing enterocolitis is thought to be the consequence of chemotherapy-induced mucosal injury that promotes local infection by enteric bacteria [230]. In a minority of cases, the neutropenia is caused by the hematological disease itself, such as aplastic anemia and multiple myeloma. Necrotizing enterocolitis has been associated with *Clostridium septicum* sepsis in several cases [237], suggesting that local infection with this gas gangrene-producing bacterium may be involved in the pathogenesis of the syndrome

in some patients. Typically, patients present with right lower quadrant pain, diarrhea, lower gastrointestinal bleeding, and fever. The syndrome should be recognized early because the characteristic cecal inflammation may progress to life-threatening necrosis and perforation, in which case surgical intervention is imperative. A clinical condition consistent with necrotizing enterocolitis also has been reported in HIV-1-infected patients, except that neutropenia may be absent [238]. However, some patients with necrotizing enterocolitis, particularly those with HIV-1 infection, have been managed conservatively with broad-spectrum antibiotics, bowel rest, fluids, and close radiographic and computed tomographic monitoring [237].

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I MISCELLANEOUS

105 Gastrointestinal manifestations of immunological disorders

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Immunodeficiency diseases and the gut

The gut epithelium represents the largest surface area where the host interacts with the environment, separating the lumen from the internal milieu. This structure is vulnerable to access by infectious material. Strategic mucosal defense requires a precisely regulated mucosal immune system supported by a variety of nonimmunological protective factors, such as gastric acidity, digestive enzymes, peristalsis, mucus secretion, epithelial cell regeneration, and the mutually competitive interactions of the gut flora [1].

The importance of effective mucosal defense is underscored by the frequency of mucosal infections. Mucosal infections are a major cause of mortality in children under 5 years of age; *Helicobacter pylori* is the most common infection in humans; and diarrheal infectious disease alone accounts for about 3 million deaths among children worldwide annually. A wide spectrum of opportunistic infections occurs in patients with immune deficiencies. Patients with milder, more selective forms of immune deficiency (e.g., immunoglobulin A [IgA] deficiency), however, are frequently free of infectious complications. The nonimmunological mucosal defenses, as well as the functional reserve of the immune system, probably account for the resistance of these patients to infection. In addition, immune deficiencies may predispose to autoimmunity and opportunistic cancers.

Classification of immunodeficiency disorders

Primary immune deficiencies (congenital or acquired) are rare and result from intrinsic defects in the cellular or secretory components of the immune system, whereas secondary immune deficiencies are common and may arise from a diversity of conditions, including immunomodulatory drugs, protein-calorie malnutrition, protein-losing enteropathy, cancer, and age-related immune senescence. The most important secondary immunodeficiency disorder is acquired immunodeficiency syndrome (AIDS), which is discussed separately in Chapter 104.

Considerable advances in basic immunology have been made by studying the primary immunodeficiencies. About 100 of these are recognized and in most cases the genetic and molecular basis is understood [2–4]. The current classification of primary immunodeficiencies is summarized in Table 105.1. Several new conditions are included, such as the hereditary periodic fevers including familial Mediterranean Fever, because these conditions have been shown to be inborn errors in the regulation of innate immunity [5]. It is noteworthy that the list of molecular defects is increasing as new immune deficiencies in mice with various targeted gene deletions are discovered. Conditions of particular gastroenterological importance are discussed in this chapter. A representative list of opportunistic or recurring gastrointestinal infections that commonly occur in patients with different primary immunodeficiency syndromes is shown in Table 105.2.

X-linked (congenital or Bruton) hypogammaglobulinemia

This is the prototypic syndrome of a pure B-cell or humoral immunodeficiency. It occurs in males, although rarely in females. The genetic defect results from mutations in the cytoplasmic signal transduction molecule, Bruton tyrosine kinase (Btk) [2,3]. Serum levels of all immunoglobulins are less than 100 mg/dL, and there is an inability to make functional serum antibodies. An intrinsic B-cell defect with a maturation block in preB-cell to B-cell differentiation is present [2,3]. The preB cells fail to express functional immunoglobulin M (IgM) on the cell surface because of an inability to rearrange immunoglobulin light-chain genes. PreB cells are

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Table 105.1 Categories of primary immunodeficiency syndromes

Category	Examples/Comments
Combined T-cell and B-cell deficiencies	Highly heterogeneous and result in a severe clinical phenotype
Predominantly antibody defects	B-cell defects that usually present with recurrent bacterial infections, e.g., X-linked agammaglobulinemia, common variable immunodeficiency, selective immunoglobulin deficiency
Defined syndromes of immunodeficiency with nonimmunological features including syndromes with defects in DNA repair	For example, Wiskott–Aldrich syndrome, ataxia-telangiectasia, chronic mucocutaneous candidiasis
Diseases of immune dysregulation	Defects in apoptosis leading to lymphoproliferation or autoimmunity
Defective phagocytic cell function/number	For example, chronic granulomatous disease, leukocyte adhesion deficiency
Defects in innate immunity	Mannose-binding protein deficiency
Autoinflammatory disorders and Blau syndrome (NOD2 mutations)	Includes the hereditary periodic fever syndromes, e.g., familial Mediterranean fever
Complement deficiencies	Defects in early components of pathway are associated with lupus-like autoimmunity; defects in terminal components are linked with infections (notably *Neisseria*); defects in the lectin pathway initiated by mannose-binding lectin are associated with recurring infections; defects in C1 esterase inhibitor cause hereditary angioedema.

Table 105.2 Gastrointestinal infections in immunocompromised hosts

Site	Infection	Examples/comment
Oroesophageal	Candidiasis	*C. albicans* and non-*albicans*
	CMV	
	HSV	
Gastric	*Helicobacter pylori*	
	Adenovirus	
	Bacillary angiomatosis	*Bartonella* spp.
Small intestine	Adenovirus	
	Mycobacteria (tuberculosis and atypical mycobacteria)	
	Fungi	*Histoplasma capsulatum*
	Parasites	*Giardia lamblia*
		Cryptosporidium spp.
		Microsporidium spp.
		Isospora belli
		Entamoeba spp.
		Strongyloides stercoralis
Large bowel	CMV	
	Clostridium difficile	
	Tuberculosis	
	Spirochesosis	*Brachyspira* spp.

CMV, cytomegalovirus; HSV, herpes simplex virus.

present in bone marrow in normal numbers, but circulating B cells are absent and peripheral lymphoid tissue is hypoplastic with rare or absent plasma cells. The thymus and T-cell function are normal.

Patients usually present with recurrent pyogenic infection during infancy (after the disappearance of maternal immunoglobulin G [IgG]) or in early childhood. The respiratory and gastrointestinal tracts are the most frequent sites of infection

[6,7]. Gastrointestinal infections occur in 30% of patients [7]. These are usually infectious diarrheal episodes commonly caused by *Campylobacter* species. Giardiasis can cause severe mucosal damage and steatorrhea in these patients, but it is responsive to therapy and is surprisingly uncommon. Perirectal abscess formation and small bowel bacterial overgrowth may also occur. In asymptomatic patients, rectal biopsy characteristically reveals an absence of plasma cells and a mild lamina propria neutrophil infiltrate with early crypt abscess formation. This may lead to an erroneous diagnosis of chronic colitis. Although cellular immunity is normal, these patients are susceptible to certain virus infections, notably hepatitis and enteroviral infections including chronic meningoencephalitis and poliomyelitis [6]. There is an increased risk of malignancy, particularly lymphomas and leukemias. Treatment consists of parenteral immunoglobulin replacement.

Selective IgA deficiency

Selective IgA deficiency is the most common primary immune deficiency with a variable prevalence in different ethnic groups, in the range of 1 in 500 to 1 in 3000 [2,3]. Its occurrence is usually sporadic, but occasionally familial. Most patients lack both serum and secretory IgA1 and IgA2. The basic defect is not known, and there may be etiological heterogeneity as well as clinical heterogeneity. Occasionally IgA deficiency may be transient, and a large number of drugs including phenytoin, penicillamine, and sulfasalazine have been linked with reversible deficiency [8].

Although IgA deficiency is associated with a wide range of conditions, mainly recurrent infections of mucosal tissues and autoimmune disorders (Table 105.3), a majority of IgA-deficient individuals are asymptomatic and are free of a complicating disease. The associations with celiac disease and pernicious anemia may be partly the result of a common linkage of IgA and such diseases with human leukocyte antigen (HLA) B8/DR3. In one case, IgA deficiency with malabsorption and villous atrophy was associated with an IgG antibody to intestinal epithelial cells and a favorable response to cyclophosphamide [9].

When infections occur in IgA deficiency, they are usually recurrent bacterial and viral sinopulmonary disorders; the gastrointestinal tract is seldom involved. Giardiasis occurs in IgA-deficient subjects, but unlike pan-hypogammaglobulinemia, its frequency is probably the same as in the general population. When persistent or recurrent infections occur, it suggests associated immunological defects, such as deficiencies of IgG subclasses, particularly IgG2 and IgG4, which have been found in some patients with IgA deficiency [8]. Jejunal biopsy is usually morphologically normal in selective IgA deficiency, with an absence or paucity of IgA-producing cells and an increase in IgM-secreting cells. This may be an important compensatory response because IgM can bind to secretory component.

Table 105.3 Disorders that may occur in association with immunoglobulin A (IgA) deficiency

Gastrointestinal manifestations and associations:
None (usually)
Infections, especially giardiasis (may not be any more common than in general population)
Gluten-sensitive enteropathy (IgA prevalence in celiac disease is 1:50)
Pernicious anemia
Vitamin B-12 deficiency secondary to bacterial overgrowth
Intrinsic factor deficiency
Nodular lymphoid hyperplasia
Food allergy (increased serum antibodies to food antigens but clinical disease apparently rare)
Crohn's disease (IgA deficiency prevalence reported to be 1:73)
Disaccharidase deficiencies (unproven association)
Extraintestinal manifestations and associations of IgA deficiency:
Non-organ-specific autoimmune disorders (e.g., collagen vascular diseases)
Atopy
Malignancy (lymphomas, carcinoma reported but extent of risk is unclear and probably low)
Risk of anaphylaxis if given IgA-containing solutions, including blood

Most patients with IgA deficiency require no treatment. Replacement with serum IgA is futile because only locally produced IgA is transported into the lumen. Parenteral administration of IgA-containing blood products may be contraindicated because of the risk of anaphylactic reactions due to anti-IgA antibodies. Whether patients should be screened routinely for IgA antibodies is unclear because the titer of antibody is not well correlated with the risk of anaphylaxis to blood products. Prophylactic IgG therapy for IgA deficiency is either hazardous or ineffective, although some clinicians have reported a beneficial effect [10]. Patients with recurring infections should be screened for IgG subclass deficiency, but can usually be managed with prophylactic or periodic antibiotics without requiring immunoglobulin replacement.

Secretory component deficiency

Secretory component is a glycoprotein receptor on the basolateral surface of the mucosal epithelial cell that is essential for the transepithelial delivery of IgA and IgM from the lamina propria to the lumen. Deficiency of secretory component is rare, and affects not only lumenal IgA levels but also any compensatory effects of IgM. In isolated case reports, it has been associated with intestinal candidiasis and diarrhea [11,12]. Administration of IgA-rich bovine colostrum may result in symptomatic improvement.

Common variable hypogammaglobulinemia ("acquired" or late-onset hypogammaglobulinemia)

Common variable hypogammaglobulinemia is second to selective IgA deficiency as the most common primary immuno-

deficiency in adults and is the most common symptomatic primary immunodeficiency. Its variable phenotype and immunological heterogeneity have hindered identification of the underlying defect(s). It comprises a group of disorders in which there is defective terminal differentiation of B lymphocytes in the majority of patients and probably represents distinct diseases with differing immunopathogeneses [8,13]. Most cases are sporadic, but the condition can be familial.

Clinically, common variable hypogammaglobulinemia may be similar to X-linked (Bruton) agammaglobulinemia, but differences include the later age of onset, less severe infections, the fact that the lymphoid tissue (tonsils, lymph nodes, and spleen) may be normal or enlarged, and the association with autoimmune and granulomatous disease in some patients [13]. The chronic granulomatous disorder resembles the noncaseating lesions of sarcoidosis but is distinct from the chronic granulomatous disease associated with defects in neutrophil function. Patients usually present in the second or third decade with either recurrent respiratory tract infections (most commonly pneumococci, staphylococci, and *Haemophilus influenzae*) or diarrhea and steatorrhea (Table 105.4). Up to 60% of patients have chronic recurrent diarrhea; two-thirds of these have malabsorption. Enteroviral infection of the central nervous system is rare. Giardiasis is a particularly common cause of symptoms in common variable hypogammaglobulinemia, but unlike in normal individuals, it may be persistent and lead to extensive mucosal damage with steatorrhea and malabsorption [14]. It is usually reversible with appropriate anti-*Giardia* therapy. Other parasitic infections, such as cryptosporidiosis and strongyloidiasis, occur less commonly. Patients with common variable hypogammaglobulinemia are susceptible to bacterial enteric pathogens including *Shigella*, *Salmonella*, and *Campylobacter* species (*C. jejuni* and *C. fetus*), which occasionally may mimic ulcerative colitis. Bacterial overgrowth may also occur.

In some patients, sprue-like changes may be found on intestinal biopsy, and giardiasis should always be ruled out. True gluten-sensitive enteropathy has not been well documented in common variable hypogammaglobulinemia. Jejunal biopsy generally shows a paucity of plasma cells in the lamina propria, unlike sprue. When the mucosa is flat, an enteropathy refractory to a gluten-free diet is likely. Other intestinal inflammatory disorders that have been reported in common variable hypogammaglobulinemia include Crohn's disease and idiopathic ulcerative jejunitis [8,13,14].

In one-third of patients with common variable hypogammaglobulinemia, atrophic gastritis and pernicious anemia develop, with an increased risk of gastric cancer [15]. Differences from classic pernicious anemia include the absence of mucosal plasma cells, lack of autoantibodies, involvement of the entire gastric mucosa, and normal rather than elevated serum gastrin levels. Defective gastrin release in response to bombesin or food has been reported as a highly specific means of distinguishing common variable hypogammaglobulinemia from other forms of hypogammaglobulinemia [16].

Chronic liver disease has occurred in 10%–15% of patients with common variable hypogammaglobulinemia due to hepatitis C or another non-A, non-B hepatitis virus acquired from previous plasma infusion. It has not been a problem with the use of the licensed intravenous immunoglobulin preparations. Other potential causes of hepatobiliary disease include autoimmune hepatitis, cholelithiasis, sclerosing cholangitis, and biliary cryptosporidiosis.

Nodular lymphoid hyperplasia is frequently found in common variable hypogammaglobulinemia, but it does not occur in X-linked hypogammaglobulinemia and is rare in selective IgA deficiency. The lymphoid nodules usually involve the small intestine but may affect the colon, rectum, and stomach. The nodules consist of large lymphoid follicles with germinal centers within the lamina propria. Plasma cells are either absent or markedly diminished. The lymphoid hyperplasia is thought to reflect B cells unable to undergo full differentiation to immunoglobulin-screening plasma cells. Localized forms of nodular lymphoid hyperplasia may occur in apparently immunocompetent individuals, particularly in the large bowel. In children and adolescents, a self-limited lymphoid hyperplasia in the terminal ileum is a frequent radiological finding. Although there is an increased incidence of lymphomas in common variable hypogammaglobulinemia, intestinal nodular lymphoid hyperplasia per se does not appear to be premalignant. Almost all patients with common variable hypogammaglobulinemia require parenteral immunoglobulin replacement therapy.

Table 105.4 Disorders that may occur in association with common variable hypogammaglobulinemia

Gastrointestinal:
Giardia lamblia infection
Small bowel bacterial overgrowth
Viral gastroenteritis (e.g., rotavirus)
Infectious diarrheas (*Campylobacter*, *Salmonella*, *Shigella*)
Secondary disaccharidase deficiency
Pernicious anemia, atrophic gastritis, gastric carcinoma
Gluten-sensitive sprue
Sprue refractory to gluten-free diet
Autoimmune enteropathy
Nodular lymphoid hyperplasia
Extraintestinal:
Increased incidence of generalized lymphomas
Increased incidence of autoimmune diseases
Recurrent respiratory tract infections
Chronic granulomatous disorder

Congenital thymic hypoplasia (DiGeorge syndrome)

DiGeorge syndrome, the most common contiguous-gene deletion syndrome in humans, is associated with defective formation of the third and fourth pharyngeal pouches during embryogenesis. It is characterized by absent T-lymphocyte function, hypoparathyroidism, and cardiovascular abnormalities, particularly of the aortic arch. The syndrome is the result of deletions in the short arm of chromosome 22, and several studies have implicated the transcription factor *TBX1* as a key candidate gene, although other genes have a contributory role [17]. Presentation is usually with neonatal tetany or seizures. There may be unusual facies. Other structures developing at the same stage of embryogenesis also may be affected; for example, esophageal atresia may occur. Other gastrointestinal manifestations include candidiasis, chronic diarrhea, and partial villous atrophy with malabsorption.

Chronic mucocutaneous candidiasis

Chronic mucocutaneous candidiasis represents a spectrum of clinical syndromes in which there is an increased susceptibility to chronic *Candida* infections of the mucosa, skin, and nails [18]. Most patients become infected with other organisms in addition to *Candida* species. There is variability in the mode of inheritance (autosomal recessive and sporadic), age of onset (early or late), and frequency of association with autoimmune disorders such as pernicious anemia and endocrinopathies, including Addison disease, diabetes mellitus, hypothyroidism, and hypoparathyroidism. The latter subset of patients comprise the autoimmune polyglandular syndrome type 1 or autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy, a recessive disorder caused by mutations in the *AIRE* (autoimmune regulator) gene [19].

Most patients develop infections before the age of 5 years, but some may have the onset of *Candida* infections in adulthood. *Candida* infection most commonly involves the oropharyngeal mucosa, but involvement of the esophagus also may occur and may be present in the absence of oral lesions. Esophageal strictures, nutritional deficits, and the endocrinopathies account for most of the morbidity and mortality associated with the disease. More commonly the skin lesion is a sharply demarcated scaly erythematous macular rash; less commonly it may be granulomatous. Nails of the hands and toes are frequently involved. The *Candida* infection is usually superficial, involving the epidermis, but does not penetrate the dermis or become systemic.

A variety of defects in T-cell function have been described. Autoantibodies may precede the development of endocrinopathies. The development of endocrine abnormalities is often insidious, and it is important to evaluate patients regularly for endocrine function. Chronic mucocutaneous candidiasis does not respond well to topical antifungal therapy; systemic agents are usually required. Immunological reconstitution with therapeutic benefit has been achieved with transfer factor alone or in combination with fetal thymus transplantation.

Severe combined immunodeficiency syndromes

Severe combined immunodeficiency (SCID) is the most severe form of primary immunodeficiency and represents a syndrome caused by several distinct genetic defects, characterized by profound deficiency of T- and B-lymphocyte function and sometimes of natural killer cells [2,3,20]. At least seven different forms of SCID are recognized in humans, and the genetic defect responsible has been identified in some of these. The most common form of SCID (60% of cases) is X-linked. It is caused by defective cytokine signaling as the result of a mutation of the common cytokine receptor γ-chain, a component of the receptors for interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15. The remaining SCID cases are autosomal recessive, most of them as the result of deficiencies of purine-degradation enzymes, such as adenosine deaminase and nucleoside phosphorylase. Mutations in the gene for Janus kinase 3 (*Jak3*), which transduces the receptor signal from the common γ-chain, also cause autosomal recessive SCID, as do mutations in the gene encoding the γ-chain of the IL-7 receptor. Another variant of SCID is reticular dysgenesis, in which there is a coexisting deficiency of granulocytes.

Clinically, the syndrome presents in early infancy with severe life-threatening infections, chronic diarrhea, malabsorption, and failure to thrive. Graft-versus-host disease (GVHD) as the result of transplacentally acquired maternal lymphocytes may occur during the neonatal period. There is increased susceptibility to viral infections, including cytomegalovirus, and chronic rotavirus infection may account for the intractable diarrhea in some patients. Jejunal biopsy reveals absent plasma cells, and in some cases there is partial villous atrophy with numerous periodic acid–Schiff-positive macrophages in the lamina propria. A high incidence of gastroesophageal reflux has been reported in association with SCID [21]. Without a bone marrow graft, the clinical course is rapidly fatal. Results with gene therapy have been encouraging, despite suboptimal initial results in adenosine deaminase deficiency [22].

Wiskott–Aldrich syndrome

Wiskott–Aldrich syndrome is an X-linked recessive condition of variable severity characterized by eczema, thrombocytopenia, recurrent infections, progressive defects in T-cell function, and a poor antibody response to polysaccharide antigens. Atopy with elevated immunoglobulin E (IgE) levels is common. The underlying defective gene encodes the Wiskott–Aldrich syndrome protein, a member of a family of proteins responsible for signal transduction from the cell membrane to the actin cytoskeleton in hemopoietic cells [23]. Gastrointestinal manifestations include hemorrhage, chronic diarrhea, malabsorption, and nonspecific colitis. Without treatment, patients die from hemorrhage or the develop-

ment of Epstein–Barr virus-induced lymphoma. Stem cell transplantation is the only curative therapy for Wiskott–Aldrich syndrome, particularly if it is performed within the first 5 years of life [24].

Ataxia telangiectasia

Ataxia telangiectasia is an autosomal recessive, multisystem disorder. There are defective DNA repair mechanisms, cell cycle abnormalities, frequent chromosomal abnormalities, increased sensitivity to ionizing radiation, and predisposition to cancer. The gene responsible for this syndrome (*ATM*) encodes a product similar to phosphatidylinositol-3-kinases, which are involved in signal transduction and cell-cycle control [24]. The most prominent clinical features are progressive cerebellar ataxia, oculocutaneous telangiectasia, chronic recurrent sinopulmonary infections, and a high incidence of malignancy. The immune deficiency is variable and affects both cellular and humoral elements. IgA deficiency occurs in over 50% of patients and may be associated with IgG2 subclass deficiency. Malignancies are the most common cause of death. They are usually lymphoreticular, but adenocarcinomas also occur. Other gastrointestinal complications are not common in ataxia telangiectasia. Mild abnormalities of liver function may occur, and levels of α-fetoprotein are elevated.

Phagocytic cell defects: chronic granulomatous disease

Intrinsic defects in neutrophils, monocytes, and macrophages include several disorders of cell locomotion, adhesion, chemotaxis, killing, and metabolism [25]. Numerically, the most important of these and the most frequently encountered in gastroenterology is chronic granulomatous disease.

Chronic granulomatous disease represents a group of disorders of phagocytic cell oxidative metabolism. There are several variants of the basic defect in oxidase function, and more than one mode of genetic transmission [25]. In its classic form, inheritance is X-linked and is caused by mutations in the gene coding for the β-subunit of cytochrome β558, which is the catalytic redox entity of nicotinamide adenine dinucleotide phosphate (NADPH) and responsible for generating superoxide in phagocytes. More rarely, chronic granulomatous disease may be transmitted in an autosomal recessive or dominant pattern caused by mutations in the β-subunit of cytochrome β558 or in cytosolic proteins required for the activation of NADPH.

The respiratory burst associated with stimulation of phagocytes is lacking. This results in defective microbicidal activity because of the failure to generate toxic oxygen metabolites such as hydrogen peroxide. There is a markedly increased susceptibility to pyogenic and fungal infections, including staphylococci, *Serratia marcescens*, *Salmonella*, gram-negative enterococci, and *Candida* and *Aspergillus* species. There is particular susceptibility to infections with catalase-positive organisms. In contrast, catalase-negative organisms such as pneumococci, streptococci, and lactobacilli are not major pathogens in chronic granulomatous disease because they do not destroy their endogenous hydrogen peroxide and thereby contribute to their own demise [25,26].

The condition classically presents during infancy, although variants of the disease may present later. Every organ is vulnerable to infection, and the associated granulomas occur throughout the body. They consist of plasma cells, lymphocytes, macrophages, and occasional multinucleated giant cells without organisms. A second type of granuloma occurs in which hyphal elements of fungi are found in giant cells. Pigmented, lipid-bearing tissue histiocytes are also found.

Hepatic and gastrointestinal disorders are a prominent feature of chronic granulomatous disease. Hepatomegaly is common, with liver abscess formation in more than 30% of patients. *Staphylococcus aureus* is the most common organism in the abscesses, which often require surgery and may be recurrent.

Gastrointestinal presentations of chronic granulomatous disease may mimic Crohn's disease, and commonly include chronic gingivitis and stomatitis, perianal abscesses with fistulae, *Salmonella* gastroenteritis, diarrhea, and malabsorption. The characteristic granulomas and pigmented histiocytes may be found in patients with no gastrointestinal symptoms. In some patients the granulomatous reaction leads to stricture formation, most commonly in the gastric antrum. Although surgical treatment may be required, resolution of strictures following antibiotic therapy is well known, and aggressive antimicrobial therapy is the treatment of first choice.

Complement deficiency: hereditary angioedema (C1 esterase inhibitor deficiency and nondeficiency syndromes)

Deficiency states involving individual complement proteins are associated with a high incidence of infectious (particularly neisserial) and autoimmune disorders [2]. Deficiency of the complement regulator C1 esterase inhibitor is the most common complement deficiency state and the major one in which gastrointestinal symptoms are prominent.

Hereditary angioedema is characterized by recurrent, self-limited attacks of circumscribed nonpitting, subepithelial edema in the skin and mucous membranes [27,28]. The skin lesions are painless, and, unlike urticaria, pruritus is absent. The onset of symptoms is variable, although most patients have their first episode in childhood. A positive family history is common. Attacks may develop over hours and may last from hours to several days, often precipitated by minor local trauma, dental extractions, infections, and surgery. There is frequently initial awareness of a tingling sensation in the affected area, and a faint macular or serpiginous erythema may precede the swelling. The most serious manifestation is laryngeal edema, which may lead to fatal airway obstruction. Gastrointestinal involvement is common and includes colicky abdominal pain, vomiting, and watery diarrhea. Significant

intestinal fluid loss can occur, leading to hypotension and shock. Fever and leukocytosis are notably absent. The abdomen may be tender but not rigid. Bowel sounds may be increased. Barium studies during an attack may show the "stacked coin" appearance of mucosal edema. The lesions consist primarily of circumscribed edema without inflammatory cellular infiltrates. The edematous segment of bowel can cause intussusception. When gastrointestinal symptoms precede other manifestations, or occur in isolation, the diagnosis is often delayed, and many of these patients have been subjected to unnecessary abdominal surgery.

Hereditary angioedema is transmitted in an autosomal dominant manner. In approximately 85% of patients, the levels of C1 esterase inhibitor (C1-INH) are low because of heterozygosity for a non-expressed C1 inhibitor allele. In a minority of patients, levels of the inhibitor protein are normal but it is functionally defective because of heterozygosity for a nonfunctional C1 inhibitor allele. A third, estrogen-dependent, inherited form of angioedema has been described with no defect in C1-INH [29]. This is transmitted in an autosomal dominant manner but may be X-linked in some cases. Episodes of angioedema are indistinguishable from those associated with other forms of the disease, but occur only during pregnancy or with the use of exogenous estrogens. Acquired forms of angioedema caused by increased catabolism of C1-INH are clinically similar to the hereditary disorders occurring particularly in patients with lymphoproliferative disorders and collagen vascular diseases [27]. Drugs such as angiotensin-converting enzyme inhibitors may also cause angioedema.

The pathogenesis of angioedema is not fully understood, but the increased vascular permeability may be mediated by kinins. C1-INH prevents autoactivation of C1 and inhibits activated Hageman factor and kallikrein. The diagnosis, in most cases, is confirmed by quantitative and qualitative analysis of C1-INH. In addition, levels of C4 are secondarily reduced between attacks and nearly absent during attacks. A consensus approach to the management of angioedema has been published [27]. Infusion of C1-INH concentrate is effective for severe attacks whereas prophylaxis includes avoidance of estrogens and prompt treatment of infections and the graded use of antifibrinolytic agents (tranexamic acid or ε-aminocaproic acid) or attenuated androgenic steroids, such as danazol and stanozolol.

Secondary immunodeficiency of gastroenterological importance

Immunodeficiency due to protein loss may be a component of any severe inflammatory condition in the gastrointestinal tract; examples include protein-losing gastroenteropathy (e.g., Ménétrier disease) and extensive Crohn's disease. However, the more severe cases of gastrointestinal protein loss occur in the setting of primary lymphangiectasia or secondary lymphatic obstruction (see Chapter 83). Protein-calorie malnutrition leads to significant depression of cell-mediated immune function. This may occur in association with malignancy, particularly lymphoreticular tumors. Iatrogenic immunosuppression is an increasingly common cause of secondary immunodeficiency (e.g., in patients undergoing organ transplantation or reversible IgA deficiency with drugs such as sulfasalazine, penicillamine, and phenytoin) The increased susceptibility to *Candida* and other infections with corticosteroid use is well established but it is less well known that corticosteroids are the most common cause of superinfection with *Strongyloides stercoralis* [30]. The signficance of mucosal immune senescence in humans is uncertain. Reduced mucosal resistance and risk of bacterial overgrowth in older adults are probably multifactorial. Nonimmunological risk factors in the elderly include motility disturbances, achlorhydria, comorbidity, polypharmacy, and antibiotic use with altered enteric flora [31].

Neutropenic enterocolitis

Neutropenic colitis, also called necrotizing enterocolitis, ileocecal syndrome, and typhlitis (from the Greek *typhlos* meaning "blind sac" or cecum), may occur with neutropenia of any cause, but is most common following chemotherapy, occurring in about 6% of patients treated for leukemia [32,33]. It usually begins 7–10 days after chemotherapy, and affects primarily the ileocecal region with clinical manifestations ranging from abdominal discomfort, vomiting, distention, diarrhea, and tenderness to local or generalized peritonitis after perforation. Secondary sepsis as the result of bacterial translocation is common. The organism traditionally associated with neutropenic colitis is *Clostridium septicum* but other clostridial and non-clostridial species from the normal flora are more frequently isolated in blood cultures [33]. Diagnosis requires a high index of suspicion – many cases are diagnosed postmortem. Radiological findings include dilated bowel loops, air–fluid levels, mural thumbprinting, and pneumatosis. Barium studies are generally avoided because of the risk of perforation. Recommended management includes fluid and electrolyte replacement, broad-spectrum antibiotic coverage, and restoration of neutrophil count with granulocyte colony-stimulating factor [34]. Depending on the severity, surgery may be required.

Food allergy (hypersensitivity)

Immunological hypersensitivity to foodstuffs was shown in the early part of the 20th century, but the issue of food allergy has become controversial and confusing for several reasons. These include the lack of adherence to standardized terminology, failure to distinguish food allergy from other forms of adverse food reaction, and widespread use of unsubstantiated or inappropriate tests for food allergy by some practitioners and commercial outlets. The development of

scientifically sound experimental approaches has advanced our understanding of the pathogenesis of food allergy, improved diagnostic management of the problem, and clarified and standardized the terminology regarding the spectrum of adverse reactions to foods [35–37].

Classification and prevalence

The terms *food allergy* and *food hypersensitivity* may be used interchangeably but should be reserved for those reactions that have been shown to be mediated by the immune system (Table 105.5). *Food intolerance* is a term that should be used to describe nonimmunologically mediated adverse reactions to food or food additives that may closely mimic acute allergic reactions. Reactions to food additives, such as sulfites, salicylates, and tartrazine, may be more common and as important as allergy to natural food components (see Table 105.5). Some foods may produce adverse reactions by multiple mechanisms in different individuals, including allergic reactions to milk proteins or, rarely, to contaminant antibiotics, or they may result from lactose intolerance or fat intolerance.

The spectrum of nonimmunologically mediated food intolerance is wide (see Table 105.5) and includes food avoidance, which may arise for various reasons, including faddism, anorexia nervosa, or even dysgeusia. Common causes of dysgeusia include medications (mostly halogen salts), menopause, and depression.

Community surveys indicate that at least 20% of adults believe that they have an adverse reaction to some food. When tested objectively, using blinded food challenges, the majority of complaints cannot be reproduced; estimates closer to 1% are more realistic [35–38]. Most adverse reactions to food are probably not immunologically mediated. The prevalence of true food allergy is more common in atopic individuals and in children, but declines with age. Estimates of the frequency of food allergies in children are about 5%, although cows' milk allergy may be more common [36]. As with other forms of allergy and immune-mediated disorders, there appears to be an increasing prevalence of food allergy in industrialized countries; this is particularly true with peanut allergy [39]. In adults, food allergy is a well-documented entity, but is uncommon. True food allergy is a most unlikely diagnosis in adults presenting with new onset of gastrointestinal symptoms in the absence of a strong atopic background or without a history of food allergy since childhood.

Table 105.5 Differential diagnosis of adverse reactions to foods[a]

Immunologically mediated:
Early: immunoglobulin E-mediated
Delayed: late-phase reactions, immune complex-mediated or cell-mediated immunity (e.g., celiac disease, most cases of cows' milk allergy)
Nonimmunologically mediated:
Idiosyncratic
Gastrointestinal (e.g., lactase deficiency)
Systemic (e.g., glucose 6-phosphate-dehydrogenase deficiency)
Pharmacological (e.g., caffeine, tyramine, serotonin, alcohol, histamine)
Toxic (e.g., aflatoxins, botulism, mushroom toxins)
Infectious (e.g., salmonellosis)
Other contaminants and additives
Antibiotics, pesticides
Dyes (e.g., tartrazine)
Flavorings and preservatives (e.g., monosodium glutamate [Chinese restaurant syndrome], sulfites, benzoate, nitrites, and nitrates)
Gastrointestinal disorders (e.g., peptic ulcer, cholelithiasis)
Psychological (e.g., phobias, avoidance, faddism)

a This list is intended to be representative and is not comprehensive.

Immune response to dietary antigens

Small amounts of antigenic macromolecules normally penetrate the gastrointestinal mucosal barrier and reach the systemic circulation, occurring more in early life and in conditions causing disruption of the mucosal defenses. Uptake of antigenic material and presentation to the mucosal immune system may occur by three main routes [40]. First, the specialized microfold or M cells that overlie lymphoid follicles are adapted to sampling lumenal antigens and delivering them to dendritic cells for presentation to T cells. Second, the surface enterocytes lining the intestine are also capable of presenting antigens, and can selectively stimulate suppressor T cells in vitro. Although the significance of enterocyte-mediated uptake and processing of antigens in vivo is unclear, preferential stimulation of suppressor cells could be an important mechanism preventing hypersensitivity to dietary antigens. Finally, antigen sampling occurs across the epithelium by dendritic cells, which are capable of extending to the lumen without disrupting epithelial tight junctions.

When dietary antigen engages the immune system, there may be a local secretory IgA antibody response, but a systemic immune response seldom occurs. Most commonly, a state of immunological tolerance develops. Oral tolerance is a state of specific immunological unresponsiveness that occurs following oral ingestion of antigen, and significantly regulates immune responses to dietary antigens [40–42]. Immune responses that are most easily suppressed or tolerated by feeding proteins are IgE-mediated and delayed-type hypersensitivity responses. Not surprisingly, these are the major immunological reactions mediating food allergy. Stated differently, food allergy or hypersensitivity represents a breakdown in oral tolerance. Mechanisms responsible for the induction and maintenance of oral tolerance appear to involve activation of regulatory T cells. The molecular mediators include suppressive cytokines, particularly transforming growth factor-β. Moreover, the liver appears to down-regulate systemic immune responses to dietary antigens, and

the enteric flora have an obligatory role in the establishment of immune tolerance [40].

Although sensitization to food allergens usually occurs after exposure through the oral route in infancy, sensitization may also occur through extraintestinal routes. This has been described in children with peanut allergy who may have been sensitized to peanut protein by topical application to skin rashes of medicines containing peanut oil [43]. Sensitization may also arise because of cross-reactivity against other protein allergens.

Susceptibility to food allergy is genetically influenced but environmental determinants also play a role. All forms of allergy, including food allergies, are more common in developed countries, linked with improved sanitation and changes in the pattern of exposure to childhood infections. Some enteric infections may have a protective effect against food allergy, although the relationship is complex because enteric infections may also act as an adjuvant for responses to ingested antigens [44].

Food allergens and pathogenesis of food allergy

Most food hypersensitivity reactions are caused by a relatively small number of foods, notably milk, soy, eggs, nuts (peanut, tree nuts such as Brazil nut and walnut), fish, shellfish, and wheat. Whereas age-related loss of sensitivity is common with milk and eggs, this is less likely with allergies to nuts or seafood which are, therefore, more common in adults. For several of these foods, the specific allergen epitope has been molecularly defined [45,46]. Many food allergens are heat-stable and acid-stable, are resistant to proteolysis, and have the appropriate size with a molecular weight in the range of 10–70 kDa for bridging IgE on the surface of mast cells. Their immunogenicity may be retained or modified with food processing, digestion, and cooking (for example peanuts appear to be more allergenic if roasted).

Since food-labeling regulations vary in different countries, allergens may be hidden in processed foods [47]. With the development of transgenic foods, it is also important to be aware that food allergens may be transferred by genetic manipulation [46].

Food-allergic or hypersensitivity reactions are either immediate (IgE-mediated, type I hypersensitivity) or delayed (late-phase IgE-mediated, immune complex-mediated, and cell-mediated). Immediate or IgE-mediated hypersensitivity reactions account for the majority of immunologically mediated adverse reactions to foods. Delayed-type hypersensitivity is involved in the pathogenesis of gluten-sensitive enteropathy; so it is distinct from food allergy in its pathogenesis (see Chapter 50).

The wheal and flare reaction that occurs in some individuals after intradermal injection of a food allergen is the result of the presence of food-specific IgE antibodies on the surface of cutaneous mast cells; adjacent IgE molecules are cross-linked by the allergen and trigger degranulation of the mast cell. This phenomenon along with the uptake of intact food allergens across the mucosal barrier was elegantly demonstrated almost 80 years ago [48]. Nonallergic subjects after intradermal injection with serum from fish-sensitive patients developed wheal and flare reactions at the injection site on ingestion of fish, showing that such antigens can reach and trigger mast cells at extragastrointestinal sites.

Studies in mast cell-deficient murine models of food hypersensitivity have confirmed the central role of mast cells and their interaction with other cells including nerves, epithelial cells, and eosinophils, in the pathogenesis of food allergy [49]. This is illustrated in Fig. 105.1. Eosinophils may partially mediate the late phase of food allergic reactions within the gut mucosa in experimental animals and may contribute to allergy-related dysmotility syndromes [36]. Interleukin-5 and the eosinophil chemokine eotaxin are the primary mediators of eosinophil recruitment to the mucosa [37,50].

Clinical features of food allergy

Symptoms usually occur within minutes of ingestion but may be delayed several hours. Systemic anaphylaxis is the most serious manifestation of food hypersensitivity and may be the first presentation. Cofactors such as aspirin ingestion or exercise may have a profound effect on the clinical expression of food hypersensitivity and postprandial, exercise-induced systemic anaphylaxis has been described. More commonly, a variety of less severe and usually transient symptoms may be experienced. The organs primarily affected are the skin (eczema, urticaria), the respiratory tract (rhinitis, asthma), and the gut [36,37,51].

Gastrointestinal symptoms are nonspecific and range from edema and pruritus of the lips, buccal mucosa, and pharyngeal mucosa to vomiting, cramping, distention, and diarrhea, depending on the level of the gut primarily affected. Symptoms primarily involving the buccal or lingual mucosa (oral allergy syndrome) are particularly common and may not be associated with other gastrointestinal manifestations. Chronic diarrhea and malabsorption may arise as a result of non-IgE-mediated delayed hypersensitivity reactions, such as in food protein enteropathies and celiac disease (see Chapter 50). Cows' milk hypersensitivity in infants may cause occult bleeding or frank colitis. Food allergy is not responsible for most cases of irritable bowel syndrome but should be considered in the small subset of patients with a strong history of atopy. The importance of taking a careful history is confirmed by studies showing that symptoms can discriminate irritable bowel from food allergy [52]. Evidence for IgG-mediated adverse reactions to foods has been found in some patients with irritable bowel syndrome [53]. There is no convincing evidence for food allergy in the pathogenesis of inflammatory bowel disease. Links between food allergy and other syndromes including esophageal reflux and dysmotility appear important in children and have been reviewed elsewhere [65] and in Chapter 33.

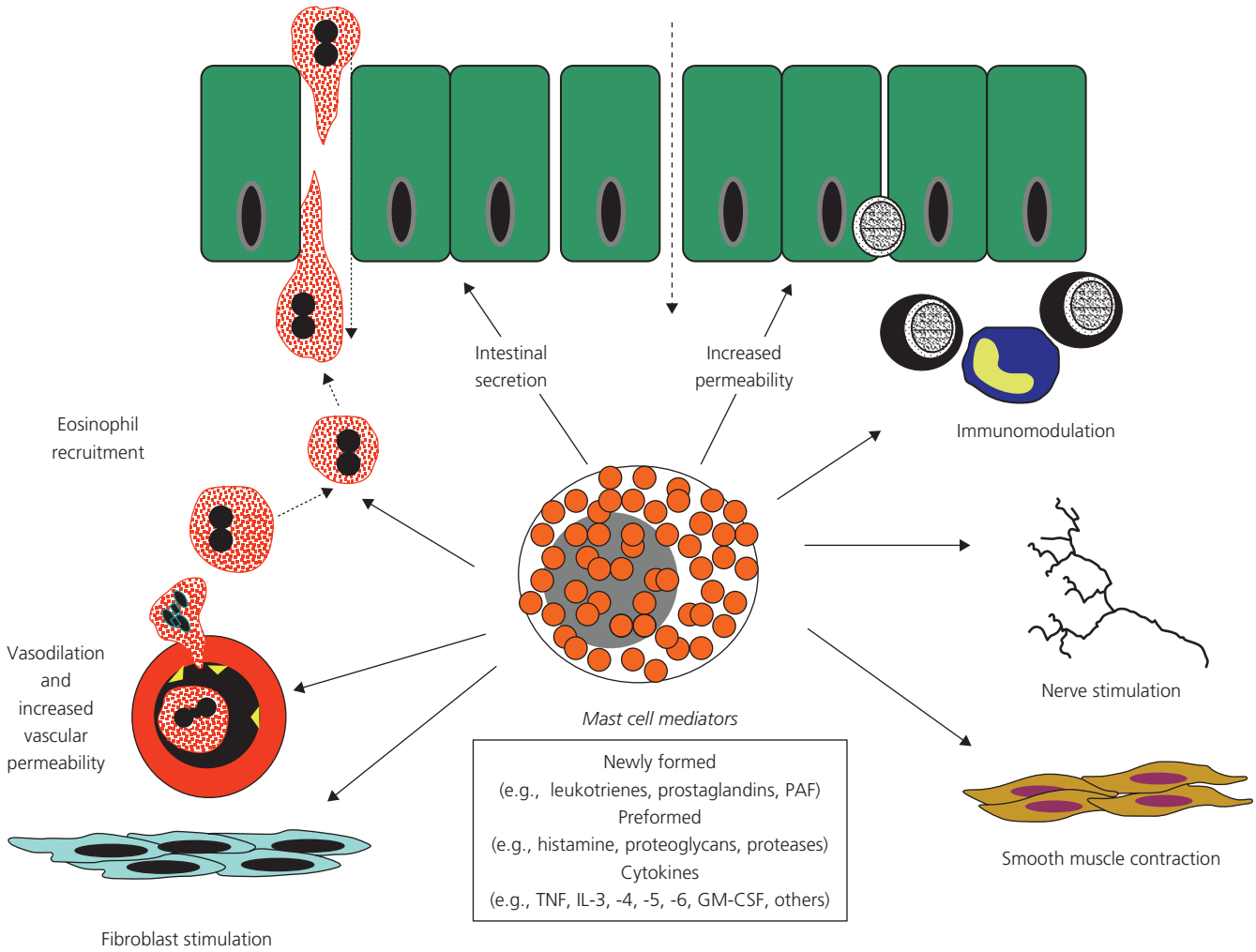
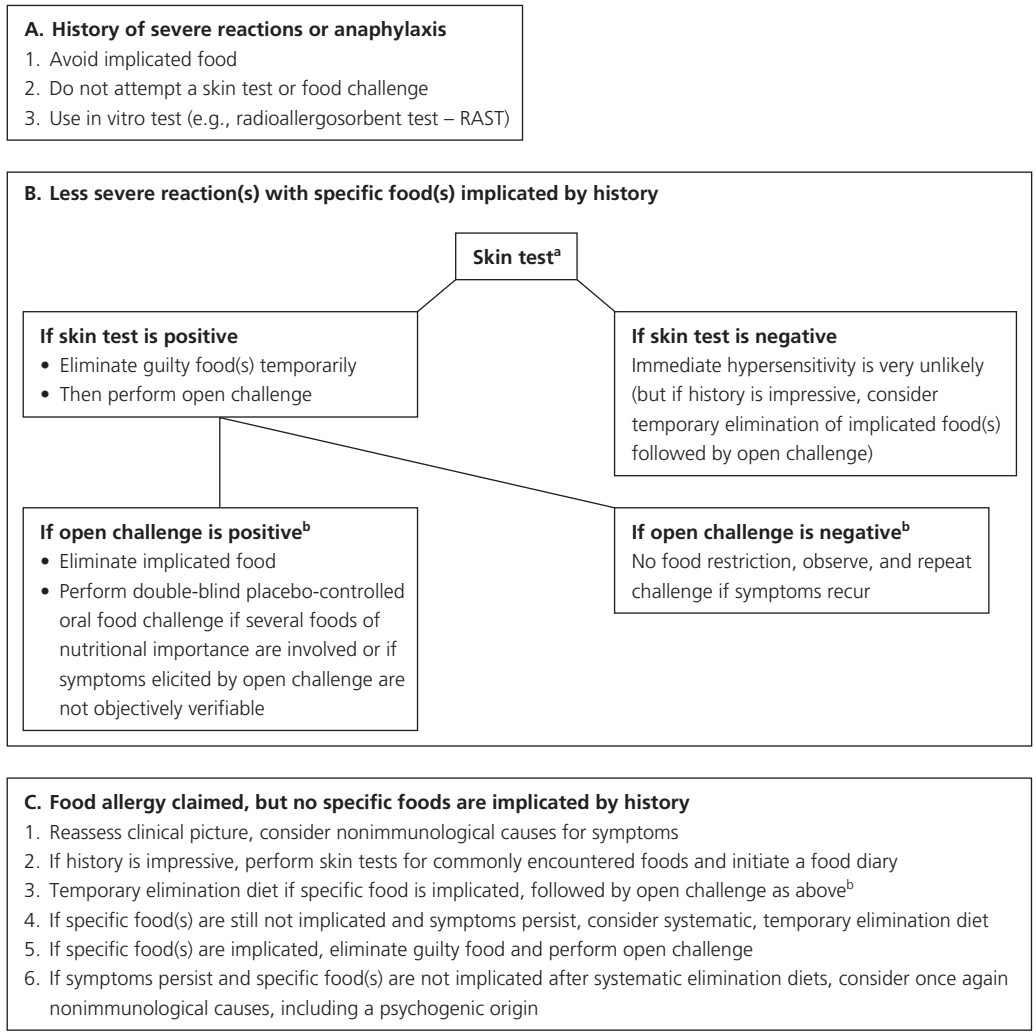


Figure 105.1 Mast cell mediators and potential consequences of their release. Mediator release is triggered when adjacent immunoglobulin E (IgE) antibodies on the surface of the mast cell are bridged by binding to an allergen. Mast cell secretion may also be stimulated by a variety of other endogenous and exogenous factors, including activated complement components (C3a and C5a), neuropeptides, bee venom, polymyxin, ionomycin, and opiates. GM-CSF, granulocyte macrophage-colony stimulating factor; IL, interleukin; PAF, platelet activating factor; TNF, tumor necrosis factor.

Evaluation of the patient with suspected food allergy

Only a small number of adult patients referred with a supposed food allergy have a reproducible, immunologically mediated adverse reaction to food [54–56]. A careful and objective assessment is important not only to detect preventable disease in such patients but also to offset inappropriate and potentially harmful dietary restriction in others. Although the differential diagnosis of food-related symptoms is broad (see Table 105.4), a detailed history and physical examination will often indicate the correct diagnostic category.

The definitive criteria for the diagnosis of food allergy are reproducible induction of the patient's symptoms after ingestion of the implicated food coupled with evidence for an immunological mechanism. A general diagnostic guideline is shown in Fig. 105.2. In practice, the diagnostic approach should be individualized, and the rigor with which one attempts to prove the diagnosis will be influenced by several factors, such as the age of the patient, the nature and nutritional importance of the food implicated, the likely reliability of the patient's history and compliance with challenge test protocols, and the severity of the symptoms. For example, patients who are thought to be allergic to several major food groups require an accurate confirmation of the diagnosis because of the inconvenience and potential danger of malnutrition with prolonged, complicated restriction diets. In contrast, patients with only episodic symptoms triggered by uncommonly encountered or nonessential foods should be instructed to avoid such foods and do not require rigorous food challenges. For reliable patients with a clear history of objectively verifiable symptoms and signs, an open challenge may suffice. The patient with vague or subjective complaints,



a Prick technique with fresh food or commercial extract.

b Depending on nature of the food and the ease with which it can be camouflaged, a single blinded challenge may be performed in preference to open challenge.

Figure 105.2 General guidelines for the investigation of adult patients with suspected food allergy.

self-diagnosed as an adverse reaction to food, poses the greatest difficulty. In adults, particularly the nonatopic individual, the likelihood of such symptoms being the result of food allergy is small. Prolonged investigative evaluation beyond simple screening tests may not be appropriate or desirable for these patients.

Food challenges and skin testing with food antigens can trigger potentially serious and even fatal anaphylaxis in a small percentage of patients. Such diagnostic tests should be performed only by experienced physicians and only in a facility equipped to address serious allergic reactions.

Skin tests and in vitro assays

For practical purposes, the demonstration that an adverse reaction to food is immunologically mediated involves the demonstration of IgE antibodies specific for the implicated food allergen. Direct skin testing with the prick technique with food extract and control solution is a simple and sensitive method of detecting specific mast cell-bound IgE antibodies [36]. If properly performed, a negative skin test indicates that immediate hypersensitivity to the food tested is highly unlikely. However, a positive test (wheal diameter > 3 mm larger than that of the control wheal) merely indicates the presence of sensitizing antibodies and not necessarily the presence of clinical food hypersensitivity, which can be confirmed conclusively only by food challenge (see Fig. 105.2).

Skin testing should not be attempted if the clinical history indicates there is a substantial risk of anaphylaxis; instead, an in vitro assay for the presence of allergen-specific IgE in the serum should be used. Assays for IgE antibodies to several of

the known food allergens include the commercially available radioallergosorbent test and enzyme-linked immunosorbent assay kits. Less commonly used is the basophil histamine release assay, which is performed mainly in research laboratories. There is no place for the nonvalidated, so-called cytotoxicity food allergy test, which is still widely advertised in the lay press [57].

Elimination diets

Systematic elimination of different foods and the use of a food/symptom diary may help identify foods to which a patient might be allergic. Resolution of symptoms and recurrence with reintroduction of selected foods is consistent with a diagnosis of food hypersensitivity. If multiple or several major food groups are implicated, confirmation should be obtained by controlled food challenge. The design of the diet, particularly the degree of food restriction, will depend on the patient's history and the number of potential food allergens thought to be involved. Ideally, the design of the diet should involve an experienced dietitian. Reintroduction of the implicated food(s) should be supervised by a physician because anaphylaxis can occur in some patients during food challenge following elimination trials.

The most restrictive elimination diet is an elemental diet. Elemental diets are unappealing but on a short-term basis they may be diagnostically useful in patients claiming to have allergies to multiple foods, or where the possibility of food allergy is a major concern but no specific food has been implicated by the clinical history. Persistence of symptoms on such a diet excludes food allergy from the differential diagnosis. If symptoms resolve on an elemental diet, various foods may be reintroduced in a systematic fashion.

Food challenge

A double-blind, placebo-controlled oral food challenge is the most reliable and unbiased method for determining if symptoms are related to a specific food. However, like direct skin testing, oral food challenge is only required in a minority of patients and is contraindicated in patients with a history of food-related anaphylaxis [54–56]. Dehydrated foods are most convenient for diagnostic challenges because they can easily be disguised in a variety of vehicles or gelatin capsules, and are commercially available. Guidelines for the performance of food challenges have been published [58]. The double-blind, placebo-controlled food challenge is time consuming and expensive so "single-blinded" or "open" challenges are more convenient initial tests to use in selected cases to rule out food allergy or to help identify those who require a double-blind challenge (see Fig. 105.2).

Direct challenge of the intestinal mucosa with food antigen extracts using colonoscopy has been used to identify patients with intestinal allergy. Mucosal wheal and flare reactions are recorded semiquantitatively and tissue biopsy specimens are examined for mast cell and eosinophil activation. As with skin testing, positive and negative controls are required and similar safety considerations must be applied [59].

Treatment and prevention of food allergy

Most patients can be reassured that they will outgrow their food allergy, although allergies to seafood and especially peanut, which is the most common cause of severe food allergy in adults, may be exceptions. The only acceptable treatment of food allergy is avoidance of the offending food. Patient education is the best means of ensuring dietary compliance. Expert instruction and supervision by an experienced dietitian reduce the likelihood of inadvertent ingestion of allergens. Attention to food labels is important and the European parliament has issued a directive regarding specific ingredients considered to be allergenic which must be indicated on food labels. Drug therapy with antihistamines and corticosteroids has only a secondary role in the management of symptoms caused by inadvertent ingestion of food allergens. Parenteral immunotherapy (hyposensitization) with food extracts has been disappointing. Monoclonal anti-IgE antibodies that bind to the Fc region of IgE, interfere with its binding to mast cells and basophils, and down-regulate the expression of the receptor, have had promising results in patients with peanut allergy [60]. There is evidence to suggest that breast-feeding until infants are 6 months old may have a protective effect against the development of food allergy [61]. The potential prophylactic role of altering the enteric microflora with probiotics is an active area of investigation [62].

Eosinophilic gastrointestinal disorders

Recognition of a spectrum of eosinophilic gastrointestinal disorders has increased remarkably over the past decade. This has been accompanied by an improved understanding of the biology of the eosinophil and its potential role in host defense and tissue injury [50]. Homing to the gut is under separate control to that responsible for other inflammatory cells and appears to be independent of the endogenous flora [63]. IL-5 is the principal eosinophil growth factor whereas the eotaxins are the chemokines responsible for tissue recruitment of eosinophils. The secretory products of eosinophils include cytotoxic cationic proteins, proteases and ribonucleases, neuropeptides such as substance P, leukotrienes, chemokines, and proinflammatory cytokines. Eosinophils are normally present in the lamina propria of all segments of the gastrointestinal tract with the exception of the normal esophagus. Therefore, it is particularly noteworthy that eosinophilic infiltration of the esophagus has been increasingly recognized, particularly in children but also in adults, in the setting of gastroesophageal reflux syndromes, food allergy, and as isolated causes of dysphagia and dysmotility. The topic of eosinophil esophagitis is dealt with separately in Chapter 33.

Eosinophilic gastroenteritis

Pathophysiology

Eosinophilic gastroenteritis is probably not a single entity but rather a heterogeneous collection of disorders of varied causes with similar clinicopathological features [50,63,64]. Allergic phenomena, particularly food hypersensitivities, have been proposed to account for eosinophilic gastroenteritis but the evidence is limited in many patients. Although food hypersensitivity (usually to milk) may be found in children with eosinophilic gastroenteritis, the response to dietary elimination is often disappointing. Some patients have no atopic symptoms, and fewer than half of all patients with eosinophilic gastroenteritis have a positive family history of allergy. Studies of experimental models of allergic intestinal disorders have demonstrated the role of the eosinophil chemokine, eotaxin, and of eosinophils in mediating intestinal injury including neuronal damage [50]. Other cytokines particularly IL-5 but also IL-3 and granulocyte–macrophage colony-stimulating factor promote the persistence of the eosinophil infiltration within the gut [64].

Clinical manifestations

The clinical manifestations of eosinophilic gastroenteritis depend on the gastrointestinal site primarily affected and the layer of bowel wall predominantly involved. The stomach and intestine are most commonly involved, but the disease may affect any part of the gastrointestinal tract as well as the pancreatic and biliary tree. When the predominant involvement is mucosal, the condition behaves like other forms of inflammatory bowel disease, with diarrhea, cramping, postprandial nausea, vomiting, and periumbilical pain. If the eosinophilic infiltration is extensive, there may be malabsorption, weight loss, protein-losing enteropathy, blood loss, and anemia. In preadolescents, growth retardation may occur. A low level of peripheral eosinophilia occurs in most cases. Stools may be positive for occult blood, although frank hematochezia is rare. Charcot–Leyden crystals secondary to lumenal extrusion of eosinophils may be seen. Detection of these distinctive hexagonal bipyramidal crystals may be easier with fluorescence microscopy than conventional light microscopy. Although characteristic of eosinophilic involvement in inflammation, they may also be derived from basophils and are not specific for allergic reactions, being found in parasitic and other causes of inflammation including idiopathic inflammatory bowel disease. Quantification of mediators such as eosinophil cationic protein and eosinophil protein X within stool samples promises to be an alternative diagnostic strategy. Definitive diagnosis of mucosal eosinophilic gastroenteritis is made by barium radiography and gastric and small bowel biopsy. A diffuse mucosal pattern with nodules or intralumenal masses and a sawtooth mucosal pattern may be noted in the small bowel. A widening of small bowel segments may be seen secondary to mesenteric nodal involvement. Gastric lesions may have a cobblestone appearance in the antrum and thickening of mucosal folds. Small bowel biopsies may be diagnostic, showing a diffuse infiltration of the mucosa with eosinophils. However, because eosinophilic gastroenteritis may present as a patchy intestinal disease, multiple biopsies at different levels of the gastrointestinal tract should be performed.

When eosinophilic gastroenteritis predominantly involves the muscularis layer of the intestine, the clinical presentation consists of intestinal obstruction with nausea, vomiting, pain, and abdominal distention. Barium studies of the upper gastrointestinal tract will usually demonstrate irregular narrowing of the distal antrum or small bowel. Endoscopic mucosal biopsy may not be helpful. Occasionally, isolated infiltration of esophageal muscle may be seen with symptoms and manometry evaluations suggestive of achalasia. Rarely, the eosinophilic infiltration is predominantly serosal and may be associated with eosinophilic ascites or pleural effusions.

Differential diagnosis

Diseases associated with peripheral eosinophilia in addition to mucosal eosinophilic infiltrates may mimic idiopathic mucosal eosinophilic gastroenteritis. The most frequently encountered is the hypereosinophilic syndrome, characterized by diffuse gastrointestinal involvement, frequent peripheral eosinophilia, and eosinophils in several other organs. Periarteritis nodosa may be associated with a peripheral eosinophilia, and abdominal pain and nodular masses in the stomach and small bowel may be seen on radiography. This condition may be distinguished from eosinophilic gastroenteritis by the systemic nature of the inflammatory process and by the eosinophilic infiltrate localized to the perivascular region. The nodular polypoid mucosal findings on radiographs in periarteritis nodosa are secondary to ischemia and inflammation rather than eosinophilic infiltration. Intestinal parasitism (hookworm, *Ascaris*, *Strongyloides*, *Toxocara*, *Trichuris*, *Capillaria*, and *Trichinella* species) causes peripheral eosinophilia and gastrointestinal symptoms, including diarrhea and abdominal pain. The diagnosis is usually suggested by the clinical history and confirmed by stool analysis and examination of a duodenal aspirate. Protozoal infections such as amebiasis and giardiasis are not associated with eosinophilia. Finally, intramural collections of mature eosinophils, most commonly in the stomach, are often seen in eosinophilic granuloma. These patients present with symptoms of gastric obstruction. The isolated nature of the granuloma may help distinguish this disorder.

The second major category of diseases that may mimic eosinophilic gastroenteritis clinically and radiologically includes inflammatory and infiltrative disorders, such as gastrointestinal lymphoma, scirrhous carcinoma of the stomach, and Crohn's disease. These conditions may be associated with a mild increase in eosinophils histologically.

Treatment

There is no specific treatment for eosinophilic gastroenteritis. An attempt to identify a specific environmental or food aller-

gen may be considered if there is a convincing history of symptoms triggered by food and if there is a strong background of atopy. In most adult patients, a specific food will not be implicated, and prolonged investigation with elimination diets is not appropriate. If necessary, a trial of an elemental diet can quickly eliminate a role for food hypersensitivity. Oral corticosteroids, 20–40 mg prednisolone/day are effective in most patients. A short course of 7–10 days usually produces clinical remission, although repeat and more prolonged courses may be required. Depending on disease distribution, a topically active corticosteroid, such as budesonide with controlled small bowel release, may be optimal. The long-term prognosis for patients with eosinophilic gastroenteritis is generally favorable, and the disease course is one of waxing and waning severity. Occasionally, the use of total parenteral nutrition is required to manage patients with severe disease and symptoms that are refractory to steroids. Segmental resection of the intestine is not curative. Promising therapeutic developments include the use of leukotriene inhibitors and monoclonal antagonists against eotaxin and IL-5.

Gastrointestinal complications of organ transplantation

Gastrointestinal complications are common in patients undergoing transplantation, particularly hemopoietic stem cell transplantation. Complications include toxicity of conditioning protocols (radiation or chemotherapy) before bone marrow grafting; acute and chronic GVHD; and opportunistic infections due to immunodeficiency associated with both the induction procedure and graft-versus-host reactions [65,66]. Some of the clinical and diagnostic characteristics that help distinguish these entities in the setting of bone marrow transplantation are shown in Table 105.6.

Intestinal disease secondary to conditioning protocols

Conditioning protocols which include both chemotherapeutics and high-dose radiation are capable of injuring rapidly dividing cell populations and producing intestinal cell death (see Table 105.6). Enterocyte death occurs immediately during the conditioning and up to 3 weeks elapses before there is mucosal regeneration. Both the small and large intestine are damaged during this process, although the epithelium of the small intestine is more susceptible to this form of cell death [67]. Anorexia, cramping, and abdominal pain, with watery diarrhea, may develop. During healing, there is atypia of cell nuclei and regeneration of crypt cells, but the surface epithelium is normal. This histopathological picture is important in helping distinguish this entity from acute GVHD or secondary viral infections [68,69]. The time of onset following bone marrow transplantation is a helpful clue, and rectal biopsy with negative stool cultures is the most important diagnostic test (see Table 105.6). Treatment consists of supportive measures and parenteral nutrition.

Graft-versus-host disease

GVHD is common after hemopoietic stem cell transplantation, occasionally occurs after organ transplantation, and is

Table 105.6 Gastrointestinal complications of bone marrow transplantation

	Secondary to induction protocol	Acute GVHD	Chronic GVHD
Time to onset after BMT (days)	0–20	20–80	>80
Clinical features	Anorexia, abdominal pain, diarrhea	Diarrhea (large volume), abdominal pain ± skin rash ± liver disease	Dysphagia, oral ulcers, diarrhea
Radiographic changes	Not helpful	Mucosal edema, mucosal ulcers, pneumatosis	Esophageal strictures and webs
Endoscopy or manometry	Nonspecific	Normal → erythema → mucosal sloughing; stomach and rectum are spared	Upper esophageal bands and webs; lower third of esophagus is spared
Histology: source and changes	Rectal: atypia of cell nuclei, crypt cell regeneration	Early → rectal; crypt cells/apoptosis Late → mucosal disintegration	Esophagus: neutrophil infiltration, basal layer necrosis
Differential diagnoses	Intestinal infection, esophagitis, early GVHD	Infection, drug effect	Peptic esophagitis, infection – *Candida*, herpesvirus, CMV
Stool findings	Negative for pathogens	Negative for pathogens, cellular debris, increased protein	Negative for pathogens

BMT, bone marrow transplantation; CMV, cytomegalovirus; GVHD, graft-versus-host disease.

rare after blood transfusion. Acute GVHD is characterized by epithelial cell death mainly in the gastrointestinal tract, liver, and skin, whereas chronic GVHD is characterized by fibrosis and atrophy of these and other organs [65,68]. The pathophysiology of GVHD is a multiphase process beginning with damage to the intestinal mucosa during the conditioning, pretransplant period. This causes increased permeability and translocation of bacterial products such as lipopolysaccharide with the generation of inflammatory cytokines that upregulate adhesion molecules and major histocompatibility complex (MHC) antigens on host tissues. This is followed by activation of $CD4^+$ donor T cells that recognize disparities with the recipient MHC proliferation and amplify the process by further release of T helper type 1 cytokines (e.g., tumor necrosis factor-α, IL-12, and interferon-γ) and activation of cytotoxic lymphocytes. Different subsets of memory T cells expressing distinct adhesion molecules home separately to the gut and skin. Activation of these alloreactive donor T cells within the tissues is followed by clonal expansion (expansion phase). This is followed by cytokine and chemokine release ("cytokine storm"), which leads to the recruitment of additional inflammatory cells (effector phase). The cytokine profile of acute GVHD is predominantly T helper type 1 but type 2 cytokines also contribute and feature more prominently in chronic GVHD, although there is considerable overlap in the immunopathogenesis of acute and chronic GVHD [65,70]. The central role of intestinal injury in the pathogenesis of GVHD is supported by evidence for reduced severity of GVHD with nonmyeloablative conditioning protocols. It also provides the rationale for use of "cytokine shields" such as keratinocyte growth factor and IL-11 to fortify the mucosal barrier [65,71].

Acute GVHD

Acute GVHD may be staged on the basis of damage to the skin (dermatitis), gut (mucositis, enteritis), and liver. A moderate to severe degree of acute GVHD develops in 30%–50% of patients with sustained bone marrow engraftment. The condition appears to be increasing although this may reflect more liberal use of endoscopic biopsy surveillance [72]. The onset or continuance of profuse, watery diarrhea 3 weeks following bone marrow transplantation is indicative of intestinal acute GVHD. The severity of the intestinal condition generally parallels that of the skin and liver involvement, although profound gastrointestinal symptoms can occur without any gross skin or liver changes.

The clinical picture, when severe, includes anorexia, vomiting, buccal mucositis, abdominal pain, intestinal bleeding, protein loss, and secondary infection (see Table 105.6). High-volume diarrhea may occur, and the amount of fluid generated is an index of extent and severity of disease activity. Extensive disease may be associated with up to 10 L of diarrheal fluid loss per day. The large stool volume may lead to distention and pain. Distention of the bowel may be exacerbated by opiate analgesics, which should be used with caution. The symptoms of GVHD are not specific so vigilance is required for other complications, particularly infections. The most common cause of gastrointestinal bleeding in these patients is esophagitis and gastric erosions. However, when a discrete ulcer develops, cytomegalovirus infection is an important consideration. Medical treatment of such lesions is often unsatisfactory, and if bleeding is persistent, surgical resection may be required.

Features that distinguish acute GVHD from the enteritis associated with the induction protocol include an erythematous, maculopapular skin rash over the palms, soles, and trunk, as well as liver test abnormalities, including hyperbilirubinemia. The diagnosis of acute GVHD may be made with confidence approximately 3 weeks following bone marrow transplantation if there is evidence of a functioning graft and if there is watery diarrhea accompanied by a diffuse skin rash and jaundice (see Table 105.6). The patient who presents with gastrointestinal symptoms but without jaundice or skin rash represents a much more difficult diagnostic challenge. Other diagnostic considerations include an opportunistic intestinal infection and the residual effects of the chemoradiotherapy conditioning lasting beyond the usual 3-week period. A combination of stool examinations, barium radiographic studies, endoscopy, and histopathology may be helpful (see Table 105.6).

The stool usually contains large amounts of cellular debris and red and white blood cells. Protein loss in the stool may be sufficiently severe to lead to profound hypoalbuminemia. The absence of pathogens in the stool is important but does not necessarily rule out an infection of the gastrointestinal tract.

Barium radiography may be helpful in the differential diagnosis and in establishing extent of disease. Widespread changes may occur, including mucosal and submucosal edema, pneumatosis cystoides intestinalis, and mucosal ulcerations. These radiological findings may also be consistent with other conditions, such as cytomegalovirus enteritis and the effects of acute radiation. The acute radiological features may return to normal, or in some cases they may progress to a chronic segmental, ribbon-like appearance, usually in the small intestine.

Endoscopic appearances may be normal or may show patchy erythema or extensive mucosal sloughing. These lesions are most prominent in the ileum, cecum, and ascending colon, with relative sparing of the gastric and rectal mucosa. Histological changes seen in biopsy samples from advanced or severe lesions may not be as diagnostically useful as those from early lesions. Therefore, endoscopic biopsy samples should be taken from intact mucosa as well as from areas involved with gross inflammation. The earliest change seen on light microscopy is apoptosis of individual cells in the intestinal crypts (Fig. 105.3). This is diagnostic, if obtained from normal-appearing mucosa at least 20 days following transplantation. Inflammatory cells or microorganisms are not present in the adjacent mucosa. Later, the histopathology

Figure 105.3 Rectal biopsy specimen taken after bone marrow transplantation from a patient with acute graft-versus-host disease shows apoptotic bodies (arrows). This necrosis of individual cells within the crypt is the first change seen on light microscopy and is characteristic of acute graft-versus-host disease if found after day 20, when damage from chemoradiation therapy has resolved.

can progress to a total denudation of the mucosa; the apoptosis lesion is no longer evident, and changes are not specific. The site of greatest diagnostic yield for endoscopic biopsy is a matter of some debate with some centers favoring colorectal biopsy and others sampling the segment of gut suggested by the patients symptoms [65,68].

Management of intestinal acute GVHD consists of nutritional support, maintenance of fluid and electrolyte balance, steroid and immunosuppressive treatment, and vigilance for secondary infectious complications. Common opportunistic pathogens include astrovirus, *Clostridium difficile*, and adenovirus [73]. For patients with severe high-volume diarrhea, subcutaneous administration of octreotide may provide relief. To improve long-term survival, doses ranging up to 2 mg/kg of prednisone, for up to 1–2 weeks, may be used. Cyclosporine (ciclosporin rINN) may be used in combination with specific anti-T-cell monoclonal antibodies and anti-tumor necrosis factor-α therapy with infliximab is effective. A subset of patients with intestinal GVHD do not progress to severe multisystem disease and can be managed more conservatively with less toxic drug strategies, including topically active steroids, thereby minimizing the risk of opportunistic infections [74]. Prevention of GVHD can be achieved by prior removal of mature donor T lymphocytes from the bone marrow graft. Unfortunately, this is associated with reduced graft survival. To circumvent this, strategies for nonmyeloablative conditioning with reduced mucosal toxicity and removal of more specific subsets of effector cells coupled with various immunosuppressive regimens are increasingly studied [65,75].

Chronic GVHD

Chronic GVHD is a multisystem disorder that occurs 80–400 days after allogeneic transplantation (see Table 105.6). The majority of patients have previously had acute GVHD. However, approximately one-fourth of the patients with chronic GVHD have had no clinical indications of any previous acute graft-versus-host illness. The pathogenesis is poorly understood but is thought to represent either an end-stage of alloreactivity in which the reactive donor T cells have evolved to a T helper type 2 phenotype (in contrast to the type 1 predominance of acute GHVD), or it might represent a T-cell-mediated autoimmune process [65].

There is a wider spectrum of disorders in chronic GVHD than in acute GVHD. The pattern of gastrointestinal involvement also differs with more proximal involvement in chronic GVHD. Skin, liver, and gastrointestinal tract are again prominently involved but the oropharynx, lungs, eyes, and musculoskeletal system may also be affected [65]. The clinical features resemble those of sicca syndrome and systemic sclerosis. Gastrointestinal involvement occurs particularly in the oral mucosa (mucositis), esophagus, and small intestine. Esophageal symptoms of dysphagia with weight loss are common presenting gastrointestinal manifestations [76]. Patients with skin involvement (hyperpigmentation and scleroderma-like changes) frequently, but not always, have esophageal involvement. Dysphagia with chronic esophageal reflux can lead to chronic lung disease. Small bowel involvement with chronic GVHD is characterized either by a patchy fibrosis of the lamina propria and submucosa, or by bacterial overgrowth as a result of stasis and dysmotility. This latter abnormality responds to oral broad-spectrum antibiotics.

As with other immunosuppressed conditions, opportunistic gastrointestinal infections are a constant threat posttransplantation and must always be considered in the differential diagnosis. The combination of radiology, esophagoscopy with biopsy, and manometry may be required to distinguish between esophageal involvement with chronic GVHD and reflux peptic disease of the esophagus. Endoscopic lesions of

chronic GVHD can range from generalized desquamation of the upper and midesophagus to web-like fibrous bands [76]. Manometry with pH monitoring shows a nonspecific motor abnormality and a decreased ability to clear acid from the esophagus. In contrast to peptic esophagitis, the distal esophagus is usually spared in chronic GVHD. Histological changes within the esophagus include infiltration with neutrophils and lymphocytes with necrosis of individual cells of the basal mucosa, similar to the changes found in the skin and oral mucosa. Submucosal fibrosis may be found, but there are no muscle or neural abnormalities. In the colon, the changes may be similar to those of ulcerative colitis but mucosal biopsies may be unhelpful in some cases because many of the fibrotic changes are submucosal [69]. A recent report describes intestinal thrombotic microangiopathy after bone marrow transplantation which presents with bleeding and which may be mistaken for chronic GVHD. The distinction is important because the treatments differ [77].

In contrast to acute GVHD, the treatment of chronic GVHD is frequently satisfactory. Early diagnosis and drug therapy have prevented much of the disability associated with this condition. Prednisone alone or in combination with azathioprine has been effective. Aggressive antireflux therapy is important for reducing the symptoms associated with esophageal involvement. However, fibrosis tends to be progressive, and esophageal dilation may be required for treatment of webs and strictures.

The liver in GVHD

In acute GVHD, liver involvement is manifested by cholestasis and mild hepatocellular necrosis. Hepatic failure is uncommon. Although liver abnormalities may be the presenting feature of acute GVHD, skin and intestinal GVHD are usually evident by the time jaundice occurs, and diagnosis is relatively clear when these multisystem manifestations arise at least 3 weeks following bone marrow transplantation. Within the first 2–3 weeks after transplantation, venoocclusive disease secondary to chemoradiotherapy may be a more important diagnostic consideration, whereas throughout the posttransplant period, GVHD must be distinguished from, or may coexist with, several other causes of hepatotoxicity. These include parenteral nutrition, viral infections, drug-induced liver damage (such as immunosuppressants, antibiotics, and antifungals), hypotension, and sepsis.

Biochemical abnormalities as the result of liver complications of GVHD include a marked elevation in serum alkaline phosphatase followed by hyperbilirubinemia, but serum transaminases are elevated to a lesser extent. Liver biopsy is hazardous because of the risk of bleeding from thrombocytopenia in bone marrow transplant patients, but may be helpful, particularly where additional causes of liver disease are suspected. Histological changes of GVHD within the first week after the onset of biochemical abnormalities are nonspecific and consist of a mild lobular hepatitis with variable eosinophilic necrosis of hepatocytes and necrosis of biliary epithelium. Subsequently, the changes become more characteristic; one of the earliest features is attachment of lymphocytes to vascular endothelium followed by lymphocytic infiltration of the portal areas, pericholangitis and then destruction of small bile ducts and proliferation of ductules. The histological picture may evolve into one of profound chronic cholestasis with portal inflammation and fibrosis. Progression to liver failure may occur but most patients with severe GVHD die of infection or other complications before this. At least half of the patients who survive severe acute GVHD develop chronic GVHD and most of these will have liver involvement. In contrast to the mixed hepatocellular–bile duct damage of acute GVHD, the liver damage in chronic GVHD is predominantly portal with dense plasmacytic infiltration, loss of small bile ducts, and cholestasis.

A variant hepatic GVHD has also been described. This presents with an acute hepatitis rather than the cholestatic picture described above. Serum transaminases are greatly elevated and liver biopsy shows that in addition to lymphocytic infiltration of the portal triad and bile duct destruction, there is loss of the limiting plate and lobular inflammation with lymphocytic infiltration, pericentral necrosis, and acidophil bodies [78].

Small bowel transplantation

Transplantation of the small bowel is a realistic therapeutic strategy for patients with intestinal failure in whom continued parenteral nutrition is no longer a safe option (see also Chapter 53). These will usually be patients with complications of parenteral nutrition such as advanced liver disease or loss of venous access as the result of catheter sepsis or venous thrombosis. Intestinal failure in adults is generally the result of short bowel syndrome following resection for conditions such as Crohn's disease or infarction associated with trauma or hypercoagulable states. In children, congenital disorders such as atresia or necrotizing enterocolitis are the most common indications for small bowel transplantation. Since the topic is covered separately under short bowel syndromes in Chapter 53, the principal immunological features will be discussed here.

Improvements in the success rate for intestinal transplantation have been largely the result of the introduction of potent immunomodulatory agents; patient and graft survival rates are comparable with those of lung transplantation and approaching those of liver and heart transplantation [79–81]. In adults, the reasons for graft loss are rejection, thrombosis/ischaemia, and sepsis whereas infection followed by rejection are the main causes of death [80,81]. Contrary to what had been expected, GVHD, albeit more common than

with solid organ transplants, has not been a major obstacle with small bowel transplantation [79–81].

The basis of graft rejection is recipient T-cell reactivity against a variety of polymorphic donor antigens (alloantigens), the most important of which are encoded within the MHC, but other non-MHC antigens are also contributory. Much of the risk of rejection of a small bowel graft is attributed to the expression of MHC antigens within the abundant lymphoid tissue of the gut. Recruitment of activated T cells into the graft leads to target cell death by several mechanisms including direct lysis, induction of apoptosis, and the generation of proinflammatory cytokines [82]. Rejection predisposes to sepsis because of loss of mucosal barrier function. This is compounded by potent immunosuppression with the risk of opportunistic infections, particularly cytomegalovirus enteritis, and a subsequent lymphoproliferative disorder that is probably related to Epstein–Barr virus infection. Distinguishing rejection from infections and other pathology may be difficult, particularly because the rejection process is often focal or patchy. Multiple endoscopic biopsies are required to monitor the progress of the graft. Crypt cell apoptosis characterizes acute rejection, whereas chronic rejection is associated with fibrosis, focal ulceration, and obliterative arteriopathy [83]. Recipient lymphocytes infiltrate the lamina propria of the graft and replace donor lymphoid cells within weeks to months. The rejection reaction is associated with local production of T helper type 1 cytokines including tumor necrosis factor-α and interferon-γ, and targets mainly the crypt epithelium and local endothelium. Neither the morphology nor the cytokine response is specific to the rejection process. Vigilance and careful attention to detail are required, and the degree of immunosuppression to prevent rejection must be balanced against the risk of opportunistic infection.

Outcomes of small bowel transplantation are likely to continue to improve with the development of strategies that promote transplantation tolerance requiring minimal immunosuppression. Promising strategies include the use of highly selective immunomodulatory agents, mixed hemopoietic chimerism, and induction of regulatory T cells, but the redundancy and reserve within the immune response implies that a multifaceted approach will be needed [82,84].

With longer survival of intestinal grafts additional complications may be anticipated. One outcome that has been particularly informative has been the recurrence of Crohn's disease in the intestinal graft [85]. Although this must be carefully distinguished from GVHD, it provides insight into the pathogenesis of Crohn's disease. In contrast to the sustained resolution of Crohn's disease following bone marrow transplantation [86], recurrence of Crohn's disease following transplantation of the end organ is consistent with the view that the fundamental defect in Crohn's disease does not reside exclusively in the gut and probably reflects a dysregulated immune response to enteric flora.

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I MISCELLANEOUS

106 Parasitic diseases: protozoa

Ellen Li, Samuel L. Stanley Jr

Extracellular protozoan parasites, 2632 Intracellular protozoan parasites, 2641

Protozoan parasitic infections of the gastrointestinal tract are being increasingly recognized as an important public health problem in the United States. They were the most frequently identified etiological agents in water-borne disease outbreaks in 1991 through 1994 [1]. The reasons for the apparent increased prevalence of intestinal parasites in the United States include the continuing immigration of people from developing countries in southeast Asia, the Caribbean, and Central America; the expanded use of day-care facilities, which are breeding grounds for any fecally-orally transmitted pathogens; a growing problem with fecally contaminated food and water supplies; the increased recognition of parasites as pathogens of patients with AIDS; and the development of improved stool examination techniques [2].

The intestinal protozoan pathogens include both parasites that are extracellular, such as *Giardia lamblia*, *Blastocystis hominis*, and *Entamoeba histolytica*, and the intracellular spore-forming parasites *Cryptosporidium parvum, Cyclospora cayetanensis*, and *Isospora belli*. This latter group of organisms have emerged as important pathogens in immunocompetent individuals as well as in patients with AIDS. The most commonly detected protozoan pathogen in stool specimens in the United States is *G. lamblia* [2] (Table 106.1), with *B. hominis* the next most commonly detected, although the latter's role in intestinal disease remains controversial. The prevalence of infection increases during the summer months [3]. This may be related to increased exposure to recreational water during these months. Because conventional microscopic examination of the stool for ova and parasites is time-consuming and expensive, special requests for microscopic examinations may need to be made to the laboratory. In some hospitals only fecal immunoassay(s) directed against *Giardia* and *Cryptosporidium* species may be performed when stool ova and parasite testing is requested. Stool microscopic examinations may be performed in clinical laboratories only if specifically requested. In some cases in which infection is suspected on clinical grounds, stool examination may not be revealing, and endoscopy may be necessary to make the diagnosis. It is important for the gastroenterologist to know what the likely organisms are and whether specific requests need to be made for stool microscopic examinations.

Table 106.1 Intestinal protozoan parasites detected by stool examination in the United States

Organism	Percentage of stool specimens containing the organism
Giardia lamblia	7.2
Blastocystis hominis	2.6
Entamoeba histolytica	0.9
Dientamoeba fragilis	0.5
Cryptosporidium spp.	0.2

The results of 216 275 stool specimens examined for intestinal parasites by state public health laboratories in 1987 are shown. Only organisms detected at a level of more than 0.1% of the stools are listed. Adapted from Kappus et al. [2].

Extracellular protozoan parasites

Amebiasis: *Entamoeba histolytica*

E. histolytica is the causative agent of human amebiasis. The clinical manifestations of human amebiasis range from asymptomatic disease to invasive disease manifested as amebic colitis or liver abscess. Although Brumpt postulated in 1925 that there were two morphologically indistinguishable species of *E. histolytica* – one that could cause disease and a second that could not [4] – only in the 1990s did molecular genetic analyses confirm data from isoenzyme studies and clearly establish that there are two genetically distinct but morphologically identical species [5,6]: *E. histolytica*, which can cause invasive amebiasis, and *E. dispar*, a harmless commensal.

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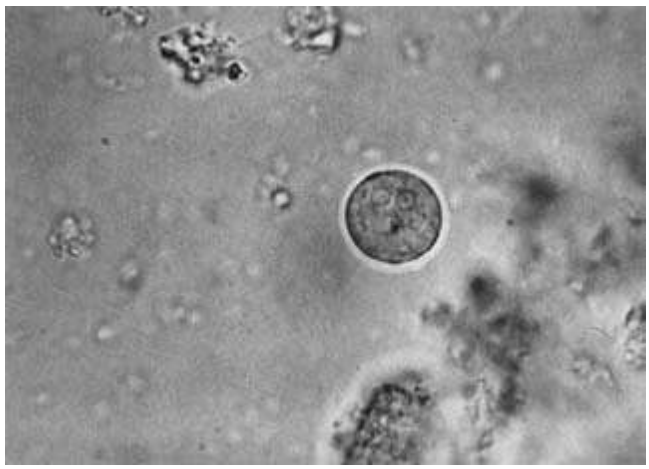


Figure 106.1 *Entamoeba histolytica* cyst from stool specimen stained with iodine. The cyst contains four nuclei and measures 9–25 μm in diameter. Courtesy of Centers for Disease Control and Prevention, Atlanta, GA.

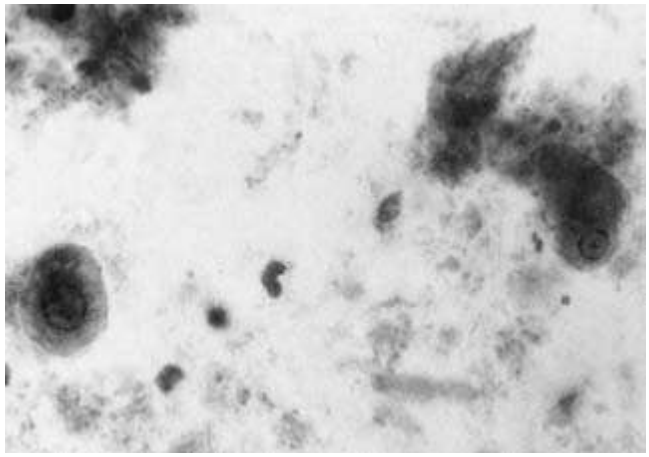


Figure 106.2 *Entamoeba histolytica* trophozoites in stool. Note ingested red cells and the single nucleus (trichrome stain). Courtesy of Centers for Disease Control and Prevention, Atlanta, GA.

The life cycle of *E. histolytica* consists of two stages: the cyst (host infective stage) (Fig. 106.1) and the trophozoite (host tissue invasive stage) (Fig. 106.2). No host other than humans is implicated in the life cycle, although natural infection of primates has been reported [7]. The disease is most commonly acquired by ingestion of food or water contaminated with feces containing the cyst form of the parasite, but venereal transmission through fecal–oral contact also occurs. Prompted by still-undefined stimuli in the intestinal tract, the cysts excyst and then form by nuclear and cytoplasmic division a total of eight trophozoites. The trophozoites are the motile feeding forms of the parasite and contain a single nucleus and pseudopodia. Trophozoites are the causative agents of invasive disease but play no role in the transmission of disease, owing to their rapid degeneration outside the body and destruction by gastric acidity. *E. histolytica* trophozoites multiply by binary fission, and they encyst, producing uninucleate cysts, which then undergo two successive nuclear divisions to form the characteristic quadrinucleate cysts.

Epidemiology

It has been estimated that 500 million people are infected with either *E. histolytica* or *E. dispar* [8]. In asymptomatic individuals, the prevalence of *E. dispar* infection is about 7- to 10-fold higher than that of *E. histolytica* infection. Forty million individuals infected with *E. histolytica* develop disabling colitis or extraintestinal abscesses, resulting in 50 000 deaths annually. Among parasitic diseases, only malaria and schistosomiasis result in more deaths than amebiasis [8].

High prevalence rates of amebiasis (both *E. histolytica* and *E. dispar*) are found in developing regions, including Central and South America, the Indian subcontinent and Indonesia, and sub-Saharan and tropical Africa [8–10]. A prospective study of a cohort of almost 300 children living in a slum region of Dhaka revealed that almost 40% acquired a new *E. histolytica* infection during the 12-month study period, but only 3% of the infections were associated with dysentery [9]. A serological study of the inhabitants of Mexico City demonstrated that 8.4% had been infected with *E. histolytica* in the previous 5–10 years [10]. The prevalence of colonic disease is equal between men and women, but amebic liver abscesses and other extraintestinal disease are 3–10 times more common in men. Children, especially neonates, and pregnant and postpartum women have an increased risk for severe disease and death. Malignancy, malnutrition, and treatment with corticosteroids are other risk factors for more severe disease.

In developed countries of Europe and North America, amebic infection is generally confined to certain high-risk groups, including recent travelers and immigrants, inmates of mental institutions, and sexually active homosexual men [11]. However, it should be noted that even in these populations, most individuals are infected with *E. dispar* rather than *E. histolytica* [12,13].

There is conflicting evidence regarding naturally acquired immunity to amebiasis. In the study of Bangladeshi children, some children showed resistance to the acquisition of a new *E. histolytica* infection that was associated with the presence of stool immunoglobulin A (IgA) antibodies to an *E. histolytica* antigen [9]. However, the effect was relatively short lived, and almost 20% of the children in the study had more than one episode of amebiasis during the 1-year study period [9]. These data, along with anecdotal reports of multiple episodes of amebic liver abscess in the same individual, suggest that naturally acquired immunity to amebiasis is, at best, short lived and may occur in only a small proportion of infected individuals [14,15].

Pathogenesis and pathology

Invasive disease is characterized by ulcerations of the intestinal mucosa, which extend into the submucosa. Trophozoites invading laterally through the submucosa, relatively sparing the overlying epithelium, give rise to the classic flask-shaped ulcer of amebiasis [16–20]. In some cases, amebic invasion through the mucosal and submucosal layers and subsequent dissemination through the portal circulation to the liver result in the formation of liver abscesses. Factors implicated in tissue invasion and destruction by the organism include:

- adhesion of amebic trophozoites to host epithelial cells through a galactose-binding lectin [21]
- release of cysteine proteases [22–26]
- amebic contact-dependent cytolysis of host cells by means of amebapore (a pore-forming protein that resembles NK-lysin from natural killer and cytotoxic T cells) [27,28]
- amebic phagocytosis and motility [29,30].

Sequencing of the *E. histolytica* genome (strain HM1:IMSS) has led to the identification of 9938 predicted genes, each averaging 1.7 kb in size, comprising ~ 49% of the genome (24 Mb) [31]. Ten percent of the sequence reads consisted of one of 25 different types of repeating transfer RNA (tRNA) unit, containing between one and five tRNA types per unit. Redundancy has also characterized many of the genes encoding suspected or proven *E. histolytica* virulence factors, including the cysteine proteinases, amebapores, and the intermediate subunit of a galactose-binding lectin. The repetitive nature and the high AT content of the genome have made it difficult to generate a map of the genome. Nonetheless, a number of key observations have been made from the genome project. First of all, although introns were thought to be rare in *E. histolytica* genes, 25% of the putative genes contained introns. The genome project has also led to the delineation of a new repertoire of signal transduction pathways in *E. histolytica*. But there was no evidence of a mitochondrial genome, consistent with the observation that trophozoites do not contain mitochondria. Instead, the parasite derives its energy from glycolysis and fermentation. Many of these genes seem to represent cases of recent prokaryotic to eukaryotic transfer. Finally, it has been noted that *E. histolytica* possesses an essentially complete complement of the genes known to be required for meiosis, although thus far there have been no confirmed sightings of amebic sex [32].

Expression profiling has been utilized to examine host response to amebic infection in the liver and the colon [33,34]. *E. histolytica* DNA microarrays have been generated to examine virulence factors and amebic responses to host factors [35–37]. Proteomic analysis has been applied to examine phagosome biogenesis in *E. histolytica* [38,39] and to identify novel virulence factors [40].

Clinical manifestations

Intestinal disease

The clinical manifestations of intestinal amebiasis range from asymptomatic infection to mild diarrhea to classic dysentery with abdominal pain, tenesmus, and bloody stools [41–43]. The presentation is generally subacute, with most individuals symptomatic for less than 1 month. Dysentery is a hallmark of invasive amebic infection, and stools that are not grossly bloody almost invariably contain occult blood, consistent with the invasive pathophysiology. Rarely, patients may present with a more fulminant colitis with severe bloody diarrhea and fever [44]. These patients have diffuse abdominal tenderness but usually do not present with a rigid surgical abdomen, although up to 75% of these patients may have peritonitis secondary to leakage through the severely diseased colon. Fulminant colitis is associated with a high (> 50%) rate of mortality. The risk for developing this syndrome is greater in children, pregnant women, and patients taking corticosteroids. Toxic megacolon is a rare but often fatal complication that is associated with administration of corticosteroids to patients with intestinal amebiasis. Unusual presentations of intestinal amebiasis include chronic non-dysenteric infection – in which symptoms of diarrhea, abdominal pain, and weight loss persist for years – and appendiceal involvement. The differential diagnosis of invasive intestinal amebiasis includes infection with *Shigella*, *Campylobacter*, and other invasive bacteria, as well as pseudomembranous colitis secondary to *Clostridium difficile*, cytomegalovirus colitis, ischemic colitis, and inflammatory bowel disease. Because of the similarities in symptoms between amebiasis and inflammatory bowel disease [45], amebiasis should be excluded by examination of stools or amebic serology in all patients before a diagnosis of inflammatory bowel disease is made and especially before corticosteroid therapy is begun.

Rarely, a localized amebic colonic infection results in a segmental mass of granulation tissue forming an ameboma. Amebomas are found, in decreasing order of frequency, in the cecum, ascending colon, rectosigmoid, transverse colon, and descending colon, and they can be detected on physical examination as a tender palpable mass. The ameboma can be mistaken for a carcinoma on barium enema. Amebic strictures are most commonly observed in the anus, rectum, or sigmoid colon and must be differentiated from those due to lymphogranuloma venereum (*Chlamydia*) or malignancy.

Extraintestinal disease

The most common manifestation of extraintestinal amebiasis is amebic liver abscess [46–49]. Patients often present with fever and right upper quadrant pain, leukocytosis, and abnormal liver test results, with an elevated alkaline phosphatase level being the most common finding (> 75% of individuals). Hyperbilirubinemia is uncommon, and severe jaundice may indicate bacterial superinfections of the abscesses. Concurrent diarrhea occurs in only one-third of the patients. In about half the patients with amebic liver abscess, hepatomegaly and point tenderness of the liver are prominent on physical examination. Most patients have a single abscess, usually in the right lobe. Multiple abscesses

are observed more commonly in patients with an acute presentation. With early diagnosis and treatment, the mortality of uncomplicated liver abscess is less than 1%. Most of the complications are related to rupture of the amebic liver abscess, which occurs most commonly into the chest (10%–20%), but also into the peritoneum (2%–7%) or pericardium (rare, often from a left lobe abscess). The development of a bronchopleural fistula presents as a cough productive of the odorless, brown anchovy-paste-like necrotic contents of the liver abscess. Amebas are usually not detectable in the necrotic material. Before the availability of effective antiamebic therapy, formation of such a fistula was a good prognostic sign for a spontaneous cure because it provided drainage of the abscess.

Amebic brain abscess occurs rarely but has a high associated rate of mortality. There have been case reports describing genitourinary involvement, including perinephric abscesses, splenic abscesses, infected rectal cysts, rectovaginal fistulae, cervical ulcers, uterine involvement, and vaginal lesions [50]. Cutaneous amebiasis is a rare complication and generally involves the perineum and genitalia as a complication of intestinal disease [51].

Diagnosis

The diagnosis of intestinal amebiasis (Table 106.2) still rests primarily on microscopic demonstration of cysts or trophozoites in the stool or in scrapings or biopsy samples obtained by sigmoidoscopy or colonoscopy. Because organisms may be excreted intermittently or may be unevenly distributed in the fecal specimen, at least three stool specimens on separate days should be examined to detect 85%–95% of the infections. Barium studies, laxatives, bismuth-containing preparations, antidiarrheal agents, and antibiotics can interfere with detection of the organism. Problems with microscopy include the misidentification as *E. histolytica* of other *Entamoeba* species and leukocytes that have ingested red cells, and the inability to distinguish between *E. histolytica* and *E. dispar*. In patients with mild bowel complaints, whose symptoms do not appear consistent with invasive amebiasis, care must be taken in the interpretation of a positive microscopic examination for amebic cysts or trophozoites. Fecal antigen detection assays that can distinguish between *E. histolytica* and *E. dispar* infections have been developed for commercial use; however, their efficacy in areas where disease is not endemic has been questioned [51–55]. Because *E. dispar* infections are not associated with seroconversion, amebic serology may be a useful adjunct in assessing the patient, especially in areas where *E. histolytica* is not endemic.

Sigmoidoscopy should generally be performed in patients presenting with dysentery. Endoscopy should be performed without previous bowel preparation to increase the chance of detecting amebas in the colonic mucus [56]. Discrete shallow-based ulcers covered with yellow or white exudate with intervening areas of edematous mucosa are often found in invasive amebic colitis. However, the endoscopic appearance of amebic colitis may be indistinguishable from inflammatory bowel disease, diffuse mucosal erythema, granularity, and friability, or from pseudomembranous colitis secondary to *C. difficile* infection with pseudomembranes [44]. Multiple biopsy samples or scrapings should be taken from the ulcer edge, exudate, and intervening mucosa [44,56]. The diagnostic yield from endoscopic biopsy samples or scrapings is high. Trophozoites are usually easily identified in these specimens by routine light microscopy, but immunohistochemical staining for *E. histolytica* may further increase the yield [44].

Serology is an important tool in the diagnosis of amebic liver abscess and can help confirm the diagnosis of intestinal amebiasis [57]. Because antiamebic antibodies may persist for months and years after the eradication of infection, a positive serology requires more rigid clinical and diagnostic correlation in endemic areas. Indirect hemagglutination is the most sensitive assay, which yields positive results in 90%–100% of subjects with liver abscess, 75%–90% of subjects with symptomatic intestinal infection, and 5%–50% of subjects with asymptomatic infection. Enzyme-linked immunosorbent assay (ELISA) kits for the serodiagnosis of amebiasis are also available. Because serological tests may be negative early in the course of an acute infection, it is important to repeat the serology 5–7 days later. Recombinant antigens have been used for diagnosis and may offer improved specificity for acute infection [58–60].

An imaging procedure should be performed in all suspected cases of amebic liver abscess. Right upper quadrant ultrasonography is usually the least expensive initial procedure. The usual appearance is a round or oval-shaped hypoechoic lesion with no wall echoes. On abdominal computed tomography (CT), the abscesses usually appear as well-defined low-density lesions. The differential diagnosis of space-occupying lesions in the liver includes bacterial (pyogenic) liver abscess, hydatid cyst, or tumor. Amebic serology usually establishes or excludes the diagnosis of amebic liver abscess. Ultrasonographic or CT-guided needle aspiration of the abscess can be performed to rule out a pyogenic abscess if the results of serology are nondiagnostic or if the clinical condition of the patient requires an immediate diagnosis. Concomitant pyogenic and amebic infections are unusual at the time of the initial presentation, but bacterial superinfection may occur after repeated aspirations or open drainage. Amebas are rarely recovered in the aspirate. Because of the risk for spillage and anaphylaxis, aspiration should be avoided if there is suspicion of a hydatid cyst.

Treatment

Patients with *E. histolytica* infection should receive antiamebic therapy, but there is no evidence that patients with *E. dispar* infection require treatment [42,61]. If assays that distinguish between infection with *E. histolytica* and *E. dispar*

Table 106.2 Morphology of human gastrointestinal protozoan parasites

	Stool	Intestinal biopsy
EXTRACELLULAR		
Ameboid		
Entamoeba histolytica	Trophozoite 10–20 μm; pale, round nucleus with small central karyosome; cyst 9–25 μm, four nuclei; morphologically indistinguishable from *E. dispar*; with ELISA, fecal antigen detection of trophozoite antigen can distinguish *E. histolytica* from *E. dispar*; serology may be useful adjunct	Trophozoites but not cysts seen invading colonic mucosa, causing colonic ulcerations
Blastocystis hominis (pathogenicity is controversial)	Organisms 6–40 μm, round with large central body or vacuole	
Flagellates		
Giardia lamblia	Trophozoite pear-shaped, 10–20 μm long, characteristic face-like image secondary to two nuclei, each with prominent karyosome; cyst oval, 7–10 μm long; direct fluorescence antibody test available in many laboratories for detection of cysts. Fecal immunoassays are routinely used to detect *Giardia* in many US hospitals	Trophozoites seen most commonly on duodenal mucosal surface but also on jejunal and ileal biopsy; histology usually normal, but villous atrophy seen in severe infections
Dientamoeba fragilis	Ameboid in shape, flagella not visible, 5–15 μm, 1–2 nuclei; no known cyst form	
Ciliates		
Balantidium coli (rare)	Trophozoite 50–200 μm in length, motile; cysts are rarely seen in stool and are 50–75 μm in diameter	Trophozoites can invade colonic mucosa, causing ulceration
INTRACELLULAR (coccidia)		
Cryptosporidium parvum	May need to submit special request to the laboratory; with modified acid-fast stain, oocysts stain uniformly, are round, 4–6 μm, contain four sporozoites, which may or may not be visible; measurement with micrometer to differentiate from *Cyclospora*; direct fluorescence assay may be helpful in detecting cysts	Intracellular forms seen as 4-μm extracytoplasmic dots on the apical surface of enterocytes; highest yield in the proximal small intestine, but also in ileum and colon
Isospora belli	May be observed on wet preparation; may need to submit special request to laboratory; with modified acid-fast stain, oocysts stain uniformly, appear oval, 20–30 μm, with two sporocysts, each containing four sporozoites	Intracellular forms seen as 20-μm intracytoplasmic inclusions within the enterocyte on light microscopy; distribution may be patchy
Cyclospora cayetanensis	May be observed on wet preparation; may need to submit special request to laboratory; with modified acid-fast stain, oocysts stain variably, resemble cryptosporidia oocysts but are larger, 8–10 μm; contains two sporocysts each with two sporozoites; the oocysts autofluoresce under UV light (365 nm); measure with micrometer to distinguish from cryptosporidia	Intracellular forms difficult to observe on light microscopy; intracellular forms have been observed on electron microscopy as intracytoplasmic inclusions

ELISA, enzyme-linked immunosorbent assay.

are unavailable, treatment decisions must be based primarily on the clinical presentation. The goals of treatment are to cure invasive disease and eradicate intestinal carriage of the organism. Metronidazole and tinidazole are the drugs of choice for patients with invasive intestinal disease or amebic liver abscess (Table 106.3). Metronidazole is highly efficacious (90% cure rate in many studies) and is relatively well tolerated. Despite widespread use of this drug, *E. histolytica* resistance to metronidazole has not been a problem to date. Intestinal disease is generally treated for 5–10 days. Antibiotic therapy of liver abscess is similar; however, 2.4 g of metronidazole taken as a single dose for 2 days has been reported to be more effective in a small group of patients [62]. Common side effects of metronidazole include nausea, headache, metallic taste, and abdominal discomfort; ataxia, confusion, insomnia, and paresthesias may occasionally occur. Patients should avoid alcoholic beverages owing to the disulfiram-like properties of metronidazole. The most serious side effects involve the central nervous system (psychosis, seizures), which mandate cessation of the drug. Rapid recurrence of these symptoms after readministering the drug has been reported [63]. The drug is available for intravenous administration

Table 106.3 Antibiotic therapy for amebiasis

Drugs	Adult dosage	Pediatric dosage
Lumenal agents[a]		
Paromomycin (Humatin)	30 mg/kg/day orally in three divided doses for 7 days	25 mg/kg/day orally in three divided doses (maximum 2 g/day) for 7 days
Iodoquinol (Yodoxin)	650 mg orally t.i.d. for 20 days	30–40 mg/kg/day in three divided doses (maximum 2 g/day) for 20 days
Agents for invasive amebiasis[b]		
Metronidazole	750 mg orally t.i.d. for 5–10 days 500 mg i.v. every 6 h for 5–10 days	30–50 mg/kg/day orally in three divided doses for 5–10 days 15 mg/kg i.v. load followed by 7.5 mg/kg every 6 h (maximum 2250 mg/day)
Tinidazole	2 g/day for 3–5 days	50 mg/kg/day (children > 3 years of age)
Dehydroemetine[c] (Mebadin)	1–1.5 mg/kg/day i.m. for 5 days (maximum 90 mg/day)	1–1.5 mg/kg/day i.m. for 5 days (maximum 90 mg/day)

a Lumenal agents are used primarily for the treatment of nondysenteric amebiasis and to eradicate intestinal colonization after treatment of invasive amebiasis (colitis and liver abscess).
b Treatment of amebic colitis, liver abscess, and other invasive disease is followed by a complete course of a lumenal agent to eliminate intestinal colonization.
c Available only through the US Centers for Disease Control and Prevention: telephone 404-639-3670 or 404-639-3356; nights and emergencies, 404-639-2888.
i.m., intramuscularly; i.v., intravenously; t.i.d., three times daily.

in patients who are unable to take medications by the oral route. Tinidazole may be more effective than metronidazole and is prescribed at a dose rate of 2 g/day for 3–5 days [64].

In the critically ill patient with fulminant amebic colitis, there may be some advantage in adding a second agent, dehydroemetine, for the first 2–3 days of therapy. Although there are no controlled trials to support such combination therapy, dehydroemetine offers a rapid amebicidal effect that could be beneficial in severe disease. The short course of therapy is designed to lessen the risk for cardiotoxicity. Dehydroemetine, which is preferred to the parent compound, emetine, because it may be less toxic, is available in the United States only from the Centers for Disease Control and Prevention. Because bacterial peritonitis resulting from leakage of the diseased colon is a frequent complication of fulminant amebic colitis, antibacterial therapy should also be initiated in these patients [42]. As in other patients with dysentery, antidiarrheal agents should not be administered.

After treatment of invasive intestinal or extraintestinal amebic disease is complete, administration of a lumenal agent is generally started to eradicate intestinal carriage of the organism. Three drugs have proven efficacy as lumenal agents: diloxanide furoate (no longer available in the United States), paromomycin, and iodoquinol (Table 106.3). The side effects of paromomycin are diarrhea and gastrointestinal side effects. Rare dose-related neurological toxicity, including optic neuritis, has been reported with iodoquinol. It is controversial whether a lumenal agent is necessary in conjunction with metronidazole treatment because courses of metronidazole of 10 days or longer may be effective in eradicating intestinal carriage [65]. Because about 10%–15% of patients treated with any of these agents fail to eradicate intestinal carriage, a follow-up stool examination after completion of therapy is recommended.

Most cases of colonic and extracolonic amebiasis can be managed medically. Even in cases of fulminant colitis, it is unclear whether these patients benefit from surgery unless toxic megacolon is present [42]. Further interventions are generally reserved for prevention of complications resulting from perforation of the colon and rupture of liver abscesses. Possible indications for aspiration of an amebic liver abscess as an adjunct to medical therapy include extremely large abscesses in which rupture is felt to be imminent, abscesses in the left lobe of the liver (because of the greater risk of rupture into the pericardium), and treatment failure, in which fever and pain persist after 3–5 days of appropriate therapy. Aspiration can generally be accomplished percutaneously. Surgical drainage should be reserved for large left lobe abscesses that cannot be reached percutaneously. It is important to note that amebic liver abscess cavity may resolve slowly and that successfully treated abscess cavities may increase in size over the first few weeks. Most abscesses resolve by 6 months, but 10% of patients may have abnormal ultrasonographic findings more than 1 year after therapy. The persistence of a cavity alone is not an indication for aspiration or for administering another course of antibiotics.

Positive ameba stool examinations may be encountered in patients with minimal bowel complaints as well as in AIDS patients with diarrhea who do not have symptoms or findings consistent with invasive disease. Most of these cases represent infection with *E. dispar*, rather than *E. histolytica*, and do not require therapy. However, it is recommended that asymptomatic cyst passage of pathogenic strains of *E. histolytica* be treated because these patients may subsequently develop invasive disease or transmit the organism to close contacts. At the present time, our recommendation is to treat all patients in areas of nonendemicity whose stool examination results are positive, particularly because stool examinations are rarely conducted in the absence of any intestinal symptoms. This practice may change after widespread acceptance of tests that can differentiate between *E. histolytica* and *E. dispar*.

Despite legitimate concerns about administering metronidazole in pregnancy, women who develop symptomatic amebic colitis during pregnancy should receive metronidazole because of their increased risk for fulminant colitis. A long-term follow-up study of women given metronidazole for therapy of *Trichomonas* during pregnancy reported no adverse outcomes [66]. However, in the case of the pregnant woman with a positive stool examination result, but without evidence of invasive disease (a setting in which infection with *E. dispar* is most likely), treatment with paromomycin, a nonabsorbable aminoglycoside, could be initiated with close follow-up of the patient [67].

Giardia lamblia

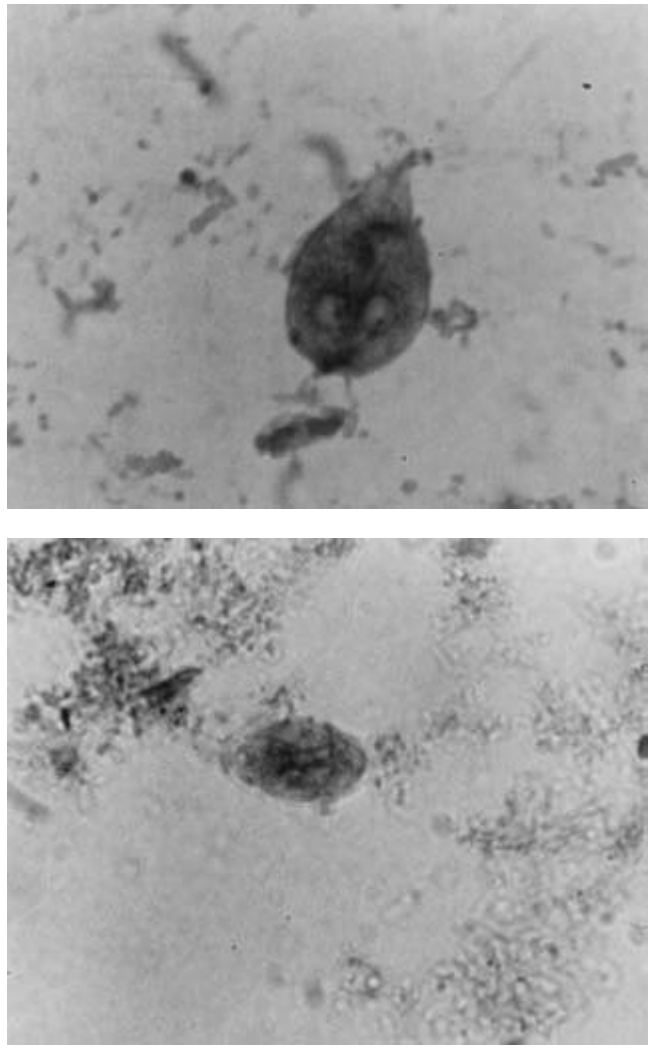
Giardia lamblia (also termed *G. intestinalis* and *G. duodenalis*) is a flagellated protozoan initially described by van Leeuwenhoek in 1681 and by Lambl in 1859. The clinical spectrum of human giardiasis includes asymptomatic carriage, acute self-limiting diarrhea, and, rarely, persistent diarrhea that may fail to respond to appropriate therapy even in immunocompetent individuals. *Giardia* infection can cause intestinal malabsorption, and in children this may be associated with retardation of growth and development.

Giardia spp. are among the most primitive of all eukaryotes [68]. The life cycle consists of two stages: the trophozoite and the cyst (Fig. 106.3). Infection is initiated by ingestion of as few as 10–100 cysts [69]. The cysts excyst in the proximal small intestine, releasing two trophozoites that multiply by binary fission. Trophozoites attach to the intestinal mucosa through an attachment organelle called the *ventral disk*. The life cycle is completed by excystation, an event that may be induced by exposure to a mildly alkalotic pH of 7.8, intralumenal conjugated bile salts, or decreasing levels of cholesterol [70,71].

Epidemiology

Giardia is cosmopolitan in distribution, and prevalence rates are estimated to be as high as 2%–5% of populations in the industrialized world and a staggering 20%–30% of individuals in some regions of the developing world. *Giardia* is one of the most frequently identified intestinal parasites in the United States and was the most commonly implicated pathogen in water-borne disease outbreaks during the past 30 years [12]. Although recognized as a common pathogen, it is less well appreciated that *Giardia* can cause illness serious enough to require hospital admission, with the number of admissions for giardiasis rivaling that for shigellosis [72].

High-risk groups for giardiasis include infants and young children, particularly those attending day-care centers, individuals who have traveled outside the United States, people exposed to infected water sources, and homosexual men [73]. Reporting of giardiasis as a nationally notifiable disease



(a)

(b)

Figure 106.3 **(a)** Trichrome stain of a *Giardia lamblia* trophozoite, demonstrating the characteristic face-like image secondary to the two nuclei, each containing a prominent karyosome and four symmetrically placed flagella. **(b)** Trichrome stain of a *Giardia lamblia* cyst, 7–10 μm in length and ovoid. Courtesy of Patrick Murray, PhD, St Louis, MO.

in the United States began in 2002 [74]. The total number of reportable cases was approximately 20 000 per year between 1998 and 2002. The highest number of case reports received were for children aged 1–9 years and adults aged 30–39 years. The peak incidence was during the early summer through early fall months coinciding with the summer recreational water season [74]. *Giardia* has been implicated in multiple outbreaks of diarrheal illness associated with ingestion of contaminated drinking water and recreational water sources [1,75]. Many of these outbreaks were associated with unfiltered water systems in which the only treatment was chlorination, a tribute to the ability of the cysts to survive in fresh water and their relative insensitivity to chlorination. Although contamination of recreational water sources may occur from animals, and humans can be infected by *Giardia* from animal sources [76], evidence does not generally support zoonotic transmission as a major risk for human infection [77]. Transmission of *Giardia* does not occur exclusively by ingestion of contaminated water; food-borne transmission has been described in several outbreaks [78].

Pathogenesis and pathology

Despite intensive investigation, the pathogenesis of human giardiasis is incompletely understood. In contrast to *E. histolytica*, *Giardia* causes disease without penetrating the epithelium or surrounding tissues, and infection is not typically associated with significant mucosal inflammation. Giardiasis causes diarrhea through multiple mechanisms, including intestinal malabsorption, hypersecretion, loss of epithelial barrier function, and intestinal hypermotility [79–81]. *Giardia* induces loss of brush-border surface area and impairment of disaccharidase activity, thus contributing to malabsorption. The observation that microvillous injury is not observed in athymic mice infected with *Giardia* suggests that this injury is not due solely to parasite attachment or virulence factors and strongly implicates the role of T cells [82]. A 58-kDa enterotoxin has been implicated in stimulating chloride secretion [83,84]. Disruption of epithelial tight junctions and increased rates of epithelial apoptosis contribute to the loss of epithelial barrier function observed in cell culture and animal models of giardiasis [79,80,85,86].

Infecting strains may differ in their virulence [87–90]. One phenotypic difference between *Giardia* strains is their susceptibility to host intestinal proteinases, and resistance may depend on the form of variant surface protein expressed by the parasite [88]. The *Giardia* human isolates can be divided into two major genotypes, assemblage A and assemblage B, based on genetic and biological differences [68]. Assemblages A and B share less than 90% nucleic acid identity at many homologous loci, and some investigators have proposed that they be considered as separate species [90]. It has been suggested that assemblage B genotypes are associated with a more severe diarrhea, but this remains to be confirmed in further studies [91–94]. Genome sequence information from the *Giardia* genome project [95] will continue to provide important insights into *Giardia* genes with respect to their biological function and the evolution of eukaryotic processes [32,95].

There is strong experimental and clinical evidence that secretory antitrophozoite antibodies are important and contribute to clearance of the parasite from the intestinal tract [96–98]. For example, individuals with immunoglobulin deficiencies are at significantly higher risk for chronic *Giardia* infection [99,100]. Although animal studies suggest that cell-mediated immunity could play a role in host defense against *Giardia* [101], children with a severe T-cell deficiency due to thymic aplasia (Di George syndrome) or purine nucleoside phosphorylase deficiency are not more susceptible to giardiasis, and their morbidity is comparable with that in immunocompetent children. The clinical course of giardiasis does not appear to be altered in patients infected with the human immunodeficiency virus (HIV) [102]. Ingestion of maternal antibody in breast milk appears to provide infants with protection from symptomatic disease but does not stop the establishment of *Giardia* infection [103].

Clinical manifestations

Studies of human volunteers suggest that the incubation period for symptomatic disease is 1–2 weeks [69,88]. It is important to note that the onset of symptoms may precede the excretion of cysts in stool by more than 1 week [104]. The clinical presentation is varied and can be classified in one of three syndromes: asymptomatic carrier state, acute self-limiting diarrhea, and chronic diarrhea. Asymptomatic infection is probably the most common outcome of *Giardia* infection [105,106]. In individuals who develop symptoms, the most common presentation is that of diarrhea with watery foul-smelling stools, often with abdominal distention, flatulence, nausea, flatus, anorexia, and vomiting. Blood and mucus in the stool are rare. Low-grade fever also may be observed, and in some individuals, fatigue and malaise or headache may be prominent.

Although infection is self-limiting in most healthy individuals, a significant proportion (25%–30%) come to medical attention because of chronic diarrhea, often with features of steatorrhea and weight loss. Malabsorption of vitamins A and B-12, proteins, and D-xylose is not unusual, and more than 50% of symptomatic individuals report weight loss of greater than 4.5 kg (10 lb) [107,108]. It is important to note that secondary lactase deficiency may persist for several weeks after eradication of *Giardia* infection, necessitating the dietary restriction of lactose. This can be confused with recurrent or relapsing infection.

Protracted disease is more frequent in young children and in those individuals with an underlying humoral immunodeficiency syndrome and nodular lymphoid hyperplasia [99,100,108,109]. This syndrome is most commonly associated with patients who have common variable immunodeficiency

and occasionally with selective immunoglobulin A deficiency. However, *Giardia* infection also has been associated with nodular lymphoid hyperplasia in the absence of overt humoral immunodeficiency. Extraintestinal disease is rare, but there are case reports of individuals with gastritis, reactive arthritis, biliary tract disease, and urticaria [110–113]. Gastric giardiasis may be seen in patients with achlorhydria.

Diagnosis

The diagnosis of *Giardia* infection (Table 106.2) should be considered in any patients with prolonged diarrhea, particularly if they have been exposed to children in day care, were exposed to potentially contaminated water, or report a history of recent travel. Stool parasitological exam has traditionally relied on microscopic examination. However, this is a labor-intensive method and relies on the availability of skilled technicians for reading stool parasite smears. Consequently many hospitals offer enzyme immunoassay methods for screening stools for *Giardia* and *Cryptosporidium*, and a microscopic exam is not performed unless the clinician specifically requests this exam or provides the laboratory with a detailed clinical history [114]. Multiple stool samples collected on different days may be required to obtain more than 90% sensitivity in detecting *Giardia* infection [115]. Barium studies are not useful and may even hamper detection of the organism in stool. The experience of the laboratory staff in the microscopic recognition of *G. lamblia* also has an important impact on the sensitivity of microscopic examination of stools. A direct fluorescent antibody test can be used as a rapid alternative confirmatory test instead of routine microscopy [114]. When the diagnosis cannot be made by examination of stool, intestinal aspiration, intestinal biopsy, or brush cytology may be indicated [116]. One advantage of biopsy is that it may provide additional information regarding associated bowel pathology, such as nodular lymphoid hyperplasia. An alternative approach is to treat empirically for giardial infection before subjecting the patient to more invasive tests. The nonspecificity of symptoms associated with chronic *Giardia* infection and the similarity to symptoms observed in irritable bowel syndrome may contribute to delayed and missed diagnoses [117]. The differential diagnosis includes infection by other protozoan organisms, enteric viruses, and noninvasive bacteria. In patients with malabsorption, the differential diagnosis includes other causes of steatorrhea and malabsorption as well as inflammatory bowel disease. Serology has not proved helpful in the diagnosis of giardial infections.

Treatment

Metronidazole and tinidazole are the drugs of choice (Table 106.4) for the treatment of giardiasis [118]. Tinidazole is superior to metronidazole when given in a single dose. Furazolidone is somewhat less effective than metronidazole, but it is available in a liquid preparation that is widely used in children in the United States. Albendazole has been shown to exhibit some efficacy in the treatment of giardiasis, and it may prove useful for administration to children in less developed countries for the simultaneous treatment of intestinal helminths and *Giardi* [119]. None of the above agents is recommended for use during pregnancy. Paromomycin, a nonabsorbable aminoglycoside with antigiardial activity, has been suggested as a possible drug for use in pregnant women. Nitazoxanide has been licensed to treat patients over 1 year of age. It has not been shown to be more efficacious than the nitroimidazoles, but may have a role in treating metronidazole-resistant giardiasis [120]. This medicine has not been studied in pregnant women, but is not expected to be harmful.

A major problem in treating patients with *Giardia* infection is the recurrence of symptoms after standard courses of therapy. This may be secondary to persistent infection because of resistant organisms, or may occur in patients in whom reinfection cannot be documented and in whom an immunodeficiency, such as hypogammaglobulinemia, is not present. One possibility is secondary lactase deficiency, which may persist despite eradication of infection. Patients who have persistent symptoms after single-drug therapy may benefit from treatment with another agent from a different class of drugs, or combination therapy [118,120].

Although symptomatic patients infected with *Giardia* should receive therapy, the treatment of asymptomatic carriers is not routinely recommended. This particularly pertains to control of infection within day-care centers. A longitudinal

Table 106.4 Drug treatment of giardiasis

	Adult dosage	Pediatric dosage
Metronidazole	250 mg t.i.d. for 5–7 days	5 mg/kg/day for 10 days
Tinidazole	2 g single dose	50–75 mg/kg single dose
Nitazoxanide	500 mg orally b.i.d. for 3 days	Age 12–47 months: 100 mg orally b.i.d. for 3 days Age 4–11 years: 200 mg orally b.i.d. for 3 days
Furazolidone	100 mg q.i.d. for 7–10 days	2 mg/kg t.i.d. for 7–10 days

b.i.d., two times daily; q.i.d., four times daily; t.i.d., three times daily.

study of asymptomatic children in a day-care center indicated that excretion of *Giardia* cysts is well tolerated, and in the setting of a high rate of reinfection, treatment of asymptomatic children may lead to recurrent courses of therapy with potentially toxic drugs [121].

Blastocystis hominis

Blastocystis hominis is a unicellular protozoan parasite detected in 10%–20% of stools examined by microscopy (Table 106.2). Its life cycle is unknown, and controversy surrounds its taxonomy and pathogenicity [122,123]. Symptomatic patients with high concentrations of *Blastocystis* parasites (more than 10 organisms per high-power [400 ×] field) were more likely to be coinfected with other enteric pathogens [124]. This may explain why the treatment of *Blastocystis* with iodoquinol and metronidazole appears to be more effective in relieving the symptoms of infected patients than in eliminating the infection [124]. Alternatively, there may be distinct strains of *B. hominis* differing in their pathogenic potential, which could explain the poor correlation between infection and symptomatic disease. One study suggested that *Blastocystis* infection was associated with disease only in immunocompromised individuals [124]. Symptomatic *Blastocystis* infection should be considered a diagnosis of exclusion, and if symptoms persist after therapy, there should be a further investigation for other causes of intestinal disease [125]. Optimum therapy has not been established, but metronidazole and trimethoprim sulfamethoxazole have been used when treatment is deemed necessary [126].

Dientamoeba fragilis

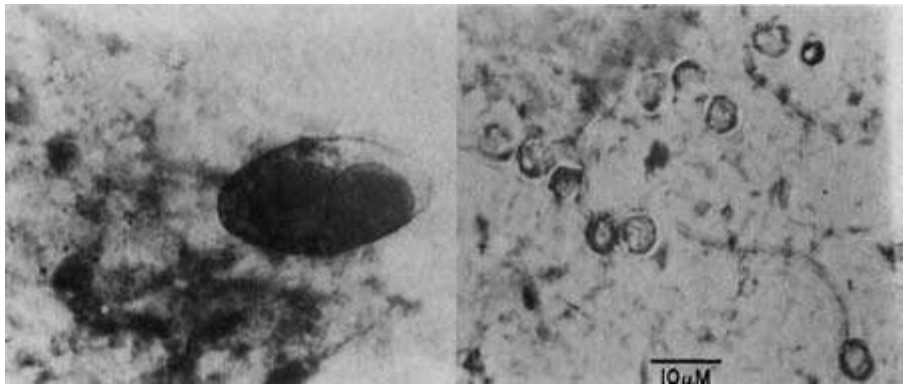
Dientamoeba fragilis is a protozoan parasite that is being increasingly identified in stool samples (Table 106.2) [127]. No known cyst form of *D. fragilis* has been identified and its life cycle and mode of transmission are unknown. Molecular phylogenetic studies indicate that *D. fragilis* is not an amoeba but a flagellate that has permanently lost its flagella, and is closely related to the trichomonads. Symptoms ascribed to *D. fragilis* infection are similar to those observed in patients with irritable bowel syndrome, including mild diarrhea, abdominal pain, anorexia, and fatigue. Fever, irritability, weight loss, nausea, and vomiting are occasionally reported, and an associated eosinophilia has been described [128]. The pathogenic potential of this organism remains uncertain, because the organism is detected in asymptomatic patients and in patients harboring other suspected pathogens. It has been proposed that *D. fragilis* is transmitted in the ova of *Enterobius vermicularis*, because some studies have observed a high level of coinfection, but subsequent studies have rejected this hypothesis. In several reports, symptomatic individuals become free of symptoms when the parasite was eradicated [127]. Attempts to introduce infections in animals or human volunteers have been unsuccessful. If symptomatic infection is diagnosed, adults should be treated with diiodohydroxyquin (650 mg three times daily for 3 weeks), or tetracycline (500 mg four times daily for 1 week) as an alternative. In children, metronidazole is recommended (30–50 mg/kg/day orally in three divided doses for 5–10 days). Paromomycin (25 mg/kg/day orally in three divided doses for 5–7 days) is also a reasonable alternative.

Balantidium coli

Balantidium coli is a ciliated protozoan parasite and is a rare cause of intestinal disease (balantidiasis) in humans (Table 106.2). Disease outbreaks in humans are associated with close exposure to pigs and their feces [129]. Outbreaks in institutions associated with poor hygiene or coprophagia have also been reported [130], as have water-borne outbreaks. Humans are probably relatively resistant to *B. coli* infection, but malnutrition, achlorhydria, and poor hygiene predispose to disease. The spectrum of disease ranges from asymptomatic carriage, through watery diarrhea, to active colitis with bloody mucus-laden stools. *B. coli* infection is also a rare cause of appendicitis [131,132]. *B. coli*, like *E. histolytica*, can invade the colonic mucosa and spread through submucosal layers, causing colonic ulcerations. Misdiagnosis as ulcerative colitis has been reported [133]. Rarely, colonic perforation occurs with resultant peritonitis [134]. Extraintestinal disease, with lung or liver involvement, is a rare complication [133]. Diagnosis is made by observing the motile trophozoites in stool saline mounts under low-power microscopy [129]. Cysts are much less frequently seen in human infections. Optimum therapy remains uncertain, but tetracycline (500 mg four times daily for 10 days) is recommended by most authorities. Iodoquinol (650 mg three times daily for 3 weeks) is an appropriate alternative.

Intracellular protozoan parasites

The AIDS epidemic has played an important role in the recognition of intracellular spore-forming parasites as important gastrointestinal protozoan pathogens [135]. Organisms from the genera *Cryptosporidium*, *Cyclospora*, and *Isospora* can cause an acute, self-limited diarrheal illness in immunocompetent hosts and a severe chronic diarrhea in patients with AIDS and other forms of immune dysfunction. The similar life cycles of these organisms may account for their similar clinical presentations. *Cryptosporidium*, *Cyclospora*, and *Isospora* are all intracellular pathogens that can replicate asexually within the enterocyte to produce a merozoite stage, which can autoinfect other enterocytes, leading to a rapid spread of the infection within the epithelium. All three pathogens also undergo a sexual stage that leads to the generation of cysts or spores, which are sloughed into the gut lumen and excreted in the stool. For *Cryptosporidium*, formation of the infectious sporozoite can take place within the host, which can autoinfect other enterocytes. However, for *Cyclospora* and *Isospora*,



(a) (b)

Figure 106.4 **(a)** Modified Kinyoun stain of *Isospora belli* cyst in stool. Note that the ellipsoid shapes are larger (20–33 μm × 12–15 μm) than the oocysts of *Cryptosporidium* or *Cyclospora* (4–10 μm). Immature oocysts contain a single sporocyst; mature oocysts contain two. **(b)** Modified Kinyoun stain of *Cryptosporidium* oocyst in stool. From DeHovitz JA, Pape JW, Boncy M, Johnson WD. Clinical manifestations and therapy of *Isospora belli* infection in patients with the acquired immunodeficiency syndrome. N Engl J Med 1986;315:88, with permission from the Massachusetts Medical Society.

formation of the infectious sporozoite must take place outside the host.

Enzyme immunoassays are routinely used to screen stools for cryptosporidia [114]. Many laboratories no longer routinely screen for *Cyclospora* or *Isospora* in their analysis of stool samples for parasites. The modified acid-fast stain may be the most useful for distinguishing among the three genera (Fig. 106.4). The distinction is important because infections with *Cyclospora* and *Isospora*, but not *Cryptosporidium*, respond well to antibiotic treatment.

Microsporidia are spore-forming protozoa that have been recognized as causing widespread infection in patients with AIDS (see Chapter 104). Because they do not appear to be significant pathogens in immunocompetent hosts, they are not discussed further in this chapter.

Cryptosporidium

Cryptosporidium species were originally described in the stomach and intestine of asymptomatic mice by Tyzzer in 1907 [136]. *C. parvum* was recognized as a cause of diarrhea in animals but was not identified as a human pathogen until 1976 [137,138]. Initially, most of the reported *C. parvum* infections were in patients with AIDS [139]. However, with improved methods of stool diagnosis and better understanding of the clinical disease, cryptosporidiosis has emerged as an important cause of diarrhea in normal hosts as well. *C. hominis* was identified in 2002 as a distinct *Cryptosporidium* species infecting humans [140]. *C. hominis* is restricted to humans, but *C. parvum* can infect other mammals. Most human *Cryptosporidium* infections involve either *C. parvum* or *C. hominis*; however, other *Cryptosporidium* species traditionally associated with animals (*C. meleagridis*, *C. felis*, and *C. muris*) have also been isolated from humans [141]. *Cryptosporidum* species were originally classified as coccidia, but subsequent evidence suggests that they should be placed in a distinct taxonomic group [142].

Epidemiology

Human cryptosporidiosis is found worldwide in both industrialized and developing countries and in both rural and urban areas. The parasite is transmitted by ingestion of oocysts that are excreted in the feces of infected humans or animals, from person-to-person spread, by ingestion of contaminated food and water, and, as a zoonosis, by contact with infected (primarily farm) animals. Direct person-to-person transmission may occur by fecal–oral spread related to sexual practices. The prevalence of *Cryptosporidium* infection is highest in young children and immunocompromised individuals. The stool positivity rate is higher in developing countries (1%–22%) than in industrialized countries, where cryptosporidia are identified in the stool of 0.3%–4% of individuals with diarrheal illnesses [143–145]. In developing nations, there is a high rate of asymptomatic carriage, and in some studies, the prevalence of stool positivity for *Cryptosporidium* in control patients approached or exceeded that observed in children with diarrhea [145]. Based on serological studies, exposure to *Cryptosporidium* is relatively common even in industrialized countries, with as many as 15%–36% of individuals seropositive [118,145–149]. *C. parvum* also infects cattle, and individuals with occupational exposures, such as dairy farmers, have a higher prevalence of seropositivity [148]. In developing countries, the seroprevalence is even higher, with 65% to nearly 100% of individuals possessing antibodies to *C. parvum* in parts of Latin America [149]. Cryptosporidiosis is among the most common causes of diarrhea in patients with AIDS, with an annual incidence of 5%–10%. Severe cases of cryptosporidiosis also can be seen in individuals with other forms of immunodeficiency, such as hypogammaglobulinemia, and in those patients who are receiving immunosuppressive drugs for malignancies, autoimmune diseases, or organ transplantation [118,145]. The reader is referred to Chapter 104 for a more detailed discussion of *Cryptosporidium* infection in immunocompromised hosts.

In developing countries, a significant portion of disease transmission may arise from person-to-person spread within families, with transmission rates comparable with those of *Shigella* [150]. In industrialized countries, day-care centers represent a source for outbreaks and sporadic infections. A cross-sectional study showed that 1.8%–3.8% of children

in day-care centers are infected, but rates are much higher (27%–59%) among children in centers experiencing an outbreak of *C. parvum* disease [151]. Infections occur primarily in diaper-wearing children, who serve as a potential nidus for secondary infection of the surrounding community. The risk for secondary transmission within a household is low (5%) when the index case involves an adult [152]. Person-to-person spread within the hospital setting also has been reported [153].

Water-borne outbreaks of *C. parvum* infection are a major threat to public health and have been associated with contaminated drinking water and recreational waters [75,143,144]. These outbreaks underscore the resistance of *Cryptosporidium* oocysts to chlorination and the difficulties in removing the organism by filtration. Food-borne outbreaks of cryptosporidiosis have also been reported, and have been associated with ingesting unpasteurized apple cider, unpasteurized milk, chicken salad, and raw produce [118,145]. Cryptosporidiosis has also been associated with travel, particularly to south-central Asia [154].

Pathogenesis and pathology

Cryptosporidium sporozoites emerge from the ingested oocysts after undergoing sexual recombination. Time-lapsed video-microscopy has demonstrated that the sporozoites invade epithelial cells within 30 s after contact [155]. However, the parasite remains at the cell surface within a membrane-bound compartment without entering deep into the cytoplasm. Parasite-induced epithelial cell apoptosis induces inflammatory mediators [156,157]. The potential mechanisms by which cryptosporidia cause diarrhea include disruption of the epithelial barrier (resulting in malabsorption), induction of inflammatory mediators, or release of parasite enterotoxins that stimulate intestinal secretion [145].

The complete genome sequences for *C. parvum* and *C. hominis* have been reported [158,159]. The two genomes are similar with only 3%–5% sequence divergence. Analysis of the protein-coding genes indicates that both aerobic and anaerobic metabolic pathways are available, but that the latter consists of an alternative electron transport system in a simplified mitochondrion. The parasite lacks the enzymes for the synthesis of key biochemical building blocks. Consistent with its need to import essential nutrients from the host cell, the genome encodes multiple transporters.

On histological examination of intestinal biopsy specimens from infected individuals, a variety of nonspecific changes can be seen, including villous atrophy (partial or complete) and crypt hyperplasia with increased numbers of lymphocytes, macrophages, plasma cells, and, less commonly, neutrophils in the lamina propria [160]. However, many patients show normal duodenal biopsy specimens, despite chronic diarrhea [160]. There appears to be an association between the extent of malabsorption and intestinal injury and the intensity of the infection (based on oocyst excretion) [161]. The prolongation of infection in immunocompromised patients and in animal models suggests that CD4 (helper) T cells and interferon are critical to the control of infection [145,157,162–164]. One of the remarkable consequences of the availability of highly active antiretroviral therapy (HAART) has been a significant reduction in the cases of cryptosporidiosis seen in HIV-infected individuals [165].

Clinical manifestations

The clinical spectrum of the disease is wide and includes asymptomatic carriage, a self-limited diarrheal illness in immunocompetent individuals, and a relentless, severe, watery diarrhea in some immunocompromised individuals. In a study of 29 healthy volunteers, the median infective dose of *C. parvum* was estimated to be 132 oocysts, with a mean incubation period of 9 days (range 5–22 days) [166]. As few as 30 oocysts caused infection in some subjects. The infectivity was therefore comparable with that of *Giardia* cysts. Subjects with diarrhea excreted more oocysts over the course of the infection than did subjects without diarrhea [167]. In a study of 21 healthy volunteers, the median infective dose of *C. hominis* was 10 and 83 oocysts using clinical and microbiological definitions of infection respectively [168]. The mean incubation period was 5.7 days (range 2–10 days). The infectivity of *C. hominis* was similar to that of *C. parvum*. In contrast to *C. parvum*, *C. hominis* elicited a serum IgG response in most infected persons.

The predominant symptom in intestinal cryptosporidiosis is diarrhea, which is often accompanied by abdominal cramps and varies greatly in severity from individual to individual. Some patients have scant intermittent diarrhea, whereas others (more often immunocompromised individuals) experience voluminous watery diarrhea that can approach the quantities seen with cholera (> 10 L/day). Mucus may be present in stools, but bloody diarrhea is rare, and white blood cells are usually not found in stools from patients with cryptosporidiosis. Accompanying symptoms may include nausea, fatigue, general malaise, anorexia, vomiting, and low-grade fever. The duration of illness in immunocompetent individuals ranged from 2 to 26 days in one study [169], with a mean of 12 days, but more prolonged illness is not unusual [150]. Transient relapse of watery diarrhea has been observed in immunocompetent hosts, particularly in the massive outbreak of water-borne *Cryptosporidium* infection that occurred in Milwaukee [152]. Extraintestinal disease is rare in immunocompetent individuals, but pancreatitis and cases of reactive arthritis have been reported in previously healthy adults [170,171]. Cholecystitis is seen in up to 10% of patients with cryptosporidiosis and AIDS but is rare in immunocompetent patients.

The differential diagnosis for cryptosporidiosis in immunocompetent hosts includes other protozoan infections (e.g., *Cyclospora cayetanensis, Giardia lamblia*, and *Isospora belli*), bacterial infection (e.g., *Salmonella, Shigella, Campylobacter, Yersinia,*

Clostridium difficile, and enterotoxigenic *Escherichia coli* in patients with a history of travel to developing countries), and viral infections (e.g., rotavirus and Norwalk viruses).

Diagnosis

The diagnosis of *Cryptosporidium* infection should be suspected in individuals with diarrhea lasting longer than 3 days. Patients should be questioned with respect to recent travel, exposure to children in day care, the state of their drinking water, ingestion of unpasteurized milk or fruit juices, exposure to recreational water, and exposure to animals, particularly cattle. Many laboratories routinely use commercial enzyme immunoassay methods to detect *Cryptosporidium* antigens in the stool [114]. At least two stools should be obtained on two different days to reach a sensitivity of over 90% [115]. In some laboratories, positive results using enzyme immunoassays are confirmed by immunofluorescent assays [116]. In the human volunteer studies, organisms could be detected using a more sensitive flow cytometric assay in stool samples that were negative by enzyme immunoassay or immunofluorescent assays [168]. The US Environmental Protection Agency has developed methods of concentrating the organisms from water involving filtration, centrifugation, and immunomagnetic separation, but these methods are not applied in clinical microbiological laboratories [172]. It is important to make a diagnosis in the interest of public health, although disease is generally self-limiting in immunocompetent hosts.

For those unusual cases in which *Cryptosporidium* infection is suspected, but the major symptoms are abdominal cramps or abdominal pain without significant diarrhea, stool specimens are more likely to be negative, and a duodenal aspirate with biopsy, combined with lower endoscopy and terminal ileum biopsy, may have a higher diagnostic yield [173].

Treatment

For immunocompetent individuals, the disease is self-limiting, and symptomatic treatment (replacement of lost fluids) is the cornerstone of therapy. Nonspecific antidiarrheal therapies, such as antimotility agents, may provide some relief for individual patients. Most antimicrobial agents investigated for cryptosporidiosis have demonstrated mixed success. The most promising agent is nitazoxanide, which is licensed for the treatment of giardiasis and cryptosporidiosis in children [174,175].

Cyclospora cayetanensis

In the early 1990s, diarrhea associated with an organism variously described as a large cryptosporidium, blue-green algae, cyanobacterium-like body, or coccidian-like body was reported [176]. The organism was identified as a species of *Cyclospora* by Ortega and colleagues [177], who successfully induced sporulation and excystation of oocysts obtained from individuals with diarrhea. Electron microscopic studies of the organisms in enterocytes revealed that they possessed morphological features of coccidians of the phylum Apicomplexa [178], and a molecular phylogenetic analysis confirmed that *Cyclospora* is a coccidian parasite closely related to protozoa of the *Eimeria* genus, which are known pathogens of birds [179].

Epidemiology

Cyclospora species are distributed widely but appear to be more common in tropical and subtropical areas. Originally, disease was described primarily in travelers to endemic areas, but with greater awareness of the parasite, indigenous disease in endemic areas and outbreaks in areas of low endemicity are being more widely recognized [180]. Prevalence rates as high as 11% have been reported in nonnative residents of an endemic area, and 18% of young children (1 month to 2 years of age) in poor regions of Peru had oocysts in their stool [180,181]. In the United States and the United Kingdom, surveys of stool specimens during nonoutbreak periods reveal that the prevalence of positive stools is less than 0.5% [176].

One of the most important biological properties affecting the epidemiology of *Cyclospora* is that the oocysts in freshly excreted stool are noninfectious! The oocysts must sporulate outside the host before infection of the next host can occur. Consequently transmission is not likely to occur by person-to-person contact. The environmental conditions required for oocysts to sporulate are not well understood. The ingestion of water or fresh produce containing sporulated oocysts of *Cyclospora* is the major mode of transmission. In North America there have been over 12 documented food-borne outbreaks involving *Cyclospora*. This number is unprecedented in the experience with other protozoan parasites. The types of fresh produce implicated in such outbreaks have included raspberries, mesclun, basil, and snow peas that were potentially imported from Central and South American countries [176,182]. Produce should be thoroughly washed before being eaten to decrease the risk of transmission.

Pathogenesis and pathology

Limited information is available on how *Cyclospora* causes disease in humans. Thus far infections have not been established in animals models or human volunteers [176,183]. Disease appears to involve primarily the small intestine, and documented impaired D-xylose absorption suggests that the proximal small intestine may be an important target for infection [184,185]. Electron microscopic studies of jejunal biopsy samples have demonstrated that the sporozoite, trophozoite, schizont, and merozoite stages of the organism are present within parasitophorous vacuoles in the cytoplasm of infected enterocytes [178]. In contrast to cryptosporidia, the *Cyclospora* are intracytoplasmic in the apical supranuclear region, rather than extracytoplasmic at the lumenal surface of the enterocyte. Villous blunting has been observed on endoscopic biopsies obtained from patients with active *Cyclospora* infection,

which could be mistaken for celiac disease or tropical sprue [186–188].

Clinical manifestations

The clinical manifestations range from asymptomatic illness to prolonged, nonbloody diarrhea associated with loss of appetite, fatigue, and weight loss [176]. Whereas infected children in endemic areas are asymptomatic or have relatively mild symptoms, infected nonimmune adults developed a more severe and prolonged illness. In untreated nonimmune patients remitting–relapsing symptoms may persist for weeks to months, with fatigue and malaise being the most persistent symptoms. The average incubation period is estimated to be 7 days, but may be as short as 1–2 days [176]. The mean duration of oocyst shedding has been found to be 23 days [176]. Symptoms of diarrhea and fatigue may persist after organisms are no longer detectable in stool samples; conversely, individuals may continue to shed oocysts after symptoms have disappeared. Although cyclosporiasis can be prolonged and frequent relapses are common, disease appears to be self-limited in immunocompetent hosts. Complications can include malabsorption, Reiter syndrome, and possibly Guillain–Barré syndrome [176].

Diagnosis

A high index of suspicion is needed to make the diagnosis because stools are not routinely processed for detection of this organism. It is important for the physician to communicate with the laboratory to determine how the stool should be collected and whether it should be sent to a reference laboratory. *Cyclospora* infection should be suspected in patients with prolonged diarrhea accompanied by marked fatigue and malaise, particularly those with a recent history of travel. Patients should also be questioned regarding their intake of fresh produce. Diagnosis depends on the detection of oocysts by the microscopic examination of stool samples using acid-fast staining methods or ultraviolet fluorescence microscopy (Fig. 106.5; see also Table 106.2). Because excretion of oocysts may be intermittent, multiple stool samples should probably be examined before excluding the diagnosis of *Cyclospora* infection. The diameter of oocysts in stained specimens can distinguish *Cryptosporidium* oocysts (4–6 μm) from the larger (8–10 μm) *Cyclospora* oocysts.

Diagnosis of cyclosporiasis also has been made by light microscopic examination of duodenal and jejunal aspirates obtained during endoscopy and examined as wet mounts, or, probably more efficiently, as acid-fast-stained samples. Smears also can be examined under ultraviolet fluorescence microscopy, looking for the characteristic blue fluorescence. Identification of the parasite by light microscopy in a duodenal biopsy specimen has been reported, but in general this has not been a high-yield approach for diagnosis. If the diagnosis is suspected endoscopic biopsies should be sent in special fixative for electron microscopy [178].

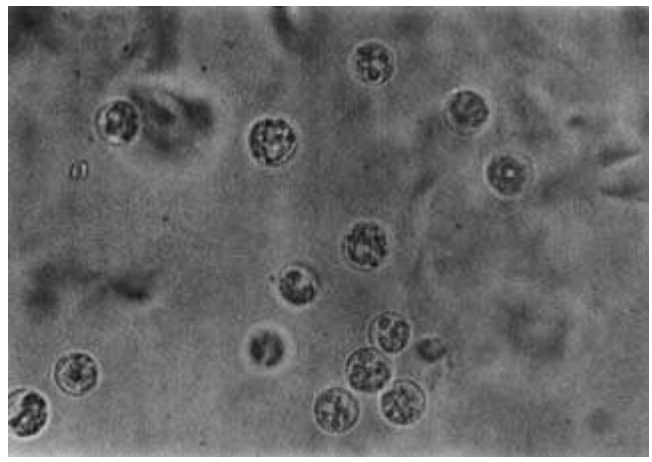


Figure 106.5 *Cyclospora cayetanensis* oocysts (saline, wet preparation). Note 8- to 10-μm diameter spheres containing numerous refractile globules. Courtesy of Earl G. Long.

Treatment

Immunocompetent patients can be treated successfully with few or no relapses with trimethoprim (TMP)–sulfamethoxazole (SMX) (i.e., co-trimoxazole; 160 mg TMP + 800 mg SMX) twice daily for 7–10 days [176]. Alternative treatments for persons allergic to sulfa drugs have not been established. Ciprofloxacin has been reported to demonstrate efficacy [189]. There is an anecdotal report that an immunocompetent sulfa-allergic patient infected with *Cyclospora* failed ciprofloxacin treatment but subsequently responded to nitazoxanide [190].

Isospora belli

Isospora belli is a coccidian protozoan that was first recognized as an intestinal parasite of humans more than 80 years ago in military forces stationed overseas and in outbreaks of diarrhea in institutionalized individuals [191,192]. Molecular phylogenetic analysis indicates that *Isospora* spp. are most closely related to *Toxoplasma* and *Neospora* [193,194].

Isospora infection is more common in individuals residing in developing countries and is relatively rare in immunocompetent individuals in the United States. Sporadic small-scale outbreaks of *Isospora* infection have been seen in mental institutions and day-care centers within the United States, but more widespread outbreaks, such as as ones seen with *Cryptosporidium* and *Cyclospora*, have not been reported [195]. *Isospora* is an important cause of diarrhea (~ 15%) in individuals with AIDS in developing countries but accounts for only 0.2% of diarrhea cases in AIDS patients within the United States [196]. *Isospora* is sensitive to TMP–SMX, and one reason for the lower prevalence of isosporiasis in AIDS patients within the United States may be the widespread use of TMP–SMX as prophylaxis for *Pneumocystis carinii* infection. There is no evidence of significant zoonotic transmission of

the organism. Like *Cyclospora* but unlike *Cryptosporidium*, oocysts are excreted unsporulated or partially sporulated. The major mode of transmission is therefore by ingestion of water or food contaminated with sporulated oocysts.

The manifestations of *Isospora* infection are similar to those seen with both *Cyclospora* infection and cryptosporidiosis: watery, nonbloody, noninflammatory diarrhea that is often accompanied by abdominal cramps, fatigue, weight loss, anorexia, and occasionally fever [195]. The incubation period is relatively short (2–3 days), and infection is generally self-limited but may be prolonged (months to years) even in immunocompetent hosts. In contrast to cryptosporidiosis and cyclosporiasis, a peripheral eosinophilia may be seen in individuals with isosporiasis. Diarrhea may be more severe in young children than in adults. Villous atrophy and crypt hyperplasia have been observed in small intestinal biopsies of immunocompetent patients. Extraintestinal infections have been observed primarily in AIDS patients, but one has been reported in an immunocompetent patient [197].

Because stools are not routinely processed for detection of this organism, the physician must specifically request the laboratory to process the stool. The diagnosis can be made by detection of the *Isospora* oocysts in feces using light microscopy (Table 106.2). Shedding of oocysts is intermittent, and multiple stool samples may need to be examined. Charcot–Leyden crystals and eosinophils are occasionally seen in stool samples from individuals with isosporiasis [195]. There are case reports of individuals who had negative stool tests but in whom organisms were detected by histological examination of small intestinal biopsy specimens [198].

The drug of choice for the treatment of *Isospora* infection is TMP–SMX (160 mg TMP + 800 mg SMX four times daily for 7 days, followed by 10 days of 160/800 mg twice daily). In patients with severe malabsorption, parenteral administration may be required to achieve therapeutic drug levels [197]. In the sulfa-allergic patient pyrimethamine alone may be used.

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107 Parasitic diseases: helminths

Carlos Seas, Martin Montes, A. Clinton White Jr

Intestinal nematodes (roundworms), 2651
Trematodes (flukes), 2659
Cestodes, 2661

Helminth parasites, or worms, are multicellular eukaryotic organisms that are common inhabitants of the human gastrointestinal track. Throughout human history, parasitism with helminths has been a normal part of the human condition [1–3]. Only with the development of improved standards of sanitation, housing, and food preparation in the 20th century did the prevalence of these infections decrease in wealthy countries. In contrast, the total number of infected individuals continued to increase throughout most of the 20th century. Data from the late 20th century highlighted the previously unrecognized burden of morbidity, focusing on the role of helminths in chronic malnutrition, anemia, and accompanying cognitive defects, leading to the recognition that intestinal helminths are neglected tropical diseases of global public health importance [4,5].

Helminths are multicellular members of the animal kingdom, large enough to be visible to the human eye at some point in their life cycle. Parasites have different morphological forms that vary with the host and environment and most have an obligate one-way life cycle. In contrast to most microorganisms, helminths do not typically reproduce within the human host. Thus, the parasite burden is largely a reflection of the infecting dose and host control of infection. Interestingly, the population structure of worm burden in communities is typically overdispersed, i.e., most of the worms live in a small portion of the population. Most of this pattern appears related to genetic predisposition [6].

Helminths are categorized into three major groups: nematodes, trematodes, and cestodes. Nematodes are roundworms with a tubular gut including both a mouth and anus. There are two groups of flatworms: trematodes and cestodes. Trematodes, or flukes, are flat leaf-shaped organisms with a blind gut. All require an obligate freshwater snail host. The cestodes, or tapeworms, typically have two distinct forms. The adult forms a tapeworm in the gut of the definitive host and possesses an attachment organ (scolex) and segments (proglottids). While not having a separate gut, the external surface shares many features with the mammalian digestive tract. The proglottids are hermaphroditic, containing both ovaries and testes. The larval forms develop as cystic lesions in the tissues of the intermediate host. Some species have a second intermediate host. Many parasites use humans as an obligate host in their life cycle. In addition, related animal parasites often infect people and cause human disease. Zoonotic infections are noted with all the major nematodes groups as well as some trematodes and cestodes. The major human intestinal helminths and their treatment are detailed in Table 107.1.

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Intestinal nematodes (roundworms)

Ascaris lumbricoides

Ascaris lumbricoides is the most common human helminth infection, with one-fifth of all humans infected. Adult females live inside the host for about 1 year, producing 200 000 ova daily. Humans acquire infection by ingestion of contaminated food or water. Larvae are released in the small intestine, penetrate the intestinal wall, and migrate to the lung and up the trachea. They are subsequently swallowed, and complete their development within the lumen of the small intestine.

Epidemiology

Approximately 1.2 billion people are infected with *Ascaris*, with 100–300 million symptomatic infections and 60 000 deaths annually [3–5,7]. Most infections occur in tropical and subtropical areas of the world, especially in Asia. The peak prevalence occurs between ages 5 and 15 years. Ascariasis is thought to cause growth deficit, lactose intolerance, malabsorption of vitamin A, malnutrition, impaired cognition, and impaired physical fitness in developing countries [5,7–9]. Much of the variability in worm burden reflects host genetic susceptibility [6].

Table 107.1 Helminth infections involving the human gastrointestinal tract and their treatment.

Nematode	Drug	Adult dose	Pediatric dose
Nematodes (roundworms)			
Ascaris	Albendazole	400 mg, single dose	400 mg, single dose
	Mebendazole	100 mg, twice daily for 3 days	100 mg, twice daily for 3 days
		500 mg, single dose	500 mg, single dose
	Pyrantel pamoate	11 mg/kg (maximum 1 g) for 3 days	11 mg/kg (maximum 1 g) for 3 days
	Levamisole	2.5 mg/kg, single dose	2.5 mg/kg, single dose
Hookworm	Albendazole	400 mg, single dose	400 mg, single dose
	Mebendazole	100 mg, twice daily for 3 days	100 mg, twice daily for 3 days
	Pyrantel pamoate	11 mg/kg (maximum 1 g) for 3 days	11 mg/kg (maximum 1 g) for 3 days
	Levamisole	2.5 mg/kg, single dose, repeat after 7 days in heavy infection	2.5 mg/kg, single dose, repeat after 7 days in heavy infection
Strongyloides	Ivermectin	200 µg/kg for 1–7 days	200 µg/kg for 1–7 days
Trichuris	Mebendazole	100 mg, twice daily for 3 days	100 mg, twice daily for 3 days
	Albendazole	400 mg for 3 days	400 mg for 3 days
Enterobius	Albendazole	400 mg, single dose, repeat in 2 weeks	400 mg, single dose, repeat in 2 weeks
	Mebendazole	100 mg, single dose, repeat in 2 weeks	100 mg, single dose, repeat in 2 weeks
	Pyrantel pamoate	11 mg/kg (maximum 1 g), repeat in 2 weeks	11 mg/kg (maximum 1 g), repeat in 3 weeks
Trichinella	Albendazole	400 mg, twice daily for 8–14 days	400 mg, twice daily for 8–14 days
	Mebendazole	200–400 mg three times daily for 3 days, then 400–500 mg three times daily for 10 days	200–400 mg three times daily for 3 days, then 400–500 mg three times daily for 10 days
Ancylostoma (cutaneous larva migrans)	Ivermectin	200 µg/kg, single dose	200 µg/kg, single dose
	Albendazole	400 mg daily for 3 days	400 mg daily for 3 days
Visceral larva migrans	Albendazole	400 mg, twice daily for 5 days	400 mg, twice daily for 5 days
	Mebendazole	100–200 mg, twice daily for 5 days	100–200 mg, twice daily for 5 days
Anisakiasis	Surgical removal is the treatment of choice. Mebendazole or albendazole for 1–5 days has been effective in some cases		
Baylisascariasis	Albendazole	25–40 mg/kg daily for 28 days	25–40 mg/kg daily for 28 days
Angiostrongylus costaricensis	Ivermectin?		
Capillariasis	Albendazole	400 mg daily for 10 days	400 mg daily for 10 days
	Mebendazole	200 mg, twice daily for 20 days	200 mg, twice daily for 20 days
Tremadotes (flukes)			
Schistosoma spp. *S. mansoni*, *S. haematobium*, *S. intercalatum*	Praziquantel	40 mg/kg in two doses on a single day	40 mg/kg in two doses on a single day
S. japonicum or *S. mekongi*	Praziquantel	60 mg/kg in three doses on a single day	60 mg/kg in three doses on a single day
Fasciola	Triclabendazole	10 mg/kg, one to two doses	10 mg/kg, single dose
Other flukes	Praziquantel	25 mg/kg three times daily for 1 day	25 mg/kg three times daily for 1 day
Cestodes			
Adult tapeworms			
Hymenolepis nana	Praziquantel	25 mg/kg, single dose	25 mg/kg, single dose
	Nitazoxanide	500 mg, twice daily for 3 days	100–500 mg, twice daily for 3 days
	Niclosamide	2 g, single dose	50 mg/kg, single dose
Taenia saginata or *T. solium* taeniasis, or other tapeworms	Praziquantel	5–10 mg/kg, single dose	5–10 mg/kg, single dose
	Nitazoxanide	500 mg, twice daily for 3 days	100–500 mg, twice daily for 3 days
	Niclosamide	2 g, single dose	50 mg/kg, single dose

Table 107.1 (*cont'd*)

Nematode	Drug	Adult dose	Pediatric dose
Larval cestodes (cysticercosis, hydatid disease)			
Neurocysticercosis	Albendazole Plus steroids or neurosurgery	400 mg, twice daily for 8 up to more than 30 days	7.5 mg/kg, twice daily for 8 up to more than 30 days
Echinococcus granulosus	Albendazole Alone, +PAIR, or +surgery	400 mg, twice daily for > 10 weeks	7.5 mg/kg, twice daily for > 10 weeks
Echinococcus multilocularis	Surgical resection plus albendazole	400 mg, twice daily for 2 years up to lifelong	7.5 mg/kg, twice daily for 2 years up to lifelong

PAIR, *p*uncture, *a*spiration, *i*njection of scolicidal agent, and *re*-aspiration.

Clinical manifestations

With low worm burdens, patients are typically asymptomatic or may note passage of an adult worm, which can reach a length of 40 cm [7]. Most symptoms attributed to *Ascaris* (abdominal pain, nausea, and diarrhea) are difficult to differentiate from those of other endemic diseases. Intestinal obstruction may result from a massive parasite burden, particularly in children, who present with abdominal distention, abdominal pain, and poor oral tolerance [10,11]. The adult *Ascaris* may migrate upward to the oropharynx to be eliminated by the nasal or oral route, or may travel to the biliary tract, causing cholangitis, hepatic abscesses or pancreatitis, particularly in adults [12–14]. During the migration of larvae through the lungs patients may experience bronchospasm, and transient pulmonary infiltrates accompanied by eosinophilia.

Diagnosis, treatment, and prevention

Typical ova found in the stools with direct saline preparations or the Kato–Katz method are sufficient to make the diagnosis (Figs 107.1 and 107.2). Adult worms may be found in the stool, biliary tract and pancreatic duct. *Ascaris* can be visualized by ultrasound or computed tomography (CT) as cylindrical shapes with a slow and nondirectional movement [15].

Albendazole and mebendazole are the first-line drugs (see Table 107.1). Levamisole, pyrantel pamoate, and nitazoxanide are alternatives. Endoscopic extraction coupled with antiparasitic thrapy is the treatment of choice for biliary tract involvement. Ascariasis can be prevented by sanitation and promoting good hygienic practices. However, these measures are difficult to implement in endemic areas. Mass community treatment programs can dramatically decrease disease prevalence and reduce transmission [7,16–18].

Hookworm

Hookworm infections are mainly caused by *Ancylostoma duodenale* and *Necator americanus*, and are considered among the most common parasitic infections in tropical countries [3,5,19,20]. Humans are infected by skin contact with the filariform infective larval stage of the parasite. The most common penetrating sites are the hands and feet; from these sites the larva migrates to the lung where it usually resides for about 10 days. The larvae migrate up the trachea from the lungs and are swallowed. The adult worms develop in the small intestine. Adult hookworms are 1 cm long and pass approximately 30 000 eggs per day [19,21]. Each adult worm attaches to an intestinal villus, aspirates a plug of tissue from the villus tip, secretes inhibitors of the host coagulation and platelet pathways, and ingests blood. Periodically, the parasites will move to other villi. The main consequence of hookworm infection is intestinal blood losses due to ingestion by the parasite or villus damage. Infections with *A. duodenale* results in loss of approximately 0.2 mL/day per adult hookworm, significantly more than noted with *N. americanus* (~ 0.03 mL/day per adult hookworm) [19]. Occasionally, filariform larvae of *A. duodenale* may be ingested causing nausea, vomiting, and pharyngeal irritation [20].

Epidemiology

Hookworms are widely distributed, with *N. americanus* more widespread than *A. duodenale*. More than 700 million humans are infected with hookworms globally, with the greatest burden of disease in sub-Saharan Africa and Asia. Infection is more prevalent in males, but women and children are more vulnerable to the chronic sequelae (anemia and iron deficiency). Approximately 44 million pregnant women are infected. Poor sanitation, walking barefoot, and the use of human

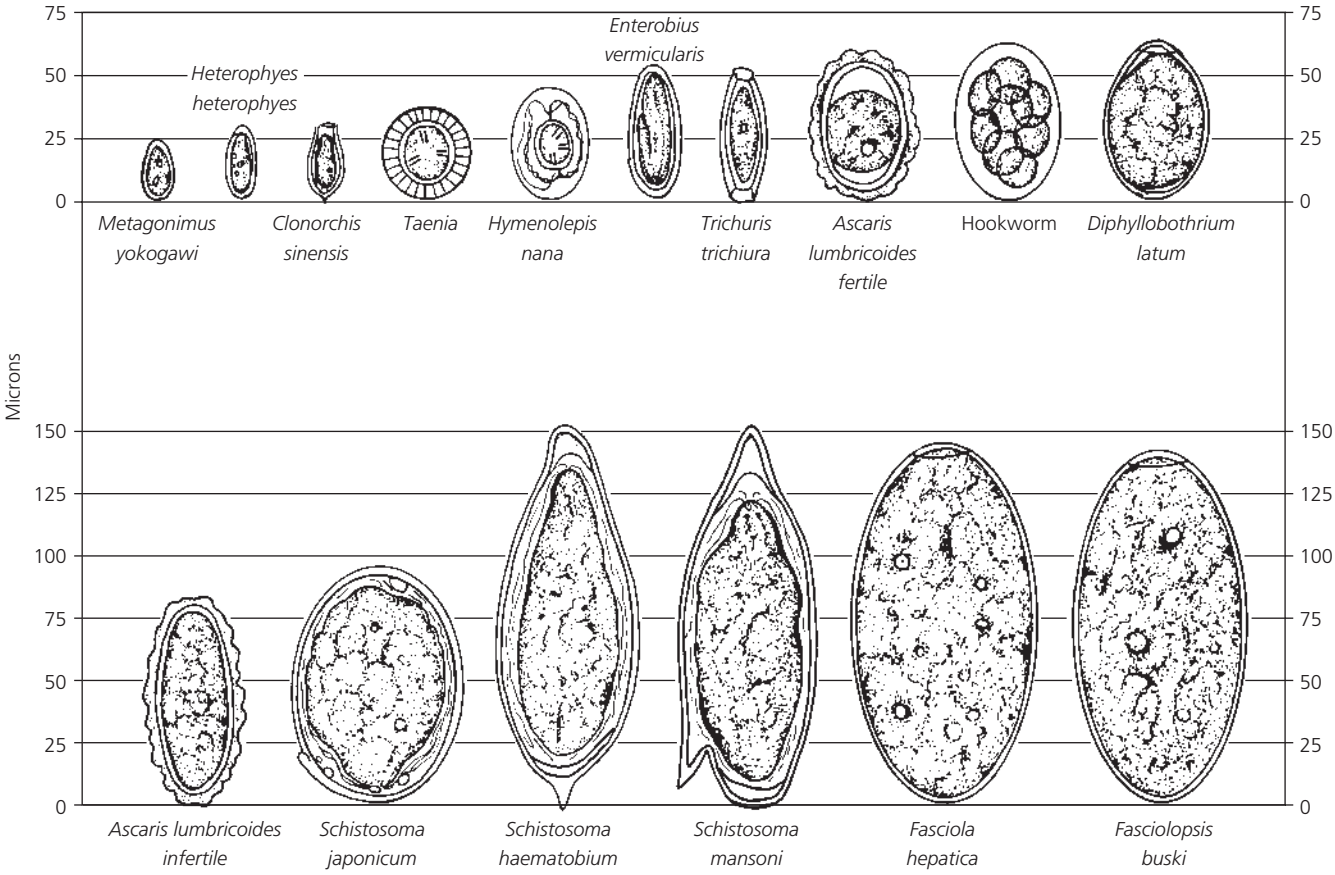

Figure 107.1 Relative sizes and shapes of helminth ova. Adapted from Smith JW, Ash LR, Thompson JH Jr, et al. Intestinal helminths. In: Atlas of Diagnostic Medical Parasitology Series. Chicago: American Society of Clinical Pathologists, 1984.

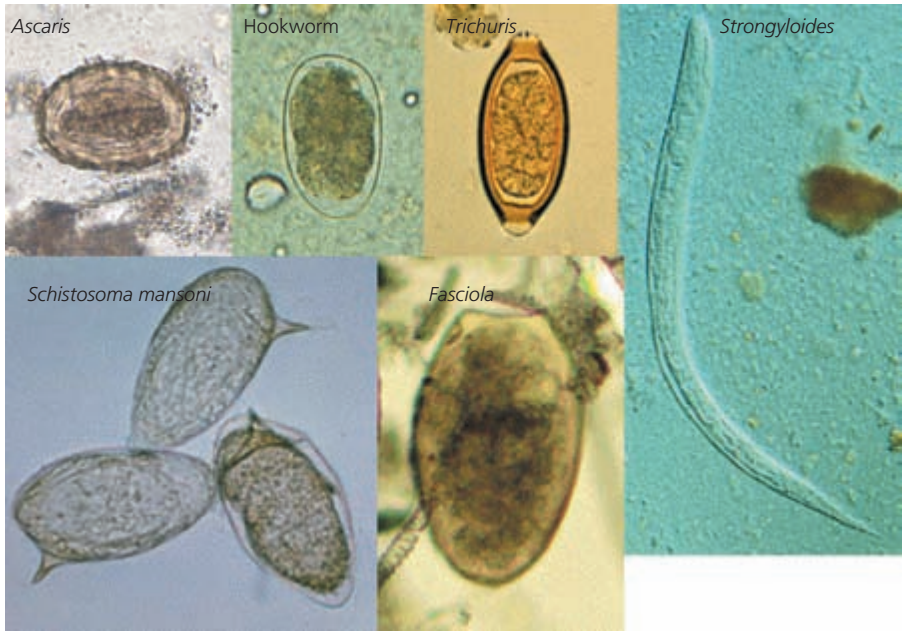

Figure 107.2 Ova of *Ascaris*, *Ancylostoma*, *Trichuris*; rhabditiform larva of *Strongyloides*; and ova of *Schistosoma mansoni* and *Fasciola hepatica*. Images from DpDx website (http://www.dpd.cdc.gov/DpDx).

excrement as fertilizers contribute to transmission in developing countries.

Clinical manifestations

The main clinical manifestation of hookworm infection is anemia due to iron deficiency, resulting from blood losses induced by parasite feeding. *Ancylostoma* induces more profound anemia than *Necator*. Hypoalbuminemia also follows the chronic infection and results in protein malnutrition. Typically, patients with chronic infection complain of severe pallor, dyspnea, fatigue, palpitations, and poor school performance. Increase maternal mortality, prematurity, low birthweight, and reduced lactation have been reported [5,20,21].

Most infections are asymptomatic or mildly symptomatic. Epigastric pain and tenderness as well as nausea are among the most commonly reported abdominal symptoms. Papular and pruritic skin eruptions may follow exposure to the filariform larvae. Mild cough, sore throat, and rarely pneumonitis may accompany larval lung migration. Eosinophilia, anemia and hypoalbuminemia are common [19].

Diagnosis, treatment, and prevention

Stool examination for eggs is usually adequate to make the diagnosis (see Figs 107.1 and 107.2). There is no clinical need to differentiate between hookworm species. Anthelminthic treatment includes albendazole, mebendazole, or pyrantel pamoate (see Table 107.1). If the patient is anemic, iron supplementation is usually needed as well. Improving sanitary conditions, promoting good hygienic practices, treatment of infected persons, and use of footwear can prevent transmission. However, the main current control strategy is mass chemotherapy of children [5,16,17,19,21]. Novel vaccines are under clinical evaluation.

Trichuris trichiura (whipworm)

Trichuris trichiura is the third most common soil-transmitted helminth, infecting 600 million with high prevalence rates in China, India, East Asia, and Central Africa [3,5]. Humans are infected by the ingestion of embryonated ova. The larvae are liberated from the eggs, migrate to the large intestine, and mature over 3 months. Each adult female produces approximately 20 000 eggs per day.

Epidemiology

Trichuris trichiura has a worldwide distribution but is especially prevalent in Africa, Asia and Latin America. The severity of infestation varies within a community resulting in an overdispersed distribution, with most of the worm burden occurring in a subgroup of individuals who are more likely to manifest disease [5].

Clinical manifestations

The adult parasite lives preferentially in the cecum, but the whole large intestine can be infected. The anterior end of the adult burrows inside the mucosa and submuocsa, leaving the posterior end visible in the lumen. Low-burden infections are typically asymptomatic [5,22]. In heavy infections, the inflammatory reaction to the attached nematode leads to colitis, dysentery, and rarely rectal prolapse. Heavy infections are most common in children below 10 years of age, who present with diarrhea and growth retardation. Colonic obstruction and perforation have been reported. Eosinophilia is the most frequent laboratory abnormality.

Diagnosis, treatment, and prevention

The diagnosis is confirmed by identifying ova in the stool (see Figs 107.1 and 107.2) [23]. Mebendazole or albendazole are first-line drugs for therapy (see Table 107.1), but should be given as a multidose regimen because single-dose therapy with albendazole is not consistently effective [24]. Mass deworming programs with single dose of albendazole fail to eliminate heavy infestations [16–18]. Adequate sanitary disposal of human feces, proper hygiene, and preparation of food reduce transmission.

Strongyloides stercoralis

Strongyloides is capable of completing its life cycle inside a single human host, allowing infection to persist for decades or to progress to life-threatening hyperinfection [25]. Adult female parasites live in the intestinal wall, where the ova hatch, releasing the rhabditiform larvae, which migrate into the lumen and pass in stools. In the environment, larvae molt into the filariform larvae, which infect the human host by penetrating through intact skin. The larvae then migrate via the bloodstream to the lungs, into the alveoli, up the tracheobronchial tree, and are swallowed. The adult female worms invade the small intestinal wall, where they produce eggs asexually. Some of the rhabditiform larvae molt into invasive forms prior to being excreted, and are capable of reinvading via the intestinal wall or perianal skin (autoinfection). The number of filariform larvae is increased in some immunocompromised individuals (e.g., those taking corticosteroids), leading to a hyperinfection syndrome. Some of the rhabditiform larvae convert to free-living adult males and females, which produce ova that hatch and mature to filariform larvae in the soil.

Epidemiology

Estimates of infected people range from 3 million to over 100 million, with a consensus estimate of approximately 60 million [26]. Endemic areas include most tropical areas of Africa, Asia, and Latin America. High prevalence areas include Southeast Asia and the Amazon basin [27–29]. Lower rates are noted in the southern USA, southern and eastern Europe, and Japan.

Strongyloides stercoralis can produce life-threatening hyperinfection in people with altered host defense, especially associated with corticosteroid treatment. A high prevalence of

S. stercoralis and increased severity of disease has been observed in people concurrently infected with human T-cell lymphotrophic virus type 1 (HTLV-1) [30–33]. Spontaneous secretion of interferon-γ by HTLV-1-infected mononuclear cells or increased regulatory T cells may suppress protective granulocyte and interleukin (IL)-5 responses [31,34].

Hyperinfection has also been reported in association with organ transplantation, malignancies (especially lymphoma and leukemia), other immunosuppressive therapy, protein-calorie malnutrition, visceral leishmaniasis, lepromatous leprosy, and other debilitating conditions [35,36]. However, hyperinfection has only rarely been associated with human immunodeficiency virus infection [37,38].

Clinical manifestations

A maculopapular rash or linear urticaria, called larva currens, may occur at the time of skin penetration or as a consequence of autoinfection [25,36,39,40]. The pulmonary phase of strongyloidiasis can be associated with cough, shortness of breath, wheezing, fever, transient pulmonary infiltrates, and eosinophilia, but is usually asymptomatic in chronic infection [41]. Eosinophilia is common in normal hosts with strongyloidiasis.

The adult worms of *S. stercoralis* often produce epigastric or diffuse abdominal pain or diarrhea [25,39,40]. This may be accompanied by occult blood in stool. With heavy infections, vomiting, malabsorption, steatorrhea, and weight loss can occur. Upper endoscopy may reveal edema or discoloration of the stomach or duodenum. The colon may have edema, ulceration, or tears [42]. Eosinophilic granulomatous enterocolitis, pseudopolyposis, and inflammatory mass lesions have also been reported.

Strongyloides hyperinfection is characterized not only by multisystem involvement [25,36]. The clinical manifestations are highly variable. Lung disease may be the only site involved clinically [43]. Pulmonary involvement results from the migration of large numbers of larvae through the lungs, causing bronchospasm, pulmonary hemorrhage, pneumonia, and respiratory failure [41,44]. Pulmonary symptoms include cough, shortness of breath, wheezing, and hemoptysis. Hyperinfection may progress to the acute respiratory distress syndrome, often with bilateral infiltrates seen on chest radiograph. Others have primarily intestinal symptoms.

As the filariform larvae penetrate the intestines and migrate, they can carry Gram-negative bacteria and *Enterococcus*, which adhere to their surface, leading to bacterial infections with enteric organisms or polymicrobial bacteremia, meningitis, peritonitis, or endocarditis [25,35,36,45]. The mortality rate with *S. stercoralis* hyperinfection may exceed 50%.

Diagnosis, treatment, and prevention

The diagnosis of strongyloidiasis is confirmed by identifying larvae in stool, tissues, or secretions (see Fig. 107.2) [25,46]. Eosinophilia is frequently present, but may be absent in immunocompromised hosts. Direct microscopic examination of stool for the organisms is insensitive (only 30% positive in a single sample) because larval output is low and intermittent. Concentration techniques such as the Baermann sedimentation method improve the sensitivity only modestly. The agar plate method is more sensitive. In hyperinfection syndrome, the number of larvae is greatly increased, facilitating the identification of larvae in stool as well as other tissues, with organisms frequently found in sputum, bronchoalveolar lavage fluid, intestinal biopsies, and biopsies of other tissues. The poor sensitivity of parasitological tests led to the development of several immunodiagnostic assays [25,46]. In endemic areas, enzyme-linked immunosorbent assay (ELISA) can be used as a screening method to rule out infection [29,39,47]. Assays using recombinant antigens appear to be more specific [48].

Ivermectin is the treatment of choice for strongyloidiasis [49]. A single dose of 200 μg/kg is adequate in uncomplicated cases. Relapses have been reported with all these drugs, especially in HTLV-1 coinfected patients, in whom a second round of therapy is recommended. In hyperinfection, patients should be treated with daily therapy for up to 1 week. Veterinary parenteral preparations can be used in severe cases. Thiabendazole, while also effective, has frequent side effects and is no longer manufactured in the USA. Comparative trials have demonstrated that albendazole is less effective than ivermectin, but albendazole may be used as an alternative if ivermectin and thiabendazole are not available [50,51]. Prevention of strongyloidiasis depends on improved standards of living and personal hygiene. Patients infected with HTLV-1 and those initiating corticosteroid therapy from endemic areas should be screened for *Strongyloides* infection.

Enterobius vermicularis (pinworm)

Enterobius vermicularis or pinworm is prevalent worldwide [52]. Humans acquire the infection by ingesting ova. The larvae escape the eggs, molt twice in the small intestines, and develop into adults in the colon. The adult female is approximately 1 cm long and lives in the large intestine. At night, the adult females crawl to the perianal area (Fig. 107.3), where they lay 10 000 eggs per night. The eggs adhere to perianal skin, causing pruritis. Transmission occurs via the hands of infected individuals.

Epidemiology

Children are at the highest risk of infection. Overcrowding, poor sanitary conditions, and household contact are factors associated with transmission. The infection is overdispersed, with the greatest burden of infection occurring in a minority of individuals [52].

Clinical manifestations

Anal pruritus is the main clinical manifestation, but dermatitis and bacterial superinfections can result from scratching

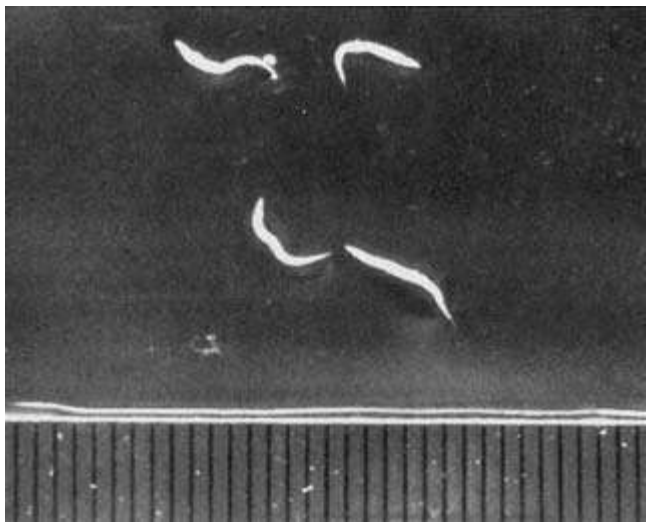

Figure 107.3 Adult female *Enterobius vermicularis* (pinworms) may be found on the perianal skin or occasionally on the surface of stools. Adult female pinworms are 8–13 mm long and 0.3–0.5 mm wide. (The scale is in millimeters.) From Smith JW, Ash LR, Thompson JH Jr, et al. Intestinal helminths. In: Atlas of Diagnostic Medical Parasitology Series. Chicago: American Society of Clinical Pathologists, 1984.

the area. Enuresis, insomnia, and rarely nausea and abdominal pain may occur. Appendicitis has been attributed to obstruction of the appendix by adult pinworms. Infection of the female genital tract is well recognized, causing vulvovaginitis in prepubertal girls; more infrequently cervical granulomas, salpingitis, and ovarian abscess have been reported. Peritonitis may result from intestinal perforation leading to ectopic localizations.

Diagnosis, treatment, and prevention

The recommended diagnostic approach is to apply adhesive transparent tape over the perianal skin area early in the morning and identifying ova microscopically (i.e., Scotch Tape test; see Fig. 107.1). The sensitivity is improved when several samples are taken. Direct stool examination is insensitive. Adult worms are occasionally identified on sigmoidoscopy or colonoscopy.

Albendazole, mebendazole, and pyrantel pamoate are the recommended antiparasitic drugs (see Table 107.1). Household members should also be treated. Reinfection is common. Hand hygiene and washing of bedclothes and underwear are recommended. Repeated treatment is often needed.

Trichinella

Trichinosis is a food-borne zoonosis of worldwide distribution. While animals are also infected, humans are particularly prone to clinical illness after eating improperly cooked meat infested with the larval stage of the parasite, mostly from pork, bear, horse and boar [53–56]. The larvae are released and mature in the small intestine over 2 weeks. Female adults deposit larvae, which invade the mucosa, travel through the bloodstream, and encyst in skeletal muscles [56]. The life span of an adult is several weeks, during which time a single female worm may produce approximately 1500 larvae.

Epidemiology

The disease is acquired only by ingestion of meat [55–57]. In Arctic North America, outbreaks are associated with the ingestion of bear meat. Molecular techniques have identified different groups: one, with five species and three genotypes, encapsulates in the host; the other, with three distinct species, does not encapsulate [55,57]. The worldwide incidence has declined, but outbreaks are still reported [53,54,58–60].

Clinical manifestations

The acute phase coincides with the intestinal and migratory phases of parasite development, with nausea, vomiting, abdominal pain, and diarrhea as the main complaints. Later, 1–3 weeks after exposure, fever, myalgia, facial or periorbital edema, and conjunctivitis are prominent symptoms [56,61–63]. Striated muscles are mainly affected during this phase, but cardiac and extraocular muscles are also infected. The central nervous system, kidney, and skin may also be infected.

Diagnosis, treatment, and prevention

The clinical picture, exposure history, and eosinophilia suggest the diagnosis. Several serological tests including ELISA are available, but antibodies may not be present until 3–5 weeks after infection [56,64]. Muscle biopsy is sometimes needed for diagnosis.

Albendazole or mebendazole is the drug of choice for the treatment of acute trichinosis [56,62]. Steroids are recommended in more severe forms of the disease. Trichinosis can be prevented by properly cooking pork or other potentially contaminated meat to at least 71°C (160°F). Meat less than 15 cm thick can be rendered safe if frozen at –23.3°C (–10°F) for 10 days.

Anisakiasis

Anisakiasis is an acute disease caused by fish ascarids (*Anisakis simplex*, *Anisakis physeteris*, *Pseudoterranova decipiens*) [65,66]. Humans are infected by eating undercooked marine fish containing the larval stage of the parasite, including cod, herring, rockfish, sardines, anchovies, salmon, tuna, mackerel, red snapper, and squid. The classical clinical presentation is with severe abdominal pain, nausea and vomiting developing minutes or hours after ingesting a contaminated fish. The diagnosis is usually confirmed by observing larva in the stomach by upper endoscopy [65–67]. Small intestinal involvement presents with localized invasion, edema, and even obstruction. Allergic manifestations may dominate the clinical picture. The diagnosis is confirmed by observing the worms at endoscopy or bowel resection. Larvae in the

stomach can be extracted with biopsy forceps. Anisakiasis is prevented by filleting freshly caught fish before larvae penetrate muscle, by cooking fish to 60°C (140°F) for at least 10 min, or by freezing for 24 h at –20°C (–4°F).

Visceral larva migrans and ocular toxocariasis

Toxocariasis is caused by two species of zoonotic ascarids of dogs and cats: *Toxocara canis* and *Toxocara catis* respectively [68,69]. The two main clinical syndromes are visceral larva migrans and ocular toxocariasis. Humans become infected by ingesting ova excreted by the definitive hosts into the environment. After ingestion of ova, the larvae penetrate the intestine and into tissues, but they are unable to enter the lung aveoli. Instead, the larvae continue to migrate through the host tissues until they eventually die. Children are most commonly affected. The disease is more frequently reported from developing countries with tropical and subtropical climates [68,69].

The clinical manifestations are characterized by fever, hepatomegaly, and eosinophilia. Other symptoms include abdominal pain, diffuse arthralgia, rash, and wheezing [68,69]. Pulmonary infiltrates are also observed. Neurological involvement is noted less frequently [70,71]. Ocular toxocariasis is characterized by unilateral involvement with retinal granulomatous lesions, causing visual defects, ocular pain and strabismus and, with chronic disease, retinal detachment [68,69,72,73]. The diagnosis is suspected on clinical grounds and confirmed by serological ELISA using parasite excretory/secretory antigens. The mainstay of treatment is the use of anti-inflammatory medications (e.g., prednisone 0.5–1.0 mg/kg). Antiparasitic drugs improve the response in visceral larva migrans, but should generally be avoided in ocular larva migrans. Albendazole is the treatment of choice. Thiabendazole and ivermectin are less effective. Ocular larva migrans requires a longer duration of therapy (4 weeks) and may require doses of albendazole of 800 mg twice a day. Control measures include deworming dogs and preventing defecation of dogs in areas where children play.

Baylisascariasis

The raccoon ascarid *Baylisascaris procyonis* can also cause a larva migrans syndrome with a predilection for the central nervous system [66,74]. Neurological involvement is characterized by fulminant eosinophilic meningoencephalitis, which is associated with very high mortality and significant neurological sequelae among survivors [75]. Visceral and ocular larva migrans have also been reported. Diagnosis is confirmed by measuring specific antibodies in serum and cerebrospinal fluid with ELISA. Thiabendazole or albendazole are recommended; ivermectin seems to be ineffective [75]. Steroids may be beneficial.

Cutaneous larva migrans

Cutaneous larva migrans or creeping eruption is a zoonosis caused by hookworms of dogs (*Ancylostoma caninum*) or cats (*Ancylostoma braziliense*). Like human hookworms, the filariform larvae penetrate the skin and cause a dermatitis characterized by serpiginous, papulovesicular, erythematous, and pruritic lesions as they migrate through the skin and subcutaneous tissues. Cutaneous larva migrans is prevalent in tropical and subtropical areas of the Caribbean, Africa, Latin America, the southern coasts of North America, and Southeast Asia. Transmission in developing countries occurs in both urban and rural settings [76]. The disease is also becoming more frequently recognized among travelers [77]. The diagnosis is made on clinical grounds. A single dose of ivermectin, or albendazole, is the drug of choice (see Table 107.1).

Ancylostoma caninum and eosinophilic enteritis

Ancylostoma caninum, the dog hookworm, has been identified as a cause of eosinophilic enteritis, generally a rare condition but quite common in northeastern Australia [67,78]. The findings include eosinophilic infiltration of the intestine, aphthous ulceration of the ileum, regional lymphadenopathy, and peripheral blood eosinophilia. Infection may be asymptomatic or cause abdominal pain. The diagnosis is made by finding the immature adult worm at endoscopy or by serological evidence of *A. caninum* infection. Albendazole or mebendazole are likely to be effective therapies.

Gastrointestinal angiostrongyliasis and other strongylids

Angiostrongylus species are strongylid nematodes whose normal hosts are rodents [79]. *Angiostrongylus cantonensis* was formerly limited to Southeast Asia and the Pacific, but has now been introduced into Jamaica and the US Gulf Coast, causing eosinophilic meningitis. *Angiostrongylus costaricensis* is endemic in rodents from California to South America. Adult worms reside in mesenteric arteries of rodents. Humans and rodents acquire infection by ingesting infected intermediate hosts (slugs, or their slime) on vegetation. Sporadic human disease is initially described among children and outbreaks in adults in Central America [79,80]. Recent studies from Brazil suggest that there may be widespread infection and underdiagnosis [81,82].

The parasites localize to the ileocecal region causing a granulomatous arteritis with eosinophilic infiltration in the ileocecal region that can result in thrombosis, local infarction, and bowel perforation [79]. The clinical manifestations vary from a visceral larva migrans-like syndrome to an acute abdomen, with nausea, vomiting, right lower quadrant pain, and fever. A right lower quadrant mass may be documented by palpation or visualized on radiographic studies of the ileocecal region. Eosinophilia and leukocytosis are common. Neither ova nor larvae are found in the stools of humans. Surgery is often necessary, and the diagnosis is often only made when adult worms or ova are identified in surgical specimens. Ivermectin, thiabendazole, and diethylcarbamazine are active against the worms in rodents, but there are

no data on their efficacy in people. Cooking slug-infested vegetables and hand washing can prevent infection.

Other strongylid parasites (*Trichostrongylus*, *Mammomonogamus*, *Oesophagostomum*, *Ternidens*) rarely cause human infection, but present with abdominal pain and eosinophilia [66].

Capillariasis

Capillariasis is an emergent zoonotic infection caused by three species of *Trichuris*-like organisms: *Paracapillaria philippinensis*, *Capillaria hepatica* and *Capillaria aerophilia* [66,67]. Humans are infected by eating uncooked freshwater or brackish fish containing infectious larvae; fish-eating birds are the definitive hosts. The parasite reproduces in humans, potentially achieving very high loads. The disease in endemic in the Philippines, Japan, Taiwan, India, Iran, and Egypt [66,67,83].

The clinical features are watery diarrhea, abdominal pain, malabsorption, and weight loss. Massive infestation may cause jejunal stricture and intestinal pseudoobstruction. The diagnosis is made by identifying the distinctive features of ova. Albendazole is the drug of choice, but mebendazole is an alternative. Proper cooking of fish prevents infection.

Trematodes (flukes)

Trematodes, or flukes, are flatworms (phylum Platyhelminthes, subphylum Trematoda) and require a freshwater snail as an intermediate host. Some use a second intermediate host. The blood flukes (genus *Schistosoma*) are among the most common human helminths. *Schistosoma mansoni* and *Schistosoma japonicum*, and the less common *Schistosoma intercalatum* and *Schistosoma mekongi*, live in mesenteric venules and cause intestinal and liver pathology. *Schistosoma haematobium* primarily affects the urinary tract. *Schistosoma* species enter through intact skin when humans come in contact with fresh water. Other pathogenic trematodes are foodborne, acquired by eating the second intermediate host (e.g., freshwater fish/crustaceans) or aquatic plants (*Fasciola* and *Fasciolopsis buski*). The liver/biliary flukes include *Clonorchis sinensis*, *Opisthorchis viverrini*, *Fasciola hepatica*, and *Metorchis conjunctus*. The intestinal flukes include *Fasciolopsis buski*, *Heterophyes heterophyes*, and *Metagonimus yokogawai*. The organisms of the genus *Paragonimus* develop primarily in the lung.

Schistosoma species

Human schistosomiasis results from infection with one of five species of blood flukes of the genus *Schistosoma*, and is a major global public health problem. Each species of *Schistosoma* infects a specific freshwater snail [84]. The cercarial forms are released from the snail into fresh water, contact potential hosts, and enter human skin when contact is made with fresh water during bathing, fishing, or other water-related activities. The cercariae then shed their tail and the resulting schistosomulae invade the vasculature and migrate to the portal circulation, or in the case of *S. haematobium* the venous drainage of the urinary tract. *Schistosoma mansoni* migrates to the superior mesenteric veins and *S. japonicum* to the inferior mesenteric veins. The adult worms live as pairs within mesenteric venules, with the adult female surrounded by the male canal. Adult worms can live as long as 30 years in humans. Beginning 2–8 weeks after infection, the females begin to shed ova (approximately 300 ova daily for *S. mansoni* and up to 3500 daily for *S. japonicum*). The ova induce a granulomatous response, which facilitate migration through the intestinal or bladder wall allowing the ova to be excreted in stool or urine. Miracidia are released when ova hatch in fresh water and complete the life cycle by penetrating the surface of the appropriate snail host.

Epidemiology

An estimated 200 million people worldwide are infected with schistosomiasis [85–88]. *Schistosoma mansoni* is endemic in Africa, parts of Latin America, and scattered foci in the Middle East. Transmission is associated with dams, reservoirs, and irrigation systems. *Schistosoma intercalatum* is found in areas of western and central Africa. *Schistosoma japonicum* is endemic in the Far East, China, coastal river valleys in Japan, numerous islands in the Philippines, and Indochina, where it has a number of nonhuman hosts, including cows, dogs, and pigs. *Schistosoma mekongi* is found along the Mekong River in Indochina. In most areas, *Schistosoma* infection usually occurs in childhood and prevalence peaks between ages 10 and 20 years [89]. Updated information on the prevalence and epidemiology of schistosomiasis is available at http://www.who.int/ctd/schisto/epidemio.htm.

Clinical manifestations

Cercarial invasion is usually asymptomatic in local populations, but can cause a mild, pruritic, papular dermatitis termed cercarial dermatitis (swimmers itch) at the site of penetration [86,88,90]. The rash may last up to a few weeks. Acute schistosomiasis, or Katayama fever, was first described among people with *S. japonicum* infection, but can occur with other *Schistosoma* species [86,88–91]. The time from infection to onset of systemic symptoms ranges from 2 to 12 weeks and coincides with the initial production of ova. Katayama fever, a serum sickness-like syndrome thought to result from a hypersensitivity reaction to egg antigens, is characterized by fever, malaise, urticaria, abdominal discomfort, diarrhea, weight loss, cough, wheezing, mild hepatosplenomegaly, lymphadenopathy, and eosinophilia [88,91,92]. Some patients present with pulmonary infiltrates, which may reflect eosinophilic pneumonia, genital involvement (e.g., hematospermia), or spinal cord disease [93–95]. Symptoms can persist for several months.

Chronic schistosomiasis results from the granulomatous inflammatory response to the schistosome ova. For species

other than *S. haematobium*, eggs are deposited in mesenteric vessels, carried to the liver or intestines through the portal circulation, or reach the lungs or other sites by way of the systemic circulation. The pathology varies with the worm burden and the host immune response to the ova. A complex mix of Th1 and Th2 cytokines leads to varying degrees of inflammation and fibrosis [96].

Inflammation, hypertrophy, and ulceration of the mucosa characterize intestinal disease caused by *Schistosoma* [86,89,97]. Intestinal involvement is often asymptomatic, but infected people may experience abdominal pain, diarrhea, and occult or gross blood in their stools. Intestinal polyps, ulceration, and strictures may be encountered late in disease. There is also increasing evidence that chronic disease even with few symptoms is associated with malnutrition [4].

Ova that reach the liver elicit a granulomatous inflammatory response. Important components of the granulomas include eosinophils, lymphocytes, macrophages, and granulocytes. Advanced liver disease may also include fibroblasts, collagen, and giant cells [86,88,89]. A subset of individuals progress to severe "pipe-stem" portal fibrosis with presinusoidal portal hypertension with relatively preserved hepatic function. Hepatomegaly and splenomegaly are common in heavily infected persons. The parenchyma and liver function are preserved until late in the disease, but patients coinfected with hepatitis viruses, especially hepatitis C, may develop frank cirrhosis. Bleeding (from esophageal and rectal varices or intestinal granulomatous lesions) and ascites are complications of advanced hepatosplenic schistosomiasis.

Portosystemic shunting of ova to the pulmonary vasculature can cause granulomas and fibrosis may progress to chronic obliterative arteritis, pulmonary hypertension, and eventually cor pulmonale. Granulomatous responses to eggs are occasionally encountered in other organs, including the gallbladder (causing cholecystitis and cholelithiasis) and genital tract (causing cervicitis) [98]. Schistosomiasis also has been associated with prolonged or relapsing *Salmonella* bacteremia [99].

Schistosoma haematobium resides primarily in venules of the vesical plexus, but on occasion it too involves the gastrointestinal tract in association with acute appendicitis or asymptomatic appendiceal calcification [88,100].

Diagnosis, treatment, and prevention

The diagnosis of schistosomiasis is usually made by identifying ova in feces, urine or biopsy material (see Figs 107.1 and 107.2) [86,88]. Katz think smears are more sensitive than direct stool examination. Diffuse smears or patchy mucosal hyperemia, friability, and occasionally schistosomal polyps may be observed at colonoscopy [101]. Rectal biopsy may be necessary to document low-level infections. Histological examination may reveal ova even in the absence of macroscopic abnormalities. Ultrasonography has been widely used to assess hepatosplenic involvement and responses to therapy [102]. With hepatic disease, ultrasound may reveal dilation of the portal vessels and characteristic periportal fibrosis (so called "clay-pipe" or Symmer fibrosis), which correlates with parasite burden and hepatic pathology. Serological assays are available and provide suggestive evidence of schistosomiasis. In acute infection, antibody can often be detected prior to excretion of ova. The main serological test used in the USA is a FAST-ELISA [103]. The sensitivity is poor for *S. japonicum* and species-specific immunoblot assays may be more sensitive and specific. Antibodies may persist and cannot distinguish active from prior infection. Antigen detection assays can also prove helpful [104].

Praziquantel 40 mg/kg in one dose is recommended for the treatment of all *Schistosoma* species, except *S. japonicum* and *S. mekongi*, which should be treated with two doses of 30 mg/kg (see Table 107.1) [49,88]. Praziquantel is effective in killing the parasite and can decrease periportal fibrosis, hepatomegaly, and splenomegaly, but treatment does not reverse all the effects of chronic schistosomiasis. The response rate is lower in early infection and some cases may require a second dose. Second-line drugs include oxamniquine (*S. mansoni*) and metrifonate (*S. haematobium*). Endoscopic sclerotherapy or surgical decompression have been used successfully to treat bleeding esophageal varices due to portal hypertension.

Control measures in endemic areas include improved sanitation and disposal of human feces, avoidance of contaminated fresh water, use of molluscicides, and mass treatment programs. Chlorination of swimming pools and letting water stand for 24 h prior to bathing can prevent infection in travelers. Recent studies suggest that mass chemotherapy, particularly when directed at schoolchildren may decrease prevalence in many endemic areas, but this approach may not work as well in areas of zoonotic disease [4,105].

Liver flukes

Clonorchis and *Opisthorchis*

Clonorchis sinensis and *Opisthorchis viverrini* are found in Japan, China, Indochina, South Korea, and Taiwan [106–109]. *Opisthorchis felineus* and *Opisthorchis guayaquilensis* are found in Russia/Central Asia, and South America respectively. *Metorchis conjunctus* is found in North America. Humans become infected when they eat raw or incompletely cooked fish containing the infectious metacercariae. The released larvae mature into adults and migrate through the sphincter of Oddi to enter the biliary tract. Adult worms reside in small and middle-sized intrahepatic bile ducts, although they can also be found in extrahepatic ducts, the gallbladder and pancreatic ducts. Flukes may have prolonged life spans of up to 30 years. Eggs are released into the bile and passed in the stool. The eggs hatch in fresh water and are ingested by the snail intermediate host. Cercarciae emerge from the snail and infect freshwater fish, the second intermediate host, and transform into the metacercarcia form in the fish musculature.

Acute infection may present with fever, abdominal pain, and eosinophilia, but is not typically noted with Asian infections [108]. Slight liver enlargement and tenderness may be observed at the onset of infection. The major pathological changes of chronic infection manifest in the bile ducts: desquamation of the biliary epithelium, followed by hyperplasia, adenomatous proliferation, and later fibrosis and stricture of the bile ducts. The clinical manifestions of chronic infections are variable, ranging from asymptomatic to dyspepsia, abdominal pain, anorexia, hepatomegaly, icterus, edema, and diarrhea. Cholangitis, cholelithiasis, chronic cholecystitis, and pancreatitis have been noted. Cholangiocarcinoma is a potential long-term complication and is more common with *O. viverrini*.

The diagnosis of biliary flukes is based primarily on the identification of ova in the stool or bile (see Fig. 107.1). Ultrasonographic examination may reveal liver enlargement; dilation, sludge, or thickening of the wall of the gallbladder; dilation of the intrahepatic bile ducts; or evidence of cholangiocarcinoma [110,111]. Endoscopic retrograde cholangiopancreatography (ERCP) may show small irregular filling defects in the common bile duct, tortuosity and irregular dilation of the intrahepatic ducts, or blunting of the terminal branches of the biliary tree [109]. Praziquantel is the drug of choice for the treatment of *C. sinensis* and *O. viverrini*, but triclabendazole may also be effective [106,109,112].

Fasciola hepatica

Fasciola hepatica is found in most sheep- and cattle-raising areas of the world [108,113]. Snails are the intermediate host. Humans become infected when they eat wild watercress, alfalfa, or other aquatic plants contaminated with encysted metacercariae. Larvae excyst in the small intestine, penetrate the gut wall, migrate through the peritoneal cavity to the liver, and enter the liver through the capsule. They burrow through the human liver for periods of 6–9 weeks before finally penetrating into the bile ducts, where they spend their adult lives. Acute infection is characterized by fever, hepatomegaly, abdominal pain, weight loss, anemia, and eosinophilia [108,114–116]. Jaundice, biliary colic, and cholangitis are characteristics of chronic disease. Diagnosis of acute infection rests on a typical clinical scenario, supported by characteristic radiological findings, and demonstration of specific antibody. Multiple low-density lesions are observed on CT. Lesions are subcapsular, arrayed in tracts, and slowly evolve [114,116]. Serological tests for antibodies to either excretory/secretory antigens or recombinant proteins can confirm the diagnosis of acute infection [108,117]. In chronic infection, ova or adult worms may be identified in bile, duodenal aspirates, or stool. Therapeutic failures are common in patients with *F. hepatica* treated with praziquantel, and triclabendazole is now the drug of choice for fasciolasis (see Table 107.1) [112,115,118]. Nitazoxanide may also work in chronic infection [119]. Cure can also be achieved by removing the adult worms by ERCP [120].

Intestinal flukes

The intestinal fluke *Fasciolopsis buski* has as its definitive hosts humans, hogs, and occasionally dogs and a snail intermediate host. It is endemic in Asia [108,112]. The adult flukes attach to the mucosa of the upper small intestine and release ova into the feces. Cercariae encyst on water plants such as the water caltrop, water hyacinth, water chestnut, or water bamboo, which are ingested raw by humans. Inflammation, ulceration, and mucosal abscesses may develop in the small intestine. Epigastric pain, nausea, and diarrhea of varying severity are reported, but many people are asymptomatic. The diagnosis is made by identifying ova in the stool. Praziquantel is recommended for treatment.

Many species of the trematode family Heterophyidae (including *Heterophyes heterophyes* and *Metagonimus yokogawai*) and Echinostomatidae (including *Echinostoma* species) are parasites of humans and other fish-eating mammals [108]. The first intermediate hosts are snails, and the second intermediate hosts freshwater or brackish-water fish. Humans become infected from eating raw or inadequately cooked fish. *Heterophyes heterophyes* is encountered in the Middle East and Asia. Colicky pain, abdominal tenderness, and diarrhea may be noted by those with heavy infections. Eosinophilia may be present. *Metagonimus yokogawai* is found primarily in the Far East, where it is associated with the formation of egg-related granuloma at distant foci, such as the heart or central nervous system. *Echinostoma* species are found in Southeast Asia, where they cause inflammation in the intestines, with abdominal pain, diarrhea, and eosinophilia. Intestinal fluke infections are typically diagnosed by finding ova in the feces. Praziquantel is the treatment of choice. Infection can be prevented by eating only thoroughly cooked fish.

Cestodes

Cestodes (phylum Platyhelminthes, subphylum Cestoda) are tapeworms with adult stages and larval stages (e.g., cysticercosis or hydatid disease) [121]. The adult tapeworms are segmented worms that contain a scolex (attachment organ), a neck region where the segments are formed, and segments termed proglottids. Chains of proglottids comprise the body of the adult worm. The external proglottid surface functions as the parasite gut. As the proglottids mature, they develop both male and female sexual organs. The terminal proglottids contain the ova, which shed into the environment.

Taenia saginata (beef tapeworm)

Taenia saginata is a common human tapeworm endemic in most of the world. When people ingest raw or poorly cooked beef containing viable cysticerci, the 1–2 mm scolex, which has four muscular suckers but no hooks, evaginates and attaches to the intestine. The chain of proglottids forms from the scolex and reaches 4–25 m in length. The proglottids

gradually enlarge; mature gravid proglottids are usually 16–20 mm in length and 5–7 mm in width. The uterine stem has 12–30 main lateral branches on each side. When bovines ingest the ova, embryos hatch and invade the intestinal mucosa, migrate through the bloodstream, and develop into larval cysticerci in the muscles. This cystic ovoid structure measures 7–10 mm by 4–6 mm and contains an invaginated scolex. Ova are shed in feces or, more commonly, as gravid proglottids actively crawl out of the anus. The ova of *Taenia solium* and *T. saginata* are morphologically indistinguishable.

Epidemiology

Worldwide, 45–60 million people are thought to be infected with *T. saginata*. Contamination of pastures with human feces or untreated sewage leads to bovine cysticercosis. Infection is most important in tropical and subtropical cattle-raising areas of Africa, the Middle East, Europe, Asia, and South America [122,123]. The prevalence of human infection is less than 1% of stool examinations in most areas, but recent studies have identified prevalence rates of over 20% in East Africa, Bali, and Tibet [123]. The incidence of taeniasis in the USA is very low but transmission does occur.

Clinical manifestations

Taenia saginata usually causes few symptoms [123]. Common symptoms include sensing gravid proglottids migrating from the anus, abdominal pain, nausea, weakness, and loss of weight [122,123]. Rarely, intestinal obstruction or symptoms related to ectopic localization of proglottids have been seen. Some patients have significant eosinophilia, but most do not.

Diagnosis

Diagnosis is made by identifcation of the ova, scolex, or proglottids. Multiple stools are required to detect ova, since they are infrequently found on a single fecal examination, even when enhanced by concentration methods. Cellulose tape swabs applied to the perianal skin, as used for the diagnosis of *Enterobius* infection, is more sensitive than fecal concentration [124]. The eggs of *T. saginata* and *T. solium* cannot be differentiated morphologically. The scolex of *T. saginata* is differentiated easily from *T. solium* by the absence of hooks. However, the scolex is usually not recovered, even after successful treatment. Polyethylene glycol salt purgatives improve recovery of the scolex [125]. Examination of gravid proglottids is the main method for determining species, by counting the main branches of the uterine stem: counts of 13 or fewer branches on one side of the stem are considered diagnostic for *T. solium*; counts of 14 or greater are diagnostic for *T. saginata*.

Antigen detection tests are sensitive for taeniasis, but are not widely available and cannot distinguish the species [126]. Polymerase chain reaction (PCR) has been useful in diagnosing *T. saginata* in field situations [123]. Occasionally, tapeworms are identified as a ribbon-like defect in the barium column during contrast studies of the gastrointestinal tract.

Treatment and prevention

Praziquantel is the primary medication used to treat *T. saginata*. Niclosamide is also effective, but currently is not widely available. Nitazoxanide can also be used to treat taeniasis [127]. *Taenia saginata* infection can be prevented by avoiding raw or undercooked beef. Meat inspection is effective in detecting all but light infections. Cooking beef to a temperature of 56°C (133°F) kills cysticerci. Vaccines to prevent bovine *T. saginata* cysticercosis are feasible, but development has been slowed by limited commercial interest [128].

Taenia asiatica (Asian pork tapeworm)

Taenia asiatica is endemic in East and Southeast Asia. The proglottids are morphologically similar to those of *T. saginata*, such that it was only recognized as a separate species in 1993 [123,129]. Unlike *T. saginata*, the scolex contains a rostellum (like *T. solium*) but no hooks (like *T. saginata*). *Taenia asiatica* cysticerci are mainly found in the liver of pigs [129]. Humans become infected when they ingest cysticerci, which develop into the intestinal adult form.

Epidemiology

Asian taeniasis has been identified in Taiwan, Korea, China, the Philippines, Vietnam, Thailand, Indonesia, and Malaysia [123]. The prevalence of infection varies among regions, e.g., zero to 21% of people in various parts of Indonesia are infected.

Clinical manifestations, diagnosis, treatment, and prognosis

The clinical manifestations, diagnosis, and treatment of *T. asiatica* appear similar or identical to those of *T. saginata*. Human *T. asiatica* infection can be prevented by avoiding ingestion of undercooked liver and other viscera.

Taenia solium (pork tapeworm and cysticercosis)

Taenia solium infection has two distinct forms in the human host: taeniasis (tapeworm infection) and cysticercosis (tissues infected with the larval cysticercus) [130,131]. Neurocysticercosis refers to cysticercosis involving the central nervous system and is now recognized as a major cause of neurological disease worldwide. Humans develop the tapeworm form after ingesting undercooked pork containing cysticerci. The scolex evaginates, attaches to the intestinal wall, and develops chains of proglottids. The tapeworms are typically several meters long. Eggs or gravid proglottids are shed intermittently into stool. Pigs acquire the infection by ingesting eggs or proglottids from human fecal material. Once ingested, the larvae emerge from the eggs, penetrate the wall of the gut, enter the bloodstream, and migrate to many tissues, where they develop into cysticerci. Taeniasis is acquired by ingest-

ing undercooked pork and is closely associated with pig-raising areas in endemic countries. Cysticercosis is acquired from human tapeworm carriers, who often harbor eggs on their hands and fingernails. The eggs can autoinfect the tapeworm carrier or infect other people.

Epidemiology

Between 50 and 100 million people are infected with cysticercosis, but far fewer with intestinal taeniasis. Nearly all areas where pigs are raised with access to human fecal material are endemic for cysticercosis [132]. Currently, porcine cysticercosis remains highly endemic in Latin America, sub-Saharan Africa, South and Southeast Asia, as well as parts of Korea, China, Indonesia, and Papua New Guinea. In the USA, porcine cysticercosis is very rare, but human neurocysticercosis is widespread among immigrants from endemic countries [133,134].

Clinical manifestations

Taeniasis typically causes few symptoms. The most specific symptom is noting proglottids passed in the stool. The proglottids appeared flattened (typically 0.3–0.5 cm wide by 1–2 cm long and 1 mm thick). Each segment has 13 or fewer uterine branches. Unlike *T. saginata*, the proglottids are usually single and are not often noted to be motile.

Cysticercosis can present with a wide range of symptoms and signs depending on the location of the parasites and the host inflammatory response. Even neurocysticercosis presents with a range of syndromes that differ in pathogenesis, clinical manifestations, prognosis, and management [131,135–138]. The most common presentations of neurocysticercosis are seizures and hydrocephalus.

Diagnosis

Taeniasis has traditionally been diagnosed by examining stool for proglottids or ova. The sensitivity of stool microscopy for ova is only 26% [139]. Species identification requires collection of the proglottids, which can be facilitated by treating the patient with polyethylene glycol [125]. The proglottids of the two species can be distinguished by counting the number of uterine branches, which are lower for *T. solium*.

Antigen detection assays are more sensitive and specific but are not widely available and cannot distinguish *Taenia* species [123,139]. ELISAs to identify serum antibodies to *T. solium* tapeworm-stage antigens may be more sensitive than stool-based tests and seem to be quite specific for the *T. solium* tapeworm stage [140]. However, the antibody may persist after treatment. PCR assays can also be used to improve sensitivity and specificity [141].

Diagnosis of neurocysticercosis requires neuroimaging studies and serological confirmation [142]. The preferred serodiagnostic test is the enzyme-linked immunotransfer blot (EITB) employing semipurified parasite glycoproteins [131,142,143].

Treatment

Experts agree that neurocysticercosis represents a spectrum of disease that differs in optimal management. Symptomatic therapy with antiepileptic drugs for seizures and surgery for hydrocephalus are the mainstay of therapy. There is an emerging consensus among experts about the proper role of antiparasitic drugs and corticosteroids in the management of neurocysticercosis [136–138,144]. Patients with parenchymal cysticerci should usually be treated with a course of albendazole plus a simultaneous course of corticosteroids [137]. If patients have a history of seizures and calcified lesions, they should generally be treated with antiepileptic therapy indefinitely. Elective endoscopic removal of the cysticercus is the treatment of choice for ventricular neurocysticercosis, but some patients require emergency diversion of cerebrospinal fluid via a ventriculostomy or placement of a ventriculoperitoneal shunt.

Prevention

Taeniasis can be prevented by adequate cooking or freezing of infected pork, but meat inspection does not work in areas where pigs are slaughtered informally. Mass chemotherapy of endemic populations for human tapeworm carriage or porcine cysticercosis has resulted in significant short-term decreases in prevalence [145], although prevalence rates rapidly increase unless there is continued mass treatment. Development of an effective vaccine against cysticercosis may be the best hope for eradication of the disease. There are promising studies that this will be possible for human or porcine cysticercosis [146].

Diphyllobothrium species (fish tapeworm)

Diphyllobothrium species typically have fish-eating birds, marine mammals, bears, dogs, and foxes as definitive hosts, but people can also be infected when they eat raw or undercooked fish [122,123]. *Diphyllobothrium latum* infects approximately 9 million individuals worldwide, mostly in Europe, South America and Asia [147]. *Diphyllobothrium pacificum* infects humans in Japan and South America, and *Diphyllobothrium nihonkaiense* has been found in Pacific salmon in Japan and Europe [123].

The scolex of *D. latum* possesses has two deep grooves that allow it to attach to the intestines. The worm may reach a length of 15 m. The gravid proglottids have a rosette-shaped central uterus. The ova are shed into the fecal stream, develop in fresh water, hatch, and are ingested by copepods. Fish ingest the infected copepod, and the larval stages develop into the plerocercoid stage in the muscles of fish. Smaller fish are eaten by larger species, and people are infected from ingesting undercooked pike, perch, turbot, lake trout, whitefish, and salmon. The prevalence of infection increases with age in endemic areas [123]. Higher endemicity is associated with eating freshwater fish raw, lightly salted, or pickled without cooking.

Clinical manifestations

Most infections are asymptomatic or patients may notice proglottids in their stool. Nonspecific gastrointestinal complaints and megaloblastic anemia may occur in infected individuals [122,123]. Megaloblastic anemia caused by *D. latum* infection is seen mainly in patients older than 50 years in Finland. Comparison of nonanemic infected patients with uninfected controls revealed an increase in the symptoms of fatigue, weakness, craving for salt, lack of well-being, dizziness, and numbness of the extremities in the infected group. Significant gastrointestinal symptoms occur infrequently. Episodes of intestinal obstruction have been associated with the vomiting of masses of tapeworms. Eosinophilia is rare.

Diagnosis, treatment, and prevention

Identification of the characteristic ova (oval in shape, operculated, about 60 µm across) or proglottids provides the diagnosis. The parasite produces nearly 1 million eggs per day, and ova are usually detected easily by fecal examination. Treatment with praziquantel is highly effective. An alternative medical treatment would be niclosamide. Tapeworm-related anemia is reversible by treating the infection, but the affected child should be supplemented initially with vitamin B-12. A significant reduction in the transmission of *D. latum* followed introduction of sewage treatment and targeted drug treatment of human infections. Raw and undercooked fish should not be consumed in areas where there is a possibility of larval infection. Larvae do not survive in fish that is either cooked for 5 min or more at no less than 55°C (131°F) or frozen for at least –10°C (14°F) for 8–72 h [147].

Dipylidium caninum (dog tapeworm)

Dipylidium caninum, the common tapeworm of dogs and cats, also infects infants and young children [122,123]. Transmission occurs through the accidental ingestion of infected fleas (*Ctenocephalides* species) or body lice (*Trichodectes* species). While usually asymptomatic, infection may be associated with decreased appetite, abdominal discomfort, or perhaps diarrhea. The gravid proglottids are excreted in feces or migrate actively from the anus. The eggs are liberated as the proglottid disintegrates. Several species of flea serve as intermediate hosts. Development of the tapeworm's larval stages takes place as the larval flea metamorphoses into an adult. When the flea is ingested by the definitive host, the adult worm develops in the small intestine. Infection of dogs and cats is common throughout the world. *Dipylidium caninum* has also been found in a variety of wild canines and felids. However, reports of dipylidiasis in humans in the USA are uncommon [148,149].

Diagnosis, treatment, and prevention

Frequently, in infants, the only evidence of infection is the finding of proglottids in stool or in an infant's diapers. Abdominal pain, diarrhea, irritability, and pruritus have been recorded in symptomatic children. The small motile proglottids that migrate from the anus can be confused with pinworms. Examination of stool may be negative since proglottids may disintegrate on the walls of the specimen container. Treatment with praziquantel is effective. An alternative treatment is niclosamide. Infected dogs and cats should also be treated. Flea control requires the use of appropriate insecticides on pets. In addition, particular attention must be given to carpets and areas where pets sleep because these are the sites of development of the larval fleas.

Hymenolepis nana (dwarf tapeworm)

Hymenolepis nana, the dwarf tapeworm, is common throughout the world and is maintained by direct fecal–oral transmission from person to person [122,123]. Adult worms measure 1–5 cm in length and are less than 1 mm in width. Gravid proglottids disintegrate within the intestine, and ova are found in the feces. The oval-shaped eggs measure 30–50 µm in diameter and contain a six-hooked embryo within two envelopes. Larval stages develop in insect intermediate hosts after ingestion of the eggs. If people ingest the ova, larval stages develop in the intestinal villi in 4–5 days and then break into the lumen and develop into adult worms. Ova appear in feces 2–4 weeks after infection. An identical organism found in rodents may be a separate species. The rodent strains are alternatively called *Hymenolepsis fraterna* or *Hymenolepsis nana* var. *fraterna* [123,150]. The rodent strain occasionally infects humans. Human infection is acquired by the ingestion of eggs shed by a tapeworm carrier. Humans serve as both intermediate and definitive hosts in this direct cycle. Poor hygienic habits, overcrowding, lack of running water, and any other factor that fosters fecal–oral transmission enhance transmission via this route.

Epidemiology

Hymenolepsis nana is a common parasite of rats, mice, and people worldwide, with 50–75 million people infected [122,123]. Prevalence is higher in some urban areas. Stool surveys from endemic areas commonly show prevalence rates of 5%–25%, which may approach 50% in children aged 4–10 [82].

Clinical manifestations, diagnosis, treatment, and prevention

Most infections are asymptomatic. Heavy worm burdens have been associated with abdominal pain, diarrhea, malnutrition, growth retardation, and lethargy [122,123]. Detecting the characteristic eggs in fecal material by examining direct saline wet mounts or by concentration techniques is not usually difficult. However, a single examination is not always adequate to rule out infection. Praziquantel is effective in treating *H. nana* infection. Nitazoxanide is a reasonable alternative therapy; efficacy is about 75%–82% [151,152]. Family mem-

bers should be examined and treated if infected. *Hymenolepsis nana* infection is rarely severe and responds well to appropriate treatment.

Transmission of *H. nana* infection could be largely eliminated if proper methods of personal hygiene and disposal of human waste could be invoked throughout the world. Infected food handlers should always be treated and monitored to ensure that treatment has been successful. Stored food should be protected from rodents and insects.

Echinococcus granulosus and related species (cystic hydatid disease)

Human cystic hydatid disease results from infection with the larval stages of parasites of the genus *Echinococcus* [153–158]. The disease in humans and the intermediate hosts is called hydatid disease and is classified based on morphology of the larval stages: cystic echinococcosis is caused by *Echinococcus granulosus* and related organisms, alveolar echinococcosis by *Echinococcus multilocularis*, and polycystic echinococcosis by *Echinococcus vogeli* or *Echinococcus oligarthrus*.

Based on morphology, all cases of cystic hydatid disease were thought to be caused by a single species. However, genetic studies have demonstrated 10 separate genotypes that infect different intermediate hosts [154,155,157]. Most human infections are caused by the sheep strain; other strains can also cause human infection. The life cycles of the species of *Echinococcus* are similar, but the geographic distribution, types of hosts, and morphology of the parasites differ significantly. The adult tapeworms of the sheep strain develop in dogs, which may host thousands of tapeworms. The adult tapeworm is 3–8 mm long and has two to five segments. When sheep or humans ingest ova from infected dogs, the embryos hatch in the intestine, penetrate the intestinal wall, and gain access to the portal circulation. The embryos most commonly develop in the liver or lungs, where they become the cystic larval structures called hydatid cysts. A hydatid cyst is composed of an outer acellular membrane that is lined by a thin cellular germinal membrane. Structures called brood capsules grow from the germinal membrane and form protoscolices. Each protoscolex has the potential to become the scolex of an adult worm if eaten by a dog or to form a daughter cyst if the cyst ruptures into tissues. Host tissues produce a pericyst of compact collagen-rich host tissue around its exterior. In older cysts, daughter cysts may develop within the primary cyst cavity.

Epidemiology

Echinococcus granulosus sheep strain is found in most areas where sheep are raised [154,155,157–159]. In the past, the impact was significantly underestimated compared with other illnesses [160]. One of the highest morbidity rates is seen in rural Africans in Kenya and Uganda, where people live in close association with their dogs. The custom of feeding sheep viscera to sheep dogs maintains foci of infection.

Clinical manifestations

Most infected with *E. granulosus* have a single unilocular cyst, but multiple cysts are seen in 15%–30% of patients [154,157,161]. The most common site for cysts is the liver. In clinical series, approximately one in five cases have a pulmonary cyst often with a concurrent liver cyst. The proportion outside the liver is lower in population-based screening [162]. Approximately 10% of cysts are found in sites other than the liver or lung, including spleen, kidney, peritoneum, genitourinary tract, bone, muscles, heart, eye, and brain. Liver cysts grow approximately 1–3 cm in diameter each year, but pulmonary cysts grow more rapidly [162].

There is a wide spectrum of symptoms in cystic hydatid disease depending on the number, size, and location of the cysts [154,157,161]. Cysts in liver or lung are often asymptomatic for years. Symptomatic intrahepatic cysts cause right upper quadrant or epigastric pain, hepatomegaly, nausea, and vomiting. Cyst rupture may be precipitated by a traumatic event and may cause a range of symptoms, including fever, abdominal pain, hypotension, and allergic manifestations, including eosinophilia, urticaria, and anaphylaxis. Rupture into the biliary tract may cause cholangitis.

Intact pulmonary cysts are typically asymptomatic [154,157,161]. Leakage may cause cough, chest pain, dyspnea, and hemoptysis. Fever may be present especially with a secondary bacterial infection. When pulmonary cysts rupture into a bronchus, the patient may describe coughing up portions of the membranes, which may resemble grape skins, or noting a salty taste.

Diagnosis

The diagnosis of cystic echinococcosis usually is suspected on the basis of clinical or radiological findings plus a history of residence in an endemic area. About half of patients with hepatic cysts have abnormal liver enzyme results. Eosinophilia is typically low grade or absent. The initial diagnosis of cystic hydatid disease is based on imaging findings. An unruptured pulmonary hydatid cyst has a sharply demarcated, round or oval border but may be surrounded by atelectasis [154,157,161]. After the cyst has ruptured into a bronchus, a crescent-shaped air layer may be seen that is virtually diagnostic. In addition to the arc of air between the parasite and the host cyst wall, air in the cyst lumen also may be present.

Cysts in the liver are usually easily visualized and measured with ultrasound, CT, or magnetic resonance imaging (MRI). A World Health Organization (WHO) staging system has been developed that has been used to define management (Fig. 107.4) [163]. Stage CL cysts are unilocular collections without a discrete wall and with uniform anechoic contents, but this is not specific for *Echinococcus*. Stage CE1 cysts display a dense wall and may have internal echos. This so-called hydatid sand is due to protoscolices within the cyst. CE2 cysts have internal septae or daughter cysts, forming a

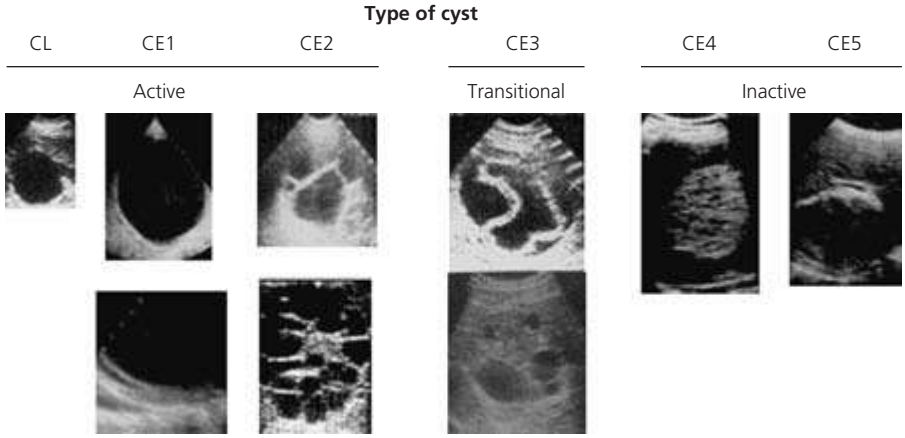

Figure 107.4 Ultrasound stages of cystic hydatid disease. From World Health Organization Informal Working Group on Echinococcosis [163], with permission from Elsevier.

honeycomb pattern, that are highly characteristic of hydatid cysts. CE3 cysts demonstrate detachment of the cyst wall or early collapse. CE4 cysts demonstrate inhomogeneous material instead of a cyst cavity. CE5 cysts demonstrate a thick rim of calcification. Radiographically apparent cyst wall calcification occurs only in liver or spleen cysts and generally takes more than 5–10 years to develop. Bone cysts typically produce radiolucencies without periosteal reaction. MRI offers little advantage over CT for the imaging of cysts except in the central nervous system.

Serological tests can be useful in confirmation of the diagnosis [154,157,161]. Antibody tests using crude antigen of either *E. granulosus* or *E. multilocularis* tested by ELISA, indirect hemagglutination, and fluorescent antibody assays are positive in 50%–90% of cases. False-negative results are more common in patients with pulmonary, brain, and splenic cysts. The available tests may cross-react with cysticercosis or other parasitic infections. Thus positive results should be confirmed by a more specific test (e.g., immunoblot assay or looking for the specific Arc 5 pattern by gel diffusion).

Treatment and prognosis

Experts now suggest that treatment should vary based on the location of the cyst or cysts, the nature and size of the cysts, the condition of the patient, and available expertise. Treatment modalities include surgery, antiparasitic chemotherapy, percutaneous drainage termed PAIR (*p*ercutaneous *a*spiration, *i*ntroduction of scolicide, and *r*easpiration), and watch and wait [154,157]. In contrast to the older reliance on surgery, a recent prospective study found that only 10% of children with hepatic cysts required surgery over the subsequent 5 years [164].

Albendazole and mebendazole have been used for chemotherapy [154,157,164–166]. While older series emphasize the low cure rate, approximately one-third of hepatic cysts will resolve in response to benzimidazoles and 30%–50% improve. Many of the residual lesions were subsequently shown to be nonviable. Albendazole is now thought to be the treatment of choice for small (< 5 cm) CL, CE1, and perhaps CE3 lesions [154,157]. Chemotherapy has also been successful for small pulmonary cysts [167]. However, there is a high failure rate with chemotherapy alone for larger lesions in either the liver or the lung. Albendazole is thought to be more effective than mebendazole. Albendazole is typically given at 10–15 mg/kg daily in two daily doses continued for 3–6 months. Side effects may include neutropenia, elevated liver enzymes, and alopecia. Prospective studies of surgically removed specimens show that protoscolices can remain viable for up to 3 months, which is the minimum duration of chemotherapy [166,168]. Treatment failures can be managed by further chemotherapy, PAIR, or surgery. In some cases, PAIR may demonstrate that protoscolices are no longer viable even with persistent lesions.

The PAIR technique is less invasive than the traditional surgical approaches [157,169–172]. Results have been similar to those of surgery but with fewer complications. PAIR is thought to be the treatment of choice for uncomplicated CL and CE1 lesions larger than 5 cm and for CE3 lesions. CE2 lesions can be treated with a modification of PAIR that employs a larger-bore catheter and drainage [171,173]. PAIR should be performed after initiation of albendazole chemotherapy. To limit spillage, aspiration should generally pass through solid tissue (e.g., liver) rather than directly into the cyst. The cyst fluid should be examined for protoscolices or hooks (to confirm the diagnosis). Prior to injection of scolicidal agents, the cyst fluid should be checked for bile staining (by dipstick or by injection of contrast medium) since scolicidal agents cause sclerosing cholangitis if injected into the biliary tract. Alcohol or hypertonic saline are used as scolicidal agents in this procedure. Contraindications to PAIR include communication with the biliary tract, inaccessibility, superficial cysts, and cysts that are free in the abdomen, heart, brain, or spine. If the cyst communicates with the biliary tract, contrast media, which are mildely scolicidal, can be used to further define the communication.

Surgery remains the treatment of choice for complicated disease and some CE3 lesions [154,157,161]. Rupture of the cyst contents at the time of surgery carries a risk of disseminated echinococcosis and a small risk of anaphylaxis at the time of operation. Surgical approaches have included pericystectomy (removal of the entire cyst and a rim of surrounding tissues), resection of the involved organ, simple drainage, omentoplasty, and capitonnage. More radical procedures are associated with fewer relapses at the cost of more perioperative complications. However, laparoscopic approaches have recently been reported to be associated with a high success rate and less morbidity [172,174]. Chemotherapy with albendazole with or without praziquantel is now routinely performed [157,166]. With chemotherapy, pressure within the cyst may be reduced and removal of cyst membranes and contents will be facilitated. In addition, treatment decreases the likelihood of secondary cyst development [168]. Pulmonary cysts have been treated successfully surgically, medically, and with PAIR.

Prevention

Control programs for cystic hydatid disease have met with variable success [156–158]. *Echinococcus granulosus* was eliminated from Iceland by banning home slaughter of sheep. Banning of home slaughter, education, arecolic purging of dogs, and treatment of infected dogs with praziquantel have led to eradication in New Zealand and Tasmania. However, these eradication programs required significant resources and have been less successful elsewhere. A recombinant vaccine has proven highly effective in field studies, but awaits implementation [158]. Other tools in development include copro-antigen tests to detect infected dogs and improved therapy for ovine infection.

Other *Echinococcus* species

Echinococcus multilocularis is the cause of alveolar hydatid disease [154,155,157,161,175]. Humans are an incidental host in a life cycle that typically involves foxes as definitive hosts (but also wolves and occasionally domestic dogs) and rodents as intermediate hosts. The larval parasite does not form a large cystic structure but grows by progressive external budding. The laminated membrane and host pericyst are thin. The larval mass slowly enlarges and replaces liver tissue much as a malignancy does. *Echinococcus multilocularis* is endemic in arctic and alpine areas, including western China, central Europe, and scattered foci in North America. In recent years, disease is emerging in much of central Europe. The larvae may invade contiguous structures and rarely metastasize. Human infection almost invariably involves the liver, but may spread to adjacent tissues or metastasize to the brain. The larval mass grows slowly, with a typical latent period of 5–15 years prior to clinical presentation. Thus, clinical disease is rare in children. Diagnosis involves imaging studies with serological confirmation. Management requires surgical resection along with prolonged chemotherapy. Comprehensive reviews of epidemiology, clinical manifestations, diagnosis, and management are available [154,155,157,161,175,176].

Echinococcus vogeli is a parasite of bush dogs and feral dogs in South America. [157]. The intermediate hosts are rodents (pacas, agoutis, spiny rats). When humans are infected, the germinal membrane grows externally to form additional cysts, and septae develop within the original cyst. This manifestation is the polycystic variety of hydatid disease. A rare cause of polycystic hydatid disease in humans has been attributed to *E. oligarthrus*, a fourth species that is found as adult tapeworms in wild felids such as pumas and jaguars. The intermediate hosts are the same as those for *E. vogeli*. Most infections occur in Central and South America. Polycystic hydatid disease is limited to South and Central America, where fewer than 100 human infections have been reported. Initial management should include albendazole.

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I MISCELLANEOUS

108 Gastrointestinal and hepatic manifestations of specific genetic disorders

Elizabeth M. Petty

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Genetic contributions to the pathogenesis of many gastrointestinal (GI) and hepatic disorders are being increasingly recognized. Recognition of a well-understood genetic contribution to a GI or hepatic disease may have an impact on clinical management and have significant implications for other family members. To recognize genetic contributions, review of a comprehensive family history, relevant medical history, and related medical records (e.g., pathology reports, laboratory studies, clinical summaries) of the patient and, in some cases, of affected family members, is needed.

The family history may reveal a specific Mendelian inheritance pattern (e.g., autosomal dominant, autosomal recessive, X-linked recessive, or X-linked dominant) or non-Mendelian mode of inheritance (mitochondrial/maternal/cytoplasmic or imprinted) that suggests a major inherited genetic contribution. Even such major genetic contributions to GI or hepatic disease may be difficult to recognize if a family is small, no history about biological relatives is available, significant genetic heterogeneity exists, common phenocopies (sporadic conditions that mimic inherited disorders) occur, variable expressivity of manifestations is present, incomplete penetrance is possible, or a new mutation causes the disease. Determining genetic contributions to GI or hepatic diseases that are multifactorial (e.g., where significant environmental or epigenetic factors interact with an individual's underlying genetic constitution) or polygenic (e.g., where several genetic factors, including multiple modifying genes, interact to contribute to disease susceptibility) can be even more challenging. Despite such challenges, it is clinically relevant to assess genetic contributions to the disease process.

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The purposes of this chapter are:

- to provide a basic framework as to when, how, and why to consider genetic factors
- to briefly review several well-known genetic syndromes, most of which are highly penetrant, single-gene disorders with Mendelian patterns of inheritance, that may present with significant GI or hepatic manifestations.

Some of these genetic conditions present in infancy (e.g., some inborn errors of malabsorption), whereas others are characterized by adult-onset presentation (e.g., some colon cancer syndromes). Awareness of these syndromes is needed to ensure appropriate diagnoses and medical management for affected individuals. For example, an adult with Ehlers–Danlos syndrome type IV (the "vascular type" of Ehlers–Danlos syndrome – an autosomal dominant syndrome characterized by type III collagen abnormalities) who presents with sudden severe abdominal pain, must be immediately evaluated and treated, when needed, for bowel rupture and arterial dissection to reduce disease-associated morbidity and early mortality.

Most diseases presented in this chapter are summarized in an abbreviated table format for easy reference. More comprehensive discussions of the pathophysiology and treatment for common GI and hepatic diseases mentioned in this chapter are covered in more detail elsewhere in this text (see the references for each topic, Part III of the textbook and the Index). For more information about less common genetic conditions, several excellent genetic textbooks and online genetic databases are referenced for additional, more detailed, information about the particular syndromes and diseases [1–5]. Specifically, the *Online Mendelian Inheritance of Man* (OMIM) National Center for Biotechnology Information database (*http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM/*), *GeneReviews* at the *GeneTests* website (*http://www.genetests.org/*), and the Center for Disease Control's HuGE Net (*http://www.cdc.gov/genomics/hugenet/reviews.htm*) are excellent online

database references that provide regularly updated information about many genetic syndromes and include more detailed up-to-date reference lists. Information for this chapter was obtained through online searches of these databases with review of their key references and genetic textbooks [4,5]. Genetic contributions to common GI and hepatic conditions that have complex multifactorial or polygenic etiologies (e.g., inflammatory bowel disease, cirrhosis) are covered in other disease-specific chapters throughout this text (see Index).

Practicing genetic medicine

Clinicians must understand basic genetic principles to provide accurate diagnostic, prognostic, and therapeutic services for individuals with genetic conditions and their at-risk relatives. Once the genetic basis of a disease is recognized, preventive screening, medical or surgical management, genetic counseling, patient and family education, and referrals can be implemented as appropriate. DNA testing is available for dozens of conditions that have prominent GI or hepatic manifestations and the list will continue to grow. With completion of the Human Genome Project and identification of the genetic codes for all of our estimated 25 000 human genes, genomic knowledge relevant to the clinical practice of gastroenterologists and hepatologists will continue to be revealed at an unprecedented pace. Genetic testing can serve various purposes, from molecular confirmation of a suspected disease to facilitation of management and recurrence risk counseling, as in the case of hemochromatosis, or, predictively, to more accurately predict one's own risk of developing colon cancer. The practice of genomic medicine with utilization of genetic testing has already become standard of care in many areas of clinical medicine.

An example of this is seen in familial adenomatous polyposis (FAP), most often a highly penetrant, autosomal dominant condition caused by deleterious mutations in the adenomatous polyposis coli gene (*APC*) located on the long arm of chromosome 5 (see Chapter 64). In its classic form, individuals with one copy of a mutated *APC* gene will develop colon cancer in early to mid adulthood after having hundreds to thousands of polyps that began developing in adolescence. At-risk individuals benefit from genetic counseling, education, and testing to better understand and reduce their risks of developing cancer. In up to 90% of affected individuals, causative *APC* mutations can be detected, allowing robust and reliable predictive testing for at-risk family members. Specific preventive management strategies (e.g., serial colonoscopies, colectomies) can greatly reduce the risk of colon cancer in mutation carriers. Individuals lacking the mutation that segregates in affected family members, are spared the high risk of developing early-onset colon cancer and do not need the surveillance and surgical interventions recommended for mutation carriers. It is therefore both clinically and economically desirable to offer predictive genetic testing to individuals at risk for a highly penetrant, late adolescent/early adult-onset disorder such as FAP, where interventional management can significantly reduce morbidity and early mortality. In contrast to the highly penetrant *APC* mutations responsible for classic FAP, recognition of a relatively common polymorphism associated with a hypermutable tract of DNA in the *APC* gene in Ashkenazi Jews confers only a mildly increased risk of colon cancer in individuals with the polymorphism. An autosomal form of adenomatous polyposis colon cancer has been recognized in association with *MYH* gene mutations. Understanding the relative risks for colon cancer associated with various gene mutations is critical for patients because it may have profound effects on clinical management and predictive risk assessment. With increased understanding of genetic mechanisms in health and disease, the availability of clinically useful genetic tests will continue to grow and, ultimately, individually targeted molecular-based therapies may become a reality for many disorders. Testing for specific genetic polymorphisms in drug-metabolizing enzymes is already being used to predict clinical outcomes of treatment with common chemotherapy agents in cancer patients.

Issues in genetic counseling, education, and testing will be raised throughout this chapter. Genetic counseling involves discussing the natural history of the condition as well as diagnostic, prognostic, recurrence risk, medical management, genetic testing, and related psychosocial issues with appropriate referral to support systems and educational resources. Board-certified medical geneticists and genetic counselors provide the bulk of genetic counseling through specialized genetic clinics, which are available through most academic medical centers. The growth of genetic knowledge, however, will require that virtually all health-care professionals become comfortable with providing some basic level of genetic counseling in the future.

Increasingly more sensitive, specific, and cost-effective genetic tests continue to be developed so detailed discussion of specific genetic tests will not be addressed here. It is important, however, to understand that each test has its own analytic validity and clinical utility and that the specificity and sensitivity will vary considerably depending on the particular type of genetic test and the disease being studied. For some disorders, such as hereditary nonpolyposis colorectal cancer or Lynch syndrome, an adult-onset, autosomal dominant disease with genetic heterogeneity, clinicians must decide between a number of different kinds of available molecular assays to identify the most appropriate, sensitive, specific, and cost-effective test for any one individual or family. Online genetic testing laboratory databases, such as *GeneTests*, for health-care professionals, provide up-to-date information on laboratories that offer research or clinical genetic testing for

diseases with a genetic component [2]. Individuals needing more information should consult with local genetic centers and gather up-to-date information provided by national disease-associated organizations.

Whereas DNA-based genetic tests may have high clinical utility when evaluating an individual for a genetic condition, this is not always the case. Consider, for instance, a disease like neurofibromatosis type I (NF1), an autosomal dominant, neurocutaneous disorder that may have significant GI symptoms as the result of the mass effect of intraabdominal plexiform neurofibromas or of vascular ischemia in the intestinal vascular bed. Current genetic testing for NF1 is about 90% sensitive – less sensitive than a good clinical genetics examination in a classically affected adult. DNA testing in an individual with known NF1 who presents for management of their GI symptoms offers no direct clinical benefit to the patient because no relevant genotype/phenotype correlations exist that would provide insights into their management. Testing for NF1 mutations, however, may be useful if the individual is interested in prenatal testing or if the patient has young children where the diagnosis cannot be firmly established by clinical examination.

In all cases, genetic testing encompasses more than a simple laboratory analysis and should include pretest counseling and education, provision of informed consent, accurate interpretation and discussion of implications, follow-up after test results, and posttest education, management, and support (Table 108.1). This is especially important when molecular testing is used to more accurately determine a healthy individual's genetic risk for developing a disease, as in predictive testing of an asymptomatic individual who, by virtue of their family history or their ethnicity, is at risk of having inherited a particular mutation. Such testing is employed in the evaluation of inherited colon cancers, as described previously.

Table 108.1 Foundations for the appropriate use of genetic tests

DNA testing encompasses more than just the laboratory test. Tests must:
Be ethically applied with respect for genetic privacy and patient autonomy
Demonstrate analytic validity, clinical utility, and high-quality (sensitivity and specificity) with a high positive predictive value
Be cost-effective and yield results that will benefit the patient from a medical or psychosocial standpoint
Be accompanied by accurate patient education, counseling, and follow-up care
Prior to testing:
Conduct a comprehensive medical evaluation including family history
Perform a risk analysis regarding the potential for the diagnosis of a particular syndrome
Review the current literature regarding the positive predictive value of genetic testing for that condition
Determine what gene should be studied
Evaluate available tests and laboratories for reported sensitivity and specificity
Provide the patient with education about the disease process
Provide risk estimates of gene inheritance, susceptibility for disease, and potential related medical problems
Determine how the test results may modify the patient's risk
Discuss test specifics including costs, benefits, risks, and limitations
Provide counseling regarding various outcomes of test results and the resulting implications for patient or family members
Discuss management options including alternative approaches to genetic testing
Explore any psychosocial concerns including impact on self-esteem, relationships, potential for discrimination, and implications for other family members
At the time of testing:
Obtain and document informed consent after patient has been adequately educated about the test and has agreed to proceed with testing knowing potential benefits, risks, and limitations related to medical and psychosocial implications
Ensure that an appropriate specimen is properly collected, and shipped or delivered to the laboratory
Determine how results will be presented to the patient
When test results are available:
Interpret tests based on knowledge about known genotype–phenotype correlations, the clinical course of affected family members, the medical evaluation of the patient, and other environmental or hormonal risk factors for the patient
Provide the patient with counseling and education regarding results
Refer patient and family for appropriate support and education
Provide follow-up and medical surveillance, chemoprevention, and management as appropriate
Offer evaluations to other at-risk family members as appropriate

Well-defined genetic disorders with prominent gastrointestinal or hepatic manifestations

Malabsorption disorders

Some genetic syndromes involve defects of intestinal absorption (Table 108.2). Most are disorders of brush border enzymes or transport proteins that transfer sugars, amino acids, electrolytes, lipids, vitamins, and minerals from the gut lumen into the circulation. There may be malabsorption of a singular, specific substance, such as in lactase deficiency, or general malabsorption of several substances, such as in Hartnup disease. The other large group of genetic malabsorption syndromes involves disorders of the exocrine pancreas. Cystic fibrosis is most common, followed in frequency by the Shwachman–Bodian syndrome and the much rarer Johanson–Blizzard syndrome; the last two have normal sweat chloride tests.

Defects in carbohydrate metabolism

Congenital lactase deficiency

Patients with congenital lactase deficiency (i.e., disaccharide intolerance II) present with watery diarrhea, usually after the first milk feed. Lactase, an enzyme with lactase and phlorizin hydrolase activity, is virtually absent in the duodenal mucosa of affected infants, but the duodenum is otherwise histologically and enzymatically normal. The human lactase-phlorizin hydrolase gene, *LCT*, maps to chromosome 2q21.

Later-onset lactase deficiency

Lactase activity is generally around 10% of maximal activity in adults with later-onset lactase deficiency. Approximately three-fourths of non-white adults are lactase-deficient and 5%–30% of white persons, depending on ethnicity, have some degree of lactase deficiency. In ethnic groups where late-onset lactase deficiency is common (e.g., > 50% prevalence), affected individuals between 1 and 5 years of age often present with diarrhea and crampy abdominal pain after the ingestion of foods containing lactose. In white persons with hypolactasia, symptoms generally arise during adolescence. The diagnosis is most often confirmed by the noninvasive techniques of oral lactose tolerance tests or breath tests.

Since adult lactase activity variation in different ethnic groups is independent of lactose intake, the differences are largely genetic. There is a decreased amount of active brush border lactase, which is probably regulated by gene expression. Two phenotypes have been described: type I is characterized by a decrease in actase-phlorizin hydrolase messenger RNA; type II is characterized by abnormal posttranslational processing of lactase-phlorizin hydrolase, with abnormal retention of the protein in the endoplasmic reticulum. Hypolactasia is an autosomal recessive syndrome but parents of affected children are commonly affected because the gene alterations for hypolactasia are present in the population at high prevalence, resulting in homozygotes marrying heterozygous carriers.

Congenital lactose intolerance

Congenital lactose intolerance is a serious autosomal dominant disorder characterized by vomiting, failure to thrive, dehydration, renal tubular acidosis, disaccharidiuria, aminoaciduria, liver damage, cataracts, and brain damage in infancy. Unlike congenital lactase deficiency, it can be fatal unless diagnosed and managed promptly. It is caused by the abnormal absorption of lactose in the stomach rather than the duodenum. Lactose is hypothesized to be toxic to the liver and brain, analogous to the toxicity of galactose-1-phosphate in galactosemia. Treatment consists of lactose avoidance. The disorder spontaneously resolves between 12 and 18 months of age.

Sucrose–isomaltose malabsorption/disaccharide intolerance I

The autosomal recessive sucrose–isomaltose malabsorption disorder (i.e., sucrase–α-dextrinase or intestinal disaccharidase deficiency) presents with symptoms similar to lactase deficiency, except that they are triggered by sugar, not milk. Symptoms range from severe diarrhea in infants to occasional stomach upset in adults. Ingestion of sweetened foods and fruits provokes symptoms but tolerance to the sugars increases with age. Starch is generally well tolerated. In general, homozygotes have severe enzyme deficiency with lifelong symptoms, but heterozygotes with intermediate enzymatic activity may have mild symptoms as infants and no symptoms as adults. The disorder affects 10% of the Inuit of Greenland but only 0.2% of North Americans. The diagnosis is made using the same techniques as are used in lactase deficiency. Ingesting fresh baker's yeast that has sucrase activity, preferably on a full stomach, can ameliorate the symptoms after sucrose ingestion.

Studies suggest that the primary defect involves sucrase with secondary effects in isomaltase. Defects in various aspects of intracellular processing of the enzyme complex have been demonstrated: endoplasmic reticulum accumulation, blocked Golgi transport, and transport of a catalytically altered enzyme to the cell surface, illustrating the heterogeneity of this relatively uncommon, autosomal recessive disorder. The sucrase–isomaltase gene (*SI*) maps to chromosome 3q25–26.

Glucose–galactose malabsorption

The autosomal recessive condition of intestinal monosaccharide transport deficiency has a similar presentation to intestinal disaccharidase deficiency. In glucose–galactose malabsorption, diarrhea and dehydration occur after ingestion of carbohydrates containing one or more of these sugars. Tolerance to these sugars improves with age. Acidic stools, containing glucose and galactose, suggest the diagnosis. Some patients

Table 108.2 Genetic disorders of intestinal malabsorption

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Defects in carbohydrate metabolism				
Congenital lactase deficiency (disaccharide intolerance II)	AR	2q21 *LCT*	Watery diarrhea within the first 10 days of life	Possibly less cardiovascular disease due to avoidance of dairy products
Adult lactase deficiency or hypolactasia (disaccharide intolerance III)	AR	2q21 *LCT*	Abdominal pain, bloating, diarrhea after lactose ingestion	Present in > 75% of non-white and about 20% of white population
Congenital lactose intolerance	AD	2q21 upstream of LCT	Vomiting and diarrhea	Failure to thrive, dehydration, renal tubular acidosis, disaccharidiuria, aminoaciduria, liver damage, cataracts, and brain damage
Sucrase isomaltase deficiency (disaccharide intolerance I)	AR	3q25–26 *SI*	Variable symptoms, from severe diarrhea in infancy to stomach upset in adults after sucrose ingestion	Start well tolerated
Glucose–galactose malabsorption	AR	22q13 *SGLT1/SLC5A1*	Bloating, abdominal pain, and diarrhea after ingestion of most carbohydrates	Mild defect in renal tubular absorption of glucose
Fanconi syndrome with intestinal malabsorption and galactose intolerance	AR	3q26 *SLC2A2*	Poor appetite, generalized malabsorption	Impaired galactose metabolism, normal glucose absorption, slow weight gain, retarded psychomotor development, and abnormal renal tubular function
Trehalase deficiency	AR	11q23 *TREH*	Diarrhea and vomiting after ingestion of trehalose-containing foods (e.g., insects, mushrooms)	Rare in white Americans, found in 10%–15% of cDNA isolated in Inuit of Greenland
Defects in amino acid transport				
Iminoglycinuria	AR	?	None	A membrane transport defect causing excess excretion of proline, hydroxyproline, and glycine – often no clinical consequence but may be mental retardation and gyrate atrophy; frequency among whites, 1 in 15 000
Hartnup disease	AR	11q13 *HND*	None	Intermittent, red, scaly, pellagra-like rash after sun exposure; cerebellar ataxia, psychiatric disorders, low or subnormal intelligence, massive aminoaciduria of neutral and aromatic amino acids; US incidence of 5 per 100 000
Cystinuria I	AR	2p16 *SLC3A1*	None	Renal tubule and intestinal epithelium transport abnormalities of all dibasic amino acids; nephrolithiasis due to insolubility of cystine in urine; US incidence of 1 in 15 000
Cystinuria II, III	AR	19q13 *SLC7A9*		
Lysinuric protein intolerance (dibasic aminoaciduria I and II)	AR	7q31 *SLC7A7*	Vomiting and diarrhea after ingestion of cows' milk	Hyperammonemia, failure to thrive, organomegaly, mental retardation, lens opacities, elastic skin, and sparse hair

Blue diaper syndrome	AR	?	None	Hypercalcemia, nephrocalcinosis, and indicanuria due to intestinal tryptophan transport defect
Oasthouse syndrome	AR	?	Diarrhea	White hair, hyperpnea, seizures, mental retardation, and an odor of an oasthouse (i.e., kiln for drying hops)
Lowe oculocerebrorenal syndrome	XLR	Xp26 *OLRL*	None	Cataracts, mental retardation, vitamin-D-resistant rickets, glaucoma, generalized aminoaciduria and organic aciduria; death from renal failure; impaired intestinal and renal absorption of lysine and arginine; mapped to *Xq*; carrier detection possible
Lysine intolerance	AR	7q31 *SLC7A7*	Episodic vomiting, rigidity	Coma in infancy relieved by low-protein diet; hyperammonemia and high lysine and arginine levels, apparently due to defective L-lysine: NAD-oxidoreductase activity in liver
Lysine malabsorption syndrome	AR	?	Impaired intestinal absorption of lysine	Physical and mental retardation; hyperlysinuria and hypolysinemia, probably due to lysine transport defect
Glutamate–aspartate transport defect (dicarboxylic aminoaciduria)	AR	9p24	None	Defective intestinal and renal transport of glutamic and aspartic acids, leading to moderate hyperprolinemia and hypoglycemia
Disorders of electrolyte absorption				
Congenital chloride diarrhea	AR	7q22–31 *SLC26A3A*	Voluminous, chloride-containing, watery stools	Polyhydramnios, premature birth, hypochloremia, hypokalemia, metabolic alkalosis, growth retardation
Congenital sodium diarrhea	AR	?	Voluminous, sodium- and bicarbonate-containing stools	Polyhydramnios, premature birth
Microvillus inclusion disease	AR	?	Severe, watery diarrhea	No polyhydramnios or premature birth
18-Oxidation defects of aldosterone	AR		Vomiting, intestinal salt wasting	Dehydration, hypernatremia, and hypokalemia; deficiencies of 18-hydroxylase and 18-hydroxysteroid dehydrogenase
Congenital adrenal hyperplasia	AR	6p21 *CYP21*	Intestinal salt wasting	Deficiency in any one of several enzymes in cortisol synthesis; virilization, renal salt losing, or hypertension
Hypomagnesemia	AR XLR	3q27 *CLDN16*	Primary intestinal malabsorption of magnesium	Hypomagnesemia, hypocalcemia, seizures, and tetany in newborn period
Defects in intestinal protein absorption				
Intestinal lymphangiectasia	AD	?	Intestinal loss of protein, dilated lymphatic spaces in small bowel and partial villous atrophy	Neonatal edema, high incidence of stillbirth, congenital malformations, lymphopenia, hypogammaglobulinemia, skin energy, dysproteinemia, leg edema and ulcers in males
Aplasia cutis congenita with intestinal lymphangiectasia	AR	?	Intestinal loss of protein	Aplasia cutis congenita of the vertex, nonpitting limb edema, hypoproteinemia, lymphopenia
Protein-losing enteropathy	AR	?	Diarrhea, abdominal pain, ascites, intestinal loss of protein	Edema, growth retardation, clubbing, hypoproteinemia, iron-deficiency anemia, no lymphopenia; hepatic vein stenosis can lead to Budd–Chiari syndrome

Table 108.2 (*cont'd*)

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Epidermolysis bullosa lethalis with pyloric atresia	AR	17q24 *ITGB4*	GI symptoms may dominate the clinical picture; pyloric atresia, diarrhea, and protein-losing enteropathy	Bilateral pyelonephrosis due to stenosis at the uterovesical junctions; prenatal diagnosis by ultrasound, demonstrating dermal–epidermal separation at the lamina lucida in the fetal skin
Vascular hyalinosis	AR	?	Diarrhea, rectal bleeding, malabsorption, protein-losing enteropathy	Progressive hyalinosis of the capillaries, arterioles and small veins of the GI tract, kidneys, and brain; poikiloderma and graying of the hair in the 20s
Hereditary lymphedema I, II	AD	5q35.3 *FLT4* 16q24.3 *MFH1/FOXC2*	Loss of albumin through the intestines	Severe edema below the waist; onset at birth; paucity of lymph nodes above the inguinal ligaments; distinct from hereditary lymphedema II, which has onset at puberty
Defects in fat absorption				
Familial amyloidosis polyneuropathy type 1	AD	18q11–12 *TTR*	Abnormal GI motility with resultant bacteria overgrowth and malabsorption of bile acids; initial constipation followed by bouts of diarrhea	Progressive sensorimotor neuropathy beginning in the lower extremities; orthostatic hypotension, and impotence
Abetalipoproteinemia	AR	4q22–24 *MTTP*	Steatorrhea with malabsorption of fat, poor weight gain, impaired absorption of fat-soluble vitamins	Beginning in the second decade; peripheral neuropathy, cerebellar ataxia, atypical retinitis pigmentosa, mild to moderate anemia, and acanthocytes; coagulopathy and low serum lipid values
Anderson disease	AR	Xq22	Steatorrhea with malabsorption of fat	Growth retardation; low serum lipid values
Familial hypobetalipoproteinemia	AD	3q22–21 *APOB* 4q24 *MTTP*	Mild fat malabsorption	Low serum levels of low-density lipoprotein and high-density lipoproteins; normal triglycerides
Wolman disease	AR	10q24–25 *LIPA*	Intestinal malabsorption	Death usually in infancy; tissue accumulation of cholesterol esters and triglycerides in liver, adrenals, spleen, lymph nodes, bone marrow, small intestines, lungs, and thymus; prenatal diagnosis made by amniocytes demonstrating enzyme deficiency
Cholesterol ester storage disease	AR	10q24–25 *LIPA*	Intestinal malabsorption	Similar to Wolman disease, with later onset and milder symptoms
Wolman disease with hypolipoproteinemia and acanthocytosis	AR	?	Intestinal malabsorption	Similar to Wolman disease; also features hypolipoproteinemia and acanthocytosis
Storm syndrome	AD	?	Fat malabsorption	Early, progressive, calcific cardiac valvular disease; loss of eyebrows and eyelashes; premature thinning and graying of hair; taut skin over hands and face

Groll–Hirschowitz syndrome	AR	?	Diminished gastric motility due to a vagal nerve abnormality; multiple diverticula with jejunoileal ulceration; malabsorption of fat and intestinal loss of protein	Progressive sensory neuropathy; nerve deafness with onset in the first three decades
Glucose-stimulated secretory diarrhea with common variable immunodeficiency	AD	?	Severe, watery diarrhea; malabsorption of fat, bile acids, vitamin B-12, and xylose	Combined immunodeficiency syndrome; perfusion studies show glucose-stimulated secretion of water, sodium, and chloride in the jejunum and ileum, with normal transport for bicarbonate and mannitol
Exocrine pancreas abnormalities				
Cystic fibrosis	AR	7q31 *CFTR*	Intestinal malabsorption, steatorrhea, pancreatitis	Thickened secretions, respiratory infections, infertility
Shwachman–Bodian/Diamond syndrome	AR	7q11 *SBDS*	Steatorrhea, intestinal malabsorption	Incidence of 1 in 250 000 births; poor growth, recurrent respiratory infections, moderate dwarfism with dysplastic metaphyseal changes
Johanson–Blizzard syndrome	AR	15q15–21 *UBR1*	Intestinal malabsorption	Aplasia or hypoplasia of the nasal alae, upswept frontal hair, widely spaced teeth, congenital deafness, hypothyroidism, postnatal growth retardation, mental retardation, midline ectodermal scalp defects, absent permanent teeth, and genitourinary and anorectal abnormalities; insulin-requiring diabetes in adulthood
Cartilage–hair hypoplasia (metaphyseal chondrodysplasia, McKusick type)	AR	9p13 *CHH*	Intestinal malabsorption, Hirschsprung disease	Dwarfing syndrome with radiographic changes called metaphyseal dysostosis; fine, sparse, light-colored hair, anemia, susceptibility to chicken pox, and malignancies
Asphyxiating thoracic dystrophy (Jeune syndrome)	AR	15q13	Intestinal malabsorption	Skeletal dysplasia with marked involvement of the rib cage causing asphyxia; polydactyly, chronic nephritis, cystic changes of liver, kidneys, and pancreas
Pearson marrow–pancreas syndrome	mt	mt	Intestinal malabsorption	Refractory anemia requiring transfusion; thrombocytopenia, neutropenia, and hepatic dysfunction
Lipase or colipase deficiency	AR	10q26 *PNLIP*	Steatorrhea with malabsorption of fat-soluble vitamins	Stature is within normal limits; responds to exogenous administration of lipase or colipase
Trypsinogen deficiency	AR	7q32qter *PRSS1*	Diarrhea	Failure to thrive, edema, hypoproteinemia with normal sweet electrolytes, imperforate anus
Enterokinase deficiency	AR	21q21 *PRSS7*	Diarrhea	Similar to trypsinogen deficiency; favorable response with pancreatic enzyme replacement
Congenital pancreatic hypoplasia	AR	13q12 *IPF1*	Diarrhea	Exocrine and endocrine insufficiency, intrauterine growth retardation, and early-onset type I (insulin-dependent) diabetes
Cystinosis	AR	17p13 *CTNS*	Diarrhea	Intralysosomal storage of cystine, leading to pancreatic exocrine and endocrine insufficiency in long-term survivors; corneal deposits with photophobia and corneal erosions, ovarian failure, hypothyroidism, confusion, short-term memory loss, cerebral atrophy, Fanconi syndrome, and renal failure; myopathy and CNS involvement; prenatal diagnosis by studies of cystine uptake and retention by chorionic villi. Heterozygotes have six times the normal free cystine concentration. Three types are known: infantile nephropathic, late-onset or adolescent nephrohepatic, and benign or adult nonnephrohepatic

Table 108.2 (*cont'd*)

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Disorders of vitamin and mineral assimilation				
Imerslund–Grasbeck syndrome	AR	10q12 *MGA1*	Malabsorption of vitamin B-12 not corrected with exogenous intrinsic factor	Chronic, relapsing megaloblastic anemia; no antibodies to intrinsic factor or gastric parietal cells; proteinuria and malformations of the urinary tract
Folic acid transport defect	AR	?	Malabsorption of folic acid	Defect in transport of folic acid into CNS, causing seizures; mental retardation and movement disorder; megaloblastic anemia corrected by parenteral administration of folic acid
Pseudovitamin D deficiency	AR	12q14	Defective calcium absorption	Several subtypes; responds to large doses of vitamin D; rickets and short stature, myopathy, hypocalcemia and hypophosphatemia; distinct from X-linked vitamin-D-resistant rickets
Familial isolated deficiency of vitamin E	AR	8q13 *TTPA*	No evidence of fat malabsorption; selective defect in vitamin E absorption	Ataxia, areflexia, loss of proprioception; increased serum lipids, xanthelasmata, and xanthomas of the Achilles tendon; neurological symptoms may improve with administration of vitamin E. Heterozygotes may manifest tendinous xanthomas and elevated serum cholesterol levels.
Menkes syndrome (kinky hair disease)	XLR	Xq12–13 *ATP7A*	Defective intestinal copper absorption	Light-colored kinky hair, growth retardation, severe neurological impairment, metaphyseal changes, tortuosity of vessels, bladder diverticula; low serum copper and ceruloplasmin levels
X-linked cutis laxa (occipital horn syndrome)	XLR	Xq12–13 *ATP7A*	Defective copper absorption	Occipital bony prominences; short, broad clavicles, fused carpal bones, hooked nose, long philtrum, multiple bladder diverticula, varicosities, inguinal hernias, joint laxity; low serum copper and ceruloplasmin levels; allelic with Menkes syndrome
Hereditary iron-handling disorder	AR	?3	Selective iron malabsorption	Microcytic anemia unresponsive to oral iron therapy, partial response to intramuscular iron dextran
Acrodermatitis enteropathica	AR	8q24.3 *SLC39A4*	Intermittent diarrhea caused by zinc malabsorption	Bullous dermatitis, failure to thrive; alopecia of scalp, eyebrows, and eyelashes; thymic hypoplasia; improvement with zinc supplementation
Hemochromatosis	AR	6p21 *HFE*	Iron overload caused by increased absorption in the intestine; symptoms relate to the degree of iron overload in specific tissues and are often milder in menstruating females. Liver disease.	Findings may include fatigue, cirrhosis, cardiomyopathy, arthritis, and darkening of skin color, and may be relieved or prevented in the earliest stages by scheduled phlebotomy, thus highlighting the usefulness of presymptomatic DNA testing.

AD, autosomal dominant; AR, autosomal recessive; CNS, central nervous system; GI, gastrointestinal; mt, mitochondrial; XLR, X-linked recessive.

also have a mild defect in renal tubular reabsorption of glucose. It is clinically distinguished from disaccharidase deficiency because there is no significant rise in plasma glucose during a glucose–galactose oral tolerance test. Treatment consists of substituting fructose for other dietary carbohydrates. The transport mechanism in the small intestine is dysfunctional where the mucosa is unable to take up glucose and galactose. Mutations in the glucose–galactose transporter gene (*SGLT1*, also known as *SLCA5A1* – solute carrier family 5), which maps to chromosome 22q13, are responsible for the condition.

Trehalase deficiency

Trehalase deficiency, an autosomal recessive disorder characterized by diarrhea and vomiting after ingestion of trehalose-containing foods, is rare in white Americans but is found in 10%–15% of the Inuit of Greenland. Trehalose is a disaccharide found in insects and mushrooms. Deficiency can be detected by oral tolerance tests. The gene encoding human renal trehalase (*TREH*) encodes a brush border membrane glycoprotein and maps to 11q23.

Defects in amino acid transport

Proteins are normally absorbed by two major mechanisms: transport of liberated free amino acids by group-specific sodium-dependent amino acid transport systems and uptake of unhydrolyzed peptides independent of the specific entry mechanisms. At least three major group-specific active transport systems exist: monoamine monocarboxylic (neutral) amino acids; dibasic amino acids and cystine; and dicarboxylic (acidic) amino acids. The genetic disorders of amino acid absorption involve deficiencies in one or more of these transport mechanisms.

Iminoglycinuria

Iminoglycinuria occurs as part of autosomal recessive inborn errors of imino acids (i.e., proline and hydroxyproline) and glycine absorption in the kidneys and consists of five subtypes, two of which also involve the intestine. It is also a benign, normal finding in infants younger than 3–6 months of age. The malabsorption condition is often asymptomatic but has been associated with mental retardation and gyrate atrophy of the choroid and retina. When any of the three amino acids is ingested, excretion of the other two increases, indicating a group-specific defect. Some homozygotes show no L-proline defect. At least four alleles for the disorder are suspected but the degree of locus heterogeneity is unknown. The reported frequency among the white population is about 1 in 15 000, giving a heterozygote frequency of about 2%. Obligate heterozygotes may be hyperglycinuric. A rare autosomal dominant form of glycinuria with oxalate nephrolithiasis, seen most often in Ashkenazi Jews, may be a distinct form of iminoglycinuria that predisposes to urolithiasis.

Hartnup disease

Hartnup disease is a rare heterogeneous autosomal recessive disorder with an incidence of 5 cases per 100 000 individuals. It causes virtually no symptoms in more than 90% of affected persons in the United States, probably reflecting the consumption of an adequate diet in this population. In symptomatic individuals, an intermittent, red, scaly, pellagra-like rash after sun exposure and attacks of cerebellar ataxia, sometimes accompanied by psychiatric disorders and seizures, develop in childhood. Low-normal or subnormal intelligence has also been reported. The main feature is massive aminoaciduria involving neutral and aromatic monoamine monocarboxylic amino acids sharing a common proximal renal tubule reabsorption mechanism. Patients have diminished capacity for jejunal absorption of these amino acids because of a brush border transport defect, causing retention of the amino acids in the intestine, which allows intestinal bacteria to convert them into dipeptide decomposition products, some of which are absorbed. Diminished tryptophan absorption causes inadequate nicotinamide synthesis, resulting in pellagra. Dipeptide decomposition is toxic to the central nervous system, and coupled with pellagra, produces the neuropsychiatric manifestations. A nutritional deficiency results from the diminished availability of essential amino acids. Liver biopsy shows extensive fatty liver without infiltration of inflammatory cells or fibrosis. Patients are treated by the administration of niacin. The gene, *HND*, maps to chromosome 5p15.

Cystinuria

Cystinuria is a rare autosomal recessive disorder characterized by cystine, lysine, ornithine, and arginine transport abnormalities in the renal tubule and intestinal epithelium. The clinical manifestations are confined to nephrolithiasis resulting from the insolubility of cystine in the urine. Expression of the disease peaks in the second and third decades. Men are more severely affected than women. Its incidence in the United States is 1 in 15 000 individuals but its incidence is 1 in 2500 among Israeli Jews of Libyan origin. Three forms of cystinuria are recognized. Identification of radiopaque stones that present as hexagonal cystine crystals in the urine leads to the diagnosis of all types. Type classification is based on excretion rates of amino acids in obligate heterozygotes. Type I heterozygotes excrete no excess amino acids, type IIIs excrete up to twice the normal amount, and type IIs (the rarest form) excrete of 9–15 times the normal amount.

Type I cystinuria maps to chromosome 2p16 where the culprit gene, *SLC3A1*, was identified. The type II and III cystinuria gene, *SLC7A9*, maps to chromosome 19q13. Treatment is directed at reducing the urinary cystine by increasing urine volume, urine alkalinization to increase solubility, and, as needed, reducing free cystine excretion by additional medical therapy.

Lysinuric protein intolerance/dibasic aminoaciduria II

Lysinuric protein intolerance, or dibasic aminoaciduria II, is a rare but serious autosomal recessive disorder beginning in infancy when cows' milk is introduced. It is characterized by abnormal transport of dibasic amino acids across the basolateral membranes of intestinal and renal tubular cells. Symptoms include vomiting, diarrhea, failure to thrive, severe growth retardation, hepatosplenomegaly progressing to cirrhosis, lens opacities, mental retardation, sparse and brittle hair, osteopenia, intermittent stupor, and sometimes hyperextensible skin and joints. It is caused by the malabsorption of arginine, lysine, and ornithine as demonstrated after oral protein loading. Reduced tubular reabsorption of these amino acids also occurs in the kidney with demonstrable lysinuria. Some heterozygotes manifest partial intestinal malabsorption after an oral protein load.

Quantitative plasma amino acids reveal low levels of arginine, lysine, ornithine, leucine, and tyrosine; normal serine and citrulline levels; and elevated glutamine and alanine levels. Dibasic aminoaciduria without cystinuria, impaired intestinal absorption of basic amino acids, and protein intolerance are diagnostic features. Hyperammonemia results from deficient hepatic uptake of the amino acids producing a deficiency of some intermediate products of the urea cycle. Treatment consists of protein restriction with citrulline and lysine supplements. Some of the abnormalities resolve on this therapy. It is caused by mutations in the amino acid transporter gene, *SLC7A7*, which maps to chromosome 14q11.

Blue diaper syndrome

Blue diaper syndrome is a rare autosomal disorder of intestinal, but not renal, tryptophan transport. Infants present with failure to thrive, recurrent fever, infections, irritability, and constipation in addition to the bluish discoloration of the diapers. Hypercalcemia and nephrocalcinosis also occur because of increased absorption of calcium. The unabsorbed tryptophan is converted to indoles by bacteria in the intestine. The indoles are absorbed and converted to indican in the liver. Indican, when oxidized, becomes indigo blue in the urine. The differential diagnosis includes the blue-green discoloration that can occur from a pigment manufactured by *Pseudomonas aeruginosa*.

Methionine malabsorption syndrome (oasthouse urine disease)

This autosomal recessive condition of methionine malabsorption is often called oasthouse urine disease because affected patients excrete urine containing α-hydroxybutyric acid, a product of the bacterial metabolism of methionine that has the odor of an oasthouse (a kiln for drying hops). The clinical features include white hair, blue eyes, hyperpnea, seizures, diarrhea, and mental retardation. Individuals will have a positive ferric chloride test on analysis of their urine in addition to α-hydroxybutyric aciduria. A methionine-free diet improves the symptoms.

Disorders of electrolyte absorption

Four genetic causes of intestinal electrolyte and water imbalances are well characterized: congenital chloride diarrhea, congenital sodium diarrhea, microvillus inclusion disease, and disorders of aldosterone metabolism causing significant salt wasting.

Congenital chloride diarrhea

Congenital chloride diarrhea is an autosomal recessive disorder that may present in utero with polyhydramnios, presumably caused by in utero diarrhea. The infant may be premature and exhibit abdominal distention. Frequent voluminous, watery, high-chloride-containing stools are produced, leading to hypochloruria, hypokalemia, and metabolic alkalosis. The secretory diarrhea in this condition results from a deficiency in intestinal brush border chloride–bicarbonate exchange. With later presentations, or inadequate replacement therapy, growth failure is noted. The family of one young boy with the disorder required donations of disposable diapers by a diaper manufacturer because this was the boy's largest nonreimbursable "medical" expense. Affected adults have a history of watery diarrhea since birth with numerous hospital admissions for dehydration and electrolyte abnormalities, glucose intolerance, gout, proteinuria, and mild renal function impairment. The chloride content of the stools and characteristic serum electrolyte changes are diagnostic. Treatment consists of administering prostaglandin inhibitors and potassium chloride. The gene for this condition, *SLC26A3*, maps to chromosome 7q22-q31.

Congenital sodium diarrhea

Congenital sodium diarrhea is an autosomal recessive disorder that presents similarly to congenital chloride diarrhea with polyhydramnios, prematurity, and voluminous, watery, high sodium- and bicarbonate-containing stools in the first days of life. Treatment consists of replacement of sodium bicarbonate. Perfusion studies show that the jejunum is in a net secretory state, with abnormal intestinal sodium–hydrogen exchange activity.

Microvillus inclusion disease

Microvillus inclusion disease is an autosomal recessive disorder involving the brush border membrane, which results in severe congenital fluid and electrolyte malabsorption and causes severe refractory diarrhea in early infancy. Unlike sodium and chloride diarrheas, polyhydramnios is not present prenatally. Microvillus inclusion disease likely results either from defective brush border assembly and differentiation or from an error of intracellular transport. Localization of the cytoplasmic inclusions to the microvillus membrane in

villus cells is revealed by electron microscopic examination. Rectal biopsy is used for early diagnosis. Therapies such as octreotide, clonidine, and epidermal growth factor have had limited success. It has been suggested that the only therapy to reduce fluid losses may be enterectomy. Survival is unusual after 2 years of age. Small bowel transplantation may become the best available treatment.

Intestinal defects in protein absorption

Many genetic syndromes demonstrate selective protein malabsorption as outlined in Table 108.2. Most involve abnormalities of the intestinal lymphatic system with intestinal lymphangiectasia, protein-losing enteropathy, and vascular hyalinosis. A rare association of protein-losing enteropathy with neurofibromatosis has been described. This patient had massive protein loss from a localized segment of small intestine with possible neurofibromatosis involvement of the mesenteric vessels.

Intestinal defects in lipid absorption

Several important genetic disorders may present in adults with lipid malabsorption. Many are caused by inborn errors of apolipoprotein or lipid metabolism. Familial amyloidosis polyneuropathy is also an important disorder with onset in adulthood that may present with lipid malabsorption. The clinical features common to most of these syndromes are caused by a deficiency of fat-soluble vitamins. Neurological symptoms may be present because of a deficiency of vitamin E, and a coagulopathy may be caused by vitamin K deficiency.

Familial amyloidosis polyneuropathy

The amyloid protein in familial amyloidosis polyneuropathy type 1 is composed of abnormal transthyretin molecules. Transthyretin is a plasma protein that transports thyroid- and retinol-binding proteins. Systemic deposition of this abnormal protein causes a progressive stocking sensorimotor neuropathy that usually begins in the lower limbs. The autonomic nervous system may also be involved, causing orthostatic hypotension, impotence, and abnormal GI motility presenting as initial constipation followed by episodic diarrhea. Malabsorption of bile acids is common and appears to be the result of GI motility dysfunction from the autonomic neuropathy, with resultant bacterial overgrowth, rather than from the amyloid deposits in the intestinal mucosa. This is an autosomal dominant condition that can be presymptomatically diagnosed by looking for mutations in the transthyretin gene, *TTR*, on chromosome 18q11-12.

Abetalipoproteinemia

The principal GI symptom of abetalipoproteinemia, an autosomal recessive disorder, is malabsorption of fat, often beginning in infancy with steatorrhea and poor weight gain. The disorder is often misdiagnosed as celiac disease. Histopathological analysis of the small bowel shows normally formed villi with lipid-engorged enterocytes. Impaired absorption of fat-soluble vitamins is responsible for many of the clinical features. Neurological symptoms usually begin in the second decade and include peripheral neuropathy and cerebellar ataxia. An atypical presentation of retinitis pigmentosa with decreased night and color vision may also develop, as does mild to moderate anemia, usually with hemolysis. There may be an iron or folate deficiency because of malabsorption and there is usually a coagulopathy caused by decreased vitamin K stores. The total cholesterol values are in the range of 20–50 mg/dL and triglyceride values are also very low, with little postprandial increase. There are no chylomicrons, very-low-density lipoproteins, low-density lipoproteins, or apolipoprotein B particles, the major structural apolipoprotein for these lipids.

Treatment consists of avoidance of fat and the administration of fat-soluble vitamins. Heterozygotes are asymptomatic with normal plasma lipids. The genetic defect is in the microsomal triglyceride transfer protein gene, *MTTP*, on chromosome 4q24 that mediates the intracellular transport of membrane-associated lipids. *MTTP* mutations cause defects in the ability of the liver and intestines to assemble or secrete apolipoprotein B.

Familial hypobetalipoproteinemia

Familial hypobetalipoproteinemia is thought to be an autosomal dominant fatty liver disorder lacking neuroocular features caused by low levels of apolipoprotein-β. Truncated versions of the normal apolipoprotein B gene, *APOB*, on chromosome 2p24 have been reported in some families where affected individuals have low levels of high-density and low-density lipoproteins, normal triglycerides, mild fat malabsorption, and defective chylomicron clearance. Genetic studies suggest that mutations in one allele of *MTTP*, which maps to 4q24, may also be responsible for some cases; confirming the genetic heterogeneity of this condition.

Anderson disease/lipid transport defect of the intestine

Individuals with Anderson disease, an autosomal recessive lipid transport defect of the intestine characterized by hypobetalipoproteinemia with accumulation of apolipoprotein B-like protein in intestinal cells, present with fat malabsorption with steatorrhea and growth retardation. They lack the significant acanthocytosis and the neuroocular symptoms of abetalipoproteinemia. Low-density lipoprotein particles are present in the plasma, but there is a failure of chylomicron formation. Enterocytes from intestinal biopsies show numerous fat droplets, and monoclonal antibody staining reveals the presence of apolipoprotein B48, the form produced by the intestine. Defective glycosylation of apolipoprotein B48 has been observed, which may underlie the absence of formation and secretion of chylomicrons.

Wolman disease and cholesterol ester storage disease/lysosomal acid lipase deficiency

Wolman disease is a severe autosomal recessive disorder, often fatal in infancy, where cholesterol esters and triglycerides accumulate in the liver, adrenal gland, spleen, lymph nodes, bone marrow, small intestine, lungs, and thymus because of lysosomal acid lipase deficiency. Death results from intestinal malabsorption. Cholesterol ester storage disease is a late-onset disorder with milder manifestations. It also involves lysosomal acid lipase deficiency and is allelic with Wolman disease. The culprit lysosomal acid lipase gene (*LIPA*) maps to chromosome 10q24-25. Prenatal diagnosis has been performed by enzyme assay in cultured amniocytes. DNA-based testing is available through a laboratory in Germany.

Exocrine pancreas abnormalities (see also Chapter 72)

Defects in protein and fat absorption are more commonly caused by abnormalities of the exocrine pancreas. Examples include deficiencies of specific pancreatic exocrine enzymes, cystic fibrosis, the Shwachman–Bodian syndrome, and the Johanson–Blizzard syndrome. Table 108.2 lists genetic syndromes manifesting with exocrine pancreas insufficiency.

Cystic fibrosis

Cystic fibrosis (CF) is a common autosomal recessive disorder in the Caucasian population, occurring with a frequency of about 1 in 2500 births. The classic disorder is characterized by viscous secretions and dysfunction of multiple exocrine glands, leading to pancreatic insufficiency, malabsorption, chronic pulmonary infection with emphysema, and a high chloride concentration in sweat. Milder mutations of the gene, *CFTR*, may result only in male infertility owing to congenital bilateral absence of the vas deferens or chronic pancreatitis. Only the GI manifestations of CF are reviewed here. CF remains a leading cause of malabsorption in white children in developed countries. More than one-half of patients with CF survive to adulthood and develop many of the GI complications with time. In fact, the majority of patients with classic CF have some degree of pancreatic insufficiency leading to achylia. Many are born with insufficiency and require enzyme replacement therapy by their first birthdays. Because the pancreatic secretions are abnormal, the pancreatic ducts become blocked, leading to destruction of the distal ducts. Proximal to these clogged ducts, autodigestion and inflammation lead to fibrosis and ultimately to exocrine insufficiency. The result is malabsorption, primarily of fats and fat-soluble vitamins, which causes malnutrition, susceptibility to hemorrhage caused by lack of vitamin K, tetany caused by hypocalcemia from losses in the stool, and neurological problems caused by vitamin E deficiency. Treatment consists of pancreatic enzyme replacement. The islets of Langerhans may become secondarily affected by fibrosis of the pancreas, leading to glucose intolerance or clinical diabetes, which is similar to non-insulin-dependent diabetes (type II), in approximately 50% of patients with CF.

Approximately 25%–30% of CF patients also develop focal biliary cirrhosis. In 2%–5% of these cases, multilobular biliary cirrhosis with portal hypertension may develop. Thick secretions plug the bile ducts, leading to inflammation and fibrosis. There is usually a history of pancreatic insufficiency in these cases.

The earliest clinical manifestation of CF may be meconium ileus, occurring in approximately 10%–15% of patients, caused by the lack of fluid in the small intestine because of a lack of pancreatic secretion or defective intestinal epithelial secretion. Gastrografin enema or oral *N*-acetylcysteine may resolve the obstruction; if not, surgery is necessary. A similar abnormality occurring later in life, called the distal intestinal obstruction syndrome, may occur in 10%–20% of CF patients, causing symptoms of colicky pain and constipation. These symptoms may be relieved with Golytely, mineral oil, an increase in pancreatic enzymes, or *N*-acetylcysteine. Rectal prolapse occurs in approximately one-fourth of patients before 2 years of age, but it usually does not occur in patients receiving pancreatic enzyme replacement therapy.

The responsible gene, *CFTR*, maps to chromosome 7q31 and encodes the cystic fibrosis transmembrane regulator protein, which forms a cAMP-regulated chloride channel. More than 1000 *CFTR* mutations have been described. The sensitivity of mutation testing varies depending on an individual's ethnicity, however, with up to 96% of mutant chromosomes detectable in affected Ashkenazi Jews and less than 70% of mutant chromosomes detected in African Americans with most commercially available mutation testing products.

The most common mutation in white patients of Northern European descent is δF508, a deletion of the amino acid residue phenylalanine at codon 508, occurring in 70% of CF patients. Most of the non-δF508 mutations are relatively rare, affecting less than 1%–10% of CF patients. Some *CFTR* genotypes are associated with pancreatic sufficiency or insufficiency, suggesting that the genotype at the *CFTR* locus is responsible for susceptibility to pancreatic insufficiency. δF508 and the common mutation among Ashkenazi Jews, *W1282X*, are examples of pancreatic insufficiency alleles. Preconceptual testing and prenatal testing are readily available. As of 2001 the American Colleges of Medical Genetics and Obstetrics and Gynecology recommend that all white couples considering parenthood and pregnant women be routinely offered DNA carrier testing.

Shwachman–Diamond/Shwachman–Bodian syndrome

The Shwachman–Diamond/Shwachman–Bodian syndrome, also called congenital lipomatosis of the pancreas, is an autosomal recessive disorder and the second most frequent pancreatic cause of malabsorption in childhood with an incid-

ence of 1 in 250 000 births. Symptoms include poor growth, intestinal malabsorption, steatorrhea, and recurrent respiratory infections, which present within the first few years of life. Dysplastic changes in the metaphyses of long bones lead to a moderate dwarfism. Patients may suffer from a pancytopenia caused by bone marrow dysfunction and are at increased risk for hematological malignancies. The pancreatic enzyme replacement required in infancy may not be necessary later. The responsible gene, *SBDS*, maps to chromosome 7q11.l

Johanson–Blizzard syndrome

In addition to pancreatic insufficiency with malabsorption, the autosomal recessive Johanson–Blizzard syndrome is characterized by aplasia or hypoplasia of the nasal alae, upswept frontal hair, widely spaced teeth, congenital deafness, hypothyroidism, postnatal growth retardation, mental retardation, midline ectodermal scalp defects, and absent permanent teeth. Genitourinary and anorectal abnormalities have also been described. Autopsy findings have shown almost complete replacement of the pancreas with fat and a paucity of islet cells. Death in childhood often results from malabsorption-related complications. Survival to adulthood is rare but possible if continuous enzyme replacement is instituted. Insulin-requiring diabetes is a feature in adulthood, similar to the situation for CF patients, presumably because of the loss of β-cells. It is the result of mutations in a gene important in the ubiquitin-ligase E3 pathway, *UBR1*, which maps to the long arm of chromosome 15.

Cartilage-hair hypoplasia/metaphyseal chondrodysplasia, McKusick type

Cartilage-hair hypoplasia (i.e., metaphyseal chondrodysplasia, McKusick type) is an incompletely penetrant autosomal recessive dwarfing syndrome (average adult height is 107–157 cm) with changes visible on radiographs called metaphyseal dysostosis. Other features include fine, sparse, light-colored hair, anemia, malabsorption caused by pancreatic insufficiency, Hirschsprung disease, and a susceptibility to chickenpox. Malignancies, specifically lymphoma, are more frequent in affected individuals. The *CHH* gene maps to chromosome 9p13.

Asphyxiating thoracic dystrophy/Jeune syndrome

Asphyxiating thoracic dystrophy, also called Jeune syndrome, is an autosomal recessive skeletal dysplasia that is often lethal in the perinatal period caused by involvement of the rib cage leading to severe asphyxia. Mildly affected individuals survive to adulthood. Other features include polydactyly, chronic nephritis, and intestinal malabsorption because of pancreatic insufficiency. Cystic changes occur in the kidney, liver, and pancreas. This syndrome shares clinical features seen in two other genetic syndromes, Ellis–van Creveld and renal–hepatic–pancreatic dysplasia syndromes.

Disorders of vitamin and mineral assimilation

This heterogeneous group of disorders displays widely variable manifestations dependent on the specific vitamin or mineral involved. Some disorders of vitamin and mineral metabolism can be appropriately treated by adequate replacement therapy.

Imerslünd–Grasbeck syndrome (see also Chapter 51)

Imerslünd–Grasbeck syndrome is an autosomal recessive disorder of chronic relapsing megaloblastic anemia caused by malabsorption of vitamin B-12. It is not corrected with exogenous intrinsic factor, differentiating it from intrinsic factor deficiency and transcobalamin II deficiency. No antibodies to intrinsic factor or gastric parietal cells are detected. The defect appears to be located between the attachment of B-12 to the surface of the ileal cell and the binding to transcobalamin II. In some cases, the defect is a selective failure of intrinsic factor transcytosis; in others, it may be the absence of an ileal receptor; and in others, the defect is unknown. Associated features include proteinuria and malformations of the urinary tract and persistent proteinuria.

The gene for this disorder, *MGA1*, maps to 10p12.2. Treatment consists of monthly intramuscular doses of 250 μg hydroxycobalamin or intramuscular doses every 2–3 months of 1000 μg hydroxycobalamin. The proteinuria does not usually respond. Mental retardation may ensue from delay of treatment so siblings of an affected person should be screened.

Menkes syndrome (see also Chapter 51)

Menkes syndrome, also called kinky hair disease, is an X-linked recessive disorder of copper metabolism caused by a defect in the intestinal absorption of copper owing to mutations in the *ATP7A* gene on chromosome Xq12-13. This affects copper-dependent enzymes in the body, producing a phenotype that is characterized by light-colored and kinky hair, growth retardation, severe neurological impairment with focal cerebral and cerebellar degeneration, changes in the metaphyses of the long bones, tortuosity of vessels, and bladder diverticula. Most patients die between 6 months and 3 years of age. Affected patients with milder allelic forms of the disease have survived to adolescence. Low serum copper and ceruloplasmin levels are reliable diagnostic findings. Heterozygotes may be detected by the finding of pili torti (i.e., kinky hair).

X-linked cutis laxa/occipital horn syndrome

X-linked cutis laxa/occipital horn syndrome mutations are caused by mutations in *ATP7A*, on chromosome Xq12-13, making it allelic with Menkes syndrome. It is characterized by occipital bony prominences (occipital horns), short broad clavicles, fused carpal bones, pectus deformities, a hooked nose with a long philtrum, multiple large diverticula of the bladder, varicosities, inguinal hernias, joint laxity, and diarrhea. Serum levels of copper and ceruloplasmin are low.

Acrodermatitis enteropathica (see also Chapter 110)
Acrodermatitis enteropathica is an autosomal recessive disorder of zinc malabsorption that presents with intermittent alopecia of the scalp, eyebrows, and eyelashes as well as diarrhea, dermatitis, and failure to thrive. Islet cell hyperplasia, absence of the thymus, and absence of germinal centers with plasmacytosis of the lymph nodes and spleen are noted at autopsy. Low serum and urinary levels of zinc and serum alkaline phosphatase (i.e., a zinc metalloenzyme) are helpful but not 100% sensitive in making the diagnosis. Oral zinc therapy has been successful in ameliorating symptoms. Ultrastructural abnormalities of the Paneth cells of the small intestine (i.e., cells that usually contain high concentrations of zinc) or a trial of oral zinc supplementation with good clinical response may provide evidence for the diagnosis in cases with normal serum zinc levels. Mutations in the intestinal zinc-specific transporter gene, *SLC39A4*, which maps to chromosome 8q24.3, have been implicated in the disease.

Hemochromatosis (see also Chapter 86)
Hemochromatosis is one of the most common autosomal recessive disorders with a carrier frequency of 1 in 10 individuals and affecting between 1 in 200 to 1 in 400 individuals of Northern European descent. An entire chapter, Chapter 86, is devoted to this condition. In homozygotes, increased absorption of iron in the small intestine results in an excess of iron deposited in many organs, ultimately resulting in organ failure in some individuals. Serious manifestations of hemochromatosis include cirrhosis, diabetes, cardiomyopathy, arthritis, hepatomas, and hypogonadotropic hypogonadism. Symptoms vary considerably among individuals. These may be minimal in individuals with chronic or episodic blood loss (e.g., menstruating women) and more severe in men with a high content of iron in their diet. Generally, symptoms begin in adulthood, after several years of increase in iron absorption.

The gene, *HFE*, maps to the human leukocyte antigen (HLA) region of chromosome 6p21, and has been cloned. One predominant mutation, Cys282Tyr, accounts for between 80% and 90% of mutant chromosomes, making DNA testing relatively straightforward. Homozygotes diagnosed early or when asymptomatic can be closely monitored and managed to prevent symptoms of iron overload. In addition to DNA diagnosis, liver biopsy along with serum iron, total iron-binding capacity, and ferritin levels may be helpful in the diagnosis. Treatment continues to include phlebotomy for individuals with increased iron levels. Other forms of hemochromatosis, caused by other factors, exist.

Chronic diarrhea syndromes not characterized by malabsorption

Several genetic disorders featuring chronic, recurrent diarrhea are not associated with a specific malabsorption syndrome. Many of the inherited immunodeficiency disorders involving T-cell antigens, T-cell antigen receptors, B cells, and immunoglobulin and complement deficiencies are characterized by a chronic diarrhea. Inherited colitis syndromes, neurological disorders, and endocrinopathies may also feature chronic or recurrent diarrhea as a common manifestation. Many of the polyposis syndromes may present with complaints of chronic diarrhea. Table 108.3 briefly summarizes those disorders that may be more commonly encountered by the clinician.

Recurrent gastrointestinal bleeding syndromes

Recurrent acute or occult GI hemorrhage should raise suspicion for a variety of genetic disorders (Table 108.4). These include structural abnormalities, such as the polyposis syndromes (e.g., Peutz–Jeghers syndrome, Gardner syndrome); vascular abnormalities, such as those that occur in vascular malformation syndromes and connective tissue disorders (e.g., hereditary hemorrhagic telangiectasia syndrome, Ehlers–Danlos syndrome); and disorders of the hematological system that may involve platelets, clotting factors, or abnormalities in fibrinolysis. The polyposis syndromes are reviewed elsewhere (see Chapter 64).

Many of the structural and vascular abnormality syndromes that cause GI bleeding belong to a group of disorders called the phakomatoses or genodermatoses, including NF1, basal cell nevus syndrome, Gardner syndrome, hereditary hemorrhagic telangiectasia, Klippel–Trenaunay–Weber syndrome, and Peutz–Jeghers syndrome. *Phakos* means spots, and these disorders have characteristic spotty or patchy cutaneous lesions. Other common features include a major vascular component to some lesions, involvement of the skin and central nervous system, and autosomal dominant inheritance patterns, often with significant variable expressivity and a high new mutation rate.

Many genetic hematological disorders predispose to GI bleeding. Deficiencies of clotting factors may result in a bleeding diathesis. von Willebrand disease and combined deficiency of factor VII and VIII are autosomal dominant disorders that may present with recurrent GI bleeding. The most common factor deficiency is factor VIII deficiency, better known as hemophilia A, an X-linked condition occurring with a frequency of 1 in 10 000. This disorder usually presents with hemarthroses that result in a chronic arthropathy, deep muscle hematomas, frank hematuria, and spontaneous intracranial bleeds. GI bleeding is not a common complication.

Abnormalities in fibrinolysis may also predispose to GI bleeding. Deficiencies of the anticlotting factors, protein C, protein S, and antithrombin III, can cause portal vein thrombosis, which can be complicated by ruptured esophageal varices.

Several genetic disorders are associated with abnormal platelet number or function predisposing to increase GI bleeding. Glanzmann thrombasthenia (i.e., platelet glycoprotein IIb-IIIa deficiency) is an autosomal recessive bleeding

Table 108.3 Diarrhea syndromes

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Immunodeficiency syndromes				
T3 T-cell antigen deficiency	AD	11q23	Nonspecific malabsorption	
T-cell antigen receptor deficiency, zeta subunit	AD	1q22–q23 *CD3Z*	Anorexia, diarrhea; small bowel shows absence of villi	Failure to thrive, pneumonia
Bare lymphocyte syndrome (BLS)	AR	19q13 *RFX1*	Severe, persistent diarrhea	A member of the severe combined immunodeficiency syndromes (SCID); mucocutaneous candidiasis, interstitial pneumonitis, and bacterial infections. In BLS type 1, the defect is the lack of HLA class I molecules. In type 2, there is a lack of HLA class I and II molecules. BLS seems to involve defects that affect gene expression.
Agammaglobulinemia, Swiss type, X-linked severe combined immunodeficiency disease	XLR	Xg13 *IL2RG*	Chronic diarrhea	Susceptibility to infections, thymic hypoplasia, and severe thrush
IgA and IgG2 deficiency	?AD	6q21	Nonspecific malabsorption	Infections, atopy, autoimmune disorders; association with HLA-BB; stronger association with neutral amino acids at the 57 position of HLA-DQ β-chain
κ-Light chain deficiency	AD	2q12 *IGKC*	Diarrhea	Recurrent respiratory infections
Deficiency of complement component 5	AD	9q34	Intractable diarrhea	General seborrheic dermatitis, recurrent local and systemic infections (gram-negative), and marked wasting
Wiskott–Aldrich syndrome	XLR	Xp11 *WAS*	Bloody diarrhea	Eczema, thrombocytopenia, susceptibility to infections; death by age 10; lymphoreticular malignancies. Therapy consists of bone marrow transplant. Carriers have a defect in platelet oxidative phosphorylation. Frequency is 4 per million male births; prenatal diagnosis possible with closely linked DNA markers.
Hereditary mucoepithelial dysplasia	AD	?	Diarrhea in infancy; esophageal stenosis or webs	Periorofacial flat, red mucosal lesions, follicular keratosis, photophobia, and nystagmus in infancy; keratitis, pannus, and cataracts; fibrocystic lung disease and cor pulmonale in adulthood; nonscarring alopecia; vaginal, oral, or urinary Papanicolaou smears show large immature cells containing vacuoles and strand-shaped inclusions. Mild mucocutaneous candidiasis, increased susceptibility to bacterial infection, possibly hypoadrenocorticism
Colitis syndromes				
Lethal cutaneous photosensitivity and colitis	AR	?	Severe colitis, refractory diarrhea	Early cutaneous photosensitivity; skin lesions are erythematous and vesicular, normal plasma zinc
Enterocolitis	AR	?	Bloody diarrhea	Death within a few weeks; very rare
Hermansky–Pudlak syndrome	AR	10q23 *HPS*	Inflammatory bowel disease, usually in Puerto Ricans	Oculocutaneous albinism with bleeding tendency, restrictive lung disease, pigmented reticular cells, defect in platelet ADP release, causing prolonged bleeding time and defective platelet aggregation

Table 108.3 (*cont'd*)

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Neuropathy syndromes				
Charcot–Marie–Tooth disease (AD CMT1A most common and several other forms exist)	AD, AR, or XLR	17p *CMT1A* 1q22 *CMT1B-MPZ*	Chronic diarrhea, nausea, and vomiting	Begins with a motor and sensory neuropathy involving the distribution of the peroneal nerve; loss of reflexes and trophic changes may occur; pes cavus is common; significant genetic heterogeneity
Fatal neonatal radiculoneuropathy	AR	?	Chronic diarrhea	Prenatal polyhydramnios, floppy infant, distal muscle weakness, areflexia, opisthotonic posturing, autonomic instability, rare variant of infantile spinal-muscular atrophy
Endocrinopathy syndromes				
Multiple endocrine neoplasia type 1 (MEN1)	AD	11q13 *MEN1*	Severe peptic ulcer disease and chronic diarrhea secondary to gastrinomas	Pituitary, parathyroid, and pancreatic adenomas; bronchial and duodenal carcinoid; malignant schwannomas
Polyglandular autoimmune syndrome type 1	AR	22q22	Diarrhea	Triad of Addison disease, hypoparathyroidism, and chronic mucocutaneous candidiasis; frequently associated with chronic active hepatitis, pernicious anemia, and primary hypogonadism; IDDM and thyroid disease are infrequent
IDDM-secretory diarrhea syndrome	XLR	Xp11–q13 *FOXP3*	Severe secretory diarrhea	Newborn presentation of brittle IDDM; death in infancy from septicemia, malnutrition, and poor diabetes control
X-linked diarrhea, poly-endocrinopathy, fatal infection syndrome	XLR	Xp11–q13 *FOXP3*	Intractable diarrhea	Eczema, hemolytic anemia, diabetes or thyroid autoimmunity; increased susceptibility to viral infections
Miscellaneous syndromes				
Systemic infantile hyalinosis	AR	4q21 *CMG2/ANTXR2*	Diarrhea in infancy	Early thickening and focal nodularity of the skin, causing reduced mobility and joint contractures; gum hypertrophy and osteoporosis, failure to thrive, and recurrent infections. Histopathology shows deposits of hyaline in the skin, muscle, GI tract, and endocrine glands.
Rapadilino syndrome	AR	8q24.3 *RECQL4*	Diarrhea	Radial and patellar aplasia or hypoplasia, absence of thumbs, dislocation of joints, long face with narrow palpebral fissures, long slender nose, small chin, unusual ears, cleft or high-arched palate, small stature, normal intelligence
Hyperphosphatemia, polyuria, and seizures	AR	?	Vomiting, diarrhea due to hyperphosphatemia	Seizures, intermittent polyuria, and hyperphosphatemia occurring together or separately; irritability, anorexia, high-pitched cry, carpopedal spasm, and overt tetany secondary to hyperphosphatemia
Dutch-type perodic fever	AR	12q24	Diarrhea during attacks; otherwise minimal abdominal symptoms; no serositis	Recurrent attacks of fever, headache, and swollen glands; leukocyte count of 10 000–20 000 cells/μL.
Fatal infantile diarrhea with abnormal hair	AR	?	Severe diarrhea preceded by excoriated buttocks	Large, low-set simple ears; flat nasal bridge, large mouth, and wooly, easily removed hair; hepatic fibrosis, hemosiderosis, and islet cell hyperplasia; increased serum galactose. Consider Menkes syndrome in differential diagnosis.
Erythroderma desquamativa of Leiner	AR	?	Severe diarrhea	Susceptibility to infection, lymphatic hypoplasia, and increase in reticular cells of the lymph nodes.

AD, autosomal dominant; AR, autosomal recessive; GI, gastrointestinal; HLA, human leukocyte antigen; IDDM, insulin-dependent (type I) diabetes mellitus; IgA, immunoglobulin A; XLR, X-linked recessive.

Table 108.4 Recurrent gastrointestinal bleeding syndromes

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Polyposis syndromes		see Chapter 64		
Neurofibromatosis type I (NF1)	AD	17q11 *NF1*	GI bleeding, secondary to ulceration of intestinal neurofibromas	Multiple café au lait macules, neurofibromas, axillary and inguinal freckling, multiple Lisch nodules, optic gliomas, bony dysplasia of the sphenoid and long bones; associated neoplasms include pheochromocytoma, meningiomas, and malignant degeneration of neurofibromas
Neurofibromatosis type III (NF3)?	AD	?17q11 *?NF1*	GI bleeding caused by ulceration of intestinal neurofibromas	None of the other features seen in NF1
Multiple endocrine neoplasia type 1 (MEN1)	AD	11q13 *MEN1*	GI bleeding caused by peptic ulcer disease secondary to gastrinomas	Triad of pancreatic (i.e., insulinomas and gastrinomas), parathyroid, and pituitary adenomas; disease gene located on chromosome 11q
Muir–Torre syndrome	AD	3p21.3 *MLH1*	GI bleeding secondary to multiple primary carcinomas of the colon, duodenum, and larynx	Keratoacanthomas of the face; multiple sebaceous tumors
Macrocephaly, multiple syndrome lipomas, and hemangiomas (see text for syndrome names)	AD	10q23 *PTEN* 10q22 *BMPR1A*	Multiple visceral hemangiomas and intestinal hamartomas predispose to GI bleeding	Macrocephaly, pseudopapilledema, multiple hemangiomas involving the skin; males may have hyperpigmented macules on the penis
Cutis laxa type	AR, AD	5q23–31 *FNBL5*	Diverticula of the GI tract that are susceptible to bleeding (X-linked cutis laxa also features of GI diverticula)	Diaphragmatic and other hernias, diverticula of the urinary tract, and pulmonary emphysema
Small intestinal diverticulosis	AR	?	Small bowel diverticula and ulcerative colitis predispose to GI bleeding	Possible increase in autoimmune disorders, including rheumatoid arthritis, thyroiditis, and nonviral hepatitis

Table 108.4 (*cont'd*)

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Diverticulosis of bowel, hernia, and retinal detachment	AR	?	Diverticula of large or small bowel	Femoral or inguinal hernias, diverticula of bladder, myopia, strabismus, and retinal detachment
Williams syndrome	AD	7q11 *ELN*	Risk for GI bleeding caused by diverticulosis; constipation	Supravalvular aortic stenosis, elfin face, dental abnormalities, infantile hypercalcemia, growth and mental deficiency; estimated frequency of 1 in 10 000
Ehlers–Danlos syndrome (many subtypes with significant genetic heterogeneity)	AD	Type IV 2q31 *COL3A1*	Bowel and large vessel rupture, more common in type IV	Classic type (I and II): soft, extensible skin; lax joints, easy bruisability, atrophic scarring, hernias, and premature birth. Vascular type (IV) caused by type III collagen defect, thin, translucent skin
Pseudoxanthoma elasticum	AR and AD	6p13 *ABCC6*	Abnormal vasculature causes GI and urinary tract bleeding; hematemesis is common	Pseudoxanthomatous changes of the skin of the flexural areas; angioid streaks of the retina
Hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu syndrome)	1-AD	9q34 *ENG* 12q13 *ALK-1*	Mucocutaneous telangiectases with acute or chronic blood loss	Epistaxis, cutaneous telangiectasias, arteriovenous malformations in the lung, liver, and brain
Klippel–Trenaunay–Weber syndrome	Sporadic/AD	5q13.3 VG5Q	Diffuse cavernous hemangiomas of the colon causing GI bleeding	Varicose veins, large cutaneous hemangiomas with hypertrophy of underlying soft tissues and bone
Blue rubber bleb nevus syndrome	AD	?	Hemangiomas of the nasopharynx and GI tract	Hemangiomatous skin lesions of the trunk and upper extremities
Idiopathic colonic varices	AD	?	GI bleeding from colonic varices in the absence of portal hypertension	
Hermansky–Pudlak syndrome	AR	10q23 *HPS*	Inflammatory bowel disease and defective platelet aggregation predispose to GI bleeding	Partial albinism, restrictive lung disease; common in Puerto Ricans
Cutaneous photosensitivity and colitis	AR	?	GI bleeding secondary to severe colitis; refractory diarrhea	Early cutaneous photosensitivity, causing erythematous, vesicular lesions of the skin

AD, autosomal dominant; AR, autosomal recessive; GI, gastrointestinal.

diathesis with deficient clot retraction and abnormal platelet morphology. Mucosal bleeding is typical, and gingival bleeding, epistaxis, and laryngeal bleeding are common features. An autosomal dominant form of Glanzmann thrombasthenia has similar features. Bernard–Soulier syndrome (i.e., platelet glycoprotein Ib deficiency) is an autosomal recessive bleeding disorder characterized by unusually large platelets and a prolonged bleeding time. The platelet membrane is abnormal and lacks a receptor, glycoprotein Ib, involved in the adhesion of platelets to subendothelium and von Willebrand factor; patients are susceptible to mucosal bleeding. Thromboxane synthase deficiency, leading to platelet aggregation abnormalities, is an autosomal recessive disorder that may present with petechiae, bruising, epistaxis, hematuria, or massive GI bleeding. The Hermansky–Pudlak syndrome is also characterized by abnormal platelets and is discussed in a later section of this chapter.

Neurofibromatosis (see also Chapter 64)

Of the two major types of neurofibromatosis, NF2 is not typically associated with significant GI manifestations. NF1 and a probable allelic variant of NF1, previously called NF3, known as familial intestinal neurofibromatosis, can be associated with GI hemorrhage because of ulceration of neurofibromas in the lumen of the bowel. Major characteristics of NF1 include multiple cutaneous café au lait macules, multiple neurofibromas or plexiform neurofibromas, axillary or inguinal freckling, optic gliomas, multiple Lisch nodules (i.e., iris hamartomas), and bony dysplasia of the sphenoid or long bones. Occasionally, an individual with NF1 may present with obstructive GI symptoms secondary to having large internal plexiform neurofibromas extending throughout their thoracoabdominal cavity. Arterial occlusive disease also has been reported in NF and may be the underlying etiology of GI complaints in affected individuals.

NF1 is an autosomal dominant disorder displaying significant intrafamilial and interfamilial variability and occurs in 1 per 4000 persons of all ethnic groups. New mutations are thought to account for up to 50% of affected individuals. The large NF1 gene maps to chromosome 17q12. DNA-based diagnostic testing detects approximately 90% of mutations. The National Neurofibromatosis Foundation (and its local chapters) provides educational materials, support, and newsletters to families and physicians.

Hereditary hemorrhagic telangiectasia/Osler–Weber–Rendu syndrome

Hereditary hemorrhagic telangiectasia (HHT) is a genetically heterogeneous autosomal dominant disorder of high penetrance characterized by mucocutaneous telangiectases. Two major genetic forms, HHT1 and HHT2, are recognized. The usual presenting feature is epistaxis occurring at puberty. The cutaneous telangiectases appear later, usually between 5 and 20 years of age. Blood loss from the mucosal lesions in the gut can be chronic or acute. Larger vessel involvement is common. Pulmonary arteriovenous malformations occur in approximately 15%–20% of individuals, especially in those families with HHT1 whose disease is caused by mutations in the endoglin gene, *ENG*, on chromosome 9q34, and may lead to embolic complications or high-output cardiac failure. HHT2 maps to 12q13 where mutations have been identified in *ALK-1*. Arteriovenous malformations occur less often, in the cerebral and hepatic vessels.

Klippel–Trenaunay–Weber syndrome

The Klippel–Trenaunay–Weber syndrome is usually sporadic, but may be autosomal dominant. It is characterized by varicose veins and large, cutaneous hemangiomas with hypertrophy of the underlying bones and soft tissues. Diffuse cavernous hemangiomas of the colon are found in 1%–12.5% of patients and can sometimes cause significant GI bleeding. Partial colectomy with endoscopic laser ablation of residual abnormal colonic mucosa has been successful in the management of this complication. Mutations in or gain-of-function translocation involving *VG5Q*, which maps to chromosome, 5q3.13, have been identified.

Blue rubber bleb nevus syndrome

The blue rubber bleb nevus syndrome is an autosomal dominant disorder characterized by the presence of easily compressible bladder-like, hemangiomatosis skin lesions that refill quickly after compression and have the look and feel of rubber nipples. The sites most commonly involved are the trunk and upper extremities, but the mucosa of the nasopharynx and GI tract, particularly the small bowel, can be affected. Bleeding is an important complication of these lesions. Nocturnal pain and regional hyperhidrosis may also be features of this disorder.

Ehlers–Danlos syndrome

Connective tissue disorders in which the integrity of structural tissues is impaired may predispose to GI bleeding. Ehlers–Danlos syndromes (EDS) are a heterogeneous group of connective tissue disorders affecting the skin, ligaments, joints, blood vessels, and internal organs, depending on the structural protein or modifying enzyme affected. There are six major subtypes of EDS that have been described, most of which are autosomal dominant, although the dermatospraxis and kyphoscoliosis forms are autosomal recessive. The nomenclature for EDS subtypes has been revised to define the "classic" forms, EDS I and EDS II (where EDS II is a milder form) as characterized by soft, velvety, hyperextensible skin, lax joints, easily bruised skin, atrophic scarring, venous varicosities, hernias, and prematurity caused by rupture of fetal membranes. GI bleeding caused by hollow organ (i.e., bowel) and arterial rupture is not a usual manifestation but may occur. Some cases of EDS I and EDS II have demonstrated alterations in the type V collagen gene, *COL5AI*, which

maps to chromosome 9q. An increased incidence of irritable bowel syndrome and diverticulitis has been observed in some individuals with the hypermobility type of EDS, also known as EDS type III, but specific risks for these GI symptoms in EDS have not been established.

The "vascular" form of EDS, also known as EDS IV, is associated with increased morbidity and early mortality caused by bowel and large vessel ruptures. Individuals most often have thin, fragile skin with easily visible veins and friable tissues. They bruise easily, have tight skin over the fingers, face, and ears (acrogeria), and hyperextensible distal interphalangeal joints. Defects in the type III collagen gene, *COL3A1*, which maps to chromosome 2q31, are responsible for the phenotype. Unfortunately, no specific treatment is available for EDS IV and it is not uncommon for individuals to die during late adolescence or early adulthood owing to rupture of the bowel, large arterial vessels, or uterus during pregnancy. Cases have been reported where the ruptured bowel could not be appropriately repaired at surgery owing to the extensive friability of the tissue that was unable to adequately hold sutures. Analysis of skin fibroblasts for type III collagen synthesis and secretion has been the diagnostic method of choice for vascular EDS although direct DNA sequencing is available. When a specific DNA mutation is identified in a family, sensitive DNA-based mutation testing can be done. Unfortunately, even with early diagnosis, no specific therapy is available to prevent rupture of hollow organs and vessels. However, appropriate genetic counseling about risks and strategies to minimize catastrophic complications, especially during pregnancy, can be offered to individuals.

Pseudoxanthoma elasticum

Pseudoxanthoma elasticum primarily affects the skin of the neck, axillae, and other flexural areas; the Bruch membrane of the eye, resulting in angioid streaks on funduscopic examination and ultimate retinal detachment with visual loss; and the arteries, producing GI and urinary tract hemorrhage, precocious calcification, and occlusive vascular changes. Recurrent, severe hematemesis was the presenting feature of one of the first reported cases. Hematemesis is much more common than melena; superficial hemorrhages and erosions may be seen in the stomach. Bleeding tends to occur as early as the first decade of life, before the skin and eye changes are evident. Pregnant women are more susceptible to bleeding.

The diagnosis is made on the basis of clinical examination but there is widely variable expression. Allelic autosomal dominant and autosomal recessive forms have been reported. The dominant types are characterized by peau d'orange skin changes, severe vascular complications, severe choroiditis, angioid streaks, retinal detachment, macular or focal changes in the skin (which may be very stretchy), myopia, a high-arched palate, blue sclerae, and loose joints. The recessive types may be clinically similar. Patients have been reported with generalized skin changes with no ocular or vascular abnormalities.

Mutations in the multidrug resistance gene, *ABCC6* (ATP-binding cassette, sub-family C, member 6), on chromosome 6p13 have been identified in individuals with both autosomal dominant and autosomal recessive forms of the disease.

Syndromes characterized by hamartomas, macrocephaly, multiple lipomas, or hemangiomas

(see also Chapter 64)

A collection of several allelic autosomal dominant syndromes, including Bannayan–Zonana syndrome, Bannayan–Riley–Ruvalcaba syndrome, Cowden syndrome, Riley–Smith syndrome, and Ruvalcaba–Myhre–Smith syndrome, present with similar features but exhibit genetic variability as well as both locus heterogeneity. Typical features include macrocephaly, pseudopapilledema, multiple lipomas, multiple hemangiomas involving the skin and viscera, thyroid nodules, and intestinal hamartomatous polyposis. The latter two features create a susceptibility to GI hemorrhage. Males may have diagnostic hyperpigmented macules on the penis. High birth weight and length are typical, but growth levels off at 6–7 years of age. There may be delayed motor development, autistic features, cognitive delays, and lifelong problems with coordination. Seizures have been reported from intracranial hemorrhage caused by arteriovenous malformations; such arteriovenous malformations may also lead to extremity overgrowth, necessitating amputation. In adults, higher incidences of breast, thyroid, colon, renal, endometrial, and skin cancers have been reported, making this a cancer predisposition syndrome for which preventive screening and management should be employed.

Mutations in the *PTEN* gene, a tumor suppressor gene first identified in association with Cowden syndrome that maps to chromosome 10q23, have also been demonstrated in individuals with Bannayan–Riley–Ruvalcaba, Bannayan–Zonana, Riley–Smith, and Ruvalcaba–Myhre–Smith syndromes, demonstrating that these are indeed, allelic conditions. Mutations in the *BMPR1A/ALK3* gene, a member of the transforming growth factor-β/*BMP* superfamily, on 10q22, have been identified in some families with these conditions. They have also been noted in some kindreds with the inherited juvenile polyposis syndrome, a similar but distinct hamartoma syndrome with an increased predisposition to colon cancer. Mutations in the *MAD4/DPC4* gene located on 18q21 have been associated with familial juvenile polyposis, although early reports suggested that juvenile polyposis can be caused by mutations in the *PTEN* gene. Atypical juvenile polyps with mixed features of hamartomas and adenomas are characterized by a variant of the juvenile polyposis syndrome known as the hereditary mixed polyposis syndrome. This form maps to chromosome 6q16. Further molecular characterization of

the hamartomatous syndromes is needed to more precisely clarify the relationships between these conditions. It has been suggested that if *PTEN* mutations are identified in a patient that the most useful diagnosis may be the "*PTEN* hamartoma syndrome" to reduce the confusion associated with the multiple allelic syndromes that have been associated with *PTEN* alterations.

Cutis laxa

Several genetically heterogeneous forms of cutis laxa have been described. Cutis laxa type I is an autosomal recessive disorder characterized by abnormalities of the elastic tissue and features diaphragmatic and other hernias; diverticula of the GI and urinary tracts, which are susceptible to bleeding; and pulmonary emphysema, which may lead to cor pulmonale. Death usually occurs in the first year of life. Mutations in *FNBL5* on the long arm of chromosome 5 have been implicated in some forms of the diease. The second less severe form is characterized by prenatal and postnatal growth deficiency, large fontanelles with delayed closure, congenital hip dislocation, and lax joints. Diminished elastin production has been verified in these patients, and quantification reveals decreased elastin messenger RNA, perhaps caused by decreased rates of transcription or degradation of unstable transcripts. A dominant form of cutis laxa also associated with mutations in the *FBLN1* gene or the elastin, *ELN*, gene, does not have the visceral manifestations characteristic of the recessive forms. X-linked cutis laxa is discussed in the section on disorders of vitamin and mineral assimilation because it is associated with copper abnormalities. All forms of the syndrome also feature diverticular disease of the GI tract, increasing the possible risk for GI hemorrhage.

Hermansky–Pudlak syndrome

Hermansky–Pudlak syndrome is an autosomal recessive disease that occurs most frequently in Puerto Rico, where the prevalence is 1 in 2000 individuals. It is a storage disorder of a ceroid-like substance and is associated with features of partial albinism, restrictive lung disease, and a platelet abnormality. There is a deficiency of the granule storage pool in platelets, causing defective ADP release, which leads to a prolonged bleeding time and defective platelet aggregation. Inflammatory bowel disease with an onset between the ages of 12 and 30 years is also common in Puerto Rican patients. The combination of inflammatory bowel disease and defective platelets contributes to the occurrence of GI bleeding in these patients. The gene for this disorder, *HPS*, maps to chromosome 10q23.

Intestinal motility or pseudoobstruction disorders

(see also Chapter 47)

Intestinal pseudoobstruction may result from intrinsic enteric myopathies and neuropathies or from generalized muscular and neurological disorders. This section reviews the genetic disorders that feature abnormal intestinal motility or pseudoobstruction in each of these categories. These disorders are summarized in Table 108.5.

Intestinal pseudoobstruction is suggested by recurrent signs and symptoms of intestinal obstruction in the absence of mechanical obstruction, as proven by appropriate radiological, endoscopic, or surgical investigations. The presence and severity of complications depend on the individual's age, site of involvement, and degree of functional impairment. In the adult, a long-standing history of symptoms suggesting GI motility dysfunction – such as dysphagia, early satiety, and constipation or diarrhea – may precede the onset of acute symptoms. Patients usually present in the second to fourth decades of life with complaints of abdominal pain, distention, and sometimes vomiting. Malnutrition is common and results from decreased food intake, impaired digestion, or malabsorption. Infants with these disorders usually present with acute, severe, obstructive symptoms of abdominal pain and distention after feeding. Reflux esophagitis and aspiration pneumonia caused by impaired gastroesophageal motility is also common in infants.

After mechanical obstruction has been ruled out, diagnostic studies to evaluate pseudoobstruction include esophageal and intestinal manometry, gastric emptying studies, and full-thickness biopsy with silver stains. Esophageal manometry shows aperistalsis and incomplete relaxation of the lower esophageal sphincter. Intestinal manometry usually shows a hypomotile pattern. Gastric emptying studies show a delay for solids and a variable pattern for liquids. Silver stains delineate neuropathic from myopathic forms. Detection of subclinically affected family members at risk can be accomplished with these diagnostic techniques.

Visceral myopathy syndromes

Clinical GI manifestations of the visceral myopathic and neuropathic forms of intestinal pseudoobstruction are similar. In the myopathic form, there may be other evidence of myopathy, such as megacystis, vesicoureteral reflux, ophthalmoplegia, ptosis, and small intestine diverticulosis. Pathogenetic mechanisms of GI manifestations include degenerative changes and thinning of intestinal smooth muscle, especially of the longitudinal layers; the enteric neurons are normal. A mitochondrial myopathy associated with intestinal pseudoobstruction caused by a visceral myopathy has been described. Some forms of visceral myopathy may be mitochondrial myopathies. Diagnosis of a mitochondrial myopathy can often be made by a Gomori trichrome-stained skeletal muscle biopsy, which shows the characteristic ragged, red fibers, or by analysis of respiratory chain enzymes. There are no well-documented reports of a mitochondrial DNA mutation responsible for intestinal pseudoobstruction. The reported familial cases are autosomal dominant or recessive, lacking the maternal pattern of transmission seen with mitochondrial

Table 108.5 Intestinal motility and pseudoobstruction disorders

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Visceral myopathies				
Visceral myopathy with megaduodenum or megacystitis	AD	?	Intermittent abdominal pain, distention, vomiting, constipation, and diarrhea, usually presenting in the 1st to 2nd decade	Megacystitis occurs without obstruction but with vesicoureteral reflux, predisposing to urinary tract infections; diagnosis often made in adults. Family study can prevent unnecessary diagnostic surgery for symptomatic relatives
Visceral myopathy with external ophthalmoplegia	AR	?	Chronic diarrhea, abdominal distention and pain, dilated duodenum and jejunum with diverticulosis	Ptosis, ophthalmoplegia, proximal muscle weakness, and atrophy; mitochondrial myopathy may accompany or be responsible for disorder, usually adult onset
Megacystitis–microcolon–intestinal hypoperistalsis syndrome	AR	15q24 *PABPN1*	Microcolon and dilated small intestine with symptoms of obstruction; abdominal distention and bilious vomiting	Symptom onset in infancy; marked dilation of the bladder, hydronephrosis, external appearance of prune belly, multiple cardiac rhabdomyomas; 4 : 1 female preponderance, possibly due to increased severity in males; prenatal diagnosis by ultrasound identifying distended GI tract and bladder; death usual by 3 years from malnutrition of sepsis
Hereditary internal anal sphincter myopathy	AD	?	Severe anal pain, constipation	Onset in 3rd to 5th decades; noncompliant, thickened internal anal sphincter
Duchenne muscular dystrophy	XLR	Xp21 *DYS*	Bloating, abdominal fullness, delayed gastric emptying, acute gastric dilation, chronic intestinal pseudoobstruction	Skeletal muscle degeneration, causing severe debilitating weakness caused by deficiency of the gene product dystrophin
Myotonic dystrophy	AD	19q13 *DM*	Problems with swallowing, aspiration, and constipation; more severe complications occur with congenital myotonic dystrophy with gastroparesis; subacute obstruction, megacolon, and constipation	Delayed muscular relaxation, ptosis, cataract, gonadal atrophy, cardiac, conduction defects, and male pattern baldness; disease gene localized to chromosome 19p; maternal transmission associated with more severe phenotype of congenital myotonic dystrophy with mental retardation, neonatal hypotonia, and facial diplegia
Oculopharyngeal muscular dystrophy	AD	14q11–13	Dysphagia	Ptosis; weakness and wasting of facial, neck, and distal limb muscles; mitochondrial abnormalities seen on electron microscopy
Kearns–Sayre syndrome	mt deletions	mt	Swallowing difficulties	Progressive external ophthalmoplegia, pigmentary retinopathy, cardiac conduction defects caused by cytochrome C oxidase deficiency; deletions in the mitochondrial DNA responsible for the phenotype; all reported cases are sporadic
Visceral neuropathies				
Deficiency of argyrophil myenteric plexus	AR ?AD	?	Intermittent abdominal pain, distention and vomiting, functional intestinal obstruction; short, small intestine, malrotation, and pyloric hypertrophy caused by failure of the development of the myenteric plexus	Ataxia, abnormal pupillary reflexes, dysarthria, absent deep tendon reflexes, peripheral neuropathy and autonomic neuropathy; eosinophilic nuclear inclusions in the myenteric neurons, brain, spinal cord, and celiac plexus ganglia

Microcolon	AR	?	Obstruction, abdominal distention, vomiting	Aganglionosis of the entire colon and distal small intestine leads to microcolon; may be secondary to congenital atresia or mechanical obstruction (e.g., meconium ileus)
Hirschsprung disease microcephaly, and iris coloboma	AD multifactorial	10q11, others *RET, others*	Aganglionic megacolon with constipation and abdominal distention	Risks to family members increase with severity and number of female relatives affected
Hirschsprung disease, microcephaly, and iris coloboma	AR	?	Aganglionic megacolon	Microcephaly, hypertelorism, iris coloboma, submucous cleft palate, short stature, learning problems; similar features described in chromosome deletion syndromes; high-resolution karyotype recommended
Hirschsprung disease with bilateral bicolored irides	AR	22q13 *SOX10* 20q13 *EDN3*	Aganglionic megacolon	Heterochromia, two distinct colors present in the same iris
Hirschsprung disease with polydactyly, renal agenesis, and deafness	AR	?	Aganglionic megacolon	Polydactyly, unilateral renal agenesis, hypertelorism, and congenital deafness
Hirschsprung disease with ulnar polydactyly, polysyndactyly of big toes, and ventricular septal defect	AR	?	Aganglionic megacolon	Ulnar polydactyly, polysyndactyly of big toes, and ventricular septal defect
Hirschsprung disease with hypoplastic nails and dysmorphic facial features	AR	?	Aganglionic megacolon	Hypoplastic nails, minor dysmorphic facial features, bilateral hydronephrosis, imperforate anus, inguinal hernia; death in infancy
Hirschsprung disease and Ondine curse	?AR ?AD	?	Short segment and total colonic aganglionosis	Idiopathic, congenital hypoventilation; possible abnormality of the neural crest
13q-syndrome	Chromosomal		Aganglionic megacolon	Growth deficiency, microcephaly, mental deficiency, abnormal facies with prominent nasal bridge, hypertelorism, ptosis, epicanthal folds, microphthalmia, colobomas, retinoblastoma, prominent maxilla, prominent, slanting, low-set ears, micrognathia, hypoplastic or aplastic thumbs, cardiac defects, club feet, hypospadias, cryptorchidism
Down syndrome	Chromosomal	Trisomy 21	Aganglionic megacolon in 5.9% of cases	Hypotonia, protruding tongue, joint laxity, short stature, mental retardation, speckling of irides (i.e., Brushfield spots), small ears, flat facial profile, upslanting palpebral fissures, redundant neck skin, single transverse palmar crease, 5th finger clinodactyly, hypothyroidism; leukemia in 1% of cases
Cartilage–hair hypoplasia (metaphyseal chondrodysplasia, McKusick type – several types of metaphyseal chondroplasia exist)	AR	9p13 *CHH*	Megacolon with constipation	Dwarfing syndrome with radiographic changes called metaphyseal dysostosis; fine, sparse, light-colored hair, anemia; susceptibility to chickenpox and malignancies
Waardenburg–Shah syndrome	AR	20q13	Intestinal obstruction with dilated proximal ileum, collapse of distal ileum and colon	White forelock, white eyebrows and eyelashes, isochronia irides (i.e., mosaic pattern in light brown irides)

Table 108.5 (*cont'd*)

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Waardenburg syndrome	AD	2q35 *PAX3*	Short segment aganglionosis megacolon	Dystopia canthorum, broad nasal root, white forelock, white skin patches, deafness. This and the Waardenburg–Shah syndrome may represent a single dominant gene with pleiotropic effects. A more severe phenotype exists in homozygotes
Familial achalasia and diffuse esophageal spasm	AR		Retrosternal discomfort, progressive dysphagia, intermittent regurgitation, diverticulosis of the esophagus	Severity varies in family members. HLA studies suggest an immunogenetic mechanism in the pathogenesis of this disorder.
Achalasia–microcephaly syndrome	AR	?	Onset of dysphagia and regurgitation due to achalasia in early childhood	Microcephaly and mental deficiency
Congenital deafness with vitiligo and achalasia	AR	?	Dysphagia and regurgitation caused by achalasia	Deafness, short stature, and vitiligo; variable expressivity; some family members with only vitiligo and achalasia
Groll–Hirschowitz syndrome	AR	?	Dysphagia, nausea, diverticula of the small bowel	Cachexia; protein and fat malabsorption, progressive sensory neuropathy, tachycardia, and abnormal GI motility secondary to vagus nerve involvement; progressive nerve deafness, with neurological deficit ascribed to a process of demyelinization
POLIP syndrome (myoneuro-gastrointestinal encephalopathy)	mt	mt	Severe GI dysmotility caused by visceral neuropathy with chronic malabsorption and malnutrition	Progressive sensorimotor peripheral neuropathy, cranial neuropathy (i.e., external ophthalmoplegia and deafness), abnormal cerebral and cerebellar white matter, endoneural fibrosis and demyelination, ragged red fibers in skeletal muscles, partial defect of cytochrome C oxidase
Familial dysautonomia (Riley–Day syndrome)	AR	9q31–33	Dysphagia, emesis, and aspiration pneumonia	Failure to thrive, dehydration secondary to GI manifestations, autonomic instability
Hereditary coproporphyria (for other porphyrias see Chapter 87)	AD	3q12 *CPO*	Nausea, vomiting, abdominal pain and distention, constipation caused by autonomic neuropathy	Pruritic skin blisters, weakness and paresthesias of the extremities caused by a peripheral neuropathy, elevated fecal coproporphyrins with normal erythrocyte uro-1-synthase and α-aminolevulinic acid dehydratase
Multiple endocrine neoplasia type 2b (MEN-2B)	AD	10q11 *RET*	Chronic constipation with megacolon or diarrhea, frequently developing before the endocrine neoplasms	Neuromas from lips to rectum; prominent lips, nodular tongue, thickened eyelids, medullary thyroid carcinoma, pheochromocytoma, marfanoid habitus
Optiz G syndrome	AD, XLR	22q11, Xp22	Esophageal dysmotility and laryngeal tracheal clefts causing swallowing difficulties; imperforate anus	Mild to moderate mental deficiency, hypertelorism, widow's peak, hypospadias, cryptorchidism, bifid scrotum, and hernias

AD, autosomal dominant; AR, autosomal recessive; GI, gastrointestinal; HLA, human leukocyte antigen; mt, mitochondrial; XLR, X-linked recessive.

DNA mutations. If a mitochondrial myopathy is involved, it is likely that the disease gene responsible in most cases resides in the nucleus.

Duchenne muscular dystrophy

Duchenne muscular dystrophy is an X-linked disorder characterized by skeletal muscle degeneration causing atrophy and weakness. The incidence in male births is about 1 in 3500. Onset of symptoms usually begins before 5 years of age, with progressive symptoms of muscle weakness, toe walking, waddling gait, pseudohypertrophy of the calves, and difficulty in rising from sitting and in climbing stairs. By puberty joint contractures and scoliosis develop and most patients require use of a wheelchair. Death usually occurs in the early 20s from respiratory infections or heart failure. Laboratory studies show elevated serum levels of aldolase, lactate dehydrogenase, and creatine kinase. Common GI symptoms include abdominal pain and distention, delayed gastric emptying, acute gastric dilation, and chronic intestinal pseudoobstruction. The large disease gene, encompassing over 2 Megabases of DNA, responsible for Duchenne muscular dystrophy maps to Xp21 and encodes a protein found in skeletal muscle called dystrophin, which helps stabilize the cytoskeleton of skeletal muscle. Dystrophin is often lacking in affected boys and partially deficient in female carriers. DNA diagnosis is readily available. The majority of mutations are large deletions that can be easily detected by polymerase chain reaction-based DNA diagnostic methods. Prenatal DNA diagnosis, in vitro fertilization with donor eggs, and preimplantation diagnosis with in vitro fertilization are viable options for families wishing to prevent the birth of an affected child. Obviously, improved treatments are desperately needed and many hope that efforts in gene therapy will lead to more human trials in the near future.

Myotonic dystrophy

Myotonic dystrophy is an autosomal dominant disorder characterized by delayed muscular relaxation, facial weakness and muscle atrophy, ptosis, cataracts, gonadal atrophy, cardiac conduction abnormalities, and male pattern baldness. Significant GI complications are caused by abnormal functioning at all levels of the GI tract, from the pharynx to the anal sphincters; this results from degenerative changes in smooth muscle and fatty infiltration, causing problems with swallowing, aspiration, and constipation. The more severe phenotype of congenital myotonic dystrophy only occurs with alleles of maternal origin. The congenital form, often inherited from a mildly affected mother, features mental retardation, hypotonia, facial diplegia, gastroparesis, subacute intestinal obstruction, megacolon, and constipation.

The disease gene maps to 19q13 and involves a CTG trinucleotide repeat in the three untranslated regions of the gene. Unaffected individuals have 5–27 copies of the repeat, whereas affected individuals have minimally 50 or more repeats with severity increasing as the repeat copy number increases to several kilobases. Expansion of the repeat can occur in subsequent generations, providing a molecular basis for the clinical phenomenon of anticipation (i.e., earlier clinical onset of disease in subsequent generations) in this disorder. DNA testing is sensitive, specific, and relatively easy to perform, thereby providing a specific way to confirm a suspected clinical diagnosis noninvasively. Other disorders presenting with myotonia caused by different genetic alterations can present with GI symptoms similar to those previously described.

Kearns–Sayre syndrome

Kearns–Sayre syndrome is a mitochondrial disorder characterized by progressive external ophthalmoplegia, pigmentary retinopathy, cardiomyopathy, and cardiac conduction defects; weakness of facial, trunk, and extremity muscles; and deafness, small stature, electroencephalographic changes, and increased cerebrospinal fluid protein. GI symptoms relate to the pharyngeal muscle weakness experienced by affected individuals, causing swallowing difficulties and dysphagia. The mitochondrial mutations characteristic of this disorder are deletions ranging in size from 2 to 7 kilobases and can be easily detected by analysis of mitochondrial DNA.

Visceral neuropathy syndromes

The visceral neuropathic forms of intestinal pseudoobstruction are characterized by abnormal pupillary reflexes, ataxia, and dysarthria; peripheral nervous system abnormalities, including absent deep tendon reflexes and impaired vibratory and position sense; and autonomic neurological abnormalities such as inappropriate blood pressure responses to phenylephrine, Valsalva maneuver, upright posture, and lack of sweating on warming. One type is also associated with basal ganglia calcification and mental retardation. Histological sections of tissues stained with a silver stain reveal degenerative changes of myenteric plexus neurons.

Hirschsprung disease (see also Chapter 56)

Hirschsprung disease is probably a multifactorial disorder that usually occurs as an isolated congenital anomaly but may be a syndromic association of several genetic conditions (see Table 108.4). With isolated Hirschsprung disease, the risk to family members increases with severity (i.e., length of GI tract involved) and the number of affected relatives, especially female relatives. Empiric risk estimates are 7.2% for the siblings of affected women and 2.6% for the siblings of affected men. Complex segregation analysis demonstrated Mendelian inheritance patterns when the length of affected bowel was considered. Cases with aganglionosis beyond the sigmoid colon had a mode of inheritance that was compatible with an autosomal dominant trait with incomplete penetrance. Cases with aganglionosis no more proximal than the sigmoid colon had inheritance patterns consistent with multifactorial inheritance or autosomal recessive inheritance

with very low penetrance. Isolated, nonsyndromic familial forms of the disease have demonstrated mutations in the *RET* protooncogene, which maps to chromosome 10q11.

Familial achalasia and diffuse esophageal spasm

Familial occurrence of achalasia, an autosomal recessive disorder, involves the esophagus, causing symptoms of progressive dysphagia, retrosternal discomfort, and intermittent regurgitation. The cause of achalasia is unknown. Diverticulosis of the esophagus may also occur. The severity of the disorder exhibits intrafamilial variability. Esophageal ganglion cells and dorsal motoneurons of the vagal nucleus are decreased, and there is Wallerian degeneration of the vagus nerve.

Familial dysautonomia

Familial dysautonomia, also called Riley–Day syndrome, is an autosomal recessive disorder, involving the autonomic nervous system that is most prevalent in the Ashkenazi Jewish population, with an incidence of about 1 in 3500 individuals. It is characterized by autonomic instability, including lack of tearing, emotional lability, paroxysmal hypertension, increased sweating, cold hands and feet, corneal anesthesia, red blotching of the skin, and drooling. GI manifestations include oropharyngeal incoordination, abnormal esophageal motility and decreased lower esophageal sphincter pressure, prolonged gastric emptying, and gastroesophageal reflux, all of which result in dysphagia, emesis, and aspiration pneumonia. Affected individuals are reluctant to eat and suffer from dehydration and failure to thrive. Fundoplication and gastrostomy has some documented benefit in these cases. Diagnosis is determined by a lack of axon flare after intradermal injection of histamine, absence of fungiform papillae on the tongue, miosis of the pupil after instillation of methacholine chloride (2.5%), absent deep tendon reflexes, and diminished tear flow. The disease gene, *IKBKAP*, maps to chromosome 9q31 and linkage disequilibrium is present within Ashkenazi Jews, with a high-risk haplotype recognized.

Mechanical obstruction and malformation syndromes

Most genetic disorders that cause mechanical obstruction are related to congenital malformations, and in general they have more importance in the pediatric population than they do for adults. However, as noted earlier in this chapter, some disorders with potential mass effects, such as large abdominal plexiform neurofibromas in NF1, may cause mechanical obstructive symptoms in rare instances. Another more common genetic cause for mechanical obstruction not caused by malformation is meconium ileus seen in 10%–15% of CF patients. The anatomic site or sites of developmental malformation in these syndromes is usually restricted to derivatives of the embryological foregut, midgut, and hindgut. In a rostral to caudal distribution, Table 108.6 reviews genetic malformation syndromes that may present with symptoms secondary to a mechanical obstruction.

Chronic abdominal pain syndromes

Many genetic syndromes can present with recurrent bouts of abdominal pain, including familial Mediterranean fever (FMF), Fabry disease, hereditary angioneurotic edema, hereditary fructose intolerance, and the hereditary pancreatitis syndromes (Table 108.7). Although these syndromes (except for FMF) are relatively rare; making the correct diagnosis in a symptomatic individual may prevent unnecessary and invasive procedures and may similarly benefit other family members. The porphyrias are also associated with significant recurrent abdominal pain as discussed in Chapter 56.

Familial Mediterranean fever

FMF is an autosomal recessive disorder with protean manifestations and an obscure cause that has a frequency of approximately 1 in 2700 in Israel, a country in which it has been studied extensively. Half of affected individuals are of Sephardic Jewish descent, about 20% are Armenian, 20% are Turkish or Arabic, and the remaining patients are Italian, Greek, or of Ashkenazi Jewish descent. FMF occurs rarely in Northern Europeans. Approximately 50% of patients do not have a positive family history.

Manifestations usually appear during childhood or adolescence and are characterized by brief episodic febrile attacks, recurring at varying intervals and associated with painful inflammation involving a variety of serosal surfaces, including the abdomen, chest, joints, and skin, lending the alternative name of recurrent familial polyserositis. Attacks last typically for 1–2 days and occur once or twice each month. The natural history of the attacks can vary considerably, even in an individual patient. Clinical features include fever, abdominal pain, and signs of peritonitis in most patients. The peritonitis is nonspecific so other acute febrile conditions must be considered, such as appendicitis, cholecystitis, pancreatitis, and intestinal obstruction. Less constant features include pleuritic pain, mild arthritis of the large joints, and a transient erysipelas-like skin lesion on the lower extremities. In 1% of patients, meningitis also occurs. Arthritis is an episodic monarthritis or oligoarthritis of the large joints, mimicking oligoarthritic forms of juvenile rheumatoid arthritis with attacks usually lasting days to weeks. They may last for months and can be associated with radiographic changes of periarticular osteopenia without erosions.

Laboratory test results are nonspecific during the attacks of pain and fever. The leukocyte count averages 16 000 cells/μL but may be as high as 40 000 cells/μL. The erythrocyte sedimentation rate increases, as do other acute-phase reactants. Albuminuria and microscopic hematuria also occur. Radiographic studies may show bowel edema and air–fluid levels in the small bowel, causing confusion with obstruction. The

Table 108.6 Mechanical obstruction and malformation syndromes

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features/comments
VATER association	Sporadic	?	Tracheoesophageal fistula with esophageal atresia and anal atresia	Vertebral defects; radial, renal, and cardiac abnormalities
Hiatal hernia and congenital short esophagus	AD	?	Gastroesophageal reflux, esophagitis	
Galloway syndrome	AR	?	Reflux with vomiting of feedings from birth	Microcephaly, large ears, nephrotic syndrome, renal microcystic dysplasia and focal glomerulosclerosis; death by age 3 years
Tracheoesophageal fistula with or without esophageal atresia	?Multifactorial	?	Aspiration, dysphagia	
Leiomyoma of vulva and esophagus	AD?	?	Dysphagia caused by an obstructing lesion (e.g., leiomyoma)	Vulvar lesions, clitoromegaly caused by leiomyoma involvement at its base
Abnormal hands, feet, short palpebral fissures, microcephaly, and esophageal or duodenal atresia	AD	?	Polyhydramnios, duodenal atresia secondary to annular pancreas, tracheoesophageal fistula	Microcephaly, upslanting, short palpebral fissures, fifth finger clinodactyly, syndactyly of fourth and fifth toes
Familial short rib syndrome, Beemer type	AR	?	Pyloric stenosis, short bowel, malrotation	Short stature, short ribs, severe respiratory failure, hydrops
Epidermolysis bullosa dystrophica, Hallopeau–Siemens type	AR	3p21 *COL7A1*	Mucosal surfaces may be involved with bullae, leading to esophageal stricture and perforation	Destructive bullae develop on hands, feet, elbows, and knees with secondary joint contractures; conjunctiva and cornea may be involved caused by excessive collagenase activity; disease gene maps to chromosome 11q
Epidermolysis bullosa lethalis with pyloric atresia	AR	17q11–q34 *ITGB4* *ITGA6*	Membranous diaphragmatic obstruction at the pylorus, diarrhea, protein-losing gastroenteropathy	Bilateral stenosis at the uterovesical junction, causing hydronephrosis; prenatal diagnosis by demonstration of dermal–epidermal separation at the lamina lucida level in fetal skin
Pyloric atresia	AR	?	Dysphagia	Congenital pyloric atresia due to reduction of the pylorus to a fibrous band or obstruction from a membranous diaphragm
Pyloric stenosis	AD, multifactorial	12q24.2 *NOS1*	Projectile vomiting at about 1 month of age	Recurrence risk of about 10% for male relatives and 2% for females; male predominance
Smith–Lemli–Optiz syndrome	AR	7q31 *SLO*	Vomiting secondary to pyloric stenosis, failure to thrive	Dysmorphic facial features, microcephaly, mental retardation, hypotonia, hypospadias, syndactyly of second and third toes, defect in cholesterol metabolism, low total cholesterol, may respond to increased cholesterol intake
Familial intrathoracic gastric volvulus	AD	?	Sharp, epigastric discomfort, occult blood loss, liquid dysphagia	? Same as hiatus hernia
Duodenal atresia	AR	?	Bilious vomiting	May be seen in Down syndrome (5.9% of cases)
Annular pancreas	Ad	?	GI obstruction with vomiting caused by duodenal stenosis	

Table 108.6 (*cont'd*)

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Jejunal atresia	AR	?	Abdominal pain and distention	Agenesis of the mesentery leading to distal small bowel coming straight off cecum and twisting around the marginal artery, creating an apple-peel appearance seen at surgery; primary abnormality may be obliteration of the superior mesenteric artery
Volvulus of midgut	AD	?	Abdominal pain, distention, and constipation	Present in infancy or early childhood; atresia of the ascending colon may be present
Fryns syndrome	AR	?	Duodenal atresia, pyloric hyperplasia, malrotation and common mesentery in about 50% of patients	Diaphragmatic defects, lung hypoplasia, cleft lip and palate, cardiac defects, renal cysts, urinary tract malformation, distal limb hypoplasia, hypoplastic external genitalia, Dandy–Walker brain anomaly, agenesis of the corpus callosum
Lissencephaly syndrome	AR vs. chromosomal deletion	17p13 *PAFAH1B1*	Midgut, malrotation, gastric volvulus	Postaxial and preaxial ray defects; renal anomalies
Multiple intestinal atresia	AR	?	Multiple atretic lesions from stomach to anus	Intraluminal calcifications seen on radiographs
Cornelia de Lange syndrome	Sporadic AD	3q26 *CDL1*	Intestinal atresia in a minority of patients	Hirsutism, synophrys, broad nasal root, micrognathia, malformed ears, abnormal hands, congenital heart disease, severe mental retardation; 2%–5% recurrence risk
Anosacral anomalies	X-linked	?	Imperforate anus	Anterior sacral meningocele, anal canal duplication cyst
Imperforate anus	X-linked	?	Imperforate or ectopic anus	Rectovaginal fistula, hypospadias, hearing loss
Townes–Brocks syndrome	AD	16q12 *SALL1*	Imperforate anus	Triphalangeal thumbs and other anomalies of the hands and feet, mild, sensorineural deafness, lop ears, renal anomalies
Cat-eye syndrome	Chromosomal	22q11	Imperforate anus	Vertical pupil caused by iris coloboma, preauricular tags or fistulae, cardiac and urinary tract abnormalities, mental deficiency; extra chromosomal material, probably a derived dicentric chromosome 22
Optiz G syndrome	AD	XLR 22q11, Xp22	Imperforate anus, pharyngeal incoordination with regurgitation	Mild to moderate mental deficiency, hypertelorism, widow's peak, hypospadias, cryptorchidism, bifid scrotum, hernias
Rieger syndrome	AD	4q25–26 *RIEG*	Anal stenosis	Iris coloboma, broad nasal root, umbilical malformation, glaucoma
Ulnar mammary syndrome	AD	12q24 *TBX3*	Anal atresia and pyloric stenosis	Abnormalities of the fourth and fifth fingers, hypoplastic ulna, small penis, delayed puberty, obesity

AD, autosomal dominant; AR, autosomal recessive; GI, gastrointestinal; VATER, vertebral defects, imperforate anus, tracheoesophageal fistula with esophageal atresia, and radial and renal dysplasia; XLR, X-linked recessive.

Table 108.7 Chronic or recurrent abdominal pain syndromes

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features/comments
Familial Mediterranean fever	AR	16p13 *MEFV*	Recurrent bouts of abdominal pain	Recurrent fever, pain in chest and joints caused by a serositis; elevated leukocyte count, erythrocyte sedimentation rate, renal amyloidosis; mapped to chromosome 16p
Fabry disease	XLR	Xq22 *GLA*	Abdominal pain	A lysosomal storage disease caused by α-galactosidase deficiency; accumulation of lipoid material in the skin, corneas, vessels, kidneys, heart, and ganglion cells of the autonomic nervous system; skin lesions called angiokeratoma characteristic but nonspecific
Hereditary angioneurotic edema	AD	11q11–13 *C1NH*	Abdominal pain	Choking, laryngeal edema; c1 esterase inhibitor deficiency is underlying defect; androgens useful in prophylaxis against attacks; mapped to chromosome 11p
Hereditary fructose intolerance	AR	9q22 *ALDOB*	Abdominal pain, vomiting after fructose ingestion	Kidney and liver damage, growth retardation, coma and death on chronic exposure to fructose; caused by aldolase B deficiency; mapped to chromosome 9q
Hereditary pancreatitis	AD	7q32-ter *PRSS1* 7q31 *CFTR*	Severe abdominal pain	Fever, elevation of serum amylase, pancreatic insufficiency, diabetes, pseudocyst; hemorrhagic pleural effusions and portal or splenic vein thrombosis possible
Deficiency of pancreatic stone protein (PSP)	AD	7q35 *PRSS1* *SPINK1*	Chronic calcifying pancreatitis	PSP inhibits $CaCo_3$ crystal growth; PSP gene maps to chromosome 2p
Familial hypertrophy of the sphincter of Oddi	AD	?	Chronic pancreatitis	Rare but a surgically correctable cause of pancreatitis
Pancreatitis, sclerosing cholangitis, and sicca complex	AR	?	Pancreatitis	Dry eyes and mouth; HLA-DR3 associated with primary sclerosing cholangitis
Lipoprotein lipase deficiency	AR	8q22 *LPL*	Recurrent attacks of abdominal pain, chronic pancreatitis	Massive hyperchylomicronemia on a normal diet; hepatosplenomegaly, eruptive xanthomas, lactescent plasma; early atherosclerosis not a feature; heterozygotes show slight hyperlipernia, maps to chromosome 8p
Apolipoprotein C-II deficiency	AR	19q13 *APOC2*	Chronic pancreatitis	A necessary cofactor for lipoprotein lipase; clinically and biochemically similar to lipoprotein lipase deficiency except xanthomas and hepatosplenomegaly are less common
Glycogen storage disease type 1 (Von Gierke disease)	AR	17q21 *G6PT*	Chronic pancreatitis caused by hyperlipidemia, hepatosplenomegaly, hepatic adenomas, which may undergo malignant degeneration	Defect in glucose-6-phosphatase; hypoglycemia, lacticacidemia and ketonemia; hyperlipidemia causing xanthomas; hyperuricemia causing gout, renal insufficiency and proteinuria. Dietary therapy consists of continuous nocturnal infusions and frequent administration of raw cornstarch; survival to adulthood is reduced
Homocystinuria (cystathionine β-synthase deficiency)	AR	21q22 *CBS*	Pancreatitis with pseudocyst formation	Marfanoid habitus, ectopia lentis, osteoporosis, arterial and venous thromboembolic events, elevated urine methionine and homocystene

AD, autosomal dominant; AR, autosomal recessive; HLA, human leukocyte antigen.

diagnosis is made in patients with the appropriate ethnic background, who have typical, self-limited, and recurrent attacks of fever and abdominal pain.

The most severe feature of this disease is the progressive accumulation of a specific protein, known as amyloid fibrillar protein, in the kidney, which manifests clinically as a nephropathy. There is considerable ethnic variation in the incidence of amyloidosis in FMF. Amyloidosis occurs least frequently in Armenians with the disorder, even though FMF occurs most commonly in Armenians (1 in 400). Amyloidosis occurs commonly in non-Ashkenazi Jews (1 in 2400) and is most common in Moroccan Jews in Israel (up to 30%). In some patients, the amyloidosis develops before any other clinical sign. The genetic or environmental factors that account for these differences in the incidence of amyloidosis are not clear. The amyloidosis appears to be a phenomenon secondary to the periodic inflammation, because its occurrence is reduced by colchicine therapy.

Colchicine (0.6 mg), administered orally, two to three times per day, is an effective treatment in the prevention of the acute febrile attacks of the disease. A low-fat diet (20 g/day) may also prevent the attacks. Colchicine does not allay symptoms if taken during attacks. Some patients use it intermittently to forestall attacks when they recognize that one is imminent.

FMF seems to involve a genetically determined defect in the regulation of inflammatory responses. Research into the cause of FMF has included studies of suppressor T cells, leukocyte chemotaxis, lysosome release from neutrophils, and immunoglobulins, all without a definitive identification of the pathophysiological defect. The FMF susceptibility gene, *MEFV* or the pyrin gene, maps to chromosome 16p13, and both diagnostic and predictive testing of at-risk family members is possible. Although clinical heterogeneity exists, as reflected by the different prevalence rates of renal amyloid in different ethnic groups, there is no evidence for locus heterogeneity, because the FMF gene is linked to the same locus in the non-Ashkenazi Jewish and Armenian populations.

Fabry disease/diffuse angiokeratoma

Fabry disease, also called diffuse angiokeratoma, is an X-linked recessive lysosomal storage disorder caused by a deficiency of α-galactosidase. Deposition of lipoid material in several tissues is responsible for the characteristic findings. These include: attacks of pain in the abdomen and extremities (probably caused by lipid changes in ganglion cells of the autonomic nervous system), vascular lesions in the fundi of the eye and the kidney, corneal opacities, a hypertrophic cardiomyopathy, and characteristic skin lesions of a vascular nature called angiokeratoma. Symptoms may present in adulthood, and death usually occurs after renal failure in midadulthood. Female heterozygotes may have renal insufficiency and proteinuria, and rarely have skin lesions.

α-Galactosidase activity is deficient in the leukocytes of male patients, and carrier females can be identified because of decreased activity. Several mutations in the α-galactosidase gene, *GLA*, at Xq22 in patients with Fabry disease have provided the definitive evidence to indicate that this is the gene for Fabry disease. Enzyme replacement therapy is available to treat affected individuals.

Hereditary angioneurotic edema

Hereditary angioneurotic edema is an autosomal dominant disorder that affects the respiratory and GI tracts. Symptoms of choking occur because of airway edema that can be precipitated by acts of trauma, such as tracheal intubation. GI symptoms include abdominal pain, nausea, vomiting, and diarrhea. Barium studies during an acute attack show bowel wall edema. Associated findings include an increased incidence of autoimmune disorders.

Deficiency of C1 esterase inhibitor is the underlying pathophysiological defect in these patients. This protein regulates the first component of complement (C1) by inhibition of the proteolytic activity of its subcomponents C1r and C1s, preventing activation of C4 and C2 by C1s and inhibiting other serine proteases, including plasmin, kallikrein, and the coagulation factors XIa and XIIa. Low levels of C4 and normal levels of C1 are characteristic of hereditary angioneurotic edema. These low levels of C4 are responsible for the increased incidence of systemic lupus erythematosus, glomerulonephritis, and vasculitis among these patients. Heterozygotes have about 10%–20% of the normal levels of C1 inhibitor rather than the expected 50%, possibly because of complex formation of the activated C1 inhibitor and activated C1 or because of increased catabolism of the protein. Androgens are effective in stimulating increased synthesis of C1 inhibitor from the normal allele and are useful for prophylaxis against attacks. Concentrates of C1 inhibitor are useful in acute attacks.

The C1 inhibitor gene, *C1NH*, maps to chromosome 11q11–q13. Two clinically indistinguishable types of hereditary angioneurotic edema are described. Patients with type 1 have a deletion of the C1 inhibitor gene or a truncated transcript, and patients with type 2 have a single base substitution.

Hereditary fructose intolerance

Hereditary fructose intolerance (HFI) is an autosomal recessive disorder caused by a deficiency of aldolase B (i.e., fructose-1-phosphate aldolase). This enzyme is expressed in tissues that metabolize fructose: the liver, kidney, and small intestine. It catalyzes the reversible cleavage of fructose-1-phosphate into dihydroxyacetone phosphate and glyceraldehyde. After fructose, sorbitol, or sucrose ingestion, patients with HFI present with severe abdominal pain, vomiting, and hypoglycemia. Liver and kidney damage, growth retardation, coma, and even death may result from chronic ingestion of fructose. Most patients with HFI learn to avoid foods containing fructose. Problems arise when they are unable to

avoid them, as during infancy, or if given intravenous fructose- or sorbitol-containing infusions for dehydration resulting from diarrhea and vomiting associated with illness, a practice carried out in some European countries. Intravenous fructose loading tests and measurement of aldolase B activity in liver biopsy specimens are methods used for the diagnosis of HFI.

The aldolase B gene, *ALDOB*, maps to chromosome 9q22. It consists of nine exons that code for 363 amino acids. At least 10 mutations have been described; three missense mutations of *A149P*, *A147D*, and *N334K* account for 87% of alleles in the European HFI population and 68% in the North American population. The *A149P* mutation is the most common and widespread, accounting for 58% of HFI alleles in Europe and 55% in North America. Linkage studies suggest that the *A149P* allele arose once during evolution and spread through the mechanism of genetic drift, rather than occurring several times in different populations. Testing for the common mutations with amplified DNA specimens against a panel of allele-specific oligonucleotides would be an appropriate initial diagnostic approach that could identify most patients in a noninvasive manner.

Hereditary pancreatitis syndromes (see also Chapter 72)

Hereditary pancreatitis is an autosomal dominant disorder that is similar clinically to nonfamilial chronic pancreatitis. Steatorrhea and recurrent attacks of severe abdominal pain, fever, and marked elevation of serum amylase levels are characteristic. The elevated amylase level differentiates the disorder from FMF. The mean age at onset is 13 years. Between 5% and 10% of affected persons have pancreatic insufficiency, diabetes, and pseudocysts. As in nonsyndromic pancreatitis, emotional stress, alcohol, or a diet that is rich in fat can precipitate an attack.

The disorder exhibits 80% penetrance. The gene for hereditary pancreatitis is the cationic trypsinogen gene, *PRSS1*, which maps to chromosome 7q32-qter. One predominant mutation in codon 117 results in an arginine to a histidine substitution that can easily be detected by restriction digestion. Codon 117 is a trypsin-sensitive site and when the arginine 117 cleavage site is replaced by histidine, trypsin continues to activate trypsinogen and other zymogens, ultimately leading to pancreatitis secondary to autodigestion of the pancreas. Mutations in the cystic fibrosis gene, *CFTR*, have also been associated with chronic pancreatitis when present in heterozygotes in association with the variable number of thymidines in intron 8, specifically the *5T* allele.

Other genetic syndromes involving obstruction of the pancreatic duct, such as secretory pancreatic stone protein deficiency, familial hypertrophy of the sphincter of Oddi, or sclerosing cholangitis, pancreatitis, and sicca complex, can present as cases of familial or recurrent pancreatitis. Recurrent pancreatitis caused by a submucosal ampullary tumor has also been described in a patient with neurofibromatosis. Cases of hereditary or recurrent pancreatitis may be caused by genetic disorders of triglyceride metabolism, leading to hypertriglyceridemia, such as lipoprotein lipase deficiency, apolipoprotein C-II deficiency, and familial hypertriglyceridemia.

Gastrointestinal neoplasm syndromes (see also Chapters 36, 54, 64, 65)

Exciting and important applications of genetics to oncology and gastroenterology have led to the elucidation of genetic contributions to many GI malignancies. Details about GI and hepatic malignancies are further described in Chapters 36, 54, 64, 65, 93. Genetic disorders have been associated with malignancies in the esophagus, stomach, small and large bowel, pancreas and rectum as summarized in Table 108.8 and the general features that suggest an inherited predisposition to cancer are presented in Table 108.9. Although most cancers are sporadic, approximately 10% are caused by the inheritance of an autosomal dominant germ-line mutation in a tumor suppressor or DNA recognition and repair gene leading to a significantly increased risk of developing cancer at a relatively early age. The clinical features of GI malignancies are covered in many other chapters of this volume and will not be discussed further here. An outline of genetic syndromes that are associated with increased risks for GI tract neoplasms is given in Table 108.10. Predictive genetic testing and early preventive management may be useful for several of these conditions (Table 108.11). Increasingly, prognostic genetic tests will be used in oncology to help direct clinical management of patients.

Syndromes associated with benign hepatic masses or cysts

Many genetic conditions are associated with benign hepatic masses or cysts. While the hepatic manifestations often do not result in significant clinical symptoms, recognition of the hepatic manifestations may be helpful in making the diagnosis of the genetic syndrome. Of the many multisystemic genetic syndromes that may be associated with benign hepatic lesions, the autosomal dominant disorders polycystic kidney disease, tuberous sclerosis complex, and von Hippel–Lindau syndrome are three conditions where clinical recognition is of paramount importance to ensure that affected patients receive appropriate medical management. von Hippel–Lindau syndrome is presented in Table 108.10.

Polycystic kidney disease

There are both autosomal dominant and autosomal recessive polycystic kidney diseases. In general, autosomal recessive polycystic kidney diseases present very early in life, whereas the autosomal dominant forms generally present later with progression to end-stage renal disease around the fifth decade of life. While the main manifestations of polycystic kidney disease involve multiple cysts in the kidneys bilaterally with

Table 108.8 Specific cancer sites in the gastrointestinal tract of the hereditary cancer predisposition syndromes[a]

Site of cancer	Genetic condition/syndromes[b]	Gene(s) or locus (loci)[c]
Colon	Familial adenomatous polyposis (FAP)	*APC*
	Hereditary breast/ovarian cancer	*BRCA1, BRCA2*
	Hereditary nonpolyposis colon cancer (HNPCC)	*MSH2, MLH1, PMS1, PMS2, MSH6*
	Juvenile intestinal polyposis syndrome	*SMAD4, PTEN*
	Peutz–Jeghers syndrome	*STK11*
Esophageal	Familial Barrett esophageal cancer	?
	Familial keratosis/tylosis with esophageal cancer	*TOC*
Pancreas	Familial pancreatic cancer	*SMAD4*
	Hereditary breast/ovarian cancer 2	*BRCA2*
	Hereditary melanoma	*CDKN2, CDK4*
	HNPCC	*MSH2, MLH1, PMS1, PMS2, MSH6*
	Li–Fraumeni syndrome	*TP53*
Small bowel	FAP	*APC*
Stomach	Familial gastric cancer	*CDH1*
	HNPCC	*MSH2, MLH1, PMS1, PMS2, MSH6*
	Li–Fraumeni syndrome	*TP53, CHK2*

a Syndromes are listed alphabetically, not in order of frequency of cancer at that site.
b Other cancer predisposition syndromes may be associated less frequently with cancer at any of the sites.
c While the main genes implicated to date for each cancer predisposition syndrome are listed, additional genes will likely be discovered that may contribute to the syndrome.

Table 108.9 Findings suggestive of an inherited cancer predisposition syndrome

A clear inheritance pattern is suggested in the family history
One particular type of cancer, or a limited number of different cancers, segregates in the family
Cancer diagnoses occur at earlier ages when compared with the onset in the general population
Family member has an identified genetic mutation associated with a cancer predisposition syndrome
There is an unusual constellation of tumor(s)
Multiple primaries in a single individual
Bilateral cancers in paired organs of an individual
Occurrence of relatively rare cancers not commonly seen in the general population clustering within a family
Multiple recognized precursor lesions are present in the individual or family members
Cancers are associated with other nonmalignant physical features or medical problems
Constellation of cancers or other features that are part of a known cancer predisposition syndrome

renal failure, hepatic cysts and colon diverticula may also develop. In addition, patients with the autosomal dominant form of adult polycystic kidney disease may have vascular complications including a high risk of intercranial aneurysms and an increased prevalence of cardiac valve disease. In general, the hepatic cysts are asymptomatic but the kidney cysts progress to end-stage renal disease in later adulthood.

There is locus heterogeneity for autosomal dominant polycystic kidney disease with mutations in the *PKD1* gene at chromosome 16p13 and *PKD2* gene at chromosome 4q21–q23. In addition, there have been some families reported that do not map to either the chromosome 16 or chromosome 4 locus, suggesting that there is at least one additional gene that predisposes to autosomal dominant polycystic kidney disease. In general, management of the disease consists of management of the renal complications with dialysis and kidney transplants.

Tuberous sclerosis complex

Tuberous sclerosis complex is a multisystemic autosomal dominant disease with significant variable expression among affected individuals, even those within the same family. Several cutaneous features, including facial angiofibromas, hypopigmented macules, Shagreen patches, subcutaneous

Table 108.10 Intestinal neoplasm syndromes

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Keratosis palmaris et plantaris with esophageal cancer	AD	17q24–25	Esophageal cancer	Palms and soles are diffusely hyperkeratotic; onset in first year of life; low serum vitamin A level in some cases; distinct from keratosis palmaris et plantaris familiaris because of the esophageal cancer association
Dyskeratosis congenita	XLR	Xq28 *DKC*	Leukoplakia of the oral mucosa and sometimes of anal mucosa	Cutaneous pigmentation, continuous lacrimation caused by atresia of the lacrimal ducts, nail dystrophy, testicular atrophy, thrombocytopenia and anemia, opportunistic infections
Neurofibromatosis type I (NF1)	AD	17q12 *NF1*	Malignant degeneration of intestinal neurofibromas, duodenal carcinoid containing somatostatin	Multiple café au lait macules, neurofibromas, axillary and inguinal freckling, multiple Lisch nodules, optic gliomas, bony dysplasia of the sphenoid and long bones; associated neoplasms include pheochromocytoma, meningiomas and malignant degeneration of neurofibromas
Neurofibromatosis type III (NF3)	AD	?17q12 ?*NF1*	Malignant degeneration of intestinal neurofibromas	None of the other features seen in NF1
Carney triad	?AD		Gastric leiomyosarcomas	Pulmonary chondromas and paraganglionomas; all cases are sporadic
Basal cell nevus syndrome (Gorlin–Goltz syndrome)	AD	9q22 *PTCH*	Hamartomas of the stomach	Many basal cell nevi usually appear at puberty, rarely at birth. Basal cell nevi may develop at sites of therapeutic amounts of ionizing radiation. Associated neoplasms include medulloblastoma, astrocytoma, and ovarian fibroma. Other features include mild prognathism, lateral displacement of the inner canthi, frontal and biparietal bossing, odontogenic keratocysts of the jaw, kyphoscoliosis, abnormal cervical spine, calcified falx cerebri, short fourth metacarpal, and palmar and plantar pits
Cowden syndrome (multiple hamartoma syndrome)	AD	10q23 *PTEN*	Hamartomatous polyps of the intestines	Multiple hamartomas of the skin, mucous membranes, breast, and thyroid; verrucous skin lesions of the face and limbs; papules of the gingiva and buccal mucosa, multiple facial trichilemmomas, macrocephaly; females have high frequency of breast cancer
Cronkhite–Canada syndrome	unknown	?	Intestinal polyposis	Diffuse skin hyperpigmentation, alopecia, fingernail changes; all cases sporadic with no proof of genetic basis; possible phenocopy for the genetic polyposis syndromes; unclear if genetic
Multiple lipomatosis	AD	12q15 ?others	Gastrointestinal lipomas	Multiple subcutaneous lipomas, cytogenetic abnormalities within the lipomas are common. If chromosome 12q is involved, there is a tendency for malignant degeneration.

Table 108.10 (*cont'd*)

Condition	Inheritance pattern	Chromosome location/gene locus	Gastrointestinal manifestations	Other features and comments
Multiple endocrine neoplasia type I (MEN1)	AD	11q13 *MEN1*	Severe peptic ulcer disease caused by Zollinger–Ellison syndrome secondary to gastrinoma; duodenal carcinoid in some cases	Triad of pancreatic (i.e., insulinomas, gastrinomas), parathyroid, and pituitary adenomas; intrafamilial uniformity of tumor types usual; MEN2 lacks pancreatic adenomas and has pheochromocytomas; MEN1 disease gene localized to chromosome 11q
Multiple endocrine neoplasia type IIb (MEN2B)	AD	10q11 *RET*	Multiple true neuromas throughout the GI tract; megacolon and colonic diverticulosis common; duodenal carcinoid in some cases	Hypertrophy of lips and thickened eyelids from neuroma, pheochromocytoma, medullary thyroid cancer; prophylactic thyroidectomy indicated; no parathyroid disease; disease gene is localized to chromosome 10
Intestinal carcinoid	AD	see *MEN*	Familial incidences of appendiceal and ileal carcinoid reported	Duodenal carcinoid seen in MEN1, MEN3, and NF1
Pancreatic carcinoma	AD	9p21 p16	Pancreatic acinar carcinoma	No history of pancreatitis or tumors at other sites
von Hippel–Lindau disease	AD	3p25–26 *VHL*	Pancreatic cysts and pancreatic carcinoma	Cardinal features are angiomas of the retina and hemangioblastoma of the cerebellum. Other features include hypertension caused by pheochromocytoma; hypernephroma-like renal tumors; polycythemia due to renal tumor or cerebellar hemangioblastoma; hemangiomas of the spinal cord, lungs, and adrenals; cysts of the pancreas, liver, and kidneys; and epididymal cystadenomas. The gene has been mapped to chromosome 3p, and direct mutational analysis is available in about 15% of cases.
Ataxia-telangiectasia (AT)	AR	11q22 *ATM*	Pancreatic carcinoma	Progressive cerebellar ataxia; telangiectases, especially of the conjunctiva; susceptibility to sinopulmonary infection, oculomotor apraxia; strong predisposition to malignancy, especially leukemias. Heterozygotes for AT are also at an increased risk for malignancy. Female heterozygotes exposed to certain types of ionizing radiation are at increased risk for breast cancer. The disease gene maps to chromosome 11q.
Muir–Torre syndrome	AD	2p22 *MSH2* (3p-MLH1)	Multiple, primary, carcinomas of the colon, duodenum, and larynx	Keratoacanthomas of the face, multiple sebaceous tumors

AD, autosomal dominant; AR, autosomal recessive; GI, gastrointestinal; XLR, X-linked recessive.

Table 108.11 Examples of the use of DNA testing and ancillary studies in the evaluation of cancer predisposition syndromes

DNA testing is standard of care in the evaluation
Example: von Hippel–Lindau (VHL) syndrome:
DNA-based testing of *VHL* gene
Very specific, sensitive with a high positive predictive value
Has become standard of care to determine if an asymptomatic individual inherited the condition
Childhood testing appropriate and important
Other studies useful in the baseline evaluation of a patient with a VHL mutation:
Imaging studies:
Abdominal ultrasound or CT examination of kidneys, adrenals, liver (teenagers, adults)
MRI of the brain and spine (teenagers, adults)
Laboratory studies:
Urinalysis (children, teenagers, adults)
BUN and creatinine (children, teenagers, adults)
Urinary catecholamine metabolites or plasma metanephrine (children, teenagers, adults)
Focused examinations in addition to general physical examination:
Testicular examination (teenagers, adults)
Ophthalmological examination (children, teenagers, adults)
Detailed neurological examination (children, teenagers, adults)
Audiology

DNA testing is widely used, readily available, and helpful in the evaluation
Example: Familial adenomatous polyposis:
DNA-based testing of *APC* gene
> 90% sensitive; false-negative test results possible, identification of a mutation in an affected family member improves predicative value of test in asymptomatic individuals
May be appropriate to do DNA testing before any other baseline evaluations of patient
Testing in children is appropriate
Other studies useful in baseline evaluation of individuals with mutation or at-risk asymptomatic individuals where mutation status is not known or cannot be determined with certainty:
Imaging studies:
Colonoscopy
Laboratory studies:
Complete blood count
Occult fecal blood test
Focused examinations in addition to general physical examination:
Detained cutaneous examination
Ophthalmological examination
Comprehensive neurological examination

DNA testing is of limited clinical value
Example: Tuberous sclerosis (TS):
DNA-based testing of *TSC1* or *TSC2* genes
Locus heterogeneity decreases test sensitivity and predictive value
Not widely employed in the diagnostic evaluation of patients given clinical diagnosis often easily recognizable
Childhood testing may be appropriate if specific mutation is detected in an affected parent
Other studies are usually needed to determine if individuals have TS and assess baseline systemic involvement in affected individuals:
Imaging studies:
Head CT or MRI (any age)
Renal ultrasound (any age)
Chest radiograph (any age)
Echocardiogram (any age)
Laboratory studies:
Urinalysis (children, teenagers, adult)
BUN and creatinine
Focused examinations in addition to general physical examination:
Wood lamp examination of the skin (all age)
Ophthalmological examination (children, teenagers, adults)
Detailed neurological examination (all ages)

BUN, blood urea nitrogen; CT, computed tomography; MRI, magnetic resonance imaging.

nodules, café au lait spots, and subungual fibromas, suggest the diagnosis. Retinal patches and astrocytomas can be identified on ophthalmology examinations. Dental examinations reveal pitted dental enamel and gingival fibromas. More serious complications include cardiac rhabdomyoma, which may present early in childhood or infancy and neurological problems as the result of subependymal nodules, cortical tubers, and intracranial calcification that can be detected with head imaging studies and may lead to infantile spasms, seizures, and mental retardation in a significant number of patients. Cysts and angiomyolipomas are often seen in the kidneys and may also be identified in the livers of patients. The lesions in the liver are usually asymptomatic. In addition, lymphangiomyomatosis can also be identified in the lung and it is important to identify them in women of childbearing age. Patients are at increased risk for renal carcinoma.

There is locus heterogeneity for this autosomal dominant condition with mutations in the *TSC1* gene on chromosome 9q34 and mutations in the *TSC2* gene at chromosome 16p13 accounting for the vast majority of cases. Although this condition is autosomal dominant, many patients are thought to possess new mutations and, therefore, there may be no family history of this disease. The *TSC* genes encode proteins that function as tumor suppressors.

Disorders of bile pigment metabolism

Several genetic disorders of bile pigment metabolism have been described. Although they vary in severity and clinical symptoms, most present with some degree of jaundice. The most commonly observed disorders are presented in Table 108.12.

Examples of inborn errors of metabolism with hepatic manifestations (see also Chapter 87)

Most of the inborn errors of metabolism are autosomal recessive or X-linked recessive disorders that present with multisystemic complications. Many are associated with a variety of hepatic findings including storage of precursor metabolic products, cirrhosis, or hepatomegaly. Classic metabolic diseases involving the GI tract and the liver are discussed in Chapter 109. Many so-called metabolic disorders have been presented earlier in this chapter (i.e., hereditary fructose intolerance, Fabry disease, hemochromatosis). A few additional diseases will be discussed subsequently to further illustrate the scope of different hepatic and GI manifestations that may be associated with inborn errors of metabolism. Although many inborn errors of metabolism present early in infancy or childhood, some do not present until later years.

Wilson disease

Wilson disease is an autosomal dominant disorder caused by mutations in the ATPase copper ion transporting β-polypeptide gene (*ATP7b*) which is associated with high nonceruloplasmin-bound serum copper levels. High urinary copper levels are also seen, as well as a low serum ceruloplasmin. Patients present with a variety of neurological and abdominal complaints. Often the onset of symptoms is in late childhood, adolescence, or early adulthood. Neurological symptoms include dementia, poor motor coordination, personality changes, dystonia, dysarthria, tremors, dysphagia, and decreased concentration. The main abdominal findings that can occur involve the liver and may include atypical hepatitis, cirrhosis, hepatomegaly, hepatic coma, and high liver copper levels. Esophageal varices can also arise. Renal tubular dysfunction and kidney stones may be seen. Loss of protein, amino acids, glucose, uric acid, phosphates, and calcium is often identified in the urine. Hypoparathyroidism and hemolytic anemia may also be observed. One characteristic ophthalmological finding is Kayser–Fleischer rings.

The diagnosis is based on analyzing serum copper levels and ceruloplasmin. *ATP7b* gene mutations lead to a dramatic build up of intracellular hepatic copper with subsequent and significant hepatic and neurological abnormalities. The gene maps to 13q14-q21. Treatment involves reducing serum copper levels. Agents including penicillamine and zinc have been successful in decreasing plasma copper levels.

Medium-chain acyl-coenzyme A dehydrogenase deficiency

Medium-chain acyl-coenzyme A dehydrogenase (MCAD) is a relatively common autosomal recessive inborn error of fatty acid metabolism frequently seen in populations with the homozygote frequency estimated to be 1 in 6500 to 1 in 20 000, depending on ethnic background. White subjects of Northern European decent exhibit the highest frequency of MCAD-deficient genotypes. Patients deficient for MCAD are at high risk of hypoglycemia, vomiting, lethargy, encephalopathy, cardiorespiratory arrest, hepatomegaly, seizures, apnea, and sudden death in infancy. In fact, patients with this condition often die undiagnosed with sudden infant death syndrome. Long-term outcomes may include developmental and behavior abnormalities, muscle weakness, cerebral palsy, and attention deficit disorder. Most often, infants are born healthy but present with symptoms after a fast or illness. It is in these times of metabolic stress caused by fasting or infection when the demands for fatty acid oxidation are high and patients may present with progressive symptoms. The MCAD gene is located on chromosome 1p31 and encodes an enzyme that is responsible for the metabolism of medium-chained fatty acids. Newborn screening for this condition is offered in several states, as recognition of the condition at the time of birth may allow for prevention of symptoms and sudden infant death syndrome by treating infections promptly and avoiding fasting conditions. Supplemental treatment, including carnitine therapy, may also be beneficial.

Table 108.12 Genetic disorders of bile pigment metabolism

Condition	Inheritance pattern	Chromosome location/gene locus	Hepatic and gastrointestinal manifestations	Other features and comments
Defects in bilirubin metabolism or processing				
Crigler–Najjar syndrome type I	AR	2q37 *UGT1A1*	Unconjugated hyperbilirubinemia with normal liver histology and routine liver function tests; no bilirubin uridine diphosphoglucuronate glucuronsyltransferase activity	Jaundice with kernicterus, rare in population, phenobarbital does not reduce serum bilirubin levels, liver transplant has been curative in affected patients
Crigler–Najjar syndrome type II	AR	2q37 *UGT1A1*	Unconjugated hyperbilirubinemia with milder elevations than in Crigler–Najjar type II with normal liver histology and routine liver function tests; up to 10% of normal bilirubin uridine diphosphoglucuronate glucuronsyltransferase activity	Usually benign jaundice without kernicterus
Gilbert syndrome	AR	Variant promoter of *UGT1A1*	Benign mild unconjugated hyperbilirubinemia with normal liver histology and routine liver function tests; up to 40% of normal bilirubin uridine diphosphoglucuronate glucuronyltransferase activity	Common (3%–7% of population) inherited form of mild chronic fluctuating unconjugated hyperbilirubinemia
Dubin–Johnson syndrome	AR	10q23–24 *MRP2* (*ABCC2*)	Predominantly conjugated hyperbilirubinemia with black liver grossly and dark pigments in centrilobular areas on histology; routine liver function studies normal except bilirubin	Chronic benign nonhemolytic mild jaundice
Rotor syndrome	AR	22q13 SGLT1 or SLC5A1	Predominantly conjugated hyperbilirubinemia with normal liver and routine liver function studies normal except bilirubin	Increased urinary coproporphyrin
Progressive familial intrahepatic cholestasis: type I, Byler disease, type II, and type III	AR	Type I: 18q21 *FIC1* Type II: 2q24 *SPGP* (*ABCB II*) Type III: *MDR3* (*ABCB4*)	Type I associated with life-threatening cholestasis first described in Amish-Mennonite community; type II has a similar phenotype but seen more commonly in European and Middle East populations; type III causes severe cholestasis due to progressive destruction of small bile ducts	All may present with very severe disease and limited treatment options are available
Alagille syndrome	AD	20p12 *JAGGED*	Congenital developmental anomaly of the hepatobiliary system characterized by paucity of bile ducts and resulting in infantile cholestasis in some cases	Other features include characteristic mild facial dysmorphisms, butterfly vertebrae, posterior embryotoxon of eye, and cardiac anomalies

AD, autosomal dominant; AR, autosomal recessive; ABC, ATP-binding cassette transporter. For a regularly updated website of human ABC transporters with links, see http://www.med.rug.ml/mdl/humanabc.htm

Tyrosinemia

Tyrosinemia type 1 is an autosomal recessive condition caused by mutations in the fumarylacetoacetate hydrolase (*FAH*) gene, which maps to chromosome 15q23–q25 and is required for the metabolism of tyrosine. Build up of tyrosine in the body can create multiple problems including failure to thrive, hypertrophic cardiomyopathy, renal Fanconi syndrome, renal failure, kidney stones, rickets, muscle weakness, episodic paralysis and peripheral neuropathy, abnormal blood coagulation studies, and a variety of abdominal problems. Hepatic problems that may occur include hepatomegaly, liver failure, cirrhosis, and associated ascites. Pancreatic islet-cell hypertrophy may also occur, as may splenomegaly. Gastrointestinal bleeding and paralytic ileus may also occur. Analysis of plasma amino acids is diagnostic. In addition, elevated α-fetoprotein, hypoglycemia, and hypophosphatemia can also be detected.

Symptoms are highly variable and patients may have a progressive course that rapidly leads to hepatic failure whereas others may have acute and chronic hepatic crises. Hepatocellular carcinomas occur at high frequency in affected patients. The highest incidence of this condition is in the Saguenay-Lac St Jean region of Quebec, Canada, and Northern Europe. Patients with this condition are often described as having an unusual "cabbage-like" odor.

α_1-Antitrypsin deficiency

α_1-Antitrypsin is an autosomal recessive condition with multiple allelic forms. Homozygotes for the *PiZ* form and some other much rarer variants are at a high risk of developing emphysema, particularly if they smoke, in early adulthood. These patients account for approximately 1% of all patients with emphysema. About 10% of the ZZ homozygotes develop liver failure that often leads to fatal childhood cirrhosis. This is the result of an abnormal polymerization of the Z antitrypsin where the abnormal molecule has been poorly secreted in forms insoluble intracellular inclusions that are presumably toxic. In general, the infantile liver abnormalities occur when there is a less than 20% deficiency of the enzyme. Laboratory findings include a low serum α_1-antitrypsin (Pi) deficiency and abnormal liver function tests. Mutations in the protease inhibitor 1 gene on chromosome 14q32 are responsible for the phenotype. Again, multiple different mutations have been identified and different alleles are associated with different degrees of severity of disease. In the most severe form with hepatic failure in infancy, liver transplant has been used to treat this condition.

Common gastrointestinal and hepatic disorders with complex genetic causes

In addition to the many monogenic, mendelian genetic disorders with GI and hepatic manifestations previously reviewed in this chapter, there are several common disorders whose genetic susceptibilities are inherited in a more complex or multifactorial mode. These disorders include conditions such as pernicious anemia, celiac disease (Chapter 50), inflammatory bowel disease (Chapter 57), cirrhosis, gallstone disease, peptic ulcer (Chapter 40), and sporadic colon cancer (Chapter 65). Common diseases often show familial aggregation caused by genetic factors or the combination of genetic and environmental factors. They also exhibit associations with certain genetic markers, often with specific HLA genotypes. Studies of family aggregation, twin data, subclinical markers, and genetic markers have been essential in demonstrating the importance of genetic determinants in the development of these common diseases. Many of the common complex gastrointestinal conditions are seen more frequently in specific genetic syndromes. For example, inflammatory bowel disease has been reported in individuals with Turner syndrome, Hermansky-Pudlak syndrome, glycogen storage disease type Ib, various immunodeficiency disorders, secretory immunoglobulin deficiency, gammaglobulinemia and hypogammaglobulinemia, selective immunoglobulin A deficiency, Chédiak–Higashi syndrome, deficiency of C2, hereditary angioedema, and chronic granulomatous disease. Further elucidation of genetic contributions to common nonmendelian GI and hepatic conditions will undoubtedly improve our understanding of their pathophysiology and may ultimately lead to susceptibility testing and preventive management for some of these conditions, at least in some populations in the near future. All of these common complex GI and hepatic conditions are discussed in great detail in other chapters of this text (see Index) and will not be further discussed here.

References

The following online genetic databases and comprehensive genetic textbooks were used as the sources for all information contained in this overview chapter that summarizes salient features. Specific detailed references for each disease are found in these sources with the *OMIM* and *GeneTests/GeneReviews* databases being most comprehensive and up to date. Information from *OMIM* and *GeneTests/GeneReviews* and its links was used to gather information and generate tables.

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I MISCELLANEOUS

109 Gastrointestinal manifestations of systemic diseases

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Systemic diseases are commonly manifest by gastrointestinal signs or symptoms. The liver or gut may be the principal targets of the disease process or simply indirectly affected; in either case, the gastrointestinal manifestations may be the cause for seeking medical attention. The purpose of this chapter is to examine the systemic disorders that have well-recognized gastrointestinal manifestations and to stress their more important effects on the digestive system.

Cardiovascular diseases

Aortic stenosis

Aortic stenosis predominantly affects men in middle to late life [1], and has been associated with an increased incidence of gastrointestinal bleeding [2], often attributed to angiodysplasias of the gut (Heyde syndrome) [3,4]. Although case series suggest that 15%–41% of patients with bleeding angiodysplasias also have hemodynamically significant aortic stenosis, no such relationship is found when aortic stenosis is sought in patients with nonbleeding colonic angiodysplasias [5]. Thus, the validity of the association between the two conditions has been questioned [6], and probably lies more with the now recognized (below) changes in blood coagulation associated with the abnormal valve that makes the angiodysplasias commonly in the elderly bleed.

Lesions identified by angiography or endoscopy (fiberoptic or capsule) can be incriminated as the bleeding source if active hemorrhage is observed. Endoscopic cautery, angiographic embolization, and intestinal resection remain standard therapy for active bleeding [7]. Aortic valve replacement has resulted in the cessation of bleeding in some cases [8], but the angiodysplasias persist [9]. A probable mechanism is that the majority of patients with severe aortic stenosis have an acquired type 2A von Willebrand syndrome characterized by a deficiency of the largest multimers of von Willebrand factor and impaired shear-induced platelet aggregation, and this is improved by porcine valve replacement [10,11]. There are no evidence-based medical alternatives to surgery.

Congestive heart failure

Impairment of the heart's ability to fill or empty the left ventricle results in inadequate perfusion of tissues and a consequent inability to maintain tissue metabolic demands. Ischemia and hypertension are the most common causes, but valvular heart disease, cardiomyopathies, anemia, and hypermetabolic states (e.g., thyrotoxicosis and sepsis) may be seen [12]. Heart failure leads to congestion of the splanchnic venous bed, causing anorexia, nausea, bloating, and abdominal pain. Mesenteric ischemia of the bowel due to low flow states is not uncommon in such patients [13]. Rarely, venous congestion is sufficiently severe to produce weight loss, diarrhea, malabsorption, protein-losing enteropathy [14,15], and the clinical picture of cardiac cachexia. Hepatic congestion secondary to right heart failure (Fig. 109.1) may mimic biliary tract disease or hepatitis because of jaundice, right upper quadrant pain, hepatomegaly, abnormal liver enzyme levels,

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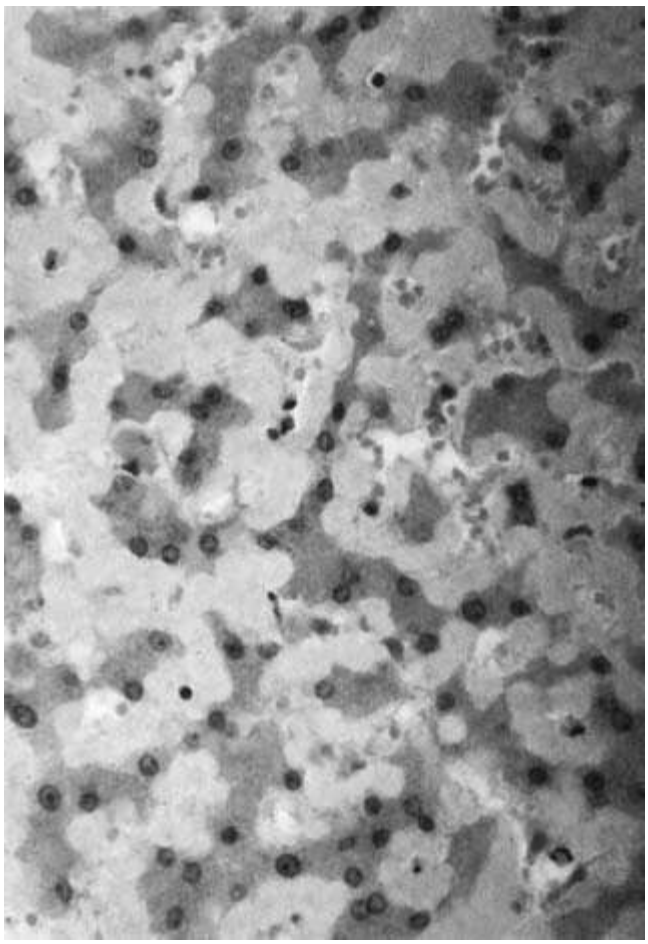

Figure 109.1 Hepatic congestion and cirrhosis secondary to congestive heart failure. (H & E stain; original magnification × 100.)

low protein ascites, and prolongation of the prothrombin time [16]. Prolonged congestion rarely progresses to cardiac cirrhosis [17].

The increasingly effective, but complex, medical therapy of left ventricular failure [18] can produce gastrointestinal side effects (Table 109.1). Mainstays are the angiotensin-converting enzyme (ACE) inhibitors, which are generally well tolerated, but may cause mild diarrhea. Rarely, ACE inhibitors have been associated with bowel obstruction due to angioedema of the intestine, caused by increased bradykinin levels [19]. Angiotensin II receptor blockers can also cause angioedema. Digoxin is less commonly used and causes anorexia, nausea, and vomiting in many patients [20]. Digoxin-associated constriction of splanchnic vessels may contribute to intestinal ischemia [13]. Severe pancreatitis has been attributed to a variety of diuretics, including thiazides, furosemide, and ethacrynic acid [21]. Oral potassium supplements have been implicated in esophagitis, ulcers, and, rarely, intestinal strictures [22].

Coronary artery disease

Although the incidence of coronary artery disease (CAD) has decreased markedly since 1990, it remains the most prevalent cause of death in the United States. It is not uncommon for the initial presenting symptoms of angina or infarction to mimic gastrointestinal disease (epigastric pain or pressure, heartburn), which at times is confusing to both patient and physician. Once the diagnosis of CAD is certain, its treatments may lead to gastrointestinal problems.

Smooth muscle relaxants (calcium channel antagonists, nitrates) improve coronary blood flow but also relax the lower esophageal sphincter and have been associated with the epidemic of gastroesophageal reflux disease (GERD) and esophageal adenocarcinoma over the past two decades [23]. Constipation is also prevalent with the calcium channel antagonists, and attention to bowel function is important. Statins are associated with constipation and elevations of transaminases, but only rarely severe hepatotoxicity [24]. There is no reason to withhold statins from patients with mild liver function abnormalities (e.g., nonalcoholic steatohepatitis [NASH], hepatitis C), but test monitoring should be more rigorous [25]. An interesting association between statin use and reduced risk of colorectal cancer needs confirmation [26].

Aspirin is a foundation in the prevention of myocardial infarction [27,28]. In all trials, there is a small but consistent increase in the risk of gastrointestinal bleeding in the aspirin-treated groups. Although the risk of gastrointestinal bleeding is dose related, even daily doses below 100 mg per day cause irreversible inactivation of platelet prostaglandin synthetase for 7–10 days [29]. Epidemiological studies suggest that the concurrent use of nitrovasodilators reduces this risk substantially [30]. Ticlopidine and clopidogrel, other antiplatelet agents, appear to be more effective than aspirin, are effective in preventing restenosis of coronary stents, are used with increasing frequency, and have a lower risk of gastrointestinal bleeding [31].

Gastrointestinal complications of cardiac bypass surgery (acalculous cholecystitis, acute pancreatitis, mesenteric ischemia, hepatic necrosis, and others) occur more commonly in the elderly and those requiring dialysis [32] Gastrointestinal complications are recognized more frequently as higher-risk patients with multiple comorbidities are chosen for the surgery.

Chromosomal abnormalities and other genetic disorders

Abetalipoproteinemia

Abetalipoproteinemia is an autosomal recessive condition that causes severe hypolipidemia, diarrhea, fat malabsorption, growth failure, retinitis pigmentosa, acanthocytosis, and cerebellar ataxia. The growth failure and diarrhea can be

Table 109.1 Gastrointestinal side effects of drugs for cardiovascular disease

Medication class	Examples	Indication	GI side effects
Selective inhibitor of α-adrenergic agonist	Doxazosin mesylate, Terazosin	HBP	Nausea (C)
Adrenergic stimulants	Clonidine, methyldopa	HBP	Nausea/vomiting (C); hepatotoxicity (R)
α/β-Adrenergic blockers	Labetalol	HBP	Nausea/vomiting (C); dyspepsia/GERD (UC); hepatoxicity (R)
Angiotensin converting enzyme inhibitors	Enalopril, lisinopril	HBP, CHF	Diarrhea/dyspepsia (UC); ileus/pancreatitis (R); hepatotoxicity (R)
Angiotensin II receptor antagonists	Losartan, irbesartan	HBP, CHF, arrhythmias	Constipation (UC); diarrhea (UC)
Antiarrhythmics			
Group 1	Mexilitene, Quinidine, Disopyramide		Nausea/vomiting (C); diarrhea (C); constipation (C)
Group 2	See Beta-blockers		
Group 3	Sotalol, Amiodarone		Nausea/vomiting (C); abnormal liver tests (C);
Group 4	See Calcium channel antagonists		hepatotoxicity (UC)
Beta blockers	Atenolol, metoprolol	CAD, HBP	Diarrhea (UC)
Calcium channel antagonists	Verapamil, Diltiazam, Nifedipine	HBP, CAD	GERD (C); nausea (C); constipation (C); hepatotoxicity (R)
Hypolipidemic agents			
Bile acid binding resins	Cholestyramine	CAD	Dyspepsia (C); flatulence (C); constipation (C)
Cholesterol absorption	Ezetimibe	CAD	Diarrhea (UC); inhibitors
Fibric acid derivative	Gemfibrozil	CAD	Dyspepsia (C); abnormal liver tests (UC); cholestatic jaundice (R)
HMG-CoA reductase inhibitors abnormal liver tests (C); hepatotoxicity (R)	Lovastatin, simvastatin	CAD	Constipation (UC);
Nicotinic acid	Nicotinic acid	CAD	Dyspepsia (C); abnormal liver tests (UC)
Inotropic agents	Digoxin	CHF, arrhythmias	Mesenteric ischemia (R)
Vasodilators, coronary	Nitrates	CAD, CHF	GERD (UC)
Vasodilators, systemic	Hydralazine, Minoxidil	CAD, HBP, nausea/ vomiting(C); diarrhea (C); constipation (UC)	

C, common; CAD, coronary artery disease; CHF, congestive heart failure; GERD, gastroesophageal reflux disease; GI, gastrointestinal; HBP, high blood pressure; HMG-CoA, 3-hydroxyl-3-methyl-glutaryl-CoA; R, rare; UC, uncommon.

improved with a diet modified to contain medium-chain triglycerides. Children with the disease can develop severe neurological disease from vitamin E deficiency, and fat-soluble vitamins must be monitored and replaced. The disorder is a result of mutations of the microsomal triglyceride transfer protein [33], and with current molecular technology it can be differentiated from a variety of hypobetalipoproteinemias caused by truncation-producing mutations of the apolipoprotein B-100 gene [34] and from chylomicron retention disease.

Down syndrome

Down syndrome (chromosome 21 trisomy) can be associated with multiple gastrointestinal anomalies, the presence of which predicts higher mortality [35]. Infants with duodenal stenosis and atresia present with vomiting, abdominal distention, and the "double bubble" sign on abdominal radiography. Less complete obstructions from fenestrated duodenal membranes may present later in life as a cause of vomiting [36]. Esophageal abnormalities include tracheoesophageal fistula, esophageal stenosis, and gastroesophageal reflux.

Imperforate anus, Hirschsprung disease, and malrotation of the intestine have been described. Celiac disease has a relatively high prevalence in these patients [37]. The high incidence of hepatitis A and B infection in patients with Down syndrome has been reduced with early vaccination.

Fabry disease

Anderson–Fabry disease, an X-linked glycosphingolipidosis, is caused by mutations in the gene encoding the lysosomal exoglycosidase α-galactosidase A (α-Gal A), with the resulting deposition of ceramide trihexose in the lysosomes of endothelial, neural, and smooth muscle cells. Diagnosis is made by skin biopsy, measurement of α-Gal A in leukocytes, and genetic studies. Gastrointestinal manifestations are present in most affected men and in 29% of female carriers and include episodic constipation, diarrhea, nausea and vomiting, and abdominal pain, and, rarely, intestinal ischemia and perforation. The cause of these symptoms may be related, in part, to impaired autonomic function and vascular insufficiency. The advent of enzyme replacement therapy with α-Gal A has afforded these patients regression of gastrointestinal symptoms and substantial improvement in functionality [38–40].

Familial Mediterranean fever

Familial Mediterranean fever (recurrent polyserositis) is a recessive disease that is common in populations of Eastern Mediterranean origin. The gene responsible for familial Mediterranean fever has recently been cloned and designated *MEFV*. Three missense mutations have been identified and can be screened for using an amplification-refractory mutation system assay [41]. The target organ appears to be the blood vessel, and vasculitis can be present. Clinical manifestations begin in childhood and include attacks of fever, synovitis, severe abdominal pain suggesting an acute surgical emergency, or pleurisy [42].

The diagnosis is suspected when patients of Mediterranean extraction with a positive family history present with recurrent episodes of chest or abdominal pain, peritoneal signs, arthritis, fevers, and leukocytosis. The patient may have undergone previous fruitless diagnostic workups, including laparotomy. Frequent complications include AA amyloidosis, degenerative arthritis, renal vein thrombosis, and narcotic addiction [42]. Treatment with colchicine has been beneficial in preventing and ameliorating acute attacks [43].

Gaucher disease

Type 1 (nonneuronopathic or "adult") Gaucher disease is a rare autosomal recessive disease caused by a deficiency of glucocerebrosidase and resulting in the amassing of an insoluble glucocerebroside in the lysosomes of macrophages. Adults present with hepatosplenomegaly, thrombocyto-penia, and pathological bone fractures. Ascites or esophageal varices, which occasionally may bleed massively, have been described (Fig. 109.2). Definitive diagnosis relies on assaying β-glucerebrosidase actitivity [42]. Improvement in hepa-tosplenomegaly and overall function can be achieved with either enzyme replacement therapy with small doses of mannose-terminated glucocerebrosidase (alglucerase) or substrate reduction therapy with an inhibitor of glucosylceramide synthetase, attenuating the synthesis of glucocerebroside the substrate of the deficient glucocerebrosidase (oral miglustat) [42,43].

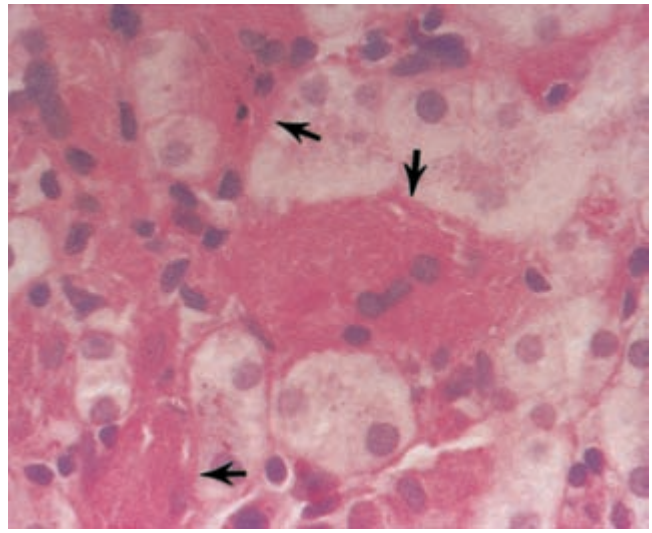

Figure 109.2 Gaucher disease of the liver. Multinucleated Gaucher cells (arrows) are present. (H & E stain; original magnification × 400.)

Hepatic porphyrias

The hepatic porphyrias are a group of inherited metabolic disorders of heme synthesis. The acute hepatic porphyrias include acute intermittent porphyria, variegate porphyria, hereditary coproporphyria, and δ-aminolevulinic acid (ALA) dehydratase deficiency. They are characterized by attacks of abdominal pain (commonly with vomiting and constipation) associated with hypertension, neuropathy, and neuropsychiatric disturbance that may last days to months. The abdominal pain is severe, may be associated with signs of peritoneal inflammation on physical examination, and can lead to unnecessary abdominal surgery. The peripheral neuropathy is manifest as pain in the extremities but may progress to a motor neuropathy. Photosensitivity occurs only in variegate and coproporphyria. Attacks can be precipitated by medicines (barbiturates, dilantin, alcohol), menstruation, malnutrition, or other medical or surgical illnesses [44].

Sequence analyses of the genes for the enzymes of heme biosynthesis have revealed the porphyrias as highly heterogeneous, with multiple mutations underlying each type. All but ALA dehydratase deficiency are inherited as an autosomal dominant trait and are associated with increased urinary excretion of porphobilinogen (PBG) and ALA (Table 109.2). In patients with ALA dehydratase deficiency excretion of PBG is normal but ALA is elevated [44]. Once the diagnosis is made, genetic counseling should be undertaken; patients

Table 109.2 The hepatic porphyrias

Disorder	Abdominal pain	Skin lesions	Biochemical markers	
			Urine	Feces
Acute intermittent porphyria	+	0	High PBG and ALA	0
Variegate porphyria	+	+	High PBG, ALA, and coproporphyrin	High protoporphyrin and coproporphyrin
Hereditary coproporphyria	+	+	High PBG, ALA, and coproporphyrin	High coproporphyrin
ALA dehydratase deficiency	+	0	High ALA with normal PBG	0
Porphyria cutanea tarda	0	+	High uroporphyrin	0

This table does not list all the biochemical abnormalities, but rather serves as a guide to indicate which measurements are most critical in evaluating a particular porphyria. ALA, delta-aminolevulinic acid; PBG, porphobilinogen. (Adapted from Bloomer JR. The hepatic porphyrias. Gastroenterology 1976;71:689)

are told to avoid precipitating factors and are placed on a high-carbohydrate diet. Acute attacks may be ameliorated by infusion of glucose, hematin (being replaced by heme arginate in Europe) [44], which suppresses ALA synthetase. Treatment of seizures has been difficult because many antiepileptic medications increase the hepatic metabolism of heme and can precipitate attacks. Gabapentin does not have this effect and seems to be safe and effective as a treatment for seizures in this setting [45].

Porphyria cutanea tarda (PCT) is inherited as an autosomal dominant trait, but its clinical presentation as a disease may depend on a number of other genetic and environmental factors. It is associated with prominent dermal photosensitivity and mechanical sensitivity without abdominal pain and also with alcoholic liver disease, hemochromatosis (or other disorders of iron overload), and hepatitis B and C. In PCT, urinary excretion of PBG is normal and the disease is differentiated from variegate and coproporphyria by the increased urinary excretion of uroporphyrins. Abstention from alcohol, phlebotomy or deferioxamine to reduce iron overload, low-dose chloroquine, and interferon–ribaviron treatment of hepatitis C all have reduced symptoms [46].

Hereditary angioedema

Hereditary angioedema is a rare autosomal dominant inherited deficiency of activated C1 inhibitor, a plasma protein that prevents uncontrolled intravascular activation of complement. Low serum C4 and an assay showing absent serum C1 inhibitor confirms the diagnosis [47].

The major clinical feature is episodic, self-limited, nonpitting swelling of the orofacial region, larynx, extremities, and gastrointestinal tract. Angioedema is distinguished from urticaria in that it is asymmetric and burns rather than itches. Edema in the cervical region can compromise the upper airway. Abdominal attacks are characterized by colic, bloating, and nausea and vomiting secondary to enteric mucosal edema, and can occur in the absence of peripheral or orofacial angioedema. Barium studies demonstrate "thumbprinting." Bradykinin is felt to be the main if not the sole mediator responsible for the edema. Treatment with synthetic androgens (e.g., danazol or methyltestosterone) increases the levels of C1 esterase inhibitor and can ameliorate or prevent attacks. Where available, infusions of C1 inhibitor concentrates are the treatment of choice for emergent attacks [47]. Premedication with C1 inhibitor concentrate or antifibrinolytic agents (e.g., ε-aminocaproic acid, tranexamic acid) should be considered before oropharyngeal manipulations such as upper endoscopy [48].

Hyperlipidemias

The familial hyperlipidemias are a collection of inherited disorders of lipid metabolism characterized by elevated plasma lipid levels. The epidemiological association with atherosclerotic vascular disease is clear. Only disorders that predispose to high levels of triglycerides (> 1000 mg/dl but usually > 2000 mg/dl) are associated with acute pancreatitis [49]. Many of these patients, particularly if overweight or diabetic, will develop fatty liver associated with abnormal serum liver enzyme levels, and will have nonalcoholic steatohepatitis on biopsy. The use of oral contraceptives during the third trimester of a pregnancy and highly active antiretroviral therapy (HAART) therapy for human immunodeficiency virus (HIV) are associated with an increased risk of developing severe dyslipidemia and pancreatitis [50].

In the past 5 years multiple studies have demonstrated the safety and beneficial effect of lipid-lowering medication on the primary and secondary prevention of coronary disease and stroke. Statins [3-hydroxy-3-methylglutaric acid (HMG-CoA) reductase inhibitors], fibrates, and niacin are commonly employed in combination in difficult to manage dyslipidemias. Hepatotoxicity is rare, but clinically unimportant elevations of aminotransferases occur in 1%–2% of the treated population. Asymptomatic elevations of aminotransferases to twice the upper limit of normal can be monitored without stopping medication. When clinically indicated, statins should not be withheld from patients with minor

transaminase elevations (e.g., NASH) but tests of liver function should be performed within the first 1–2 months of initiating therapy and every 6 months thereafter [24,25].

Bile acid-binding resins such as cholestyramine cause abdominal fullness, flatulence, and constipation in 30% of patients. These symptoms usually respond to dose reductions or the addition of psyllium or prune juice. Nicotinic acid, especially at doses > 2000 mg/day, has been implicated in more common hepatotoxic reactions than statins. Gemfibrozil may cause severe abdominal pain, and is associated with increased frequency of gallstones [51].

Niemann–Pick disease

Niemann–Pick disease is a rare, inherited lipid storage disease that principally afflicts people of Jewish ethnicity. The enzyme sphingomyelinase is absent or nonfunctional, leading to the accumulation of sphingomyelin in the lysosomes of reticuloendothelial cells. Affected infants manifest severe neurological disease, hepatosplenomegaly, and hepatic failure and do not survive to adulthood. Survival of patients with type B Niemann–Pick disease to adulthood is common but may be associated with cirrhosis and portal hypertension [52].

Tangier disease

Tangier disease is a rare codominant inherited deficiency of plasma α-lipoprotein. The genetic defect has been localized to chromosome 9q31. ABCA1, a cell membrane protein, mediates the active efflux of excess cholesterol from cells and is mutated in Tangier disease. Affected patients exhibit reduced total cholesterol, low high-density lipoproteins, and elevated plasma triglycerides. Accumulation of cholesterol in the reticuloendothelial system results in enlarged orange or red tonsils, lymphadenopathy, peripheral neuropathy, hepatosplenomegaly, and yellow–orange patches in the colonic mucosa [53,54].

Turner syndrome

Turner syndrome (X chromosome monosomy, or mosaicism) occurs in 1:2500 live female births and presents with a variety of external somatic features, of which short stature and gonadal dysgenesis are almost invariably present. Gastrointestinal hemorrhage resulting from intestinal vascular malformations, an increased incidence of inflammatory bowel disease and celiac sprue, and a greater risk of anorexia nervosa have been described [55]. Mild elevation of alkaline phosphatase may be seen. This cholestasis is not related to the chronic use of estrogens for the gonadal dysgenesis [56].

von Hippel–Lindau disease

von Hippel–Lindau disease (VHL) is an autosomal dominant inherited disorder characterized by hemangioblastomas of the retina and central nervous system, renal cell carcinoma, pheochromocytoma, and endolymphatic sac tumors with marked phenotypic variability. Heterogeneous germline mutations lead to the absence of the VHL protein, resulting in uncontrolled production of vascular endothelial growth protein and the formation of blood vessels [57]. Pancreatic disease is found in three-quarters of the affected individuals. The most common lesions found were cysts, serous cystadenomas, and neuroendocrine tumors. These lesions rarely cause symptoms and require surgical intervention only when very large [58].

Connective tissue diseases

Ehlers–Danlos syndrome

Ehlers–Danlos syndrome is a collection of inherited diseases affecting collagen synthesis. Nine different subtypes are described, although gastrointestinal involvement is common only in types I (gravis) and IV (arterial). Patients with type I disease have fragile, hyperelastic skin and hyperextensible joints. They often complain of bleeding gums and easy bruising after even minor trauma. Severe gastrointestinal bleeding from mucosal lesions occasionally occurs [59].

Ehlers–Danlos syndrome type IV is unique in that hyperelastic skin is lacking and joint hyperextensibility is less prominent. Ecchymoses, thin skin with visible vessels, and "cigarette paper" scars are common, however. Cultured fibroblasts that synthesize abnormal type III procollagen molecules or the identification of or a mutation in the gene for type III procollagen (*COL3A1*) confirms the diagnosis. Most complications occur after age 20. The defect in type III collagen synthesis leads to multiple aneurysms, including in splanchnic arteries, rupture of which results in intraabdominal bleeding. Spontaneous perforation of the bowel, particularly the sigmoid colon, is common and a cause of death. Tissue fragility and poor wound healing with dehiscence contribute to surgical mortality. Repeated perforations are seen [60].

Mixed connective tissue disease

Mixed connective tissue disease (MCT), or overlap syndrome, is a heterogeneous autoimmune disorder which may present with clinical features of lupus, scleroderma, and polymyositis. Gastrointestinal manifestations may resemble any of those described (see below) for the aforementioned rheumatoid diseases. However, the most common manifestations are esophageal symptoms of heartburn and dysphagia, with either distorted proximal or distal esophageal motility. Treatment with corticosteroids may improve the swallowing abnormality [61].

Polymyositis and dermatomyositis

Polymyositis and dermatomyositis are rheumatological diseases characterized by inflammation of striated and, to a lesser extent, smooth muscle. Muscle biopsy is needed for diagnosis. A characteristic skin rash accompanies dermatomyositis. The gastrointestinal tract may be affected through-

out its entire length, but the proximal esophagus is usually involved. Gastric and esophageal emptying is compromised in many patients, who may complain of dysphagia, aspiration, regurgitation, early satiety, and bloating [62].

Colonic pseudodiverticula and pneumatosis intestinalis may develop. Constipation is a common complaint. Acute inflammation of smooth muscle may result in gut wall edema, ulceration, or perforation. There is an association between dermatomyositis and malignancy – colon cancer seems to be prevalent, and colonoscopic screening is therefore appropriate [63].

Progressive systemic sclerosis

Progressive systemic sclerosis (PSS) or scleroderma is characterized by vasculitis of small arteries and fibrosis of skin and other organs. Gastrointestinal manifestations are found in most patients. Tightening of the perioral skin with restricted ability to open the mouth and impaired taste sensation may contribute to malnutrition. Esophageal involvement, present in 70%–80% of patients, is associated with more severe Raynaud phenomenon, skin abnormalities, and autonomic dysfunction [64,65]. Smooth muscle atrophy leads to absent or low-amplitude esophageal contractions that are most prominent in the mid and distal esophagus and weakening of the lower esophageal sphincter, leading to both reflux of acid and retarded clearance of the refluxed material. In addition, gastric emptying is commonly delayed, further increasing acid reflux [66]. The result is the most severe type of symptomatic reflux and erosive esophagitis. Evaluation of dysphagia may be difficult in advanced cases as it may be impossible to distinguish primary dysmotility from stricture. Endoscopy should be the primary diagnostic modality. Dilation of a stricture can substantially improve swallowing. The risk of developing strictures is high, and patients should be maintained indefinitely on an adequate amount of proton pump inhibitor to suppress heartburn.

In those PSS patients with upper gastrointestinal bleeding, endoscopy may reveal telangiectasia [67] or gastric antral vascular ectasia (watermelon stomach). Chronic iron deficiency anemia, persistent occult blood in the stool, and recurrent melena and hematemesis are common manifestations. Cautery with heater probe or laser may reduce recurrent bleeding [68].

The small intestine is frequently involved, causing severe morbidity. Hypomotility, dilation, pseudodiverticula, and thickening of the bowel are common [69]. Stasis, pseudoobstruction, and bacterial overgrowth may produce anorexia, abdominal distention, postprandial periumbilical pain, malabsorption, diarrhea, steatorrhea, and weight loss. The bacterial overgrowth may be worsened by the need for effective inhibition of acid secretion because of concomitant gastroesophageal reflux. Erythromycin, cycled antibiotics, and octreotide may reduce symptoms, but many patients require home parenteral nutrition.

Figure 109.3 Wide-mouthed pseudodiverticula are present in a patient with scleroderma.

Delayed colonic transit and impaired anal sphincter function have been demonstrated in patients with PSS [70]. Pseudodiverticula (Fig. 109.3) and telangiectasias are common. Constipation, pseudoobstruction, and volvulus may develop. Fecal incontinence and rectal prolapse are particularly disabling symptoms. Pneumatosis cystoides intestinalis may be found and is probably due to excessive hydrogen production by intestinal bacteria, altering the partial pressure of nitrogen in the intestinal wall [69]. Careful attention to bowel function has an important role in extending the patient's functionality. Patients with the CREST (calcinosis, Raynaud phenomenon, esophageal dysmotility, sclerodactyly, telangiectasias) variant of PSS are at risk for the development of primary biliary cirrhosis [71].

Pseudoxanthoma elasticum

It has been found that mutations in the *ABCC6* gene encoding for the multidrug resistance-associated protein 6 cause pseudoxanthoma elasticum [72]. The disease is characterized by plaques resembling xanthomata on the skin and angioid streaks on the fundus. Degeneration of elastic fibers in visceral blood vessels results in ineffectual vasoconstriction and vessel retraction after injury, predisposing to gastrointestinal bleeding [73]. Yellow raised plaques similar in appearance to the skin lesions have been identified in the stomach during endoscopy [74]. The best therapy for bleeding is uncertain.

Rheumatoid arthritis

Rheumatoid arthritis is a chronic systemic disease characterized by a deforming symmetrical polyarthritis and the presence

of serum rheumatoid factors. It most commonly afflicts women between the third and seventh decades of life and has a prevalence of about 1% in the United States [75]. Gastrointestinal manifestations are common and protean. Involvement of the temporomandibular joint impedes chewing. Sicca syndrome, when present, is accompanied by stomatitis and gingivitis. Heartburn and dysphagia are not as common as in PSS or MCT disease. Secondary amyloidosis may occur in patients with long-standing rheumatoid arthritis, may involve any portion of the gastrointestinal tract, may be manifest by chronic diarrhea, and can be diagnosed by endoscopic gastroduodenal biopsies [76].

Vasculitis is less common than in other rheumatic diseases; however, both small and large vessels may be involved in the setting of severe arthritis, subcutaneous nodules, high titers of rheumatoid factor, and depressed serum complement levels. Necrotizing vasculitis of the mesenteric vessels may result in intestinal ischemia, bleeding, and infarction [77]. Cholecystitis, appendicitis [78], perisplenitis, splenic infarction, pancreatitis, and hepatic arteritis have been described.

Iatrogenic illness results in the most common gastrointestinal manifestations in patients with rheumatoid arthritis. Chronic administration of salicylates or nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit both cyclooxygenase 1 and 2 (COX-1 and COX-2) produce gastroduodenal erosions and ulcers, as well as hypocoagulability. Although these lesions can be associated with anorexia, nausea, and abdominal pain, many patients are asymptomatic [79]. Significant gastrointestinal bleeding or perforation occurs in about 1%–2% each year [80]. Of these adverse events, 40% are the consequence of lower gastrointestinal (GI) ulceration or perforation [81]. The risk of bleeding is increased substantially (up to 9%–10%) in frail older patients who (a) are taking certain NSAIDs (e.g., piroxicam), (b) are taking higher doses of any NSAIDs, (c) have a previous history of gastrointestinal bleeding or ulcers, (d) are using steroids or aspirin with an NSAID, or (e) have concomitant cardiopulmonary disease [82].

Under ideal circumstances, a patient with rheumatoid arthritis would be treated with joint-sparing immunotherapy alone (e.g. methotrexate, anti-tumor necrosis factor [TNF]) and would not require chronic NSAIDs. In practice, however, many continue to have pain and inflammation that respond only to NSAIDs. In such patients, the use of selective COX-2 inhibitors (celocoxib) has been associated with decreased dyspepsia and bleeding. Because of the availability of inexpensive generic proton pump inhibitors (PPIs), which will also protect the stomach, and the concerns about increased cardiovascular events with the coxibs, many patients are prescribed a PPI along with non-selective NSAIDs.

Methotrexate is commonly used to modify the disease progression of rheumatoid arthritis. Nausea and vomiting are common side effects that during the first year of therapy limit the use of this efficacious medication. Decreased renal function (because of either kidney disease or aging) increases the risk of side effects, and high-dose folic acid supplementation is protective [83]. Although chronic therapy with low-dose methotrexate can result in hepatic fibrosis, the risk of developing cirrhosis is small in young nonobese patients who do not abuse alcohol. Hence, the clinical value of obtaining periodic liver biopsies is controversial [84,85]. As many patients with rheumatoid arthritis also have chronic hepatitis C, it is comforting to know that using the TNF-α compounds does not appear to worsen the viremia or liver biochemistries [86]. The toxic effects of gold preparations are discussed in the section on heavy metal toxicity.

Seronegative spondyloarthropathies

The seronegative spondyloarthropathies are a collection of rheumatic diseases including ankylosing spondylitis, psoriatic arthritis, the reactive arthritides (e.g., Reiter syndrome), arthritis associated with inflammatory bowel disease, and Whipple disease. Serum rheumatoid factor is lacking, but most patients are human leukocyte antigen (HLA) B27 positive, and many have recurrent oral and genital ulcers [87]. Patients with reactive arthritis frequently have an antecedent gram-negative enteric or chlamydial infection [88]. Many have chronic gastrointestinal symptoms (e.g., mild diarrhea, abdominal cramps), and most patients have endoscopic or histological evidence of ileocolonic inflammation that sometimes resembles Crohn's disease [89].

Sjögren syndrome

Sjögren syndrome results from immune-mediated destruction of exocrine glands and is characterized by keratoconjunctivitis sicca (dry eyes and mouth). Atrophy of the salivary glands leads to a lack of alkaline saliva, which can worsen acid reflux. In addition, esophageal dysmotility and mucosal webs are found [90]. Gastric atrophy, protein-losing enteropathy, and celiac disease [91] are reported in these patients. There is a strong association with the development of primary biliary cirrhosis [92]. An association with autoimmune pancreatitis has been reported [93].

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE) is a systemic autoimmune disease that is characterized by arthritis, myalgias, fever, rash, lymphadenopathy, renal disease, polyserositis, and central nervous system involvement. Abdominal pain is common in SLE and has a broad differential diagnosis. Inflammation of serosal surfaces may produce lupus peritonitis [94], which is usually self-limited but may be associated with ascites. Self-limited peritonitis is difficult to distinguish from intestinal ischemia or perforation secondary to mesenteric vasculitis [95]. Pancreatitis, sometimes severe, and perhaps related to vasculitis, has been associated with SLE. Lupus enteritis is a common cause of admission to hospital [96], and SLE may present with terminal ileitis, and can

be confused with Crohn's disease [97]. Malabsorption and protein-losing enteropathy with lymphangiectasia are documented [98]. Although clinically significant liver disease is uncommon in SLE, many patients have hepatomegaly and abnormal liver enzyme levels. Drug-induced hepatotoxicity is probably the most common underlying cause [99]. Differentiation from autoimmune hepatitis with systemic features of SLE can be difficult. Other drugs, such as azathioprine, may produce similar abnormalities. Hepatic vasculitis is a rare cause of liver disease, which in extreme cases has resulted in liver rupture [100].

Dermatological diseases

Blue rubber bleb nevus syndrome

Blue rubber bleb nevus syndrome is characterized by rubbery, blue nevi on the skin and gastrointestinal hemangiomas. The gastrointestinal hemangiomas can result in hemorrhage and iron deficiency anemia, may be the lead point of intestinal intussusceptions, and are commonly identifiable at the time of upper or lower endoscopy. Actively bleeding lesions can be cauterized, or the vessel can be occluded during angiography [101–103].

Cowden syndrome

Cowden syndrome is an autosomal dominant disease characterized by ectodermal, mesodermal, and endodermal hamartomatous neoplasms. Patients manifest small papular lesions on the skin and oral mucosa. Diffuse esophageal glycogenic acanthosis may be characteristic of the syndrome [104]. There is a strong propensity for thyroid goiter, thyroid cancer, and, in women, breast cancer. Mutations of the tumor-suppressor gene *PTEN* have been found [105]. Multiple hamartomatous polyps of various sizes can develop from the esophagus to the colon, but have a low potential for malignant degeneration. The hamartomas can cause chronic gastrointestinal blood loss and partial intestinal obstruction [106] (see Chapter 64).

Epidermolysis bullosa

Epidermolysis bullosa is a group of diseases in which minor trauma disrupts the cohesion between epidermis and dermis, resulting in the formation of vesicles or bullae. Adult forms of this entity include epidermolysis dystrophica with either dominant or recessive inheritance and epidermolysis simplex of dominant inheritance.

Lingual scarring and microstomia occur as a consequence of injury to the oropharyngeal mucosa during the eating of solid foods [107]. Clinically significant esophageal disease is seen more commonly in recessive epidermolysis dystrophica. Odynophagia and dysphagia are common because esophageal bullae lead to erosions, ulcers, pseudodiverticula, shortened esophagus, webs, strictures, and obstruction, most commonly in the cervical esophagus. Eating a soft or puréed diet may limit mechanical injury to the esophagus, but endoscopic placement of a gastrostomy tube may be needed. Strictures respond to repetitive ballon dilation. Bougienage should probably be avoided because the shearing forces could perpetuate injury [108].

Hereditary hemorrhagic telangiectasia

Hereditary hemorrhagic telangiectasia (HHT, Osler–Weber–Rendu disease) is an autosomal dominant condition found in 1–2 per 100 000 population. Mutations in the endoglin and ALK-1 genes cause the two recognized disease types, HHT1 and HHT2. HHT is associated with the formation of telangiectasias, aneurysms, and arteriovenous malformations (AVMs) throughout the body. Cutaneous telangiectasia usually appears after the second or third decade of life and is located most frequently on the face, lips, nares, tongue, ears, and hands. Pulmonary AVMs occur only in HHT1 while HHT2 has milder disease manifestations [109].

Epistaxis is the most common form of bleeding, with gastrointestinal hemorrhage developing in 10%–40% of patients [110]. Upper and lower GI endoscopy have been amplified by capsule endoscopy in localizing lesions [111,112]. Hepatic vascular malformations cause arterioportal and intrahepatic portosystemic shunts, resulting in high-output congestive heart failure. Hepatomegaly, hemobilia, portal hypertension, esophageal varices, hepatic encephalopathy, bruit, and cardiomegaly have been noted. Elevated alkaline phosphatase and bile duct lesions similar to Caroli disease or sclerosing cholangitis can be encountered [113].

Iron deficiency anemia is common and may require continuing oral or parenteral iron replacement or even transfusions. Endoscopic coagulation can effectively control life-threatening hemorrhage from a visible intestinal vascular malformation. In many cases, however, the bleeding site cannot be localized, and, in the absence of an evidence-based medical therapy, chronic transfusion or iron therapy is initiated. When large intrahepatic arteriovenous fistulae result in high-output congestive failure, embolization of these vessels or ligation of select branches of the hepatic artery may result in improvement of the failure, but infarction of the liver. Liver transplantation has been employed in severe cases [113].

Neurofibromatosis

Neurofibromatosis (von Recklinghausen disease) is a dominantly inherited disease. The gene frequency is 1 in 3000 births. Cutaneous diseases comprise café au lait spots and neurofibromas, which rarely transform into sarcoma [114]. Polypoid neurofibromas can be present throughout the gastrointestinal tract, and it has been suggested that the Auerbach myenteric plexus may be the source of the tumors [115].

Neurofibromas occasionally cause gastrointestinal bleeding and obstruction presenting as melena and abdominal

pain. Gastrointestinal stromal tumors are commonly found in patients with neurofibromatosis [116].

Pemphigus

Pemphigus is a group of diseases characterized histologically by acantholysis, resulting in bullae formation. Pemphigus vulgaris, the most common variant, is characterized by circulating immunoglobulin G (IgG) antibodies to desmoglein 3 [117]. D-Penicillamine, which is used in the management of Wilson disease, can cause pemphigus, although this is rare. Pemphigus vulgaris can involve the esophagus, endoscopy showing blisters or erosions in the upper esophagus and linear red streaks along the entire organ, but treatment of the pemphigus with steroids regresses the esophageal disease [118].

Psoriasis

Psoriasis is not associated with gastrointestinal disease; however, difficult psoriasis is often controlled by prolonged methotrexate administration. At the doses used, this drug carries a risk of potentially serious hepatotoxicity, including fatty liver, hepatic fibrosis, and cirrhosis. This risk has probably been overstated, with only 8% developing advanced fibrosis with cumulative doses > 6000 mg [119]. Where available, monitoring with assays of type III procollagen has replaced serial liver biopsies to detect early hepatic damage [120].

Stevens–Johnson syndrome

Stevens–Johnson syndrome is an uncommon, but severe, hypersensitivity reaction characterized by a diffuse rash (erythema multiforme) that may progress to an exfoliative dermatitis. It is fatal in about 5% of cases [121]. This syndrome is often accompanied by fever and mucositis of both the gut and the respiratory tract. Etiological agents include drugs (e.g., sulfonamides, allopurinol, anticonvulsants, NSAIDs) and infectious agents. The oropharyngeal mucosa frequently exhibits erosions and mucosal sloughing, although the entire gut may be involved in severe cases. Oropharyngeal pain may be severe enough to limit oral intake. Mucosal injury may result in dysphagia, odynophagia, abdominal pain, and gastrointestinal bleeding [122]. Esophageal stricture has been reported as a sequela of the Stevens–Johnson syndrome [123]. The syndrome is commonly associated with abnormal liver tests and hepatotoxicity.

Management is similar to that provided for major burns, and patients with extensive loss of skin should be managed in intensive care. Treatment includes identification and elimination of the inciting factors, rehydration, nutritional support, antibiotics, and vigilance for respiratory problems and secondary infections. Complete recovery may take several weeks [121–123].

Sweet syndrome

Sweet syndrome is an uncommon condition, seen in women more than men, characterized by the acute eruption of tender, erythematous, nonulcerating plaques and nodules. Nodules on the legs can mimic erythema nodosum. Diagnosis is defined by the histological presence of neutrophilic infiltration and leukocytoclasis on skin biopsy. The latter findings also are found in pyoderma gangrenosum, which, in contradistinction, is represented clinically by ulcerating plaques with violacious edges. As with pyoderma, a strong association of Sweet syndrome with both ulcerative colitis and Crohn's disease has been noted. Also reported are mouth ulcerations and an association with myeloma, myeloid leukemia, and adenocarcinoma of the colon [124].

Tylosis

Tylosis is a rare autosomal dominant disorder characterized by thickening of the skin on the palms and soles (focal nonepidermolytic palmoplantar keratoderma) (Fig. 109.4). Late-onset tylosis (type A) is associated with squamous cell carcinoma of the esophagus. The causative locus, located on chromosome 17q25.1, has been termed the tylosis esophageal cancer (TOC) gene [125], and has also been associated with sporadic esophageal cancer [126]. The esophageal mucosa forms papillomas. Affected persons have about a 90% probability of developing esophageal carcinoma by the age of 65, and should be screened with endoscopy and biopsies [127].

Figure 109.4 Hyperkeratosis of the palm and leg may be indicative of tylosis.

Urticaria

Urticaria is a reactive inflammatory vascular dermatosis characterized by vasodilation, increased vascular permeability, and extravasation of protein and fluids into the dermis, resulting in the characteristic peau d'orange wheals. Urticaria of less than 6 weeks' duration is usually related to inciting allergic factors. Chronic cases are usually idiopathic. Only a minority of patients with chronic urticaria will have systemic mastocytosis, which may be signaled by the more classical urticaria pigmentosa, flushing, and diarrhea [128]. Intial reports of an association with *Helicobacter pylori* infection appear unfounded [129]. Implicated in the pathophysiology of both acute and chronic urticaria is the release of histamine and other inflammatory mediators. Gastrointestinal manifestations have been reported in a number of patients suffering from chronic urticaria and include abdominal pain, gastroduodenitis, peptic ulcer, and enteric mucosal "hives" [128].

Endocrinological disorders

Acromegaly

Acromegaly is a disorder whose clinical manifestations result from excessive growth hormone (GH) and insulin-like growth factor 1 production, usually a result of a pituitary adenoma. Rarely, carcinoid and pancreatic islet cell tumors can secrete a GH-releasing factor that leads to acromegaly. Although patients manifest enlargement of the tongue, enlargement of the other organs of the digestive system is unusual, and hepatosplenomegaly thus would warrant further investigation [130]. It has become accepted that patients with acromegaly are at greater risk for colorectal neoplasia, although this idea is controversial, and they should therefore undergo routine screening guidelines by colonoscopy after the age of 50 [131].

When GH secretion cannot be normalized by ablation of the adenoma, long-acting octreotide can control symptoms. As a consequence of the disease and the chronic use of the somatostatin analog, patients are at increased risk of developing gallstones. It has been suggested that this may be accounted for by (a) slow-transit constipation, leading to increased formation of deoxycholic acid, and (b) impairment of postcibal cholecystokinin release and gallbladder contraction [132,133]. For this reason, reducing the lithogenicity of the bile with ursodeoxycholic acid may be considered.

Addison disease

Addison disease results from insufficient production of both mineralosteroids and corticosteroids by the adrenals. Gastrointestinal symptoms are common in untreated Addison disease. The prominent clinical features include anorexia, vomiting, weight loss, abdominal pain, apathy, hypotension, hyponatremia, and hyperkalemia [134]. Addison disease of autoimmune etiology is associated with atrophic gastritis, antibodies against H^+,K^+-ATPase and intrinsic factor, and uncommonly achlorhydria and pernicious anemia. Subclinical Addison disease may present with increased transaminase levels [135].

Autoimmune polyendocrine syndromes

The autoimmune polyendocrinopathies are a diverse group of inherited diseases that share a common immunonological theme: the loss of immunological tolerance to a peptide within a molecule found in a specific organ. Hypoparathyroidism is present in most patients with the autosomal recessively inherited autoimmune polyendocrine syndrome type I, as is candidiasis, and Addison disease [136]. Mutations in *AIRE* (an autoimmune-suppressor gene) cause the syndrome, with presentation early in life. Associated manifestations include gonadal failure, type IA diabetes, gastric parietal cell atrophy and pernicious anemia, and malabsorption with steatorrhea [137]. The type II autoimmune endocrinopathy is more common, occurs later in life, presents as idiopathic hypotension (adrenal failure), and results in the development of hypothyroidism. As more is understood about the nature of these illnesses, the larger the number of patients that are classified under a broad diagnostic umbrella [136].

Cushing syndrome

Overproduction of adrenal corticosteroids caused by oversecretion of adrenocrticotrophic hormone (ACTH) from a pituitary adenoma, or by ACTH ectopically produced in islet cell or small cell lung cancers, leads to the typical redistribution of fat (buffalo hump, central obesity, Cushingoid facies) that identifies the Cushing syndrome. Gastrointestinal manifestations, other than transaminase elevations from hepatic steatosis, are unusual. However, an uncommon type of an ACTH-independent Cushing syndrome, which is food dependent, deserves comment. Hypercortisolism in these patients is induced by an overexpression of gastric inhibitory polypeptide (GIP) receptors in the adrenal cortex. Eating stimulates GIP secretion, GIP binds to the receptors in the adrenal gland, and abnormal cortisol synthesis and secretion is initiated [138].

Diabetes mellitus

Diabetes mellitus is a common metabolic disease resulting in primary hyperglycemia. Insulin-dependent diabetes mellitus (IDDM) is caused by a genetically mediated immune destruction of the pancreatic β-cells, whereas the eight times more common non-insulin-dependent diabetes mellitus (NIDDM) is caused by insulin resistance at the effector cell. Non-insulin-dependent diabetes mellitus is more prevalent in Hispanic groups of Mexican origin, in Native Americans, and in some Asian–Pacific Island groups. For a variety of inadequately understood socioeconomic, genetic, and cultural reasons, diabetes-related morbidity and mortality are also more common among minorities [139]. Abdominal complaints (Table 109.3) are common, nonspecific to diabetes, and may not relate directly to the diabetes [140].

Table 109.3 Gastrointestinal manifestations of diabetes mellitus

Organ	Symptoms	Pathophysiology
Esophagus	Odynophagia	Candidiasis; autonomic neuropathy, causing dysmotility
Stomach	Bloating, nausea/vomiting	Transient dysmotility due to hyperglycemia or fixed gastroparesis
Small bowel	Bloating, diarrhea, weight loss	Celiac sprue, bacterial overgrowth caused by autonomic neuropathy and dysmotility
Colon	Constipation	Autonomic dysfunction
Biliary system	Increased frequency of cholelithiasis	Altered bile acids due to dysmotility and/or bacterial overgrowth; genetic concordance of diabetes, obesity, and cholelithiasis
Liver	Abnormal liver tests, hepatomegaly	Fatty liver with or without steatonecrosis
	Abdominal pain	
	Acute pain	Diabetic neuropathy, pancreatitis, cholecystitis, mesenteric ischemia
	Chronic pain	Diabetic neuropathy, mesenteric angina

Acute abdominal pain associated with nausea and vomiting is a frequent accompaniment of acute diabetic ketoacidosis and resolves with normalization of blood sugar. Persistent, severe abdominal pain may signal that the ketoacidosis is the consequence of some other serious intraabdominal process. Acute pancreatitis, cholecystitis, intestinal ischemia, appendicitis, and diverticulitis must always be considered.

Later stages of the disease may entail gastrointestinal symptoms of a chronic and irreversible nature. Many of the chronic symptoms are believed to result from visceral autonomic neuropathy, which is thought to be a consequence of a metabolic dysfunction of peripheral nerves caused by prolonged hyperglycemia. When blood sugar is maintained within a normal range by frequent daily administration of insulin, the onset of neuropathy is delayed and established neuropathy is improved [141].

Although clinical manifestations of esophageal dysfunction, often mild pyrosis and dysphagia, are unusual, abnormal esophageal motor function is common in both IDDM and NIDDM [142]. Esophageal dilation, reflux, and delayed esophageal emptying may be seen radiographically. Changes demonstrated manometrically may include feeble peristaltic pressure waves that are at times multipeaked or multiphasic, increased frequency of nonperistaltic contractions, and decreased lower esophageal sphincter pressure.

New-onset odynophagia in the diabetic patient should suggest the presence of *Candida* esophagitis. Oral thrush accompanies *Candida* esophagitis in no more than 50% of patients, but when present it is an indication for an empirical trial of an antifungal agent such as fluconazole. If odynophagia persists, or if oral thrush is not present, diagnostic upper endoscopy should be undertaken. The most common visual finding is that of cream-colored plaques scattered throughout the esophagus. The diagnosis is made through endoscopic biopsy and brushing of the plaques. Nausea and vomiting are common in patients with uncontrolled hyperglycemia and do not necessarily mean that the patient has the fixed vagal autonomic neuropathy seen in IDDM patients with gastroparesis diabeticorum. Hyperglycemia, even that induced in normal volunteers using the glucose clamp technique, disrupts normal myoelectric activity in the stomach. There is an inverse relationship between gastric emptying and blood glucose concentration [143]. These alterations in gastric myoelectric activity result in gastric dilation, slow peristalsis, and delayed gastric emptying. Increased pyloric resistance and small intestinal dysmotility [144] also slow transpyloric flow. The patient's diabetes must be under optimal control before investigations are undertaken for gastroparesis.

Symptoms of gastroparesis include early satiety, bloating, heartburn, persistent nausea, and paroxysmal vomiting of undigested food 3–4 h after eating. Physical examination may demonstrate a succussion splash as well as findings of peripheral neuropathy. Patients are predisposed to the formation of gastric bezoars from indigestible food. Yet most diabetic patients who have diabetes with abnormalities of gastric emptying have no symptoms [145]. Classical gastroparesis is defined by finding more than 1000 mL of gastric contents after awakening in the morning or by demonstrating a massively dilated stomach on routine abdominal radiographs or upper gastrointestinal series. Endoscopy supports the diagnosis by confirming the absence of structural gastric outlet obstruction and revealing a substantial gastric residue after an overnight fast. Other causes of gastroparesis that may be more treatable, such as hypocalcemia, hypothyroidism, or medications (e.g., narcotics, tricyclic antidepressants), must be excluded. Subtle gastric emptying dysfunction can be documented by gastroscintiscanning but must be interpreted with caution as abnormalities are found commonly in asymptomatic diabetics [145].

Central to the treatment of diabetic gastric emptying disorders is the optimal normalization of blood glucose, modification of concomitantly prescribed medications, and correction of any electrolyte abnormalities. In young women with IDDM eating disorders are common, and are associated with

noncompliance, underdosing of insulin to achieve weight loss, and poor metabolic control. Symptoms can mimic gastroparesis. If the eating disorder is not treated progression of microvascular complications is more common [146].

Therapy with prokinetic agents can be disappointing in advanced gastroparesis. The central dopaminergic and peripheral cholinergic effects of metoclopramide to accelerate gastric emptying may be short-lived because of tachyphylaxis. Although not available in the United States, domperidone (up to 20 mg four times daily) can result in longer-lasting improvement in symptoms despite a variable effect on tests of emptying, without the risk of the parkinsonian side effects of metoclopramide [147]. Although erythromycin improves gastric emptying in diabetic patients, it has not been helpful in the long-term management of gastroparesis. Surgery to improve gastric drainage should not be considered, but gastric pacing may be helpful in selected cases. Repeated measurements of gastric emptying are not useful because they do not discriminate well between symptomatic and asymptomatic patients and do not predict the response to treatment [148].

Diarrhea and fecal incontinence occur commonly in advanced IDDM [149]. The diarrhea is watery, painless, and commonly nocturnal. It can remit after days or months, but recurrence is frequent. Although intestinal autonomic neuropathy may be the cause in as many as 50% of cases, other, more treatable, causes must be excluded. Celiac disease, which is found more commonly in patients with IDDM, can be screened for with tissue transglutaminase antibodies. Pancreatic insufficiency and bacterial overgrowth should also be considered in the differential diagnosis. Fecal incontinence is commonly due to anal sphincter dysfunction and decreased rectal sensation [150], but a flexible sigmoidoscopy will exclude proctitis.

Once other causes have been cleared, idiopathic diabetic diarrhea is treated with good glucose control, opiates, and bulk-forming agents containing psyllium. Some patients may improve with clonidine hydrochloride, an α_2-adrenergic agonist, which possibly stimulates an increase in intestinal fluid absorption by enhancing adrenergic activity [151]; however, this medication may induce hypotension. Other patients with suspected bacterial overgrowth may respond to cycled antibiotic therapy. Diarrhea control and biofeedback training [152] may provide complete or partial relief of fecal incontinence.

Severe constipation associated with colonic motor abnormalities [153] occurs in 20% of patients with diabetes and neuropathy. The onset of constipation is often insidious. Management includes the use of a variety of nonstimulant laxatives.

Patients with diabetes can develop chronic abdominal pain secondary to thoracic radiculopathy. The pain is present constantly, is unilateral, and may or may not be associated with paresthesia or hypesthesia. Antidepressant drugs such as amitriptyline or desipramine may help control painful diabetic neuropathy [154].

Diabetes may lead to a large, poorly functioning gallbladder that has a predilection for cholelithiasis [155]. Contrary to previous assumptions, patients with diabetes with acute cholecystitis may not have a substantially increased risk of complications compared with the general population [156]. Thus, diabetes mellitus is not an indication for prophylactic cholecystectomy.

Hepatomegaly and fatty liver are frequent findings, especially in patients with poorly controlled blood sugar, hyperlipidemia, and obesity. In a minority of such patients, nonalcoholic steatonecrosis can develop, presenting with mild elevation of transaminases. Progression to cirrhosis is recognized, but weight reduction and diabetes control can normalize liver tests [157].

Endometriosis

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterine cavity and musculature. The disorder is common to women during the years they menstruate, and it is associated with severe perimenstrual lower abdominal pains and infertility. Since bowel function is commonly abnormal around the period, the pains can be difficult to distinguish from irritable bowel syndrome or diverticulitis. Most endometriosis takes the form of plaques of tissues on the serosal surface of the bowel lying in the pelvis in close proximity to the uterus and ovaries.

When intestinal endometriosis infiltrates into the deeper layers of the bowel, it can cause symptoms. Deeper infiltration can lead to obstruction of either the small or large bowel. Penetration of the mucosa can cause lower gastrointestinal bleeding, which may occur only during menstruation, but most patients do not characterize symptoms as cyclic. Treatment is surgical and bowel resection may be necessary if implants are deep [158,159].

Hyperparathyroidism

Hyperparathyroidism is a disorder of calcium and phosphate metabolism due to the excess secretion of parathyroid hormone. Primary hyperparathyroidism is caused by adenomatous or hyperplastic change in the parathyroid gland and may be associated with inherited multiple endocrine adenopathies (see below). Primary hepatic tumors have been reported to do this more commonly [160]. Secondary forms of the diseases occur when there is a tendency toward hypocalcemia, such as chronic renal failure. Symptoms that are probably consequences of high serum calcium concentrations include anorexia, nausea, vomiting, constipation, and abdominal pain. Hypercalcemia induces gastric hypersecretion, and likely predisposes to peptic ulcer formation [161]. Pancreatitis can develop when hypercalcemia is moderate to severe [162].

Hypoparathyroidism

Hypoparathyroidism, or low serum ionized calcium from whatever cause, can lead to neuromuscular irritability. Gastrointestinal manifestations can include abdominal pain, intestinal tetany, diarrhea, steatorrhea, and intestinal pseudoobstruction [163]. The steatorrhea may be secondary to pancreatic insufficiency due to insufficient endogenous cholecystokinin release following a meal.

Hyperthyroidism

Excessive circulating thyroid hormone causes hyperthyroidism. Although several disorders can induce this syndrome, 60%–80% of the cases are a consequence of Graves disease, an autoimmune disease. The symptoms of hyperthyroidism include nervousness, palpitations, heat intolerance, excessive perspiration, fatigue, weakness, dyspnea, and eye complaints. Despite having an enhanced appetite and hyperphagia, patients usually experience weight loss because of their hypermetabolic state [164]. The most common physiological effect on the gastrointestinal system is an alteration in gastric and intestinal motility, resulting in dyspepsia and shortened small bowel and colonic transit time [165]. This, as well as the increased consumption of fat, may result in steatorrhea and increased stool frequency, but rarely frank watery diarrhea. Hyperthyroidism should be excluded in all patients with new-onset diarrhea. There is also a high incidence of atrophic gastritis and hypochlorhydria or achlorhydria. Moderate to severe hyperthyroidism can be associated with hepatic dysfunction. Increases in blood alkaline phosphatase, transaminases, and bilirubin have been attributed to liver injury. In many cases, passive congestion of the liver secondary to heart failure could be the underlying mechanism [166]. Autoimmune-type thyroid diseases can flare up when chronic hepatitis is treated with interferon [167]. Propylthiouracil and methimazole, used in the treatment of thyrotoxicosis, can induce serious liver damage [168,169].

Hypothyroidism

Hypothyroidism results from a deficiency of thyroid hormone. Most cases are a consequence of thyroid gland dysfunction resulting from Hashimoto thyroiditis or therapy of hyperthyroidism. Symptoms include weakness, dry skin, swelling of the extremities, mental aberrations, coarse voice, paresthesias, and muscle cramps. Patients have a decrease in metabolism and experience anorexia with moderate weight gain. Motility is slowed throughout the gastrointestinal tract [165]. Intestinal transit is slowed, usually manifest as constipation and abdominal distention. Rarely, intestinal pseudoobstruction or megacolon results [170]. The gallbladder also may dilate and contract poorly.

Ascites, usually having a high protein concentration, is a rare complication of myxedema. It can be associated with histological changes in liver architecture suggestive of central congestive fibrosis, without congestive heart failure [171]. The ascites resolves after appropriate thyroid replacement therapy.

Granulomatous diseases

Granulomas are dynamic, usually focal inflammatory processes composed of macrophages and other inflammatory cell types. Many factors such as infectious agents, silica, metals complexed to macromolecules, and immune complexes can evoke a granulomatous response. Even when there is a known nidus, the cause of a granuloma is not always evident on histological examination.

Many infectious and noninfectious diseases are associated with granuloma formation in the liver and intestines (Table 109.4) [172,173]. The significance of these associations is complicated by the observation that granulomas are present in 3%–10% of liver specimens obtained at abdominal surgery or autopsy. The etiology of granulomas can, at times, be ascertained when bacteria, ova, or mineral crystals are identified within individual lesions. Some granulomas have distinctive morphology, such as the ring granuloma that is commonly found in hepatic Q fever, although ring granulomas have been described in other conditions [174]. Serological testing, culture of the involved tissue, or clinical presentation may provide the diagnosis in other circumstances.

In industrialized societies, the illnesses most commonly associated with hepatic granulomas are sarcoidosis and tuberculosis (see below) (Table 109.5). Primary biliary cirrhosis, drug-induced liver disease, and occasionally other primary liver diseases account for some of the remaining cases. In many patients, it is not possible to identify an etiological factor or an associated disease. Patients may be asymptomatic or may present with fever, fatigue, hepatosplenomegaly, and jaundice. Granulomatous hepatitis is suggested by a disproportionately high alkaline phosphatase with a normal biliary system on imaging. Diagnosis requires a liver biopsy that is cultured and examined histologically. The process rarely progresses to extensive hepatic fibrosis and cirrhosis, although sarcoidosis can lead to portal hypertension.

Causes of granulomatous inflammation of the intestines are less well characterized (see Table 109.4). In the United States, Crohn's disease and lymphoma are overwhelmingly the most frequent diagnoses, but a rare case of intestinal tuberculosis may be encountered. All these diseases can have a chronic course, presenting with abdominal pain, fever, fatigue, weight loss, diarrhea, and gastrointestinal bleeding. All have a predilection for the terminal ileum and can result in intestinal strictures [175].

When a specific cause of the granulomatous inflammation is defined, treatment is directed at eliminating the offending agent. Lesions that defy diagnosis or which are of unknown cause present special problems. Many patients are asymptomatic and require no therapy. Unusual conditions such as

Table 109.4 Some conditions associated with granulomatous inflammation of the liver (L) and/or intestines (I)

Infections

Bacterial

Atypical myocobacteria (L)
Brucellosis (L)
Cat scratch disease (L)
Leprosy (L)
Tuberculosis (L, I)
Tularemia (L)
Yersinia (I)
Chlamydiał
Lymphogranuloma
venereum (L, I)

Fungal

Blastomycosis (L, I)
Coccidiodomycosis (L, I)
Cryptococcosis (L)
Histoplasmosis (L, I)
Nocardiosis (L)
Systemic candidiasis (L)
Trichosporosis (L)

Helminthic

Ascariasis (L)
Capillariasis (L)
Enterobiasis (L)
Strongyloidiasis (L, I)
Tongue worm (L)
Toxocariasis (visceral larva migrans) (L)

Protozoan

Toxoplasmosis (L)
Visceral leishmaniasis (L)

Rickettsial

Q fever (L)
Spirochetal
Secondary syphillis (L, I)

Viral

Cytomegalovirus (L)
Mononucleosis (L)

Drug hypersensitivity

Allopurinol (L)
Amoxicillin-clavulanate (L)
Diphenylhydantoin (L)
Halothane (L)
Hydralazine (L)
Methyldopa (L)
Nitrofurantoin (L)
Phenylbutazone (L)
Procainamide (L)
Quinidine (L)
Quinine (L)
Sulfasalazine (L)
Sulfonamides (L)
Sulfonylurea (L)

Malignancy

Hodgkin disease (L)
Non-Hodgkin lymphoma (L)

Metals

Barium (I)
Beryllium (L)
Copper (L)

Vasculitis

Giant cell arteritis (L)
Lupus erythematosus (L)
Polyarteritis nodosa (L)
Polymyalgia rheumatica (L)
Rheumatoid arthritis (L)
Wegener granulomatosis (L, I)

Miscellaneous

Appendiceal granuloma (I)
Bacile Calmette-Guérin immunotherapy (L)
Crohn's disease (L, I)
Idiopathic granulomatous gastritis (I)
Idiopathic granulomatous hepatitis (L)
Immunodeficiency (L)
Jejunoileal bypass surgery (L)
Mineral oil (L, I)
Primary biliary cirrhosis (L)
Sarcoidosis (L, I)
Silica (L)
Whipple disease (L)

idiopathic granulomatous hepatitis frequently respond to corticosteroid administration.

Sarcoidosis

Sarcoidosis is a systemic disorder that is characterized by noncaseating granulomas in pathological specimens. The age-adjusted annual incidence of sarcoidosis in the United States is 10.9/100 000 for whites and more than three times higher in African Americans. In addition, the disease affects blacks more acutely and more severely than people of other races. Genetic factors appear to play a part in the pathogenesis as it is likely that the host develops an exaggerated cellular immune response and the formation of granulomas when exposed to a variety of antigenic stimuli [176].

Although the most common sites of involvement are the lungs and the intrathoracic lymphatic system, evidence of the disease in the liver will be found in 40%–70% of cases. Hepatic sarcoidosis is usually manifest by fever, hepatomegaly, and

Table 109.5 Etiologies of hepatic granulomas in several reported series from around the world

Diagnosis	Number with listed diagnosis in indicated series								Total (%)
	1	2	3	4	5	6	7	8	
Sarcoidosis	15	6	92	8	28	11	217	81	395 (35)
Tuberculosis	6	13	51	34	15	5	70	30	224 (20)
Undetermined diagnosis	4	10	11	13	15	18	45	13	129 (11)
Primary biliary cirrhosis						3	59		62 (5)
Other cirrhosis							4	51	55 (5)
Schistosomiasis				9	1		19		29 (2)
Lymphomas				1	3	3	11	4	22 (2)
Brucellosis		1	1	4	1		4	8	19 (2)
Drug-induced or toxic hepatitis							13	10	13 (1)
Acute viral hepatitis								13	13 (1)
Fungal infections					3	1	6		10 (1)
Other infectious causes*	3	7		1			14	27	52 (5)
Other noninfectious causes†	3		45	1			49	6	104 (9)
Unspecified etiology			2						
Total	32	38	214	64	60	50	565	106	1129

* Including Mediterranean fever (Olmer disease), 13 cases; typhoid fever, 7; infectious mononucleosis, 5; syphilis, 5; unspecified bacterial infections, 4; unspecified viral infections, 4; leprosy, 3; toxoplasmosis, 3; cytomegalovirus, 2; lymphogranuloma, 2; actinomycosis, 1; influenza B, 1; visceral larva migrans, 1; and bacille Calmette-Guérin infection, 1.

† Including primary liver disease, 44 cases; biliary and pancreatic disorders, 17; berylliosis, 9; malignancy, 9; fatty liver; 8; chronic active hepatitis, 4; temporal arteritis, 3; regional enteritis, 2; Wegener's granulomatosis, 2; erythema nodosum, 1; eosinophilic granuloma of the lung, 1; starch granuloma of the peritoneum, 1; hypogammaglobulinemia, 1; and celiac disease, 1.

Adapted from Harrington PT, Gutierrez JT, Ramirez-Ronda CH, et al. Granulomatous hepatitis. Rev Infect Dis 1982;4:639.

elevated alkaline phosphatase, and may be the most obvious clinical manifestation when pulmonary disease is subtle [176,177]. Typically the noncaseating granulomas are scattered throughout the liver, but confluent granulomas may be present. It is in the patient with severe hepatic sarcoidosis and confluent granulomas that scarring, portal hypertension, and hepatic dysfunction can result. A chronic cholestatic syndrome mimicking primary biliary cirrhosis is due to a granulomatous cholangiopathy with consequent ductopenia. Imaging studies may be normal, show a diffuse hepatopathy, demonstrate nodular lesions that can be mistaken for malignancy, and rarely show extrahepatic biliary obstruction due to lymphadenopathy or bile duct strictures [178,179]. Treatment commonly uses steroids, but progression to cirrhosis and liver failure many ensue. Liver transplantation can be considered, but recurrence of sarcoidosis in the allograft is the norm [180].

Although symptomatic disease in the liver, spleen, and abdominal lymphatics is common in sarcoidosis, involvement of the gastrointestinal tract is rare. The stomach is the most common site and may be defined by thickened gastric folds, nodularity, ulcers, or erosions [181]. As small bowel and colon disease is so rare, the demonstration of typical granulomatous ileocolitis in a patient with known sarcoidosis should still be followed and treated as presumed Crohn's disease.

Tuberculosis

Approximately one-third of the world's population is infected with the causative organism of tuberculosis (TB), *Mycobacterium tuberculosis*, with over 8 million new cases annually. Despite an average annual decline in incidence in the United States of 5.8%/year from 1953 to 1985, the number of new cases of tuberculosis increased 20% over the next 7 years as a result of the HIV/AIDS epidemic and immigration, and diminished TB control [182]. Although extrapulmonary TB occurs in about 20% of cases, in the United States the gastrointestinal tract is involved in only 2% [183].

Tuberculosis may involve any portion of the gastrointestinal tract. At presentation, abdominal pain, fever, and weight loss are the most common symptoms, and have usually been present for months. A mass is palpable in the right lower quadrant in 25% of cases. In the United States, Crohn's disease, colon cancer, lymphoma, and even amebiasis will be considered before TB. As the therapy for Crohn's disease (e.g., anti-TNF-α therapy) can suddenly worsen TB, the importance of considering TB in the differential diagnosis is evident. The diagnosis of tuberculosis should be considered if the disease has been documented elsewhere in more typical locations (e.g., the chest radiograph shows characteristic lesions, exudative ascites and peritoneal disease are recognized, tubercular tuboovarian disease is present) in a high-risk patient (e.g., malnourished, immigrant from a developing

country, a patient with AIDS, an alcoholic patient). Tubercular granulomas may have distinct histological features: They tend to coalesce and are larger than 400 μm; they often contain giant cells that are numerous and large; and caseation, although rare in the liver and intestine, should suggest tuberculosis. In the unusual case, histological staining, polymerase chain reaction (PCR), or culture may identify acid-fast bacilli. Treatment follows accepted protocols [184–186].

Peritoneal disease with ascites is common in countries in which TB is endemic. In the United States, TB peritonitis is associated with poverty, homelessness, alcoholism, and malnutrition. Abdominal distention, fever, weight loss, and abdominal pain in a patient with the appropriate risk factors should raise the suspicion for the disease. The chest radiograph will be abnormal in 50%, but active pulmonary TB will be present in only 15%. Paracentesis will usually show a high protein (> 2.5 g/dL) fluid with a low albumin gradient (< 1.1 g/dL). Examination of the fluid for acid-fast bacilli is positive in less than 3% of cases, and cultures are positive in 20%. Adenosine deaminase activity > 33 U/L has excellent sensitivity and specificity. In nonendemic countries, laparoscopy with peritoneal biopsies may be needed for diagnosis, whereas a therapeutic trial is commonly employed in areas with a higher prevalence of TB [187].

Heavy metal toxicity

Arsenic poisoning

Arsenic poisoning is endemic in many parts of the world, including North America, as a result of environmental contamination. Arsenic compounds continue to be used commercially, particularly in the preservation of wood, mining, and the manufacture of glass and metal [188].

Acute arsenic poisoning results in a reversible inactivation of sulfhydryl-containing enzymes. Cellular oxidative processes are blocked and tissue hypoxia ensues. Patients present with severe colicky abdominal pain, nausea, vomiting, profuse diarrhea, malodorous ("garlicky") breath, dysphagia, hepatomegaly, jaundice, and circulatory collapse. Quantities of 100–300 mg may be fatal in an adult. Quantitative measurement of 24-h urinary arsenic excretion is the only reliable test to confirm arsenic poisoning. Treatment includes gastric lavage, chelation therapy (e.g., British anti-Lewisite [BAL, dimercaprol] or D-penicillamine), and cardiorespiratory support [188].

Chronic arsenic toxicity often manifests as nonspecific symptoms, including weakness, nausea, diarrhea, or constipation. Macular skin pigmentation, leukoderma, keratotic lesions of the palms and soles, pancytopenia, edema, peripheral vascular disease, neuropathy, cirrhosis, and accompanying presinusoidal portal hypertension may appear alone or in combination [189,190]. The diagnosis of chronic arsenic intoxication is made by measuring arsenic levels in tissue samples (e.g., hair, nails). An association with cutaneous, hematological, respiratory, and hepatic malignancies (angiosarcoma) has also been suggested [191].

Gold

Gold preparations (e.g., gold sodium thimalate, auranofin) have long been used as disease-modifying treatment of rheumatoid arthritis. Adverse effects include aplastic anemia, proteinuria, rash, stomatitis, and diarrhea [192]. The last effect is particularly common with auranofin, an oral preparation that increases intestinal permeability and decreases intestinal transit time.

Rarely, gold can induce a severe enterocolitis, with markedly ulcerated small and large intestinal mucosa. The clinical picture can mimic ulcerative jejunoileitis seen in celiac sprue, or small intestinal lymphoma. The clinical course may be protracted and, if the relationship to gold therapy is not appreciated, there may be a need for laparotomy. Gold should be withdrawn as soon as the condition is recognized. Severe, reversible cholestatic hepatitis has also been reported [193].

Lead poisoning

Lead poisoning remains the most common form of heavy metal intoxication, often producing vague, nonspecific, and transient symptoms. Inner-city children in major metropolitan areas are at particularly high risk of lead-based paint ingestion. The diagnosis should be considered in patients with abdominal complaints and a history of occupational or environmental exposure, such as ingestion or removal of lead-based paints, manufacture of batteries or jewelry, welding, automobile radiator repair, and eating from painted dishes acquired abroad [194].

Common symptoms include recurrent severe abdominal pain (lead colic), oral ulcers, constipation, stocking–glove paresthesias, psychiatric symptoms, and a metallic taste in the mouth. Physical findings include a gingival lead line (absent in edentulous patients) and peripheral neuropathy. Anemia and renal dysfunction also may be evident at presentation [195]. Lead inhibits enzymes involved in the synthesis of heme, and secondary porphyria, characterized by elevated ALA and other porphyrin metabolites in the urine, may result.

Blood lead concentrations are an unreliable predictor of body lead stores, as they reflect only recent exposure. The diagnosis of lead intoxication is best established by measuring 72-h urine lead levels after administration of calcium disodium edetate [196]. Treatment of lead poisoning with parenteral or oral [190] chelating agents is usually successful if the diagnosis is made in time.

Hematological disorders

Hemoglobinopathies

Sickle cell anemia is an autosomal recessive disease of hemoglobin synthesis (hemoglobin SS). Eight percent of the

African American population of the United States are heterozygotes; hence, as many as 15 of every 100 000 African Americans may be affected. Under conditions of low oxygen tension, erythrocytes containing hemoglobin SS deform (sickle), and become lodged in capillary beds, causing widespread venous congestion, thrombosis, and microinfarction and leading to the clinical vasoocclusive "crisis" [197].

In addition to diffuse pains throughout the long bones of the body, abdominal pain and intestinal ileus are common during the acute crisis. It is often difficult to distinguish a sickle cell crisis from other gastrointestinal emergencies such as cholecystitis, appendicitis, bowel infarction, and bowel obstruction. Moreover, these intraabdominal illnesses themselves may provoke a sickle cell crisis. The absence of bone or pleuritic pain is suggestive of a primary intraabdominal cause. Sickle cell crises often resolve in less than 4 days. Hence, abdominal symptoms of longer duration also should raise the possibility of a primary abdominal illness [198].

Acute crisis can selectively affect the liver with hepatic infarcts, causing pain, fever, and transaminase elevation. Chronic sickling causes cholelithiasis, cholecystitis, and chronic passive congestion. The common use of hypertransfusion programs to lower the risk of recurrent stroke places these patients at high risk of hemosiderosis [199]. A combination of these factors can lead to progressive liver injury, with significant fibrosis and cirrhosis developing by adulthood [200]. Splenomegaly is frequent in the early stages of the disease, but "autosplenectomy," caused by repeated splenic infarctions, often results in a shrunken, fibrotic, or calcified spleen. Hyposplenism will be documented by the presence of Howell–Jolly bodies in the peripheral blood smear and signals an increased risk from encapsulated organisms, such as pneumococci and bacteroids [197].

Patients with beta-thalassemia are characterized by a relative overproduction of alpha-globin chains, consequent ineffective hematopoiesis, and severe anemia. Current therapy has depended on transfusion therapy. Iron overload of tissue, which is fatal if not treated, is the most important complication of this disease. In patients not receiving transfusions, the enormous erythroid expansion leads to abnormally regulated iron absorption and increases in body iron burden ranging from 2 to 5 g per year. Regular transfusions may double this burden [201]. Iron-induced liver disease, often aggrevated by chronic hepatitic C, is a common cause of death in adults. This can be delayed by adequate deferoxamine therapy monitored by frequent quantitative measurement of liver iron stores with liver biopsy [202].

Hemolytic uremic syndrome/thrombotic thrombocytopenic purpura

The hallmarks of the hemolytic uremic syndrome (HUS) are hemolysis, thrombocytopenia, and acute renal failure. Although most common in children, all age groups are affected because thrombotic thrombocytopenic purpura (TTP) is likely the same illness manifesting with fever and central nervous system sequelae in patients over 40 years of age. Both are now grouped under the thrombotic microangiopathies. Antecedent gram-negative enteric infections with *Salmonella*, *Shigella*, and *Campylobacter* species have been associated with HUS/TTP [203]. In the past decade, verotoxin-producing strains of *Escherichia coli*, especially serotype O157:H7, appear to be responsible for most reported cases. It must be remembered that only a small minority (5% of patients with symptomatic enteritis in a recent outbreak) of patients with these foodborne illnesses develop HUS/TTP [204]. Thrombotic thrombocytopenic purpura has also been reported in some patients undergoing solid organ transplantation, associated with the use of cyclosporine (ciclosporin) or tacrolimus [205].

The pathophysiology connecting enteric infections and medications with the hematological consequences is poorly understood. The cellular pathogenesis of both HUS and TTP appears to involve diminished activity of the von Willebrand factor-cleaving protease (ADAMTS13), leading to an accumulation of ultralarge von Willebrand factor multimers [206,207]. Endothelial cell injury (perhaps mediated by endotoxins or cytokines) followed by thrombotic microangiopathy in the glomerulus and the gastrointestinal mucosa are consistent features (Fig. 109.5) [208,209]. Similar thrombotic angiopathy has been reported in gastric antral vascular ectasia syndrome (GAVE) (see Chapter 112). Thus, TTP should be included in the differential diagnosis of patients with endoscopic changes of GAVE and in whom biopsies show microscopic thromboses [210].

The spectrum of gastroenterological symptoms ranges from gastroenteritis, through segmental colitis mimicking Crohn's disease or ischemia, to fulminant colitis with toxic megacolon or colonic perforation. Bowel disease almost always

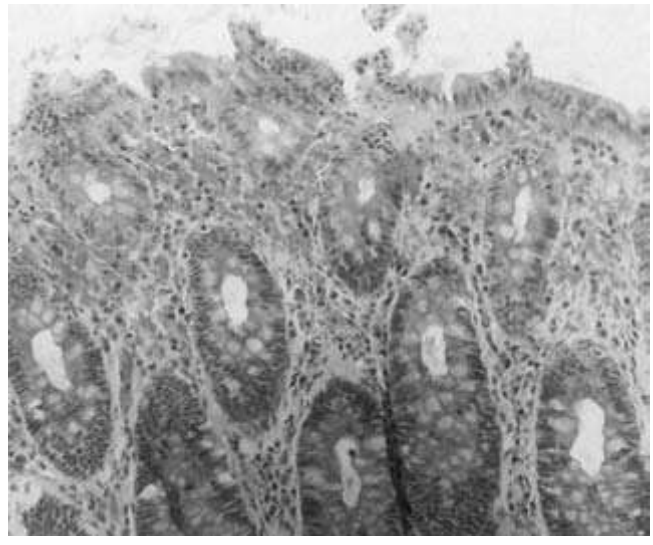

Figure 109.5 Colonic biopsy specimen from a patient with hemolytic uremic syndrome shows intravascular coagulation and thrombotic microangiopathy. (H & E stain; original magnification × 16.)

precedes the development of HUS [209]. The disease is usually self-limited, although the course may be prolonged, and fatalities occur. There is now evidence that early treatment of the enteric infection with antibiotics increases the risk of developing HUS/TTP, and should be avoided [211]. Treatment focuses on general support and dialysis when needed. In adults with neurological changes (e.g., TTP) plasmapheresis can be life-saving [208,209].

Hypercoagulable states

Hypercoagulability is an important cause of gastrointestinal morbidity. Spontaneous thrombosis of the mesenteric veins can cause intestinal ischemia and infarction (see Chapter 114) while thrombosis of the portal vein can lead to portal hypertension and variceal hemorrhage (see Chapters 32 and 128) and of the hepatic veins can result in ascites and the Budd–Chiari syndrome (see Chapter 44). Oral contraceptives, pregnancy, SLE, myeloproliferative disorders (see below), cancer, and surgery all are associated with a hypercoagulable state and a consequent propensity for venous thrombosis. Antithrombin III deficiency [212] and protein C deficiency are inherited (autosomal dominant) disorders associated with recurrent venous thrombosis. Factor V Leiden mutation, resulting in thrombophilia attributable to activated protein C resistance, has been associated with small bowel infarctions [213], hepatic vein occlusion during pregnancy [214], and an increased risk of venous thrombosis in patients with inflammatory bowel disease [215]. Once the cause is identified, most affected patients require lifelong therapy with warfarin to prevent recurrent vascular catastrophe.

Hypocoagulable states

Deficits of coagulation factors

Deficiencies of coagulation factors may be inherited or acquired and when severe are associated with bleeding tendencies. The best known of the inherited factor deficits is hemophilia A, an X-linked disorder of factor VIII synthesis. Other inherited forms include hemophilia B (Christmas disease or factor IX deficiency) and factor XI deficiency. Gastrointestinal bleeding occurs in all these diseases. It may be mucosal, intramural, or intraabdominal and may involve virtually any part of the gastrointestinal tract. Bleeding can be spontaneous or follow trauma or surgery. Upper gastrointestinal hemorrhage has multiple potential causes in these patients, including varices, because of the increasing incidence of chronic liver disease from blood-borne hepatitis viruses. If deemed important in the management of such patients, transjugular liver biopsy can be performed safely [216]. Endoscopy can be undertaken safely in hemophiliac patients after correction of factor VIII level to 0.4 U/mL [217,218].

Platelet abnormalities

Platelet defects also may result in severe bleeding diatheses that manifest as gastrointestinal bleeding. von Willebrand disease, the Bernard–Soulier syndrome, and Glanzman thrombasthenia are inherited diseases of platelet adhesion and aggregation associated with prolonged bleeding times. Aspirin and other drugs (e.g., penicillins, cephalosporins) interfere with platelet function by irreversibly inhibiting COX-1 activity and prolong the bleeding time for up to 5–7 days [219]. The importance of these drugs in reducing morbidity and mortality after heart attack, stent placement, and stroke is clear, and they should be stopped prior to endoscopy only with extreme caution.

Plummer–Vinson–Kelly syndrome

The Plummer–Vinson–Kelly syndrome is characterized by dysphagia, esophageal webs, and iron deficiency anemia. Middle-aged women are most commonly affected. Glossitis, dyspepsia, atrophic gastritis, diarrhea, hoarseness, and paresthesias are frequently present [220]. An association with postcricoid esophageal cancer has been reported. The syndrome is rarely seen, which may reflect the improved identification and treatment of iron deficiency anemia.

Metabolic disorders

Capillary leak syndrome

Capillary leak syndrome is a rare episodic illness of variable severity characterized by increased small vessel permeability and fluid shifts [221]. Its association with systemic toxicity, sepsis, and administration of a number of recombinant protein medications, including interleukin 2 and granulocyte–macrophage colony-stimulating factor (GM-CSF), supports the contention that it is mediated directly by cytokines [221,222]. In addition to edema, hemoconcentration, hypotension, and a monoclonal gammopathy, patients can experience severe nausea, vomiting, and diarrhea secondary to impaired fluid absorption in the gastrointestinal tract. Therapy with aminophylline and terbutaline has been reported to ameliorate the syndrome in some patients [223].

Systemic amyloidoses

The systemic amyloidoses are a group of diseases characterized by the widespread extracellular deposition of insoluble fibrillar proteins. Classification of amyloidosis is based on the precursor plasma proteins that form the fibril deposits. Two types of primary systemic amyloidosis are recognized: AL (immunoglobulin light chain related in patients with plasma cell dyscrasia) and ATTR (familial transthyretin associated). Secondary amyloidosis is formed from serum amyloid A, an acute-phase reactant formed in response to inflammation. It is termed AA and caused by chronic infectious or inflammatory diseases, such as tuberculosis and rheumatoid arthritis [224].

The range of organs infiltrated is greatest in AL amyloidosis, with the kidney and heart most commonly involved. The

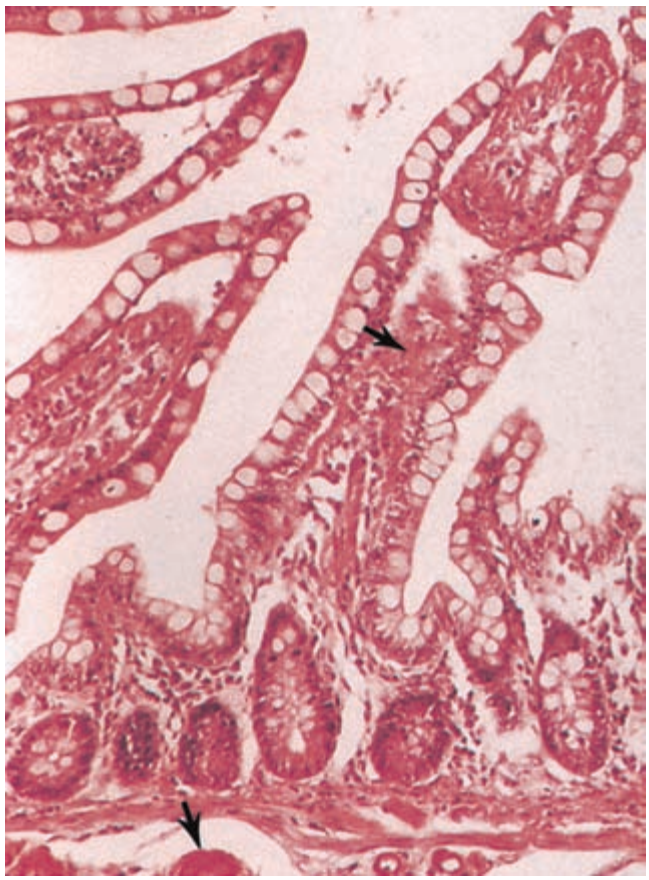

Figure 109.6 Amyloid infiltration of the small intestine (arrows). (Congo red stain; original magnification × 200.)

gastrointestinal tract may be involved at any site. Infiltration of the tongue results in macroglossia, a feature that is uncommon in the ATTR and AA forms of the disease. Gastric infiltration can produce prominent gastric folds, but resulting gastric outlet obstruction, ulcer, and bleeding are uncommon [225].

Amyloid can accumulate in the intestine and mesentery, causing coarsening of the valvulae conniventes (Fig. 109.6), retroperitoneal fibrosis, protein-losing enteropathy, and intestinal obstruction. Autonomic dysfunction can lead to stasis, bacterial overgrowth, and malabsorption [226]. This is also a common feature of the ATTR type. Intestinal ischemia has also been reported [227].

High alkaline phosphatase can signal amyloid deposition in the liver. Hepatomegaly but not splenomegaly is common in AL amyloidosis [228]. Hepatosplenomegaly is more common in AA amyloidosis, but liver failure and portal hypertension are uncommon [229]. Ascites may result from nephrotic syndrome-induced hypoproteinemia. Pancreatic involvement occasionally produces exocrine pancreatic insufficiency and steatorrhea.

The diagnosis of amyloidosis is based upon clinical suspicion and tissue biopsy of the affected organ. A safe and simple procedure, obtaining a sample of subcutaneous fat, has replaced the blind rectal biopsy recommended in the past. Treatment of the underlying disease process, if present, may result in amyloid regression, but this is not the rule. Stem cell transplant after high-dose chemotherapy is helpful in selected patients [230].

Neoplastic disorders

Cancer cachexia

Cancer cachexia, a common manifestation of advanced neoplasms, is characterized by marasmus with anorexia, early satiety, weight loss, anemia, and edema (see Chapter 49). Nutritional evaluations find an elevated basal metabolic rate and increased energy expenditure associated with systemic catabolism of muscle and adipose tissue [231]. Such tumor-induced, treatment-related malnutrition is the proximate cause of death in 20%–30% of patients with cancer, but it also complicates the delivery of adequate antineoplastic therapy and markedly impairs the quality of life [232].

In exploring the cellular mediators of cancer cachexia, two groups of compounds have been suggested. Materials produced by tumors with hormone-like properties that result in the direct catabolism of host tissues, such as angiotensin II and proteolysis-inducing factor (PIF), form one group [233,234]. The second group is the proinflammatory cytokines such as tumor necrosis factor alpha, interleukin 6 (and 1), and gamma-interferon [235].

The literature regarding the efficacy of nutrition support in cancer patients has been critically reviewed, and no clear benefit was found [236]. Megestrol acetate [237] and eicosapentanoic acid [234] have been shown to improve appetite and modestly increase weight.

Consequences of chemotherapy

Constipation

Patients with cancer commonly develop constipation as a consequence of factors such as decreased physical activity, reduced fluid intake, depression, drugs used in treatment, and changes in diet. Effective pain management may lead to the use of potent narcotics, all of which lead to constipation. Close attention to bowel function as opioids are being started will reduce the number of later problems with obstipated and impacted patients, who occasionally require hospital admission [238]. Metabolic disorders and intestinal luminal narrowing secondary to tumor growth or radiotherapy can also cause constipation. Constipation may result from hypercalcemia related to ectopic production of parathyroid-like hormone by neoplasms. Rarely, malignancy, particularly small cell carcinoma of the lung, produces a paraneoplastic visceral neuropathy that results in aberrant motor activity throughout the gastrointestinal system [239].

Although attention to diet is important, there are limitations to the amount of fibrous food the cancer patient will

tolerate. Reversal of established opioid constipation requires agents that increase peristalsis, such as senna derivatives (see Chapter 41), but polyethylene glycol can also be effective.

Diarrhea

Diarrhea is common immediately after potent chemotherapy. In adults, diarrhea may be a self-limited consequence of mucositis, but other causes need to be considered. *Clostridium difficile* colitis is a common cause. The spore-forming organism is found on the environmental surfaces of oncology units [240], and antibiotics are ubiquitous. Because sigmoidoscopy is avoided in neutropenic patients, identification of the toxin-forming *C. difficile* in stools should lead to treatment with metronidazole or vancomycin. In uncomplicated cases (no fever no blood in stool, negative cultures and toxin assays) loperamide up to 16 mg a day can reduce the frequency of diarrhea [241].

Mucositis

Damage of the oral and gastrointestinal mucosa (mucositis) is a common accompaniment of current chemotherapy regimens, and frequently leads to severe pain, inanition, diarrhea, and increased risk of severe infection. The pathobiology of the lesion is unknown, although a number of growth factors are currently under study and show promise. Currently, standard therapy includes close attention to oral hygiene, analgesia, infection identification and management, and the provision of parenteral nutrition when required [242].

Nausea and vomiting

Nausea and vomiting, common after chemotherapy, exacerbate inanition and malnutrition. Inadequate management of these symptoms is a common reason for discontinuation of potentially effective chemotherapy or the unwanted conversion of outpatient to inpatient chemotherapy. Cancer chemotherapy causes two main phases of vomiting: an intense, acute phase of emesis that occurs almost immediately following administration of anticancer drugs; and a delayed phase of nausea and vomiting of longer duration [243]. It appears that the acute vomiting is mediated by serotonin (5-hydroxytryptamine, 5-HT) binding to 5-HT3 receptors in the central nervous system, triggering emesis. High doses of intravenous 5-HT3 receptor antagonists (e.g., ondansetron, granisetron, dolasetron, palonosetron) with dexamethasone are most effective in treating acute-phase vomiting [244]. The addition of an oral neurokinin-1 antagonist (aprepitant) to this regimen has been found to decrease delayed vomiting and improve response with subsequent cycles of a highly emetogenic cisplatin-based chemotherapy [245]. A wide variety of antiemetics of differing effects and costs is available to the clinician [246]. Because the cost of newer pharmaceuticals needs to be matched against the increased total cost of therapy, and new drugs outpace the publishing industry, readers may find this frequently updated approach to acute and delayed emesis at the website of the National Comprehensive Cancer Network (www.nccn.org/professionals/physician_gls/PDF/antiemesis.pdf) valuable.

Anticipatory vomiting can be difficult to control once initiated. The development of emesis induced by the sights and sounds of the setting in which chemotherapy is provided is more likely to occur in patients with pretreatment anxiety, posttreatment dizziness, delayed nausea and vomiting, and severe nausea and vomiting [247]. Behavioral therapy has been shown to be effective once anticipatory vomiting has developed [248].

Hematological malignancies

Heavy-chain diseases

The heavy-chain diseases are rare lymphoplasma cell proliferative disorders associated with the production of abnormal monoclonal heavy chains without associated light chains. α-Heavy-chain disease (Mediterranean lymphoma), the most common of these entities, is seen in two forms: pulmonary and enteric. The former is more common in the United States, and it rarely exhibits gastrointestinal manifestations. The latter is found principally in the Mediterranean region. Infiltration of the small intestine and abdominal lymph nodes by malignant B cells and α-heavy chains produces abdominal pain and masses, vomiting, weight loss, malabsorption, steatorrhea, and hypocalcemia [249]. Additional complications include obstruction, intussusception, and intestinal perforation. Diagnosis is established by small bowel biopsy. If diagnosed early in the course of disease antibiotics can be helpful [250].

Leukemias

Both acute and chronic leukemias can involve the gastrointestinal tract. The gastrointestinal manifestations result chiefly from leukemic infiltration of the luminal gut and liver, or they are a consequence of therapy. Gingivitis is caused by leukemic invasion of the gums and causes oral pain and bleeding. Leukemic infiltration of the esophagus, stomach, intestine, or colon produces mucosal or intramural lesions that may cause dysmotility, obstruction, bleeding, or enterocolitis [251]. Hepatosplenomegaly and portal hypertension are additional complications of leukemic infiltration. Rarely, an illness indistinguishable from fulminant hepatitis occurs [252]. Subcapsular splenic hemorrhage and splenic rupture have been reported, particularly in acute lymphoblastic leukemia [253].

Induction chemotherapy for leukemia can induce an often fatal syndrome characterized by acute abdomen, paralytic ileus, diarrhea, and gastrointestinal bleeding. Development of this syndrome often is attributed to neutropenic enterocolitis or typhlitis, a severe inflammatory process that involves the distal small bowel, cecum, or appendix (Fig. 109.7) [254]. The etiology of the disease is unknown. Diarrhea (sometimes bloody), right lower quadrant pain or tenderness, nausea, and vomiting are the usual presenting features, developing

Figure 109.7 Typhlitis involves terminal ileum and cecum in a patient with acute myelogenous leukemia.

over hours to days. The differential diagnosis includes infectious colitis caused by *C. difficile*, cytmegalovirus (CMV), and other organisms, appendicitis, Ogilvie syndrome, and ischemia. Patients at risk must be observed carefully for evidence of perforation.

Lymphoma

As many as 10% of patients with non-Hodgkin lymphoma originating from a nongastrointestinal site develop involvement of the gastrointestinal tract [255]. Obstruction, bleeding, abdominal masses, and perforation are among the more frequently recognized clinical features. Invasion of the liver and spleen is frequent, commonly presenting with fever and elevated alkaline phosphatase. Lymphoma in the liver can mimic acute hepatitis and fulminant hepatic failure [256]. Mantle cell lymphomas can present as multiple lymphomatous polyposis and resemble the familial adenoma syndromes until biopsy is obtained [257]. Primary digestive tract lymphoma of the stomach (see Chapter 67) and the intestine (see Chapter 78) are discussed elsewhere.

Clinically evident gastrointestinal involvement in Hodgkin disease is infrequent, although a substantial proportion of patients who die from this illness are found at autopsy to have hepatic involvement (Fig. 109.8) [258]. Presinusoidal portal hypertension can result from either increased intrahepatic blood flow as a consequence of splenomegaly or intrahepatic infiltration with malignant cells. When the luminal gut is involved, thickened mucosal folds are common (Fig. 109.9).

Multiple myeloma

Multiple myeloma results from a monoclonal neoplastic proliferation of plasma cells and usually affects middle-aged and older people. Osteolytic lesions of the skeleton, hypercalcemia, anemia, monoclonal gammopathy, and renal disease are characteristic findings. When the gastrointestinal tract is invaded, plasmacytomas form, with attendant abdominal pain, ulceration, bleeding, or obstruction. The lesions can be detected radiographically or endoscopically [259] and may look like peptic ulcer disease or colon cancer. Amyloidosis is a well-recognized complication of myeloma (see section on metabolic diseases). Involvement of the liver and spleen results in hepatosplenomegaly and, rarely, portal hypertension. Jaundice can be caused by infiltration of the liver parenchyma or obstruction of the biliary tree [260].

Figure 109.8 Hodgkin disease infiltrating the liver. Reed–Sternberg cells are present (arrows). (H & E stain; original magnification × 25.)

Myeloproliferative disorders

Myelofibrosis with myeloid metaplasia is associated with bone marrow fibrosis and extramedullary hematopoiesis in the spleen and liver, and presents with marked hepatosplenomegaly and progressive anemia. Portal hypertension, associated with ascites or variceal hemorrhage, occurs in about 7% of patients. The portal hypertension can be

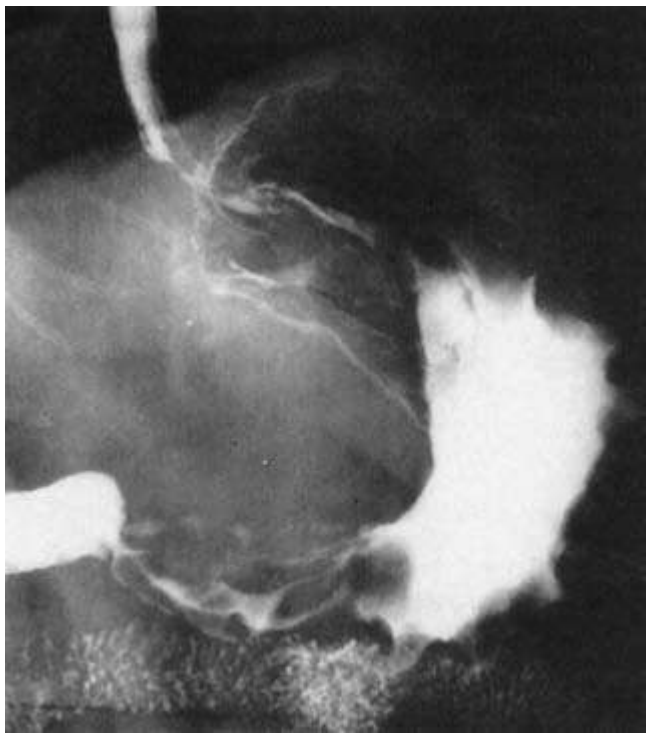

Figure 109.9 Hodgkin disease infiltrating the stomach. Thick folds in antrum and fundus.

due to the increased portal flow through the enlarged spleen and obliteration of small portal venules, and is resolved with splenectomy. Patients can also develop acute left upper quadrant abdominal pain due to splenic infarction [261].

Paroxysmal nocturnal hemoglobinuria is characterized by intravascular hemolysis that is manifest by episodes of hemoglobinuria and life-threatening venous thrombosis. In a large cohort of such patients seen over a 30-year period at one hospital [262], the most common sites of venous thrombosis were the hepatic and mesenteric veins. This was also the most common cause of death. All such patients should be on long-term anticoagulation.

Polycythemia vera affects middle-aged people and is characterized by an increased red cell mass. Many patients have associated thrombocytosis. The erythrocytosis and hyperviscosity syndrome lead to plethora, headache, vertigo, dizziness, visual disturbances, vascular ischemia, and venous thrombosis [263]. Budd–Chiari syndrome may develop as a consequence of hepatic vein thrombosis. Hepatosplenomegaly resulting from extramedullary hematopoiesis and bleeding esophageal varices secondary to portal vein thrombosis and increased blood flow through the giant spleen are described.

Primary or essential thrombocytosis is characterized by platelet counts greater than $10^6/mm^3$ but without an increase in the red cell mass. This condition must be differentiated from the secondary thrombocytosis that accompanies inflammation and iron deficiency. Platelet aggregation defects predispose to gastrointestinal bleeding or thrombosis. Hepatosplenomegaly is evident in many patients [264] and is caused by extramedullary hematopoiesis or Budd–Chiari syndrome.

Waldenström macroglobulinemia

Waldenström macroglobulinemia is an IgM-secreting variant of multiple myeloma that is characterized by the presence of macroglobulin (monoclonal IgM) in the serum. Osteolytic lesions are seen less commonly than in myeloma, whereas lymphadenopathy and hyperviscosity are more prominent. Massive hepatosplenomegaly results from infiltration by plasma cells. The deposition of large amounts of IgM in the intestinal lamina propria and mesenteric lymph nodes can impair absorption [265], resulting in diarrhea and steatorrhea.

Nonhematological malignancies

Gynecological malignancies

The treatment of cancer of the cervix, depending on stage of disease, usually involves a combination of a hysterectomy, chemotherapy, and radiation therapy. Because any invasion of the bowel can abort attempts at exenteration procedures [266], a change in bowel habit or blood in the stool should lead to a colonoscopy before operation. Most gastrointestinal consequences of cervical cancer relate to the sequelae of radiation therapy. The risk of more severe radiation-induced bowel complications, such as obstruction or fistula formation, is doubled when adjuvant radiotherapy follows, rather than precedes, hysterectomy [267]. Although most radiation-induced bowel disease occurs in the first 2 years after radiotherapy, illness can occur at any time in the next several decades (see Chapter 115).

Ovarian cancer can produce gastrointestinal complications by local extension and frequently encases the abdominal viscera. Intestinal obstruction, either because of direct invasion of bowel by peritoneal metastases or as a consequence of radiotherapy, is a common and disabling clinical complication [268]. In many terminally ill patients, palliation of vomiting can be a distressing management problem that is beyond the capabilities of reconstructive surgery. Options for palliation include the use of a percutaneous endoscopic gastrostomy for venting [269] and the use of intraluminal stents to bypass extrinsic compression [270]. Refractory malignant ascites also can be an important cause of ovarian cancer morbidity, requiring repeated paracentesis.

Metastases to the ovary from gastrointestinal malignancies (Krukenberg tumors) may be difficult to distinguish from primary ovarian cancer by noninvasive imaging modalities. Typically, these tumors are bilateral, asymmetrically large, and solid. Almost all are mucinous signet ring tumors from the stomach, pancreas, and colon, all with poor prognosis [271].

Malignant melanoma

Malignant melanoma is a common malignancy whose prevalence is increasing. Although metastases to the gastrointestinal

tract are commonly revealed at autopsy, fewer than 9% of melanoma patients are diagnosed with gastrointestinal involvement while living [272]. The small intestine, stomach, and colon are most commonly involved, but the liver, pancreas, and gallbladder also can be affected. Although most patients remain asymptomatic, complications include abdominal pain, anorexia, gastrointestinal hemorrhage, and intestinal perforation. In appropriate circumstances, surgical intervention to alleviate significant gastrointestinal complications is warranted and may prolong survival [273].

Mastocytosis

Mastocytosis is a rare disorder characterized by the clonal proliferation of mast cells into the skin (urticaria pigmentosa) and other organs, including the intestines and liver. Common symptoms are headache, pruritus, flushing, dizziness, wheezing, and tachycardia. Serum tryptase and urinary histamine excretion are elevated. Gastrointestinal dysfunction develops more commonly than previously thought, resulting in abdominal pain, nausea, diarrhea, fecal urgency, malabsorption, gastritis, and peptic ulcers [274]. Rubbing cutaneous lesions or drinking alcohol may precipitate symptoms. Symptoms probably develop secondary to the release of many mast cell products, such as histamine, leukotrienes, and platelet-activating factor. Many patients have liver disease, manifested by hepatosplenomegaly, portal hypertension with ascites, and abnormal liver tests. Liver biopsy shows portal fibrosis, nodular regeneration, and venoocclusive disease. Diagnosis and therapy are controversial and should be performed in centers capable of genetic analysis of bone marrow [275].

Metastatic disease to the gastrointestinal tract

Intestinal metastasis from tumors originating in locations outside the gastrointestinal system can induce significant symptoms. Abdominal pain, obstruction, and bleeding are common consequences. Other than melanoma, malignancies derived from organs such as the breast, lung, and thyroid [276] can metastasize to the intestines and on occasion are diagnosed by endoscopic biopsies. In approximately half of patients with Kaposi sarcoma, an accompaniment of AIDS, intraoral or intestinal involvement develops (Fig. 109.10) [277].

Renal cell carcinoma

Renal cell carcinoma (hypernephroma) can directly invade into, or metastasize to, the intestinal tract, causing bleeding [278] and obstruction. Resection of the primary tumor has been associated with regression of metastatic lesions. Nonmetastatic hepatic dysfunction (Stauffer syndrome) is usually manifest by abnormal alkaline phosphatase and prolongation of the prothrombin time [279], and it is associated with hepatic sinusoidal dilation [280]. It is possible that in some cases the elevated phosphatase derives from the tumor itself and does not denote hepatic dysfunction.

Figure 109.10 Kaposi sarcoma of the lower extremities.

Neuromuscular disorders

Autonomic dysfunction

The autonomic nervous system supplies every organ in the body, and dysfunction is associated with orthostatic hypotension, erectile problems, urinary retention, and temperature deregulation. Abnormal gastrointestinal function of the sympathetic and parasympathetic pathways in primary neurological disorders such as Parkinson disease, pandysautonomia, and paraneoplastic syndrome is manifested by vomiting, small bowel bacterial overgrowth, intestinal pseudoobstruction, fecal incontinence, and constipation (Table 109.6) [281,282]. A number of gastrointestinal diseases can produce dysautonomic symptoms, particularly advanced liver disease [283]. Antibodies to ganglionic acetylcholine receptors have been found in a group of patients with idiopathic dysautonomia, suggesting an autoimmune etiology for some [284].

A common association with the neurological diseases characterized by autonomic dysfunction is postprandial hypotension. Marked reduction in blood pressure after a meal may result in syncope, weakness, angina pectoris, nausea, and falls. The pathophysiology is complex and includes inadequate sympathetic nervous system compensation for meal-induced splanchnic pooling of blood, impaired baroreflex

Table 109.6 Gastrointestinal manifestations of neuromuscular disorders

Disorder	Symptoms	Causes
Amytrophic lateral sclerosis	Dysphagia with aspiration and inanition	Oropharyngeal dysfunction
Autonomic dysfunction	Vomiting; bloating	Decreased gastric emptying
Small bowel bacterial overgrowth	Constipation	Small bowel/colonic pseudoobstruction
Dementia syndromes	Dysphagia, aspiration, feeding problems	Oropharyngeal dysfunction
Migraine	Vomiting, abdominal pain, diarrhea	Secondary to headache attack
Multiple sclerosis	Dysphagia with aspiration; constipation	Oropharyngeal dysphagia
Prolonged transit time, decreased gastrocolic reflex, difficulty in transferring for toileting	Fecal incontinence	Decreased perception of rectal distention, decreased anal sphincter function
Muscular dystrophies, oculopharyngeal	Dysphagia with aspiration; GERD; fecal incontinence	Oropharyngeal dysfunction; LES dysfunction; decreased anal sphincter tone
Duchenne muscular dystrophy	Nausea/vomiting; abdominal distention; constipation	Decreased gastric emptying; prolonged transit time; pseudoobstruction
Myasthenia gravis	Dysphagia	Oropharyngeal dysfunction
Myotonic dystrophy	Dysphagia/aspiration; bloating, constipation; fecal incontinence	Myotonic contractions of the tongue and pharynx; prolonged transit time; decreased anal sphincter tone
Parkinson disease	Dysphagia, constipation	Oropharyngeal dysfunction
Decreased colonic motility, impaired defecation, anticholinergic medications		
Spinal cord disorders	Heartburn, bloating; constipation; fecal incontinence	High number of lesions impairs gastric and small bowel transit; decreased rectal sensation; impaired voluntary control of defecation and anal sphincter

GERD, gastroesophageal reflux disease; LES, lower esophageal sphincter.

function, and peripheral vasodilation secondary to vasodilatory gastrointestinal peptides and insulin secretion. Similar symptoms are also seen in the elderly without more global dysautonomia [285].

Dementia syndromes

Patients with dementia syndromes, such as Alzheimer disease, multiinfarct dementia, and Huntington disease, exhibit gastrointestinal symptoms related to oropharyngeal dysfunction, with dysphagia for solids and liquids and an increased risk of aspiration and progressive inanition and malnutrition. Diminished recent memory and disorientation impede rapid diagnosis of common and often easily recognized medical problems.

In advanced terminal neurological diseases, difficult decisions must be made pertaining to providing or withholding enteral feedings that must be delivered by nasogastric tube, gastrostomy, or jejunostomy. These techniques of enteral alimentation permit more effective, longer administration of defined enteral diets that can prolong the end stage of patients in a severely demented or "vegetative" state. Enteral support through feeding tubes is a form of medical treatment that is not necessarily mandatory for all patients in such condition, and indeed would not be the choice of most competent patients living in a nursing home [286–288]. Percutaneous endoscopic gastrostomy is an easily applied, safe (but not perfectly so [289]), and popular method of administering enteral feeding. Jejunostomies should be used only in patients with severe gastroesophageal reflux, in whom they may provide additional protection against aspiration pneumonia.

Hiccups

Hiccups (or singultus) are intermittent, involuntary, spasmodic contractions of the diaphragm and accessory muscles of respiration that lead to abrupt ending of inspiration by airway closure at the glottal level. Transient hiccups are a common phenomenon often associated with overindulgence in food or drink, and they can be dealt with effectively by a variety of home nostroms and traditional remedies. Hiccups become a medical problem when they are prolonged and unresponsive to the usual approaches. Prolonged hiccups lasting for weeks or months are seen more commonly in men and can lead to insomnia, inanition with weight loss, and occasionally suicide [290].

Particularly if the hiccups continue while the patient is sleeping, an organic cause should be sought. The potential causes of protracted hiccups are extensive and include foreign bodies in the ear canal, cervical tumors or adenopathy, central nervous system disease, metabolic disease (diabetes, uremia, hyponatremia), alcoholism, and chest (tumors or inflammation in the mediastinum, pericardium, pleural space, or diaphragm) disease. Gastrointestinal diseases associated

with protracted hiccups include gastroesophageal reflux, gastric obstruction with distention, and stomach or esophageal tumors. Many of these problems can be defined with a careful history and physical, plain films of the chest or abdomen, and routine blood work. Further diagnostic studies may require computed tomography (CT) of the chest, abdomen, and central nervous system, endoscopy, and otolaryngology and neurology consultations. For many patients, a specific causal etiology may not be found [291].

In a practical sense, the most immediate problem confronting the clinician is how to abort the protracted hiccups. Assuming that the usual home remedies (e.g., breath holding, drinking water) have been unsuccessful, local stimulation of the pharynx with a nasogastric tube might be tried. Continued hiccups would lead to sequential trials of medications, none of which has been evaluated in randomized trials. Baclofen, a centrally acting antispasticity agent, has been effective in dosages of 5–60 mg a day orally [292]. The dopaminergic antagonists (chlorpromazine, metoclopramide, haloperidol), amitriptyline, carbamazepine, diphenylhydantoin, quinidine, and nifedipine have all successfully stopped protracted hiccups [293].

Migraine

Migraine is a periodic headache syndrome that usually begins early in life. Well-defined visual, sensory, or motor dysfunction may precede or accompany the headache. Gastrointestinal manifestations are almost universal and include nausea, vomiting, crampy abdominal pain, and diarrhea. The nausea and vomiting may impede the use of oral medications during an attack. Sumatriptan, a commonly employed antimigraine medication, and a selective 5-hydroxytryptamine-1 receptor agonist, has been associated with the development of ischemic colitis [294].

Migraine equivalents such as cyclic vomiting [295] and abdominal migraine [296] are encountered, particularly in childhood. The diagnostic criteria include recurrent stereotypic attacks of either intractable vomiting or upper abdominal pain, the absence of abdominal symptoms between attacks, a family history of migraine, and response to specific migraine therapy. Only occasionally are these symptoms accompanied by headache or prodrome. Nortriptyline has been used with some efficacy in adults to prevent attacks of vomiting.

Multiple sclerosis

Multiple sclerosis (MS) is a focal demyelinating disorder of the central nervous system. Whipple disease can mimic the clinical presentation of MS. Epidemiological studies suggest that patients with inflammatory bowel disease (IBD) have an increased risk of developing demyelinating diseases [297], as do patients receiving anti-TNF therapy [298]. Oropharyngeal dysphagia is common, but in population-based studies constipation and fecal incontinence are the most serious and disabling problems for MS patients. Constipation is a problem for more than two-thirds of MS patients, while about 25% experience fecal incontinence weekly [299]. Causes of constipation are multiple, including difficulty in transferring to commode, increased segmenting contractions that lead to prolonged transit time [300], and diminished gastrocolic response to a meal. Abnormalities of anal sphincter function and diminished perception of rectal distention contribute to the incontinence [301].

Careful attention to bowel function can markedly improve the quality of life of a patient with an MS. Having an occupational therapist evaluate the home environment to improve access to a commode, judicious use of fiber in the early stages of disease, and a strict bowel regimen that uses digital stimulation or glycerin suppositories and enemas to avoid impaction are all useful. Stimulant laxatives may increase bowel spasm and cause unpredictable bowel explosions that always are associated with incontinence and further lack of control.

Muscular dystrophies

Muscular dystrophies are inherited myopathies featuring progressive weakness and muscle wasting. Although generally thought of as involving only the skeletal muscle, several forms have been associated with motor disturbances of the gastrointestinal system. Ptosis and dysphagia are the principal manifestations of oculopharyngeal muscular dystrophy. Radiographic abnormalities reflecting the global degeneration of skeletal muscle include altered clearance of barium from the pharynx, delayed initiation of swallowing, aspiration, and nasopharyngeal regurgitation [302]. The external anal sphincter is also nonfunctional.

Duchenne muscular dystrophy is an X-linked recessive disorder that usually presents with initial proximal skeletal muscle weakness by the age of 5 years, progressing to incapacitation and death by the third decade of life. Gastrointestinal symptomatology includes nausea, vomiting, abdominal distention, and constipation. Acute gastric dilation [303] and intestinal pseudoobstruction have been reported [304].

Myotonic dystrophy

Myotonic dystrophy is an autosomal dominant, slowly progressive condition, with symptoms presenting in the third decade of life. The distinctive features are delayed relaxation of muscle after initial contraction, muscle wasting of a characteristic pattern, frontal baldness in men, testicular atrophy, dysarthria, and cataracts. Studies have found abnormal muscle function throughout the gastrointestinal tract. Common symptoms are abdominal pain, dysphagia, emesis, chronic or episodic diarrhea, and fecal incontinence. One-fourth of the patients consider their gastrointestinal symptoms the most disabling consequence of the disease [305].

Weak or myotonic contractions of the tongue and pharynx can result in oropharyngeal dysphagia and aspiration. Delayed gastric emptying, prolonged intestinal transit, and abnormal anal sphincter function also may develop [306,307].

Parkinson disease

Parkinsonism is characterized by progressive rigidity, tremor at rest, bradykinesia, and diminished postural reflexes. Autonomic dysfunction is common. Although 10%–15% of cases occur in the third and fourth decades of life, the prevalence of the disease increases exponentially in the elderly, being higher than 3% among those over the age of 65. Asians and African Americans are less likely to develop the disease than the white population. Regularly observed pathological changes include degeneration of pigmented brain stem nuclei, which contain distinctive eosinophilic intracytoplasmic inclusions termed Lewy bodies. Dementia is common and loss of sympathetic ganglia and parasympathetic neurons in the enteric nervous system has been documented [308].

Gastrointestinal dysfunction is common and clinically important in Parkinsonian patients. Dysphagia, bloating, nausea, constipation, and defecatory dysfunction are common symptoms. Over 50% of patients have swallowing disorders. For many, pharyngeal swallowing dysfunction documented on swallowing studies is similar to that seen after stroke or with amyotrophic lateral sclerosis. Gastric emptying is disordered in many patients, and is associated with abnormal myoelectric activity [309]. The gastroparesis may lead to malnutrition, it may also be responsible for some of the motor fluctuations seen with levodopa therapy. The therapy that appears to be most effective in reducing these symptoms, domperidone [310], is not available in the United States.

Constipation characterized by colonic inertia is associated with depletion of dopaminergic neurons in the muscularis externa, decreased colonic motility, and alteration of puborectalis and anal sphincter function. Constipation is aggravated by the anticholinergic effects of antiparkinsonian medications. The use of stimulant laxatives is common. Pelvic floor dyssynergia is frequently documented, and straining to defecate complicates the constipation [311]. A daily bowel regimen with the use of glycerin suppositories can be helpful.

Spinal cord disorders

Acute spinal cord injury is commonly associated with ileus, and patients are at increased risk of gastrointestinal bleeding [312]. Nasogastric suction and prophylaxis with continued acid suppression or sucralfate are warranted. After surviving the acute injury, constipation and fecal incontinence are almost universal and are a major source of disability and depression [313]. Over time, laxative dependence and dilated colon develop, and the increased time to generate a movement occupies a great deal of the patient's day. Rectal sensation, voluntary control of defecation, and normal anal sphincter function are lost [314]. Loss of sympathetic coordination leads to slow transit [315]. A carefully designed bowel program that includes laxatives, manual distention of the rectum to stimulate the intact defecation reflex, and careful attention to assistance in transfer to a commode or low toilet can be helpful. Although a few patients may be aided by anterior sacral root stimulators [316], in many patients a colostomy can substantially improve the quality of life [317].

Other neuromuscular diseases

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive loss of motor neurons (perhaps as a consequence of mitochondrial injury [318]), muscle atrophy, and weakness. Dysphagia will ultimately be present in all patients and is due to discoordination of the entire pharyngeal and upper esophageal swallowing mechanisms. Rapid nutritional disintegration is common, speeding the potential for respirator dependence. Studies demonstrate that placement of a percutaneous endoscopic gastrostomy (PEG) is safe, rapidly improves nutrition, and seems to improve quality of life in ALS patients [319]. Delayed gastric emptying has been reported, and if present might necessitate the use of jejunal feeding.

A variety of mitochondrial DNA defects have been identified that lead to a global and progressive degeneration in muscles and gastrointestinal symptoms that are not dissimilar to ALS. These have been termed the mitochondrial myopathies and, as with ALS, management of dysphagia and bowel dysfunction are central to the quality of life of the patients [320,321].

Residual swallowing problems are common in patients who have had a stroke. The consequences are impaired nutritional intake, decreased quality of life, and a risk of aspiration pneumonia. Pharyngeal motor dysfunction and a delay in swallowing initiation are common after acute stroke. Vocal cord mobility is reduced, with resulting reduction in airway protection. Swallowing therapy may help maintain oral intake in some cases [322]. Commonly, a choice must be made with dysphagic stroke patients as to whether and when to provide nutrition via nasogastric feeding or percutaneous gastrostomy. A recent randomized trial confirmed that early enteral feeding marginally increases early survival, but at the expense of increasing the proportion of patients surviving with a poor functional outcome. Outcomes after endoscopic gastrostomy were not as favorable as with nasogastric feeding [323].

Myasthenia gravis is an autoimmune disorder of the neuromuscular junction that is a treatable cause of oropharyngeal dysphagia [324]. Some patients have coexisting immunodeficiency states and may have intestinal villus atrophy and increased susceptibility to enteric infections [325]. Stiff man syndrome consists of symmetrical, progressive stiffness and painful spasm of axial musculature. Dysphagia may develop, possibly related to muscle spasm of the cricopharyngeus and upper esophagus [326].

Nutritional disturbances

Malnutrition

Malnutrition results from famine, war, poverty, natural disasters, poor dietary habits, and chronic disease. Kwashiorkor

identifies the protein-deficient patient with an adequate intake of calories, exmplified in developed countries by the catabolic patient after trauma, infection, or burn. Typically such patients are characterized by a persistent edematous state. Marasmus syndromes are causally related to a deficiency in total caloric intake, the starved patient [327]. Diarrhea and infection are common manifestations of the terminal malnutrition. Malnutrition causes both humoral and cellular immunodeficiency, which renders the mucosa susceptible to bacterial overgrowth and infection by various pathogens, such as parasites, toxigenic bacteria, and viruses. Atrophy of the intestinal mucosa and pancreas contributes substantially to development of the diarrheal state. Hepatomegaly may develop in conjunction with fatty liver, and pancreatic enzyme secretion may be reduced by over 80% [328]. In Western countries, chronic alcoholism and chronic liver disease are common causes of malnutrition [329,330].

The indications for the delivery of nutrients by the enteral and parenteral route as well as the efficacy of nutritional supplementation in malnourished, hospitalized patients are unproved [331]. Recent weight loss of more than 10% of body weight, the inability to eat for 5–7 days in an undernourished patient or for 10–14 days in a well-nourished patient, and a hypercatabolic state (e.g., burn) are generally proposed criteria for initiation of nutritional supplementation [236], especially in critically ill patients. Both parenteral and enteral formulations can provide all essential nutrients, but the enteral route is preferred whenever the gastrointestinal tract is functional [332].

Refeeding of extremely malnourished patients should be done gradually to permit mucosal and metabolic adaptation and to avoid exacerbation of diarrhea. Many patients will need pancreatic enzyme supplementation. Too rapid refeeding with high-calorie solutions that are rich in carbohydrates can lead to death by precipitating severe hypophosphatemia, hypokalemia, and hypomagnesemia and by intensifying other metabolic disturbances [333].

Obesity

Over 50% of adult Americans weigh more than 10% above ideal body weight, and obesity-related conditions are estimated to contribute to more than 300 000 deaths annually. Detailed evaluation of this topic can be found in Chapter 22.

Organ transplantation and complications

Bone marrow transplantation

Most of the gastrointestinal complications that occur after bone marrow transplantation (BMT) relate to graft-versus-host disease (GVHD) and the consequences of chemotherapy (see above). Overt gastrointestinal bleeding is uncommon (7.4%) in such patients when sucralfate or histamine H_2 antagonists are given prophylactically. Endoscopy usually does not define an isolated lesion and carries a 19% risk of inducing clinically relevant bacteremia [334]. Antibiotic prophylaxis should be given, and the risk and benefit of endoscopy should be considered before initiating the procedure.

A suppressed immune system predisposes the BMT recipient to life-threatening infections. Bacterial and fungal infections of the gastrointestinal tract are more common during the initial 30 days, whereas viral and parasitic infections are observed more often after day 30 [335]. Oropharyngeal and esophageal thrush occur frequently. Herpesvirus infections and CMV are common and may involve any portion of the gastrointestinal tract. Acute diarrhea is most commonly caused by GVHD, but *C. difficile* infection can cause severe, prolonged diarrhea [336]. Diagnosis depends on appropriate stool studies.

Graft-versus-host disease

Allogeneic stem cell BMT preceded by myeloablative high-dose chemotherapy conditioning can cure hematological malignancies but is associated with a high mortality. Acute GVHD (see Chapter 129) is the major cause of death. Recently, the use of nonmyeloablative conditioning (spurred by the concept that the curative potential of BMT relies on the killing of the recipient's malignant cells by the graft's T-cells and that newer highly immunosuppressive conditioning regimens cause less epithelial damage) have demonstrated reduced GVHD without reducing the curative potential of stem cell BMT [337,338].

The earliest sign of GVHD is the onset of a severe watery, secretory diarrhea. Anorexia, nausea, vomiting, jaundice, gastrointestinal bleeding, and abdominal pain may also be present, and 10%–20% have either severe hemorrhage or perforation [339]. Although less common, chronic GVHD may also cause gastrointestinal symptoms. The diagnosis is best made by endoscopy with biopsy, a relatively safe and simple procedure (Fig. 109.11). Tissue typically demonstrates apoptosis and gland destruction, sparse inflammatory infiltrate, and granular eosinophilic debris in dilated glands. The granular debris may be the most specific feature but is not seen commonly [340]. Oral beclomethasone dipropionate and prednisone have been found to be more effective than prednisone alone in treating intestinal GVHD [341].

Hepatic venoocclusive disease

Liver damage secondary to cytoreductive therapy is common after BMT. The most prominent site is the terminal hepatic venule. Venoocclusive disease is manifested by hyperbilirubinemia, hepatomegaly or right upper quadrant pain, and sudden weight gain within 20 days of transplantation without other cause. Pretransplant aminotransferase elevations, high-dose cytoreductive therapy, and persistent fever are independent risk factors. There is no clearly defined therapy for venoocclusive disease, which, when severe, is associated with multiorgan failure and death [342].

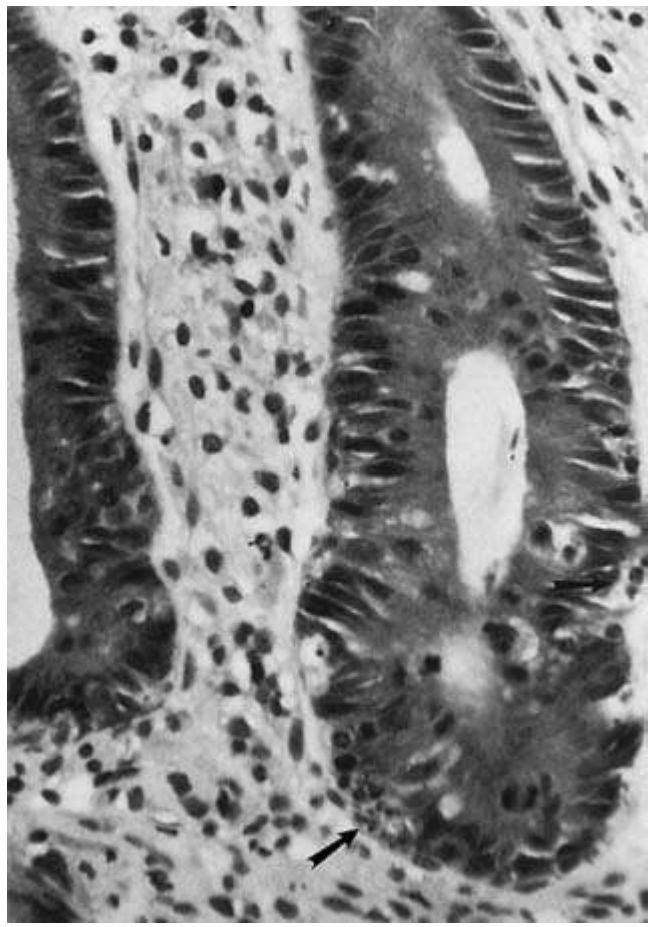

Figure 109.11 Rectal biopsy specimen shows crypt cell degeneration, which is the characteristic lesion of graft-versus-host disease. (H & E stain; original magnification × 100.)

Heart transplantation

The most common severe gastrointestinal complications after heart transplant include upper gastrointestinal bleeding, pancreatitis, cholecystitis, and bowel perforation. An increased risk for pancreaticobiliary disease persists for 1 year after the operation [343]. Although there is no evidence of an increased risk of colorectal cancer or adenomatous polyps in patients post transplant, nor any evidence-based guideline to follow, the chronic immunosuppression dictates a vigilant approach to colorectal cancer screening.

Lung transplantation

The risks of lung transplantation are similar to those noted after other solid organ transplants; however, several reports have described a high frequency of postoperative colonic perforation [344,345]. Because mortality is related directly to the duration of delay in diagnosis after symptoms are noted and symptoms may be blunted secondary to immunosuppression, careful evaluation for abdominal distention with plain films of the abdomen may help to guide clinical decisions. Because of the extensive mediastinal dissection required during heart–lung transplants, vagal injury can occur, leading to dysphagia and gastric stasis. Fortunately, there are few important clinical sequelae of the nerve injury [346]. Fatal hyperammonemia has been reported to occur uncommonly after lung transplantation, and is associated with hepatic glutamine synthetase deficiency [347].

Renal transplantation

Patients who undergo renal transplantation commonly develop gastrointestinal complications [348]. Peptic ulcer disease with hemorrhage or perforation was common when high doses of prednisone were used, but is much less common in the cyclosporine era. More effective medical therapies exist for ulcers, making surgical intervention less common [349]. Intestinal ischemia, diverticulitis with perforation, CMV-induced gastroduodenitis and colitis, intestinal tuberculosis, and intraabdominal abscesses are increasingly common [350]. Infection with hepatitis C and B is common in dialysis patients who develop chronic renal failure. After transplantation, the immune suppression may accelerate the course of chronic hepatitis (particularly hepatitis B) and fulminant hepatitis may occur [351].

Pregnancy

Pregnancy is accompanied by a variety of physiological changes, many of which have been attributed to the effects of progesterone or α-human chorionic gonadotropin on gastrointestinal motility and displacement of the viscera by the gravid uterus. Common gastrointestinal symptoms of pregnancy include altered appetite, pica, ptyalism, gingivitis, vomiting, dyspepsia, heartburn, constipation, and hemorrhoids. Management of symptoms with pharmaceuticals is complicated by a paucity of clinical studies in humans demonstrating safety for the fetus. These issues are dealt with in detail in Chapter 50.

Liver disease

Although the liver functions normally in pregnancy, palmar erythema and spider nevi are often observed, perhaps as a result of elevated steroid hormones. Serum alkaline phosphatase and leucine aminopeptidase levels are increased, attributable to placental production. Other liver enzyme levels are normal. Jaundice is infrequent, but when it occurs it is most often the result of intrahepatic cholestasis of pregnancy (ICP) [352]. Cholestasis presents in the second or third trimester as severe generalized pruritus that worsens at night. Marked increases in alkaline phosphatase and serum bile acids with normal γ-glutamyltransferase (GGT) are characteristic. A subgroup with raised GGT is associated with heterozygous *MDR3* mutations [353]. In Chile, ICP has been associated with a decrease in blood selenium level, suggesting

environmental causes [354]. There is an increased risk of stillbirths and premature births. Ursodiol can help control the pruritus, but its effects on the outcome of pregnancy are uncertain [355]. Prophylactic vitamin K should be administered parenterally to avoid postpartum bleeding [352].

The HELLP syndrome is a variant of severe preeclampsia characterized by hemolysis, elevated liver enzymes, and low platelets. Its features suggest a thrombotic microangiopathic process, and it must be distinguished from acute fatty liver of pregnancy, hemolytic–uremic syndrome, and thrombotic thrombocytopenic purpura [356]. Acute fatty liver and HELLP have been associated with the presence of long-chain 3-hydroxyl coenzyme A dehydrogenase deficiency in the infants of these mothers [357]. Marked elevations of transaminases are unusual and may suggest complicating hepatic vein thrombosis or fulminant liver failure, or spontaneous intrahepatic hemorrhage and hepatic rupture with an increased mortality [358]. Despite studies suggesting high fetal and maternal mortality with the HELLP syndrome [356,359], recent studies have noted greatly improved outcomes when interdisciplinary teams (that included a hepatologist) have managed the patients aggressively with expeditious delivery [360,361].

Acute fatty liver of pregnancy resembles Reye syndrome and usually occurs in young primigravida or multiparous women in the third trimester. Vomiting, right upper quadrant pain, and a viral-like syndrome are common early. Jaundice, encephalopathy, and acute renal failure then supervene. In contrast to the HELLP syndrome, hypofibrinogenemia, hypoglycemia, and hypoprothrombinemia are common [360]. Microvesicular fat accumulated in the central zone is seen on liver biopsy, but the diagnosis is based on clinical presentation and biopsy is rarely needed. When acute fatty liver is present, the pregnancy should be terminated promptly. Maternal and fetal mortality has been reduced to less than 5%, and survivors have no long-term liver disease [360,361].

Conception and viable pregnancy in patients with chronic liver disease is uncommon and fraught with complications. Patients with established portal hypertension have an increased risk of variceal hemorrhage during the third trimester of pregnancy with consequent increase in both fetal and maternal mortality. Prophylactic variceal ligation with ablation of varices or transvenous intrahepatic portal shunt should be considered [362].

Psychological disorders

Eating disorders

Anorexia nervosa

Anorexia nervosa is a potentially fatal eating disorder characterized by severe voluntary weight loss, altered body image, and morbid fear of obesity that primarily affects adolescent females [363]. Anorexia nervosa may carry a mortality of up to 9%. Gastrointestinal complaints are seen in more than half of patients, but multiple systems are involved secondary to the starvation. Delayed gastric emptying that contributes to a feeling of bloating and early satiety is common, but it usually resolves with a return to normal body weight [364]. Serum levels of trace metals such as zinc and copper are commonly depressed, and hypophosphatemia may supervene during refeeding. In severe cases, parenteral feeding may be needed. Details of evaluation and management are found in Chapter 35.

Bulimia

Bulimia is a distinct syndrome characterized by cyclic binge eating, followed by self-induced vomiting, fasting, laxative abuse, or excessive exercising to promote weight loss [365]. In contrast to anorexia, severe weight loss and multisystem involvement are rare. Common gastrointestinal symptoms include bloating, flatulence, constipation, abdominal pain, borborygmi, and nausea. Binge eating causes acute gastric dilation, which can result in gastric rupture. Vomiting is associated with dental enamel erosion, parotitis, esophagitis, Mallory–Weiss tears, and esophageal rupture [366]. Although pancreatitis may occur, hyperamylasemia in patients with bulimia is most often caused by increased salivary-type amylase. Treatment is directed at the underlying psychiatric diagnosis.

Rumination

Although frequently reported in children, rumination is uncommon in adults who are not developmentally disabled. It is characterized by daily effortless regurgitation of undigested food and may be associated with prominent weight loss. Screening for other eating disorders is important. Delays in diagnosis and the concomitant overuse of diagnostic tests are common. Esophageal and upper gastrointestinal studies are normal. There is no clearly effective medical therapy other than reassurance and psychiatric consultation [367].

Munchausen syndrome

Munchausen syndrome is the repeated seeking of medical care for factitious and purposefully self-generated illness. In contrast to hysteria, the symptoms are subject to "conscious" control. Patients have feigned many gastrointestinal diseases, including abdominal emergencies, hematemesis, hematochezia, and inflammatory bowel diseases [368]. Satisfactory treatment of this syndrome is rare.

Pulmonary disorders

α_1-Antitrypsin deficiency

α_1-Antitrypsin (AAT) deficiency is a recessively inherited condition that is the most common genetic cause of liver disease in children. The most frequent mutation leading to AAT

arises in the *SERPINA 1* gene [369]. It is also associated with emphysema (the most common cause of death), chronic liver disease, and hepatocellular cancer in adults. The ZZ phenotype has been associated most frequently with severe disease [369]. Adult patients can be screened effectively by serum AAT levels. Those with an AAT level below 80 mg/mL should undergo AAT phenotyping [370]. Liver disease tends to manifest late in life, and liver biopsy reveals inclusion bodies containing entangled polymers of mutant Z AAT in the endoplasmic reticulum of hepatocytes in the periportal areas, a finding unrelated to the severity of the liver disease [371]. Liver transplantation is an effective form of therapy for patients with severe liver disease [369].

Asthma/chronic cough

Asthma is a common cause of disability and death that is characterized by varying degrees of bronchial constriction. GERD is present in three-fourths of asthmatic patients [372] and may be worsened by the use of asthma medications (theophylline, β-adrenergic agonists) that lower esophageal sphincter pressure, although GERD also may substantially complicate the course of the lung disease. Worsened wheezing is thought to result from the aspiration of gastric contents, although reflex bronchial constriction has also been suggested as a mechanism [373]. Pyrosis as well as other symptoms of GERD, asthma onset in middle age, the presence of nocturnal gasping, cough, and wheezing, and the absence of coincident allergy or eosinophilia are clues to a possible association with GERD [374]. When GERD-induced asthma is suspected, 24-h ambulatory pH monitoring can confirm severe reflux [375]. Therapy of GERD in this setting usually requires the strict exclusion of eating before bedtime and prolonged potent acid inhibition with proton pump inhibitors [374,376]. Because of uncertainties in demonstrating a causal relationship between asthma and GERD, antireflux surgery should be considered carefully [377].

Chronic idiopathic cough is commonly associated with GERD [378]. Even in the absence of heartburn, acid may trigger nocturnal cough or laryngospasm. Laryngospasm may be mistaken for asthma unless appropriate pulmonary function tests are employed. Evaluation and management are the same as for asthma.

Consequences of mechanical ventilation and respiratory failure

Respiratory failure and mechanical ventilation may be complicated by gastrointestinal bleeding from erosive gastritis and ulcer disease. Associated coagulopathy further increases the risk of overt hemorrhage. It is important to emphasize the infrequency of this complication when it is monitored prospectively in large populations [379]. This said, prophylactic suppression of acid secretion with H_2 antagonists or proton pump inhibitors in critically ill patients at high risk of bleeding (expectation of remaining on mechanical ventilation for > 48 h) reduces the incidence of stress ulcerations [380] and should be instituted. It has been suggested, however, that raising the gastric pH might permit colonization of the stomach by pathogenic bacteria, which, in turn, may predispose to nosocomial ventilator-associated pneumonia. This is controversial, and careful attention to a general preventive strategy is likely to be more helpful than arbitrarily switching to sucralfate [381]. Administering sucralfate alone through a nasogastric tube may expose the patient to an increased risk of erosive esophagitis because gastric pH remains low [382].

Acute exacerbation of chronic respiratory failure is a common cause of ischemic hepatitis. In contrast to the severe hypertransaminitis seen after circulatory arrest, of which severe hypotension is the proximate cause, in patients with acute on chronic respiratory failure the cardiac index is increased. The cause of the hepatic damage is a combination of severe arterial hypoxemia and elevated central venous pressure [383]. This has also been noted in a patient with severe obstructive sleep apnea [384].

Cystic fibrosis

Cystic fibrosis (CF) is the most common inherited disease among Caucasians in North America, with an incidence of about 1 per 2000. Mutations in the CF transmembrane conductance regulator gene [385] on the long arm of chromosome 7 lead to an underlying defect in mucosal cAMP ion and water transport. A net shift in fluid from the lumen results in viscous secretions and duct obstruction in the respiratory tract, salivary glands, pancreas, intestinal lumen, and biliary tree [386].

The presence of viscous secretions in the intestinal lumen has important clinical consequences. Affected infants may present with meconium ileus, intussusception, distal intestinal obstruction syndrome (DIOS), intestinal atresia, volvulus, and perforation. Chronic constipation is common and may lead to rectal prolapse. Adults with CF who have a severe genotypic expression of the regulator gene commonly present with DIOS [387]. Mineral oil or acetylcysteine may be effective. Recurrent use of gastrointestinal lavage with Golytely also may be helpful in reducing the symptoms of bloating and distention that characterize DIOS [388].

Symptomatic gastroesophageal reflux is common in children who have CF. Contributing factors are decreased saliva production, gastrointestinal hypomotility, chronic cough, and the need for postural drainage of pulmonary secretions [389]. Standard antireflux therapy is usually effective. As more patients survive to adulthood, complications of GERD, such as stricture and Barrett esophagus [390], will be seen with greater frequency.

Essentially all patients have severe nutritional impairment caused by pancreatic insufficiency and manifested by steatorrhea and malabsorption of nonfat nutrients. Protein–calorie malnutrition is aggravated by the catabolic state induced by chronic respiratory tract infections but may also contribute to

worsening lung disease. Improvement of exercise tolerance and pulmonary function can result from enteral/parenteral nutritional support, pancreatic enzyme replacement, and fat-soluble vitamin supplementation [391,392]. Although the use of large doses of high-potency pancreatic enzymes has caused a fibrosing colitis manifested by bloody diarrhea associated with diffuse narrowing of the colon with rectal sparing [393], the bulk of evidence supports the use of pancreatic enzyme supplementation to treat pancreatic insufficiency in CF [394]. As survival to adulthood becomes more common, new problems are recognized. Symptomatic osteomalacia is a common sequela of chronic steatorrhea, 25-hydroxyvitamin D levels should be monitored, supplemental calcium and vitamin D should be provided [395], and medications for osteopenia considered. A greater than expected incidence of malignancies of the digestive tract has been found [396]. Thus, persistent or unexplained gastrointestinal symptoms should be investigated carefully.

Focal biliary cirrhosis is common and asymptomatic, but about 10% of children with CF develop confluent biliary cirrhosis and progressive portal hypertension. If variceal bleeding occurs, banding or portosystemic shunting would be the therapeutic modalities of choice. Ursodiol improves liver function tests, but it is unclear whether fibrosis is retarded. Improvements in surgical techniques have allowed CF patients with end-stage cirrhosis to undergo liver or combined liver and lung transplantation successfully [397].

Renal disorders

Chronic renal failure

Chronic renal failure is associated with a number of gastrointestinal disturbances. Dysgeusia, a metallic taste in the mouth, and fetor uremia are often noted by uremic patients. Parotitis and sicca syndrome commonly occur in uremic patients, further limiting compliance with fluid restriction and impairing nutrition.

Renal failure is commonly associated with anorexia, nausea, vomiting, and epigastric pain. Endoscopy should be carried out to exclude ulcer or gastritis. Because uremia is associated with gastric emptying abnormalities [398], however, symptoms may be alleviated by using agents such as metoclopramide. Gastrointestinal bleeding has multiple possible causes [399], but, in contrast to patients with normal renal function, arteriovenous malformations are particularly prevalent. Peptic ulcer disease, gastritis, esophagitis, and duodenitis may be managed as in patients without renal failure. The diagnosis of *Helicobacter pylori* infection is difficult in uremic subjects and relies on histological methods rather than the breath test [400]. Duodenal pseudomelanosis is an idiopathic condition in which pigmentation develops in the proximal duodenal mucosa (Fig. 109.12). There are no known symptoms attributable to this syndrome.

Figure 109.12 Duodenal biopsy specimen from a patient with pseudomelanosis duodeni shows the deposition of pigment within lamina propria macrophages.

Uremic patients may develop abdominal pain, constipation, pseudoobstruction, and intussusception. Important factors in the development of constipation include oral fluid restriction and the use of antacids that contain aluminum hydroxide. Diarrhea secondary to a variety of causes is also common. Fecal impaction must be excluded. Although conditions such as bacterial overgrowth and pseudomembranous colitis must be considered, the cause of many cases remains idiopathic. The incidence of pancreatitis in chronic renal failure is unknown [398]. Elevation of serum amylase is common but should not necessarily be attributed to impaired renal clearance, especially if the level exceeds twice the upper limit of normal [401]. Intestinal ischemia has been associated with systemic calciphylaxis [402].

Uremic patients can develop hepatic friction rubs without other manifestations of liver disease. Elevated alkaline phosphatase in such patients can be of intestinal origin and may not be indicative of bone disease or cholestasis. Chronic hepatitis B and C are common, and may complicate the ability to provide effective dialysis and kidney transplantation [403].

Treatment with lamivudine for hepatitis B and interferon for hepatitis C can be undertaken with caution in such patients.

Hemodialysis

Diarrhea and the inability to gain weight may be manifestations of inadequate dialysis. A few patients on long-term hemodialyis can develop severe obstipation, colonic pseudo-obstruction, and spontaneous perforation of the colon [404]. Serum lipase levels can increase significantly after heparin administration and thus have reduced specificity for the diagnosis of pancreatitis in this setting [405]. Idiopathic refractory exudative ascites occurs in a few patients, is difficult to treat medically, and commonly resolves with kidney transplantation [406].

Peritoneal dialysis

Patients undergoing continuous ambulatory peritoneal dialysis are commonly subject to an easily treatable form of bacterial peritonitis. Differentiating this condition from peritonitis associated with important intraabdominal pathology (bowel perforation, ischemic bowel, appendicitis) can be difficult because polymicrobial infections can occur without important gastrointestinal pathology [407], and pneumoperitoneum is common after dialysis. Pneumoperitoneum not associated with perforated viscus was found in 4% of one series, and may take weeks to resolve [408].

Polycystic kidney disease

Polycystic kidney disease (PKD) is an autosomal dominant disorder associated with abnormal genes (*PKD1*, *PKD2*) that have been localized to chromosome 16. Polycystic kidney disease accounts for about 10% of the chronic dialysis population [409]. Significant inter- and intrafamilial differences in the severity of phenotypic expression appears to relate to modifier loci on the gene [410]. Hepatic cysts are an important manifestation of PKD. The frequency of coincident hepatic cysts increases with age, and the large cysts are more common in women who have had multiple pregnancies. Despite large cysts, hepatic parenchymal volume is preserved, as is liver function [411]. Hepatic cysts can become infected, large cysts can obstruct the biliary tree, and early intervention with both antibiotics and percutaneous drainage is needed [412]. An association with congenital hepatic fibrosis has been reported [413].

Substance abuse

Alcohol

Alcohol consumption can increase lower esophageal sphincter (LES) pressure, reduce the frequency of esophageal peristaltic contractions, and delay esophageal emptying. High-amplitude peristaltic contractions have also been noted (nutcracker esophagus) [414]. These alterations in function may result in chest pain and esophagitis. Chronic alcohol ingestion increases the risk of esophageal cancer.

Alcohol produces acute and chronic gastritis by disruption of the gastric mucosal barrier. Binge drinking can result in gastric erosions and severe hemorrhage. Chronic use, however, seems to protect against development of serious mucosal injury. Recent epidemiological studies suggest that the concomitant use of alcohol and NSAIDs [415], or of alcohol and warfarin [416], potentiates the risks of severe GI bleeding or perforation compared with the use of either of these drugs alone.

Alcoholism may result in diarrhea and malabsorption. Alcohol increases small intestinal transit, decreases brush border enzyme activity, and impairs absorption of nutrients. Acute exposure of the small bowel to alcohol causes altered mucosal and microvascular permeability [417]. Chronic pancreatitis, the spectrum of chronic alcoholic liver disease, protein–calorie malnutrition, and vitamin deficiencies are contributing factors (see Chapter 94).

Amphetamines

The social use of ecstasy (methylenedioxymethamphetamine, MDMA) and amphetamines has become widespread in the United States. These drugs induce euphoria, intensify emotions, and have become a staple of the "rave" culture. In excess, these drugs cause anxiety, hallucinations, chest pain, palpitations, coma, subarachnoid hemorrhage, and death [418]. A panarteritis that is similar to polyarteritis nodosum (but antineutrophil cytoplasmic antibody [ANCA] negative), with associated mesenteric ischemia, is reported [419]. As with cocaine hepatotoxicity, liver dysfunction is common, caused by both immune-mediated mechanisms and hyperthermic liver injury, and therapy for severe cases is largely supportive [420].

Cocaine

The use of cocaine and related metabolites is widespread. Anorexia and diarrhea are common. Cocaine use can result in gastric ulcerations with perforation [421], retroperitoneal fibrosis, and intestinal ischemia with infarction [422]. Severe liver injury is almost always associated with rhabdomyolysis and hyperthermia. The zone of hepatic necrosis will vary depending on other drugs being used [423]. The practice of smuggling cocaine in condoms concealed in the digestive system can result in intestinal obstruction and death.

Narcotics

Narcotic administration for abuse or for pain relief frequently results in physical dependency, with resulting physical symptoms on withdrawal. Gastrointestinal symptoms of withdrawal include anorexia, nausea, vomiting, and abdominal pain. These symptoms may be difficult to distinguish from structural gastrointestinal disorders. Narcotics delay gastric emptying and intestinal transit, and suppress intestinal secretion

of water and electrolytes [424]. Slow colonic peristalsis frequently induces constipation, which can be a long-standing problem [425]. If the narcotics must be continued, stimulant laxatives are frequently required.

Parenteral administration of illicit drugs can result in chronic viral hepatitis, cirrhosis, and hepatic abscess. Injected contaminants such as talc can cause hepatic granulomas.

Vasculitides

Behçet disease

Behçet disease is a systemic illness of unknown etiology characterized by necrotizing vasculitis and most commonly described in people of Middle Eastern and Japanese extraction. Common manifestations include orogenital ulcerations, eye inflammation, skin lesions, arthritis, and migratory thrombophlebitis. Gastrointestinal tract involvement is uncommon [426]. A curious and virtually pathognomonic sign is the development of pustules at the site of needle punctures.

Oral lesions resemble aphthous ulcers and may be single or multiple. Esophageal ulcerations are common, but infrequently cause symptoms. The most frequent extraoral manifestations of gastrointestinal involvement are segmental inflammation and focal deep ulceration of the ileocecal region and colon, resembling Crohn's disease. Intestinal bleeding or perforation may result. Therapy is similar to that of Crohn's disease, with immunomodulating medications often ameliorating the disease. Resection of severely diseased segments of the gut may be required, but postoperative recurrence is common [427,428].

Other than symptomatic Budd–Chiari syndrome, involvement of the liver and spleen is rare. Typically, affected patients have cavernous transformation of the portal vein, associated with portal vein, inferior vena caval, and hepatic venous thrombosis. Definable coagulopathies have not been defined as the cause of the thrombosis, suggesting the primary importance of vasculitis. Once established, the presence of Budd–Chiari syndrome in the patient with Behçet disease indicates a poor prognosis [429,430].

Churg–Strauss syndrome

Churg–Strauss syndrome is a systemic vasculitis characterized by the presence of asthma, hypereosinophilia, and necrosis with extravascular eosinophil granulomas. Common symptoms other than the asthma are weight loss, fever, myalgias, arthralgias, and vasculitic skin lesions. Small to medium-sized vessels are involved, and ANCAs are commonly detected. Corticosteroids, alone or in combination with cyclophosphamide, will usually induce clinical remission, but recurrence is common [431,432].

Gastrointestinal manifestations are common, and when present are associated with a poor prognosis [431]. They include nausea, vomiting, abdominal pain, gastrointestinal hemorrhage, perforation, cholangiopathy, and cholecystitis, are related to mesenteric vasculitis, and are similar to those found in polyarteritis nodosa. Mesenteric ischemia with ulceration and perforation is reported. On occasion a case may be identified on endoscopic biopsy [433].

Cryoglobulinemia

Cryoglobulinemic vasculitis is caused by immune complexes of mixed cryoglobulins in the walls of venules, capillaries, and arterioles, which incites acute inflammation. Mixed cryoglobulins, rheumatoid factor, and low levels of C4 are typically found in the serum. The most frequent clinical manifestations are purpura, arthralgias, and nephritis (the main cause of morbidity). Cryoglobulinemia occurs in a variety of clinical settings, such as lymphoproliferative disorders, infections, and autoimmune diseases, or as an idiopathic process. However, cryoglobulinemic vasculitis is almost always associated with chronic hepatitis C [431].

Gastrointestinal illness, such as recurrent episodes of crampy abdominal pain, enterocolitis, and, rarely, small or large bowel ischemia, has been reported. When possible, the effective treatment of the hepatitis C infection with interferon-ribaviron will abolish the vasculitis [434].

Giant cell arteritis

Giant cell arteritis is a granulomatous disease that usually involves large-sized arteries, including the aorta and the temporal arteries. The disease usually occurs in the elderly and is associated with the clinical syndrome of polymyalgia rheumatica. Disseminated fibrinoid necrosis of smaller vessels can result in visceral ischemia, abdominal pain, nausea, anorexia, weight loss, bleeding, and perforation. Disturbances of liver function are common and have been attributed to hepatic artery involvement [435] and hepatitis associated with fibrin ring granulomas [436].

Henoch–Schönlein purpura

Henoch–Schönlein purpura is a vasculitis syndrome with a peak age of onset in childhood, characterized by systemic deposition of IgA-dominant immune complexes in small vessels. The classic triad of symptoms includes a palpable purpuric rash, abdominal cramping, and hematuria that typically begins after an upper respiratory infection [431]. In Henoch–Schönlein purpura, ANCAs are absent. Most children have a self-limited disease without chronic sequelae. Any part of the gastrointestinal tract may be affected. Gastrointestinal symptoms include abdominal pain, vomiting, diarrhea, intestinal obstruction, and intussusception and can precede the onset of the characteristic purpuric rash [437]. The bowel may be edematous and show evidence of submucosal or subserosal hemorrhage. Radiographic findings include thickening of small bowel folds (Fig. 109.13), colonic wall edema, and intussusception. Whether corticosteroids actually improve outcomes is controversial [438].

Figure 109.13 Henoch–Schönlein purpura. Thickened folds in the second part of the duodenum.

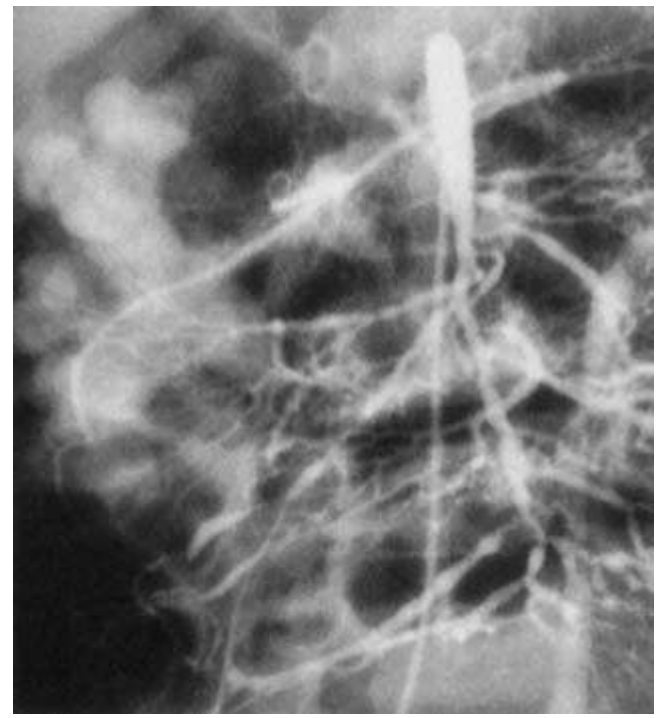

Figure 109.14 Polyarteritis nodosa. Typical aneurysmal dilation of small and medium-sized arteries.

Polyarteritis nodosa

Polyarteritis nodosa (PAN) is a systemic necrotizing vasculitis that affects small and medium-sized arteries of nearly every organ system, giving rise to a wide spectrum of clinical findings. Recently distinguished from PAN, microscopic polyangiitis is an ANCA-positive systemic vasculitis of small-size vessels, smaller than arteries, characterized by rapidly progressive glomerulonephritis and pulmonary involvement that is usually absent in PAN. The absence of necrotizing granulomatous inflammation separates this entity from Wegener granulomatosis and Churg–Strauss syndrome. The gastrointestinal manifestations of the two disorders are similar [431].

Many cases of PAN are associated with hepatitis B infection. Fever, weight loss, arthralgias, subcutaneous nodules, hypertension, and peripheral neuropathy are common. Gastrointestinal complaints are present in about one-third of patients, usually secondary to visceral ischemia. Polyarteritis nodosa is the most common cause of localized vasculitis of the gastrointestinal tract, and when present carries a poor prognosis [439]. The most common clinical manifestations are epigastric pain, nausea, anorexia, mucosal ulceration, intestinal bleeding, and diarrhea [440]. Appendicitis, cholecystitis, pancreatitis [441], cataclysmic intraabdominal hemorrhage, bowel obstruction, and liver infarction have been reported. Typical aneurysmal dilation of small and medium-sized arteries on arteriography (Fig. 109.14) may provide a diagnosis. Corticosteroid and cyclophosphamide therapy have improved the overall survival of these patients dramatically [442].

Wegener granulomatosis

Wegener granulomatosis is an ANCA-positive, small-vessel vasculitis characterized by granulomatous inflammation involving the respiratory tract and necrotizing vasculitis of small to medium-sized vessels. Glomerulonephritis is common. Manifestations of upper airway disease are sinus pain and purulent drainage, nasal mucosal ulceration, and otitis media. Tracheal inflammation causes stridor and may lead to dangerous airway compromise. Chest radiographs commonly show nodular radiographic densities, and, unlike Churg–Strauss syndrome, there is no asthma [431].

Odynophagia secondary to esophageal vasculitis, manifest as punched-out ulcers in the esophagus, has been reported [443]. Granulomatous inflammation of the stomach may be mistaken for Crohn's disease [444]. The associated vasculitis may result uncommonly in intestinal or colonic ischemia, bleeding, and perforation [445].

The reader is directed to Chapter 45, Skin lesions associated with gastrointestinal diseases; Chapter 48, Approach to gastrointestinal problems in the immunocompromised patient; Chapter 50, Approach to gastrointestinal disease in the female patient; Chapter 51, Approach to the patient requiring nutritional supplementation; and Chapter 112, Gastrointestinal manifestations of immunological disorders.

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I MISCELLANEOUS

110 Skin lesions associated with gastrointestinal and liver diseases

Matilde Iorizzo, Joseph L. Jorizzo

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Many diseases can involve both the skin and the gastrointestinal tract. Primary disorders of the gut such as inflammatory bowel disease can initially manifest in the skin, and primary skin disorders such as pemphigus vulgaris may present with mucosal lesions. It is important that specialists in both disciplines be aware of how cutaneous lesions may be reflective of gastrointestinal diseases. This chapter reviews the skin lesions that may be associated with specific gastrointestinal complaints or known pathological processes. If a dermatological lesion or diagnosis is suspected, dermatological consultation is suggested to assist in diagnosis and management.

Inflammatory bowel disease

Chronic nonspecific ulcerative colitis and Crohn's disease are associated with a host of immunological abnormalities. Although the role of circulating immune complexes in producing extraintestinal manifestations has been debated (see Chapters 7, 50, 52, 57 and 59), it is not surprising that numerous reactive inflammatory vascular dermatoses occur in patients with these diseases.

Erythema: generalized, localized and annular

Reactive inflammatory vascular dermatoses comprise a spectrum of inflammatory blood vessel reactions that occur in association with internal disease [1]. These range from generalized or local erythema (macular blanchable lesions characterized histologically by simple vasodilation and mild inflammation) to urticarial lesions, erythema multiforme, and vasculitis characterized by palpable purpuric lesions with histological leukocytoclasis and fibrinoid necrosis of blood vessel walls. Table 110.1 summarizes the various types of vessel-based inflammatory lesions that may occur in patients with gastrointestinal disease.

Urticaria and angioedema

Urticaria is a raised, peau d'orange, cutaneous vascular reaction characterized by dermal edema and erythema in which, by definition, individual lesions resolve within 24 h [1]. Similar lesions occurring in a deep dermal or subcutaneous location are called angioedema. In patients with inflammatory bowel disease [2], urticaria is usually drug induced or results from some cause other than bowel disease. Hereditary or acquired C1 esterase deficiency is associated more with angioedema than urticaria; abdominal pain caused by mucosal edema may occur. Chronic urticaria affects primarily middle-aged women and is characterized by the occurrence of daily hives (individual lesions still resolve within 24 h) for longer than 6 weeks. Two specific conditions, urticarial vasculitis and a syndrome of urticarial lesions occurring in the setting of a serum sickness-like illness lasting longer than 24 h, are not, by definition, true urticaria. A skin biopsy should be performed on urticarial lesions that last longer than 24 h to exclude vasculitis. The cause of chronic urticaria in an individual patient may be difficult to establish. Several practical approaches have been published [3]. Treatment of urticaria consists of an attempt to eliminate the cause and relieve signs and symptoms with around-the-clock antihistamine therapy.

Erythema multiforme

Erythema multiforme is an acute, self-limiting, mucocutaneous syndrome characterized by targetoid cutaneous lesions

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Table 110.1 Erythematous vessel-based eruptions in patients with gastrointestinal disease

Clinical pattern	Common associations
Generalized erythema	
Morbiliform (macules and papules) Scarlatiniform (confluent)	Drug or transfusion reaction; viral exanthem
Palmar erythema	Nonspecific: oral contraceptive therapy; pregnancy, chronic liver disease, collagen-vascular diseases
Annular erythema	
Erythema migrans: often a single expanding lesion following a tick bite	Lyme disease
Erythema annulare centrifugum: multiple lesions, central clearing with rim of scale	Underlying infection; inflammatory disease
Erythema gyratum repens: rare "wood grain" pattern	Occult carcinoma: lung, stomach, esophagus
Urticaria	
Transient red wheals	Idiopathic, drug, food hypersensitivity; underlying infection; collagen-vascular disease
Erythema multiforme (EM)	
EM minor: target lesions	
EM major: mucous membrane	Drug reaction; infections (*Mycoplasma* species, herpes simplex, other); underlying inflammatory disease
Erythema nodosum	
Tender red nodules on legs and elsewhere	Underlying inflammatory disease (e.g., bowel disease, sarcoidosis); drug reaction

that may be accompanied by serum sickness-like signs and symptoms (e.g., fever, arthralgias) [4]. Erythema multiforme minor refers to a form of the syndrome in which patients have a finite number of acrally located typical target lesions and, in general, lack mucosal lesions and other symptoms. Erythema multiforme major represents a more severe disease, and is termed *Stevens–Johnson syndrome* if the patient has significant mucosal involvement [4]. Patients with toxic epidermal necrolysis, which may overlap with severe Stevens–Johnson syndrome, have a burn-like appearance. Toxic epidermal necrolysis is usually drug induced and may be associated with a 30% rate of mortality.

Well-documented causes of acute erythema multiforme include drugs (e.g., sulfonamides, phenytoin, penicillin), herpes simplex, *Mycoplasma* infections and other infections (e.g., tuberculosis), as well as a host of underlying diseases [4]. Typical erythema multiforme has been described in patients with inflammatory bowel disease [2]. Complications of erythema multiforme major may affect the gastrointestinal tract and may be severe in toxic epidermal necrolysis. Esophagitis and esophageal stricture have been reported. There is considerable evidence that most cases of recurrent erythema multiforme minor are induced by recurrent herpes simplex infections [4].

Therapy for erythema multiforme has not been assessed in a double-blind trial. Systemic corticosteroids are often prescribed for adults with erythema multiforme major in whom infection has been excluded. Ophthalmological consultation for eye involvement and aggressive topical care are required to prevent secondary infection.

Erythema nodosum

Erythema nodosum is characterized by the occurrence of tender nonulcerative nodules, most often on the legs, resulting from inflammation in the subcutaneous fat (Fig. 110.1). Usually, these nodules last 3–6 weeks and affect the anterior lower legs in a symmetric distribution. Patients may have associated signs and symptoms, including fever, malaise, arthritis, and arthralgias [5].

Erythema nodosum has been well established as an extraintestinal manifestation of ulcerative colitis, Crohn's disease, and infectious colitis such as that caused by *Yersinia enterocolitica* [1]. The incidence of erythema nodosum in patients with ulcerative colitis was 7% in one large series, but the incidence in Crohn's disease is lower [5]. Other well-documented conditions associated with erythema nodosum include drugs (e.g., estrogen, sulfonamides), infections (e.g., bacteria, viruses, fungi, chlamydia, acid-fast bacteria, spiro-

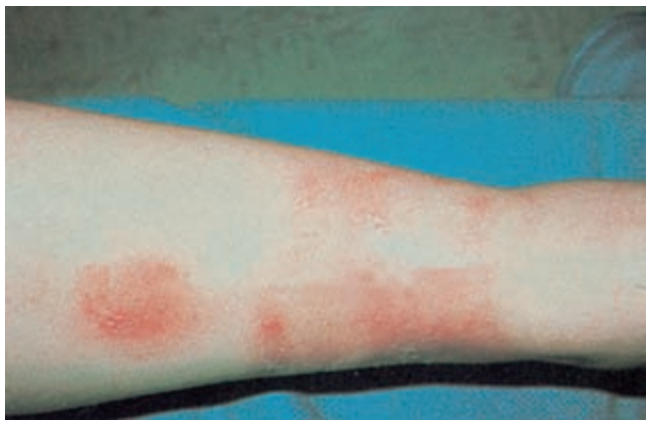

Figure 110.1 Tender nodules of erythema nodosum occurring in association with ulcerative colitis.

chetes), malignant diseases, and other diseases, including Behçet disease and sarcoidosis [5]. An immune complex-mediated mechanism affecting vessels in the septal panniculus has been proposed, although the exact pathogenesis of erythema nodosum remains unknown.

Treatment of erythema nodosum in patients with inflammatory bowel disease is directed at the underlying disease. Nonsteroidal antiinflammatory drugs or even acetaminophen may alleviate the systemic symptoms.

Vasculitis

The term *cutaneous small vessels vasculitis* (CSVV) has been used to refer to a form of vasculitis affecting postcapillary venules with the histopathological findings of endothelial swelling, neutrophilic invasion of blood vessel walls, leukocytoclasis (karyorrhexis of nuclei of neutrophils), extravasation of erythrocytes, and fibrinoid necrosis of blood vessel walls [6]. It presents as palpable purpura and may go by other names such as leukocytoclastic vasculitis, Henoch–Schönlein purpura (which is actually a subgroup with IgA-containing immune complexes), allergic angiitis, and hypersensitivity vasculitis. Larger-vessel involvement characterizes polyarteritis nodosa, granulomatous vasculitis (e.g., Wegener syndrome, Churg–Strauss syndrome), and giant-cell arteritis.

CSVV has been proposed as a cutaneous model for circulating immune complex-mediated vessel damage [6]. The central and peripheral nervous system, synovium, pleura, pericardium, gastrointestinal tract, and kidney may act as filters for complexes with the similar end result of necrotizing lesions [6].

Between 39% and 61% of patients with CSVV do not have a precipitating cause that can be identified. Drugs, infections (e.g., hepatitis B, streptococcal infection), and diseases associated with immune complexes (e.g., collagen vascular diseases, cancers, chronic active hepatitis) account for most of the identified associations. Crohn's disease [7] and ulcerative colitis [8] have both been clearly described in association with necrotizing venulitis and occasionally with granulomatous vasculitis [9].

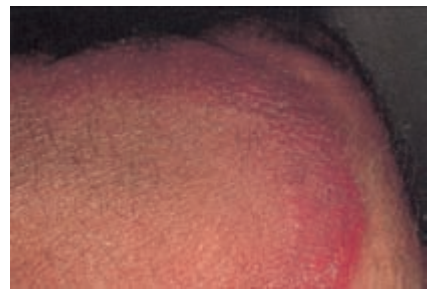

Figure 110.2 Typical lesions over joints in a patient with erythema elevatum diutinum.

Prognosis in all forms of vasculitis depends on the internal organ involvement. Treatment should be directed at the underlying disease. Severe vasculitis may require acute therapy with high-dose systemic corticosteroids, or pulse cyclophosphamide, or both. Low-dose weekly methotrexate, azathioprine or, for larger-vessel vasculitis, cyclophosphamide may be important adjuvant therapies. Oral colchicine or sulfone drugs (e.g., dapsone) may be used for primarily cutaneous vasculitis [6].

Erythema elevatum diutinum

Erythema elevatum diutinum is a chronic fibrosing dermatosis characterized by juxtaarticular, persistent, symmetric, firm, tender, red to yellow-brown papules and plaques on the extensor surfaces of extremities (Fig. 110.2). It is believed to be caused by immune complexes. Early histological features include leukocytoclastic vasculitis, subepidermal fibrin deposition, and dermal papillary edema. A storiform proliferation of dermal spindle cells with fibrosis and foci of neutrophils or neutrophilic vasculitis characterize older lesions. Besides inflammatory bowel disease and celiac disease, it has been associated with rheumatoid arthritis, pyoderma gangrenosum, diabetes mellitus, paraproteinemias, and dermatitis herpetiformis, as well as chronic infections including streptococcal, mycobacterial, and human immunodeficiency virus (HIV) infections [10]. Oral dapsone therapy is the treatment of choice.

Pyoderma gangrenosum

Pyoderma gangrenosum is a misleading designation for an idiopathic ulcerating cutaneous disease. Theories range from a forme fruste of vasculitis to a cell-mediated reactive process [6]. Typically, fully evolved lesions have a dusky purple, undermined border and heal with cribriform scarring (Fig. 110.3) [6]. Lesions may demonstrate the pathergy phenomenon: the rapid extension of the lesion after trauma such as surgical debridement.

The diagnosis of pyoderma gangrenosum is a clinical diagnosis of exclusion. Bacterial, fungal, mycobacterial, and other infections, as well as vasculitis, squamous cell carcinoma, iododerma, bromoderma, and factitial disease, must be

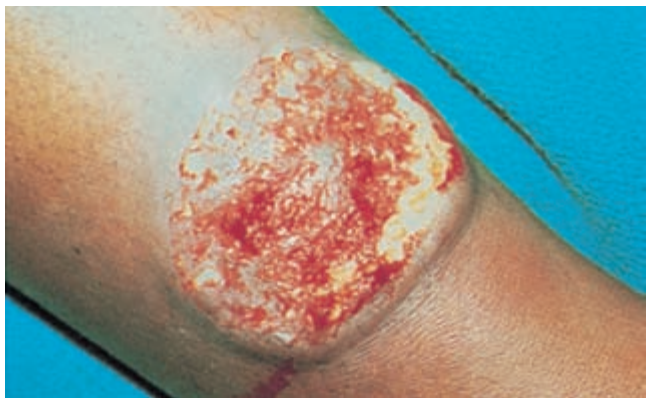

Figure 110.3 Pyoderma gangrenosum in a patient with Crohn's disease.

excluded histologically with appropriate special stains and cultures from biopsy specimens. More than 50% of patients with typical pyoderma gangrenosum do not have an associated disease. However, the association with inflammatory bowel disease is one of the best known and should be considered and excluded in every patient with pyoderma gangrenosum [11]. Other associated conditions include chronic active and chronic persistent hepatitis, polyarthritis including rheumatoid arthritis, and myeloproliferative disorders.

Aggressive therapy is often required because the disease can spread rapidly and can predispose patients to sepsis or inanition and even death. Systemic corticosteroids in high doses, immunosuppressive agents, and sulfones are the mainstays of treatment. Antibody therapy with antitumor necrosis factor α may be useful [12].

Oral manifestations

As many as 30% of patients with ulcerative colitis have oral lesions, including aphthae and angular stomatitis [2], whereas specific lesions (i.e., granulomas) of Crohn's disease affect the mouth and lips more rarely. Granulomatous lip lesions of Crohn's disease may be confused with Melkersson–Rosenthal syndrome, a condition in which granulomatous cheilitis occurs in association with facial nerve palsy. Granulomatous oral lesions may be nodular or show a cobblestone pattern [13]. *Aphthae* (canker sores) are nonspecific mucosal lesions that may be the most common oral lesions in patients with inflammatory bowel disease (Fig. 110.4). Simple aphthae occur in up to 25% of healthy persons. Patients with numerous, almost constant, oral aphthae (complex aphthosis) should be evaluated to exclude inflammatory bowel disease and Behçet disease [14].

Angular cheilitis (perléche and angular stomatitis) may occur as a manifestation of nutritional deficiency or, more commonly, as a manifestation of oral candidiasis. *Pyostomatitis vegetans* is a papular eruption of the oral mucosa that produces a cobblestone and eroded appearance. This distinctive oral disease is associated with Crohn's disease or ulcerative colitis in most patients [15].

Figure 110.4 Tender erythematous papules and plaques on the upper extremities in a patient with Sweet syndrome.

Sweet syndrome

Sweet syndrome, also known as acute febrile neutrophilic dermatosis, is characterized by the abrupt onset of tender cutaneous erythematous papules and plaques (Fig. 110.5). Fever and peripheral blood neutrophilia may also occur. Disease onset frequently follows a nonspecific infection of the respiratory or gastrointestinal tract and is believed to represent a hypersensitivity reaction. Sweet syndrome has been reported in association with acute infections, myelodysplastic and other malignant diseases, and inflammatory bowel disease [16]. Histological specimens show a neutrophil vascular reaction with dermal inflammatory neutrophilic infiltrates and leukocytoclasis with no fibrinoid change in vessel walls.

Cutaneous lesions are typically located on the upper extremities, head, and neck. They may ulcerate, become purulent, and resemble lesions of pyoderma gangrenosum. Many dermatologists view Sweet syndrome and pyoderma gangrenosum as representing a continuum along a spectrum of disease, and considerable overlap between these two diseases occurs. Like pyoderma gangrenosum, the disease responds to therapy with prednisone or other immunosuppressive agents, although often therapy with oral dapsone alone is adequate.

Figure 110.5 Aphthous ulcer involving the tongue in a patient with Behçet disease.

Figure 110.6 Finger clubbing.

Nail abnormalities

Finger clubbing is a nail disorder caused by enlargement of the soft tissues of the distal digit. The nail plate is enlarged and excessively curved (Fig. 110.6), with greater than 180° widening of the angle between the proximal nail fold and the nail plate (angle of Lovibond). Secondary bilateral clubbing has been associated with pulmonary, cardiac, gastrointestinal, infectious, and endocrine diseases. In Crohn's disease and ulcerative colitis, the hyponychial angle can be significantly correlated with disease activity [17]. Finger clubbing in patients with Crohn's disease has been reported to regress after resection of macroscopic disease. Clubbing usually develops over years but sometimes may develop subacutely, generating pain.

Miscellaneous skin lesions

Although certain reactive dermatoses occur more commonly in patients with inflammatory bowel disease, many reports exist of single-case associations of a given dermatosis with Crohn's disease or ulcerative colitis. Metastatic Crohn's disease, which is the occurrence of noncaseating granulomas remote from the gastrointestinal tract in patients with Crohn's disease, has been well described at a host of cutaneous sites [18,19]. Lesions are usually ulcers, papules, or nodules. Pustular vasculitis, which can occur with inflammatory bowel bypass syndrome, is discussed in the section Bowel-associated dermatosis/arthritis syndrome. Details of other less common associations can be sought in review articles [2,7], as well as in classical reports of large patient population surveys [20,21]. Table 110.2 provides an overview.

Table 110.2 Some cutaneous associations of Crohn's disease and ulcerative colitis

Erythemas (including annular erythemas)
Urticaria
Erythema nodosum
Cutaneous small vessel vasculitis
Larger vessel vasculitis
Pustular vasculitis
Pyoderma gangrenosum
Oral lesions
Specific granulomas (Crohn's disease only)
Aphthosis
Angular cheilitis
Pyostomatitis vegetans
Metastatic Crohn's disease
Finger clubbing
Acquired acrodermatitis enteropathica (zinc deficiency)
Striae
Epidermolysis bullosa acquisita
Psoriasis
Exfoliative erythroderma
Vitiligo
Lichen nitidus
Lichen planus

Rheumatological diseases

Rheumatoid arthritis, systemic lupus erythematosus, and dermatomyositis

A review of skin manifestations of rheumatoid arthritis, systemic lupus erythematosus, and dermatomyositis [22,23] is beyond the scope of this review. Gastrointestinal involvement is usually related to vasculitis and is similar to that described earlier. Chapter 109 describes the gastrointestinal manifestations of rheumatic diseases.

Antiphospholipid syndrome

This disorder is characterized by recurrent venous or arterial thrombosis with typical laboratory abnormalities (elevated levels of antibodies against membrane anionic phospholipids or their associated plasma proteins, or evidence of a circulating anticoagulant). It can occur as a primary disease or in association with systemic lupus erythematosus or another rheumatic or autoimmune disorder. Dermatological manifestations include livedo reticularis, purpura, and infarcts/ulceration. Gastrointestinal involvement is usually related to vasculitis.

Scleroderma

Scleroderma is a cutaneous or multisystem disease of unknown origin. A localized cutaneous form (morphea) is characterized by plaque, gyrate, generalized, or linear dermal sclerosis. The systemic form may be considered as a continuum of disease severity, the extremes of which are the milder CREST variant (*c*alcinosis, *R*aynaud's phenomenon, *e*sophageal dysmotility, *s*clerodactyly, and *t*elangiectasia) and a more severe progressive systemic sclerosis [24]. Most patients with systemic scleroderma have the CREST variant. Although patients with CREST can become critically ill with

any of the varied manifestations of systemic scleroderma, they generally are middle-aged or older women who have a chronic course and die with, not of, their disease. Esophageal reflux should be treated to reduce esophageal fibrosis. Sclerosis occurs acrally and periorally and can affect the trunk. A salt-and-pepper postinflammatory pigment change is particularly noticeable over sclerotic areas in darker-skinned patients. Telangiectasias are described as being box-like or mat-like and occur on the hands, face, oral mucosa, and other sites [25]. The progressive systemic sclerosis type of systemic scleroderma occurs with an explosive onset. The dermal sclerosis is dramatic, but CREST features are often absent or are present to a lesser degree. The systemic and gastrointestinal features of scleroderma are discussed in Chapters 31, 47, and 109.

Behçet disease

Behçet disease is a complex multisystem disease first described by and named after a Turkish dermatologist. Because there is no pathognomonic laboratory test for the diagnosis, several sets of diagnostic criteria have been proposed. The O'Duffy criteria require that patients without inflammatory bowel disease or collagen vascular disease must have oral aphthosis (canker sores) plus at least two of the following: genital aphthae, synovitis, posterior uveitis, cutaneous pustular vasculitis, and meningoencephalitis [26]. The exclusion of patients with inflammatory bowel disease is crucial because patients with bowel disease may have recurrent aphthosis and (especially those with HLA-B27 antigen) are best considered as having the Reiter disease/HLA-B27 spectrum of diseases rather than Behçet disease [27].

The oral lesions in Behçet disease are aphthae and can easily be distinguished from the psoriasiform oral lesions of Reiter disease [27]. Genital aphthae resemble oral aphthae (Fig. 110.7). Recurrent herpes simplex must be excluded by culture or by immunoperoxidase study of biopsy material. Cutaneous pustular vasculitis lesions show either leukocytoclastic vasculitis or a neutrophilic vascular reaction histologically [28]. Other lesions have been described that are similar to those seen in erythema nodosum, Sweet syndrome, or pyoderma gangrenosum.

Certain ocular changes have been described in these patients, although posterior uveitis (i.e., retinal vasculitis) is the most important manifestation. The synovitis produces an asymmetric, migratory, nonerosive oligoarthritis. Many neurological manifestations have been reported. Vascular involvement such as aneurysms, arterial occlusions, venous occlusions, and varices may occur, and they can prove fatal. The principal gastrointestinal manifestations are aphthae, which can occur at any gastrointestinal site. Changes similar to those in inflammatory bowel disease have been described but, as has been discussed, these may occur in HLA-B27-positive patients with a Reiter-like illness and aphthae.

Bowel-associated dermatosis/arthritis syndrome

Approximately 20% of patients in one report developed a characteristic syndrome after jejunoileal bypass surgery [29] that included pustular vasculitic cutaneous lesions, arthritis, and serum sickness-like features. Clinically and histopathologically, the skin lesions resembled the pustular vasculitis lesions seen in Behçet disease. This operation has essentially

Figure 110.7 Early genital aphtha showing features of pustular vasculitis in a patient with Behçet disease.

Figure 110.8 Bowel-associated dermatosis/arthritis syndrome. Pustular vasculitis lesions in a patient with a blind loop after Billroth II surgery.

Figure 110.9 Amyloidosis. Note the perirectal amyloid nodules in this patient with multiple myeloma.

been abandoned; however, we expanded the concept to include patients who have not had bypass surgery but who have either inflammatory bowel disease or the occurrence of a blind loop after Billroth II surgery [30].

The cutaneous lesions occur in crops, with each episode lasting 1–2 weeks and occurring at intervals of 1 month to several months (Fig. 110.8). Lesions similar to those seen in erythema nodosum may occur. Fever, myalgias, chills, flu-like symptoms, gastrointestinal upset, and arthralgias or nonerosive arthritis are often associated. Circulating immune complexes have been postulated to mediate this syndrome [29] and serum enhancement of neutrophil migration may mediate the cutaneous lesions [31].

The bowel bypass syndrome can be cured by restoration of normal bowel anatomy (see Chapter 53). Treatment of the underlying disease is necessary to control the syndrome in patients with inflammatory bowel disease. Systemic antibiotic therapy with metronidazole, tetracycline, or erythromycin has proved beneficial in controlling cutaneous and systemic manifestations of this syndrome. Systemic corticosteroid therapy and oral dapsone are also effective therapies.

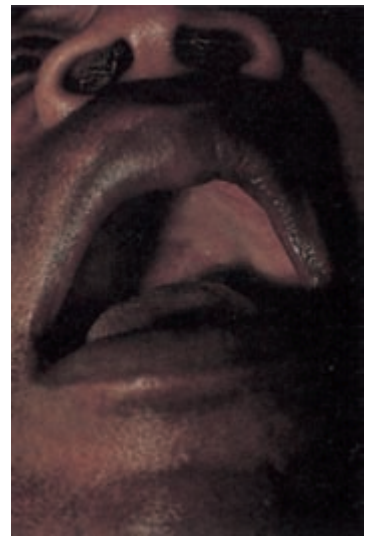

Figure 110.10 Typical telangiectasia of hereditary hemorrhagic telangiectasia.

Amyloidosis

Amyloidosis is a group of syndromes characterized by the extracellular deposition of an abnormal protein with certain staining properties and electron microscopic features. Primary amyloidosis, also called light chain-related systemic amyloidosis [32], often occurs in patients with multiple myeloma. Macroglossia, cutaneous papules, plaques, and nodules occur with pinch purpura (Fig. 110.9). Patients with primary amyloidosis have frequent gastrointestinal, cardiac, renal, and other multisystem involvement. Skin biopsy of a cutaneous lesion in primary amyloid is virtually diagnostic of the disease.

Patients with secondary (AA) amyloidosis do not have cutaneous involvement [33]. This pattern is associated with underlying disease, such as chronic inflammatory bowel disease, tuberculosis, leprosy, chronic osteomyelitis, or familial Mediterranean fever. Biopsy of normal skin reveals amyloid deposits in fewer than one-third of patients, whereas small bowel or colonic mucosal biopsy samples are positive in more than 75%–85% of patients.

Skin disease and gastrointestinal bleeding

Vascular abnormalities

Hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease)

This autosomal dominant trait is characterized by telangiectasis, aneurysms, and arteriovenous malformations that can affect mucocutaneous areas as well as internal organs [34]. The mucocutaneous hallmark for the syndrome is 1–3 mm telangiectatic mats resembling those seen in patients with scleroderma (Fig. 110.10). These lesions can affect the lips, tongue, face, hands, chest, and feet [34] (see Chapter 112).

Blue rubber bleb nevus syndrome

Blue rubber bleb nevus syndrome is a rare autosomal dominant condition with blue, subcutaneous, rubbery, compressible, sometimes painful nodular vascular malformations that are up to 10 cm in diameter [35]. These vascular malformations are not true hemangiomas. They occur in the small intestine or colon but have also been observed in the oropharynx, nasopharynx, esophagus, stomach, peritoneal cavity, mesentery, liver, lung, glans penis, eye, and central nervous system [36]. Gastrointestinal bleeding and intussusception may occur (see Chapter 112).

Kaposi sarcoma

Most publications since the early 1980s regarding Kaposi sarcoma have focused on the epidemic form of the disease, associated with acquired immunodeficiency syndrome (AIDS) [37] (see Chapter 104). An endemic form has long been recognized in Africa, and the third variant is the classic type, which occurs primarily in persons of Mediterranean ancestry.

The tumor in all groups is derived from proliferating endothelial cells. The cutaneous lesions vary from reddish-purple macules to large vascular tumors. In the slowly progressive classic form of the disease, lesions begin on the feet or lower legs of affected patients (who are typically elderly men) and extend proximally, becoming associated with severe peripheral edema. The most common site of internal involvement, but usually not a prominent feature, is the gastrointestinal tract. Sites affected, in decreasing frequency of involvement, include the small intestine, stomach, esophagus, and colon [38]. Extensive hemorrhage or partial bowel obstruction can occur. Other organs that may be affected include lymph nodes, larynx, spleen, liver, and lungs. Most patients with the classic form of Kaposi sarcoma die with, and not of, their disease.

Malignant atrophic papulosis (Degos disease)

Malignant atrophic papulosis (Degos disease) is a rare, idiopathic, usually lethal disease with characteristic lesions that affect the skin, gastrointestinal tract, and central nervous system [39]. The cutaneous lesions are discrete painless papules with umbilicated porcelain-white centers surrounded by telangiectatic rims. Histologically, these lesions reveal an atrophic epidermis with a wedge-shaped dermal scar that is broader at the top than at the bottom [40]. Gastrointestinal involvement may lead to massive gastrointestinal bleeding and to death (see Chapter 112). Current evidence is against an immunopathogenesis in this disease [40]. There is no effective therapy, and visceral involvement may be fatal.

Diseases of connective tissue

Pseudoxanthoma elasticum

Pseudoxanthoma elasticum is a genetic disorder characterized by cutaneous and visceral manifestations that result from alterations to elastic fibers. Four genetic subtypes have been described, including both autosomal recessive and dominant inheritance patterns [41]. These types have a variety of ocular, cardiovascular, and gastrointestinal manifestations.

Figure 110.11 Pseudoxanthoma elasticum. Note the chicken skin-like appearance of the axillary skin.

The cutaneous lesions consist of yellow papules and plaques that resemble chicken skin (Fig. 110.11). These lesions begin early in life and may progress to affect the buccal mucosa, neck, axillae, and other flexures. Histologically, lesions show calcification of middermal elastic fibers. Angioid streaks are the characteristic retinal finding in patients affected with pseudoxanthoma elasticum but are not specific.

Gastrointestinal involvement may present with recurrent upper or lower gastrointestinal hemorrhage (see Chapter 112). Endoscopy reveals the characteristic yellow cobblestone changes in the bowel. The pathogenesis remains unknown. The treatment is aimed at complications, but no therapy exists to alter the connective tissue abnormality. Prognosis depends on the subtype of disease.

Ehlers–Danlos syndrome

Ehlers–Danlos syndrome is a heterogeneous group of genetically inherited syndromes characterized by defective collagen. More than 10 distinct subtypes exist, with various autosomal or X-linked inheritance patterns and with diverse abnormalities of collagen production [42].

The skin may be both hyperextensible and fragile. Wound healing is delayed and may produce cigarette burn-like scars or fishmouth-like scars as well as pseudotumors over joints. Purpura caused by easy bruising and hyperextensibility of joints are prominent features of Ehlers–Danlos syndrome.

Visceral involvement is varied. Gastrointestinal perforation is prevalent in patients with type IV Ehlers–Danlos syndrome who have defective type III collagen and translucent skin with numerous ecchymoses. In addition to bowel perforation and gastrointestinal bleeding, these patients have a predisposition toward rupture of blood vessels and therefore the prognosis is particularly grave [43] (see Chapter 112).

Cutis laxa (generalized elastolysis)

Cutis laxa is a heterogeneous group of autosomal and X-linked disorders characterized primarily by abnormalities of elastic fibers [44]. The skin appears to be too large for the body. It sags and has a wrinkled aged appearance. Gastrointestinal diverticula and hernias occur [44].

Neurofibromatosis (Von Recklinghausen disease)

Neurofibromatosis is inherited in an autosomal dominant fashion. Cutaneous features such as café au lait macules, axillary freckles, and cutaneous neurofibromas are hallmarks of this disease. Neurofibromas may occur throughout the gastrointestinal tract in 25% of patients [45]. The tongue, gallbladder, stomach, and jejunum are more commonly affected than the esophagus or colon. Ulceration, bleeding, volvulus, obstruction, perforation, and intussusception may occur. Malignant transformation of neurofibromas in the gut is not common, but is well reported. Surgery is often required for symptomatic gastrointestinal involvement.

Syndromes associated with gastrointestinal polyps

Gardner syndrome (familial adenomatous polyposis)

In 1953, Gardner refined his description of a dominantly inherited triad of osteomas, epidermoid cysts, and colorectal polyposis that predisposed to colonic adenocarcinoma [46]. Gardner syndrome occurs as part of a spectrum that includes familial polyposis coli (no extracolonic lesions) and Turcot syndrome (features of Gardner syndrome plus central nervous system neoplasms) (see Chapter 64).

Patients with Gardner syndrome develop multiple epidermoid cysts in early teenage years [47]. These cysts occur in a generalized distribution, especially on the scalp, face, and extremities [48] (Fig. 110.12). Desmoid tumors, which represent locally aggressive but nonmetastasizing tumors of fibrous tissue, often affect the mesentery or the abdominal wall.

Figure 110.12 Typical epidermal inclusion cyst of Gardner syndrome. This patient had many other cystic nodules, particularly on the scalp.

Peutz–Jeghers syndrome

Peutz and, later, Jeghers described a condition with an autosomal dominant inheritance pattern characterized by small intestinal hamartomatous polyps and hyperpigmented macules of the lips [49]. Although most polyps occur in the jejunum, they also occur in the ileum, stomach, duodenum, and colon (see Chapter 64). Polyps are present in childhood and may predispose to intussusception or to gastrointestinal bleeding. The melanotic macules of Peutz–Jeghers syndrome begin in infancy. They affect the lips most commonly, but also may affect the palms, soles, digits, periorbital skin, anus, and buccal mucosa. Histologically they resemble ephelides (freckles). All except the buccal lesions fade with age [50].

Cronkhite–Canada syndrome

Cronkhite–Canada syndrome is a sporadically occurring disorder of adults defined by the tetrad of cutaneous hyperpigmentation, alopecia, onychodystrophy, and intestinal polyposis [51]. The gastrointestinal signs and symptoms (e.g., diarrhea, abdominal pain, weight loss, anorexia) often precede the onset of the hair, nail, and cutaneous manifestations by several months (see Chapter 64).

The predominant cutaneous lesions are hyperpigmented macules that coalesce to form plaques that are distributed primarily on the upper extremities but which may occur on most cutaneous sites [52]. Vitiligo may occasionally occur. Onycholysis, onychoschizia, or onychomadesis may affect all 20 nails. Widespread alopecia is nonscarring and may resemble alopecia areata [52]. The tongue may be fissured.

Cowden disease

Cowden disease, also called multiple hamartoma syndrome, is inherited in an autosomal dominant fashion [52]. Mucocutaneous markers for the syndrome include facial and oral mucosal papules that histologically represent tricholemmomas (benign hair-derived tumors). The oral lesions produce a cobblestone appearance. A scrotal tongue is often associated. Acral keratoses resemble acrokeratosis verruciformis. Patients may also have lipomas, hemangiomas, neuromas, and café au lait macules.

Hamartomatous polyps occur throughout the gastrointestinal tract, with the majority affecting the rectum and sigmoid colon. The polyps are not premalignant; however, up to one-third of patients with Cowden disease develop a malignant disease, usually of the breast or thyroid [53].

Muir–Torre syndrome

Muir and Torre each reported the association of sebaceous neoplasms and multiple visceral carcinomas [54,55]. The inheritance pattern is autosomal dominant. Cutaneous markers for the syndrome include sebaceous neoplasms (hyperplasia,

adenoma, epithelioma, and carcinoma), basal cell carcinomas, and keratoacanthomas [52].

Adenomatous colonic polyps are not a universal feature of the syndrome, but half or more of these patients do have polyps, and some develop colonic adenocarcinomas. Urogenital, hematological, breast, and other malignant diseases may occur [56]. Patients should be screened frequently for colonic polyps, colonic carcinoma, and other malignant diseases.

Acrochordons and colonic polyps

In 1982, Klein and colleagues reported an increased incidence of acrochordons (skin tags) in patients with acromegaly observed for colonic polyps [57] and suggested that patients with more than six acrochordons could reasonably be targeted for evaluation for colonic polyps [58]. Several subsequent studies confirmed the correlation between acrochordons and colonic polyps [59], although this was refuted in an autopsy study [60]. Most agree that the association is of no prognostic significance (see Chapter 63).

Cutaneous signs of gastrointestinal malignancy

Metastases and direct extension

Metastatic nodules of gastrointestinal adenocarcinoma are usually firm, pink, dermal to subcutaneous masses that most likely occur on the abdomen or pelvic area. The primary lesion has usually already been diagnosed by the time metastases develop. Biopsy of such a lesion helps to diagnose the primary site in fewer than 50% of patients [61]. An indurated nodule of the umbilicus (the Sister Mary Joseph nodule) may be the initial clinical presentation of an underlying (usually advanced) abdominal malignant disease, most commonly adenocarcinoma of the stomach or large bowel. Rarely, direct involvement of the lymphatics in the abdominal or thigh region draining a primary site can lead to extensive dermal infiltration, presenting as a sclerotic plaque. Occasionally, such a plaque can be quite erythematous and can mimic cellulitis (carcinoma erysipeloides).

Cutaneous lesions that should raise suspicion of possible gastrointestinal malignancy

Extramammary Paget disease

Extramammary Paget disease is considered to be a cutaneous adenocarcinoma, probably of apocrine gland origin [62]. It presents insidiously as an erythematous scaling or lichenified patch, often with surface erosion or crusting. Typically, the site has been treated with topical corticosteroids or antifungals without response, and the diagnosis is made by clinical suspicion and skin biopsy. If it occurs in the perianal area, it should raise suspicion of a possible underlying rectal or cloacogenic carcinoma [63] (see Chapters 65 and 66). The frequency of underlying visceral carcinoma has been reported at up to 86%, although a more recent review showed that only 25% of patients with perianal Paget disease had a concurrent carcinoma of rectal origin [63]. Treatment of Paget disease is usually by surgery (Mohs micrographic or wide excision) or radiation therapy, but the lesion frequently recurs.

Acanthosis nigricans

Acanthosis nigricans presents as a smooth, thickened, and dark appearance to the skin in body fold areas, most often in the axilla and around the neck (Fig. 110.13). If extensive, it may lead to a striking appearance, with thickening of the palms and soles and oral lesions of hyperkeratosis. Acrochordons (or skin tags) may also occur in affected sites. This clinical presentation is most often seen without malignant disease in obese patients, in certain insulin-resistant diabetes syndromes, as a familial trait, and with certain medications.

Suspicion for malignancy-associated acanthosis nigricans should arise if the condition occurs with recent onset in a nonobese patient who does not have diabetes. Biopsy of the skin is not helpful. Attention should be focused on the possibility of gastric adenocarcinoma, although other intraabdominal and pelvic sites should also be evaluated for possible malignant disease [64,65]. The malignant disease appears concomitantly in 61% of patients, but in almost 20% the acanthosis nigricans may precede the diagnosable tumor by years. In these patients, frequent surveillance is necessary. In a patient known to have had an abdominal adenocarcinoma who then develops acanthosis nigricans, a vigorous search for recurrence or metastatic disease should be undertaken. The mechanism of malignant acanthosis nigricans is thought to be related to peptide growth factors released by the tumor

Figure 110.13 Acanthosis nigricans in a nonobese adult patient.

[66]. Remission of the skin lesions occurs with removal of the tumor. There is no good dermatological or cosmetic treatment for acanthosis nigricans.

Keratoderma of the palms and soles

Keratoderma of the palms and soles (tylosis) is a nonspecific presentation of diffuse or punctate yellow hyperkeratosis of the palmar and plantar surfaces. There are multiple causes, and it is only in patients with long-standing skin lesions and a family history of cancer that the question of gastrointestinal malignant disease should be raised. The strongest association has been with esophageal squamous cell carcinoma, and in the Howel-Evans syndrome it may occur in an autosomal dominant pattern, with up to 95% of family members developing esophageal carcinoma by 65 years of age [67] (see Chapter 36). In such kindreds, regular evaluations with barium swallow and esophagoscopy are warranted [68]. Other kindreds have been described with punctate keratodermas and carcinoma of the colon or pancreas. There are individual case reports of tylosis occurring in association with esophageal or bronchial carcinoma without a family history. In most of those cases, the palmar–plantar thickening was of recent onset without any other skin disease. The diagnosis of malignant disease was usually apparent at the time of initial presentation and evaluation.

Generalized erythroderma

Generalized erythroderma is a nonspecific cutaneous reaction pattern with a large differential diagnosis of possible causes, including primary skin diseases, adverse drug reaction, and internal malignant disease (especially lymphoma). The possibility of esophageal or other occult carcinoma should be raised in an erythrodermic patient with compatible symptoms [69].

Hypertrichosis lanuginosa acquisita

Hypertrichosis lanuginosa acquisita refers to the sudden development of diffuse, fine, downy hair on the face and trunk. It is extremely rare but is strongly associated with internal malignant disease. It has been seen most often with carcinoma of the lung or colon and, less commonly, of the pancreas and gallbladder [70].

Paterson–Brown–Kelly (Plummer–Vinson) syndrome

Patients with the Paterson–Brown–Kelly (Plummer–Vinson) syndrome present with mucocutaneous findings of brittle spoon-shaped nails (koilonychia), atrophic tongue, and angular stomatitis (see Chapter 111). Dysphagia and iron deficiency complete the syndrome. Carcinoma of the postcricoid area of the esophagus occasionally occurs in long-standing cases [71].

Bazex acrokeratosis

Bazex acrokeratosis is a rare paraneoplastic manifestation of malignant epitheliomas of the upper respiratory or digestive tracts, reported for the first time by Bazex in 1965. It may appear several months before the first signs of the malignancy with erythematous, keratotic and irregular lesions symmetrically distributed in hands, feet, ears, and nose. The nails are generally early involved, becoming fragile, thin, and with the free edge raised from subungual hyperkeratosis. Skin manifestations disappear when the tumor is removed, even if the nails do not always benefit from total recovery.

Carcinoid syndrome

Flushing episodes may be the presenting sign of carcinoid syndrome, which has been seen primarily with carcinoid tumors of the small intestine with liver metastases. The flushing is a bright red color on the face and upper chest, occasionally involving the entire trunk and extremities, lasting 10–30 min. After repeated episodes, permanent rosacea-like telangiectasia and edema develop. Episodes may be provoked by food intake, alcohol, and emotional stress. The mechanism of the flushing is related to contributions by various vasoactive peptides and neuropeptides (discussed in more detail in Chapter 54). The best treatment for carcinoid flush is octreotide, which is effective in up to 90% of cases [72]. Similar flushing episodes can occur in patients with multiple endocrine neoplasia. Pellagra-like skin lesions and cutaneous metastases have rarely been reported in patients with carcinoid tumors.

Necrolytic migratory erythema

Necrolytic migratory erythema is the specific descriptive term for the cutaneous lesions of the glucagonoma syndrome, which occurs with a glucagon-secreting islet cell tumor of the pancreas (see Chapter 71). The cutaneous eruption typically affects perioral, lower abdominal, and perineal sites with a configurative erythema, erosions, and superficial necrosis and scale. Clinically, the condition resembles zinc deficiency. Patients often have angular cheilitis and a red swollen tongue. The clinical presentation consisting of glucose intolerance, weight loss, psychological changes, and anemia is more important than skin biopsy in making the diagnosis [73]. Skin lesions may respond to intravenous amino acid supplementation, to octreotide or its analog, or to treatment of the underlying tumor [74,75]. Necrolytic migratory erythema has occasionally been reported in the absence of a pancreatic tumor, but a vigorous search for a tumor is needed whenever typical skin lesions occur in a patient with other suggestive symptoms.

Bullous diseases of the skin that can directly affect the gastrointestinal tract

Epidermolysis bullosa

Epidermolysis bullosa (EB) is a spectrum of inherited mechanobullous diseases in which blisters occur spontaneously

or at sites of friction or trauma. Onset of skin lesions occurs at birth or shortly thereafter, and a dermatological diagnosis of EB has usually been made before the patient presents to a gastroenterologist. Rarely, pyloric atresia has been found in infants with junctional or recessive dystrophic EB, apparently resulting from mucosal blistering and scarring in utero. The major sites of gastrointestinal involvement, most often occurring in the recessive dystrophic form, are the oral cavity, esophagus, and anal areas [76,77] (see Chapter 37).

Esophageal lesions may become symptomatic at any time in the first three decades of life. Dysphagia is the most common symptom and may reflect the presence of esophageal bullae or web-like scarring, both of which can cause partial or total obstruction. Most such lesions occur in the cervical esophagus near the cricopharyngeal area. Anal involvement, with bullae and subsequent scarring, may lead to constipation. EB acquisita is an uncommon late-onset disease with skin blistering and scarring at trauma sites and sometimes causing extensive scarring oral and esophageal lesions [78]. There is no family history of EB in these patients, which has occurred occasionally in association with inflammatory bowel disease.

Pemphigus vulgaris

Pemphigus vulgaris is an immunological blistering disease that commonly presents with oral erosions before the onset of cutaneous lesions. Skin or mucosal biopsy findings of an acantholytic blister and evidence of IgG in the intraepidermal space on direct and indirect immunofluorescence microscopy confirm the diagnosis. Symptomatic esophageal involvement is rare (see Chapter 37) [79]. Pemphigus involving the lower gastrointestinal tract can present with hemorrhage [80].

Bullous pemphigoid

Bullous pemphigoid is another immunological blistering disease in which older patients present with tense bullae. A biopsy specimen of the lesion shows a subepidermal blister, and IgG is found in the basement membrane zone in a linear pattern on direct and indirect immunofluorescence microscopy. Mucosal involvement is less common in bullous pemphigoid than in pemphigus (see Chapter 37), occurring in the mouth in about one-third of pemphigoid patients and less often in the esophagus or anus [81]. There have been case reports of bullous pemphigoid occurring coincident with gastrointestinal malignant disease, particularly carcinoma of the colon.

Skin conditions associated with abdominal pain

Porphyrias

Variegate porphyria is the most common porphyria in which patients present with skin lesions and episodic abdominal pain. The typical skin lesions (the same as are seen in porphyria cutanea tarda; see section Cutaneous signs associated with liver disease) are noninflamed blisters and erosions at sun-exposed sites, particularly the backs of the hands. Scarring with milia formation, hyperpigmentation, facial hypertrichosis, and sclerosis may occur. Photoprotection is of minimal benefit because the patients are exquisitely sensitive to light, and avoidance of inducing drugs is important. Hereditary coproporphyria is even more rare but may also have photosensitivity as a component.

Fabry disease

The skin lesions in Fabry disease, a rare lysosomal storage disease, are punctate, 1-mm, red to blue angiokeratomas that do not blanch. They are most densely distributed from the midabdomen to the knees. Lesions have their onset from childhood to adolescence. Decreased or absent sweating may also occur. The gastrointestinal manifestations of this disease are covered in Chapter 109.

Mastocytosis

Mastocytosis is an uncommon disorder of mast cell proliferation. The most common skin lesions are urticaria pigmentosa, with childhood onset. These are usually reddish-brown macules, but solitary nodules may occur. On stroking, these lesions typically itch, and a raised wheal or vesicle occurs (the Darier sign). Much less common is a telangiectatic variant or an erythroderma that occurs in adults. Skin biopsy confirms the increased numbers of mast cells in the dermis. Cutaneous symptoms may be controlled with antihistamines. Topical cromolyn and systemic psoralen in combination with ultraviolet A irradiation (PUVA) have been reported to be helpful in some patients. The gastrointestinal manifestations of mastocytosis [82] are discussed in Chapter 109.

Herpes zoster

T7–L1 dermatomal involvement of herpes zoster may cause abdominal pain before the onset of typical grouped vesicular lesions. Involvement of sacral nerve roots can produce constipation and pain during defecation [83]. Postherpetic neuralgia can cause persistent recurrent pain in the involved dermatome.

Rosacea

Rosacea is a chronic inflammatory condition characterized by central facial flushing, telangiectasias, acneiform lesions, and possible complication of nasal thickening called rhinophyma (Fig. 110.14). Several reports have described a possible relationship between *Helicobacter pylori* infection of the gastric mucosa and rosacea; however, no large case–control studies have been performed to prove a causal relationship [84]. Rosacea is commonly treated with topical or oral antibiotics, with the highest response rate usually obtained with oral tetracycline.

Figure 110.14 Characteristic telangiectasias, acneiform papules, and rhinophyma in a patient with rosacea.

Cutaneous signs associated with liver disease

Jaundice

This yellow discoloration of the skin, mucous membranes, and sclerae occurs because of the accumulation of bilirubin and its metabolites in these areas. In liver disease, it usually results from impaired excretion of conjugated (direct) bilirubin and becomes clinically evident at blood levels greater than 2.5 mg/dL. It can occur in almost any type of liver disease [85]. Treatment of the underlying liver condition will cause the jaundice to resolve. Ultraviolet light can resolve neonatal hyperbilirubinemia.

Pruritus

Pruritus, which is often severe and debilitating, can occur in cholestasis, cirrhosis, chronic hepatitis C, primary sclerosing cholangitis, and other forms of liver failure [86]. Patients with liver disease-associated itching often have elevated bile acids, bilirubin, alkaline phosphatase, and cholesterol, but the degree of itching does not generally correlate with the degree of elevation of these parameters. In fact, as the liver disease progresses, the itch may disappear, and this sign often heralds liver failure.

Pruritus may be the only symptom of cholestasis of pregnancy, which has associated increased fetal morbidity and mortality. A study of eight women with 13 pregnancies complicated by cholestasis revealed only one normal delivery, eight stillbirths, and three cases of fetal distress, one of which resulted in fetal death. Three of these women had subsequent pregnancies, with cholestasis treated with ursodeoxycholic acid and had healthy deliveries [87].

Treatment of liver-related itch involves measures to decrease the pruritus as well as measures to target the underlying liver disease (e.g., interferon for hepatitis C or surgery for common duct stones). Topical and systemic corticosteroids, PUVA therapy, and sedating antihistamines can decrease pruritus. Opiate antagonists such as naloxone, nalmefene, and naltrexone decrease itching, suggesting that a central opioid may be involved in the mechanism of pruritus. Symptoms resembling those of opiate withdrawal may occur with use of these drugs, and naltrexone can be hepatotoxic [88].

Other forms of cholestatic itch are treated empirically, because the pathogenesis is unknown. First-line agents are the bile acid sequestering agents such as cholestyramine and colestipol, which are resins that bind bile acids in the bowel and thereby reduce blood levels. They are moderately effective in decreasing itching, but are poorly tolerated because of gastrointestinal discomfort. These agents may work by a means other than just decreasing bile acids, because they also decrease itching associated with uremia and polycythemia vera. It is typical for patients to respond initially, but many become somewhat resistant thereafter. Ursodeoxycholic acid is actually a bile acid that lowers endogenous bile acid secretion into the gut. It is the only specific therapy that causes both a biochemical and survival benefit in patients with primary biliary cirrhosis [89]. Rifampicin can decrease the pruritus of cholestasis, but it must be used with care because it can be hepatotoxic [90]. Serotonin antagonists such as ondansetron also work in some cases [91].

Polyarteritis nodosa

Polyarteritis nodosa is a reaction pattern characterized by tender palpable purpuric nodules and livedo (Fig. 110.15) most commonly on the anterior legs, often with associated fever, arthralgias, malaise, and fibrinoid necrotizing arteritis histologically. Renal insufficiency and microaneurysms may occur. Hepatic arteritis was seen in 100% of patients with polyarteritis nodosa at autopsy in one study [92]. Intrahepatic hemorrhage is also reported [93]. Polyarteritis nodosa can occur in association with hepatitis B and, less commonly, hepatitis C and infections with parvovirus B19 and HIV. It is usually treated with immunosuppressive medications such as corticosteroids, which could exacerbate the underlying infection.

Figure 110.15 Polyarteritis nodosa with characteristic livedo reticularis and tender nodules.

Figure 110.16 Cryoglobulinemia often results in acral vasculitic infarcts.

Cryoglobulinemia

Cryoglobulins are immune complexes that can form as a reaction to drugs or infections. They can deposit within blood vessels and can create palpable purpuric nodules similar those seen in polyarteritis nodosa, with similar systemic complaints. Signs and symptoms are worsened by cold exposure and may include the Raynaud phenomenon or acral skin infarcts (Fig. 110.16). Hepatitis C is probably the leading cause of essential mixed cryoglobulinemia (type II or III forms) [94]. Patients with hepatitis B infections have detectable cryoglobulins, usually of mixed type, in around 15% of cases [95]. Persistent chronic hepatitis A infection with cholestasis may result in cryoglobulinemic vasculitis [96]. Cryoglobulins resolve with treatment of the underlying liver disease.

Papular acrodermatitis of childhood

This condition, also known as Gianotti–Crosti syndrome, is associated with hepatitis B, Epstein–Barr virus infection, and other viral infections in children [97]. It is characterized by asymptomatic erythematous papules that arise on the extremities, may occur on the face, and typically spare the trunk. These lesions tend to last 2–4 weeks and resolve on their own.

Lichen planus

Lichen planus is a mucocutaneous condition characterized by pruritic, purple, polygonal papules with surmounting Wickham striae and lacy reticulate white mucosal plaques. It can occur in conjunction with hepatitis C in significantly higher prevalence than in normal controls [98]. Lichen planus can also be seen in primary biliary cirrhosis and many other liver diseases. Most often, however, there is no underlying disease. Treatments include systemic or topical corticosteroids, dapsone, PUVA therapy, and methotrexate.

Porphyria cutanea tarda

Porphyria cutanea tarda is characterized by subepidermal bullae, milia, and scarring most often on the dorsum of the hands, with hypertrichosis and hyperpigmentation of the face, photosensitivity to visible light (Soret band, 410 nm), and iron overload. It is often acquired in chronic liver diseases such as alcoholic or viral hepatitis and cirrhosis. Hepatitis C RNA has been detected in up to 91% of patients with porphyria cutanea tarda [99]. Most often, the predisposition is an inherited dysfunctional enzyme known as uroporphyrinogen decarboxylase, which is important in porphyrin metabolism, and the liver disease potentiates expression of porphyria cutanea tarda. Treatments include phlebotomy, antimalarials, deferoxamine, and thalidomide.

Hypermelanosis

There are several hypotheses to explain why patients with chronic liver disease such as cirrhosis may develop hypermelanosis, which usually manifests as a diffuse or patchy grayish mucocutaneous pigmentation. It may occur because of increased levels of adrenocorticotropic hormone or melanocyte-stimulating hormone. As a result, the melanin content of the skin may be increased, and giant melanosomes may be present. Hemosiderin may also be directly deposited in the skin in iron overload conditions such as hemochromatosis.

Vascular changes

Some of the vascular changes of liver disease are thought to occur because of altered estrogen metabolism. Spider angiomas, which are small arteriovenous malformations characterized by a central feeding vessel and radiating telangiectasias, are often seen in chronic liver disease. They are most common on the face, upper trunk, and distal upper extremities. Palmar erythema may be patchy or diffuse.

Nail abnormalities

Terry nails is an opaque whitish discoloration of the nail plate, with the distal third being pink. All nails are uniformly involved. This discoloration can be seen in cirrhosis, occurring in up to 82% of patients, but it is not specific to liver disease.

Azure lunula (blue discoloration of the half-moon distal part of the nail matrix) is seen in hepatolenticular degeneration (Wilson disease), which is the result of abnormal copper metabolism. Clubbing may also occur in liver disease.

Other cutaneous manifestations

Urticaria, urticarial vasculitis, other forms of vasculitis, erythema multiforme, erythema nodosum, and pyoderma gangrenosum (all covered in this chapter) can occur in liver disease, especially in patients with hepatitis B infection. Acute hepatitis B infection can produce a serum sickness-like reaction with fever, arthralgias, polyarthritis, urticaria, and vasculitis.

Pancreatic disease

Periumbilical purpura (Cullen sign) and left flank purpura (Grey Turner sign) are caused by dissection of blood along

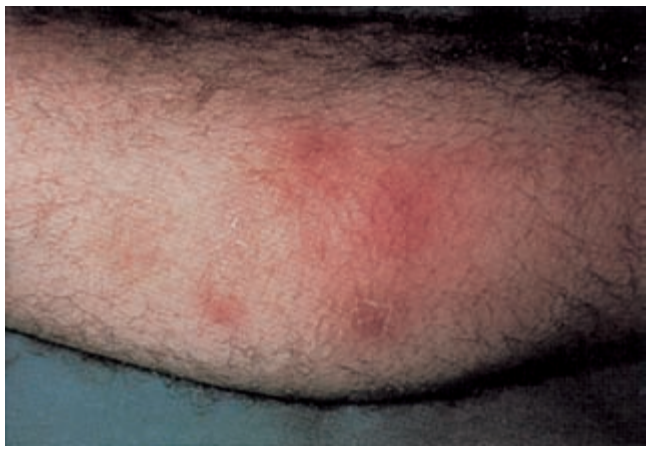

Figure 110.17 Panniculitis presenting as tender erythematous nodules in a patient with pancreatitis.

Figure 110.18 Dermatitis herpetiformis. Both elbows are involved. Note the intact vesicles and multiple crusted (i.e., excoriated) lesions.

fascial planes from retroperitoneal bleeding of acute hemorrhagic pancreatitis. Presentation of tender red subcutaneous nodules, occurring at any site but most commonly on the pretibial region of the extremities, should raise the diagnostic possibility of panniculitis (Fig. 110.17). Pancreatic fat necrosis may feel fluctuant, but lesions rarely ulcerate or discharge fluid [100]. Pancreatic fat necrosis may be the presenting sign in 22% of patients with pancreatitis or in up to 65% of patients with pancreatic carcinoma. Excisional biopsy (not punch biopsy) is indicated to confirm the diagnosis of panniculitis. The clinical differential diagnosis includes superficial migratory thrombophlebitis, which may be seen in pancreatic, gastric, or other cancers and in Behçet disease.

The eruption of multiple yellow-red papules, predominating over extensor surfaces or buttocks, suggests a diagnosis of eruptive xanthomatosis. These lesions occur in the presence of hypertriglyceridemia and type IV or V hyperlipoproteinemia and may develop in a patient with pancreatitis, particularly in the setting of diabetes mellitus.

Dermatological disorders associated with malabsorption

Dermatitis herpetiformis is an uncommon blistering disease characterized by intense pruritus and multiple grouped small vesicles distributed symmetrically over the body (Fig. 110.18). The scalp and extensor surfaces are prominently involved, and the itching and excoriation may be so severe that intact blisters are not conspicuous. Diagnosis is made by skin biopsy, which shows a subepidermal blister with a neutrophilic infiltrate of the dermal papillae. Direct immunofluorescence of perilesional skin confirms the diagnosis of dermatitis herpetiformis with the demonstration of IgA at the dermal–epidermal junction or within the dermal papillae [101,102].

Gastrointestinal manifestations may not be clinically prominent in patients with dermatitis herpetiformis who initially present to a dermatologist. Nevertheless, almost all patients have gluten sensitivity of the small intestine (see Chapter 50). Steatorrhea is observed in 20%–30% of patients, and anemia may occur secondary to malabsorption (e.g., of folate or iron). Even in the absence of gastrointestinal symptoms, strict observance of a gluten-free diet leads to improvement of the skin disease (which may take 6–24 months of careful diet restriction) and minimizes the need for medication [103,104]. Gluten challenge may cause a flare of the skin lesions in patients whose condition was previously controlled by diet.

Therapy of dermatitis herpetiformis with one of the sulfones, usually diaminodiphenylsulfone (dapsone) or with sulfapyridine, leads to prompt relief of symptoms and cessation of lesion formation (within hours to a few days). Oral dapsone is often started at a dose of 100–200 mg daily in adults and is titrated according to the clinical response and with the goal of minimizing side effects.

Malabsorption can occur with other skin diseases. Patients with extensive inflammatory skin disease, such as exfoliative erythroderma or atopic eczema, develop secondary malabsorption, termed *dermatopathic enteropathy* [105]. This is usually manifested as mild steatorrhea and is often asymptomatic; resolution is prompt with treatment of the skin disease. The mechanism is unclear, but it does not appear to result from gluten sensitivity.

Whipple disease is another cause of malabsorption that can be accompanied by diffuse pigmentation and subcutaneous nodules. This disorder generally presents with arthralgia and general malaise. The causative agent has been identified as *Tropheryma whippelii*.

Malabsorption can also occur as a result of abnormal motility or vascular compromise in systemic sclerosis and systemic

Table 110.3 Nutritional deficiencies and skin manifestations

Vitamin A	Multiple keratotic follicular papules over the extremities (phrynoderma) Generalized dry skin Sparse and fragile hairs
Vitamin B-1 (thiamin)	Glossitis Edema of the mucosae
Vitamin B-2 (riboflavin)	Seborrheic dermatitis Diffuse eczema Depapillated glossitis Hair loss, hypopigmentation, hair curling
Vitamin B-3 (niacin)	Painful erosive and pigmented scaling patches in sun-exposed areas Perianal lesions Cheilitis Red atrophic glossitis
Vitamin B-6 (pyridoxine)	Stomatitis Glossitis
Vitamin H/B-8 (biotin)	Eczema Hair loss
Vitamin B-9 (folic acid)	Lemon-yellow skin color Cheilitis Glossitis
Vitamin B-12 (cobalamin)	Generalized hyperpigmentation Vitiligo Hair loss
Vitamin C (ascorbic acid)	Follicular hyperkeratosis, perifollicular hemorrhages, perifollicular pigmentation Hair loss, corkscrew body hair Purpura
Vitamin K	Purpura and/or massive hemorrhages
Zinc	Erythematous, scaling, vesiculopustular or eroded plaques acrally distributed Stomatitis Hair loss Nail dystrophy
Iron	Cheilitis Hair loss Koilonychia
Copper	Light, sparse, brittle and kinky hair Pili torti
Proteins	
Total starvation	Dry loose skin Loss of subcutaneous fat Hair loss, hair fragility, slow hair growth rate, occasionally facial lanugo Purpura
With carbohydrate excess	Hypo-hyperpigmented scaling patches with peeling of the epidermis over edematous sites, joints and flexural areas Hair depigmentation diffuse or banded (flag sign) Purpura
Essential fatty acids	Erythematous scaling patches diffusely distributed Dry skin Intertriginous erosions Alopecia Purpura

vasculitides. Skin manifestations of chronic malabsorption are largely nonspecific and reflect altered nutritional status.

Nutritional disorders

See Table 110.3 [106–108].

Perineal skin lesions

There are several skin lesions that occur in the perianal or perineal areas that may be encountered in the course of examination or endoscopy procedure (Table 110.4) (see Chapter 66). Vesicles or erosions on an erythematous base should suggest herpetic lesions. Herpes simplex is often recurrent in this site, and in immunocompromised patients lesions may be persistent crusted erosions or ulcers. Herpes zoster usually presents acutely as dermatomal grouping of vesicles. Perianal ulcers and erosions may also occur in Crohn's disease, amebiasis, primary or secondary syphilis, or nontreponemal venereal disease. Flesh-colored pedunculated papules in the perineal area are often condylomata acuminata (venereal warts). Lesions, if they are few, usually respond well to therapy with cryosurgery, electrofulguration, surgical or laser excision, or application of topical podophyllin. Refractory lesions may respond to intralesional or systemic interferon.

Table 110.4 Perineal skin lesions (practical list of common causes)

Erythema with scale or maceration
Contact dermatitis
Seborrheic dermatitis
Psoriasis
Candidiasis
Dermatophytosis
Secondary syphilis
Extramammary Paget disease
Nutritional deficiencies
Bowen disease
Vesicles, erosions, and ulcers
Herpes simplex
Herpes zoster
Impetigo (streptococcal, staphylococcal)
Syphilis (primary or secondary)
Chancroid
Deep fungal, acid-fast bacilli, protozoal infections
Bullous pemphigoid
Pemphigus
Ecthyma
Nodules, tumors, and ulceration
Condylomata acuminata (i.e., warts)
Hidradenitis suppurativa
Squamous or basal cell carcinoma
Crohn's disease
Carcinoma: metastatic or direct extension
Kaposi sarcoma
Granulomatous herpes simplex

Erythema with scaling or maceration in the gluteal fold and perianal area raises the major considerations of candidal infection, psoriasis, seborrheic dermatitis, irritant contact dermatitis (from fecal soiling), or bacterial (streptococcal or staphylococcal) impetigo. Clinical presentation, potassium hydroxide preparation of skin scrapings, and culture are helpful in differentiating these diagnoses. If lesions do not respond to appropriate therapy for one of these diagnoses, biopsy may be indicated to rule out extramammary Paget disease. Idiopathic pruritus ani is a diagnosis that should be made only after a primary dermatosis, such as those just listed, has been excluded. It is a common complaint, particularly in middle-aged men, and psychological factors have been emphasized in some reviews [109,110]. The key to effective therapy of pruritus ani is eliminating underlying triggering factors, including infections, irritants, allergies, and anorectal disorders (e.g., rectal fissures, prolapse). Careful cleaning measures, dietary changes, and treatments ranging from topical corticosteroids to intralesional corticosteroids or cryosurgery have been used successfully. Treatment is most successful if the symptoms have been present for less than 2 years.

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I MISCELLANEOUS

111 Oral manifestations of gastrointestinal diseases

John C. Rabine, Timothy T. Nostrant

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Many gastrointestinal disease states are associated with oral findings (Table 111.1). This chapter briefly addresses those disorders in which oropharyngeal pathology is most common.

Gastroesophageal reflux disease

The supraesophageal manifestations of gastroesophageal reflux disease (GERD), including reflux laryngitis and reflux-associated asthma, have gained increasing attention. Dental injuries, predominantly erosions, are also very common. Erosions are defined as circumscribed, punched-out, and discolored regions on the surface of a tooth, corresponding to enamel loss (Fig. 111.1), and can be differentiated from caries, which are dark defects with jagged edges [1]. Gastric acid exposure has been proposed as the mechanism of dental erosions because of its association with salivary loss [2], bulimia [3], and hiatal hernias [4]. Reflux has been documented in 83% of patients with erosions referred by their dentists for pH monitoring [5]. Parotid saliva analysis has also been proposed to be useful as GERD patients tend to have lower flow rates than control patients and have higher salivary calcium levels than bulimic patients [6].

The true incidence of dental erosions in GERD patients is unclear, but at least 20% of reflux patients have been found to have erosions [7,8] and more than 50% of patients with proven reflux by pH monitoring have been shown to have dental erosions [5]. Extrinsic acid exposure (citrus fruits, soft drinks, vinegar, chewable vitamin C tablets, etc.) is more likely to induce erosions on the labial surfaces of upper anterior teeth, whereas intrinsic acid from GERD is more likely to harm the lingual surfaces of teeth [1,9–12].

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The treatment of GERD-induced dental erosions is obviously aimed at limiting reflux and oral acid exposure. Patients should avoid acidic foods and drinks, they should allow antacids to dissolve in the mouth during symptomatic reflux events, and they should avoid brushing the teeth immediately after a reflux event. Chewing gum (sugarless) can be used to stimulate salivary flow between meals, and medications that reduce salivary flow should be avoided. In patients with xerostomia from medications, previous surgery, or previous radiation therapy, water should be consumed frequently or a saliva substitute should be used. Once the underlying reflux disorder has been addressed by the gastroenterologist, the patient should be under the care of a dentist, who can provide a polyethylene mouth guard at night to limit dental exposure to acid, topical fluoride pastes, and restorative dental procedures [13].

Laryngeal abnormalities are also very common with supraesophageal acid reflux. Symptoms that suggest reflux laryngitis include hoarseness, throat clearing, globus sensation, halitosis, or prolonged vocal cord warm-up [14]. Barium esophagraphy, upper endoscopy, and standard esophageal manometry are diagnostic in less than 25% of patients with reflux laryngitis, whereas 24-h pH monitoring is positive for distal reflux in 80% and for proximal reflux in 30%. Reflux laryngitis may be induced with minimal acid exposure. Small amounts of acid applied to the vocal cords only 3 times per week will induce significant cord edema and inflammation [15]. Therefore, microaspiration of small undetectable amounts of acid may cause laryngitis and explain both the need for strong acid suppression for effective treatment and the low number of abnormal pH studies in those patients who do respond to treatment [16,17]. Proximal acid reflux is cleared more slowly than distal acid reflux and may reflux into the oral cavity when no acid is present distally. If pH monitoring is considered in patients with supraesophageal symptoms, dual-channel monitoring should be performed.

Table 111.1 Oral lesions associated with gastrointestinal diseases

Ulceration/erosions
Crohn's disease
Ulcerative colitis
Sprue
Iron deficiency anemia
Behçet disease
Malabsorption
Acrodermatitis enteropathica
Amyloidosis
Pernicious anemia
Pyridoxine, B-6, folate deficiencies
Steroid use
Gastroesophageal reflux disease (GERD)
Lead poisoning
Graft-versus-host disease
Bulimia
Acquired immunodeficiency syndrome (AIDS)

Glossitis
Crohn's disease
Alcoholism
Sprue
Iron deficiency anemia
Pernicious anemia
Malabsorption
Carcinoid syndrome
Kwashiorkor amyloidosis
Pernicious anemia
Pyridoxine, B-6, B-2, niacin, folate deficiencies
Plummer–Vinson syndrome
Cowden disease
AIDS

Cheilitis
Crohn's disease
Alcoholism
Sprue
Iron deficiency anemia
Malabsorption
Acrodermatitis enteropathica
Plummer–Vinson syndrome
Folate, B-6, B-2 deficiencies

Vascular
Osler–Weber–Rendu syndrome
Blue rubber bleb nevus
Scleroderma
Kaposi sarcoma
Pseudoxanthoma elasticum

Pigmentation
Peutz–Jeghers syndrome
Addison disease
Acanthosis nigricans
Pseudoxanthoma elasticum
Scurvy
GERD
Lead poisoning
Gardner syndrome

Gingivitis
Crohn's disease
Anorexia, bulimia
Sprue
Kwashiorkor

White patches
Vitamin A deficiency
Acrodermatitis enteropathica

Candidiasis
Steroid use
Kwashiorkor
Iron deficiency anemia

Tooth decay
Anorexia, bulimia
AIDS
GERD
Gardner syndrome
Collagen vascular disease (sicca complex)
Plummer–Vinson syndrome

Oral burning
Sprue
Iron deficiency anemia
Malabsorption
Niacin deficiency
Pernicious anemia
Carcinoid syndrome

In a recent study, significant differences were noted with proximal esophageal pH monitoring in patients with suspected supraesophageal reflux compared with control subjects and patients with typical GERD symptoms [18].

The gastroenterologist may be able to identify reflux laryngitis during routine upper endoscopy as the larynx is well visualized [19,20]. The posterior glottis, the posterior edges of the vocal cords and the mucosa overlying the arytenoid cartilage are seen best during inspiration when the vocal cords are abducted. The vocal cords are better examined during phonation when the cords are adducted. Classic posterior laryngitis consists of red arytenoid tissue and "piled-up" interarytenoid mucosa, but these findings are seen in the minority of reflux laryngitis patients (Fig. 111.2) [15]. Laryngeal edema is the most common finding but may easily be overlooked. Table 111.2 summarizes the laryngoscopic findings of reflux disease [19]. It is important to note that there is great controversy as to the specificity of these

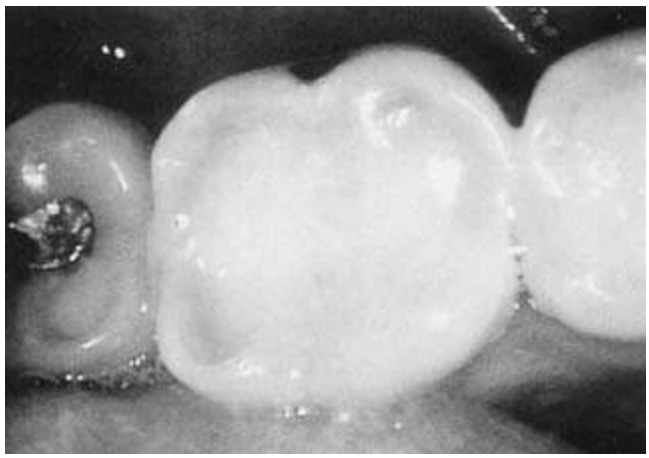

Figure 111.1 Dental erosion. Circumscribed regions correspond to exposed dentin in areas of enamel loss from repeated exposure to gastric contents in a woman with bulimia.

findings. One study suggests that many patients with presumed supraesophageal reflux based on laryngeal symptoms and findings do not respond to proton pump inhibitor (PPI) therapy or even have abnormal pH monitoring studies [21]. In another study, 52 nonsmokers with no GERD symptoms underwent laryngoscopic examinations that were independently judged by three ENT (ear, nose, and throat) specialists; approximately 50% of these patients had interarytenoid bars and medial arytenoid erythema, signs that are often attributed to supraesophageal GERD by ENT providers but appear to have questionable specificity when so common in asymptomatic patients [22].

Several studies have defined the benefits of acid suppression for reflux laryngitis. A 12-week course of omeprazole clearly improved laryngoscopic findings except for granulomas [23]. An initial response rate of 65% was seen in 22 patients treated with omeprazole 40 mg q.i.d. for 8 weeks. Relapse occurred in 38% after omeprazole was stopped. Abnormal pH studies were documented in the majority of nonresponders and there was no improvement in response with twice-daily omeprazole [24]. In another trial, 20 patients with dysphonia and laryngoscopically documented laryngitis were treated with omeprazole b.i.d. and demonstrated a 63% laryngoscopic (not clinical) response at 12 weeks [25]. Neither baseline GERD symptoms nor endoscopic findings predicted response, but pharyngeal reflux on pH monitoring (seen in only 4 patients) was predictive of response. In another study of 22 patients with idiopathic laryngitis, 50% of patients had a total symptomatic response after 3 months of lansoprazole b.i.d. therapy compared with a 10% response in the placebo group [26]. Three months of PPI therapy is considered the minimum to assess for response in supraesophageal GERD, but one study suggested that response rates improved from 48% to 70% if the PPI was continued for at least 4 months [27]. There are very limited data on the role of fundoplication for reflux laryngitis. In one study, 10 patients in whom 4 months of PPI therapy was ineffective underwent fundoplication; 80% showed improvement in laryngoscopic findings, but only 10% of patients had symptomatic improvement [28]. In short, it is reasonable to use PPI therapy empirically when reflux laryngitis is suspected, but at least 4 months of PPI therapy at b.i.d. dosing is necessary to assess response. Symptoms correlate with quality of life better than laryngoscopic findings, also supporting an empiric PPI trial [29]. The role of pH monitoring in documenting supraesophageal reflux is limited, though the longest reflux episode while supine and the fraction of supine time with pH less than 4 have been proposed to be good markers of reflux in patients with dysphonia [30].

Table 111.2 Laryngeal findings of gastroesophageal reflux disease

Reflux laryngitis
Vocal cord nodules
Polypoid degeneration
Contact ulcers/granulomas
Laryngeal stenosis
Proximal laryngospasm
Laryngeal cancer

Polyposis syndromes

Gardner syndrome

The oral manifestations of diseases associated with intestinal polyposis, such as Gardner syndrome, may be the first clue to the diagnosis.

(a) (b) (c)

Figure 111.2 **(a)** Edema and erythema of the vocal cords and posterior glottis (reflux laryngitis). **(b)** Vocal cord granuloma. **(c)** Distal reflux esophagitis in the same patient as in (a) and (b).

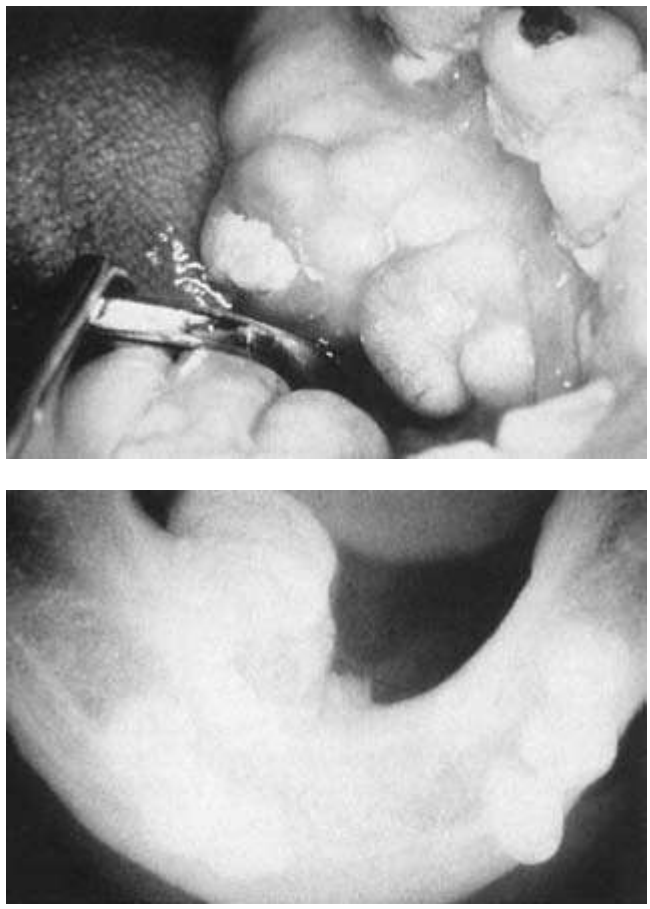

Figure 111.3 **(a)** Mandibular osteoma of Gardner syndrome. **(b)** Radiograph of the mandible in the same patient. From Bresalier RS. Neoplasia of the colon and rectum. In: Gastroenterology and Hepatology: The Comprehensive Visual Reference. Philadelphia: Current Medicine, 1997, with permission.

Multiple mutations of the adenomatous polyposis coli (APC) gene result in multiple forms of Gardner syndrome. Bone tumors, soft-tissue tumors, lipomas, desmoids, and sebaceous cysts may be seen (Fig. 111.3). Dental abnormalities including unerupted teeth, supernumary teeth, and dentigerous cysts may predispose these patients to early and profound tooth loss [31].

Peutz–Jeghers syndrome

Small intestinal hamartomatous polyps are the classic finding in Peutz–Jeghers syndrome, an autosomal dominant disease with variable penetrance. Pigmentation abnormalities with melanin deposition on mucosal surfaces are the visual hallmark. Typically appearing in the first year of life, small black or blue-gray macules form on the lips, perioral region, and buccal mucosa but may occur on any mucocutaneous surface including the fingers and toes. Perioral lesions often fade with age while those in the mouth or on the extremities may become more apparent [32]. Overall, the risk for malignancy in multiple organs can be as high as 30% and requires periodic surveillance.

Cowden disease

Multiple hamartoma syndrome, or Cowden disease, is a rare polyposis syndrome with facial trichilemmomas and oropharyngeal papillomatous growths appearing in the second decade [33,34]. Skin-colored papules, histologically neuromas, have also been described in younger patients [35]. Gastrointestinal tract malignancy is not increased but there is a higher incidence of extraintestinal malignancy, particularly breast and thyroid cancers. The overall risk for malignancy and the need for surveillance are similar to Peutz–Jeghers syndrome.

Bleeding disorders

Osler–Weber–Rendu syndrome

Hereditary hemorrhagic telangiectasias, or Osler–Weber–Rendu syndrome, is an autosomal dominant disorder characterized by widespread mucocutaneous telangiectasias of the face, ears, lips, oral cavity, nasopharynx, and hands. Oral bleeding has been reported in children, even before these vascular lesions can be seen [31]. Similar telangiectasias can be seen with scleroderma and CREST syndrome (*c*alcinosis cuti, *R*aynaud phenomenon, *e*sophageal dysmotility, *s*clerodactyly, and *t*elangiectasia), so the clinical features of these disorders can be used to differentiate them from Osler–Weber–Rendu syndrome [36]. Major bleeding sources are the nose and stomach, although colonic bleeding is recorded. Hemobilia secondary to vascular rupture can also occur. Varices are a rare source of bleeding with vascular transformation of the liver and associated cirrhosis.

Blue rubber bleb nevus syndrome

Blue rubber bleb nevus syndrome is characterized by multiple cavernous hemangiomas of the skin, oropharynx, and gastrointestinal tract. The angiomas have a blue color and smooth compliant surface, and rupture can lead to severe bleeding. The skin and gastrointestinal tract are affected more commonly than the mouth [31,37]. Histologically, these lesions consist of large, thin-walled vascular spaces with vessels lined by hyperplastic endothelium and separated by a scant network of elastin-deficient connective tissue [38].

Pseudoxanthoma elasticum

Pseudoxanthoma elasticum is a rare form of elastic tissue degeneration producing thin vascular abnormalities in the gastrointestinal and genitourinary tracts that bleed. Yellow papules on the neck may also be seen.

Table 111.3 Immunodeficiency states (AIDS)

Type	Organism	Oral findings	Diagnosis	Treatment	Reference(s)
Fungal	*Candida albicans* Others	Whitish adherent exudates (friable)	Biopsy/brushing (yeast forms)	Topical nystatin Fluconazole or ketoconazole	41,42,45,46
Viral	Herpes simplex (HSV)	Painful vesicles, superficial ulcers	Epithelial biopsy (Cowdry type A inclusions)	Acyclovir, Famciclovir maintenance treatment	39–44
	Cytomegalovirus (CMV)	Serpiginous ulcers	Blood vessel inclusions	Ganciclovir, Foscarnet maintenance	39–44
	Varicella zoster (VZV)	Vesicles, confluent ulcers	Cultures, immunohisto-chemistry	High-dose acyclovir	
Bacterial	Oral organisms	Necrotizing gingivitis, necrotizing periodontitis	Culture	Antibiotics, debridement	44–45
Idiopathic	HIV	Painful ulcers	Visual diagnosis	Steroids	47–48
	Epstein–Barr virus	Oral hairy leukoplakia (whitish verrucous tongue coating)	Visual diagnosis	No known treatment	49–50
	Human papillomavirus				51
Neoplastic	Kaposi sarcoma	Purple nodules (oral/perioral)	Spindle cell hyperplasia Vascular proliferation	Laser excision Radiation	52–53
	Lymphoma	Firm/painless nodules-ulcerated	B-cell in origin	Chemotherapy	

AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

Immunodeficiency states

Acquired immunodeficiency syndrome (AIDS)

Human immunodeficiency virus (HIV)-associated oral infections are common and produce significant disability in affected patients causing anorexia, decreased oral intake, weight loss, and malnutrition. Common oral findings are given in Table 111.3.

Graft-versus-host disease

Graft-versus-host disease (GVHD) is a significant source of morbidity in bone marrow transplantation patients and generally occurs within the initial 3–4-week period after transplantation and may present as mucositis, dermatitis, enteritis, and hepatic dysfunction. Chronic GVHD often develops in patients with acute GVHD and typically presents at 3 months from the time of transplantation. Clinically, patients have symptoms suggestive of sicca complex and scleroderma. Painful oral mucositis and upper gastrointestinal dysmotility may develop, the latter resulting in gastroesophageal reflux and in bacterial overgrowth. In addition to more aggressive immunosuppression, patients with GVHD require nutritional and fluid support as well as therapy for any infectious complications.

Collagen vascular disease

Scleroderma is the classic collagen vascular disease associated with oral manifestations. Perioral skin tightening, telangiectasias of the lips and tongue, and dysgeusia are common findings. Sclerosis of the esophagus and small intestine may respectively result in the sequelae of reflux disease and in malabsorption from bacterial overgrowth. The sicca complex, characterized by decreased salivary and lacrimal gland secretion, may be seen in numerous connective tissue diseases but is classically associated with rheumatoid arthritis (Sjögren syndrome). Dental caries, tongue and buccal fissures, and swallowing difficulties may all develop. Human T-lymphocyte virus-1 (HTLV-1), has been proposed as part of the pathogenesis of Sjögren syndrome, based on the presence of serum and salivary antibodies in HTLV-1 [54]. Dermatomyositis is not commonly associated with oral manifestations, but oral ulcers, leukoplakia-like lesions, and gingival telangiectasias have been reported in association with this disorder [55].

Nutritional deficiencies and malabsorption

As summarized in Table 111.4, vitamin deficiencies may result in numerous epithelial changes in the oropharynx (Figs 111.4–111.6). Such deficiencies are usually associated with malabsorption or with inadequate intake in the setting of chronic illness. Rarely, a deficiency stems from competition for normal body stores such as with B-12 deficiency in *Diphyllobothrium latum* infestation. The malabsorptive states typically cause oropharyngeal manifestations based on the specific nutrient deficiency. A sore mouth (niacin deficiency),

Table 111.4 Vitamin/mineral/trace metal deficiencies

Type	Oral finding	Special considerations	Reference
B-2/B-6	Inflammation/superficial denudation (angular cheilitis) Engorged granular tongue (glossitis)	Look for secondary infection (staphylococcus/candida)	31
B-12	Painful glossitis (smooth/shiny), aphthous ulcers, glossal atrophy	Similar to B-2/B-6	31
C	Red, spongy, friable gums (violet in color)	Tooth loss	31 Secondary infections
A	White patches Petechiae, purpura	Associated night vision loss	31
D	Unerupted teeth (children) Poor mandible development (children)	Osteopenia Osteomalacia	31
K	Petechiae, purpura	Skin bruising	
Iron	Cheilitis, gingivitis, glossitis, ulceration, palatal pallor	Anemia	31
Zinc	Red, scaly, plaques vesicles Whitish tongue coating	Inherited (acrodermatitis enteropathica) Total parenteral nutrition, cirrhosis	31 31

Figure 111.4 Angular cheilitis. Inflammation at the angles of the mouth is commonly associated with B vitamin deficiencies such as deficiencies of riboflavin (B-2), niacin (B-3), and pyridoxine (B-6). Secondary *Candida* or staphylococci infection may develop. From Habif TP. Superficial fungal infections. In: Baxter S (ed.) Clinical Dermatology: a Color Guide to Diagnosis and Therapy, 3rd edn. St Louis: Mosby, 1996:400, with permission from Elsevier.

cheilitis and glossitis (B vitamin and folate deficiencies), stomatitis and oral ulceration (B vitamin, folate, niacin deficiencies), and bleeding (vitamin K deficiency) may all develop. White patches on the tongue reflect vitamin A deficiency. The hypermetabolism of tryptophan to serotonin with decreased niacin production can produce pellagra in patients with carcinoid syndrome.

Figure 111.5 Swollen, inflamed, bleeding gums and poor dentition may be seen in scurvy. From Briggaman R. Clinical Nutrition I Slideset. American Gastroenterological Association, 2000, with permission from Milner-Fenwick.

Inflammatory bowel states

Celiac disease

Cheilitis, gingivitis, and small vesicular ulcers of the tongue and oral mucosa are seen in celiac sprue [31]. The oral cavity may burn. Numerous but small white ulcerations can develop on the dorsum of the tongue, and swelling of the tongue can result in a "strawberry" appearance. Dental enamel loss has also been described in children [56].

Crohn's disease and ulcerative colitis

Oral lesions in inflammatory bowel disease (IBD) are extremely common with a prevalence of 4%–30%, and such lesions are more likely to be seen with Crohn's disease than with ulcerative colitis [57,58]. A prevalence of 0.5% was

Figure 111.6 Zinc deficiency may present as a scaly, erythematous rash at the angles of the mouth and the nasolabial folds in addition to stomatitis and tongue coating. From Grossman M. Clinical Nutrition I Slideset. American Gastroenterological Association, 2000, with permission from Milner-Fenwick.

Figure 111.7 Aphthous ulcer. Aphthae appear as minute, shallow, white ulcers along mucous membranes. Though they may occur in healthy individuals, multiple or persistent lesions suggest an underlying diagnosis such as inflammatory bowel disease or Behçet disease. From Allison MC. Diagnostic Picture Tests in Gastroenterology. London: Mosby-Wolfe, 1991, with permission. Courtesy of Dr MC Allison.

seen in a tertiary care center specializing in oral dermatology [59]. In addition to extraintestinal inflammation in IBD, patients may develop malabsorption and subsequent malnutrition due to rapid transit, stricture formation, enterocolonic fistulas, previous severe mucosal loss, and bacterial overgrowth. Folate, iron, B-12 and fat-soluble vitamin deficiencies are most common. Trace metal deficiencies can be seen in IBD patients on total parenteral nutrition (TPN) without adequate replacement.

Crohn's disease

The oral lesions of Crohn's disease typically reflect active intestinal disease, though they may precede or follow clinically evident intestinal flares [31]. Aphthous linear labial ulcers, vestibular ulcers, pseudopolyps, and labial or buccal swelling have all been reported [31,59,60]. The various lesions frequently heal spontaneously but may also persist for several years, making it difficult to define a correlation between oral manifestations and intestinal disease activity [61,62]. Nonetheless, some have proposed that an eruption of aphthous ulcers actually predicts an impending Crohn's flare [63]. The pathogenesis of aphthous lesions is unclear, but it has been suggested that lymphocytes activated against enterobacterial toxins may release inflammatory mediators; diminished immunoglobulin A (IgA) secretion may also be involved [61–65]. Lip swelling, cheilitis, and Melkerson–Rosenthal syndrome (orofacial swelling, facial paralysis, and fissured tongue) have been described as initial presentations for Crohn's disease, particularly in children and adolescents [66,67].

Although not specific for IBD, aphthous ulcers are the most common oral manifestation of Crohn's disease and are seen in up to 20% of Crohn's patients [31]. These distinct shallow, white ulcers may be singular or widespread throughout the oral cavity (Fig. 111.7). Gingival hyperplasia and nodularity are not uncommon, and a cobblestone pattern may appear on the palate. The buccal mucosa may also show this cobblestone appearance owing to the presence of smooth nodules. Oral candidiasis has been seen in up to 5% of Crohn's patients, a finding not found in ulcerative colitis patients within the same trial [58]. One trial assessing the treatment of Crohn's oral lesions demonstrated complete remission of oral lesions with systemic steroids or with azathioprine in only 50%, whereas topical steroids induced a remission in 58% of cases [68]. Although data are limited, infliximab has also been reported as effective for oral lesions [69].

Ulcerative colitis

Apthous stomatitis and ulcers may be seen in ulcerative colitis patients, though ulcers are more typical of Crohn's disease. Cheilitis may also be seen and has been associated with scaling perioral skin and cervical adenopathy [31,57]. Pyostomatitis, also known as pyoderma vegetans, involves deep pustules that may progress to deep ulcers. This classically occurs on the lower lip but may also develop on the buccal mucosa, and peripheral eosinophilia has been reported. Like many oral manifestations of IBD, this lesion often develops during a colitis flare and resolves with aggressive IBD management; pyostomatitis may also predate a colitis flare by months or years [70]. Although not classically thought of in the oral cavity, pyoderma gangrenosa of the tongue has been reported and is characterized by deep ulceration and necrosis. It has been suggested that all of these lesions involve an immunological reaction such as circulating antigen–antibody complexes [31]. Unfortunately, many oral manifestations may persist or recur even after total colectomy. Although systemic steroids may help these lesions, topical or

locally injected steroids may lead to more rapid and profound resolution.

Behçet disease

Behçet disease is characterized by orogenital ulceration, ocular manifestations, and arthritis. Common problems include diarrhea, anorexia, bloating, and colorectal ulceration. Oral lesions consist of small or very large aphthous ulcers or of herpetiform-like ulcers. These ulcers tend to involve the palate, pharynx, and esophagus and can cause significant pain and dysphagia. In fact, recurrent oral lesions may be the only manifestation of Behçet disease for several years [71]. Based on clinical symptoms and ulcer appearance, IBD, herpes simplex virus (HSV) infection, and Reiter syndrome all need to be considered in the differential diagnosis. Periodontal disease is more common in patients with Behçet disease than in patients with ulcerative colitis or Crohn's disease and is a marker of disease activity; it is not clear if periodontal disease promotes a systemic inflammatory response that induces Behçet activity or if it is a result of the Behçet disease itself [72]. Treatment of oral Behçet lesions consists of topical steroids, nystatin, and tetracycline [31]. Thalidomide has been suggested as an alternative therapy, with an excellent response rate in one early trial [73].

Disorders of taste

There are numerous etiologies for the total loss of taste (ageusia), diminished taste (hypogeusia), and altered taste (dysgeusia). The human tongue contains approximately 10 000 taste buds, each of which contains 50–150 nerve receptors [74]. These receptors sense five primary tastes: sweet, sour, bitter, salty, and umami (taste of glutamate). It has also been proposed that there may be receptors for fatty acids as well. As summarized in Table 111.5, a variety of neurological conditions, systemic diseases, and medications are associated with altered taste. A full discussion of these processes is beyond the scope of this text [74–77]. Medications do deserve a brief mention as they impair taste by a variety of mechanisms. Some medications directly affect certain taste receptors; for example, ampicillin and pentamadine impede salty taste. Others inhibit cytochrome P450-dependent enzymes at the receptor level of the tongue, and some lead to silver sulfate deposition on the tongue. Medications can decrease turnover and regeneration of taste buds, and they may also impede taste by interfering with prostaglandin synthesis. Finally, some medications may contribute to a zinc deficiency or may alter calcium influx in taste receptors. Radiation therapy is another common cause of impaired taste, whether it be direct damage to receptors or the induction of xerostomia, which limits receptor exposure to ingested foods. Indeed, radiation therapy has a significant impact on quality of life and can cause weight loss as a result

Table 111.5 Etiologies of altered taste

Medications
Carbamazepine
Phenytoin
Baclofen
Levodopa
Esmolol
Amiodarone
Nifedipine
Diltiazem
Acetazolamide
Penicillamine
Glipizide
Losartan
Protease inhibitors
Angiotensin-converting enzyme inhibitors
Amlodipine
Clonidine
Ampicillin
Pentamadine
Acyclovir
Terbinafine
Doxazosin
Gemfibrozil
Simvastatin
Pravastatin
Peripheral nervous system
Bell palsy/central nervons (CN) VII palsy
CN IX/X damage (surgical, local mass)
Neuritis (e.g., herpes zoster)
Cerebellopontine angle mass (e.g., meningioma)
Polyneuropathies (lupus, amyloidosis, porphyria)
Submandibular mass
Cervical artery dissection (ischemia)
Iatrogenic (laryngoscopic trauma)
Central nervous system
Cerebrovascular accident
Multiple sclerosis, demyelination
Epilepsy
Mass/neoplasm (cortical, thalamic)
?Migraine headaches
?Schizophrenia, depression
?Eating disorders
?Dementia
Miscellaneous
Familial dysautonomia
Craniofacial trauma
Xerostomia
Radiation therapy
Renal failure
Heavy metal toxicity
Guillain–Barré syndrome
Rabies
Lyme disease
Influenza
Hepatic failure
Cigarette smoke
Altitude sickness
B-12, zinc deficiences
Diabetes mellitus
Hypothyroidism
Cushing syndrome

Table 111.6 Miscellaneous disorders

Disorder	Oral finding	Special consideration	Ref
Lead poisoning	Oral ulcers Gingival lead line Metallic taste	Poor socioeconomic status Police/pistol shooters Occupational exposure	31
Amyloidosis	Jaw pain Numbness Swelling Macroglossia Bleeding	Gingival/rectal biopsies (deep)	31
Plummer–Vinson syndrome	Glossitis Hypopharyngeal/esophageal webs Glossal atrophy Cheilosis Tooth loss	Patterson–Kelly syndrome Middle-aged women Hypopharyngeal/esophageal cancer	31

of altered taste. It typically takes 6–12 months for taste to recover after radiation therapy, though it can take several years in some patients [75]. Treatment for taste disturbances is directed at the underlying etiology whenever possible. Though data are limited, zinc gluconate supplementation at a dose of 140 mg per day has been proposed as a reasonable approach in cases of idiopathic dysgeusia [74]. The use of vitamin A supplementation and steroids has also been reported.

Miscellaneous disorders

The oral findings and diagnostic considerations in lead poisoning, amyloidosis and Plummer–Vinson syndrome are listed in Table 111.6.

There are some features of amyloidosis that are worth mentioning. This disease may present as oral burning, jaw pain, and xerostomia [78]. Oral findings include papules, small raised ulcers, and macroglossia. Oral amyloidosis usually involves the tongue and is commonly felt to represent a paraneoplastic process, though local amyloidosis of the tongue has been reported [79]. Multiple myeloma is one condition in which oral amyloidosis can be a paraneoplastic manifestation. Hardening of the soft palate, the loss of facial expression, and difficulty in opening the mouth are other symptoms of oral amyloidosis associated with multiple myeloma [80].

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I MISCELLANEOUS

112 Gastrointestinal vascular malformations or neoplasms: arterial, venous, arteriovenous, and capillary

Mitchell S. Cappell

Dieulafoy lesion, 2785
Vascular Ehlers–Danlos syndrome, 2790
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Hemangiomas, 2800
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Hemangioendotheliomas, 2804

Vascular lesions of the gastrointestinal tract are clinically highly relevant, and are a common cause of gastrointestinal bleeding. Among these lesions, gastrointestinal angiodysplasia is frequently encountered, whereas Dieulafoy lesion and gastric antral vascular ectasia (GAVE) are less common. An understanding of the other described lesions is important, despite their rarity, because they are frequently misdiagnosed and mistreated. For example, historically most rectal hemangiomas were misdiagnosed and mistreated as internal hemorrhoids [1].

The classification of gastrointestinal vascular lesions has been confusing because of variable classification schemes and inadequate consideration of anatomical and pathophysiological principles [2]. A simple classification based on anatomical and pathophysiological principles is illustrated in Fig. 112.1. Vascular lesions are divided into neoplastic lesions and non-neoplastic malformations, the latter including inflammatory (vasculitis) and structural malformations. Structural malformations are divided anatomically according to the most affected vascular component: arteriovenous, arterial, capillary, and venous. Neoplasms are, likewise, categorized according to the predominantly affected vessel and the cell of origin. Only the entities highlighted in color in Fig. 112.1 are discussed in this chapter. Table 112.1 summarizes the predominant clinical presentation, anatomical or histological abnormalities, endoscopic appearance, and characteristic features of the discussed lesions.

The nomenclature of these lesions has also been confusing owing to redundancy, application of erroneous pathophysiological mechanisms, use of the same name for different diseases, and inconsistent application of names to different diseases. For example, the Dieulafoy lesion has been called an exulceratio simplex, cirsoid aneurysm, caliber persistent artery, and submucosal arterial malformation; but, the Dieulafoy lesion is not an aneurysm [3]! The term phlebectasia has been used to describe both small hemangiomas or isolated venous varicosities [4]. The most commonly used name is employed in this chapter, provided the name is pathophysiologically reasonable.

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Dieulafoy lesion

Pathology and pathophysiology

Although uncommon, the Dieulafoy lesion is important because it tends to bleed repeatedly and massively. Arteries normally narrow progressively in the arterial tree as successive branches approach the mucosa to terminate as submucosal end arteries. In the Dieulafoy lesion, the submucosal end artery is abnormally large; the alternative name of "caliber-persistent artery" is based on this abnormality. The lesion usually, but not always, occurs in the stomach, within 6 cm of the gastroesophageal junction and near the lesser curvature. The propensity for this location is explained by the absence of the usual submucosal arterial anastomotic gastric plexus in this region [5]. In three large clinical series, this lesion was in the proximal stomach in 75% of patients, the distal stomach in 13%, and the duodenum in 12% [6,7]. It rarely occurs in the esophagus [8].

The Dieulafoy artery is vulnerable to relatively minor mechanical trauma because it is large and superficial. Pulsations of the artery, peristaltic contractions, or passage of the food bolus may induce gastrointestinal bleeding [9]. Alternatively, the

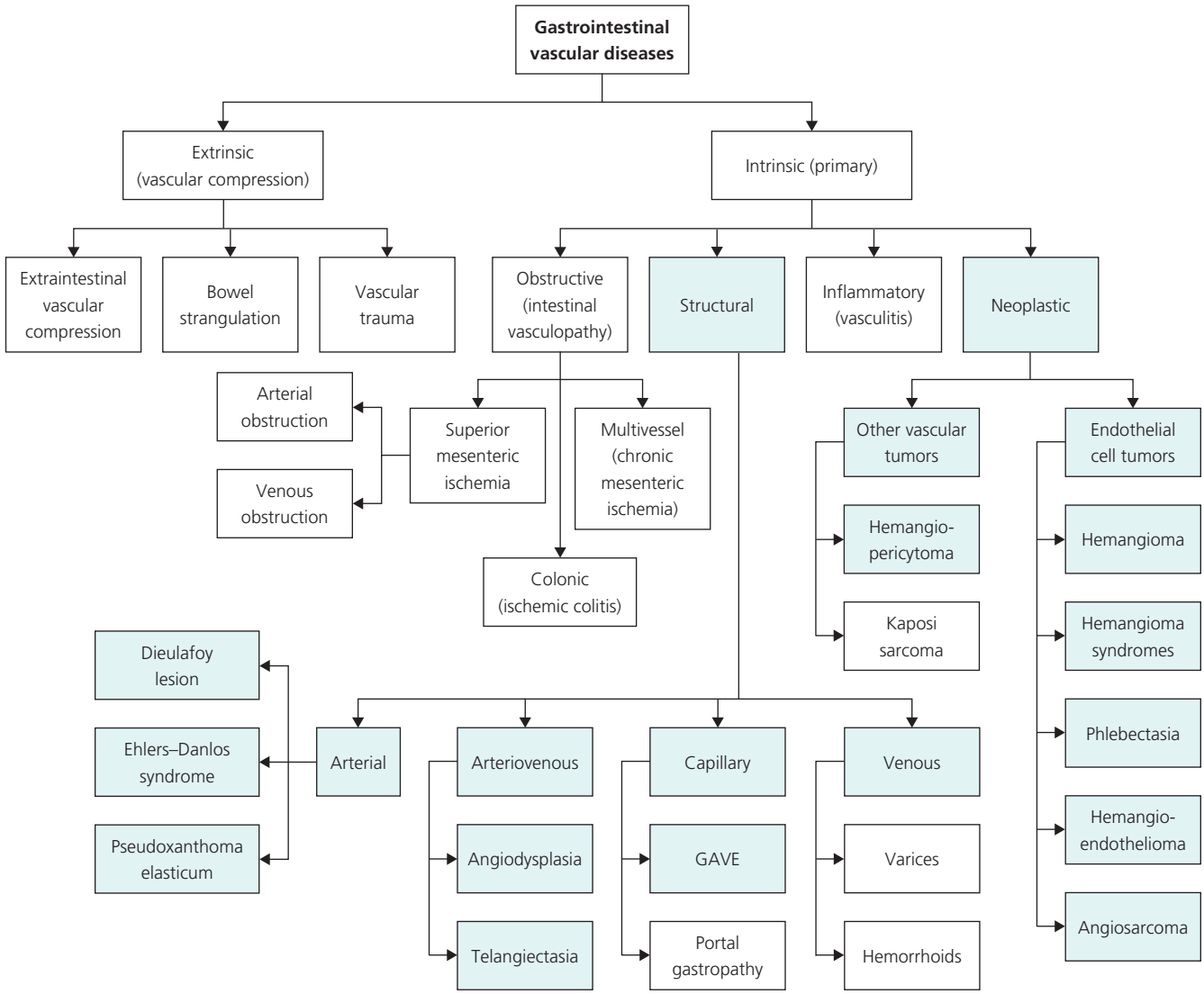


Figure 112.1 Classification of vascular gastrointestinal lesions. Diagrammatic outline of a new classification of vascular gastrointestinal lesions according to pathophysiology and according to the most affected vascular structure, whether arterial, venous, arteriovenous, or capillary. Diseases highlighted in green are discussed in this chapter. GAVE, gastric antral vascular ectasia.

large artery might cause hypoperfusion, ischemia, and erosion of overlying mucosa by shunting of blood (steal phenomenon). Both theories are compatible with the pathological findings of a thrombus attached to a large superficial artery overlying a small nonexcavated mucosal defect, with a normal vascular endothelium, without atherosclerosis, inflammation, or aneurysm [3,9,10]. Whether the lesion is congenital or acquired is unknown [3,11].

Upper gastrointestinal lesions

Epidemiology and clinical presentation

The incidence is about 1.5% among patients with upper gastrointestinal bleeding [6,12–14]. The male:female ratio is about 2:1 [7,10,14], and the mean age at diagnosis is 65 years, with a range of 1–95 years [7,10,15]. The lesion is not associated with use of alcohol, nonsteroidal antiinflammatory drugs, or tobacco, nor with the presence of peptic ulcer disease or *Helicobacter pylori* infection [7,10,16,17].

Patients present with acute, usually severe, gastrointestinal bleeding, often associated with manifestations of hemodynamic compromise such as hypotension, orthostasis, tachycardia, and prerenal azotemia. The bleeding is rarely occult and usually presents as hematemesis, melena, or hematochezia; about 50% of patients manifest both hematemesis and melena [10]. Other gastrointestinal complaints, such as abdominal pain, are atypical, and their presence should suggest another diagnosis, such as peptic ulcer or esophagitis [7].

Table 112.1 Gastrointestinal vascular malformations or neoplasms: clinical presentation, histology, endoscopic appearance, and other diagnostic features

Lesion	Predominant clinical problem	Anatomical/histological abnormality	Endoscopic appearance	Characteristic features on other tests
Arterial lesions				
Dieulafoy lesion	Severe arterial GI bleeding due to unusually large superficial artery that is vulnerable to minor trauma	Unusually large-caliber submucosal end artery, most commonly on lesser curve of proximal stomach	Pigmented, small (several millimeters wide) protuberance representing the vessel with minimal surrounding erosion and no ulceration	Angiography – extravasation of contrast from a point source in proximal stomach Endosonography – abnormally large (2–3 mm wide), high-flow, pulsatile submucosal artery
Vascular Ehlers–Danlos	GI bleeding, especially colonic, from spontaneous arterial rupture due to vascular and perivascular connective tissue weakness	Arterial wall weakness due to disordered collagen synthesis arising from mutation of *COL3A1* gene	Colonic diverticular bleeding, intramural hematomas, and other forms of arterial GI bleeding	Angiography – relatively contraindicated because of vascular fragility. MRA is the preferred test because of its greater safety
Pseudoxanthoma elasticum	GI bleeding attributed to elastin degeneration in small gastric arteries	Elastin degeneration of arterioles because of mutation of *ABCC6* gene	Bleeding from yellowish xanthomatoid papules (pseudoxanthomas) and petechiae in gastric fundus	Gastric lesions best diagnosed by EGD. Has cutaneous pseudoxanthomas, excessive skin folds, and papyraceous cutaneous scars
Arteriovenous communication or capillary lesion				
Angiodysplasia	GI bleeding from high pressure in thin-walled arteriolized veins and capillaries	Arteriovenous communications	Mucosal or submucosal, 2–8 mm wide, intensely red, dense, macular, reticular network of vessels (vascular tuft); abrupt and irregular (stellate) border	Angiography – vascular tuft, and early-filling, late-draining vein
Hereditary hemorrhagic telangiectasia	GI bleeding as a result of high pressure in thin-walled arteriolized veins and capillaries	Arteriovenous communication resulting from mutation of genes involved in blood vessel growth and repair	Endoscopic appearance identical to nonsyndromic angiodysplasia except that lesions distributed in all layers of the bowel wall	Angiography – identical to nonsyndromic angiodysplasia except for numerous lesions distributed in all layers of bowel wall
Gastric antral vascular ectasia (watermelon stomach)	Iron deficiency anemia consequent to chronic GI bleeding from dilated, tortuous, thin-walled vessels	Hypertrophied mucosa; dilated tortuous mucosal capillaries often occluded by bland fibrin thrombi; dilated tortuous submucosal veins; minimal inflammation	EGD – multiple parallel, prominent, intensely erythematous, longitudinal folds that traverse the antrum to converge on the pylorus	Endoscopic appearance with pathological analysis of endoscopic biopsy is characteristic

Table 112.1 (*cont'd*)

Lesion	Predominant clinical problem	Anatomical/histological abnormality	Endoscopic appearance	Characteristic features on other tests
Venous lesions				
Cavernous hemangiomas	GI bleeding from large, dilated, thin-walled vessels	Large, dilated, thin-walled vessels	Moderately well-circumscribed, violet-blue, sessile, compressible polypoid lesions, most commonly in distal colon or rectum	CT – large, thickened mesentery with large vacuoles next to an abnormal intestinal segment with a thickened wall that contains phleboliths
Blue rubber bleb nevus syndrome	GI bleeding from variant of GI hemangiomas with similar skin lesions	Multiple intestinal hemangiomas and similar skin lesions	Endoscopic appearance like that of cavernous hemangiomas. May have numerous and diffuse GI lesions	Capsule endoscopy is recommended if EGD and colonoscopy do not reveal the cause of GI bleeding
Klippel–Trenaunay syndrome	GI bleeding from extensive hemangiomas of the distal colon	Colonic hemangiomas; cutaneous hemangiomas; and atretic deep veins and bony hypertrophy of one lower limb	Colonoscopy – extensive infiltrative cavernous hemangioma of distal colon	CT – similar to findings with nonsyndromic colonic hemangiomas, except lesions tend to be larger and more locally invasive
Phlebectasias	Typically asymptomatic and incidental findings	Markedly dilated, tortuous vein with normal vascular wall. No portal hypertension	Colonoscopy – many dark-bluish, soft, compressible submucosal veins	Usually visualized by capsule endoscopy or colonoscopy
Neoplastic lesions				
Angiosarcomas (hemangiosarcomas)	GI bleeding, anemia, or abdominal pain. May present with diffuse metastases	Numerous interconnecting vascular channels (vasoformative structures) irregular in size and shape, atypical cells with mitosis	Hemorrhagic, ulcerated lesions	Histological markers – immunoreactive for vimentin, factor VIII-related antigen, CD34, or CD31. Immunonegative for endothelial cell markers, e.g., S-100
Hemangiopericytoma	GI bleeding from this highly vascular tumor, or constipation from a bulky GI mass	Tightly packed, spindle-shaped, neoplastic pericytes, surrounding interconnecting vascular channels	Endoscopy – solitary, circumscribed, submucosal polyp, covered by normal or ulcerated mucosa	CT – mass with multiple foci of low attenuation Angiography – richly vascular mass with a capillary blush
Hemangioendothelioma	GI bleeding from this vascular tumor, or abdominal pain, or GI obstruction	Borderline malignancy with more cellularity and mitoses than hemangiomas, but less than for angiosarcomas	Intestinal mass	Histological markers – immunoreactive for factor VIII-related antigen, CD34, or CD31

CT, computed tomography; EGD, esophagogastroduodenoscopy; GI, gastrointestinal; MRA, magnetic resonance angiography.

Figure 112.2 Endoscopic appearance of Dieulafoy lesion. Videophotograph shows a gastric cardial Dieulafoy lesion as a small, pigmented protuberance, which represents the vessel stump, with minimal surrounding erosion and no ulceration. From Lee JG, Leung JWC. Stigmata of recent hemorrhage in Dieulafoy's lesion. Gastrointest Endosc 2000;51:191, with permission.

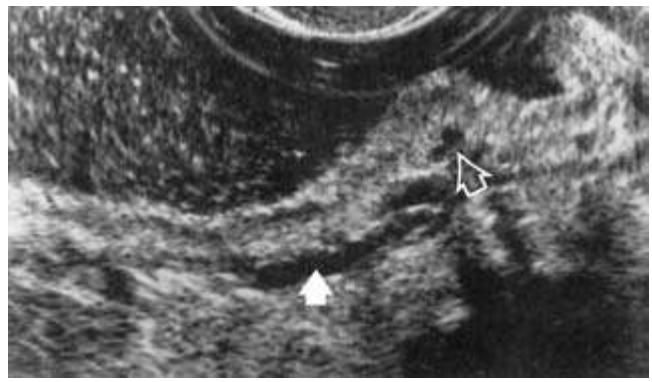


Figure 112.3 Videoendosonographic image of a Dieulafoy lesion. A relatively large gastric vessel courses outside the muscularis propria **(left**: *solid arrow***)**, penetrates the muscularis propria **(middle)**, and runs tortuously through the submucosa **(right**: *open arrow***)**. From Fockens & Tytgat [6], with permission from Gastrointest Endosc Clin North Am.

Diagnosis

The lesion is usually diagnosed by endoscopy, which reveals a pigmented protuberance, representing the vessel stump, with minimal surrounding erosion and no ulceration (Fig. 112.2). A pigmented protuberance within an ulcer represents a visible vessel in a peptic ulcer, not a Dieulafoy lesion. The Dieulafoy lesion is typically small (2–5 mm diameter); sometimes only a tiny necrotic spot is found in the proximal stomach. Stigmata of recent hemorrhage, such as an adherent clot or active bleeding, commonly occur. Active bleeding is often spurting or micropulsatile, as expected from an arterial source. The lesion should be suspected when a fresh, densely adherent clot in the proximal stomach has a narrow point of attachment to a small mucosal defect. The lesion may be missed at endoscopy because of its small size; careless gastric retroflexion; and concealment between gastric rugae, underneath blood in the gastric lake, or underneath a clot [18]. Indeed, in one large series, the lesion was detected in only 73% on the initial esophagogastroduodenoscopy (EGD) and then detected in a further 21% of cases on repeat EGD [10]. Meticulous endoscopic technique – including careful examination during gastric retroflexion, aspiration of the gastric lake, cautious irrigation of gastric clots, and adequate gastric insufflation to efface the gastric rugae – should increase the sensitivity of endoscopy. Endoscopic biopsy of the lesion is not recommended because of the risk of inducing bleeding.

Angiography is indicated for ongoing upper gastrointestinal bleeding after a nondiagnostic EGD; it was found to be diagnostic in 11 of 14 patients [10]. Angiography is suggestive of this lesion when extravasation of contrast is detected from a point source in the proximal stomach after a nondiagnostic EGD. Angiography may also demonstrate arterial tortuosity in the territory of the left gastric artery without other vascular abnormalities [19]. Angiographic findings of arteriovenous shunting, a vascular tuft, an early-filling vein, a late-draining vein, or a large draining vein are consistent with angiodysplasia, not a Dieulafoy lesion. The Dieulafoy artery is usually too small in caliber to be recognized as enlarged by angiography and, besides, is often obscured by extravasated contrast. Computed tomographic (CT) angiography or magnetic resonance angiography (MRA) may replace conventional angiography for the diagnosis [20].

Endoscopic Doppler ultrasound can confirm the diagnosis. The classic findings are an abnormally large (2–3 mm wide), high-flow, pulsating submucosal artery located along the lesser cuve near the gastroesophageal (GE) junction [21–23] (Fig. 112.3).

Therapy

Most patients require vigorous fluid resuscitation and transfusion of three or more units of packed erythrocytes for the severe bleeding. The lesion is not related to gastric acidity, and gastric acid suppression has no proven therapeutic role.

Endoscopic therapy for this point source of bleeding is particularly attractive, and fatal hemorrhage may occur without it. Endoscopic therapies include injection therapy, bipolar electrocoagulation, thermocoagulation, laser photocoagulation, endoscopic band ligation, and hemoclips [24–26]. In four large reviews, long-term hemostasis was achieved

in about 90% of patients by various endoscopic therapies [6,10,15,27]. The most experience has accumulated with endoscopic injection therapy with epinephrine (adrenaline) or polidocanol, which has been shown to achieve hemostasis in 53 of 56 patients [13,28,29]. There may be a higher risk for gastric perforation from sclerotherapy for a Dieulafoy lesion than for a gastric ulcer [30], owing to a lack of inflammation and fibrosis around a Dieulafoy lesion [6]. Endoscopic studies have reported high rates of successful hemostasis with endoscopic band ligation [31]. Two reports suggest that endoscopic hemoclips may be superior to endoscopic injection therapy [32,33]. Endoscopic epinephrine injection to reduce lesion vascularity followed by mechanical hemostasis or electrocoagulation appears to be a prudent strategy [34]. The major complication of endoscopic therapy is ulceration at the treated site.

Surgery was the treatment of choice before the development of endoscopic therapy, but surgery is now restricted to patients who fail endoscopic therapy. Surgical therapy has included electrocoagulation with vessel ligation, wedge resection, or proximal partial gastrectomy. Bleeding may recur after electrocoagulation or limited wedge resection because of the long span of the abnormal artery [35]. However, gastrectomy is theoretically unnecessary because the abnormal artery is usually restricted to the proximal stomach [36]. A wide wedge resection is a reasonable compromise between resecting too little or too much [7,16].

Therapeutic angiography with selective left gastric artery embolization before surgery for ongoing bleeding is supported by anecdotal evidence, but the hemostasis may only be transitory [37]. Although this lesion used to have a grave prognosis, the mortality decreased to about 25% with the advent of endoscopic therapy in the 1980s [7], and has since declined to about 10% [38].

Lower gastrointestinal lesions

Dieulafoy lesions of the jejunum, ileum, colon, and rectum are being increasingly reported. In one review, this lesion occurred in 0.3% of about 3000 patients with gastrointestinal bleeding [39]. About 10% of all Dieulafoy lesions are colonic. Colonic lesions also tend to bleed suddenly and severely [40–43], manifesting as bright-red blood per rectum, rather than melena, with hemodynamic instability [39].

Dieulafoy lesions of the jejunum, ileum, and colon are diagnosed preoperatively by endoscopy or angiography [44]. Lesions are more commonly detected in the colon and rectum than the jejunum and ileum, probably because of the more frequent use of colonoscopy than enteroscopy and the easier identification of lesions at colonoscopy than at enteroscopy because of a wider endoscopic field. The incidence of jejunal and ileal lesions may therefore be underestimated [45]. Videocapsule endoscopy may play an increasingly important role in diagnosing jejunoileal lesions beyond the reach of an enteroscope. The colonoscopic diagnosis is often complicated by severe lesion hemorrhage, which increases the risk and decreases the yield of colonoscopy [46]. Rectal lesions, however, are readily diagnosed by sigmoidoscopy [46,47]. The endoscopic appearance and histology of these lower gastrointestinal lesions are similar to those of gastric lesions [45].

Traditionally, surgery has been the therapy for lower gastrointestinal lesions [46,47], but colonoscopic hemostasis is being increasingly attempted, with the same modalities as are used for gastric lesions. Endoscopic therapy should be performed initially for a bleeding colonic lesion and surgery should be reserved for refractory bleeding [39,47]. The colonic Dieulafoy lesion is not a polyp and should not be removed by snare polypectomy because of risks of massive postpolypectomy hemorrhage [48].

Vascular Ehlers–Danlos syndrome

The Ehlers–Danlos syndrome is a rare heterogeneous group of six genetic disorders of collagen metabolism that cause skin hyperextensibility, articular hypermobility, and tissue fragility. Vascular Ehlers–Danlos syndrome (type IV) is the most common type associated with vascular lesions [49,50]. It results from mutation in the *COL3A1* gene that encodes for type III procollagen [51]. Patients often have a positive family history, in combination with thin transparent skin, wide thin (papyraceous) cutaneous scars overlying bony prominences, easy bruisability, and cutaneous ecchymoses from spontaneous arterial rupture as a consequence of vascular and perivascular connective tissue friability [52,53]. They have increased risks of intramural intestinal hematomas [52], colonic diverticular bleeding [52,54], other gastrointestinal bleeding [55,56], and, rarely, intestinal perforation [52,57] due to dissection or rupture of intestinal arteries. There is also increased risk of abdominal wall hernias because of connective tissue weakness [51]. About 75% of bowel complications occur in the colon [58]. The syndrome does not predispose to peptic ulcer disease, but patients with this syndrome who have coincidental peptic ulcers often bleed severely because of weakness of perivascular supportive tissue [50]. Type IV disease is diagnosed by biochemical analysis of collagen or genetic analysis [55], and leads to high mortality from repeated gastrointestinal bleeding [56]. Arteriography entails increased risks due to vascular fragility; noninvasive imaging of visceral vessels, such as MRA, is a preferred alternative. Gastrointestinal surgery produces poor results owing to tissue fragility and a bleeding diathesis; surgical anastomoses are vulnerable to disruption.

Pseudoxanthoma elasticum

Pseudoxanthoma elasticum is a rare genetic disorder of elastin synthesis, caused by mutation of the *ABCC6* gene [59]

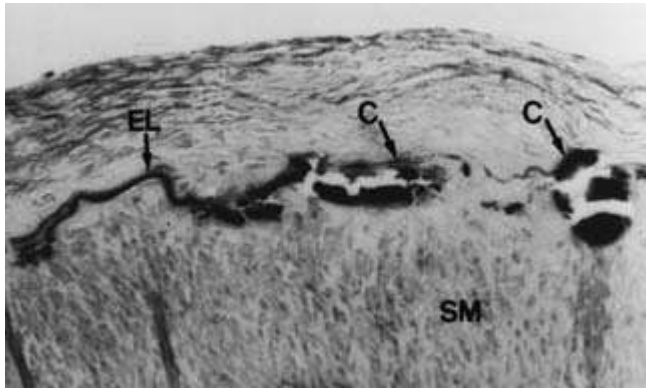


Figure 112.4 Histology of pseudoxanthoma elasticum. An elastin stain of a cross-section of the proximal stomach from a patient with pseudoxanthoma elasticum, who underwent total gastrectomy for massive refractory gastric bleeding, reveals a submucosal gastric artery with focal intimal thickening, fragmentation of the elastin lamina (EL), and intramural calcifications (C). SM, smooth muscle. From Kundrotas et al. [63], with permission from Blackwell Publishing.

and characterized by yellowish xanthomatoid papules (pseudoxanthomas) on the skin, excessive skin folds in flexural areas, angioid streaks in the ocular fundi, and intermittent claudication and weak peripheral pulses due to vascular occlusion [60]. Gastrointestinal bleeding occurs frequently; a rate of 13% has been observed in 200 patients [60]. The bleeding typically commences in young adulthood [60,61]. Most commonly, patients bleed from a characteristic lesion: numerous petechiae or erosions as well as yellowish xanthomatoid nodular lesions, that resemble the cutaneous lesions, are found predominantly in the gastric fundus [62–65]. Bleeding is attributed to elastin degeneration in small gastric arteries (Fig. 112.4) and is frequently severe and recurrent [66]. Bleeding arterioles do not vasoconstrict normally in response to hemorrhage, owing to intimal calcification and elastin degeneration, and are refractory to available pharmacological therapy [63]. Lesions may also not be amenable to endoscopic therapy because of their diffuseness. Partial or subtotal gastrectomy is the primary therapy.

Angiodysplasia

Epidemiology

Angiodysplasia is an important cause of acute and chronic gastrointestinal bleeding, particularly from the lower gastrointestinal tract. About 1% of asymptomatic healthy subjects have colonic angiodysplasia [67], whereas about 3%–6% of lower gastrointestinal bleeders have angiodysplasia [68–71]. The frequency of asymptomatic upper gastrointestinal angiodysplasia in healthy subjects is unknown, but angiodysplasia accounts for about 2%–5% of acute upper gastrointestinal bleeding [72–74]. About 3%–5% of gastrointestinal bleeding arises from the jejunum and ileum, and angiodysplasia is the most common cause of bleeding in this location [75,76]. The recognition of colonic angiodysplasia has increased markedly because of greater use of colonoscopy, causing the incidence to increase more than six-fold during the 1980s at one university hospital [77], and the recognition of small intestinal angiodysplasia has increased markedly since 2002 because of greater use of capsule endoscopy [75,78].

The incidence is unaffected by gender or race [71,77]. Angiodysplasia is not familial, but it may be confused with hereditary hemorrhagic telangiectasia (HHT), which is familial. Patients with bleeding from colonic angiodysplasia are generally elderly, with an average age of about 70 years [67,69,77,79,80], but the bleeding occasionally occurs in infants, children, and young adults [81].

Pathology

Colonic angiodysplasia occurs most commonly in the right colon, especially the cecum [82], but nearly 40% of colonic lesions occur distal to the hepatic flexure [67,83,84]. The predilection for the cecum is explained by the greater cecal mural tension owing to its larger lumenal diameter, according to the LaPlace law [85]. Upper gastrointestinal lesions occur most commonly in the stomach, sometimes in the duodenum, and rarely in the esophagus [72,77,82,86].

Angiodysplasias are often multiple [87] and tend to be clustered [80] (Fig. 112.5). Despite clustering, 15%–20% of patients with colonic angiodysplasia have upper gastrointestinal angiodysplasia, and vice versa [80,88].

Unlike the lesions of HHT, which occur in all layers of the bowel wall, angiodysplasia is restricted to the mucosa and submucosa [75]. Histologically, angiodysplasia consists of dilated, distorted, tortuous, and thin-walled vessels lined by endothelium with little or no smooth muscle and no inflammation, fibrosis, or atherosclerosis [72,79,89]. Initially, only submucosal veins are dilated, but in older lesions, mucosal veins and capillaries and even arteries become dilated [85,89] (Fig. 112.6). Congenital arteriovenous malformations, in contrast to angiodysplasia, typically have thick-walled arteries.

Associations and pathophysiology

Angiodysplasia is the most common cause of upper gastrointestinal bleeding (19%–32%) in patients with *chronic renal failure*, as compared with only about 3%–5% in the general population [90–93]. Similarly, colonic angiodysplasia is a frequent cause of lower gastrointestinal bleeding in patients with chronic renal failure [94].

The association with chronic renal failure is, however, somewhat controversial because several studies report no association [95–97]. The reported discrepancies could arise from several sources. First, the risk of angiodysplasia appears to increase with the duration and severity of renal dysfunction [90], and studies showing no association may include

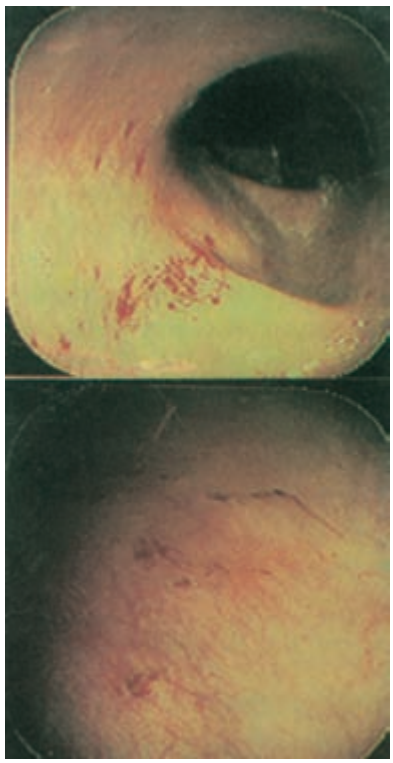


Figure 112.5 Endoscopic photo of cluster of angiodysplasia. **(a)** Colonoscopic videophotography demonstrates two adjunct clusters of angiodysplasia in the descending colon in an elderly female with iron deficiency anemia; colonoscopy revealed no other angiodysplasia in the rest of the colon. **(b)** Close-up views demonstrate the characteristic endoscopic appearance of angiodysplasia: an intensely red color, an intricate reticulonodular structure, and communication with prominent feeding or draining vessels. From Cappell [80], with permission from Springer Science and Business Media.

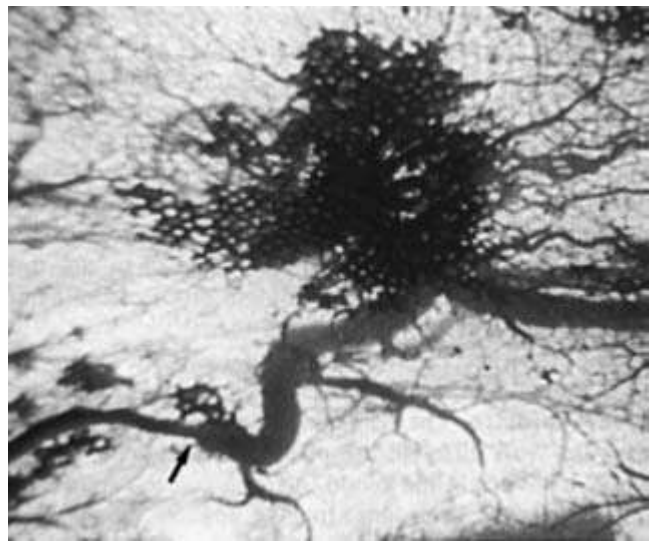


Figure 112.6 Transilluminated view of angiodysplasia. Photomicrograph through a dissecting microscope of a transilluminated, cleared colon after intravascular silicone injection reveals a mucosal ectatic vascular tangle surrounded by normal colonic crypts. The vascular tangle is drained by ectatic venules that lead to a large, distended, and tortuous underlying submucosal vein (arrow). From Boley SJ, Brandt LJ, Mitsudo SM. Vascular lesions of the colon. Adv Intern Med 1984;29:301, with permission from Elsevier.

patients with a short duration and mild severity of renal insufficiency. Second, many studies that show a lack of association were published in the early 1980s when angiodysplasia was an obscure diagnosis. Third, the presence of an association may be artifactual: platelet dysfunction with renal insufficiency may promote bleeding from and detection of otherwise clinically silent angiodysplasia [90]. In conclusion, angiodysplasia appears to be associated with chronic renal failure, but a prospective, controlled, investigator-blinded endoscopic study is needed to resolve the controversy.

The pathophysiology of this apparent association is speculative. Chronic, intermittent fluid overload in patients with renal failure may result in venous hypertension, microvascular venous dilation, and retrograde capillary dilation [92]. Older renal failure patients might have asymptomatic angiodysplasia, which could clinically manifest as a result of the bleeding diathesis associated with renal failure [93]. Hypertension and vascular disease, common in renal failure, could exacerbate bleeding from angiodysplasia [72].

After an association between *aortic stenosis* and unexplained gastrointestinal bleeding was proposed in 1958 [98], several retrospective studies showed that up to 25% of patients with angiodysplasia had aortic stenosis [82,85,87,91,99,100]. In many of these studies, aortic stenosis was diagnosed by clinical criteria without confirmation by echocardiography or cardiac catheterization [101]. An association was further strengthened by several case reports of cessation of bleeding from angiodysplasia after successful aortic valve replacement [102–104]. In two prospective endoscopic studies of 29 or 40 patients with gastrointestinal angiodysplasia, none had aortic stenosis detected by two-dimensional Doppler echocardiography [68,105]. By contrast, another study reported 32% of 73 patients with angiodysplasia had aortic stenosis on echocardiography, a much higher rate than expected [106]. Although somewhat controversial [107], the weight of evidence supports an association between aortic stenosis and gastrointestinal bleeding from angiodysplasia.

The association is likely the result of bleeding from previously clinically silent angiodysplasia caused by loss of large multimers of von Willebrand factor from high shear forces across a stenotic aortic valve. These multimers normally prevent bleeding at high-flow and high-shear-stress lesions such as angiodysplasia [108]. After successful aortic valve replacement, there is restoration of normal multimer levels and cessation of bleeding from angiodysplasia [109]. Types 2 or 3 von Willebrand disease are associated with severe bleeding

from intestinal angiodysplasia even when congenital and not associated with aortic stenosis [87,107,110].

A number of case reports have noted an association between gastrointestinal angiodysplasia and *scleroderma* or the *CREST syndrome* (calicinosis cutis, Raynaud phenomenon, esophageal dysfunction, sclerodactyly, and telangiectasia) [111–114]. Although this association has not been proven in a large controlled trial, the known occurrence of cutaneous telangiectasia in scleroderma renders an association with gastrointestinal telangiectasia both credible and likely. Several cases of angiodysplasia have been reported in association with *Turner syndrome* [115,116] and *portal hypertension* [117,118]. These associations, however, are unproven.

The pathogenesis of angiodysplasia is unknown and may vary according to the associated condition and the age of onset [119]. A popular theory is that angiodysplasia is a degenerative lesion of aging caused by chronic intermittent low-grade obstruction of veins, capillaries, and arterioles that supply the mucosa [85] (Fig. 112.7). A neurovascular (or hormonal) mechanism could contribute to angiodysplasia [102]. In response to low-grade local hypoperfusion, sympathetic nerves may stimulate intestinal vascular smooth muscle relaxation to increase flow and reverse the hypoxia. With chronicity, local vascular overload, dilation, and ectasia develop pathologically [102]. This theory could explain the disappearance of angiodysplasia with intravenous meperidine injection (a problem with endoscopic diagnosis) [120] or cold water colonic irrigation [105] and the reappearance with naloxone administration. Increased expression of angiogenic factors [121] and deficient production of type IV collagen [122] have also been proposed as pathogenetic factors. Conceivably, the same genetic lesion that occurs in germ cells in HHT, as discussed later, may occur focally in somatic cells in sporadic angiodysplasia. This hypothesis, although speculative, is easily tested by molecular genetic analysis of tissue from sporadic angiodysplasia for *ENG* or *ACVRL1* mutations (see "Hereditary hemorrhagic telangiectasia" below).

Clinical presentation

Most angiodysplasias are asymptomatic and incidental. Bleeding from angiodysplasia is usually low grade and recurrent, and always painless. About 10% of patients present with acute severe hemorrhage [99]. The bleeding pattern may depend on the lesion stage, with more advanced lesions causing more frequent and more severe bleeding [79], and may depend on hemostatic parameters, with acquired von Willebrand deficiency causing severe bleeding [110]. The clinical presentation depends on the bleeding site, chronicity, and severity. Acute upper gastrointestinal bleeding most commonly manifests as melena, and occasionally as hematemesis [77]; acute jejunal and ileal bleeding as melena [75]; and acute lower gastrointestinal bleeding as hematochezia, and rarely as melena [72,77]. Chronic bleeding presents with fecal occult blood or iron deficiency anemia. Patients bleeding from angiodysplasia tend to have a longer history of gastrointestinal bleeding than other patients owing to greater difficulty in diagnosis [74,123]. Patients sometimes present with prior gastrointestinal bleeding without an endoscopic

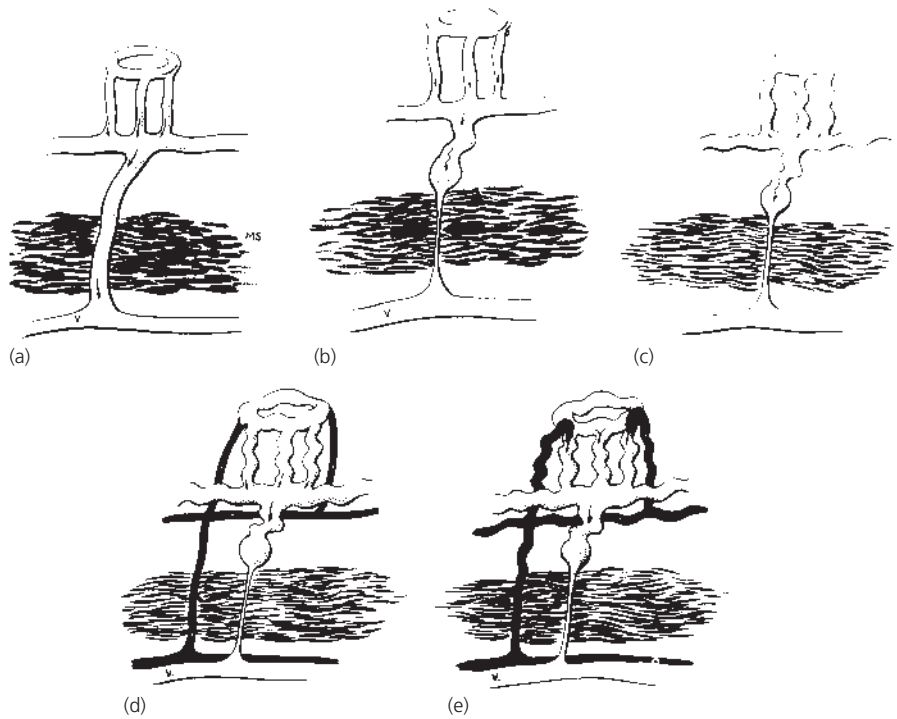


Figure 112.7 Diagrammatic illustration of a popular theory of angiodysplasia pathogenesis. **(a)** Normally patent distal veins within the muscular layer of gut drain proximal mucosal vessels, including the capillary ring. **(b)** These distal veins become partially obstructed due to muscular contractions or increased intralumenal pressure. **(c)** Chronic repeated intermittent obstruction of these distal veins results in proximal venous dilation and tortuosity. **(d)** With time, gut veins become progressively more dilated and tortuous, and the lesion propagates proximately. **(e)** Ultimately, the capillary ring becomes dilated, the precapillary sphincter becomes incompetent, and a small arteriovenous communication occurs through the ectasia. From Boley et al. [85], with permission from Elsevier.

diagnosis, although this phenomenon is becoming less frequent with increasing endoscopic lesion recognition [77,119].

Physical examination is unrevealing except for signs of gastrointestinal blood loss. Although, in the past, angiodysplasia was associated with severe gastrointestinal bleeding [82], angiodysplasia is increasingly associated with mild bleeding, probably owing to earlier identification and recognition of milder cases [77].

Diagnosis

Endoscopy is the diagnostic procedure of choice [83,123]. In a study of 80 patients, colonoscopy had a sensitivity of 81% when compared with angiography and pathological examination of the resected colon [124]. Angiodysplasia may be missed behind folds, at sharp turns, or when covered by stool. True angiodysplasia can be carelessly dismissed as endoscopic erosions or fresh blood [86]. Angiodysplasia may be inconspicuous in a patient with hypotension or severe anemia and may become obscured after meperidine administration for endoscopy [120]. Although endoscopy should generally be performed after correction of hypotension or anemia with fluid resuscitation or blood transfusion, this principle is particularly important when angiodysplasia is suspected. When colonic angiodysplasia is suspected, complete colonoscopy with careful cecal examination is important. Although endoscopy is generally superior to barium studies in the evaluation of any cause of chronic gastrointestinal bleeding, this is particularly true when angiodysplasia is suspected because angiodysplasia is mucosal and macular and not visualized with barium contrast, and barium studies preclude subsequent angiography [125].

Endoscopy is a fairly specific test provided strict diagnostic criteria are applied. At endoscopy, angiodysplasia appears as a dense macular and reticular network of vessels (vascular tuft), which is typically 2–8 mm wide and is intensely red because of the high oxygen content in erythrocytes within vessels supplied by arteries without intervening capillaries (Fig. 112.8; see also Fig. 112.5). Sometimes a prominent feeding artery or draining vein is observed [68,124,126]. Some endoscopists note a pale (anemic) mucosal halo surrounding angiodysplasia due to shunting of blood (vascular steal) from surrounding tissue by the low-resistance angiodysplastic shunt [100].

It is important to assess at endoscopy whether observed angiodysplasia is the source of blood loss. In two large studies, 30%–44% of patients with angiodysplasia had other significant gastrointestinal lesions, such as colonic polyps, colon cancer, inflammatory bowel disease, or peptic ulcer disease [70,126]. Bleeding is attributed to angiodysplasia when it is actively bleeding or has an overlying clot. Otherwise, the diagnosis is made only by exclusion of other causes.

The hot biopsy technique is preferred to reduce the risk of bleeding from endoscopic biopsy, but endoscopic biopsy is generally not recommended because of the low diagnostic yield and the increased risk of provoking hemorrhage [72,87]. Pathological examination of an endoscopic biopsy supports the endoscopic diagnosis in only about 40% of cases because endoscopic biopsies are mostly mucosal, and the most consistent and characteristic histological abnormalities are submucosal.

Small intestinal angiodysplasia is the most common diagnosis (30% of cases) of capsule endoscopy performed for persistent, obscure, gastrointestinal bleeding (i.e., no etiology determined by EGD or colonoscopy), whether overt or occult [127,128]. Endoscopic stigmata of recent hemorrhage are frequently present. The endoscopic appearance and criteria for angiodyslpasia are the same at capsule endoscopy as for conventional tube endoscopy. Capsule endoscopy, however, lacks the capability for endoscopic biopsy or therapy of detected lesions.

The angiographic hallmarks of angiodysplasia are:

- a vascular tuft or tangle resulting from the local mass of irregular vessels, best visualized in the arterial phase
- an early and intensely filling vein resulting from a direct arteriovenous connection without intervening capillaries
- persistent opacification beyond the normal venous phase (slowly emptying vein), possibly resulting from venous tortuosity [129] (Fig. 112.9).

About 60%–90% of patients have each of these angiographic signs [84,129]. Angiodysplasias bleed only intermittently, and extravasation of contrast material from angiodysplasia is detected in only about 10% of angiograms [79]. The advantages of angiography are that it can detect angiodysplasia of the small intestine as well as the large intestine, and can be performed during severe hemorrhage when colonoscopy is ineffective [72]. Small intestinal angiodysplasia can, however, be missed at angiography because of misidentification as normal vascular arcades [130]. Helical CT angiography may be a relatively noninvasive alternative diagnostic test for colonic angiodysplasia, as demonstrated in a study of 26 patients [131].

Angiodysplasia is seldom detected at laparotomy by visual inspection or manual palpation of the serosal surface of gut because of its small size and mucosal location [72]. Richardson and colleagues [84] correctly identified angiodysplasia in only one of 39 laparotomies. Thus, preoperative diagnosis is critical to avoid blind bowel resection for gastrointestinal bleeding. Intraoperative enteroscopy can help localize angiodysplasia to minimize the extent of small bowel resection [132].

Differential diagnosis

At endoscopy, angiodysplasia can be differentiated from other red lesions, such as gastritis, Barrett esophagus, inflammatory bowel disease, tumor, phosphosoda enema-induced lesions, and other vascular lesions. Phlebectasia and hemangiomas are blue or wine-colored submucosal vascular masses that collapse with endoscopic air insufflation. Kaposi sarcoma

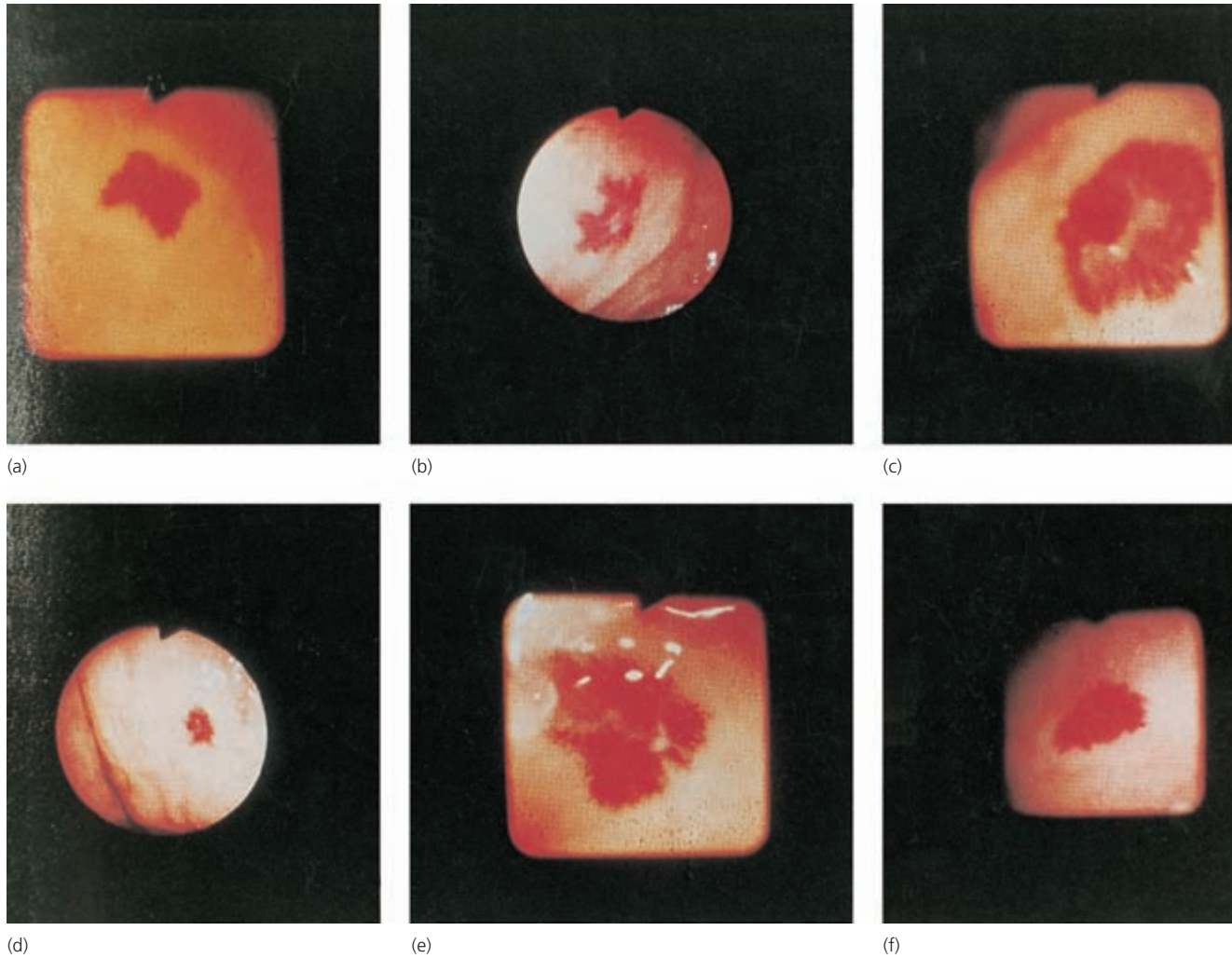


Figure 112.8 Spectrum of endoscopic appearance of upper gastrointestinal angiodysplasia. The characteristic endoscopic findings of angiodysplasia are intense erythema, well-demarcated margin, irregular stellate (star-like) margin, fine internal reticular (fern-like) structure, and macular mucosal location. Lesions range from 2 × 3 mm to 5 × 7 mm in size. All angiodysplasias are from the stomach, except for **(a)**, which is from the duodenum. From Clouse et al. [91], with permission from JAMA.

presents as a violaceous submucosal nodule or mass. A Dieulafoy lesion is typically elevated with one visible vessel. The characteristic antral location and arrangement of red lesions on longitudinal ridges distinguishes GAVE from angiodysplasia. Portal gastropathy is distinguished from angiodysplasia by a distinctive pale reticular border surrounding polygonal, intensely erythematous patches located in the proximal stomach.

The endoscopic appearance of gastrointestinal telangiectasias of HHT may be identical to that of angiodysplasia, except that the lesions of HHT tend to be greater in number, and occur in all bowel wall layers and all bowel segments. Patients with HHT may also have cutaneous telangiectasia, a positive family history, and a history of epistaxis. The diagnosis of scleroderma, CREST, or renal failure in association with angiodysplasia is based on the clinical manifestations of these conditions. The endoscopic findings in radiation colitis, aside from telangiectasia, include fibrosis, exudation, erosions, and mucosal friability, findings that are incompatible with angiodysplasia [133].

Red mucosal lesions resembling angiodysplasia when seen at endoscopy can be created iatrogenically by nasogastric aspiration or by endoscopic abrasion or suction. Lesions from nasogastric aspiration are round, colinear, equidistant, and relatively uniform in size, owing to the geometric arrangement of the nasogastric tube apertures, and are at the same stage of lesion evolution because of their simultaneous creation [134]. Lesions from endoscopic trauma are typically not seen during endoscopic intubation, but identified during endoscopic extubation. Compared with endoscopic trauma, angiodysplasia has a finely reticular (fern-like) internal structure owing to a vascular tuft, an irregular (stellate)

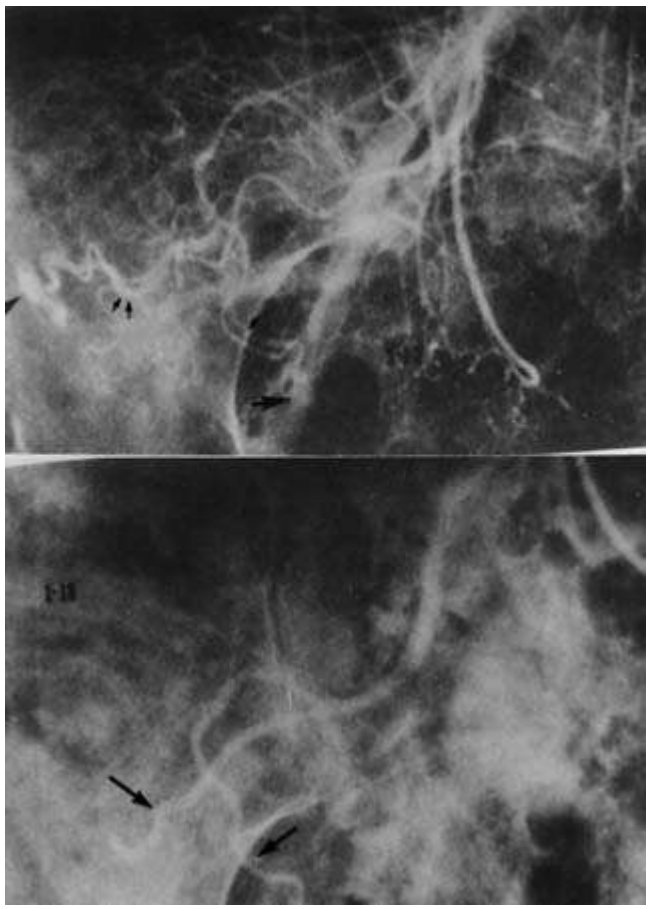


Figure 112.9 Angiographic findings in angiodysplasia. **(a)** Arterial phase shows a vascular tuft (large arrow) and two early filling veins (small arrows) that have filled with contrast before other veins. **(b)** Late phase reveals two densely opacified, dilated, and tortuous cecal veins (arrows) that still retain contrast after other veins have cleared. From Boley et al. [129], with permission from the Radiological Society of North America.

border as opposed to a round border with trauma, an abrupt lesion margin as opposed to an indistinct margin with trauma, and lesions lying flush (coplanar) with mucosa (see Figs 112.5 and 112.8). Thus, angiodysplasia is best diagnosed by detailed mucosal examination during endoscopic insertion with minimal use of suction [99]. The angiographic findings of angiodysplasia are occasionally mimicked by tumor or inflammatory bowel disease [72], but these other conditions are differentiated by endoscopy.

Natural history

Knowledge of the natural history of angiodysplasia provides a rational basis for deciding which angiodysplasias merit therapy based on their risk of future bleeding. Many angiodysplasias are asymptomatic and incidentally found at endoscopy, as, for example, during screening for colon cancer. Elderly patients with no history of bleeding frequently have small angiodysplasias detected in resected colonic specimens removed for unrelated colonic disease [85]. In a review of 41 patients with upper gastrointestinal angiodysplasias, 21 (51%) were incidental endoscopic findings [100]. In two series of 9 or 15 subjects with asymptomatic angiodysplasia, none experienced gastrointestinal bleeding during a mean follow-up of 2–3 years [67,126]. Thus small angiodysplasias discovered incidentaly at colonoscopy are unlikely to bleed in the near future.

Angiodysplasia is also associated with fecal occult blood, iron deficiency anemia, or gross bleeding. In a retrospective comparison of angiodysplasia and other causes of gastrointestinal bleeding, patients with angiodysplasia had a milder hospital course with fewer transfusions of packed erythrocytes, shorter hospitalizations, and lower mortality [77]. In three moderately large studies of patients with angiodysplasia presenting with bleeding or anemia, and treated conservatively by blood transfusions alone, 40%–50% rebled within 3 years [126,135,136].

Therapy

Endoscopic therapy

Therapy for angiodysplasia is stratified according to the likelihood of future bleeding as indicated by the medical history, findings on rectal examination, presence of iron deficiency anemia, and endoscopic findings. Asymptomatic angiodysplasias, incidentally found at endoscopy, are generally not endoscopically treated because of a low likelihood of subsequent bleeding [72,137]. This dictum holds particularly in the cecum and ascending colon because of the greater risks entailed by endoscopic therapy with the thinner colonic wall. Endoscopic therapy may, however, be considered when an angiodysplasia is exceptionally large or when prior bleeding from an angiodyplasia is suspected but undocumented. In other clinical situations, a graded approach to therapy is predicated on the likelihood of further bleeding from angiodysplasia. Actively bleeding angiodysplasia or angiodysplasia with recent prior bleeding, as evidenced by stigmata of recent hemorrhage, should be treated at endoscopy in the absence of contraindications, such as coagulopathy. Isolated angiodysplasias identified at endoscopy performed for recent acute bleeding that has stopped should likewise be treated if they are the suspected souce of the bleeding (e.g., no other gastrointestinal lesions identified) because of a high risk of future bleeding. Angiodysplasia associated with otherwise unexplained iron deficiency anemia may be treated at endoscopy depending on the clinical scenario. When multiple angiodysplasias are present, the endoscopist should concentrate on the largest and most accessible lesions, especially on lesions with endoscopic stigmata of recent hemorrhage.

At EGD, colonoscopy or enteroscopy, isolated, actively bleeding angiodysplasia is treated by endoscopic thermocoagulation, electrocoagulation, or photocoagulation [138]. Bleeding angiodysplasias are sometimes first injected with

alcohol or epinephrine (adrenaline) to slow the bleeding. These endoscopic therapies are relatively safe and highly successful at achieving short-term hemostasis. Gostout and colleagues [88] reported cessation of bleeding in 72 of 83 patients (87%) after laser photocoagulation during a mean follow-up period of 12 months. These therapies are also relatively effective in the long term: among 47 patients receiving laser photocoagulation, 61% had no significant gastrointestinal bleeding during 54 months of follow-up [139]. Laser therapy has a reportedly higher perforation rate of up to 4%, compared with other endoscopic therapies, because of the greater depth of tissue destruction [88,119,139]. The highest risk for perforation is in the thin-walled cecum, the most common location of angiodysplasia. Endoscopists generally prefer thermocoagulation or electrocoagulation over photocoagulation because of lower equipment costs and simpler technique, with equal efficacy.

Argon plasma coagulation (APC) is becoming the endoscopic therapy of choice because of its relatively low risks consequent to the shallow depth of tissue injury, and high efficacy given the superficial, mucosal location of angiodyslasia. Among 100 patients undergoing APC therapy of angiodysplasia for either overt gastrointestinal bleeding or iron deficiency anemia and fecal occult blood, 90 patients (90%) did not require any transfusions during a mean of 20 months of follow-up [140]. The two reported postprocedure complications were minor and without clinical sequelae. Other large studies have reported similarly high efficacy with few complications [141]. Double-balloon enteroscopy may permit APC therapy of jejunoileal lesions beyond the reach of an enteroscope [142]. Endoscopic band ligation has been reported to be effective for short-term hemostasis, but may have a higher long-term failure rate than APC [143].

Angiographic therapy

At angiography, active bleeding may be arrested by intraarterial vasopressin infusion or by transcatheter embolization. This may obviate the need for surgery or may slow bleeding enough to permit elective rather than emergent surgical resection. Vasopressin infusion has an initial success rate of about 50%–90%, with a roughly 33% chance of rebleeding [144,145]. Embolization produces a high rate of hemostasis [146,147]. Formerly, embolization had a high rate of intestinal infarction, but the frequency of this complication has decreased markedly as a result of improved catheter design and application of distal embolization. Angiography offers an alternative nonoperative therapy for jejunoileal angiodysplasia when conventional or double-balloon enteroscopy is unavailable.

Surgical therapy

Surgery is reserved for severe bleeding from well-characterized and localized lesions that are refractory to endoscopic or angiographic therapy. Endoscopy of both the upper and the lower gastrointestinal tract should be performed preoperatively, as well as capsule endoscopy, to exclude distant synchronous angiodysplasia or other lesions [80]. Right hemicolectomy is indicated for refractory bleeding from cecal angiodysplasia [99]. Even if angiodysplasia is identified only in the cecum, the entire right colon should be resected to ensure that no angiodysplasia are left behind. In right-sided angiodysplasia, none of the left colon should be removed, even if colonic diverticulosis is present, to avoid the increased morbidity and mortality of subtotal colectomy [99]. In two studies of 16 and 17 patients, this resection strategy yielded a significant but manageable long-term risk of postoperative rebleeding of 24%–37% during several years of follow-up [82,123]. Early rebleeding, which accounts for half or more of the cases, is usually the result of unappreciated lesions in the remaining colon and is usually successfully treated by subtotal colectomy [99].

Medical therapy

After reports of their use to control epistaxis from intranasal telangiectasia in HHT, estrogen and progesterone were used to control chronic recurrent bleeding from gastrointestinal angiodysplasia. These hormones are believed to promote vascular integrity [148]. Several small initial studies showed benefits from this therapy for angiodysplasia [149,150]. However, two studies of 64 [148] and 72 patients [135] reported no difference between patients treated with estrogen and progesterone versus untreated controls in the rate of bleeding cessation or in the number of units transfused.

Hormonal therapy for angiodysplasia, without HHT, is strictly reserved for patients with chronic bleeding from numerous angiodysplasias, for patients with jejunoileal angiodysplasias that are inaccessible to endoscopic therapy, and for patients who fail or cannot tolerate endoscopic or surgical therapy [148,151]. Hormonal therapy can cause feminization in male patients [152]. Other side effects include vaginal bleeding, fluid retention, thromboembolism, and congestive heart failure [153].

Hereditary hemorrhagic telangiectasia

The term telangiectasia denotes vascular ectasia, which is found as part of a systemic disease or syndrome, whereas angiodysplasia is nonsyndromic [4]. Gastrointestinal telangiectasia, which appears nearly identical to angiodysplasia, is associated with HHT, scleroderma, CREST syndrome, and possibly Turner syndrome. The latter two were mentioned above under "Angiodysplasia."

HHT is a genetic vascular disorder caused by mutation of the *ENG* (endoglin) gene (type I HHT) or of the *ACVRL1* gene (type II HHT) [154]. These mutations impair blood vessel

growth and repair, resulting in irregular, tortuous blood spaces lined by a single thin layer of endothelial cells [155]. These mutations produce a syndrome of multiple orocutaneous telangiectasias, especially on the face, lips, tongue, oral mucosa, and hands, together with multiple mucosal telangiectasias, especially in the nose and gastrointestinal tract (see Chapter 110). The cutaneous lesions are usually a minor cosmetic problem, whereas the nasal and gastrointestinal lesions frequently bleed significantly and repeatedly [156]. This bleeding tendency is explained by the thin and fragile vascular wall that lacks an elastic lamina or muscle layer, vulnerability to trauma because of vessel superficiality, and perhaps intralesional venous hypertension due to arterial shunting of blood.

The disease prevalence is about 1 per 10 000 population [157], and the gender distribution is equal [158,159]. More than 80% of patients experience epistaxis, spontaneously or after minimal trauma, which typically begins during the teenage years [157]; some 25% of patients with epistaxis require blood transfusions [153]. About 15% of patients have pulmonary arteriovenous malformations or fistulae, which can cause hypoxemia or hemoptysis [160], and about 10% have cerebrovascular malformations, which can cause a hemorrhagic stroke or brain abscess [161]. Symptomatic hepatic involvement is uncommon but important in that it can produce high-output heart failure, portal hypertension, biliary disease, or liver failure [162,163].

About 25% of patients experience clinically significant gastrointestinal bleeding, which typically begins during middle age [164,165]. This risk increases with age as patients acquire more gastrointestinal telangiectasia [166]. Chronic blood loss may result in iron deficiency anemia, whereas acute blood loss may cause hypovolemia and hypotension. Upper gastrointestinal bleeding is more common than lower gastrointestinal bleeding because upper gastrointestinal telangiectasias are more common [165]. Gastrointestinal bleeding typically manifests as melena and occasionally as hematemesis. The differential diagnosis of melena in these patients includes swallowed blood from epistaxis. The gastrointestinal bleeding is typically painless, chronic, episodic, and progressive [159]. Of 25 patients with gastrointestinal bleeding, half required blood transfusions, and one-fourth required transfusion of more than six units [164].

The diagnosis is straightforward in patients with the triad of telangiectasia, recurrent epistaxis, and a compatible family history [167]. Patients with sporadic angiodysplasia are differentiated from patients with HHT by clinical presentation in old age, a paucity of lesions, a negative family history, and exclusively gastrointestinal involvement without epistaxis. Cutaneous telangiectasias, unlike spider nevi, only partly blanch by diascopy because of vessel tortuosity. The site and source of gastrointestinal bleeding are diagnosed by endoscopy, with EGD for upper gastrointestinal bleeding, colonoscopy for lower gastrointestinal bleeding, and, increasingly, capsule endoscopy for small intestinal lesions [168]. The endoscopic appearance of telangiectasia resembles that of nonsyndromic angiodysplasia or of cutaneous telangiectasia occurring in this syndrome. Lesions tend to be widespread throughout the gastrointestinal tract. As for angiodysplasia, other causes of gastrointestinal bleeding should be excluded at endoscopy [159,165], barium contrast radiography should not be used for diagnosis [159], and angiography may be useful to diagnose the extent of bowel telangiectasia [169].

Patients generally require iron supplementation to replete iron stores. The endoscopic therapy resembles that for nonsyndromic angiodysplasia, using thermocoagulation, electrocoagulation, photocoagulation, or APC [88,139], but endoscopic therapy is complicated by lesion multiplicity, dissemination, and progression. Isolated actively bleeding telangiectasias are usually successfully treated, but patients often rebleed from other untreated gastrointestinal telangiectasias and therefore require multiple endoscopic sessions [170]. Segmental bowel resection is reserved for active, severe, and well-localized bleeding refractory to medical or endoscopic therapy because of the tendency to rebleed subsequently from telangiectasias at other sites [123,126,158]. When available, angiographic embolization can obviate the need for surgery.

In theory, systemic or generalized therapy should be better than local therapy because of the typically large number and widespread extent of the telangiectasias. A few small studies have reported a markedly decreased incidence in chronic gastrointestinal bleeding after estrogen and progesterone therapy [150,171]. Estrogen–progesterone therapy should be considered before performing gastrointestinal surgery for chronic bleeding, but decisions concerning this therapy are controversial [166].

Gastric antral vascular ectasia

Clinical presentation

This condition is relatively uncommon. It was reported in only three cases among 10 000 consecutive EGDs in 1984 [172], but is being increasingly recognized. The incidence may be as high as one per several hundred EGDs if early, but characteristic, endoscopic findings are recognized (M.S. Cappell, unpublished). The mean age at presentation is 70 years (range 50–90 years) [173–175], with a female-to-male ratio of about 4:1 [172,174–177]. Patients typically present with iron deficiency anemia from chronic occult gastrointestinal bleeding [172,175,178], but occasionally have melena from acute bleeding [173,174]. Hematemesis is unusual. The occult bleeding and anemia may be longstanding because of delayed diagnosis [174], and patients have often had multiple previous blood transfusions. The lesion is painless, but physical examination reveals pallor and fecal occult blood [175]. Patients with early endoscopic findings

tend to have less severe and less frequent symptoms and laboratory abnormalities (M.S. Cappell, unpublished).

Diagnosis

Endoscopy reveals multiple parallel prominent longitudinal folds that traverse the antrum and converge to the pyloric sphincter. The folds contain intensely erythematous linear streaks at their apices [172] (Fig. 112.10). Occasionally the streaks extend into the proximal stomach [179,180]. The term *watermelon stomach* derives from the resemblance of these erythematous linear streaks to the stripes on a watermelon rind. These lesions should not be confused with focal antral gastritis [181,182]. These erythematous lesions differ from those in antral gastritis by virtue of their location on folds radiating to the pylorus, blanching on pressure, and sharp lesion demarcation [177]. Capsule endoscopy may also reveal this gastric lesion [182]. Despite lesion vascularity, endoscopic biopsy appears safe in that it can cause slight, but not severe, bleeding [172,183]. Histological analysis of endoscopic biopsy samples taken from the apices of the longitudinal folds reveals hypertrophied mucosa, dilated and tortuous mucosal capillaries that are often occluded by bland fibrin thrombi, dilated and tortuous submucosal veins, and a lack of inflammatory infiltration [172,177,183,184]. Endosonography may demonstrate dilated ectatic vessels in antral mucosa and submucosa, whereas focal antral mural thickening may be revealed by computed tomography. Double-contrast upper gastrointestinal series may reveal prominent, scalloped antral folds radiating to the pyloric sphincter [185], and angiography may reveal mild antral hypervascularity [186]. However, these radiological procedures are seldom necessary because the endoscopic appearance and histological findings are generally diagnostic.

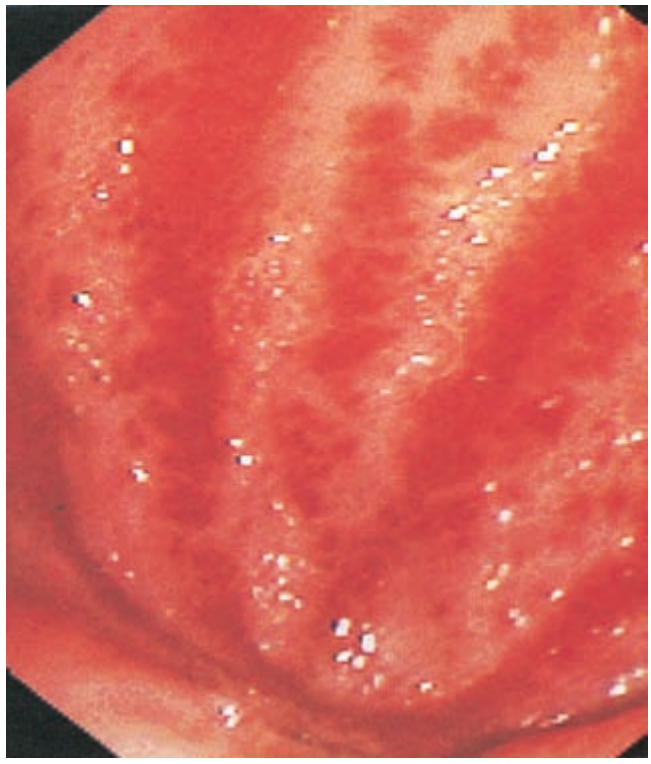


Figure 112.10 Videophotograph showing the characteristic endoscopic finding in gastric antral vascular ectasia of linear, intensely erythematous lesions at the apices of longitudinal antral folds radiating to the pylorus. The alternative lesion name of *watermelon stomach* derives from the resemblance of these erythematous linear streaks to the stripes on a watermelon rind. From Clouse RE. Vascular ectasias, tumors, and malformations. In: Yamada et al. (eds). Atlas of Gastroenterology, 2nd edn. Philadelphia: Lippincott Williams & Wilkins, 1999:550.

Etiology

The etiology is unknown. The disease is associated with atrophic gastritis [176], renal failure [187], bone marrow transplantation [188], and scleroderma [180,189]. There is also an association between GAVE and cirrhosis [190] but not portal hypertension [191–193]. These associations do not appear to provide a clue to the etiology. A trophic etiology has been proposed [194] because of the finding of elevated blood or mucosal tissue concentration of gastrin [195], prostaglandin E_2 [196], or 5-hydroxytryptamine (serotonin) [197], but these abnormalities have not been proven [190].

Alternatively, the lesion may arise from repeated antral mucosal trauma resulting from pyloric prolapse [172]. The lesion is generally restricted to the antrum, the only gastric region subject to peristaltic contractions, and generally occurs on longitudinal folds, the antral area subjected to the most trauma during peristaltic contractions [175]. Affected antral mucosa appears to be abnormally mobile and loosely attached to the underlying muscularis propria, as expected from repeated prolapse [172]. The vascular ectasia resembles that seen in other traumatic gastrointestinal lesions, such as the solitary rectal ulcer syndrome, prolapsed hemorrhoids, or intestinal intussusception [175,198,199]. Cirrhotic patients might develop the lesion as a consequence of antral dysmotility [200].

Therapy

Patients should avoid gastrotoxic or antiplatelet medications that could exacerbate the bleeding, and should receive transfusion for acute gastrointestinal bleeding, and iron replacement for chronic gastrointestinal bleeding. Patients with renal failure may require erythropoietin. Pharmacological therapy, employing variable agents, has had variable and inconsistent results [177,201]. Histamine H_2 receptor antagonists and proton pump inhibitors are ineffective because this lesion is not acid-related and patients often have hypochlorhydria from atrophic gastritis [181]. Endoscopic therapy is the initial treatment. Endoscopic ablation requires multiple endoscopic sessions because of lesion size. Lesion eradication is endoscopically recognized by complete blanching. Laser therapy

was commonly used because it requires fewer endoscopic sessions than most other modalities [174,180,202,203], but the heater probe has also been successfully used [175]. From 87% to 100% of patients have stable hematocrits without transfusions for up to 2 years after endoscopic therapy [174,180,204]. Endoscopic complications, such as perforation, stenosis, ulceration, and hemorrhage, are infrequent [173,174]. Argon plasma coagulation may become the preferred endoscopic therapy because of the diffuse nature and superficial location of the lesion. This modality entails a low risk of endoscopic complications because of the shallow depth of tissue injury [141]. Analysis of the combined results of four studies shows that 50 of 55 transfusion-dependent patients required no transfusions after APC ablation therapy, during a mean follow-up of approximately 2 years [191,205–207]. Patients should receive acid suppressive therapy after endoscopic ablation to prevent iatrogenic ulcers.

Antrectomy removes the lesion and nearly always cures the disease [172,176], but is reserved for endoscopic therapy failures because of significant surgical morbidity and a small, but significant, mortality of about 5% [177,202]. Among 45 patients undergoing surgery, only two required transfusions during a mean follow-up of 2 years; both bled from other gastrointestinal lesions [177]. Transjugular intrahepatic portosystemic shunt does not stop bleeding from GAVE in cirrhotic patients [208].

Hemangiomas

Capillary and cavernous hemangiomas

Intestinal hemangiomas are rare, with an incidence of about 1 per 15 000 patients [209]. About half of these hemangiomas are associated with cutaneous hemangiomas, most commonly in the blue rubber bleb nevus syndrome and rarely in Klippel–Trenaunay syndrome, as described later [210]. Intestinal hemangiomas are either capillary, cavernous, or mixed. *Capillary hemangiomas* consist of small (capillary-sized), thin-walled, closely packed vessels; *cavernous hemangiomas* consist of large, dilated, blood-filled, thin-walled vessels; and *mixed hemangiomas* contain both types of vascular lesions [211,212]. Capillary hemangiomas tend to be small and well circumscribed, whereas cavernous hemangiomas tend to be medium-to-large and poorly circumscribed. In both types of hemangiomas, vessels are lined by well-differentiated, flattened, and hyperplastic endothelial cells. Capillary hemangiomas tend to be located in the perianal skin, whereas cavernous hemangiomas tend to be located in the rectum and distal colon [1,213].

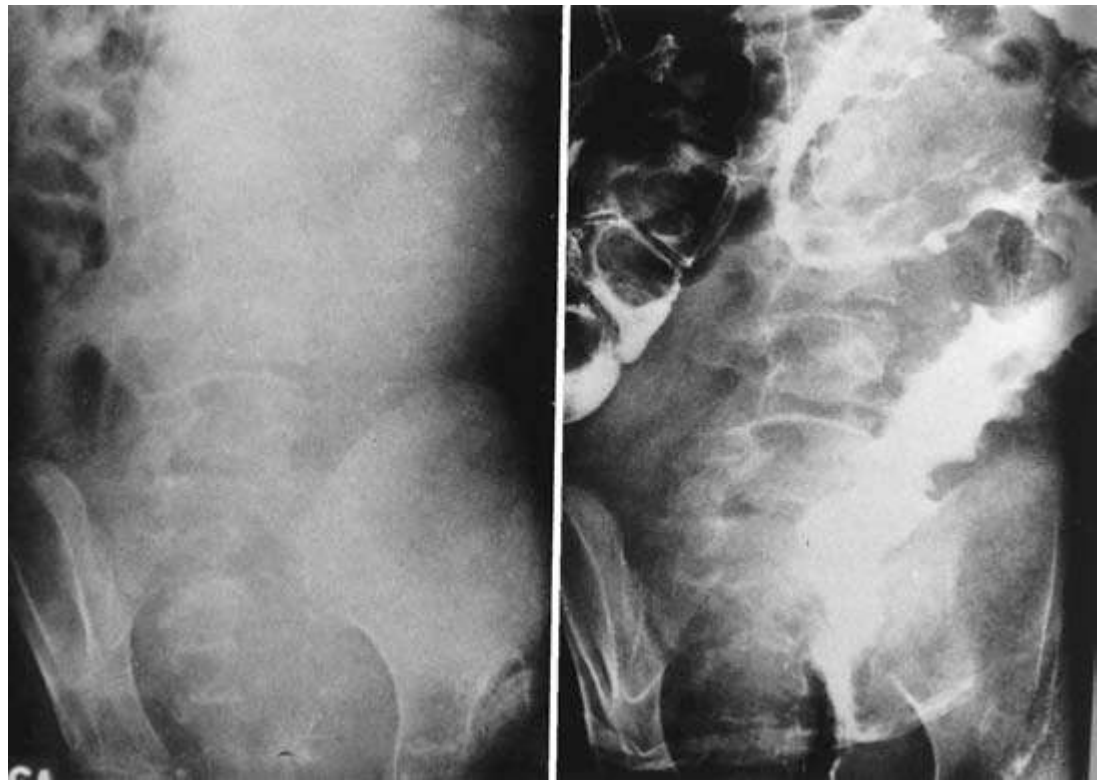
Cavernous hemangiomas are clinically more significant owing to their propensity to bleed because of their frequent large size, large blood volume, and consumption of coagulation factors and platelets [214,215]. Multiple lesions often occur within the gastrointestinal tract [216].

Colonic cavernous lesions may be asymptomatic (10%), but commonly produce acute gastrointestinal bleeding that manifests clinically as hematochezia or melena (72%) [213,217,218], or chronic gastrointestinal bleeding that manifests clinically as iron deficiency anemia [219–222]. Bleeding is typically episodic, recurrent, and progressive, and usually begins in childhood or young adulthood [223]. Bleeding lesions are generally large and cavernous. Gastrointestinal lesions can cause gastrointestinal obstruction from lumenal encroachment or intestinal intussusception [224,225]. Rectal hemangiomas may produce tenesmus and rectal urgency [212,226]. Hemangiomas occasionally extend into adjacent organs or occur simultaneously in other organs, where they clinically manifest as hematuria, epistaxis, hemoptysis, or menorrhagia, according to their location [221,223,227].

Abdominal radiographs often demonstrate phleboliths from thrombosis and calcification within hemangiomas [1,228] (Fig. 112.11a). Barium contrast studies typically reveal multiple polypoid filling defects in the stomach, small bowel, or colon, but occasionally show an annular intestinal constriction [217,227,229–231] (Fig. 112.11b). Computed tomography may demonstrate a large, thickened mesentery containing large vacuoles next to an abnormal intestinal segment with a thickened wall that contains phleboliths [1]. Magnetic resonance imaging may demonstrate intensely bright signals from these highly vascular lesions [232]. Frequently, selective angiography poorly delineates these venous lesions as a result of vascular thrombosis [233].

Esophagogastroduodenoscopy and colonoscopy have high diagnostic sensitivity in patients with upper and lower gastrointestinal lesions, respectively. Small intestinal lesions are being increasingly diagnosed by caspsule endoscopy, or even double-balloon enteroscopy [234]. The endoscopic diagnosis is straighforward in patients with the characteristic dermatological syndromes (see below), but in the absence of these the lesion can be misdiagnosed endoscopically. At endoscopy, intestinal hemangiomas appear as violet-blue, sessile, compressible, polypoid lesions [220] (Fig. 112.12a,b). It is often difficult to determine at endoscopy which lesion bled because of lesion multiplicity and multifocality [230]. Endoscopic biopsy entails a risk of profuse hemorrhage [1]. Pathological examination reveals findings typical of a capillary or cavernous hemangioma: numerous ectatic, dilated, blood-filled spaces lined by a thin layer of cuboidal endothelium and surrounded by scant fibrous stroma with occasional smooth muscle cells [221]. Lesions lack cellular atypia or dysplasia and do not undergo malignant transformation.

The differential diagnosis includes colon cancer, adenomatous polyps, hemorrhoids, and ulcerative colitis [235]. Colonic hemangiomas are distinguished from colonic polyps at endoscopy by their bluish color, typical submucosal location, compressibility, and poorly defined margins [236]. Rectal hemangiomas are distinguished from internal hemorrhoids



(a) (b)

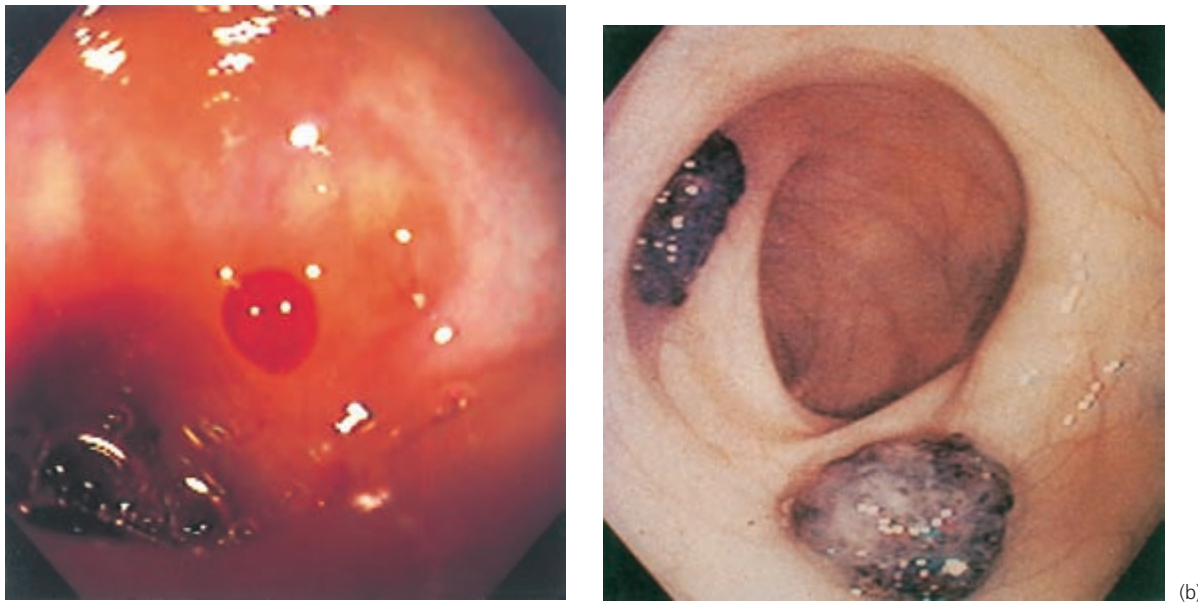
Figure 112.11 Radiographic appearances of intestinal hemangiomas. **(a)** Abdominal radiograph of a 16-year-old white male patient with an extensive distal colonic cavernous hemangioma demonstrates a cluster of radiopaque phleboliths partly outlining the distal colonic wall. **(b)** Barium enema demonstrates irregular narrowing in the rectosigmoid and "thumbprinting" in the sigmoid and descending colon caused by intramural hemangiomatous masses. Although the rectosigmoid narrowing resembles an apple-core lesion found in colon cancer, the radiographic findings of phleboliths and intramural masses point to the diagnosis of cavernous hemangioma. From Ghahremani et al. [247], with permission from the Radiological Society of North America.

by a nodular, as opposed to serpiginous, shape; submucosal versus superficial location; and extension beyond the anorectum. Colonic hemangiomas are differentiated from pseudopolyps of ulcerative colitis by the presence of a bluish color, submucosal location, and lesion compressibility, and by the absence of mucosal granularity, blunting of the vascular pattern, and continuous involvement on colonoscopy [210]. The correct diagnosis is crucial for proper management. Polypectomy may be inadvisable because of the risk of postpolypectomy hemorrhage from a cavernous hemangioma. Hemorrhoidectomy is ineffective therapy for bleeding from rectal hemangiomas [235].

Patients typically require frequent blood transfusions for chronic gastrointestinal bleeding. Surgery for gastrointestinal bleeding is limited by the tendency for lesions to be multiple and widespread and by frequent development of new lesions [237]. Segmental resection is reserved for severe well-localized bleeding [227,229]. Endoscopic hemostasis by photocoagulation [218,238], band ligation [239], or endoscopic polypectomy [237,239] has been reported without complications.

Blue rubber bleb nevus syndrome

Although rare, this syndrome should be suspected when characteristic dermatological lesions occur in conjunction with gastrointestinal bleeding (see Chapter 110). Patients present with multiple violet-blue, slightly raised, well-circumscribed, and elastic cutaneous hemangiomas resembling a rubber nipple [240], and ranging from 0.5 to 5 cm in diameter, together with multiple gastrointestinal hemangiomas, which are commonly located from the stomach through the rectum and rarely in the esophagus or oropharynx [219,224,229] (Fig. 112.12a,b). The cutaneous lesion has a wrinkled surface and can be emptied of blood by manual pressure, leaving a wrinkled blue or white sac that slowly refills when the pressure is released [220,223]. Lesions usually begin in early childhood, and they tend to increase progressively in number and sometimes increase in size with age [227,230,239]. Most cases are sporadic, but several families have been reported with genetic transmission in an autosomal dominant pattern [227]. A candidate gene has been identified on chromosome 9 [241].



(a) (b)

Figure 112.12 Endoscopic appearance of colonic hemangiomas. **(a)** Videophotograph of a 3-mm colonic capillary hemangioma adjacent to a mid-sigmoid diverticulum. Courtesy of T.G. Tietjen, MD, Petoskey, Michigan. **(b)** Videophotograph from the colon showing the characteristic endoscopic appearance of a cavernous hemangioma as a bluish, round, smooth, and well-circumscribed sessile polypoid lesion. From Bak et al. [239], with permission from Elsevier.

Only aspects unique to these gastrointestinal lesions are discussed here; the clinical presentation, diagnosis, and therapy were mentioned in the previous section "Capillary and cavernous hemangiomas." Significant gastrointestinal bleeding in a patient with this syndrome should be investigated by capsule endoscopy if EGD and colonoscopy are unrevealing [242]. In a clinical series of 10 patients, surgical removal by wedge resection and polypectomy of all gastrointestinal lesions was feasible and highly effective in conjunction with intraoperative enteroscopy [243]. On average, however, 137 gastrointestinal lesions were surgically resected per patient, with a mean operative time of 14 h. Nine of the 10 operated patients did not require transfusions during a mean follow-up of 5 years. Cutaneous lesions seldom require treatment because they rarely bleed and never become malignant [229].

Intestinal hemangiomas in Klippel–Trenaunay syndrome

Klippel–Trenaunay syndrome is characterized by port-wine cutaneous hemangiomas, bony and soft tissue hypertrophy, superficial varicose veins, and abnormal – atretic, hypoplastic, or occluded – deep veins, usually affecting one lower extremity but occasionally affecting both lower extremities or one or both upper extremities [244,245] (Fig. 112.13). A family history of similar lesions is usually lacking [244]. In *Parkes Weber syndrome*, patients additionally have arteriovenous fistulae.

This syndrome is associated with extensive infiltrative cavernous hemangiomas of the distal colon [246–249] and, rarely, the small intestine [250]. Patients present with chronic intermittent hematochezia and iron deficiency anemia in

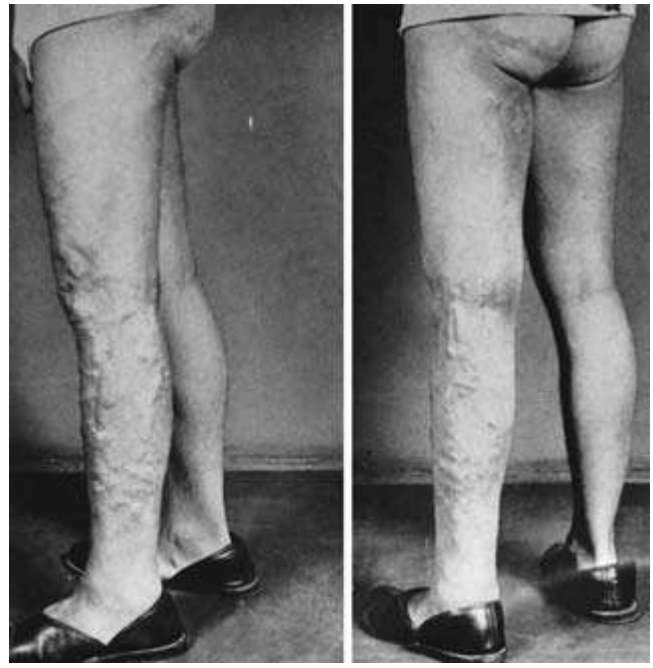


Figure 112.13 Leg lesions in Klippel–Trenaunay syndrome. A 23-year-old man has the characteristic physical findings in one lower extremity of superficial varicose veins (see left calf), an increased girth (compare size of ankles and calves in the two legs), an increased length, and a port-wine cutaneous hemangioma (see left calf). From Lindenauer [244], with permission from Lippincott Williams & Wilkins.

childhood or young adulthood [247,251]. Thrombocytopenia due to platelet sequestration and consumption may contribute to the bleeding [248], as may mesenteric venous hypertension caused by congenital obstruction of lower extremity veins and consequent overload of the internal iliac veins [249]. The rate of rectal bleeding associated with this syndrome is poorly characterized [247], but is reported as 1%–13% [249,252]. Diagnostic features found on plain abdominal radiographs, barium enema, colonoscopy, computed tomography, and selective inferior mesenteric angiography are essentially the same as those for multiple cavernous hemangiomas (see "Capillary and cavernous hemangiomas" above) [246,247,250]. Abdominoperineal resection of the colonic hemangioma is the definitive therapy. Endoscopic hemostasis may provide a nonsurgical alternative therapy in the future.

Disseminated hemangiomatosis

Rarely, patients present with more than 50 intestinal hemangiomas, which range in size from 2 to 20 mm, as part of a syndrome of disseminated hemangiomatosis involving three or more organ systems [253]. Patients present at birth with numerous cutaneous hemangiomas. Untreated infants have a 60% mortality from massive gastrointestinal bleeding, hemorrhagic stroke, or high-output cardiac failure due to arteriovenous shunting [254,255]. Corticosteroids and interferon-α have been successfully used to promote lesion regression [254,255].

Phlebectasias

Intestinal phlebectasias are venous varicosities, without portal hypertension, consisting of a markedly dilated tortuous vein, with a normal vascular wall and scant connective tissue stroma [256]. Endothelial cells within the vessel appear normal. Phlebectasias are generally classified as multiple, small hemangiomas, but this classification is controversial [212,214]. They occur primarily in elderly men [257]. Phlebectasias may also occur in the oral cavity, mostly at the base of the tongue, where they are called caviar spots (varices) or sublingual phlebectasia, and in the genitalia, where they are called Fordyce lesions or scrotal phlebectasia [256]. These orocutaneous signs are clues to the presence of intestinal lesions in elderly men with gastrointestinal bleeding.

Intestinal lesions are characteristically dark bluish-black, ranging from several millimeters to 10 mm in size, soft and compressible, blanch with pressure, are multiple, and located in the submucosa [257]. Although usually asymptomatic and incidental, they occasionally produce chronic occult or acute gross intestinal bleeding [256–258]. Because of their location, small intestinal phlebectasias are diagnosed by enteroscopy [258]. Angiography can occasionally demonstrate the site of bleeding by contrast extravasation but does not delineate these lesions [256]. Surgery is the only definitive therapy, but is reserved for severe ongoing bleeding because of the multiplicity and extent of the small bowel lesions.

Angiosarcomas

Angiosarcomas are rare, highly vascular malignancies derived from endothelial cells, representing less than 1% of sarcomas. They are divided into hemangiosarcomas and lymphangiosarcomas [259]. They commonly arise in cutaneous and subcutaneous tissue, and rarely in the gut [209]. Chronic lymphedema and prior local radiotherapy are risk factors for cutaneous angiosarcomas [260], and exposure to thorium dioxide (Thorotrast) during angiography, arsenic in insecticides, or polyvinyl chloride in manufacturing plastics are risk factors for hepatic angiosarcomas [261]. Intestinal lesions are associated with prior abdominopelvic radiotherapy [262].

Grossly, lesions appear as a hemorrhagic and often ulcerated mass. Histologically, angiosarcomas contain numerous interconnecting vascular channels (vasoformative structures), irregular in size and shape, that contain either atypical cells with sparse mitotic activity or sheets of pleomorphic cells with prominent mitotic activity. Poorly differentiated angiosarcomas are distinguished immunohistochemically from poorly differentiated adenocarcinomas by immunoreactivity for vimentin, and endothelial markers including factor VIII-related antigen, CD34, and CD31, and by immunonegativity for endothelial cell markers, such as S-100 [263,264].

Patients are generally elderly [265,266]. Lesions have been reported in the esophagus, stomach, small intestine, colon, and rectum [209,265–267]. Half of intestinal lesions are primary and half are metastatic [263]. Symptoms are similar to those with cavernous hemangiomas [266,267]. Patients commonly present with gastrointestinal bleeding, anemia, or abdominal pain, occasionally present with intestinal obstruction, and rarely present with intestinal perforation [268]. Endoscopy may demonstrate an ulcerated mass or tight asymmetrical stricture. Surgical resection is recommended. Angiosarcomas are often multifocal at diagnosis and typically biologically aggressive [265,266]. The prognosis appears to be poor as a result of early metastasis [260].

Hemangiopericytomas

Hemangiopericytomas are uncommon, richly vascular neoplasms composed of pericytes, the mesenchymal cells normally apposed to endothelial cells. The stomach is the most commonly involved gastrointestinal organ [269,270], but the small intestine [271–275], rectum [276], and even mesentery or retroperitoneum [277] can be involved. The neoplasm most commonly occurs in early middle age. Gastrointestinal tumors can present with gastrointestinal bleeding as a result of this highly vascular tumor eroding into the lumen, or with constipation as a consequence of lumenal impingement by the mural mass [278]. Other symptoms include abdominal pain, abdominal distention, and nausea and vomiting

[269,274,275]. Intestinal intussusception occasionally occurs [271–274]. An abdominal mass may be palpable because of the tumor's bulk [277]. Large tumors are rarely associated with hypoglycemia [279].

Barium studies typically reveal a smoothly contoured polypoid lesion [269,270]. Angiography demonstrates a richly vascular mass with a diffuse capillary blush [279]. Computed tomography may demonstrate multiple areas of low attenuation from necrosis, hemorrhage, or cystic degeneration within the tumor. Endoscopy may reveal a solitary, circumscribed, submucosal mass or polyp covered by normal or ulcerated mucosa. Pathological examination of endoscopic biopsy specimens reveals tightly packed, spindle-shaped, neoplastic pericytes surrounding intercommunicating vascular channels of variable caliber and shape [274,280]. The vascular channels usually resemble capillaries [281].

Most tumors are benign and slow growing [269,274,279], and many are bulky. Malignancy is characterized histologically by increased cellularity, prominent mitotic activity, hyperchromatism, cellular pleomorphism, and foci of necrosis or hemorrhage [279,282]. Wide local surgical resection is the primary therapy [282].

Hemangioendotheliomas

Hemangioendotheliomas are rare, borderline malignancies with a histological appearance between benign hemangiomas and malignant angiosarcomas. Hemangioendotheliomas display more cellularity and mitotic activity than hemangiomas, but less mitotic activity and cellular atypia than angiosarcomas. The most important subtype is epithelioid hemangioendothelioma, in which neoplastic endothelial cells focally line small extracellular vascular spaces or contain small intracellular spaces or vacuoles [283]. The tumor often arises from a vein. Immunohistochemistry shows the presence of at least one endothelial marker among factor VIII-related antigen, CD34, or CD31 [284]. This vascular tumor most commonly presents as a gastrointestinal mass with gastrointestinal bleeding, but may present with abdominal pain, intestinal obstruction, or intestinal perforation [285,286]. Hemangioendotheliomas are sometimes locally invasive but rarely metastasize. Wide local resection is the recommended therapy.

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I MISCELLANEOUS

113 Intestinal ischemia

Julián Panés, Josep M. Piqué

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Intestinal ischemia is not rare, accounting for 0.1% of hospital admissions [1]. The increase in the incidence of these disorders in recent years is secondary to multiple factors such as heightened awareness for the diagnoses, the advanced mean age of the population, and the increasing number of critically ill patients. Despite advances in knowledge of pathophysiology, laboratory diagnosis, and imaging techniques, acute intestinal ischemia is still associated with mortality rates of more than 60% and continues to be a challenging diagnostic problem [2]. *The key to a better outcome, and the main problem in clinical practice, is early diagnosis.* The clinical setting of acute intestinal ischemia is determined by the combination of the need for rapid and aggressive diagnostic and therapeutic interventions in an often elderly and multimorbid patient, thus making clinical management a challenging task. The objectives of this chapter are to provide knowledge of the basic anatomic and pathophysiological mechanisms underlying intestinal ischemic damage, the guidelines for identification of patients who require prompt and aggressive evaluation, and delineation of the optimal diagnostic workup and type of therapy for each patient.

Anatomy of the intestinal circulation

Generally, the involved segment of ischemic intestine has a linear correspondence to the location and extent of vascular occlusion. Knowledge of the vascular anatomy is therefore crucial for the understanding of the pathophysiology, clinical presentation, and therapy of intestinal ischemia.

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Celiac artery

The celiac artery arises from the abdominal aorta at about the level of the T12 or L1 vertebral body. It usually courses anteriorly and slightly inferiorly 1–2 cm before branching into the left gastric, common hepatic, and splenic arteries. The left gastric artery supplies the stomach and provides small branches to the esophagus that anastomose with aortic esophageal branches, and small branches that anastomose with the right gastric artery, a branch of the hepatic artery. The common hepatic artery divides into the hepatic artery, which supplies the liver; the right gastric artery, which supplies the stomach along the lesser curvature; and the gastroduodenal artery, which divides into right gastroepiploic and superior pancreaticoduodenal arteries to supply the antrum, duodenum, and pancreas. The splenic artery supplies the spleen and body and tail of the pancreas [3].

Superior mesenteric artery

The superior mesenteric artery (SMA) arises from the aorta approximately 1 cm below the celiac artery, usually at the level of L1. It courses inferiorly and toward the right to terminate at the level of the cecum as the ileocolic artery (Fig. 113.1a). The inferior pancreaticoduodenal artery is one of the first branches of the SMA; it supplies the pancreatic head, uncinate process, and descending duodenum, and anastomoses with the corresponding superior branches from the celiac axis. Generally there are more SMA branches to the distal small bowel than to the more proximal portions, thus providing greater potential for distal anastomotic communication. The middle colic artery arises from the proximal SMA to supply the transverse colon and communicates with branches of the IMA. The right colic artery may arise as a common trunk with the middle colic artery or have a separate origin from the SMA; it supplies the distal ascending colon and right colonic (hepatic) flexure. The ileocolic artery is the terminal branch of the SMA and supplies the distal ileum, cecum, and ascending colon.

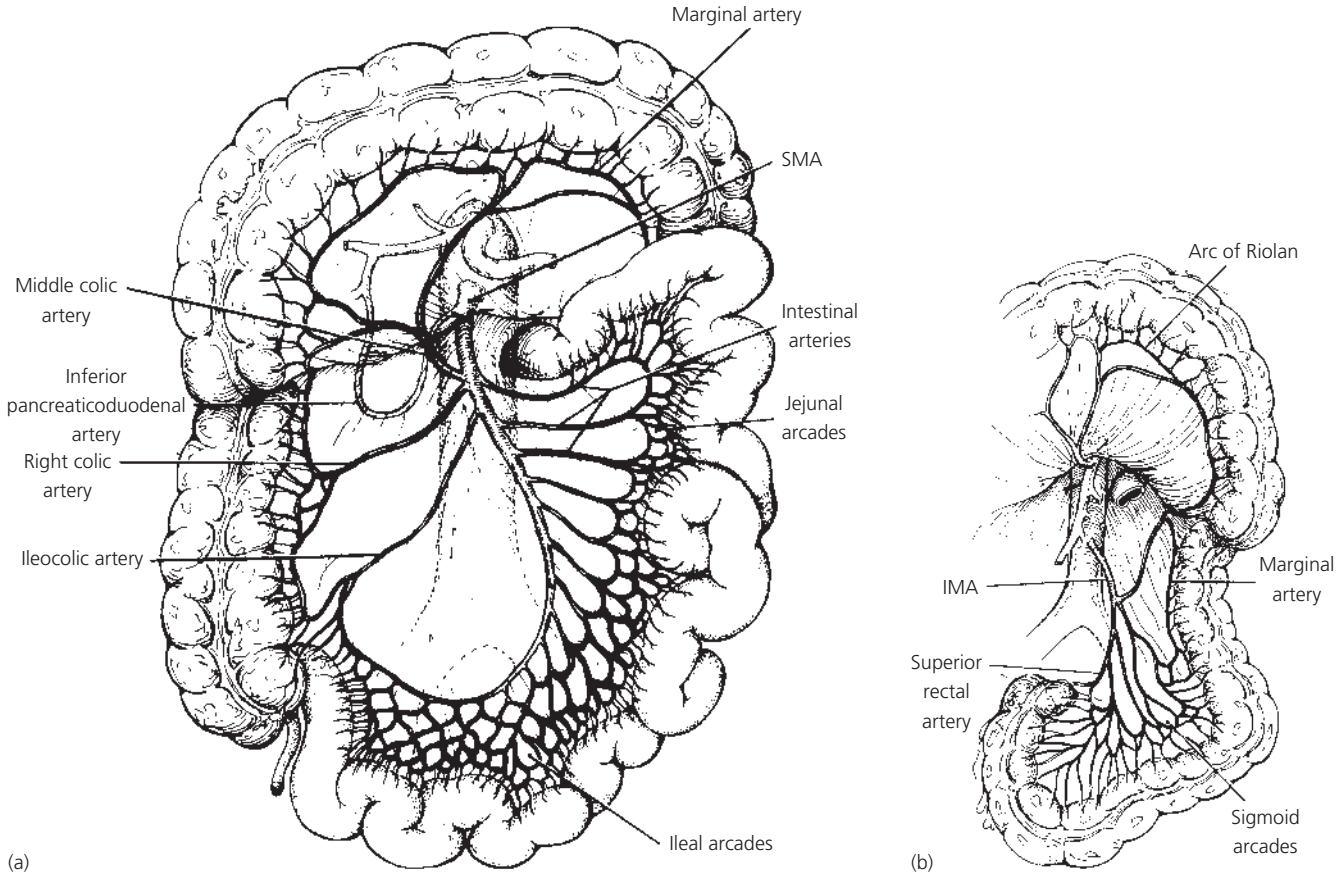

Figure 113.1 Mesenteric arterial circulation. **(a)** The superior mesenteric artery (SMA) exits the aorta 1 cm below the celiac artery. It supplies the entire small intestine except for the superior part of the duodenum, and supplies the right colon and part of the pancreas. Branches include the inferior pancreaticoduodenal artery, numerous jejunal and ileal branches, the ileocolic artery, and the middle colic artery. **(b)** The inferior mesenteric artery (IMA) arises from the aorta 3 cm proximal to the aortic bifurcation. The artery branches into the left colic, several sigmoid (inferior left colic) arteries, and the superior rectal (hemorrhoidal) artery. The arcades of the SMA and IMA interconnect at the base and border of the mesentery. The connection at the base of the mesentery provides a potential collateral channel between SMA and IMA called the "arc of Riolan." The connection along the mesenteric border provides another potential collateral channel called the marginal artery of Drummond. From Marston A, Pegington J. Macroscopic anatomy. In: Marston A, Bulkley GB, Fiddian-Green RG, Haglund UH (eds). Splanchnic Ischemia and Multiple Organ Failure. London: Edward Arnold, 1989:13, with permission from Hodder Education.

Inferior mesenteric artery

The inferior mesenteric artery (IMA) is the smallest of the mesenteric vessels and arises from the ventral aorta approximately 6–7 cm below the SMA at the level of L3 (Fig. 113.1b). The artery branches into the left colic, which supplies the left (distal) transverse colon and splenic flexure; several sigmoid (inferior left colic) arteries, which supply the sigmoid and descending colon; and the superior rectal (hemorrhoidal) artery, which supplies a large part of the rectum. The inferior rectal (hemorrhoidal) arteries derived from the internal pudendal arteries, and the small and inconstant middle rectal (hemorrhoidal) arteries, derived from the internal iliac arteries also supply the rectum.

Arterial collaterals

Extensive anastomotic and collateral circulation exists within each and between the three major intestinal vessels, as well as between the mesenteric vessels and the nonmesenteric systemic circulation. Major branches of the celiac artery interconnect in the stomach and duodenum. A series of arcades interconnect neighboring branches of the SMA [4]. A similar series of arcades interconnect neighboring branches of the IMA. The primary potential pathway of collateral flow between the celiac artery and SMA is through the gastroduodenal and pancreaticoduodenal arteries (Fig. 113.2), with the inferior pancreaticoduodenal being the most common. Additionally, omental branches of the SMA can communicate with branches of the celiac artery by the arc of Barkow [3]. An uncommon but well-described communication is the arc of Bühler, a direct communication between the celiac artery and SMA that represents a persistence of the embryonic ventral segmental arteries.

The arcades of the SMA and IMA interconnect at the base (away from the colon) and border (near the colon) of the

Figure 113.2 (a) Selective digital subtraction angiography (DSA) of the superior mesenteric artery (SMA), showing a long and high-grade stenosis of the proximal artery (arrow). **(b)** DSA injection of the celiac axis (large arrow) of the same patient demonstrating collateral filling of the distal SMA through pancreaticoduodenal collaterals (small arrow).

mesentery (Fig. 113.1b). The connection at the base of the mesentery provides a potential collateral channel between the SMA and IMA, called the meandering mesenteric artery, or arc of Riolan [4]. The connection along the mesenteric border provides another collateral channel called the marginal artery of Drummond.

Despite the presence of collateral channels, the vasculature has several points susceptible to poor perfusion (watershed areas). The middle part of the small intestine, in the middle of the area perfused by the SMA, is far away from collaterals from the celiac axis to the proximal SMA, and from collaterals from the IMA to the distal SMA. This area is the most vulnerable to develop ischemia from SMA occlusion. Narrow terminal branches of the SMA supply the splenic flexure, and the rectosigmoid junction is supplied by terminal branches of the IMA. These two watershed areas are most vulnerable to develop ischemia during systemic hypotension if the collateral anastomoses are small or tenuous [5].

Venous anatomy

The venous system generally parallels the arterial distribution. The inferior mesenteric vein (IMV) drains the rectum through the superior rectal vein, the sigmoid through the sigmoid veins, and the descending colon through the left colic vein. The superior mesenteric vein (SMV) drains the small intestine, cecum, ascending colon, and transverse colon via the jejunal, ileal, ileocolic, right colic, and middle colic veins [6]. The SMV also drains the greater omentum and distal stomach with the right gastroepiploic vein and drains part of the pancreas and duodenum via the inferior pancreaticoduodenal vein. The IMV drains into the splenic vein, which then joins the SMV to form the portal vein.

Mechanisms of ischemic injury

When blood flow to the intestine decreases below a critical point, important metabolic and structural alterations appear in a variety of intestinal cells that can eventually cause cell destruction and death. Despite the fact that restitution of blood flow is necessary to limit the progression of cellular injury associated with decreased oxygen and nutrient delivery, it has become apparent that restoration of blood flow and oxygenation to the ischemic intestine can result in a paradoxical enhancement of tissue injury, a process that has been termed "reperfusion injury." The mechanisms that are believed to participate in ischemic and reperfusion injury include the infiltration of postischemic tissues by inflammatory cells, an increased production of reactive oxygen species (ROS), alterations in vascular permeability, and inhibition of local cytoprotective mechanisms [7] (Fig. 113.3). For a detailed description of the structure and functions of intestinal microcirculation under physiological conditions the reader is directed to Chapter 22, Gastrointestinal blood flow.

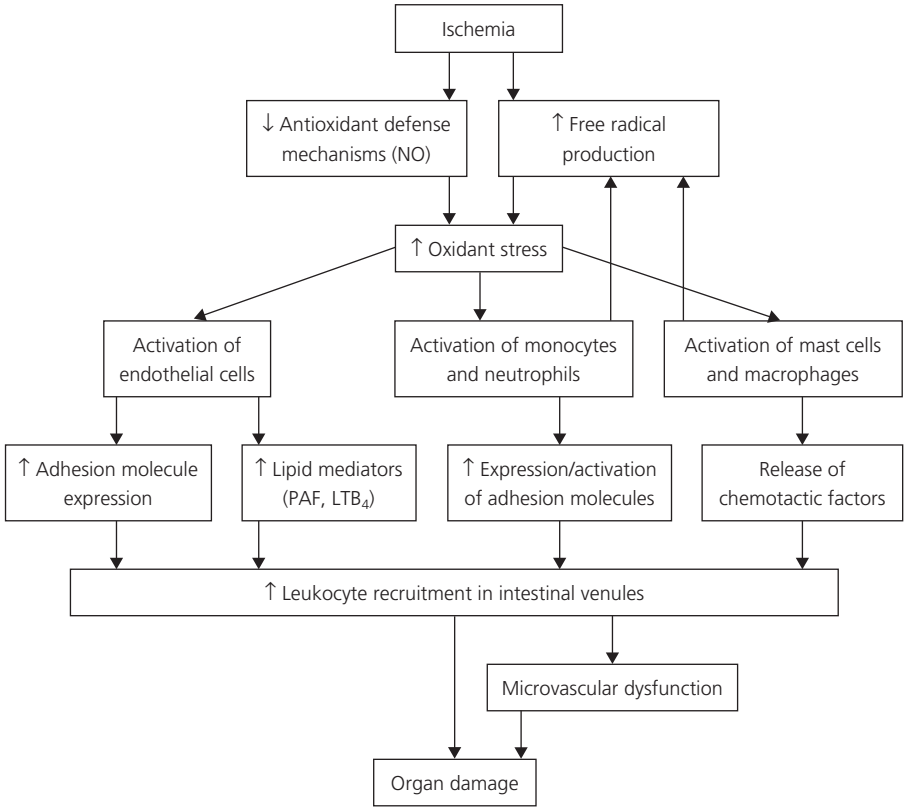

Figure 113.3 Mechanisms involved in ischemia-induced intestinal damage. Ischemia results in an increased production of oxidants, with a corresponding reduction in the synthesis of nitric oxide (NO) by endothelial NO synthase. The enhanced generation of oxidants results in the activation of endothelial cells and leukocytes. Firm adhesion of leukocytes, which is mediated by β2-integrins (CD11/CD18), is induced by engagement of activated LTB_4, and PAF with their receptors on rolling leukocytes. Sustained rolling and adhesion of leukocytes on endothelial cells are ensured by an oxidant-dependent synthesis of endothelial cell adhesion molecules, such as P-selectin and ICAM-1. The inflammatory responses to ischemia are further amplified by oxidants derived from NADPH oxidase in leukocytes, and by mediators released from mast cells and macrophages that normally reside in close proximity to postcapillary venules. ICAM-1, intercellular adhesion molecule-1; LTB_4, leukotriene B_4; PAF,

Inflammatory response

Recruitment of circulating leukocytes toward the ischemic intestine is a key event in the pathophysiology of intestinal ischemic injury. Both leukocytes and endothelial cell adhesion molecules (CAMs) participate in slowing the leukocyte as it exits the capillary and enters the postcapillary venule, which is the major site of leukocyte–endothelial cell adhesion [8]. The initial event, known as the rolling phenomenon, consists in a transient adhesive interaction and is mainly mediated by the selectin family of adhesion molecules, L-selectin, expressed on all leukocytes, P-selectin, detected on activated platelets and endothelial cells, and E-selectin, exclusively found in the latter. Several studies have reported an important upregulation of P-selectin in the intestine submitted to ischemia [9]. L-selectin has also been implicated in the process of tissue infiltration and organ failure resulting from ischemia [10].

Firm leukocyte adhesion and eventual emigration to the perivascular interstitium is mediated mainly by the intercellular adhesion molecule 1 (ICAM-1), a constitutively expressed immunoglobulin located on endothelial cells, and by its counter-receptors on neutrophils, the β_2-integrins CD11a/CD18 and CD11b/CD18. Endothelial expression of ICAM-1 has been found to increase in tissues subjected to ischemia [11] and also in distant organs, suggesting a possible role for this molecule in the pathogenesis of multiple organ failure [12]. Moreover, the administration of an antibody against ICAM-1 or against the common β_2-subunit CD18 significantly prevents leukocyte accumulation and microvascular dysfunction during reperfusion [13]. Certain conditions such as diabetes and hypercholesterolemia, which are associated with a higher risk of ischemic damage, have been shown to enhance the inflammatory response associated with ischemia and reperfusion [14–18].

Reactive oxidants

Increased production of ROS by both endothelial cells and leukocytes in postischemic tissues appears to be a key determinant of injury to the microvasculature. Ischemia promotes the conversion of the nicotinamide adenine dinucleotide (NAD)-reducing dehydrogenase form of the enzyme to the oxygen-reducing oxidase form. The accumulation of hypoxanthine during ischemia allows for a burst of superoxide and hydrogen peroxide production by the enzyme when oxygen is reintroduced into the blood vessel at the time of reperfusion. While xanthine oxidase contributes to the initial oxidant stress elicited in venules after a few minutes of reperfusion, adherent leukocytes account for the substantially greater oxidant stress that occurs thereafter [19]. This contention is supported by studies that demonstrate a diminished oxidant stress in postischemic venules of animals in which postischemic leukocyte recruitment is abrogated by

means of blocking monoclonal antibodies directed against endothelial or leukocyte adhesion molecules [20].

Imbalance of proinflammatory and protective mechanisms

There are several lines of evidence that implicate nitric oxide (NO) bioavailability as an important determinant of the inflammatory responses observed after ischemia. These include (1) a reduction in the activity of the Ca^{2+}-dependent isoform of NO synthase in postischemic tissues; (2) the ability of NO synthase inhibitors to mimic most of the microvascular alterations normally produced by ischemia, i.e., leukocyte recruitment, platelet–leukocyte aggregation, mast cell degranulation, and an increased albumin leakage; and (3) the protection against ischemia-induced leukocyte recruitment and microvascular dysfunction that is afforded by NO-releasing compounds, which replenish tissue NO levels in postischemic tissues [21,22].

Under normal conditions, the flux of NO greatly exceeds the rate of superoxide production. This allows for NO to effectively scavenge the low intracellular levels of superoxide. However, within minutes after reperfusion of ischemic tissues, the balance between NO and superoxide is tipped in favor of the latter. This imbalance results from a profound increase in the production of superoxide by endothelial cells and adherent leukocytes with a corresponding decline in the synthesis of NO from endothelial NO synthase. The relatively low levels of NO are insufficient to oppose leukocyte–endothelial cell interactions and to maintain optimal tissue blood flow.

The accumulation of superoxide that occurs in the absence of NO after ischemia allows for an enhanced generation of hydrogen peroxide. The two reactive oxygen metabolites (O_2^- and H_2O_2) can activate nuclear transcription factors such as NF-κB and AP-1, which in turn activate genes that encode adhesion molecules such as P-selectin, E-selectin, and ICAM-1 [23] (see Fig. 113.3).

Definitions of clinical syndromes

The intestinal ischemia includes a number of clinical disorders in which blood supplied to the intestine and mesentery is impaired, thus limiting tissue oxygenation and nutrient availability. This can be a consequence of reduced splanchnic or mesenteric blood flow in the overall territory or in a regional bed.

The spectrum of intestinal ischemia comprises a number of syndromes including acute mesenteric ischemia (AMI) as a result of emboli, arterial or venous thrombi, vasoconstriction secondary to low-flow states, or small bowel loop strangulation, chronic mesenteric ischemia (also named intestinal angina) due to transient, recurrent episodes of inadequate intestinal blood flow to sustain metabolic needs or to support increased metabolic demands such as that associated with digestion, and ischemic colitis, in which a circulatory insufficiency of the colon results in varying degrees of local tissue necrosis and systemic manifestation.

Acute mesenteric ischemia

Acute insufficiency of the blood supply to the intestine can result from emboli, arterial and venous thrombi, or vasoconstriction secondary to low-flow states [24,25].

Pathophysiology

Mesenteric arterial embolism

Embolization to the SMA constitutes about 5% of peripheral emboli and accounts for roughly 50% of all cases of AMI. The SMA is anatomically susceptible to embolism because it has a large caliber and arises from the aorta at a narrow angle [26]. In contrast, the IMA rarely suffers embolic occlusion because it has a small caliber, and the celiac axis rarely suffers embolic occlusion because it arises from the aorta at a right angle. Risk factors for mesenteric arterial embolism are listed in Table 113.1. The majority of mesenteric arterial emboli originate from the left side of the heart, commonly from a thrombus in the left atrium or left ventricle, or a lesion on the mitral or aortic valves [24]. Less commonly, emboli originate from an ulcerated aortic atherosclerotic plaque or a thrombosed aortic aneurysm. The most common precipitant of thrombus dislodgment and embolization is a cardiac arrhythmia, especially atrial fibrillation. Anticoagulation markedly decreases the risk of embolization from atrial fibrillation [27]. Myocardial dyskinesia, cardioversion, and cardiac catheterization are other clinical settings for embolization. The sudden occurrence of abdominal pain in these settings should prompt evaluation for SMA embolization. A substantial fraction of patients have prior emboli, and about 20% have synchronous emboli [24].

About 15% of emboli impact at the origin of the SMA, whereas the majority lodge distally 3–10 cm in the tapered segment of the SMA, past the origin of the middle colic artery [28]. Emboli tend to obstruct mesenteric flow acutely and completely and provide insufficient time to develop protective mesenteric collaterals. Intestinal ischemia due to embolic arterial occlusion can be compounded by reactive mesenteric vasoconstriction, further reducing collateral flow and worsening the ischemic insult [25].

Acute mesenteric arterial thrombosis

Arterial thrombosis accounts for about 15% of cases of acute intestinal ischemia. Thrombosis of the SMA or celiac artery is generally associated with a preexisting stenosis, usually at the origin of the arteries [29]. Typically, the SMA plaque slowly progresses to a critical stenosis over years and the residual lumen suddenly thromboses during a period of low

Table 113.1 Factors predisposing to intestinal arterial ischemia

Embolus (55% of cases)
Cardiac arrhythmia (atrial fibrillation)
Myocardial dyskinesia
Prosthetic valve
Cardioversion
Cardiac catheterization
Recent myocardial infarction
Prior/simultaneous emboli
Thrombus (15% of cases)
Prior arterial insufficiency
Coronary
Cerebrovascular
Peripheral
Old age
Low-flow state
Diabetes
Hypercholesterolemia
Hypertension
Congestive heart failure
Hypercoagulable states
Vasculitides
Aortic/mesenteric artery aneurysm
Trauma
NOMI (30% of cases)
Cardiogenic shock
Hypovolemic shock
Congestive heart failure
Pulmonary edema
Aortic insufficiency
Major cardiac/abdominal surgery
Dialysis
Vasoconstrictive drugs

NOMI, nonocclusive mesenteric ischemia.

flow. Patients usually have diffuse atherosclerotic disease, with prior coronary, cerebrovascular, or peripheral arterial insufficiency (Table 113.1). About 30% of patients have histories consistent with chronic mesenteric ischemia, including postprandial pain, malabsorption, and weight loss before the acute episode [25].

Nonocclusive mesenteric ischemia (NOMI)

NOMI causes 20%–30% of acute mesenteric ischemia episodes [24,25]. Mesenteric ischemia without anatomic arterial or venous obstruction is due to mesenteric vasospasm, which can occur during periods of relatively low mesenteric flow, especially if there is underlying arterial atherosclerotic disease. Such low-flow states can result from heart failure (due to myocardial pump failure or arrhythmia), hypotension (shock, sepsis), or hypovolemia (dehydration, bleeding, treatment with diuretics) (Table 113.1). The vasoconstriction is produced by sympathetic activity, possibly mediated by vasopressin and angiotensin [30]. Moreover, mesenteric vasospasm may persist and perpetuate the ischemic injury even after correction of the precipitating event [25]. Vasoconstrictive drugs, particularly alpha-adrenergic agents, vasopressin, ergotamine, diuretics, and digitalis glycosides, can contribute to NOMI. Digitalis, even at nontoxic levels, can cause paradoxical and sustained contraction of both arterial and venous smooth muscle cells in the mesenteric circulation [31].

To make the diagnosis of NOMI, physicians must have a high index of suspicion because these patients may not present with the classic symptom complex of severe abdominal pain. Predisposing factors include myocardial infarction, congestive heart failure, pulmonary edema, aortic insufficiency, dialysis [32], and major cardiac or intraabdominal surgery [33]. The mortality of this specific subset of patients is relatively high irrespective of treatment due to the underlying medical conditions and the frequent delays in diagnosis.

Acute mesenteric venous thrombosis

Thrombosis of the SMV causes 5%–10% of the causes of AMI [34,35]. Symptomatic SMV thrombosis is about 20-fold more common than symptomatic IMV thrombosis because of the larger caliber and flow of the SMV [36]. The mean reported patient age ranges from 47 to 60 years [6], which is younger than that for superior mesenteric arteriopathy because of the association of arterial disease with atherosclerosis [37].

Although previously less than 50% of patients had a recognized cause, etiological factors are currently identified in about 80% of cases [35]. Risk factors for mesenteric venous thrombosis are listed in Table 113.2; they include hypercoagulable syndromes (protein C deficiency, protein S deficiency, factor V Leiden mutation, antithrombin III deficiency, antiphospholipid syndrome), portal hypertension, abdominal infections, perforated viscus, pancreatitis, and trauma [38]. The life-threatening complications of this syndrome are induced by the resulting bowel wall edema and increased outflow resistance secondary to venous thrombosis and increased blood viscosity, which can impede arterial inflow, leading to submucosal hemorrhage, venous capillary congestion, and bowel infarction [37].

Clinical presentations

Abdominal pain out of proportion to physical findings is a cardinal symptom in patients with AMI and is commonly manifest after embolic or thrombotic occlusion of the SMA [39]. The pain may initially be colicky but becomes constant with progression of ischemia. The pain may be localized or diffuse. The duration of pain is typically short. A history of chronic postprandial abdominal pain is present when acute arterial thrombosis is superimposed on chronic mesenteric ischemia [24]. Abdominal pain may not be appreciated in confused or critically ill patients [38].

Table 113.2 Causes of superior mesenteric venous thrombosis

Hypercoagulable states
Factor V Leiden mutation
Antithrombin III deficiency
Hyperfibrinogenemia
Antiphospholipid syndrome
Protein S deficiency
Protein C deficiency
Hormones/pregnancy
Neoplasms
Hyperviscosity syndromes
Myeloproliferative disorders
Sickle cell disease
Infection/inflammation
Pancreatitis
Appendicitis
Diverticulitis
Intraabdominal abscess
Portal hypertension
Trauma

If the clinical picture of occlusive intestinal ischemia may often be blurred and misleading, this is even truer for NOMI. Patients will often already be in intensive care for other reasons and, quite often, clinical presentation is dominated by one of the underlying diseases. Gastrointestinal symptoms may be nonspecific and present as constipation, loss of appetite, nausea, and vomiting. Abdominal pain is usually gradually increasing or discontinuous and may be colicky. Diarrhea with blood, mucus, and melena may ensue. A diagnosis of NOMI should be considered in all patients with risk factors, if the patient complains about gradually increasing abdominal discomfort with no other obvious cause, or if sedated intensive care patients develop signs of abdominal disease.

The symptoms of acute mesenteric venous thrombosis are usually less severe than those of acute arterial ischemia. Typically, these patients have a diffuse, intermittent abdominal pain of several days' or even weeks' duration.

In the absence of pain, unexplained abdominal distention and gastrointestinal bleeding may be the earliest signs of ischemia and impending intestinal infarction. Fever, diarrhea, nausea and vomiting, and diminished bowel sounds are other common but nonspecific manifestations of AMI. Diffuse or localized abdominal tenderness, rebound, and rigidity are ominous signs and usually herald transmural bowel infarction [40]. Infarction also leads to hypotension from fluid loss and septicemia, decreased urine output from hypovolemia and renal hypoperfusion, and hyperventilation from hypoxia and acidosis [38].

Diagnosis

Laboratory tests

Serum laboratory tests are not very helpful in making the diagnosis of AMI; no serum marker is sensitive or specific enough to establish or exclude the diagnosis. Moreover, elevations in the levels of serum markers most suggestive of intestinal ischemia usually occur only after transmural bowel infarction develops and, therefore, cannot be used to diagnose AMI at its early stages, when improved survival would be possible.

Leukocytosis, neutrophilia, and a shift to immature leukocytes in the leukocyte differential are common [28,41], but these laboratory findings are nonspecific markers of inflammation and infection. About 50% of patients have metabolic acidosis, an ominous finding suggestive of intestinal infarction, and 25% have hyperamylasemia [42]. Hemoconcentration may develop from fluid loss intramurally as interstitial edema, intraperitoneally as ascites, and intraluminally from fluid transudation [28]. Intestinal necrosis is associated with elevations of serum lactate, phosphate, and alkaline phosphatase levels as well as prerenal azotemia, hypoxemia, and bacteremia [26,28]. In prospective clinical studies including a reduced number of patients, increases in serum D(–)-lactate [43] or in a human homologue of intestinal fatty acid-binding protein (I-FABP) [44] have been detected. The diagnostic value of the fibrinolytic marker D-dimer, which has been studied extensively in the diagnosis of thromboembolism, has been assessed also in a clinical pilot study showing that patients suffering from mesenteric ischemia had significantly higher D-dimer levels than those with nonischemic acute abdominal events [45]. However, there are no reliable data on sensitivity and specificity of these serum markers.

Plain X-ray films of the abdomen

Plain abdominal radiography is most useful to exclude other potential causes of abdominal pain, rather than confirming the diagnosis of acute mesenteric ischemia. Completely normal plain radiographs have been reported in more than 25% of patients with mesenteric ischemia [46]. Subtle signs of AMI on plain abdominal radiographs include adynamic ileus and distended, air-filled loops of bowel, but these abnormalities are most commonly due to other causes such as pancreatitis, mechanical obstruction, or colonic pseudoobstruction. More specific radiographic findings occur in 25% of patients, usually those with advanced disease. These findings include mural thumprinting resulting from edema or hemorrhage (Fig. 113.4). In advanced stages of ischemia, pneumatosis of the bowel wall can be detected. Specifically, portal vein gas on abdominal radiography portends an extremely poor prognosis [47].

Intraluminal barium contrast evaluations are contraindicated because residual contrast can limit visualization of the

Figure 113.4 Thumbprinting. Detail of a plain abdominal radiograph showing semiopaque projections of the wall of the left colon into the lumen (arrows) resulting from submucosal edema and sometimes hemorrhage. Although thumbprinting is highly suggestive of intestinal ischemia, it occurs in relatively advanced lesion.

mesenteric vasculature during diagnostic angiography. On the rare occasion when barium studies are performed in a patient with abdominal pain not initially thought to have AMI, thumbprinting, stricture, or ulcerations can be found.

Ultrasonography

B-scan ultrasonography is widely practiced as the first diagnostic procedure in patients with acute abdominal pain. In patients with AMI, ultrasonography may reveal a thickening (> 45 mm) of bowel walls, signs of ileus with distended bowel loops, and hypoperistalsis. In severe cases, ultrasound may show intraperitoneal fluid or even air in the portal vein as a sign of massive intestinal necrosis. However, the main task of B-scan ultrasonography is to rule out other underlying abdominal pathology (e.g., aortic aneurysm).

Duplex ultrasonography may be of some benefit in visualizing flow in the SMA and celiac axis. With expert technical assistance, these tests can document proximal stenosis in the SMA or celiac axis or complete occlusion of these vessels with a high specificity (92%–100%), but relatively low sensitivity (70%–89%) [48]. Unfortunately, duplex sonography is of no value in detecting emboli beyond the proximal main vessel or in diagnosing NOMI. Moreover, identification of significant arterial stenosis does not establish the diagnosis of intestinal ischemia because total occlusion of two or even all three splanchnic vessels can be present in asymptomatic patients.

Angiography

Selective catheter angiography has long been the gold standard for diagnosis of AMI. In a review of mesenteric angiography in the diagnosis of AMI, sensitivities in five of six studies have ranged between 90% and 100%; specificity was reported in two of these studies to be 100% [2]. Although early angiography will produce a considerable number of negative results, it is essential if diagnoses are to be made early enough to improve survival [49,50]. However, prompt laparotomy should be performed in patients with suspected AMI in whom expeditious angiography is not available.

More controversial is the need for angiography in a patient with suspected AMI and signs of peritonitis. Since the latter indicate the presence of infarcted bowel, the most compelling reason for angiography, i.e., diagnosis while the effects of intestinal ischemia are still reversible, is no longer a consideration. However, angiography may still provide valuable information in these patients since it may establish a definitive diagnosis of AMI and its cause, identify all vascular lesions, which will guide revascularization procedures, and offer the possibility of administering intraarterial vasodilators in cases of NOMI. Disadvantages of traditional angiography are its limited availability and potential renal toxicity as well as its expense and time constraints.

In cases of SMA embolization, most emboli are impacted 3–10 cm from the origin of the SMA, distal to the origin of the middle colic artery. The classic meniscus sign can often be visualized at the point of occlusion, which is different from the planar defect produced by a thrombus [51]. However, when angiography is performed more than 24 h after embolization, the embolic filling defect becomes less sharp and less round because of proximal and distal clot propagation. Contrast is typically poorly visualized distal to emboli because the rapidity of embolic occlusion provides insufficient time to develop collateral flow. Other angiographic findings favoring embolus over thrombus include minimal atherosclerosis in mesenteric vessels, multiple lesions in mesenteric arterial branches, and simultaneous extramesenteric emboli [51].

Symptomatic thrombosis of the SMA is generally associated with a high-grade stenosis or occlusion of the celiac axis. The stenosis usually slowly progresses and permits reconstitution of vascular flow because of the development of collaterals (Fig. 113.2). Aortography is essential in these patients to evaluate potential inflow and outflow sites for bypass

grafts as well as to clarify the extent and location of other atherosclerotic lesions in the iliac and inferior mesenteric arteries. When the SMA distal to thrombotic occlusion fills from large collaterals, the SMA thrombus is probably chronic and not the cause of acute symptoms.

In patients suffering NOMI, angiography usually reveals multiple areas of narrowing and irregularity in major branches. The small and medium arterial branches may be decreased or absent producing a "pruned" arterial tree, and there is also impaired intramural vascular filling [50]. Acute mesenteric embolic or thrombotic obstruction may also produce mesenteric vasospasm, so NOMI is diagnosed only when acute vascular obstruction is absent. The diagnosis of NOMI is strongly favored over the diagnosis of arterial thrombosis by an absence of extramesenteric atherosclerosis and by an increase in SMA caliber after transcatheter vasodilator therapy [51].

In mesenteric venous thrombosis, selective angiograms may reveal reflux of contrast material back into the aorta due to extremely slow flow and heightened outflow resistance. A prolonged arterial phase with accumulation of contrast and thickened bowel walls is also characteristic. Extreme cases may demonstrate contrast extravasation into the bowel lumen, which may be indicative of active bleeding. The definitive diagnosis of mesenteric venous thrombosis is made during the venous phase; either a filling defect is noted within the portal vein or, in more extensive thromboses, the entire venous phase is absent [37].

Computed tomography and magnetic resonance angiography

Whereas single-detector helical computed tomography (CT) is of limited use in the diagnosis of AMI [52], except in patients suspected of having superior mesenteric vein thrombosis (Fig. 113.5) [46], the spread of spiral CT equipment has certainly increased the potential of this investigation, which now claims 92% specificity and 64% sensitivity [53]. Multidetector-row CT is the most recent evolution, which provides an even better chance of recognizing mesenteric ischemia, thanks to increased spatial resolution, the high-quality three-dimensional (3D) reconstructions (Fig. 113.6), and the shorter examination times. It has thus confirmed a fundamental role for CT in the diagnosis of AMI, achieving a sensitivity of 80%–96% and a specificity of 94%–98% [53–55], and therefore has become the test of choice in the diagnosis of AMI. Unenhanced scans can show atherosclerotic calcification and, in cases where the onset of thrombosis is of recent date, the hyperdense aspect of the vascular structure involved. In cases of advanced infarction, diagnosis can be made on the basis of the baseline scans alone, if parietal pneumatosis or air at the level of the mesenteric vessels or portal branches can be seen. In all other cases, intravenous (i.v.) injection of contrast medium is essential in order to be able to identify the characteristic causes and signs of the ischemia that have been described previously with angiography. In occlusive, embolic or thrombotic forms, CT enables the site of the vascular obstruction to be visualized, appearing as a site of defective opacification of the lumen of the vessel (Fig. 113.7). It is most easily recognized when the occlusion

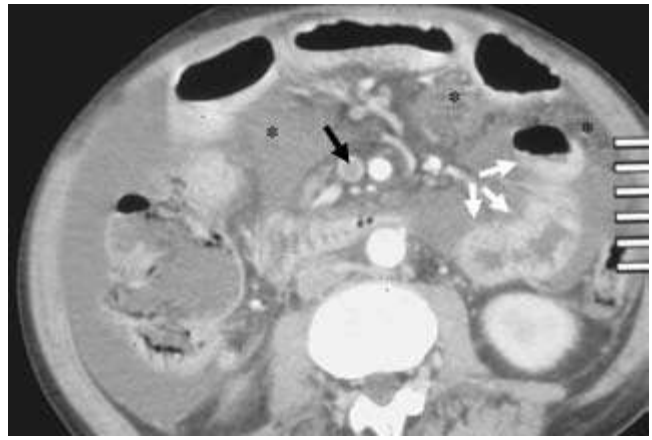

Figure 113.5 Mesenteric venous thrombosis in a patient with acute pancreatitis. Contrast-enhanced computed tomography (CT) scan shows small bowel wall thickening (thick arrows) and a hypoattenuating thrombus of the superior mesenteric vein (thin arrow). Ascites is also present (asterisks).

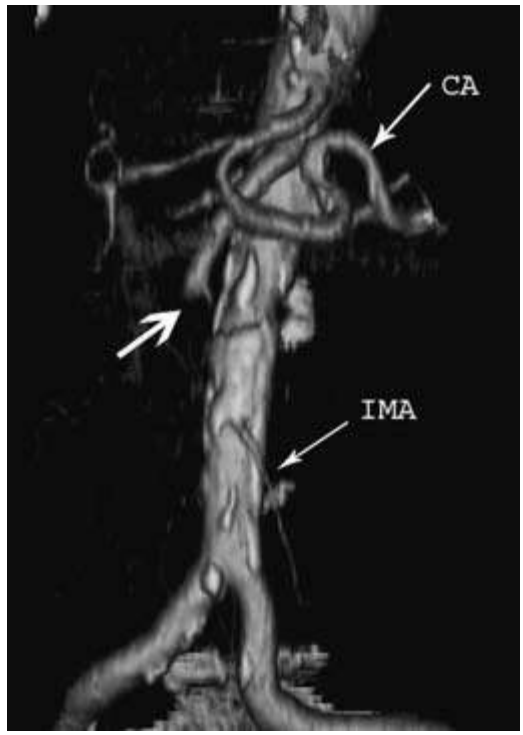

Figure 113.6 Volume-rendered three-dimensional computed tomography image of abdominal aorta in frontal projection showing occlusion of the superior mesenteric artery (SMA) (large arrow) with patency of the celiac axis (CA) and inferior mesenteric artery (IMA).

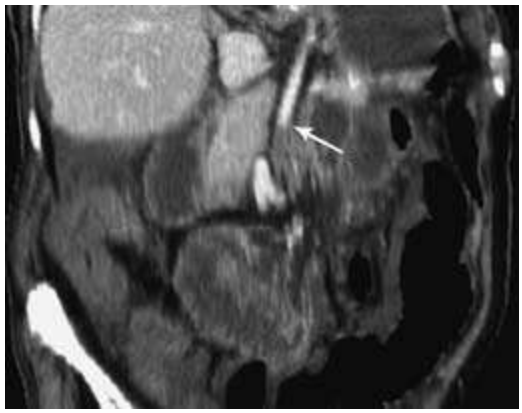

Figure 113.7 Contrast-enhanced computed tomography (multiplane reformat reconstruction). Oblique projection showing embolic occlusion of the superior mesenteric artery (arrow).

is at the level of the main trunks. Thrombosis of the main vessels can easily be seen at axial imaging, often with associated collateral vessels, depending on its chronicity. For evaluation of more distal branches of the mesenteric vessels and of narrowing or plaque at the origin of the arteries, 3D imaging offers a distinct advantage. Three-dimensional images allow better representation of complex collateral vessels [53,54]. A systematic review has demonstrated a high sensitivity of CT for ischemia in the setting of small bowel obstruction and suggests that a CT scan finding of partial bowel obstruction is likely to reflect a clinical condition that will resolve without surgical intervention [56].

Magnetic resonance imaging with angiography is another noninvasive modality that rivals conventional angiography. In mesenteric venous disease, excellent visualization of the vascular anatomy is possible in addition to assessment of portal venous patency, flow direction, splanchnic thrombosis, and changes suggestive of portal hypertension. Three-dimensional gadolinium enhanced reconstruction of vascular anatomy with a single breath hold and ultrafast scanning with digital subtraction angiography are also available [57]. High sensitivity and specificity were found for MRI in the detection of severe stenoses or occlusion of the origins of the celiac axis and SMA [58]. In patients with thrombosis of the portal or mesenteric veins, the overall sensitivity, specificity, and accuracy of MRI angiography for the detection of thrombosis have been reported to be 100%, 98%, and 99%, figures similar to those of digital subtraction angiography, suggesting that noninvasive contrast-enhanced MRI angiography has the potential to replace subtraction angiography as the standard method to assess the whole intestinal venous system [59]. However, this modality is limited in its ability to identify more peripheral occlusions and NOMI. Functional MRI angiography also has been studied: the normal increase in postprandial SMV flow compared with SMV flow in the fasting state has been shown to be less in patients with chronic mesenteric ischemia than in control subjects [60]. Additionally, the ratio of postprandial SMV flow increase to SMA flow increase is less in patients with chronic mesenteric ischemia than in control subjects [61]. While not yet routinely available, functional MRI may hold promise for the future in helping to diagnose chronic mesenteric ischemia. The major advantage of MRI over CT angiography or conventional angiography is its lack of renal toxicity.

Therapy

General measures

Initial treatment of patients suspected of having AMI is aimed at resuscitation. Fluids are aggressively administered to replace intravascular fluid lost into the interstitium as edema, into the peritoneum as ascites, or into the lumen from fluid exudation or hemorrhage. Adequate hydration and optimization of cardiac function are particularly important in NOMI because hypovolemia and hypotension exacerbate mesenteric vasoconstriction. In patients with hypotension or hypovolemia, there will always be mesenteric vasoconstriction; thus, if there is a strong suspicion of NOMI (and arterial embolus or thrombosis are considered unlikely), angiography before correction of hypovolemia or hypotension will not be of diagnostic help. Medications that cause vasoconstriction, such as vasopressin or digitalis, should be discontinued. Electrolyte abnormalities and acid–base disturbances should be corrected before surgery. Broad-spectrum antibiotics are administered intravenously because intestinal ischemia promotes bacterial translocation and sepsis. Gastrointestinal decompression via a nasogastric tube is recommended because increased intraluminal pressure may decrease mucosal perfusion. Urine volume should be monitored with a bladder catheter. In critically ill patients, pulmonary pressures and cardiac output should be monitored with a Swan–Ganz catheter to optimize fluid and drug therapy. Measurements of arterial oxygen concentration will guide the need for oxygen supplementation or mechanical ventilation.

Treatment of emboli

Various therapeutic approaches have been proposed for SMA emboli, including surgical revascularization, intraarterial perfusion of thrombolytic agents or vasodilators, and systemic anticoagulation. The therapeutic option should be chosen taking into account the presence or absence of peritoneal signs, whether arterial occlusion is partial or complete, and whether location of the embolus is above the origin of the ileocolic artery or in more distal branches.

There is uniform agreement that exploratory laparotomy is mandatory when signs of peritonitis are present and that embolectomy and resection of any infarcted bowel should be performed as necessary. When abdominal contamination

is absent, primary anastomosis is generally performed. When extensive bowel segments are questionably viable, only unambiguously necrotic bowel is resected, and a second-look operation is performed about 24 h later to permit demarcation between viable and nonviable bowel and to minimize the extent of bowel resection [62]. Anticoagulation with heparin is administered postoperatively to prevent recurrent embolization [2].

Several case reports and small series have reported successful nonoperative treatment of acute mesenteric arteriopathy using heparin [63] and thrombolytic agents including urokinase, streptokinase, and recombinant tissue plasminogen activator [64,65]. Data from that experience suggest that thrombolytic therapy is most likely to be successful when the embolus is partially occluding, or is in one of the branches of the SMA or in the main SMA distal to the origin of the ileocolic artery, and the treatment is applied within 12 h of the onset of symptoms. An important question is the long-term reocclusion rate after these therapies. Patients treated medically require close surgical observation to detect failure of medical therapy and the rapid progression to infarction. If signs of intestinal infarction develop, immediate surgery is indicated [2].

There is some controversy as to the use of vasodilators as a complementary treatment in patients with SMA embolus. There is evidence showing that vasoconstriction of both the unobstructed and the obstructed branches occurs with an SMA embolus [50]. Such vasoconstriction resolves spontaneously if the embolus is removed soon after it develops. However, if the vasoconstriction has existed long enough, it can become persistent. In these cases, intraarterial infusion of a vasodilator such as papaverine might be beneficial [2].

Treatment of arterial thrombosis

If a diagnosis of acute thrombosis of the SMA is made, emergency surgical revascularization is recommended. Simple surgical thrombectomy is unlikely to be successful in the long term, and it has to be accompanied by revascularization performed via bypass [29]. In a contaminated operative field, an autogenous aortovisceral graft is required, but polyester fluoroethylene prosthesis may be used when contamination is absent [1]. The proximal SMA should be opened through a longitudinal arteriotomy. If thrombectomy is successful, then a temporary intraarterial SMA shunt can be placed while definitive revascularization is considered. The longitudinal arteriotomy in the SMA can serve as the distal anastomotic site for an antegrade bypass originating in the supraceliac aorta or for a retrograde bypass from the infrarenal aorta or iliac vessels. As for treatment of an embolus, bowel viability is assessed after revascularization to minimize bowel resection.

Treatment of NOMI

Management of NOMI is essentially pharmacological and is readily achieved by selective infusion of vasodilators into the SMA. Splanchnic vasodilators include papaverine, tolazoline, nitroglycerin, glucagon, prostaglandin E, phenoxybenzamine, and isoproterenol [24]. The greatest clinical experience is with papaverine, which is administered as a continuous infusion into the SMA at 30–60 mg/h. This approach has resulted in a reduction in mortality rates from 70% to 90% observed through the 1980s and to 50%–55% during the last decade [25,50,62].

Subsequent management is dictated by the patient's clinical response to vasodilator therapy. Angiography may be repeated after 30 min to document the relief of vasospasm. Papaverine infusion is usually continued for approximately 24 h. A repeat angiogram should then be performed, prior to which the intraarterial infusion is changed to saline for 30 min. Depending on the clinical course and the presence or absence of vasoconstriction in the angiogram, papaverine is discontinued or maintained for another 24 h. Repeat angiograms are performed at 24-h intervals until radiographic signs of vasoconstriction have disappeared and the patient's clinical symptoms and signs have vanished. Generally, infusions can be discontinued after 24 h, but they have been maintained for as long as 5 days in some cases [25]. Certain precautions specific to the use of intraarterial papaverine are noteworthy. The patient's hemodynamic status should be carefully monitored, as significant hypotension can result if the indwelling catheter migrates into the aorta and papaverine is infused into the peripheral circulation. In this event the papaverine infusion should be replaced by with saline and the position of the catheter confirmed with plain film.

Immediate laparotomy is indicated during papaverine therapy if peritoneal signs do not rapidly remit with onset of infusion, if peritoneal signs develop during infusion, or if the patient deteriorates clinically as evidenced by increasing leukocytosis, sepsis, gastrointestinal bleeding, or unstable vital signs [25]. At surgery, gangrenous bowel is resected according to the surgical principles described for an embolus. Intraoperative peritoneal lavage with warm saline and maintenance of a warm operating room temperature moistens and warms the intestine and helps reduce mesenteric vasospasm. The SMA catheter is left in place to provide vasodilator therapy during and after surgery.

Treatment of mesenteric venous thrombosis

In asymptomatic individuals in whom the diagnosis has been made on a CT scan obtained for reasons other than abdominal pain, either no therapy or a 3- to 6-month course of anticoagulation is reasonable [2]; there are no studies to aid in this therapeutic decision. In symptomatic patients in whom an acute thrombosis of the SMV is diagnosed, by CT scan, MRI, or angiography, treatment is determined by the presence or absence of peritoneal signs. As in all patients with AMI, signs of peritonitis mandate laparotomy and resection of infarcted intestine. Successful thrombectomy of the SMV has been described [35,66]. However, it is not recommended

unless the thrombus in the proximal SMV is fresh (within 1–3 days), since most patients have diffuse venous thrombosis with distal extension, which is not suitable for thrombectomy. If the diagnosis of SMV thrombosis is established intraoperatively, anticoagulation should start immediately. Intravenous anticoagulation with heparin has been shown to prevent thrombus propagation and recurrence, and to improve survival [63–67]. In the absence of peritoneal signs, heparin should be immediately initiated, with an initial bolus of 5000 units followed by continuous infusion of 1000 units/h. Heparin dosage is then adjusted according to the prolongation of the partial thromboplastin time. Heparin is contraindicated in the presence of acute gastrointestinal bleeding from mesenteric ischemia. Heparin is administered for 7–10 days; then prolonged anticoagulation with warfarin for 3–6 months is recommended [37].

Thrombolytic therapy is rarely used for SMV thrombosis. Thrombolytic agents have been administered successfully in an antegrade fashion via the SMA [68], retrograde via the internal jugular vein [69], and transhepatically via the portal vein [70]. Nevertheless, these forms of therapy should be considered experimental for SMV thrombosis.

Prognosis

Until the early 1980s, the mortality of acute mesenteric arterial ischemia ranged from 70% to 90% [71,72]. Early diagnosis and aggressive therapy have resulted in improved survival. In a report of 21 patients with SMA embolus, intestinal viability was achieved in 100% of patients if the duration of symptoms was less than 12 h, in 56% if it was between 12 and 24 h, and in only 18% if symptoms were more than 24 h in duration before diagnosis [73]. During the last decade a 50%–60% survival has been reported in large clinical studies [73,74]. More than 90% survive when acute mesenteric arteriopathy is diagnosed early by conventional, CT scan or MRI angiography before peritoneal signs occur [28]. Variables that are independently associated with a poor prognosis include previous cardiac illness, high urea plasma levels, and small and large bowel involvement, whereas resection surgery is associated with a better prognosis [75]. Causes of death include extensive intestinal necrosis, recurrent SMA thrombus or embolus, emboli to other sites, cardiopulmonary failure, and intestinal hemorrhage.

The mortality for acute SMV thrombosis, 25%–30%, is lower than that for acute mesenteric arterial ischemia [67]. The mortality rate depends on the grade of venous occlusion, the presence of collaterals, diagnostic delays, and comorbidity [76]. In a report of 53 patients with SMV thrombosis, 55% had major postoperative complications, such as short bowel syndrome, wound infection, sepsis, and recurrent SMV thrombosis [35]. Recurrences usually involve infarction of bowel adjacent to previously resected bowel.

Chronic mesenteric ischemia

Chronic mesenteric ischemia (intestinal angina) is a clinical syndrome characterized by recurrent abdominal pain and weight loss as a result of repeated transient episodes of insufficient intestinal blood flow, usually related with the increased metabolic demand associated with digestion.

Intestinal angina is an uncommon process that occurs only when severe atherosclerotic narrowing of a major splanchnic vessel exists in association with occlusion of one or two of the remaining vessels. This is because the very efficient collateral circulatory network in the small bowel and colon can successfully overcome the single occlusion of a major vessel. In fact, intestinal angina is very uncommon despite the finding at autopsy, performed in unselected cases, of significant stenosis of any of the three major splanchnic vessel in 29% in the overall series and in 67% in those patients over 80 years of age at the time of death [77].

This ischemic syndrome is seen in middle-aged and elderly people and predominantly in women (3:2). Direct or indirect evidence of arthrosclerosis is common in these patients, with more than one-third having hypertension, coronary artery disease, peripheral arterial disease, and/or cerebrovascular disease. Patients with diabetes and smokers are at increased risk.

Clinical manifestations are related to limitations of the ability of the celiac artery or superior mesenteric artery to supply increased blood flow in response to metabolic demands after a meal, mainly because of atherosclerotic vascular lesions. Alternatively, it has been suggested that a steal phenomenon from the intestinal to the gastric circulation occurs when food reaches the stomach.

Clinical presentation

Recurrent abdominal pain and weight loss are the two primary characteristic symptoms. Abdominal pain is epigastric or periumbilical. It eventually radiates to the back and appears shortly after meals, lasting for 1–3 h. The intensity of the pain is related to the quantity of the meal or to the fat content, is crampy in nature, and has to be distinguished from pancreatic, biliary, or ulcer-related pain. As a consequence of the pain, food intake tends to diminish (sitophobia) and, with time, weight loss becomes obvious. Other unspecific symptoms such as nausea, vomiting, diarrhea and/or constipation are present in one-third of patients. Isolated cases of malabsorption associated with chronic mesenteric ischemia have been documented [78], and resolved gastroparesis has been reported after revascularization of occluded mesenteric vessels [79].

Physical findings are not specific. Weight loss with signs of malnutrition is the most common alteration, although some patients maintain their weight despite the high frequency and intensity of the pain. Abdominal tenderness without rebound may be present during an episode of severe pain.

Indirect signs of atherosclerotic vascular disease may be found, mainly diminished peripheral pulses, carotid or femoral bruits, or stigmata of past stroke.

Diagnosis

The diagnosis is mainly based on a characteristic clinical picture, the presence of an occlusive lesion in the splanchnic vessels documented by angiography, and the absence of other common causes of abdominal pain. In most cases, patients undergo an extensive workup for obscure chronic abdominal pain, including routine laboratory evaluation, plain abdominal radiographs, upper and lower GI endoscopy, small bowel barium series, and ultrasonography or abdominal CT. Laboratory abnormalities related to malnutrition, e.g., anemia, hypoalbuminemia, and hypocholesterolemia, may be present. When common causes of abdominal pain have been ruled out and suspicion of intestinal ischemia is well established from epigastric pain after meals and substantial weight loss, a CT angiographic study of the splanchnic arteries is warranted, especially in patients with risk factors. In approximately 90% of patients with intestinal angina, at least two major splanchnic vessels are found to be occluded or stenosed, and, in up to 50% of patients, blood flow in all three vessels is severely compromised [80]. In contrast, isolated occlusion of a single vessel is uncommon in documented intestinal angina.

Duplex ultrasonography can show high flow velocity and poststenotic turbulence reflected by modifications on spectral waveform and mixture of colors in the duplex image. Retrograde hepatic artery blood flow suggests stenosis of the celiac artery. In stenosis of the superior mesenteric artery greater than 50%–70%, the sensitivity of the technique is above 90%, with marginal improvement with postprandial study [81]. Sensitivity for lesions in celiac axis is slightly lower, 85%–87%. When performed by skilled operators, duplex ultrasonography may be a useful screening tool.

Treatment

For many years, surgical revascularization has been the treatment of choice for chronic mesenteric ischemia. The techniques used include antegrade and retrograde bypass grafting, transaortic mesenteric endarterectomy, and aortic reimplantation of the SMA. In the last 20 years, percutaneous transluminal mesenteric angioplasty with or without stenting has been introduced.

Patients with symptomatic chronic mesenteric ischemia should be considered for surgery with the goals to relieve symptoms, improve nutrition, and prevent mesenteric infarction. Asymptomatic patients submitted to aortic reconstruction for aortoiliac occlusive disease, in whom significant mesenteric occlusion is also present, should also be considered for bypass grafting because they have an increased risk of mesenteric infarction after surgery [82]. The indication for surgery in asymptomatic patients in the absence of a need for aortic reconstruction is not clear. It has been proposed by some authors that surgery should be considered in such patients when three-vessel ischemia is documented because of the high risk of developing ischemic symptoms or death [83]. Complete revascularization has been recommended by many surgeons, but several authors have recently proposed that bypass grafting of the superior mesenteric artery alone is an effective and durable procedure for treatment of intestinal ischemia [84]. Series published in the last 20 years have reported a wide range of outcome figures for surgical revascularization, but results of more recent publications show mortality rates from 0% to 4%, success rates (defined as relief of symptoms) over 90%, and recurrence rates under 15% [2]. Advancing age, cardiac disease, hypertension, and additional occlusive disease influence overall mortality, and concomitant aortic replacement, renal disease, and complete revascularization are associated with high postoperative morbidity rates [85]. In patients with severe weight loss, parenteral nutrition before revascularization may reduce surgical morbidity.

Chronic mesenteric ischemia has been effectively treated by the endovascular route [86]. In a series of 19 patients, successful dilation of at least one stenosed vessel was achieved in 100% of cases using balloon angioplasty, which was followed by stenting in seven cases. Significant amelioration of symptoms was observed in all cases, and three patients suffered a restenosis during a mean follow-up of 3 months [87]. A remarkable feature of this series is the success obtained by dilating only a single artery. Success and mortality rates seem not to be significantly different from those of surgical revascularization, but recurrence of symptoms has been slightly higher than after surgery in some series [88].

Based in the current information, it seems reasonable at the present time to offer surgical revascularization to low-risk patients and to perform angioplasty in those with a higher surgical risk. If it can be demonstrated that the addition of stenting to angioplasty can decrease the recurrence rate of symptoms better than angioplasty alone, then this procedure might be a valid alternative to surgery, even in low-risk patients. Surgery rather than angioplasty is indicated for suspected bowel necrosis.

In patients who survive surgical revascularization, the prognosis is excellent; 5-year survival rates approach 80%, and most patients become free from symptoms, gain weight, and resume normal eating habits [80].

Ischemic colitis

Ischemic colitis is the most common form of ischemic injury to the gut and occurs more frequently in elderly people. The true incidence in general population is not well known, but it has been estimated in the range of 4.5–44 cases per 100 000 person-years [89]. The disease can result from either

occlusive or nonocclusive events, mainly in the territory of the inferior mesenteric artery, in colonic branches of the superior mesenteric artery and in the superior and inferior mesenteric veins. Thus, ischemic colitis is predominantly seen in the left colon [90]. The splenic flexure and rectosigmoid junction, where low perfusion exists (watershed areas), are commonly affected, while the rectum is not usually compromised because of excellent collateral perfusion. Right colon ischemic colitis is rare [91], is mainly due to low-flow states, and is associated with poor prognosis [92].

Large arterial vessel occlusion may be caused by thrombi or atheromatous lesions [90]. Embolization of the inferior mesenteric artery is uncommon because most emboli are too large to enter this narrow vessel. Aortic surgery can cause ischemic colitis because of unnoticed ligation of the IMA, the occurrence intraoperative hypoperfusion in the presence of inferior mesenteric artery occlusion [93], or embolization of atheromatous debris to the hypogastric artery's tissue beds during endovascular manipulations [94]. Blood flow in the small segmental arterioles may also be compromised by small vessel disease induced by diabetes, radiation or autoimmune inflammatory arteritis, and by chronic renal failure treated by hemodialysis [95,96]. Recently, irritable bowel syndrome, constipation, and chronic obstructive pulmonary disease have also been identified to increase the risk for ischemic colitis [89,97]. Venous occlusion may occur as a consequence of hypercoagulability states (Table 113.2), pancreatitis, or portal hypertension [95]. Vessel occlusion may also be secondary to colonic obstruction from colon cancer, benign colonic strictures, adhesions, volvuli, or hernias [98]. Nonocclusive ischemia may be present in the colon due to low-flow states or vasoconstriction induced by several drugs [99,100] (Table 113.3). Ischemic colitis has been reported in long-distance runners [101].

Clinical manifestations

A wide spectrum of disorders can be seen as a consequence of colonic ischemia. These include acute, transient, self-limited ischemia (reversible colopathy characterized by mucosal and submucosal hemorrhage), acute fulminant ischemia (which is transmural and progresses to necrosis), and chronic ischemic colitis (partially reversible vascular disease usually manifested by late colonic stenosis).

Table 113.3 Drugs associated with intestinal ischemia

Estrogen–progesterone	Diuretics	Alosetron
Cocaine	Pseudoephedrine	Barbiturates
Crack	Cyclosporine	Methylsergide
Ergotamine	α-Adrenergic agonists	
Vasopressin	β-Adrenergic antagonists	
Digitalis	Psychotropic drugs	

Abdominal pain is present in more than two-thirds of patients [24]. The pain is moderately severe, crampy, mostly localized in the left lower quadrant, and sometimes associated with diarrhea. Rectal bleeding manifested by either bright-red blood in the stools or hematochezia is another common symptom. Bleeding is usually mild and does not require transfusion in most cases [102]. Abdominal distention is also frequent and can be accompanied by nausea and vomiting. Physical signs during the acute episode include abdominal distention, with mild-to-moderate tenderness, fecal occult blood, tachycardia, or moderate fever. In comparison with AMI, the majority of patients presenting with ischemic colitis have hemodynamic stability with no signs of peritonitis. However, in a small proportion of patients, massive colon necrosis may occur with obvious signs of peritonitis and shock. Hypertension and a history of cancer have been identified as independent prognostic factors of colonic necrosis in elderly people suffering an episode of ischemic colitis [103]. A minority of patients have subclinical acute ischemic disease and develop a colon stricture which can be asymptomatic or manifested by constipation, diarrhea, or recurrent abdominal pain [104].

Diagnosis

All patients, especially elderly people with any of the well-known precipitating factors, who develop abdominal pain, bloody diarrhea, and abdominal distention should be investigated for ischemic colitis.

Plain abdominal radiograms usually show nonspecific, air-filled bowel dilation. The specific finding of thumbprinting (Fig. 113.4) is found in less than 25% of cases [105]. Colonoscopy or flexible sigmoidoscopy is the method of choice for the diagnosis of ischemic colitis, as it allows direct visualization of the mucosa and tissue sampling [106]. Colonoscopy is preferable to sigmoidoscopy because 50% of the ischemic lesions are proximal to the sigmoid colon, except in the case of ischemic injury after aortic surgery, when lesions always involve the distal colon. The endoscopic examination has to be performed with caution and minimizing air insufflation in order to avoid perforation. The mucosa of the affected segment usually appears edematous, hemorrhagic, friable, and ulcerated. When bowel necrosis is present, colonoscopy reveals cyanotic, gray or black mucosa.

Biopsies often demonstrate nonspecific findings of vascular congestion, submucosal hemorrhage, interstitial edema, inflammatory infiltration, loss of superficial cells, and intravascular platelet thrombi (Fig. 113.8). Hyalinization of the lamina propria, full-thickness mucosal necrosis, the presence of atrophic microcrypts, and hemorrhage in the lamina propria are also microscopic signs suggestive of acute ischemic injury, whereas hemosiderin deposition, along with transmural fibrosis and mucosal atrophy, is a pathognomonic finding of the disease in its chronic phase [107].

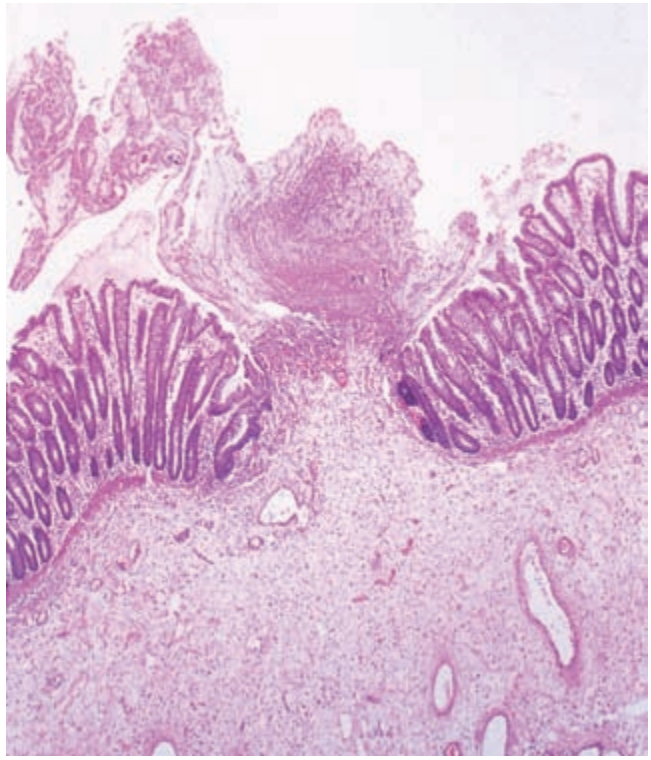

Figure 113.8 Intestinal ischemic damage. Well-delimited ulceration of the mucosa, with formation of a pseudomembrane. The remaining mucosa is normal. The submucosa has a marked edema and discrete infiltration by inflammatory cells.

Barium enema may be helpful also for the diagnosis of ischemic colitis, with a sensitivity that approaches 80% [108]. Thumbprinting is the most characteristic finding, but ulcerations, mural deformity, sacculation, and transverse ridging may also be seen. CT scan is not usually performed for diagnosis of ischemic colitis, but it can show significant alterations of the bowel wall such as circumferential, segmental wall thickening with fold enlargement, lumenal narrowing, and polypoid filling defects, and it may be helpful to exclude other diseases [109].

Angiography is not usually indicated for the evaluation of ischemic colitis because, by the time of clinical presentation, colon blood flow may be restored. However, in cases in which distinction between ischemic colitis and AMI is not clear, when only the right side of the colon is affected, and when a more generalized ischemic disorder is suspected, CT or MRI angiography must be performed. In some cases, color duplex ultrasonography is able to distinguish ischemia from inflammatory bowel disease [110], and absence of arterial flow on Doppler sonography may be a better early predictor of outcome than clinical and laboratory findings [111].

Differential diagnosis

It is not uncommon that the diagnosis of ischemic colitis is established only when repeated clinical, roentgenographic, or endoscopic studies are performed after symptoms appear. This is because the signs and symptoms of ischemic colitis overlap with those of other colonic diseases or other mesenteric vascular diseases. Infectious colitis, inflammatory bowel disease, colon cancer, radiation colitis, diverticulitis, NSAID-induced colonic lesions, and pancreatitis should be considered.

Some pathogens, such as *Escherichia coli* O157:H7 and cytomegalovirus, may cause vascular damage in the colon leading to hemorrhagic colitis, which can be easily misdiagnosed as ischemic colitis [112,113], i.e., ischemia may be a condition associated with these infections [114]. Pseudomembranous colitis caused by *Clostridium difficile* may also be mistaken for ischemic colitis [115]. Therefore, stool analysis for bacteria, ova, and parasites and the assay for *C. difficile* toxin should be performed in some cases.

The differential diagnosis between ischemic colitis and inflammatory bowel disease can be difficult in some cases [116]. Data supporting the diagnosis of ischemic colitis are age of onset, segmental distribution of injury, abrupt transition between damage and undamaged mucosa, rectal preservation (useful only for distinction from ulcerative colitis), and rapid resolution of lesions in subsequent colonoscopies [117]. In elderly people, the initial distinction between ischemic and ulcerative colitis may be more difficult. Because of its segmental involvement and rectal sparing, differentiation between ischemia and Crohn's colitis can also be difficult, although terminal ileum involvement and the presence of fistulas or colonic granulomas may help to diagnose Crohn's disease.

Radiation colitis can be easily suspected in patients with antecedents of such treatment, and colon cancer is differentiated mainly by pathological examination of colonic biopsy specimens [118]. Diverticulitis can mimic ischemic colitis, but CT is helpful to identify colon diverticula as well as mural thickening, pericolonic inflammation and eventually fistula or abscess induced by diverticulitis [119].

In the last decades, NSAID-induced colonic lesions have been identified more frequently than previously suspected. Abdominal pain and diarrhea with or without blood loss is the most common complaint of patients with such colonic lesions. Nonspecific inflammatory changes and/or erosions and diaphragm-like strictures may be seen at endoscopy, and histology may reveal findings similar to those of ischemic colitis [120].

Treatment

Patients with reversible or transient ischemic colitis manifested by mild symptoms and signs do not need specific therapy because the event usually resolves spontaneously. In patients with more severe or persistent symptoms, bowel rest and general supportive measures, including parenteral fluids, are recommended. Precipitating factors should be corrected. This includes stopping any medication with potential vasoconstrictor effects on the mesenteric vasculature or

narcotics that may worsen colonic distention. Measures should be implemented to correct low-flow states, which can be due to either hypovolemia or cardiac dysfunction. Intravenous infusion of broad-spectrum antibiotics are usually recommended [121], although no definitive clinical evidence is available of the beneficial effects of such a policy. Antibiotic use is based in the theoretical protection against bacterial translocation that occurs in severe colonic ischemia, and for early treatment of sepsis that follows massive colon gangrene [122]. In patients treated medically, endoscopy should be repeated during hospitalization to confirm diagnosis and to document recovery.

Patients showing evident signs of peritonitis require urgent surgery. Surgery is also warranted in patients in whom severe symptoms, such as abdominal tenderness or fever, or laboratory findings, such as leukocytosis or metabolic acidosis, persist or deteriorate despite medical treatment [104]. Other infrequent indications for surgery are massive bleeding, persistent ischemic lesions with protein-losing colopathy, and recurrent sepsis in a patient apparently recovered from an acute episode of ischemic colitis. In any case, segmental colectomy of the involved area should be performed and the resected specimen should be opened in the operative room to assess the extent of mucosal injury. If surgical margins are involved, an additional segment should be resected until margins appear normal. Areas of questionable viability in the colon should also be resected [90,95]. The use of intraoperative laser Doppler flowmetry has been proposed to ascertain the viability of colonic segments [123].

In patients submitted to abdominal aortic graft surgery, several strategies have been tested to improve rectocolonic microcirculation during and after surgery, hoping to prevent postoperative colonic ischemia. These include intravenous infusion of prostaglandin E_1 [124], and rectal administration of short-chain fatty acids [125].

In cases of chronic evolution of ischemic colitis, surgery is required only when colonic stenosis becomes symptomatic [126]. Segmental resection is the procedure of choice in such cases, although transendoscopic ditation may be an alternative in selected patients with symptomatic colonic stenosis in whom surgery would be high risk.

Unusual causes of mesenteric ischemia

Obstructing diseases

An uncommon and controversial cause of mesenteric ischemia is the compression of the celiac artery and eventually the superior mesenteric artery by the median arcuate ligament, especially in young, thin, female patients. It is manifested by upper abdominal pain induced by food intake and associated with weight loss. Diagnosis is made by angiography, duplex sonography, CT, or MR angiography. Surgical division of the obstructing diaphragmatic fibers and denervation of the celiac ganglion, with or without celiac artery dilatation or reconstruction, is the recommended treatment [127].

When a segment of the large bowel is obstructed, the adjacent vasculature could be compromised, thus producing regional colonic ischemia. This phenomenon, which can be manifested as an acute or chronic event, may be observed in some cases of sigmoid adenocarcinoma or small bowel carcinoid tumors, incarcerated hernia, sigmoid or cecal volvulus, intussusception, peritoneal adhesions, retroperitoneal fibrosis, neurofibromatosis, or cancer infiltrating the mesentery [90,128].

Vasculopathies

Several vasculitides may induce mesenteric ischemia. Polyarteritis nodosa (PAN) is characterized by necrosis of small or medium-sized muscular arteries with the eventual development of aneurysms. PAN may commonly involve the mesenteric circulation. In patients with PAN, gastrointestinal bleeding and bowel infarction with or without perforation are common events with very poor prognosis (mortality approximately 75%–100%). Recurrent abdominal pain and steatorrhea with weight loss are also manifestations of this syndrome. The erythrocyte sedimentation rate is usually significantly increased and diagnosis is confirmed by characteristic angiographic findings [129]. Treatment with corticosteroids and cyclophosphamide provide the best results [130]. Bowel rest, nasogastric decompression, and parenteral nutrition are often needed. Early surgery is indicated when perforation or obstruction occurs.

Unusual cases of mesenteric ischemia have been described in systemic lupus erythematosus and in the antiphospholipid syndrome [131]. Although infrequent, intestinal ischemia may be seen also in some cases of Takayasu and Buerger diseases [132,133], the latter usually presenting in young men who are heavy smokers. An increased risk of thrombotic events has been documented in Behçet and Crohn's diseases [134,135], and primary or secondary amyloidosis may be associated with intestinal ischemia [136].

Hematological disorders

Mesenteric ischemia can be associated with hypercoagulability states, mainly thrombocytosis, polycythemia, antithrombin III deficiency, antiphospholipid syndrome, proteins C or S deficiencies, 20210A prothrombin gene mutation, and factor V Leiden mutation [137–141]. The diagnosis of hypercoagulability in the presence of mesenteric ischemia is crucial to prevent further episodes by means of anticoagulation.

Drugs

Several drugs may precipitate or promote mesenteric ischemia. Estrogen–progesterone combinations used as contraceptives have been described as precipitating factors for intestinal ischemia [142]. However, the frequency and the severity of the contraceptive-related ischemic events have decreased

over time, probably because of more careful use of these drugs in patients at risk or because of a lower dose of estrogen included in the new generation of contraceptives. If mesenteric ischemia occurs in a young woman without any other risk factors, past or current use of oral contraceptives should be investigated.

During the last decade, an increased frequency of gastrointestinal events associated with cocaine use has been reported, including gastric ulcerations, intestinal ischemia, perforations, and retroperitoneal fibrosis [100]. Similar complications have also been documented in crack users (the free-base form of cocaine) [143]. The mechanism by which cocaine produces ischemia is uncertain, but it is known that it blocks the reuptake of norepinephrine and enhances the flux of calcium across the endothelial cell membrane, two pathways leading vasoconstriction [143,144]. The interval between drug ingestion and onset of symptoms varies from 1 h to 2 days, and abdominal pain, tenderness, and bloody diarrhea are the most common forms of presentation. Surgery is required in most cases to correct perforations or remove necrotic bowel segments.

Vasopressin used as a therapeutic agent for bleeding esophageal varices is associated with significant risk of systemic and regional ischemic events [145]. Indirect signs of transient intestinal ischemia are seen in some patients treated with vasopressin infusion as a consequence of its vasoconstrictor effect in the splanchnic vascular bed. This drug also reduces gastrointestinal oxygen extraction [146].

Ischemic colitis has been described associated with the treatment of irritable bowel syndrome with alosetron, a selective 5-HT3 antagonist [147]. Ischaemic jejunal vasculitis has been observed during treatment with peginterferon alfa-2b and ribavirin for hepatitis C virus [148]. Other drugs with possible effects promoting intestinal ischemia include ergotamine, digitalis, methylsergide, pseudoephedrine, barbiturates, and amphetamines [90].

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I MISCELLANEOUS

114 Radiation injury in the gastrointestinal tract

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The first reports of radiation-induced injury to the gastrointestinal tract date to the early 20th century shortly after the discovery of X-rays in 1895 [1]. The use of ionizing radiation for therapy of malignancies also dates back to the early 20th century and arose out of the observations of Bergonie and Tribondeau, who suggested that "X-rays are more effective on cells which have a greater reproductive activity; the effectiveness is greater on those cells which have a larger dividing future ahead . . ." [2,3]. They went on to reason that radiation could be effective in destroying tumors while sparing many normal tissues. Although this concept may be somewhat simplistic in today's terms, it led to the expectation that side effects would occur in tissues, such as the gastrointestinal epithelium, that undergo rapid cell turnover.

The use of multimodality therapies that include radiation has become commonplace in treating many malignancies; in fact, about half of patients with cancer receive radiation therapy as a component of their treatment. Modern techniques for tomographic localization and fractionation of radiation therapy have significantly reduced short-term and long-term gastrointestinal morbidity resulting from radiation therapy. Nevertheless, most patients experience gastrointestinal symptoms associated with acute radiation therapy, such as diarrhea, abdominal pain, bloating, tenesmus, and bleeding [4]. Chest pain, dysphagia, and odynophagia may be seen when the radiation fields involve the upper gastrointestinal tract. Generally, the acute side effects of therapy can be treated with antispasmodics, antidiarrheals, and topical anesthetics. As a rule, these symptoms resolve shortly after radiation treatment ends. However, up to one-fourth of patients who receive radiation therapy also develop some form of chronic injury, defined as symptoms presenting more than 3 months after completion of therapy [4]. Symptoms are usually evident within the first 2 years after initiation of therapy; however, some patients do not develop symptoms for years or even decades [4]. Thus, it is likely that gastroenterologists will continue to encounter and treat patients with acute or chronic radiation injury in their practice.

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Radiation pathobiology

High-energy photons from gamma irradiation, which is used for most types of radiation therapy, can interact with atoms in a number of ways [5]. The most prominent effect leading to radiation-induced injury is Compton scattering, first described by A.H. Compton in 1922. Compton scattering occurs when a gamma photon collides with an outer orbital electron, loses energy, and is deflected so that the energy lost by the photon is imparted to the electron, which is then ejected from the atom. These ejected Compton electrons subsequently cause many of the biological sequelae of irradiation by secondary ionizations of other atoms or through generation of other highly reactive species, such as free radicals. Such radiochemical interactions can damage a variety of cellular constituents, including DNA, proteins, and lipids. Of these, induction of single- and double-stranded breaks in DNA appears to mediate most of the lethal effects of radiation on cells. Although many cellular pathways for repairing such potentially lethal genetic damage have evolved in humans, failure to repair even a single double-stranded break in DNA may result in the death of that cell.

The amount of radiation-induced damage produced in a tissue or tumor is dependent on the energy of the gamma rays or X-rays, on the absorbed dose of radiation, and on the fractionation schedule of the administered dose of irradiation [3,5,6]. The gray (Gy) has supplanted the rad as the accepted unit of absorbed radiation dose and is defined as 1 joule of energy deposited per kilogram of absorbing material. One gray is a equivalent to a dose of 100 rad. The dose of radiation absorbed by a biological tissue is variable and related to the biochemical properties of the tissue, including tissue oxygenation, the activity of pathways that detoxify reactive

oxygen species or scavenge free radicals, and the position of cells within the cell cycle. Cells in early S phase or those undergoing mitosis appear to be most sensitive. A minority of DNA damage occurs through direct interaction with high-energy Compton electrons. However, these electrons can interact with other molecules present in the cell, including water and molecular oxygen, to generate peroxides, free radicals, and other reactive oxygen intermediates that are highly reactive with DNA and other cellular macromolecules. Unfortunately, malignant cells that are relatively hypoxic within solid tumors may be resistant to the lethal effects of photon irradiation.

Physical characteristics of the patient, previous abdominal surgeries, and concurrent medical illnesses can also influence the amount of radiation-induced damage to the alimentary tract. Predisposing factors for radiation injury have been identified: higher doses of radiation, patients whose intestines are fixed within the exposed region by adhesions from previous surgery, and those with a narrow anteroposterior diameter are at greater risk [7]. Preexisting vascular disease within the radiation field may predispose patients to increased radiation-induced vascular injury and subsequent ischemia (see "Pathology" below). Thus, patients with diabetes, hypertension, and atherosclerotic disease are all more prone to radiation enteritis [8].

Inflammation may also modify the acute response to irradiation through growth factors, cytokines, and other inflammatory mediators produced at sites of tissue injury. For example, fibroblast growth factor-2 (FGF-2), prostaglandin E_2, tumor necrosis factor-α (TNF-α), TGF-β3, interleukin-1 (IL-1), and IL-11 all decrease the sensitivity of tissues to radiation injury, suggesting that these mediators may have an adaptive function in cell survival [9–15]. Interleukin-12 has a mixed effect, protecting bone marrow-derived cells but sensitizing gut epithelial cells to radiation injury [13,16,17]. Activation of nuclear factor kappa B (NFκB), an intracellular regulator of many inflammatory responses driven by bacterial-derived macromolecules, appears to protect selectively the small intestinal epithelium against ionizing radiation-induced damage [18]. A study has suggested that the lumenal microbiota can also exacerbate radiation injury through induction of an epithelial derived protein (Fiaf) that increases sensitivity of endothelial cells and lymphocytes to radiation-induced apoptosis [19]. Thus, the interplay of the lumenal microbial environment with the mucosal immune system is complex and the effects of subsequent inflammatory responses on the biological response to therapeutic irradiation are difficult to predict.

Concurrent pharmacological therapy may also alter the response of both normal and neoplastic tissue to gamma irradiation. Tissue is more susceptible to radiation-induced injury when the radiation is administered together with chemotherapeutic agents or other pharmaceuticals that sensitize the gut. For instance, the combination of gamma irradiation with administration of chemotherapeutic agents that alter cell cycle kinetics or lead to synchronization of the replicating cell populations may potentiate damage to normal tissues or to tumor cells because the degree of radiation-induced damage varies with cell-cycle phase (see above). Therefore, the timing of irradiation in relation to administration of these chemotherapeutic agents is an important factor in determining the relative effects on tumor versus normal tissue. Halopyrimidines, such as fluorouracil, fluorodeoxyuridine, and iododeoxyuridine, may sensitize tumors both by inhibiting effective DNA repair and by increasing the amount of radiation-induced DNA damage [20,21]. Adjuvant therapy combining fluorouracil and radiation therapy for resected gastrointestinal tumors has improved survival in patients with pancreatic, rectal, and esophageal cancers [21–24]. Actinomycin D, doxorubicin, and bleomycin also sensitize cells to radiation-induced DNA damage and cell death.

Drugs with radioprotective activity are under investigation. These compounds have the potential to improve the therapeutic index for radiation therapy. For example, the prostaglandin analogues misoprostol and dimethyl-prostaglandin E_2 both decrease radiation-induced apoptosis in rapidly replicating intestinal epithelial cells and enhance epithelial stem cell survival after irradiation [9,12]. Data for aminosalicylates give mixed results [25].

A number of growth factors and cytokines, including FGF-2, HST-1 (FGF-4), keratinocyte growth factor, transforming growth factor-β3 (TGF-β3), and IL-11, also have radioprotective activity in the intestine if administered before irradiation [11,15,18,26–28]. Free radical scavengers, such as amifostine (WR-2721), have been shown to be radioprotective in both experimental systems and clinical trials [29]. Whether these radioprotective agents will find a role in routine clinical practice remains to be established.

Because DNA damage is thought to be the primary mechanism mediating the lethal effects of radiation [5,6], a number of complex intracellular systems have evolved to repair DNA damage induced by radiation as well as damage induced by chemotherapeutic agents. Some of these proteins are specifically involved in recognition and repair of DNA damage. Other enzymes, such as DNA-dependent protein kinase, are involved in DNA replication and V(D)J recombination during immunoglobulin gene rearrangement in normal lymphocytes. These enzymes also play a central role in rejoining double-stranded DNA breaks induced by radiation injury in epithelial cells in the gastrointestinal mucosa [6,30,31].

The fate of injured cells after irradiation is a complex process. Cells that cannot repair radiation-induced DNA damage adequately will eventually undergo "mitotic cell death" or mutation because of genomic instability as a consequence of the unrepaired DNA strand breaks and other DNA damage [6]. However, in rapidly replicating cell populations, DNA damage induced by relatively small doses of irradiation can also trigger apoptotic cell death, an active, genetically pro-

grammed type of cell death. In both humans and rodents, a low frequency of physiological apoptosis occurs continuously within the lower regions of the crypt epithelium of the normal small intestine and colon, and may be an inherent part of the regulatory mechanism determining stem cell numbers in the normal adult tissue [32,33]. After even relatively low doses (about 1 Gy) of ionizing radiation, many damaged cells within the rapidly dividing crypt epithelium of the small intestine and colon undergo apoptosis [32–34]. The highest rates of radiation-induced apoptosis occur in the lower two-thirds of the crypt, a region containing the epithelial stem cells and the more rapidly replicating progenitor or "transit" cell population; this apoptosis reaches a maximum 6–12 h after irradiation. Many of the remaining cells within the proliferative zone that do not undergo apoptosis arrest at checkpoints in G1 or in late G2 phases of the cell cycle [6,34,35]. These cell-cycle checkpoints are thought to provide time for repair of DNA damage and prevent cells from entering S phase or mitosis, when the presence of even a few double-stranded DNA breaks may result in death of the cell or generation of cells with chromosomal abnormalities.

The tumor suppressor gene, *p53*, plays an important role in recognition of DNA damage and coordinating the subsequent cellular response to radiation injury, leading some investigators to describe *p53* as the "guardian of the genome" [36–38]. It is normally expressed at low levels except during DNA synthesis. Following DNA damage, expression of *p53* is upregulated and the p53 protein is translocated to the nucleus, where it binds to DNA and regulates the transcription of a number of genes, including $p21^{CIP1,WAF1}$, *GADD45*, *MDM2*, and *BAX* [39–41]. These genes are involved in regulating apoptosis or cell-cycle arrest at checkpoints at the G1–S boundary or in G2/M [42–44]. Activation of *p53* occurs rapidly in the intestinal crypt epithelium following even low doses of gamma irradiation (0.5–1 Gy). This activation results in a rapid and sustained increase in the transcription of *BAX*, a proapoptotic member of the *Bcl-2* oncogene family that mediates the apoptotic response. The ultimate fate of a cell with DNA damage also depends on the expression of other counterregulatory genes that affect apoptosis. Expression of these counterregulatory genes varies along different regions of the alimentary tract and may also depend on extracellular signals. For example, antiapoptotic members of the Bcl-2 family, including BCL-2 and BCL-X_L, suppress apoptosis by formation of heterodimers with BAX. *BCL-2* appears to be expressed in the colonic crypts, but not in small intestinal crypt epithelial cells, which explains regional differences in the sensitivity of crypt epithelial cells to radiation-induced apoptosis.

Activation of *p53* in response to DNA damage causes cells to arrest at critical checkpoints [6,36,37,43], a process that is mediated by induction of $p21^{CIP1,WAF1}$, *GADD45*, and other gene products. This arrest allows time for the cell to make a decision as to its fate (repair vs cell death) before committing itself to S phase or mitosis. Entry into S phase with damaged DNA or into mitosis in the presence of single-stranded or double-stranded DNA breaks could lead to daughter cells with unstable chromosomal structure, allowing mutations or chromosomal rearrangements to occur. Other genes, such as the gene responsible for ataxia telangiectasia (*ATM*), are thought to regulate the response to DNA damage upstream of *p53*. Thus, cells from these patients have diminished activation of *p53*, decreased cell cycle arrest at the G1–S cell-cycle checkpoint, an increased sensitivity to ionizing radiation, and increased malignant transformations.

The clonogenic stem cell populations within the intestine and colon appear to be more resistant to radiation-induced cell loss than the more rapidly replicating transit cells. Furthermore, the survival of these clonogenic stem cells may be determined by a mechanism independent of *p53* function [45]. The increased survival and proliferation of the stem cells with radiation-induced DNA damage probably enhances the capacity for restitution of the epithelial function in the short term but also may contribute to the potential for secondary malignancies many years after therapeutic irradiation (see "Pathology" below).

Pathology

The histopathological changes in the alimentary tract follow a predictable time course and may be functionally divided into early and delayed clinical syndromes [46]. The early effects primarily involve the mucosa, which is lined by rapidly proliferating epithelial cells that are sensitive to the acute effects of radiation injury. Clinical symptoms include odynophagia, diarrhea, nausea, vomiting, or gastrointestinal bleeding, depending on the location of the radiation field, the dose of radiation, and the fractionation schedule. The delayed effects are more likely to be chronic diarrhea, fibrosis, ulcer formation, or bleeding and are secondary to damage to the vasculature of the organs involved. Although the histological hallmarks seen in tissue specimens are often characteristic of acute or delayed radiation injury, no histological feature is pathognomonic for radiation-induced damage [46].

The histological and pathological features of acute radiation injury in the human alimentary tract are poorly defined because the acute effects of radiation therapy are generally well tolerated and tissue samples are rarely obtained. Thus, much of what is known about the acute effects of irradiation has been derived from a few studies in humans and more extensive experiments using animal models of radiation injury [46–51]. The earliest recognizable histological features following radiation injury to the gastrointestinal tract occur within hours, and include apoptosis of lamina propria lymphocytes and epithelial cells, the cessation of epithelial cell replication, and damage to vascular endothelial cells. This results in loss of replicating cells in the squamous epithelium

of the esophagus and the columnar epithelial cells in the gastric, small intestinal, and colonic epithelium. Mature, differentiated epithelial cells continue to be lost by extrusion, and in the absence of replacement by replication of the progenitor cells, there is subsequent loss of mucosal function: attenuation and damage to the squamous epithelium in the esophagus; reduced numbers of parietal and chief cells in the gastric epithelium, which will decrease gastric secretion; and progressive loss of the small intestinal villous epithelium, which decreases absorptive surface area, and in some patients leads to transient malabsorption of fats and other macromolecules, or acute diarrhea. In the colon, there are reduced numbers of mitoses and epithelial cell atypia with loss of nuclear polarity in the columnar epithelium lining the crypts. Swelling and loss of goblet cells leads to decreased mucus production. Microabscesses composed of granulocytes and eosinophils may be present. Mucosal and submucosal edema may also be observed in both the small intestine and colon.

An increase in the replicating crypt epithelial cell population in the small intestine and colon, through proliferation of stem cells located near the base of the crypts, is observed beginning about 3 days after low doses of irradiation. This increase in crypt epithelial proliferation can compensate for the initial loss of differentiated epithelial cells [34]. However, the survival of the stem cell population is compromised at higher single doses of gamma irradiation, limiting epithelium repair. Fractionated irradiation protocols enable higher total doses to be administered by allowing repair and recovery of replicating epithelial cells and stem cells between fractions [6]. If damage to the stem cell population is severe after high doses of radiation, then epithelial integrity and barrier function are lost, leading to translocation of lumenal bacteria and eventual death from loss of fluids and serum proteins, hemorrhage, and sepsis 7–10 days after irradiation. Although rarely, if ever, seen in therapeutic applications of gamma irradiation, death from this syndrome has been observed after exposure of individuals to a single whole-body dose of 10–20 Gy as a consequence of radiation accidents or atomic warfare [46,52].

Liver biopsies are rarely obtained from patients with acute hepatic injury secondary to therapeutic irradiation for solid neoplasms. However, hepatic venoocclusive disease can be seen in bone marrow transplant recipients after cytoreductive therapy with combined chemotherapy and irradiation (see "Liver" below) [53–55], usually occurring before day 30 posttransplantation [54]. Hepatic histology shows vascular congestion (which is most prominent in centrilobular areas), subendothelial edema, endothelial destruction, sinusoidal dilation, and centrizonal hepatocyte necrosis with attenuation of the hepatocellular cords [53]. Deposition of clotting factors in the walls of hepatic venules suggests that microthrombus formation may be the primary pathogenic mechanism [56]. Occlusion of central veins by thrombus is sometimes observed. As the disease progresses, deposition of collagen in hepatic sinusoids, fibrosis of hepatic venules, and sclerosis of venular walls may be seen [53,54]. One proposed mechanism for the increased sensitivity to combined chemotherapy and irradiation in this setting involves depletion of glutathione stores in sinusoidal endothelial cells and hepatocytes [54,57–59].

The delayed effects of radiation injury most commonly occur from 6 months to 5 years after radiation therapy; however, symptoms may appear as early as 3 months or as late as 30 years after radiotherapy in a small number of patients [46,60,61]. Vascular injury and regeneration are characteristic of chronic radiation injury and are often observed in pathological specimens [46]. These findings are sometimes focal and may be missed unless multiple levels through the specimen are examined. Myointimal proliferation in medium-sized muscular arteries may lead to chronic ischemic injury because of the marked decrease in the lumenal diameter of these vessels (Fig. 114.1). Ulceration of the overlying mucosa occurs in areas of localized ischemia (Fig. 114.2). Thrombosis is sometimes seen associated with these lesions and is more frequent in vessels with fibrinoid necrosis adjacent to deep ulcerations. The presence of lipophages or foamy macrophages in the intima of small arterioles is also a characteristic finding of delayed radiation injury, although this lesion may also result from other etiologies (Fig. 114.3). Telangiectatic vessels are frequently found in the submucosa and probably account for the diffuse bleeding sometimes observed in radiation enteritis (Fig. 114.4). Sclerosis or medial fibrosis indicative of healing vasculitic lesions may also be observed.

Changes in the submucosa are frequently observed in delayed radiation injury and are thought to be secondary to the chronic vascular damage described earlier [46]. Increased collagen deposition and fibrosis are often observed with

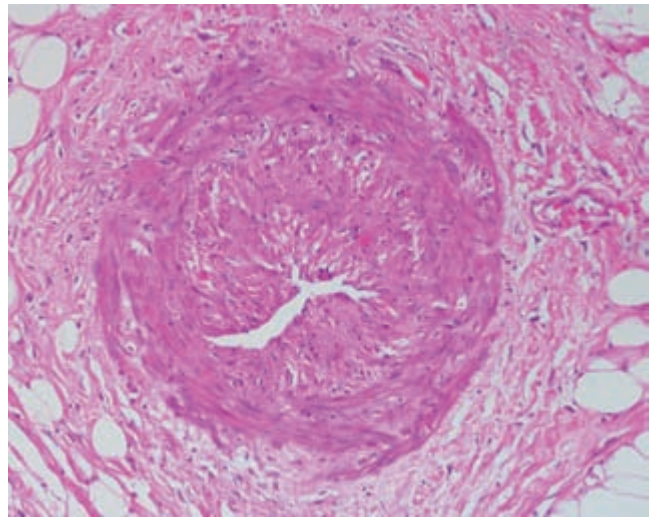
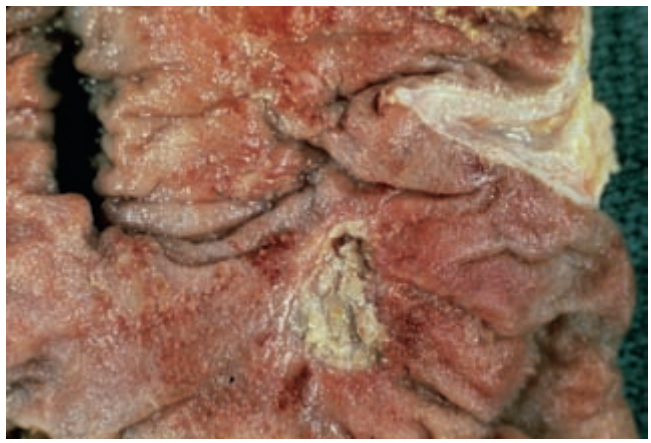
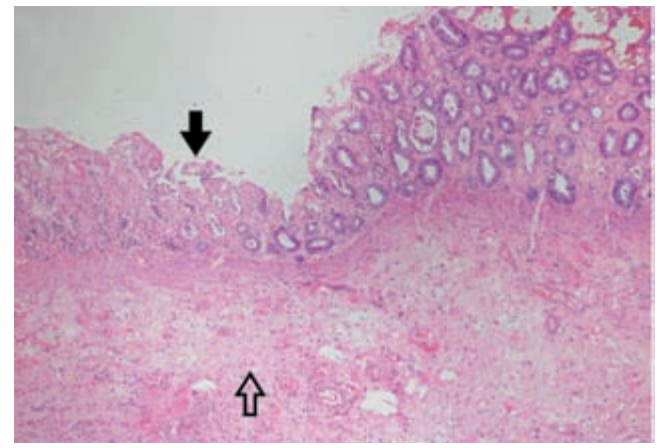


Figure 114.1 Myointimal proliferation nearly occludes the lumen of a moderate-sized mesenteric artery. This process usually occurs over several years after radiation injury and may lead to chronic ischemic injury to the intestine. (Original magnification × 100.) Courtesy of Dr Christopher Moskaluk.

(a)





(b)

Figure 114.2 Radiation-induced ulceration in the colon. **(a)** Gross appearance of a well-demarcated ulcer present in the rectum years after external radiation for an adjacent neoplasm. **(b)** Histological appearance with chronic ulceration, mucosal necrosis (solid arrow), and dense submucosal fibrosis (open arrow) similar to that seen in ischemic injury. The lesion is notable for the absence of a prominent inflammatory infiltrate. (Original magnification × 20.) Courtesy of Dr Christopher Moskaluk.

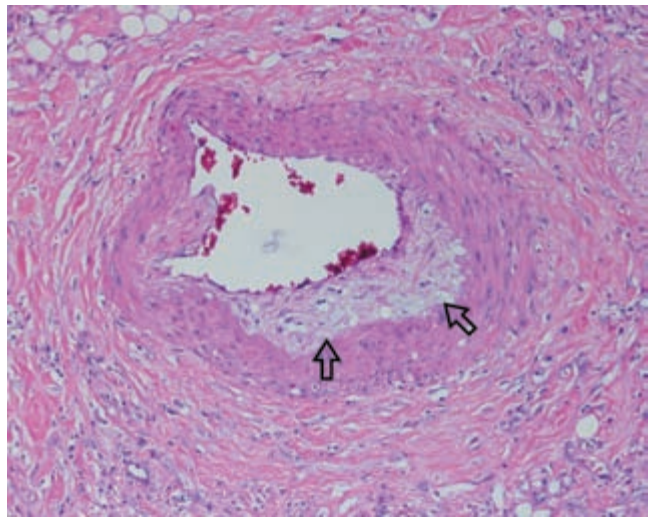


Figure 114.3 Intimal lipophage accumulation in intestinal arteriole after irradiation (arrows). These foam cells may be seen in the intima of small arteries and arterioles of the intestine several years after irradiation and may lead to lumenal narrowing of these vessels. (H & E stain; original magnification × 100.) Courtesy of Dr Christopher Moskaluk.

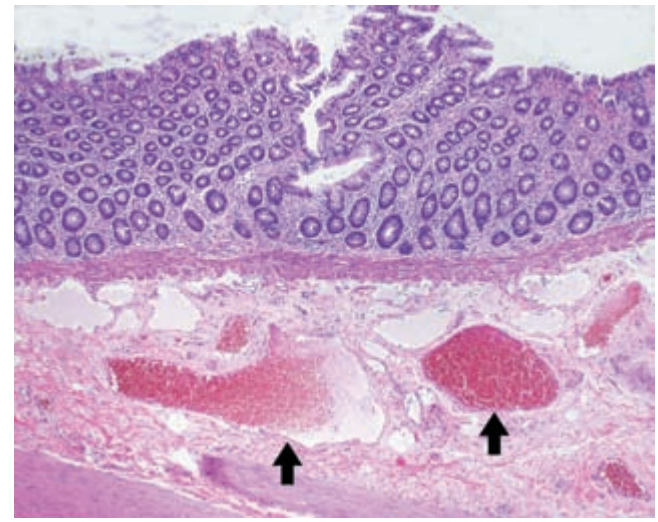


Figure 114.4 Submucosal telangiectasias (arrows) are seen in delayed radiation injury. Dilated venules and lymphatic channels are seen in the submucosa underlying relatively normal-appearing colonic epithelium. (H & E stain; original magnification × 40.) Courtesy of Dr Christopher Moskaluk.

minimal cellular inflammatory infiltrate. The fibrosis may be confined to the submucosa or may extend through the muscularis propria and account for the thickening or strictures that may occur within any irradiated region of the alimentary tract. Atypical fibroblasts with bizarre nuclei are sometimes found in areas of fibrosis (Fig. 114.5). The appearance of these fibrotic lesions in the small intestine may resemble that of Crohn's disease, both on gross inspection and on radiological examination, although fistulae and creeping fat are rarely observed.

Mucosal alterations in the small intestine include villous atrophy, flattening of epithelial cells, and fibrosis of the lamina propria [46]. These findings are often observed in patients with or without symptomatic radiation enteritis and may result in malabsorption when these changes are extensive. Superficial erosions and mucosal ulceration are common, sometimes penetrating deep into the submucosa and muscularis propria and occasionally perforating into the peritoneal cavity. Chronic changes in the mucosa of the colon are similar to those seen in the small intestine.

Colitis cystica profunda is a rare delayed complication of radiation injury [46]. The importance of this lesion is in distinguishing it from invasive adenocarcinoma. Its symptoms are similar to colitis cystica profunda secondary to other

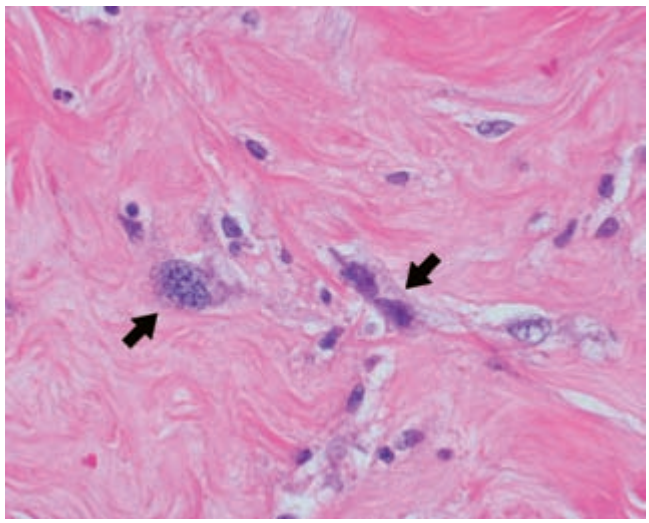


Figure 114.5 Atypical fibroblasts in radiation injury (arrows). Bizarre-appearing, pyknotic fibroblasts are frequently seen in the delayed phase of radiation injury in the alimentary tract. Although these atypical fibroblasts are frequently observed, their presence is not specific for radiation injury. (H & E stain; original magnification × 400.) Courtesy of Dr Christopher Moskaluk.

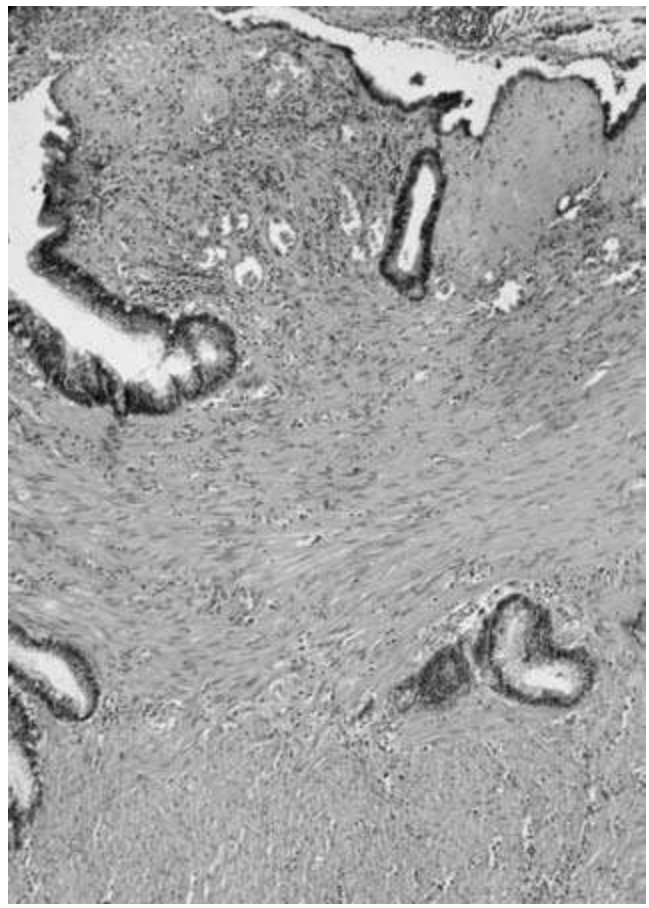


Figure 114.6 Colitis cystica profunda. Colonic glands are seen penetrating the muscularis mucosa into the submucosa and muscularis propria. This lesion is distinguishable from carcinoma by the normal cytological characteristics of the epithelium lining the penetrating glands. (Original magnification × 40.) Courtesy of Dr Christopher Moskaluk.

etiologies. However, its histological appearance may differ from colitis cystica profunda of other causes in that colonic glands extend deep into the submucosa and sometimes penetrate into the muscularis propria [46,61] (Fig. 114.6). The presence of glands lined by normal epithelium usually makes it easy to distinguish colitis cystica profunda from adenocarcinoma in pathological specimens.

The development of secondary epithelial or stromal malignancies is also a potential complication of radiation therapy [46,62–64]. However, the number of documented cases is relatively small, and most of these cases generally have occurred after lower doses of gamma irradiation (< 10 Gy). For example, the frequency of colon cancer is increased in atom bomb survivors 30 years after their exposure (relative risk of 2.4 compared with a matched control population), and an increased risk for colon cancer has been documented in a cohort of women irradiated with relatively low doses (6.25–10.5 Gy) for benign uterine bleeding [46,65–67]. Thus, whereas exposure of the colon to radiation may increase the risk for developing a secondary malignancy, the interval between therapeutic irradiation and development of secondary malignancies may be relatively long [46,65,66].

Specific organ involvement

Esophagus

Radiation therapy for thoracic tumors and hematological malignancies can result in both acute and long-term esophageal injury. The field required to treat thoracic, hematological, and some pharyngeal malignancies frequently (25% in one series [68]) exposes the esophagus to sufficient radiation to produce pathological changes, but clinical syndromes generally require doses of 60 Gy [69]. This threshold may be lower in patients whose tissue is sensitized by chemotherapeutic agents. Three major syndromes related to esophageal irradiation have been described:

- acute inflammation with odynophagia or dysphagia
- fibrosis with stricture and dysphagia
- fistula formation with aspiration into the trachea, or lethal bleeding from the aorta.

During the acute phase of radiotherapy, symptoms are usually related to mucosal injury and the inflammatory response. Patients typically present with mucositis, which causes odynophagia or dysphagia beginning after the second week of treatment. Frank bleeding is unusual [70]. Acute dysphagia may also reflect a motor disturbance. A pattern

of ineffective peristalsis at and distal to the radiation field may occur 4–8 weeks after initiation of radiation therapy.

The diagnosis of acute-phase radiation-induced esophagitis is usually evident from the patient's history and symptoms, although other conditions need to be excluded. Acute infection must be considered, particularly for those whose immune systems are impaired by the underlying disease or its treatment. Pill ulceration and reflux esophagitis are also in the differential diagnosis. Endoscopic evaluation is the test of choice, allowing characterization of the lesion and tissue sampling where appropriate. Barium swallow may offer some information in unusual circumstances when endoscopy is not clinically feasible, but these radiological studies are normal or non-diagnostic most of the time [71].

Prevention of acute radiation injury should be considered the first line of therapy. Limiting the total radiation exposure and careful selection of the radiation field are important preventive measures. Pharmacotherapy and dietary modifications have also been advocated [72]. If symptoms of odynophagia and dysphagia are sufficiently severe, temporary interruption of radiation therapy for several days to 1 week or enteral nutritional support may be necessary. Pharmacological therapy is often administered on an empirical basis and is directed at controlling symptoms; for example, topical lidocaine is generally given for odynophagia and may be compounded into a variety of "stomatitis cocktails" along with antacids, antihistamines (diphenhydramine), or antifungal (nystatin) ingredients. Antisecretory therapy is frequently used but, like these cocktails, suffers from the same lack of substantiating data [70,73].

Patients with strictures or fistulae typically present some time after radiation therapy has been completed (i.e., 6 months or later after initiation of treatment). Diagnosis and therapy of strictures and fistulae typically require a combination of radiological and endoscopic techniques. Fluoroscopic evaluation is needed to determine the site, whereas either fluoroscopic or combined endoscopic and fluoroscopic methods can be used for subsequent dilation or stent placement. The initial approach to treatment of late esophageal stenosis is careful dilation. Balloon dilation devices may be preferable to rigid dilators because they allow isolated radial expansion of the stricture with minimal axial pressure. The complication rate for the dilation of strictures induced by radiation is no greater than that for dilation of strictures with other etiologies [74]. However, stent migration of conventional plastic endoprostheses was substantially higher in the radiation group (21%) than in the nonradiation group (3%) [74].

The introduction of the self-expanding metallic stent (SEMS) has substantially changed the therapy of esophageal cancer and radiation-induced stenosis and fistula formation. Typically, a SEMS is a cylinder of wiremesh or a coil with spring memory for a particular configuration. The first was introduced in 1983 [75]; coated and uncoated varieties are available, and a variety of materials, attachments, and delivery devices are under investigation. The compromised circulation associated with radiation injury can be further worsened by the radial forces from these stents. This may account for the increased perforation rates seen in radiation strictures compared with esophageal cancers stented before or without associated radiotherapy (36% vs 2.5%), and may contribute to the increased mortality (22% vs 0%) seen in the same series [76].

The coated stent is useful for patients suffering tracheoesophageal fistulae. However, enteral nutritional support may be necessary in patients with severe acute symptoms or with difficult-to-manage chronic strictures or fistulae. In patients with uncoated SEMS, later placement of a percutaneous endoscopic gastrostomy is generally feasible because the wiremesh typically embeds securely in the esophagus. Coated SEMS may not be amenable to the same wait-and-see approach.

Stomach

Gastric damage following radiation therapy is rare, but injury can occur when the stomach lies within the radiation field of an adjacent extragastric tumor [46,77]. Gastric irradiation was used in the past as therapy for peptic ulcer disease because modest doses of radiation could significantly deplete the parietal and chief cell populations, inducing gastric atrophy [46,78,79]. In the era before effective medical therapy was available, this approach was taken for patients with refractory ulcers. A total dose of 1600–1700 cGy given in 10 divided doses resulted in atrophic gastritis with associated decreases in acid and pepsin production. However, in most, acid production returned to normal basal levels within 2 years [69,79,80].

Most patients receiving gastric irradiation are asymptomatic, although hemorrhage due to multiple gastric erosions has been reported as an early complication [46]. Gastric injury from greater exposures can present more dramatically, with stenosis, fistulae, and perforation [81]. Strictures are more common in the antrum and cardia than in the body or fundus. Delayed ulceration may also occur in this setting and is difficult to distinguish from peptic ulcer disease. Radiation-induced ulcers heal poorly because of obliterative arteriolitis within the ulcer bed. Ulceration and gastric strictures usually occur at doses exceeding 4500–5000 cGy, and about half of patients experience clinically significant gastric injury at doses greater than 5500 cGy [46].

The diagnosis is usually made on the basis of clinical presentation and patient history. Endoscopy may reveal an irregular thickened mucosa similar to the appearance of a gastric neoplasm or an appearance indistinguishable from peptic ulcer disease. Fluorographic upper gastrointestinal studies or computed tomography (CT) may be useful in demonstrating the presence of strictures. However, these studies may only show thickening of the gastric wall with an appearance similar to that seen in gastric malignancies. Data are lacking to guide therapy for this uncommon condition. In

patients exposed to less than 20 Gy, simple passage of time may suffice.

Small bowel

The small intestine is particularly susceptible to radiation injury. Significant epithelial cell death may be observed as early as 4–6 h after irradiation, even with the relatively small 1–2-Gy fractions commonly used in some radiation therapy regimens. Radiation injury to the small intestine most often occurs in the clinical setting of radiotherapy for gynecological, urological, rectal, or retroperitoneal malignancies. The degree of injury appears proportional to the radiation dosage delivered to the segment of small intestine lying within the radiation field [82,83]. The relatively fixed position of the duodenum, distal ileum, and cecum within the abdominal cavity make these regions particularly vulnerable to radiation-induced injury [6,46]. Ninety percent of patients have significant lesions at doses of more than 3000 cGy, compared with 40% at 10–30 Gy, and 20% at less than 10 Gy [84]. Previous abdominal surgery that may fix loops of bowel in place is another important risk factor [7,85,86].

Radiation injury to the small bowel can also be divided into acute injury and a chronic syndrome. Nausea, diarrhea, and abdominal discomfort are cardinal manifestations of acute injury. Although many of these symptoms are attributable to epithelial cell death and subsequent loss of mucosal function, symptoms such as nausea and vomiting occurring within a few hours of irradiation may be mediated by the production of inflammatory cytokines. Fortunately, the epithelium regenerates, and function is restored within 2 weeks, with corresponding resolution of the acute symptoms [84]. This acute syndrome may foreshadow the occurrence of chronic radiation enteritis, at least in children [87]. It is not clear whether this holds for adults, of whom about 5% develop chronic enteritis after exposure [47].

Symptoms of chronic radiation enteritis typically occur 1–2 years after exposure, but presentation 20 years after radiotherapy has been described [88–90]. The central mechanism of injury is vascular damage with progressive localized ischemia (see "Pathology" above). This leads to ulceration of the mucosa, fibrosis with stricturing, and, less frequently, fistula formation or even perforation. Radiographs of the small intestine or colon may reveal evidence of single or multiple strictures or mucosal ulceration, a pattern similar to that observed in Crohn's disease [4,91] (Fig. 114.7). The condition may also be detected with wireless capsule endoscopy, but caution is needed because of the risk of lodging the capsule in a stricture [92]. The risk of capsule lodging may be lessened by employing a capsule designed to check patency [93]. Life expectancy is poorer in patients whose enteritis is complicated by fistulae rather than bleeding [94]. Loose stools are the most common symptom and may be caused by a number of abnormalities: bile salt malabsorption in 65%, bacterial overgrowth in 45%, severe fat malabsorption in 40%, rapid intestinal transit in 35%, and lactase deficiency from villous blunting with consequent lactose intolerance in up to 20% [8,95].

Figure 114.7 Barium small bowel radiograph showing an ileal stricture (arrowheads) in a patient with diarrhea several years after radiation therapy for cervical cancer. Courtesy of Dr Hubert Schaffer.

Prostheses or mesh material may be used as a preventive measure to displace or protect the small intestine during radiation therapy [96–98]. Acute radiation injury is treated supportively and with dose adjustment [99]. Antispasmodics and medications that decrease bowel motility must be used cautiously given that many of these patients are at risk for outright obstruction from underlying tumor or surgical adhesions. Perforation may have a bland presentation in this setting; typical signs of peritonitis, such as fever and severe abdominal pain, may be absent, and adhesions may wall off free air [100,101]. The data are contradictory for the use of aminosalicylates as a preventative measure. In a prospective double-blind study of sulfasalazine (2 g/day) given to 87 patients undergoing radiation, diarrhea occurred in 55% of the sulfasalazine and 86% of the placebo groups, respectively [102]. However, in another trial, mesalazine at 4 g/day was not beneficial [103].

The treatment of chronic radiation enteritis requires patience and long-term pragmatism. Once symptomatic, this condition is persistent at best and worsens in many, even after surgical resection of diseased intestine [4]. Cholestyramine is often a good choice as both a nonspecific antidiarrheal agent and for targeted therapy of bile salt malabsorption [95]. Other antidiarrheals, such as loperamide and diphenoxylate, may also be useful. A peripheral opiate antagonist, loperamide-*N*-oxide, was used successfully in a series of 18 patients with radiation enteritis [104]. Treatment was associated with fewer bowel movements and improved bile salt absorption. Bacterial overgrowth can be treated with antibiotics, such as amoxicillin clavulanate, ciprofloxacin, or tetracycline. The immunomodulatory effects of these agents may

play a role along with their antimicrobial action, but this has not been studied in radiation enteritis.

Specialized enteral formulas, such as elemental diets, are available for treating moderate malnutrition. However, they are costly, and their superiority to standard formulas has not been established [105]. Total parenteral nutrition may be required for severe malnutrition or when enteral feeding is not feasible. Support with total parenteral nutrition allows correction of malnutrition, but recurrent symptoms are a problem in up to 47% of patients [106]. Methylprednisolone may provide additional benefit, but the long-term role of this relatively toxic agent is unknown [107].

It is prudent to avoid surgery whenever possible in chronic radiation enteritis, given the likelihood of intraoperative and postoperative difficulties. Surgery is required in about one-third of patients for management of strictures causing small bowel obstruction or for perforation. Complications are common (30%), and the mortality rate can be particularly high (> 5%) for emergency operations [108]. Outright resection of the involved small bowel segment is considered preferable to bypass because of the high incidence of complications, such as perforation, bacterial overgrowth, and fistulization [109].

Colon and rectum

Radiation proctosigmoiditis is among the most common complications of radiation injury. Many gynecological and urological tumors are treated with radiation, and the relatively fixed location of the rectum makes it more susceptible to radiation injury than the rest of the colon [110]. There is a clear dose-related incidence of injury, with acute symptoms common at doses of 30–40 Gy and with more than one-third of patients suffering injury at 60 Gy [111]. Complication rates from patient reports, rather than record abstraction, are higher still [112–115]. When a series of patients undergoing 6 weeks of radiotherapy for nongastrointestinal pelvic tumors was studied at 2-week intervals using sigmoidoscopy, biopsies, and symptom scores, histological and endoscopic abnormalities were both most pronounced after 2 weeks of therapy and improved subsequently. However, symptomatic complaints climbed consistently to the end of the 6-week observation period [116]. One-third to one-half of patients suffer acute injury with diarrhea and tenesmus during the first months of or after therapy. As a rule, these symptoms resolve over the following 6 months, but about 15% of patients continue to suffer chronic proctitis, manifested by rectal pain, diarrhea, and bleeding [114]. The degree of bleeding has some prognostic value: those patients who do not require transfusion have a high rate of spontaneous remission (70%), low rate of surgery (5%), and low mortality rate (25%); and patients who require blood transfusions or who suffer significant bowel disturbance have a low rate of spontaneous remission (< 20%), often require surgery (50%), and have substantial mortality (60%) [117].

Many medical approaches have been tried for treatment of patients with acute or chronic radiation injury involving the colon and rectum. Most attention has been paid to the unfortunate 15% of patients who suffer from chronic radiation proctitis. Unfortunately, no therapy stands out as clearly superior, and the risks of each must be weighed against possible benefits. Oral aminosalicylic acid preparations by themselves do not appear effective, although they may help if combined with steroid treatment [118]. Sucralfate enemas by themselves appear effective and well tolerated [119]. Oral sucralfate was beneficial in a series of three patients, but a larger series is needed to evaluate the oral approach [120]. Sucralfate prophylactically administered orally or rectally has not been shown to prevent radiation injury [121,122]. Short-chain fatty acids are nutrients that promote colonocyte survival and proliferation and have been advocated to accelerate healing in radiation proctitis. In one double-blind study, placebo-treated patients ultimately reached the same level of recovery at 6 months [123]. A crossover study of 20 patients showed encouraging results, but the logistics of drug preparation and the odor of short-chain fatty acids present obstacles to this therapy [124]. The potential of antioxidant therapy has also been investigated: vitamin E (400 IU three times daily) and vitamin C (500 mg three times daily) were helpful in an open-label series of 20 patients [125]. The application of formalin has been used to treat refractory bleeding from radiation proctitis through a presumed mechanism of obliteration of the telangiectatic vessels through coagulative necrosis. In one series, 50 mL of 4% formalin solution was introduced into patients under sedation and kept in contact with the mucosa for 30 s and then cleared away using saline irrigation. Five to six aliquots were used in each session. However, complications were often encountered [126]. In another series of five patients (one treated with topical application of formalin-soaked gauze), severe pain occurred in one patient and another experienced acute colitis requiring hydration and antibiotics. In another prospective study examining 11 patients treated with four 20-mL aliquots and a total of 15 minutes contact time, all patients had decreased bleeding without reported complications [127]. There is anecdotal information on outpatient instillation of dilute formalin solution using a lubricated slip-tip syringe, followed by prompt evacuation into a bedpan. Hyperbaric oxygen has been used successfully to improve the healing of ulcers in radiation proctitis [128,129].

Surgical and endoscopic treatments may be tried when bleeding from telangiectasia is the predominant manifestation of radiation injury (Fig. 114.8). It is critical to weigh potential benefit against risk in this setting. Patients who do not require transfusion generally do not require an interventional approach. Laser therapy can be associated with nonhealing ulcers and may aggravate bleeding or cause outright ulceration. Argon plasma coagulation is designed to create a more controlled level of treatment-induced injury and is

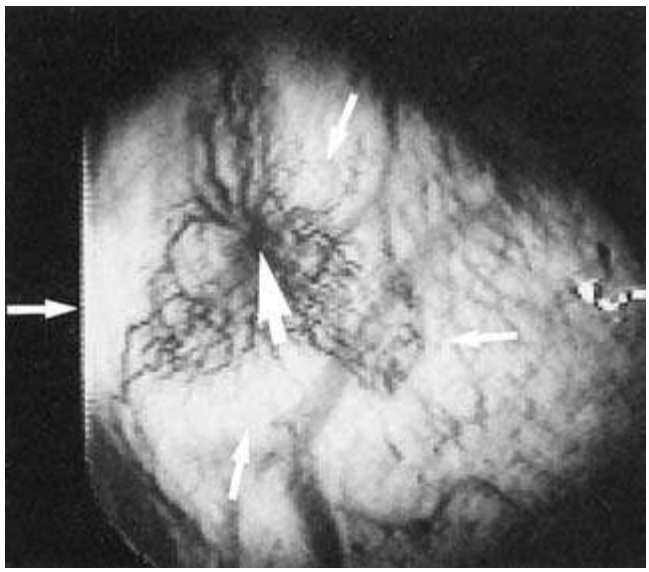


Figure 114.8 Endoscopic appearance of telangiectasia (small arrows) with prominent central arteriole (large arrow) and surrounding pallor in a patient with radiation proctitis.

used to obliterate natural gastrointestinal telangiectasias. Its introduction as an endoscopic treatment led to some enthusiasm about its use in radiation-related bleeding. Compared with treatment of classic telangiectasias from other causes, argon plasma coagulation of telangiectasias associated with radiation injury appears effective on the evidence of several small series [130–133]. Less appears to be more, as higher wattage can be associated with greater complications [134]. Settings of 25–40 W appear to work well [135,136].

Individual episodes of bowel obstruction may be treated conservatively with bowel rest, intravenous fluids, and nasogastric decompression, but recurrent obstruction or an unresponsive episode may require surgery. Surgical intervention for stricture generally consists of resection of the diseased segment, with primary anastomosis. Outcome is best if sufficient resection is performed to ensure that one margin is free from radiation injury [4,137].

Liver

Older administration techniques made liver injury common when therapeutic radiation was directed at tumors within the hepatic parenchyma. The therapeutic index using these older techniques was unfavorable, with doses of 3000–3500 cGy offering consistent injury but inadequate therapeutic effect. Thus, hepatic irradiation waned in popularity until more modern technology, such as nonaxial planes of administration and sophisticated modeling, made it possible to deliver higher doses safely [138].

Hepatic injury following irradiation typically manifests about 4–8 weeks after exposure, but may occur as early as 2 weeks or as late as 7 months. In reports from the 1960s, more than one-fourth of patients with radiation hepatitis had a clinical presentation consistent with Budd–Chiari syndrome or obstruction of suprahepatic veins [139,140]. Nonspecific symptoms, such as malaise, fatigue, and weight gain, are common. Ascites is often present but may be below the threshold for bedside detection. Right upper quadrant pain is also common, but jaundice is not, in keeping with the characteristic laboratory findings in which alkaline phosphatase levels are elevated with minimal increases in bilirubin and mild elevation of transaminases [141,142]. The acute illness is generally well tolerated and usually resolves with supportive care, but development of chronic radiation-induced hepatitis has also been described [143]. Computed tomography findings may be helpful. In one series, 23 of 31 patients (74%) showed a low-attenuation area adjacent to the hepatic tumor on tomographic scans of the targeted radiation field. A sharp, straight interface was rarely seen at the treatment margin. These findings were maximal 2–3 months after completion of therapy and persisted for up to 3 months. Atrophy in the treated segment or lobe and hypertrophy of the untreated liver were seen in several patients [144]. With proton-beam irradiation, abnormalities noted on CT scans may persist as long as 42 months [145]. Given the setting and frequency with which combined modalities of treatment are used, the differential diagnosis for this condition must always include metastatic disease and drug toxicity.

Liver injury is a particular concern with bone marrow transplantation, in which the combination of total-body irradiation and high-dose chemotherapy for initial cytoreductive therapy is commonly used. Venoocclusive disease, presenting with weight gain (fluid retention), jaundice, and tender hepatomegaly, is common, occurring in 10%–60% of patients within several weeks after transplantation [54,146,147]. Weight gain and increasing liver size with tenderness may herald the onset of venoocclusive disease and usually precede the development of hyperbilirubinemia [54]. Serum aminotransferases may also be elevated. Mortality rates from venoocclusive disease in this setting range from 0% to 67% [54]. Supportive treatment with diuretics and careful fluid and electrolyte management are usually effective in mild to moderately severe venoocclusive disease. Treatment of severe cases of venoocclusive disease has been less successful.

Chemotherapeutic regimens that potentiate hepatic injury when combined with therapeutic irradiation include mitomycin C, MOPP (nitrogen mustard, vincristine, procarbazine, and prednisone), CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone), CCNU [2-chloroethyl-3-cyclohexyl-1-nitrosurea (lomustine)], and pro-MACE (prednisone, methotrexate, doxorubicin, cyclophosphamide, etoposide) [148,149]. Injury from combined therapy is clearly more severe than that seen from radiation alone and carries an overall mortality rate of 30%–50%.

Treatment of patients with isolated radiation injury to the liver is generally supportive. Salt restriction and diuretics are often helpful. Improvement over months is the rule, with

a mortality rate of less than 20%, although those patients who become jaundiced may have a worse course [139]. With combined injury the treatment is similar, but more aggressive therapy may be required.

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I MISCELLANEOUS

115 Complementary and alternative medicine in gastroenterology

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One of the recent trends in health care has been the marked growth in the use of complementary and alternative medicine (CAM). Two widely used definitions of CAM are "those health care and medical practices that are not currently an integral part of conventional (Western) medicine" and "those interventions neither taught widely at U.S. medical schools nor generally available in U.S. hospitals." During the 1990s, the federal government created the National Center for Complementary and Alternative Medicine (NCCAM), as well as the Office of Dietary Supplements. Moreover, versions of *Physicians' Desk Reference* were created and are now updated for both herbal therapies and dietary supplements. The NCCAM has grouped CAM into five major areas. The greatest proportion of human as well as experimental animal and in vitro research relating CAM to gastrointestinal (GI) diseases has evaluated biologically based therapies (the major focus of this chapter). Other forms of CAM include manipulative and body-based methods, energy therapies, mind–body interventions, and alternative medical systems (Table 115. 1).

The increasing interest in CAM was highlighted in studies by Eisenberg and colleagues, demonstrating a high prevalence of CAM use and a large sum of out-of-pocket dollars spent on CAM in the United States [1,2]. Indeed, in 1997, approximately 15 million adults took prescription medicines in conjunction with herbal remedies or high-dose vitamins, and total out-of-pocket costs for CAM were estimated to be at least $27 billion. A 2001 survey found that approximately half of American adults were taking nonprescription dietary or mineral supplements [3]. Patients reported equal confidence in their CAM providers and their traditional medical doctors [2]. Of great importance, almost 40% of patients did not disclose their CAM use to their traditional physicians. In spite of this high use and cost of CAM, there are few data evaluating/supporting its cost-effectiveness [4].

CAM users tend to be highly educated, are often white (non-Hispanic), and frequently use CAM therapy for chronic diseases. Use of CAM in chronic illness may result from frustration with the lack of efficacy of conventional therapy and the fact that CAM allows patients some control over their therapy. Thus, it should not be surprising that CAM is frequently used by patients with chronic GI diseases. For example, a recent British survey found that patients with inflammatory bowel disease used CAM as often as those with functional GI disorders; both groups used CAM with greater frequency than the general population [5].

In spite of important data generated on CAM over the past 10–15 years, students in most traditional Western medicine training programs continue to receive limited education about CAM therapies, and therefore may be reluctant to use them. However, recent studies suggest that practitioners are beginning to recognize gaps in effectiveness of conventional therapies and may be more willing to accept CAM treatments for patients with chronic complaints [6]. Because of all these factors, it is important that health-care providers be knowledgeable about CAM, including both potential benefits and side effects. This chapter reviews CAM use and its role in major GI disease processes.

CAM use in liver disease

Use of CAM in liver disease appears to parallel overall widespread CAM use in the United States [7]. We will review the use of milk thistle (silymarin), glutathione prodrugs, *S*-adenosylmethionine (SAM), betaine, vitamin E, zinc, lecithin, glycyrrhizin, and herbals in liver disease.

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Table 115. 1 Types and examples of complementary and alternative medicine

Type of CAM	Examples
Biologically based therapies	Herbal therapies Special diets to promote health Orthomolecular therapies (e.g., megavitamins) Biological therapies (e.g., shark cartilage)
Alternative medical systems	Oriental medicine Maintaining proper balance of Qi Traditional Indian medicine, Ayurveda Homeopathy (dilute treatments more potent)
Mind–body interventions	Meditation Prayer Mental healing Art, dance, and music therapy
Manipulative and body-based methods	Osteopathy Massage therapy Chiropractic
Energy therapies	Qigong Touch therapy to maintain body's energies and balance Bioelectromagnetically based therapies

CAM, complementary and alternative medicine.

Silymarin (milk thistle)

Silymarin is the active ingredient extracted from *Silybum marianum* (milk thistle). The plant is a member of the daisy family, and the leaves have prominent white "milky" veins [8]. This agent protects animals from multiple types of experimental liver injury induced by carbon tetrachloride, acetaminophen, iron overload, bile duct obstruction, alcohol, and, very importantly, mushroom poisoning [8–10]. Its hepatoprotective effects are highlighted by its use in the treatment of *Amanita* mushroom poisoning, with studies in several animal species documenting the efficacy of this agent both in pretreatment phases and following mushroom ingestion [8,10]. Moreover, cases have been reported in which positive results were achieved in patients up to 3 days after accidental ingestion.

In the United States, silymarin is probably the most widely used form of CAM in the treatment of liver disease. It has antioxidant activities, protects against lipid peroxidation, beneficially modulates cytokines, has antifibrotic effects, and promotes liver regeneration [8,9]. Large controlled trials of silymarin have been performed in Europe, with variable results. Ferenci and colleagues [11] evaluated 170 patients with cirrhosis in a treatment program (140 mg three times daily) that had a mean duration of 41 months. They observed a positive beneficial effect, especially in patients with alcoholic cirrhosis and in those with milder disease (Child class A). On the other hand, a study by Pares and colleagues [12] found no beneficial effects (150 mg of silymarin three times daily) in a study of 200 patients with alcoholic cirrhosis, some of whom also had hepatitis C. A recent Cochrane metaanalysis of 915 patients found modest improvement in some liver tests, but no improvements in liver-related mortality in high-quality studies [13]. In spite of these issues, silymarin is likely to remain one of the most popular forms of CAM therapy for liver disease because it has a good safety profile and has been extensively investigated in multiple forms of experimental liver injury in animals. Results of recently initiated National Institutes of Health (NIH)-funded randomized trials of Silymarin are eagerly awaited.

Glutathione and glutathione prodrugs

Glutathione is a tripeptide that is synthesized from glutamate, cysteine, and glycine. Glutathione, in its reduced form, is the main nonprotein thiol in cells and has an important role in the detoxification of electrophils and protection against reactive oxygen toxicity [14]. Glutathione cannot be taken up by hepatocytes, but a number of pharmacological agents have been devised to enhance intracellular pools. The most widely used include *N*-acetylcysteine (NAC) and 2-oxothiazolidine-4-carboxylic acid (OTZ), with NAC available as a supplement [14].

Glutathione prodrugs have been used extensively in virtually every known experimental model of hepatotoxicity with beneficial results [14]. The glutathione prodrug NAC (given

orally and now available intravenously) is the standard therapy for acetaminophen toxicity in humans [15]. Maintaining adequate hepatocyte glutathione levels has been documented to prevent acetaminophen liver injury. However, information on glutathione prodrugs in prospective randomized studies in other forms of liver disease is very limited. A recent phase I clinical trial of antioxidants, including oral and i.v. glutathione, showed improvement in liver enzymes and quality of life in hepatitis C virus (HCV) patients, but this was a nonrandomized study using multiple antioxidants [16].

Glutathione prodrugs can not only directly affect the hepatocyte, they can decrease proinflammatory cytokine production also, presumably through inhibition of the oxidative stress–sensitive transcription factor nuclear factor-κB (NF-κB) in Kupffer cells/macrophages [17]. For example, Pena and colleagues [18]. demonstrated that the glutathione prodrug procysteine increased whole-blood glutathione and inhibited monocyte tumor necrosis factor (TNF) and interleukin 8 (IL-8) production when given intravenously to patients with stable cirrhosis. Similarly, procysteine was shown to protect against alcohol-induced liver injury in the intragastric ethanol feeding model [19]. Thus, glutathione prodrugs can, in theory, decrease oxidative stress in hepatocytes and attenuate proinflammatory cytokine production in Kupffer cells to inhibit hepatotoxicity.

S-Adenosylmethionine

SAM is an obligatory intermediate in the conversion of methionine to cysteine in the hepatic trans-sulfuration pathway (Fig. 115.1). The conversion of methionine to SAM involves transfer of the adenosyl moiety of adenosine triphosphate (ATP) to the sulfur atom of methionine and is catalyzed by methionine adenosyltransferase (MAT). SAM is a precursor for the synthesis of polyamines, choline, and glutathione, and is also the major methylating agent for a vast number of molecules via specific methyltransferases [20].

Patients with many types of both acute and chronic liver disease have elevated plasma methionine levels, markedly delayed clearance of an oral methionine load, and decreased hepatic MAT activity. Subnormal hepatic SAM levels have been reported in various experimental models of liver injury, and SAM therapy in rats attenuates liver injury secondary to cholestasis, ethanol, carbon tetrachloride, fatty liver, acetaminophen, or galactosamine [20,21]. Liver-specific *MAT* (*MAT1A*) knockout mice have been generated [22]. Initially, they are highly susceptible to another "stress," such as a choline-deficient diet. Later, fatty liver and periportal inflammation develop, which may have a great relevance not only to alcoholic liver disease but also to nonalcoholic steatohepatitis (NASH). Lastly, very late in life, hepatic cancer develops in some mice. Deficiency of MAT modulates the expression of many genes, especially those involved in proliferation (growth), and normal *MAT1A* activity appears to be important for maintaining the hepatocyte in a "differentiated" state [20,22]. Studies from our laboratories have shown that SAM down-regulates production of the cytotoxic proinflammatory cytokine TNF, as well as increasing the antiinflammatory cytokine IL-10 [20]. Mato and colleagues reported that patients with alcoholic liver cirrhosis who were randomized to receive SAM (1200 mg daily orally) for 2 years had lower rates of liver mortality/liver transplantation (16% vs 30%) than a placebo-treated group [23]. SAM has also been used successfully in various types of cholestatic liver disease. Thus, substantial animal studies and emerging clinical data indicate that SAM may be useful in several forms of liver disease. Some potential beneficial effects of

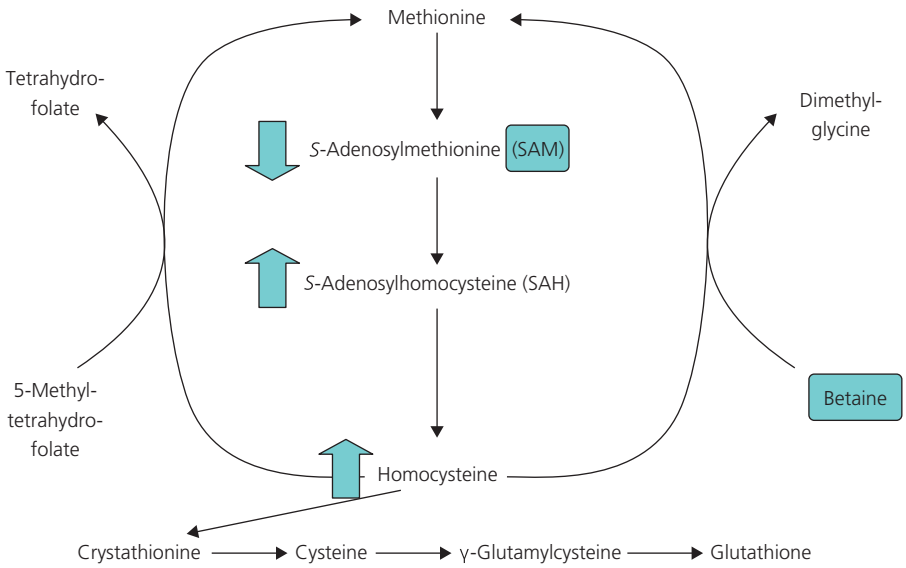

Figure 115.1 Hepatic methionine metabolism. Chronic alcohol consumption and several forms of liver disease cause *S*-adenosylmethionine (SAM) deficiency, but enhancement of homocysteine and *S*-adenosylhomocysteine (SAH). SAM can act as a therapeutic agent by replacing inadequate endogenous SAM, improving the SAM/SAH ratio and stimulating crystathionine β-synthase activity and subsequent reduced glutathione (GSH) production. Betaine can help remove SAH and homocysteine with subsequent regeneration of methionine.

SAM include increased glutathione production, improved mitochondrial function, decreased proinflammatory cytokine production, and enhanced methylation reactions [20].

Betaine

Betaine (trimethylglycine – initially discovered in the juice of sugar beets) acts as an organic osmolyte to protect cells from stress and functions as a major methyl donor in the methionine cycle (see Fig. 115.1) [24]. Thus, SAM and betaine together are involved in linked vital metabolic processes in the liver. In the transmethylation pathway, SAM is converted to *S*-adenosylhomocysteine (SAH). While SAM levels tend to be low in most forms of liver disease, SAH levels are often elevated, as are homocysteine levels [25]. SAH can sensitize hepatocytes to TNF-induced hepatotoxicity, and homocysteine is a major inducer of fatty liver [25]. Increases in SAH and/or decreased SAM to SAH ratios inhibit most transmethylation reactions. Thus, it is important to be able to remove excess levels of both SAH and homocysteine from the liver. Betaine facilitates the conversion of homocysteine back to methionine and helps remove both SAH and homocysteine (Fig. 115.1).

Betaine is known to be protective against a variety of forms of experimental hepatotoxicity such as those induced by endotoxin, carbon tetrachloride, chloroform, alcohol and others [24,26]. As noted previously, low levels of SAM are observed in most forms of liver disease and SAM is hepatoprotective. Betaine functions by removing SAH and homocysteine from the liver, as well as regenerating SAM [27].

Abnormalities in SAH and homocysteine levels are now being recognized in both experimental forms of liver injury and in human injury. Our group, Barack's laboratory, and others have documented elevated SAH levels in experimental models of liver disease, and studies by several groups document elevated homocysteine levels in animal models of liver injury [25–27]. As noted previously, the SAM/SAH ratio and SAH levels critically regulate transmethylation reactions (with increased SAH inhibiting transmethylation reactions) [25]. Studies from Ji and Kaplowitz reported that hyperhomocysteinemia induced endoplasmic reticulum stress with subsequent liver injury in alcohol-fed mice, and removal of homocysteine by betaine supplementation attenuated fatty liver and liver injury [26]. Studies from Barack and coworkers clearly indicated that betaine removes not only homocysteine but also SAH and protects against alcoholic liver injury in animals (see Fig. 115.1 – betaine facilitates methionine regeneration from homocysteine) [27]. Our findings suggest that elevated SAH levels increase sensitivity to TNF-induced hepatotoxicity, and we postulate that this is a clinically relevant cause of TNF-induced hepatotoxicity in patients with alcoholic liver disease [25]. Betaine has been used with success in a human pilot study in NASH patients, and larger trials are ongoing in NASH, with promising initial results [28].

Vitamin E

Vitamin E is a potent chain-breaking antioxidant that is widely used as a nutritional supplement. Depressed serum and hepatic levels of vitamin E have been documented in several types of liver disease [29].

Vitamin E has been used extensively with hepatoprotective effects in experimental models of liver injury, such as that induced by carbon tetrachloride or ischemia [29]. Vitamin E has been used in human liver disease with some success, with probably the most compelling data generated in children with NASH [30]. Obese children were given vitamin E (400–1200 IU) in an open-label pilot study, and all children with NASH demonstrated an improvement in liver enzymes without losing weight. Multiple subsequent studies have been performed in NASH patients, with most showing some beneficial effects of vitamin E [8]. Vitamin E has multiple potential beneficial effects, including membrane stabilization, reduced NF-κB activation and TNF production in monocytes/Kupffer cells, and decreased collagen production [17]. Vitamin E treatment has also been reported to decrease serum levels of the profibrotic cytokine transforming growth factor beta (TGF-β) in NASH patients [31]. Some of the initial enthusiasm for vitamin E use in liver disease has been tempered by a recent metaanalysis suggesting a potential for toxicity with high doses (see section on safety issues), and the results of NIH trials of vitamin E and NASH are eagerly awaited.

Polyenylphosphatidylcholine (lecithin)

Polyenylphosphatidylcholine (PPC) is a lipid extract from soybeans that has been shown to have hepatoprotective effects, especially in alcoholic liver disease [32]. PPC (specifically, dilinoleoylphosphatidylcholine, or DLPC) has been shown to prevent the development of septal fibrosis and cirrhosis in alcohol-fed baboons and has also been shown to stimulate the release of collagenase activity by cultured hepatic stellate cells. Stellate cell activation in rats has also been found to be decreased by DLPC. These animal studies of DLPC led to a large Veterans Administration cooperative study in patients with early alcoholic liver disease. No significant difference was found between the active agent and placebo; however, alcohol intake was markedly decreased in both groups and projected disease progression was improved [32].

Glycyrrhizin

Glycyrrhizin is an extract made from licorice roots that has been used in a variety of GI disease processes, ranging from peptic ulcer disease to liver disease. Glycyrrhizin has been reported to improve liver enzymes in a variety of patients with chronic hepatitis, including hepatitis C [8]. While it stimulates endogenous interferon production, it does not appear to decrease viral load in hepatitis C [8]. A retrospective study of intravenous glycyrrhizin therapy in patients with chronic hepatitis C found an approximately a 2.5-fold reduction in the risk of hepatocellular carcinoma [33].

Although glycyrrhizin has potential beneficial effects, it also has a variety of side effects, including aldosterone-like properties that predispose to electrolyte imbalance and fluid retention, and therefore we do not recommend that our patients use this drug [8,34].

Zinc

Zinc is an essential trace element, and zinc deficiency is well documented in many types of liver disease, including alcoholic liver disease [35]. Potential functional consequences of zinc deficiency of relevance to liver disease include neurosensory defects, skin lesions, hypogonadism, immune dysfunction, altered protein metabolism, and depressed wound healing [35]. Zinc deficiency has been best studied in patients with alcoholic liver disease, in whom there is not only decreased serum zinc levels but also increased urinary losses of zinc and poor zinc absorption [35]. Recent studies with experimental animals have shown that zinc supplementation markedly attenuates the development of alcohol-induced hepatotoxicity [36]. Beneficial actions of zinc include suppression of alcohol-induced oxidative stress, inhibition of hepatocyte apoptosis, maintenance of intestinal integrity, and a reduction in TNF production, to name only a few [36]. In humans, zinc supplementation has been shown to reverse or attenuate some of the functional consequences of zinc deficiency in alcoholic liver disease, such as skin lesions, immune dysfunction, and impaired night vision [35]. Zinc also has been used as adjunct therapy in hepatic encephalopathy with some success [37]. Lastly, zinc is now widely used for the treatment of Wilson disease [38]. Zinc induces intestinal metallothionein, which binds copper in the gut mucosa, preventing its absorption and resulting in its excretion in the stool. The dose of zinc used in Wilson disease is usually at least 150 mg of elemental zinc (50 mg orally three times a day) in order to effectively induce intestinal metallothionein.

Oriental herbals

Herbals are widely used to treat a variety of chronic inflammatory processes, such as rheumatoid arthritis, as well as in the chemoprevention of cancer. Green tea and green tea polyphenols down-regulate proinflammatory cytokine production (e.g., TNF) and block endotoxin lethality and endotoxin-induced hepatotoxicity in galactosamine-sensitized rats [39]. Other major polyphenols are made from grape seeds; they also have antioxidant/antiinflammatory properties and have been used to protect experimental animals from liver injury.

TJ9 is commonly used in China and Japan to treat hepatitis B and C and comes from an aqueous extract from the roots of a variety of plants. This agent has been shown to be hepatoprotective/antifibrotic in certain animal models of liver injury and to down-regulate proinflammatory cytokine production [8,40]. Compound 861 is an aqueous extract of 10 defined herbs that is reported to have very potent antifibrotic activity. It has been used extensively in China as an antifibrotic agent and has been reported actually to decrease fibrosis in sequential liver biopsy specimens from patients with hepatitis B [8,41]. It has also been shown to be effective in carbon tetrachloride-induced liver injury and to have antiproliferative effects on stellate cells in vitro. Selected other herbals used to treat liver disease include *Phyllanthus amarus*, *Picrorrhiza kurroa*, CH-100, and ginseng. One of the major problems with herbals is that they are actually a combination of agents that are poorly characterized and not highly reproducible from one lot to the next. The potential risks of herbals have recently been reviewed and are discussed in the section on safety issues [7,8].

CAM use in pancreatic disease

Currently, effective therapies for pancreatic disease, especially chronic pancreatitis, are limited. Relief of abdominal pain, malabsorption, and diabetes are the main areas of focus, with abdominal pain being the most difficult symptom to treat. Because pancreatic enzyme replacement therapy is ineffective in controlling pain [42] and large randomized studies evaluating endoscopy or surgery are lacking [43], patients and providers are actively seeking alternative therapies for chronic pancreatitis, including acupuncture, herbal therapies, and antioxidant therapy.

Acupuncture

Acupuncture has been used for decades in traditional Chinese medicine. The Chinese believe that the interaction of yin and yang creates the vital life force known as Qi (pronounced chee) and that diseases occur when the flow of Qi is disrupted (excessive or deficient). Qi flows through the body in meridians or channels and can be accessed via acupoints. Modern functional magnetic resonance imaging studies have shown reproducible, regionally specific, and quantifiable effects on relevant structures in the brain when acupoints are stimulated [44]. However, in the only randomized prospective study of 23 patients with nonalcoholic chronic pancreatitis, neither electroacupuncture nor transcutaneous electrical nerve stimulation was as effective as medical therapy [45].

Antioxidants

The increased use of antioxidants as an alternative therapy for chronic pancreatitis is based on the compelling evidence of persistent oxidative stress in patients with chronic pancreatitis [46], in addition to increased lipid peroxidation and altered glutathione metabolism [47]. Furthermore, regardless of the cause of injury, oxidative stress is felt to be pivotal in the progression to parenchymal pancreatic tissue destruction [48]. Compared with control subjects, the serum antioxidant profiles of patients with acute and chronic pancreatitis, and of those with hereditary pancreatitis, reveal lower plasma

concentrations of selenium, vitamin A, vitamin E, xanthine, β-carotene, β-cryptoxanthine, and lycopene [49,50]. Additionally, selenium deficiency in chronic pancreatitis has been well documented, with some patients experiencing relief of chronic pain after selenium supplementation [51].

The histological hallmarks of chronic pancreatitis include loss of parenchyma, fibrosis, blockage of pancreatic ducts with protein plugs that calcify, and variable degrees of inflammatory cell infiltration with a predominance of lymphocytes. Cytokines and chemokines released from these cells promote ongoing inflammation. Elevated proinflammatory cytokines have been found in the serum [52], peripheral blood monocytes [53], and more recently in the pancreatic tissue [54] and pure pancreatic juice [55] from patients with acute and chronic pancreatitis. Cytokine response predicts disease severity and outcome, supporting the rationale for the antioxidant- and cytokine-blocking therapies now under development. In a prospective trial of 10 patients with chronic and acute recurrent pancreatitis of various causes, supplementation with a complex containing vitamin E, vitamin C, and organic selenium resulted in significant pain relief and a decreased need for hospital admission during the year of treatment in comparison with the year before treatment [56]. In a 20-week, double-blinded, placebo-controlled, switch-over trial of supplementation with selenium, β-carotene, vitamin C, vitamin E, and methionine, most patients reported fewer attacks with significantly less background pain while on treatment [57]. A retrospective cross-sectional audit of 94 consecutive patients with chronic pancreatitis on antioxidant therapy followed for an average of 30 months showed a decrease in the requirements for surgical intervention, in the number and intensity of painful attacks, and in absences from work [58]. Large prospective placebo-controlled trials are needed to confirm these preliminary observations and guide practitioners on the best doses for these over-the-counter preparations.

CAM use in peptic ulcer and nonulcer dyspepsia

Peptic ulcer disease remains prevalent despite the recognition of its association with *Helicobacter pylori* infection and the availability of potent antisecretory medications. For thousands of years, alternative therapies were used to provide symptom relief and promote ulcer healing, especially in Indian and Chinese traditional medicine.

Acupuncture

Acupuncture has been used extensively in China and the former Soviet Union for the treatment of peptic ulcer. The physiological basis of acupuncture effects on acid secretion has been well studied in animals and humans [59]. In a prospective randomized study of human volunteers, electroacupuncture, but not sham acupuncture or simple acupuncture, significantly reduced vagally stimulated acid secretion [60]. Clinical trials reporting efficacy for acupuncture in humans with gastric or duodenal ulcer have been criticized for poor study design and lack of randomization.

Acupuncture may be useful for persons with nonulcer or functional dyspepsia. These patients have been shown to be hypersensitive to gastric distention, and somatic stimulation with transcutaneous electric stimulation can reduce the discomfort associated with gut distention [61]. Electroacupuncture modulates gastrointestinal motility by significantly increasing the percentage of regular gastric slow waves as measured by electrogastrography (EGG) [62], and by influencing duodenal peristalsis. Several small uncontrolled trials claim efficacy for acupuncture in subjects with nonulcer dyspepsia [63]. Future controlled randomized trials are needed to clarify whether patients with nonulcer dyspepsia or documented gastroduodenal dysmotility can benefit from acupuncture.

Probiotic/herbal therapies

Yogurt containing appropriate bacteria (probiotic therapy) has been shown to suppress *H. pylori*, and combining yogurt with antibiotics can improve *H. pylori* eradication. Moreover, recent research has shown that prior treatment with *Lactobacillus acidophilus*- and *Bifidobacterium*-containing yogurt significantly improved the efficacy of quadruple therapy in eradicating residual *H. pylori* after failed triple therapy [64].

Sangre de grado is derived from the sap of several Amazonian trees of the *Croton* genus (*C. dracanoides*, *C. planostigma*, *C. lechleri*). It is commonly used in tropical South America to treat a variety of GI illnesses, including infectious diarrhea, gastritis, and peptic ulcer, and it is also used topically for wounds, bites, and insect stings [65]. It is inexpensive and widely available, unlike many of the Western pharmaceuticals. Its mechanism of action is unknown, but its efficacy has been attributed to potent antibacterial properties. Despite the high prevalence of *H. pylori* infection in South America, no current studies have focused on sangre de grado and *H. pylori* eradication per se.

In a randomized clinical trial of 508 patients with endoscopically proven gastric or duodenal ulcers and documented *H. pylori* infection, the Chinese herb Chuanjia Weidan was compared with cimetidine. Ulcer healing rates and symptom reduction were similar, but the *H. pylori* clearance rate was 75% with Chuanjia Weidan therapy, versus 6% for the cimetidine-treated group. Ulcer recurrence rates were also higher for the cimetidine treated group [66]. A preliminary screening of more than 200 different Chinese herbal medicines revealed that 38 of the preparations had growth-inhibiting effects on *H. pylori* organisms cultured from 136 subjects with antral infection [67].

Other botanicals and herbals with reported efficacy and extensive use in the treatment of humans with peptic ulcer

include flavonoids (quercetin, silymarin, naringin, anthocyanosides, sophoradin derivatives), saponins, tannins, gum guar and mucilages (myrrh), licorice, aloe gel, goldenseal, and capsicum (chili) [68]. Proposed mechanisms of action include inhibition of prostaglandins, cytoprotection, antiinflammatory or immune modulatory effects, altered nociception, and suppression of *H. pylori*. Well-designed randomized controlled trials supporting many of these claims are lacking. For nonulcer dyspepsia, there are several randomized controlled trials that support the efficacious use of peppermint oil alone or in combination with caraway oil for symptom relief [69]. The effects of these agents are most likely due to their ability to relax smooth muscle.

CAM use in irritable bowel syndrome

Irritable bowel syndrome (IBS) is one of the functional bowel disorders characterized by chronic or recurrent abdominal pain and disturbed defecation without structural or biochemical abnormalities. Although the pathogenesis of IBS remains unknown, the documented altered bowel motility patterns and exaggerated pain perception to visceral distention (visceral hyperalgesia) suggest neurohormonal or autonomic dysfunction as a likely cause [14,70].

Treatments for IBS generally focus on symptom reduction; however, no single treatment is reliably effective. Rather, multiple regimens combining dietary and lifestyle modification, normalization of bowel habits, antispasmodics when indicated, and anxiolytics or antidepressants are used. Often, an initial approach is to increase dietary fiber; however, clinical controlled studies of fiber therapy have revealed conflicting results [71]. Because of the limited efficacy of conventional treatments for IBS, many patients seek alternative therapies. A questionnaire completed in a physician's waiting room in Britain revealed that one in five patients with IBS had used some form of alternative therapy. Also, 41% were agreeable to consultation with alternative medicine practitioners, compared with only 6% of patients with "organic" upper GI diseases. Of alternative medicines used at home, herbal remedies were most common [72]. However, most people with IBS symptoms do not seek formal medical care, and the use of alternative therapies in these individuals will remain difficult to quantify unless surveys of large cross-sections of the population are developed and administered.

Acupuncture

Acupuncture therapy may benefit patients with IBS through a variety of mechanisms, including its effects on GI motility and autonomic function. Coffin and colleagues [61] examined the effects of transcutaneous electrical nerve stimulation of the hand during balloon distention of the stomach and duodenum and found that it induced somatosensory stimulation and raised the discomfort threshold. Kunze and colleagues [73] found a greater relief of symptoms with real (31%) than with sham (17%) acupuncture in patients with IBS. Among 10 patients with IBS, Diehl and colleagues [74] reported a good response to acupuncture in four and a partial response in five, with one nonresponder. In another small study of seven patients given acupuncture for IBS, all reported an improved sense of general well-being and less abdominal bloating, but no change in abdominal discomfort or bowel patterns [75].

Commonly used herbal therapies

Essential oils are known for their carminative and antispasmodic properties, and thus have been used for various GI ailments. Peppermint oil (*Mentha* × *piperita* L.) is derived from steam distillation of the aerial parts of flowering peppermint (*Mentha* × *piperita*). Its active component is menthol, which has pharmacological properties similar to those of calcium channel antagonists [76]. Peppermint oil has been shown to relax smooth muscle from isolated human colonic cells. In a metaanalysis of eight randomized controlled trials, peppermint oil use found to be beneficial for relief of IBS symptoms; however, only one trial selected patients based on the Manning diagnostic criteria for IBS, illustrating the need for more scientifically rigorous methodology in future studies. Additionally, not all studies reported the doses used, so that practical recommendations for use cannot be reliably made [77]. Peppermint oil use may increase gastroesophageal reflux disease (GERD) owing to relaxation of the lower esophageal sphincter, but otherwise it has no known harmful effects such that patients may self-administer to seek their own dose–response needs.

Ginger root (*Zingiber rhizoma*, *Zingiber officinale*) has been used for thousands of years in Asia to treat multiple GI ailments, including nausea, dyspepsia, cholera, and diarrhea, as well as motion sickness, postoperative vomiting, and hyperemesis gravidarum. The pharmacological properties of ginger include antioxidant effects and inhibition of prostaglandins. It also increases intestinal tone and peristalsis. Ginger supplementation enhances charcoal meal transport in mice, with effects felt to be similar to or slightly weaker than those of metoclopramide and domperidone [78]. For practical uses, the dose of ginger needed to relieve symptoms would need to be self-assessed by users; ginger is believed to be nontoxic.

Licorice (*Glycyrrhiza glabra*, *Glycyrrhiza uralensis*) is used to treat functional dyspepsia, bronchitis, and peptic ulcer. It has expectorant properties and antiinflammatory and antiplatelet effects, and it increases prostaglandin levels. Overuse can result in salt and water retention, hyperkalemia, hypertension, and edema. Many drug–drug interactions are also possible.

Derived from the plant *Hypericum perforatum*, St. John's wort is the herb most commonly used worldwide to treat depression and anxiety. In clinical studies comparing St.

John's wort with various serotonin reuptake inhibitors, it was equally effective for the treatment of depression and had fewer side effects [79]. The active ingredient of St. John's wort, hypericin, acts by inhibiting the reuptake of norepinephrine, serotonin, and dopamine. Controlled trials of St. John's wort use in IBS have not been reported.

Perhaps because of their agreeable flavor and aroma, or the ceremony of making and sharing a beverage with others, the commercial use of herbal teas is increasing. The following have reported benefits for the GI tract and their use is felt to be safe, but controlled clinical trials of efficacy have not been published. Chamomile (*Matricaria camomilla*, *Chamaemelum nobile*) is felt to have GI antispasmodic, antiinflammatory, and antimicrobial activities, in addition to anxiolytic and slight sedative effects. The licorice-tasting herbs fennel (*Foeniculum vulgare*) and anise (*Pimpinella anisum*) are touted to help expel gas and aid digestion. Lemon balm (*Melissa officinalis*) has mild sedative, carminative, and antispasmodic effects, and fragrant raspberry leaf (*Rubus idaeus*, the "pregnancy tea") is reported to relieve morning sickness, nausea, and dyspepsia [80].

Chinese herbal agents

In traditional Chinese medicine, rather than single agents being used, multiple herbs are combined for their synergistic properties. In a randomized placebo-controlled trial of 176 patients with IBS according to the Rome criteria, those receiving treatment with individualized herbs ($n = 58$) or a standard formulation of Chinese herbs ($n = 63$) reported significant relief of bowel symptoms and quality of life in comparison with those receiving placebo ($n = 55$). No greater benefit for those receiving individualized herbs rather than standard herbs during the 4-month study was found. However, only the subjects who had received individualized herbs showed a sustained relief of symptoms 14 weeks after completion of the study. The authors concluded that further investigation of Chinese herbal medicine as a treatment for IBS should be undertaken [81].

Indian herbal agents

The bilva (*Aegle marmelos correa*) plant from the Indian Ayurvedic system of medicine has been used to treat chronic constipation, flatulence, diarrhea, and colic. Yadav and colleagues [82] randomized patients with IBS to one of three groups: standard treatment (clidinium bromide, chlordiazepoxide, and ispaghula), herbal therapy (bilva plus the sedative herb *Bacopa monniere* L.), or placebo. Both active treatment groups significantly improved compared with the placebo group. A subanalysis revealed that the standard therapy was most beneficial for patients with pain as a predominant symptom, whereas the Ayurvedic herbs were most beneficial for those with diarrhea. At 6-month follow-up, relapse rates did not differ between the active treatment and placebo groups.

CAM use in inflammatory bowel disease

Inflammatory bowel diseases (IBDs) exhibit a large degree of heterogeneity, both in disease expression and in response to established therapies. The toxicity and relatively low efficacy of many standard medical therapies impel up to 50% of IBD patients in some populations [5] to seek complementary or alternative therapies, including probiotics, nutritional supplements, and botanical products. Clinical evaluations of alternative therapies suggest therapeutic benefits, but definitive clinical trials demonstrating objective evidence of efficacy remain sparse.

Probiotics

Publications concerning probiotics (viable microorganisms with putative health effects) have proliferated recently, while clinical trials have not kept pace. Probiotic bacteria stimulate nonspecific host resistance to microbial pathogens, enhance humoral immune responses to pathogens, and enhance antiinflammatory cytokine profiles [83,84]. They also alter the composition of the lumenal flora by producing lactic acid, bacteriocins, and other antimicrobial peptides that are efficacious against pathogens.

Lactobacterium species, in general, perform well against pathogenic bacteria without inhibiting other probiotic organisms. Most species adherent to the small bowel belong to the *Lactobacillus* genus and perform well as potential probiotics. *Lactobacillus salivarius* competes exceptionally well against pathogenic bacteria without inhibiting other beneficial strains of lactobacilli or bifidobacteria isolated from the human ileum [85].

Many IBD patients exhibit imbalances of the intestinal microflora [86,87]. The current concept of dysbiosis, or altered balance between pathogenic and protective bowel flora, has enhanced the popularity of probiotic therapy. The most convincing evidence to date regarding the benefits of probiotic therapy comes from trials in ulcerative colitis (UC). One randomized, double-blind, placebo-controlled (RDBPC) trial demonstrated significant improvement in patients with mild to moderate UC ingesting a *Bifidobacterium*-containing fermented milk product [88]. *Escherichia coli* Nissle 1917 benefits the maintenance of 5-aminosalicylic acid (5-ASA)-induced remission [89,90]. In an open-label trial with VSL#3 (a combination of probiotic organisms), 69% of UC patients with mild to moderate activity despite 5-ASA therapy experienced remission of symptoms, and 23% responded [91]. Evidence for a probiotic benefit in Crohn's disease has been less dramatic. When used in a complementary fashion in a small RDBPC trial, *Lactobacillus rhamnosus* GG administered to patients who are tapering off remitive medications did not improve duration of remission [92]. Two other maintenance of remission trials fared better: 70% of patients in remission on corticosteroids maintained remission with *E. coli* Nissle

1917 compared with 30% receiving placebo [93], while the addition of *Saccharomyces boulardii* to mesalamine maintained remission at 6 months in 94% of patients receiving both compared with 62% in patients receiving mesalamine alone [94].

Studies in mice reveal that probiotic administration leads to the competitive displacement of intestinal pathogens and the engagement of cell membrane receptors that modulate signaling events for cytokine synthesis [95]. A variety of organisms, including *Lactobacillus* and *Bifidobacterium* species possess the ability to modulate cytokine production and immune function in an antiinflammatory fashion in several models of intestinal inflammation [95–98].

Helminthic parasites

A theory recently advanced suggests that tolerance to lumenal bacterial antigens (helper T cell type 2 [Th2] mucosal conditioning) may be conferred by exposure to helminthic parasites [99]. The decline in helminthic infestations in the United States during the past 60 years has been offered as an explanation for the rise in the incidence of Crohn's disease during the same period [99]. Antigen characteristics determine whether an antigen-presenting cell (APC) influences a naive T cell to assume either a Th1 (interferon-γ, TNF-α) or Th2 profile (IL-4, IL-5, IL-10, IL-13) [100,101]. Immune response to helminthic antigens generally elicits a strong Th2-type reaction, whereas patients with Crohn's disease mount a Th1 response to lumenal antigens. Persons harboring helminths are more apt to mount a diminished Th1 response when challenged with proinflammatory antigens [99]. Mice exposed to *Schistosoma mansoni* before trinitrobenzene sulfonic acid and ethanol were protected from the development of experimental colitis [102]. IL-10 deficient mice inoculated with the helminths *Heligmosomoides polygyrus* and *Trichuris muris* were far less susceptible to inflammation caused by bacterial exposure than were those given a sham inoculation [99]. Based on these data, researchers conducted an open-label clinical trial involving 29 Crohn's disease patients with persistent inflammation despite conventional medical therapy [103]. Patients received 2500 live *Trichuris suis* eggs every 3 weeks for 24 weeks. Seventy-nine percent of patients experienced a drop in Crohn's disease activity index (CDAI > 100), and 72% met remission criteria. An RDBPC study by the same group using *T. suis* on medically refractory mild to moderate UC demonstrated a 43% response to therapy compared with 17% response to placebo [104].

Prebiotics/short-chain fatty acids

Prebiotics, described as "nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of beneficial bacteria in the colon, thereby improving the host's health," [105] provide an additional mechanism for influencing the population dynamics of the intestinal microflora. The most beneficial prebiotics reach the cecum, where they can promote the growth of lactic acid-producing microorganisms. Recovery of a high proportion of the chicory *Fructans inulin* and its hydrolysate, oligofructose, from ileostomy outputs supports their ability to reach the cecum [106]. Prebiotics are present in significant amounts in a wide variety of fruits and vegetables and are fermented by colonic bacteria to lactic acid and short-chain carboxylic acids [107]. Other oligomers with prebiotic potential are lactulose and such sugar alcohols as mannitol and xylitol. Additionally, prebiotics such as oligofructose may function in a primary antibacterial role by augmenting the growth of beneficial probiotic strains, as seen in competition experiments between strains of *E. coli, Clostridium perfringens*, and *Bifidobacterium infantis* [106]. Colonic aerobic bacteria act to salvage energy for the host by fermenting undigested food materials to short-chain fatty acids, which in turn are absorbed by colonocytes [97]. Butyrate has been shown to inhibit the production of cytokines as well as the activation of NF-κB [108]. Initial trials of short-chain fatty acids demonstrated efficacy, but subsequent trials showed mixed results, possibly because of poor compliance. An open-label study investigated the effects of *Plantago ovata* (an alternative source of butyrate) on 12-month remission rates, either alone or in combination with standard 5-ASA therapy. Remission rates were similar for the groups [108]. A subsequent abstract evaluated butyrate yields from a variety of fibers, comparing *P. ovata* seeds, husks, and other forms of fiber. All produced significant amounts of butyrate, but butyrate production from the seeds tended toward a higher output [109]. Germinated barley foodstuff, a by-product of beer fermentation, was evaluated as first an inductive therapy in patients with mild to moderate UC not responding to standard UC therapy [110]. Patients receiving germinated barley foodstuff had significantly lower activity scores, reduced levels of C-reactive protein, and endoscopic improvement compared with subjects receiving standard medical therapy. Bacterial composition and butyrate levels changed during therapy [110]. A follow-up study determined that long-term open-label administration provided an improvement in 6 months in patients entering the trial with mild–moderate symptoms [111].

Transforming growth factor-β

Therapy with enteral nutrition has been shown to be efficacious in Crohn's disease. A specific dietary component, TGF-β, has been studied individually for its applicability as an IBD treatment. TGFs are multifunctional polypeptides found in milk. An oral polymeric diet rich in TGF-β was studied in 29 children with Crohn's disease [112]. Seventy-nine percent achieved remission within 8 weeks of therapy, with two treatment failures that required surgical intervention or steroid therapy. Laboratory indicators of inflammation decreased with therapy, and a follow-up colonoscopy revealed endoscopic improvement in about 50% of cases. Moreover,

this same TGF-β-rich enteral formula attenuated intestinal inflammation in the IL-10 knockout mouse [113].

Green tea

Green tea extracts contain high levels of antioxidants. The polyphenol fraction accounts for most of the antioxidant potential and includes the compounds (–)-epicatechin (EC), (–)-epigallocatechin (EGC), (–)-epicatechin-3-gallate (ECG), and (–)-epigallocatechin-3-gallate (EGCG) [35]. Green tea polyphenols are more potent antioxidants than either vitamins E or C and have potent antiinflammatory properties [114]. Green tea polyphenols have been shown to inhibit intestinal inflammation in the IL-2 knockout mouse model of IBD. An important potential mechanism for treating IBD, independent of antioxidant properties, relates to the effects of the green tea polyphenol (GrTP) fraction on NF-κB, a nuclear activating protein [114]. EGCG inhibits, in a dose-dependent fashion, the effects of inflammatory mediators on NF-κB by inhibiting the phosphorylation of inhibitor of κB (IκB) by IκB kinase (IKK). When bound to IκB, NF-κB cannot migrate to the nucleus and bind with cytokine promoter sequences, interrupting the proinflammatory cascade [114]. The antioxidant properties of GrTP were evaluated in a model of intestinal oxidative stress induced by dextran sodium sulfate (DSS) administered to Balb/C mice [115]. GrTP administration significantly improved the colon length and blood antioxidant levels, while lowering TNF-α and serum amyloid A (SAA) levels when compared with DSS-treated animals that had not received any antioxidant therapy.

Fish oil

Arachidonic acid metabolites, particularly leukotriene B_4 (LTB_4), are important components in the inflammatory processes associated with IBD. Arachidonic acid metabolites cause increased vascular permeability, vascular dilation, and neutrophil recruitment. Serum LTB_4 levels are increased in patients with ulcerative colitis [116]. Eicosapentaenoic acid (EPA), a component of fish oil, can reduce the formation of LTB_4 by serving as a competitive substrate for 5-lipoxygenase, thereby producing a less potent metabolite, LTB_5. Controlled trials of fish oil in patients with ulcerative colitis have demonstrated trends toward efficacy. The data of Hawthorne and colleagues [117] suggested a steroid-sparing effect from 4.5 g of EPA per day in patients with ulcerative colitis. Belluzi and colleagues [118] found that an enteric-coated preparation of 1.8 g of EPA per day maintained remission in patients with Crohn's disease. An RDBPC trial in 38 pediatric Crohn's disease patients who had achieved remission with a 2-month course of steroids demonstrated that coadministration of encapsulated ω-3 fatty acids resulted in remission being maintained for longer, and in a greater percentage of subjects at 12 months (39% vs 5%), than administration of 5-ASA alone [119].

Botanical derivatives

A specific inhibitor of 5-lipoxygenase is contained in the gum resin from *Boswellia serrata*. Indian patients with chronic colitis were treated with either *Boswellia serrata* preparation or sulfasalazine [120]. Gum therapy was associated with a higher rate of response than sulfasalazine therapy. A pilot study evaluating purified curcumin (550 mg) as an adjunct therapy in a mixed cohort of patients with UC or Crohn's disease demonstrated a clinical response to the botanical therapy in both types of IBD as well as a marked reduction in sedimentation rate [121]. The authors of both studies called for formalized testing of these products.

Nicotine

Tobacco use has a well-described dichotomous effect in IBD. Although smoking may aggravate Crohn's disease, patients with ulcerative colitis may experience disease onset or a flare of their existing disease with smoking cessation [122]. Investigators have attempted to exploit this clinical effect by administering nicotine supplements to patients with UC. In an RDBPC study of nicotine patches (average 17 g daily) for the treatment of active UC, a statistically significant response was observed in comparison with placebo [123]. Two subsequent studies with the nicotine patch as monotherapy showed no statistical improvement in remission rates [124], but a study by Sandborn and colleagues [125] did demonstrate a significant improvement in clinical parameters. Other routes of administration, including oral therapy and rectal enemas, continue to be plagued by either side effects or poor efficacy [126,127]. The use of nicotine therapy will likely benefit only a small, select population of UC patients.

CAM use in diarrheal states

Antibiotic-associated diarrhea occurs in approximately 20% of hospitalized patients receiving antibiotics, with up to 33% of cases related to *Clostridium difficile* [128,129]. The other 66% experience symptoms related to alterations in the bacterial production of short-chain fatty acids [130]. Antibiotic-associated diarrhea was reduced from 27% in a placebo group to 9% in a group taking the probiotic *Enterococcus faecium* [131]. Other organisms shown to be efficacious in antibiotic-associated diarrhea include *Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* administered in combination, and *Saccharomyces boulardii* [130]. Worldwide, infectious diarrhea remains a leading cause of morbidity and mortality in infants and children. Numerous studies have investigated the potential of many probiotic and prebiotic agents for the treatment of these conditions. Trials with *L. rhamnosus* GG and *S. boulardii* used as prophylactic therapy for diarrheal infections demonstrated small overall reductions in diarrhea in comparison with the placebo, although some study populations experienced substantial

benefit [132]. The evaluation of probiotics as abortive agents for diarrheal illnesses has been carried out mainly in children, who are at the greatest risk for serious illness and death. *E. faecium* and *S. boulardii* relieve symptoms in acute diarrheal illnesses, and *L. rhamnosus* GG decreases the rate of rotaviral diarrhea and viral shedding [133].

Traditional remedies for diarrhea include the ingestion of a variety of plant extracts. Pectin, from green bananas, serves as a source of amylase-resistant starch [134]. Pectin passes through the small intestine undigested, then undergoes fermentation in the colon into short-chain fatty acids [135]. The presence of short-chain fatty acids in the colon stimulates salt and water absorption and also has a trophic effect on colonic and small bowel mucosa [136]. When dietary pectin and green bananas were blended into a rice-based diet and used to treat children with persistent diarrhea resulting from a variety of infectious and noninfectious causes, a statistically significant reduction in stool weight and relief of symptoms were noted in the treated children in comparison with the children on control diets [134]. Many other antidiarrheals derived from plant sources are undergoing preclinical testing. Recent clinical trials have demonstrated a significant benefit from wood creosote, a derivative of seirogan (an herbal antidiarrheal) [137], and tormentil root extract, which has been used in Eastern Europe as a traditional medicine [138].

Safety issues

The safety of CAM agents is not well defined, in large part because of a lack of regulatory guidelines. In 1994, Congress passed the Dietary Supplement Health Education Act, which exempted dietary supplement producers from many Food and Drug Administration (FDA) regulations. Consequently, dietary supplements are under less government scrutiny than over-the-counter nonprescription drugs. Thus, it is critical that both consumers and health-care workers understand that the labeling of CAM products is not necessarily correct, that products may not be pure, and that the advertised dose may not be correct.

Concerns about safety relate to both product content and potential harm. Multiple studies have documented great variability in the composition of herbal products/supplements, with some products containing only a small fraction of the advertised dose [139–141]. This product variability is probably an unavoidable aspect of many Chinese herbal compounds, and traditional prospective clinical trials are difficult to design because of the inherent variability in the therapeutic agent. Toxicity induced by CAM products has been reported [140,142]. Hepatotoxicity is one of the most frequently reported side effects of CAM products (e.g., germander- and pyrrolizidine alkaloid-containing compounds), sometimes leading to liver transplantation or death [142–144]. Recently reported cases of hepatotoxicity due to kava-kava and to various weight loss agents have heightened awareness of this problem and have reinforced the need to carefully question patients with elevated liver enzymes about CAM use (see Tables 115.2 and 115.3 for examples of CAM hepatotoxicity) [8]. A recent report from one medical center highlighted the risk of fulminant hepatic failure requiring liver transplantation following CAM ingestion [145].

Some CAM products are contaminated with drugs/toxins [139]. A classical example is the report of a young woman who presented with nausea, vomiting, and an irregular heart

Table 115.2 Selected complementary and alternative medicine agents and related hepatotoxicity

Herb/supplement	Description
Aristolochia	Contains highly toxic aristolochic acid
Bajiaolian	Used in treatment of tumors and snakebites, but elevation of liver enzymes within hours of ingestion has been reported
Black cohosh	Popular herbal remedy used for treatment of menopausal symptoms, but multiple reports of hepatotoxicity
Cascara sagrada	Laxative, has caused cholestatic hepatitis
Celandine	Can cause acute hepatitis
Chapparral	Used especially for respiratory tract infections, but liver damage has been reported
Eternal life	Chinese herbal remedy has been associated with hepatotoxicity
Germander	Used for its antimicrobial and anti-inflammatory activity; can cause severe liver damage; removed from most markets
Kava-kava	Documented to have caused hundreds of cases of hepatic damage worldwide
LipoKinetix	Used for weight loss; can cause acute hepatitis and fulminant hepatic failure
Ma huang	Used in weight loss and increasing energy, can cause acute hepatitis
Pennyroyal	Has been associated with severe hepatotoxicity, including one fatality
Pyrrolizidine alkaloids	Has been reported to induce portal hypertension, venoocclusive disease, ascites, and eventual hepatic failure
Senna	A laxative, has been reported to cause toxic hepatitis after overdosing
Skullcap	Reported to cause venoocclusive disease and liver failure

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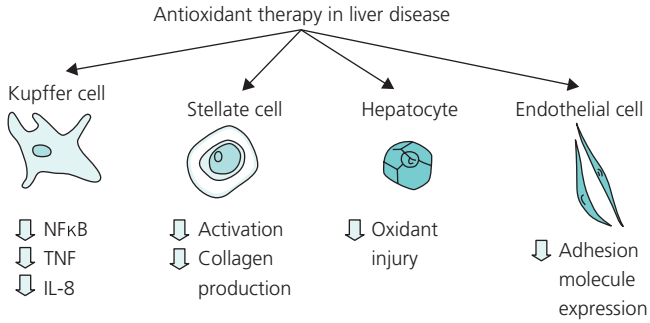

Figure 115.2 Most forms of complementary alternative medicine therapy in liver disease have antioxidant effects. These theoretically increase proinflammatory cytokine production, decrease stellate cell production and subsequent collagen production, attenuate oxidant injury in hepatocytes, and decrease endothelial cell adhesion molecule expression. IL-8, interleukin 8; NF-κB, nuclear factor κB; TNF, tumor necrosis factor.

beat after she initiated an oral regimen of dietary supplements for "internal cleaning." The patient was found to have an elevated digoxin level, and her herbal medications were contaminated with plant extracts containing a cardiac glycoside (*Digitalis lanata*) [146]. Importantly, some Chinese patent medicines purposely contain small amounts of prescription drugs (e.g., antibiotics, ephedrine, methyltestosterone) [139,147]. These drugs may also be contaminated with heavy metals (e.g., lead, arsenic, mercury) [148]. CAM agents can interact adversely with traditional medications (Fig. 115.2 and Table 115.4). An example is the potential interactions of St. John's wort with cyclosporine (cyclosporin) and indinavir [149]. This interaction was highlighted when cardiac transplant patients underwent rejection shortly after starting St. John's wort, presumably because of the induction of cytochrome P450 3A4 by the St. John's wort [150]. It is important to instruct all transplant patients (and other patients on immunosuppressive therapy) to inform their physician before starting any CAM agent. Other, more common, side effects also can be seen with relatively safe agents, such as St. John's wort, including photosensitivity, especially in virally infected patients. A variety of herb–anesthesia interactions are possible (e.g., garlic, gingko, St. John's wort), such as bleeding, and the American Society of Anesthesiologists recommends that patients discontinue all herbal medicines 2–3 weeks before elective surgery and inform their physicians about their CAM use [151]. Indeed, the public can be easily misinformed about CAM agents and inadvertently take an unintended product with a subsequent adverse outcome. On the other hand, some studies may raise

Table 115.3 Selected other adverse effects of herbal remedies on the gastrointestinal tract

Herb	Main use	Adverse effect
Aloe	Constipation, IBS	Diarrhea, abdominal cramps
Black cohosh	Joint pains, menopausal symptoms	Nausea, vomiting
Ephedra	Weight loss	Hypertension, stroke, myocardial infarction, death
Gentian	Appetite stimulant, digestive aid	Nausea, vomiting
Sassafras	General well-being	Carcinoma

IBS, irritable bowel syndrome.

Table 115.4 Clinically proven interactions of complementary alternative medicine (CAM) with conventional medicines

CAM agent	Drug interaction	Consequence
Ginger, ginko	Iron	Reduced absorption of iron
Ginseng, garlic	Warfarin	Increased warfarin levels, increased risk for bleeding
St. John's wort	Cyclosporine, indinivar	Reduced cyclosporine and indinivar serum levels
Chili/capsiacin	ACE inhibitors	Cough
Psyllium	Lithium	Reduced lithium levels
Liquorice	Spironolactone	Reduced effectiveness
	Prednisolone	Hypokalemia, salt and water retention
SAM	Serotonin reuptake inhibitors	Enhanced serotonin elevations

ACE, angiotensin-converting enzyme; SAM, *S*-adenosylmethionine.

inappropriate concerns about CAM agents. A recent meta-analysis on high-dose vitamin E reported a minimal, but significant, increase in mortality [152]. Only a select number of studies were included in this metaanalysis, and it is unclear whether the conclusions can be extrapolated to the population at large. However, many patients taking high-dose vitamin E for reasons such as NASH have discontinued therapy. This study reinforces the need for careful interpretation of the CAM literature.

Conclusion

In conclusion, CAM is already being widely used by our patients with chronic gastrointestinal symptoms and liver disease, either alone or in conjunction with conventional therapies. Today, many types of CAM therapy are based on substantial scientific rationale (e.g., SAM for liver disease, certain green tea polyphenols as antiinflammatory therapy). Some forms of CAM have yielded very positive clinical trial data (e.g., acupuncture and ginger for nausea and vomiting, peppermint oil for dyspepsia, and probiotics for diarrhea). However, because a great potential for toxicity or drug interactions also exists, both the patient and health-care provider need to be optimally educated about CAM. Clinicians may best be served by subscribing to the *PDR for Herbal Medicines* and the *PDR for Nutritional Supplements*. Most of the information in these references originates in publications of Commission E, a European scientific advisory panel with functions similar to those of the FDA, whose focus is to collect and publish information regarding herbal preparation, dosages, purity, uses, drug–drug interactions, and deleterious effects. In addition to the previously mentioned versions of *Physicians' Desk Reference*, the websites of the Cochrane Database System reviews (*http://www.cochrane.org*) and the NCCAM (*http://nccam.nih.gov*) are valuable sources for updated information.

Future directions

CAM therapies hold promise for treating a variety of gastrointestinal and liver diseases, especially those ailments with gaps in effective "traditional" therapies. Research in CAM should continue to focus on scientific rationale, and randomized controlled trials should be designed with sound methodology in order to yield practical and safe application of CAM in clinical practice. Leaders in health care education need to include CAM training in their school curricula as well as continuing medical education programs, and practice guidelines need to incorporate CAM where there is evidence of efficacy. It should be irrelevant whether therapies are categorized as traditional or complementary or alternative, but they should be effective, safe, and available to those who need them.

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Diagnostic and therapeutic modalities in gastroenterology

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A ENDOSCOPIC

116 Reprocessing of gastrointestinal endoscopes and accessories

Gregory Zuccaro Jr

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The risks of gastrointestinal endoscopy are generally discussed in terms of those directly related to the procedure (e.g., oversedation, bleeding after polypectomy, and perforation of the gastrointestinal wall). However, another theoretical risk of endoscopy involves the introduction of pathogens from the endoscope or accessories into patients undergoing a procedure. Because the endoscope and accessories often come into contact with potentially infectious fluids (e.g., blood) from one patient, and then are reused in subsequent patients, pathogens could be passed from patient to patient if not completely removed after each use. Nonpathogenic organisms could theoretically be transmitted to immunocompromised hosts by the same mechanism. Another potential mechanism whereby infection could be introduced via endoscopy is colonization of the endoscope or accessories by organisms in the environment after proper reprocessing, with introduction of these organisms into a subsequent patient. Although apparently rare, clinically significant infections have been documented in the literature by each of these scenarios. In most cases, a breakdown in accepted practices of endoscope and accessory reprocessing can be identified.

Endoscope and accessory reprocessing involves a series of steps that result in the generation of a patient-ready system: a system free of any organisms that might pose a risk of infection to the subsequent patient undergoing endoscopy. In keeping with the concept of universal precautions, each patient is assumed to be potentially infected with a pathogen.

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Transmission of infection through endoscopy

The risk of transmission of infection from patient to patient via endoscopes and accessories appears to be quite small. An American Society for Gastrointestinal Endoscopy (ASGE) review identified 28 cases of transmission of infection via endoscopy since the implementation of standard guidelines for endoscope reprocessing. In each case the reviewers felt that some breach in endoscope reprocessing techniques occurred (although this may not be true because some cases apparently occurred in the face of accepted reprocessing techniques). They estimated that the risk of infection from endoscopy was approximately 1 in 1.8 million [1]. This estimate may be understated, in view of the many reports of infection cited in the following sections.

Hepatitis virus

Transmission of hepatitis C virus (HCV) from a carrier undergoing colonoscopy to two subsequent patients has been documented [2]. HCV genotyping and nucleotide sequencing established that all three patients were infected with the same isolate. Accepted reprocessing techniques were not performed between each procedure. Transmission of hepatitis B virus (HBV) from patient to patient has been reported [3]. Acute HBV infection developed 96 days after endoscopy and therapy for a bleeding ulcer. The endoscope was used immediately before to treat bleeding esophageal varices in a patient with HBV. In this case, however, the endoscope and accessories had been reprocessed carefully following accepted protocols. Serotyping of the HBV showed the same subtype in both patients. The patient with the bleeding ulcer did receive blood transfusions; nevertheless, the investigators (and subsequent reviewers [4–6]) feel that this case most likely represents transmission of HBV. Several clinical series indicate

that the likelihood of HBV transmission from patient to patient is extremely low [7–17]. In many of these series, the endoscope and accessories were not reprocessed following currently accepted protocols, yet no infection in subsequent patients was evident. Transmission of hepatitis virus from patient to patient via endoscopy, although possible, is exceedingly rare.

Human immunodeficiency virus

Transmission of HIV as a result of gastrointestinal endoscopy or bronchoscopy has not been reported, but the virus can be recovered from these instruments after procedures in infected patients. Sampling of the suction and the air/water channels immediately after use in patients with HIV infection revealed HIV-specific DNA by polymerase chain reaction (PCR) in 7 of 20 endoscopes [18]. In a similar study, all seven bronchoscopes tested were positive for HIV-specific DNA [19]. HIV was readily eradicated from all contaminated endoscopes and bronchoscopes using standard reprocessing techniques.

Salmonella species

Numerous reports implicate endoscopy in transmission of *Salmonella* infections [20–27]. A group of patients developed infection with *Salmonella* serotype Newport after undergoing colonoscopy subsequent to this procedure being done in a patient with acute infection [20]. Organisms identical to those recovered from the index case were identified in 8 of 48 patients who had procedures before the transmission was recognized. The apparent source of infection was not a colonoscope but the spring from a biopsy forceps. In all these series, the technique of endoscope reprocessing was less rigorous than currently recommended.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a ubiquitous organism. It can be found in tap water and can quickly colonize any damp area, including the channel of a reprocessed endoscope, water bottle, or endoscopic automated reprocessor. *Pseudomonas* species have even been identified as contaminants within hospital disinfectants, including chlorhexidine [28]. Therefore, although the cases mentioned so far involved transmission of organisms from patient to patient via a contaminated endoscope, pseudomonal infections involve the introduction of this organism from the environment into a patient.

In one clinical series seven patients had *P. aeruginosa* bacteremia after endoscopic retrograde cholangiopancreatography (ERCP) in a 1-year period, with one death related to the infection [29]. In each case the infected patient was the first case of the day in which that duodenoscope was used. In six of the seven cases, the endoscope had been reprocessed more than 48 h prior to the endoscopy. Cultures from these endoscopes after prolonged storage were positive for *P. aeruginosa*, *Escherichia coli*, and *Serratia marcescens*. Similar culture results were obtained from other devices in the endoscopy suite, including wash basins, water irrigation bottles, and irrigation syringes. Infection control measures, including alcohol flushing of endoscope channels after reprocessing prior to compressed air drying to eliminate moisture that would serve as the medium for bacterial growth, resulted in elimination of further cases. The endoscope has been identified as the source of infection in several other series as well [30–40]. Patients may become infected even when the pancreatobiliary tree is normal at the time of ERCP [35].

There may be sources of *P. aeruginosa* infection other than the operating channel of an endoscope. Struelens and colleagues noted an increase in post-ERCP bacteremia after the implementation of automated reprocessors in the endoscopy suite [41]. *Citrobacter* and *Pseudomonas* species were among the organisms identified in patients and cultured from duodenoscopes, and the reprocessor also showed evidence of bacterial contamination. Additional steps, including alcohol and air flush of the duodenoscope channels after reprocessing, and disinfection of the automated reprocessor, remedied the situation. Although the literature on transmission of *P. aeruginosa* involves primarily duodenoscopes, other endoscopes may be similarly colonized. Alvarado and colleagues noted a significant increase in the colonization rate of upper gastrointestinal endoscopes with *P. aeruginosa* after reprocessing in a recently purchased endoscopic automated reprocessor [42]. When endoscopes that had been manually reprocessed were tested, none were found to be contaminated with *P. aeruginosa*. Certain areas of the endoscopic automated reprocessors, including the water inlet hose and air vents, were not accessible to the disinfectant solution and served as a reservoir for the organism. This design flaw in the original endoscope automated reprocessor has been corrected. The problem was corrected in the endoscopy suite by implementation of additional steps to the disinfection process, including rinsing of endoscope surfaces and channels with 70% isopropyl alcohol after machine disinfection, then drying with forced air for 10–20 min [43].

Serratia marcescens

Serratia marcescens has been reported as an infectious complication of ERCP, sclerotherapy, and bronchoscopy. Two consecutive patients developed complications of ERCP related to the organism: acute hemorrhagic pancreatitis (patient died) and acute cholecystitis [44]. Cultures from the duodenoscope biopsy channel and intraoperative cultures for both patients grew *S. marcescens*. A case of with *S. marcescens* mediastinitis and empyema after variceal sclerotherapy was reported, although the organism was not recovered from the endoscope or endoscopy suite [45]. Transmission was also reported via bronchoscopy to six patients after the instrument had been used on a patient with *S. marcescens* pulmonary infection [46]. The organism was recovered from the bronchoscope on subsequent testing. Prior to this episode, the disinfection process

involved only mechanical cleaning, suctioning water and alcohol through the operating channel, wiping the scope with alcohol, and drying the operating channel by aspirating ambient air. After the consecutive infections were identified, Betadine was added to the operating channel cleaning regimen, and gas sterilization was performed. These additional steps eliminated the organism.

Helicobacter pylori

There are multiple clinical series documenting patient-to-patient transmission of *Helicobacter pylori.* In 1979, a group of volunteers undergoing studies of gastric acid secretion developed rapid hypochlorhydria [47]. Endoscopic biopsy revealed histological gastritis. Although no organism was recovered, the investigators suspected an infectious etiology. A similar report noted gastritis and hypochlorhydria in four of six volunteers undergoing gastric acid analysis for a clinical study [48]. Contaminated pH probes rather than endoscopes were the source of infection in these series, emphasizing that proper disinfection must be performed on all gastrointestinal devices (e.g., manometry catheters, pH probes). Gastritis with *H. pylori* developed in an individual with normal baseline histology who underwent serial gastric analysis and endoscopy as part of a research protocol [49]. Presumably the endoscopic procedure was the source of the infection, although no information is offered as to whether the source was the endoscope, the biopsy forceps, or other unidentified agent.

A retrospective review of patient-to-patient endoscopic transmission of *H. pylori* (with restriction enzyme analysis of bacterial DNA) suggested the rate of transmission due to endoscopy and biopsy was 1.1% over 3 years [50]. The method of endoscope disinfection involved mechanical cleaning, a 30-min wash with 2% glutaraldehyde, sterile water rinse, and hot air dry. Biopsy forceps were not always sterilized between use, but sometimes "cleaned and disinfected." All the patients infected with *H. pylori* in the series underwent mucosal biopsy, raising the possibility that the forceps was the source of infection. In a study of nine patients with recurrent infection after *H. pylori* eradication, two had recurrent infection introduced via endoscopy [51]. The investigators reported that "improved" reprocessing techniques eliminated further transmission. The source of transmission (endoscopy, forceps) was not specified.

Mycobacterium species

The contamination of a flexible endoscope with *Mycobacterium* organisms has been regarded primarily as a problem for bronchoscopists, not gastrointestinal endoscopists. However, with the possibility of transmission of atypical mycobacteria to immunocompromised hosts, a review of this literature is relevant to gastrointestinal endoscopists.

Early reports describe patient-to-patient transmission of *Mycobacterium tuberculosis* via bronchoscopy when disinfection was performed using an iodophor, a chemical disinfectant currently felt to be inadequate for endoscope reprocessing [52,53]. However, use of a bronchoscope colonized with *M. tuberculosis* does not necessarily lead to cross-infection. In one series 19 patients had bronchoscopy using a bronchoscope colonized with *M. tuberculosis*. None of the patients developed symptoms or signs of tuberculosis or positive skin tests.

Continual colonization of bronchoscopes with *Mycobacterium chelonae* led to clinically significant respiratory infection in two immunocompromised patients [54]. Glutaraldehyde disinfection of the bronchoscopes did not remedy the problem, nor did ethylene oxide sterilization. When the bronchoscopes were sent for structural analysis, punctures of the operating channels were found, with gross contamination of proteinaceous material within. The bronchoscopes were still functional despite these internal defects. Clinical experience such as this is the basis for current recommendations regarding leak testing of flexible endoscopes with each use.

Mycobacterium tuberculosis and *M. avium* were transmitted via contaminated bronchoscopes, despite use of a rigorous reprocessing protocol involving mechanical cleaning and 2% glutaraldehyde [55]. The endoscope channels were not the source of contamination, but persistent mycobacterial contamination was present on the suction valve. This contamination could not be eradicated with glutaraldehyde, but was eradicated with steam autoclaving. Persistent colonization of endoscopes and bronchoscopes with *M. chelonae* has also been linked to persistent colonization within an automated reprocessor [56]. Attempts to disinfect the reprocessor were not successful because of the formation of biofilm within a portion of the reprocessor. Addition of 70% alcohol rinsing to the endoscopes after reprocessing eliminated the problem. A similar case of *M. abscessus* transmission was traced to persistent colonization of an automated reprocessor [57].

Other organisms

Other microorganisms that have been reported to be transmitted via endoscopy or bronchoscopy include *Listeria*, *Strongyloides*, *Rhodotorula*, *Trichosporon*, *Enterobacter*, and *Citrobacter* [5,38,58–61].

Sterilization and levels of disinfection

The Association for Professionals in Infection Control and Epidemiology (APIC) has proposed definitions helpful in a discussion of endoscope reprocessing (Table 116.1) [62]. Varying levels of disinfection and sterilization may be achieved when reprocessing an endoscope and accessories. In fact, the same agent may achieve sterilization or high-level disinfection of an endoscope depending on factors such as the exposure time and the degree to which debris and organic material were removed prior to exposure to the agent. It is reasonable therefore to ask if an endoscope and accessories

Table 116.1 Definitions in endoscope reprocessing

Sterilization
Complete elimination of all forms of microbial life

Disinfection
Elimination of most or all pathogenic organisms
- High level: all microorganisms except high levels of bacterial spores are destroyed
- Intermediate level: eliminates most microorganisms, except some viruses, fungi, and bacterial spores
- Low level: eliminates most bacteria, but not more resistant organisms such as mycobacteria and spores

Cleaning
Removal of foreign material (e.g., blood, tissue) from the endoscope and accessories; includes wiping, brushing channels, enzymatic detergents, ultrasonic cleaners

should be sterilized between each use, or if a lower standard, such as high-level disinfection, is acceptable.

In 1968, Spaulding proposed a classification scheme that has been accepted and retained (with minor modifications) by experts in infection control, including the APIC [63]. A device that enters sterile tissue or the vascular system is called a critical device, and must be sterilized prior to use. Noncritical devices involve contact with intact skin, and intermediate or low-level disinfection is acceptable. A device that comes into contact with mucous membranes, but not sterile tissue or the vascular system, is called semicritical, and high-level disinfection is deemed sufficient for these items. However, this produces somewhat of a double standard for endoscopy. In many cases, endoscopes simply contact mucosal surfaces, fitting the Spaulding classification scheme nicely. However, a biopsy forceps, sclerotherapy needle, or papillotome may come in contact with the vascular system. Because these devices are passed through the operating channel of the endoscope, should the endoscope be considered a critical device? Although there is no simple answer to this question, most experts in infection control, including APIC, have recommended high-level disinfection for the endoscope, but sterilization of accessories that come into direct contact with the vascular system [6].

Problems specific to the endoscope and the endoscopy unit

The endoscope is a complex structure, with multiple channels and interior surfaces in its internal construction. Areas that cannot be accessed with a cleaning device or disinfectant/sterilant cannot be reprocessed reliably. The operating channel of the endoscope is accessible to a cleaning brush, and manual cleaning of this channel is essential in endoscope reprocessing. The operating channel may become damaged, forming a nidus of persistent colonization. This damage may not be recognizable to the operator because the endoscope may perform normally until the damage is advanced and fluid invasion of the system causes overt malfunction. The air/water channel of the endoscope is smaller and in many endoscopes cannot be brushed manually; forcing air through the channel after the procedure and use of liquid or gas disinfectants/sterilants that can insinuate themselves into these small areas are used to disinfect the air/water channel of the endoscope. The newer, totally immersible endoscopes lend themselves to this aspect of reprocessing. Some newer endoscopes do allow for mechanical cleaning of the air/water channel.

Another aspect of disinfection is the time allotted for the process. Most endoscopy suites have a limited number of endoscopes available and therefore require reasonable turnaround times. A highly effective technique for sterilizing or providing high-level disinfection that requires several hours is not practical and cannot be implemented in most endoscopy suites.

Organisms and their resistance to chemical disinfectants and sterilants

Organisms are not equal in their resistance to destruction by chemical disinfectants or sterilants. Among the most susceptible are viruses such as HBV and HIV. These viruses are readily inactivated (i.e., rendered unable to cause infection if injected into a susceptible host) by a number of chemical disinfectants and sterilants [62]. Among the agents effective in HBV inactivation are glutaraldehyde at 1% and 0.1% concentrations (5-min exposure), 80% ethyl alcohol (2-min exposure), and 98°C heat for 2 min [64]. Hanson and colleagues reported that 2% glutaraldehyde was effective in inactivating HIV in serum within 2 min, but 1% glutaraldehyde was ineffective after 15 min of exposure, and 70% ethanol did not inactivate the virus after a 10-min exposure [65]. Resnick and colleagues reported that HIV was rendered noninfective with exposure to solutions of 0.5% sodium hypochlorite (1 min), 70% alcohol (1 min), and 0.08% quaternary ammonium chloride (10 min) [66]. Van Bueren and colleagues also found inactivation of cell-free and cell-associated HIV with 10-min exposure to 70% alcohol [67].

In general, the bacteria that may lead to cholangitis or other infections from endoscopy, including *P. aeruginosa*, *S. marcescens*, and *Salmonella* species, are also destroyed by common disinfectants and sterilants [62]. There is a report of isolation of a strain of *P. aeruginosa* resistant to disinfection by gluteraldehyde [68]. Small viruses, including poliovirus and hepatitis A virus (HAV), may be more difficult to inactivate. Chemical disinfectants, including 70% ethanol, 3% and 6% hydrogen peroxide, and dilute aldehydes, are only variably effective against poliovirus [69]. Solutions of 2% glutaraldehyde and high-concentration hypochlorite are necessary to inactivate the virus reliably. Only 0.5% sodium hypochlorite, 0.3% organochlorine, 2% glutaraldehyde, and modified quaternary ammonium compounds effectively inactivated coxsackievirus, adenovirus, parainfluenza virus, and coronavirus [70]. Only 2% glutaraldehyde, quaternary ammonium compounds with high concentrations of HCl, and sodium hypochlorite were effective in HAV inactivation [71]. Among the less efficacious compounds were iodines, alcohols, acetic acids and peracetic acids, and hydrogen peroxide. Peracetic acid and 2% glutaraldehyde are among the chemical sterilants able to inactivate human rotavirus [72]. Among the less efficacious agents were 70% ethyl alcohol and sodium hypochlorite. Protozoan species are also relatively more resistant to destruction. Campbell and colleagues found that *Cryptosporidium* oocysts were resistant to many common disinfectants but were inactivated by some agents, including formol saline and ammonia compounds [73]. The commonly used agents in reprocessing endoscopes were not tested in this series.

Among the most resistant microorganisms are mycobacteria and bacterial spores. Several agents, including quaternary ammonium compounds, dilute sodium hypochlorite, 70% ethanol, povidone iodine, iodophors, and chlorhexidine, are ineffective against *M. tuberculosis*; more concentrated sodium hypochlorite, and 2% glutaraldehyde, are effective [74]. Contact times are important: 2% glutaraldehyde did not destroy the organism in 1 min but did in 10–30 min. A study with spores of *Bacillus subtilis* and *Cryptosporidium sporogenes* found that 6% hydrogen peroxide solution was extremely effective against the spores, while 2% glutaraldehyde solutions reduced the number of viable spores but did not completely eradicate all spores, even with prolonged exposure times [75]. Glutaraldehyde, peracetic acid, and 10% hydrogen peroxide were the most effective agents in a study of *B. subtilis* spores, but none totally eradicated all spores [76]. Some mycobacterial species have been reported to be resistant to even prolonged exposure to glutaraldehyde solutions [77].

Common chemical disinfectants and sterilants

A large number of chemical disinfectants and sterilants may be used in hospitals or endoscopy suites. Several factors determine the suitability of a given agent for endoscope reprocessing:

- the agent must be effective against a broad range of viruses, fungi, and bacteria, including mycobacteria
- the time of exposure necessary for this activity must not be prohibitively long
- the agent must not damage the endoscope or accessories
- the agent should not be toxic to staff or patients
- the cost must not be prohibitively high.

Gas sterilization of endoscopes has long been established as an effective method. Ujeyl and colleagues found that only a 20-min exposure rendered endoscopes free of microorganisms with no apparent damage [78]. Alfa and colleagues raised concerns about the ability of sterilizers to completely eradicate organisms from the narrow lumens of endoscope channels. Multiple bacteria, including *P. aeruginosa*, mycobacteria, and bacterial spores, were inoculated into plastic tubing 3.2 mm in diameter and sterilized with several agents, including ethylene oxide. None of the tested methods completely sterilized the narrow lumen when serum and salt were also present [79]. This result emphasizes the need for mechanical cleaning prior to disinfection and sterilization with any gas or chemical.

One commonly used agent is glutaraldehyde. In the exposure time typical for endoscopy suites, it is an effective high-level disinfectant. If allowed sufficiently long contact times, sterilization is possible. Effective killing of bacteria, tubercle bacilli, and viruses occurs within 10 min, and vegetative spores in less than 3 h [80]. Kovacs and colleagues found that 2% glutaraldehyde achieved high-level disinfection from *M. chelonae* in endoscopes with only a 10-min exposure, but only if mechanical cleaning and alcohol rinsing were also performed [81]. However, not all clinical series support effective killing of mycobacteria within 10 min. In one study, the length of exposure with 2% alkaline glutaraldehyde required to achieve 99% eradication of all mycobacterial species ranged from 10 to 40 min (10 min for *M. tuberculosis*) [82]. These data have led to differing recommendations for the exposure time necessary for 2% glutaraldehyde to achieve high-level disinfection. The US Food and Drug Administration (FDA) had required labeling of Cidex (2% glutaraldehyde; Johnson & Johnson) as effective as a high-level disinfectant only after a 45-min exposure at 25°C [83]. However, Rutala and Weber [83] and prior APIC guidelines [6] argued that this amount of exposure time was only necessary if no mechanical cleaning of the endoscope had occurred prior to glutaraldehyde treatment, and that exposure times of 20 min or more were sufficient to achieve high-level disinfection if mechanical cleaning was performed. Guidelines of several professional societies support a 20-min exposure time for high-level disinfection [84,85], as do the most recent APIC guidelines for infection prevention and control in flexible endoscopy [86]. Testing of endoscopes contaminated with *Enterococcus* or *H. pylori* confirm these recommendations [87,88].

Rhinitis, dermatitis, nausea, headache, and respiratory difficulties are among the reported adverse effects on staff of

glutaraldehyde exposure [89–93]. Epidemiological studies have suggested that spontaneous abortions are higher among staff performing sterilization with ethylene oxide but not glutaraldehyde [94]. Adequate ventilation is necessary in areas where this agent is handled, staff should wear appropriate protective equipment, and the atmosphere should be periodically tested to ensure that concentrations are within appropriate parameters.

Glutaraldehyde has been linked to episodes of colitis in patients undergoing endoscopy. In one series [95], six patients experienced low-grade fever, tenesmus, and bloody diarrhea, with symptoms lasting approximately 5 days. It was felt that the colitis was due to a gradual build-up of glutaraldehyde in the post-reprocessing rinse water, and failure of the staff to use forced air to dry the channels after reprocessing. In a similar outbreak most of the cases were in patients whose sigmoidoscope was reprocessed by a new staff member who did not flush residual glutaraldehyde from the endoscope channels after reprocessing [96].

Ortho-phthalaldehyde is a high-level disinfectant whose use decreases the time of endoscope reprocessing compared with glutaraldehyde (at least 12-min manual soak, at least 5 min in automated reprocessor). In contrast to glutaraldehyde, there appear to be no issues with odor or air handling.

Peracetic acid is an effective chemical sterilant, active against all organisms, including bacterial spores [97,98]. Bradley and colleagues evaluated the Steris System 1 Processor (0.2% peracetic acid) in the reprocessing of endoscopes contaminated with vegetative bacteria and spores. All bacteria were killed within 1 min and spores within 5 min [97]. The investigators noted that the cost per cycle was higher than for reprocessing with glutaraldehyde. Whitbourne and Preston confirmed the efficacy of the Steris process on 10 medical devices contaminated with high titers of bacterial spores (approximately 10^7 colony-forming units/mL) [99]. The process involves exposure of the medical device to the peracetic acid for 12 min, followed by several automatic rinses with water sterilized by passage through a membrane filter, for a total cycle time of approximately 25 min. Fuselier and Mason reprocessed cystoscopes for 1 year using the Steris System 1 processor, and then used the Voluntary Hospital Association Plus Glutaraldehyde HLD system the next year. They reported no "clinical differences" between the two systems, but no microbiological data were presented. Yearly operating costs for the Steris processor totaled $6037, compared with $445 for the HLD system [100].

Hydrogen peroxide is another agent active against a wide range of microorganisms. Vesley and colleagues tested the efficacy of 2% glutaraldehyde, 6% hydrogen peroxide, and ethylene oxide in endoscopes contaminated with *Pseudomonas* or *Bacillus* species [101]. Ethylene oxide was the most effective agent overall. Hydrogen peroxide was more effective than 2% glutaraldehyde against the *Bacillus* organisms. However, the exposure times for these liquid chemicals was only 10 min, not the minimum 20 min recommended by APIC for glutaraldehyde. As with glutaraldehyde, hydrogen peroxide may cause iatrogenic colitis [102].

Other FDA-cleared sterilants and high-level disinfectants include various concentrations or combinations of the above-mentioned agents, as well as hypochlorite and hypochlorous acid 650–675 ppm and active free chlorine, and glutaraldehyde in combination with phenol/phenate or isopropanol.

Essential concepts in reprocessing of endoscopes and accessories

The overriding principle in endoscope reprocessing is that each patient must be assumed to potentially possess an infectious agent that could be passed via the endoscope to a subsequent patient. It is inappropriate for an endoscopy unit to reserve an endoscope or set of endoscopes for patients identified with some known pathogen. Rather, processes must be in place where all endoscopes are reprocessed in such a way that all potential pathogens are eliminated.

Several professional societies, including the APIC, the Society of Gastroenterology Nurses and Associates (SGNA), and the ASGE have published guidelines for proper reprocessing of endoscopes and accessories [84–86]. Essential aspects of these guidelines are presented below and summarized in Table 116.2. These are not intended to be a substitute for the step-by-step detail presented in the guidelines.

Table 116.2 Some essential concepts in the reprocessing of endoscopes and accessories

Inspection for damage should be performed after each case. Leakage testing may identify internal damage not apparent from endoscope performance
Mechanical cleaning prior to exposure to the disinfectant/sterilant is essential
High-level disinfection is the current standard for endoscope reprocessing
Endoscopic accessories that may contact the vascular system must be sterilized if reused; mechanical cleaning alone or high-level disinfection is inadequate
Channels must be adequately dried after high-level disinfection to prevent colonization with microorganisms
Duration of use and monitoring of effective concentration for high-level disinfectants/sterilants must follow manufacturer's recommendations
Continuous quality improvement is essential for each endoscopy unit

Inspection for damage

Leaks, tears, and other disruptions to the external or internal integrity of the endoscope may be a nidus of continual colonization by microorganisms [54]. Internal damage to the endoscope may not be apparent to the endoscopist until it is far advanced because the endoscope may still perform well. The endoscope must be carefully inspected prior to reprocessing. Leak testing may detect internal structural damage at an early stage.

Mechanical cleaning

Removal of organic material prior to exposure to chemical disinfectants or sterilants is the most important step in endoscope reprocessing. Scrupulous cleaning of external surfaces, use of enzymatic detergents on external surfaces and internal channels, passing a brush through the operating channel, brushing or forcing air through the more narrow air/water channel, and manual or ultrasonic cleaning of detachable parts of the endoscope are all part of this process. Special attention must be given to difficult-to-access portions of the endoscope, such as elevators and elevator channels of the duodenoscope. The elevator should be mechanically cleaned and the channel flushed.

Removal of organic and foreign material is necessary to achieve high-level disinfection. Alfa and colleagues studied the suction channels of 10 bronchoscopes, duodenoscopes, and colonoscopes immediately after procedures. They identified multiple compounds and organisms, including protein, hemoglobin, carbohydrate, endotoxins, and bacteria. Mechanical cleaning eliminated a good deal of this material, but protein and some bacteria remained [103]. Lewis and Arens noted persistence of HIV and vegetative bacteria after high-level disinfection of dental devices when the organism was entrapped in commonly used lubricants [104]. The necessary exposure time of the liquid chemical sterilants to achieve high-level disinfection is decreased by prior mechanical cleaning.

Mechanical cleaning of the endoscope, without exposure to chemical sterilant, eliminated microorganisms in one study from 66 of 68 sites tested [18]. Other studies document the efficacy of mechanical cleaning in inactivation of HBV [105], HCV [106], *H pylori* [107–110], Gram-negative bacteria [111], and *Clostridium difficile*. Thorough mechanical disinfection plus use of an approved chemical disinfectant/sterilant is highly effective in eliminating bacteria and viruses from endoscopes, but is not effective when mechanical cleaning is ineffective [113].

Use of a recommended disinfectant or sterilant for high-level disinfection

Manual high-level disinfection may still be performed but, with the advent of totally submersible endoscopes, automated reprocessors have become more common. These machines lessen the amount of time staff members must devote to the overall process, and lessen exposure to glutaraldehyde, if this is the agent of choice. Vesley and colleagues showed that automated reprocessors were more effective than manual high-level disinfection in killing *B. subtilis* [101]. However, Fraser and colleagues found no difference in overall endoscope contamination rates when endoscopes disinfected manually were compared with those disinfected in automated reprocessors [114]. Automated reprocessors do have potential drawbacks. Persistent infection of the endoscope reprocessor with resultant contamination of the endoscope has been documented [42]. The endoscope must be properly positioned within the machine, with all adapters and covers correctly placed. Furthermore, with endoscopic automated reprocessors using glutaraldehyde, the effective concentration of the disinfectant will decrease with repeated use [115]. Dilute glutaraldehyde is less effective in its action against microorganisms [116,117]. A test strip or other device must be used at least daily to monitor the concentration of the liquid disinfectant, possibly more frequently depending on procedure volume. Solutions must be changed in accordance with the manufacturer's recommendations. *o*-Phthalaldehyde appears to be stable for up to 14 days of use; just as with glutaraldehyde, test strips to monitor concentration should be used routinely [118]. Again, solutions must be changed in accordance with the manufacturer's recommendations. For all high-level disinfectant/sterilant agents, the duration of use and monitoring of effective concentration should follow the manufacturer's recommendations.

Rinsing and drying

The chemical disinfectant or sterilant must be rinsed from the internal and external surfaces of the endoscope. If tap water (which may be colonized with microorganisms) is used, a rinse with 70% alcohol should be performed. Drying with compressed air (or drying cycles on some automated washers) will also decrease the likelihood of residual moisture, which can be a source of colonization. Drying of endoscopes prior to prolonged storage significantly decreases the rate of bacterial colonization [119], and forced air drying adds effectiveness to the disinfection process [120]. Alcohol rinse prior to prolonged storage may also decrease the likelihood of colonization. Endoscopes should be stored with the insertion tubes uncoiled, and in a well-ventilated cabinet to facilitate continued air drying, and with valves and buttons removed.

Reprocessing of endoscopic accessories

Because biopsy forceps and other accessories may come into contact with the vascular system, these items should be sterile prior to each use. Both disposable (single use) and reusable forceps are commercially available. Improperly reprocessed biopsy forceps may be the source of transmission of infection. Single-use disposable forceps do not expose patients to this risk. Two recent trials support the use of disposable over reusable forceps in terms of cost and function [121,122].

Most accessories for therapeutic ERCP, including papillotomes, guidewires, and extraction balloons, are labeled as single-use items. These items incur a significant cost for the endoscopy suite and are not entirely compensated by technical room reimbursements [123]. Reusable papillotomes are available. These devices function well after multiple uses and sterilizations, but cost more than single-use papillotomes [124,125]. The cost associated with endoscopic accessories has led some centers to reprocess items labeled as single use. Kozarek and colleagues found that the majority of single-use papillotomes performed well with multiple uses and sterilizations. No organisms were recovered from the devices after manual cleaning followed by ethylene oxide sterilization [126]. In a follow-up study, this group found that the "single-use" papillotomes could be used an average of 3.4 times, and such reuse saved approximately $66 000 in 1 year for their practice. No infectious complications were attributed to the reuse [127]. This group has also studied the reuse of other single-use items such as argon plasma coagulation probes, and assert that sterilization could be achieved and up to 10 uses from a single probe obtained [128]. In a 1995 survey of hospitals in Australia, 58% were reusing at least some medical devices labeled as single use [129], and a more recent survey documented reuse is common in the Asia–Pacific region [130]. There are no data to indicate how common this practice is in the USA. A recent APIC guideline states only that this practice is "controversial," and that implementation of such a program "requires a major institutional commitment, including a monitoring committee with clearly defined protocols" [86].

Continuous quality improvement

Proper reprocessing of endoscopes and accessories requires an organized plan, motivated and educated staff, and a continuous quality improvement process that monitors each step of the process. Breakdowns in reprocessing techniques can lead to clinically significant infections, and even death. The process can fail due to technical or human error. Staff and processes must be assessed and improved continually. Each unit should develop its own plan and should generate data on factors including competence of staff and results of random cultures from endoscopes, reprocessors, and accessory equipment.

Compliance with recommendations for endoscope reprocessing

One concern about endoscope reprocessing is the complexity of the process and the potential that some steps might be omitted or performed inadequately in a busy endoscopic practice. Several investigators have examined the actual practice of endoscope reprocessing, either with surveys or site visits. A 1988 survey of 74 endoscopy suites in western Europe revealed that once a procedure was completed in a patient with no known infectious disease, high-level disinfection was not performed after upper endoscopy in any center, in only 13% of centers after colonoscopy, and in only 30% of centers after ERCP [131]. In a 1989 survey only 45% of Australian hospitals responding followed a process for high-level disinfection after each case [132]. Two years later, a repeat survey indicated that high-level disinfection was satisfactorily performed by 75% of respondents [133]. A 1991 survey in 107 endoscopy units in North Carolina found that glutaraldehyde was the chemical disinfectant used in 91% of units. Only 51% exposed the endoscope to glutaraldehyde for at least 20 min (a 10-min cycle was used in most other centers), and 54% rinsed with only tap water (not sterile water or alcohol after tap water) after disinfection [134]. A US survey of SGNA members and nonmembers with an interest in endoscope reprocessing found that 93% performed chemical disinfection after each case; glutaraldehyde was the disinfectant used in 88% of respondents' practices [135].

A more sobering report came from Kaczmarek and colleagues, who directly studied endoscopic reprocessing practices in 26 endoscopy suites: 24% of endoscopes cultured after high-level disinfection were positive for bacterial growth. A lack of sterilization of all biopsy forceps was observed in 78% of units. Several other basic errors in the disinfection process were observed [136]. Variability was also observed in 18 endoscopy areas in eight Massachusetts hospitals; however, high-level disinfection was practiced in 17 of 18 endoscopy areas. Glutaraldehyde soak times varied from 10 to 45 min, and not all areas performed sterilization of biopsy forceps [137]. In a more recent survey, a significant minority of US centers did not completely conform to established society guidelines for reprocessing [138]. In a 1996 survey of 20 Japanese endoscopy suites, endoscopes in all centers were used in consecutive patients with mechanical cleaning but no high-level disinfection [139].

Future trends

Proper reprocessing of endoscopes demands time and careful attention, is not foolproof, and is associated with significant cost. Therefore, the concept of a sheathed or disposable endoscopic system is very attractive. A new endoscopic system in which the working surfaces are covered with a disposable sheath has been assessed in clinical trials [140,141]. In a prospective trial, the length of time required to perform endoscopy was slightly longer than with the conventional endoscope, while set-up and reprocessing times were far shorter [142]. Recent reports indicate promise for new techniques of endoscope reprocessing using super-oxidized or acidic electrolytic water [143–146]. A complete list of FDA-cleared sterilants and disinfectants, along with their specific attributes, can be found at the FDA website [147].

Summary

Introduction of infection into patients undergoing endoscopy is rare but may occur. Infection may be due to patient-to-patient transmission or from introduction of microorganisms from the environment into patients. Potential sources of infection include the endoscope, accessories, and automated reprocessors. High-level disinfection is recommended for reprocessing endoscopes, but sterilization is indicated for accessories that may come into contact with the vascular system. The process of high-level disinfection requires close attention to multiple steps. Several clinical series have demonstrated breakdowns in this process, with resultant morbidity and mortality. A continuous quality improvement system is essential for each endoscopy center.

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A ENDOSCOPIC

117 Sedation and monitoring for gastrointestinal endoscopy

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Introduction

Sedation and analgesia improves patient tolerance to endoscopic procedures by alleviating anxiety and pain [1]. Adequate and comfortable sedation improves the public reputation of endoscopy, enhances adherence with colonoscopic screening, and increases the likelihood of patients returning for follow-up examinations [2]. Sedation prevents or blunts potentially harmful autonomic stress responses, e.g., hypertension and tachycardia [3,4], although some studies found that sedation did not reduce cardiac stress [5,6]. Sedation is critical to accomplish procedures in children and uncooperative adults. Patient comfort allows the endoscopist to focus on the technical performance of endoscopy.

Some patients tolerate esophagogastroduodenoscopy (EGD) and colonoscopy with little or no sedation. Tolerance of unsedated procedures is more likely with older age, lack of anxiety, male gender, absence of abdominal pain history [7–11], and higher levels of education [12]. Ultra thin endoscopes facilitate EGD without sedation [13–15]. Cultural differences in pain perception and expectations of patients and physicians produce great variation in sedation practice between countries. Very high percentages of unsedated colonoscopy have been reported from Finland [16], Turkey [17] and Northern Italy [18]. Sedation is used less in Asia and Europe compared with America [19]. However, general anesthesia with propofol is used commonly in France [20].

Sedation is associated with several cardiac and respiratory adverse events. For these reasons, it is important for endoscopists to assure effective as well as safe sedation during endoscopy. Endoscopists and their nurses, therefore, need a strong familiarity with the different levels of sedation, adverse events as well as patient resuscitation maneuvers, including reversal agents. Continuous hemodynamic monitoring, oximetry, and periodic check of the depth of sedation during the procedure are crucial.

Continuum of depth of sedation

Table 117.1 shows the levels of sedation as defined by the American Society of Anesthesiologists (ASA) [21] and Joint Commission on Accreditation of Healthcare Organizations (JCAHO) [22].

It is important to realize that sedation is a continuum and patients may slip quickly through the various stages. Continuous monitoring of depth of sedation is critical. Individuals administering moderate or deep sedation should be trained adequately to rescue patients from deep sedation and general anesthesia, respectively. A set of standards of practice specific to monitoring and sedation for gastrointestinal endoscopy by nonanesthesiologists has been issued by ASA [21] and by the American Society for Gastrointestinal Endoscopy (ASGE) [23].

Presedation preparation

Clinicians should perform a sedation-oriented history including a history of experience with previous sedation, sleep apnea, major comorbidities, drug allergies, current medications, smoking, and alcohol or substance use. A complete assessment of airway, baseline vital signs, respiratory rate and effort, and general neurological status should be done. Patients should fast for a minimum of 6 h before the procedure, though clear liquids can be consumed up to 2 h prior;

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Table 117.1 Continuum of depth of sedation

	Minimal sedation (anxiolysis)	Moderate sedation/analgesia (conscious sedation)	Deep sedation/analgesia	General anesthesia
Responsiveness	Normal response to verbal stimulation; cognition and coordination may be impaired	Depressed; purposeful response to verbal commands or light tactile stimulation	Depressed; not easily arousable; purposeful response to repeated or painful stimulation	Loss of consciousness; unarousable even by painful stimulation
Airway	Maintained	No intervention needed to maintain patent airway	Intervention may be needed to maintain patent airway	Intervention often needed to maintain patent airway
Spontaneous ventilation	Maintained	Adequate	May be inadequate	Frequently inadequate; positive pressure ventilation may be required
Cardiovascular function	Maintained	Usually maintained	Usually maintained	May be impaired

Modified from the American Society of Anesthesiologists Task Force on Sedation and Analgesia by Non-anesthesiologists, 2002 [21], with permission from Lippincott Williams & Wilkins.

this would be increased to 8 h and 4 h respectively for elderly patients [24]. In emergency situations, when fasting is not practical, either the target level of sedation should be modified or endotracheal intubation should be considered. Appropriate preprocedure counseling must be done regarding risks and benefits of, and alternatives to sedation. Intravascular access should be obtained and maintained through recovery. Routine sedation does not require the presence of an anesthesiologist, however in case of severely compromised patients or those with a difficult airway or with previous adverse reaction to sedation, such consultation may be warranted.

Medication regimens

The choice of sedatives depends on the endoscopist's preference for and familiarity with different regimens as well as the type of procedure. Brief procedures can be completed with minimal amounts of sedation in some cases, whereas therapeutic procedures may require deep sedation. For moderate sedation benzodiazepines are typically used either alone or in combination with an opiate. Propofol is an emerging agent for endoscopy. It may be used either alone for deep sedation, or it can be combined with benzodiazepines or opiates and targeted to minimal, moderate, or deep sedation. Sedatives are given intravascularly for endoscopy and must be administered slowly and titrated in small increments. Another approach is patient-controlled analgesia which allows patients to administer their own sedation [25,26]. The pharmacology of the commonly used agents is summarized in Table 117.2.

Benzodiazepines

Benzodiazepines induce relaxation, anxiolysis, and amnesia. Additional doses are given according to the desired level of sedation as well as patient tolerance. The most commonly used benzodiazepines for endoscopy are midazolam [27–29] and diazepam, both of which are comparable in efficacy [30]. Midazolam is preferred because of better efficacy and tolerability [31], shorter duration, better amnesic properties, and 2–3 times higher potency than diazepam [32]. Also, it has a decreased incidence of local pain and phlebitis compared with diazepam. It does not significantly affect the gag reflex when given in recommended doses. Midazolam crosses the placenta and also enters breast milk. Both midazolam and diazepam are pregnancy category D. However, birth defects have been associated with chronic oral use of diazepam during pregnancy [33–36]. Short-term use for endoscopic procedures during pregnancy is widely considered safe.

Benzodiazepines do not have analgesic properties and usually require coadministration of opiates for endoscopy. Dosages must be reduced in elderly or debilitated patients and in those receiving narcotics or other CNS depressants concomitantly. Underlying liver disease must also be considered as benzodiazepines are metabolized by the liver.

Adverse effects include paradoxical agitation and severe respiratory depression, which is greatly potentiated by coadministration of opioids. Inadvertent overdose can cause severe respiratory depression, hypotension, general anesthesia and apnea. Management is supportive and administration of the reversal agent flumazenil (see below).

Opiates

Meperidine and fentanyl are among the most commonly used opiates for gastrointestinal endoscopy. They have sedative as well as analgesic properties. Meperidine affects both the central and the peripheral nervous systems and its metabolites have neurotoxic potential. Renal impairment can lead to accumulation of metabolites, which may cause seizures.

Table 117.2 Pharmacology of commonly used sedatives

Sedative		Mechanism of action	Properties	Onset/peak effect	Duration of action	Side effects	Dosing
Benzodiazepines	Midazolam	Binds to benzodiazepine receptors on postsynaptic GABA neuron in CNS, including the limbic system, reticular formation and enhances the inhibitory effect of GABA on neuronal excitability	Induces sedation and amnesia. No analgesic properties	Onset 1–5 min	1–3 h	Respiratory depression (decreased tidal volume or respiratory rate); potentiated by concomitant use of opiates; respiratory arrest; apnea hypotension CNS depression; drowsiness; oversedation. Paradoxical agitation	i.v. Initial: 0.5–2 mg over 2 min; repeat doses every 2–3 min; usual total dose: 2.5–5 mg. Decrease doses in elderly and by 30% if opiates are administered concomitantly. Maintenance: 25% of dose used to reach sedative effect
	Diazepam	Same as midazolam	Less potent and amnesic than midazolam	Onset 3–10 min	2–8 h	Same as midazolam	i.v. 2.5–5 mg; incremental doses of 2.5 mg at 3–4 min intervals
Opiates	Meperidine	Binds to opiate receptors in CNS, causing inhibition of ascending pain pathways; produces generalized CNS depression	Analgesic with mild sedative properties. Slow onset and longer duration than other opioids. Used for longer procedures	Onset 1–3 min Peak 10–15 min	2–3 h	Respiratory depression, hypotension, nausea, vomiting, CNS depression	i.v. Initial: 15–50 mg; redose 25 mg at 5 min intervals as needed
	Fentanyl	Binds with receptors within CNS, increases pain threshold, alters pain reception, inhibits ascending pain pathways	Analgesic with mild sedative properties. Rapid onset and recovery; well-suited for outpatient endoscopy	Onset 30 s; peak effect 5–8 min	0.5–1 h	Respiratory depression, hypotension, bradycardia, CNS depression, nausea, vomiting	i.v. Initial: 25–100 µg; redose 25–50 µg/dose every 1–2 min as needed
Propofol		Hindered phenolic compound with i.v. general anesthetic properties	Rapid onset. Fast recovery. Smaller doses are required when used with narcotics. No analgesia, weak amnesic effect	Onset 30–60 s	3–10 min	Hypotension, apnea, injection site stinging or pain	i.v. Initial 20–40 mg slow injection. Incremental boluses of 10–20 mg every
Promethazine		Blocks postsynaptic dopaminergic receptors in the brain; alpha-adrenergic blocker; competes with histamine for the H1-receptor; reduces stimuli to the brainstem reticular system	Synergizes sedation; combats nausea	Onset 3–5 min	2–6 h	Hypertension, bradycardia, confusion, disorientation. Rapid administration may cause transient hypotension	i.v. 12.5–50 mg/dose. Infused at a maximum rate of 25 mg/min
Droperidol		Butyrophenone neuroleptic tranquilizer. Mild alpha-adrenergic inhibitory action	Exerts anti-emetic and anti-anxiety effect. Mild sedative ECG monitoring for 2–3 h after administration is suggested	Onset 3–10 min	2–4 h	Q–Tc prolongation and torsades de pointes (dose dependent). Hypotension, tachycardia, restlessness, anxiety. Extrapyramidal symptoms	i.v. Initial 2.5 mg; redose 1.25 mg to achieve desired effect; administered as a rapid IVP, over 30–60 s. Administer additional doses with caution. Maximum dose 5 mg

CNS, central nervous system; ECG, electrocardiogram; GABA, gamma-aminobutyric acid or γ-aminobutyric acid; i.v., intravenous; IVP, intravenous pyelogram; Q–Tc, Q–T interval corrected for rate (the Q–T interval is measured from the Q point to the end of the T wave on an ECG).

The dose should be reduced to 75% of normal for creatinine clearances of 10–50 mL/min and to 50% for creatinine clearances < 10 mL/min. The initial starting dose is 0.4 mg/kg; however, this should be reduced in the elderly. Titration should be done using half this dose, keeping in mind that onset of action may be 5–10 min. Fentanyl is a morphine-like compound, although much more potent. It produces profound analgesia and suppresses respiratory and cough centers. It has rapid onset and clearance and reduced incidence of nausea compared with meperidine. In high doses, it can cause chest wall rigidity and respiratory difficulty. One disadvantage of fentanyl is cumulative respiratory depression with successive doses which may result in dissociation between analgesia and respiratory depression [37]. Both meperidine and fentanyl are pregnancy category C.

All narcotics should be used cautiously in patients taking other CNS depressants. They should be avoided in patients taking monoamine oxidase inhibitors. Caution must be taken with liver disease, just as with benzodiazepines.

Propofol

Propofol is a substituted isopropyl phenol, which promotes gamma-aminobutyric acid or γ-aminobutyric acid (GABA) activity in the brain. It is a relatively new addition to sedation regimens in endoscopy. In comparison to benzodiazepines/opioids, the mean time to onset of sedation is shorter: depth of sedation is greater, with faster recovery [38] and discharge sooner. Propofol induces amnesia when titrated to deep sedation or general anesthesia [39,40]. Rapid onset of sedation and rapid recovery may improve efficiency of endoscopy units [41]. Safety and efficacy of propofol has been demonstrated for colonoscopy, EGD, EUS, and ERCP [38,42–44]. Surveys of patient satisfaction found that propofol compared with regular sedation had no significant impact on or improvement in patient satisfaction [31,42,45].

Propofol has been administered by intermittent bolus [46,47], continuous infusion [46,48], and patient-controlled analgesia [49–53]. Intermittent bolus allows faster onset of sedation and has been preferred for endoscopy [54]. Initial boluses when the drug is used as a single agent are typically 30–50 mg and are adjusted for age, weight, and comorbidities. Subsequent boluses are 10–20 mg. When used as a single agent, propofol is typically targeted to deep sedation for endoscopy. Avoidance of respiratory depression, as with most sedatives, is critically dependent on gradual titration [55,56]. Propofol, when used as a single agent for moderate sedation, often precipitates withdrawal responses with coughing occurring during upper endoscopy. Deep sedation is usually required when the drug is given alone.

Propofol has been safely administered by nonanesthesiologists for endoscopy in more than 200 000 patients without reported death and only one case requiring endotracheal intubation [57]. In a meta-analysis, propofol was less likely than narcotics and benzodiazepines to produce hypoxemia [58]. A registered nurse supervised by the endoscopist is the most common model [54,59–62]. The registered nurse must have no duties except drug administration and patient monitoring. A technician should assist the endoscopist with technical performance of the procedure. The rationale for administration by nonanesthesiologists is cost reduction. Anesthesia groups have opposed nonanesthesiologist administered propofol [63–65], citing the package insert [66,67] and safety concerns [68], but the evidence from clinical studies overwhelmingly supports the safety of nonanesthesiologist administered propofol [45,69–74].

Successful training programs for nonanesthesiologists incorporate didactic training, testing and typically 2 weeks of observation and supervised administration [69,75,76]. Nonanesthesiologists should not pursue administration of any sedative for endoscopy without appropriate training. Propofol administration by nonanesthesiologists in nonintubated patients is prohibited in 12 states by state laws or nursing practice acts [77].

Nonanesthesiologist administration of propofol as a single agent should be performed with caution in patients at increased risk of aspiration (e.g., achalasia, acute upper GI bleeding, delayed gastric emptying), increased risk of airway obstruction (e.g., sleep apnea, high risk airway assessment), and multiple comorbidities. Propofol is contraindicated with allergy to eggs or soybeans [66,67].

Coadministration of benzodiazepine or opioids enhances the sedative effect of propofol. They also block withdrawal responses and coughing and allow titration of propofol to moderate sedation [78]. Combination regimens targeted to moderate sedation would be logically expected to reduce the safety concerns of anesthesiologists. Combination therapy allows total propofol doses to be reduced by about 50% [79,80] and make propofol administration easier by prolonging the interval between doses [47,81]. Patients who are candidates are usually ASA class I to III.

The major drawback of propofol is the potential for respiratory depression, the risk of which far outweighs the risk of cardiovascular compromise [58]. Burning at the site of injection is common and lidocaine may block the effect. Monitoring patients receiving propofol is discussed below.

Antihistamines

Phenergan and diphenhydramine can be used as sedatives adjunct to opiates or benzodiazepines and to treat nausea induced by these medications. Phenergan is pregnancy category C, and diphenhydramine is B.

Droperidol

Droperidol is a neuroleptic and is used as an adjunct for complex endoscopic procedures and in patients who have paradoxical reactions to standard sedation or in whom narcotics and benzodiazepines are likely to be inadequate [82,83] (history of alcoholism, prescribed or illicit benzodiazepine or opiate

use, or prior difficulty with conscious sedation). Droperidol exerts a calming and antiemetic effect, has a mild sedative and α-adrenergic inhibitory action, and can cause prolonged postprocedure drowsiness. It does not exacerbate opioid-induced respiratory depression. Additional boluses should not be administered for agitation once the procedure has started because, by the time dosages take maximal effect, the procedure may be complete and recovery may be prolonged. Safety and efficacy have been demonstrated in several studies [84–86]. It prolongs the Q–Tc interval (Q–T interval corrected for rate) on the electrocardiogram and can induce torsades de pointes which prompted FDA to issue a black box warning. Cardiac events, some fatal, have been reported in patients without known risk factors for Q–T prolongation [87]. Droperidol is contraindicated if the Q–Tc is prolonged > 440 ms in men and > 450 ms in women. Caution must be exercised in patients with risk factors for Q–T prolongation such as congestive heart failure, bradycardia, cardiac hypertrophy, hypokalemia, hypomagnesaemia, or concomitant use of drugs that prolong Q–T interval. The heart rhythm should be monitored for 3 h after administration. Droperidol is pregnancy category C. Guidelines for its use has been suggested by ASGE [83].

Combination regimens

Combinations of benzodiazepines and opiates are often used [88] and are efficacious and well tolerated [42,45,47,49,89,90]. Combination therapy risks increased cardiorespiratory complications and the dose of each drug should be reduced. Addition of benzodiazepines to opioids enhances sedation and amnesia and addition of opioids to benzodiazepines adds analgesia. Their combination has synergistic effects. According to one study, pain experienced during colonoscopy was similar whether or not a combination was used. [91] Similar results for upper endoscopy were demonstrated in another study [92]. However, this study also showed that endoscopists preferred combinations of benzodiazepine and opiates. Ideally, each drug should be administered separately to achieve the desired effect and the dose of each should be correspondingly reduced [21]. Opiates should be administered first because of the slower onset of action of meperidine.

In situations where high doses of narcotic and benzodiazepines provide inadequate sedation, Phenergan or diphenhydramine may be used in addition to synergize sedative effects.

The combination of propofol with a benzodiazepine or an opioid enhances the sedative effects of propofol and allows propofol to be targeted to minimal or moderate sedation [78]. Propofol has variable amnesic effect, which can be improved by coadministration of a benzodiazepine. Also it has no analgesic properties, so addition of a narcotic is recommended for painful procedures targeted to moderate sedation. The quality of sedation and recovery profile were similar to those reported with standard dose propofol.

Adjuvant drugs

These drugs increase tolerance and reduce doses of sedatives required.

Pharyngeal anesthesia

Topical anesthetics subdue the gag reflex during upper endoscopy. Commonly used topical anesthetics include benzocaine, tetracaine, Cetacaine and lidocaine. They may not improve upper endoscopy in patients already getting conscious sedation [93–95]. Benzocaine has a rapid onset and short duration. One study suggested that topical anesthetics are most useful in patients undergoing EGD for the first time, who are less than 40 years of age, or who are anxious [96]. Methemoglobinemia is an important adverse effect associated with the use of topical benzocaine [97–100] as well as Cetacaine [101]. It presents with clinical cyanosis with a normal arterial Po_2 [102]. Treatment is intravenous methylene blue [2 mg/kg). Topical anesthetics may also cause an idiosyncratic anaphylactic reaction [103]. Systemic effects such as arrhythmia and seizures owing to absorption have been observed rarely [104,105]. Topical anesthetics have been implicated in the development of pneumonia after EGD [106].

Reversal agents

Reversal agents should be used when patients develop severe respiratory depression including hypoxia and decreased ventilation that does not respond to supplemental oxygen or gentle stimulation.

Flumazenil is a benzodiazepine antidote and competitively inhibits the activity at the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex, resulting in reversal of central nervous system (CNS) depression without reversing the amnesia. The dosage of 0.2 mg intravenously may be repeated at 1 min intervals to a maximum dose of 3 mg. It has limited efficacy in reversing respiratory depression. For this reason, naloxone should be tried before flumazenil in patients who develop severe respiratory depression after being given a combination of opiates and benzodiazepines. Re-sedation may occur owing to the shorter half life of flumazenil compared with benzodiazepines. However, in practice this rarely is a problem unless large doses of benzodiazepines or additional agents have been used [107]. Flumazenil also may be used to treat paradoxical reactions to benzodiazepines. Flumazenil administration can precipitate acute withdrawal in patients on prolonged benzodiazepine therapy. The duration of action is 1–2 h and the drug is pregnancy category C.

Naloxone is a pure opioid antagonist that competes and displaces narcotics at opioid receptor sites. It is used to reverse sedation and respiratory depression caused by opiates and the degree and extent of reversal is dose dependent. The usual dose is 0.4–2 mg intravenously over 30 s. It may be cleared faster than meperidine, thus in patients who receive

high doses of meperidine, resedation may occur. Repeat doses may be given at 2–3-min intervals to a maximum of 10 mg. Acute withdrawal and severe pain may occur in chronic opiate users. Acute reversal may cause tachycardia, hypertension, or pulmonary edema due to release of catecholamines. Therefore, caution must be exercised in elderly patients and those with cardiovascular disease. Too rapid reversal may cause nausea, vomiting, diaphoresis and circulatory distress. The duration of action is 1–4 h and the drug is pregnancy category B.

Elective use of reversal agents for quicker recovery is not recommended at this time [21] and is only marginally supported by literature [108,109].

General anesthesia

General anesthesia may be appropriate in patients with paradoxical agitation to benzodiazepines, for difficult to sedate patients, for children [110], and for emergency therapeutic procedures that are anticipated to be long and complicated [111]. It is also an option for patients who are obtunded or have high aspiration risk, including active hematemesis.

Pregnancy and lactation

For sedation, meperidine alone is preferred with small doses of midazolam if needed. Fentanyl is preferred over meperidine for breast-feeding mothers. Breast-feeding should be delayed by at least 4 h after maternal midazolam administration and for 24 h after propofol exposure.

Procedural monitoring

Owing to the potentially dangerous cardiopulmonary complications associated with sedation, careful patient monitoring is mandatory. For moderate sedation, periodic blood pressure monitoring, continuous pulse oximetry, and pulse measurements are mandatory. For deep sedation or propofol titrated to any level of sedation, continuous electrocardiogram (EKG) is mandatory and capnography may be considered [21,76,83]. Continuous clinical monitoring of respiratory effort is essential with deep sedation. Vital signs, oxygenation and level of consciousness should be assessed before the beginning of the procedure, after administration of sedatives, at regular intervals during the procedure, during initial recovery and just before discharge. Depth of sedation is continuously monitored clinically in all patients.

Oxygen tanks, oral suction devices, oral airways, reversal agents and fully equipped code carts must be available in the endoscopy unit. Cardiac defibrillators should be immediately available for patients with cardiovascular disease undergoing moderate sedation and for all patients undergoing deep sedation. It is also recommended that an individual with advanced life support skills be within 1–5 min away for moderate sedation and within the procedure room for deep sedation.

Oximetry

Respiratory depression is a major cause of sedation associated morbidity. Hence pulse oximetry is an established standard protocol for procedural monitoring [21,112,113]. Pulse oximetry provides early warning of impending respiratory failure and hence allows intervention before clinically dangerous hypoxemia occurs. Early detection of hypoxemia decreases adverse events such as cardiopulmonary arrest and death. The pulse oximeter is a spectrophotometric device that detects and calculates the differential absorption of light by oxygenated and reduced hemoglobin to produce an estimate of oxygen saturation. Pulse oximetry does not substitute for clinical monitoring of respiratory rate and effort [114]. Supplemental oxygen has been shown to prevent or reduce desaturation when given during endoscopic procedures [115–120]. Supplemental oxygen administration will maintain normal oxygen saturations in the face of prolonged hypoventilation or apnea.

Motion may cause false alarms owing to probe dislodgement. A waveform is essential to distinguish true desaturations from motion artifact. Readings may be inaccurate with shock or vasoconstriction.

Capnography

Hypoventilation may be masked in patients who are receiving supplemental oxygen and may lead to CO_2 retention and respiratory acidosis. Capnography is the continuous measurement of inhaled and exhaled CO_2 concentration, typically displayed as a numeric value and a graphical tracing. Carbon dioxide absorbs light in the infrared region of the electromagnetic spectrum and quantification of the absorption generates a curve. Capnography provides breath-to-breath feedback and generates a respiratory rate that is measured at the airway [121] (Fig. 117.1). There is evidence that capnography may detect ventilatory compromise that would otherwise not be detected by routine monitoring [122]. It is superior to oximetry for evaluation of ventilation [123,124] and it accurately detects apnea [125]. It may be considered in all patients receiving deep sedation (or moderate sedation involving propofol) and for those receiving moderate sedation if the procedure is long, the patient is older or with comorbidities or if adequate observation of ventilatory effort cannot be ensured [126]. However, there is no evidence that routine use of capnography improves outcomes in patients undergoing sedation with propofol.

Bispectral monitoring

Bispectral monitoring (BIS) assesses brain activity and expresses it as a value on a linear scale from 100 (fully awake) to 0 (no cortical activity). Values below 60 correlate with general anesthesia. When used for benzodiazepine or opiate sedation, BIS values have been shown to track the sedative effect (parallel to OAA/S) and correlate with patient recovery and endoscopist satisfaction [127–129]. During

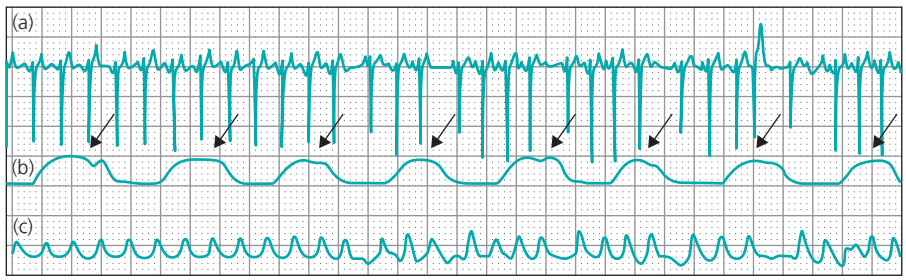

Figure 117.1 A baseline recording of the electrocardiogram **(tracing a)**, respiratory activity with capnography **(tracing b)** and pulse oximetry curve, which corresponds to the arterial pressure waveform **(tracing c)**, in a patient before receiving propofol. **Tracing b** is a normal capnography curve demonstrating a stable respiratory pattern. The peaks of the curve (arrows) represent the carbon dioxide tension at end exhalation. The rapid downstroke after the peaks represents the inhalation phase of the respiratory cycle. (Note: *y*-axis represents the partial pressure of carbon dioxide, expressed in mmHg.) Reproduced from Vargo JJ [48], with permission from Elsevier.

propofol sedation, the BIS scores lag behind the actual sedation level during both induction and recovery, but correlates fairly well with sedation level during the maintenance phase [130]. Currently, BIS is not a standard monitoring tool in routine endoscopy.

Hemodynamic monitoring

Blood pressure should be measured before and after the procedure and at 5–10 min intervals during the procedure. More frequent monitoring may be done (every 3–5 min) in patients with acute gastrointestinal bleeding and if hemodynamic disturbances are anticipated and in patients with underlying cardiopulmonary disease.

It has been recommended that EKG monitoring should be used during deep sedation [76] and in patients with significant cardiovascular or pulmonary disease undergoing moderate sedation. It is also recommended in elderly patients, those with acute gastrointestinal bleeding and those in whom prolonged procedures are anticipated [21]. However, the necessity of EKG monitoring has not been shown conclusively.

Postprocedure monitoring

Because of duration of the action of sedatives, patients are at risk for complications during recovery. Therefore, continuous monitoring is recommended until patients recover to a level of minimal sedation. The clinician should be in proximity until the patient is hemodynamically stable and arousable [76]. Patients may be discharged when their vital signs have stabilized and they are fully alert.

Sedatives may cause a prolonged period of impaired cognition, so patients are advised against driving, operating heavy machinery and making important decisions until the following day. Discharge instructions must be written because of the amnesic effects of the medicines.

Complications of sedation and their management

Morbidity and mortality owing to cardiopulmonary complications continues to be a major problem in gastrointestinal endoscopy. Risk factors for sedation related complications include older age [24,131], comorbid conditions [132] (particularly pulmonary disease), emergency and therapeutic procedures [21,23,133–135], upper GI bleeding [136–138], compromised airway [139], obesity [139], dementia, and anemia [23].

Cardiopulmonary complications constitute about 40–50% of all endoscopic complications [140,141]. The most common are aspiration, hypoventilation and airway obstruction [88,142–144]. The incidence of cardiopulmonary events with narcotics and benzodiazepines has ranged from of 2 per 1000 [106] to 5.4 per 1000 procedures [145]. However, only 0.01–0.03% cases are significant such as myocardial infarction or life-threatening arrhythmias [114,146,147]. The incidence of sedation-related mortality with narcotics and benzodiazepines has ranged from 1 per 3000 to 1 per 11 000 cases [106,145,148,149]. Seventy-three deaths were reported from over-sedation from midazolam within the first 4 years of its introduction [145]. There are no reported cases of endotracheal intubation or death from nonanesthesiologist administered propofol for endoscopy over an experience exceeding 200 000 cases [38,42,43,56,59,61,69,71,72,78,81,150–157]. Thus nonanesthesiologist administered propofol by trained personnel is as safe, if not safer than administration of narcotics or benzodiazepines by endoscopists.

Respiratory depression

Respiratory depression and airway obstruction are the primary causes of drug-induced morbidity and may manifest as hypoxemia or CO_2 retention, usually but not always together

[124,133]. Hypoventilation risk is increased in patients receiving high doses of benzodiazepines and opiates [133], not only because of their sedative effect but also depression of respiratory drive due to decreased central responsiveness to CO_2.

Hypoxia

For EGD, the physical presence of the endoscope may contribute to hypoxemia [158,159] with higher degree of desaturation occurring with large diameter endoscopes [132] and in procedures performed by inexperienced endoscopists [160]. The need for bag–mask ventilation during nonanesthesiologist administration of propofol is substantially greater during upper endoscopy compared with colonoscopy [54]. Looping during colonoscopy may cause transient desaturation [146], however it is generally mild and transient. Severe and prolonged hypoxemia, particularly if it causes respiratory arrest, is usually drug-induced [124,133]. Sustained desaturation to less than 90% may prove dangerous in elderly and those with ischemic heart disease [161]. It may lead to cardiac ischemia [162–164] and both atrial and ventricular arrhythmias [132,165]. Although risk factors exist (see above), most studies have shown that it is impossible to predict by medical illness or age which patients will become hypoxemic [124,133,166,167].

Carbon dioxide retention reflects alveolar hypoventilation and may occur during endoscopy [124,133]. Although, oxygen supplementation readily corrects hypoxemia, it does not correct underlying hypoventilation. Severe hypoventilation may go undetected by pulse oximetry once supplemental oxygen is applied.

Patients with respiratory depression and hypoxia should be given supplemental oxygen by increasing the amount of oxygen delivered or changing the delivery route from nasal cannula to face mask. If the patient is snoring and breathing is labored, tilting the head and lifting the jaw may open the airway. The oropharynx should be suctioned and an oral airway inserted if necessary. If these maneuvers do not help or if the patient is apneic or hypoventilating, breathing should be assisted with a bag–valve–mask device.

Cardiac and hemodynamic complications

Myocardial ischemia and infarction may occur during endoscopy, particularly in patients with cardiac disease [163,164]. It correlates better with periods of tachycardia than periods of hypoxemia [162]. Hemodynamic disturbances during endoscopy include vasovagal reactions and fluctuations in blood pressure and pulse. Vasovagal reactions usually manifest as diaphoresis and bradycardia and frequently are a result of overinsufflation and pain during colonoscopy. Atropine is effective in treating bradycardia. Significant increases or decreases in blood pressure occur commonly during endoscopy [146,168,169]. Hypotension may respond to fluid administration if the underlying cause is hypovolemia. Most hypotension during endoscopy is well tolerated, but prolonged or severe hypotension should lead to consideration of reversal agents and vasopressor agents such as ephedrine or dopamine in addition to fluid administration. Hypertension may be treated with giving more sedatives or analgesics if the patient is under sedated or by decreasing pain, e.g., reducing endoscope loops or by suctioning air.

Aspiration

Aspiration may result in pneumonia and adult respiratory distress syndrome as well as cardiopulmonary arrest and carries a high risk for mortality [106]. The risk is increased with obtundation, dementia, excessive esophageal or gastric fluid or food and in those with active upper gastrointestinal bleeding. Prophylactic endotracheal intubation is indicated with active hematemesis, especially from variceal bleeding [170] or those who are unstable, obtunded, or uncooperative. When excessive gastric contents are encountered, excessive insufflation should be avoided and the procedure should be terminated if the material cannot be readily cleared by suction. Elderly patients are at increased risk for aspiration as a result of an increase in the sensory stimulus threshold required for reflexive glottic closure. It is advisable to avoid topical inhibition of the gag reflex in high risk patients. A catheter for oropharyngeal suction should be by the bedside at all times.

Cardiac arrhythmias

The risk of arrhythmias increases with advanced age, underlying cardiovascular disease, arterial desaturation and, in case of EGDs, the diameter of the endoscope used [132]. The most common arrhythmia was sinus tachycardia which may occur as a result of hypoxemia or painful stimuli.

Procedure termination

Procedure termination as a result of inadequate or excessive sedation may result in morbidity owing to delayed therapy, extended hospitalization, repeat or alternative procedures such as surgery and increased cost. Diab et al. [171] found a 7% incidence of procedure termination during EGD using midazolam alone for sedation. In a prospective multicenter study, 4.1% of all endoscopic retrograde cholangiopancreatographies (ERCPs) had to be terminated prematurely owing to difficulty with sedation [124]. If difficulty is anticipated with sedation for a therapeutic procedure, general anesthesia by an anesthesiologist may be considered.

Training for use of sedatives

Several guidelines have been published regarding training for personnel administering sedatives [21,23,172,173]. Recommendations for safe administration of conscious sedation are emphasized in reviews [88,142]. Although there are no strict requirements, it is obvious that education in pharmacology

Box 117.1 Skills and training required for use of sedatives

The individual administering sedation should:

- be familiar with pharmacology and adverse effects of all sedatives to be used
- be aware of the contraindications to use of each sedative and analgesic
- be able to assess for sedation risk and airway risk
- be able to determine sedation level and recognize complications
- allow for adequate time between dosages to prevent cumulative overdose
- be familiar with antagonists if available
- be trained in rescue of patients from oversedation, including basic life support skills such as cardiopulmonary resuscitation, techniques to open airway, and bag–mask ventilation
- undergo training in sedative administration, including a period of observation of experts followed by supervised administration

of sedatives reduces risk of complications (Box 117.1). It is also recommended that an individual with advanced life support skills be within 1–5 min away for moderate sedation and within the procedure room for deep sedation.

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A ENDOSCOPIC

118 Upper gastrointestinal endoscopy

William R. Brugge

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Upper gastrointestinal (GI) endoscopy is the basis for many of the diagnostic and therapeutic procedures offered by endoscopists and gastroenterologists. This chapter reviews the current status of endoscopic procedures performed in the esophagus, stomach, and duodenum. Diagnostic endoscopy will be reviewed in detail, including standard and advanced imaging techniques, diagnostic specimen collection, and criteria for endoscopic diagnoses. Therapies applied during upper GI endoscopy will be examined, but will not be described in detail.

The diseases that are often diagnosed and managed with upper GI endoscopy will also be reviewed, particularly those diseases that lend themselves to further understanding with a knowledge of upper GI endoscopy. This chapter will also examine the risks and contraindications for endoscopy to help the reader understand the cost–benefit analysis of a procedure compared with alternative diagnostic or therapeutic approaches (Box 118.1). The final section considers speculations about endoscopy trends in the future and improvements in diagnostic and therapeutic modalities.

History and background

The father of modern endoscopy is Rudolf Schindler [1]. He pioneered the use of gastroscopy through the use and development of a semirigid gastroscope. The first endoscopic device was introduced in 1806 by Philip Bozzini, who developed a "Lichtleiter" (light conductor) "for the examinations of the canals and cavities of the human body." However, the Vienna Medical Society disapproved of such a device, and the development stalled.

An endoscope was apparently first introduced into a human in 1853. The use of an electric light was a major step in the improvement of endoscopy; these lights were at first external. Later, smaller bulbs became available, making internal light possible. Jacobeus has been credited with early endoscopic explorations of the abdomen and the thorax with laparoscopy (1912) and thoracoscopy (1910). One of the major developmental milestones for endoscopy was the invention of a superior glass fiber for flexible endoscopes by Basil Hirschowitz. The technology not only resulted in the first useful medical endoscope, but revolutionized of endoscopic uses, and led to practical fiberoptics.

Technical considerations

Upper GI endoscopy is a highly technical procedure that requires a close cooperative arrangement between physicians and nurses. A well-organized facility will optimize patient safety and the ability to provide the appropriate techniques. With only a few exceptions, upper GI endoscopy should be performed in a hospital or a medical care facility that can reliably provide highly trained personnel and specialized equipment. In addition to a wide array of endoscopes and processors, the procedure unit should be equipped with the appropriate accessories suitably organized.

In addition to procedure rooms, it is critical to have a travel cart that will enable endoscopists to carry out endoscopic procedures at sites remote from an endoscopy unit. Well-maintained, controlled-access storage is essential for the endoscopic accessories. There must be a well-designed area for endoscope disinfection and preparation of accessories for sterilization. It is also critical to have preparation and recovery areas for the evaluation and monitoring of patients.

Electrocautery devices are critical for the performance of many endoscopic procedures. In many units, the devices are not installed in each procedure room. As these devices are frequently used, it is critical that they be readily available during procedures and properly maintained by qualified personnel.

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Box 118.1 Indications and contraindications for esophagogastroduodenoscopy

EGD is generally indicated for evaluating:

- upper abdominal symptoms that persist despite an appropriate trial of therapy
- upper abdominal symptoms associated with other symptoms or signs suggesting serious organic disease (e.g., anorexia and weight loss) or in patients > 45 years old
- dysphagia or odynophagia
- esophageal reflux symptoms that are persistent or recurrent despite appropriate therapy
- persistent vomiting of unknown cause
- other diseases in which the presence of upper GI pathological conditions might modify other planned management (examples include patients who have a history of ulcer or GI bleeding who are scheduled for organ transplantation, long-term anticoagulation, or long-term nonsteroidal antiinflammatory drug therapy for arthritis, and those with cancer of the head and neck)
- familial adenomatous polyposis syndromes
- for confirmation and specific histological diagnosis of radiologically demonstrated lesions
  1 suspected neoplastic lesion
  2 gastric or esophageal ulcer
  3 upper tract stricture or obstruction
- GI bleeding
  1 in patients with active or recent bleeding
  2 for presumed chronic blood loss and for iron deficiency anemia when the clinical situation suggests an upper GI source or when colonoscopy results are negative
- when sampling of tissue or fluid is indicated
- in patients with suspected portal hypertension to document or treat esophageal varices
- to assess acute injury after caustic ingestion
- treatment of bleeding lesions, such as ulcers, tumors, vascular abnormalities (e.g., electrocoagulation, heater probe, laser photocoagulation, or injection therapy)
- banding or sclerotherapy of varices
- removal of foreign bodies
- removal of selected polypoid lesions
- placement of feeding or drainage tubes (peroral, PEG, percutaneous endoscopic jejunostomy)
- dilation of stenotic lesions (e.g., with transendoscopic balloon dilators or dilation systems using guidewires)
- management of achalasia (e.g., botulinum toxin, balloon dilation)
- palliative treatment of stenosing neoplasms (e.g., laser, multipolar electrocoagulation, stent placement)

EGD is generally not indicated for evaluating:

- symptoms that are considered functional in origin (there are exceptions in which an endoscopic examination may be done once to rule out organic disease, especially if symptoms are unresponsive to therapy)
- metastatic adenocarcinoma of unknown primary site when the results will not alter management
- radiographic findings of:
  1 asymptomatic or uncomplicated sliding hiatal hernia
  2 uncomplicated duodenal ulcer that has responded to therapy
  3 deformed duodenal bulb when symptoms are absent or respond adequately to ulcer therapy

Sequential or periodic EGD may be indicated:

- surveillance for malignancy in patients with premalignant conditions (i.e., Barrett esophagus)

Sequential or periodic EGD is generally not indicated for:

- surveillance for malignancy in patients with gastric atrophy, pernicious anemia, or prior gastric operations
- surveillance of healed benign disease such as esophagitis or gastric or duodenal ulcer
- surveillance during repeated dilations of benign strictures unless there is a change in status

EGD, esophagogastroduodenoscopy; GI, gastrointestinal; PEG, percutaneous endoscopic gastrostomy.

Videoendoscopes

Videoendoscopy, introduced in the mid-1980s, has dramatically improved and expanded the field of endoscopy. Videoendoscopy is now used almost universally. The endoscopic image is generated electronically, using a charge-coupled device (CCD) located in the tip of the endoscope [2]. Endoscope processors manage the images and display them on video monitors. Prior to videoendoscopy, fiberoptics generated the images on small hand-held eye-pieces.

The first videoendoscopes used black and white CCDs that required a color wheel. Green, red, or blue light was sequentially sent down the illumination bundle of the endoscope and activated the CCD at the tip [3]. A color image was reconstructed using the three sets of images generated by the colored lights. The videoprocessor displayed a full-color image of the GI tract lining, although with apparent image flickering during rapid movement. Most current videoendoscopes use a color CCD that obtains the image in color on the tip of the endoscope [4]. These devices provide 30 000–850 000 pixels of resolution. By incorporating high-pixel-density

charged-coupled devices, high-resolution endoscopes provide images that display vivid mucosal detail. High-resolution endoscopes are capable of discriminating objects 10–70 μm in diameter; in comparison, the naked eye is capable of discriminating objects 125–165 μm in diameter. The videoendoscope has controls for air, water, and suction and knobs for moving the tip up, down and to the right and left. The instrument channel is shared for the passage of accessories and suction. There are also buttons on the videoendoscope control handle to activate digital video recording, image capture and recording of video images.

Videoendoscopy has greatly expanded the viewing capabilities of procedures. Multiple monitors in the procedure room provide bright vivid images than enable many personnel to participate in procedures. The live video images can also be distributed remotely to sites within an institution, or to remote sites for teaching, research, and demonstration [5]. Endoscopy training has improved dramatically with the use of videoendoscopy. Documentation of procedures is provided by the saving, retrieval, and reviewing of stored digital images [6]. Stored images can be recalled from a central image storage system and sent to any location in the endoscopy service. The storage drives can be used for image processing and management, as well as enabling reliable storage of endoscopic images and information on PACS (picture archiving and communication system) [7]. Hardware and software are now available for the capture, editing, and storage of video clips. This type of material will further improve teaching and patient care [8].

Introducing the endoscope

Most small-diameter videoendoscopes can be easily passed under direct vision through the upper esophageal sphincter. The tip of the instrument is advanced in the midline into the direction of the closed cricopharyngeal sphincter. The patient is asked to swallow, and under direct vision the tip of the instrument is passed from the epiglottis and larynx into the proximal esophagus. In the past, endoscopes were passed blindly aided by the swallowing action. The direct vision technique allows an inspection of the pharynx, epiglottis, and vocal cords prior to insertion. Furthermore, direct imaging may decrease the risk of the inadvertent passage of the endoscope into a proximal esophageal diverticulum. Small-diameter videoendoscopes can also be passed transnasally and may provide the opportunity to perform unsedated endoscopy [9].

Endoscopes are designed to enable the endoscopic controls (e.g., air, water, and suction valves – tip direction wheels) to be controlled with the left hand. The right hand is used to advance the instrument and to manipulate the tip control knob. Torque of the endoscope is accomplished by rotating the instrument control handle with the right hand, which results in rotation of the entire shaft and tip of the endoscope. A central instrument channel is used for a wide variety of devices and accessories. The instrument channel is variable in diameter and some instruments have two instrument channels.

The normal upper GI endoscopic examination

The endoscopic examination of the upper GI tract begins with an examination of the upper esophagus as the endoscope is passed through the upper esophageal sphincter. Since the proximal esophagus is best examined on withdrawal, the endoscope is usually passed to the mid-esophagus, where the formal examination begins. At 40 cm lies the ora serrata, which is the junction between the pearly stratified squamous mucosa and the gastric columnar epithelium. The esophageal folds can be seen to change with air distention. It is possible to recognize extrinsic pressure on the esophagus from adjacent structures, such as the aorta and the left main stem bronchus. The impingement by the diaphragm is apparent in the upper stomach and defines the presence of a hiatal hernia.

With slight angulation to the left and anteriorly, the endoscope is passed into the stomach. Air is insufflated to distend the stomach. It is important to notice the presence of food, bile or blood in the gastric lumen. Gastric fluid in the cardia should be aspirated to improve endoscopic inspection and to reduce the likelihood of regurgitation and aspiration during the procedure. The gastric mucosa is inspected, and observations are made about the color, texture, and size of folds. The gastric folds begin in the upper portion of the stomach and extend down to the entrance into the antrum. With gentle distention of the stomach, these folds often flatten. The size, number, and depth of mucosal defects are noted. Indentation of the lumen by mural lesions, or extrinsic compression by extrinsic organs, can be observed.

A complete examination of the stomach requires a retroflexed view of the stomach. The maneuver takes place in the antrum with a 180° flexion of the endoscope in an inflated stomach. The gastroesophageal (GE) junction, cardia, and fundus can be examined by pulling the endoscope. Retroflexion also permits inspection of a hiatal hernia. Lesions in a hiatal hernia and the GE junction are best viewed in a retroflex position.

The entry into the antrum of the stomach is marked by the incisura, a fold on the lesser curve of the stomach. The antrum can be easily identified by the lack of folds and a conical shape. The antrum is the site of the initiation of peristalsis. Vigorous contractions begin in the proximal antrum and progress through the antrum at a frequency of approximately three per minute. Antral peristalsis terminates in the pylorus. The viewing of peristalsis enables the endoscopist to examine the antral mucosa in detail, particularly for infiltrating lesions. Peristalsis may be weak or absent in a heavily sedated patient, but quantification of peristalsis is not possible.

Once the examination of the body and antrum has been completed, the endoscope is advanced to the pylorus. It is sometimes necessary to create a loop along the greater cur-

vature before the tip of the endoscope can progress through the pylorus. With a small-caliber endoscope, it is possible to inspect the bulb in detail. The endoscope is passed beyond the apex of the duodenal bulb into the second descending portion of the duodenum. Passage from the tip of the bulb into the descending duodenum is readily performed with a turn of the endoscope to the right. The duodenal bulb is usually free of folds; duodenal Kerkring folds begin in the descending portion of the duodenum. A tangential view of the ampulla is usually appreciated.

After completion of the examination in the duodenum, the endoscope is withdrawn, during which time the endoscopist can examine the mucosa of the duodenum, stomach, and esophagus. If there is any suspicion of a lesion in the stomach, it is important to inflate the stomach, flattening the folds and allowing a detailed examination of the gastric mucosa for small ulcers, angioectasia, and early gastric cancer. After completion of the examination of the stomach, air is withdrawn. During the withdrawal of the endoscope through the esophagus, it is important to examine upper esophagus, the upper esophageal sphincter (UES), and the larynx because these may not have been examined in detail during the initial entry.

In transnasal esophagogastroduodenoscopy (EGD), a small-caliber endoscope is passed via the most patent side of the nasal cavity after local application of lidocaine gel and a nasal decongestant (Boxes 118.1 and 118.2) [10]. After inspection of the pharynx, the scope is introduced into the esophagus after traversing the UES under direct vision. Following evaluation of the esophagus, the stomach and duodenum are entered and evaluated.

The use of endoscopic accessories

The use of endoscopic accessories has greatly expanded the number of procedures that can be performed with endoscopy. Accessory devices are used to obtain tissue, inject agents, direct cautery, deliver diagnostic and therapeutic light, and provide additional imaging probes (Table 118.1).

Biopsy forceps

Biopsy forceps are used to retrieve mucosa specimens for histology or culture (Table 118.2). The size of the forceps, often determined by the size of the instrument channel, will determine the size of the specimen obtained. The standard forceps used in a 2.8-mm biopsy channel have a span of 8 mm and can retrieve a full-thickness biopsy of the mucosa. Large cup forceps provide larger specimens with less crush artifact. Forceps are available for single or multiple biopsies. A small central knife, an optional feature, is used to hold multiple specimens. "Hot forceps" (with cautery) should not be routinely used as they provide inferior histological specimens [11]. Reusable and disposable forceps are available [12].

Biopsy specimens are retrieved with tweezers or by flushing, but care should be taken to avoid fragmentation of the specimen. It is not necessary to orient mucosal specimens; this is usually performed in the pathology laboratory. Large biopsy forceps should be used to obtain mucosal specimens for the detection of malignancy, in order to maximize the

Box 118.2 Summary of proposed quality indicators for esophagogastroduodenoscopy

1. Accepted indication(s) is provided before performance of EGD
2. Informed consent is obtained, including specific discussion of risks associated with EGD
3. Prophylactic antibiotics are given in patients with cirrhosis with acute upper GI bleeding who undergo EGD
4. Prophylactic antibiotics are given before placement of a PEG
5. Complete examination of the esophagus, stomach, and duodenum, including retroflexion in the stomach
6. Biopsy specimens are taken of gastric ulcers
7. Barrett esophagus is measured when present, with the location of the gastroesophageal junction and squamocolumnar junction in centimeters from the incision being documented
8. Biopsy specimens are obtained in all cases of suspected Barrett esophagus
9. Type of upper GI bleeding lesion is described and location is documented. For peptic ulcers, at least one of the following stigmata is noted: active bleeding, nonbleeding, nonbleeding visible vessels (pigmented protuberance), adherent clot, flat spot, cleaned base
10. Unless contraindicated, endoscopic treatment is given to ulcers with active bleeding or with nonbleeding visible vessels
11. In cases of attempted hemostasis of upper GI bleeding lesions, whether hemostasis has been achieved is clearly documented
12. When epinephrine injection is used to treat nonvariceal upper GI bleeding or nonbleeding visible vessels, a second treatment modality is used (e.g., coagulation or clipping)
13. Variceal ligation is used for endoscopic treatment of esophageal varices
14. Written instructions, which include particular signs and symptoms to watch for after EGD, are provided to the patient on discharge
15. In patients undergoing dilation for peptic esophageal strictures, PPI therapy is recommended
16. Patients diagnosed with gastric or duodenal ulcers are instructed to take PPI medication for an H_2 antagonist
17. Patients diagnosed with gastric or duodenal ulcers have documented plans to test for the presence of *H. pylori* infection
18. Rebleeding rates after endoscopic hemostasis are measured

EGD, esophagogastroduodenoscopy; GI, gastrointestinal; PEG, percutaneous endoscopic gastrostomy; PPI, proton pump inhibitor.

Table 118.1 Methods for esophageal stenosis dilation

Type of stenosis	Maloney dilation	TTS-CRE balloons	Insight dilator	Catheter-based balloon dilator
Benign esophageal stenosis	Highly effective	Highly effective	Not well tested	Not indicated
Malignant esophageal stenosis	Transient effect	Transient effect	Not well tested	Not indicated
Achalasia	Not effective	Transient effect	Not tested	Effective
Benign pyloric channel stenosis	Not indicated	Effective	Effective	Not indicated
Malignant duodenal stenosis	Not indicated	Transient effect	Not indicated	Not indicated

TTS-CRE, through the scope controlled radial expansion.

Table 118.2 Endoscopic methods for tissue sampling

	Forceps	Brush	Needle	Cap-assisted EMR	Snare resection
Barrett with dysplasia	Large particle forceps in four quadrants	Possibly effective	Not indicated	Highly diagnostic	Not indicated
Esophageal cancer	Large particle forceps	Highly diagnostic	Not indicated	Not necessary	Not indicated
Gastric cancer	Resection biopsy	Rarely used	Not indicated	Highly diagnostic	Not indicated
Gastric lymphoma	Highly diagnostic	Highly diagnostic	Possibly	Not necessary	Not indicated
Gastric GIST	Not effective	Not effective	Method of choice for tissue acquisition	Curative for small lesions	Curative for small lesions
Duodenal polyp	Consider resection	Not indicated	Not indicated	For resection therapy	Diagnostic and curative
Ampulla adenoma	4–6 specimens	Not indicated	Not indicated	For resection therapy	Diagnostic and curative

EMR, endoscopic mucosal resection; GIST, gastrointestinal stromal cell tumor.

quality and quantity of tissue. The risk of bleeding from biopsies is negligible.

Most endoscopic forceps obtain mucosa and, occasionally, a small amount of submucosa. To sample more deeply into a lesion, such as a submucosal tumor, needle cytology may be applied, or repetitive sampling with large biopsy forceps should be made in the same area ("well biopsy") (Table 118.2). The acquisition of diagnostic material from the esophagus is more difficult than from the stomach because of the orientation of the esophagus. Angled forceps will improve the biopsy of the esophagus [13].

As an alternative to pinch biopsies, a snare may be used to obtain a large sample of tissue from abnormal mucosa (Table 118.2) [14]. The snare is placed over the fold or polypoid area, tightened, and gently pulled away from the wall. Prior injection of saline or diluted epinephrine in the submucosa may lift the mucosa to facilitate snaring and to prevent deep mural damage. As the snare is pulled through the lesion, the electrosurgical current aids in the cutting through the tissue and prevents bleeding.

The snare biopsy technique can be extended by lifting the targeted mucosa prior to snaring. In this technique, a two-channel endoscope is used with the snare in one channel and lifting forceps in the second channel [15]. The snare is opened and the forceps is directed through the snare. The tissue is grasped by the forceps and pulled up into the snare. As the snare is tightened, the tissue can be excised with electrocautery. This technique may result in unexpectedly large, deep biopsy specimens and is not recommended for the inexperienced endoscopist.

Tissue acquisition may also be achieved using techniques associated with endoscopic mucosal resection (EMR). Typically, EMR techniques are employed for the resection of a flat neoplastic lesion, such as early gastric cancer. Many EMR techniques incorporate a submucosal injection to separate the lesion from the muscularis propria. Epinephrine diluted with saline is the commonly used injectant because it provides transient hemostasis as well as separation of the wall layers. The most commonly performed method for EMR is cap-assisted EMR. In this technique, a translucent plastic cap is placed on the tip of an endoscope and the target tissue is aspirated into the cap. A small snare placed inside the rimmed cap provides the ability to resect a considerable amount of tissue. The lesion is suctioned into the cap and the snare tightened around the lesion. The snare is then advanced out of the cap to enable the extent of the captured

mucosa to be confirmed prior to transection. Newer methods for EMR use the application of a band to select and lift mucosa or lesions for resection [16]. Large surface area mucosal malignancies can be resected using endoscopic knives. This technique has been used to resect superficial gastric malignancies of the stomach in Japan and in the Western world; the technique is used to resect Barrett mucosa.

Cytology brushes

Retrieval of specimens for cytological evaluation is easier than using biopsy forceps for tissue acquisition (Table 118.2) [17]. Brushing of mucosal lesions is usually performed for the diagnosis of infectious processes in the upper GI tract. However, cytology brushing is also important in lesions that are inaccessible for biopsy, such as tight strictures in the esophagus. Cytology brushes are often protected by a plastic sheath, which aids in the retrieval of diagnostic material. After removal of the cytology brush from the endoscope biopsy channel, the specimen is placed on a glass slide and into preservative solution. For the diagnosis of *Candida* infections, a simple smear can be submitted for fungal staining.

Cytological material from the upper GI tract can also be retrieved with the use of an aspiration needle [18]. Aspiration cytology is usually performed for cystic lesions, submucosal tumors, or infiltrating malignancy in the gastric wall. A small-gauge needle is used to obtain small amounts of tissue or fluid from the lesion using suction. Needle aspiration cytology is usually performed with endoscopic ultrasonography (EUS) guidance because of the ability of EUS to direct the needle into areas rich in cytological material.

Measuring devices

Occasionally it may be important to know the actual size of an identified lesion. Endoscopic estimation of size is often inaccurate and underestimates the size of lesions. The closer the tip of the endoscope is to the target, the larger the lesion appears. Measuring methods include using open biopsy forceps with a known distance between the tips of the biopsy cups, using a spring-type measuring probe with gradations of known length, and using a projected grid [19].

Measuring mucosal blood flow

Endoscopic imaging of the upper GI mucosa is not a reliable method for the measurement of mucosal blood flow or the detection of abnormal blood vessels. The measurement of blood flow may be used in focal lesions or for a mucosal process. Doppler ultrasound probes can detect the flow in abnormal blood vessels below the mucosa, such as varices, or in an artery in the base of an ulcer [20]. Doppler devices can be used before and after endoscopic hemostatic therapy, such as injection or thermal therapy for an artery in the base of an ulcer [20,21]. It is also possible to use a laser Doppler device to measure mucosal red blood cell velocity, a parameter thought to correlate with mucosal blood flow [22]. The assessment of mucosal blood may be predictive of ulcer healing [23]. Mucosal blood flow is normally increased in the mucosa adjacent to ulcers and attenuated blood flow is predictive of slow healing. Paradoxically, it appears that blood flow is decreased in areas of portal hypertensive gastropathy [24].

Endoscopic imaging can be expanded through the use of spectroscopy probes that can be placed through the instrument channel to image the mucosa. Advances in fiberoptics, light sources, and detectors have led to the development of several novel methods for tissue evaluation in situ [25]. The term "optical biopsy" refers to methods that use the properties of light to enable the operator to make a diagnosis at endoscopy, previously possible only by using histological or cytological analysis. Promising imaging techniques include fluorescence endoscopy, optical coherence tomography, confocal microendoscopy, and molecular imaging (Table 118.3). Point detection schemes under development include light scattering and Raman spectroscopy. Such advanced diagnostic methods go beyond standard endoscopic techniques by offering improved image resolution, contrast, and tissue penetration and providing biochemical and molecular information about mucosal disease. The detection of dysplasia arising in Barrett esophagus would be an obvious goal of optical spectroscopy [26].

Endoscopic ultrasonography

One of the major advances in endoscopy is the coupling of ultrasound with endoscopy (endoscopic ultrasound or EUS). There are two approaches to this combination of technologies. In the first approach, the endoscope has an ultrasound device built into the tip of the endoscope. The ultrasound transducer can be oriented radially and provide cross-sectional images of the GI tract or in a linear orientation. In the linear orientation, fine-needle aspiration can be directed with ultrasound imaging of the GI tract and for the major organs adjacent to the esophagus, stomach, and duodenum. The alternative to using dedicated ultrasound endoscopes is to use small ultrasound probes that can be passed through the instrument channel. Probe endosonography provides high-resolution radial images of wall lesions. EUS imaging has dramatically improved tumor staging, acquisition of tissue from the pancreas, and will provide injection therapies. See Chapter 129 for more on EUS.

Chromoendoscopy

Chromoendoscopy is an in vivo technique in which the mucosa or a lesion is washed with an agent designed to enhance mucosal imaging (Table 118.3). Chromoendoscopy agents include those that enhance the mucosal detail or in which the agent is absorbed by certain cell types (vital staining). The dye agent is usually sprayed against the mucosa using a specially designed catheter passed down the biopsy channel of the endoscope. The various dyes are used for specific clinical settings and provide specific information regarding mucosal abnormalities. Before using any of these

Table 118.3 Summary of endoscopic image enhancement techniques used for detection of gastrointestinal malignancy

Endoscopic technique	Agent or device used	Mechanism	Clinical application
High-resolution endoscopy	High-resolution endoscope with high pixel density (e.g., 850 000)	Increased resolution by the CCD on the endoscope	Endoscopic identification of surface details, such as crypts, glands, and pit pattern
Magnification endoscopy	Magnification endoscope with manual (lens change) or electronic control	Magnification of the endoscopic image	Endoscopic identification of surface details enhanced with dyes
Combined high-resolution and magnification endoscopy	Magnification endoscope with manual (lens change) or electronic control	Magnification of the endoscopic image	Endoscopic identification of surface details enhanced with dyes
Chromoendoscopy	Topical agents often used in conjunction with magnification endoscopy		
	Lugol solution	Contrast dye, staining glycogen in squamous epithelium	Detection of early squamous cell carcinoma of the esophagus
	Methylene blue	Absorptive dye, staining absorptive epithelium (small intestine and colon) including Barrett	Detection of Barrett esophagus and early malignancy arising from Barrett (lack of staining)
	Indigo carmine	Contrast dye, outlining mucosal surface	Detection and mapping of gastroduodenal malignancies
	Acetic acid	Reversible intracellular protein denaturation	Detection of Barrett epithelium
Narrow band imaging	Electronic light source with narrow-band filters	Enhancement of surface capillary patterns, pits, and villi	Detection and mapping of early gastric and esophageal cancer
Fluorescence endoscopy	Image-processing module that provides real-time fluorescence images 5-Aminolevulinic acid (topical or systemic) HAL	Sensitizers accumulate selectively in malignant lesions and induce fluorescence upon illumination with light of the appropriate wavelength	Detection of early gastric and colonic adenomas and malignancies
Confocal microscopy	Dedicated combined endoscope and microscope image processing unit Acriflavine hydrochloride or fluorescein sodium	Fluorescent agents are absorbed by tissue and provide cellular and histological details	Broad applications in detection of mucosal neoplasia

CCD, coupled chip device; HAL, hexaminolevulinate.

dyes, it is necessary to wash away adherent mucus. This is often accomplished using a solution of dimethyl polysiloxane, sodium bicarbonate, and pronase. A 10% solution of *N*-acetylcysteine can also be used before spraying the dye.

Contrast chromoendoscopy

Contrast agents are used to accentuate minute alterations in mucosal architecture by accumulating in normal or abnormal mucosal structures and accentuating small lesions. This is best achieved using indigo carmine, which pools between mucosal grooves and provides a three-dimensional image of the mucosa. Similar effects have been reported with acetic acid [27]. Contrast chromoendoscopy is often used in conjunction with high-resolution endoscopy [28].

Vital staining

Vital dyes, such as methylene blue, are absorbed by epithelial cells, and the differential absorption by a mucosal process may aid in the detection of dysplasia (Table 118.3). Absorptive staining takes place over a few minutes during the application of the dye to the tissue. After staining is achieved, the solution is washed away. Lugol solution stains intracellular glycogen in nonkeratinized squamous epithelium and therefore normally stains the entire esophageal mucosa (Table 118.3). Early esophageal cancer and high-grade squamous dysplasia do not stain with Lugol solution [29]. Lugol staining is useful in looking for plaques overlying varices, evaluating high-risk patients, or to precisely map a neoplasm of the esophagus. Methylene blue dye is used to enhance the detec-

tion of dysplastic tissue arising from Barrett epithelium as the dye is not absorbed by the dysplastic tissue [30]. Biopsies are directed to areas of Barrett mucosa that lack staining.

Congo red is a reactive stain that maps the acid-producing parietal cell mucosa, staining from red to dark blue in the presence of acid secretion. Lesions associated with hereditary diffuse gastric cancer can be detected by Congo red and methylene blue staining [31].

Advanced imaging procedures

Upper GI endoscopy provides an opportunity to guide the passage of imaging instruments in close proximity to lesions or areas of interest [32]. Endoscopic ultrasound is the most commonly performed advanced imaging procedure. Small ultrasound catheters may be passed through the endoscope in order to provide high-resolution imaging of mucosal lesions. Using water as a medium, the high-frequency probe offers a detailed examination of mucosal and subepithelial lesions and their relationship to the wall structure of the GI tract. Probe endosonography may provide staging of early malignancy as well as differentiate between cystic, solid, and lipomatous lesions (Table 118.4). The disadvantages of probe endosonography include the need for water immersion and the inherent inability to provide imaging of histological structure.

Optical coherence tomography (OCT) is an infrared-based imaging technique that provides high-resolution imaging of mucosal details. Although OCT is used clinically in cardiology and ophthalmology, it is not a standard imaging technique in endoscopy. OCT is ideally equipped to differentiate between the basic epithelial structures, such as squamous and columnar epithelia [33]. Extensive testing in the setting of Barrett esophagus has demonstrated the ability to diagnose Barrett esophagus [34]. Furthermore, there is some evidence that OCT may be able to detect early changes of dysplasia and guide mucosal biopsies [35,36].

Magnification endoscopy is often used to provide higher-resolution images of the epithelium in concert with chromoendoscopy (Table 118.3). Most videoendoscopes provide optional magnified or "zoom" imaging. The most obvious application of this relatively simple technique is in the detection of duodenal villous atrophy. Using saline or contrast agents to separate the villi, high-resolution endoscopy can be used to reliably and accurately diagnose sprue (Table 118.3) [37]. Similar techniques have been applied for the diagnosis of Barrett esophagus. The finding of villi arising from the esophageal epithelium is diagnostic of intestinal metaplasia [38]. Changes in the villi morphology have enabled endoscopists to diagnose early gastric cancer [39].

Narrow band imaging (NBI) involves the use of interference filters to illuminate a target in narrowed red, green and blue (R/G/B) bands of the spectrum (Table 118.3). The imaging modality is built into commercial endoscopy processors and no catheters or probes are necessary. NBI provides imaging of the surface of epithelium, particularly of the surface vascular pattern. Many early malignancies have surface pattern changes in vascularity, such as the corkscrew pattern

Table 118.4 Radiological and endoscopic imaging used to stage gastrointestinal malignancies

Imaging modality	Device used	Agent used	Detection of malignant tissue	Availability	Clinical applications
EUS	Dedicated echoendoscope; high-frequency ultrasound probes can be used through the instrument channel; fine-needle aspiration device can be used with linear EUS	Water is used as a coupling agent. Levovist and Definity are intravenous ultrasound contrast agents rarely used in EUS	The vast majority of malignancies are relatively less echoic (hypoechoic) than normal tissue	Widely available	Frequently used to stage esophageal, gastric, pancreatic, and rectal cancer
OCT	Laser infrared light source, semiconductor optical amplifier, catheter probe	None	Changed architecture of tissue (e.g., loss of goblet cells) is used as a sign of malignancy	Not commercially available; prototypes used in research centers	Staging of superficial malignancies
CT	An X-ray beam is emitted in a fan shape as the rotating frame spins the X-ray tube and detector around the patient. Multidetector CT with computer-generated images	Intravenous and oral iodine-based contrast agents	Using ionized radiation, the vast majority of malignant tissue have a different attenuation, accentuated by the use of intravenous contrast	Helical CT is widely available. MDCT (multidetector CT) will become widely distributed	Broad applications in staging of advanced GI malignancies

CT, computed tomography; EUS, endoscopic ultrasound; GI, gastrointestinal; OCT, optical coherence tomography; MDCT, multidetector computed tomography.

seen in early gastric cancer [40]. When NBI is used in conjunction with magnification endoscopy, the diagnosis of intestinal metaplasia of the esophagus can be secured with a high degree of accuracy [41]. In comparative trials, NBI has produced results similar to high-resolution chromoendoscopy [42].

Endoscopic confocal microscopy provides the highest resolution images of the GI tract of any imaging modality (Table 118.3) [43]. The instrument is a dedicated endoscope with an independent processor and is not yet widely available. Confocal endomicroscopy uses blue laser light and is often used in conjunction with an intravenous and a topical fluorescent agent. The images obtained from this technique provide near real-time cellular details, including individual epithelial cells to a depth of 1–2 mm. The crypts of the colonic mucosa, the villi of the terminal ileum and duodenum, the gastric pits of the stomach, and the squamous epithelium of the distal esophagus can be clearly visualized. The technique has been demonstrated to improve the detection of dysplasia arising from the colonic mucosa in patients with inflammatory bowel disease [44]. The high-resolution imaging has demonstrated *Helicobacter pylori* organisms in the gastric mucosa [45].

Removal of foreign bodies

In removing a foreign body, the endoscopist should carefully evaluate the patient for any possible evidence of airway obstruction or perforation prior to the endoscopic procedure. During removal of a foreign body, an overtube provides a degree of protection of the airway by preventing dropping of the foreign body during removal, but the only way to ensure complete airway patency is to use a cuffed endotracheal tube.

The mucosa can be protected, especially in the removal of sharp objects, by using an overtube to protect the intestinal lining during removal of a sharp foreign body [46]. It is possible to remove foreign bodies with rigid and flexible endoscopes. Foreign bodies in the area of the hypopharynx and very proximal esophagus are often best removed with an open laryngoscope and grasping forceps. For other foreign bodies, flexible endoscopes are used, preferably with a large instrumentation channel (2.8–3.5 mm). The larger channel allows a variety of instruments to be used to grasp and hold the foreign body. These include baskets of the Dormia type, forceps for grasping the foreign body, and polypectomy snares. Alligator forceps with teeth are useful for grasping coins and other types of smooth objects. If an object is irregular or soft, other devices, such as baskets, snares, and graspers with three prongs, can be used. An irregular, sharp object, an object with a ragged surface, or an object that is impacted in the wall (e.g., dental plate) may require a rigid laryngoscope or esophagoscope to be passed under general anesthesia for removal of the foreign body.

If the foreign body is sharp, a plastic overtube should be used to protect the esophagus (Fig. 118.1) [34]. The endoscope can be withdrawn into the overtube while grasping the foreign body, which protects the mucosa from injury by the sharp foreign body as it is being removed. This also eliminates the possibility of dropping the foreign body in the hypopharynx, causing pulmonary aspiration of the object. The overtube is useful if the endoscope must be passed several times for removal of a foreign body, such as a piece of meat. An alternative to a plastic overtube is a simple latex protector hood fastened to the tip of the endoscope [35].

Most experienced endoscopists practice before attempting foreign body removal. A foreign body similar to that swallowed by the patient is manipulated with the type of instrument available to grasp the foreign body during endoscopy. This time spent in forethought and planning makes the foreign body removal safer and easier [36].

Endoscopic control of upper gastrointestinal bleeding

Upper GI bleeding is one of the most common indications for upper GI endoscopy. For approximately 85%–95% of these patients, a precise diagnosis of the cause of the bleeding can be obtained. For a few patients with massive hemorrhage, endoscopic control of bleeding may not be possible. In most

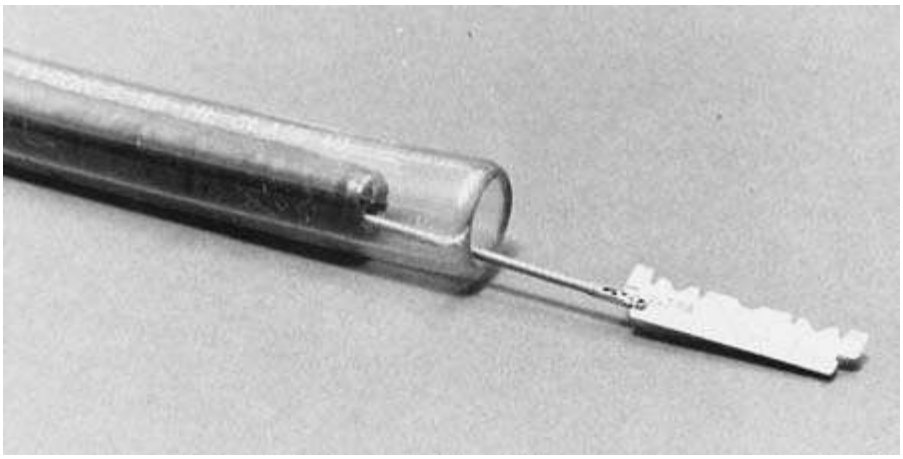

Figure 118.1 A plastic overtube around the endoscope allows safe retrieval of sharp or pointed foreign bodies.

Figure 118.2 In polypectomy, the snare loop is positioned around the sessile, adenomatous, gastric polyp.

patients with bleeding, endoscopy can be performed as soon as the patient is stabilized hemodynamically. Endoscopic treatment of nonvariceal and variceal bleeding is discussed in Chapters 123 and 124.

Polypectomy

Although polyps in the upper GI tract are relatively common, removal is not necessary in the vast majority of patients [47]. Removal of a polyp or polypoid lesion is indicated in neoplastic lesions or those lesions that cause bleeding or symptoms [48]. Polypectomy is accomplished using a wire snare placed over the polyp in a fashion similar to colonoscopy (Fig. 118.2). Snares of a variety of sizes and shapes are available, including a symmetric ellipse, a hexagon, and a slightly hooked configuration. After the handle is closed, the tip of the snare should retract at least 1 cm into the plastic overtube. As the snare is tightened on the lesion, an electrocoagulative current is applied, alone or in combination with a cutting current, during the transection of the polyp.

The complications with gastric polypectomy include hemorrhage and perforation. Because of the thickness of the gastric wall, perforation is rare, but hemorrhage may be a significant problem. The risk of bleeding is probably greater in large polyps with thick stalks. Injection of the stalk with epinephrine is recommended, but may not always prevent bleeding. Mechanical hemostatic devices, such as endoloops or clips, can prevent bleeding after polypectomy [49].

Mucosal resection

Endoscopic mucosal resection is a group of techniques designed to remove superficial malignancies of the GI tract. The lesions are most commonly located in the stomach, but an increasing number of dysplastic lesions are being discovered in Barrett esophagus. Usually, the lesion is first examined with endosonography to prove its superficial nature. In principle, the lesion should be limited to the mucosal layer, but lesions involving the submucosa can also be resected. After proper localization with chromoendoscopy or fluorescence endoscopy, the lesion is lifted after submucosal injection of an agent designed to separate the mucosa from the deep layers [50]. Thereafter, the lesion is aspirated, usually into a small translucent cap located on the end of an endoscope. The size, shape, and flexibility of the cap will determine the amount of tissue that is resected [51]. More advanced freehand techniques are being investigated for the removal of large portions of malignant gastric mucosa [52]. Transection is performed with cautery, allowing the specimen to be retrieved for histopathological examination. Although the primary purpose of EMR is resection of malignancy, the procedure also provides detailed histological evidence of the tumor stage (Table 118.2) [53]. Mucosal specimens obtained with EMR techniques provide a higher accuracy for the detection of mucosal malignancy [54]. When used for the resection of mucosal adenocarcinoma in Barrett esophagus, cap-assisted EMR provides high rates of remission {Conio, 2005 #6}.

Dilation

Esophageal stricture dilation may be performed using Maloney dilators, wire-guided dilators, or balloons (Table 118.1) [55]. Flexible endoscopy has an increasingly important role in dilating strictures through the use of through-the-scope (TTS) balloons or placement of a guidewire [56]. However, the overall need for esophageal stricture dilation has decreased with the increasing use of proton pump agents [57]. TTS or controlled radial expansion (CRE) balloon dilation of esophageal strictures is highly successful and safe [58]. The radial force provided by a CRE balloon is greater than the radial force generated by bougie dilation. Stricture dilation has been eased by the use of CRE balloons, dramatically improving patient tolerance. In a complex stricture, the balloon should be guided with a guidewire. However, in most strictures, the balloon is easily placed in the stricture and held in place during pneumatic dilation. Balloons used for CRE vary from 4 to 20 mm in diameter when fully inflated with a liquid, and many provide graduated dilation over 3 mm. The risk of perforation is low in benign strictures, but malignant strictures can be safely dilated only to 14 mm [59].

Semirigid esophageal dilating devices are guided through strictures using a guide wire placed endoscopically. The guidewire with a spring tip is passed through an instrument channel across the stricture. With the guidewire in place, the endoscope is removed. Confirmation of the positioning of the guidewire below stricture can be obtained with fluoroscopy, but is rarely needed. Increasing diameter dilators are passed over the guidewire and across the stricture. The guidewire

and the spring tip should remain below the area to be dilated throughout the procedure. Thermoplastic Savary dilators are the most commonly used wire-guided dilator [56].

Using the same principles for esophageal stricture dilation, TTS balloons can be used in the pylorus and duodenum [60]. Benign strictures of the pylorus are easily dilated with lasting effects [61]. Passage of the balloon and its inflation can be monitored with fluoroscopy to be certain that the waist of the balloon, representing the stricture, has been obliterated. Balloon dilation of benign strictures is highly effective.

Stents

Esophageal stent placement is a highly effective procedure for the palliation of malignant dysphagia. The timing of stent placement is very important in order to provide optimal benefit to the patient and avoid complications, such as stent migration. Prior to stent placement, endoscopy is used to evaluate the degree of narrowing, measure the length of the tumor narrowing, and determine the distance from the top of the tumor to the incisor teeth, an important factor in placing the prosthesis. Patients and family should be fully informed of the risks and complications of esophageal stent placement.

Prior to the placement of an esophageal stent, the esophageal lumen must be large enough to accommodate a stent. Dilation of the malignant stricture can be accomplished with CRE balloons or bougies. After the length of the stricture is determined, the stent length should be long enough to include 2.5–3 cm proximal to the narrowing and the same distance distal to the narrowing to prevent early restenosis by tumor. The proximal and distal sites of the stricture should be marked with an injection of contrast or the placement of a clip.

Expandable esophageal stents that are placed across a malignant stricture readily provide lumenal patency. Self-expanding esophageal stents are provided in a covered or a noncovered format [62]. These devices are passed over a guidewire through the malignant narrowing, within a sheath covering the compressed stent. After the constraining sheath is released, the stent is left in place and expands. The expanding stents have the advantage of producing a wide lumen through the stenosing tumor mass. However, these stents are difficult to remove. Stents covered by a silicone membrane may prevent tumor ingrowth and offer relief from symptoms associated with fistulas and leaks [63]. One variety of covered esophageal stents are designed to be used in benign strictures and be removed [64]. The long-term efficacy of the use of stents in benign strictures has not been defined.

Laser tumor ablation

An endoscope can be used to guide a neodymium-doped yttrium aluminum garnet (Nd:YAG) laser to provide long-term patency of a malignant esophageal stricture [65]. Repeated ablative sessions are required in order to eliminate sufficient malignant tissue for symptomatic relief. Long-term success, in terms of relief of dysphagia, is relatively modest, about 66% [65]. This approach has largely been replaced with the placement of self-expanding metal stents [66]. Stenting can provide rapid relief from dysphagia with only one endoscopic procedure and is associated with low risk. Endoscopic laser therapy with homogentisate oxidase (HGD) has been used in small numbers of patients with Barrett esophagus [67]. In addition, laser therapy has been used to ablate low-grade malignancies of the esophagus, such as

Table 118.5 Endoscopic methods for ablation of malignancy

	Argon plasma coagulation	Photodynamic therapy	RFA	Ethanol	Laser
Primary clinical indication	Ablation of vascular lesions	Barrett esophagus with dysplasia	Ablation of hepatocellular carcinoma	Ablation of hepatocellular carcinoma	Ablation of esophageal malignancy
Method of application	Topical application via catheter	Intravenous photosensitizer and catheter-guided light exposure	Percutaneous needle with RFA wire	Needle injection	Catheter-directed laser light
Primary oncological application	Barrett esophagus	Barrett esophagus with dysplasia	Hepatocellular carcinoma, hypernephroma	Hepatocellular carcinoma	Esophageal malignancy
Additional targets	Adenomas	Gastric malignancy	Hepatic metastases, pancreatic cancer	Pancreatic cystadenomas	Gastric malignancy
Mechanism of action	Coagulation and heat	Cell death induced by singlet oxygen	Heat	Protein denaturation	Heat

RFA, radiofrequency ablation.

granular cell tumors [68]. An alternative method of applying laser photocoagulation is to use argon plasma coagulation (APC) (Table 118.5), which is highly effective in the ablation of Barrett epithelium [69].

Photodynamic therapy

Photodynamic therapy (PDT) is an ablative therapy that can be delivered through an endoscope. An appropriate photosensitizing agent, such as a hematoporphyrin derivative, is injected intravenously and is taken up in high concentration in the area of gastric or esophageal malignancy. A standard endoscope is used to place a flexible fiber bundle to the tumor site and red laser light therapy is administered. Light activation results in singlet oxygen release and superficial tumor necrosis. PDT can be used in conjunction with EMR for the elimination of focal dysplastic lesions of the esophagus [70]. PDT may also be used for T1 malignancies of the esophagus, although the long-term success has not been determined [71]. In a prospective blinded trial, PDT eliminated high-grade dysplasia in 77% of patients [72].

Safety of upper GI endoscopy

Before endoscopy is performed, a thorough history should be obtained from the patient. The history should include previous surgery, endoscopic procedures, and anesthesia history. A list of the patient's medications should be generated. A review of active medical problems will help the endoscopist assign an ASA (anesthesia risk) score and decide whether the patient will safely tolerate conscious sedation. A history of airway or upper esophageal surgery, procedures, or of radiation, will help decrease the risk of perforation.

Knowledge of bleeding tendencies in the patient or the patient's family and any history of drug allergies should be obtained. If there is a history of bleeding abnormalities, a clotting screen with a prothrombin time, partial thromboplastin time, platelet count, and bleeding time is indicated. If there is evidence of chronic liver disease or other hematological disorder, it is important to check the degree of coagulopathy.

Diagnostic endoscopy with biopsy is usually safe in patients who are taking oral anticoagulants or aspirin. However, for a patient who is to be treated with electrocoagulation for polypectomy or hemostasis, anticoagulants should be discontinued before the endoscopic procedure and for 7–10 days after the procedure to prevent delayed bleeding. Aspirin-containing compounds and other antiplatelet agents, such as nonsteroidal antiinflammatory drugs (NSAIDs) and ticlopidine, should be avoided for an appropriate interval (i.e., aspirin for 7–10 days; NSAIDs for 2–3 days) before an endoscopic procedure that may involve removal of tissue.

Most patients begin fasting after midnight the evening before the procedure. The patient should have no liquids for 4 h before endoscopy and no solids for at least 6–8 h beforehand. Patients with gastric outlet obstruction may be kept on a clear liquid diet for 24–48 h, and lavage of the stomach with a large-bore tube may be necessary to remove retained stomach contents.

Informed consent is an important risk management technique and should be obtained by a physician prior to every procedure. The physician should provide a realistic assessment of the risk of the procedure and the consequences of the complications. A discussion of risks should include a statement of the risks of conscious sedation.

Topical anesthesia to the pharynx is often provided in order to minimize the gag reflex during endoscopy. The need for the topical anesthetic in the setting of intravenous conscious sedation is controversial [73]. Excessive amounts of topical lidocaine may result in methemoglobinemia [74]. Intravenous conscious sedation is the most commonly provided anesthesia for patients undergoing endoscopy [75].

For endoscopic procedures that are expected to be brief and in which small-caliber or pernasal endoscopes are used, no intravenous sedation may be necessary unless the patient is anxious [9]. For therapeutic procedures that involve larger-diameter instruments and longer periods, the patient is usually sedated to deeper levels of sedation [76]. In a small percentage of patients, the administration of a benzodiazepine will result in a paradoxical reaction [77]. General anesthesia may be required in patients who cannot tolerate conscious sedation when there are airway issues, such as removal of foreign bodies in small children [78,79]. Propofol anesthesia can be provided safely with properly trained personnel in the endoscopy unit [80].

Bacteremia may be associated with endoscopic procedures. In a patient with a preexisting heart condition, there may be a risk of developing infectious endocarditis, although this risk is small (Table 118.6). The efficacy of antibiotic prophylaxis for endocarditis is unproven, although prophylaxis appears to be effective in patients with prosthetic valves, surgically constructed systemic–pulmonary shunts, and hypertrophic cardiomyopathy (Table 118.6). Prophylactic antibiotics are advised for patients with known endocarditis or prosthetic cardiac valves. For gastrointestinal endoscopic procedures, the standard regimen is 2 g of ampicillin, administered intravenously or intramuscularly, plus gentamicin (1.5 mg/kg, not to exceed 80 mg), administered intravenously or intramuscularly 30 min before the procedure, followed by 1.5 g of amoxicillin taken orally 6 h after the initial medication. Alternatively, the parenteral regimen may be repeated once 8 h after the initial dose.

The most common complications of upper GI endoscopy are related to the cardiopulmonary effects of sedative drugs [78]. Benzodiazepines cause dose-related hypoxia that can be readily treated with administration of oxygen [81]. In randomized trials, propofol is a safe and well-tolerated anesthetic for upper GI endscopy [82]. Recovery after propofol

Table 118.6 Antibiotic prophylaxis for endoscopic procedures

Patient condition	Procedure	Antibiotic prophylaxis
High risk		
Prosthetic valve	Stricture dilation	Recommended
History of endocarditis	Variceal sclerotherapy	Recommended
Systemic pulmonary shunt	ERCP/obstructed biliary tree Other endoscopic procedures, including EGD and colonoscopy (with or without biopsy/ polypectomy), variceal ligation	Recommended Recommended
Synthetic vascular graft (<1 year old)		Optional
Complex cyanotic congenital heart disease		Optional
Moderate risk		
Most other congenital abnormalities	Esophageal stricture dilation	Optional
Acquired valvular dysfunction (e.g., rheumatic heart disease)	Variceal sclerotherapy	Optional
Hypertrophic cardiomyopathy	Other endoscopic procedures, including EGD and colonoscopy (with or without biopsy/polypectomy), variceal ligation	Not recommended
Mitral valve prolapse with regurgitation or thickened cordae		Not recommended
Low risk		
Other cardiac conditions (CABG), repaired septal defect or patent ductus, mitral valve prolapse without valvular regurgitation, isolated secundum atrial septal defect, physiological functional, heart murmurs, rheumatic fever without valvular dysfunction, pacemakers, implantable defibrillators)	All endoscopic procedures	Not recommended
Obstructed bile duct	ERCP	Recommended
Pancreatic cystic lesion	ERCP, EUS-FNA	Recommended
Cirrhosis, acute GI bleed	All endoscopic procedures	Recommended
Ascites, immunocompromised patient	Stricture dilation Variceal sclerotherapy Other endoscopic procedures, including EGD and colonoscopy (with or without biopsy/polypectomy), variceal ligation	No recommendation
All patients	Percutaneous endoscopic feeding tube	Recommended (parenteral placement cephalosporin or equivalent)
Patients with prosthetic joints	All endoscopic procedures	Not recommended

Cardiac prophylaxis regimens (oral 1 h before, i.m. or i.v. 30 min before procedure).
Amoxicillin by mouth or ampicillin i.v.: adult 2.0 g; child 50 mg/kg.
Penicillin-allergic patients: clindamycin (adult 600 mg; child 20 mg/kg) or cephalexin or cefadroxil (adults 2.0 g, child 50 mg/kg) or azithromycin or clarithromycin (adult 500 mg, child 15 mg/kg) or cefazolin (adult 1.0 g, children 25 mg/kg i.v. or i.m.) or vancomycin (adult 1.0 g, child 10–20 mg/kg i.v.).
CABG, coronary artery bypass graft EGD, esophagogastroduodenoscopy; ERCP, endoscopic retrograde cholangiopancreatography; EUS-FNA, endoscopic ultrasound-guided fine needle aspiration; GI, gastrointestinal.

is quicker than after standard conscious sedation. Sedation with propofol during therapeutic endoscopy provides a rapid onset of deep sedation with a desaturation rate of 15% [83].

Perforation is the most directly assignable complication of upper GI endoscopy, but this occurs in less than 0.1% and is increasingly rare. The most common cause of perforation is the passage of the blind tip of the endoscope into a malignant stricture. If these perforations are recognized early, immediate surgery and repair will provide closure with the minimum of complications. The performance of EMR and resection of a subepithelial malignancy is also associated with esophageal or gastric perforation. However, in the case of EMR, the perforations are usually small and may be managed with endoscopic clipping. The third most common cause of perforation is the performance of large-balloon dilation of a stricture or achalasia. These types of perforations are more accurately referred to as tears, and their management is much more difficult. Uncomplicated, small perforations may be managed endoscopically and with supportive care. Large tears require urgent surgical resection and/or repair.

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A ENDOSCOPIC

119 Capsule and small bowel endoscopy

C. Paul Swain

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Introduction

The small intestine has been relatively inaccessible to flexible endoscopy until recently [1]. Its length is a challenge to endoscopy. The small intestine accounts for 75% of the total length and 90% of the surface area of the gastrointestinal (GI) tract. In adults it measures about 570 cm at postmortem [2], which is substantially longer than conventional videogastroscopes. Colonoscopes and gastroscopes measure 100–180 cm. There are features about its position and anatomy that limit the endoscopist's chance of passing longer endoscopes much further than a few centimeters into it, either through the mouth and duodenum or through the ileocecal valve. The tight curve from the bulb around the head of the pancreas and its relatively fixed retroperitoneal posterior position as it crosses the spine to the ligament of Treitz, whence it passes downwards as a loosely supported much-looped structure on a mesentery, is a challenge. If a colonoscope is passed through the mouth into the jejunum and a surgeon pleats the small intestine over the endoscope by hand at laparotomy, the stiffness of the endoscope tends to stretch the mesentery, which is attached posteriorly, as the endoscope follows the loops of small intestine and increases the friction exerted by the intestine on the endoscope. Colonoscopists can usually enter the terminal ileum for a few centimeters. Either way, the distance and the curved path that a conventional endoscope has to pass to reach the small intestine mean that the force for effective forward propulsion which can be exerted on the tip of an endoscope is small. Loops tend to form and enlarge in the stomach or in the colon, which can make deep small intestinal intubation difficult.

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Recent technical developments in the design of longer flexible instruments, specifically for push enteroscopy, have made this examination much more successful.

The advent of wireless video-capsule endoscopy [3] has released the endoscopist from the requirement to exert force on a long floppy cable-type endoscope to examine relatively short segments of small intestine. This device exploits peristalsis to propel the videoendoscope through the small intestine and can usually acquire images from the whole of the small intestine, from the pylorus to the cecum.

The development of a double-balloon insertion method for push enteroscopy has substantially increased the length of small intestine that can be reached by flexible endoscopes with an accessory channel. It is now possible to examine the whole of the small intestine in a majority of patients, although this requires passing the enteroscope, overtube, and balloon apparatus into the mouth and the anus on separate occasions.

Technical advances

There has been a substantial increase in small intestinal endoscopy in the last 10 years, which has followed two technical advances (Table 119.1). The first was the development of specific endoscopes for push enteroscopy [1,4–6] (Fig. 119.1). Careful attention to the design allowed better transmission of force to the tip and shaft stiffness for use at flexible endoscopy. These instruments were longer than colonoscopes, measuring 200–240 cm (Fig. 119.2), and slimmer, usually measuring 11 mm in diameter, but with a 2.8-mm accessory channel. They were initially designed for use with an overtube to aid deep intubation, which usually needed X-ray control for safe insertion. The overtube appeared to cause some of the complications associated with push enteroscopy, for example sometimes causing esophageal trauma [7]. The addition of video imaging [4–6] to these instruments allowed very high-quality images to be obtained from the esophagus

Table 119.1 New and available video methods of small intestinal imaging

What is new?
Wireless capsule endoscopy
Double-balloon push enteroscopy
What is available?
Push enteroscopy
Pediatric/adult colonoscopes to be used for push enteroscopy
Intraoperative enteroscopy using a colonoscope
Laparoscopic assisted enteroscopy
Ileoscopy at colonoscopy
What is no longer used?
Sonde enteroscopy
New nonvideo methods for small intestinal imaging
Computed tomography enteroclysis and virtual enteroscopy
Ultrasound
Magnetic resonance enterography

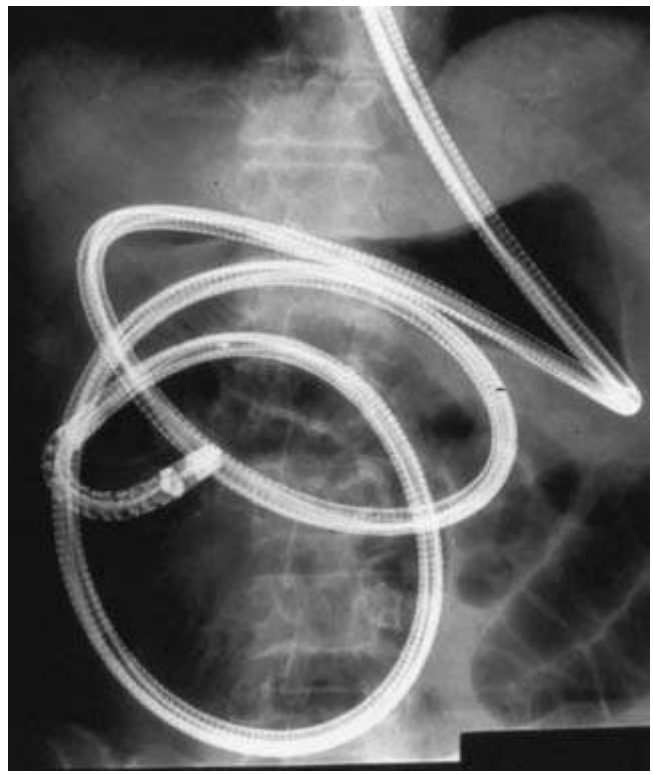


Figure 119.2 Fluoroscopic image of 240-cm-long push enteroscope, which is introduced to its maximum length into the jejunum.

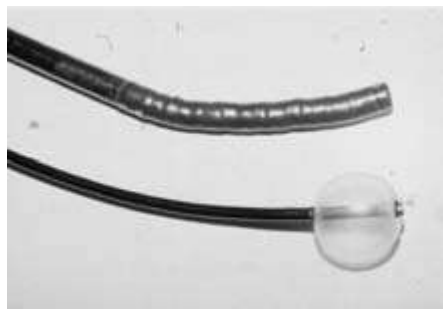
to the jejunum. Despite some evidence that 240-cm enteroscopes do not reach further or find more pathology than those of 200 cm [8], some of the newer instruments are longer than the earlier enteroscopes (240–280 cm). Variable-stiffness enteroscopes have recently become available [9].

Most [9–16] endoscopists [8] found that these instruments could be used almost as well without an overtube and thus dispensed with the need to perform this procedure under X-ray control. There was evidence from two studies that the use of an overtube was associated with a significant increase in depth of insertion into the small intestine by 10–15 cm [17,18] but did not increase pathology findings in either of these studies. The whole of the duodenum and some of the jejunum can be examined by video-colonoscopes [19,20], especially with pediatric colonoscopes, which are more widely available than push enteroscopes and of the same diameter but substantially shorter (135 cm vs 200–240 cm) [21].

It is difficult to be sure how far push enteroscopes are usually advanced into the small intestine because there are no landmarks and it is easy to overestimate the distance examined. A few groups have attempted to measure the distance with or without X-rays, and obtained results that seem rather short (45, 70, or 100 cm) [11,17,18] given the length of the small intestine (about 570 cm in adults at postmortem).

Wireless capsule videoendoscopy was first used in humans in 1999 [3]. It was able to provide video images from the whole of the small intestine and received Food and Drug Administration (FDA) approval in 2002, following the demonstration of safety and efficacy as an adjunct to the investigation of patients with suspected small intestinal disease, especially obscure gastrointestinal bleeding. Although this

(a)



(b)

Figure 119.1 Types of enteroscopes: **(a)** push enteroscope with bending section at the tip and **(b)** Sonde enteroscope with smaller diameter and balloon at the tip.

Table 119.2 Comparison of push and capsule enteroscopy*

	Push enteroscopy	Capsule endoscopy
Image lesions	xxx	xx
Biopsy	Yes	No
Therapy	Yes	No
Image quality	xxx	xx
Real-time viewing	Yes	No (Yes with new software)
Entire small bowel visibility	No (Yes with double balloon enteroscopy, from both ends)	Yes
Sedation	Yes	no
Patient acceptance	x	xxx
Image acquisition time	30 min (more for double balloon)	8 h
Image assessment time	30 min (as above)	1 h
Value in gastrointestinal bleeding	xx	xxx
Manual skills needed	Yes	No
Clinical experience needed	Yes	For interpretation only

* In area examined: xxx, very good; xx, good; x, acceptable.

technology has been available for a relatively short period, it has been widely adopted and used in more than 750 000 patients. Table 119.2 compares the differences between this technology and push enteroscopy.

Over this period, another technology – Sonde-type enteroscopy – has atrophied and been largely abandoned. This method was usually carried out with a thin (5–7 mm), very long flexible transnasal endoscope with a balloon and a guide thread attached to its tip (Fig. 119.1). Once in the stomach, a conventional transoral gastroscope was used to catch the guide thread and push the Sonde enteroscope through the pylorus. The balloon was inflated and the endoscope was propelled by peristalsis through the small intestine. After about 6 h, a radiograph was taken to see how far it had passed, and the Sonde enteroscope was withdrawn, sometimes recording the image on video [21,22].

There has been another recent advance in the ability to image the small intestine – using flexible endoscopy. Yamamoto et al., in conjunction with Fujinon [23], have developed a double-balloon enteroscope system which can be advanced much further into the small intestine than other push enteroscopes. The balloons are used to pleat the small intestine on the overtube, which is cautiously advanced over the enteroscope. This technique takes much longer to perform than conventional push enteroscopy and needs X-ray screening, but it can sometimes examine even the whole of the small intestine, though usually with both anal and oral insertion on different days. The system [24,25] may well be an important advance in enteroscopy because it may allow endoscope-directed therapy, such as biopsy, snare polypectomy, and thermal treatment, of any area in the small intestine.

A side-viewing duodenoscope is occasionally helpful in viewing periampullary or duodenal abnormalities [26], which are not well seen with a forward-viewing endoscope, or for assessing whether bleeding is coming from the biliary or pancreatic duct [27].

Diagnostic laparoscopy can be especially valuable in children with suspected small intestinal bleeding [28] and probably should be used early in young males with recurrent bleeding because a Meckel diverticulum is the cause in two-thirds of male patients under 25 [29]. It is also of some value in middle-aged patients when a small intestinal tumor is suspected [30]. There has been some interest in using laparoscopy to pleat the small intestine over a push enteroscope in order to advance the enteroscope to the ileocecal valve [31,32].

Open surgical exploration with an endoscope passed through the mouth or through an enterotomy, for example in the cecum, continues to be an important last resort investigation in some patients with difficult GI bleeding [33].

Push enteroscopy

Technique

Push enteroscopes are heavier and longer than standard gastroscopes. They are slightly more difficult to handle during esophageal intubation and passage across the pylorus, but the push characteristics and increased flexibility allow for better handling in the jejunum. The bending section of push enteroscopes is longer, to allow increased angulation in all directions (Fig. 119.1).

A complete examination of the esophagus and stomach should be performed with the instrument before entering the duodenum; looping of the instrument in the stomach may produce red marks, usually on the greater curve, which may be difficult to distinguish from vascular ectasias or erosions

on pullback. Reducing air insufflation and consequent distention of the stomach, by sucking out the air in the stomach before entering the duodenum, may lead to deeper insertion of the enteroscope. The patient may feel discomfort as the instrument is advanced, distending the greater curvature of the stomach. Paradoxical or arrested tip motion occurs if the instrument loops within the fundus. By exerting pressure and clockwise torque, the endoscopist can generally push through the loop. Care should be taken not to form a loop within the stomach. Once the scope has been advanced into in the second part of the duodenum, pulling the endoscope back will produce paradoxical advancement, as is the case during endoscopic retrograde cholangiopancreatography (ERCP). The ligament of Treitz is usually encountered 85–110 cm from the incisors, and usually requires full tip deflection to find the lumen. Once around the ligament, the first jejunal loop can be identified by a straight configuration, which points in a caudal direction if seen on a radiograph.

Overtube

A variety of methods have been used to try to reduce the tendency of the enteroscope to loop in the stomach. These include the use of abdominal pressure, internal stiffening devices [11], and overtubes [11,17,18] of 60–100 cm in length. Some have two ring-shaped metal tip markers to facilitate placement under screening and to prevent the overtube tip from being compressed. Others have a more pliable Gore-Tex tip of 10 cm in length, which may limit mucosal trauma when advancing the overtube over the endoscope. The overtube is initially backloaded onto the shaft of the endoscope, and is advanced down the endoscope through the esophagus until its distal tip rests within the second portion of the duodenum or beyond the ligament of Treitz. Fluoroscopy is helpful for overtube use as prepyloric placement does little to aid deep intubation. The softer Gore-Tex-tipped overtube may buckle in the stomach and fail to provide sufficient rigidity. As the overtube is advanced, the endoscope shaft needs to be straightened by twisting the endoscope shaft clockwise while pulling it backward. Some commercially available enteroscopes are too floppy to be used without an overtube. Recently, a shape-lock overtube, which has interlocking rings that can be pulled tight with wires to lock the device into a fairly rigid conformation, and has an external and internal hydrophilic coated sheath, has been used to aid insertion of push enteroscopes deep into the small intestine.

Diagnostic applications

The main indication and pressure for improvements in enteroscopy has been obscure gastrointestinal bleeding in patients with a negative gastroscopy and colonoscopy. Push enteroscopy has been performed with published diagnostic yields of 30%–50% [1,11]. One surprise has been that in a proportion of such patients having a push enteroscopy – about 25% – the cause has been found in the esophagus, stomach or duodenum within reach of a gastroscope and was missed on a previous gastroscopy. A list of common missed causes of obscure bleeding is given in Table 119.3.

Table 119.3 Common missed causes of bleeding in the upper gastrointestinal tract that may be found during enteroscopy

Large hiatus hernia with Cameron erosions
Watermelon stomach
Dieulafoy exulceratio simplex
Varices
Ulcers
Angiodysplasia

Table 119.4 Small bowel tumors to be detected during enteroscopy

Lymphangiectatic cysts
Gastrointesinal stromal cell tumors
Neuroendocrine tumors, e.g., carcinoid, Zollinger–Ellison syndrome
Polyps
Adenomas
Hamartomatous polyps
Lipoma/angiolipoma
Neurofibromas
Duplication cysts
Adenocarcinomas
Lymphomas
Sarcomas
Metastases, e.g., melanoma, renal cell carcinoma

Push enteroscopy has helped in the diagnosis, biopsy, and management of small intestinal tumors (Table 119.4). Other indications include malabsorption including coeliac disease; assessment of Cohn's disease, nonsteroidal antiinflammatory drug (NSAID)-induced strictures and erosions, abnormalities on barium or computed tomography (CT), and worm infestation.

Therapeutic applications

Push enteroscopes include a standard 2.8-mm biopsy channel that has been used for several therapeutic functions within the proximal small intestine, as well as for directed biopsy [11]. The more the instrument becomes looped as it advances into the small bowel, the greater the difficulty in getting instruments to emerge from the biopsy channel. This particularly applies to instruments with a long rigid tip, such as a heater or bipolar probes and balloons, rather than snares and laser fibers.

Biopsy

Endoscopic forceps-type biopsy has now largely replaced the use of a suction-fired biopsy capsule as a means of taking

small bowel biopsies [34]. The advantages of the endoscopic method are speed, ease, patient comfort, and reliability. Enteroscopic biopsies can reduce the chances of proximal duodenal inflammation and shorter villi in the bulb causing confusion when examining for celiac disease. They might offer advantages in the workup of patients with diarrhea or malabsorption, particularly if focal specific mucosal abnormalities have been seen in the proximal jejunum on small bowel radiograph contrast series [14]. In countries such as the UK, where celiac disease is the most common cause of malabsorption, enteroscopic biopsy does not add much information that is not acquired by duodenal biopsies taken with a gastroscope because celiac disease mainly affects the proximal jejunum. It is more useful in assessing celiac-like syndrome patients with unresponsive sprue or celiac patients with weight loss when a lymphoma is suspected [35–37]. The cause of diarrhea in patients with chronic human immunodeficiency virus (HIV)-related diarrhea is more likely to be detected by jejunal biopsy than by duodenal biopsy [38].

Jejunal feeding tube placement

Push enteroscopy has also been used in a variety of ways to place jejunal feeding tubes. It has been used to carry a transgastric jejunal tube through a previous gastrostomy into the jejunum [39–41]. Nasojejunal feeding tubes have been placed using the Seldinger over-the-wire technique [42]. The push enteroscope is placed into the jejunum initially and then a guidewire is advanced. The endoscope is removed, leaving the guidewire in place. A feeding tube is advanced over the wire and then rerouted from the mouth through the nose. This method has been combined with a sewing method for attaching the jejunal feeding tube to the stomach to prevent displacement [11].

Direct percutaneous endoscopic jejunostomy placement

By using push enteroscopy, the pull technique for the placement of percutaneous gastrostomies has been extended to permit direct percutaneous jejunostomies [11,41,42]. This technique has also been used to place jejunal tubes for small bowel enemas [43] as well as to obtain cholangiograms in patients after Roux-en-Y hepaticojejunostomy [11,44].

Polypectomy

Polypectomy can be performed in the small intestine via the push enteroscope using snares [11,45]. Surveillance of patients with a polyposis syndrome can be performed with push enteroscopy, allowing biopsy and polypectomy of larger lesions. Removal of large jejunal hamartomatous polyps in the Peutz–Jegher syndrome has become part of the screening of this autosomal dominant condition with a malignant potential (Fig. 119.2). The more proximal lesions are relatively easy to remove, but endoscopic removal may require an enteroscope and snare used at open surgery: this technique can find and deal with polyps that cannot be identified by surgical palpation and transillumination [46].

Treatment of gastrointestinal bleeding

In addition to diagnosis, push enteroscopy allows cauterization of bleeding sites. Using bipolar cautery, Foutch was able to fulgurate angiodysplasias in 11 of 12 patients [19]. Control of bleeding was obtained in 8 of the 11 treated patients. Askin and Lewis [47] assessed the long-term effectiveness of push enteroscopic cauterization of bleeding arteriovenous malformations in 89 patients. Of these patients, 61 were cauterized and 28 were not. The group that was cauterized required significantly fewer blood transfusions than the noncauterized group. In another series of 50 patients undergoing therapeutic push enteroscopy, bleeding was terminated in all patients with isolated angiomas and reduced in more than half of the patients with multiple jejunal angioma, as well as in some malignant tumors [48]. In addition, in 9 of the 50 patients the bleeding source was found within the reach of a gastroscope [48] and successfully treated. This group had a significantly reduced transfusion requirement compared with their precauterization status. Heater probes and bipolar probes, as well as monopolar probes and laser fibers, are available in lengths to fit the newer longer push enteroscopes. Argon plasma probes are just long enough to reach beyond 240-cm enteroscopes. Power levels should be reduced for cautery of flat lesions in the small intestine, as it is thinner than the stomach and colon.

Injection of small intestinal varices

Small intestinal varices, especially in the duodenum and ileum, around anastomoses or adhesions in patients with mesenteric thrombosis are an uncommon cause of bleeding but can be misdiagnosed and biopsied and may cause torrential bleeding. Successful treatment of bleeding jejunal varices using push enteroscopy to inject a sclerosant has been described [49].

Complications

Complications are uncommon with push enteroscopy, which is probably nearly as safe as gastroscopy, and most series report that no complications occurred. Some of the complications appear to be associated with the use of a metal-tipped overtube. These include a Mallory–Weiss tear, pancreatitis most likely secondary to papillary trauma, a pharyngeal tear, and three cases in which long strips of gastric mucosa were torn off during advancement of the overtube [7,12,21]. These complications with overtubes occurred prior to the addition of a Gore-Tex tip. Duodenal perforation has been described [50] but appears less common than with ERCP or endoscopic ultrasound. Some series describe severe patient discomfort, which is usually in relation to stretching of the stomach as a loop is formed along the greater curve while trying to transmit forward pressure to advance the tip in the small bowel.

Intraoperative video-enteroscopy

Technique

The endoscope needs to be cleaned, but not sterile, because the technique involves either peroral intubation of the small intestine or placement of the endoscope into a nonsterile bowel through an enterotomy, in which case it can help to place the endoscope in a sterile plastic sheath. The endoscope is advanced into the proximal jejunum through the mouth prior to laparotomy; once the abdomen is open it may be difficult to advance the instrument around the ligament of Treitz. With oral intubation using an adult colonoscope, the endotracheal tube cuff may need to be deflated to permit passage of the wide-caliber endoscope. Once the endoscope has been positioned in the proximal jejunum, laparotomy is performed. It can be helpful to place a noncrushing clamp across the ileocecal valve to prevent distention of the colon with insufflated air, because colonic distention can lead to difficulties with subsequent abdominal closure.

The examination is carried out by an endoscopist and a surgeon; the endoscopist looks at the endoscopic image using minimal air insufflation while the surgeon holds the endoscope tip inside the small bowel so as to inspect a short segment ahead of the endoscope. The surgeon then advances the endoscope into another segment. The view is best seen by dimming the overhead lights, also allowing the surgeon to look at the transilluminated bowel, which can be helpful in finding angiodysplasia. Once examined both internally and externally, the small bowel is pleated onto the shaft of the endoscope and the next section of bowel is examined. Care has to be taken not to overstretch the mesentery on colonoscopies or push enteroscopes, which are rather stiff for this use. Active bleeding within the small bowel may diminish the effectiveness of this examination. Visual examination and interpretation has to be performed during intubation and not on pull-back because mucosal trauma occurs with pleating, causing artifact that may be confused with the appearance of angiodysplasia [51]. A technique of reverse transillumination to help differentiate trauma from vascular ectasias in this setting and to allow visualization of the feeding vessel has been described [52]. At the end of the examination, the endoscope is withdrawn and the surgeon can resect the abnormalities identified by the sutures.

An alternative technique is to perform an enterotomy through which a "sterilized" endoscope or an endoscope covered by a sterile plastic sheath is placed. The enterotomy is generally performed in the mid-small bowel, allowing both proximal and distal intestinal intubation, but it can be performed in the cecum if vascular ectasias are suspected in this area, or in the distal small bowel. Retrograde intubation of the small bowel can alternatively be performed after the cecum has been inspected. The colonoscope is advanced through the ileocecal valve by the surgeon and up the small intestine.

Diagnostic and therapeutic utility

Intraoperative endoscopy has been used for a variety of indications [46,47,52–58], but until recently was probably the endoscopic method most widely used in identifying small intestinal sites of bleeding beyond the proximal jejunum. It can be successful in identifying the site of blood loss in selected patients with reported yields of 83%–100% [53–58]. Vascular ectasias are the most common nonpalpable cause of bleeding, but radiation enteritis, ulceration, malignancies, strictures, and polyps (e.g., multiple polyps in Peutz–Jegher syndrome) may require intraoperative endoscopic identification. Hemostasis can be achieved at intraoperative enteroscopy, either by resection of the bleeding lesion or by transendoscopic ablation of the bleeding lesion, usually with a thermal endoscopic method.

Complications

Prolonged ileus, sepsis, and perforations (the surgeon should listen for a hissing sound that indicates that perforation has occurred) are common sequelae of operative enteroscopy.

Outcome studies of the value of video-enteroscopy with flexible instruments

Several studies have looked at the number of diagnoses, the influence on management, and the subsequent clinical path of patients who have enteroscopy for suspected small intestinal outcome. Recent prospective studies have suggested that management was altered in more than 50% who had push enteroscopy [57,58]. One study has shown that the costs of investigating and managing patients who bleed from the small intestine are different and substantially more expensive than the costs of patients who bleed from other gastrointestinal sites [59]. Outcome studies of the value of push enteroscopy in nonbleeding conditions suggest that it can be of value in malabsorption [14,60], Crohn's disease [61], NSAID toxicity [62], and worm infestation [63]. It can be usefully performed in nonspecialist, district general hospitals [64].

It may be helpful to have some knowledge of the clinical patterns and appearances of the many less common or rare diseases that can affect the small intestine [65]. Access to an atlas [66] and a book-length review on enteroscopy (in the *Gastrointestinal Endoscopy Clinics of North America*) [67] may help.

Wireless capsule videoendoscopy

The development of wireless capsule endoscopy [3,68–72] has changed videoendoscopy of the small intestine into a much less invasive and more complete examination [3,71,73–83]. It has been particularly successful in finding the cause of obscure gastrointestinal bleeding of suspected small intestinal origin [73–83] (Fig. 119.3).

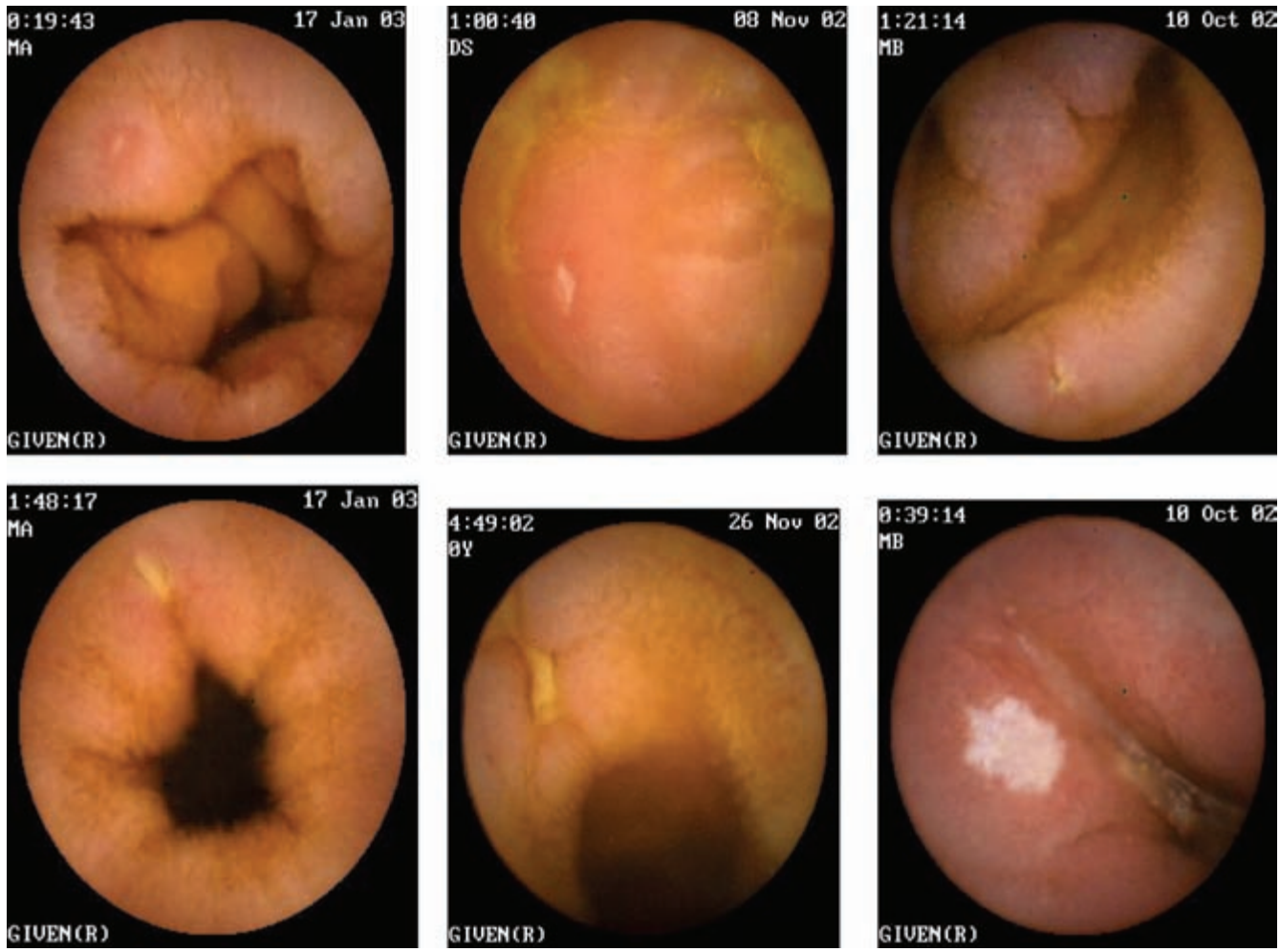


Figure 119.3 Aphthous ulceration.

Figure 119.4 Capsule endoscope with six light-emitting diodes.

The capsule endoscope (M2A, Given Imaging, Yokneam, Israel) contains a video-imager, transmitter, light source, and batteries in a rounded cylindrical plastic container measuring 11 × 26 cm [3]. There is a clear optical dome-shaped window that allows the intestine to be illuminated by an array of six (formerly four) light-emitting diodes (Fig. 119.4). The image is captured through a short focal length lens with a wide angle as the optical window of the capsule sweeps past the gut wall, without requiring air inflation of the gut lumen. The video images are transmitted using radiotelemetry (operating in an ultra high frequency (UHF) of 432 MHz) to an array of eight aerials attached to the body. They allow image capture and enable calculation and indication of the position of the capsule in the body. The capsule transmits images at a rate of two (or more recently four) frames per second for over 7 h, allowing the acquisition of over 50 000 images of the gastrointestinal tract.

These images are subsequently downloaded from the portable recorder for analysis off-line. Currently, the time needed to evaluate the video sequence takes between 45 min and

2 h and varies with the experience and concentration of the examiner as well as the number of pathological abnormalities present.

Wireless capsule endoscopy can provide about 7–8 h of continuous video imaging of the gastrointestinal tract. This usually allows image acquisition from the esophagus, stomach, small intestine and right colon. The capsule is propelled by peristalsis and is passed through the anus into the toilet, usually 1 or 2 days after ingestion. Because the gut is a hollow tube it is unimportant as to whether the capsule points forward or backward as it passes through. This form of endoscopy is painless, does not require sedation, and, in consequence, the patient is ambulant and does not need to be confined to a hospital during the examination [3,73–83].

Procedure and technique

To obtain consent, an explanation of the procedure should be provided that specifically includes the facts that the capsule may become stuck in the small intestine, requiring surgical removal; that capsule endoscopy does not always image the whole of the small intestine; that a plain abdominal radiograph may be performed if colonic images are not seen and the capsule is not seen in the toilet by the patient; and that MRI scans should not be done if the capsule is not known to have emerged. The patient should have an overnight fast or be nil by mouth for 12 h prior to ingesting the capsule. Preparation, as for colonoscopy, may improve the quality of images in the lower small intestine, but there are few data to support one regime over another. This is necessary if images of the right colon are important, for example in patients with incomplete colonoscopy.

The battery packs need to be fully charged and the patient details registered on the workstation with the recorder before starting the examination. The aerial cable should be securely attached to the recorder and placed in the belt. The patients should be shown how to manage the belt and lift it in the event of having to go to the toilet and to lay it carefully to the side while lying down in bed during the examination period. The capsule can then be removed from its packaging, which contains a magnet. This action will activate the capsule by releasing an internal magnetic switch and the capsule will start flashing. The patient can then swallow the capsule, usually with some water. Most patients can swallow it with the first gulp, but some children and a few adults find it difficult. Of these, some may be helped by asking them to place it in the mouth and then to try to swallow it while drinking out of a bottle. It is helpful to ask the patient to keep an eye on the flashing light on the recorder and the connection between the aerial and the recorder and to report back if it stops flashing over the next 2 or 3 h.

Patients should be asked not to eat for 3 h after swallowing the capsule but told that they can drink clear water. They can have a light meal after this time. The belt and aerial can be removed 8 h after swallowing the capsule, or when the recorder has stopped flashing. Patients should return the recorder, aerials, and belt but do not need to find and return the disposable capsule.

The images on the recorder need to be downloaded and processed prior to interpretation. Currently, image analysis of the 50 000 or so images acquired over the 8-h period takes about an hour or more to complete. It is probably unwise to read all the images at the fastest of the three available speeds. It is helpful to store any unusual images as well as definite abnormalities as thumbnails for more careful subsequent review. The points of entry into the stomach, duodenum, and cecum need to be marked to enable the localization software to image the passage of the capsule inside the abdomen. Abnormalities detected as blood by the blood-sensing algorithm need to be checked. Newer software places two adjacent images side by side to allow faster image analysis. The recording, with marked thumbnails, needs to be saved to the hard drive and to a CD-ROM. A typed report with an interpretation of the findings needs to be generated.

It may help to take breaks while reading the images as a loss of concentration may lead to missing important pathology, which may be seen only on a single frame. Interpretation of small intestinal images is not always easy and it may help to go backward and forward over some image sequences and to ask another endoscopist for an opinion. There is a wide range of pathologies, and numerous rarities are found that can affect the small intestine. The visual appearances are not specific, especially for the wide range of tumors found in the small intestine (Table 119.4). Benign conditions, including Crohn's disease, amyloidosis, and lymphangiectasia, have a wide range of appearances. It can be difficult to be sure when a red spot is really an angiodysplasia. In young males, it may be helpful to review the lower small intestinal images for a double-lumen suggestive of a Meckel diverticulum and to run over some of the images in the light of the patient's clinical history.

Most patients think that wireless capsule endoscopy is great and find it preferable to conventional endoscopy or colonoscopy [74,82]. It has been at least as successful as, and sometimes significantly more successful [75,82] than, push enteroscopy in finding the cause of obscure gastrointestinal bleeding (Table 119.5) in published series with yields of 55%–70% (Table 119.6). A blood-sensing algorithm may assist in alerting the physician to an area of bleeding pathology (Fig. 119.5) [84]. It has been successful in identifying small intestinal tumors (Table 119.4), even though multiple other investigations have failed [74,82,85], but may miss other tumors [86]. It has been significantly more successful than barium studies [87] in finding pathology in the small intestine, and has outperformed barium meal as well as state-of-the-art enteroclysis [88,89] and computed tomographic enteroclysis [90] in fully published recent studies.

Table 119.5 Possible causes of small bowel bleeding

Angiodysplasia and vascular malformations
NSAID enteropathy
Erosive jejunitis
Diverticula
Crohn's disease
Jejunal or ileal ulceration: idiopathic, nonsteroidal antiinflammatory drug induced, etc.
Intussusception
Small intestinal tumors (see Table 119.4)
Ischemic enteropathy
Graft versus host disease
Cytomegalovirus infection
Aortoenteric fistula
Blue rubber bleb syndrome

NSAID, nonsteroidal antiinflammatory drug.

Table 119.6 Comparison of the diagnostic yield of capsule endoscopy and push enteroscopy in the diagnosis of gastrointestinal bleeding

	Capsule (%)	Enteroscopy (%)
Mata 2003 [111]	66	19
Mylonaki 2003 [82]	68	32
Saurin 2003 [112]	69	38
Lewis 2002 [74]	55	30
Ell 2002 [75]	66	28

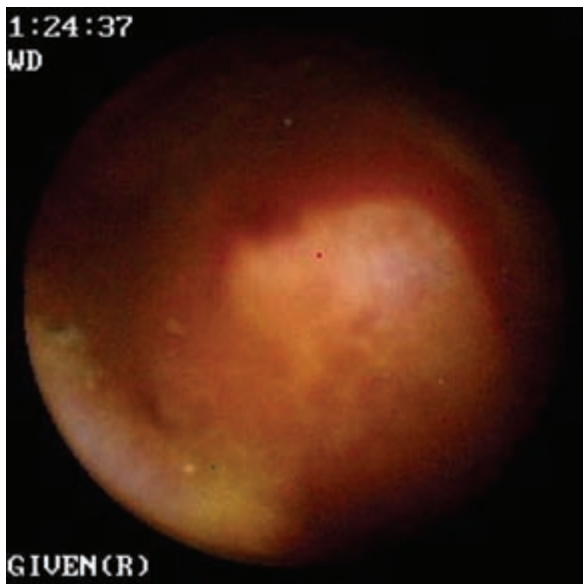


Figure 119.5 Bleeding from a jejunal adenocarcinoma.

Limitations

The images from the esophagus, stomach and colon are not as good as those acquired during conventional endoscopy, but may show pathology in these areas. The quality of images in these areas is likely to improve as specific wireless capsule devices and methods for image acquisition in these areas are developed. The images from the small intestine are somewhat poorer than enteroscopic views because the capsule cannot wash or be pulled back to re-examine a possible abnormality. At present, wireless capsule endoscopes cannot take biopsies from or provide therapy for a patient. With its current acquisition time of 7–8 h, the capsule fails to provide images from the cecum about 15% of the time. As a consequence, an uncertain length of small intestine remains unexamined. A few patients, especially children, have difficulty swallowing capsule endoscopes. Occasional patients retain it for prolonged periods in the stomach since there is a very wide range of "normal" gastric emptying times. Images may be poor in patients with morbid obesity. It can take a long time to interpret the images. Skill and experience are required. Many of the findings can be of unknown significance. Pathology may only be seen on a single frame. Because it can travel looking forward or backward, it may not look at pathology in an expected direction. Images in the lower small intestine are often rather dark. The recurring cost of the capsule as a disposable and the relatively lengthy examination times require specific funding and organizational strategies.

The main complication is that the capsule may become impacted in strictures [91] or diverticula [83]. Most commonly, capsule impaction is symptomless. Sometimes capsule impaction may be a good outcome, leading to identification of strictures, diverticula or ulcers, which can be resected. If the capsule can be reached by an endoscope, a snare with a net is the most useful retrieval tool since the capsule is more difficult to grasp with a stone basket or simple snare. Inadvertent MRI might cause complications as the capsule will respond to a magnetic field. If a capsule examination does not provide colonic images and the patient does not see the capsule in the toilet it might be sensible to take a plain abdominal radiograph to check for passage. Pacemakers are probably of no contraindication. Capsule endoscopy has been approved by the FDA for use in children over 10 years of age.

Double balloon enteroscopy

Double-balloon enteroscopy, sometimes called push-and-pull enteroscopy, is a new method that may allow complete visualization, biopsy, and treatment in the small bowel.

The double-balloon enteroscope features two balloons, one attached to the distal end of the 200-cm endoscope (9 mm in diameter) and the other attached to a transparent tube sliding over the endoscope (Fig. 119.6). The enteroscope

Figure 119.6 Double-balloon enteroscope.

is thinner and shorter than most push enteroscopes. When inflated with air, the balloons can grip sections of the small intestine and shorten the small intestine by pleating it over the endoscope. Sequential shortening of the small intestine over the endoscope onto the overtube and advancement of the endoscope allows the endoscope to be advanced further into the small intestine than push enteroscopes, and may examine up to more than half of the small intestine. If passed through the anus first, as in Japan, or through the mouth first, as in Europe, and then through the other route, usually on another day, it appears to be possible to examine the whole of the small intestine in more than two-thirds of patients.

Yamamoto, whose groups developed this method in Japan with Fujinon, reported that the double-balloon endoscopy system was used to perform 178 enteroscopies (89 by the anterograde approach and 89 by the retrograde approach) in 123 patients [92]. Insertion of the endoscope beyond the ligament of Treitz or ileocecal valve was possible in all 178 procedures. It was possible to observe approximately one-half to two-thirds of the entire small intestine by each approach, and observation of the entire small intestine was possible in 24 (86%) of 28 attempts. The source of bleeding was identified in 50 (76%) of 66 patients with GI bleeding, scrutiny of strictures was possible in 23 patients, and a tumor was examined endoscopically in 17 patients. Two complications (1.1%) occurred. Endoscopic therapies in the small intestine, including hemostasis (12 cases), polypectomy (one case), endoscopic mucosal resection (one case), balloon dilation (six cases), and stent placement (two cases), were performed successfully.

In a recent European series reported by May, 248 consecutive double-balloon enteroscopies (push-and-pull enteroscopies) were performed in a prospective study in 137 patients with suspected small bowel disease, most with chronic GI bleeding (66%) [93]. On average, 240 ± 100 cm of the small bowel was visualized by using the oral route, and 140 ± 90 cm was visualized by using the anal route. The investigation time averaged 73.5 ± 25 min. The overall diagnostic yield was 80% (109/137 patients). The main diagnosis was angiodysplasia (40/109; 37%); erosions and ulcerations of various etiologies were found in 27% (29/109). Polyps and tumors, including malignancy, were identified in 25% (27/109). No relevant pathology was found in 20%. Subsequent treatment was influenced by the results in 104 patients (76%): endoscopic therapy in 57 (41.5%), medical treatment in 23 (17%), and surgery in 24 (17.5%).

Feasibility and diagnostic/therapeutic yield in patients with suspected small bowel disease

It seems clear that double-balloon enteroscopy can reach further than push enteroscopy without balloons. It can allow a variety of therapeutic procedures to be performed as well as biopsy. The accessory channel in most of the enteroscopes used with the double-balloon technique is only 2 mm, which limits therapy, although experience is being reported with prototypes with a larger 2.8-mm channel.

A comparison between wireless capsule endoscopy and double-balloon enteroscopy in 35 patients by Hadithi et al. [94] showed that small bowel abnormalities were detected using wireless capsule endoscopy in 28 (80%) of the 35 patients with obscure gastrointestinal bleeding, compared with 21 (60%) of the 35 patients using double balloon enteroscopy ($P = 0.01$). Both examinations were well tolerated, but capsule endoscopy was more acceptable to patients. No major adverse event occurred after either examination. Biopsies ($n = 27$), argon plasma coagulation ($n = 19$), tattoo injection ($n = 8$), and polypectomy ($n = 2$) were feasible with double-balloon enteroscopy when indicated in 27 of the 35 patients (77%).

The identification of a Meckel diverticulum [95] and endoscopic resection of a duodenal carcinoma in a patient with a Roux-en-Y gastrectomy [96] suggests that this method can reach parts of the small intestine that could not be reached by more conventional endoscopes. Therapy of bleeding from some small bowel sources has been reported using this method [92–94]. Double-balloon enteroscopy has also allowed the removal of capsules caught by strictures in the small intestine [97,98], treatment of strictures [99,100], and resection of Peutz–Jeger hamartomatous polyps throughout the small intestine without laparotomy [101].

There is a price to pay for this type of enteroscopy. It is uncomfortable for patients, can cause ileus [102], and takes much longer to perform than conventional push enteroscopy. The most experienced group admit all their patients for 2 days so that they can enteroscope from the mouth and the anus on consecutive days. There is a recurring cost for the disposable double balloons. Even so, it is likely that double-balloon enteroscopy will come to replace a good deal of push enteroscopy. It seems to be complementary to capsule endoscopy, allowing detailed review of lesions found by capsule endoscopy offering biopsy and therapeutic options.

Clinical management of patients with obscure gastrointestinal bleeding

The most common causes for small bowel investigations are patients with obscure gastrointestinal bleeding and a suspected small intestinal bleeding source. It is assumed that these patients have been investigated with a negative gastroscopy and colonoscopy.

If the review of the gastroscopy and colonoscopy findings suggest that this examination was incomplete or of poor quality, it may be appropriate to repeat upper endoscopy and colonoscopy prior to any other examinations. The clinical history and any other investigations should be reviewed for hints about the possible location of the obscure bleeding. Age, location of any discomfort, length of history, color of blood seen by patient, presence of skin abnormalities, unexplained abnormal blood tests, weight loss, and previous history of malignancy (e.g., melanoma) should be used to focus the search. Investigation pathways can be constructed based on the age and symptoms of the patients.

If a male between 10 and 25 years of age presents with obscure GI bleeding, a Meckel scan should be performed. If positive, surgery should be undertaken without further imaging. Because the scans are frequently negative in such patients, and bleeding from a Meckel diverticulum accounts for bleeding in two-thirds of this group of patients, capsule examination could be the next step to look for this and other causes of bleeding, such as Crohn's disease. If the capsule examination is negative, an early laparoscopy should still be considered.

In all higher age groups, specific questioning about aspirin or NSAID use, site of abdominal pain if any, history of previous treatment for malignancy (e.g., melanoma), duration of bleeding, and time since last bleed should influence the choice and order of investigations.

If the patient is aged between 40 and 65, with a short history of recurrent anemia, continuous bleeding or transfusion requirement, or weight loss and raised inflammatory markers, a more intensive investigational algorithm should be followed with early push enteroscopy or capsule examination. If the patient is a female, consider a gynecological opinion, including pelvic ultrasound and fecal occult blood testing, prior to intensive small intestinal investigation.

If the history is short, or in the case of active bleeding, transfusion requirement, alarming symptoms, or an anxious patient, then capsule examination should be the next examination. Double-balloon enteroscopy should be considered for patients with uncertain abnormalities on capsule enteroscopy or incomplete examinations, for therapy. An algorithm of examinations in these patients is given in Box 119.1.

Conclusion

Video-enteroscopy is changing the management of patients with small intestinal disease. It is of proven value in the diagnosis of patients with obscure GI bleeding. There is a high incidence in most enteroscopic series of lesions being missed or their significance not being understood on initial gastroscopy (Table 119.3). A case could be made for a repeat gastroscopy by an experienced endoscopist before performing push enteroscopy or wireless capsule examination. There is also a well-documented miss rate [103] for colonoscopy of polyps and cancers, which may be due to failure in reaching the cecum (10%–22% in large colonoscopy series) or to incomplete examination on rapid withdrawal of the colonoscope.

In patients with documented malabsorption, push video-enteroscopy with biopsy is sometimes of value in establishing a diagnosis when duodenal biopsies are unhelpful. It is more valuable in assessing compliant patients with celiac disease who have weight loss and diarrhea, when a lymphoma is suspected, and in assessing ulcerating jejunitis.

Push enteroscopy is frequently disappointing in assessing patients with negative barium studies in whom Crohn's disease is suspected [11,14], perhaps because Crohn's disease less commonly affects the proximal jejunum. Wireless capsule endoscopy has been, perhaps surprisingly, good at finding evidence of early Crohn's disease [104–106] in patients with negative colonoscopy and barium studies, and probably is the most important advance in imaging for this disease. It has shown that negative ileoscopy does not exclude the diagnosis of Crohn's disease. The fairly common finding of one or a few aphthous ulcers in the small intestine at capsule endoscopy requires further assessment because not all of these patients have Crohn's disease nor is the ulceration likely to be the cause of chronic abdominal pain in all of them. In patients with chronic abdominal pain, the yield of wireless capsule endoscopy is low but not zero [107]. Wireless capsule studies have been able to visualize ileal tuberculosis [108] and worm infestation, and have helped in the management of small bowel transplantation [109]. It has been suggested that abnormal delays in capsule transit can indicate the presence of small intestinal pathology [110].

Video-enteroscopy has opened up a new world of diagnoses and possibilities to the gastroenterologist. It is a privilege to

Box 119.1 Algorithm for investigation of patients with obscure gastrointestinal bleeding and suspected small intestinal bleeding.[a]

- Review clinical history: If male and under 25 consider following Meckel diverticulum algorithm. Presence of liver disease, the patient's or the endoscopist's report on color of blood seen if any, specific questioning about aspirin or NSAID use, site of abdominal pain if any, history of previous treatment for malignancy (e.g., melanoma), length of patient history, and time since last bleed should influence the choice and order of investigations.
- If aged 40–65 and with a short history of recurrent anemia, or with continuous bleeding, a transfusion requirement or weight loss and raised inflammatory markers, follow more intensive investigational algorithm.
- If premenopausal females, consider gynecological opinion, pelvic ultrasound, and fecal occult blood testing prior to intensive small intestinal investigation.
- Review endoscopy and colonoscopy findings. The finding of a large hiatus hernia, blood in the stomach with no obvious cause, an examination by an inexperienced operator, the suggestion that a colonoscopy was incomplete may suggest that a repeat gastroscopy or colonoscopy by a more experienced operator should be performed prior to push or wireless capsule enteroscopy. If the patient describes bright red rectal bleeding then anoscopy and repeat colonoscopy may be helpful prior to enteroscopy.
- Review ultrasound (US), computed tomography (CT), and technetium bleeding – scanning images if any. If there is weight loss or anxiety about cancer, consider US or contrast enhanced CT. If active bleeding is seen in duodenum but no source found, consider using side-viewing endoscope and/or contrast enhanced CT.
- If blood is seen in the stomach, hiatus hernia is known or suspected, known liver disease or if first endoscopy is performed by a relatively inexperienced person, then investigate with repeat gastroscopy by an experienced endoscopist or a push enteroscopy with careful examination of esophagus and stomach prior to small intestinal examination. If negative consider capsule examination next.
- If short history, active bleeding, transfusion requirement, alarm symptoms, anxious patient then capsule examination should be the next examination.
- If male between 10 and 25 years old, a Meckel scan should be performed. If positive then laparoscopic examination and removal should be undertaken. Because the scans are frequently negative in such patients and bleeding from a Meckel diverticulum accounts for bleeding in two-thirds of this group of patients, early laparoscopy, perhaps following a capsule examination, should be considered.

a It is assumed that these patients have been investigated with a negative gastroscopy and colonoscopy.

see images of small intestinal abnormalities at videoendoscopy, such as an ulcerated Meckel diverticulum or active bleeding from a tumor in the middle of the small intestine, which were not possible until recently. The increasing use of this resource and the comfort and ease with which some of these examinations can be performed make it likely that video imaging will have a substantial impact on the management of small intestinal disease.

In the last few years, enteroscopy has been the most rapidly evolving form of endoscopic imaging, necessitating a complete rewrite of this chapter. It is likely that the pace of evolution will remain rapid in this area.

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A ENDOSCOPIC

120 Colonoscopy and flexible sigmoidoscopy

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Instrumentation

Flexible fiberoptic technology began to be used for visualization of the colon several years after fiberoptic gastroscopy was introduced in 1957. The first commercially available fiber-colonoscope, the Overholt Coloscope, appeared in the early 1960s. Modern colonoscopes, whether fiberoptic or video, give a brilliant, high-resolution, color view of the mucosa through wide-angle (120–140°) optics. The 20–30-fold magnification at 5 mm distance rivals the close-up view of a dissecting microscope, but colonoscopes provide excellent distant views as well. Tip angulation in four directions to 160–180° allows angulation around most acute flexures. Retroflexion (i.e., J configuration) is possible in the proximal colon or rectum, thus minimizing the risk of missing significant lesions in capacious areas that could not be seen with older, less maneuverable instruments. The length of the instruments varies from the 65-cm flexible sigmoidoscope to the 165-cm colonoscope. The instruments range in shaft diameter from 1-cm pediatric colonoscopes to 15-mm therapeutic instruments. Combined instrumentation/suction channel sizes range from 2.8 to 3.8 mm in diameter with some therapeutic colonoscopes having two accessory/suction channels. A colonoscope is similar in design to a gastroscope, but it has a more flexible shaft to accommodate the various colonic bends encountered. Smaller-diameter pediatric colonoscopes or standard gastroscopes, which are more maneuverable in the colons of babies and small children, also have a place in examining ileostomies and fixed or strictured adult colons. Their ability to retrovert in the capacious portions of the colon can also make some difficult polypectomies easier. Zoom colonoscopes have the capability of image magnification to levels similar to a dissecting microscope (150× original). Enhanced maneuverability is possible with a variable-stiffness instrument [1]. High-resolution optical chips afford greater definition of the visual image. Several colonoscopes have an integral pressurized water jet to wash the colon surface, whereas others may be pushed through the colon by an air-lock system [2]. A shape-locking overtube has been developed to prevent excess looping in the sigmoid area [3].

A full range of endoscopic accessories is available for colonoscopes, including biopsy forceps, electrocoagulating hot-biopsy forceps, cytology brushes, washing and spraying catheters, sclerotherapy needles, various sizes of dilating balloons, and a full range of polypectomy snares and retrieving devices. Other standard techniques can be applied, such as the introduction of balloons or tubes over a guide wire and insertion of self-expanding stents for obstructing cancer. Thermal therapeutic devices such as the heater probe, bipolar electrocoagulation, argon plasma coagulator, or laser are as applicable to the lower gastrointestinal tract as they are to the upper gastrointestinal tract. Some endoscopists use an overtube as a splinting or stiffening device to control looping in the sigmoid colon or as a temporary stent to facilitate rapid withdrawal and reinsertion of the colonoscope.

Blind rectal ultrasound probes can assess invasion in tumors of the anorectal area. Direct-vision, ultrasound-bearing colonoscopes may also have a role in tumor staging. Small ultrasound probes may be passed through the instrument channel of the colonoscope to provide ultrasound as desired at any point throughout the colon [4] (e.g., before removal of sessile lesions, to check the relation of the colon wall to surrounding organs before electrocoagulation).

Fluoroscopy is rarely employed for passage of the colonoscope, but a nonradiological computer-generated image of

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the scope position and configuration is available. This technology uses a magnetic field locator to read the force from tiny electromagnets embedded in a dedicated scope (or from a probe carrying electromagnets inserted into the accessory channel) to reconstruct an exact model on a monitor [1].

Anatomical basis of colonoscopy

The ease or difficulty of colonoscopy in a given patient is related to the degree to which the supporting mesenteries fuse to the posterior abdominal wall during fetal development. Although the descending and ascending colon become fixed retroperitoneally, in about 10%–15% of subjects they remain mobile on mesocolons that are usually tethered to the sigmoid and transverse parts of the colon. The consequence for the endoscopist is that these colons, being mobile, can move around freely and unpredictably within the abdomen, largely uncontrollable by the various straightening or rotation maneuvers that are effective with conventional colonic anatomy.

There are no fixed anatomical landmarks between the anus and the ileocecal valve, but there are geographical clues that often are helpful (but may also be misleading) to the endoscopist. At about 17 cm, the colonoscope leaves the capacious rectum and enters the narrower, extraperitoneal part of the sigmoid colon (Fig. 120.1). The sigmoid has frequent circular haustral infoldings – which may be especially exaggerated with diverticular disease – into which the instrument tip impacts if steering is not accurate.

In contrast to the zigzag path of the sigmoid colon, the descending colon is a short, tubular segment. An acute angulation of the instrument tip at the junction of the sigmoid and descending colon may afford a clear view of the tubular descending colon, but forward progress may be impeded by loops in the instrument caused by the tortuous and convoluted sigmoid colon.

The transverse colon (Fig. 120.2) frequently shows a triangular configuration as a result of its three longitudinal muscles, or taeniae coli, that act as limiting boundaries to air distention. The liver is a fairly predictable landmark with the flat, bluish-gray surface and sharply defined edges caused by the balloon-like, air-filled colon contacting the hepatic surface (Fig. 120.3).

On entering the ascending colon, the superior lip of the ileocecal valve may appear in the distance as a short, flat segment on the most proximal circular haustral fold (Fig. 120.4). The upper lip may have a notched bulge or may appear thickened by fat accumulation. Most often the opening into the ileum is slit-like on the inferior side of the fold invisible to the endoscopist advancing toward the cecum. The appendiceal orifice, a less definite landmark, is seen as a crescent-shaped slit or a circular convolution of folds at the blind saccular cecal pole (Fig. 120.5). Multiple, pale-reddish, 1–2-mm circular

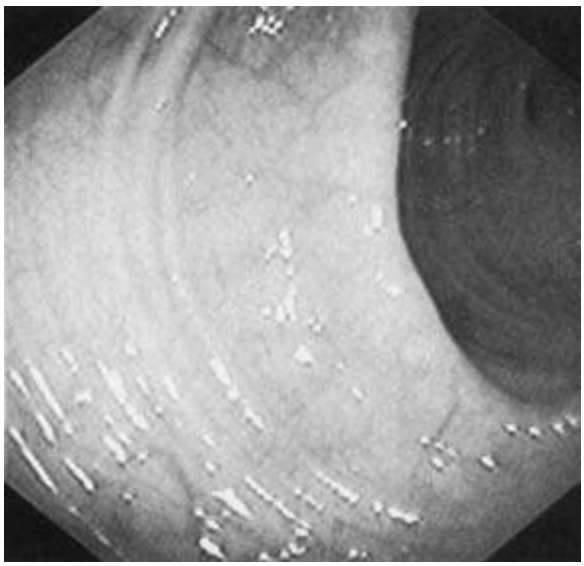

Figure 120.1 In the sigmoid colon, the circular outline with the light reflex over arcs of circular musculature clearly indicates the center of the lumen for close-up endoscopic steering purposes.

Figure 120.2 Transverse colon shows the characteristic triangular outline resulting from the relative thickness of the three longitudinal taeniae coli. The circular muscle is much thinner.

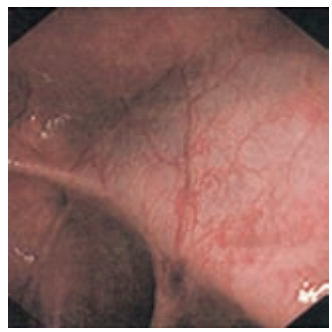

Figure 120.3 Hepatic flexure shows the dark impression of the liver. Similar discoloration can be caused by other extracolonic viscera at the splenic flexure or in the distal colon. Note the longitudinal impression of a taenia and the transverse haustral folds.

Figure 120.4 Ileocecal fold shows the characteristic notch on a flattened fold. The notch denotes the superior lip of the ileocecal valve. The opening is just proximal to and below the notch, and cannot be seen without marked angulation of the instrument tip.

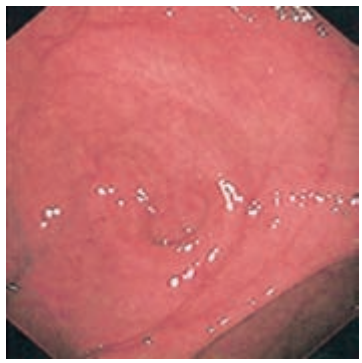

Figure 120.5 The appendix orifice is variable in configuration. Here, the orifice is round, but it may also be seen as a crescent-shaped slit. It is usually rather insignificant in appearance. The internal aspect remains the same after appendectomy.

halos are often seen in proximity to the appendix when using the videoendoscope. These represent lymphoid follicles.

The ileal mucosa has a granular appearance when the ileum is air-filled, and short frond-like villi are visible in pools of fluid. The granular appearance is caused by multiple light reflections from the individual villi. Nodules of lymphoid tissue measuring 1–3 mm, and sometimes aggregated into 10–15-mm plaques of Peyer patches, are normal in the terminal ileum, and particularly prominent in children. By contrast, the normal colorectal mucosa is smooth and shiny, although with deflation, fine transverse innominate grooves may show, especially with surface dye spray.

Throughout the colon, a fine interlacing vascular pattern is visible through the transparent overlying epithelium. When the normal vascular pattern is hidden by mucosal congestion, visible as diffuse redness of the surface, a pathological process must be considered, but biopsies are needed to differentiate between inflammatory change and traumatic or reactive hyperemia. Opacity of the surface can also be caused by

Figure 120.6 Retroversion of the endoscope in the rectum is a useful maneuver to find or remove small polyps in the distal part of the rectal ampulla. The dentate (pectinate) line is serpiginous at the junction of the squamous mucosa of the anal canal and the columnar rectal mucosa.

thickening that occurs after chronic colitis or by edema. In the rectum, the vascular pattern is more pronounced, with larger vessels. Hemorrhoidal veins may appear wide and tortuous. A U-turn (i.e., retroflexion) maneuver in the rectum should always be attempted to afford a view of the rectal ampulla, the dentate line (Fig. 120.6), and internal hemorrhoids [5], but the rigid anoscope gives a superior view of this area by dilating it maximally.

Localization during colonoscopy

The position of the colonoscope can be judged approximately by a combination of factors, principally the length of instrument inserted and the characteristic appearances of certain areas, including the sharply defined blue color of the liver, the fluid-filled splenic flexure area, and the triangular air-filled transverse colon. Most instruments are bright enough to transilluminate the abdominal wall where the bowel approximates to the surface at the splenic flexure (left midaxillary line), transverse colon, or cecum.

The transabdominal impression of the endoscopist's palpating fingers may often be seen from within. Transillumination or endoscopically visible indentation by finger palpation in the right iliac fossa, however, does not necessarily indicate that the tip of the instrument is located in the cecum, because the instrument tip may stretch almost any portion of the colon into the right lower quadrant during colonoscopic intubation. For instance, mobile portions of the bowel, such as the sigmoid or the midpoint of the transverse colon, can be pushed toward the right iliac fossa.

All localizing judgments that depend on landmarks or palpation can be mistaken. For instance, if using the length of scope inserted, in a long or mobile colon, 80 cm of shaft may be looped in the proximal sigmoid, whereas in the same patient during withdrawal, the instrument may be straightened so that the splenic flexure is 40 cm from the anus, and the hepatic flexure (or even the cecum) can be reached with

50–60 cm of instrument. The only definitive landmarks are the appendiceal orifice, the ileocecal valve, and the ileum seen through the ileocecal valve, but even this may be difficult to locate, and the valve can be effaced after severe or long-standing inflammatory disease.

Because of the absence of absolute landmarks throughout the colon, even if the endoscopist has reported success in total colonoscopy with identification of the ileocecal valve and cecal pole, there is still the possibility of being grossly mistaken about the location of the instrument tip and the anatomical localization of pathological findings. Experienced endoscopists can occasionally mistake the splenic flexure for the hepatic flexure, or the hepatic flexure for the cecum, and thereby mislead the surgeon as to the site of an obstructing tumor [6]. In spite of this, fluoroscopic guidance is rarely used, but some find it helpful in the endoscopist's learning phase and in a few difficult cases. The magnetic imager [1] is a localizing device whereby small electromagnets are inserted into the colonoscope shaft, either during manufacture or embedded in a catheter-type wand. External sensors can detect the magnetic energy and plot a computer image of the shape and location of the endoscope. There is no radiation, and the computer-generated image is similar to that obtained fluoroscopically.

Any lesion seen by colonoscopy can be marked with an intramural deposit of carbon particles using sterilized India ink or a pure carbon suspension, injected through a long, flexible, injector needle; these techniques enable subsequent surgical localization or enable the endoscopist to find the area on a subsequent examination [7]. A gray-blue submucosal stain of carbon particles permanently remains at the injection site. Vital dyes, such as methylene blue, indocyanine green, or indigo carmine, are rapidly absorbed, disappearing within hours or days. It is possible to mark a lesion or the proximal extent of tip progress with metal clips [8], but this technique is not often clinically useful.

Bowel preparation

Total colonoscopy is rarely possible in the unprepared colon. Patients with profuse watery diarrhea are sometimes mistakenly thought not to require preparation and are often found to have an opaque mucosal coating of fecal material that obscures adequate view of the surface. Even patients with a surgically defunctionalized bowel produce inspissated mucoid residue and require cleansing. A patient with massive colonic bleeding may effectively cleanse the colon of fecal material, but often a residual coating of blood obscures mucosal events during the colonoscopic examination.

Limited preparation

When it is necessary to examine only the distal colon segments, an adequate preparation can consist of one or two evacuant enemas of tapwater, saline, or, more usually, a small-volume hypertonic phosphate or other proprietary enema, given 15–60 min before the examination begins. This allows enough time for proper bowel clearance but is not so long that proximal contents move distally. Limited bowel preparation presumes normal colonic ability to empty the whole left colon rapidly by mass action. This may not happen in patients with diverticular disease or strictures, for whom even limited examination must usually be preceded by full bowel preparation. A limited preparation does not remove potentially explosive gases from the proximal colon, and distal electrosurgery should not be instituted unless these gases have been removed by purge or by air exchange.

Full preparation

A wide variety of bowel preparations are available [9]. Ideal attributes for bowel preparation include pleasant taste and avoidance of excessive volume, cramps, or the need for enemas. Certain modifications of medication and diet are necessary. Oral iron medications must be stopped 4–5 days before colonoscopy, because the formation of organic iron tannates in combination with dietary green-vegetable residue renders colon contents black, sticky, and difficult to clear. Constipating agents such as codeine or loperamide should be discontinued 12 h before colonoscopy. It has been suggested that aspirin and other antiplatelet drugs or anticoagulants be discontinued 7 days before examination to reduce the risk of immediate or delayed bleeding should polypectomy be performed, but this may not be necessary because clinical experience has not borne out any excess bleeding risk in patients on aspirin as noted in a guideline prepared by the American Society for Gastrointestinal Endoscopy [10]. No matter what the preparation regimen, colon cleansing is enhanced by a clear or full-liquid diet for 24–48 h before the examination.

Purgative regimens

Purgative regimens are still the most commonly used form of bowel preparation. Their advantage is that the colon, after a contact laxative such as senna, or bisacodyl, is stimulated to evacuate most of the bowel contents. Disadvantages are that the evacuation stimulus can cause severe cramping abdominal pain, that the cecal region is often poorly cleared, and that some solid residue often remains distally. Therefore, enemas or oral osmotic agents may be required to supplement the cleaning effect of the purgative. Contemporary bowel cleansing methods do not require enemas.

Osmotic purges can be used as an alternative to enemas, either adding to or supplementing the oral purgative with up to 2 L of an agent such as magnesium salts (e.g., citrate, sulfate) or a nonabsorbed carbohydrate (e.g., mannitol, lactulose, sorbitol). Magnesium sulfate has strong purgative and osmotic effects but also an unpleasant taste, so that the milder but citrus-flavored magnesium citrate is generally preferred. Mannitol has been the most widely used of the carbohydrate

solutions. Its sweet taste can be reduced by cooling, and the likelihood of nausea is prevented by preadministration of an oral antiemetic prokinetic agent such as domperidone or metoclopramide 30 min before administration. Five-percent mannitol is isotonic, and 2 L is drunk slowly over 1 h or more. The use of mannitol or other carbohydrate preparations risks explosion during electrosurgical procedures, because of the production of hydrogen gas by the fermentation of intestinal bacteria. This risk can be effectively eliminated by insufflation of carbon dioxide during the procedure or by meticulously aspirating gas and reinsufflating air before electrosurgery. A low-volume sodium phosphate preparation is commonly used for endoscopy preparation and has been well received by patients [9]. The evening before and 4 h before colonoscopy, 45-mL aliquots of sodium phosphate taken orally produce a vigorous catharsis. Cleansing enemas are not required. Fluid balance shifts, absorption of sodium, or hyperphosphatemia may cause deleterious effects, especially in the presence of cardiac or renal disease. The noxious taste may be circumvented by dehydrated sodium phosphate in pill form. Studies published in 2005 [11] implicated the precipitation of phosphate crystals in the renal tubules as a cause of chronic renal insufficiency. This may be related to relative dehydration from inadequate fluid intake during the preparation phase, and the water loss from the powerful cathartic effect.

Balanced electrolyte solutions based on polyethylene glycol (PEG) as an osmotic agent, administered in a volume of 4 L orally or by nasal tube; result in an acceptable bowel preparation. The salty, oily taste and required large volume cause about 10% of patients to stop drinking the solution, thereby failing to prepare their colon adequately. Newer formulations with flavoring have improved palatability and may improve patient compliance. Although package instructions advise minimal dietary restrictions, better results can be expected from a 24-h full-liquid diet. Simple saline solution (0.9% sodium chloride) is effective and arguably safe for preparation of the majority of patients, but potassium losses and electrolyte fluxes can be avoided by appropriate additional ingredients. Other cathartic regimens have been introduced that require ingestion of only 2 L of PEG solution with adequate bowel cleansing [12].

The regimen chosen for whole-colon preparation necessarily varies according to circumstance, patient preference, and clinical indication. Patients need to sleep and must be able to travel without fear of incontinence, so that the timing of preparation must also be adjusted according to the time of examination and travel arrangements. Frail, ill, and elderly patients may require modified preparation, sometimes on an inpatient basis if cooperation is uncertain. If obstruction is a possibility, oral preparations may be dangerous or at least should be spread out over a longer period. In almost all circumstances, a patient fit for total colonoscopy is fit for full-bowel preparation. Compromise in reducing the regimen may result in poor preparation and in only being able to perform a limited examination or a less accurate colonoscopy.

Whichever of the different purgative regimens is chosen, it must cause profuse, clear, fluid diarrhea. A more vigorous preparation may be required for the patient with chronic constipation or severe diverticular disease or a patient whose bowel was not properly cleansed with a previous regimen. It is desirable that patients continue to drink fluids up to a few hours before the examination.

Medication

Sedation

Sedation is unnecessary for some examinations, especially those that are limited, or in patients after sigmoid colon resection or in whom a previous procedure is known to have been easy. A great deal also depends on the patient's attitude and on local custom. Endoscopists in some countries rarely sedate, whereas those in other countries almost always do. Some routinely use general anesthesia administered by an anesthesiologist or, more rarely, by the endoscopist [13,14], although this may add to the risk of complications by removing feedback from the patient relating to pain from loops or difficult electrosurgical procedures. Using less sedation makes for easier change of position and greater safety. Using no sedation allows the patient to leave rapidly and unescorted after the procedure. Endoscopists who never use sedation may have a lower success rate in total colonoscopy [14,15].

Some stretching of peritoneal attachments, at least transiently, is inevitable during colonoscopy. This causes gnawing or acute unpleasant visceral pain. Coupled with air distention and the sensation of rectal fullness simulating the desire to defecate, this can make a slow examination difficult to bear for some patients who are mildly or not sedated, although others tolerate it easily. Pain during colonoscopy can usually be rapidly diminished or eliminated by reducing the loops responsible for stretching the mesentery, or by aspirating air, but sometimes further doses of analgesic agents are needed. Normal diet, activities, and medication can usually be resumed as soon as the mild stress of the examination and the effects of medication are over.

Antispasmodics

Antispasmodics (glucagon 0.5–1 mg i.v.; hyoscine-*N*-butyl bromide 20–40 mg i.v.) are thought by some to increase the difficulty of insertion by rendering the colon atonic and therefore more likely to stretch and loop, but they eradicate circular muscle spasm and may increase the accuracy of examinations for small lesions such as polyps or angiodysplasias [16]. Some endoscopists use antispasmodics routinely, and others not at all.

Antibiotics

A prosthetic heart valve, history of endocarditis, surgically constructed or congenital systemic–pulmonary shunt, or synthetic

vascular graft less than 1 year old places a person at high risk for infection with various procedures. Even in these persons, however, there are insufficient data strongly to justify antibiotic prophylaxis for colonoscopy with or without biopsy or polypectomy [17,18]. Antibiotics are not recommended for colonoscopy with or without biopsy, polypectomy, or esophageal variceal ligation in patients with cirrhosis and ascites unless they present with an acute bleeding episode. Nor are antibiotics recommended for immunocompromised patients, those with prosthetic joints or pacemakers, or patients with a history of rheumatic valvular dysfunction, mitral valve prolapse with insufficiency, hypertrophic cardiomyopathy, or for most congenital cardiac malfunctions. If antibiotics are deemed necessary, the recommended dose is amoxicillin 2.0 g p.o. 1 h before the procedure, or ampicillin 2.0 g i.v. or i.m. within 30 min before the procedure.

Colonoscopy procedure

Position and position changes

The left lateral position is most commonly used for flexible sigmoidoscopy and colonoscopy, mainly because it gives convenient access to the perineum for insertion. However, colonoscopic examinations can be, and in some centers are, performed in other positions, including lithotomy, knee-chest/elbow, and right lateral positions. Especially if a large-volume fluid preparation regimen has been used, position changes can modify both the configuration of the colon and of the air and fluid within it because of the effects of gravity. When forward progress can no longer be accomplished, a change in the patient's position will aid tip advancement in two-thirds of cases [19].

Insertion of the endoscope and flexible sigmoidoscope

Digital examination of the anorectum is used to lubricate the area generously and to palpate any lesion. A local anesthetic gel may reduce anal discomfort. When there is rectal stenosis, a fistula, or abnormal anal sensitivity, it may be desirable to use a small-diameter pediatric instrument or a gastroscope. The technique for flexible sigmoidoscopy is the same as for the first part of a colonoscopy, except that it is less important to avoid looping, because the proximal colon is not to be intubated. Characteristically, there is a red-out when the instrument enters the rectum, because the tip abuts on the rectal mucosa. Simultaneous air insufflation and instrument withdrawal are required to disimpact it, and angulation or rotational movements are useful to find the rectal lumen. Movements of the instrument tip should thereafter be slow and deliberate, taking care to steer toward the lumen before pushing inward, so as to avoid constantly losing the view. When the lumen cannot be seen, continuing to push the instrument shaft into the rectum stretches the sigmoid colon into unnecessary and painful loops and rarely results in tip advancement, because once impacted against a haustral fold the tip tends to remain stationary while the rest of the scope flexes into a loop with its concomitant traction on the mesentery.

Slide-by is a term describing the maneuver when the tip is against the mucosal surface, no part of the lumen can be seen, and the instrument is pushed forward causing the mucosal vessel pattern visibly to slide across the lens. This maneuver should be avoided but is permissible for short distances and only when the instrument is known to be pointing in the correct direction; if tip movement stops and the mucosa blanches, additional pressure risks perforation, and the instrument must be withdrawn to locate the lumen. Withdrawal and disimpaction of the tip is a routine procedure that should be performed every time the view is lost for more than a few seconds.

Diverticular disease is often associated with thickened rings of circular muscle or fixation of the sigmoid colon by pericolic adhesions from previous inflammatory episodes. The resulting distortion and angulations can make it difficult to locate the lumen and coax the instrument through, because the tip is not free to maneuver normally. In a few cases, the angulations are so acute that a small-diameter pediatric colonoscope or a standard gastroscope with their shorter radius of tip deflection may allow passage through diverticular disease where the standard colonoscope cannot be passed [20]. Despite the technical problems in intubation, endoscopic examination of diverticular disease can be clinically valuable, mainly because the distorted appearances seen on barium enema may make it impossible to exclude malignancy on radiological grounds. Similar bowel fixation and angulation may occur after hysterectomy as a result of postoperative adhesions between the sigmoid colon and anterior aspect of the pelvic cavity.

If any pathology is seen during flexible sigmoidoscopy, a biopsy can be easily and safely performed. However, electrosurgery for polyp removal is contraindicated after the limited bowel preparation for flexible sigmoidoscopy because of the significant hazard of explosive gas mixtures.

Total colonoscopy

The insertion technique is fundamental to rapid, relatively pain-free, and accurate total colonoscopy. Difficulty during intubation of the proximal colon or passage around the hepatic flexure is invariably related to failure to straighten the colonoscope in the distal colon before attempting the last part of the insertion. Looping in the sigmoid colon is a continual and recurrent problem throughout a colonoscopy but especially when it is necessary to push harder to transmit force around a looped colonoscope to reach the cecum.

During a colonoscopy, there are only a limited number of options available to the endoscopist. These include:

- pushing the instrument in or pulling it out
- twisting the shaft to the right or the left

- insufflation or withdrawal of air
- using tip controls to steer up, down, right, or left
- use of abdominal pressure to reduce loops
- changes of patient position.

The difference between the slow endoscopist who reaches the cecum infrequently after a traumatic examination and a fast endoscopist who examines the whole colon in over 95% of patients within an average time of 10 min is the ability to use the various options logically, quickly, and sequentially without repeating a nonproductive maneuver over and over again.

During the intubation phase, the instrument controls must be continuously manipulated while using torque to keep an adequate view of the lumen. The endoscopist must concentrate on visual detail to locate and find the direction when it is briefly lost, and back off quickly if the tip impacts into a bend or fold. The endoscopist can feel when the instrument is passing freely and can be advanced or when it is becoming snarled and should be withdrawn to straighten the shaft before pushing in again. The biggest mistake of the inexperienced endoscopist is to think that a good view of the lumen is an unqualified signal to push, especially when looped in the sigmoid with a view of the descending colon; pushing under these conditions often results in an expanding sigmoid loop with resultant pain but without tip progress.

Some sigmoid loops, such as the spiral alpha loop configuration that may form in the sigmoid colon, are favorable, because they allow the instrument to proceed into the descending colon without forming acute angulations; others, such as the N loop, which more frequently occurs in the sigmoid, put the tip into an acute angulation at the descending colon junction, making this area difficult and painful to pass through. Fluoroscopy is not necessary to ascertain the presence of a loop, because it is apparent that a loop is forming whenever the shaft is pushed in more than the tip advances. Additionally, once a loop is stretched on its mesentery, additional pushing hurts the patient. Conversely, when the colonoscope shaft is straight, with the colon shortened or pleated over it rather than stretched, only a light touch is needed on the instrument with 1:1 correspondence between shaft and tip during push–pull and rotational movements. When there are no excessive loops, tip angling using the dial controls is also easy and full. Conversely, when the tip is maximally deflected using both control knobs and the lumen is still at an acute angle, this indicates that the scope is in a loop configuration. The best maneuver is to withdraw and straighten the shaft, and if that cannot be accomplished, short in-and-out thrusts of the scope often result in forward tip movement.

Because of the multiple bends, folds, and twists of the large bowel, it is inevitable during a colonoscopy that the instrument will loop or start to loop on many occasions. After each advance, by pulling back and restraightening the shaft, the colon is effectively shortened and pleated over the instrument, which is progressively advanced on a "three-steps-forward-and-two-steps-back" basis.

It is usually not possible to keep the instrument straight or the patient completely comfortable all the time because of the unpredictability of the sigmoid colon. There may be a period of difficulty or looping before the tip reaches the fixed retroperitoneal part of the descending colon or the splenic flexure, where the shaft can be fully straightened by pulling back with clockwise rotation to only 50 cm from the anus. Unfortunately, because of the variability of the anatomy and attachments of the sigmoid and descending colon, this first part of a colonoscopy is the most difficult part of the procedure.

Passing the splenic flexure into the transverse colon is usually easy if the sigmoid has been straightened and can be held straight. After maximal shaft withdrawal, the combination of gentle shaft advancement with clockwise twist maintained, simultaneous abdominal pressure over the sigmoid, and avoidance of tip overdeflection usually allows progress into the transverse colon. Overaggressive pushing tends to reform the loop in the sigmoid, and overangulation impacts the tip into the flexure (i.e., the inverted "J" or "walking stick handle" effect). If, after several attempts, the tip does not advance, the instrument should be withdrawn and restraightened; rotating the patient into the right lateral position almost invariably causes the splenic flexure to drop downward, allowing the instrument to slide more easily around to the midtransverse colon. With the patient in the right lateral position, the midtransverse colon often sags down into a rather acute bend, providing a good point at which to withdraw and restraighten the colonoscope once again and also to rotate the patient onto the back or into the left lateral position before insertion into the proximal colon.

In the proximal transverse colon or hepatic flexure region, and thereafter in the even more capacious ascending colon, deflation is extremely helpful in advancing the instrument and shortening the distance to the ileocecal region. Aspiration of air causes the colon to deflate concentrically so that the diameter is smaller, but it also results in shortening the colon longitudinally. A few additional maneuvers may be helpful in assisting total intubation, such as rolling the patient into the prone or supine position or asking the patient to inspire to lower the diaphragm. The more difficult it is to reach up or around the hepatic flexure and down to the cecal pole, the less likely it is that aggressive forward pushing will work. The longer and more mobile the colon is, the more certain it is that force will generate loops that will absorb all the motive power applied. With attention to technique and patience, even a redundant colon often shortens to only 50–60 cm at the hepatic flexure, permitting the tip to slip down the last few centimeters with ease as air is aspirated. Advances in technology enable electromagnetic imaging devices to visualize, in real time, the three-dimensional localization of the colonoscope without radiographic involvement, which may have a significant impact on practice [1].

Ileocecal region

Even experienced endoscopists can be mistaken in thinking that a capacious hepatic flexure is the cecal pole, or that tonic contraction of the ileocecal fold represents the appendiceal orifice. The appendiceal orifice is seen often merely as a crescent-shaped slit or a circular convolution of folds. When a crescent orifice is seen, the apex of the crescent almost invariably points toward the ileocecal valve. With the tip in the cecum and the crescentic orifice clearly visible, deflection of the tip in the direction indicated by the apex of the curve will align the scope with the ileocecal valve orifice. Withdrawal of the shaft and deflection of the up/down control dial in the direction of the appendix curve when the first large fold is encountered will often result in ileal intubation. Alternatively, withdrawing the colonoscope 10 cm or so back up the ascending colon and looking for the tell-tale inward bulge of the valve on the ileocecal fold, which is the first encircling fold back from the cecal pole, is another method to permit the endoscopist to determine where to angulate and enter the ileum following reintubation of the cecum. Even so, ileal intubation can be difficult and may require several minutes and not a little skill and patience. The anatomy is variable, and both colon and colonoscope can rotate or move during attempted insertion. Some ileocecal valves show prominent pouting lips; others, however, are the merest slit on the reverse side of the ileocecal fold, which may require visualization by attempting retroversion of the bending section of the colonoscope within the cecal pole.

Terminal ileum

A skilled endoscopist can enter the terminal ileum with reasonable ease in approximately 80% of cases [21] and can almost invariably obtain a blind forceps biopsy through the valve in the remaining patients. Failures arise mainly when deformity or scarring make angulation impossible or the opening too narrow, but poor bowel preparation and loops in the instrument can be contributory problems. Endoscopic assessment of the mucosa of the terminal ileum is straightforward. Although the polypoid lymphoid follicles that are frequently present may cause the radiologist mistakenly to diagnose Crohn's disease and consider cobblestoning, these nodules and the surrounding mucosa are covered with normal pink mucosa with a granular surface in air or with small villi visible underwater.

The endoscopic view of the terminal ileum is usually limited to 5–10 cm. There is rarely a serious indication to attempt further insertion, because ileal Crohn's disease almost invariably involves the terminal part of the ileum, other lesions such as ileal angiodysplasia are exceedingly rare, and a Meckel diverticulum is not endoscopically accessible because it is located 1 m proximally. If deep ileal intubation is attempted, 30–50 cm is usually the limit of what can be seen because of the acute angulations that occur in the small bowel.

Role of the assistant

Colonoscopy must not be performed by one person alone. A properly trained and observant gastrointestinal assistant is an absolute necessity for the proper performance of colonoscopy. The assistant's functions extend beyond the confines of the endoscopic procedures itself to include:

- setting up the room before the procedure
- recording endoscopic photographs
- noting biopsy specimens
- filling out requisition forms for the histopathologist.

All equipment that may possibly be necessary for the procedure must be assembled and available before the examination. The endoscopic assistant monitors the patient and assists with accessories. If polyps are encountered, the snare and patient return-plate must be plugged into the electrocautery apparatus, which requires setting the proper diathermy levels and testing to ensure that all equipment is functioning properly. During the examination, additional lubricant, gloves, and abdominal pressure may be provided from time to time during various phases. The assistant orders and prepares the necessary preendoscopic medications and is responsible for maintenance of the emergency cart, which must include antidotes for the various medications used, cardiac drugs, resuscitation equipment such as an Ambubag, endotracheal tubes and laryngoscopes, intravenous solutions, and oxygen. The cleaning, care, and maintenance of all the endoscopy equipment is in the hands of the assistant, who is responsible for mechanical cleaning and disinfection procedures. Most colonoscopists prefer to advance and withdraw the shaft of the instrument with the right hand, while the left hand manipulates the dial controls as well as the air/water and suction buttons. However, some colonoscopists prefer a two-person technique, whereby the assistant advances the instrument while the endoscopist handles the control portion of the colonoscope.

Therapeutic procedures

Colonoscopy makes possible a range of therapeutic procedures. Polypectomy is the most obvious, but electrocoagulation, laser photocoagulation, or injection therapy of vascular lesions (e.g., telangiectasias, angiodysplasias, hemangiomas, and radiation changes) are all routine [22]. The argon plasma coagulator is a monopolar device that delivers thermal energy to the colon wall in a noncontact fashion. It is useful for arteriovenous malformations, radiation telangiectasias, and fulguration of residual adenomatous tissue at the base of piecemeal polypectomy sites [23].

Snare polypectomy of pedunculated or sessile polyps is efficient, rapid, and relatively safe. By constricting the tissue within the closed snare wire, low electrical power (often only 15–20 W) is sufficient for transection and heat sealing of

blood vessels, with localized tissue heating. Because the colon mucosa is insensitive to thermal injury, the patient should feel nothing during polypectomy or electrocoagulation unless full-thickness heating is occurring, which causes the patient to warn of peritoneal pain at an early stage, before actual damage has occurred. If small (2–5-mm) polyps are found, simultaneous biopsy and electrocoagulation with electrically insulated hot-biopsy forceps can be performed. This rapid procedure ensures that a histological specimen is obtained. An alternative technique for small, sessile polyps is cold resection, or cheese-wiring across the base by closing the snare in the absence of current [24]. Snared tissue can be retrieved for pathological analysis by aspiration through the instrument channel into a filtered suction-trap or into a gauze tissue placed at the connection of the suction tubing with the endoscopic umbilical cord. Larger polyps can be retrieved whole using the snare loop or other grasping devices, although this may necessitate several reinsertions of the endoscope for multiple polyps. A metal basket or nylon-mesh net allows retrieval of several polyps or fragments from the right colon.

Except for polyps with an extremely broad base, almost all benign-appearing polyps encountered can be removed endoscopically. Pedunculated polyps are removed with snare application around the pedicle. Pure coagulating current at low power is used by most endoscopists following snare-loop tightening, providing controlled and localized tissue heating for blood vessel obliteration [25]. Thicker stalks with increased risk of immediate or delayed bleeding can be preinjected with an epinephrine (adrenaline) solution to ensure hemostasis before snaring. Both flat and broad-stalked polyps with early invasive colon carcinoma may be successfully resected by the submucosal injection polypectomy (SIP) technique, whereby the polyp is elevated by a fluid injection (3–10 mL [but possibly up to 40 mL] of saline or diluted epinephrine solution) into the submucosa prior to transection [26]. Various solutions have been used to prolong the duration of the submucosal cushion including a viscous solution of sodium hyaluronate [27] and the proprietary "artificial tears" whose active ingredient is hydroxyl-propyl-methylcellulose. If the polyp is exceptionally large and snare placement is difficult, it may be useful to remove segments sequentially until the base can be easily visualized and complete transection can be accomplished. Even extremely large pedunculated polyps may be removed in piecemeal fashion. Polyps that usually cannot be removed endoscopically are those that cover a substantial area of the colonic surface (i.e., > 30% of the circumference), extend over two interhaustral folds, are obviously malignant, or involve the appendiceal orifice [28].

Every effort should be made to retrieve all the resected portions of each polyp and to submit them to the pathologist for histological evaluation. Because of the force of gravity, polyps have a tendency to fall into fluid pools or crevices, which hide them from view making retrieval difficult. There are various techniques for polyp retrieval. Although in clinical circumstances, only about 94% of transected colon polyps are recovered for full histopathological interpretation, those most likely to be lost are small and of little clinical significance.

Prior to the introduction of neostigmine therapy, tube decompression in patients with pseudoobstruction (Ogilvie syndrome) or postoperative ileus was accomplished by placing a tube through the colonoscope channel, sliding it over an endoscopically placed guide wire [29] or by carrying the tube up alongside the endoscope during insertion. Immediate decompression occurs, but because of the frequency of recidivism, the tube is left until it is expelled spontaneously, signaling restoration of normal propulsive contractions. Volvulus can be reduced by placing a wide-caliber tube beyond the twisted segment, provided the colon is not gangrenous – in which case surgery is mandatory. Foreign bodies can be successfully removed endoscopically from all levels of the large bowel. Balloon dilation is possible, usually with through-the-scope (TTS) balloons inserted under direct vision into a stricture, with or without prior guide wire placement [30]. The controlled radial expansion (CRE) balloons exert maximal dilating force over a range of sizes according to the degree of pressure applied. An appropriate-sized balloon gives sufficient dilation for most circumstances with minimal risk of splitting the stricture. These balloons have the advantage of allowing passage over an endoscopically positioned guide wire, with correct insertion and dilation observed either endoscopically or on fluoroscopy. Other techniques for stricture dilation include incision into the stricture with electrocoagulation by a needle-knife, the slightly protruded tip of a polypectomy snare, or a papillotome; these methods are, however, useful only for relatively thin, short strictures.

Short strictures, such as those after right hemicolectomy for Crohn's disease or when postoperative complications result in localized anastomotic narrowing, respond well to endoscopic dilation. A single dilation can be permanently effective or can last for a few weeks to many months depending on the presence of any inflammatory condition likely to cause restenosis [28]. Postoperative anastomotic stenoses may be dilated widely during the first examination, because there is usually considerable fibrosis around the anastomotic site, making the occurrence of free perforation unlikely. Primary strictures in inflammatory bowel disease unrelated to surgery, however, do not have the same surrounding cicatricial reaction, and the initial dilation should be limited to not greater than twice the diameter of the stricture to avoid perforation. Frequently, the lumenal narrowing in Crohn's disease is related to active inflammation with circular muscle spasm and so is rarely ameliorated by attempts at endoscopic dilation techniques. Self-expanding metal stents effectively dilate malignant strictures, and are usually permanently

embedded into the tissue once inserted. They may be used for decompression prior to surgery or for symptom palliation. New removable plastic stents have been developed for esophageal lesions [31], and may be adapted to benign colon strictures.

Indications

Flexible sigmoidoscopy

Flexible sigmoidoscopy should be the procedure of first choice for examination of the rectum and sigmoid colon. It is useful for evaluating complaints most probably caused by conditions affecting the lower bowel, and for population-based screening for colon and rectal cancer. It should replace rigid proctosigmoidoscopy except when the rigid instrument is used for screening examinations of the rectum on grounds of cost, expediency, and the ease of taking larger biopsy specimens. The rigid proctoscope (i.e., anoscope) is preferred for assessment of hemorrhoids and anal canal pathology, which are often poorly seen with a flexible instrument, even in retroversion.

Flexible sigmoidoscopy alone may be considered sufficient examination in patients with minor disturbance of bowel habit, obvious hemorrhoidal bleeding such as blood dripping into the toilet bowl following defecation, or localized left iliac fossa pain. With minimal discomfort and no sedation, the rectum, sigmoid colon, and often the descending colon or splenic flexure can be examined.

Colonoscopy

Colonoscopy can be performed in any patient fit for barium enema who can tolerate a full-colon preparation (Table 120.1) [32]. With the increasingly widespread availability of endoscopic skills and improvements in instrument technology, colonoscopy can often be regarded as a first-line procedure not proceeded by radiological imaging. A prior computed tomography (CT) scan or barium enema rarely helps the endoscopist, because the configuration on radiographic films gives little indication of how easy or difficult the colonoscopy will be. Most patients find radiological imaging a more unpleasant experience than colonoscopy because of the sustained inflation required in the radiological procedure [33] and perhaps also because of the sedative/analgesic medications often given for colonoscopy.

High-yield indications

Abnormal CT scan

The highest diagnostic yield of endoscopic pathology is in patients with tumor, polyps, stricture, or mucosal disease diagnosed on radiological imaging. Colonoscopy provides histological confirmation or confident exclusion of a questionable abnormality and allows immediate therapy where appropriate.

Table 120.1 Indications for colonoscopy

Evaluation of abnormality on barium enema likely to be clinically significant, such as a filling defect or a stricture
Evaluation of unexplained gastrointestinal bleeding (including occult bleeding)
Unexplained iron deficiency anemia
Screening and surveillance for colonic neoplasia
Chronic inflammatory bowel disease of the colon if more precise diagnosis or determination of the extent or activity of disease will influence immediate management
Clinically significant chronic diarrhea of unexplained origin
Intraoperative identification of a lesion not apparent at surgery (e.g., polypectomy site, location of a bleeding site)
Treatment of bleeding from such lesions as vascular malformation, ulceration, neoplasia, and polypectomy site
Foreign body removal
Excision of colonic polyp
Decompression of acute nontoxic megacolon or sigmoid volvulus
Balloon dilation of stenotic lesions (e.g., anastomotic strictures)
Palliative treatment of stenosing or bleeding neoplasms
Marking of a neoplasm for surgical localization

Modified from American Society for Gastrointestinal Endoscopy [32].

Colon polyps: benign and malignant

Nearly all polyps can be snare-resected during the procedure, and some radiologically diagnosed carcinomas prove to be benign lesions that are manageable endoscopically. Even with an apple-core lesion on radiographic films, colonoscopy can confirm the histology of the lesion and in the absence of obstruction, also rule out synchronous polyps and cancers.

Five percent of pedunculated or broad-based polyps removed endoscopically are found histologically to contain invasive carcinoma, with malignant cells traversing the muscularis mucosae into the submucosal layer. The biologically benign entity in which malignant cells are present only superficial to the muscularis mucosae should not be described as "carcinoma in situ," but rather by the more innocuous term "severe dysplasia." Surgical resection is not necessary in pedunculated polyps with invasive carcinoma if there is a sufficient margin (1–2 mm) between the limit of invasive carcinoma and the resection line on multiple sections, if the carcinoma is well or moderately well differentiated, and if there is no lymphatic or venous invasion [34]. There is controversy about whether these endoscopic and histological principles should be applied to the local removal of sessile malignant polyps or whether surgery should be recommended unless the patient is a poor operative risk. Because malignant polyps and polypoid carcinomas tend to occur in older, frail patients, and surgery rarely yields resectable lymph nodes without evidence of distant metastases, there is an increasing tendency to use conservative endoscopic

management, even in lesions failing to meet the above criteria, unless the patient is an excellent surgical risk. The use of a permanent colonic tattoo makes it possible to localize a malignant polypectomy site with accuracy in the event that surgical resection is necessary or for subsequent surveillance after an interval of a few months before entering a normal follow-up regimen.

Rectal bleeding of undetermined etiology

Rectal bleeding, especially if sustained, dark, or mixed in the stools, is frequently caused by tumor or mucosal pathology. In the subgroup of patients with visible rectal bleeding in whom a barium enema and sigmoidoscopy are normal, about 10% of referral patients have cancer, 15%–20% have polyps (Fig. 120.7), and up to 50% have some kind of a visible abnormality, including traumatized, inflamed, or ulcerated mucosa. Blood loss or anemia, particularly in elderly persons, remains the highest-yield indication for colonoscopy. Total colonoscopy should be performed rather than double contrast barium enema (DCBE) in the assessment of rectal bleeding, whether overt or occult, because of the obvious bonus of color view in seeing blood, altered blood, or bleeding points, which can be flat and totally invisible on radiographic films. It may also be possible to treat the cause of bleeding endoscopically [35].

Acute colonic bleeding

Acute colonic bleeding of overwhelming proportions may require surgery and perioperative bowel irrigation with on-table colonoscopy, but bleeding of lesser severity may stop spontaneously. Ten percent of patients admitted to an intensive care unit with a tentative diagnosis of acute colonic bleeding will have an upper gastrointestinal source [36], which must be considered in all instances. In most cases of acute bleeding, an attempt at conventional colonoscopy examination is indicated before resorting to angiography, scintigraphy, or other investigational techniques. If possible, oral or nasal tube electrolyte bowel preparation is started immediately after the patient presents, because blood coating makes localization of the bleeding site difficult without preparation or if enemas reflux blood proximally. Colonoscopy should be performed as soon as practicable, because the aim is to examine the bowel while fresh bleeding continues. Waiting even an hour or two may miss the opportunity for diagnosis and allow degradation of blood into a dark unpleasant mess. Changes of patient position during the examination, while bleeding is active, help to maintain adequate visualization. Expertise is needed in this situation, but a cause for bleeding should be apparent in more than half of cases, and treatment may be possible by polypectomy, electrocoagulation, injection therapy, or other means. Postpolypectomy hemorrhage should be managed by immediate colonoscopy without preparation, because the site of bleeding is known [37]. An algorithm for the approach to acute lower gastrointestinal (GI) bleeding has been published [38].

Figure 120.7 A smooth sessile adenoma is located in the descending colon.

Anemia

Iron deficiency anemia or a positive fecal occult blood test should be evaluated by colonoscopy rather than DCBE, because flat lesions such as angiodysplasia or minor inflammatory change cannot be diagnosed radiologically. Even if radiographs appear normal, endoscopy is known to find significant numbers of radiologically missed lesions such as cecal carcinomas. In anemic patients, gastroscopy and colonoscopy can be performed at the same visit. The object of endoscopy in anemic patients mainly is to rule out carcinoma or vascular anomalies with certainty; about 80% have negative examinations, but the endoscopic opinion of normality is considerably more certain than a negative radiographic film result.

Chronic diarrhea

Patients with long-standing diarrhea require an accurate examination with histological biopsies routinely taken and other specimens as appropriate. Rectal evaluation is adequate in many patients, but total colonoscopy and ileal examination complete the investigation and sampling process in one definitive test. Microscopic or collagenous colitis can occur despite normal endoscopic appearances; therefore, biopsy specimens must be taken in any patient with chronic diarrhea or increased bowel frequency [39]. However, the normal colonic mucosa frequently harbors inflammatory cells, and great care should be taken to avoid misinterpretation and overdiagnosis of inflammatory bowel disease when none is truly present, based on an inexperienced pathologist's report of acute or chronic inflammation on biopsy specimens. Collagenous colitis is diagnosed histologically from a thickened intraepithelial collagen layer. Ischemic colitis has a range of appearances, from slight reddening and friability, to varying degrees of ulceration, to frank gangrene; the diagnosis is often possible by combining the typical history of sudden onset of pain, bleeding, and diarrhea with endoscopic evidence of a perisplenic distribution.

Inflammatory bowel disease

Often, the mucosal appearances are sufficient to make immediate assessment of the extent and type of colitis [40]. Crohn's disease, in particular, shows a rather characteristic pattern of small aphthoid ulcers with intervening normal mucosa unlike the generalized redness of early ulcerative colitis. More advanced inflammatory diseases of any type, including ulcerative colitis, Crohn's disease, tuberculosis, or amoebic colitis, can be virtually indistinguishable from each other because of the limited range of responses of the colon to various diseases that affect the mucosal surface. Once the diagnosis of inflammatory bowel disease has been established, there are only a few indications for follow-up colonoscopy besides malignancy surveillance, unless there is a marked change in symptoms. Dye spray in the colon has been reported to increase the yield of dysplastic lesions. This technique is becoming increasingly used during surveillance colonoscopies. Methylene blue or indigo carmine are applied with a specialized spray catheter [41].

Cancer surveillance or prevention

The long period preceding cancer during which a focal adenomatous polyp is visible gives the opportunity for visualization, biopsy, and destruction of precancerous lesions, even at sizes of 1–2 mm. Accuracy is possible, because color makes it easy to distinguish which small excrescences are fecal, which are air bubbles, and which are polyps. A syndrome of flat adenomas has been described [42]; these adenomas may have a tendency to degenerate into carcinoma while still relatively small in diameter. Detecting their presence may be enhanced by zoom colonoscopy or by dye spraying or "chromoendoscopy."

The recommendations of an expert multidisciplinary panel, published in 1997 [43], included offering colonoscopy every 10 years to asymptomatic average-risk persons aged 50 years or older. In addition, the panel suggested annual fecal occult blood tests in this population, with colonoscopy as the investigation of choice if the occult blood test is positive. A flexible sigmoidoscopy is recommended every 5 years, followed by colonoscopy to remove polyps, biopsy cancers, and to examine the rest of the bowel should an adenoma or cancer be seen. Colonoscopy is indicated for any patient with a positive fecal occult blood test and in those who are at increased risk for polyp and cancer formation, including those with first-degree relatives with either colorectal cancer or an adenomatous polyp – in whom screening should begin at age 40. People with a family history of familial adenomatous polyposis whose genetic testing is positive or indeterminate should be offered flexible sigmoidoscopy every 12 months beginning at puberty. A family history of hereditary nonpolyposis colorectal cancer (HNPCC) should trigger an examination of the entire colon every 2–3 years starting between the ages of 20 and 30 years.

The interval between surveillance examinations after removal of adenomatous polyps or an operation for colorectal cancer is a matter of debate [44], but the expert panel has made the following recommendation: removal of an adenomatous polyp larger than 1 cm in diameter or of multiple polyps requires repeat colonoscopy 3 years after the initial examination, and if that is negative or reveals only a single small tubular adenoma, the surveillance interval can be increased to 5 years. In special circumstances, such as a polyp with invasive cancer, large sessile adenomas, or numerous adenomas, a shorter follow-up interval may be necessary. In patients who have had a colorectal cancer surgically resected with a curative intent, if total colonoscopy had not been performed preoperatively, a complete colon examination should be completed within 1 year of the resection. If this or the preoperative examination was normal, subsequent colonoscopy is indicated in 3 years, and if normal, every 5 years.

Surveillance examinations in extensive ulcerative colitis [45] and Crohn's colitis patients normally start 8 years after onset of disease and are repeated at 1- or 2-year intervals, with 10 or more biopsy specimens taken at representative sites around the colon. The endoscopist is alert for any nodular, indurated, or plaque-like lesions in the colon, but varying degrees of low- or high-grade dysplasia may be found in relatively normal-looking mucosa, a finding that is enhanced with chromoendoscopy.

Intraoperative colonoscopy

The most common indication for intraoperative colonoscopy is to localize the site of a previously endoscopically resected malignant polyp (not necessary when preoperative tattooing is performed) during laparoscopy [46]. The site may heal completely in 3 weeks, leaving no external sign of its location, but it can often be endoscopically identified as a scar on the mucosal surface. Another indication is to assist the surgeon in identifying which colonic segment is the source of massive lower GI hemorrhage.

Low-yield indications and circumstances when colonoscopy is not indicated

Conditions manifesting with functional symptoms such as long-standing constipation, bloating, or chronic abdominal pain, may be investigated by CT or radiographic examination if there is no occult or overt colonic bleeding (Table 120.2) [32]. However, the elderly patient may be unable to cooperate with the need to retain contrast and air, resulting in a poor radiographic examination. Bowel symptoms in patients older than 70 years of age may be better investigated by colonoscopy than radiographic examinations.

A flexible sigmoidoscopy may be the examination of choice when the patient presents with an acute diarrheal syndrome, because the view of the distal colonic mucosa often (but not always) mirrors the findings in the remainder of the colon. Excepting surveillance for malignancy, repeated colonoscopy for routine follow-up of patients with established inflammatory bowel disease provides little clinically

Table 120.2 Circumstances in which diagnostic colonoscopy is usually not indicated or has a low diagnostic yield

Chronic, stable, irritable bowel syndrome or chronic abdominal pain
Acute diarrhea
Metastatic adenocarcinoma of unknown primary site in the absence of colonic signs or symptoms when it will not influence management
Routine follow-up of inflammatory bowel disease
Upper gastrointestinal (GI) bleeding or melena with a demonstrated upper GI source

Modified from American Society for Gastrointestinal Endoscopy [32].

useful information. Colonoscopy need not be performed in the patient with overt GI bleeding in whom an active upper GI source has been demonstrated. Similarly, there is no indication for colonoscopy in the patient with an adenocarcinoma where the primary site of the tumor is unknown, unless the result of colonoscopy will influence the management of the patient.

Contraindications

Colonoscopy is a relatively stressful physiological experience and a strong vagal stimulus that can produce dysrhythmias, minor electrocardiographic disturbances, and a degree of hypotension; therefore, it is contraindicated for several weeks after myocardial infarction [47]. Both the air pressure involved in distention of the colon and the unavoidable stretching during passage around loops and bends have the potential to increase any existing risk of perforation. Because of this, colonoscopy is contraindicated in the acute or abscess phase of diverticulitis. Severe acute episodes of ulcerative, Crohn's, ischemic, or infective colitis have generally been considered a contraindication for colonoscopy, but some reports suggest that, with due expertise and care, it is safe and may be of value in therapeutic decision-making in these situation [48].

Therapeutic maneuvers increase the hazards of colonoscopy. Balloon dilation in a sick patient may justify antibiotics. Polypectomy carries a small risk of immediate primary bleeding or of delayed secondary hemorrhage for up to 29 days after the procedure. The likelihood of delayed bleeding is especially increased in patients with a coagulopathy or in those taking anticoagulants or antiplatelet therapy. When medical conditions prohibit discontinuation of Coumadin (warfarin), the patient may be admitted to the hospital for the substitution of intravenous heparin for oral anticoagulation. When the coagulation parameters have returned to normal levels, the intravenous heparin dosage should be stopped 4 h before colonoscopy with polypectomy. If a clean polypectomy has been performed without bleeding, heparin may be restarted 4 h after the procedure, and oral anticoagulants reinstituted 12–24 h later although the possibility of delayed hemorrhage will remain. The patient must remain on heparin until the international normalized ratio (INR) levels have returned to the therapeutic level. If polypectomy is associated with bleeding, an attempt should be made to withhold heparin for 8–12 h if it is considered that the patient can safely remain off anticoagulants for that period. Low molecular weight heparin has been used during periods of Coumadin discontinuation but this has not been subject to clinical investigations [10].

Polypectomy can result in perforation by cutting through the bowel wall or by applying sufficient thermal energy to burn through and necrose the full thickness of the colon, resulting in a perforation delayed for between several minutes and many days after the polypectomy. The colon wall is extremely thin – the normal mean colon wall thickness is about 2.2 mm [49]. Some features of a full-thickness thermal injury to the colon wall, including localized pain, fever, and leukocytosis, may occur 6–24 h after polypectomy. This is known as the postpolypectomy or transmural burn syndrome [26], and must be distinguished from a perforation. Transmural damage is localized and self-sealing when bowel loops, mesentery, omentum, or other adjacent peritoneal surfaces become adherent, so that conservative management with antibiotics and bed rest is usually sufficient.

When signs of frank perforation occur after colonoscopy, it is safest to advise immediate surgery. If only a small amount of free air is present on a postendoscopy radiographic film and the patient has no symptoms, it may be safe to treat the patient conservatively, with antibiotics but without surgery. In any event, a surgeon should always be involved in observation of the patient and in deciding whether or not surgery is indicated [50].

Morbidity and mortality rates for colonoscopy are unknown but reviews from the late 1980s and 1990s indicate that the perforation rate is about 1 in 2500 colonoscopies [51], with a mortality of 1 in 15 000 colonoscopies; most deaths occur after inappropriate conservative management of suspected perforation [52]. An institutional review [53] found that there may be one perforation per 1200 colonoscopies, with almost half due to mechanical trauma and the rest resulting from polypectomy or electrocautery. Most perforations occurred at the rectosigmoid junctional area. Conservative management was successful in one-fourth of those with polypectomy perforations and in 10% of cases of mechanical injury.

The bleeding rate after polypectomy varies from 0.26% to 1.5% [51], but probably can be reduced with better attention to hemostatic techniques such as the sole use of coagulating current and slow transection. The use of cutting current may be associated with a higher immediate bleeding incidence [54]. Injection or other means of endoscopic therapy for immediate control of bleeding should avoid the small number of cases in which angiographic or operative intervention was

previously necessary. Unexpected radiographic demonstration of free air in the abdominal cavity (pneumoperitoneum) of symptomless patients submitted to routine abdominal radiography after colonoscopy has been reported. Surgeons also report the existence of hematomas, serosal splits, and manifestations of local colonic trauma in patients coming for laparotomy soon after colonoscopy. These facts serve as a reminder to all endoscopists, particularly those in the learning phase, to be cautious, to be considerate to the patient, to avoid oversedation, and to be prepared to abandon a colonoscopy that appears unreasonably traumatic.

Virtual colonoscopy (CT or MR colography)

A computer reconstruction of a rapidly performed abdominal spiral CT scan provides a simulated tubular view of the intestinal lumen. Infoldings (haustra), outpouchings (diverticula), and intralumenal protuberances (polyps) can be identified. The process is noninvasive, requiring a single breath-hold while the colon is maximally air-inflated. Cancers and most polyps over 10 mm in diameter can be identified, but smaller polyps may be overlooked when compared with findings at conventional colonoscopy [55]. Retained stool may give a false-positive reading, and flat mucosal lesions are difficult to identify. Technological developments have increased the accuracy of this tool, which will take a place among the procedures for evaluating the colon. Work is in progress for a computer software system capable of finding polyps using dedicated algorithms [56]. The reader is referred to Chapter 130 for detailed information on CT colonoscopy, and to Chapter 131 for detailed information on MR colography.

Colonoscopy has become the procedure of first choice for most colonic investigations. Barium enema for radiographic evaluation of the large bowel has been supplanted by virtual colonography. It is estimated that it is unusual but possible to miss a polyp equal to or exceeding 1 cm in diameter during colonoscopy [57].

Limitations of colonoscopy

It is pertinent to mention that the endoscopist is capable of gross errors in localization and occasionally of missing large lesions. Blindspots for the endoscopist occur in certain areas, such as the rectal ampulla and flexures (especially the mobile sigmoid–descending colon junction), behind acute bends, and in regions of spastic muscle contractions. Adhesions or strictures can render the endoscope tip immobile and make a proper view for targeted biopsies impossible. Large polyps on long stalks can be missed by the endoscopist because they move around and may spring out of the field of view as the colonoscope is pulled back. Poor preparation and redundant or mobile colons also make the endoscopic examination less accurate.

Careful inspection for possible abnormalities is important during intubation as well as during the withdrawal phase of a colonoscopy, because the view of the stretched colon is different from that obtained when the bowel is shortened. Several passes may be needed to see all aspects of an angulated or convoluted area properly. There is no room for complacency by the endoscopist over the accuracy of colonoscopy, because it takes dexterity and integrity to be sure that an optimum examination has been performed. It can be assumed that 5%–10% of the mucosal surface is not seen during colonoscopy. This explains the significant pick-up of lesions up to 5–10 mm in diameter during the check colonoscopies usually performed within 1 year (or even on the same day) [58] of a previous colonoscopy and polypectomy to establish a "clean colon" that is free of adenomas. It has been estimated that 27% of adenomas in the size range 1–5 mm are missed on colonoscopy, as are 13% of adenomas in the range 6–9 mm, and 6% of those over 10 mm [58]. Colonoscopy can miss colonic cancer in the intramucosal lesions of chronic ulcerative colitis, within strictures, or where there is submucosal involvement by extrinsic neoplasm or metastases.

In addition to diagnostic difficulties, the mechanical limitations of colonoscopy are relevant. An expert manages up to 98%–99% total colonoscopy, especially if there is no stricture or stenosing lesion to prevent insertion. Less expert endoscopists or those with a smaller case load may reach the cecum [59] less than 90% of the time and are more likely to be slow, inaccurate in diagnosis and localization, and more traumatic and liable to complications. The lack of proper means of teaching colonoscopy skills limits its clinical application worldwide.

Costs

Colonoscopy is highly cost-effective. The instrumentation involved is inexpensive compared to radiography. A colonoscope should last for at least 500 examinations before major repair or replacement becomes necessary. The actual physician time involved per procedure is greater for colonoscopy than for the virtual CT scan, during which the examination may be performed by a technician. The overall cost is approximately equal if a radiography and flexible sigmoidoscopy-first schema is compared with a colonoscopy-only approach to the investigation of patients with lower GI symptoms. Many patients with an abnormal sigmoidoscopy or virtual CT scan demonstrating a lesion or probable pathology will require a subsequent colonoscopy for confirmation, polypectomy, or biopsy; whereas only a few with the colonoscopy-first approach will require a virtual CT scan [56].

Future developments

Videocolonoscopy will allow improvements in image resolution as well as potential for computer enhancement and related developments that should improve diagnostic accuracy, localization, and ease of instrument handling. Advances in bowel preparation should permit comfortable clearance in a few hours instead of the 24–48-hour preparations used at present. Developments in safe, short-lived analgesia should make insertion easily tolerated while allowing normal activities soon afterward, such as driving or return to work. Anesthesiologists and some endoscopists administer propofol for colonoscopy, during which period the patient is in a deep stage of anesthesia, but recovery is reasonably rapid. Computerized interactive teaching models will permit education in manipulative and interpretive skills without imposing an undue burden on and risk to patients during the endoscopist's early training phase [60].

Development of other diagnostic and therapeutic modalities will allow the endoscopist to apply miniaturized imaging probes to areas of interest and to ablate lesions more completely and safely than can be performed at present. For instance, ultrasound probes passed through the instrumentation channel help to determine the presence and depth of penetration of malignancy [61]. Optical coherence tomography (OCT) uses light instead of sound waves to achieve an "optical biopsy" of the mucosa and submucosa [62]. A new technology is emerging to record spectrophotometric measurements of tissue fluorescence by a sensing device passed through the accessory channel [63]. Differentiation between neoplastic and normal tissue by their inherent differences in level of tissue excitation by laser stimulation may be sufficient for diagnostic purposes or will enable directed biopsy of abnormal areas [64]. With improved photosensitizing agents and laser photocoagulation or locally injected immunotherapeutic agents, colonoscopy should be able to make further inroads into territory that is traditionally considered the domain of the surgeon.

Wireless "capsule" endoscopy [65] has been developed for small bowel exploration, where peristalsis is the propulsive force for movement, and the narrow-caliber lumen is sufficiently illuminated by the light to produce a diagnostic visual image. A capsule has been developed for the colon and studies are continuing for its assessment [66] in spite of several differences between the large and small bowel such as:

- the diameter is wide and folds may hide lesions
- peristalsis is relatively slow in the colon
- the capsule tends to tumble and its movements cannot be controlled
- there is no therapeutic capability.

Some or all of these factors can be overcome in the future, which may see the development of robotic therapeutic capsules armed with snare, forceps, scissors, and clips.

The future prospects for GI imaging seem limitless; already the colonoscope is the best imaging system for the colon, and has the additional ability to perform therapy including injection, biopsy, decompression, and polypectomy.

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A ENDOSCOPIC

121 Endoscopic retrograde cholangiopancreatography, endoscopic sphincterotomy and stone removal, and endoscopic biliary and pancreatic drainage

Tony E. Yusuf, Daniel K. Mullady, David L. Carr-Locke

Ampulla of Vater (major duodenal papilla), 2933
Sphincter of Oddi dysfunction, 2934
Biliary system, 2935
Pancreas, 2945

Endoscopic retrograde cholangiopancreatography (ERCP) is an advanced endoscopic procedure that has been available for over three decades. It encompasses the use of a side-viewing endoscope and various accessories to gain access to the biliary and pancreatic ductal system under fluoroscopic guidance. First described in 1968 by McCune et al. [1], ERCP has evolved into a complex endoscopic procedure for the diagnosis and therapy of pancreatobiliary diseases with many defined indications. ERCP requires dedicated and supervised endoscopy training to acquire the different techniques, which include endoscopic papillectomy, sphincter of Oddi manometry, biliary sphincterotomy, pancreatic sphincterotomy, stone removal, tissue sampling, stenting, and drainage of peripancreatic fluid collections. ERCP has an overall complication rate of 4.95%. Complications includes pancreatitis (1.8%), hemorrhage (1.13%), cholangitis (0.57%), perforation during sphincterotomy (0.57%), others (0.57%), and death (0.12%) [2]. This chapter summarizes the clinical applications and techniques used during ERCP.

Ampulla of Vater (major duodenal papilla)

Ampullary adenoma and carcinoma

Small intestinal tumors represent 2.4% of all gastrointestinal malignancies and 55.2% of these are found in the duodenum [3,4]. Duodenal adenomas are common in patients with familial adenomatous polyposis (FAP), with 50%–100% occurring in a periampullary location [5–7]. Sporadic adenomas of the papilla are being increasingly recognized, often as an incidental finding during a routine upper endoscopy. In postmortem studies, the incidence of adenomas of the ampulla of Vater is 0.04%–0.12% [8]. The frequency of carcinoma in papillary adenomas has been reported to range from 30% to 65% [9,10].

Because histologically proven adenomas of the papilla are premalignant and follow the adenoma–carcinoma sequence similar to that seen in the colon [11], resection of these lesions is recommended. Traditionally, the approach to these lesions was wide radical resection with pancreaticoduodenectomy, as the presence of malignant foci can be missed on preoperative biopsies. This was accompanied by significant morbidity and a mortality rate as high as 30% [12,13]. Even with improvements in surgery, local resection is currently the method of choice, with lower morbidity and mortality but an association with a known recurrence rate [14–16].

Endoscopic resection of the papilla (endoscopic papillectomy or ampullectomy) is gaining popularity and wider acceptance as a less invasive treatment modality for ampullary lesions and, in particular, for those patients who are poor operative candidates. One study concluded that ampullectomy is the procedure of choice for resecting benign lesions smaller than 3 cm, small neuroendocrine tumors, and T1 carcinomas of the ampulla [17]. The technique involves gross inspection of the papilla with a side-viewing duodenoscope. The margins of the lesion can be highlighted by chromoendoscopy with spraying dye such as methylene blue or indigo carmine; however, this step is not often required as the lesion is usually protuberant (Fig. 121.1). A cholangiogram and a pancreatogram are carried out to define intraductal tumor extension and to opacify the ducts with contrast to aid in identifying the ductal orifices after the procedure is completed. One approach is to perform a small pancreatic sphincterotomy

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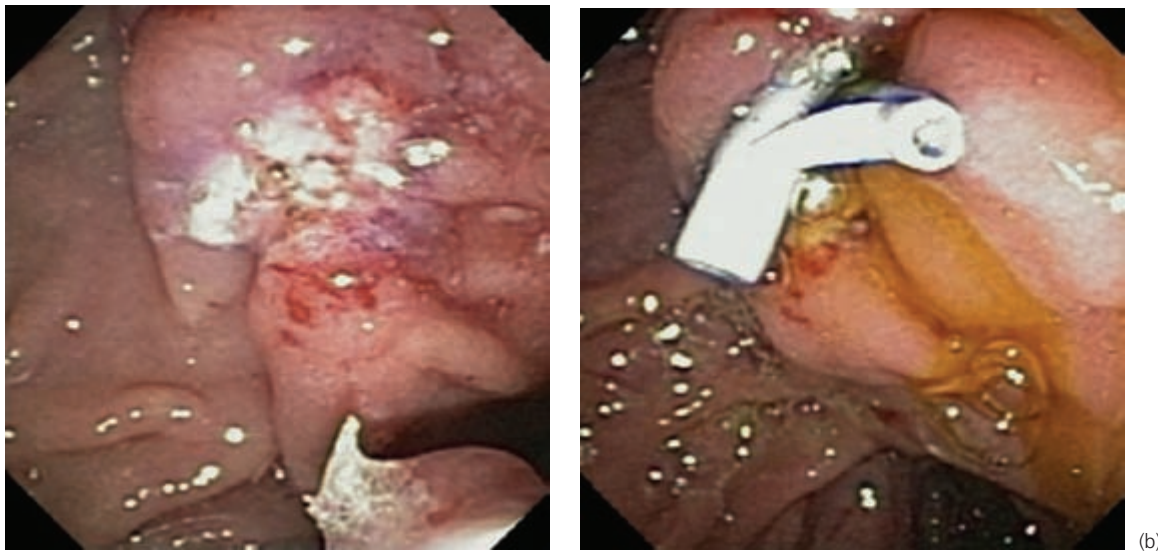
Figure 121.1 A 48-year-old man with hereditary nonpolyposis colon cancer (HNPCC) found to have ampullary adenoma on screening upper endoscopy.

and to place a small pancreatic stent prior to piecemeal snare polypectomy [18]; however, most commonly an en-bloc snare excision of the entire lesion is performed using electrosurgical current and the specimen is retrieved for histopathological examination. Residual adenomatous tissue at the margins of the resection site can be ablated with contact thermal or noncontact argon plasma coagulation. At the end of the procedure, a short polyethylene pancreatic stent is placed to minimize the risk of postprocedural pancreatitis. Occasionally, a biliary stent is also placed to optimize bile drainage (Fig. 121.2). In a prospective randomized controlled trial of endoscopic ampullectomy in 19 patients with ampullary adenoma, the risk of postprocedural pancreatitis was 0% in the stented group compared with 33% in the unstented group [19]. The stents are removed endoscopically at 1 month and the site inspected for residual adenoma. Surveillance endoscopy with a duodenoscope is carried out every 6–12 months for benign lesions to assess for recurrent adenomatous tissue.

Accurate preoperative diagnosis and staging of papillary tumors is essential in planning the therapeutic intervention. Endoscopic biopsies of papillary adenomas cannot exclude the presence of carcinoma [10,20]. Adenocarcinoma was found at surgery or follow-up in 30%–60% of patients with a primary preoperative diagnosis of papillary adenoma [10,11]. ERCP with biopsies and endoscopic ultrasound (EUS) is the current accepted approach for diagnosis and staging of ampullary tumors. EUS has been shown to be an accurate endoscopic imaging modality for local staging of ampullary tumors and assessment of lymph node status [21,22]. In one study, EUS T-staging and failure to adequately lift the ampullary lesion with a submucosal saline injection were predictive of malignancy by univariate analysis [23]. Patients with inoperable ampullary cancer causing biliary obstruction will have a self-expandable metal stent placed into the main bile duct for adequate biliary drainage. Metal stents provide long-term palliation of malignant biliary obstruction, with a median stent patency period of 8 months, and are more cost-effective than plastic stents in patients with a life expectancy of 6 months or more [24–26].

Sphincter of Oddi dysfunction

The sphincter of Oddi is a muscular structure made of smooth muscle fibers that surrounds the distal pancreatic duct, bile



(a) (b)

Figure 121.2 **(a)** The same patient as in Figure 121.1 after papillectomy. **(b)** The same patient after stenting of the bile duct and pancreatic duct.

duct, and ampulla of Vater. This sphincter mechanism lies mostly within the duodenal wall and measures 6–10 mm in length [27–32]. Functionally, the sphincter of Oddi is independent from the duodenal smooth muscle system and it serves to prevent reflux of duodenal contents into the ductal system and to control the flow of bile and pancreatic juice into the duodenum [32].

Sphincter of Oddi dysfunction, commonly known as SOD, refers to an abnormality within the sphincter, either motility related (dyskinesia, spasm) or structural (stenosis), related to chronic inflammation or fibrosis. SOD can involve the biliary sphincter, the pancreatic sphincter, or both. Patients typically present with episodic epigastric pain radiating to the back and associated with hepatic or pancreatic enzyme abnormalities. Postcholecystectomy abdominal pain similar to the preoperative pain of suspected biliary origin occurs in 10%–20% of patients [33]. In two studies, SOD was diagnosed in 9%–11% of patients during evaluation of postcholecystectomy pain [34,35].

SOD is classified into a biliary type according to the Milwaukee classification of Hogan and Geenen [36], and a pancreatic type according to Sherman et al. [37]. Each classification system has three types: biliary I patients have biliary-type pain, abnormal liver function tests (alanine aminotransferase [ALT] and aspartate aminotransferase [AST] levels > 2 × normal) documented on two or more occasions, delayed drainage of ERCP contrast of more than 45 min, and a dilated common bile duct of more than 12 mm diameter; biliary II patients have biliary-type pain but only one or two of the above criteria; and biliary III patients have only biliary-type pain and no other abnormalities. Pancreatic I patients have recurrent pancreatitis or typical pancreatic pain, elevated pancreatic enzymes 1.5–2 × the upper limit, a dilated pancreatic duct of more than 6 mm in the head and 5 mm in the body, and delayed contrast during ERCP of more than 9 min; pancreatic II patients have pancreatic pain and one or two of the above criteria; and pancreatic III patients have only pancreatic-type pain.

Sphincter function can be evaluated by invasive and noninvasive methods [38]. Noninvasive methods include the morphine–prostigmin provocative test; hepatobiliary scintigraphy, which evaluates radionuclide filling and drainage of the bile duct; and ultrasound assessment of the pancreatic duct and bile duct after secretin or cholecystokinin stimulation [39–44]. The gold standard diagnostic test for SOD is manometric assessment of basal sphincter pressure by sphincter of Oddi manometry (SOM). A baseline ERCP is usually performed to exclude pancreatobiliary diseases such as congenital anomalies, stones or malignancy. SOM involves the use of solid-state or perfusion low-compliance catheters that can measure the biliary and pancreatic sphincter pressures through ports located at the distal end [45]. Multiple station pull-throughs are performed and graphic recordings of the pressures are displayed on a dedicated computer station. Selective cannulation of the bile duct or pancreatic duct for isolated sphincter pressure measurement is guided by clinical suspicion for the origin of symptoms. The patient must be sedated for the procedure and smooth muscle relaxants are avoided as they may interfere with the sphincter mechanism and interpretation of results. A basal sphincter pressure greater than 40 mmHg above duodenal pressure is considered abnormal and sphincterotomy is the current standard treatment of choice for SOD in the appropriate clinical setting. Most of the data on the effect of endoscopic therapy of SOD refer to biliary sphincterotomy. Symptomatic patients with type I SOD benefit from sphincterotomy (90%–100%) irrespective of the manometry findings; thus, manometry is not required for this group of patients [38,46]. In type II SOD, 85%–95% of patients with an elevated basal sphincter pressure benefited from sphincterotomy and reported improvement of symptoms after 2–4 years of follow-up [47,48]. Patients with type III SOD and an elevated basal sphincter pressure on SOM may benefit from sphincter ablation in 55%–65% of cases [38].

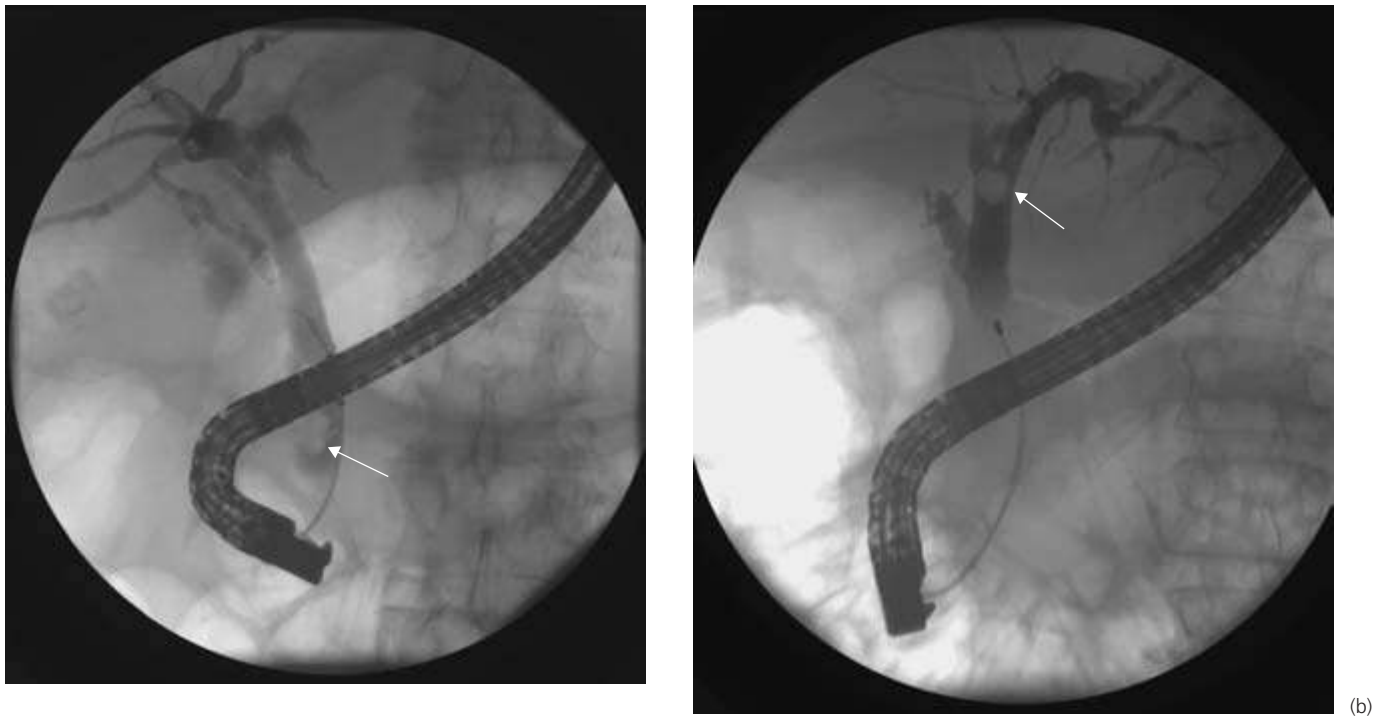
The rate of complications is high in patients undergoing endoscopic sphincterotomy for SOD and varies from 10.8% to 21.7% [49,50]. Pancreatitis is the most common complication and occurs in up to 20% of patients. The risk of pancreatitis may be minimized by stenting of the pancreatic duct. Patients with suspected pancreatitis following SOM and sphincterotomy should be admitted to hospital for observation and treatment.

Biliary system

Bile duct stones

Bile duct stones occur in 6%–15% of cases of symptomatic cholelithiasis and in 1%–2% of cholecystectomies performed for acalculous biliary disease [51–55]. Patients may present with abdominal pain of biliary origin (erroneously termed biliary colic), cholangitis, jaundice, pancreatitis, transient elevation of hepatic aminotransferases, or a filling defect with or without biliary ductal dilation on imaging studies such as transabdominal ultrasound, computed tomography (CT), and magnetic resonance cholangiopancreatography (MRCP). When choledocholithiasis is suspected clinically or on imaging studies, the ultimate therapy is extraction of the stones from the biliary tree to minimize serious complications such as severe pancreatitis, sepsis, and death. Stone extraction can be achieved endoscopically, surgically, or radiologically using the transhepatic approach.

Endoscopic removal of bile duct stones is the mainstay of treatment and first-line therapy of choice in centers that have expertise in this technique (Fig. 121.3). Since the first descriptions of endoscopic sphincterotomy by Classen and Demling [56] and Kawai et al. [57] in 1974, the role of ERCP in the management of bile duct stones has witnessed a



(a) (b)

Figure 121.3 (a) Choledocholithiasis in the distal common bile duct (arrow). **(b)** Choledocholithiasis in the proximal common hepatic duct (arrow).

tremendous growth. By using standard techniques of stone extraction (see below), up to 90% of bile duct stones can be removed after a biliary sphincterotomy [58]. The remaining 10% of cases require more advanced techniques (see below).

Patients are sedated before therapeutic ERCP performed for bile duct stone removal. In most medical centers, conscious or moderate sedation is administered. Alternatively, general anesthesia is used in selected groups of patients such as children, the elderly, the severely ill, those with serious cardiopulmonary problems or difficult airways, and those who are difficult to sedate based on previous history. In one study that retrospectively analyzed 1056 ERCPs performed on patients under conscious sedation (82%) or general anesthesia (18%), the latter was given more frequently to patients with primary sclerosing cholangitis, liver transplant recipients, and those undergoing painful dilations whereas conscious sedation was more frequently given to patients with neoplasia and choledocholithiasis. More interventions per ERCP were performed with the patient under general anesthesia than with the patient under conscious sedation ($P < 0.001$), and with conscious sedation the ERCP failure rate was double that with general anesthesia (7% vs 14%, $P = 0.012$) [59].

Access to the bile duct by deep cannulation is essential to achieve endoscopic therapy of bile duct stones. Biliary access can be difficult in some patients and there are a number of different techniques that can be used in this situation to gain entry into the bile duct. Once stones are identified on a cholangiogram, the decision should be made as to what accessory instruments need to be used. A biliary sphincterotomy (incision of the intraduodenal portion of the common bile duct sphincter muscle) is usually performed to the maximum extent of the endoscopic landmarks and under direct endoscopic guidance prior to stone removal (Fig. 121.4). This is achieved with the use of a sphincterotome catheter that has a cutting electrosurgical wire at the distal end. Sphincterotomy is associated with some complications. A retrospective analysis of complications in 535 patients who underwent endoscopic sphincterotomy found a complication rate of 5.4%, which included bleeding (2.4%), retroduodenal perforation (0.4%), pancreatitis (0.6%), cholangitis (1.3%), and cholecystitis (0.7%); 14% of these patients required an operation. Overall mortality was 0.3% [60]. A multicenter study by Freeman et al. [49] identified, in a multivariate analysis, five significant risk factors for complications occurring within 30 days of endoscopic biliary sphincterotomy. These were suspected SOD, cirrhosis, difficult bile duct cannulation, precut (access) papillotomy, and use of a combined percutaneous–endoscopic procedure. The rate of complications was highest when the indication for the procedure was suspected SOD (21.7%) and lowest when the indication was removal of bile duct stones within 30 days of laparoscopic cholecystectomy (4.9%). Compared with endoscopists who performed fewer ERCP procedures, those who performed more than one sphincterotomy per week had lower rates of all complications (8.4% vs 11.1%, $P = 0.03$) and severe complications (0.9% vs 2.3%, $P = 0.01$).

Since the first description in 1983 [61], endoscopic balloon dilation of the papillary sphincter, or balloon sphincteroplasty,

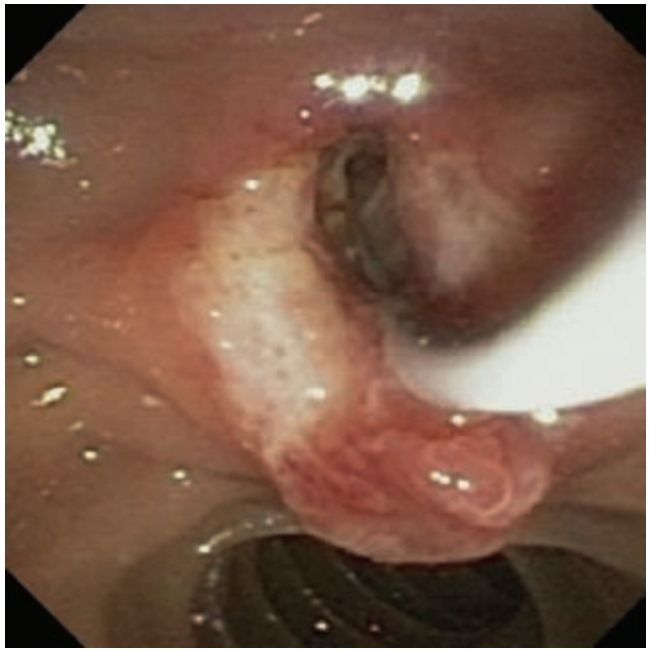


Figure 121.4 Endoscopic appearance of a widely patent biliary sphincterotomy.

has been proposed as an alternative to endoscopic sphincterotomy for the treatment of papillary stenosis and management of bile duct stones [62–66]. This involves the use of a hydrostatic balloon positioned across the papilla and inflated under high pressure while dilation is monitored fluoroscopically. The potential advantages over endoscopic sphincterotomy are preservation of the sphincter mechanism and possible minimization of short- and long-term complications.

A metaanalysis of eight prospective randomized trials comparing endoscopic balloon dilation with sphincterotomy in the treatment of bile duct stones found similar outcomes in overall complications and success rate in stone removal [67]. There was less bleeding but higher rates of postprocedural pancreatitis with balloon dilation than with sphincterotomy (0% vs 2.0% and 7.4% vs 4.3% respectively). There were no significant differences in the rates of perforation or infection. Mechanical lithotripsy was required more frequently in the group of patients who had papillary balloon dilation than in the sphincterotomy group (20.9% vs 14.8%, $P = 0.014$). The authors recommend the use of endoscopic balloon dilation of the papillary sphincter in the group of patients with coagulopathy to minimize the risk of bleeding. A multicenter study of 237 patients with bile duct stones randomized to balloon dilation and sphincterotomy found that dilation is associated with increased short-term morbidity and death secondary to pancreatitis compared with sphincterotomy [68]. As there seems to be an increased incidence of adverse events in Western populations, balloon dilation of papilla cannot be recommended as the routine first-line option in the management of bile duct stones. Balloon dilation of a previous sphincterotomy may, however, be safer than repeated sphincterotomy.

An attempt at stone extraction should be made after a biliary sphincterotomy is performed unless the patient condition mitigates in favor of drainage only. The most commonly used accessories for this purpose are balloon catheters and baskets made of metal wire. The choice between a balloon or a basket depends on the size of the stones and endoscopist preference [69]. For large and multiple stones (≥ 10 mm), a basket is preferable (Fig. 121.5). A balloon is commonly used

(a)

(b)

Figure 121.5 **(a)** Basket extraction of a proximal common bile duct stone. **(b)** Basket extraction of a distal common bile duct stone.

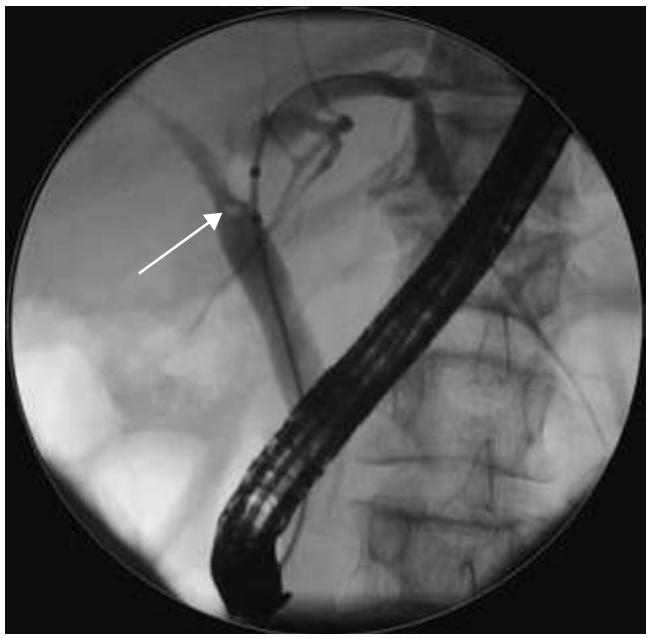


Figure 121.6 A 40-year-old woman with sickle cell anemia with pigment stones in the common bile duct (arrow), one being removed by balloon extraction.

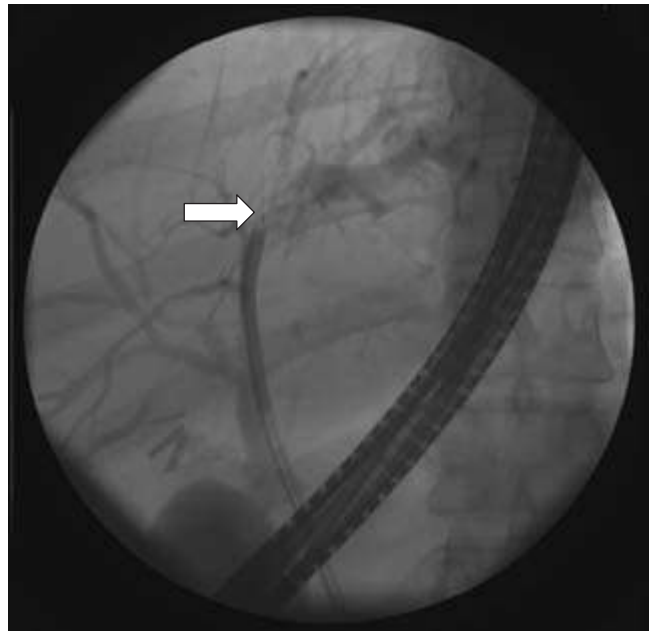


Figure 121.7 Choledochoscope in the left hepatic duct with electrohydraulic lithotripsy probe extended (arrow) prior to lithotripsy (stone not shown).

for smaller stones and sludge by virtue of its ability to inflate to the bile duct diameter and occlude the lumen. By this method, the stones can be swept outside the duct and into the duodenal lumen (Fig. 121.6). Stones larger than 15 mm present a challenge to the endoscopist and often require alternative and complex endoscopic techniques such as the use of a mechanical lithotripter or intraductal shock waves generated by electrohydraulic or laser techniques. A mechanical lithotripter consists of a basket similar to the standard basket used to extract stones but with an additional outer metal sheath against which stones are crushed using a manual cranking device. Shock waves used to fragment bile duct stones are delivered by a thin catheter probe introduced into the channel of a cholangioscope (ultrathin endoscope), which is in turn advanced into the bile duct lumen through the working channel of a duodenoscope (Fig. 121.7). The source of energy used to generate shock waves can be electrical (electrohydraulic) or a laser [70,71]. The procedure is performed under combined endoscopic and fluoroscopic guidance. Intermittent bolus of saline infusion into the bile duct through the cholangioscope is necessary to create a medium for shock wave transmission and to optimize visualization of the duct lumen. Newer lasers that integrate a stone detection system can be used under fluoroscopic guidance only.

In rare situations, the standard basket can become entrapped with a stone in the bile duct and this can be managed with a special lithotripter designed for this urgent condition. If this method fails a number of other techniques are available, and rarely is surgical intervention required. In addition, when complete stone removal fails, insertion of a biliary endoprosthesis or stent provides temporary biliary drainage, minimizing the risk of biliary sepsis before further endoscopic attempts at duct clearance are made [72,73]. Long-term biliary drainage, especially in old and frail patients, has now been questioned as a safe alternative to clearance [74].

Hepatolithiasis, the presence of stones in the intrahepatic biliary tree above the hepatic duct confluence, is difficult to treat and poses a major challenge to the endoscopist. A study from Japan reported the effectiveness and long-term results of peroral cholangioscopic lithotomy for hepatolithiasis in 36 patients over 11 years [75]. Large stones refractory to mechanical lithotripsy were fragmented using electrohydraulic or laser lithotripsy. Extracorporeal lithotripsy was used when the endoscopic approach was difficult. Complete stone removal was achieved in 64% of patients, of whom 78% remained symptom-free. The cumulative recurrence rate for patients who had complete stone removal was 21.7% at 164 months.

Cholelithiasis and choledocholithiasis

When patients present with the combined problem of gallstones in the gallbladder and bile duct simultaneously, there are two questions to answer: (1) what is the best method for clearing the bile duct and (2) what should be done with the gallbladder? The sequential options are: (1) laparoscopic cholecystectomy with laparoscopic bile duct exploration; (2) laparoscopic or open cholecystectomy followed by postoperative ERCP; (3) preoperative ERCP followed by cholecystectomy (laparoscopic or open); (4) open cholecystectomy with open exploration of the bile duct; (5) ERCP and no cholecys-

tectomy; and (6) in special circumstances, a range of additional and much less commonly used surgical and nonsurgical techniques such as percutaneous cholecystostomy, percutaneous access to the bile duct including percutaneous cholangioscopy, techniques for Mirizzi syndrome, management of hepatolithiasis, and approaches to recurrent stones after biliary and nonbiliary upper gastrointestinal surgery. It is unlikely that one option will be appropriate for all clinical circumstances in all centers in all countries as the variables of disease states, patient demographics and risk stratifications, available endoscopic, radiological, and surgical expertise, patient preferences, and health-care economics will all have significant influences on practice [76].

In patients with contemporaneous cholelithiasis and choledocholithiasis, it is usually the latter that dominates the acute clinical presentation and leads to intervention for pain, obstructive jaundice, cholangitis, pancreatitis, or any combination thereof. Less commonly, asymptomatic bile duct stones are discovered incidentally on noninvasive imaging for nonbiliary indications, during evaluation of symptomatic cholelithiasis, or intraoperatively during cholecystectomy. Since the universal adoption of laparoscopic cholecystectomy as the primary method for treating cholelithiasis in the early 1990s, only a minority of surgeons have mastered the techniques required for laparoscopic transcystic or direct choledochal exploration to treat choledocholithiasis, and there has been some reluctance to convert to open bile duct exploration for this indication. Since ERCP became the standard approach for acute presentations of choledocholithiasis in the 1980s and with the overall success of endoscopic bile duct clearance, the question of treating cholelithiasis surgically became a secondary issue.

Although there is evidence that elective and emergent clearance of the bile duct by ERCP had advantages over open bile duct exploration [77–79], the comparison of ERCP therapy with laparoscopic bile duct clearance is much more equivalent for most stones in the elective setting [78,80]. Unfortunately, this surgical option is not always available and the evidence is therefore tempered by local expertise. Following endoscopic bile duct clearance, the decision to leave the gallbladder in situ and follow the patient expectantly (option 5 above) compared with routine laparoscopic cholecystectomy has been studied [80,81]. In a prospective randomized multicenter trial by Boerma et al. [80], 27 out of 59 patients allocated to a "wait and see" policy had recurrent biliary symptoms, of whom 22 underwent cholecystectomy for biliary pain or acute cholecystitis. In a study of Chinese patients over the age of 60 years, Lau et al. [81] reported a 7% occurrence of biliary events (cholangitis, pain) after endoscopic bile duct clearance and laparoscopic cholecystectomy in contrast to 24% after endoscopic duct clearance and leaving the gallbladder in situ. Although their patient recruitment was not consecutive and the very-high-risk surgical patients were excluded, these observations are valuable.

Where the expertise is available, elective management of cholelithiasis and choledocholithiasis by the laparoscopic approach should be offered. The role of ERCP in the management of acute calculous bile duct disease remains central and optimal. The majority of patients after successful endoscopic bile duct clearance should undergo laparoscopic cholecystectomy as early as possible to reduce the risk of a further biliary event or to treat cholecystitis if already present. There will still be a small population of patients, however, considered at high risk for laparoscopic cholecystectomy and, in these, judicious conservative management of the in situ gallbladder is justifiable.

Mirizzi syndrome

First described by Mirizzi [82] in 1948, Mirizzi syndrome, a rare complication of gallstone disease, refers to stone impaction in the neck of the gallbladder or the cystic duct and subsequent inflammation and extrinsic compression of the main bile duct leading to obstructive jaundice and cholangitis. Mirizzi syndrome is classified into two types, type I and type II, based on cholangiographic findings [83]. Type I refers to extrinsic compression of the main bile duct secondary to a stone in the cystic duct or gallbladder and type II occurs when there is a cholecystocholedochal fistula. Type II has been further subdivided into three subtypes based on the size of the defect in the common duct [84] (Fig. 121.8).

Transabdominal ultrasound and CT are often the initial imaging studies performed in suspected cases of Mirizzi syndrome but these studies are limited by regional inflammation and bowel gas [85,86]. Direct cholangiography during ERCP or via the percutaneous transhepatic route is the diagnostic method of choice. A distended gallbladder, extrinsic compression of the main bile duct, and a dilated intrahepatic biliary tree are seen in type I Mirizzi syndrome. A fistulous tract between the gallbladder and bile duct may be seen in type II Mirizzi syndrome. A stone that is fixed in the region of the cystic duct or gallbladder should also raise the possibility of this diagnosis. MRCP is an alternative noninvasive imaging modality that can be utilized to diagnose Mirizzi syndrome [87].

Treatment of Mirizzi syndrome has traditionally been surgical. Endoscopic management consists of drainage and decompression of the bile duct and/or gallbladder [88,89]. This is essential in relieving jaundice and treating cholangitis before surgical intervention. Binmoeller et al. [90] reported the use of electrohydraulic lithotripsy in 14 patients with Mirizzi syndrome who were at high risk for surgery. Stones were impacted in the cystic duct and ranged in diameter from 1.5 cm to more than 3 cm. Insertion of the cholangioscope along with the lithotripsy probe and stone fragmentation was successful in all patients without mortality. One patient had a cystic duct leak of contrast that was attributed to pressure necrosis from an impacted stone. This resolved spontaneously with conservative management. A study of 25

Figure 121.8 Mirizzi syndrome: large stone in the neck of the gallbladder causing compression of the common bile duct.

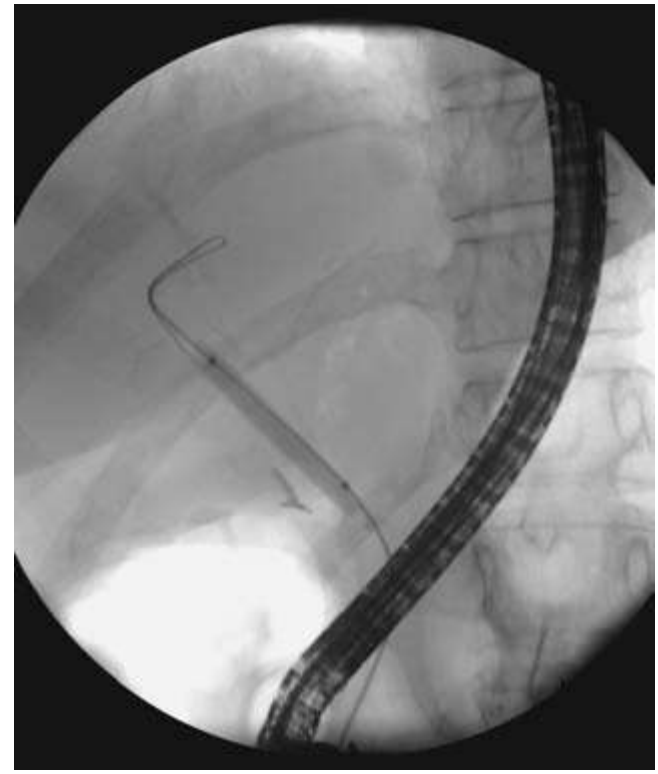
patients with Mirizzi syndrome in whom 13 were managed only by endoscopic therapy such as endoscopic sphincterotomy, bile duct stenting, mechanical lithotripsy, and extracorporeal shockwave lithotripsy achieved complete stone removal in three. Nine patients were treated with long-term biliary stenting [91]. The authors concluded that endoscopic therapy of Mirizzi syndrome is an effective temporizing intervention before surgery and can be the only therapy for patients who are poor surgical candidates. The use of extracorporeal shockwave lithotripsy to fragment stones in the cystic duct in patients with Mirizzi syndrome has also been reported [92].

Bile duct strictures

Benign biliary strictures result from many different etiologies of which the commonest include anastomotic, operative injury and chronic pancreatitis. Patients often present with cholestasis, cholangitis, or secondary biliary cirrhosis [93]. In the past surgical management was the standard approach, with associated morbidity and mortality, but currently endoscopic therapy with dilation and stenting offers a less invasive and an effective line of therapy (Fig. 121.9). The insertion of multiple biliary stents for the treatment of benign strictures has gained wide acceptance in the endoscopy community. In one study [94], 46 patients with chronic pancreatitis and distal common bile duct stricture who presented with obstructive symptoms were divided into two groups: group 1 ($n = 34$) had single stent placement exchanged at 3- to 6-month intervals and group 2 ($n = 12$) had multiple simultaneous stent placement at 3-month intervals [94]. Patients were followed up for a mean period of 4 years. At the end of the treatment period, liver function tests nearly normalized in all patients in group 2, whereas only marginal benefit was noted for patients in group 1. In total, 12% of patients in group 1 had recurrent cholangitis compared with none in group 2. Distal common bile duct stricture diameter increased from a mean of 1.0 mm to 3.0 mm in patients after treatment with multiple stent placements (group 2); no change in diameter was noted in patients treated with a single stent (group 1). Bergman et al. [95] reviewed ERCPs performed on 74 patients with postoperative bile duct stenosis over a 10-year period. The most common surgical procedure performed was cholecystectomy with and without bile duct exploration. Two 10F stents were inserted for a maximum of 12 months and exchanged every 3 months to avoid stent occlusion and risk of cholangitis. Of the 47 patients treated according to protocol, stents were eventually removed in 44 patients who were followed up for a median of 9.1 years. Restenosis occurred in nine patients (20%) at a median of 2.6 months (range 1 week–2 years) after stent removal. Patients presented with cholestasis, jaundice, and cholangitis. Of these patients, four went for surgery and five had repeated stenting. Costamagna et al. [96] reviewed the records of 45 patients with postoperative strictures treated with placement of an increasing number of stents. The maximum number of stents were placed relative to the stricture tightness and distal bile duct diameter until the stricture resolved. Previous surgery in these patients included cholecystectomy (open and laparoscopic), liver transplantation, hepatic trauma with biliary repair, and hepatic resection. The success rate for stent placement was 89% and early complications occurred in 9% (cholangitis, pancreatitis) with stent occlusion requiring early exchange in 18%. In total, 42 patients completed the protocol (mean number of stents 3.2 +/– 1.3; range 1–6) over a treatment period of 12.1 +/– 5.3 months (range 2–24 months). Among 40 patients followed up for a mean of 48.8 months (range 2–11.3 years), there was no recurrence of symptoms caused by recurrent biliary stricture. The authors concluded that this aggressive approach of biliary stenting may improve long-term results for patients with postoperative biliary strictures. The sequential insertion of an increasing number of plastic biliary stents with larger diameters was also found to be an effective therapy for distal common bile duct strictures that developed as late complications of endoscopic sphincterotomy [97]. In addition, the use of self-expandable metal stents (SEMS) in benign bile duct strictures secondary to chronic pancreatitis has been shown to provide successful and prolonged biliary drainage in a selected group of patients [98].

Biliary obstruction secondary to malignant bile duct stricture of pancreatic, biliary or metastatic origin is usually

(a)



(b)

(c)

Figure 121.9 **(a)** Benign right hepatic duct stricture after cholecystectomy. **(b)** Balloon dilation of benign right hepatic duct stricture. **(c)** Plastic stent across benign stricture after balloon dilation.

palliated by stent placement. Polyethylene plastic stents are placed into the bile ducts of patients with tumors that may be surgically resectable, and during initial evaluation when histology or cytology is not yet available. Locally advanced and metastatic tumors of biliopancreatic origin and those patients who are poor surgical candidates are suitable candidates for metal stents (Fig. 121.10). SEMS are commonly used for ease of deployment, high patency rate, and cost-effectiveness despite the high cost of individual stents [99,100]. The patency of metal stents is significantly prolonged compared with polyethylene stents, a median of 273 days vs 126 days in one study [99]. A cost-effectiveness analysis by Arguedas et al. [101] found that initial metal stent placement for palliation of obstructive jaundice in patients with pancreatic cancer was associated with a slightly lower cost per patient and slightly higher quality-adjusted life months compared with a plastic stent (US$13 466 vs US$13 879 per patient and 1.832 vs 1.799 quality-adjusted life months respectively). A prospective multicenter study of Diamond stents (one type of metal stent) in 126 patients with malignant biliary obstruction secondary to inoperable tumor had technical and clinical success rates of 96% and 98% respectively. There were no major procedure-related complications. Stent occlusion occurred in 22% and all were treated with plastic stent placement. Median stent patency was 477 days. Overall median survival was 173 days and the 30-day mortality rate was 13% [102].

Malfunction of SEMS can be attributed to tumor ingrowth or overgrowth, tissue hyperplasia at the ends of the stent, and migration. SEMS can be covered or uncovered by a thin plastic coat. Uncovered stents have a higher occlusion rate but a lower migration rate than covered ones. Some types of covered stents are potentially removable endoscopically [103]. Comparative trials are ongoing.

Tissue sampling of bile duct strictures is important in defining their nature (benign vs malignant) and can be achieved by forceps biopsy, brush cytology and needle aspiration. Biliary brush cytology is the most common method of sampling but has a low sensitivity of 30%–60% and a specificity of over 90% [104–106]. The sensitivity of brush cytology is higher for primary bile duct tumors (80%–86%) and when combined with biopsies of the stricture (63%) [104,106]. One study compared a new long cytology brush with rigid bristles oriented at 45 degrees to the standard brush and found improved cellularity with the long brush; however, cancer detection rates were not improved [107]. Additional molecular techniques such as fluorescence in situ hybridization, digital image analysis, and flow cytometry may improve accuracy [108–110].

Bile leak

Iatrogenic injury to the bile duct is a known complication of cholecystectomy. Postoperative bile leak occurs in 0.1%–0.5% of patients after open cholecystectomy and in 0.8%–1.1% of patients after laparoscopic cholecystectomy [111–114]. Injury to the cystic duct is the most common type of injury. Patients may present with abdominal pain, abdominal tenderness, fever, and continued drainage through the Jackson-Pratt drain when present. ERCP is the most accurate technique for delineating the site of the leak and can offer endoscopic therapy (Fig. 121.11) [115]. A study of 64 patients with postcholecystectomy bile leak over a 5-year period found a 1.1% leak incidence among those who had a laparoscopic approach [113]. Patients presented a mean of 5.3 ± 4.2 days (range 0–21 days) postcholecystectomy with abdominal pain (89%), abdominal tenderness (81%), fever (74%), jaundice (43%), and nausea and vomiting (43%). A biloma was detected by ultrasound in 73% of patients and by CT in 95%. The leak site was identified by hydroxyiminodiacetic acid (HIDA) scanning in 19% of patients and by ERCP in 98% of patients. Leak sites were the cystic duct (77%), accessory duct of Luschka (15%), and the common bile duct, common hepatic duct, and an aberrant intrahepatic duct (8%). Bile duct stones were found in 22% of patients. Rates of intraoperative complications and conversion to open surgery were greater among patients who developed leaks (5.2% vs 0.6% and 33% vs 6.3% respectively, $P < 0.00001$).

In addition to delineating the site of bile duct leak, ERCP can offer endoscopic therapy such as sphincterotomy alone, stent placement alone, a nasobiliary drain, or any combination of these. The purpose of endoscopic therapy is to decrease the pressure gradient between the bile duct and duodenum, allowing preferential flow of bile through the transpapillary route and sealing of the leak site. The optimal endoscopic approach is not well established but most endoscopists insert a single 10F stent across the major papilla. The stent does not have to cross the leak site unless there is a major bile duct injury. One study concluded that endoscopic placement of short transpapillary stents without sphincterotomy is a temporary, effective, and technically simple method of pressure equalization [116]. A retrospective study by Kaffes et al. [117] of 100 patients referred for ERCP for suspected postcholecystectomy bile leak evaluated the outcome of different endoscopic treatment. A total of 93 patients had ERCP therapy: stent alone ($n = 40$), endoscopic biliary sphincterotomy alone ($n = 18$), sphincterotomy and stent ($n = 31$), papillary dilation and stenting ($n = 1$), and no endoscopic therapy ($n = 3$). In the stent alone group, one patient required additional ERCP but not surgery to control the leak. In the sphincterotomy alone group, four patients required surgery and two needed repeat ERCP to control the leak. No additional intervention (endoscopic or surgical) was required for the other groups of patients. The authors concluded that the optimal endoscopic therapy for postcholecystectomy bile leak is stent placement.

Bile duct cysts

Bile duct cysts or choledochal cysts are congenital anomalies typically seen in children and young adults, although they

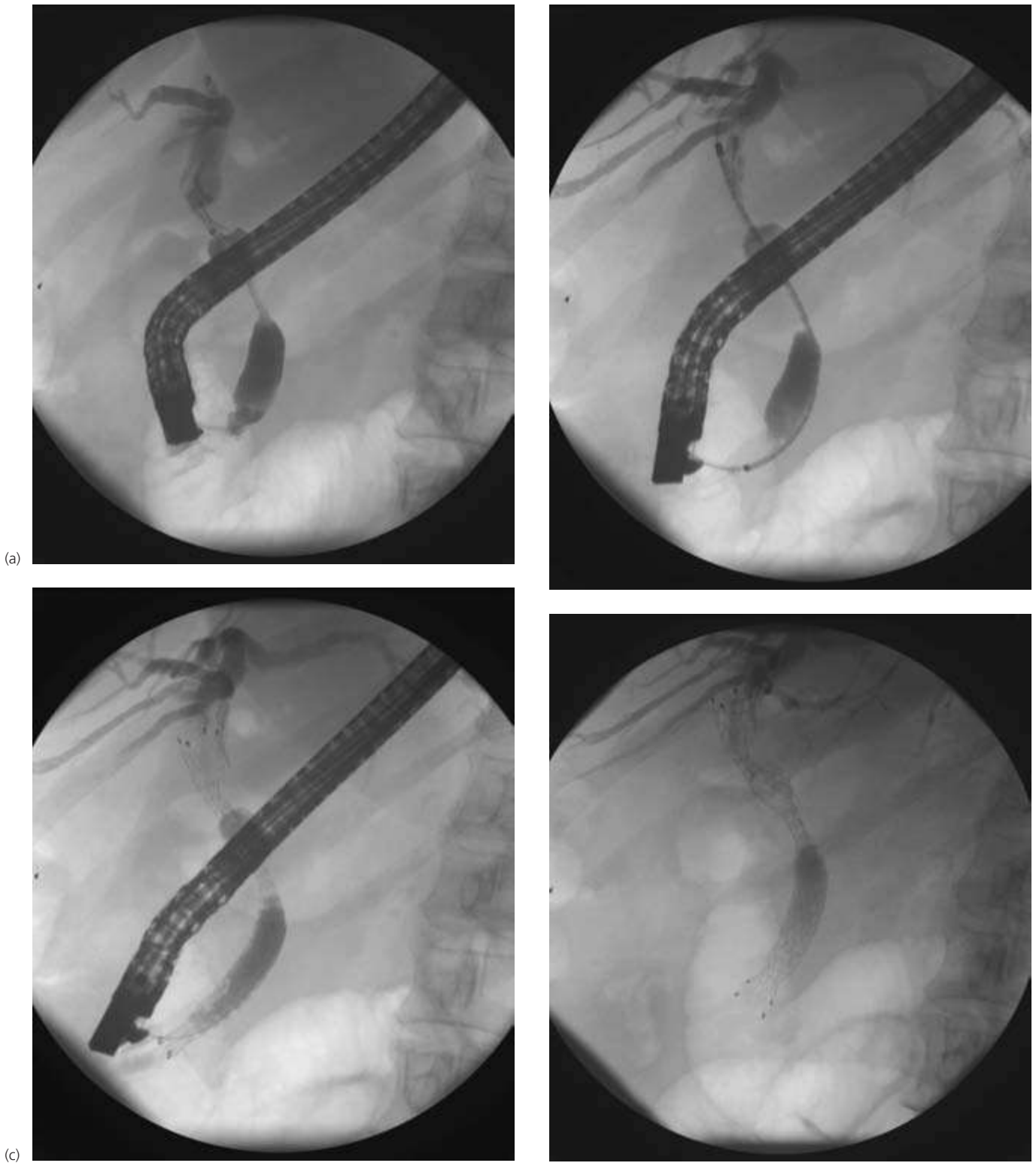


Figure 121.10 **(a)** Gallbladder cancer with two discrete biliary strictures. **(b)** The same patient during placement of an uncovered metal stent. **(c)** Appearance immediately after release of the uncovered metal stent. **(d)** Final position of the uncovered metal stent.

(a)

(b)

(c)

Figure 121.11 **(a)** Leak from cystic duct stump after cholecystectomy. **(b)** Transpapillary stent placed to treat leak. **(c)** Cholangiogram 6 weeks later revealing no leak.

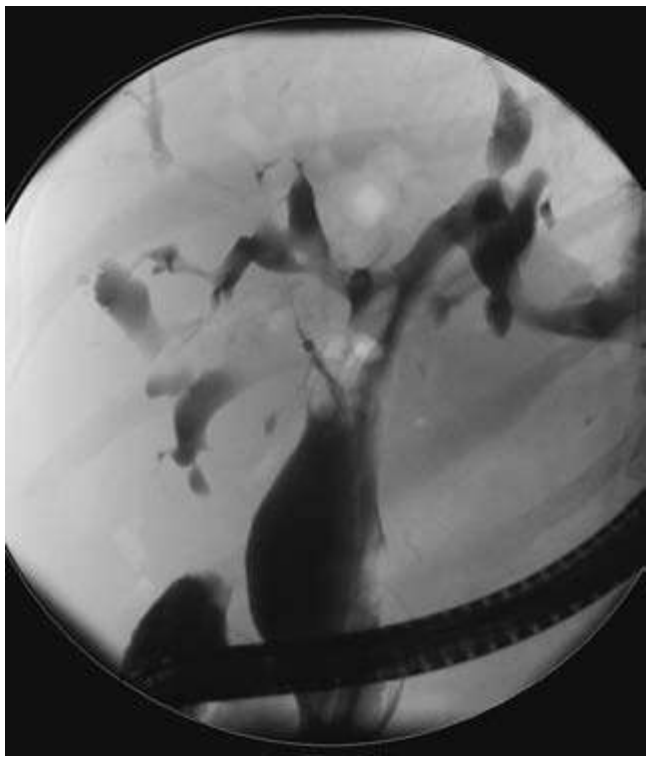


Figure 121.12 Caroli disease.

can be diagnosed at any age. They may affect the intrahepatic biliary tree, extrahepatic biliary tree, or both. They occur with an incidence of 1:13 000–1:2 000 000 live births and are three times more common in females than in males [118–120]. Choledochal cysts are commonly classified into five types according to Todani et al. [121], with type I the most common type and type V the least common [120,122–125]. Type I (80%–90%) involves the extrahepatic bile duct and is subclassified into IA (common type), IB (segmental dilation), and IC (diffuse dilation). Type II (2%) is a diverticulum in the main bile duct. Type III cysts (1.4%–5%) involve the intraduodenal portion of the bile duct and are also referred to as choledochocele. Type IV (13%) is the second most common type and is more frequently seen in adults. Type IV is subclassified into IVA (multiple intra- and extrahepatic cysts) and IVB (multiple extrahepatic cysts). Type V (less than 1%) or Caroli disease refers to single or multiple dilations of intrahepatic bile ducts (Fig. 121.12).

The etiology of choledochal cysts is not clear but the most accepted theory is an association with anomalous pancreatobiliary junction (APBJ) with a long common channel (> 15 mm) [126,127]. In this condition, the junction of the bile duct and pancreatic duct is located outside the duodenal wall and reflux of pancreatic juice can result in inflammation and cystic dilation of the bile duct. The risk of malignancy of the biliary tree (gallbladder and bile duct) is increased when APBJ is present, with an overall risk of 14% in patients with choledochal cysts and 50% in those without cysts [128,129]. Classic presenting symptoms and signs are jaundice, abdominal mass, and pain, which are more common in children than adults (82% vs 25% respectively) [130]. Adults often present with vague abdominal pain, cholecystitis, cholangitis, and pancreatitis [119,130,131]. Choledochal cysts are often suspected on imaging studies such as abdominal ultrasonography and CT scan carried out for nonspecific abdominal complaints. ERCP usually establishes the diagnosis, classifies bile duct cysts, and detects the presence of APBJ. MRCP is being increasingly used as a noninvasive imaging modality for various pancreaticobiliary disorders and has been shown to have an overall detection rate for choledochal cysts of 96% [132].

Surgical management of choledochal cysts (cyst excision and biliary reconstruction based on the type of cyst) is the recommended approach to avoid the risk of biliary tract malignancy. Several surgical techniques have been reported such as complete resection of affected bile ducts with Roux-en-Y reconstruction, cyst enterostomy, cyst excision and hepaticojejunostomy, cyst excision and choledochoduodenostomy, and extrahepatic cyst excision with a Roux-en-Y hepaticojejunostomy [133–135]. Management of choledochocele is controversial and endoscopic therapy by biliary sphincterotomy has been recommended [136,137]. In one study, 15 symptomatic patients with choledochocele were identified out of 1019 ERCP referrals over a 7-year period [137]. In total, 12 were treated by endoscopic sphincterotomy and 3 by surgery. Of the 14 patients who had a complete follow-up, 11 were asymptomatic and had normal liver biochemical tests at a median of 18 months (range 12–97). One patient died of a misdiagnosed ampullary carcinoma arising in the choledochocele. The authors concluded that choledochocele, when recognized, should be treated by endoscopic sphincterotomy and that an ampullary carcinoma may develop in a choledochocele.

Pancreas

Endoscopic retrograde cholangiopancreatography and gallstone pancreatitis

The goal of therapeutic ERCP in the setting of acute gallstone pancreatitis is to clear the bile duct of stones that, if left in place, may worsen the severity of pancreatitis and increase the risk of further attacks. Urgent ERCP and sphincterotomy in gallstone pancreatitis has clearly been shown to improve morbidity and mortality in patients with severe attacks but has little or no benefit in patients with mild pancreatitis as the majority of these patients pass the stones spontaneously. The most cited randomized controlled trials are from the United Kingdom, Hong Kong, Poland, and Germany [138–141]. A metaanalysis of these studies has shown that ERCP with endoscopic sphincterotomy reduces morbidity and mortality

Table 121.1 Endoscopic retrograde cholangiopancreatography (ERCP) in biliary pancreatitis for patients with predicted mild attacks

		Morbidity (%)		Mortality (%)	
Country/ref.	*n*	ERCP	Conventional	ERCP	Conventional
United Kingdom [138]	68	11.8	11.8	0	0
Hong Kong [139]	69	17.6	17.1	0	0
Poland [140]	155	10.0[a]	25.3	0	5.3
Germany [141]	160	41.7	47.4	2.4	0

a $P < 0.05$ compared with conventional treatment.
Adapted from ref. 143.

Table 121.2 Endoscopic retrograde cholangiopancreatography (ERCP) in biliary pancreatitis for patients with predicted severe attacks

		Morbidity (%)		Mortality (%)	
Country/ref.	*n*	ERCP	Conventional	ERCP	Conventional
United Kingdom [138]	53	24.0[a]	60/7	4/0	17/9
Hong Kong [139]	58	13/3[a]	53/6	0	17/9
Poland [140]	50	39/1[a]	74/0	4/3[a]	33/3
Germany [141]	46	65.4	70.0	23.1	14.3

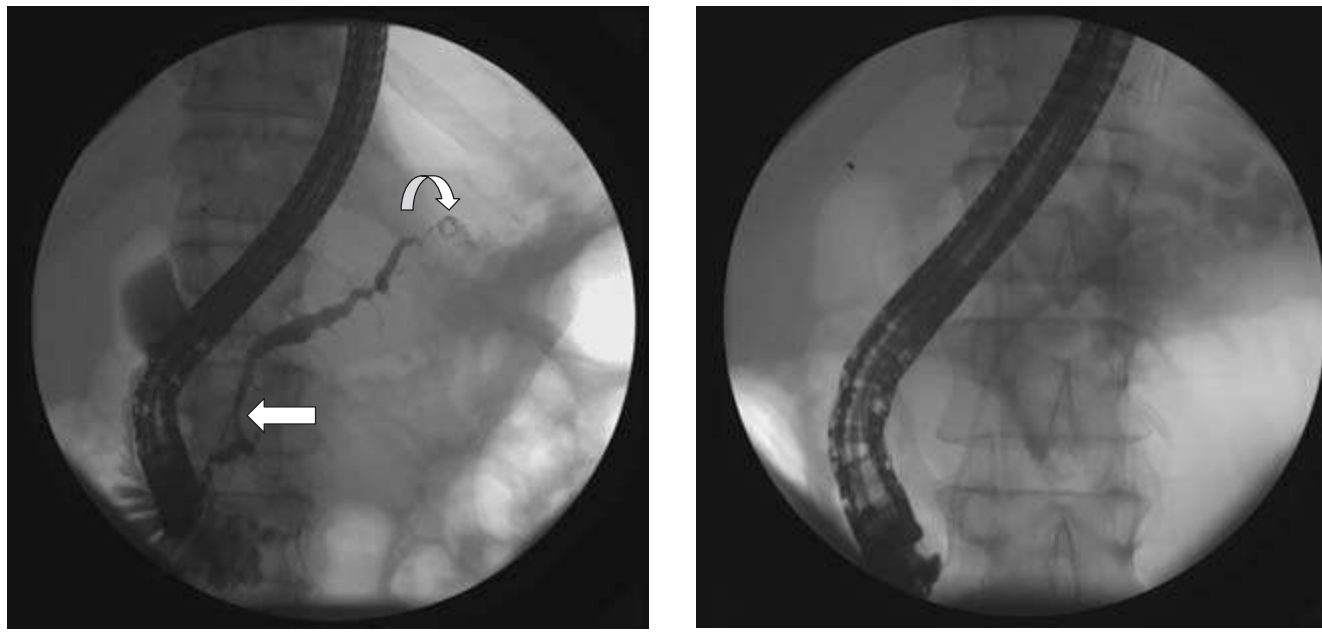
a $P < 0.05$ compared with conventional treatment.
Adapted from ref. 143.

of patients with acute biliary pancreatitis, and treating 26 such patients is predicted to save one life [142]. Tables 121.1 and 121.2 summarize the results of these studies with regard to morbidity and mortality rates in patients who underwent therapeutic ERCP vs conventional treatment in the setting of predicted mild and predicted severe pancreatitis [143]. A prospective randomized controlled trial has shown decreased morbidity in patients who underwent early ductal decompression by ERCP and sphincterotomy (within 48 h) for gallstone pancreatitis compared with those who had therapy after 48 h of the onset of symptoms [144]. An algorithm has been proposed by the senior author [143] which indicates that those patients presenting and continuing with mild pancreatitis require supportive therapy and a subsequent cholecystectomy, preferably during the same admission, whereas those patients in whom evidence of biliary obstruction develops, with or without cholangitis, those deteriorating after an initial mild attack, or those presenting with severe pancreatitis from the outset should be considered for urgent ERCP and sphincterotomy for removal of bile duct stones. Cholecystectomy should be planned for the same admission, if possible, but can be delayed after sphincterotomy because there is a degree of protection from further pancreatitis in this circumstance.

Chronic pancreatitis

Chronic pancreatitis is a progressive disease with persistent inflammation, fibrosis, atrophy of the gland, and ductal abnormalities. Chronic pancreatitis causes significant morbidity related to chronic abdominal pain, loss of exocrine and endocrine function, and complications such as stone disease and pancreatic fluid collection. The severity of chronic pancreatitis is graded according to Cambridge criteria that classify the disease into equivocal, mild, moderate, and severe based on main duct and side branch abnormalities and other findings such as cystic cavities and stones [145]. The pancreatic duct pressure may be elevated secondary to strictures, stones, or outflow obstruction at the level of the major papilla (Fig. 121.13). The goal of therapeutic ERCP is to decompress the pancreatic ductal system and relieve the obstruction with the hope of minimizing attacks of recurrent pancreatitis and alleviating chronic abdominal pain.

Benign strictures and stone disease of the main pancreatic duct can result in ductal obstruction and hypertension, which may be the basis of relapsing pain and recurrent pancreatitis. The strictures are treated by balloon or catheter dilation and insertion of a plastic stent that is exchanged every 2–4 months for up to 1 year [146]. In about 75% of patients symptomatic improvement is immediate, but ERCP-related



(a) (b)

Figure 121.13 (a) Chronic pancreatitis with stricture in the head (straight arrow) and spontaneous leak in the tail (curved arrow). **(b)** Stent placement to treat tail leak.

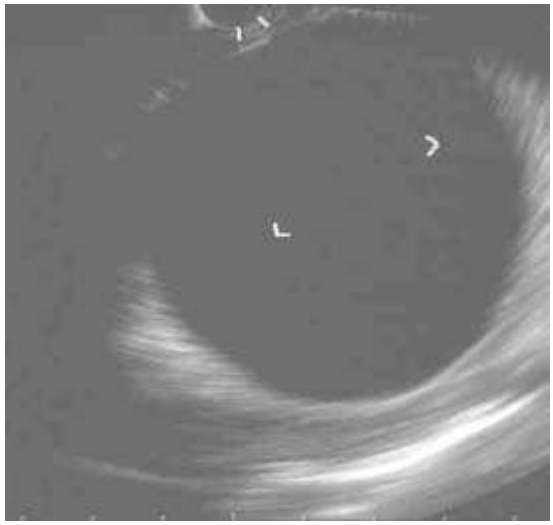
pancreatitis and symptomatic stent occlusion are common [147,148]. Management of pancreatic duct stones in patients with symptomatic chronic pancreatitis is a challenging issue to the endoscopist. Stone extraction is technically feasible if the stones are small, few in number, located in the head of the pancreas, and not impacted [146]. A pancreatic sphincterotomy is often required and the stones are removed with a basket or balloon. Stones located in the upstream duct proximal to a stricture require stricture dilation before extraction. The use of endoscopic balloon dilation of the pancreatic sphincter before stone extraction in patients with chronic pancreatitis has been shown to be safe and effective [149]. Pancreatoscopy and electrohydraulic lithotripsy of pancreatic duct stones has been advocated as an adjunct option to conventional methods and extracorporeal shock wave lithotripsy in the treatment of refractory stones [150]. In a study of six patients with pancreatic duct stones, complete or partial stone clearance was successful in five patients, who experienced complete relief of abdominal pain for at least 6 months following the procedure [150]. No complications were reported.

In patients with recurrent abdominal pain and pancreatic ductal obstruction, the mainstay of therapy is ductal decompression to decrease the pancreatic duct hypertension and maintain outflow of pancreatic secretions. In a multicenter study of 1000 patients with painful chronic pancreatitis and pancreatic ductal obstruction secondary to strictures or stones treated endoscopically and followed up for 2–12 years, relief of pain was seen in 65% and surgical intervention was performed in 24% [151]. A prospective randomized trial of 72 patients with pancreatic ductal obstruction and pain comparing surgery (resection, drainage) and endoscopic therapy (sphincterotomy and stenting and/or stone removal) found, at the 5-year follow-up, complete absence of pain to be more frequent after surgery (37% vs 14%, respectively), with the rate of partial relief being similar (49% vs 51%, respectively) [152]. In a study of 22 patients with pain and a dilated pancreatic duct who underwent endoscopic therapy, pain disappeared and analgesics could be discontinued in all patients immediately after endotherapy. Six patients were pain-free after a mean period of 5.5 years. Four patients underwent surgery for frequent pain relapses after a mean period of 2.5 years. Six patients were lost to follow-up [153].

Pancreatic stent placement has been shown to be effective in reducing pain in two small series of patients with malignant pancreatic duct strictures [154,155].

Pancreatic duct disruption and fluid collection

Pancreatic duct disruption and subsequent fluid collection formation can be the result of acute or chronic pancreatitis. A pseudocyst is a collection of amylase-rich pancreatic juice enclosed by a wall of nonepithelialized, fibrous, or granulation tissue [156]. Symptomatic pseudocysts may be drained percutaneously (radiologically), surgically, or endoscopically [157,158]. Endoscopic drainage of pancreatic pseudocysts and pancreatic necrosis can be performed by using transpapillary or transmural (transgastric, transduodenal) placement of endoprostheses [159]. The transpapillary approach is utilized for pseudocysts communicating with the main pancreatic duct, with the tip of the stent positioned in the cyst cavity



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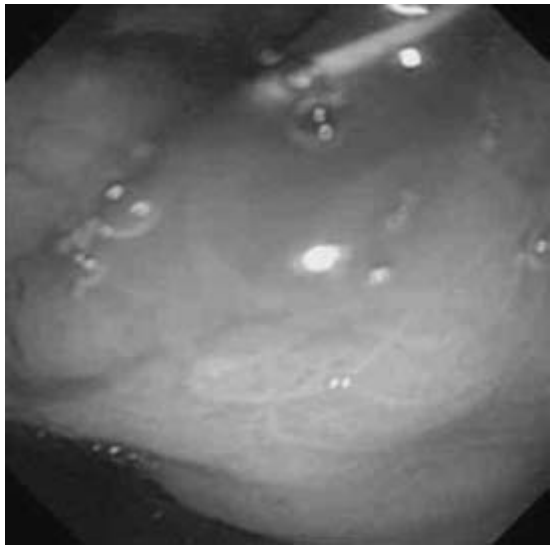

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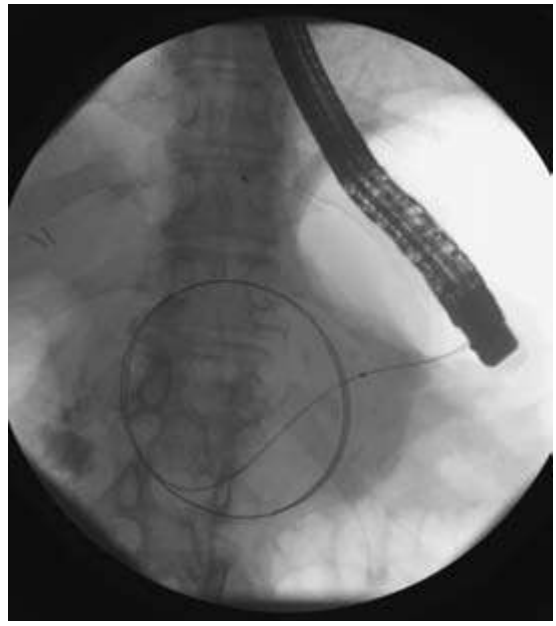

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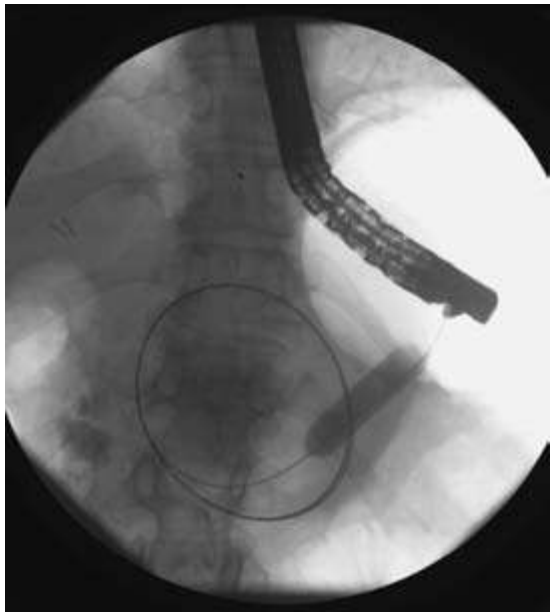

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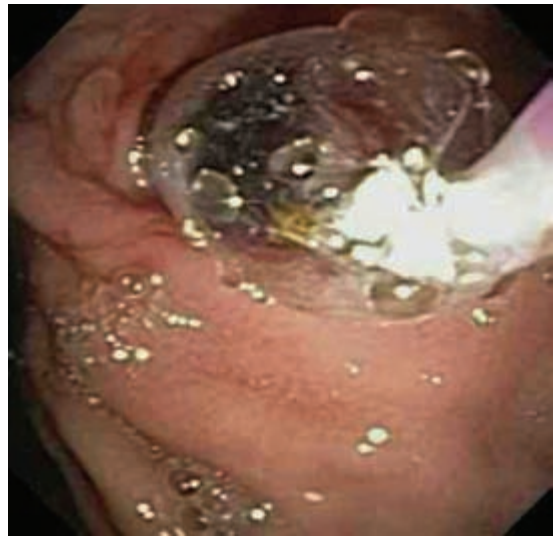

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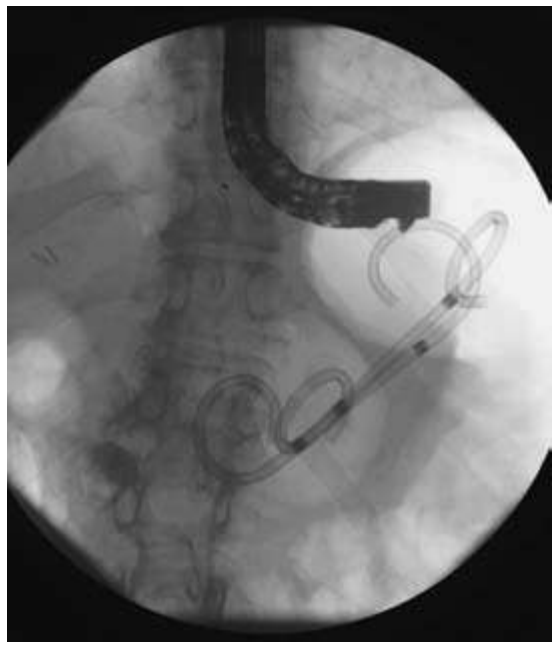

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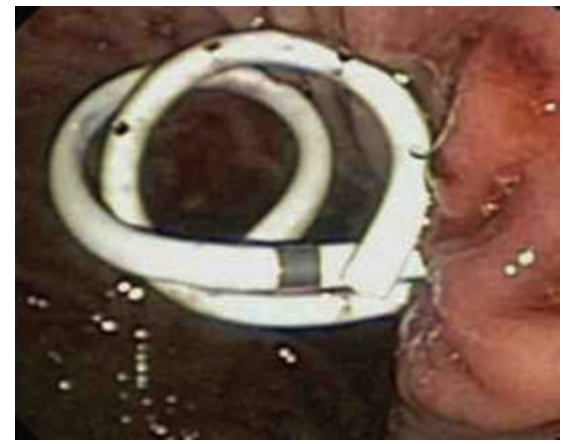

(h)

Figure 121.14 **(a)** Endoscopic ultrasound (EUS) image of a 5-cm pseudocyst. **(b)** EUS-guided puncture of the pseudocyst with a 19G needle. **(c)** Guidewire advanced through the needle across the gastric wall. **(d)** Guidewire coiled within the pseudocyst. **(e)** Balloon dilation of the cystogastrostomy to 12 mm. **(f)** Endoscopic view of cystogastrostomy balloon dilation. **(g)** Two double pigtail stents deployed across the cystogastrostomy. **(h)** Endoscopic view of the two double pigtail stents.

after a pancreatic sphincterotomy [160]. Fluid collections not communicating with the pancreatic duct are often drained using the transmural approach. The goal is to establish a communication between the cyst cavity and gastric or duodenal lumen for continuous drainage of pancreatic juice. The major prerequisites for such a procedure are a distance between the cyst and gastrointestinal tract of 10 mm or less and an obvious bulge on endoscopy [160] or a window for needle puncture demonstrated on EUS. The steps for the standard procedure are summarized as follows: entry into the cyst cavity, placement of a guidewire within the cyst, dilation of the track between the cyst and gastric or duodenal lumen, and double pigtail stent placement (Fig. 121.14).

Endoscopic transmural drainage of pancreatic pseudocysts was first described more than 20 years ago [161,162]. There are a variety of entry methods into the pseudocyst cavity [163–166]. Transmural entry is obtained by entering the collection at the point of maximal endoscopically visible extrinsic compression, by using a modified papillotome, dedicated cystotome, or needle-knife with or without electrocautery, which in turn may be performed at the site of prelocalization with a sclerotherapy needle [167,168]. Other endoscopists have described both localization and entry into the collection by using a large-caliber needle without electrocautery using the Seldinger technique [166].

The use of EUS before the procedure or EUS-guided drainage has gained popularity and importance. EUS can be used before considering drainage of a pancreatic fluid collection for four reasons. First, in a patient with a pancreatic collection following a documented episode of pancreatitis, EUS allows assessment of the collection for the presence of significant solid debris, which may alter the management strategy. Second, if the endoscopist is uncertain whether the collection in question represents a true pseudocyst or other noninflammatory cystic lesion, EUS allows one to obtain a definitive diagnosis by analyzing both the ultrasonographic features and cyst contents aspirated during EUS [169]. Third, EUS can detect the presence of varices between the gastric and cyst wall and, finally, EUS can mark a site for needle puncture. Once the decision has been made to proceed with endoscopic drainage, EUS may be used to guide transmural drainage. It has been shown that endosonographic imaging of pancreatic pseudocysts before drainage provides important information, such as the intervening vessels or distance between the cyst and gut wall, and may change management in a subset of patients [170]. Although there is no definite evidence that transgastric drainage is superior to transduodenal drainage, the long-term patency of the transduodenal drainage track may reduce the recurrence rate of pancreatic pseudocysts [171].

To explore the practice of pseudocyst drainage among different endoscopists, a web-based survey was distributed by the author to endoscopists who are members of the American Society for Gastrointestinal Endoscopy (ASGE) and who

practice in the United States and abroad [172]. Of the 3054 endoscopists to whom the survey was sent, 266 (8.7%) replied; 198 performed pseudocyst drainage (103 [52%] from the United States and 95 [48%] international members). The median of the total number of drainages per physician was 15 (range 1–364). The transgastric route was the most commonly used drainage route (65%). Transmural entry was performed using a needle-knife in 53% of transmural drainages that were not EUS guided. The number of stents placed ranged from one to five and these remained in place for 2–30 weeks. CT scan was used before drainage by 95% of all respondents. EUS imaging was used before drainage by 72 of 103 (70%) US endoscopists compared with 56 of 95 (59%) international endoscopists ($P = 0.1$). EUS-guided drainage was used by 56% of the US endoscopists compared with 43% of the international endoscopists ($P = 0.06$).

Pancreas divisum

Pancreas divisum, the failure of fusion of the dorsal and ventral pancreatic ductal systems during embryogenesis, is the most common variant of pancreatic ductal anatomy and occurs in about 10% of individuals (Fig. 121.15) [173]. The relationship between pancreas divisum and pancreatitis remains controversial. In a retrospective review of ERCPs carried out in 6850 patients, pancreas divisum was found in 0.5% [174]. Acute pancreatitis was associated with 17.1% of those with pancreas divisum and chronic pancreatitis with 28.6%.

Endoscopic therapy of pancreas divisum in patients with recurrent pancreatitis and abdominal pain aims to decompress the dorsal pancreatic duct by stent insertion and minor papillotomy. In a prospective trial of patients with pancreas divisum and recurrent pancreatitis randomized to dorsal pancreatic duct stent placement ($n = 10$) or control ($n = 9$) and followed for a mean of 28.6 months and 31.5 months, respectively, statistically significant fewer hospitalizations and emergency room visits were seen in the stented group [175]. Lehman et al. [176] evaluated the outcome of minor papillotomy in 52 patients with pancreas divisum and acute recurrent pancreatitis, chronic pancreatitis, and chronic pain. The mean duration of symptoms was 5.1 years before endoscopic therapy. A short stent was placed in the dorsal duct and a sphincterotomy was performed using a needle-knife over the stent. The stent was removed after 2 weeks and patients were followed for a mean of 1.7 years. Patients with acute recurrent pancreatitis had a significant reduction in the mean symptom score and number of hospital days per month compared with the group of patients with chronic pain and chronic pancreatitis. In addition, the group of patients with acute recurrent pancreatitis had significant improvement postpapillotomy compared with those with chronic pancreatitis (76.5% vs 27.3%, $P = 0.01$) or chronic pain (76.5% vs 26.1%, $P = 0.002$). Stent-induced ductal changes were seen in 15% of patients. The authors of the study concluded that minor papillotomy should be considered for the group of patients with pancreas divisum and acute recurrent pancreatitis. A retrospective study of 53 patients with pancreas divisum treated with endoscopic minor papillotomy and followed for a median of 29 months reported an overall long-term improvement in 32.1%, more favorable for the group of patients with recurrent pancreatitis [177]. Injection of botulinum toxin into the minor papilla of five patients with pancreas divisum and idiopathic recurrent pancreatitis

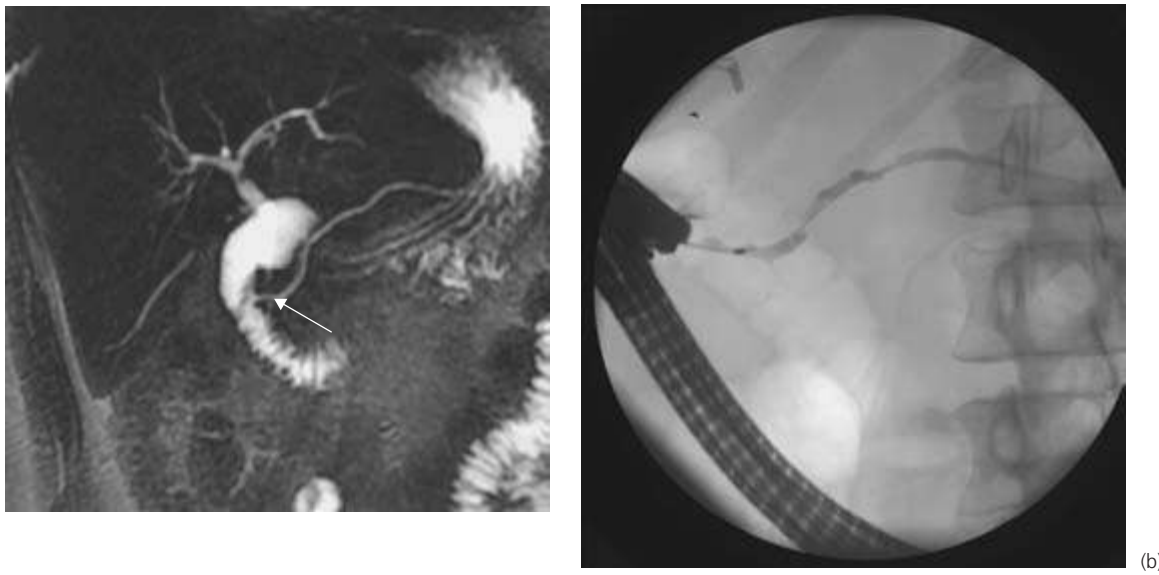

Figure 121.15 **(a)** Magnetic resonance cholangiopancreatography (MRCP) showing the dorsal pancreatic duct crossing the bile duct (arrow) characteristic of pancreas divisum. **(b)** Endoscopic retrograde cholangiopancreatography (ERCP) in pancreas divisum with contrast injected via the minor papilla and the guidewire in the dorsal pancreatic duct.

was found to be technically feasible and safe and to offer short-term relief of symptoms [178].

Autoimmune pancreatitis

With the report of a case of chronic inflammatory sclerosis of the pancreas with a possible underlying autoimmune mechanism in 1961 by Sarles et al. [179], awareness was brought to this relatively new entity, and the concept of "autoimmune pancreatitis" (AIP) was proposed by Yoshida et al. [180] in 1995. The accepted terminology for autoimmune pancreatitis is lymphoplasmacytic sclerosing pancreatitis, although the former designation is more in common usage.

The epidemiology of AIP is not well understood but a prevalence of 5% has been reported among patients with chronic pancreatitis [181]. The disease is more common in males with a mean age of incidence of 58 years. The coexistence and association of AIP with other autoimmune diseases such as primary sclerosing cholangitis, Sjogren syndrome, and diabetes mellitus have been reported [182–185]. Diagnostic criteria have been proposed by the Japan Pancreas Society [186], including: (1) pancreatic imaging studies show diffuse narrowing of the main pancreatic duct with irregular walls and diffuse enlargement of the pancreas; (2) laboratory data demonstrate abnormally elevated levels of serum gamma-globulin and/or IgG, or the presence of autoantibodies; and (3) histopathological examination of the pancreas shows fibrotic changes with lymphocyte and plasma cell infiltration. For diagnosis of AIP, criterion 1 must be present along with 2 and/or 3 (Fig. 121.16).

Several imaging modalities are utilized to assist in the diagnosis of AIP. A retrospective study of 29 patients (25 males, mean age 57.1 years) with a histopathological diagnosis of AIP reviewed findings on different imaging techniques [187]. CT scan showed focal and diffuse pancreatic enlargement. Similar findings were seen on magnetic resonance imaging (MRI), along with a rim-like enhancement in one patient. On MRCP, pancreatic duct strictures were seen in two patients and a sclerosing cholangitis-like appearance was seen in one patient. EUS demonstrated diffuse enlargement of the pancreas ($n = 13$) and a mass in the head region ($n = 6$). ERCP showed a distal common bile duct stricture ($n = 12$), irregular narrowing of intrahepatic ducts ($n = 6$), diffuse and irregular narrowing of the pancreatic duct ($n = 9$), and focal stricture in the proximal duct ($n = 6$). Follow-up CT scan performed within 6 months of steroid therapy (40 mg/day of prednisolone for 4–6 weeks) showed resolution of the common bile duct stricture and CT abnormalities in three patients. ERCP showed similar findings in three patients. One patient developed a proximal pancreatic duct stricture after 12 months and another had diffuse progression of pancreatic duct strictures within 2 months.

Multiple bile duct strictures with morphological features similar to those seen in primary sclerosing cholangitis (PSC) have been associated with AIP. This entity of atypical PSC or sclerosing pancreaticocholangitis has been shown to respond dramatically to steroids [188–192]. The cholangiographic appearance of the bile duct strictures has been proposed to allow differentiation between strictures of PSC (band-like, beaded or pruned appearance, diverticulum-like) and those strictures associated with AIP (segmental, long with prestenotic dilations, and distal common bile duct strictures) [193].

Intraductal papillary mucinous neoplasm

Intraductal papillary mucinous neoplasm or IPMN is a cystic neoplasm of the pancreas that involves the main pancreatic duct, side branches or both. It is characterized by intraductal growth of mucin-producing epithelium, segmental or diffuse ductal dilation, and gaping or fish-mouth papilla from which large amounts of mucin flow. Around 75% of IPMN arise from the main pancreatic duct in the head of the pancreas whereas the branch duct type arise from the uncinate process (most common) or tail of the pancreas [194]. The true incidence of IPMN is not known but it was found in 19% of pancreatic neoplasms in one surgical series [195]. IPMN occurs more commonly in elderly men who often present with recurrent pancreatitis. Jaundice and weight loss can be the presenting symptom in cases with malignant transformation.

A number of imaging modalities are used in clinical practice to diagnose IPMN including CT, EUS, MRI, and ERCP. A patulous and gaping papilla (major, minor) with excretion of copious amounts of mucin is the hallmark for IPMN [196,197] and is seen on endoscopic views during ERCP (Fig. 121.17). A pancreatogram is remarkable for an ecstatic and dilated pancreatic duct and intralumenal filling defects secondary to mucin or mural nodules [198]. During ERCP, pancreatic juice can be aspirated for cytology and transpapillary brushings and biopsies can be obtained from suspicious lesions seen on pancreatograms. In one study, pancreatic juice cytology had a sensitivity of 40% for malignant IPMN and it was more useful in cases with main duct disease with mucin hypersecretion and mural nodules [199]. In another study, pancreatic juice cytology had a higher sensitivity for IPMN when collected by peroral pancreatoscopy than with a catheter (62.2% and 38.2% respectively) [200].

Pancreatoscopy involves the use of an ultrathin endoscope (pancreatoscope, baby scope) that is introduced through the working channel of a duodenoscope (mother scope) and advanced into the pancreatic duct through the major papilla. The ductal epithelium can be examined and directed biopsies of suspicious mural nodules or masses can be performed. Pancreatoscopy has been increasingly used as an adjunct to other imaging modalities for diagnosis and determining the extent of IPMN. In one series of 30 patients with IPMN, the diagnosis after surgery and histological confirmation was adenocarcinoma ($n = 6$), adenoma ($n = 20$), and hyperplasia ($n = 4$) [201]. ERCP findings were enlarged papilla (63%), dilated main pancreatic duct (87%), and cystic lesion (73%). On EUS and intraductal ultrasonography (IDUS), four lesions were greater than 10 mm and all had adenocarcinoma. None

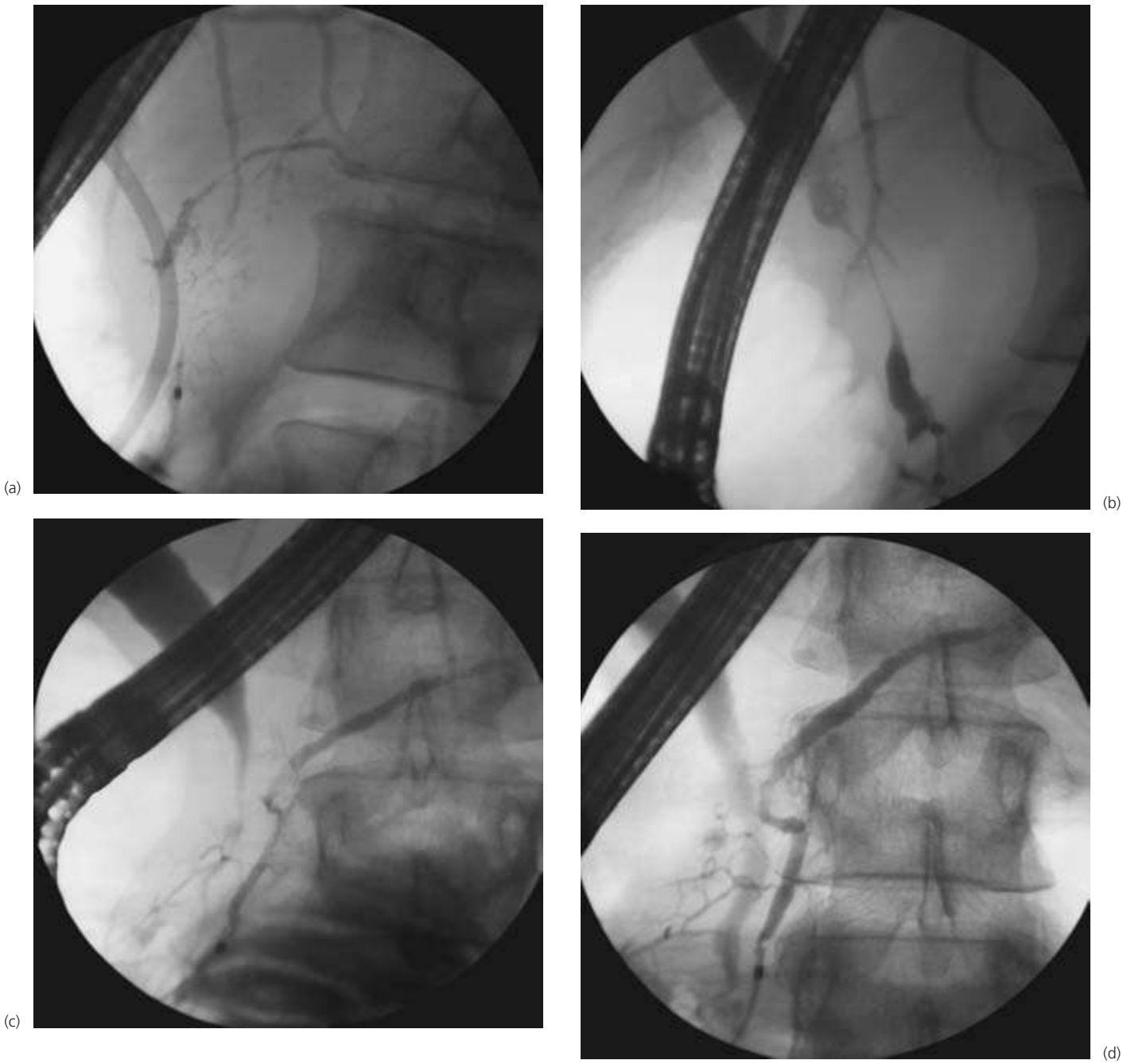

Figure 121.16 (a) A 36-year-old with jaundice and recurrent pancreatitis. The pancreatogram shows an irregular, nondilated pancreatic duct with multifocal stricturing consistent with autoimmune pancreatitis. **(b)** The same patient with a high-grade common bile duct stricture simulating malignancy. **(c)** Improvement in common bile duct stricture and pancreatogram after 1 month of steroid therapy. **(d)** Marked improvement on endoscopic retrograde cholangiopancreatography (ERCP) after 2 months of steroids.

of the lesions of less than 3 mm had cancer. Peroral pancreatoscopy was performed in 26 patients and papillary tumor was found in 100% of the cases of carcinoma and 13% of the cases of adenoma. Redness and proliferation of blood vessels were seen in 83% of carcinomas, 31% of adenomas, and not at all in hyperplasia. Fish egg-like appearance was noted in 31% and 25% of the cases of adenoma and hyperplasia respectively. The sensitivity for detecting lesions greater than 3 mm compared with histology was 67% (92% by EUS and 100% by IDUS). Biopsies through the pancreatoscope and pancreatic juice cytology were positive in 50% of adenocarcinomas. The results of this study suggest that pancreatoscopy is an adjunct to other diagnostic modalities for the diagnosis of IPMN. Therapy remains operative in good surgical candidates.

(a)

(b)

Figure 121.17 **(a)** Gaping "fishmouth" papilla with mucus pathognomonic of main duct intraductal papillary mucinous neoplasm (IPMN). **(b)** Markedly dilated main pancreatic duct with filling defects consistent with mucus in the same patient with main duct IPMN.

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A ENDOSCOPIC

122 Gastrointestinal dilation and stent placement

James D. Lord, Richard A. Kozarek

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The primary goal of treatment of any stenosis is lumenal enlargement and amelioration of obstructive symptoms. Such symptoms depend on the stricture etiology and site and may include dysphagia, nausea and vomiting, abdominal pain and obstipation, or frank bowel obstruction. This chapter compares the various technologies available for the treatment of stenoses with regard to ease and site of application, patient tolerance, cost–benefit ratio, and available safety and efficacy data.

Historically, the dilation of gastrointestinal strictures was limited to accessible anatomical areas, primarily the esophagus or anorectum. With the advent of endoscopically or radiographically placed polyethylene balloons, a variety of gastric, small bowel and colonic strictures also became amenable to dilation. Likewise, prosthetic stent placement was initially limited to the esophagus and subsequently expanded into the pancreaticobiliary tree. However, expandable metallic stent technology has allowed treatment of previously inaccessible and more central (e.g., gastric, duodenal, colorectal) stenoses.

Whereas all the current dilating systems achieve efficacy by either stricture stretch or fracture, data are sparse regarding the specific mechanism of action by which each individual dilating system works. The few existing small prospective studies comparing the efficacy and side effects of various dilating systems or techniques has failed to demonstrate consistent or significant differences in outcomes. Similarly, the few available randomized studies comparing different expandable prostheses in the esophagus have shown little difference in efficacy or safety between different stent brands. However, technological improvements incorporated into some prostheses do appear to reduce the risk of certain complications, such as stent migration or overgrowth, or gastroesophageal reflux. Data regarding central stent placement has been more limited, but existing randomized trials agree that prostheses compare favorably with surgery for malignant colorectal or gastroduodenal obstructions.

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Dilation

Theoretical considerations

The basic goals of stricture dilation include safe and effective lumenal enlargement plus the prevention of restenosis [1]. Efforts to achieve the latter goal may include proton pump inhibitors after esophageal bougienage for a reflux-induced stricture [2], placement of a prosthesis after dilation of an esophageal malignancy, or elective surgical resection, plasty, or bypass of a dilated area after dilation or stent placement for an obstructing rectosigmoid malignancy.

Although the exact mechanisms by which dilation results in lumenal enlargement remain uncertain, circumferential stretch and stricture split are two likely possibilities. The former presupposes considerable elasticity in circumferential fibrous tissue, and the latter an inherent rigidity in which dilation results from one or several longitudinal tears. It is unlikely that a pylorus of 3 mm diameter can be dilated to 10 or 15 mm without significant laceration of scirrhous tissue and, potentially, of muscle. Indeed, gross longitudinal tears and histological disruption of collagen and circular muscle have been described after esophageal bougienage for fibrous stenoses and achalasia [3]. Most perforations associated with dilation, in turn, appear to be an extension of these tears.

Among the earliest dilation techniques, bougienage was first used by Fabricius d'Acquapendente, who used a wax dilator for a food impaction [4]. The term *bougienage* is derived from the Algerian town of Boujiyah, the medieval capital of the wax candle trade. Cork and woven silk dilators have also been used, the latter since at least the 16th century, when Thomas Willis used a cork-tipped whalebone to treat a probable achalasia patient.

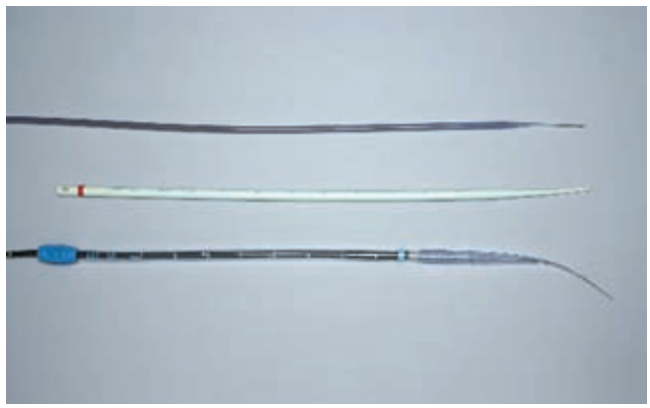


Figure 122.1 Dilating systems: Maloney **(top)**, Savary–Gilliard **(middle)**, and Optical **(bottom)** dilators. The latter two are wire-guided, with the Optical dilator also admitting an endoscope for direct visualization.

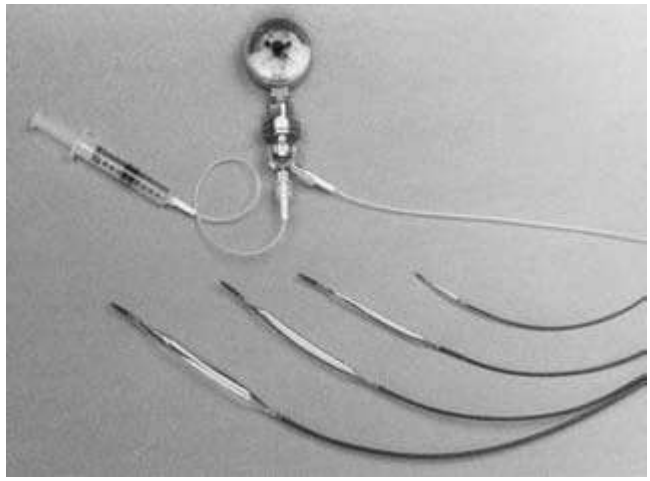


Figure 122.2 Polyethylene balloon dilating kit includes mercury manometer and injection syringe. Balloons are passed over an endoscopically or radiographically placed guidewire.

Table 122.1 Dilators for gastrointestinal stenoses

Mercury bougies
Blunt-tip (Hurst)
Tapered-tip (Maloney)
Guidewire directed
Metal olives (Jackson–Plummer, Eder–Puestow)
Hollow-core polyvinyl (Savary–Gilliard, American)
Neoplex stepped-diameter (Celestin)
Spindle-shaped (Keymed Advanced dilator)
Polyethylene balloons
Through the scope (TTS)
Guidewire facilitated
Controlled radial expansion (CRE)
Latex balloons
Brown–McCardy
Mosher
Miscellaneous
Woven silk (Jackson, Phillips)
Graded, plastic oversheath
Tapered-tip endoscope
Balloon or tape affixed to endoscope shaft
Electronic/mechanical (Starck variant)
Tapered transparent oversheath (Optical dilator)

Dilating modalities can be divided into mercury bougies, guidewire-directed dilators (Fig. 122.1), polyethylene balloons (Fig. 122.2), and miscellaneous types (Table 122.1) [1]. Mercury-filled dilators, ranging in size from 3 to 20 mm (10–60Fr), can be subdivided into the original, blunt-tipped Hurst bougies, and tapered-tip variants called Maloney bougies. Originally passed without fluoroscopic control, these dilators are now used infrequently by many practitioners.

Historically, guidewire-directed dilators have included Jackson–Plummer bougies and Eder–Puestow dilators. These include a triple olive variant, in which multiple metal olives of increasing diameter are placed on the same dilating shaft [5]. Five additional wire-guided dilating systems have subsequently been marketed: the KeyMed Advanced dilator (KeyMed Ltd, Southend-on-Sea, UK), consisting of three spindle-shaped silicone bougies on stainless steel shafts; the stepped neoplex Celestin dilator; two types of hollow-core polyvinyl systems, the Savary-Gilliard and the American; and the transparent Optical dilator. The Celestin dilating system consists of two tapered dilators that reach a maximum diameter of 12 and 18 mm respectively. The Savary-Gilliard system (Wilson-Cook Medical, Winston-Salem, NC) consists of bougies ranging in size from 5 to 18 mm (15–54Fr). The American Dilating System (CR Bard Inc., Billerica, MA) ranges in size from 7 to 20 mm (21–60Fr). In contrast to the barium-impregnated American dilators, Savary-Gilliard dilators are longer and have a more gradually tapered tip. The fifth type of tapered dilator, called the Optical dilator (Inscope, Cincinnati, OH), is a hybrid device that has only recently been marketed. Actually three separate dilators with variable diameters ranging from 14 to 20 mm, these hollow devices fit over an endoscope to allow direct visualization at the time of bougienage, and thus theoretically improve procedural safety.

After wire-guided bougies, the second major advance in dilation technology was the development of polyethylene balloons for use in the gastrointestinal tract [6,7]. Ranging in diameter from 4 to 40 mm [6], dilating balloons are fixed on 5–7Fr catheter shafts that range between 100 and 200 cm in length. They can be passed over an endoscopically or radiographically placed guidewire, or directly through the scope (TTS) to allow dilation of previously inaccessible strictures in the stomach, small bowel, and colon. A full dilation set includes balloons of variable length and diameter, 5-mL to 30-mL syringes, guidewires, and a manometer to delineate balloon pressure during inflation. A dilating gun to maintain pressure

and stopcocks to ensure a constant pressure during inflation are optional. More recent advances in balloon technology have included the development of low-profile or high-compliance balloons. The latter can withstand a dilating pressure threefold to fourfold higher than previously marketed balloons to potentially improve results in recalcitrant strictures, although balloon compliance has not been found to affect dilation success in achalasia [8,9]. A newer development is the controlled radial expansion (CRE) balloon (Boston Scientific, Natick, MA) [10]. These balloons can be passed over a guidewire and inflated with variable pressure to predictably increase balloon diameter. A single CRE balloon, depending on the pressure used to inflate it, may increase in diameter from 6–8, to 8–10, to 10–12, to 15–18, or to 18–20 mm.

Technical applications

In general, radiologically or endoscopically directed dilation should be safer and more effective than blind dilation [7,11], particularly for sharply angulated, extremely tight, or proximal esophageal stenoses, as well as more central stenoses in the stomach, duodenum and colon. These general principles must be balanced against the availability of fluoroscopy, the added cost of endoscopy or fluoroscopy, and the physician's previous experience with a particular dilating modality. Endoscopically facilitated guidewire placement and subsequent bougienage of the esophagus need not always require fluoroscopic control if copious guidewire has been placed into the stomach or a marked guidewire is used and attention is given to ensure that guidewire displacement does not occur with endoscope withdrawal [1].

Of importance technically is the degree of lumenal enlargement that can be undertaken safely in a single dilating session. There remains a maxim in esophageal bougienage that one should increase a lumenal stenosis by no more than 2 mm (6Fr) in a single dilation session [1]. Although it is based on common sense and an attempt to avoid such complications as bleeding and perforation, this adage does not necessarily hold true for most rings or webs and some pliable reflux-induced strictures. The degree of lumenal enlargement should be contingent not only on the nature of the stricture itself (i.e., membranous or fibrotic) but also on the degree of associated lumenal ulceration and the risks and benefits of alternative treatment modalities. These decisions cannot be made by a review of the scant existing literature discussing side effects of dilation, but require a great deal of common sense on the part of the physician [1,11,12].

Esophageal strictures

Most patients who present with dysphagia have had previous esophagoscopy, although a few with lower esophageal rings may have had barium studies alone. With rings, webs, and mild reflux stenosis, mercury bougienage after a 6-h fast and pharyngeal anesthesia can often be done using a single 16–18 mm (48–54Fr) Maloney dilator [13]. Such dilators can be passed in the upright or lateral decubitus position. Although ideally done under fluoroscopic control to avoid kinking or retroflexion, many esophageal dilations can be performed safely without these capabilities.

Long, angulated, or eccentric esophageal strictures, as well as severe (< 7 mm) stenoses, are best handled with a guidewire dilating system. Patients require an initial endoscopy to define the stricture's cause and characteristics, for example length, diameter, pliability, eccentricity, and associated pseudodiverticula. Because a biopsy of the entire length of the stricture can be performed after dilation, it is sometimes best to delay tissue sampling until immediately after the dilation. Polyvinyl dilators, which have supplanted Eder–Puestow metal olives in most centers, always requires guidewire placement [1,11,14]. This is usually done in conjunction with initial endoscopy, at which time a fluoroscopically monitored wire with a spring tip can be passed through the stricture and fed freely into the stomach. Alternatively, the wire can sometimes be passed radiographically without endoscopy. After baseline endoscopy and stricture sizing, a dilator approximately the size of the stricture is passed, making sure the guidewire is fixed and the patient's neck is flexed forward. This is followed by one or two additional dilators, for an increment of up to 3 or 4 mm (10–12Fr), contingent on the stricture, before repeat endoscopy and stricture biopsy. Because of their gradual taper, polyvinyl dilators can pass through most stenoses with relative ease. The hesitation felt with these systems is related more to dilator friction over the guidewire than to the stricture itself.

Moderate stenoses (7–13 mm) can be dilated using either mercury bougies under fluoroscopic control or wire-guided polyvinyl dilators. The latter systems can be used without radiographic monitoring in some, although it is imperative that the guidewire is advanced far into the stomach and not inadvertently withdrawn at the time of endoscope removal. This is best done by feeding a marked guidewire forward in conjunction with endoscope or bougie withdrawal and having an assistant fix the wire at the level of the patient's mouth [1]. In addition, polyethylene balloons have been used to dilate moderate esophageal strictures [15]. The claimed advantages of balloon dilators over other systems have included ease of passage, dilation of the stricture alone, and radial as opposed to vector force applied to a stenotic wall [1], suggesting that such balloons should be safer and more effective. However, randomized trials comparing balloons to bougienage in benign esophageal strictures have demonstrated little consistent advantage to warrant the higher cost of balloon dilation in most cases [16–20]. If fluoroscopy is used to ensure waist dilation, total costs using this technology in our institution are threefold higher than dilation with polyvinyl dilators and eightfold higher than mercury bougienage [21]. Given this and the fact that such balloons are marketed as one-time use devices, balloon technology is better limited to areas of the gut not accessible to other types

of dilators. One notable exception is achalasia, where forceful disruption of the lower esophageal sphincter is usually performed with a balloon to afford a larger diameter and avoid blindly passing a dilator through the redundant and often tortuous esophagus associated with this condition (see Chapter 31). The technique of balloon dilation is described in the next section.

Nonesophageal strictures

Most nonesophageal strictures require balloon dilation, although stenotic gastric stapling orifices and anastomotic strictures of the rectosigmoid can be enlarged with polyvinyl dilators or even electrocautery [22–24]. Although both transendoscopic and wire-guided balloons have shown comparable safety and efficacy for benign colonic anastomotic strictures, wire guidance allows the use of larger balloons, which appears to reduce the need for repeat dilations in this setting [25]. The use of TTS or CRE balloons provides certain advantages, including direct stricture visualization, improved placement control, and immediate evaluation of the dilated stenosis [6]. TTS or CRE dilators require endoscopic estimation of stricture size for selection of a balloon that is 1–2 mm (3–6Fr) larger. Both balloon and dilator shaft should be coated with silicone, and negative pressure should be applied to the balloon using a 10- or 20-mL syringe. These measures, as well as avoiding excessive angulation of the endoscopic tip, allow dilator passage until all or part of the balloon is visualized. In the case of pyloric stenosis dilation, the balloon should be centered in the stenotic pylorus under a combination of endoscopic and fluoroscopic control. The latter also helps prevent damage of the bulb apex or C loop wall related to excessive pressure of the balloon tip or extreme balloon angulation.

Although air can be used for inflation, 10%–25% contrast solution allows better visualization fluoroscopically and a more uniform balloon dilation. Placement of the endoscope tip against a transparent balloon during inflation additionally provides a transendoscopic view of the stricture from within the balloon throughout dilation. Obliteration of the balloon waist is required with pressures up to 1212 kPa (12 atmospheres). Although larger balloons may increase the risk of perforation [26]. Balloon compliance has not been found to affect dilation safety or efficacy in achalasia [8,9]. Similarly, randomized trials of balloon dilation in achalasia have not found the duration of balloon inflation or number of inflations in a session to significantly impact procedural efficacy or safety [27,28]. In the case of most strictures, 30 s of dilation, repeated a second or third time after repositioning of the balloon, usually produces good results. After dilation has occurred, the balloon must be completely evacuated and the endoscope tip must be straightened to allow balloon retrieval. Additional, larger dilating balloons can then be used, although the degree of lumenal enlargement to be attempted in a single session remains a matter of common sense and is contingent on size of the initial stenosis, presence and degree of active ulceration, and patient discomfort with initial dilation. Using pyloric dilation again as an example, the ultimate goal is to dilate to 15–18 mm and follow up with complete endoscopic inspection of the pylorus and duodenum. This goal sometimes requires two or three dilating sessions separated by an interval of several days if the obstruction is acute, or several weeks if it is chronic.

Indications and contraindications

Indications for dilation are contingent on the anatomical area involved (Table 122.2). In the esophagus, symptoms are most often dysphagia and food impaction, although atypical chest pain, aspiration, and odynophagia may also be seen [19,29–31]. Indications for pyloric dilation are usually recurrent nausea and vomiting, weight loss, abdominal pain, and severe reflux [32,33]. Small bowel and distal colonic stenoses may require dilation for intractable obstipation, progressive diminution in stool size, pain, and recurrent bowel obstruction [22–24,34]. The causes of the above and of a variety of miscellaneous stenoses are listed in Table 122.2.

The only absolute contraindication to esophageal dilation mentioned in guidelines for esophageal dilation published in 1998 by the American Society for Gastrointestinal Endoscopy (ASGE) was acute or incompletely healed esophageal perforation [11]. Common sense dictates that contraindications to dilation also include a lack of informed consent, an acute abdomen, or a deeply ulcerated stenosis for which the risks of dilation outweigh the benefits. Indeed, relative contraindications listed by the ASGE include bleeding disorders, cardiopulmonary instability, recent esophageal perforation or

Table 122.2 Indications for gastrointestinal dilation

Esophagus
Ring or web
Reflux stricture
Malignancy
Miscellaneous (e.g., caustic ingestion, motility disorders)
Stomach
Pyloric stenosis
Anastomotic stricture
Gastric stapling/bypass stenosis
Miscellaneous (e.g., caustic ingestion, proximal malignancy)
Small bowel
Duodenum (web, acid peptic stricture, anastomotic)
Ileum (Crohn's disease, NSAID-induced stenosis)
Colon
Anastomotic stenosis
Inflammatory stricture (IBD, diverticular, radiation induced)
Miscellaneous
Stenotic gastrostomy, enterostomy, or colostomy stoma

IBD, inflammatory bowel disease; NSAID, nonsteroidal antiinflammatory drug.

surgery, and a large aortic aneurysm, but specifically excluded concomitant radiation therapy or forceps biopsy. For extraesophageal dilation, similar contraindications presumably apply, but there may also be lesion-specific contraindications, such as colonic stenosis associated with acute diverticulitis.

Risks and alternatives

Risks of gastrointestinal dilation include problems associated with all endoscopic procedures. The risks associated with the addition of dilation have usually been defined as an increased incidence of bleeding, perforation [35], and bacteremia [36,37], although the incidence of these complications has not been well defined for gastroduodenal and colorectal dilations. Most data on gastrointestinal dilation derive from esophageal bougienage with a combination of Puestow and mercury dilating systems in over 850 patients with benign esophageal strictures compiled from various series [7,13,38]. Reported complications in these patients included 16 perforations, five major bleeding episodes, two aspirations, and one death. This compares with perforation rates of 0.4% for mercury dilators and 0.6% for metal olives reported in an ASGE survey [7]. Such perforations are usually in the cervical esophagus and relate to improper dilator introduction or in the thoracoabdominal esophagus just proximal to the stenosis related to dilator or guidewire kinking. Less frequently, the stricture itself splits, as may be seen with achalasia (1%–5% perforation rate) [1]. Overall, the reported risk of perforation with esophageal dilation ranges from 0.1% to 0.4%, with the greatest risk associated with longer, narrower, angulated or irregular strictures [39]. While scant bleeding is not uncommon after esophageal dilation, significant hemorrhage was identified in less than 0.5% of dilations in 1976 [40] and is presumably even less common with modern techniques.

However, bacteremia is quite common, occurring after 22.8% of esophageal dilations, and more frequently than with nearly any other endoscopic procedure [41]. While this probably represents no significant infectious risk in the vast majority of patients, there is sufficient concern about endocarditis in patients with cardiovascular structural abnormalities to support the use of prophylactic antibiotics in this population. Specifically, the American Heart Association (AHA) and ASGE recommend that patients with a history of prosthetic cardiac valves, prior endocarditis, complex cyanotic congenital heart disease, a surgical systemic–pulmonary shunt, or a recent (< 12 months old) vascular graft should receive ampicillin or, if penicillin allergic, clindamycin, vancomycin, or a macrolide antibiotic 30 min prior to dilation [42,43]. The AHA also recommends gentamicin coadministration, although this may reasonably be reserved for colonic or gastroduodenal dilations. In patients with other congenital heart disease, valvular dysfunction, or hypertrophic cardiomyopathy, antibiotic prophylaxis is optional.

Long-term pharmacological implications of dilation must also be considered in any discussion of risks. Reflux-related esophageal strictures and many pyloric stenoses require long-term proton pump inhibition and possibly use of a prokinetic agent. Dilation of a stenosis from Crohn's disease usually requires adjustment of immunosuppressive medications to minimize the inflammatory response and stricture reformation [44].

The benefits and risks associated with lumenal dilation must be considered in relation to alternative treatment modalities, including other endoscopic therapies, surgery, or conservative management. Direct comparisons between dilation and these alternatives are sparse, but include several trials in which biopsy forceps or needle knife-mediated incision of Schatzki rings was at least as safe and effective as dilation [45] and possibly superior [46,47]. Furthermore, use of alternative approaches, such as electrocautery, in conjunction with dilation may enhance the latter's clinical efficacy [48]. Dilation should not be the sole treatment in individuals who require bougienage so frequently that cumulative risk and expense become prohibitive. In this setting, intralesional steroid injection may reduce the frequency with which dilation is required [49,50]. Alternatively, definitive management with surgical resection may be more appropriate.

Efficacy

Esophagus

Although dilation results in immediate symptomatic improvement in the majority of patients with benign esophageal strictures, multiple dilation sessions are often required. Strictures commonly recur, resulting in a return of dysphagia and necessitating repeat dilation. Using sequential bougies over a period of days to months, Puestow and mercury dilating systems have been associated with a 70%–100% improvement in dysphagia in a number of series [7,13,38]. Other studies have reported symptomatic improvement in excess of 90% [21,51,52].

A number of series have assessed balloon dilators for esophageal stenoses. In an early series, 93% of dilations of benign and malignant esophageal strictures in 88 patients were technically successful, with a 3% minor complication rate and 91% symptomatic improvement over a mean of 10 months [53]. Additional series and trials comparing balloon technology to Eder–Puestow or Savary dilators have demonstrated comparable efficacy between these technologies for benign esophageal stenoses [51,53–55]. Similarly, in a randomized prospective trial there was no significant difference between Eder–Puestow and Celestin dilators in long-term symptom relief of 72 patients, although the Celestin system was thought to be quicker and cause less pharyngeal trauma [56].

A number of series have reviewed experiences with hollow-core polyvinyl dilating systems [4,21,52,54,55,57]. Dumon and colleagues claimed efficacy in all 300 patients treated for benign or malignant esophageal stenosis, with only one perforation [57]. Our group successfully dilated 432

patients with 716 courses of dilation therapy; 89% were dilated with polyvinyl dilators and only 8% required fluoroscopic monitoring [21]. Approximately 80% of the dilation sessions were undertaken with a single large dilator or employed incremental dilators of 6 mm or more in a single session. There were no complications directly related to the bougienage. Additional large series have been published with polyvinyl dilators [52], but there have been no published series using the Optical dilator, so claims about improved procedural safety with this device remain to be confirmed.

Stomach

The only large series of gastric dilation are limited to polyethylene balloon dilation, and series with long-term follow-up report conflicting results. Since the initial description of successful hydrostatic dilation in a patient with pyloric stenosis and gastric outlet obstruction [58], several series have been published using this technique. McLean and colleagues radiographically dilated 94 gastrointestinal strictures in 92 patients, with a mean follow-up of 389 days in 80 patients [59]. Of these patients, 33 had various forms of gastric stenosis. Technically, 25 of the 33 were successfully dilated, and 70% were symptom free at 1 year. Perng and colleagues reviewed 42 patients with benign gastric outlet obstruction [32]. At a median follow-up of 2 years, two-thirds of patients had sustained improvement and one-third required surgery. The symptom-free rates at 1, 2, 3, and 4 years were 85%, 78%, 69%, and 69%, respectively, and the independent prognostic factor for failure was need for more than two courses of dilation for symptom relief. Lau and colleagues reviewed 45 patients who had successful gastric outlet obstruction dilation, four of whom had rapid reobstruction and were found to have malignancy [33]. At a median follow-up of 39 months, more than 50% of patients required surgery, 18 for recurrent obstruction, two for interval perforations, and one for bleeding. Nevertheless, a 60%–65% good-to-excellent symptomatic response at 1–2 years has been reported [32,33], and this should be compared with the results of drainage or resective therapy for benign gastric outlet obstruction [29]. A cost analysis comparing pyloric balloon dilation with vagotomy and pyloroplasty at our institution [73] showed balloon dilation to be cost-effective in the short term at one-tenth of the total costs of vagotomy and pyloroplasty. However, long-term acid suppression was not considered.

Small bowel

Because of the problems of access to the small intestine, there are few data regarding dilation of proximal small bowel strictures [62]. However, there are multiple series using balloon technology to dilate ileal or anastomotic ileocolonic strictures in Crohn's disease [44]. Dilation of 27 anastomotic strictures in patients with Crohn's disease led to no complications, and after 7–38 months 18 patients remained free of obstructive symptoms [63]. A prospective study of 55 patients with Crohn's disease with 59 ileocolonic strictures noted 90% technical success with complications in 11% (six perforations) [34]. Complete long-term symptom relief was noted in 34 patients (62%). Corticosteroid injections into inflamed small bowel following anastomotic balloon dilation may decrease restenosis rates further [64–66]. However, results remain anecdotal and data should be placed into the perspective of surgical resection or stricturoplasty.

Colon

Most reported colonic dilations have been used for rectal or anastomotic stenoses [22,23]. Linares and colleagues dilated 33 anorectal strictures in patients with Crohn's disease with a variety of techniques; half experienced short- or long-term symptomatic relief [67]. Pietropaolo and colleagues dilated postoperative strictures in 42 patients, using either balloons or bougies, with a 2.4% failure rate but no morbidity or mortality [68]. A combination of various types of dilators with endoscopic electrosection in 39 patients with benign anastomotic strictures resulted in no complications or symptoms in any patient at a mean follow-up of 25 months [23]. Solt and colleagues performed 133 balloon dilations over 17 years in 57 patients with benign (primarily anastomotic) strictures, with a 30% eventual reoperation rate but no procedural complications [69]. Finally, Virgilio and colleagues used a 30–40 mm achalasia dilator in 18 patients with anastomotic strictures following resection for colorectal cancer [70]. A total of 45 dilating sessions was performed in 17 of the patients. Long-term symptom relief was described in 16 patients (94%) and complications were limited to one episode of bleeding and a punctiform bowel perforation. Remaining unanswered questions include the relative efficacy of balloons as opposed to polyvinyl dilators for rectosigmoid stenoses, and the rapidity with which stenoses at various sites can be safely dilated.

Stent placement

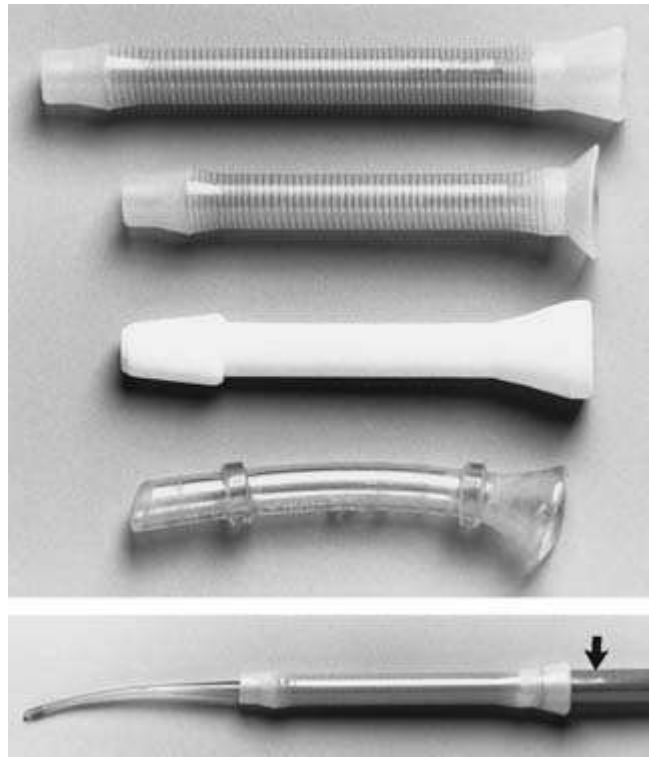
Theoretical considerations

In contrast to dilation, stent placement attempts to reestablish permanent continuity to the lumen of the gastrointestinal tract. The fact that such prostheses are placed primarily in the setting of malignancy suggests that the technology is imperfect: gastrointestinal stents occlude, migrate, erode, and may allow reflux of potentially noxious gastrointestinal contents depending on their location [71]. Symonds is credited with the first prosthesis placement in the esophagus in 1887. Initial stents were fabricated of ivory or boxwood and held in place by a variety of external fixation devices until the development of the inverted funnel by Gootskin in the early 1900s. Since then, a variety of homemade and commercially available esophageal prostheses have been used, the latter usually constructed of latex or silicone molded over

Table 122.3 Prostheses for malignant gastrointestinal stenoses

Conventional stents
Tygon tubing (homemade)
Silicone/latex-coated steel spiral[a]
Neoprene coated (Wilson-Cook, Inc.)
Expandable stents
Esophageal
Wallstent
Covered/uncovered
Flamingo variant
Wallflex
Z stent
Gianturco–Rösch variant
Dua stent with antireflux valve
Songstent variant
FerX-Ella variant
Esophacoil
Ultraflex
Covered/uncovered
Memotherm
Choostent
Niti-S
Alimaxx-E
Polyflex
Gastroduodenal
Enteral Wallstent
Wallflex
Hanaro
Niti-S
Enter-Ella
Colorectal
Enteral Wallstent
Colonic Z-stent
Colonic Memotherm

a Multiple types/manufacturers.



(a) (b)

Figure 122.3 **(a)** Conventional esophageal prostheses. Conventional and low-profile Wilson–Cook stents **(top)**, Atkinson prosthesis **(second from bottom)**, and homemade stent fashioned from Tygon tubing **(bottom)**. **(b)** Arrow demonstrates pusher tube used to seat prosthesis, which is stabilized over a Savary dilator.

a stainless steel core (Table 122.3 and Fig. 122.3). Inserted surgically or pushed into place using bougies, a small-caliber endoscope, or an expandable metal olive fixation device (Nottingham introducer), the design of these stents and insertion devices precluded placement across central or sharply angulated stenoses. Their application was also primarily limited to esophageal or gastroesophageal malignancies.

Expandable stents have largely supplanted rigid stents in the esophagus and have also made gastroduodenal and colorectal stenoses amenable to prostheses (Table 122.3) [72]. Although they differ in expansile force and delivery systems, all these prostheses are deployed in a compressed state, with subsequent spontaneous or balloon-assisted expansion. Uncovered stents, typically consisting of a cylindrical metal mesh tube, become embedded in the mucosa, which permanently anchors the stent in place but can allow stent occlusion from tumor ingrowth [73]. Alternatively, stents covered with a membrane reduce stent reocclusion, but may increase rates of stent migration [74]. Consequently, a wide variety of stent technologies have arisen, employing flared stent ends, partial coverage, and novel materials to simultaneously minimize both reocclusion and migration. At least three major types of metal stents are currently marketed in the USA, and variations of these stents are marketed around the world (Fig. 122.4).

The Wallstent (Boston Scientific, Natick, MA) was initially introduced as a two-layer stainless steel, double-dog bone prosthesis with an interposed layer of silicone. Uncovered or partially silicon-covered stents have subsequently been marketed that range between 18 and 25 mm in diameter and which are constrained by an 18Fr delivery system. A version of this prosthesis (Flamingo stent, Boston Scientific International) has also been marketed in Europe and has a pronounced taper to prevent distal migration when placed across esophagogastric junction tumors [75]. More recently, an uncovered nitinol mesh version, called the Wallflex stent (Boston Scientific), has been marketed; it is available with

Figure 122.4 Expandable prostheses **(left to right)**: Flamingo and covered Wallstent II, uncovered Wallstent, uncovered Ultraflex, partially covered Ultraflex, covered Z stent with and without Dua antireflux valve, small intestinal submucosa-covered Z stent (prototype device), Esophacoil, and Polyflex stent.

diameters up to 25 mm and requires only a 10Fr delivery system, thus facilitating transendoscopic deployment.

The Gianturco Z stent available in the USA (Wilson-Cook, Inc., Winston-Salem, NC) is a 20-mm diameter urethane-covered stainless steel prosthesis that flares to 22–25 mm at the ends. Ranging from 4 to 14 cm in length, the prosthesis must be back-loaded into a 28Fr compression catheter at time of placement [76]. In contrast, the Z stent marketed in Europe and Asia has a different coating and delivery system and also has small barbs on the shaft to minimize migration. The Songstent (M.I. Tech Co. Ltd, Seoul, Korea), also not available in the USA, resembles the Z stent [77] but includes a variant with a thread attached to the wire frame to facilitate retrieval [78]. Another variant of the Z stent contains an antireflux "windsock" valve (Dua Anti-Reflux Valve, Wilson-Cook) designed to minimize the regurgitation commonly associated with stent placement across the distal esophagus or gastric cardia [79]. The FerX-Ella (Radiologic, UK Medical Ltd, Sheffield, UK) and Choo (M.I. Tech Co. Ltd) stents have also been marketed with antireflux valves. The latter, marketed in Asia and Europe, can also be retrieved by a thread attached to its nitinol frame [80], much like the Songstent.

The Ultraflex prosthesis (Boston Scientific) is a 10 or 15 cm long, 18–25 mm diameter stent also made of weaved nitinol, with or without a covering, which is released by pulling a long suture used to constrain the stent on an insertion shaft. The Memotherm stent (CR Bard Inc., Billerica, MA) is also made of a nitinol weave [81], is uncovered, and is available in a version dedicated to colonic applications [82]. Niti-S stents (TaeWoong Medical, Seoul, Korea) represent a diverse array of additional nitinol mesh stents available outside the USA. Finally, the EsophaCoil (Medtronic Inc., Eden Prairie, MN) is a spiral nitinol coil, constrained on an insertion shaft by three trip wires, which is no longer available in the USA [83].

All the above differ not only in design and delivery system but also in physical characteristics. Although all are more malleable than conventional prostheses, Ultraflex and Wallflex stents can often be used for angulated stenoses. Fully covered Z stents, Song, and Choo stents can, at least in theory, be removed and may ultimately play a role in refractory benign strictures. Wallstents and Ultraflex prostheses imbed deeply into the esophageal wall and may have a role to play in patients with esophageal–airway fistulae without a tumor shelf. Each prosthesis generates a different amount of radial force (EsophaCoil > Wallstent > Z stent ≈ covered Ultraflex stent), which may help define the type of stent chosen for a given application [84].

An advantage of expandable metal prostheses over their conventional counterparts in the esophagus is their ability to be inserted under combined endoscopic and fluoroscopic control. Additionally, because expandable prostheses have a limited need for dilation for placement [72], they may be associated with less risk of bleeding or perforation during insertion. Moreover, their flexibility allows placement beyond acutely angulated malignancies, and their ability to imbed into the esophageal wall should minimize subsequent stent migrations. Their use in central stenoses offers a previously unavailable therapeutic option to patients with recurrent, metastatic, or unresectable obstructing malignancies. Examples include obstructing sigmoid cancer with widespread disease, or advanced pancreatic cancer causing gastric outlet obstruction [85–87].

In addition to the various expandable metal stents described above, a self-expanding plastic stent called the Polyflex stent (Willy Rusch GmbH, Kernen, Germany) [88] has become available. These 16–21 mm diameter, 9–15 cm long polyester mesh stents are fully covered by a silicone membrane. The inert nature of this coating appears to reduce the hyperplastic tissue reaction at the ends of the stent that is commonly seen within weeks to months of metallic stent placement, preventing removal of the latter. Initial studies thus indicate that Polyflex stents can be removed from most patients months after placement with variable ease. As such, their use has been expanded to include the treatment of benign or temporary problems, such as peptic strictures [89–91] or esophageal leaks [92–94]. Unfortunately, the large size (36–42Fr) of the insertion catheter required by these stents precludes transendoscopic placement, and thus restricts their use primarily to esophageal lesions. Additionally, their polyester mesh does not generate as much radial force as metal stents, which may limit their efficacy with tighter or stiffer stenoses, and may contribute to their documented higher rate of migration [88]. The Alimaxx-E stent (Alveolus, Charlotte, NC), available in Europe and marketed in the USA, is a nitinol mesh stent fully embedded in silicone. This prosthesis may overcome some of the limitations of the Polyflex stent while

remaining potentially removable. However, its efficacy and side-effect profile remain to be defined.

Considerable progress has been made toward increasing the safety, efficacy, and applicability of stent technology. What remains ill-defined, however, is the role this technology should play compared with conventional treatments such as surgery or percutaneous endoscopic gastrostomy/jejunostomy (PEG/J) placement. Moreover, where this technology fits into our therapeutic armamentarium of ablative therapies (Nd:YAG laser, bipolar cautery, caustic therapy, argon plasma coagulation, photodynamic therapy, and endoscopically assisted brachytherapy) requires continued evaluation [95].

Technical application

Stent placement requires stricture dilation to a size that admits a conventional prosthesis or the delivery system of an expandable stent [71]. Strictures are commonly dilated to 30–36Fr even with small (18Fr) delivery systems. This precludes stent dislodgement when the delivery system is retrieved through a partially expanded prosthesis and may avoid a need for stent dilation, which can itself result in dislodgement. Perhaps the most important technical aspects of stent placement are accurate placement and adequate measurement of the stenosis with selection of a prosthesis 4–6 cm longer than the neoplasm. While a subset of expandable prostheses can be placed either through or alongside an endoscope without radiographic control [96], most endoscopists use concomitant fluoroscopy, marking the proximal and distal stricture margins with either external markers or injections of contrast material. This allows more precise prosthesis placement and may allow repositioning of the prosthesis prior to full stent deployment.

Esophageal strictures

Esophageal prosthesis placement requires adequate dilation both to allow passage of various delivery devices and to localize and measure the length of the neoplasm [71]. Necessary dilator sizes range from 48 to 51Fr for conventional prostheses and from 24 to 36Fr for expandable prostheses. Preoperative bronchoscopy may be required, particularly for bulky extrinsic neoplasms, as stent placement may be associated with airway compression and acute respiratory distress. Tumor localization can be done by using external radiopaque markers taped to the patient's chest but is more commonly accomplished by injecting water- or lipid-soluble contrast material into the proximal or distal tumor margins [71]. Alternatively, endoscopic placement of radiopaque clips can be used.

Prosthesis placement requires fluoroscopic control, although some of the smaller delivery systems (Ultraflex, single-braid Wallstent) allow simultaneous endoscopic visualization during delivery [71]. Stents should be 4–6 cm longer than the neoplasm, contingent on local anatomy (e.g., esophagogastric junction, cricopharyngeus, contralateral gastric wall; stricture angulation). Conventional prostheses are pushed into place over a guidewire using a variety of devices to stabilize the stent (e.g., small-caliber endoscope, Savary-type dilator, Nottingham expandable olive fixation shaft) and various types of pusher tubes. These devices encounter considerable resistance at the level of the cricopharyngeus and may require neck hyperextension for passage. After conventional stents are pushed into place, the pusher tube and delivery system are retrieved and an immediate repeat endoscopy should be performed to document correct prosthesis position. Expandable prostheses have variable delivery systems (Fig. 122.5) [71]. Polyflex stents, Z stents and Wallstents are deployed by pulling back a compression catheter. Ultraflex stents are constrained by a single long suture that can be pulled off the delivery shaft. Although Z stents essentially retain their preexpansion length after deployment, there is significant foreshortening of both Wallstents and Ultraflex prostheses, making proper placement crucial.

Despite the plethora of prostheses now available, certain practices are followed after placement of all prostheses. The most important is the need for immediate endoscopy

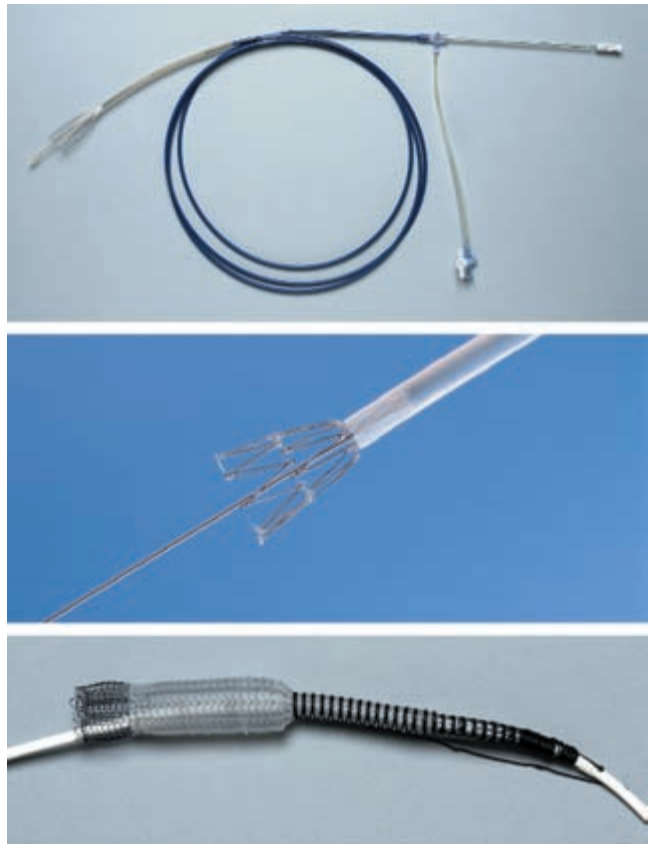


Figure 122.5 Stent deployment devices: Wallstent **(top)** and Z stent **(middle)** are deployed by withdrawing a compression catheter sleeve. Ultraflex stent **(bottom)** is released by uncoiling a suture from the proximal or distal end.

after insertion to assure that the prosthesis has accomplished the treatment goal, e.g., correct location, full expansion, or occlusion of tracheoesophageal fistula. Problems occur when the prosthesis is too long or short, abuts into the cricopharyngeus or contralateral gastric wall, or has inadequate radial force to open a tight neoplastic stricture. The latter may preclude retrieval of the delivery system. Airway compression and immediate migration at the time of placement are additional potential problems. The endoscopist has to be prepared to deal with these problems with balloon dilators, as well as additional expandable and conventional prostheses to correct problems associated with inadequate length, acute angulation, or migration. Excessive stent material extending too far beyond a stricture's margin may cause occlusion or mucosal injury, although it may be trimmed from metallic stents with an argon plasma device [97]. Once expandable stents have fully deployed, the coated Z stent and Polyflex stent are the only prostheses available in the USA with the potential to be repositioned. The uncovered Ultraflex prosthesis, in turn, can be completely removed, at least in theory, up to several weeks after placement.

Nonesophageal strictures

Expandable prostheses can be placed in the proximal stomach and distal colorectum using conventional delivery systems and techniques similar to those described for the esophagus. Stents approved in the USA specifically for colonic application, including the colonic Z stent (Wilson-Cook), Enteral Wallstent (Boston Scientific) and colonic Memotherm stent (CR Bard), share a wider diameter of 20–30 mm to reduce the risk of obstruction. However, strictures in the more proximal colon, as well as those in the duodenum or proximal jejunum, require either a longer insertion system or a stent mounted on a 3–4 mm shaft that can be placed through a large channel endoscope [95]. A variety of stents have been developed for transendoscopic enteral placement, including the Enteral Wallstent and Wallflex stents (Boston Scientific), the Hanaro enteral stent (M.I. Tech), the Niti-S duodenal and pyloric stents (TaeWoong Medical), and the EnterElla duodenal stent (Radiologic). These stents require balloon dilation of the malignant stenosis, delineation of stricture length, localization with contrast injection at the tumor margins, and concomitant endoscopic and fluoroscopic control to ascertain proper placement and prosthesis expansion. If biliary and duodenal stents are both required, as may be the case in locally advanced pancreatic cancer, the permanent biliary stent should be placed first whenever possible, as it is technically very challenging to cannulate the biliary system through the fenestration of a duodenal stent.

Indications and contraindications

For the most part, conventional and metal expandable prostheses are difficult or dangerous to remove once placed, and thus should be reserved for obstructing or fistulizing neoplasms that cannot be cured by resection, or in potentially curable patients with prohibitive operative risk (Table 122.4) [71,72]. The most experience has accrued in the proximal gut for malignant dysphagia or esophago-airway fistulae. The latter can be a consequence of primary esophageal carcinoma, lung cancer, or mediastinal metastases. Gastric outlet obstruction from widespread pancreatic cancer has traditionally been treated by gastrojejunostomy or PEG/J in infirm patients, but the latter might be ideal candidates for stent insertion into the C loop [86,98]. Likewise, patients who present with malignant colon obstruction usually have far advanced disease (stage III [40%] or IV [60%]) [87]. Traditionally treated with palliative resection or bypass, colorectal prosthesis placement appears to be a useful alternative, both to allow adequate preoperative bowel preparation in surgical candidates [99,100] and as long-term palliation in a subset of patients with malignant ascites or liver metastases, and thus a higher operative risk [101–103].

While removable stents, such as the plastic Polyflex stent, can also be used for malignant stenoses [88,91,104], their ability to be removed months after deployment expands the indications for prostheses to include benign conditions as well. In the esophagus, temporary placement of a Polyflex stent for 6 weeks to 13 months has been successfully used

Table 122.4 Indications for prostheses

Conventional stents
Esophagus
Malignant dysphagia (esophagus, esophagogastric junction, lung, mediastinal tumor)
Esophago-airway fistula
± Benign disease, e.g. caustic ingestion/anastomotic stricture or leak
Expandable stents
Permanent prostheses
Esophageal
Malignant dysphagia
Esophago-airway fistula
Gastroduodenal (high surgical risk)
Malignant pyloric/C-loop obstruction
Gastric/pancreatic/biliary tumor
Colorectal (high surgical risk)
Obstructing/malignant stenosis
? Benign strictures: radiation, Crohn's disease
Removable prostheses
Esophageal
Refractory peptic stricture or ring
Anastomotic leak
Gastroduodenal/colorectal (theoretical)
? Anastomotic stricture
? Radiation or Crohn's stricture

to provide durable relief from a variety of benign causes of esophageal stenosis, including peptic, caustic, anastomotic and radiation-induced strictures [89,90]. Additionally, this covered stent allows temporary occlusion of nonmalignant esophageal perforation [92], particularly postsurgical anastomotic leaks [93,94]. Although minimal literature exists on the extraesophageal application of removable stents, these prostheses may soon become indicated for benign strictures of the colorectum, stomach, or even small bowel in the near future.

With this notable exception of removable stents, prostheses are generally contraindicated in the low-risk and potentially curable patient [71,95], as permanent stents have a long-term potential for erosion, occlusion, and migration. Conventional prostheses should not be placed in the setting of a tracheoesophageal fistula unless there is an adequate shelf to seat the stent. Esophageal stents should not be used if placement of a dilator approximating the stent diameter results in significant airway compression by a bulky neoplasm. Stents may be contraindicated if placement cannot be undertaken without impingement on the cricopharyngeus muscle or anal sphincter, or if the opening of a placed stent would abut a contralateral lumenal wall, thus occluding it. An absolute contraindication for prosthesis placement is an inability to endoscopically or radiographically define both the proximal and distal stricture margin.

Risks and alternatives

Esophageal strictures

Complications with the early rigid esophageal stents were not uncommon, and so provide a paradigm for possible omplications with more modern stents. Acute procedure-related complications included perforation, bleeding, tracheal compression, and tube malposition. Total complications with these conventional stents have approximated 20%, with a mean procedure-related mortality of 8.6% [71]. Subacute complications include erosion with bleeding or fistula development, stent migration, food bolus impaction, and tumor overgrowth. Variable degrees of reflux are inevitable if the esophagogastric junction has been stented, and florid regurgitation or aspiration may be noted in patients who have delayed gastric emptying as a consequence of vagal denervation or gastric infiltration by tumor.

Complications of expandable prostheses are variable and contingent on prosthesis design and endoscopist experience, but are generally similar to those of conventional prostheses, except less common [105–108]. For example, a Swedish nationwide review of 152 recipients of expandable metal esophageal stents revealed such complications as transient chest and pharyngeal discomfort (in nearly all patients), stent migration (in 5% of cases), bleeding (in 1%), incomplete stent expansion (in 1%), perforation (in 1%), and stent occlusion (in 10%) from food impaction or tumor overgrowth or ingrowth [109]. The latter is a complication unique to expandable stents, and can be reduced with covered stents, although this increases the risk of stent migration [110–112]. As with conventional stents, gastroesophageal reflux can complicate expandable stents placed across the lower esophageal sphincter, prompting some manufacturers to incorporate valves or sleeves into versions of their prostheses. While some of these modifications have appeared ineffective in randomized trials [113], others have demonstrated as much as an eightfold lower rate of subjective [114] or objective reflux [79,115] than unmodified prostheses, with rare cases of potentially fatal reflux aspiration pneumonia seen only with the valveless stents in these studies. The ability to place expandable esophageal stents more proximally than conventional prostheses [116] has also associated them with rare complications such as cervical discitis [117] and stent perforation into the common carotid artery [118].

The risk of life-threatening complications from both conventional and expandable stents may be increased in esophageal cancer patients with prior chemotherapy and radiation. Multiple series have reported increased rates of bleeding, perforation, sepsis, or tracheoesophageal fistula formation in such patients [107,119–122]. However, a number of reports have also found no significant association between these complications and chemoradiation therapy [123–125], raising the question of whether these patients are particularly sensitive to variations in stent design, physician technique, or institutional experience.

Tumor ablative technologies have also been studied as alternatives to stent placement. In randomized trials, endoscopic laser therapy by itself was less effective than covered or uncovered expandable stents at relieving dysphagia [110], although it increased median survival and quality of life, as well as treatment cost, in one study [126]. However, the cost and survival with laser therapy were found to be comparable, and the quality of life actually worse, relative to stents in a Greek case series [127]. The addition of external beam radiotherapy to laser increased serious complications, hospitalization time, and cost, but did not result in more long-term efficacy than stents [128]. A major limitation of laser therapy is the need for repeated endoscopic treatments.

In contrast, single-dose esophageal brachytherapy has compared favorably with partly covered Ultraflex stent placement in a randomized trial of 209 patients [129]. Although stents resulted in more immediate relief of dysphagia, these symptoms were better in brachytherapy recipients after about 5 months. Cost, survival, and quality of life were not significantly different between the two groups. Similar results have also been reported with multiple sessions of endolumenal brachytherapy relative to Ultraflex stent placement [130].

Nonesophageal strictures

Although few randomized trials have been conducted with extraesophageal stents, a plethora of case series have been summarized by metaanalyses concerning gastroduodenal

[131] and colorectal [132,133] malignancies. According to these analyses, stent migration and occlusion are among the most common complications, each occurring in 5%–17% of placements. While occlusion is usually from tumor ingrowth or overgrowth, 10%–25% of colonic stent obstructions can also be from fecal impaction. Life-threatening complications, namely perforation or severe bleeding, are rare, with each occurring in less than 1% of gastroduodenal stent placements. However, the rate of perforation is higher (up to 5%) with colorectal stent placement, particularly if balloon predilation was employed, and it constitutes the major etiology of the 0.5%–1% mortality observed with this procedure. Unique to gastroduodenal prostheses, biliary obstruction, with jaundice or cholangitis, complicates 1.3% of placements. Pain is less commonly a complication with extraesophageal stent placements than with esophageal stents, being reported in only 2.5%–5% of cases, and usually as transient or minor pain.

Surgery is the principal alternative to stent placement for extraesophageal obstructions, and has historically been indicated for emergency colorectal obstructions, even in patients for whom a surgical cure is not possible. Recent randomized trials and case series have concurred that while stents and surgery for malignant colorectal obstruction have comparable morbidity and mortality, colectomy is associated with longer hospitalization and usually necessitates colostomy [99,100,102,103,134]. Similarly, surgical treatment of malignant gastric outlet obstruction is comparable to stent placement in terms of morbidity, mortality, and ultimate clinical efficacy, but takes longer to achieve that efficacy and results in longer hospitalization [102]. Thus, reports have suggested that prostheses represent a more attractive approach to malignant extraesophageal obstructions than their surgical alternatives. There are few data that compare prostheses to less invasive palliative alternatives such as a jejunal feeding tube or a diverting cecostomy.

Efficacy

Esophagus

Conventional stents can be successfully placed in approximately 90%–95% of patients with malignant esophageal obstructions, with mean patient survival of 2–4 months [71]. Terminal events frequently include tumor cachexia or subacute stent-related problems such as aspiration or stent erosion into major vessels.

Numerous series defining long-term results of expandable stent placement for malignant dysphagia have been published, revealing technical and clinical success rates comparable to conventional stents [71,75,135–137]. As noted above, these results are contingent, in part, on both stent design and initial successful palliation of dysphagia, but usually reveal fewer complications and shorter hospitalization with expandable stents. Nevertheless, in most series, survival appears identical to that following conventional prosthesis insertion [71], underscoring the degree to which mortality is dictated more by the underlying malignancy than the complications of these palliative prostheses. Our study comparing 85 patients in whom conventional versus expandable prostheses were placed for malignant dysphagia documented comparable levels of post-stent dysphagia (0.4 vs 0.9), fistula occlusion (13/15 vs 13/14), and survival (87 vs 90 days) [123]. Comparable palliation and complication rates have been noted in studies comparing Z, Ultraflex and Flamingo prostheses for malignant dysphagia [138,139].

Benign esophageal strictures have been successfully treated with temporary Polyflex stents, with 80% of recipients remaining symptom-free many months after stent removal [89,90]. These stents have also successfully occluded 78%–89% of esophageal anastomotic leaks in some small series [92], with less mortality and shorter hospitalization than surgical or conservative management [93]. Furthermore, placement of a Polyflex stent inside a previously placed, and normally permanent, metal stent has been reported to facilitate the removal of the latter, presumably through local tissue ischemia [90].

Nonesophageal strictures

Numerous series relating to stent placement in malignant stenoses of the stomach, small bowel, and colon have been summarized in the aforementioned metaanalyses [131–133]. Technically successful stent deployment was reported in 97% of gastroduodenal and about 93% of colorectal placements, with most failures due to inability to cannulate a very high grade obstruction with a guidewire. Curiously, the technical success rate was not increased in colorectal strictures when predilation was performed to facilitate guidewire passage [133], suggesting that the increased risk of colonic perforation these analyses associated with predilation [132,133] may not be warranted. Factors that predict a lower likelihood of technical success with colorectal prostheses include lesions proximal to the rectosigmoid, where only about 85% of placements were successful [132]. Extrinsic colorectal compression from noncolorectal primary malignancies were also less amenable to stent placement, with only 78% of these cases achieving technical success [132].

About 88% of extraesophageal prostheses achieved clinical success, namely improved oral intake for gastroduodenal stents [131] and colonic decompression for colorectal stents [132,133]. The majority (61%) of gastroduodenal stent failures were due to disease progression, with most of the remainder reflecting stent migration (20%) or incorrect deployment (15%) [131]. Although stent malposition or migration also caused about half the clinical failures of colorectal prostheses, perforation was another significant limitation, accounting for 15%–30% of failures in this group [132,133]. In a subset of patients who received a colorectal prosthesis as a bridge to surgery, 72% were able to undergo a single-stage colectomy and thus avoid colostomy [132].

Future applications

The future of gastrointestinal dilation is contingent on further experience with the newer dilating systems, such as CRE balloons, in comparison to polyvinyl dilators. Both expanded experience and controlled clinical trials should allow improved definition of the indications, benefits, procedure costs, and risks for individual dilating systems, and for specific stenotic lesions. New technologies, such as the transparent over-the-scope Optical dilator, may facilitate endoscopic guidance and visualization during dilation to improve the safety of dilation, and extend the applicability of solid dilators beyond esophageal and anorectal stenoses. If the latter proves true, solid dilators could ultimately provide a more cost-effective alternative to balloons for central stenoses. The future of gastrointestinal dilation may also include the development of dilators that can detect a fall in wall tension or resistance within a tissue during dilation, as an indication that the dilation is adequate. By thus identifying a point beyond which further lumenal enlargement may be unnecessary or hazardous, devices such as these should improve safety.

There are also likely to be additional technological combinations using dilators and various thermal modalities for malignant stenoses. Just as bipolar tumor probes have conjoined Eder–Puestow dilators with multipolar electrocautery to treat concentric esophageal or rectal neoplasms, similar electrodes have been implanted on balloons or polyvinyl dilators. A new approach is to take a quartz laser fiber and surround the tip with a balloon so that the balloon keeps the fiber tip in the center of the lumen. These catheters may ultimately allow treatment of nonresectable biliary, gastroesophageal, or rectal neoplasms by transmission of laser energy. Photodynamic therapy, in turn, may play an increasing role in the treatment of malignant stenoses, while argon plasma coagulation or endoscopic cryotherapy may be used in lieu of Nd:YAG laser to open a lesion or facilitate stent placement.

Finally, as noted already, dilation will be increasingly used in conjunction with expandable stent technology. Permanent expandable stents, undergoing refinement both in the endoprostheses themselves and in their delivery systems, hold at least the potential of prolonged stricture patency and decreased stent migration. A Niti-S esophageal stent impregnated with a β-emitting radionuclide has been tested in dogs in an effort to combine the benefits of brachytherapy and stent technology in a single device [140]. Z stents covered with small intestinal submucosa have facilitated reepithelialization of vascular and airway stents in animal models [141,142] and may hold similar potential in the alimentary canal. At the same time, as the technology improves for removable prostheses, their role in the treatment of benign conditions may expand to obviate repeated dilation sessions, and potentially provide a novel future treatment for benign central stenoses and leaks.

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A ENDOSCOPIC

123 Percutaneous endoscopic gastrostomy

Brian J. Dunkin, Jeffrey L. Ponsky

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Percutaneous endoscopic gastrostomy (PEG) was introduced in 1980 as an alternative to operative gastrostomy for those patients requiring long-term intubation of the stomach [1]. It was devised as a safe and effective nonoperative means of providing enteral nutrition. The method has become widely practiced, with approximately 230 000 PEGs placed in patients in the USA annually [2].

The concept of a surgically created fistula between the stomach and abdominal wall was first conceived by Egeberg in 1837 and attempted by Sellidot in 1849. A variety of surgical approaches were developed, culminating in the more popular Janeway and Stamm methods [3]. Both were effective in creating a gastrocutaneous fistula, but use of these techniques was somewhat limited because the patients were frequently poor surgical candidates and these procedures required a laparotomy, often with general anesthesia.

Thus, delivery of enteral nutrition was most often by means of nasoenteric tubes. This was effective but associated with numerous frustrations and complications. These tubes were frequently large in caliber and constructed of stiff rubber or plastic, making them uncomfortable. Frequent plugging of the tubes necessitated replacement, and it was not unusual for them to become dislodged, accounting for up to 60% of tube removals [4,5]. Esophagitis also developed from irritation of the esophageal mucosa by the tube, and gastroesophageal reflux was common because the tube rendered the esophagogastric junction incompetent [6,7]. This reflux frequently led to the development of aspiration pneumonia. Long-term use of nasoenteric tubes also caused parotitis and erosion of the nasal cartilage.

In 1980, the technique of PEG was introduced. This method allowed the formation of a tube gastrostomy without laparotomy or general anesthesia. Safety of the procedure was ensured by endoscopic visualization of tube insertion, and placement directly into the stomach reduced the risk of complications encountered with the nasoenteric tube. The original method described by Ponsky and Gauderer has been called the pull technique [8]. Two modifications of the original method have been introduced and have gained some popularity. These are referred to as the push or Sacks–Vine technique and the introducer or Russell technique [9,10]. All these methods have been shown to be safe and effective.

Complications of percutaneous gastrostomy, although not frequent, are known to occur and are well documented. Careful attention to patient selection and the details of the method may help to reduce the incidence of undesirable outcomes. The increasing ease by which enteral access is established with percutaneous gastrostomy has led to tremendous growth in the application and use of this procedure. Moral and ethical dilemmas arise as the technique is applied to an increasingly debilitated population of patients. Further study of the technique and its long-term outcome has provided answers to some of these problems.

Methods

Patient preparation

Proper preparation can help avoid complications. The patient should not be fed for 6 h prior to the procedure. If an antibiotic with good Gram-positive coverage is not already being administered, then a single intravenous dose should be given [11]. The most common organisms cultured from PEG site infections are *Staphylococcus aureus* and β-hemolytic streptococci. Cefazolin is very good at covering these and other commonly encountered organisms [12]. The patient should be positioned supine with the head elevated to reduce the risk of aspiration. Many practitioners forgo the use of topical anesthetic to the oropharynx in these supine patients because it may increase the risk of aspiration and has not been shown to improve patient tolerance of upper endoscopy [13]. Because

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it can be difficult to intubate the esophagus in this position, it is sometimes preferable to start the endoscopy in the left lateral decubitus position and then reposition the patient supine. Suction should be available to manage oropharyngeal secretions, and the patient should be maintained on supplemental oxygen. In addition, blood pressure and pulse oximetry should be monitored by a nurse in attendance throughout the procedure. A full endoscopic survey, including inspection of the duodenum, should be conducted prior to PEG placement. Such a survey has led to a change in patient management in 4%–36% of patients [14,15].

Standard techniques

Pull (Ponsky–Gauderer) technique

Once positioned, the patient is given intravenous sedation and the abdomen is washed and draped in a sterile fashion. The room lights are dimmed as the gastroscope is passed into the stomach. Transillumination of the abdominal wall by the light of the scope is an indication that intervening structures have been displaced and that the stomach lies in contact with the abdominal wall. The site selected for placement of the gastrostomy should be one at which transillumination is well seen. Finger pressure by the assistant at this point produces a clear indentation of the gastric wall as seen by the endoscopist (Fig. 123.1). This indentation should be covered by an open polypectomy snare passed through the endoscope. To enhance the safety and accuracy of PEG placement, use of the *safe tract technique* described by Foutch is very helpful. The technique begins with identification of the best site of finger indentation or transillumination. A syringe with local anesthetic is advanced only a small distance beneath the skin. Suction is applied to the syringe barrel as it is advanced more deeply. The appearance of air in the syringe barrel and visualization of the needle tip in the stomach by the endoscopist should occur simultaneously. Should air appear in the syringe barrel before the needle tip appears in the stomach, an alternative site should be selected as this usually indicates that an intervening air-containing viscus, such as colon or small bowel, has been punctured.

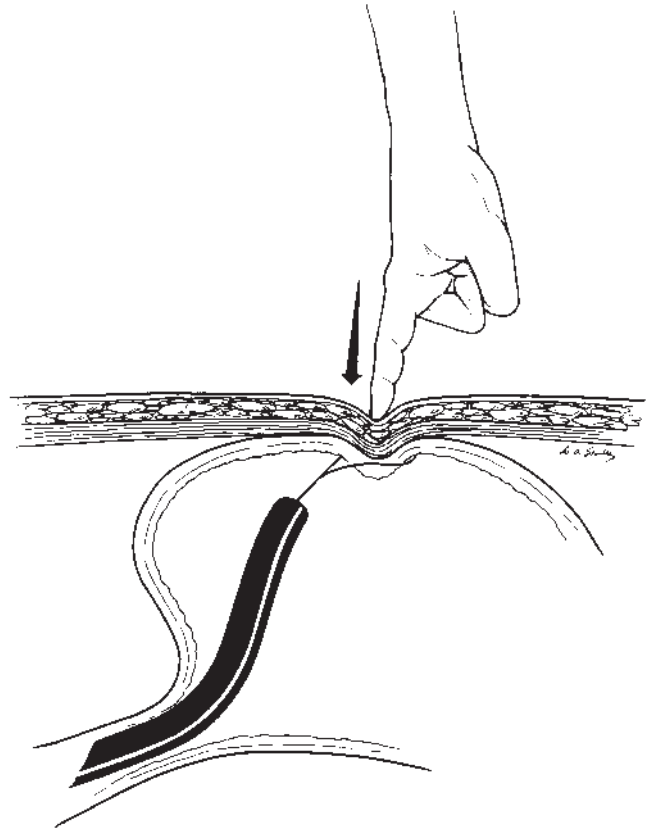

Figure 123.1 The endoscopist observes a distinct indentation of the gastric wall as the assistant applies finger pressure to the abdominal wall at the proposed site of puncture.

Local anesthetic is injected into the abdominal wall at the proposed area of puncture, and a 1-cm incision is made in the skin. An intravenous cannula is thrust through the incision and into the gastric lumen. If the polypectomy snare is correctly positioned, the thrusting needle will enter the center of its loop. If not, the snare is quickly looped around the cannula and tightened near the point where the cannula exits the gastric mucosa (Fig. 123.2). A long stout suture or wire is

Figure 123.2 The snare is looped around the cannula and tightened near the point where the cannula exits the gastric mucosa.

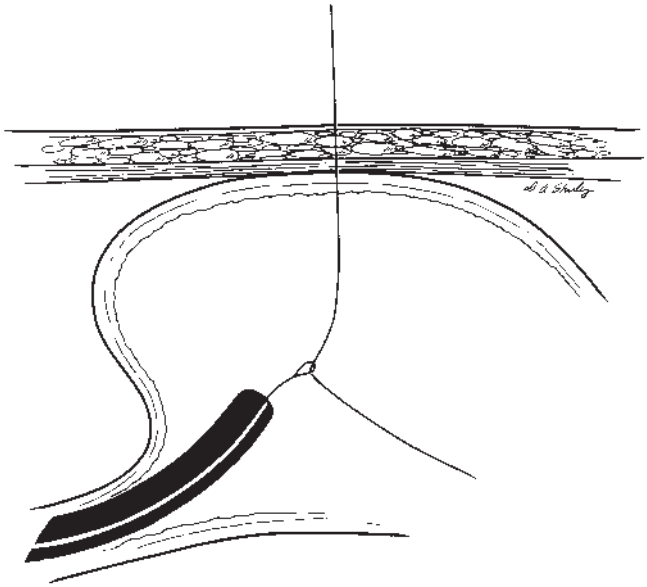

Figure 123.3 The snare is tightened around the suture and pulled out of the patient's mouth using the gastroscope.

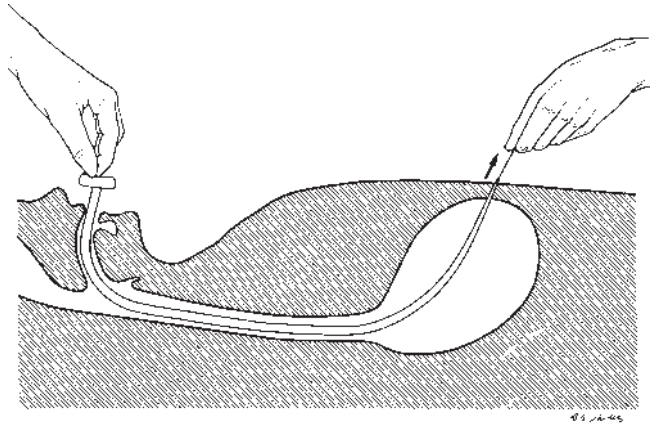

Figure 123.5 The assistant pulls on the abdominal end of the suture, and the gastrostomy tube proceeds down through the esophagus and stomach to exit the abdominal wall.

passed through the needle into the gastric lumen, and the snare is moved from around the cannula to tighten around the suture itself (Fig. 123.3). The suture is pulled with the endoscope from the stomach and out of the mouth. The drainage end of the gastrostomy tube is affixed to the suture at the patient's mouth (Fig. 123.4). The tube is well lubricated, and pull is begun on the abdominal end of the long suture so that the gastrostomy tube moves down the esophagus and exits the abdominal wall (Fig. 123.5). An outer bolster is applied, which holds the gastric and abdominal walls in close contact. Many endoscopists reinsert the gastroscope at this point to check the position of the head of the tube and adjust the external bumper to avoid excessive tension on the head of the catheter. If there is no suspicion of a technical problem, however, this step may be omitted at the discretion of the endoscopist [17].

Push (Sacks–Vine) technique

This method differs little from the pull technique. The patient is prepared, the puncture performed, and a guidewire grasped and pulled from the patient's mouth in the same manner (Fig. 123.6). In this procedure, however, a special gastrostomy tube with a long tapered dilator end is pushed over the guidewire. Both ends of the wire must be held under tension as the tube is pushed down the esophagus and exits the abdominal wall (Fig. 123.7). Once the tube emerges from the abdominal wall, it may be grasped and pulled the rest of the way into its final position. The gastroscope is reinserted (at the discretion of the endoscopist as described above) to ensure that the head of the catheter lies in contact with the gastric mucosa. An outer crossbar or faceplate is added to fix the abdominal and gastric walls together.

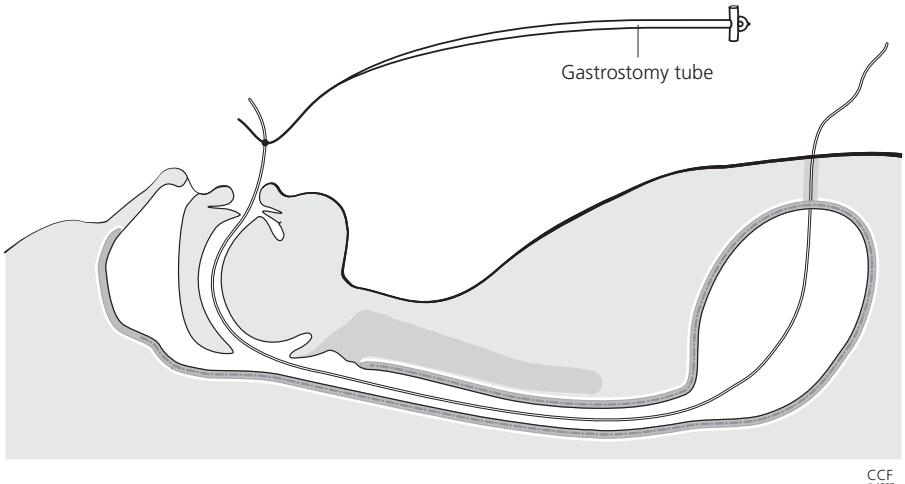

Figure 123.4 The gastrostomy tube is affixed to the suture at the patient's mouth.

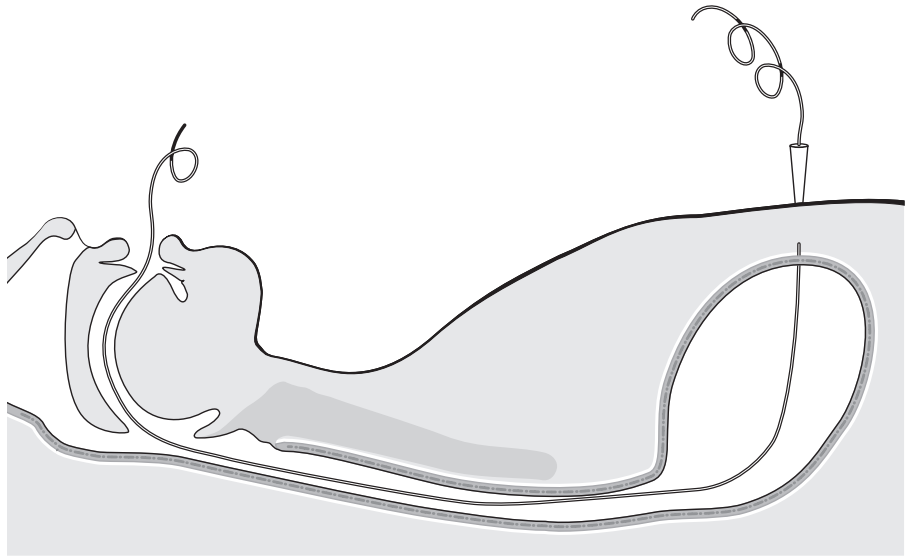

Figure 123.6 As in the pull technique, the cannula is inserted into the stomach, a guidewire is passed through it, and the wire is grasped and pulled out of the mouth using the gastroscope.

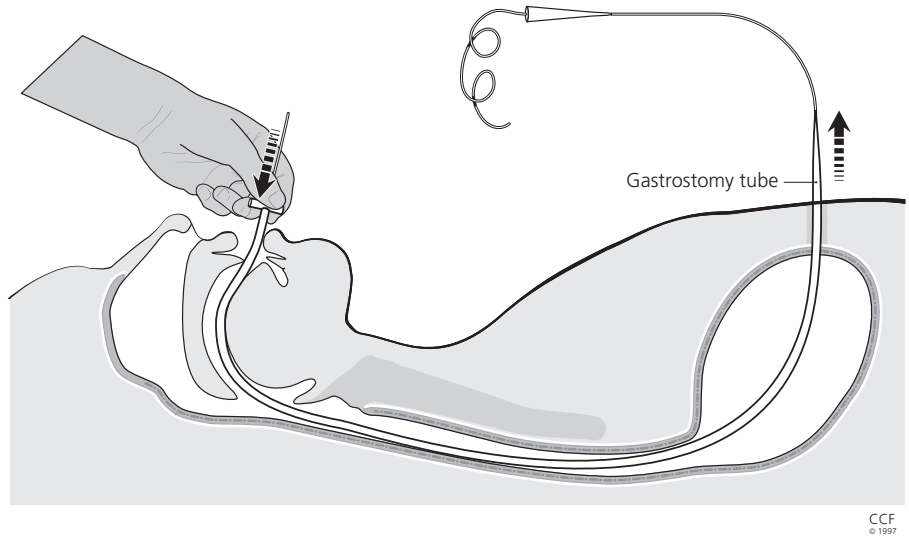

Figure 123.7 Both ends of the wire are held under tension while the gastrostomy tube is pushed down the esophagus and out the abdominal wall. Once the tube emerges from the abdominal wall, it is grasped and guided into position.

Introducer (Russell) technique

The percutaneous method for insertion of a central venous catheter as described by Seldinger has been adapted to the performance of gastrostomy placement in the introducer method. Developed by Russell and colleagues [10], this method allows percutaneous placement of a gastrostomy tube without passing the tube through the oropharynx and with only a single insertion of the endoscope. The gastroscope is passed into the stomach, and the room lights are dimmed. Transillumination of the abdominal wall and finger pressure at the point of best transillumination are used to identify the proper site for puncture. Once the site for insertion has been established, the skin is anesthetized and a small incision is made. A needle is introduced into the gastric lumen, and a guidewire is passed through the needle. The needle is removed, and an introducer with an outer peel-away sheath is passed over the guidewire into the stomach (Fig. 123.8). The wire and introducer are removed, leaving only the sheath in the stomach. A balloon catheter is introduced through the sheath and inflated (Fig. 123.9). The sheath is removed, and the balloon of the catheter is pulled up to the gastric mucosa. The catheter is either sutured to the skin, or an outer faceplate is applied to hold the gastric and abdominal walls in approximation.

Comparison of standard methods

All these techniques for the performance of percutaneous gastrostomy have been widely used and found effective. Each provides benefits that should be considered in choosing a method for a particular patient [18].

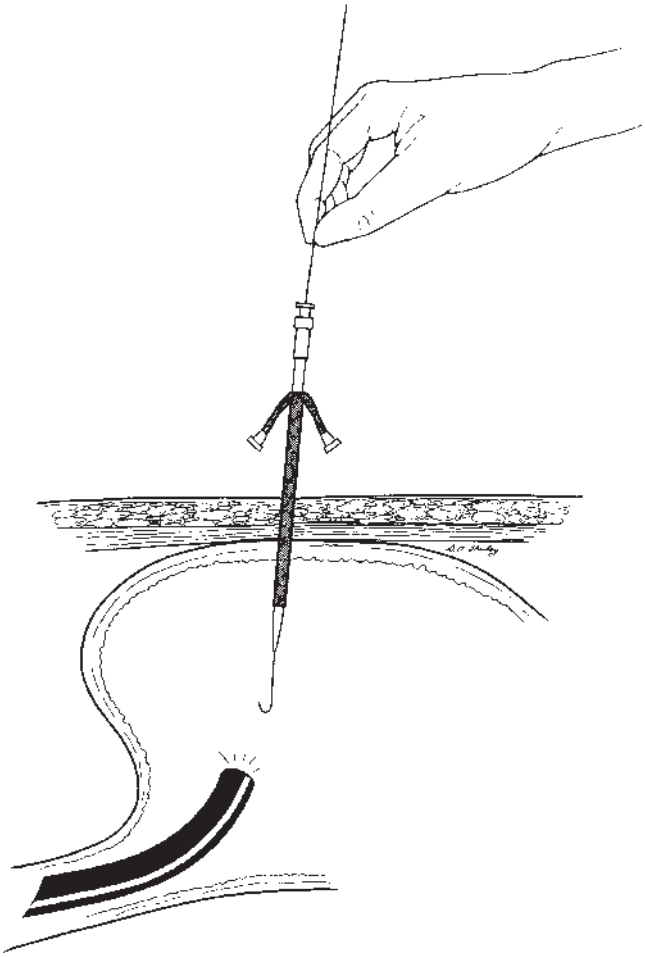

Figure 123.8 The introducer and its outer sheath are passed over the guidewire into the gastric lumen.

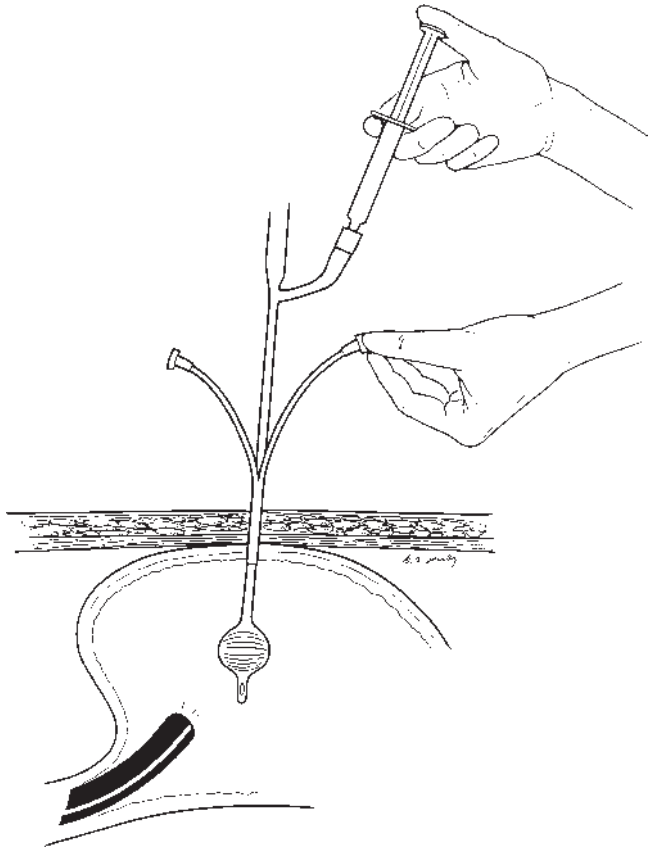

Figure 123.9 The introducer and guidewire are removed, and the balloon catheter is inserted through the sheath. The balloon is inflated and the sheath is peeled away.

The pull technique provides the physician with a great deal of control. The puncturing needle is stabilized with the surrounding snare, ensuring that loss of air from the gastric lumen does not result in loss of the puncturing needle. The gastrostomy tube is pulled down the esophagus and out of the abdominal wall in a direction that apposes the gastric and abdominal walls. This should minimize leakage around the puncture site. The soft button of the pull PEG trailing free behind the tube may be more deformable than that of the push PEG, thus allowing it to traverse a narrowed esophagus more readily. The push method adds the stability of a guidewire to act as a trolley on which the tube can ride as it passes through the alimentary tract. Both these methods have the potential of introducing oral bacteria or tumor cells from a lesion in the oropharynx or esophagus into the tissues of the abdominal wall. Thus, infection and metastasis of carcinoma are potential complications with these techniques.

The introducer method enjoys the advantage of direct puncture insertion of the tube. This avoids the potential risk of infection from oral flora and possibly reduces the risk of cancer seeding to the abdominal wall. The method permits insertion of a gastrostomy in patients with narrow lesions of the esophagus without fear of disrupting such lesions while the catheter is positioned. In the introducer technique, the endoscope serves only an observatory function. However, the forces of insertion with the introducer method push the stomach away from the abdominal wall and may lead to the creation of a submucosal tunnel in the stomach without puncture of the mucosa. Loss of control of the punctured stomach and leakage of gastric contents is also more likely to occur. Finally, the tubes used for this procedure are of small diameter and suffer from premature extrusion secondary to deflation of the balloon catheter.

In practice, each of these methods has been demonstrated to be effective and to have a low rate of complications. Experienced endoscopists are familiar with all these techniques and select the one with which they are most comfortable and that best suits the needs of the patient.

Alternative techniques

Incision omission PEG

The traditional method of pull PEG placement includes making a skin incision large enough to permit bacterial egress. It was thought that this technique would reduce stoma site

infections. Sedlack and colleagues have called into question the validity of this step of the procedure and advocate simply pulling the tapered end of the PEG tube directly through the skin without the creation of an incision [19]. The force required to pull the tube into position is 47% greater when compared with standard pull PEG (some tube tips pulled off during the process), but stoma infection rates were lower and patient comfort after the procedure was similar. The authors state that there may be additional advantages of eliminating the scalpel from the PEG kit such as improved safety for the procedure team, lower cost of the kits, and less creation of hazardous waste.

SLiC technique

This is a modification of the introducer technique and is named after the describing authors Sabnis, Liu, and Chand [20]. Endoscopic access to the stomach is gained in the usual fashion and the site for PEG placement chosen. However, in place of the introducer and outer peel-away sheath, a 7–8 mm radially expanding Step trocar (United States Surgical, Inc., Norwalk, CT) is used to traverse the abdominal wall and gain access to the lumen of the stomach. The PEG tube is placed through this trocar which is then removed to allow securing of the tube in the usual fashion. The advantage of the trocar is that it maintains a secure airtight access and allows placement of a large 20Fr Malecot tube. Such a tube is more resistant to plugging or inadvertent removal. Other advantages of the procedure include minimizing the chance for tumor seeding to the PEG site and the ability to place large-bore tubes even in patients with lesions of the oropharynx or esophagus since the tube is not pulled through these areas.

Percutaneous transesophageal gastrostomy tube

Percutaneous transesophageal gastrostomy tube (PTEG) was developed in Japan as an alternative route of access into the gastrointestinal tract [21]. It is placed via the cervical esophagus thus avoiding traversal of the abdominal cavity and gastric wall. This has potential advantages in those patients who have a contraindication to standard PEG placement such as ascites, carcinomatosis, or obstructed oropharynx. The procedure is usually performed under conscious sedation in the endoscopy unit. The patient is in semirecumbent position. A guidewire is inserted through the nose and into the esophagus and its position confirmed by fluoroscopy. A rupture-free balloon (RFB) is passed over this guidewire distal to the cricopharyngeus and inflated with dilute contrast material. The RFB can be punctured with a needle without collapse or rupture. Traction is maintained on the RFB to keep it in the cervical esophagus. Ultrasound is used to locate the inflated RFB and confirm that it has displaced the carotid artery and jugular vein medially and the thyroid medially and superiorly. Color Doppler is a useful addition to the visualization. Once the puncture site has been chosen, the area is anesthetized with a local anesthetic and, under ultrasound guidance, a straight needle with an outer sheath is advanced through the left side of the neck until the tip enters the RFB. Needle placement is confirmed by aspiration of contrast from the balloon. The inner needle is then removed and a second guidewire is inserted through the sheath and into the RFB. The sheath is removed, the wire secured at skin level, and the RFB deflated and advanced distally in the esophagus. This allows the cervical guidewire to fall out of the lumen of the balloon but remain within the esophagus. The RFB and nasal guidewire are then removed. An incision is made over the guidewire in the neck and a 16Fr dilator with outer peel-away sheath is inserted under fluoroscopic guidance over the wire and into the esophageal lumen. The dilator and wire are removed and the PTEG balloon catheter advanced via the sheath into the proximal stomach. The balloon is inflated and pulled upward so that the tip of the catheter rests just below the esophagogastric junction. Its position is confirmed by the injection of contrast under fluoroscopy. The peel-away sheath is then removed and the catheter secured at skin level.

PTEG has been placed most often in patients with malignant obstruction of the gastrointestinal tract and massive malignant ascites [22]. It avoids the complication of peritonitis in these patients and provides more comfortable and complete decompression of the stomach than a nasogastric tube or standard PEG.

Radiologically placed gastrostomy

Gastrostomy tubes may be placed under radiological guidance without endoscopy using a Seldinger technique like that described for the introducer method. A number of studies have examined these methods and found them to compare favorably with endoscopically guided insertion [23]. Common indications for radiologically placed gastrostomy (RPG) tubes are esophageal stenosis due to malignant or benign stricture or any situation in which endoscopy is contraindicated for the patient. Some have argued that patients with oropharyngeal cancer should have RPG tubes as well to avoid seeding cancer to the abdominal wall. A more recent common indication is to access the remnant stomach after Roux-en-Y gastric bypass surgery for morbid obesity [24]. Gastric bypass patients have a small gastric pouch completely separated from the majority of the remnant stomach and directly connected to the jejunum. Post-bypass access to the gastric remnant for decompression, imaging, or to serve as a conduit for endoscopy have all been described using RPG tubes [25]. Interestingly, a common method of initial access is to percutaneously pass a needle through the left lobe of the liver and into the lumen of the remnant stomach which is then filled with air and contrast. This route of access avoids traversing an intervening loop of bowel. Once the stomach is maximally inflated and visualized with contrast, a more direct anterior abdominal wall approach is used for placement of the gastrostomy tube. One disadvantage of the RPG tube is its small caliber, which makes it prone to clogging.

This can be avoided by sequentially up-sizing the tube over time after the access tract has matured.

Mini-laparoscopically guided percutaneous gastrostomy

In some patients PEG tube placement is complicated by inability to transilluminate, absence of a stomach after prior resection, or failure of radiological placement. In these situations mini-laparoscopy has been used to assist in placement of the gastrostomy tube [26]. Using conscious sedation and local anesthesia, a 2.3-mm diameter Veres needle (Richard Wolf GmbH, Knittlingen, Germany) is percutaneously passed into the intraperitoneal space and used to instill 1.5 L of nitrous oxide. The Veres needle is then exchanged for a 1.9-mm diameter laparoscope (Richard Wolf GmbH). Upper endoscopy is performed and the appropriate insertion point of the stomach or jejunum is chosen using direct laparoscopic visualization. The standard pull technique is then used to place the gastrostomy or jejunostomy tube. The pneumoperitoneum is subsequently evacuated and the laparoscope withdrawn leaving only a small 1.9-mm diameter incision on the abdominal wall.

Indications

PEG was first conceived as a means of providing long-term enteral nutrition to those patients unable to ingest food by mouth but who have a functioning gastrointestinal tract. Nutritional support should be initiated in all patients anticipated to be without nutrient intake for two or more weeks. If the patient has a functional gastrointestinal tract and is expected to require enteric feeding for less than 30 days, then a nasogastric or nasoenteric tube should be used. If it is anticipated that enteric feeds will be required for more than 30 days, however, then a tube gastrostomy or enterostomy should be considered. Such patients may have severe neurological or developmental disorders, obstruction of the oropharynx from trauma or tumor, or a critical illness requiring prolonged endotracheal intubation.

As the safety and efficacy of PEG has been demonstrated, the population of patients benefiting from it has expanded. PEG tubes have been placed in burn patients (even through burned skin) to facilitate enteric feeding of high caloric density formulas [27]. They have also been placed in patients about to receive neoadjuvant therapy for esophageal cancer [28] and head and neck carcinoma [29,30]. Victims of severe maxillofacial injury have benefited from PEG tube placement as well [31].

Non-alimentation-related applications have also been described. These include decompression of the stomach for gastric atony, uncorrectable obstruction, carcinomatosis, and gas bloat following Nissen fundoplication [18,32,33]. The method has also been used for the administration of unpalatable medications to children [34] and the refeeding of bile to patients with external biliary fistula [35]. Multiple PEG tubes have been placed in patients with paraesophageal gastric herniation and gastric volvulus as a method of gastropexy [36]. Finally, RPG tubes have been used to gain access to the remnant stomach in Roux-en-Y gastric bypass patients for decompression or to allow for gastroscopy or endoscopic retrograde cholangiopancreatography via the PEG tract [25].

Despite the excellent overall safety profile of PEG tube placement, careful consideration should be given prior to insertion. Controversy exists as to whether PEG tubes should be placed in patients with advanced dementia or incurable metastatic cancer [37]. Some have even questioned whether PEG tubes decrease the risk of aspiration when compared with use of a nasogastric tube, although more recent data show that patients with a nasogastric tube have a significantly greater risk of aspiration than patients with PEG tubes (40.7% vs 20.3%) [38]. The endoscopist must act as more than a technician and provide reasonable expectations to the patient, the patient's family, and the referring provider when making a decision about PEG tube placement.

Contraindications

As experience has grown with PEG tube placement the number of absolute contraindications to placement has become few. Uncorrectable coagulopathy, peritonitis, or any process that precludes endoscopic access to the gastrointestinal tract are the only absolute contraindications to standard PEG tube placement.

Other disease processes raise concern about PEG tube placement and must be managed cautiously. Massive ascites is one such process. Patients with massive ascites are at major risk for complication from PEG tube placement. However, successful placements have been described using preprocedure treatment with β-blockade, diuresis, nitrate administration, and paracentesis [39,40]. Avoiding access via the peritoneal cavity with PTEG has also been successfully used in these difficult patients (see Methods). Patients on peritoneal dialysis, with gastric varices, or on immunosuppression were also once considered not to be candidates for PEG. However, successful placement has been accomplished in all these patients with careful preparation and rigorous adherence to excellent technique.

Inability to transilluminate the abdominal wall, usually because of morbid obesity or the presence of an intervening structure between the stomach and the abdominal wall, is another scenario that requires caution. Endoscopic and transabdominal ultrasound have been used to ensure that no intervening structures are present and to guide the passage of a spinal needle through a thick abdominal wall [41]. Alternatively, a cut-down through the skin and subcutaneous tissue under local anesthesia has been used in an obese

patient so that fascia could be visualized and a PEG tube safely placed [42]. The safe tract technique described by Foutch has been advocated as more important to the safe placement of a PEG tube than transillumination, especially with modern endoscopes that use lower levels of light intensity for visualization [43]. Without these additional maneuvers, however, the inability to transilluminate the abdominal wall is a contraindication to PEG tube placement.

Other situations where PEG tube placement should be performed with caution include prior surgery, distended loops of small bowel, presence of a ventriculoperitoneal shunt, or severe cardiac disease. Previous abdominal surgery is not a contraindication to PEG tube placement if there is meticulous adherence to the details of the procedure, including transillumination of the abdominal wall [44]. As described above, a safe tract should be identified in these patients by looking for aspiration of air from only the stomach during passage of the angiocatheter [45]. These same principles apply when treating a patient who has distended loops of small bowel. The presence of a ventriculoperitoneal shunt is not a contraindication to PEG tube placement as long as care is taken to avoid the catheter [46]. A preprocedure abdominal radiograph can be helpful. Severe underlying heart disease increases the risk of major complication in PEG tube placement, possibly related to use of intravenous sedation [47]. However, Cappell and Iacovone have shown that a PEG tube can be safely placed within 1 month following myocardial infarction if it is considered essential and the patient is otherwise stable [48]. Severe malnutrition has been identified as a risk factor for complications from PEG tube placement [49]. A serum albumin of less than 3 g/dL is an independent risk factor for 30-day mortality in hospitalized patients. Although these data have not resulted in the use of minimum serum albumin level as a selection criterion for PEG candidates in most institutions, it does warrant careful counseling of the patient and referring physician as to the potential for complications following PEG tube placement in severely malnourished patients.

Complications

Large cumulative retrospective studies have reported that 10%–16% of both adult and pediatric patients have at least one complication following PEG tube placement [50]. The overall mortality rate is 1%, with a major complication rate of 3% and a minor complication rate of 13% [51]. This section reviews the most commonly encountered complications.

Wound infections

Wound infections account for up to 30% of all minor complications. These are caused by multiple organisms, but most commonly by *S. aureus* and β-hemolytic streptococci. Gossner and colleagues [12] have shown that a single prophylactic dose of cefazolin covers 72% of the organisms encountered in PEG infections and will reduce the rate of infection following PEG placement from 28.6% to 7.4%. In contrast, cefoxitin is only effective against 33% of the organisms encountered. Infection usually becomes evident as erythema around the gastrostomy tube site several days after the procedure, with local tenderness and slight edema of the skin. The patient often demonstrates a low-grade fever and leukocytosis. Early recognition and incision and drainage of the area under local anesthesia frequently resolves the problem. Failure to identify and treat this problem at an early stage can result in necrotizing infections of the abdominal wall and death. Necrosis of the tissue of the abdominal wall that is interposed between the head of the tube and the outer bolster may play a major role in the occurrence of infection at a PEG site. Excessive tension applied to the bolster acts as a tourniquet, producing ischemia of the underlying tissue. Loose contact is all that is required between the outer bolster and skin. If a dressing is required, it should be placed over the bolster, not under it, for the same reason. It is a good idea to begin systemic antibiotics for all infections around the tube and to continue them until the infection is resolved. Also, an adequate skin incision in the abdominal wall has been advocated to allow room around the gastrostomy tube for the egress of bacteria. However, experience with incision omission PEG has called into question the validity of this tenet for reducing PEG site infections (see Methods).

Buried bumper syndrome

Extrusion of the head of the tube from the gastric lumen into the subcutaneous tissue (the buried bumper syndrome) has been encountered. This is almost certainly caused by excessive tension applied to the tube. To prevent leakage of gastric contents and ensure adhesion of the stomach to the abdominal wall, the physician may pull up on the gastrostomy tube and push downward on the outer bolster. Although this fixes the stomach to the abdominal wall, it also produces ischemia of the intervening abdominal wall tissue, with subsequent necrosis. This can be avoided by ensuring that no tension is present when the head of the catheter is placed in contact with the gastric mucosa and that the outer bolster only loosely approximates the skin. Such approximation of the gastric and abdominal walls results in satisfactory adhesion without tissue necrosis.

Leakage or inadvertent early removal

Leakage of feedings into the peritoneal cavity may occur after percutaneous gastrostomy. This is usually the result of separation of the gastric and abdominal walls and is often due to necrosis of the abdominal wall tissue caused by excessive tension on the catheter. Patients who develop abdominal tenderness, fever, or leukocytosis should be evaluated for leakage and the resultant peritonitis. This may be done by instilling water-soluble contrast material into the gastrostomy

tube under fluoroscopic guidance. Intraperitoneal extravasation indicates that something has gone awry. If the contrast study results indicate that the head of the tube remains in the stomach and that the extravasation is around the tube, the clinician may pull up on the tube, providing a bit more tension to seal the leak, and place the tube to drainage; intravenous fluids and antibiotics should be administered. If the contrast study results reveal complete separation of the gastric and abdominal walls with dislodgement of the tube from the stomach, the tube should be pulled from the abdominal wall, a nasogastric tube inserted to effect gastric drainage, and intravenous fluids and antibiotics begun. A patient whose PEG tube has been pulled out inadvertently less than 2 weeks from the time of insertion should be treated similarly. If at any time the patient's condition begins to deteriorate or signs of peritonitis worsen, exploratory laparotomy or laparoscopy with operative repair should be performed.

Gastrocolic fistula

Gastrocolic fistula has rarely been known to occur after percutaneous gastrostomy. This type of fistula may be caused by puncture of the colon at the time of gastrostomy or pinching of the colon between the gastric and abdominal walls with subsequent necrosis of the colonic wall and fistula formation. This complication usually becomes apparent after several weeks, with the development of severe diarrhea following feedings. It may be documented with an upper gastrointestinal series or barium enema. In nearly all cases, the condition may be treated by removing the gastrostomy tube. The fistula closes rapidly once the tube is removed.

Progressive enlargement of gastrostomy stoma

Progressive enlargement of the gastrostomy stoma around the gastrostomy tube may occur in some patients. Although this, too, may be the result of excessive tension on the tube, it is also occasionally seen in patients in whom the gastrostomy tract is well established. Poor nutritional status may play a part in the development of this problem, as may excessive movement of the tube at the skin level. The temptation in addressing this problem is to remove the gastrostomy tube and replace it with a larger one that fills the hole. This solves the problem for a short time, but the tract soon enlarges again. A better solution is to remove the tube entirely and allow the tract to close. After it has closed a bit, a new smaller tube may be inserted. Also, minimizing movement at the skin by securing the tube properly or placing it through a baby bottle nipple or a similar stabilizing device is effective.

Pneumoperitoneum

Pneumoperitoneum is a frequent occurrence after percutaneous gastrostomy. This may be the result of air escaping around the puncturing needle. Routine radiographs of the abdomen are unwarranted. Air in the abdominal cavity has been shown to last for up to 5 weeks after PEG tube placement. Patients found to have pneumoperitoneum after gastrostomy must be clinically evaluated. In the absence of abdominal tenderness, leukocytosis, or fever, there is no need for further evaluation. However, the patient who demonstrates any of these should be evaluated with a water-soluble contrast study through the gastrostomy tube for signs of separation or intraperitoneal extravasation. One case was reported of tension pneumoperitoneum during PEG tube placement causing hemodynamic compromise, but this is exceedingly rare [52].

Neoplastic seeding to the abdominal wall

Neoplastic seeding to the skin from both oropharyngeal and esophageal cancers has been reported following PEG tube placement [53]. If the gastrostomy is being placed for palliation, this may not be an issue. However, if placement is part of the patient's therapy, as in preparation for neoadjuvant radiation therapy for esophageal carcinoma, then this must be considered. The mode of tumor spread to the abdominal wall remains controversial, but direct inoculation from disruption of the tumor by instrumentation at the time of PEG tube placement seems most likely. This hypothesis is supported by the observation that metastasis of head and neck cancer to the skin rarely occurs spontaneously and that abdominal wall metastasis following PEG tube placement has only been observed using the pull technique [54]. The incidence of such metastasis is low (< 1%), but ominous since the average survival after development is 7 months and the 1-year survival is zero [55]. Given this fact there may be advantages to using an introducer technique in this setting.

Aspiration

Aspiration with the development of pneumonitis or pneumonia is a potentially major complication of PEG tube placement, occurring either at the time of insertion or afterward during feeding. It is associated with a very high mortality. In an effort to minimize this risk, some have advocated placing feeding tubes further down the gastrointestinal tract either via the PEG tube (percutaneous endoscopic gastrojejunostomy, PEG/J) or directly as a percutaneous endoscopic jejunostomy (direct PEJ). There has been controversy regarding the efficacy of this technique, but prospective data have shown that the incidence of gastroesophageal reflux and aspiration significantly reduces as the level of infusion descends from the stomach down through the first, second, and third portions of the duodenum [56]. Heyland and colleagues also showed in a metaanalysis of seven studies that shifting the level of infusion of tube feeds from the stomach to the small bowel was associated with a 24% reduction in aspiration pneumonia [57].

Stoma problems

Good skin care is important after gastrostomy. It is common to see a foreign body reaction around the tube with some

exudate or granulation tissue. This is usually easy to treat. The exudate is merely swabbed away with hydrogen peroxide. The site should be left open to the air. Granulation tissue may be cauterized with silver nitrate. Occlusive dressings should be avoided because they lead to maceration of the underlying skin.

Feeding

Once the PEG tube has been placed without complication, the next issue is how to feed through it. Intermittent feeding is preferred because it is easy to administer (no pump required), well tolerated, and physiological. The formula is allowed to flow in by gravity over a period of 30–60 min. Rapid bolus feeds should be avoided because they decrease lower esophageal sphincter pressure to incompetent levels and are associated with free gastroesophageal reflux. Patients should be placed in a semirecumbent position to reduce the risk of aspiration. Most endoscopy units start feeding 24 h after PEG tube placement with the initiation of a continuous infusion of water at 50 mL/h. Once this has been tolerated for 4 h, the infusion of a full-strength polymeric formula is begun at 50 mL/h and advanced by 25 mL/h every 12 h as tolerated until the goal rate is reached. This method is anecdotal, however, and some clinicians have demonstrated that a continuous infusion of full-strength formula at 30 mL/h can be safely initiated only 3 h after PEG tube placement [58]. The choice of formula is based on the patient's caloric needs, tolerance, and associated medical conditions. Residual volumes are often checked on a regular basis until tolerance of the regimen has been demonstrated. This practice is controversial, however, and McClave and colleagues have shown a poor correlation between gastric residual volume and risk of aspiration even at volumes greater than 400 mL [38]. In addition, tubes smaller than 10Fr in size have been shown to be unreliable for the determination of residual volume [59].

Endoscopic jejunostomy

Endoscopic jejunostomy is a modification of PEG intended to accomplish feeding into the jejunum. There are two methods. The first places the jejunal feeding tube through the gastrostomy tube (PEG/J). This method is similar to that of PEG, with the gastric tube acting to anchor the apparatus and decompress the stomach while a longer enteric tube is passed through the PEG and positioned in the distal duodenum or proximal jejunum [60]. The advantage of this system is that use of the PEG to gain access to the gastrointestinal tract minimizes the risk of traversing another loop of intestine during placement as well as leakage from around the tube or inadvertent displacement of the tube tip from within the jejunal lumen. The disadvantage of this system is that correct placement of these tubes has proved difficult. Traditionally, a suture is attached to the tip of a jejunal tube prior to passing it through the PEG and into the stomach. The suture is grasped with a biopsy forceps loaded through the endoscope and dragged under direct vision into the duodenum. Deep passage is often impossible, however, and the tube frequently drags back into the proximal duodenum or stomach during removal of the endoscope. A pediatric colonoscope or enteroscope may be used to ensure passage of the jejunal tube into the distal duodenum or proximal jejunum. More recently, various guidewire techniques have been described to assist in placement. Duckworth and colleagues [61,62] have described passing a guidewire through a 24Fr PEG tube and grasping it with a biopsy forceps passed through the endoscope. The wire is dragged under direct vision into the distal duodenum or proximal jejunum. While still holding it with the forceps, tension is applied to the free end of the wire to straighten it. A 12Fr jejunal feeding tube is then passed over the wire. The endoscope is removed by a rocking motion followed by removal of the guidewire. Tube placement is confirmed by abdominal radiograph. An initial placement success rate of 100% has been reported, but there is a 20% long-term tube failure rate due to malpositioning or occlusion. Chaurasia and Chang have described passing a pediatric bronchoscope through the PEG and into the distal duodenum to gain access to the bowel [63]. A wire is passed through the scope and guided beyond the ligament of Treitz using fluoroscopy. The jejunal feeding tube is then passed over the wire and into position. Finally, Leichus and colleagues [64] have described passing the endoscope into the stomach and through an open polypectomy snare that has been passed into the stomach via the PEG. The tip of the endoscope is placed in the distal duodenum and a guidewire passed through it and beyond the ligament of Treitz. The wire is kept in position while the endoscope is withdrawn, leaving the peroral wire to be grasped at the intragastric segment by the trans-PEG polypectomy snare. The snare is pulled so that the oral end of the wire is retrieved from the PEG. A jejunostomy tube is then passed over the wire and into position confirmed by fluoroscopy.

To overcome some of the placement difficulty with PEG and PEG/J systems, Shike and colleagues [65] described placing a feeding tube directly into the jejunum using a similar technique to that of PEG (direct PEJ). This was initially used only in patients with partial or total gastrectomy but has subsequently been used in patients without gastric resection. Such placement may require use of a push enteroscope or pediatric colonoscope, and fluoroscopic guidance is useful. When successful, this procedure obviates the problem of tube migration and ensures placement beyond the ligament of Treitz. It is also easier to place larger-caliber tubes that are less likely to fail from occlusion. When used in combination with a PEG, better decompression of the stomach can be obtained

because the PEG tube is not partially occluded by the PEG/J system. There is a greater potential for complications, however. The mobility of the jejunum and the higher likelihood for intervening structures between it and the abdominal wall make strict adherence to technique mandatory. The thin wall of the jejunum is also more likely to allow leakage of enteric contents around the tube. Finally, the direct PEJ tube can act as a "maypole" around which the small bowel can twist and obstruct.

The proposed advantage of jejunal feeding is a decrease in the risk of aspiration. Whether this is a true benefit is controversial, with early studies failing to demonstrate an advantage over PEG. Many of these studies were small and the tip of the jejunal tubes were often only in the proximal duodenum. Later studies have confirmed placement of the tube into the distal duodenum or proximal jejunum using an over-the-wire technique and have demonstrated virtual elimination of aspiration associated with feeding [61].

The indications for PEG/J or direct PEJ are tracheal aspiration, reflux esophagitis, gastroparesis, insufficient stomach from previous resection to perform PEG placement or to tolerate gavage feeding, or enteral access in patients with partially obstructing lesions from gastric or pancreatic carcinoma.

Removal and replacement of PEG tubes

PEG tubes may require replacement when the tube has become frayed, obstructed, or develops a leak. Additionally, in some patients, an enlarging stoma may be the result of excessive tube motion at the skin level. In such cases, removal and replacement with a skin-level device may be advantageous. Elective tube removal should not be performed until a secure tract has formed, usually at least 10 days to 2 weeks from the time of insertion.

When PEG tubes are inadvertently removed prematurely, peritonitis is a threat. If discovered immediately, such patients can often be treated with nasogastric suction and intravenous administration of antibiotics. With close observation and steady improvement, the PEG may be repeated in about 1 week. Should the patient demonstrate signs of peritonitis or sepsis, urgent laparotomy is indicated.

All currently manufactured PEG tubes have deformable or partially deflatable internal bolsters that allow for removal by means of external traction without the need for endoscopy [66]. Some have advocated cutting the tube at skin level and letting the internal bumper pass spontaneously. In adults this is associated with a relatively low risk of complication [67], but in children there is a particularly high risk of small bowel obstruction, retained internal components, or perforation and the internal bumper should be removed with the endoscope [68].

Although PEG tubes may be replaced with balloon tip catheters, the balloons generally last only a short time and these tubes have been known to migrate and obstruct the pyloric channel. Use of a mushroom-tip catheter or a skin-level device is preferable. Gauderer and colleagues developed the concept of a skin-level gastrostomy device [69]. This device has an antireflux valve that permits a cosmetic solution to gastrostomy feedings. It may be placed after removal of a previous gastrostomy tube or at the time of the creation of the initial PEG. A standard tube is often preferable to the skin-level device in patients who require custodial care because it is more easily accessed by nursing personnel.

Conclusion

Percutaneous gastrostomy is a simple procedure with significant advantages over open gastrostomy and few severe complications. Proper patient selection, close attention to detail during placement, and rapid assessment of potential problems minimizes unsatisfactory outcomes.

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A ENDOSCOPIC

124 Management of upper gastrointestinal hemorrhage related to portal hypertension

Paul J. Thuluvath

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Gastroesophageal variceal bleeding is an unpredictable complication of portal hypertension with an associated mortality of 20%–40% with each bleeding episode in patients with cirrhosis [1,2]. About one-third of all deaths in patients with cirrhosis can be directly attributed to acute variceal bleeding. The mortality can be minimized by effective primary prophylaxis in high-risk subjects, optimal management of acute bleeding, and universal secondary prophylaxis after the index bleed. This chapter will briefly discuss the anatomy of the portal venous system and pathophysiology of portal hypertension, and discuss in more detail the prevention and management of upper gastrointestinal bleeding secondary to portal hypertension.

Portal venous anatomy and physiology, and causes of portal hypertension

The anatomy of the portal venous system is shown in Fig. 124.1. The portal vein (PV) is about 5 cm long and formed behind the neck of the pancreas by the confluence of the splenic vein (SV) and superior mesenteric vein (SMV). The inferior mesenteric vein drains into the SV before the confluence of SV and SMV. The left gastric vein (also known as the left coronary vein) and right gastric vein (the smaller of the two) usually drain into the PV immediately after the confluence of the SV and SMV, but they may also drain at the confluence or into the SV. The short gastric vein, which drains the fundus of the stomach, enters the spleen above the origin of the SV. Usually remnants of the umbilical vein drain

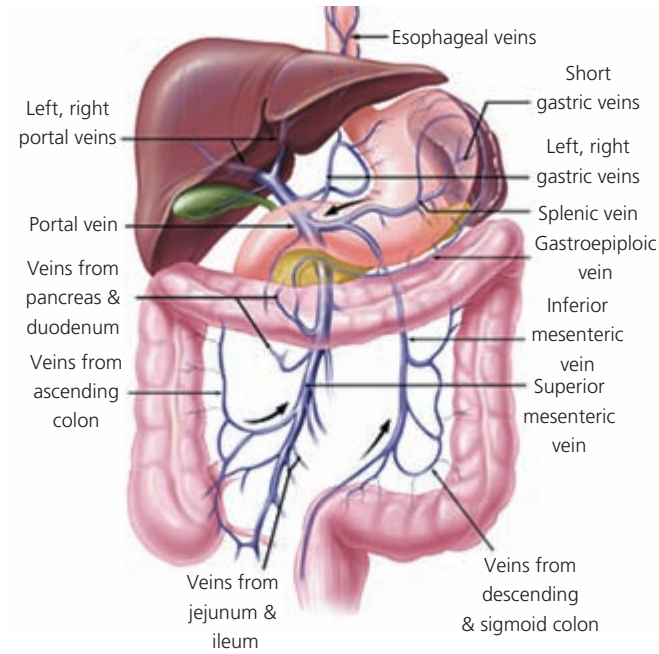

Figure 124.1 Anatomy of the portal venous system.

into the left portal vein and cystic vein from the gall bladder communicate with the right portal vein. About 70% of blood flow into the liver is through the portal vein and the rest comes from the hepatic artery. Three hepatic veins drain blood away from the liver into the inferior vena cava (IVC), but the caudate lobe drains directly into the IVC. The blood vessels near the cardia of the stomach are drained by the azygos vein. Normal azygos blood flow is between 150 and 200 mL/min, but this increases significantly in the presence of portal hypertension.

Normal PV pressure is 5–10 mmHg, and the gradient between PV and IVC is 1–5 mmHg. Under physiological

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Table 124.1 Causes of portal hypertension

Presinusoidal	Sinusoidal	Postsinusoidal
Schistosomiasis[a]	Cirrhosis from most causes[b]	Budd–Chiari syndrome
Idiopathic[a]	Severe hepatitis	IVC malformation
Portal vein thrombosis/stenosis[a]		Constrictive pericarditis
Compression of portal vein		Right heart failure
Splenic vein thrombosis		
Splanchnic arteriovenous fistula		
Primary biliary cirrhosis[a]		

a Relatively common causes.
b Most common cause.
Other rare causes include nodular regenerative hyperplasia, partial nodular transformation, congenital hepatic fibrosis, sarcoidosis, polycystic liver, and hereditary hemorrhagic telangiectasia.
IVC, inferior vena cava.

conditions, hepatic sinusoids have tremendous compliance and will maintain the pressure within these ranges even when the blood flow increases significantly. Portal hypertension is diagnosed when the portal venous pressure gradient (difference between PV and IVC pressure) is elevated above 5 mmHg. However, complications of portal hypertension usually do not occur until the pressure gradient is above 10 mmHg.

Portal hypertension occurs for a variety of reasons, but the most common cause is cirrhosis. In the majority of patients with cirrhosis, the resistance to flow is at the sinusoidal level. Portal hypertension may also occur due to portal vein occlusion or compression (presinusoidal) or due to obstruction in hepatic veins or the suprahepatic IVC (postsinusoidal). The common causes of portal hypertension are shown in Table 124.1. In primary biliary cirrhosis and schistosomiasis, the resistance is predominantly at presinusoidal level. Worldwide, schistosomiasis is a common cause of presinusoidal portal hypertension. This is caused by a granulomatous inflammation in response to the eggs laid by *Schistosoma mansoni* or *S. japonicum* in the presinusoidal portal venules. Idiopathic portal hypertension, common in Asia, is also presinusoidal. It is important to note that presinusoidal portal hypertension cannot be assessed indirectly by measuring the hepatic venous wedge pressure gradient (HVWPG). Rare causes of portal hypertension include nodular regenerative hyperplasia, congenital hepatic fibrosis, hereditary hemorrhagic telangiectasia (Fig. 124.2), and splanchnic arteriovenous fistula.

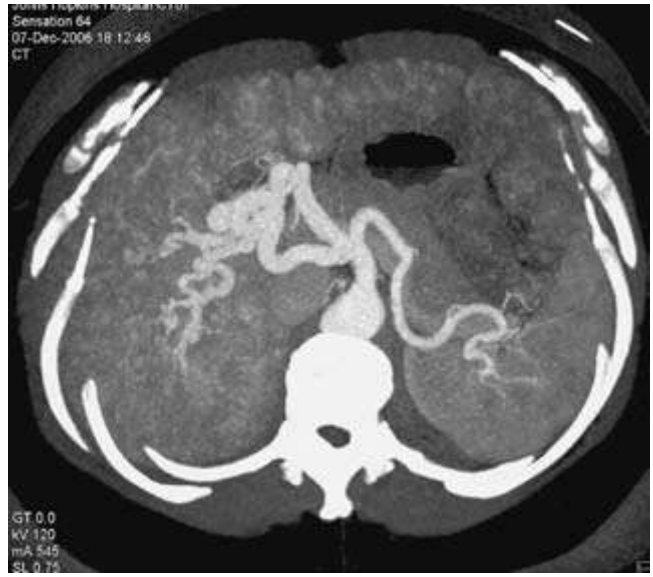


Figure 124.2 3D CT scan of a patient with hereditary hemorrhagic telangiectasia.

Portal venous imaging and measurement of portal pressure

The portal vein can be imaged in many ways, including ultrasound with Doppler, three-dimensional computed tomography (3D-CT) scan (Fig. 124.3), magnetic resonance imaging (MRI), endoscopic ultrasound (EUS), and angiogram. Of these, ultrasound with Doppler is commonly used to assess PV and hepatic vein patency. However, this investigation is decidedly operator dependent. A CT scan along with 3-D construction is a valuable test to assess liver parenchyma, liver masses, PV patency, splenic size, and to some degree the severity of collaterals. A magnetic resonance venogram (MRV) or angiograms are performed only when a firm assessment cannot be made by other methods.

Measurement of portal pressure is not performed routinely in clinical practice. However, it is an extremely valuable test to assess the risk of variceal bleeding. Portal pressure can be measured by direct puncture (transhepatic) or by a trans-

Figure 124.3 3D CT scan is a sensitive and reliable way to assess portal venous anatomy.

jugular intrahepatic route. Direct measurement is preferred only when presinusoidal portal hypertension (PHTN) or portal vein stenosis is suspected. In clinical practice, portal pressure is assessed indirectly by measuring hepatic venous wedge pressure (HVWP), where a balloon catheter (it can also be done by wedging a catheter into a hepatic vein) is passed into the hepatic vein and the balloon inflated to create a stationary and continuous column of fluid between the hepatic vein and the sinusoids. Total occlusion is confirmed by injection of contrast into the occluded hepatic vein. Free hepatic venous pressure (FHVP) is then measured by deflating the balloon. The difference (HVWPG) between HVWP and FHVP (HVWPG = HVWP – FHVP) is equal to the portal venous pressure gradient (portal pressure minus intraabdominal IVC pressure).

Studies have consistently shown that the risk of variceal bleeding is low (rarely gastric varices may be an exception) when HVWPG is less than 12 mmHg. Reduction of HVWPG below 12 mmHg or a 25% reduction from baseline significantly reduces the risk of variceal bleeding [3,4].

Pathophysiology of portal hypertension

The pathophysiology of portal hypertension is complex and incompletely understood despite the advances in our knowledge at the molecular level. Portal pressure is dependent on portal blood flow and resistance to portal venous flow (Ohm's law). In addition to the fixed defect caused by cirrhosis, the reversible contractile component in the hepatic vascular bed and the hyperdynamic splanchnic circulation, induced mainly by nitric oxide and to some extent by other local and systemic neurohumoral mechanisms, aggravate the portal hypertension and lead to complications including gastroesophageal variceal bleeding, ascites, hyponatremia, and hepatorenal syndrome [5–14]. A detailed discussion of the pathophysiology of portal hypertension and its complications is beyond the scope of this chapter, but important aspects are illustrated in Fig. 124.4 and briefly discussed here. Increased resistance to blood flow in the liver and increased splanchnic blood flow are two critical components of advanced portal hypertension.

The increased resistance in the liver in cirrhosis is due to "fixed" defects in the liver (including collagen deposition in the space of Disse, "capillarization" of sinusoids, thrombosis of portal venules, and compression of portal vein by large cirrhotic nodules), and increased, but potentially reversible, contractility of smooth muscle and hepatic stellate cells. The early event in the development of portal hypertension is chiefly mechanical obstruction caused by the "fixed" defects. The reversible contractile component in the hepatic vascular bed is important because this aspect may be amenable to pharmacological therapy. An imbalance between vasoconstrictors (endothelin-1, angiotensin, leukotrienes, norepinephrine [noradrenaline]) and vasodilators (nitric oxide, carbon monoxide, prostaglandins) may partly determine the resistance to portal venous flow within the liver by the effect of that imbalance on hepatic vasoregulation [6].

Although the collateral circulation decompresses the portal system in the early stages, it becomes inadequate with disease progression because of increased portal blood flow caused by changes in the splanchnic bed and the development of resistance in the collateral circulation. A hyperdynamic circulation, characterized by an increased cardiac output, markedly reduced systemic vascular resistance, and reduced mean arterial pressure, is a common cardiovascular manifestation of patients with cirrhosis and advanced portal hypertension [5,6,11]. The marked reduction in vascular resistance predominantly involves the splanchnic arterioles [5]. Local paracrine and autocrine vasodilators, including nitric oxide (perhaps most important), prostacyclin, and carbon monoxide, play a role in the pathogenesis of splanchnic vasodilation. It seems that nitric oxide (NO) is the most important mediator of splanchnic vasodilation [5,6,8–14]. Unlike in the hepatic circulation, NO is upregulated in the splanchnic bed. The precise events that lead to increased NO production remain unclear. Moreover, it is unclear whether increased NO production precedes or develops as a consequence of the hyperdynamic circulation. The activation of nitric oxide synthase (eNOS) in the endothelial cells is multifactorial and may include mechanical stimuli such as "shear stress" and other stimuli, including vascular endothelial growth factors, endotoxemia, or inflammatory cytokines including tumor necrosis factor-α (TNF-α). These stimuli may result in increased NO production through pathways that include eNOS phosphorylation and interaction with other proteins. Although our understanding of these pathways is incomplete, the

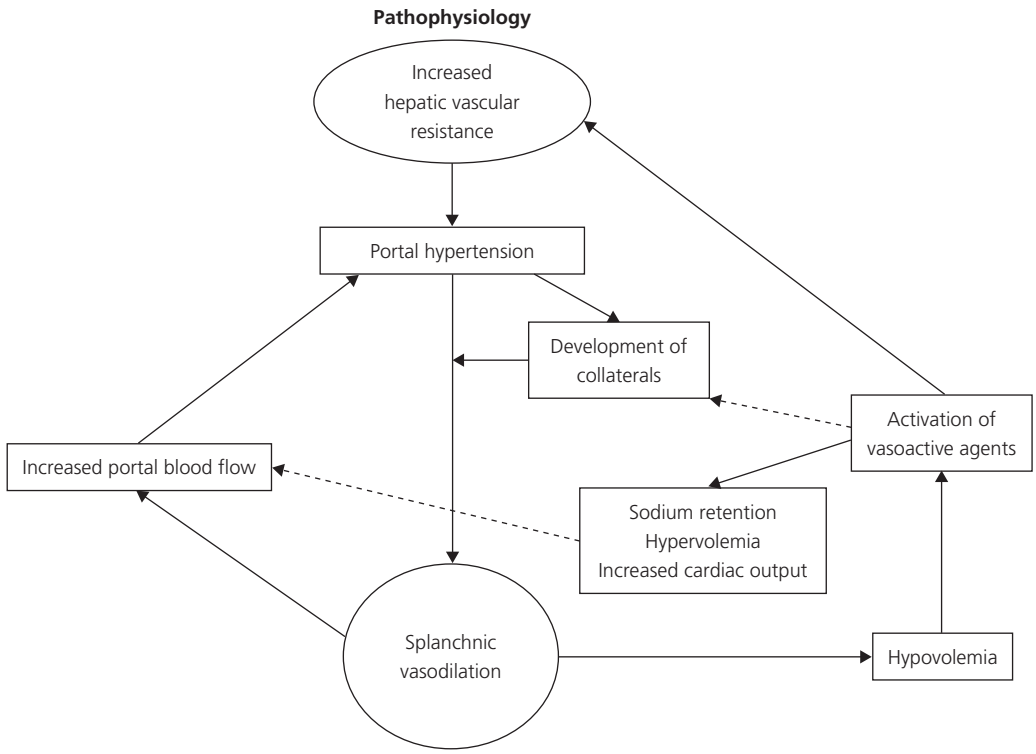


Figure 124.4 Pathophysiology of portal hypertension.

accumulating circumstantial evidence favors a key role for NO in the pathogenesis of splanchnic vasodilation in patients with advanced cirrhosis and portal hypertension [8–13,15,16]. Other alleged factors that contribute to the splanchnic vasodilation include increased levels of circulating vasodilators such as prostacyclin (prostaglandin I-2, PGI-2), vasoactive intestinal peptide (VIP), anandamide (an endogenous vasodilatory cannabinoid), substance P, platelet-activating factor, and glucagon [17–19]. Endogenous vasodilators, PGI-2 and PGE-2, counterbalance the vasoconstrictor effects of angiotensin-II, norepinephrine, vasopressin, and the augmented sympathetic tone to maintain renal blood flow and glomerular filtration rate (GFR). Increased vascular synthesis of prostacyclins contributes to increased circulating levels of prostacyclin; however, the vasodilating effect of prostacyclin is probably localized to the vascular bed where it is synthesized. Endotoxemia may be a causative factor for increased prostacyclin levels [18]. In contrast to increased systemic prostacyclin, renal prostaglandin synthesis decreases with advanced liver disease, which may contribute to the marked renal vasoconstriction found in the hepatorenal syndrome. For this reason, use of nonsteroidal antiinflammatory drugs (NSAIDs) is discouraged in patients with cirrhosis.

Experimental evidence has shown that when one of the vasoactive mediators, such as NO or prostacyclin, is inhibited, other vasoactive pathways are upregulated thereby preventing the correction of the splanchnic vasodilation. The complex relationship among these vasoactive systems implies that no one factor is likely to be solely responsible for the splanchnic vasodilation seen in patients with portal hypertension. Despite our increased understanding of the pathogenesis of portal hypertension, we have not been able to develop effective pharmacological agents to manage portal hypertension and its complications because of the complex relationship of the vasoactive system, as discussed above. However, this understanding has helped us to explore various pharmacological options, and hopefully we will see significant progress in this field in the coming decade. Drugs used for the management of portal hypertension include nonselective β-blockers, isosorbide mononitrate (ISMN), somatostatin (and its analogue octreotide), and vasopressin (and its analogue Glypressin). Drugs undergoing evaluation include angiotensin receptor blockers. Isosorbide mononitrate (which acts as a NO donor) and potentially angiotensin receptor blockers reduce intrahepatic resistance, whereas other drugs mainly reduce portal inflow. Somatostatin may also act by inhibiting endothelin-1-dependent hepatic stellate cell contraction. Octreotide and β-blockers may constrict collateral vessels. The mechanism of action of these drugs is discussed in more detail later in this chapter. The remainder of this chapter will focus on the management of gastrointestinal bleeding secondary to portal hypertension.

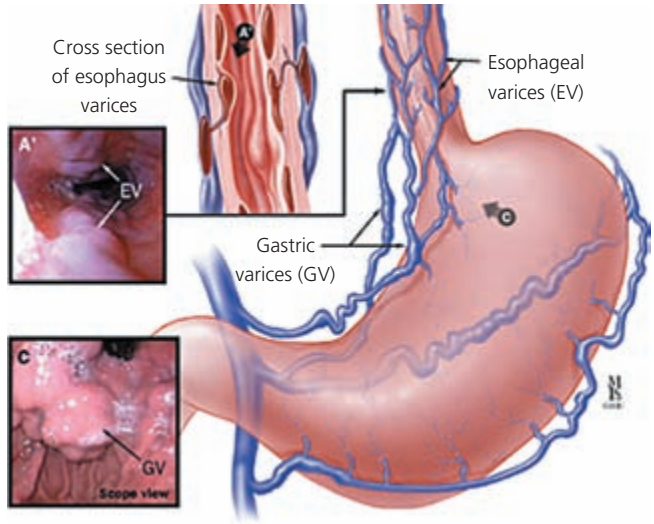


Figure 124.5 Collateral circulation in portal hypertension.

Figure 124.6 MRI showing large fundal varices.

Gastroesophageal varices

Varices are seen mainly at the gastroesophageal junction (GEJ) largely because of the unique anatomy [20]. Four zones (gastric, palisade, perforating, and truncal) of venous drainage are identified at the gastroesophageal junction. The *gastric zone* is 2–3 cm distal to the GEJ, and here longitudinal submucosal and lamina propria veins drain into short gastric and coronary veins. Most of the collateral circulation is seen between left and right gastric veins, and these collaterals appear as esophageal or gastric varices in continuation with esophageal varices (Fig. 124.5). Above this zone is the *palisade zone*, where veins appear more superficial with no communications between these veins and periesophageal veins that run parallel but outside the lumen. This zone extends for 2–3 cm and is the most common site for bleeding. Above this zone is the *perforating zone*, where there is an excellent venous plexus between submucosal and periesophageal veins. Bleeding is less common here, but these perforating veins continue to feed submucosal veins, which may explain why varices recur after band ligation or sclerotherapy. The *truncal zone* extends for another 10 cm as longitudinal veins with excellent venous connections with periesophageal veins. The endoscopic treatment of varices is mainly aimed at obliterating the varices in the palisade zone and to some extent the perforating zone.

In addition to gastroesophageal varices, large varices can also be seen in the retroperitoneum, rectum (collaterals between the IMV and pudendal vein), around the umbilicus (collateral between the remnant of the umbilical vein and left portal vein), and around the ileostomy stoma (as in patients with primary sclerosing cholangitis). Rarely, varices are seen in the colon, small intestine, and duodenum.

In cross-sectional studies, gastroesophageal varices were seen in only one-third of patients with cirrhosis, with a higher prevalence in those with decompensated cirrhosis. In a longitudinal surveillance study, only 31% (83/265) patients with primary biliary cirrhosis developed varices during a median follow-up of 5.6 years [21]. In this study, serum bilirubin and histological staging were independent predictors for the development of varices, and among those who developed varices, 1-year and 3-year bleeding rates were 33% and 41%, respectively.

Gastric varices

The prevalence of gastric varices in published reports varies from 6% to 16%, and the incidence appears to increase after esophageal variceal obliteration. Gastric varices are usually seen in association with esophageal varices or, rarely, in isolation as in splenic vein thrombosis (segmental portal hypertension). The gastric varices may be seen as a continuation of esophageal varices along the lesser curve or in the fundus, or as isolated varices in the fundus or antrum. Lesser curve varices are formed by the deep submucosal veins that arise from the left gastric vein. Fundal varices develop from the short gastric or posterior gastric veins; direct anastomotic veins between gastric and retroperitoneal veins also may be involved. These varices can be readily seen on 3D-CT scan or MRI as large collaterals (Fig. 124.6).

Ectopic varices

Ectopic varices other than rectal varices are rare, and generally occur in the duodenum, ileostomy stoma, small intestine, colon, or rectum. Duodenal and stomal varices are more often seen in portal vein thrombosis. Rectal varices are commonly seen in patients with cirrhosis, but bleeding is uncommon. Rectal varices are dilated superior and middle hemorrhoidal veins (Fig. 124.7).

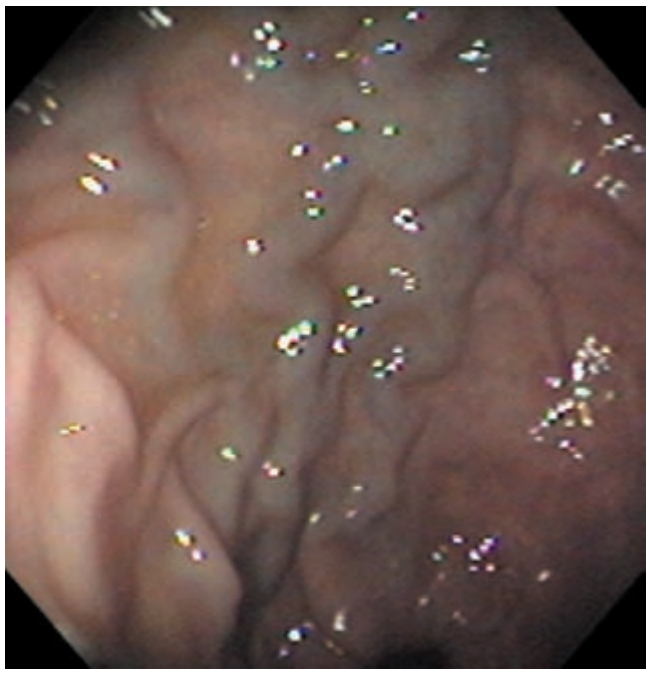


Figure 124.7 Rectal varices.

Risk of variceal bleeding and associated mortality

The evidence suggests that approximately one-third of patients with documented esophageal varices bleed within 2 years of the time of diagnosis. Bleeding from varices is an unpredictable complication of cirrhosis, with an associated mortality of 20%–40% with each bleeding episode [23–26]. A study has shown that in-hospital mortality of acute variceal bleeding has remained at around 20% since the mid-1990s despite advances in the management of acute variceal bleeding [2]. Many patients who survive the immediate post-bleeding period subsequently die from the complications of bleeding including sepsis, bacterial peritonitis, or renal failure. The mortality of variceal bleeding is directly related to the severity of liver disease, with a relatively low mortality in Child A patients (~ 10%–20%) and high mortality (~ 70%) in Child C patients [22–25] (Table 124.2). It is important to note that only one-third of all deaths in patients with cirrhosis can be directly attributed to variceal bleeding. This is an important consideration when the results of clinical trials are analyzed. Unfortunately many interventional studies have lacked the adequate sample size needed to show a difference in overall mortality or bleeding-related mortality. Nevertheless, these studies have helped us to define the management of these patients in a more objective manner.

The risk of bleeding from gastric varices is not well characterized, but the overall mortality from bleeding gastric varices exceeds 50%. In one study, 74 of 293 patients who presented to a center were found to have bleeding from gastric varices [26]. In other studies, the reported bleeding rates from gastric varices range from 14% to 36%. In one prospective study of fundal varices, 15/132 had previous variceal bleeding at the time of enrollment [27]. During follow-up, the cumulative risk of bleeding at 1, 3, and 5 years was 16%, 36%, and 44%, respectively. Large varices, red signs, and advanced liver disease were independent predictors of bleeding on multivariate analysis.

Bleeding from nongastroesophageal sites varies from 1.6% to 5% in the large reported series. The common ectopic sites, usually seen in association with esophageal varices, are

Table 124.2 Modified Child–Turcotte–Pugh (CTP) score

	Points		
Parameter	**1**	**2**	**3**
Albumin (g/dL)	> 3.5	2.8–3.5	< 2.8
Prothrombin time:			
Seconds prolonged	< 4	4–6	> 6
INR	< 1.7	1.7–2.3	> 2.3
Bilirubin (mg/dL)	< 2	2–3	> 3
Ascites	Absent	Slight	Moderate/tense
Encephalopathy	None	Grade I–II (slight/moderate)	Grade III–IV (moderate/severe)

Child–Turcotte–Pugh (CTP) class	**Points**
A	5–6
B	7–9
C	10–15

INR, international normalized ratio.

duodenum, colon, anorectum, and enterostomy. Rarely, these varices may be localized, especially in the colon, and result from superior or inferior vein thrombosis, tumor infiltration, adhesions, or congenital malformation.

Primary prophylaxis

The rationale for primary prophylaxis (prevention of first bleeding episode) is based on the high mortality associated with each variceal bleeding episode. The aim of primary prophylaxis is to treat high-risk patients with minimal complications. The treatment should be relatively cheap and effective, and should reduce bleeding-associated mortality and improve overall survival. In this section, we will discuss the risk factors for the first variceal bleed, the optimal treatment and its efficacy, and the cost-effectiveness of primary prophylaxis [28].

Endoscopic screening

It is difficult to show any benefit with primary prophylaxis in unselected patients with cirrhosis because of the low bleeding rates in patients without the risk factors for bleeding. Although β-blockers are considered effective in preventing the first bleed, studies in unselected patients with cirrhosis have failed to demonstrate any significant benefit with β-blockers. In a study where 319 "unselected" (not selected based on risk factors) patients with chronic liver disease were randomized to either propranolol or placebo, the overall incidence of bleeding within the first year was only 3.5% (11/319), with a similar incidence of bleeding in both groups [29]. The findings of this study emphasize the importance of endoscopic screening and staging of esophageal varices before primary prophylaxis because prophylaxis is likely to benefit only those with risk factors.

Independent risk factors that have been reported to predict the presence of esophageal varices include:

- elevated prothrombin time
- low platelet count
- splenomegaly
- portal vein (PV) diameter greater than 13 mm by ultrasound [30–33].

Based on these findings, it has been suggested that screening might be restricted to cirrhotic patients with a prothrombin activity less than 70%, a platelet count less than 100×10^9/L, splenomegaly, or ultrasonographic PV diameter greater than 13 mm [30–32]. However, the existing recommendation is screening for esophageal varices by endoscopy in all patients with cirrhosis because of the high mortality associated with variceal bleeding. The cost-effectiveness of such a strategy will be discussed later in this section. Patients who are unlikely to benefit from prophylactic therapies and those with low life expectancy may be excluded from screening [33]. When no varices or small varices without stigmata (low-risk varices) are found on initial screening endoscopy, repeat endoscopy is recommended every 2 years. A new development in this field is the use of capsule endoscopy for screening. Although small preliminary studies are encouraging, larger studies that are in progress will determine the sensitivity and specificity of this technique to diagnose and stage esophageal and gastric varices.

Staging of esophageal varices

Because only 30%–40% of patients with varices bleed in the first 2 years after the diagnosis of esophageal varices, it is important to identify the subgroup (those with risk factors) that is most likely to bleed and offer the most effective treatment to those patients [34]. Reproducible staging of varices is important for many reasons, including accurate description (common terminology), surveillance (reproducibility), treatment, and research (identify high-risk subjects) purposes. However, there are too many classifications described in the literature and many of them are subjective with significant intra- and inter-observer variation [34–37]. Based on the author's own experience, a minor modification of the Japanese Research Society for Portal Hypertension classification for staging varices is recommended for routine clinical purposes [35,38]. In this classification, esophageal varices are described by:

- the extent (distance from GE junction in cm)
- size (F0 – no varices; F1 – small and nontortuous; F2 – tortuous, but less than 50% radius; F3 – large and tortuous)
- color (blue or white)
- presence of red signs (red wale markings, cherry-red spots, hematocystic spots) (Fig. 124.8a,b).

Staging of gastric varices

Gastric varices are described by their location (cardia or fundus) and size (small and large) as well as whether they are in continuation with esophageal varices (Fig. 124.9). The most commonly used classification, described by Sarin et al. [39], divides gastric varices into four groups:

- GOV1 – when esophageal varices extend into the lesser curvature
- GOV2 – when esophageal varices extend into the fundus
- IGV1 – isolated fundic varices without esophageal varices
- IGV2 – isolated antral varices (Fig. 124.10).

Predictors of first variceal bleeding

Many risk factors have been identified as predictors of first bleeding in patients with esophageal varices. There is a general consensus that patients with advanced cirrhosis (Child B and C), large varices, varices with red signs (red spots, wale sign, hematocystic spot), and those with high portal pressure (HVWPG ≥ 12 mmHg) are more likely to bleed [34,35,40–42]. It has been shown that the risk of bleeding is higher with:

- proximal extension of varices (63% with the proximal third vs 26%% when seen only in the distal third)
- larger varices (F1, 15%; F2, 32%; F3, 68%)

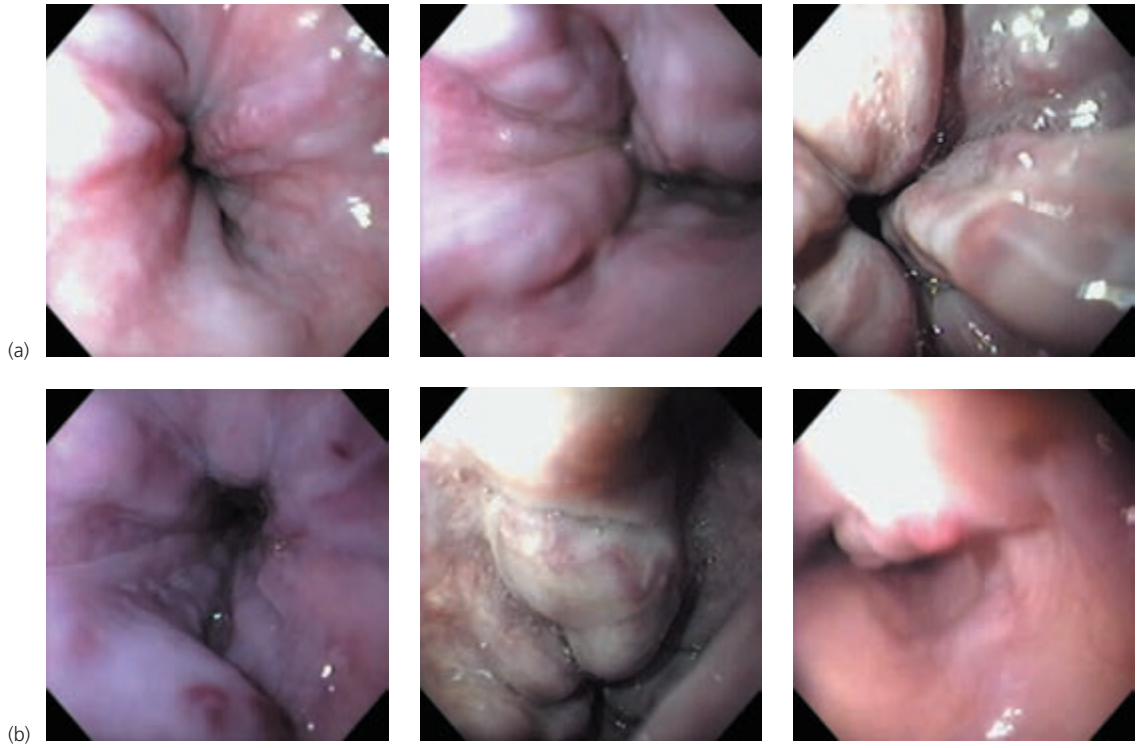


Figure 124.8 **(a)** Grading of esophageal varices based on their size. **(b)** "Red signs" of esophageal varices.

Figure 124.9 Small and large gastric varices.

- blue varices (80% vs 45% with white)
- red signs (76% vs 17% without)
- hematocystic spots (100% vs 49% when absent) [35].

Many studies have confirmed that HVWPG is directly related to the risk of bleeding [41–44]. It has been consistently shown that patients do not bleed if the HVWPG is less than 12 mmHg [41,43]. Pharmacological intervention studies have confirmed that the risk of bleeding is absent or markedly diminished (bleeding risk reduced by 80%) if the HVWPG is reduced below 12 mmHg or more than 25% below the baseline pressure respectively [1,3,42]. Other risk factors for variceal bleeding include continued alcohol use and hepatocellular carcinoma [34,45].

Therapeutic options for primary prophylaxis

In the 1960s, before the endoscopic era, a number of randomized, controlled surgical shunt trials were conducted for primary prophylaxis of variceal bleeding. Although these trials consistently showed a significant reduction in variceal and gastric mucosal bleeding in patients who had shunts, the

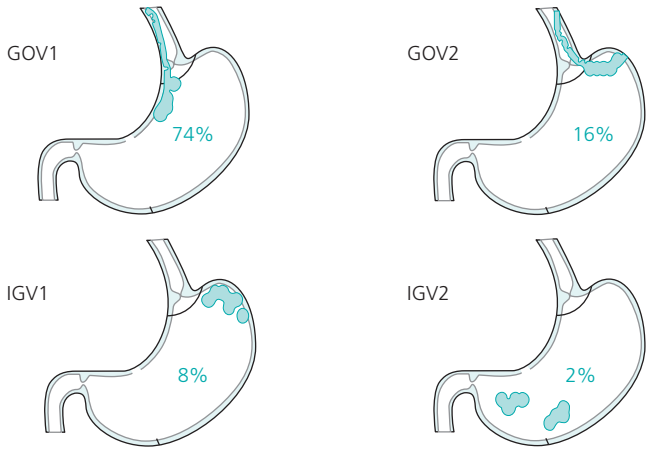


Figure 124.10 Sarin's classification of gastric varices.

mortality and the incidence of chronic hepatic encephalopathy were significantly higher in the shunted patients compared with the control group [46]. These poor results and the advent of endoscopy led to the abandonment of shunt surgery for primary prophylaxis. Esophageal transection with devascularization has been tried for primary prophylaxis. There is no convincing evidence to recommend this form of surgery for primary prophylaxis, and such surgery may make future liver transplantation technically more difficult. Transjugular intrahepatic portosystemic shunt (TIPS) has not been studied for primary prophylaxis, but based on secondary prevention trials; it is unlikely to be beneficial and may be even be detrimental [47]. On the basis of the evidence, there is no role for shunt, nonshunting surgery, or TIPS for the primary prophylaxis of variceal bleeding.

Sclerotherapy

Prophylactic sclerotherapy has been the subject of many small and large randomized controlled studies. Early reports claimed a reduced bleeding rate and improved survival in patients who had sclerotherapy compared with controls, but subsequent studies have not confirmed these results. A large multicenter trial ($n = 281$) involving men with moderate to severe alcoholic liver disease was terminated prematurely (after 22.5 months) because of higher mortality in the sclerotherapy arm compared with the sham therapy arm (32.2% vs 17.4%, $P = 0.004$) [48]. It has been argued that inclusion of patients with a lower risk of bleeding in these trials may have masked the benefits for patients who had a higher risk of bleeding. To test this hypothesis, a number of trials were conducted where only those patients with high risk of bleeding were enrolled. These studies also failed to show a consistent improvement in survival in the treated group compared with the control group. A metaanalysis of eight controlled studies showed that prophylactic sclerotherapy may reduce mortality by 11% (95% confidence interval [CI] 4%–19%) [49]. The results of this metaanalysis should be interpreted with caution because the difference was only marginal and the analysis included pooled data from heterogeneous studies [49,50]. The evidence suggests that, even in high-risk patients, the benefits from prophylactic sclerotherapy may be marginal and the procedure is not cost-effective.

β-Blockers

Nonselective β-blockers reduce portal pressure by reducing cardiac output, and by reducing portal inflow through antagonism of the β_2 receptors on the splanchnic vessels. β-Blockers also may increase portocollateral resistance and hence reduce collateral blood flow. Many trials have assessed the efficacy of nonselective β-blockers such as propranolol or nadolol for primary prophylaxis. Although some earlier studies did not show significant differences in bleeding rates in patients treated with propranolol [29,51,52], a majority of studies have shown that nonselective β-blockers reduced the risk of first bleeding by about 50% in patients with large varices [52,53]. Metaanalyses of these trials have also confirmed a benefit in the treated group with regard to bleeding (about 50% reduction) and mortality (25%–45% reduction) [46,53,54].

Patients with varices are unlikely to bleed if the HVWPG is maintained below 12 mmHg [42]. Unfortunately, with the available drugs this can be achieved only in a small group of patients. Unless a reliable noninvasive method is developed, it is difficult to justify repeated HVWPG measurements to determine whether or not a drug therapy is effective. So for all practices, the end point of treatment is to achieve a resting heart rate of less than 60 beats/min or a 25% reduction from the baseline heart rate. The major disadvantage of β-blockers is poor tolerance and compliance. Despite these limitations, the use of nonselective β-blockers is cost-effective and should be the treatment of choice for primary prophylaxis for patients with advanced liver disease and large gastroesophageal varices. Use of long-acting preparations, gradual increments in dose, and better patient education may improve the compliance. In patients with cirrhosis the practice is to continue administration of β-blockers indefinitely or until liver transplantation. Although there are no convincing data to support this strategy, a small study seems to indicate that the risk of bleeding returns to that expected in the untreated population when propranolol is discontinued [55].

It has been suggested that the hemodynamic response to β-blockers may predict the risk of subsequent bleeding. Merkel et al. measured the HVWPG before and 1–3 months after treatment with nadolol or nadolol plus ISMN in 49 patients with large varices and no previous variceal bleeding [56]. A good response – defined as a reduction in HVWPG to ≤ 12 mmHg ($n = 12$), or a reduction in HVWPG by more than 20% from baseline ($n = 18$) – was seen in 30 (61%) patients. During a follow-up of 5 years, seven (7/19) "poor-responders" and two (2/30) "good-responders" had variceal bleeding. The probability of bleeding at 3 years was 41% in

poor-responders and 7% in good-responders ($P < 0.001$). As in many previous studies, none of the 12 patients who had a reduction in HVWPG to ≤ 12 mmHg had variceal bleeding. Although this is perhaps the "optimal" treatment strategy and the best predictor of bleeding, the costs may be prohibitive for routine clinical care. In addition, the invasive nature of the process (twice in the same patient) may increase the risks and reduce the compliance.

An alternative strategy was tested in another study where HVWPG was measured before and 90 min after a single dose of 80 mg of propranolol in 33 patients [57]. Of the 22 patients who had a reduction in HVWPG equal to or greater than 20%, only one had variceal bleeding during a follow-up of 9–38 months, and none of the patients whose HVWPG was reduced below 12 mmHg had bleeding. Although this was a small study, if these results are reproduced in larger studies, a similar strategy could be recommended, provided cost is not an issue, to all patients with large varices before initiating primary prophylaxis with β-blockers. Those who do not show a reduction in HVWPG could then be offered alternative treatments.

The cost-effectiveness of measuring HVWPG has been examined in many studies using hypothetical models. Hicken et al. examined the role of HVWPG in patients with varices at risk for bleeding [58]. The authors compared three protocols:

- β-blocker therapy without measurement of HVWPG
- a single measurement 4 weeks after the initiation of treatment
- two measurements (one before and another 4 weeks after initiation of treatment).

Compared with the standard practice (no measurement of HVWPG), the incremental cost with one HVWPG measurement was $108 185 per bleeding prevented and $355 200 per death averted. The corresponding figures for two measurements of HVWPG were $202 796 and $719 300, respectively. Although these costs may not be a true reflection of real costs, nevertheless HVWPG may be prohibitive for routine use. Cheaper and less invasive techniques need to be developed to measure portal pressure for "optimal" and effective primary prophylaxis.

Nitrates

About one-third of patients with cirrhosis do not tolerate β-blockers because of side effects including hypotension and severe fatigue. Of the other vasodilators that also been studied for primary prophylaxis, nitrates have been the most promising. Although not clearly understood, it is thought that nitrates may reduce intrahepatic resistance by acting as a NO donor, and thereby relaxing the actively contracting hepatic stellate cells [59]. However, there is evidence to suggest that the response of stellate cells to NO may be impaired in cirrhosis [60]. Another study suggested that ISMN may act "by means of reflex splanchnic arterial vasoconstriction in response to vasodilatation in other vascular beds" [61].

Isosorbide mononitrate has been tried in patients for primary prophylaxis and the results have been mixed. In a double-blind, randomized, controlled trial, Garcia-Pagan et al. compared ISMN ($n = 67$) and placebo ($n = 66$) in 113 consecutive cirrhotic patients with varices who could not tolerate or had contraindications for β-blockers [62]. The variceal bleeding rate was similar at 1 year and 2 years in both groups. In multivariate analysis, presence of the variceal red sign was the only independent predictor of bleeding (relative risk 3.4, $P < 0.01$). Based on this study, the authors concluded that ISMN does not have any role for primary prophylaxis. Another study comparing ISMN with placebo found an increased mortality in patients above 50 years of age [63]. In addition, a tendency for ascites to increase in patients treated with nitrate monotherapy has been reported.

Endoscopic variceal band ligation

Primary prophylactic sclerotherapy failed to show any distinct advantage over no treatment because the benefits of sclerotherapy were negated by the local complications of sclerotherapy. Banding, with fewer local complications, was a logical alternative to sclerotherapy [64] (Table 124.3) (Fig. 124.11). Many studies have determined the role of endoscopic variceal ligation (EVL) for primary prophylaxis [65–72].

In a small study, Sarin et al. randomized 35 patients to banding and 33 to no treatment and found a lower bleeding rate in the banding group (3/35, 8.6%) compared with the no treatment group (13/33, 39.4%, $P < 0.01$) during mean follow-up of 14 months [65]. Although overall mortality was similar (11.4% vs 24.2%), there was a trend toward lower bleeding-related mortality in the banding group (2.9% vs 15.2%, $P = 0.08$). Another prospective, randomized trial comparing prophylactic EVL with no treatment found that EVL was associated with a significantly decreased risk of variceal hemorrhage and overall mortality [66]. In this study, EVL was more effective in Child A patients compared with those with decompensated disease (Child B and C). A prospective, randomized trial conducted in Taiwan found that patients with Child–Pugh class B cirrhosis and high-risk varices (F2 or F3 with red color signs) had a significantly lower risk of variceal bleeding than untreated patients [67]. In addition, a trend toward reduced mortality and less blood transfusion requirements was seen in patients undergoing banding. Apart from the small sample size, the major concern of these studies was the "no-treatment" arm because the standard of care for primary prophylaxis during the study period was β-blocker therapy, and one may consider that it was unethical to have a "no treatment arm" for such studies.

Band ligation has also been compared with sclerotherapy for primary prophylaxis of variceal bleeding; no difference in efficacy was found [68]. As in secondary prevention trials, band ligation required fewer sessions to eradicate varices in patients, but the recurrence rate of varices was higher in those treated with ligation.

Table 124.3 Complications of endoscopic treatment

Treatment	Common complications	Uncommon complications
Sclerotherapy	Retrosternal chest pain Ulcers Bleeding from ulcers Mediastinitis Transient dysphagia Stricture[b]	Esophageal perforation Bacterial peritonitis/sepsis[a] Motility disorder Portal/mesenteric vein thrombosis Aspiration pneumonia[a] Pleural effusion
Band ligation	Retrosternal chest pain Ulcers Transient dysphagia	Stricture Perforation Aspiration pneumonia[a] Bacterial peritonitis/sepsis[a] Bleeding from ulcers

a More common in acute variceal bleeding.
b Late complication.

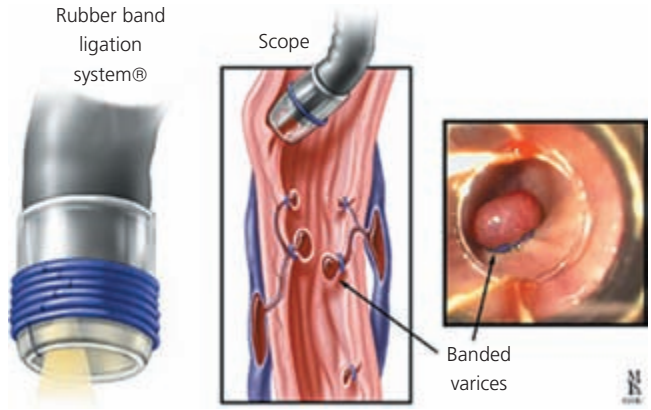


Figure 124.11 A cartoon showing esophageal variceal banding.

Because the "best available" treatment for primary prophylaxis was nonselective β-blockers, any new treatment modalities needed to be tested against that treatment. Sarin et al. addressed this by comparing banding to propranolol in a study (high-risk patients with large varices and red signs) where 44 patients were randomized to propranolol and 45 to endoscopic banding [69]. During a mean follow-up of approximately 14 months, 12 patients in the propranolol group and 4 in the ligation group had variceal bleeding, with an actuarial probability of bleeding of 43% in the propranolol group and 15% in the ligation group ($P = 0.04$). Three of four patients in the banding group experienced their first bleeding before varices were completely obliterated. The overall mortality and bleeding-related mortality were similar in both groups. In this study, 7 of 89 patients had noncirrhotic portal hypertension. Moreover, the study has been criticized for inadequate dosage of propranolol (mean propranolol dose was 70 mg vs 123 mg in other studies [46,52–54]). The higher bleeding rate in the propranolol group (43% vs 22% in other trials) may have been due to inadequate β-blocker dosage. Despite some of the criticisms and the small sample size, it was an important and provocative study that stimulated further research into the role of variceal banding for primary prophylaxis.

In a three-arm study, Lui et al. compared endoscopic banding ($n = 44$), propranolol ($n = 66$), and ISMN ($n = 62$) in 172 patients with grade II to III varices over a 6-year period [70]. During a mean follow-up of 20 months, by intention-to treat analysis, variceal bleeding occurred in 7% in the banding group, 14% in the propranolol group, and 23% in ISMN group. The 2-year actuarial risk of first bleeding was 6.2%, 19.4%, and 27.5%, respectively. The incidence of side effects was significantly greater in the propranolol (45%) and ISMN (42%) groups compared with the banding group (2%, $P < 0.001$), and this resulted in withdrawal of treatment for 30% of patients in the propranolol group and for 21% in the ISMN group. When patients were analyzed based on the treatment received, the actuarial bleeding rate was significantly different only between the banding and ISMN groups (7.5% vs 33%, log rank test $P = 0.03$), and there was no difference between the banding and propranolol groups [70]. A trial comparing band ligation and β-blockers in patients with large varices and a HVWPG greater than 12 mmHg was abandoned when investigators realized that they would require 424 patients in each arm to show a difference in bleeding rates between the groups [71]. Subsequently, a small study suggested that propranolol-treated patients had higher bleeding rates compared with those who were treated by banding [72]. Because the difference in bleeding rates between endoscopic therapy and β-blocker therapy for the prevention of first variceal bleeding is small, a large multicenter trial with an estimated sample size of 500 patients in each arm will be

required to demonstrate a statistically significant difference between these two treatment options [71].

A metaanalysis of 601 patients from five randomized controlled trials comparing banding with "no treatment" confirmed that banding reduced first variceal bleeding (relative risk [RR] 0.36, CI 0.26–0.5), bleeding-related mortality (RR 0.20, CI 0.11–0.39), and overall mortality (RR 0.55, CI 0.43–0.71) [73]. However, when banding was compared with β-blockers in 283 patients from four randomized trials, the only benefit with variceal banding was in the reduction of first variceal bleeding (RR 0.48, CI 0.24–0.96). Bleeding-related mortality and overall mortality were similar with banding and propranolol, suggesting that the role of variceal banding may be in patients who cannot tolerate β-blockers because of side effects, hypotension, or noncompliance.

If noninvasive, reliable methods for measuring portal pressure are identified, it will be appropriate to offer banding to those who fail to show significant reduction (< 25% reduction from baseline) in portal pressure with pharmacological therapy. However, methods of measuring HVWPG are not applicable or cost-effective for the majority of patients with large esophageal varices.

Combination treatment

Sclerotherapy plus β-blocker

Combination treatment with endoscopic sclerotherapy and β-blockers has not shown any advantage over β-blockers alone for primary prophylaxis. In one study, 86 patients with high intravariceal pressure (> 18 mmHg) were randomized to either propranolol alone ($n = 42$) or propranolol plus sclerotherapy ($n = 44$) [74]. There was a trend toward a higher bleeding rate (23%) in the combination group compared with those who received only propranolol (14%, P = not significant [NS]) during a median follow-up of approximately 24 months. There was a significantly higher complication rate in the combination group (52% vs 19%, $P = 0.002$), but mortality rates were similar (14% vs 18%, P = NS). Predictably, this study showed that there is no role for sclerotherapy in combination with β-blockers for primary prophylaxis.

β-Blocker plus isosorbide mononitrate (ISMN)

Although β-blockers reduced the risk of bleeding in patients with large varices, about 50% of patients who were on β-blockers experienced variceal bleeding. This was perhaps the result of a combination of factors including poor compliance, intolerance to maximal treatment, and, more importantly, inadequate reduction in portal pressure. Because it is not practical or cost-effective to monitor HVWPG before and after treatment with β-blockers to assess the treatment response, many investigators have studied the effect of a combination of β-blockers and ISMN. The combination treatment of nitrates with β-blockers has produced conflicting data.

In one study, Merkel et al. compared nadolol alone ($n = 74$) and nadolol plus ISMN ($n = 72$) in 146 patients with large esophageal varices [75]. Nadolol was started at 40 mg daily and increased every second day to achieve a 20%–25% reduction in resting heart rate. In the combination group, ISMN was started with nadolol, and the dose of ISMN was increased from 10 mg twice daily to 20 mg twice daily if patients could tolerate it. During a median follow-up of 30 months, 11 (11/74) from the nadolol-alone group and 4 (4/72) in the combination group had their first episode of variceal bleeding ($P = 0.03$ by Kaplan–Meier analysis). The cumulative risk of bleeding was 18% for the nadolol-alone group and 7.5% for the combination group. The mortality was not statistically significantly different (14/74 vs 8/72) between the groups, but there was a trend favoring the combination group. As a sequel to this study, the authors reported the long-term results (up to 7 years of follow-up) of the same cohort [76]. During the study period 16 patients in the nadolol group and 8 in the combination group had variceal bleeding ($P = 0.02$), with a cumulative bleeding risk of 29% and 12%, respectively. However, the mortality remained similar in both groups. By contrast, a multicenter, prospective, double-blind, randomized controlled trial ($n = 349$) comparing propranolol plus placebo ($n = 174$) to propranolol plus ISMN ($n = 175$) found no significant difference in the risk of first variceal bleed at 1 year (8.3% vs 5%) and 2 years (10.6% vs 12.5%) [77]. Moreover, there were significantly more frequent adverse side effects in the combination group secondary to a higher incidence of headache. These observations suggest that combination treatment of β-blockers plus ISMN is a reasonable option for primary prophylaxis if patients cannot tolerate maximal β-blockers. There is no role for routine combination treatment of β-blockers and ISMN for primary prophylaxis.

Nadolol plus spironolactone

Other drugs, including spironolactone, have been tried for primary prophylaxis. In one such study, combination therapy using nadolol plus spironolactone for primary prophylaxis of variceal hemorrhage did not show any additive effect [78].

Cost-effectiveness of screening and primary prophylaxis

There is overwhelming evidence to suggest that primary prophylaxis with β-blockers is beneficial in patients with cirrhosis and large varices with stigmata of imminent bleeding (Table 124.4). However, screening is not routinely performed in the majority of patients with advanced cirrhosis. In one study, only 46% of patients referred for liver transplantation to a tertiary center had either endoscopy or barium studies to assess the presence of esophageal varices [79]. We are unlikely to make a major reduction in the incidence of first bleeding without better use of screening and prophylaxis among the community of gastroenterologists. Although there are no rigid criteria, it has been suggested that all patients

Table 124.4 Variceal bleeding rates (%) in primary prophylaxis trials

Authors	Control	ES	β-Blocker	EBL	*P* value
VA study [48] (*N* = 281)	13.8	7.0			< 0.05
Pascal et al. [52] (*N* = 230)	61		26		< 0.05
Lay et al. [66] (*N* = 126)	60			19	0.0001
Lo et al. [67] (*N* = 127)	22.2			12.5	0.22
Sarin et al. [69] (*N* = 89)			12	15	0.04
Lui et al. [70] (*N* = 172)			19.4	6.2	NS
Thuluvath et al. [71] (*N* = 31)			7	13	NS
Jutabha et al. [72] (*N* = 62)			19	0	

ES, endoscopic sclerotherapy; EBL, endoscopic band ligation; NS, not significant.

with cirrhosis should have a screening endoscopy. If there are large varices, they should be offered prophylactic therapy. If varices are absent, they should have surveillance endoscopy every 2 years. If the varices are small, they should have repeat endoscopy every 1–2 years based on the severity of liver disease.

Many studies have examined the cost-effectiveness of primary prophylaxis for variceal bleeding. Using a Markov model, Teran et al. compared the cost-effectiveness of propranolol, sclerotherapy, and shunt surgery after stratifying patients by their risk of bleeding [80]. Whereas sclerotherapy had no advantage for quality of life, shunt surgery was associated with decreased quality of life. Propranolol was most cost-effective (cost saving $450–14 600 over a 5-year period) followed by sclerotherapy and shunt surgery.

In another study, using Markov modeling, Spiegel et al. examined whether it was cost-effective to treat all patients with cirrhosis without screening endoscopy [81]. The authors compared six different strategies:

- strategy 1: universal screening followed by β-blockers if there are varices
- strategy 2: screening followed by banding
- strategy 3: selective screening only in high-risk patients followed by β-blockers if varices are present
- strategy 4: selective screening followed by banding
- strategy 5: empirical β-blocker therapy in all patients
- strategy 6: no prophylactic therapy.

Cost estimates were from a third-party payer perspective and the outcome measure was the cost of first variceal bleeding prevented by the above six strategies. As expected, strategy 6 was the least expensive and, in comparison, the cost of strategy 5 was $12 408 per additional variceal bleeding prevented. Strategies 2 and 3 were most expensive ($175 000 per additional bleed prevented), whereas the costs of strategies 4 and 5 were in between. Based on this mathematical modeling, the authors recommended universal β-blocker therapy for all patients with cirrhosis. Another strategy that was not examined in this study is the use of screening endoscopy in only those who do not tolerate β-blockers.

Other investigators have also addressed the cost-effectiveness of screening and no prophylaxis or prophylaxis with either β-blockers or banding using Markov models [82,83]. Saab et al. recommended universal prophylaxis with β-blockers because it was associated with lower costs ($34 100) and higher quality-adjusted life years (6.5) [82]. By contrast, costs and quality adjusted life-years were $36 600 and 4.84 for "no prophylaxis" and $37 300 and 5.72 for screening and β-blockers for those with large varices. In another study, a Markov simulation model was created in two cohorts of 50-year-old patients with compensated or decompensated cirrhosis [83]. Four strategies were examined in this study:

- no screening or prophylaxis
- screening followed by β-blockers
- screening followed by banding
- universal prophylaxis with β-blockers.

In compensated cirrhosis, screening followed by β-blockers was cost-effective ($3605 per year of life saved), and in decompensated cirrhosis universal β-blockers was cost-effective ($1154 per year of life saved).

The results based on Markov models are sensitive to the prevalence of esophageal varices, risk of bleeding, and cost of endoscopy and primary prophylaxis. In the absence of clinical trials, I believe that it is premature to abandon screening or surveillance endoscopy before starting prophylactic β-blockers because of the high incidence of side effects associated with such therapy and the low risk of bleeding in patients with small varices [29]. Moreover, the awareness of the presence of large esophageal varices may improve compliance among the patients. In addition, the physician may offer alternative treatment (variceal banding) to the high-risk subjects who do not tolerate β-blockers. In future, less invasive tools such as capsule endoscopy may be used for screening if ongoing studies corroborate the excellent sensitivity and specificity shown in small preliminary studies.

Table 124.5 Overall mortality (%) in primary prophylaxis trials

Authors	Control	ES	β-Blocker	EBL	*P* value
VA study [48] (*N* = 281)	17.4	32.2			0.004
Pascal et al. [52] (*N* = 230)	49		28		< 0.05
Lay et al. [66] (*N* = 126)	58			28	0.0011
Lo et al. [67] (*N* = 127)	36.5			25	0.19
Sarin et al. [69] (*N* = 89)			11	11	0.77
Lui et al. [70] (*N* = 172)			27.3	25	NS

ES, endoscopic sclerotherapy; EBL, endoscopic band ligation.

Gastric and ectopic varices

In general, gastric variceal bleeding is often more severe than esophageal variceal bleeding, requiring more blood transfusion. Similarly, the overall mortality from bleeding gastric varices is also higher, reaching close to 50% in some series with significantly higher risks when fundal varices bleed [39]. Because there are no controlled studies for primary prophylaxis of gastric or ectopic varices, it is difficult to recommend any special strategy. However, based on the data from esophageal varices, it is recommended that all patients with large gastric or ectopic varices are treated with nonselective β-blockers.

Primary prophylaxis: summary of recommendations

Nonselective β-blockers should be used for primary prophylaxis in cirrhotic patients with large varices and those with risk factors (red signs, HVWPG ≥ 12 mmHg) for bleeding. A summary of clinical trials is shown in Tables 124.5 and 124.6 (see also Table 124.4). Nitrovasodilators are possibly beneficial in combination with β-blockers in those who cannot tolerate maximal β-blocker therapy. Endoscopic sclerotherapy has no role for primary prophylaxis. Endoscopic banding may be an option for those with high risk of bleeding who do not tolerate, or are noncompliant or show poor response to β-blockers. An algorithm for primary prophylaxis is shown in Fig. 124.12.

Table 124.6 Bleed-related mortality (%) in primary prophylaxis trial with banding

Authors	Control	β-Blocker	EBL	*P* value
Lay et al. [66] (*N* = 126)	16		19	< 0.05
Lo et al. [67] (*N* = 127)	11.1		12.5	0.15
Sarin et al. [69] (*N* = 89)		9	7	NS
Lui et al. [70] (*N* = 172)		6.1	2.3	NS

EBL, endoscopic band ligation; NS, not significant.

Acute variceal bleeding

Patients with acute variceal bleeding should be managed in an optimal fashion to reduce early mortality. The past decade has seen significant progress in the management of acute variceal bleeding. A large number of randomized controlled studies have been published on this topic comparing various treatment options. Although these studies have uniformly lacked the sample size to show an improvement in survival, metaanalyses of these studies have been able to circumvent this problem to some extent. However, inclusion of heterogeneous studies has been a major weakness of such analyses. Despite these limitations, careful observations based on individual studies as well as metaanalyses have made it possible for clinicians to devise a more successful strategy to manage patients who present with acute variceal bleeding. A broad outline of the management is shown in Fig. 124.13.

Resuscitation and immediate management

Management of acute variceal bleeding includes general resuscitative measures and specific treatment aimed at arresting the variceal bleeding and prevention of early rebleeding. Airways must be protected in an encephalopathic patient, particularly during endoscopic procedures and balloon tamponade, because aspiration pneumonia is a major cause of morbidity and mortality in these patients. Measurement of central venous pressure is a useful guide to the blood volume status but caution should be exercised in the presence of tense ascites because diaphragmatic compression of the right atrium by the ascites may lead to an overestimation of the readings. Pulmonary capillary wedge pressure is an accurate guide to control blood volume and may be invaluable in the presence of major ongoing bleeding. Replacement of blood volume should be prompt and as accurate as possible to protect vital organs, particularly renal function. Although the ideal replacement fluid is blood, colloids (preferably) or crystalloids may be used for immediate infusion until blood becomes available. Isovolemic replacement should be the

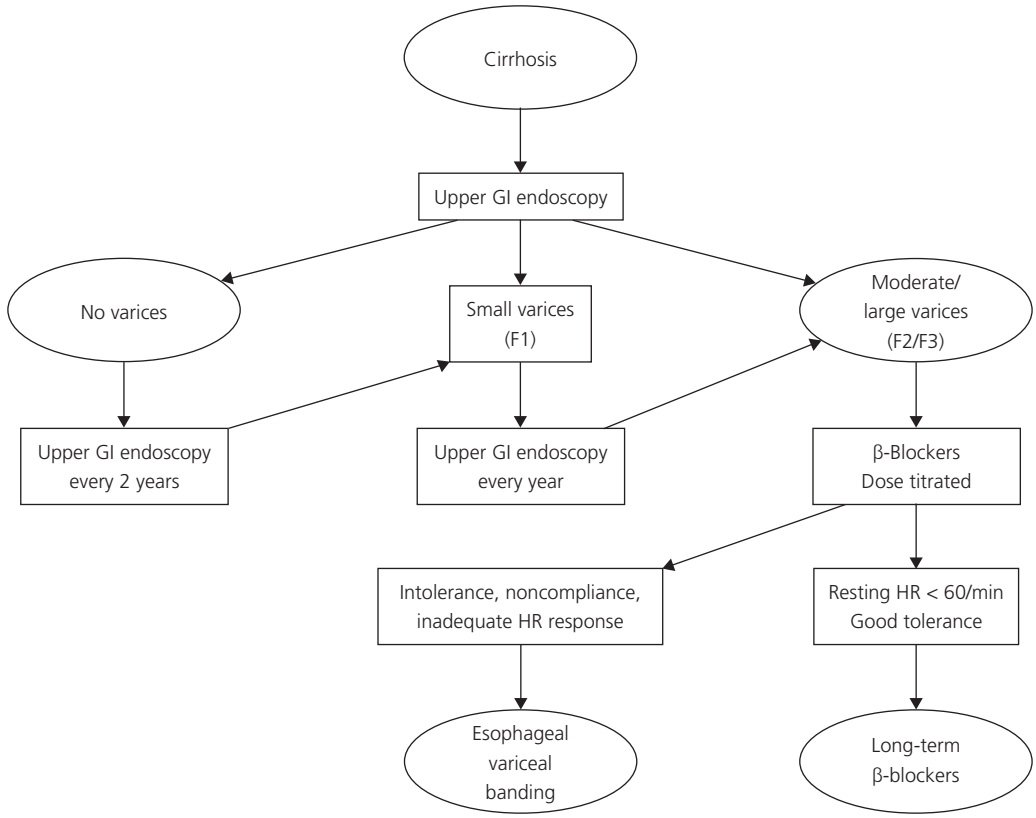


Figure 124.12 Algorithm for primary prophylaxis. HR, heartrate.

aim, with care taken to avoid major overexpansion of the circulation that may precipitate further bleeding due to the associated increase in portal pressure. Correction of clotting abnormalities may also be helpful in major bleeding.

There is convincing evidence to support routine prophylactic antibiotics, especially in those with ascites and in those with active bleeding at the time of endoscopy. Bacterial infection has been associated with higher rates of failure to control variceal bleeding [84]. Patients should be carefully observed for the development of aspiration pneumonia, spontaneous bacterial peritonitis, and other opportunistic (e.g., fungal infection) infections. These infections should be treated at the earliest opportunity with appropriate antibacterial or antifungal agents, with extreme care taken to avoid nephrotoxic drugs because these patients are susceptible to renal toxicity and failure. If variceal bleeding is suspected in patients who present with acute upper gastrointestinal bleeding, pharmacological treatment should be initiated while the patient is resuscitated, and this may improve the endoscopic diagnosis, efficacy of treatment, and survival. Because bleeding from a nonvariceal source is common in chronic liver disease, endoscopy should be performed as soon as the patient is stabilized.

Pharmacological therapy

The major benefit of pharmacological therapy is that it can be given without any specialized training to most patients. The safety margin and efficacy of some of the vasoconstrictor drugs used for this purpose have been less than desirable in the past, but improvements in these areas have been made.

Vasopressin and terlipressin

Vasopressin, a peptide hormone, causes splanchnic arterial vasoconstriction, thereby reducing portal blood flow and portal pressure. The main side effects of vasopressin are secondary to nonselective arterial vasoconstriction. These include ischemia of the myocardium, abdominal viscera, and lower limbs; left heart failure; hypertension; and arrhythmia. In randomized controlled studies, between 30% and 60% experienced one or more complications and about 25% of patients were withdrawn because of a major complication [85–87]. Selective infusion of vasopressin into the superior mesenteric artery has not been shown to overcome these adverse effects or improve efficacy. In a number of studies, a combination of vasopressin and nitroglycerine has been shown to reduce the hemodynamic side effects of vasopressin without attenuating the effect on portal inflow [88,89]. The transdermal route may be preferred because of ease of administration [88]. The addition of nitroglycerine to vasopressin is of proven benefit in reducing the side effects and improving hemostasis [88,89]. The reported efficacy of vasopressin is around 50%–60%, compared with 40% with placebo [46,85–89]. Because of the poor safety profile and

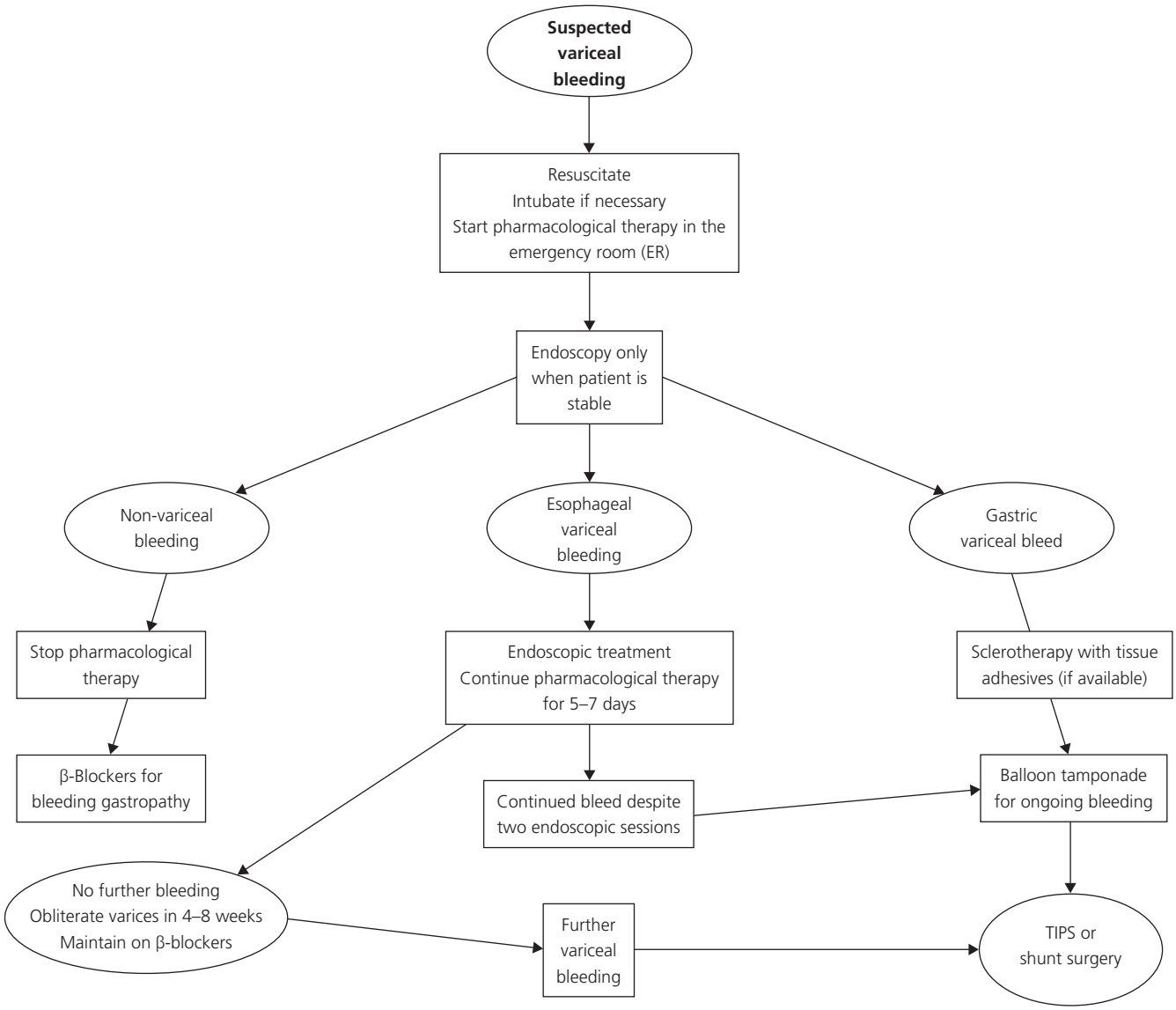


Figure 124.13 Broad outline of the management of acute variceal bleeding.

the availability of safer alternatives, vasopressin is not recommended for the management of acute variceal bleeding. However, if used because of its cost advantages, it should be used in combination with nitroglycerin.

Terlipressin (or Glypressin) (triglycyl lysine vasopressin) is a prodrug that is converted to lysine vasopressin in vivo; it is not approved for use in the United States. Terlipressin has properties similar to those of vasopressin, but unlike vasopressin, terlipressin can be given as bolus doses through a peripheral line. For unexplained reasons, terlipressin does appear to offer enhanced control of bleeding (efficacy around 80%) with fewer side effects compared with vasopressin or vasopressin used with nitroglycerin [90–94]. Repeated administration of terlipressin over a period of 6 days is as effective as sclerotherapy to control acute bleeding and to prevent early rebleeding [95].

Somatostatin and octreotide

Somatostatin and octreotide, a cyclic octapeptide analogue of somatostatin, cause splanchnic vasoconstriction by a direct effect on vascular smooth muscle and possibly by inhibiting the release of vasodilatory peptides such as glucagon [96]. Both drugs reduce portosystemic collateral blood flow as measured by azygos blood flow, but in cirrhotic patients the effect on portal pressure has been variable. Continuous infusion of somatostatin has been shown to produce a sustained decrease in HVWPG and blunt the rise in HVWPG in response to a test meal or blood transfusions [97]. However, to obtain optimal results, these drugs may need to be given as repeated bolus doses in addition to a continuous infusion. Although the standard dose of somatostatin is 250 μg bolus followed by 250 μg/h, a higher dose (500 μg/h) may benefit those who demonstrate active bleeding during emergency endoscopy

[98]. The addition of ISMN to somatostatin has not been shown to have any advantage over somatostatin alone [99].

A number of studies have compared somatostatin and octreotide with placebo, endoscopic treatment, or tamponade. These studies have clearly shown that both drugs are free of any major side effects, even when they were used as continuous infusion over 5–7 days. These studies also have shown a significant trend favoring somatostatin and octreotide (~ 85% efficacy) compared with either placebo or vasopressin [93,100–104]. Infusion of somatostatin or octreotide has also been shown to be comparable with balloon tamponade or endoscopic treatment [105–108]. A metaanalysis has shown that the control of bleeding was 40% better with octreotide (RR 0.6, CI 0.4–0.8) compared with vasopressin/terlipressin (bundled together), and comparable with sclerotherapy [109]. More importantly, the side-effect profile with octreotide was similar to placebo and significantly better than vasopressin/terlipressin (RR 0.3, CI 0.11–0.87).

Because of their excellent safety margin, these drugs can be used in the emergency department while the patient is resuscitated, and their use continued over the first few days after the bleeding to prevent early rebleeding. In a randomized controlled trial, the active bleeding rate was lower when vapreotide, another analogue of somatostatin, was administered prior to endoscopy, reinforcing the utility of early pharmacological therapy [110]. A combination of continuous infusion of these drugs for 5–7 days along with either sclerotherapy or band ligation has been shown to be superior to either sclerotherapy or band ligation alone in reducing early rebleeding and mortality [110–114]. In addition, the blood transfusion requirement is lower when these drugs are administered for 5–7 days after the bleeding. The combination treatment appears to benefit particularly those patients with shock and ongoing bleeding [114,115]. Another advantage of continuous pharmacological therapy is its efficacy in reducing bleeding from sclerotherapy- or banding-induced esophageal ulceration [115]. In fact, a Cochrane metaanalysis of randomized controlled trials suggested that there is no real advantage of endoscopic treatment as first-line therapy in patients with acute variceal bleeding [116]. The accumulating evidence favors the use of a combination of endoscopic treatment with either octreotide (or other analogues) or somatostatin. These drugs should be started prior to endoscopy and continued for 5–7 days after the bleeding episode.

Endoscopic therapy

The control of bleeding with either endoscopic sclerotherapy or band ligation is around 75%–90% [117,118]. The control of bleeding with endoscopic banding is comparable with sclerotherapy in patients with active variceal bleeding [119]. However, no specific benefits have been shown for band ligation over injection sclerotherapy during active bleeding [119,120]. Band ligation has also been found to be effective and probably safer than sclerotherapy in children with acute variceal bleeding [121]. Although endoscopic treatment was found to be marginally better than somatostatin/octreotide in some metaanalytical studies [120], many carefully performed studies and a metaanalysis have shown efficacy comparable with either form of treatment when used alone [116]. However, sclerotherapy was associated with more adverse events than somatostatin/octreotide [116,117]. About 15%–20% of patients treated with endoscopic sclerotherapy develop complications such as bleeding postsclerotherapy ulcers, esophageal stenosis, or perforation of esophagus. The complication rates are lower with band ligation.

Tissue adhesives, such as *n*-butyl-2-cyanoacrylate, have also been used by intravariceal injection to obliterate varices [122]. Tissue adhesives are not approved for intravariceal injection in the United States, but studies from elsewhere have claimed control of bleeding in approximately 90% of cases. The specific role for this treatment may be to obliterate fundal or ectopic varices that respond unreliably to intravariceal injection of sclerosant or band ligation [26,122]. There is no convincing evidence to recommend the use of tissue adhesives for esophageal variceal obliteration.

The optimum timing of endoscopic treatment with respect to the bleeding episode remains unknown. Formerly, it was generally believed that immediate endoscopic treatment is the optimum strategy for active variceal bleeding. The success rate of endoscopic treatment is operator dependent, and expertise varies widely among different centers [117]. The options are immediate treatment with associated technical difficulties and less than optimal expertise, or delayed treatment after temporary hemostasis is achieved using vasoconstrictive drugs. Because the efficacy of somatostatin, octreotide, or terlipressin is comparable with endoscopic treatment, it is reasonable to postpone endoscopy, for treatment as well as to confirm the diagnosis, until the patient is stabilized with pharmacological intervention [104–110,123,124]. Immediate endoscopic treatment could be reserved for nonresponders to pharmacological intervention. To reduce early rebleeding, it is advisable to continue vasoconstrictors such as somatostatin or octreotide (or terlipressin) for the first 5–7 days after the bleeding episode [110–114,123,124] (Table 124.5).

Predictors of failure to control acute variceal bleeding

A reliable model that can predict the failure of hemostasis after an acute variceal bleeding episode would be useful for risk stratification, management, and research. Many studies have been done, but small sample sizes, the retrospective nature of the studies, heterogeneity of offered therapies, and different patient characteristics make it difficult to draw any definite conclusions. However, active bleeding at the time of endoscopy has been shown independently to predict failure to control bleeding and early (30-day) mortality in many studies [125]. Other factors include higher HVWPG, encephalopathy, low platelet counts, alcoholic liver disease, shorter

interval between bleeding and hospitalization, and bacterial infections [97,125,126]. Mortality is clearly a reflection of the severity of liver disease and, therefore, particular attention should be given to providing "optimal" treatment to patients with more advanced liver disease. If bleeding continues despite a combination of pharmacological therapy and endoscopic treatment, or if endoscopic treatment is not feasible because of massive bleeding, the patient should be stabilized with balloon tamponade and considered for either transjugular intrahepatic portosystemic shunting (TIPS) or surgery.

Balloon tamponade

Balloon tamponade is a highly effective method of controlling active variceal bleeding. In experienced hands, it is equivalent to endoscopic treatment for the immediate control of bleeding. However, it is effective only when applied; when the balloon is deflated the rebleeding rate is extremely high. Moreover, in inexperienced hands (usually the case in most situations), the morbidity and mortality may be unacceptably high. Therefore, balloon tamponade is best reserved for the management of life-threatening bleeding when a risk of exsanguinations exists and endoscopic intervention is unlikely to be feasible. Balloon tamponade should not be applied for more than 24 h consecutively because of the risk of ischemic mucosal ulceration, and should be followed immediately by more definitive treatment. Depending on the situation, the definitive treatment may include endoscopic ligation or sclerotherapy (only in those who had no effective endoscopic treatment prior to balloon tamponade), surgery, or TIPS.

Role of surgery or transjugular intrahepatic portosystemic shunting (TIPS)

Although endoscopic therapy at the time of diagnostic endoscopy probably is the optimum approach to an episode of variceal bleeding, TIPS or surgical intervention (shunt or non-shunt procedure) has an important role, particularly in patients who continue to bleed despite early injection sclerotherapy or band ligation and pharmacological therapy (Fig. 124.14). It is reasonable to repeat endoscopic therapy on a second occasion before considering it to have failed, but the decision to progress to TIPS or surgery should not be further delayed, thereby preventing the inevitable escalation of complications or risks associated with TIPS or surgery in a deteriorating patient. Patients with ongoing variceal bleeding should be stabilized with balloon tamponade prior to TIPS or surgery. The choice between TIPS and surgery depends on local expertise, patient choice, patency of the portal vein, and the severity of the liver disease as determined by Child–Pugh score or Model for End-stage Liver Disease (MELD) score. Surgery should be reserved for patients with a Child A or low MELD score, or those with portal vein thrombosis.

Although the role of TIPS in the management of acute variceal bleeding is not well defined by controlled trials, it has evolved as the major "rescue" procedure for the management of acute variceal bleeding in most centers. It is being increasingly used in the United States as a rescue procedure because of the relative inexperience with emergency shunt surgery in many centers. The morbidity and mortality with

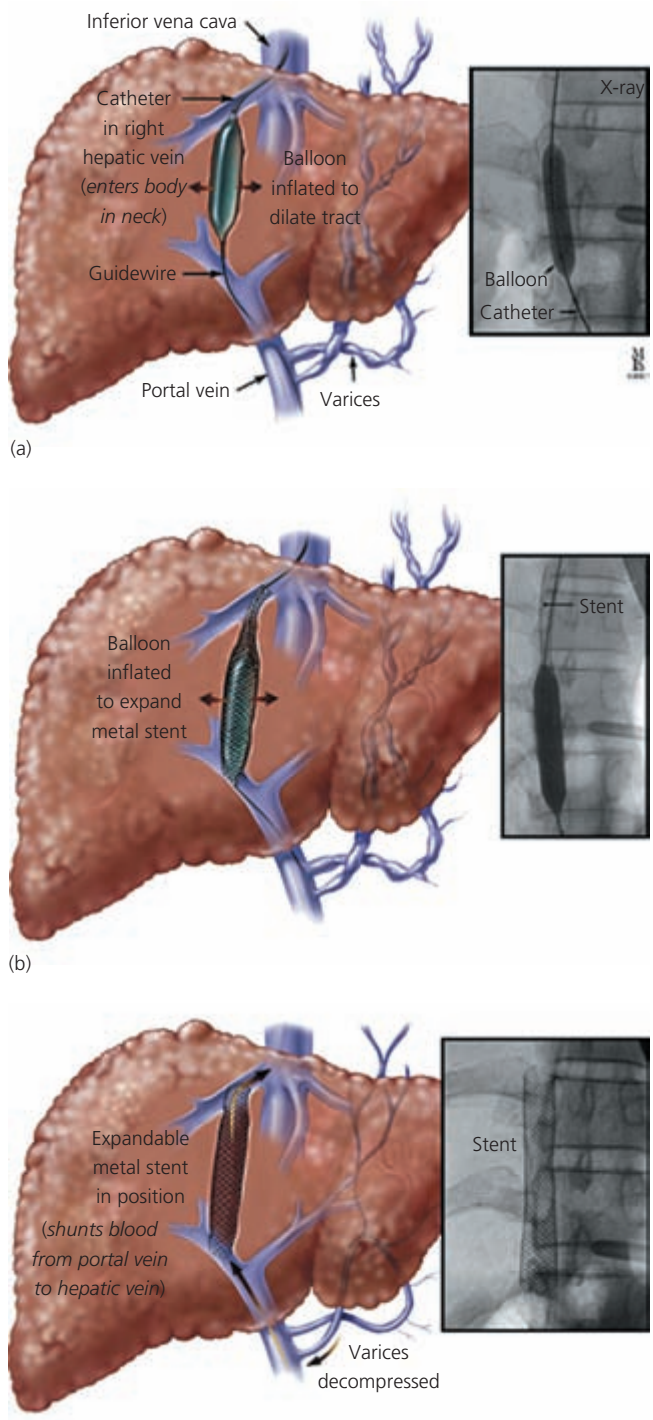


Figure 124.14 A cartoon showing the technique of transjugular intrahepatic portosystemic shunt (TIPS).

TIPS in patients with advanced cirrhosis and ongoing bleeding are similar to those of surgical procedures. In patients with ongoing bleeding and advanced liver disease (Child–Pugh C), the mortality with TIPS may be close to 30%–50%, which is more of a reflection of the severity of liver disease.

If surgical shunt was the choice of "rescue" procedure, the type of surgery chosen is dependent on the local expertise. Selective distal splenorenal shunt requires more operating time compared with either mesocaval or portacaval shunt, which makes it generally unsuitable as an emergency procedure (Fig. 124.15). Shunt surgery does not preclude subsequent transplantation, but could be associated with hepatic encephalopathy (~ 30%) in a significant number of patients. The most commonly used nonshunting surgery is esophageal transection with varying degrees of devascularization, but it is rarely done in Western countries because of better options [24]. In trials comparing sclerotherapy with esophageal transection or portacaval shunting, the survival rates were not different between the groups [1]. These studies have confirmed the utility of esophageal transection and shunt surgery as "rescue" procedures for patients who fail combined endoscopic and pharmacological therapy. The evidence suggests that TIPS may be the best option in patients who fail combined treatment and have ongoing bleeding. The shunt surgery may be reserved for secondary prophylaxis in Child A patients who bleed despite β-blockers and endoscopic treatment (Fig. 124.16) [127].

Bleeding from gastric varices

Although the management of gastric varices is similar to that of esophageal varices, endoscopic sclerotherapy using sclerosant should be reserved only for lesser curve varices or varices within a hiatus hernia [128]. Tissue adhesives (*n*-butyl-2-cyanoacrylate) may be used to inject fundal varices during active bleeding or preferably after the initial bleeding has stopped [25,129]. These adhesives polymerize rapidly in contact with blood, and plug the injection site as well as the bleeding site. However, it is technically difficult to inject fundal varices when there is active bleeding.

In one randomized controlled trial, 32 (GOV1 stage, 21; GOV2 stage, 7; IGV1 stage, 4) patients were randomized to butyl cyanoacrylate injection, and 29 (GOV1 stage, 20; GOV2 stage, 8; IGV1 stage, 1) to variceal banding; 15 and 11 patients had active bleeding at the time of randomization. When compared with band ligation, hemostasis (13/15, 87% vs 5/11, 45%; $P = 0.03$), and rebleeding rates (31% vs 54%, $P < 0.001$) were better with cyanoacrylate [25]. Complications (19% vs 38%, $P = 0.05$) and mortality (29% vs 48%, $P = 0.05$)

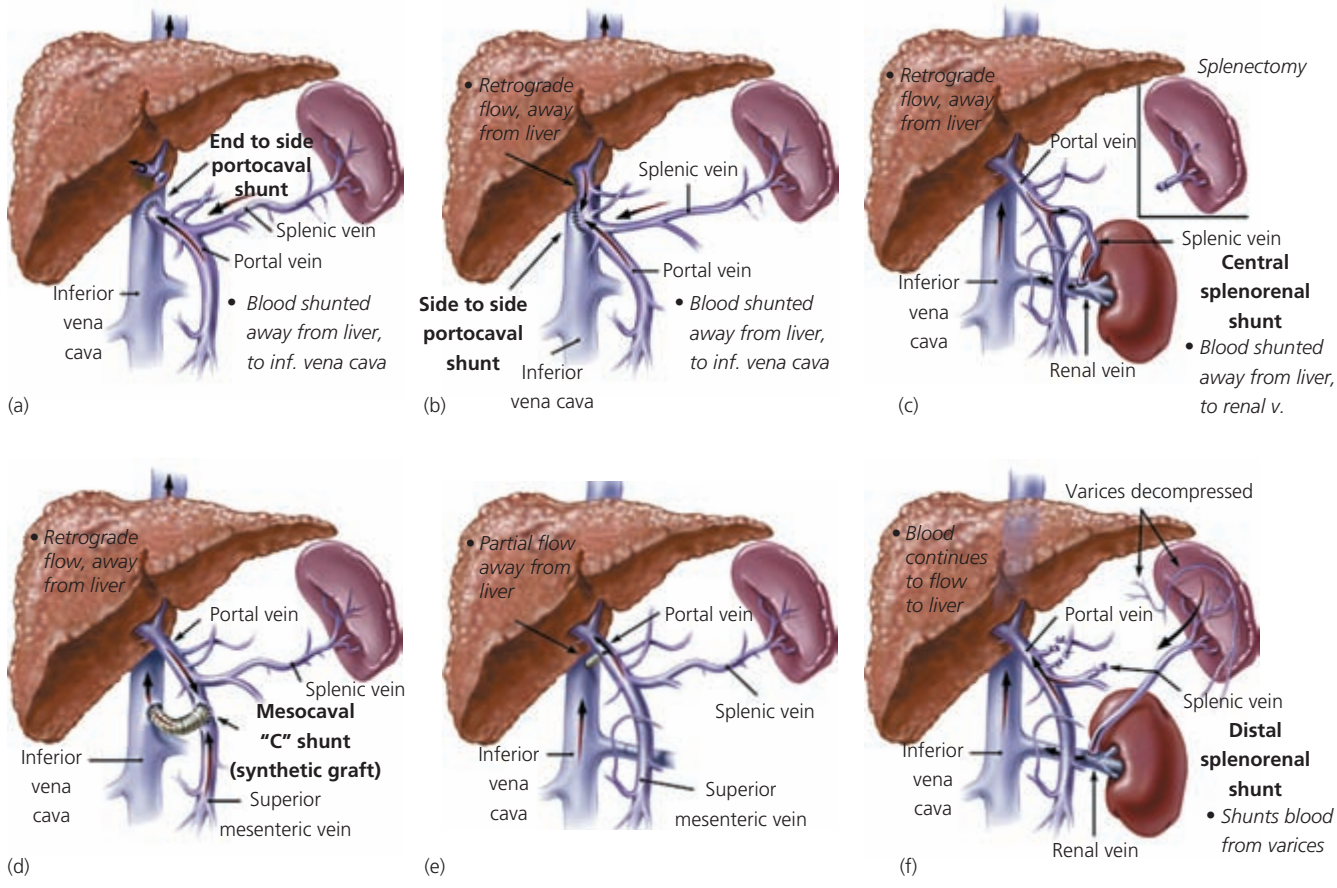


Figure 124.15 Various types of shunt surgeries for the management of portal hypertension.

Table 124.7 Variceal bleeding control (%) with pharmacological and endoscopic treatment in acute bleeding

Authors	Endo therapy + octreotide	Sclerotherapy	Somatostatin	Octreotide
Shields et al. [105] (*N* = 80)		83	77	
Planas et al. [106] (*N* = 70)		83	80	
Sung et al. [107] (*N* = 98)		74		69
Jenkins et al. [108] (*N* = 150)		82		85
Besson et al. [111] (*N* = 199)	87*	71		
Sung et al. [112] (*N* = 100)[a]	91*	62		

a Endoscopic ligation.
* Statistically significant (< 0.05).

were higher in the banding group. Bleeding from ulcer was the most common complication (6% vs 28%, $P = 0.03$). One patient developed a cerebrovascular event 12 h after cyanoacrylate injection – this is one of the dreaded complications of cyanoacrylate injection. This study included predominantly GOV1 varices and may not be applicable to fundal varices. Of the four patients with isolated fundal varices treated with cyanoacrylate, two patients rebled after 2–3 sessions, one died, and the fourth one had to be rescued by TIPS. To my knowledge, there are no other controlled studies that have compared cyanoacrylate with variceal banding.

The FDA has not approved the use of *n*-butyl-2-cyanoacrylate for variceal treatment, although it is available for other purposes. If a decision was made not to treat gastric varices with "glue," the next best option for bleeding fundal varices is balloon tamponade for temporary hemostasis followed by more definitive therapy such as TIPS or shunt surgery. If balloon tamponade is unsuccessful in patients with bleeding fundal varices, the surgeon should proceed immediately to transjugular intrahepatic portosystemic shunting (TIPS) or shunt surgery [130–132]. In one case series, bovine thrombin was used effectively for bleeding gastric varices [133]. However, in the absence of controlled trials, this form of treatment is not recommended.

Barange et al. reported their experience with TIPS in 32 (GOV1 stage, 22; GOV2 stage, 10) consecutive patients who did not respond to pharmacological therapy ($n = 26$) or sclerotherapy ($n = 18$) [131]. Active bleeding was controlled by TIPS in 18 of 20 patients. Rebleeding rates for the entire cohort after TIPS were 14%, 26%, and 31% respectively at 1 month, 6 months, and 1 year. Other studies have also reported that TIPS is effective for gastric variceal bleeding [132]. Other options for bleeding gastric varices may include transhepatic embolization. However, rebleeding rates are high after this procedure. When isolated fundal varices are seen in association with splenic vein thrombosis, the treatment is splenectomy. Injection of tissue adhesive is another option in these patients if they are not candidates for splenectomy.

Bleeding from other ectopic varices

Management of the bleeding depends on the site of bleeding, the severity of liver disease, and the local expertise. Because there are no controlled studies, it is difficult to recommend any special strategy. Shunt surgery may be the best line of management for bleeding varices in the duodenum, jejunum, ileum, and enterostomy. If the portal vein is patent and the expertise for TIPS is readily available, TIPS should be considered as the first line of management before other forms of shunt surgery. Anecdotal reports suggest that duodenal varices and ileostomy varices can be managed successfully by sclerotherapy or feeding vessel embolization, but serious consideration must be given to the postsclerotherapy complications like duodenal perforation and stoma dysfunction, and the high recurrence rate after embolization. Colonic varices are usually localized and can be managed by local resection. When colonic varices are seen in association with esophageal varices, TIPS may be a safer alternative. Anorectal varices can be safely treated with sclerotherapy or by underrunning with an absorbable suture.

Acute variceal bleeding: summary of management recommendations

Optimal resuscitation in a timely fashion is crucial for a better outcome in patients with acute variceal bleeding. Pharmacological intervention while the patient is resuscitated is safe and beneficial, and pharmacological treatment should be continued for 5–7 days to prevent early rebleeding. Endoscopic diagnosis and therapy should be performed when the patient is adequately resuscitated and stabilized (Table 124.7). Shunt surgery or TIPS should be reserved for nonresponders to a combination of pharmacological and endoscopic therapy, and should not be delayed when endoscopic therapy fails on two separate occasions. Bleeding from fundal varices is managed by either early surgery or TIPS. If tissue adhesives are available, endoscopic treatment with these agents may be an alternate option for bleeding fundal varices before considering surgery or TIPS.

Secondary prophylaxis

Rebleeding is common after the first bleed; 50%–80% of those who survive the first bleed may rebleed within 2 years, and most rebleed within 6 weeks after the first bleed. A number of options are available to prevent recurrent esophageal variceal bleeding, including endoscopic sclerotherapy or banding, nonselective β-blockers, shunt or nonshunt surgery, TIPS, and liver transplantation [134–145].

Secondary prevention studies comparing propranolol with placebo have shown that propranolol reduces both the bleeding rate and the mortality rate by about 40% [1,145]. It has been shown that a 20% reduction in HVWPG can reduce the rebleeding rate significantly, and reduction to below 12 mmHg may completely abolish recurrent bleeding [3,136,145]. Trials comparing propranolol or nadolol with sclerotherapy have confirmed that both forms of treatment are similar in efficacy [1,137].

The recurrence of bleeding after the initial obliteration of varices is related to the development of new esophageal variceal columns; other sources include portal hypertensive gastropathy and gastric varices. Although it may appear logical to perform long-term follow-up endoscopic treatment, there is no convincing evidence to suggest that long-term follow-up treatment is superior to short-term treatment aimed at obliteration of varices, especially if patients are maintained on β-blockers. Based on the limited data, it appears that addition of β-blockers to short-term endoscopic therapy may reduce the bleeding rate compared with either treatment alone. This was to be expected because a significant number of patients on β-blockers do not attain the desired reduction in HVWPG. Combination treatment may be useful especially in patients who are less likely to comply with medical treatment or in those who cannot tolerate maximal medical treatment.

Shunt surgery (preferably mesocaval shunt or distal splenorenal shunt) is reserved for patients with good synthetic function (Child A) and recurrent variceal bleeding who fail endoscopic and pharmacological treatment [127] (Fig. 124.16). Although TIPS is effective in preventing recurrent rebleeding (50% reduction compared with endoscopic treatment), it is associated with high rates of shunt stenosis and occlusion [138–144] (Table 124.6). Periodic monitoring and revision of shunts is therefore necessary to obtain optimal results, and this may turn out to be expensive in the long run. In addition, TIPS is associated with high rates of hepatic encephalopathy (29%–55%). It has not been shown that TIPS reduces overall mortality compared with endoscopic treatment. The

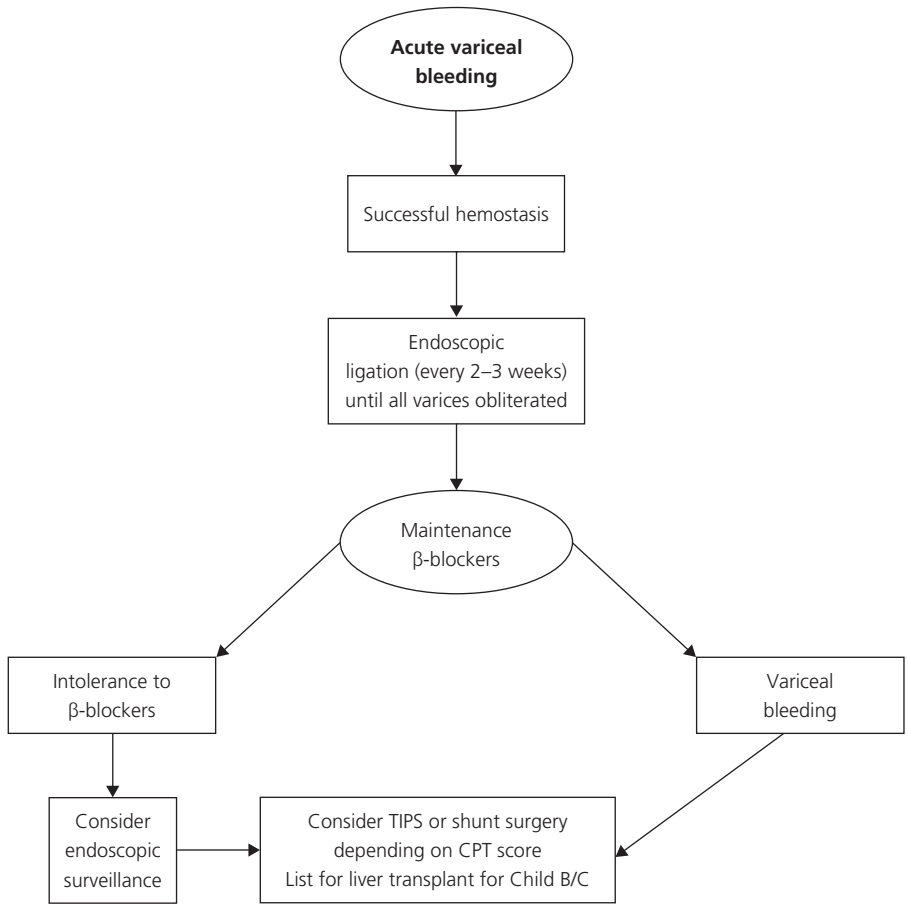


Figure 124.16 An algorithm for secondary prophylaxis.

Table 124.8 Summary of controlled trials comparing TIPS and endoscopic treatment

	Recurrent bleeding		Encephalopathy		Mortality	
Authors	**TIPS**	**Endoscopy**	**TIPS**	**Endoscopy**	**TIPS**	**Endoscopy**
Cabrera et al. [138] (*N* = 63)	23	52	33	13	20	16
Cello et al. [139] (*N* = 49)	12	48	30	44	21	16
Merli et al. [140] (*N* = 81)	24	51	55	26	24	19
Sanyal et al. [141] (*N* = 80)	24	26	31	13	29	18
Jalan et al.[a] [142] (*N* = 58)	10	52	36	33	42	37
Sauer et al.[b] [143] (*N* = 83)	23	57	29	13	29	27
Rossle et al.[b] [144] (*N* = 126)	21	52	36	18	13	12

a Endoscopic banding.
b Endoscopy + β-blocker.
TIPS, transjugular intrahepatic portosystemic shunt.
All data expressed as percentages.

indication for TIPS is as a bridge to transplantation in patients with advanced cirrhosis (Child B or C) who present with recurrent rebleeding despite endoscopic and pharmacological treatment. The mortality after variceal bleeding and the long-term survival following the index bleed are directly related to the severity of liver disease. It is difficult to imagine that the various treatment options discussed here are likely to improve liver function and hence survival. Liver transplantation, with a 5-year survival rate reaching 85%–90% percent, is the treatment of choice for patients with advanced liver disease.

The in-hospital mortality has remained high, even in tertiary care hospitals, despite advances in the management of acute variceal bleeding [2]. Moreover, there is a wide variation in mortality among various hospitals. One of the main reasons for this high mortality may be lack of a "critical pathway" for the management of acute variceal bleeding in most hospitals, leading to nonuniform and often suboptimal treatment regimens for these patients depending on the physician in charge. This could be improved with well-defined protocol-based management in the emergency room, in the intensive care unit, and in the wards, followed by a discharge summary with clear instructions for outpatient management to both the patient and the family physician.

Recommendations

A combination of β-blockers and endoscopic treatment (preferably banding) may be a logical approach for secondary prophylaxis. The objective of endoscopic therapy is to obliterate the varices over a period of 6–8 weeks after the index bleed. There is no evidence to suggest that long-term endoscopic surveillance is beneficial. Shunt surgery or TIPS should be reserved for patients who rebleed despite a combination of endoscopic and pharmacological therapy (Table 124.8; Fig. 124.16). Liver transplantation is the treatment of choice for patients with advanced liver disease and recurrent variceal bleeding.

Portal hypertensive gastropathy

Portal hypertensive gastropathy (PHG) is the term used to describe the endoscopic appearance of gastric mucosa seen in patients with cirrhotic or noncirrhotic portal hypertension, characterized by a mosaic-like pattern with or without red spots (Fig. 124.17) [146]. The "mosaic" pattern appears as a white reticular network separating areas of raised red or pink mucosa resembling the skin of a snake (also often described as snake-skin appearance). Portal hypertensive gastropathy is seen mainly in the body and the fundus of the stomach, but also is seen rarely in the gastric antrum. These changes are not unique to the gastric mucosa; similar changes are also rarely seen in the small intestine and colon (Fig. 124.18), especially in the presence of extrahepatic portal hypertension. When PHG is severe, it can include discrete cherry-red spots, fine pink speckling, or scarlatina-type rash, collectively called red marks [147]. The characteristic histological finding of PHG is dilated capillaries and venules in the mucosa and submucosa without erosion, inflammation, or fibrinous thrombi [148].

Classification

There is no general consensus on the endoscopic classification of PHG [146–151]. The most widely used classification is the one recommended by McCormack et al. [148], who classified PHG into mild and severe. Its popularity relates in part to its simplicity and also its ability to predict the risk of bleeding, with an increased risk of gastric hemorrhage in severe (38%–62%) compared with mild cases (3.5%–31%) [146]. However, often the observed endoscopic findings are of an intermediate severity and are not well represented as

(a) (b) (c)

Figure 124.17 Grading of portal hypertensive gastropathy.

Figure 124.18 Portal hypertensive colopathy (cecum).

being either mild or severe. Tanoue et al. and New Italian Endoscopy Club (NIEC) have subsequently described a more detailed scoring system [152]. Tanoue et al. classified PHG into four grades:

- Grade 0: none
- Grade 1: mild
- Grade 2: moderate
- Grade 3: severe.

Grading PHG as mild, moderate, and severe permits more informative description of the observed endoscopic findings. However, Yoo et al. found substantial limitations with regard to both specificity and reproducibility with both classification systems [149]. A simpler classification system as recommended by McCormack et al. had a relatively better intra- and inter-observer agreement and reproducibility as determined by kappa values [148,149].

Prevalence

The reported prevalence of severe PHG ranges from 9% to 46%, and that of mild PHG from 29% to 57%. Estimates of the overall prevalence of PHG vary from 51% to 98% (Table 124.7). The wide variation in the reported prevalence is perhaps related to patient selection, absence of uniform criteria and classification, and, more importantly, inter- and intra-observer variation [146,153,154].

The development of PHG correlates with the duration and severity of liver disease, presence and size of gastroesophageal varices, and previous variceal eradication by endoscopic variceal sclerotherapy or banding [152–156]. Although PHG is usually seen in association with either esophageal or gastric varices, there is no direct linear correlation between portal pressure and the presence or severity of PHG. The cause of portal hypertension is unlikely to be a significant factor. Sarin et al. reported a low prevalence of PHG (2.6%–37.1%) in studies from India, where many patients had noncirrhotic portal hypertension [155–157]. However, another large series from India reported a prevalence of 62% suggesting that the cause of portal hypertension may not play an important role in the development of PHG [157]. The prevalence of PHG does not appear to have a linear relationship with the severity of liver disease, as evidenced by a study that showed the lowest prevalence of severe PHG in Child C patients [151]. This may suggest that other hitherto unidentified factors may play a role in the pathogenesis of PHG. However, there is general agreement, based on longitudinal studies, that the prevalence of PHG does increase with variceal obliteration.

Pathogenesis

Portal hypertension, and not liver disease, seems to be the key factor for the development of PHG, because PHG is equally common in portal hypertensive patients with or without liver disease [158,159]. Moreover, the improvement or disappearance of PHG in many patients after TIPS and shunt surgery suggests that there is a significant association between PHG and portal hypertension. However, the severity and the presence of PHG do not have a linear correlation with the

severity of portal hypertension [154]. Chronic elevation in portal pressure and increased splenic circulation may increase gastric mucosal blood flow, and this may be one explanation for the development of PHG, although actual measurement of gastric blood flow using Doppler ultrasound has shown variable results, suggesting that there may be other explanations for the pathogenesis of PHG [159–164]. The severity of PHG may increase with more advanced liver disease, as shown in some studies, but the results are not consistent [151,153,154]. There have been many other explanations for the pathogenesis of PHG. In experimental models, noxious agents such as aspirin, bile acids, or alcohol have been shown to produce gastric mucosal damage in animals with portal hypertension compared with controls [165]. Circumstantial evidence in humans has shown similar results [166]. There is experimental evidence to suggest that gastric mucosal defense mechanisms are impaired in the presence of portal hypertension [165–173]. In addition, activation of cytokines and growth factors in the gastric mucosa may induce nitric oxide and endothelin-1, which in turn may further contribute to hyperdynamic circulation. In addition, endothelin-1 and peroxynitrite overproduction may also increase susceptibility of gastric mucosa to damage [174]. Kawanaka et al. [175] have shown that oxidative stress-induced ERK2 – a mitogen-activated protein (MAP) kinase – is defective in PHG, and this is mediated by over-expression of MAP kinase phosphatase 1 (MKP-1). Interestingly, in this study, MKP-1 over-expression was normalized after supplementation with vitamin E, and this may have therapeutic implications for the management of PHG.

Gastric antral vascular ectasia (GAVE)

An area that has not been resolved completely is the association of gastric antral vascular ectasia (GAVE) and PHG [146,166,176]. "Watermelon stomach," or GAVE, is a term used for the typical endoscopic findings of red stripes separated by normal mucosa, most commonly seen in the gastric antrum or proximal stomach [177] (Fig. 124.19). Watermelon stomach, or GAVE, has been reported in a variety of autoimmune diseases and connective tissue diseases including atrophic gastritis, scleroderma, sclerodactyly, hypothyroidism, pernicious anemia, and primary biliary cirrhosis [178]. Something similar to watermelon stomach is also seen in recipients of bone marrow transplants [179,180]. The typical histological appearance of GAVE includes marked dilation of capillaries and collecting venules in the gastric mucosa and submucosa with areas of intimal thickening characterized by fibromuscular hyperplasia, fibrohyalinosis, and thrombi [166,177,181,182]. The pathogenesis of GAVE is poorly understood and there is no single unifying hypothesis. Possible mechanisms include humoral factors (such as gastrin, prostaglandin, serotonin, or vasoactive intestinal peptide [VIP]), cellular growth factors (especially in bone marrow transplant recipients), and mechanical causes [166,181,183,184].

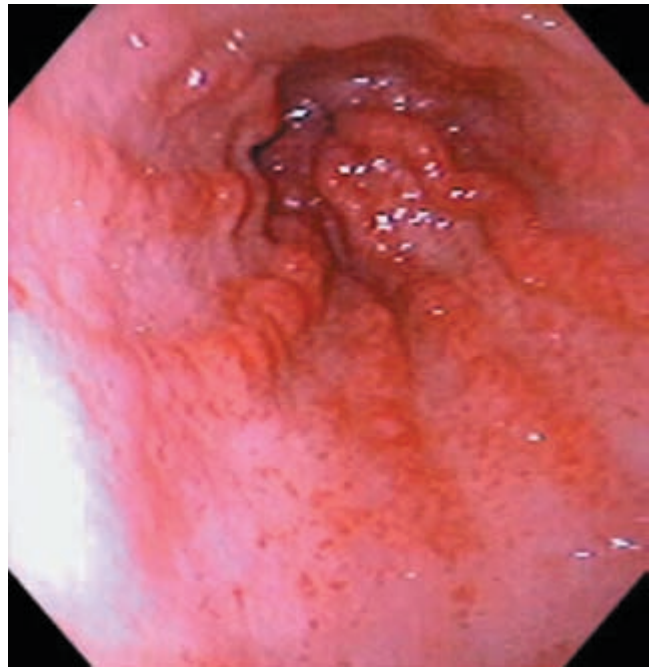


Figure 124.19 Gastric antral vascular ectasia (GAVE).

Proponents of the "mechanical" theory have suggested that chronic intermittent venous obstruction associated with powerful muscular contractions of the antrum and pylorus, or repeated trauma associated with a loosely attached antral mucosa and prolapse of the antral mucosa through the pylorus, may cause GAVE [177,182,185]. It is reasonable to suggest that despite the absence of a unifying hypothesis, GAVE is a distinct entity and the presence of GAVE is associated with intermittent bleeding in a significant number of subjects.

When the red marks seen in severe PHG become confluent, they may have a striped appearance producing a close resemblance to GAVE (Fig. 124.20). A "GAVE-like" appearance is not uncommon in patients with portal hypertension; indeed, in my experience, a "GAVE-like" appearance is more common in patients with portal hypertension than in those without. The dilemma is whether this appearance is called GAVE or severe PHG. The histological appearance of GAVE is different from PHG, but mucosal biopsies are rarely deep enough to make a firm diagnosis. Moreover, it may not be completely safe to do repeated biopsies in patients with portal hypertension and severe PHG. Bleeding from GAVE, unlike PHG, is not known to respond to nonselective β-blockers [166,186]. However, such a therapeutic challenge is not clinically useful to differentiate these two entities because the response to β-blockers is variable in patients with PHG. Kamath et al. showed that patients with GAVE, in the absence of a background mosaic appearance, did not respond to TIPS compared with those with PHG [187]. On the basis of their study, the authors suggested that the GAVE-like appearance seen in patients with portal hypertension is distinct from severe

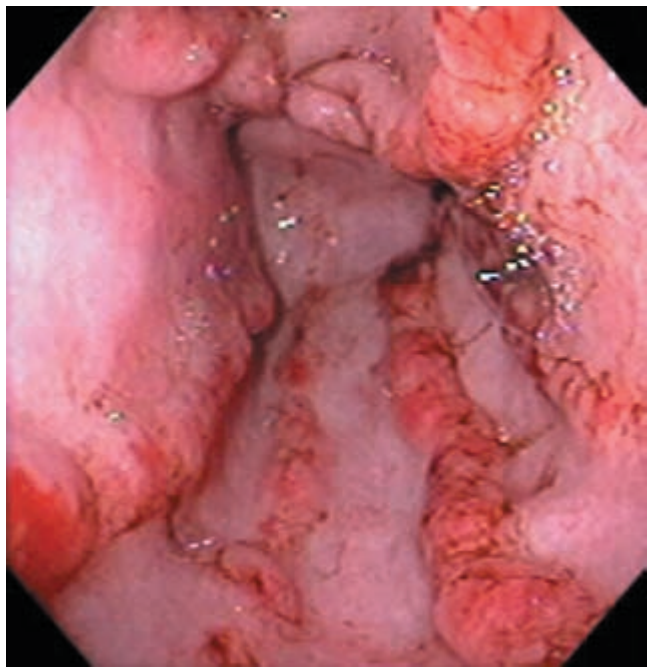
(a)  (b)

Figure 124.20 GAVE-like appearance in portal hypertensive gastropathy. Note the typical background portal hypertensive gastropathy.

PHG. Although their observation merits further consideration, in their study TIPS was done for the prevention of recurrent gastric variceal bleeding and not for prevention of bleeding from PHG. The response of PHG bleeding to TIPS has been variable in anecdotal reports and this may suggest that a lack of response after TIPS is not evidence for a different pathogenesis.

There is circumstantial evidence to suggest a possible association between PHG and GAVE. Although GAVE is seen in patients without portal hypertension [178], it is seen more commonly in patients with cirrhosis and portal hypertension [181,182,187]. It has been suggested that the same gastric vascular alteration related to portal hypertension may appear different, grossly and histologically, depending on the anatomical location in the stomach [188]. The hemodynamics of venous drainage of the antrum are different from the body or fundus of the stomach. It can be argued that the "typical" histological changes, fibrosis and thrombosis, seen in GAVE may also be seen with more chronic and severe PHG. The variable response to β-blockers and TIPS may be a reflection of the advanced histological changes [166]. I believe that the term GAVE should be reserved for the presence of typical, linear red lesions in the gastric antrum without the "mosaic"-like appearance of mucosal background in the rest of the stomach. When there is evidence of a mosaic-like pattern or evidence of more severe PHG in the body and fundus, I favor classifying it as a spectrum of PHG (Fig. 124.17).

Natural history and complications of PHG

The overall incidence of acute bleeding from PHG is low, and bleeding is seen mostly in those with more severe PHG. Bleeding is often mild, and transfusion requirement is usually limited to one or two units of blood. The prevalence of chronic bleeding cannot be reliably estimated because of the uncertainty of making a firm diagnosis. D'Amico et al., in a long-term follow-up of 137 patients, reported PHG in 33 patients (mild 28, severe 5) on their initial endoscopy, and, during a mean follow-up of 46 months, 35 additional patients developed PHG (mild 33, severe 2) [189]. The frequency of bleeding was higher in those with severe PHG (38%–62%) compared with those with mild PHG (3.5%–31%) [156,189]. The wide variation in the reported incidence of acute bleeding from PHG is mainly related to the difficulty in making a firm diagnosis and the inaccuracy of classification of the severity of PHG. Unless an obvious bleeding point is identified, it is difficult to confirm that the source of bleeding is PHG in most patients. Moreover, if a drop in hematocrit is used to diagnose chronic bleeding, it is likely grossly to overestimate bleeding from PHG. The mortality associated with bleeding from PHG is low because most bleeds are minor compared with variceal bleeding. Death resulting from PHG is unusual and bleeding from PHG is not an independent risk factor for survival.

The presence and severity of PHG appear to change with time. In one study, during a follow-up of 2 years, the severity of PHG fluctuated with time in 25%, improved in 23%, remained stable in 29%, and deteriorated in 23% [151]. In the same study, 46% (65 mild PHG and 7 severe PHG) of patients with cirrhosis developed PHG during a 5-year follow-up. Endoscopic sclerotherapy appears to worsen the severity of PHG and increases the risk of bleeding [148,152,155,190]. In

one study, 44% of patients developed PHG following variceal eradication by sclerotherapy compared with only 9% before sclerotherapy [156]. Major determinants of bleeding from PHG are duration of the PHG, and the extent and severity of lesions. Aggravation of PHG after sclerotherapy might be caused by alterations in local hemodynamics following obliteration of the esophageal varices [155]. Increase in pressure and flow, and the differential hemodynamics within gastric veins are potential causes for the development of PHG after variceal obliteration [191–193]. Hepatofugal flow velocity is increased in the left gastric vein in direct relationship to the enlargement of esophageal varices. Gastric mucosal blood flow is higher in cirrhotic patients whose extrahepatic collaterals are predominantly through esophageal varices. Therefore, obliteration of the esophageal collateral venous network may cause higher flow velocity in the gastric vascular bed. It has been speculated that the extravasation of sclerosant into the mediastinum during sclerotherapy may result in chemical vagotomy, and this may cause esophageal dysmotility and delayed gastric emptying. Delayed gastric emptying, from the chemical vagotomy, has been proposed as a possible mechanism for the development of PHG [194]. However, there is no direct evidence to suggest that delayed gastric emptying causes PHG.

Management

Clinically significant bleeding is seen only when PHG is severe. Nonselective β-blockers such as propranolol and nadolol have been shown to reduce portal pressure and gastric mucosal blood flow. In small studies, propranolol has been shown to reduce bleeding related to PHG [195,196]. These observations were confirmed in a randomized controlled trial of 56 patients with PHG [183]. In this study, the bleeding was significantly lower in the treated group compared with the placebo group at 12 months (35% vs 62%) and 30 months (48% vs 93%). Multivariate analysis showed that absence of propranolol treatment was the only predictive variable for rebleeding. Other medications such as prednisone, estrogen, and progesterone are ineffective for bleeding related to PHG [197].

Somatostatin has been shown to reduce gastric mucosal blood flow by decreasing splanchnic blood flow [198]. Similar effects are also seen with vasopressin and Glypressin. In patients with acute bleeding, an uncontrolled study has shown therapeutic efficacy with both somatostatin and octreotide [199]. However, it has been shown that the decrease in portal pressure is only transient, therefore use of somatostatin or octreotide is unlikely to benefit patients with chronic PHG bleeding [200]. The use of these agents should be limited to patients with acute bleeding.

Endoscopic treatment does not have a significant role in the management of PHG bleeding because the bleeding is often mild and diffuse. If an active bleeding site is identified, it can be managed by injection of sclerosant, or by cauterization using the heater or bipolar probe. However, there are no controlled studies to suggest that these forms of treatment are either efficacious or safe. Although laser therapy has been shown to be safe and effective in reducing GAVE-associated bleeding, it is not known whether laser therapy is effective or safe in patients with severe PHG when the predominant lesion is in the gastric antrum [201]. A study reported success with argon plasma coagulation in eight patients with GAVE and cirrhosis [202]. The safety and efficacy of this treatment needs to be confirmed in large studies before it can be recommended for patients with portal hypertension. Both TIPS and shunt surgery have been shown to be effective for PHG bleeding in anecdotal reports [203]. In a small study, TIPS improved severe PHG in six of eight patients, and in mild PHG 23 of 27 patients showed improvement [186]. Despite these encouraging anecdotal reports, I believe that TIPS or shunt surgery should be used only as the last resort in these patients because the risks outweigh the benefits. The only treatment that can be recommended for prophylaxis of PHG bleeding is administration of nonselective β-blockers such as propranolol or nadalol.

Portal hypertensive gastropathy: summary

Portal hypertensive gastropathy has characteristic endoscopic features of a mosaic pattern with or without red spots, and is found most frequently in the fundus and body of the stomach. The classification systems have many limitations, but a simple grading system may assist inter- and intraobserver agreement. The pathogenesis of PHG is not clearly defined, but there is a close relationship between portal hypertension and development of PHG. Gastric antral vascular ectasia (GAVE) is a separate entity from PHG, but patients with severe PHG may have a GAVE-like appearance in the gastric antrum. If a "mosaic-like" pattern is seen in the rest of the gastric mucosa, I believe that a GAVE-like appearance should be considered as a spectrum of PHG. Acute bleeding from PHG is usually mild and seen in the presence of severe PHG. The only treatment that can be recommended for prophylaxis of bleeding from PHG is nonselective β-blockers.

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A ENDOSCOPIC

125 Endoscopic diagnosis and treatment of nonvariceal upper gastrointestinal hemorrhage

David J. Bjorkman

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Upper gastrointestinal (UGI) bleeding is a common clinical disorder. It is responsible for over 250 000–300 000 hospital admissions and $2.5 billion in costs in the USA each year [1–3]. The most common cause of nonvariceal UGI bleeding is peptic ulcer disease, but other disorders may have an identical presentation [4]. Gastrointestinal endoscopy remains the diagnostic and therapeutic procedure of choice for UGI bleeding. Despite progressive advances in the diagnosis, the mortality from acute UGI bleeding remains 10%–15% [3].

Initial evaluation and preparation for endoscopy: treat shock

The initial evaluation of patients with acute GI bleeding should focus on the hemodynamic status. Blood hemoglobin concentration and hematocrit are unreliable markers of acute blood loss. Intravenous access and vigorous volume replacement improves patient outcomes and should be immediately initiated in all patients with hemodynamically significant GI bleeding [5]. This requires large-bore vascular access in at least two locations and immediate infusion of isotonic electrolyte solutions until vital signs become stable. The use of blood products should be determined by the patient's clinical condition and the rapidity of blood loss [6]. Other diagnostic and treatment decisions should not take precedence over volume resuscitation.

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Assessing the level of bleeding

Endoscopy is the optimal method for establishing the level of bleeding. A history of hematemesis with frank blood or coffee grounds-like material is sufficient to confirm the level of bleeding to be above the ligament of Treitz. A prior history of bleeding ulcers, nonsteroidal antiinflammatory drug (NSAID) use, or variceal bleeding is suggestive, but not sufficient, to establish the presence of a UGI bleeding source. A history of melena is also suggestive of UGI bleeding, but dark, melena-like, stool can occur with slow bleeding from the small bowel or proximal colon. Hematochezia is more suggestive of a bleeding site in the lower GI tract, but may also result from vigorous UGI bleeding in 10% of cases.

Nasogastric aspiration is 80% sensitive for an actively bleeding UGI source when it produces fresh or altered blood. False-negative aspirates occur when the tube is improperly positioned, or the reflux of blood from a duodenal source is prevented by pylorospasm or obstruction [7]. Continuous nasogastric suction can identify continued or recurrent bleeding prior to changes in vital signs or laboratory values. When vigorous bleeding is identified, gastric lavage using a large-bore orogastric tube can clear the stomach of blood and clots and improve visualization for endoscopic evaluation and treatment [8]. Intravenous erythromycin has also been demonstrated to promote passage of blood and clots from the stomach, improving visualization [9].

Role of endoscopy in diagnosis

After the patient has become hemodynamically stable, endoscopy is the diagnostic procedure of choice in the setting of

Table 125.1 Contraindications for upper gastrointestinal endoscopy

Absolute contraindications
Refusal of informed consent
When the risks of the procedure outweigh the potential benefits
Endoscopic information or therapy will not alter the outcome or care
Relative contraindications
Suspected gastroduodenal perforation
Hemodynamic instability
Combative, uncooperative patient
Severe coagulopathy
Unprotected airway

UGI bleeding. The contraindications to endoscopy are listed in Table 125.1. Absolute contraindications include refusal of the procedure by the patient, a situation where the risks of the procedure outweigh the potential benefits of the procedure (such as shock), and where the procedure will not alter the outcome or care of the patient [10]. The relative contraindications can often be overcome by appropriate patient preparation. For example, airway protection may be achieved by endotracheal intubation prior to endoscopy and coagulation disorders may be mitigated by the infusion of blood products.

Endoscopy has a sensitivity of 92% when the entire stomach and proximal duodenum can be carefully visualized and has a specificity that approaches 100% [11]. By comparison barium radiography has a sensitivity of only 54%. Residual blood can be removed from the stomach by nasogastric lavage. Intravenous erythromycin has been demonstrated to stimulate gastric emptying of clots prior to endoscopy [9]. Visualization of the bleeding lesion can be aided by local application of 3% hydrogen peroxide through the endoscope [12,13]. The ability to perform biopsies to diagnose malignancy and the presence of *Helicobacter pylori* infection is not available with barium studies. Most importantly, prior barium radiography prevents potential endoscopic therapy that can improve morbidity and mortality. As a result, barium radiography should be avoided in the setting of acute UGI bleeding.

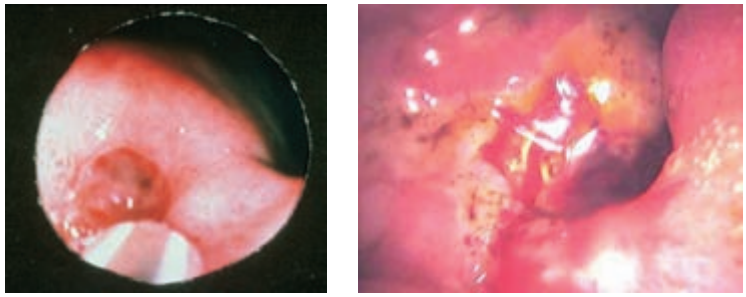
Not only will a careful endoscopic examination identify the source of UGI bleeding, it can also provide predictive information about the prognosis (probability of rebleeding, morbidity and mortality). Clinical prognostic factors, including age, shock, volume of bleeding, need for transfusion, onset of bleeding in the hospital, and comorbid conditions, have been identified [14,15]. Since the advent of endoscopy, however, it has been demonstrated that the endoscopic appearance of bleeding ulcers more accurately predicts the risk of rebleeding morbidity and mortality. Shortly after the availability of endoscopy, Forrest recognized specific endoscopic findings that were associated with an increased risk of rebleeding [16]. The original classification by Forrest (in order of risk of rebleeding and increased mortality) includes the following features: ulcers with a clean base, the presence of flat pigmented spots in the base, an adherent clot resistant to vigorous flushing, a nonbleeding visible vessel, and active bleed (either oozing or vigorous bleeding) (Fig. 125.1a–e). This classification has been repeatedly shown to accurately predict the rate of rebleeding, morbidity, and mortality in multiple studies [1,17]. A review of the data from various studies by Laine and Peterson [1] is presented in Table 125.2, demonstrating that patients with high-risk stigmata for hemorrhage (active bleeding or a nonbleeding visible vessel) have a high rebleeding rate that is associated with a correspondingly high morbidity and mortality. Patients without high-risk stigmata (those with a clean ulcer base or flat pigmented spots) have an extremely low rate of rebleeding and a negligible mortality [1]. In a large study in the UK, Rockall and colleagues combined the above noted clinical predictors with the endoscopic appearance of lesions into a scoring system to predict the outcome of acute UGI bleeding (Table 125.3) [18]. This validated scoring system demonstrates the utility of endoscopy in predicting the outcome of acute UGI bleeding. Patients with a score of 2 or less have a risk of rebleeding of less than 5% and virtually no mortality [18]. The addition of endoscopic findings to the other clinical criteria dramatically increased the accuracy of prognosis prediction based on clinical factors alone. In another study Jaramillo and colleagues evaluated 1567 consecutive patients with acute UGI bleeding using multivariate analysis. None of the patients had endoscopic therapy. The rebleeding rate was 20%. The presence of endoscopic high-risk stigmata was the most powerful predictor for rebleeding (odds ratio [OR] 3.1, $P < 0.001$) [19].

Other endoscopic findings have been suggested to increase the risk of recurrent bleeding. These include the size and location of the ulcer [20]. Both of these findings relate to the proximity of the base of the ulcer to a major artery or arteriole. Doppler ultrasound probes have been used in an attempt to identify large vessels in or near ulcers to predict the risk of rebleeding. These attempts have met with moderate success in targeting endoscopic therapy, but have limited sensitivity and specificity [21,22]. The technology has not been widely accepted.

Diagnostic role of urgent endoscopy (endoscopic triage)

While it may seem intuitively obvious that endoscopy would improve patient outcomes, randomized trials have indicated that diagnostic endoscopy alone does not improve mortality, rebleeding rates, need for surgery, or hospital stay [23–25]. The overwhelming majority (75%–80%) of patients with bleeding ulcers stop bleeding spontaneously, limiting the impact of early endoscopy for diagnosis [17,19,25]. Based on these data, patients are often initially admitted to hospital for volume resuscitation and observation, then undergo diagnostic

(a) (b) (c)



(d) (e)

Figure 125.1 Endoscopic photos of stigmata of recent hemorrhage. **(a)** Clean base. Duodenal ulcer with a clean base. No evidence of protruding or pigmented areas is present. The rebleeding rate in this lesion is near zero. **(b)** Flat spot. Pyloric channel ulcer with a flat black spot in the center. The risk for rebleeding in this lesion is less than 10%. **(c)** Adherent clot. Duodenal ulcer with a large adherent clot in the base that could not be removed with vigorous irrigation. The nature of the lesion beneath the clot is unknown. The risk for rebleeding in this lesion is intermediate (up to 25%). **(d)** Nonbleeding visible vessel. A prominent protruding visible vessel with a bipolar probe in the foreground. This lesion has a high risk for recurrent bleeding (40%–50%) without endoscopic therapy. **(e)** Active bleeding. A duodenal ulcer with blood vigorously flowing. This is the lesion with the highest risk for continued or recurrent bleeding (approaching 60%). Endoscopic hemostasis can control active bleeding in most patients and can reduce the risk for rebleeding.

Table 125.2 Rate of rebleeding and mortality in multiple studies

Endoscopic finding	Risk of rebleeding (%)	Mortality (%)
Active bleeding	55	11
Visible vessel	43	11
Adherent clot	22	7
Flat spot	10	3
Clean base	5	2

Adapted from Laine & Peterson [1].

endoscopy electively. Because urgent endoscopy improves morbidity and mortality only in the subset of patients with persistent or recurrent bleeding, both the National Institutes of Health and the American Society for Gastrointestinal Endoscopy have recommended that endoscopy be urgently performed in the subset of patients for whom endoscopic therapy is ancitipated [26,27].

Other data have raised the possibility of endoscopically identifying patients with acute UGI bleeding who have a low risk of rebleeding and require limited medical care. Laine and colleagues demonstrated that patients with clean ulcer bases or Mallory–Weiss esophageal tears had only a 2% risk of rebleeding and could be safely fed and immediately discharged from hospital [28]. Rockall and colleagues applied the previously mentioned scoring system to 2531 consecutive patients with acute UGI bleeding in the UK [29]. They identified 29.4% of the patients who had a score of 2 or less and a negligible risk of rebleeding or morbidity. Based on these data the authors suggested that a large number of patients without clinical risks or endoscopic stigmata for rebleeding could be safely managed without hospital admission, stating that "hospital admission has usually been regarded as obligatory until the risk of further hemorrhage has receded. This policy means that some patients at low risk stay in hospital for longer than is necessary especially when diagnostic endoscopy is delayed" [18]. Kodali and colleagues demonstrated that 21% of patients with bleeding from peptic ulcer disease had a clean ulcer base at endoscopy. These patients had a 3% risk of rebleeding [30]. The authors concluded that this group of patients could be safely managed as outpatients.

Table 125.3 Rockall classification of risk in acute upper gastrointestinal (UGI) bleeding

Variable	Score 0	Score 1	Score 2	Score 3	Points for this variable
Age (years)	< 60	≥ 60 and ≤ 79	≥ 80		Maximum 2
Shock	HR < 100 Systolic BP ≥ 100	HR ≥ 100 Systolic BP ≥ 100	Systolic BP < 100		Maximum 2
Comorbidity	None		Cardiac failure Cardiac ischemia Malignancy (undisseminated) Other comorbidity	Renal failure Liver disease Disseminated malignancy	Maximum 3
Diagnosis	Mallory–Weiss tear, no lesion	All other diagnoses	Malignancy of UGI tract		Maximum 2
Endoscopic stigmata	Clean base or flat spot		Active bleeding, adherent clot or nonbleeding visible vessel		Maximum 2
Total score					Maximum 11

BP, blood pressure (mmHg); HR, heart rate.
Adapted from Rockall et al. [18].

Two trials have retrospectively reviewed hospitalizations for UGI bleeding, and then developed criteria for same-day discharge (endoscopic triage) that were prospectively applied with success [31,32]. In a single-center study, Lee and colleagues demonstrated a decrease in costs of care associated with decreased resource utilization in patients undergoing endoscopic triage for nonvariceal UGI bleeding, with no increase in morbidity [33]. However, another similar-sized prospective multicenter study demonstrated no difference in the length of stay or clinical outcomes of patients undergoing urgent (preadmission) endoscopy or elective endoscopy after admission [34]. The major difference between these studies was the number of patients discharged from the emergency department. In the first study, 46% of the patients randomized to receive urgent endoscopy were discharged from the emergency room by the endoscopist, while in the second study the decision regarding admission was left to the attending physician (not the endoscopist) and only 4 of 47 eligible patients avoided hospitalization. These studies demonstrate the difference between efficacy and clinical effectiveness. In a situation where the endoscopic findings drive subsequent clinical care, endoscopic triage can significantly decrease hospitalization and costs of care for patients with a low risk of rebleeding. If the endoscopic findings do not affect subsequent care, then the information will not affect resource utilization.

Additional support for the effectiveness of endoscopic triage is provided by two studies by Hay and colleagues [35,36]. In the first study they developed a treatment guideline for UGI bleeding retrospectively, demonstrating the potential for substantial cost savings using endoscopy and three other factors as predictors for low risk and possible discharge [35]. In the second study they used an alternating time approach to evaluate implementation of the guideline [36]. During the intervention periods they aggressively modified physician behavior in the emergency department, promoting the previously developed clinical guideline that included preadmission endoscopy and discharge of patients with a low risk of rebleeding. During the control periods they allowed physicians to make the admission decisions. Compliance with the guideline was 70% during the period of aggressive promotion, but only 30% during the other periods. Aggressive implementation of the guideline resulted in substantially decreased hospitalization and costs without compromising patient care or satisfaction. The cost savings were lost during the control periods.

It appears that urgent endoscopy can substantially reduce the costs of care for UGI bleeding by identifying a group of patients that can be safely treated as outpatients only if the endoscopic findings, in conjunction with other clinical information, can alter the subsequent care of the patient.

A nonendoscopic scoring system based on data available at the time of presentation can distinguish between patients at high or low risk of needing further treatment for bleeding [37]. A retrospective study comparing this scoring system to that of Rockall with and without endoscopy showed that early endoscopy identifies more low-risk patients who may be treated as outpatients (30% compared with 8% using the Blatchford score) [38].

Endoscopic therapy for bleeding peptic ulcers

As previously noted, approximately 20% of patients with nonvariceal UGI bleeding have persistent or recurrent bleeding.

There is compelling evidence that endoscopic therapy reduces both the morbidity and mortality in these patients [26,27,39]. All available endoscopic techniques appear to have similar results, but vary in the approach to sealing the bleeding vessel and maintaining hemostasis. The methods may be divided into two major groups: thermal coagulation and mechanical tamponade.

Thermal coagulation

Thermal therapy of bleeding peptic ulcer disease is based on the understanding that the ulcer erodes into a submucosal vessel. Therapeutic interventions using heat have the goal of coagulating or sealing this vessel to stop active bleeding and prevent rebleeding [8].

Tissue has a characteristic response to thermal energy. When heated to 50–60°C, intracellular proteins become denatured, destroying the tissue and forming a white coagulum. This also produces a surrounding area with a zone of sublethal damage manifest by erythema and edema [40]. When heated to 100°C the water in cells is vaporized, resulting in cavitations and tissue ablation. The goal of endoscopic therapy is to coagulate the bleeding lesion and create a ring of edema that reduces blood flow and promotes hemostasis without causing tissue vaporization or cavitation that may exacerbate the bleeding. There are multiple methods of delivering different types of energy capable of raising tissue temperature, including light energy (lasers), electrical energy (monopolar or multipolar cautery), and direct application of a heated surface.

Laser therapy

Laser therapy was extensively used in the past for endoscopic hemostasis. While different lasers produce wavelengths of light with specific effects on tissue, the most commonly used laser was the neodymium:yttrium–aluminum–garnet (Nd:YAG) laser. The light from this laser (1060 nm wavelength) can be delivered through a flexible quartz fiber that is introduced through the working channel of a standard endoscope (Fig. 125.2). As the light strikes the tissue it is converted to thermal energy, creating the heat necessary for coagulation. Tissue contact is avoided while coagulating a ring of tissue surrounding the bleeding site. Other lasers (argon, KTP) commonly used in endoscopic therapy produce light of a shorter wavelength with more limited tissue penetration. As a result most work in acute UGI bleeding has been done with the Nd:YAG laser. This laser has been shown to stop bleeding lesions in the stomach and duodenum in over 90% of cases and reduce rebleeding to less than 20% [41].

Disadvantages of laser therapy include variable control of the depth and type of tissue injury, with the potential for overheating, vaporization, and charring of tissue that exacerbates bleeding. There is a small risk of perforation (< 2%) when used in the stomach. When coaxial gas is used around the quartz fiber, abdominal distention can result. The equipment necessary for laser therapy is expensive and requires special expertise for its use and maintenance. Most lasers are not portable, and require special safety precautions and training. Because of these logistical issues, and the availability of equally effective alternatives, lasers are now rarely used in the setting of acute UGI bleeding.

Electrocautery

Electrocautery uses an electrical current passed through tissue to generate thermal energy to coagulate the target tissue. The earliest use of electricity was with electrified endoscopic biopsy forceps touched to the tissue. In this case the electrical current flowed from the tip of the forceps through the patient to an electrical pad placed on the hip or thigh. In this method the degree of tissue injury depends on electrical current density at the site of contact with the forceps and local tissue factors that are poorly controlled. The result is an unpredictable depth of injury. For this reason, monopolar electrocautery is usually avoided in the treatment of acute UGI bleeding [26].

To avoid the problems associated with monopolar electrical current, endoscopic probes have been developed that have both the positive and negative electrical poles included in a circular design at the end of a catheter (Fig. 125.3). The result is limited delivery of electrical current within the diameter of the probe, using the electrical resistance of the tissue to heat only that area where the probe is placed. The depth and degree of tissue injury are well controlled. The probe also allows direct pressure to be applied to the bleeding lesion to seal the vessel (coaptive coagulation) [42].

This method was evaluated in a randomized prospective trial of patients with acute UGI bleeding by Laine [43]. Hemostasis was achieved in 90% of the patients treated with the multipolar probe, compared with 13% treated with

Figure 125.2 A quartz laser fiber with covering plastic sheath. Fibers may also have a sheath that provides coaxial gas flow. The quartz fiber conducts the laser light but is flexible enough to be passed through an endoscope channel. Similar fibers are used for argon, Nd:YAG, and KTP lasers.

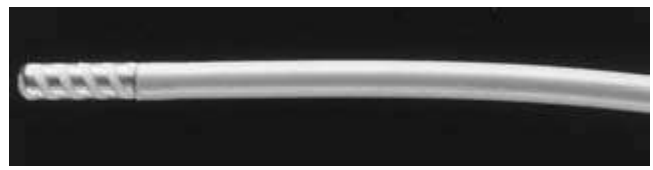


Figure 125.3 Bipolar electrocoagulation catheter. Electrodes circle the end of the catheter, and a central channel provides vigorous water irrigation. Some electrodes have a retractable central injection needle to allow combined electrocoagulation and injection therapy.

Figure 125.4 Heater probe. A flexible catheter tipped with a heating element that is placed directly on the tissue for coagulation. No electrical current passes through the patient. Vigorous water irrigation is possible through the central channel of the catheter.

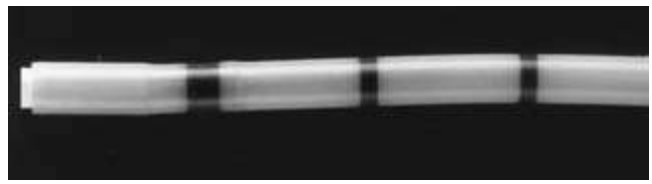


Figure 125.5 Argon plasma coagulation catheter. This hollow catheter produces a flow of electrically charged argon gas when activated. The catheter should not come in contact with the tissue. It allows rapid electrocoagulation of larger areas of tissue, or areas that are difficult to reach with a contact probe.

medical therapy [43]. There was also a significant reduction in transfusion requirement, hospital stay, morbidity, and costs for the patients receiving endoscopic therapy. In a similar trial limited to patients with nonbleeding visible vessels, the rebleeding rate was reduced by over 50% in the treatment group (18% vs 41%) [44]. There was a similar improvement in transfusion requirements, hospital stay, need for surgery, and cost in the treatment group. Many other trials have demonstrated similar results when using multipolar electrocautery [45,46].

Heater probe

Direct application of thermal energy can be applied to bleeding lesions using a heater probe. This device uses a catheter with a heating element at the tip that can be passed through the endoscope channel (Fig. 125.4). The heating element delivers a prespecified amount of energy by adjusting the time of the energy burst. The tissue effect is limited to the area in contact with the probe. As with the multipolar electrode, pressure can be applied directly to the bleeding site allowing coaptive coagulation of bleeding vessels.

The efficacy of this method is very similar to that of the multipolar probe. Initial randomized trials demonstrated that hemostasis could be achieved in actively bleeding lesions in 78% of patients, compared with none in a sham treatment group [47]. In a study limited to patients with nonbleeding visible vessels there was, again, a greater than 50% reduction in the rebleeding rate (10% vs 26%) in the treatment group [48]. As with multipolar electrocautery, many other studies have confirmed the ability of heater probe therapy to control active bleeding and reduce the risk of rebleeding from high-risk lesions [8].

Argon plasma coagulation

Another method of endoscopic hemostasis is the argon plasma coagulation (APC) device. This uses a flow of ionized argon gas to conduct electricity from the power generator to the tissue (Fig. 125.5). It is a type of monopolar electrocautery and should not be confused with the argon laser. Few controlled studies have evaluated the efficacy of this method of hemostasis. The distribution of tissue injury depends on the flow of gas and density of the electrical current at the tissue surface. One small study of 41 patients suggested no difference in results when comparing APC to heater probe therapy in acute UGI bleeding [49]. However, this methodology has not undergone the rigorous evaluation of other thermal hemostasis methods [50,51].

Technique of thermal therapy

The technical approach to thermal treatment of bleeding lesions is different in contact methods (multipolar electrocautery, heater probe) and noncontact methods (laser, APC). Sapphire tips can also be attached to laser fibers, creating a light-heated tip for contact therapy. Bleeding in peptic ulcer disease occurs when the ulcer erodes into a vessel. Either the vessel wall or the clot in the vessel can be identified endoscopically as a *visible vessel* (Fig. 125.6). In the noncontact method the vessel feeding the bleeding site is cauterized below the surface by treating circumferentially around the clot or point of bleeding (Fig. 125.7). This has the advantage of not disrupting any clot that may have formed in the lesion, but does not seal the vessel with coaptive coagulation. Contact probes, on the other hand, are used to apply direct pressure to the bleeding site, using the water jet capability of the

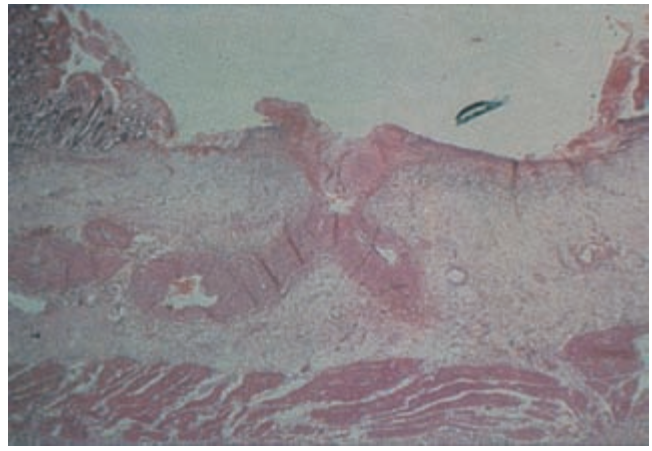


Figure 125.6 Visible vessel. This classic image demonstrates the histology of a bleeding vessel in the base of an ulcer. The submucosal vessel is seen extending to the ulcer surface, with a thrombus present in the "hole" in the arterial wall. Courtesy of C. Paul Swain MD.

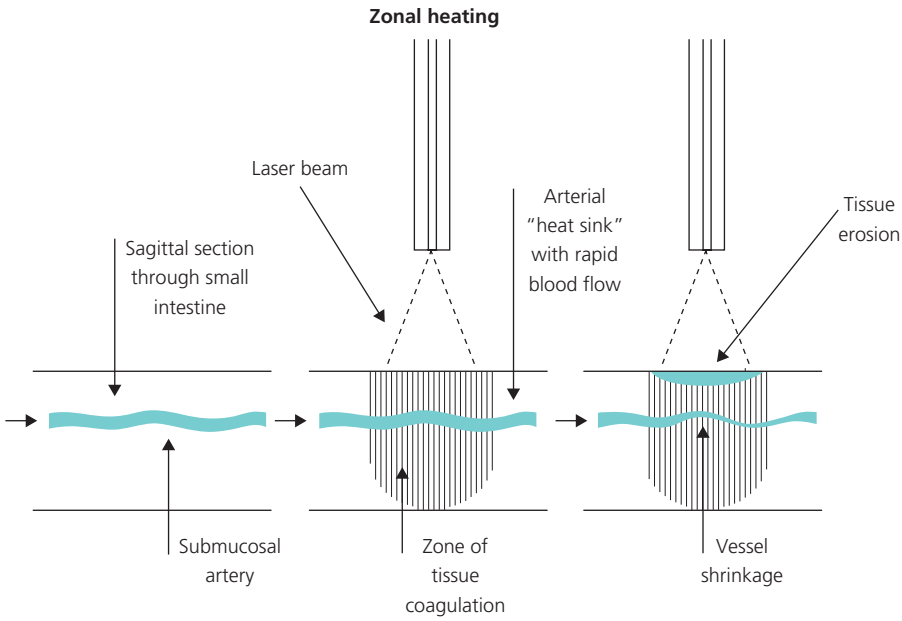


Figure 125.7 Noncontact therapy diagram. Coagulation of tissue with noncontact methods, such as laser therapy or argon plasma coagulation. The area surrounding the bleeding site is treated with the aim of coagulating the submucosal vessel. Even when the vessel is not coagulated, the resulting circumferential edema results in enough tamponade to control bleeding.

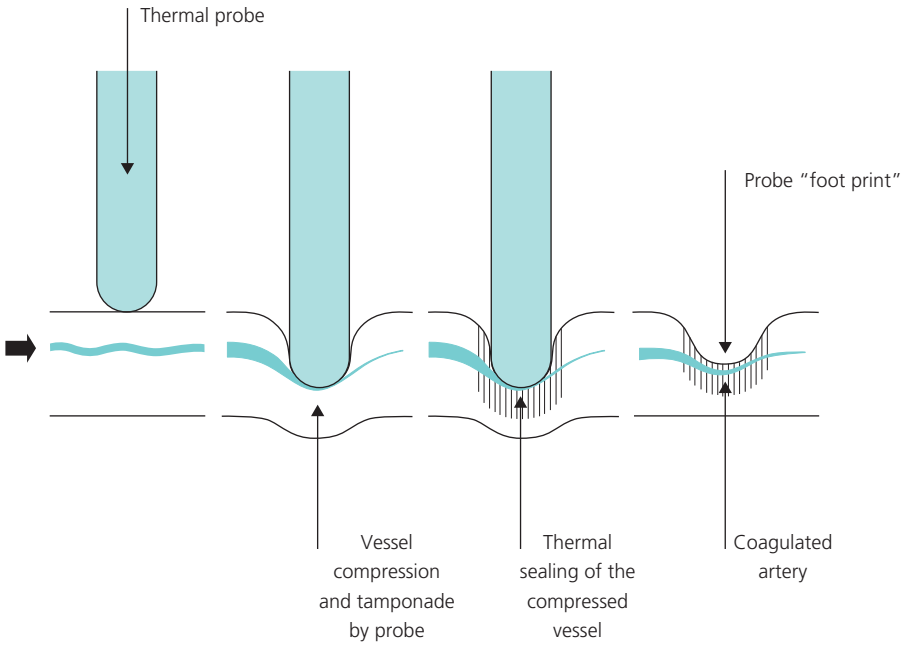


Figure 125.8 Contact therapy diagram. Treatment with contact probes focuses on coaptive coagulation of the bleeding site. Considerable pressure is applied with the probe to tamponade the bleeding vessel. Multiple bursts of energy are then delivered to coagulate the lesion and seal the bleeding vessel. Care must be taken to avoid dislodging coagulated tissue, which can adhere to the catheter tip. From Johnston JH, Jensen DM, Auth D. Experimental comparison of endoscopic yttrium–aluminum–garnet laser, electrosurgery, and heater probe for canine gut arterial coagulation. Importance of compression and avoidance of erosion. From Gastroenterology 1987;92:1101, with permission from Elsevier.

probes to clear away surrounding blood and debris. Multiple bursts of energy are applied to the lesion until hemostasis or adequate coagulation has been achieved (Fig. 125.8). Larger probes are more effective, as are longer pulses of lower energy [52]. The design of contact probes also allows tangential treatment of lesions in difficult locations. The disadvantage of contact probes is tissue adherence to the probe. When the probe is moved, the clot and coagulated tissue are dislodged, leading to more vigorous bleeding. This occurred in 18% of patients treated with contact probe therapy in one randomized trial [45].

The approach to patients with adherent clots in the base of ulcers remains controversial. Adherent clots are those which cannot be dislodged by prolonged application of a vigorous water jet. Unless the clot is removed the nature of the underlying lesion cannot be determined. A metaanalysis that compared six studies of endoscopic and medical therapy for adherent clots suggested that endoscopic therapy reduced rebleeding (pooled OR 0.35) but did not affect mortality or hospital stay [53]. In another study not included in the above metaanalysis, Laine and colleagues evaluated 46 patients with adherent clots [54]. They vigorously tried to remove

the clot with a water jet for 5 min. If the clot was dislodged, they treated the underlying lesion. In those where the clot remained no endoscopic therapy was applied. Only one of the patients rebled.

When clot removal is contemplated it should be done only if the endoscopist is prepared to deal with any underlying lesion, including vigorous bleeding. Freeman has advocated injection therapy of the base of the clot prior to attempted mechanical removal to reduce the risk of vigorous bleeding [17].

It appears that the clinical outcome of patients with adherent clots found at endoscopy depends on the lesion under the clot and the stability of the hemostatic plug formed by the clot. The removal of such clots should be determined by the patient's condition and the availability of appropriate therapy should clot removal precipitate vigorous bleeding.

Injection therapy

The most convenient and least expensive method of endoscopic therapy for UGI bleeding is the use of a simple needle catheter to inject the bleeding site with a compound selected to achieve and maintain hemostasis. There are different classes of materials used for injection therapy, including hemostatic agents, sclerosants and thrombogenic agents.

The most commonly used solution is diluted epinephrine (adrenaline), usually in the readily available 1 in 10 000 dilution. This is injected submucosally around the bleeding site in aliquots of 1–2 mL to a total volume of 5–10 mL (Fig. 125.9). Hemostasis is postulated to occur due to a combined effect of vasoconstriction by the epinephrine and tamponade of the bleeding vessel by the volume effect of the injection [55,56]. The efficacy of this method was demonstrated by Chung and colleagues in a study where 68 patients with actively bleeding ulcers were randomized to receive either injection therapy with 1 in 10 000 epinephrine or medical therapy [57]. Initial hemostasis was achieved in all patients in the injection therapy group. There were fewer patients who required surgery (5 vs 14), fewer blood transfusions, and a shorter hospital stay in the epinephrine-treated group. These findings were validated in a larger prospective trial of 555 patients with acute UGI bleeding. In this study, 98 patients were randomized to receive either 1 in 10 000 epinephrine injected in 1–2 mL aliquots around the lesion and 1–2 mL of ethanolamine into the bleeding site, or medical therapy alone. The rebleeding rate was 17% in the injected group and 47% in the control group ($P = 0.011$). There was also a reduced mortality, need for surgery, transfusion requirement, and length of hospital stay in the endoscopic treatment group [58].

Figure 125.9 Dilute epinephrine injection of an oozing ulcer using a metal-tipped injection catheter. Large volumes of dilute epinephrine or saline can be injected circumferentially around the ulcer to cause tamponade of the vessel and slow the bleeding. Definitive thermal or clip therapy should then be applied, as injection therapy alone is not sufficient to provide definitive hemostasis.

Other agents have also been used for subcutaneous injection to tamponade bleeding UGI lesions. These include normal saline, 3% saline, distilled water and 50% glucose in water. All these solutions have an effect similar to that of 1 in 10 000 epinephrine [59,60].

Sclerosing agents include absolute ethanol, polidocanol, ethanolamine, and sodium tetradecyl sulfate. An important difference between these compounds and those that act by local tamponade is that sclerosants cause tissue desiccation or destruction. As a result, much smaller volumes must be used (0.1–0.2 mL/injection, total volume < 2 mL) [55]. Even with these volumes there is a risk of extending tissue destruction and complications [61,62]. Initial trials demonstrated that ethanol injection could control active UGI bleeding [63], reduce the rate of rebleeding, and improve clinical outcomes [64] to a degree similar to that seen with epinephrine injection. Other sclerosants have mainly been used in conjunction with epinephrine injection. There are no convincing data to suggest that the combination of a sclerosant with epinephrine has a better outcome than epinephrine alone [65–67].

Two thrombogenic compounds, fibrinogen and thrombin, can be combined to form a glue-like substance that has been compared with polidocanol in a large European trial [68]. There was a significant improvement in the treatment group, but this required repeated endoscopy with multiple injections and is of limited utility in practice. Thrombin alone has also been investigated, but has not been widely adopted.

As the practice of endoscopic therapy progresses it has become clear that the combination of injection therapy and thermal therapy can be very useful. In the setting of an actively bleeding lesion initial therapy with injected 1 in 10 000 epinephrine, or other agent, around the bleeding site to tamponade the lesion helps slow the rate of bleeding and clears the field, facilitating subsequent thermal coagulation [55]. It has also been suggested that this injection pretreatment also creates a cushion of fluid that reduces the complication of a transmural thermal injury. Trials have demonstrated

that combination injection and thermal therapy is superior to injection therapy alone for the prevention of rebleeding in patients with high-risk endoscopic stigmata [69].

In summary, injection therapy using large volumes (10–20 mL) of nonsclerosing solutions is similar in efficacy to thermal therapy in patients with acute UGI bleeding from ulcer disease. There appears to be a significant advantage of combined injection/thermal therapy in the setting of active bleeding.

Hemostatic clips

The use of hemostatic clips was first reported in 1975 by Hayashi and colleagues [70]. The technique was based on the use of clips to halt blood flow in a specific vessel in both surgery and radiology. As the logistics of clip placement were improved, the use of clips was shown to be effective in the treatment of active bleeding in uncontrolled studies (Fig. 125.10) [71,72]. Binmoeller and colleagues treated 88 patients with either active bleeding ($n = 78$) or nonbleeding visible vessel ($n = 10$) with endoscopic clips [72]. Initial hemostasis was achieved in all patients with an average of three clips. With a mean follow-up of over 1 year, only five patients had rebled, all of whom were successfully retreated with clips. In randomized studies comparing the use of clips to injection therapy, thermal therapy, and injection therapy plus clips there was no difference in the outcomes between the three approaches [73,74]. One study compared the use of endoscopic clips to heater probe in ulcers with major stigmata of hemorrhage. The rebleeding rate was significantly lower in the patients treated with clips (1.8% vs 21%) [75]. However, this study has been criticized because the rebleeding rate in the heater probe group is at the high end of that reported in other studies, and the rebleeding rate in the clip-treated group is remarkably low [76]. Nonetheless, the study noted that logistical issues prevent adequate treatment of lesions that are in specific locations (lesser curve of the stomach, posterior gastric wall) where it is difficult to achieve an en face endoscopic approach for clip placement. A more recent study comparing epinephrine injection alone with injection plus clip placement in high-risk bleeding ulcer showed a reduction in rebleeding with combined therapy (3.8% vs 21%) [77]. Rare complications have been reported with clip placement [78].

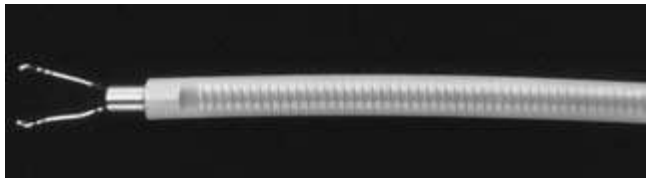


Figure 125.10 A hemostatic clip with its delivery catheter. The clip can be rotated to achieve optimal position, then closed and released from the delivery device. Multiple clips are often required to achieve the appropriate hemostatic effect.

Summary of the efficacy of endoscopic therapy

It is clear that endoscopic therapy for bleeding peptic ulcers can control active bleeding and reduce the risk of recurrent bleeding. Two large metaanalyses combining studies of endoscopic hemostasis in acute UGI bleeding demonstrate the efficacy of thermal therapies and injection techniques [39,46]. Very similar outcomes are achieved regardless of the treatment method used (laser, multipolar, heater probe or injection) [39]. In one analysis thermal endoscopic therapy reduced the rate of rebleeding (OR 0.38), surgery (OR 0.36), and mortality (OR 0.55). The overall complication rate, requiring surgery or causing perforation, was less than 1% for each method. Subgroup analysis showed no significant differences in the outcomes between the various thermal methods of therapy. However, the benefit of therapy was limited to patients with endoscopic stigmata, suggesting a high risk for rebleeding (visible vessels, active bleeding), but not in patients with low-risk stigmata (clean ulcer base, flat pigmented spots, adherent clots). This confirms the importance of endoscopic criteria in the selection of patients for therapy. As mentioned previously, rebleeding occurs in only 20%–25% of patients. Those with a low risk for rebleeding do not require endoscopic therapy. In the subset of patients with a high risk of rebleeding, however, endoscopic therapy improves clinical outcomes.

Nonulcer upper gastrointestinal bleeding

Upper gastrointestinal bleeding may also occur from sources other than peptic ulcers. The most common of these is a tear at the gastroesophageal junction, called a Mallory–Weiss tear (Fig. 125.11), which accounts for 5%–14% of UGI bleeding

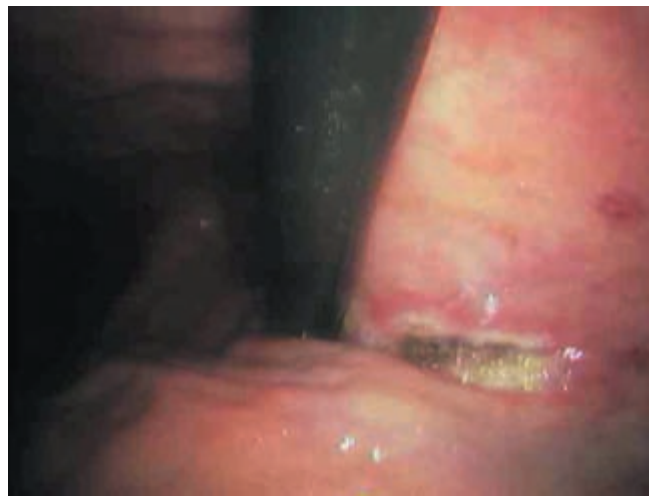


Figure 125.11 A deep Mallory–Weiss tear is adjacent to the shaft of the endoscope at the gastroesophageal junction. Bleeding from these lesions is often self-limited, but hemostasis can be achieved using the same methods applied to ulcers (injection, thermal therapy, clips).

[79,80]. This lesion should be treated in a similar manner as bleeding from peptic ulcers. Similar endoscopic stigmata also predict the risk for rebleeding in this lesion. Arterial bleeding in these patients is unlikely to stop spontaneously. On the other hand, patients who have self-limited bleeding have a good prognosis [28,81]. Active bleeding responds to the same methods outlined for peptic ulcers, including thermal therapy, injection therapy, and endoscopic clips [82,83]. Another method of hemostasis reported for therapy of a bleeding Mallory–Weiss tear is band ligation [84]. This method is commonly used for treatment of esophageal varices; a rubber band is placed around the bleeding lesion with excellent efficacy and safety [85].

Dieulafoy lesion is an aberrant submucosal artery that erodes into the lumen of the stomach and often presents as intermittent massive UGI bleeding [86]. While more common in the cardia, these lesions can occur anywhere in the stomach. The overall prevalence of Dieulafoy lesion in UGI bleeding is probably less than 5% [80]. Because these lesions are not accompanied by ulceration, they are often difficult to identify unless seen while actively bleeding. The infrequent nature of Dieulafoy lesion has prevented randomized trials on different types of endoscopic therapy. Combining uncontrolled experience, it appears that endoscopic therapy is effective for initial hemostasis in 96% of patients and achieves permanent hemostasis in 85%–90% [86,87]. As with Mallory–Weiss tears, ligation of the lesion, with either a rubber band [88] or endoscopically placed loop [89], has been reported to be successful.

Malignant and benign tumors are rare (< 1%) causes of acute UGI bleeding [89]. They more commonly present with other symptoms and chronic low-grade blood loss. When the cause of acute bleeding, the bleeding lesion usually responds to endoscopic therapy as outlined for peptic ulcer disease [8,89].

Another rare cause of acute UGI bleeding is a vascular ectasia. These lesions may occur as part of the hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu) syndrome or sporadically [90]. The sporadic lesions are a common cause of lower GI bleeding, but rarely present with hematemesis. These lesions also respond to standard thermal or injection therapy [90,91]. An uncommon cause of acute UGI bleeding is a diffuse antral form of vascular ectasia, often called a "watermelon stomach" or gastric antral vascular ectasia [92]. As with other vascular ectasias, chronic blood loss is a more common presentation of this lesion. Endoscopic laser therapy has been demonstrated to control acute bleeding and decrease transfusion requirements [93]. Other thermal methods have been demonstrated to also be effective, but may require more time than noncontact methods [94–96].

Diffuse bleeding from diffuse gastric erosions, as seen in critically ill patients, does not respond to endoscopic therapy and should be avoided by primary medical prevention [97–99].

Repeat endoscopic therapy

The issue of routine second-look endoscopy with repeated treatment remains controversial [100]. One randomized study of 40 patients with bleeding peptic ulcers showed that those who underwent a second session of endoscopic therapy with a heater probe 24 h after the initial therapy had a reduced transfusion requirement and rebleeding rate (0% vs 24%) [101]. Preliminary results of other studies have produced conflicting data on the value of routine retreatment of bleeding lesions [102,103]. When retreatment is provided only to those patients with persistent stigmata for rebleeding, the rates of permanent hemostasis can be improved [103].

It is clear that not all initial attempts at endoscopic therapy are equal. Patient condition, the presence of blood or clots in the stomach or duodenum, and other logistic factors can impact the efficacy of the treatment. The endoscopist often has a perception for how adequate the initial therapy was. In cases where the therapy was felt to be suboptimal, a second procedure timed to provide optimal therapy may be appropriate. However, routine second-look endoscopy for all patients requires further evaluation before the benefits can be demonstrated to outweigh the costs and risks to the patient.

On the other hand, it is clear that repeated endoscopic therapy is indicated in the setting of rebleeding. Lau and colleagues randomized 92 patients who had recurrent bleeding after endoscopic thermal therapy to either endoscopic retreatment or surgery [104]. The second endoscopic therapy session produced sustained hemostasis in 35 of 48 patients randomized to endoscopy; 13 patients went to surgery, 11 of whom had failure of the second endoscopic therapy and two because of a perforation resulting from the repeat endoscopy. The 30-day mortality rates were similar in the two groups, but complications were less in the endoscopy group, where seven patients (six of whom ultimately went to surgery) had complications compared with 16 of 44 patients in the surgery group. The authors concluded that a second attempt at endoscopic therapy can avoid surgery, with its associated complications, in many patients and does not increase mortality.

In most cases of rebleeding after endoscopic therapy a second attempt at endoscopic therapy is indicated before resorting to surgical therapy [105]. Thermal therapy in this situation may be associated with a higher rate of perforation.

Prevention of rebleeding

The prevention of recurrent bleeding after endoscopic therapy has been the source of considerable controversy. It is clear that the ulcerogenic conditions that led to peptic ulcer bleeding should be ameliorated. In most cases this means the discontinuation of NSAIDs or treating *Helicobacter pylori* infection [106]. However, there are no data to show that this

improves the short-term (72 h) outcome of the acute bleeding episode.

Normal hemostatic mechanisms are inhibited in the low pH of the stomach. Increasing the pH can improve coagulation and platelet aggregation [107]. In an attempt to increase gastric pH, intravenous histamine H_2-receptor antagonists have been routinely used in the setting of acute UGI bleeding. Unfortunately, they have been demonstrated to be no better than placebo in reducing the rate of recurrent bleeding [108]. This is probably because these agents have a decreased effect on acid reduction over time [109].

Despite the failure of H_2-receptor antagonists, it is clear that profound acid suppression can reduce the rebleeding rate of peptic ulcers with high-risk stigmata. Khuroo and colleagues randomized 220 patients to receive either oral omeprazole (40 mg b.i.d. for 5 days) or placebo [110]. No endoscopic therapy was provided. The rebleeding rates were 12 of 110 in the omeprazole group and 40 of 110 in the placebo group ($P < 0.001$). Subgroup analysis showed the benefit of omeprazole to be limited to those patients with nonbleeding visible vessels. In a prospective study of patients with either active bleeding or nonbleeding visible vessels (high-risk stigmata), Lau and colleagues randomized patients to either intravenous omeprazole (8 mg/h for 72 h) or placebo [111]. All patients received endoscopic therapy prior to randomization. Rebleeding occurred within 72 h in five of the patients receiving omeprazole and 24 of the patients receiving placebo ($P < 0.001$). This difference persisted through the 30-day follow-up.

Metaanalysis of randomized controlled trials comparing high-dose proton pump inhibitor (PPI) therapy with placebo or H_2-receptor antagonist therapy has demonstrated a reduction in rebleeding with PPI therapy [112,113]. The results vary somewhat with the route (intravenous or oral) and dose of the PPI. A post hoc analysis of one study demonstrated a more profound effect in studies performed in Asia compared with those performed in North America or Western Europe [114]. Mortality was only decreased in the Asian studies. The reason for this differential effect is not clear, but may be due to genetic differences in PPI metabolism.

The combination of endoscopic therapy with high-dose PPI administration is superior to PPI use alone. Sung and colleagues randomized 156 patients with nonbleeding visible vessels or adherent clots to receive intravenous PPI therapy and either endoscopic therapy or sham endoscopy [115]; 9% of the patients treated with the PPI alone rebled compared with none in the endoscopic therapy group. Based on these data it is clear that profound acid suppression using a PPI is an appropriate adjunct to endoscopic therapy of acute UGI bleeding. Intravenous therapy appears to have a greater effect, but oral administration also decreases rebleeding.

Somatostatin, or its analog octreotide, are standard therapy for bleeding esophageal varices. It has been suggested that the reduction in splanchnic blood flow caused by these agents could also treat acute UGI bleeding from other causes. In a metaanalysis Imperiale and Birgisson demonstrated a modest advantage of somatostatin/octreotide compared with H_2-receptor antagonists in the prevention of recurrent bleeding from peptic ulcer disease [116]. The authors suggest a possible role for these agents in patients awaiting endoscopy or when endoscopy is not available.

Summary

Acute UGI bleeding is a major medical problem, both in terms of the burden of disease and mortality of an individual episode. Endoscopy is the gold standard for diagnosis of the cause of bleeding and provides important prognostic information for the risk of continued bleeding or recurrent bleeding. Early endoscopy may also identify a group of patients with a very low risk of rebleeding who can be treated safely as outpatients. Endoscopic therapy using thermal, injection or mechanical methods is effective in controlling active bleeding, reducing the risk for recurrent bleeding, and decreasing morbidity and mortality from bleeding peptic ulcers. It has a similar efficacy in bleeding from most nonulcer lesions in the UGI tract. Endoscopic diagnosis and therapy is the standard of care for acute UGI bleeding.

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A ENDOSCOPIC

126 Endoscopic therapy for polyps and tumors

Sergey V. Kantsevoy

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Endoscopic resection of gastrointestinal polyps and tumors has become an important addition to traditional surgical therapy. At the same time endoscopic lesion removal has multiple advantages over open and laparoscopic surgery.

- Endoscopic lesion resection preserves the affected organ and is less traumatic and less risky than surgery.
- Endoscopic resection is usually done as outpatient procedure under sedation, obviating the need for general anesthesia and hospital admission with significant cost saving.
- Endoscopic procedures provide faster patient recovery and early return to work and normal physical activity compared with traditional open and laparoscopic surgery.

This chapter reviews currently available methods and tools for endoscopic therapy of polyps and tumors, the selection of candidates for endoscopic treatment, complications of endoscopic therapy, and endoscopic treatment of specific lesions in the esophagus, stomach, duodenum, and colon. Endoscopic therapy for tumors in the pancreas and biliary tree is covered in Chapter 121.

Methods of endoscopic therapy

Polypectomy

Polypectomy is the most commonly used method of endoscopic removal of lesions that are confined to the mucosal layer of the gastrointestinal tract and which have a stalk or are attached to the organ's wall with a relatively narrow base (7 mm or less). Other endoscopic techniques (endoscopic mucosal resection, endoscopic submucosal dissection) should be used for removal of lesions with a broad base or those originating from or expanding into the submucosal layer. Tumors extending into the muscularis propria or originating from it are usually not amenable to curative endoscopic resection [1].

Polypectomy can be done without use of electrical current ("cold" snare/forceps) or with electrical current ("hot" forceps/diathermic snare).

Cold snare/forceps polypectomy

Small (< 4 mm) polyps can be removed endoscopically without electrocautery using cold biopsy forceps [1,2]. Minor immediate bleeding is common, but it usually does not require any treatment and stops spontaneously within 1–2 min of observation. Delayed bleeding is uncommon in patients who are not anticoagulated and who do not have a bleeding tendency. Despite the apparently complete polypectomy, residual polypoid tissue was still detected on subsequent endoscopy in 29% of patients treated with cold biopsy [2].

Cold snare polypectomy (without electrocautery) is usually used for polyps 5 mm or less in size [3]. The polypectomy snare is placed around the base of a polyp which is then guillotined by mechanical closure of the snare [1]. Minor immediate bleeding is typical and usually stops spontaneously without any therapy within 1–2 min of observation. The major advantage of both cold forceps and cold snare polypectomy is a reduction in the risk of delayed bleeding when electrocautery is not used (delayed hemorrhage is usually caused by sloughing off of coagulated tissue or extension of coagulation-induced necrosis into larger diameter submucosal vessels) [2,3]. Cold snare polypectomy is not recommended for polyps larger than 7 mm in size [1].

Hot forceps polypectomy

Hot biopsy forceps are also used to remove polyps 1–3 mm in size [4]. Proper use of the hot biopsy forceps involves grasping the top of the polyp with the forceps and stretching or *tenting* the polyp to produce a stalk and then applying electrocautery (Fig. 126.1) [1,5]. Application of electrical current causes endoscopically visible whitening (coagulation) of the polypoid tissue

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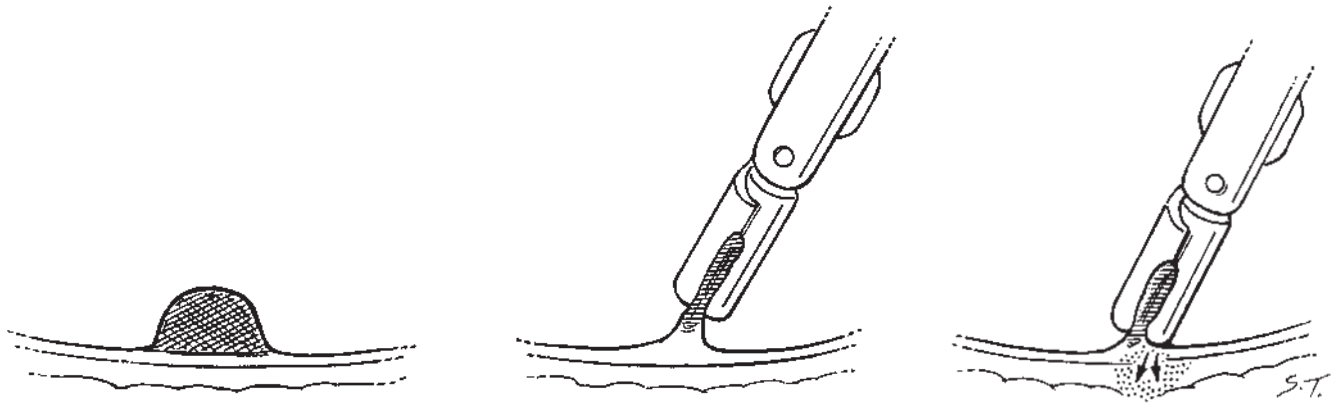

(a) (b) (c)

Figure 126.1 Use of hot biopsy forceps for removal of a diminutive polyp. **(a)** Diminutive polyp. **(b)** Correct use of hot biopsy forceps: the polyp is grasped with the forceps and pulled away from the bowel wall while cautery is applied. Notice how proper positioning of the forceps prevents coagulation injury to the colonic wall. **(c)** Wrong use of hot biopsy forceps: grasping too much tissue (i.e., too close to the colonic wall) or failure to "tent" the polyp by pulling it away from the wall may allow coagulation damage to the normal colonic wall.

starting at the tip of the forceps. After destruction of the polyp the forceps are withdrawn and the part of the polyp preserved in the forceps is sent for histological assessment [1]. Unfortunately, the extent of tissue coagulation by hot forceps is difficult to control and it may cause deep ulcers with subsequent delayed bleeding and even perforation [6–8].

Weston and Campbell summarized the results of removal of 1963 diminutive polyps and demonstrated that the risk of complications after hot forceps polypectomy was five times greater with right than left colon polyps [9]. Bipolar forceps limit the spread of electrical current and can potentially decrease the risk of a deep burn, although greater safety remains unproven and many gastroenterologists no longer use hot biopsy forceps [10,11]. Use of mini-snares is advocated instead of hot forceps for diminutive (< 6 mm) colorectal polyps, especially in the right colon [11,12].

Polypectomy with diathermic snare

Diathermic snares are usually used for polyps larger than 7 mm in size [4]. A variety of sizes (jumbo, standard, mini) and shapes (oval, crescent) of diathermic monopolar snares are used for endoscopic polypectomy. A snare is a wire loop contained inside an electrically isolating catheter. The diathermic snare is placed around the base or stalk of a polyp with subsequent application of electrocautery followed by mechanical transection of the polypoid tissue. Electrocautery heats the tissues to 45–100°C, which results in tissue coagulation and endoscopically visible coagulation necrosis (tissue whitening), reflecting denaturation of proteins and cell death [1]. Heat generated by the snare also causes desiccation and shrinkage of coagulated tissues with blood vessel constriction and thrombosis and thus prevents postpolypectomy hemorrhage and facilitates subsequent mechanical tissue transection.

Three modes of electrosurgical current are available: pure cut, pure coagulation, and a mixture (blend) [8]. In pure-cut mode, the electrical current waveform continuously oscillates between peak positive and negative voltage producing a local heating effect [8]. In pure-coagulation mode, the current wave is turned on in powerful bursts for short intervals of time [8]. For the blend mode, the periods of time when the duty cycle is off are attenuated compared with the cutting mode [8]. At the same power setting the peak power in a coagulation mode is five times higher than in blend mode and 10 times higher than in pure-cut mode, causing a wide coagulation zone [8].

Monopolar electrocautery should be used with caution in patients with implanted electrical devices (pacemakers, defibrillators) due to the risk of inactivation or electrical damage to these devices by the electrical current traveling from the monopolar snare to a grounding pad placed on the patient's skin. Bipolar snares decrease the energy required for polypectomy, limit the depth of coagulation and reduce damage to the underlying bowel wall, and potentially provide an added safety margin during polypectomy [10,13]. However, clinical studies have not shown significant advantages of bipolar snares over monopolar snares [14,15].

Special diathermic snares with barbs (Olympus Optical Ltd, Tokyo, Japan), spikes or needle at the tip of the snare (Wilson-Cook Medical Inc., Winston-Salem, NC) are designed to prevent snare slippage during polypectomy and are particularly useful for removal of large flat lesions [16].

When a pedunculated polyp is removed with a diathermic snare, most endoscopists recommend leaving a short portion (approximately half or one-third) of the stalk not involved with polypoid tissue. This remnant of the stalk, in case of postprocedure bleeding, can be injected, cauterized, or used for mechanical hemostasis with application of clips or detachable loops.

Endoscopic mucosal resection

Endoscopic mucosal resection (EMR) refers to removal of sessile or flat neoplasms confined to the mucosa or penetrating but not expanding beyond the submucosa [17]. There are multiple techniques of EMR: injection-assisted EMR, suction-assisted EMR, ligation-assisted EMR, and traction-assisted EMR.

Injection-assisted EMR

Injection-assisted EMR (also sometimes called saline-assisted polypectomy) was introduced by Rosenberg in 1955 for rigid sigmoidoscopy [18] and by Deyhle and colleagues in 1973 for flexible colonoscopy [19]. This procedure starts with injection of a solution into the submucosal space under the lesion to create a cushion. This cushion lifts the lesion, facilitating its removal and preventing damage to the deep layers of the gastrointestinal tract wall during EMR. Nonlifting of the tumor after submucosal injection is the best predictor of deep invasion and in this case endoscopic resection of the mass should not be attempted [20].

Various endoscopic needles are used for injection, for example 25G Carr-Locke injection needle (US Endoscopy), 23G Interject needle (Boston Scientific, Natick, MA), and VIN-23 (Wilson-Cook Medical Inc., Winston-Salem, NC). Various solutions can be used for injection including normal saline, hypertonic solution of sodium chloride, glycerol, dextrose, albumin at various concentrations, hydroxypropylmethylcellulose, fibrinogen mixture, and hyaluronic acid [21–23].

The ideal agent for submucosal cushion creation should be cheap, readily available, easy to inject, and should provide a long-lasting submucosal cushion without damaging the tissues at the site of injection [24]. A cushion created with normal saline, even with addition of epinephrine (adrenaline), lasts only a short time and is not suitable for removal of large lesions. Several studies have shown that hyaluronic acid, hydroxypropylmethylcellulose and glycerol provide a long-lasting submucosal cushion [25,26]. Unfortunately, these agents are expensive, not readily available in most endoscopy units, and difficult to store and inject [21,27,28]. Other studies have demonstrated tissue damage and local inflammatory reactions at the site of injections of hydroxypropylmethylcellulose and hypertonic solutions of sodium chloride (3.75%) and dextrose (≥ 20%) [27,29].

Autologous blood taken from the patient before the injection appears to be a new promising solution for submucosal injection [24,29]. The cushion created with autologous blood lasted seven times longer than the cushion created with normal saline [24]. Autologous blood did not impair visualization during EMR and facilitated subsequent endoscopic resection by separating the muscularis layer from the submucosal layer and promoting local hemostasis [24,29].

The volume of injected fluid depends on the size of the lesion. Usually between 5 and 50 mL of injected solution is required to create an adequate submucosal mound. After creation of the submucosal cushion, two different techniques can be used.

- Inject-and-cut technique (Fig. 126.2) is used with a single-channel endoscope: the endoscopic diathermic snare is placed around the lesion and electrical current is used to cut it off.
- Inject-lift-and-cut technique (Fig. 126.3) requires a double-channel endoscope: the resected lesion is grasped and lifted with endoscopic forceps through one channel of the endoscope [30]. Then through the second channel a diathermic snare is placed around the lesion to cut it off with electrical current.

Suction-assisted EMR

Suction-assisted EMR was originally performed using suction through an overtube [31,32]. This technique is now rarely performed and instead most physicians use specially designed caps (oval or round shape) that are frontloaded onto the tip of the endoscope (Fig. 126.4) [33]. This procedure requires several steps.

1 A specially designed crescent-shaped snare is placed around the margin of the cap. The lesion and surrounding normal mucosa are suctioned into the cap.
2 The snare is tightened around the lesion.
3 The suction is stopped to release the lesion from the cap (but still captured by the snare).
4 The snare is pulled up several times to ensure that only the lesion, not the entire wall, is moving; this prevents full-thickness resection of the gastrointestinal wall.
5 The EMR is completed with the snare using electrical current.
6 The resected lesion can then be aspirated into the cap to facilitate its removal for pathological examination.

Ligation-assisted EMR

A variceal ligation band can be placed around the mucosal lesion using commercially available variceal band ligators (Fig. 126.5) [34,35]. The lesion is then removed with electrical current using a diathermic snare above or below the band. The Duette Multi-Band Mucosectomy device (Wilson-Cook Medical Inc., Winston Salem, NC) is a combination of a specially designed six-band ligator with a large channel. This device allows banding and passage of a diathermic snare for subsequent resection of the banded lesion without the need to remove the banding device from the endoscope.

Traction-assisted EMR

Traction-assisted EMR uses countertraction of the lesion with grasping forceps placed via percutaneous endoscopic gastrostomy or anterior abdominal wall and gastric wall puncture [36]. This technique was successfully performed in eight patients with large gastric lesions (mean size 50 mm), facilitating endoscopic resection and shortening the procedure time [36].

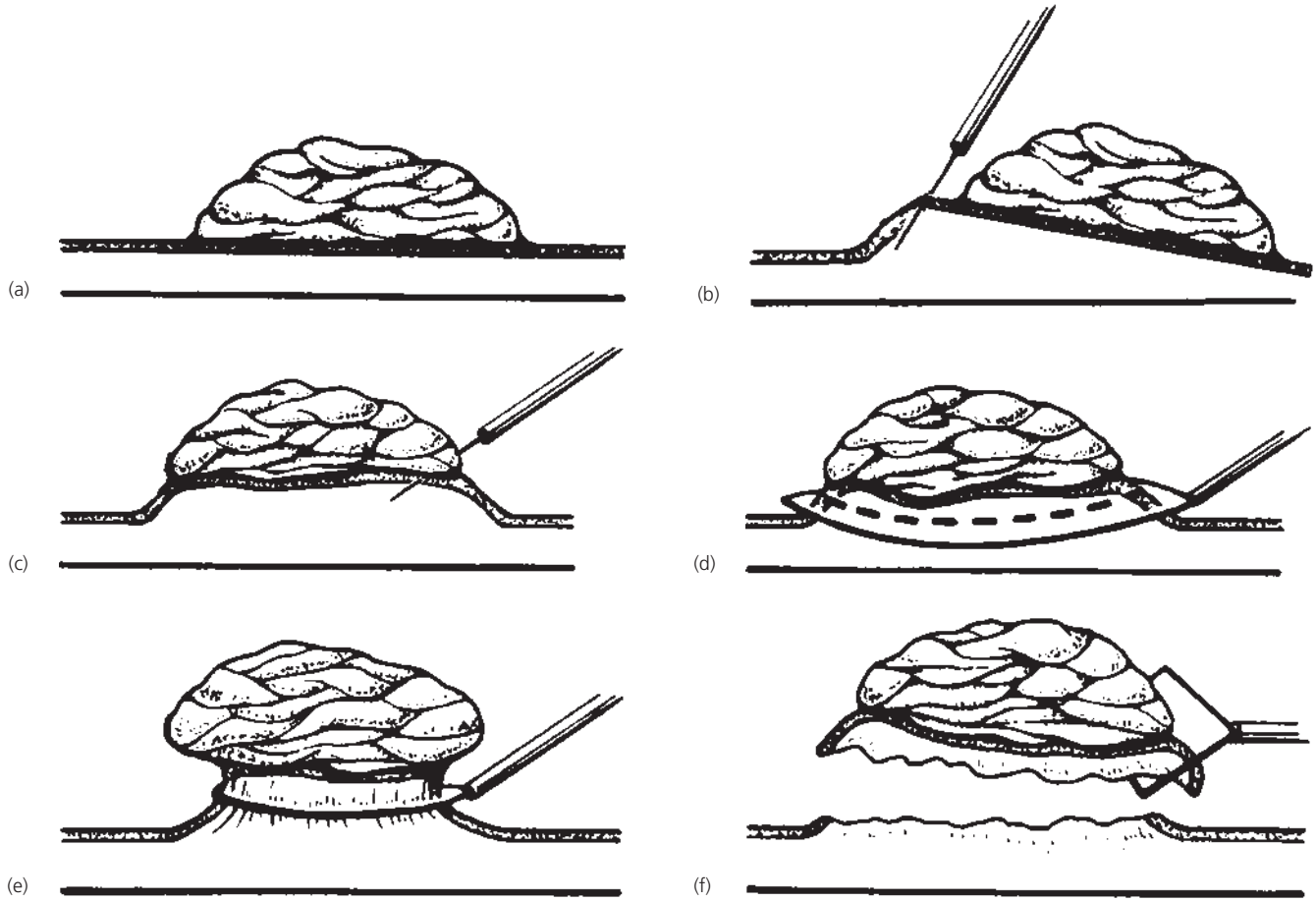

Figure 126.2 Endoscopic mucosal resection: inject-and-cut technique. **(a)** Sessile lesion limited to the mucosa. **(b)** Initial submucosal injection of fluid is recommended under the proximal edge of the lesion. **(c)** Subsequent submucosal injection of fluid is performed under the distal edge of the lesion to complete creation of a submucosal cushion. **(d, e)** The lesion is grasped and resected using a diathermic snare. **(f)** The entire specimen is retrieved for pathological examination.

Endoscopic submucosal dissection

Endoscopic submucosal dissection (ESD) has been developed for removal of large, flat, benign, and malignant gastrointestinal tract lesions [37–40]. The procedure is usually performed in several steps.

1 The margins of the lesion are marked by electrocautery (to facilitate its visualization when the submucosal injection changes the local anatomy).

2 The submucosal injection is made to lift the lesion.

3 A circumferential incision is made around the lesion to divide it from surrounding normal tissues.

4 The lesion is separated from underlying deep layers of the gastrointestinal tract wall, removed en bloc, and sent for pathological examination.

EMR and ESD have dramatically expanded the spectrum of endoscopically removable lesions. Large sessile, very flat, and even depressed lesions can now be removed endoscopically. EMR and ESD should not be attempted for lesions that do not "rise" during submucosal injection [41,42]. Failure to raise (the nonlifting sign) indicates fixation of the deep layers of the gastrointestinal wall (deep submucosal and muscularis) by the tumor [20,42]. Patients with such lesions should be referred for laparoscopic or open surgical resection.

Submucosal endoscopy

Submucosal endoscopy is a new promising approach for removal of gastrointestinal tumors. This procedure has only been tested in a live porcine model [43–45]. The first step of this procedure is creation of submucosal tunnel. Then the endoscope is advanced into the submucosal space under the lesion and the lesion is resected en bloc from submucosal space toward the lumen of the organ. Further human studies are necessary to validate and improve this promising technique, which may expand future indications for endoscopic removal of gastrointestinal tract lesions.

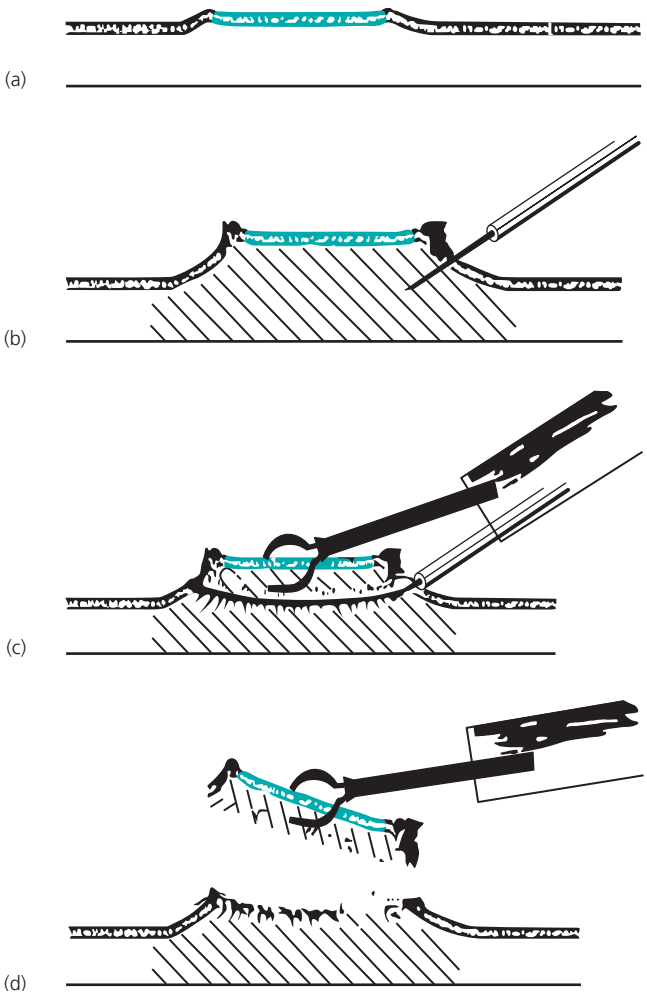

Figure 126.3 Endoscopic mucosal resection: inject-lift-and-cut technique. **(a)** Flat mucosal lesion (indicated by blue color). **(b)** Submucosal injection of fluid has lifted the mucosal lesion, separating it from the deep muscularis layer. **(c)** The lesion is grasped and lifted with a biopsy forceps, and a snare is advanced through the second channel of a dual-channel endoscope and placed around the lesion. **(d)** The lesion is resected and retrieved for pathological examination.

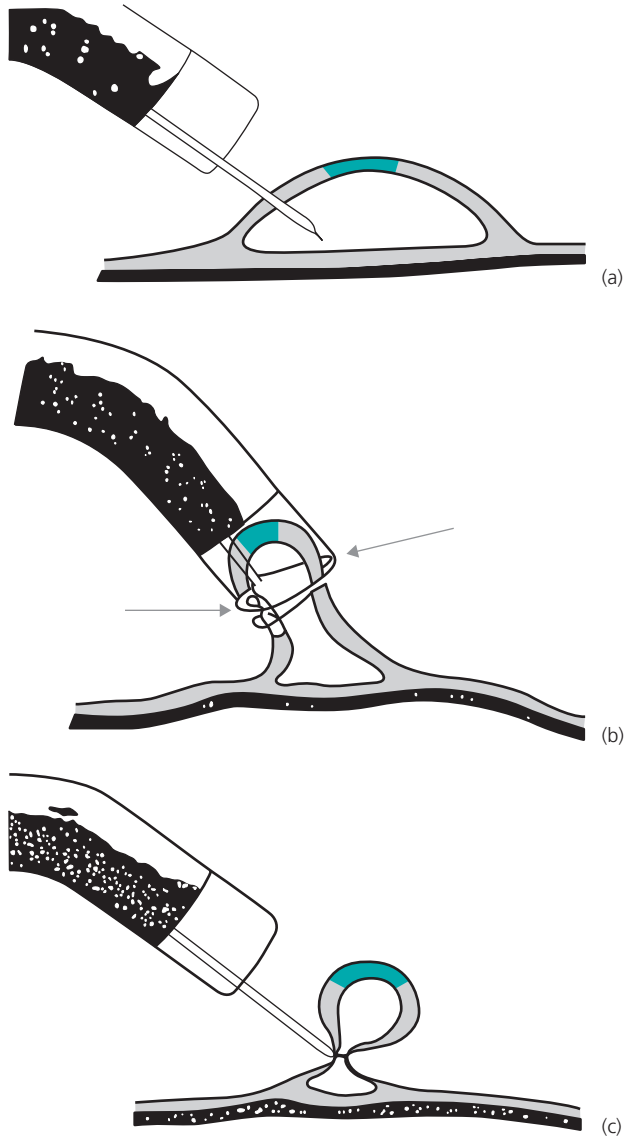

Figure 126.4 Suction-assisted endoscopic mucosal resection. **(a)** Submucosal injection of fluid to create a cushion under the mucosal lesion (indicated by blue color). **(b)** The mucosal lesion is aspirated into a cap mounted on the tip of the endoscope and grasped with a snare (arrows). **(c)** Suction is stopped so that the lesion is released from the cap. The lesion will be removed by application of electrical current and further tightening of the snare.

Additional tools for endoscopic lesion removal

Endoscopic clips

Endoscopic clips were developed to facilitate hemostasis and tissue approximation after endoscopic removal of gastrointestinal tract lesions [46–50]. The first commercially available endoscopic clips were introduced by Olympus Optical Ltd (Tokyo, Japan) and required preassembly with a reusable clip applicator before use [51,52]. Various sizes and shapes of clips were available, but because of the need to clean and reassemble the applicator prior to each use, this device has never become popular among endoscopists and gastroenterology nurses.

The first preassembled and ready-to-use endoscopic clip (HX-200L/U-135 or QuickClip) was also developed by Olympus Optical Ltd. Shortly afterwards other single-use endoscopic clips were introduced to the market by Wilson-Cook Medical Inc. (TriClip; Winston Salem, NC) and Boston Scientific Microvasive (Resolution; Natick, MA). The QuickClip and TriClip can only be closed once and cannot be repositioned after closure. The Resolution clip is gaining popularity among gastroenterologists because it can be opened and closed multiple times before its final application, which

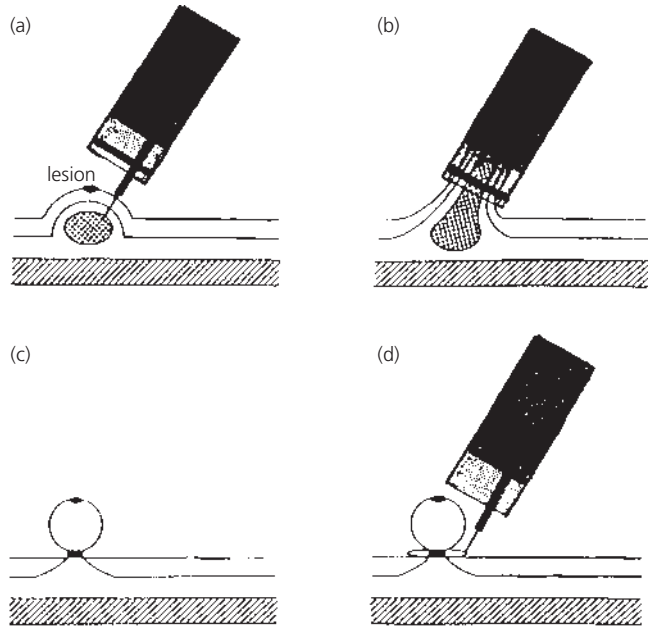

Figure 126.5 Ligation-assisted endoscopic mucosal resection. **(a)** Submucosal injection of fluid to create a cushion under the mucosal lesion. **(b)** The lesion is suctioned into the banding device mounted on the tip of the endoscope. **(c)** The rubber band is applied, ligating the mucosa below the lesion. **(d)** The ligated lesion will now be cut off using diathermic snare above or below the rubber band.

facilitates precision. A multiple clip applicator (MultiClip Applier) is under development by InScope (Cincinnati, OH) and will allow sequential application of up to four endoscopic clips without the need to reload the instrument [53].

In patients with endoscopically resected polyps and lesions of the gastrointestinal tract, endoscopic clips have been highly successful for prevention and control of complications [54]. Cipoletta and colleagues reported clipping of the large polyp stalk, preventing bleeding after polypectomy [55]. Other authors have reported successful use of endoscopic clips for control of bleeding and closure of perforations after endoscopic resection in a porcine model and in humans [56–58].

Detachable loops

Endoscopic detachable loops are also very useful during endoscopic removal of polyps and lesions of the gastrointestinal tract. As with clips, endoscopic detachable loops (Olympus Optical Ltd) first required a multiple use applicator and assembly prior to use. This model was quickly replaced by a preassembled single-use device. The loop can be applied to the base of the lesion or stalk of a polyp to prevent bleeding (Fig. 126.6) [59,60]. In a large randomized controlled trial of 488 consecutive patients with pedunculated colorectal polyps, application of a detachable loop prior to polypectomy helped to decrease the risk of bleeding by four times (from 7.9% to 1.8%) [61].

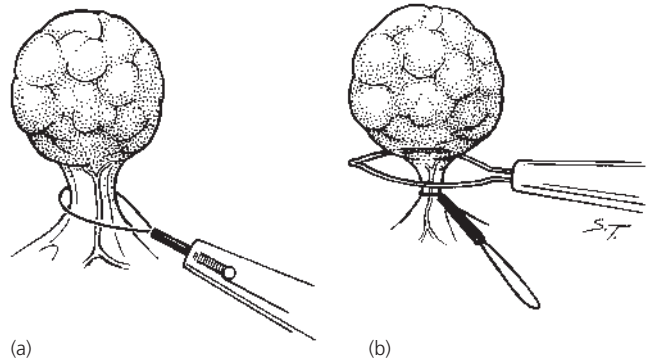

Figure 126.6 Use of a detachable loop for endoscopic resection of a pedunculated polyp. **(a)** A detachable loop is placed around the stalk of the polyp, tightened, and then released from its delivery device. **(b)** A cautery snare is placed above the loop (closer to the head of the polyp) to transect the stalk of the polyp.

Ablative (destructive) therapies

Ablative techniques result in destruction of tissue and are usually used for palliation of malignant tumors that cannot be resected surgically or endoscopically. The most common indications for endoscopic tumor ablation are restoration of lumenal patency and control of bleeding. Various endoscopic modalities are available for ablative therapy.

Bipolar electrocoagulation

Bipolar electrocoagulation uses a specially designed bipolar cautery probe with two active electrodes located at the tip of the device. Electrical current travels between these electrodes without the need for a grounding plate [62]. Compared with monopolar devices, the path of electric current in bipolar electrocautery is restricted to the distance between the electrodes, limiting the depth of tissue injury [63].

Bipolar probes are mostly used for eradication of very small polyps or ablation of residual flat neoplastic tissue that cannot be resected with a snare. These probes are rarely used for ablation of large benign or malignant tumors because of inadequate depth of injury and the need for repeated treatments and prolonged procedures with frequent cleaning of coagulated tissue from the working electrode [64–67].

Heater probe

The heater probe contains a thermal element and causes tissue coagulation at the point of contact with the heated tip. The heat is transferred to tissues regardless of the degree of coagulation, and the depth of injury tends to be deeper with the heater probe compared with bipolar electrocoagulation probes [1]. Because the depth of heat injury is difficult to control, the primary role of the heater probe has been limited to hemostasis rather than ablation of neoplastic lesions [68].

Laser

Energy generated by a laser (*l*ight *a*mplification by *s*timulated *e*mission of *r*adiation) can be delivered to tissues via specially designed light-transmitting fibers advanced through the biopsy channel of an endoscope. Laser energy is an effective modality for palliation of malignant obstruction, control of hemorrhage from tumor, and curative ablation of benign growths [69]. The use of endoscopically delivered laser energy has declined for the treatment of benign and malignant gastrointestinal tumors, mostly because of the high cost of equipment, prolonged duration of endoscopic procedures, difficulties in controlling depth of tissue penetration, and the need for repeated treatment [70,71].

Argon plasma coagulation

Argon plasma coagulation (APC) is another method for noncontact thermal tissue coagulation. APC is based on the conduction of high-frequency electric current to the treated tissue through the argon gas emitted from the tip of the APC probe. The depth of injury is determined by power settings and the duration of application. Inadvertent transmural injury with application of APC has been reported [72].

To decrease the risk of transmural injury in the cecum and right colon, power settings of 40–45 W and short bursts are usually used [1]. In the rectum and stomach, settings of 60–75 W and more prolonged applications are appropriate for benign tumor ablation [72]. For palliation of cancers, power settings of 90–100 W and repeated applications are usually necessary [1].

APC is widely used to treat actively bleeding diffuse mucosal lesions (arteriovenous malformations, radiation proctitis), eradicate gastrointestinal tumors and Barrett esophagus, restore stent patency in cases of tumor ingrowth or overgrowth, and treat residual flat neoplasia after snare resection of the main lesion [73–77].

Injection of chemicals

Small published series have reported palliation of malignant obstruction and control of bleeding through endoscopic injection of agents causing chemical necrosis [70,78]. Relief of obstruction occurs via tissue sloughing, and hemorrhage is controlled via thrombosis, edema, and vasoconstriction [1]. These techniques are usually inexpensive, easily performed, and the necessary equipment and agents are widely available [70]. Despite the obvious advantages of injection therapy, this endoscopic modality has not become popular, probably because of the absence of an immediate tissue destructive effect and difficulties in controlling and predicting the final extent of tissue destruction. Effective agents previously reported for injection therapy include sclerosants (ethanol, sodium morrhuate) and chemotherapeutic drugs (cisplatin) [70,71,78–81].

Other therapeutic modalities

Additional palliative modalities (photodynamic therapy, dilation and stent placement) are covered in other chapters of this book.

Endoscopic tissue tattooing

Tattooing refers to the local labeling of a lesion or site where a lesion was endoscopically resected by intramural injection of a pigment for future identification during subsequent surgery or repeat endoscopy. India ink and suspension of pure carbon particles (Spot, GI Supply, Camp Mill, PA) are the most commonly used solutions, producing a blue-black submucosal stain visible from serosal and lumenal surfaces [82–84]. A 1 in 10 or 1 in 100 dilution of ink saline can be autoclaved or filtered. Commercial preparations of both India ink and Spot for endoscopic injection are also available [84–86].

The tattoo mark is made by direct injection of 1–2 mL of India ink or Spot into each site through a sclerotherapy needle inserted tangentially to make a submucosal bleb. Injections into three or four quadrants are usually sufficient and ensure subsequent intraoperative or endoscopic identification of the lesion. Many endoscopists prefer Spot over India ink for marking of colonic polyps because transmural (intraperitoneal) injection of India ink can cause significant patient discomfort or even pain and can result in colonic abscess and focal peritonitis [84,87,88].

Several techniques have been developed to prevent transmural (intraperitoneal) injection of tattooing agents and to improve marking of the lesion. In the two-step method, a submucosal bleb is formed under the tumor with subsequent injection of the tattooing agent into this bleb through a separate needle [89]. A second method is called the sequential injection technique and starts with the creation of a submucosal bleb with injection of normal saline followed by injection of a tattooing agent (through the same needle without its withdrawal from the bleb) into this submucosal bleb [90,91]. Both methods ensure injection of the tattooing agent into the correct layer of the gastrointestinal wall, decrease the amount of agent needed, and prevent transmural puncture with subsequent injection of the tattooing agent into the peritoneal cavity.

Patient selection for endoscopic treatment

The ability to remove gastrointestinal tract lesions endoscopically depends on multiple factors: nature of the lesion, experience of the endoscopist, presence of all necessary equipment, local situation, and patient's condition.

Malignant lesions confined to the mucosal layer of the gastrointestinal tract can usually be removed endoscopically. If

the malignant lesion invades the submucosal layer, endoscopic removal is usually contraindicated. Inability to raise the lesion with submucosal injection (nonlifting sign) is generally a very reliable indication of possible invasion into the submucosal layer [20].

Endoscopic experience is very important for successful and safe completion of lesion removal. Most practicing endoscopists are experienced in polypectomy, especially removal of pedunculated polyps. EMR and ESD are more difficult than polypectomy and usually performed in specialized centers equipped with the necessary instruments. If an important piece of equipment is missing or malfunctioning, then endoscopic removal should not be attempted.

Local situation (shape, size, location of the lesion) can make endoscopic removal of the tumor impossible, especially if the lesion is located behind a fold or in an anatomically inaccessible portion of the gastrointestinal tract (e.g., in the duodenal bulb immediately after the pylorus or just proximal to the hepatic flexure). Similarly, very flat and broad-based lesions can be difficult to grasp with the diathermic snare and should not be removed endoscopically.

Endoscopic lesion removal should not be attempted if the patient is not adequately sedated or is agitated or uncooperative. If the physician who discovered the endoscopic lesion is not comfortable with its removal, the best course of action is to biopsy the lesion, mark it with a tattoo, and refer the patient to a more experienced colleague or for a surgical consultation.

Complications of endoscopic therapy

The most serious immediate complications of endoscopic lesion removal are perforation and bleeding. Perforations occur in 0.3% of patients after polypectomy [92]. Small perforations can be closed with application of endoscopic clips and treated conservatively, but larger perforations require immediate surgery [93,94].

The reported frequency of postpolypectomy bleeding ranges from 0.2% to 1.0% [95–100]. In a recently published large prospective study of 5152 patients with 9336 polyps immediately after polypectomy, bleeding was documented after removal of 262 polyps (2.8%) in 215 patients [101]. This study revealed nine factors associated with immediate postpolypectomy bleeding: age older than 65 years, comorbid cardiovascular or chronic renal disease, anticoagulant use, polyp size greater than 1 cm, gross morphology of polyps such as pedunculated polyp or laterally spreading tumor, poorer bowel preparation, cutting mode of electrosurgical current, and inadvertent cutting of a polyp before current application [101]. Most cases of active bleeding during endoscopic procedures can be successfully controlled by injection, local application of coagulation (bipolar, thermal or APC), or mechanical hemostasis (band ligation, endoscopic clips and detachable loops).

The most common delayed complications of endoscopic therapy for gastrointestinal tumors are formation of strictures, delayed bleeding, and postpolypectomy syndrome [102,103]. In general, the risk of stricture formation is higher when the lesion occupies more than half of the lumenal circumference [93,104,105].

Aspirin and other nonsteroidal antiinflammatory drugs in standard doses do not increase the risk of significant immediate or delayed bleeding after colonoscopy with biopsy and polypectomy [106,107]. However, the risk of bleeding is definitely higher in patients who have bleeding disorders, low platelet count, or who are therapeutically anticoagulated [107,108]. Friedland and Soetikno have demonstrated that in therapeutically anticoagulated patients with polyps less than 10 mm in diameter, prophylactic application of endoscopic clips after removal of the polyp can prevent postpolypectomy bleeding [109].

Electrocoagulation injury to the bowel wall during polypectomy can cause a transmural burn in approximately 0.51%–3.7% of patients, which may result in the postpolypectomy (postcoagulation) syndrome [97,103,110]. Patients with this syndrome usually present 1–5 days after colonoscopy with symptoms of localized abdominal pain, fever, peritoneal signs, and leukocytosis [111]. Abdominal radiography and computed tomography are not suggestive of perforation (no free air or extravasation of contrast into a free peritoneal cavity) and conservative therapy (bowel rest, antibiotics) is usually sufficient for resolution of the symptoms [111].

Esophageal lesions

EMR is indicated for early (M1 [intraepithelial] and M2 [cancer limited to lamina propria]), moderately differentiated, and well-differentiated squamous cell esophageal cancer without venous or lymphatic invasion [93]. EMR will not be curative for cancers involving the muscularis mucosa and submucosal layer [93]. These patients will have a high rate of metastases and endoscopic resection should be offered only to those who are not surgical candidates or who have declined surgery [112]. Inoue and colleagues have reported excellent results of endoscopic therapy for esophageal cancer with low complication rates and 5-year survival of 95% [112,113]. In another study, Ciocirlan and colleagues confirmed a disease-specific 5-year survival rate of 95% after EMR in patients with esophageal superficial squamous neoplasms [114].

Endoscopic therapy of Barrett esophagus and early esophageal adenocarcinoma is less established than treatment of esophageal squamous cell cancer [93]. Esophageal adenocarcinoma patients who are not surgical candidates can be

treated endoscopically (EMR, photodynamic therapy, balloon-based radio-ablation device) with attempt of cure (T1 disease) or palliation (dilation, photodynamic therapy, laser therapy, bipolar thermocoagulation, APC, stent placement) to restore esophageal patency and to improve swallowing [115,116].

Several authors have reported high rates of success with few complications after EMR of high-grade dysplasia and superficial esophageal adenocarcinoma. Using EMR, Conio and colleagues successfully treated 36 of 39 consecutive patients with high-grade dysplasia or intramucosal cancer arising from Barrett esophagus [117]. Bleeding complicated the procedures in 10.3% of patients and was controlled endoscopically in all cases. After a median follow-up of 34.9 months all patients were still in remission.

In another study, EMR was used to treat 41 patients with high-grade dysplasia and cancer (23) arising from Barrett esophagus [118]. The neoplastic lesions were removed on initial procedure and residual Barrett epithelium was resected 1 month later. After a mean follow-up period of 31.6 months, Barrett epithelium was completely replaced in 76.6% and recurred in 24.4% of cases. Recurrent or metachronous early cancer was diagnosed in five patients (12.2%) and again was treated endoscopically in four of the five patients. Postprocedure complications (bleeding and perforation) occurred in 14.3% of patients. Despite the circumferential removal of Barrett areas, only one patient developed esophageal stricture.

Overholt and colleagues randomized 485 patients with high-grade dysplasia originating from Barrett esophagus to photodynamic therapy versus omeprazole [119]. There was a statistically significant difference in complete ablation of high-grade dysplasia in patients treated with photodynamic therapy (77% vs 39%). Encouraging results of balloon-based endoscopic radio-ablation of Barrett esophagus in 100 patients have also been published [116]. A complete response to therapy (all biopsies negative for Barrett esophagus at 12 months follow-up) was achieved in 70% of patients. There were no strictures and no buried glandular mucosa was found during the entire period of follow-up. Conversely, a high recurrence rate of Barrett epithelium was found during long-term follow-up after APC ablation [120]. Twenty five patients were followed prospectively for 51 months and continuously treated with high-dose proton pump inhibitor. Barrett epithelium recurred in 66% of patients.

Gastric lesions

The most common gastric lesions found during endoscopy are gastric polyps (fundic gland, hyperplastic, and adenomatous), malignant neoplasms, and submucosal tumors (gastrointestinal stromal cell tumor, lipomas).

Fundic gland polyps are usually small, multiple, have negligible malignant potential, and do not need endoscopic removal [121,122]. Hyperplastic gastric polyps are typically located in the antrum, can be solitary or multiple, and are more common in elderly patients. If a hyperplastic polyp reaches significant size, it may cause gastrointestinal blood loss and iron deficiency anemia and can occasionally undergo malignant transformation [123–125]. Gastric adenomatous polyps are considered premalignant lesions, with an incidence of malignancy of 24% in polyps larger than 2 cm but only 4% in polyps smaller than 2 cm [125,126].

No clinical or endoscopic finding can reliably distinguish the variants of gastric polyps before histological examination [121]. At the same time, the histological examination of specimens obtained by forceps biopsy sampling of gastric polyps is of limited accuracy, and their management on this basis is controversial [127]. Therefore gastric polyps should be fully resected by EMR for a final diagnosis and (depending on lesion size and type) possible definitive treatment [127,128]. If the resected specimen reveals carcinoma with deep invasion into the submucosal layer or lymphatic vessels, surgical resection should be recommended [129,130]. A large study of 1177 patients with gastric polyps followed over a period of 7 years demonstrated recurrence of benign gastric polyps in 6.1% of patients (most within 1 year after polypectomy) and development of gastric carcinoma in 1.7% of patients [131]. Based on these results, the authors recommended yearly follow-up endoscopy after removal of benign gastric polyps [131].

The techniques of gastric and colonic polypectomy, EMR and ESD are similar (see above). However, the risk of perforation during endoscopic lesion removal is lower in the stomach, while the risk of bleeding is lower in the colon [127,132,133]. Special caution should be exercised during retrieval of the specimen from the stomach to protect the patient's airway [1]. After endoscopic resection of esophageal and gastric lesions many physicians recommend a liquid diet for 12–24 h followed by a mechanically soft diet for an additional 24 h and then regular diet. Proton pump inhibitors and liquid carafate are often prescribed for several weeks to promote local healing of ulcers caused by lesion removal.

The largest experience in endoscopic resection of early gastric cancer has been accumulated in Japan, where screening for early gastric cancer has been active since 1957 [93]. Approximately 50% of gastric cancers (10 000 cases yearly) in Japan are now discovered at an early stage [36,134–137]. EMR and ESD are now accepted as minimally invasive treatment of early gastric cancer in Japan. Kojima and colleagues summarized the outcomes of endoscopic therapy for early gastric cancer published in the Japanese literature by 1998: 1832 patients had EMR and ESD for early gastric cancer [138]. Endoscopic resection was complete in 73.9% and incomplete in 26.1% with a combined complication rate of 1.9% (1.4% bleeding, 0.5% perforation).

Duodenal lesions

Duodenal adenomas can be sporadic or part of a genetic syndrome (familial adenomatous polyposis, Peutz–Jeghers syndrome) [139]. Duodenal adenomas have potential for malignant transformation and should be removed surgically or endoscopically. The choice of an endoscope for removal of duodenal adenomas is largely determined by location of the lesion: polyps originating from the duodenal bulb are usually better visualized with a forward-viewing endoscope, whereas tumors located in the second and third portion of the duodenum are best evaluated with a side-viewing endoscope.

Various polypectomy and EMR techniques can be used to remove duodenal polyps. Periampullary lesions are usually removed with standard polypectomy technique without submucosal injection. There is no consensus in the literature regarding what type of electrical current should be used during ampullary polyp removal [139]. Some authors advocate pure cutting current (to prevent coagulation damage to the pancreatic duct), whereas others recommend blended current to avoid bleeding [140,141]. Endoscopic papillectomy is associated with an increased risk of postprocedure pancreatitis and several studies have demonstrated reduction in the incidence of pancreatitis after prophylactic stenting of the pancreatic duct after papillectomy [142,143]. APC is frequently used after papillectomy to destroy residual adenomatous tissue and to control postprocedure bleeding [144,145]. Large, firm, ulcerated, and nonlifting ampullary lesions are suspicious of malignancy [139]. Endoscopic removal of malignant ampullary tumors is usually inadequate and these patients should be referred for surgical evaluation [144,146,147].

Duodenal adenomas located outside the major duodenal papilla are usually flat and should be treated similarly to flat colonic polyps. These lesions are most commonly removed with inject-and-cut or suction-assisted EMR. Polyps that cannot be raised with submucosal injection should be referred for open surgery or laparoscopic removal.

Colorectal polyps and tumors

Most polyps are diagnosed in asymptomatic patients during routing screening colonoscopy. Larger polyps are found in patients evaluated for anemia, heme-positive stool, or overt lower gastrointestinal bleeding. Colorectal polyps should be removed to halt adenoma–carcinoma progression [148,149]. Several studies have demonstrated the effectiveness of colonoscopy with polypectomy in reducing the incidence of colorectal cancer [150–155].

Diminutive polyps

Diminutive polyps are lesions less than 6 mm in diameter [156–158]. In the right colon and transverse colon diminutive polyps are more likely to be adenomatous, but in the left colon most of the small polyps are hyperplastic [9]. Use of chromoendoscopy with magnification helps to differentiate adenomatous from hyperplastic polyps [159]. In a study of 240 small colonic polyps, the diagnostic accuracy of regular colonoscopy was only 76.3% compared with an accuracy of 95.4% achieved when chromoendoscopy with magnification was used [160]. This may be especially important when large numbers of diminutive polyps are present in the colon and endoscopic removal of all of them for histological examination is not feasible. A large study of 6722 polyps, of which 4281 (76%) were smaller than 6 mm, demonstrated unfavorable histology in 4.4% of diminutive polyps, emphasizing the clinical significance of small colorectal polyps [161].

Several endoscopic techniques can be used for removal of diminutive colonic polyps. Hot forceps polypectomy has a high rate of bleeding complications especially in the right colon and is rarely used [9,11]. Although cold forceps polypectomy appears safer than hot forceps polypectomy, residual polypoid tissue was detected on subsequent endoscopy in 29% of patients treated with cold biopsy [2]. Many experts advocate use of tiny snares instead of cold or hot forceps for eradication of diminutive colorectal polyps [11,12].

Pedunculated polyps

Pedunculated polyps are attached to the colonic wall by stalks of various sizes. Histologically, the head of a pedunculated polyp usually represents colonic adenoma, sometimes with dysplasia or cancer. Adenomatous changes rarely involve the stalk of pedunculated polyps. Large polyps may be fed with significant caliber blood vessels. Pedunculated polyps can be found in different areas of the colon, but most commonly in the left colon.

Most pedunculated polyps are removed with diathermic snare. It is advisable to leave a short portion (approximately half or one-third) of stalk not involved with polypoid tissue. This prevents extension of coagulation necrosis to the colonic wall and the remnant of the stalk in cases of postpolypectomy bleeding can be injected, cauterized, or grasped with endoscopic snares, clips or detachable loops for mechanical hemostasis. If malignant transformation of the polyp is suspected, the snare should be positioned as far from the polyp head as possible to avoid residual polypoid tissue within the resection margins [1].

Endoscopic polypectomy of large pedunculated polyps, especially those in the tortuous sigmoid colon, can be technically difficult [162–164]. Large polyps may partially occlude the colonic lumen, preventing the manipulation of colonoscope and endoscopic snare [55,163]. Polyps on a long stalk can possess additional difficulties due to their excessive mobility [162].

Several maneuvers can be used when placement of a snare over the polyp cannot be easily performed [165]. If the optimal position for snare application cannot be achieved by

manipulation of the endoscope, changing the patient's position is recommended to move the polyp to a better view. Sometimes even retroflexion of the endoscope may be needed to gain the best conditions for polypectomy [166]. Changing the colonoscope to a double-channel endoscope or attachment of an additional improvised channel to a regular colonoscope and use of a second grasping instrument can significantly facilitate polypectomy [162,163,165]. Piecemeal reduction of the polyp can be performed if the head of the polyp is too large and will not fit even into the largest polypectomy snare or into a detachable loop [167]. Finally, clip application to the stalk with subsequent needle-knife transection of the stalk can be performed if placement of a diathermic snare around the stalk is impossible [55].

To prevent electrical damage to the colonic wall during polypectomy when monopolar snares are used, special attention should be paid to avoid touching the intact colonic wall with any part of the snare or polyp (Fig. 126.7). Most endoscopists recommend lifting the polyp into the colonic lumen with the snare and gently shaking the polyp while the electrosurgical unit is activated to dissipate the electrical current at sites of inadvertent contact with the colonic wall and to decrease the possibility of prolonged unwanted electrical contact. Electrical current during polypectomy should be applied continuously, as intermittent application allows cooling of the snare and may seal it to the tissue ("stuck snare") [1].

Even large pedunculated polyps can be removed endoscopically with a low rate of complications [110,168]. The risk of perforation during removal of pedunculated polyps is lower compared with removal of sessile lesions. Cold snare polypectomy is not recommended in polyps with a sizable stalk: application of electrical energy should precede complete closure of the snare to allow adequate coagulation of blood vessels inside the stalk [169]. Many authors recommend saline and epinephrine injection into the stalk or application of detachable loops to prevent postpolypectomy bleeding from polyps with a large stalk [59,170].

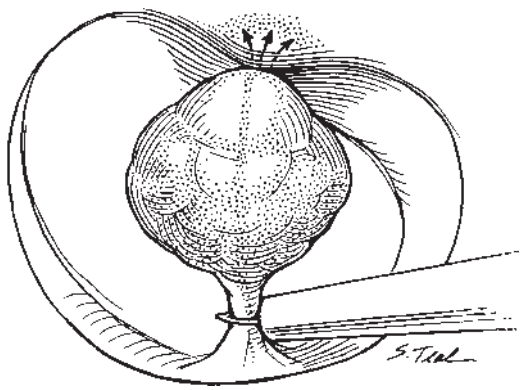

Figure 126.7 Current dissipation through the contralateral colonic wall during polypectomy. The electrosurgical snare is tightened around the stalk of a pedunculated polyp. The polyp head is touching the contralateral wall of the colon. Electrical current can spread through this unwanted contact and may cause thermal injury to the contralateral wall (arrows). This injury to the colonic wall can be easily avoided by moving the polyp head away from the opposite colonic wall during application of electrocautery.

Ideally all resected polyps should be sent for pathological examination [11,171]. In reality, some of the resected polyps are lost, but more than 95% of polyps can be retrieved using suction and special retrieval instruments [172,173]. J.D. Waye, a world-renowned polypectomy expert, has a very practical recommendation: "Remove small polyps in any position on the way in, remove medium size polyps (up to 1 cm) if they are in good position during intubation, and do not remove large polyps during instrument insertion, because most of the time they are readily seen upon withdrawal" [11]. Suction through the working channel of the endoscope allows transfer of small polyps and pieces of large polyp to a trap connected to the colonoscope [173]. Various constructions of trap devices are currently available [174]. Traps should be prepared in advance. Larger polyps can be regrasped for removal by endoscopic snare, graspers with multiple prongs, or specially designed retrieval nets and instruments combining a snare and a removal basket [175,176].

Sessile polyps

Sessile polyps with a base larger than 5 mm in diameter are usually removed with hot snare polypectomy or EMR. Polyps larger than 20 mm in diameter can present a significant challenge for endoscopic therapy, especially those located in the right colon [110]. Endoscopic resection of large polyps can be done en bloc or piecemeal according to the size and location of the lesion [110]. The piecemeal technique is used most frequently, but is associated with a higher risk of recurrence [177].

The inject-and-cut EMR technique is most commonly used for piecemeal removal of sessile colonic lesions. After the submucosal injection, the snare is placed around the polyp and gently pressed against the mucosa. Excess air is aspirated to decrease colonic distention and facilitate grasping of the polyp [110]. If the polyp is very flat and regular and the snare cannot grasp it, use of barbed snares or snares with spikes or a needle on the tip can prevent slippage [1,16]. A circumferential mucosal cut around the polyp with the tip of the polypectomy snare or a needle-knife can create a groove that allows the snare to grasp the polyp [178]. After the polyp is resected the colon should be redistended with air to visualize any residual polypoid tissue, which is then removed in similar fashion until all polypoid tissue is resected and the muscularis propria is visualized [179]. If EMR is technically difficult due to the unfavorable position of the polyp (behind a fold or colonic turn), colonoscopic polypectomy in retroflexion can facilitate the procedure [166,180].

A high adenoma recurrence rate (up to 46%) has been documented after piecemeal removal of large colonic polyps

even without any visible adenoma remnants after EMR [181]. When endoscopically visible remnants of adenoma remain after EMR, APC has been used to reduce the frequency of residual adenoma by 50% [177]. Brooker and colleagues randomly assigned patients with large sessile colonic polyps to either EMR or EMR followed by application of APC to the rim and any residual mucosal or submucosal tissue in the base of the polypectomy site [177]. They observed statistically significant reduction of adenoma recurrence (1 of 10) in patients treated with both EMR and APC compared with those who had only EMR (recurrence in 7 of 11 patients; $P = 0.02$).

All resected tissue should be collected and sent for pathological examination. If malignancy with submucosal infiltration is detected in the resection specimens, surgery should be recommended [110]. It is estimated that about 10% of submucosally invasive cancers have metastasized to lymph nodes and endoscopic resection alone is not adequate for tumors invading the deep submucosal layers of the colon [182].

Endoscopic treatment of colorectal cancers

Flat adenomas, rectal carcinoids, adenomas with high-grade dysplasia, and early-stage colon cancers can be successfully removed by EMR [183–186]. Invasive carcinoma is found in 2%–4% of colonic polyps removed endoscopically [186]. The growth pattern of colorectal tumors can be divided into three main types: protruding (exophytically) growing, depressed (endophytically) growing, and laterally spreading lesions [185,187]. Laterally spreading tumors are further subdivided into a granular (homogeneous) type, a nodular (mixed) type, and a nongranular type [187]. Laterally spreading and depressed lesions are more difficult to recognize endoscopically and these tumors have malignant potential up to 10 times greater than polypoid adenomas [188–191].

Several techniques have been introduced into clinical practice to improve visualization of the flat colonic lesions. Magnification endoscopy, topical spraying of the lesion with 0.4% indigo carmine or 0.05% crystal violet, and use of high-frequency endoscopic ultrasound probes (20–30 MHz) allow adequate identification of lateral margins of the tumor and depth of invasion into the colonic wall [187,192–194]. Accurate determination of invasion depth is crucial when making a decision about possible endoscopic removal of the lesion. Lesions with deeper submucosal invasion should not be treated with EMR due to a higher risk of lymph node metastases [20,195]. After analysis of 511 colorectal laterally spreading tumors, Uraoka and colleagues recommended endoscopic piecemeal resection (with the large nodule resected first) for the granular type of laterally spreading tumors and en bloc resection of the nongranular type of laterally spreading tumors [195].

Polypectomy or EMR with curative intent is feasible in carefully selected patients with only superficially invasive colon cancers. The rate of lymph node metastases is directly proportional to the depth of tumor invasion into the submucosa. Colorectal tumors invading the upper third, middle third, and lower third of the submucosa have 2%, 9%, and 35% rates of lymph node metastases, respectively [196]. Several factors are associated with a high rate of lymph node metastasis and local cancer recurrence after endoscopic resection of malignant polyps confined to the submucosa: poorly differentiated histology, vascular or lymphatic invasion, and incomplete resection with positive resection margins [186,196–198].

There is a consensus in the literature that, after endoscopic removal of large premalignant and early malignant colorectal tumors, patients should have endoscopic surveillance but the recommendations about the optimal follow-up differ from publication to publication. Some authors recommend follow-up endoscopic evaluation at 3 months post endoscopic removal with subsequent endoscopies at 1, 3, and 5 or more years if no residual or recurrent polyps are detected [187]. Other experts advocate follow-up colonoscopy at 3, 6, and 12 months and then yearly [110]. This schedule was able to detect early local recurrence of a polyp in 21.9% of resected polyps (which were then easily treated with APC) [110].

In patients with large sessile tumors, especially if piecemeal removal was performed or if the endoscopist is unsure about the completeness of resection, repeat colonoscopy should be performed within 3–6 months to rule out residual neoplastic tissue at the polypectomy site [186]. If residual tissue is again found and removed during repeat colonoscopy, then a follow-up examination should be performed again within another 3–6 months to verify complete resection of the lesion [186]. If the polyps cannot be removed completely within one to three examinations, surgery should be recommended [199,200].

If curative endoscopic or surgical resection is not possible, tumor palliation with ablative techniques (laser, APC, photodynamic therapy) or restoration of lumenal patency with endoscopic stents can be recommended [201–209].

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A ENDOSCOPIC

127 Laparoscopy and laparotomy

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Laparoscopy and laparotomy are surgical techniques that provide direct access to the abdominopelvic cavity for diagnosis and therapy. The word "laparoscopy" was compounded from two Greek roots, *lapara* (meaning the soft parts of the body between ribcage and hips) and *skopein* (*scope* in English, meaning to see or examine). Similarly, laparotomy was compounded from the Greek roots *lapara* and *tome* (meaning to cut). Since most gastrointestinal (GI) organs reside in the abdominopelvic cavity, laparotomy has traditionally been the gold standard in diagnosing and treating GI conditions. However, the dominance of laparotomy has been challenged by laparoscopy. Nowadays, laparoscopy has replaced laparotomy as the standard approach for gallbladder surgery, antireflux surgery, and weight-loss surgery. In colon surgery and hernia surgery, laparoscopic approaches are rapidly gaining popularity. In this chapter, we trace the history of laparoscopy and laparotomy. We also describe the techniques of laparoscopy and laparotomy as they are applied in modern GI surgical practice. We end by projecting future developments of laparoscopy and laparotomy.

Laparoscopy

History

The history of endo-laparoscopy dates back to the Greco-Roman period, when Hippocrates used an anoscope to diagnose anorectal diseases. Over the next 2000 years, however, very little progress was made in this technique because of inability to provide adequate illumination. The invention of incandescent light bulbs and the three-lens optical system facilitated development of rigid endoscopes in the late 19th century. These were first introduced through natural orifices to examine the bladder, esophagus, and rectum and subsequently the peritoneal cavity. In the abdominopelvic cavity, controlled carbon dioxide insufflation was subsequently added to create a pneumoperitoneum, which improved visualization and working space. Laparoscopic surgery first became popular in gynecological surgery. In 1990s, the development of videoendoscopes allowed images to be projected on video screens in real time. For the first time, a laparoscopic surgeon and his assistant could see the same image and work as a team with both hands free to manipulate surgical instruments. In 1987, Mouret in France reported the first laparoscopic cholecystectomy. Since then, the number of GI operations performed laparoscopically has increased exponentially.

Techniques

Equipment

The practice of modern laparoscopic surgery requires special equipment. A rod-like laparoscope combined with a charge-coupled device (CCD) video camera acquires images that are displayed on one or more video monitors. High-intensity light is transmitted through the laparoscope to illuminate the peritoneal cavity. Carbon dioxide is used to insufflate the abdomen and an insufflator maintains a pneumoperitoneum at a set pressure (usually 10–15 mmHg). Trocars (or ports) are hollow plastic tubes that allow instruments to be repeatedly inserted into and withdrawn from the abdominal cavity without releasing the pneumoperitoneum. A side port on the trocars also allows carbon dioxide to be pumped continuously into the abdomen. Laparoscopic instruments are thin and long to allow for access into the abdomen through the trocars. Laparoscopic stapling devices have been developed for intracorporeal tissue division and approximation. Electrocautery, ultrasonic shears, and the bipolar cautery are energy sources used for tissue dissection and hemostasis. Other instruments specially made for laparoscopy include intracorporeal suturing devices, preformed knots, tacking devices, specimen removal bags, and laparoscopic ultrasound probes. While some of these devices have been adapted from their open surgical counterparts, others have been engineered to overcome the limitations of working through small ports. Their development has been a key factor in the widespread adoption of laparoscopic surgery.

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Access to the peritoneum

The Veress needle is the most commonly used device for initially accessing the peritoneal cavity. The Veress needle is a large-bore hollow needle with a retractable, spring-loaded inner stylet. When pushed against the abdominal wall, the blunt-tipped stylet retracts, allowing the sharp needle to engage the layers of anterior abdominal wall. Once inside the abdominal cavity, the stylet springs out to protect viscera from the sharp needle sheath. After a saline drop test to confirm the needle tip is in a free space, carbon dioxide is infused into the peritoneal cavity through the Veress needle. Once a pneumoperitoneum is established, a trocar can then be placed without risk of injuring internal organs. Although it is theoretically safe, the Veress needle is a "blind" technique: the surgeon has no knowledge of the precise location of the tip and inserts the needle unsighted. Reports of visceral and vascular injuries resulting from Veress needle placement led to the development of an alternative technique. The so-called open technique is in fact a mini-laparotomy during which the surgeon incises the skin, fascia, and peritoneum under direct vision. A blunt trocar is then inserted and secured to the fascial edge and pneumoperitoneum is established through the trocar. A metaanalysis concluded that the Veress needle technique results in a higher incidence of vascular injuries (1 in 1333) than the open technique, while both techniques result in similar incidences of visceral injuries [1]. The choice of access technique is therefore left up to the surgeon, who must exercise utmost vigilance in entering the abdomen to avoid troublesome, and at times devastating, complications. Newer "optical" trocars with transparent tips have become available. By inserting the laparoscope into the trocar, the surgeon can watch as the trocar traverses the anterior abdominal wall layer by layer. The widespread use of these newer devices may help further reduce the incidence of access-related injuries in laparoscopy.

Exploration

After gaining initial access to the abdomen and establishing pneumoperitoneum, additional trocars are placed under laparoscopic vision. The positioning of these additional trocars is critical to the ease or even success of the subsequent operation. It is ideal to place the working trocars in front, and to either side, of the camera port, much like the diamond design of the baseball field with the camera port at home base and the target organ at second base (Fig. 127.1). There should be a minimal distance of 8–10 cm between trocars to avoid collisions between instruments. It is advisable to avoid placing trocars low in the abdomen since the bony pelvis and thighs may limit the movement of instruments. The use of an angled laparoscope such as a 30° laparoscope aids in visualization of the entire abdomen. Gravity is used to help with exposure, especially when retraction is limited. When viewing the upper abdomen, the operating table should be placed in reverse Trendelenburg (head-up) position so that omentum, small bowel, and transverse colon will fall caudally, exposing the stomach, liver and spleen. Conversely, the operating table needs to be in Trendelenburg (head-down) position when viewing the pelvis. The operating table can also roll sideways to expose structures in the flank better (e.g., right and left colon). Because of the frequent position changes, patients need to be well secured to the operating table with multiple straps, wide tapes, and a footboard.

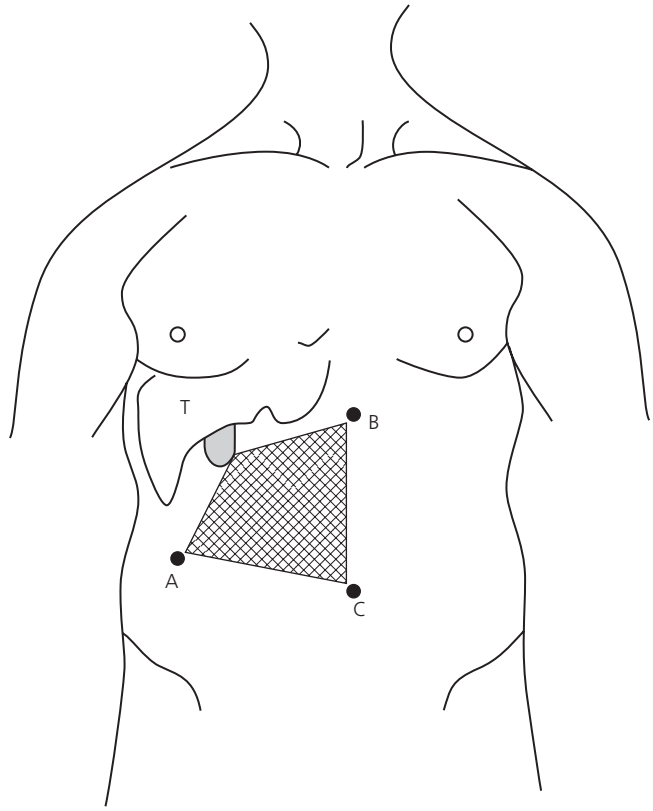

Figure 127.1 Optimal trocar placement in laparoscopic surgery: T, target organ, in this case the gallbladder; C, camera port; A and B, ports for laparoscopic instruments.

Specimen extraction

Small specimens such as the gallbladder or appendix may be extracted directly through large trocar sites. Alternatively, they may be placed in a plastic bag before extraction to prevent contamination of the wound. Larger specimens such as the spleen require morcellation in order to remove them through a small incision. Morcellation can be accomplished either manually with a pair of long clamps or mechanically with a specially designed morcellator. In bowel surgery, after mobilizing a segment of small bowel or colon intracorporeally and dividing the mesentery, most surgeons exteriorize the bowel by enlarging one of the trocar incisions. An impermeable wound protector should be used in all cancer cases to prevent tumor implantation. Specimen extraction and

anastomosis are then carried out before the bowel is returned to the abdomen.

Closure

At the completion of a laparoscopic procedure, the surgeon must ensure that all foreign materials (including tapes, drains, vessel loops, and gauze) have been removed from the abdominal cavity. The trocars are then slowly withdrawn under laparoscopic vision. Bleeding may be encountered on withdrawal of the trocars, particularly when they are placed in the vicinity of epigastric vessels. If bleeding is detected, electrocautery may be used to stop it, or a transfascial figure-of-eight suture may be placed to ligate the bleeding vessel. Routine closure of all trocar sites larger than 5 mm should be performed to prevent trocar site hernias. It is important to ensure that the pneumoperitoneum is completely released before closing the trocar sites since retained carbon dioxide may cause postoperative diaphragmatic irritation. It is also important to avoid trapping the omentum or small bowel as the trocar closure sutures are tied. The skin and fascia at the trocar sites should be injected with long-acting local anesthetics to reduce postoperative pain.

Therapeutic laparoscopy

Laparoscopic cholecystectomy and common bile duct exploration

Since the initial introduction of laparoscopic cholecystectomy to the surgical community in 1991 [2], it has rapidly emerged as the procedure of choice in treating gallbladder diseases [3]. Nowadays, in the USA over 95% of cholecystectomies are performed laparoscopically. The indications have expanded from symptomatic cholelithiasis and chronic cholecystitis to include gallstone pancreatitis and acute cholecystitis.

The American technique of laparoscopic cholecystectomy uses four trocars, one at the umbilicus, one in the epigastrium, and two in the right upper quadrant. After incising any adhesions or omental attachments to the gallbladder, the gallbladder fundus is grasped and retracted cephalad, thereby elevating the right lobe of the liver and exposing the porta hepatis. The dissection begins in the triangle of Calot. With lateral retraction of the infundibulum of the gallbladder, the cystic duct is put on stretch at a right angle to the common bile duct (CBD) and dissected circumferentially at the cystic duct–infundibulum junction. The cystic duct can be partially transected at this point for the insertion of a cholangiogram catheter or it can be divided between clips. The cystic artery is divided between clips. The gallbladder is then detached from its liver bed in a retrograde direction and extracted in a plastic bag.

Postoperatively, patients are discharged on the day of surgery or the next morning. Major complications include bleeding, bile leak, biliary obstruction, and pancreatitis. Bile leaks result from cystic stump leak, unrecognized injury to major bile duct, or injury to a subcholecystic duct. Biliary obstruction may be caused by either retained CBD stones or iatrogenic clipping of the CBD.

Patients with postoperative severe right upper quadrant pain, fever, or hyperbilirubinemia should be suspected of having a bile duct injury. They should be promptly evaluated radiographically. Computed tomography (CT) may demonstrate intraabdominal fluid collections (bile leak) and biliary dilatation (biliary obstruction). Percutaneous drainage should be pursued to treat symptomatic biloma, followed by endoscopic retrograde cholangiopancreatography (ERCP) to delineate the anatomy of the biliary tree and identify the site of leak. A sphincterotomy with or without stent placement usually decreases the volume of the leak if injury is to the cystic duct stump or distal bile duct. Most small leaks will seal with this approach and can be managed without a reoperation. Patients who develop postoperative biliary obstruction should be suspected of having CBD injury. In patients with dilated bile ducts, percutaneous transhepatic cholangiograms should be performed urgently to assess the bile duct anatomy and the level of injury. If an injury is identified, the cholangiogram catheter should be externalized to provide temporary drainage. Patients diagnosed of such injury should be promptly referred to specialized tertiary centers for biliary reconstruction. Results for biliary reconstruction following injury are superior at high-volume centers compared with community practices [4].

When the technique of laparoscopic cholecystectomy was first introduced, CBD injuries occurred at a much higher rate than seen previously with open cholecystectomies. Since then, the incidence of CBD injury has decreased and now occurs in approximately 4 in 1000 cases. Nevertheless, such an injury is devastating to both the patient and the surgeon involved. Way and colleagues analyzed 252 cases of such complications and concluded that 97% were caused by a visual perceptual illusion; technical error accounted for only 3% of the cases [5]. Therefore, it is crucial to obtain what surgeons call the *critical view* prior to dividing the cystic duct. The key to achieving the critical view is proper cephalad–lateral retraction of the gallbladder to open up the angle between cystic duct and CBD. It is especially important in cases where the gallbladder is acutely inflamed and the cystic duct appears foreshortened. Some surgeons routinely perform intraoperative cholangiogram (IOC) during laparoscopic cholecystectomy, arguing that it prevents bile duct injury, while others perform IOC on a selective basis. The clinical indications for IOC include gallstone pancreatitis, elevated liver enzymes, or the presence of CBD stones on imaging studies. A metaanalysis by Metcalfe and colleagues did not support the routine nonselective use of IOC. They noted that a policy of routine use of cholangiogram did not significantly decrease the incidence of bile duct transection. It did increase the detection rate of CBD stones, but only 15% of those CBD stones detected would go on to cause symptoms [6]. A positive IOC should be

followed up with either operative exploration of the CBD (see below) or a postoperative ERCP to interrogate and clear the duct.

The most straightforward, least invasive method of bile duct exploration for a patient already in the operating room is laparoscopic transcystic CBD exploration. Under laparoscopic vision, the cholangiogram cannula is exchanged for a guidewire. A balloon over the wire is then used to dilate the cystic duct to a size sufficient to accommodate a choledochoscope. A choledochoscope is then advanced over the guidewire transcystically into the CBD. Once a CBD stone is visualized, a wire basket is deployed via the working channel of the choledochoscope and the stone is engaged. The stone is then extracted under direct vision along with the choledochoscope. The process is repeated until all the CBD stones have been removed and the duodenum is reached. Usually a completion cholangiogram is obtained to document clearance of the CBD. If a CBD stone is too large to be extracted through the cystic duct, the CBD can also be dissected out laparoscopically and accessed through a longitudinal choledochotomy. The stones can then be retrieved as described above, with the choledochotomy closed over a T tube at the end.

Compared with postoperative ERCP, laparoscopic cholecystectomy with loparoscopic common duct exploration spares patients a second procedure. Operative clearance of the CBD is also associated with a lower incidence of pancreatitis than ERCP. Some clinicians are concerned that long-term duodenobiliary reflux and bacteriobilia can eventually lead to biliary neoplasia [7,8]. Therefore, a good case can be made to preserve the biliary sphincter especially in young patients with gallstone disease. Laparoscopic CBD clearance negates the need for a sphincterotomy.

Laparoscopic Nissen fundoplication

Gastroesophageal reflux disease (GERD) is the most common chronic condition of the GI tract. While most patients respond well to acid suppressive therapy, some are either intolerant of medications or do not desire to stay on lifelong medication. In these patients surgical antireflux procedures provide excellent symptom control. The current surgical antireflux procedure of choice is laparoscopic Nissen fundoplication (LNF). The efficacy of Nissen fundoplication was first validated against standard medical therapy in 1992 [9]. Most recently in a randomized trial against proton-pump inhibitors (PPIs), LNF led to significantly less acid exposure of the lower esophagus at 3 months and significantly greater improvement in quality of life as measured by both condition-specific and general well-being instruments after 12 months [10].

Patient selection is paramount to the success of LNF. Esophagogastroduodenoscopy is essential to assess the presence of erosive esophagitis and Barrett esophagus, and to rule out a stricture or malignancy. Before recommending surgery, there must be proof that the patient's symptoms are caused by acid reflux. Patients who respond to PPIs and have endoscopic evidence of esophagitis are good candidates for LNF. However, in patients with nonerosive disease or atypical symptoms, a 24-hour pH study is the gold standard for confirming the diagnosis of GERD and should be routinely performed. Esophageal manometry should be routinely utilized to exclude primary esophageal motility disorders such as achalasia and diffuse esophageal spasm. Many surgeons find an upper GI series to be useful as it locates the gastroesophageal junction and alerts the surgeon to the presence of a paraesophageal hernia or short esophagus. In patients with chronic GERD, an esophagus shortened by chronic inflammation may require a concomitant lengthening procedure such as a Collis gastroplasty.

LNF uses five trocars with one placed supraumbilically for the camera, and the other four placed in a semicircle just beneath the costal arch. The left lobe of the liver is elevated to expose the gastroesophageal junction. The right and left crus are completely dissected away from the esophagus and both vagi are identified and preserved. The esophageal hiatus is reinforced from posterior to anterior with either permanent sutures or a prosthetic mesh to restore it to a normal diameter. This crural closure step prevents the fundoplication from herniating into the mediastinum. It also causes cephalad transposition of the esophageal hiatus, which lengthens the intraabdominal segment of the esophagus. The short gastric vessels are divided to allow the gastric fundus to be passed posterior to the esophagus toward the lesser curvature. A short plication of 2 cm is then constructed over a 56Fr Maloney bougie passed down the esophagus orally to construct a loose wrap.

Postoperatively, patients are observed overnight and discharged home the following day if they can tolerate liquids. They are maintained on a liquid diet for 1 week to allow edema at the gastroesophageal junction to subside before they are advanced to an unrestricted diet. Intraoperative complications include bleeding (which may necessitate conversion to open surgery if visualization is impaired), esophageal injury, vagal nerve injury, and pneumothorax. Postoperative complications include dysphagia due to perioperative hematoma or edema or an improperly constructed tight wrap. Fundoplication failure is associated with obesity, Barrett esophagus, and surgeon inexperience. The most common anatomical cause of failure is wrap herniation through the crural closure. A slipped Nissen is an intussusception of the body of the stomach through the wrap that leads to a two-compartment stomach. A slipped Nissen can present with symptoms of both dysphagia and heartburn. Failed LNF can be redone laparoscopically, but should be attempted by experienced laparoscopists since repeat operations are quite challenging and the risk of vagal nerve injury and esophageal injury is substantial.

The results of LNF are excellent as reported in several large series [11–14]. Early clinical studies document successful

relief of reflux symptoms in nearly 90% of patients [15]. At 5 years after the operation, 12% of patients experienced recurrence of reflux-type symptoms, but only 3% of patients required repeat surgery [16]. A recent study from England showed that LNF becomes cost saving after 8 years compared with maintenance PPI therapy for the treatment of GERD [17].

With long-standing GERD, about 10% of patients will develop Barrett esophagus or intestinal metaplasia of the distal esophagus. Without reduction in acid exposure, Barrett esophagus may progress to dysplasia and eventually adenocarcinoma of the esophagus. Therefore, the presence of Barrett esophagus, especially the presence of low-grade dysplasia, mandates effective antacid reflux treatment [18]. For patients already on maximum medical therapy but who still demonstrate pathological acid reflux, LNF should be performed. Many believe that bile or nonacid reflux is an essential element in the development and progression of Barrett esophagus, but thus far there is no convincing proof that antireflux surgery is more effective than adequate medical therapy in preventing the development of esophageal carcinoma.

Other commonly performed laparoscopic foregut procedures include laparoscopic Heller myotomy for achalasia and laparoscopic repair of paraesophageal hernias. These techniques are similar to LNF with almost identical trocar placements and dissection at the esophageal hiatus. Laparoscopic foregut surgery affords patients less operative trauma and faster, easier postoperative recovery compared with either a thoracotomy or a large upper midline laparotomy incision.

Laparoscopic colorectal surgery

Laparoscopic colon resections are performed for both benign and malignant conditions. The most common benign conditions are diverticular disease, ulcerative colitis, and large colonoscopically unresectable adenomatous polyps. Several large randomized controlled trials have demonstrated the safety and efficacy of laparoscopic colorectal surgery (LCRS) for cancer. One of them, the Clinical Outcomes of Surgical Therapy Study Group (COST) trial, showed that LCRS is both surgically and oncologically sound, as measured against the benchmark of traditional open surgery [19]. This report galvanized a new wave of interest in LCRS for the treatment of benign and malignant colorectal diseases alike.

The technique of laparoscopic right colectomy uses three or four trocars. It begins with mobilization of the cecum and right colon from its lateral attachments. The hepatic flexure is then mobilized. The ileocolic vascular pedicle is then identified and divided intracorporeally with a vascular stapler. The right colon is externalized through a small incision made by enlarging one of the trocar sites. Bowel resection and ileotransverse colon anastomosis is carried out extracorporeally before the bowel is returned to the abdomen.

Laparoscopic left colectomy is usually performed through three or four trocars placed in the midline and right lower quadrant of the abdomen. It also begins with mobilization of the left colon at the white line of Toldt (lateral peritoneal attachment). The splenic flexure is then taken down with the spleen carefully preserved. Pelvic dissection then begins with identification and preservation of the left ureter. The presacral plane is entered by incising the peritoneum from both sides. For cancer operations, the presacral plane is followed down toward the pelvic floor and levator muscles so the mesorectum can be resected en bloc. Once a distal margin is selected, the rectal wall is cleared circumferentially and transected with a stapler. The colon is then exteriorized through a small incision where resection is carried out. The anvil of an EEA (circular) stapler is secured to the proximal colon before it is returned to the abdomen. The EEA stapler is then advanced transanally and coupled with the anvil intraperitoneally. An end-to-end colorectal anastomosis is then created by firing the EEA stapler.

Over the years multiple studies have compared LCRS with open surgery. Intraoperatively, laparoscopy takes longer but loses less blood; postoperatively, LCRS patients require less narcotics, have faster return of bowel function, and shorter length of stay [20]. Studies have also shown that LCRS causes less of a systemic inflammatory response than open surgery [21]. Over long-term follow-up, LCRS reduces the incidence of incisional hernias and adhesive small bowel obstruction compared with open surgery [22].

LCRS has also been evaluated in special patient groups. It has been found to be safe and effective for elderly patients over 65 years old, resulting in shorter hospitalization and less complication [23]. Obese patients with body mass index (BMI) over 30 may also undergo LCRS, with shorter postoperative recovery than open surgery [24]. Nevertheless, LCRS is a difficult procedure that requires specialized training to perform. Tekkis and colleagues demonstrated a fairly lengthy learning curve for the procedure [25]. They found that surgeons reduced their operative time, conversion rate, and complication rate significantly only after 55 cases of laparoscopic right colectomies and 62 cases of laparoscopic left colectomies. Based on this and other studies with similar findings it is recommended that novice surgeons first successfully complete 20–30 cases of LCRS for benign indications such as diverticular disease before performing LCRS for colorectal cancer.

In experienced hands, LCRS for colon cancer is oncologically sound. The best evidence comes from the COST trial in which 872 patients from 48 institutions with colon cancer participated. At 3 years, the primary outcome (rate of recurrence) was similar in the laparoscopic and open groups. Recurrence rate in surgical wounds and trocar sites was similar, less than 1% in both groups, alleviating the concern for so-called port-site metastasis. Overall survival, intraoperative complication rate, 30-day surgical mortality, postoperative complication rate, readmission rate, and reoperation rate were also not significantly different between the two groups [19]. Another randomized controlled trial conducted in

Barcelona actually found better cancer-related mortality in the laparoscopic group compared with the open group [26]. This difference was due to improvement in survival of patients with stage III tumors. The long-term results of another large-scale European trial, the COLOR trial (Hazelbrook EJ, Color Study Group. COLOR: a randomized clinical brial comparing laparoscopic and open resection for colon cancer. Surg Endosc 2002;16:949).

Laparoscopic weight loss (bariatric) surgery

About two-thirds of Americans are overweight, and of these almost half are obese (BMI > 30). Obesity can both cause and exacerbate preexisting conditions such as diabetes, high blood pressure, hyperlipidemia, heart disease, obstructive sleep apnea, arthritis, and acid reflux disease. These comorbidities are responsible for over 2.5 million deaths per year worldwide. It does not come as a surprise that a morbidly obese person has a life expectancy 12 years shorter than a normal weight individual [27]. Unfortunately, dietary and medical treatments for moderate to morbid obesity do not have long-lasting effect. In 1991, the National Institutes of Health (NIH) acknowledged surgery as an accepted treatment modality for morbid obesity. The guidelines stated that patients with a BMI over 40 (or BMI of 35 with comorbidities) should be considered for weight loss (bariatric) surgery [28]. As a consequence, the volume of bariatric surgery has increased steadily since 1991. Nowadays, two types of weight-loss operations are performed regularly in the USA. The Roux-en-Y gastric bypass, which has a long track record, has remained the gold standard of bariatric surgery. However, the original technique has been modified and adapted into a laparoscopic version with great success. The laparoscopic adjustable gastric banding procedure, popular in Europe for many years, has now been introduced into the USA. Compared with gastric bypass, it is much easier to perform but the rate of weight loss is slower.

The laparoscopic Roux-en-Y gastric bypass is a technically demanding procedure and has quite a steep learning curve for the surgeon. The first step is to create a 30-mL gastric pouch by dividing the stomach between the lesser curvature and the angle of His. Following that, the small bowel is divided 60–100 cm distal to the ligament of Treitz. An enteroenterostomy is created 100–150 cm further distally, hence reestablishing bowel continuity. The Roux limb is then brought to the upper abdomen. A gastrojejunostomy is then constructed between the small gastric pouch and the Roux limb by hand sewing, linear stapling, or EEA stapling techniques. The gastrojejunostomy is then tested for leaks by upper endoscopy, and all the mesenteric defects are closed to prevent future internal hernia and intestinal obstruction. By creating a long Roux limb, this procedure allows for weight loss through both restrictive (i.e., small gastric pouch) and malabsorptive means (Fig. 127.2).

Postoperatively, patients typically stay in the hospital for 2–3 days. They are allowed scheduled advances of diet after a Gastrografin swallow study on the first postoperative day confirms the integrity of the gastrojejunostomy. The morbidly obese patient has extremely limited cardiopulmonary reserve and usually decompensates rather quickly. Major complications, such as leaks from suture lines, intestinal obstruction, gastric remnant distention and postoperative bleeding, are not well tolerated. Therefore, exceptional vigilance during the initial postoperative course is warranted. Every sign or symptom of possible complication such as tachycardia, tachypnea, fever, or excess pain should be thoroughly investigated with imaging studies (Gastrografin swallow, CT, chest X-ray). Reexploration via laparoscopy or laparotomy may ultimately be required for diagnostic and

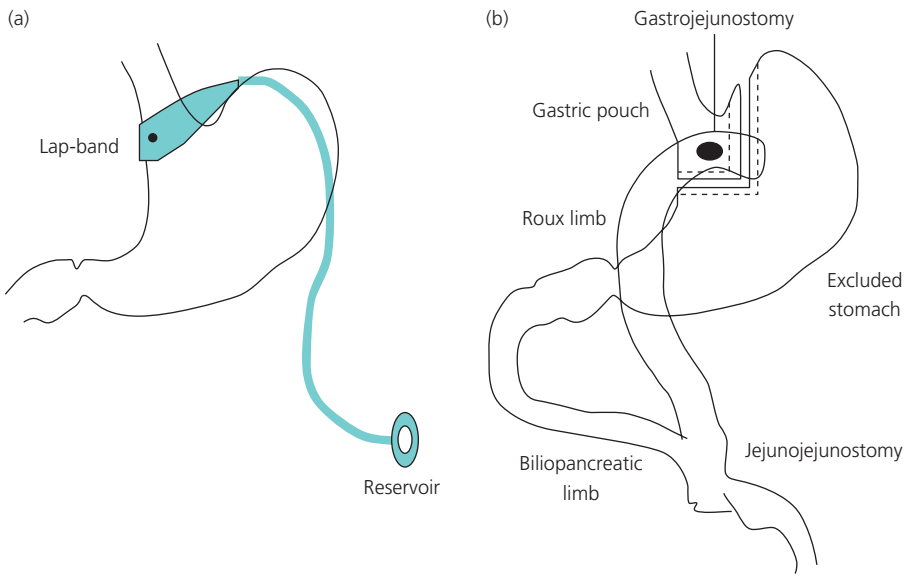

Figure 127.2 Weight loss surgery: **(a)** laparoscopic adjustable gastric band; **(b)** Roux-en-Y gastric bypass.

therapeutic purposes when there are persistent clinical signs and symptoms despite negative radiographic studies.

The 30-day mortality rate for gastric bypass surgery is quite low (0.5%–1.0%). Overall complication rates are similar between laparoscopic and open methods. In general, laparoscopic gastric bypass has a slightly higher leak rate at the gastrojejunostomy. Open gastric bypass has a much higher abdominal hernia rate (15% vs 1%), while laparoscopic gastric bypass has a higher internal hernia or bowel obstruction rate (5% vs 1%). Wound infection rate is also higher in open gastric bypass. The length of stay is shorter for laparoscopic gastric bypass by 1–2 days [29].

In 2004, Buchwald and colleagues performed a systematic review and metaanalysis of bariatric surgery that included a total of 22 094 patients [30]. The average excess weight loss was 61.2% for all patients, 47.5% for gastric banding patients, and 61.6% for gastric bypass patients. The 30-day mortality was 0.1% for gastric banding procedure and 0.5% for gastric bypass. Diabetes completely resolved in 76.8% of patients and improved in 10% of patients. Interestingly, the changes in insulin resistance seen after gastric bypass, which are responsible for the resolution or improvement of diabetes, occur within 6 days of surgery, before any appreciable weight loss has occurred [31]. Hyperlipidemia improved in 70% of patients. Hypertension resolved in 62% of patients and improved in another 17% of patients. Obstructive sleep apnea resolved in 86% of patients. In addition, patients surviving at least 1 year following the procedure had a 33% reduction in actuarial death rate [32]. The results speak for the safety and efficacy of bariatric surgery.

A Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) position paper advocated laparoscopic gastric bypass based on its improved short-term recovery [33]. Nevertheless, the learning curve is formidable so only surgeons skilled in advanced laparoscopy and bariatric surgery should be performing it. As an example, Shikora and colleagues reported that the morbidity and mortality of their patients were reduced by 50% after the first 100 cases of laparoscopic gastric bypass. Specifically, mortality rate fell from 1% to 0.3%, leak rate from 3% to 1.1%, small bowel obstruction from 5% to 1.1%, overall complication rate from 26% to 13%, and operative time from 212 min to 132 min [34].

As surgeons gain more experience with this procedure, laparoscopic gastric bypass can be offered to super-obese patients with BMI over 50 and 60 with good results [35]. This procedure is also offered to increasingly older patients (> 60 years) and to those with major comorbidities (diabetes, renal failure, post transplant).

Laparoscopy in the management of common surgical emergencies

Appendectomy

Appendectomy is one of the most commonly performed emergency surgical procedures. The standard technique of laparoscopic appendectomy (LA) uses three trocars, one at the umbilicus, one suprapubically, and another in either the left lower quadrant or right upper quadrant. Once the appendix is identified laparoscopically, it is retracted anteriorly revealing the cecal base. A window between the base of the appendix and the mesoappendix is created bluntly. The appendix and its mesentery are transected with separate firing of the staplers or divided between clips/endo-loops. The appendix is retrieved through the trocar if uninflamed or in a plastic bag if engorged.

A recent metaanalysis of 45 studies showed that LA carried a lower rate of wound infection (odds ratio 0.47) but a higher intraabdominal abscess rate (odds ratio 2.77) than traditional open appendectomy. Based on these data, the authors cautioned against using laparoscopy to treat complicated (i.e., gangrenous or perforated) appendicitis. The other differences between the two techniques are much more subtle. The operative time of LA is longer, but only by 14 min. Hospital stay after LA is shorter, but only by 0.7 days. Patients also have less pain after LA and return to normal activity, work and exercise earlier by 6, 3, and 7 days, respectively [36].

Another advantage of LA is that any intraabdominal organ can be inspected at the time of surgery. This feature is particularly helpful in young female patients in whom gynecological conditions such as ruptured ovarian cyst, tuboovarian abscess or pelvic inflammatory disease could be misdiagnosed as appendicitis.

Peptic ulcer disease

With the advent of effective acid suppressive medication and antibiotic eradication of *Helicobacter pylori*, peptic ulcer disease rarely requires surgical intervention. An expedient laparotomy with either Graham patching of a perforation or oversewing of a bleeding vessel is still a life-saving operation for a critically ill, unstable patient. For patients who present with signs and symptoms of perforated gastric or duodenal ulcer, and who are hemodynamically stable, laparoscopic exploration and closure can be performed.

As in the open operation, the site of perforation is identified first, usually on the anterior wall of the duodenum. A healthy, soft, well-vascularized omental pedicle is mobilized to cover the defect and secured with sutures. The abdomen is then thoroughly irrigated laparoscopically and the patient treated with PPIs as well as an antibiotic cocktail for *H. pylori*. Patients with perforated gastric ulcers should have the ulcer excised and a wedge excision of the surrounding stomach performed to rule out cancer.

Laparoscopic treatment of peptic ulcer disease has been more commonly performed in Europe and Asia than the USA. Series from Hong Kong, India, Russia, and France report success rates ranging from 79% to 86%. The reported complication rate ranges from 6% to 16%, with less than 2% of patients requiring reexploration. Both operative time and postoperative recovery compare favorably with control open series [37–40].

Obstructive disease of the small bowel

Although obstructive disease of the small bowel is a relatively common occurrence in most surgical wards, laparoscopic treatment is quite difficult. Franklin and colleagues reported a series of 167 patients who were treated for intestinal obstruction with laparoscopy. All patients had the site of obstruction diagnosed laparoscopically, most of whom (92%) were also treated laparoscopically without conversion to open surgery [41]. In another series, laparoscopy was successful in treating only 48% of the patients. Laparoscopic lysis of adhesion was also associated with higher incidence of intraoperative complications including bowel perforation [42]. The dilation of the small bowel that occurs with obstruction makes the safe procurement of a reasonable working space for the insertion of laparoscopic instruments very difficult. The dilated and sometimes friable bowel wall is difficult to handle safely with the available laparoscopic instruments. Therefore, the current use of laparoscopy in patients with intestinal obstruction is quite limited.

Future of laparoscopy

Laparoscopic surgery can be used for more complex procedures such as pancreatectomy and hepatectomy. While feasibility is established, their safety and efficacy compared with standard open techniques is not yet clear.

The future of laparoscopy rests in further refinement of special equipment. Most of the current laparoscopic instruments are crude prong-like adaptations of their open counterparts. They lack the degree of freedom and fluidity of movement afforded by human hands and fingers. They fail to provide the tactile feedback that is taken for granted in open surgery. Minute tasks such tying knots or placing sutures take a long time to complete, especially for the novice. The future generation of laparoscopic instruments will need to be flexible and allow for added levels of articulation. Surgical robotics provides a glimpse of what is on the horizon for laparoscopy in the future. However, much work is still needed to make the technology affordable and easily adaptable to the everyday surgical practice.

Laparotomy

History

For centuries, laparotomies were only performed in trauma or emergency situations. The outcome of these heroic efforts was predictably poor, although a few miraculous success stories were recorded. In 1474, for example, a French prisoner was operated on for an abdominal "colic." The operation was permitted because he had been condemned to death for robbery and was awaiting execution. The exploratory laparotomy was a success and the patient made a good recovery, after which his punishment was commuted [43]. It was the discovery of the three As, namely anesthesia (Morton, 1844), antisepsis (Lister, 1867), and antibiotics (Fleming, 1929) that made elective abdominal surgery or laparotomy possible.

Techniques

Incisions

Laparotomy incisions vary by surgical indication and surgeon preference. The most versatile incision is a midline incision. In a midline incision, the linea alba is opened longitudinally before the peritoneum is entered (Fig. 127.3). It is at the midline that the aponeuroses of the external oblique, internal oblique, and transversus abdominis muscles all fuse into one layer, which makes entry and closure of the abdomen fast and secure. The length and location of the incision vary with the type of surgery performed. Because only the terminal branches of blood vessels and nerves reach the linea alba, vascular and nerve damage caused by a midline incision is limited. Therefore a midline incision is also well suited for anticoagulated patients. In situations where a diagnosis is uncertain, a midline incision allows for maximal versatility and stoma placement if needed.

The abdominal cavity can also be accessed through a paramedian incision (Fig. 127.3). This vertical incision is made on the side of anticipated pathology but, compared with a

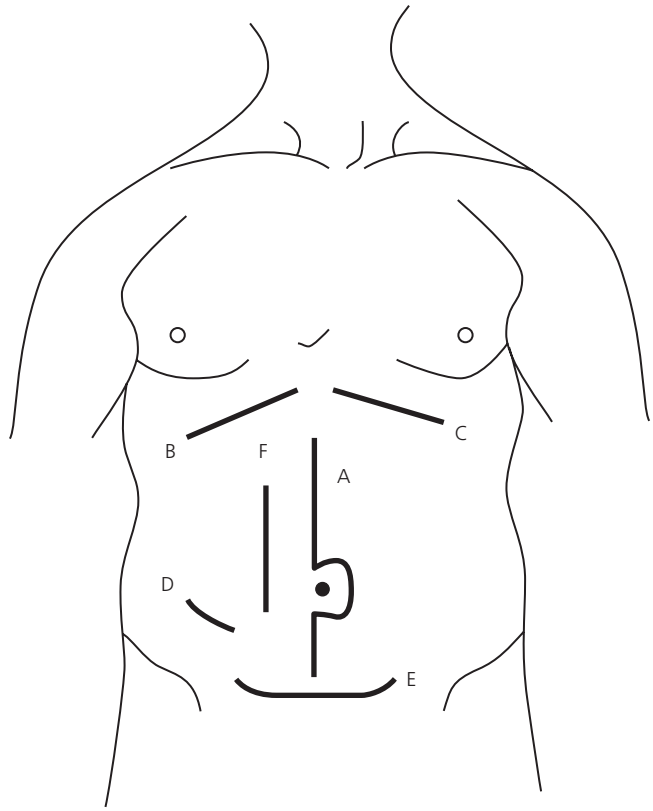

Figure 127.3 Laparotomy incisions: A, midline; B, right subcostal; C, left subcostal; D, McBurney; E, Pfannenstiel; F, right paramedian.

midline incision, takes longer to perform, poses a greater risk of injury to the epigastric vessels, and provides less versatility if unexpected findings are encountered.

Transverse incisions provide excellent cosmesis because they can be placed parallel to Langer lines and natural skin folds. The most popular transverse incision is the Pfannenstiel incision. This transverse incision is made 2–5 cm above the pubic symphysis and is commonly used for gynecological procedures (Fig. 127.3). In females, this skin incision is just above or at the pubic hairline and can be easily hidden under clothing. While the Pfannenstiel incision provides nice exposure of the pelvic organs, the fascial incision cannot be extended above the umbilicus so is not suited for surgery involving the middle and upper abdomen. Another popular transverse incision is one that traverses the rectus sheath in the midabdomen. Such an incision on the right side is frequently used for colectomies or creating stomas.

Surgery in the upper abdomen can also be performed through subcostal incisions (Fig. 127.3). These provide excellent exposure for hepatic and biliary procedures. In addition the presence of two strong fascial layers (i.e., both anterior and posterior rectus sheath) provides for a very secure closure with low rates of incisional hernia formation.

The McBurney incision (and its variant the Rockey–Davis incision) is an oblique muscle-splitting incision in the right lower quadrant used primarily for appendectomy (Fig. 127.3). These incisions can be placed directly over the target organ, so can be made quite small. Closure and healing are facilitated by muscle contraction and return to full activity is faster than with a midline or transverse incision since there is little fascial disruption.

Exploration

Once the abdominopelvic cavity is entered via one of the incisions detailed above, a thorough exploration is usually first carried out. One advantage of laparotomy compared with laparoscopy is the ability to carry out a very thorough abdominal examination and this should be a standard opening maneuver in all laparotomies. Any free fluid (e.g., ascites, succus, or feculent material) is noted, cultured, and sent for cytology if malignancy is suspected. The liver is inspected and palpated for cirrhosis, mass, or abscess. The gallbladder is examined for inflammation or gallstones. The stomach and duodenum are examined for perforation, mass, or ulcer. The nasogastric tube position is confirmed. The spleen is visualized and palpated with the aid of retraction. The omentum is then lifted from the pelvis and delivered into the upper abdomen. Any omental "caking" or evidence of tumor metastasis is noted. The ligament of Treitz is located and the small bowel is examined in its entirety to the ileocecal valve. Any perforation, mass, stricture, or change in caliber (transition point in small bowel obstruction) is noted and marked with a suture for later identification. Attention is usually also paid to the small bowel mesentery for lymphadenopathy or mass. Certain mass lesions such as carcinoid or desmoid tumors tend to grow in the root of the small bowel mesentery. The total length of small bowel should be estimated in patients whom have undergone prior surgery, especially in patients with Crohn's disease. The appendix is examined for inflammation or perforation. The colon and rectum are then examined for perforation, mass, diverticula, or stricture. In females, the uterus, ovaries, and fallopian tubes are also carefully inspected and palpated for mass or cyst. Retroperitoneal organs such as the pancreas, portions of the duodenum, and kidneys are palpated to rule out mass lesion. Visual inspection of the retroperitoneum is also required to discover any hematomas that would suggest aortoiliac artery or inferior vena caval injury.

Closure

At the completion of the procedure, the laparotomy incision must be reapproximated in a secure fashion. A considerable amount of research has been done to find the optimal method of closing the incision [44,45]. These studies show that most abdominal incisions are best closed with a continuous mass closure of slowly absorbable monofilament sutures placed 1 cm from the fascial edge and 1 cm apart. The peritoneum layer does not need to be closed separately. The suture to wound length ratio should be at or above 4 : 1 to prevent hernias. Most surgeons have incorporated these principles of abdominal wound closure into their daily practice with minor modifications based on individual preference and patient characteristics. During the first postoperative week, the abdominal wound has less than 5% of normal tissue strength and is therefore almost entirely held together by sutures. Abdominal wounds achieve 80% tensile strength by the sixth postoperative week and 90% tensile strength at 6 months. Therefore most patients are instructed to avoid vigorous physical activity for 6 weeks following a laparotomy, although activities of daily living such as driving are permitted as soon as patients no longer require pain medication.

Complications

Wound complications

Wound-related complications after laparotomy include seroma, hematoma, and infection. Small seromas and hematomas are generally managed expectantly. Larger fluid collections in the wound need to be drained to relieve symptoms and prevent superinfection. The occurrence of wound infection is affected by a multitude of patient and surgery-related factors. Obesity, poor preoperative nutritional status, diabetes, tobacco abuse, and immunocompromise all increase the risk of wound infection. It has been shown that administration of one dose of appropriate antibiotics prior to skin incision decreases the incidence of wound infection. This has become a universal practice by all surgeons. All wound infections need to be promptly opened, drained, and

cultured for offending organisms. Initially, debridement-type dressings (e.g., normal saline wet-to-dry) should be applied and changed frequently to remove pus and necrotic tissues. After the wound base is clean, moisture barrier-type dressings (e.g., calcium alginate, hydrogel) can then be placed to promote granulation. In large open wounds, a negative-pressure wound vacuum dressing (VAC dressing) can also be applied to speed up wound contraction. During operations where there is gross contamination of the wound, the surgeon may elect to leave the subcutaneous tissue and skin open for either delayed primary closure or healing by secondary intention. This is done to prevent the development of necrotizing fasciitis, a rare but lethal complication. If necrotizing fasciitis develops, the wound (including the underlying fascia) needs to be widely debrided in the operating room. Broad-spectrum antibiotics should be instituted based on microbiological data. The abdomen should also be reexplored at the time of debridement to rule out any intraabdominal source of sepsis (leak, fluid collection).

Fascial dehiscence and hernia

Fascial dehiscence and hernia are early and late manifestations of wound disruption at the fascial level. The etiologies include underlying conditions (advanced age, obesity, comorbidities, poor nutrition, radiation, wound infection) and surgeon factors (length and orientation of incision, suture material and length, wound closure technique). Most dehiscences occur within the first 2 weeks of the operation. Drainage of large amounts of serous fluid through the incision usually heralds a wound dehiscence. If left unattended, evisceration may follow with severe consequences. Dehiscence or evisceration are surgical emergencies and mandate immediate return to the operating room. Limited exploration should be undertaken to rule out an intraabdominal source of sepsis. Mass closure of the abdominal wall should be done with interrupted permanent sutures. Retention sutures may also be placed if the patient's underlying conditions are judged prone to recurrence of fascial disruption. The skin can be either closed loose or left open to heal by secondary intention.

Incisional hernias develop in as many as 11% of surgical patients. Postoperative wound infection doubles the risk of developing an incisional hernia [46]. Some patients with incisional hernias need to undergo emergency surgery for bowel incarceration or strangulation [47]. Others are forced to make lifestyle modifications because of their hernias [48]. The traditional open repair of incisional hernias that did not employ mesh carried recurrence rates as high as 30%–50%. Repeat repairs led to pain, suffering, dense adhesion formation, and eventually significant loss of domain at the anterior abdominal wall. Most surgeons have now adopted the Rives–Stoppa technique that uses large sheets of mesh placed deep to the rectus sheath. This repair has had a success rate in excess of 90% [49].

Nerve injuries

The risk of nerve injury in a midline incision or a short transverse incision is low because only terminal nerve fibers are transected as a result of the incision. Longer incisions, especially at the level of the anterior superior iliac spine, may risk injuring the ilioinguinal or iliohypogastric nerves. Similarly, long subcostal incisions invariably transect branches of the intercostal nerves and create areas of diminished sensation inferior to the incision. Neuromas, although rare, may develop. Rarely, the femoral nerve can be compressed by retractor blades in pelvic surgery. Up to 10% of patients who undergo pelvic surgery develop symptoms due to femoral nerve compression [50]. Fortunately, the resultant neurapraxia is generally mild and transient.

Adhesive small bowel obstruction

Another unintended consequence of laparotomies is intraabdominal adhesion formation and resulting small bowel obstruction. In a large series, 12.4%–17% of patients developed symptoms consistent with small bowel obstruction 2 years after laparotomy and 2.6%–3.1% of patients required adhesiolysis operatively [51]. This translates into 300 000 admissions, 800 000 days of inpatient care, and $1.3 billion of expenditure per annum in the USA [52]. For patients who are admitted for adhesive small bowel obstruction, the risk of recurrence is 34% by 4 years and 42% by 10 years [53]. It is estimated that three-quarters of small bowel obstructions are caused by postoperative adhesions. Adhesion barriers have been developed and used in laparotomies to prevent the formation of dense adhesions (e.g., Interceed, Seprafilm). When properly used, they have been shown to decrease postoperative adhesion formation [54]. Unfortunately, there are no data to show that this reduces the rate of small bowel obstruction since these adhesive barriers only prevent adhesions to the abdominal wall and pelvic side wall. Because of cost and lack of reduction of the important consequences of adhesion formation, they are not commonly used. In comparison, however, laparoscopy induces much less adhesion formation than laparotomy. With the gradual replacement of laparotomy by laparoscopic procedures, it is anticipated that the incidence of postoperative adhesive small bowel obstruction will decline.

Future of laparotomy

Increasingly sophisticated imaging modalities and the advent of modern laparoscopic surgery have limited the role of laparotomy to complex procedures. Nowadays, it is rare to perform a laparotomy without an a priori radiographic diagnosis. The majority of those diagnoses are confirmed at the time of laparotomy. Staging laparotomies and exploratory laparotomies are rarely performed nowadays. Despite this trend, laparotomy is still the gold standard in many situations. A laparotomy is fast. A well-trained surgeon can gain entry to the abdominopelvic cavity in minutes without injur-

ing any internal organ. For this reason, laparotomies are still performed routinely for trauma patients in whom rapid control of bleeding vessels and perforated viscus is paramount. A laparotomy is inexpensive. Most, if not all, instruments used to perform laparotomies are reusable. For this reason, laparotomies will have advantages in countries where health-care dollars are limited or expertise in advanced laparoscopy is lacking. Laparotomy is versatile. A large midline incision affords surgeons direct, hands-on access to all intraabdominal and pelvic organs. This versatility makes laparotomy the safe fallback procedure if other less invasive procedures fail. Laparotomy will always be performed when a laparoscopic procedure is unable to be completed or a percutaneous intervention is unsuccessful. Laparotomy and traditional open surgery are still taught at every surgical training program, and for a good reason. Patients undergoing complex operations such as liver transplantation and Whipple procedures are unlikely to be treated with laparoscopic approaches. Hence it is unlikely that laparoscopy will replace laparotomy as the workhorse of complex abdominal surgery.

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B IMAGING

128 Plain and contrast radiology

Marc S. Levine, Stephen E. Rubesin, Hans Herlinger, Igor Laufer

Abdominal radiographs, 3061 Contrast studies, 3065

Abdominal radiographs

Abdominal radiographs often play an important role in the evaluation of patients with abdominal pain or distention or clinical signs of an acute abdomen [1]. The purpose of this section is to review radiographic findings such as bowel dilation, pneumoperitoneum, and pneumatosis, and to discuss their significance. However, computed tomography (CT) is increasingly recognized as a more sensitive technique than abdominal radiography for diagnosing a host of conditions in patients with acute abdominal symptoms.

Technique

In patients with acute abdominal findings, both supine and upright radiographs of the abdomen should be obtained. The upright radiographs should be centered to include the diaphragms. This permits assessment of not only free intraperitoneal air beneath the diaphragms but also air–fluid levels within the bowel that can be detected only on horizontal-beam views. If patients are too sick or debilitated to stand, left lateral decubitus views should be obtained instead to detect free air between the liver and the right lateral abdominal wall.

Abnormalities

Bowel dilation

Bowel dilation is usually caused by obstruction or ileus. The small bowel is a more common site of obstruction than the colon. Other patients may develop an adynamic ileus without evidence of mechanical obstruction. These conditions are considered separately in the following sections.

Small bowel obstruction

Because of the great increase in abdominal surgery in recent decades, most small bowel obstructions are caused by postoperative intraperitoneal adhesions [1]. Other less common causes include incarcerated hernias, metastases, radiation, Crohn's disease, intussusception, and gallstone ileus.

In a mechanical small bowel obstruction, supine abdominal radiographs usually reveal multiple loops of dilated small bowel and a paucity of colonic gas (Fig. 128.1a); multiple air–fluid levels are almost always seen on upright or decubitus views (Fig. 128.1b). Dilated small bowel can usually be differentiated from dilated colon by its more central location in the abdomen as well as the presence of tightly spaced folds or valvulae conniventes that completely traverse the diameter of the bowel.

While advanced small bowel obstructions are easily recognized on abdominal radiographs, the diagnosis can be more difficult in patients who do not swallow a large amount of gas. In such cases, gaseous distention of bowel can be minimal or absent. Nevertheless, accumulation of fluid still occurs, and horizontal-beam views may still demonstrate numerous air–fluid levels in nondilated bowel. Occasionally, a row of tiny gas bubbles or "string of pearls" may be seen on upright or decubitus radiographs due to small amounts of gas trapped along the superior margin of small bowel loops almost completely filled with fluid [2]. In patients who swallow virtually no air whatsoever, abdominal radiographs may reveal a gasless abdomen with multiple fluid-filled loops of bowel appearing as tubular or sausage-shaped densities in the bowel that may be indistinguishable from true abdominal masses [2].

A simple mechanical obstruction does not usually cause bowel ischemia. However, a closed-loop obstruction due to an incarcerated hernia, volvulus, or other cause may occasionally produce a strangulating obstruction with ischemia or necrosis of the involved segment of bowel. Recently, CT has been recognized as a sensitive technique for detecting strangulation of bowel in patients with closed-loop obstruction [3].

It is important to recognize that advanced cecal carcinomas may obstruct the ileocecal valve, mimicking the radiographic appearance of a distal small bowel obstruction [1]. More distal colonic obstructions may also be masked by an incompetent ileocecal valve that allows gas to reflux from the obstructed

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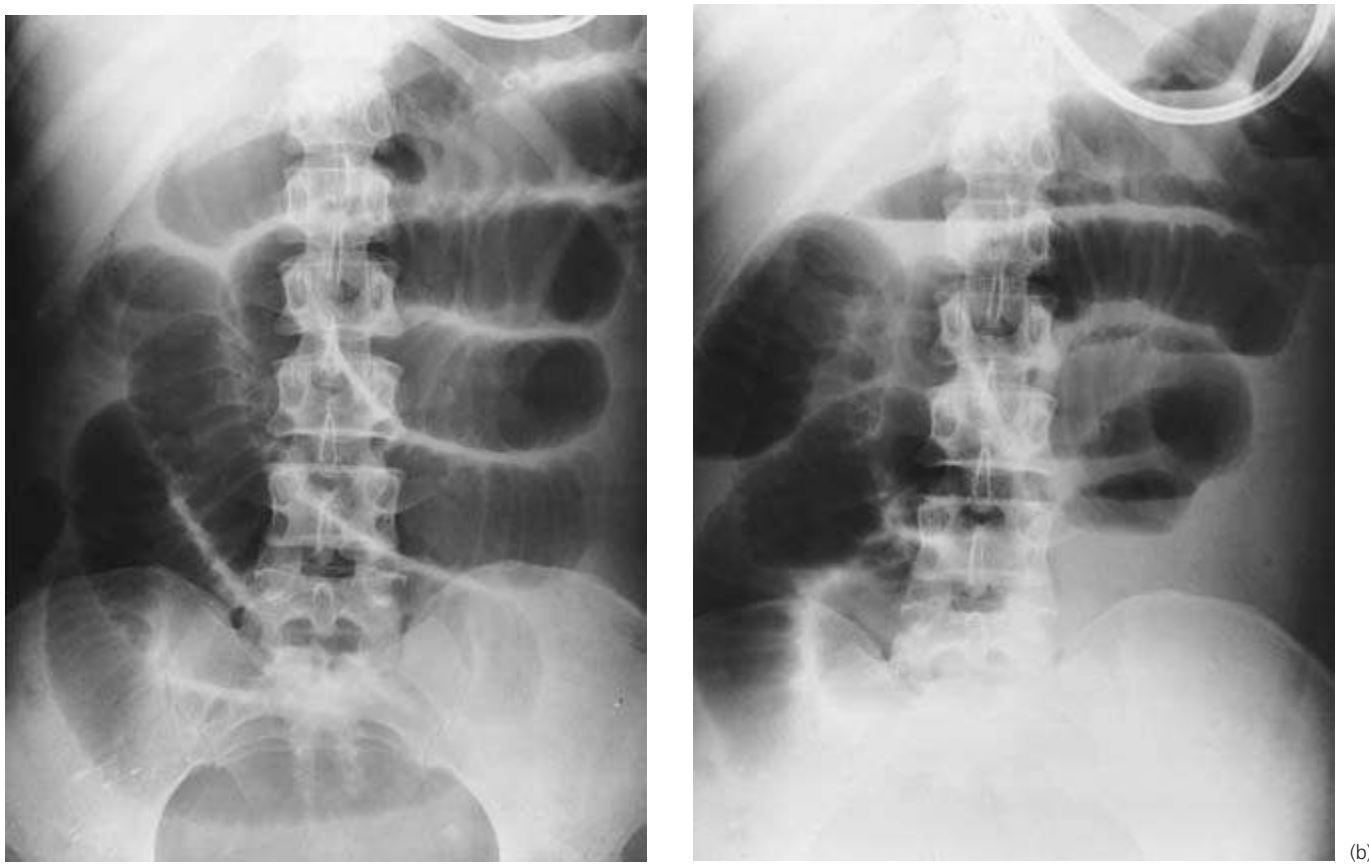


(a) (b)

Figure 128.1 Small-bowel obstruction due to postoperative adhesions. **(a)** Supine abdominal radiograph shows multiple loops of dilated small bowel and a paucity of colonic gas. **(b)** Upright radiograph shows multiple air–fluid levels in dilated small bowel. These findings are characteristic of small-bowel obstruction. From Levine [1], with permission from Elsevier.

colon into the small bowel. A barium enema may be performed on patients with radiographic evidence of a distal small bowel obstruction to rule out an unsuspected colonic carcinoma, particularly when there is no history of prior abdominal surgery.

Colonic obstruction

In colonic obstruction, supine abdominal plain radiographs usually reveal disproportionate colonic distention proximal to the obstructing lesion, with air–fluid levels in the dilated bowel on upright radiographs. The transition from dilated to nondilated bowel can often be recognized and most commonly occurs in the sigmoid colon because of an obstructing carcinoma. Primary colonic carcinoma accounts for 80%–90% of all colonic obstructions, but diverticulitis, metastases, and volvulus are other less common causes [1].

The most devastating complication of colonic obstruction is cecal perforation. Because of the high mortality associated with cecal perforation, colonic obstruction should be considered a surgical emergency. In general, a cecal diameter greater than 10–12 cm on abdominal radiographs is thought to be an indication for urgent colonic decompression because of the high risk of perforation [4].

Colonic volvulus should be distinguished from a simple colonic obstruction because it results from twisting of the colon around a fixed point on its mesentery, producing a closed-loop obstruction. The vast majority of cases involve the sigmoid colon or cecum [5]. Sigmoid volvulus is an acquired condition that often occurs in elderly patients who have chronic constipation or a high-residue diet [6]. In contrast, cecal volvulus tends to occur in younger patients due to congenital failure of retroperitoneal fixation of the cecum and ascending colon [6]. As a result, the right side of the colon has a persistent mesentery on which a volvulus can take place.

In sigmoid volvulus, abdominal radiographs usually reveal a massively dilated sigmoid colon that extends out of the pelvis into the upper abdomen with some degree of proximal colonic distention (Fig. 128.2) [1,7]. In contrast, cecal volvulus usually produces a massively dilated cecum that flips into the left upper quadrant with a single air–fluid level on upright or decubitus horizontal-beam views [1,7]. Because colonic volvulus is a closed-loop obstruction, twisting of the

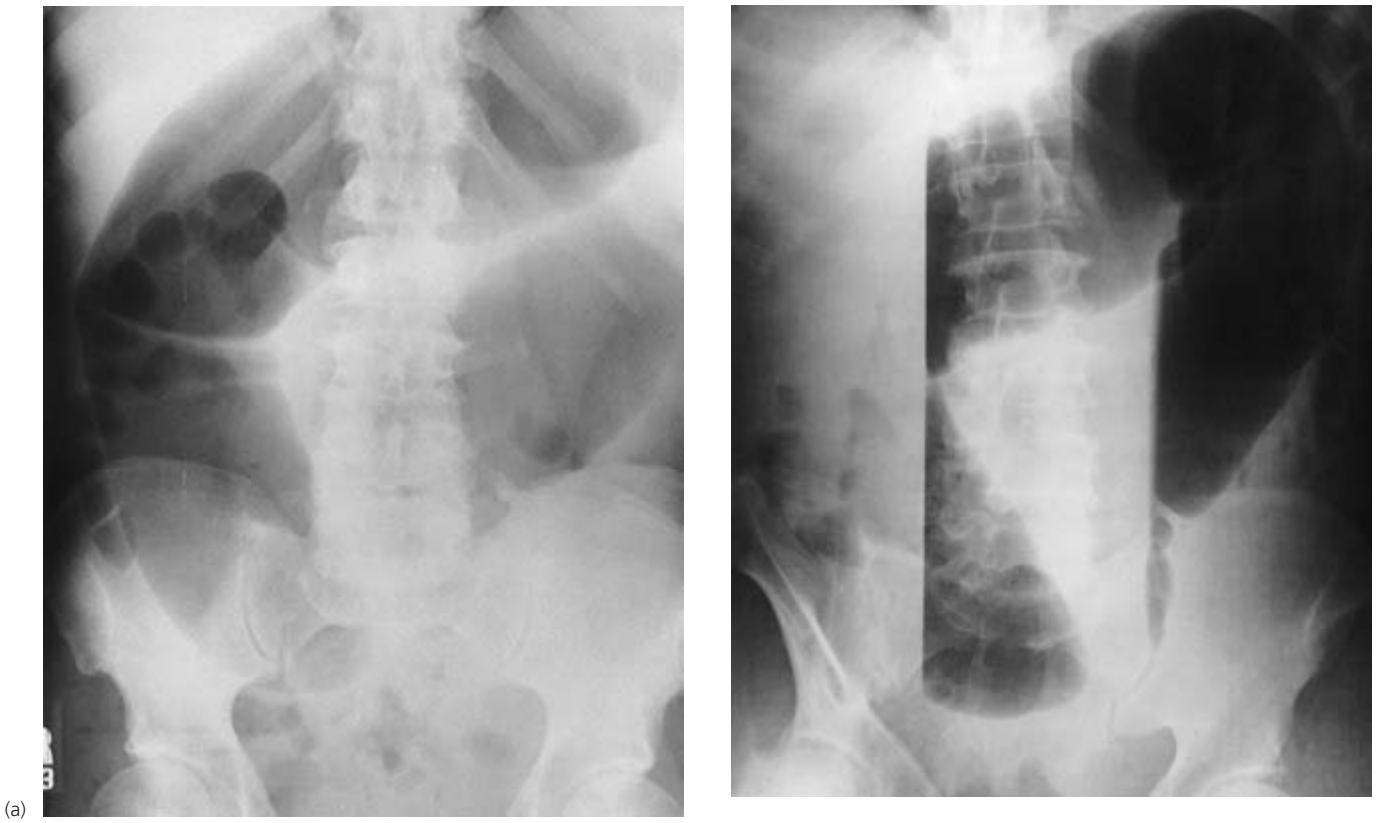


Figure 128.2 Sigmoid volvulus. **(a)** Supine abdominal radiograph shows a massively dilated sigmoid colon rising out of the pelvis into the abdomen. **(b)** Right lateral decubitus radiograph shows long air–fluid levels in both limbs of this dilated sigmoid loop. From Levine [1], with permission from Elsevier.

mesentery may compromise the vascular supply of the bowel, leading to strangulation, infarction, and perforation of the involved loop. Early diagnosis is therefore essential so that colonic volvulus can be treated before strangulation occurs.

Because of the risk of cecal perforation in patients with colonic obstruction, a barium enema may be required as an emergency procedure to determine whether an obstruction is present and to delineate the nature and site of the obstructing lesion. Carcinoma of the colon typically manifests as an annular lesion with shelf-like overhanging borders, whereas diverticulitis produces a tapered area of narrowing with intact but distorted mucosal folds. In colonic volvulus, the barium enema reveals a typical "bird-beak" deformity at the site of the volvulus.

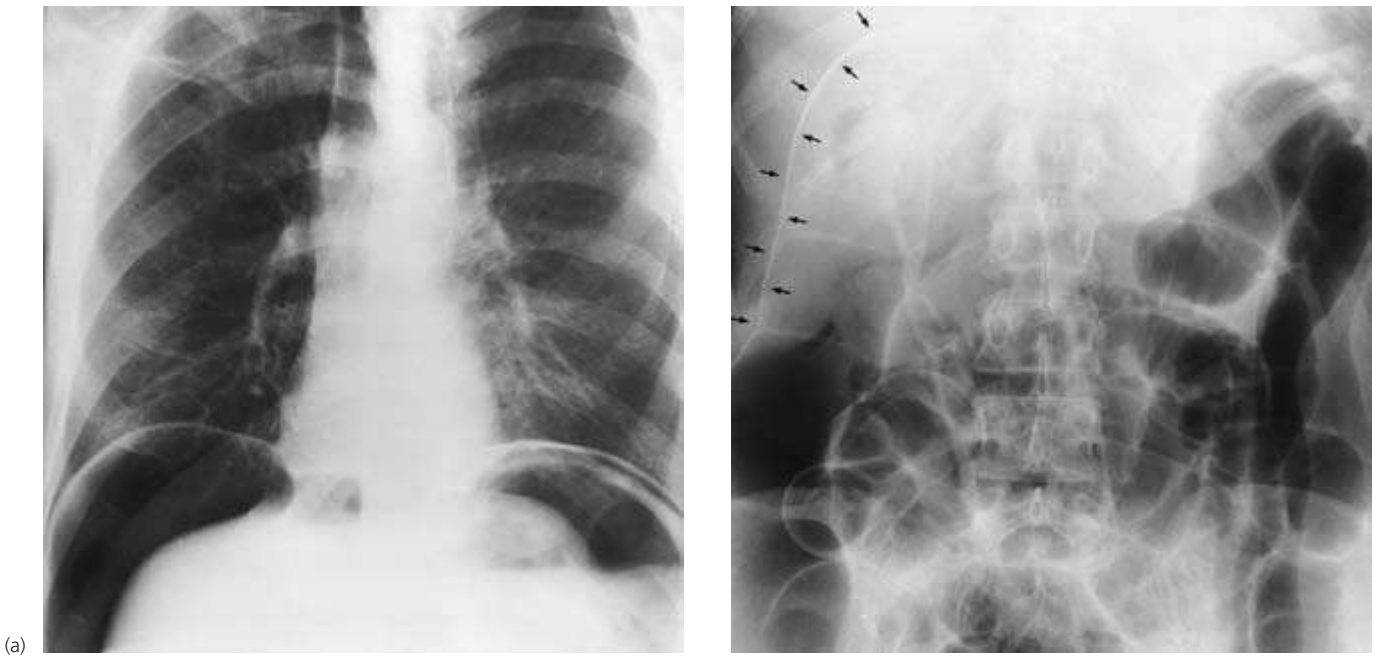
Adynamic ileus

In patients with an adynamic or paralytic ileus, there is interference with intestinal transport without an actual mechanical obstruction. As a result, supine abdominal radiographs classically show diffusely dilated small and large bowel. Although the degree of distention depends on the degree of air swallowing, horizontal-beam views usually demonstrate air–fluid levels throughout the bowel [8]. For reasons that are unclear, however, abdominal radiographs sometimes reveal an isolated small bowel or colonic ileus. In such cases, the findings may be indistinguishable radiographically from a mechanical small bowel or colonic obstruction [1,8]. When the colon appears dilated, the presence of a significant amount of rectal gas on left lateral views of the rectum should favor an ileus [9]. If it is unclear whether the patient has a distal colonic obstruction or an ileus, a single-contrast barium enema may be performed to differentiate these conditions.

Pneumoperitoneum

Upright chest and abdominal radiographs and left lateral decubitus radiographs of the abdomen are extremely sensitive for detecting free intraperitoneal air (i.e., pneumoperitoneum) and may demonstrate collections of intraperitoneal air as small as 1 mL [10]. In patients with an acute abdomen, the presence of free air almost always indicates a perforated viscus with subsequent peritonitis. The most common cause is a perforated duodenal ulcer [1]. Colonic perforation due to ischemic bowel disease, toxic megacolon, and diverticulitis is much less common but should be suspected in any patient with pneumoperitoneum and colonic distention on abdominal radiographs [11].

Pneumoperitoneum can readily be documented on upright chest radiographs or upright or left lateral decubitus



(a) (b)

Figure 128.3 Pneumoperitoneum. **(a)** Upright chest radiograph shows large amounts of free intraperitoneal air beneath the diaphragm in this patient with a perforated duodenal ulcer. **(b)** Supine abdominal radiograph in a different patient shows an indirect sign of pneumoperitoneum with air on both sides of the bowel wall (Rigler sign) (arrows) following inadvertent perforation at colonoscopy. From Levine [1], with permission from Elsevier.

radiographs of the abdomen by the presence of free intraperitoneal air directly beneath the diaphragms (Fig. 128.3a) or between the liver and the right lateral abdominal wall. Unfortunately, some patients are too ill or debilitated to stand or lie on their side; supine abdominal radiographs may be the only images obtained in these patients. However, pneumoperitoneum can be recognized on supine radiographs by the presence of air on both sides of the bowel wall (i.e., Rigler sign) (Fig. 128.3b), by linear or triangular collections of gas in the subhepatic space, or by air outlining the falciform ligament as a linear density in the right upper quadrant [1,2]. In one study, one or more signs of free intraperitoneal air were present on supine abdominal radiographs in 59% of patients with pneumoperitoneum [12].

Pneumatosis

In patients with intestinal ischemia or necrosis, gas may dissect into the wall of the affected bowel, producing intramural bowel gas or pneumatosis. This finding is recognized on abdominal radiographs by mottled or linear gas shadows that have a characteristic radiographic appearance (Fig. 128.4) [1,13]. CT has been recognized as a more sensitive technique than abdominal radiography for detecting pneumatosis [14]. It has classically been taught that the presence of pneumatosis indicates bowel infarction in the clinical setting of intestinal ischemia. However, studies have shown that pneumatosis can be detected on CT in patients with ischemic bowel disease who have viable bowel with partial mural

Figure 128.4 Pneumatosis due to an infarcted descending colon following abdominal surgery. Close-up view from supine abdominal radiograph shows tiny, mottled, and linear collections of gas in the wall of the descending colon.

ischemia rather than transmural infarction, so these individuals may recover without need for surgery [15,16].

In patients with intestinal necrosis, tiny, linear, peripherally branching gas shadows may occasionally be identified over the liver due to gas within the portal venous system [13]. This finding usually indicates severe disease with an extremely ominous prognosis. However, portal venous gas should be distinguished radiographically from biliary gas (i.e., pneumobilia), which tends to collect centrally in the common bile duct due to the opposite direction of bile flow. Pneumobilia can result from a variety of causes, including gallstone ileus, emphysematous cholecystitis, and a surgical or spontaneous biliary–enteric fistula [1].

Pneumatosis can also occur as a benign condition in which multiple gas-filled cysts or blebs are present in the wall of the bowel [1,13]. These patients are rarely ill, and the condition is usually discovered accidentally on abdominal radiographs obtained for other reasons. Radiographically, these gas-filled blebs are typically recognized as cystic or grape-like collections of gas that appear quite different from the linear gas shadows associated with intestinal necrosis.

Contrast studies

Contrast studies of the gastrointestinal tract can be performed using single- or double-contrast techniques. Single-contrast studies entail filling the lumen with a relatively low-density barium suspension and evaluating for contour abnormalities or filling defects in the barium pool. In contrast, double-contrast studies utilize a smaller amount of high-density barium and gas to evaluate the en face appearance of the mucosal surface. As a result, double-contrast techniques have dramatically improved the radiologist's ability to diagnose a variety of inflammatory and neoplastic diseases throughout the gastrointestinal tract. A major advantage of these techniques is their ability to demonstrate superficial mucosal abnormalities that cannot easily be recognized on conventional single-contrast examinations. In other cases, double-contrast studies may detect lesions that are missed or misinterpreted at endoscopy. Double-contrast radiography is also less expensive and less invasive than endoscopy. Thus, it is a valuable technique for evaluating patients with suspected gastrointestinal disease. All radiologists should strive for excellent double-contrast studies to maximize the information available from the procedure.

Pharynx

Indications

With increased survival of the elderly, pharyngeal disorders have become an increasingly frequent problem in modern medical practice. Approximately 35% of nursing home patients have some form of swallowing dysfunction [17]. Aspiration pneumonia and choking are particularly common causes of morbidity and mortality. Radiographic examination of the pharynx is now recognized as a valuable tool in the diagnostic workup of this large group of patients with pharyngeal disorders.

Contrast studies of the pharynx are most frequently performed on patients who have difficulty swallowing. However, disorders of the pharynx may also be manifested by respiratory and speech problems. Laryngeal penetration or overflow aspiration may lead to recurrent pneumonia, asthma, chronic bronchitis, coughing, or choking. In other patients, soft-palate insufficiency may result in nasal regurgitation or may give the voice a nasal quality. A pharyngoesophagram may be helpful in patients who have a wide spectrum of respiratory, speech, and swallowing difficulties. Barium studies are also useful in assessing pharyngeal function and morphology in patients with a history of neuromuscular disease, stroke, pharyngeal tumor, or prior head and neck surgery or radiation.

Normal anatomy

The pharynx is a complex muscular tube suspended superiorly from the skull base and styloid process, posteriorly from the cervical spine, and anteriorly from the mandible and hyoid bone. At least 26 muscles and six cranial nerves participate in pharyngeal function [18,19].

The pharynx can be arbitrarily divided into three portions: the nasopharynx, oropharynx, and hypopharynx. The soft palate separates the nasopharynx from the oropharynx, and the pharyngoepiglottic fold separates the oropharynx from the hypopharynx. The tongue forms the anterior wall of the oropharynx. The larynx, with its associated epiglottic, thyroid, cricoid, and arytenoid cartilages, forms the anterior wall of the hypopharynx. This laryngeal complex often protrudes into the lower hypopharynx as an apparent mass.

The mucosal surface of the pharynx is thrown into a series of folds by underlying lymphoid and muscular tissue. The vertical surface of the base of the tongue often has a nodular appearance due to the circumvallate papillae and lingual tonsil. Nodular lymphoid tissue or linear webs may also interrupt the normally smooth surface of the valleculae. Although the anterior border of the hypopharynx usually has a smooth contour, close apposition of the longitudinal muscles of the pharynx to the overlying squamous mucosa results in longitudinal striations of the lateral and posterior walls of the hypopharynx [16]. Horizontal mucosal striations are seen in the redundant mucosa overlying the arytenoid processes and cricoid cartilages [19].

Technique

A complete radiographic examination of the pharynx includes a cine or video pharyngoesophagram to evaluate motility and a series of spot images to evaluate morphology [20–22]. In patients with suspected foreign body, fistula, or abscess, frontal and lateral radiographs of the neck should also be obtained.

The barium study is initially performed with a high-density barium suspension for optimal visualization of the pharynx. The patient is asked to swallow barium in frontal, lateral, and oblique projections. A video recording of each swallow permits a frame-by-frame or slow-motion analysis of the various parameters of deglutition. Movement of the tongue, soft palate, and epiglottis as well as laryngeal closure and cricopharyngeal opening are best evaluated in the lateral projection. However, symmetry of tongue motion, pharyngeal peristalsis, and epiglottic tilt are best evaluated in the frontal projection.

After individual swallows of barium, double-contrast spot images of the pharynx are obtained in frontal and lateral projections. The spot images are obtained during suspended respiration and during a modified Valsalva maneuver or phonation to optimally distend the pharynx [23]. The frontal view is best for demonstrating the contours of the valleculae and piriform sinuses, the lateral walls of the tonsillar fossae and hypopharynx, and the superior border of the base of the tongue. The lateral view is best for demonstrating the inferior border of the base of the tongue, the soft palate, the posterior pharyngeal wall, the anterior hypopharyngeal wall, the epiglottis, and the cricopharyngeus.

After the pharyngeal examination has been completed, upright double-contrast and prone single-contrast views of the esophagus are obtained to rule out associated esophageal disease.

Abnormalities

Laryngeal penetration and aspiration

Laryngeal penetration occurs when barium enters the laryngeal vestibule during swallowing. Penetration may be limited to the region of the subepiglottic space or may extend as far as the true vocal cords or trachea. Laryngeal penetration occurs because of poor timing of oral and pharyngeal events associated with swallowing or pharyngeal dysmotility due to neuromuscular disorders such as amyotrophic lateral sclerosis, multiple sclerosis, or cerebrovascular accidents. Inflammatory or neoplastic diseases that restrict pharyngeal motility may also cause penetration. One study found that patients with epiglottic carcinoma may present with signs and symptoms of aspiration because of decreased epiglottic excursion when this structure is involved by tumor [24].

Aspiration occurs when barium enters the laryngeal vestibule during normal breathing. Aspiration results from stasis and retention of pharyngeal contents because of tumor, diverticula, or neuromuscular disease in the pharynx. Aspiration may also be caused by gastroesophageal reflux or reflux of esophageal contents above an obstructing esophageal lesion such as a stricture or carcinoma. Penetration is primarily associated with dysmotility, whereas aspiration is associated with stasis. Some patients can have "silent" tracheobronchial aspiration that fails to elicit a cough reflex. It has been shown that the risk of developing aspiration pneumonia correlates directly with the degree of pharyngeal dysfunction on barium studies [25].

Cricopharyngeal prominence

The pharyngoesophageal segment, the radiographic equivalent of the manometrically defined upper esophageal sphincter (UES), is formed by the inferior portion of the cricopharyngeus and the proximal cervical esophagus [26,27]. Although the UES is tonically contracted at rest, initiation of swallowing causes the sphincter to relax ahead of the oncoming bolus. The UES also acts as part of the pharyngeal peristaltic wave, functioning in sequence with the constrictor musculature. The pharyngoesophageal segment is best evaluated on a dynamic recording of the pharynx in a lateral projection.

During swallowing, a prominent cricopharyngeus appears as a smooth, 1-cm high, bar-like protrusion of the posterior pharyngeal wall into the barium column on lateral projections (Fig. 128.5). The cricopharyngeus may show delayed opening, incomplete opening, or early closure. A prominent cricopharyngeus is detected on barium studies in about 5% of asymptomatic individuals [28,29]. However, some patients

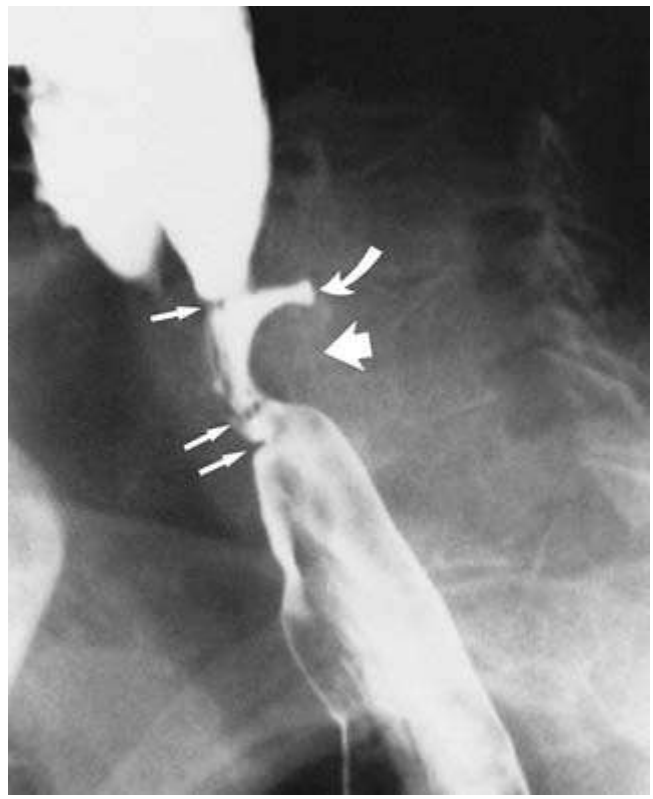


Figure 128.5 Incomplete opening of cricopharyngeus, manifested by a posterior indentation on the pharyngoesophageal segment (large arrow). Notice the associated webs anteriorly (small arrows) and pseudo-Zenker diverticulum posteriorly (curved arrow) that is seen as a result of trapping of barium above a prominent cricopharyngeus. Cricopharyngeal dysmotility is often related to underlying gastroesophageal reflux or pharyngeal paresis.

with this finding complain of dysphagia. In symptomatic patients, a prominent cricopharyngeus is often associated with pharyngeal paresis or occurs as a compensatory response to gastroesophageal reflux or esophageal obstruction.

Lateral pharyngeal pouches and diverticula

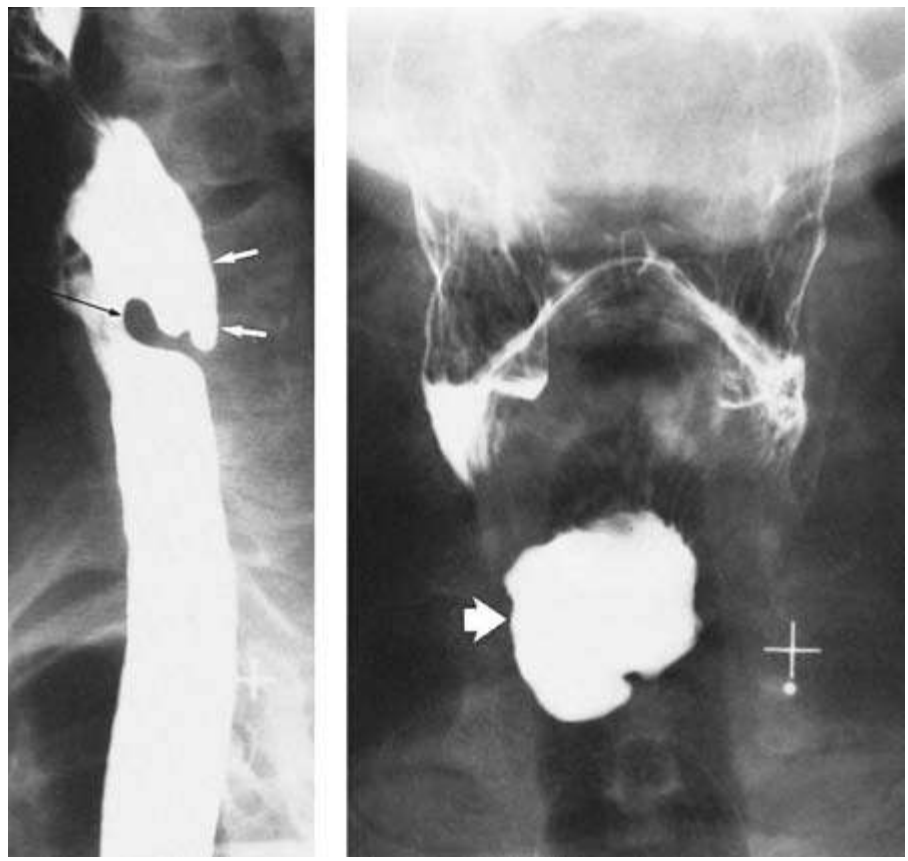
Lateral pharyngeal pouches are transient protrusions of the lateral pharyngeal wall at sites of anatomical weakness, such as the posterior thyrohyoid membrane and tonsillar fossae after a tonsillectomy [30]. These pouches are common findings, usually occurring as normal variants in asymptomatic patients. In contrast, lateral pharyngeal diverticula are persistent protrusions from the tonsillar fossae or region of the thyrohyoid membrane. These diverticula are much less common than pharyngeal pouches, occurring primarily in individuals who have markedly elevated pharyngeal pressure, such as glassblowers and tuba players. If stasis occurs in pharyngeal pouches or diverticula, subsequent spillage of pouch contents into the hypopharynx may result in aspiration into the larynx or tracheobronchial tree. Stasis with delayed spill into the hypopharynx may also cause mild neck discomfort or dysphagia after swallowing. Diverticula may also be manifested by neck masses and may occasionally be sites of ulceration or neoplasia.

Lateral pharyngeal pouches appear on frontal views as transient hemispheric protrusions of mucosa in the upper hypopharynx above the calcified edge of the thyroid cartilage. These pouches can be recognized on lateral views as ovoid barium collections or rings anteriorly in the upper hypopharynx just below the hyoid bone. In contrast, lateral pharyngeal diverticula appear as persistent protrusions in these areas.

Zenker diverticulum

Zenker diverticulum or posterior hypopharyngeal diverticulum is an acquired mucosal herniation through an area of anatomical weakness in the region of the cricopharyngeus (i.e., dehiscence of Killian). This area of anatomical weakness is located between the thyropharyngeus and cricopharyngeus or between the oblique and horizontal fibers of the cricopharyngeus [26,27]. Most patients with Zenker diverticulum have an associated hiatal hernia or gastroesophageal reflux. Rarely, these diverticula are complicated by ulceration or malignancy.

During swallowing, a Zenker diverticulum appears radiographically as a posterior bulging of the distal pharyngeal lumen above an anteriorly protruding cricopharyngeal bar (Fig. 128.6a). At rest, the barium-filled diverticular sac often



(a) (b)

Figure 128.6 **(a)** Zenker diverticulum seen as a posterior outpouching of the hypopharyngeal wall (white arrows) above a prominent cricopharyngeus (black arrow) during swallowing. **(b)** A frontal view shows retention of barium in the diverticulum (arrow) at completion of the swallow.

extends below the level of the cricopharyngeus posterior to the proximal cervical esophagus (Fig. 128.6b).

Inflammatory conditions

Barium studies are of limited value in patients with viral, bacterial, or fungal infection of the pharynx [31]. Such patients usually have normal pharyngograms or nonspecific lymphoid hyperplasia of the palatine tonsil or base of the tongue. Occasionally, however, *Candida* or herpes pharyngitis may be manifested on double-contrast radiographs by plaques or ulcers in the pharynx, particularly in patients with acquired immunodeficiency syndrome (AIDS). Barium studies may also be helpful in a patient who has a chronic sore throat in order to determine whether there is underlying gastroesophageal reflux or reflux esophagitis.

Tumors

Double-contrast pharyngography has an important role in the initial detection and subsequent workup of pharyngeal tumors [32,33]. Double-contrast radiographs of the pharynx can accurately define the size, level, and extent of the lesion. Radiological examination is particularly helpful in demonstrating regions of the pharynx (e.g., valleculae, lower hypopharynx, cricopharyngeus) that are difficult to visualize at endoscopy. It also enables detection of submucosal masses that are easily missed at endoscopy. Although its accuracy is limited in the region of the palatine tonsil, the double-contrast examination is capable of detecting more than 95% of all mucosal neoplasms in the pharynx below the level of the pharyngoepiglottic fold [32].

Squamous cell carcinoma is by far the most common malignant tumor of the pharynx. With an overall 5-year survival rate of approximately 20%, this tumor has a somewhat better prognosis than esophageal carcinoma. These lesions may be manifested on double-contrast radiographs by an intralumenal mass, mucosal irregularity, or loss of distensibility [34]. An intralumenal mass may cause asymmetry or obliteration of the normal pharyngeal contour, barium-coated lines in unusual locations, or a superimposed radiodensity. Mucosal irregularity may be manifested by an irregular, lobulated, nodular, or granular surface pattern. Loss of distensibility may be associated with fixation of pharyngeal structures by infiltrating tumor. When malignant lesions are detected in the pharynx, the esophagus should be carefully evaluated radiographically because of the increased incidence of synchronous esophageal cancers in these patients [35].

Upper gastrointestinal tract

The development of routine double-contrast techniques for examining the upper gastrointestinal tract has dramatically improved our ability to diagnose a variety of inflammatory and neoplastic diseases in the esophagus, stomach, and duodenum. Despite increasing acceptance of this technique, some investigators advocate endoscopy as the initial screening study in patients with dyspepsia or other upper gastrointestinal symptoms. Although endoscopy has been recognized as a highly accurate technique for examining the upper gastrointestinal tract, it is also an invasive technique with a small but measurable risk of gastric perforation or other complications. Furthermore, it is an expensive technique, costing three to four times more in the USA than double-contrast upper gastrointestinal examinations. Because barium studies are safer and less expensive than endoscopy, radiological evaluation of the upper gastrointestinal tract remains a viable alternative as long as its accuracy approaches that of endoscopy for clinically significant disease. We believe that a carefully performed double-contrast examination provides the best opportunity for radiology to be competitive with endoscopy as a diagnostic modality.

Technique

The routine double-contrast upper gastrointestinal examination should be performed as a biphasic study in which double-contrast and single-contrast views of the esophagus, stomach, and duodenum are obtained [36]. In the double-contrast portion of the study, a series of maneuvers is required to achieve adequate gaseous distention of the lumen while a thin layer of high-density barium is spread on the mucosa. The double-contrast examination is facilitated by the use of pharmacological agents (e.g., glucagon 0.1 mg i.v.) to induce gastric hypotonia. After the double-contrast portion of the study has been completed, prone or upright single-contrast views of the esophagus, stomach, and duodenum are obtained with a low-density barium suspension and various degrees of compression to supplement the double-contrast study. Because of its greater diagnostic yield, this biphasic study has been advocated as the best radiological technique for examining the upper gastrointestinal tract.

Indications

Esophagus

In patients with reflux symptoms, barium studies have traditionally been advocated to document the presence of a sliding hiatal hernia or gastroesophageal reflux, to detect complications such as strictures, and to exclude other abnormalities in the esophagus that can mimic reflux esophagitis. However, by permitting a more detailed assessment of the esophageal mucosa, double-contrast radiographic techniques have made it possible to detect superficial ulceration and other changes of esophagitis before the development of deep ulcers and strictures.

Because modern medical care is prolonging the survival of patients who are immunosuppressed, infectious esophagitis has become an increasingly frequent problem. When infectious esophagitis is suspected on clinical grounds, double-contrast esophagography may be performed to confirm the diagnosis and differentiate the various underlying organisms. The radiologist's ability to differentiate fungal and viral

esophagitis is particularly important for patients with AIDS, because some gastroenterologists are reluctant to perform endoscopy on these individuals because of fear of contaminating their endoscopic instruments or exposing themselves to human immunodeficiency virus (HIV). However, endoscopy should be performed if the radiographic findings are equivocal.

Dysphagia is another important indication for performing barium studies. If the sensation of dysphagia is localized to the pharynx, a careful pharyngeal examination should be obtained. However, some lesions involving the distal esophagus or cardia may cause dysphagia referred to the upper esophagus or pharynx. Thus, the gastric cardia and esophagus should be carefully evaluated radiographically in all patients with unexplained pharyngeal dysphagia to rule out a distal lesion masquerading as a pharyngeal disorder.

Stomach and duodenum

The most common indications for performing a double-contrast examination of the stomach and duodenum include epigastric pain or discomfort, bloating, belching, early satiety, and signs or symptoms of upper gastrointestinal bleeding such as hematemesis, melena, and guaiac-positive stool. If gastritis, duodenitis, duodenal ulcers, or unequivocally benign-appearing gastric ulcers are diagnosed radiographically, the patient can be treated medically without need for endoscopic intervention. However, if the double-contrast examination demonstrates a gastric ulcer or other lesion that is equivocal or suspicious for malignancy, endoscopy and biopsy should be performed for a more definitive diagnosis. If the double-contrast examination is normal, the decision for endoscopy should be based on the severity of symptoms, age, and overall health of the patient.

Contraindications and risks

Because oral barium sulfate is contraindicated in patients with suspected esophageal or gastric perforation, water-soluble contrast media should be used if there are any clinical or radiographic signs of mediastinitis or peritonitis. Otherwise, the risks of the barium study are negligible. Nevertheless, a double-contrast examination may be difficult to perform on elderly or debilitated patients who cannot undergo the turning maneuvers required for this examination. These patients may be evaluated by conventional single-contrast barium studies.

Abnormalities

Gastroesophageal reflux disease

In patients with suspected gastroesophageal reflux disease, barium studies may be performed not only to detect gastroesophageal reflux but also to look for the morphological sequelae of reflux, including reflux esophagitis, peptic strictures, and Barrett esophagus. Conventional single-contrast esophagography has been considered an unreliable technique for diagnosing reflux esophagitis, with an overall sensitivity of only 50%–75% reported in the literature. However, the use of double-contrast esophagography has increased the radiographic sensitivity to almost 90% [37]. The single most common sign of mild reflux esophagitis on double-contrast studies is the presence of a nodular or granular mucosa due to mucosal edema and inflammation [37,38]. Other patients may have shallow ulcers and erosions appearing as one or more tiny collections of barium in the distal esophagus near the gastroesophageal junction (Fig. 128.7). In more severe disease, the esophagus may have a grossly irregular contour, with serrated margins and decreased distensibility due to extensive ulceration, edema, and spasm.

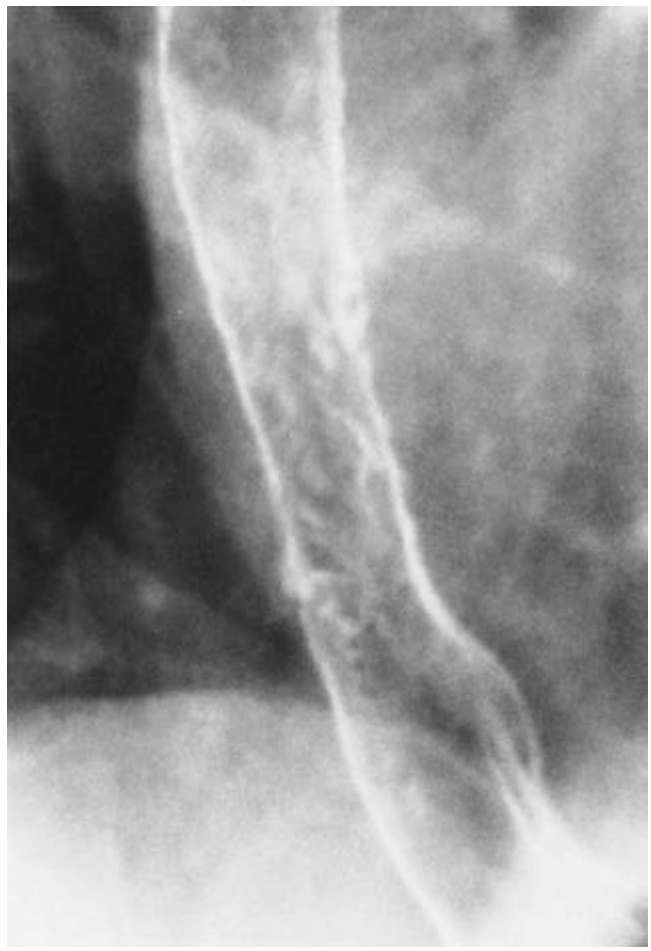


Figure 128.7 Reflux esophagitis with multiple areas of shallow ulceration seen en face and in profile in the distal esophagus. From Levine et al. [36], with permission from the Radiological Society of North America.

Scarring from reflux esophagitis may lead to the development of reflux-induced or peptic strictures, most commonly seen as tapered areas of concentric narrowing in the distal esophagus above a hiatal hernia. Less frequently, peptic strictures may appear as ring-like areas of narrowing that can be difficult to differentiate from Schatzki rings [39].

Barrett esophagus is a well-recognized complication of reflux esophagitis that is associated with a significantly increased risk

of developing esophageal adenocarcinoma. Unfortunately, the classic radiological features of Barrett esophagus (i.e., a high esophageal stricture or ulcer or a reticular mucosal pattern) occur in only a minority of patients [40]. However, data suggest that patients without reflux esophagitis or peptic strictures on double-contrast examinations rarely have Barrett esophagus at endoscopy [41]. Thus, double-contrast esophagography may be a useful screening study for Barrett esophagus to determine the relative need for endoscopy and biopsy in patients with reflux symptoms.

Infectious esophagitis

Esophagography has traditionally been considered an unreliable technique for diagnosing *Candida* esophagitis, with an overall sensitivity of less than 50% [42]. With double-contrast techniques, however, esophagography has a sensitivity as high as 90% in diagnosing *Candida* esophagitis [43]. The major advantage of this technique is its ability to demonstrate mucosal plaques that cannot easily be recognized on conventional single-contrast studies. These discrete plaque-like lesions tend to be longitudinally oriented, appearing on double-contrast radiographs as linear or irregular filling defects with normal intervening mucosa (Fig. 128.8a).

The AIDS epidemic has led to the development of a more fulminant form of *Candida* esophagitis, manifested by a "shaggy" esophagus with a grossly irregular contour due to multiple plaques, pseudomembranes, and ulcers (Fig. 128.8b) [44]. Because this degree of esophagitis rarely occurs in other immunocompromised patients, the possibility of AIDS should be suspected when a shaggy esophagus is detected on barium studies, particularly in young homosexual men.

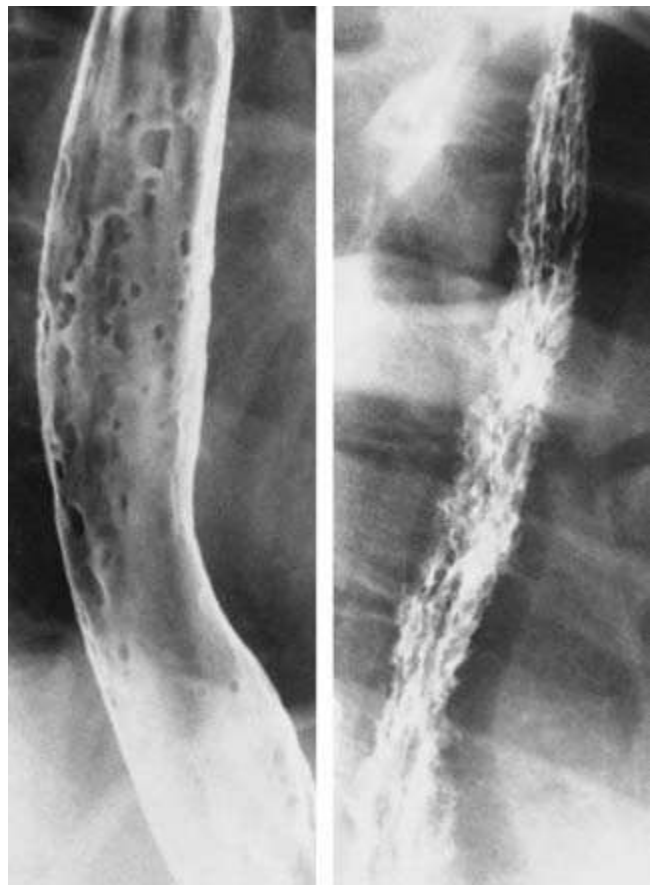
Herpes and, less frequently, cytomegalovirus (CMV) esophagitis also occur in immunosuppressed patients with odynophagia; these conditions should be suspected in the same clinical setting as *Candida* esophagitis. More than 50% of patients with herpes esophagitis have discrete superficial ulcers on the esophageal mucosa that are readily detected on double-contrast radiographs (Fig. 128.9) [45]. In contrast, CMV esophagitis, which occurs primarily in patients with AIDS, is often manifested by the development of one or more large, relatively flat ulcers in the esophagus [44].

HIV itself has also been associated with the development of giant esophageal ulcers in HIV-positive patients with odynophagia. The lesions typically appear radiographically as giant flat ulcers indistinguishable from those caused by CMV [46]. Because HIV-related ulcers may respond dramatically to treatment with oral steroids, endoscopy is required to differentiate HIV from CMV infection in the esophagus before initiating treatment.

Esophageal carcinoma

Esophageal carcinoma is a deadly disease with an overall 5-year survival rate of only 5%–10%. This dismal prognosis is primarily related to the advanced stage of the disease at the time of clinical presentation. Occasionally, however, esophageal cancer may be discovered at an early stage. Unlike advanced esophageal carcinoma, early esophageal cancer is a readily curable lesion with reported 5-year survival rates approaching 90%. In Western countries, detection of these lesions is best accomplished by some form of radiological or endoscopic surveillance of patients known to be at increased risk for developing esophageal cancer.

Early esophageal cancers classically appear on double-contrast esophagrams as protruded lesions less than 3.5 cm in diameter [47]. They may be plaque-like lesions or small sessile polyps with smooth or slightly lobulated contours. Other superficial spreading carcinomas may be manifested radiographically by tiny coalescent nodules or plaques, causing localized nodularity or granularity of the mucosa [47]. In contrast, advanced esophageal carcinomas appear as poly-



(a) (b)

Figure 128.8 *Candida* esophagitis. **(a)** Double-contrast esophagram shows discrete plaque-like defects with normal intervening mucosa. This appearance is characteristic of candidiasis. From Levine et al. [43], with permission from the Radiological Society of North America. **(b)** Severe *Candida* esophagitis in AIDS patient. Notice the shaggy contour of the esophagus, caused by multiple plaques, pseudomembranes, and ulcers.

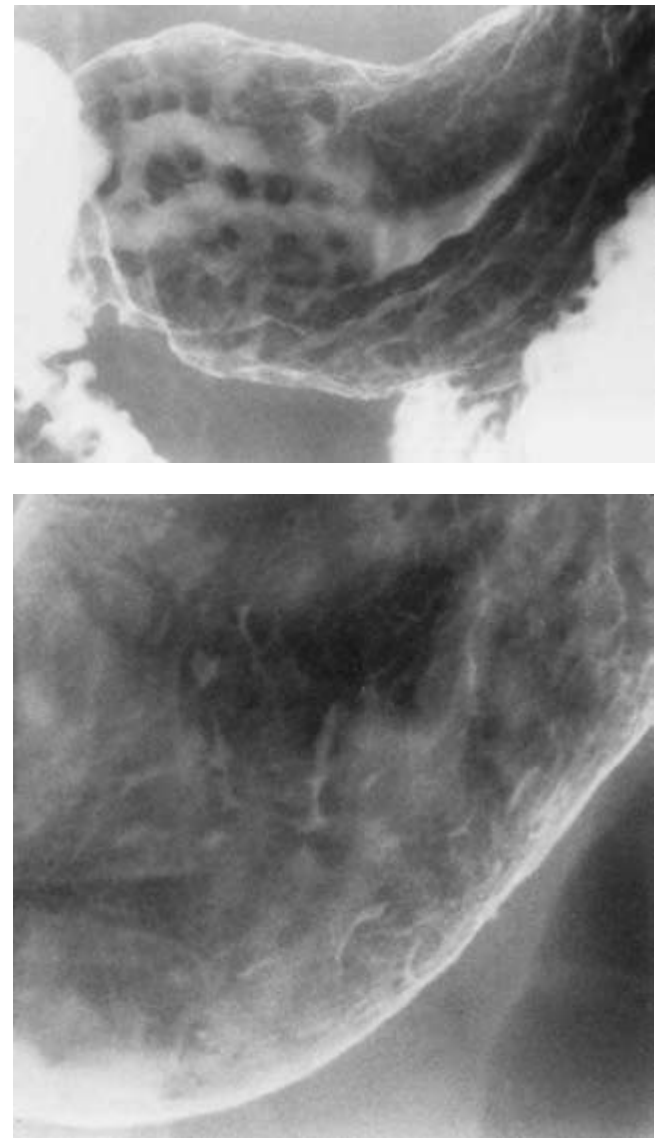
Figure 128.9 Herpes esophagitis with discrete superficial ulcers (arrows) on a relatively normal background mucosa. Radiolucent halos of edematous mucosa surround the ulcers. From Levine et al. [44], with permission from the Radiological Society of North America.

poid, ulcerated, or infiltrating lesions with mass effect, ulceration, or irregular narrowing of the lumen.

Some authors believe that endoscopy is warranted for all patients with dysphagia who have negative esophagrams because of the risk of missing esophageal cancer on barium studies. In one study, however, carcinoma of the esophagus or esophagogastric junction was diagnosed or suspected on double-contrast esophagography in 96% of patients with proven cancers [48]. In the same study, endoscopy was recommended to rule out malignant tumors of the esophagus or esophagogastric junction in only about 1% of all patients who had barium examinations. Thus, a high sensitivity can be achieved in the radiographic diagnosis of these tumors without exposing an inordinate number of patients to unnecessary endoscopy.

Erosive gastritis

Gastric erosions may be classified radiographically as complete or incomplete erosions. Most patients have complete or varioliform erosions in which a punctate or slit-like collection of barium is surrounded by a radiolucent halo of edematous mucosa (Fig. 128.10a) [49]. Varioliform erosions are most commonly found in the gastric antrum and are often aligned on the crests of the rugal folds. In the majority of patients, this finding is caused by ingestion of aspirin or other nonsteroidal antiinflammatory drugs (NSAIDs) [50]. In contrast, incomplete or "flat" erosions appear as dots or streaks of barium without an edematous halo. Because the surrounding mucosa is normal, incomplete erosions have been extremely difficult to detect radiographically, accounting for less than 5% of all erosions seen on double-contrast studies.



(a)

(b)

Figure 128.10 Erosive gastritis. **(a)** Typical varioliform erosions in the gastric antrum. The erosions are aligned on the rugal folds. **(b)** Linear and serpiginous erosions in the body of the stomach near the greater curvature are the result of recent ingestion of indomethacin.

Although erosions caused by aspirin or other NSAIDs are usually located in the gastric antrum, they sometimes occur in the gastric body, appearing as distinctive linear or serpiginous erosions that tend to be clustered together on or near the greater curvature (Fig. 128.10b) [51]. In such cases, it has been postulated that these erosions result from localized mucosal injury as the dissolving tablets collect by gravity in the most dependent portion of the stomach.

Helicobacter pylori gastritis

Helicobacter pylori gastritis can be diagnosed on barium studies by the presence of thickened folds or thickened polypoid folds in the antrum, body or, less commonly, fundus of the stomach [50,52]. It is important to recognize that fold thickening is a nonspecific radiographic finding. Nevertheless, *H. pylori* gastritis should be a leading consideration in the differential diagnosis of thickened gastric folds (particularly in the gastric antrum) in patients with dyspepsia, epigastric pain, or other upper gastrointestinal symptoms [50].

Patients suspected of having peptic ulcer disease could be evaluated by a noninvasive *H. pylori* test combined with a double-contrast upper gastrointestinal examination. If the barium study reveals a gastric or duodenal ulcer, a noninvasive *H. pylori* test could be performed to determine whether antibiotic therapy should be initiated as well as conventional antiulcer therapy. Such an approach might avoid the need for endoscopy in many symptomatic patients.

Gastric ulcers

In the past, some researchers have advocated endoscopy and biopsy of all radiographically diagnosed gastric ulcers to rule out cancer in these patients. However, several studies have found that virtually all gastric ulcers with an unequivocally benign appearance on double-contrast upper gastrointestinal examinations are benign lesions [53,54]. In these studies, about two-thirds of all benign ulcers had a benign radiographic appearance. As a result, unnecessary endoscopy could be avoided in the initial evaluation of most gastric ulcers diagnosed on double-contrast examinations. Instead, typically benign gastric ulcers could be followed radiographically to complete healing without need for endoscopic intervention. Because endoscopy is a considerably more expensive procedure than double-contrast radiography, the potential financial savings are enormous.

Benign gastric ulcers classically appear en face as round or ovoid collections of barium, often surrounded by a smooth mound of edema or thin straight folds that radiate directly to the edge of the ulcer crater [54]. When viewed in profile, benign ulcers project beyond the contour of the adjacent gastric wall and are often associated with an ulcer mound or collar. In contrast, malignant gastric ulcers classically appear en face as irregular ulcer craters within a discrete tumor mass, sometimes associated with nodularity or clubbing of adjacent folds due to infiltration of the folds by tumor. When viewed in profile, malignant ulcers project inside the gastric lumen within a tumor mass that forms acute angles with the adjacent gastric wall rather than the obtuse, gently sloping angles expected for a benign mound of edema.

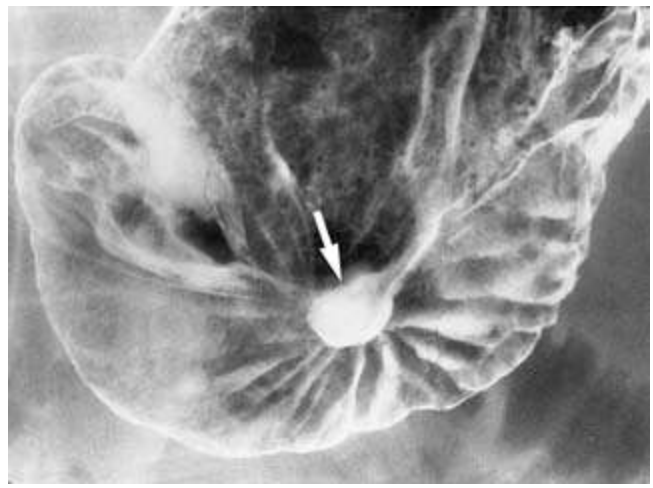


Figure 128.11 Benign gastric ulcer. Round symmetric ulcer (arrow) is seen on the posterior gastric wall, with smooth folds radiating to the edge of the crater. This ulcer has the typical radiological features of a benign gastric ulcer.

Most gastric ulcers detected on double-contrast studies are less than 1 cm in diameter [54]. Although some benign ulcers are round and symmetric, others have a rod-shaped or linear appearance. Almost all benign ulcers occur in the antrum or body of the stomach, and most are located on the lesser curvature or posterior wall (Fig. 128.11) [54]. Occasionally, benign gastric ulcers may be found on the greater curvature of the distal stomach. The latter ulcers are almost always caused by ingestion of aspirin [54]. As these aspirin-induced greater curvature ulcers enlarge, they have a tendency to penetrate inferiorly into the gastrocolic ligament, occasionally leading to the development of gastrocolic fistulae [55].

Ulcer healing may be manifested radiographically by a decrease in the size of the ulcer and by a change in its shape. In most cases, ulcer healing produces a radiographically visible scar with a central pit or depression, radiating folds, or retraction of the adjacent gastric wall [54].

Gastric carcinoma

Gastric carcinoma has a relatively bleak prognosis, with 5-year survival rates of only 10%–30%. Advanced tumors may appear radiographically as polypoid or infiltrating lesions or, less frequently, as scirrhous lesions with diffuse narrowing of the stomach, producing a linitis plastica appearance [56]. In contrast, early gastric cancer is a curable disease, with reported 5-year survival rates as high as 95%. The Japanese have developed an elaborate system for classifying these tumors based on whether they are predominantly elevated, flat, or depressed lesions. Unfortunately, most patients in the USA with gastric carcinoma already have advanced lesions

at the time of clinical presentation. As a result, early gastric cancer is unlikely to be detected on double-contrast studies or endoscopy as long as these examinations are performed predominantly on symptomatic patients [57].

The average sensitivity of single-contrast barium studies for the diagnosis of gastric carcinoma has only been about 75% [58]. Concern about missing gastric cancers on barium studies has therefore been used as a rationale for performing endoscopy as the initial diagnostic test in patients with upper gastrointestinal signs or symptoms. In one study, however, gastric carcinomas were diagnosed or suspected on double-contrast examinations in 96% of patients with proven lesions [58]. In a separate part of the study, only 3.5% of all patients who had double-contrast examinations during this period underwent endoscopy because of findings that were equivocal or suspicious for gastric carcinoma. Thus, a high sensitivity can be achieved in the radiographic diagnosis of gastric cancer while referring only a small percentage of symptomatic patients for endoscopy.

Gastric lymphoma

It is well recognized that chronic *H. pylori* gastritis leads to the development of mucosa-associated lymphoid tissue (MALT) in the stomach. This lymphoid tissue is thought to be the precursor of low-grade B-cell gastric MALT lymphomas which, if untreated, may undergo transformation to more high-grade lymphomas [59]. Gastric MALT lymphomas can sometimes be recognized on double-contrast upper gastrointestinal examinations by variably sized, rounded, often confluent nodules in the stomach (Fig. 128.12) [60]. However, focal gastritis due to *H. pylori* or other causes may produce similar findings, so that endoscopic biopsy specimens are required for a definitive diagnosis. In contrast, advanced gastric lymphoma may be manifested on barium studies by thickened folds, multiple submucosal masses, centrally ulcerated bull's-eye lesions, or giant cavitated lesions [61].

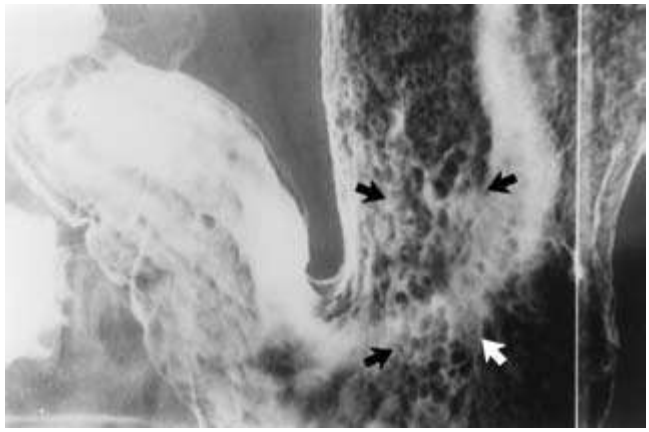


Figure 128.12 Low-grade gastric MALT lymphoma. Multiple confluent nodules of varying sizes (arrows) are seen in the gastric body. From Yoo et al. [60], with permission from the Radiological Society of North America.

Other gastric tumors

Leiomyomas are the most common benign submucosal tumors found in the stomach. These tumors usually appear on barium studies as smooth submucosal masses (with or without central ulceration) that form right angles or slightly obtuse angles with the adjacent gastric wall. Most leiomyomas are less than 3 cm in diameter. In contrast, malignant gastrointestinal stromal tumors usually appear as larger, more lobulated submucosal masses, often containing one or more ulcers or giant areas of cavitation [61]. Ectopic pancreatic rests are usually located on the greater curvature of the distal antrum, appearing as small submucosal masses, sometimes with central umbilication or ulceration. Rarely, barium may reflux into rudimentary ductal structures. Gastric carcinoids may also be manifested on barium studies by one or more small submucosal masses, occasionally containing central ulcers.

Duodenitis

Duodenitis may be manifested radiographically by thickened folds, mucosal nodules, erosions, or deformity of the bulb [62]. Duodenal erosions are detected less frequently than gastric erosions on double-contrast studies because of the difficulty in differentiating these lesions from normal mucosal pits. However, erosive duodenitis can be diagnosed when double-contrast radiographs reveal central barium collections surrounded by radiolucent halos of edematous mucosa.

Duodenal ulcers

Unlike gastric ulcers, which occur primarily on the lesser curvature or posterior wall of the stomach, as many as 50% of duodenal ulcers are located in the anterior wall [36]. Because most double-contrast radiographs are obtained with the patient in a supine or supine oblique position, the anterior wall of the duodenum is not optimally coated with barium, and anterior wall ulcers can be missed on the double-contrast portion of the study. For this reason, double-contrast views of the duodenum should be supplemented with prone compression views obtained with a low-density barium suspension to demonstrate ulcers on the anterior wall. Thus, the biphasic technique is particularly important for evaluating the duodenum.

Small bowel

The conventional small bowel follow-through examination usually consists of a series of overhead abdominal radiographs obtained at regular intervals after ingestion of barium. With this technique, the small bowel is evaluated fluoroscopically only if the overhead radiographs appear abnormal. Because this technique is extremely unreliable in detecting pathology in the small bowel, this form of examination should have no place in modern radiological practice. Instead, adequate evaluation of the small bowel requires more detailed methods of examination, such as the small bowel meal and

enteroclysis. However, these techniques require time, effort, and interest by the radiologist. Barium studies of the small bowel should be requested only if there are legitimate clinical indications of small bowel disease.

Techniques

Small-bowel meal

The dedicated or fluoroscopic small-bowel meal (SBM) includes overhead radiographs but uses fluoroscopy as the primary means of examining the small bowel. Unless contraindicated, metoclopramide (20 mg) is often administered orally 20 min before the examination to accelerate transit of barium through the stomach and small bowel. The patient then ingests 500–600 mL of an appropriate 35%–40% (weight/volume) suspension of barium sulfate. After a brief examination of the upper gastrointestinal tract, intermittent fluoroscopy is performed until all small bowel loops have been demonstrated. Periodic spot images are obtained to document the fluoroscopic findings.

Enteroclysis (small-bowel enema)

A more detailed study of the entire small bowel may be performed by enteroclysis [63]. If not contraindicated, the patient swallows 20 mg of metoclopramide to accelerate small-bowel transit. Surface anesthesia to the throat precedes intubation of the distal duodenum or proximal jejunum. Between 200 and 250 mL of a 75%–80% suspension of barium is then injected, followed by infusion of 1–2 L of a 0.5% solution of methylcellulose in water. These biphasic examinations first demonstrate the small bowel in single contrast (Fig. 128.13a) and then in lumen-distended double contrast (Fig. 128.13b). The radiologist must be present throughout the study to assess individual bowel loops by graded compression and to document the fluoroscopic findings with spot images. An overhead radiograph of the entire small bowel completes the examination.

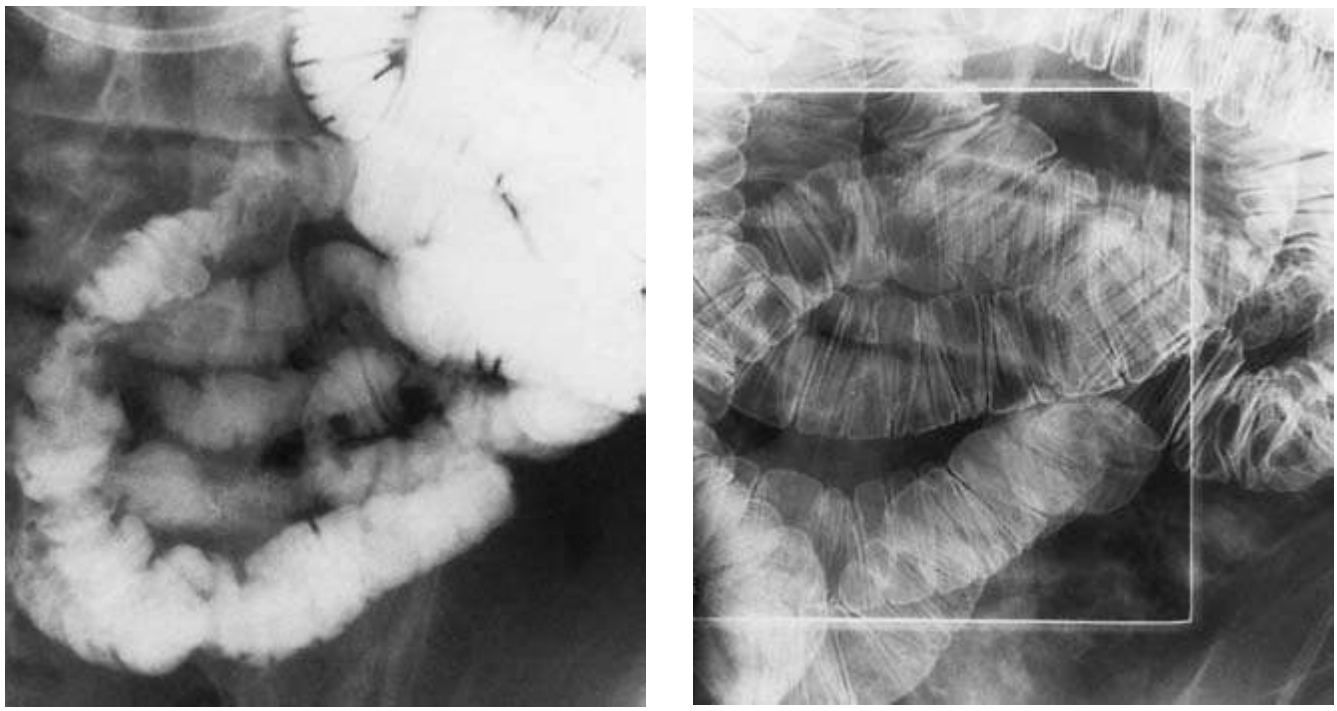
Application of techniques

Small-bowel meal

The major advantage of an SBM is that it does not require intubation. Transit acceleration by oral metoclopramide is an important component of the examination because it decreases flocculation of barium and the time needed to perform the study. However, distention of the bowel lumen cannot be achieved by this technique. The SBM is an appropriate examination for evaluating established diseases of the small bowel, such as Crohn's disease. It is also adequate for investigating pathological conditions not associated with lumenal distention such as radiation enteritis, ischemia, and hematomas. This technique can be used to demonstrate the level and degree of small-bowel obstruction, although it is less successful in determining the cause.

Enteroclysis

The major advantage of enteroclysis is that it combines transit acceleration with lumenal distention. Enteroclysis virtually



(a) (b)

Figure 128.13 Normal small-bowel enema. **(a)** Distal jejunum seen on a preliminary single-contrast examination. **(b)** Same jejunal loops on double-contrast examination.

prevents flocculation of barium. As a result, the double-contrast images permit assessment of mucosal surface detail, even through overlapping small bowel loops. The straightening of mucosal folds that occurs with lumenal distention permits detection of small, relatively subtle lesions. Normality becomes measurable in terms of wall thickness; fold thickness, shape, and height; and distance between folds.

The major indications for enteroclysis include the following:

- Small-bowel obstruction, particularly intermittent obstruction: enteroclysis often permits differentiation of the various causes of obstruction [64].
- Malabsorption states: enteroclysis can identify features of celiac disease, lymphangiectasia, Whipple disease, morphological changes associated with bacterial overgrowth, and other conditions associated with malabsorption.
- Crohn's disease: enteroclysis can delineate the full extent of disease, including skip lesions and fistulae and can be used to distinguish fibrous strictures from stenotic disease.
- Small-bowel tumors, particularly carcinoids, other primary and secondary malignancies, and lymphoma.
- Meckel diverticulum: increased lumenal distention greatly improves the detection rate [65].
- Normality: enteroclysis is the most reliable radiological examination for establishing morphological normality of the small bowel.

Abnormalities

Small-bowel obstruction

Concern has been expressed regarding the possible inspissation of barium within obstructed small bowel. Although animal studies and clinical experience have shown that such inspissation does not occur [66], it is often recommended that barium-based techniques be replaced by CT or by CT enteroclysis in patients who have high-grade or complete small-bowel obstructions [63]. With lower grades of obstruction, barium enteroclysis remains a useful test for delineating the site and cause of obstruction, particularly in patients with intermittent obstruction. If a decompression tube has already been positioned beyond the duodenum, it can be used to perform the enteroclysis examination.

Adhesions

Adhesions account for almost 75% of small-bowel obstructions. Enteroclysis can identify features that favor obstruction by a single band, multiple bands, or extensive adhesions. Single bands are more likely to produce high-grade obstruction. A closed-loop obstruction may be caused by prolapse of a bowel loop beneath an adhesive band. This type of obstruction is associated with twisting of bowel, with a high risk of ischemia and necrosis. Although barium studies may be helpful in the early stages of a closed-loop obstruction, CT is essential for establishing the degree of vascular compromise in these patients.

Hernias

Barium studies are not required for small-bowel obstructions caused by clinically evident external hernias. However, enteroclysis is useful in detecting small-bowel obstructions caused by hernias of developmental or postsurgical origin, including incisional, peristomal, Spigelian, or Richter hernias.

Malignancy

In patients who have undergone laparotomy for abdominal malignancy, small-bowel obstruction may be caused by adhesions, metastases, radiation, or residual tumor. These conditions can be differentiated by enteroclysis in more than 80% of patients [63]. This information can facilitate surgical management by providing a preoperative choice between resection of tumor, bypass of radiation-damaged bowel, or lysis of adhesions.

Malabsorption states

Malabsorption is usually diagnosed by clinical and laboratory criteria. The purpose of barium studies is to determine the most likely cause of a clinically diagnosed malabsorption state or to document complications. In many conditions associated with malabsorption, abnormal accumulation of intralumenal fluid causes flocculation of barium on SBM. Because flocculated barium prevents accurate depiction of mucosal surface detail, enteroclysis is the preferred radiological examination in these patients.

Celiac disease

The diagnosis of celiac disease must be firmly established to justify placing the patient on lifelong dietary restrictions. A confident diagnosis can be made in patients who have characteristic mucosal changes on duodenal biopsy and have shown a satisfactory response to gluten withdrawal. However, more than 50% of patients have atypical clinical presentations. As a result, the diagnosis may first be suggested by enteroclysis, which can demonstrate a measurably increased separation of folds in the distended proximal jejunum (Fig. 128.14). In one study, zero to three folds per 2.5 cm of jejunum were found by enteroclysis in 73% of celiac patients but in only 2% of controls [67]. Conversely, patients with five or more folds per 2.5 cm of jejunum rarely had celiac disease.

Enteroclysis is especially important in celiac patients who relapse after an initial dietary response; this technique can aid in the diagnosis of ulcerative jejunoileitis, T-cell lymphoma, and intestinal carcinoma as complications of long-standing celiac disease.

Bacterial overgrowth syndrome

Barium studies can accurately depict many structural abnormalities in the small bowel that cause stasis or contamination and lead to bacterial overgrowth. These abnormalities include strictures, blind pouch and blind loop syndromes, jejunoileal diverticulosis, and coloenteric fistulae. Other patients with

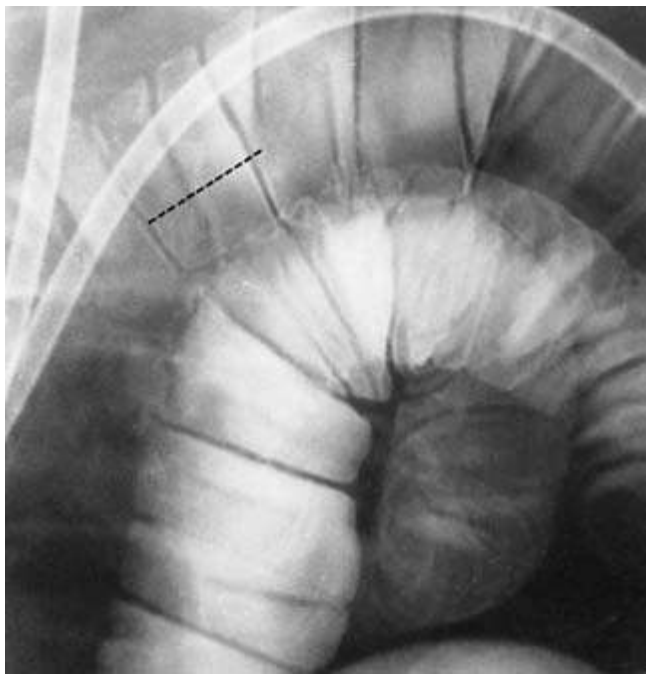


Figure 128.14 Diagnosis of celiac disease on a small-bowel enema. The distended proximal jejunum shows an increased separation of folds, with only three folds per 2.5 cm (dotted line).

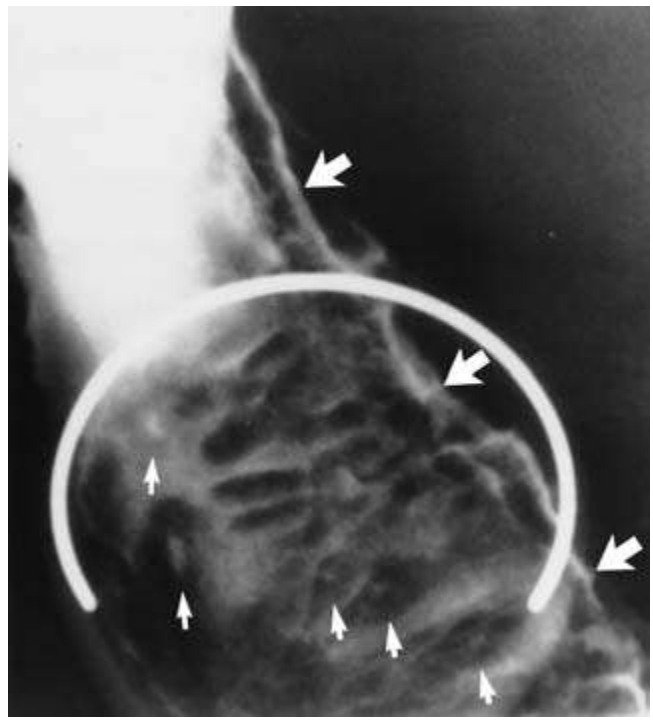


Figure 128.15 Small-bowel Crohn's disease. Spot image from enteroclysis examination shows a long linear ulcer (large arrows) on the mesenteric border of the bowel with multiple aphthous ulcers (small arrows) and thickened, interrupted folds. The linear mesenteric border ulcer is characteristic of Crohn's disease.

bacterial overgrowth syndrome may have chronic intestinal pseudoobstruction due to scleroderma or other causes. Small-bowel involvement by scleroderma may lead to intestinal dilatation with crowded folds, producing the "hide-bound" sign that is virtually pathognomonic of this condition [68].

Intestinal lymphangiectasia

The primary and secondary forms of intestinal lymphangiectasia are manifested by nonspecific changes of fold thickening and fluid increase on conventional barium studies. With enteroclysis, it is possible to demonstrate 1–2 mm micronodules representing villi distended by dilated lacteals. Whipple disease involving the small bowel may also be manifested by tiny nodules due to villous distention by the enlarged lamina propria laden with foamy macrophages, bacilli, and periodic acid–Schiff (PAS)-positive material. A similar nodular pattern may be demonstrated in patients with *Mycobacterium avium* complex enteritis, macroglobulinemia, IgA deficiency, and other unusual conditions.

Crohn's disease

Early radiological findings of Crohn's disease in the small bowel include a coarse villous pattern of the mucosa, thickened folds, and aphthous ulcers. The presence of linear ulcers along the mesenteric border of the bowel with pleating or sacculation of the uninvolved antimesenteric border is virtually diagnostic of Crohn's disease (Fig. 128.15). With progression of disease, transaxial extension of linear ulcers and fissures produces a cobblestone appearance with narrowing of the lumen. Further progression of Crohn's disease leads to complications such as fibrous strictures, fistulae, inflammatory masses, and abscesses.

Many features of Crohn's disease can be demonstrated as effectively by SBM as by enteroclysis. Disease of the terminal ileum is particularly well shown by combining SBM with a peroral pneumocolon (i.e., introducing barium by mouth and air via the rectum) [69]. However, enteroclysis is the optimal radiological examination for detecting early lesions, particularly the more proximal skip lesions. It is also a better examination for distinguishing Crohn's disease from other pathological conditions such as lymphoma, tuberculosis, ischemia, and metastatic disease.

Malignant tumors

Adenocarcinoma

Most primary adenocarcinomas of the small bowel are advanced tumors with regional lymph node involvement at the time of clinical presentation. In the absence of obstruction, these tumors can be missed on conventional small bowel follow-through examinations. Enteroclysis is the best radiological technique for demonstrating these lesions (Fig. 128.16).

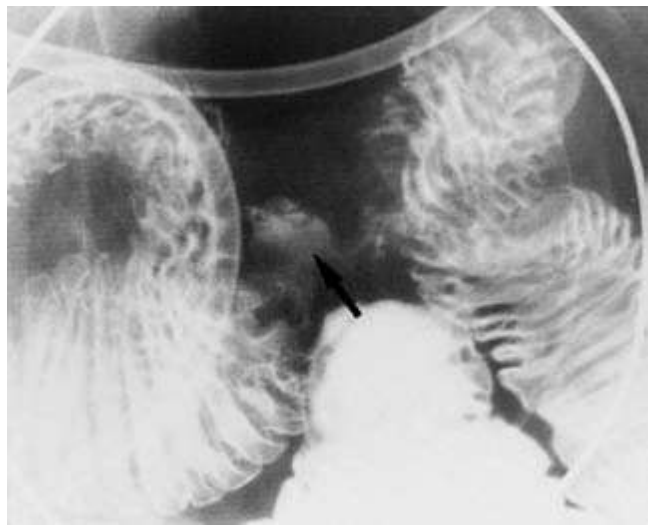


Figure 128.16 Annular adenocarcinoma of the proximal jejunum with a central ulceration (arrow).

Non-Hodgkin lymphoma

Primary small-bowel lymphomas typically appear as cavitary lesions extending into the mesentery or as segmental infiltrating lesions. In other patients with disseminated lymphoma, barium studies may demonstrate submucosal nodules of various sizes. An enlarging mass of mesenteric nodal lymphoma also can secondarily infiltrate a loop of small bowel [63]. CT can be used to better delineate the extent of mesenteric and lymph node involvement.

Other malignancies

Small-bowel carcinoids can be diagnosed by enteroclysis at the stage of incipient transmural extension of the primary tumor or tumors (Fig. 128.17). More advanced carcinoids are typically associated with distorted folds. Enlarging mesenteric metastases produce mass effect and secondary fibrotic alterations in adjacent bowel loops. Early carcinoids may appear as small polypoid nodules, more often in the terminal ileum.

Both hematogenous and intraperitoneal-seeded metastases to the small bowel tend to occur as multiple lesions, often associated with signs of obstruction. Gastrointestinal stromal tumors may appear as excavated exoenteric masses.

Meckel diverticulum

Enteroclysis is the best radiological technique for diagnosing Meckel diverticulum, with an overall detection rate comparable to that found at autopsy [65]. The diverticulum arises from the antimesenteric border of the distal ileum, forming a blind sac, with a characteristic mucosal fold pattern at its site of origin. Occasionally, it is possible to identify a defect in the diverticulum due to ectopic gastric mucosa. It is also possible to identify an inverted Meckel diverticulum acting as the lead point for a small-bowel intussusception [70].

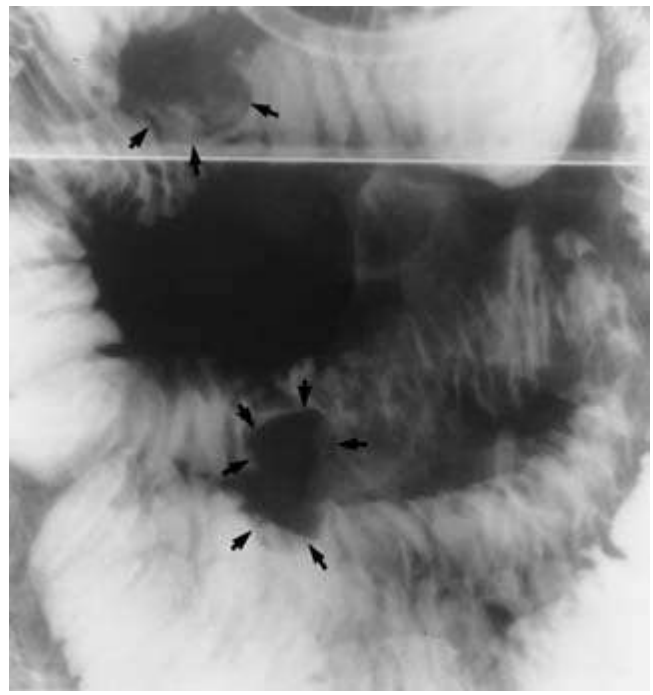


Figure 128.17 Small-bowel carcinoids. Discrete submucosal masses (arrows) are seen in the mid small bowel. Also note separation of small bowel loops caused by spread of tumor into the mesentery.

Obscure blood loss

In patients with unexplained gastrointestinal blood loss, enteroclysis is indicated when radiological and endoscopic examinations of the upper gastrointestinal tract and colon fail to demonstrate a source of bleeding. Enteroclysis has a diagnostic yield of 21% in identifying the presumptive source of obscure gastrointestinal bleeding [71]. The majority of these patients are found to have tumors involving the small bowel; other causes include ischemia, arteriovenous malformations, Meckel diverticulum, and celiac disease.

Colon

Since the 1920s, the barium enema has been used as the primary nonsurgical method for investigating the colon. Since the 1970s, colonoscopy has become an important diagnostic modality for evaluating the colonic mucosa and for removing polyps. Cross-sectional imaging techniques, particularly CT, have also become important for evaluating the extramucosal extent of disease. CT and ultrasound may also be used to assess bowel wall thickness. However, because of its simplicity, low cost, safety, and accuracy, the barium enema remains a useful diagnostic technique for investigating the colon.

Single versus double contrast

Radiological examination of the colon can be performed by single- or double-contrast technique. In single-contrast studies, the entire colon is filled with a low-density barium suspension. The examination is performed under fluoroscopic

control, with extensive palpation and compression of the colon as it fills. A postevacuation radiograph is usually obtained to demonstrate additional mucosal detail. In double-contrast studies, a smaller volume of high-density barium is introduced into the colon, followed by insufflation of air. With this technique, the mucosal surface is coated by a thin layer of high-density barium, and the lumen is distended with air.

The double-contrast examination is considered to be an extremely useful radiological technique for showing fine mucosal lesions such as small polyps and the early changes of inflammatory bowel disease (IBD) [72,73]. It can also better demonstrate segments of the large bowel that are inaccessible to palpation, such as the rectum and the hepatic and splenic flexures. The single-contrast barium enema is the preferred technique when careful control of the flow of barium is required in patients with suspected obstruction, acute diverticulitis, fistulae, or Hirschsprung disease. This technique is also frequently used in patients who are too old or debilitated to tolerate a double-contrast study.

Radiology versus colonoscopy

There is ongoing controversy about the relative roles of radiology and endoscopy in investigating colonic disease. In general, the choice of technique depends on the clinical setting and the relative skill and experience of the examiner. Nevertheless, it should be recognized that colonoscopy is primarily of value for detecting mucosal lesions. It should not be used as the primary diagnostic modality when the patient's symptoms suggest an intramural or extrinsic lesion involving the bowel.

Colonoscopy is probably more accurate than radiology in demonstrating subtle mucosal abnormalities in the colon. When compared with high-quality double-contrast barium enemas, this benefit applies primarily to the detection of polypoid lesions less than 1 cm in diameter and the early preulcerative changes of IBD. However, this benefit must be balanced against the higher cost and complication rate of colonoscopy. The colonoscopist is unsuccessful in advancing the endoscope to the cecum in about 10% of patients [74], but the cecum is almost always visualized on barium enema studies.

We recommend contrast examination of the colon for patients with symptoms that could be caused by diseases of the colon (e.g., abdominal pain, constipation, change in bowel habit, anemia). Colonoscopy is generally the examination of choice for the evaluation of uncertain radiological findings, for removal or biopsy of lesions found at barium enema, and for the evaluation of patients at high risk for developing colorectal cancer or who have occult blood in the stool.

Indications

Although radiological examination of the colon may be performed for a wide variety of indications, the most common include detection of colorectal polyps and cancer; diagnosis of IBD and assessment of the type, extent, and severity of disease; diagnosis of diverticular disease and its complications; evaluation of extrinsic mass lesions involving the colon; and evaluation of the rectum.

Colorectal polyps and cancer

The barium enema is a valuable diagnostic test for symptomatic patients with colorectal cancer. It is useful not only for diagnosing the primary lesion but also for detecting synchronous cancers elsewhere in the colon. The double-contrast barium enema has an overall accuracy of about 95% in detecting colorectal cancer. However, there appears to be an irreducible minimum error rate of approximately 5%, primarily the result of perceptive error [75].

The double-contrast barium enema is also used to evaluate patients who have overt rectal bleeding or occult blood in the stool on routine screening tests for colorectal cancer. In such cases, the goal is to detect invasive carcinomas as well as small adenomas that are precursors of colonic carcinoma (Fig. 128.18). These adenomatous polyps should be removed endoscopically to prevent the subsequent development of cancer.

Contrast examination of the colon may also have a role in the routine screening of average-risk patients for colorectal cancer. Eddy and colleagues suggested that annual fecal occult

Figure 128.18 Example of a coexisting colonic carcinoma and benign adenoma in the same patient. Note the polypoid carcinoma (black arrows) in the transverse colon and small tubulovillous adenoma (white arrow) in the distal sigmoid colon.

blood testing and barium enema every 5 years may be the most cost-effective way of reducing mortality from colorectal cancer [76]. Recently, CT colonography (virtual colonoscopy) has also been widely advocated as a noninvasive radiological test for colorectal cancer screening [77], although the ultimate value of this technique remains uncertain.

Inflammatory bowel disease

Radiological examination of the colon serves a variety of purposes in patients with known or suspected IBD. It can establish the presence of disease in patients who have not had prior sigmoidoscopy or in those with negative sigmoidoscopy because the disease did not involve the rectosigmoid colon. It can also define the extent of disease in the colon. This information is particularly important in patients with ulcerative colitis, because the risk of developing carcinoma is related to the extent of colonic involvement.

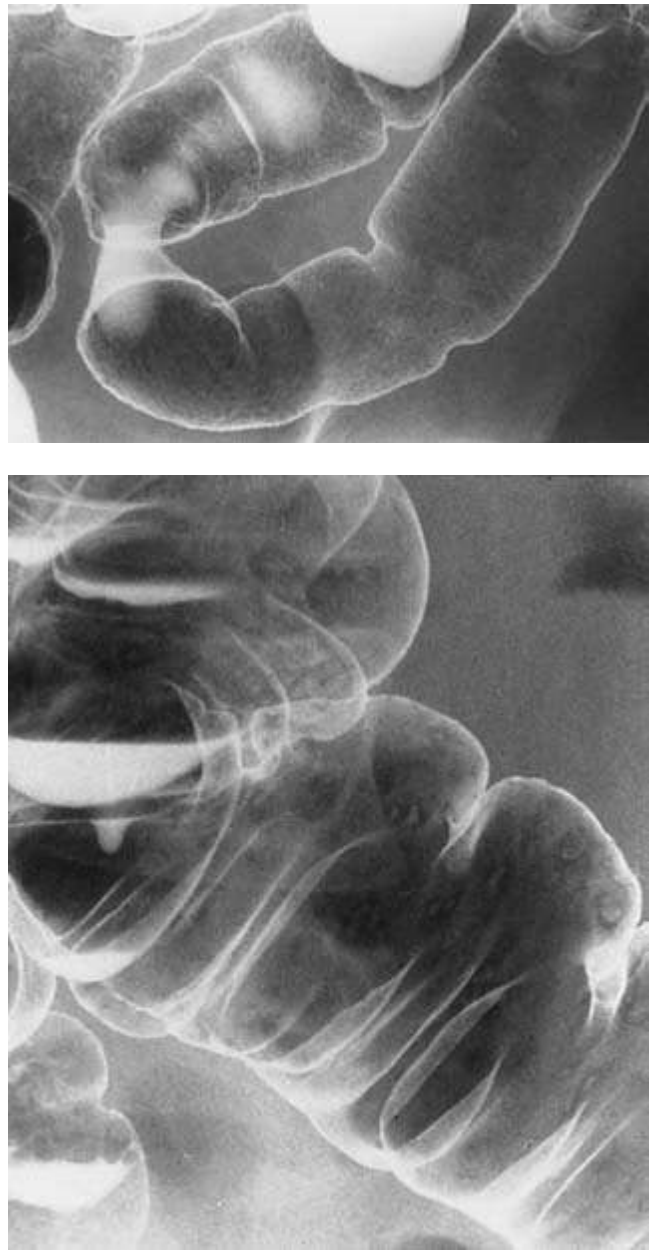
Radiological examination of the colon can differentiate ulcerative from granulomatous colitis. In typical cases, ulcerative colitis is characterized on double-contrast radiographs by a granular mucosa involving the rectum and extending proximally to a variable degree (Fig. 128.19a). In contrast, granulomatous colitis is characterized by progression from discrete aphthous ulcers (Fig. 128.19b) to transmural disease with deep ulcers, fissures, fistulae, and abscesses. The rectum is often spared, and colonic involvement tends to be discontinuous and patchy.

Radiological examination can also detect complications of chronic ulcerative or granulomatous colitis, including strictures, abscesses, fistulae, and inflammatory or post-inflammatory polyps. In patients who develop carcinoma as a complication of chronic ulcerative colitis, the tumor can be diagnosed and in some cases the development of macroscopic dysplasia can also be recognized on double-contrast studies [78].

Diverticular disease

Diverticular disease is one of the most common afflictions of Western society. The presence of massive diverticulosis poses a particular dilemma in interpreting double-contrast studies, because the multiplicity of ring shadows makes it difficult to differentiate diverticula from polyps. As a result, single-contrast studies may be easier to interpret than double-contrast studies in patients with severe diverticulosis.

Diverticulitis is a complication of diverticulosis in which a diverticular perforation leads to the formation of a pericolic abscess. Because barium may extravasate into the abscess, it is important to control the flow of barium into the colon in these patients. We therefore prefer a single-contrast technique when a barium enema is performed for suspected diverticulitis (Fig. 128.20). In recent years, however, CT has increasingly been used as the primary radiological modality in patients with suspected diverticulitis because of its relatively high sensitivity and specificity for diagnosing this condition [79].



(a)

(b)

Figure 128.19 Early findings of inflammatory bowel disease on double-contrast barium enemas. **(a)** Ulcerative colitis with typical granular mucosa in the sigmoid colon. **(b)** Granulomatous colitis with discrete aphthous ulcers in the transverse colon.

Extrinsic mass lesions

In patients with abdominal or pelvic masses, the barium enema is a useful technique for determining whether the colon is displaced, compressed, or actually invaded by these lesions. The barium enema can also detect intraperitoneal seeding of the colon by metastatic tumor, inflammatory lesions, or endometriosis. In many cases, the barium study must be correlated with CT or other cross-sectional imaging

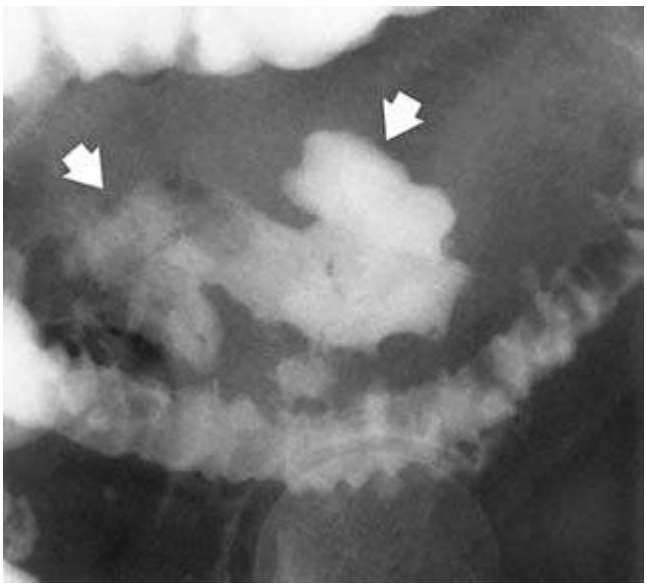


Figure 128.20 Diverticulitis with pericolic abscess. Barium has extravasated from a perforated diverticulum into an abscess (arrows). Notice the diverticula in the sigmoid colon.

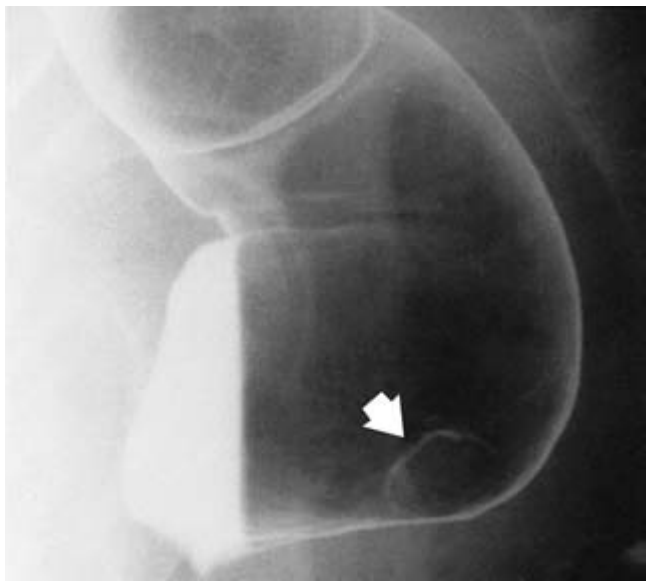


Figure 128.21 Villous carcinoma (arrow) of the posterior wall of the rectum that had been missed on prior digital and proctoscopic examinations.

modalities to determine the true extent of disease in the abdomen.

Rectum

When barium enemas were performed primarily by single-contrast technique, the rectum was not considered to be the province of radiology. With the use of double-contrast technique, however, the rectum has become one of the simplest portions of the large bowel to evaluate on barium studies.

The anatomy of the rectum, including the columns of Morgagni and the valves of Houston, can be demonstrated in exquisite detail [80]. Radiological evaluation of the rectum is particularly important for the diagnosis of rectal tumors, IBD, and other unusual conditions such as the solitary rectal ulcer syndrome [81]. The double-contrast examination can also detect rectal carcinomas that are missed by digital rectal examination and proctoscopy (Fig. 128.21) [82]. The routine double-contrast barium enema should therefore include a careful examination of the rectum.

Contraindications

The barium enema examination is generally a safe procedure, but there are several conditions where the study is contraindicated or should be performed with extreme caution. These conditions include toxic megacolon, ischemic colitis, pseudomembranous colitis, or other diseases in which the bowel wall is friable and more likely to perforate during the procedure. When a colonic perforation is suspected, the radiological examination should be performed with water-soluble iodinated contrast material that can be reabsorbed from the peritoneal cavity if extravasation occurs.

Complications

The most feared complication of the barium enema is colonic perforation [83]. When this occurs, the perforation usually involves the intraperitoneal portion of the colon. Extravasated barium in the peritoneal cavity may be seen outlining loops of bowel. Surgical treatment is usually required. On some double-contrast barium enemas, a colonic perforation may be manifested only by a pneumoperitoneum without extravasation of barium into the peritoneal cavity. If these patients are aggressively treated with intravenous fluids and antibiotics, surgery may be avoided in some cases.

Retroperitoneal perforations usually result from laceration of the rectum by an inflated retention balloon on the enema tip in patients with diffuse rectal disease such as ulcerative or radiation proctitis. In such cases, barium may be observed in the rectal wall or in the perirectal soft tissues. Because of the risk of rectal laceration, it is our practice not to routinely inflate the retention balloon. When the balloon is required in patients who have poor anal sphincter tone, it should be inflated under careful fluoroscopic control after barium has been instilled into the rectum.

Despite occasional complications, the barium enema examination is a safe, simple, and relatively inexpensive procedure. It provides reliable information about the nature and extent of mucosal disease and about disease within and outside the bowel wall. Most patients undergo barium enemas to rule out colonic neoplasm or IBD, and a double-contrast study is the best radiological technique for evaluating these individuals. In other patients with suspected diverticulitis or colonic obstruction, a single-contrast barium enema may be performed. Whether a single- or double-contrast technique

is used, the barium enema examination has few contraindications, and the complications can be minimized by careful attention to the clinical history and examination technique. In complicated or difficult cases, correlation of the radiological and endoscopic findings may be necessary to clarify the nature and extent of colonic disease.

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B IMAGING

129 Diagnostic sonography

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Basic principles of diagnostic ultrasound

Sonography is complex and technically challenging. It requires knowledge of physics, instrumentation, ultrasound anatomy, pathology, and, most important, scanning technique. Small handheld scanners make the concept of sonography as an extension of the physical examination a real possibility. But those wishing to use sonography must learn the technique adequately, otherwise erroneous and misleading results are inevitable. At least 4 months of full-time intensive training is needed for those wishing to perform even the simplest sonographic evaluation.

Ultrasound refers to the frequencies of sound above the audible range – more than 20 000 cycles per second (i.e., > 20 000 hertz [Hz]). Diagnostic sonography uses sound frequencies far higher than this, in the 1–20 million Hz range (i.e., 1–20 megahertz [MHz]). Diagnostic ultrasound systems transmit sound pulses into the body. The echoes reflected from tissue are then processed to produce ultrasound images. The heart of a modern transducer is a complex array of specially manufactured piezoelectric elements that convert electrical energy into sound waves when transmitting and then convert the faint returning sound into electrical signals that can be processed into images.

In sonograms, the depth of any tissue is determined by timing how long it takes an echo to return, then calculating depth (distance from the transducer) based on an assumed average velocity of sound in human tissue. This is the pulse–echo principle. The earliest returning echoes arise from tissue reflectors closer to the transducer; later echoes come from progressively deeper reflectors. The exact anatomical location of each reflector may be calculated from the known direction of the sound beam in the body and the measured time of arrival of its echo. Repeated pulses are used to generate a two-dimensional image of the region interrogated.

Acoustic interfaces reflect sound and generate the echoes that make up the returning ultrasound information. Acoustic interfaces occur where two tissues of different acoustic impedance are in contact. Acoustic impedance is related to tissue density and the speed of sound in the tissues. The greater the acoustic impedance difference between the two tissues at an interface, the greater the percentage of incident sound reflected and the stronger the returning echo. This explains why ultrasound cannot "see through" gas or bone. The soft tissue–bone and soft tissue–gas interfaces have huge acoustic impedance differences, causing nearly all sound to be reflected. Clearly, if nearly all the sound beam is reflected, tissues deep to the bone or gas will not be imaged.

The amplitude (loudness) of echoes reflected from any given tissue is represented by different shades of gray. Most ultrasound images use a black background display. With this display convention, whiter shades of gray represent higher amplitude echoes. Descriptively, echo-free structures are said to be anechoic. Isoechoic means identical in echogenicity to another tissue. Hypoechoic means lower in echogenicity than another tissue, whereas hyperechoic (or echogenic) means higher in echogenicity than another tissue.

The ultrasound beam is progressively attenuated (weakened) as it travels deeper into the body. Soft tissues are more attenuative than fluid-filled structures. Cystic structures cause little attenuation of sound so tissues deep to the gallbladder or liver cysts appear more echogenic (brighter echoes) than do neighboring areas at a similar depth. This is called *distal acoustic enhancement* or *through transmission* of sound.

There is a tradeoff between the use of high-frequency and low-frequency transducers in ultrasound imaging. Low-frequency sound penetrates tissue effectively, facilitating good images of deep tissues (e.g., large livers). Higher-frequency transducers produce higher-resolution images, but cannot penetrate sufficiently well to produce images deeper than relatively superficial structures. As a practical matter, abdominal

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organ sonography is done with lower-frequency transducers (3–7 MHz). When the bowel itself (e.g., appendiceal ultrasound) is evaluated, higher-frequency transducers (5–10 MHz) are generally used. It is generally best to choose the highest-frequency transducer that allows adequate penetration.

Artifacts are a significant problem in ultrasound imaging. They may be related to equipment problems, but unavoidable artifacts caused by the interaction of sound and tissues are the most troublesome. Speckle is the result of interference patterns caused by the sound beams sent into tissue. Reverberation of sound between tissue and the face of the transducer can obscure detail, most notably in the near field. Blockage of sound results in acoustic shadowing – a "void" in the image deep to the structure or tissue that causes the shadowing. Acoustic shadowing may be complete or partial. Reflection, refraction, or absorption of the sound beam may cause acoustic shadowing. Shadowing occurs deep to obstructing objects (such as gallstones, bone, or gas), or deep to curved interfaces at the margin of cysts. Shadowing is one of the diagnostic criteria for gallstones; it can be useful in distinguishing gas or calcification from other echogenic reflectors. The mirror image and multipath reflection artifacts can cause tissue to appear at an incorrect depth or location, simulating, for example, liver tissue above the diaphragm, or an apparent double inferior vena cava (IVC). Side lobe and partial thickness artifacts may lead to spurious hazy echoes that are most evident when superimposed on echo-free structures (e.g., the gallbladder). Comet-tail artifacts appear as highly echogenic "tails" deep to small intense reflectors such as small air bubbles, surgical clips, and small cholesterol crystals.

Tissue harmonic imaging is a technique that results in less image artifact. It uses the nonlinear sound produced by the ultrasound beam passing through tissue to produce images. This is distinct from conventional ultrasound, which produces images from echoes (reflected sound). Because of the decrease in artifact, tissue harmonics are becoming more popular as a way to obtain grayscale images.

Doppler sonography

Doppler sonography is a routine component of most modern sonographic examinations. It adds dynamic real-time flow information to the morphological information provided by grayscale imaging. Flowing blood is detected by its Doppler frequency shift. Stationary soft tissue lacks a Doppler frequency shift; echoes without a Doppler shift are ignored in the production of flow images.

Color Doppler sonography passively and automatically displays color-coded flow information superimposed on all or a selected portion of the grayscale image, all in real time. It detects flow, and displays both flow direction and frequency shift (a rough estimate of flow velocity). Flow direction is usually displayed as red or blue; velocity estimates are depicted as different hues or a change in color saturation. Color Doppler sonography is invaluable when flow detection or global anatomical information about blood flow is needed. The ability of color Doppler sonography passively to display flow in real time minimizes the chance of missing flow in an unexpected area, and facilitates comparisons of flow in different anatomical locations. Color Doppler sonography has the advantage of displaying both flow and anatomical information in a real-time image, which is much easier to understand than a combined grayscale/spectral "duplex Doppler" display.

Spectral Doppler acquires detailed flow information from a small area (the sample volume or range gate). Spectral Doppler is often erroneously called *duplex Doppler*. In fact, both color and spectral Doppler are "duplex" – both display flow and morphological information simultaneously. Spectral Doppler is useful when detailed quantitative information about flow velocity or pulsatility is important (e.g., liver transplants). The information is displayed graphically as a "time–velocity waveform," scrolled out across the screen. Spectral Doppler information can also be processed into an audible signal that is useful to experienced examiners. For example, arteries can be distinguished from veins audibly, and the higher pitched sound of stenosis can be useful in detecting arterial narrowing. In many situations, color Doppler sonography is first used to survey a vessel to facilitate fast and accurate spectral Doppler sample volume placement.

Ultrasound contrast agents

No ultrasound contrast agent (USCA) has so far received the approval of the US Food and Drug Administration (FDA) for radiological imaging. Limited approval has been granted for cardiac sonography. However, significant contrast research, as well as daily clinical use, is occurring worldwide. Use of these agents improves the diagnostic power of ultrasound imaging and may expand ultrasound's role into diagnosis of conditions where computed tomography (CT), magnetic resonance imaging (MRI), and nuclear medicine are dominant. Most USCAs are intravenously injected microbubbles smaller than a red blood cell and thus capable of passing through capillary beds.

Microbubble ultrasound contrast agents enhance the useful ultrasound signal detected by the ultrasound transducer. The increased signal is depicted as an increase in echogenicity [1] or increase in signal strength. Ultrasound contrast agents thus affect grayscale imaging as well as color or spectral Doppler imaging.

Vascular enhancement with USCAs may improve examination quality by improving flow visualization and analysis. Stenosis and thrombus determinations are made with more confidence [2]. Smaller, deeper vessels and greater lengths of vessels may be visible, and the Doppler signal from vessels is increased.

Although some USCAs do not enter the tissues, all traverse both large vessels and small capillaries. Thus, on grayscale imaging, they can appear to enhance an entire organ rather

than merely its large arteries and veins. Grayscale enhancement is a new method of ultrasound imaging, which may dramatically change sonographic tumor evaluation and diagnosis [3]. Grayscale enhancement with harmonic imaging dramatically increases the conspicuity of the ultrasound signal from microbubbles. Harmonic imaging takes advantage of the microbubbles' property of resonance during insonation [4]. Benefits of harmonic imaging and other techniques include a significantly increased signal-to-noise ratio of the image [4].

Strengths and weaknesses compared with other modalities

When sonography can image the area of clinical interest, it is a near ideal modality. The ability of diagnostic sonography to display flow and soft tissue in real time is unique among imaging techniques. Its spatial resolution is superior to CT and MRI. It is safe and tolerated well by patients. When needed, ultrasound can be performed quickly and at the bedside. Unfortunately, there are circumstances in which optimal, or even adequate images cannot be obtained. The single most important problem is sonography's inability to see beyond gas–soft tissue or bone–soft tissue interfaces. This makes comprehensive survey scanning essentially impossible. Computed tomography especially, and MRI are better survey modalities. Thus, some abnormalities displayed automatically on whole-body CT or MRI are either impossible or difficult to see sonographically. Lastly, sonography is technically challenging – scanning to obtain optimal images is more of an art than a science. Producing acceptable diagnostic images sonographically is usually harder than obtaining acceptable CT or MRI images. Sonography, however, is often superior to CT and MRI in patients who are uncooperative, unable to hold their breath, or unable to remain relatively still during examination. Sonography is often superior in patients with little body fat. This makes sonography useful in pediatric imaging, for example. Patient factors that impede optimal sonography include extreme obesity and limited cutaneous access, such as burns, incisions, dressings that cannot be removed, and cutaneous gastrointestinal (GI) enterostomies.

Liver

Indications for sonography

Primary indications include suspected or known cirrhosis, suspected liver abscess, suspected liver tumor, suspected fatty liver, suspected vascular abnormalities, trauma, and transplantation. Sonography can also be used to characterize abnormalities found on other imaging examinations. Its safety and relatively modest cost make it an ideal means to assess the therapeutic response of known lesions, or sequentially to follow liver lesions of unknown significance. Sonography is often a simple and effective way to guide percutaneous aspiration, drainage, biopsy, or tumor ablation.

Patient preparation and technique

Hepatic sonography can be performed without special preparation. As the other upper abdominal organs are often evaluated with the liver, an overnight fast or 8–12 h of a clear liquid diet is recommended to decrease bowel gas and distend the gallbladder. In some patients, it may be difficult to image the entire liver. Potentially difficult areas include the superficial liver above the costal margin, the left tip of the lateral segment of the left lobe, and the ventral subdiaphragmatic regions [5].

The liver is best imaged with the patient in the supine and left lateral decubitus positions, starting with 3–8-MHz curved linear array transducers. A subcostal acoustic window should be used first, supplemented with intercostal scans. Small sector transducers should be used to image areas inaccessible with the larger curved linear transducers. The liver surface (usually the ventral left lobe in the subxyphoid region) should be evaluated for nodularity with a near field-optimized 7–15-MHz linear array transducer. It is easier to appreciate nodularity during real-time examination than on hard copy images. Routine color Doppler imaging is useful in patients with suspected liver pathology. Optimal color Doppler and spectral Doppler sonography of the liver generally require relatively low frequency (2–3-MHz) scanning and good acoustic access.

Normal anatomy

The liver parenchyma is of uniform medium echogenicity, interspersed with vessels and fissures. The liver parenchyma is slightly less echogenic than the spleen, equal to or slightly more echogenic than the renal cortex, and isoechoic or somewhat less echogenic than the pancreas.

The liver has three lobes – the large right lobe, the left lobe, and the small caudate lobe. Sonographic landmarks can be used to segregate the liver into lobes and segments (Figs 129.1 and 129.2). Fissures and ligaments can be identified because they are echogenic, fat-containing structures. Portal veins, with their accompanying bile ducts and hepatic arteries, make up the portal triad. These triads are encased in echogenic fibrofatty tissue. This associated echogenic tissue is, as a practical matter, the feature that allows differentiation from hepatic veins (HVs). Portal veins, HVs, hepatic arteries, and bile ducts can also be identified with Doppler imaging. Within the porta hepatis, the left and right bile ducts lie anterior to the portal veins. More peripherally this relationship is not constant. A transverse oblique sonogram through the porta hepatis is one of the simplest methods for determining the presence of intrahepatic biliary dilation and obstruction.

Diffuse disease

Diffuse liver disease does not always cause distortion of liver anatomy or architecture. This can make sonographic detection difficult. Liver surface nodularity or atrophy of the right lobe, when present, can be useful signs of cirrhosis.

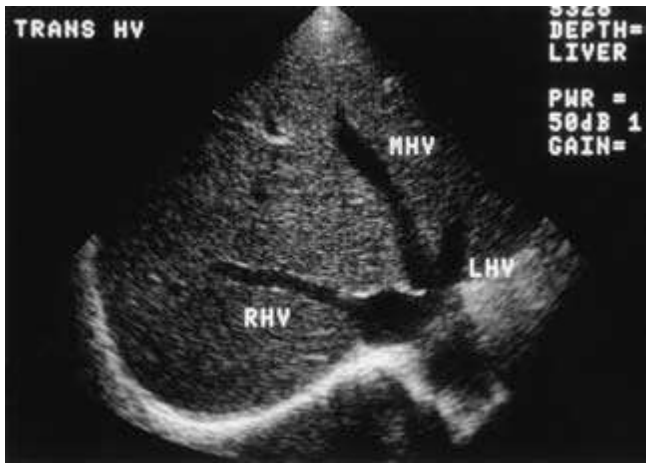


Figure 129.1 Normal hepatic veins. Transverse ultrasound of the upper portion of the liver shows the right hepatic vein (RHV), middle hepatic vein (MHV), and left hepatic vein (LHV) entering the inferior vena cava. Note the relative paucity of echogenic material around the hepatic veins, a characteristic that distinguishes them from portal veins, which have peripheral echogenic fibrofatty tissue. The middle hepatic vein separates the right and left lobes. The right hepatic vein and left hepatic vein separate the segments of the left and right lobes.

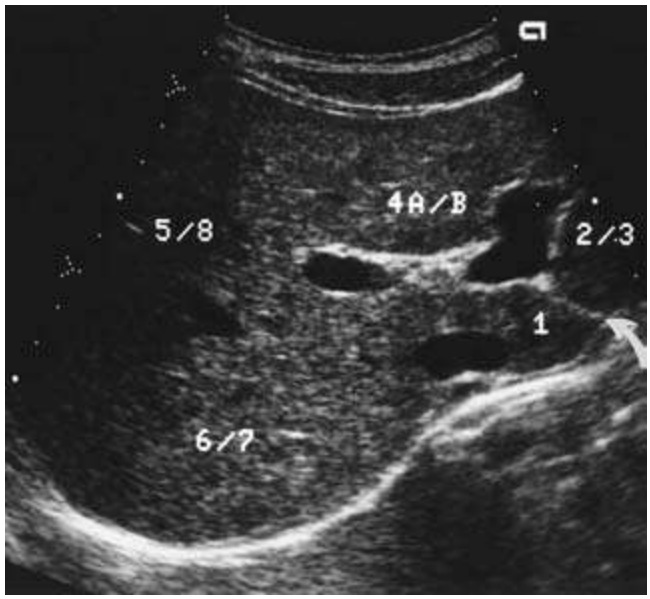


Figure 129.2 Couinaud segments. Transverse sonogram through the left and right portal veins, the axis through which the liver is divided into Couinaud segments. Segment 1 is the caudate lobe, bordered ventrally by the fissure for the ligament of venosum (curved arrow) interposed between the caudate lobe and the lateral segment of the left lobe (Couinaud segments 2 and 3). The medial segment of the left lobe is composed of Couinaud segments 4A (cephalically) and 4B (caudally). The anterior segment of the right lobe is composed of segments 5 and 8, and the posterior segment of the right lobe of segments 6 and 7. Couinaud segmental anatomy is important in planning hepatic surgery.

Parenchymal echogenicity may be increased in diffuse disease but is difficult to evaluate, as no absolute echo amplitude standard exists (analogous to CT attenuation numbers). Liver echogenicity is judged by comparison with adjacent organs, usually the kidney.

Hepatomegaly

Hepatomegaly can be difficult to diagnose objectively with sonography. The normal span of the adult liver is 15–17 cm. Single measurements are imperfect, but the sagittal dimension from the dome to the tip of the right lobe, measured at the midclavicular line, is the most frequently used. If this exceeds 15.5 cm, the liver is usually enlarged [6]. Hepatomegaly can be diagnosed qualitatively when the liver extends caudal to the right kidney and the left lobe is of normal size or larger. Better, volumetric measurements using 3D techniques may be feasible in the future.

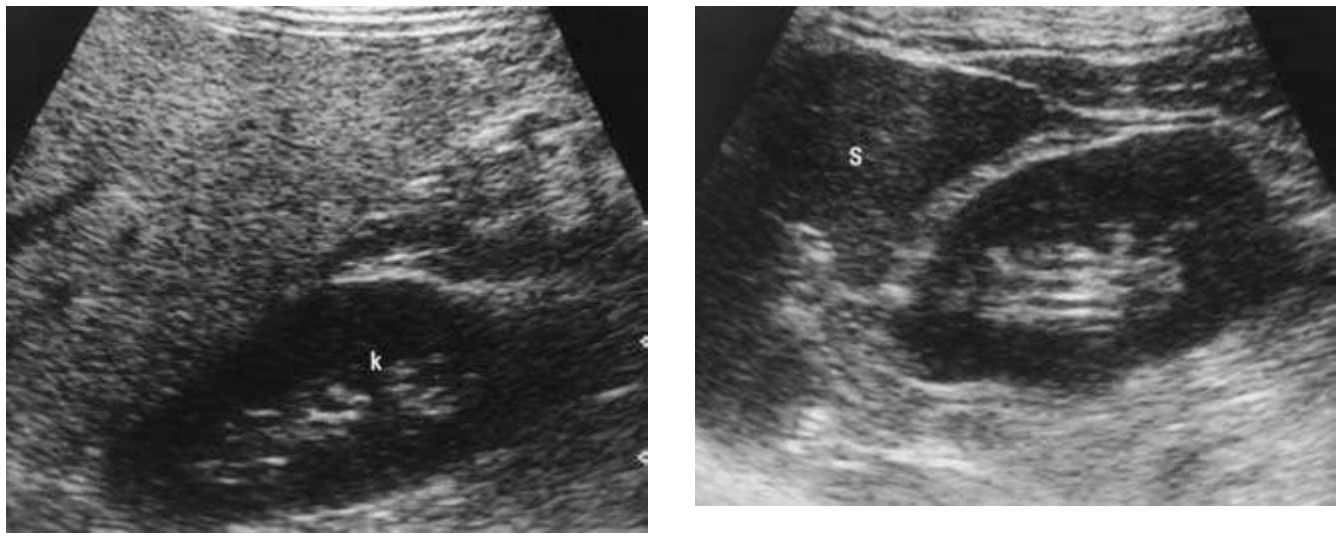
Hepatitis

Because many different diseases cause hepatic inflammation, sonographic findings are variable. The liver is generally normal in acute viral hepatitis. Although the "starry sky" pattern (increased periportal echoes with decreased parenchymal echogenicity) has been described in older studies [7,8], only 19 of 791 patients in a large series had this pattern [9]. In the same study, there was no difference in ultrasound findings between a normal control group and patients with acute viral hepatitis. Striking irregular gallbladder wall thickening is sometimes present in patients with acute hepatitis, especially hepatitis A [10]. Direct inflammation and edema cause wall thickening, sometimes reaching 20 mm (normal < 3 mm).

Hepatomegaly and inhomogeneous patchy or diffuse increased echogenicity are common in chronic hepatitis and are related to the amount of fatty infiltration and fibrosis present. The liver surface is smooth, unless cirrhosis is present. Enlarged arteries are noted on color Doppler sonography when there is increased arterial flow. This may cause a "double channel" sign on grayscale images that can potentially be confused with biliary dilation. Enlarged lymph nodes may be seen in the hepatoduodenal ligament [11]. Ultrasound findings in alcoholic hepatitis depend on the amount of fibrosis or fat present. Patchy, irregular increased parenchymal echogenicity is often present. The liver is often enlarged but the surface is usually smooth, in contrast to the nodularity sometimes detected in cirrhosis. Similar to chronic hepatitis, the arteries may be enlarged.

Fatty liver disease

The hallmark of fatty infiltration of the liver is increased echogenicity, usually diffuse. Severe fatty infiltration often results in an enlarged, echogenic liver (Fig. 129.3) with decreased acoustic penetration that may result in indistinct vessels or diaphragm. The liver surface is smooth. Fatty infiltration is often patchy and sometimes focal. Focal fat appears



(a) (b)

Figure 129.3 Fatty infiltration of the liver. Longitudinal sonogram **(a)** of the liver shows that the liver is much more echogenic than the right kidney (k). The echogenicity of the splenic parenchyma (S) and left renal cortex sonogram **(b)** are nearly equivalent. This internal comparison of the liver and spleen with adjacent kidneys allows a more reliable determination of the increased liver echogenicity associated with fatty infiltration.

as an area of increased echogenicity. A less affected region ("spared" area) may appear as a conspicuous hypoechoic area. Both focal fat and spared areas have a tendency to be pyramidal, with flame-shaped tapered margins. Frequent locations include the region of the porta hepatis, near the falciform ligament, the dorsal left lobe, and the caudate lobe. Although both focal fatty infiltration and focal sparing can simulate neoplasm [12–14], an appreciation of the usual appearance and location generally suffices to avoid confusion. Rarely, noncontrast CT scanning, MRI, or even biopsy may be required to clarify the diagnosis. Geographic fatty infiltration typically has well-defined margins between areas of greater and lesser involvement. No image features are known that can distinguish nonalcoholic steatohepatitis from other conditions in which there is fatty infiltration of the liver.

Cirrhosis

Sonographic findings of cirrhosis include changes in the shape of the liver, parenchymal inhomogeneity, and nodularity of the liver, notably at the surface. Intrahepatic vessels may be indistinct. Unfortunately, these signs are both insensitive and insufficiently specific to diagnose cirrhosis reliably. The caudate and left lobes tend to be relatively less affected by cirrhotic scarring than the right lobe. This sometimes results in a small right lobe with left and caudate lobe hypertrophy, especially in hepatitis B. Ratios comparing the size or volume of the caudate to the shrunken right lobe have been used to diagnose cirrhosis [15,16]. These ratios are not always useful; a large series revealed a sensitivity of 43% and an accuracy of 79% for the caudate-to-right lobe ratio [17].

Evaluation of the smoothness or nodularity of the liver surface using a high-resolution linear array transducer is useful [18–20]. Surface nodularity may be the only sonographic sign of cirrhosis. Some studies have not confirmed the usefulness of evaluating surface nodularity [20]. We believe that the specificity for macronodular cirrhosis is good, although micronodular cirrhosis is often missed. The only other common cause of surface nodularity is multiple subcapsular tumor nodules, usually from metastasis. Occasional causes are carcinomatosis with liver surface implants and acute massive liver necrosis.

Color Doppler sonography may detect portal vein flow reversal or portal collaterals, prompting the diagnosis of portal hypertension. These may be the only findings indicating severe liver disease in an otherwise normal study. Enlarged tortuous arteries are sometimes imaged by color Doppler sonography in cirrhotic livers. This finding, similar to "corkscrew" arteries seen angiographically, occurs because of increased arterial flow that occurs when portal venous flow decreases [21] and tortuosity of arteries from liver atrophy related to cirrhosis. Focal fatty infiltration, hepatocellular carcinoma (HCC), regenerating nodules (adenomatous hyperplastic nodule), or other focal lesions may occur in a cirrhotic liver. A liver mass in a cirrhotic patient strongly suggests the possibility of HCC.

Hepatic vascular disease

Portal hypertension

Sonographic evaluation of portal hypertension includes evaluation of the portal venous system and a search for portosystemic collaterals. The normal main portal vein measures slightly more than 1 cm in diameter. Although absolute portal vein size is not useful, lack of respiratory variation in size can be helpful in diagnosing portal hypertension [22].

Normally, blood flows toward the liver (hepatopetal) in portal veins. Reversed (hepatofugal) flow, although often associated with collaterals [21], may be the sole indication of portal hypertension. Other portal flow abnormalities include bidirectional flow and, rarely, nearly static blood flow. Ultrasound contrast agents can be useful in aiding the detection of flow in the main portal vein and other hepatic vessels. Detection of portal collaterals may prompt diagnosis of unsuspected portal hypertension, or reinforce it when suspected. Portal collaterals should be sought with color Doppler sonography, as they are often invisible on grayscale images.

The most common collaterals are left gastric (coronary) and paraumbilical veins. Left gastric collaterals, although the most common, are often difficult to image because of their deep location. Paraumbilical veins are easier to image, as they are superficial. They arise from the ventral tip of the left portal vein and flow caudally through the ligamentum teres, where they communicate with superficial peritoneal collaterals. Other types of portal collaterals occur, including retroperitoneal, splenorenal, splenoretroperitoneal, short gastric, and omental.

Therapeutic portosystemic shunts

Therapeutic surgical shunts may be seen with color Doppler, even when not apparent on grayscale images [23]. Shunt thrombosis can be reliably diagnosed only when no color flow is seen in the shunt. Secondary signs of shunt thrombosis, such as hepatopetal intrahepatic portal flow, may be misleading [24].

Candidates for transjugular intrahepatic portosystemic shunt (TIPS) stents can be evaluated by spectral and color Doppler before, after, and sometimes during the procedure. Doppler is imperfectly accurate in diagnosing shunt dysfunction, but is a useful screening tool to select patients for repeat venography and potential maintenance. We feel that the most useful of the many parameters [25,26] employed to evaluate stent function are intrastent velocity, flow direction in the native portal veins, and overall clinical judgment of the radiologist performing the study. Mid and distal stent velocity should be between 90 and 200 cm/s, and blood flow in the native portal veins should be toward the stent.

Venous thrombosis

Sonographically, intralumenal echoes are suggestive of clot, but not diagnostic. When hypoechoic or anechoic, clot may be virtually invisible without color Doppler. Anechoic thrombus is rare in neoplastic invasion but is fairly common with bland clot. Color Doppler sonography highlights clot by displaying flow around the clot and in adjacent patent vessels [21]. Common causes of portal venous thrombosis include neoplastic invasion, septic portal thrombosis (pylephlebitis), and pancreatitis. We concur with other investigators [21,27,28] that thrombosis in portal hypertensive patients is considerably more common than the 1% previously reported [29]. Color Doppler sonography is unrivaled in diagnosing partially occluded vessels. Small residual flow channels are automatically displayed in color. Clot resolution or progression can be documented on serial examinations. Contrast agents may be useful in difficult cases.

Cavernous transformation of the portal vein, resulting from main portal vein clot, consists of prominent hepatopetal portal collaterals. In acute portal vein thrombosis, color Doppler sonography may reveal small, acute hepatopetal portal collaterals, invisible on grayscale sonography. This may be a precursor to cavernous transformation of the portal vein.

Budd–Chiari syndrome

Sonography, especially with color Doppler imaging, is the preferred screening technique for evaluating patients with suspected Budd–Chiari syndrome [30]. Grayscale findings include HV abnormalities: absence, stenosis, dilation, irregularity, and abnormal or absent junction with the IVC. The IVC may be narrowed or occluded. Less specific changes include caudate hypertrophy and atrophy/enlargement of the other lobes and segments, and parenchymal inhomogeneity. Absent or reversed hepatic venous flow and intrahepatic HV to HV "spider-web" collaterals are diagnostic of Budd–Chiari syndrome. A flat HV waveform, lacking the normal cardiac-related phasic fluctuation, indicates HV compression – a finding that, although nonspecific, supports the diagnosis of Budd–Chiari syndrome. Other collaterals seen in Budd–Chiari syndrome include HV to portal vein collaterals with portosystemic shunting (e.g., through enlarged paraumbilical veins, esophageal varices) and HV to systemic veins.

Other vascular diseases

Sonographic findings in hepatic venoocclusive disease are nonspecific changes related to fibrous obliteration of microscopic central hepatic lobular veins. These findings, not universally present, include thickened gallbladder wall, abnormal or reversed portal venous flow [31], and increased hepatic artery peripheral resistance [32]. Hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu), characterized by telangiectases, arteriovenous (AV) fistulae, and aneurysms, involves the liver in about one-third of patients. Grayscale findings include enlarged hepatic arteries, and increased echogenicity from fatty infiltration or cirrhosis [33,34]. Color Doppler sonography reveals enlarged, tortuous, arteries with high-velocity flow, and also clarifies the potentially confusing grayscale appearance that may mimic biliary dilation. Visualization of hepatic AV fistulae is variable, and probably depends on their size.

Color Doppler sonography can be used to diagnose and follow hepatic pseudoaneurysms and AV fistulae, to assess the results and adequacy of vascular interventional procedures without recourse to angiography, and to demonstrate portal and HV malformations. Thrombosis or occlusion of the hepatic artery is the most common cause of hepatic

infarction [35]. Sonographically, infarcts are usually peripherally located hypoechoic wedge-shaped lesions. Others are round and centrally located. In the acute phase, the infarcts are ill-defined hypoechoic lesions that evolve into more anechoic or cystic areas [36]. After hepatic infarction, cystic collections of bile (bile lakes) may develop.

Focal liver disease

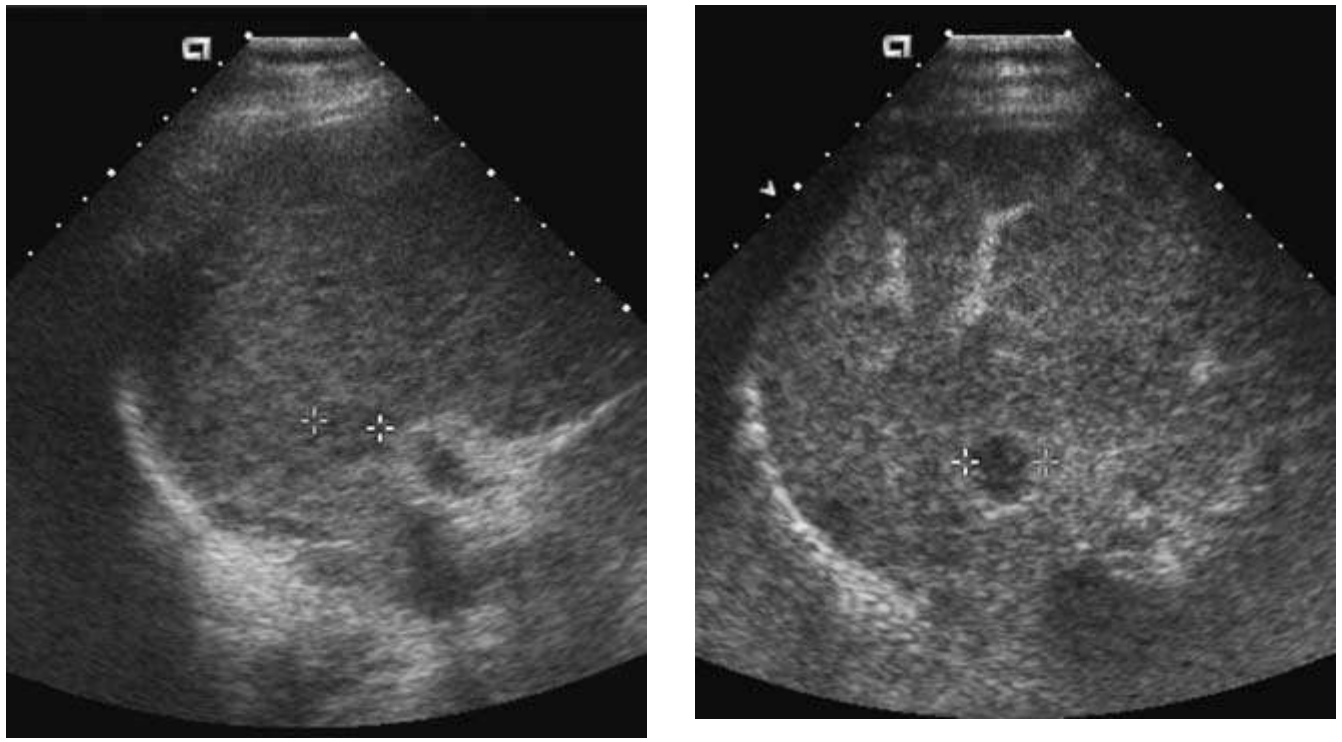
All standard noninvasive imaging modalities are imperfect detectors of focal hepatic disease, despite the improvement in detection rates achieved with multidetector CT, fast-scan contrast-enhanced MRI, and PET imaging [37–39]. Focal hepatic lesions are still missed with one modality, and then detected with another. Although sonography is less sensitive in detecting metastatic lesions than CT and MRI [40–43], modern sonography can perform well in detecting focal liver disease, outperforming CT, MRI, and PET in HCC detection in liver transplant candidates [43]. Arterial and portal venous phase imaging with ultrasound contrast agents can increase both the detection and characterization of lesions in patients with cirrhosis or liver metastasis [2]. Ultrasound contrast enhancement may prove useful in differentiating benign from malignant tumors [3,44] (Fig. 129.4).

Sonography's main strengths are its abilities to guide biopsy and characterize common benign lesions (cysts, hemangiomas), its safety, and its low cost. Its weaknesses include its inability to image the entire liver in some patients and its inferiority to CT in detecting extrahepatic disease. Ultrasound-guided biopsy is efficient and cost-effective, even when lesions are initially detected with some other modality. Sonography may be used to evaluate resectability of primary or metastatic liver tumors. Color Doppler sonography improves detection of focal lesions, especially anechoic or hypoechoic lesions. Some tumors are more conspicuous because of increased flow compared to normal liver: these include focal nodular hyperplasia (FNH), most HCCs, some metastases, and occasionally other masses [45–47]. Color and spectral Doppler can be used to help discriminate between benign and various types of malignant liver tumors [46,48–51].

Simple cysts

The prevalence of simple cysts increases with age [52]. When a cyst is found in a young patient, other diseases should be considered, including: autosomal dominant polycystic disease, von Hippel–Lindau disease, and conditions simulating simple cysts (e.g., echinococcal disease, residua from trauma or infection, and cystic neoplasia). Sonography is usually superior to CT or MRI for evaluating cysts.

Simple cysts are usually well-defined, echo-free round or oval lesions with imperceptible walls and good



(a) (b)

Figure 129.4 Transverse ultrasound images through the medial segment of the left lobe in a patient with prior right hepatectomy for metastatic disease from adenocarcinoma of unknown primary tumor. **(a)** Precontrast grayscale image shows a mass that is difficult to see (cursors). **(b)** Postcontrast grayscale image shows a small hypoechoic metastasis (cursors). Note that the surrounding liver is hyperechoic, consistent with increased vascularity with respect to the metastasis.

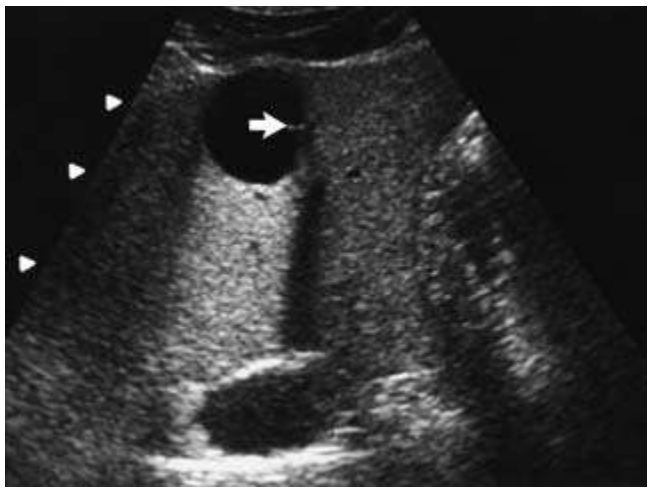


Figure 129.5 Simple liver cyst. This 2.5-cm cyst is anechoic, has imperceptible walls, and has prominent through-transmission (whiter area, deep to the cyst). There is a thin septation within the cyst (arrow). Thin (but not thick) septations are perfectly compatible with diagnosis of a classic simple cyst.

through-transmission (Fig. 129.5). Thin septations are frequent, and should not suggest a different diagnosis. Thicker septations are generally a sign of a complicated cyst or of a cystic neoplasm.

Hepatic polycystic disease

Sonographic findings include multiple, often contiguous simple cysts. Irregular shape and septae are common. Hemorrhage or infection may cause debris-like echoes within one or more cysts [53]. About one-half of patients have renal cysts. von Meyenburg complex [54] is a rare disorder characterized by multiple small cystic hamartomas of the bile ducts, which may be detected with CT, MRI, or sonography.

Other cystic lesions

Acquired cysts may occur as a result of trauma or infection [55–57]. Neoplasms that simulate simple cysts are unusual; metastases from squamous cell carcinoma, GI stromal tumors, and cystic adenocarcinomas occasionally do this. Hepatobiliary cystadenoma is a rare neoplasm probably arising from bile duct epithelium. These lesions are predominantly multicystic with varying amounts of solid tissue [58–60].

The liver is the most frequently involved organ in hydatid (echinococcal) disease. A spectrum of sonographic findings from purely cystic to solid-appearing pseudotumors occurs. Internally, wavy bands of delaminated endocyst (the "water lily" sign) may be noted [61]. Daughter cysts, sometimes surrounded by echogenic debris ("matrix"), are frequent. Calcifications, varying from tiny to massive, are often present. A densely calcified cyst usually indicates a dead, inactive lesion. Previously considered contraindicated, percutaneous drainage and treatment are gaining wider acceptance [62].

Figure 129.6 Pyogenic liver abscess. This transverse sonogram of the right lobe of the liver shows a mixed-echogenicity, predominantly hypoechoic liver abscess at the hepatic dome. The lesion is not well defined medially, a feature of pyogenic liver abscess on ultrasonography.

Pyogenic liver abscess

When liver abscess is suspected clinically, sonography is the preferred screening modality. Sonographically, pyogenic liver abscesses are variable in appearance (Fig. 129.6). Typically, features include a mixed echogenicity, primarily hypoechoic mass with irregular margins. Irregular areas of increased echogenicity are frequent. On occasion, a diffusely hyperechoic appearance may be noted, owing to microbubbles from gas-forming organisms. Diffuse microabscesses, often associated with biliary obstruction, may cause a confusing sonographic pattern of increased irregular hepatic echogenicity. Clusters of small low-echogenicity lesions may also be noted [63]. Septic portal venous thrombosis (pylephlebitis), which may be imaged sonographically, may lead to liver abscess [64].

Amebic liver abscess

Amebic liver abscesses tend to have a round or oval shape and hypoechoic appearance with fine, homogeneous, low-level echoes throughout. Image findings alone, however, are insufficient to distinguish amebic from pyogenic liver abscesses [65]. Amebic liver abscess can have sonographic patterns that are bizarre, including diffuse increased echogenicity, debris levels, and prominent heterogeneity. When it is unclear clinically if an abscess is amebic or pyogenic, percutaneous aspiration should be performed (necessary in 15% of patients in our institution). In contrast to pyogenic liver abscesses, percutaneous drainage of amebic liver abscess is rarely indicated [66]. Therapeutic aspiration provides no

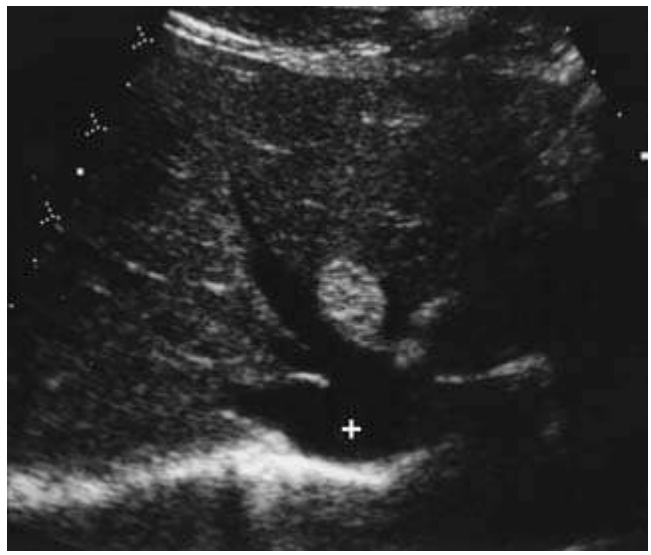


Figure 129.7 Small hepatic hemangioma. This transverse sonogram at the level where the hepatic veins enter the inferior vena cava (+) reveals a well-defined, white, echogenic focus that is virtually diagnostic for hepatic hemangioma.

objective benefit when compared with the use of oral amebicidals alone [67].

Candidiasis

Candidiasis, occurring in leukemic and other immunocompromised patients, causes multifocal hypoechoic lesions typically smaller than 2 cm. Four distinct sonographic patterns of hepatic microabscesses from candidiasis have been described by Pastakia and colleagues [68]. These include discrete hypoechoic nodule, a "bull's eye" configuration, a discrete echogenic focus with variable degrees of acoustic enhancement, and a "wheel within a wheel" appearance. Internal echogenic foci produce the target or "wheel within a wheel" appearance [69,70]; echogenic lesions and lesions larger than 2 cm are much less common.

Benign neoplasms

Cavernous hemangioma

Cavernous hemangioma, the commonest benign hepatic neoplasm (prevalence 1%–4%), rarely causes clinical symptoms. Classic hemangiomas are well-defined, echogenic lesions sometimes exhibiting enhanced through-transmission (Fig. 129.7) [71]. Another typical appearance is of variable internal echogenicity surrounded by a complete echogenic margin [72] (Fig. 129.8). Larger hemangiomas frequently diverge from these classic patterns. Mixed-echogenicity lesions and even hypoechoic hemangiomas may occur. Hemangiomas rarely change on serial exams [73].

Classic hemangiomas in low-risk patients (patients without malignancy or chronic liver disease) do not need further evaluation with imaging [74]. In patients with malignancy or cirrhosis, hemangiomas must often be evaluated further to exclude more significant lesions. Preliminary results suggest that addition of ultrasound contrast agents may be sufficient confidently to diagnose incidentally detected hemangiomas at US (Fig. 129.9). Computed tomography and MRI may fail to identify small (< 1 cm) hemangiomas detected with ultrasound. If needed, thin-needle biopsy is a reasonable and safe way to evaluate hemangiomas [75,76], provided skilled cytological consultation is available.

Focal nodular hyperplasia/liver cell adenoma

Focal nodular hyperplasia (FNH) is a clinically insignificant lesion, whereas liver cell adenoma (LCA) can cause morbidity and mortality because of hemorrhage and, occasionally

(a) (b) (c)

Figure 129.8 Atypical hemangioma (stable for 11 years): transverse grayscale ultrasound image through the right lobe. **(a)** Precontrast shows large ill-defined heterogeneous mass (cursors). **(b)** Postcontrast, normal liver (black arrow) and the medial wall of the hemangioma (white arrow) enhanced. **(c)** At 90 s postcontrast administration, a larger volume of tissue with delayed enhancement is seen (arrows).

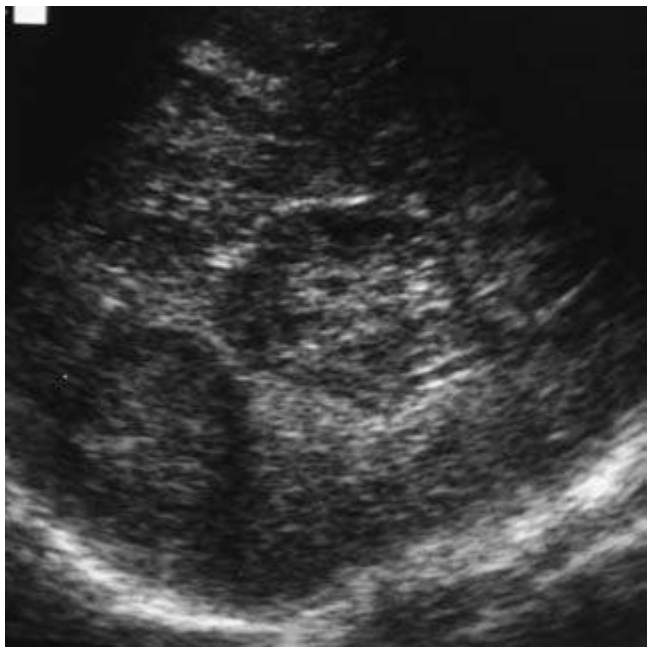


Figure 129.9 Multifocular hepatocellular cancer. A transverse sonogram shows multiple tumor nodules in the right lobe of the liver. Some of these are hypoechoic whereas others have echogenic centers with hypoechoic rims.

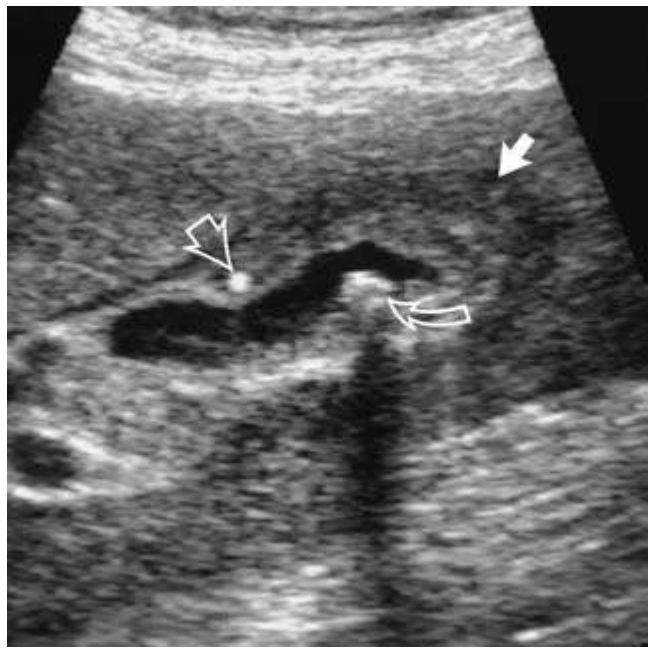


Figure 129.10 Gallstones in a patient with adenomyomatosis. Note the intralumenal gallstone (curved open arrow). There is an intramural gallstone (straight open arrow) within a diffusely thickened gallbladder wall (arrows) as a result of adenomyomatosis.

malignant degeneration. The sonographic features of FNH and LCA are variable. There is a tendency for FNH to be more homogeneous than LCA. Focal nodular hyperplasia often has mildly increased echogenicity compared with normal parenchyma, whereas LCAs are usually hypoechoic and more inhomogeneous [77–81]. On color Doppler imaging, FNH usually has markedly increased flow, sometimes with vessels radiating peripherally from a central feeding artery.

Malignant neoplasms

Hepatocellular carcinoma

Advanced HCC is almost always multifocal, making it difficult to distinguish from metastatic disease (Fig. 129.10). Small HCCs (< 5 cm) are often (75%) hypoechoic [82]. As HCCs grow, they tend to develop hypoechoic peripheral rims [83]. With further progression, lesions become more numerous and heterogeneous. Fatty tissue within the lesion may cause increased echogenicity and confusion with hemangioma [84]. Portal vein or hepatic vein invasion should suggest the diagnosis of HCC, although other liver tumors also invade veins [85]. Rarely, HCC invades the bile duct. About three-fourths of HCCs have identifiable internal color flow compared with only one-third of metastases. With ultrasound contrast agents, most HCCs reveal abnormal tortuous tumor vessels on the periphery, a pattern distinct from benign tumors. Fibrolamellar HCC, which accounts for 2% of HCCs, but 25%–50% of HCCs in young adults, is typically a single well-circumscribed lesion in an otherwise normal liver. Other features include a "central scar" and calcification, both of which also occur in other subtypes of HCC and lesions of other histology [81].

Hepatic metastasis

Metastatic disease, the most common liver malignancy in North America, is multifocal in 90% of patients. Virtually any sonographic appearance may occur. Metastatic lesions, whether diffuse or focal, are usually heterogeneous. Hypoechoic halos, target, or bull's-eye patterns, with rings of varying echogenicity, are common. Ill-defined infiltration with focal nodularity is another frequent pattern. Metastases that simulate simple cysts or "classic" hemangiomas are rare. Predominantly fluid-filled, presumably necrotic metastases occur most frequently with squamous cell carcinoma, sarcomas, and ovarian and testicular carcinoma. Calcified lesions, especially mucinous adenocarcinoma, may occur. Although sonographic appearance is a poor predictor of the primary tumor, certain patterns are suggestive. Large to moderate-sized hyperechoic metastases should suggest the possibility of a colonic primary. Lesions with fluid-fluid levels (representing intralesional necrosis and hemorrhage) are often found with metastatic GI stromal tumor.

Distinguishing between metastasis and HCC occasionally requires biopsy. Invasion of the portal or HVs suggests HCC rather than metastasis. Although portal venous invasion in metastatic disease has been reported in as many as 8% of patients [86], our experience suggests a lower frequency.

Human immunodeficiency virus infection

In patients with human immunodeficiency virus (HIV) infection the liver is often involved by neoplasm – non-Hodgkin lymphoma, Kaposi sarcoma (KS) – or by opportunistic infection. Sonographic findings of uncertain cause, such as decreased periportal echogenicity or focal hyperechoic areas, are frequent.

High- and intermediate-grade HIV-related B-cell lymphomas cause focal liver lesions more frequently than other lymphomas. These are usually hypoechoic masses that may exhibit increased peripheral color flow. Kaposi sarcoma usually infiltrates the liver, causing little or no sonographic abnormality; it may cause focal masses and increased periportal echogenicity [87].

Multiple echogenic foci, often imaged more clearly sonographically than with CT, may give a "starry sky" pattern with disseminated *Pneumocystis carinii* infection [88]. This pattern also occurs with mycobacterial or cytomegalovirus infection [89]. On occasion, disseminated *Pneumocystis* infection results in larger calcifications or large hypoechoic lesions.

Other focal lesions

Hepatic lymphoma and leukemia are usually microscopic. Only about 5% cause sonographically detectable focal liver lesions [90]. The lesions are usually anechoic or hypoechoic, although other patterns occur [91]. Chloroma of the liver, associated with myelogenous leukemia, can be hypoechoic or echogenic. Lymphomas related to HIV cause more frequent and conspicuous focal lesions than other lymphomas.

Hepatic sarcomas are rare. Angiosarcomas and undifferentiated embryonal sarcomas are usually large, internally inhomogeneous masses [92]. Hepatic epithelioid hemangioendothelioma (EHE), a rare vascular sarcoma [93], starts as a multinodular tumor, coalescing as it grows. It is usually hypoechoic compared with normal liver. Hypertrophy of the uninvolved normal liver may occur.

Adenomatous hyperplastic nodules in cirrhotic livers (regenerating nodules) are premalignant lesions that, when visualized, are usually hypoechoic and may have a thin echogenic rim [94]. Intrahepatic extramedullary hematopoiesis may cause inhomogeneous, mainly hypoechoic masses [95]. Mesenchymal hamartoma of the liver is usually a large, mainly cystic mass with septae of variable thickness and solid internal tissue [96]. Intrahepatic hematomas may cause sonographically visible masses.

Trauma

Computed tomography is the mainstay for evaluating hemodynamically stable patients with suspected blunt hepatic trauma. Sonography can be used effectively to detect hemoperitoneum, but it has a rather limited role in the initial diagnostic evaluation of hepatic trauma. A high percentage of liver and other parenchymal injuries are missed sonographically [97]. Ultrasound contrast may improve the usefulness of ultrasound in trauma [98]. Sonography may be valuable in the follow-up of nonoperated patients [99] and in diagnosing posttraumatic complications including biloma, vascular pseudoaneurysms and arteriovenous (AV) fistulae, and abscesses.

Liver transplantation

Sonography, especially Doppler imaging, is important in evaluating liver transplant candidates and potential donors [100]. Sonography can be useful in defining portal hypertension-related collaterals, detecting portal vein clot, delineating spontaneous or surgical portosystemic shunts, and detecting HCC in the native liver.

After transplantation, sonography can screen for hepatic arterial occlusion [101]. Absent flow on color Doppler or decreased peripheral resistance, measured by resistive index (RI) [102], are signs of arterial compromise. The use of ultrasound contrast agents may improve the accuracy and speed of examination in transplant patients [103]. When abnormalities are noted, arteriographic evaluation and intervention are often indicated [104]. Hypoechoic infarcts or intrahepatic fluid collections may result when arterial occlusion occurs. Cholangiography is generally needed to detect bile duct injuries reliably, as sonography may miss 50% of bile duct complications [105]. Posttransplant fluid collections include abscess, biloma, seroma, lymphocele, and hematoma [106].

Overview of hepatic imaging

Hepatic sonography's main strengths are its ability to characterize common benign lesions (cysts, hemangiomas) and guide percutaneous procedures, plus its safety, excellent patient tolerance, and low cost. In the near future, the application of ultrasound contrast agents to the liver may extend ultrasound's utility from a screening examination into a lesion detection and diagnosis modality. Its weaknesses include an inability to image the entire liver in some patients and inferiority to CT in detecting extrahepatic disease. When liver abscess is suspected clinically, sonography is the preferred screening modality [41]. Sonography's ability to image in any oblique plane often makes it superior to CT and MRI in localizing lesions to an anatomical hepatic segment when evaluating resectability of a liver tumor.

In experienced hands, sonographically guided liver biopsy is often quicker and easier than CT guidance. Sonography can directly visualize the needle tip as it is placed in the lesion, facilitating biopsy of small lesions and lesions in uncooperative patients. Ultrasound-guided biopsy is more efficient and cost-effective, even when lesions are initially detected with some other modality. Despite sonography's advantages, CT-guided biopsy is often more popular with radiologists because it almost always shows needle location. Sonographic needle visualization may be difficult or impossible when the liver is echogenic or when acoustic access is imperfect. Newer sonographic techniques that enhance needle-tip visualization

and improved biopsy guides promise to make sonographically guided biopsies easier.

Ultrasound contrast enhancement also may be useful in delineating residual tumor after ablation. Viable tumor can be identified and targeted for retreatment with ultrasound contrast during or after ethanol [107] or radio-frequency ablation. Interestingly, US failed to demonstrate flow in residual tumor prior to contrast enhancement, an indication of the potential usefulness of ultrasound contrast agents in these patients.

Gallbladder

Indications for sonography

Sonography is the imaging method of choice for the initial evaluation of all suspected diseases of the gallbladder. It is particularly valuable in patients with acute right upper quadrant pain and possible acute cholecystitis [108,109]. Sonography is highly reliably in detecting tiny gallstones and is useful for evaluating focal or diffuse abnormalities of the gallbladder wall [110]. Biliary sonography has several distinct advantages compared with scintigraphy and CT. Sonography is less expensive and can be performed rapidly without patient preparation or contrast agents. Unlike biliary scintigraphy, sonography is not "organ specific" and may provide important diagnostic information regarding the liver, pancreas, and peritoneal cavity. Sonography can readily be performed in patients with abnormal liver function that may preclude scintigraphy. Finally, sonography may be used at the bedside to guide percutaneous cholecystostomy for critically ill patients.

Patient preparation and technique

In patients undergoing sonography for acute right upper quadrant pain and possible acute cholecystitis, no prior preparation is necessary and patients can be imaged directly from the emergency room. Patients undergoing elective sonography to evaluate the gallbladder should be fasted for a minimum of 6 h. This will ensure adequate distention of the gallbladder to visualize small stones and facilitate imaging of the gallbladder wall.

Normal anatomy

Except in the rare instance of agenesis, the normal gallbladder can be seen in virtually all fasting patients [111,112] as a pear-shaped, fluid-filled structure along the inferomedial aspect of the right lobe of the liver. The gallbladder is typically less than 12 cm in length and is up to 3 cm in anteroposterior diameter. The normal gallbladder wall measures less than 3 mm. In addition to agenesis, other rare anomalies of the gallbladder that may be diagnosed with sonography include duplication and septations between the fundus and body (phrygian cap) [113].

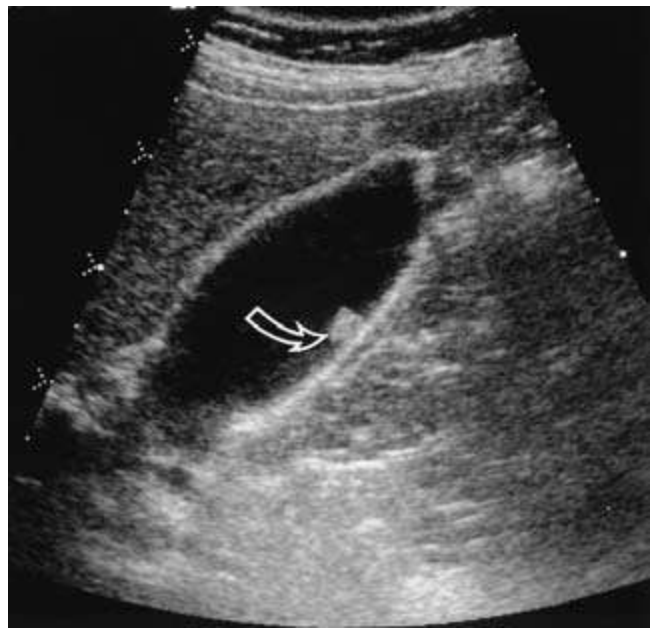


Figure 129.11 Gallbladder polyp. Note the echogenic mucosal mass without an acoustic shadow (arrow).

Gallstone disease

Stones as small as 3–5 mm can be easily seen sonographically. The characteristic sonographic appearance is an intralumenal echogenic focus casting a distal acoustic shadow (Fig. 129.11). It may be difficult to document acoustic shadowing in all patients with small gallstones. By varying the patient's position, mobility of the gallstones may be demonstrated in a high percentage of patients. Demonstrating mobility helps to differentiate small stones from polyps arising from the gallbladder wall that do change in position.

Although sonography is accurate for detecting stones within the gallbladder, once stones migrate into the cystic duct they are often difficult to see [114]. It is important to perform parasagittal images of the neck, gallbladder, and cystic duct region in patients with right upper quadrant pain. The identification of impacted cystic duct stones strongly suggests cystic duct obstruction.

Sometimes, echogenic abnormalities within or adjacent to the gallbladder may sonographically mimic gallstones. Such abnormalities include polyps, tumefactive sludge, surgical clips, and air bubbles. Gallbladder polyps are echogenic mucosal lesions that do not shadow. Sludge within the gallbladder is never a normal finding. It generally indicates lack of gallbladder emptying with precipitation of cholesterol or calcium bilirubinate crystals. At times sludge crystals may adhere together as "tumefactive" sludge and mimic a gallstone or mucosal mass. Gas within the gallbladder may be due to surgically created biliary–enteric anastomoses, endoscopic biliary procedures, or gas-forming infection from emphysematous cholecystitis.

Acute cholecystitis

The most important sonographic findings to confirm the diagnosis of acute cholecystitis are gallstones, gallbladder wall thickening, and a positive sonographic Murphy sign [108,109]. The sonographic Murphy sign differs significantly from the clinical sign. It refers to focal, unequivocal tenderness directly over the visualized gallbladder when the transducer applies pressure. In a prospective study of 497 patients with acute right upper quadrant pain, the combination of gallstones and a positive Murphy sign had a 92% positive predictive value for acute cholecystitis [108]. Gallbladder wall thickening (> 3 mm) and pericholecystic fluid in the appropriate clinical setting are secondary findings of acute cholecystitis, but both nonspecific findings may be due to a variety of other disorders. Biliary scintigraphy should be performed whenever the sonographic findings are equivocal.

In a small percentage of patients (5%–10%), acute cholecystitis develops in the absence of gallstones or cystic duct obstruction [115]. Conditions implicated in acalculous cholecystitis include trauma, major surgical procedures, hyperalimentation, burns, sepsis, and other severe intercurrent illnesses. The sonographic findings in early acalculous cholecystitis are often subtle and nonspecific. In a small percentage of patients, the gallbladder may be normal early in the clinical course [116]. Generalized symmetric thickening of the gallbladder wall is frequently not due to cholecystitis and must be interpreted with caution. It may be caused by such diverse conditions as hepatitis, fluid overload, portal hypertension, or hypoalbuminemia. In the appropriate clinical setting, gallbladder wall thickening with a positive sonographic Murphy sign is suggestive of acute acalculous cholecystitis. The sensitivity of sonography for the diagnosis of acalculous cholecystitis, however, is substantially less than with calculous cholecystitis, in the range of 63%–67% [117]. Interval follow-up studies performed at 24–48 h may be valuable in demonstrating progressive gallbladder wall edema or the development of fluid.

In patients without acute right upper quadrant pain, stones within a contracted gallbladder often indicate chronic cholecystitis. Shrinking of the gallbladder wall is a common associated finding. Marked contraction of the gallbladder around a large stone resultsin the wall-echo shadow sign and there is prominent acoustic shadowing from the gallbladder fossa.

There are several sonographic features that, in the appropriate clinical setting, may suggest gangrenous cholecystitis. These include pericholecystic fluid, intralumenal membranes (due to sloughed mucosa or fibrinous debris), and asymmetric thickening of the gallbladder wall [117] due to intramural microabscesses or hemorrhage. A high percentage of patients with gangrenous cholecystitis do not have a positive sonographic Murphy sign [118]. In critically ill patients with suspected gangrenous cholecystitis, sonography may be useful to guide percutaneous cholecystostomy even at the bedside.

Perforation of the gallbladder with pericholecystic abscess is a major cause of sepsis and morbidity from acute cholecystitis [119]. Pericholecystic abscesses appear sonographically as complex fluid collections in direct contiguity with the gallbladder. Mural necrosis may be identified sonographically as a focal loss of the normal reflectivity of the gallbladder wall. Inflamed omental and pericholecystic fat has been noted adjacent to pericholecystic abscesses. These fatty structures "wall off" the abscesses from the greater peritoneal cavity.

Hyperemia of the inflamed omental fat may be demonstrated with color Doppler imaging [120].

Gallbladder tumors

The vast majority of benign neoplasms of the gallbladder originate from the mucosal surface of the gallbladder and are either adenomatous or cholesterol polyps (Fig. 129.12) [121,122]. Small polyps are often incidental findings that, when less than 5 mm in diameter, are of no cause for clinical concern. Enlarging gallbladder polyps on serial sonograms or polyps greater than 1 cm in diameter are usually an indication for cholecystectomy due to the slight increased risk for carcinoma.

The sonographic findings of gallbladder carcinoma are variable and include a polypoid mucosal lesion or focal or irregular thickening of the gallbladder wall, sometimes associated with an extramural mass [123]. In advanced cases of gallbladder carcinoma patients may present with a large mass arising from the gallbladder fossa [123]. Direct hepatic invasion and nodal metastases may also be demonstrated sonographically. However, in many instances CT is required to delineate more precisely the extent of tumor and to detect distant spread of the disease.

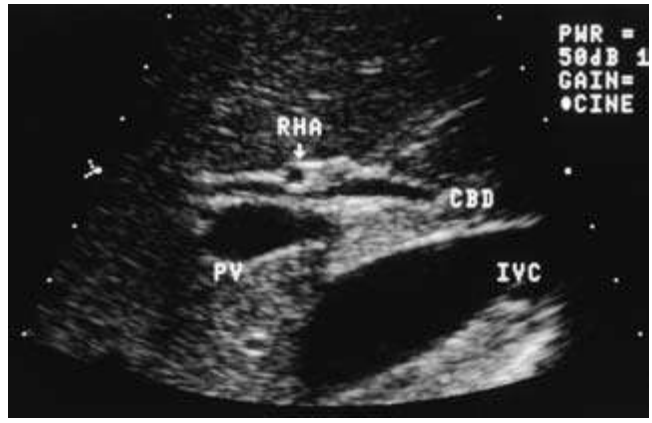


Figure 129.12 Normal common bile duct. This long-axis view of the normal common bile duct (CBD) shows the relationship of the bile duct to the portal vein (PV). Note that the right hepatic artery (RHA) passes ventrally to (above) the bile duct. This occurs in 10% of patients. In 90% of patients, the right hepatic artery passes between the portal vein and the bile duct. The common bile duct enters the pancreatic parenchyma. IVC, inferior vena cava.

Other conditions

Adenomyomatosis and cholesterolosis are benign disorders of the gallbladder that are classified as hyperplastic cholecystoses [121,122]. Cholesterolosis typically results in either single or multiple small mucosal polyps. It is due to the subepithelial accumulation of cholesterol-laden histiocytes that ultimately extends to the mucosal surface. Adenomyomatosis causes either focal or diffuse hypertrophy of the normal Rokitansky–Aschoff sinuses and smooth muscle within the gallbladder wall. Crystals and debris may be trapped within the sinuses of the hypertrophied wall and produce echogenic foci with echogenic "comet-tail" artifacts.

Bile ducts

Indications for sonography

Sonography's ability to detect dilated bile ducts and the level of biliary obstruction makes it the technique of choice for evaluating jaundiced patients. Sonography can also detect the cause of obstruction, albeit with less accuracy. Infectious cholangitis and conditions such as HIV cholangiopathy are indications for sonography. Sonography can detect and assess, with the help of color Doppler, resectability of cholangiocarcinoma and other tumors affecting the bile ducts.

Patient preparation and technique

Biliary sonography is best performed after an overnight fast or 8–12 h of a clear liquid diet. Because distention of the gallbladder is desirable, fatty foods should be avoided. The biliary tree is best imaged with the patient in the supine and right anterior oblique positions, starting with 3- to 7-MHz curved linear array transducers. A subcostal acoustic window should be used first, supplemented with intercostal scans. Sector or other small-footprint transducers are often useful to image the extrahepatic bile duct and the cystic duct of the gallbladder. Transverse compression views of the pancreatic head region with large-footprint curved linear transducers are useful to visualize the intrapancreatic bile duct, where about 90% of symptomatic common duct stones are located [124]. Color Doppler imaging is useful to differentiate the bile ducts from vessels and in detecting hyperemia in and near the gallbladder.

Normal anatomy

Sonography routinely displays the normal intra- and extrahepatic ducts. The normal extrahepatic bile duct ("common duct") runs ventral to the main portal vein (Fig. 129.13) and to the left of the proper hepatic artery. The internal diameter of the normal extrahepatic bile duct is 5 mm or less, although elderly patients and patients with cholelithiasis may have ducts of internal diameter 6–9 mm. The distal common duct is usually seen within the pancreatic head, and has a somewhat smaller caliber. The intrahepatic bile ducts may be seen ventral to the left and right portal veins. The normal internal diameter of the main left and right intrahepatic bile ducts is about 1 mm.

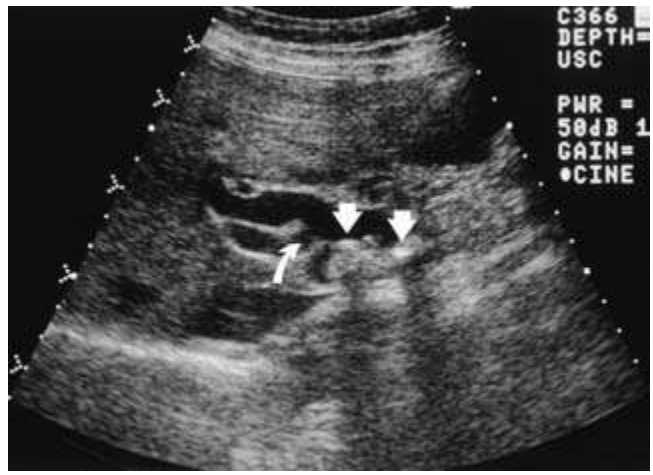


Figure 129.13 Common duct stone. This long-axis view of the intrahepatic and extrahepatic bile ducts reveals two echogenic common duct stones (arrows). Note the cystic duct (curved arrow) entering the common duct dorsally.

Bile duct obstruction

Stones are the most common cause of distal bile duct obstruction. Tumors are a more frequent cause of proximal bile duct obstruction. Sonographically, dilated intrahepatic bile ducts are tubular anechoic structures that demonstrate posterior acoustic enhancement. Centrally, in the porta hepatis, dilated ducts are usually ventral to the portal veins. Peripheral ducts have a variable relationship to the portal veins. The "too many tubes" sign is the classic pattern indicative of intrahepatic biliary dilation. Color Doppler is useful to distinguish veins and enlarged arteries from dilated bile ducts, simplifying detection of abnormal ducts and preventing misidentification of vessels as bile ducts.

Surprisingly, "normal" bile duct size is controversial. Obstruction is rarely present when the extrahepatic bile duct is 5 mm or less in maximal internal diameter. Nondilated obstruction may be seen in cholangiocarcinoma or sclerosing cholangitis. Bile ducts with maximal internal diameters of 10 mm or more are almost always obstructed. In most patients, 6–9-mm ducts ("gray zone" size ducts) are enlarged not because of obstruction but because of breakdown of the elastic fibers within the duct wall. Patients over 50 years old are "allowed" 1 mm per decade in duct diameter. For example, a 7-mm duct should be considered normal in a 70-year-old. Patients with cholelithiasis often have 6- to 9-mm-diameter ducts. We postulate that this is caused by degeneration of elastic tissue within the duct wall from transient obstruction caused by recurrent passage of stones. This is analogous to the increased residual diameter of a deflated balloon that has

been repeatedly blown up and deflated. Rarely, an unobstructed duct measuring 10 mm or larger may be found. Administration of a fatty meal may aid in evaluating "gray zone"-sized ducts. A decrease of 2 mm in duct size after a fatty meal indicates normal bile dynamics and excludes obstruction. If the extrahepatic duct enlarges after the fatty meal, obstruction is likely.

Distal bile duct obstruction causes extrahepatic bile duct dilation and often intrahepatic bile duct dilation. Intrahepatic duct dilation is almost always a sign of obstruction, although rare exceptions are seen. Thus, intrahepatic dilation is specific for obstruction, but it is less sensitive than extrahepatic bile duct dilation. Obstruction at sites other than the distal duct results in proximal dilation, with a normal-caliber distal duct.

Choledocholithiasis

Choledocholithiasis occurs in at least 15% of patients with cholelithiasis. Conversely, 95% of patients with common duct stones also have gallstones [125]. The prevalence of choledocholithiasis in patients with gallstones increases with age [126]. A shadowing echogenic focus in the bile duct is virtually diagnostic of cholelithiasis (Fig. 129.14). Shadowing may be difficult to demonstrate in some impacted stones. Potential pitfalls include the Mirizzi syndrome, air in the bile duct, extrabiliary calcifications, or surgical clips. On occasion, a completely stone-filled duct may be confused with a gas-containing segment of bowel. Extrahepatic biliary dilation is present in 70%–94% of patients with choledocholithiasis [127,128]. Although stones in normal-sized ducts can be identified, these are probably harder to detect than stones in dilated ducts.

Figure 129.14 Cholangiocarcinoma. Transverse sonogram in the region of the porta hepatis shows biliary dilation and a hypoechoic mass (M).

Bile duct stones are harder to detect than gallstones, with optimal reported sensitivity of 75%–80% [129]. The difficulty relates to gas sometimes obscuring the distal duct and the lack of echo-free bile around impacted stones. About 90% of proximal, suprapancreatic bile duct stones (approximately 10% of cases) are detected [130]. Meticulous technique is necessary to achieve optimal sensitivity. A prospective Finnish trial reported detection rates of only 22% for sonography and 25% for CT in patients with choledocholithiasis [130].

Infectious cholangitis

Contrast CT is probably the best screening examination in patients with severe cholangitis [131]. Sonography is the study of choice in patients with clinically less severe cholangitis. Diffuse bile duct wall thickening is often present [132]. Debris in the bile duct suggests the presence of pus and, hence, infectious cholangitis. Liver abscesses can be identified.

Recurrent pyogenic cholangitis

Recurrent pyogenic cholangitis (also known as Oriental cholangiohepatitis) is characterized by recurrent bacterial cholangitis promoted by strictures and primary bile duct stones that may occur in any part of the biliary tree. Gallstones and liver abscesses are frequent [133]. Sonography is the preferred initial imaging [134,135]. Most patients have huge stones with dramatic shadowing that can obscure the biliary tree. The soft, mud-like stones are often of medium echogenicity, however, and may lack acoustic shadowing. This appearance can mimic sludge, pus, blood, or even neoplasm within the biliary tree. Markedly dilated intra- and extrahepatic ducts are the rule. It is not unusual for duct size to exceed 2 cm [136]. Sonography is limited in patients with prior biliary enteric anastomosis, as intraductal gas obscures both the ducts and intrahepatic calculi. Gas may also make detection of liver abscesses difficult.

HIV-related cholangiopathy

HIV-related cholangiopathy is a diffuse biliary tract disease, affecting the gallbladder and the bile ducts. More than 80% of patients have alkaline phosphatase greater than twice normal, but bilirubin is usually normal or minimally elevated [136–138]. Sonography, although less sensitive than endoscopic retrograde cholangiopancreatography (ERCP), is useful in evaluating patients suspected of having HIV-related cholangiopathy [138]. Smooth or irregular thickening of the bile duct wall (45%) and biliary dilation (about 70%) are frequent abnormalities [139]. Biliary dilation often occurs in association with ampullary stenosis. An echogenic nodule at the end of the common duct, thought to represent an edematous ampulla of Vater, has been described. Striking thickening of the gallbladder wall with prominent wall striations suggestive of acute acalculous cholecystitis, may occur [136,138,140].

Tumors

Cholangiocarcinoma

Primary bile duct tumor, or cholangiocarcinoma, has two forms, peripheral cholangiocarcinoma and hilar cholangiocarcinoma (Klatskin tumor) [141,142]. In the United States, peripheral cholangiocarcinoma is three times more common than hilar cholangiocarcinoma, whereas in Japan hilar and peripheral cholangiocarcinomas occur with equal frequency [143]. Peripheral cholangiocarcinoma is usually a large tumor. Hilar cholangiocarcinomas present earlier with ductal obstruction and jaundice and are more difficult to image, as the tumors are smaller. Cholangiocarcinoma is usually hypoechoic (Fig. 129.15), but isoechoic and hyperechoic tumors may occur [144].

Both color and spectral Doppler sonography are useful in determining if cholangiocarcinoma is resectable [145,146]. Cholangiocarcinoma is unresectable when one of the following is present in both the left and the right lobes: lobar atrophy, vascular invasion, or obstruction of two or more sectorial (segmental) ducts. Vascular invasion, narrowing, and tumor contiguity are readily demonstrated with color Doppler sonography. Duct involvement can be diagnosed when a duct is obstructed by a mass, or when wall thickening or an intraductal mass is identified. When two or more ducts peripheral to the left or right hepatic duct are involved by the mass, sectorial (segmental) duct involvement is present. Sectorial ducts are those that arise from different liver segments and join to form the main left and right hepatic ducts.

The sonographic evaluation of the bile ducts should be performed before stenting, if at all possible. Stenting may cause mural and periductal hemorrhage or edema that can obscure or simulate tumor. It may also interfere with identification of dilated sectorial ducts by decompressing them.

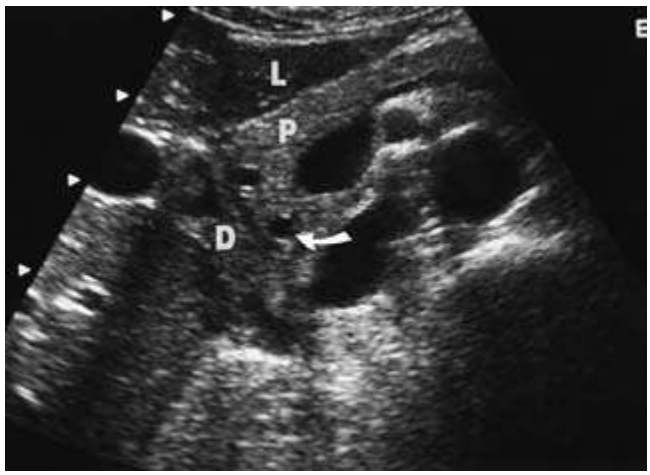


Figure 129.15 Normal pancreatic head. The pancreas (P) is more echogenic than the hepatic parenchyma (L). Note the anatomical relationship of the duodenum (D) to the pancreatic head. The rounded anechoic areas within the pancreatic head are the gastroduodenal artery (anteriorly) and the common bile duct (curved arrow).

Biliary cystic neoplasms

Biliary cystic neoplasms (formerly called biliary cystadenoma and cystadenocarcinoma) are rare. Lesion morphology ranges from completely cystic with a few thin septations to cystic with thick septations and large amounts of internal soft tissue. Thick, sometimes papillary septations and larger amounts of internal soft tissue characterize the mesenchymal variant, which has a greater potential for malignant behavior.

Other conditions

Biliary parasites

Biliary parasites such as *Ascaris* species or *Clonorchis sinensis* may infest the biliary tree and cause secondary biliary tract obstruction [147]. Sonographically, the adult *Ascaris* worm may be seen as a tubular structure within the bile ducts or gallbladder. The flukes of *Clonorchis sinensis* are small and not generally visible with sonography. However, aggregates or clumps of parasites may be identified within the extrahepatic bile duct or within the gallbladder.

Mirizzi syndrome

Mirizzi syndrome is biliary obstruction caused by a gallstone impacted in the cystic duct or gallbladder neck. Extrinsic compression and inflammation obstruct the extrahepatic bile duct. Sonography may suggest the diagnosis when a stone is impacted in the neck of the gallbladder, proximal bile duct dilation is present, and the distal common bile duct is normal.

Choledochal cysts

Of the four main types of choledochal cysts, about 90% of cases involve dilation of the extrahepatic bile duct (type I). Sonographically, there is obvious dilation of the extrahepatic duct, often associated with varying degrees of intrahepatic dilation. Dilation may be cystic (IA), focal (IB), or fusiform (IC). Other types include choledochal diverticula (type II), choledochocele (type III), and multiple intra- or extrahepatic cysts (type IV) [148].

Caroli disease

Caroli disease is cystic dilation of the intrahepatic bile ducts. Some cases are associated with a choledochal cyst (Todani type 4A). Sonographically, multiple small cystic areas that may or may not appear to communicate with the unaffected bile ducts characterize Caroli disease. A central vessel identified with Doppler sonography supports the diagnosis. Other findings include renal cysts, cirrhosis, or liver abscess.

Bilomas

Bilomas are encapsulated bile collections caused by rupture, usually traumatic; of the biliary tree [149]. Large asymptomatic bilomas are not rare. Sonographically, bilomas are usually well-defined anechoic collections with striking through transmission of sound. Detection of a lesion with this appearance 2–6 weeks after liver trauma strongly suggests the diag-

nosis [150]. Occasionally, bilomas are more complex, with internal debris and septations, and less pronounced through transmission, which are features that may also occur with abscesses. In these cases, needle aspiration can exclude infection and confirm the presence of bile by laboratory analysis for bilirubin.

Bilomas discovered incidentally in asymptomatic patients generally do not require treatment; they resolve spontaneously within a few weeks. Imaging studies alone cannot always distinguish bilomas from abscesses, chronic hematomas, or hepatic cysts. Percutaneous drainage, when required, is often an effective alternative to surgery [149,151].

Pancreas

Indications for sonography

Although CT remains the most sensitive means of evaluating pancreatic disease, modern ultrasound technology and new scanning techniques (oral contrast, compression scanning) are reestablishing sonography as a useful and clinically relevant pancreatic imaging technique. Sonography is indicated in all patients with acute pancreatitis, not to evaluate the pancreas itself but rather to detect gallstones and biliary dilation. Computed tomography is superior to sonography in evaluating potential complications of pancreatitis, but sonography may be used to follow pancreatitis-associated fluid collections and can guide biopsy, drainage, or aspiration of selected pancreatic lesions. Sonography is the primary imaging method to screen patients with jaundice or abdominal pain. Thus, sonography may detect acute or chronic pancreatitis or reveal pancreatic masses. Sonography may be more effective than CT in determining whether a lesion is pancreatic or contiguous to the pancreas. Sonography is occasionally useful to characterize abnormalities noted on CT, determining, for example, whether a lesion is cystic or solid.

Patient preparation and technique

Pancreatic sonography is easier after the patient has fasted overnight or for at least 6 h. This improves visualization of the gallbladder, and minimizes GI gas. Sonography's spatial resolution is better than CT and MRI when conventional scanning parameters are used. In some patients, however, CT is needed to visualize the pancreas adequately. With sonography, optimal pancreatic visualization requires that intervening GI content (gas and fluid) be displaced by compression or by filling the gut with water or GI contrast material. For the head and body, we prefer compression, using 3- to 7-MHz curved array transducers. Compression results in better resolution – the transducer is closer to the pancreas. The left lateral decubitus position may be useful, with visualization of the pancreatic head through the gallbladder or liver. Oral administration of water may be helpful if compression is unsuccessful.

The tail of the pancreas is more difficult to image. Routine pancreatic scanning should include coronal and axial views through the spleen and left kidney. The normal tail is usually not seen, but lesions invisible on other views, especially pseudocysts, are occasionally delineated. Water or contrast in the stomach is useful for imaging the tail. Right anterior oblique positioning is useful to view the tail through the water-containing stomach. Color Doppler imaging can assess vascular complications of pancreatitis and delineate vascular relationships to pancreatic neoplasms.

Normal anatomy

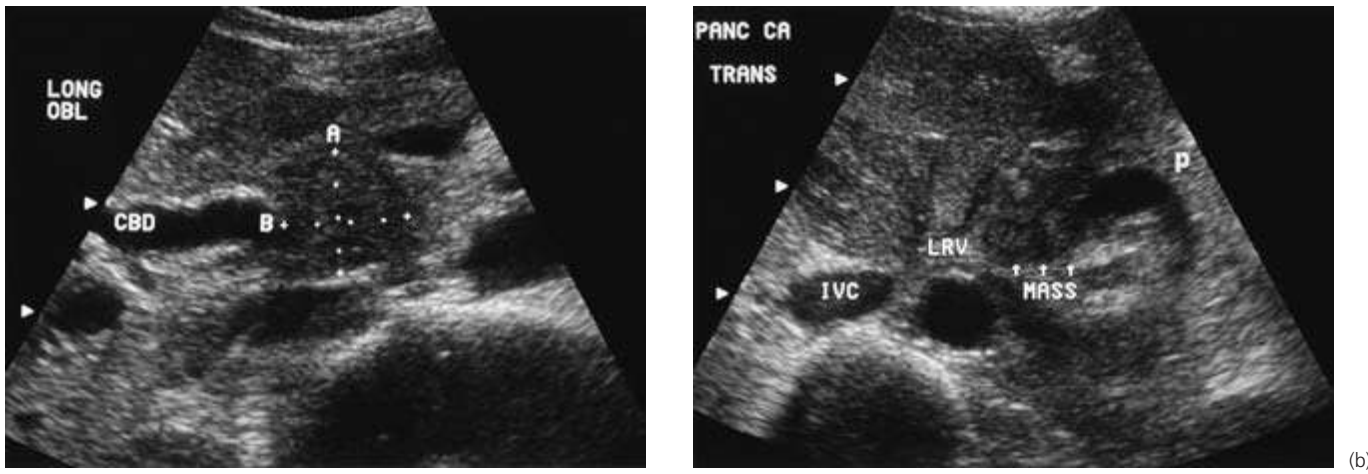
The normal pancreatic parenchyma is finely homogeneous and isoechoic to hyperechoic compared with liver parenchyma. The pancreas is a retroperitoneal organ that lies draped over the spine, aorta, and IVC. The pancreatic head is medial to and intimately related to the sweep of the second and third portions of the duodenum. The head is ventral to the IVC and medial to the superior mesenteric artery and vein (Fig. 129.16). In cross-section, the round and sonolucent common bile duct is seen in the posterior head, sweeping caudally and to the right as it passes into the duodenum. The body lies ventral to the aorta, splenic vein, and superior mesenteric artery, behind the stomach (with the collapsed potential space of the lesser peritoneal sac between). The pancreatic tail nestles into the splenic hilus and is closely related to the upper pole of the left kidney.

Acute pancreatitis

Evaluation with sonography is mandatory for all patients, even alcoholics, during their first attack of acute pancreatitis. Sonography in patients with acute pancreatitis focuses on the gallbladder and bile ducts – the gallbladder for stones (as a cause of pancreatitis) and the bile duct for choledocholithiasis and obstruction.

The reported prevalence of acute pancreatitis-associated abnormalities varies widely, from 33% to 90% of patients. Pancreatic echogenicity typically decreases in acute pancreatitis because of interstitial edema, but can be considered abnormally hypoechoic only when echogenicity is less than liver parenchyma. As the normal pancreas may be more echogenic than the liver, echogenicity is normal in many patients with acute pancreatitis. In patients with echogenic livers from fatty infiltration, the normal pancreas may be relatively hypoechoic, simulating the findings of pancreatitis. This is called pseudopancreatitis. Rarely, echogenicity may actually increase with acute pancreatitis, owing to hemorrhage, necrosis, or fat saponification. With newer sonographic systems, inhomogeneity of the parenchyma is often evident in acute pancreatitis.

Enlargement of the pancreas in acute pancreatitis is probably almost universal. Unfortunately, enlargement may be difficult to judge, as pancreatic size before the onset of pancreatitis is unknown and varies widely from individual to



(a) (b)

Figure 129.16 Pancreatic carcinoma with obstruction of the bile duct. **(a)** The long-axis view of the common bile duct shows a 3.5-cm mass (highlighted by calipers A and B) that obstructs the common bile duct (CBD). **(b)** A transverse sonogram obtained with the patient in the right anterior oblique position shows the mass (arrows). The mass is hypoechoic, compared with the normal pancreatic parenchyma (p). IVC, inferior vena cava; LRV, left renal vein.

individual. It is not unusual to detect extrapancreatic inflammatory changes even when the pancreas appears normal. Thus, extrapancreatic abnormalities may be the only sonographic findings that suggest the diagnosis of acute pancreatitis. Hypoechoic inflammation is often seen around the pancreas, in the anterior retroperitoneal spaces bilaterally, in the transverse mesocolon, around splanchnic vessels, and elsewhere [152]. Acute fluid collections, pseudocysts, and abscesses may be imaged. Color and spectral Doppler sonography may be useful in detecting pancreatitis-associated vascular complications, such as pseudoaneurysms and portal thrombosis.

Acute pancreatic fluid collections and pseudocysts

Acute pancreatic fluid collections and pseudocysts are the most common complications of pancreatitis. Pancreatic fluid collections consist of a combination of pancreatic juice, edema fluid, hemorrhage, and necrotic tissue resulting from acute pancreatic inflammation, enzymatic autolysis, and pancreatic ductal disruption. Two features distinguish a pancreatic pseudocyst from an acute fluid collection: persistence past 4–6 weeks and a well-defined wall (fibrous pseudocapsule) [153]. Acute fluid collections occur in approximately 30%–50% of patients with severe pancreatitis, but more than half of these regress spontaneously.

Computed tomography is more sensitive than sonography in diagnosing acute pancreatic fluid collections and pseudocysts [152]. When an acute pancreatic fluid collection is identified with CT or sonography, serial studies should be performed to document either resolution or persistence as a pseudocyst. If sonography shows the pancreatic fluid collection well, it is the most cost-effective method for serial evaluation. But CT should be performed immediately before any surgical drainage of a pseudocyst, to identify any lesions not detected by sonography.

Acute fluid collections and pseudocysts have a variable sonographic appearance depending on the associated internal hemorrhage and debris. Acute fluid collections may be anechoic to hyperechoic, but are generally hypoechoic with internal debris. Enhanced through transmission of sound is almost always present, but is variable in degree. Color Doppler sonography should be used to ensure that all discrete "cystic" areas are not pseudoaneurysms.

Pancreatic abscess and infected pancreatic necrosis

The two major infectious complications of acute pancreatitis are infected pancreatic necrosis and pancreatic abscess. Pancreatic abscess is a circumscribed collection of pus that contains little or no necrosis. Infected pancreatic necrosis is a region of infected nonviable pancreatic parenchyma. Pancreatic abscess is associated with a lower mortality (7%–33%) than infected pancreatic necrosis (~ 40%) [154]. Necrosis is diagnosed by contrast CT – no enhancement of the pancreatic parenchyma corresponds to necrosis. Ultrasound cannot diagnose pancreatic necrosis. Contrast CT, because of its superiority to sonography as a survey modality, is preferred when infectious complications are sought. Unfortunately, image findings alone cannot distinguish infected from uninfected collections [155], so aggressive use of CT- or US-guided needle aspiration is mandatory to diagnose infection.

The treatment of infectious complications of pancreatitis is controversial. Localized pancreatic abscess responds well, albeit imperfectly, to percutaneous drainage. vanSonnenberg et al. [156] reported successful cure of 69% of patients with pancreatic abscess by percutaneous drainage alone.

Hemorrhage and vascular complications

Erosion of small vessels is the most frequent cause of pancreatitis-associated hemorrhage. It may result in bleeding

into the pancreatic bed, the pancreatic duct, or a pseudocyst. Peripancreatic pseudoaneurysm [157] is rare, but potentially catastrophic. Pseudoaneurysms occur most often in the splenic artery (42%), the gastroduodenal artery (22%), and small peripancreatic arteries (25%). Contrast CT and Doppler sonography can identify pseudoaneurysms, but smaller pseudoaneurysms (i.e., < 1 cm) are more reliably diagnosed angiographically.

Inflammation from acute pancreatitis may cause venous occlusion. The splenic vein is most commonly affected and may result in gastric varices. The superior mesenteric or portal veins are also occasionally involved. Color Doppler sonography and contrast CT may be useful to diagnose pancreatitis-related thrombosis in the portal venous system.

Chronic pancreatitis

Image findings are poor predictors of the clinical severity of chronic pancreatitis [158], although patients with severe exocrine insufficiency are more likely to have abnormal pancreatic size and contour irregularity [159]. Multiple calcified stones located within the main or branch pancreatic ducts are the hallmark of chronic pancreatitis, usually alcohol related. Sonography is less sensitive than CT in detecting pancreatic calcifications. On occasion, the pancreas is diffusely hyperechoic. This may be difficult to judge sonographically, as the pancreas is normally more echogenic than adjacent parenchymal organs like the liver. Bolondi et al. believe that dilation of the main pancreatic duct is the most reliable sign of chronic pancreatitis [160]. The "double-duct sign" (dilation of pancreatic and bile ducts) may occur not only in pancreatic carcinoma but also in chronic pancreatitis [161]. Glandular atrophy or fatty infiltration may occur, making evaluation of the pancreas difficult sonographically. Chronic asymptomatic pseudocysts may be found in patients with chronic pancreatitis.

Focal masses that can simulate neoplasm occur in about one-third of patients with chronic pancreatitis. Calcification within the mass is strongly suggestive, but not definitive, for chronic pancreatitis. Uncalcified isoechoic or hypoechoic masses may require ERCP or biopsy to exclude malignancy [158].

Neoplasms

Sonography is the primary imaging method to screen patients with jaundice or abdominal pain. Thus, sonography often detects pancreatic masses. Sonography may be useful to characterize the internal architecture of masses, information that can be useful in classifying neoplasms. Sonography may detect a carcinoma as an area of decreased echogenicity within the gland when no morphological or attenuation abnormality is noted on CT [162].

Ductal adenocarcinoma

Sonographically, pancreatic carcinoma is typically a hypoechoic mass that deforms the gland's morphology (Fig. 129.17).

Figure 129.17 Acute appendicitis. Note distended appendix (A) with dilated tip (cursors).

Occasionally, pancreatic carcinoma is echogenic. A few scattered calcifications or cystic areas may occur occasionally (about 5% each) [163]. Secondary findings of carcinoma include ductal dilation (Fig. 129.17) (biliary and pancreatic), vascular and extraglandular invasion, and metastatic disease. Pseudocysts, related to obstruction of a pancreatic duct, have been reported in up to 11% of patients [164], although in our experience carcinoma-related pseudocysts are less frequent.

Most discussions of imaging in pancreatic and periampullary neoplasms have virtually ignored sonography [165,166], despite a 1988 study showing that grayscale sonography was useful in assessing resectability [167]. We and others have shown that color Doppler sonography can effectively assess resectability, specifically vascular involvement by tumor, with accuracy comparable with that of CT [168–171].

Ductal adenocarcinoma variants

Many histological variants of pancreatic ductal adenocarcinoma are indistinguishable on imaging from tumors with the usual histological features. Acinar center cell carcinoma and pleomorphic giant cell carcinoma can be larger and exhibit central necrosis [172]. Mucinous ductectatic carcinoma and mucin-hypersecreting carcinoma are variants of ductal adenocarcinoma [172,173]. These lesions have markedly dilated, mucin-filled, pancreatic branch ducts, usually in the pancreatic head. Sonographically, mucinous ductectatic carcinomas show multiple sonolucent, dilated ducts. A discrete mass may not be evident. Mucin-hypersecreting carcinoma usually has more extensive ductal dilation.

Cystic pancreatic lesions

The most common cystic pancreatic neoplasms are serous cystic neoplasm (microcystic adenoma), and mucinous cystic

neoplasms [174]. Morphologically, microcystic adenomas comprise many tiny cysts, most smaller than 2 mm. The most distinctive feature, present in about one-half of tumors, is a central radial fibrotic scar that frequently calcifies. Sonographically, microcystic adenomas are echogenic in regions where there are many tiny cysts. Through transmission is frequent. Larger cysts (2–3 cm) may be seen at the periphery of the lesion. Calcifications are imaged more often with CT than sonography. Microcystic adenomas are almost universally benign [175].

Mucinous cystic neoplasms consist of large cysts, usually easily imaged with CT or sonography. Most are located in the tail or body of the pancreas and are much more common in females. Calcifications occur in approximately 20% of these neoplasms (compared with about one-half of serous/microcystic neoplasms). Sonography usually reveals a mass with internal cysts of variable size. Unilocular lesions may occur. The septations may be few or many, thin or thick and polypoid. The internal architecture is often shown to better advantage by sonography than CT. Mucinous cystic neoplasms may be confused with rarer cystic neoplasms such as papillary cystic tumor, cystic islet cell tumor, lymphangioma, cystic metastases, or nonneoplastic lesions such as pseudocyst, abscess, and echinococcal disease [176].

Solid and papillary epithelial neoplasm, a low-grade malignancy found most frequently in young females, tends to be large and well encapsulated. Tumors can be mostly solid, mixed cystic and solid, or almost entirely cystic. Less common cystic neoplastic tumors include papillary cystic tumor, cystic islet cell tumors, and acinar cell cystadenocarcinoma. Simple cysts of the pancreas are uncommon. They occur sporadically or in association with von Hippel–Lindau syndrome and, rarely, in renal polycystic disease.

Endocrine tumors

It is difficult to image functional islet cell tumors; they are usually small when the patient presents with hormonal abnormalities. Insulinomas and gastrinomas are frequently less than 2 cm in diameter. In contrast, "nonfunctional" islet cell tumors can be large at the time of presentation. Sonographic detection rates are 25%–60% for insulinomas [177] and approximately 20% for gastrinomas [178]. Sonographically, islet cell tumors are usually hypoechoic, well defined, and round or oval in shape. Intraoperative sonography is useful in localizing occult neoplasms.

Gastrointestinal tract

Indications for sonography

The most common indication for GI tract sonography is evaluation of the patient with right lower quadrant pain and possible appendicitis. Sonography may also be useful in the diagnosis of diverticulitis, small bowel obstruction, and bulky mesenteric or GI neoplasms. One of the more useful applications of sonography of the GI tract is endolumenal ultrasound to stage the depth of GI tract neoplasms. This is discussed in Chapter 154.

Patient preparation and technique

Patients undergoing sonographic evaluation for possible GI tract abnormalities typically require no special preparation. Patients with right lower quadrant pain and possible appendicitis can be imaged immediately after initial assessment in the emergency room. Having the patient drink sufficient water to distend the upper GI tract may facilitate evaluation of the stomach and duodenum. A water enema may be useful in selected patients to evaluate pelvic masses.

Graded compression sonography is the most important technique for evaluation of possible appendicitis and other focal GI tract abnormalities [179]. This technique takes advantage of the fact that lesions infiltrating the bowel wall alter its compressibility. The normal GI tract can be readily compressed by the sonologist when moderate pressure is applied with the transducer. However, inflammatory or neoplastic disorders of the bowel alter its compressibility and render it relatively noncompressible [180,181].

Sonography has a number of important advantages compared with CT in evaluating the GI tract. Real-time observation of bowel peristalsis is possible with sonography. Delineation of layers of the bowel wall (most importantly, the echogenic submucosal layer) is also readily apparent with sonography. Rapid multiplanar imaging can be performed and vascular flow can be assessed without contrast agents with the use of color and power (the most sensitive form of color Doppler sonography) Doppler [182,183]. Finally, sonography is an interactive technique and the patient can be scanned at the site of maximal pain and tenderness to correlate symptoms with anatomical abnormalities. It should be noted, however, that sonography is often unable to image all the lumenal GI tract mesentery and subperitoneal space reliably because of bowel gas.

Graded compression is generally performed with a high-resolution linear array transducer (5–10-MHz) depending on the depth of the structures to be imaged. For most other GI studies, a 5-MHz curved array transducer yields the best results.

Normal anatomy

Standard abdominal transducers do not delineate all five layers of the bowel wall. The echogenic submucosal layer, however, is clearly visible and serves as a constant anatomical feature that is an extremely useful landmark in identifying an intraabdominal structure as a bowel loop. Pathological processes that cause ulceration and necrosis of the bowel lead to either focal or global loss of visualization of the echogenic submucosa [184]. Primary neoplasms involving the bowel wall may result in focal bowel wall thickening referred to as the target sign or "pseudokidney" sign [185]. Tumor infiltrat-

ing the bowel wall appears as a hypoechoic mass (mimicking the cortex of the kidney). The echogenic mucosal surface lumen is preserved mimicking the fat-containing hilum of the kidney. Gas trapped within ulcerating lesions involving bowel wall may result in high-amplitude echoes with acoustic reverberation artifacts within the submucosal layers of the bowel wall.

Color Doppler sonography may be a useful adjunct to grayscale imaging in evaluation of patients with focal bowel wall thickening [182,183]. Increased arterial flow within the involved segment suggests either inflammation or infection; diminished or absent flow suggests intramural hemorrhage, ischemia, or infarction. The vascularity of GI tract tumors is variable but, in general, adenocarcinomas are hypovascular.

Acute appendicitis

In patients with an uncertain clinical diagnosis of appendicitis, sonography is preferred to CT in women of childbearing age, in pediatric patients, and in thin adult male patients. Computed tomography is preferred in patients who are obese or if appendiceal perforation is suspected [186]. The sonographic criteria for diagnosing acute appendicitis include a noncompressible appendix measuring 7 mm or more in greatest anteroposterior diameter (Fig. 129.18) [187]. An appendix measuring 5 mm or less is virtually always normal. Appendices measuring 5–6 mm are equivocal for appendicitis, and clinical observation or repeated sonography should be considered [187]. In a patient with right lower quadrant pain, the presence of an appendicolith within a noncompressible appendix generally indicates acute appendicitis regardless of the size of the appendix. In some patients, early acute appendicitis is confined to the distal tip of the appendix [188]. It is therefore important always to visualize the entire length of the appendix to evaluate for distal appendicitis.

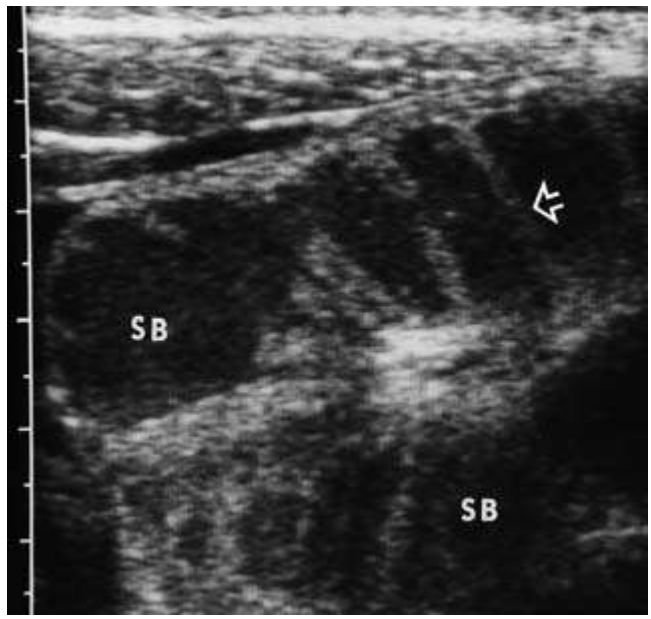


Figure 129.18 Closed-loop small bowel obstruction. Dilated U-shaped loop of small bowel (SB) represents closed-loop obstruction (arrow). Small bowel can be distinguished from other gastrointestinal viscera by identification of the valvulae conniventes (open arrow).

Diverticulitis

In most patients, CT is the imaging method of choice to evaluate patients who present with left lower quadrant pain and possible sigmoid diverticulitis [189]. This is particularly true in septic patients with possible paracolonic abscesses that may require percutaneous or surgical drainage [190]. Nevertheless, sonography may play a useful role as a screening examination in patients with pelvic pain and unclear signs and symptoms of diverticulitis. The sonographic findings in acute diverticulitis include focal mural thickening of the bowel and increased echogenicity of the mesenteric fat with hyperemia on color Doppler imaging [191]. In some patients, the infected colonic diverticulum may be identified as an echogenic intramural focus due to either gas or echogenic pus within the diverticulum [191]. On occasion, ectopic gas bubbles may be identified in the sigmoid mesentery due to their characteristic high-amplitude linear echoes and distal reverberation artifacts.

Enteritis and colitis

The sonographic findings of enteritis or colitis are often nonspecific [192]. Typical findings include focal areas of mural thickening of the bowel, often with associated adjacent adenopathy. There is lack of compressibility of the involved segment with graded compression. Increased flow on color Doppler may be demonstrated with high-frequency probes. Fibrofatty mesenteric masses may be identified as focal echogenic areas in Crohn's disease. In general, CT is superior to sonography in identifying mesenteric abscesses, colovesicular fistulae, and retroperitoneal abnormalities that accompany Crohn's disease [192].

Mesenteric adenitis

In the presence of a normal appendix or sonography, the identification of enlarged mesenteric lymph nodes is highly suggestive of mesenteric adenitis. There may also be associated thickening of the terminal ileum [193]. Once this diagnosis is suggested, patients should be observed clinically without surgical intervention. Symptoms of mesenteric adenitis typically resolve spontaneously without specific therapy. Color Doppler may be useful to highlight the hypoechoic lymph nodes adjacent to the flow in mesenteric vessels.

Intestinal obstruction

Sonography and CT may play an important role in the patient with possible small bowel obstruction [194]. The characteristic sonographic features are dilated fluid-filled loops with increased peristalsis on real-time examination. Fluid-filled levels and echogenic intralumenal debris may also be seen

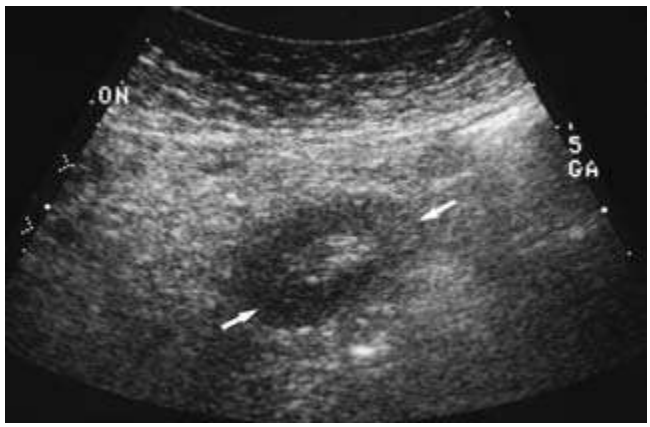


Figure 129.19 Pseudokidney sign of gastrointestinal tumor. Sagittal sonogram of midabdomen demonstrating a mass (arrows) that resembles a kidney.

within the dilated, obstructed bowel. In any patient with possible obstruction, a specific attempt should be made to identify a transition zone between dilated and nondilated bowel. This is often the most specific finding to confirm the diagnosis of obstruction. On occasion, sonography may identify GI tract tumors, mesenteric masses, abscesses, and other intra-abdominal pathology that caused the obstruction. The appearance of intussusception is characteristic on sonography as there is a target appearance of the invaginated intussusceptum. A crescent-shaped intralumenal area of echogenic fat representing invaginated mesentery is identified at the level of the lead point.

Gastric outlet obstruction may be diagnosed whenever marked dilation of the stomach is noted with a collapsed duodenum. In selected patients, however, gastroparesis may have a similar appearance. Closed-loop obstruction characteristically results in a single U-shaped aperistaltic loop of dilated bowel (Fig. 129.19). Hyperperistaltic bowel is noted proximal to this loop.

Small bowel infarction

In most patients with suspected mesenteric ischemia and small bowel infarction, CT or mesenteric angiography are performed emergently. However, in selected patients, sonography may be performed because of confusing signs and symptoms on clinical presentation. Mural thickening of the small bowel wall resulting from either intramural hemorrhage or edema may be visualized as a noncompressible area of mural thickening [182,183]. In the appropriate clinical setting, absence of vascular flow on color and more specifically with power Doppler suggests the diagnosis of ischemia.

Gastrointestinal tract tumors

In patients with palpable abdominal masses, sonography is often valuable in identifying the organ of origin of the lesion. As previously noted, the pseudokidney sign may be due to tumors infiltrating through muscular elements of the bowel wall (Fig. 129.20). Caution must be observed, however, in that focal inflammatory or ischemic processes may mimic neoplasms on sonography. Endoscopy, barium studies, or CT are often required to establish a more precise diagnosis. Both lymphoma and GI stromal tumors result in bulky, exophytic masses that are typically hypoechoic on sonography. Areas of liquefied hemorrhage or necroses are more typical of GI stromal tumor.

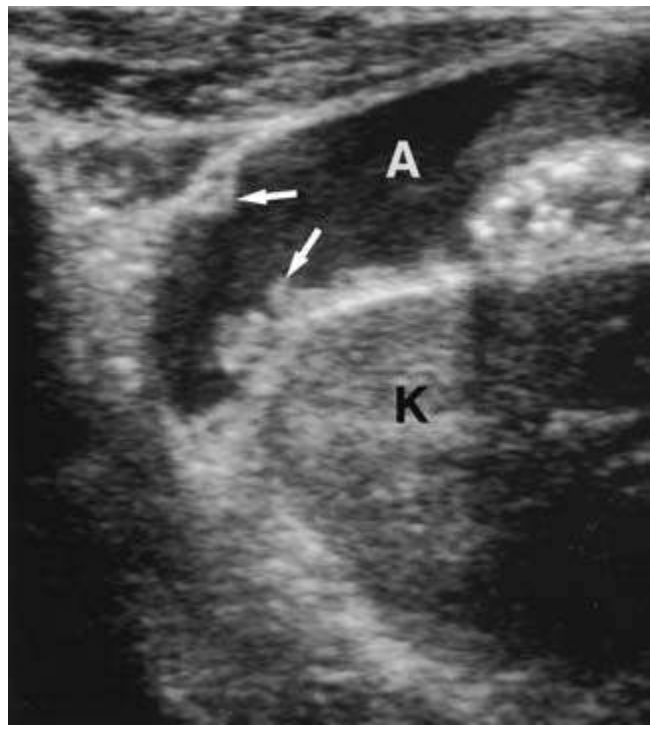


Figure 129.20 Peritoneal implants from mesothelioma. Transverse scan of right lower quadrant demonstrates ascites (A) and plaque-like peritoneal implants (arrows). K, right kidney.

Peritoneal cavity

Indications for sonography

The most common indication for evaluating the peritoneal cavity with sonography is to search for intraperitoneal fluid collections such as ascites, abscesses, or hemorrhage. Sonography is useful not only to identify intraperitoneal fluid collections but also to guide percutaneous needle aspiration for definitive diagnosis. Solid peritoneal masses representing either primary or metastatic tumors may also be detected on occasion with sonography. However, CT is the preferred imaging modality in patients with a clinical suspicion for peritoneal metastases.

Patient preparation and technique

Scanning of the pelvic peritoneal spaces is easier when the bladder is full. The distended bladder provides an excellent

acoustic window to evaluate the cul-de-sac or retrovesical recess. However, the remainder of the peritoneal cavity can readily be imaged with little or no patient preparation. In general, a curved 3.5- or 5-MHz transducer provides adequate penetration for a general abdominal survey to detect intraperitoneal fluid in the upper peritoneal compartments. Where access is difficult, sector transducers may be useful. High-resolution linear arrays (5–7.5 MHz) are best for evaluation of superficial peritoneal implants or omental abnormalities.

Intraperitoneal fluid collections

Sonography excels in demonstrating even minute quantities of intraperitoneal fluid (Fig. 129.21). With endovaginal scanning, small amounts of intraperitoneal fluid may routinely be demonstrated in the cul-de-sac as a normal finding in ovulating women. In the upper abdomen, free intraperitoneal fluid first collects in the hepatorenal fossa, which is the most dependent portion of the supramesocolic peritoneal cavity [195]. As fluid increases within the peritoneal cavity, it involves the subphrenic, subhepatic, and intraloop compartments [196]. Massive ascites typically displaces bowel loops centrally unless tethered by adhesions [197].

Uncomplicated serous fluid collections are typically anechoic. On sonography, complex fluid characteristically demonstrates internal debris, septations, and low-level echoes. The differential diagnosis of complex intraperitoneal fluid includes hemorrhage, infection, or carcinomatosis. On occasion, longstanding bland ascites may contain internal septations and be loculated. However, ascites that is not infected generally has no mass effect and passively conforms to its intraperitoneal components. Intraperitoneal abscesses, by contrast, typically exhibit mass effect and may displace adjacent bowel and or adjacent solid organs. It is not always possible to differentiate loculated bland ascites from an abscess by sonographic criteria alone. Therefore, diagnostic needle aspiration is often required.

Intraperitoneal abscesses

Computed tomography and sonography have greatly facilitated the diagnosis of intraabdominal abscesses [198–201]. With both modalities the predominant finding is a localized fluid collection with mass effect. Gas bubbles or an air–fluid level may be present in some patients, but are not identified in the majority of intraabdominal abscesses. Sonography is the imaging method of choice for hepatic, pericholecystic, and pelvic abscesses, whereas CT is superior to sonography in patients with pancreatic, retroperitoneal, paraspinous, intraloop, or gas-forming abscess [199]. In the postoperative setting, CT is often preferred to sonography because of frequent problems with access as a consequence of open wounds and surgical drains.

The sonographic appearance of intraabdominal abscesses is variable and depends on the presence or absence of septations or solid debris [201]. Also, there is considerable overlap with the sonographic appearance of other complex intraperitoneal fluid collections such as lymphoceles, bilomas, pancreatic pseudocysts, postoperative seromas, and hematomas. Because of the attenuation of sound by proteinaceous debris, many intraabdominal abscesses demonstrate little if any enhanced through transmission of sound. Thus, an intraabdominal abscess may sonographically mimic a solid hypoechoic mass [201]. This pitfall should always be kept in mind in a febrile patient. Color Doppler may be of value in resolving this dilemma as liquefied abscesses lack internal vascularity. Intraabdominal enteric abscesses are often "walled-off" by adjacent mesenteric or omental fat. In some patients, it may be possible, with sonography, to identify adjacent abnormal bowel to identify the cause of the abscess (i.e., appendicitis or diverticulitis). Both CT and sonography may be valuable for guidance of percutaneous needle aspiration for confirmation of an abscess and subsequent catheter insertion for definitive therapy [198].

Tumors of the peritoneum, mesentery, and omentum

The most common intraperitoneal neoplasms are metastatic carcinomas. The majority of these lesions are tiny plaque-like implants on the surface of the bowel or mesentery that cannot be imaged with any diagnostic techniques. In the presence of ascites, peritoneal implants may be detected by sonography as solid masses arising from the peritoneum or mesentery surrounded by peritoneal fluid (Fig. 129.21). Thickened septations may also be demonstrated sonographically. Diffuse infiltration of the omentum is a characteristic finding in patients with ovarian metastases, carcinomatosis from GI tract malignances, and primary omental tumors. Primary neoplasms involving the omentum, such as mesothelioma, can be imaged with graded compression sonography using high-frequency linear array transducers [202]. Diffuse omental infiltration ("omental caking") typically results in a broad band-like mass anterior to the transverse colon. Because of their superficial location, these abnormalities are often best imaged with high-resolution linear array transducers.

Pseudomyxoma peritonei, an unusual form of peritoneal carcinomatosis caused by mucinous adenocarcinomas of the appendix, ovary, or colon [203], results in gelatinous intraperitoneal masses that cause subtle scalloping of solid viscera such as the liver and spleen. Echogenic intraperitoneal masses containing debris may be detected sonographically.

Mesenteric masses may often be best imaged with sonography using a graded compression technique and color Doppler imaging. Adenopathy with the dorsal and ventral leaves of the mesentery may be noted to encase the mesenteric vessels highlighted by color Doppler. Primary mesenteric and peritoneal tumors such as mesothelioma cannot be distinguished from the more common metastatic peritoneal tumors.

Intraoperative ultrasonography

The information obtained by intraoperative ultrasonography (IOUS) and laparoscopic ultrasonography (LUS) often has a direct impact on clinical management and choice of surgical procedures at the time of surgery [204]. The demand for IOUS has grown steadily, and the proliferation of minimally invasive surgical techniques has led to increased utilization of LUS. The latter can provide both diagnostic information as well as guidance for interventional procedures, such as biopsy, aspiration, and tumor ablation [205].

Indications

In gastroenterology, IOUS is most often used in evaluating patients who are candidates for surgical resection of primary or metastatic hepatic malignancies [206]. Formerly, IOUS was far superior to conventional preoperative imaging, including CT and MRI, routinely detecting 25%–30% more lesions than on preoperative studies [207]. However, with the development of faster MRI, and multidetector CT and PET scanning, the percentage of additional lesions picked up by IOUS has diminished to approximately 10% [37–39]. Intraoperative ultrasound of the liver is important in defining the precise anatomical location of tumors to help decide the extent of resection. It is used in adult living related liver transplantation to define the avascular plane of resection when harvesting the donor right lobe.

Laparoscopic ultrasound is often used to assess pancreatic neoplasms, chiefly to look for signs of unresectability (e.g., vascular invasion, liver metastases) prior to commitment to open laparotomy. Intraoperative ultrasound may be helpful in localizing nonpalpable islet cell tumors, evaluating cystic pancreatic malignancies, and helping with surgical drainage procedures in chronic pancreatitis. Moreover, LUS is useful in evaluating choledocholithiasis, inflammatory and neoplastic biliary tract diseases. Both IOUS and LUS can provide accurate guidance for interventional procedures, including biopsies, aspiration of cysts, drainage of pseudocysts or abscesses, and guidance for a variety of tumor ablation techniques.

Patient preparation and technique

No patient preparation is needed for IOUS beyond that required for surgery itself. For the liver and intrahepatic bile ducts, a 5-MHz T-shaped side-fire probe is best because it can penetrate the entire liver, often 12–15 cm in depth. The liver is usually imaged from the anterior surface because it is accessible and provides a smooth scanning surface. The pancreas and extrahepatic biliary tract are better imaged with end-fire probes with frequencies up to 7.5 MHz, because the depth is much less than with the liver. Laparoscopic ultrasound probes can be either linear or curvilinear arrays, and must be small enough to fit through a standard 10–11-mm laparoscopic port; they range from 5 to 7.5 MHz, depending on the target organ being scanned.

All intraoperative transducers must either be sterilized or covered with sterile sheaths, which must cover the entire electrical cord [208]. Direct sterilization (if allowed by the manufacturer) is preferable to the use of sheaths, which take time to apply and may tear during the procedure. Sterile gel or sterile saline must be applied inside the sheath for acoustic coupling with the transducer. Natural moisture on the intraabdominal organs is usually sufficient for acoustic coupling, but warmed sterile saline can be also used.

Liver

The principal uses of liver IOUS include:

- detection of occult primary or metastatic tumors
- demonstration of lobar and segmental anatomy, vascular supply, and any anomalous or accessory vasculature
- determination of resectability and guidance for planning appropriate lobar, segmental, or nonsegmental wedge resections
- guidance for biopsy, aspiration, or therapeutic ablation.

The superb spatial resolution of IOUS and LUS allows for consistent imaging of cysts as small as 1–3 mm and solid nodules as small as 3–5 mm. This spatial resolution is slightly better than the usual lower limit of 5–10 mm for detection of solid lesions by preoperative imaging techniques. Most of the additional IOUS-detected lesions will be extremely small, subcentimeter nodules that frequently cannot be visualized or palpated by the surgeon.

Many lesions that IOUS detects are unrelated to the patient's primary or metastatic neoplasm. These include hemangiomas, cysts, bile duct hamartomas, focal nodular hyperplasia, and focal areas of sparing in fatty livers. Usually, these lesions are distinguishable from malignancy, but if questions persist, IOUS-guided biopsy can be readily obtained.

Demonstration of the relationship of tumors to the hepatic vasculature by IOUS may be important in determining tumor resectability. Hepatocellular carcinoma frequently invades the portal or hepatic veins. This can be demonstrated by IOUS and confirmed by IOUS-guided biopsy. Surgeons strive for a 1-cm tumor-free resection margin, and the demonstration by IOUS of the tumor boundaries may influence the extent of resection. Demonstration of standard or anomalous hepatic vasculature may be of critical importance during donor hepatectomy for adult living related transplantation procedures.

Guided biopsies and aspirations are usually performed freehand using the IOUS images to define the accurate placement of the tip of the biopsy or aspiration devices. Guidance by IOUS or LUS is indispensable for successful intraoperative tumor ablation techniques [209]. For cryosurgery, guidance is important both for placement of the cryoprobes as well as real-time monitoring of the expanding cryolesion to ensure that the cryoablation margin extends beyond the tumor into

the surrounding liver. For ethanol ablation and hyperthermic ablation techniques, IOUS guidance is critical for accurate placement of the needles and probes [210], although the microbubbles generated during the actual ablation make it more difficult accurately to assess the ablation margins because of scatter of the ultrasound waves.

Pancreas

As with liver imaging, improvements in CT, MR, and radionuclide imaging have changed the role of pancreatic IOUS. Whereas formerly, IOUS was by far the most accurate technique for localizing islet cell tumors [211], the vast majority of these tumors are nowadays accurately identified and localized with preoperative imaging. Most islet cell tumors are homogeneous and hypoechoic when compared with the more highly echogenic background of the pancreas [212]. These lesions are usually hypervascular, and color Doppler imaging may be helpful in lesion detection. Insulinomas are usually solitary, benign, and hypoechoic [213], but when performing IOUS for evaluation of islet cell tumors, a complete study should also involve imaging of the liver and the periportal and peripancreatic beds for evidence of metastatic disease. Metastases are more frequently found in other forms of islet cell tumors, such as gastrinomas, glucagonomas, and VIPomas. Gastrinomas may frequently be found in extrapancreatic locations, in the so-called "gastrinoma triangle" between the head of the pancreas, duodenal sweep, and root of the mesentery, including locations within peripancreatic lymph nodes and even within the wall of the adjacent duodenum [214]. The role of IOUS is primarily to identify those nonpalpable small islet cell tumors for the surgeon to facilitate an appropriate and complete tumor resection.

For pancreatic ductal adenocarcinoma, preoperative imaging techniques are fairly accurate in defining unresectability as a consequence of invasion of critical vascular structures. Laparoscopy is often performed to search for small peritoneal or surface liver metastases to avoid unnecessary open surgical procedures for those with incurable pancreatic carcinoma. Laparoscopic ultrasound can evaluate the liver for occult metastases beneath the surface, and search for signs of unresectability due to vascular invasion [214,215].

Intraoperative ultrasound is also useful for assessing cystic neoplasms of the pancreas and intraductal papillary mucinous neoplasms (IPMN). Imaging findings may be helpful in distinguishing serous microcystic cystadenomas from the more likely malignant mucinous cystadenomas and cystadenocarcinomas [216]. The mucinous lesions are more likely to have thick septations, mural nodules, or other solid components. In IPMN, intraductal papillary masses can be demonstrated, as well as small side branch cysts, which may help to define the total extent of the neoplasm and help select the best resection plane [217]. For more benign-appearing cystic neoplasms, IOUS may demonstrate the relationship of the tumor to the common bile duct or pancreatic duct and may allow for conservative wedge resections if the lesion is sufficiently distant from these structures.

In patients undergoing surgical drainage procedures for pancreatitis, IOUS may be helpful to guide pseudocyst drainage [218]. The size, extent, internal contents, and site of origin of pseudocysts and other peripancreatic fluid collections can easily be demonstrated, and real-time guidance may facilitate drainage of these collections. The pancreatic duct can be well visualized by IOUS. Color Doppler imaging may also be important in evaluating fluid collections for the presence of pancreatitis-associated pseudoaneurysms.

In patients with chronic pancreatitis and focal masses, IOUS may allow for definition of "a mass within the mass," which would be suggestive of concurrent pancreatic carcinoma. These masses may be difficult to palpate because of surrounding fibrosis, and IOUS-guided biopsy can be important. Patients with chronic pancreatitis refractory to medical treatment may undergo a Puestow (pancreaticojejunostomy) drainage procedure. Identification of the dilated pancreatic duct and selection of the appropriate site for pancreatotomy can be difficult because of fibrosis and calculi. Intraoperative ultrasound can facilitate placement of a needle, guide wire, or surgical instrument into the pancreatic duct [219], which can then be used as a visual guide to assist in the surgical drainage procedure.

Biliary tract

In patients undergoing resection for gallbladder carcinoma, IOUS is useful to evaluate the depth of invasion of tumor into the contiguous liver, thereby helping to decide the extent of hepatectomy. Careful scanning of the liver and contiguous lymph node bed is important to identify metastatic disease, which would obviate curative surgery [220].

Intraoperative ultrasound of the bile ducts is at least as good as standard intraoperative cholangiography [221], and LUS may be preferable to laparoscopic cholangiography, in both its ease of performance and accuracy [222]. In most instances choledocholithiasis is diagnosed with preoperative imaging and frequently treated with endoscopic sphincterotomy and stone retrieval. As a result, the routine use of IOUS or LUS for common duct stones is not favored because of the low yield of positive studies [223].

In patients with the Klatskin-type cholangiocarcinoma, IOUS can provide indispensable information regarding tumor resectability. With IOUS defining the full extent of tumor, appropriate decisions can be made regarding radical hepatic resections, in contrast with conservative biliary drainage procedures in patients with bilobar disease [224]. When bilobar tumor prevents surgical resection and hepaticojejunostomy, IOUS can be used to identify the largest dilated peripheral bile ducts, which may be suitable for internal drainage. Similar information can be obtained by laparoscopic ultrasonography,

thereby avoiding unnecessary laparotomies in patients who are unresectable because of the extent of disease [225]. These patients can then be better palliated by percutaneous catheter biliary drainage. We have also found IOUS useful in the surgical approach to patients with segmental Caroli disease and recurrent pyogenic cholangitis, by accurately defining the extent of disease and affected ducts and segments, including visualization of tiny intraductal stones, which may be missed with preoperative imaging studies.

Miscellaneous

Some authors have advocated laparoscopic ultrasound for staging tumors of the esophagogastric junction and stomach. Laparoscopy is useful in these patients to visualize peritoneal metastases or small surface liver metastases [226], and a small percentage of this group had subsurface liver metastases that could only be discovered by LUS [227]. However, the discovery of deep liver lesions on LUS requires biopsy confirmation to diagnose metastatic disease, because incidental benign liver nodules are frequently encountered. Laparoscopic ultrasound-guided liver biopsies are technically feasible but difficult and not always successful. An analysis of the contributions of LUS for staging gastrointestinal malignancies concluded that the additional value of LUS to laparoscopy was small [228].

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B IMAGING

130 Endoscopic ultrasonography

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Endoscopic ultrasonography (EUS) is an established diagnostic and therapeutic modality in gastroenterology with increasing widespread use. Although most gastroenterologists are not trained in the practice of EUS, it is important that they all understand the principles of the technique and the indications (diagnostic and therapeutic) for EUS to offer optimal patient care.

This chapter outlines the principles of EUS, provides a brief overview of the equipment, and details the indications for EUS in commonly encountered clinical scenarios. Particular attention is paid to the role of EUS and EUS-guided tissue sampling in relation to other imaging modalities. This is not a comprehensive chapter on the practice of endosonography and the authors recommend that anyone interested in learning EUS should review textbooks dedicated to the practice of EUS and to seek out an established EUS training program.

Technical considerations

Basic properties of sound waves

EUS is the addition of an ultrasound transducer to the tip of an endoscope, which allows ultrasound-generated imaging of the gastrointestinal tract and adjacent anatomical structures. The ability to image in real time from inside the gastrointestinal tract has led to diagnostic and therapeutic indications. A basic knowledge of the principles of ultrasonography is required to understand EUS. Chapter 129 is a detailed review of sonography, and this section reviews those ultrasound principles related to EUS generation and interpretation.

Sound is mechanical energy generated in the form of vibrations as they propagate through a medium such as air, water, or tissue [1]. The frequency of a sound wave is measured in Hertz (1 Hz = 1 cycle/s) and the ultrasound frequency used in medical technology is in the range 1–30 MHz (1 MHz = 1 000 000 cycles/s). The velocity of an ultrasound wave is a product of the frequency and wavelength, and the velocity in a specific tissue depends on the acoustic impedance of that particular tissue. Hence, each tissue, based on its density and elastic properties, propagates sound waves at a particular velocity. Tissues with similar acoustic impedance will propagate similar amounts of sound waves, whereas tissues with different acoustic impedance will reflect the majority of sound waves. Air, bone, and fat have different acoustic impedances compared with soft tissue [2].

Ultrasound waves interact with tissue in four ways: reflection, refraction, scattering, and absorption.

- Reflection is when the sound wave strikes a surface at a nonperpendicular angle and is thus reflected at an angle equal to the angle of incidence. If a sound wave encounters a medium interface with different acoustic impedance, a proportion of the ultrasound waves will be reflected back to the transducer while the remainder of the waves will be transmitted to the second medium.
- Refraction is the amount of bending the transmitted wave experiences. This occurs when there is a difference in the acoustic velocities of the incident and transmitted mediums, and is analogous to light traveling though a prism.
- Scattering occurs when a propagating ultrasound wave interacts with different components in a tissue that are smaller than the wavelength and with different acoustic impedance than the propagating medium. When scattering occurs, only a small portion of the scattered wave is reflected back to the transducer. Examples of scatterers include collagen, fat goblets, and individual cells [1].
- Absorption is when tissue assimilates the ultrasound wave and generates heat. Higher frequencies cause more tissue vibration resulting in greater absorption of the ultrasound energy and more heat generation.

Components of an echoendoscope

The main components of an EUS system include the transducer, processor, and display monitor. A transducer is a device

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that generates the ultrasound pulse by converting electrical energy into mechanical energy. The piezoceramic element is the component responsible for the conversion of energy. The piezoelectric crystals vibrate when an electrical impulse is applied to them, generating an ultrasound wave that is propagated toward the surrounding tissue. A proportion of the ultrasound waves are reflected back to the transducer and subsequently converted back into an electrical impulse that is processed and displayed as an image on the monitor. The intensity of the returning echoes determines the brightness of the pixels on the screen, thus generating shades of black and white [2].

There are two types of the transducers, single element and phased array. Single-element transducers are mechanically rotated 270°–360° on an axis that is perpendicular to the axis of the endoscope; these are referred to as mechanical echoendoscopes. This is in contrast to phased-array transducers, where the piezoceramic crystals are stimulated by electric pulses sweeping along the long axis of the endoscope; these are referred to as electronic echoendoscopes [2].

The processor controls the transducer and amplifies the returning signal. When a transducer is perpendicular to the reflected waves, the greatest quantity of echoes are garnered by the transducer and the resulting image is optimized. As a result of scattering and absorption (see previous section for details), echoes that are returning to the transducer from greater distances are attenuated. These weak signals may require amplification, i.e., time gain compensation (TGC). TGC allows the EUS operator to adjust the amplification (gain) of the signal at varying distances from the transducer. Increasing the gain will increase detection of weak echoes while sacrificing resolution [2]. The operator can also increase the frequency of the ultrasound, which improves the resolution of the image. However, higher frequencies result in greater ultrasound wave absorption and decreased penetration, which leads to a reduced range of imaging.

Principles of imaging

It is important that the endosonographer has a basic understanding of the characteristics involved in determining image quality and the various modes available for imaging. These characteristics include resolution and artifacts of imaging, and modes such as A-mode and B-mode scanning, and Doppler.

Spatial resolution (the ability to discriminate two points as separate) is vital for quality imaging. Axial resolution is the ability to discriminate between two points along the beam path and is dependent on the frequency of the ultrasound wave. Axial resolution is the most important property in imaging the layers of the gastrointestinal tract. Lateral resolution is the ability to discriminate between two points in the same plane perpendicular to the transmitted ultrasound wave [1]. To achieve optimal lateral resolution, the ultrasound beam from the object of interest must lie in the narrowest portion, termed the focal zone. Radial echoendoscopes have a fixed focal zone (1.5–3.5 cm), which requires that the operator manipulate the EUS endoscope such that the target being imaged is located within the focal zone. Electronic array echoendoscopes have a variable fixed focal zone that is adjusted using the processor [2].

Since EUS imaging and interpretation are performed in real time, the endosonographer must be able to recognize image artifacts. Ultrasound waves can interact with adjacent tissue and fluid to create artifacts of imaging. An artifact is any phenomenon that does not accurately represent the target being imaged. The most common image artifacts are listed in Table 130.1, and several of these are reviewed below. A trained endosonographer must accurately identify and interpret such artifacts, so as to provide an accurate assessment of the ultrasound image and procedure.

Reverberation can occur when a single ultrasound beam is bounced back and forth between the transducer and a highly reflective surface until the signal is attenuated beyond detection of the processor. This can result in a false image deep to the true image; this artifact can be mitigated using TGC [1,2]. Acoustic shadowing occurs when a large impedance mismatch is encountered by the ultrasound wave, and there is intense hyperechoic reflection at the surface associated with an anechoic signal beyond the interface. Acoustic shadowing is a phenomenon often seen when imaging calcified lesions, such as in chronic pancreatitis or gallstones. Acoustic enhancement is the relative increase in ultrasound transmission in structures beyond a fluid-filled structure, and is due to less attenuation of the ultrasound wave as it travels through a fluid-filled structure in comparison to the surrounding tissues. This is commonly seen in the gallbladder, urinary bladder, vascular structures, and cysts [2]. The mirror image

Table 130.1 Artifacts of ultrasound imaging

Reverberation: multiple reflections between transducer and a strongly reflective surface
Reflection (mirror image): an air–water interface acts as a mirror, placing image at deeper depth than in reality
Refraction: difference in the acoustic velocities of the incident and transmitted mediums, resulting in objects placed erroneously at locations different from their true location
Acoustic shadowing: large impedance mismatch results in hyperechoic signal at interface and anechoic signal beyond interface
Acoustic enhancement: enhancement of structure beyond a fluid-filled structure
Side-lobe artifacts: a hyperechoic signal within an anechoic structure that disappears with scope repositioning

artifact occurs in the setting of a highly reflective surface behaving as a mirror. The EUS machine places the image at a deeper depth than in reality, and is commonly seen when visualizing the rectum or intralumenal gas in the rectum.

Scanning is the processing of the received signal, which can be visualized in several imaging modes. The received signal is amplified (A-mode, or amplitude mode) by the processor to yield an A-mode signal. Most clinicians do not image using A-mode but it forms the basis of the most common type of imaging, B-mode. B-mode (brightness mode) scanning is created by processing a series of A-mode signals, the amplitude of the signal being reflected by the brightness of the dot on the display monitor. EUS systems generate images from a compound B-mode scan [1].

Doppler is a feature available on all EUS processors and takes advantage of the Doppler shift phenomenon. This states that an object in motion relative to the transducer will reflect an ultrasound wave at a different frequency relative to the frequency transmitted by the transducer. Hence, the returning wave reflected from an object moving toward the transducer will contain a higher frequency, and vice versa. The most common application of Doppler is imaging of blood flow, and assisting in the identification of blood vessels. Continuous-wave Doppler is the simplest configuration of Doppler ultrasound, consisting of a receiving and transmitting transducer. However, continuous-wave Doppler does not provide information about the depth at which the movement is occurring. Pulsed-wave Doppler consists of a single transmitting and receiving transducer that emits and receives a lengthy pulse wave and uses electronic gating to calculate depth information. Using pulse-wave Doppler with B-mode imaging is called duplex scanning. Color Doppler combines the principles of pulse-wave Doppler and shades of red or blue to reflect the relative velocities of blood flow. Immobile objects are represented on gray-scale B-mode imaging, and on this background, information regarding direction and relative velocity of blood flow are obtained. Power Doppler is the most sensitive Doppler for detecting blood flow, providing information on the presence of blood flow but no information on the relative velocity or direction of blood flow [1].

Equipment

Echoendoscopes can be broadly classified into two types, radial (or sector) and linear (convex array); each type is available with either a mechanical or electronic transducer. A variety of EUS catheter probes are available for imaging of small submucosal lesions and from within the biliary and pancreatic ducts. Along with the echoendoscope, the standard equipment must include an EUS processor, balloons, and needles for tissue sampling if fine-needle aspiration (FNA) is planned. This section focuses on the main types of echoendoscope and their uses. It does not focus on the specific competing products available for purchase in the market.

Radial echoendoscopes

Radial echoendoscopes provide circumferential views at right angles to the echoendoscope. The mechanical radial echoendoscope has been the workhorse of EUS by providing cross-sectional anatomical images with a 270°–360° field of imaging coupled with an oblique-viewing endoscope. Newer generations of radial echoendoscope include electronic array with forward-viewing endoscopic optics. The electronic technique allows for the addition of Doppler to the radial echoendoscope. A balloon is situated over the transducer tip to allow for water-filled acoustic coupling to the wall of the gastrointestinal tract, and imaging can be performed at a variety of frequencies from 5 to 20 MHz with a range of up to 12 cm (Fig. 130.1a).

Linear echoendoscopes

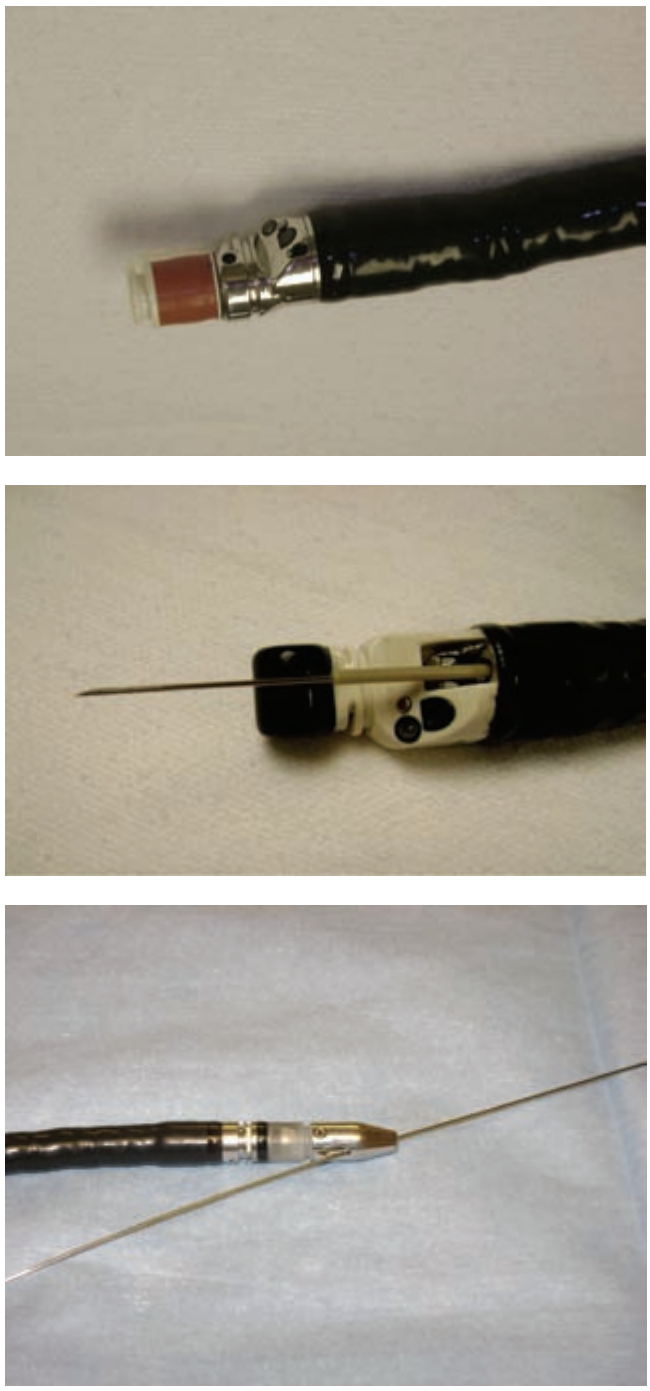
The linear echoendoscope provides an imaging view analogous to transabdominal ultrasonography. The ultrasound waves are transmitted in the same axis as the long shaft of the transducer, which allows for real-time therapeutic intervention, most commonly FNA. By advancing the FNA needle in the plane of the ultrasound wave, the endosonographer can visualize the needle in real time as it is inserted into the lesion for cellular acquisition. The linear echoendoscope has a 120°–180° field of imaging, and biopsy channel diameters range from 2.0 to 3.8 mm (therapeutic) (Fig. 130.1b).

Catheter probes

Ultrasound probes vary in their design and capabilities. They consist of a flexible shaft with a central wire that drives the rotation of the mechanical transducer at the tip. The transducer is surrounded by oil, which provides the acoustic interface with tissue, providing a 360° view. Techniques to improve probe-based imaging include close apposition of the probe to tissue with air aspiration, instillation of liquid into the gut lumen, use of a condom over the tip of the endoscope, and use of a balloon sheath over the probe. Catheter probes are available in various diameters (2–2.9 mm) and frequencies (12–30 MHz) [3–5]. A 2.9-mm guidewire-based EUS probe is available with imaging at 20 MHz that can be advanced via a side-viewing duodenoscope into either the biliary or pancreatic duct for intraductal imaging. The most common applications for catheter-based probes include assessment of small superficial lesions of the gastrointestinal tract, evaluation of submucosal nodules, and examination of pancreaticobiliary ductal pathology including choledocholithiasis and ductal strictures.

FNA needle

Various needles are available for use, ranging from 19G to 25G. The 22G needle is most often employed for FNA sampling



(a)

(b)

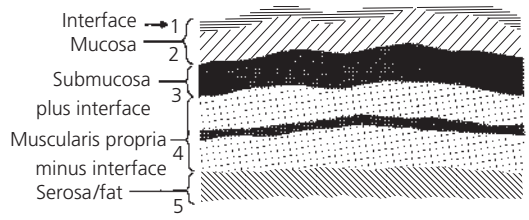
(c)

Figure 130.1 Types of echoendoscopes. **(a)** Radial imaging electronic echoendoscope. **(b)** Linear (convex array) echoendoscope. **(c)** Over-the-wire esophagoprobe.

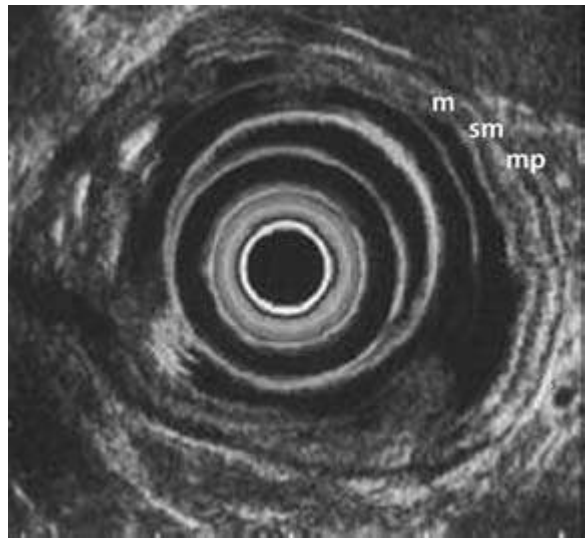
and the 19G needle is most often employed for therapeutic interventions, such as celiac plexus neurolysis and pancreatic pseudocyst drainage. There is no single needle diameter or technique that is proven to result in higher cytological yield [6]. The EUS Tru-Cut biopsy needle (Quickcore, Wilson-Cook, Winston-Salem, NC) is designed to provide core tissue sampling through a linear echoendoscope. Overall, studies demonstrate an equivalent diagnostic accuracy between the Tru-Cut needle and the traditional FNA needles; however, widespread experience with the Tru-Cut needle is lacking [7–11].

EUS imaging: general considerations

The ultrasound image of the normal gastrointestinal tract is dependent on the ultrasound frequencies used. Frequencies of 5–12 MHz produce five layers, while frequencies of 20–30 MHz depict seven to nine layers (Fig. 130.2) [12]. Most commonly, there are three echogenic layers separated by two echo-poor layers. The first echogenic layer, beginning at the mucosal surface, is thin and is produced by the interface between lumenal fluid or the balloon and the mucosa. The second layer is echo-poor and represents the remainder of the mucosa. This layer was formerly attributed to the muscularis mucosae; however, the normal muscularis mucosae is



(a)



(b)

Figure 130.2 The five layers of the stomach wall. **(a)** Normal five-layer endoscopic ultrasonography image of the stomach wall: m, mucosa; sm, submucosa; mp, muscularis propria. **(b)** The layers of the normal gastrointestinal wall as seen on ultrasound images. The ultrasound image comprises echoes arising from the anatomical layers and from the acoustic interfaces between tissue layers.

too thin to account for entire second layer [12]. The muscularis mucosae is usually obscured by an echo occurring at its interface with the lamina propria so that the location of the muscularis mucosae corresponds to the most superficial part of the third (submucosal) layer. If the muscularis mucosae is thicker than the interface echo, a separate thin hypoechoic layer is seen between this interface echo and the underlying submucosal layer [13]. The third ultrasound layer is easily recognizable because it is the most echogenic. This layer corresponds to the submucosa but is thicker because it also includes the interface between the submucosa and muscularis propria [12]. The fourth layer is echo-poor and corresponds to the muscularis propria. In areas with a well-developed inner circular and outer longitudinal muscle component, a small amount of connective tissue between the muscle layers may produce a line of echoes within the muscularis propria [12,13]. The fifth layer is echogenic but of variable thickness. If no subserosal fat or inflammation is present, this echogenic layer corresponds to the serosa and the interface between the serosa and the surrounding tissue. The layer can be thick in the rectum, where there is often abundant echogenic perirectal fat.

The appearance of organs in the mediastinum and abdomen is roughly similar to that seen by transcorporeal ultrasound, but often in greater detail. The posterior mediastinum, the aortopulmonary and subcarinal regions, portions of the heart, and the great vessels can be visualized with the transducer in the esophagus. The liver and spleen are homogeneous and of intermediate echogenicity; the liver can usually be differentiated from the spleen by the bright echoes adjacent to the intrahepatic portal venous branches.

The normal pancreas has a characteristic "salt and pepper" appearance (Fig. 130.3). The kidneys are easily recognized by the characteristic appearance of the urinary collecting system. In most patients, the left adrenal gland can be seen cephalad to the upper pole of the kidney as a seagull-shaped echo-poor structure. Large arteries and veins in the upper abdomen are key landmarks for orienting EUS images and include the aorta, celiac and superior mesenteric arteries, and the inferior vena cava and portal, splenic, and superior mesenteric veins.

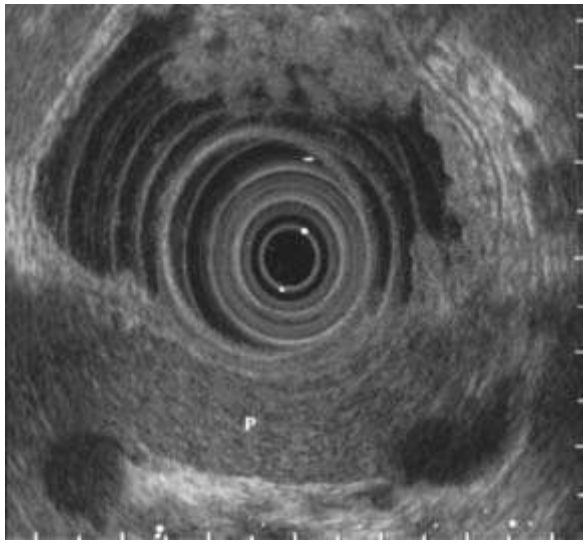
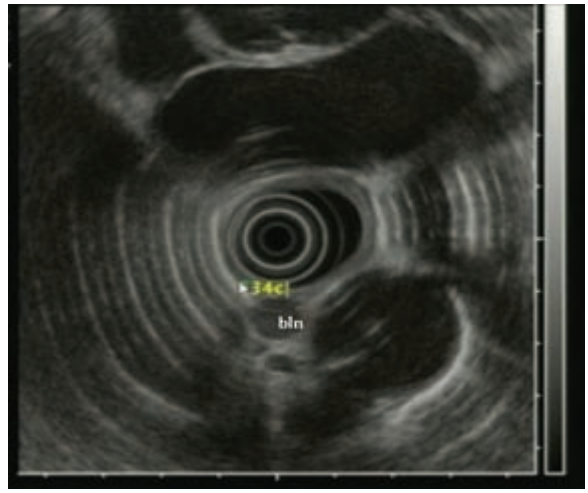
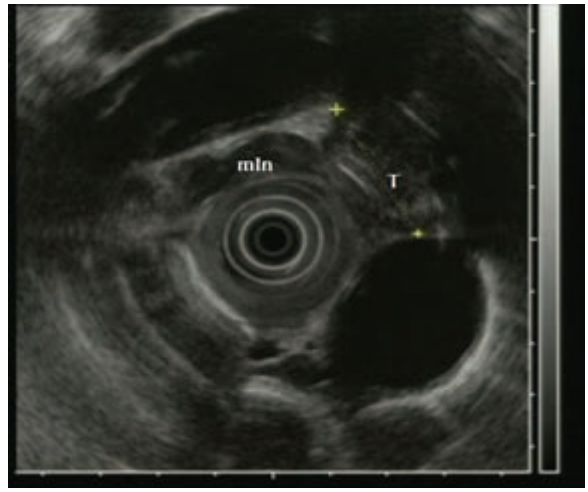


Figure 130.3 Normal pancreas. Radial endoscopic ultrasonography image showing characteristic "salt and pepper" pattern of the pancreas (P).

Efforts to differentiate malignant from benign lymph nodes by analysis of ultrasound features such as size, shape, echogenicity, internal echo pattern, and the character of their outer margin have been reported [14–16]. Malignant lymph nodes are usually echo-poor with sharply defined borders. The internal echoes from the nodes can be homogeneous or inhomogeneous. Benign nodes tend to have poorly defined boundaries and may be hyperechoic (Fig. 130.4). When nodes are over 1 cm in diameter, hypoechoic, and have a rounded distinct border, there is an 80%–100% probability of malignant involvement [17,18]. However, it is increasingly clear that the diagnosis of a malignant lymph node by



(a)



(b)

Figure 130.4 Benign and malignant lymph nodes. **(a)** Endoscopic ultrasonography (EUS) showing a benign lymph node (bln). **(b)** EUS of mediastinum showing a malignant lymph node (mln) and an adjacent tumor (T) arising from the lung.

EUS appearance alone has limitations, which has led to the increased use of EUS-guided needle aspiration for pathological confirmation of suspicious nodes [6,19].

EUS in common clinical scenarios

Lung cancer

The annual incidence and mortality of lung cancer worldwide is estimated to be 1.35 million and 1.18 million respectively [20]. The prognosis and treatment depends on histology (small cell vs nonsmall cell), accurate assessment of mediastinal lymph node spread, and the presence or absence of T4 disease (tumor invasion into adjacent organs, central vessels, or vertebrae). Nearly half of patients diagnosed with nonsmall cell lung cancer (NSCLC) present with advanced disease. It is clinically relevant to differentiate malignant spread to the ipsilateral lymph nodes or subcarinal lymph nodes (N2, stage IIIA) from spread to the contralateral lymph nodes (N3) or T4 disease (stage IIIB). Although 5-year survival in stage III NSCLC is dismal, neoadjuvant chemoradiation for stage IIIA improves median survival [21–23].

In patients with suspected lung cancer, EUS can visualize the following lymph node stations: station 4L (left paratracheal), station 5 (aortopulmonary window), station 7 (subcarinal), station 8 (lower paraesophageal), and station 9 (pulmonary ligamentum). Since ultrasound cannot penetrate the air-filled trachea, EUS cannot reliably assess anterior mediastinal lymph nodes at levels 2R (upper paratracheal), 4R (lower paratracheal), 3 (prevascular, retrotracheal), and 6 (paraaortic) [24,25].

Posterior mediastinal assessment

The most common clinical indication for EUS in NSCLC is for mediastinal staging. Prior to the advent of EUS-FNA, patients underwent various procedures including bronchoscopy with transbronchial FNA, computed tomography (CT), positron emission tomography (PET), video-assisted thoracoscopy, and mediastinoscopy to accurately assess metastatic disease. EUS-FNA (as opposed to EUS alone) [18,26–28] provides accurate evaluation of the posterior mediastinum, and is complementary to those imaging modalities that assess the anterior mediastinum.

The performance characteristics of EUS-FNA have been reported in numerous studies [28–44] and a metaanalysis of over 1200 patients [25]. EUS-FNA for mediastinal staging or in suspected mediastinal malignancy has a pooled sensitivity of 83%, specificity of 97%, negative predictive value (NPV) of 78%, and positive predictive value (PPV) of 98%. EUS-FNA is more sensitive (88% vs 57%) and specific (91% vs 82%) than CT for the detection of suspected malignant lymph nodes [26,27], and detects malignant lymphadenopathy in 35% of patients without lymphadenopathy on prior CT. This reduces the number of patients requiring mediastinoscopy and exploratory thoracotomy [25,33,35,39,43] (Fig. 130.5).

Figure 130.5 Lung cancer. Endoscopic ultrasonography fine-needle aspiration of a lung mass.

Fluorodeoxyglucose (FDG)-PET and CT are comparably sensitive for the detection of enlarged mediastinal lymph nodes; however, in cases where lymph nodes are not enlarged on CT, FDG-PET had a higher sensitivity than both CT and EUS-FNA (82% vs 58%). Due to the false-positive rate of benign granulomatous disease on PET, a positive PET should be confirmed with tissue diagnosis. When available, EUS-FNA is a more accurate and a preferential alternative to "blind" transbronchial FNA [26,28,35,42,45].

EUS-FNA can assess intrapulmonary lesions in the posterior mediastinum. The accuracy of EUS-FNA for such lesions is 97%–100% [9,46], and EUS is more accurate than CT or PET for assessing T4 disease (sensitivity 88%, specificity 98%, PPV 70%, NPV 99%) [47]. CT and PET have limited sensitivity and specificity for T4 detection [48,49].

Impact on clinical care

The clinical impact of EUS-FNA in patients with NSCLC is its ability to accurately stage the posterior mediastinum, subsequently reducing the number of invasive mediastinoscopies and thoracotomies. EUS-FNA prevents further invasive staging procedures in up to 70% of cases [34,38,40,41,50,51]. The role for EUS-FNA in the clinical algorithm for NSCLC patients continues to evolve, but the general consensus is that it should be utilized early in the clinical staging algorithm since EUS, by itself, can demonstrate T4 disease or mediastinal metastases in 25% of patients regardless of CT findings [39,43]. PET, if available, should be performed before EUS-FNA.

The future staging of NSCLC is likely to involve endobronchial ultrasound (EBUS). Compared with CT and PET, EBUS with transbronchial needle aspiration has high sensitivity and specificity for mediastinal and hilar lymph node evaluation [52,53]. The challenge will be for bronchoscopists and endoscopists to integrate the two techniques efficiently and to work together to achieve complete mediastinal staging.

EUS-FNA plays a vital role in the management of patients with suspected NSCLC. Given that it is minimally invasive,

safe, more accurate than CT and transbronchial FNA, and more specific than FDG-PET, EUS-FNA impacts patient care by reducing the number of invasive diagnostic surgical procedures. Indications for EUS-FNA in the diagnosis and staging of NSCLC include the evaluation of mediastinal lymphadenopathy, identification of adjacent intrapulmonary lesions and diagnosis of T4 disease, and mediastinal FNA of PET-positive lesions, and should be the first invasive procedure performed after CT [54]. Since EUS cannot visualize the anterior mediastinal structures, it is most appropriate to proceed to mediastinoscopy if EUS-FNA is nondiagnostic. Mediastinoscopy provides evaluation of the upper and lower paratracheal regions (stations 2 and 4). In a comparison of EUS-FNA and mediastinoscopy, both procedures demonstrated a comparable accuracy rate of 90%, and the combination detected significantly more patients with lymph node metastases than either technique alone [40]. The role of EBUS remains to be clearly defined.

Esophageal cancer

It is estimated that in 2007, esophageal cancer will affect over 14 000 individuals in the USA and result in over 13 000 deaths [55]. The 5-year survival from esophageal cancer is 10%–15% [56]. Most patients present with locally advanced disease because there are few early signs or symptoms of the tumor, because the esophagus lacks a serosa, and because there is early spread to the lymph nodes since the esophageal submucosa has a rich plexus of lymphatic vessels.

The International Union Against Cancer and American Joint Committee on Cancer updated the guidelines in 2002 for staging esophageal cancer. The involvement of cervical or celiac axis lymph nodes is classified as metastatic disease (M1a or M1b, depending on tumor location). Tumor of the lower esophagus with celiac axis lymph node spread is classified as M1a disease, as is tumor of the upper esophagus with cervical lymph node involvement. Tumor of the mid esophagus with either cervical or celiac axis lymph node spread is classified as M1b disease, and also stage IV disease [57]. In general, early lesions (T1N0, T2N0) undergo surgery while presence of any lymph nodes usually mandates neoadjuvant therapy. Esophageal cancer patients who undergo EUS are associated with improved survival, probably due to accurate staging resulting in the use of stage-appropriate adjuvant therapy and surgical resection. [58].

Early esophageal cancer

Early esophageal cancer (carcinoma-in-situ or T1N0Mx) is most commonly detected in patients undergoing aggressive surveillance. When such lesions are identified, high-frequency EUS demonstrates excellent cross-sectional gastrointestinal tract images to assess T and N stage. In patients with Barrett esophagus with high-grade dysplasia or intramucosal carcinoma, EUS is more sensitive than CT for T and N stage. It can detect previously unknown submucosal involvement (sensitivity 100%, specificity 94%) and lymph node metastasis (sensitivity 100%, specificity 81%), and the absence of these findings on EUS is reassuring to physicians that the patient has early esophageal cancer or carcinoma-in-situ (NPV 100%) [59,60]. EUS confirmation of early malignant lesions allows nonoperative curative endoscopic ablative techniques, if desired.

Advanced esophageal cancer

Patients with locally advanced esophageal cancer should initially be evaluated with CT (and FDG-PET if available), searching for distant metastatic disease. The accuracy of CT ranges from 63% to 90% [61,62], and is not as sensitive in detecting small hepatic lesions or peritoneal metastases compared with diagnostic laparoscopy [63,64]. EUS can complement CT by improving the accuracy for M-staging by detecting cervical or celiac axis lymphadenopathy (M1a or M1b disease) [61], adrenal gland or liver metastases, or malignant ascites. FDG-PET is superior for detecting distant metastasis and nonregional lymph nodes compared with CT plus EUS (82% vs 64%), and may up-stage patients to stage IV [65–67] (Fig. 130.6).

The locoregional staging of a malignant esophageal stricture is a common indication for EUS. The overall accuracy of EUS for T-stage is 80%–85%, with highest accuracy in staging T3 and T4 disease [61,68–76]. EUS is superior to CT and PET for assessing T-stage of esophageal malignancy, and should be the test of choice.

Although less than 5% of T1m tumors are associated with nodal spread, 60% of T2 and more than 80% of T3/T4 lesions are associated with malignant nodal spread [77–79]. The presence and number of malignant lymph nodes portends poor prognosis and often shifts the treatment paradigm toward neoadjuvant therapy [80,81]. CT and FDG-PET are inferior to EUS in the evaluation of locoregional lymphadenopathy. The accuracy of CT is estimated to be only 51%–70% [61,82], and FDG-PET lacks the spatial resolution to differentiate local nodal involvement from adjacent primary tumor, and reactive inflammatory lymph nodes can lead to false-positive results. The sensitivity of PET for detection of N1 disease is approximately 33% [65].

EUS assessment of lymphadenopathy is accomplished by visualization and FNA. The accuracy of EUS in classifying a lymph node as malignant based on appearance is approximately 80% [17,18,57] and the overall sensitivity of EUS in detecting malignant lymphadenopathy ranges from 50% to 75% with an accuracy of 65% [17,18,61,75]. The accuracy is increased to 85%–93% with the addition of FNA [31,83]. It should be noted that when performing FNA of a lymph node, a false-positive sample can be obtained if the needle tract penetrates through primary tumor, thus contaminating the FNA sample. Hence, FNA of peritumoral lymph nodes may not be always feasible. Common hiding places for metastases from esophageal malignancies are the region above the aortic

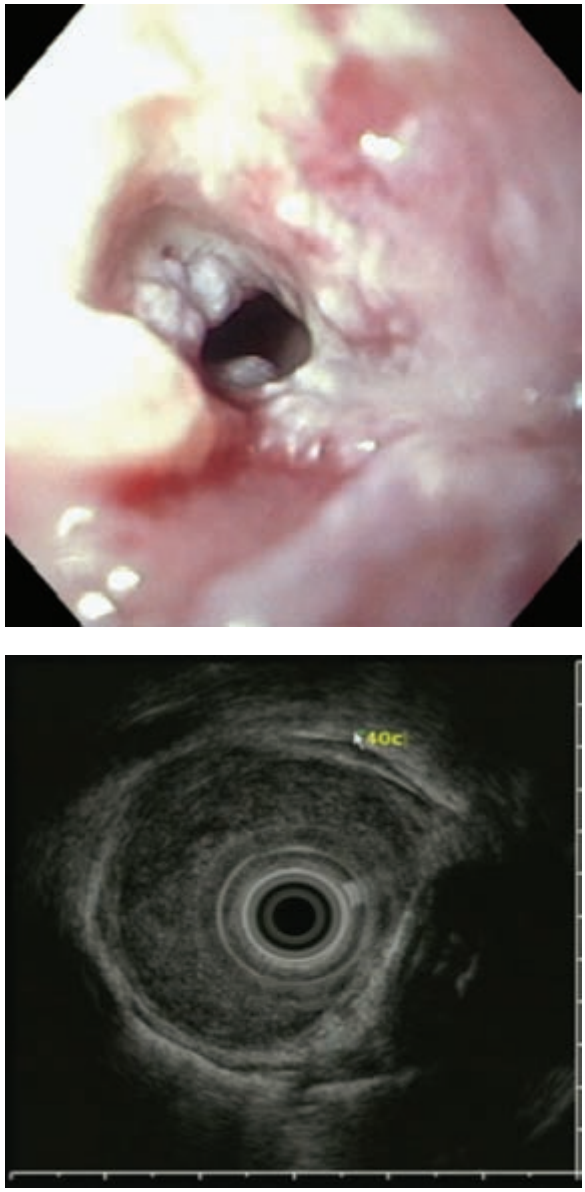


Figure 130.6 Esophageal cancer. **(a)** Carcinoma of the esophagus causing a malignant stricture. **(b)** Endoscopic ultrasonography image of esophageal carcinoma (T4) with pleural invasion.

arch (outside the cervical esophagus), the gastrohepatic ligament, and the celiac axis region.

A common clinical scenario is the nontraversable malignant esophageal stricture, where the standard echoendoscope is unable to pass beyond the stricture without prior dilatation to 14–16 mm. Although perforation is a risk, dilation can be performed safely, and is indicated. Wallace and colleagues reported that 32% of their 132 patients required dilation for adequate staging, and only one patient suffered a perforation. In addition, 19% of patients would have been under-staged if dilation had not been performed [84]. In cases when the stricture is so tight that dilation and passage of the standard echoendoscope is not feasible, a catheter ultrasound probe or an over-the-wire esophagoprobe (Olympus MH-908, Tokyo, Japan) can be used [57]. Unfortunately, if celiac lymphadenopathy is detected, FNA cannot be performed.

Submucosal lesions

A submucosal lesion is an endoscopically visible, often incidental finding (i.e., a "bump") with normal overlying mucosa. The estimated prevalence of gastrointestinal submucosal findings is less than 1%, and is most commonly found in the stomach [85,86]. EUS is well suited for evaluating submucosal lesions because of its ability to visualize gut wall layers and abdominal structures and to safely perform FNA. Table 130.2 lists the common etiologies of submucosal gastrointestinal lesions and their descriptive EUS features.

Causes of extrinsic compression include adjacent organs, bowel, blood vessels, retroperitoneum, omentum, and lymph nodes [85,87–95]. The frequency is approximately 30%. The accuracy of CT and transabdominal ultrasonography for differentiating between extrinsic compression and a true submucosal lesion is 22% and 28% respectively compared with an EUS accuracy of 100% [93].

After excluding extrinsic compression, the approximate frequency of gastrointestinal submucosal lesions is as follows: smooth muscle neoplasm (53%), aberrant pancreas (8%), carcinoid (6%), cystic lesion (7%), granular cell tumor (4%), lymphangioma or hemangioma (4%), Brunner gland hyperplasia (1%), malignant lymphoma (1%), and other (8%) [85]. Most smooth muscle neoplasms are gastrointestinal stromal cell tumors (GISTs), but older studies do not differentiate GISTs from other mesenchymal tumors.

When evaluating a submucosal lesion by EUS, a variety of clinical issues need to be addressed. In many cases, the endosonographic appearance and characterization of which gut layer(s) the lesion originates from will narrow the differential. However, issues regarding differentiation between benign and malignant lesions, role of FNA and surveillance of lesions need further clarification. Several of the submucosal lesions are discussed in further detail.

Gastrointestinal stromal cell tumor

Gastrointestinal stromal tumors, the most common mesenchymal tumors, arise from the interstitial cells of Cajal and often express a protooncogene called c-*kit*, which confers a gain-of-function mutation to such lesions. Immunohistochemical staining can differentiate GISTs from other related lesions [96]. Approximately 80% of gastrointestinal mesenchymal tumors are GISTs, and 10%–30% of GISTs are malignant [96,97]. All GISTs are considered to be potentially cancerous, and are stratified into low-, medium-, or high-risk category depending on tumor size and mitotic count. However, GISTs can be unpredictable, and low-risk lesions have been reported to metastasize [85,98].

Table 130.2 Etiology of submucosal lesions

Etiology	EUS layer	EUS appearance
Extrinsic compression	None	Normal gastrointestinal tract
Stromal cell tumor	Third/fourth	Hypoechoic, well circumscribed
Lipoma	Third	Hyperechoic
Carcinoid	Second/third	Homogeneous, hypoechoic
Granular cell tumor	Second/third	Homogeneous
Varices	Third	Anechoic, tubular
Cysts	Third	Anechoic, duplication of wall layers
Inflammatory polyp	Second/third	Hypoechoic, indistinct margin
Glomus tumor	Third/fourth	Hypoechoic, smooth margin
Metastatic deposits	Second/third/fourth	Hypoechoic
Lymphoma	Second/third/fourth	Distortion of wall layers
Heterotopic pancreas	Second/third/fourth	Hypoechoic, anechoic ductal structure

EUS, endoscopic ultrasonography.

Most GISTs are located in the stomach or duodenum [99] and under EUS appear as a homogeneous, hypoechoic, well-circumscribed lesion that originates from either the muscularis mucosae or muscularis propria (Fig. 130.7). EUS features suggestive of malignancy include tumor size more than 4 cm, an irregular extralumenal border, echogenic foci, and cystic anechoic spaces (suggestive of necrosis). If two of the four features are present, the sensitivity ranges from 80% to 100% for detecting a malignant GIST [100]. Additional notable features suggestive of malignancy include malignant-appearing lymph nodes, ulcerated mucosa, and nonoval shape [87,100–103]. The lack of these features does not rule out malignancy. FNA of suspected GISTs should be performed, when possible, and immunohistochemical staining is the only reliable method for diagnosing GIST. Approximately 90% of GISTs express CD117 (c-*kit*) and its presence confirms the diagnosis but does not assess malignant potential [104].

The question remains of how to manage an asymptomatic mesenchymal lesion, particularly if it is less than 1 cm in size, where the risk of malignancy is believed to be low. It may be useful to perform FNA on a small suspected GIST for immunohistochemical confirmation. Medications that target CD117-positive GISTs are available. There is no proven EUS-based strategy for optimal surveillance of suspected GISTs; however, a reasonable strategy may be annual EUS surveillance for small asymptomatic lesions without any features of malignant transformation.

Lipoma

Lipoma, a benign tumor composed of mature lipocytes, is most often asymptomatic. It appears as a well-circumscribed, homogeneous, hyperechoic lesion arising from the submucosa (third layer). FNA is not required to confirm the diagnosis as its appearance is pathognomonic [105,106].

Carcinoid

Carcinoids are slow-growing, premalignant, neuroendocrine tumors primarily found in the lung and gastrointestinal tract. Approximately 17% of carcinoids are found in the rectum and 3% are located in the stomach, both sites amenable to EUS evaluation. Sporadic gastric carcinoids tend to behave more aggressively than the multifocal gastric carcinoids seen in hypergastrinemic states.

The EUS appearance is a well-circumscribed, mildly hypoechoic or isoechoic mass arising from either the mucosa (second layer) or submucosa (third layer). EUS identifies carcinoid lesions amenable to endoscopic versus surgical resection [107–109].

Granular cell tumor

Granular cell tumors are uncommon lesions of neural derivation that appear endoscopically as yellowish mucosal or submucosal nodules. The majority of these lesions are less than 2 cm in size [110] and typically considered to be benign, although approximately 2%–3% of lesions are malignant [111]. Under EUS, these tumors appear as homogeneous, hypoechoic, well-circumscribed lesions originating from the mucosa (second layer) or submucosa (third layer) [112,113].

Stomach

Gastric cancer

Worldwide, gastric cancer accounts for approximately 10% of all malignancies, and in 2006 over 21 000 patients were diagnosed with gastric cancer in the USA [55]. Despite a reduction in mortality during the 1980s and 1990s, gastric cancer remains the second leading cause of cancer-related mortality worldwide [114]. A major clinical challenge is to diagnose gastric cancer in its early curable stages, a feat unfortunately rarely accomplished in the absence of a screening program. The Japanese have the most experience with early gastric cancer.

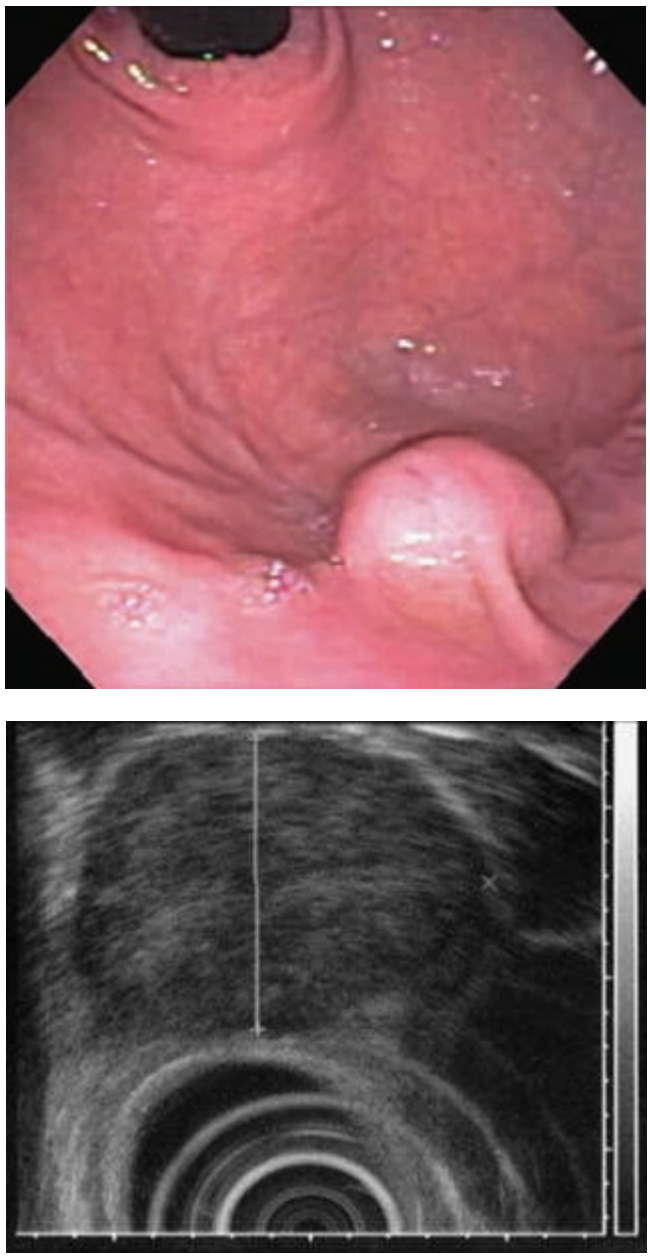


Figure 130.7 Gastrointestinal stromal cell tumor (GIST). **(a)** A submucosal lesion visualized on upper endoscopy. **(b)** Typical endoscopic ultrasonography appearance of a GIST.

They use the Japanese classification for management while worldwide the TNM system is most commonly used.

Because gastric cancer confined to the mucosa and submucosa can be treated with surgical resection, endoscopic mucosal resection (EMR), or endoscopic submucosal dissection (ESD), EUS plays a role in local staging. Cancers with involvement of only the mucosa (m) and submucosal invasion less than 500 μm (sm1) have negligible risk of metastasis and are ideal for EMR. Some conservative authorities advocate EMR and ESD only for lesions with mucosal involvement and less than 2 cm in diameter [115]. Stage T2 or T3 tumor is treated with surgical resection and T4 lesions are managed with neoadjuvant chemotherapy and radiation.

EUS is an accurate method for local staging of gastric cancer: it can visualize infiltrating scirrhous malignancies, which appear as diffuse thickening of the normal layers of the gastric wall, or assess tumor invasion based on disruption of wall layers [116–126] (Fig. 130.8). The accuracy of dynamic CT and magnetic resonance imaging (MRI) for T-staging is 50%–70% [127–131], whereas the overall accuracy of EUS for T-stage ranges from 70% to 93% [61,117,125,132–138]. In particular, the accuracy for detecting submucosal invasion has been reported to be as high as 90% with a highfrequency catheter probe [133,139]. EUS T-staging is lowest for T2 lesions (accuracy 60%–70%), and the difficulty is in differentiating between subserosal (T2) from serosal (T3) involvement, particularly since the entire stomach is not covered by a serosa.

EUS has long been the test of choice for assessing T-stage in gastric cancer; however, with the advent of multidetector CT and the advanced imaging techniques of MRI, the accuracy of T-staging among the different modalities has been reevaluated. Table 130.3 shows the performance characteristics of EUS, multidetector CT, and MRI in assessing tumor stage in gastric cancer. These imaging modalities appear to be equivalent, although the greatest expertise remains with EUS and further experience needs to be gained with multidetector CT and MRI [73,125,126,132,135,137,138,140–54]. A limitation of EUS is that depth of invasion may be overestimated if there is an ulcer scar or inflammatory reaction below the cancer (peritumorous inflammation) or a protruding lesion, and underestimated if there is microinvasion [123].

The overall accuracy of EUS nodal staging in gastric cancer ranges from 50% to 87% [73,124,125,132,136,137,140–143,145,151,155,156], and is highest for T3 and T4 lesions, for lymph nodes located within 3 cm of the tumor, and along the lesser curvature. Only 15% of T1 lesions are associated with malignant lymph nodes, but detection of these lymph nodes is critical, particularly if EMR or ESD is contemplated. The overall accuracy of EUS for detection of lymph nodes is approximately 69%, and FNA should be employed, if needed. Nodal staging with CT and MRI is not clearly superior to EUS [131,138,157,158].

Gastric wall layer abnormalities

EUS is indicated in the presence of thickened gastric folds. By definition, the gastric folds are considered thickened if they fail to flatten with endoscopic insufflation or if an upper gastrointestinal series or CT suggests a thickness greater than 1.5 cm [159]. The normal thickness of the gastric wall ranges from 0.8 to 3.6 mm [12] and is considered to be thickened when the diameter of the five-layer EUS image of the gastric wall is greater than 4 mm [160,161]. The differential diagnosis (Table 130.4) includes benign and malignant conditions. A clear diagnosis may not always be possible; however, based on the EUS origin of the lesion, the differential can be narrowed.

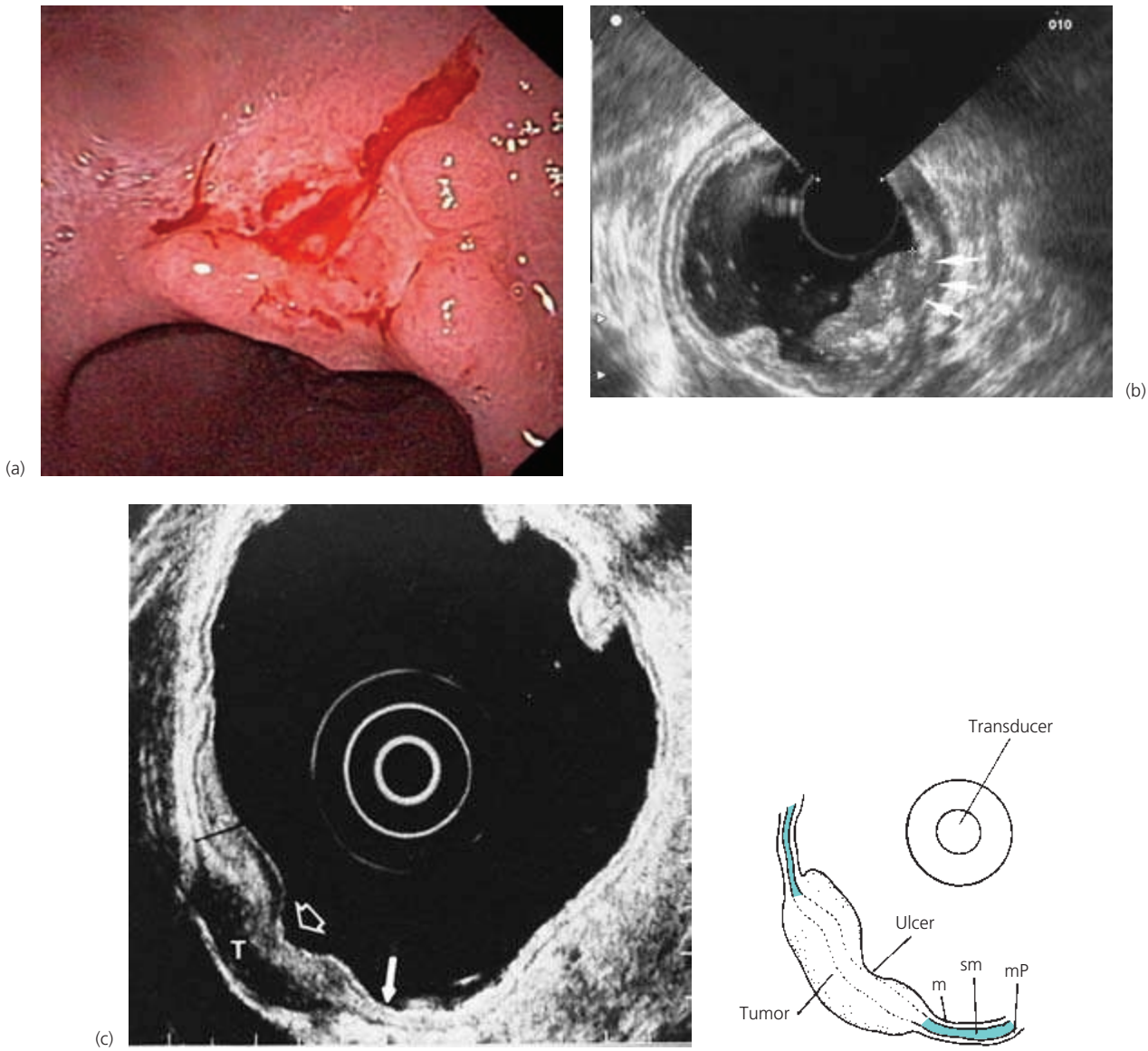


Figure 130.8 Cancer of the stomach. **(a)** Shallow gastric ulcer on upper endoscopy. **(b)** Endoscopic ultrasonography (EUS) of early gastric cancer. **(c)** EUS of advanced gastric cancer, showing raised borders with central ulceration (open arrow). The tumor mass (T) arises from the normal wall (arrow), where layers corresponding to the mucosa (m), submucosa (sm), and muscularis propria (mP) can be seen. The cancer invades the fat and other tissue around the gastric wall.

Table 130.3 Performance characteristics of various imaging modalities to assess T-stage in gastric cancer

Imaging test	Sensitivity (%)	Specificity (%)	Accuracy (%)
EUS	77.8–100	67.9–100	65–92.1
MDCT	82.8–100	80–96.8	77.1–88.9
MRI	89.5–93.1	94.1–100	71.4–82.6

EUS, endoscopic ultrasound; MDCT, multidetector computed tomography; MRI, magnetic resonance imaging.
Adapted from Kwee & Kwee [138].

Multiple studies have reported EUS findings in patients with large gastric folds [162–165]. The presence of gastric varices is diagnostic and biopsy specimens can be avoided. If EUS demonstrates abnormalities in layers 3 or 4, malignancy needs to be strongly considered, even if biopsies are negative [162,163]. Most cases with a scirrhous carcinoma have a thickened third and fourth layer [163], and the second and third layers can be thickened in benign or malignant conditions [163]. Endoscopic biopsy of an abnormality in layer 2 should be diagnostic and if biopsies are negative for cancer, follow-up studies report that malignancy will often not develop [162] (Fig. 130.9).

Table 130.4 Differential diagnosis of thickened gastric folds

Benign
Gastritis
Ménétrier disease
Zolliger–Ellison syndrome
Gastritis cystica profunda
Hyperrugosity
Vascular conditions
Gastric varices
Portal hypertensive gastropathy
Infections
Secondary syphilis
Tuberculosis
Cytomegalovirus
Helicobacter pylori
Herpes simplex virus
Histoplasmosis
Aspergillosis
Anisakiasis
Infiltrative conditions
Sarcoidosis
Amyloidosis
Crohn's disease
Malignant conditions
Adenocarcinoma
Linitis plastica
Lymphoma
Metastatic disease

EUS can diagnose early infiltrative gastric lymphoma, which is typically a non-Hodgkin lymphoma. An early-stage lymphoma can be subtle in its endoscopic and endosonographic appearance, revealing only a thickened second layer with preservation of the gastric layers and endoscopically normal-appearing mucosa [166,167]. More advanced stages show a diffuse hypoechoic thickening with distortion of the gastric layers. The accuracy of EUS for staging gastric lymphoma is approximately 95% [141,168], and EUS can also be used to evaluate tumor response to chemoradiation [169–172].

Ampullary and pancreatic neoplasms

The role of EUS in the management of suspected ampullary or pancreatic neoplasms is to identify the lesion, assess for surgical resectability, and acquire cytological confirmation if needed.

Ampullary neoplasm

Ampullary adenomas, the most common tumors of the ampulla of Vater, can be sporadic or part of a genetic polyposis syndrome. They follow the adenoma–carcinoma sequence for neoplastic progression, [173] and identification of an asymptomatic precancerous lesion mandates further endoscopic intervention. The two major roles of EUS in this clinical scenario are to confirm the diagnosis of an ampullary tumor and to stage the adenocarcinoma.

Endoscopically, ampullary neoplasms appear polypoid or ulcerated, and can be associated with choledocholithiasis and marked inflammation [174–176]. The latter may result in false-negative biopsies or, in cases where the tumor is intramural, the inability to accurately diagnose the malignancy [175,176]. In as many as 19%–30% of cases, biopsies may not reveal the presence of adenocarcinoma [175–178] (Fig. 130.10).

EUS is more sensitive than CT or transabdominal ultrasonography for diagnosing ampullary neoplasm and more accurate than CT and MRI for staging early ampullary lesions [179–183]. Endosonographic features specific for malignancy include presence of tumor invasion (at least infiltration of the duodenal muscularis propria) or growth into the biliary or pancreatic ductal system. In the absence of clear invasive features, EUS cannot differentiate between malignant and nonmalignant conditions (e.g., odditis, inflammation, normal ampulla) [184]. EUS sensitivity is higher in symptomatic patients and patients at risk for adenomas.

Accurate staging is critical in the management of ampullary neoplasms, since endoscopic options are feasible in selected cases. According to the TNM classification used to stage ampullary neoplasms, T1 corresponds to tumors not extending beyond the sphincter of Oddi, T2 to tumors invading the muscularis propria of the duodenal wall, T3 to tumors with less than 2 cm of invasion into the adjacent pancreas, and T4 tumors deeply invade the pancreas or adjacent vasculature [185].

EUS is the most reliable modality for local preoperative staging of ampullary neoplasms, including assessing for portal venous involvement. It is superior to MRI, CT, transabdominal ultrasound, and angiography, regardless of whether the radial or linear echoendoscope is used [182,184,186–188]. Peritumoral pancreatitis due to the desmoplastic reaction of malignant cells can lead to under-staging of true T3 lesions or over-staging of true T2 lesions, but typically the surgical treatment for both lesions is identical [181]. The accuracy of EUS in determining whether endoscopic resection for a T1 lesion can be performed with curative intent ranges between 87% and 94% [180,181,186,189,190]. The Japanese staging system subdivides T1 lesions into do (tumors limited to the sphincter of Oddi) and d1 lesions (tumors extending into the submucosa). The prevalence of malignant lymph nodes varies from 0 to 30% in T1 tumors, with the highest risk associated with tumors that invade the submucosa [180,181,187,191].

Intraductal ultrasonography (IDUS) complements radial or linear EUS. Imaging at 20 MHz demonstrates the muscle

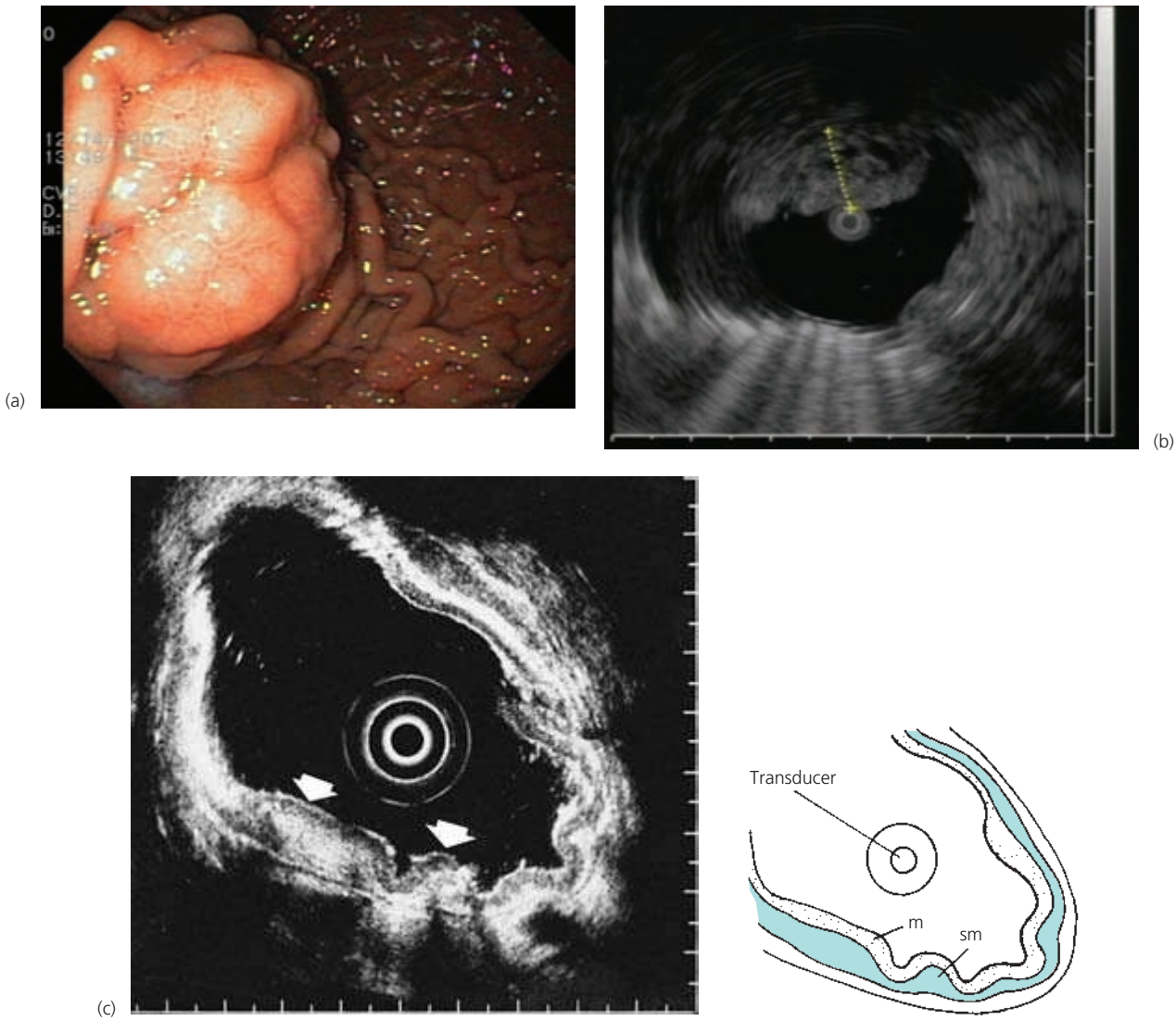


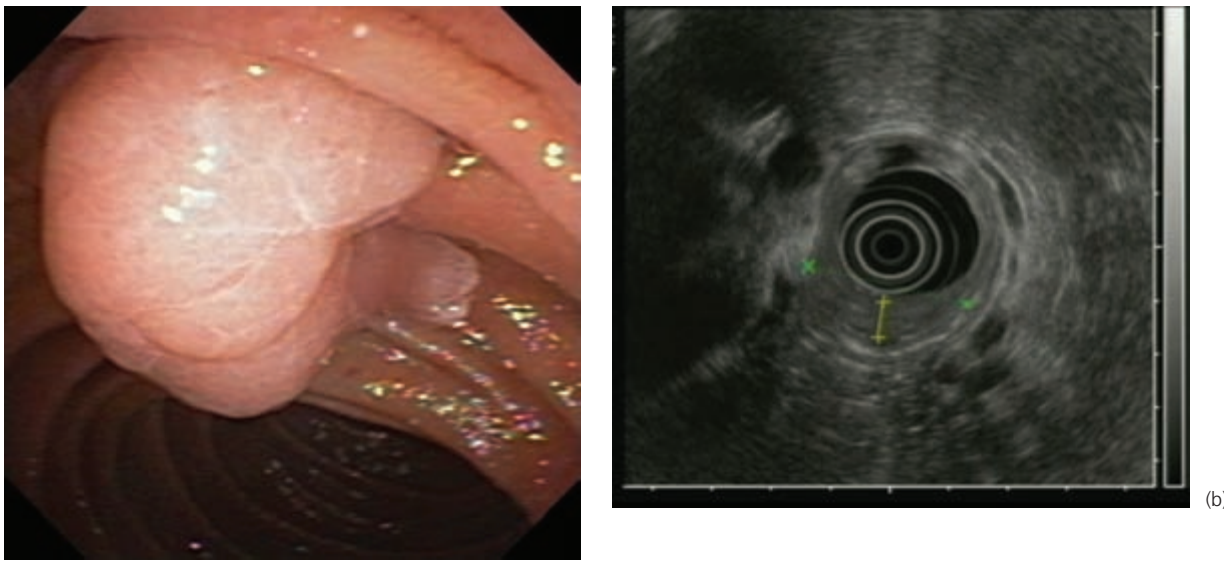
Figure 130.9 Gastric lymphoma. **(a)** A "mass-like" thickening of gastric folds is noted on upper endoscopy. **(b)** Catheter-based endoscopic ultrasonography demonstrates heterogeneous infiltrative lesion of the gastric mucosa and submucosa. Pathology confirms lymphoma. **(c)** EUS image showing thickening of ultrasound layers corresponding to mucosa (m) and submucosa (sm) in the region under arrows, caused by gastric lymphoma.

layer of the sphincter of Oddi as a distinct hypoechoic layer, which allows greater tissue layer resolution and the ability to discern d0 lesions from d1 lesions with an approximate accuracy of 89% [190]. IDUS is nearly 100% accurate in visualizing tumor invasion into the pancreaticobiliary ductal system [190]. The combination of EUS and IDUS is believed to provide the most accurate staging information, and reliably select candidates eligible for curative endoscopic ampullectomy versus surgical treatment.

Pancreatic neoplasms

One of the first targets for EUS imaging was pancreatic cancer. The early detection of this usually lethal neoplasm has eluded other diagnostic abdominal modalities such as transabdominal ultrasound. The increased spatial resolution of EUS allows detection of smaller resectable neoplasms in patients with vague symptoms [192] and is the most sensitive imaging modality for the detection of a pancreatic mass. Numerous studies have documented the high sensitivity (98%–100%) of EUS for detection of pancreatic tumors [193–204]. EUS may play a role in the screening of asymptomatic high-risk populations for early cancer detection [205–208].

As an imaging modality, EUS is not as widely available as transabdominal ultrasound, CT, and PET. Transabdominal ultrasound is inferior to EUS for detection of pancreatic masses, particularly those located in the pancreatic head or uncinate [184]. Despite the advent of multidetector CT [209,210], studies suggest that EUS remains a superior imaging modality, particularly for small lesions. Compared with multidetector CT, EUS is more sensitive (98% vs 86%) [201] and more



(a) (b)

Figure 130.10 Ampullary mass. **(a)** Endoscopic appearance of ampullary mass. **(b)** Endoscopic ultrasonography of ampullary mass limited to mucosa. Lesion treated with endoscopic ampullectomy.

accurate (94% vs 74%) [203]. In patients with indeterminate findings on CT, EUS has an accuracy of 92% for detection of a pancreatic tumor and an NPV of nearly 100% for excluding a focal pancreatic mass [211,212]. A normal EUS of the pancreas in the setting of subtle radiological findings, nonspecific symptoms, or laboratory values effectively rules out a pancreatic neoplasm.

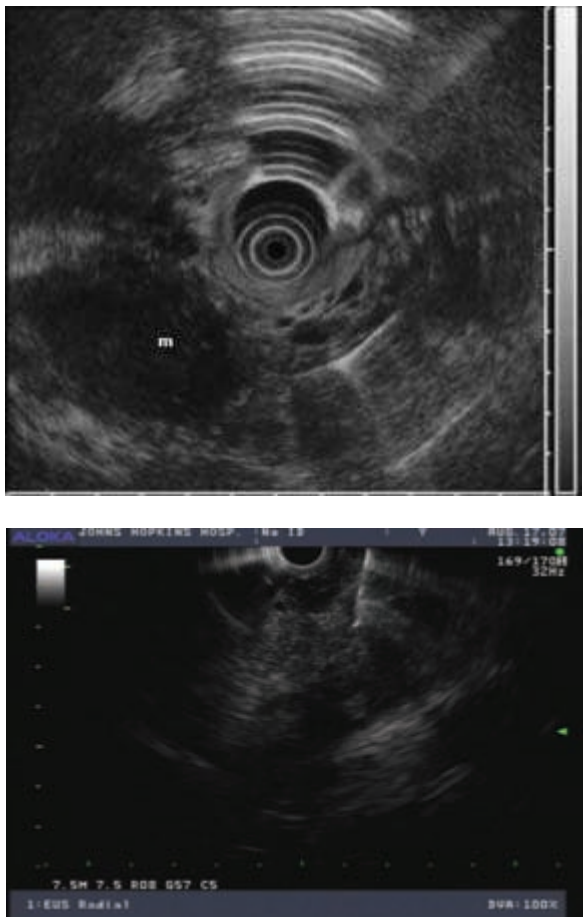
MRI and PET may detect small pancreatic tumors; however, inflammatory conditions such as chronic pancreatitis can result in glucose uptake and false-positive PET [213,214]. The anatomical resolution from PET is inferior to CT and MRI for determining locoregional tumor spread; however, PET can identify distant metastases that can alter clinical treatment in up to 40% of patients with pancreatic cancer [215–217].

For locoregional staging of pancreatic cancer, multidetector CT and MRI provide excellent assessment of tumor staging, vascular involvement, and nodal staging [188,218,219]. Both modalities are superior to EUS for detection of metastatic disease, but EUS can provide information suggesting metastatic disease by detecting previously unknown hepatic metastases [220–224], small pockets of ascites [225], and malignant mediastinal lymphadenopathy [226]. These findings preclude surgical resection.

Vascular invasion generally precludes surgical intervention, with the possible exception of portal vein and superior mesenteric vein involvement [218]. The accuracy of EUS in assessing for vascular invasion ranges from 62% to 100% [182,202,218,219,227,228]. EUS is most accurate (> 80%) when assessing for invasion of the portal vein or portal vein/superior mesenteric vein confluence [188,192,229–231], and least accurate when assessing invasion of the superior mesenteric artery or vein and celiac axis [184,187,227]. On a practical level, optimal assessment of tumor invasion is accomplished with multidetector CT or MRI, and EUS if needed for clarification of the portal vein or superior mesenteric vein [232].

A common indication for endosonography is to obtain tissue diagnosis of an unresectable pancreatic mass (Fig. 130.11). If available, EUS-guided FNA of a pancreatic mass should be the preferred method for tissue acquisition. It has an approximate sensitivity of 85%, specificity of 98%, and accuracy of 88%. However, the NPV of EUS-guided FNA is 55%–80% [30,31,83,203,233–247]. Hence, a negative or nondiagnostic aspirate does not rule out the possibility of malignancy. This is of particular concern in patients who have underlying chronic pancreatitis, where the sensitivity of EUS for diagnosis of malignancy drops to approximately 55% [239]. In comparison with CT- or transabdominal ultrasound-guided FNA, EUS-FNA is equivalent in sensitivity but perceived advantages of the EUS approach include reduced risk of needle-track seeding [248–251] and improved patient comfort.

An on-site cytopathologist or technician should be present for FNA, which can improve diagnostic accuracy and reduce the number of unsatisfactory samples and repeat procedures [252]. If a cytopathologist is unavailable, the EUS physician should obtain five to seven specimens for cytological interpretation to maximize diagnostic yield [253,254]. The overall risk of complications from an EUS-FNA of a pancreatic mass is 0.5%–2% [30,83,245,255–257], with a higher complication rate for cystic lesions (14%) compared with solid tumors (0.5%) [83]. Complications of EUS-FNA include pancreatitis, perforation, bleeding, and infection.



(a)

(b)

Figure 130.11 Pancreatic neoplasm. **(a)** Endoscopic ultrasonography (EUS) showing pancreatic mass (m). **(b)** EUS fine-needle aspiration of the pancreatic mass seen in (a).

Islet cell neoplasm

The anatomical localization of hormone-producing pancreatic tumors is important for guiding their surgical resection. Most of these neoplasms arise from islet cells within the pancreas, and conventional transcorporeal imaging with ultrasound and CT detects less than one-third of tumors. In particular, insulinomas are less likely to be detected by CT. MRI and selective arteriography may also fail to localize the neoplasm.

Careful examination of the pancreas with EUS detects about 77%–94% of islet cell neoplasms within the pancreas not found on CT or MRI [258–268]. Islet cell neoplasms are usually round, well-circumscribed, homogeneous, and hypoechoic compared with the surrounding parenchyma, and lesions as small as 5 mm in diameter have been imaged by EUS [258,268,269]. All patients diagnosed with a resectable pancreatic neuroendocrine tumor should undergo preoperative EUS and octreotide scanning to localize the neoplasm and facilitate surgical planning [270,271]. EUS-FNA is not required in the setting of a well-defined clinical syndrome and documented excessive hormone secretion.

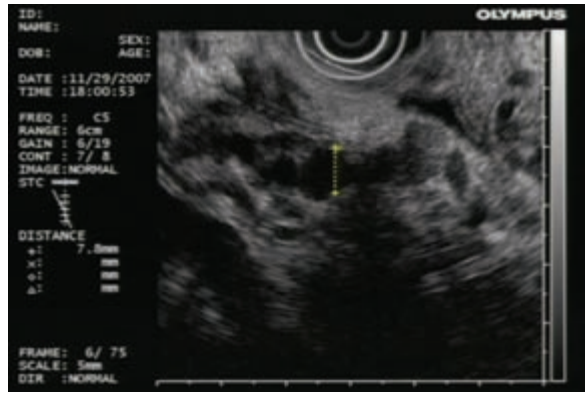


Figure 130.12 Chronic pancreatitis. Endoscopic ultrasonography image showing features of chronic pancreatitis and dilated main pancreatic duct.

Chronic pancreatitis

Chronic pancreatitis is an ongoing inflammatory process that results in irreversible destruction of pancreatic tissue leading to exocrine and endocrine insufficiency. It can be a diagnostic challenge, particularly in nonsevere cases when radiological imaging tests and functional diagnostic tests are equivocal or negative. There is no perfect test (imaging or functional) that accurately diagnoses early chronic pancreatitis, but EUS offers several advantages because of its minimal risk of causing pancreatitis and its ability to characterize both pancreatic parenchymal and ductal abnormalities (Fig. 130.12).

The endosonographic criteria and the histological correlates for chronic pancreatitis are listed in Table 130.5 [272–274]. Additional proposed criteria include gland contour (lobular vs smooth), echogenic foci with and without shadowing, and accentuation of the gland's lobular pattern [273,275]. Despite the specific terminology of these EUS

Table 130.5 Histological correlates for endoscopic ultrasonography (EUS) findings as related to chronic pancreatitis

EUS finding	Histological correlate
Parenchymal features	
Hyperechoic foci	Focal areas of fibrosis
Hyperechoic strands	Linear areas of fibrosis
Pseudolobule	Linear fibrosis containing focal edema
Cysts	Cysts or cyst side branches
Ductal features	
Main pancreatic duct dilation	Dilated duct
Side-branch dilation	Dilated side branches
Irregular duct contour	Periductal fibrosis
Hyperechoic duct wall	Periductal fibrosis
Stones	Calcified stones

features, the interobserver agreement for these features and the collective assessment of an EUS examination is only moderate [274] and clinical bias exists in the interpretation of the examination as it applies to each patient [276].

When evaluating the performance characteristics of EUS for the diagnosis of chronic pancreatitis, the best reference standard is pathological confirmation of the presence of inflammation, atrophy, or fibrosis, but only a few studies have tried to correlate EUS features of chronic pancreatitis with microscopic findings. Most studies have compared EUS with morphological tests such as CT, MRI/magnetic resonance cholangiopancreatography (MRCP) and endoscopic retrograde cholangiopancreatography (ERCP) and with the functional secretin test [276–280].

EUS has an approximate sensitivity and specificity of 85% and 75% respectively when ERCP is used as the gold standard to diagnose chronic pancreatitis [272,273,275,276,281]. Wiersema and colleagues enrolled 69 patients with suspected pancreatic pain and noted that the sensitivity and specificity of EUS was 80% and 86% respectively when three or more criteria were used [275]. Sahai and colleagues [272] performed EUS in a prospective blinded study of patients with unexplained pain who were referred for ERCP. They noted that patients with five or more EUS features correlated with at least moderate chronic pancreatitis on ERCP. Fewer than three features essentially ruled out moderate or severe chronic pancreatitis (NPV > 85%), and three to four EUS features was equivocal. However, three to four EUS features in patients with history of alcohol intake or pancreatic pain symptoms may be significant [272,282]. When compared with secretin testing, the overall sensitivity and specificity of EUS is 80% and 70% respectively [273,275,276]. Catalano and colleagues [273] compared EUS with ERCP and secretin testing in patients with recurrent pancreatitis. There was 100% agreement between EUS findings for normal pancreas (no EUS features of chronic pancreatitis were seen) and severe chronic pancreatitis (more than five EUS features) when compared with ERCP and secretin testing. For moderate chronic pancreatitis (three to five EUS features), there was 92% agreement with ERCP and 52% agreement with secretin testing [273].

It is unclear if focal chronic pancreatitis is a normal variant, a sign of focal fibrosis, or early malignancy. When the clinical suspicion and EUS features of moderate chronic pancreatitis are present throughout the gland, performing FNA does not improve the accuracy of the examination [283]. However, EUS-FNA should be considered in patients with focal chronic pancreatitis, despite the relatively low sensitivity of EUS-FNA in chronic pancreatitis (54%) [284]. A nondiagnostic FNA may eliminate an underlying malignancy, and careful follow-up is mandated in 1–3 months.

The EUS diagnosis of chronic pancreatitis depends on the threshold set for the number of positive criteria required to establish a definitive diagnosis. Changing the threshold affects the performance characteristics of EUS. In general, if less than two criteria are present, chronic pancreatitis is highly improbable. If three or four criteria are present, the test outcome is equivocal. If more than five criteria are present, the probability of chronic pancreatitis is high.

Pancreatic cysts and intraductal papillary mucinous neoplasms

Asymptomatic pancreatic cysts are increasingly recognized due to widespread imaging of the abdomen. While the majority of cysts are benign, approximately 10% represent cystic neoplasms [285,286]. Accurate characterization of the malignant potential of a pancreatic cyst remains challenging. EUS-FNA is often used in the management of such lesions. This section reviews the diagnostic accuracy of the various imaging modalities and recommends a current clinical algorithm.

The potential advantages of EUS and FNA include the ability to evaluate lesions for high-risk stigmata (e.g., thick septae and mural nodules) and to obtain specimens for cytological analysis and fluid for cyst fluid analysis. The EUS examination should note the following cyst characteristics: size; location (including relationship to adjacent vasculature); wall thickness; presence of focal wall irregularity, papillary projections, associated mass, or septae, echogenic debris or mucus; and dilation of the main pancreatic duct. Some of these findings are considered high-risk EUS features suggestive of malignant transformation; however, they do not reliably differentiate between benign and malignant cysts [287–291]. In addition, the absence of these features does not exclude the presence of malignancy.

When performing FNA, the recommendation is to undertake a single puncture, aspirate the cyst dry, describe the appearance of the aspirated cyst fluid, and analyze the cyst fluid for specific proteins, tumor markers, or genetic changes. All patients should receive antibiotic prophylaxis prior to FNA followed by 1–3 days of oral antibiotic treatment to theoretically reduce the risk of infection.

A multicenter trial [292] of 341 patients showed the accuracy of EUS morphology to be 51%, with the addition of FNA increasing the accuracy to 59%. A smaller trial of 67 patients found the overall EUS accuracy to be 73% (range 43%–88%). Cytology improved the sensitivity for mucinous lesions (94%), malignant mucinous lesions (100%), serous lesions (100%), and pseudocysts (100%), with a specificity of 98%–100% [293]. Additional findings were that a low carcinoembryonic antigen (CEA) level (< 5 ng/mL) was predictive of a serous cystadenoma, and high amylase or lipase concentration was associated with pseudocysts. Studies have reported a wide accuracy of EUS with or without FNA, ranging from 40% to 96% [287–289,291–296]. Studies have evaluated a variety of cyst fluid tumor markers or cyst fluid properties (CEA, CA19-9, CA72-4, CA125, amylase, lipase, viscosity, mucin stain). CEA is found in high levels in mucin-

ous tumors, whereas levels are low in pseudocysts (unless infected) and serous cystadenomas [294,297,298]. A large, prospective, multicenter trial reported that a CEA level of 192 ng/mL or more was 79% accurate for differentiating mucinous neoplasms from other types of cysts. In this study, no combination of morphological features, cytology results, and cyst fluid analysis improved the accuracy rate over that obtained with CEA alone [292]. Other studies have reported variable accuracies with CEA [293,299,300]. It has also been reported that an elevated CEA (≥ 480 ng/mL) and viscosity (> 1.6) accurately predicted mucinous cystic neoplasm from serous cystadenoma and pseudocysts. Mucinous cystadenocarcinoma lesions had a CEA level greater than 6000 ng/mL in one study [301].

CA19-9 (> 50 000 units/mL) has been associated with sensitivity of 86% and specificity of 85% in distinguishing cystadenocarcinoma from other lesions. However, CA19-9 is often elevated in inflammatory conditions and biliary obstruction, which limits its usefulness [294,297]. Amylase has been found in high concentrations in pseudocysts and intraductal papillary mucinous neoplasms (IPMNs), and amylase levels greater than 5000 U/L are associated with varying sensitivity (61%–94%) and specificity (58%–74%) in differentiating pseudocysts from other cystic lesions [293,298,302].

The ideal diagnostic approach to patients with pancreatic cysts is unclear and not validated, and is variable among medical institutions. Management often depends on local expertise. An international consensus guideline for management of IPMNs and mucinous cystic neoplasms has been published [303], and this algorithm should serve as a general framework in the management of patients with pancreatic cysts.

Biliary tract indications and cholangiocarcinoma

The common bile duct and gallbladder are in close proximity to the antrum and duodenum, allowing EUS to image these organs. EUS is a safe and accurate alternative imaging modality to ERCP, the traditional method most often used to evaluate the biliary tract. In many cases, EUS-guided therapy can be performed and can nearly eliminate the need for diagnostic ERCP.

A common indication for endoscopic biliary intervention is for suspected choledocholithiasis. An ERCP is often performed in such cases, but the risk of complications is significant [304–307] and it should be performed only in cases requiring therapeutic intervention.

Choledocholithiasis

EUS is superior to other imaging modalities for evaluation of the extrahepatic biliary tree for suspected choledocholithiasis. Transabdominal ultrasound detects approximately 30% of common bile duct stones [308,309] and helical CT has a reported diagnostic accuracy of 86%–94% [310,311] but with reduced accuracy for small bile duct stones. EUS and MRCP are both accurate imaging modalities for detection of common bile duct stones, with EUS slightly more accurate (approximately 96% vs 90%) [312–320]. EUS has greater resolution than MRCP, and hence EUS can detect stones as small as 1–2 mm without the loss of accuracy seen with MRCP [313,321]. EUS is the most accurate imaging test available for detection of microlithiasis [318,321].

Although long considered the gold standard for detection of choledocholithiasis, ERCP is less accurate and more invasive and has a higher failure rate compared with EUS. The NPV (the ability to rule out a common bile duct stone) of EUS is nearly 100%, with up to 32 months of patient follow-up [322–326].

When managing patients with suspected choledocholithiasis, adopt a treatment algorithm of performing EUS on patients with low or intermediate risk for choledocholithiasis, and proceed directly to ERCP in patients with a high clinical suspicion for choledocholithiasis. The EUS-based approach reduces the number of unnecessary ERCP procedures and is cost-effective [313,322,327].

Indeterminate biliary strictures

The critical question in the characterization of an indeterminate bile duct stricture is whether the stricture is malignant. Brushings or forceps biopsy obtained at ERCP has an accuracy that ranges from 30% to 70%, primarily due to the desmoplastic reaction associated with such tumors [328–333]. EUS (radial, linear, and IDUS) can visualize the extrahepatic biliary tree and extrinsic masses [334,335]. A prospective study of 40 patients with an indeterminate biliary stricture who underwent various imaging and endoscopic techniques found that the combination of MRCP and EUS had the highest specificity for diagnosis of an indeterminate biliary duct stricture [336].

For bile duct lesions, radial or linear EUS provides excellent visualization and ability to obtain tissue [337,338]. For lesions located in the porta hepatis region, the 20-MHz IDUS miniprobe provides accurate images of the bile duct wall and surrounding structures. The IDUS miniprobe is advanced to the stricture without a prior papillotomy. The first hyperechoic layer corresponds to the mucosa in addition to a border echo, the second hypoechoic layer is smooth muscle fibers with fibroelastic tissue, and the third hyperechoic layer is the thin and loose connective tissue with a border echo [339]. IDUS findings concerning for malignancy include disruption of the bile duct wall, sessile lesions, and tumor size greater than 10 mm. The accuracy of IDUS in differentiating between benign and malignant strictures ranges from 76% to 92% [340–344]. The presence of two of these three features is considered specific for the diagnosis of malignancy, and the absence of all these features carries a very high NPV for ruling out malignancy [343]. However, IDUS is not accurate when evaluating for metastatic lymphadenopathy [342]. The linear EUS echoendoscope is still required for FNA. Studies have shown that EUS-FNA may aid in the diagnosis of a hilar

cholangiocarcinoma when other standard methods of tissue acquisition have failed [345].

Cholangiocarcinoma

When cholangiocarcinoma is diagnosed, EUS can assist in staging and assessment of surgical resectability. Bile duct cancer T-staging is as follows: T1, limited to the wall of the common bile duct; T2, invasion beyond the wall of the common bile duct; and T3, invasion to adjacent structures. The accuracy of IDUS is higher than EUS for assessing T-staging (77.7% vs 54.1%) [346], particularly for hilar or common hepatic duct strictures.

When assessing for surgical resectability, the key question is longitudinal extent of tumor spread and involvement of adjacent structures, particularly the portal vein and right hepatic artery. The accuracy of IDUS for assessment of longitudinal tumor spread ranges from 72% to 86%, when using notching of the outer margins and asymmetric wall thickening as visualized endosonographic features. However, thickening due to inflammation can reduce the accuracy [347]. IDUS is highly accurate at visualizing portal vein and right hepatic artery involvement, and is more accurate than angiography [348,349].

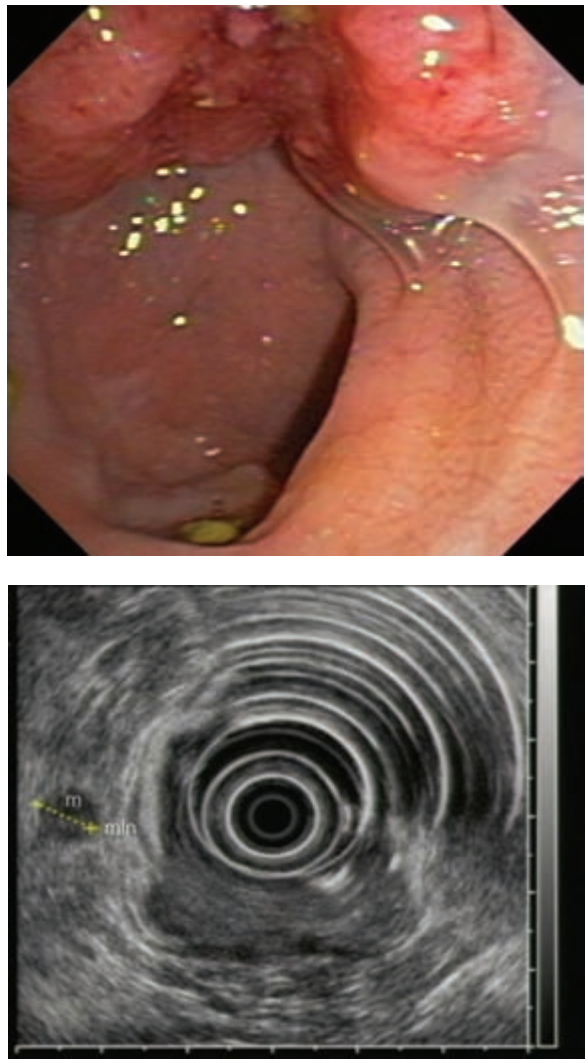
Anorectal EUS

Common indications for rectal EUS include evaluation of suspicious polyps and locoregional staging of rectal cancer, assessment of submucosal lesions, and evaluation of incontinence (for possible anal sphincter defects). Most patients can be adequately prepared for this unsedated procedure with enemas.

Malignancy often appears as an irregular hypoechoic lesion with disruption of the rectal wall layers (Fig. 130.13). Since neoadjuvant therapy in locally advanced rectal cancer is associated with an improvement in recurrent-free survival, the accurate preoperative staging of such lesions is critical [350–357]. EUS is indicated in the locoregional staging of patients with rectal cancer and in the evaluation of patients with recurrent malignancy [358,359].

When M0 rectal cancer has been established, EUS is the next step in staging because it has superior performance characteristics to CT and MRI [360]. The accuracy of EUS for T-staging ranges between 80% and 95%, with a mean sensitivity of 87.5% and specificity of 83.5%; this compares with an accuracy of 65%–75% for CT and 75%–85% for MRI [359,361–366]. The accuracy of EUS for determining nodal metastases is approximately 70%–75%, whereas that for CT is 55%–65% and MRI 60%–70% [359,362,364]. The presence of perirectal lymph nodes often warrants FNA, which can be performed safely with low risk of infection [367,368]. EUS has been shown to alter the treatment plan in nearly one-third of rectal cancer cases [369].

A common submucosal rectal lesion may be rectal carcinoid, often an incidental finding [370,371]. Poor prognosis is associated with larger tumor size, deep invasion of tumor, and lymphovascular invasion [371]. EUS can assess lesions to determine eligibility for local excision versus surgical resection [372].



(a)

(b)

Figure 130.13 Rectal carcinoma. **(a)** Endoscopic image of ulcerated rectal mass. **(b)** Endoscopic ultrasonography image demonstrates T3N1 stage: m, mass; mln, malignant lymph node.

Anorectal EUS also plays a role in detecting sphincter defects in patients with fecal incontinence, and helping to assess whether the defect is amenable to surgical correction [373]. EUS findings have been correlated accurately with operative findings [374]. EUS may also be useful in assessing perianal inflammatory conditions [375].

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B IMAGING

131 Applications of computed tomography to the gastrointestinal tract

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Since its introduction in the late 1970s computed tomography (CT) has made unparalleled technical advancements in both scanner hardware and software. Today's multidetector CT (MDCT) scanners allow submillimeter collimation (0.6 mm) and fast scanning speeds (0.33 s per rotation) [1]. The resultant temporal and spatial resolution have greatly improved our ability to image the body. For example, 64-slice MDCT scanners can image the entire abdomen and pelvis in less than 10 s using submillimeter slices. This narrow temporal window has directly impacted protocol design, enabling data acquisition during the phase of maximum lesion conspicuity. Consequently, specific acquisition phases have been delineated for pancreatic tumor and hepatocellular carcinoma detection using multislice technology [2,3]. Following data acquisition, easy-to-use three-dimensional postprocessing software is now widely available, which enables the radiologist to view the scan data in any imaging plane. Beyond improved image quality and diagnostic efficacy for existing applications, these advancements have facilitated the development of new CT applications such as virtual colonoscopy and coronary CT angiography.

In this chapter, we review the current state of CT imaging of the gastrointestinal tract, including the hollow viscera and the hepatobiliary tree and pancreas. New CT innovations such as three-dimensional rendering and virtual imaging are also discussed.

Hollow viscera of the gastrointestinal tract

Techniques

Oral contrast

Identifying abnormalities of the lumenal gastrointestinal tract, whether it be neoplastic or inflammatory, relies on the ability to adequately visualize the bowel wall [4,5]. Optimal distention of the bowel is necessary for evaluation of the wall. There are a variety of CT contrast agents that can be administered orally or rectally. These are categorized as positive agents, neutral agents, or negative agents [6–8].

Positive agents appear "white" on CT and usually consist of diluted (1.5%–2%) iodinated water-soluble contrast or diluted (2%) barium suspensions. These are the agents traditionally used for routine CT scans of the abdomen.

Neutral contrast agents appear "gray" on CT and usually have a density similar to water. Currently used neutral agents include water, methylcellulose compounds, and commercially available products such as Volumen (EZ-Em; Lake Success, NY), which has a density slightly greater than that of water [8]. Megibow and colleagues have compared the neutral oral contrast agents Volumen and methylcellulose in 60 patients undergoing CT of the pancreas [8]. Half of the patients consumed 1200 mL of Volumen over 30 min and the other half consumed a 3 : 1 mixture of water and methylcellulose. Volumen, which has an attenuation of 20–40 Hounsfield units (HU), resulted in superior distention of the bowel and better demonstration of mural features.

More studies are needed to determine which oral agent is best for specific CT applications [8]. Neutral agents may be beneficial for detailed imaging of the stomach and small bowel because they allow excellent visualization of the enhancing wall that may otherwise be obscured by positive agents [8,9–11]. In addition, if three-dimensional imaging is performed, it is beneficial to use either neutral or negative contrast agents because they still allow three-dimensional visualization of the vasculature without extensive postprocessing to segment the bowel loops from the volume [12,13]. When positive oral agents are used, these "white" loops need to be removed, as they obscure the adjacent contrast-enhanced vessels, particularly with maximum intensity projection.

Negative agents appear "black" on CT and usually consist of either gas granules administered orally to distend the stomach or insufflation of the colon with air or carbon dioxide for

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virtual colonoscopy. Other negative agents such as oil-based products (peanut oil) are not used routinely [14]. In clinical practice, the choice of oral contrast depends on the clinical indication and is reviewed in the appropriate subsections of this chapter.

Intravenous contrast

Intravenous contrast-enhanced images expand the diagnostic range of CT and have become essential for most clinical indications. Intravenous contrast enhancement improves conspicuity of pathological processes in evaluation of the alimentary canal by increasing the level of confidence in diagnosing a thickened bowel loop as being truly diseased, as well as allowing assessment of bowel wall enhancement and detection of small-bowel masses. This refines the differential diagnosis by highlighting attenuation differences within the thickened loop, or by accentuating the "vascularity" of a bowel-related mass, thereby facilitating the determination of histological type and neoplastic potential [15]. Variations in blood supply of pathological processes demand a tailored approach to intravenous contrast administration for evaluation of the alimentary tract.

Nonionic iodinated contrast agents are typically utilized for CT. The protocol for administration will vary depending on the clinical indication. For example, in patients with suspected mesenteric ischemia, it is important to acquire images in both the arterial and venous phases to ensure adequate visualization of both the mesenteric arteries and veins in addition to assessment of bowel wall enhancement [16]. The radiologist will tailor the intravenous contrast injection rate and scan delay based on the clinical question. Therefore, it is essential that appropriate clinical information be provided by the ordering physician in order for scans to be performed correctly.

Scanning protocol

In general, CT imaging of the abdomen, and the alimentary tract in particular, requires high-resolution volumetric datasets. Data acquisition parameters are dictated by the clinical question. For example, if three-dimensional imaging is performed, it is helpful that the CT scan be performed using the thinnest possible detectors. State-of-the-art 64-slice scanners have detectors as narrow as 0.6 mm, which can be used to create 0.75-mm overlapping sections reconstructed every 0.5 mm to produce a volume dataset that is ideal for three-dimensional imaging. The data may also be reconstructed with 3–5-mm sections for axial review of the abdomen and pelvis. With respect to voltage and tube current, 120 kV and 270 mAs (effective) are usually adequate for three-dimensional studies. For studies not requiring three-dimensional imaging, thicker collimator settings can be used (e.g., 1.2 mm) and the tube current can be reduced appropriately.

Three-dimensional imaging

A comprehensive CT examination in patients with suspected gastrointestinal tract pathology may be aided by review of the dataset with three-dimensional software. The interpretation is tailored to fit the specific clinical question, and different rendering algorithms may be needed to optimally display specific findings. Current three-dimensional imaging software varies by vendor, but typically includes a combination of multiplanar reconstruction (MPR), volume rendering and maximum intensity projection (MIP) [17–19]. MPR is the simplest to use and allows scrolling through the dataset in any plane. However, for full analysis of gastrointestinal tract and abdominal vasculature, volume rendering is the most robust three-dimensional imaging tool. Volume rendering allows the brightness, opacity, window width, and window level to be adjusted in real time in order to accentuate certain tissue types or selective viewing of the vasculature [19]. Manipulating trapezoidal transfer functions interactively modifies the image contrast and the related pixel attenuations in the final image. This function allows color and opacity assignments to each voxel and can be adjusted to alter the display. Although initial volume rendering software was somewhat labor intensive, software packages are now easy to use and can be adjusted in real time. Also, the process can be simplified by creating pre-sets, which can be applied quickly and then only minor adjustments are needed. MIP is a projection technique in which the brightest voxel is displayed along a ray [19]. This can be valuable using thin slabs of data to accentuate small vessels. The radiologist often utilizes a combination of these three-dimensional tools to optimally display the relevant anatomy and pathology. Specialized three-dimensional software has been developed for specific CT applications, including virtual colonoscopy.

Esophagus

Neoplasms

For evaluation of suspected or known esophageal masses, CT aids in identification of the mass, assessment of local extension, detection of local and regional lymphadenopathy, and identification of sites of metastases. Esophageal neoplasms usually appear on CT as focal, asymmetric, soft tissue masses arising from the wall or as concentric areas of wall thickening. However, these findings are nonspecific and can be seen in benign and malignant tumors as well as in inflammatory conditions [20].

Carcinoma of the esophagus typically appears on CT as a focal eccentric mass or as concentric thickening of the esophageal wall, neither of which is a specific finding [21] (Fig. 131.1). CT provides no histological specificity; squamous cell carcinoma, adenocarcinoma, lymphoma, and metastatic disease to the esophagus all have similar CT appearances. However, esophageal stromal tumor should be considered when there is a prominent exophytic component to the mass [22].

It is important to note that early superficial esophageal lesions may not be visible on CT, and therefore CT is typically not used to screen for esophageal cancer [23]. Also, despite technical advances in CT scanners and three-dimensional

Figure 131.1 **(a)** Axial intravenous contrast-enhanced multidetector computed tomography (MDCT) image reveals a large circumferential esophageal mass (arrows). The mass splays the carina. An esophageal stent is in place (arrowhead). **(b)** Axial intravenous contrast-enhanced MDCT image demonstrates bulky mediastinal adenopathy (arrows). The esophageal stent is again noted (arrowhead).

imaging software, CT cannot visualize individual layers of the esophageal wall and therefore endoscopy with endoscopic ultrasound (EUS) is definitely more valuable for T-staging [23]. EUS may also be more valuable than CT for identifying locoregional nodes [23]. However, EUS alone is not sufficient to stage patients with esophageal cancer. It should be combined with CT and possibly positron emission tomography (PET) for comprehensive staging [23].

The real value of CT in patients with esophageal carcinoma includes staging the extent of disease (highest use in detecting extraesophageal disease), evaluating patients after therapy, and evaluating the complications of surgical therapies. Therapy success or failure can be based on documentation of the development of enlarged mediastinal or upper abdominal lymphadenopathy, lung nodules, or liver or adrenal metastases. Recent studies suggest that PET may also be useful in detecting metastatic disease, and in a study by Pfau and colleagues PET identified more patients with metastatic disease than did CT [23].

CT criteria for extensive esophageal cancer include demonstration of local invasion (aorta, diaphragm, tracheobronchial tree), distant lymphadenopathy, and distant metastases. Local extension is the most difficult parameter to accurately diagnose. Contact angles of more than 45° have been suggested to accurately predict aortic invasion, although several studies have shown this finding to result in frequent over-staging [24]. In an interesting study by Wayman and colleagues, the authors performed supine and prone CT imaging in 39 patients with esophageal carcinoma. Results revealed that the combination of supine and prone imaging improved accuracy for determining the presence of aortic invasion compared with the supine images alone [25]. Tumor invasion of the pericardium is suspected if the normal surrounding fat plane is obliterated. The addition of MPR, three-dimensional imaging, or CT esophagography may help improved accuracy [26,27].

Pathological lymphadenopathy can be more reliably determined when regional adenopathy of more than 5 mm in the short axis is used as a criterion [28]. However, it should be noted that even nodes smaller than 5 mm can harbor malignancy. Therefore, PET or EUS may play a role here [23].

CT has been shown to be a helpful addition to fiberoptic bronchoscopy to identify tracheobronchial invasion [29]. For this indication three-dimensional imaging is especially helpful for demonstrating the relationship of the esophageal mass to the airway, as well as for identifying tumor extension. The addition of virtual bronchoscopy viewing of the airway may also be beneficial for this indication [29].

Postsurgical complications after esophagectomy are not uncommon, and can occur in up to 50% of patients [30]. The most frequently noted complication on CT is an anastomotic leak, which may not be detected by standard contrast esophagography [30]. In some cases it may be beneficial to perform CT immediately after contrast esophagography to increase sensitivity in detecting small leaks that may be subtle at fluoroscopy. In patients who have undergone esophageal stent placement, CT is also valuable for documenting patency and detecting complications [31].

Varices

Esophagogastric varices are easily detected with contrast-enhanced CT and diagnosis is improved using three-dimensional CT techniques [32]. CT is particularly useful in demonstrating extraserosal varices, which may be underestimated by EUS [32]. Thickening of the esophageal wall, a scalloped contour, and enhancing intralumenal protrusions after a bolus intravenous contrast injection are seen [33]. Low attenuation in the esophageal wall in patients with other stigmata of portal hypertension should suggest a history of sclerotherapy.

Esophagitis

Esophagitis may produce thickening of the distal esophageal wall. CT is neither sensitive nor specific for this finding. CT

does not aid in the differentiation of distal esophageal abnormalities and cannot distinguish carcinoma from severe esophagitis. CT may be useful in recognizing distal esophageal changes in immunocompromised patients, prompting endoscopic evaluation to determine the specific etiology.

Achalasia

CT is not the imaging modality of choice in patients with suspected achalasia. However, CT is sometimes the first modality to suggest the diagnosis in patients with nonspecific symptoms. The CT appearance of achalasia can be dramatic, showing a markedly distended esophagus with retained fluid and food [34].

Stomach

CT can evaluate the position of the stomach, the lumenal contour, the gastric wall, surrounding lymph nodes, peritoneal ligaments, and surrounding viscera. When the stomach is distended adequately, the gastric wall should measure less than 5 mm. However, at the esophagogastric junction, the wall may be up to 1 cm thick and still be normal. This is related to the scanning plane and the orientation of the lower esophageal sphincter. Similarly, because of muscle orientation in the antrum as well as antral contraction, it has been shown that in this region the wall commonly exceeds 5 mm in thickness and can measure up to 13 mm and still be normal [35]. Using absolute gastric wall measurements is not always valuable for distinguishing malignant from benign disease. In a study by Insko and colleagues, the authors noted that a gastric wall thickness of 1 cm had a sensitivity of 100% for detecting malignant or potentially malignant lesions, but had a specificity of only 50% [36]. The authors also noted that focal eccentric enhancing wall thickening was always malignant but was only present in a small percentage of cases [36].

Changes in positioning may be necessary to maximally distend regions of the stomach where the wall is questionably thickened. Several studies have confirmed the value of three-dimensional imaging to improve evaluation of the stomach and its perigastric relationships [37].

The gastric wall displays uniform enhancement following intravenous contrast administration. The use of dual-phase imaging may be helpful for evaluating differences in enhancement in a variety of gastric pathologies [38]. Administration of neutral or negative oral contrast allows assessment of the distribution of the rugal fold pattern in the proximal stomach as well as optimal visualization of mural enhancement [6,39–41].

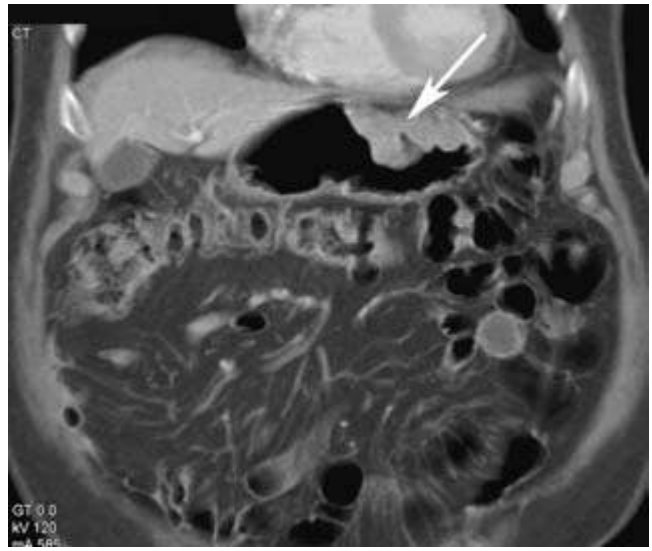
Neoplasms

Gastric adenocarcinoma

Gastric carcinoma appears as a region of focal gastric wall thickening or mass (Fig. 131.2). Polypoid and scirrhous forms (with or without ulceration) are encountered. Scirrhous tumors (linitis plastica) can be difficult to diagnose as the degree of wall thickening is less than in polypoid tumors [42].

CT is typically used for staging once the diagnosis has been made by endoscopy. Overall staging accuracy has been reported to be as high as 94%, with better results for advanced tumors than early gastric cancers [43]. Studies have shown that EUS is more accurate than CT for determining depth of mural invasion and for local lymph node staging [43]. Because CT cannot resolve the individual gastric wall layers, superficial early gastric cancers may not be detected [43].

(a)



(b)

Figure 131.2 (a) Axial intravenous contrast-enhanced multidetector computed tomography image in a patient with gastric adenocarcinoma shows an infiltrating mass in the gastric body (arrows). There is extension into the gastrohepatic ligament (arrowhead). **(b)** Coronal volume-rendered image nicely demonstrates the bulky gastric mass (arrow).

However, the CT detection rates and T-staging of gastric cancer have improved with the incorporation of MDCT three-dimensional imaging [44].

There has also been some interest in the use of CT virtual gastroscopy for improving gastric cancer detection and perhaps even for screening high-risk patients [43,45,46]. To perform virtual gastroscopy, the stomach is distended with air. Thin collimation CT is then performed and virtual software is used to image the stomach. The computer can travel through the gastric lumen to provide images similar to those seen during conventional endoscopy. The exact role for virtual gastroscopy in gastric cancer detection is not yet known.

As with esophageal tumors, CT is not consistently accurate in T-staging (especially T1 vs T2 lesions) due to its inability to resolve the bowel wall layers. However, three-dimensional imaging has been shown to improve T-staging compared with axial images alone [43,44]. With single-slice helical CT, using thin sections and intravenous contrast, resultant density discrimination improves staging accuracy over conventional CT. In a Japanese study, overall T-staging was correct in 66% (27 of 41), despite a large number of "early" carcinomas that might bias the sample against CT accuracy [47], implying that results should be improved in populations where patients present with more advanced disease. Dedicated imaging protocols have yielded detection of lesions in greater than 95% of patients and accurate detection of lymph node disease in approximately 71% [38].

Gastric cancer spreads along the perigastric peritoneal reflections through intramural lymphatics, by peritoneal seeding, and hematogenously. Lymphatic submucosal spread into the esophagus occurs in approximately 30% of patients with tumors in the gastric fundus. Extension into the serosa allows tumor access to the supporting mesenteries of the stomach, providing pathways into omentum, kidney, liver, pancreas, transverse colon, and spleen. Proximal lesions can extend into the diaphragm and the aorta. Again, these complex pathways of tumor spread are best appreciated with the ability to visualize the CT data in three dimensions.

Lymphoma

Gastric lymphomas produce significantly greater wall thickness than carcinoma, although this finding is of limited value for differential diagnosis in individual cases [48,49]. Following intravenous contrast enhancement, lymphoma masses minimally enhance and remain homogeneous, as opposed to the heterogeneous appearance typical for adenocarcinoma. In cases presenting with fold hypertrophy, there may be demonstrable clefts between the thickened folds extending through the wall into the extraserosal tumor [50].

Gastrointestinal stromal tumors

Gastrointestinal stromal tumors (GISTs) are mesenchymal tumors that arise within the muscularis propria of the gastrointestinal tract wall. Approximately 60% of GISTs occur in the stomach, although they only represent 1–3% of all gastric malignancies [51]. CT plays an important role in the detection and staging of these tumors, as CT can detect the tumor and assess for local spread and distant metastases [11]. When small, GISTs are confined to the gastric wall, and they usually appear as rounded intramural masses (Fig. 131.3). Lesions as small as 1 cm have been detected on MDCT [52]. In a series of patients described by Nishida and colleagues, all GISTs over 2 cm were visible on four-slice CT [52]. As all tumors 2 cm or greater in size were detected with MDCT, these findings suggest that MDCT can be useful in screening symptomatic patients suspected of having GIST.

Large tumors can either protrude into the gastric lumen or be exophytic [11], often demonstrating central necrosis or ulceration. Three-dimensional imaging is particularly helpful for demonstrating the relationship of the mass to the stomach. When very large and exophytic, a gastric GIST may be misdiagnosed as a mass from the adrenal, pancreas or colon using only axial sections for interpretation [11].

Inflammatory diseases

CT has no primary indication for the evaluation of patients with suspected gastric inflammatory diseases. However, because CT is so frequently ordered in patients with abdominal pain, it may be the first modality to suggest the presence of gastritis or peptic ulcer disease [53]. In patients with gastritis, CT may show focal or diffuse wall thickening. The thickened wall will display some degree of low attenuation, reflecting intramural edema [54]. This would be an uncommon finding in a patient with a gastric neoplasm. Although CT is not sensitive for the detection of ulcers, they can occasionally be seen, especially when large [53]. However, the

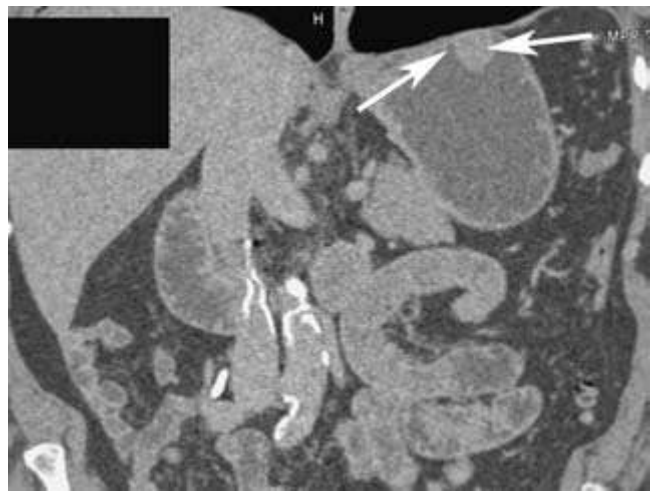


Figure 131.3 Intravenous contrast-enhanced coronal multiplanar reconstruction using water as oral contrast demonstrates a 1.3-cm intramural gastric mass (arrows) compatible with a gastrointestinal stromal tumor. This was an incidental finding.

CT appearances of inflammatory and malignant conditions overlap.

Small intestine

The key to evaluation of the small intestine lies in adequate opacification with orally administered contrast material [7]. Typically, 750 mL of oral contrast material should be administered approximately 1 h before examining the abdomen to opacificy the small bowel. An additional 250 mL is administered just prior to the scan to ensure adequate distention of the stomach and duodenum. The choice of neutral or positive agent will depend on the clinical indications. Some investigators advocate the use of oral metoclopramide to aid in gastric emptying and filling of the terminal ileum, while others utilize glucagon to help improve small bowel distention by decreasing peristalsis.

Neoplasms

Early diagnosis of small-bowel tumors continues to pose a significant challenge for both clinicians and radiologists. This is because small-bowel neoplasms are relatively uncommon, representing less than 25% of all gastrointestinal neoplasms and less than 2% of all malignant tumors [55]. Also, small-bowel tumors tend to be small initially and may be difficult to diagnose by conventional radiological studies [56]. In addition to contrast fluoroscopy studies, CT is now recognized as an important tool for evaluation of these patients. CT has the advantage of visualizing both the entire gastrointestinal tract and extralumenal structures such as lymph nodes [10].

Adenocarcinoma

Adenocarcinoma of the small intestine produces soft tissue attenuation, wall thickening and irregular narrowing of the lumen, which can lead to obstruction [10]. These lesions most frequently arise in the periampullary duodenum, but may occur anywhere along the mesenteric small bowel (Fig. 131.4). Malignant lesions appear as exophytic or intramural masses with central necrosis and ulceration [57]. However, ulceration is not always well demonstrated on CT [58].

Lymphoma

Four major patterns of small-bowel lymphoma have been identified on radiographic studies [59].

- Lymphoma can appear as multiple nodules within the small bowel. Involvement of multiple sites along the small intestine aids in differentiation of lymphoma from adenocarcinoma and carcinoid tumor.
- Lymphoma can appear as a single mass lesion, which varies in size. These isolated lesions can cause small-bowel intussusception.
- Lymphoma can be infiltrating and appear as wall thickening with destruction of the normal small-bowel folds. Unlike adenocarcinoma, these tumors are typically soft and are unlikely to result in obstruction. In addition, as the tumor infiltrates the musculature of the wall, it can inhibit peristalsis and result in aneurysmal dilatation of the involved loop, as opposed to obstruction [59].
- Lymphoma can appear as an exophytic mass, which can ulcerate.

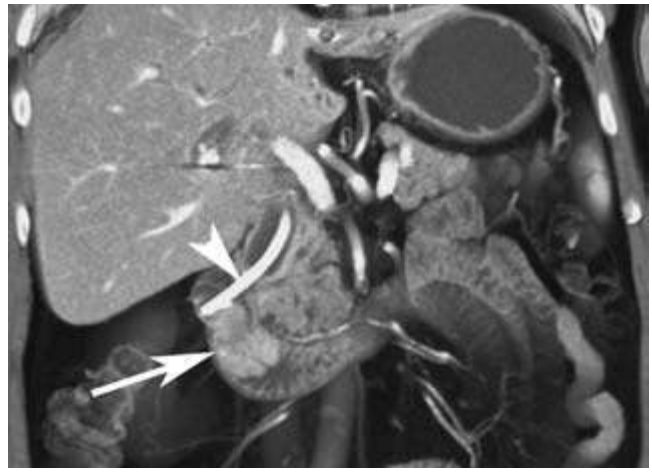


Figure 131.4 Intravenous contrast-enhanced coronal volume-rendered image using water as oral contrast demonstrates a 2-cm lobulated mass (arrow) in the second portion of the duodenum. A common bile duct stent is in place (arrowhead). The patient underwent Whipple surgery and pathology revealed adenocarcinoma arising in a tubular adenoma.

The morphological patterns of nodular, ulcerative, and aneurysmal disease due to lymphoma are best visualized with CT [48]. Acquired immunodeficiency syndrome (AIDS)-related lymphomas display two main patterns on CT: single or multiple segments with homogeneous circumferential wall thickening, or single or multiple cavitary lesions. The gross morphological features, distribution pattern, degree of wall thickening, and length of involvement are similar in AIDS-related and non-AIDS-related lymphomas [60].

Carcinoid tumors

Small-bowel carcinoid tumors are neuroendocrine neoplasms. In early stages, the tumor is small, confined to the bowel wall, and appears as an enhancing intramural lesion [61]. At this time small-bowel series and enteroclysis may be more sensitive for detection than CT or magnetic resonance imaging (MRI). As the tumor grows, it can extend beyond the involved bowel loop causing infiltration of the mesentery and desmoplastic reaction (Fig. 131.5). This results in a characteristic appearance on small-bowel contrast examinations and cross-sectional imaging studies. The CT appearance is characterized by radially oriented linear densities in the perienteric fat. A lymph node mass (frequently calcified) is present centrally [62]. Bowel loops are drawn toward the center. The mesenteric desmoplasia may produce ischemic thickening of the involved loops. In addition to contrast studies and CT, nuclear medicine techniques utilizing indium 111- (or iodine 123)-labeled octreotide or iodine 131-labeled

Figure 131.5 (a) Axial intravenous contrast-enhanced multidetector computed tomography image shows a 1.5-cm infiltrating mass (arrow) in the root of the mesentery, compatible with carcinoid. **(b)** Coronal maximum intensity projection nicely demonstrates encasement of the superior mesenteric artery branches (arrowhead) by the infiltrating tumor (arrows).

metaiodobenzylguanidine (MIBG) are useful tools to diagnose and localize carcinoid tumors and to identify their metastases.

Gastrointestinal stromal cell tumor

The fourth most common small-bowel malignancy is GIST, which arises from smooth muscle cells within the wall of the gastrointestinal tract and most commonly involves the stomach. Small-bowel GISTs more likely arise in the jejunum or ileum, as compared to the duodenum. It is usually not possible to distinguish benign from malignant GISTs radiographically unless obvious metastases are present. As with gastric GISTs, small-bowel lesions, when small, may go undetected on CT. However, as they enlarge, they typically become exophytic and often ulcerate, producing a characteristic appearance on CT [11]. These tumors can be bulky. Central necrosis or ulceration is also common. These tumors may be so large that it may be difficult to appreciate the site of origin. The communication of the tumor with the intestinal lumen may sometimes be difficult to appreciate. However, three-dimensional imaging can be helpful to make this determination. Tumor spread is usually through direct extension into adjacent organs or hematogenously to the liver. Liver metastasis can appear low-density or cystic [11].

Metastatic disease

Metastatic disease to the small bowel results from hematogenous spread, peritoneal seeding, or direct extension of a contiguous lesion. Each has a recognizable CT appearance. Hematogenous metastases are commonly seen from melanoma, Kaposi sarcoma, and breast carcinoma. Discrete masses eccentrically localized along the bowel circumference are present. Tethering of the mesenteric border of individual bowel loops in the presence of peritoneal carcinomatosis is indicative of seeded metastases. Variable degrees of partial bowel obstruction are common. Nodular thickening of the mesenteric reflections is observed. These findings are seen in metastatic ovarian, colonic, breast, and lung carcinoma.

Nonneoplastic diseases

Crohn's disease

One of the earliest applications of CT in the alimentary tract was the evaluation of Crohn's disease [63]. Markedly expanded contrast resolution compared with plain film and barium radiography make CT ideal in displaying the transmural inflammation, effects in local fat, and global assessment of disease [64]. The affected bowel wall displays variable degrees of mural thickening. When intravenous contrast is administered, submucosal hypoattenuation, reflecting submucosal edema, may be seen. This "target sign," although nonspecific for Crohn's disease, was first described with this entity [65]. This appearance may be mimicked in long-standing disease from deposition of submucosal fat. The bowel may become isolated in a "sea" of hypertrophied fat. CT definitively establishes this fatty proliferation as a cause of separation of bowel loops. The fatty proliferation is accompanied by hypervascularity in the local mesentery [66].

Linear nonvascular densities are correlated with the presence of sinus tracts or fistulae. When these are identified on CT, barium studies are sometimes necessary to precisely map their relationships, although, 3D imaging can also be helpful. Local lymphadenopathy (generally not greater than 15 mm) is associated with these findings. Increased density in the fat reflects inflammatory exudate and, when contained, frank abscess. Intraabdominal abscess is not uncommon, with severe steroid-refractory relapses of Crohn's disease being present in about one-third of patients. CT is superior to ^{111}In-labeled leukocyte scintigraphy, which is sensitive for detecting inflammation but poor at documenting extent. Nonetheless, ^{111}In-labeled leukocyte scintigraphy is the most specific method for imaging active inflammation.

Figure 131.6 Coronal volume-rendered image from an intravenous and oral contrast-enhanced computed tomograph in a patient with Crohn's disease demonstrates small bowel wall thickening, mucosal hyperemia, and engorgement of the vasa recta. This is an example of the comb sign, indicating active disease.

With advancements in CT technology resulting in improved resolution and speed, investigators are starting to assess if CT can provide more useful information regarding disease activity in Crohn's patients. In a recent study by Bodily and colleagues, the authors retrospectively obtained quantitative information from CT scans of 96 patients with Crohn's disease, including measurements of small-bowel mural attenuation and thickness [67]. Mural attenuation correlated significantly with active Crohn's disease, although small-bowel wall thickness by itself was not a significant factor [67]. In a study of 143 patients with CT studies, Colombel and colleagues found that endoscopic score correlated significantly with CT bowel enhancement, comb sign (vascular enlargement of the vasa recta), and increased density in the fat [68] (Fig. 131.6). C-reactive protein correlated with increased fat density, but not when the inflammation was limited to the bowel wall [68]. This is currently a topic of active research.

Vascular diseases

Isotropic volumes from submillimeter MDCT scanners facilitate routine use of CT angiography, and dedicated CT of the mesenteric vessels is now possible [9,69]. Detailed imaging of the mesenteric vessels requires rapid intravenous contrast administration combined with appropriately timed dual-phase scanning to ensure adequate visualization of both the mesenteric arteries and veins. Also, if evaluation of mesenteric vessels is requested, low-density oral contrast is administered in order to allow evaluation of bowel enhancement as well as to facilitate three-dimensional imaging of the vasculature without the need to edit high-density oral contrast-filled bowel.

In many cases, CT angiography can replace initial diagnostic angiography. CT angiography can be used to evaluate a variety of vascular pathology including splanchnic artery aneurysms, median arcuate ligament syndrome, and superior mesenteric artery syndrome [9,70,71]. A common clinical indication requiring detailed CT imaging of the mesenteric vessels and bowel is the evaluation of suspected mesenteric ischemia (Fig. 131.7). Dual-phase three-dimensional CT can evaluate the mesenteric arteries and veins and identify potential causes of ischemia such as superior mesenteric artery or vein thrombus or tumors such as small-bowel carcinoid or pancreatic cancer, both of which can result in small-bowel ischemia [16,69,72]. Even in the absence of obvious pathology of the mesenteric vessels, CT can suggest the presence of mesenteric ischemia based on the appearance of the small bowel. Delayed or absent enhancement can indicate underlying ischemia. Pneumatosis may reflect infarcted bowel, but can also occur in benign conditions and should therefore be correlated with clinical findings.

Small bowel obstruction

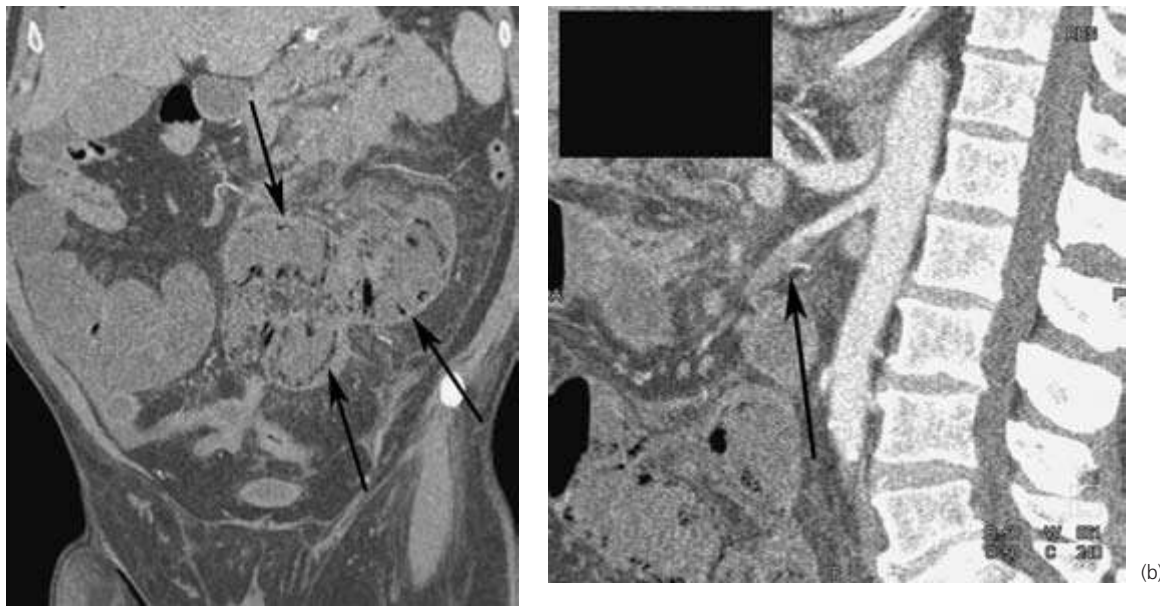
CT findings of obstruction are similar to traditional radiographic findings: disparate dilation of proximal bowel loops compared with more distal bowel (Fig. 131.8). Comparative studies have shown that CT is superior to plain film radiography in detecting intestinal obstruction and in determining the cause of obstruction [73]. Studies have shown that CT sensitivities reportedly range from 90% to 95% in detecting obstruction. CT establishes the etiology in a majority of cases [74]. The major contribution of CT in these patients is the ability to detect signs of strangulation. These signs include thickening and edema in affected loops (ischemic signs) and mesenteric clouding, ascites, or infiltration (mesenteric signs) [75]. The combination of multiple findings has improved reliability in predicting obstruction as compared to any single sign in isolation [76].

CT can change management by accelerating surgical treatment in obstructed patients, resulting in verifiable improvements in outcome [77,78]. CT efficacy decreases in patients with chronic obstructive symptoms and low-grade obstruction. For these patients, barium radiography, especially enteroclysis, is the method of choice [79].

The ability to view the CT data in more than one plane can be useful in the evaluation of patients with small bowel obstruction, as the exact site of transition may be difficult to detect on axial images. The use of MPR for this clinical indication has been shown to increase reader confidence [80].

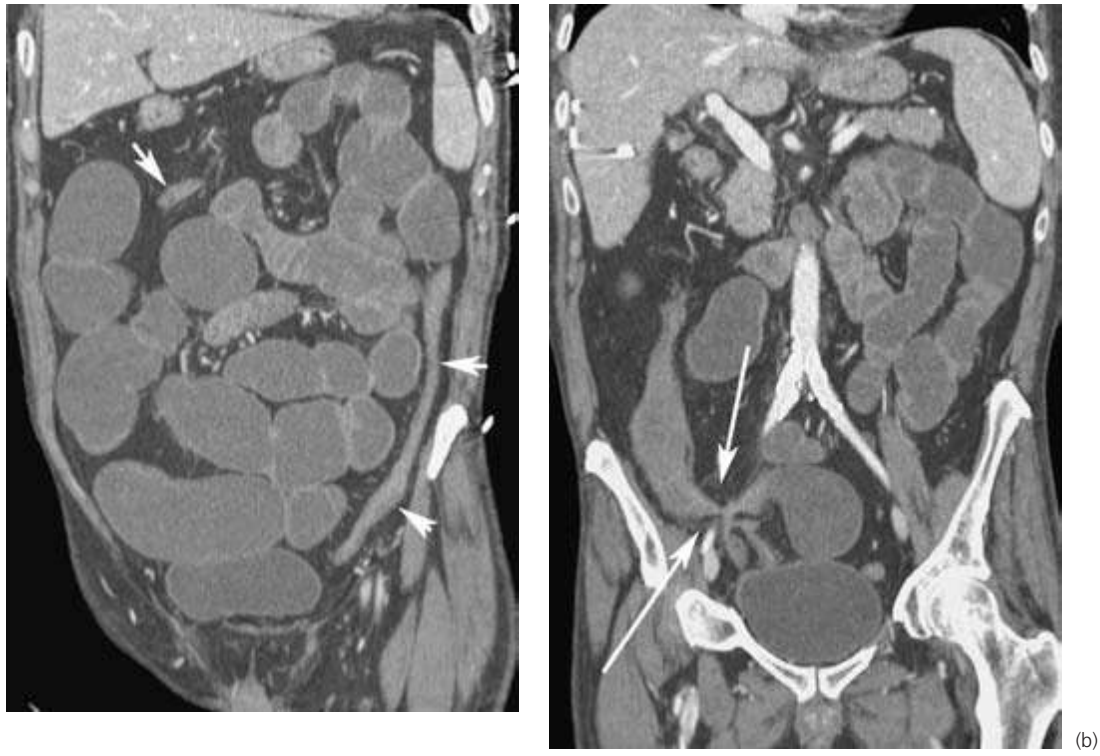
Colon

CT is useful in the evaluation of many colonic disorders. Because CT shows both the colonic wall (generally < 3 mm) and surrounding pericolic fat, it can identify extension of pathological processes into the surrounding tissues. This results in improved staging of tumors and preoperative assessment



(a) (b)

Figure 131.7 (a) Coronal multiplanar reconstruction (MPR) from intravenous contrast-enhanced computed tomography demonstrates dilated small bowel with pneumatosis (arrows). **(b)** Sagittal MPR demonstrates thrombosis in the superior mesenteric artery (arrow).



(a) (b)

Figure 131.8 (a) Intravenous contrast-enhanced coronal multiplanar reconstruction (MPR) using water as oral contrast demonstrates moderate small bowel dilatation. The colon is decompressed (arrows). **(b)** Intravenous contrast-enhanced coronal MPR using water as oral contrast demonstrates multiple fistulae (arrows) in the distal small bowel and colon. This was the source of the small bowel obstruction in this patient with Crohn's disease.

of complex inflammatory reactions with a single examination, thereby facilitating both diagnosis and treatment decisions.

Neoplasms

Malignant neoplasms

A thorough preoperative evaluation is essential in selecting appropriate operative therapy and for sequencing surgery with available adjuvant treatments [81]. These decisions are based on the anatomical location of the primary tumor(s) and the presence or absence of metastatic disease. An additional element in rectal cancer patients is assessment of transmural penetration and the distance from the anal verge. Although EUS excels in local staging of accessible lesions, CT provides the most efficient methodology by which all these components can be comprehensively evaluated. Results from a multiinstitutional study reported 74% accuracy for CT assessment of wall invasion; the sensitivity in evaluating lymph node metastases was only 48%. CT demonstrated 85% accuracy and 97% specificity in detection of liver metastases [82].

The detection of colorectal carcinoma with CT depends on the degree of colonic distention and adequacy of the preparation [83]. In the unprepared bowel, all but the largest lesions will be overlooked. Adenocarcinoma appears as a soft tissue attenuation focal mass, which focally or circumferentially thickens the bowel wall (Fig. 131.9). Using air insufflation and a colon preparation, detection rate was increased from 68% to 95% [83].

Transmural invasion is suggested when streaky, spike-like, soft tissue attenuation changes are seen in the peritumoral fat. The main limitation of CT staging is under-staging the extent of local disease, with few over-staging problems. CT is useful in identifying complications of colorectal cancer such as perforation, obstruction, as well as synchronous lesions. These may be clinically silent but their presence impacts long-term survival. The negative predictive value of CT has been estimated at 90% [84]. Although most surgeons have come to rely on preoperative CT to survey the abdomen and pelvis, there is no routine agreement regarding widespread use [85].

Figure 131.9 Axial intravenous and oral contrast-enhanced multidetector computed tomography image demonstrates a subtle mass (arrows) in the right colon. Colonoscopy and biopsy revealed adenocarcinoma.

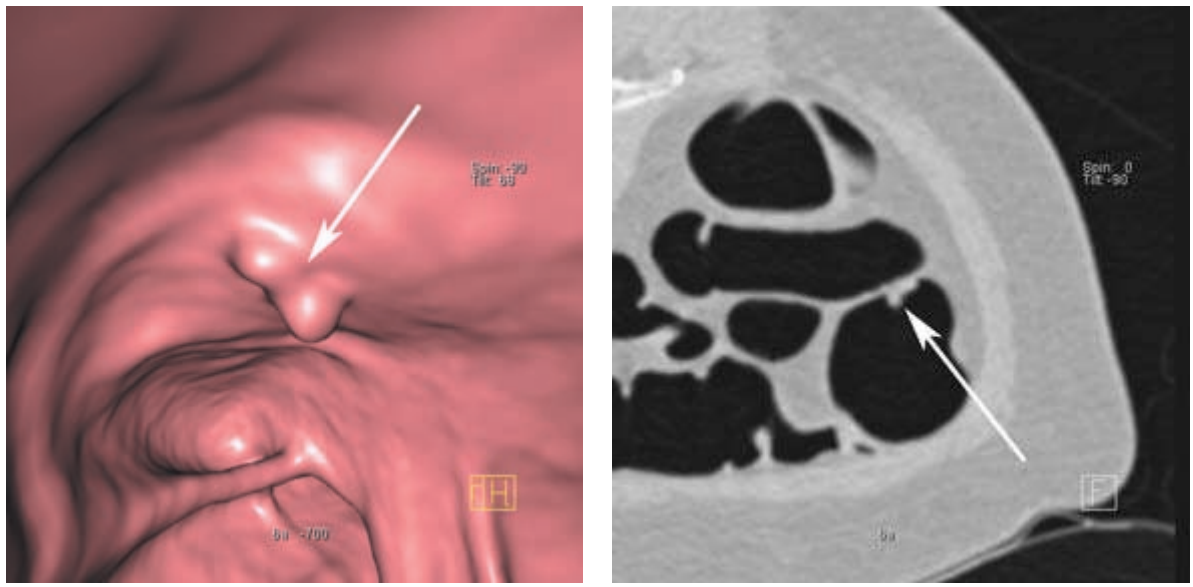
CT plays an adjunctive role in evaluating recurrent disease after abdominoperineal resection or colonic resection. CT is capable of demonstrating locally recurrent pelvic masses, lymphadenopathy, and liver metastases. Locally recurrent rectal cancer appears as spiculated densities or a fibrous soft tissue layer around the rectum; when the abnormal tissue is attached to the perirectal fascia or extends beyond it, positive predictive value is high [86]. CT or ultrasound imaging combined with colonoscopy have been shown to detect recurrent colon cancer at a lower total cost per patient than routine use of carcinoembryonic antigen, physical examination, and chest X-ray [87].

CT colonography (virtual colonoscopy)

The ability of helical CT to acquire a continuous volumetric dataset allows application of rendering techniques that result in display of the lumenal (mucosal) surface of the colon, a process known as CT colonography or virtual colonoscopy [88] (Fig. 131.10). The method was first introduced in the mid 1990s but was initially limited by slow scanner speeds, relatively thick 3–5-mm slice collimation, and the lack of easy-to-use software. The introduction of thin-slice MDCT now allows submillimeter collimation, which is crucial to high-quality virtual colonoscopy. Scanner speeds have also improved.

The study requires adequate bowel cleansing, which can be achieved with any of the traditional conventional colonoscopy agents such as polyethylene glycol, sodium phospate or magnesium citrate. For CT colonography, the patient also drinks special tagging agents such as dilute barium suspensions and iodinated solutions. The tagging agents mix with the residual solid and liquid stool, which will then appear "white," easily distinguishing stool from soft tissue polyps. Sophisticated software is now available from a variety of manufacturers and allows time-efficient review of the datasets using a combination of MPR and endolumenal fly-through views that simulate conventional colonoscopy. Most expert readers can review a study in 15 min.

The largest study of virtual colonoscopy in a screening population was published by Perry Pickhardt and colleagues in 2003 [89]. This study included 1233 symptomatic adults who underwent both CT colonography and conventional colonoscopy in the same day. For adenomas more than 10 mm in size, the sensitivity for virtual colonoscopy was 94% compared with 88% for conventional colonography. Per-patient sensitivity for virtual colonoscopy was 96% [89]. These results were very encouraging, especially when one considers that the study included only four- and eight-detector row CT



(a) (b)

Figure 131.10 (a) Endolumenal fly-through view from virtual colonoscopy shows 8-mm polyp (arrow). **(b)** Axial prone image from virtual colonoscopy demonstrates an 8-mm polyp (arrow) in the right colon.

scanners. A smaller screening virtual colonoscopy study of 615 patients was subsequently published with disappointing results [90]. This study showed less than ideal results for virtual colonoscopy, but included relatively inexperienced radiologists as readers [90]. It is clear that virtual colonoscopy requires training, and the American College of Radiology (ACR) has recommended training of at least 50 cases for radiologists.

Results of the multicenter ACRIN National CT Colonography trial, which was completed in late 2006, are pending. This trial included over 2600 patients from 15 institutions. Inexperienced readers (less than 500 cases in practice) were required to undergo training and pass a competency test before joining the study. All scans were performed on either 16- or 64-slice scanners such that data analysis will reveal the utility of currently implemented CT technology.

Nonneoplastic diseases

Diverticulitis

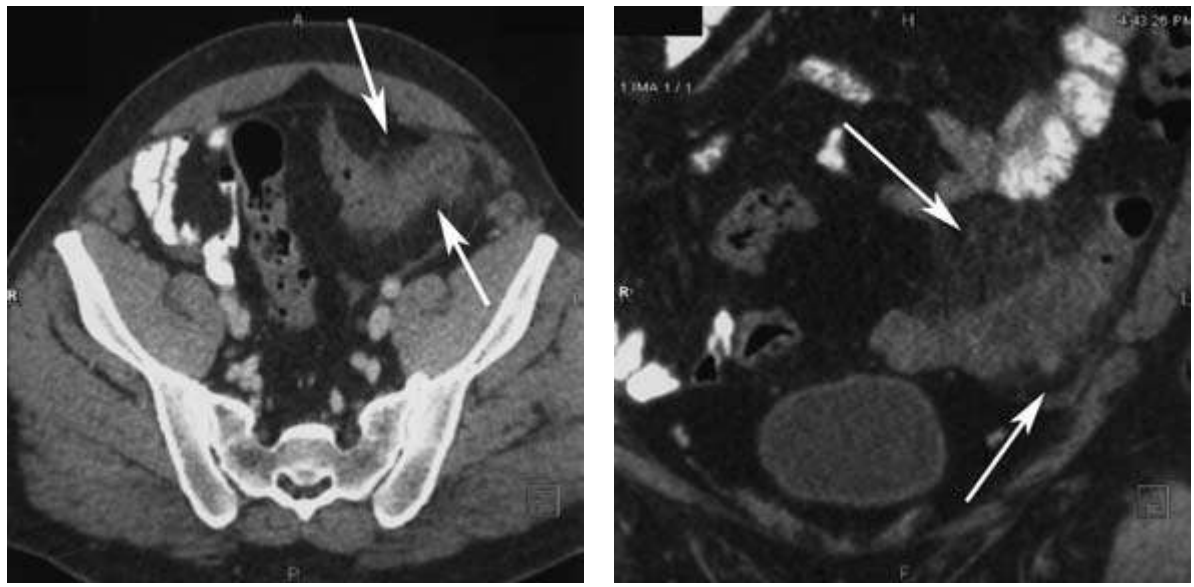
In those patients in whom the clinician requires imaging clarification or confirmation of a diagnosis of diverticulitis, CT is the primary and, in most instances, the only necessary test [91,92] (Fig. 131.11). Although CT and contrast enemas have equal accuracy in establishing the diagnosis, CT is clearly superior in determining whether abscess is present and in documenting the extent of the inflammatory mass [93]. When abscess is present, resection of the perforated segment with anastomosis is desirable; however, if the field is contaminated by pus, temporary colostomy may be necessary. Percutaneous abscess drainage reduces surgical morbidity and allows a primary anastomosis [94].

CT is excellent in diagnosing diverticulitis as a cause of fistulae arising from the colon, particularly those to the urinary bladder or the vagina [95]. The major limitation of CT is the inability to assess the integrity of the colonic mucosa, and perforated neoplasm may be overlooked. This is estimated to be a problem in approximately 10% of cases [96]. In these individuals, lumenal evaluation with either endoscopic or contrast radiography is indicated [97]. Of note, underlying colon cancers may also result in an increased number of pericolonic nodes as compared with diverticulitis, and this has been reported as a potentially useful finding to suggest the possibility of an underlying neoplasm [98,99].

Appendicitis

CT has emerged as the primary imaging modality in patients suspected of acute appendicitis [100]. Outcome-based studies have documented an impact on negative appendectomy rates in patients who have undergone CT [101,102]. CT has similar accuracy to ultrasound, although the negative predictive value of CT is significantly higher [103]. Local expertise and body habitus (thinner patients for ultrasound and larger patients for CT) should dictate the use of ultrasound or CT.

The diagnosis is dependent on visualizing an abnormal appendix (Fig. 131.12). The collapsed normal appendix usually measures 6 mm or less in cross-section; when distended, the appendix can measure up to 10 mm in diameter in normal patients [104]. A dilated appendix, as an isolated finding, can be suspicious but is not necessarily diagnostic of acute appendicitis. Defining criteria include a dilated thick-walled tubular structure that enhances following intravenous contrast [105]. If present, periappendiceal inflammation, phlegmon,



(a) (b)

Figure 131.11 (a) Axial oral and intravenous contrast-enhanced multidetector computed tomography image reveals thickening of the sigmoid colon with pericolonic stranding (arrows) compatible with diverticulitis. **(b)** Coronal multiplanar reconstruction shows extensive inflammation and minimal fluid in the pericolonic fat (arrows) typical of diverticulitis.

Figure 131.12 Axial intravenous contrast-enhanced multidetector computed tomography in a patient with right lower quadrant pain demonstrates an enlarged fluid-filled appendix (arrow) with moderate periappendiceal inflammation, compatible with acute appendicitis.

or abscess may also be identified. However, periappendiceal inflammatory changes are not always present in the setting of appendicitis [106], or may be minimal, even when the appendix is found to be gangrenous at surgery. Although it is possible to recognize findings of acute appendicitis on unenhanced studies [107], we strongly believe that intravenous contrast and oral contrast and acquisition using thin sections [108] are essential in patients referred for evaluation of right lower quadrant pain. In urgent cases, rectal contrast can be substituted for oral contrast.

Colitis

CT is useful in patients with nonspecific abdominal symptoms, directing the workup to an unsuspected colitis [109]. In an attempt to suggest a specific diagnosis, one evaluates mural thickness and enhancement pattern, distribution of bowel involvement, and associated mesenteric and small-bowel disease.

The mean colon wall thickness in Crohn's colitis, for example, is significantly greater than in ulcerative colitis [110]. Exclusive involvement of the right colon and small bowel is more frequent with Crohn's and infectious colitis rather than ulcerative colitis. Abscess is associated almost exclusively with Crohn's colitis rather than ulcerative colitis. In Crohn's colitis, CT has additional use in that pericolic disease is frequent. CT can also evaluate for perianal complications [111].

CT is not always needed in the workup of patients with ulcerative colitis, but may be obtained in symptomatic patients. On CT, the disease is recognized by a continuously thickened segment of colonic wall, displaying a target-like appearance. Fat hypertrophy in the presacral region is seen. In patients with long-standing colitis, fat deposition within the wall may occur [110].

An accordion-like appearance of the colon can be seen in a variety of acute colitides but was first described in pseudomembranous colitis (*Clostridium difficile*) [112]. In the hospital setting, CT may be the first test to suggest the possibility of pseudomembranous colitis in debilitated patients with suspected infection. The condition can involve the entire colon or may be segmental (Fig. 131.13). CT findings in

Figure 131.13 Axial intravenous and oral contrast-enhanced multidetector computed tomography demonstrates marked diffuse colonic thickening compatible with pseudomembranous colitis.

pseudomembranous colitis include dramatic wall thickening. CT can be used to help guide patient management, as some patients will ultimately require colectomy [113].

Solid organs of the gastrointestinal tract

Liver

Techniques

The CT technique used to examine the liver is based on whether the study is to be performed to exclude the presence of hepatic disease, determine the precise disease burden for preoperative planning, or characterize masses previously identified on other imaging studies. Separating these imaging tasks allows the radiologist to optimize contrast administration, acquisition speed, and timing.

Hepatic parenchymal enhancement following a rapid bolus infusion of intravenous contrast is characterized by three distinct phases: a hepatic artery phase, a portal phase, and an equilibrium phase. The hepatic artery phase, useful in detection of hypervascular lesions including hepatocellular carcinoma (HCC), lasts between 30 and 50 s following the initiation of injection. All primary hepatic lesions are supplied by the hepatic artery, and it is during this time that hypervascular lesions are brightly enhanced, because background parenchyma has not reached peak enhancement. Rapid scanning with MDCT enables subdivision of the arterial phase and the ability to acquire data during the early portion or the late portion of the arterial phase. The early arterial phase is used for arterial vascular imaging, and can be timed with a test-bolus technique or bolus tracking to correspond to peak aortic enhancement. The late arterial phase has been shown be superior to the early arterial phase for identification of hypervascular HCCs [3,114–116]. At approximately 70–90 s, peak hepatic enhancement is achieved (portal phase; also called the hepatic venous phase [3]). Peak enhancement is maintained for approximately 30 s and then slowly decays. For many liver tumors, including hypovascular metastases, scanning is performed during this portal phase because this is the time when there are maximal differences in attenuation between normal liver parenchyma and the tumor. Delayed scanning during the equilibrium phase usually results in decreased lesion conspicuity and is therefore avoided when imaging for hypovascular metastases. However, a delayed phase acquisition (180s) is used by some in conjunction with other acquisitions for HCC imaging, as a comparative investigation revealed that a small percentage of HCCs, which are hypovascular, increase in conspicuity during the delayed phase in cirrhotic patients [117].

Unenhanced scans may be useful in patients with suspected hypervascular lesions, including metastases from renal, thyroid, breast, islet-cell, and carcinoid tumors and HCC [118]. The clinician should communicate a specific clinical history or suspicion of these diagnoses to the radiologist for proper examination [119].

With MDCT, images of the entire liver can be acquired in a single breath-hold. Using 16- or 64-slice CT, the liver can be scanned in under 15 s or less than 5 s, respectively. This allows the arterial acquisition timing to be tailored to the clinical question. Early arterial acquisitions are used to perform three-dimensional CT angiography for tumor mapping and preoperative determination of arterial anatomy, while late arterial acquisitions are optimal for HCC detection. Following hepatic arterial acquisition, the liver can be immediately rescanned to capture peak parenchymal enhancement during the portal vein phase [120]. Imaging for hypovascular metastases typically requires only the portal venous phase. Combination of arterial and portal phases, referred to as dual-phase helical CT [121], is used for hypervascular lesion detection, or an early arterial phase can be combined with portal phase if CT angiography is indicated. A three-phase study can include unenhanced scans [118,122]. In order to achieve a diagnostic arterial-phase image, contrast must be power injected at a rate exceeding 4 mL/s. High infusion rates have been shown to increase both visualization of small hepatic arterial branches and conspicuity of HCC [116,123,124].

Intravenous, portal-phase, contrast-enhanced, single-detector helical CT was shown to be sensitive (91%) for detecting malignant hepatic tumors measuring larger than 1 cm, but relatively insensitive (56%) for tumors smaller than 1 cm [125]. Technological advances have not succeeded in dramatically improving sensitivity for small metastases. A mean sensitivity of 31% has been reported for lesions smaller than 1 cm in an investigation using four-slice or eight-slice MDCT [126], increasing to 41% with 16-slice technology

[127]. The availability of multislice CT does facilitate simultaneous lesion characterization and detection, because both phases of hepatic enhancement are accessible from the same dose of intravenous contrast [128]. Additionally, the high-quality three-dimensional display resulting from isotropic datasets improves localization.

If documenting the exact number and location of liver metastases is imperative, such as before contemplating resection of apparently isolated metastases, angiographic-assisted CT improves sensitivity. CT portography (CTAP) has the highest reported sensitivity for detecting focal liver masses, approaching that of intraoperative ultrasound, but is considerably more invasive [129]. The improved sensitivity may lead to better patient selection for resection of metastases [130]. The volumetric dataset from helical and particularly multislice acquisitions allows angiographic and three-dimensional volume-rendered data displays; these aid in lesion localization and surgical planning [131]. Improvements in contrast sensitivity afforded by MRI, with similar sensitivity for identifying colorectal metastases compared with CTAP [132], have significantly reduced the use of CTAP in most clinical practices compared with the late 1990s. For detection of HCC larger than 1.0 cm in cirrhotic patients, combined approaches using MDCT and MRI resulted in similar detection rates to those of CTAP with CTHA (CT during hepatic arterigraphy) in one investigation [127]. In our practice, which includes a large number of transplant patients in whom hepatic surgery is dependent on accurate demonstration of entire tumor burden, CTAP is almost never performed.

Diffuse diseases

Fatty infiltration

Fatty infiltration of the liver may occur in a diffuse or focal distribution. This intracellular fat lowers the CT attenuation of the liver, reversing the relation to splenic density as judged on unenhanced scans [133]. Fatty infiltration of the liver is best appreciated on noncontrast scans and may be difficult to detect accurately once intravenous contrast has been administered. Measurement of the liver attenuation only on unenhanced CT has been shown to be best for prediction of pathological fat content [134].

Focal fatty deposition can be confused with hepatic space-occupying lesions. Lack of displacement of adjacent vessels, location, and rapid change with time can be helpful in differentiation of focal fat from a true mass lesion [135]. When problematic, the presence of focal fat can be established by biopsy or "chemical shift" MRI [136].

Cirrhosis

Characteristic CT features can be seen in patients with advanced cirrhosis. The right lobe and medial segment of the left lobe are often decreased in size, but the lateral segment of the left lobe and caudate lobe are enlarged [137] (Fig. 131.14). Additionally, cirrhotic livers demonstrate irregular nodular liver margins, with apparent widening of the intrahepatic fissures and the porta hepatis as the liver retracts and shrinks. Associated changes of portal hypertension, including varices and venous collaterals, commonly occur. Enhancement of the cirrhotic liver is similar to normal liver during the arterial phase, but may be slightly less dense during the portal phase [138]. A normal CT does not exclude the diagnosis of cirrhosis as fibrosis may still be severe on liver biopsy.

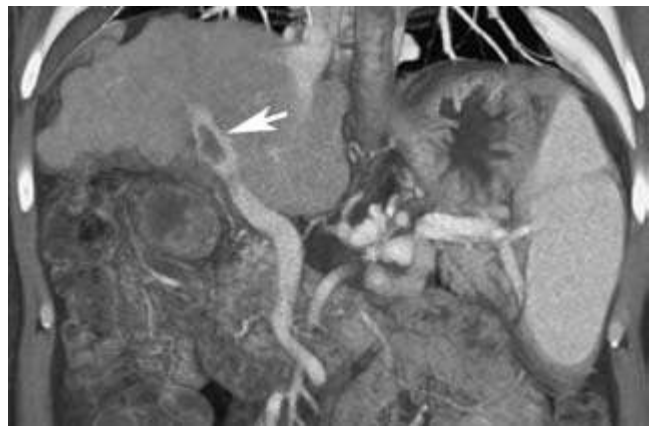


Figure 131.14 Coronal volume-rendered image from intravenous contrast-enhanced computed tomography demonstrates a small shrunken nodular liver compatible with cirrhosis. Splenomegaly reflects portal hypertension. Thrombus is present in the intrahepatic portal vein (arrow).

The distorted liver appearance caused by cirrhosis obscures many small tumors on CT. Considering the worldwide frequency of hepatitis C, the number of cases is expected to increase. Using conventional CT imaging, Miller and colleagues [139] reported detection of HCC in only 68% of cirrhotic patients with tumor. Incorporating arterial-phase helical CT scanning improves detection of HCC tumor nodules over portal venous-phase imaging alone [140]. Correlation with subsequent transplant specimens has shown that arterial-phase imaging for detecting HCC in cirrhotic patients is a significant improvement over conventional or portal venous contrast imaging. However, using multiphasic MDCT, sensitivity for small lesions remains low (47%–57% for those ≤ 1 cm) [141]. The addition of a delayed phase (after portal venous) to a biphasic acquisition protocol has been shown to improve diagnostic performance for identifying lesions 2 cm or less in size in cirrhotic patients [142]. Nonetheless, despite the combination of arterial, portal, and delayed acquisitions, the prospective and retrospective sensitivity of MDCT for detection of HCC lesions in cirrhotics has been reported to be 64% and 73%, respectively, and false positives resulted in disease overestimation [141].

Comparison of MDCT with MRI has produced variable results, with individual studies reporting superior sensitivity of MDCT [143] or MRI [144] for lesions 1 cm or less in size. However, the accuracy of the two technologies was comparable in both studies [143,144]. While the significance of dysplastic

nodules remains a controversial issue, it is known that CT is poor at detecting dysplastic nodules in cirrhotic livers [145].

Other diffuse diseases

Iron deposition and hemochromatosis, whether primary or secondary, increase the attenuation of the liver and spleen on CT [146]. CT is valuable in evaluating long-term iron deposition in the reticuloendothelial system from chronic transfusion therapy. The hepatic density may also be increased in patients receiving amiodarone therapy, those with glycogen storage disease, and following gold therapy for rheumatoid arthritis. Despite high serum copper levels, CT of the liver does not show significant elevations over normal attenuation measurements in patients with Wilson disease.

Masses

Cysts

Cysts appear as homogeneous, low-attenuation (0–10 HU) masses having smooth sharp margins without a perceptible wall. Cysts less than 1 cm in size may cause concern, particularly in patients with cancer, because of artifactually higher attenuation measurements from volume averaging. Comparison with prior studies is necessary [147], and MRI can be more specific for confirming that a small lesion is a cyst as opposed to a solid mass [148].

Benign neoplasms

Hepatic hemangioma is the most common tumor of the liver. Taking advantage of the improved contrast and temporal sensitivity of helical CT, hemangioma may be accurately and definitively diagnosed in over 90% of cases by CT alone [149]. On noncontrast studies, hemangiomas are usually hypoattenuating relative to normal liver. Their density is similar to that of the unenhanced blood vessels. After intravenous contrast administration, the lesions demonstrate peripheral globular enhancement and a centripetal fill-in pattern (Fig. 131.15). The enhancing areas have attenuation values identical to that of the aorta. Globular enhancement, isodense with the aorta, is 67% sensitive and 100% specific in differentiating cavernous hemangiomas and hepatic metastases. When demonstrated, no further workup is needed. However, hemangiomas can have atypical CT appearances [150]. For example, small hemangiomas can be hypoattenuating, especially on early-phase scans and may demonstrate no identifiable enhancement. Alternatively, due to arterioportal shunting, hemangiomas can show rapid enhancement [150]. Atypical hemangiomas can therefore mimic metastases in cancer patients.

Hepatic adenomas and focal nodular hyperplasia (FNH) are vascular lesions that can present with a variety of CT appearances that are often indistinguishable, unless there is hemorrhage (indicating adenoma). Both lesions usually demonstrate homogeneous increased attenuation during the hepatic arterial phase of enhancement, rapidly becoming isodense or slightly hypodense as peak portal enhancement is achieved. The identification of a central, stellate, low-density area is helpful in the diagnosis of FNH, but is seen only in a minority of patients [151] (Fig. 131.16). The introduction of MDCT has resulted in better visualization of the angioarchitecture of these lesions, useful for diagnosis [152]. CT angiography evaluation of FNH can reveal the presence of large feeding arteries and draining veins and helps to better visualize the pseudocapsule and central scar [152]. These features can be used to help distinguish FNH from hepatic adenomas on CT.

Figure 131.15 Intravenous contrast-enhanced axial multidetector computed tomography demonstrates a 2.5-cm mass (arrows) in the right lobe of the liver with peripheral nodular enhancement characteristic of a hemangioma.

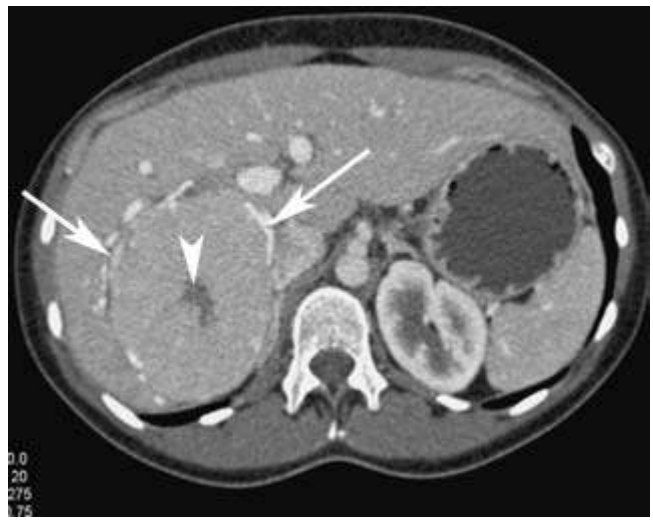


Figure 131.16 Axial intravenous contrast-enhanced multidetector computed tomography during portal venous phase of enhancement demonstrates a large mass in the right lobe of the liver (arrows), with a central scar (arrowhead). This is a typical CT appearance of focal nodular hyperplasia.

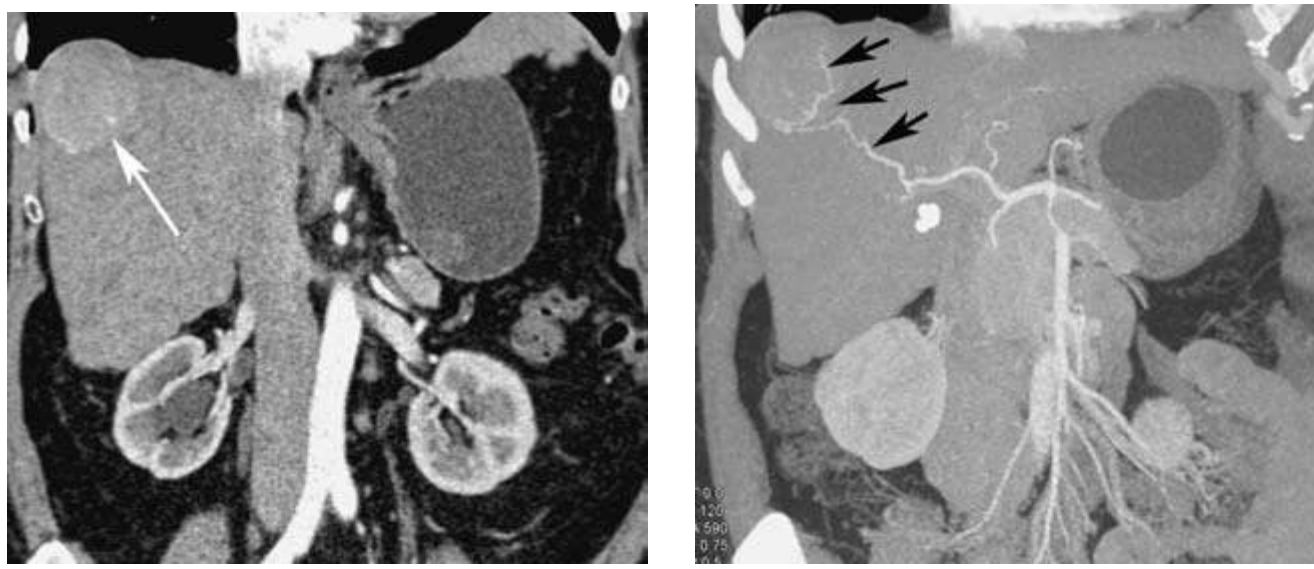


Figure 131.17 (a) Coronal intravenous contrast-enhanced multiplanar reconstruction from an arterial phase acquistion demonstrates a 4-cm enhancing hepatoma (arrow) in the dome of the liver. The liver is shrunken, compatible with cirrhosis. **(b)** Coronal maximum intensity projection demonstrates the arterial vessel (arrows) feeding the hepatoma.

Malignant neoplasms

HCC can appear on CT as a focal solitary mass, a multifocal process, or a diffuse infiltrating process. These lesions appear hypodense or isodense to normal liver on noncontrast-enhanced images. Following contrast administration, smaller masses are typically hypervascular on arterial phase acquisitions [142,153] (Fig. 131.17). Hypovascular HCC, which accounts for a small percentage of these tumors, becomes increasingly conspicuous as lowdensity lesions on portal or delayed-phase acquisitions [142,153]. However, the imaging appearance varies according to histology, with most (93%) moderately or poorly differentiated lesions demonstrating hypervascularity during the arterial phase, as opposed to 44% of well-differentiated HCCs [142]. During the portal phase, regardless of histology, smaller lesions are often hypodense, or may be isodense (and are rarely hyperdense) [142,154]. However, if a lesion is hyperattenuating on arterial phase and isodense on the portal phase, possible etiologies are broad. Differential diagnosis for lesions that are apparent exclusively during the arterial phase includes HCC, dysplastic nodule, hemangioma, FNH, arterioportal shunt, and aberrant venous drainage, among others [153].

Characteristic appearances of HCC include fibrous capsules, fibrous septa, and mosaic appearances. The fibrous capsules of HCCs are readily detected by CT and MRI [155]. The mosaic pattern is a result of variable tissue composition of HCC. In most patients, enhancing nodules indicate viable tumor cells, and low-attenuation areas represent necrosis, fibrosis, or hemorrhage [156]. With the increase in prevalence and incidence of hepatitis B and C viruses worldwide, interest in imaging surveillance of these high-risk populations is gaining momentum. Sonography is not adequately sensitive in this role [157], whereas CT and MRI are the imaging modalities of choice.

There is considerable interest in regional therapy for HCC, including chemoembolization and radiofrequency ablation. CT imaging aids in localization of foci of tumor for which angiographic administered chemoembolization can be applied [158]. Smaller lesions are amenable to percutaneous alcohol ablation [159].

Liver metastases demonstrate a variety of CT appearances. The majority typically appear hypodense compared with adjacent enhanced liver parenchyma on portal renous phase images, although vascular lesions can appear hypodense, isodense, or hyperdense, depending on the timing of contrast administration and scanning. Hypervascular metastatic lesions, as found in renal cell carcinoma, islet-cell tumors, leiomyosarcoma, and thyroid carcinoma, may be demonstrated on noncontrast-enhanced CT [118]. Metastatic lesions do not have a pathognomonic appearance and can be simulated by HCC, hemangiomas, abscesses, complicated cysts, or hematomas.

Abscesses

The diagnosis of liver abscess depends heavily on radiological imaging, particularly ultrasound and CT [160]. CT has become the screening procedure of choice for detecting pyogenic liver abscesses, with detection rates as high as 97%. The typical CT features of a pyogenic abscess include a mass hypodense to normal liver parenchyma, often with a contrast-enhanced rim. Gas collections are seen in approximately 20% of cases; internal septations may be present. The mass is usually of higher density than a simple cyst, but overlap in these densities can make it difficult to differentiate a simple cyst from an abscess [161]. Some malignant lesions

can simulate liver abscess. Clinical correlation and percutaneous aspiration are necessary to differentiate the entities.

Fungal abscesses usually present on CT as multiple, small, low-density, nonenhancing lesions spread throughout the liver and spleen. Although CT is sensitive for detecting these lesions, active disease can evade CT visualization [162]. Almost always caused by *Candida albicans*, biopsy is the only way to establish the specific etiological diagnosis.

An amebic abscess appears similar to a pyogenic lesion, as a low-density mass, usually solitary, but with 20% of patients demonstrating multiple lesions [163]. Echinococcal infection (i.e., hydatid cysts) also appears as low-density cysts, often with internal septations and daughter or inclusion cysts, creating the appearance of a cyst within a cyst. Calcification may be seen in the cyst wall or septations.

Vascular diseases

Increasing awareness of imaging findings secondary to altered hepatic perfusion is the direct result of growing use of helical CT and improvements in technology that have facilitated the synchronization of contrast infusion with data acquisition. Hepatic perfusion disorders are related to obstruction of arterial or venous flow, focal liver lesions, inflammatory disease, or portosystemic shunts. MDCT, especially with three-dimensional imaging, allows comprehensive evaluation of the hepatic vasculature.

Portal vein

Portal vein thrombosis can be detected on CT by visualizing clot within the portal vein, accompanied by peripheral enhancement from opacification and thickening of the vasovasorum (see Fig. 131.14). Chronic extensive collateral formation involving the porta hepatis is called cavernous transformation and is readily visible on MDCT. Intrahepatic portal vein thrombi produce slow flow peripherally in the liver, resulting in paradoxical hyperattenuation in the periphery related to increased hepatic arterial supply. The changes appear similar whether caused by a primary vascular abnormality with intrinsic thrombosis or secondary tumoral obstruction of the portal venous system, such as HCC.

Zonal perfusion abnormalities may be seen in radiation hepatitis. The radiated liver demonstrates decreased perfusion in the portal venous phase. In fatty livers, the radiated portions appear to have normal attenuation because the damaged hepatocytes cannot undergo fatty metamorphosis [164]. Similar changes may be seen in hepatic parenchyma surrounding masses that alter the regional portal vein flow.

Hepatic veins

CT reveals a variety of findings in hepatic venoocclusive disease (Budd–Chiari syndrome). Because of their noninvasive nature and optimal demonstration of vessel morphology and flow without the use of intravenous contrast, MRI and Doppler ultrasound are preferred for screening examinations of suspected patients. Noncontrast-enhanced CT findings include hepatomegaly with a hypodense parenchyma that spares the caudate lobe and perihilar regions of the left lobe. After intravenous contrast administration, the liver parenchyma demonstrates a mottled enhancement pattern. Initially, the greatest contrast enhancement is seen in the caudate lobe and the periportal regions of the left lobe, but a reversal of this pattern is seen on later images. Delayed images generally show a homogeneous enhancement pattern of the liver. Although direct visualization of thrombus within the hepatic vein or inferior vena cava can be demonstrated, in most cases the hepatic veins are not well seen and their nonvisualization may be the clue to the diagnosis. A similar mottled enhancement pattern is present in patients with chronic passive congestion, regardless of etiology, although, in this instance, the hepatic veins are patent [165].

Hepatic arteries

MDCT and CT angiography is playing an increasing role in evaluation of the hepatic vasculature. Contrast-enhanced CT with three-dimensional imaging can visualize both the intrahepatic and the common hepatic arteries and is useful for a variety of indications. For example, CT is helpful for identifying vascular anatomy and anatomical variants in both liver donors and recipients. CT is also useful for detecting vascular complications after liver transplant, which may include infarcts, stenosis, occlusion, or pseudoaneurysm of the hepatic artery [166].

Pancreas

Pancreatic CT requires thin collimation (preferably submillimeter), overlapped reconstruction, bolus injection of iodinated contrast material (best performed with a power injector), and rapid scanning to improve tumor detection, visualize relationships of peripancreatic vessels, and display findings three-dimensionally for optimized surgical and therapeutic planning. Properly timed contrast-enhanced studies will image the pancreas when its parenchyma is maximally enhanced and the peripancreatic vessels optimally opacified [167]. Accuracy in depicting variations in peripancreatic arterial anatomy, as well as clear depiction of vessel encasement, has resulted in the elimination of invasive angiography [168,169]. MDCT facilitates the use of multiphase imaging. For pancreatic CT, arterial, pancreatic or portal phases can be acquired [2,170]. The early arterial phase is used for CT angiography, because the arteries are optimally enhanced. However, maximum parenchymal and venous enhancement during the pancreatic and portal phases facilitates lesion detection and also aids in the determination of resectability [2].

Anatomical variants

Pancreas divisum is the most common congenital anomaly of the pancreas. Although this often goes unrecognized on CT, the pancreatic head can appear enlarged and simulate a mass lesion. Occasionally, the dorsal duct can be visualized

Figure 131.18 Oral and intravenous contrast-enhanced axial computed homography demonstrates pancreatic tissue (arrows) surrounding the second portion of the duodenum (arrowhead), compatible with annular pancreas.

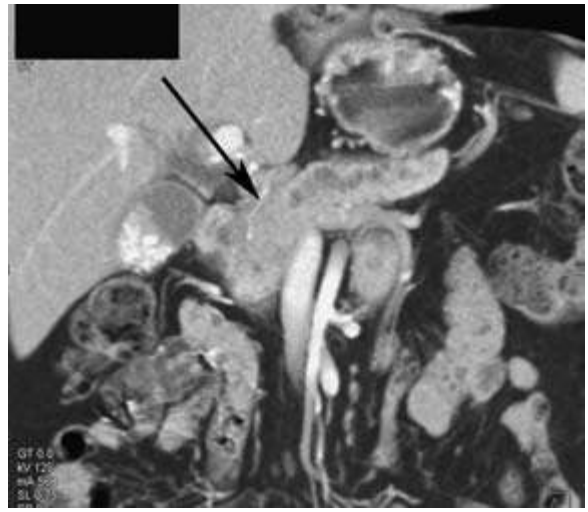


Figure 131.19 Intravenous contrast-enhanced coronal volume-rendered image demonstrates 1.5-cm pancreatic mass (arrow) with distal pancreatic ductal obstruction. At surgery this was an adenocarcinoma.

emptying into the accessory duct, confirming the diagnosis. In a study of 77 patients using four-detector MDCT with 3.2-mm slice thickness and endoscopic retrograde cholangiopancreatography (ERCP), pancreatic ductal anatomy was visualized adequately in 95% of patients [170]. ERCP detected pancreatic divisum in 10 patients, nine of whom were also identified on MDCT. For the radiologists, there were a total of three false-positive diagnoses [170]. Submillimeter MDCT, which is now possible with current 64-slice MDCT and three-dimensional imaging with minimum intensity projection, may allow more reliable visualization of aberrant pancreatic duct anatomy. However, magnetic resonance cholangiopancreatography (MRCP) may still be a more sensitive noninvasive method for diagnosis [171].

Agenesis of the dorsal pancreatic duct is identified with CT by visualizing only the head of the pancreas without a body or tail portion, and the condition may be confused with a mass in the head of the pancreas with distal pancreatic atrophy [172].

Annular pancreas is an anomaly formed during embryogenesis by the ventral anlage of the pancreas as the duodenum rotates, mainly due to hypertrophy of the left ventral bud. According to the fusion pattern of the ventral and dorsal pancreatic ducts, annular pancreas can be divided into three types: type I, divisional annular pancreas; type II, branch annular pancreas; and type III, main duct annular pancreas. Final diagnosis is based on the evidence of ERCP combined with CT. The CT diagnosis is suggested by visualizing a collar of pancreatic tissue surrounding the descending duodenum [173] (Fig. 131.18).

Neoplasms

Adenocarcinoma

The most common CT finding in adenocarcinoma is a focal mass isodense with the pancreas on noncontrast images and hypodense to the pancreas after intravenous contrast administration [174] (Fig. 131.19). This is more obvious using dual-phase helical CT protocols, which are now considered to be standard of care [175]. With improvements in CT resolution, faster contrast administration, and widespread availability of three-dimensional imaging, it is now possible to detect even smaller tumors using current technology. Studies using MDCT and three-dimensional imaging have an accuracy for detection of pancreatic cancer in the 95% range [2,176]. However small tumors less than 2 cm in size may nonetheless be difficult to detect. In a study by Bronstein and colleagues, the sensitivity of triple-phase helical MDCT for the detection of pancreatic masses less than 2 cm in size was only 77% with a specificity of 100% [177].

Even if the mass itself is not well visualized on CT, the presence of secondary findings can be used to infer the presence of an underlying tumor. For example, segmental pancreatic duct obstruction is a crucial finding and may be the only indicator of an obstructing mass. The pancreas distal to the tumor also usually appears atrophic. Lesions located in the pancreatic head will result in obstruction of the common duct as well as the pancreatic duct.

In addition to detecting the primary pancreatic mass, the major role of imaging is the determination of potential surgical resectability. CT is the modality of choice for preoperative staging and for identifying patients for a possible curative resection. Nonresectability is defined as the presence of either major arterial or venous encasement, hepatic (or other distant) metastases, or distant lymph node metastases [178] (Fig. 131.20). Realistically, at the time of initial diagnosis, 50% of patients have distant metastases to the liver or peritoneal surface, and more than 80% of the remaining patients have locally advanced tumors. However, it is the role of CT to

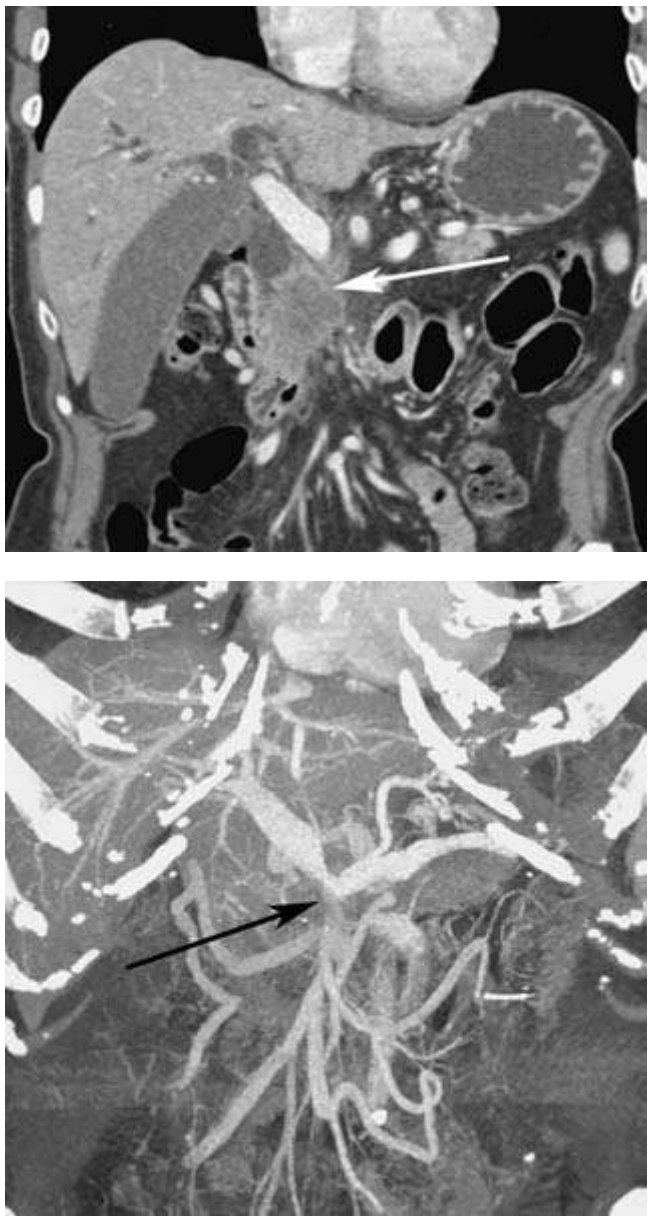


Figure 131.20 **(a)** Intravenous contrast-enhanced coronal MPR image demonstrates a 4-cm mass (arrow) in the head of the pancreas causing common bile duct obstruction. The gallbladder is also distended. Biopsy revealed pancreatic adenocarcinoma. **(b)** Coronal maximum intensity projection demonstrates encasement of the superior mesenteric vein (arrow) with resulting collaterals. The patient was deemed unresectable.

identify that small percentage of patients who might benefit from attempt at curative resection.

CT has been shown to have a high predictive value for unresectability (90%–100%) with a slightly lower predictive value of resectability (76%–90%) [176,179,180]. This is usually due to its inability to detect tiny liver metastases or minimal peritoneal spread [181]. These values are a marked improvement from conventional CT, which yielded accuracy in the range of 44%–73% [182,183]. The key to evaluation of the peripancreatic vasculature is high-resolution thin-collimation scans performed during the arterial and venous phases [184,185]. The ability to visualize the celiac axis, superior mesenteric artery, superior mesenteric vein, and portal vein in three dimensions with a combination of MPR, MIP and volume rendering is essential for detecting vascular encasement. CT angiography has replaced the need for preoperative conventional angiography for this indication.

In addition to assessing potential vascular encasement in patients with pancreatic cancer, CT can also identify adenopathy. Radiologists usually rely on nodal measurements to identify potentially malignant nodes. However, this is not always accurate, as even tiny nodes can harbor malignancy and large nodes can simply be reactive. In a study by Roche and colleagues, 62 patients with pancreatic cancer underwent detailed nodal analysis [186]. Applying a threshold of greater than 10 mm to identify malignant nodes, this study showed a sensitivity of only 14% with specificity of 85% [186]. This resulted in a positive predictive value of 17% and a negative predictive value of 82% [186]. Therefore, when determining resectability of ductal adenocarcinoma, CT is not necessarily helpful in the prediction of nodal involvement. Hence, in a patient thought otherwise to be resectable, the presence of peripancreatic lymph nodes should not prevent attempted surgical resection.

CT is the modality of choice for detecting distant metastasis, most commonly to the liver. These typically appear low density on the portal venous phase of the study. There is a size limitation for CT detection of metastasis in the liver. Even normal patients commonly have a few small-density lesions in the liver, thought to represent tiny cysts, hemangiomas, or bile duct hamartomas. Therefore, the presence of a few tiny lesions in the liver would not necessarily indicate metastases. Larger lesions in the liver can be confidently diagnosed as metastasis. However, small liver and peritoneal implants below the resolution of current CT scanners can be seen at surgery [181].

Islet cell tumors

Pancreatic islet cell tumors are neuroendocrine neoplasms that produce and secrete hormones to a variable degree. Depending on the clinical presentation, they can be classified as syndromic or nonsyndromic.

Syndromic islet cell neoplasms have clinical evidence of hormone production and produce a recognizable endocrinopathy. These include insulinoma and gastrinoma, glucagonoma, vipoma, and somatostatinoma. Such neoplasms usually present at a relatively smaller size compared with nonsyndromic neoplasms, because of the symptoms produced by the associated hormone production. Nonsyndromic islet cell tumors typically represent half of all islet cell tumors and present at a larger size than functioning tumors. Nonsyndromic islet cell tumors show no clinical evidence of hormone production, although the tumors are still hormonally active.

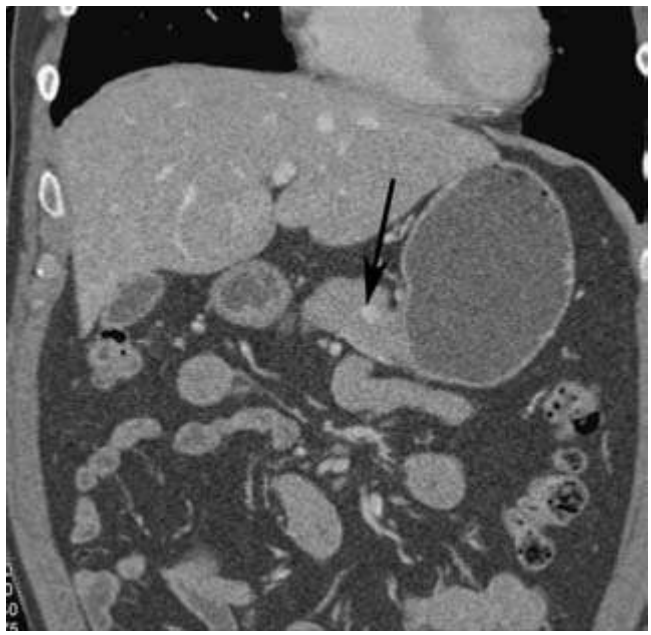


Figure 131.21 Coronal intravenous contrast-enhanced multiplanar reconstruction demonstrates a 5-mm enhancing lesion (arrow) in the body of the pancreas. This was an insulinoma.

MDCT plays an important role in the diagnosis and staging of both syndromic and nonsyndromic islet cell neoplasms [187]. In general, syndromic islet cell tumors are less than 3 cm in size (Fig. 131.21). Typically they are hyperenhancing and usually can best be seen on the arterial phase, although some are better visualized during the portal venous phase [188]. Nonsyndromic islet cell neoplasms tend to be larger at presentation than syndromic islet call neoplasms and are more likely to be cystic or necrotic [189]. Unlike pancreatic adenocarcinoma, these neuroendocrine tumors usually do not obstruct the pancreatic duct.

Multiple radiological techniques can be used for tumor localization, including preoperative and intraoperative ultrasound, EUS, CT, MRI, radionuclide scanning, angiography, and venous sampling [190]. There are conflicting claims as to the relative accuracies of angiography and venous sampling, and many of these investigations are difficult to justify because of their high cost, degree of invasiveness, or lack of precise anatomical information obtained [191]. If surgical resection of a neuroendocrine tumor is planned, intraoperative ultrasound is the best method for detecting nonpalpable masses and discerning the relationship of the tumor to vital adjacent pancreatic ductal anatomy.

The choice of preoperative imaging is more controversial, and depends on the clinical problem, local expertise, and availability of imaging techniques. Ultrasound and contrast-enhanced helical CT using two-phase helical CT in both the arterial and the parenchymal phase are the most commonly used preoperative imaging methods [192] because of their relatively low cost and widespread availability. Radionuclide scanning with ^{111}In-labeled DTPA-D-octreotide may be valuable in patients with equivocal CT scans or those with symptoms of tumor recurrence.

Cystic neoplasms

Cystic lesions of the pancreas are being identified with increasing frequency, due partly to the dramatic improvements in CT resolution. Most of these lesions are discovered incidentally [193]. While many will prove benign, some represent the precursors of pancreatic malignancy. A considerable amount of research has focused on whether imaging modalities (CT, MRI, EUS) can distinguish benign from premalignant or malignant cystic lesions, and the proper management for lesions that are too small to characterize [194,195].

Three cystic masses with distinguishing CT features arise in the pancreas: microcystic or serous cystic neoplasms; macrocystic or mucinous cystic neoplasms; and intraductal pancreatic mucinous neoplasms. The earliest pathological differentiation of microcystic and macrocystic lesions remains a useful framework, but considerable variation and overlap is becoming more obvious [196–198]. Currently, lesion characterization can be performed with MRI, CT, or EUS [194,198,199]. The ability of CT and MRI to accurately differentiate between the different classes of cystic neoplasms is limited, even in the setting of high diagnostic confidence [198,200,201]. Advances in technology have not resulted in a definitive means of pathological characterization through imaging.

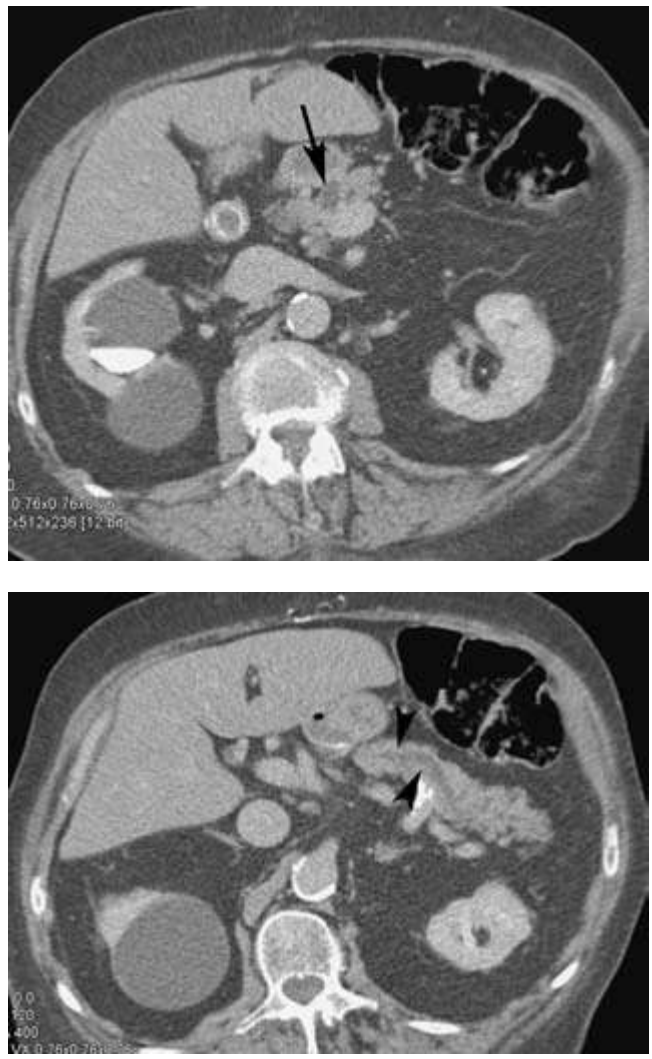
Most older published series intimated that while distinctions can be suggested on CT, surgical management was considered the standard of care [202,203]. However, new algorithms are evolving, and management decisions take into consideration patient characteristics (age, surgical risk, aspiration risk, symptoms), imaging findings, and analysis of endoscopically guided fine-needle aspiration [194,195,198]. Small lesion size (< 4 cm) does not ensure benignity [201], and neither imaging findings nor aspiration can prove that a lesion is benign or malignant in all cases [195,198]. Nonetheless, surgery remains indicated in the appropriate patient for lesions that appear malignant on imaging, for those which are symptomatic, or for those with tumor markers, mucin or malignant cells identified in aspirated fluid [194]. For patients who do not undergo surgery, follow-up is performed with serial imaging.

Cyst size is accurately determined by imaging and aids in distinguishing microcystic from macrocystic lesions [204,205]. Microcystic (≤ 1 cm) lesions are almost always serous. The classic appearance is a focal mass with innumerable cysts interspersed within a dense fibrous honeycomb. However, up to 25% of serous cystadenomas appear as a single cyst [198]. Central calcification occurs in approximately 30% of the lesions [206]. Serous microcystic cystadenoma is considered benign; there are scattered reports of metachronous

adenocarcinoma arising at a distant site in the pancreas of the same patient [207].

Mucinous lesions present as macrocystic (≥ 2 cm) unilocular cysts or as intraductal neoplasms. Peripheral soft tissue nodules, peripheral calcification (in < 5% of lesions), and minimal septation aid in radiological differentiation from microcystic adenoma. Reports of serous macrocystic adenomas reflect the lack of precision of absolute diagnosis from imaging alone [208]. Using thin collimation, mural nodules can often be resolved in malignant lesions. However, it is impossible to definitively distinguish benign from malignant mucinous lesions using imaging criteria. Most authorities recommend needle aspiration of the cyst fluid for preoperative diagnosis [209].

Intraductal pancreatic mucinous neoplasms are becoming increasingly recognized [210] (Fig. 131.22). These lesions have a wide range of clinical presentation, from small foci of duct ectasia with hyperplastic epithelium to frank carcinoma. The most frequent feature of intraductal papillary mucinous tumors is a lobulated multilocular cystic mass located in the uncinate process in contiguity with the dilated main pancreatic duct [211]. Demonstration of a connection with the duct aids in making this diagnosis [199]. In some patients, a bulging papilla and papillary projections in the ducts, which are specific findings, can be visualized on CT. When these lesions are branch type, under 2.5 cm in size, and have no solid components, clinical behavior appears nonaggressive [212]. Main duct-type lesions are often malignant. The possibility of a sequential change from nonpapillary and papillary hyperplasia, via adenoma, to carcinoma in intraductal papillary mucinous neoplasms associated with mucinous ductal ectasia has been speculated [213]. Investigations have delineated MDCT findings that reflect invasion or malignancy, including larger tumors, greater degrees of pancreatic and biliary ductal dilatation, and the presence of a solid lesion in the adjacent pancreatic parenchyma [214,215].



(a)

(b)

Figure 131.22 (a) Axial intravenous contrast-enhanced multidetector computed tomography demonstrates a 1-cm cystic lesion (arrow) in the pancreatic neck. **(b)** Axial image also demonstrates pancreatic ductal dilatation (arrowheads). These findings are very suspicious for intraductal pancreatic mucinous neoplasm.

Pancreatitis

Acute pancreatitis presents clinically and on imaging with a variety of forms. Interstitial edematous pancreatitis and necrotizing pancreatitis are the most frequent clinical manifestations; pancreatic pseudocyst and pancreatic abscess are late complications after necrotizing pancreatitis, developing after 3–5 weeks [216]. CT has become integrated into the evaluation of virtually all patients suspected of acute pancreatitis, and is considered mandatory in patients suspected of pancreatic necrosis [217,218]. CT offers excellent anatomical and morphological representation of the pancreas and peripancreatic tissue [219,220].

The value of CT is based on its ability to show the presence and distribution of pancreas-related fluid collections and the presence or absence of pancreatic necrosis. These two prognostic factors are evaluated in the Balthazar severity index. The presence of acute pancreatitis is graded as normal (grade A), gland enlargement (grade B), enlarged gland with peripancreatic edema (grade C), or single (grade D) or multiple (grade E) fluid collections [221]. Pancreatic necrosis on CT is recognized by regional nonenhancement on contrast-enhanced CT [222,223]. The degree of necrosis, combined with CT grading of the appearance of the gland and fluid collections, has greater prognostic implication than either manifestation alone [224].

The detection of necrosis is one of the primary goals of the imaging study. Intravenous contrast is required to make this assessment. Studies in laboratory rats have shown that intravenous iodinated contrast may increase cellular damage and potentially increase the severity of acute pancreatitis by promoting further necrosis and higher mortality [225]. These

findings are not reproducible in other animal models [226]. Most clinicians believe that the importance of establishing the presence of necrosis justifies the preferred use of intravenous contrast. In addition, pseudoaneurysm formation occurs in 2%–5% of cases. Intravenous contrast and rapid MDCT scanning are required for detection, which is improved with helical CT supplemented by angiographic renderings [70]. Treatment is aimed at drainage of periarterial fluid collections and possibly embolization of the affected vessel.

CT in patients with documented chronic pancreatitis reveals findings in over 90% of cases. These findings, in decreasing order of frequency, include dilation of the main pancreatic duct, parenchymal atrophy, pancreatic calcifications, fluid collections, focal pancreatic enlargement, biliary ductal dilation, and alterations in peripancreatic fat or fascia [227]. Focal mass-like chronic pancreatitis can result in pancreaticoduodenectomy for presumed neoplasm. In a Mayo Clinic series [228], 22 of 603 pancreaticoduodenectomies performed between 1956 and 1990 ultimately proved to be for chronic pancreatitis. When a patient with a pancreatic mass has a history of alcoholism and pancreatitis, and normal serum levels of CA19-9, mass-forming pancreatitis should be kept in mind in the differential diagnosis of a tumor suspicious for pancreatic carcinoma [229].

Pseudocyst formation is a well-recognized sequela of chronic pancreatitis. Percutaneous catheter drainage of both infected and noninfected pancreatic fluid has a reported average success rate of 80% and a complication rate of about 15% [230]. Cyst "maturity" is difficult to diagnose by imaging. A visible wall and lack of change over several weeks indicate the patient is most likely a candidate for successful cystogastrostomy.

Biliary tract

CT is good at documenting the presence and extent of biliary tract malignant disease and, because of its use as a general survey tool in the abdomen, varying forms of bile duct disease are frequently encountered on abdominal CT examinations. Ultrasound and MRI are superior in the evaluation of calculous disease.

Using current CT technology and intravenous contrast enhancement, normal intrahepatic bile ducts may be visualized in over 40% of normal patients; these should not be confused with ductal dilation [231]. The extrahepatic bile ducts are identified in 80% of normal patients and usually have diameters of 4–10 mm [232]. The extrahepatic biliary tree may be up to 10 mm in diameter in patients who have undergone cholecystectomy.

Studies have shown that CT is comparable to ultrasound in its ability to detect biliary obstruction and generally exceeds ultrasound in the ability to predict the level and cause of biliary obstruction, although some controversy still exists [233–235]. If there is a high degree of suspicion for obstruction and if malignancy is strongly suspected, CT is the examination of choice because it allows the staging of disease extent in a standard reproducible fashion with excellent depiction of all regions surrounding the duct and distant sites as well.

Three-dimensional helical CT cholangiography following intravenous infusion of a biliary contrast agent has been reported and rivals direct cholangiography for displaying biliary tract anatomy and pathology [236,237]. This technique, which uses postprocessing reconstructions (maximum intensity and shaded surface display projections), has not achieved widespread use due to the invasive nature of intravenous biliary contrast agents and the availability of noninvasive MRCP. However, advances in postprocessing have led to new display methods for the biliary system (in coronal and sagittal orientations) without cholangiographic contrast. These include two-dimensional MPRs, and specialized three-dimensional rendering using minimum intensity projection and volume rendering [17]. The minimum intensity projection algorithm selects the lowest attenuation voxels along rays projected through the dataset, to display bile-filled ducts in any plane [237]. With volume rendering, the settings can be adjusted to depict the biliary tree as a high-attenuation structure in a three-dimensional display from any viewing orientation [238,239].

Congenital anomalies

Congenital anomalies of the biliary tract are diagnosed definitively with direct cholangiography, but CT and ultrasound can suggest their presence. Congenital anomalies of the gallbladder are rare, consisting mostly of positional variants. The gallbladder can be seen in the left abdomen or in suprahepatic, intrahepatic, and other rare locations. CT can identify these anomalies if the gallbladder is not seen in its usual location at ultrasound.

Congenital cystic diseases of the biliary tree, including choledochal cysts, choledochoceles [240], and Caroli disease, can often be diagnosed by demonstrating the dilated biliary tree with appropriate patterns of dilation. Choledochal cysts appear as focal dilations of the extrahepatic bile ducts that may be mild and simulate a dilated duct or may be as large as 15 cm in diameter [241].

Gallbladder disease

Ultrasound is the modality of choice for evaluating the gallbladder. However, CT may demonstrate gallbladder wall abnormalities. Thickening of the gallbladder wall, although nonspecific, can be a clue to underlying pathology. Unusual right upper quadrant abdominal calcifications can be clarified with CT. CT can document the location of these as stones within the gallbladder calcification, within the gallbladder wall (e.g., porcelain gallbladder), or calcifications extrinsic to the gallbladder. CT is able to demonstrate gallstones in 74%–79% of patients with cholelithiasis; the remainder are isoattenuating with bile and cannot be delineated [242,243]. This lower sensitivity limits the usefulness of CT in screening for gallbladder stone disease.

Neoplasms

Gallbladder carcinoma is usually far advanced when patients are first seen by a physician, and CT findings will reflect these changes. Early gallbladder cancer has been classified on the basis of its CT appearance [244]. In the more commonly seen advanced disease, total replacement of the gallbladder lumen by a soft-tissue-density mass usually invading adjacent hepatic parenchyma characterizes this lesion. Less common appearances include focal gallbladder wall thickening or polypoid intralumenal masses, similar to those seen with ultrasound [245]. The extent of tumor can be depicted on CT in the common regions of spread, including liver, lymph nodes, peritoneal metastases and invasion of adjacent organs such as liver and duodenum.

The gallbladder can be secondarily involved by tumor from other organs. Direct invasion from adjacent organs is usually by means of porta hepatis metastases, most often from gastric or pancreatic carcinoma. Blood-borne metastases to the gallbladder are uncommon but can be seen in patients with melanoma.

Cholecystitis

The most common CT findings in cholecystitis are gallbladder wall thickening (> 3 mm) and cholelithiasis [246]. These findings are neither specific nor sensitive indicators and are also found with gallbladder carcinoma and hyperplastic cholecystosis. Other CT findings suggestive of the diagnosis include increased attenuation of the bile (> 20 HU) and loss of clear definition of the gallbladder wall. Increased attenuation in the adjacent hepatic parenchyma is a useful indicator of acute inflammation [247]. With more advanced cases, streaky inflammatory changes infiltrating adjacent pericholecystic fat can be seen. Air within the gallbladder wall or lumen in the absence of a history of prior enteric anastomosis or sphincterotomy is virtually pathognomonic of complicated emhysematous cholecystitis [248]. A low-attenuation halo around the gallbladder may indicate edema or minimal fluid collections and is a useful clue in differentiating complicated cholecystitis from carcinoma on CT [249].

Bile duct diseases

Neoplasms

Primary bile duct cancers take three forms: mass-forming, periductal infiltrating, and intraductal [250]. Hilar cholangiocarcinoma usually presents centrally near the porta hepatis, producing obstruction of the main hepatic ducts and intrahepatic branches. Peripheral intrahepatic cholangiocarcinoma will present as a hypodense hepatic lesion with peripheral enhancement, biliary dilation, capsular retraction, and contrast enhancement on delayed images [251]. In cirrhotic patients, small peripheral cholangiocarcinomas may be indistinguishable from HCC [252]. Hilar cholangiocarcinomas are generally seen on well-timed arterial phase images, and most will be seen during portal venous phase imaging. Using 16-slice MDCT with a multiphasic protocol, the diagnostic accuracy for hilar cholangiocarcinoma detection (64%) was similar to that of MRI (71%) [253]. A characteristic CT feature of cholangiocarcinoma is the delayed retention of iodine diffusely throughout the tumor during equilibrium phase imaging at 8–20 min following contrast initiation [254]. This retention is seen in tumors with a marked fibrous stroma and can be helpful in delineating the extent of hilar cholangiocarcinoma and in differentiating it from HCC [255]. CT is useful in the preoperative assessment of extent of disease and resectability of cholangiocarcinoma; CT sensitivity for resectability was approximately 60% with single-detector helical CT [256]. In an investigation that used 16-slice MDCT with 0.75-mm detector thickness, Park and colleagues evaluated accuracy for TNM staging of extrahepatic bile duct cholangiocarcinoma. Using axial sections and MPRs, accuracy was 77% for T-staging, 63% for N-staging, and 97% for M-staging [257].

Inflammatory diseases

Acute cholangitis is usually found in patients with underlying biliary tract obstruction. Most patients demonstrate dilated intrahepatic and extrahepatic bile ducts. Suppurative material within the bile ducts may be seen on CT images as high-attenuation debris within the duct lumen. Bile duct wall thickening may be present and appears diffuse and concentric, often demonstrating marked contrast enhancement [232]. Gas can be seen within the biliary tree in cases of infection with gas-forming organisms. Acute suppurative cholangitis can progress to frank liver abscesses, demonstrated on CT as low-attenuation areas in the liver in contiguity with the biliary tree.

The characteristic findings seen by means of cholangiography in primary and secondary sclerosing cholangitis can also be demonstrated on CT images [258]. Intrahepatic duct stenoses, dilated peripheral ducts with no apparent connection to the central ducts, and irregular intrahepatic duct dilation with a beaded appearance are characteristic CT findings of sclerosing cholangitis. CT findings in the extrahepatic ducts in this condition include duct wall thickening and irregularity, as well as duct wall enhancement after intravenous contrast.

Primary sclerosing cholangitis should be considered as premalignant, with more than 15% of patients at risk [259]. Careful search for masses, delayed contrast enhancement, progressive biliary dilation, and thickening of the bile duct wall is required to recognize complicating cancer [260].

Other less common causes of infection can produce characteristic CT images. Recurrent pyogenic cholangitis (also known as oriental cholangiohepatitis) is found in patients of lower socioeconomic status from Asia. The CT findings include marked intrahepatic and extrahepatic bile duct dilation, sharp tapering of peripheral extrahepatic bile ducts with loss of arborization, giant ductal calculi, and intraductal debris.

Organisms such as cytomegalovirus and *Cryptosporidium* can cause inflammation of the biliary tract in patients with

AIDS, resulting in changes on CT, ultrasound, and cholangiography similar to those of sclerosing cholangitis, with bile duct wall thickening, multiple strictures, and duct wall contrast enhancement [261].

Choledocholithiasis

The noninvasive diagnosis of common bile duct stone disease has been simplified with the development of MRCP, which has a sensitivity and specificity similar to ERCP [262]. Most patients with obstructive jaundice will undergo ultrasound, with CT used as a problem-solving modality [263]. Reported CT sensitivity for common duct stone detection varies from 45% to 97% [264–266]. The higher figures (up to 90%) reflect series with selected patient populations, often patients with known dilated ducts in whom it is easier to visualize stones, the highest sensitivity (97%) being reported using submillimeter MDCT [264]. The ability to use thin collimation and overlapping image reconstruction available with volume data acquisition of helical CT scanners improves stone detection rates [267]. However, even with submillimeter MDCT (0.75 and 0.625 mm), small stones are missed [264,268]. Postprocessing with multiplanar display is critical [264,268] (Fig. 131.23). Using MPRs and multiphasic acquisitions with 40 × 0.625 mm detector configuration, Kim and colleagues reported 97% sensitivity and 96% specificity for choledocholithiaisis in 34 patients with stones confirmed by ERCP or percutaneous transhepatic cholangiography [264].

CT is often performed without contrast, as it may improve the recognition of choledocholithiasis to more than 90% [269]. However, a 16-slice MDCT study has shown that combining intravenous contrast-enhanced axial sections with MPRs resulted in diagnostic accuracy comparable to the use of combined pre- and post-contrast CT (both techniques 89% sensitive) [268]. These results suggest that a single intravenous contrast-enhanced acquisition with two-dimensional postprocessing should be considered to obviate the need for multiple acquisitions and the additional radiation exposure to the patient [268].

The CT appearance of common duct stones parallels that of gallstones and depends on the chemical composition of each stone. Densely calcified stones can be seen as high-attenuation structures within the duct lumen, but most duct stones are isoattenuating with soft tissue or bile on CT [266].

Several processes can simulate duct stones on CT, and care must be taken to avoid misdiagnosis. Critically placed pancreatic calculi, oral contrast in an adjacent duodenal diverticulum, residual contrast material from a prior cholangiogram, and papillary ductal neoplasms can simulate choledocholithiasis.

Figure 131.23 **(a)** Axial intravenous contrast-enhanced multidetector computed tomography in a patient with obstructive jaundice shows a subtle filling defect in the common duct (arrow). **(b)** Coronal multiplanar reconstruction better demonstrates the stone (arrow) in the distal common bile duct.

Peritoneum

Many gastrointestinal diseases can have associated peritoneal findings. Many of these are subtle; recognition of abnormalities requires a systematic evaluation of the peritoneal structures based on understanding the spread of disease processes within the abdominal cavity. The majority of ligaments and mesenteries in the abdomen are formed from remnants of the ventral and dorsal mesenteries, which suspend the primitive gut. The pelvic ligaments are mainly formed by reflections of peritoneum over the pelvic organs or structures. The mesenteries and ligaments form the boundaries of the peritoneal spaces. Accurate localization of fluid collections and detection of neoplasms is facilitated when the

pathway of spread through adjacent ligaments and mesenteries is understood [270]. For purposes of radiological diagnosis, the peritoneal cavity can be thought of as an "end organ," in that the response to a pathological process is similar regardless of etiology.

Neoplasms

The radiological appearances of the more common peritoneal abnormalities may be thought of in three basic patterns: solid but relatively well-defined masses; cystic-appearing masses; and ill-defined or infiltrative processes. The most common solid masses to affect these anatomical regions are secondary neoplasms. The various cystic-appearing masses (including duplication cyst, cystic lymphangioma, cystic mesothelioma, teratoma, and loculated ascites) and infiltrating masses (e.g., peritoneal mesothelioma, retractile mesenteritis, desmoid, and carcinoid) must be differentiated on the basis of clinical findings and additional imaging findings (e.g., CT depiction of fat and calcium in teratomas and the radiating appearance of carcinoid) [271]. However, there is overlap in the CT appearance of inflammatory and neoplastic disease, which may necessitate biopsy for definitive diagnosis. Examples include retractile mesenteritic fibrosis (a form of sclerosing mesenteritis) and carcinoid tumor, which can be indistinguishable on CT [272]. Similarly, tuberculous peritonitis can be difficult to distinguish from peritoneal carcinomatosis.

Peritoneal carcinomatosis is a form of metastatic disease most frequently associated with primary lesions in the ovary, large bowel (including appendix), and stomach; however, any primary malignancy may disseminate through the peritoneal cavity. Peritoneal lymphoma (not unique to AIDS patients) [273], peritoneal leiomyosarcoma [274], and melanoma have also been reported. Loculated ascites is the most common finding. Bowel wall thickening and irregularity with or without obstruction may occur, the most common site of involvement being at the rectosigmoid junction. Additional findings include parietal peritoneal thickening and enhancement [275].

Tumor involvement of the omentum is visible as soft-tissue-attenuation nodular or reticular densities within the peritoneal fat. This can coalesce into an omental cake [275]. Implants may be present without ascites [276] and careful inspection of the omentum and sites of peritoneal ligamentous attachments is necessary to detect these subcentimeter nodules [277]. An MDCT investigation showed that use of MPR increases lesion detection rates in patients with ovarian cancer metastatic to the peritoneum, including lesions involving the infracolic omentum [278]. However, in patients with single-site disease and no ascites, small isolated metastatic lesions were still missed [278].

Pseudomyxoma peritonei has a unique appearance of large low-attenuation masses distributed within the peritoneal cavity (Fig. 131.24). Calcifications may occur along the periphery. When pseudomyxoma peritonei is suspected, careful attention should be given to the appendix or ovaries as the potential source.

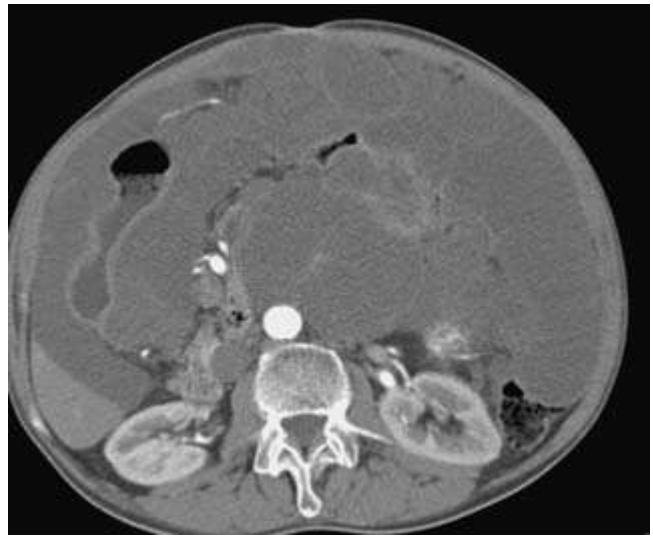


Figure 131.24 Intravenous contrast-enhanced axial computed tomography in a patient with pseudomyxoma peritonei demonstrates extensive low-density implants filling the peritoneal cavity.

Primary peritoneal tumors include a variety of benign mesenteric cysts, stromal neoplasms, lymphoid neoplasms (such as Castleman disease), mesothelioma, and desmoid tumors.

Nonneoplastic peritoneal diseases

Enteric inflammatory processes will cause local increased attenuation within the adjacent mesenteric and intraperitoneal fat [279]; however, one should not diagnose acute generalized peritonitis unless there is evidence of free fluid. A variety of intraabdominal pathologies can give rise to secondary peritonitis, including appendicitis and bowel perforation. In chronic peritonitis, such as in *Mycobacterium tuberculosis* peritonitis, one may see retraction of the parietal peritoneum from the abdominal wall and apparent enhancement of the peritoneal surfaces [280]. In all other ways, the findings may be identical to those of peritoneal carcinomatosis.

For patients with an acute abdomen, CT is useful for diagnosing a range of possible etiologies. In the setting of bowel perforation, CT has a higher sensitivity for the detection of pneumoperitoneum than plain radiography, including upright chest films [281]. When subhepatic free air tracks into the porta hepatis intrahepatic reflections of the peritoneal cavity, it may resemble biliary air [282]. The prevalence of pneumoperitoneum in the postoperative period based on CT findings is greater than that previously reported. Half of patients may demonstrate free intraperitoneal air 6 days after surgery [283].

In the setting of blunt abdominal trauma, pneumoperitoneum does not necessarily indicate hollow viscus injury. It may be secondary to other etiologies, especially dissection of interstitial air from the chest [284]. When free air is due to

bowel perforation (traumatic and nontraumatic), the precise location of bowel rupture may be difficult to delineate on CT. The site of nontraumatic bowel perforation was "directly visualized" in 12%–41% of patients in a study by Ghekiere and colleagues [285]. Findings that indicated the region of bowel disruption in a separate MDCT investigation included the location of clustered extralumenal air bubbles, thickening of the bowel wall, or a focal bowel wall defect, enabling identification of the point of disruption in 86% of subjects [286].

Hemoperitoneum is seen most frequently in trauma [287], acute gynecological disease, or hemorrhage from neoplasm [288]. Unclotted intraperitoneal hemorrhage typically measures 30–45 HU, higher than water and bile [289]. The CT attenuation of hemoperitoneum may be falsely low in the upper abdomen, because the blood products have settled to the dependent portion of the peritoneum (hematocrit effect). When blood is clotted, the density is in the range 45–70 HU. Detection of a site of high-density hemorrhage, the sentinel clot, can lead to identification of the injured organ [289,290]. In patients with blunt abdominal trauma, scanning through the lower pelvis, regardless of the site of injury, can detect hemoperitoneum in the cul-de-sac, even without signs of hemorrhage in the upper abdomen.

Hernias

The diagnosis of abdominal wall hernia is aided by CT [291]. Although most hernias are diagnosed clinically, they may be occult, particularly in obese patients, and they can be a source of abdominal pain. A wide variety of abdominal wall hernias have been described, including lumbar, spigelian, obturator, and perineal. When a hernia is suspected as the cause of bowel obstruction, it is important to be able to identify a transition zone within the hernia; if both afferent and efferent limbs are dilated, then a downstream etiology should be sought. Complications of hernias include incarceration and strangulation (Fig. 131.25). The CT findings are similar to those seen in strangulated small bowel obstruction [292].

The CT appearance of internal hernias, when bowel protrudes through an opening or defect in the mesentery or peritoneum, has been described [293]. Predisposing factors that can cause such a defect include surgery, trauma and inflammatory disease, or the opening may be congenital [294]. Of the six types described, paraduodenal hernias are most common, followed by pericecal, transmesenteric, foramen of Winslow, intersigmoid and, least commonly, paravesical hernias [293]. CT findings of right paraduodenal hernia are encapsulation of small bowel loops in the right midabdomen with looping of arterial and venous jejunal branches behind the superior mesenteric artery. The findings of left paraduodenal hernia, which is more common than right paraduodenal hernia [293], are less specific, but include clustering of small bowel loops or encapsulation of bowel

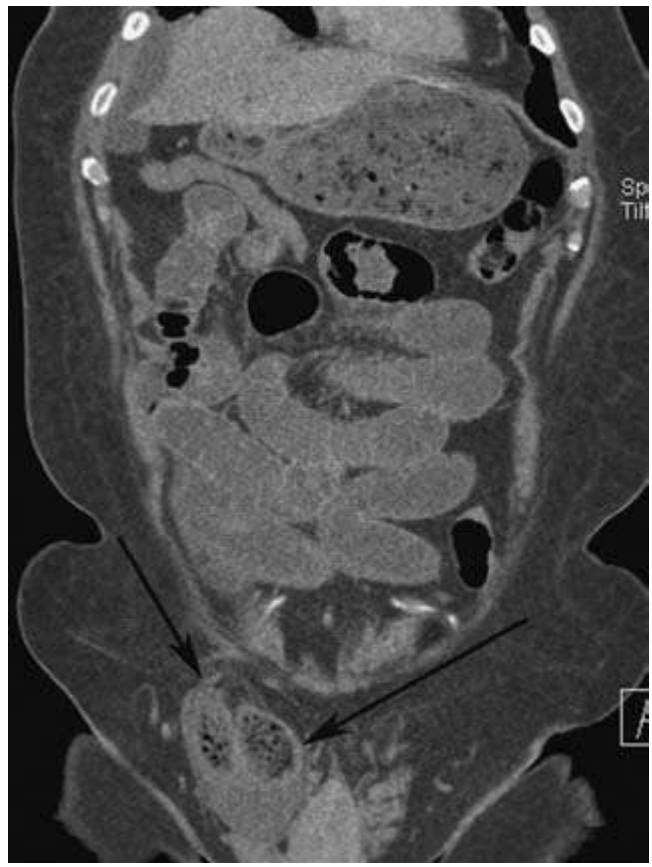


Figure 131.25 Coronal multiplanar reconstruction shows moderate dilatation of small bowel loops due to an obstructing right inguinal hernia (arrows).

loops at or above the level of the ligament of Treitz with intermittent dilation, depression of the duodenal–jejunal junction, and compression of the posterior gastric wall [295,296].

Traumatic diaphragmatic hernia may cause diagnostic difficulty. Left-sided diaphragmatic hernia is easier to diagnose than right [297]. CT can supplant barium studies and nuclear scans. However, in a helical CT study, the diagnosis was missed using subjective prospective interpretation in up to 50% of cases. Identification of numerous predefined criteria increased sensitivity for detecting blunt diaphragmatic rupture to 100% [298]. Continuity of the diaphragm should be assessed to aid in differentiation of ectopic organ position due to hernia or diaphragmatic paralysis. Multiplanar or reconstructions or volume tendering are especially helpful. A careful history of trauma should be sought.

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B IMAGING

132 Magnetic resonance imaging

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Magnetic resonance imaging (MRI) is a powerful imaging tool for the evaluation of disease processes of the abdomen. It has an established role as a primary diagnostic technique in abdominal imaging, with evidence showing MRI to have advantages over computed tomography (CT) with regard to diagnostic sensitivity and specificity for many pathological processes of solid organs, bile and pancreatic ducts, bowel, peritoneum, and retroperitoneum.

Since the development of MRI, applications for its use in the gastrointestinal tract have expanded rapidly. The evaluation of the abdomen by MRI was initially hampered somewhat because of artifacts associated with respiratory and bowel motion. However, the development of newer fast imaging techniques has overcome these motion effects, enabling examination of structures and organs that were previously not reliably imaged. These new techniques have improved the results of MRI of the abdomen, particularly when compared with CT.

MRI has certain advantages over CT. CT relies on the single variable of X-ray attenuation for tissue contrast. All image contrast with CT relies on attenuation, and vascularity of structures can be inferred by the changes in attenuation imparted by intravenous contrast agents. Basic differences that can be distinguished include air, calcification, bone, soft tissue, fat, and changes in soft-tissue attenuation imparted by intravenous contrast agents. However, MRI incorporates several parameters that can distinguish pathological tissues. These include T1, T2 (see following section), lipid content, the magnetic susceptibility imparted by metal ions such as iron in the liver, and specific characteristics of flowing blood. In addition, various types of contrast agents, some of them targeted receptor agents, have been developed for imaging the abdomen. Finally, newer techniques such as diffusion and perfusion imaging, are being developed for imaging the abdomen. Compared with CT, MRI has a range of tissue characteristics with which to build the image, and therefore has a greater potential for tissue characterization.

The risks of CT derive from the dose of radiation and from the risk of nephrotoxicity with intravenous contrast agents. The risks of radiation dose may be significant, particularly in younger patients. The probability of a radiation-induced cancer in a young adult undergoing CT of the abdomen and pelvis is about 1 in 2000 or greater. The cost of this one-time radiation dose has been estimated at $30/mSv, or $300 per abdominal CT. These considerations have led one author to suggest that MRI in younger patients may be cost-effective compared with CT based on considerations of radiation risk alone. Costs associated with irradiation of unsuspected pregnancies have not been assessed.

Risk of nephrogenic systemic fibrosis (NSF) has been reported following intravenous gadolinium contrast agent in patients with severe renal failure (stage 4 or 5; glomerular filtration rate <30 mL/min/1.73 m^2) [1]. Nephrogenic systemic fibrosis (NSF) is a rare and serious syndrome that involves fibrosis of skin, joints, eyes, and internal organs. Its cause is not fully understood, but it is associated with exposure to gadolinium in patients with severe kidney failure. If, after review of clinical indications, gadolinium enhancement is judged to be important for diagnosis in patients with severe renal failure, the lowest effective dose of contrast agent should be used. Current reports on NSF suggest that MRI using 20 mL or less of gadolinium contrast agents poses negligible risk of NSF. Most NSF has occurred using more than 30 mL of gadolinium contrast agent, and most have been following gadodiamide [2]. For patients treated with hemodialysis, a hemodialysis session within a few hours following gadolinium contrast injection is likely to further reduce risk of NSF.

CT of the abdomen routinely employs iodinated contrast agents for delineation of vascular anatomy, and to increase

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the tissue contrast between tumors and normal organs. Iodinated contrast agents pose three dangers to patients: anaphylactic reaction, renal nephrotoxicity, and osmotic load. Certain patients, such as those with diabetic nephropathy or renal insufficiency from any cause, are at increased risk from renal nephrotoxicity from iodinated contrast agents. It is safer and cost-effective for these patients to be imaged with MRI. MRI poses significant risk only to patients with certain types of implanted devices such as cochlear implants, cerebral aneurysm clips, or cardiac pacemakers. The risk of anaphylactic reaction associated with currently available gadolinium agents is very low. These agents have no known cross-reactivity with the iodinated contrast agents used for CT, so these patients may be safely imaged with MRI.

In this chapter, we explain some of the tissue characteristics and basic principles of imaging and discuss specific applications and techniques in MRI.

Basic principles (Table 132.1)

Differences in tissue contrast with CT are measured by Hounsfield units, which are a measure of X-ray attenuation. In MRI, the difference in tissue contrast (white, gray, black) depicted on the image is termed its signal intensity. High signal intensity refers to structures which are white on the image and low signal intensity to structures which are dark on the image. Unlike Hounsfield units in CT, signal intensity units are arbitrary and have no real meaning as absolute numbers.

The terms T1 and T2 refer to specific tissue properties that describe the way protons (mostly in water and lipids) behave after being excited by a radiofrequency pulse in a strong magnetic field. Each type of tissue will show a typical T1 and T2. When the image tissue contrast is based mostly on the differences in T1 between the tissues, the image is termed a T1-weighted image. T1 is termed the longitudinal relaxation rate and T2 the transverse relaxation rate or spin-relaxation rate. Structures or fluids that are bright on T1-weighted images have a short (lower) T1 because of greater longitudinal magnetization, and images which are dark have a long T1. Structures containing no water or fat, such as bone or air, also will appear black on a T1-weighted image.

The specific parameters chosen to acquire the image determine whether an image is T1-weighted or T2-weighted or some other type of image. TE and TR refer to the echo time and repetition time, respectively, and are the parameters chosen by the operator to acquire a T1-weighted or T2-weighted image. The specific parameters chosen by the technician determine exactly what kind of image is obtained and are termed the pulse sequence. This sequence therefore describes a set of images covering a specific anatomical area, with identical imaging parameters such as TE, TR, and resolution. T1-weighted images have a short TE and a relatively short TR.

Images designed to show contrast based mostly on the T2 values of the tissues are called T2-weighted. Such images generally have a long TE and a long TR. Structures which are high signal intensity (white) on the T2-weighted image are generally fluid-containing structures such as cysts, intestinal contents, or gallbladder bile. Most tissues in the abdomen, such as liver, pancreas and intestinal wall, have low signal intensity (dark) on T2-weighted images. Structures which have a high fluid content or contain more fluid than normal tissues are brighter on the T2-weighted image. Examples are cysts and hemangiomas of the liver. Structures such as malignant tumors contain higher water content than normal tissues, and therefore show higher signal intensity than normal tissues. Because of their long repetition time (2–3 s), T2-weighted images usually take longer to acquire than T1-weighted images, and therefore are somewhat more susceptible to motion artifact.

Within a strong magnetic field, protons (hydrogen atoms in water or lipid) have a physical property termed spin (angular momentum). These spins can be perturbed by a radiofrequency pulse at a specific frequency, the resonant frequency of the spin. After this excitation pulse or tip, the spins will emit a radiofrequency wave that can be registered by a receiver called the receiver coil. A radiofrequency pulse of sufficient magnitude to tip the spins 90° away from the main magnetic field is referred to as a 90° pulse. Similarly, a radiofrequency pulse that tips the spin 180° is called a 180° pulse or an inversion pulse. After tipping the spin 90°, the spins emit a signal termed the free-induction decay (FID). This radiowave signal is the basis of the magnetic resonance signal.

Because it occurs soon after the initial radiofrequency pulse, the FID is not received normally. The FID rapidly decays in signal amplitude because of dephasing of the spins. This means the spins precess out of phase relative to each other because of small differences in the magnetic field strength in the tissues and therefore have no net signal. This effect of signal amplitude decay, because of dephasing, can be overcome by the application of the 180° pulse after the 90° pulse, in order to refocus the spins and bring the spins back into phase with a corresponding rapid increase in signal amplitude. This process is termed the spin echo and is used in spin echo sequences, which are the most frequently used sequences in MRI. Spin echo sequences can be T1-weighted, T2-weighted, or intermediate-weighted. Intermediate-weighted images use a short TE and a long TR, and are sometimes called proton density-weighted images. They generally have limited usefulness for abdominal imaging.

The signal from the spin echo is localized in space within the body by applying magnetic field gradients. These gradients change the resonant frequency and phase of the spins linearly across the body so that the source of the radio signal can be localized. These gradients are termed the frequency-encoding and phase-encoding gradients. A map of the location of the signal and the strength of the signal strength linearly

Table 132.1 Terms used in magnetic resonance imaging

Term	Synonym	Definition
Longitudinal magnetization		Amplitude of magnetization (and potential signal intensity) that is oriented along the main magnetic field
Transverse magnetization		Amplitude of magnetization (and actual signal intensity) that is oriented perpendicular to the main magnetic field in the plane of the receiving coil
Tissue characteristics		
T1	Longitudinal relaxation time or spin-lattice relaxation time	Measurement of the rate at which the longitudinal relaxation returns to the direction of the main magnetic field after perturbation by a radiofrequency pulse
T2	Transverse relaxation time or spin-spin relaxation time	Measurement of the rate at which spins dephase relative to each other in the transverse plane which cannot be refocused by a 180° refocusing pulse
Magnetic susceptibility		Local distortion of the magnetic field induced by paramagnetic compounds, metal, air–tissue interfaces, etc., which decrease the signal intensity
Chemical shift		Reference to the different resonant frequencies of certain compounds such as fat or silicone relative to water, which allows their selective saturation
User-controlled parameters		
TE	Time to echo or echo time	Time interval between the alpha pulse (a 90° pulse in a spin echo pulse sequence) and the center of the received signal
TR	Repetition time	Time interval between the phase-encoding steps
TI	Inversion time	Time interval between an inversion pulse in an inversion recovery sequence. The time interval between the inversion pulse and the TE
Pulse sequence	Sequence	Complex set of commands that control the magnetic resonance system, gradients and radiofrequency generator in a specific manner, to generate the received signal
In-phase image		Image in which fat and water in the image are in-phase with one another and therefore their signal intensity is additive in the image
Opposed-phase image		Image in which the fat and water signals are out of phase with one another and therefore subtractive or cancel in the image
Acquisition matrix	Matrix	Number of frequency-encoded steps and phase-encoded steps, which determines the resolution of the image
Magnetic resonance angiography	MRA	Set of images, usually gradient echo images, which are designed to emphasize flow within blood vessels. These are displayed in a format via computer reconstructions to resemble conventional angiograms
Magnetic resonance cholangiopancreatography	MRCP	Set of images that are heavily T2-weighted and designed to demonstrate fluid within the biliary tree and pancreatic duct
Contrast agent		Chemical compound injected or ingested to change the tissue contrast on the magnetic resonance image
Paramagnetic (e.g., gadopentetate dimeglumine)		Chemical compound that alters the relaxation properties of water, generally shortening T1 (producing brighter signal intensity on T1-weighted images) more than T2
Superparamagnetic (e.g., iron oxides)	SPIO	Chemical compound that alters the relaxation properties of water, generally shortening T2 (producing low signal on T2-weighted sequences) more than T1

across the body is the image. The complex radio signal received by the receiver coil from the body is decoded into the spatial location of the signals and the strength of the signals by a mathematic algorithm called the Fourier transform.

Another way of refocusing the signal after the 90° pulse is by the application of bipolar gradients, which are gradients that are switched on in one direction and then switched on rapidly in the opposite direction. This will cause refocusing of

the FID signal in a manner that is appropriate for spatial localization of the signal. These sequences are termed gradient refocused echoes or gradient echo (GRE) sequences. The advantage of GRE sequences is that they are acquired very rapidly, allowing acquisition within a breath-hold period. GRE images designed specifically to image blood flow are termed magnetic resonance angiography sequences, because flowing blood appears bright on these sequences.

The resonant frequencies of water differ from the protons in lipid molecules, such as adipose tissue or fat. Because of this difference in resonant frequencies, these two populations of protons in the body can be imaged separately, sometimes termed chemical shift imaging. The most frequently used method is the application of a radiofrequency pulse which saturates the signal selectively from fat or water. If this is performed at the resonant frequency of fat, it is called a fat saturation sequence or fat saturation image.

On GRE images, fat and water may be out of phase with one another and thus cancel their signal. Whether the fat and water spins are out of phase with one another depends on the TE. If fat and water are out of phase relative to each other, small volumes of tissue containing both fat and water (such as fatty infiltrated liver) will cancel their signal and appear dark on the image. This type of image is called an opposed-phase image. In contrast, an in-phase image shows the lipid and water protons in the image as bright. In-phase and opposed-phase images are very sensitive to the presence of small amount of lipid in tissues. These sequences are used to detect fatty liver and the lipid that commonly occurs in adrenal adenomas.

Magnetic resonance angiography (MRA) refers to a family of pulse sequences that image flow within blood vessels. These pulse sequences take advantage of the fact that flowing blood is bright (high signal intensity) on certain GRE sequences. Blood flowing into a slice being imaged is fully unsaturated (i.e., has greater longitudinal magnetization) because it has not experienced prior excitation pulses. This effect renders the blood flowing into the slice as very high signal intensity on the image. Blood flowing through the slice shows less signal intensity because it experiences the excitation pulses used to acquire the data for the image. If thin slices are obtained continuously, preferably with the blood vessel of interest in cross-section, the resulting data can be reconstructed into a dataset representing the entire volume of tissue and then projected into images which resemble conventional angiograms. The principle described here is called *time-of-flight MRA*.

There are other forms of MRA that rely on different physical principles, such as phase contrast angiography, bolus tracking, black blood techniques, and three-dimensional gadolinium-enhanced MRA. Description of these techniques is beyond the scope of this chapter. However, these techniques have in common the principle of imaging flowing blood, while not necessarily imaging the vascular structures themselves. They are all susceptible to certain artifacts that give the impression of occluded or stenotic vessels, such as slowly flowing blood and turbulent flow, and artifacts from adjacent structures such as metallic surgical clips. The basic goal of these sequences is to increase the contrast between the vessel and surrounding structures dramatically. Thus, the vessels are selectively displayed with markedly higher signal intensity than surrounding stationary tissue.

A magnetic resonance technique that simulates MRA but which differs in principle is *magnetic resonance cholangiopancreatography* (MRCP). The goal of MRCP is to selectively image fluid within the biliary tree and pancreatic duct. Very heavily T2-weighted images are used to achieve this effect. With the very long TEs used for these heavily T2-weighted images, virtually all solid tissue has low signal (dark on the image). By virtue of its long T2, fluid within the biliary tree, gallbladder, and pancreatic duct retain signal intensity and therefore appears bright on the image. Like MRA, multiple projections in a manner similar to conventional cholangiography or endoscopic retrograde cholangiopancreatography (ERCP) can be obtained.

Current pulse sequences for imaging the liver are designed for very fast acquisition of images so that motion artifacts are limited or absent. These very fast images generally require improved (high performance) gradient systems. For T1-weighted images, fast GRE sequences are used. T1-weighted images that acquire the whole liver as a volume (three-dimensional imaging) can be used for dynamic perfusion studies, or MRA studies, or both. For T2-weighted images, subsecond pulse sequences have been developed so that motion-free images are obtained, whether or not the patient is holding their breath. These sequences are called HASTE (half-Fourier acquisition single-shot turbo spin echo), EXPRESS, or single-shot fast spin echo (FSE) sequences, depending on the manufacturer of the system. Echo planar imaging is not a new development but has been adapted to average clinical scanners with improved gradient systems and can also acquire subsecond images.

Injected contrast agents identify vascular structures and areas of abnormality within organs by differences in their vascularity. Contrast agents for use during MRI are available and have similar functions to the iodinated contrast agents used in CT. The most frequently used agent in MRI is gadopentetate dimeglumine. This fills the arteries and capillary beds and rapidly diffuses into the interstitium and the sinusoids in the liver. Because of this sequence of events, it is important to rapidly image abdominal organs to capture this temporal progression. In the liver, for example, it is advantageous to image an arterial phase, followed by a portal phase, followed by a delayed phase. This imaging of the arterial and venous phases is important in the differential diagnosis of liver tumors. Specifically, hypervascular tumors such as hepatocellular carcinoma will be seen better on an arterial phase and may be invisible on delayed phases. Similarly, other liver tumors such as hemangiomas can be characterized accurately based on their temporal sequence of enhancement with the

contrast agent. Gadolinium chelate-enhanced images are important for detecting and characterizing vascular abnormalities.

Agents that are accumulated selectively by hepatocytes and which undergo some biliary excretion have been developed. These show prolonged liver enhancement on T1-weighted images and increase the signal intensity difference between metastases and liver.

Superparamagnetic iron oxide agents have been approved for imaging the liver by the Food and Drug Administration. These agents are taken up by normal Kupffer cells in the liver and spleen. Most tumors, such as metastases, contain no Kupffer cells and therefore do not accumulate the agent. Thus, the signal intensity difference between liver and tumors is increased, making tumors more conspicuous on T2-weighted images. Greater sensitivity to metastases using these agents has been reported. Hepatocellular tumors such as focal nodular hyperplasia and hepatic adenoma take up these agents, providing a valuable diagnostic tool for evaluating liver tumors.

Liver (Table 132.2)

Various comparisons of contrast-enhanced CT and MRI have shown that MRI has significantly better capacity to characterize liver lesions [3–5]. MRI consistently characterizes over 95% of detected hepatic lesions and compares favorably with percutaneous biopsy, which typically has a diagnostic yield of only 61%–67% for liver masses, regardless of the imaging modality used for percutaneous needle guidance [3,4]. Given that about one-fourth of liver lesions detected in patients with a history of malignancy are subsequently determined to be malignant, it may be cost-effective to image those patients requiring tumor staging directly using MRI. In patients with malignancy, the ability to characterize a benign liver lesion can be as important as identifying metastases. For example, small liver cysts are common in adult patients and are frequently too small for accurate density measurement on CT, but can be resolved and characterized down to 1 mm on MRI because of the very high signal produced on T2-weighted images [6]. Generally, the advantages of MRI over CT arise from the greater contrast resolution, and the variety of different soft tissue contrast achieved through implementation of multiple sequences.

Benign lesions

Cysts

Benign lesions of the liver are common in the adult, and cysts represent the most common benign lesion. Pathology typically shows a wall comprising a single layer of epithelial cells. Etiologies are mostly idiopathic, but cysts may be seen in association with developmental disorders such as polycystic kidney disease or von Hippel–Lindau disease, infections including *Echinococcus*, or hemorrhage. Cysts appear uniform and high in signal intensity on T2-weighted images and low in signal intensity on T1-weighted images, with well-defined margins (Fig. 132.1) and no evidence of enhancement on gadolinium-enhanced spoiled gradient echo (SGE) images [6,7]. Benign cysts may appear slightly complicated with lobulation of borders and septations, and may have elevated signal on SGE T1-weighted images, typically in association with protein or related to prior hemorrhage. As complexity becomes more prominent, other possibilities should include biliary cystadenoma (Fig. 132.2), biliary cystadenocarcinoma, and mucinous cystadenocarcinoma of ovarian or other origin, usually bowel or pancreas. These tumors typically show perilesional gadolinium enhancement. These lesions may also have internal septations and enhancing mural nodules. These lesions will also increase in size on follow-up imaging.

Table 132.2 Classical MRI features of common liver lesions

Hepatic lesion	T1	T2	Opposed-phase GRE	Arterial enhancement	Portal venous enhancement	Delayed enhancement
Benign cyst	Low	High	NA	None	None	None
Hemangioma	Low	High	NA	+/– peripheral nodular	+/– peripheral nodular	Yes
Hepatic adenoma	Intermediate/high	Isointense	+/– reduced signal	Yes	Isointense	Isointense
Focal nodular hyperplasia	Isointense	Isointense/slightly increased	NA	Yes	Isointense	Isointense with delayed enhancement of central scar
Hepatocellular carcinoma	Low	Increased	+/– drop	Yes	Isointense/reduced	Reduced/washout
Cholangiocarcinoma	Low	Slightly increased	NA	Isointense	Isointense/increased	Delayed ill-defined

GRE, gradient echo; MRI, magnetic resonance imaging; NA, not applicable.

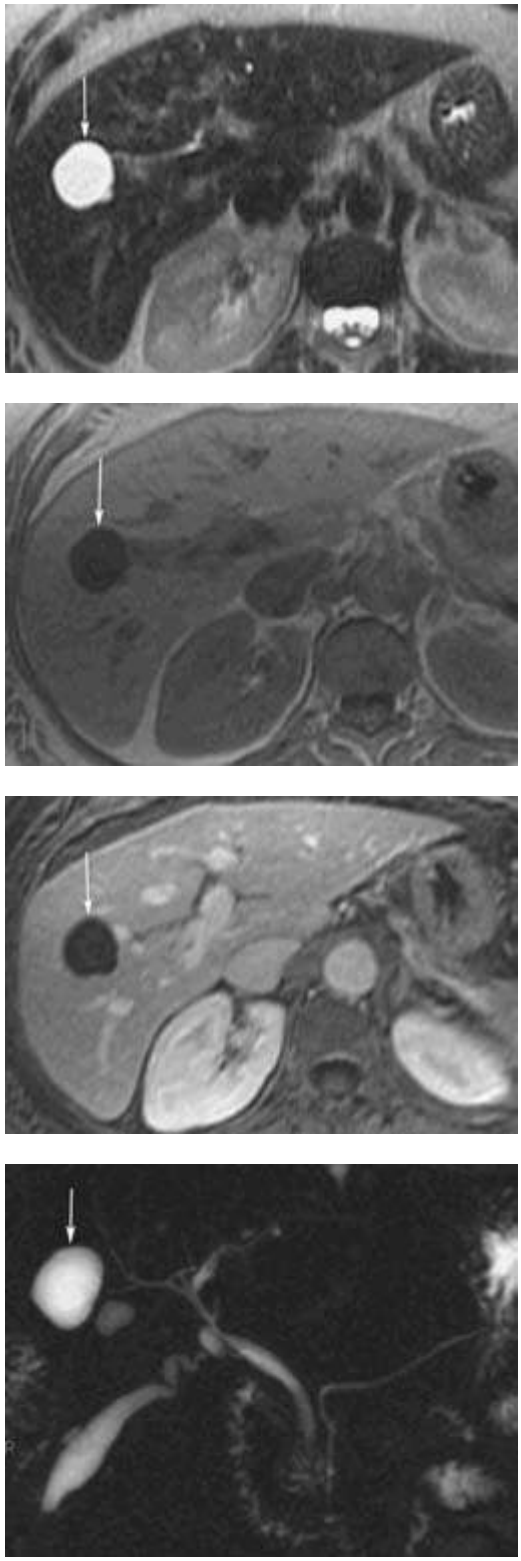


Figure 132.1 Simple hepatic cyst. **(a)** Axial T2 image, **(b)** axial T1 image, **(c)** postcontrast three-dimensional gradient echo image, and **(d)** magnetic resonance cholangiopancreatography demonstrate high T2 signal lesion (arrow on **a** and **d**) and low T1 signal nonenhancing lesion (arrow on **b** and **c**) consistent with simple hepatic cyst.

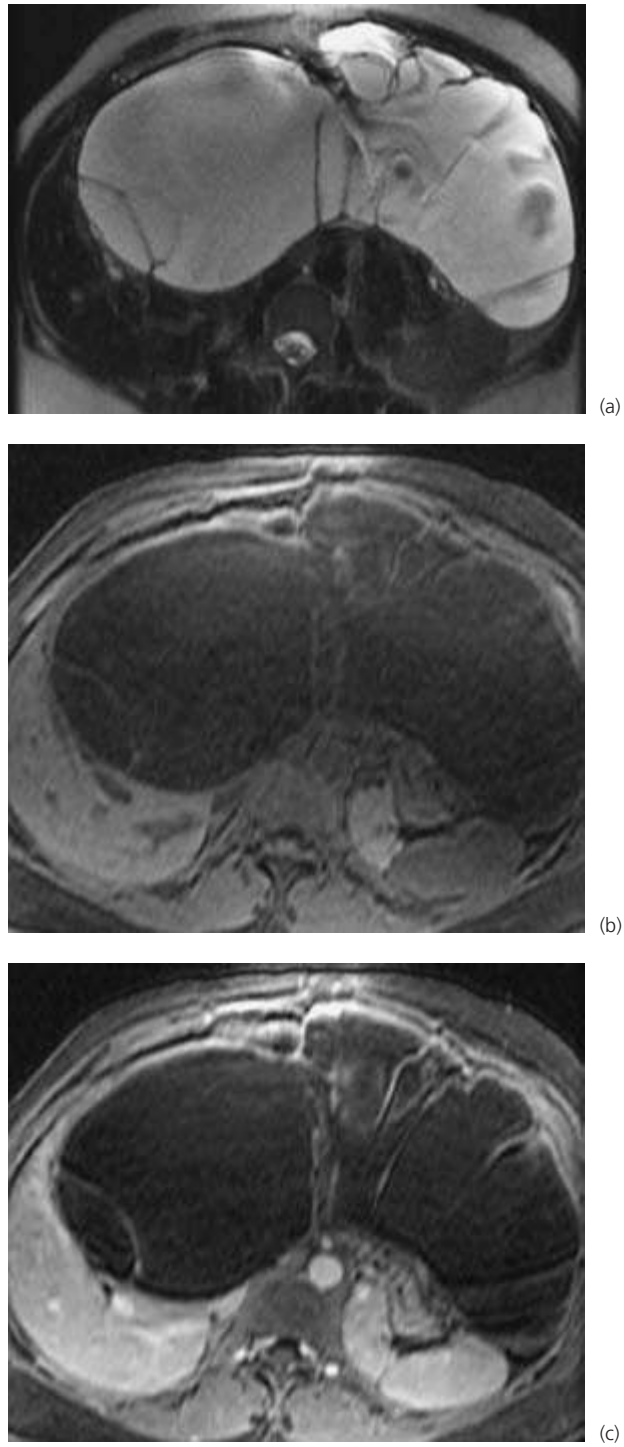


Figure 132.2 Biliary cystadenoma. Axial T2 image **(a)** and corresponding axial three-dimensional gradient echo images of the liver **(b)** before and **(c)** after contrast demonstrate large cystic lesion in the left and right lobes of the liver with internal enhancing septations that was surgically proven to be biliary cystadenoma.

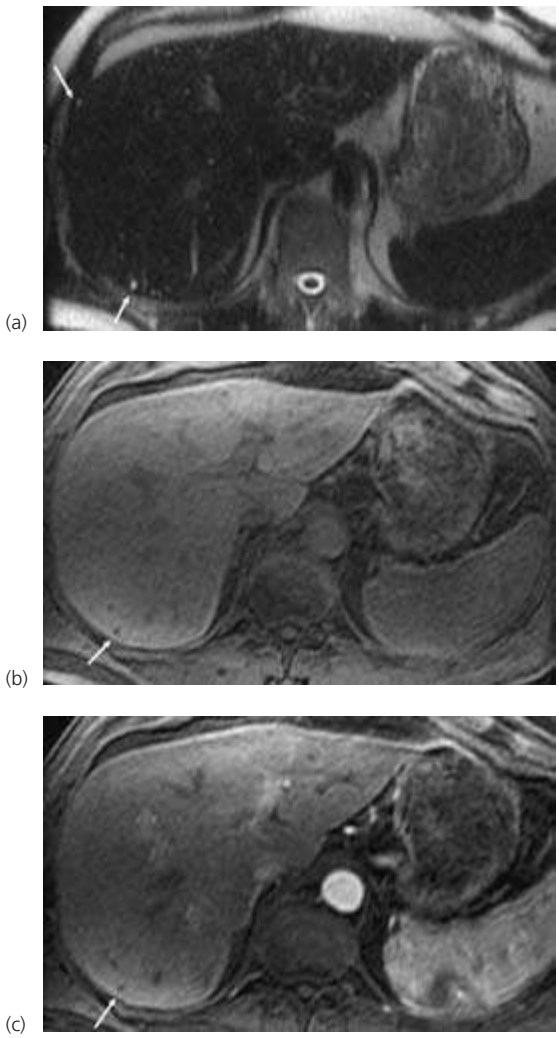


Figure 132.3 Bile duct hamartomas. Axial T2 image **(a)** and corresponding axial three-dimensional gradient echo images of the liver **(b)** before and **(c)** after contrast demonstrate scattered, less than 1-cm cysts (arrows) in the liver with thin peripheral rim of enhancement consistent with biliary hamartomas.

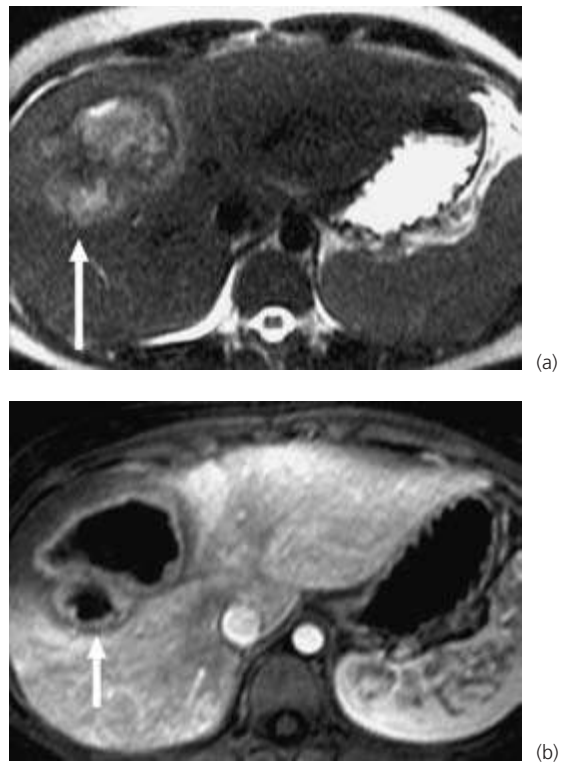


Figure 132.4 Hepatic abscess. **(a)** Axial T2 image and **(b)** postcontrast three-dimensional gradient echo image of the liver demonstrate large, peripheral, enhancing, thick-walled abscess (arrow).

Bile duct hamartomas

These lesions are relatively common, occurring in 3% of the population, and comprise irregular branching bile-dilated bile ducts. Bile duct hamartomas are frequently peripheral, multiple, and less than 1 cm in size. These lesions have features identical to cysts on T2- and unenhanced T1-weighted images, with the exception of demonstrating a peripheral thin and uniform rim of gadolinium enhancement (Fig. 132.3). Metastases show perilesional enhancement, and hypervascular tumors show central enhancement on arterial phase [7,8].

Abscess

Pyogenic abscesses are associated with sepsis, recent bowel surgery, diverticulitis, Crohn's disease, and appendicitis. Fungal microabscesses are more common in immunocompromised patients. Typically, abscess (Fig. 132.4) has high central increased signal and intermediate peripheral rim on T2-weighted images. The central portion is low on T1-weighted images and does not enhance on postcontrast images [9]. The peripheral rim shows persistent late enhancement without centripetal progression of enhancement [10]. There is indistinct perilesional enhancement on the early phase because of hyperemia [11].

Hemangiomas

Hemangiomas are the most common benign liver neoplasm, are multiple in up to 70% of cases, and are found with highest incidence in young adult women. Histology shows a series of vascular lakes and channels, with larger lesions developing areas of thrombosis and fibrosis. Imaging shows moderately elevated signal on single-shot T2-weighted images, typically less intense than demonstrated by simple cysts, and low signal on T1-weighted images [6,12]. Enhanced images show peripheral interrupted nodules on arterial phase (Fig. 132.5), and this finding is pathognomonic for this pathology. Venous and delayed phases may show progressive enlargement and coalescence of the peripheral nodules with variable degrees of central filling [6,13]. Smaller lesions generally fill more quickly, and larger lesions are progressively more likely to show slower central filling. Giant hemangiomas, usually larger than 5–10 cm, typically develop central areas that fail

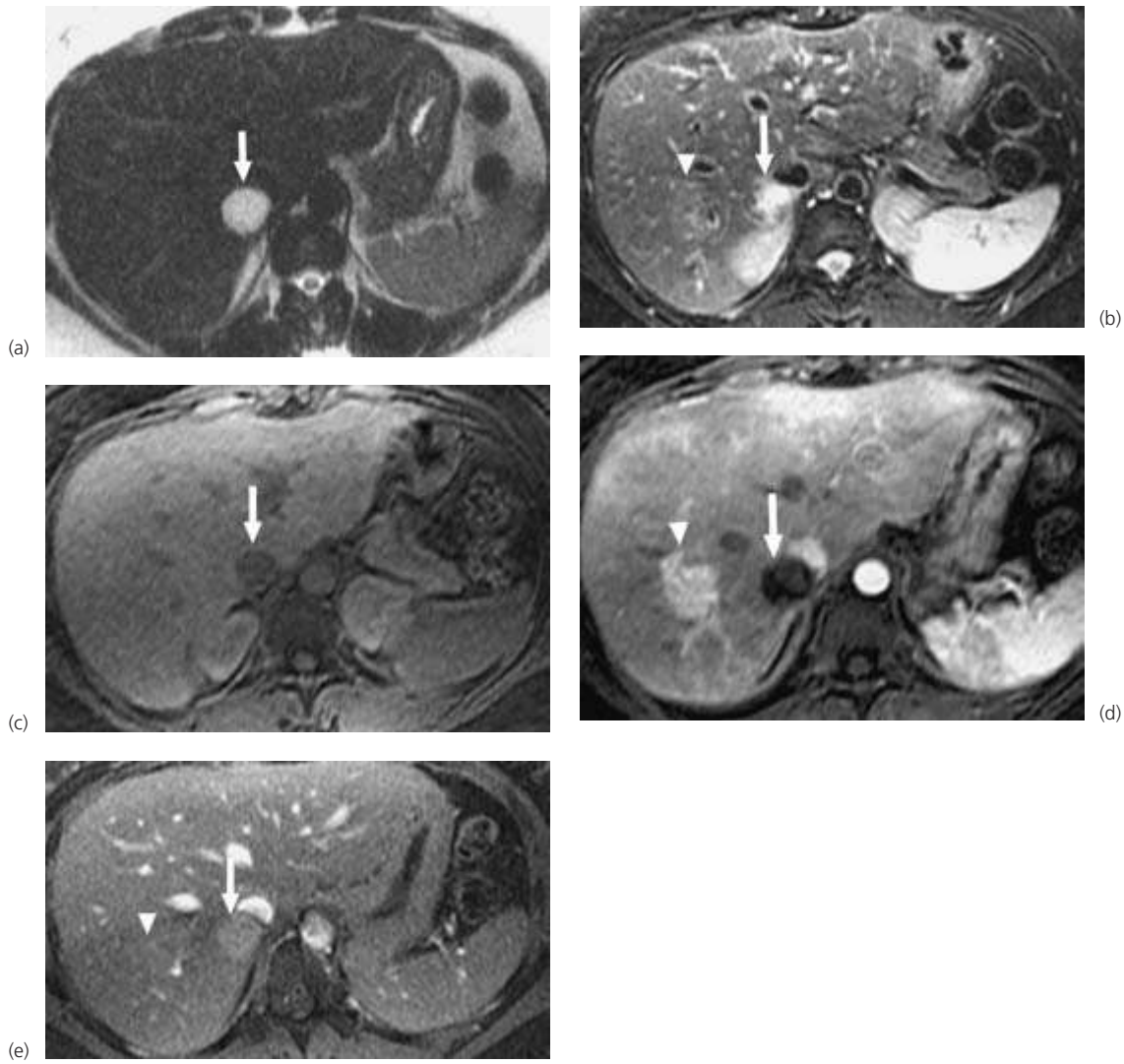


Figure 132.5 Hepatic hemangioma (arrow) and focal nodular hyperplasia (arrowhead). **(a)** Long TE (180 ms) and **(b)** fat-suppressed intermediate TE (80 ms) axial T2-weighted images of the liver demonstrate a high T2 signal lesion (arrow) adjacent to the inferior vena cava. Of note, a second lesion (arrowhead) is also seen on **(b)** but not on **(a)**. Three-dimensional gradient echo images **(c)** before contrast, **(d)** during arterial phase, and **(e)** delayed post contrast demonstrate peripheral enhancement of the paracaval lesion (arrow) with progressive delayed enhancement consistent with a hemangioma. The second lesion (arrowhead) enhances avidly in the arterial phase and becomes isointense to hepatic parenchyma on delayed imaging, consistent with focal nodular hyperplasia.

to fill in on delayed enhanced images, and may show central cystic areas that are as bright as simple fluid, such as cerebrospinal fluid, with well-defined margins and no enhancement. Small (< 1 cm) lesions may fill quickly and be difficult to delineate from other arterial-phase enhancing neoplasms, such as a small hepatocellular carcinoma (HCC) or hypervascular metastases [14]. In such cases, the distinguishing features can be found on venous and delayed images, where the other hypervascular neoplasms may typically show washout whereas hemangiomas may demonstrate persistent enhancement with signal above that of the adjacent liver parenchyma. Metastases have been described as enhancing progressively from outside-in; however, the pattern of enhancement does not show peripheral interrupted nodules with coalescence, as is typical of hemangiomas [15,16]. An optimally timed arterial-phase enhanced image typically provides the most critical diagnostic information [16].

Focal nodular hyperplasia

Focal nodular hyperplasia (FNH) is most commonly seen in young adult women and seems to represent a hamartomatous lesion with a disorganized growth pattern of hepatocytes and ducts that can form an unencapsulated mass with abnormally structured vessels and bile ducts. These lesions may appear isointense to mildly hyperintense on T2-weighted images, and isointense to mildly hypointense on T1-weighted

images (Fig. 132.5). An important, and unique, characteristic of FNH is the presence of a central fibrovascular core, which may produce a high signal on T2-weighted images [6,7,12,16]. On gadolinium-enhanced imaging the fibrovascular core may demonstrate slowly progressive enhancement, with no perceived enhancement on arterial phase, and becoming maximally conspicuous on delayed-phase images [16,17]. This is in contrast to the bulk of the mass surrounding the core that typically enhances uniformly and intensely in the arterial phase, and which becomes isointense or slightly hyperintense to surrounding liver on venous and delayed phases. Small lesions (less than 1–2 cm) may appear more uniform in enhancement and a fibrovascular core may not be perceived. Other distinguishing features on gadolinium-enhanced imaging from other arterial-phase enhancing tumors include the lack of capsule enhancement, as is observed with adenomas and HCC [16–19]. Fibrolamellar HCCs are typically large, greater than 10 cm at presentation, but may share features of FNH with the exception that the central scar of fibrolamellar tumors is typically lower than surrounding tumor on T2-weighted images and shows radiating enhancing bands on postgadolinium images [16,20]. Alternative contrast agents may be helpful in a small fraction of cases that remain ambiguous. Gadolinium-EOB-DTPA may demonstrate early arterial-phase tumor enhancement and late hepatocellular uptake caused by uptake by hepatocytes within the mass and excretion into poorly draining malformed bile ducts. Manganese or superparamagnetic iron oxide particles (SPIO) may be taken up by hepatocytes within FNH and darken the lesion similar to surrounding normal liver [15,16].

Adenomas

Adenomas are benign neoplasms of epithelial origin, with predominant incidence in young women. They are associated with oral contraceptives, and rarely with exogenous anabolic steroids, galactosemia, and glycogen storage disease type Ia. Spontaneous hemorrhage may be related to larger masses, typically greater than 4–5 cm in diameter, and can result in presentation with abdominal pain, with a risk of extrahepatic extension and intraperitoneal bleeding. Hepatic adenomas comprise sheets of hepatocytes and form a pseudocapsule related to compression of adjacent hepatic parenchyma; however, in contrast to FNH, they do not form bile ducts. T2-weighted images show isointense to slightly hyperintense signal and T1-weighted images show mildly hypointense to mildly hyperintense signal (Fig. 132.6) [5]. Opposed-phase SGE T1-weighted images may demonstrate signal drop related to lipid accumulation in approximately half of adenomas [21]. Blood products may result in irregular foci of mixed high or low signal on T1- and T2-weighted images. Gadolinium enhancement is maximal during arterial phase and seen as an arterial-phase blush with rapid fading in the venous and delayed phases to hypointensity or isointensity to adjacent liver, with development of a persistent

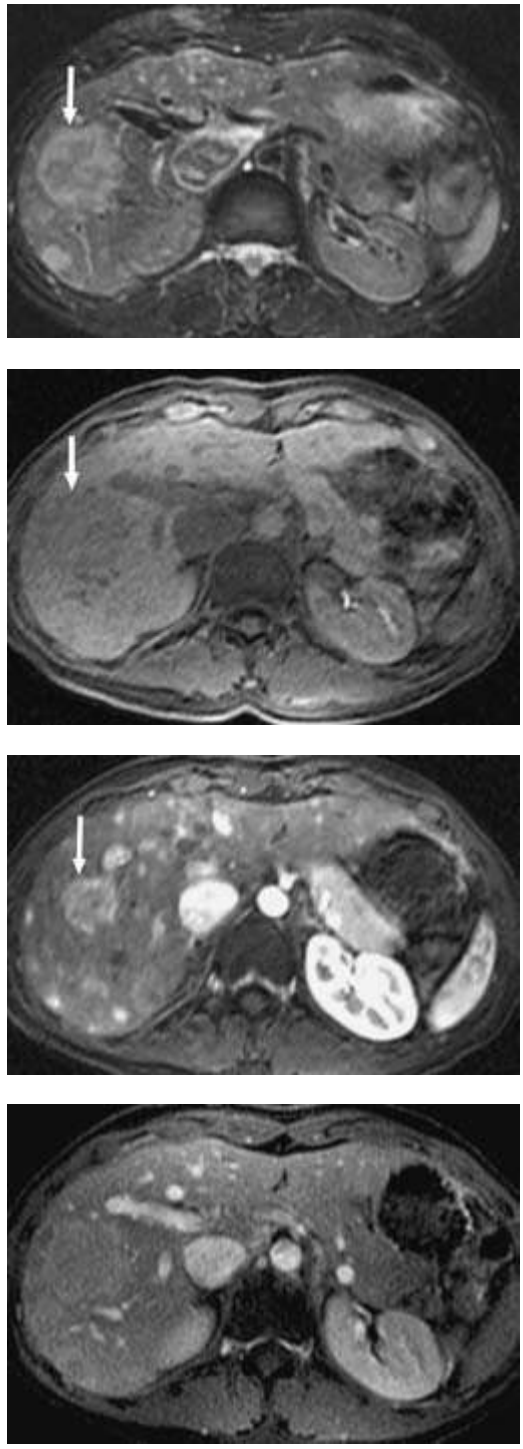


Figure 132.6 Hepatic adenoma. **(a)** Axial, fat-suppressed, intermediate TE, T2-weighted image shows scattered mildly increased T2 signal lesions (largest indicated by arrow). Three-dimensional gradient echo images **(b)** before contrast and **(c)** during arterial phase demonstrate arterial enhancing hepatic adenomas (largest indicated by arrow) that become near isointense on delayed postcontrast image **(d)**.

enhancing rim related to the pseudocapsule [5,19]. An enhancing scar may be observed in a small subset, but such a scar does not produce high signal on T2-weighted images as seen in FNH. Although most features of adenoma mimic HCC, HCC is usually associated with a background of chronic liver disease with evidence of cirrhosis [5,19]. However, in patients with HCC risk factors where the liver is normal and tumor markers are negative, differentiation may be difficult. The presence of portal venous involvement helps differentiate HCC, and the presence of blood products helps differentiate adenoma. Hepatic adenomatosis is a rare and separate entity that involves numerous adenomas scattered throughout the liver, which have malignant potential [22].

Malignant lesions

Primary HCC is the most common primary liver malignancy and usually occurs in the setting of cirrhosis [23]. These tumors are thought to arise from premalignant dysplastic nodules, and dysplastic nodules are thought to progressively dedifferentiate from low- to high-grade histology [24]. HCC occurs as a solitary lesion in 50% of cases, is multifocal in 40%, and diffuse in 10%, and is typically greater than 2 cm in diameter, in contrast to dysplastic nodules, which are typically less than 2 cm [25]. Imaging features are variable, but the pattern of elevated signal on T2-weighted images and diminished signal on T1-weighted images increases the likelihood of HCC as compared with dysplastic nodules (Fig. 132.7) [5]. In the setting of cirrhosis, however, highly specific imaging features for HCC are reliably found on gadolinium-enhanced SGE images, which show irregular marked arterial-phase enhancement with rapidly diminished enhancement on venous and delayed-phase images, and development of a peripheral enhancing rim resulting from a pseudocapsule [26]. High-grade dysplastic nodules may show enhancement, but do not develop a pseudocapsule [27]. Diffuse HCC shows irregular T2 and T1 signal, with heterogenous enhancement. Regions within the tumor may washout on delayed images while enhancement in other areas persist. Associated portal venous tumor thrombosis typically enhances in the arterial phase [27].

Hypovascular metastases

Most common malignant hepatic tumors can be further classified as hypovascular or hypervascular based on imaging features. Hypovascular metastases are the most common and usually arise from colon adenocarcinoma. These tumors tend to have mixed arterial and portal venous supply, and usually develop central necrosis presumably because of poor vascular perfusion to the center of enlarging tumors [28]. Hypovascular

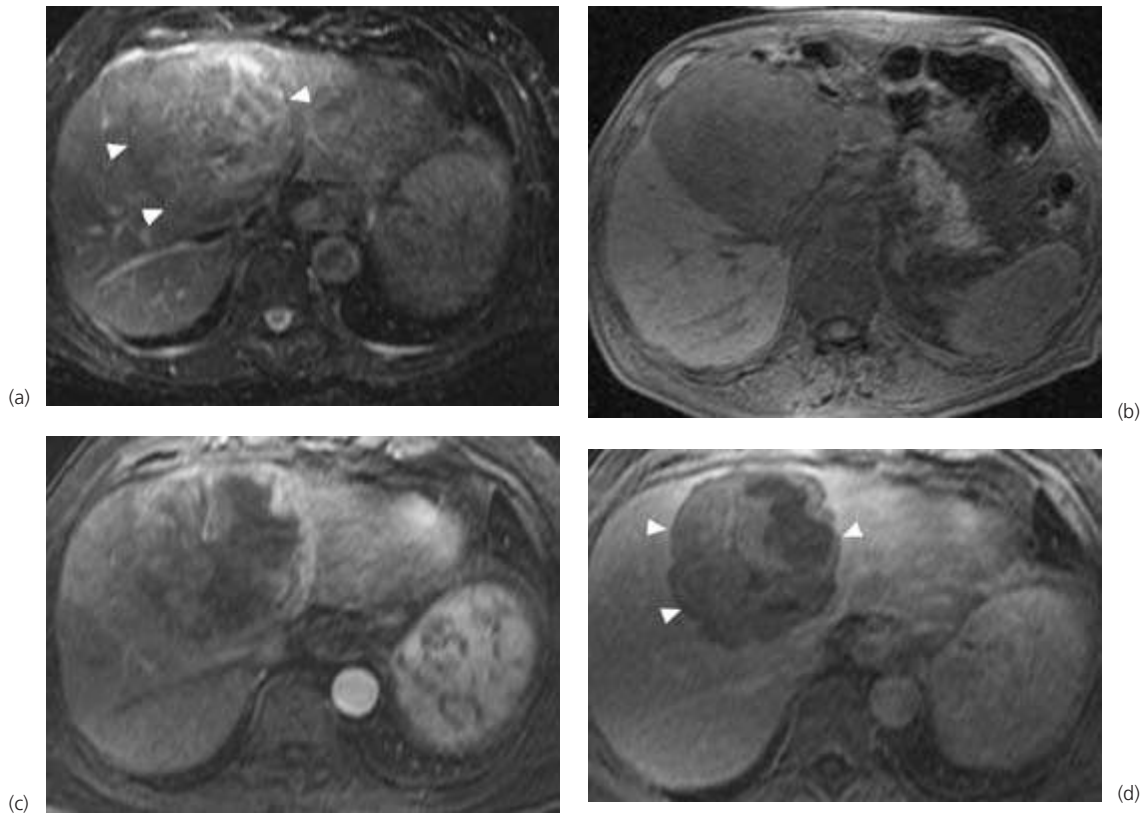


Figure 132.7 Hepatocellular carcinoma. Axial, fat-suppressed, intermediate TE, T2-weighted image **(a)** and three-dimensional gradient echo images **(b)** before contrast, **(c)** during arterial phase, and **(d)** delayed post contrast demonstrate increased T2 signal lesion with preferential arterial enhancement and washout on delayed images following contrast. Note low T2 signal and hypoenhancing peripheral pseudocapsule (arrowheads) of this lesion.

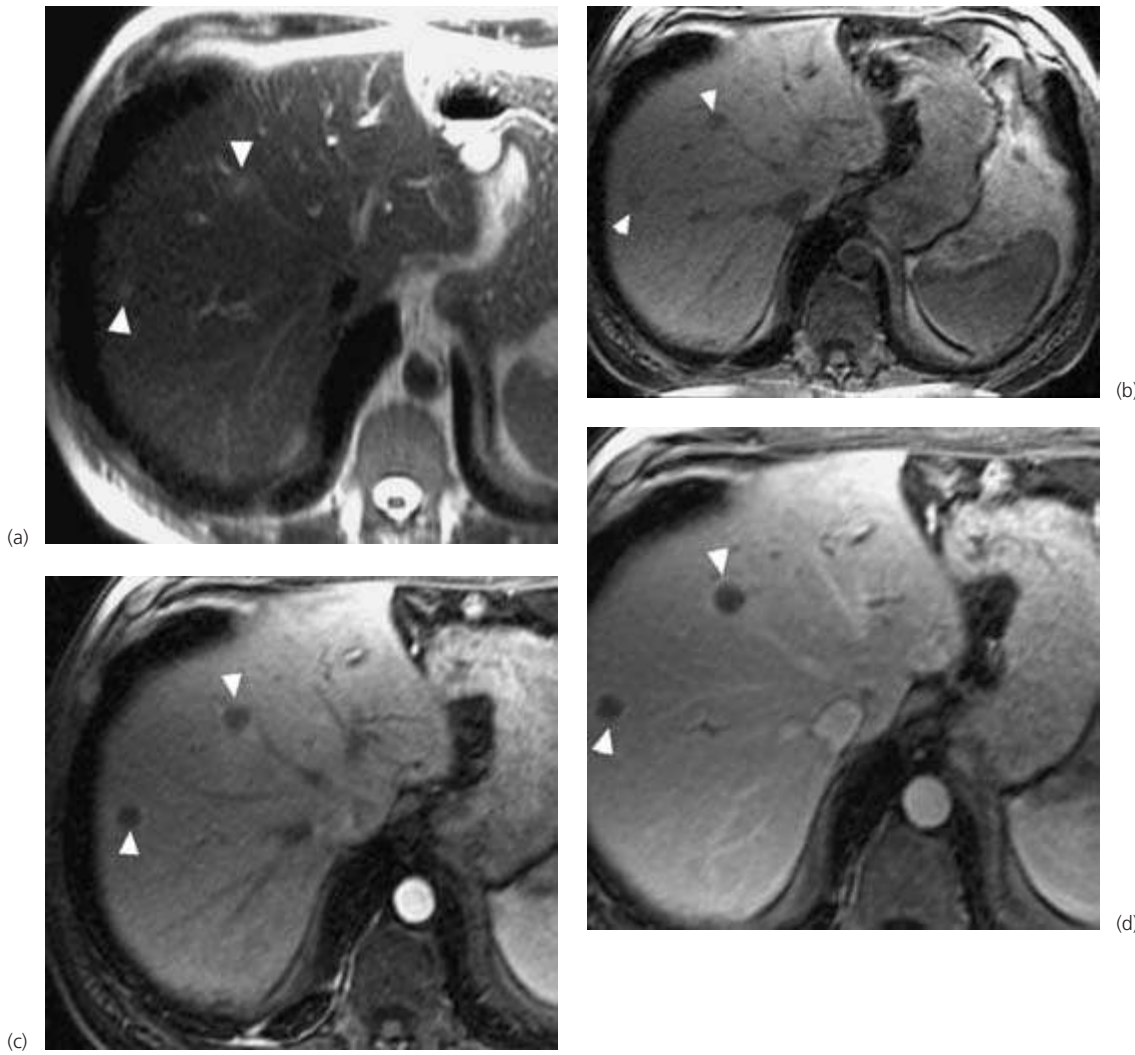


Figure 132.8 Hypovascular hepatic metastases. Axial T2 image **(a)** and three-dimensional gradient echo images **(b)** before contrast, **(c)** during arterial phase, and **(d)** after contrast demonstrate hypoenhancing metastatic lesions (arrowheads) with rim enhancement in a patient with metastatic pancreatic carcinoma.

metastases may have variable appearance on T2-weighted images and are usually most conspicuous on pregadolinium- and postgadolinium-enhanced SGE T1-weighted images, showing slowly progressive irregular enhancement from outside-in (Fig. 132.8) [29]. Unlike hemangioma, the enhancement on arterial phase is less and does not show peripheral interrupted nodular enhancement. Arterial-phase perilesional enhancement may be observed, and is typically seen with colon, pancreatic, or stomach adenocarcinoma metastases [30].

Hypervascular metastases

Hypervascular metastases are frequently neuroendocrine, renal, melanoma or breast in origin. T2-weighted images show variable signal, usually moderately elevated, and low signal on T1-weighted images [30]. Melanoma is an exception, where melanin accumulation, having T1 shortening properties, may result in elevated signal on T1-weighted images (Fig. 132.9). Characteristic gadolinium enhancement shows that the lesions demonstrate maximal conspicuity on arterial-phase images [16,29].

Posttherapeutic imaging

Cross-sectional imaging has largely relied on anatomical depiction of disease, including tumors. There is a large subset of patients with malignancy being treated by nonsurgical approaches, including chemotherapy and radiation, where the ability to follow tumor response is valuable [31]. Current methods have mostly relied on anatomical depiction of tumor and measurement of tumor size to evaluate tumor response [32]. This is believed to require a relatively long follow-up period in most applications, and other measures of tumor response sensitive to cellular and vascular reactions may be more useful [33]. A multitude of approaches are being evaluated using MRI [34]. One simple test that may be helpful is the evaluation of perfusion by gadolinium enhancement pattern within a treated malignant mass [34]. Demonstration of decreased

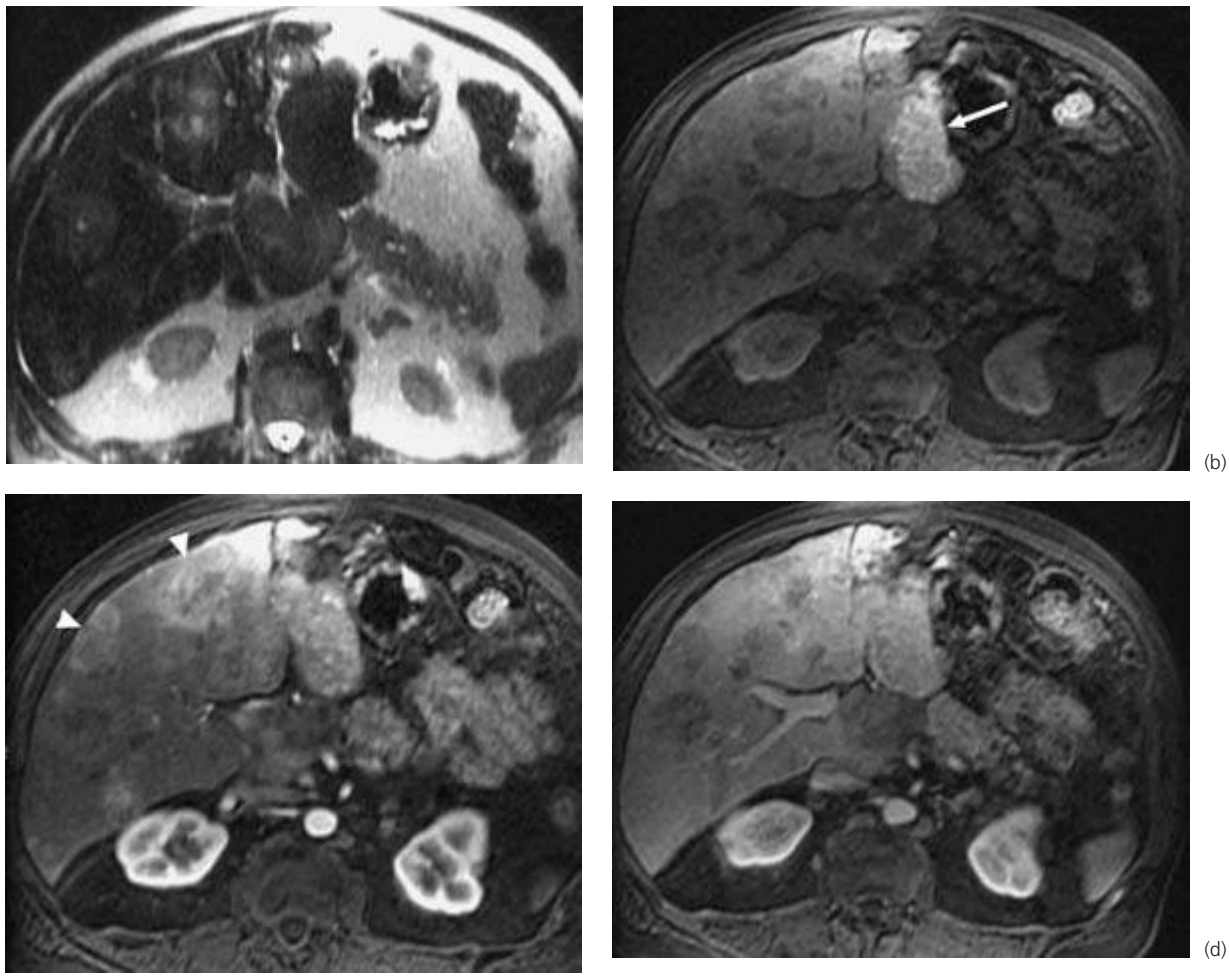


Figure 132.9 Hypervascular hepatic metastases. Axial T2 image **(a)** and three-dimensional gradient echo images **(b)** before contrast, **(c)** during arterial phase, and **(d)** during portal venous phase demonstrate multiple arterial enhancing lesions (arrowheads) in this patient with melanoma metastases. Note increased signal on precontrast T1-weighted images in lesion in the lateral segment of the left lobe of liver (arrow) secondary to melanin or hemorrhage.

vascularity after therapy, reflected by reduced enhancement, reduced intermediate T2 signal, and necrosis, has been associated with tumor response (Fig. 132.10) [35,36].

Diffuse liver diseases

MRI of the liver may also be used for the evaluation of diffuse liver diseases, including abnormal lipid metabolism, iron deposition disease, and perfusion abnormalities related to inflammation, fibrosis, vascular occlusion, or infarction and hemorrhage [37].

Fatty liver

Lipid accumulation in hepatocytes can occur as a result of impaired liver function secondary to a variety of etiologies. In the USA, fatty liver can be detected in over 20% of the population, and the most common association with fatty liver is obesity [38,39]. There has been further association of fatty liver with development of hepatitis, resulting from nonalcoholic acute steatohepatitis, and potential progression to cirrhosis [40]. In the setting of liver transplantation, a living related donor liver assessment must include evaluation for the presence of fatty infiltration, which is considered a contraindication to transplantation when severe and can lead to failure of the transplant. Abnormal lipid accumulation in liver can be detected on MRI, and can be evaluated by comparing liver signal on SGE images acquired in-phase and out-of-phase [40,41]. Protons in a voxel containing 100% fat precess 220–230 Hz slower than a voxel comprising 100% water at 1.5 T. That means every 4.4 ms the fat protons migrate 360° and regain in-phase orientation relative to water protons, whereas at 2.2 ms, or at half this time, the fat and water protons are 180° out-of-phase [38]. Current generation MRI systems have incorporated dual echo breath-hold SGE sequences that can acquire two sets of k-space filled to obtain two sets of images, one set in-phase, the other out-of-phase, with spatially matched slices. Liver containing lipid results in image voxels with a physical mixture of water and lipid, which when imaged out-of-phase results in phase

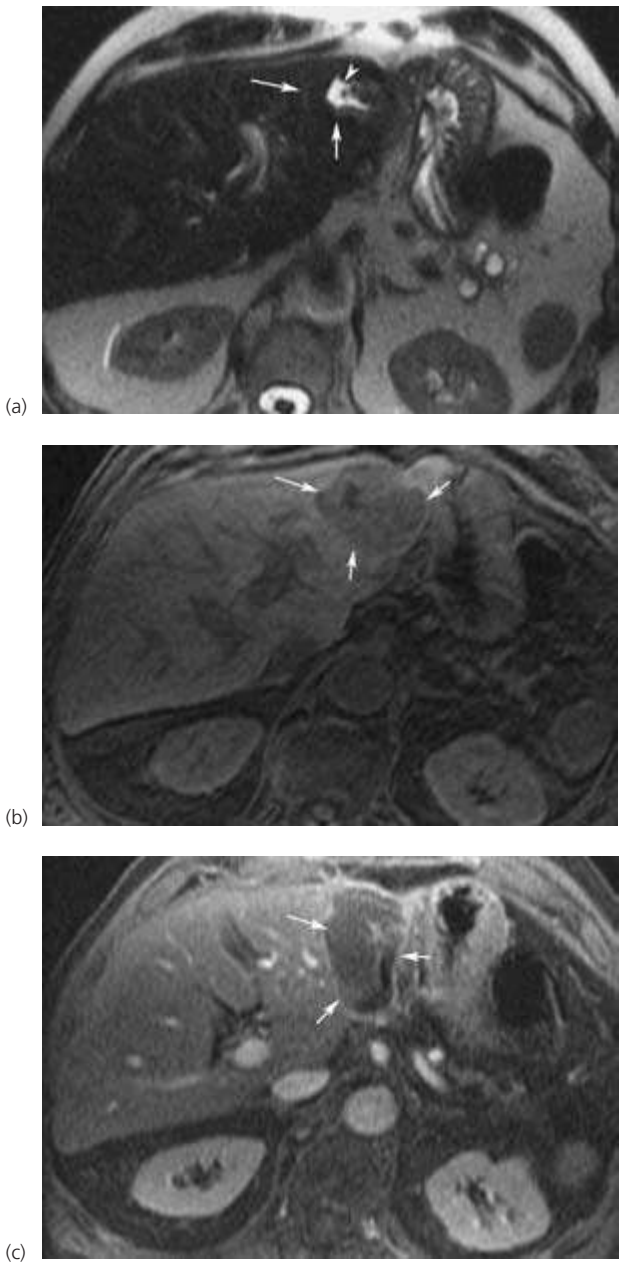


Figure 132.10 Radiofrequency-ablated lesion. **(a)** Axial T2 image demonstrates fluid signal consistent with cavitation (arrowhead) of lesion (borders of lesion indicated by arrows) treated with radiofrequency ablation. Three-dimensional gradient echo images **(b)** before and **(c)** after contrast demonstrate minimal peripheral enhancement of lesion consistent with granulation tissue. There is some residual central enhancement suspicious for persistent viable tumor and will warrant continued surveillance.

cancellation and diminished signal (Fig. 132.11) [37,38]. Spleen is an organ that does not accumulate fat, and can be used as a control against which liver signal can be assessed as a ratio to test for relative diminishment in liver signal on out-of-phase images. Spleen signal can change as a result of iron deposition, and use of kidney or skeletal muscle within the image may be more reliable for assessment of relative liver

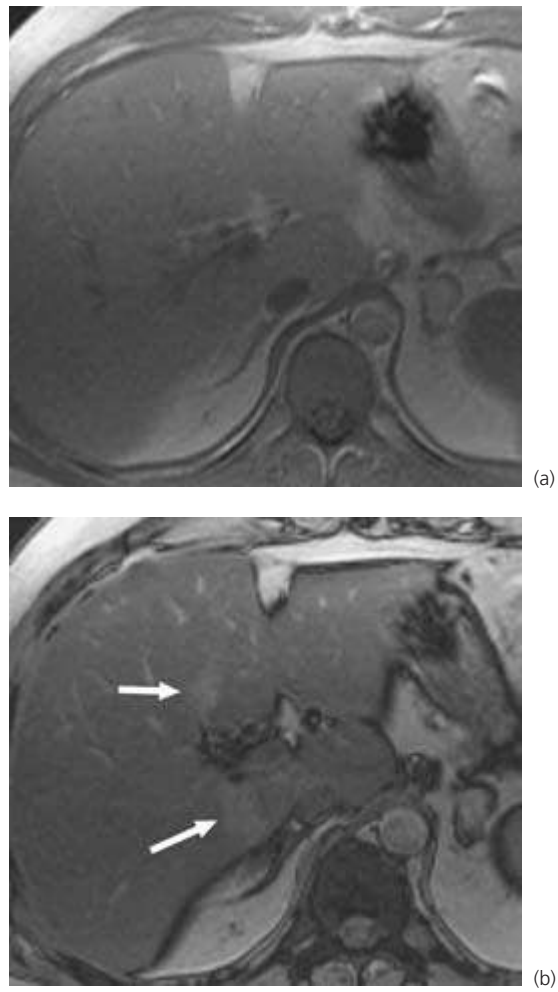


Figure 132.11 Hepatic steatosis. **(a)** Axial in-phase gradient echo (GRE) image (TE 4.6 ms) and **(b)** opposed-phase GRE image (TE 2.2 ms) of the liver demonstrates drop in signal of the liver parenchyma on opposed-phase images consistent with hepatic steatosis. There is relative lack of drop in signal near the gallbladder fossa (arrows) consistent with focal fatty sparing.

signal changes between in-phase and out-of-phase images. Fat accumulation in liver can be diffuse, diffuse with focal sparing, or focal. Typical regions affected by focal fatty accumulation occur around the falciform ligament, gallbladder fossa, and inferior vena cava [39]. One possible explanation is that these are areas of liver prone to irritation or stimulation, resulting in local changes in carbohydrate–lipid metabolism. Contrast-enhanced CT and standard ultrasound are relatively nonspecific and less sensitive for assessment of fatty liver, and can confuse irregularly accumulated lipid with a mass [39]. Fatty liver can lead to reduced CT density, and diminish contrast between a low-density mass and adjacent liver, making the mass less conspicuous.

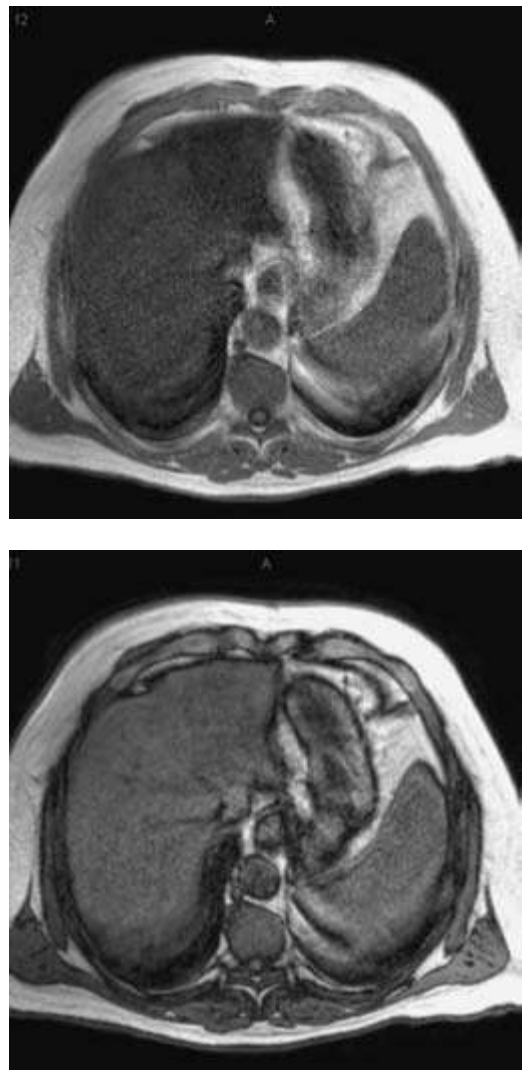
Iron deposition disease

Iron can accumulate within the liver by two basic mechanisms: accumulation within hepatocytes through normal

metabolic chelation mechanisms; or uptake within the phagocytic Kupffer cells, representing part of the reticuloendothelial system. Serum iron and transferrin saturation are poorly correlated with the degree of iron overload. Hepatocytes chelate the iron that accumulates within the cytosol. Pancreas also has chelation mechanisms within acinar cells, and can accumulate excess intracellular iron. To some degree, however, iron accumulation can occur in most tissues, typically as a late feature occurring after hepatic stores have reached high levels. Important examples include the pituitary and heart, where this can result in impaired pituitary function and fatal cardiac arrhythmias and congestive heart failure. Patients presenting with a first-time diagnosis of primary hemochromatosis with the combined findings of elevated liver and cardiac iron deposition, and congestive heart failure, have a poor prognosis with a 6-month life expectancy. There is now a clinically available genetic test that can be used for screening purposes and which is relatively inexpensive.

In secondary hemochromatosis, iron overload occurs secondary to excess red cell turnover from exogenously derived red cells as a result of blood transfusion therapy, as seen in patients with underlying red cell or bone marrow abnormalities such as thalassemia, mastocytosis, or myelofibrosis. Alternatively, endogenously derived excess iron from red cell turnover can result from polycythemia rubra vera or from myoglobin in rhabdomyolysis, or from siderosis related to alcoholic liver disease. In secondary hemochromatosis, the mechanism of iron accumulation is different from that in primary hemochromatosis: increased uptake of iron derived from hemoglobin from dying or abnormal red cells taken up by the reticuloendothelial system leads to iron accumulation in Kupffer cells within liver sinusoids and in splenic tissue. In contrast to primary hemochromatosis, the pancreas does not typically accumulate iron. The clinical significance of primary hemochromatosis includes the observation that many patients develop cirrhosis and approximately 25% of patients develop HCC.

The presence of iron deposition within the liver may be detected by MRI of the liver [42]. Liver biopsy has been used for biochemical determination of iron overload and has been used as the basis for therapy management in patients treated by periodic phlebotomy and iron chelation therapy; however, this method has bleeding risks associated with the invasive procedure, and is susceptible to sampling error in patients with heterogeneous iron deposition in the liver. The sensitivity of CT is insufficient, with a minimum threshold for liver iron more than five times above the normal liver iron load, particularly in cases with fatty liver. MRI is sensitive to iron concentration in the liver because of the paramagnetic properties of iron, resulting in T2 or T2* effects that diminish the signal intensity on both single-shot breath-hold T2 images and breath-hold T1-weighted multiecho SGE images (Fig. 132.12) [42]. Quantitative assessment of liver iron concentration based on MRI has been demonstrated using both SGE and spin echo sequences, relying on measurements of T2* and T2 decay. Coronal breath-hold T2-weighted single-shot FSE images, which should be obtained as part of a routine abdominal MRI examination, are very useful for rapid visual evaluation, providing slices that include liver, psoas muscle, and spleen within the same image [43]. Normally, liver signal intensity is near the midpoint between the lower signal intensity of muscle and the higher signal intensity of spleen. In iron overload disease, the liver signal intensity becomes as low as or lower than skeletal muscle. In secondary iron overload, spleen similarly becomes dark. In cases where there is bone marrow abnormality, such as in myelofibrosis, normal high-signal marrow fat becomes replaced with low-signal cellular marrow hypertrophy and


(a)

(b)

Figure 132.12 Secondary hepatic hemosiderosis. **(a)** Axial in-phase gradient echo (GRE) image (TE 4.6 ms) and **(b)** opposed-phase GRE image (TE 2.2 ms) demonstrate relatively decreased signal on the in-phase images of the hepatic and splenic parenchyma consistent with secondary hemosiderosis.

sclerosis [43]. Chronic iron overload can lead to cirrhosis and increased risk for HCC, complications that can be assessed on MRI. For more sensitive and potentially quantitative noninvasive measurement of liver iron, T2* and T2 decay rate measurements can be performed. T2* decay is a measure of how quickly protons lose phase coherence without use of refocusing pulses, and T2 decay is a measure of proton-dephasing rates after application of refocusing pulses, measuring only dephasing effects that are not correctable [44]. Generally, the T2* decay rate is more sensitive to lower levels of intracellular iron accumulation [42]. Intracellular iron accumulation can cause localized magnetic field distortion that leads to susceptibility effects, which result in more rapid loss of phase-dependent signal [42].

One method used to detect this effect is based on multiecho GRE imaging to measure T2* decay, whereas a spin echo single-shot or echo-planar technique with increasing echo times may be used for T2 decay measurements [42]. When performing imaging dedicated to iron measurement, a series of GRE images are acquired with increasing increments of TE [45]. As TE lengthens, the proton dephasing leads to progressively increased loss of signal intensity, and this process has been shown to be proportionately increased in relation to intracellular liver iron concentration. When performing a dedicated T2* analysis of liver iron, TEs may be selected to correspond to in-phase echoes to avoid potential out-of-phase effects from fat, a potentially spurious effect in the setting of fatty liver infiltration [42]. At minimum, however, routine imaging of the abdomen and liver should include a dual-echo SGE acquisition that can be used in conjunction with the coronal single-shot T2. The longer second echo image (TE 4.4 ms) should show darkening of the liver compared with the shorter echo image (TE 2.2 ms) in the setting of elevated liver iron concentration (Figs 132.12 and 132.13) [42]. The sensitivity to liver iron may be increased on SGE imaging by increasing the echo time to include, for example, echoes at 8.8 and 13.2 ms. If only routine shortest possible dual-echo out-of-phase (TE 2.2 ms) and in-phase (TE 4.4 ms) imaging is used, then the relative sensitivity of the single-shot spin echo technique is more sensitive. With this approach, demonstration of low liver signal on single-shot spin echo alone indicates a relatively lower liver iron concentration, and demonstration of low liver signal on both single-shot spin echo and on the longer dual-echo SGE indicates relatively higher liver iron burden. Others have shown that liver iron concentration may be calculated and that noninvasive measurement of tissue iron concentration may be feasible. This represents the only noninvasive technique available for liver iron quantitation, and could be used for example for following hemochromatosis patients on therapy, minimizing the need for liver biopsy. Although it is tempting to suggest the use of MRI for genetic hemochromatosis screening, it has been noted that a genetic marker test is accurate and relatively inexpensive [45]. Regardless, the

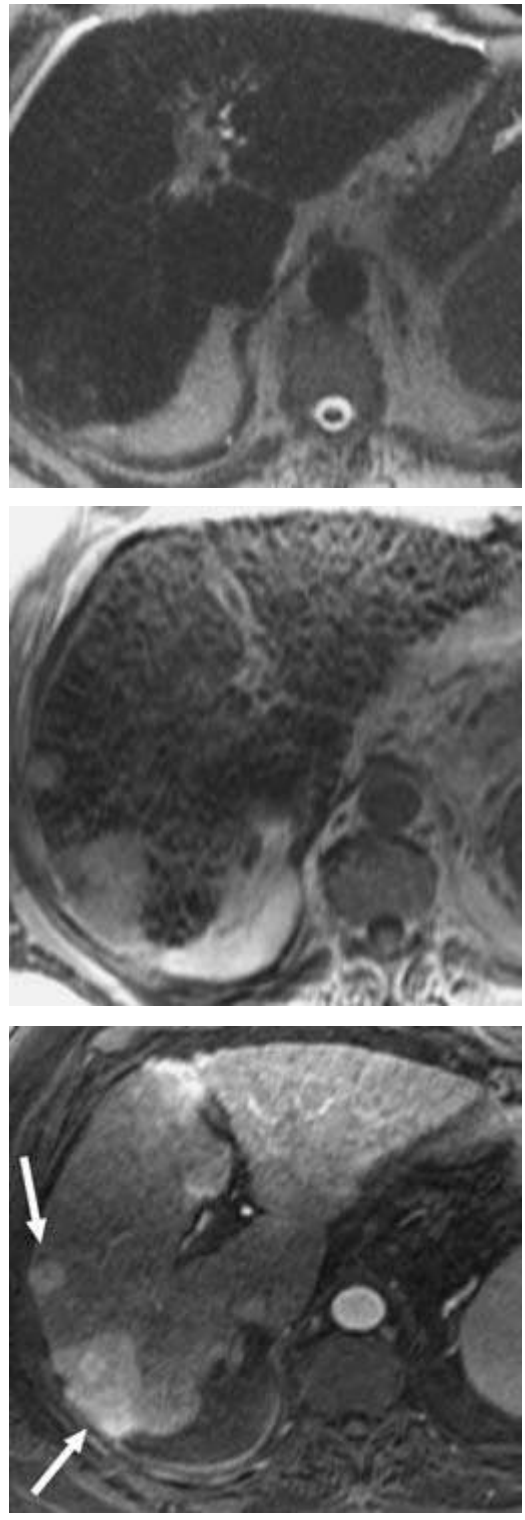


Figure 132.13 Primary hemochromatosis with hepatocellular carcinoma. **(a)** Axial T2 image and **(b)** axial T1 gradient echo (GRE) image demonstrate low signal in the hepatic parenchyma consistent with iron deposition. **(c)** Axial three-dimensionalGRE image during arterial phase after contrast administration demonstrates arterial enhancing lesions (arrows) in the right lobe of liver consistent with multifocal hepatocellular carcinoma in patient with hemochromatosis.

ability to determine tissue iron concentration quantitatively by MRI is a clinically valuable test and there are attempts to develop useful methodology for routine clinical imaging [46].

Acute hepatitis

Inflammatory liver disease can result from a large number of etiologies, including idiopathic, drug induced, viral, alcoholic, and gallstone bile duct obstruction. It has been noted that MRI may be sensitive to acute hepatitis [47]. On MRI, the most sensitive images are the postgadolinium breath-hold SGE images acquired during arterial phase. It has been reported that this abnormal enhancement becomes more marked and can persist into the venous and delayed phases as the severity of disease increases, and can resolve in cases when hepatitis resolves. Furthermore, the arterial-phase timing critically determines sensitivity to mild acute hepatitis. By performing SGE imaging every 5 s after administration of gadolinium in a patient with mild acute hepatitis, irregular liver enhancement is detectable only during the time when the portal veins are filling with contrast, and the hepatic veins are still unenhanced. In mild hepatitis, images acquired before portal venous filling are too early, and liver images acquired when the hepatic veins are filled are too late. For most patients, optimal timing falls between 18 and 22 s after initiation of the gadolinium injection into an antecubital vein, administered at 2 mL/s, followed by a 20-mL saline wash-in bolus. No other imaging technique has been shown to be sensitive for detection of acute hepatitis. MRI could be used as a diagnostic aid in patients with equivocal liver enzyme elevation and nonspecific symptoms, and in patients presenting with fatty infiltration. The reasons for heterogeneous liver enhancement in acute hepatitis have not been fully determined. Hypothetically, it may be that the areas of relative arterial-phase hyperenhancement represent regions of abnormality [48]. In this case, periportal inflammation may differentially compress the lower pressure portal vein intrahepatic branches, leading to preferential segmental hepatic arterial perfusion. Alternatively, the inflammation may lead to altered vascular regulatory effects, with vasodilation and increased hepatic arterial flow to the involved regions. Pathological correlation is challenging given that histopathological correlation lacks the ability to determine pathophysiological in-vivo processes involved in hemodynamics, an advantage inherent to contrast-enhanced imaging.

Findings suggestive of acute hepatitis (seen as irregular arterial-phase gadolinium enhancement) in the setting of fatty liver (seen as signal drop on opposed-phase GRE images) are consistent with steatohepatitis and raise the possible diagnosis of nonalcoholic steatohepatitis or nonalcoholic acute steatohepatitis. Nonalcoholic acute steatohepatitis is a recognized disease entity thought to represent a hepatitis that is directly related to excess intracellular fat accumulation within hepatocytes [48,49]. This disease has a strong association with obesity and has a risk of progression to chronic hepatitis and cirrhosis. Given that obesity is an epidemic in the USA, the health concerns regarding nonalcoholic acute steatohepatitis is significant. MRI has greater sensitivity and specificity for detection of fatty liver, and is the only imaging test sensitive for milder cases of hepatitis as compared with CT or ultrasound. Multiple causes of transient hepatic perfusion abnormalities have been described; however, in cases presenting clinically with right upper quadrant pain and abnormal liver arterial-phase perfusion, as described in this study, acute hepatitis should be the major diagnostic consideration [48]. It may be argued that patients with right upper quadrant abdominal symptoms should be preferentially examined by MRI rather than CT. MRI has potential relative strengths with regard to contrast sensitivity, and can provide excellent temporal resolution because of the small contrast volume used, in combination with the acquisition of contrast data for the entire liver over approximately 4–5 s, usually in the center of a two-dimensional T1-weighted GRE sequence using interleaved phase acquisition. The safety profile of gadolinium agents and nonionizing radiation imaging for a multiphase examination are also attractive characteristics of MRI.

Chronic hepatitis and cirrhosis

A major complication of chronic hepatitis is cirrhosis. In Western nations, alcohol-induced hepatitis used to be the most common etiology, but viral hepatitis is now the most common cause. Globally, viral hepatitis is the most common association with chronic hepatitis, cirrhosis, and HCC. The feature of fibrosis associated with cirrhosis is progressive enhancement on delayed MRI, resulting from leakage of gadolinium contrast agent from the intravascular into the interstitial space within the fibrotic regions [50]. The typical patterns of cirrhosis include fine reticular and coarse linear bands, with these fibrotic bands outlining foci of regenerative nodules. If active hepatitis is present, the fibrotic tissue bands may have edema, and appear high in signal on T2-weighted images, and the liver tissue may develop irregular patchy areas of enhancement seen mostly on arterial-phase images [50]. Regenerative nodules occur in the setting of cirrhosis, and represent relatively more normal hepatic parenchyma that derives the major blood supply from the portal venous system [51]. These nodules maximally enhance during portal venous-phase postgadolinium SGE images, and are usually less than 1 cm in diameter. These nodules can accumulate iron, and appear low in signal on both SGE T1- and single-shot FSE T2-weighted images, with little enhancement appreciated on postgadolinium SGE images (Fig. 132.14) [49].

Dysplastic nodules are premalignant and are believed to have the potential to transform into progressively higher grades of dysplasia, and finally into HCC [51]. Dysplastic nodules are typically larger than regenerative nodules, and can be seen to grow over a period of weeks or months [50,51]. These lesions can show overlap with HCC, and may show mildly elevated T1-weighted signal and low T2-weighted

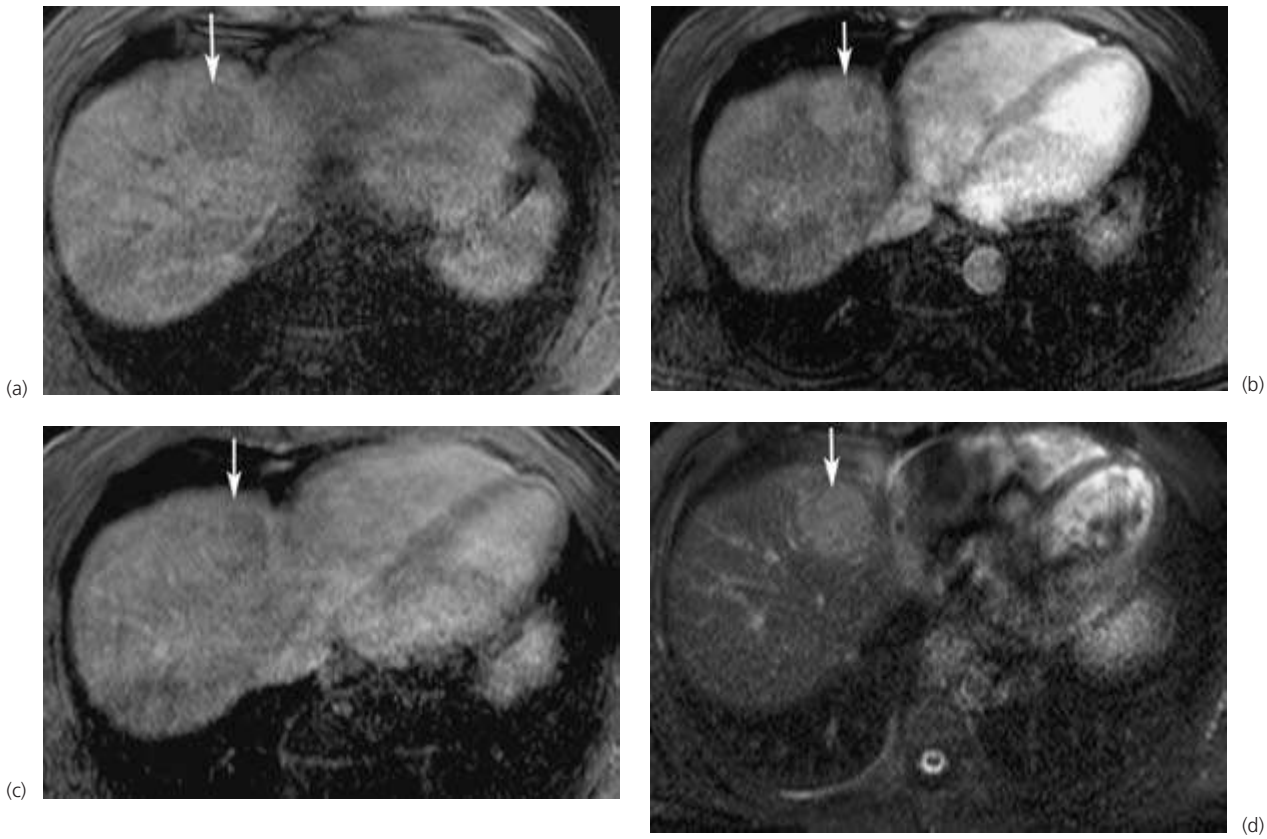


Figure 132.14 Hepatic cirrhosis with hepatocellular carcinoma. **(a)** Axial, fat-suppressed, intermediate T2-weighted image demonstrates cirrhotic liver with nodular contour. Lesion (arrow) in the hepatic dome shows increased signal on the intermediate T2-weighted image **(a)**, is isointense to liver on precontrast, fat-suppressed, three-dimensional gradient echo (GRE) image **(b)**, and enhances avidly during arterial phase on postcontrast, fat-suppressed, three-dimensional GRE image **(c)**. It "washes out", becoming isointense relative to adjacent liver parenchyma on delayed fat-suppressed, three-dimensional GRE image.

signal. Features that help distinguish HCC include increased T2-weighted signal, transient marked arterial-phase post-gadolinium enhancement, capsular peripheral rim enhancement on venous- and equilibrium-phase images, relative washout on delayed images, and size greater than 2–3 cm. It may be that higher-grade dysplastic nodules overlap more with the HCC features; however, this distinction may be of small clinical significance because higher-grade dysplasia has the potential to transform to HCC rapidly (Fig. 132.14). HCC frequently invades vessels and a small subset may contain fat.

Portal hypertension may result from obstruction at presinusoidal, sinusoidal, postsinusoidal, or a combination of these sites, corresponding to abnormalities of portal venous, hepatic fibrosis, hepatic venous, or mixed disease. Images optimal for visualizing changes related to portal hypertension are obtained on equilibrium-phase SGE images with fat suppression. In early or mild portal hypertension images show dilation of the portal vein, and possibly splenic vein. In more severe and chronic cases, the portal vein can occlude and become thin or not visualized, with development of multiple smaller-caliber collaterals seen within the porta hepatis, so-called cavernous transformation. Furthermore, portosystemic collaterals can form and be seen as increased number and size of retroperitoneal vessels in the region of the splenic hilum, gastrohepatic ligament, paraesophageal region, and with demonstration of splenorenal venous connections. A patent periumbilical vein can be seen as a vessel, sometimes massive, extending from the left portal vein anteriorly along the falciform ligament toward the anterior abdominal wall umbilical region. Ascites is commonly seen in combination with more advanced portal hypertension as simple uniform high-signal T2-weighted fluid in the free intraperitoneal space.

Biliary tree

MRI has evolved to become a valuable imaging tool for the pancreas and biliary tree. Applications in this area have been aided greatly by developments in MRA and MRCP, which allow selective visualization of the vascular structures and biliary and pancreatic ducts. MRCP is useful for the detection of biliary obstruction, calculi, and normal variants of biliary

anatomy that have surgical importance [52]. The bile ducts appear as high signal intensity with this technique. Calculi appear as low signal intensity filling defects. Unlike CT, where the attenuation of calculi is variable and may approximate that of surrounding soft tissue, calculi identified by MRI are almost universally low signal intensity on T2-weighted images. MRCP has been shown to be accurate for the detection of calculi in the common bile duct [52].

Cholangiocarcinoma

Because of its ability to image bile ducts, vascular structures, and soft tissue of the hepatic parenchyma in one examination, MRI is useful for the detection of cholangiocarcinoma [53]. Cholangiocarcinoma shows a characteristic pattern of enhancement on dynamic-enhanced images. Classically it shows little uptake of contrast on the arterial and portal phases and increased enhancement on delayed images (see section Magnetic resonance cholangiopancreatography) [53,54]. With hilar cholangiocarcinomas (Klatskin tumors), MRCP can show the multiple obstructed ductal segments. Unlike percutaneous transhepatic cholangiography, which requires separate injection of the different ductal segments if they are completely obstructed, MRCP visualizes all the obstructive ductal segments simultaneously [32]. Therefore, MRCP can assist in treatment planning for these tumors [32].

Pancreas

Developments in faster imaging systems with improved contrast resolution continue to enhance the role of MRI in imaging pancreatic abnormalities with equivocal features on CT. In addition, MRCP is accepted as a noninvasive and accurate method of imaging the pancreatic duct and is now considered an important imaging technique for the diagnosis of chronic pancreatitis. Endoscopic ultrasound (EUS) and positron emission tomography (PET) have emerged as important complementary imaging modalities for imaging of pancreatic abnormalities.

MRI of the pancreas is optimally performed with a high-performance gradient system (1.5 T) using phased-array torso coil to improve the signal-to-noise ratio with a smaller field of view and thin-slice profile [55]. Breath-hold GRE or FSE sequences are most commonly used. Moderately T2-weighted FSE and single-shot FSE (HASTE) sequences, followed by T1-weighted in-phase GRE and T1-weighted opposed-phase GRE images are included in the MRI protocol. To evaluate cystic lesions of the pancreas or pancreatic ducts in patients with chronic pancreatitis, coronal and axial MRCP with single-shot FSE or HASTE are obtained. For gadolinium-DTPA-enhanced MRI (0.1 mmol/kg body weight), triple-phase, breath-hold, fat-suppressed, three-dimensional, fast SGE sequences are acquired. Fat-suppressed, high-resolution, T1-weighted images acquired 10 min after administration of mangafodipir trisodium (5 mol/kg, in a slow bolus over 1–2 min) are useful for lesion detection in equivocal cases. The basis for utilization of mangafodipir is that normal pancreatic parenchyma enhances following mangafodipir administration and becomes hyperintense on T1-weighted images, whereas tumors do not enhance. Mangafodipir is usually reserved for equivocal cases for tumor detection and differentiation from benign inflammatory masses of the pancreas.

MRCP with single breath-hold, thick (2–5 cm) section can provide excellent selective display of the whole extrahepatic biliary tract and pancreatic duct with no respiratory artifacts and few susceptibility artifacts. Some authors have stressed the value of secretin administration in improving pancreatic ductal details in MRCP. The basis of secretin-enhanced MRCP is that exogenous administration of secretin stimulates secretion of pancreatic juice, notably fluid and bicarbonate, which consequently increases the volume of stationary fluid in the pancreatic ducts. Secretin (1 mL per 10 kg body weight) administration 10–15 min prior to MRCP improves pancreatic duct and side-branch delineation and permits evaluation of pancreatic flow dynamics and assessment of pancreatic exocrine function.

Normal anatomy and variants

On MRI, the normal pancreas has signal intensity similar to that of the liver. It has relatively high signal intensity compared with the liver on in-phase T1-weighted images. In opposed-phase T1 images, a hypointense rim surrounding the pancreas is observed due to etching artifact. On fat-suppressed images, the relative signal intensity of pancreas increases, thus facilitating detection of pathological conditions with decreased signal intensity. Being a very vascular organ, the pancreas shows intense contrast enhancement in the arterial phase followed by rapid washout of contrast agent (Fig. 132.15). This makes optimum arterial-phase imaging crucial for accurate detection of most focal pancreatic lesions, which usually enhance less than the normal parenchyma and hence are rendered more conspicuous.

The head and neck regions of pancreas reveal lateral contour anomalies in up to 35% of individuals, the most common of which is discrete lobulation of pancreatic tissue lateral to the gastroduodenal or anterior superior pancreaticoduodenal artery. Pancreas divisum results from failure of fusion of ventral and dorsal pancreatic buds [56]. In this anomaly, the dorsal pancreatic duct drains the neck, body, and tail regions through the minor papilla of duodenum, whereas the ventral pancreatic duct drains the head and uncinate process through the major papilla (Fig. 132.16) [52]. The dorsal duct is prone to obstruction, with associated increased incidence of pancreatitis. Bret and colleagues have reported an accuracy of 100% for MRCP in the diagnosis of pancreas divisum. In patients with recurrent episodes of acute pancreatitis, MRI and MRCP are extremely useful for detecting anatomical variants such as pancreas divisum [56].

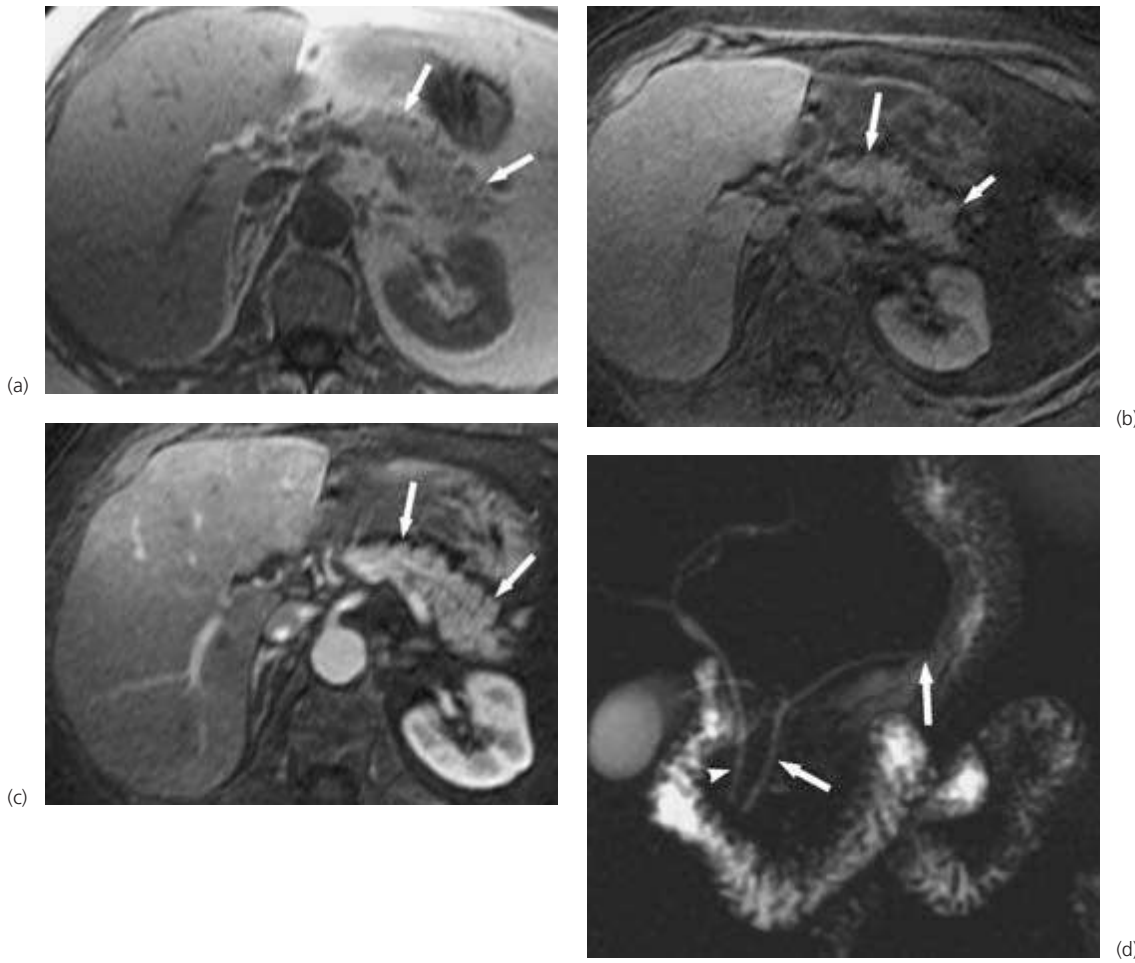


Figure 132.15 Normal pancreas. **(a)** Axial, two-dimensional, T1-weighted and **(b)** precontrast, three-dimensional, fat-suppressed, T1-weighted gradient echo (GRE) images demonstrate normal nonenhanced T1 signal of the pancreatic parenchyma of the body and tail of pancreas that is isointense or higher in signal than liver parenchyma. **(c)** Axial, three-dimensional, postcontrast GRE image demonstrates normal peak enhancement of the pancreatic parenchyma during the arterial phase. **(d)** Magnetic resonance cholangiopancreatography demonstrates normal relative anatomy of the common bile duct (arrowhead) and the pancreatic duct (arrows).

Annular pancreas is an anomaly characterized by pancreatic tissue partially or completely encircling the duodenum. In annular pancreas, MRI shows apparent thickening of the posterior, anterior, and lateral walls of the descending duodenum caused by tissue having signal intensity and enhancement pattern similar to that of pancreatic parenchyma. In addition, MRCP demonstrates the aberrant pancreatic duct encircling and extending lateral to the duodenum. Anomalous union of the pancreatic and bile ducts has been postulated to allow reflux of pancreatic enzymes into the bile duct, thus weakening the bile duct wall. This mechanism has also been postulated as a predisposing factor for the development of a choledochal cyst [56].

Acute pancreatitis

Acute pancreatitis is an acute inflammatory process of the pancreas that may include suppuration, necrosis, and hemorrhage of pancreatic tissue with variable involvement of other regional and remote tissues. The diagnosis can often be made with serum amylase and lipase levels, and cross-sectional imaging techniques are immensely useful for staging, detecting complications such as infection, necrosis and pseudocyst formation, and guiding percutaneous and surgical interventions. At the International Symposium on Acute Pancreatitis held in Atlanta, Georgia in 1992, an acceptable series of clinical definitions for classifying the disease and its complications was described [57]. In patients with mild acute pancreatitis, interstitial and peripancreatic edema predominates.

The severe form of acute pancreatitis is characterized by extensive pancreatic necrosis and inflammation of the peripancreatic fat tissues that may lead to variable degrees of tissue necrosis, pancreatic and peripancreatic abscess, and parenchymal hemorrhage. Pancreatic gland necrosis has been defined as focal or diffuse areas of nonviable parenchyma typically associated with peripancreatic fat necrosis. Acute fluid collections occur in about 50% of patients in the early

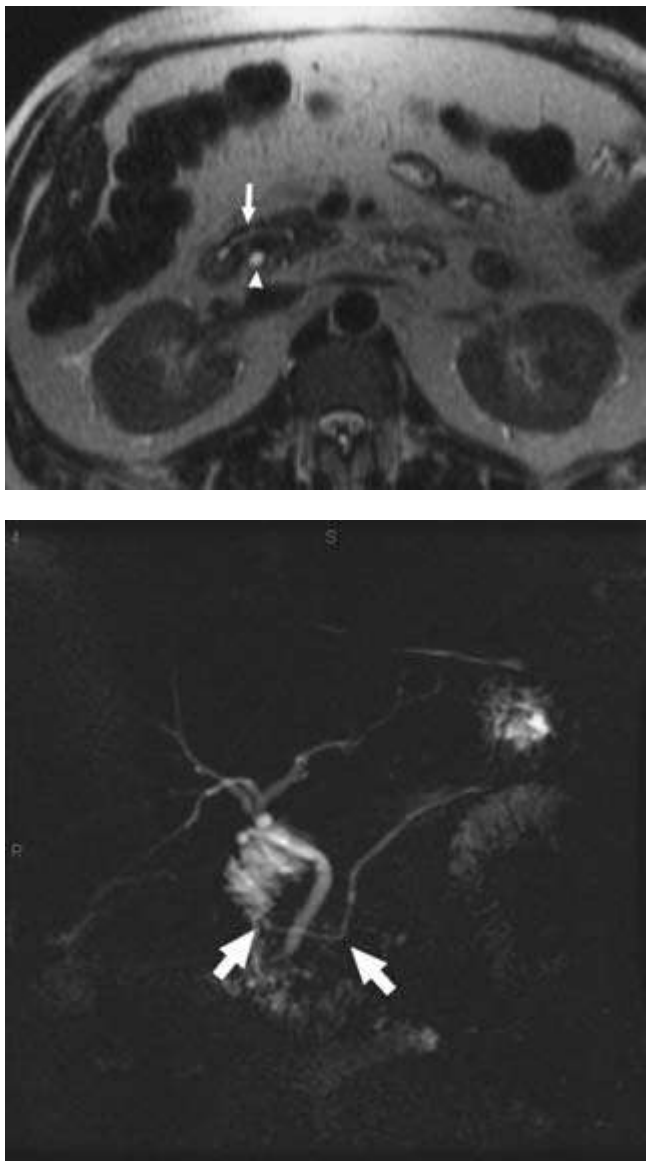


Figure 132.16 Pancreas divisum. **(a)** Axial T2-weighted image and **(b)** magnetic resonance cholangiopancreatography demonstrate pancreas divisum with primary draining pancreatic duct of Santorini (arrow) crossing the common bile duct (arrowhead) to empty via the minor ampulla.

course of the disease and contain enzyme-rich pancreatic juice. Encapsulated collections of pancreatic fluid called pseudocysts evolve from the persistent peripancreatic fluid collections. Peripancreatic abscesses represent circumscribed pus collections that occur in areas of limited necrosis with secondary infections. The frequency of development of sepsis increases with time after onset of symptoms of pancreatitis. Infected necrosis is associated with high mortality and represents infected pancreatic or peripancreatic necrotic tissue.

CT is the imaging modality of choice for diagnosis and staging of suspected acute pancreatitis, follow-up evaluations, and guidance for percutaneous and surgical intervention. Some reports have raised concerns about possible aggravation of pancreatic injury with the use of iodinated contrast agents in experimental and human studies. In these circumstances and in patients who have other contraindications to iodinated contrast, such as renal dysfunction, MRI with gadolinium offers a safer alternative and excludes the radiation burden associated with CT [57]. MRCP using T2-weighted and gadolinium-enhanced, fat-suppressed, T1-weighted sequences also helps therapeutic planning and demonstrates the size, location, and possible communication of a pseudocyst with the pancreatic duct, possible choledocholithiasis, presence of ductal distention, or disruption of the pancreatic duct. Normal pancreatic parenchyma shows high signal intensity on T1-weighted fat-suppressed images and possesses a uniform capillary blush in mild pancreatitis. More severe cases of pancreatitis are associated with variable diminution of signal intensity on T1-weighted fat-suppressed images and immediate postgadolinium GRE images (Fig. 132.17) [58]. Nonenhancing pancreatic parenchyma suggests the possibility of necrosis or suppuration. MRI is at least as sensitive as CT in depiction of necrosis and peripancreatic fluid collections and is superior to CT in demonstration of the internal consistency and drainability of these collections. Some authors have recommended that predrainage MRI should be performed in patients with pancreatic collections to differentiate pancreatic abscess from pancreatic necrosis, which is not usually amenable to percutaneous management, to avoid infectious complications [57].

Chronic pancreatitis

Chronic pancreatitis is an irreversible inflammatory disease of the pancreas characterized by replacement of glandular acini, ducts, and blood vessels by fibrous tissue that causes ductal strictures, obstruction, and dilatation leading to parenchymal atrophy and stone formation. Early changes of chronic pancreatitis are difficult to recognize with CT. MRI and MRCP have been widely accepted as the primary imaging modalities for the diagnosis of chronic pancreatitis [59]. Findings with MRI and MRCP include atrophy of the gland, changes in signal intensity of pancreatic parenchyma with reduced signal on T1-weighted images, irregular dilatation of the pancreatic duct, pancreatic calcification, and chronic pseudocysts [60]. Pancreatic parenchyma shows decreased signal intensity on T1-weighted fat-suppressed images attributed to fibrosis and reduced concentration of soluble proteins. On T2-weighted images, the signal intensity of the pancreas may be normal because of the presence of fibrosis with variable degrees of inflammation and residual pancreatic tissue (Fig. 132.18) [60]. Differentiation between pseudotumorous lesions in cases of chronic pancreatitis and pancreatic carcinoma may be difficult due to similar mild contrast enhancement of focal lesions. However, MRI with mangafodipir may improve the detection rate and characterization of focal pancreatic lesions because of better delineation of the abnor-

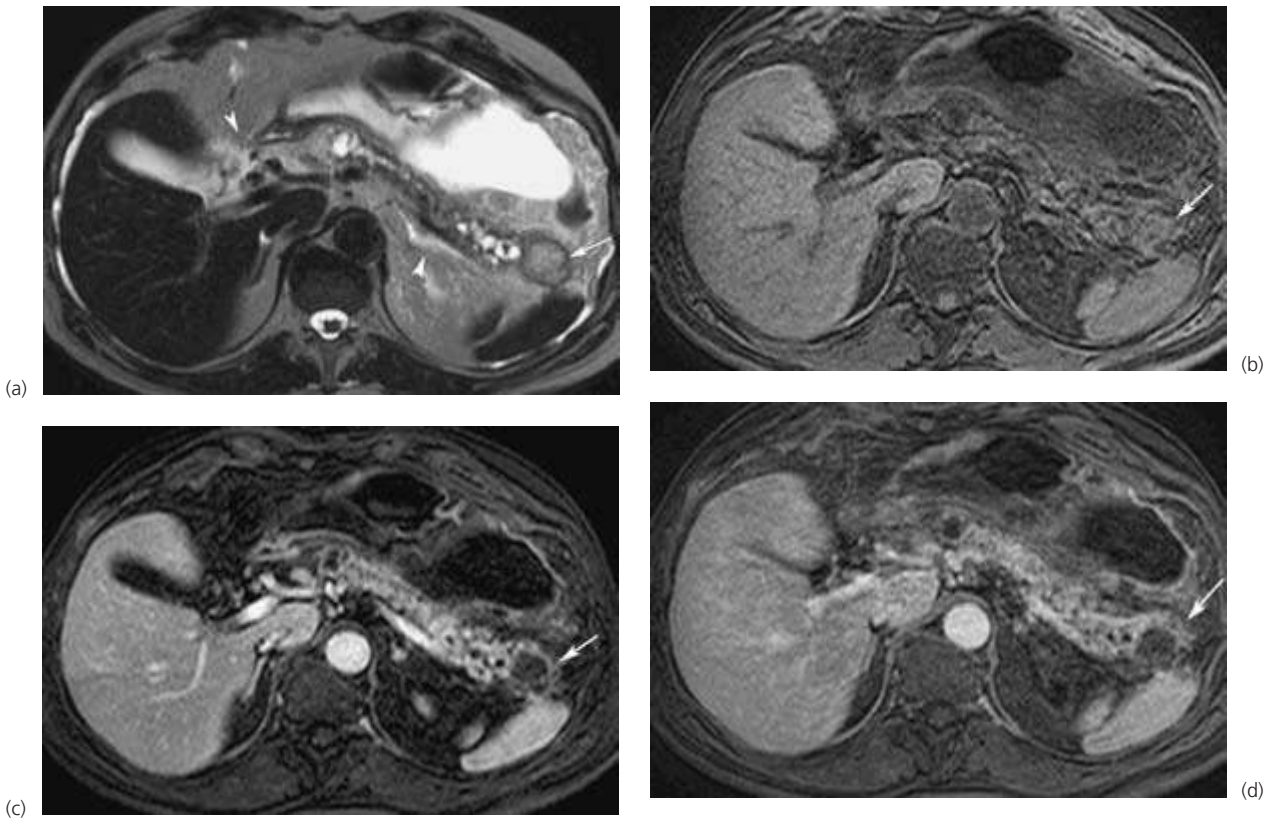


Figure 132.17 Acute on chronic gradient echo pancreatitis. Axial T2 image **(a)** and three-dimensional precontrast images **(b)** before contrast, **(c)** during arterial phase, and **(d)** delayed post contrast demonstrate peripancreatic edema and fluid (arrowheads), relatively low T1 signal in the pancreatic parenchyma, with focal area of necrosis and evolving pseudocyst formation at the tail of the pancreas (arrow) secondary to pancreatic necrosis. There is mild diffuse pancreatic ductal dilation likely reflecting change of prior pancreatitis.

mal area. MRCP demonstrates pseudocysts in or near the pancreas and ductal abnormalities, including segmental dilatation, narrowing, ductal filling defects representing calculi, or mucinous casts in pancreatic ducts [61]. In some instances, the ductal communication of pseudocyst can be demonstrated with MRCP, which can frequently be difficult with ERCP. Prior studies have reported significant increase in pancreatic duct visibility after secretin injection ($P < 0.05$) [62]. These authors have also reported significantly higher fluid filling of the duodenum in healthy persons and in those with pancreas divisum compared with those with chronic pancreatitis or pancreatic tumors [62]. Focal pancreatitis has been reported to occur in 20% of cases and typically involves the pancreatic head. It often simulates pancreatic malignancy, and differentiation, though critical, is often difficult. Although signal intensity of chronic pancreatitis is decreased compared with the normal pancreas on T1-weighted images, there is considerable overlap of signal characteristics and contrast enhancement patterns with pancreatic malignancies. MRCP following secretin may help in demonstrating an associated stricture of the pancreatic duct and the altered filling dynamics of pancreatic fluid that is generally absent in pancreatic malignancy [62]. A positive duct-penetrating sign, seen on MRCP as a smoothly stenotic or normal main pancreatic duct penetrating through the apparent tumor, may be useful in the distinction from pancreatic malignancies. Autoimmune pancreatitis is defined as a special form of chronic pancreatitis caused by an autoimmune mechanism or associated with autoimmune and related diseases such as primary sclerosing cholangitis and ulcerative colitis. Typical MRI characteristics include focal or diffuse pancreatic enlargement, absence of parenchymal atrophy, and significant pancreatic duct dilatation proximal to the site of stenosis. In patients with cystic fibrosis, the pancreatic glandular tissue is significantly reduced in size and is replaced with fat. In older cystic fibrosis patients, complete or incomplete lipomatosis of the pancreas is the most frequent pattern and correlates with pancreatic exocrine dysfunction. In addition, pancreatic calcification and cystic and macrocystic pancreas may be seen on MRI.

Trauma

CT plays an important role in the detection and management of pancreatic trauma. In some instances, MRCP may show the duct disruption and associated fluid collections. MRCP offers a useful alternative to invasive ERCP in assessing delayed complications secondary to pancreatic duct injury [63].

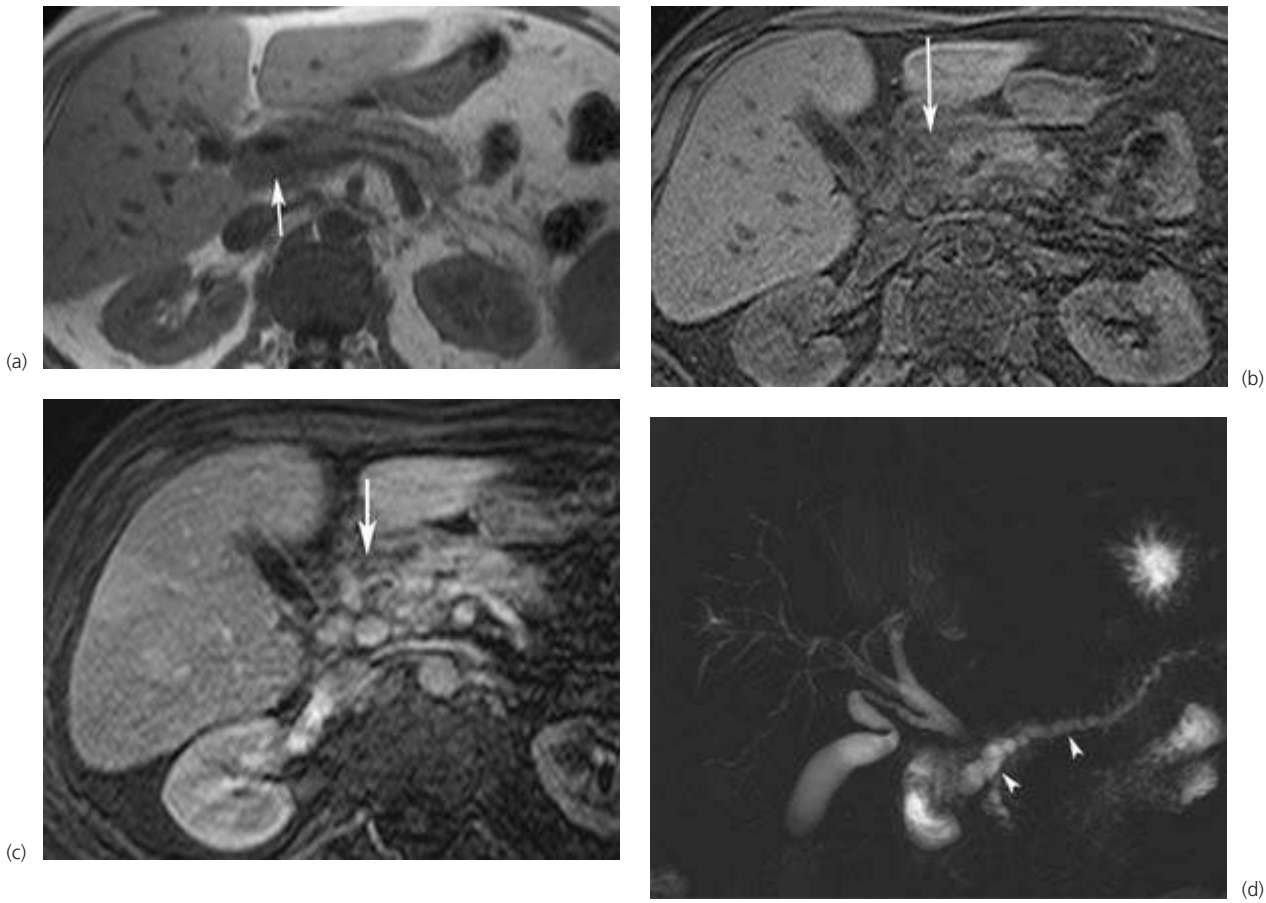


Figure 132.18 Chronic pancreatitis. **(a)** Axial T1 image and **(b)** precontrast three-dimensional gradient echo image demonstrate relatively low signal (arrow) in the region of the head of the pancreas with relative hypoenhancement following contrast **(c)**. **(d)** Magnetic resonance cholangiopancreatography demonstrates associated diffuse dilation of the pancreatic duct (arrowheads).

Neoplasms

Adenocarcinoma

Pancreatic adenocarcinoma is the fourth most common tumor in the USA. About 90% of pancreatic tumors are ductal adenocarcinomas. Neuroendocrine tumors and acinar cell carcinomas constitute about 2%–5% of all pancreatic tumors. The major aims of imaging pancreatic malignancies are detection and staging, which determine the appropriate management options and ultimate prognosis of the disease. Most commonly, adenocarcinoma appears as a mass distorting the contour of the gland with associated findings such as dilated pancreatic duct and common bile duct, atrophy of the gland, vascular invasion, and metastases to regional nodes, liver, and peritoneal cavity. On MRI, T1-weighted spin-echo images with and without fat suppression and immediate post-gadolinium SGE images have been found to be superior to spiral CT for detecting small lesions. Because of their scirrhous character from dense fibrotic tissue, pancreatic adenocarcinomas are generally slightly hypointense relative to the pancreas on T2-weighted images but are difficult to visualize unless there is substantial necrosis [64]. Being relatively hypovascular, ductal adenocarcinomas enhance to a lesser extent than normal pancreatic tissue on early postcontrast images. Hence, they appear distinctly hypointense to the maximally enhanced pancreas during the arterial phase of dynamic contrast enhancement (Fig. 132.19) [65]. However, a thin rim of greater enhancing pancreatic tissue is commonly observed in patients with pancreatic cancers and may help to establish the focal nature of the disease process. Normal pancreatic tissue becomes hyperintense on T1-weighted images after intravenous administration of the tissue-specific contrast agent mangafodipir trisodium [66–69]. Because pancreatic adenocarcinomas do not take up manganese, they are well delineated in the background of enhanced normal pancreatic parenchyma on T1-weighted fat-suppressed images. T1-weighted spin-echo imaging has been reported to be superior to dynamic contrast-enhanced CT for the determination of vascular encasement. Gadolinium-enhanced T1-weighted SGE is extremely useful for evaluating arterial and venous patency. Three-dimensional, contrast-enhanced,

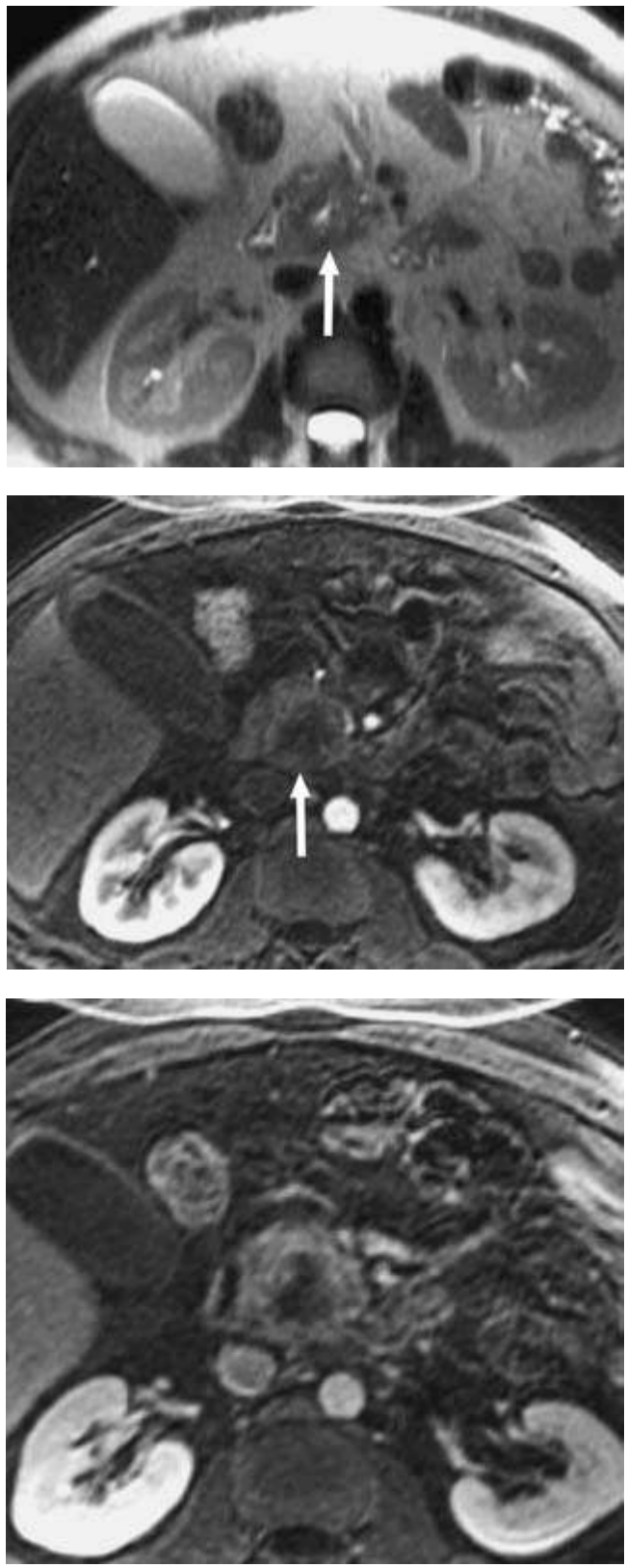


Figure 132.19 Pancreatic carcinoma. **(a)** Axial T2 image demonstrates mass in the head of the pancreas (arrow) with some central necrosis that hypoenhances relative to the remainder of the pancreas during arterial phase **(b)** and portal venous phase **(c)** on postcontrast three-dimensional gradient echo images.

dynamic MRA with fat suppression exquisitely delineates vascular encasement or occlusion for the determination of nonresectability and regional vascular anatomy. MRCP features of pancreatic head adenocarcinoma include encasement and obstruction of the pancreatic duct or bile duct. Dilatation of biliary and pancreatic ducts (double-duct sign) occurs in 77% of cases of pancreatic head carcinoma, biliary duct dilatation occurs alone in 9%, and pancreatic duct dilatation in 12%. MRCP readily demonstrates this double-duct pattern of obstruction, strongly suggesting a pancreatic or ampullary carcinoma. When performed in conjunction with abdominal MRI, MRCP is useful in detecting pancreatic malignancies and in establishing resectability and preventing unnecessary preoperative ERCP. In addition, MRCP is helpful in planning percutaneous biliary drainage and radiation therapy. Metastases to peripancreatic and paraaortic lymph nodes and the lymph nodes in mesenteric and celiac chains can be well depicted on T2-weighted, fat suppressed, spin-echo images and gadolinium-enhanced, T1-weighted, fat-suppressed images. However, detection of small peripancreatic lymph nodes can be difficult on MRI. Gadolinium-enhanced MRI has greater accuracy for detection and characterization of liver metastasis compared with helical CT. MRI is more sensitive than helical CT in the detection of subcentimeter-sized liver metastases and visualization of peritoneal implants [66]. In patients with a focal pancreatic lesion, Diehl and colleagues have documented a significant increase in contrast-to-noise ratio with mangafodipir-enhanced MRI without (106%) and with (82%) fat saturation [67]. In their study, qualitative image analysis also demonstrated a significant improvement of mangafodipir-enhanced fat-suppressed MRI in delineating pancreatic parenchyma ($P < 0.01$) and pancreatic tumors ($P < 0.01$). Romijn and colleagues have reported that MRI following mangafodipir trisodium administration provides better delineation of pancreatic tumors but does not significantly improve the detection rate and staging accuracy of focal pancreatic lesions over MRI without this contrast medium [68].

Neuroendocrine tumors

Pancreatic neuroendocrine tumors or islet cell tumors are believed to originate from the neuroendocrine cells of the pancreas [70,71]. They are uncommon, slow-growing pancreatic or peripancreatic masses that either result in symptomatic hormonal overproduction (functional) or show no clinical findings of hormone secretion (nonfunctional). On MRI islet tumors are well depicted, being hypointense relative to normal high-signal pancreatic parenchyma on precontrast T1-weighted images and typically hypervascular following contrast enhancement (Fig. 132.20) [70,71]. On fat-suppressed T2-weighted images, tumors may be high signal compared with adjacent pancreatic parenchyma. Lesions with increased fibrous component may be T2 hypointense or isointense to pancreas [70,71].

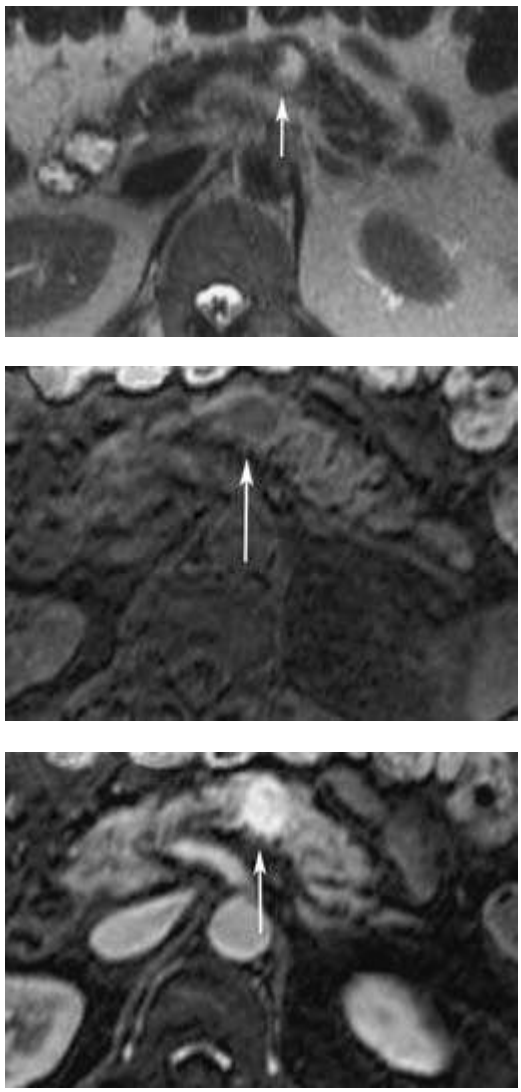


Figure 132.20 Pancreatic islet cell tumor. **(a)** Axial T2 image demonstrates high-T2 signal lesion in the body (arrow). Three-dimensional gradient echo images **(b)** before contrast and **(c)** during arterial phase show prominent enhancement in the arterial phase characteristic of an islet cell tumor (arrow).

Cystic tumors

The differential diagnosis of cystic pancreatic lesions includes both congenital true cysts and acquired cysts.

- Congenital cysts: simple cysts, cysts associated with polycystic disease of pancreas or other disorders.
- Acquired cysts: pseudocysts, hydatid cysts, retention cysts, angiomatous cysts, and cystic neoplasms, i.e., serous cystadenoma, mucinous cystadenoma, mucinous cystadenocarcinoma, papillary cystic neoplasm, intraductal papillary mucinous neoplasm [IPMN], ductal adenocarcinoma with cystic necrosis, teratomatous cysts, cystic choriocarcinoma, and neuroendocrine cystic tumors of pancreas [72].

The most common types of cystic neoplasms of the pancreas are serous and mucinous tumors and IPMN [73]. Serous tumors are benign and typically contain more than six cysts that are smaller than 20 mm and which have a central stellate scar. These tumors typically occur between the ages of 34 and 88 years and have a strong female predilection. Accurate radiological diagnosis and differentiation from mucinous cystic tumors are crucial to avoid aggressive surgical intervention in the case of an asymptomatic serous cystadenoma, which has low malignant potential [73]. On MRI, serous cystadenoma appears as a well-defined lesion that does not show invasion of fat or adjacent organs. On T2-weighted images, the small cysts and intervening septations may look like a cluster of small grape-like hyperintense cysts, best depicted on breathing-independent, single-shot, echo-train, spin-echo sequences. Cyst components are typically smaller than 1.5 cm. Tumor septa usually enhance minimally on early and late postgadolinium images. Delayed enhancement of the central scar may occasionally be observed. Mucinous cystic neoplasms are most often located in the body or tail of the pancreas and have a strong female predilection [74]. These lesions are unilocular or have larger internal (> 1.5 cm) cystic components. These tumors are more sinister neoplasms with malignant potential. They display cysts that are less numerous and larger in size than typically seen with serous cystadenoma. The higher inherent soft-tissue contrast of MRI allows better differentiation between serous and mucinous cystic neoplasms than CT. Mucin produced by mucinous cystic tumors may result in high signal intensity on T1- and T2-weighted images of the primary tumor [75]. If present, liver metastases frequently follow the appearance of the primary pancreatic lesion on T1- and T2-weighted and contrast-enhanced images. Thick irregular septa and solid portions or papillary excrescences may be seen on gadolinium-enhanced, T1-weighted, fat-suppressed images (Fig. 132.21) [76]. Mucinous cystadenoma and cystadenocarcinoma do not reveal a central scar.

IPMN is recognized by dilatation of the main pancreatic duct or branch ducts associated with mucin overproduction. IPMN originates from the main pancreatic duct and its collateral branches and may be differentiated from other mucinous cystic tumors that typically originate from peripheral ducts. IPMN is divided into main duct type (Fig. 132.22), branch duct type, or a combination, depending on the major site of involvement. Cross-sectional imaging of main-duct IPMN shows either diffuse or segmental dilatation of the main pancreatic duct, associated with parenchymal atrophy, especially in more advanced cases [77,78]. Intraductal fungating lesions or mucin deposits may cause small intraductal areas with solid signal intensity seen on MRI. MRCP can demonstrate the dilated ducts as hyperintense collections that are not frequently conspicuous on ERCP [77,78]. Branch-duct IPMN is characterized by cystic ectasia of the branch ducts, forming a mass with lobulated contours that usually

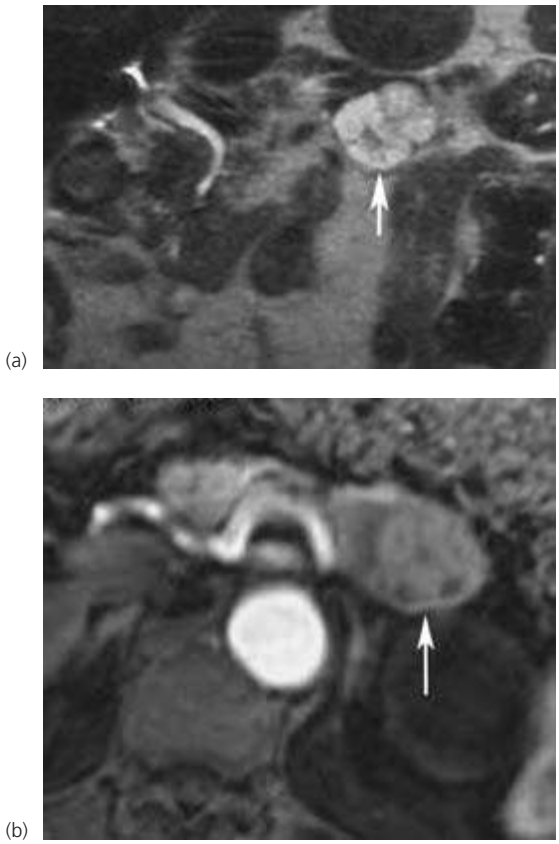


Figure 132.21 Pancreatic mucinous cystadenocarcinoma. **(a)** Axial T2 image demonstrates septated cystic lesion (arrow) in the tail of the pancreas with **(b)** internal nodular enhancement (arrow) consistent with mucinous cystadenocarcinoma.

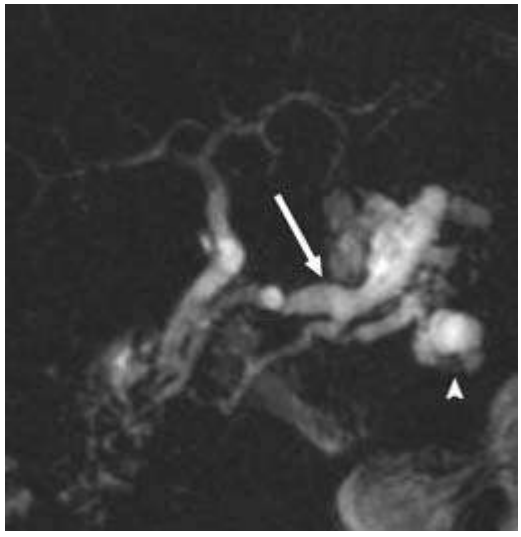


Figure 132.22 Diffuse intraductal papillary mucinous neoplasm. Magnetic resonance cholangiopancreatography demonstrates diffuse dilation of pancreatic duct (arrow) with focal cystic area (arrowhead).

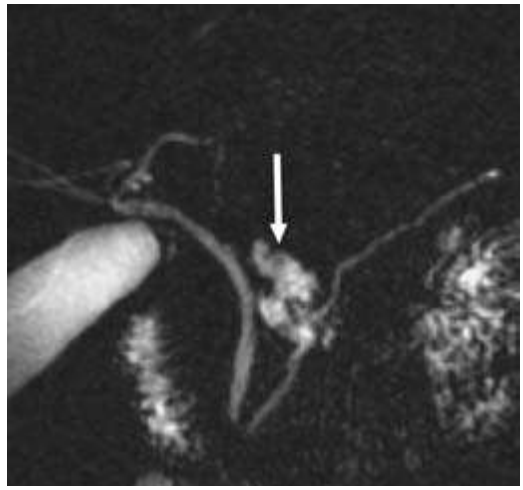


Figure 132.23 Branch-duct type intraductal papillary mucinous neoplasm. Lobulated grape-like cystic lesion in the head of the pancreas (arrow) communicating with the main pancreatic duct.

involves the uncinate process (Fig. 132.23). MRCP demonstrates a grape-like appearance of the lesion and its wide communication with the main pancreatic duct. Rarely, ectasia of a single duct communicating with the main pancreatic duct through a long pedicle may be seen [77,78].

Solid pseudopapillary tumor

Solid pseudopapillary tumor of pancreas is a rare low-grade malignant neoplasm typically occurring in young women, particularly of African American or Asian descent. The mean age of presentation is 25 years. The tumor is usually large (mean 9 cm) and well circumscribed with internal solid and cystic components. MRI shows a complex mass that may have hemorrhagic necrosis with high signal on T1-weighted images and heterogeneous variable signal on T2-weighted images. The differential diagnosis is nonfunctioning cystic islet cell tumor.

Lymphoma

Pancreatic lymphoma is almost invariably of the non-Hodgkin B-cell type and may be associated with concomitant infection with a retrovirus or Epstein–Barr virus (Burkitt lymphoma). It is usually associated with peripancreatic and retroperitoneal lymphadenopathy or splenic, hepatic, renal, and epidural space lesions. Presence of intact fat planes between the nodes and the pancreas, and anterior displacement of the pancreas help to distinguish peripancreatic lymphadenopathy from a primary neoplasm [79]. Diffuse pancreatic enlargement often noted in pancreatic lymphoma may be due to diffuse pancreatic tumor, tumor-induced pancreatitis, or pancreatitis associated with tumor lysis from chemotherapy. MRI demonstrates diffuse or focal pancreatic enlargement that is isointense to normal pancreatic parenchyma on T1- and T2-weighted images. Contrary to the

enhancement of the normal pancreas after mangafodipir administration, these lesions do not show any uptake of mangafodipir [80].

Pancreatic metastases

The most common primary tumors that metastasize to the pancreas are from the lung, breast, kidney, and melanoma. There are three different patterns of pancreatic involvement, namely localized, multifocal, or diffuse enlargement. Pancreatic metastases should be suspected in the presence of multiple pancreatic masses in a patient with a known primary carcinoma. On MRI most pancreatic metastases appear hypointense on T1-weighted images and hyperintense on T2-weighted images [81]. The majority of metastases are hypointense to a background of high-signal pancreas on T1-weighted fat-suppressed images. Contrast-enhanced MRI may be useful in the evaluation of pancreatic metastases by showing features such as increased vascularity in the presence of a hypervascular primary malignancy [81].

Gallbladder

Magnetic resonance sequences used to evaluate the gallbladder are similar to other imaging protocols used in the abdomen, including axial T1-weighted breath-hold GRE chemical shift sequences, T2-weighted sequences, MRCP, and contrast-enhanced T1-weighted sequences. Both T2-weighted and MRCP sequences are essential in the evaluation of the gallbladder and surrounding structures. Gallstones are low signal intensity against the higher signal intensity of bile. Gallbladder wall edema and pericholecystic fluid are hyperintense on both sequences because of their fluid content.

Contrast-enhanced, breath-hold, fat-suppressed, T1-weighted sequences are used to evaluate the gallbladder and adjacent hepatic parenchyma. Dynamic gadolinium-enhanced MRI may also play a role in differentiating benign and malignant gallbladder masses based on early and delayed enhancement patterns [82].

Hepatocyte-specific T1-shortening agents such as mangafodopir trisodium have biliary excretion. These agents may provide temporal information on hepatobiliary excretion (Fig. 132.24). Lack of excretion into the gallbladder lumen implies cystic duct obstruction, which in conjunction with additional findings may be used to diagnose acute cholecystitis.

The normal gallbladder contents show high signal intensity on T2- and heavily T2-weighted images (MRCP) because of static fluid content of bile. The T1-weighted signal intensity of bile varies depending on patient's fasting status, bile viscosity, and bile salt concentration. In the fasting state, 90% of water is removed from the bile with a resultant increase in concentration of phospholipids, cholesterol and bile salts. Concentrated bile can therefore be hyperintense to liver and fat on T1-weighted images due to T1 shortening [82].

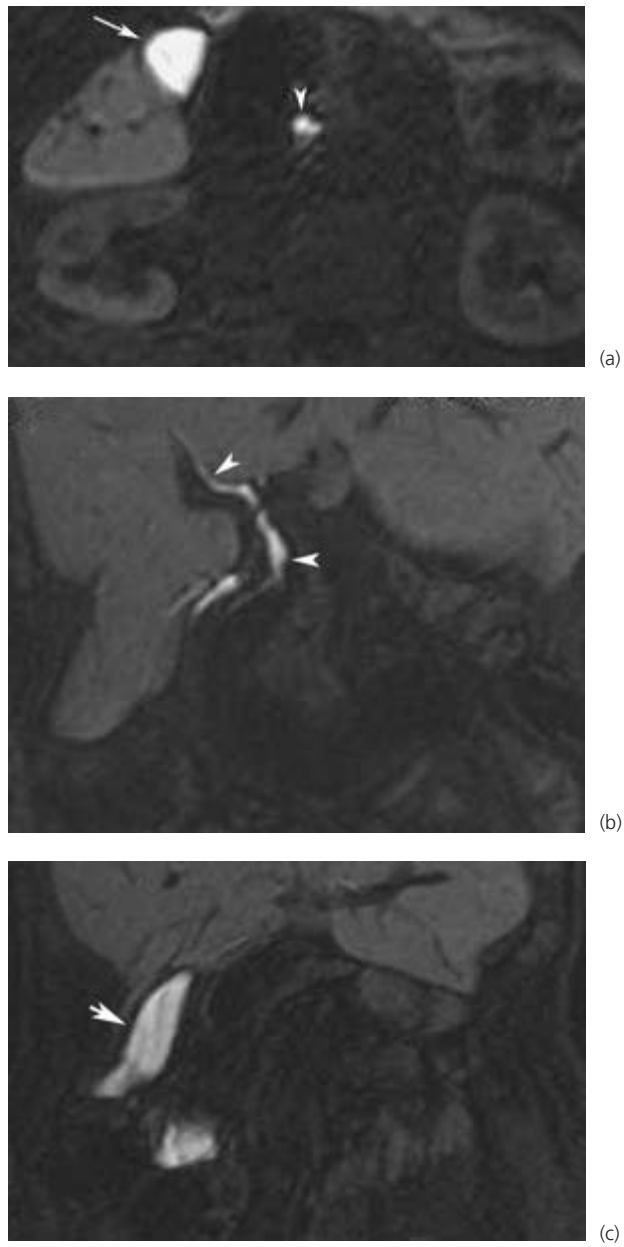


Figure 132.24 Functional magnetic resonance cholangiograms. Increased signal consistent with secreted mangafodopir trisodium in the normal common hepatic duct, common bile duct (arrowheads), and gallbladder (arrow) on axial **(a)** and coronal **(b, c)** images.

Although MRI is as sensitive as ultrasound for the detection of gallstones, it is not used as a primary modality for the diagnosis of cholelithiasis, since sonography is the imaging modality of choice [83]. Cholesterol is the main component in 80% of stones and makes up less than 25% of bilirubin pigment stones. Gallstones have low signal intensity on T1- and T2-weighted images compared with surrounding bile. High signal intensity stones on T1-weighted images have also been attributed to a high fatty acid content or concentrated bilirubin.

Acute cholecystitis occurs when the outflow of bile is prevented by occlusion of the cystic duct, often by a gallstone impacted in the gallbladder neck or cystic duct. Although not used as a screening test for acute cholecystitis, MRI may be used as a primary modality for diagnosis in certain patients with a confusing clinical presentation or equivocal sonographic or cholescintigraphic findings. The presence of cholelithiasis, pericholecystic fluid in the absence of ascites, gallbladder thickening (< 3 mm), and gallbladder edema on T2-weighted images suggest a diagnosis of acute cholecystitis. Gallbladder thickening and pericholecystic fluid in themselves are nonspecific findings and can also occur in the setting of hypoalbuminemia, hepatitis, chronic cholecystitis, cirrhosis, and renal disease. Contrast-enhanced MRI can help confirm a diagnosis of acute cholecystitis by showing hyperemia (> 80% enhancement) of the gallbladder wall and transient hyperenhancement of the adjacent hepatic parenchyma (Fig. 132.25) [84]. Pericholecystic enhancement is present in more than 70% of patients with acute cholecystitis and may extend into the medial segment of the left hepatic lobe. Pericholecystic enhancement is secondary to increased hepatic arterial flow in response to local inflammation of the gallbladder. The finding of segmental absence of mucosal enhancement on contrast-enhanced MRI can suggest a diagnosis of complicating gangrene in patients with suspected acute cholecystitis [84,85]. Chronic cholecystitis can present as a small and irregularly shaped gallbladder with a thickened gallbladder wall. Fibrosis in the gallbladder wall shows delayed enhancement. The degree of wall enhancement is significantly lower than that present in acute cholecystitis [84].

Gallbladder adenomyomatosis is characterized by overgrowth of mucosa, hypertrophy of the muscularis layer, and extension of mucosa into the thickened gallbladder wall, forming intramural diverticula known as Rokitansky–Aschoff sinuses. Adenomyomatosis is found incidentally in 20% of patients who have cholecystectomy. There are three subtypes: localized (usually in the gallbladder fundus), diffuse, and segmental (segmental stricture composed of a thickened wall that divides the gallbladder lumen into separate interconnecting compartments) [83]. The MRI diagnosis of adenomyomatosis is based on the demonstration of Rokitansky–Aschoff sinuses. The thickened gallbladder wall appears hypintense on T2-weighted images, while the intramural diverticula are revealed as discrete, linearly arranged, smoothly marginated, intramural cyst-like structures that are isointense to bile on T2-weighted images and MRCP. The appearance of the intramural cysts in a circumferential distribution has been described as the "pearl necklace" sign.

Gallbladder polyps are elevated lesions of the mucosal surface of the gallbladder wall and are often incidental findings on imaging evaluation of the abdomen. The majority of resected polyps are cholesterol polyps that have no malignant potential. Polyp size is a significant predictor of malignant potential, with a 40%–88% prevalence of malignancy in polyps larger than 10 mm. On MRI, polyps are discrete wall-based lesions that contrast against the high T2 signal of the adjacent bile. They enhance following contrast administration, best appreciated on fat-suppressed T1-weighted images. They are distinguished from stones because of their fixation to the gallbladder wall and their enhancement. Size greater than 10 mm, solitary polyp, age greater than 60 years, sessile morphology, coexistence of gallstones, and rapid growth increase the risk of polyps being malignant.

Gallbladder carcinoma is a rare but aggressive malignancy. It predominantly affects females (3 : 1 over males) and gallstones are present in 90% of patients with gallbladder cancer. Symptoms occur with advanced disease: anorexia, weight loss, jaundice, and abdominal pain. The most common mode of spread is direct invasion into the liver. Tumor can also directly invade the duodenum, colon, and pancreas. On MRI, the primary gallbladder cancer is hypointense to liver on T1-weighted images and slightly hyperintense to liver on T2-weighted images [86]. T2-weighted and MRCP images

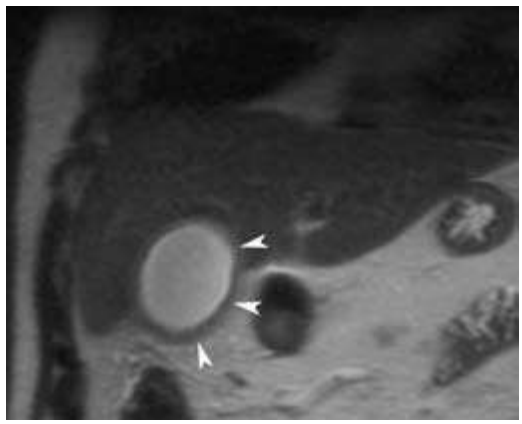
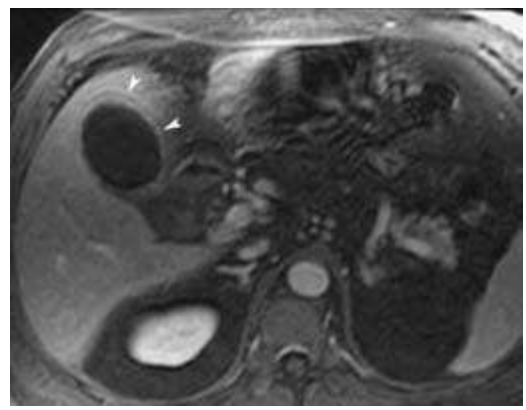
(a)   (b)

Figure 132.25 Acute cholecystitis. **(a)** Coronal T2 and **(b)** axial postcontrast images demonstrate thickening of the gallbladder wall (arrowheads) and mild pericholecystic edema consistent with acute cholecystitis.

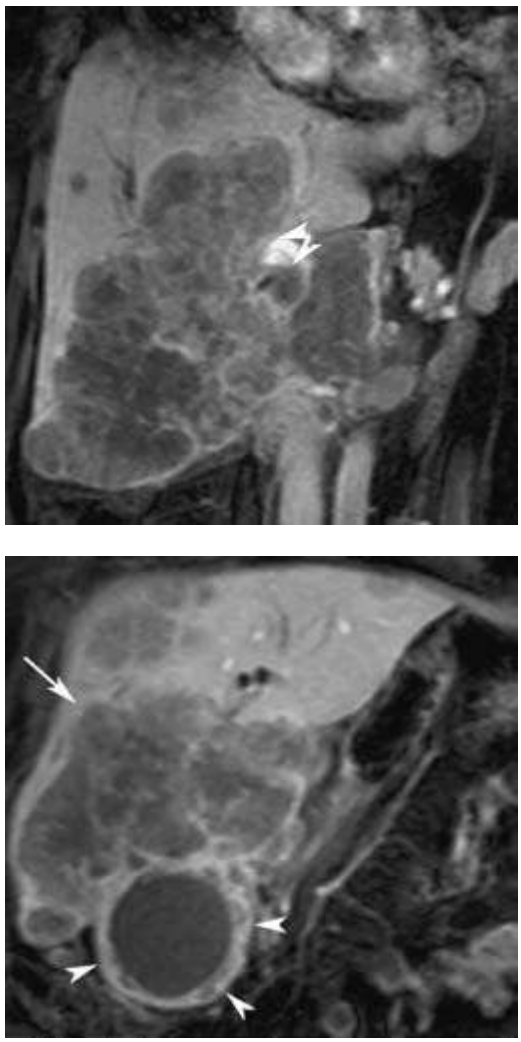


Figure 132.26 Gallbladder carcinoma. Coronal postcontrast images **(a, b)** demonstrate circumferential thickening of the gallbladder wall (arrowheads) inseparable from the liver with tumor extension into the right hepatic lobe (arrow). Note hypoenhancing metastatic lesions in the right heptic dome and left lobe.

typically depict gallstones surrounded by a tumor mass. Direct liver invasion, when present, is typically an ill-defined mass in the adjacent hepatic parenchyma contiguous with the gallbladder mass (Fig. 132.26). Dynamic contrast-enhanced MRI can demonstrate the margin of tumor extension, facilitating accurate staging. The identification of regional lymph nodes larger than 10 mm with ring-like or heterogeneous enhancement suggests lymphatic spread.

The gallbladder is very rarely the site of involvement by other malignancies. Metastatic breast or metastatic melanoma can occasionally involve the gallbladder, the latter being more frequent [87]. Primary non-Hodgkin lymphoma of the gallbladder is also very rare and has a very poor prognosis. This presents with diffuse thickening of the gallbladder wall with moderate enhancement.

Magnetic resonance cholangiopancreatography

In the initial evaluation of biliary and pancreatic disorders, MRCP has replaced the use of diagnostic ERCP at many institutions. This technique uses MRI to visualize stationary or slow-moving fluid, such as bile, displaying them as high signal intensity. Heavily T2-weighted sequences are generally used for MRCP with the single-shot, echo-train, spin-echo technique achieving the most widespread use. Because of the heavy T2 weighting of this sequence, signals from the fluid in the biliary system and pancreatic duct are hyperintense, whereas the signal of background tissue is rendered hypointense, enabling excellent depiction of the biliary system and pancreatic duct (see Fig. 132.15). Single-shot, echo-train, spin-echo sequence can be performed as single-slab acquisition or multiple thin-slice acquisitions. In the single-slab approach, a thick-collimation (30–70 mm) single section is acquired in an oblique coronal plane, obtained in 2–3 s. The single slab can be acquired in various rotations to view the biliary system and pancreatic duct from different angles. ERCP-like images can be acquired without maximum intensity projection (MIP) post processing. It is important also to acquire a multiple-slice thin-collimation sequence to examine the details of the intraductal structures. A slice thickness of 3–4 mm is needed to detect small intraductal stones. Three-dimensional reconstruction may be performed by MIP postprocessing from the thin-collimation source images. However, volume-averaging effects can obscure small stones and subtle mural irregularity, so source thin-section images must always be reviewed. With these images, however, it is not possible to evaluate periductal structures, such as tumors, which may cause narrowing or obstruction of the ducts. Also, fluids with relatively short TE, such as concentrated bile or mucinous fluid, may produce very little signal with long effective TE sequences and may obscure small bile ducts or mucinous lesions. To overcome those drawbacks of MRCP with long effective TE, an intermediate effective TE (80–100 ms) can be used. This produces images where not only all fluid including concentrated bile and mucinous fluid is bright, but also periductal structures are well depicted. This combination of MRCP with intermediate effective TE and ERCP-like images gives detailed evaluation of both intraductal and periductal structures.

Studies show that MRCP is comparable with or more useful than other techniques, such as ultrasound, CT, and ERCP, for studying choledocholithiasis, malignant obstruction of the biliary or pancreatic ducts, congenital anomalies, and chronic pancreatitis [88]. MRCP is noninvasive and safe because it does not require anesthesia or injection of intraductal or intravenous contrast agent. Using current MRI systems, high-quality images can be obtained consistently. MRCP is useful in patients after incomplete or unsuccessful

ERCP. In some patients, such as those who have undergone surgery with biliary enteric anastomosis or Billroth II, it may not be possible to perform ERCP, so MRCP is the modality of choice for evaluating these postsurgical patients. Unlike ERCP, MRCP produces images of the ducts in their natural state, because it does not involve distention of the ducts by injected contrast medium. ERCP cannot evaluate extraductal structures directly, whereas MRCP can be combined with conventional MRI for the evaluation of extraductal disease, such as tumors [62]. ERCP has advantages over MRCP, including the ability to perform direct therapeutic interventional procedures concurrently with diagnostic imaging. ERCP is generally a safe procedure, but is still associated with nonnegligible morbidity and mortality rates. Also, technical failures occur in up to 10% of cases because of unsuccessful cannulation of the common bile duct (CBD) or pancreatic duct. In some institutions MRCP is gradually replacing ERCP as a primary diagnostic imaging modality to evaluate the biliary system and pancreatic duct.

Benign cystic disease

MRCP can be effective and comparable with ERCP for the evaluation of congenital cystic lesions of the bile duct. Also, the combination of MRCP and gadolinium-enhanced T1-weighted images is useful for diagnosing associated findings, such as gallstone disease and cancer. MRCP has been demonstrated to be effective in evaluating choledochal cyst (Fig. 132.27), choledochocele, and Caroli disease.

Congenital variants of the biliary system

Anatomical variants of the cystic duct have received much attention because of the higher risk of complications during cholecystectomy. In a study evaluating anatomical variants of the biliary tree, MRCP could accurately demonstrate a range of variants, such as low cystic duct insertion, medial cystic duct insertion, parallel course of the cystic and hepatic ducts, and aberrant right hepatic duct [56].

Pancreas divisum

Pancreas divisum is the most common anatomical variant of the pancreas and results from failure of fusion of the duct of the embryonic dorsal pancreas and the duct of the ventral pancreas. As a result, the pancreas has two separate ductal systems (see Fig. 132.16). The incidence of this anomaly is reported to be 1.3%–6.7% of the general population. In patients with pancreas divisum, the minor papilla may cause impaired pancreatic drainage and result in chronic pancreatitis [56]. Another clinically relevant issue with pancreas divisum is that at ERCP only the ventral duct can be cannulated through the major papilla, and a small ventral duct may be misdiagnosed as an obstructed pancreatic duct. On MRCP images, pancreas divisum can be diagnosed by finding a dorsal dominant pancreatic duct running anteriorly to the CBD and draining into the minor papilla (see Fig. 132.16). A study that evaluated 108 patients who underwent both ERCP and MRCP demonstrated exact correlation between the two techniques for the depiction and exclusion of pancreas divisum [56].

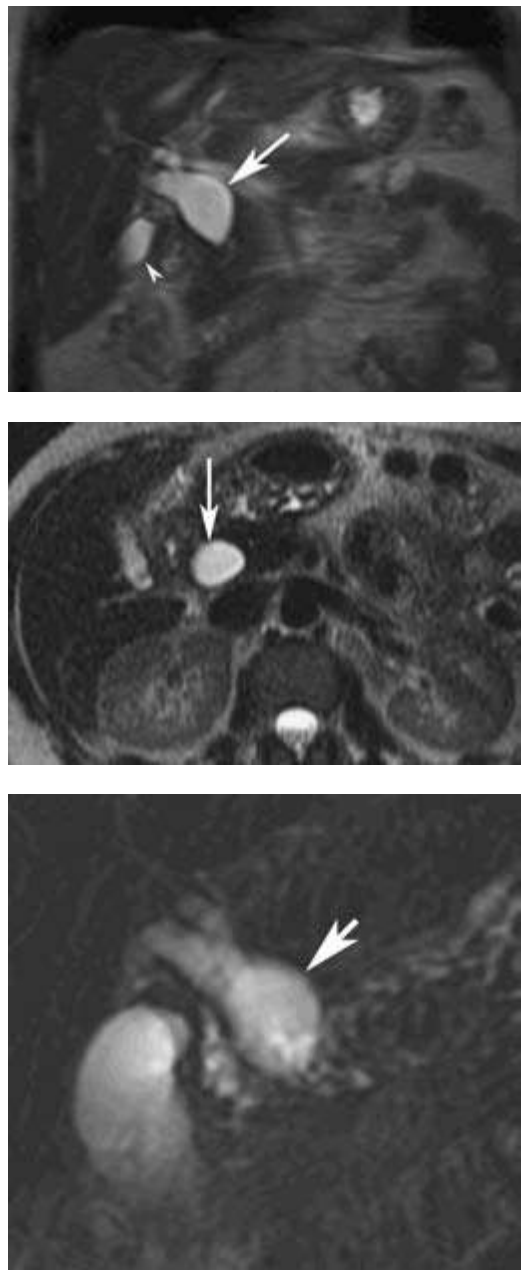


Figure 132.27 Choledochal cyst. **(a)** Coronal T2 image, **(b)** axial T2 image, and **(c)** magnetic resonance cholangiopancreatography demonstrate fusiform dilation of the common bile duct (arrow) consistent with choledochal cyst. Arrowhead indicates gallbladder.

Cholelithiasis

The primary imaging modality for cholelithiasis is sonography. However, MRCP is highly sensitive and accurate in diagnosing cholelithiasis and can outperform ultrasound and

CT. The most reliable approach for detecting gallstones is with the use of single-shot T2-weighted sequences, such as MRCP [89].

Choledocholithiasis

Accurate diagnosis of stones in the biliary ducts is crucial because their presence is a difficult challenge for cholecystectomy. Ultrasound and CT show relatively low sensitivity and accuracy for the diagnosis of bile duct stones. ERCP is considered the gold standard procedure for the evaluation of the biliary system and has a major advantage over other imaging modalities because it can be used to perform therapeutic interventions and diagnosis [89]. However, even in diagnostic ERCP alone, the rate of major complications or death is not negligible and the rate of failed ERCP is 5%–20%. MRCP has been shown to be an excellent method for detecting bile duct stones [90]. It is superior to CT or ultrasound and comparable or superior to ERCP in detecting bile duct stones. On thin-slice source images, stones appear as signal-void lesions, with stones as small as 2 mm being detected in dilated and nondilated ducts (Fig. 132.28). On thick-slab images, large or medium-sized stones in normal-caliber ducts are easily detectable, but small stones that are completely surrounded by fluid may be obscured and difficult to detect because of volume-averaging effects [91]. There are several pitfalls and mimickers of stones with MRCP. Intraductal air bubbles (pneumobilia) may mimic the appearance of stones (Fig. 132.29) [89,91]. An important differentiating feature from

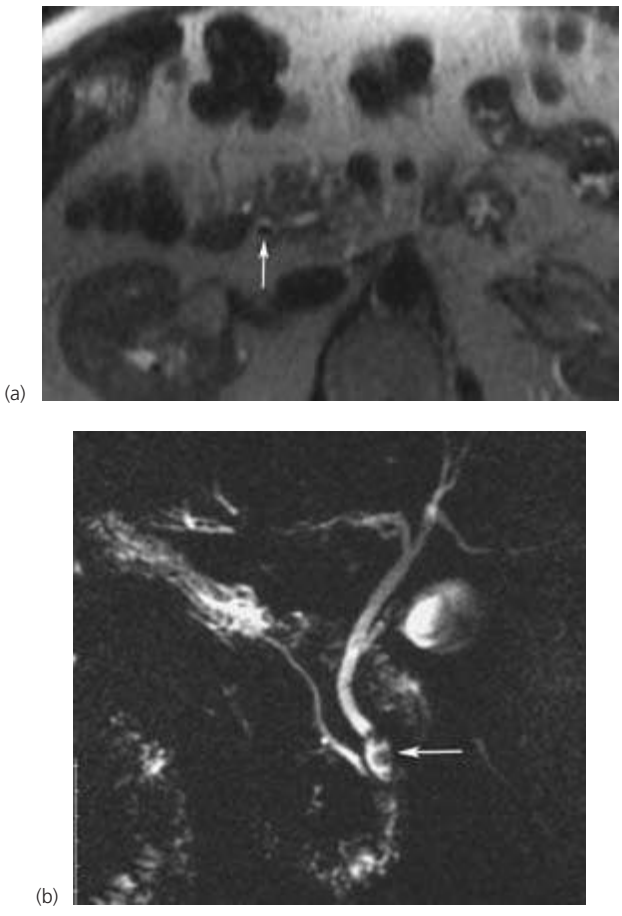


Figure 132.28 Choledocholithiasis. **(a)** Axial T2 image and **(b)** magnetic resonance cholangiopancreatography show low signal void (arrow) consistent with common bile duct calculi.

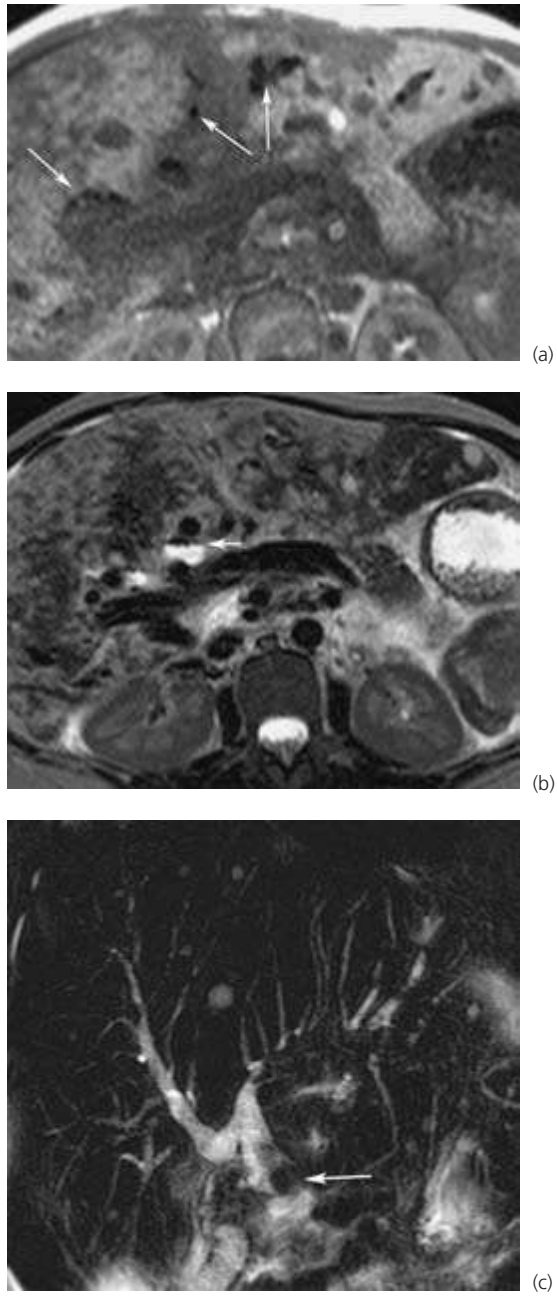


Figure 132.29 Pneumobilia. **(a)** Axial T1 image, **(b)** axial T2 FSE image, and **(c)** magnetic resonance cholangiopancreatography demonstrate low signal (arrows) in the intrahepatic and extrahepatic biliary ducts. This low signal is nondependent in the axial T2 image in the common bile duct consistent with pneumobilia.

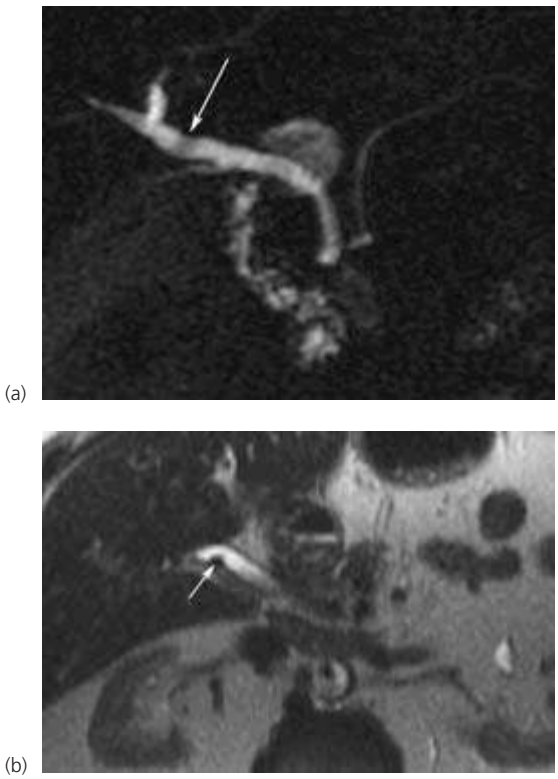


Figure 132.30 Pseudodefect of the common hepatic duct secondary to crossing vessel. Low signal void in the common hepatic duct on magnetic resonance cholangiopancreatography **(a)** secondary to adjacent crossing vessel (arrow) confirmed on axial T2 image **(b)**.

stones is that air bubble filling defects lie on the nondependent portion of the bile duct against the wall on axial images. Blood clots may appear indistinguishable from bile duct stones [92]. Other pitfalls that may mimic bile duct stones include:

- tortuosity of the bile duct running in and out of the imaging plane
- merging of the cystic duct into the CBD when observed en face on coronal images, which may result in a round hypointense focus
- metallic clips
- extraductal compression from the right hepatic or gastroduodenal artery, which may result in a signal-void focus (Fig. 132.30).

Correct diagnosis can usually be achieved by careful attention to the exact location of these foci and interpretation of thick-slab MRCP or MIP-reconstructed images in conjunction with the thin-slice source images [92].

Primary sclerosing cholangitis

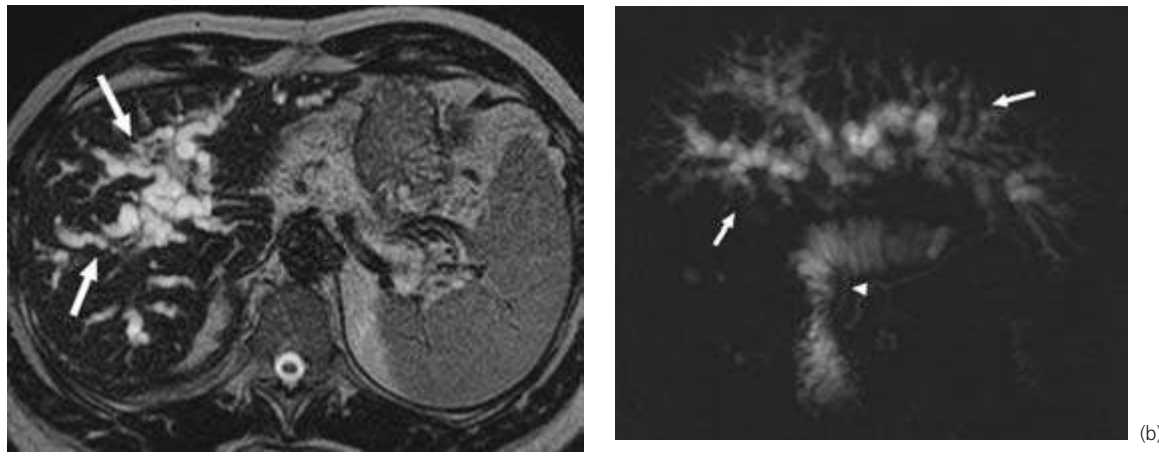
Primary sclerosing cholangitis is characterized by chronic fibrosing inflammation of the biliary system of unknown etiology. The diagnosis of primary sclerosing cholangitis is made by cholangiographic findings supported by histological results. The imaging appearance of primary sclerosing cholangitis is characterized by multiple irregular strictures and saccular dilatations of the intrahepatic and extrahepatic bile ducts, producing a beaded appearance. The conventional imaging modality for the diagnosis of primary sclerosing cholangitis is ERCP. However, complications from ERCP may result in progression of cholestasis in patients with primary sclerosing cholangitis. MRCP has been shown to be useful for the diagnosis and follow-up of primary sclerosing cholangitis [93]. A study evaluating MRCP in patients with primary sclerosing cholangitis demonstrated a sensitivity of 85%–88% and specificity of 92%–97% [93]. However, diagnostic challenges remain: subtle changes of mild primary sclerosing cholangitis may be difficult to detect by current MRI techniques, and cirrhosis may cause distortion of the intrahepatic bile ducts and mimic primary sclerosing cholangitis [94]. A major strength is that MRCP provides visualization of bile ducts proximal to even severe stenoses, which may not be evaluable by ERCP. The characteristic feature is of intrahepatic ductal dilation with areas of dilation and stenosis characterized as "beading" (Fig. 132.31).

Postsurgical biliary complications

The most common postsurgical biliary complication is benign biliary stricture. MRCP can visualize the biliary tree distal and proximal to a high-grade stricture or complete obstruction. However, the bile ducts distal to a stenosis may be collapsed and nonvisualized on MIP-reconstructed images, leading to overestimation of the stricture. Thin-section source images must be used to evaluate the extent of high-grade stenoses, because even small amounts of fluid in collapsed ducts can be depicted on these images. Other postsurgical biliary complications include retained bile duct stones, biliary leak, and biliary fistula. These conditions can be evaluated effectively by MRCP. In patients with biliary–enteric anastomoses, it may be difficult or impossible to perform ERCP. On the other hand, MRCP is very effective in evaluating the anatomy of the anastomosis, strictures of the anastomosis, strictures of the biliary ducts, and biliary stones proximal to the anastomosis in up to 100% of patients. Thin-section source images should be examined thoroughly because the biliary–enteric anastomosis and stones may be obscured on thick-slab and MIP-reconstructed images by the high signal intensity of surrounding bile and bowel fluid. Also, metallic surgical clips and pneumobilia can also produce artifacts that should not be mistaken as stones or strictures.

Chronic pancreatitis

On ERCP, typical findings of chronic pancreatitis include dilatation, narrowing or stricture, or irregularity of the pancreatic duct. Prominent dilatation of side branches is a feature of chronic pancreatitis that helps distinguish this entity from obstructed pancreatic duct caused by pancreatic cancer (see Fig. 132.18). A study evaluating 30 patients with chronic pancreatitis undergoing ERCP and MRCP demonstrated



(a) (b)

Figure 132.31 Primary sclerosing cholangitis. **(a)** Axial T2 image and **(b)** magnetic resonance cholangiopancreatography demonstrate marked intrahepatic duct dilation with "beaded" morphology (arrow) and normal bile duct (arrowhead).

sensitivity and specificity of 91% and 92%, respectively, and excellent correlation between ERCP and MRCP was reported.

Cholangiocarcinoma

Cholangiocarcinoma can be classified into three types according to anatomical location: peripheral type, originating from peripheral bile ducts in the liver; hilar type (Klatskin tumor), originating from the confluence of the right and left hepatic ducts; and extrahepatic type, originating from the main hepatic ducts, common hepatic duct, or CBD. Ductal obstruction is observed in all cases of Klatskin tumor and extrahepatic cholangiocarcinoma. Evaluation of the level of obstruction is important for treatment planning. In a study evaluating malignant perihilar biliary obstruction in 40 patients including 26 Klatskin tumors, it was reported that MRCP was as effective as ERCP at detecting the presence and the level of biliary obstruction (40 of 40 cases on MRCP and 38 of 38 cases on ERCP). On MRI, cholangiocarcinoma is poorly defined with low to intermediate signal on T2-weighted images and demonstrates delayed enhancement following contrast (Fig. 132.32). The disadvantage of ERCP is that it may result in sepsis caused by overdistention of an obstructed biliary duct with stagnant bile colonized by bacteria; additionally, ERCP may be unable to provide sufficient biliary opacification to adequately evaluate the region of narrowing. However, MRCP can demonstrate the bile duct proximal to the obstructing site safely and efficiently. T1-weighted fat-suppressed SGE acquired 2–5 min after gadolinium administration is the most consistent technique for demonstrating cholangiocarcinoma, which appears as moderately enhancing tissue (Fig. 132.32).

Pancreatic cancer

Typical pancreatographic features of pancreatic cancer include irregular narrowing or obstruction of the main pancreatic duct and dilatation proximal to the lesion. Pancreatic head tumors also result in obstruction of the CBD. MRCP is able to evaluate the pancreatic duct proximal to an obstructing site that ERCP may be unable to demonstrate. In a study evaluating 124 patients with suspicion of pancreatic cancer, MRCP was as effective as ERCP for the detection of the pancreatic cancer, with sensitivity and specificity of 84% and 97% respectively for MRCP, and 70% and 94% respectively for ERCP. As with other malignant tumors, when pancreatic ductal adenocarcinoma is suspected clinically routine MRI sequences are also performed. T1-weighted SGE acquired immediately after gadolinium administration is the most consistent technique for demonstrating pancreatic cancer (see Fig. 132.19).

Additional uses of MRCP

In a study evaluating 31 patients with chronic pancreatitis and 84 patients with suspicion of pancreatic disease, MRCP after secretin stimulation showed reduced duodenal filling in patients with severe chronic pancreatitis. Another advance in MRCP is the use of contrast agents that are hepatocyte-selective and eliminated, at least in part, by the biliary system. With these agents and faster acquisition with thin-section, three-dimensional, T1-weighted images of the biliary system, demonstration of smaller intrahepatic biliary branches is feasible. This approach may also facilitate detection of functional obstruction or bile duct leak or injury.

Intestine

MRI has had limited value in evaluating the bowel compared with CT and conventional barium studies. This is mostly because motion of the bowel, caused by either respiratory motion or peristalsis, degrades the magnetic resonance images. Newer techniques such as single-shot FSE have reduced these problems to some extent. Antiperistaltic agents are also

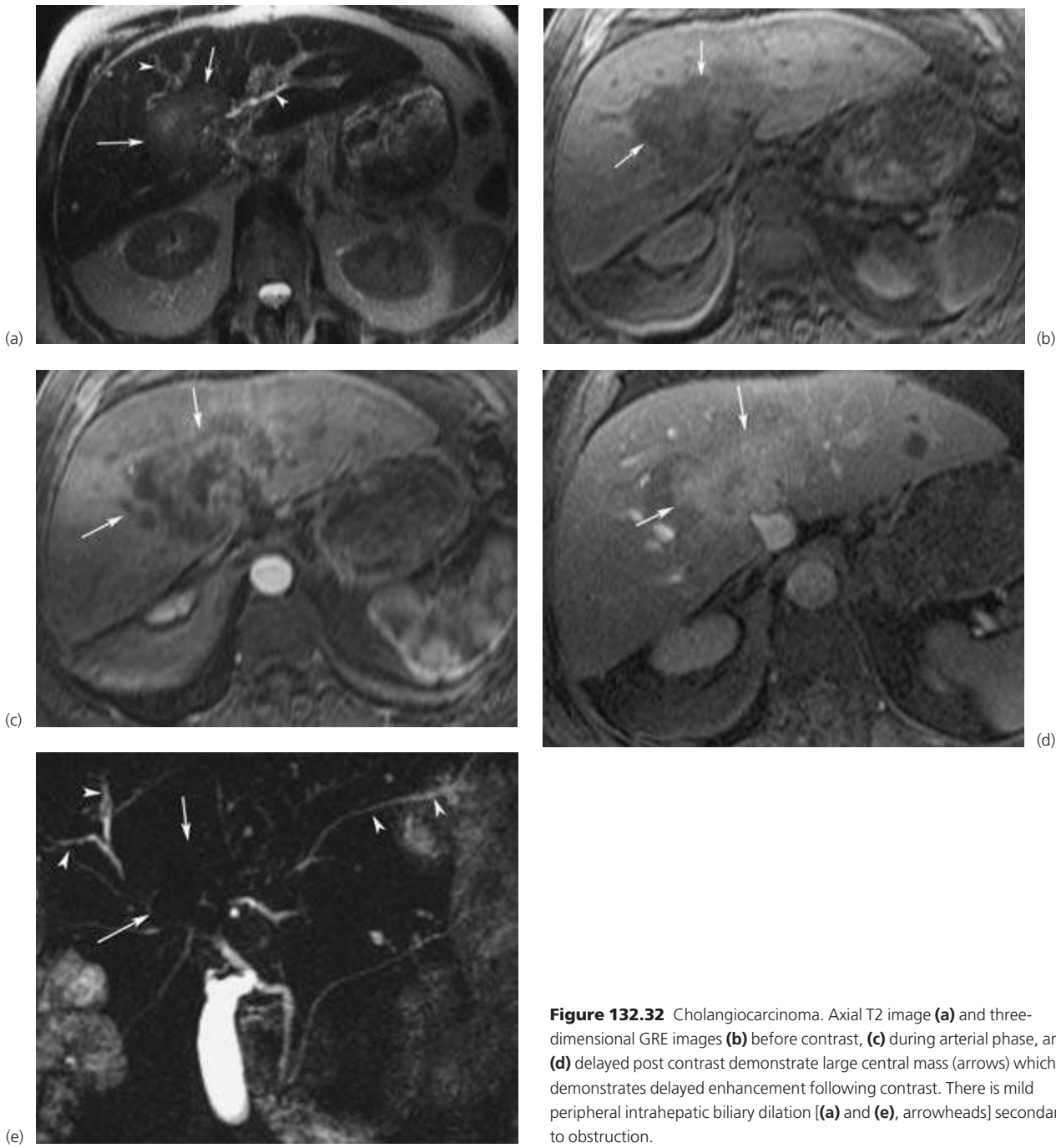


Figure 132.32 Cholangiocarcinoma. Axial T2 image **(a)** and three-dimensional GRE images **(b)** before contrast, **(c)** during arterial phase, and **(d)** delayed post contrast demonstrate large central mass (arrows) which demonstrates delayed enhancement following contrast. There is mild peripheral intrahepatic biliary dilation [**(a)** and **(e)**, arrowheads] secondary to obstruction.

helpful for reducing bowel-related motion artifacts. Now MRI has greater potential for evaluating the small bowel and colon, particularly for judging the extent of wall involvement with various pathological processes. MRI has been shown to be valuable in assessing the severity and extent of ulcerative colitis and Crohn's disease [95,96] (Fig. 132.33).

MRI is useful for showing the extent of pelvic fistulae from any cause. T2-weighted images display the muscularis propria of the bowel as low signal intensity. Breaks in the muscularis due to fistulae are seen as discontinuities in this layer. Fistulae can be seen without the administration of contrast material. The high signal intensity of granulation tissue is seen surrounded by low signal intensity fibrosis (Fig. 132.34). Because of the intrinsic soft-tissue contrast of T2-weighted images, MRI has shown promise for staging gastrointestinal tract tumors, particularly rectal carcinoma. With high

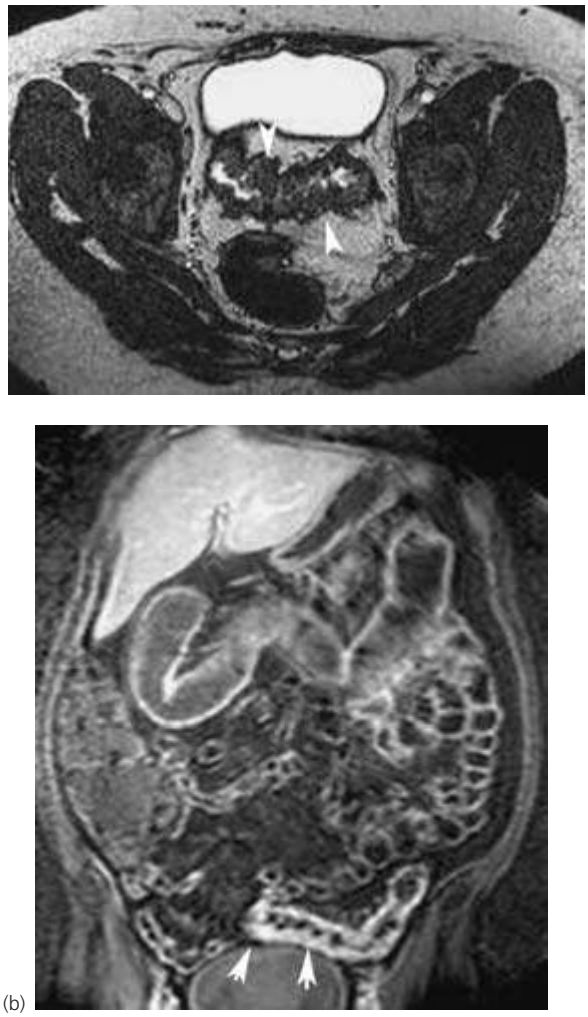


Figure 132.33 Crohn's colitis. **(a)** Axial fat-suppressed T2 image and **(b)** coronal postcontrast three-dimensional GRE image demonstrate circumferential wall thickening of the sigmoid colon (arrowheads) consistent with reactivation of colitis in this patient with history of Crohn's disease. Courtesy of Thomas Lauenstein MD, Atlanta, GA.

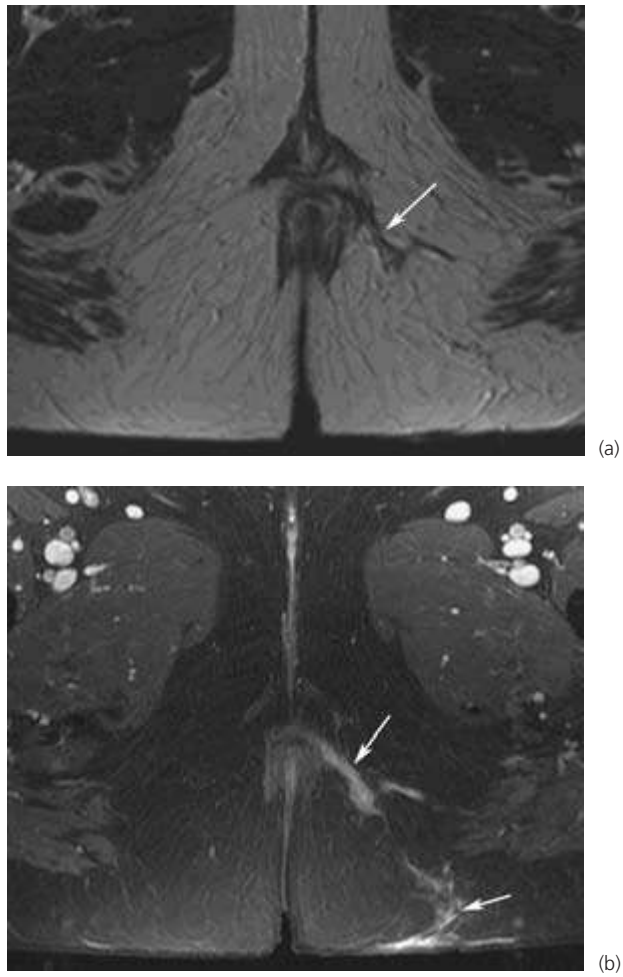


Figure 132.34 Perianal fistula. **(a)** Axial T2 image and **(b)** postcontrast three-dimensional GRE image demonstrate fluid-containing and rim-enhancing rectoanal fistulous tracts (arrows).

resolution, layers of the rectal wall can be visualized discretely. The muscularis propria appears as low signal intensity, whereas the submucosa appears as higher signal intensity. This has led to preliminary attempts to locally stage rectal carcinoma, with good results reported. MRI is an excellent method for evaluating the pelvis in general and to determine the local extent of tumors.

In addition, MRI can be used to evaluate for recurrent tumor after surgery or after radiation therapy. Radiation edema and radiation fibrosis are differentiated from tumor on T2-weighted images and dynamic gadolinium-enhanced images. Fibrosis appears as low signal intensity on T2-weighted images. Recurrent tumor shows nodules of higher-signal tissue. Recurrent tumor enhances rapidly during dynamic injection of gadolinium, whereas postradiation changes enhance more slowly.

Magnetic resonance enteroclysis

Because of its excellent soft-tissue contrast and multiplanar imaging capabilities, MRI may be used for evaluation of the small bowel. However, long acquisition times, peristalsis, and respiratory motion artifacts were severe limitations and MRI was rarely used for evaluation of small bowel diseases. With the development of ultrafast sequences, there is increasing interest in small bowel evaluation with MRI [97], although problems related to small bowel distention and to functional information remain limitations. Collapsed bowel loops may hide or simulate small bowel disease. Adequate distention of the entire small bowel is a key requirement of any small bowel imaging method. Different methods have been used to achieve adequate distention of the entire small bowel for MRI. Magnetic resonance enteroclysis was introduced to overcome the individual deficiencies of MRI and enteroclysis and to combine the advantages of both into one technique. Magnetic resonance enteroclysis consists of two parts: magnetic resonance fluoroscopy and cross-sectional imaging. Mag-

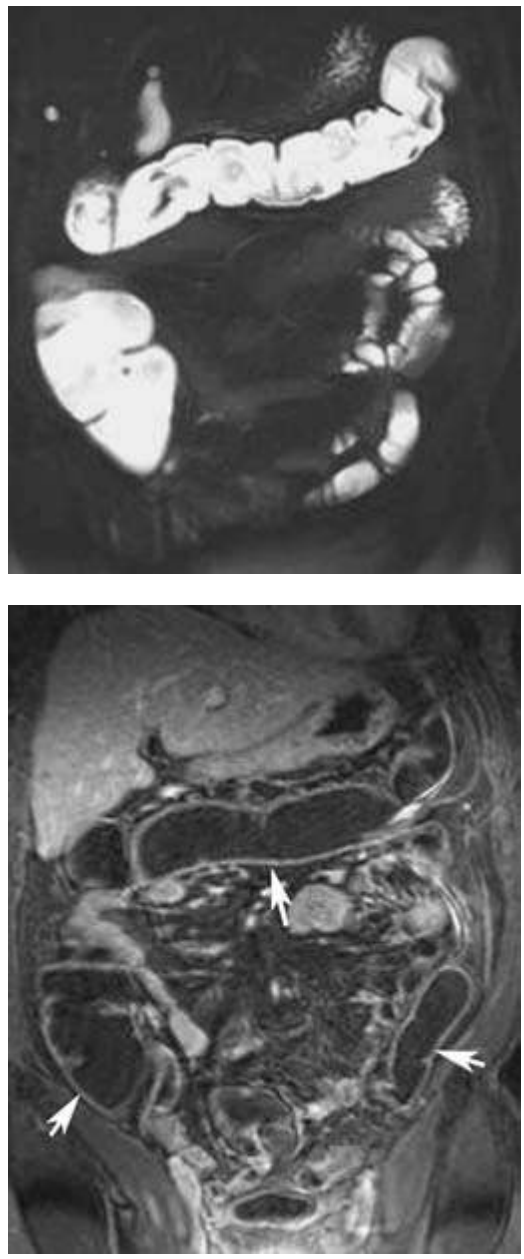
netic resonance fluoroscopy allows real-time control and monitoring of small bowel filling during the small bowel enema and provides functional information. Cross-sectional imaging sequences are used for morphological evaluation of the distended small bowel.

Magnetic resonance colonography

Magnetic resonance colonography was introduced after CT colonography. First reports described breath-hold, three-dimensional, GRE sequences that covered the entire enema-filled colon in the coronal plane. Air and carbon dioxide have been used as colonic distention agents, but most reports address liquid enema techniques.

Three different liquid enema techniques are used: bright lumen, black lumen, and fecal tagging. Both bright and black lumen methods are used after bowel cleansing. The bright lumen is based on a gadolinium–water enema with a gadolinium concentration of at least 10 mmol/L (i.e., 40 mL of 0.5 mol/L gadolinium in 2 L of water) (Fig. 132.35). Because of the lack of radiation, a magnetic resonance fluoroscopy sequence can be applied to monitor filling of the colon. After complete filling, a thin-section, three-dimensional, T1-weighted SGE sequence (3.7/1.1; flip angle 40°–50°) is performed. With this sequence, the lumen appears bright because of the presence of gadolinium, and polyps are visible as filling defects. A two-dimensional HASTE sequence can be used for delineation of the colonic wall. The black lumen technique is based on a water enema for luminal distention and an intravenous infusion of gadolinium for enhancement of the colonic wall (Fig. 132.35). After complete filling, a three-dimensional T1-weighted SGE sequence is performed before and after the intravenous administration of gadolinium. The lumen appears dark with this T1-weighted sequence, and enhancement of the colonic wall and possible inflammatory changes and polyps can be seen. A combination of three-dimensional T1-weighted SGE sequence and balanced GRE sequence makes bright and black lumen colonography possible with one protocol. The fecal tagging technique is based on a diet that contains barium to give stools the same signal intensity as water on T1-weighted GRE images. Patients are instructed to avoid fiber-rich foods and foods with a high concentration of manganese, such as fruit and chocolate. Magnetic resonance colonography starts with filling of the colon with water [96,98]. Intravenous gadolinium is administered, and a three-dimensional T1-weighted SGE sequence is performed. The lumen appears black, and enhancement of the colonic wall and possible polyps are seen.

In symptomatic patients, this new technique shows promising results for the detection of polyps equal to or larger than 1 cm in diameter. Its value in polyp detection still needs to be determined in large studies. Before this technique can be implemented in daily practice, it must show the same accuracy as colonoscopy and should be cost-effective in both high-risk and screening patients.



(a)

(b)

Figure 132.35 Magnetic resonance colonography. **(a)** Coronal T2 image and **(b)** coronal three-dimensional GRE image (T1) demonstrate fluid-distended normal colon (arrows). Courtesy of Thomas Lauenstein MD, Atlanta, GA.

MRI of perianal fistula

MRI has been shown to accurately demonstrate the anatomy of the perianal region. On CT the attenuation values for the sphincters, levator ani, fibrotic fistulous tracks, and active fistula are so similar that it is difficult to characterize these structures accurately, unless the track contains gas or leaked contrast material. The MRI appearance of perianal fistula shows greater concordance with surgical findings than any other imaging evaluation. Many different MRI techniques

have been described. MRI in the coronal and axial planes demonstrates fistulous tracks in relation to the sphincter complex, ischiorectal fossa, and levator ani. Imaging in the sagittal and oblique planes is helpful in selected cases such as anovaginal and presacral disease [99]. MRI using pelvic surface coil requires no patient preparation and is well tolerated. Use of endoanal coils was initially hoped to further improve the MRI evaluation of perianal fistula. However, this technique is poorly tolerated in symptomatic patients, and although it provides excellent anatomical detail of the anal sphincters, it fails to provide the overview required for surgical management. The anatomy of the perianal region is well demonstrated on coronal and axial T1-weighted images. In normal subjects, the internal and external sphincters are not separately resolved on MRI obtained with a pelvic surface coil, but the sphincter complex, ischiorectal fossae and levator sling are clearly seen. Unenhanced T1-weighted images provide an excellent anatomical overview of the sphincter complex, levator plate, and ischiorectal fossae. However, fistulous tracks, inflammation, and abscesses appear as areas of low to intermediate signal intensity and may not be distinguished from normal structures such as sphincters and levator ani muscles. On T2-weighted and STIR (short tau inversion recovery) images, pathological processes including fistulae, secondary tracks, and fluid collections are clearly depicted. They appear as areas of high signal intensity in contrast with the lower signal intensity of the sphincters, muscles, and fat (especially on STIR images). The only comparative study of imaging sequences suggested for use in this condition showed that STIR imaging has certain limitations [99]. In some cases, STIR imaging failed to demonstrate secondary tracks, and in others it did not reveal small residual abscesses within edematous inflammatory change [99]. Furthermore, spurious incidences of high signal intensity in inactive tracks were also observed. Fat-suppression techniques used with T2-weighted imaging have also been proposed. We use GRE, T1-weighted, dynamic, intravenous contrast-enhanced MRI combined with T2-weighted imaging to assess perianal fistulae and their complications. With use of this technique, active fistulous tracks, secondary ramifications, and abscesses are clearly demonstrated (see Fig. 132.34). The walls of the tracks as well as the abscess cavities enhance. This breath-hold technique is rapid, noninvasive and well tolerated, a particular advantage in patients with acutely inflamed perianal regions.

Contrast agents

The primary goal of MRI contrast agents is to improve the detection of focal liver lesions. This is achieved by creating a larger difference in signal intensity between hepatic lesions and surrounding parenchyma. Although a variety of liver-targeted contrast agents have been developed for MRI, gadolinium chelate remains the most widely accepted. There are three types of contrast agents commonly used in clinical practice: hepatocyte-selective agents; reticuloendothelial system (RES)-specific agents; and agents combining perfusion and hepatocyte-selective properties, namely gadolinium ethoxybenzyl diethylenetriaminepentaacetic acid (Gd-EOB-DTPA) and gadolinium benzyloxypropionictetraacetate (Gd-BOPTA). Nonspecific extracellular gadolinium chelate is a paramagnetic substance causing increased signal intensity within targeted tissues on T1-weighted images. After intravenous injection, these agents are distributed in the intravascular compartment and subsequently diffuse throughout the extracellular space. The gain of information provided by this contrast agent is dependent on tumor perfusion and enables assessment of tumor vascularity. For instance, liver metastases may be characterized as hypovascular, hypervascular, or isovascular based on the degree and pattern of lesion enhancement after gadolinium administration. The MRI assessment of vascularization is critical in the management and follow-up of liver metastases. For instance, hypovascular metastases as characterized by MRI may reflect lesion response to systemic therapy. Although liver metastases demonstrate variable degree and pattern of enhancement, the presence of ring enhancement observed on immediate postcontrast images with peripheral washout on subsequent images is the most characteristic feature. The relatively low cost, safety, good patient tolerance, and ability to detect and characterize liver metastases justifies the use of gadolinium contrast enhanced MRI for the assessment of oncology patients.

Hepatocyte-selective contrast agents (e.g., mangafodipir trisodium) are specifically captured by hepatocytes and excreted by bile ducts. After intravenous injection of mangafodipir trisodium, the normal liver parenchyma shows an increase in signal intensity on T1-weighted images, which becomes brighter compared with the spleen or muscle. However, hepatic lesions that do not contain hepatocytes (e.g., metastases) will maintain identical signal intensity prior to and after contrast injection. Therefore, the difference in signal intensity between liver parenchyma and metastases are accentuated after contrast injection. Unlike metastases, HCC displays variable enhancement patterns after injection of mangafodipir trisodium due to variability in contrast uptake and excretion, with more pronounced uptake in well-differentiated tumors. RES-specific agents (e.g., SPIO) are selectively captured by organs containing RES, such as the liver (Kupffer cells), spleen, and bone marrow. The administration of RES-specific contrast agents results in signal loss (darkening) of liver, spleen, and bone marrow on T2-weighted sequences due to the presence of iron particles in the contrast composition. As liver metastases do not take up RES-specific agents and thus maintain identical signal intensity after contrast injection, the signal difference between liver metastases and background parenchyma is increased. This improves the detection of liver metastases after contrast injection. Like metastases, most HCCs do not take up

RES-specific agents. The required long injection time (approximately 30 min) represents a drawback for the use of RES-specific agents, because the acquisition of nonenhanced and enhanced sequences in the same study is not feasible.

Imaging recommendations

There is a wide range of MRI systems in the community which, both in terms of hardware and software capabilities, provide clinicians with a corresponding range of diagnostic capabilities. In addition, there is a greater range of technical expertise and experience among radiologists in the performance and interpretation of MRI studies compared with CT. For these reasons, specific recommendations about the appropriateness of MRI examinations are difficult to make. The types of examinations well suited to MRI and which most radiologists in the community can perform include the following.

- Characterization of a liver mass identified on ultrasound, CT, or other study. Most frequently this would involve distinguishing hemangiomas or cysts from solid liver masses, although MRI diagnosis of other liver masses, such as FNH or HCC, is possible.
- Evaluation of the complications of cirrhosis. Specific techniques can identify ascites, collaterals and varices, vascular occlusions, and HCC.
- Identification of diffuse liver disease such as steatosis or iron deposition. Transfusional iron overload can be distinguished from hemochromatosis.
- Identification of small pancreatic masses or exclusion of tumor in the large or bulbous pancreas.
- Identification and characterization of a pelvic fistula, specifically perianal fistula and the degree of levator muscle involvement.
- Identification of biliary calculi.
- Evaluation of any disorders of the liver, biliary tree, or pancreas when the patient is allergic to iodinated contrast medium or is at risk from the nephrotoxic effects of iodine (e.g., creatinine > 1.5 mg/dL, diabetic nephropathy).

Clinical situations in which CT may be more effective than routine MRI include:

- routine evaluation of the abdomen and pelvis in an oncologic patient to search for metastases of the liver, lymph nodes, etc.
- initial evaluation of obstructive jaundice
- evaluation of primarily peritoneal or bowel abnormalities
- abdominal trauma.

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B IMAGING

133 Positron emission tomography

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Positron emission tomography (PET) allows noninvasive imaging of physiological parameters within the body, such as metabolic activity and perfusion, using tracers labeled with positron-emitting radioisotopes. In contrast with imaging techniques that reflect primarily anatomical structure, such as computed tomography (CT) and magnetic resonance imaging (MRI), PET reflects biological functions in tissue. In PET, the spatial distribution and concentration of administered positron-emitting compounds are used to generate cross-sectional images through the body that reflect pathological states or elucidate physiological processes, leading to applications in clinical practice as well as in research. The principal clinical uses of PET in gastroenterology are in the area of oncology. Functional and anatomical imaging are increasingly being used together, as in combined PET/CT scanners. The potential uses of PET in research are greatly varied. Functional imaging of the central nervous system with PET as well as functional MRI (fMRI) has been applied in research on visceral pain and perception in health and in functional gastrointestinal disorders.

Principles and technical considerations

PET relies on detecting the radiation produced by a systemically administered radiotracer labeled with a positron emitter, and determining the distribution of the positron emitter within the subject under study [1,2]. A small number of proton-rich radioisotopes can decay by emitting a positron (sometimes referred to as a positive electron or antielectron), whose existence is fleeting. After emission, a positron travels a short path and ultimately combines with an ambient electron. This combination of a positron and electron undergoes a process referred to as annihilation, during which the masses of the positron and electron are converted into electromagnetic radiation in the form of two photons (γ-rays) of equal energy (approximately 511 keV) that travel in nearly opposite directions to each other (Fig. 133.1). PET scanners are designed to detect the photons produced by the annihilation of emitted positrons. Using a computer-assisted image reconstruction method similar to that used with CT, this information is ultimately used to construct transaxial images depicting the concentration of the positron emitter within the object of interest. Owing to the thinner slice thickness of current PET and PET/CT scanners, three-dimensional images of the target body area (transverse, coronal, and sagittal views) can be created from the reconstructed images.

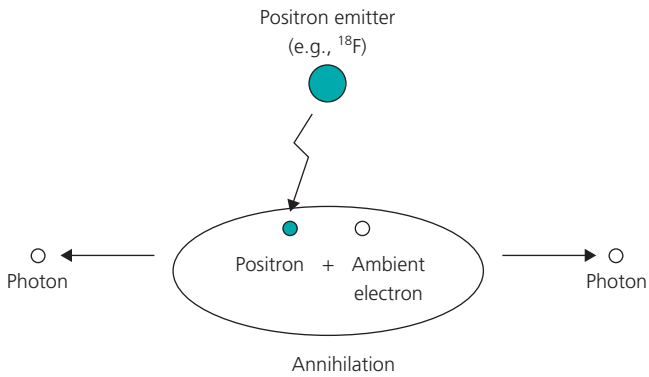

Figure 133.1 Positron emission and annihilation. Certain radioisotopes decay by emitting a positron, which travels a short path in tissue and ultimately combines with an ambient electron. This combination of particles undergoes annihilation, during which the masses of the positron and electron are converted into electromagnetic radiation in the form of two photons of equal energy that travel in nearly opposite directions to each other.

Radionuclide tracers

The first step in PET imaging consists of labeling a suitable molecule with a positron-emitting radionuclide. Positron-emitting isotopes exist for carbon, nitrogen and oxygen, three prominent atoms in biochemistry. The radionuclides

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Table 133.1 Examples of radionuclides used in positron emission tomography.

Radionuclide	Half-life (min)	Positron decay (%)	Daughter atom after decay
^{11}C	20.4	99.8	^{11}B
^{13}N	9.96	100	^{13}C
^{15}O	2.07	99.9	^{15}N
^{18}F	109.7	96.9	^{18}O
^{68}Ga	68.1	90	^{68}Zn
^{75}Br	101	76	^{75}Se (radioactive)

Adapted from Wolf & Fowler [4].

carbon 11, nitrogen 13, and oxygen 15 are commonly used in PET imaging in what is sometimes called *isotopic labeling* [3]. ^{11}C-methionine, ^{13}N-ammonia, and ^{15}O-water are examples of PET tracers labeled with these isotopes. In contrast, hydrogen, another important constituent of biochemical molecules, has no positron-emitting isotope. However, positron-emitting isotopes of halogens, most commonly fluorine 18, can be substituted for a hydroxyl group in what is termed *analogical labeling* [3]. The PET tracer most commonly used in clinical applications is a glucose analogue, 2-deoxy-2-[^{18}F]fluoro-D-glucose (^{18}F-fluorodeoxyglucose or ^{18}F-FDG). Other positron-emitting isotopes have been used in clinical and research settings, such as rubidium 82 for clinical myocardial perfusion studies and iodine 124 for emerging research applications.

Examples of radionuclides used in PET and their properties are listed in Table 133.1 [4]. Due to the relatively short half-lives of these compounds, a small amount of tracer with high specific activity can be administered, translating into relatively low radiation exposure during the imaging session [3]. Because the quantity of tracer used is very small, there is no risk of pharmacological activity or chemical toxicity. Preparation of PET tracers requires the generation of a positron-emitting radionuclide followed by incorporation of the radionuclide into a molecule of choice. In theory, an unlimited variety of molecules could be labeled. Hundreds of tracers have been labeled with positron emitters, including simple molecules used to measure blood volume and blood flow, amino acids to study protein synthesis, fatty acids and sugars to study metabolic pathways, and specific receptor ligands including pharmaceuticals to map receptor distribution. Positron emitters are generated in accelerators, commonly small medical cyclotrons [4]. The positron-emitting atoms must be incorporated quickly into a tracer molecule as a purified, sterile, isotonic, pyrogen-free solution [3]. This underscores the importance of cooperation among experts in multiple disciplines including physics, chemistry, computer science, imaging, and medicine in the original development of PET and in the maintenance of an active PET program. The short half-life of many radiotracers mandates that they be produced on site, as with ^{15}O-water. However, the comparatively longer half-life of ^{18}F-FDG allows it to be produced at a regional facility, possibly in close proximity to several PET centers. Currently, ^{18}F-FDG is commercially available and distributed for PET imaging in the USA. There have been active discussions regarding commercial distribution of other ^{18}F-labeled compounds.

2-Deoxy-2-[^{18}F]fluoro-D-glucose

The utility of ^{18}F-FDG for PET applications in oncology rests on the high demand for glucose by many tumors [5] and the similarities in the initial cellular metabolism of glucose and ^{18}F-FDG [6,7]. A great deal of research has been conducted to characterize the cellular transport and utilization of glucose. Briefly, cells take up glucose and ^{18}F-FDG through specific transporters, and both undergo phosphorylation by hexokinase inside the cell. Whereas glucose-6-phosphate can be a substrate for subsequent reactions in the glycolytic pathway, deoxyglucose-6-phosphate and fluorodeoxyglucose-6-phosphate cannot be metabolized further and are trapped in the cell, where the activity of the reverse enzyme glucose 6-phosphatase is limited. The contrast in ^{18}F-FDG uptake between tumors and metastases compared with surrounding tissue is often detectable by PET imaging and serves as the basis for PET cancer detection and staging.

Data acquisition and processing

The second key step in PET imaging comprises detection of photons emitted by the annihilation of positrons, followed by processing of these data to form cross-sectional images. The fact that positron annihilation produces photons traveling in nearly opposite directions serves as a sort of natural collimation and forms the basis for PET detector design and arrangement.

The typical PET scanner design includes multiple pairs of photon detectors oriented opposite each other in a circular arrangement around a field of view where the subject is placed [7]. *Coincidence detection* refers to the method of accepting photons as indicative of a true positron annihilation only when a pair of photons is detected nearly simultaneously (current time windows are approximately 12 ns) by an

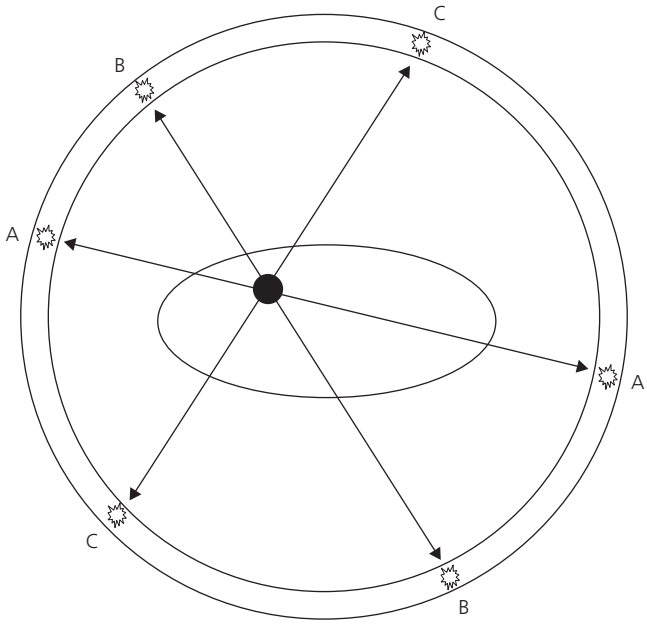


Figure 133.2 Coincidence detection is the basis for positron emission tomography (PET) detector design. Coincidence detection refers to the method of accepting photons as indicative of a true positron annihilation only when a pair of photons is detected nearly simultaneously by an oppositely aligned pair of detectors. In this schematic diagram, accumulation of a PET radiotracer within a lesion (black circle) in the PET subject (oval) produces three pairs of coincident detections (A, B, and C) in one ring of detectors surrounding the subject.

oppositely aligned pair of detectors (Fig. 133.2). Such coincident detection indicates that the photons originated somewhere near the imaginary line connecting the pair of detectors. Current PET scanners contain more than 40 adjacent rings and some 18 000 detector elements in the scanner. Each detector is connected in coincidence with an arc of detectors on the opposite side of the ring. In addition, rings can be connected in coincidence with each other. Standard two-dimensional image acquisition treats individually each pair of adjacent rings, or each "slice" through the subject. Three-dimensional acquisition has also been developed, in which all possible coincident detections across rings are used in generating image volume data. However, three-dimensional data acquisition increases the scatter fraction of noise, and it has been debated if three-dimensional imaging is well suited for abdominal PET imaging. The axial fields of view of current scanners are on the order of 15–20 cm, but whole-body scanning can be performed clinically with axial movement of the scanner table.

Corrections to coincident detection counts are often performed before image generation to account for a number of factors, including random coincidences at detector pairs and attenuation of photons inside the subject [7]. Attenuation refers to the scattering or absorption of emitted γ-rays within the subject's body. Attenuation correction is achieved by directly measuring tissue attenuation through the subject using an external radiation source equipped in the scanner. External rotating rods of germanium 68, which are shielded when not in use, are commonly used to perform a *transmission scan* before PET tracer administration. This transmission scan is equivalent to a CT scan that visualizes differences in tissue attenuation within the body for diagnostic purposes. In PET/CT scanners, CT data are used for attenuation correction as well as anatomical localization. Because of the speed of CT scanners combined with PET, PET/CT scanners can significantly reduce total imaging time.

Computerized mathematical algorithms can be applied for image reconstruction. These usually rely on filtered back-projection, the same technique used in X-ray CT. The image produced by this technique represents the solution to the question of what spatial and temporal distribution of positron emitter can account for the pattern of coincident detections recorded by the PET scanner. An iterative image reconstruction method based on statistical modeling and segmented transmission data has now become available for commercial scanners. This method generally produces better image quality, particularly when count rates are low and when large patients are scanned. However, it is important to recognize that noise distribution and potential artifacts introduced by iterative image reconstruction are quite distinct from those seen with conventional filtered back-projection reconstruction techniques. Due to a variety of factors, including the short path that positrons can travel before annihilation and the slight deviations in photon direction that can result from residual angular momentum in a positron or electron before annihilation, the intrinsic resolution of modern PET is on the order of 4–6 mm.

Clinical applications of PET in gastroenterology

In recent years, the recognized clinical uses of PET have expanded, particularly in the imaging of tumors. Regarding gastrointestinal cancers, the United States Health Care Financing Administration (HCFA), currently the Centers for Medicare and Medicaid Services (CMS), first announced Medicare coverage in 1999 for ^{18}F-FDG PET in the evaluation of patients with colorectal cancer and rising carcinoembryonic antigen (CEA) levels. In December 2000, HCFA issued a decision memorandum broadening the indications for ^{18}F-FDG PET covered by Medicare to include diagnosis, staging and restaging of nonsmall cell lung cancer, colorectal cancer, esophageal cancer, lymphoma, melanoma, and head and neck (excluding thyroid and brain) cancers; myocardial viability studies; and presurgical evaluation of refractory seizures [8]. A survey of 22 institutions found no adverse reactions in over 80 000 positron-emitting radiopharmaceutical administrations, highlighting the extraordinary safety record of PET [9]. As of 2006, head and neck cancers, eso-

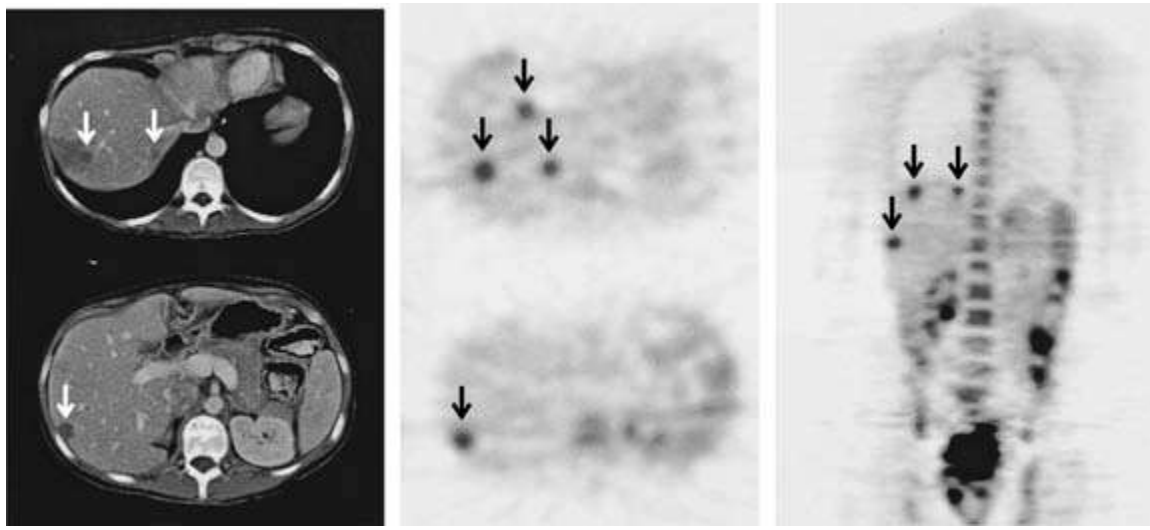
phageal cancer, colorectal cancer, nonsmall cell lung cancer, melanoma, breast cancer, lymphoma, cervical cancer, thyroid cancer, and solitary pulmonary nodule are covered by CMS reimbursement for one or more of the indications of diagnosis, staging, restaging, or monitoring. In response to the CMS proposal to expand further PET coverage for cancer workups, the National Oncologic PET Registry (NOPR) was developed for ^{18}F-FDG PET to include cancers and indications not presently eligible for CMS reimbursement.

Because anatomical information is often necessary in evaluating patients, including determining the potential resectability of the tumor, ^{18}F-FDG PET cannot be considered a substitute for anatomical imaging. However, it can provide important additional information in a variety of clinical situations. A major advance has been the development of combined PET/CT scanners, which can provide both functional and anatomical imaging [10,11], with performance that is superior to that of PET alone or that of software fusion of independently acquired PET and CT images [12]. Serious incorrect lesion localization with PET/CT is rare (2%) [13]. Evidence is accumulating that PET imaging alters medical decision making in a significant minority of cases with respect to surgical interventions and radiation therapy.

Colorectal cancer

Increased ^{18}F-FDG uptake in hepatic metastases from colorectal cancer was demonstrated at a very early stage in PET technology development [14]. Later studies suggested that ^{18}F-FDG PET could be useful in differentiating recurrent rectal cancer from surgical scar or a nonmalignant mass [15,16]. When anatomical imaging techniques could not reliably distinguish malignant from nonmalignant tissue, the enhanced glycolytic activity of malignant tissue could be detected by ^{18}F-FDG PET. Subsequently, ^{18}F-FDG PET has been investigated in the evaluation of patients with suspected colorectal cancer recurrence, as well as in preoperative staging of patients with known recurrence and in staging of primary disease. Recent studies have focused on the incremental value of PET/CT. Figure 133.3 shows an example of ^{18}F-FDG PET in a patient with rectal cancer metastatic to the liver.

In suspected metastatic or recurrent colorectal cancer, the reported sensitivity of whole-body or local/pelvic ^{18}F-FDG PET is 87%–100% and specificity 67%–100%, with sensitivity for hepatic disease of 89%–100% and specificity of 67%–100% [17–28]. In all studies that compared PET with CT with respect to hepatic metastases detection, PET performance was better (CT sensitivity 65%–89% and specificity 62%–89%). A metaanalysis of 11 studies yielded overall estimates (and 95% confidence intervals [CI]) for the sensitivity of whole-body ^{18}F-FDG PET in recurrent colorectal cancer of 97% (CI 95%–99%) and specificity of 76% [29]. For local/pelvic disease, the estimated sensitivity was 95% (CI 91%–98%) and specificity 98% (CI 96%–99.7%). For hepatic involvement, estimated sensitivity was 96% (CI 94%–99%) and specificity 99% (CI 98%–100%). Another metaanalysis reported per-patient sensitivities for nonhelical CT, helical CT, MRI, and ^{18}F-FDG PET of 60%, 65%, 76%, and 95%, respectively [30]. Another systematic review reported sensitivity of 88% and specificity of 96% for hepatic disease and 92% and 95% for extrahepatic disease using ^{18}F-FDG PET, compared with 83% and 84% for hepatic disease and 61% and 91% for extrahepatic disease using CT [31].



CT PET (slice) PET (whole body)

Figure 133.3 Imaging with contrast computed tomography (CT) and ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET) in a patient with rectal cancer metastatic to the liver. Contrast CT shows at least three hepatic hypovascular lesions (white arrows). Corresponding PET slice images show a total of four hepatic lesions (black arrows). Whole-body PET shows at least three of the hepatic lesions (black arrows). A large focus of uptake in the pelvis is excreted ^{18}F-FDG in the bladder. Uptake is also visible in the right kidney next to the lumbar spine and in the descending colon.

While in many investigations ^{18}F-FDG PET images were interpreted visually, some authors have reported higher signal intensity in malignant compared with benign lesions using semiquantitative methods, such as the standardized uptake value (SUV) or SUV corrected for lean body mass (SUL) [32], which normalizes signal intensity by radioactive dose injected and body weight, and the target-to-background ratio, which compares signal intensity within and outside a region of interest [18,21,25,28].

^{18}F-FDG PET/CT performed better than ^{18}F-FDG PET alone in detecting recurrent colorectal cancer, with sensitivity of 89%, specificity of 92%, and overall accuracy of 90%, compared with 80%, 69%, and 75%, respectively [33]. ^{18}F-FDG PET/CT has also been reported to be superior to ^{18}F-FDG PET alone in detecting recurrence of rectal cancer after abdominoperitoneal resection [34]. Dedicated interpretation of the CT portion of the ^{18}F-FDG PET/CT examination improves performance (sensitivity, specificity, and accuracy of 99%, 100% and 98% compared with 91%, 63% and 83% for the PET/CT report alone; $P < 0.05$) [35].

Patients may be suspected of having colorectal cancer recurrence on the basis of symptoms, physical findings, rising CEA levels, or anatomical imaging studies. In a study of 22 patients with previous colorectal cancer resection who had abnormal CEA but normal anatomical imaging studies, 15 patients were ultimately considered to have recurrent disease. ^{18}F-FDG PET detected abnormalities in 17 patients, including the 15 with proven recurrence, yielding a positive predictive value of 89% and a negative predictive value of 100% [24]. ^{18}F-FDG PET may predict which patients with abnormal CEA might benefit from laparotomy [36]. It may help triage patients with CEA elevations under 25 ng/mL into potentially resectable rather than unresectable subgroups, while in those with higher elevations it may primarily confirm advanced disease [37]. The additional value of ^{18}F-FDG PET was examined in 103 patients with suspected or proven colorectal cancer recurrence who also underwent conventional imaging including CT [26]. In 12 of 60 patients considered to have resectable disease on conventional imaging, ^{18}F-FDG PET detected additional disease in nine and excluded disease in three, yielding additional diagnostic value in 20%. In 13 patients with inconclusive conventional imaging or isolated elevated CEA, ^{18}F-FDG PET was of additional diagnostic value in 62%.

Accurate staging is of paramount importance in guiding potential surgical intervention, including resection of hepatic metastases. An important contribution of ^{18}F-FDG PET appears to be demonstration of hepatic and extrahepatic foci of disease not identified on conventional imaging [38,39]. However, a study directly comparing ^{18}F-FDG PET results with surgical resection specimens found that ^{18}F-FDG PET detected only 25% of hepatic lesions smaller than 1 cm, compared with 85% of lesions larger than 1 cm [40]. A prospective evaluation of 53 patients with potentially resectable hepatic metastases revealed comparable sensitivities (75% vs 76%) and accuracies (88% vs 86%) for ^{18}F-FDG PET and CT, with ^{18}F-FDG PET providing additional information in 10% of patients [41], comparable to the incremental information in 11% of patients reported in an earlier study [42].

One study has assessed ^{18}F-FDG PET in the staging of primary colorectal cancer [43]. The sensitivity and specificity of ^{18}F-FDG PET for the primary tumor were 100% and 43% respectively. For liver metastases, the sensitivity and specificity were 88% and 100%, compared with 38% and 97% for CT. For lymph node metastases, the sensitivity and specificity of ^{18}F-FDG PET were 29% and 96%, compared with 29% and 85% for CT. Thus, the utility of ^{18}F-FDG PET appears greater in detecting distant metastases than local nodal involvement. Another study found that ^{18}F-FDG PET was not superior to multidetector helical CT in the initial staging of colorectal cancer [44]. Whole-body ^{18}F-FDG PET/CT colonography has been proposed for initial staging of patients with colorectal cancer [45].

The ultimate value of a diagnostic test is manifest in its impact on patient outcomes. Several investigators have reported the impact of ^{18}F-FDG PET on medical decision making. In the metaanalysis by Huebner and colleagues, it was estimated that ^{18}F-FDG PET affected management in 29% (95% CI 25%–34%) of patients undergoing evaluation for recurrent colorectal cancer [29]. Subsequent studies have reported similar results, with ^{18}F-FDG PET affecting management in 23% of patients undergoing evaluation for possible resection of hepatic metastases [40], 20%–61% of patients suspected of having metastatic or recurrent colorectal cancer [28,46–49], and 17% of patients with primary rectal cancer [50]. In a study of 76 patients, ^{18}F-FDG PET/CT and contrast-enhanced CT provided similar information about hepatic metastases, whereas PET/CT was superior in detecting recurrent intrahepatic tumors after hepatectomy, extrahepatic metastases, and local recurrence, thus affecting management in 21% of cases [51]. ^{18}F-FDG PET was more accurate than conventional imaging tests in predicting the resectability of the recurrent disease (82% vs 68%; $P = 0.02$) [52]. Wiering and colleagues reported a change in management due to ^{18}F-FDG PET in 32% of patients in all studies in their systematic review, and a somewhat lower 25% in the highest quality studies [31]. ^{18}F-FDG PET/CT was reported to affect patient management in 7 of 23 patients with suspected hepatic metastases or recurrent disease [53]. More important than any impact on decision making is the observation that patients who underwent hepatic resection for metastases after staging with ^{18}F-FDG PET had a 5-year actuarial survival of 58% compared with a median of 30% in historic controls without preoperative PET examination [54].

The ability to monitor response to therapy or to assess a patient's candidacy for chemotherapy based on tumor characteristics are potentially of great clinical relevance. Decreases in ^{18}F-FDG uptake after chemotherapy have been shown to

correlate with response to chemotherapy [55,56]. In contrast, because inflammatory tissue may show similar glycolytic activity to that of tumor, ^{18}F-FDG PET may not be able to accurately assess the response to radiation therapy in the short term [57,58]. However, one pilot study has suggested that ^{18}F-FDG PET might be of incremental value in selecting patients with rectal cancer for sphincter-preserving surgery after preoperative radiation and chemotherapy [59]. ^{18}F-FDG PET may be useful in predicting the response of liver metastases to treatment with bevacizumab and irinotecan [60].

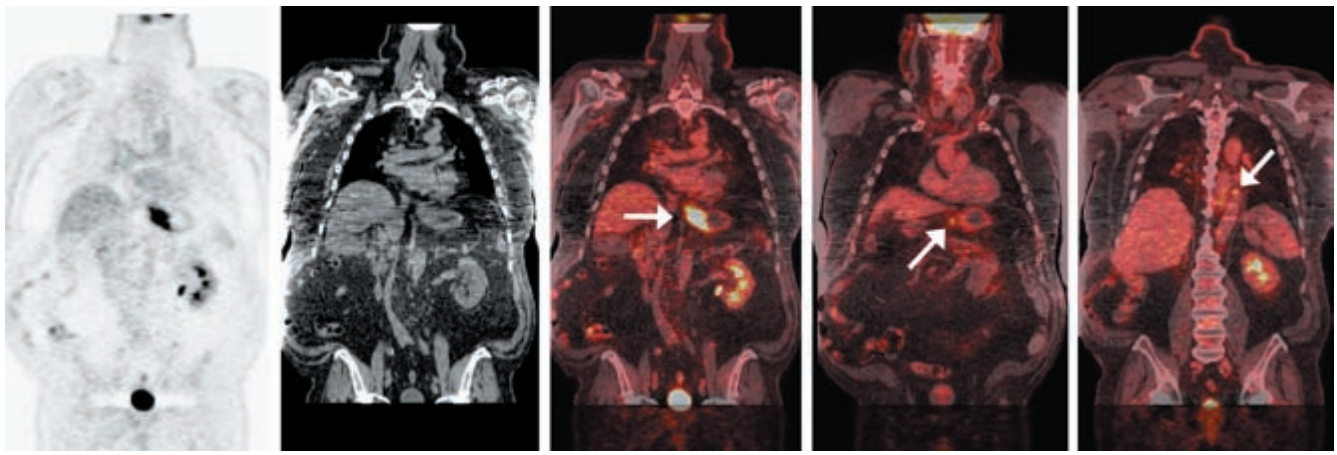
In patients with unresectable liver metastases, PET has been performed with ^{18}F-fluorouracil before 5-fluorouracil chemotherapy to determine its potential role in predicting prognosis [61]. Overall, patients with higher values for ^{18}F-fluorouracil uptake were more likely to achieve stabilization of disease with chemotherapy, but trapping of ^{18}F-fluorouracil varied among metastases even within the same patient. Quantitative modeling of ^{18}F-fluorouracil uptake has been investigated as a way to optimize treatment schedules for individual patients [62]. Specific kinetic parameters of ^{18}F-FDG PET may be useful in determining prognosis with 5-fluorouracil/folinic acid/oxaliplatin chemotherapy [63].

van Kouwen and colleagues reported increasing sensitivity of ^{18}F-FDG PET for colorectal adenomas as a function of size (e.g., 21% for 1–5 mm vs 72% for > 11 mm) and grade of dysplasia (e.g., 33% for low-grade vs 76% for high-grade dysplasia vs 89% for carcinoma) [64]. Others have reported limited sensitivity of ^{18}F-FDG PET for flat or less than 3 cm protruded premalignant lesions, and for cancers under 2 cm [65].

Esophageal cancer

Various studies have compared ^{18}F-FDG PET and CT for staging of esophageal cancer [66–70]. The specificity of the two tests appears to be comparable in the evaluation of nodal disease (PET 79%–94%, CT 73%–100%), while the sensitivity of ^{18}F-FDG PET appears to be superior (PET 69%–92%, CT 28%–46%). For both techniques, detecting local nodal involvement is problematic. In one study, the overall accuracy in determining resectability was 88% for ^{18}F-FDG PET and 65% for CT ($P = 0.04$), but neither modality could assess the extent of wall invasion [68]. Differences in accuracy of similar magnitude have been found in other studies [66,69,70]. As with colorectal cancer staging, an important contribution of ^{18}F-FDG PET appears to be its ability to detect distant metastases not visible on anatomical imaging, and thus alter management. One group has reported additional diagnostic value in 22% of patients, with up-staging in 15% and down-staging in 7% [71]. Another study reported a change in management based on ^{18}F-FDG PET results in 17% of patients [66]. PET/CT delineated tumor better than CT alone in another study, contributing to radiotherapy treatment planning [72]. Figure 133.4 shows an example of ^{18}F-FDG PET/CT staging of esophageal cancer.

^{18}F-FDG PET has been compared with the combination of CT and endoscopic ultrasound (EUS) in staging potentially operable esophageal cancers [71]. EUS demonstrated a higher sensitivity than ^{18}F-FDG PET for local nodal involvement (81% vs 33%; $P = 0.027$). In evaluating regional and distant nodal involvement, ^{18}F-FDG PET was more specific than CT and EUS combined (98% vs 90%; $P = 0.025$), with both testing strategies having similar sensitivity (43% vs 46%). PET/CT was more accurate than EUS with fine-needle aspiration (FNA) and CT in detecting nodal disease and response to neoadjuvant chemoradiotherapy [73]. The role of PET in staging adenocarcinoma of the esophagus or gastroesophageal junction may be primarily to detect organ metastases [74].



(a) (b) (c) (d) (e)

Figure 133.4 ^{18}F-fluorodeoxyglucose positron emission tomography (^{18}F-FDG PET)/computed tomography (CT) staging of esophageal cancer. Coronal ^{18}F-FDG PET scan **(a)**, corresponding CT scan **(b)**, and fused image **(c)** demonstrate primary cancer in the distal esophagus extending to the gastric fundus (arrow in **c**). ^{18}F-FDG PET/CT detected two small lymph node metastases: left gastric node (arrow in **d**) and paraesophageal node (arrow in **e**).

The combination of ^{18}F-FDG PET and ^{11}C-choline PET, which relies on uptake of choline by tumors for integration into phospholipids, has been reported to improve the accuracy of PET in staging esophageal cancer [75]. ^{11}C-choline PET had a sensitivity of 88% for mediastinal lymph nodes, compared with 34% for ^{18}F-FDG PET. However, ^{11}C-choline PET demonstrated 0% sensitivity in the upper abdomen due to high ^{11}C-choline uptake by the liver, compared with 79% for ^{18}F-FDG PET. The combination of both modalities detected 85% of all lymph node metastases.

Differences in survival have been reported based on stratification by initial ^{18}F-FDG PET result [69]. Survival at 30 months was 60% in patients with localized disease compared with 20% in patients with distant disease ($P = 0.01$), suggesting that ^{18}F-FDG PET results may be of prognostic significance. The number of PET abnormalities at baseline is an independent predictor of survival [76]. Serial ^{18}F-FDG PET appears to have a strong correlation with pathological response to neaoadjuvant chemoradiation therapy in locally advanced esophageal cancer, with PET major responders surviving a median of 16.3 months compared with 4 months for PET nonmajor responders [77]. The reported 3-year survival after surgery was 57% for patients with SUV maximum greater than 4.5, and 95% for those with SUV maximum 4.5 or less [78].

Pancreatic cancer

The initial HCFA decision memorandum of December 2000 did not include pancreatic imaging as an approved indication for ^{18}F-FDG PET under Medicare. However, pancreatic imaging is considered a recognized application of ^{18}F-FDG PET by some experts [38] and is now eligible for NOPR coverage as of 2006.

Various studies have evaluated ^{18}F-FDG PET in the diagnosis of pancreatic cancer and its differentiation from chronic pancreatitis [79–87]. The reported sensitivity of PET in this setting is 85%–96% with specificity of 78%–100%, compared with 65%–89% and 62%–89% for CT. ^{18}F-FDG PET may be useful in assessing pancreatic masses that are indeterminate for malignancy on conventional imaging, with sensitivity of 68%–96% and specificity of 78%–100% [88]. Beyond visual interpretation of images, semiquantitative analysis has demonstrated differences between signal intensity, such as SUV, in tumor and chronic pancreatitis [80,81,84,86,89]. In one study, ^{18}F-FDG PET suggested potential alterations in clinical management in 43% of patients [85].

Two important factors may affect the results of ^{18}F-FDG PET in pancreatic imaging. First, hyperglycemia can result in false-negative studies in pancreatic cancer [83,90]. The cancer itself may cause endocrine insufficiency, and hyperglycemia results in greater competition between glucose and ^{18}F-FDG for uptake into cells. In patients with normal serum glucose, for instance, the sensitivity and specificity of ^{18}F-FDG PET in diagnosing pancreatic cancer were reported as 98% and 84%, compared with 63% and 86% in hyperglycemic patients [83]. Second, inflammation can produce ^{18}F-FDG uptake as intense as that seen in tumor, either in the setting of pancreatic masses without clear evidence of acute pancreatitis, or during and after attacks of acute pancreatitis [91].

Multiple imaging modalities can be used in evaluating patients with suspected pancreatic malignancy. One study reported sensitivity and specificity for four different modalities in patients with suspected pancreatic cancer: 94% and 82% for ^{18}F-FDG PET, 89% and 73% for CT, 97% and 64% for EUS, and 89% and 45% for abdominal ultrasound [79]. Subsequent studies found sensitivity for detecting pancreatic cancer of 87% for ^{18}F-FDG PET, 53% for CT and 93% for EUS [92] and 87% for ^{18}F-FDG PET, 87% for MRI and 98% for EUS [93]. Overall accuracy values (defined as area under receiver operating characteristic curves, with 1 reflecting a perfect test) for differentiating malignant from benign pancreatic masses have been reported for various imaging strategies [90]. Overall accuracy was 0.86 for ^{18}F-FDG PET, 0.93 for endoscopic retrograde cholangiopancreatography (ERCP), 0.82 for CT, and 0.95 for the combination of ^{18}F-FDG PET and ERCP. In the subgroup of euglycemic patients with contradictory or indeterminate CT and ERCP results, ^{18}F-FDG PET was accurate in 84% of cases.

^{18}F-FDG PET has also been studied for staging of metastatic disease. One study of ^{18}F-FDG PET in pancreatic cancer found sensitivity and specificity of, respectively, 49% and 63% for lymph node staging and 70% and 95% for hepatic metastases [90]. As in colorectal cancer, ^{18}F-FDG PET may miss smaller metastases (< 1 cm). A study designed specifically to assess the detection of hepatic metastases of pancreatic cancer found sensitivity of 90% and specificity of 91% for ^{18}F-FDG PET, compared with 69% and 100% for CT, and 82% and 100% for abdominal ultrasound [94]. In another investigation, the sensitivity of ^{18}F-FDG PET for hepatic and extrahepatic disease was 78% compared with 33% for CT [92].

^{18}F-FDG PET has also been used to monitor patients after therapy [95,96]. Survival in patients without measurable pancreatic ^{18}F-FDG uptake 1 month after chemotherapy was improved compared with patients with residual ^{18}F-FDG uptake (319 vs 139 days; $P = 0.034$). When used for detecting malignancy in cystic tumors of the pancreas, ^{18}F-FDG PET had a sensitivity of 94% and specificity of 97% compared with 65% and 87% for CT [97].

Hepatic imaging

Liver and bile duct cancers are a part of the NOPR coverage as of 2006. ^{18}F-FDG PET has been evaluated in the setting of hepatocellular carcinoma as well as in metastatic disease to the liver. The sensitivity of ^{18}F-FDG PET for hepatoma is poor [98]. In one study, only 50% of sonographically detectable hepatomas demonstrated increased ^{18}F-FDG uptake, whereas 79% were detectable by CT [99]. None of the well-differenti-

ated tumors was visualized by ^{18}F-FDG PET compared with 88% of the moderately or poorly differentiated tumors. Similar results were reported in a second study, with ^{18}F-FDG PET showing a sensitivity of 55% in the diagnosis of hepatoma compared with 90% for CT [100]. In patients with rising α-fetoprotein levels after hepatoma treatment but normal conventional imaging, ^{18}F-FDG PET had 73% sensitivity and 100% specificity for detecting recurrent disease [101].

In contrast to poor results in the diagnosis of hepatoma, ^{18}F-FDG PET can be useful in evaluating metastatic disease to the liver [102], as discussed above for primary gastrointestinal tumors. One study of patients with various primary tumors and possible liver involvement found sensitivity of 97% and specificity of 88% for ^{18}F-FDG PET compared with 93% and 75% for CT (not statistically different) [103]. ^{18}F-FDG PET yielded new information for 23% of patients, including demonstrating liver metastases in 17% when other imaging was equivocal or negative. In patients undergoing CT-guided FNA of liver lesions, ^{18}F-FDG PET was positive for malignancy in 96% of cases in which malignancy was diagnosed by FNA, and was negative in 86% of cases where FNA was negative [104].

^{18}F-FDG PET has been examined in the setting of hepatic lesions requiring further characterization. In a series of 110 patients with hepatic lesions 1 cm or larger on CT, all liver metastases from adenocarcinomas and sarcomas and all cholangiocarcinomas showed increased ^{18}F-FDG uptake values, whereas only 70% of hepatomas showed increased uptake [105]. All benign lesions had poor uptake, and abscesses showed equivocal or increased uptake. A series of 78 patients with various liver tumors has confirmed that ^{18}F-FDG uptake tends to be lower in hepatocellular carcinoma compared with metastatic disease or cholangiocarcinoma [106]. Case reports have suggested that ^{18}F-FDG PET might be useful in detecting cholangiocarcinoma in patients with primary sclerosing cholangitis [107,108]. ^{18}F-FDG PET has also been used to monitor response to chemoembolization [109] and local ablative therapies [110]. ^{18}F-FDG PET/CT may have a role in monitoring response to radiofrequency ablation [111,112] or yttrium 90 glass microsphere treatment [113] of unresectable metastases.

Neuroendocrine tumors

Carcinoid tumors synthesize serotonin from 5-hydroxytryptophan (5-HTP). An early study with seven patients suggested that hepatic metatstases from carcinoid tumors could be imaged with ^{11}C-5-HTP PET [114]. In 22 patients with neuroendocrine tumors, ^{11}C-L-dihydroxyphenylalanine (^{11}C-L-DOPA) PET detected tumor in 50% [115]. In those imaged with both ^{11}C-L-DOPA and ^{11}C-5-HTP, results were similar. One study has reported similar overall results for ^{11}C-5-HTP PET and CT in 16 patients with gut carcinoids and two with endocrine pancreatic tumors [116]. Although ^{11}C-5-HTP detected more lesions than CT in 10 patients, it is not clear that this is of clinical significance. Carbidopa pretreatment improves tumor visualization [117]. ^{18}F-DOPA PET had 65% sensitivity for carcinoid tumors, and performed better than CT or MRI for primary lesion localization and lymph node staging [118]. In patients with carcinoid tumors, PET with a carbohydrated ^{18}F-labeled somatostatin receptor ligand revealed more lymph node or ossesous metastases than CT or MRI, but MRI revealed more hepatic metastases, making PET/MRI a promising approach [119].

Somatostatin receptor scintigraphy (SRS) is commonly used for imaging neuroendocrine tumors. In 15 patients with neuroendocrine tumors, SRS was found to be more useful overall than ^{18}F-FDG PET, although SRS was negative and ^{18}F-FDG PET positive in two patients with less differentiated tumors [120]. A second study with 16 patients found an overall sensitivity of 85% for SRS in neuroendocrine tumor imaging. ^{18}F-FDG PET did not seem to provide much additional value, even though it was able to image aggressive tumors [121]. Notably, in this study, CT scans were abnormal in all patients with abnormal ^{18}F-FDG PET and in all but one patient with abnormal SRS. However, a later study reported better performance for whole-body ^{11}C-5-HTP PET than for SRS or CT [122].

The difficulty in detecting pancreatic islet cell tumors is highlighted by a study in which the sensitivity of ^{18}F-FDG PET was 53%, compared with 53% for ultrasonography, 50% for CT, and 53% for MRI [123]. ^{18}F-FDG PET was negative or equivocal in all cases where the other modalities were negative.

Gastrointestinal stromal tumors

The degree of ^{18}F-FDG uptake may be useful in predicting the malignant potential of gastrointestinal stromal tumors [124]. In addition, ^{18}F-FDG PET can detect response to imatinib mesylate treatment in patients with malignant gastrointestinal stromal tumors [125–127] as early as 1 week after starting therapy [128]. PET/CT delineated lesions better than the combination of PET and contrast-enhanced helical CT [129,130].

Other diseases

^{18}F-FDG PET appears to have reasonable sensitivity and specificity for inflammation in Crohn's disease in adults [131] and in Crohn's disease and ulcerative colitis in children [132] but its clinical role is not yet established. It may be useful in selecting patients with familial adenomatous polyposis for further investigations to rule out malignancy [133]. ^{18}F-FDG PET may have a role in some cases of gastrointestinal lymphoma [134]. ^{18}F-FDG PET facilitated early detection of hereditary diffuse gastric cancer in an asymptomatic carrier of a germline E-cadherin mutation [135], but it is generally not a first-line test for staging gastric cancer [136,137]. ^{18}F-FDG PET results may predict response to preoperative chemotherapy in gastric cancer, and patients with a metabolic

response appear to have improved survival compared with metabolic nonresponders [138].

Incidental gastrointestinal uptake may be seen in approximately 3% of PET or PET/CT studies performed for pulmonary or other imaging, and often reflects clinically significant lesions, with pathology including unsuspected gastrointestinal malignancy [139–142].

Applications of PET in gastroenterology research

The ability of PET to use a theoretically unlimited variety of molecules radiolabeled with different positron emitters to image biological processes in vivo opens numerous possibilities for PET applications in research. The following studies illustrate the broad range of questions that has been addressed in PET research studies relevant to gastroenterology. An anticolorectal carcinoma monoclonal antibody labeled with copper 64 (radioimmuno-PET) has shown 71% sensitivity for imaging tumor foci [143]. ^{11}C-methionine uptake by the pancreas has been investigated by PET as a possible reflection of pancreatic exocrine function [144]. Biliary bicarbonate secretion in humans has been studied after intravenous injection of sodium ^{11}C-bicarbonate [145]. In cholestatic conditions, an impaired response to secretin was observed. Glucose utilization has been studied with ^{18}F-FDG PET in a patient with hepatoma and hypoglycemia [146]. The mechanism and kinetics of percutaneous ethanol injection of hepatomas has been studied with ^{11}C-ethanol [147].

Neurological abnormalities can accompany various forms of liver disease. ^{18}F-FDG PET of the brain in patients with Wilson disease has demonstrated decreases in regional cerebral glucose metabolism in the cerebellum, striatum, cortex, and thalamus compared with controls [148]. In this study, striatal metabolism was most affected in those patients who had received therapy for the shortest time. Altered cerebral blood flow and metabolism have been observed in patients with severe liver disease and low-grade encephalopathy [149]. Normalization of brain glucose metabolism has been observed following liver transplantation [150]. In patients with hepatic encephalopathy, central benzodiazepine receptor binding has been studied with ^{11}C-flumazenil, with significant increases shown in the thalamus, cerebellum and pons but not in the cortex or whole brain, when modeling corrected for altered hepatic metabolism [151]. In patients with cirrhosis, ^{13}N-ammonia and ^{15}O-water have been used to demonstrate regional differences in cerebral ammonia metabolism and blood flow that correlate with the distribution of histopathological changes and clinical features of hepatic encephalopathy [152].

In animal studies, liver regeneration has been assessed with ^{11}C-thymidine PET [153], the trafficking of radiolabeled tumor cells in vivo has been assessed with PET [154], ^{11}C-octanoate PET has been investigated for assessing liver function [155], and ^{11}C-labeled cholecystokinin receptor antagonists have been synthesized and evaluated [156].

Functional brain imaging and visceral sensation

One application that has received intense interest is functional imaging of the central nervous system as a tool for studying visceral sensation, particularly in the context of visceral hypersensitivity in functional gastrointestinal disorders [157]. In brain activation studies with PET, regional cerebral blood flow (assessed with ^{15}O-water) during a task of interest is compared with regional cerebral blood flow during baseline to identify cerebral regions with increased activation during the particular task. This widely used technique is based on the assumption that regional blood flow is coupled with regional neuronal activity under physiological conditions. In visceral sensation research, the stimulation task has consisted most often of visceral distention with a balloon.

In an early study, rectal distention in healthy volunteers led to activation in the precentral and postcentral gyrus and thalamus [158]. In one of the first studies of functional brain imaging in patients with functional gastrointestinal disease, rectal distention in controls but not patients with irritable bowel syndrome (IBS) produced activation of the anterior cingulate cortex, a region implicated in the affective response to pain [159]. A subsequent study with fMRI reported activation in the anterior cingulate cortex, prefrontal cortex, insular cortex, and thalamus in most IBS and control subjects, with increased anterior cingulate cortex activation in IBS subjects compared with controls [160]. Compared with female subjects with IBS, male subjects with IBS have demonstrated increased activation in the insula, considered a visceral sensory cortex [161]. In this study, insular activation was associated most strongly with distending pressure, whereas anterior cingulate activation was associated more with subjective discomfort. Activation patterns differ by gender during both actual and anticipated rectal distention [162]. Reduction in symptoms in patients with IBS treated with alosetron correlated with reductions in blood flow in regions believed to mediate emotion [163]. During alosetron treatment, male subjects showed greater levels of serotonin synthesis in several brain regions than did female subjects, as assessed by ^{11}C-methyl-L-tryptophan PET [164]. IBS patients have shown altered brain responses to anticipated as well as actual rectal distention [165]. Changes in activity of various cortical–limbic regions have been reported in patients with severe IBS after cognitive therapy [166].

The cerebral representation of esophageal distention has also been studied with ^{15}O-water PET [167]. During nonpainful stimulation, activation was seen bilaterally along the central sulcus, in the insula, and in the frontal/parietal operculum. Painful stimulation led to greater activation in the same regions, with additional activation of the right anterior insula and the anterior cingulate gyrus, leading the authors

to suggest that these latter structures are involved in esophageal pain processing. The activation pattern during volitional swallowing has been studied with ^{18}F-FDG PET [168].

Cerebral activation has been investigated with ^{15}O-water PET during gastric distention producing bloating, pain, and nausea [169]. Paralleling increases in distention stimulus and symptoms, progressive increases in activation were observed in the thalami, insulae, anterior cingulate cortex, caudate nuclei, brainstem periaqueductal gray matter, cerebellum, and occipital cortex. This activation pattern showed important similarities to activation patterns reported in studies of painful somatic stimulation. Proximal gastric distention has been reported to activate the postcentral gyri, left superior temporal gyrus, right inferior frontal and midanterior cingulate gyri and anterior insula, and left cerebellum, with increasing activation observed as a function of increased stimulus intensity [170].

Many functional brain imaging studies concerning visceral pain and intestinal sensation are now carried out by fMRI. The advantages of fMRI over PET include general accessibility, no involvement of radiation, repeatability, and time and spatial resolution.

Future directions

PET has been called the most sensitive and specific means of studying, through imaging, the molecular processes in humans in vivo [171]. The development of a micro-PET scanner for small animals, combined with rapidly growing experimental capabilities in molecular biology, presents the opportunity to carry out molecular imaging assays in vivo, including studies of gene expression [172].

In the clinical arena, the number of clinical PET facilities has grown over time, and PET is becoming an essential diagnostic tool for the management of certain patients with cancer. Given the ability to produce ^{18}F-FDG off-site and its commercial availability in the USA, not all clinical PET centers will require an on-site cyclotron. This may facilitate further the widespread use of PET. Newer tracers, including ^{18}F-labeled radiopharmaceuticals that can be prepared and delivered commercially owing to their relatively long half-life, may ultimately find clinical applications [173]. The availability of PET/CT scanners is likely to increase, providing both functional and anatomical information simultaneously with minimal incremental burden to patients.

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B IMAGING

134 Applications of radionuclide imaging in gastroenterology

Harvey A. Ziessman

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Radionuclide diagnostic methods have been used by gastroenterologists for decades. For example, the first publication on the use of radionuclides to quantify gastric emptying was reported in the *Lancet* in 1966 [1]. The advantage of methods employing nuclear medicine is that interpretation is made based on physiology and function, information that is often markedly different from that of anatomical imaging methods, such as ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI).

Radiopharmaceuticals and radiation detection instrumentation

Radionuclide methodology requires a radiopharmaceutical (radioactive chemical or drug), which is administered to the patient, and radiation detection or imaging instrumentation.

*Radionuclide*s are used as "radiotracers," that is, a radioactive label that allows for the detection, localization, and quantification of the chemical/drug to which it is bound. Radionuclides are unstable isotopes that emit radiation as they decay to a more stable isotope. Different radionuclides decay at different rates. For example, technetium 99m (^{99m}Tc) has a 6-h half-life. If 10 millicuries (mCi) (370 MBq) is administered to a patient, 5 mCi (185 MBq) will remain undecayed 6 h later. Thus, images are usually performed early after administration, before it decays away – for ^{99m}Tc, within 24 h.

When a radionuclide is chemically bound to a chemical, for example, ^{99m}Tc in sodium pertechnetate ([^{99m}Tc]pertechnetate) and iodine 123 in sodium iodide (sodium [^{123}I]iodide), or to a pharmaceutical, for example, fluorine 18 in fluorodeoxyglucose ([^{18}F]FDG) or indium 111 in octreotide ([^{111}In]octreotide), it is referred to as a radiopharmaceutical. The pharmaceutical or chemical determines its distribution in the body. Different routes of administration result in different distributions within the body. Technetium 99m sulfur colloid (^{99m}Tc-SC) administered intravenously will be extracted by the liver and spleen (liver–spleen scan), but when given orally is not absorbed and transits according to gastrointestinal motility (gastric emptying study). Diagnoses are made based on physiological deviations from the normal ranges in uptake, localization, distribution, and clearance. [^{18}F]Fluorodeoxyglucose, a glucose analogue, distributes throughout the body to all cells that metabolize glucose. Because malignant tumors have increased glucose metabolism compared with most nonmalignant tissues, cancers have increased uptake of [^{18}F]FDG.

Radiopharmaceuticals are administered in subpharmacological doses; that is, they have no pharmacological effect in humans. Thus, allergic reactions are rare. Radiation exposure to the patient from radiopharmaceuticals is generally low, often greater than a chest radiograph but usually less than a CT scan, depending on the radiopharmaceutical's distribution, total body clearance, and decay rate.

Three general types of instrumentation are used to detect radiotracers in the body. First, nonimaging radiation detectors, for example, specialized probes, are used for thyroid uptake measurements or intraoperative tumor localization. Second, well-counters are used to detect radiation in body tissues or fluids, for example red blood cell volume or Shilling tests. Third, imaging instrumentation includes gamma cameras and positron emission tomography (PET) cameras. Gamma cameras image radionuclides that give off one photon per atomic disintegration (single-photon radionuclides), e.g., ^{99m}Tc, ^{123}I, ^{111}In, and gallium 67 (^{67}Ga) [2]. Positron emission tomography cameras image radionuclides that give off two photons per disintegration (dual-photon radionuclides), e.g., ^{18}F, carbon 11 (^{11}C), oxygen 15 (^{15}O), or nitrogen 13 (^{13}N). Gamma cameras and PET cameras have radiation detectors that are positioned close to the patient's body. The radiation is detected by scintillation crystals within the detector head.

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When radionuclides interact with the crystals, light is emitted. The greater the radiation detected, the brighter the signal.

Traditional nuclear medicine images are two-dimensional, for example, the bone scan or a Meckel study. The camera is placed adjacent to the patient and an image is acquired in one or multiple views. Images can also be acquired in a movie mode to view movement, as in a gastric emptying study. However, technical developments have enabled three-dimensional images, similar to CT or MRI, to be commonly acquired and viewed for various indications. For the gamma camera, this is referred to as single photon *emission* computed tomography (SPECT). The radiation is emitted from an internal source of radiation. By comparison, conventional CT is *transmission* computed tomography, that is, the radiation comes from an external source that transits from one side of the body to the other, where it is detected. During SPECT there is acquisition of approximately 100 two-dimensional images at incremental angles around the patient. The multiple images are reconstructed by computer software into cross-sectional images (transverse, coronal, and sagittal views) by the same mathematical algorithms used for CT. In PET, many scintillation detectors encircle the patient.

The decay of PET radionuclides, for example, ^{18}F, ^{11}C, ^{15}O, and ^{13}N, is different than single-photon decay [2]. Positrons are antimatter, that is, positive electrons. With decay, positron particles are emitted, and they interact with negatively charged particles, namely electrons. Both particles are annihilated in the process, producing two gamma photons 180° apart. Hence, PET imaging is dual-photon emission tomography. The conversion of mass (positron/electrons) to energy (gamma photons) was predicted by Albert Einstein with his famous equation, $E = mc^2$. The potential of PET is unlimited if one appreciates that positron radionuclides include carbon, oxygen, nitrogen, and fluorine (fluorine can replace hydrogen), making possible tracing and imaging of any biological molecule.

The two PET photons are detected by scintillating crystal detectors that surround a region of the body. For a region larger than the brain or heart, the bed position can move in 30–43-cm (12–15-inch) sequential increments along the length of the body. Specialized electronics (coincidence circuitry) determines if the two photons detected are from the same decay event – that is, 180° apart and emitted simultaneously.

Images from PET are processed as cross-sectional images, which can be reformatted into rotating three-dimensional displays (MIPS, or maximal intensity projection images). and some SPECT cameras have CT scanners as part of the imaging system. The two cameras (the PET or SPECT camera, and the CT camera) are situated back to back. The studies are acquired sequentially with the patient in the same position the same table that moves from one instrument to the other. After acquisition, the images are registered and fused. The purpose is to combine the functional, physiological, and biochemical information from the PET or SPECT images with the superior anatomical localization of the CT scans.

Clinical nuclear medicine studies

This chapter will review radionuclide studies most pertinent to the modern practice of gastroenterology. There have been many advances since the previous edition of this textbook. Some of the diagnostic studies reviewed in the previous edition are uncommonly performed today and many studies that are in common use today are new to clinical practice since the previous edition.

Hepatobiliary imaging: cholescintigraphy

Iodine 123-labeled rose Bengal was the first radiopharmaceutical used for hepatobiliary imaging (cholescintigraphy). In 1976 the [^{99m}Tc]HIDA (hepatic iminodiacetic acid)-derived radiopharmaceuticals were introduced, and have subsequently entered routine use. Two HIDA radiopharmaceuticals are approved by the US Food and Drug Administration (FDA) for clinical use in the United States:

- [^{99m}Tc]disofenin (FDA name), DISIDA (diisopropyl-IDA), or Hepatolite (commercial name)
- [^{99m}Tc]mebrofenin, bromotrimethyl-IDA, or Choletec.

After intravenous injection, the radiopharmaceutical is extracted by hepatocytes and travels the same metabolic pathway as bilirubin, except it is not conjugated. [^{99m}Tc]mebrofenin has somewhat higher liver extraction (98% vs 89%) and more rapid hepatic clearance (17 vs 23 min half-time) than [^{99m}Tc]disofenin and is increasingly the agent of choice, particularly for patients with hepatic dysfunction, and in children. Less than 2% of [^{99m}Tc]mebrofenin is excreted in the urine in patients with normal serum bilirubin levels.

Technetium 99m IDA radiopharmaceuticals make possible physiological imaging of hepatic function and biliary flow. Because Tc-IDA travels the same pathway as bilirubin, it is subject to competitive inhibition by elevated levels of serum bilirubin. However, diagnostic studies can usually be obtained with serum bilirubin levels as high as 20–30 mg/dL. With severe hepatic dysfunction, normal pharmacokinetics is altered, with delayed uptake and clearance. In this situation there is increased percentage of the administered radiopharmaceutical cleared by the kidneys.

Preparation for cholescintigraphy requires that the patient has nothing by mouth for 3–4 h prior to starting the study. This allows time for the gallbladder to relax after contraction from endogenous cholecystokinin (CCK) stimulation produced by the recent ingested meal. A contracted gallbladder may not allow radiotracer to enter and could result in a false-positive study for acute cholecystitis (nonfilling of the gallbladder). However, if the patient has fasted for more than 24 h, CCK is often administered prior to the start of the study to empty the gallbladder of viscous bile, which can prevent

HIDA tracer entry. It is recommended that all opiate drugs be withheld for at least 6 h prior to the study because they produce a functional partial biliary obstruction by contracting the sphincter of Oddi, complicating interpretation of biliary duct obstruction.

The standard cholescintigraphic study lasts 1 h (1-min sequential frames) after radiopharmaceutical injection. By 60 min, the normal gallbladder fills and bile clears from biliary ducts into the intestinal tract. However, if the study is not normal at 60 min, modifications of the protocol require delayed imaging, or the administration of morphine or CCK. All of these ancillary procedures will be discussed.

Acute cholecystitis

The most common clinical indication for cholescintigraphy (nuclear medicine hepatobiliary imaging) is suspected acute cholecystitis. Cholescintigraphy reveals the specific underlying pathophysiology, namely, obstruction of the cystic duct, manifested on cholescintigraphy as nonfilling of the gallbladder [3]. Although ultrasonography is valuable in the workup of patients with suspected hepatobiliary disease, it is both insensitive and nonspecific for the diagnosis of acute cholecystitis. Gallstones are common, frequently asymptomatic, and may be unrelated to the patient's pain. Gallbladder wall thickening and pericholecystic fluid have numerous causes. And the sonographic Murphy sign is operator dependent and not always reliable. Cystic duct stones are rarely seen because of their small size. The more sonographic findings that present on ultrasonography, the greater the likelihood that the patient has acute cholecystitis; however, many patients with acute cholecystitis have only evidence of stones or other nonspecific findings. However, ultrasonography can detect other causes for pain, such as biliary dilation caused by biliary obstruction.

A stone obstructing the cystic duct is the usual initiating event of acute cholecystitis. This results in the gallbladder wall becoming edematous, followed by inflammatory cell infiltration, then hemorrhage and necrosis, and finally gangrene and perforation and other complications. Cholescintigraphy becomes positive for acute cholecystitis as soon as the cystic duct is obstructed.

Many published investigations have validated the accuracy of cholescintigraphy for making the diagnosis of acute cholecystitis. The sensitivity of the test is high, greater than 95%, and the specificity is 90% [4]. False-positive studies are uncommon, but can occur in sick hospitalized patients, those who have been fasting for a prolonged time or are receiving hyperalimentation, and some patients with chronic cholecystitis.

Nonvisualization of the gallbladder at 60 min is abnormal. However, delayed imaging is required to confirm the diagnosis of acute cholecystitis. Continued nonvisualization at 3–4 h after injection is diagnostic. Visualization of the gallbladder excludes the disease. The majority of patients with delayed gallbladder nonvisualization (between 1 and 4 h) have chronic cholecystitis. An alternative to delayed imaging is the use of morphine sulfate 0.04 mg/kg intravenously (Fig. 134.1). Morphine contracts the sphincter of Oddi, increases intrabiliary pressure, and produces preferential bile flow toward and through the cystic duct, if it is patent. The study can be completed and a diagnosis made in a total of 90 min, rather than the 3–4 h required with delayed imaging. The diagnostic accuracy is similar [5].

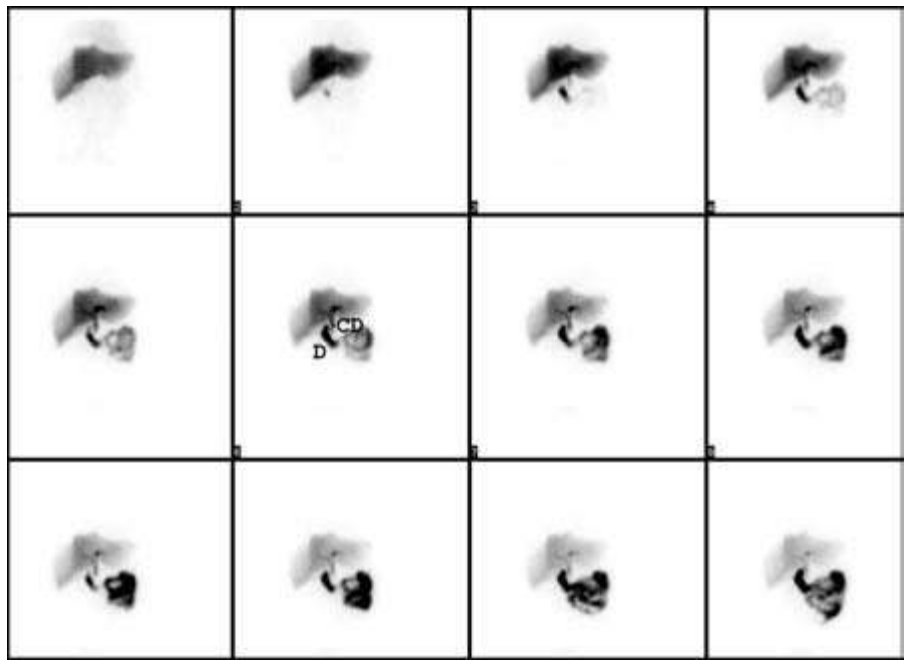
The scintigraphic "rim sign" is seen in 25%–35% of patients with acute cholecystitis. This cholescintigraphic sign is manifested as increased radiotracer uptake in the liver adjacent to the gallbladder fossa (Fig. 134.2). Although insensitive for the diagnosis of acute cholecystitis, it is highly specific. This finding has important clinical implications because patients who have this scintigraphic sign have an increased incidence of complications, such as, gangrene and perforation. The rim sign is the result of severe inflammation spreading from the gallbladder to the adjacent liver. Blood flow to this region is increased, resulting in higher than normal delivery and extraction of the HIDA tracer in the region of the inflamed liver.

Acute acalculous cholecystitis occurs in seriously ill hospitalized patients, for example, those with extensive burns, fractures, or postoperative complications. The disease has a high morbidity and mortality. As the name suggests, it is acute cholecystitis but without stones. The pathophysiology is poorly understood. Although the majority of patients have cystic duct obstruction due to inflammatory debris, inspissated bile, or local edema rather than cholelithiasis, a minority of patients do not have an obstructed duct but rather have direct inflammation of the gallbladder wall due to sepsis, ischemia, or toxemia. The lack of cystic duct obstruction makes the preoperative imaging diagnosis more difficult.

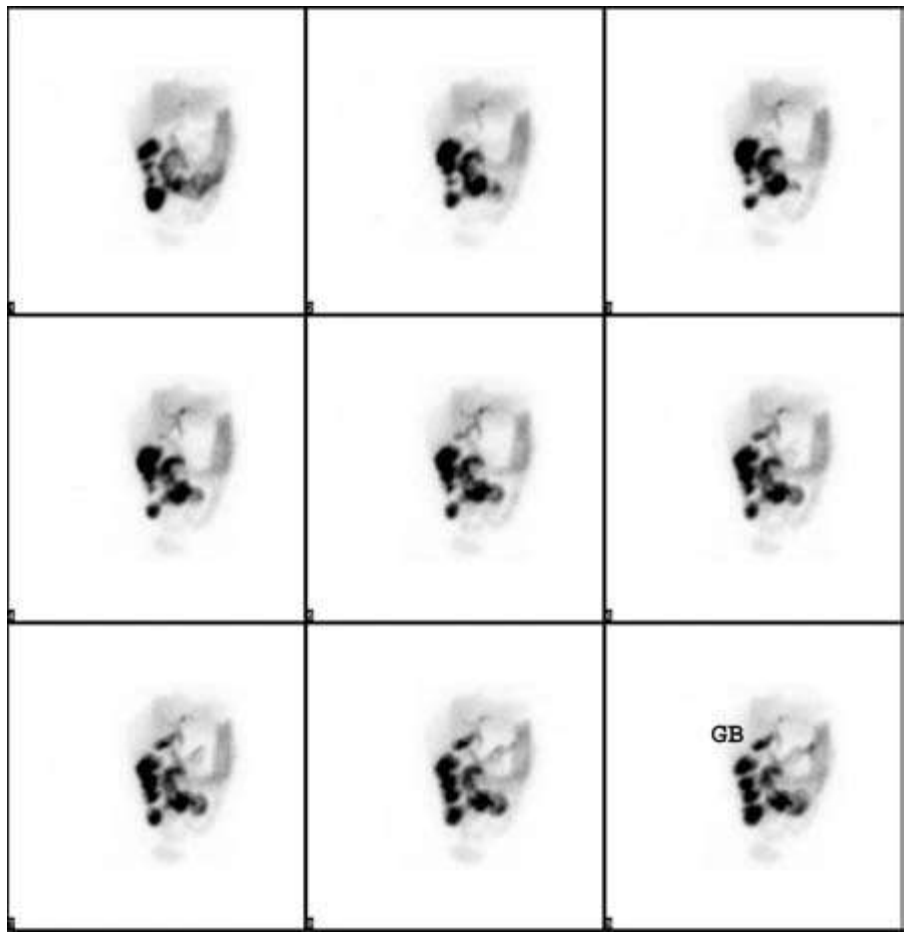
Because the majority of patients have obstruction of the cystic duct, cholescintigraphy is often diagnostic. However, the false-negative rate (gallbladder filling in patients with acute cholecystitis) is higher in acute acalculous disease than in acute calculous cholecystitis. Instead of over 95% sensitivity for the acute calculous disease, the sensitivity of HIDA imaging for acute acalculous cholecystitis is probably in the range of 75%–85%. If a patient is suspected of having a false-negative study, two approaches may be helpful. First, after the gallbladder has filled, CCK can be administered. A diseased gallbladder will not contract well. Good contraction rules out gallbladder disease; however, poor gallbladder contraction occurs with both acute and chronic disease. An ^{111}In-labeled leukocyte study can confirm acute inflammation of the gallbladder.

Chronic cholecystitis

Chronic calculous cholecystitis is commonly diagnosed in patients who have symptoms of recurrent biliary colic and imaging evidence of cholelithiasis, leading to cholecystectomy. Postoperative gallbladder histopathology shows evidence of



(a)



(b)

Figure 134.1 Delayed gallbladder visualization with intravenous morphine on a 45-year-old female referred for suspected acute cholecystitis. **(a)** Sequential images over 60 min. The right lobe has an abnormal appearance due to a loculated pleural effusion. There is normal hepatic uptake and biliary clearance of the radiotracer into the common duct (CD) and duodenum (D) and more distal small bowel. However, there is no filling of the gallbladder. **(b)** Thirty minutes of additional imaging after administration of morphine sulfate. The gallbladder (GB) progressively fills. This rules out acute cholecystitis. Transit of radiotracer can be seen in the ascending colon.

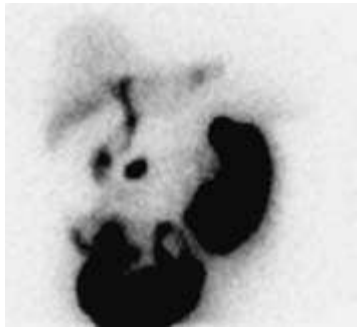


Figure 134.2 Rim sign in acute cholecystitis. This 40-year-old female was referred for suspected acute cholecystitis. The technetium 99m-labeled hepatic iminodiacetic acid ([^{99m}Tc]HIDA) study showed nonfilling of the gallbladder at 1 h (not shown). Further delayed images were obtained at 3 h. The image shows a "rim" sign with increased uptake in the liver adjacent to the gallbladder fossa along the inferior aspect of the right lobe. There is no gallbladder filling and normal transit to the small bowel. The rim sign is additional evidence that this is acute cholecystitis and suggests that there is severe inflammation and an increased likelihood of complications such as gangrene and perforation.

chronic inflammation and fibrosis. If these patients were studied preoperatively, they would be found to have poor gallbladder contraction. Patients with asymptomatic cholelithiasis have normal gallbladder contraction. Thus, in patients with atypical abdominal pain, a study of gallbladder contraction can sometimes be helpful. A poorly contracting gallbladder suggests that the pain and gallstones are related and the patient has symptomatic chronic cholecystitis. If gallbladder contraction is normal, the pain and stones are probably unrelated, and another cause for pain should be sought.

Chronic acalculous gallbladder disease is reported in approximately 5%–10% of patients who have had cholecystectomy for symptoms of chronic cholecystitis, and the laparoscopic literature suggests that the incidence may be higher [6]. Chronic acalculous gallbladder disease is clinically and histopathologically indistinguishable from chronic calculous cholecystitis, except for the absence of gallstones. Over 90% of patients with acalculous disease have evidence of chronic inflammatory changes in the gallbladder, similar to patients with chronic calculous cholecysitis. A small minority of patients do not have inflammatory changes. They also improve with cholecystectomy. The clinical problem is how to make the diagnosis noninvasively and preoperatively.

More than 20 published investigations have confirmed the value of CKK (sincalide) cholescintigraphy for the preoperative diagnosis of chronic acalculous gallbladder disease [7]. One particularly well-designed prospective investigation randomized patients referred for suspected disease who had a low gallbladder ejection fraction (GBEF), to either surgical or nonsurgical therapy [8]. Of those patients who had cholecystectomy, over 91% had resolution of their symptoms and histopathological evidence of chronic cholecystitis. Those patients with a low GBEF who did not have surgery continued to be symptomatic. Several demanded surgery and postoperatively had resolution of their symptoms. Although a few reports have not found the test useful, the overwhelming evidence is that CCK cholescintigraphy can predict which patients will benefit from cholecystectomy.

Unfortunately, the methodology for CCK cholescintigraphy is not standardized between imaging clinics and hospitals. The prospective randomized study described above used a 45-min infusion of sincalide with the GBEF calculated at 60 min. The authors established normal values in 40 normal controls. Abnormal was determined to be less than 40% (mean ± 3 SD). In another investigation of 23 normals, using a 60-min CCK infusion, normal gallbladder contraction was also found to be greater than 40% (Fig. 134.3) [9]. Other methods used include a 15- and 30-min infusion [10,11]. Normal values will vary somewhat depending on the methodology used, with a GBEF in the range of 30%–40%. Any method used should have published validated normal values.

A 1–3-min CCK infusion method is sometimes used on the strength of a retrospective report with good clinical outcome results. However, normal values have never been established with these shorter infusion methods because of the wide range in response in normals. Two published investigations have found that approximately one-third of normal subjects have poor gallbladder contraction when CCK is given as a 3-min infusion; however, they have normal gallbladder contraction when CCK is given as a 30-min or 60-min infusion [9,10]. It was noted in the early 1970s that when CCK is infused rapidly, it can cause spasm of the neck of the gallbladder and result in ineffective contraction. Infusions of 1–3 min have a similar effect in a subset of normals and patients. Thus, 30- to 60-min infusions are recommended.

A persistent misconception is that reproduction of the patient's pain during CCK infusion is a diagnostic sign of gallbladder disease. Although some older data from the 1970s suggested this, other data at that time did not support it, and subsequent investigations have not confirmed the diagnostic utility of this observation [12]. At that time CCK was administered as a bolus. Cholecystokinin has physiological effects other than gallbladder contraction and sphincter of Oddi relaxation, such as intestinal motility, which can cause intestinal abdominal cramping. Approximately one-half of normal subjects have abdominal cramping when given a 3-min or shorter CCK infusion [9,10]. When these same patients are given the dose as a slow infusion over 30 or 60 min, none has pain. Also, the symptoms of irritable bowel syndrome can be exacerbated by CCK [13]. Thus, abdominal cramping during CCK infusion is related to the method of infusion and not to the presence or absence of disease.

Cholecystokinin cholescintigraphy should be requested and interpreted in the proper clinical setting. Published studies confirming the utility of CCK cholescintigraphy to diagnose chronic acalculous gallbladder disease were performed in patients with recurrent biliary colic-type pain who had

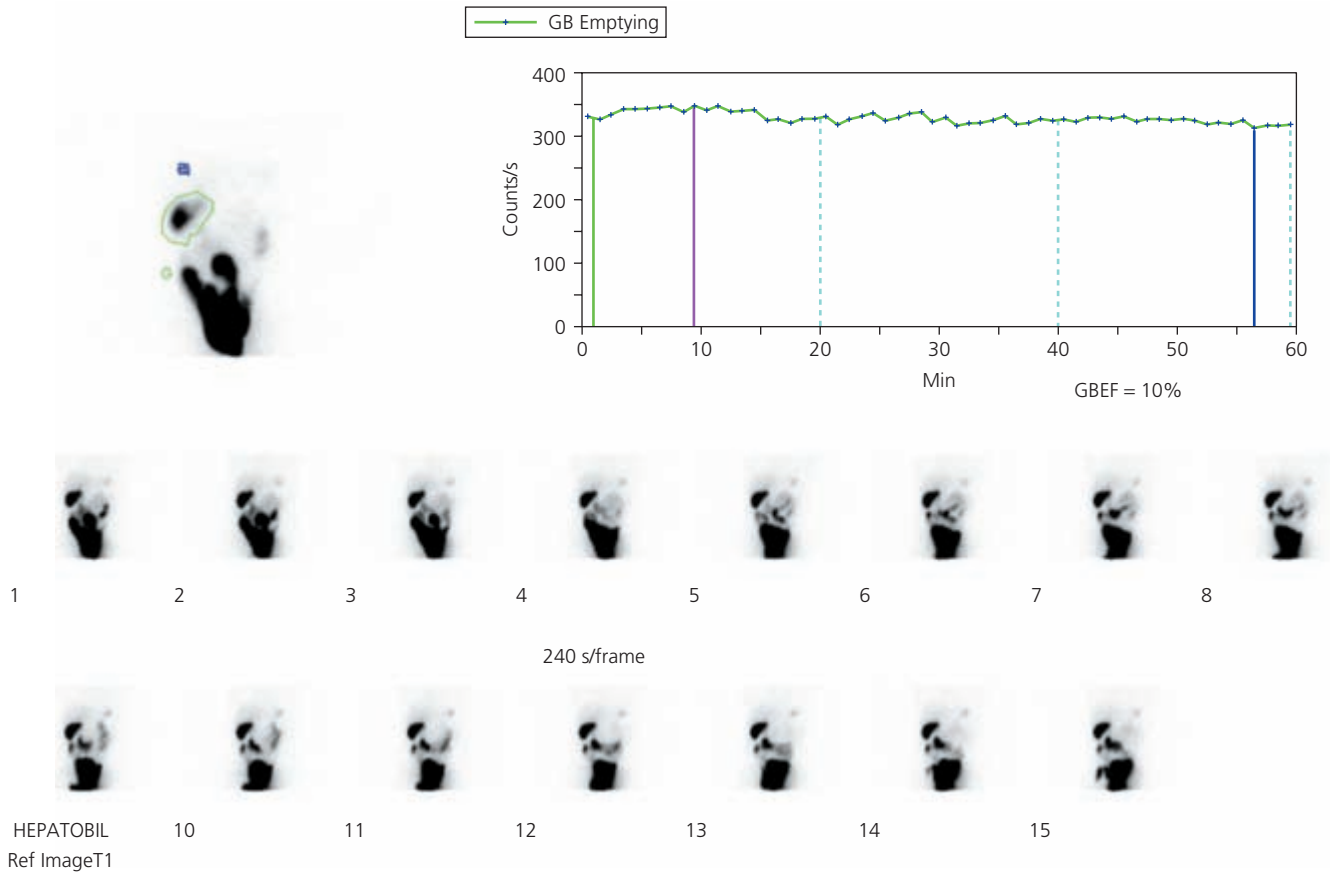


Figure 134.3 Chronic acalculous gallbladder disease in a 52-year-old patient with recurrent biliary-colic-like pain. Ultrasonography showed no stones. The gallbladder filled during the first 60 min of the study (not shown). Shown are sequential 2-min images made during the 60-min infusion of sincalide (cholecystokinin, CCK). Images show poor gallbladder (GB) contraction. A region of interest is drawn on the computer and a time–activity curve generated. The calculated gallbladder ejection fraction (GBEF) was 10% (normal ± 40%). The patient became asymptomatic postcholecystectomy and histopathology showed a chronically inflamed gallbladder.

had medical workup to exclude other diseases and were followed for months to years prior to CCK cholescintigraphy, allowing time for other diseases to manifest. Some clinicians, convinced of the utility of the test, are requesting the study after a first episode of pain and with minimal other medical evaluation [14]. The likelihood of disease in this new referable group is lower than in the published investigations, thus the accuracy of the test in this population is less certain. The posttest probability of a test is affected by the pretest likelihood of the disease (Bayes theorem). Thus, in a high-likelihood population, a positive test is likely to be a true positive, but, in a low-likelihood population, a positive test is likely to be a false positive. Cholecystokinin cholescintigraphy is a confirmatory test, not a screening test.

To confirm the diagnosis of chronic gallbladder disease, CCK cholescintigraphy should be done in an outpatient setting, not during an emergency room visit or while the patient is hospitalized. Acute illnesses and various drugs may affect gallbladder function. For example, calcium channel blockers, progesterone, octreotide, theophylline, and benzodiazepine can cause reduced gallbladder contraction.

Biliary obstruction

In patients who are clinically suspected of having biliary obstruction, anatomical imaging is often confirmatory, demonstrating dilated biliary ducts. However, early after the acute onset of obstruction caused by cholelithiasis, bile ducts may not be dilated. Dilation sometimes takes 24–72 h to become apparent. Cholescintigraphy can confirm the diagnosis early after onset because it demonstrates the pathophysiology of obstruction.

In high-grade biliary obstruction, cholescintigraphy demonstrates a severe reduction in bile flow. The diagnostic scintigraphic pattern of acute high-grade obstruction is that of good hepatic extraction but no biliary clearance, that is, a persistent hepatogram (Fig. 134.4). The accuracy of cholescintigraphy for the diagnosis of high-grade obstruction is greater than 97% [15,16]. Cholestatic jaundice, for example,

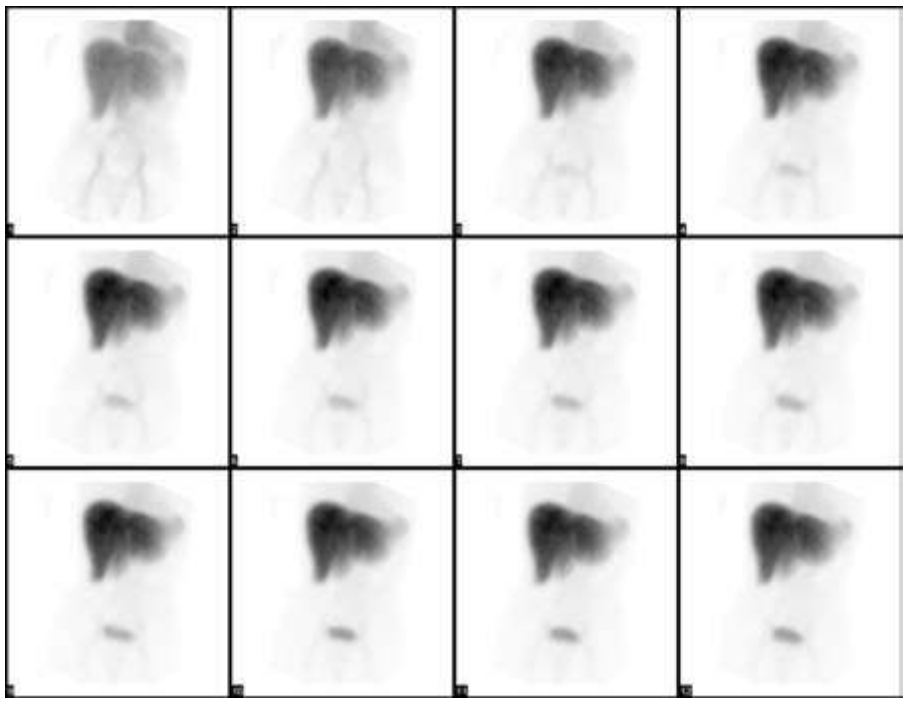


Figure 134.4 High-grade biliary obstruction. Technetium 99m-labeled hepatic iminodiacetic acid ([^{99m}Tc]HIDA) study for patient with recent onset of upper abdominal pain. Ultrasonography showed normal-sized biliary ducts. Sequential images over 1 h show prompt radiotracer extraction, but no biliary excretion into biliary ducts, consistent with high-grade biliary obstruction.

drug-induced or a resolving hepatitis, may rarely have a similar pattern, but the clinical presentation is usually different.

Partial biliary obstruction presents differently. With low-grade or intermittent obstruction, ducts are often not dilated because of the lower intraductal pressure and the intermittent nature of the process. The cholescintigraphic pattern shows good hepatic function, prompt secretion into biliary ducts, but retention of radiotracer in biliary ducts and delayed transit into the small intestines. Opiate drugs produce a functional partial biliary obstruction and should be withheld for 6 h prior to the study.

The accuracy of diagnosis for partial obstruction is also high [17]. In the largest published study, the sensitivity for cholescintigraphy was 97%, versus 78% for sonography. Specificity was similar (86%). Patients who have had prior obstruction may have persistent ductal dilation after successful therapy. Thus sonography would not be able to distinguish obstructed from nonobstructed ducts. Physiological imaging with cholescintigraphy is required to make the diagnosis. Of 125 patients thought to have early, partial, or intermittent biliary obstruction, sonography and scintigraphy disagreed in 23%. Scintigraphy diagnosed obstruction in 13 patients with no evidence of dilated ducts, whereas 7 patients with dilation from prior obstruction showed normal clearance [17].

The longer an obstruction goes untreated, the greater the likelihood of hepatic dysfunction developing. Poor hepatic function on cholescintigraphy is seen as delayed extraction and clearance from the liver. In late obstruction, the scintigraphic findings of hepatic dysfunction and obstruction can overlap, making interpretation more difficult. Good extraction and function is most consistent with obstruction, and poor hepatic uptake is more likely due to hepatic dysfunction. The two can usually be distinguished.

Postcholecystectomy syndrome

Cholescintigraphy can provide valuable diagnostic information in many patients with the postcholecystectomy syndrome. The most common biliary cause for recurrent pain after cholecystectomy is recurrent or residual biliary duct stones. Biliary stricture and sphincter of Oddi dysfunction are less common but important causes. All of these produce a scintigraphic picture of partial biliary obstruction.

Sphincter of Oddi dysfunction is a partial biliary obstruction at the level of the sphincter of Oddi. It is the cause of pain in up to 14% of patients with the postcholecystectomy pain syndrome. Clinically it presents as recurrent biliary colic-like pain and transient liver function abnormalities. In the past, endoscopic retrograde cholangiopancreatography (ERCP) was used to exclude stones, tumor, and biliary stricture as the cause. Magnetic resonance cholangiopancreatography (MRCP) has largely replaced ERCP for detection of dilation and tumor. Sphincter of Oddi manometry is considered the diagnostic gold standard of sphincter dysfunction. However, it is technically demanding, not widely available, and associated with a high incidence of adverse effects (e.g., pancreatitis).

A noninvasive screening test would be valuable to determine which patients require invasive workup. Cholescintigraphy permits physiological assessment of duct drainage, which correlates well with the washout of contrast material

from the biliary tract observed on ERCP. The scintigraphic findings are those of delayed biliary duct clearance and delayed biliary-to-bowel transit, the findings of partial biliary obstruction [18].

Quantitative cholescintigraphy has been used to improve the accuracy over image analysis alone. Various methods have been published [8,19,20]. Using a computer, regions of interest are drawn around biliary ducts or the hepatic hilum, time–activity curves are generated, and quantitative parameters of bile clearance are determined. Of the numerous methods reported, it is not certain which is most accurate. Some of the quantitative studies have relied on quantification without image analysis [7]. One investigation found a high level of accuracy by combining image analysis and quantitative analysis [20]. A unique aspect of this protocol is the infusion of sincalide prior to the study to increase bile flow and "stress" the biliary ducts, bringing out an otherwise more subtle obstruction. The authors of this study reported 100% accuracy in 23 patients, and this is the method that we use routinely (Fig. 134.5).

It is stated in the medical literature that a characteristic finding of sphincter of Oddi dysfunction is a paradoxical response of the sphincter to CCK, that is, contraction rather than relaxation, and that this finding can be used diagnostically and scintigraphically to confirm the disease. This paradoxical response was first reported using manometry, albeit in a minority of patients with sphincter dysfunction [21]. In those investigations, CCK was infused as a bolus. It has never been shown that a paradoxical response of the sphincter occurs when CCK is given at physiological doses. Unlike elevated sphincter pressure, a paradoxical response to CCK at manometry is not predictive of a good response to sphincterotomy. Scintigraphic investigations have not noted a paradoxical response when CCK was infused at physiological rate [22].

Biliary leak

Biliary leaks occur usually as a complication of cholecystectomy or biliary tract surgery. Although ultrasonography and CT can detect a fluid collection, its etiology and origin is often uncertain. Cholescintigraphy can determine whether the fluid collection is of biliary origin, rather than due to ascites or abscess, for example. Cholescintigraphy can also provide an estimate of the rate of leakage. Slow bile leaks often resolve spontaneously with conservative therapy, whereas more rapid leaks require intervention. Before percutaneous drainage of a biloma, cholescintigraphy can ensure that proximal biliary obstruction is not the underlying cause. With obstruction, bile leakage cannot be effectively treated by percutaneous drainage without addressing the underlying cause of obstruction. Follow-up cholescintigraphy can confirm resolution or persistence of the leak.

Bile leakage is seen on cholescintigraphy as progressively increasing radiotracer collection in the region of the gallbladder fossa or hepatic hilum. The radioactivity may move into the subdiaphragmatic space, over the dome of the liver, into the colonic gutters, or diffusely as free bile within the abdomen. Peritoneal tubing, drains, and collection bags may exhibit accumulation and may be the only evidence of leakage.

Other indications for cholescintigraphy

Biliary scintigraphy is useful for evaluating the patency of *surgical biliary diversion procedures*, particularly choledochojejunostomy. Scintigraphy can confirm bile leakage, determine functional patency of the anastomosis, and show evidence of recurrent obstruction. Cholescintigraphy is the only noninvasive method that can distinguish obstructed dilated ducts from those that are chronically dilated but not obstructed. In Billroth II anastomoses, afferent loop patency can be evaluated.

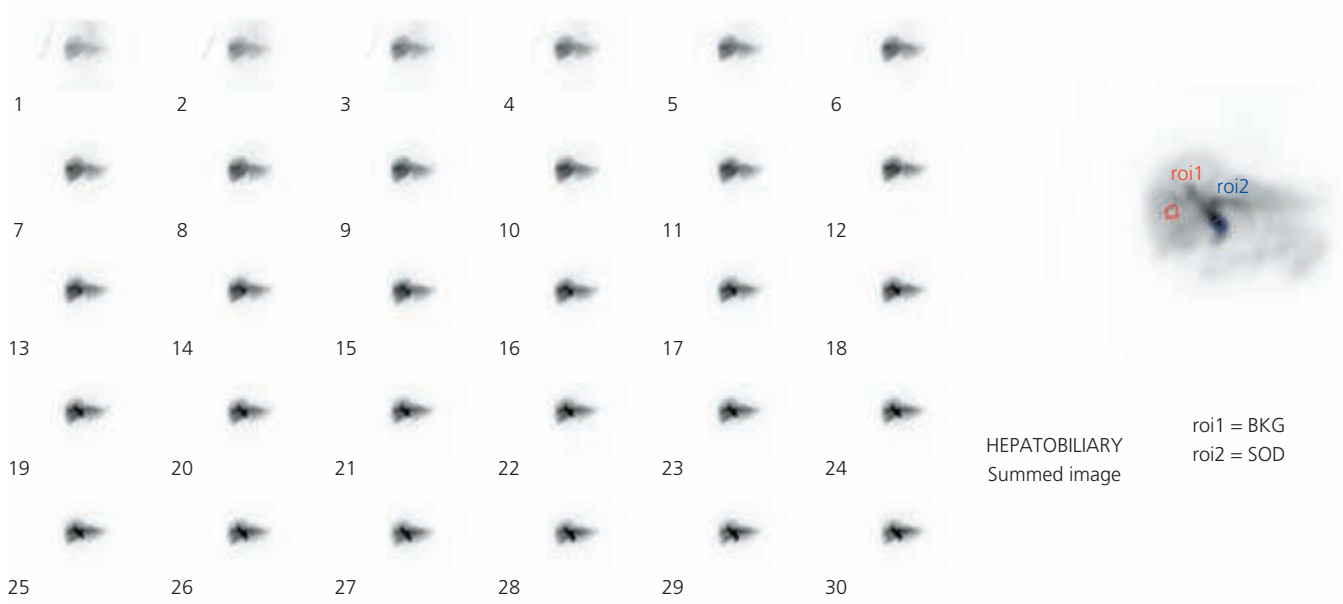
Cholescintigraphy can be an important diagnostic aid in evaluation of postoperative complications of liver transplatation, for example, in differentiating bile leaks and obstruction from rejection [23]. Scintigraphy has also been used to diagnose enterogastric bile reflux.

Cavernous hemangioma of the liver: ^{99m}Tc-labeled red blood cells

Technetium 99m-labeled red blood cell (RBC) scintigraphy has been used for decades to diagnose cavernous hemangiomas of the liver. However, MRI has become more commonly ordered for this purpose because of its superior anatomical resolution and high sensitivity for detecting small lesions and hemangiomas adjacent to vascular structures. However, its specificity is not as high as the ^{99m}Tc-labeled RBC method, because numerous benign and malignant lesions may have findings similar to hemangioma on MRI. Specificity of the ^{99m}Tc-RBC method is extremely high, with only a handful of false-positive studies reported in as many decades. With multiheaded SPECT, sensitivity approaches 100% for hemangiomas greater than 1.4 cm in size [24]. The sensitivity decreases for small hemangiomas, although ones measuring only 0.5 cm have been detected. The patient's own RBCs are radiolabeled using standard methodology similar to that for gastrointestinal bleeding studies and cardiac radionuclide rentriculograms or multi-gated acquisition (MUGA) studies.

Technetium 99m sulfur colloid liver–spleen scanning

Before the advent of CT, ^{99m}Tc-sulfur colloid (^{99m}Tc-SC) was routinely used for liver–spleen imaging. Although CT has replaced ^{99m}Tc-SC for routine liver–spleen scans for most indications because of its superior image resolution, the ^{99m}Tc-SC liver–spleen scan is still valuable for answering some specific clinical questions [2]. Technetium 99m-SC is extracted by the reticuloendothelial cells of the liver (Kupffer cells). Because most benign and malignant tumors do not have Kupffer cells, most liver masses have no uptake and are



Statistics	Curve A	Curve B
Integral 1.–>3.m	9.7 Kc*s	3.1 Kc*s
Integral percent	75.72%	24.28%
Integral ratio	A/B = 3.119	B/A = .321
Time to 1/2 max	Not reached	24.9 min
Time to peak	14.5 min	36.5 min
T 1/2	39.2 min	Not reached

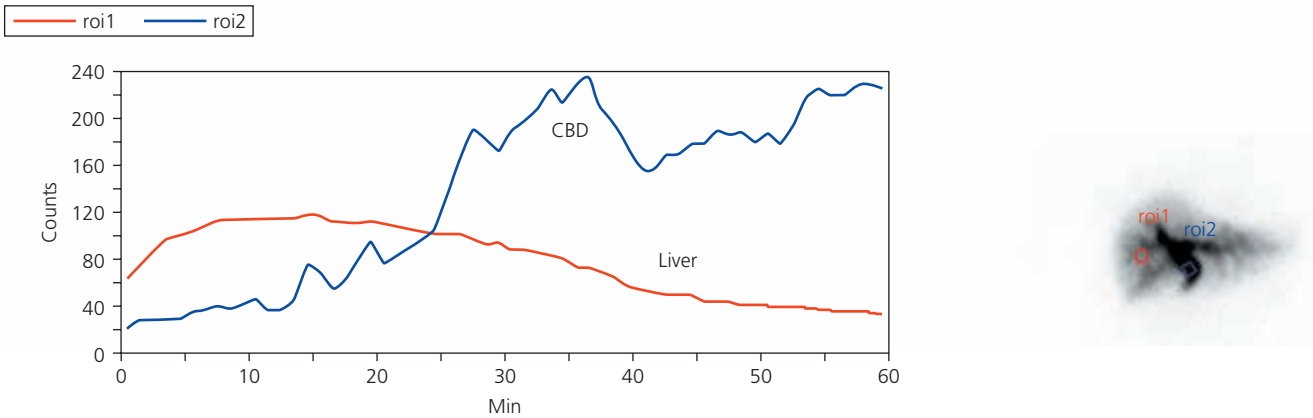


Figure 134.5 Sphincter of Oddi obstruction in a 38-year-old female with chronic recurrent biliary colic starting 5 months postcholecystectomy. Sequential technetium 99m-labeled hepatic iminodiacetic acid ([^{99m}Tc]HIDA) images over 1 h show persistent prominent activity in biliary ducts (best seen on image to right of time–activity curve) and little bile transit to the intestines (best seen on large image to right of sequential images). The common bile duct (CBD) time–activity curve shows a progressive rise. This is consistent with a partial biliary obstruction. No stones or biliary stricture were found on further evaluation and sphincterotomy was performed with subsequent symptom resolution.

cold on liver–spleen scans, with one exception, *focal nodular hyperplasia* (FNH).

Because FNH has all three liver cell types, that is, hepatocytes, biliary ducts, and Kupffer cells, the majority of these tumors show ^{99m}Tc-SC uptake. About two-thirds have normal or increased uptake and one-third have decreased uptake. Normal or increased uptake is consistent with FNH, and this differentiates FNH from other liver tumors, including hepatic adenoma, which almost always is cold. Hepatic adenoma and FNH have different prognoses and therapy.

Technetium 99m-IDA cholescintigraphy also reveals characteristics diagnostic of FNH, including increased blood flow, normal or increased uptake relative to adjacent liver, and delayed clearance of the tracer. This pattern has an accuracy matching or even exceeding that of ^{99m}Tc-SC. Other tumors with hepatocytes, for example, hepatic adenoma and hepatocellular carcinoma, have decreased uptake.

The *Budd–Chiari syndrome* (hepatic vein thrombosis) also has a characteristic pattern with ^{99m}Tc-SC, that is, reduced uptake in the liver, except for the caudate lobe. The impaired venous drainage of the liver results in poor hepatic function and radiotracer uptake. The caudate lobe retains good function because it has direct venous drainage into the inferior vena cava. Patients with *chronic hepatic dysfunction* often have a characteristic pattern based on the abnormal pathophysiology of portal hypertension, called "colloid shift," that is, decreased hepatic uptake due to poor function, and increased uptake in the spleen and bone marrow.

Technetium 99m-SC is useful in patients thought to have splenosis or splenic remnants. Because of its high target-to-background ratio, small foci of splenic tissue can be detected. Detectability is further increased by SPECT imaging. Splenic tissue next to the liver may be difficult to see because of the adjacent intense liver uptake. In that case, damaged RBCs (i.e., heat or chemically treated) will have specific splenic uptake without much liver uptake. Although perhaps superior, this latter technique is not routine because of its increased technical complexity. Most splenic tissue is detected by ^{99m}Tc-SC.

Gastrointestinal motility

The radionuclide gastric emptying study is generally considered the gold standard for measuring gastric motility and is a frequently requested clinical study. Esophageal radionuclide motility studies have been around for several decades; although in less demand because of alternative methods used, they still have a limited role. Small and large bowel transit studies are performed at a relatively small number of centers, but the methodology is underutilized and growth is expected.

Esophageal motility

In the evaluation of suspected esophageal motility disorders, a barium swallow or endoscopy is often performed initially to exclude an anatomical lesion. Esophageal manometry is commonly used to evaluate primary esophageal motor disorders. However, it has limitations. For example, in achalasia, quantification of the volume of retained food is not possible with manometry, limiting its ability to assess the effectiveness of therapy.

Esophageal transit scintigraphy is noninvasive, relatively simple to perform, and can provide clinically useful physiological information [25]. Dynamic rapid sequence swallowing scintigrams are acquired during a patient's swallow of a small volume of water mixed with 200 mCi ^{99m}Tc-SC or [^{99m}Tc]diethylenetriamine pentaacetic acid (DTPA). The computer acquisition rate must be rapid, for example, one second per frame. Qualitative analysis with cinematic display is often sufficient to diagnose abnormal esophageal motility; however, a strength of the radionuclide method is quantification. This is particularly valuable for evaluating the effectiveness of therapy. Different methods of quantification have been described [26]. An esophageal transit time or the residual esophageal activity is measured. Semisolid food swallows have been reported to be more sensitive for abnormal motility than clear liquid swallows, although less commonly used (Fig. 134.6).

The sensitivity for detection of abnormal esophageal transit is reported in the older literature to be 50%–75%. Subsequent studies have reported higher sensitivity and all reports have shown a high sensitivity for achalasia [27]. Esophageal scintigraphy, being noninvasive and quantitative, is felt to be underutilized by some; others feel it is not an ideal screening test. Most agree that its strength lies in evaluating response to therapy [28].

Gastric motility

The radionuclide gastric emptying study has been the gold standard for the evaluation of gastric motility for decades. The method is relatively straightforward, noninvasive, results in low radiation dose to the patient, and, importantly, is quantitative [29].

Although various radiolabeled meals have been used over the years, some form of egg meal, often a sandwich, is commonly used. Good binding of the radiopharmaceutical (^{99m}Tc-SC) to the meal is mandatory for accurate determination of solid or semisolid emptying. During cooking, ^{99m}Tc-SC binds tightly to the albumen in egg white. Simultaneous solid and liquid emptying studies are possible using two different radionuclides with different photopeak energies, for example ^{99m}Tc (140 keV) for the solid meal and [^{111}In]DTPA (175 and 225 keV) for the liquid meal. Solid emptying studies are reported to be more sensitive for detection of delayed emptying than liquid meals. Thus, the main indication for a clear liquid study has been for a patient who cannot tolerate solid food. Recent data suggest that liquid emptying can be abnormal when solid emptying is normal [30].

Solids empty differently than liquids. Tonic contraction of the fundus is responsible for liquid emptying. Clear liquids empty rapidly and exponentially with a half-time of less than 20 min (Fig. 134.7). When calories are added to a clear liquid meal, emptying slows. Phasic contraction of the antrum is responsible for solid emptying. With solid emptying, there is a delay before emptying begins. This *lag phase* is the time required for the antrum to grind up food into small enough particles so that they can pass through the pylorus. Emptying is generally linear after that, at least through 2 h (Fig. 134.8).

Normal values used must be based on the specific meal and methodology. Normal values must be established either in the clinic using the same methodology, or by following carefully a published protocol and using its normal values.

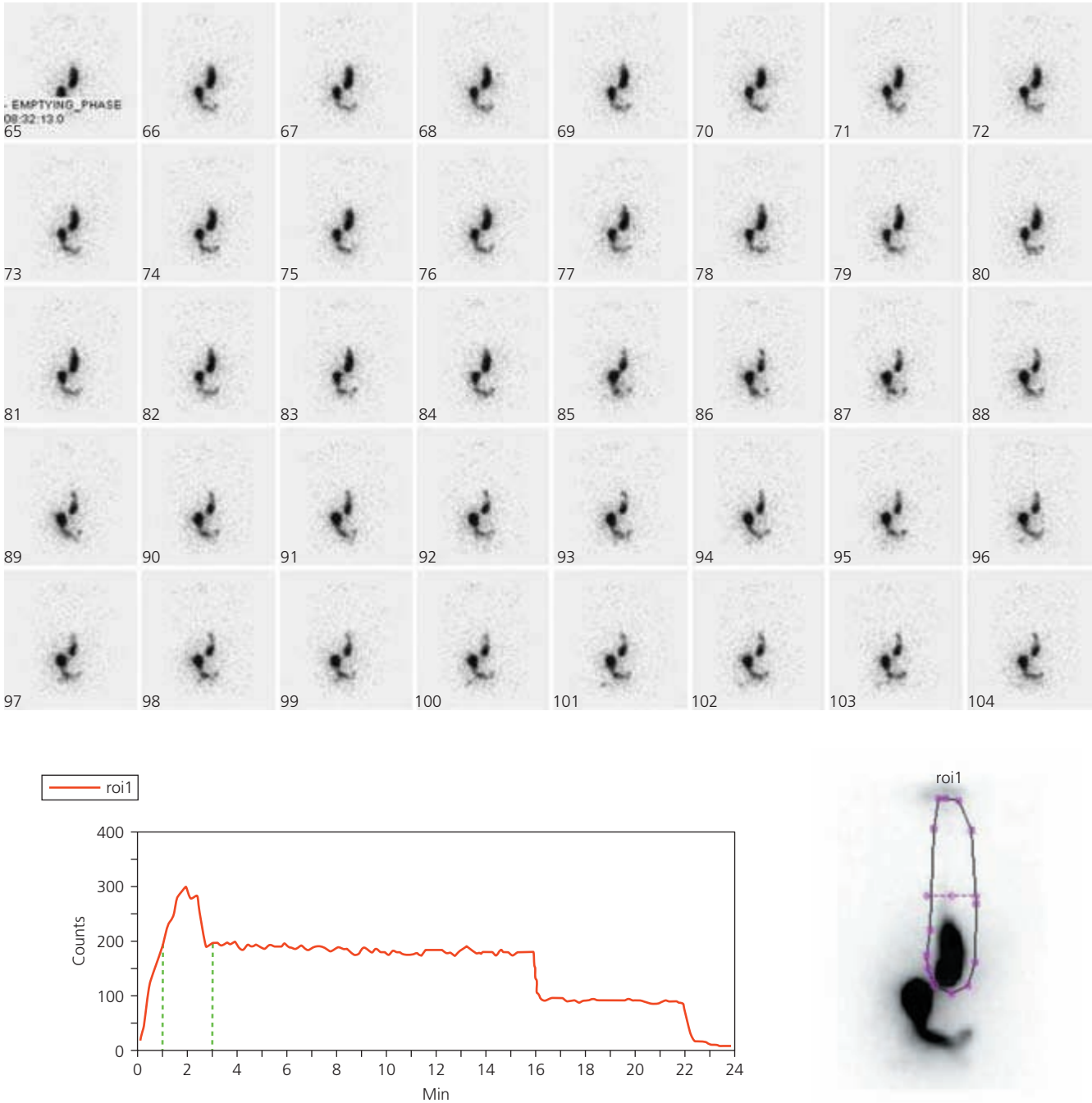


Figure 134.6 Achalasia. Esophageal swallow study of 24-min duration. Sequential 15-s images (posterior view) of ingestion of a cornflake and milk meal demonstrates delayed clearance from the esophagus. A region of interest (ROI) drawn around the esophagus generates a time–activity curve confirming the slow clearance and permits quantification. The drop in counts at 16 and 24 min is due to the ingestion of additional clear liquids.

Correction for radioactive decay and soft tissue attenuation is mandatory. Overlying soft tissue will decrease detectable counts in the stomach, and this attenuation effect varies as the stomach contents move through the stomach. The standard method for correcting for attenuation is mathematical, that is, calculation of the geometric mean (square root of the product of the anterior and posterior detector counts). If attenuation correction is not performed, gastric emptying will be underestimated. The left anterior oblique view is an acceptable alternative if only a single-headed camera is available. In this view the stomach contents move roughly parallel to the head of the camera, compensating for varying attenuation, and mathematical correction is not necessary.

In spite of its wide acceptance, the methodology used for

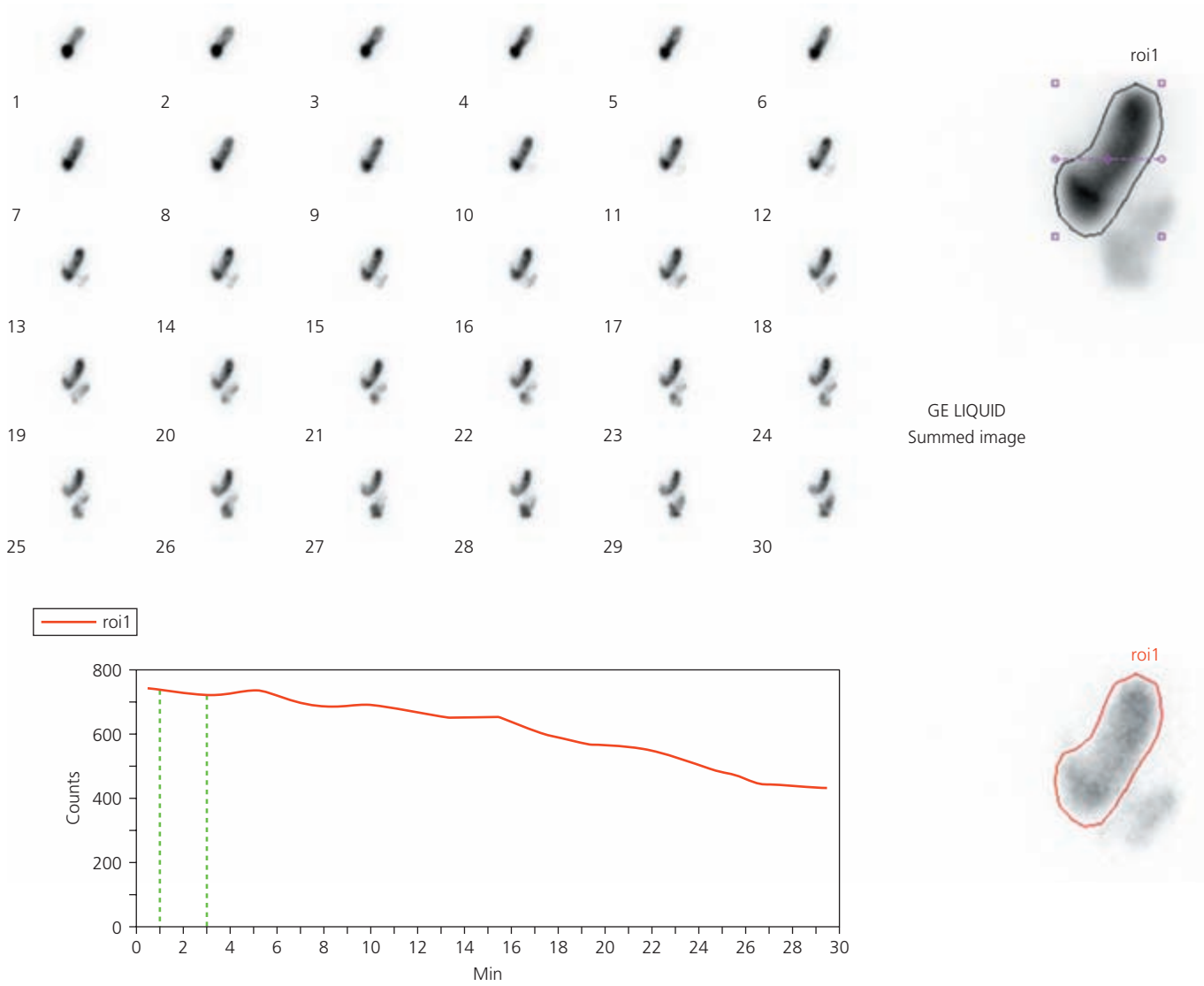
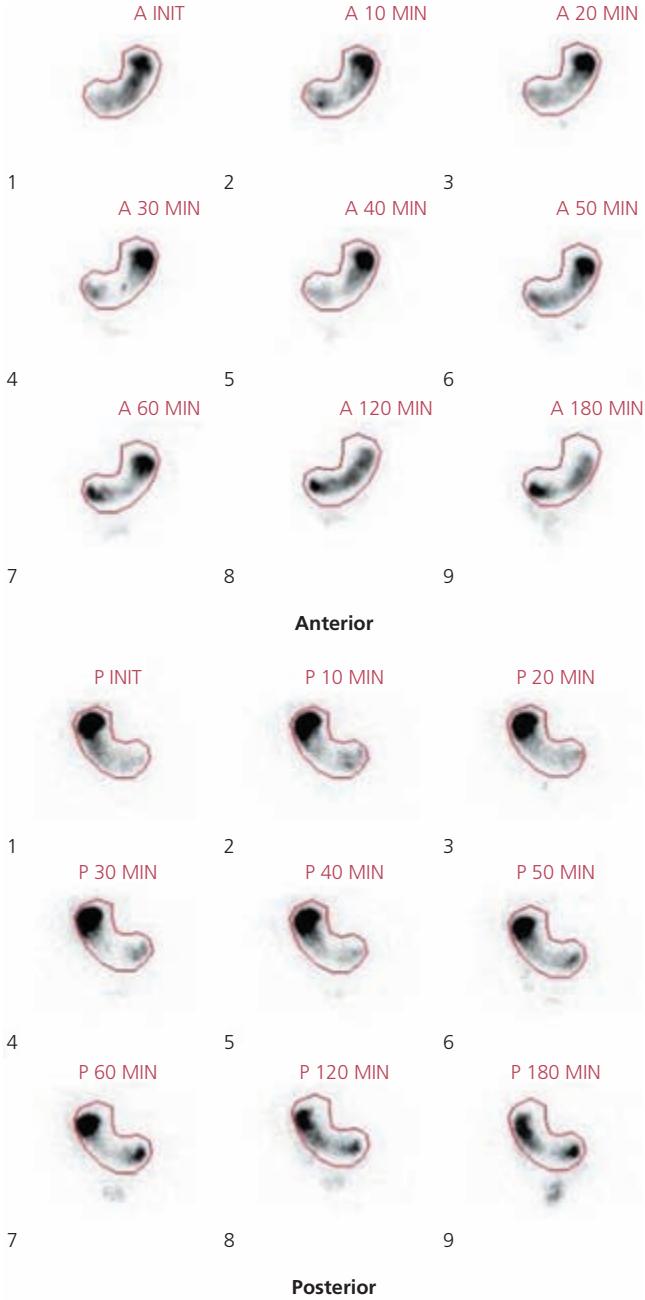


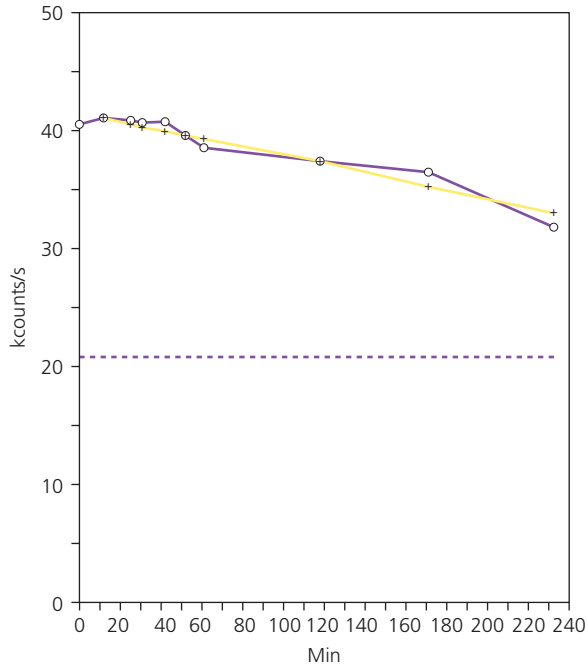
Figure 134.7 Delayed liquid gastric emptying. The patient could not tolerate solid food. Water (500 mL) mixed with technetium 99m-labeled sulfur colloid was ingested and images acquired every minute for 30 min. A region of interest (roi) was drawn around the stomach. A time–activity curve shows delayed emptying. The extrapolated emptying half-time is approximately 45 min (normal 6–18 min).

gastric emptying is not standardized between different hospitals and clinics. Various radiolabeled meals, acquisition, processing, and quantitative methods are used. Study length varies between 1 h and 4 h, although 90–120 min is most common. Normal values vary between institutions, which would be expected because normal values are affected by all the above methodological factors. Moreover, results are reported in different ways; for example, the percent emptying at the end of the study, or a half-time of emptying; also, sometimes, the length of the lag phase, and a rate of emptying. This can be confusing to referring physicians and makes it difficult to compare results between institutions.

There have been attempts to standardize gastric emptying methodology [31]. A collaboration between the American Motility Society and the Society of Nuclear Medicine is spearheading efforts to find a consensus for a generally accepted standardized protocol. One protocol of particular interest is that of Tougas et al., which was published as a multicenter, multinational normal database of 123 subjects [32]. This was designed as a simplified screening test with only four imaging times, at 0, 1, 2, and 4 h. The meal is a ^{99m}Tc-labeled egg substitute sandwich with jam and water. The study length is based on published evidence that a 4-h study detects more abnormal emptying than a 2-h study [33,34].

However, this simplified protocol does not allow analysis of the physiology of gastric emptying. With shorter length studies of 90–120 min, frequent image acquisition is standard. This allows for sophisticated qualitative and quantitative analysis. Images can be reviewed in cinematic mode. Movement of the radiolabeled meal from the fundus to the antrum





Frame/time		Fit/raw	% Empty	Kcounts**	Tc_99m ** Geometric mean Anterior	Posterior
1	0.0	0	0	40.55	55.74	29.50
2	12.0	1	–1	41.06	58.58	28.78
3	25.0	2	–1	40.87	58.48	28.56
4	31.0	3	0	40.65	54.86	30.13
5	42.0	4	–1	40.77	56.50	29.42
6	52.0	5	2	39.62	60.76	25.83
7	61.0	5	5	38.56	58.91	25.24
8	118.0	10	8	37.42	60.56	23.12
9	171.0	15	10	36.58	56.53	23.67
10	232.0	21	21	31.85	54.30	18.68

Figure 134.8 Delayed solid gastric emptying. Anterior and posterior images were acquired every 10 min during the first hour, then once per hour for 4 h. The purpose of early frequent imaging was to get an estimate of the lag phase, which is delayed. The horizontal dashed line represents 50% emptying. Overall emptying in this patient is markedly delayed with only 21% emptying at 4 h (normal > 90%). Without the images at 10, 20, 30, 40, and 50 min and the 3-h image, this would be similar to the Tougas et al. protocol [32].

and through the pylorus into the intestines can be evaluated and a lag phase (time delay before emptying begins) and a rate of emptying (linear or exponential emptying rate), as well as other emptying parameters, can be determined. However, with the Tougas et al. protocol, only delayed emptying at 2 and 4 h is determined (Fig. 134.8). Furthermore, rapid emptying has not been defined. Further work is needed to devise a standardized protocol that will provide the clinical information needed and is flexible enough to accommodate modification for research purposes [35].

Intestinal motility

Small and large bowel radionuclide intestinal transit studies are not widely performed, although methods have been

described. The Mayo Clinic and Temple University groups have been the most active in this area. Some limitations of the methodology have hindered its more widespread use. The Mayo Clinic approach has used a radiopharmaceutical that does not break down until it reaches the small bowel [33]. This is physiologically appealing; however, the radiopharmaceutical is not approved for clinical use nor is likely to be in the foreseeable future. The Temple approach is to use two isotopes with different photopeaks that can be imaged simultaneously, that is, the ^{99m}Tc-SC egg meal for gastric emptying and [^{111}In]DTPA for intestinal transit [36]. However, the test requires 3 days of intermittent imaging, at 0, 1, 2, and 4 h for gastric emptying, at 6 h for small bowel transit determination, and at 24, 28, and 72 h for large bowel transit.

Quantification is not as straightforward for intestinal transit as it is for gastric emptying. With gastric emptying, the percent emptying is determined by the amount of radioactivity in the stomach at time zero immediately after ingesting the radiolabeled meal minus the activity at end of the study, divided by the amount at time zero. However, unlike the stomach, the small and large bowel do not have a time zero where all the activity is in one place, but rather the activity enters the small and large intestines over a period of time. This requires some form of semiquantification or index of transit. One method has defined an approach for calculating the geometric center of activity [36].

Gastroesophageal reflux

The radionuclide gastroesophageal reflux study is a sensitive, noninvasive, and relatively straightforward method for detecting reflux. In the past, the methodology used for adults mimicked the radiographic method, that is, a stepwise increase in abdominal pressure using an inflatable binder to produce detectable reflux events. However, this method is not physiological. A method developed and successfully used for pediatric patients is also applicable to adults. This method has proven to be sensitive for detecting gastroesophageal reflux events, due to the rapid framing rate used for acquisition (10-s frames for 1 h). Early reports of low sensitivity for detecting reflux events date back to days when framing rates of 30 s or longer were used, which resulted in poor temporal resolution and consequent inability to detect repetitive events (Fig. 134.9). The rapid acquisition method has superior sensitivity for detecting reflux [37].

For children, formula or milk mixed with ^{99m}Tc-SC is administered. For adults, orange juice is commonly used. Various

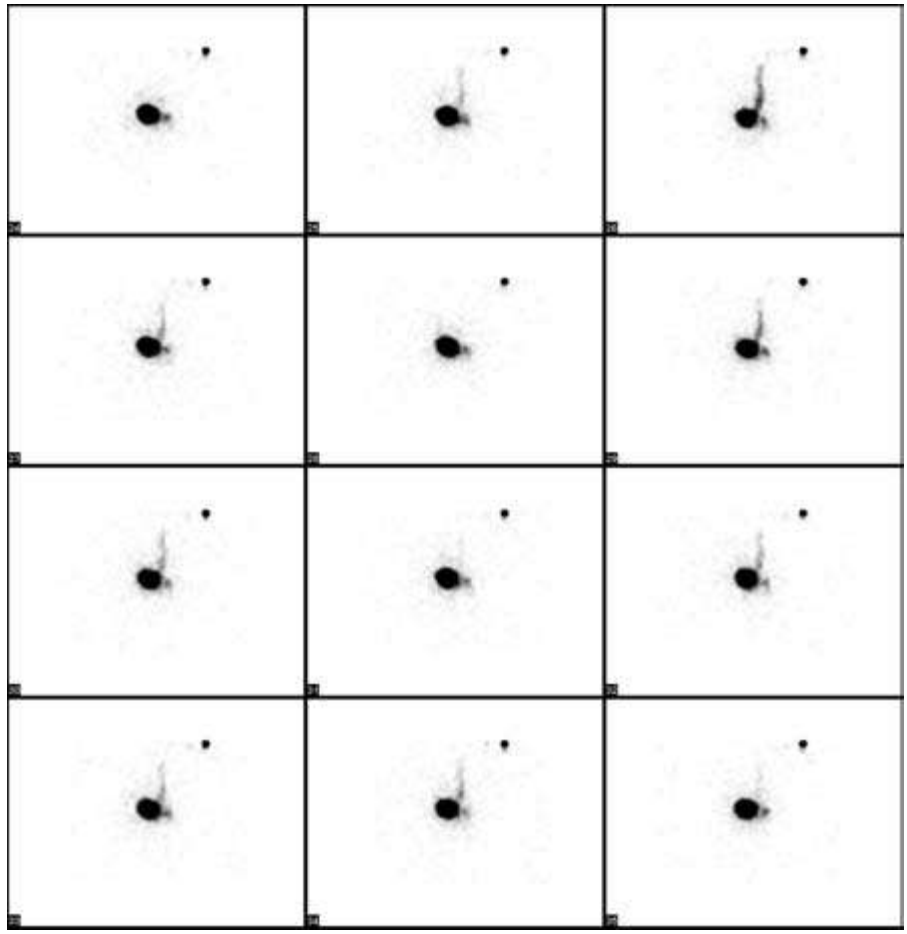


Figure 134.9 Gastroesophageal reflux. A neonate with failure to thrive ingested the usual formula meal with technetium 99m-labeled SC. Ten-second sequential frames show frequent episodes of reflux of varying duration, but mostly high level. The hot dot in the left upper corner is a radioactive marker to show the level of the mouth.

different methods have been described for quantification. A simple method is to count the reflux events and to note whether they are long events (> 10 s) or short, and high (greater than half the distance to the mouth) or low events, and to then sum the results. Evidence for aspiration is routinely sought on these studies.

Gastrointestinal bleeding

The radionuclide gastrointestinal bleeding study was described several decades ago and continues to be a commonly requested study [38]. The standard methodology is to radiolabel the patient's own RBCs with ^{99m}Tc. In order for ^{99m}Tc to bind to the β chain of the hemoglobin, it must be reduced with stannous ion (tin). High labeling efficiency is important because free (unlabeled) ^{99m}Tc is secreted by the salivary glands and stomach, then moves distally, and may be misinterpreted as bleeding. Several different radiolabeling methods have been used over the years. The in vitro commercial kit method is the preferred method because of its high labeling efficiency, greater than 97%. Because free unlabeled ^{99m}Tc is also taken up by the thyroid, routine imaging over the thyroid can confirm whether or not there is a significant amount that might confuse interpretation of the study.

The test is used primarily for lower gastrointestinal bleeding. Its sensitivity for detection of active bleeding is high. Bleeding rates as low as 0.1 mL/min can be detected. For comparison, contrast angiography can detect bleeding rates of 1.0 mL/min, a 10-fold difference. Scintigraphic images are routinely obtained at a framing rate of 1 min per frame for 60–90 min, and delayed imaging can be obtained as needed for up to 24 h [2]. Gastrointestinal bleeding is typically intermittent and the clinical signs and symptoms often are noted after an acute bleeding episode has resolved. The ^{99m}Tc-RBC bleeding study allows for continuous imaging, routinely for 60–90 min, as well as delayed imaging for up to 24 h without relabeling the patient's blood.

Angiographers often request the radionuclide study prior to performing a contrast media angiographic study, for two reasons. First, if the radionuclide study is negative, a contrast study is unlikely to detect bleeding. Second, if the radionuclide study is positive, it can guide the angiographer to the approximate bleeding site, thus saving time and contrast media.

There has been some controversy over the years regarding the accuracy of the radionuclide method, particularly its sensitivity and specificity for the detection of bleeding. Evidence can be found in the literature that its accuracy is good, mediocre, or poor, thus supporting any shade of prior bias in the reader. The discrepancies in the published literature can be explained and attributed to several factors. First, there is the problem of a gold standard used in investigations. Contrast angiography that localizes the site of active bleeding is best; however, only a small number of subjects in any reported series have this direct correlation. Surgery is also a good standard, but these correlations are usually limited to a minority of patients. Thus, contrast gastrointestinal radiography and colonoscopy become a common standard. However, colonoscopy, cannot easily be performed during active bleeding, and the finding of polyps, ulcers, arteriovenous malformations, or angiodysplasia, for instance, does not ensure that they were the source of bleeding unless active bleeding is seen at the time of the procedure. The length of time between the radionuclide study and the standard becomes a factor. Thus, a large number of patients are without confirmatory correlation for the radionuclide study. Good investigational studies are difficult to perform and evaluate.

A major cause of negative gastrointestinal bleeding studies is the clinical method of evaluation and workup of these patients. A not uncommon clinical approach is to admit patients to the hospital, put them to rest, stabilize them with fluids and transfusion, and cleanse the colon for colonoscopy the following day. The problem with this approach is that the radionuclide gastrointestinal bleeding study is most likely to be positive early after the onset of bleeding. What often happens at some centers is that if no site of bleeding is found at colonoscopy and other subsequent workup, then the scintigraphic study is considered and ordered. However, in many cases the bleeding has ceased, and the yield at this time point will be low. Using this approach, the bleeding study will have a low sensitivity. If the bleeding study is performed soon after the patient's arrival in the emergency room or admission, a positive bleeding study is much more likely and this can lead to successful angiography and appropriate therapy. This approach does not preclude stabilizing the patient and prepping them for colonoscopy the next day.

Concerns about a high incidence of false positives, although perhaps valid in the past, should no longer be problematic because of improved methodology and interpretative criteria. Regarding methodology, a 1-min continuous framing rate is standard. In the past intermittent static imaging was performed. Bleeding can move rapidly, anterograde or retrograde, and intermittent imaging can miss the site of origin.

The criteria used to diagnose active bleeding are as follows: new onset of radioactivity where there was none at first; increasing radionuclide activity with time; and movement of the activity in a pattern consistent with bowel anatomy. The images should be obtained for a duration sufficient that the pattern of movement can distinguish small from large intestine and localization is as certain as possible. The images should always be viewed on the computer in movie mode. Finally, if delayed images are performed, the acquisition method – for example, 1-min frames × 30 min – should be similar to the initial methodology and the diagnostic criteria the same. In the past the presence of blood on delayed imaging at a site was sometimes called positive, but it turned out to be false positive. Today, it is better appreciated that a single static image only reveals evidence of blood, which could have come from anywhere above or below that site. Finally,

radiolabeling is superior today than in the past, minimizing the problem of free [^{99m}Tc]pertechnetate.

Suspected upper gastrointestinal bleeding is not an indication for the radionuclide study. However, upper gastrointestinal bleeding may occasionally be diagnosed, although seemingly adequate workup had excluded it prior to the study.

Use of modern methodology and interpretative criteria and prompt performance of the test when the patient presents will ensure a reliable test for detecting active bleeding and localization of the site of bleeding.

Meckel diverticulum and ectopic gastric mucosa

Gastric mucosa takes up [^{99m}Tc]pertechnetate whether the mucosa is in the stomach or ectopic. For decades, the [^{99m}Tc]pertechnetate *Meckel scan* has been an accurate and accepted methodology to confirm the diagnosis preoperatively. The accuracy of the study is high, with a sensitivity of approximately 85% [39]. Cimetidine administered prior to the study can increase diagnostic yield. The drug inhibits release of ^{99m}Tc from the gastric mucosa, making detection more likely. Data are limited regarding its effectiveness, but 20 mg/kg orally in divided doses for 2 days prior to the study is usually without side effects. The diagnosis is made by detecting uptake, usually in the right lower quadrant. The uptake of the ectopic tissue occurs at a rate similar to stomach uptake. The most common cause for a false-positive study is urinary tract activity, a normal excretory route of the radiotracer, misinterpreted as ectopic mucosa. False positives are occasionally seen due to gastrointestinal duplications or local inflammatory hyperemia, increased permeability, or obstruction, for example, occurring with regional enteritis.

Shilling test

The Shilling test is a time-tested standard radionuclide technique for the diagnosis of vitamin B-12 malabsorption. The study is initially performed by using oral administration of cobalt 57-labeled B-12. Intramuscular B-12 is given prior to the study so that all binding sites are saturated, and thus all orally administered B-12 will clear through the kidney. Urine is collected for 48 h, and the percent excretion is calculated. If B-12 clearance is low, a second-stage study is performed with intrinsic factor and radiolabeled B-12. If the clearance improves substantially, pernicious anemia is diagnosed. If it does not improve, intestinal malabsorption is the cause. Alternative second stages can be done with pancreatic enzymes for suspected pancreatic insufficiency, or with antibiotics for suspected overgrowth syndromes.

Somatostatin receptor imaging

Somatostatin receptor imaging has an important role for imaging neuroendocrine tumors, for example, gastroenteropancreatic and carcinoid tumors. Somatostatin receptors are integral membrane glycoproteins located on normal cells of neuroendocrine origin, such as pancreatic islet cells. Five somatostatin receptor types have been described. The receptors are also found in neuroendocrine tumors derived from neural crest cells belonging to the amine precursor uptake and decarboxylation (APUD) system. [^{111}In]pentetreotide (octreotide) binds with high affinity to type 2 somatostatin receptors. Only gastroenteropancratic (gastrinomas, insulinomas) and carcinoid tumors will be discussed.

Gastroenteropancreatic tumors are highly differentiated, slow growing, and often small. Most have high concentrations of somatostatin receptors [40]. Symptoms are often secondary to the hormonal activity expressed, for example, hypoglycemia, gastric ulcers, severe diarrhea, and flushing. Frequently the tumors have metastasized by the time of diagnosis, to liver, lymph nodes, bone, lungs, and skin. *Gastrointestinal carcinoid tumors* are derived from the foregut, midgut, or hindgut. The midgut carcinoids with liver metastases cause the carcinoid syndrome, with symptoms and signs of flushing, diarrhea, wheezing, and valvular right-sided heart disease.

Although ^{99m}Tc-labeled somatostatin receptor imaging agents have been developed, ^{111}In is preferable because of its longer physical half-life (2.8 days vs 6 h), allowing time for background clearance and improved target-to-background ratio for optimal diagnostic images. Normal distribution after intravenous injection of [^{111}In]pentetreotide (OctreoScan) includes prominent uptake in the kidneys and spleen. The liver has less uptake and gallbladder clearance is variably seen. Whole body and SPECT imaging is performed 24 h after injection of the radiopharmaceutical.

Sensitivity for detection of carcinoid tumors and gastrinomas is high, at 80%–90%, but somewhat lower for VIPomas and glucagonomas (75%), and lowest for insulinomas (50%). Many of these tumors are small and difficult to detect with conventional imaging, for example, ultrasonography, MRI, and CT, which have considerably lower tumor detection rates. [^{111}In]OctreoScan is superior to [^{123}I]meta-iodobenzylguanidine (MIBG), although the latter is taken up by some tumors. Because the tumors are usually highly differentiated, FDG PET imaging, described in the next section, is often negative. Uptake of FDG PET is more likely with poorly differentiated tumors. New somatostatin receptor radiopharmaceuticals are being deeveloped for SPECT and PET.

Somatostatin receptor imaging is sometimes used to select patients likely to respond favorably to octreotide therapy. Scintigraphy is most useful for detection of metastases and can direct patients with inoperable tumors to other forms of therapy (Figs 134.10 and 134.11). [^{111}In]OctreoScan imaging positively impacts patient management in 25%–45% of patients [41].

[^{18}F]FDG PET imaging of gastrointestinal malignancy

The use of [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) PET and PET-CT is growing rapidly, and these techniques play an important

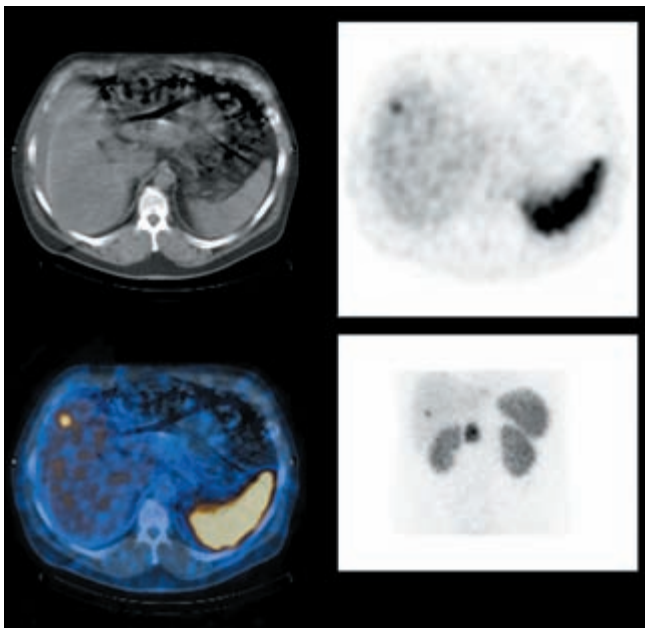


Figure 134.10 OctreoScan single photon emission computed tomography (SPECT) for a pancreatic islet cell tumor. This patient was imaged with indium 111-labeled OctreoScan on a SPECT–CT system, combining both imaging modalities. CT slice **(upper left)**, SPECT image **(upper right)**, fused SPECT–CT image **(lower left)**. The MIPS (maximal intensity projection images) image, a 3-D reconstructed abdominal image **(lower right)**, shows normal high uptake in spleen and kidneys and focal hot uptake in a midline mass, which is the patient's islet cell tumor. Also a small focus of uptake due to a metastasis is see in the anterior inferior portion of the liver on the MIPS view and the SPECT and fused images.

role in the diagnosis, staging, restaging, and monitoring of response to therapy of various gastrointestinal malignancies.

[^{18}F]fluorodeoxyglucose is a radiolabeled PET glucose analog. It is transported into the cell and phosphorylated by the same mechanism as glucose but cannot be metabolized further and is therefore trapped intracellularly. Most malignant tumor cells have increased glucose metabolism compared with normal tissue cells and thus have increased uptake of [^{18}F]FDG. Patients must have fasted for 6–8 h prior to injection, otherwise the radiopharmaceutical will distribute primarily to muscle and soft tissue and not to tumor. This is due to competition with serum glucose and the effect of insulin. This is a particular problem in diabetics. Insulin should not be injected within 2 h of the study. The 2-h half-life of ^{18}F requires imaging at 1 h after injection.

Whole-body cross-sectional imaging is performed. Most PET scanners are PET-CT cameras. The PET and CT images are obtained sequentially with the patient lying on a table that moves between the two adjacent scanners, and then the images are registered and fused (overlayed) for functional and anatomical correlation (Fig. 134.12).

Colorectal cancer

Cancer of the colon is the third leading cause of death after lung, breast, and prostate cancers. Although FDG PET has little role in initial tumor staging, it is routinely used for restaging, preoperative evaluation prior to hepatic tumor resection, and for evaluating response to therapy [42].

[^{18}F]fluorodeoxyglucose uptake is usually high in colorectal cancer, with a sensitivity for tumor detection greater than 95%. The one exception is mucinous adenocarcinoma, for which the sensitivity is considerably less, at approximately 60%. Because most patients with newly diagnosed colorectal cancer require surgery to prevent obstruction and the incidence of unsuspected distant metastases is not high, FDG PET is not routinely required preoperatively. Postoperatively, one-third of patients whose tumors are thought to be resectable with curative intent at the time of surgery, have recurrence within 2 years. Serial serum carcinoembryonic antigen (CEA) levels have sensitivity for recurrence of less than 60% and, importantly, provide no localizing information. A common clinical problem is the patient with a rising CEA level but negative findings on conventional imaging, including CT. The sensitivity of FDG PET for detecting recurrence is approximately 95%, compared with CT sensitivity of 70% [43].

Twenty percent of colorectal recurrences occur in the liver, where FDG PET has a high accuracy – 92%, compared with 78% for CT. Patients with a single liver metastasis are potentially curable. However, in the past, only 25% of patients were cured because of the presence of unknown extrahepatic metastases. Preoperative detection of distant metastases is poor by conventional methods, so FDG PET plays an important role in selecting patients for surgical resection.

Patient management is changed by FDG PET. It is reported to have a clinical impact in 30%–40% of patients, often obviating the need for unnecessary surgery. Also, FDG PET can determine the effectiveness of chemotherapy and radiation therapy. With successful therapy, FDG uptake markedly decreases. There is a growing body of data to suggest that response to chemotherapy can be predicted by FDG PET within 3 weeks after initiation of chemotherapy. Furthermore, FDG PET can differentiate postsurgical changes of necrosis and fibrosis from recurrent tumor with high accuracy, which is often not possible with CT or MRI.

Cancer of the esophagus

Early-stage esophageal cancer is potentially curable with surgery; however, up to 80% of patients have local metastases and 50% have distant metastases at initial diagnosis. Prognosis depends on the extent of the primary tumor (TNM classification). Regional metastases occur anywhere from the cervical chain to mediastinal, gastrohepatic, and celiac nodes. Neoadjuvant chemotherapy is increasingly used in patients thought to have resectable disease because it improves tumor control, prevents distant disease, and decreases tumor burden, allowing for more complete resection.

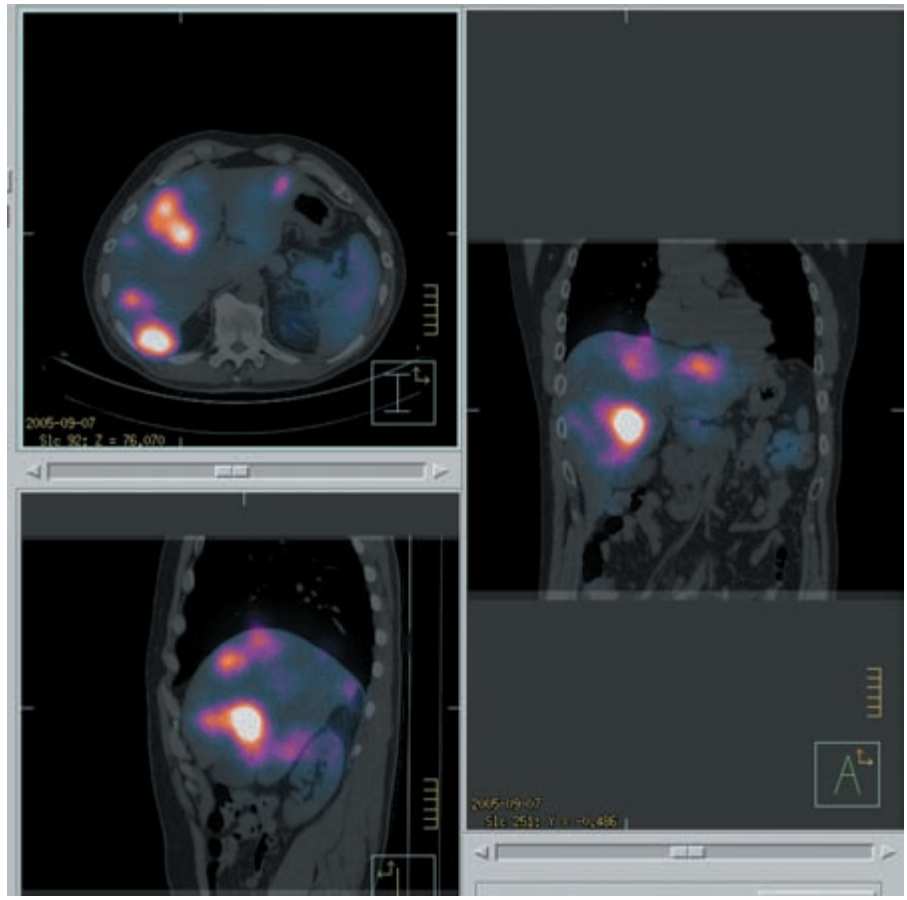


Figure 134.11 OctreoScan single photon emission computed tomography (SPECT)–computed tomography (CT) of carcinoid tumor. Fused SPECT and CT images with transverse **(left upper)**, sagittal **(left lower)**, and coronal **(right)** slices. Intense uptake is seen in multiple large carcinoid tumors metastatic to the liver.

Uptake of FDG is high in primary colorectal cancer, with detection sensitivity greater than 95%. However, FDG PET cannot provide staging information on the depth of invasion and periesophageal tissue invasion (T staging), and has relatively poor sensitivity (50%–75%) for determining nodal involvement (N stage). Five-year survival in patients without nodal involvement reaches 75%; survival is 10% for those with nodal disease.

An important role of FDG PET in primary staging is detection of distant metastases, commonly found in the liver and lung. Sensitivity for distant metastases is 80%–90%, compared with 50%–75% for CT. Imaging using FDG PET upstages as many as 15% of patients, and has a major impact by improving detection of occult stage IV disease. Exceptions to this high sensitivity are its poorer sensitivity for brain metastases compared with CT or MRI, and lower sensitivity than CT for lung metastases.

The effectiveness of therapy can be monintored using FDG PET [44]. Resolution of uptake is seen with a complete response, reduced uptake with a partial response, and persistent uptake with no response. The accuracy of FDG PET is high for detecting recurrence, and posttherapy changes can be differentiated from residual tumor. Chemotherapy responders can be differentiated from nonresponders within a few cycles of chemotherapy.

Pancreatic carcinoma

Although early diagnosis and resection can make pancreatic cancer potentially curative, it is unresectable in most patients at the time of diagnosis. The sensitivity of FDG PET for primary tumor detection is greater than 90% [45], and the technique is reported to have good accuracy for differentiating benign from malignant disease; however, pancreatitis can produce false-positive studies and hyperglycemia can result in false negatives [46].

Anatomical imaging is required for T and N staging. The main role of FDG PET is in the detection of liver and distant metastases (M staging). Overall accuracy is approximately 83% for PET and 63% for CT [47]. Management can be altered by FDG PET in 45% of patients.

For detecting recurrent disease, FDG PET is better than other imaging modalities and can differentiate postoperative changes from recurrence. Its major impact is in the identification of recurrent nodal or distant metastatic disease. Positron emission tomography can clarify uncertain regions seen on CT, detect new hepatic lesions, and evaluate patients

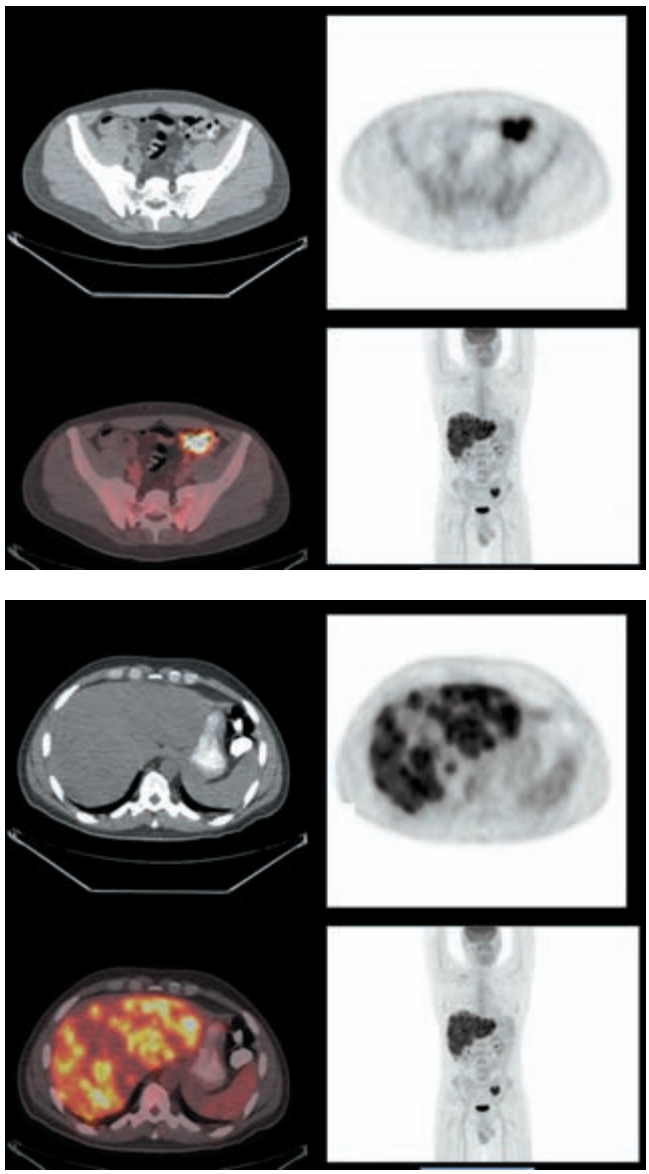


Figure 134.12 Fluorine 18-labeled fluorodeoxyglucose ([^{18}F]FDG) positron emission tomography (PET) of metastatic colon cancer: newly diagnosed adenocarcinoma of sigmoid colon. **(a)** Whole-body MIPS view **(lower right)** shows the primary cancer in the left lower quadrant and extensive metastases to the liver. There is normal bladder clearance. Transverse cross-sectional FDG PET slice **(upper right)** shows intense FDG uptake in the primary malignancy that corresponds to the CT lesion **(upper left)** and fused CT and FDG images **(lower left)**. **(b)** Multiple regions of uptake in the liver consistent with metastases.

with rising serum tumor markers but negative findings on conventional imaging. The absence of FDG uptake 1 month following chemotherapy is an indicator of improved survival.

Gastrointestinal stromal tumors

Thirty percent of these rare mesenchymal gastrointestinal tract tumors are malignant and 70% are benign. The malignant tumors tend to recur and metastasize to the liver and peritoneum, less commonly to the lungs, pleura, retroperitoneum, bone, and subcutaneous tissue. These tumors are generally resistant to chemotherapy and radiation therapy. However, many patients have a dramatic response to the tyrosine kinase inhibitor, imatinib mesylate (Gleevac, Novartis).

Both CT and FDG PET have high sensitivity and specificity for primary tumor detection, greater than 90%. In approximately 25% of patients, FDG PET can detect a good response to therapy earlier than CT [48]. In 5% of patients, CT detects the response first. Response to therapy is correctly characterized by FDG PET in 95% of patients at 1 month, and in 100% of patients at 3 and 6 months.

[^{14}C]urea breath test for *Helicobacter pylori* infection

Although the initial diagnosis of *Helicobacter pylori* infection is usually made by gastric biopsy, this approach is not practical for routinely evaluating response to antibiotic therapy. Also, serological markers fall too slowly to be diagnostically helpful in determining the effectiveness of therapy. The [^{14}C]urea breath test is accurate for this purpose. The test is noninvasive, inexpensive, relatively simple to perform, and generally available.

In the presence of the bacterial enzyme urease, orally administered urea is hydrolyzed to carbon dioxide and ammonia. The ^{14}C radioactive beta particle emitter is detected in the breath as labeled CO_2. The test has high accuracy. False-negative results may occur because of recent use of antibiotics or bismuth-containing medications. False-positive results occur in patients with achlorhydria, contamination with oral urease-containg bacteria, and colonization with another *Helicobacter* species.

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B IMAGING

135 Angiography

Kyung J. Cho

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Since the introduction of the newer imaging modalities, including ultrasound (US), computed tomography (CT), and magnetic resonance imaging (MRI), diagnostic angiography has been used less frequently in the evaluation of gastrointestinal (GI) disease. Angiography is currently used to establish a specific diagnosis of mesenteric vascular disease, localize GI bleeding, evaluate portal hypertension, assess tumor extension, demonstrate traumatic arterial injury, and obtain the necessary vascular information before percutaneous or surgical intervention. This chapter reviews the vascular anatomy as well as the techniques and role of angiography in the diagnosis and treatment of GI disease.

Technical considerations

Intraarterial digital subtraction angiography (DSA) is used in GI angiography. DSA requires a smaller amount of dilute contrast medium, causes less discomfort to patients, and reduces the risks of renal toxicity and fluid overload. DSA images may be viewed on a video monitor during and after injection of iodinated contrast material.

Two types of iodinated contrast material have been used for angiography: the conventional high osmolar (ionic) contrast agent has been replaced by the low osmolar (nonionic) or isosmolar (iodixanol, Visipaque, GE Healthcare, Princeton, NJ, USA) contrast agent. The nonionic contrast agents are more expensive but cause less pain and burning sensation. Patients with a history of contrast media allergy and renal failure will benefit from the use of low osmolar contrast media [1–3].

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Carbon dioxide (CO_2) gas is used as an alternative contrast agent in both the arterial and venous circulations. CO_2 is approximately 20 times more soluble than air, allowing intravascular injection of the gas without causing clinically significant gas embolism. CO_2 angiography is useful for patients with a history of contrast allergy or renal failure [4]. CO_2 can be used as a contrast agent for visualization of the abdominal aorta and renal, mesenteric, and peripheral arteries. CO_2 is used as a contrast agent for the diagnosis of GI bleeding, and traumatic hepatic and splenic bleeding. Because of the buoyancy of the gas, CO_2 is useful in visualizing the origins of the celiac and superior mesenteric arteries that arise from the ventral surface of the abdominal aorta. CO_2 is a safe contrast agent in the venous circulation. An injection of 30–50 mL of the gas into a peripheral or central vein causes no significant changes in vital signs. Because of its low viscosity, CO_2 is the preferred contrast agent for wedged hepatic venography to demonstrate the portal vein. CO_2 can also be used as a contrast agent for splenoportography [5,6]. CO_2 is also used as a contrast agent to guide a variety of vascular interventions.

Percutaneous catheterization

Gastrointestinal angiography is performed using the technique described by Seldinger in 1953 [7]. The arteries used for catheterization of the visceral arteries are the femoral, axillary, and brachial arteries. Of these, the femoral approach is preferable. When the needle is introduced into the artery, the guidewire is inserted through the needle into the abdominal aorta and a diagnostic catheter is introduced over the guidewire. The technique used for femoral vein catheterization is similar to that used for arterial introduction. The guidewire is inserted into the inferior vena cava (IVC) and the catheter is advanced over the guidewire. If thrombosis has occurred in the femoral veins or IVC, the catheter is introduced by way of an antecubital, brachial, or jugular vein.

General angiographic approach

Gastrointestinal angiography can be performed as an outpatient procedure. On the day of the procedure, the patient is allowed to take fluids by mouth. Patients usually receive conscious sedation with a narcotic analgesic (fentanyl) and a benzodiazepine central nervous system depressant (midazolam) immediately before and during the procedure. For the evaluation of mesenteric ischemia and median arcuate ligament compression, lateral aortography is performed in both full inspiration and expiration. Otherwise, visceral angiography begins with catheterization of the celiac and superior mesenteric arteries. Selective catheterization of the branches of the visceral arteries is performed as needed: splenic, dorsal pancreatic, and gastroduodenal arterial catheterization for evaluation of pancreatic disease, left gastric and gastroduodenal arterial catheterization for gastric and duodenal bleeding, superior mesenteric branch catheterization for small bowel and right-sided colonic bleeding, and inferior mesenteric artery catheterization for left-sided colonic and rectosigmoidal bleeding.

Visualization of the portal venous system is essential in angiographic study for evaluating intraabdominal masses, vascular diseases, and portal hypertension. The presence or absence of a venous abnormality plays an important role in determining whether the arterial abnormality is neoplastic or arteriosclerotic in nature. Arterial involvement by a neoplasm is usually associated with narrowing or occlusion of the adjacent vein. Visualization of the portal vein (indirect portography or arterial portography) requires the injection of a large volume of contrast medium into the superior mesenteric or splenic artery. Portal vein visualization can be enhanced by the intraarterial injection of a vasodilator (nitroglycerin 150 μg) before the injection of contrast medium.

Under normal conditions, puncture site hemostasis can be achieved with manual compression for 5–10 min following GI angiography. No pressure dressing is required. The patient may resume a normal diet after the procedure and ambulate after 4 h of bed rest.

Transhepatic portal vein catheterization

The indications for percutaneous transhepatic portal vein catheterization include evaluation of portal hemodynamics with manometry; venous sampling to localize islet cell tumor; coronary vein embolization for control of variceal bleeding; portal vein embolization prior to hepatic resection [8]; and recanalization of stenotic or occluded portal vein [9]. Ultrasound, CT, or magnetic resonance angiography (MRA) is used to verify portal vein patency prior to the procedure. The venous phase of a celiac or superior mesenteric angiogram is also useful in visualizing the portal vein. Wedged hepatic venography with CO_2 may be performed from the jugular or femoral vein to visualize the portal vein. CO_2 (20–30 mL) may be injected into the liver parenchyma using a 22G needle to fill the portal vein. After the procedure has been completed, the catheter track is sealed near the hepatic capsule with a gelatin sponge or coil to arrest bleeding from the puncture site.

Transjugular portal vein catheterization

The transjugular approach to the portal venous system is commonly used to create a transjugular intrahepatic portosystemic shunt (TIPS) [10,11]. The right or left internal jugular vein is punctured under US guidance. A 10Fr vascular sheath is advanced into the right atrium. A 16G Colapinto-type needle is used to puncture the right portal vein near the portal vein bifurcation under fluoroscopic control. When the portal vein is entered, a guidewire is introduced into the portal vein. Over the guidewire a 5Fr catheter is advanced into the portal vein and manometry is performed. A splenoportogram is performed to demonstrate portal vein anatomy and portosystemic collateral veins. For a TIPS procedure, the parenchymal tract is dilated with an 8-mm angioplasty balloon catheter and covered with 10-mm metallic stents such as Wallstent or covered stents such as VIATORR.

Balloon occlusion hepatic venography

The balloon occlusion method is useful in hepatic venography, facilitating measurement of the wedged hepatic venous pressure. Injection of contrast medium (4–5 mL/s for a total volume of 10–20 mL) or CO_2 (30–50 mL) into the hepatic vein distal to balloon occlusion results in visualization of the portal vein [12]. When the portal vein is not seen after either wedged hepatic or balloon occlusion venography, CO_2 (15–20 mL) is injected into the liver parenchyma using a 21G needle, and digital subtraction venography is performed to visualize the portal vein.

Splenoportography

Since the advent of the imaging modalities and the development of indirect portography (arterial portography), splenoportography is rarely performed. CO_2 is a useful contrast agent for splenoportography. Because of its low viscosity, the gas can be injected using a 22G or 25G needle. Because of the relative safety of the small needle and the lack of nephrotoxicity of CO_2, CO_2 splenoportography is useful in pediatric patients [5,13].

Risks and contraindications

The overall complication rates of transfemoral and transaxillary angiography are 1.73% and 3.29%, respectively [14]. The complications of transfemoral angiography include puncture-site complications, complications related to catheter manipulation, contrast material reactions, contrast material toxicity (renal failure), and systemic complications (cardiac and neurological). Brachial nerve injury is the most serious complication of transaxillary arterial catheterization. Nerve injury is

less likely to occur with a brachial artery puncture because the brachial plexus is not as close to the brachial artery as it is to the axillary artery. The use of intraarterial nitroglycerin and calcium-channel blocking drugs is helpful in preventing arterial spasm. Mortality related to angiographic procedures other than reactions to contrast agents is extremely rare.

The overall mortality associated with the intravenous use of ionic contrast agents is 1 in 40 000 [15]. The risk factors involved in the use of contrast material are renal failure, a history of previous reactions (major and minor), and allergic diathesis. Before the administration of contrast medium for angiography, risk factors for contrast nephropathy should be identified. These include preexisting renal insufficiency, diabetes mellitus, intravascular volume depletion, congestive heart failure, repeat contrast procedures, and multiple myeloma [16]. Recommendations for prevention of contrast nephropathy include use of alternative contrast such as CO_2, discontinuation of potential nephrotoxic agents 48–72 h before the procedure, adequate hydration before and after the procedure, use of low-osmolar or isosmolar contrast material, and use of minimal volume of contrast material. Pretreatment with prednisone 50 mg at 13, 7, and 1 h before contrast administration has been advocated to prevent reaction to contrast agents for patients with a history of hypersensitivity to iodinated contrast medium. Before the procedure, Benadryl 50 mg is administered. For emergency cases, hydrocortisone 100 mg is administered intravenously before the procedure.

There are no absolute contraindications to GI angiography. Relative contraindications include severe coagulopathy, recent myocardial infarction, congestive heart failure, renal failure, and pregnancy. Patients undergoing brachial or axillary artery puncture or a percutaneous transhepatic procedure are at increased risk for hemorrhagic complications in the presence of coagulopathy or hypertension. Depending on the urgency and nature of the procedure, coagulopathy should be reversed with appropriate treatment. Prothrombin time should be less than 18 s for femoral artery puncture and less than 13 s for brachial artery puncture. Platelets should be greater than 50 000/mL. In heparinized patients, heparin should be discontinued at least 2 h before the arterial puncture. In patients on warfarin, the warfarin should be stopped several days before any arterial puncture. If urgent GI angiography is needed, the patient should be treated with fresh-frozen plasma and vitamin K 25–50 mg i.m. Gastrointestinal angiography cannot be performed if the patient is unable to lie flat on the bed.

Vascular anatomy of the abdominal viscera

A thorough knowledge of vascular anatomy of the abdominal viscera is essential in performing and interpreting an angiogram and in planning therapeutic intervention for GI disease.

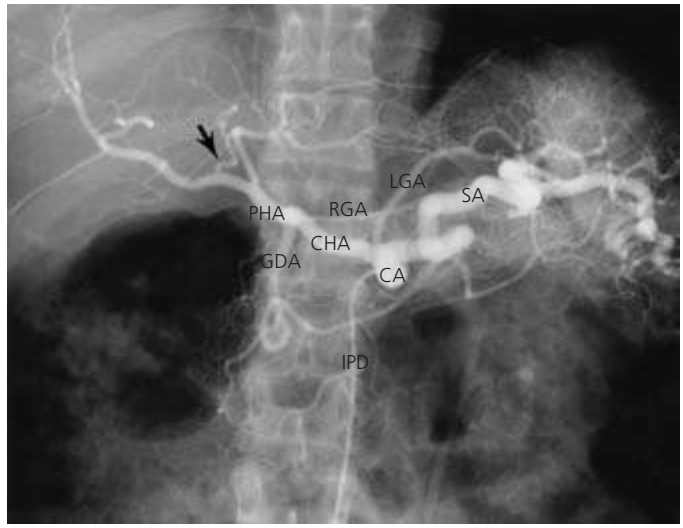
Arterial vasculature

The abdominal viscera receive their blood supply from the celiac, superior, and inferior mesenteric arteries. The superior mesenteric artery arises from the ventral aspect of the abdominal aorta 1–2 cm below the celiac artery and above the renal artery. It courses anterior to the third portion of the duodenum and the left renal vein into the mesentery. The celiac artery gives off the left gastric, splenic, and common hepatic arteries (Fig. 135.1). Occasionally, the inferior phrenic or dorsal pancreatic artery originates from the celiac axis. The inferior phrenic artery may arise from the left gastric artery or renal artery, or directly from the aorta. One or more branches of the celiac artery may originate from sources other than the celiac axis: the left gastric artery from the aorta, the splenic artery from the superior mesenteric artery, and the hepatic artery from the aorta, superior mesenteric artery, gastroduodenal artery, or left gastric artery.

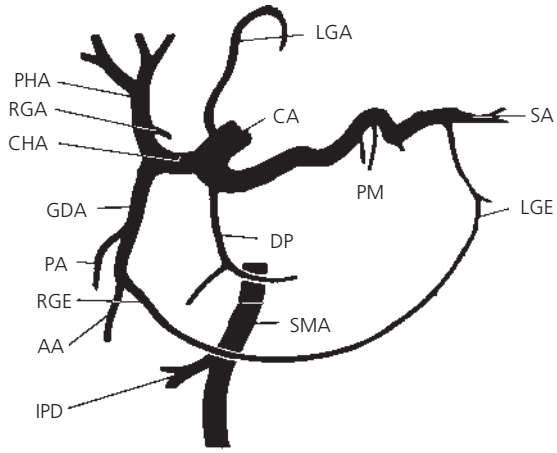
The common hepatic artery divides into the proper hepatic and gastroduodenal arteries. The proper hepatic artery ascends in the hepatoduodenal ligament for a variable distance and divides into a right and a left hepatic artery. The right hepatic artery usually courses behind the common hepatic duct and divides into an anterior and posterior segmental artery. Each of the segmental arteries gives off arterial branches to the superior and inferior subsegments of the liver. The left hepatic artery divides into a medial segmental (middle hepatic) and a lateral segmental artery. Each segmental artery gives rise to superior and inferior subsegmental branches. The middle hepatic artery may arise from a right hepatic, a left hepatic, or a proper hepatic artery. The proper hepatic artery also gives off arterial branches to the bile duct (peribiliary artery), the portal vein (vasa vasorum), and the subcapsular branches. These nonparenchymal branches may provide collateral circulation to the liver in hepatic arterial occlusion distal to the gastroduodenal artery.

In about half of cases, one or more branches of the hepatic artery arise from sources other than the celiac/hepatic artery. Two types of aberrant hepatic artery may occur: replaced (the origin of the artery is "replaced" and the aberrant artery serves as a substitute) or accessory (additive to the celiac/hepatic artery). According to Michels' dissection of 200 cadavers [17], aberrant right hepatic arteries occur in 26% of patients (18% replaced, 8% accessory), most frequently originating from the superior mesenteric artery (17%); aberrant left hepatic arteries occur in 27% of patients (15.5% replaced, 11.5% accessory), most frequently from the left gastric artery (13%).

The gastroduodenal artery usually originates from the common hepatic artery. In 25% of the population, it may have an aberrant origin. The three main branches originating from the gastroduodenal artery are the posterior superior pancreaticoduodenal (posterior arcade), anterior superior pancreaticoduodenal (anterior arcade), and right gastroepiploic arteries. The posterior and anterior arcade arteries join



(a)



(b)

Figure 135.1 Normal celiac trunk. **(a)** Celiac arteriogram showing standard arterial anatomy. The left gastric (LGA), splenic (SA), and common hepatic (CHA) arteries arise from the celiac trunk (CA). The proper hepatic artery (PHA) divides into the right and left hepatic arteries. The middle hepatic artery (arrow) arises from the right hepatic artery. The right gastric artery (RGA) arises from the PHA. **(b)** Line drawing of branches of the celiac trunk. DP, dorsal pancreatic artery; PM, pancreatica magna artery; LGE, left gastroepiploic; PA, posterior arcade; AA, anterior arcade; RGE, right gastroepiploic; IPD, inferior pancreaticoduodenal; SMA, superior mesenteric artery; GDA, gastroduodenal artery.

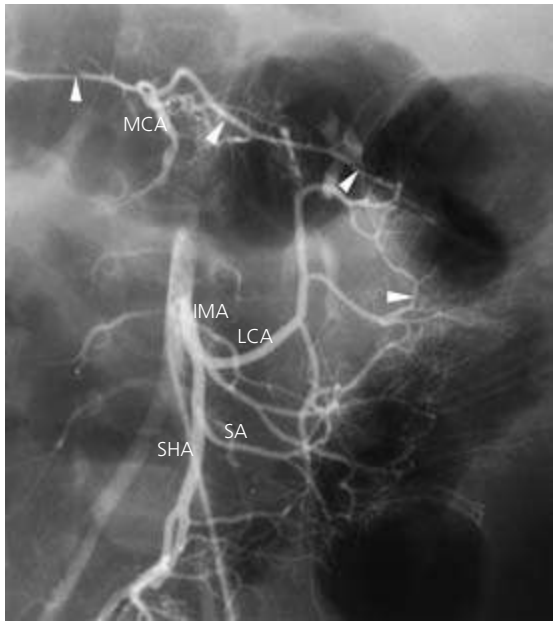
inferomedially and anastomose with the inferior pancreaticoduodenal artery, a branch of the superior mesenteric artery. The gastroduodenal artery supplies arterial blood to the stomach, duodenum, pancreas, and bile duct. In the presence of a celiac artery occlusion, the pancreaticoduodenal arcade arteries function as the major collateral pathway to the liver from the superior mesenteric artery. Normally, the gastroduodenal blood flows away from the liver, but with stenosis or occlusion in the celiac or common hepatic artery the blood flow is reversed. Recognition of flow reversal in the gastroduodenal artery is important in planning resection of pancreatic head tumors, for placement of a hepatic artery infusion catheter for chemotherapy, and for intraarterial stimulation with calcium or secretin for the localization of insulinoma or gastrinoma respectively.

The superior mesenteric artery supplies the pancreas, duodenum, small intestine, cecum, ascending colon, and the proximal half of the transverse colon. The inferior pancreaticoduodenal and occasionally the dorsal pancreatic arteries originate from the proximal portion of the superior mesenteric artery. The other branches of the superior mesenteric artery are the middle colic, jejunal, ileal, right colic, and ileocolic arteries (Fig. 135.2). A true right colic artery is an inconstant branch (present in 13% of people), and the ascending colon often receives its blood supply from a paracolic arcade fed from the middle and ileocolic arteries [18]. However, Michels asserted that any branch proximal to the paracolic arcade that supplies the ascending colon is a right colic artery [19]. With this definition, the right colic artery arises from the superior mesenteric artery in 38%, from a common right colic/middle colic trunk in 52%, and from an ileocolic/right colic trunk in 8% of individuals. The right colic artery is absent in 2% and an accessory right colic artery is present in 8% of individuals.

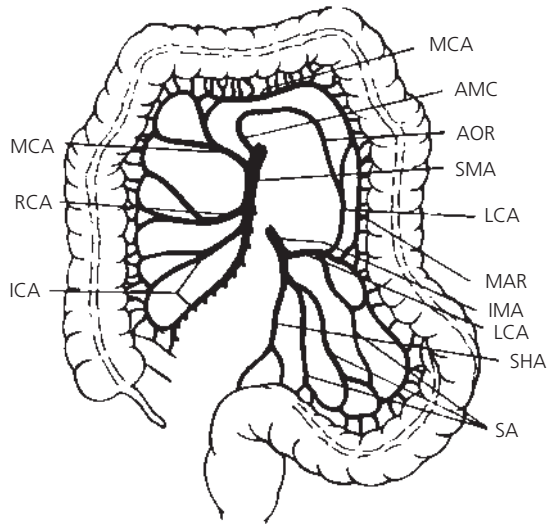
The inferior mesenteric artery arises from the anterolateral aspect of the aorta near the level of the L3–4 interspace, and courses caudally and to the left for up to 5 cm before giving off the left colic artery (see Fig. 135.2). The ascending branch of the left colic artery supplies the descending colon and a variable amount of the splenic flexure. The inferior mesenteric artery gives off several additional branches to the descending and sigmoid colon before terminating in the superior hemorrhoidal artery. The inferior mesenteric artery communicates through the left colic/middle colic anastomosis to the superior mesenteric artery. It also communicates through the superior hemorrhoidal/middle and inferior hemorrhoidal arterial anastomoses to the internal iliac artery. They function as collaterals in mesenteric arterial or distal aortic occlusion.

Portal venous system

The portal vein is formed by the junction of the splenic and superior mesenteric veins behind the head of the pancreas (Fig. 135.3) and ascends toward the hepatic hilus in the hepatoduodenal ligament, dorsal and to the left of the bile duct, and to the right of the hepatic artery. The portal vein is joined by the left gastric, right gastric, posterior superior pancreaticoduodenal, and cystic veins. These tributaries provide the



(a)



(b)

Figure 135.2 Arterial supply to the colon. **(a)** Arterial phase of an inferior mesenteric angiogram. The inferior mesenteric artery (IMA) gives off the left colic (LCA), sigmoidal (SA), and superior hemorrhoidal (SHA) arteries. The middle colic (MCA) branch of the superior mesenteric artery anastomoses with the left colic branch of the inferior mesenteric artery via the marginal artery of Drummond (arrowheads). Opacification of the middle colic artery is due to forceful injection of contrast medium into the inferior mesenteric artery. **(b)** Line drawing of the arterial supply to the colon. RCA, right colic artery; ICA, ileocolic artery; AOR, arc of Riolan; MCA, middle colic artery; SMA, superior mesenteric artery; LCA, left colic artery; IMA, inferior mesenteric artery; SHA, superior hemorrhoidal artery; SA, sigmoidal artery; AMC, accessory middle colic artery; MAR, marginal artery of Drummond.

portosystemic collaterals in patients with cirrhosis and portal hypertension. The right branch of the portal vein divides into the anterior and posterior segmental branches. The left portal branch divides into the superior and inferior subsegmental branches after giving off branches to the caudate and quadrate lobes. The umbilical vein joins the left portal vein at its bifurcation into the subsegmental branches. It is normally obliterated but may be recanalized in the presence of portal hypertension. The inferior mesenteric vein joins the splenic vein or, less frequently, the superior mesenteric vein. Portosystemic collaterals develop from the inferior mesenteric vein via the superior hemorrhoidal vein to the branches of the internal iliac vein and via the retroperitoneal vein to the IVC.

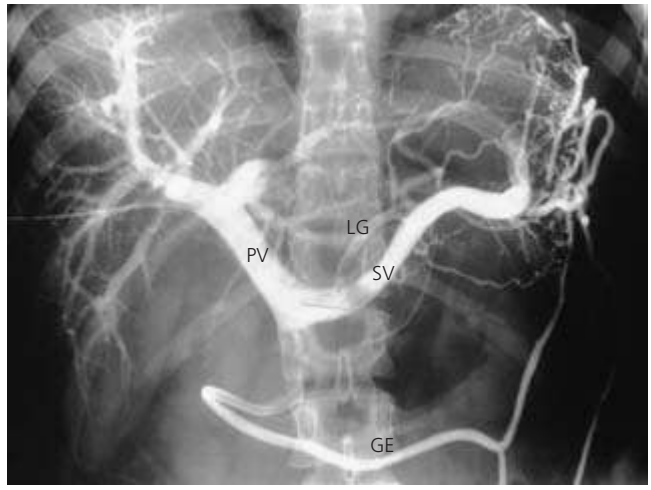
The portal venous system receives venous blood from the GI tract, the pancreas, the spleen, the gallbladder, and the omentum. Normally the portal vein blood flows toward the liver (hepatopetal). Reversal in flow (hepatofugal) in any tributaries of the portal venous system indicates the presence of portal hypertension; visualization of the coronary vein or inferior mesenteric vein in the portal venous phase of a superior mesenteric angiogram indicates reversal in flow of these veins functioning as portosystemic collaterals. Reversal of portal vein flow may be partial or complete. Partial reversal of portal flow may occur in the extrahepatic or intrahepatic branches of the portal venous system. Complete reversal of intrahepatic portal flow may occur in some patients with severe cirrhosis, hepatic vein obstruction, surgical portosystemic shunts, and TIPS. In such cases, the portal vein is the principal outflow conduit of the liver, and the hepatic artery blood leaves the liver through the portal vein. Blood flow direction in the intrahepatic and extrahepatic portal veins can be assessed by Doppler US, transhepatic portal vein catheterization, superior mesenteric and celiac angiography, and wedged hepatic venography. When the main portosystemic collateral develops from the umbilical vein, the extrahepatic portal blood flow can be hepatopetal in the presence of hepatofugal intrahepatic portal flow.

CT, US, and magnetic resonance venography (MRV) can be used to accurately visualize the portal venous system [20–22]. Portal and hepatic venous imaging can be obtained immediately following MRA with gadolinium enhancement for arterial examination (see Fig. 135.3). The method is useful when a TIPS procedure, liver transplantation, or resection of intraabdominal tumors is contemplated. CO_2 wedged hepatic venography is used to visualize the portal vein during TIPS or transjugular liver biopsy, or when hepatic vein outflow obstruction is suspected (Fig. 135.4).

Hepatic veins

The hepatic veins begin in the center of the hepatic lobules as intralobular veins. These veins join together to form sub-

(a)



(b)

Figure 135.3 Portal and hepatic veins. **(a)** Magnetic resonance angiography and three-dimensional contrast magnetic resonance venography. The celiac and superior mesenteric arteries were normal (not shown). Imaging of the upper abdomen in the coronal plane was performed following intravenous administration of gadolinium. The superior mesenteric, portal, and hepatic veins are visualized. SMV, superior mesenteric vein; MPV, main portal vein; RPV, right portal vein; LPV, left portal vein; RHV, right hepatic vein; LHV, left hepatic vein; MHV, middle hepatic vein. **(b)** Percutaneous transhepatic splenoportogram with injection of contrast medium into the proximal splenic vein. LG, left gastric vein; GE, gastroepiploic vein; SV, splenic vein; PV, portal vein. Venous anastomoses are seen between the short gastric and left gastric veins, and between the left and right gastroepiploic veins.

lobular veins. The hepatic veins are intersegmental or interlobar in course: the right hepatic vein lies in the intersegmental fissure of the right hepatic lobe, dividing it into the anterior and posterior segments; the middle hepatic vein lies in the

Figure 135.4 CO_2 wedged hepatic venogram: 30 mL of CO_2 was injected into the 5Fr end-hole catheter wedged in a branch of the right hepatic vein (arrow). CO_2 filled the right hepatic vein (hv) and both intrahepatic and extrahepatic portal vein (pv).

interlobar fissure; and the left hepatic vein lies between the medial and lateral segments of the left hepatic lobe. The hepatic veins converge posteriorly and run near the hepatic capsule before emptying into the IVC (see Fig. 135.3).

The accessory hepatic veins, which originate from the right hepatic and caudate lobes, are small and empty into the IVC between the main hepatic and renal veins. They function as collaterals in hepatic vein occlusion and occlusion of the hepatic portion of the IVC. Diagnostic imaging of the hepatic veins may be obtained by spiral CT [23], US [24], or MRA [25].

Arterial disease

Arteriography remains the gold standard in the diagnosis of visceral arterial disease. It can provide a specific diagnosis, and the necessary information on the vascular anatomy and arterial hemodynamics before endovascular intervention or surgery.

Acute mesenteric ischemia

Early angiography in patients with suspected acute mesenteric ischemia permits early differentiation between occlusive (thrombotic or embolic) and nonocclusive types that allows appropriate treatment [26] (Fig. 135.5). In clinical practice, contrast-enhanced CT is frequently performed in patients with acute abdomen. It can demonstrate the signs of acute mesenteric ischemia, mesenteric arterial or venous thrombosis, intramural gas, portal vein gas, and focal lack of bowel-wall enhancement [27]. Angiography is superior to CT for identifying

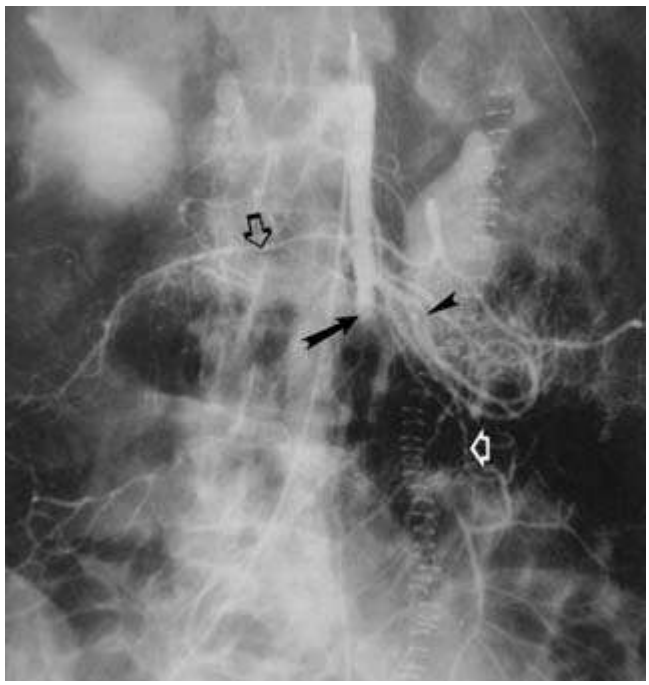


Figure 135.5 Superior mesenteric artery embolus. Superior mesenteric angiogram in an elderly woman with atrial fibrillation and abdominal pain. There is an embolus in the superior mesenteric artery (black arrow) distal to the second jejunal artery. A small embolus is seen in the jejunal artery (arrowhead). The distal mesenteric branches reconstitute through collaterals from the middle colic (open black arrow) and jejunal arteries (open white arrow).

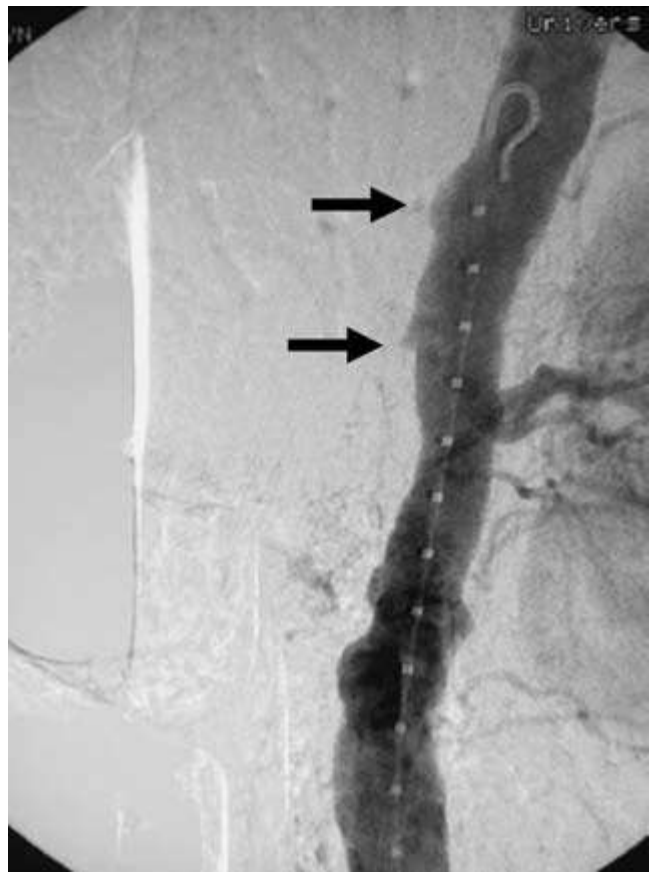


Figure 135.6 Celiac and superior mesenteric artery occlusion. Lateral aortogram in a 66-year-old woman with abdominal pain and 9-kg weight loss. Both the celiac and the superior mesenteric arteries are occluded at the origins (arrows). There is poor collateral circulation into the mesenteric arteries. The inferior mesenteric artery is also occluded. Computed tomography showed ileus, pneumatosis intestinalis, and gas in the portal. At surgery diffuse bowel infarction was found.

mesenteric artery branch occlusions or mesenteric artery spasm in patients with nonocclusive mesenteric ischemia.

After percutaneous catheterization of the femoral artery, a lateral aortogram is performed to demonstrate the origin of the celiac and superior mesenteric arteries. Occlusion of the celiac and superior mesenteric arteries with poor collateral circulation indicates acute occlusive mesenteric ischemia, and urgent surgery should be performed (Fig. 135.6). When colonic ischemia is suspected, an inferior mesenteric arteriogram is performed. When the celiac, superior and inferior mesenteric arteries are occluded, the collateral blood supply to these arteries comes primarily from the pelvic branches of the internal iliac arteries through the inferior and middle hemorrhoidal/superior hemorrhoidal arterial anastomoses. The collaterals to the superior hemorrhoidal artery and branches of the superior mesenteric artery can be demonstrated by injecting contrast medium into the distal abdominal aorta or internal iliac arteries.

Acute mesenteric ischemia may be occlusive or nonocclusive in type. Occlusive lesions include embolism, atherosclerotic plaques, aortic dissection, neoplasms, and vasculitis. Arteriography can accurately identify the level of occlusion and evaluate collateral circulation. In superior mesenteric artery embolism, the occlusion may be proximal or distal to the middle colic artery. Arteriosclerosis usually involves the origin of the superior mesenteric artery, allowing collateral development through the left colic and middle colic anastomosis, and through the posterior and anterior pancreaticoduodenal arcades and the gastroduodenal artery. Inferior mesenteric artery stenosis or occlusion causes collaterals to develop through the middle colic/left colic anastomosis. If both superior mesenteric and inferior mesenteric arteries are occluded, collaterals develop from the middle and inferior hemorrhoidal arteries of the internal iliac arteries.

Nonocclusive mesenteric ischemia is caused by severe and prolonged intestinal vasoconstriction from various causes, including systemic shock usually secondary to reduced cardiac output, cocaine ingestion, ergot poisoning, digoxin toxicity, and use of α-adrenergic agonists. The typical angiographic findings include diffuse mesenteric arterial constriction, slowing of mesenteric arterial flow, and decreased intestinal mucosal staining. When mesenteric vasospasm is identified, intraarterial infusion of papaverine is used to relieve mesenteric vasoconstriction. In patients with occlusive mesenteric ischemia and positive peritoneal signs, the infusion of papaver-

ine may be continued before, during and even after surgical intervention.

Colonic ischemia

Ischemic colitis is the most common form of mesenteric ischemia, frequently affecting the elderly. In most patients, colonic ischemia is transient and resolves without sequelae. Only some patients develop bowel necrosis or persistent ischemia that results in a stricture. The common causes of colonic ischemia are ligation of the inferior mesenteric artery during surgery [28] and low flow after cardiopulmonary bypass, myocardial ischemia, or sepsis. The other etiological factors include idiopathic, shock, colonic obstruction, digitalis, cocaine abuse, mesenteric artery thrombosis or embolism, vasculitis, radiation injury, and hematological disorders.

CT is a useful tool for the diagnosis of ischemic colitis. The CT findings for colonic ischemia include mural thickening, lumenal narrowing, polypoid defect, decreased mucosal perfusion, pneumatosis, and pneumoperitoneum. Colonoscopy is the most sensitive examination for colonic ischemia and can demonstrate the mucosal changes. Biopsies can be obtained if necessary. The angiographic findings may be nonspecific. In the early stage, the mesenteric arteries are constricted and blood flow is slowed with decreased parenchymal vascularity. In the late stage, the colon may appear hypervascular with prominent intramural arteries and increased accumulation of contrast material in the wall of the bowel. Treatment depends on the severity of colonic ischemia and includes medical therapy and surgical intervention.

Intestinal angina

Intestinal angina, also called chronic mesenteric ischemia, is characterized clinically by a combination of postprandial abdominal pain and weight loss. At least two of the three splanchnic arteries (celiac artery, superior and inferior mesenteric arteries) usually have significant occlusive disease before the syndrome of intestinal angina occurs [29]. Duplex US with spectral analysis is used for screening patients thought to have chronic mesenteric artery occlusive disease. Gadolinium-enhanced three-dimensional MRA is sensitive in assessing patency of the origins of the celiac and superior mesenteric arteries, but its resolution is inadequate for evaluation of mesenteric artery branch stenosis. Angiography provides information regarding the hemodynamic significance of mesenteric artery stenoses and the circulation distal to the stenosis necessary for endolumenal intervention (usually angioplasty and stent placement) [30] or surgical revascularization. Demonstration of collateral circulation distal to a superior mesenteric artery stenosis indicates that the stenosis is hemodynamically significant.

Celiac axis compression

Celiac axis compression syndrome (median arcuate compression syndrome) is controversial as a cause of abdominal pain. The celiac axis may be narrowed or, in severe cases, occluded by the median arcuate ligament of the diaphragm. Surgical correction of the lesion by decompression or reconstruction of the vessel usually ameliorates the pain. Because balloon dilation is not helpful in eliminating the compression, stent placement is required [31]. Celiac axis stenosis is frequently an incidental finding, with a reported prevalence of 12%–49% [32,33]. A lateral aortogram demonstrates a concave impression on the cranial aspect of the celiac axis. The compression is usually accentuated during deep expiration. A superior mesenteric angiogram will demonstrate collateral circulation to the hepatic and splenic arteries through the gastroduodenal and pancreatic arcade arteries from the superior mesenteric artery.

Superior mesenteric artery syndrome

The superior mesenteric artery can compress the third portion of the duodenum, causing duodenal obstruction. Such compression is most often found in patients with chronic immobilization with weight loss and a body cast [34,35]. The upper GI barium examination shows a characteristic oblique indentation toward the right lower quadrant on the third portion of the duodenum, corresponding to the course of the superior mesenteric artery. A narrow aortomesenteric angle corresponding to the site of obstruction is usually found on a lateral aortogram.

Vasculitis

The causes of mesenteric vasculitis include Wegener granulomatosis, systemic lupus, polyarteritis nodosa, Henoch–Schönlein purpura, Buerger disease, and giant cell arteritis. Mesenteric vasculitis may cause acute or chronic mesenteric ischemia. The angiographic findings vary with the type of vasculitis. In polyarteritis nodosa, celiac and superior mesenteric angiograms demonstrate occluded small- and medium-sized arteries and microaneurysms (Fig. 135.7). In ergot or digitalis toxicity, the mesenteric artery branches are narrowed or occluded with collaterals. In lupus mesenteric vasculitis, angiography demonstrates diffusely decreased small bowel vascularity. Angiography is the most sensitive method of visualizing microaneurysms. The angiographic examination should include the renal, celiac, and mesenteric arteries.

Splanchnic artery aneurysms

Splanchnic artery aneurysms and pseudoaneurysms are rare and often found incidentally during angiographic studies for other indications. Angiography is usually necessary to identify the exact site of the aneurysm and its relationship to adjacent vascular structures, as well as to plan surgical resection or embolization to prevent catastrophic hemorrhage [36]. Aneurysms occur in virtually all splanchnic arteries.

Splenic artery aneurysms account for 60% of all visceral artery aneurysms [37]. They occur four times more frequently in women than in men and are multiple in 20% of patients.

Figure 135.7 Multiple microaneurysms in polyarteritis nodosa. Arterial phase of a celiac angiogram shows numerous aneurysms involving the hepatic and pancreatic arteries.

Causes include atherosclerosis, medial fibrodysplasia, multiple pregnancies, pancreatitis, portal hypertension, polyarteritis nodosa, Ehlers–Danlos syndrome, and trauma. In portal hypertension, aneurysms tend to occur at the bifurcation of the intrasplenic branches of the splenic artery. Most atherosclerotic splenic artery aneurysms are asymptomatic, and calcified aneurysms less than 2 cm in diameter require no treatment. On the other hand, aneurysms occurring in pregnant women and those associated with pancreatitis require treatment because of their propensity to bleed. Splenic artery aneurysms can be treated with embolization by the transcatheter approach or percutaneous puncture with thrombin injection.

The hepatic artery is the second most common site for splanchnic aneurysms, accounting for 20% of all splanchnic aneurysms [37]. Most hepatic pseudoaneurysms are traumatic in origin and are secondary to blunt or penetrating abdominal trauma, liver biopsy, liver surgery, biliary stent placement, pancreatitis, or placement of a hepatic arterial infusion catheter. Most spontaneous aneurysms occur in the common hepatic or right hepatic artery. Clinical presentations include right upper quadrant pain, hemobilia, GI bleeding, or obstructive jaundice. Rarely hepatic artery aneurysms rupture into the peritoneal cavity, portal vein, or pancreatic pseudocyst. The diagnosis can be made by US, contrast-enhanced CT, MRA, or arteriography. Treatment includes embolization by transcatheter approach or percutaneous puncture with thrombin injection.

Mesenteric aneurysms are arteriosclerotic and may cause GI bleeding or mesenteric ischemia. Celiac axis aneurysms account for 4% of all visceral aneurysms and should be differentiated from pseudocysts when encountered during US or unenhanced CT scanning. A lateral aortogram is necessary to confirm the diagnosis of aneurysms arising from the origins of the celiac and superior mesenteric arteries.

Gastroduodenal and pancreatic arterial aneurysms are associated with pancreatitis and pseudocysts. They may cause GI and intraperitoneal hemorrhage. Aneurysms rarely rupture into the pancreatic duct or a pseudocyst. Contrast-enhanced CT usually differentiates pseudocysts and aneurysms. Angiography is required to delineate the vascular anatomy and the exact origin of the aneurysm before surgical treatment or transcatheter embolotherapy.

Abdominal trauma

Abdominal trauma can be due to penetrating or blunt injuries. The patient with signs of intraabdominal injuries should undergo exploratory laparotomy. Most traumatized patients require radiological studies to assess the extent of the injury. Contrast-enhanced CT should be the first imaging modality for the evaluation of abdominal trauma. Angiography is indicated when CT shows a high-grade injury of solid organs, contrast extravasation, pseudoaneurysm, or vascular injuries.

Venous disease

Occlusion of the portal, mesenteric, splenic, and hepatic veins may be asymptomatic or associated with ascites, hepatic failure, or GI hemorrhage. Contrast-enhanced CT and MRV can diagnose occlusion of the portal and splenic vein by demonstrating intraabdominal varices and aneurysms in the portal venous system [21]. Duplex scanning is useful in the assessment of patency and direction of blood flow of the portal vein and portosystemic shunts [38]. The portal venous phases of the celiac, superior mesenteric, and splenic angiograms are used to evaluate the patency of the splenic, mesenteric, and portal veins. An inferior vena cavogram and hepatic venogram are performed in patients with suspected Budd–Chiari syndrome.

Portal vein occlusion

The pattern of portal vein occlusion usually varies with its etiology [39]. Idiopathic intrahepatic portal vein occlusion (hepatoportal sclerosis) usually occurs in children. Occlusion of the main portal vein is the most common type and is probably of congenital etiology. Thrombosis of the main and superior mesenteric veins may be caused by intraabdominal sepsis. Extensive thrombosis of the portal venous system commonly occurs in patients with hypercoagulable states.

Angiographic findings include large collateral veins originating from the superior mesenteric vein, ascending in the hepatoduodenal ligament in the venous phase of the superior mesenteric angiogram (Fig. 135.8). The collateral vessels reconstitute the patent intrahepatic portal vein. If the intrahepatic portal venous branches are occluded, the collateral

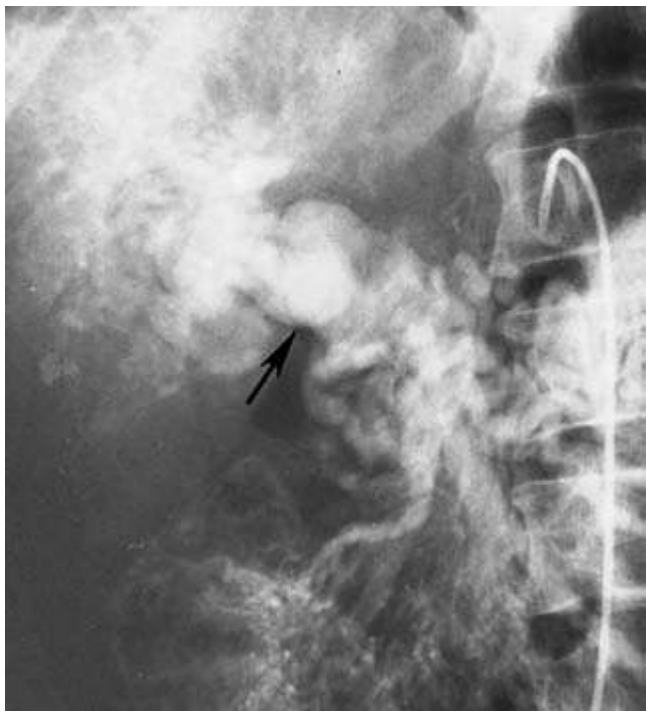


Figure 135.8 Portal vein occlusion. Portal venous phase of a superior mesenteric angiogram (oblique view) demonstrates tortuous venous collaterals (arrow) running along the hepatoduodenal ligament adjacent to the occluded portal vein. The collateral veins reconstitute intrahepatic portal venous branches. From Reuter SR, Redman HC, Cho KJ. Gastroenterology Angiography, 3rd edn. Philadelphia: WB Saunders, 1986:121.

vessels continue to run along the intrahepatic bile ducts, giving the appearance of railroad tracks [40]. In mesenteric vein thrombosis, numerous tiny collateral veins are demonstrated throughout the mesentery without visualization of the superior mesenteric vein in the venous phase of the superior mesenteric angiogram.

The angiographic abnormality of portal vein occlusion due to neoplasms varies with the type of neoplasm. Pancreatic and biliary cancers cause localized narrowing or occlusion of the portal vein without associated tumor vessels. In contrast, portal vein invasion by hepatocellular carcinoma has a characteristic appearance, with abnormal vascular channels coursing within the portal vein branches in the vicinity of the tumors. Arteriovenous shunting is often associated with portal vein invasion by tumors [41].

Splenic vein occlusion

Splenic vein occlusion is usually clinically silent but may cause hypersplenism or gastric variceal bleeding [42,43]. The diagnosis can be made by dynamic CT, duplex US, and three-dimensional contrast MRV. Angiography should be performed when noninvasive studies are inconclusive or when additional vascular information is needed. A celiac or splenic arteriogram is performed for the diagnosis; the angiographic findings include splenoportal collaterals through the short gastric/coronary and gastroepiploic veins, as well as non-opacification of the splenic vein during the venous phase. The treatment of choice for bleeding gastric varices associated with splenic vein occlusion is splenectomy. If surgical intervention is contraindicated, partial splenic artery embolization by transcatheter approach is an effective alternative treatment.

Budd–Chiari syndrome

In Budd–Chiari syndrome, a significant portion of the hepatic venous system is obstructed. CT is usually used as the initial test and may reveal nonuniform contrast enhancement of the liver parenchyma and enlarged caudate lobe. Increased central venous pressure from right heart failure may produce a contrast enhancement pattern similar to that of Budd–Chiari syndrome. MRI is useful in identifying the underlying lesions, such as hepatic and vena caval thrombi, and congenital membrane.

Angiography is the most important procedure for the diagnosis of Budd–Chiari syndrome. Superior mesenteric and celiac angiograms are obtained to vizualize the portal vein and exclude hepatic neoplasms. An inferior vena cavogram is obtained to exclude occlusion of, or membrane in, the hepatic portion of the IVC. Demonstration of hepatic vein patency excludes the diagnosis of Budd–Chiari syndrome. When the hepatic vein is occluded, the catheter is wedged into the occluded hepatic vein, and contrast medium is injected to visualize the collateral channels (Fig. 135.9). If the right hepatic vein cannot be entered, catheterization of the accessory hepatic vein should be performed. If any of the hepatic veins cannot be catheterized, a 22G needle is inserted into the liver parenchyma using the same technique used for percutaneous transhepatic portal vein catheterization. Injection of iodinated contrast medium or CO_2 usually demonstrates hepatic vein occlusion and collateral veins [44].

Percutaneous translumenal angioplasty and stent placement has been used to treat Budd–Chiari syndrome caused by obstruction of the hepatic vein and hepatic segment of the IVC [45,46]. Restenosis or reocclusion occurs frequently at the angioplasty site. Patency of the vein may be improved with use of metallic stents after unsuccessful angioplasty. The transjugular or transhepatic approach is used for angioplasty and stent placement for right hepatic vein occlusion causing Budd–Chiari syndrome [47,48]. The TIPS procedure is used to treat intractable ascites and bleeding varices from hepatic venous occlusive disease and as an effective bridge to transplantation for hepatic failure associated with Budd–Chiari syndrome [49].

Gastrointestinal disease

When evaluating GI disease, angiography is indicated for the diagnosis of vascular disease, visualization of vascular anatomy,

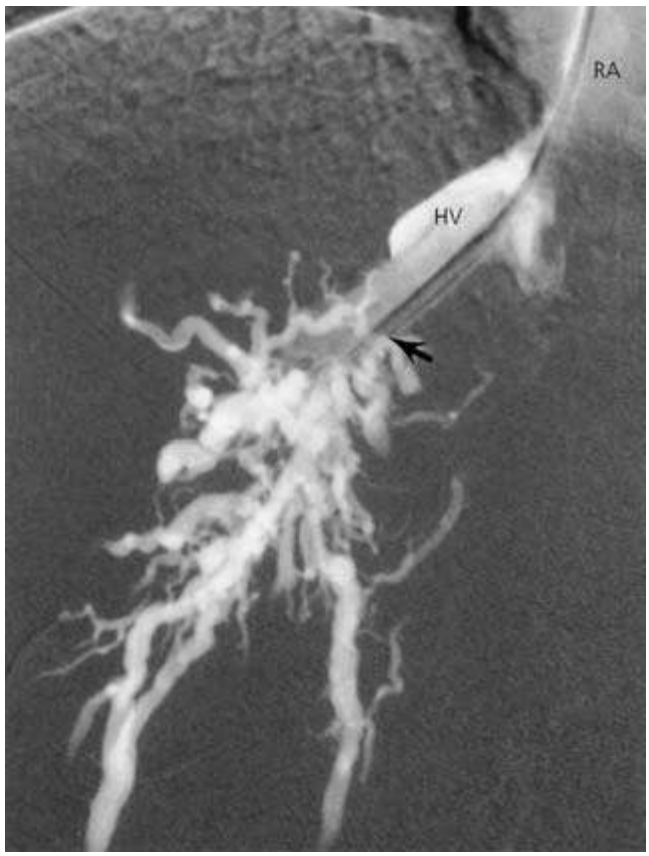


Figure 135.9 Budd–Chiari syndrome. Digital subtraction hepatic venogram was performed with contrast injection into the right hepatic vein (HV), demonstrating numerous collateral veins pathognomonic of hepatic vein occlusion. The arrow indicates the level of hepatic vein occlusion. RA, right atrium.

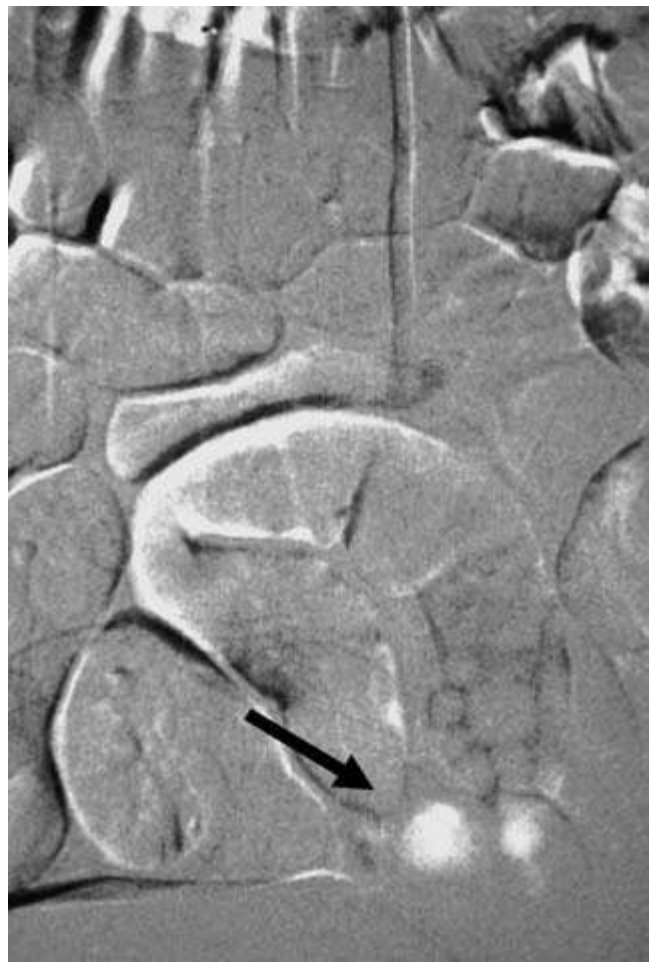


Figure 135.10 Active small bowel bleeding. A superior mesenteric angiogram with CO_2 demonstrates extravasation of the gas into the ileum (arrow). The bleeding was controlled with superselective embolization of the bleeding branch from the superior mesenteric artery.

and diagnosis and treatment of GI bleeding. If active GI bleeding is diagnosed, angiography should be urgently performed for localization of the bleeding site as well as control of the bleeding. The rate of bleeding should be at least 0.5 mL/min to be detected by angiography. Angiography is insensitive in detecting capillary or venous bleeding because of the dilution of the contrast material. Helical CT angiography with intraarterial or intravenous injection of contrast medium is useful in detecting active GI bleeding as well as GI bleeding of obscure origin, and facilitates angiographic localization of the bleeding [50,51]. CO_2 is a sensitive contrast agent in detecting active bleeding that may not be seen with contrast medium (Fig. 135.10). Once the bleeding site has been identified by CO_2, contrast medium is injected for a vascular "roadmap" before superselective catheterization of the bleeding artery for embolotherapy. Angiography is useful in identifying vascular malformations and vascular neoplasms. A catheter may be placed in the mesenteric branch supplying a vascular malformation preoperatively to facilitate intraoperative localization of the lesion.

Upper gastrointestinal bleeding

Endoscopy is the primary procedure for the diagnosis and treatment of upper GI bleeding. If endoscopy has failed to localize or control the bleeding, celiac and superior mesenteric angiograms are obtained with CO_2 and contrast medium. Because of its lower viscosity, CO_2 is a useful contrast agent in detecting GI bleeding (see Fig. 135.10). If a bleeding site is identified, the bleeding artery is selectively catheterized to control the bleeding by embolization [52]. If a bleeding site has not been demonstrated, left gastric and gastroduodenal arteriograms are obtained. If the source of bleeding has still not been identified and nasogastric aspiration reveals dark blood, a catheter may be placed in the left gastric artery for the next 6–12 h. The bleeding may be precipitated by intraarterial administration of heparin, vasodilators, or thrombolytic agents, allowing accurate localization of the bleeding site and prompt treatment [53,54]. Prophylactic embolization (left gastric artery embolization for gastric bleeding and

gastroduodenal artery embolization for duodenal bleeding) is an acceptable alternative when the massive bleeding has ceased at the time of angiography [55].

Angiography is usually not helpful in determining the cause of upper GI hemorrhage. Hemorrhagic gastritis is the most common cause of capillary bleeding and imaging may demonstrate a hypervascular stomach with dilated gastric arteries and increased parenchymal staining. Peptic ulceration is the most common cause of arterial bleeding and usually produces no angiographic abnormality. Gastric bleeding usually arises from the left gastric artery and occasionally from the short gastric, right gastric, or gastroepiploic artery. Duodenal bleeding may originate from the celiac or superior mesenteric artery or from both arteries. Angiographically the arterial bleeding appears as a localized accumulation of contrast material extravasation during the arterial phase of the angiogram. The escaped contrast material persists throughout the venous phase and may outline the mucosa. Selective arterial embolization is an effective means of controlling bleeding from peptic ulceration.

Upper GI endoscopy should be obtained in all patients suspected of bleeding from the biliary tract (hemobilia) and pancreatic duct (hemosuccus pancreaticus). Once bleeding from the ampulla of Vater is documented, angiography is performed to demonstrate the underlying lesion and rarely contrast extravasation into the bile duct. Hemobilia can be treated by embolizing the feeding artery. Bleeding into the pancreatic duct is amenable to selective arterial embolization of the associated arterial lesions. Aortoenteric fistulae rarely cause GI bleeding, and the duodenum is the most common site of fistulae. Biplane aortography may show a false aneurysm at the aortic anastomotic site.

Percutaneous transhepatic coronary vein embolization may be used to treat variceal bleeding when endoscopy is not available or fails to control the bleeding. Variceal embolization may be necessary if the varices continue to fill after creation of TIPS. TIPS is an effective method of controlling variceal bleeding and is currently indicated when the bleeding is unresponsive to endoscopic therapy [56,57]. A transfemoral retrograde approach may be used to obliterate gastric varices. The gastrorenal or splenorenal shunt is cannulated through the renal vein and then occluded with an occlusion balloon catheter before injecting sclerosing agents or glue into the gastric varices.

Lower gastrointestinal bleeding

Once lower GI bleeding is diagnosed, a superior mesenteric angiogram is performed to detect the bleeding from the small intestine and the right side of the colon. If no bleeding site is seen, a celiac arteriogram is obtained because massive gastroduodenal bleeding may present as lower GI bleeding. If a radionuclide scan has localized the bleeding to the left side of colon, inferior mesenteric arteriograms are obtained with coverage from the rectum to the splenic flexure of the colon. Active bleeding shows a localized accumulation of escaped contrast material during the arterial phase of the mesenteric angiogram, which usually persists through the venous phase (Fig. 135.11). CO_2 should be used as a contrast agent for the superior and inferior mesenteric angiograms if contrast angiograms have failed to demonstrate the bleeding site.

Figure 135.11 Superior mesenteric angiogram in an elderly man who developed massive colonic bleeding. Extravasation of contrast medium is seen from the hepatic flexure of the colon (arrow). The bleeding was controlled with embolization of the bleeding branch from the right colic artery (arrowheads).

Selective infusion of vasopressin is usually effective in controlling the bleeding in up to 80% of patients, but the recurrence rate is as high as 50% [58]. Patients with coronary artery disease should not be treated with vasopressin because of the risk for myocardial infarction. Selective embolization is an acceptable alternative when vasopressin is contraindicated. Superselective catheterization and embolization of the bleeding artery can be achieved with use of the 3Fr coaxial catheter system with minimal risk for intestinal necrosis [59–61].

Angiodysplasia

Angiodysplasia may occur in a variety of pathological types: vascular ectasia, arteriovenous malformations, and capillary telangiectasia [62]. Clinical presentation and angiography allow identification of each type of angiodysplasia.

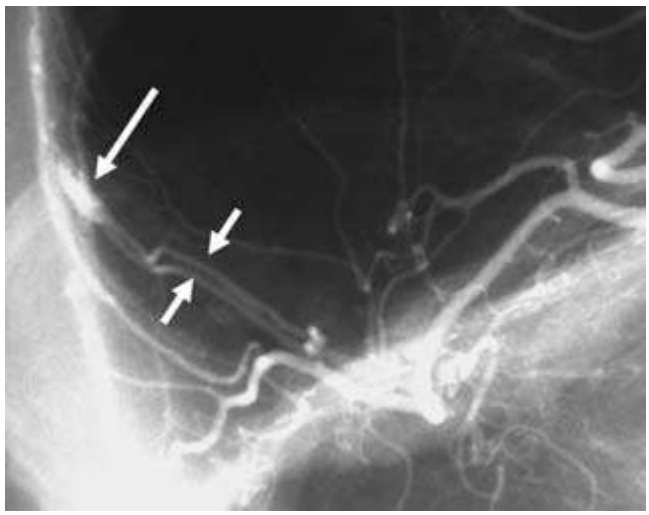


Figure 135.12 Vascular ectasia of the cecum. Superior mesenteric angiogram in a 60-year-old woman with recurrent lower gastrointestinal bleeding. A 1-cm mucosal vascular ectasia is seen on the antimesenteric surface of the cecum (longer arrow). The feeding artery and vein (shorter arrows) are opacified during the arterial phase.

Vascular ectasia usually occurs in the cecum and ascending colon. Most patients are older than 60 years of age. The angiographic findings include a small vascular cluster and blush in the wall of the colon and early dense opacification of the draining vein (Fig. 135.12) [63]. Normal mesenteric angiography is usually accepted for exclusion of vascular ectasia, although data supporting this assumption are not available. Two or more vascular ectasias are frequently present at microscopic examination of the injected specimen, although most patients demonstrate a single vascular lesion at angiography. Endoscopy is superior to angiography in identifying vascular ectasia. If active extravasation is demonstrated, selective arterial embolization is an effective treatment. Arteriovenous malformations are of developmental origin and usually affect people younger than 50 years of age. The angiographic findings include tortuous dilated arteries with early prominent veins. The lesions are frequently found in the small intestine and vary in size. Vascular malformations should be differentiated from vascular tumors, such as leiomyomas.

Capillary telangiectasia may occur in any part of the GI tract and may be associated with hereditary hemorrhagic telangiectasia (Osler–Weber–Rendu disease). The angiographic findings usually include tiny areas of blush without arteriovenous shunting. Hepatic angiography may demonstrate dilated hepatic arteries and increased hepatic parenchymal staining [64,65].

Meckel diverticulum

Angiography is obtained only when active bleeding occurs. Angiographically, a Meckel diverticulum appears as a short segment of bowel with increased contrast accumulation supplied by an abnormal branch (a remnant of the omphalomesenteric artery) from the superior mesenteric artery [66]. Superselective catheterization of the distal ileal branches of the superior mesenteric artery may be necessary to identify the abnormal artery. Superselective arterial embolization may be performed to control life-threatening GI bleeding from a Meckel diverticulum before surgical resection [67].

Rectal trauma

Inferior mesenteric angiography is performed for localization and treatment of the bleeding from rectal trama. Infusion of vasopressin into the inferior mesenteric artery is effective in controlling the bleeding. If the patient has coronary artery disease or if vasopressin fails, selective embolization is performed with Gelfoam, Ivalon particles, or microcoils [68].

Intestinal varices

Intestinal varices represent varices at an unusual site in patients with cirrhosis and portal hypertension. Contrast-enhanced CT and MRV are used to diagnose intestinal varices. Angiography provides the necessary information about the location and extent of the varices, and the status of the portal venous system. The varices are demonstrated in the portal venous phase of a superior mesenteric angiogram. Percutaneous transhepatic or transjugular portal vein catheterization may be necessary for evaluation of portal hemodynamics and embolization. TIPS is effective in treating stomal or rectal varices unresponsive to conservative treatment [69].

Neoplasms of the small intestine

Leiomyomas

Leiomyomas (GI stromal tumor) is the most common tumor of small bowel. CT is the primary imaging modality for the detection of GI stromal tumors [70]. Angiography is useful in diagnosing small bowel leiomyomas. The tumors are usually hypervascular with abundant tumor vessels and dense blush (Fig. 135.13). The draining vein usually is densely opacified. Angiographic differentiation between benign and malignant tumors is not possible unless venous invasion or metastasis is demonstrated [71]. When active bleeding occurs from the tumor, a radionuclide bleeding scan is performed to localize the bleeding site. Angiography is then performed to localize and control the bleeding. Once the bleeding has been controlled by selective embolization, the tumor should be resected.

Carcinoid tumor

Carcinoid tumors arise from enterochromaffin cells that are present in the GI tract, lung, ovary, pancreas, and other organs. They produce various hormones, including serotonin, histamine, dopamine, and tachykinins. Angiography may be used for diagnosis and staging of carcinoid tumors. The angiographic findings include retraction, kinking, and occlusion

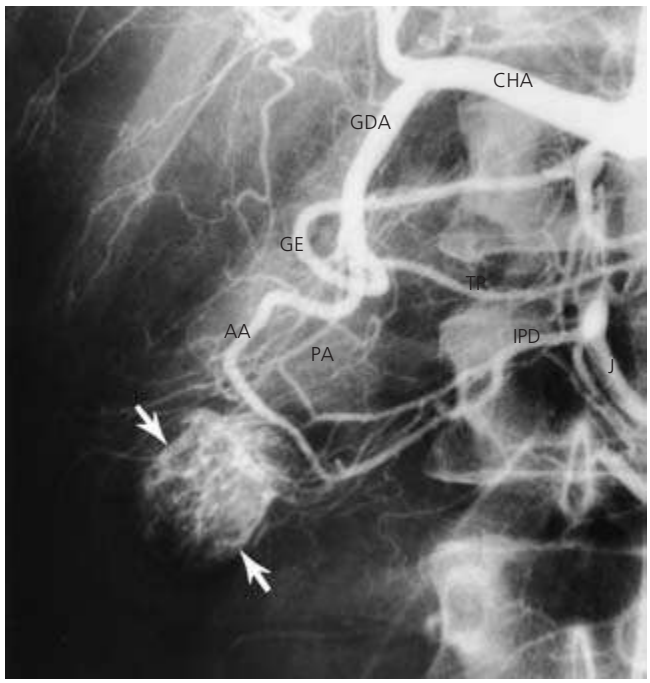


Figure 135.13 Duodenal leiomyoma. Late arterial phase of a gastroduodenal arteriogram in a young woman with upper gastrointestinal hemorrhage. A vascular mass (arrows) in the second portion of the duodenum receives blood supply from the dilated anterior arcade artery (AA). CHA, common hepatic artery; GDA, gastroduodenal artery; GE, gastroepiploic; IPD, inferior pancreaticoduodenal; J, jejunal; PA, posterior arcade artery; TP, transverse pancreatic. Surgical removal of the mass confirmed the diagnosis.

of the mesenteric arteries. Carcinoid tumors metastatic to the liver are usually hypervascular with abundant tumor vessels. Venous sampling with hormone assay may be necessary for localizing occult carcinoid tumors, especially those occurring in the ovary and lungs. When the patient with liver involvement has severe carcinoid syndrome, hepatic artery embolization is an acceptable palliative treatment [72,73]. Carcinoid crisis is a potentially life-threatening condition that can occur spontaneously or at the time of hepatic artery embolization. The clinical manifestations of carcinoid crisis vary depending on the hormones released from the tumors and include sudden flushing, sweating, hypotension, hypertension, and even cardiac arrest. Treatment should include intravenous administration of Sandostatin [72].

Other tumors

Hemangiomas of the small bowel are an uncommon cause of GI bleeding. Angiography may be used to identify small hemangiomas. The angiographic findings include small areas of abnormal vessels with contrast accumulation in the capillary phase, with or without early draining veins. Angiography is not useful in diagnosing adenomas, lipomas, carcinomas, or Kaposi sarcoma.

Pancreatic disease

Pancreatic tumors have been extensively studied by angiography, both to identify the actual tumor by its angiographic features and to assess the extent of the lesion before surgery. The newer imaging modalities are used to both diagnose and stage the lesion [74]. Angiography is performed to obtain a specific diagnosis, to determine the extent of the tumor, and to demonstrate vascular anatomy. In patients with pancreatitis, pseudocysts or vascular lesions, angiography is necessary when surgery or endovascular therapy is contemplated.

Adenocarcinoma

Current imaging techniques, including helical CT, multidetector CT, MRI, endoscopic ultrasound (EUS), and positron emission tomography are important in early diagnosis and staging of pancreatic cancer [75,76]. Angiography is performed to evaluate vascular anatomy and involvement of any major peripancreatic artery (splenic, hepatic, or superior mesenteric artery) or vein (superior mesenteric, splenic, or portal vein). When any major peripancreatic artery or vein is involved, the tumor may be unresectable (Fig. 135.14). Surgical resection offers the only potential cure if pancreatic cancer is confined within the pancreas.

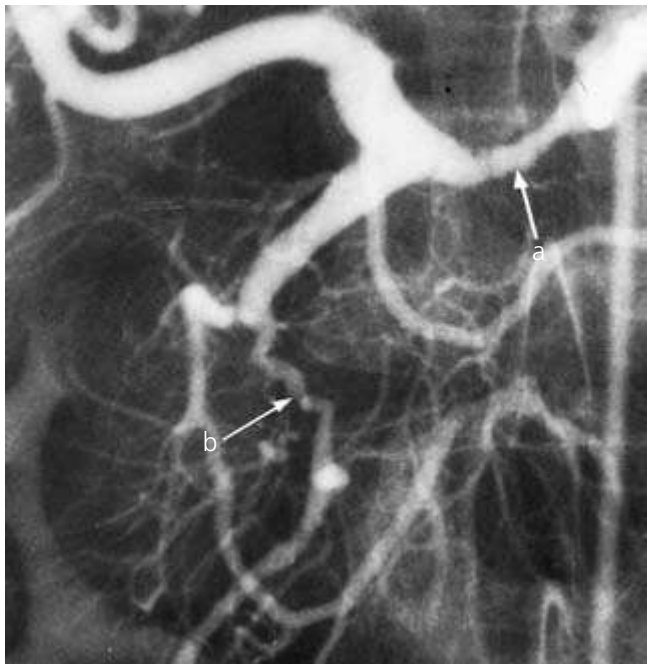
Cystic tumors

Cystic lesions may be found on imaging studies of patients without symptoms. A wide variety of cystic lesions occurs in the pancreas including cystic neoplasms and pseudocysts. CT, US, and MRI should be used to diagnose cystic lesions of the pancreas [77,78].

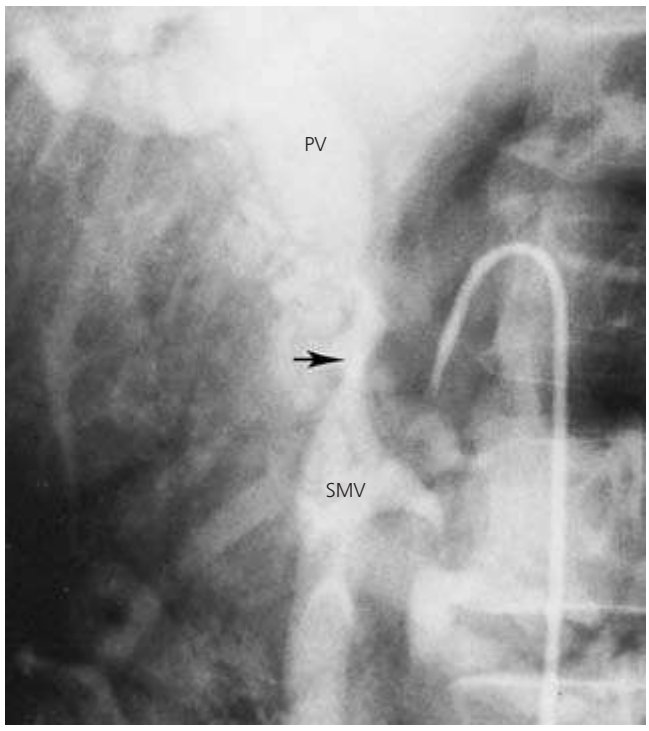
Angiography is reserved for determining the extent of the tumor and vascular anatomy before surgical resection. Angiographically, microcystic adenomas are usually hypervascular with abundant tumor vessels and blush, whereas mucinous cystic tumors are hypovascular with sparse tumor vessels mimicking a pseudocyst.

Pancreatic endocrine tumors

CT, MRI, and EUS have all been used to demonstrate endocrine tumors of the pancreas with varying success. Angiography is infrequently used for localization of islet cell adenomas. Angiographic technique in the examination of the pancreas requires meticulous catheterization of the superior mesenteric artery and celiac artery, and its branches. Angiographically, islet cell tumors appear as a localized area of contrast material accumulation (tumor blush) with or without tumor vessels (Fig. 135.15). Malignant islet cell tumors may grow into the portal vein. Most hepatic metastases from islet cell carcinomas are hypervascular and are readily detected by hepatic angiography. Angiography is sensitive in localizing insulinomas (accuracy 60%–90%) but is less sensitive in detecting gastrin-producing tumors. Other endocrine



(a)



(b)

Figure 135.14 Unresectable pancreatic cancer. **(a)** Hepatic arteriogram (magnification technique). The common hepatic (a) and proximal gastroepiploic (b) arteries are encased by a tumor in the head of the pancreas. **(b)** Portal venous phase of a superior mesenteric angiogram (oblique view). The junction of the superior mesenteric and portal veins is invaded by the tumor (arrow). PV, portal vein; SMV, superior mesenteric vein. From Shields JJ, Porter DJ, Brady TM, Cho KJ. Angiography of pancreatic disease. In: Dent TL (ed.) Pancreatic Disease. New York: Grune & Stratton, 1981:93 with permission from Elsevier.

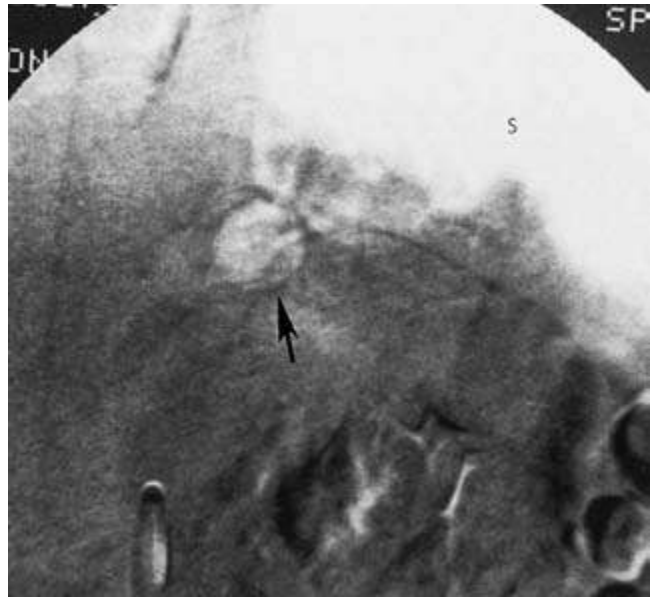


Figure 135.15 Islet cell adenoma in a patient with hyperinsulinism. Parenchymal phase of a splenic angiogram (digital subtraction technique) demonstrates a 1-cm-diameter tumor blush (arrow) in the distal body of the pancreas. S, splenic parenchymal staining.

tumors, such as vasoactive intestinal peptideproducing tumors (VIPoma), glucagonoma, and somatostatinoma, are usually hypervascular with abundant tumor vessels.

Percutaneous transhepatic venous sampling with hormone assay is a useful method for localizing occult islet cell tumors. This method may be used to localize the source of abnormal hormone secretion to the head, body, and tail of the pancreas, as well as to the liver. This is helpful in avoiding blind resection of the pancreas [79]. Simultaneous arterial and venous sampling from the splenic, superior mesenteric, and portal veins can detect localized elevation of hormones near the tumor site; adenomas of the body and tail of the pancreas produce increased hormone concentrations in the splenic vein, and those of the pancreatic head produce increased hormone concentrations in the superior mesenteric and portal veins. Simultaneous blood sampling from the hepatic and portal veins helps to determine the presence of hepatic metastases from gastrinomas [80].

Selective intraarterial injection of secretin with assay of hepatic venous gastrin has been useful in localizing gastrinomas and hepatic metastases [81,82]. It involves injection of 30 units of secretin into the splenic, gastroduodenal, proper hepatic, and superior mesenteric arteries, as well as blood sampling from the hepatic vein to measure the changes in gastrin concentration. An increase (> 50%) in gastrin concentration in the hepatic vein after secretin injection localizes the tumor to the part of the pancreas supplied by the artery injected: the gastroduodenal and superior mesenteric arteries for the head of the pancreas and the splenic artery for the

body or tail. The selective arterial secretin injection test is more sensitive than transhepatic portal venous sampling for localizing occult gastrinomas [83]. An increase in hepatic venous gastrin concentration of at least 25% at 20 s or 50% at 30 s after hepatic arterial injection of secretin indicates the presence of hepatic metastases [84].

In patients with suspected insulinomas, calcium gluconate is injected intraarterially and the level of insulin is measured in the hepatic vein for localization of insulinomas [85]. The patient is admitted the night before the procedure to stabilize the glucose level with intravenous administration of 10% dextrose. Blood samples are obtained from the hepatic vein before and at 30, 60, 90, and 120 s after the injection of calcium gluconate (Ca^{2+} 0.015 mEq/kg body weight) into the superior mesenteric, gastroduodenal, splenic and hepatic arteries, respectively. Calcium injection should be separated by 20–30 min to allow the insulin level to return to baseline. A 1.5–2.0-fold rise in insulin level in the hepatic vein at 30 and 60 s after intraarterial injection of calcium localizes the source of insulin to the region of the pancreas supplied by the artery stimulated.

Pancreatic arteriovenous malformations

These rare vascular lesions may bleed directly into the intestine, the pancreatic duct, or the bile duct [86–88]. Portal hypertension and varices associated with the malformation may be the cause of the bleeding. Angiography is important in the preoperative diagnosis and localization of the lesion (Fig. 135.16). The lesion may mimic chronic pancreatitis or a vascular tumor.

Vascular lesions associated with pancreatitis

Angiography has little diagnostic use in patients with pancreatitis because the diagnosis is well established clinically. When vascular complications occur, angiography is necessary for the diagnosis of vascular involvement and treatment. In about half of cases of pseudocyst, peripancreatic venous narrowing or occlusion is found. Bleeding associated with pancreatitis may be venous or arterial in origin. Venous bleeding usually results from gastric varices associated with splenic or portal vein occlusion. Venous bleeding rarely results from duodenal varices caused by superior mesenteric or portal vein occlusion. Arterial hemorrhage is a rare but life-threatening complication of pancreatitis, usually secondary to rupture of intrapancreatic or peripancreatic aneurysms. Arteriography is indicated for the diagnosis of arterial bleeding and pseudoaneurysm, and selective embolization should be used as a definitive or preoperative temporizing procedure [89–91].

Hepatic disease

Radionuclide scintigraphy, US, CT, and MRI have all been used for demonstration of liver masses [92–95]. Angiography still is important in evaluating liver tumors for the specific diagnosis, resectability of the tumor, and before therapeutic hepatic artery embolization.

Knowledge of the hepatic circulation is important in the performance and interpretation of hepatic angiograms and in planning for hepatic arterial chemotherapy. The liver receives a dual blood supply: about 75% of the total hepatic blood flow comes from the portal vein, and the remaining 25% comes from the hepatic artery. The relationship between the portal vein and hepatic artery is reciprocal: a decrease in portal blood flow results in an immediate compensatory increase in hepatic arterial flow. When portal blood flow is diminished in cirrhosis or obstructive jaundice, hepatic arterial embolization for bleeding or tumors must be undertaken cautiously because the liver in these conditions depends primarily on arterial blood.

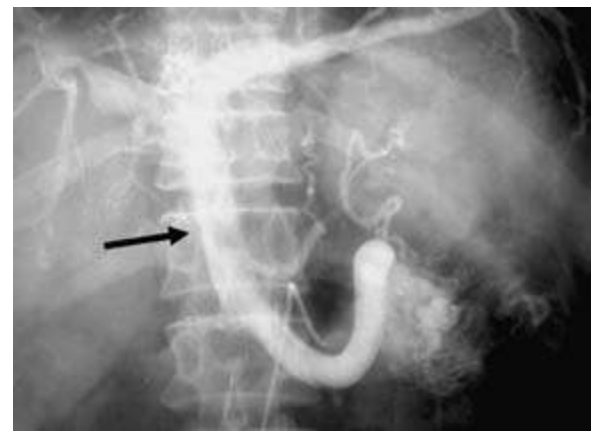
(a)  (b)

Figure 135.16 Pancreatic arteriovenous malformation (AVM). **(a)** Splenic angiogram, arterial phase, shows a tangle of vessels in the tail of the pancreas (longer arrow). Early draining splenic vein is beginning to opacify (shorter arrow). **(b)** Dense filling of the splenic and portal (arrow) veins indicates the presence of arteriovenous shunting through the AVM.

Cavernous hemangiomas

Cavernous hemangiomas, the most common benign hepatic tumors, are composed of large sinusoidal spaces with fibrosis and thrombosis. CT, MRI, and technetium-99m scintigraphy with the use of single photon emission computed tomography (SPECT) are useful for obtaining a specific diagnosis [96,97]. The lesions usually appear well defined and hypodense on unenhanced CT. When contrast medium is administered, the lesions initially show nodular peripheral enhancement, which becomes confluent gradually. On MRI, the lesions usually show bright signal intensity on T2-weighted images. The angiographic findings are characteristic, with normal feeding arteries and dense persistent pooling of contrast material through the venous phase of a hepatic angiogram.

Adenoma

Hepatic adenoma usually occurs in young women with a history of oral contraceptive use, and rarely in patients with glycogen storage disease [98,99]. Because of the risk of bleeding, hepatic adenoma must be resected. Hepatic adenomas are nonspecific at ultrasonography, and are usually evaluated by CT or MRI. Angiographically, hepatic adenomas are usually vascular, with abnormal vessels and tumor blush. The definitive diagnosis cannot be established on the basis of the angiographic abnormality. When an adenoma bleeds, angiography is necessary to evaluate the extent of bleeding and identify the tumor. If active contrast extravasation is identified, selective arterial embolization is an acceptable alternative treatment. When the patient becomes stable, resection should be performed. Adenomas associated with bleeding may become hypovascular or avascular, making their angiographic localization difficult.

Focal nodular hyperplasia

Focal nodular hyperplasia (FNH) is a benign liver tumor, often found incidentally on imaging studies performed for other indications. Asymptomatic FNH rarely bleeds and therefore no treatment is necessary. CT and MRI have been used to differentiate FNH from other hepatic tumors [100]. Because of the difficulty in differentiating from hepatic adenoma and fibrolamellar hepatocellular carcinoma, FNH is often resected. Angiographically, FNH is usually hypervascular with abnormal vessels and granular blush. Multiple septa radiating from the central area of scar is usually demonstrated.

Biliary cystadenoma

Biliary cystadenomas are benign multiloculated cystic tumors arising from the intrahepatic or extrahepatic bile duct. US and CT can demonstrate the lesions [101]. Angiography is performed to define hepatic arterial anatomy and determine the extent of the tumor before surgery. The angiographic findings are usually nonspecific and may demonstrate subtle neovascularity and rim-like staining in the wall of the tumor, mimicking a hepatic abscess, echinococcal cyst, or congenital hepatic cyst.

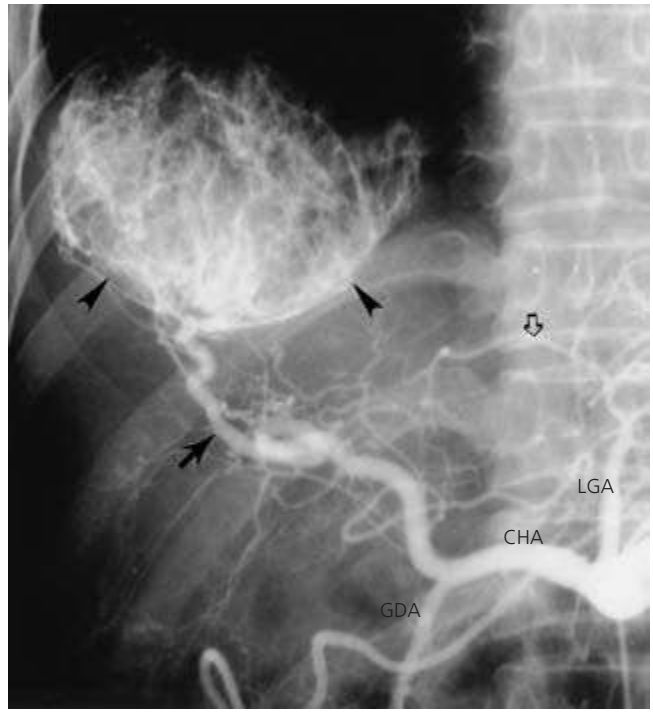


Figure 135.17 Hepatoma. Hepatic arteriogram shows a hypervascular mass (arrowheads) with tumor blush. Numerous tumor vessels supplied by a dilated right hepatic artery branch (arrow) are seen. The left hepatic artery (open arrow) arises from the left gastric artery (LGA). CHA, common hepatic artery; GDA, gastroduodenal artery.

Hepatocellular carcinoma

CT and MRI are the primary imaging methods used for the diagnosis of hepatocellular carcinoma (HCC) [102–104]. Angiography is necessary to define the extent of the tumor, blood supply, and portal venous anatomy. Most hepatomas are hypervascular angiographically, with coarse tumor vessels (Fig. 135.17). Involvement of the extrahepatic portal vein by HCC indicates that the tumor is unresectable. Angiography is also performed for a vascular "roadmap" before hepatic artery chemoembolization for HCC.

Cholangiocarcinoma

Angiography has a limited role in the diagnosis but may be performed to provide a vascular "roadmap" and evaluation of portal vein patency when surgical resection is contemplated. The angiographic findings include encasement of the hepatic arterial branches, which may be associated with encasement of the portal vein.

Figure 135.18 Hepatic carcinoid metastases. Celiac angiogram, lateral arterial and parenchymal phase, in a patient with carcinoid syndrome. Hypervascular metastases are present in the liver.

Metastatic hepatic neoplasms

Hepatic metastases from renal cell carcinomas, islet cell carcinomas, carcinoid tumors, and medullary thyroid carcinomas are hypervascular, and other metastases are less vascular. Angiography is sensitive in detecting hypervascular metastases (Fig. 135.18). Angiography is performed to determine resectability of hepatic metastases and the presence of aberrant hepatic arteries before the surgical placement of a hepatic arterial catheter for chemotherapy.

Panhepatic angiography

Increased resistance to the flow of portal vein blood is the principal cause of portal hypertension. The location of the blockage in portal blood flow may be intrahepatic or extrahepatic. Cirrhosis is the most common cause of intrahepatic sinusoidal block. Hepatic vein (postsinusoidal) and portal vein (presinusoidal) occlusion produce an extrahepatic block to portal vein flow. An increase in portal vein blood flow secondary to splenomegaly or arterioportal fistulae rarely causes portal hypertension (hyperkinetic) [105]. Transcatheter embolization may be used to occlude the arterioportal fistula and lower portal pressure.

The term *panhepatic angiography* is used for the angiographic study performed for the evaluation of portal hypertension. It includes celiac, superior mesenteric, hepatic, and splenic angiograms; wedged hepatic and left renal venograms; and manometry. Panhepatic angiography determines hepatic arterial variations, assesses portal hemodynamics, and excludes hepatoma. Visualization of aberrant hepatic arteries is important because accidental ligation of the hepatic artery in the presence of portal hypertension may result in hepatic necrosis. A right hepatic artery that originates from the superior mesenteric artery poses technical difficulty for portacaval shunt surgery.

A hepatic angiogram is necessary to exclude hepatomas and helps determine the hemodynamics of intrahepatic portal flow. In advanced cirrhosis with reversed portal flow, the portal vein may be visualized in the venous phase of the hepatic arteriogram. High-dose superior mesenteric and splenic angiograms are necessary to see the superior mesenteric, splenic, and portal veins, as well as the portosystemic collaterals. In alcoholic cirrhosis, a pressure measurement from the catheter wedged in the hepatic vein reflects portal vein pressure and is useful in determining whether the location of the obstruction is intrahepatic or extrahepatic. However, wedged hepatic vein pressure is not a useful determinant for selecting the type of shunt surgery. It is also a poor predictor of prognosis and survival after shunt surgery. The TIPS procedure has largely replaced surgical shunt in recent years. Wedged hepatic venography is the most accurate means of assessing the morphological features of the hepatic sinusoids and parenchyma. CO_2 is used as a contrast agent for most wedged hepatic venography to visualize the portal vein. Manometry of the right atrium, hepatic vein, and IVC helps determine the level of hepatic venous outflow obstruction.

Transcatheter therapy

Diagnostic angiography should be performed before any transcatheter intervention for GI disease. Interventional radiological procedures are reviewed in detail in Chapter 160.

Percutaneous translumenal angioplasty and stent placement

Surgical therapy (endarterectomy and bypass surgery) for superior mesenteric artery obstruction has significant mortality and recurrence rates. Percutaneous translumenal angioplasty (PTA) is a safe and effective alternative to surgery in treating intestinal angina resulting from arteriosclerotic occlusive disease of the visceral arteries. Stent placement is required for ostial stenosis or complete occlusion of the celiac and superior mesenteric artery, failed PTA because of elastic recoil, and mesenteric arterial compression by the false lumen of an aortic dissection [106–109]. Celiac axis stenosis caused by median arcuate ligament compression is not amenable to angioplasty and thus requires stent placement or surgery.

PTA is effective in treating Budd–Chiari syndrome caused by a membrane in the IVC and a stenosis of the hepatic vein [110,111]. The method may not be technically possible in patients with diffuse hepatic vein thrombosis and sclerosis. TIPS is used for the treatment of severe hepatic venoocclusive disease [112]. Percutaneous transhepatic angioplasty

and stent placement has been used to treat portal vein stenosis and postoperative stricture of the portal vein complicating liver transplantation [113–115].

PTA should be performed for hepatic artery stenosis in liver transplant recipients because of a significant risk of thrombosis of untreated stenosis [116].

Transcatheter embolotherapy

Transcatheter embolotherapy is an acceptable alternative to surgery in treating massive arterial GI bleeding, visceral aneurysms, hypersplenism, isolated gastric varices associated with splenic vein occlusion, and traumatic hepatic and splenic bleeding [117–122]. Occlusion of the hepatic artery by embolization for control of hepatic bleeding is usually tolerated because the hepatic sinusoidal perfusion can be maintained by collateral circulation and the portal venous blood. Hepatic arterial embolization is used for the treatment of traumatic hepatic bleeding, hemobilia, and symptomatic arterioportal fistula. However, hepatic artery embolization in patients with symptomatic hepatic arteriovenous malformations associated with hereditary hemorrhagic telangiectasia has a high risk for hepatic infarction and death [123]. The spleen receives adequate collateral blood flow through the pancreatic, left gastric/short gastric, inferior phrenic/short gastric, and gastroepiploic arteries after occlusion of the splenic artery. Superselective catheterization of the splenic artery is performed using the coaxial catheterization method using a 3Fr microcatheter. Microcoils are the most frequently used occluding agent and produces arterial occlusion equivalent to surgical ligation. Injection of thrombin into an aneurysm promotes clot formation. A 3Fr coaxial catheter system is extremely useful in catheterization and embolization of a mesenteric branch artery. If an aneurysm cannot be catheterized transarterially, a 22G needle is used for percutaneous puncture, and embolization of the lesion is accomplished with microcoils and thrombin.

Thrombolytic therapy

Thrombolytic therapy may be used for superior mesenteric artery embolism and portal vein thrombosis. A report of thrombolytic therapy for superior mesenteric artery embolism indicates that the method is safe and effective, with technical success in 90% and clinical success in 70% [124], but the treatment requires at least 6–8 h of infusion. A percutaneous transhepatic or transjugular approach is necessary for thrombolysis of mesenteric and portal vein thrombosis. The technique involves placement of a catheter into the embolus or thrombus, and infusion of a lytic agent. During thrombolysis, the patient should be monitored for bleeding and evidence of bowel infarction in the intensive care unit. The transcatheter use of a mechanical thrombectomy device, such as AngioJet (Possis Medical Inc., Minneapolis, MN) may be used for removal of thrombi from the mesenteric and portal vein thrombosis.

Hepatic arterial infusion chemotherapy

Regional delivery of antineoplastic agents through a selectively placed arterial catheter produces higher tumor response than systemic infusion [125,126]. The success of hepatic arterial chemotherapy depends on accurate evaluation of the hepatic arterial anatomy and hemodynamics, and correct catheter placement. To ensure total liver perfusion and drug delivery to the tumor site, the hepatic arterial anatomy is meticulously evaluated by celiac and superior mesenteric arteriograms. Multiple hepatic arteries can be converted to a single hepatic artery using the transcatheter embolization technique to facilitate catheter placement [127]. For example, if an aberrant left hepatic artery from the left gastric artery is embolized, the left hepatic lobe receives blood supply through intrahepatic collaterals from the right hepatic artery. If the infusion catheter cannot be placed in the proper hepatic artery, the gastroduodenal artery may be occluded by embolization to allow infusion of drugs with a catheter placed in the common hepatic artery [128]. Hepatic arterial catheters can be placed through a percutaneous approach from the femoral or brachial artery, or surgically. The introduction of the totally implantable pump has improved regional chemotherapy with surgical catheter placement [129]. Radionuclide flow study using ^{99m}Tc-labeled macroaggregated serum albumin is the most accurate means of assessing hepatic perfusion pattern after catheter placement. The complications of percutaneous catheter placement are brachial artery thrombosis, hepatic artery thrombosis, catheter displacement, and puncture-site bleeding. The complications of surgical catheter placement are hepatic artery thrombosis, incorrect catheter placement, catheter occlusion, and pump malfunction. Complications related to the toxicity of chemotherapeutic agents are chemical hepatitis, biliary sclerosis, chemical cholecystitis, and gastroduodenal inflammation and ulceration.

Hepatic tumor embolization

Transarterial chemoembolization (TACE) is used to palliate hepatic tumors including hepatoma, metastatic neuroendocrine tumors, and metastatic colon cancer if the tumors cannot be treated by the percutaneous ablation method (Fig. 135.19). Generally, TACE has been shown to be beneficial and improve survival [130–133]. TACE has also been used to prevent tumor progression or shrink the tumor while awaiting liver transplantation in order to improve clinical outcome [134–137].

CT or MRI of the liver must be reviewed for evaluation of location of tumor, extent of disease, and any associated findings (portal vein occlusion, biliary obstruction). Adequate portal venous flow should be available to maintain hepatic parenchymal vitality. If the intrahepatic portal flow is reversed (hepatofugal), embolization should not be performed or limited to the subsegment containing tumor.

Hepatic arterial embolization results in selective destruction of the tumors exclusively dependent on arterial supply

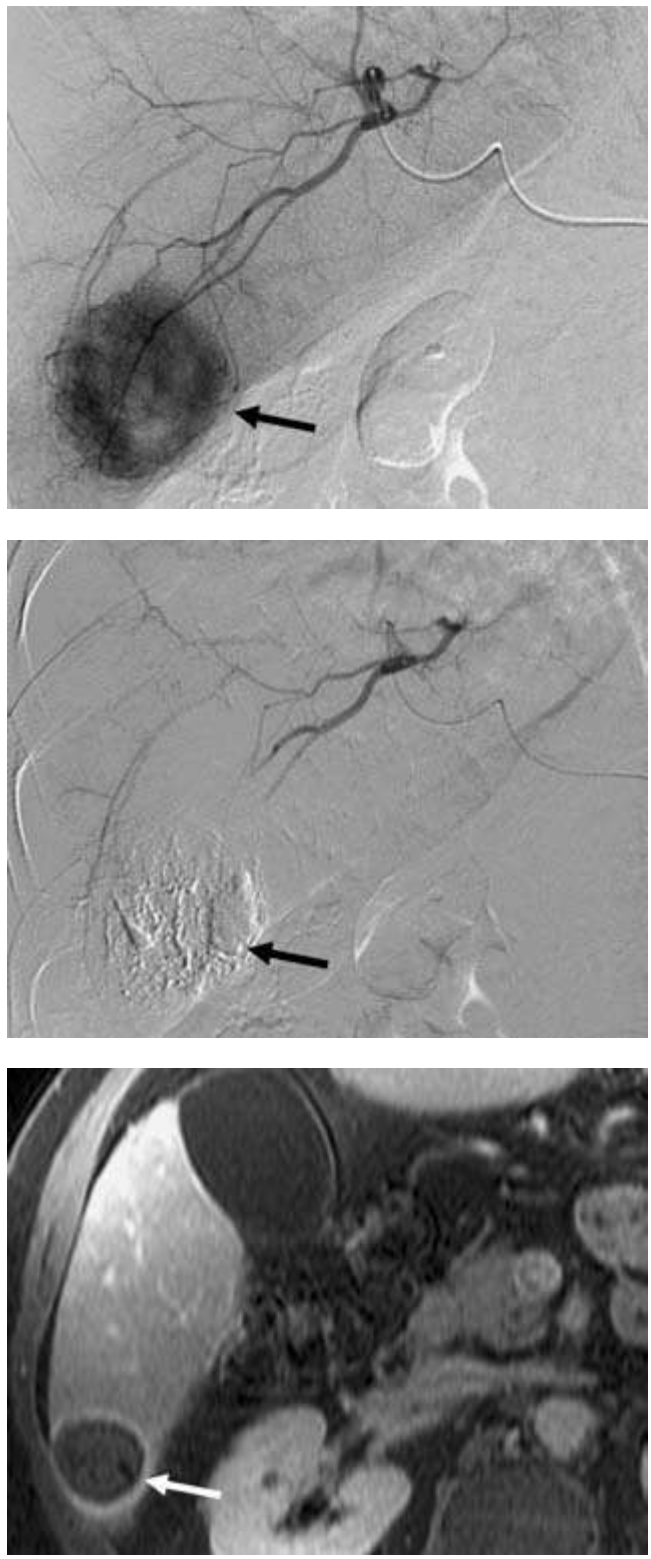


Figure 135.19 Hepatic artery chemoembolization in a 63-year-old patient with hepatitis C cirrhosis and hepatocellular carcinoma (HCC). **(a)** Right hepatic arteriogram. Branches of the right posterior segmental hepatic artery supply a hypervascular mass in the liver (arrow). **(b)** Hepatic arteriogram performed shortly after embolization showing complete devascularization of the HCC (arrow). **(c)** Contrast-enhanced magnetic resonance image obtained 6 weeks after chemoembolization confirmed complete necrosis of HCC (arrow).

while maintaining the vitality of the normal liver with portal vein blood. Small particles such as polyvinyl alcohol (Ivalon 150–250 μm) are used to occlude peripheral arteries to decrease the development of collateral blood flow. Concurrent embolization increases the effect of the drug by prolonging exposure of the tumor to the drug and producing tumor anoxia while minimizing systemic drug effects. Injection of iodized oil into the hepatic artery results in selective accumulation of the oil within most vascular hepatic tumors. This phenomenon has been exploited to selectively deliver antineoplastic agents and radioactive isotopes to hepatic cancers, with intraarterial injection of a mixture of iodized oil and antineoplastic agents or radioactive iodized oil solution. Yttrium-90 microspheres (TheraSphere) have been used for treatment of HCC and metastatic liver tumors [138–140]. TheraSphere is injected into the hepatic artery to deliver radiation directly to tumors with concurrent ischemia from microsphereres. Another new agent, DC Bead, has been used for hepatic artery chemoembolization. The DC Bead is an embolic agent loaded with chemotherapeutic agents [140,141]. When introduced into the tumors, it eludes drug over weeks.

Hepatic artery embolization may be associated with the postembolization syndrome: abdominal pain, nausea, vomiting, and fever lasting 2–7 days. Mildly abnormal liver tests are common. Other complications include hepatic necrosis, gallbladder infarction, and gastroduodenal ulcers. Follow-up CT or MRI is obtained at 3-month intervals and repeated embolizations are performed for recurrent tumors.

Forthcoming advances

Noninvasive imaging modalities (spiral CT, three-dimensional contrast MRA, color Doppler imaging, EUS and duplex US) will play an increasing role in the evaluation of suspected GI diseases. Angiography will be used less frequently but will continue to be used in the preoperative evaluation of vascular disease, portal hypertension, neoplasms of the pancreas and liver, and GI hemorrhage. Therapeutic applications of angiography will expand in the treatment of lower GI bleeding, vascular lesions, hepatic neoplasms, mesenteric vascular obstruction, variceal bleeding unresponsive to endoscopic therapy, intractable ascites, and Budd–Chiari syndrome.

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B IMAGING

136 Interventional radiology

Kyung J. Cho

Vascular intervention, 3269
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The field of interventional radiology has evolved following the introduction of the percutaneous transarterial technique devised by Seldinger in 1953 [1]. Interventional radiologists perform a variety of diagnostic and interventional procedures of the circulatory system and nonvascular tracts under imaging guidance. The imaging guidance includes fluoroscopy, ultrasound (US), computed tomography (CT), magnetic resonance (MR) imaging, and digital subtraction angiography (DSA). The interventional radiological procedures can be divided into vascular and nonvascular procedures. The vascular procedures include infusion, embolization, angioplasty, stent placement, transjugular intrahepatic portosystemic shunt, and foreign body retrieval. The nonvascular procedures include biopsy, percutaneous drainage, dilation, and ostomy formation.

Vascular intervention

Control of arterial gastrointestinal bleeding

Angiographic management is indicated for endoscopic failures, especially in poor operative candidates and for inaccessible sites of bleeding, such as hemobilia and bleeding into the pancreatic duct, and bleeding associated with aneurysms or pseudoaneurysms. Angiographic therapy for massive gastrointestinal (GI) bleeding includes intraarterial infusion of vasopressin and selective arterial embolization. The success of angiographic treatment depends on accurate localization of the bleeding site and selective catheterization of the bleeding artery.

Vasopressin infusion

Vasopressin is effective for bleeding from Mallory–Weiss tears, hemorrhagic gastritis, and small bowel and colonic bleeding [2,3], but it is less effective for bleeding from a peptic ulcer because ulcers rupture larger arteries. Vasopressin infusion can control lower GI tract bleeding in 80%–90% of patients, but 40%–50% of these patients rebleed and eventually require surgery. Therefore, vasopressin infusion is infrequently used for control of lower GI bleeding.

When the bleeding site has been demonstrated, a catheter is selectively placed in the bleeding artery. Vasopressin is infused into the left gastric artery for gastric bleeding, into the superior mesenteric artery for small bowel and right colonic bleeding, and into the inferior mesenteric artery for left colonic and rectosigmoid bleeding. Vasopressin infusion may be initiated at the rate of 0.2 U/min. After 20 min of infusion, a repeat arteriogram is performed to assess treatment effectiveness. If no bleeding is demonstrated and vasoconstriction is moderate, the infusion is continued for the next 24 h at the same rate. When the bleeding has stopped, the rate should be reduced to 0.1 U/min and continued for an additional 6–12 h before termination of the infusion. If the bleeding is not controlled after vasopressin infusion of 0.4 U/min for 20 min, increasing the rate is not recommended; embolization or surgery should be considered.

Serious side effects from vasopressin infusion occur infrequently. Most side effects are dose dependent. They are coronary ischemia, arrhythmia, bradycardia, hypertension, bowel ischemia, peripheral vascular ischemia, and oliguria. In patients with a history of coronary artery disease, the infusion should be done with caution and concomitant administration of nitroglycerin.

Selective arterial embolization

Selective arterial embolization is an effective method of therapy for control of massive upper GI hemorrhage when endoscopic therapy has failed. Selective occlusion of the bleeding artery with embolic agents such as gelatin sponge, polyvinyl alcohol (Ivalon), microcoil, and *n*-butyl cyanoacrylate (*n*-BCA) results in prompt cessation of the bleeding, and it usually obviates the need for surgical intervention [4,5] (Fig. 136.1). It is important to localize the bleeding site before embolization (Fig. 136.2). Prophylactic embolization of the left gastric

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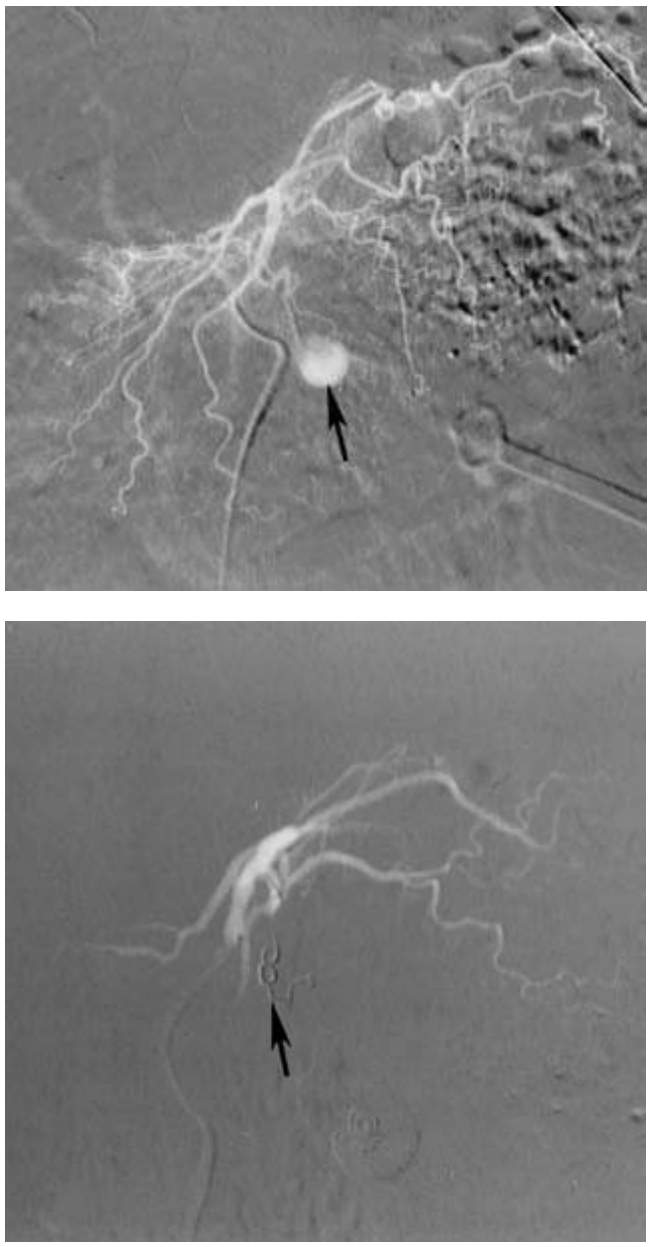


Figure 136.1 Massive arterial bleeding from a branch of the left gastric artery. **(a)** Superselective left gastric arteriogram (digital subtraction technique). Active bleeding is seen from a branch of the left gastric artery in the body of the stomach. The extravasated contrast medium has formed a pseudoaneurysm (arrow). **(b)** The bleeding has stopped following microcoil embolization (arrow).

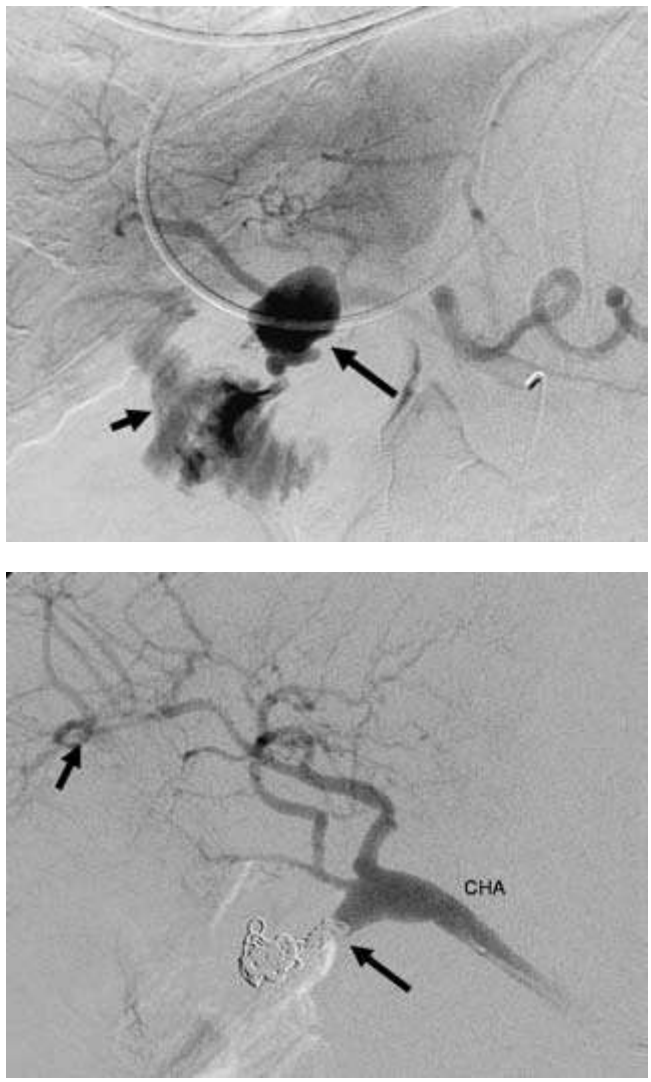


Figure 136.2 Control of duodenal hemorrhage by coil and thrombin embolization. Celiac angiogram in an 83-year-old man with a gastroduodenal artery pseudoaneurysm 1 month after a Whipple procedure for pancreatic cancer. No source of bleeding was seen at upper gastrointestinal endoscopy. **(a)** Celiac angiogram. Contrast medium (shorter arrow) extravasates from the large pseudoaneurysm (arrow) of the proximal gastroduodenal artery (GDA). The right hepatic artery arises from the GDA. **(b)** Common hepatic angiogram after embolization. The gastroduodenal artery and pseudoaneurysm are occluded. The right hepatic artery (arrow) reconstitutes distally through the hilar collateral from the middle and left hepatic arteries. CHA, common hepatic artery.

or gastroduodenal artery for upper GI bleeding can be performed, but endoscopic localization of the bleeding is necessary before embolization [6,7]. Correction of coagulopathy before and after the embolization reduces the risk for bleeding recurrence [8,9]. The use of coils as the sole embolic agent, and concurrent coagulopathy in patients with upper GI bleeding are associated with a high risk of early recurrent hemorrhage. The stomach and duodenum have sufficient collateral pathways; hence, infarction is unlikely to occur after embolization. On rare occasions, patients with previous surgery or those receiving vasopressin therapies after the embolization may develop infarction of the stomach. Duodenal ischemia, duodenal stenosis, and pancreatic necrosis are the potential complications of gastroduodenal artery embolization.

The localization procedures for lower GI bleeding before angiography include colonoscopy, technetium 99m (^{99m}Tc)-

labeled red blood cell scintigraphy, and helical CT [10]. Colonoscopy is usually performed when the bleeding has ceased. Selective arterial embolization is a safe and effective method of therapy for control of lower GI bleeding [11,12] (Fig. 136.3). Gelfoam pledgets and Ivalon particles are the commonly used embolization agents. The use of a 3-Fr coaxial catheter allows catheterization of the extravasating arterial branch for embolization with microcoil. Rectal hemorrhage is usually diagnosed and treated by interventional endoscopy. When the rectum is filled with fresh blood, endoscopic identification of the bleeding site may be difficult. Regardless of the underlying etiology, embolization can be effective in controlling superior hemorrhoidal arterial hemorrhage.

Lower GI bleeding is frequently intermittent and ceases spontaneously before and during an angiographic localization procedure. Surgical intervention without angiographic localization of the bleeding may be difficult on the basis of the results of scintigraphy. When the standard angiography has failed to demonstrate the bleeding site, provocative angiography may be used to induce bleeding; hence embolization or surgery can be performed. The agents used for the provocative angiography are heparin and intraarterial infusion of a vasodilator such as nitroglycerin and a thrombolytic agent (alteplase or reteplase) [13,14].

If superselective catheterization of the bleeding artery is not possible due to technical difficulty, vasopressin should be infused into the superior mesenteric artery proximal to the bleeding artery [15].

Variceal embolization

In 1974, Lunderquist and Yang [16] reported the technique of percutaneous transhepatic coronary vein embolization as a means of controlling gastroesophageal variceal bleeding. Portal vein catheterization can also be done using the transjugular, umbilical, and intestinal veins after minilaparotomy. In the presence of massive ascites, coagulopathy, or liver tumors, the transjugular approach is used. Coronary vein embolization can effectively control variceal hemorrhage in 80% of cases, but recurrent bleeding is common because of the development of new gastric and esophageal varices. Coronary vein embolization is infrequently performed because endoscopic therapy is more effective in preventing recurrent hemorrhage.

Balloon-occluded retrograde transvenous obliteration (BRTO) [17,18] is a safe and effective treatment of gastric varices. The technique involves the retrograde catheterization of the portosystemic collateral vein through the left renal and adrenal veins from the femoral vein (Fig. 136.4). An occlusion balloon is wedged in the collateral vein, and a sclerosing agent (i.e., 5% ethanolamine oleate mixed with contrast medium) is injected through the shunt into the gastric varices. After the injected sclerosing agent is allowed to sclerose the varices for 20–50 min, the residual sclerosing agent is aspirated through the catheter and the balloon is deflated. Alternatively *n*-butyl cyanoacrylate mixed with lipiodol can be injected through a 3-Fr microcatheter to achieve prompt occlusion of the varices. If complete obliteration of the varices is not possible with the retrograde approach, the varices can be embolized by the transhepatic approach or the transjugular intrahepatic portosystemic shunt (TIPS) if present.

Rarely, bleeding may occur from the portosystemic collaterals at unusual sites including the small bowel, colon, rectum, and enterostomy [19,20]. If the bleeding is unresponsive to conservative therapy, variceal embolization can be performed through the transhepatic or transjugular approaches [21,22]. The TIPS usually is preferable for the treatment of bleeding from varices at unusual sites owing to the risk of rebleeding with embolization alone [23–25] (Fig. 136.5).

Another reported unusual indication for variceal embolization is chronic recurrent hepatic encephalopathy [26,27]. Embolization of portosystemic shunts can decrease diversion of portal blood flow through the shunts, resulting in an increase in portal blood flow. Portosystemic shunts amenable to such embolic therapy are splenorenal shunts, gastrorenal shunts, hepatic portal vein–hepatic vein shunts, and ileal vein–gonadal vein communications. Embolization can be done through the transfemoral or transhepatic approaches, depending on the site of shunts.

Budd–Chiari syndrome

Patients with hepatic vein outflow obstruction (Budd–Chiari syndrome) usually present with abdominal pain, hepatomegaly, jaundice, and ascites. Treatment must be directed to relieve the hepatic outflow obstruction and to provide a bridge to liver transplantation in patients with acute hepatic failure. Depending on the extent, level, and nature of the hepatic venous outflow obstruction, various combinations of interventions are used, including catheter-directed thrombolysis, mechanical thrombectomy, recanalization, angioplasty, stent placement, and TIPS [28–31].

Percutaneous translumenal balloon angioplasty (PTA) is an effective therapy in patients with the Budd–Chiari syndrome secondary to obstruction of the inferior vena cava (IVC) or hepatic vein. However, recurrence is common, and therefore stent placement is usually performed. The transfemoral or transjugular approach usually is used for IVC and hepatic vein obstruction. A percutaneous transhepatic approach is used for the treatment of hepatic vein occlusion if a transjugular approach fails to cannulate the occluded hepatic vein (Fig. 136.6). When the hepatic venous outflow obstruction extends into the peripheral hepatic veins, requiring portal decompression, a TIPS procedure is a viable alternative to surgical shunting and provides a bridge to liver transplantation [32–34]. When portal, splenic, or mesenteric venous thrombosis is associated with Budd–Chiari syndrome, the thrombi can be dissolved by catheter-directed thrombolysis or removed by a mechanical thrombectomy device before placement of a TIPS. The TIPS stent may be

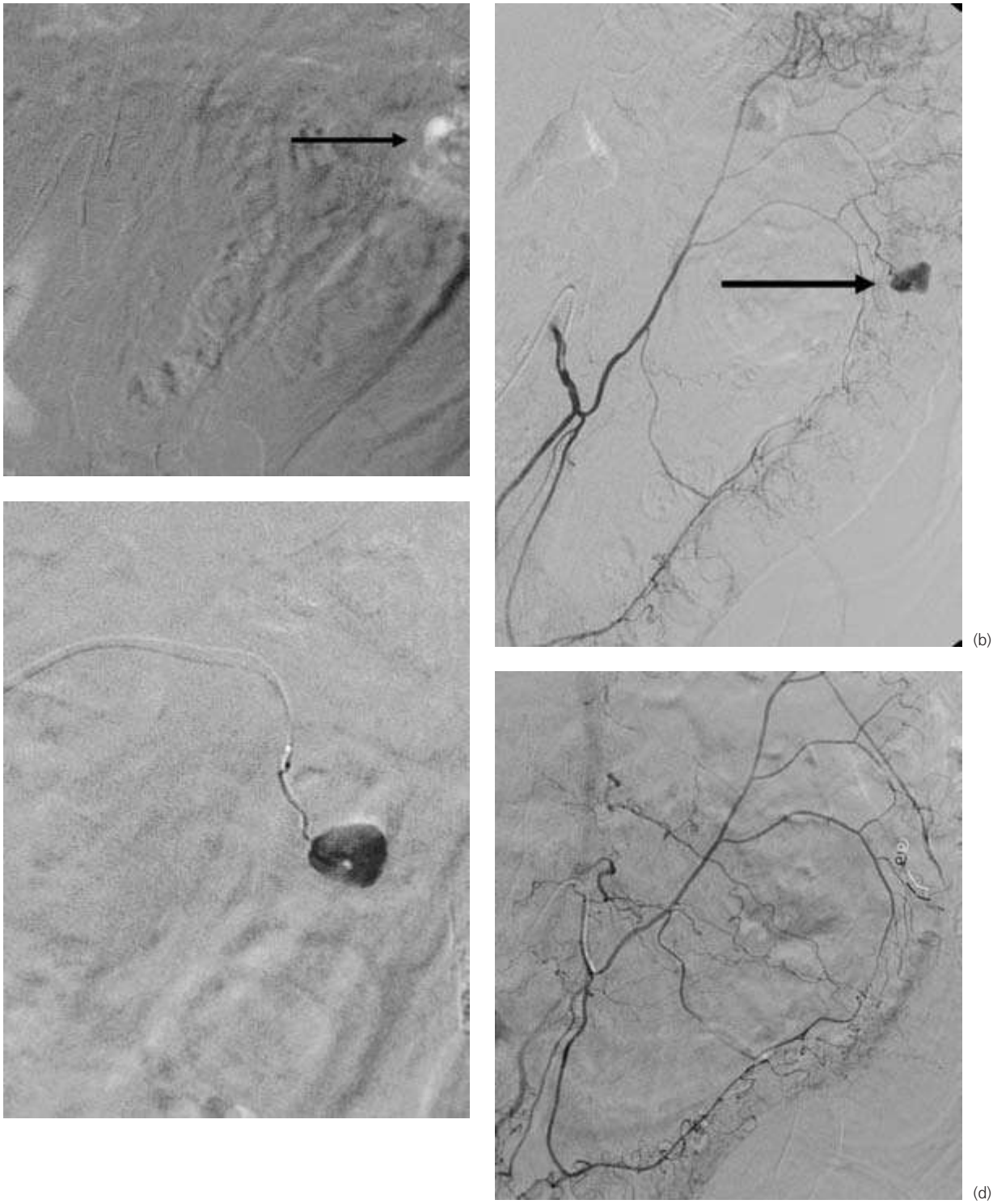


Figure 136.3 Control of left colonic hemorrhage by microcoil embolization. Inferior mesenteric arteriogram in an 82-year-old man with atrial fibrillation, diabetes mellitus, congestive heart failure, and massive lower gastrointestrial bleeding. The patient was on aspirin and Plavix. Despite transfusion of four units of packed red blood cells, his blood pressure was 90 mmHg. Neither colonoscopy nor radionuclide scintigraphy was performed. **(a)** Inferior mesenteric angiogram with CO_2. Carbon dioxide extravasates into the proximal descending colon (arrow). **(b)** Inferior mesenteric angiogram with contrast medium demonstrates extravasation (arrow) from a branch of the left colic artery. **(c)** Superselective angiogram with the injection of contrast medium into the bleeding arterial branch from the terminal arch. A dense accumulation of contrast medium is seen, likely representing a diverticulum. **(d)** Inferior mesenteric arteriogram following the embolization with microcoil. The bleeding artery is occluded and no bleeding is seen.

Figure 136.4 Balloon-occluded retrograde transvenous obliteration of gastric varices. Splenoportogram in a 52-year-old woman with alcoholic cirrhosis and hepatitis B who had undergone a transjugular intrahepatic portosystemic shunt (TIPS) procedure 14 months previously, and in whom esophagogastroduodenoscopy now revealed bleeding gastric varices. A TIPS stent stenosis was treated with balloon dilation. **(a)** Injection of the splenic vein that was catheterized through the TIPS stent demonstrates gastric varices. **(b)** Embolization of the left gastric varices with *n*-butyl cyanoacrylate through the balloon-occluded inferior phrenic vein (arrow) that was catheterized from the femoral vein.

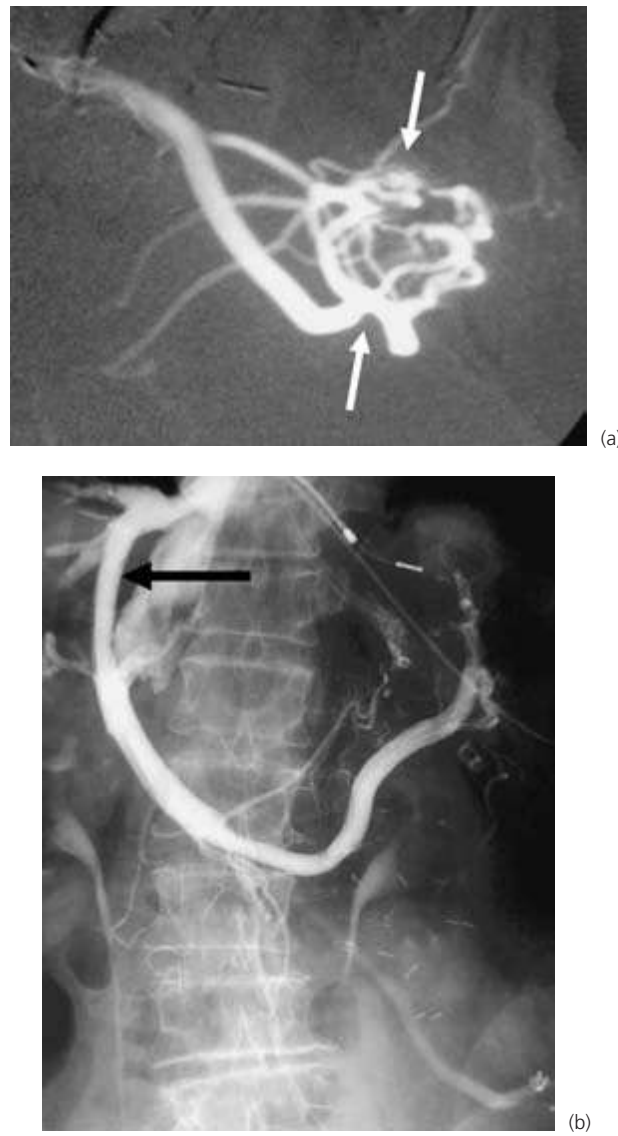


Figure 136.5 Transjugular embolization of stomal varices and placement of a transjugular intrahepatic portosystemic shunt (TIPS) in a 75-year-old woman with a 6-month history of recurrent bleeding from colostomy varices. The bleeding began 12 years after colostomy following resection of colorectal cancer. **(a)** Transjugular catheterization of the middle colic vein. Contrast medium fills the stomal varices (arrows). **(b)** A TIPS (arrow) was placed following coil embolization of the stomal varices. Stomal bleeding had not recurred 6 years after the embolization and placement of a TIPS.

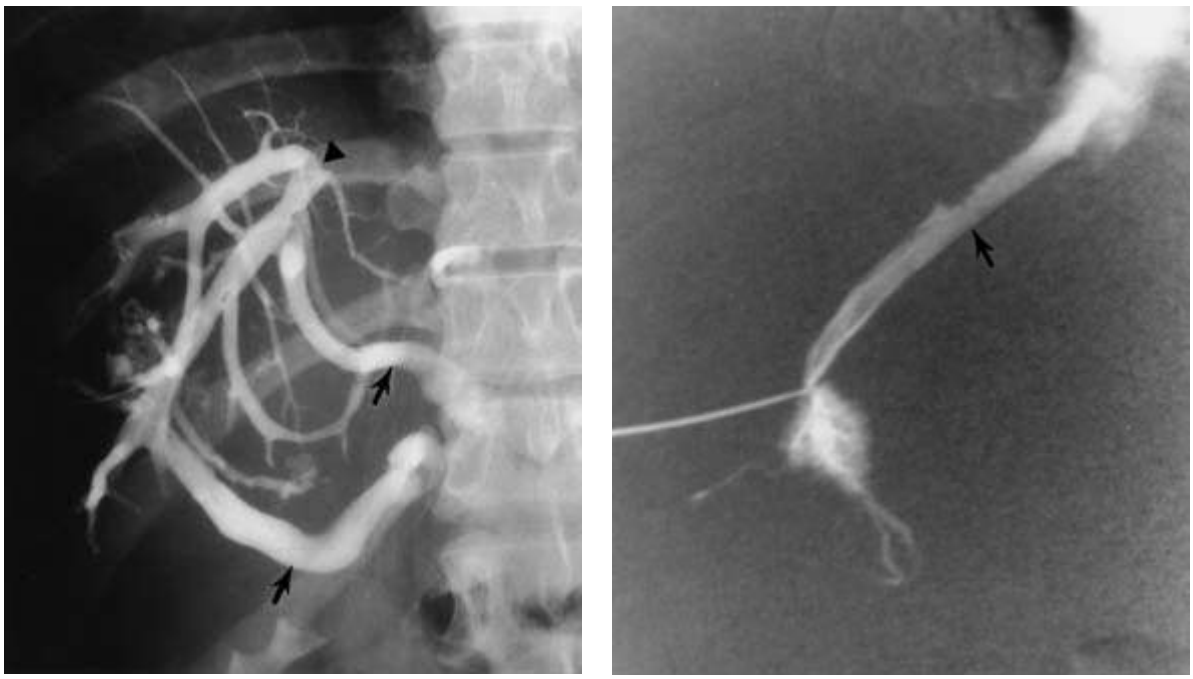
extended into the splenic or mesenteric vein to achieve adequate inflow.

Portal vein stenosis

Percutaneous translumenal balloon angioplasty and stent implantation using the percutaneous transhepatic and transjugular approaches are safe and effective in the therapy of portal vein stenosis, including cases complicating liver transplantation [35–38]. Although most cases of portal vein stenosis or occlusion are amenable to percutaneous techniques, the long-term efficacy of these techniques is not known; therefore, follow-up with imaging studies is needed to detect restenosis of the treated lesion.

Mesenteric vein thrombosis

Mesenteric vein thrombosis (MVT) may cause a severe mesenteric ischemia. It has been associated with portal hypertension, abdominal infection, inflammatory disease, trauma, surgery, and hypercoagulable states. It may occur following a TIPS placement. An endovascular technique plays an important role in managing symptomatic MVT. A thrombolytic agent is administered directly into the mesenteric vein



(a) (b)

Figure 136.6 Hepatic vein stent placement for Budd–Chiari syndrome. Transhepatic hepatic venogram in a 23-year-old woman with right upper quadrant pain and tenderness, ascites, and liver failure. **(a)** Transhepatic hepatic venogram. The hepatic vein is occluded (arrowhead). The contrast medium fills hepatic venous collaterals (arrows), draining toward the inferior vena cava. **(b)** Transhepatic hepatic venogram following recanalization of the hepatic vein and placement of a 10-mm-diameter WALLSTENT. The hepatic vein is patent with excellent blood flow (arrow). The venous collaterals are no longer filled. The hepatic vein pressure decreased from 29 mmHg to 7 mmHg after stent placement. One year later, the patient received an orthotopic liver transplant because of occlusion of the stent.

through the transjugular or transhepatic approach [39,40]. Mechanical thrombectomy using a thrombectomy device or a Fogarty catheter can be used for removal of acute thrombus. This technique can be used for recanalization of acute portal vein thrombosis complicating TIPS. Portal vein reconstruction with recanalization and stent placement may be performed in patients with symptomatic chronic splenic-mesenteric-portal venous thrombosis.

Visceral arterial stenosis

Atherosclerotic stenoses of the celiac and superior mesenteric arteries are a common cause of chronic mesenteric ischemia in elderly patients. Surgical revascularization is the standard treatment for clinically significant stenoses of the celiac and superior mesenteric arteries. Percutaneous translumenal balloon angioplasty and stenting is a safe and effective alternative to surgical treatment [41,42]. In a review of 83 reported patients who had PTA of visceral arteries, the initial technical success was 88%; the initial clinical success rate was 92%; the recurrence rate was 24%; the primary long-term clinical success rate was 76%; the rate of successful repeat PTA was 92%; secondary long-term clinical success was 92%; and major complications affected 6% [42]. Stent placement has been shown to be safe and clinically effective as an adjunctive treatment to PTA or as a primary therapy for patients with chronic mesenteric ischemia caused by calcified ostial stenoses, high-grade eccentric stenoses, chronic occlusions, and flow-limiting dissection of mesenteric arteries [43].

Percutaneous translumenal balloon angioplasty and stent placement are usually done through the femoral approach. If the femoral artery cannot be used, the left axillary approach is used. Before the procedure, lateral aortography and selective mesenteric arteriography are performed to evaluate the arterial lesions and visceral arterial circulation. The origin of the artery to be dilated is then catheterized and the stenosis is crossed using either a soft-tipped guidewire or a hydrophilic guidewire. The patient is given an intravenous bolus of 5000 units of heparin. Over a 0.035-inch (0.89-mm) heavy-duty guidewire, a PTA balloon catheter of appropriate balloon size is placed across the stenosis and dilated to pressures of 7–10 atm (102.9–147.0 psi; 1 psi = 6894.7 PA). If a gradient exceeds 10 mmHg after balloon dilation or if a hemodynamically significant dissection is seen, a metallic stent is placed. Stents may be used as the primary treatment for mesenteric artery stenosis (Fig. 136.7). A balloon-expandable stent is used for a focal proximal stenosis of the superior mesenteric or celiac stenosis. Nitinol self-expanding stents are used for longer lesions. Neither PTA nor stent placement is effective in treating celiac stenosis caused by median arcuate ligament compression. Systemic anticoagulation is not usually necessary after the procedure. Percutaneous translumenal balloon angioplasty should have a limited role in the patient with acute mesenteric ischemia.

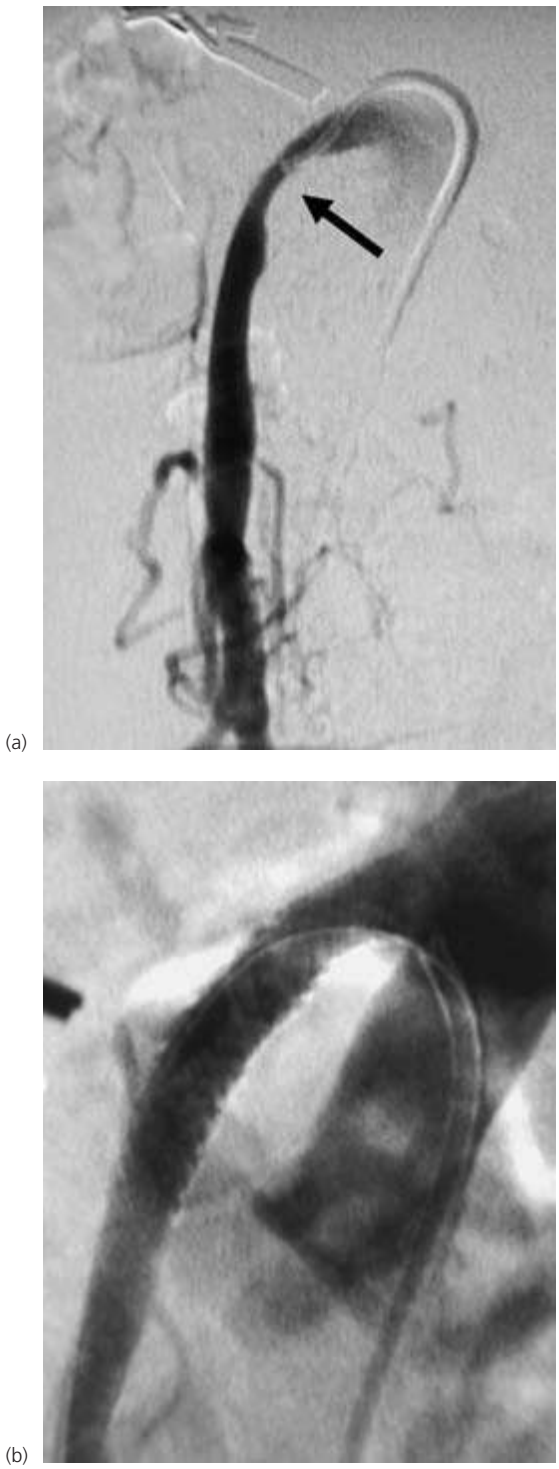


Figure 136.7 Angioplasty and stent placement for chronic mesenteric ischemia caused by superior mesenteric artery stenosis in a 65-year-old woman. **(a)** Superior mesenteric arteriogram in the lateral projection shows high-grade stenosis of the superior mesenteric artery (arrow). **(b)** Following angioplasty and stent placement the artery is widely open.

Superior mesenteric artery embolism

Embolism of the superior mesenteric artery is an important cause of acute intestinal ischemia, with high mortality rates [44]. Survival is often directly related to early diagnosis by emergency mesenteric arteriography. If an embolus is found in the proximal superior mesenteric artery, surgical treatment usually is indicated. In selected patients with an early diagnosis of peripheral embolism of the mesenteric arteries, intraarterial thrombolysis may be a therapeutic alternative [45].

Splanchnic artery aneurysm

Selective arterial embolization is safe and effective in treating visceral arterial aneurysms in poor surgical risk patients, particularly those with pancreatitis-associated aneurysms and pseudoaneurysms [46,47]. The embolization is usually done using the transfemoral approach. Celiac and superior mesenteric arteriograms are performed to evaluate the lesion and arterial anatomy. A 5-Fr catheter is advanced into the feeding artery as close to the lesion as possible. A 3-Fr coaxial catheter system is usually required to place the tip of the catheter close to or in the aneurysm. Coils are the most commonly used embolic agent. Thrombin may be added to the coils to promote clot formation.

If transarterial access to the aneurysm cannot be obtained owing to unfavorable arterial anatomy, the aneurysm is punctured percutaneously using a 22-gauge, skinny needle under ultrasonic or angiographic guidance. After confirmation of the position of the needle in the lesion, microcoils are deposited through the needle using a 0.018-inch (0.457-mm) guidewire, or thrombin is injected into the aneurysm [48,49].

Vascular lesions are uncommon complications of laparoscopic cholecystectomy. Most bleeding complications that occur intraoperatively are treated during the procedure. Any delayed vascular complications, such as hepatic artery pseudoaneurysm and arteriobiliary fistula, can be treated by selective embolization [50].

Transjugular intrahepatic portosystemic shunt

In patients with variceal bleeding who fail medical and endoscopic treatment, TIPS is invaluable and has largely replaced surgical shunts [51]. Transjugular intrahepatic portosystemic shunt is a nonsurgical method of achieving portal decompression in patients with portal hypertension and variceal hemorrhage. The indications for TIPS are variceal bleeding unresponsive to endoscopic therapy, recurrent variceal bleeding despite endoscopic therapy, portal hypertensive gastropathy, refractory ascites, hepatic hydrothorax, Budd–Chiari syndrome, stomal variceal hemorrhage, and portal venous thrombosis [52–58]. Transjugular intrahepatic portosystemic shunt may also be a treatment option for liver transplant

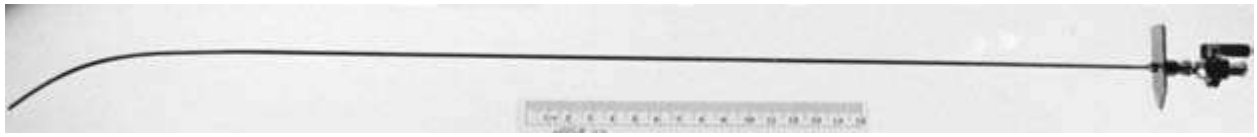


Figure 136.8 Colapinto needle used in transjugular intrahepatic portosystemic shunt procedure.

recipients with refractory variceal bleeding and intractable ascites [59]. There are no absolute contraindications for creating TIPS. The relative contraindications include elevated right heart pressure, heart failure, fulminant hepatic failure, severe hepatic encephalopathy, uncontrolled systemic infection or bacteremia, unrelieved biliary obstruction, multiple hepatic cysts, extensive primary or metastatic hepatic malignancy, and severe uncorrectable coagulopathy.

Technique

Before a TIPS procedure, patency of the portal vein should be demonstrated by imaging methods. Ultrasound is the most commonly used method for evaluation of portal vein patency. The other methods include CT arterial portography, MR venography, indirect portography (arterial portography), and carbon dioxide (CO_2) wedged hepatic venography.

Preprocedure, fresh-frozen plasma or a platelet transfusion is given if needed. The procedure can be performed under conscious sedation or a general anesthesia for uncooperative patients. The patient lies on his or her back and the neck is slightly extended backward and turned to the left for the right jugular approach or to the right for the left jugular approach. The right or left internal jugular vein is catheterized using a 21-gauge, single wall, puncture needle under US guidance. A 10-Fr vascular sheath is advanced into the right atrium. A 5-Fr catheter is then introduced coaxially and pressures are measured of the right atrium, IVC, and free and wedged hepatic veins. Carbon dioxide digital subtraction wedged hepatic venography is performed to see the portal vein.

A 16-gauge Colapinto needle (Fig. 136.8) is advanced over a 0.035-inch (0.89-mm) Amplatz, super stiff guidewire into the right or middle hepatic vein. The guidewire is removed and the needle is advanced with fluoroscopic guidance into the expected position of the right main portal vein 3 cm from the portal vein bifurcation (Fig. 136.9). The needle is withdrawn until blood can be aspirated, and contrast medium is injected to see the vascular structure entered. When the portal vein is entered, a guidewire is introduced into the superior mesenteric or splenic vein. The 9-Fr catheter is advanced over the Colapinto needle and guidewire into the portal vein. The needle is removed, and a 5-Fr catheter is advanced into the portal vein. Pressure in the portal vein is measured, and a portal venogram is performed (Fig. 136.9) to see the portal venous system and portosystemic collaterals. The parenchymal tract is injected with CO_2 and contrast medium to see whether the needle tract has passed through the bile duct or hepatic artery. The tract is then dilated with an 8-mm PTA balloon catheter, and a 10-mm diameter metallic stent is placed in the tract bridging the distance from the portal to the central hepatic vein (Fig. 136.9). Sometimes more than one stent must be used to cover the tract. The stents are then dilated with a 10-mm PTA balloon to ensure their maximal expansion. The use of covered stents, such as the GORE VIATORR TIPS (Tempe, AZ) Endoprosthesis, is preferable to bare stents, such as the WALLSTENT (Boston Scientific Corp., Natick, MA) Endoprosthesis, because the former with its expanded polytetrafluoroethylene (ePTFE) graft lining minimizes transmural permeation of bile and mucin, which are known to cause early stent occlusion, The covered stents are used in both de novo and revision TIPS procedures. Portal and systemic venous pressures are measured and another portal venogram is obtained (Fig. 136.9). If the gastroesophageal varices or gastrorenal or splenorenal shunts continue to fill despite a satisfactory portal–right atrial gradient, the collaterals are embolized with coils, alcohol, or *n*-butyl cyanoacrylate.

A portosystemic gradient of less than 12 mmHg after creation of a TIPS is deemed satisfactory. The hemodynamic alterations caused by TIPS are similar to those caused by H-mesocaval and side-to-side portacaval shunts, but there appears to be slightly better preservation of hepatic portal perfusion following the TIPS. The flow through the shunt can be controlled by modifying the size of the shunt. Intrahepatic portal vein blood flow is usually reversed after creation of TIPS, and when injected into the portal vein, contrast medium will flow directly toward the shunt into the IVC and right atrium. The intrahepatic portal venous branches will not fill. No benefit in terms of shunt patency and survival has been demonstrated by using a 12-mm stent [60].

The transfemoral approach is used in patients with severely stenotic, angulated, or thrombotic hepatic veins, which make the standard jugular approach impossible. The procedure involves the placement of loop snares in the right portal vein and hepatic segment of the IVC and puncture of the IVC from the portal vein side using a sheathed needle. A guidewire is then advanced into the IVC and is snared and withdrawn through the transjugular sheath. A sheathed, 16-gauge Colapinto needle is advanced over the guidewire into the right portal vein. The parenchymal tract is dilated, and a stent is deployed from the main portal vein into the IVC.

Surveillance

Doppler US is an effective noninvasive screening tool for assessing TIPS patency and detecting an increased portosys-

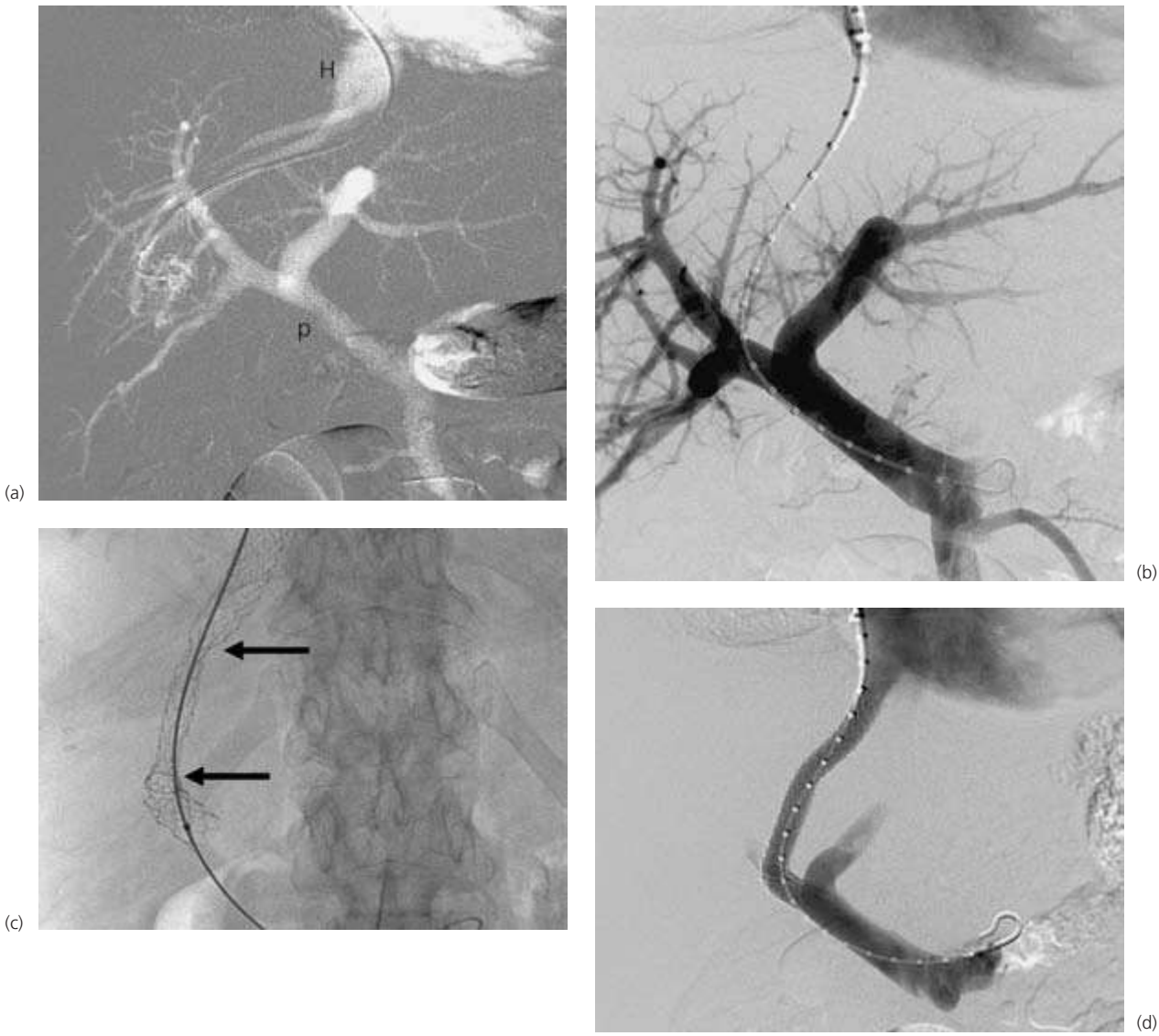


Figure 136.9 Transjugular intrahepatic portosystemic shunt (TIPS) in a patient with portal hypertension and variceal bleeding unresponsive to endoscopic therapy. **(a)** CO_2 wedged hepatic venogram shows hepatic (H) and portal (p) veins. **(b)** Transjugular catheterization of the portal vein. The transjugular portal vein catheter has been introduced into the portal vein from the right hepatic vein. Contrast medium fills the portal vein. The portal venous blood flows toward the liver (hepatopetal). **(c)** A covered stent (GORE VIATORR Endoprosthesis) is placed from the right portal vein to the right hepatic veins (arrows). **(d)** Following balloon dilation of the stent to a diameter of 10 mm, contrast injection fills the TIPS stent. The portal blood flows through the shunt directly into the right atrium. The intrahepatic portal veins are not seen due to reversal of portal blood flow. The variceal bleeding has stopped after TIPS.

temic gradient. Ultrasound examination after placement of TIPS is undertaken before discharge from the hospital and 3, 6, 9, and 12 months after the shunt. If US indicates shunt stenosis or occlusion, portal venography and pressure measurements are performed through the right internal jugular vein. Midshunt velocities of less than 60 cm/s, main portal vein velocities less than 40 cm/s, or interval change from hepatofugal to hepatopetal intrahepatic portal flow at US examination should suggest shunt stenosis [61,62]. Because US screening of TIPS is not 100% sensitive or specific, angiographic evaluation may be required if clinical suspicion for stenosis is high. Helical CT angiography has been used to identify both normal and abnormal TIPS and to image focal or diffuse stenoses of the stents and hepatic vein [63].

Therapeutic procedures can be performed easily on a failing TIPS in the outpatient setting. Patients whose shunts are occluded at US should undergo revision of their shunts. The shunt is catheterized from the jugular or femoral vein, and pressures are measured at the right atrium, IVC, hepatic vein, shunt, and portal vein. A portosystemic gradient of greater than 15 mmHg usually indicates a malfunctioning shunt, and a portal venogram will disclose a stenosis anywhere from

the portal vein to the hepatic vein. An angioplasty balloon catheter with the same size of the shunt can be used to dilate a narrowed shunt. If this is unsuccessful, a metallic stent or a covered stent is placed across the lesion.

Complications

The risks of TIPS vary greatly with the patient's bleeding status at the time of shunt creation. Data suggest that the 30-day survival of patients undergoing a TIPS procedure is approximately 95%. However, in patients who are actively bleeding and who undergo TIPS, a 55% mortality rate has been seen [64]. Major complications include hemoperitoneum, gallbladder puncture, stent malposition, hemobilia, hepatic infarction, renal failure requiring dialysis, hepatic artery injury, accelerated liver failure, severe encephalopathy, cardiac perforation, intraperitoneal hemorrhage, and death [65–68]. The reported incidence of new or worsened encephalopathy after TIPS is 20% [53,54]. In almost all cases, the encephalopathy occurring after TIPS creation can be controlled medically. A balloon catheter can be used to occlude the shunt temporarily in a patient who develops refractory hepatic encephalopathy. Balloon occlusion of the shunt for more than 12 h produces shunt thrombosis and improves encephalopathy [69] (Fig. 136.10). If the patient develops variceal bleeding after TIPS occlusion using an occlusion balloon catheter, shunt recanalization can be performed. The flow through the shunt can also be reduced by placing a WALLSTENT with constriction [70]. Minor complications include transient contrast-induced renal failure, encephalopathy controlled by medical therapy, transient pulmonary edema, and puncture site hematoma.

Outcome and prognosis

Patients with TIPS require intensive follow-up for at least 2 years after shunt placement, with shunt revision frequently being required. Primary shunt patency at 1 year after placement is only 25%–60% [52,71,72]; however, with careful monitoring and treatment of shunt stenoses with balloon dilation, additional stent placement, or both, a primary-assisted patency rate of 83%–96% can be achieved 1 year after placement. Some patients require more than one reintervention.

Acute and subacute TIPS thrombosis occurs in up to 10% of cases. Rarely, TIPS thrombosis may extend into the portal, splenic, and mesenteric veins. Percutaneous management in such cases includes thrombolysis, balloon embolectomy, suction embolectomy, and basket extraction of clot. Long-term results of each of the treatments are unknown, but a combination of methods may be required to restore mesenteric blood flow through the TIPS [73].

Inadvertent traversal of bile ducts during creation of the intrahepatic tract results in biliary-to-TIPS fistulae that result in intimal hyperplasia and eventual shunt occlusion. Testing the parenchymal tract with injections of contrast material is useful in detecting inadvertent traversal of the bile duct before stent placement. If stent occlusion is associated with biliary fistula, stent-grafts such as VIATORR can be used to isolate the shunt from the fistula, and to help prevent reocclusion [74,75].

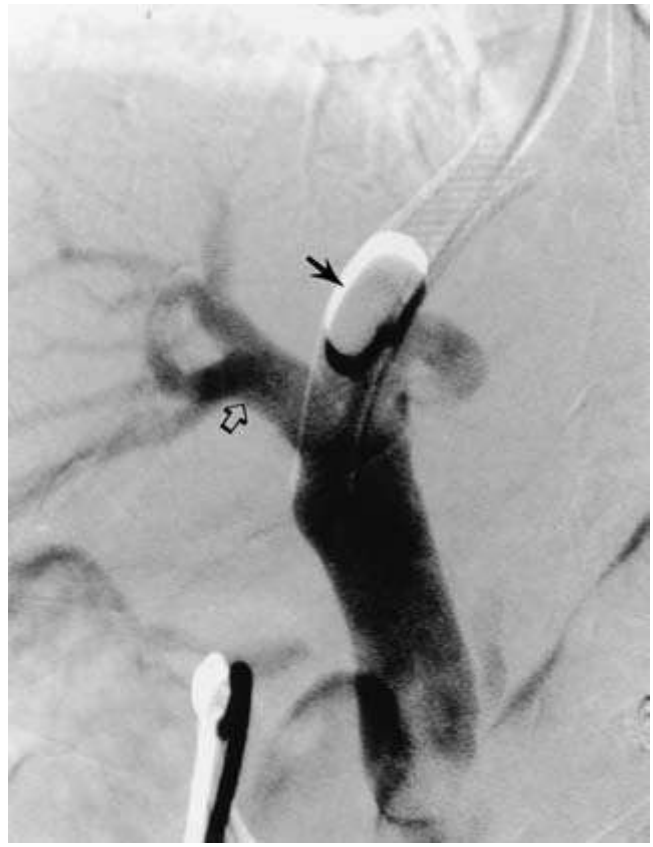


Figure 136.10 Temporary balloon occlusion of transjugular intrahepatic portosystemic shunt (TIPS) for refractory hepatic encephalopathy complicating TIPS placement. Balloon occlusion of the shunt (arrow) for 24 h improved hepatic portal perfusion and encephalopathy. The portal venogram shows hepatopetal blood flow in the right portal vein (open arrow). Subsequently, a smaller shunt (7-mm diameter) was placed. One month later, the patient underwent successful hepatic transplantation.

The long-term patency rate of TIPS remains to be determined. Unless the stenosis rate of TIPS can be reduced, either with covered stents or pharmacological therapy, TIPS will have to be thought of as a temporary treatment of portal hypertension. Even as a temporary treatment, TIPS can effectively control variceal bleeding and stabilize patients who will undergo liver transplantation. As a method to control bleeding in Child class C patients when other therapy has failed, TIPS is useful. In these patients, who have short life expectancies, a durable procedure is not required.

The role of endoscopic therapy and TIPS in elective treatment of variceal bleeding remains to be determined. Transjugular intrahepatic portosystemic shunt is more effective in preventing recurrent bleeding than endoscopic therapy but may result in encephalopathy. Both procedures require repetitive reintervention, and survival rates are similar.

Biliary intervention

Percutaneous transhepatic cholangiography

Diagnostic information about the biliary tree, bile leakage, or intraductal lesions should first be obtained by noninvasive tests – CT, US, MR imaging, biliary scintigraphy, or endoscopic US. When further diagnostic information or the opportunity for therapy is required, endoscopic retrograde cholangiopancreatography (ERCP) or percutaneous transhepatic cholangiography (PTC) are indicated. The choice between ERCP and PTC depends on local expertise, indication for the procedure, and the patient's anatomy.

The indications for PTC include:

- differentiation of obstructive from nonobstructive jaundice
- identification of the cause of ductal obstruction
- delineation of congenital anomalies
- determination of the site of biliary leakage
- documentation of communication of hepatic abscess with the biliary tree
- visualization of ductal anatomy before percutaneous transhepatic biliary drainage (PTBD).

Contraindications include:

- uncorrectable coagulopathy
- history of severe reaction to iodinated contrast medium
- uncooperative patient (may require general anesthesia)
- massive ascites
- the presence of a vascular tumor or arteriovenous malformations.

Risks include bleeding, sepsis, bile leakage, and death, with a 3% morbidity rate and a 0.1% mortality rate [76].

Technique

Coagulation tests are performed on all patients, and significant abnormalities are corrected by the administration of fresh-frozen plasma, platelets, or both. Because of the high incidence of bacterial colonization of the biliary tree in patients with biliary obstruction, broad-spectrum intravenous antibiotics, such as ceftriaxone, are administered before and for 48 h after the procedure [77].

The imaging studies should be reviewed for the presence of intrahepatic masses and the distribution of intrahepatic biliary dilation. The puncture site is chosen in the midaxillary line midway between the costophrenic angle and the lower margin of the liver. Selection of a puncture site caudal to the 10th rib usually helps avoid the pleural space. In certain situations, an anterior approach to the left hepatic duct is used. After the puncture site is selected, the area is washed with 10% povidone-iodine antiseptic and isolated with drapes. The skin and planned puncture tract is anesthetized with 2% lidocaine. The skin nick is made in the caudal portion of an intercostal space to avoid the intercostal vessels and nerves.

Under fluoroscopic control or US guidance, a 22-gauge, skinny needle is directed, in a plane parallel to the table top, to a position 3 cm cephalad to the hilum of the liver. The position of the hilum is estimated fluoroscopically by observing the position of the dome of the liver and the air in the first and second portions of the duodenum. The stylet is removed and the needle is connected through a connecting tube to a contrast-filled syringe. The needle is slowly withdrawn while small amounts of nonionic contrast medium are injected. Alternatively, contrast medium may be injected as the needle is slowly advanced through the parenchyma to avoid portal or hepatic vein branches and rarely hepatic artery [78].

When the needle tip is in the hepatic parenchyma, the contrast agent remains adjacent to the needle tip or opacifies the hepatic sinusoids. When the tip of the needle is in a hepatic artery or portal vein, the contrast agent washes out promptly to the liver periphery. When the tip of the needle is in a hepatic vein, the contrast agent washes out promptly toward the IVC. When the tip of the needle is in a biliary duct, the contrast material flows away slowly to reveal branching ducts as well as the central bile ducts. When the tip is in a lymphatic duct, the contrast material enhances small channels that run toward the liver hilum, often filling the lymph nodes and thoracic duct.

If no bile ducts have been encountered by the time the needle has been withdrawn to within 2–3 cm of the liver edge, the stylet is reinserted and the needle readvanced to a slightly different position. With dilated ducts, the biliary tree can be punctured almost always within several needle passes. When the intrahepatic ducts are not dilated, additional needle passes frequently are required.

For optimal visualization of the left hepatic ducts, or if a left-sided drainage is anticipated, a skin puncture site is chosen below the xiphoid process with the help of real-time US. A peripheral location in a duct of the lateral segment of the left lobe is chosen to be the puncture site so that if drainage is desired, the catheter will have a long segment with multiple side holes within the hepatic duct above the obstruction. The needle is angled parallel to the duct in the axial plane toward the hilum of the liver and at a 30°–50° angle to the table top. The needle is advanced under real-time US guidance and its position is confirmed with injection of contrast medium. If a left-sided drainage is needed after a right-sided drainage, air or CO_2 is injected into the right hepatic duct to fill the left hepatic duct for a target for a puncture.

If the site of obstruction has previously been determined by cross-sectional imaging and biliary drainage is to be performed, only a small amount of contrast agent is injected to assist guidewire placement. If a diagnostic study is to be performed, first an attempt is made to aspirate as much bile as possible, although typically, little or no bile can be aspirated through the fine needle because of its viscosity. Any bile obtained is sent for a Gram stain, and culture and sensitivity tests.

Iodinated contrast medium is slowly injected, and the contrast medium can be seen to flow into the most dependent ducts. Distention of the biliary tree is minimized to avoid

causing bacteremia. By tilting the table, the radiologist takes advantage of the higher specific gravity of contrast material, allowing it to flow to opacify ducts not seen with the patient in the supine position. Radiographs are obtained in multiple obliquities, and the needle is removed. The patient is placed on bed rest for 6 h, and vital signs are monitored.

Fine-needle PTC is successful in 98% of patients with dilated ducts and in 70% of patients with nondilated ducts [76]. Major complications of PTC are sepsis, cholangitis, bile leak, hemorrhage, or pneumothorax, occurring in 2% of cases [77].

Percutaneous transhepatic biliary drainage

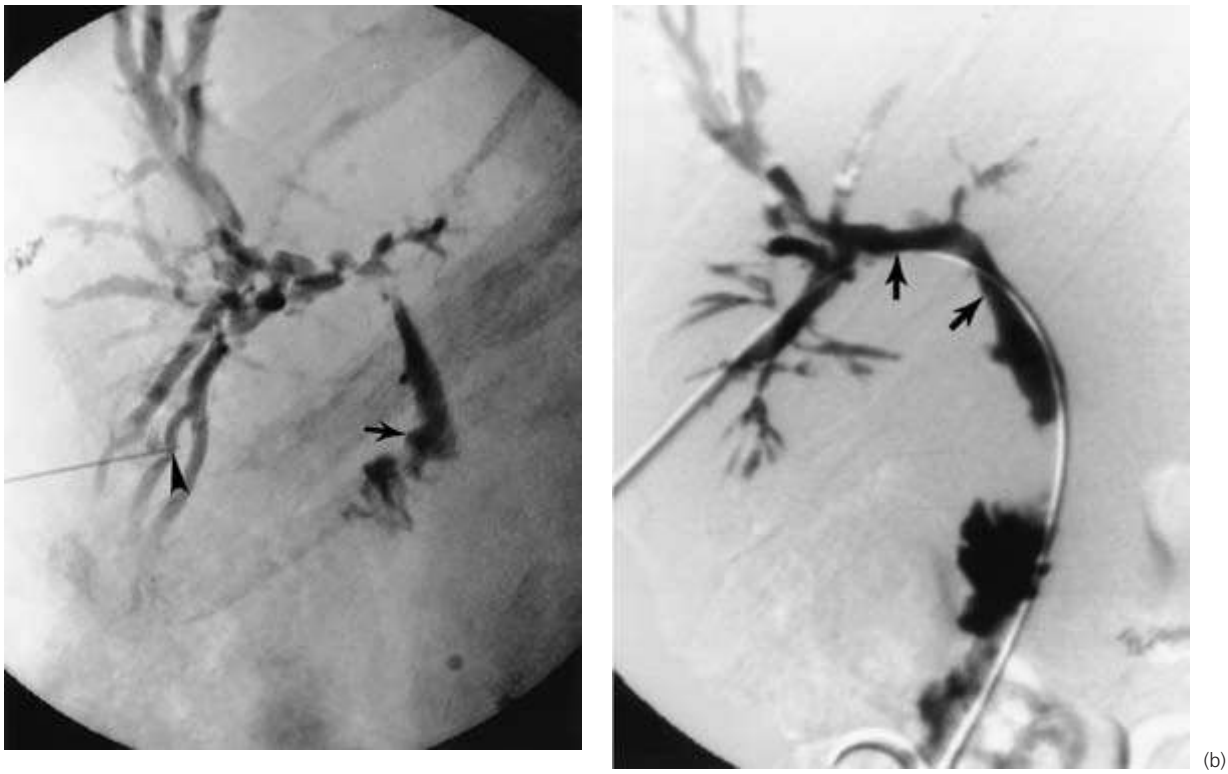
Nonoperative biliary drainage can be performed percutaneously or endoscopically in patients with biliary obstruction. Endoscopic techniques should be used when possible because the morbidity of an endoscopic approach is lower than that of PTBD. With biliary obstruction at or above the hilar level, ERCP has a higher failure rate, making PTBD frequently necessary in these patients (Fig. 136.11). In technically difficult situations in which endoscopic access has failed, a guidewire can be placed into the duodenum transhepatically to facilitate repeat ERCP and intervention. These "combined procedures" are associated with increased complications but have high success rates.

The indications for PTBD include:

- palliation of obstruction of the biliary tree by tumor
- treatment of cholangitis
- nonoperative removal of common bile duct or intrahepatic stones when ERCP is unsuccessful
- access for dilation of benign biliary strictures
- diversion of bile for treatment of biliary leaks or fistulae.

An uncorrectable coagulopathy is a contraindication to PTBD. Prominent ascites is a relative contraindication because the liver is displaced from the abdominal wall. This displacement can cause buckling of guidewires and catheters between the abdominal wall and liver, making the procedure difficult. Also, in patients with ascites, a risk exists of creating a peritoneal–cutaneous fistula with chronic leakage of ascitic fluid. Paracentesis can be performed before PTBD to minimize these problems.

The complications of PTBD include bleeding, sepsis, cholangitis, bile leakage, hemorrhage, pancreatitis, peritonitis, and death. Immediate significant complications are seen in 5%–10% of patients [79]. Patients who have chemotherapy-induced biliary sclerosis and obstructive jaundice have a higher incidence of bleeding complications with development of central pseudoaneurysm following PTBD [80]. After access to the bile duct has been made, the tract may be



(a) (b)

Figure 136.11 Percutaneous transhepatic cholangiography and cholangioplasty in a 46-year-old woman who 9 years earlier had undergone orthotopic liver transplantation for primary sclerosing cholangitis. **(a)** Contrast medium was injected into a peripheral branch of the right hepatic duct through a 22-gauge needle (arrowhead) showing multiple intrahepatic and hilar biliary strictures. Contrast medium flows through the choledochoenterostomy (arrow) into the bowel. **(b)** Cholangioplasty with a 6-mm-diameter balloon catheter improved biliary strictures (arrows). The left hepatic duct has been occluded.

injected with contrast medium to exclude communication with large vascular structures. If vascular communication is seen, the access should be abandoned and a new puncture of a peripheral bile duct is made. This technique reduces the incidence of hemobilia [81]. The overall mortality reported with PTBD is 1.7%.

Technique

The puncture of a bile duct for biliary drainage is the same as that for PTC. After a small injection of contrast medium confirms the needle position in a bile duct, a 0.018-inch (0.46-mm) wire is passed through the needle into the bile duct. Over the guidewire, a 6.3-Fr introducing catheter with a stiffening cannula is advanced into the common bile duct. The stiffening cannula and the wire are removed, and bile is aspirated. After the biliary tree has been decompressed, a cholangiogram is performed with injection of contrast medium in the bile duct near the obstruction. The introducer is then exchanged for a 50-cm catheter with an angle tip (Kumpe catheter, Cook Inc., Bloomington, IN). A 7-Fr sheath may be inserted into the right hepatic duct to prevent escape of bile into the circulation during catheter manipulation within the bile duct.

The catheter is then advanced to the point of obstruction, and a hydrophilic guidewire is manipulated through the obstruction into the duodenum. The catheter is advanced into the duodenum over the guidewire. The parenchymal tract is then dilated using sequential dilators over a 0.035-inch (0.89-mm), stiff guidewire, and an 8.5-Fr (outer diameter 2.8 mm) internal–external drainage catheter is advanced into the duodenum, its distal retention loop is formed, and the drawstring is locked at the catheter hub to prevent catheter displacement.

The drainage catheter is connected to a drainage bag to allow maximal drainage, which reduces the risk of sepsis. On the second day, the catheter is capped to allow internal drainage only. Once the internal drainage has been established, placement of a biliary endoprosthesis, if desired, is performed. If the guidewire does not traverse the occlusion, an external drainage catheter is placed above the obstruction, and the catheter is allowed to drain externally for 2–3 days. After a period of decompression, the obstruction usually can be crossed, and an internal–external drain can be placed. If the drainage is being performed for the treatment of biliary sepsis, no attempt is made to traverse the obstruction. Rather, an external drainage catheter is placed above the obstruction for drainage, and the obstruction should be crossed in several days after sepsis has resolved. Catheter and guidewire manipulation in purulent bile should be minimized because of the profound bacteremia that can ensue.

The success rate of PTBD is high, and most malignant biliary obstruction can be accessed. In patients with malignant biliary obstruction, bilirubin levels fall quickly (2 mg/day) after decompression and patients drained for sepsis improve within 24 h [82].

Percutaneous transhepatic cholangioplasty

An angioplasty balloon catheter can be used to dilate benign biliary strictures through endoscopy, a transhepatic access (Fig. 136.11), a mature T-tube tract, or a percutaneous transjejunal route [83]. Endoscopic dilation is generally preferred by the patient owing to the lack of an external appliance and has long-term success rates of 70%–85%, although it requires two to five ERCP sessions [84,85]. The usefulness of temporary stent placement after cholangioplasty, for both the endoscopic and percutaneous routes, is debated, and even among those who favor using stents the stent size and appropriate duration of placement is controversial. Long-term patency rates of percutaneous cholangioplasty are 67%–93% for anastomotic strictures [86–88], 76%–88% for iatrogenic strictures [86,87], and 42%–54% for strictures of primary sclerosing cholangitis [86,89]. Balloon dilation works well, is less expensive than placing stents, and will not interfere with a future operation, as might a metallic stent. Maccioni and colleagues [90] report a 69% 3-year patency rate in a series of patients composed of anastomotic stenoses and iatrogenic bile duct injuries.

Transhepatic balloon dilation is a safe and effective treatment for biliary stricture in liver transplant recipients [91]. The dilation procedure usually requires multiple sessions (one to six sessions; mean, three). Balloon sizes to be used depend on the estimated size of the duct and are usually 25%–30% larger than the duct. Balloon size is increased by 1–2 mm at each session and the balloon is inflated two to five times to a pressure of 10–12 atm (147–176 psi) for 10–20 min. After dilation, an 8.5–14-Fr drainage catheter is placed. The complications associated with cholangioplasty of biliary strictures include cholangitis, hematoma, bile leak, hemobilia, and pseudoaneurysm of the hepatic artery.

Percutaneous transhepatic biliary stent placement

Internal stents (endoprostheses) can be placed at ERCP or PTC to provide biliary drainage without an external catheter and thus improve quality of life. The problem with internal stents is limited patency, which ranges from 4 months to 1 year [92–96]. Endoscopically placed metallic biliary stents have longer patency than plastic stents owing to larger diameters but are not indicated in benign disease or in patients with operable tumors because they are permanent [95]. Endoscopic placement of internal stents is preferable to the percutaneous route because it can be achieved in a single session without the need for temporary percutaneous drainage. Percutaneous endoprosthesis placement is usually reserved for patients who have failed ERCP or who already have percutaneous access [97].

Hilar biliary stricture formation is a common complication of orthotopic liver transplantation, occurring in 10% of transplants [98]. Intrahepatic strictures unresponsive to balloon dilation have been treated with metallic stents with relatively poor long-term patency. Culp and colleagues [99]

reported on placement of 61 metallic stents in 36 liver transplant recipients with biliary stricture. Their primary patency was 44% at 3 years, with a secondary patency rate of 88%. Major stent-related complications occurred in 26% of their patients. Obstructed stents often require percutaneous drainage and additional stent placement to maintain patency [100]. Therefore, metallic stents play a limited role in the treatment of biliary stricture in the liver transplant patient. Metallic stents are not generally used for treatment of other benign biliary strictures [101]. In patients with malignant biliary strictures, a significant decrease in bilirubin level occurs in 83% following metallic stent implantation, and 30% of patients develop recurrent jaundice after an average of 97 days [102].

A variety of plastic and metal stents are in clinical use. Plastic stents require 12–14-Fr sheaths for placement, whereas metal stents can be placed through 7-Fr sheaths (Figs 136.12–136.14). Stent placement is usually done through the existing percutaneous transhepatic tract (Fig. 136.15) or endoscopically. The most common size of metallic stents is 10 mm diameter (4–8 cm in length), longer than the 10–14-Fr plastic stents. After the stent has been placed, an external drainage catheter is left above the stent for 48 h and with-

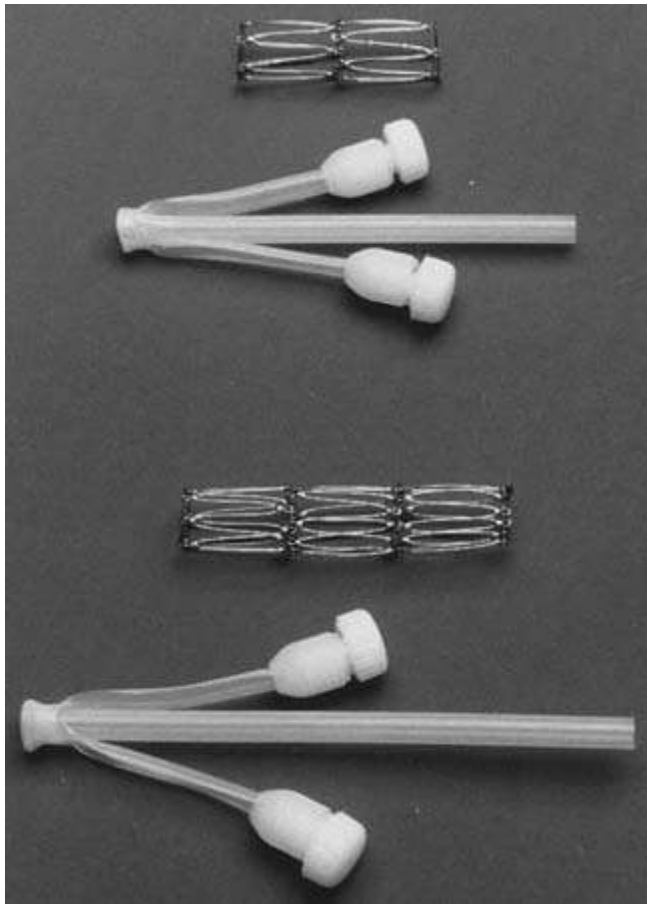


Figure 136.12 Two- and three-body Gianturco-Rösch stents and their loading devices.

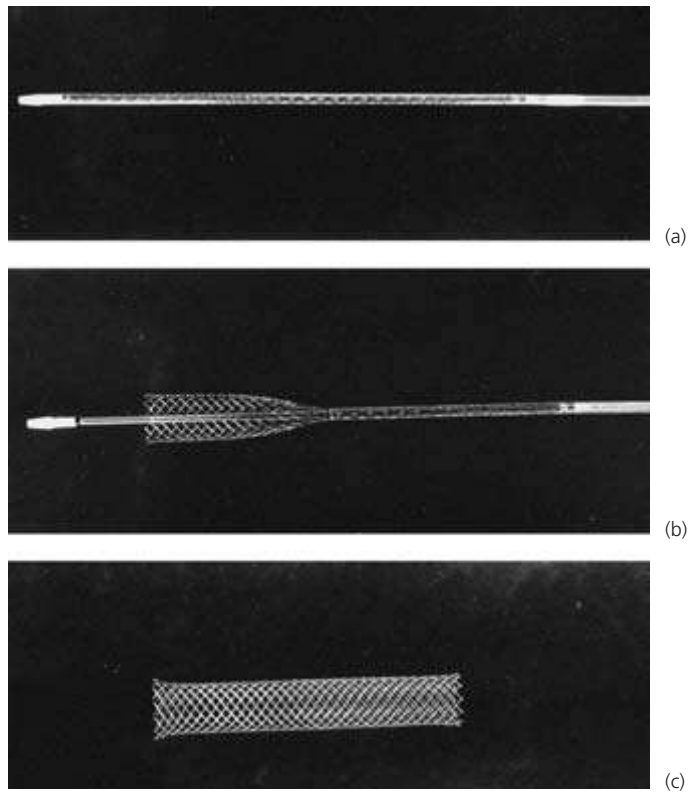


Figure 136.13 The WALLSTENT **(a)** in its constrained, elongated form, between two coaxial catheters and **(b)** partially deployed with the outer catheter partially pulled back over the inner catheter. **(c)** The fully deployed stent.

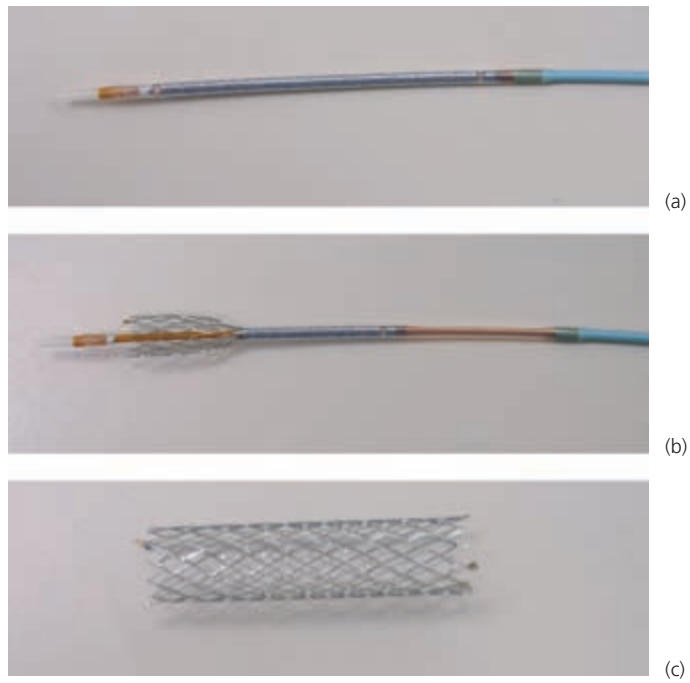


Figure 136.14 The Nitinol stent **(a)** in its constrained form and **(b)** partially deployed. **(c)** The fully deployed stent. Unlike the WALLSTENT, the nitinol stent has minimal shortening following deployment.

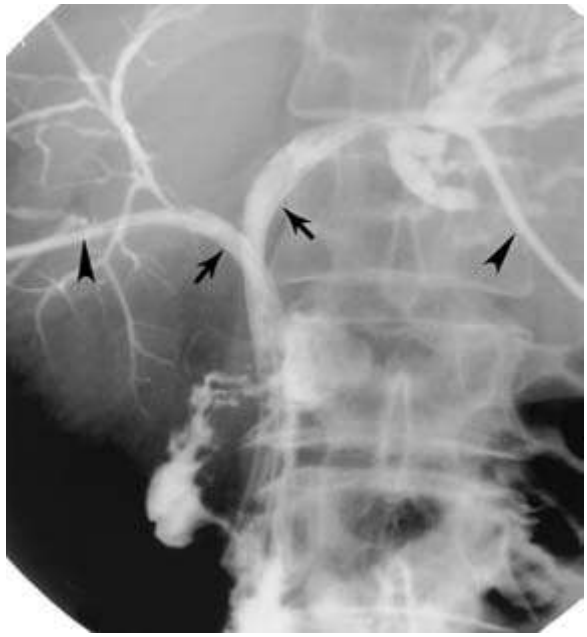


Figure 136.15 Transhepatic biliary stent placement in a 61-year-old man with Klatskin tumor. After bilateral percutaneous transhepatic drainage catheter placement for 6 months for hilar duct obstruction, 8-mm-diameter WALLSTENTS (arrows) were deployed simultaneously in both hilar ducts. The percutaneous catheters (arrowheads) were removed after demonstration of patency of the stents.

drawn following demonstration of stent patency. If there is no bleeding or other complications associated with stent placement, the external catheter is removed following stent placement.

The complications of endoprostheses are occlusion, migration, cholangitis, bowel obstruction, and perforation. The occlusion is usually caused by either tumor overgrowth or ingrowth or clogging of the stent by sludge or debris [103]. Covered stents may reduce the rate of tumor ingrowth in patients with malignant biliary obstruction, but their application should be limited to the common bile duct to avoid obstruction of the hepatic ducts [104].

Combined therapy with stent placement and brachytherapy with iridium 192 wire in patients with malignant biliary obstruction appears to extend stent patency to a mean of 19.5 months for cholangiocarcinoma and 4.8 months for metastatic disease [105,106]. The combination of arterial chemoembolization and brachytherapy following stent placement may result in additional palliation for biliary obstruction. Covered Gianturco stents have been used for malignant biliary obstruction, achieving longer patency rate without increased complication rates.

In patients with a Hutson loop (afferent limb of a Roux-en-Y choledochojejunostomy or hepaticojejunostomy sutured to the anterior abdominal wall), repeat access to the bile duct can be done for biliary drainage, cholangioplasty, or placement of biliary endoprostheses [107–109].

Percutaneous cholecystostomy

Percutaneous cholecystostomy (PC) has become an effective technique in the management of acute cholecystitis. The indications for PC include:

- suspected cholecystitis in severely ill patients unlikely to tolerate cholecystectomy [110]
- sepsis of unknown cause and a distended gallbladder in critically ill patients
- acalculous cholecystitis [111]
- obstructive jaundice because of distal common bile duct obstruction when PTBD and endoscopic drainage are unsuccessful [112]
- diagnostic cholecystocholangiography in patients without dilated intrahepatic ducts or in whom PTC and ERCP are unsuccessful
- gallstones requiring percutaneous removal or dissolution, in selected patients.

The relative contraindications for PC include the visualization of a necrotic or perforated gallbladder wall on US examination and an uncorrectable bleeding diathesis.

Once the PC tract is matured, percutaneous techniques – including contact dissolution using methyl-tert-butyl ether or monooctanoin, mechanical lithotripsy, electrohydraulic lithotripsy, and laser lithotripsy – can be used to remove gallstones. Aside from the cost and multiple sittings required for many of these procedures, the main failing is that the gallbladder remains in situ to reform stones in 20%–50% of patients [113], with an increased risk of gallbladder carcinoma [114]. The rapid evolution of laparoscopic cholecystectomy has supplanted these percutaneous techniques in most circumstances, because laparoscopic cholecystectomy does not leave the gallbladder in place. Percutaneous cholecystostomy should be performed in severely ill patients who may not tolerate laparoscopic cholecystectomy.

Technique

Intravenous antibiotics are given and coagulopathies are corrected before the procedure. Ideally, the procedure is performed in the angiography suite with a combination of US guidance for gallbladder puncture and fluoroscopy for guidewire and catheter placement. Extremely ill patients may be treated at their bedside with the entire procedure guided by US. The procedure is performed under local anesthesia.

Initially, PC was performed using a transhepatic approach in an attempt to puncture the gallbladder through the liver and avoid the possible complication of bile peritonitis [115]. Transhepatic transperitoneal punctures are common [116]. Subhepatic puncture has been shown to be safe and avoids the risk of bleeding from hepatic injury [117–119].

A variety of techniques are used for PC, including the standard Seldinger technique with needle puncture, guidewire placement, removal of the needle, tract dilation, and advancement of a drainage catheter over the guidewire into the gallbladder. Another technique more suitable for use at the

bedside involves the use of a trocar. A tapered catheter with an inner stiffener and a central mandril with a sharp point are advanced into the gallbladder under US guidance. Another technique appropriate for bedside use involves a long, 22-gauge needle with a 6-Fr Teflon catheter loaded on the back of the needle. The needle is placed into the gallbladder under US guidance and its mandril is removed. Injection of a small volume of saline and observation with US can be used to confirm the intralumenal position of the needle tip if the bile is too viscous to be aspirated through the long 22-gauge needle. A 0.046-cm guidewire is advanced through the needle and the needle tip is positioned in the center of the gallbladder. The catheter is advanced over the needle–wire unit into the gallbladder. The needle and guidewire are removed, and the monofilament suture present within the catheter is tightened to accordion the catheter into a Z-shaped self-retaining configuration.

Bile is obtained for aerobic and anaerobic bacterial cultures, and the catheter is secured to the skin. Although a small amount of contrast medium may be injected to confirm catheter position, if the PC is being performed for cholecystitis, a cholecystogram should not be performed at this time to reduce the risk for gallbladder distention and bacteremia. The catheter then is connected to gravity drainage. The use of a self-retaining catheter is essential to reduce the risk of catheter dislodgment and bile leakage.

A diagnostic cholecystocholangiogram can be performed to evaluate cystic duct patency and cholelithiasis after 48 h and after the patient's condition stabilizes (Fig. 136.16). Options for definitive therapy include surgical or laparoscopic cholecystectomy [119], percutaneous stone removal, or, if the patient had acalculous cholecystitis and cystic duct patency is confirmed, eventual removal of the catheter with no further treatment. The catheter cannot be removed until the tract is mature or bile leakage can occur. Patients with transhepatic PC catheters have mature tracts at 20 days. If a question exists about the maturity of the tract, it can be evaluated under fluoroscopy with injection of contrast medium. For patients with severe nonbiliary problems, the catheter frequently is left in place until other medical problems resolve.

Figure 136.16 Percutaneous cholecystostomy in a 52-year-old man with cardiomyopathy and acute cholecystitis. A hepatobiliary scan showed no filling of the gallbladder. Percutaneous ultrasound-guided cholecystogram shows partially filled gallbladder (arrow). The cystic duct did not fill.

Complications

The complication rate is 5%–8%; complications include bile leakage, vasovagal reaction, catheter dislodgment, hemorrhage, and a 0.5% procedure-related mortality rate [120]. In comparison, emergency surgical cholecystostomy has a mortality rate of 8% [121].

Results

Percutaneous cholecystostomy for presumed cholecystitis in critically ill patients is useful for diagnosis and treatment. Although initially it was thought that Gram stain and culture of aspirated gallbladder contents would be useful to diagnose cholecystitis, many of these patients are already on broad-spectrum antibiotics. The 48% sensitivity rate of Gram stain and 38% sensitivity rate of bile culture are sufficiently low to make gallbladder aspiration a procedure of limited value [120]. However, PC is useful as a clinical diagnostic test. Essentially all patients who have cholecystitis without a necrotic gallbladder wall will improve and normalize their leukocyte count after drainage [111,122].

Severely ill patients who have ultrasonographic evidence of a necrotic gallbladder wall are less likely to respond to PC and should undergo immediate operation or a trial of PC with operation to follow if no clinical improvement is seen in 24 h, depending on the patient's risk for surgery [123]. In patients without a necrotic gallbladder wall and no response to PC, cholecystitis is excluded, and the search for a non-gallbladder source of sepsis should continue.

Biliary duct biopsy

The most difficult diagnostic problem of bile duct lesions is differentiating between malignancy and inflammatory disease in the patient with biliary strictures. Bile aspiration yields only 35% positive cytology. Brush biopsy, clamshell forceps biopsy, and shave biopsy are somewhat more sensitive, with sensitivities of 44%–67% reported [124–126].

Endoscopically, a combination of tissue sampling techniques, such as direct forceps biopsy, brush cytology, or aspiration cytology has the highest yield [127]. An advantage of endoscopic over percutaneous tissue sampling is that all techniques can be performed during the initial procedure.

For the percutaneous route, in the absence of bacteremia or hemobilia following drainage, biopsy can be performed 1–2 days after the procedure [128]. Broad-spectrum antibiotics are administered before the procedure. The transhepatic drain-

age catheter is exchanged over a guidewire for an introducer, which is advanced distal to the stricture or to the duodenum, and a second safety guidewire is placed into the duodenum. A 3-mm bronchoscopy brush or a removable ureteral biopsy brush is advanced through the sheath or an introducing catheter beyond the lesion. After brushing of the lesion has been performed, the brush is pulled into the introducing catheter or the sheath before withdrawal. The brush is cut from the wire and submitted for cytological examination.

Another technique for biliary biopsy involves the use of forceps (Fig. 136.17). After placing a safety wire in the duodenum, a 7-Fr peel-away sheath is placed immediately proximal to the lesion, a 5.2-Fr or a 7-Fr flexible clamshell forceps is advanced to the lesion, and biopsy is performed. Clamshell forceps biopsies can also be performed through a choledochofiberscope.

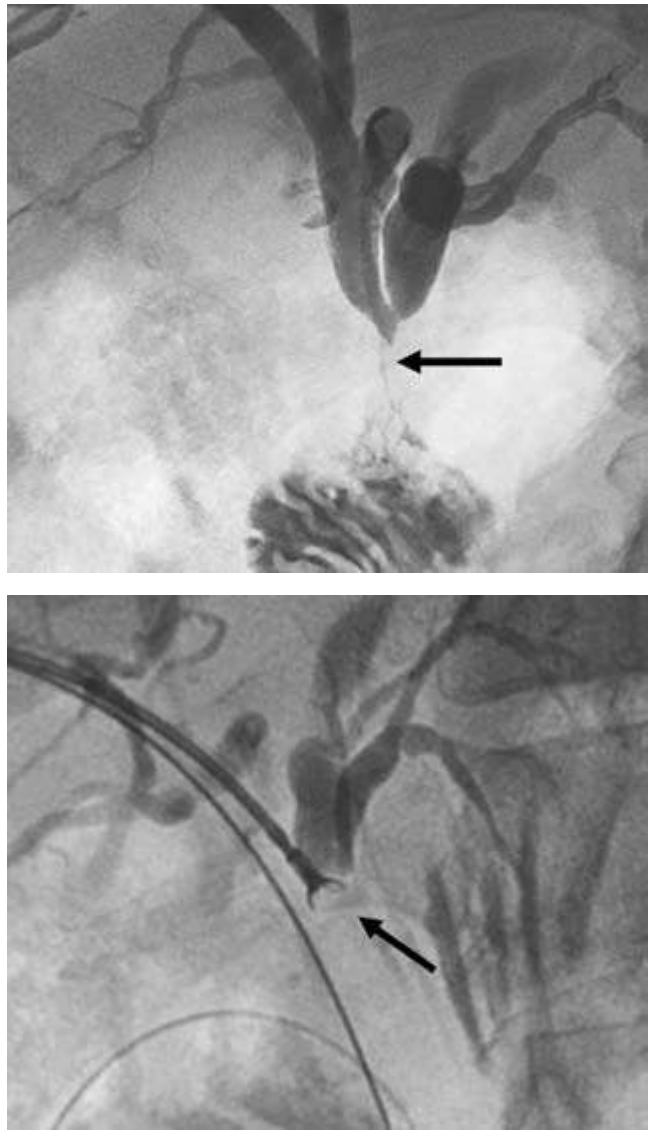


Figure 136.17 Forceps biopsy of the bile duct in a 43-year-old man with bile duct stricture after a Whipple procedure for pancreatic cancer. **(a)** Percutaneous cholangiogram shows stricture at the choledochal–enteric anastomosis (arrow). **(b)** Forceps biopsy (arrow) was performed through the transhepatic route. The result of the biopsy showed scar. A safety wire is seen crossing the stricture into the bowel.

Laparoscopic injuries to the biliary system

Both endoscopic and interventional radiological management plays an important role in the treatment of patients with bile duct injuries resulting from laparoscopic cholecystectomy. The injuries include biliary obstruction from bile duct transection or stricture, biliary leakage with formation of biloma, and retained stones. Ultrasound or CT is the initial imaging method used for the diagnosis of the biliary complications; ERCP is then performed to obtain the necessary anatomical information for therapy. If ERCP is unsuccessful or unable to cross the obstruction secondary to inadvertent ligation of the bile duct, a drainage catheter must be placed above the obstruction.

Biliary strictures that are not amenable to surgical intervention may be treated with percutaneous or endoscopic balloon dilation. Before the procedure, the patient is given broad-spectrum antibiotics. The procedure can be done on an outpatient basis with conscious sedation and local anesthetic. After placing a 0.035-inch (0.89-mm) guidewire across the stricture, a balloon catheter (usually 8–10 mm in diameter) is placed over the guidewire and inflated two to three times for 3–5 min. A drainage catheter, such as an 8.5-Fr Cope loop catheter, is left across the stricture for external drainage, and the treatment effectiveness is then assessed in 2 weeks. If a satisfactory response is demonstrated by a follow-up cholangiogram, a catheter is placed above the stricture and capped to allow internal drainage for 1–2 weeks before withdrawal of the catheter. Metallic stents should be reserved for patients with a recurrent biliary stricture following multiple balloon dilations and who are not candidates for surgery.

Bile leak is treated with temporary diversion of bile through endoscopic biliary intubation or transhepatic biliary drainage. After 3–4 weeks of bile diversion, the plastic stent is removed over a guidewire, and a cholangiogram is performed through a sheath to show healing of the leak. If no leak is demonstrated, a drainage catheter is placed above the area of leakage and capped for 1–2 weeks to reassess bile duct leak before removal of the catheter. If the leak is associated with significant bile collection, percutaneous drainage is performed under CT or US guidance.

Retained common bile duct stones may be found after laparoscopic cholecystectomy. Endoscopic extraction after sphincterotomy is the preferred method in such patients. If an endoscopic approach is unsuccessful, a percutaneous transhepatic approach may be used. Stone extraction should be deferred for 4–6 weeks to allow the tract to mature after placement of a transhepatic catheter. Alternatively, combined ERCP and PTC, with the PTC wire facilitating endoscopic access, may be used.

Biliary manometry

Biliary manometry is used to assess bile pressure and flow dynamics. This is different from the sphincter of Oddi manometry performed at ERCP, which measures sphincter muscle function. Measurement of bile pressure at PTC has been shown to be useful in predicting long-term success after treatment of biliary strictures with balloon cholangioplasty [129].

The patient is treated with prophylactic antibiotics before the examination. In patients with transhepatic biliary intubation, the indwelling tube is removed over a guidewire and an end-hole catheter is placed through a sheath above the treated stricture and connected to a manometer. Pressure is measured at the level of the midaxillary line. Continuous pressure measurements are obtained before and during perfusion of 30% contrast medium at the rate of 5 mL/min for 5 min, then 10 mL/min for a further 5 min. If the biliary pressure still remains less than 30 cmH_2O, the perfusion rate can be increased to 15 or 20 mL/min for 2 min. The perfusion should be discontinued if the patient experiences right upper abdominal pain or chills, or if the biliary pressure increases to more than 30 cmH_2O. In the absence of a significant stricture, the biliary pressure will remain below 20 cmH_2O during the perfusion.

Percutaneous biliary stone extraction

Endoscopic sphincterotomy or papillary balloon dilation with stone extraction is the primary mode of treatment for choledocholithiasis [130]. However, if a T-tube tract or transhepatic access is available, radiologically guided stone extraction may be performed. Intrahepatic stones occasionally are inaccessible at ERCP and require the percutaneous transhepatic route. Choledoscopy facilitates stone extraction [131]. Percutaneous stone extraction is a radiologically guided procedure for the treatment of biliary stones not amenable to surgical, endoscopic, or extracorporeal shock-wave therapy [132]. Stone removal can be done through the preexisting, mature transhepatic biliary drain tracts, T-tube tracts, cholecystostomy tube tracts, and hepaticocutaneous enterostomy.

Basket removal of retained stones through the T-tube tract is a safe and simple method of removing retained stones. With the advent of the steerable catheter system, stone extraction has become a technically easy procedure. The Dormia-type basket is most commonly used for stone extraction. The steps for stone extraction are:

1. removal of the T-tube over a guidewire
2. insertion of a steerable catheter through the T-tube tract
3. extraction of the stone using a wire basket.

After the stone has been extracted, a drainage catheter is placed in the common bile duct. When no residual stones are present, the catheter is capped for 1–2 weeks of "clinical trial" before withdrawal of the catheter. Large stones must be fragmented by using crushing baskets or an electrohydraulic lithotriptor before extraction [133]. Extracorporeal shock-wave lithotripsy is effective in fragmenting bile duct stones but often requires extraction of fragments [134,135]. Percutaneous techniques may be used for removal of gallstones in patients with high surgical or general anesthetic risk [136]. Because the percutaneous method is a gallbladder-sparing procedure, it carries the risk for recurrence of stone (40%) and symptoms (12%).

Percutaneous management of biliary fistula

Biliary fistulae that originate in the liver may result from trauma, surgery (cholecystectomy, resection of hepatic tumor, and liver transplantation), or local infection, such as hydatid disease. Small biliary fistulae may close spontaneously, and no specific treatment is required. When a biliary fistula is associated with distal biliary obstruction, however, biliary decompression is usually required by using endoscopic, percutaneous transhepatic, or transbronchial approaches [137–140].

The nonoperative methods for managing biliary fistulae include external biliary drainage, nasobiliary catheter drainage, bile duct stent placement, and sphincterotomy. Selective embolization through a percutaneous transhepatic or endoscopic approach may be used for the treatment of biliary fistulae unresponsive to biliary decompression [141].

Percutaneous biopsy

Percutaneous image-guided sampling of abdominal lesions is a safe and effective method of obtaining material for cytological or histological analysis and for microbiological and chemical analysis of fluid collections. Advances in CT and US have allowed accurate localization of smaller and more subtle lesions, and experience has demonstrated the safety of percutaneous biopsy through windows previously thought to be unsafe.

Technique

The optimal imaging modality for guiding a needle biopsy depends on the position and visualization of the lesion, the patient's body habitus, the ability to introduce radiopaque contrast medium adjacent to the lesion, and the experience of the radiologist. Patients undergoing biopsy have their prothrombin time, activated partial thromboplastin time, and platelet count checked and corrected if necessary. Patients who are to receive intravenous contrast medium are hydrated and have their blood urea nitrogen and creatinine levels checked. Most patients do not require a sedative, but those who are unusually anxious or find it painful to lie in the required position are given midazolam, fentanyl, or both.

Fluoroscopy in multiple planes, most frequently with a C-arm, provides easy and efficient guidance for biopsy of large, superficially located lesions. Fluoroscopic landmarks, such as distance from a particular bony structure or position adjacent to a radiopaque stent, can be used to position the needle. If an indwelling biliary catheter is present, contrast medium

can be introduced to allow fluoroscopically guided biopsy of biliary duct lesions. Fluoroscopy also is useful for biopsy of abnormal lymph nodes identified at lymphangiography that still contain lymphangiographic contrast medium.

Real-time US has many advantages for intraabdominal biopsy. Perhaps its greatest advantage is that, unlike CT, it allows continuous monitoring of the course of the needle while the needle is being advanced, allowing instant readjustment of the needle path as necessary. Ultrasound guidance may be applied when the lesion is ultrasonographically visible and the needle path avoids gas-filled bowel – the soft tissue–gas interfaces reflect most of the sound waves and preclude visualization of the needle tip and the lesion. Ultrasound is also advantageous when the ideal needle path is at a steep angle to the axial plane because such an angle makes CT guidance more difficult. However, US guidance is difficult in obese patients because fat degrades the US image.

Computed tomography is most frequently used to guide biopsies that cannot be performed reliably with fluoroscopy or US, and CT usually is the imaging modality of choice for small, deep-seated lesions. Oral, intravenous, and rectal contrast medium is administered as needed for lesion visualization. To perform CT-guided biopsies, a localizing radiopaque grid is placed on the skin covering the region of the skin entry site as estimated from prior scans, and a scan at the level of the lesion is repeated. A needle path is chosen, preferably in the axial plane, and the skin puncture site is marked on the skin. The depth of the lesion and the angle between the needle path and a vertical line through the lesion are measured.

The types of needles available for percutaneous biopsy are nearly as numerous as the types of diseases for which biopsy is performed. Needles can be classified into those that obtain material for cytological analysis and those that obtain tissue for histological analysis. Needles from 25-gauge to 14-gauge are used: the smaller sizes predominantly for aspiration cytological study and the larger ones for obtaining a core of tissue. Aspiration cytological study with a skinny needle (20–25 gauge) is the most frequently performed percutaneous biopsy. When a tissue core is desirable, the spring-loaded, mechanically triggered biopsy needles (guns) are used. These needles have a Tru-Cut-type design, and once in position, the inner or outer parts (depending on the manufacturer) of the needle are advanced rapidly forward by a preloaded spring mechanism. These guns offer the benefit of producing less pain than the Tru-Cut needle, and some 18-gauge guns produce a specimen as good as that obtained with a 14-gauge Tru-Cut needle [142].

A needle path is chosen that avoids lung, gallbladder, spleen, and, if possible, bowel and vascular structures. Passing 21-gauge needles through the stomach and small and large bowel is safe [143], and some institutions routinely place 18-gauge needles through bowel without complication. It appears judicious to avoid puncturing the bowel in immunocompromised patients and those with ascites.

Local anesthetic and a small skin nick precede needle placement. The needle may then be advanced directly into position. If a larger needle is being used, a skinny needle may be first positioned within the lesion, then the larger needle may be placed parallel to the smaller needle. This allows the needle repositioning that is common with CT guidance to take place with the smaller needle, and allows the larger needle to be properly positioned on the first pass. A coaxial needle technique also is useful for small or difficult lesions. The technique involves placement of a larger needle up to the lesion with the biopsy performed through a smaller inner needle. This allows simple, repetitive sampling by removing only the smaller inner needle, assessing its yield and, if necessary, replacing the inner needle and repeating the biopsy.

Indications

In most cases, an aspiration biopsy is adequate to make the diagnosis of malignancy, although specific tumor characterization may require a core of tissue. Aspiration biopsy is useful in diagnosing acute liver transplant rejection, but core biopsy is more useful in diagnosing chronic rejection [144]. Core soft tissue should be obtained routinely whenever the cellular architecture is important for microscopic diagnosis (e.g., diffuse liver disease, likely benign focal liver lesions, lymphoma).

Contraindications and risks

A patient who will not lie still is an absolute contraindication to image-guided needle biopsy. It is impossible to target a moving lesion, and there is the risk for visceral injury. For biopsy of abdominal lesions, patients must be capable of holding their breath for a minimum of 10 s so that respiratory excursion of the lesion is suspended during needle placement. A bleeding diathesis is a relative contraindication depending on the severity of the diathesis, the location of the lesion, lesion vascularity, and the size of the needle required. If a coagulopathy would otherwise contraindicate biopsy and the lesion is intrahepatic, transvenous biopsy or biopsy with embolization of the needle tract (plugged biopsy) can be performed. Ascites does not affect the major or minor complication rate of image-guided hepatic biopsy [145].

Suspected hemangiomas have been listed as a contraindication to needle biopsy; however, after unintended aspirations of such lesions were reported, several authors reported series of safe biopsies of these two entities [146,147]. With use of a 20-gauge cutting needle, a percutaneous hepatic biopsy can be safely performed for histological diagnosis of hemangioma [147]. A needle path traversing normal liver before reaching the lesion is recommended to reduce the possibility of hemorrhage.

Percutaneous needle biopsy is a relatively safe, invasive procedure with low morbidity and mortality. Severe pancreatitis can occur after pancreatic biopsy and the incidence of pancreatitis increases when biopsy is performed on a normal pancreas. Needle tract tumor seeding after intraabdominal

biopsy is a rare occurrence. Traversing normal parenchyma before entering a lesion may reduce the risk for seeding. Hemorrhage is the most common complication of liver biopsy. Hemorrhage may occur as intraabdominal bleeding or hemobilia after creation of an arteriobiliary fistula. Severe hemobilia after needle biopsy is best managed with selective embolization of the bleeding artery. Severe intraabdominal bleeding may be more common if the needle does not initially traverse normal parenchyma.

Results

Image-guided hepatic biopsy using skinny needles is accurate in the diagnosis of malignancy in more than 90% of patients [148]. Accurate diagnosis of hemangioma can be made 100% of the time using a 20-gauge coring needle [147]. Skinny needle biopsy of the pancreas has been reported to be accurate 60%–90% of the time. The coexistence of pancreatic carcinoma and pancreatitis may result in biopsy of the inflammatory area, which frequently cannot be distinguished by CT or US from the neoplastic area. The possibility of sampling error suggests that an initial negative result from percutaneous pancreatic biopsy should not be accepted, and either a repeat percutaneous biopsy or an operative biopsy should be performed as warranted by clinical circumstances.

Percutaneous image-guided aspiration biopsy of lymph nodes has been performed more often than large-core biopsy for evaluation of lymphadenopathy. Fine-needle aspiration of lymph nodes using cytological examination for detection of metastatic carcinoma is highly accurate [149], and the accuracy of detecting lymphoma is about 80% [149]. Accurate typing of lymphoma by fine-needle aspiration remains problematic, but advances in immunocytochemistry [150] and flow cytometry may prove helpful. The accuracy of percutaneous core biopsies for classifying lymphomas remains to be determined.

Alternative techniques to biopsy

A number of catheter-based techniques have evolved that are useful in specific situations when percutaneous needle biopsy is difficult or contraindicated.

Transjugular liver biopsy

If coagulopathy or marked ascites are present, transjugular liver biopsy provides an alternative to percutaneous biopsy because the needle does not traverse the liver capsule, thereby avoiding hemorrhage [151]. An 18-gauge, 60-cm-long automated biopsy device has been designed for obtaining liver histological samples through a jugular venous approach.

The technique is similar to that used for the TIPS procedure. The right or left internal jugular vein is punctured using a 21-gauge, single-wall access needle under US guidance, and a 9-Fr sheath is inserted. Through the sheath a 5-Fr catheter is advanced through the right atrium into the IVC. Pressures are measured in the right atrium, IVC, hepatic, and wedged hepatic veins. A CO_2 wedged hepatic venogram is obtained to demonstrate the portal vein and to identify the right or left hepatic vein. A 7-Fr, 49-cm-long curved catheter is then advanced into the right hepatic vein over an Amplatz super-stiff guide wire. Through this sheath, a 14-gauge stiffening cannula is advanced into the right hepatic vein and directed anteriorly against the wall of the vein. Liver biopsy is performed near the IVC (Fig. 136.18). Biopsy from a peripheral hepatic vein may result in perforation of the hepatic capsule and increases the possibility of bleeding. This technique is successful in more than 90% of patients [151,152]. Major complications occur in 2% of patients and include cardiac arrhythmias and intraperitoneal hemorrhage from inadvertent capsular puncture. Transjugular liver biopsy with hepatic venography and manometry can provide the anatomical, hemodynamic, and histological information on the liver and hepatoportal venous system.

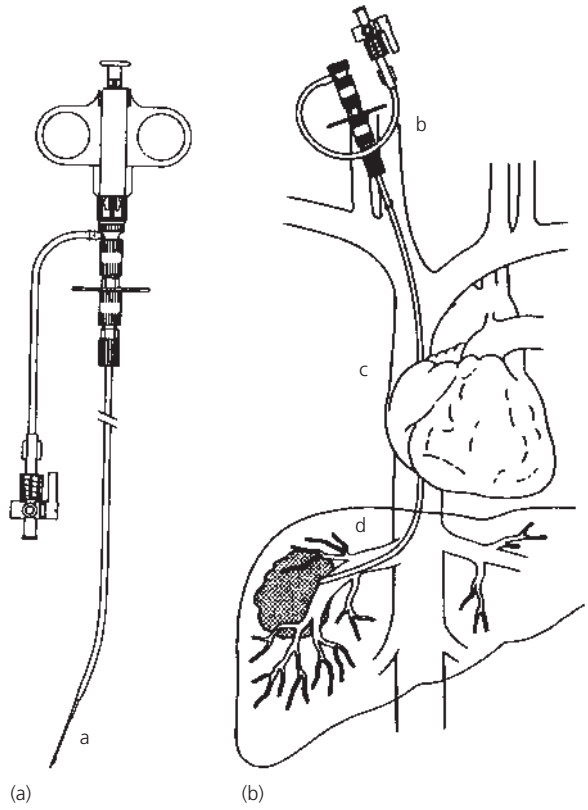


Figure 136.18 Transjugular liver biopsy. **(a)** Liver access set assembled with Quick-Core biopsy needle. **(b)** The preassembled liver access set has been introduced into the right hepatic vein over the guidewire from the internal jugular vein. The directional arrow on the hub of the stiffening cannula is used to direct the needle anteriorly and gentle pressure is applied to the wall of the central hepatic vein. The Quick-Core biopsy needle is advanced through the sheath into the liver tissue. The stylet is then advanced to expose the specimen notch (*a*), and the cutting cannula is fired to obtain tissue within the specimen notch. The biopsy needle is withdrawn and the tissue specimen is removed from the notch and placed in formalin. *b*, right internal jugular vein; *c*, superior vena cava; *d*, right hepatic vein.

Transhepatic liver biopsy with tract embolization

When a histological core of liver tissue is needed and coagulopathy or marked ascites preclude standard percutaneous liver biopsy, embolization of the needle tract within the liver can prevent bleeding from the liver capsule puncture site. Embolization of the tract is performed with a Gianturco coil [153] or with gelatin sponge plugs. The occluding materials may be introduced through the outer part of a Tru-Cut-type needle after the inner obturator and specimen have been removed, or through a 4-Fr (1.3-mm diameter) vascular sheath fitted over the needle before biopsy [154]. Introducing the embolus through the vascular sheath is useful because the sheath is much more flexible than the needle cannula and is less likely to cause injury while the patient breathes and the liver moves. The potential disadvantage of using the sheath is that a larger hole is made in the liver capsule than would be made by the needle alone.

Plugged biopsy is faster and usually less costly, and it does not require the same level of technical skill and experience required for transvenous biopsy. Plugged biopsy can be performed with imaging guidance to obtain a core sample of a focal lesion. Transvenous biopsy is not useful for most focal hepatic lesions because of the inability to steer the transvenous needle precisely. The published experience with plugged liver biopsy is too small to allow an accurate comparison of the risks of plugged liver biopsy with transvenous biopsy. Transvenous biopsy is considered safer for patients with severe coagulation disorders.

Percutaneous abscess and fluid collection drainage

Percutaneous abscess drainage (PAD) is effective in treating abdominal abscesses and fluid collections [155]. Percutaneous needle aspiration is helpful in determining the nature of any intraabdominal fluid collections, including abscess, biloma, lymphocele, seroma, loculated ascites, hematoma, and urinoma. Percutaneous catheter drainage is indicated for the treatment of infected fluid collections (e.g., hepatic and diverticular abscesses) and symptomatic fluid collections (e.g., pancreatic pseudocyst and lymphocele). Contraindications include absence of a safe access route to the fluid collection and coagulopathy.

Technique

Percutaneous needle aspiration and catheter drainage usually require CT or US guidance. Real-time US is most useful if the fluid collection is easily seen and a safe access route can be found, such as retroperitoneal, hepatic, or splenic drainage. C-arm-supported CT fluoroscopy is useful in percutaneous abscess drainage procedures [156]. Computed tomography guidance is preferred for drainage of deep fluid collections, and is helpful in differentiating bowel loops from abscess. All patients undergoing abscess drainage receive intravenous broad-spectrum antibiotics prior to the catheter placement, and in anticipation of possible drain placement, bleeding diatheses are corrected.

The methods in general use for percutaneous drainage of the intraabdominal abscesses include the Seldinger method using guidewires, dilators, and a drainage catheter; and the trocar method using a drainage catheter with an inner stiffener and central pointed trocar. The trocar catheter is advanced in a single pass, parallel to the initial needle, and to the same depth. The trocar technique obviates guidewire exchange and tract dilation, and also theoretically reduces the risk for leakage of pus into a contaminated space during guidewire exchange. When the acceptable window of access is narrow, the Seldinger technique may be preferred, so as to avoid unintentional passage of the trocar catheter into a vital structure.

Single- or double-lumen (sump) catheters (8–16 Fr) may be used. Single-lumen catheters are connected to gravity drainage. Sump catheters may be left to gravity or low suction. The catheter is flushed with 10 mL of saline every 4–6 h for the first day and once daily after the first day. The effectiveness of the drainage should be assessed with a repeat scan. The determination of successful therapy is made on the basis of abatement of symptoms and signs of infection and less than 10 mL/day of drainage, radiographic evidence of cavity closure with contrast injection, and absence of fluid collection by a repeat scan.

Hepatic cysts and abscess

Congenital cysts may be solitary or multiple, and can be associated with polycystic kidney disease. Cysts that cause symptoms such as pain or jaundice may require treatment. Percutaneous drainage and injection of sclerosing agent into the cyst is an effective alternative to surgical treatment [157,158]. The technique consists of percutaneous puncture of the cyst under US guidance and insertion of a 5-Fr pigtail catheter into the cyst using the Seldinger technique. After complete aspiration of the cyst, the cyst is filled with 95% ethanol in a volume of 30% of the cystic fluid aspirated. After 20 min of sclerosis with changes of the patient's position, the residual cystic fluid is aspirated.

Hepatic abscesses may be bacterial, echinococcal, or amebic in origin. When the diagnosis of pyogenic abscess is established, parenteral broad-spectrum antibiotic coverage is required 1 h before percutaneous aspiration or catheter drainage. Sedation and use of analgesia with appropriate monitoring of vital signs are required during the procedure. Percutaneous drainage is safe and succeeds in about 90% of patients [155]. Effective drainage can be achieved in a loculated hepatic abscess. Computed tomography or US is required for accurate puncture of the lesion. The pleural reflection extends to the 10th intercostal space laterally. To avoid transgression of the pleural space, an angled approach, guided by US and CT, should be used for drainage of high lesions.

The traditional treatment of hydatid cysts has been surgical because of the risk for percutaneous puncture (e.g., peritoneal seeding and anaphylaxis). Although hydatid cysts were previously considered a contraindication to percutaneous aspiration, safe and successful drainage has been achieved with the use of transhepatic puncture and injection of alcohol or hypertonic saline into the cyst after aspiration [159,160].

The role of percutaneous aspiration or catheter drainage for the treatment of hepatic amebic abscess remains controversial. Nearly all hepatic amebic abscesses can be cured by antimicrobial drugs alone, although percutaneous drainage may be useful for patients with abscesses of more than 6 cm and those who have failed one course of medical therapy. After a drainage catheter is placed, the catheter is irrigated with normal saline every 8 h. The volume of irrigating saline is about 25% of the volume of the original cavity. Excessive amounts of fluid should be avoided to prevent rupture of the cavity and leakage of the fluid. Follow-up imaging of the abscess is performed at 5–7 days to evaluate for the effectiveness of the drainage and communication with bile ducts or bowel. When the patient is afebrile with a normal white blood cell count, the 24-h drainage volume is less than 10 mL, and a cavity has no communication with a bile duct, the tube is removed. The patients should receive antibiotics for 2–6 weeks.

Pancreatic abscess and fluid collections

Pseudocysts, whether infected or not, are cured by percutaneous drainage in 70%–90% of patients [161–164]. The response rate may be higher in infected than in noninfected pseudocysts [163]. Indications for percutaneous drainage include pseudocyst infection, pain associated with a pseudocyst larger than 4–5 cm, and a pseudocyst causing bile duct or gastric outlet obstruction. An asymptomatic pseudocyst larger than 10 cm also warrants percutaneous drainage to avoid serious complications. Somatostatin analogues reduce output from pseudocyst drainage catheters and may prove useful in reducing the duration of catheter drainage. The technique for percutaneous drainage of pancreatic pseudocysts is the same as that for other intraabdominal fluid collections. Various access routes to pseudocysts have been used, including transgastric, transhepatic, transsplenic, and transhepatic–transduodenal approaches.

Long-term external drainage is often required for percutaneous transgastric decompression of large pancreatic pseudocysts. The initial external catheter can be converted to internal drainage by placing an internal double-mushroom stent after 14 days, eliminating the need for an external catheter [165]. When early catheter occlusion occurs, replacement or insertion of a second internal stent is done through the gastrostomy tract. When the cyst has resolved, the internal stent is retrieved by endoscopic snare.

The technique for the percutaneous cystogastrostomy involves insertion of a nasogastric tube, percutaneous transgastric puncture of the pancreatic fluid collection under CT guidance, and placement of a 10-Fr percutaneous transgastric catheter through an 11-Fr peel-away sheath into the cyst. The percutaneous gastrostomy is left to gravity drainage for 2–3 days and, when no leakage is seen with injection of contrast medium, the tube is capped. After 14 days of internal drainage, the percutaneous drainage is converted to internal drainage with placement of a stent between the cyst and the stomach under lateral fluoroscopy. Initial results in 19 patients show low complication and recurrence rates [165].

Diverticular abscess

Percutaneous abscess drainage plays an important role in treating certain patients with complications of diverticulitis [166,167]. Patients with phlegmonous changes around the colon can be managed medically. Patients with small pericolic abscesses can be treated with en bloc resection at the time of their colectomy, and patients with peritonitis require a two-stage operation. Patients who have larger pericolic, pelvic, or retroperitoneal abscesses may benefit from PAD. This subset of patients would require staged operations if PAD were not available. Percutaneous abscess drainage provides for resolution of septic complications and healing of the abscess such that a delayed single-stage operation can be performed. As with all cases of PAD, if signs of sepsis do not improve within 24–48 h of PAD and intravenous antibiotics, immediate operation should be undertaken unless catheter repositioning or replacement has a high likelihood of effecting improvement.

Abscesses with fistulae

The identification of fistulae associated with an abdominal or pelvic abscess frequently is not made on imaging studies before drainage or even at the time of drainage [168]. After several days, if the quantity of drainage remains more than 50 mL/day or if the character of the drainage changes and visually or chemically resembles stool, small bowel secretions, pancreatic juice, bile, or urine, then identification of the fistula can be attempted by injecting contrast medium into the abscess cavity under fluoroscopic observation. If this is unsuccessful, an attempt can be made to identify the fistula from its other end by filling the suspected organ of origin with contrast medium; for example, a barium enema is performed if a colonic origin is suspected, or a PTC or ERCP if biliary communication is present.

The presence of a fistula does not preclude definitive PAD in most circumstances. If a downstream stenosis is present in the hollow viscus or if a foreign body or neoplasm is present at the site of perforation, then closure of the fistula will not occur without surgical intervention [169]. Low-output fistulae of less than 200 mL/day appear to be more likely to heal than high-output fistulae. High-output fistulae may be treated more effectively by converting them to low-output

fistulae with nasoenteric intubation and suction, total parenteral nutrition, and histamine H_2-receptor blocker therapy. Catheterization of the fistula itself and advancement of a drainage catheter into the viscus is extremely useful in diverting drainage from the fistulous tract and allowing resolution [170].

When a fistula is present in association with a percutaneously drained abscess, the duration of catheter drainage usually is longer than if no fistula is present. Drainage for several months may be necessary; however, most of these patients can be managed as outpatients. If a patient's social situation precludes outpatient treatment, operative treatment should be considered to avoid the costs and risks associated with prolonged hospitalization.

Abscesses and Crohn's disease

The role of PAD in Crohn's disease has been debated [171], but several points are clear. Postoperative abscesses with or without fistulae, and hepatic abscesses in patients with Crohn's disease respond well to PAD [172,173]. Abscesses in association with transmural disease of the bowel but without visible fistulae also appear to respond [174]. It is unclear what the response rate is for abscesses with fistulae from transmural disease. Nevertheless, these patients may benefit from palliation with PAD before definitive operation. Percutaneous abscess drainage may control septic complications and allow improvement of nutritional status with a course of parenteral nutrition before operation. As in treatment of diverticular disease, PAD may allow conversion of a two-stage operation into a single-stage operation.

Percutaneous gastrointestinal intervention

Both endoscopic and interventional radiology techniques are used for gastrostomy, gastrojejunostomy, and direct jejunostomy placement. Morbidity is low and the success rate is high with both approaches; hence, the choice between techniques depends on local expertise and availability.

Percutaneous, radiologically guided ostomy formation is performed in both adults and children with use of conscious sedation and local anesthesia. General anesthesia is required in uncooperative patients. The procedure is performed on a C-arm fluoroscopic table. Before gastrostomy tube placement, US may be used to identify the liver and spleen.

Percutaneous gastrostomy and gastrojejunostomy

Indications for percutaneous radiological gastrostomy (Fig. 136.19) and gastrojejunostomy (Fig. 136.20) include:

- inadequate oral intake due to esophageal obstruction or neurological disorders
- decreased gastric motility (for gastrojejunostomy)
- decompression in chronic small bowel obstruction.

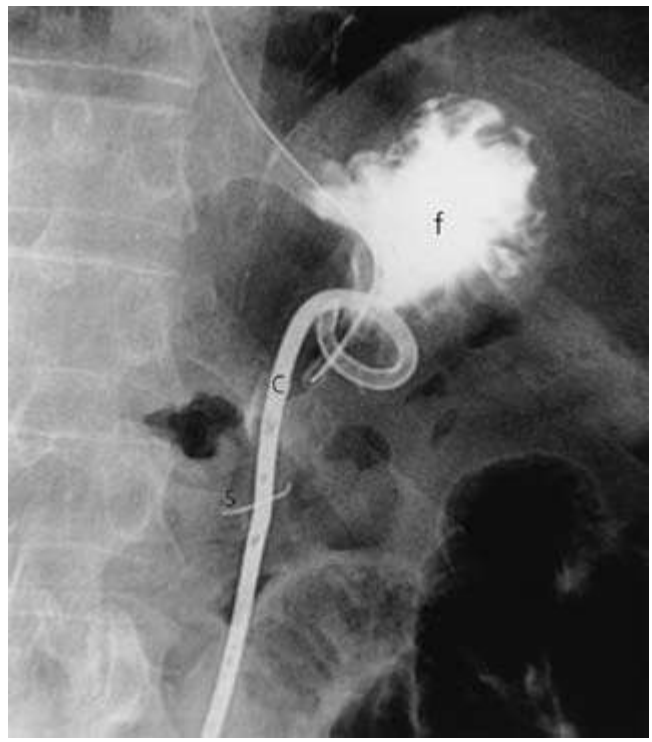


Figure 136.19 Percutaneous gastrostomy. A 12-Fr Cope loop gastrostomy tube (c) was placed following placement of a Cope suture anchor (s), and injection of contrast medium confirms the catheter loop in the fundus of the stomach (f). The nasogastric tube has been introduced into the stomach for air insufflation during puncture of the stomach.

The contraindications for gastrostomy or gastrojejunostomy tube placement include:

- uncorrectable bleeding diathesis
- inaccessible stomach from prior gastric pull-up, total gastrectomy, or partial gastrectomy
- clinical instability
- limited life expectancy
- presence of a ventriculoperitoneal shunt.

Ascites has been considered a contraindication to gastrostomy, although successful placement in patients with ascites has been reported [175]. Pericatheter ascitic leak may be avoided with preprocedural paracentesis and, if necessary, postprocedural paracentesis to promote skin healing. Gastropexy sutures are used in patients with marked ascites to avoid catheter dislodgment by preventing increasing amounts of ascites from displacing the stomach from the anterior abdominal wall. Two to three suture anchors may be placed before tract dilation. The suture may be left in place for 1–2 weeks, or the suture may be cut after placement of a gastrostomy tube. Bacterial seeding of the ascitic fluid remains a potential problem.

Technique

A nasogastric tube is placed the evening before the procedure, or, if a nasoenteric feeding tube is present, it is left in place. The patient is given nothing by mouth or through the

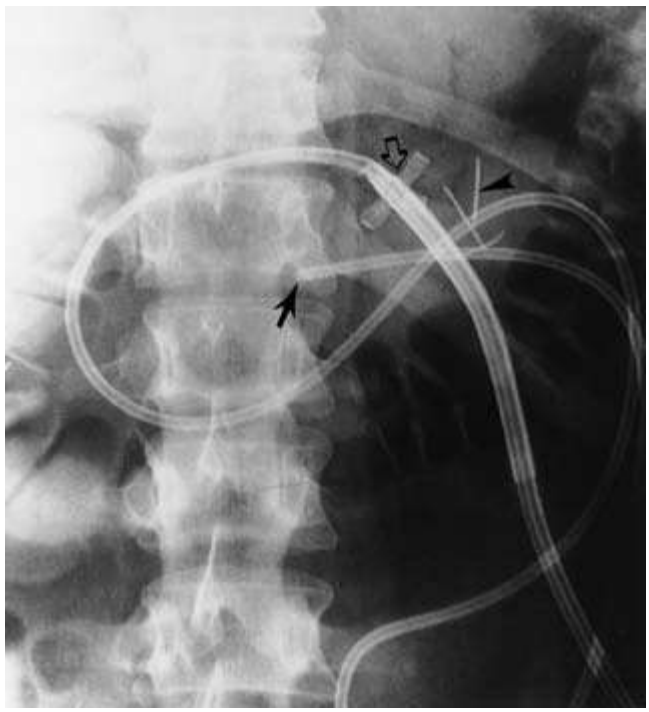


Figure 136.20 Percutaneous gastrojejunostomy. After gastrostomy, the pylorus was crossed using a guidewire and catheter. The tract was dilated and a 16.5-Fr Carey–Alzate–Coons gastrojejunostomy catheter was introduced through a peel-away sheath. The distal catheter tip is positioned in the jejunum (arrow) beyond the ligament of Treitz, allowing enteral feeding immediately following tube placement. Three anchor sutures (arrowhead) have been placed in the stomach before tract dilation. The friction-lock Malecot tip (open arrow) prevents tube dislodgment.

nasogastric tube (except for medications), beginning at midnight before the procedure. Glucagon is administered immediately before the needle puncture of the stomach to reduce the egress of air from the stomach. Distention of the stomach brings its anterior wall closer to the anterior abdominal wall, displaces the small and large bowel, and brings the stomach out from under the costal margin to facilitate puncture. Inflation also allows fluoroscopic visualization of the stomach. If no nasoenteric tube can be passed blindly because of esophageal obstruction, in nearly all cases a small-bore tube or catheter can be placed through the obstruction using standard angiographic techniques. For patients who cannot be transported to the angiography suite or who are unable to remain in supine position, percutaneous gastrostomy can be performed at bedside under portable real-time US guidance [176].

The radiological technique in general use for ostomy formation is the Seldinger method, using a guidewire, needle, dilators, and catheters. The puncture site is chosen fluoroscopically so that the needle enters the body of the stomach away from the greater curvature, avoiding the gastroepiploic vessels, epigastric vessels, the colon, and the left lobe of the liver. Oblique fluoroscopy is useful to confirm that the stomach abuts the anterior abdominal wall without intervening structures at the puncture site. The chosen puncture site is anesthetized with lidocaine, and a skin incision, large enough to admit the selected tube, is made. Gastropexy sutures may be placed with a modified 18-gauge needle [177].

Under fluoroscopic guidance, an 18-gauge needle attached to a 10-mL syringe half-filled with water-soluble contrast medium is advanced into the stomach with a brisk motion during aspiration with the syringe. Entry into the stomach is observed by free aspiration of air and is confirmed by injection of contrast medium and visualization of rugal folds. A 0.038-inch (0.97-mm) guidewire is advanced into the stomach and the tract is dilated. If a gastrojejunostomy tube is to be placed, the catheter and a long torqueable guidewire are used to traverse the pylorus and advance the guidewire through the duodenum into the proximal jejunum. The gastrojejunostomy catheter then is advanced over the guidewire into the jejunum through a peel-away sheath.

Catheters from 2.64 to 8.58 mm (8–26-Fr) are used for gastrostomy, with the smallest catheters used in the pediatric population for feeding, and the largest catheters used in adults for gastric decompression. Retention devices include balloons, Cope loops, and Malecot tips. Injection of contrast medium confirms the final position of the gastrostomy or gastrojejunostomy tube. Large-bore gastrostomy tubes and endoscopic-type gastrostomy tubes may be safely placed using the same radiological technique [178].

The gastrostomy or gastrojejunostomy tube is secured at the skin with a Molnar disk or friction fitting to stoma adhesive. If for any reason there is a high risk of catheter dislodgment, the tube may be sutured to the skin. The patient is observed for 6 h for signs of bleeding or peritoneal irritation. If a gastrostomy tube has been placed, it is left open to gravity drainage overnight and tube feedings begin the following morning. If a gastrojejunostomy has been placed, tube feedings may begin immediately.

The stitches (and gastropexy fasteners, if used) are removed after 10 days. The tract usually is mature enough by 7–10 days so that, if the tube is accidentally dislodged, the tract can be recatheterized and a de novo gastrostomy avoided if replacement is performed within 24 h.

Feeding gastrostomy versus feeding gastrojejunostomy

Because adequate data are not available, radiologists remain divided into two camps: those who place gastrostomy tubes in the majority of patients and gastrojejunostomy tubes only when gastroesophageal reflux is proven, and those who place gastrojejunostomy tubes in all patients. The most serious complication of tube feedings is aspiration pneumonia. Some evidence exists that surgical gastrostomies induce reflux; however, percutaneous radiological gastrostomy does not induce reflux. No study has demonstrated that endoscopic or radiological percutaneous gastrojejunostomy results in less aspiration than percutaneous gastrostomy.

Gastrostomy tubes are superior for the majority of patients. Most of the aspiration in these patients is due to oropharyngeal aspiration. Gastrojejunostomy tubes require greater maintenance than do gastrostomy tubes (Fig. 136.20). Gastrojejunostomy tubes have occluded more frequently than gastrostomy tubes because the jejunostomy portion of the tubes has been limited in size to 3.3–3.96 mm (10–12 Fr). Poorly crushed pill fragments or concretions of tube feedings may block the smaller tube and necessitate tube replacement. The greater length of gastrojejunostomy tubes may also contribute to this problem. Gastrojejunostomy tubes are harder to manage at home or at chronic-care facilities because they require mechanical pump infusion to achieve controlled infusion rates in the small intestine. In contrast, gastrostomy tubes can be infused with intermittent boluses or by gravity drips.

If gastroesophageal reflux and aspiration occur in a patient with a gastrojejunostomy tube, or in a patient with intractable gastroparesis, a better result may be achieved with two tubes – one placed into the jejunum for feeding and one placed into the gastric fundus for decompression.

Complications and results

A compilation of four major published series of radiological gastrostomy and gastrojejunostomy tube placements [179] in a total of 635 patients revealed a procedure-related mortality rate of 1% and a major complication rate of 5%. Major complications included peritonitis requiring laparotomy, bleeding requiring transfusion, external leakage, aspiration, gastric or duodenal perforation, and deep abdominal wall infection. Minor complications occur in 5% of patients.

A comparison of radiological gastrostomy with endoscopic gastrostomy and surgical gastrostomy revealed that radiological gastrostomy is associated with a higher success rate than is percutaneous endoscopic gastrostomy (PEG) and with less morbidity than either PEG or surgery [180]. Patients were not randomized in this study, however, making comparisons difficult. Percutaneous radiological gastrostomy or gastrojejunostomy can be performed in essentially all patients in whom the procedure is not contraindicated. Large-bore 20–24-Fr gastrostomy and gastrojejunostomy tubes can be placed under fluoroscopy. The technical success, morbidity, and mortality rates are similar to those of tubes placed surgically or endoscopically [181].

In pediatric patients with neurological or oropharyngeal impairment, fluoroscopically guided gastrostomy provides a safe and effective means for enteral nutrition and gastric decompression. Colonic interposition, microgastria, and hepatosplenomegaly are the causes of access failure of 1.2% [182]. The procedure can be performed with use of sedation and local anesthesia in nearly 90% of children.

Percutaneous jejunostomy

When percutaneous gastrostomy is not possible because of previous gastrectomy or an abnormal stomach position, direct percutaneous jejunostomy can be performed for enteral feeding [183,184]. Under fluoroscopic guidance, a nasogastric tube is passed into the stomach and through the pylorus. One milligram of glucagon is administered intravenously to decrease peristalsis. The small bowel is then filled with room air. Using a C-arm fluoroscope, a loop of bowel adjacent to the anterior abdominal wall is identified and a 21-gauge needle is inserted into the jejunal lumen; injection of contrast medium confirms the needle position within the jejunal lumen. A 0.018-inch (0.46-mm) guidewire is inserted and a 6.3-Fr introducer catheter is advanced into the jejunum. Over a 0.038-inch (0.97-mm) guidewire, the tract is dilated and an 8-Fr Cope loop nephrostomy catheter is placed in the jejunal loop. Pericatheter leakage is the main complication. The use of Cope suture anchors facilitates a feeding tube insertion by securing the jejunal loop to the anterior abdominal wall.

In the absence of a previous surgical jejunostomy scar, this procedure is considered risky and is not widely performed. In patients who have undergone esophagectomy and a previous surgical jejunostomy, the jejunal loop that has been surgically fixed to the anterior abdominal wall can be punctured using the metal clips as a guide [185]. The skin mark or a dimple at the site of the previous surgical jejunostomy is used to guide the needle puncture into the bowel loop.

Percutaneous cecostomy

Percutaneous cecostomy is an alternative to surgical and endoscopic decompression of the cecum in cases of colonic obstruction and pseudoobstruction (Ogilvie syndrome) [186,187]. Small 2.31–2.97-mm (7–9-Fr) catheters can be placed through the peritoneum with a Seldinger or trocar technique for decompression of the colon, and large 7.92–9.9-mm (24–30-Fr) catheters are placed for drainage of liquid stool after placement of anchoring sutures as used for gastrostomy. This technique is applicable for patients with Ogilvie syndrome in whom endoscopic decompression fails, and in patients with colonic obstruction whose medical condition precludes operation.

Balloon dilation and stent placement for gastrointestinal obstruction

Balloon dilation or metallic stents may be used as alternatives to surgery for malignant and benign strictures in the esophagus and GI tract (stomach, pylorus, duodenum, colon, and rectum) using radiological or endoscopic techniques [188]. Generally balloon dilation is used for benign strictures whereas stents are reserved for patients with malignant strictures.

The radiology-guided technique is similar to that used for percutaneous translumenal angioplasty. It can be performed on outpatients under sedation and local anesthetic. The strictured site is identified by injection of contrast medium or endoscopy. After placing a 0.035-inch (0.89-mm) guidewire across the stricture, an angioplasty balloon catheter is

advanced over the guidewire, placed across the stricture, and inflated two to three times for 3–5 min. Water-soluble contrast medium should be injected after dilation to rule out any perforation.

Metallic stents have been used for treatment of esophageal obstruction, gastric outlet stenosis, duodenal stenosis, and malignant colorectal stricture [189–195]. The technique for stent placement is similar to that used for angioplasty. Use of metallic stents in patients with colonic obstruction caused by malignant neoplasms eliminates the need for emergency surgical intervention on unprepared bowel, reducing mortality and morbidity [196]. The procedure can be performed on an outpatient basis or with minimal hospitalization, permitting single-stage surgery. The procedural steps for colonic stent placement are as follows: the patient is placed in the supine position on a fluoroscopic table and the combination of a 0.038-inch (0.97-mm) stiff-angled hydrophilic guidewire and a catheter are advanced through the rectosigmoid colon under fluoroscopy to the area of obstruction. After identification of the obstruction by injecting water-soluble contrast material, the catheter and guidewire are advanced through the lesion. The length of the obstruction is measured and a self-expanding WALLSTENT is deployed over a guidewire or through an endoscope if desired. The correct position of the stent and its patency are assessed by a barium examination. Complications occur in 10% of patients, including perforation, migration, hemorrhage, and sepsis.

Malignant tracheoesophageal fistula

A fistula may develop between the esophagus and the tracheobronchial tree from invasion of mediastinal structures by esophageal carcinoma. This may lead to pneumonia, lung abscess, and death. Covered esophageal stents can provide effective palliation of symptoms caused by the tracheoesophageal fistula. Simultaneous placement of a tracheal stent may be required for tracheal compression by the stent and tumor invasion [197,198]. Although radiological placement has been reported, the majority of these stents are placed endoscopically.

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C PATHOLOGY

137 Endoscopic mucosal biopsy: histopathological interpretation

Elizabeth Montgomery, Anthony N. Kalloo

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General principles of gastrointestinal biopsy interpretation

The key principle of gastrointestinal (GI) biopsy interpretation is that the GI tract has a limited repertoire of responses to a host of injuries, and diagnosing the type of injury in any given biopsy often requires correlation with clinical details. When dealing with mucosal biopsies of the GI tract, it should also be noted that they only display the mucosa (and possibly a small amount of submucosa), a feature that is obvious but sometimes forgotten by endoscopists and pathologists alike. Of course, mucosal biopsies should not include muscularis propria. When they do, it is good practice for the pathologist to contact the endoscopist and discuss the possibility of endoscopic perforation. A discussion of every entity that can be encountered on mucosal biopsies is beyond the scope of this chapter, but we attempt to describe those most commonly encountered and certain classical conditions.

Overview of the gastrointestinal tract

The innermost layer of the GI tract is the mucosa, which consists of three components.

- Epithelium: protective, secretory, or absorptive properties.
- Lamina propria: this is a loose connective tissue zone supporting the avascular epithelium. In the esophagus, stomach and small bowel, it is rich in lymphatics whereas it is less so in the lower tract. This is of clinical significance in evaluating carcinomas as they can attain lymphatic access with minimal invasion in the upper tract but not in the colon. In the upper tract there are few immune cells (lymphoid and plasma cells) in the lamina propria, whereas in the small bowel and colon lamina propria lymphocytes and plasma cells are more abundant. Neutrophils are abnormal when found in either the lamina propria or the epithelium. Eosinophils are a normal constituent of the lamina propria but mucosal biopsies from all sites must be assessed for prominence of normal inflammatory components.
- Muscularis mucosae: this is a slender double layer of smooth muscle separating the mucosa from the submucosa. It has an inner circular and outer longitudinal arrangement and in this respect can be regarded as a miniaturized muscularis propria.

The submucosa is composed of connective tissue and houses the Meissner nerve plexus as well as large-caliber vessels. The muscularis propria is the main wall of the GI tract and is composed of an inner circular and outer longitudinal layer of smooth muscle. Between these layers is the Auerbach nerve plexus. The outermost component is either adventitia or serosa. The former lacks a mesothelial membrane lining.

Parasympathetic ganglion cells are found in both the Meissner and Auerbach nerve plexuses but the submucosal Meissner plexus contains neuronal cell bodies of the intrinsic sympathetic nervous system that function on local areas of the gut. These are the neurons that have chemoreceptors and mechanoreceptors. They synapse on both other ganglion cells and muscle or secretory cells.

In assessing any biopsy from the GI tract, the pathologist should note the gender, age, and race/ethnicity (if known) of the patient and the site of the biopsy. This information is useful in directing interpretation and the endoscopist should provide these details as well as a brief synopsis of the indications for the biopsy and the clinical concerns. Examples of the utility of mentioning simple demographics include celiac disease, which is found in about 1% of all Americans but

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virtually never in Southeast Asians, and collagenous colitis, which is typically a disease of older women. Assessments of all biopsies from infants should include a systematic review of each compartment of the tissue. For example, the epithelial surface is reviewed with an eye to malabsorptive diseases (such as microvillous inclusion disease in the small intestine), the lamina propria is scanned for the presence of plasma cells (their absence suggests common variable immunodeficiency), and the muscularis mucosae is assessed for eosinophils (which should be absent).

The type of mucosa in the biopsy is compared with the recorded biopsy site to address metaplasias (or "switched" specimens) and the normal architectural structures are noted, such as villous contours of the small intestine. Before making a diagnosis, the features are compared with the history to assure that the interpretation makes sense in light of the clinical information. If the information is scant but required for or inconsistent with the microscopic findings, this too must be addressed.

With a consistent approach, the pathologist interpreting GI biopsies can provide important information that can occasionally be life-saving (in certain instances of ischemic disease) and can often be reassuring to the ever-increasing group of patients undergoing GI tract mucosal biopsies. The gastroenterologist who remembers that diseases have many overlapping histological features will be better served by supplying clinical details to increase the possibility of the most accurate interpretation.

Sampling

The most important sampling issues concern sampling error and knowing where to biopsy. Whenever feasible, jumbo forceps provide better information than smaller ones. There are certain general principles that apply to many settings. For example, when sampling an ulcer where there is clinical suspicion of cytomegalovirus (CMV) infection, it is prudent to sample areas expected to harbor granulation tissue since the cytopathic effect of CMV is usually found in endothelial cells. In contrast, if the cytopathic effect of herpes simplex virus (HSV) is sought, intact epithelium must be present on the slides for review. Consequently both types of tissue should be sampled when assessing for viral lesions in erosions or ulcers in immunosuppressed patients. Similarly, in attempting to establish a cancer diagnosis in an ulcerated zone, it is essential to obtain more rather than fewer biopsies as sampling error is often a factor. With regard to celiac disease, biopsies should be obtained from beyond the bulb, as the bulb is prone to villous blunting from chronic damage by gastric contents. When obtaining biopsies to address the possibility of microscopic colitis, it is important to remember that inflammation is best seen in the right and transverse colon; a negative rectal biopsy does not exclude microscopic colitis. Lastly, gastric polyps (other than fundic gland polyps) seldom occur in isolation but rather in the context of gastric pathology in the surrounding flat mucosa, so it is helpful to sample this when investigating a polyp in the stomach, especially if the polyp is in the antrum [1,2]. Of course, this contrasts with the colon, where polyps are usually isolated findings; sampling the background flat mucosa is only necessary in the setting of inflammatory bowel disease (IBD).

Esophagus

Nonneoplastic conditions

Inlet patch (cervical gastric heterotopia)

Inlet patches are believed to be embryological vestiges from early gestation when the esophagus is lined by columnar epithelium. They are found in about 1% of patients undergoing upper endoscopy assessed retrospectively through biopsy records, but incidences of up to 10% have been recorded [3,4]. They are found in the cervical esophagus and are usually asymptomatic. However, there are occasional complications: local injury (webs, strictures, ulcers, fistulae) can result in symptoms (pain and dysphagia). Rarely, carcinomas complicate inlet patches.

Inlet patches range in size from 0.2–0.3 cm up to about 4 cm. They typically consist of oxyntic gastric-type mucosa (containing parietal cells), although a transitional or cardiac pattern (lacking oxyntic glands and negative for gastrin on immunohistochemistry) may also be found. Lesions are often inflamed and may occasionally harbor *Helicobacter pylori*. Intestinal metaplasia in inlet patches is occasionally found. Some studies have explored a link between inlet patches and Barrett esophagus and indeed these conditions are found together more than coincidence would suggest. Of course some studies are difficult to interpret since the criteria for Barrett esophagus have changed with the recent requirement for intestinal metaplasia to diagnose lesions as Barrett esophagus (see section on Barrett esophagus). Pancreatic tissue may also be a component of inlet patch.

Glycogenic acanthosis

Glycogenic acanthosis of the esophagus is a common benign entity [5–19] found in up to 15% of esophagi (but overall probably closer to 3% [19]). It is characterized by single or multiple plaques of hyperplastic squamous epithelium with abundant intracellular glycogen deposits. Lesions appear as slightly raised grey-white plaques that are usually 2–10 mm in diameter and which may be confluent. They cause a finely nodular or cobblestone mucosal pattern demonstrable on double-contrast views of the well-distended esophagus. The findings are not associated with mucosal ulcerations, lumenal narrowing, or mobility disturbance, although some patients may have coexistent hiatal hernia and gastroesophageal reflux. There is also an association between glycogenic acan-

thosis and Cowden disease. Glycogenic acanthosis does not appear to be linked with diabetes and does not regress with acid suppression when associated with reflux disease. In one Japanese study, it was commonly found in adjacent nonneoplastic mucosa in patients with squamous cell carcinoma of the esophagus [12].

In general, it is essentially an incidental finding. The endoscopist notes a white plaque and a biopsy displays prominent intraepithelial glycogen extending to within one cell layer of the basement membrane with just a single row (or two) of basal cells between the glycogenized squamous cells and the basement membrane. For the endoscopist, the differential diagnosis is with *Candida* esophagitis, whereas pathologists often do not notice glycogenic acanthosis. However, probably the most important reason for the pathologist to diagnose patients with glycogenic acanthosis is to provide the endoscopist with an explanation for the abnormal gross appearance of the biopsied lesion.

Findings in mechanical/anatomical disorders (webs, rings, achalasia)

Typically, biopsies from webs, rings, and from the squamous mucosa in patients with achalasia display normal squamous mucosa unless the condition has resulted in long-term reflux. There may be parakeratosis, a response to the process of food passing over the protuberant ring or the constricted segment, or erosions may appear where retained food presses on the static segment proximal to the attenuated lumen.

Reflux disease

Gastroesophageal reflux disease (GERD) is the costliest GI disorder affecting citizens of the USA. In 2002, the direct cost of GERD was estimated at over $9 billion [20] so, not surprisingly, many patients undergo endoscopic biopsies in the course of evaluation for reflux symptoms. Findings in these can range anywhere from classical reflux changes (basal cell hyperplasia, elongation of vascular papillae, and intraepithelial eosinophils), to ulcers, intraepithelial lymphocytosis (which correlates poorly with pH studies), or balloon cells (which appear distended and have pale abundant pink cytoplasm).

Reflux esophagitis is an example of an "-itis" that often lacks a prominent component of inflammation. The pathology reflects injury to the squamous epithelium, followed by attempts of the epithelium to regenerate. Mild features of cellular injury include balloon cells (squamous cells with ballooned cytoplasm from accumulation of plasma proteins) and vascular lakes (dilated small blood vessels in the mucosa, often seen endoscopically as erythema or redness). Severe injury can result in mucosal sloughing with erosions or ulcers. Regenerative changes include hyperplasia of the basal zone to greater than 15%–20% of the epithelial thickness and elongation of the vascular papillae to greater than two-thirds of the epithelial thickness. Inflammation is typically mild and

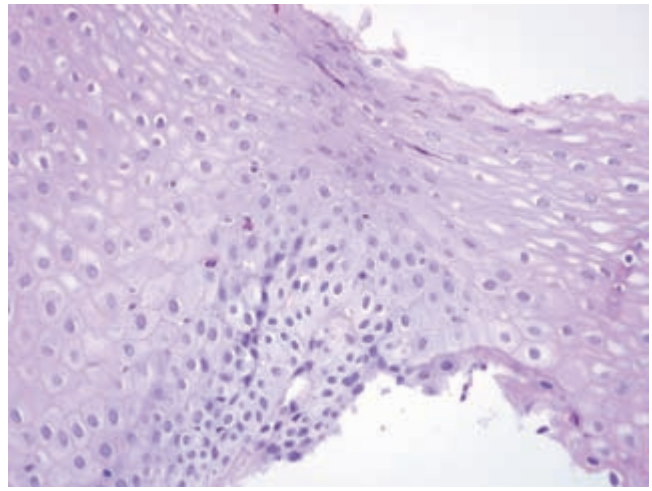

Figure 137.1 Reflux esophagitis.

includes scattered eosinophils (Fig. 137.1). Less commonly, scattered neutrophils are present; neutrophils can be more prominent in cases with more severe injury including erosions and ulcers.

The severity and extent of the histological changes on biopsy do not necessarily correlate well with the severity of the patient's symptoms. Severe complications of GERD are unusual. These include ulcer, hemorrhage, and stricture formation. The complication of Barrett esophagus occurs in approximately 10% of patients with symptomatic reflux.

Injury caused by chemical or physical agents

A few agents result in injury that leaves a footprint that can be recognized on biopsies, although often there is no microscopic clue.

Iron

In our patient population, in which inpatients are well represented, mucosal iron (ferrous sulfate) is found in about 1% of patients undergoing upper tract endoscopic biopsies. Iron is well known for its capacity to cause corrosive injury in the esophagus. While it can be argued that such a phenomenon is a result of a prior injury in which an iron tablet becomes embedded, the corrosive and toxic nature of iron itself suggests that the iron pill has caused the injury. Abraham and colleagues [21] studied the clinical and histological features of 36 upper GI tract biopsies from 33 patients (24 gastric, 9 esophageal, 1 gastroesophageal junction, 2 duodenal) containing characteristic brown crystalline iron material, and evaluated the amount and tissue distribution of the iron. The biopsies typically displayed lumenal crystalline iron adjacent to the surface epithelium or admixed with lumenal fibrinoinflammatory exudates (Fig. 137.2). Most biopsies (83%) showed crystalline iron deposition in the lamina propria, either covered by an intact epithelium, subjacent to small superficial erosions, or admixed with granulation tissue. Three

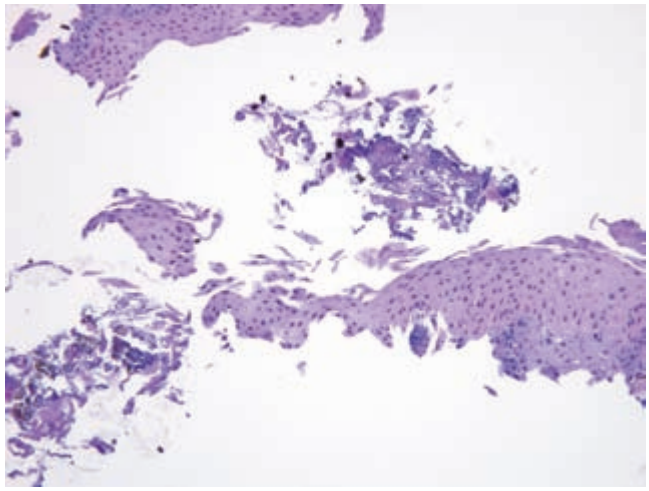

Figure 137.2 Iron pill esophagitis. The brown material in the image is iron pigment. The associated squamous epithelium shows reparative features.

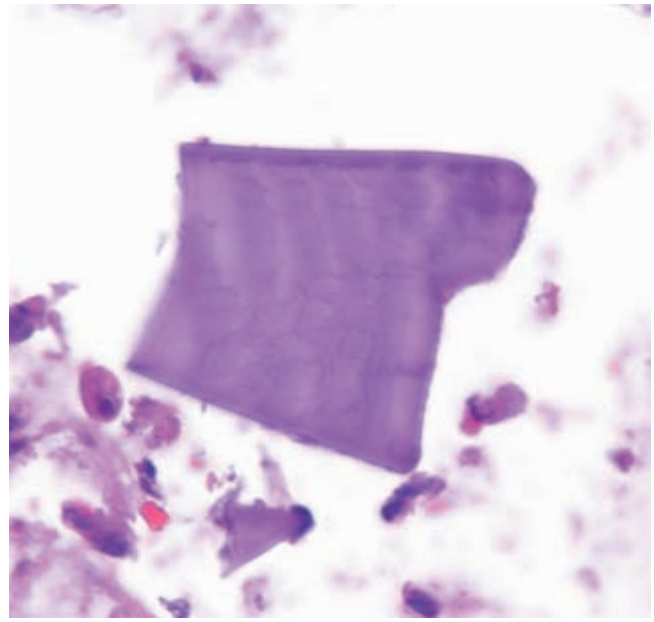

Figure 137.3 Kayexalate (sodium polystyrene sulfonate). Note the fish scale-like appearance of the crystalline material.

biopsies (8%) demonstrated iron-containing thrombi in mucosal blood vessels. Erosive or ulcerative mucosal injury was present in the majority of biopsies (83%). The amount of iron accumulation in cases with mucosal injury was greater than in cases without mucosal injury. Iron medication (usually ferrous sulfate) was confirmed in 25 of 33 patients (76%). However, as an argument for iron causing injury as a secondary event, half of the patients (17 of 33) also had underlying infectious, mechanical, toxic, or systemic medical conditions that could have initiated or exacerbated tissue injury.

Kayexalate

The use of Kayexalate (sodium polystyrene sulfonate) for the management of hyperkalemia was approved for use in the USA in 1975. It is a cation-exchange resin that can be instilled into the lower GI tract as an enema preparation or into the upper GI tract either orally or by nasogastric tube. When administered orally or by nasogastric tube, sodium cations are first released from the resin and exchanged for hydrogen ions in the acidic milieu of the stomach. As the resin passes through the intestines, hydrogen is exchanged for potassium, which is then eliminated in the feces along with the remainder of the altered resin, thereby lowering the serum potassium concentration.

During the early use of Kayexalate, the resin was administered as a suspension in water. Although generally well tolerated, some patients were reported to develop gastric and bowel opacifications as a result of concretions of resin. It therefore became increasingly popular to administer Kayexalate in a suspension with hypertonic sorbitol, which reduces the frequency of bezoar formation and colonic impaction by promoting an osmotic diarrhea. Subsequently, Lillemoe and colleagues reported five uremic patients who developed colonic necrosis temporally associated with the use of Kayexalate in sorbitol that contributed to death in four of them [22]. This study also provided experimental (murine) evidence implicating sorbitol as the agent responsible for colonic necrosis.

It has become apparent that Kayexalate can be associated with severe mucosal injury in the upper GI tract as well [23]. In most instances, the medication is easy to recognize in endoscopic biopsies (Fig. 137.3). Kayexalate crystals are lightly basophilic on hematoxylin and eosin (H & E) stain, red on periodic acid–Schiff (PAS)/Alcian blue and acid-fast stains, and blue on Diff-Quik staining. Crystals display a characteristic crystalline mosaic pattern resembling fish scales. It is this mosaic pattern that allows the distinction between Kayexalate crystals and histologically similar cholestyramine crystals. Kayexalate crystals are refractile but not polarizable.

Taxol and colchicine

Taxol, an antineoplastic agent with a novel mechanism of action, can cause striking mitotic arrest (Fig. 137.4) associated with epithelial necrosis and ulceration of the esophagus [24]. The mitotic arrest is associated with bundling of intermediate filaments secondary to accumulation of polymerized microtubules. Thus the histological correlate is the presence of arrested mitoses with ring forms. With taxol, the findings tend to be striking in the esophagus, whereas in colchicine toxicity, which has similar histological findings, the small bowel is more likely to be severely altered.

Fosamax

The bisphosphonates prevent osteoclast-mediated bone resorption and have therefore been found effective in the treatment of osteoporosis, Paget disease, and the hypercalcemia of malignancy [25].

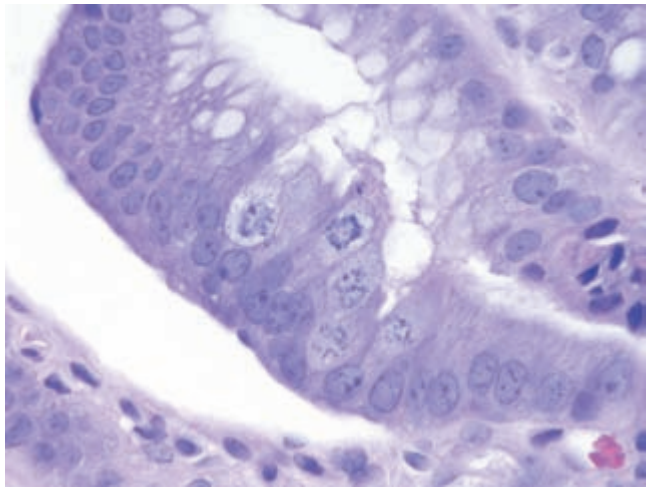

Figure 137.4 Taxol effect. This process involves metaplastic columnar mucosa in the esophagus. The ring mitoses are an indication of mitotic arrest.

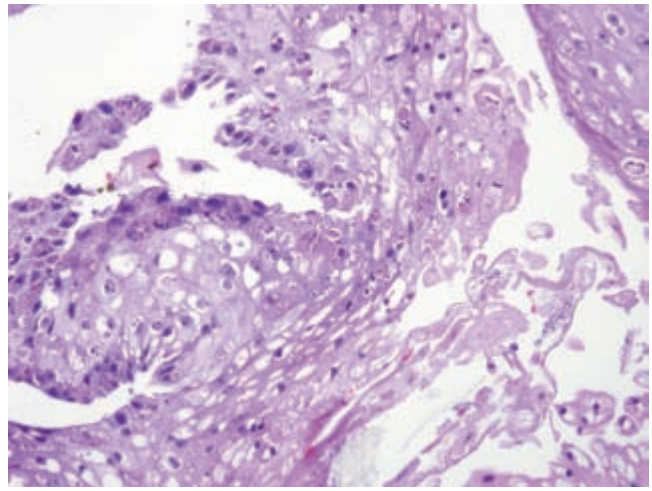

Figure 137.5 Graft-versus-host disease. There is extensive squamous epithelial apoptosis such that nuclei resemble specks of dust.

Ingestion of alendronate sodium (Fosamax) and related medications by osteoporotic patients has been associated with esophagitis and esophageal ulcer. Alendronate can damage the esophagus both by toxicity from the medication itself and by nonspecific irritation secondary to contact between the pill and the esophageal mucosa (pill esophagitis). Biopsies show inflammatory exudate and inflamed granulation tissue as characteristic of any ulcer site [26]. Polarizable crystalline foreign material is often found. There is no specific histological finding but it is worthwhile to be aware of this complication. However, it is now known that modifications in dosing schedules of this medication [27] can reduce mucosal injury and it is less likely to be encountered in practice.

These agents should be avoided in patients with achalasia and other motility disorders of the esophagus, esophageal stricture, or preexisting severe reflux esophagitis. However, patients with reflux disease can probably be treated with a proton pump inhibitor (PPI) and then safely undergo bisphosphonate therapy.

Corrosive ingestion (e.g., lye, bleach)

Like the esophageal injury associated with Fosamax, corrosive injury does not result in a specific pattern of injury (although saponification may accompany lye ingestion) and cannot be identified directly (like iron or Kayexalate). However, it can usually be correlated with an ingestion history in a pediatric or psychiatric patient. Endoscopically, severe ulceration is seen and endoscopists have described a strong bleach-like odor even when the patient has ingested lye. Microscopically, extensive necrosis is found. Those who survive are likely to have severe stricturing disease with all its complications. Such patients require lifetime follow-up based on their proclivity to develop squamous cell carcinoma [28].

Graft-versus-host disease

Esophageal biopsies from patients with graft-versus-host disease (GVHD) show intraepithelial lymphocytosis, basilar vacuolization, epithelial apoptosis (Fig. 137.5), and necrosis in severe disease. Many patients manifest a characteristic endoscopic appearance [29–37], and a bullous presentation akin to that of bullous pemphigoid is one of the possible presentations. Probably the most important goal in patients in whom GVHD is a clinical consideration is to exclude infectious etiologies since generally such patients are empirically treated with steroids.

Dermatological disease affecting the esophagus

Dermatological conditions that are well known to involve the esophagus include pemphigus vulgaris, mucous membrane bullous pemphigoid, epidermolysis bullosa, lichen sclerosis, lichen planus, and toxic epidermal necrolysis. Esophageal involvement by cutaneous diseases is not well recognized by pathologists or clinicians, resulting in delayed diagnosis and continued esophageal inflammation. In the case of lichen planus for example, dysphagia and esophageal strictures are frequently initially attributed to reflux disease.

Lichen planus

Lichen planus [38] is a subacute to chronic mucocutaneous disorder of unknown etiology. It can involve the skin, nails, and mucosal surfaces. Lichen planus of the skin affects both genders with equal frequency at any age, but most patients are middle-aged adults. Cutaneous lichen planus is characterized by eruptions of violaceous scaling papules and plaques. These plaques typically are intensely pruritic and most commonly localized to the extensor surfaces of the forearms and legs. Mucosal lichen planus may affect the perineum, oral mucosa, and pharynx. Unlike cutaneous disease,

mucosal lichen planus predominantly affects middle-aged women. Oral involvement in particular coexists with skin lesions in approximately 30%–50% of patients, but can be the only manifestation.

Esophageal lichen planus is a rarely described manifestation. It is an important diagnosis because of its tendency to cause persistent dysphagia resulting from esophagitis and stricture formation. Further trauma resulting from therapeutic dilation can lead to exacerbation of oral lichen planus in a Koebner-like phenomenon.

The pathological features of esophageal lichen planus differ from those in skin. The epidermal reaction in cutaneous lichen planus typically includes hypergranulosis, hyperorthokeratosis, acanthosis, and saw-tooth elongation of the rete pegs. In contrast, the esophageal epithelium, which does not normally contain orthokeratin or a granular layer, frequently shows parakeratosis rather than orthohyperkeratosis and frequently lacks hypergranulosis. In addition, the epithelium may be atrophic. The histological features therefore more closely resemble those of oral rather than cutaneous lichen planus. All sites of involvement reveal the typical band-like inflammatory infiltrate with a predominance of mature T cells and basal layer degeneration, including characteristic Civatte bodies (Fig. 137.6).

Lymphocytic infiltration alone is not diagnostic of esophageal lichen planus. Medications (gold, thiazides, antimalarials) can induce lichen planus-like lesions and must be excluded by clinical history. Nonspecific lymphocytic infiltrates can appear adjacent to esophageal ulcer sites from other causes and a lymphocytic esophagitis pattern is often a feature of esophageal Crohn's disease. Although infectious agents are usually easily excluded on biopsy material, exclusion of esophagitis due to pill ingestion or caustic substances requires clinical correlation. In addition, lymphocytic infiltrates are occasionally prominent, although often focal, in patients with gastroesophageal reflux. Reflux can usually be excluded on the basis of other criteria such as the lack of clinical symptoms, the results of pH probe studies, and the failure of the inflammatory condition to respond to intensive antireflux therapy. Other histological criteria for gastroesophageal reflux, including intraepithelial eosinophils, are also usually absent. Furthermore, the typical upper or middle esophageal location of the pathological findings and stricture sites would be unusual in GERD. However, it is difficult to positively diagnose lichen planus on histology; it can only be suggested and correlated with other features. Systemic treatment is usually required to forestall stricturing disease, whereas topical treatment is often sufficient for oral lesions.

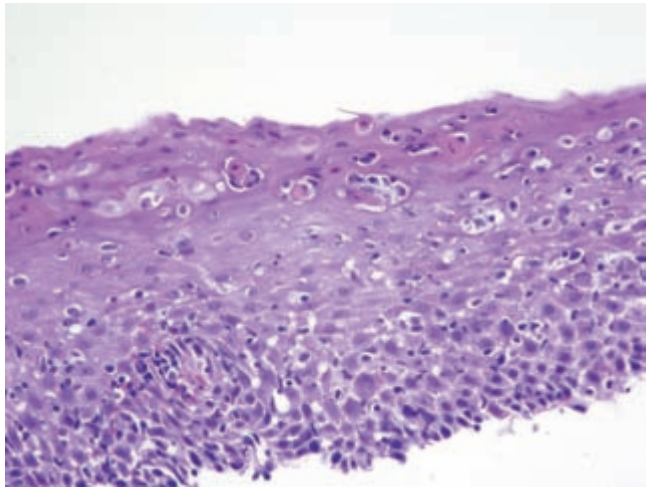

Figure 137.6 Lichen planus involving the esophagus. This field shows prominent intraepithelial lymphocytosis and necrotic squamous cells.

Bullous diseases

The bullous diseases are similarly challenging to diagnose specifically in the esophagus and must also be correlated with clinical findings. When esophageal biopsies show bullae, in the absence of an infectious explanation, additional biopsies can be submitted fresh for immunofluorescence studies to arrive at a more specific diagnosis.

Pemphigus vulgaris (PV), the most common form of pemphigus, affects the sexes equally, most commonly during the fourth and fifth decades. Skin and mucous membrane involvement are typical. Although any mucosal surface can be involved, oral lesions are the hallmark; these occur in almost every case and are the presenting sign in half of affected patients.

There are a few reports of esophageal involvement in PV, which was believed to be rare in early literature but is found in most patients if sought [39]. Because patients with esophageal involvement may be asymptomatic, such involvement has been under-recognized. Although esophageal involvement may be frequent in active mucocutaneous PV, its occurrence in the absence of other manifestations of the disease is rare [40].

Odynophagia and dysphagia are the usual symptoms of esophageal PV, as with other mucosal blistering diseases, including herpetic or esophageal candidiasis. Endoscopy is required for diagnosis; it allows evaluation of the appearance of the lesions and tissue sampling. Essentially normal esophageal mucosa may be seen on initial passage of the endoscope, followed by the appearance of erosions and sheets of sloughed mucosa on withdrawal of the instrument. Histopathological findings of suprabasal clefting, tombstone cells, and acantholysis are features of PV; immunofluorescence shows intercellular deposition of IgG and C3. Indirect immunofluorescence detects pemphigus antibodies in about 75% of cases; a negative result can occur if the disease is in remission or in an early localized stage.

Bullous pemphigoid (BP) affects the esophagus more rarely than other skin diseases [41,42]. It is a chronic, autoimmune, subepidermal, blistering skin disease that rarely involves mucous membranes. It primarily affects elderly individuals

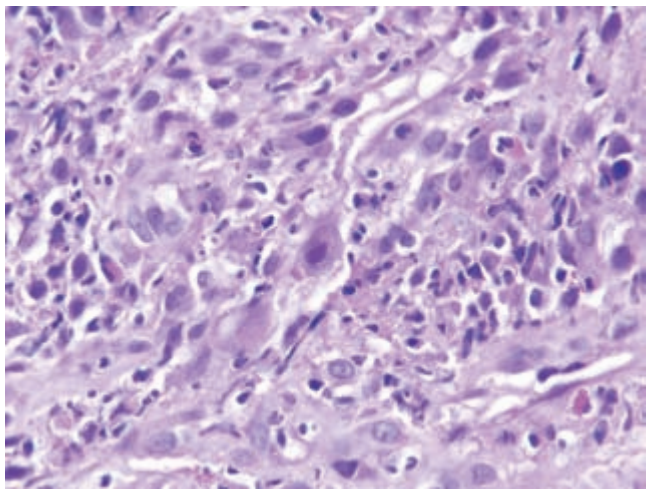

Figure 137.7 Cytomegalovirus esophagitis. An endothelial cell in the center of the field is affected. There is a large intranuclear inclusion.

in the fifth through seventh decades of life (average age at onset 65 years). In France and Germany, the reported incidence is 6.6 per million per year. In Europe, BP was identified as the most common subepidermal autoimmune blistering disease. The mucosa is affected in 10%–25% of patients.

Bullous pemphigoid is characterized by IgG autoantibodies specific for the hemidesmosomal BP antigens BP230 (BPAg1) and BP180 (BPAg2). IgG autoantibodies bind to the epithelial basement membrane and activate complement and inflammatory mediators. Eosinophils are characteristically found in bullae, although their presence is not an absolute diagnostic criterion. Serum levels of autoantibodies against BPAg2 correlate with disease activity in some studies.

Esophagitis caused by infectious agents

When an infectious etiology is suspected in erosive esophagitis, the endoscopist should biopsy the base of the erosion/ulcer to detect CMV (Fig. 137.7) whereas the epithelium is more likely to demonstrate HSV (Fig. 137.8). Greenson and colleagues have demonstrated that a prominent mononuclear infiltrate adjacent to the infected epithelium often accompanies HSV esophagitis and should provoke a search for the organism [43], whereas the presence of macrophages in a perivascular distribution in granulation tissue should spur a search for CMV [44]. In the case of HSV, the background infiltrate may suggest the need to perform immunohistochemistry for HSV as the organism may not be apparent on routine H & E stains. Zones of parakeratosis, however small, are worth study at high magnification to exclude *Candida* (Fig. 137.9). Pseudohyphal forms must be sought since budding yeast alone might be a reflection of oral contamination or colonization. Bacterial esophagitis is poorly understood. Walsh and colleagues [45] proposed a definition of "histopathologically demonstrable bacterial invasion of esophageal mucosa or deeper layers with no concomitant fungal, viral, or neoplastic involvement or previous surgery of the esophagus." Bacterial esophagitis should be considered in immunocompromised patients presenting with odynophagia and may not be accompanied by an inflammatory response but simply by numerous bacterial forms seen embedded in the squamous mucosa. Apparently, bacterial esophagitis can be a source of occult sepsis [46].

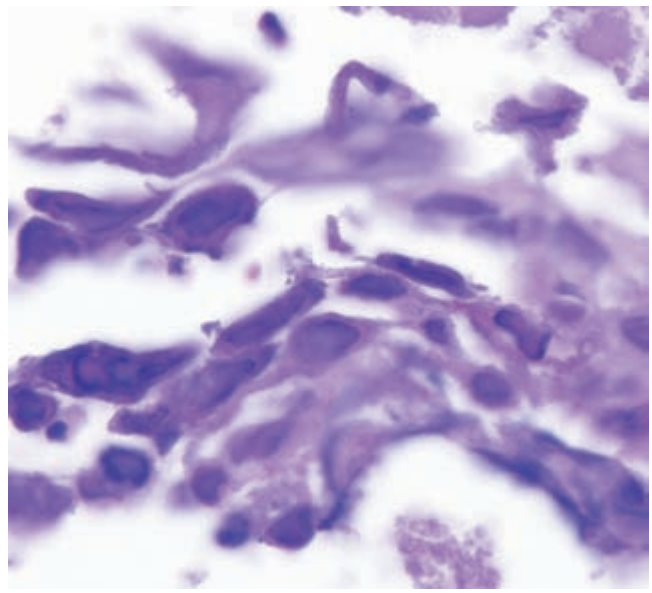

Figure 137.8 Herpes simplex virus esophagitis. The infected cells are multinucleated, with "smudged" nuclei.

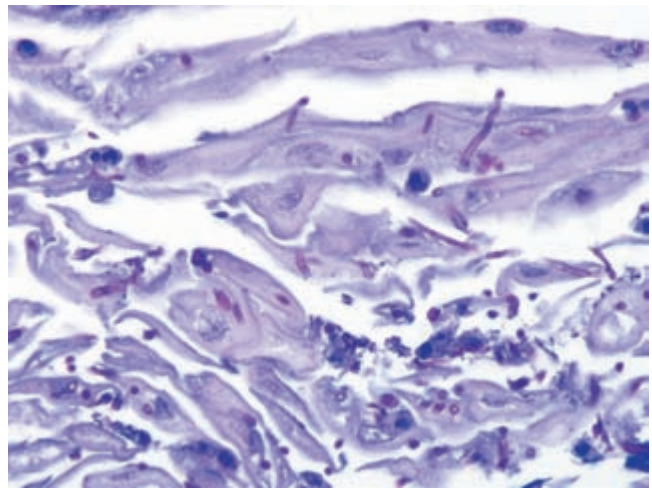

Figure 137.9 *Candida* esophagitis. This periodic acid–Schiff stain highlights pseudohyphal forms.

Allergic/eosinophilic esophagitis

Overall, eosinophilic esophagogastroenteritis is an uncommon benign inflammatory condition characterized by eosinophilic infiltration of the GI tract. The diagnostic criteria include GI symptoms, eosinophilic infiltration of the GI tract usually with intraepithelial eosinophils, and no evidence of parasitic infestation. Most patients (in addition to their GI involvement)

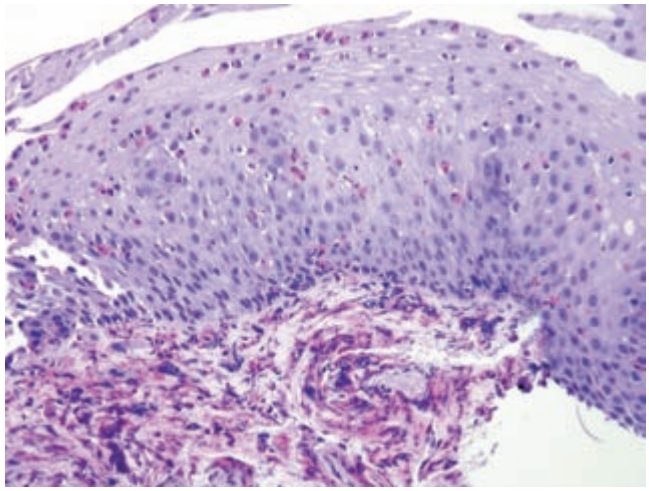

Figure 137.10 Eosinophilic esophagitis. Numerous eosinophils, some degranulating, are seen in both the epithelium and the lamina propria. Far fewer eosinophils are seen in reflux esophagitis. Compare with image shown in Fig. 137.1.

also have a history of allergy, asthma, drug sensitivities, peripheral eosinophilia, and increased IgE levels. Although food intolerance has been postulated as an etiological factor, most cases lack a specific allergen and are attributed to multiple allergens. Eosinophilic esophagogastroenteritis predominantly affects patients in the third to sixth decades. However, 15%–20% of cases are seen in the pediatric age group and, in some cases, milk allergy may be demonstrated.

Any part of GI tract from the esophagus (Fig. 137.10) to the rectum can be involved; the stomach and small bowel are commonly involved. Eosinophilic esophagogastroenteritis can show preferential involvement of the mucosa, muscularis propria, or serosa. Symptoms depend on the site and extent of eosinophilic infiltration. Mucosal disease can present as diarrhea, malabsorption, and protein-losing enteropathy. Submucosal disease presents as obstruction and abdominal pain, and patients can develop eosinophilic ascites with serosal involvement. Rarely patients can present with an acute abdominal emergency necessitating emergency laparotomy.

Patients with eosinophilic esophagitis (limited to esophagus) present with dysphagia and strictures. The differential diagnosis is with reflux esophagitis. However, unlike in reflux esophagitis, the upper and mid esophagus are commonly affected with relative distal sparing. In striking cases, the esophagus can display rings on endoscopy, an appearance termed *feline esophagus* since this appearance resembles that seen in cats [47–48].

On histological examination, superficial epithelial clusters of eosinophils that slough into the lumen are more common in eosinophilic esophagitis and there may be infiltration deep in the esophageal wall and not just limited to the mucosa. The eosinophilic infiltrate can be patchy and multiple, localized or diffuse. In 10% of cases, mucosal biopsies can be nondiagnostic due to the patchy nature of the disease or mucosal sparing. In daily practice, there are cases in which a distinction between allergic/eosinophilic esophagitis and reflux esophagitis cannot be made on histological grounds and the diagnosis requires correlation with pH monitoring studies or with other stigmata of allergic disease. However, some have proposed that an estimate of the number of eosinophils is a helpful clue in some cases: fewer than eight eosinophils per 40× field favors a diagnosis of reflux esophagitis, whereas a count of more than 24 eosinophils per 40× field better supports eosinophilic esophagitis. For cases with intermediate eosinophilic counts, clinicopathological correlation is required. We avoid reporting counts in our practice routinely since they can be meaningless [49], but we do attempt to compare biopsies to previous ones and estimate whether more or fewer eosinophils are present to help assess response to treatment. The distinction between reflux and eosinophilic esophagitis is worth attempting as patients with eosinophilic esophagogastroenteritis show dramatic response to steroids.

Crohn's disease affecting the esophagus

Reports on the prevalence of esophageal Crohn's disease vary widely in both adults and children. Decker and colleagues [50] reported that only 20 of 9900 (0.2%) adults with Crohn's disease seen over a period of 22 years had esophageal involvement. A higher prevalence was reported by D'Haens and colleagues (11%) [51]. Both studies were retrospective and used only macroscopic endoscopic evidence that likely resulted in an underestimation of the prevalence. In contrast, a prospective study of esophageal Crohn's disease in adults yielded a prevalence of 5.1% [52]. There have been few pediatric studies. In one prospective study, endoscopy identified esophageal Crohn's disease in 2 of 40 (5%) children with Crohn's disease and a total of 17 of 40 (42.5%) had histological evidence of esophageal Crohn's disease [53]. In a large cohort of 210 children with Crohn's disease, the lowest estimate of prevalence for endoscopic esophageal Crohn's disease was 7.6% and the highest estimate 17.6% when including histology findings as diagnostic criteria [54]. These findings suggest that the prevalence of esophageal Crohn's disease in adults and children may be similar and is at least 5%. There is wide variability in the clinical manifestations of esophageal Crohn's disease and many patients do not have specific esophageal symptoms. Isolated esophageal Crohn's disease is uncommon.

Esophageal Crohn's disease is usually associated with advanced ileocolonic disease but upper GI tract symptoms do not always correlate well with endoscopic and biopsy findings. Cameron [54] reported that upper GI symptoms were present in only 15% of children with Crohn's disease but found that 42% had endoscopic lesions. Alcantara and colleagues found that 17% of 41 adults had upper GI symptoms but only 5% had endoscopic esophageal Crohn's disease [52]. In the pediatric age group, there is often an overlap

of symptoms (such as nausea, anorexia, and abdominal pain) between esophageal and small bowel Crohn's disease. It is therefore difficult to identify an upper GI site of disease based on clinical evaluation alone. Thus upper endoscopy and biopsies are important in the evaluation of pediatric IBD, even in the asymptomatic child.

The presence of histological abnormalities in radiologically and endoscopically normal mucosa is not ususual. Hence, endoscopy with biopsy is generally considered the gold standard for diagnosis of early asymptomatic lesions. The spectrum of esophageal injury in Crohn's disease varies from mild esophagitis with small erosions to transmural involvement with perforation and fistulization to adjacent organs. In addition to aphthous ulcers, other findings in esophageal Crohn's disease are erythema, ulceration, erythematous nodules, and polypoid lesions. Pseudomembrane formation and progressive esophageal narrowing resulting in strictures and formation of multiple mucosal bridges have been described in advanced stages.

Epithelioid granulomas in the correct clinical setting are considered diagnostic for Crohn's disease (Fig. 137.11). Focal infiltration with mononuclear cells and histiocytes into the lamina propria that extends to the muscular layer is in keeping with Crohn's esophagitis. Unfortunately, nonspecific upper tract lesions can also be found in pediatric patients with classic ulcerative colitis [55].

The clinical course of esophageal Crohn's disease has been described by D'Haens and colleagues [51], who showed that 8 of 14 (57%) patients had no recurrence of esophageal lesions or symptoms during subsequent disease exacerbations. None of the children in the study by Ramaswamy and colleagues [56] had repeat endoscopy for recurrence of upper GI symptoms. Considering the high prevalence of histological esophageal Crohn's disease in children and the low prevalence of endoscopic esophageal Crohn's disease in adults, it seems reasonable to suggest that esophageal involvement is unlikely to progress to a more overt disease over time.

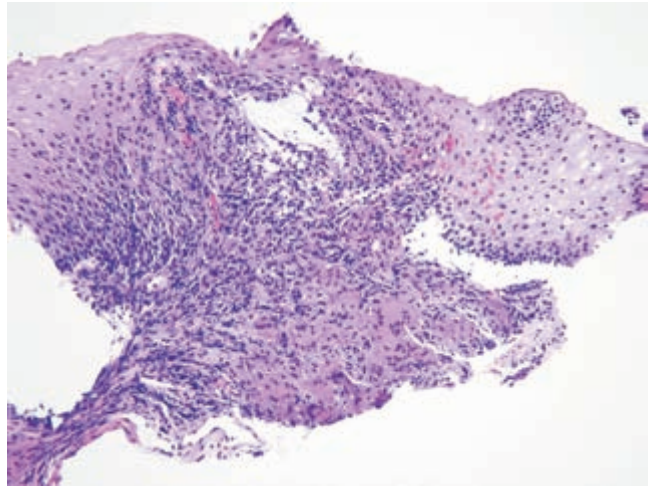

Figure 137.11 Crohn's disease involving the esophagus. There is a prominent lymphoplasmacytic infiltrate. A granuloma is seen in the lower center portion of the field.

Benign polyps

Fibrovascular polyps of the esophagus

These lesions are only rarely encountered in practice. Fibrovascular polyps are submucosal tumors of the esophagus. They have been classified as lipomas, fibromas, and fibrolipomatous polyps. There is only one example in the electronic surgical pathology files at the Johns Hopkins Hospital [57]. However, the dramatic presentation of these polyps has resulted in over 25 individual patient reports in the English literature since 1969, but the largest case series includes only 16 examples referred to the Armed Forces Institute of Pathology (AFIP) [58]. Most arise in middle-aged or elderly men. Presenting complaints include dysphagia, substernal discomfort, and sensation of a mass. In the AFIP series, all 16 patients with giant fibrovascular polyps were symptomatic; dysphagia was the most common complaint (present in 87%), followed by respiratory symptoms (25%) and regurgitation of the polyp into the pharynx or mouth (12%). This series did not include instances of asphyxiation, but it is a feared complication. In the AFIP series, the mean duration of symptoms was 17 months, and only 44% of the patients reported symptoms for 6 months or less.

Most fibrovascular polyps of the esophagus are giant lesions, some reaching 17 and 25 cm in length. They usually arise in the cervical esophagus, near the region of the cricopharyngeus muscle, which accounts for their tendency to prolapse into the mouth and their ability to impinge on the larynx. Chest radiography reveals a right-sided superior mediastinal mass or anterior tracheal bowing. Barium studies show smooth but variably lobulated intralumenal masses originating in the lower cervical esophagus with variable sizes and distal extension, with an average length of about 15 cm. Depending on the amount of fat and fibrovascular tissue in the lesion, computed tomography (CT) reveals a heterogeneous appearance in most cases with some lesions consisting of predominantly fat density and other of predominantly soft-tissue density.

Grossly, these are sausage-like pedunculated polyps arising from the wall of the esophagus. Their surface is covered by squamous mucosa and their cores consist of an admixture of grossly identifiable adipose tissue and firm whitish connective tissue.

Fibrovascular polyps are histologically rather uniform lesions composed of variable admixtures of mature adipose tissue lobules, collagenous and sometimes myxoid tissue, and prominent vasculature, all surrounded by mature squamous epithelium. Giant fibrovascular polyps of the esophagus are uniformly benign lesions that are cured by excision. Secondary tumors such as squamous cell carcinoma arising within a fibrovascular polyp are rare.

Squamous papillomas

These are uncommon lesions usually found incidentally at endoscopy. The largest series is of 25 patients [59,60]. All patients in whom race was known were white with a female predominance, and the majority of lesions occurred in the distal esophagus. Five patients had multiple lesions. Many cases showed endoscopic evidence of hiatal hernia, gastroesophageal reflux, or esophagitis. No examples of Barrett esophagus were found. In our own material from a busy gastroenterology practice, about 70 cases have been biopsied at our hospital in the past 20 years.

Squamous papillomas are seen as tiny polyps at endoscopy. On histological evaluation, they consist of bland polypoid squamous mucosa with fibrovascular cores (Fig. 137.12). Exceptional cases display viral cytopathic effects. Several studies have evaluated the presence of human papillomavirus (HPV) in these lesions by both immunohistochemistry and in situ hybridization [59–62]. They are negative in most cases and the possibility of polymerase chain reaction (PCR) contamination would seem likely in one of the studies [62]. However, a subset of patients with HPV-associated laryngeal papillomatosis has HPV-related squamous papillomas of the esophagus and occasional esophageal examples have been reported unassociated with laryngeal lesions.

Squamous papillomas of the esophagus do not appear to degenerate into esophageal malignancies, and no dysplasia is seen histologically. Evidence of recurrence is unusual, but synchronous or metachronous carcinomas of the ororespiratory tract have been described. Many reports suggest a role for mucosal injury and regeneration in the pathogenesis of these lesions. The association with other malignancies may be significant but, in our material, these have been incidental lesions.

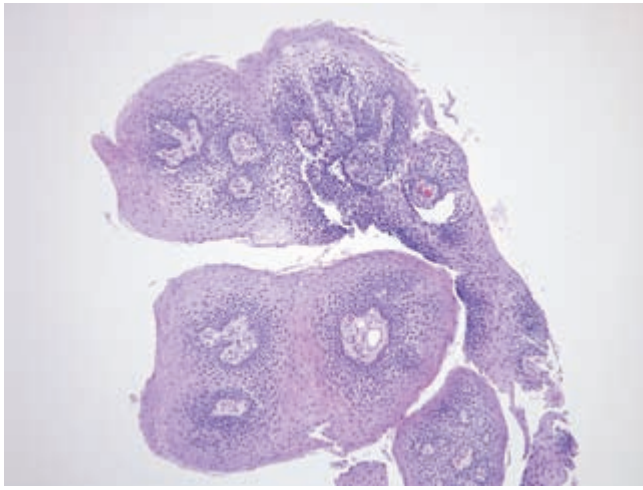

Figure 137.12 Squamous papilloma of the esophagus. Squamous mucosa coats fibrovascular cores.

Barrett esophagus

Since GERD has been shown on epidemiological grounds to be a strong risk factor for adenocarcinoma of the esophagus [63], evaluation of patients for the presence of histological precursor lesions (Barrett esophagus and columnar epithelial dysplasia in this setting) is best performed by a large number of upper endoscopic biopsies.

According to the American College of Gastroenterologists, Barrett esophagus is a change in the esophageal mucosa of any length that is visible at endoscopy and which contains intestinal metaplasia on biopsy [64]. Consequently, if an endoscopist is not certain whether a biopsy is from the esophagus, a pathologist cannot be sure whether the goblet cells found in it reflect cardiac intestinal metaplasia or Barrett esophagus. In the past, all columnar mucosa in the esophagus, whether of gastric cardiac type, oxyntic type or showing intestinal metaplasia, was diagnosed as Barrett esophagus. Currently, when biopsies are derived from the tubular esophagus and have columnar mucosa, the American College of Gastroenterologists requires the presence of unequivocal intestinal metaplasia in an endoscopically abnormal esophagus before an interpretation of Barrett esophagus is made [64]. However, it is well known that intestinal metaplasia can often be found at the gastroesophageal junction when no endoscopic lesion is apparent.

Endoscopically, Barrett mucosa appears as tongues and patches of reddish salmon-colored mucosa (in contrast to the normal pearly grey-pink color of the squamous epithelium) that extend from the gastroesophageal junction for varying distances up into the tubular esophagus. Short-segment Barrett esophagus is defined as less than 3 cm of metaplastic columnar epithelium and long-segment Barrett esophagus as more than 3 cm of metaplastic columnar epithelium.

Some authors have suggested immunophenotyping to separate cardiac intestinal metaplasia from true Barrett mucosa. Ormsby and colleagues evaluated cytokeratin (CK)7 and CK20 immunoreactivity patterns in resection specimens with long-segment Barrett esophagus and compared them to distal gastric resection specimens with intestinal metaplasia [65–67]. Virtually all cases with long-segment Barrett esophagus were characterized by superficial and deep CK7 immunoreactivity in the intestinalized mucosa with only superficial CK20 staining in the intestinalized zones. In contrast, distal gastric intestinal metaplasia was characterized by patchy superficial and deep CK20 staining in areas of incomplete intestinal metaplasia, strong superficial and deep CK20 staining in areas of complete intestinal metaplasia, and patchy or absent CK7 staining in either type of gastric intestinal metaplasia. As an extension of this work, this same group of researchers evaluated the utility of CK subsets in distinguishing intestinal metaplasia of the distal esophagus from that found in the gastric cardia in endoscopic biopsy specimens. Patients with intestinal metaplasia of the cardia all had

a normal-appearing squamocolumnar junction, and biopsies were obtained less than 5 mm from the esophagogastric junction with the endoscope in a retroflexed position. The Barrett CK7/CK20 pattern (described above) was found in all cases of Barrett esophagus but was absent in all 13 cases of cardiac intestinal metaplasia. However, other observers have questioned the utility of CK7/CK20 staining. CK7 is certainly the more useful of the two and it is key only to stain areas with a full gland profile with intestinal metaplasia. Piazuelo and colleagues found use of CK7 helpful in this distinction [68]. However, a study of carcinomas believed to arise in the esophagus and the cardia failed to find differences in CK7 and CK20 in these two types of cancers [69].

Use of MUC polypeptides has also been explored [68]. A significantly higher number of patients with Barrett esophagus ($P < 0.05$) showed goblet cell staining for MUC1 (55%) or MUC6 (32%) compared with patients with carditis with intestinal metaplasia (MUC1 14%, MUC6 7%) or antritis with intestinal metaplasia (MUC1 6%, MUC6 0%). Patients with Barrett esophagus also showed a higher frequency of MUC1 and MUC6 positivity in nongoblet columnar cells compared with carditis and antritis cases with intestinal metaplasia. Only cases of Barrett esophagus showed combined MUC1 and MUC6 staining (sensitivity 23%, specificity 100%). The sensitivity and specificity of MUC1 staining for Barrett esophagus are 55% and 96%, respectively, and for MUC6 staining 30% and 96%, respectively. Interestingly, normal gastric cardia mucosa also showed a significantly higher prevalence of MUC2 and MUC3 expression in glandular epithelium (29% and 38%, respectively) compared with the antrum (0% for both markers) ($P < 0.05$).

Das-1 antibody (named after one of the authors [70]) is also believed to stain Barrett metaplasia strongly, with less staining in gastric intestinal metaplasia.

Dysplasia in Barrett esophagus

Dysplasia refers to a neoplastic change in epithelium that remains confined to the neoplastic gland in which it arose. In other organ systems, the term *intraepithelial neoplasia* is usually applied, but old-fashioned terminology remains in place for the hollow viscus organs. Criteria in the esophagus were established at a consensus meeting held in 2001 [71].

Grading dysplasia in Barrett esophagus

Algorithm

The algorithm is based on four mucosal features in Barrett esophagus:

- surface maturation compared with underlying glands
- architecture of the glands
- cytological features
- inflammation and erosions/ulcers.

The algorithm presupposes that the biopsy is taken from the esophagus, that compatible endoscopic features of Barrett esophagus are found, and that intestinal metaplasia is found. Applying the algorithm, the classification of dysplasia is as follows.

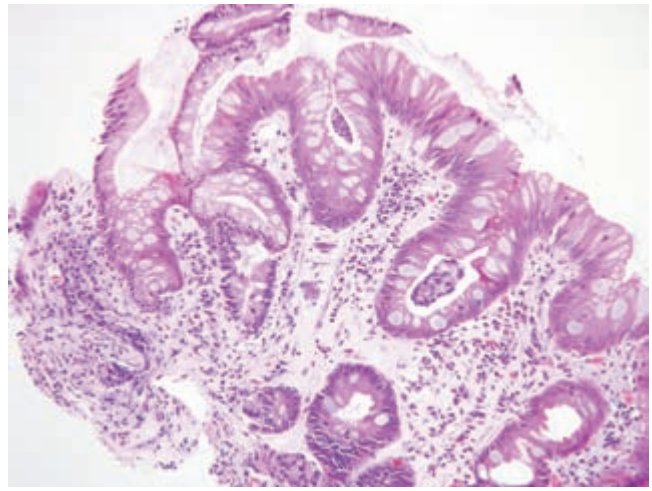

Figure 137.13 Barrett esophagus, negative for dysplasia. Note the goblet cells. There is surface maturation of the metaplastic cells and abundant lamina propria.

Barrett esophagus, negative for dysplasia

In Barrett esophagus without dysplasia (Fig. 137.13), the surface appears more mature than the underlying glands in that the nuclear-to-cytoplasmic ratio of surface cells is lower than that of the deeper glands. The architecture is normal, with abundant lamina propria between glands. The cytological features are normal, noting that mitoses may be present in deeper glands as well as nuclear stratification. The individual nuclei should have smooth nuclear membranes, and nucleoli, if present, should be small with smooth outlines. Nuclear polarity should be maintained in deep and superficial aspects of the biopsy. If inflammation is a component, reparative features may be present. In this setting, nuclear membranes should remain smooth, although the cells may display nuclear-to-cytoplasmic enlargement and nucleoli may become more prominent but retain smooth contours. The surface should show maturation compared with the deeper glands but there may be some loss of surface mucin.

Barrett esophagus, indefinite for dysplasia

Cases showing deeper cytological changes suggestive of dysplasia but with surface maturation are included in the indefinite category; other observers have used the indefinite category as a "wastecan" [72]. Cases in the indefinite category can show normal architecture or some degree of glandular crowding. On cytological evaluation, lesions can show hyperchromasia, nuclear membrane irregularities, and increased mitoses in the deeper aspects, and these mature to the surface. Loss of nuclear polarity is not a feature of the

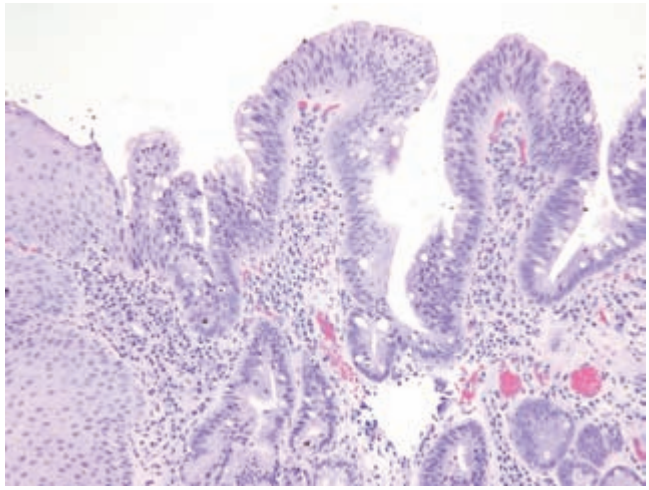

Figure 137.14 Low-grade dysplasia in Barrett esophagus. The epithelial changes are seen both in deep glands and on the surface but the nuclei are aligned perpendicularly to the basement membrane (maintained nuclear polarity).

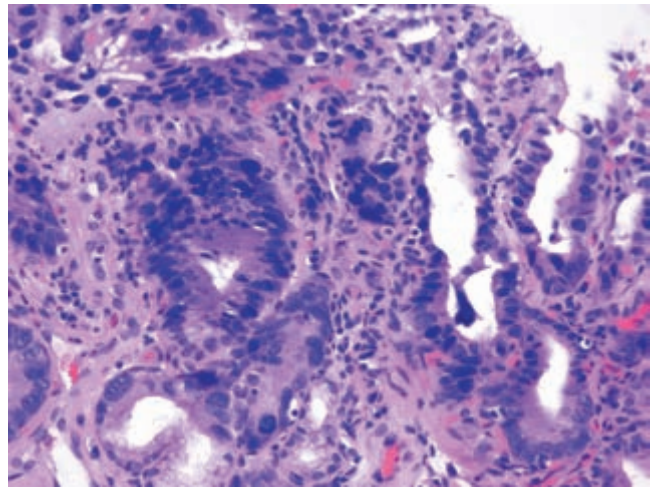

Figure 137.15 High-grade dysplasia and intramucosal carcinoma in Barrett esophagus. Hyperchromatic nuclei have lost their polarity (relation to the basement membrane).

indefinite category. In the presence of inflammation, more striking architectural abnormalities have been included in the indefinite category. This interpretation can also be applied when tangential embedding does not allow assessment between the glands and the surface. Some cases display peculiar morphology and it is unclear whether they are neoplastic or reparative. We often offer an explanation for resorting to this category when it is assigned.

Barrett esophagus, low-grade dysplasia

In Barrett esophagus with low-grade dysplasia (LGD) (Fig. 137.14), the surface appears similar to the underlying glands at low magnification or displays only slight maturation. The architecture may be mildly to markedly distorted with glandular crowding, although lamina propria should be identifiable between glands. The cytological features are important and the changes should extend at least focally to the surface. These include nuclear hyperchromasia with some chromatin clumping. The nuclei show nuclear membrane irregularities but not pronounced nuclear enlargement, although there may be focal marked enlargement if the other features support an interpretation of LGD. Nucleoli are not typically prominent in LGD. Loss of nuclear polarity is not a feature of LGD, although nuclear stratification similar to that seen in colonic adenomas is within the spectrum of LGD and may be present at the surface. Inflammation is typically minimal; cases with abundant inflammation and the other features of LGD are usually best classified in the indefinite category. If tangential embedding precludes evaluation of the surface, LGD can be diagnosed if there are dysplastic features in the deep aspects in the absence of inflammation provided that the features of high-grade dysplasia (see below) are lacking.

Barrett esophagus, high-grade dysplasia

As in LGD, surface maturation is lacking in high-grade dysplasia (HGD) (Fig. 137.15). The architecture may show crowding of cytologically abnormal glands or be markedly distorted with prominent glandular crowding and little intervening lamina propria. If the cytological features are sufficiently dysplastic, lesser architectural distortion is acceptable. Nuclei are hyperchromatic and nuclear membranes irregular. Most examples are similar to dysplasia in the uterine cervix in not displaying prominent nucleoli which, as in the cervix, tend to be present in either marked repair or when invasion has begun, both situations that are associated with ulcers. Cells may have either delicately clumped dark heterchromatin and inconspicuous nucleoli or prominent irregular nuclei with irregularly clumped chromatin and irregular nucleoli. Markedly enlarged hyperchromatic cells are a feature of HGD and these may extend to the surface. Loss of nuclear polarity is seen in HGD. Mitoses are readily identifiable. Inflammation is typically minimal. There is some evidence to suggest that HGD accompanied by an ulcer is a worrisome feature for an associated unsampled invasive carcinoma and we advise additional biopsies or endoscopic ultrasound when we see this pattern [73].

Intramucosal carcinoma

The distinction between HGD and the earliest intramucosal carcinoma (defined as invasion through the basement membrane into the lamina propria or muscularis mucosae but not beyond) remains difficult and observer variation between HGD and intramucosal carcinoma is not good. In general, these cases begin to demonstrate effacement of lamina propria architecture and a syncytial growth pattern, extensive back-to-back microglands, and an intermingling of single cells and small clusters within the lamina propria. Typically,

desmoplasia is absent or incompletely developed at this stage, and hence its recognition is difficult and subjective. In carcinoma which has invaded more deeply (into the submucosa), desmoplasia and a clearly infiltrative growth pattern become readily apparent, although tangentially embedded and scarred tissue can pose diagnostic problems. In the upper GI tract, invasion into the lamina propria is more significant than in the colon since the colon lamina propria lacks significant lymphatic access. In the colon, invasion into the lamina propria is biologically equivalent to HGD; in contrast, in the esophagus, invasion into the lamina propria can lead to metastatic disease.

Ancillary studies

Since the purpose of surveillance is to detect the earliest lesions, it is important for pathologists handling these biopsies to recognize them. Unfortunately, biopsy interpretation is plagued by problems with intraobserver and interobserver reproducibility [71,72], which probably accounts in part for the wide range of progression rates in various series, particularly at the lower ends of the spectrum. Among many surrogate markers explored to improve prognostication, flow cytometry and assessment of p53 may be useful adjuncts in identifying patients at highest risk [74] or even in identifying endoscopic foci of interest for follow-up in patients undergoing endoscopic mapping. Hopefully, newer technology will allow better clarification of high-risk patients and offer novel treatment options. However, pathologists do an excellent job in identifying high-risk lesions (HGDs) if these are biopsied. Sampling error and selection of the best patients to screen are probably more serious problems than diagnostic reproducibility in monitoring these patients. Despite substantial limitations inherent in interpretation of biopsies, grade of dysplasia correlates well with progression to invasive carcinoma, and it remains the foundation of clinical decision making. For example, in our series and others, grade on initial biopsy correlated significantly with progression to invasive carcinoma [75]. As a general rule, about half of patients with HGD ultimately progress to invasive carcinoma and no independently validated molecular test selects them. Estimates are poorer for LGD, but it results in progression rates of up to 15%.

Tumors

Adenocarcinoma

Since Barrett mucosa begins at the gastroesophageal junction and extends for varying distances into the tubular esophagus, adenocarcinomas are also most common in the distal third of the esophagus (in contrast to squamous cell carcinomas, which are most commonly found in the middle third).

Esophageal adenocarcinomas are seen at esophagectomy as firm whitish lesions usually involving the distal third. Often residual Barrett mucosa can be recognized by its salmon color and velvety appearance. When staging adenocarcinomas close to the esophagogastric junction, an attempt should be made to determine whether the tumor is esophageal or gastric [76]. The tumor is classified as esophageal if the epicenter is in the esophagus and gastric if the epicenter is in the stomach. The presence of Barrett mucosa is used as evidence that the tumor is of esophageal origin. This is of significance as esophageal carcinomas are staged as N0 or N1 whereas gastric ones are staged as N0, N1, or N2. It should also be noted that T1 includes tumors invading the lamina propria of the submucosa whereas T2 encompasses those invading the muscularis propria.

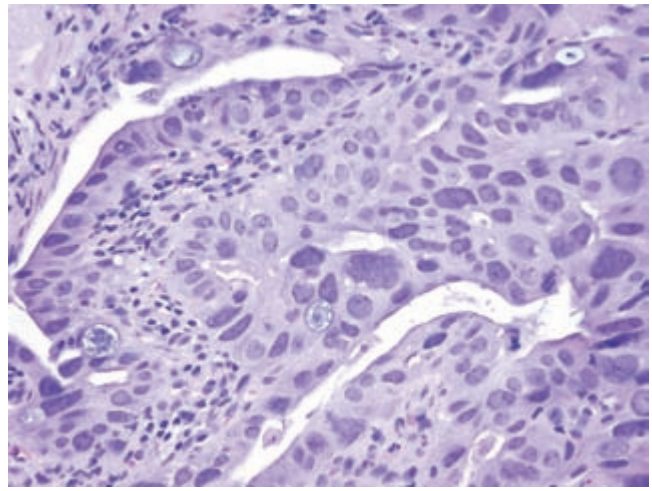

Figure 137.16 Esophageal adenocarcinoma. In this field, the adenocarcinoma undermines adjoining squamous mucosa.

Recognizing esophageal adenocarcinomas on biopsies is generally not a challenge and they appear as other adenocarcinomas, being composed of malignant cells with glandular differentiation either in the form of ductal structures or mucin production (Fig. 137.16). However, finding an in situ/HGD component is useful in assuring that the esophagus is indeed the primary site (rather than the lung). A subset of poorly differentiated carcinomas is essentially impossible to distinguish from high-grade lymphomas and requires immunohistochemistry for diagnosis. Poorly differentiated carcinomas may also assume spindle cell morphology (a more common scenario with squamous cell carcinomas) and, in the absence of an in situ component, an immunohistochemical panel is again advised.

Preoperative neoadjuvant chemotherapy and radiotherapy followed by esophagectomy is the preferred treatment; there is an especially poor prognosis when the patient presents with symptoms in the absence of surveillance for early detection.

Squamous cell carcinoma

Squamous carcinomas are most common in the middle third of the esophagus. On imaging studies, the presence of an esophageal mass in the middle third of the esophagus is in

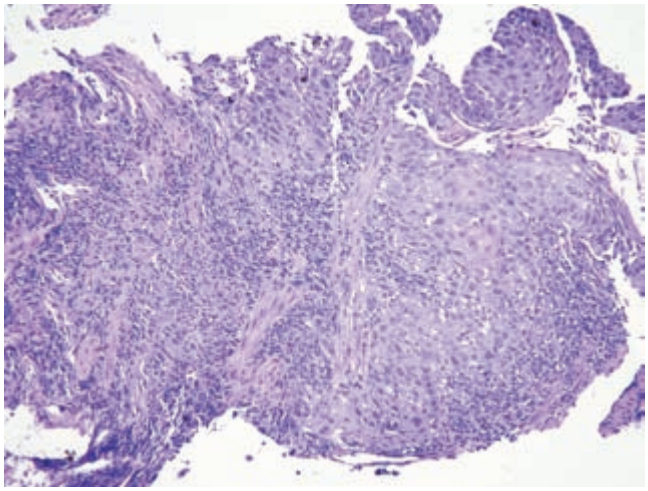

Figure 137.17 Esophageal squamous cell carcinoma. There is a squamous pearl toward the right of the field. In this biopsy, the lesion has invaded the muscularis mucosae, seen as slender pink strips.

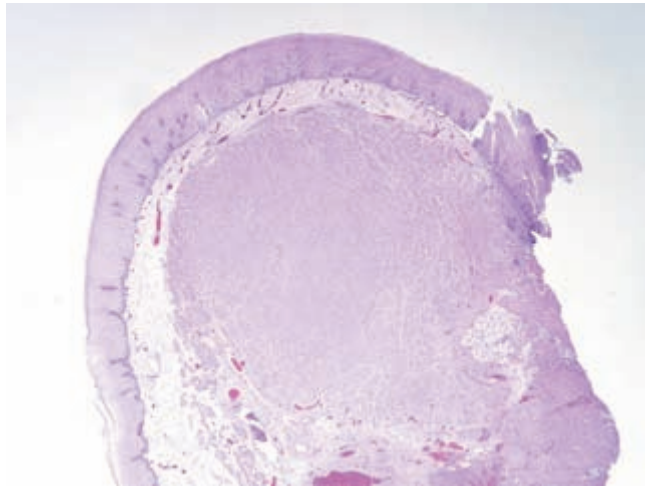

Figure 137.18 Granular cell tumor of the esophagus. At low magnification, a well-marginated nodule is seen in the lamina propria.

keeping with squamous cell carcinoma but there are no specific radiological features of this type of tumor.

On biopsies, squamous carcinoma of the esophagus appears similar to squamous carcinomas elsewhere (Fig. 137.17). They are usually well-differentiated squamous carcinoma with prominent keratinization. Background squamous epithelial dysplasia (intraepithelial neoplasia) including carcinoma in situ (HGD/intraepithelial neoplasia) is frequent at the periphery of invasive tumors. Dysplasia alone without invasion is uncommon. Some squamous carcinomas assume a prominent spindle cell appearance. This latter subtype tends to present as a polypoid mass. Short-term survival of such tumors is better than that for flat typical carcinomas owing to their exophytic growth, but long-term follow-up erases an apparent survival advantage. Squamous cell carcinomas of the esophagus, like squamous carcinomas elsewhere, express CK5/6 and a host of epithelial markers. Most examples are not a diagnostic problem. When spindled, melanoma and sarcomas must be excluded. The best way to do this is by sampling as much of the overlying squamous mucosa as possible in order to detect an in situ component.

Reported molecular genetic abnormalities include p53 mutations and deletion, deletion of Rb and p16, amplification of epidermal growth factor receptor, c-*myc*, int-2/hst-1 and cyclin D1, and overexpression of epidermal growth factor and insulin-like growth factor receptor-1. There is no important role for *ras* gene mutations.

Unfortunately, squamous carcinomas usually present late clinically, after extension into periesophageal soft tissue is already present. Esophageal obstruction, extension of carcinoma into mediastinal and intrathoracic structures (tracheobronchial tree, aorta, lung), and mediastinal lymph node metastases are more frequent clinical problems than distant metastases.

Radiotherapy or chemotherapy is the usual treatment, sometimes with esophagogectomy; this is usually palliative rather than curative. Esophageal squamous carcinoma has a dismal prognosis: the 5-year survival rate is 5%–10%.

Granular cell tumors

Granular cell tumors are generally rare. The most common sites are the tongue and skin [77]. A subset is multicentric and rare examples are malignant [78]. Granular cell tumors of the esophagus comprise about 1%–2% of all granular cell tumors and the esophagus is the most common GI site [79]. There are fewer than 100 reports of esophageal granular cell tumors in the literature. We have only encountered 12 esophageal granular cell tumors at our institution in the last 15 years. Most arise in the distal esophagus and about 5%–10% are multicentric. There is a female predominance and these tumors are relatively more common in African Americans than in whites. Rare malignant esophageal granular cell tumors have been reported [80,81]. Esophageal granular cell tumors are most likely to be identified incidentally at endoscopy but some patients are symptomatic. Most appear as well-marginated masses on imaging studies. Associated concentric narrowing can also be found.

On microscopic examination, tumors are centered in the submocosa with minor extensions into mucosa and muscularis propria and are well marginated but not encapsulated (Figs 137.18 and 137.19). They are composed of plump neoplastic cells with abundant lightly amphophilic granular cytoplasm which displays retention of PAS staining on diastase digestion. The cells are typically closely packed. Nuclei are small to pyknotic with occasional nucleoli. About half display squamous (pseudoepitheliomatous) hyperplasia in the overlying mucosa, which can raise the possibility of squamous cell carcinoma on superficial biopsies. Based on this hyperplastic squamous pattern, mucosal biopsies often

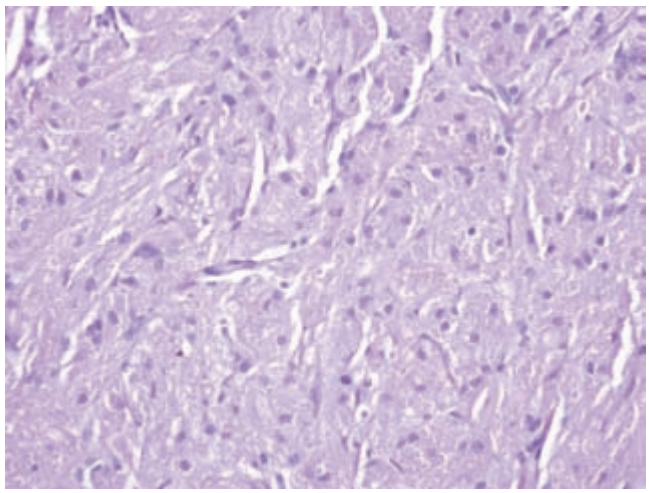

Figure 137.19 Granular cell tumor. Note the granular appearance of the eosinophilic proliferating cells.

contain predominantly the proliferated squamous epithelium and only a handful of lesional cells in the superficial lamina propria.

Granular cell tumors are reactive with S100 protein, calretinin, inhibin α, myelin basic protein, and CD68/Kp1 but lack muscle markers and melanoma markers.

The vast majority of granular cell tumors behave in a benign fashion. Those rare examples that have metastasized typically display prominent cytological alterations or mitotic activity.

Leiomyoma

This is the most common spindle cell tumor of the esophagus. It displays smooth muscle differentiation. Esophageal leiomyomas occur in a relatively young population (median age 35 years) [82] and there is a male predominance. Leiomyomas of the esophagus are, of course, benign. They show eosinophilic cytoplasm, and are positive for desmin and smooth muscle actin (SMA) and negative for CD117/c-kit and CD34. Rarely, leiomyosarcomas arise in the esophagus and are typically large high-grade tumors that show muscle cell markers but no CD117. These tumors are seldom encountered on biopsy as they tend to be intramural in location.

Gastrointestinal stromal tumors

Leiomyomas are the most common esophageal mesenchymal neoplasms even though they are distinctly uncommon in the remainder of the GI tract, despite the abundance of mural smooth muscle. In contrast, gastrointestinal stromal tumors (GISTs) predominate in the stomach and intestines but are truly rare in the esophagus. Using the files of the AFIP and the Haartman Institute of the University of Helsinki, Miettinen and colleagues were only able to accumulate 17 examples of esophageal GISTs [82]. The esophageal GISTs occurred in 12 men and five women with a median age of 63 years (range 49–75 years). All tumors were from the lowest third of the esophagus, and the most common complaint was dysphagia, whereas two tumors were detected incidentally. Histologically the tumors had the usual appearance of GISTs, displaying an overall basophilic appearance and combinations of solid, myxoid, and perivascular collar-like patterns with a spindle cell histology in 13 patients and epithelioid histology in four patients. All tumors were positive for CD117 and CD34, whereas two patients were also positive for α-SMA and three patients were positive for desmin. Nine patients died of disease, including all who had a tumor larger than 10 cm, and also one patient whose tumor showed five mitoses per 50 high-power fields.

Melanoma

Primary melanoma of the esophagus is quite rare, with fewer than 300 cases reported. The affected patients are adults, with a mean age of about 60 years [83–87]. They are more common in men than women but there is no racial predominance. Lesions predominate in the distal esophagus. At endoscopy, they are polypoid and pigmented in about 85% of cases. Imaging studies show bulky polypoid masses that bulge intralumenally without resultant obstruction. When biopsied, the findings in esophageal melanomas are similar to those elsewhere. Examples regarded as primary may display an in situ component. Obviously, finding this is extremely useful in establishing the esophagus as the primary site. Otherwise, cells are spindled to epithelioid with variable pigment, prominent nucleoli, and prominent intranuclear pseudoinclusions.

Primary esophageal melanomas have a dismal prognosis. Only rare patients whose tumors present early can be cured.

Stomach

The stomach has four main zones with divergent types of mucosa. The simplest way to consider the different parts of the stomach is that the ends (cardia and antrum) protect the esophagus and duodenum from acid and enzymes whereas the middle of the stomach (body and fundus) produces the acid and enzymes.

The empty stomach is thrown into rugae (folds). Tiny surface invaginations termed gastric pits are the conduits of secretions. Histologically the entire stomach has a surface of foveolar cells, which secrete neutral mucin that appears magenta to pink on PAS stain. In the isthmus region of the body and fundus, parietal cells (oxyntic cells) dominate but mucous neck cells are also present. Mucous neck cells are seen in the neck, and chief cells dominate in the base. Endocrine cells are in the deep isthmus towards the base. The cardia and antrum are simpler since the layers all predominantly produce mucin, although the antrum has G cells that produce gastrin. Gastrin is secreted by the antrum but not the body and fundus. The lamina propria of the stomach should

contain only a small population of lymphocytes, plasma cells, mast cells, and eosinophils, in contrast to the intestines, where these constituents are more abundant. Bacteria are usually not seen in the normal gastric mucus (in contrast to the colon, where they are normally present).

When pathologists consider biopsies from the stomach, features that help in diagnosis often involve correlation with clinical information. The endoscopist will be better served by providing information since the histological features of gastric diseases display considerable overlap. Even the gender and ethnicity can be of some value. For example, autoimmune metaplastic atrophic gastritis typically affects older white females and is unlikely (but not impossible) in African Americans. On a busy GI biopsy service, there are frequently many biopsies to review and time efficiency is critical so it is useful for the pathologist to have an idea which biopsies require the greatest attention. In gastric biopsies, the snake in the grass is signet cell carcinoma, which is often focal and subtle in mucosal biopsies. Any clinical concern (such as a stomach that distends poorly on insufflation by the endoscopist) merits a careful review of all biopsy fragments from gastric biopsies; this concern should be emphatically conveyed to the pathologist.

Inflammatory conditions

Acute hemorrhagic gastritis

Acute hemorrhagic gastritis is prototypically associated with alcohol abuse [88,89] but other agents that are injurious to the gastric mucosa can also be responsible [90,91]. In the early literature, it was difficult to separate the effects of *Helicobacter* gastritis from pure alcohol injury but we presently believe that the acute injury from alcohol is similar to a corrosive injury with little inflammation but with abundant edema, hemorrhage, and reactive epithelial changes.

Chemical gastritis/chemical gastropathy/reactive gastropathy

This type of injury, which is seen mostly in the antrum, is common as it is usually attributed to bile reflux and thus is usually seen in patients being evaluated for GERD (which in reality is duodenogastroesophageal reflux disease) [92,93]. Bile reflux is injurious to the gastric antrum as well as to the esophagus. In patients in whom the antrum has been removed, bile reflux injures the gastric body and contributes to the development of so-called "stump" cancers found in the postantrectomy setting. The other typical scenario for reactive gastropathy/chemical gastritis is seen in patients taking nonsteroidal antiinflammatory drugs (NSAIDs), a significant percentage of individuals. The general features of reactive gastropathy are foveolar hyperplasia, lamina propria edema, muscular stranding, and vascular ectasia (Fig. 137.20). Active and chronic inflammation are minimal, and hence some observers prefer the term *reactive gastropathy* rather than chemical gastritis.

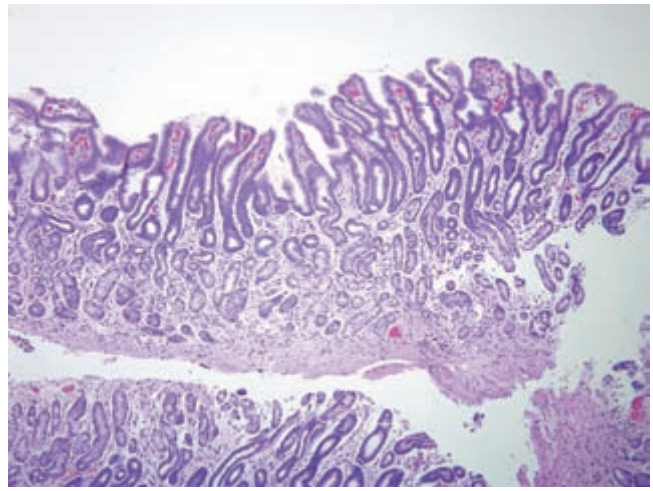

Figure 137.20 Chemical gastritis/reactive gastropathy. The antral mucosa has a villiform appearance and there is mucin loss in the epithelium. There is very little inflammation.

Mucosal calcinosis

Mucosal calcinosis is seen in some patients with renal failure and has been attributed to antacid use [94] but probably mostly reflects disorders of calcium metabolism that are typical in such patients [95]. Regardless, mucosal calcinosis seems to be of no significance in and of itself but an incidental finding in patients with renal failure.

Proton pump inhibitors

Proton pump inhibitors are commonly taken by patients having gastric biopsies. Examples of these include Prilosec (omeprazole), Prevacid (lansoprazole), and Nexium (esomeprazole). These medications reduce acid secretion by gastric parietal cells and have a morphological correlate of apocrine-like cytoplasmic swelling of parietal cells.

Injury due to chemical agents

Iron

Iron pill gastritis appears similar to iron pill esophagitis [21] as discussed above but is of particular note, since striking epithelial changes in the setting of iron pill gastritis sometimes mimic gastric carcinoma.

Kayexalate

Kayexalate can be found associated with gastric mucosa and gastric erosions/ulcerations just as in the esophagus. In the stomach, kayexalate bezoars may formed [23].

Colchicine

Colchicine is an alkaloid with antimitotic activity used to treat a variety of medical conditions, especially gout. Colchicine toxicity can result in multiorgan failure and death. If patients are taking colchicine at therapeutic doses, there are no abnormal biopsies. However, in patients who are colchicine

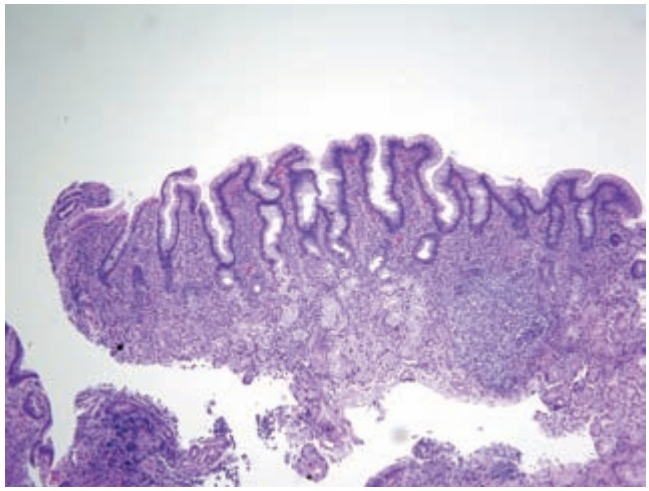

Figure 137.21 Active chronic *Helicobacter pylori* gastritis. Note the lymphoid follicles in this low-magnification field.

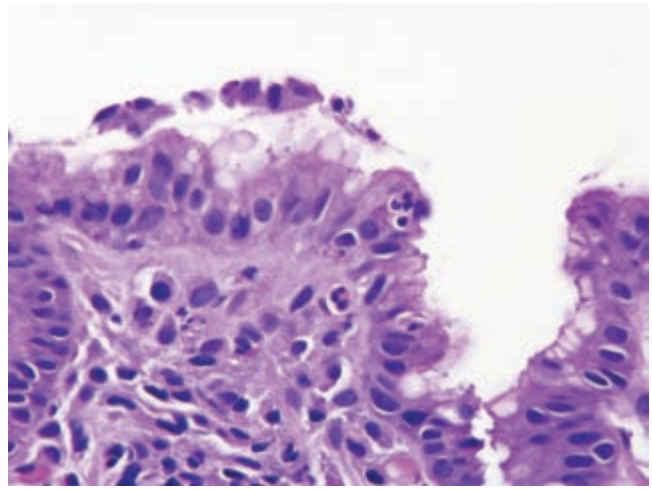

Figure 137.22 Active chronic *Helicobacter pylori* gastritis. At high magnification, neutrophils are seen in the epithelium. Organisms are easily identified on this hematoxylin and eosin stain but it is usually best to apply one of several other stains to detect them.

toxic (typically in the setting of renal insufficiency), distinct morphological changes (metaphase mitoses, epithelial pseudostratification, and loss of polarity, and abundant crypt apoptotic bodies) are seen in biopsy material [96]. These morphological features are best seen in biopsies from duodenum and gastric antrum, with relative sparing of the gastric body in the upper GI tract. Ki67 staining demonstrates an expansion of the proliferating region. Recognition of these features is important because colchicine toxicity can be fatal if undiagnosed clinically.

Helicobacter gastritis

Helicobacter gastritis has been a source of gastritis for years but was not recognized until Marshall and Warren identified the organism [97]. Marshall ultimately fulfilled Koch's postulates by swallowing a pure culture of the organism and acquiring gastritis [98]. There have been over 20 000 publications devoted to this organism in the ensuing 20 years and Marshall and Warren have been awarded a Nobel prize for their observations. *Helicobacter pylori* is now recognized as a curved flagellated Gram-negative rod that has variant forms (especially a coccoid form seen in treated patients) that is now known to be associated with both gastric carcinoma and mucosa-associated lymphoid tissue (MALT) lymphoma. Another variant, *Helicobacter heilmannii*, is more tightly coiled and rare (< 1% of *Helicobacter* isolates) but cross-reacts with the immunohistochemical stain for *H. pylori* and is fully capable of producing gastritis and associating with MALT lymphoma and gastric adenocarcinoma. Like *H. pylori*, *H. heilmannii* produces antral-predominant gastritis but this gastritis is less severe than *H. pylori* gastritis [99]. *Helicobacter* gastritis was formerly known as diffuse antral gastritis, type B gastritis (Figs 137.21 and 137.22) (autoimmune gastritis was type A), and superficial gastritis. In the modern practice of pathology, all gastric biopsy reports should include a statement on *Helicobacter* status. At our hospital, all gastric biopsies are thus stained with Diff-Quik (Fig. 137.23) but any such stain is appropriate. Hematoxylin and eosin staining alone is not regarded as sufficient, although these organisms can certainly be recognized on routine H & E stains [100]. Immunohistochemistry is interesting in that it highlights organisms embedded in tissue, specifically in parietal cells. It is also of value in identifying small coccoid forms found in partially treated patients. We seldom use immunohistochemistry as it is not cost-effective in our practice (serology is less expensive) but some observers perform immunohistochemistry in every case, a practice that may reflect economic factors.

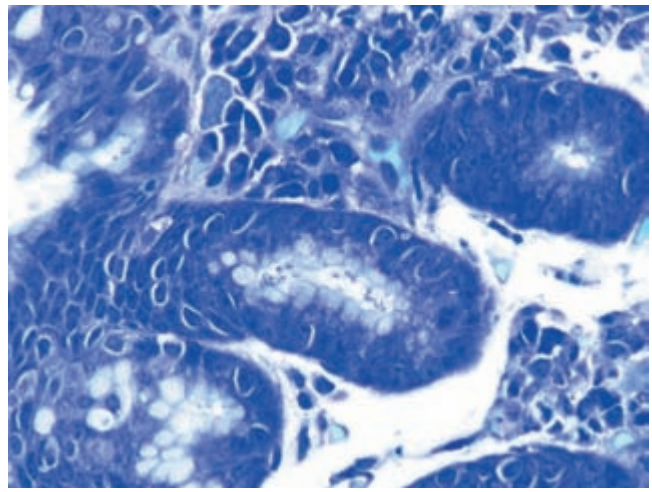

Figure 137.23 Active chronic *Helicobacter pylori* gastritis. A Diff-Quik stain highlights the organisms, seen as curved bacilli in the gland at the center of the field.

The endoscopic findings in *H. pylori* gastritis, and in assessing gastritis in general, correlate imperfectly with pathological findings, although some observers have learned to recognize a mamillated endoscopic corpus appearance and an abnormal antral appearance [101].

Histologically, *H. pylori* organisms are seen in gastric mucus and overlying surface and foveolar epithelium. They do not colonize nonsurface or foci of intestinal metaplasia. There is typically abundant lymphoplasmacytic chronic inflammation of the lamina propria with lymphoid follicles (MALT). There is often abundant active inflammation (neutrophils). The inflammation is concentrated in the upper mucosa, hence the old term of superficial gastritis. Following treatment, the active inflammatory component resolves but the chronic inflammatory component persists for months to years.

Helicobacter seems to have a particular preference for damaging D cells in the antrum, which secrete somatostatin. Since somatostatin usually keeps gastrin secretion in check, loss of these D cells causes excess gastrin secretion, which stimulates excess acid secretion in acutely infected patients. This is, in turn, responsible for the duodenal ulcers that often accompany *Helicobacter* gastritis [102].

Inactive chronic gastritis

There is an extensive literature on grading chronic gastritis, complete with published pictograms [103,104]. However, pictograms for chronic gastritis are seldom valued in daily practice but are a useful construct for standardization in research studies. Chronic gastritis is generally attributable to prior *Helicobacter* infection based on serological studies and is a common finding in biopsy material. However, the threshold for diagnosing it is not entirely clear. In a fashion that is more practical than evidence-based, we diagnose inactive chronic gastritis at 4× magnification and confirm at higher magnification. At low magnification, the lamina propria of the stomach should have only a few inflammatory cells, which appear as small dots. We essentially use a slight prominence in "dot density" as the criterion for low-magnification diagnosis of inactive chronic gastritis.

Metaplastic atrophic gastritis

Metaplastic atrophic gastritis occurs in two distinct types and, because of this, it is best if biopsies are obtained from both the gastric antrum and the body. Some authors have published studies with cartoons to be used as templates for grading the degree of inflammation and atrophy in gastritis but these are of little value in daily practice [103,104].

Autoimmune metaplastic atrophic gastritis

Autoimmune metaplastic atrophic gastritis (AMAG) was called type A gastritis in the past (before the role of *Helicobacter* was elucidated). This is a disease in which antibodies attack parietal cells. It is a disease typically affecting older women, usually with pale skin and blue eyes (e.g., Nordic ancestry). Patients have antibodies to parietal cells and to intrinsic factor (and thus are prone to severe vitamin B-12 deficiency/pernicious anemia). They are at threefold increased risk of developing gastric cancer but are not at risk for ulcers since their parietal cells are reduced in number or absent and thus little or no acid is produced. Since parietal cells are the type affected, the changes occur only in zones where parietal cells are found. Thus there is metaplasia and atrophy only in the body and fundus. An affected patient has no significant inflammatory disease in the antrum (hence the importance of taking biopsies from both the antrum and the fundus). Fundus biopsies show extensive loss of parietal cells and metaplasia. In some cases there are residual nests of parietal cells with chronic inflammatory cells (T cells) in the process of destroying them. In fact, the residual more normal mucosa sometimes appears as polyps to the endscopist in a sea of severe atrophy [105]. Metaplasia may be of the intestinal type or the pseudopyloric type. In pseudopyloric metaplasia, the oxyntic mucosa resembles antral mucosa in that it lacks parietal and chief cells (oxyntic glands) but differs from it by not secreting gastrin. Only the true antrum secretes gastrin. Thus, immunohistochemistry for gastrin in biopsies from the body lacks reactive cells, a finding that can be exploited to establish a diagnosis or exclude inaccurate sampling (i.e., the endoscopist has biopsied the antrum but labeled it "body"). In AMAG, there is no metaplasia in the antrum, which often shows hyperplastic features, possibly secondary to bile reflux (Figs 137.24 and 137.25). Since the patients are hypoacidic based on loss of parietal cell mass, a feedback loop causes antral G cells to produce abundant gastrin to stimulate acid secretion. As there are few or no parietal cells (Fig. 137.26), gastrin levels become progressively elevated in these patients and this stimulates proliferation of endocrine cells in the gastric body as a side effect. Thus chromogranin stains show linear and nodular endocrine cell hyperplasia (Fig. 137.27)

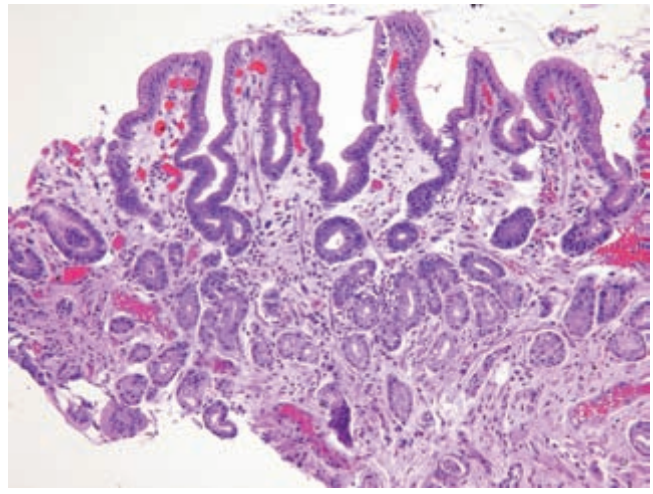

Figure 137.24 Autoimmune metaplastic atrophic gastritis. The antrum from this patient shows chemical gastritis. There are few inflammatory cells.

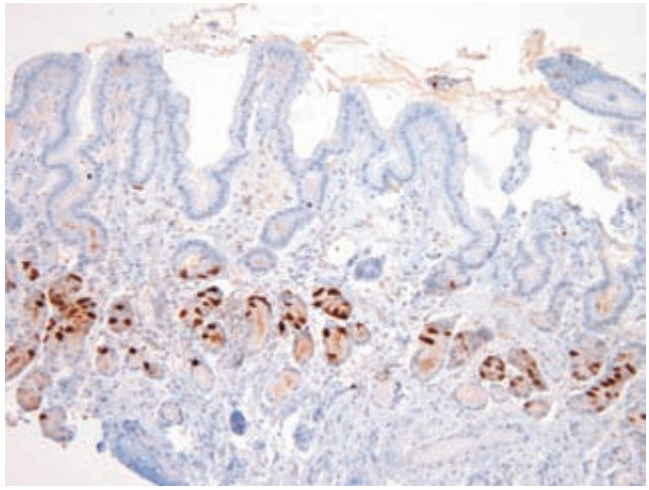

Figure 137.25 Autoimmune metaplastic atrophic gastritis. This is a gastrin stain from the field depicted in Fig. 137.24 There are many gastrin-producing cells. The patient was probably hypergastrinemic.

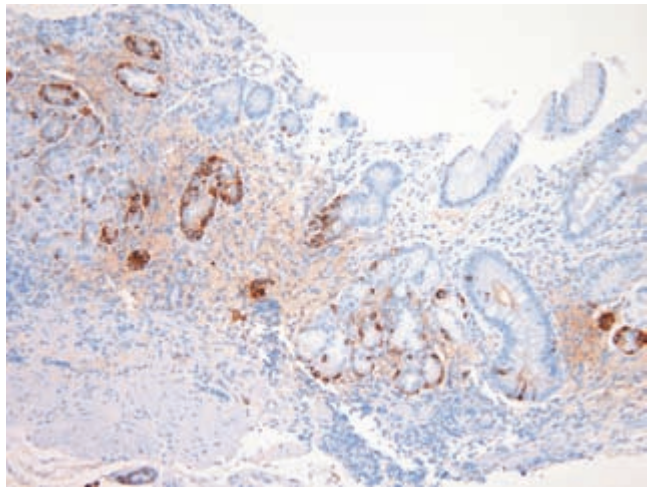

Figure 137.27 Autoimmune metaplastic atrophic gastritis. This chromogranin stain highlights endocrine cell hyperplasia in the area seen in Fig. 137.26 Such proliferation can lead to type 1 carcinoid tumors.

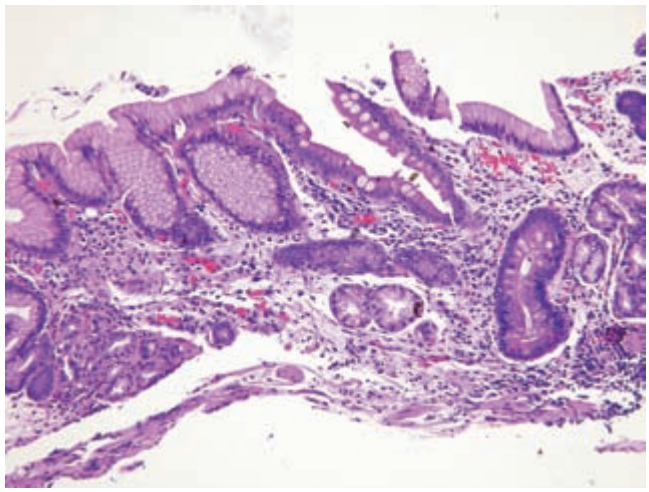

Figure 137.26 Autoimmune metaplastic atrophic gastritis. This biopsy is from the gastric body but there are no acid-producing cells. There is intestinal metaplasia in the center portion of the field (goblet cells).

in the body, none of which is attributable to G cells. If this condition persists, small indolent carcinoid tumors can result.

Environmental metaplastic atrophic gastritis

Helicobacter gastritis is probably the most important cause of environmental gastritis but is probably not the cause of all cases. Other associations include various dietary factors such as excessive salt, smoked foods (nitrites), paucity of green vegetables and fruits (lack of vitamins C and E, β-carotene, selenium), and nitrosamines. Nitrosamines can be produced from nitrites in the setting of colonization by anaerobic bacteria in a hypoacidic stomach. This type of gastritis has been called type B gastritis and multifocal atrophic gastritis.

The key feature is that environmental atrophic gastritis is most marked in the antrum. Multiple foci first appear in the transition zone between antrum and body at the area of the lesser curvature. Over time the entire antrum is affected but the body is relatively spared. There is less disease in the body, first in the distal body, but over many years affecting more of the body. The body can display pseudopyloric and intestinal metaplasia as in autoimmune gastritis, but of course the antrum is affected as well. These patients retain enough parietal cell mass that they seldom develop pernicious anemia. They can become hypochlorhydric but achlorhydria is rare. Their serum gastrin is usually normal and they can have ulcers and develop cancers. In the USA, it is usually seen in patients older than 50 years but in certain parts of the world atrophy appears in patients in their third or fourth decades (e.g., Japan, Andean South America).

Gastric antral vascular ectasia

Gastric antral vascular ectasia (*watermelon stomach*) was first described by Jabbari in 1984 and refers to an endoscopic appearance [106]. In this condition, longitudinal antral folds have visible reddened vessels radiating from the pylorus in a distribution resembling a watermelon rind. Patients present with iron deficiency secondary to chronic gastric bleeding. The typical patient is an elderly woman with achlorhydria, chronic liver disease, CREST syndrome, or lymphoma. Some cases are associated with scleroderma [107]. In some cases the endoscopic appearance is not prototypic but instead consists of diffuse gastric erythema. The etiology is not well known but is probably an acquired lesion. Some patients have antral prolapse [106].

Histological examination discloses features that reflect a mucosal prolapse component: there is foveolar hyperplasia with dilated mucosal capillaries, focal thrombi, and fibromuscular

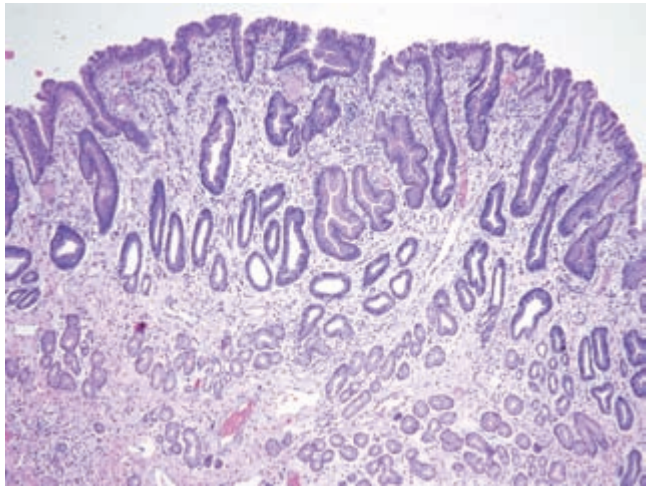

Figure 137.28 Gastric antral vascular ectasia (watermelon stomach). Even at low magnification, many vascular thrombi are apparent.

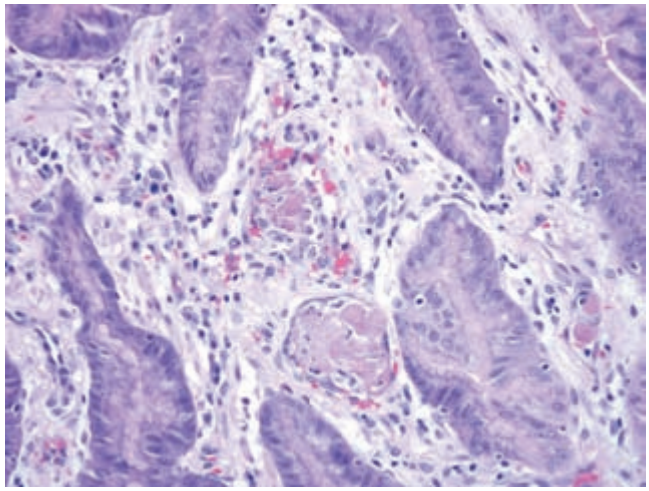

Figure 137.29 Gastric antral vascular ectasia (watermelon stomach). High magnification of fibrin thrombi.

hypertrophy [108] (Figs 137.28 and 137.29). Similar features may be seen in patients who have portal hypertension but the dilated capillaries in portal hypertension lack fibrin thrombi; however, patients with watermelon stomach may also have portal hypertension [109]. Treatment of watermelon stomach often entails mucosal ablation since patients have uncontrolled blood loss. Endoscopic therapy, including contact and noncontact thermal ablation of the ectatic vascular lesions, is the mainstay of conservative therapy. However, many patients fail endoscopic therapy and develop recurrent acute and chronic GI bleeding episodes. Surgical resection may be the only reliable method for achieving a cure and eliminating transfusion dependency. Traditionally, surgery was used only as a last resort after patients failed prolonged medical or endoscopic therapy. However, based on the experience garnered from the literature some authors have recommend a more aggressive surgical approach in patients who fail a short trial of endolumenal therapy [110].

Lymphocytic gastritis

Lymphocytic gastritis is a term that simply describes a pattern of inflammation rather than a specific etiology. It is yet another example of the limited repertoire of responses that the GI tract can mount to various types of injury. Lymphocytic gastritis is an uncommon form of chronic gastritis characterized by lymphocytosis of foveolar and surface epithelium. Lymphocytic gastritis is associated with celiac disease, *H. pylori* gastritis, and a varioliform gastritis pattern (an endoscopic form of gastropathy comprising enlarged folds, nodules and erosions, also called "octopus sucker" appearance [111]). Wu and Hamilton [112] studied 103 patients with lymphocytic gastritis classified according to the associated entities, including the distribution and severity of the gastritis in the 70 patients from whom biopsy specimens of both antrum and body were available. In 84 patients (82%), a distinct associated entity was identified, including 39 with celiac disease, 30 with *H. pylori* infection, four with varioliform gastritis, two each with inflammatory polyp, Crohn's disease, human immunodeficiency virus (HIV) infection, lymphoma, and esophageal carcinoma, and one with lymphocytic gastroenterocolitis. Lymphocytic gastritis was found in 33% of patients with celiac disease and 4.1% of histopathologically defined *H. pylori* gastritis. Lymphocytic colitis was common (5 of 13, 38%) in patients with celiac disease and lymphocytic gastritis. The authors concluded that lymphocytic gastritis most commonly occurs in celiac disease and *H. pylori* infection, but rarely with other entities. There is no need to count lymphocytes to arrive at a diagnosis of lymphocytic gastritis; they should simply be prominent and the diagnosis is made by pattern.

Collagenous gastritis

This describes a rare pattern of injury in which prominent subepithelial collagen, intraepithelial lymphocytosis, and surface damage is present in the stomach. It has been associated with lymphocytic and collagenous colitis and with celiac disease. There is a female predominance and the changes may be found throughout the stomach [113–121].

Gastric Crohn's disease

In the absence of granulomas, it is difficult to know in gastric biopsies whether inflammatory changes reflect unspecified gastritis or are a manifestation of the patient's systemic disease. However, some observers have used the term *focally enhanced gastritis* to refer to a pattern of gastritis seen in Crohn's disease [122]. This refers to a pattern in which most of the biopsy has little or no gastritis and only a single area displays a more striking inflammatory infiltrate. We have also found that identification of this pattern of focally enhanced gastritis is helpful in identifying upper tract Crohn's disease but it is not wholly specific, especially in children [123].

Granulomatous gastritis

Granulomatous gastritis is explained by Crohn's disease in about half of cases seen in Western populations and has been attributed to *Helicobacter* in populations with prevalent infection [124] but its etiology often remains unexplained and it seems to be of little clinical significance. In daily practice, when confronted with isolated granulomas in a gastric biopsy, we make an attempt to correlate with history or Crohn's disease or sarcoidosis and exclude infectious etiologies, including *Helicobacter* gastritis. Rare cases of granulomatous gastritis have been associated with adenocarcinoma, so this is always sought [125]. If a necrotizing appearance is present rather than isolated small granulomas, vasculitis is considered but we are reluctant to diagnose vasculitis definitively on any mucosal biopsy.

Other inflammatory conditions

A discussion of every cause of gastric inflammation is beyond the scope of this chapter, but some such conditions merit brief mention. Like the esophagus, the stomach can also be the center of GI tract allergic disease (eosinophilic gastritis) and prominent eosinophilia involving epithelium and muscularis is sought (since scattered eosinophils are a normal lamina propria constituent). Again, we do not count eosinophils but simply note whether they are prominent and suggest correlation with other stigmata of allergic disease.

CMV gastritis is not uncommon in inpatient material, where it can be found in both epithelial and endothelial cells. A pitfall in diagnosis can be the monocytic reaction it can invoke, which can be mistaken for lymphoma. This is also a potential pitfall with rare examples of Epstein–Barr virus (EBV) gastritis, which appears similar to large cell lymphoma in the manner that infectious mononucleosis simulates leukemia.

Gastric graft-versus-host disease

Gastric GVHD shows the same features as GVHD elsewhere in the GI tract, with epithelial apoptosis and ulcers/erosions to a varying degree [29]. We make an attempt to grade these changes as mild, moderate, and severe (where severe changes include ulcers/erosions) and to compare current biopsies with prior ones when possible to assess improvements (or lack thereof). Such biopsies are carefully studied for CMV inclusions as well.

Hyperplastic gastritis

A simplistic approach to hyperplastic gastritis (or *giant folds*) is to consider whether the cell type resulting in the thickened folds is foveolar hyperplasia or hyperplasia of oxyntic glands.

Foveolar hyperplasia

The syndrome of hypertrophic gastric folds, oxyntic gland loss (and thus achlorhydria), and protein loss is also termed Ménétrier disease [126–131]. However, this term often describes a phenotype rather than an etiology when used to encompass the host of lesions that can display hyperplastic mucosa [126–144]. When used this way, it is another example of the limited repertoire of mucosal response to a variety of insults and in fact the original cases reported by Ménétrier were probably not a single entity. However, some observers suggest isolating those cases that display massive diffuse foveolar hyperplasia in the absence of inflammation as the "true" disease (massive foveolar hyperplasia) [127]. The mucin in this condition has a profile that differs from the mucin extracted from normal stomachs, but this pattern does not explain the etiology [141].

Ménétrier disease is a rare disorder that has been described in adults as well as children (average fourth to sixth decades). It is three times more common in men than women. The course is usually chronic with an unfavorable prognosis. Patients present with hypoproteinemia and peripheral edema with loss of immunoglobulins, albumin, and transferrin. Symptoms appear insidiously and become progressive, consisting of epigastric pain, dyspepsia, anorexia, peripheral edema, hematemesis, and vomiting. The risk of developing carcinoma with Ménétrier disease is subject to debate. Approximately 15% of Ménétrier disease cases in the literature have been associated with carcinoma. In contrast to adults, children have a self-limited course for hyperplastic gastropathy, and CMV infection has been frequently implicated. Overexpression of transforming growth factor (TGF)-α has a possible role in the pathogenesis of Ménétrier disease. Transgenic mice that overproduce TGF-α in the stomach have many features of Ménétrier disease such as foveolar hyperplasia, increased mucin content, decreased parietal cell mass, and reduced acid production.

On mucosal biopsy, this condition appears no different from a gastric hyperplastic polyp, consisting of hyperplastic foveolar epithelium arranged in a disorderly fashion with loss of oxyntic mucosa in biopsies from the body or fundus (Fig. 137.30). Making a diagnosis of this sort of hyperplastic gastropathy on mucosal biopsies is impossible without correlation with the endoscopic and imaging findings.

Hyperplasia of oxyntic glands

Giant folds may be imparted by marked hyperplasia of the oxyntic component of the mucosa as well as by the surface component (Fig. 137.31). This happens in the setting of hypergastrinemia. Of course hypergastrinemia can result from an interrupted feedback loop when there is loss of oxyntic mucosa in autoimmune gastritis, but of course there cannot be hyperplasia of oxyntic mucosa if it has been destroyed by an autoimmune process. However, if there is intact gastric oxyntic mucosa, hypergastrinemia can be the result of a gastrin-producing neoplasm (gastrinoma) and the resultant hypertrophic gastropathy is referred to as a component of Zollinger–Ellison syndrome. Since the gastrin in Zollinger–Ellison syndrome also stimulates endocrine cells, endocrine cell hyperplasia is also a component of this

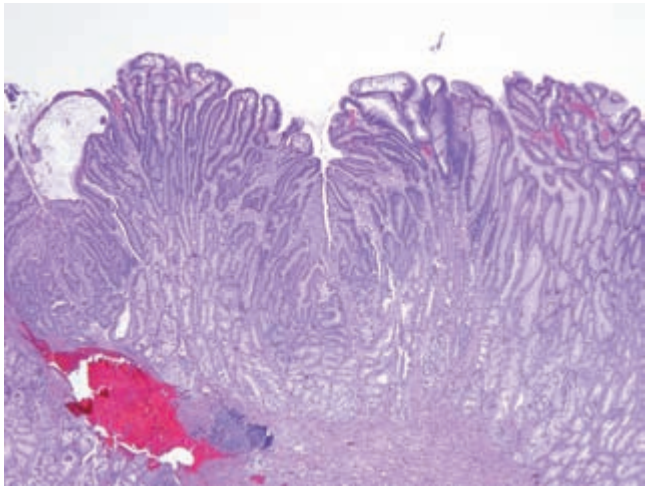

Figure 137.30 Hypertrophic gastropathy/Ménétrier disease. There is striking hyperplasia of foveolar (mucin-producing) cells.

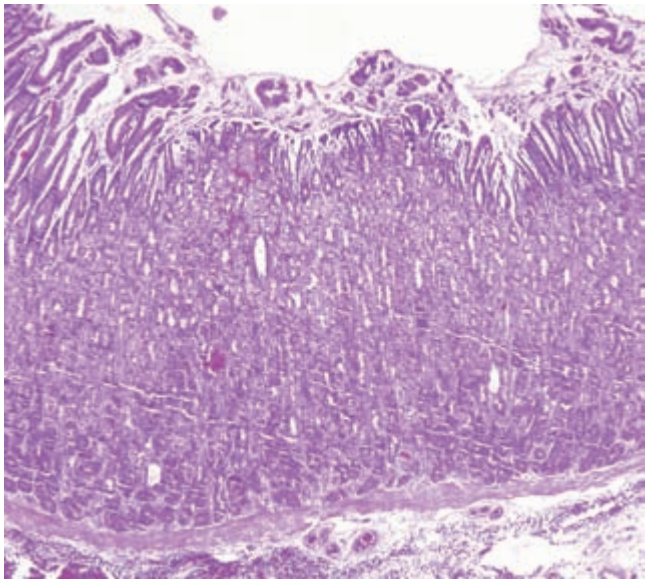

Figure 137.31 Gastric mucosa in Zollinger–Ellison syndrome. There is striking hyperplasia of parietal cells. Contrast this with the image in Fig. 137.30.

condition. On mucosal biopsy, the oxyntic component is markedly expanded and thick but the key feature is that parietal cells are seen insinuated in the epithelium nearly or even at the surface between residual foveolar cells instead of nested down deeper in the mucosa.

Nonneoplastic polyps

Endoscopically and pathologically, gastric polyps are defined simply as projections above the adjacent mucosal surface. The polyp itself may be reactive/inflammatory, hamartomatous, or neoplastic. Gastric polyps can arise from or be present in tissue anywhere in the layers of the stomach wall;

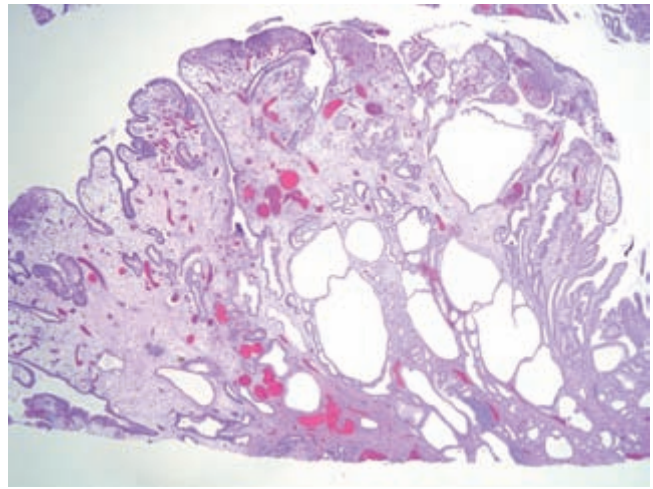

Figure 137.32 Gastric hyperplastic polyp. There is prominence of mucin-producing epithelium and cystically dilated glands. This lesion has overlap with hypertrophic gastropathy and diagnosis of either condition requires correlation with the endoscopic appearance.

however, polyps that are endoscopically biopsied are most commonly one of a wide variety of epithelial lesions.

The classification of gastric epithelial polyps can be challenging histologically, but has important consequences for clinical management of the polyp itself as well as implications about the remainder of the patient's gastric mucosa. Unlike colonic polyps (most of which are isolated findings in an otherwise normal background mucosa) many gastric polyps arise in association with either inflammatory/atrophic gastritides or inherited polyposis syndromes. Therefore, correct classification of gastric polyps, even innocuous-appearing polyps, may sometimes provide important clues about abnormalities in the surrounding stomach and patient.

Hyperplastic polyps

Hyperplastic polyps are one of the most common gastric epithelial polyps [145]. These polyps can range from a few millimeters to many centimeters in size, and as such may be mistaken endoscopically for carcinoma. They are composed of characteristic hyperplastic, elongated, and dilated foveolae within an edematous inflamed stroma (Figs 137.32 and 137.33). The lining of the hyperplastic foveolae is generally that of mature gastric mucin cells, but foci of intestinal metaplasia are found in about 15%. The lining cells can be markedly reactive in appearance (especially when surface erosions are present), but true dysplasia arising in hyperplastic polyps is uncommon [146,147]; in a review of 160 patients with gastric hyperplastic polyps biopsied at our hospital, dysplasia was found in only 4% [1]. Similarly, adenocarcinomas are occasionally reported to occur in these polyps but this is an unusual; Abraham and colleagues found adenocarcinoma within a hyperplastic polyp in only 1 of 160 (0.6%) patients [1].

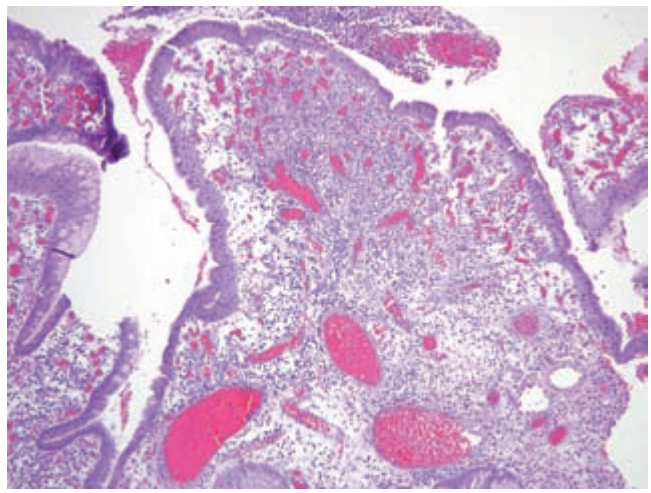

Figure 137.33 Gastric hyperplastic polyp. It is not uncommon for large hyperplastic polyps to display surface erosions/ulcers and reparative epithelial changes.

Hyperplastic polyps may arise anywhere in the stomach, with a slight preference for the antrum, and are multiple in approximately 20%. One of the major reasons for diagnosing hyperplastic polyps histologically is their significant association with a wide range of background gastric mucosal abnormalities. It is unusual for hyperplastic polyps to arise in normal stomachs. They are most strongly associated with atrophic gastritis of either autoimmune or environmental (e.g., *H. pylori*-associated) types, but many types of acute and chronic gastritides have been reported with hyperplastic polyps including the postantrectomy state, chemical/reactive gastropathy, and following therapy for gastric antral vascular ectasia (watermelon stomach). The frequent association between hyperplastic polyps and autoimmune gastritis has been extensively documented. Hyperplastic polyps are essentially a response to injuries. As a result, patients with hyperplastic polyps are at increased risk (approximately 6%) for synchronous or metachronous adenocarcinomas arising in the stomach outside of the polyp.

When we diagnose a hyperplastic polyp in a patient who has not had corresponding biopsies of the surrounding flat mucosa, we add a note in the report underscoring the relationship between hyperplastic polyps and background gastric atrophy or inflammatory gastritis, and indicate that biopsies of the flat antrum and body may be worthwhile.

The pathogenesis of hyperplastic polyps is not known, but they are thought to represent an exaggerated regenerative response to mucosal injury. The histological appearance of hyperplastic polyps overlaps significantly with conditions of generalized gastric mucosal hyperplasia (Ménétrier disease, Cronkhite–Canada syndrome) and with hamartomatous polyps and syndromes involving the stomach. As such, diagnosis requires correlation with the endoscopic findings and clinical history.

Hamartomatous polyps

Hamartomas are polypoid lesions formed from disorganized tissue elements native to that site. Hamartomatous syndromes that may involve the stomach include Peutz–Jeghers syndrome and juvenile polyposis, and less commonly Cowden disease. Peutz–Jeghers syndrome is an autosomal dominant condition caused by germline mutations in the *LBK1/STK11* gene on chromosome 19p13.3 and is characterized by polyposis and distinctive melanin pigmentation around the lips, buccal mucosa, and sometimes hands and eyelids. The pigment may fade after puberty and thus the syndrome is not excluded if it is absent in an adult presentation. The polyps of Peutz–Jeghers syndrome primarily occur in the small bowel (65%) and are slightly less common in the colon and stomach [148–152]. Importantly, unlike the small bowel polyps, which show prominent arborization of the muscularis mucosae, gastric Peutz–Jeghers polyps are composed mostly of dilated or branching mucus-filled pits and may have relatively inconspicuous smooth muscle. Occasional examples of gastric Peutz–Jeghers polyps have the classic arborizing architecture with strands of smooth muscle, but most have less specific features (but some degree of smooth muscle proliferation). Essentially they are best distinguished from hyperplastic polyps by correlation with the history. Dysplasia is rare in these polyps; however, patients with Peutz–Jeghers syndrome are at significant risk for gastric and other adenocarcinomas developing outside of the hamartomas [148].

Juvenile polyposis is a genetically heterogeneous condition in which some families have autosomal dominant germline mutations in the *DPC4* gene on chromosome 18q21. The polyps in juvenile polyposis can be limited to the colon or can be generalized, involving the colon, small bowel, and stomach. In addition, patients who appear to have juvenile polyposis predominantly confined to the stomach have been described. Juvenile polyps in the stomach frequently show a rounded surface contour with superficial mucosal erosions and an abundant, edematous, and inflamed lamina propria. The foveolae are frequently hyperplastic and dilated. Superimposed epithelial dysplasia or even mixed adenomatous/juvenile polyps occurs in up to one-third of juvenile polyps. Cowden disease is an autosomal dominant condition that is relatively poorly characterized, but some families have germline mutations in the *PTEN* gene on chromosome 10q22–23. The GI polyps in Cowden disease are usually a minor component of the syndrome (affected individuals typically show more prominent facial trichilemmomas and oral papillomas, as well as being at increased risk for breast and thyroid carcinomas). Detailed reports of the gastric polyps in Cowden disease have not been made, but they are reported to be histologically indistinguishable from small hyperplastic polyps [153]. Dysplasia is not reported in these polyps [151].

There are some clinical and pathological clues that help to distinguish between gastric hyperplastic polyps and hamartomatous polyps.

• The patient may have a previously characterized polyposis syndrome.
• Biopsies of the nonpolypoid gastric mucosa may show an atrophic or inflammatory gastropathy of the type associated with the development of hyperplastic polyps.
• Hyperplastic polyps frequently show a more lobulated or villiform surface compared with the often rounded surface of juvenile polyps.
• Hyperplastic polyps often contain a more prominent edematous inflamed lamina propria compared with Peutz–Jeghers polyps, which can sometimes but not always show smooth muscle arborization.

However, we do not believe that it is possible to distinguish between a hyperplastic or hamartomatous polyp based solely on the histological features of a polyp that is resected or biopsied. In the absence of clinical information and knowledge of the histology of the background gastric mucosa, we state in the diagnostic report that the polyp is histologically consistent with a hyperplastic polyp and give the differential diagnosis listed above.

Fundic gland polyps

Fundic gland polyps (FGPs) occur in two forms: sporadic and familial adenomatous polyposis (FAP) associated. FGPs were originally described in patients with FAP, believed to be a manifestation of that syndrome, but they are now recognized to be one of the most common gastric polyps (along with hyperplastic polyps) in individuals without FAP. Sporadic FGPs are found in 1%–2% of routine upper endoscopic examinations and are most common in middle-aged females. They are typically small (a few millimeters and only rarely more than 1 cm), sessile, and dome-shaped. Sporadic FGPs may be single but are commonly multiple (usually a few polyps). Rarely patients without FAP have numerous FGPs proliferating in a manner that resembles a polyposis syndrome. Unlike hyperplastic polyps, FGPs are not particularly associated with any type of inflammatory or atrophic background mucosal pathology and are essentially an incidental finding. However, some studies do indicate a temporal association between use of proton pump inhibitors and the development of FGPs [154]. This potential link is controversial and has not been demonstrated in any controlled prospective trial [155].

The morphology of FGPs overlaps considerably with that of proton pump inhibitor effects. An endoscopic nodule correlates with dilated oxyntic glands, some of which contain cells with apocrine-like snouts (Fig. 137.34). It is sometimes difficult to distinguish these two processes and the reality is that it is essentially done by correlation with the presence of an endoscopic lesion.

FGPs associated with FAP syndrome differ from sporadic FGPs on several epidemiological and clinicopathological points: FAP-associated FGPs occur in a majority of patients with FAP (reported frequencies range from 12.5% to 84% of FAP patients, depending on the age at endoscopy) and show a more equal gender distribution. They are more numerous than sporadic FGPs, and hence patients with FAP are more likely to have fundic gland "polyposis." They occur at younger ages, including children (FGPs are vanishingly rare in the non-FAP pediatric population). In addition, approximately 25% of FAP-associated FGPs demonstrate low-grade epithelial dysplasia [156]. Dysplasia in sporadic FGPs can occur but is distinctly unusual. A retrospective histological evaluation of several hundred sporadic FGPs at our hospital revealed fewer than 1% with low-grade dysplasia, and one of these cases was subsequently determined to be a member of an attenuated FAP family [156].

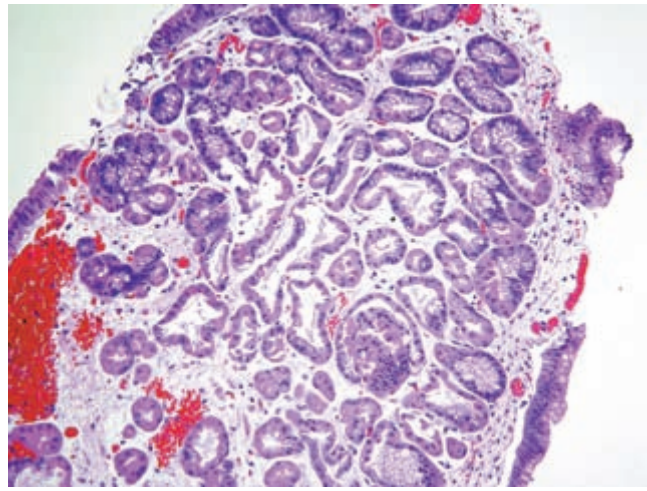

Figure 137.34 Fundic gland polyp. The cystically dilated oxyntic (fundic) glands are the key feature.

The natural history of FGPs is interesting because both sporadic and FAP-associated FGPs can increase, decrease, or remain constant in number when patients are followed with serial upper endoscopic examinations. Sporadic FGPs, even fundic gland polyposis, have never been reported to progress to gastric adenocarcinoma. Several cases of adenocarcinoma associated with fundic gland polyposis in patients with FAP exist, but this is a reportable occurrence [157,158]. Currently, upper endoscopic surveillance in patients with FAP is performed to address the increased risk for duodenal (> 300-fold) and particularly periampullary adenomas and adenocarcinomas [159] rather than to address gastric pathology. The presence of dysplastic or nondysplastic FGPs is regarded an incidental finding and would almost never require surgical resection. Even surveillance of dysplastic FGPs is controversial and we regard them as low-risk lesions.

As their name implies, FGPs develop only in the gastric body and fundus and not in the antrum. In patients with FAP, FGPs are more common than gastric adenomas, despite the fact that the colorectal and duodenal polyps in these patients are adenomas. When gastric adenomas do develop in the FAP setting, they are most frequently antral. Despite

the increased frequency of dysplastic FGPs and gastric adenomas in FAP, the risk of gastric adenocarcinoma is only slightly increased (approximately twofold in one study) and not statistically significantly increased in Western FAP patients [159]. This contrasts with studies of Asian patients with FAP, among whom the risk of gastric adenocarcinoma is increased approximately 10-fold. This low risk in Western FAP patients also contrasts with the much higher risk of gastric adenocarcinoma in non-FAP patients who have adenomas. This is most likely a reflection of the fact that germline mutations in the *APC* gene on chromosome 5q21 leading to FAP do not predispose patients to background gastritis or gastric atrophy. Most Western patients with FAP have normal gastric mucosa outside of any antral adenomas or body FGPs. In contrast, most patients with sporadic gastric adenomas have chronic atrophic gastritis (particularly *H. pylori*-mediated) that predisposes to gastric adenocarcinoma in the intestinalized background gastric mucosa outside of the adenomas.

Inflammatory fibroid polyps

These lesions were described first by Vaněk in the American Journal of Pathology in 1949 [160] although there were prior case reports. Helwig and Ranier coined the present term in the early 1950s [161] but these lesions have been called gastric submucosal granuloma with eosinophilic infiltration, eosinophilic granuloma, granuloblastoma, neurofibroma, and hemangiopericytoma. The vast majority arise in the stomach, accounting for about 3%–4% of all gastric polyps, but they have been reported throughout the GI tract [145,161–181]. In Stolte's large series, patients with gastric examples were typically 60–80 years old, but examples are recorded in young adults and the elderly. Most are found in the antrum, but other gastric sites are known. Their endoscopic appearance is that of a smooth submucosal lesion that can be pedunculated or sessile with surface ulceration/erosion in about one-third of cases. Presentation is site specific, in that small intestinal examples can lead to intussusception or obstruction and gastric examples are found in patients with pain and nausea and vomiting. Inflammatory fibroid polyps are probably reactive in nature, but an interesting family with these lesions in females for three generations has been reported (Devon polyposis) [164,165]. They lack alterations of a host of oncogenes [182]. These benign lesions seldom recur after excision. Japanese examples have been found in association with gastric dysplasia/carcinoma (presumably coincidence) [170] but not Western ones.

Histologically, these tumors are well marginated but nonencapsulated and affect the mucosa and submucosa. They are composed of uniform spindled cells, mixed inflammatory cells, and prominent vasculature. The spindle cells have amphophilic elongate cytoplasm and pale ovoid to spindle-shaped nuclei with variable collagen deposition. Most examples display a whorled "onion-skin" proliferation around vessels and all examples are punctuated by abundant background eosinophils, lymphocytes, and plasma cells (Figs 137.35 and 137.36). Mitoses are infrequent.

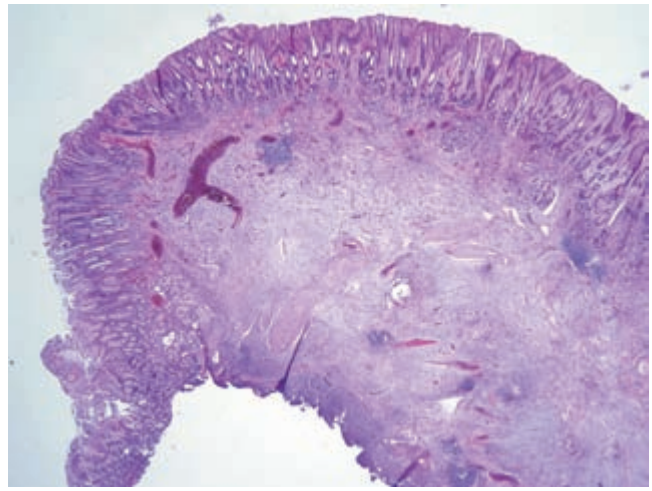

Figure 137.35 Inflammatory fibroid polyp. These tumors have their epicenters in the superficial submucosa.

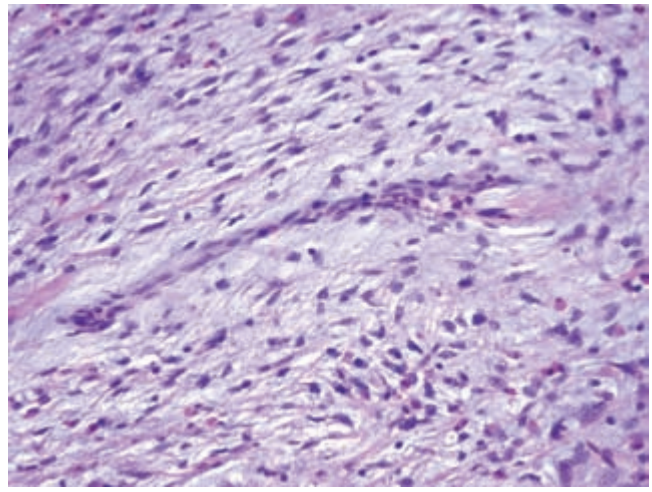

Figure 137.36 Inflammatory fibroid polyp. At higher magnification, there is a spindle cell lesion punctuated by many eosinophils.

The immunohistochemical and ultrastructural profile of the proliferating cells is that of modified fibroblasts/myofibroblasts, with variable actin but no S100 protein or epithelial markers [175,178]. Some authors believe that their consistent expression of cyclin D1 and fascin suggests that they are of dendritic cell origin [175] but their key feature is consistent CD34 reactivity in small tumors and less consistent staining in larger lesions [170]. This latter finding naturally raises the differential diagnostic consideration of GISTs, but the morphology is different and inflammatory fibroid polyps lack CD117/c-kit [175].

Pancreatic acinar cell metaplasia/heterotopia

In the esophagus this finding is generally incidental, and is in the stomach as well, but we have occasionally seen it

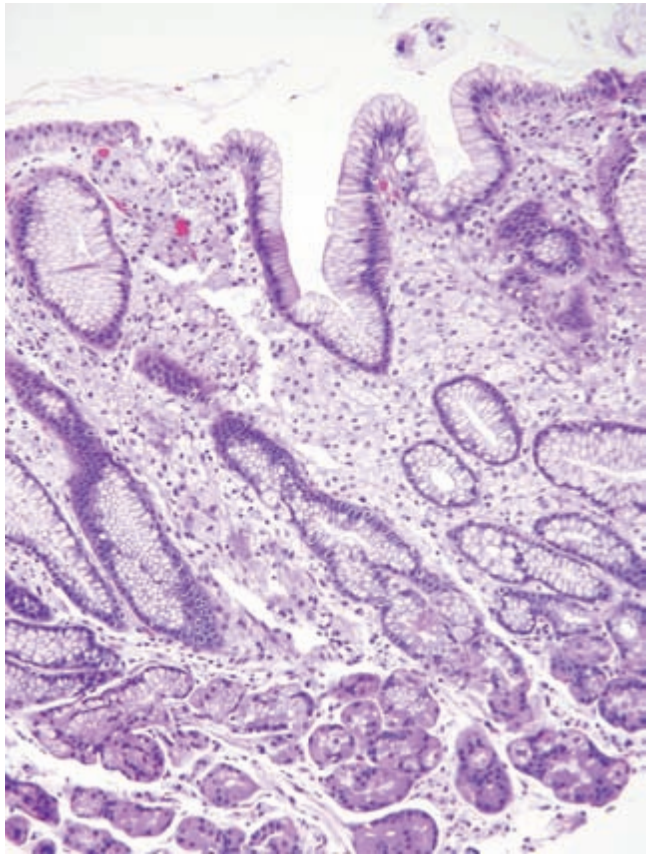

Figure 137.37 Gastric xanthoma. Numerous lipid-laden macrophages are seen in the lamina propria.

mistaken for carcinoid tumor and the pathologist is confused by the lack of expression of neuroendocrine markers. Occasionally, heterotopic pancreatic tissue can produce a symptomatic mass in the gastric antrum that results in gastric outlet obstruction [183–185]. Other than such occurrences, it is of little clinical significance. The heterotopic tissue may contain an admixture of pancreatic acinar tissue, ducts, and islet cells in varying proportion. Since the lesion is often submucosal, superficial biopsies containing only the overlying mucosa may be nondiagnostic.

Gastric xanthomas

Gastric xanthomas are pale yellow nodules or plaques, usually less than 3 mm in size, in the gastric mucosa. They are frequently found in groups, most often along the lesser curvature and pyloric regions. They consist of lipid-laden histiocytes in the lamina propria occasionally extending into the submucosa (Fig. 137.37). There is no correlation between gastric xanthomas and hypercholesterolemia. The lesion is of little significance, except that it may be misdiagnosed as carcinoma [186]. However, in any suspicious case, performing a keratin and CD68 stain can be reassuring. Some authors have associated gastric xanthomas with atrophic gastritis and *Helicobacter* gastritis [187].

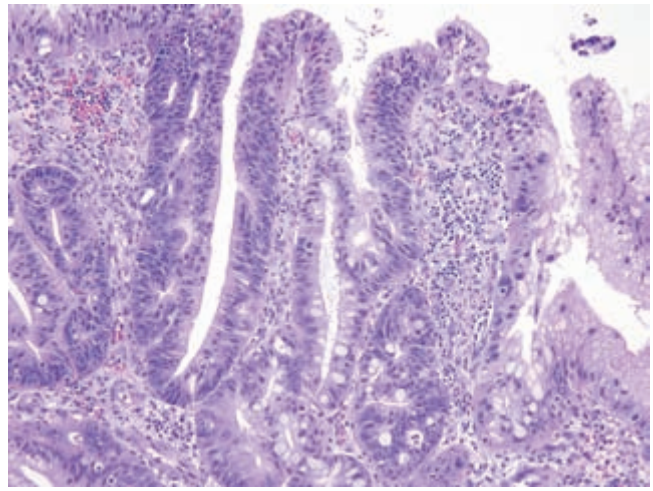

Figure 137.38 Gastric adenoma, intestinal type. This example shows both intestinal metaplasia and high-grade dysplasia.

Neoplasms

Gastric adenomas and gastric dysplasia

Literature in this area has been difficult to interpret in the past. There is massive experience with gastric cancer and its precursors in Japan but Japanese pathologists have applied different diagnostic criteria from Western pathologists. This was pointed out by Lauwers and colleagues in 1999 [188]. The observation that Japanese pathologists did not require invasion to diagnose carcinoma (and "invasion" was not listed as a criterion in the 1990 WHO classification) has presumably informed the far better cure rates in Japan for early carcinomas compared with results in Western countries. Based on these observations, an international panel convened in Vienna in 2000 and published consensus definitions of gastric epithelial neoplasia [189], which distinguished between noninvasive and invasive lesions.

Essentially, at present, if such a lesion appears as a polyp, it is referred to as an adenoma (Fig. 137.38) whereas flat lesions are termed dysplasia and are graded using criteria similar to those for the esophagus. This is not particularly scientific but is a convention based on practicality. Both types of lesions confer risk and both require mapping of the entire stomach to exclude invasive carcinoma. As in the case of gastric hyperplastic polyps, the background pathology is important. To illustrate this, Abraham and colleagues studied 61 gastric adenomas from 51 patients between 1985 and 2001 [2]. The adenomas were classified as intestinal type (containing at least focal goblet cells or Paneth cells) (Fig. 137.38), gastric type (Fig. 137.39), or indeterminate. The histological features of both the adenomas (location, multiplicity, degree of dysplasia, presence of adenocarcinoma within the polyp) and the surrounding gastric mucosa (presence of gastritis, intestinal metaplasia, and adenocarcinoma) were evaluated. Gastric adenomas were distributed equally throughout the stomach, were most frequently solitary (82%), and con-

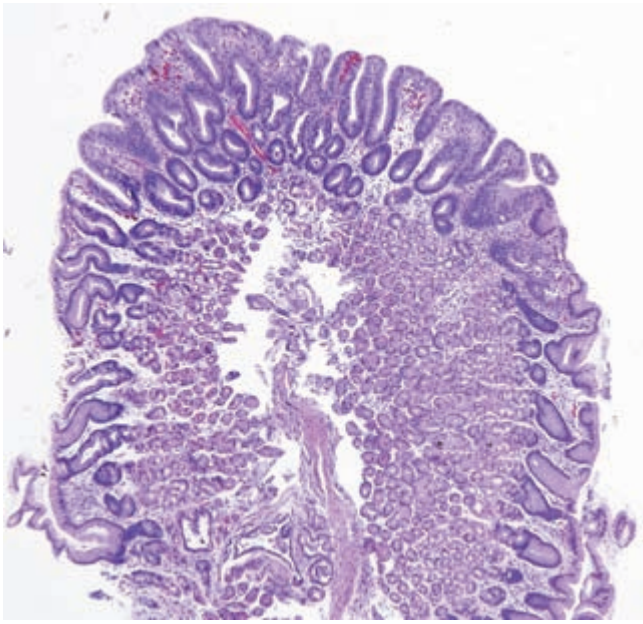

Figure 137.39 Gastric adenoma, gastric type. Such a lesion, arising in normal background mucosa, is a low-risk lesion unlikely to be associated with either high-grade dysplasia or invasive carcinoma.

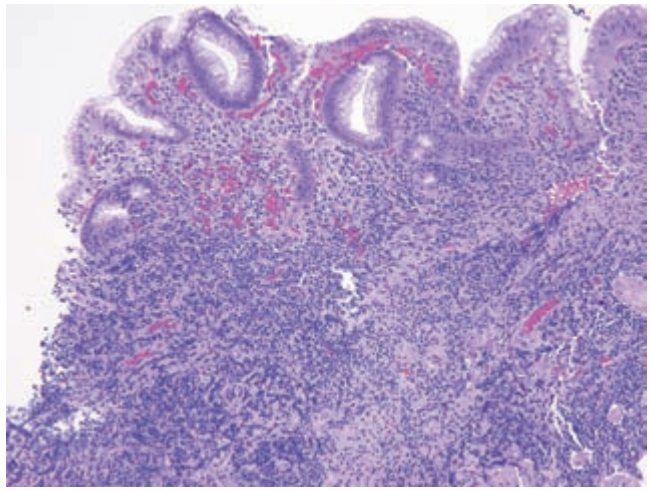

Figure 137.40 Mucosa-associated lymphoid tissue (MALT) lymphoma, also called extranodal marginal zone B cell lymphoma. Note the bottom-heavy distribution of the lymphoid infiltrate.

tained adenocarcinoma in nine cases (14.8%). There were 34 intestinal-type adenomas (56%) in 31 patients, 25 gastric-type adenomas (41%) in 18 patients (including 10 patients with FAP), and two of indeterminate type (3%). Intestinal-type adenomas were significantly more likely than gastric-type adenomas to show high-grade dysplasia ($P < 0.0001$), adenocarcinoma within the polyp ($P = 0.016$), intestinal metaplasia in the surrounding stomach ($P < 0.000001$), and gastritis ($P = 0.002$). Patients with intestinal-type adenomas were also more likely to have separate adenocarcinomas (five cases vs zero cases), although this did not reach statistical significance. Gastric adenomas are rarely truly sporadic lesions. In any individual patient, complete removal of the adenoma should be performed, and thorough biopsy of the surrounding gastric mucosa is essential to understand the clinicopathological context of the adenoma and exclude associated carcinoma. Similarly, when gastric flat dysplasia in the absence of a mass is found, the stomach should be mapped with multiple biopsies. Most cases are easily diagnosed, but some patients harbor unusual deceptively bland lesions.

MALT lymphoma

Low-grade B-cell lymphomas of MALT are believed to arise in organized lymphoid tissue in the gastric mucosa that is usually acquired in response to *H. pylori* infection. They are also termed extranodal marginal zone lymphomas. The close association between *H. pylori* and the lymphoma is further reflected by the demonstration that proliferation of the lymphoma cells can be driven by the presence of *H. pylori* organisms through a complex path of cellular interactions involving specific T cells [190–195]. From these observations it was suggested that removal of one of the proliferative drives to the neoplastic cells in the form of eradication of the organism might induce remission of the tumor [194]. Long-term remissions can indeed be induced in low-grade MALT lymphomas in 70%–80% of cases. The lymphomas that are most likely to respond to *H. pylori* eradication are those located superficially within the gastric mucosa. It has been suggested that certain genetic abnormalities, such as t(11;18) and the *Bcl-10* mutation, may be associated with lack of response to this therapy. Recurrences of low-grade lymphoma are encountered in patients treated by *H. pylori* eradication, but these appear to be infrequent and may be self-limiting and spontaneously regress without further therapy.

In assessing such a case at low magnification, the lamina propria is expanded with small uniform cells, some of which have a halo around them. We always assess the muscularis mucosae at low magnification (with confirmation at high magnification) as physiological lymphoid collections typically do not infiltrate the muscularis mucosae whereas MALT lymphomas do (Fig. 137.40). The classic lymphoepithelial lesion, in which lymphocytes are seen peppered in residual gland epithelium (Fig. 137.41), is not present in every case. At high magnification, before wasting resources on immunohistochemical stains, the cell composition is assessed: numerous plasma cells are more often an indicator of a reactive lesion (*Helicobacter* gastritis). We perform an abbreviated immunophenotyping panel consisting of CD20 (B-cell marker), CD3 (T-cell marker), and CD43 (normally found in T cells) and assess for CD20-reactive B cells that aberrantly express CD43. The CD3 stain is necessary since it is normal for T cells to express CD43 and the various stains must be compared. Unfortunately not every MALT lymphoma coexpresses CD43 (about half do) but the diagnosis can still be made on a

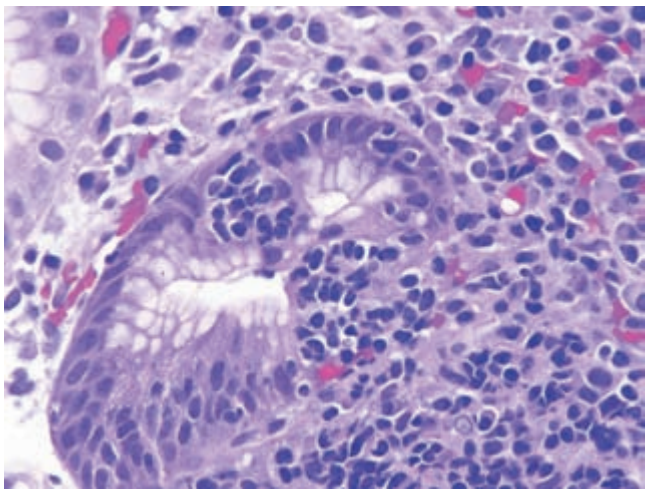

Figure 137.41 Mucosa-associated lymphoid tissue lymphoma. This field shows a lymphoepithelial lesion in which lymphoid cells proliferate in the epithelium itself.

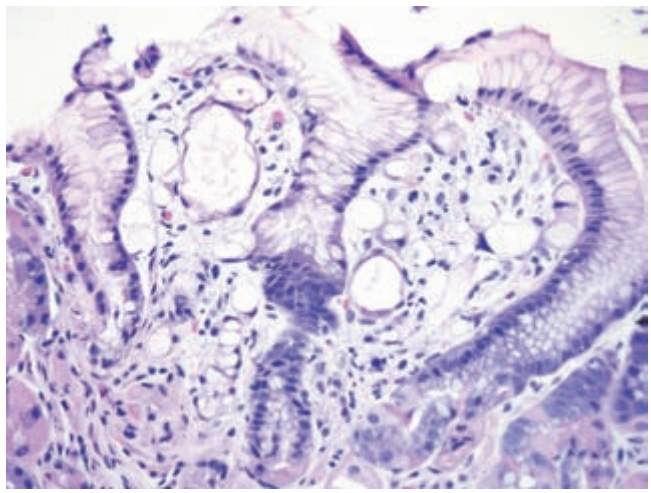

Figure 137.42 Gastric adenocarcinoma. This subtle early lesion is seen only in the lamina propria of the stomach (intramucosal carcinoma).

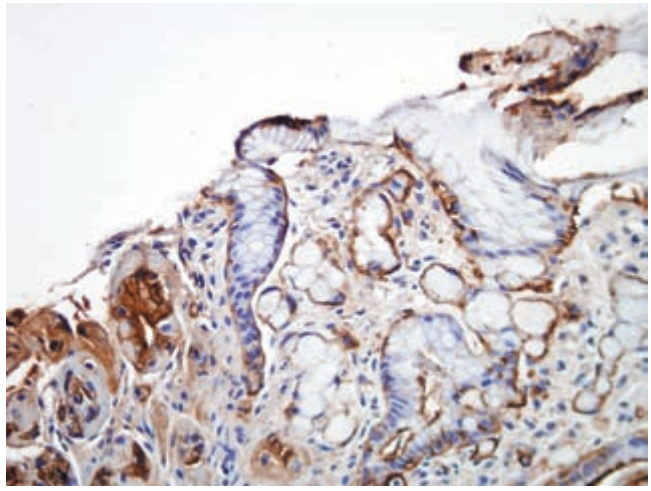

Figure 137.43 Gastric adenocarcinoma. A keratin stain highlights the cancer cells from the field seen in Fig. 137.42.

B-cell-rich infiltrate with typical morphology. The pathologist should always make an attempt to document the presence (or absence) of *Helicobacter*. It is most important for both the endoscopist and pathologist to remember that these lesions regress slowly over time on initiation of therapy and can linger for up to 18 months.

Adenocarcinoma

Gastric adenocarcinoma is the second leading cause of cancer death worldwide [196]. There are an estimated 21 500 new cases of gastric cancer annually in the USA, and about 10 880 of these patients will die of their disease [197]. Over the past several decades, the epidemiological and topographical profile of gastric cancer has changed dramatically. The incidence of gastric adenocarcinoma outside the gastric cardia is decreasing, but the incidence of gastric cardia adenocarcinoma is increasing [198].

Survival after resection of gastric adenocarcinoma has been studied in multiple series with patients stratified by stage of disease, Lauren tumor type (diffuse vs intestinal) [199,200], tumor location, time period, and administration of adjuvant therapy. All studies uniformly show an association between stage and survival. The Lauren classification correlates significantly with survival, in that intestinal-type tumors are associated with longer survival than diffuse-type tumors [201]. The Lauren classification, viewed simply, separates gastric cancers into those that have intestinal differentiation, form a large mass, and arise on a backdrop of intestinal metaplasia (intestinal type) and those that apparently arise de novo in otherwise unremarkable mucosa and diffusely infiltrate the tissues (diffuse type, subsuming signet ring carcinomas/linitis plastica). It is the diffuse type that causes problems on biopsies. According to some data analyses, tumor location is significantly associated with survival, tumors away from the gastric cardia having an improved prognosis over gastric cardia adenocarcinomas [198]. In many studies, both operative mortality and 5-year survival have improved over the past several decades [202]. Improved survival with adjuvant chemoradiotherapy has also been reported [203]. However, in our material, gastric carcinoma remains highly lethal and important to recognize in biopsy material [204].

Gastric carcinoma is often easy to recognize on biopsies but can also be extremely subtle (Figs 137.42 and 137.43). When there is a high index of suspicion, we examine each fragment carefully, concentrating on any focus that appears distinct or fibrotic at low magnification. Biopsies from patients who have undergone partial gastrectomy should be reviewed attentively as these are high-risk patients based on their continuous bile reflux. In doubtful cases, a keratin stain is helpful but should not be ordered in ulcerated cases as regenerative mucosa can give a false impression of a single cell

pattern. Carcinomas of the intestinal type can be extremely subtle on superficial biopsies but there is the correlate of a mass. Diffuse carcinomas are very difficult to spot on biopsies but there is the correlate of a stomach that does not distend on insufflation. It always behooves the endoscopist to provide information concerning these features. Some carcinomas are so poorly differentiated that they require keratin stains for diagnosis. Occasional gastric carcinomas have a clear cell pattern. Rare gastric carcinomas have squamous differentiation and still rarer ones have hepatoid differentiation. Some observers have suggested that carcinomas having parietal cell differentiation have an improved prognosis over other gastric carcinomas but this seems doubtful based on our material. We do not make an exercise of typing gastric carcinomas in our practice.

Mesenchymal tumors

Gastric stromal tumors

The stomach is the most common site for GISTs and they are occasionally diagnosed on mucosal biopsies. Typically, those diagnosed on biopsies are aggressive lesions that have invaded the mucosa. GISTs are mesenchymal tumors arising in the GI tract and occasionally within the abdomen with no GI connection. The earlier literature attempted to classify them as smooth muscle or nerve sheath tumors, but even in the benign tumors evidence for such differentiation was difficult to find. Mazur and Clark introduced the term *stromal tumor* in 1983 [205]. GISTs show differentiation toward (and supposedly arise from a precursor of) interstitial cells of Cajal, which are normally concerned with motility of the gut [206, 207]. The availability of specific antibodies and clarification of their immunohistochemical profile has facilitated diagnosis.

About 25% of GISTs (from all sites) are malignant, representing about 1% of all GI malignancies. They are most common in adults aged 50–60 years. These tumors vary in differentiation and prognosis according to their location within the GI tract. Esophageal GISTs are rare, but 50%–70% involve the stomach, 25%–40% the small intestine (of which 10%–20% arise in duodenum, 27%–37% in jejunum, 27%–53% in ileum), and less than 10% are colorectal (50% colonic, 50% rectal). They are rare in the esophagus (where true smooth muscle tumors are more usually found), colon and rectum. GIST-type tumors arising in omentum, peritoneum and retroperitoneum have been identified, comprising 6.7% of the large AFIP series of 1008 GISTs.

Malignant GIST are rare, with an incidence of about 5 per million (compared with 25 per million for soft tissue sarcomas). There is an increased incidence of GIST including multiple tumors in small intestine in patients with neurofibromatosis (NF)1, and GIST is occasionally familial (associated with a germline c-*kit* mutation). Some patients have second cancers, and some epithelioid gastric stromal tumors are associated with paraganglioma and pulmonary chondroma in Carney's triad.

About 20% of gastric GISTs are malignant. The consensus is that five mitoses per 50 high-power fields and size greater than 5 cm are adverse prognostic factors. The 5-year survival of gastric GISTs is about 40%, with improvement in completely resected cases. There is no evidence that radical surgery improves survival, so that the least extensive surgical procedure compatible with complete excision is advisable. Gastric GISTs are more frequent in males, but young patients, especially female, have an improved outcome.

Most GISTs are spindle cell tumors with variable palisading, peculiar paranuclear vacuoles, and collagen fibrils (Fig. 137.44). On a practical note, it is epithelioid GISTs (Fig. 137.45) that are most likely to cause diagnostic problems on mucosal biopsies as they are readily mistaken for a host of epithelioid and epithelial neoplasms. It is advisable to perform an immunohistochemical panel in assessing them to

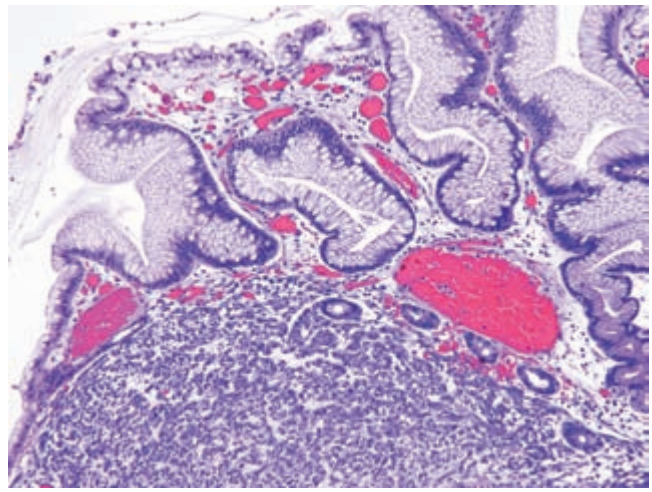

Figure 137.44 Gastrointestinal stromal tumor (GIST), spindle cell type. This lesion displays mucosal invasion, a feature of malignant GIST.

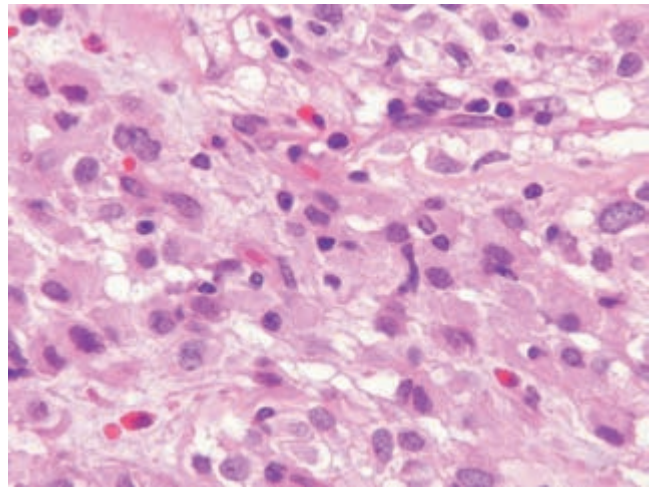

Figure 137.45 Gastrointestinal stromal tumor, epithelioid type. Note the eosinophilic appearance of the lesional cells. Such tumors were referred to as leiomyoblastoma in the past.

Table 137.1 Suggested prognostic criteria for resected gastrointestinal stromal tumors from all anatomical sites [211]

Risk assessment	Size (cm)	Mitotic counts per 50 high-power fields
Very low risk	< 2	< 5
Low risk	2–5	< 5
Intermediate risk	< 5	6–10
	5–10	< 5
High risk	> 5	> 5
	> 10	Any
	Any size	> 10

Table 137.2 Suggested prognostic criteria for resected gastrointestinal stromal tumors specific to stomach [214]

Risk assesment	Size (cm)	Mitotic counts per 50 high-power fields
Benign	No larger than 2	No more than 5
Probably benign	> 2, ≤ 5	No more than 5
	> 5, ≤ 10	No more than 5
Uncertain	No larger than 2	> 5
Low to moderate malignant	> 10	Not more than 5
potential	> 2, ≤ 5	> 5
High malignant potential	> 5, ≤ 10	> 5
	> 10	> 5

include CD117/c-kit, S100 protein (to address melanoma), and a cytokeratin stain to address signet cell carcinoma. The vast majority of GISTs have *kit* mutations and are CD117/c-kit stain positive, but about 5% lack *kit* mutations and many in the c-kit-negative subset have alternate mutations of platelet-derived growth factor α instead [208–210]. Since about 70% of GISTs express CD34, this can also be included in a diagnostic panel. On mucosal biopsies, it is difficult to assess tumor size and mitotic counts to prognosticate but it is possible to make a diagnosis in many cases and allow the patient to be scheduled for an operation to remove the tumor. Evaluation of resection specimens is not the focus of this book, but guidelines for this have been published [211] (Table 137.1). Some observers have taken issue with this as it assumes that all GISTs are potentially malignant and does not take site into account but is still a useful construct for attempting a prognosis. Trupiano and colleagues have published criteria that they believe allow separation of benign gastric stromal tumors from those they regard as not benign [212]. We have attempted to apply these to a series of gastric GISTs and had one outlier case [213] so we continue to add a note of caution to reports on large gastric stromal tumors that appear otherwise benign.

The AFIP group probably has the largest accumulated series of gastric stromal tumors, and have reported a large series of 1765 GISTs confined to the stomach with good follow-up information [214]. There was a slight male predominance (55%) and a median age of 63 years. Only 2.7% of tumors occurred before the age of 21 years and 9.1% before the age of 40 years. The tumors varied from 0.5 to 44 cm (median 6.0 cm) and most commonly presented with GI bleeding; 12% were incidentally detected. Histological variants recognized among the spindle cell tumors included sclerosing, palisaded/vacuolated, hypercellular, and sarcomatous and among the epithelioid tumors, sclerosing, dyscohesive, hypercellular, and sarcomatous. Outcome was strongly dependent on tumor size and mitotic activity. Only 2%–3% of tumors less than 10 cm in size and with less than five mitoses per 50 high-power fields metastasized, whereas 86% of tumors more than 10 cm in size and with more than five mitoses per 50 high-power fields metastasized. However, tumors more than 10 cm in size with less than five mitoses per 50 high-power fields and those less than 5 cm in size with more than five mitoses per 50 high-power fields had a relatively low metastatic rate (11% and 15%). A small number of patients survived intraabdominal metastasis for over 20 years. Tumor location in fundus or gastroesophageal junction, coagulative necrosis, ulceration, and mucosal invasion were all unfavorable factors ($P < 0.001$), whereas tumor location in antrum was favorable ($P < 0.001$). Probably the key feature of this very large series is that it allowed separation of a "benign" category of gastric GISTs based on large numbers of cases [214]. The AFIP group has proposed malignancy criteria specific to the stomach (Table 137.2). Another important feature of the AFIP study is that mucosal extension by gastric GISTs was evidence that the tumor would behave badly. This feature remained equivocal based on several earlier smaller studies (including our own [213]). However, based on these data from Miettinen and colleagues [214], if we encounter a gastric GIST on mucosal biopsy, it is likely malignant and we suggest this in our reports. Based on the large AFIP series of gastric GISTs, CD117/c-kit is found in 91% of gastric GISTs, CD34 in 82%, SMA in 18%, and desmin in 5%; the latter two tend to be focal.

Gastric neural tumors

These are not typically encountered on gastric mucosal biopsies. Most gastric nerve sheath tumors have been classified as schwannomas, although there is no reason that a patient with syndromic nerve sheath tumors (neurofibromas, neuromas, and ganglioneuromas) cannot manifest gastric lesions. Most GI schwannomas occur in the stomach involving submucosa and muscularis propria. They rarely arise in the esophagus or colon. They typically do not affect the mucosa but are surrounded by a lymphoid cuff, which is more likely to be biopsied than the underlying mesenchymal lesion.

GI tract schwannomas are not encapsulated, a feature that distinguishes them from schwannomas in the peripheral

nervous system [215]. They can also be plexiform. They have a lymphoid cuff with germinal centers, and intralesional lymphocytes can be seen. They are composed of interlacing bundles of spindle cells that are only loosely palisaded (in contrast to GISTs which, ironically, often display striking palisading). They appear quite similar to GISTs but the lymphoid cuff is a hint that these are Schwannian. These benign tumors are strongly S100 protein positive and lack muscle markers and CD117. They may have CD34 as well. The differential diagnosis is with GIST. The inflammatory backdrop is the key morphological feature that should prompt consideration of a nerve sheath tumor.

Other gastric mesenchymal tumors

Other gastric mesenchymal tumors include lipomas and glomus tumors. Glomus tumors are usually deep in the gastric muscularis propria and not encountered on mucosal biopsies. In the present era of better control of HIV disease, we seldom encounter Kaposi sarcoma in our GI material but when it is seen, there is a history of extreme immunosuppression and proliferation of CD34-reactive spindle cells. Antibodies to human herpesvirus 8 are also available to confirm this impression.

Carcinoid tumors

A tripartite classification system for gastric carcinoid tumors is now in common use:

- tumors associated with chronic atrophic gastritis with hypergastrinemia
- tumors associated with Zollinger–Ellison syndrome or multiple endocrine neoplasia (and thus with hypergastrinemia)
- sporadic lesions (without hypergastrinemia).

These subsets have been classified as types I, II, and III respectively. Gastric carcinoids associated with hypergastrinemia (types I and II) are relatively benign, while sporadic lesions require aggressive surgical management. However, gastric carcinoids can be managed initially by endoscopic excision of accessible tumors, with evaluation of the setting in which they have arisen, followed by regular endoscopic surveillance. Modlin and colleagues cite a striking increase in gastric carcinoids in the past 50 years, including an 800% increase in gastric carcinoids in white females [216,217]. Patients were not routinely endoscoped 50 years ago and tiny carcinoids are not uncommonly found in white women as a result of autoimmune gastritis if they are sought.

Essentially, in the two settings where the trophic effects of hypergastrinemia on gastric endocrine cells produce a tumor composed of these cells, the tumors are essentially a nuisance with negligible metastatic risk. Cutoffs for the minimal size that determines what is endocrine cell hyperplasia and what is neoplasia (often set at 5 mm but sometimes 0.5 mm) are of no clinical importance since these patients do not experience metastases. In our practice, we apply the term "carcinoid" when the endoscopist sees a nodule. It is more important to determine the source of the excess gastrin than to quibble about the size of the endocrine cell proliferation. This is done by attention to the background gastric changes: atrophy versus hyperplasia of the acid-producing cells. Hyperplasia of intact parietal cells is the hallmark of Zollinger–Ellison syndrome, in which there is a gastrin-secreting neoplasm, often in the pancreas. There are only a few large studies of the outcome of such tumors, but in one study it was noted that in patients having sporadic (type III) carcinoid tumors, size greater than 2 cm was a poor prognostic indicator [218].

Gastric carcinoids consist of small round proliferating cells and the diagnosis can be confirmed by performing endocrine stains (Figs 137.46–137.48). Some such tumors display cytological atypia and there are examples of gastric small-cell carcinoma, but experience with large numbers of these tumor types is lacking, as are good criteria for their separation from typical carcinoid tumors. It is worthwhile attempting to distinguish small-cell carcinomas, but those analogous to atypical carcinoids in the lung remain poorly characterized.

Metastatic neoplasms

Although theoretically any tumor might metastasize to the stomach, those seen in practice are metastatic lobular breast cancer, renal cell carcinoma, melanoma, and hepatobiliary carcinoma. These are best separated from de novo gastric primaries by history, but immunohistochemistry is important as well. Melanomas can be separated from gastric carcinomas by S100 staining. As a word of caution, melanomas can label with CD117 antibodies so a panel approach is warranted to exclude epithelioid GISTs. Breast cancers are separated by the identification of hormone receptors (estrogen/progesterone receptor) and gross cystic fluid disease protein since only rare gastric carcinomas express hormone receptors in material from the USA. Gastric cancers lack cytokeratin 34 β E12 (CK903) [219], whereas hepatobiliary cancers express it.

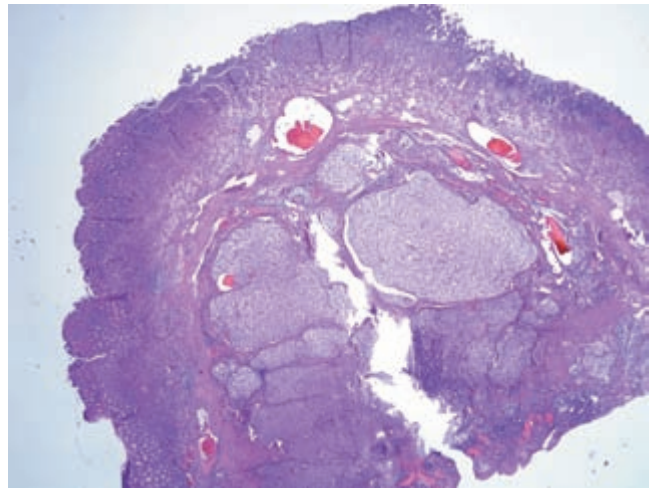

Figure 137.46 Gastric carcinoid tumor. This tumor is centered in the submucosa.

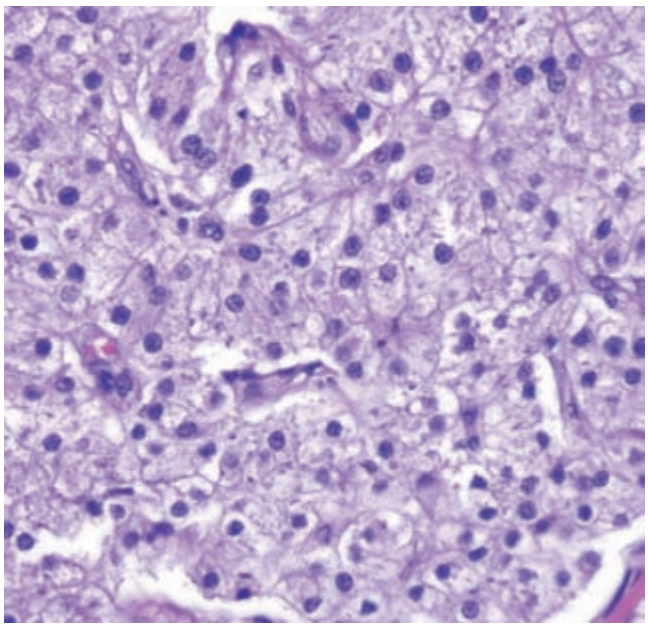

Figure 137.47 Gastric carcinoid tumor. Note the uniform nuclear features.

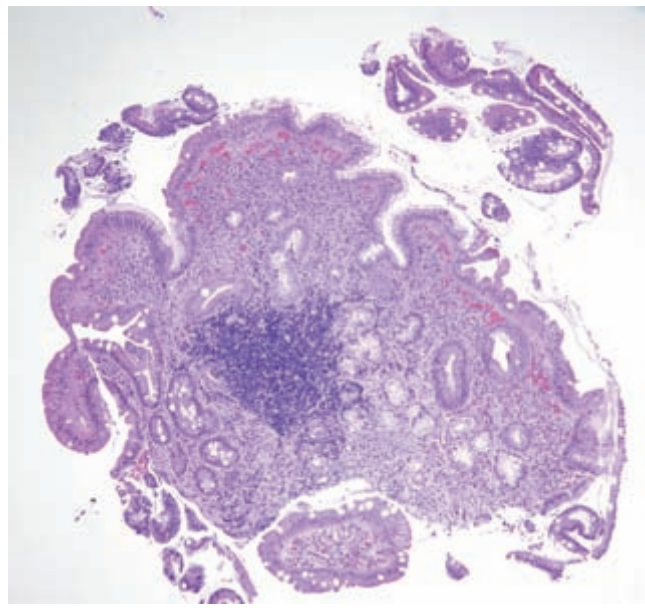

Figure 137.49 Chronic peptic duodenitis. At low magnification, normal duodenal mucosa is seen at the left (goblet cells are present) but the central portion shows gastric-type epithelium that is metaplastic.

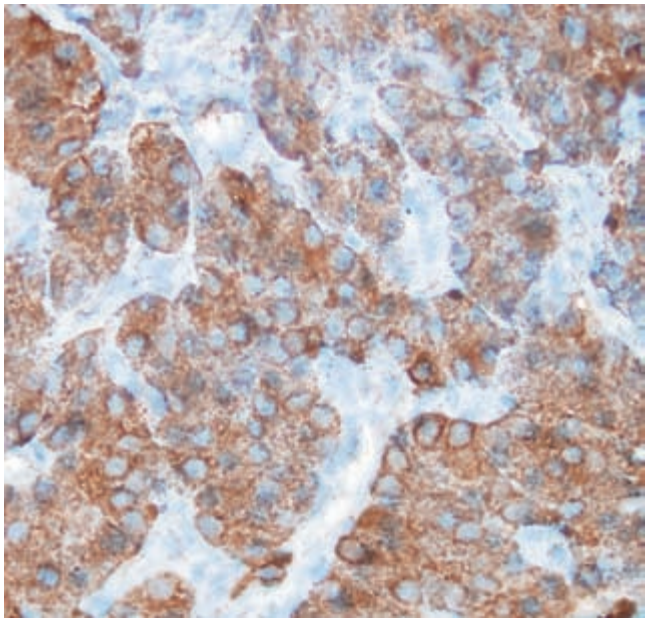

Figure 137.48 Gastric carcinoid tumor. The lesional cells are reactive with synaptophysin antibodies.

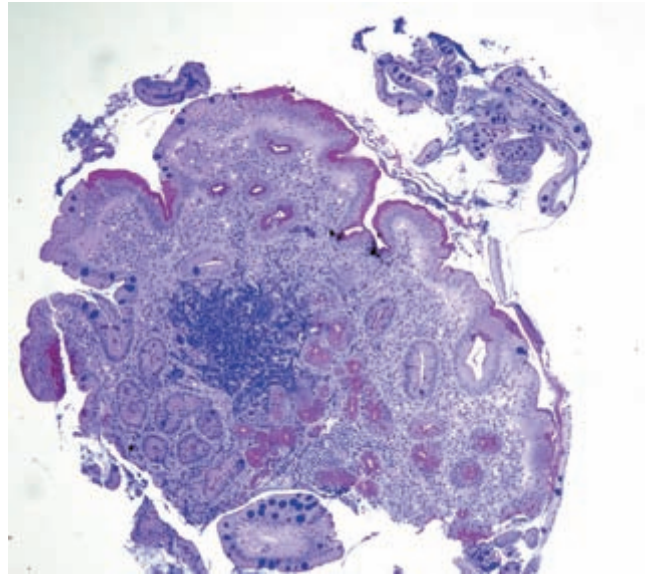

Figure 137.50 Chronic peptic duodenitis. Periodic acid–Schiff with Alcian blue shows the gastric metaplasia to advantage; it appears magenta.

Small bowel

Nonneoplastic conditions

Reactive lesions (gastric metaplasia of the duodenum)

When the esophagus and stomach are injured, metaplasia ensues, often of intestinal type. The duodenum can also undergo metaplasia when injured, but to gastric-type mucosa (Figs 137.49 and 137.50). *Helicobacter* gastritis was originally strongly associated with duodenal ulceration [97,220] since *H. pylori* infection up-regulates gastric acid secretion by damaging D cells that secrete somatostatin (somatostatin normally reduces gastric secretion). It is predominantly the bulb that is damaged. Gastric mucin cell metaplasia supervenes together with Brunner gland hyperplasia. In addition, the area

of metaplastic duodenum is then able to support *H. pylori*, which further stimulates ulceration in the duodenum. This cycle of events may attract inflammation and impart a nodular appearance to the duodenum (nodular peptic duodenitis) [142,221]. There may be striking reparative epithelial changes that are occasionally mistaken for adenomas. If PAS staining is done, these foci usually have at least some gastric mucin cell metaplasia as a clue that the process is reparative rather than neoplastic. In any doubtful case, repeat biopsy can be performed following treatment. Peptic duodenitis appears similar to gastric heterotopia, which occurs as a nodule in uninjured small bowel and often consists of oxyntic mucosa. It must be noted, however, that in some cases it is impossible to suggest whether the endoscopically noted nodule consists of a heterotopic or metaplastic process; fortunately both are benign.

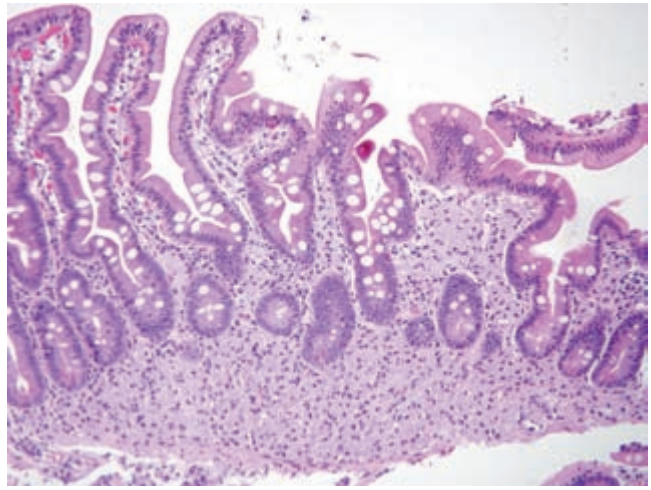

Figure 137.51 *Mycobacterium avium–intracellulare* duodenitis. The villi are expanded with macrophages.

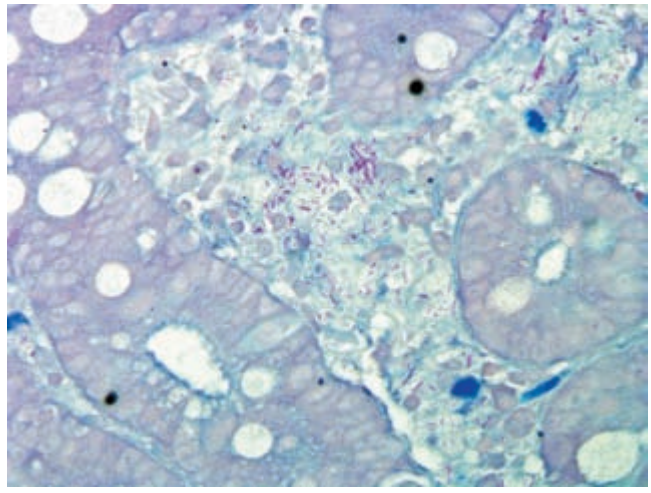

Figure 137.52 *Mycobacterium avium–intracellulare* duodenitis. This acid-fast stain highlights the organisms.

Infectious enteritis

A host of etiological agents may affect the small bowel mucosa. It is impossible to be exhaustive, but some merit comment.

Mycobacterium avium–intracellulare

In immunocompetent hosts, atypical mycobacteria show little virulence. However, *Mycobacterium avium–intracellulare* (MAI) is a significant cause of opportunistic infection in immunocompromised patients, especially patients with AIDS. Patients with $CD4^+$ cell counts of less than 100/μL are at risk of developing MAI. Symptoms include abdominal pain, fever, weight loss, chronic malabsorption, and diarrhea. Disseminated GI tract infection with MAI is common in AIDS patients. Clarithromycin or azithromycin are the preferred agents for MAI prophylaxis.

MAI usually affects both the small and large bowel. Endoscopically, the small bowel mucosa either appears normal or shows a coarse granularity without significant exudate (implying an attenuated local immunological response). The granular mucosa corresponds to massive infiltration of small intestinal villi by histiocytes loaded with acid-fast bacilli (Figs 137.51 and 137.52).

Unlike for *Mycobacterium tuberculosis*, well-defined granulomas are not a feature of MAI. Instead loose clusters of foamy histiocytes appear with little accompanying inflammation. Apart from the GI tract, MAI infection can affect a wide range of organs including lung, lymph nodes, spleen, liver, bone marrow, and brain.

Nematode infection

Strongyloides stercoralis is a nematodal infection with a worldwide distribution affecting 30–100 million people. It is endemic in Africa, Southeast Asia, South America and parts of the USA (e.g., Kentucky and eastern Tennessee).

The life cycle of this worm is as follows. The female lays eggs (and can do so without fertilization, i.e., parthenogenesis) in small intestinal crypts. The embryonated eggs hatch into rhabditiform larvae that pass into the lumen and are expelled with feces. The rhabditiform larvae develop into filariform larvae (infective form) that penetrate human skin, passing through blood vessels to the lungs and into the upper airways. The patient coughs and the larvae are swallowed and finally reach the small intestine where they mature into adult worms. The males are rapidly expelled after fertilizing the females. The female worms can remain in the intestine for decades, and in some cases have been known to be present for up to 30 years. In immunosuppressed patients, autoinfection is a major threat as internal rhabditiform larvae undergo transformation, penetrate intestinal mucosa and disseminate throughout the body. Autoinfection/hyperinfection carries a high mortality rate (up to 80%). Unlike hookworm infection, for which eggs are used for identification in stool examinations, eggs are not present in the

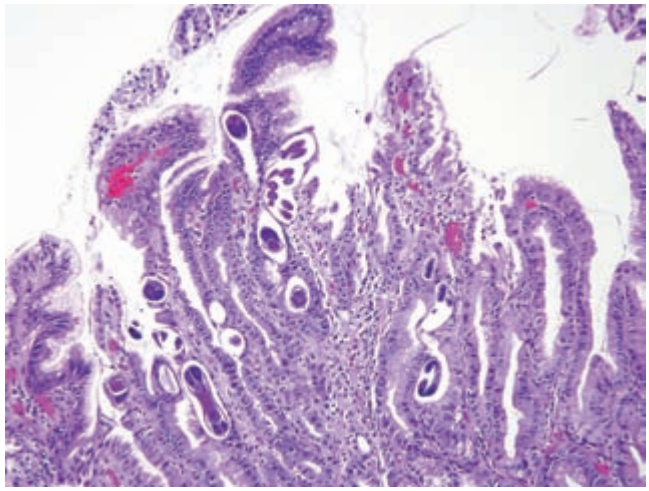

Figure 137.53 Strongyloidiasis. Multiple larval forms are seen in the small intestinal mucosa.

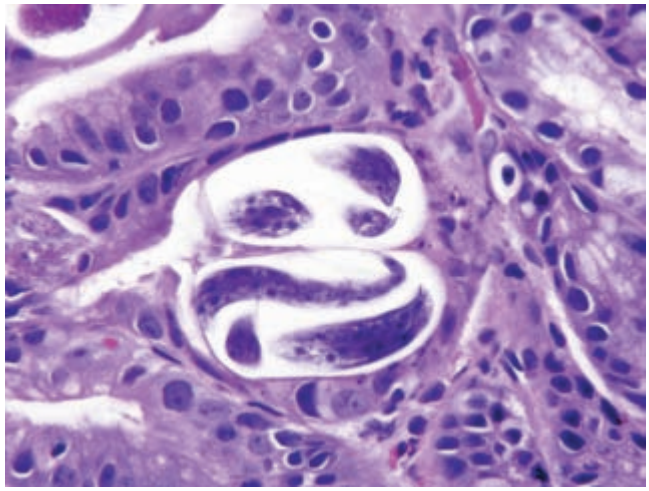

Figure 137.54 Strongyloidiasis. Higher magnification of Fig. 137.53.

stool in *Strongyloides* infection. Diagnosis is based most frequently on identification of rhabditiform larvae in stool. Intestinal biopsies are used less often for diagnosis. Characteristic findings in the biopsy are the presence of all stages of the worm embedded in mucosal crypts (Figs 137.53 and 137.54). Thus larvae, eggs and rarely adult worms (recognized by one intestinal tract and a pair of reproductive tubes) can all be seen. Additional changes include villous flattening, crypt hyperplasia, and an inflammatory reaction consisting of lymphocytes, plasma cells, and eosinophils. Finding a prominent eosinophilic infiltrate and Charcot–Leyden crystals should prompt a search for parasitic infestation. Treated worms invoke a brisk immunological response. The small intestine is the most common affected site but rarely stomach and colon can also be involved.

Patients can remain asymptomatic or present with nausea, vomiting, diarrhea, constipation, malabsorption, abdominal pain, and weight loss; the presentation can be varied depending on worm burden and site of infestation. Thiabendazole and more recently ivermectin (current drug of choice according to the WHO) are used to treat strongyloidiasis.

Whipple disease

Whipple disease is a rare systemic bacterial infection caused by *Tropheryma whippelii* (an actinomycete). Whipple disease was first described by Dr George Whipple at Johns Hopkins in 1907 as "intestinal lipodystrophy" due to the prominent accumulation of lipids in intestinal mucosa and lymph nodes. The bacterial etiology of this condition was confirmed by electron microscopy in 1961 [222,223]. There is a striking male predominance of 8–10 : 1, with white men aged between the fourth and fifth decades most commonly affected. Patients present with diarrhea, low-grade fever, weight loss, malabsorption, abdominal pain, arthralgia, anemia, lymphadenopathy (50%), and cardiac and central nervous system symptoms (10%). Most patients respond dramatically to antibiotics (trimethoprim and sulfamethoxazole). However, some patients can have a chronic relapsing course, with organisms persisting in affected tissues for a long time despite extended antibiotic treatment.

Whipple disease usually involves the postbulbar duodenum and jejunum. Thus biopsies should be taken both from proximal and distal duodenum and also jejunum. The characteristic appearance of small intestinal biopsy consists of blunted and rounded villi with distention of lamina propria by foamy pink macrophages. The macrophages contain PAS-positive, diastase-resistant, rod- or sickle-shaped bacterial inclusions. The bacterial inclusions can also be present in the extracellular space of lamina propria, and in epithelial cells, fibroblasts, endothelial cells, and smooth muscle. Lymphatic obstruction gives rise to dilated lacteals containing lipid deposits, a helpful feature which suggests the diagnosis of Whipple disease on H & E stain (Fig. 137.55). Endoscopically, the lesions can present as yellowish-white plaques or as pale yellow shaggy mucosa, or have an erythematous friable appearance. Thus the classic histological features of intensely PAS-positive foamy macrophages and dilated lacteals with large lipid droplets are characteristic of Whipple disease.

Apart from histology and special stains, it is now possible to detect the Whipple antigen by immunohistochemical analysis using a polyclonal rabbit antibody produced against a cultured strain of *T. whippelii* [224] (Fig. 137.56). The antigen is a component of the bacterial cell wall. This is a sensitive and specific method of detecting Whipple disease. Other tests consist of PCR assays for 16S ribosomal RNA genes of *T. whippelii*, and electron microscopy. The organism responsible has also been successfully grown in human fibroblast cell lines using shell-vial assays.

With electron microscopy, PAS stain, and Whipple immunostain it is still possible to see retained bacterial products months and even years after initiation of therapy despite

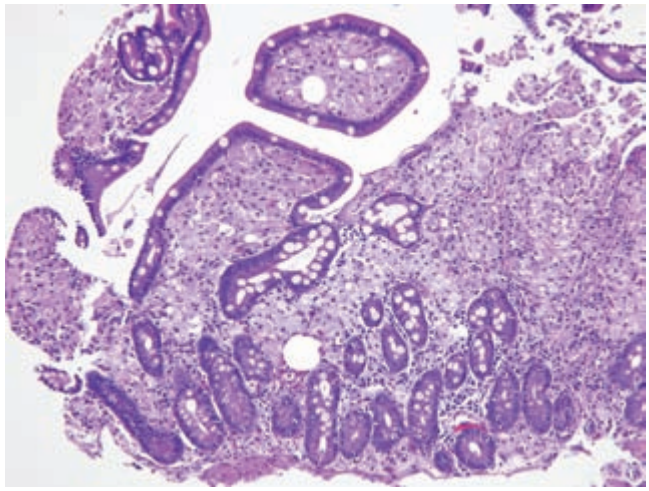

Figure 137.55 Whipple disease. Like Fig. 137.51, this image shows many foamy macrophages expanding the villi. In contrast, dilated lacteals are a feature of Whipple disease.

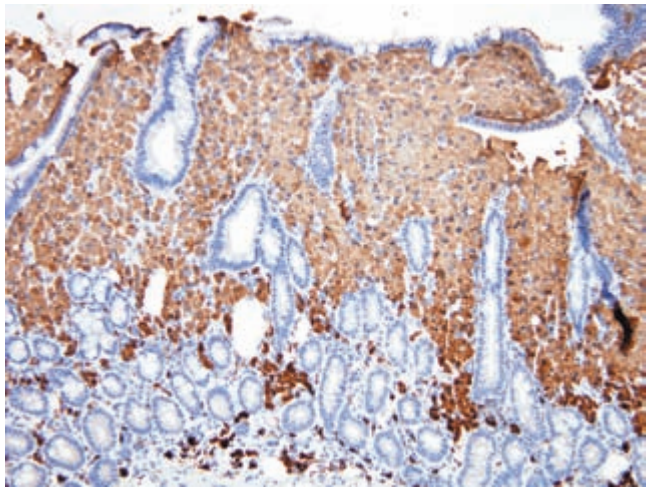

Figure 137.56 Whipple disease. This is an immunohistochemical preparation using an antibody directed against the organism.

clinical improvement. Thus, PCR may be a better tool to monitor treatment response as the bacterium has been shown to become undetectable shortly after initiation of antibiotic treatment.

Other infectious agents

Other infectious agents known to affect the small bowel include *Yersinia* spp., which produces necrotizing granulomas, and adenovirus, which results in marked lymphoid hyperplasia that can lead to obstruction or intussusception [225].

NSAID-associated injury

NSAIDs are well known to be associated with mucosal damage in the small intestine and may even lead to a peculiar form of strictures called *diaphragm disease* based on its macroscopic appearance [226–228]. The typical injury consists of ulcers which may lead to strictures. Unfortunately, the histological features of NSAID-associated ulcers are not specific, although they are usually not associated with abundant chronic inflammation.

Graft-versus-host disease

Patients with GVHD present with secretory diarrhea, abdominal pain and, at times, hemorrhage. There is a syndrome of upper GI GVHD that presents clinically as anorexia, dyspepsia, food intolerance, nausea, and vomiting. These syndromes were first recognized in the early 1990s [36,229,230] and endoscopic criteria for recognizing the lesions of GVHD are now available [29]. Of course patients who have had bone marrow transplants are prone to many infectious causes of enteritis, but many have upper tract GVHD. The original grading criteria were published by Snover and colleagues [229] and are summarized as follows: grade 1, increased crypt apoptosis; grade 2, apoptosis with crypt abscess; grade 3, individual crypt necrosis; grade 4, total denudation of areas of mucosa. These criteria are simple to apply and correlate well with clinical findings. Unfortunately, chronic GVHD results in nonspecific features of lamina propria fibrosis and mucosal atrophy. When confronted with such biopsies, we make an attempt to grade the active component and note the features of the chronic component. We also compare the biopsies to any prior ones. Papadimitriou and colleagues have reported a GVHD-like response to CellCept (mycophenolate mofetil) in colon biopsies [231]. This medication is widely used for maintenance immunosuppression in solid organ transplantation. Gastrointestinal toxicity, usually manifested as diarrhea, is its most common side effect. The authors reported findings in the colon, and we have seen them in the upper tract as well in our own material.

Common variable immunodeficiency

Daniels and colleagues have reported common GI complaints in patients with common variable immunodeficiency and related disorders [232]. Small bowel biopsies from these patients display villous atrophy and may have prominent intraepithelial lymphocytes as well as crypt apoptosis and even occasional granulomas, all features that suggest celiac disease or Crohn's disease. They differ from these other conditions by lacking plasma cells in about two-thirds of cases and being likely to harbor infectious agents (particularly *Giardia*). Of course, *Giardia* may be found in immunocompetent hosts as well; there are often fewer organisms.

Malabsorption

Celiac disease

Celiac disease is a relatively common (far more common than previously realized) systemic autoimmune disorder induced by gluten proteins found in wheat, barley, and rye. Injury to the small intestine is the hallmark of disease, but

Table 137.3 Schemes for assessment of biopsies in patients with celiac disease

Lesion category	Rostami modification [243,244]	Marsh classification [242]
Marsh 0	Same as original	Preinfiltrative: normal mucosal and villous architecture
Marsh I	Same as original	Infiltrative: normal mucosal and villous architecture; prominent intraepithelial lymphocytes
Marsh II	Same as original	Hyperplastic: same as infiltrative but also with enlarged crypts and increased crypt mitoses
Marsh III	Partial villous atrophy Subtotal villous atrophy Total villous atophy	Destructive lesion: flat mucosa (complete loss of villi); lymphocyte infiltration; enlarged hyperplastic crypts
Marsh IV	Same as original	Hypoplastic: total villous atrophy; normal intraepithelial lymphocyte count; believed to reflect severe malnutrition and be nonspecific

manifestations are systemic. Sites of extraintestinal injury include, but are not limited to, the skin (classically with dermatitis herpetiformis), joints, and uterus such that clinical presentations can be subtle and not GI tract-related. Certain HLA types confer the highest risk (HLA-DQ2 and HLA-DQ8) [233] and certain subsets of patients similarly are at high risk (e.g., family members of those with celiac disease, type 1 diabetics, children with Down syndrome [234], individuals with iron deficiency anemia). Prevalence of celiac disease has been reported as high as 1.5% in Finnish schoolchildren [235] and only slightly less in the general population of the USA [236] such that some observers suggest that there might be some utility in screening large numbers of individuals for this condition [237]. The incidence of celiac disease appears nil to low in Southeast Asians, but there are endemic populations in the Sahara with a high incidence of celiac disease [238]. In fact, pockets of prevalent celiac disease are found throughout the Middle East [239]. Undiagnosed celiac disease is responsible for growth retardation in some children [240] and has been associated with fetal wastage, infertility, and osteoporosis. An NIH conference was held on this topic and a statement from this is available at http://consensus.nih.gov and reported in 2005 [241].

When evaluating patients for celiac disease, it is worthwhile to remember that anti-gliadin tests are no longer recommended as they produce too many false positives. For example, patients with Crohn's disease may have both false-positive anti-gliadin tests as well as prominent intraepithelial lymphocytes, a combination likely to result in misdiagnosis. Presently, anti-tissue transglutaminase or anti-endomysial tests are recommended [241]. These are IgA-based tests in general so they yield false-negative results in IgA-deficient patients. IgG-based tests exist for this situation.

It is best to evaluate biopsies from beyond the bulb (first portion) since these are less prone to injury from gastric contents. Several biopsies should be obtained since characteristic features may be patchy. Review of these is easiest in well-oriented samples. There is a range of appearances in biopsies obtained from patients with celiac disease and the constellation of findings has been classified by Marsh [242] and further modified by Rostami and colleagues [243,244] (Table 137.3). What is important about these grading schemes is that they provide a framework for recognizing celiac disease lesions of that fall short of mucosal atrophy. Since these observers originally published their findings in the gastroenterology literature rather than the pathology literature, many pathologists have remained unaware of subtle findings that suggest celiac disease [245,246], although such findings are by no means specific [247,248]. Essentially, subtle or treated lesions display only intraepithelial lymphocytosis, similar in density or more prominent in the tips of the villi than the bases, where intraepithelial lymphocytes are physiological. As lesions progress, the lamina propria becomes expanded with numerous lymphocytes and plasma cells, the villi become progressively attenuated with epithelial damage, and the crypts become hyperplastic (Figs 137.57–137.59). The cells accounting for the infiltrate are T cells, and there is

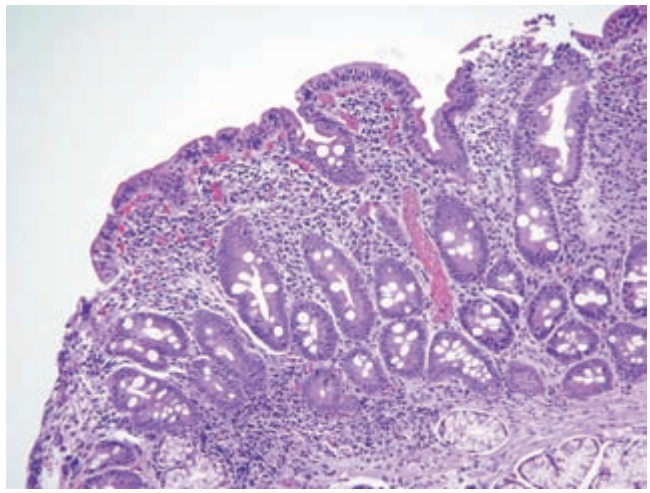

Figure 137.57 Celiac disease. The villi in this duodenal biopsy are wholly attenuated.

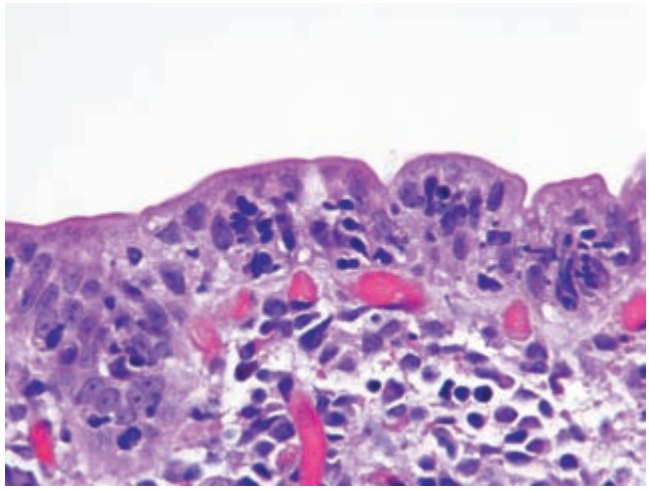

Figure 137.58 Celiac disease. Prominent intraepithelial lymphoctes are a key diagnostic feature.

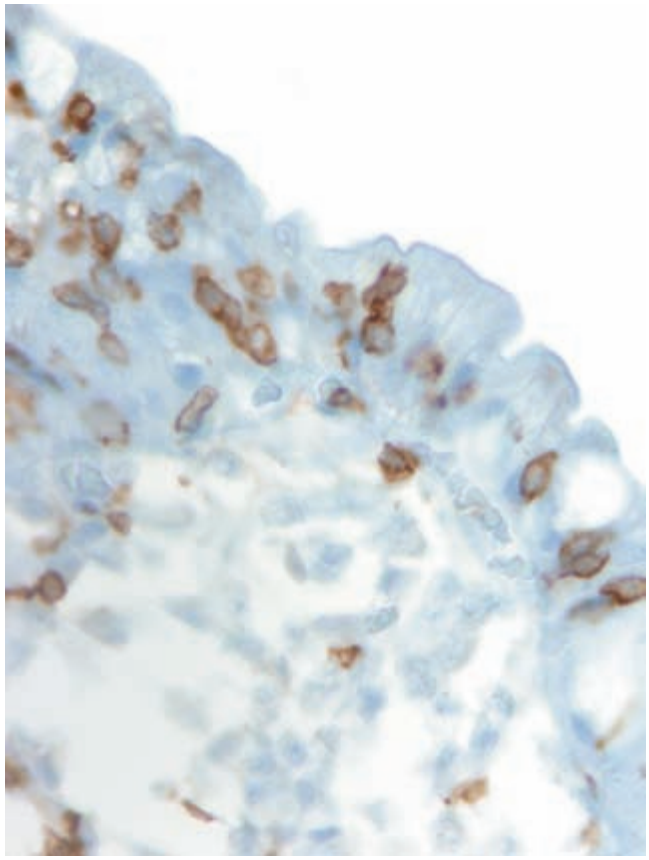

Figure 137.59 Celiac disease. This CD3 immunostain highlights the T cells in the epithelium.

extensive literature devoted to the phenotype of these T cells, most of which is of value only for research purposes.

Of course celiac disease is not unique in resulting in villous atrophy. Diseases that may produce villous atrophy include celiac disease and the related condition dermatitis herpetiformis, cows' milk protein intolerance (pediatric), giardiasis, peptic duodenitis, Crohn's disease, small bowel bacterial overgrowth (stasis and overgrowth usually of anaerobes), eosinophilic gastroenteritis, radiation enteritis, tropical sprue, severe malnutrition, lymphoma, GVHD, hypogammaglobulinemia/common variable immunodeficiency syndrome, and α-chain disease. A rule of thumb in considering atrophic enteritis is that neutrophils and histiocytes are not a typical inflammatory constituent in celiac disease and their presence should prompt other considerations, particularly bacterial overgrowth (stasis changes) and Crohn's disease. In reality, there are biopsies for which it is difficult to offer a precise etiology and in this situation a descriptive report is indicated together with dialogue with the endoscopist.

There remains a troubling subset of cases that defies current medical management and has a presentation resembling celiac disease and these are sometimes termed *refractory sprue* [249–251]. Robert and colleagues reported 10 cases of refractory sprue [252] and found that five of these patients ultimately developed collagenous sprue as a distinct histological marker of refractory disease. Additional distinctive findings in small bowel biopsies in the refractory group were subcryptal chronic inflammation (10 of 10) and marked mucosal thinning in three patients. Other nonspecific findings included acute inflammation and gastric metaplasia. One patient with collagenous sprue developed a B-cell lymphoma of the ileum, and in general collagenous sprue was associated with a poor prognosis. Two of five patients died whereas two others required total parenteral nutrition for survival. In other studies and in our experience, such cases may be clonal by gene rearrangement studies and related to T-cell lymphomas but these cases are also heterogeneous and rare so are difficult to study well [253]. Some patients respond to steroids but others are managed with antineoplastic regimens.

IgA deficiency

The incidence of IgA deficiency ranges from 1 in 400 to 1 in 3000 in healthy blood donors and most IgA-deficient individuals are healthy, although this condition is associated with protean manifestations [254]. It is believed that these people have a compensatory increase in secretory IgM. However, for the purposes of GI biopsy interpretation, disorders associated with IgA deficiency include giardiasis, nodular lymphoid hyperplasia, celiac disease (in around 2%), milk intolerance, and IBD. When reviewing biopsies from IgA-deficient individuals (serum IgA levels < 7 mg/dL), IgA-producing cells may be identified on immunohistochemistry but are usually reduced in number. It may be that, at least in some cases, the secretory (J chain) component is defective so that IgA-reactive cells are seen despite a lack of detectable serum IgA. However, since the number of IgA-labeled plasma cells is variable in any population of healthy individuals, the disease can only be suggested by the pathologist when the associated disorders are detected.

Tropical sprue and small bowel bacterial overgrowth

Aside from infectious intestinal diseases with known etiology, there is a group of GI disorders mainly affecting the small intestine of individuals living predominantly in, and less often visiting or returning from, the developing world, usually the tropics. These disorders range from asymptomatic structural or functional abnormalities of the GI mucosa (subclinical enteropathy) to a fully symptomatic condition highlighted by malabsorption of nutrients with associated nutritional deficiencies responsive to folate and broad-spectrum antibiotic treatment (tropical sprue). An infectious etiology is strongly suspected because of the occurrence of tropical sprue in epidemics, particularly in rural areas with poor sanitation, the susceptibility of visitors to endemic regions from developed countries, and its favorable response to treatment with antibiotics. In addition, patients with tropical sprue have also been associated with bacterial contamination of the small bowel. These patients have prolonged transit time through the small intestine, akin to that observed in obstruction [255], and evidence of bacterial overgrowth. The appearance of biopsies from these patients is very much like that in patients in Western countries with small bowel bacterial overgrowth. The findings are similar to those in celiac disease in terms of villous attenuation but with the addition of active inflammation and, at times, features of chronicity such as gastric mucin cell metaplasia and crypt distortion. Both conditions have overlapping features with upper tract Crohn's disease. Small bowel bacterial overgrowth is relatively common and occurs when small bowel motility is altered and patients present with diarrhea [256,257]. This alteration may be the result of underlying medical conditions such as diabetes or scleroderma (which results in sclerosis of the bowel wall) or from surgery. In addition, many patients with so-called irritable bowel syndrome are believed to have this condition. It is best diagnosed by small bowel aspirate and quantitative cultures and specific antibiotic therapy is determined by sensitivity testing of the cultured agents, which are generally anaerobes. A hydrogen breath test, which exploits hydrogen production from excess bacteria, is also effective in diagnosing this condition [258,259]. Sadly, the morphological features in small bowel bacterial overgrowth range from normal to Crohn's disease-like but usually do include a component of neutrophilic inflammation. The pathologist reviewing biopsies may be able to raise this possibility and suggest confirmation but it is difficult to diagnose in isolation of the clinical findings.

Microvillous inclusion disease

Microvillous inclusion disease is an uncommon congenital enteropathy that produces intractable secretory diarrhea in early infancy [260–267]. It is inherited as an autosomal recessive genetic trait. There are several synonyms used for this disease, including Davidson disease [261], familial microvillous atrophy, congenital microvillous atrophy, and intestinal microvillous dystrophy (a variant of microvillous inclusion disease). The outcome is typically poor, with most patients requiring small bowel transplantation. Rare cases have resolved.

Biopsies of duodenal mucosa show moderate villous blunting without active inflammatory reaction or intraepithelial lymphocytosis. The optimal specimen is a small intestinal biopsy; duodenal biopsy is most commonly used. (Rectal biopsy has been proposed as a simple and reliable method for early diagnosis although the findings may be subtle.) Architecturally, diffuse intestinal villous atrophy with no inflammatory reaction is a characteristic feature. Due to increased crypt cell apoptosis, either crypt hypoplasia or hyperplasia can be found. Subtle histological changes in the enterocytes are confined to the villous tips and distal villous lateral borders. Changes are difficult to discern at the base of the villus and in the crypt epithelium; indeed the enterocytes appear normal in these regions. Cytologically, a bubbly vacuolated appearance of the apical cytoplasm with extensive or patchy absence of the brush border is a specific diagnostic sign. Typical targetoid cytoplasmic inclusions are occasionally identified in the surface enterocytes.

Instead of a sharp linear brush border that stains with PAS or alkaline phosphatase histochemically, a bright apical cytoplasmic blush is present and is the most easily recognized change of microvillous inclusion disease. Brush border biomarkers, such as carcinoembryonic antigen (CEA) and particularly CD10 [264], are also useful in the diagnosis of microvillous inclusion disease. CEA and CD10 stains demonstrate the characteristic apical cytoplasmic staining pattern in the enterocytes. Ultrastructurally, identification of apical microvillous inclusions in surface enterocytes in intestinal tract biopsies makes a definitive diagnosis. A variant of this condition (possibly an incomplete form) is called *tufting enteropathy* or *intestinal epithelial dysplasia*; adhesion molecule defects have been implicated [260,266,267].

Colchicine toxicity

Colchicine is an alkaloid with antimitotic ability used to treat a variety of medical conditions. Iacobuzio-Donahue and colleagues reported findings in 21 GI mucosal biopsies from nine patients receiving oral colchicine therapy [96]. All patients had a history of gout. Four patients with chronic renal failure also had clinical evidence of colchicine toxicity, but the other five patients did not. Distinct morphological changes, seen as metaphase mitoses, epithelial pseudostratification, and loss of polarity, were seen in biopsy material from four of four (100%) patients with clinical colchicine toxicity. Three of these four cases (75%) also contained abundant crypt apoptotic bodies. These morphological features were best seen in the biopsies from duodenum (Figs 137.60 and 137.61) and gastric antrum, with relative sparing of the gastric body in the upper GI tract. Probably the most important finding in this study was that the distinctive mor-

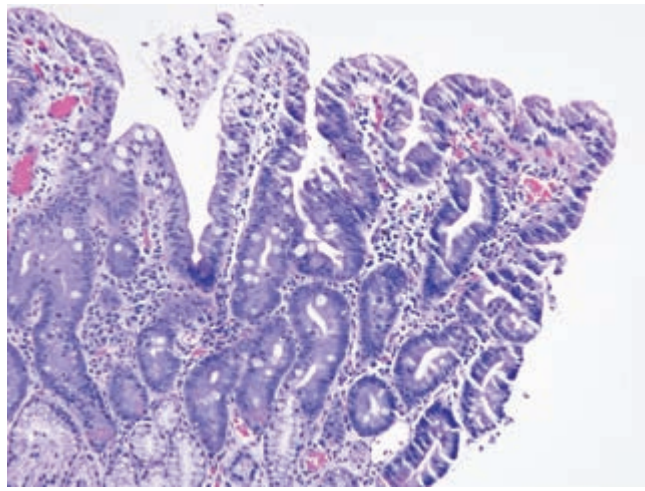

Figure 137.60 Colchicine toxicity. This duodenal biopsy shows attenuated villi and an expanded proliferative compartment. Mitotic arrest is seen as ring mitoses.

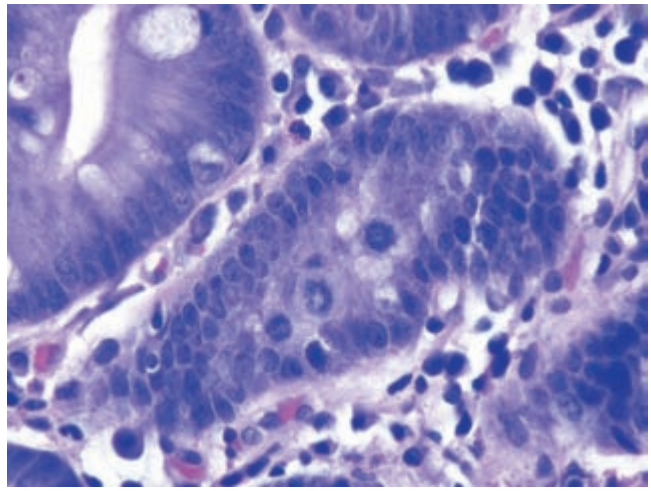

Figure 137.61 Colchicine toxicity. Higher magnification of arrested mitoses.

phological features were not seen in the five patients without clinical colchicine toxicity.

Lymphangiectasia

Primary intestinal lymphangiectasia is usually diagnosed before the age of 3 years. Most patients have growth retardation. Gastrointestinal complaints that vary in severity consist of diarrhea, vomiting, abdominal pain, and steatorrhea. If large segments of the gut are involved, secondary edema resulting from protein-losing enteropathy and malabsorption may occur. The edema is usually generalized, but asymmetric edema is not uncommon. Small biopsies taken in these patients may be nondiagnostic but they simply display multiple dilated lacteals, a finding easy for the pathologist to overlook without knowledge of an often dramatic CT appearance. The long-term course is variable, but is usually slow in progression with intermittent clinical remissions. The reported medical treatments for lymphangiectasia include a high-protein, low-fat diet with added medium-chain triglycerides. Octreotide has been reported to decrease intestinal protein losses.

Crohn's disease

The clinical features of Crohn's disease are well summarized elsewhere in this book. It is also referred to as regional enteritis, granulomatous enterocolitis, and terminal ileitis, and is a chronic relapsing and remitting inflammatory disease of unknown etiology that is often multifocal and can affect any portion of the GI tract. However, the classic disease site is the terminal ileum. It is typically characterized by foci of glandular destruction, aphthous erosions, serpiginous ulcers as well as areas of transmural inflammation, fibrosis and sometimes granulomas. Because of its transmural nature, fissures, sinuses, and fistulae may occur.

The gross endoscopic appearance of Crohn's disease is most consistently characterized as a multifocal process. Aphthous erosions (Greek *aphtha*, spot), longitudinal ulcers (tram-track or rake ulcers), and adjacent areas of erythematous or grossly unremarkable mucosa are hallmarks. Additional endoscopic findings include cobblestoning (irregular mucosa due to alternating ulcers and edematous mucosa), strictures, and fissures. The ileum typically shows focal or asymmetric involvement, which may include erosions, ulcers, or strictures. The rectum is often not involved (unlike in ulcerative colitis). Inflammatory polyps may be present. Rarely, Crohn's disease may become confluent, involving the entire bowel, which can be diagnostically difficult for the endoscopist. In both Crohn's disease and ulcerative colitis, the normal submucosal vascular network is lost in the involved areas, assisting the endoscopist in the diagnosis of chronic colitis. There is little correlation between the endoscopic appearance and the clinical symptoms of the patient.

Mucosal biopsies, although superficial, can show features suggestive of Crohn's disease (Fig. 137.62). Discrete foci of inflammation often associated with neutrophils within crypts (cryptitis) and adjacent to histologically normal crypts are common. Aphthous erosions or ulcers, characterized by focal surface epithelial necrosis associated with a mixed chronic inflammatory infiltrate, sometimes associated with underlying lymphoid aggregates are typical early lesions. Variability of inflammation within a single biopsy and among several biopsy fragments from the same anatomical location is also typical. The areas of inflammation show architectural changes of chronic crypt destructive colitis whereas adjacent crypts may appear totally normal. Granulomas are infrequently seen but when present are poorly formed and associated with chronic inflammation.

Terminal ileal involvement includes increased lamina propria inflammation, aphthous erosions, and flattened and broad

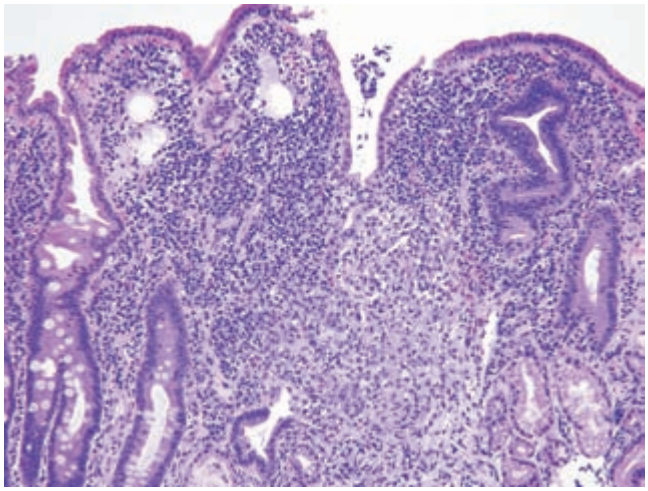

Figure 137.62 Crohn's disease. A granuloma is seen in the center of the field.

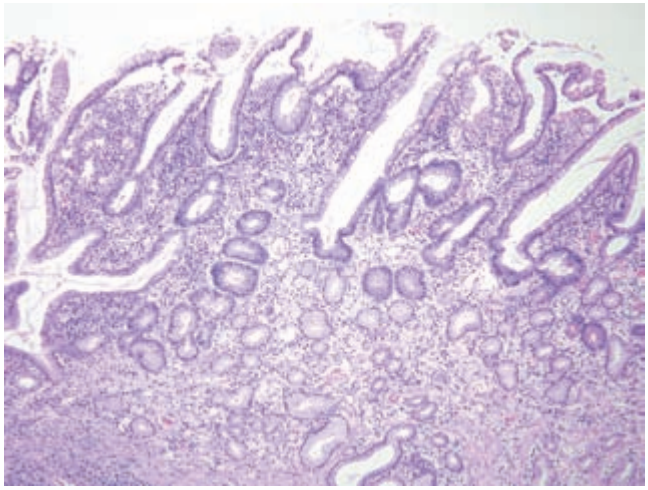

Figure 137.63 Crohn's disease. This ileal biopsy shows pyloric metaplasia: the metaplastic glands in the deep portion of the field lack goblet cells.

villi. Pyloric metaplasia (mucinous glands, reminiscent of the antral or pyloric mucosa) (Fig. 137.63) indicates repeated bouts of inflammation and repair. One must remember that focal acute ileitis is most commonly caused by medications, particularly NSAIDs, rather than Crohn's disease and finding the features of chronicity is critical; medication-induced injury is usually not associated with increased lamina propria inflammation or pyloric metaplasia. Submucosal fibrosis can also be a sequel to NSAID damage. Peyer patches are a normal component of the terminal ileum, particularly in young individuals, and should not be diagnosed as chronic inflammation. The mucosa over Peyer patches may be distorted with flattened villi due to underlying expansion of lymphoid nodules in nonspecific reactive hyperplasia. Yersiniosis can also result in prominent Peyer patches.

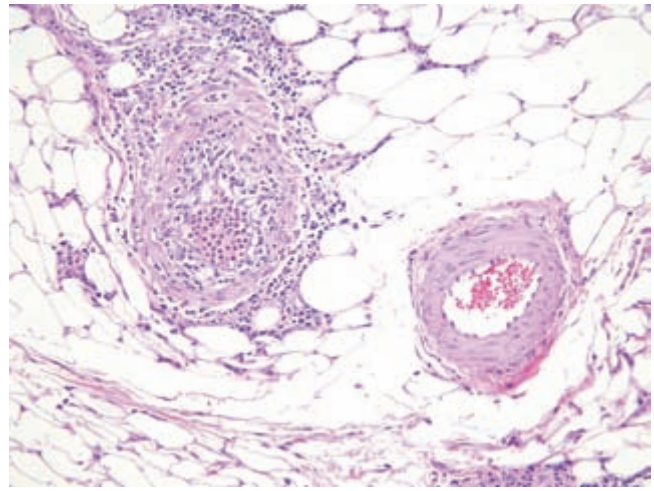

Figure 137.64 Behçet disease. Vasculitis primarily affecting mesenteric veins is the hallmark.

Behçet disease

Behçet disease is a systemic disorder consisting of oral ulceration plus any two of genital ulceration, typical defined eye lesions, typical defined skin lesions, or a positive pathergy test (an exaggerated response to a minor injury of skin) [268–270]. The GI tract may be affected anywhere, where deep penetrating ulcers lacking specific histological features are found, but the commonest sites are the terminal ileum and cecum. Establishing the diagnosis requires clinicopathological correlation and surgical management is often required. Vasculitis is one of the hallmarks, particularly involving veins (Fig. 137.64).

Small intestinal findings in ulcerative colitis

Generally the small intestine is unaffected by ulcerative colitis. Unfortunately, there is a subset of patients who have severe pancolitis that extends a short distance into the ileum as well, and the term *backwash ileitis* has been applied and used with some dissatisfaction by surgeons, gastroenterologists, and pathologists alike. It is associated with pouch complications in some studies [271] but not others [272] and is believed to reflect the severity of the ulcerative colitis. Backwash ileitis is sometimes described as active chronic and sometimes as minimal active but no real consensus exists. In daily practice we simply describe what is seen in the ileum. A group of patients with classic ulcerative colitis and severe confluent duodenitis has also been described [273]. These patients had otherwise classic ulcerative colitis and had a good clinical outcome with continent pouches and thus presumably had a rare form of ulcerative colitis with diffuse duodenal involvement rather than Crohn's disease. Whether these patients had a peculiar small bowel bacterial overgrowth complicating their ulcerative colitis was not clarified in the report.

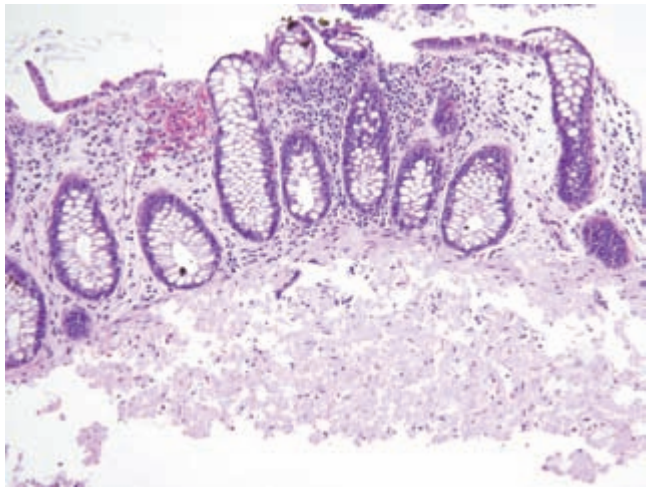

Figure 137.65 Amyloidosis. This field shows prominent submucosal deposits. This is from a colonic biopsy.

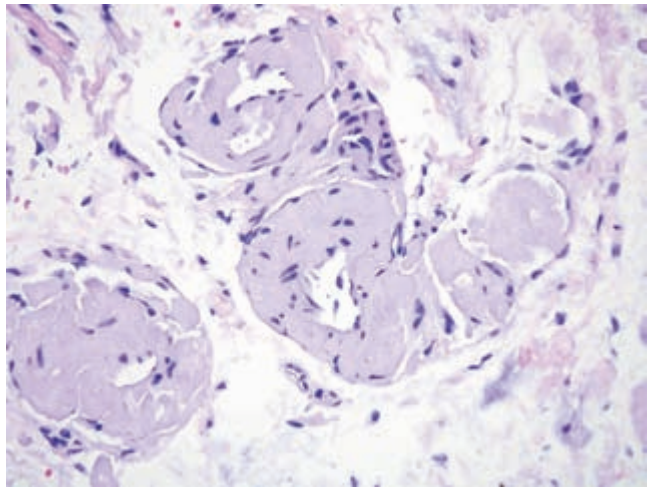

Figure 137.67 Amyloidosis. This vascular deposition was from a gastric biopsy.

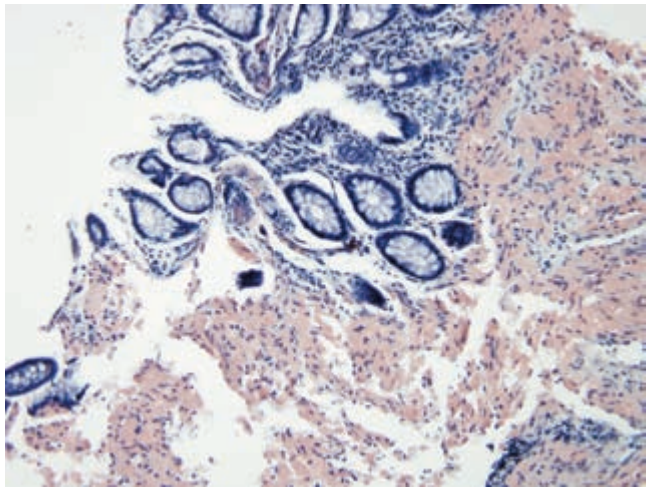

Figure 137.66 Amyloidosis. The Congo red preparation imparts an orange to brown color.

Amyloidosis

Gastrointestinal amyloidosis is an almost universal finding in systemic amyloidosis and consists of extracellular deposition of β-pleated protein sheets in the mucosa, submucosa, muscularis propria, or serosa (Figs 137.65–137.67).

The older classification divided amyloidosis into localized and generalized, or primary and secondary amyloidosis. Localized amyloidosis usually does not involve the GI tract. However, systemic amyloidosis almost universally involves the gut. Other common sites of amyloid deposition include heart, kidney, liver, and spleen, with restrictive cardiomyopathy, nephrotic syndrome, or hepatosplenomegaly depending on the site of amyloid deposition. The newer classification of amyloidosis is based on the biochemical composition of amyloid:

- AL amyloid (immunoglobulin light chain), associated with B-cell lymphoma, multiple myeloma, and Waldenström macroglobulinemia
- AA amyloid (serum amyloid-associated protein), seen in chronic illnesses such as rheumatoid arthritis, tuberculosis, bronchiectasis, Crohn's disease, and familial disease such as familial Mediterranean fever
- β_2-microglobulin, associated with renal failure and long-term hemodialysis
- β-precursor protein, associated with Alzheimer disease
- transthyretin (usually involves the heart).

Except for the familial forms, amyloidosis usually affects older adults in their sixties. Gastrointestinal manifestations consist of hemorrhage, nausea and vomiting, weight loss, malabsorption, gastric outlet obstruction, gastroparesis, stasis-related change such as bacterial overgrowth, or diarrhea. Amyloid deposits in blood vessels, which become fragile and leaky leading to hemorrhage. Deposits in the muscularis propria and nerve bundles cause impaired motility. Mucosal deposits lead to malabsorption and diarrhea. Rarely amyloid may present as a tumoral mass and can be mistaken for a carcinoma.

The endoscopic appearance is not specific, consisting of mucosal erosions, ulcers, thickened folds, or even a tumoral mass. Resection specimens with massive amyloid deposition have thick and rigid walls with a waxy appearance. Diagnosis is achieved through tissue biopsy of the GI tract, rectum and stomach being the preferred sites with a high yield. Biopsy samples must be adequate and deep enough to contain submucosal vessels in order to increase the diagnostic yield.

Biopsies often show intact and slender villi and few intramucosal Brunner glands. The lamina propria and muscularis mucosae contain extensive dense, eosinophilic, slightly globular extracellular deposits that produce the characteristic apple-green birefringence on Congo red stain, consistent

with amyloidosis. Amyloid deposits can also be seen in submucosal vessel walls.

The distribution of amyloid in the gut can be patchy or diffuse. Amyloid presents as dense, homogeneous, acellular, eosinophilic extracellular deposits in the lamina propria, submucosa, muscularis propria, nerve trunks, vascular intima and media. Amyloid deposits often show cracking with slit-like spaces as a result of tissue processing artifacts. Amyloid in the lamina propria often appears globular. Massive amyloid accumulation in the muscularis propria and submucosa is usually associated with AL amyloidosis.

Ultrastructurally, amyloid consists of nonbranching fibrils 7.5–10 nm in diameter. The fibrils are composed of β-pleated sheets of amyloid proteins. Cotton dyes such as Congo red, when polarized, impart a characteristic apple-green birefringence in the presence of amyloid. Other useful stains include Sirius red, Sirius supra scarlet and Thioflavin T (fluorescent dye). Immunolabeling for κ and λ chains is useful in AL amyloidosis, with the majority showing a λ restriction.

Arteriosclerosis of vessels with resulting hyalinization may be misdiagnosed as amyloid deposits. Systemic sclerosis can result in fibrosis of muscularis and submucosa which may be mistaken for amyloid. Ischemic injury resulting in lamina propria hyalinization and microcrypts can resemble amyloid. However, amyloid deposits in the lamina propria appear as globular deposits.

Polyps

Adenomas

Small intestinal adenomas are usually found in the duodenum and, like their colon counterparts, have three major histological types: tubular, tubulovillous, and villous. Adenomas in this site appear essentially the same as those in the colon. In patients with FAP, endocrine cells are often a prominent component. Most adenomas occur singly; the presence of multiple adenomas in the small intestine is unusual in the absence of FAP. In FAP patients, after colectomy, the main cause of death is upper GI malignancy [274]. The majority of patients with FAP also develop upper GI polyps. Those in the gastric antrum and duodenum are usually neoplastic. Therefore, surveillance of FAP patients through upper endoscopy plus biopsy is recommended. Because of their potential to undergo malignant transformation, adenomas should be removed via endoscopic polypectomy for pedunculated tumors and endoscopic mucosal resection or surgical resection for large sessile lesions.

Probably the biggest pitfall for pathologists in interpreting adenomas in the small bowel is the proclivity of reparative lesions to mimic them. When peptic duodenitis has a nodular configuration, it is easily confused with an adenoma. One clue is that peptic duodenitis often has surface gastric mucin cell metaplasia in the atypical focus and adenomas have their initiation point just beneath the surface epithelium [275] whereas reparative lesion have theirs at the base of the mucosa. Unfortunately, there are occasional cases in which it is impossible to distinguish adenomas from reparative processes and we designate them "indefinite for dysplasia" and additional samples are obtained. This distinction is not trivial in the small intestine, particularly in the area of the ampulla; there is a precedent for treating large ampullary adenomas with pancreatoduodenectomy based on their high likelihood of harboring an occult invasive carcinoma [276].

The ampulla itself is often a source of difficulty for pathologists since it normally has ampullary glands, with their features akin to biliary epithelium, interspersed with disorganized bundles of smooth muscle. When inflammation is a feature, great caution is advised. The ampulla is not typically biopsied without a compelling reason since pancreatitis may be a severe consequence of performing such biopsies.

When a clearly neoplastic small bowel lesion displays peculiar features, it is always important to consider that the process is a metastasis or extends directly from the pancreatobiliary tree.

Peutz–Jehgers polyps

The WHO has defined Peutz–Jeghers syndrome as an inherited cancer syndrome characterized by mucocutaneous melanin pigmentation and hamartomatous intestinal polyposis preferentially affecting the small intestine [277]. Extraintestinal neoplasms include those of the ovary (sex cord tumors with annular tubules), cervix (adenoma malignum), testis (Sertoli cell tumors), pancreas (carcinomas [278,279]), and breast (carcinomas). This syndrome is about one-tenth as common as FAP (incidence of FAP is between 1 in 7000 and 1 in 30 000). The diagnostic criteria include three or more histologically confirmed Peutz–Jeghers polyps; any number of Peutz–Jeghers polyps in an individual with this syndrome; appropriate melanin pigmentation in an individual with affected relatives; or any number of polyps with the appropriate mucocutaneous pigmentation. Since the pigmentation tends to fade with age, it may not be found in some adult patients with the syndrome. Recognition of the syndrome is important based on the associated 10–18-fold increase in cancers of both the intestines and extraintestinal sites [148,280]. This syndrome is an autosomal dominant trait with virtually 100% penetrance. Alterations in an involved gene, *LKB1/STK11* [281], are found in about half of affected patients, so doubtless there is more than one active locus that produces disease [282].

In their classical appearance, Peutz–Jeghers polyps are easy to recognize on endoscopic biopsies. They are hamartomas and thus display the type of mucosa typical for the site in which they are found. Thus, in the stomach they have gastric mucosa and in the small bowel they have small intestinal mucosa. The problems arise when biopsies are superficial or when ulceration distorts them. However, Peutz–Jeghers polyps in the small intestine are far more likely than those from the stomach to display the classic arborizing smooth

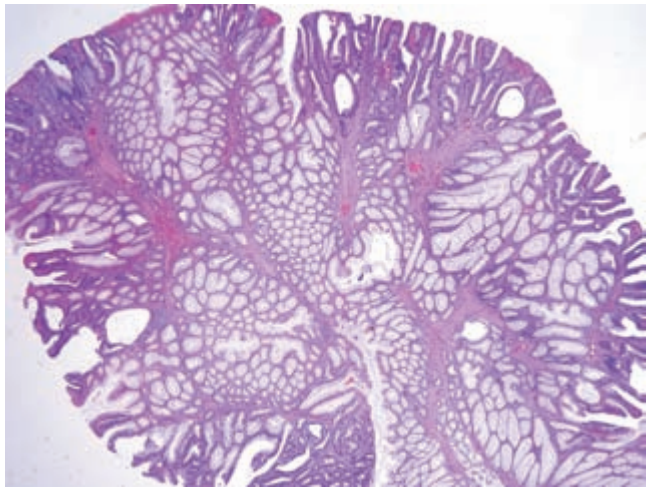

Figure 137.68 Peutz–Jehgers polyp. Note the striking muscular arborizing cores.

muscle cores from which the mucosa leafs out and must always be assessed for dysplasia (Fig. 137.68).

Neoplasms

Carcinoma

Adenocarcinomas are the most common malignancies of the small intestine (30%–50% of small bowel malignancies). However, primary adenocarcinomas are still rare lesions, accounting for only 2% of GI tract tumors or 1% of GI tract cancer deaths [274]. They present in older adults (median age 67 years), with a male predominance and are more common in African Americans than whites.

The majority are sporadic and share with sporadic colorectal adenocarcinomas both clinical risk factors and development from adenomatous polyps. The remaining minority of cases arise on a background of certain predisposing conditions, including several of the polyposis syndromes (primarily FAP but also hereditary nonpolyposis colon carcinoma syndrome, Peutz–Jeghers syndrome, and juvenile polyposis syndrome), Crohn's disease, gluten-sensitive enteropathy [283], ileostomy, and ileal conduits among others. The greatest risk of small intestinal adenocarcinomas is seen with FAP. In these patients, the relative risk of duodenal adenocarcinoma is striking (relative risk 330.82; 95% confidence limits 132.66 and 681.49; $P < 0.001$), as is that for ampullary adenocarcinoma (relative risk 123.72; 95% confidence limits 33.65 and 316.72; $P < 0.001$) [159]. As an aside, there seems to be no significant increased risk for gastric or nonduodenal small intestinal cancer in FAP patients. The risk of small intestinal carcinoma in Crohn's disease and celiac disease are each about 50–100-fold.

Lesions are most common in the duodenum, where 65% are periampullary, and their prevalence decreases progressively through the rest of the small intestine. An important exception to the proximal location occurs in patients with Crohn's disease, where 70% of adenocarcinomas are ileal, corresponding to the primary site of the inflammatory process.

Most adenocarcinomas are moderately differentiated, and one-third are poorly differentiated. Degree of differentiation and special histological subsets (mucinous, adenosquamous) have little bearing on prognosis and as such we mention them only as comments. The majority of small bowel adenocarcinomas have invaded through the bowel wall by the time of diagnosis.

Residual adenomatous epithelium is found with the majority of resected proximal tumors (those likely to be biopsied prior to resection), but often cannot be demonstrated in large distal small intestinal adenocarcinomas, presumably due to tumor overgrowth. Adenomatous epithelium can be mimicked by tumors metastatic to the GI mucosa. The main differential diagnostic consideration is metastatic disease, as the small intestine is the most common GI site for metastatic disease. Features favoring a metastatic tumor include the presence of multiple lesions, the absence of a precursor adenoma, a histological appearance of tumor being "bottom heavy" or encroaching from below, and lack of ulceration. Immunohistochemical studies are also helpful in most circumstances. Common metastatic lesions include colonic adenocarcinoma, melanoma, and breast and gynecological malignancies. Endometriosis is relatively common in the colon, but can also be seen in the small intestine and mimic metastatic adenocarcinoma.

Lymphomas

The GI tract houses more lymphoid tissue than any other anatomical site so it is not surprising that many lymphoid lesions are found here. In fact, the GI tract is the most common extranodal site of non-Hodgkin lymphoma. Secondary disease is common, especially in patients with advanced disease. Primary small intestinal lymphomas, in contrast to gastric lymphomas, are uncommon in the Western world, but still comprise 30%–50% of malignant tumors at this site. Primary GI tract lymphomas are defined as an extranodal lymphoma arising in the small bowel with the bulk of disease localized to this site and with the primary clinical manifestations and subsequent therapy related to this site. Lymphomas with a predilection for the small intestine include diffuse large B-cell lymphoma, followed by MALT lymphomas (usual and immunoproliferative small intestinal disease), secondary involvement by low-grade lymphomas (primarily mantle cell lymphoma and rarely follicular lymphoma), and enteropathy-like T-cell lymphoma. There is also evidence of rare follicular lymphomas primarily involving the duodenum. Hodgkin's disease can involve the GI tract primarily or secondarily but this is an exceedingly rare event [284].

Predisposing conditions include autoimmune diseases, immunodeficiency syndromes (e.g., AIDS), long-standing immunosuppressive therapy (e.g., post transplantation), Crohn's disease, and radiation therapy.

Marginal zone B-cell (MALT) lymphoma

Lymphomas of MALT are the most frequent primary lymphomas of the small intestine in the Western world but are much less frequent than gastric MALT lymphoma. There is a unique form of MALT lymphoma seen in Mediterranean areas and the Middle East referred to as immunoproliferative small intestinal disease (IPSID), which includes a spectrum of diseases including α heavy chain disease.

Small intestinal MALT-type lymphoma typically occurs in patients over the age of 50 years, with a slight male predominance. It is an indolent lymphoma; disease is often localized at diagnosis and may include mesenteric nodal involvement but typically no systemic spread. Long-term disease-free survival and cure are common. Unlike their gastric counterparts and IPSID, small intestinal MALT lymphomas have no clear infectious etiological associations. Presenting symptoms typically include abdominal pain and weight loss. Aggressive, diffuse, large B-cell lymphomas also develop in the intestine both as de novo lesions and through transformation from MALT lymphoma. Most of these large-cell lymphomas occur in older adults, but they can also rarely occur in children.

Small intestinal MALT lymphomas typically appear as ulcerated, exophytic, or annular mass lesions but rarely as small polyps. Deep mural invasion is typical although disease may be confined to the mucosa. The distal small intestine, particularly the ileocecal region, is the most frequent site but any part of the small intestine may be involved. The histology of small bowel MALT lymphoma is similar to that of gastric MALT lymphoma except lymphoepithelial lesions are often less prominent. The lesion typically progresses from an infiltrate between preexisting lymphoid follicles to one in which neoplastic cells erode and eventually overrun the lymphoid follicles, resulting in a vague nodularity or a completely diffuse infiltrate composed of marginal zone B cells that have a variable appearance even within the same tumor. They are intermediate size, with irregular nuclei and a rim of variable amounts of pale cytoplasm, giving them an overall appearance that ranges from a mature small B lymphocyte to a monocytoid appearance. Plasma cell differentiation is not uncommon and may be striking. The presence of moderate nuclear atypia, Dutcher bodies, and prominent lymphoepithelial lesions characterized by clusters of neoplastic cells infiltrating and destroying glands are features highly suggestive of lymphoma.

The presence of a large-cell component may represent transformation of a MALT lymphoma but criteria for diagnosing transformation to large-cell lymphoma are not well defined. When a large-cell component is prominent with clusters and sheets of large cells, a diagnosis of large-cell transformation or composite tumor has been suggested. When only focal large cells are present it should be noted in the report, but is a more dubious finding. If the tumor is composed exclusively of large cells, a diagnosis of extranodal diffuse large B-cell lymphoma should be made.

Immunohistochemistry is very useful in the diagnosis of MALT lymphoma; the labeling pattern is $CD20^{+}CD10^{-}CD5^{-}$. Coexpression of CD43 in CD20-reactive cells is a helpful finding when present but is only seen in about half of cases. The lesional cells express surface, and to a lesser extent cytoplasmic, immunoglobulin (usually IgM or IgA, rarely IgG) and show light chain restriction. Cytokeratin antibodies may highlight lymphoepithelial lesions, and follicular dendritic cell markers (CD21, CD23 ands CD35) help demonstrate the underlying follicular dendritic cell networks in those cases in which the lymphoid follicles have been obliterated by lymphoma. Ki-67 labeling index is low and can be useful for distinguishing from large-cell lymphoma.

Overall, long-term disease-free survival and cure are the norm. Primary therapy is en bloc resection of the bowel and contiguous lymph nodes in addition; radiation therapy and chemotherapy are often used postoperatively. Patients most likely to relapse include those with nodal involvement, extension beyond the bowel wall, and high-grade histology.

Immunoproliferative small intestinal disease

Immunoproliferative small intestinal disease (α heavy chain disease, Mediterranean lymphoma, and diffuse small intestinal lymphoma), a special form of MALT lymphoma restricted to a limited geographic distribution, is characterized by synthesis of α heavy-chain immunoglobulin. IPSID typically afflicts young adults and is slightly more common in males. It is seen almost exclusively in the Middle East and Mediterranean regions [285]. Common risk factors include low socioeconomic status, endemic parasitic infestation, poor sanitation, and infantile infectious enteritis. Some genetic factors have also been implicated. Presentation is typically severe, with malabsorption and chronic severe intermittent diarrhea and weight loss. α Heavy chain paraproteinemia (α heavy chain immunoglobulin without associated light chain) is present in up to 70% of IPSID cases; blood levels are at their highest early in the course and may diminish with progression of disease.

IPSID is generally a diffuse infiltrating lesion; initially the mucosa may appear normal then develop a cobblestone appearance and eventually may form lymphomatous masses. Mesenteric lymph node involvement occurs early in the course. The primary site of involvement is the proximal small intestine, but any part of or the entire small intestine may be affected. The stomach and colon can also be involved.

IPSID demonstrates a histological spectrum ranging from low- to high-grade histology, subclassified into stages A–C. All stages exhibit at least some features typical of other MALT lymphomas but usually with more striking plasma cell differentiation.

- Stage A is characterized by a lymphoplasmacytic infiltrate with features of a typical MALT lymphoma (reactive follicles with parafollicular clusters of clear cells and lymphoepithelial lesions) that is confined to the mucosa and which expands

the lamina propria causing broad villi, but may involve mesenteric lymph nodes.

• In stage B disease, the infiltrate becomes nodular, goes beyond the mucosa into the submucosa, and has mild cytological atypia. The macroscopic appearance at this stage is typically abnormal, with thickened mucosal folds. In MALT lymphoma with follicular colonization, the neoplastic cells may completely replace the follicle making distinction from a follicular lymphoma difficult.

• Stage C is characterized by the presence of large masses and transformation to large-cell lymphoma with numerous centroblasts and immunoblasts. Plasmacytoid differentiation is still evident but there is marked cell atypia including Reed–Sternberg cells. Mitotic activity is increased.

The lymphoma cells are $CD20^{+}CD5^{-}CD10^{-}CD23^{-}$. Immunoglobulin α heavy chains can be demonstrated in the cytoplasm of the infiltrating plasma cells, centrocytes and transformed blast cells.

The differential diagnostic considerations depend on the stage of IPSID. Early disease (stage A) may have a similar appearance to that of gluten-sensitive enteropathy (GSE). Unlike IPSID, GSE has prominent intraepithelial lymphocytes, true villous blunting, and crypt hyperplasia and clinically GSE is usually seen in northern Europeans and responds to a gluten-free diet. Intestinal T-cell lymphoma, while diffuse like IPSID, infiltrates the surface epithelium and is clearly is defined by immunohistochemistry ($CD3^{+}CD20^{-}$).

The nodular lesions of stage B disease require distinction from reactive follicular hyperplasia, and mantle cell and follicular lymphoma. Reactive follicular hyperplasia is unusual in the proximal small bowel and lacks prominent lymphoepithelial lesions and the typical MALT-type neoplastic cell of IPSID with pale/clear cytoplasm. Compared with other low-grade lymphomas, IPSID demonstrates monocytoid cells as well as plasmacytoid differentiation, and lymphoepithelial lesions and lacks the monotonous appearance of mantle cell lymphoma as well as the cleaved cells of follicular lymphoma. Immunohistochemical staining is very helpful in separating IPSID from the other low-grade lymphomas as IPSID is positive for α heavy chain and negative for CD5, CD10, and CD23.

The differential diagnosis of stage C disease is that of other high-grade malignancies, especially diffuse large B-cell lymphoma, but can be differentiated by the presence of residual MALT lymphoma with characteristic features and α heavy chain staining.

There is no well-established regimen for optimal treatment strategies. Early-stage disease responds to antibiotics. Later in the course as the tumor becomes more invasive and develops high-grade histology, radiation therapy or combination chemotherapy with nutritional support are typical treatments. Surgery is rarely indicated because intestinal involvement is generally diffuse. Prognosis has been traditionally considered poor but more recent studies note 5-year survival of 70%.

Mantle cell lymphoma

Mantle cell lymphoma (MCL) is a disease of older adults (mean age 55 years) with a male predominance (at least 2 : 1). Typically, it is a nodal-based disease but extranodal disease is common, particularly in the spleen and bone marrow. Gastrointestinal tract involvement is found in about one-third of MCL patients. Any part of the GI tract can be involved, but the ileum is favored.

When MCL involves the GI tract nearly all cases (~ 90%) have both distal small bowel and colon involvement. Involvement of the stomach and duodenum are also very common, seen in about half of patients [286]. Frequently MCL manifests as an isolated mass or small nodular polypoid tumors (2 mm to more than 2 cm in size), with or without normal intervening mucosa. The manifestation of multiple polypoid masses is termed *lymphomatous polyposis*. This pattern is most commonly associated with MCL but not specific for it. Mesenteric node involvement often accompanies GI tract disease.

The lymphoma cells are small to medium sized with scant cytoplasm; the nuclei have irregular outlines and indistinct nucleoli. Large transformed cells and proliferation centers are not typically present giving MCL a much more monotonous appearance than other low-grade lymphomas. Mitotic figures are easily identified. The most common architectural pattern is diffuse; however, both a nodular pattern and true mantle zone pattern can also be observed. Reactive germinal centers may be intermixed with and compressed by the lymphoma. Although displacement or obliteration of the glands may also be evident, lymphoepithelial lesions characteristic of MALT-type lymphomas are absent.

The tumor cells are mature B cells that express both CD19 and CD20, and aberrantly express CD5 and CD43. They lack CD10 and CD23. Cyclin D1 (BCL-1) is virtually always present. Surface light chains are present (usually IgM or IgD) and are typically λ restricted.

Although systemic chemotherapy is the treatment of choice, MCL is presently considered incurable, with a median survival of 3–5 years. Aggressive chemotherapy followed by autologous stem cell transplantation may benefit younger patients. Surgery has a relatively small role in the management of this disease, but may be necessary to relieve bowel obstruction.

Burkitt lymphoma

Burkitt lymphoma (BL) is a highly aggressive B-cell neoplasm that occurs in three major clinical forms: endemic, sporadic, and immunodeficiency associated [287–290]. All three types present primarily in extranodal sites including the GI tract.

Endemic BL is found primarily in Africa where it is the most common childhood malignancy and has a male predominance (male/female ratio 2 : 1). It typically presents with jaw, orbit or paraspinal lesions and is strongly associated

with EBV infection. It is unusual for African endemic BL to affect the GI tract. However, in other endemic regions (particularly the Middle East) BL frequently presents with intestinal obstruction or intussusception due to ileocecal involvement. Sporadic BL presents throughout the industrialized world, where it exhibits a wider age distribution. Half of cases involve children and the remainder occur in young adults but, as for endemic BL, the male to female ratio is about 2 : 1 and it is associated with EBV infection, particularly early in life. Sporadic BL frequently presents as an abdominal mass due to primary intestinal involvement (usually ileocecal), typically causing abdominal pain and obstructive symptoms. In adults, tumors can arise at any site in the GI system, but are most common in the ileocecal region and the rectum. Immunodeficiency-associated BL is primarily seen with HIV infection, often as the first manifestation of AIDS. EBV infection is found in up to half of cases. BL can be seen in other non-HIV immunodeficiency states, but not as often.

Gastrointestinal BLs of all clinical types have a predilection for the ileocecal region and less often rectum and stomach. They are bulky tumor masses that typically replace areas of involvement. They have a solid glistening white cut surface (fish flesh appearing) that is often associated with hemorrhage and necrosis. Lymph nodes are often surprisingly uninvolved but instead enveloped by tumor.

The morphology varies, with three common patterns: classic BL, BL with plasmacytoid differentiation, and atypical BL. The imprint morphology is distinct and well described, and thus touch preps and smears are helpful in the diagnosis. All the patterns share extremely high proliferation rates (manifest by numerous mitotic figures and very high Ki-67 labeling index) and high rates of spontaneous cell death (manifest by numerous macrophages with ingested apoptotic tumor cells, so-called "tingible body" macrophages). Classic BL morphology is seen in endemic BL and in most sporadic BL, and is characterized by a starry sky appearance, composed of diffuse monotonous pattern of infiltration of medium-sized cells (the "sky") admixed with frequent tingible body macrophages (the "stars"). The cells may have squared-off borders that make them appear cohesive at times. The nuclei are round with clumped chromatin and contain three to four centrally located small basophilic nucleoli. The cytoplasm is deeply basophilic and cytoplasmic lipid vacuoles are readily apparent. The atypical BL pattern has more pleomorphism than the monotonous classic BL morphology, and fewer but more prominent nucleoli. BL with plasmacytoid features has eccentric nuclei with only a single nucleolus; this morphology is more common in immunodeficiency-associated BL.

The tumors cells are positive for CD20 and CD10, and negative for CD5, BCL-2, and terminal deoxynucleotidyltransferase. Ki-67 labeling is seen in nearly 100% of cells. CD3-positive T cells are typically less common than the numbers seen infiltrating diffuse large B-cell lymphoma.

BL is a highly aggressive tumor but is potentially curable in the majority of patients. Prognosis depends on stage and is generally better in children. The mainstay of treatment is intensive combination chemotherapy regimens that result in 90% cure in low-stage disease and 60%–80% cure rate in advanced-stage disease. The prognosis for HIV-associated Burkitt-like lymphomas is related more to the underlying HIV syndrome than the lymphoma itself. Even Burkitt leukemia and those with central nervous system involvement can be cured. Surgical therapy has a role in treatment when debulking is required.

T-cell lymphoma

Intestinal T-cell lymphomas are far less common than those of B-cell origin. The majority of T-cell primary GI lymphomas occur in the setting of GSE and are specifically referred to as enteropathy-type T-cell lymphoma (ETL). ETL is rare (~ 5% of all GI tract lymphomas). Refractory GSE and particularly ulcerative enteritis (jejunitis) probably represent the first step toward ETL, although not all cases appear to progress to ETL [251]. The mean age of patients with ETL is 60 years with no gender predilection. The geographic distribution of ETL follows that of GSE, with the highest incidence in northern Europe. Immunodeficiency states are seldom associated with ETL. Since many patients presenting with ETL appear to have subclinical GSE, all patients with T-cell lymphoma of the gut should probably be tested for occult celiac disease.

ETL occurs most commonly in the jejunum, alone or in combination with other sites in the GI tract. The affected bowel segment is often dilated and edematous with large circumferential ulcers, ulcerated plaques and strictures with intervening areas of normal mucosa. Bulky exophytic or infiltrating masses are not typical but may be seen on occasion. Mesenteric lymph node involvement is common.

The histological appearance of ETL is quite variable between individual cases and between different sites in the same patient. Three basic types can be discerned, but transition between morphologies is common in the same case. The most common histological pattern is that of an infiltrate of pleomorphic medium to large cells followed by an anaplastic type with marked pleomorphism, both of which are associated with fibrosis and admixed inflammatory cells. The third type is composed of monomorphous small to medium-sized cells lacking background inflammation and fibrosis. The pleomorphic medium to large cell type is the most frequent morphology seen and is characterized by irregular nuclei with small nucleoli and moderate amounts of often pale cytoplasm. All the subtypes are associated with destruction of the overlying epithelium by the lymphoma cells. The areas of epithelium most affected are the upper and intermediate villous regions, or in the presence of villous atrophy the upper aspect of the crypts. In the majority of cases, endoscopically normal background mucosa shows changes

of GSE (intraepithelial lymphocytosis, villous blunting and crypt hyperplasia).

Most cases are positive for CD3 and CD7 and negative for CD4, CD8, CD5, and CD56 and express cytotoxic granule-associated protein TIA-1 often with granzyme B. CD56 has been used to describe two variants of ETL, type A ($CD56^-$) and type B ($CD56^+$ and $CD8^+$). The immunohistochemical labeling pattern of ETL correlates to some extent with the histological pattern. Both the medium to large pleomorphic and the anaplastic variants of ETL are usually type A ($CD56^-$) often with $CD30^+$, but are always negative for ALK1. Most of the type B ($CD56^+$) lymphomas are the small to medium-sized variant.

The prognosis is dismal, with a median survival of only 3 months and 5-year survival rates of approximately 10%. Patients suffer complications of peritonitis and malnutrition initially and later from progressive disease. A significant number of patients cannot tolerate chemotherapy due to underlying malabsorption. Factors associated with long-term survival include absence of a previous diagnosis of GSE and tolerance of a complete course of chemotherapy.

Carcinoid tumors

Carcinoid tumors (well-differentiated neuroendocrine tumors) of the small intestine account for about one-third of small intestine neoplasms. They seem to be overrepresented in African Americans [216,217,291]. Small intestinal carcinoids derive from two separate embryonic divisions of the alimentary tract, the foregut (duodenum) and the midgut (jejunum and ileum); these different origins correlate with distinct neuroendocrine differentiation and clinical behavior of these tumors.

Duodenal carcinoids are the second most common tumor in that site after adenocarcinomas and represent about 20% of GI tract carcinoids [292]. Overall, duodenal carcinoids are more common in men (male/female ratio 1.5 : 1) with a median age of 59 years (range 33–90 years). Two-thirds of duodenal carcinoids are gastrinomas (G-cell tumors); of the remaining one-third most are somatostatinomas (D-cell tumors) with a small percentage of undefined type. Only one-third of gastrinomas are functional and cause Zollinger–Ellison syndrome; these duodenal tumors account for half of cases of Zollinger–Ellison syndrome, the remainder being caused by pancreatic endocrine tumors. One-third of somatostatinomas are associated with von Recklinghausen disease. The somatostatinoma syndrome (diabetes mellitus, diarrhea, cholelithiasis), associated with pancreatic islet cell tumors, is virtually never seen in its entirety with these intestinal tumors. Proximal small intestinal carcinoids virtually never produce the carcinoid syndrome. Functional gastrinomas and somatostatinomas present in slightly younger patients than nonfunctioning gastrinomas and somatostatinomas are slightly more common in females. Nonfunctioning gastrinomas are usually located in the bulb, while their functional counterparts are found equally in all parts of the duodenum. Somatostatinomas are typically found in the periampullary region [293]. The carcinoid syndrome (watery diarrhea, flushing, endocardial fibrosis) is seen in 5%–7% of cases and jejunoileal carcinoids account for the majority of carcinoid syndrome cases overall. Nearly one-third of patients diagnosed with a small intestinal carcinoid have a synchronous or metachronous carcinoma (e.g., colon, stomach, lung, and breast).

Duodenal tumors can be readily biopsied and diagnosed by endoscopy. The location of jejunoileal tumors makes diagnosis more problematic as they are often not amenable to endoscopy and conventional imaging techniques (CT and barium contrast studies) are not particularly sensitive. In these circumstances, use of radiolabeled somatostatin analogues (octreotide and lanreotide) in scintigraphy studies has proven a sensitive detection method of both primary and metastatic lesions. This is also an area for which capsule endoscopy offers promise [294].

Duodenal carcinoids are typically small polypoid lesions less than 2 cm in size. They are submucosal lesions with an overlying mucosa that may be focally ulcerated. Infiltrative larger tumors can occur but are infrequent. Gastrinomas tend to be smaller than somatostatinomas. A minority (15%) are multicentric.

Jejunoileal carcinoids are twice as likely to be multicentric (30%), tend to be large (> 2 cm), and commonly are locally advanced, with deep bowel wall and mesenteric tumor involvement causing significant mesenteric fibrosis and subsequent obstruction and kinking of the bowel. Frank intestinal ischemia may also be seen due to tumor-induced angiopathy. Like duodenal carcinoids, jejunoileal tumors are typically centered in the deep mucosa or submucosa, and the overlying mucosa is typically intact or only slightly eroded.

The distinction between benign and malignant carcinoid is based on the presence or absence of metastasis rather than histology alone, since even clinically malignant tumors show little or no cellular pleomorphism, hyperchromasia, or increased mitotic activity. Carcinoids are characterized by a monotonous proliferation of small bland polygonal cells with moderate amounts of cytoplasm, and round regular nuclei with "salt and pepper" chromatin. Pleomorphism and significant mitotic activity are conspicuously uncommon. Morphological features of the cells do not differ by cell of origin (immunohistochemistry is required for this distinction). Carcinoids demonstrate variable architectural growth patterns. These do not have prognostic significance, but may be characteristic of certain carcinoid subtypes. The main architectural patterns are insular or nested (type A), trabecular (type B), and acinar (type C). These patterns often overlap and multiple patterns are typically seen within any single tumor. Since these categories offer no prognostic information, there is little to be gained by reporting them and many pathologists do not.

Duodenal G-cell tumors can manifest a variety of architectural growth patterns, whereas somatostatinomas often have characteristic features of prominent acinar growth pattern with frequent intraluminal psammoma bodies. Jejunoileal carcinoids, like G-cell tumors, do not display any consistent characteristic growth patterns. They typically display prominent nested growth, with more trabecular and acinar growth peripherally. These tumors are often larger and extend deeply into the bowel wall and can be associated with significant fibrosis.

Although carcinoid tumors can show immunohistochemical evidence of multiple hormone production, this information is typically not clinically significant. The vast majority of small intestinal carcinoids are positive for chromogranin and synaptophysin, and these markers are useful in confirming the diagnosis of carcinoid. Specific cell types (e.g., G-cells) can be identified by immunohistochemical labeling but the utility of this is limited as functional status correlates poorly with immunohistochemistry.

Duodenal carcinoids are generally indolent (overall 4% mortality); however, two-thirds of somatostatinomas and half of functional gastrinomas behave aggressively. Poor outcome in duodenal tumors is best predicted by invasion beyond the submucosa and lymph node or distant metastases. Jejunoileal carcinoids have a worse prognosis than those of the duodenum, with a 21% mortality rate. Survival has been negatively correlated with distant metastases (liver), tumor multiplicity, mitotic rate, invasion beyond submucosa, and female gender. Pathological features that have been associated with behavior are size, depth of invasion beyond submucosa, mitotic activity, and lymph node involvement.

Mesenchymal tumors

Gastrointestinal stromal tumors

Overall, GISTs of the small bowel should be regarded with caution compared with those in the stomach, which more often behave in a benign than malignant fashion [295,296]. They are not typically diagnosed on biopsies. When biopsies are evaluated, it is prudent to perform an immunohistochemical panel that includes CD117, CD34, SMA, desmin, keratin, and S100 protein.

Gangliocytic paraganglioma

These are rare and fascinating tumors, the vast majority of which are found in the duodenum in adult patients (average age about 54 years) [297–302]. The typical presentation involves abdominal pain, gastric outlet obstruction, or bleeding. There are isolated reports of an association with neurofibromatosis, but most cases are sporadic. These lesions typically have their epicenter in the submucosa with minor extensions into the mucosa that are occasionally biopsied. Excised tumors are 3–4 cm in size with a soft yellowish cut surface. They have infiltrative borders.

These tumors display a histological constellation of three cell types: spindle cells with the appearance of nerve sheath cells, ganglion-like cells, and epithelioid cells arranged in nests (endocrine pattern), trabeculae or papillary structures. The proportion of the cell types is variable and hence these lesions are prone to causing diagnostic problems.

On immunohistochemistry, the tumors are reactive with S100 protein in spindle and supporting/sustentacular cells, with synaptophysin in ganglion-like cells, and with neuron-specific enolase in all three cell types [297,300]. About half of cases display keratin in epithelioid cells. A variety of hormones can be demonstrated in various fractions of gangliocytic paragangliomas, including somatostatin, human pancreatic polypeptide, serotonin, gastrin, glucagon, insulin, and vasoactive intestinal peptide.

In lesions dominated by spindle cells, the differential diagnosis is with GISTs and nerve sheath tumors: positivity for S100 protein excludes GIST; finding the admixture of other cell types excludes nerve sheath tumors. Epithelioid lesions are distinguished from carcinoid tumors and carcinomas by the admixture of other cell types.

Gangliocytic paragangliomas are benign in the majority of cases. There are rare reports of regional metastases [298,299] but not of tumor-associated deaths.

Colon

Nonneoplastic conditions

In the USA, the most common cause of chronic diarrhea is irritable bowel syndrome (IBS), a condition with a prevalence of 6%–20% depending on inclusion criteria. Diagnosis is based on a set of criteria that include continuous or recurrent abdominal discomfort, bloating or distention, sometimes associated with a change in stool frequency or appearance. The American Gastroenterological Association advocates colonoscopy as part of the evaluation of patients with presumed IBS who are over 50 years old or in whom there are symptoms that might suggest another disease (diarrhea predominance or weight loss) [303]. Thus, most of the colonic biopsies reviewed as part of the evaluation for chronic diarrhea can be expected to have essentially normal histology and should be reported as such. This requires an understanding of the histological features of normal mucosa from all parts of the colon and rectum, as well as the ability to recognize histological "abnormalities" that can be ignored.

Normal histology in the colon reflects function of the specific site. Liquid stool from the small intestine enters the cecum and water is progressively absorbed such that the colonic contents has the consistency of feces by the time it has reached the descending colon. The colonic mucosa produces abundant mucus that lubricates the surface and facilitates the passage of fecal material. The differences in the normal histology of the right and left colonic mucosa reflect

these functions of absorption and lubrication. In the crypts of the right and transverse colon, absorptive cells predominate, although the larger goblet cells are more conspicuous. The lamina propria in these proximal segments of the colon is more cellular than that of the distal colon and rectum, and includes abundant plasma cells (involving the full thickness of the mucosa), lymphocytes, and eosinophils. Paneth cells may be found in the cecum and right colon. In contrast, goblet cells increase sharply in number, and absorptive cells are fewer in the left colon and rectum. The lamina propria is much less cellular, with fewer eosinophils. Plasma cells are present only beneath the surface epithelium more distally.

Lymphoid aggregates are normal and prominent intraepithelial lymphocytes are normally found over lymphoid aggregates. Macrophages are normally present, and play a role in the processing and presentation of antigens. They aggregate in the superficial lamina propria, where they phagocytose cellular debris from normal apoptosis of surface epithelial cells. Surface apoptosis is more prominent in the left colon and rectum than in the proximal colon.

Although eosinophils are present in the lamina propria of nearly all right colonic biopsies, their numbers vary greatly, particularly between geographic regions, being generally more numerous in warmer areas in the USA [304]. Paneth cells are abnormal outside of the right colon (although we accept them up to the splenic flexure); their presence in the left colon is metaplastic, usually in response to chronic inflammation or injury.

Effects of bowel preparation and other artifacts

Bowel preparation for sigmoidoscopy and colonoscopy may induce a number of mucosal injuries that must be recognized lest they be misinterpreted as pathological. Hypertonic enemas, generally used before sigmoidoscopy, may injure the mucosal surface, resulting in flattened or absent surface epithelium, loss of epithelial mucin, or slight neutrophilic inflammation. The lamina propria may be edematous or hemorrhagic [305].

The two most commonly used preparations for full colonoscopy are sodium phosphate and balanced electrolyte solutions containing polyethylene glycol (PEG). Sodium phosphate bowel preparations (e.g., Fleet enema) are preferred by many patients, and thus are currently the most common bowel preparation in adult patients, the exception being those who cannot tolerate the potential fluid and electrolyte shifts. Oral sodium phosphate preparations are strong osmotic laxatives that induce a number of endoscopic and histological changes. The most common endoscopic change is the presence of what appear to be small aphthous lesions: small foci of pale mucosa surrounded by erythematous rings that look like shallow erosions. These aphthous-like lesions have been described in up to one-fourth of patients after sodium phosphate bowel preparation. Histologically, these are usually large lymphoid aggregates, although some authors have described edema, hemorrhage, or acute inflammation [306–308]. The lesions disappear when colonoscopy is repeated with an alternate bowel preparation, and so are clinically unimportant. Oral sodium phosphate bowel preparation is also believed to induce foci of neutrophilic cryptitis (focal active colitis) in about 3% of patients. Whereas apoptosis is normal in the surface epithelium, scattered apoptotic bodies at the bases of crypts are found after sodium phosphate bowel preparation. These apoptotic bodies have been counted at about 1 per 10 well-oriented crypts [308].

Other endoscopic and biopsy-related artifacts

During the course of colonoscopy, gas is induced to insufflate the bowel for better visualization of the mucosa. Small amounts of this gas may infiltrate tissue and appear as small, clear, bubble-like spaces in the mucosa or superficial submucosa, often in or near lymphoid aggregates. Because these spaces resemble adipocytes, this phenomenon has been termed *pseudolipomatosis* [309]. Biopsy forceps can also induce tissue trauma, resulting in mucosal hemorrhage, crushing of epithelium that may resemble dysplasia, and an artifact in which the epithelium is squeezed entirely out of the crypts leaving empty crypts. This squeeze artifact is common on the edges of mucosal biopsies and usually readily recognized. However, when a large area is involved, it may be mistaken for ischemic injury.

Focal active colitis

Focal active colitis (FAC) is the term used to describe focal neutrophilic infiltration of colonic crypts. It may consist of one focus in a single biopsy, or multiple foci. Since focal inflammation is a feature of Crohn's disease, studies of FAC have examined whether it antedates subsequent development or diagnosis of Crohn's disease. In two studies of adult patients with FAC in colon biopsies, only a very few developed Crohn's disease [310,311]. Many seemed to have a clinical course that resembled infectious diarrhea, although this may have had no relationship to the FAC and may simply have reflected a common indication for endoscopy. However, in a group of pediatric patients with FAC, a higher proportion (27.6%) went on to develop Crohn's disease [123]. In Greenson's studies, no adult patient with FAC developed Crohn's disease and the only endoscopic abnormalities were aphthous or aphthous-like ulcers, possibly artifacts of bowel preparation. In contrast, in the pediatric group, 2 of 17 patients with normal colonoscopy developed Crohn's disease, compared with 6 of 11 with abnormal colonoscopy. Thus, it seems that in adult patients without history or endoscopic features suggestive of Crohn's disease, FAC can be safely ignored whereas it should probably be followed when it is found in pediatric biopsies.

Lamina propria macrophages

Macrophages are a normal constituent of the colonic lamina propria, where they serve roles in antigen processing and

phagocytosis of surface epithelial cells that have undergone apoptosis. CD68 immunostaining highlights a gradient in their distribution, with more macrophages in the upper lamina propria and fewer deeper [312]. Pigmented macrophages are referred to as melanosis coli and those containing mucin as muciphages.

When muciphages are present in otherwise normal biopsies, the usual gradient is reversed, as they tend to accumulate in the lower part of the mucosa. Muciphage aggregates are found associated with crypt rupture from any cause and so are seen in many settings [312]. Ultrastructural study supports degenerated epithelial cells as the source of the mucin [313]. Muciphages must be distinguished from the macrophages of Whipple disease and mycobacterial infection and from carcinoma, but it is not usually necessary to perform a full workup in any given case since aggregates of muciphages are very common in colorectal mucosa, present in 40% of otherwise normal rectal biopsies [314]. They are presumed to reflect occult and clinically unimportant epithelial injury, such as trauma related to stool passage, or subclinical infection.

The term melanosis coli is a misnomer. The brown pigment that accumulates in lamina propria macrophages in this condition is lipofuscin not melanin (Fig. 137.69). Although it is most often seen in right colonic biopsies, it can be found throughout the colorectum, appendix, and terminal ileum. It is extremely common and detected in the majority of elderly individuals. In one study of 200 patients, of men and women in the age group 20–54 years, 32% and 44% were affected; above the age of 75 years, 76% and 67% respectively were affected [315]. Lipofuscin accumulates by phagocytosis of apoptotic epithelial cells [316–318]. There is a strong association with anthraquinone and other laxatives because they induce apoptosis [319–321], but melanosis may be a result of any process associated with apoptosis, such as NSAID use.

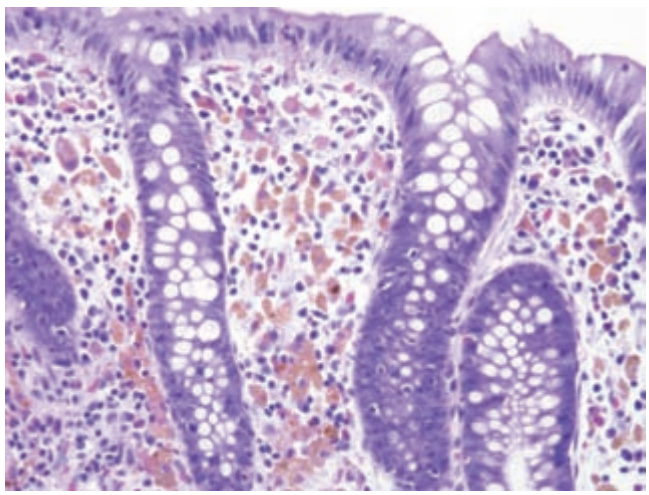

Figure 137.69 Melanosis coli. The pigment in the lamina propria macrophages is lipofuscin rather than melanin.

Many patients with melanosis have not used laxatives, including those with ulcerative colitis and Crohn's colitis [322].

Ulcerative colitis

As discussed in detail elsewhere in this text, ulcerative colitis is a chronic crypt-destructive inflammatory process of unknown cause characterized by predominantly mucosal-based disease and clinically associated with exacerbations and remissions of bloody diarrhea. The initial presentation may be indolent in onset or may be severe and acute presenting with toxic hemorrhagic colitis. In children, initial biopsies may be nondiagnostic since they are likely to be brought to medical attention before histological features of chronicity are evident (whereas adults wait until they have been symptomatic for months to years before seeking attention) [123,323,324].

The gross endoscopic appearance of ulcerative colitis varies with the degree of activity, duration of disease, and response to therapy. It is characterized by diffuse disease with rectal involvement extending proximally. In the more active phases the mucosa is typically erythematous, bloody, and friable with a granular appearance. Quiescent disease may appear granular with areas of punctate erythema. Pseudopolyps (mucosal remnants) and inflammatory polyps may be seen in active disease and postinflammatory polyps in active or quiescent disease. In all cases, the normal submucosal vascular network is lost and for the endoscopist is an important clue indicating chronic colitis. In patients who have had repeated bouts of colitis, the normal haustral folds may be absent. The transition between normal and abnormal mucosa is generally gradual but may be abrupt. Except for the occasional presence of focal cecal or periappendiceal involvement ("skip areas"), there is no loss of disease continuity and no skip lesions are seen in ulcerative colitis.

On biopsies, crypt architectural distortion and increased mucosal chronic inflammation are the two characteristic histological findings (Figs 137.70–137.72). Neutrophils, the hallmark of active disease, are located within crypt epithelium (cryptitis) and crypt lumina (crypt abscesses) with fewer within the lamina propria around the crypts. Architectural distortion results from prior mucosal destruction and imperfect repair, resulting in decreased numbers of crypts and branched, malformed, regenerated crypts (crypt-destructive colitis refers to the distortion of regenerated crypts rather than active destruction of crypts due to cryptitis). The chronic inflammatory constituents consist of variable numbers of plasma cells, eosinophils, and lymphocytes. Eosinophils may predominate. Lymphoid aggregates are commonly seen at the mucosal–submucosal interface. The regenerating epithelium is immature and as such does not contain mucin, typical of a mature goblet cell (mucin depletion). Biopsy fragments from the same topographic area typically show similar findings. Inflammation is usually more severe distally. Of course granulomas are lacking but occasionally a foreign-body re-

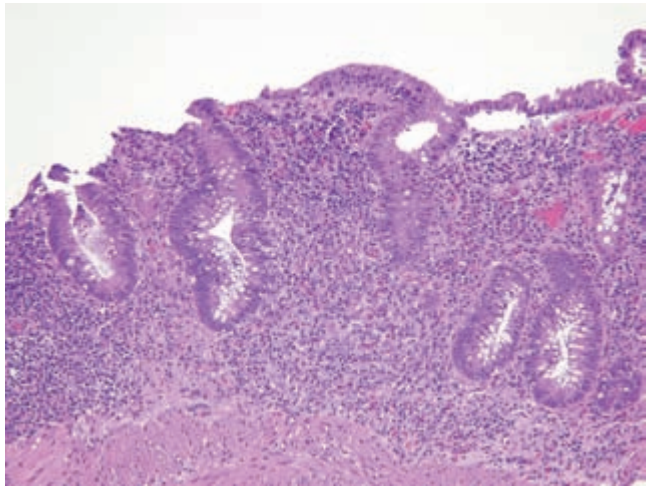

Figure 137.70 Ulcerative colitis. In this field, the crypts are distorted and do not reach the muscularis mucosae at the bottom of the field.

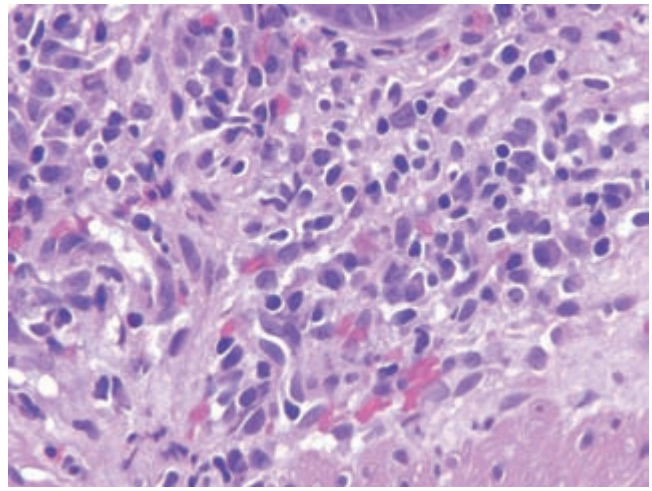

Figure 137.72 Ulcerative colitis. This field shows basal plasmacytosis, a feature of chronic injury. Plasma cells are seen between the base of the crypts and the top of the muscularis mucosae.

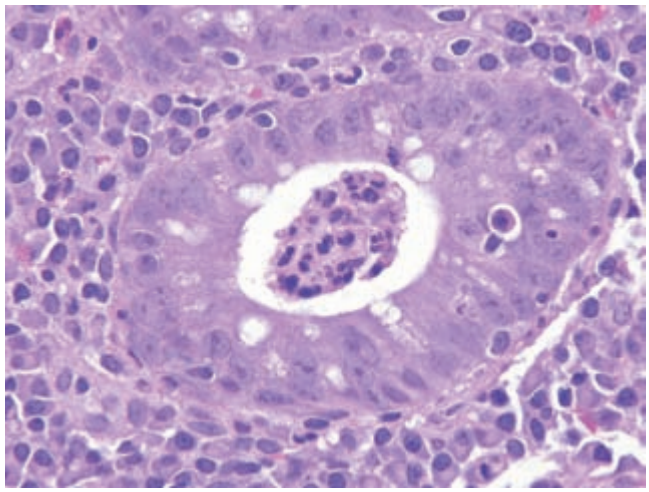

Figure 137.71 Ulcerative colitis. This field shows a crypt abscess (a collection of neutrophils within a crypt).

sponse to a ruptured crypt may be found. Usually the damaged crypt that has invoked a foreign body response can be found on additional sections. Pyloric metaplasia is not typical in ulcerative colitis but is occasionally found. Of course, knowledge of upper tract findings is important since ulcerative colitis theoretically involves only the colon and not the upper tract.

Ulcerative colitis must be distinguished from infectious colitis, medication-induced colitis, ischemic colitis, and Crohn's disease. Long-standing infectious colitis may display the features of ulcerative colitis including increased chronic inflammation, neutrophils, basal plasmacytosis, and diffuse colonic involvement. A predominance of neutrophils within the lamina propria rather than within the crypt epithelium and overall architectural preservation supports infectious over ulcerative colitis. The initial episode of ulcerative colitis may appear histologically similar to infectious colitis and thus infectious etiologies should be excluded prior to confirming a diagnosis of ulcerative colitis. Medications, particularly NSAIDs, may present with focal acute colitis or ulcers throughout the colon. Histologically, NSAID ulcers are typically abrupt with no significant inflammation in the adjacent mucosa and minimal chronic inflammation. Chronic ischemic colitis can be mistaken for ulcerative colitis particularly because ischemia due to low vascular flow often affects the left colon and because it may be intermittent. As such, abrupt episodes of ischemia can result in mucosal ulceration which, following reperfusion, may become inflamed and show all the architectural features of ulcerative colitis. Features that support ischemia include age of the patient, location, involvement of the muscularis propria and, importantly, the lack of neutrophils destroying crypt epithelium. Crohn's lesions characterized by crypt destruction are typically patchy, with areas that appear involved and uninvolved within single biopsies and among different fragments from one area. Crohn's colitis typically lacks the diffuse crypt destruction characteristic of ulcerative colitis.

Once a patient is treated, the characteristic distribution of ulcerative colitis may become altered. Thus, a patient may display iatrogenic rectal sparing, something that should be remembered by pathologists interpreting biopsies from treated patients (the usual case). Most patients have mild to moderately active disease that does not require colectomy.

Long-standing ulcerative colitis is associated with an increased risk of dysplasia (Fig. 137.73) and adenocarcinoma, even in patients with well-controlled quiescent disease. Periodic colonoscopic examinations are used to survey for dysplasia and adenocarcinoma. Routine surveillance with biopsies is recommended annually for patients with extensive disease of

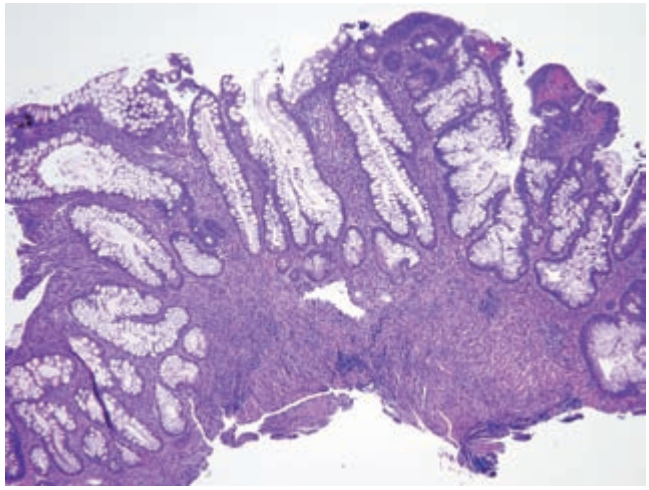

Figure 137.73 Ulcerative colitis. A zone of low-grade dysplasia is seen at the upper right portion of the field.

more than 8 years' duration because there is an increasing annual incidence of adenocarcinoma. Biopsies are generally taken from the cecum, ascending, splenic flexure, transverse, hepatic flexure, descending, and sigmoid colon, and rectum. The pathologist should record evidence of colitis, activity, and the presence or absence of dysplasia. As noted above, these surveillance biopsies often lack the typical distribution of ulcerative colitis but this should not prompt a diagnosis of Crohn's disease but rather of treated ulcerative colitis [325–327].

Crohn's disease

The biopsy features of Crohn's disease are addressed above as the small intestine is often the epicenter of disease, but Crohn's colitis is well known.

Mucosal biopsies, although superficial, can show features suggestive of Crohn's disease. Discrete foci of inflammation often associated with neutrophils within crypts (cryptitis) adjacent to histologically normal crypts are common. Aphthous erosions or ulcers, characterized by focal surface epithelial necrosis associated with a mixed chronic inflammatory infiltrate, sometimes associated with underlying lymphoid aggregates, are typical early lesions. Variability of inflammation within a single biopsy and among several biopsy fragments from the same anatomical location is also typical. The areas of inflammation show architectural changes of chronic crypt-destructive colitis while adjacent crypts may appear normal. Fissures may be apparent. Granulomas are infrequent but, when present, are usually poorly formed and associated with chronic inflammation.

If submucosa is present in the biopsy, there may be significant submucosal chronic inflammation while the overlying mucosa may be only slightly expanded by chronic inflammatory cells, a feature absent in infectious or ulcerative colitis. Pyloric metaplasia (mucinous glands, reminiscent of the antral or pyloric mucosa) indicates repeated bouts of inflammation and repair.

Crohn's colitis must be distinguished from infectious colitis, medication-associated colitis, and ulcerative colitis. The endoscopic finding of focal aphthous erosions of early Crohn's colitis may be similar to erosions caused by medications, bowel preparation, and infections. Erosions of Crohn's disease are associated with a chronic inflammatory infiltrate. Infectious colitis is characterized by a predominance of neutrophils rather than lymphocytes and plasma cells. Medication (NSAIDs and others)-induced erosions usually have negligible acute inflammation, typically only a few neutrophils in the area of erosion. The more advanced changes of Crohn's colitis, particularly discrete ulcers and masses seen endoscopically, must also be distinguished from those induced by medications and infection. NSAID ulcers may be multiple and deep. They tend to be on the top of mucosal folds and are circumferential rather than longitudinal. Biopsies often show fibrosis with only minimal inflammation. NSAID strictures may form; when these are circumferential, they are termed "diaphragms." Bowel wall thickening and heaped-up masses may be seen in *Yersinia* infection, particularly in the terminal ileum and right colon. The presence of marked inflammation histologically with numerous granulomas and the clinical information of a mass or stricture may lead to a diagnosis of Crohn's in a patient with yersiniosis or histoplasmosis. Features that suggest infection over Crohn's disease include numerous granulomas, granulomas centered in lymphoid follicles, and granulomas with central necrosis. Any specimen with numerous granulomas should suggest infection over Crohn's disease.

Fulminant Crohn's colitis is difficult or impossible to distinguish from ulcerative colitis in a mucosal biopsy specimen since many of the distinguishing features are deep to the mucosa. Furthermore, in fulminant (toxic) ulcerative colitis inflammation may become transmural. In resection specimens, the most important features are the presence of transmural lymphoid aggregates and the presence of occasional poorly formed granulomas at all levels of the bowel wall. The effects of treatment can also cause diagnostic difficulty as treated ulcerative colitis may show areas of involved and uninvolved mucosa and variable degrees of disease activity. It is important to ensure that there is re-review of original pretreatment material, if possible, to help distinguish between ulcerative and Crohn's colitis since patients with ulcerative colitis are candidates for ileal pouch anal anastomosis and those with Crohn's disease are not, because of the high risk of pouch failure due to anastomotic breakdown.

Pouchitis

Although technically pouchitis should be included under nonneoplastic small bowel conditions, it is included here because the continent pouch plays the role of a rectum. When Kock explained the creation of an ileal continent

pouch, he also described an inflammatory lesion that followed and this has been referred to as *pouchitis* [328–331]. Pouchitis is different from diversion colitis, which is found in blind pouches (Hartmann pouches) in contrast to continent ones such as Kock pouches or ileal pouch anal anastomosis. In a nutshell, the problem in pouchitis is that there really are no absolute criteria to determine whether the inflammation in a pouch is a reflection of the response of the ileal mucosa to serving as a rectum or a manifestation of recurrent inflammatory disease. When the pouch has been created after colectomy for a neoplasm rather than inflammatory disease, it is easier to dismiss the possibility of recurrent inflammatory disease but this is the minority of cases. In one study, pathological features that were associated with the subsequent development of pouchitis included the presence of severe colitis that extended into the cecum (severe pancolitis), early fissuring ulcers, active inflammation of the appendix, and appendiceal ulceration [272]. No significant differences in patient gender or age, depth or extent of ulceration, or the presence or absence of backwash ileitis were identified in patients with pouchitis compared with those without it. However, other observers have found an association between backwash ileitis and subsequent pouchitis [271].

The classic histological description includes neutrophilic inflammation in acute pouchitis and classic features of chronicity with villous atrophy in the chronic setting, all features that are identical to those of ulcerative colitis or Crohn's disease [332,333]. In fact, pyloric metaplasia is even seen in some examples and appears identical to that in the ileum in Crohn's disease [334–337]. However, these criteria certainly do not exclude infection or that the presumed ulcerative colitis patient actually has Crohn's disease. In some studies, pouchitis is classified as type A, B, or C:

- type A is defined as normal mucosa or mild villous atrophy with no or mild inflammation
- type B mucosa shows transient atrophy with temporary moderate inflammation followed by normalization of architecture
- type C mucosa is defined as a pattern of persistent atrophy with severe inflammation [338,339].

Some patients (type C) experience striking villous atrophy, and this may be the precursor to the small number of patients who develop dysplasia in their pouches [338–341]. In daily practice, we do not list response types as above but do make some attempt to report the degree of inflammation and the presence of extensive atrophy and allow our clinical colleagues to form the correlation with the endoscopic findings. A pouchitis activity disease index has been developed that combines clinical, endoscopic, and microscopic findings [342].

A more distressing concern in interpreting biopsies from patients believed to have ulcerative colitis at the time of resection involves the occasional patient who develops pouch complications that are indistinguishable from Crohn's disease. The conclusion of surgical and endoscopic colleagues is generally that the pathologist has misdiagnosed the resection specimen with ulcerative colitis when the patient in fact has Crohn's disease. In some instances this may be the case. Since patients with Crohn's disease in whom a continent pouch is attempted have terrible complications about half the time, most surgeons avoid creating continent pouches in these individuals (severe pouch complications are documented in about 20% of patients with indeterminate colitis and in about 8%–10% of those with ulcerative colitis). However, there is a small set of patients with classic ulcerative colitis in the view of all participating in their care who develop an idiosyncratic Crohn's-like disease in their continent pouch and it requires revision. This phenomenon was well described by Goldstein and occasionally it pays to be aware of this possibility [343].

Infectious colitis

The term acute self-limited colitis is used here to invoke features of colitis secondary to various bacteriological etiologies, with the exception of enterohemorrhagic *Escherichia coli* (mentioned below since it produces an ischemic colitis appearance). Such cases display neutrophils and crypt abscesses that tend to appear in the upper half of the mucosa and prominent neutrophils in the lamina propria [344]. The basal plasmacytosis seen in ulcerative colitis and Crohn's colitis is lacking [345]. Glandular architecture is preserved. Erosions and lamina propria edema may be seen. The course is self-limited and correlates with pertinent stool cultures. As acute self-limiting colitis heals (often when biopsies are taken), there are reactive epithelial changes but crypt architecture remains intact.

As for specific infectious agents affecting the colon, bacterial colitis is often a result of *Campylobacter* sp. or *Aeromonas* sp. but culture is required for precise typing. Of course, CMV infection may be encountered in many settings in which patients are immunosuppressed as can various parasites. The ova of schistosomes can be encountered (Figs 137.74 and 137.75) and *Strongyloides* is occasionally found on colon biopsies.

Colonic spirochetosis is a peculiar condition in which numerous unusual Warthin–Starry reactive organisms carpet the colon surface (Figs 137.76–137.78). It has been associated with abdominal pain, appendicitis, chronic diarrhea, and rectal bleeding in some cases, but in the majority of cases spirochetosis is an incidental finding with no clear clinical correlates. The anaerobic intestinal spirochetes *Brachyspira aalborgi* and *Brachyspira pilosicoli* seem to be responsible for most cases of spirochetosis. *Brachyspira pilosicoli* colonizes the intestinal tract of many animal species, especially pigs, and can be found in approximately 30% of feces from persons in developing countries. Koteish and colleagues have reported a retrospective analysis of colonic spirochetosis in 14 cases including four children and 10 adults seen at our hospital [346]. Two men had HIV infections. All children and both HIV-infected men had abdominal complaints, diarrhea, or

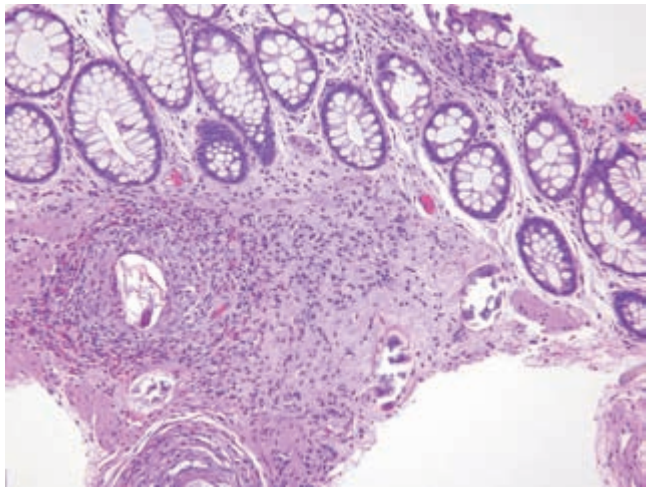

Figure 137.74 Schistosomiasis. Ova surrounded by eosinophils are seen in the submucosa.

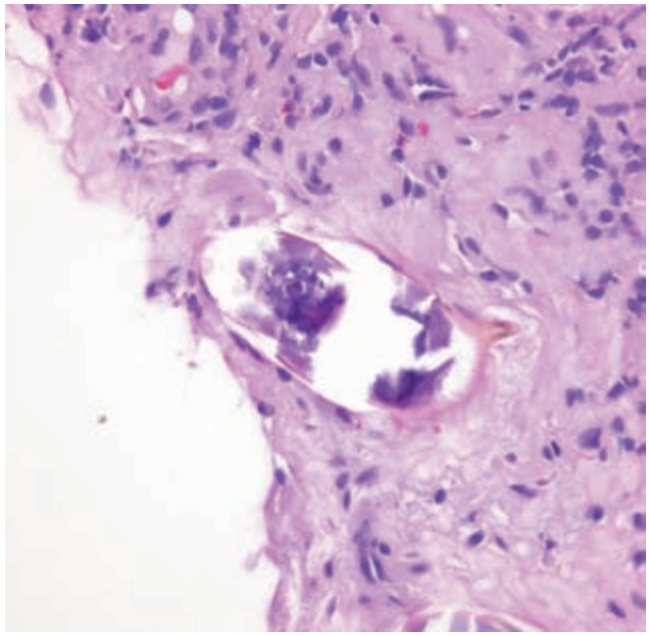

Figure 137.75 Schistosomiasis. The lateral spine of this ovum is in keeping with *Schistosoma mansoni*.

both. Most other adults underwent colonoscopy for polyp screening (four) or follow-up of Crohn's disease (one). Histologically, spirochetosis was identified in all parts of the colon and was not strongly associated with active inflammation, mucosal injury, or changes of chronicity. Genotype analysis of 13 cases showed that 11 resulted from *B. aalborgi* and two from *B. pilosicoli* infections. Only two patients were treated specifically with antibiotics, with complete resolution of abdominal symptoms in one patient with follow-up. Follow-up biopsy results were available for two patients who did not receive treatment; one showed persistent spirochetosis and the other was negative. Spirochetosis in this series

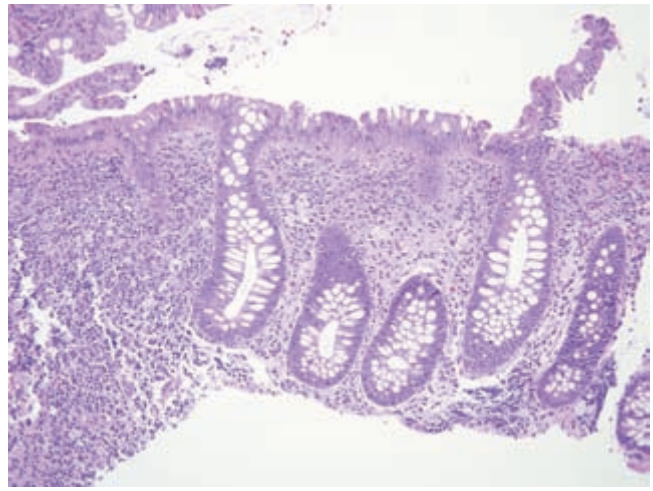

Figure 137.76 Spirochetosis. This biopsy was from an immunosupressed person with diarrhea. The biopsy shows features of acute self-limiting colitis in that there is cryptitis without crypt distortion.

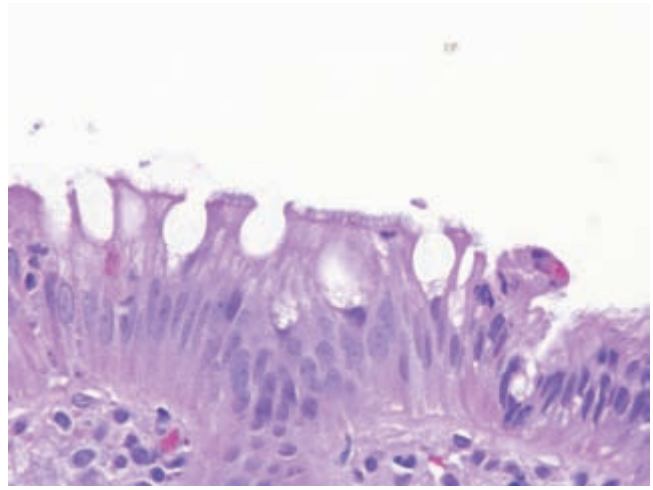

Figure 137.77 Spirochetosis. The hair-like structures emanating from the surface are the organisms.

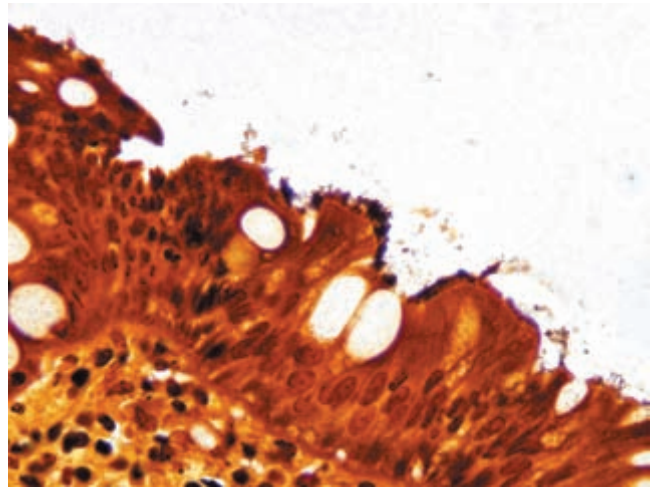

Figure 137.78 Spirochetosis. Warthin–Starry silver stain highlights the organisms.

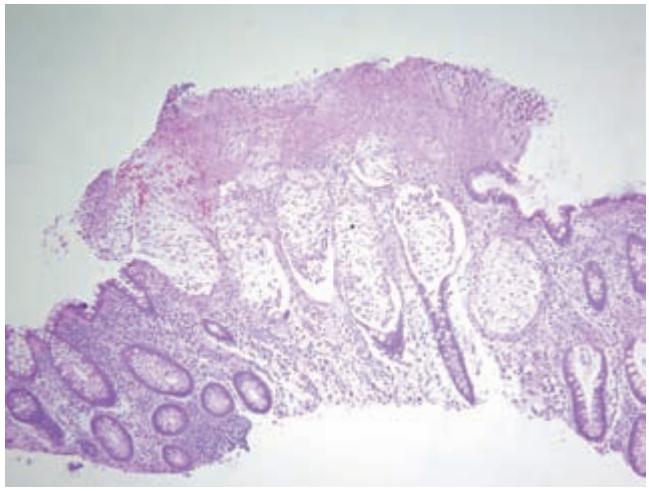

Figure 137.79 Pseudomembranous colitis. A pseudomembrane is seen at the center of the field.

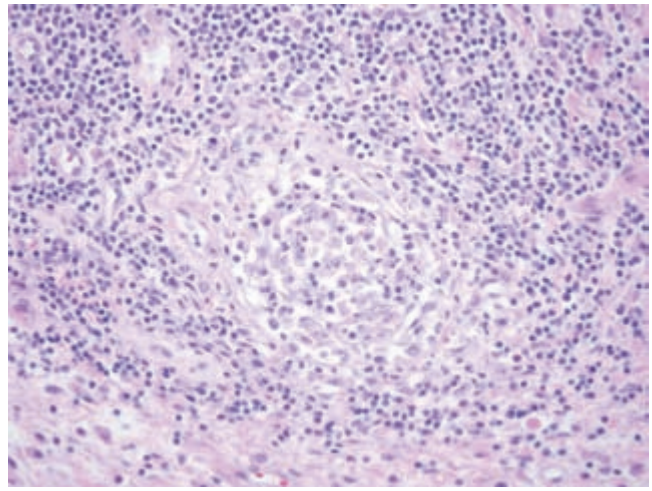

Figure 137.80 Histoplasmosis. This poorly formed necrotizing granuloma was found in an immunosuppressed person and contains the organisms.

had a male predominance, was generally caused by *B. aalborgi*, and occurred in two distinct clinical settings: children (who often have abdominal symptoms) and adults (who typically are asymptomatic). While treatment information remains limited, treatment seemed to ameliorate symptoms in some cases.

It is well known that a large number of infectious processes can mimic idiopathic inflammatory disease. Generally, the features of *Campylobacter*, *Salmonella*, and *Shigella* are distinguished from IBD by the proclivity of responding neutrophils to involve the lamina propria more than the glands and the lack of architectural distortion and basal plasmacytosis, but overlapping features do occur. Epithelioid granulomatous inflammation may accompany *Yersinia pseudotuberculosis* and aggregates of macrophages may accompany *Yersinia enterocolitica*. To the extent that the morphological features of pseudomembranous colitis (Fig. 137.79) and enterohemorrhagic *E. coli* colitis mimic IBD, these must also be considered (although these mimic ischemic effects more than they mimic IBD). The proclivity for GI histoplasmosis to mimic IBD has been emphasized by Lamps and colleagues [347] (Figs 137.80 and 137.81). The presentation of IBD and GI histoplasmosis can be identical (fever, malaise, GI bleeding, diarrhea, nausea and vomiting, ulcers, fissures, perforation, colitis) and the histological presence of granulomas and full-thickness inflammation can lead to an interpretation of Crohn's colitis. The disease may be seen in nonendemic areas and in immunocompetent hosts. Ulcerated areas should always be screened for the presence of *Entamoeba histolytica* and CMV. The former tends to appear in the exudate itself whereas the latter is found frequently in endothelial cells. Adenovirus may involve the bowel in immunocompetent individuals where it invokes an impressive lymphoid response, particularly in the terminal ileum, accompanied by loss of nuclear polarity and subtle intranuclear inclusions

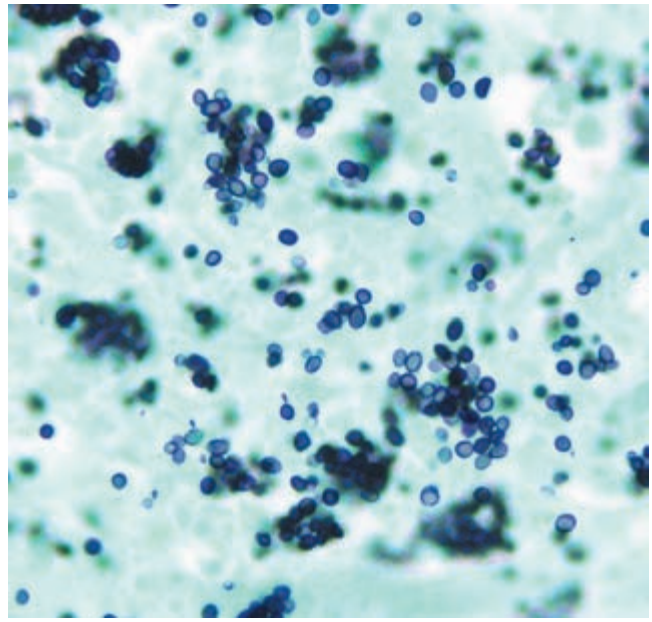

Figure 137.81 Histoplasmosis. Gomori–methenamine silver stain from the image seen in Fig. 137.80 shows budding yeast forms.

[225]. The necrotizing granulomas associated with *Yersinia* spp. are lacking in adenovirus infection. *Mycobacterium avium* complex and *Cryptosporidium* (Fig. 137.82) can be found in immunosuppressed hosts. We have also sometimes noted prominent lymphoid aggregates in biopsies from patients with a course clinically in keeping with viral gastroenteritis.

Of course malakoplakia, while usually in the genitourinary tract, can also be found in the GI tract and reflects a curious response to degenerating bacteria which assume a targetoid appearance when ingested by macrophages (Michaelis–Gutmann bodies).

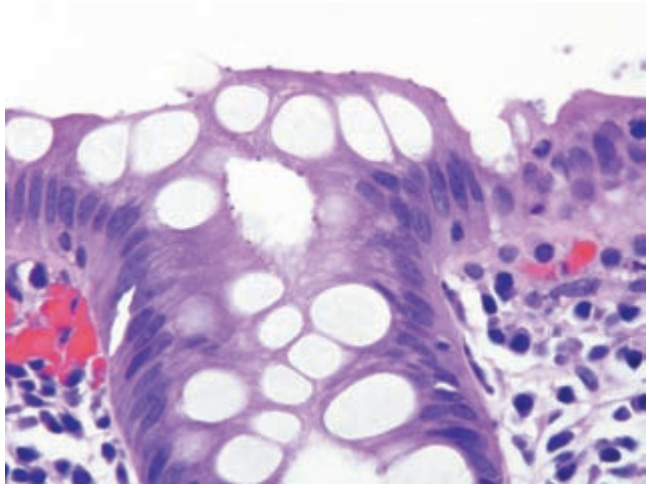

Figure 137.82 Cryptosporidiosis. Organisms appear as small beads at the epithelial surface.

Ischemic colitis

The biopsy features classically associated with ischemic colitis include marked surface injury, mucin loss in crypts, atrophic microcrypts, and hyalinization of the lamina propria such that the normal loose connective tissue punctuated with plasma cells, lymphocytes, and eosinophils is replaced with dense eosinophilic matrix and the residual glands become more closely spaced (lamina propria collapse) [348] (Fig. 137.83). In older patients, ischemic disease is typically attributable to atherosclerotic mesenteric vascular disease. In the setting of global hypoperfusion or splanchnic constriction the watershed zones are prone to vascular insufficiency. The classic watershed zone is at the splenic flexure, an area supplied by the most distal reaches of both the superior mesenteric artery and the inferior mesenteric artery but not by more proximal branches of either source. As such, any vascular compromise from hypovolemia or splanchnic shunting during exercise or global hypotension (such as after trauma) can affect this zone (point of Griffith) more substantially than others in the colon. A similar watershed zone is present in the ileocecal region (point of Sudeck) [349].

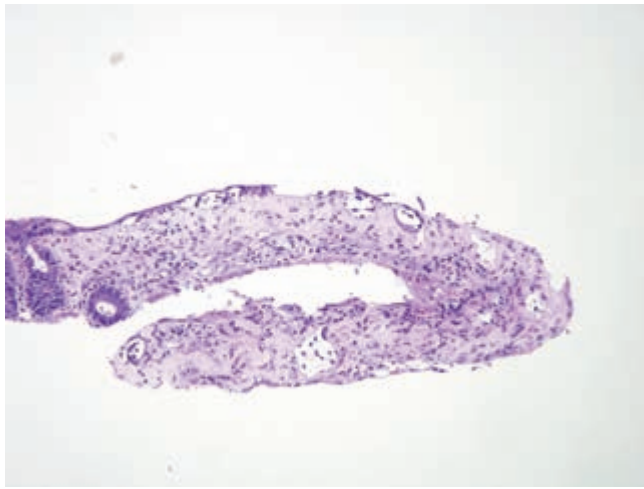

Figure 137.83 Ischemic colitis. This example is from an individual who was receiving Kayexalate but the features are similar regardless of etiology. This biopsy shows atrophic microcrypts and lamina propria hyalinization.

There are many etiologies for ischemic colitis. For example, distance running is known to be associated with varying degrees of colonic ischemia. It is known that about one-fourth of both male and female runners who previously lacked it have occult blood in their stool following participation in a marathon [350] and more extensively documented colonic ischemia has also been reported in such athletes [351–353]. In contrast to the effect of splanchnic constriction in athletic settings, however, ischemic colitis due to hypovolemia in young trauma patients is unusual [354].

Certain infectious agents are also known to produce an ischemic picture, classically enterohemorrhagic (verotoxin-producing) *E. coli* [355–371] and *Clostridium difficile*. The prototype infectious agent for producing ischemic colitis is *E. coli* 0157:H7, which first came to attention in the early 1980s [360,372]. It is a primary cause of severe hemorrhagic diarrhea and one of its complications, hemolytic–uremic syndrome, which develops in up to 20% of cases, is a serious consequence. Ground beef and other bovine products have been implicated as sources. The largest outbreak of this organism occurred in upstate New York in association with an agricultural fair [373]. Since *E. coli* is ubiquitous in stool, it must be specifically sought by stool cultures using sorbitol MacConkey medium from which sorbitol nonfermenting colonies are selected and assayed for the specific organism using either an enzyme immunoassay or a latex agglutination assay [374]. Unfortunately, about 3%–18% of toxin-producing *E. coli* are able to ferment sorbitol (and thus appear as ordinary *E. coli* on culture plates) and may also be responsible for outbreaks but are not detected by routine screening protocols for these organisms [361].

The key pathogenetic mechanism underlying the manifestations of enterohemorrhagic *E. coli* is its direct cytotoxic effect on endothelium [375], which results in GI manifestations if the process is limited but in hemolytic–uremic syndrome if it becomes systemic. Endoscopic biopsies taken from infected patients may display ischemic colitis features with prominent fibrin thrombi, findings that allow the pathologist to suggest pertinent laboratory evaluation [362] but which are by no means specific. An immunohistochemical test has been developed although there is limited experience with it in tissue diagnosis [365].

In younger patients with ischemic colitis, a variety of drug associations can be invoked, including illegal drugs (cocaine [376–381]), over-the-counter decongestant medications [382–385], oral contraceptives [386,387], NSAIDs [388,389], kayexalate (given for hyperkalemia in renal patients and suspended in a sorbitol solution that induces osmotic

ischemia) [390], the migraine headache medication sumatriptan [391,392], and alosetron (Lotronex) [393–401].

Other causes of ischemic intestinal disease in young adults include coagulopathies [402], anorexic behavior [403], and vasculitides (see below). A 2001 report from the Mayo Clinic of 39 young adults with ischemic bowel disease included 13 (of 25) women taking oral contraceptives, four patients with vascular thrombi, four taking vasoactive drugs, four with hypovolemia, and two with vasculitis. No etiology was demonstrated in the remaining 19 patients [404].

In older patients, the differential diagnosis is often between pseudomembranous and ischemic colitis. The diagnosis of ischemic colitis often requires careful clinicopathological correlation. The process may resolve spontaneously. Involvement is segmental and the splenic flexure area is particularly vulnerable. In early cases there is desquamation and necrosis of the mucosa, particularly the superficial mucosa. Pseudomembranes are often seen microscopically, although the endoscopic appearances of ischemia and pseudomembranous colitis differ. Hemorrhage and hemosiderin-laden macrophages are seen in the lamina propria. Fibrin thrombi may be seen as the process progresses. Chronicity is evidenced by crypt atrophy and hyalinized lamina propria. Dignan and Greenson have published helpful criteria to distinguish ischemic colitis and pseudomembranous colitis [348]. These criteria allow distinction of most but not all cases where this differential diagnosis arises. *Clostridium difficile* is often the etiological agent in pseudomembranous colitis, but not all pseudomembranes are caused by this bacterium and not all infestations with *C. difficile* result in pseudomembranes. Cases are typically associated with antibiotics.

Findings in systemic diseases

Scleroderma

This connective tissue disorder can result in hypomotility and intestinal pseudoobstruction, classically associated with sclerosis of the inner circular layer of the muscularis propria. Since the pathological process is mural, there are no specific biopsy changes. However, the stasis resulting from the obstruction can produce bacterial overgrowth.

Vasculitis

It is treacherous to attempt diagnosis of vasculitis on colonic biopsies since if ischemic ulcers are present, any inflammation in walls of small vessels may be secondary rather than primary. The systemic vasculitides are clinically diverse multisystem diseases and their signifying pathological feature is inflammation of blood vessel walls. Vasculitis may be categorized according to vessel size. Small-vessel vasculitides include systemic lupus erythematosus (SLE), microscopic polyangiitis, Wegener granulomatosis, Churg–Strauss syndrome, Henoch–Schönlein syndrome, Adamantiades–Behçet disease, and rheumatoid vasculitis. Medium-sized vessel vasculitides include SLE, polyarteritis nodosa (PAN), thromboangiitis obliterans, and Kawasaki disease, whereas large-vessel vasculitides include Takayasu arteritis and giant-cell arteritis. However, there may be considerable overlap among the various syndromes. In addition, both Crohn's disease and ulcerative colitis may result in vasculitis. The type of IBD most often associated with vasculitis is Crohn's disease, which usually affects arteries.

Intestinal vasculitis may accompany a variety of systemic vasculitides such as PAN, Churg–Strauss syndrome, Henoch–Schönlein purpura, lupus, and rheumatoid arthritis [404–409]. Among the angiitides, PAN, SLE, and Henoch–Schonlein syndrome are those most commonly accompanied by GI complications. Key features of the vasculitides affecting the GI tract are shown in Table 137.4.

Isolated venulitis of the GI tract has been reported using several different names: lymphocytic phlebitis, necrotizing and giant-cell granulomatous phlebitis, idiopathic myointimal hyperplasia of mesenteric veins, mesenteric inflammatory venoocclusive disease, intramural mesenteric venulitis, idiopathic colonic phlebitis [407,410]. The trouble with this entity, as is frequently the case in GI tract lesions, is it describes a pattern of injury but the etiology remains unknown.

On biopsies, vasculitis involving the GI tract vessels results in ischemic changes that cannot be distinguished from other causes of ischemia, and clinical correlation by working closely with the endscopist is crucial. For example, the angiographic features of PAN are characteristic and the diagnosis can be made prospectively by noting ischemic features on mucosal biopsy. If steroid therapy is promptly initiated, surgery can be avoided.

Amyloidosis

Amyloidosis can affect the colon just as much as the remainder of the GI tract (see Figs 137.65 and 137.66). Occasionally, since it imparts a thickened basement membrane, it may have overlapping features with collagenous colitis. In contrast to the latter, in which collagen is punctuated with nuclei of fibroblasts and entrapped endothelial cells, the thick basement membrane in amyloidosis contains no nuclei.

Common variable immunodeficiency

Common variable immunodeficiency is the second most common immunodeficiency syndrome after selective IgA deficiency. Manifestations in the colon are similar to those in the small bowel. The hallmark presentation is persistent sinonasal infections, but many such patients have GI tract complaints attributable to small intestinal atrophy, chronic giardiasis, pernicious anemia, and colitis. Such patients are at risk for development of small bowel lymphoma and gastric carcinoma. Since common variable immunodeficiency is a chronic disease, injury to the colon in patients with this condition is lifelong. As such, although they do not have plasma cells (Figs 137.84 and 137.85), other features of their disease mimic quiescent ulcerative colitis (they can display marked crypt distortion). Apoptosis is often a prominent feature, mimicking GVHD (Fig. 137.86), and these patients are prone

Table 137.4 Summary of the vasculitides affecting the gastrointestinal tract

Type	Definition	Vessel size typically affected	Microscopic features
Henoch–Schönlein syndrome (also called anaphylactoid purpura, Schönlein–Henoch syndrome)	Most common vasculitis in children characterized by purpuric skin eruption, fever, arthralgia, visceral symptoms. Seasonal incidence (peaks in winter). Renal involvement in up to half. Skin purpura, intestinal, renal, or pulmonary hemorrhage	Small	Necrotizing vasculitis (small vessel), histologically indistinguishable from small-vessel forms of polyarteritis. IgA immune deposits in vessel walls
Polyarteritis nodosa (also called periarteritis nodosa, polyarteritis)	Necrotizing vasculitis (medium- and small-sized arteries). Involvement of the aorta is unknown. Can involve any organ. In descending order: kidneys, muscles, nerves, viscera, heart. In children, heart most common site	Medium (arteries)	Focal segmental necrotizing arteritis with fibrinoid necrosis is hallmark of acute lesions, which have a proclivity for microaneurysm formation. Healing lesions have vessel wall sclerosis. Characteristic feature is coexisting acute and healing lesions with intervening segments of unaffected artery
Lupus vasculitis	Vasculitis affecting patients known to have lupus	Medium	Variable and nonspecific histology
Wegener vasculitis	Necrotizing granulomatous vasculitis that in classical form affects the lungs and kidneys. Most affected (75%) are c-ANCA (anti-neutrophil cytoplasmic antibody) positive	Small	Lesions have geographic necrosis, granuloma formation, and vasculitis. All three components are only found in 20%–30% of extrapulmonary cases, so diagnosis requires clinicopathological correlation
Churg–Strauss vasculitis (also called allergic granulomatosis, allergic granulomatous angiitis)	A subset or overlap syndrome of polyarteritis nodosa. The pathological diagnostic criteria require systemic and pulmonary necrotizing vasculitis with an eosinophilic infiltrate and extravascular granulomas. Clinical diagnostic triad: history of asthma/allergy, peripheral blood eosinophilia, and pulmonary and systemic vasculitis with eosinophilic infiltrate	Medium	Like polyarteritis nodosa with an eosinophilic infiltrate. When changes are limited to an isolated organ system, the condition is known as limited/isolated Churg–Strauss syndrome
Buerger disease (also called thromboangiitis obliterans)	Nonarteriosclerotic segmental inflammatory occlusive disease of medium-sized and small arteries and veins usually affecting limbs with occasional visceral involvement. Strong association with smoking, male sex (9 : 1). Usually in young men (< 40 years). Begins with Raynaud phenomenon	Medium	Diagnostic only in early stages with clinically evident thrombophlebitis. Inflammatory thrombi of arteries and veins with endovascular microabscesses with scattered giant cells. Later stages less diagnostic and organizing thrombi are seen. The end stage is nondiagnostic with fibrotic occluded vessels

to a host of infections. Common variable immunodeficiency is also associated with lymphocytic colitis.

Radiation colitis/proctitis

Radiation colitis may be acute or chronic. The acute phase occurs hours to days following irradiation and at endoscopy shows an edematous dusky pattern with loss of vascular pattern. On biopsy, nuclear pyknosis, karyorrhexis and enlarged nuclei are seen with diminished mitotic activity and mucin. These changes are reversible over 1–2 months. Chronic radiation colitis develops weeks to years after therapy. Hyalinized connective tissue is seen in any of the bowel wall layers accompanied by atypical fibroblasts, telangiectasia, vessel wall hyalinization, phlebosclerosis, and atrophy on the muscularis propria. The changes may be best seen in the submucosa and thus are not detected by mucosal biopsy [411–413]. Of course stromal changes may be striking and occasionally there may be epithelial changes [413]. We have noted an appearance where hyalinized vessels are seen aligned parallel to the surface epithelial basement membrane, a finding that is very common in rectal biopsies from men who have had irradiation for prostate cancer (Fig. 137.87).

Lymphocytic and collagenous colitis

The term *microscopic colitis* has been applied to biopsies of patients with symptomatic colitis in whom there are no endoscopic (gross) anomalies but in whom specific microscopic features are found. It was coined by Read in 1980 to describe patients who had chronic diarrhea, normal colonoscopy, normal barium enema, and mucosal inflammation in their

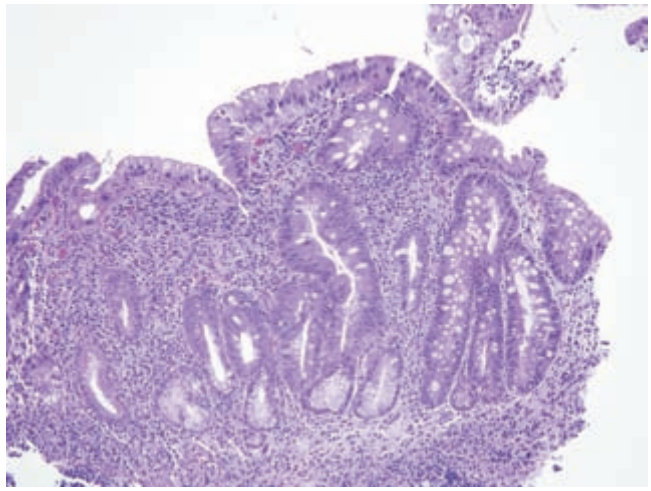

Figure 137.84 Common variable immunodeficiency. The features are similar to those of ulcerative colitis and it is easy for pathologists to make an incorrect diagnosis in such cases.

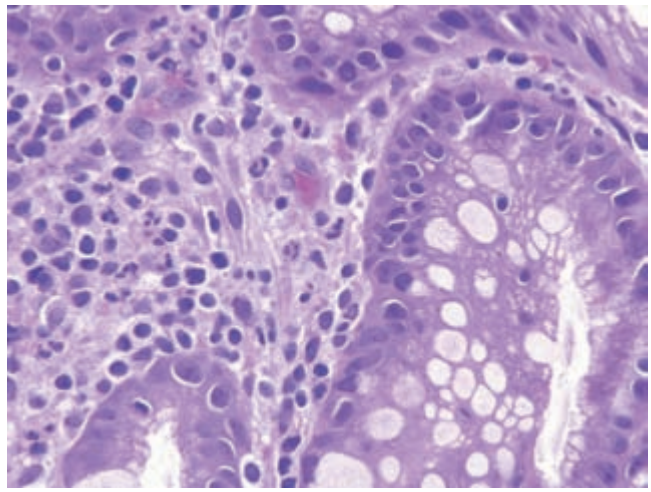

Figure 137.85 Common variable immunodeficiency. The key is the absence of plasma cells in the lamina propria; they would be a prominent constituent in the inflammatory backdrop of ulcerative colitis. The prominent lamina propria neutrophils in this biopsy are in keeping with an acute infection.

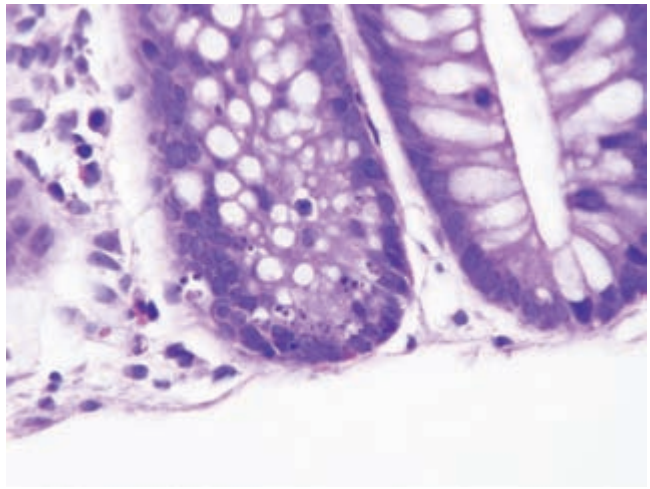

Figure 137.86 Graft-versus-host disease. Note the crypt apoptosis. Crypt apoptosis may also result from phosphasoda bowel preparation so this method is not advised if assessing for graft-versus-host disease.

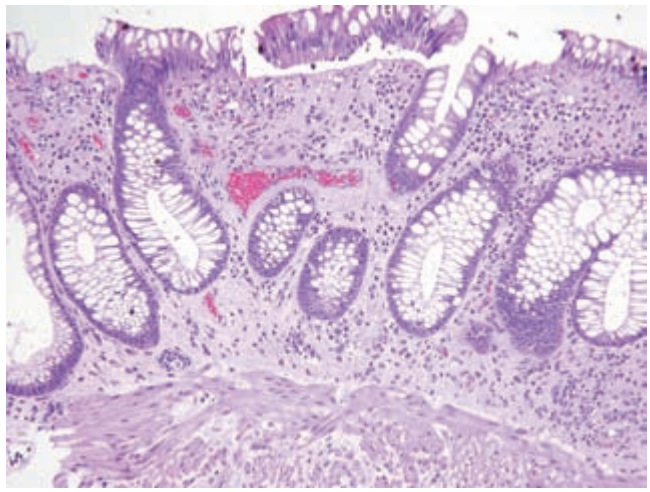

Figure 137.87 Radiation proctitis. Note the prominent vessel parallel to the epithelial surface.

colonic biopsies [414]. There are two types of microscopic colitis that account for most such patients: collagenous and lymphocytic colitis. Both entities feature an inflamed lamina propria and surface injury but seem to be distinct entities that differ in their demographics, disease associations, and clinical course.

Collagenous colitis was described in 1976 [415]. Patients have chronic watery diarrhea that may have an insidious onset or develop abruptly. In addition to diarrhea, many patients report abdominal pain, fatigue, or weight loss. Collagenous colitis is more common in women, with a female to male ratio of 6–8 : 1, and a median age at diagnosis of 55 years. However, the age range is broad, and many cases are diagnosed in patients under the age of 45 years. Associated disease is seen in 40% of patients. The most common of these include rheumatoid arthritis, thyroid disorders, celiac disease, and diabetes mellitus. There may also be an association with the use of NSAIDs [416–418].

Lymphocytic colitis was separated as a specific form of microscopic colitis by Lazenby and colleagues in 1989 [416,419]. Like those with collagenous colitis, patients with lymphocytic colitis have chronic watery diarrhea and normal colonoscopy. There is a broad age range at diagnosis, ranging from young adults to elderly patients, with a mean in the sixth to seventh decades. In sharp contrast to collagenous colitis, men and women are equally affected. Some patients report abdominal pain or weight loss. Lymphocytic colitis has a

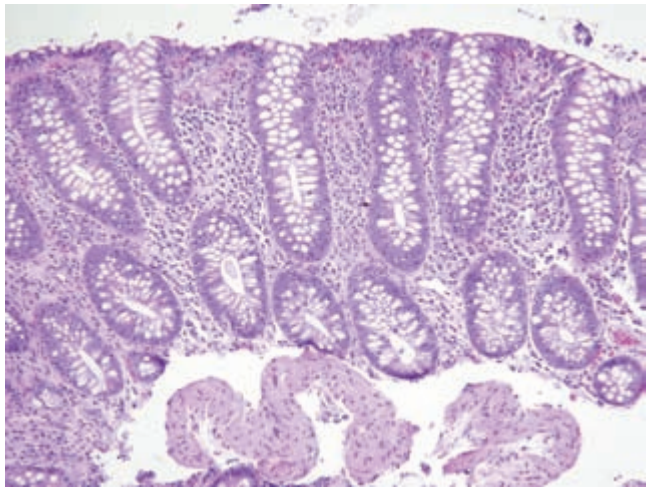

Figure 137.88 Lymphocytic colitis. Note the intraepithelial lymphocytosis.

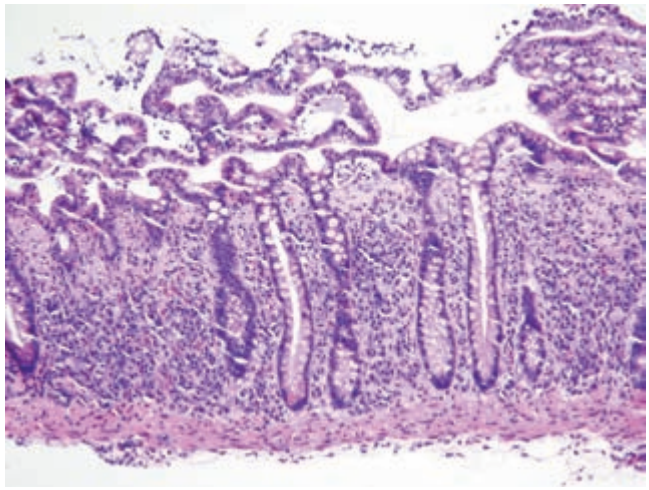

Figure 137.89 Collagenous colitis. Subepithelial collagen is the hallmark.

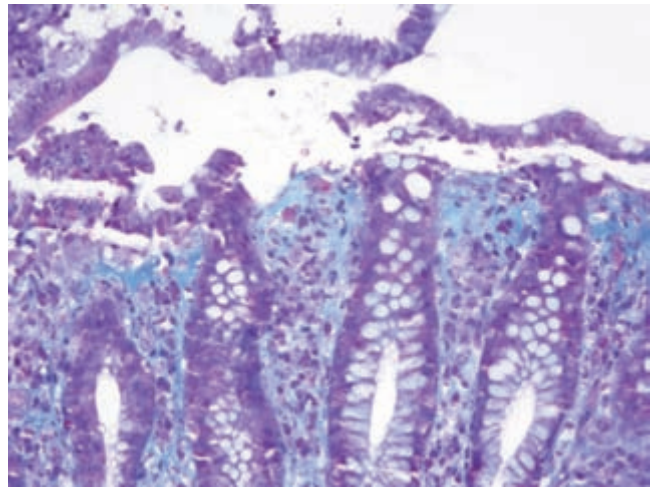

Figure 137.90 Collagneous colitis. This trichrome stain results in a blue color in the subepithelial collagen.

stronger association with celiac disease than collagenous colitis. Of patients with celiac disease, about one-third have histological features of lymphocytic colitis on biopsy. Of patients with lymphocytic colitis, as many as one-fourth may have celiac disease.

These two microscopic colitides share many histological features, including increased lamina propria cellularity, primarily attributable to plasma cells, but also lymphocytes and eosinophils (Figs 137.88–137.90). The crypts are not distorted or minimally distorted. There is surface epithelial injury associated with infiltration by lymphocytes, and degenerative features such as cytoplasmic vacuoles, loss of mucin, nuclear irregularity, pyknosis, and flattening.

The key diagnostic distinction between collagenous colitis and lymphocytic colitis is the deposition of an abnormal layer of collagen between the surface epithelium in the former. This can be variable, and tends to be more pronounced in the proximal colon and less so in the rectosigmoid. The lower border is irregular, with strands of collagen extending into the underlying lamina propria and incorporating dilated capillaries and fibroblast nuclei. Eosinophils are quite often prominent in collagenous colitis, both in the lamina propria and within surface and crypt epithelium [419]. Scattered neutrophils are often found in collagenous colitis but are not a feature of lymphocytic colitis. When neutrophils are prominent, it suggests superimposed infectious colitis. In collagenous colitis, the surface epithelium tends to separate from the basement membrane beneath, leaving stretches in which abnormal collagen is exposed on the surface. In lymphocytic colitis the surface lymphocytosis is usually more prominent, eosinophils are less common, and prominent crypt epithelial lymphocytosis is often a feature.

The abnormal subsurface collagen that is diagnostic of collagenous colitis is not always thick enough to be appreciated on routine H & E stain. There is no minimal thickness required for the diagnosis of collagenous colitis. In normal colonic mucosa, the trichrome stain marks the basement membrane lying at the base of the surface epithelium, forming a smooth thin line. When collagen is deposited beneath this epithelium, the trichrome stain has an irregular lower border, with strands of collagen extending into the adjacent lamina propria and trapping capillaries. It is this abnormal pattern rather than the absolute thickness of the collagen that is the key feature in collagenous colitis. There is no reason to measure the deposited collagen to diagnose collagenous colitis. The abnormal collagen deposition is not always evident on routine stains and we generally order a trichrome stain when we plan to diagnose lymphocytic colitis to detect subtle collagenous colitis. An abnormal collagen band alone is insufficient for the diagnosis of collagenous colitis, but must be present in the appropriate context. Occasionally, thick bands of collagen may be seen in the colonic mucosa as

a result of healed ischemia, radiation injury, ulcerative colitis, or mucosal prolapse [420].

Graft-versus-host disease

The features of GVHD of the colon are similar to those elsewhere, with varying degrees of apoptosis and crypt drop-out. It can be graded for comparison with prior biopsies (grade 1, increased crypt apoptosis (see Fig. 137.86); grade 2, apoptosis with crypt abscess; grade 3, individual crypt necrosis; grade 4, total denudation of areas of mucosa). Like GVHD elsewhere, it can have a chronic phase in which crypt distortion appears but it lacks the active inflammation and prominent basal plasmacytosis that characterize ulcerative and Crohn's colitis. It typically accompanies bone marrow rather than solid organ transplantation. Of course, GVHD is always diagnosed in the proper clinical setting so correlation with the clinical impression is critical. The differential diagnosis is with entities resulting in apoptosis, such as viral infection, autoimmune disease, NSAID use, and medications. For example, mycophenolate mofetil (CellCept) administered to recipients of solid organ transplants can result in colon biopsy findings that are identical to those of GVHD [231], and chemotherapy or other prolonged injury can produce similar features. The endoscopist should remember that phosphasoda bowel preparation itself can result in prominent apoptosis and this preparation should be avoided when GVHD is an issue.

Diversion colitis

Sometimes, when a segment of bowel is resected, the surgeon is forced to create an ileostomy or colostomy as there is simply too much inflammation (or tumor) to reanastomose the remaining bowel without clinical compromise. In this case the remaining bowel (often the rectum) has no passing fecal stream. If the rectum is left as a blind opening continuous with the anus, it is called a Hartmann pouch and the pathologist should consider that there might be diversion colitis. Colitis following diversion of the fecal stream is usually mild. However, in severe cases, the features may perfectly mimic both ulcerative and Crohn's colitis. Regression of the colitis generally follows restoration of the normal continuity of the fecal stream. Diversion colitis is common, occurring as subclinical disease in about 70% of diverted patients. Some authors have reported that lymphoid follicle hyperplasia is a typical finding, although others have reported variable histology [421–426]. If diverted colon is reconstructed into another anatomical structure (such as a neovagina), it is still prone to diversion colitis [427–429]. The hypothesis that the condition results from the absence of lumenal short-chain fatty acids (the preferred metabolic substrate of colonic epithelium) secondary to a change in the bacterial milieu is based on reversal of the condition in many cases on instillation of these compounds into the diverted segment [427,430].

Diverticular disease-associated conditions

In the early 1990s, clinicians became aware of a small subset of patients with diverticular disease (usually involving the sigmoid colon) who developed segmental colitis in the area of diverticular disease [431,432]. The pathological findings were subsequently described in detail and it was noted that cases sometimes perfectly mimicked ulcerative colitis [431] or Crohn's disease [433] and that there is a minority of patients who later manifest ulcerative colitis or Crohn's disease following their episode of diverticular disease-associated colitis. The key principle is that these cases may mimic either Crohn's disease or ulcerative colitis flawlessly and the pathologist is cautioned against diagnosing idiopathic inflammatory disease affecting only the sigmoid colon in patients with diverticular disease. Cases may display features of chronicity, including basal plasmacytosis and crypt distortion, although most show mild active colitis. Those cases of diverticular disease displaying granulomas may even have granulomas in resected lymph nodes [434]. In general, most patients with diverticular disease simply have "floppy" mucosa that is quite prone to prolapse changes [435,436] but one should always exercise caution when colitis is confined to the sigmoid colon. Patients with diverticular disease-associated colitis usually respond to antibiotics.

Polyps

Elastosis/elastofibromatous change

There is a subset of polyps of the GI tract in which the submucosa and muscularis mucosae exhibit a focal or diffuse increase of elastin fibers. This elastosis or elastofibromatous change most commonly manifests as a colonic polyp and is usually found during screening colonoscopy. Gastric and small intestinal cases are less frequent and associated with ulcers or an inflammatory process. Hobbs and colleagues [437] summarized the literature and found reports of 13 GI elastotic lesions with a topographic distribution similar to their own series of 13 cases. Histologically, elastosis appears as finely granular or fibrillar amphophilic material, sometimes with a fibrous component (elastofibromatous change) (Figs 137.91 and 137.92). The changes occasionally appear centered around blood vessels and are often mistaken for amyloid but are negative for Congo red stain and strongly positive for elastin stain. These lesions are probably incidental and of no clinical consequence.

Filiform polyps/postinflammatory polyps

Filiform polyps (also called postinflammatory polyps) are essentially a subtype of polyp associated with prior mucosal injury so they are not unusual in patients with IBD but can be found in patients who have had any type of prior ulceration. They consist of finger-like projections of submucosa covered by mucosa on all sides. They reflect healing of undermined mucosal and submucosal remnants and ulcers and are typically multiple. To the endoscopist, they appear

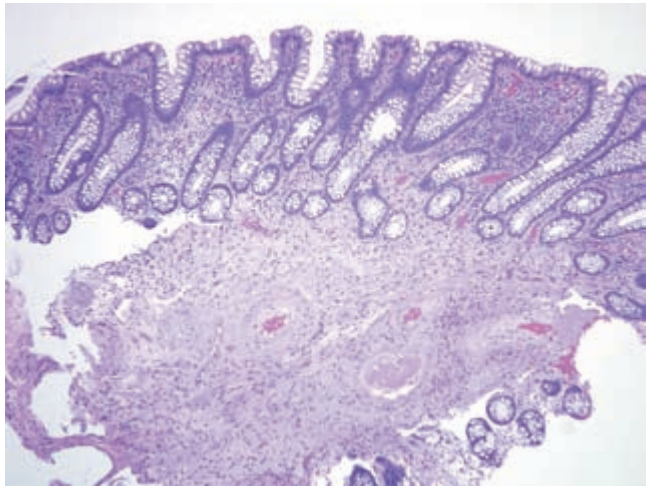

Figure 137.91 Elastosis. This process produced an incidental polyp.

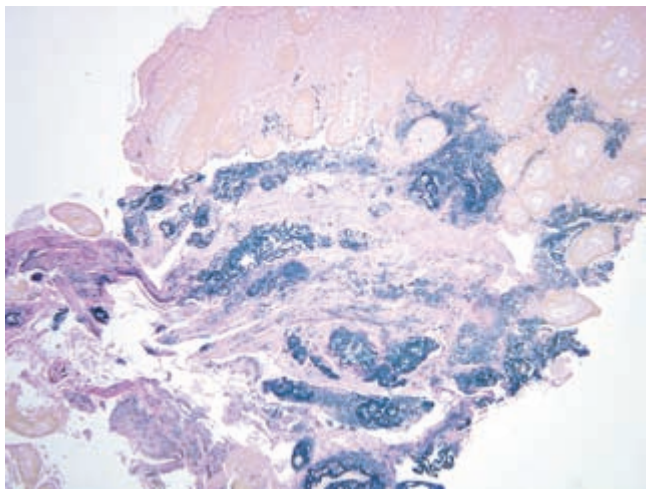

Figure 137.92 Elastosis. Elastic stain of the polyp seen in Fig. 137.91.

long and finger-like [438]. They are diagnosed by noting that they consist of two protruding layers of mucosa plastered together with only one intervening layer or no intervening layer of muscularis mucosae. This is because they reflect regrowth of mucosa over an area of ulcer that has damaged the muscularis mucosae.

Mucosal prolapse conditions

This category of lesion encompasses a host of processes in diverse GI tract sites, but prolapses often yield an endoscopic polyp when they occur in the colon. Well-defined colonic prolapse conditions include solitary rectal ulcer syndrome, localized colitis or proctitis cytstic profunda, inflammatory cloacogenic polyp, prolapsing folds associated with diverticular disease of the colon, and fibrin cap polyps of the colon. So-called myoglandular polyps are presumably part of the same spectrum [439,440]. In some GI sites, prolapse is virtually physiological. For example, the ileocecal valve is prone to prolapse and if there is abundant submucosal fat in the prolapsed area, this is termed lipoma of the ileocecal valve but is probably not neoplastic. These conditions are all benign and are all occasionally mistaken both clinically and microscopically for carcinomas.

Solitary rectal ulcer syndrome

Solitary rectal ulcer syndrome describes a pattern of mucosal changes (some of which are polyps without ulceration) localized to the terminal rectum and imparted by mucosal prolapse. It occurs at all ages, with a peak incidence between 20 and 40 years. The classic history is of a young woman who strains when defecating. There may be hematochezia, pain, tenesmus, and sometimes lower abdominal pain. Inability to evacuate the rectum or sensation of a foreign body is described. At endoscopy ulcers are seen in 20%–70% of patients, usually on the anterior or anterolateral rectal wall but a mass-like lesion can also be found, raising the possibility of a neoplasm. Sometimes defecation studies are used to evaluate these patients as they are believed to have difficulty coordinating the smooth muscle during the defecation process such that the puborectalis sling does not relax at the proper time.

The pathological changes on biopsy consist of hypertrophy of the muscularis propria with splaying of fibers which course into the mucosa and are seen throughout the lamina propria. The proliferated smooth muscle is accompanied by variable fibrosis and glands become entrapped and distorted. As the process continues, there is surface ulceration and glands can herniate into the submucosa, accompanied by wisps of lamina propria (a theme in adenomas as well) [441–445]. Thus lesions can have a polypoid phase or an ulcerated phase. Often crypts become diamond-shaped [446,447]. A caveat is that mucosal prolapse changes adjacent to carcinomas are the same as those of isolated mucosal prolapse, so we suggest multiple biopsies of large solitary rectal ulcers to exclude sampling error [448]. Colitis cystica profunda is part of the same spectrum of disease and implies that glands have prolapsed into the submucosa.

Inflammatory cloacogenic polyp

Inflammatory cloagogenic polyp refers to a mucosal prolapse polyp arising at the anorectal transition and thus has both squamous and columnar mucosa (Figs 137.93 and 137.94) [444,449]. Patients with such polyps present with hematochezia and the polyps are typically found on the anterior wall of the anal canal. These polyps display a tubulovillous growth and surface ulceration. Displaced clusters of glands are found in the submucosa, and abundant fibromuscular stroma extends into the mucosa.

Juvenile polyps

Juvenile polyps are the most commonly encountered colorectal polyps in children, and in one-third to half of instances more than one polyp is found [450–454]. Sporadic juvenile

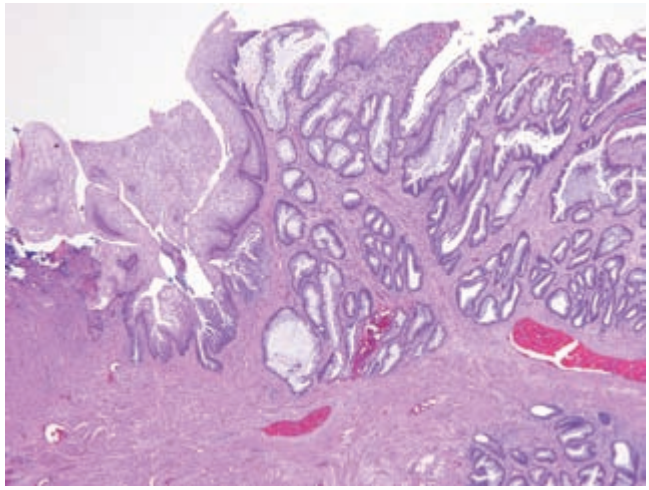

Figure 137.93 Inflammatory cloacogenic polyp. This prolapse lesion is found at the junction of anal squamous (left part of field) and rectal mucosa.

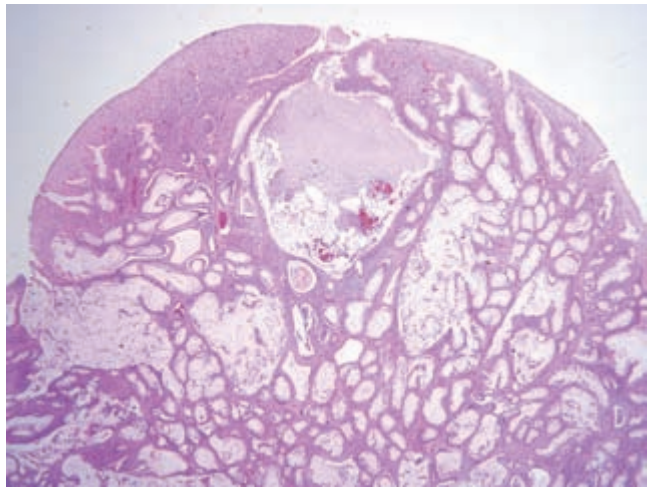

Figure 137.95 Juvenile polyp. This polyp features cystically dilated glands, an expanded lamina propria, and an eroded surface.

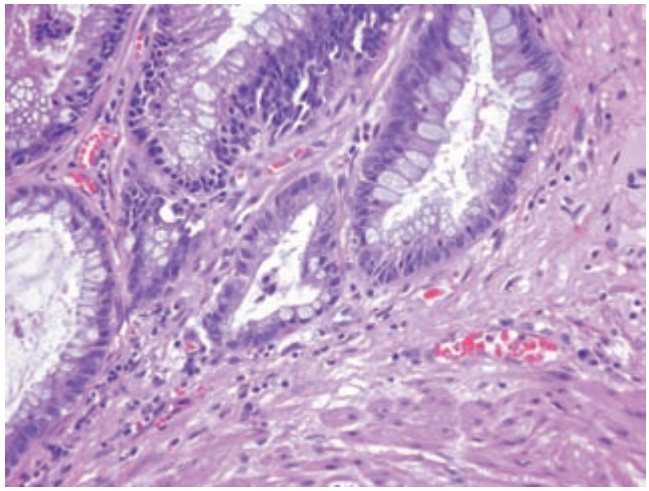

Figure 137.94 Inflammatory cloacogenic polyp. Diamond-shaped glands are a characteristic finding of colorectal prolapse lesions.

polyps usually have a spherical lobulated surface that is often eroded. These polyps are considered hamartomatous so in the colon they display colonic-type mucosa and have irregularly shaped and dilated glands accompanied by lamina propria that is expanded with edematous granulation tissue (Fig. 137.95). Dysplasia is rare in sporadic juvenile polyps.

Juvenile polyposis has been recognized since 1975 [455] and criteria for diagnosis include more than five juvenile polyps of the colorectum, juvenile polyps throughout the GI tract, or any number of juvenile polyps in a patient with a family history of juvenile polyposis. The incidence is about 0.5–1 per 100 000 in the West. The polyps may be found anywhere in the GI tract and the patients are at increased risk for colorectal, gastric, duodenal, and pancreatobiliary carcinomas. In addition, juvenile-type polyps are a component of several genetic syndromes [456–463], including classic juvenile polyposis, Cowden syndrome, and Bannayan–Riley–Ruvalcaba syndrome. Germline mutations in *MADH4* and *BMPR1A* predispose to juvenile polyposis, both genes being involved in TGF-β superfamily signaling pathways. In Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome, juvenile polyps are a less consistent feature. Cowden syndrome patients are at risk for breast and thyroid cancers. Mutations of the tumor suppressor gene *PTEN* have been found in the germline of patients with Cowden syndrome and Bannayan–Riley–Ruvalcaba syndrome. Despite different underlying genetic mechanisms, these and other syndromes share the same phenotypic feature of juvenile polyps. These syndromes are summarized in Table 137.5.

When juvenile polyps are biopsied from syndromic patients, smaller ones are identical to the typical sporadic ones. However, larger ones display an increase in the relative amount of epithelium compared with stroma, are multilobulated with rounded or finger-like lobes, and are more likely to display true dysplasia. Both types of juvenile polyp are prone to surface erosions with attendant reactive epithelial changes. In general, the pathologist is cautioned against diagnosing dysplasia when active inflammation and erosions are a feature.

Peutz–Jehgers polyps

Peutz–Jehgers polyps are most common in the small intestine. Their manifestations in the colon are similar to those in other sites in that they are characterized by site-specific (colonic) mucosa with arborizing smooth muscle. Unfortunately, since mucosal prolapse is so common in the colon, it is difficult to prospectively diagnose Peutz–Jehgers syndrome on the basis of a colonic polyp in isolation. When we encounter a colon polyp with features of a Peutz–Jehgers polyp we issue a descriptive diagnosis and suggest correlation with other stigmata of Peutz–Jehgers syndrome.

Table 137.5 Summary of syndromes associated with multiple juvenile polyps

Syndrome	Components	Genetic defect	Cancer risk
Juvenile polyposis	More than five colorectal juvenile polyps or juvenile polyps throughout the GI tract, or any juvenile polyp in a patient with family history of juvenile polyposis	Autosomal dominant: germline mutations in *SMAD4/DPC4* gastrointestinal	Colorectal, stomach, small intestine, pancreatobiliary
Cowden syndrome	Hamartomas involving organs from all three germ layers. Classic skin tumor (trichilemmoma), thyroid abnormalities, fibrocystic disease, juvenile polyps, uterine leiomyomas, macrocephaly, mental retardation, dysplastic gangliocytoma of the cerebellum	Autosomal dominant: germline mutations in *PTEN/MMAC1* gene	Breast and thyroid cancer
Bannayan–Riley–Ruvalcaba syndrome	Microcephaly, lipomatosis, hemangiomatosis, speckled penis, polyps. Overlap with Cowden syndrome	*PTEN* germline mutations	Presumed at risk

Cronkhite–Canada polyps

Unfortunately, this is a type of polyp that is virtually impossible to diagnose prospectively based on microscopic features. In 1955, Cronkhite and Canada reported a series of patients who had polyposis, pigmentation, alopecia, and onychotrophia [464]. There have been few subsequest reports of this condition (other than case reports), although Burke and Sobin [465] were able to amass polyps from nine patients from the consultation files of the AFIP for histological analysis, and Ward has provided excellent reviews of the literature [466–468].

Cronkhite–Canada syndrome is characterized by diffuse polyposis in patients with unusual ectodermal abnormalities, including alopecia, onychodystrophy, and skin hyperpigmentation. Europeans and Asians are most frequently affected, with a mean age at onset of 59 years. Several hundred cases of Cronkhite–Canada syndrome have been reported worldwide, with 75% of these reports originating from Japan. The male to female ratio is 3 : 2. Potentially fatal complications, such as malnutrition, GI bleeding and infection, often occur and the mortality rate has been reported to be as high as 60%. Neither a familial association nor a genetic defect is known.

The most common presenting symptoms include diarrhea, weight loss, nausea, vomiting, hypogeusia and anorexia. Paresthesias, seizures and tetany, apparently related to electrolyte abnormalities, have also been reported. Nail dystrophy (thinning, splitting and separation from the nail beds), essentially a reflection of protein loss, is one of the features. Both scalp and body hair alopecia may be present. Diffuse hyperpigmentation of the skin, manifested by light- to dark-brown macular lesions, is seen most frequently on the extremities, face, palms, soles and neck. Microscopic examination of biopsied skin reveals abnormally increased melanin deposition with or without increased melanocyte proliferation.

A number of complications may occur with Cronkhite–Canada syndrome and can contribute to poor outcomes in patients with this disease. These include potentially fatal GI bleeding, intussusception, and prolapse. Electrolyte abnormalities, dehydration, protein-losing enteropathy, and other nutritional deficiencies due to malabsorption can complicate the course of the disease. Cronkhite–Canada syndrome patients are prone to recurrent infections, but it is not known whether this is related to malnutrition or is a primary immunological deficiency.

Cronkhite–Canada syndrome is distinguished by the diffuse distribution of polyps throughout the entire GI tract, except for characteristic sparing of the esophagus. The question of whether polyps in Cronkhite–Canada syndrome possess malignant potential remains controversial. The Cronkhite–Canada polyp is characterized by its broad sessile base, expanded edematous lamina propria, and cystic glands [465] (Figs 137.96 and 137.97). Similar features are found in the lesions of

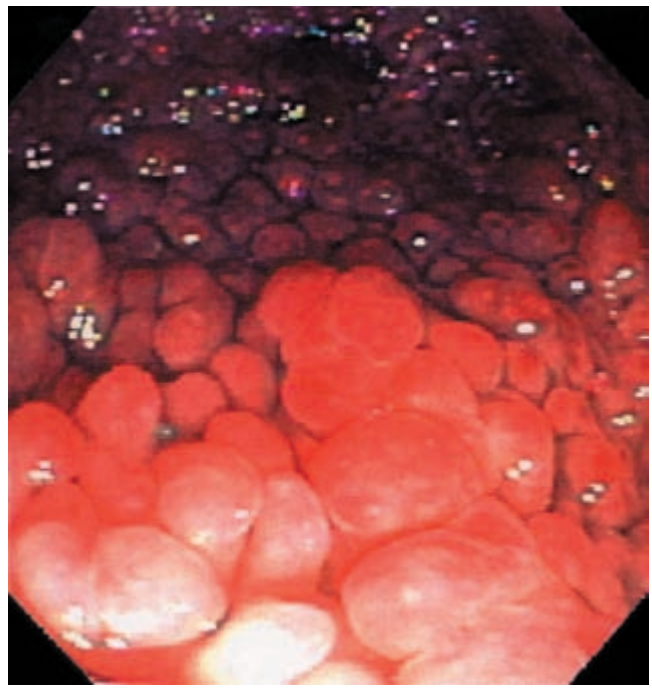

Figure 137.96 Cronkhite–Canada polyposis. This endoscopic image is from the patient's cecum. He had polyps throughout his gastrointestinal tract, sparing only his esophagus.

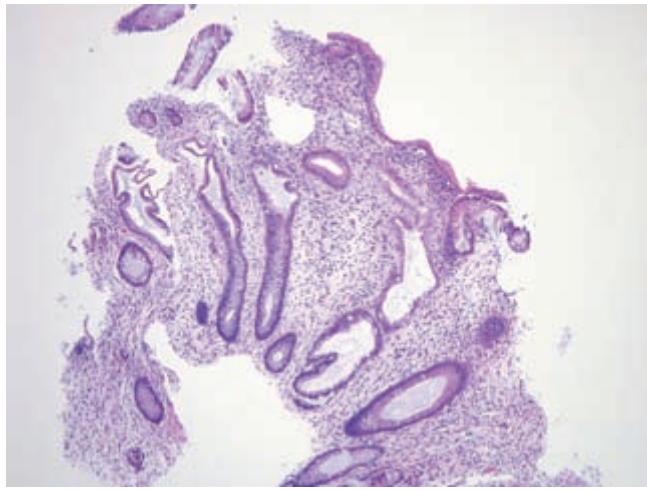

Figure 137.97 Cronkhite–Canada polyposis. This histological appearance of a colonic polyp is hardly specific: the polyp appears similar to a juvenile polyp. The distinction is made on clinicopathological grounds and by attention to the background flat mucosa, which is abnormal in Cronkhite–Canada polyposis but normal in juvenile polyposis.

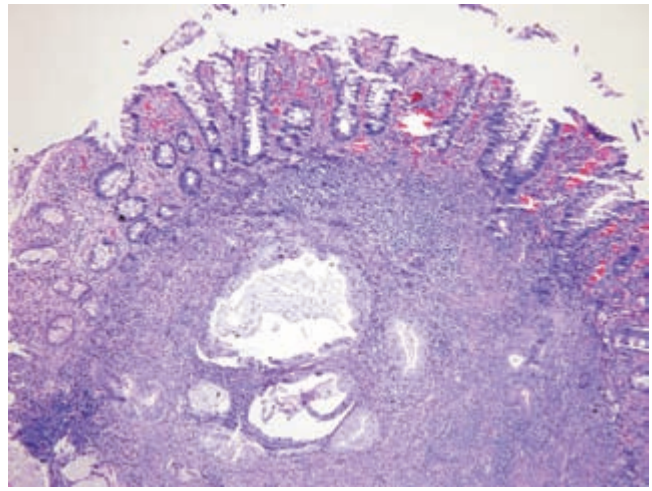

Figure 137.98 Colonic endometriosis. This is a diagnostic pitfall for endoscopists and pathologists alike. The key for the pathologist is to note the background stromal tissue accompanying the glands.

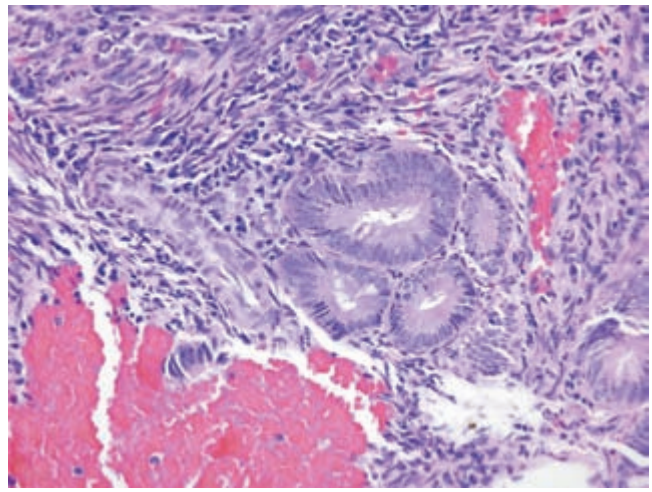

Figure 137.99 Colonic endometriosis. Higher magnification of the lesion seen in Fig. 137.98.

juvenile polyposis. The only distinguishing feature reported between Cronkhite–Canada and colonic juvenile polyposis was the pedunculated growth of the latter [465], a feature that did not hold for gastric lesions. Unlike Cronkhite–Canada polyps, juvenile polyps sometimes have areas of dysplasia, but this is not typical. Therefore the diagnosis of Cronkhite–Canada polyps (especially in the stomach) requires correlation with the presence of the ectodermal changes characteristic of this syndrome.

Other conditions

Endometriosis

Endometriosis is well known to present in a variety of sites and affects the GI tract in up to 40% of patients with pelvic endometriosis and about one-third of these patients have mucosal lesions amenable to biopsy [469]. The sigmoid colon is the most common site.

On gross examination, endometriosis appears as it does in other sites, as firm areas that may contain cysts filled with brown fluid. Most examples of endometriosis affect the serosa or muscularis propria and are accompanied by abundant fibrosis and adhesions, although submucosal examples are also reported. Endometriosis of the colon resembles examples found elsewhere, consisting of endometrial-type glands and stroma associated with hemosiderin deposition and a fibroblastic response (Figs 137.98 and 137.99). The endometrial-type epithelium changes with the menstrual cycle. A stromal decidual reaction may be found in endometriotic foci in pregnant patients.

Vascular lesions

As biopsy of vascular ectasias in not always a wise procedure, these are not often seen in biopsy material. When they bleed, they are managed with various obliterative techniques but not sampled for histology. However, there are a variety of vascular lesions occasionally encountered on colonic biopsies. In HIV patients, of course, Kaposi sarcoma is sometimes encountered. Other lesions occasionally seen include incidental hemangiomas and lymphangiomas, Dieulafoy lesions (usually found in the stomach but rarely in the colon), and vascular malformations. Angiosarcomas tend to be deeper and diagnosed on resections.

Neoplasms

Adenomas

It is estimated that over half the Western population will develop a benign colorectal tumor (adenomatous polyp) during their lifetime, and that about 10% of such tumors will

progress to malignancy [470]. Patients with FAP have germline defects in the adenomatous polyposis coli (*APC*) gene, the gatekeeper of colorectal neoplasia [470], and have hundreds to thousands of colon adenomas and essentially all develop colon cancers if no prophylactic colectomy is performed. Adenomas of the colorectum generally pose few diagnostic problems to the pathologist and endoscopists usually recognize and remove them readily [471]. The situations that can be difficult involve differential diagnosis between reparative conditions with epithelial changes simulating adenomas and adenomas themselves, and dysplasia in the setting of IBD.

Adenomas have dysplasia by definition. It is usually low grade with regular nuclei showing maintained nuclear polarity (their long axes are perpendicular to the basement membrane) (Fig. 137.100). When this is the case, we do not offer a grade of dysplasia but simply diagnose an adenoma and comment on cauterized margin status if the specimen is not tiny or fragmented. Typical adenomas display elongated pencillate nuclei that are similarly hyperchromatic throughout, in contrast to normal mucosa that shows reduced nuclear hyperchromasia at the surface. Adenomas may contain foci of clear cell change, squamous-like morules [472], and Paneth cell differentiation, none of which matter in an adenoma but which may inform some of the variation in the appearances of invasive carcinomas. Of course, small-cell carcinomas may arise in the background of ordinary-appearing adenomas but this is rare.

Adenomas have their initiation point near the surface of the mucosa such that tiny adenomas only affect the upper half of the mucosa, growing in a top-down fashion [275]. Genetically altered cells in the superficial portions of the mucosa spread laterally and downward to form new crypts that first connect to preexisting normal crypts and eventually replace them. This is a useful feature to remember in separating them from both reactive lesions and colitis-associated dysplasia, both of which seem to display bottom-up growth. Adenomas also usually display prominent apoptosis and may contain scattered neutrophils. The presence of prominent apoptosis can be a helpful diagnostic feature.

Adenomas may undergo striking mucosal prolapse changes, which can cause a host of diagnostic problems since neoplastic glands can herniate into the submucosa and, similarly, strands of muscularis mucosae can proliferate into the lamina propria and simulate submucosa. When neoplastic glands from adenomas prolapse into the submucosa, this can occasionally impart an appearance similar to that of invasive carcinoma, especially if the glands become obstructed and inspissated mucus dissects into the surrounding connective tissue, so-called *pseudoinvasion* [473]. The following clues are used to separate this artifact from true invasive carcinoma.

- In pseudoinvasion, lamina propria is dragged along with the neoplastic glands into the submucosa.
- There is often accompanying hemosiderin.
- The proliferation in the submucosa has the same cytoarchitectural features as that which can be recognized as adenoma only in the portion clearly in the mucosa; even high-grade dysplasia may be among these cytological features.

When an adenoma has high-grade dysplasia (Fig. 137.101), we mention this in our report but point out that the lesion still has no metastatic potential to forestall overly aggressive treatment in the event that the reader of the report is not familiar with this basic principle. When carcinomas arise in adenomas of the colon, invasion of the lamina propria is considered biologically equivalent to high-grade dysplasia (since the lamina propria of the colon is believed to lack lymphatic access). Intramucosal carcinoma in the colon is staged as Tis rather than T1 [474] so some observers do not report

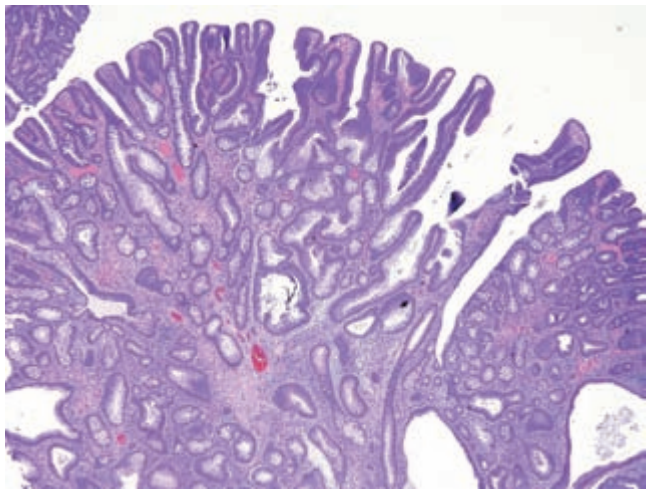

Figure 137.100 Tubular adenoma. By definition, the epithelium is dysplastic (neoplastic) but no invasive carcinoma is seen here.

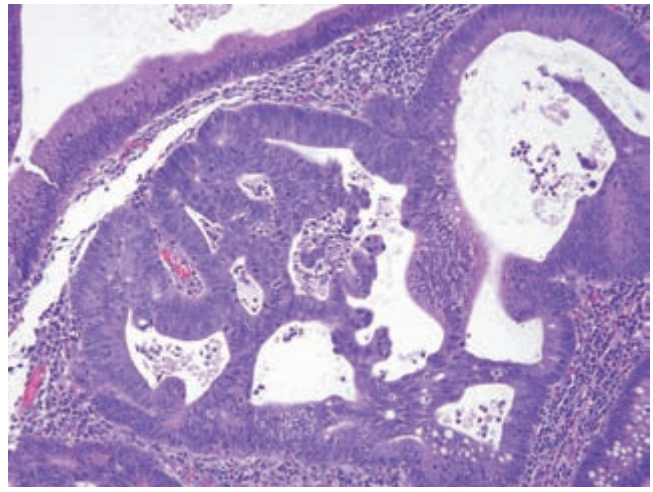

Figure 137.101 High-grade dysplasia in a tubular adenoma. The gland in the center is complex with a cribriform architecture. However, the basement membrane around it is intact.

this invasion. We do choose to report intramucosal carcinoma in adenomas as such but always include a note stating that it is biologically equivalent to high-grade dysplasia and that complete polypectomy should be curative. We provide this information as a precaution in the event that additional sampling might disclose deeper invasion.

Pathological evaluation of early colorectal cancers (pT1) diagnosed and treated by endoscopic polypectomy (malignant polyps)

Carcinomas develop in around 1 in 25 adenomas left in situ. When these are found in endoscopically removed polyps, a management decision must be made and the role of the pathologist is important. To justify colectomy, the risk of the patient having a metastasis must be higher than the risk to the patient of undergoing surgery to remove a segment of colon. Criteria to inform this decision have appeared in the literature [475,476] and have largely stood the test of time. Early studies indicated that there were levels of invasion akin to those in a melanoma [475] but other protocols have proved better.

The diagnosis and treatment of colorectal cancers by endoscopic polypectomy has become commonplace. Malignant polyps are adenomas that contain any amount of invasive carcinoma, which is defined as tumor that has penetrated the muscularis mucosae into the submucosa. They also include polypoid carcinomas, in which the entire polyp head is replaced by carcinoma. By definition, malignant polyps exclude adenomas containing intraepithelial carcinoma or intramucosal carcinoma because these polyps lack biological potential for metastasis. Polyps containing invasive carcinoma comprise about 5% of all adenomas. The chance that any given adenoma contains invasive carcinoma increases with polyp size, and the incidence of invasive carcinoma in adenomas greater than 2 cm in greatest dimension ranges from 35% to 53% [477]. Therefore, any polyp greater than 2 cm in diameter should be approached with suspicion that it might harbor an invasive cancer. When technically possible, these polyps are best removed intact rather than piecemeal, with as great a margin as possible at the base or stalk. Identification of the resection margin is necessary for determining both the adequacy of the excision and the closest approach of the tumor, a parameter that predicts the risk of tumor recurrence.

Malignant polyps often constitute a form of early carcinoma (pathological category pT1) curable by endoscopic polypectomy alone [478–480]. However, the incidence of an unfavorable outcome (lymph node metastasis or local recurrence from residual malignancy) for malignant polyps treated by polypectomy alone varies from about 10% to 20% [476,481–483]. Pathological evaluation is critical in defining polyps with an increased risk of residual or recurrent disease, and the subsequent clinical management of the patient may be based partly on the findings. The histopathological parameters that are known to be associated with a significantly increased risk of adverse outcome are listed below.

- High tumor grade (or histological type that is, by convention, always considered high grade, such as poorly differentiated adenocarcinoma, signet ring cell carcinoma, small-cell carcinoma, or undifferentiated carcinoma). It remains unclear in the literature whether poorly differentiated carcinomas apparently confined to the lamina propria have the biological potential to metastasize. In a limited series, patients with such tumors did well, but we would hesitate to suggest polypectomy alone for a poorly differentiated intramucosal carcinoma in a young patient [484].
- Tumor at or less than 1 mm from the resection margin (some authors advise 2 mm). We assess this by making two small dots (one at the leading edge of the tumor and the other at the nearest cauterized margin) and measuring the distance between them with a ruler. It is important to remember that cauterized tissue contracts, a process that can pull the normal tissue margins together, giving a false impression of positive margins.
- Small (thin-walled) vessel (presumed lymphatic) involvement by tumor (Figs 137.102 and 137.103).

In the presence of one or more of these features, the risk of an adverse outcome following polypectomy is estimated to be about 10%–25%. Therefore, if one or more of these high-risk features are found on pathological examination, further therapy may be indicated. Optimal management is decided on an individual basis, but segmental resection of the involved colonic segment, local excision (e.g., transanal disc excision for a low rectal lesion), or radiation therapy may be considered. In the absence of high-risk features, the chance

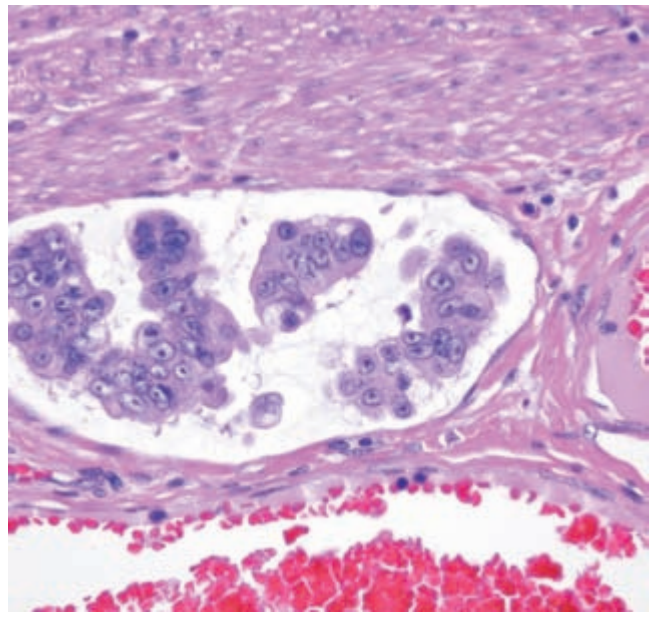

Figure 137.102 Carcinoma arising in association with an adenoma; vascular space invasion. This is a feature that most authors believe should prompt resection following diagnosis of cancer in a polyp.

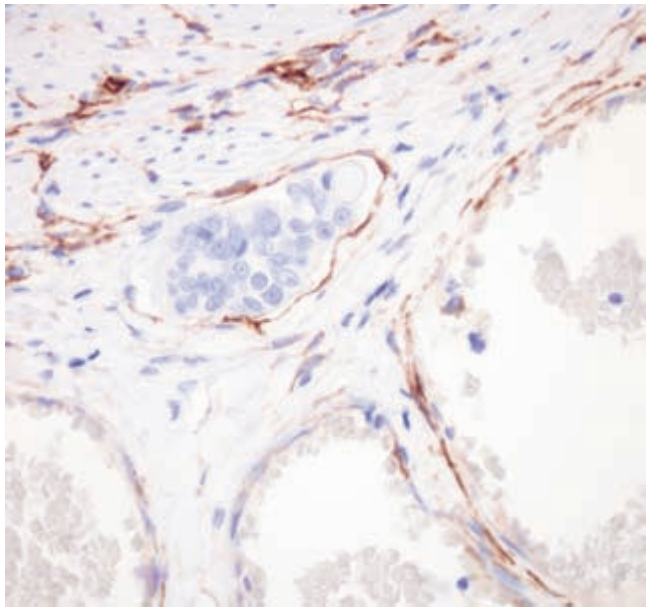

Figure 137.103 Carcinoma arising in association with an adenoma; vascular space invasion. This CD34 stain highlights endothelial cells lining the invaded vessel from the lesion seen in Fig. 137.102.

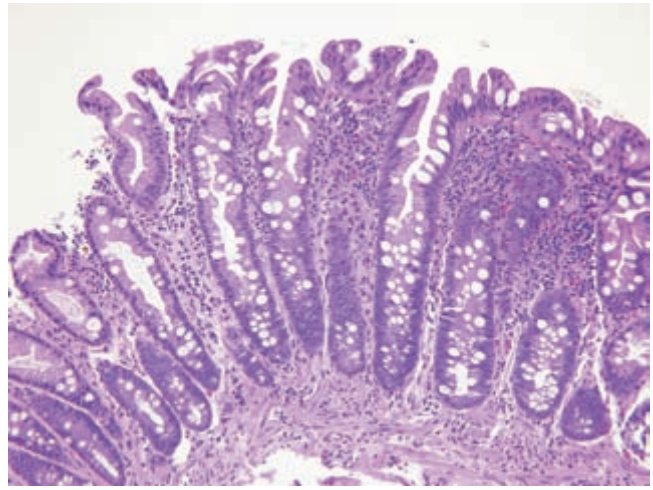

Figure 137.104 Hyperplastic polyp. This serrated polyp shows cells with eosinophilic cytoplasm. Note that the bases of the glands are narrow.

of adverse outcome is extremely small, and polypectomy alone is considered curative.

In the pathological evaluation of malignant polyps, assessment of small-vessel invasion can be difficult with interobserver variability [476]. In fact, small-vessel invasion may be impossible to diagnose definitively in some cases and ultimately may be judged as being indeterminate. An absolute diagnosis of vessel invasion is dependent on finding carcinoma cells within an endothelial-lined space. Contraction artifact in the tissue, tumor-induced stromal sclerosis, and extracellular pools of mucin secreted by tumor cells may all complicate the evaluation of vessel invasion. Examination of additional tissue levels of the specimen, review by a second observer, or immunohistochemical staining for endothelial markers may or may not resolve the dilemma. In published cases in which malignant polyps lacked definitive evidence of high-risk features but where the patients eventually died of their disease, lymphatic invasion had been judged (on blinded review) as indeterminate because of a lack of interobserver agreement [476]. This suggests that even the suspicion of small-vessel invasion on pathological examination should be considered as potentially important.

Small-cell carcinomas of the colon

These tumors are morphologically identical to those in the lung. They are typically found in the right colon where they are often associated with an ordinary adenoma or typical colorectal adenocarcinoma. They are not found associated with carcinoid tumors. The prognosis is poor and patients often have metastases at the time of surgery. The small-cell component usually proliferates off the lower portions of the lesion and is thus not detected on biopsies. Like small carcinomas in other sites, these express neuroendocrine markers (chromogranin and synaptophysin). Additionally, like small-cell cancers elsewhere in the body, about one-fourth stain using CD117/c-kit antibodies [485] but lack *kit* mutations. Carcinoid tumors and conventional colorectal cancers do not display CD117/c-kit labeling.

Serrated polyps (hyperplastic polyps, traditional serrated adenomas, sessile serrated adenomas)

For many years, physicians have believed that there was a simple set of polyps called hyperplastic polyps that could be ignored as they posed no risk. Pathologists recognized them by their serrated microscopic architecture. However, much new information has now been gained about a subset of polyps that superficially resemble hyperplastic polyps, but one with "teeth" among the serrations. The concept of a *serrated pathway* to colorectal neoplasia is very exciting but its effect on our daily practice remains unclear. We are now confronted with a new set of preneoplastic lesions to classify and manage.

Serrated polyps traditionally included hyperplastic polyps, mixed hyperplastic/adenomatous polyps, and serrated adenomas. It is now apparent that there are more groupings that need to be addressed, and an updated classification might include traditional left-sided hyperplastic polyps (Fig. 137.104), sessile serrated adenomas (Fig. 137.105), sessile serrated adenomas with superimposed dysplasia (formerly mixed adenomatous and hyperplastic polyps) (Figs 137.106 and 137.107), and traditional serrated adenomas (Fig. 137.108) [486]. It now appears that sessile serrated adenomas are the precursor to some colorectal cancers that exhibit high-level microsatellite instability (MSI-H) and which have unique genetic features (Table 137.6), including alterations in mis-

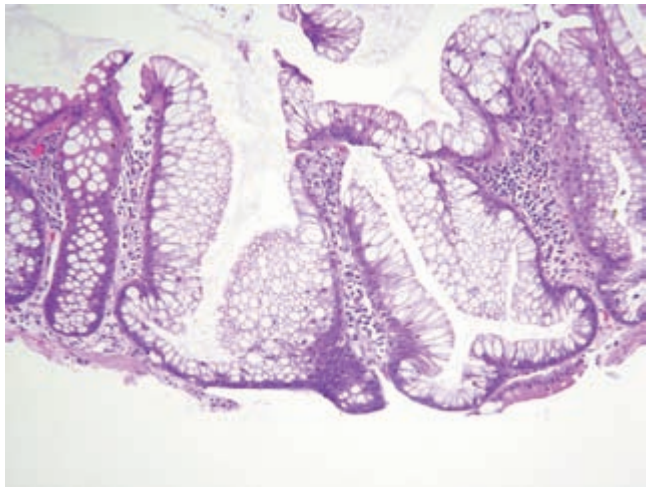

Figure 137.105 Sessile serrated adenoma. This lesion superficially resembles a hyperplastic polyp but differs by having broad-based glands (compare with Fig. 137.104). It lacks conventional dysplasia but is fully capable of progressing to invasive carcinoma.

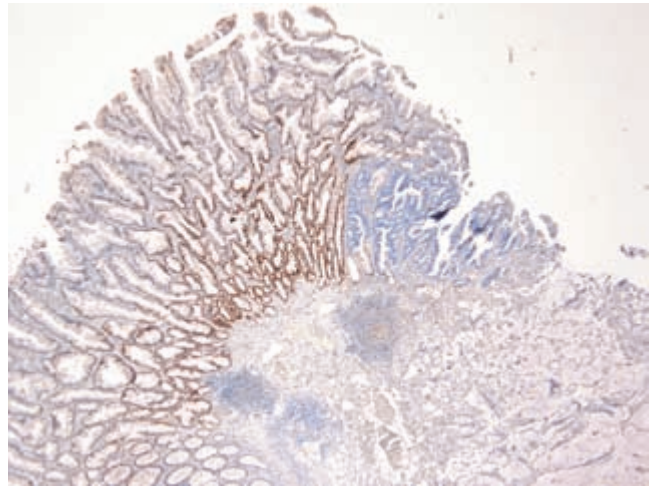

Figure 137.107 Sessile serrated adenoma with associated dysplasia and invasive carcinoma. In this immunohistochemical preparation for the mismatch repair protein MLH1, there is (nuclear) loss in the high-grade dysplasia and carcinoma component.

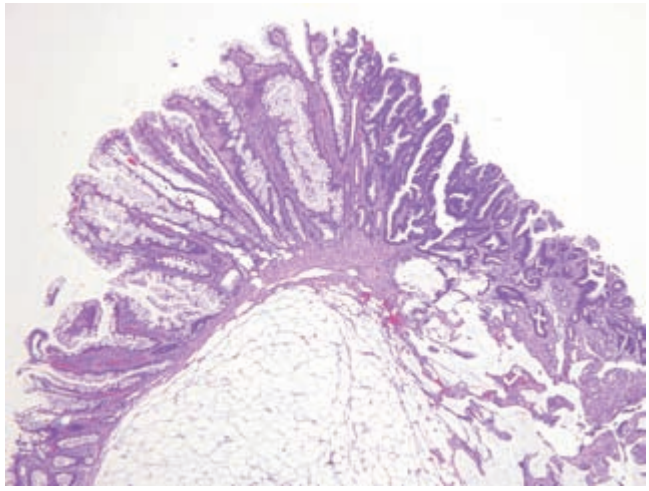

Figure 137.106 Sessile serrated adenoma with associated dysplasia and invasive carcinoma. The high-grade dysplasia and carcinoma component is at the right of the field.

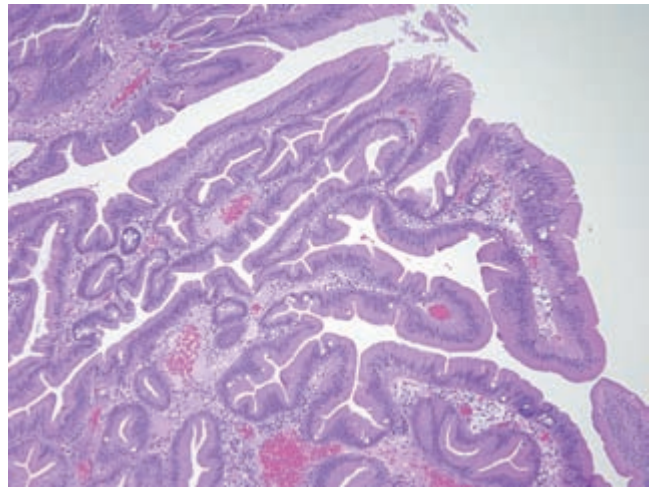

Figure 137.108 Traditional serrated adenoma. There is serrated architecture of the epithelial cells as well as traditional epithelial dysplasia like that of an ordinary adenoma.

match repair genes and *BRAF* alterations, both of which relate to MSI-H.

An important set of observations was published in the American Journal of Surgical Pathology that has set the stage for a radical change in how pathologists address colorectal polyps [487], although this paper had earlier underpinnings. Traditionally, the two global categories of colorectal epithelial polyps have been adenomatous and hyperplastic polyps. It is well recognized that adenomatous polyps are premalignant lesions. The molecular pathway most often seen in sporadic colorectal cancers but also in FAP is thus called the adenomatous polyposis coli (or APC) pathway. Subsequently, another molecular pathway to colorectal cancer, the MSI pathway, has also been described. Like the APC pathway, this can be seen in either sporadic colorectal adenocarcinomas or an inherited condition called hereditary nonpolyposis colon cancer (HNPCC). The key feature of this pathway is dysfunctional mismatch repair enzymes and subsequent accumulation of mutations. Colon cancers in these patients have a tendency to arise on the right side of the colon. In the work published by Torlakovic and colleagues [487], it was noted that there is a subset of polyps that tends to occur on the right side of the colon. These polyps superficially resemble hyperplastic polyps of the left colon but differ by larger size, a higher proliferative index, the presence of serrations extending to their bases, dilated architecture of the glands at

Table 137.6 *BRAF* mutations in polyps and cancers of the colorectum

Type of lesion	Morphology and anatomical location	Patients with *BRAF* mutations (%)[a]
Conventional hyperplastic polyp	Serrated architecture, crypts slender at bases with serrations in the superficial portions, many endocrine cells, left-sided location, small size	19
Sessile serrated polyp (also called sessile serrated adenoma)	Serrated architecture with serrations to the bases of polyps, higher proliferative index, loss of endocrine cells, usually right-sided location, larger size	75
Mixed hyperplastic/adenomatous polyp (sessile serrated polyp with dysplasia)	Similar to sessile serrated polyp but with foci of adenomatous change, namely surface hyperchromatic nuclei aligned in a pencillate fashion	89
Traditional serrated adenoma	Serrated architecture and adenomatous-type epithelium with hyperchromatic pencillate nuclei	20
Conventional adenoma	Tubular or tubulovillous architecture with dysplastic epithelium with pencillate nuclei and top-down neoplastic growth [275]	0
Sporadic MSI-H cancers	Poorly differentiated or mucinous appearance, often with prominent tumor-infiltrating lymphocytes, tendency for right side, reduced CK20 expression [570]	76
HNPCC cancers	Medullary or mucinous pattern, tendency to right side. Reduced CK20 expression [570]	0
Conventional MSS cancers	Moderately differentiated carcinomas with ample glandular lumenal necrosis, $CK20^{+}CK7^{-}$ keratin profile	9

a Mutations in *BRAF* have been linked with colorectal cancers showing high-level microsatellite instability.
HNPCC, hereditary nonpolyposis colorectal cancer; MSI-H, high-level microsatellite instability; MSS, microsatellite stable.

the bases, and a tendency to have mismatch repair defects. The authors had previously grouped such polyps with so-called serrated adenomas but this is somewhat unsatisfactory as the lesions lack the pencillate nuclei that typify traditional serrated adenomas [488]. Thus, although these polyps lack conventional dysplasia, they are regarded as adenomas.

Initially hyperplastic polyps were regarded as benign and metaplastic. However, there is now considerable evidence implicating at least a subset of what have traditionally been called hyperplastic polyps in the development of a subset of colorectal carcinomas [489–492]. Initially, reports and small series of adenocarcinomas associated with giant or large hyperplastic polyps (usually defined as > 1 cm) appeared [493,494]. It also became apparent that the presence of multiple hyperplastic polyps in the form of hyperplastic polyposis (also known as giant hyperplastic polyposis [495] or serrated adenomatous polyposis [496]) is clearly associated with the development of adenocarcinoma [495]. Even in patients lacking criteria for hyperplastic polyposis, some hyperplastic polyps are associated with colorectal cancers. Some patients with MSI colorectal cancers have an increase in serrated polyps (hyperplastic polyps and serrated adenomas) but not adenomas in the background mucosa [497]. A large series of more than 90 MSI colorectal cancers in which hyperplastic polyps had been diagnosed near the site of the colorectal cancer at earlier examination further implicates hyperplastic polyps in a subset of colorectal carcinoma [498].

Molecular data now provide convincing evidence for a pathway from hyperplastic polyps (or hyperplastic-like polyps) to colorectal carcinoma. As such, the serrated pathway to colorectal cancer is a hot topic in the GI pathology literature [486]. Our understanding of this pathway is under evolution. Early steps appear to involve reduced cell death (apoptosis) in serrated polyps leading to prolonged cell life, an increased concentration of epithelial cells resulting in a serrated appearance, and a presumed susceptibility to DNA methylation.

Thus, in summary, lesions that we have traditionally diagnosed as hyperplastic polyps are heterogeneous lesions, a subset of which is now regarded as the precursor of some colorectal cancers with MSI. Some authors have distinguished this subset from classic hyperplastic polyps and have introduced the term *sessile serrated adenoma*. These lesions frequently show *BRAF* mutations and DNA methylation [499–501]. It is estimated that around 25 hyperplastic polyps of the proximal colon exist for every colorectal cancer with MSI [502]. Further research is required to identify additional risk factors for hyperplastic polyps other than anatomical location. These may be demographic, clinical, morphological, or molecular.

The key practical issue is how to manage patients with the various serrated polyps, the main clinical decisions being how to address incompletely excised polyps, whether to perform pan-colonoscopy when index lesions are identified

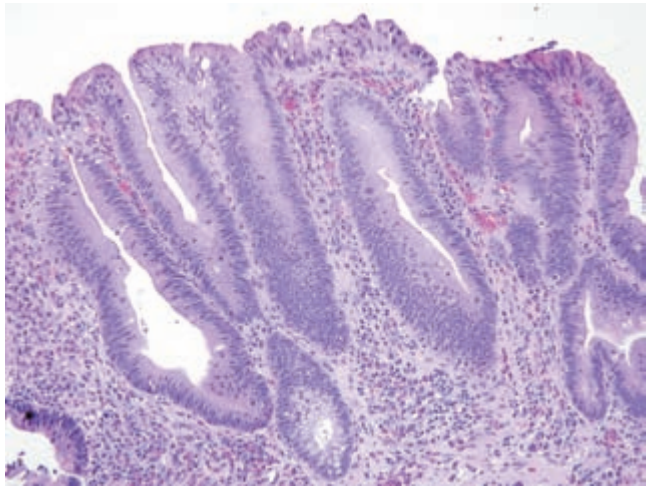

Figure 137.109 Dysplasia-associated lesion or mass. This lesion has low-grade dysplasia and presented as an elevated visible lesion.

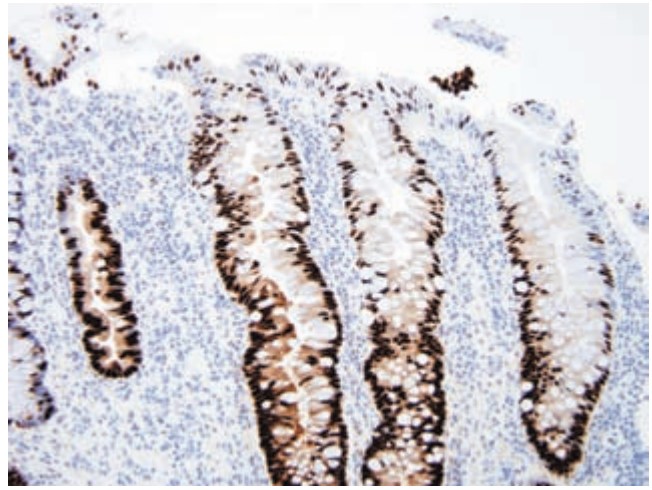

Figure 137.110 Dysplasia-associated lesion or mass (DALM). A p52 stain can be useful in confirming an impression of DALM.

on procotoscopy or sigmoidoscopy, and whether long-term endoscopic surveillance is needed. Currently, straightforward guidelines exist for the management of adenomatous polyps but guidelines for the serrated family of polyps are lacking. Snover and colleagues have suggested the following guidelines [486].

- Right-sided sessile serrated adenomas: complete endoscopic removal. If this is not feasible, additional sampling, probably at yearly intervals, to monitor for dysplasia. If this appears, surgical excision should be considered.
- Left-sided lesions: complete removal. If not feasible, additional sampling to monitor for dysplasia. A higher threshold for suggesting colectomy.
- The interval for surveillance remains unknown. It is probably reasonable to follow as per adenomas.

Colitis-associated dysplasia and colon carcinoma

Criteria for grading dysplasia in the setting of inflammatory disease were developed in 1983 [503] and remain useful. Dysplasia in ulcerative colitis is separated into low and high grades (Figs 137.109–137.111) and cases in which the findings are unclear are diagnosed as indefinite for dysplasia. The criteria for grading dysplasia in colitis are summarized in Table 137.7 [504]. Essentially, these are similar to other sites except that many patients have striking active inflammation, so it is difficult to make definitive diagnoses of low-grade dysplasia. A diagnosis of low-grade dysplasia requires nuclear alterations that extend onto the mucosal surface without loss of nuclear polarity whereas high-grade dysplasia displays surface loss of nuclear polarity. Some literature supports colectomy in the presence of any degree of dysplasia; others recommend resection only in the setting of high-grade dysplasia in flat mucosa. Dysplasia-associated lesion or mass (DALM) is typically irregularly shaped and raised, without a discrete stalk. The dysplasia may be found at any location

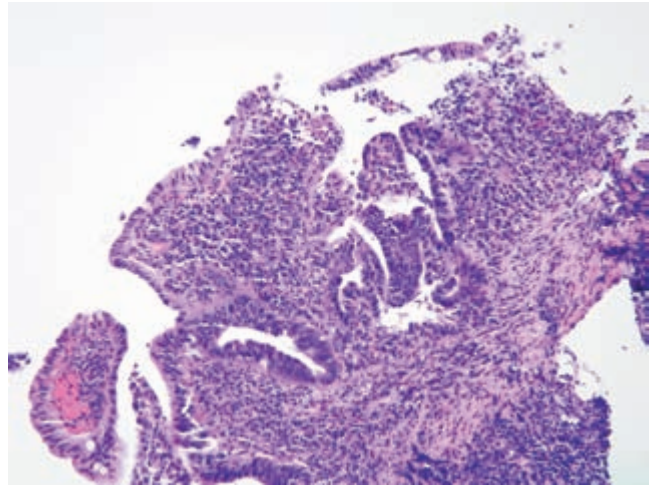

Figure 137.111 Dysplasia-associated lesion or mass. This area shows high-grade dysplasia.

in the crypt, a difference from an adenoma in which the dysplasia begins at the surface and moves down the crypt. Endoscopically, DALMs are often difficult to remove, in some instances because they are contiguous with an underlying invasive adenocarcinoma. Because sessile irregular DALMs are likely to harbor an adenocarcinoma, colectomy is sometimes recommended. While distinguishing between an adenoma and a DALM is important, in some cases this distinction is difficult to impossible. Happily, emerging evidence supports treating the lesion in the same fashion as a sporadic adenoma (i.e., polypectomy) if it is discrete and endoscopically adenoma-like [505]. Criteria that some observers have applied for distinguishing between colitis-associated dysplasia and adenomas are summarized in Table 137.8 but unfortunately there are no wholly reliable criteria for this distinction [506–509]. In daily practice, we report such lesions as

Table 137.7 Grading criteria[a] for ulcerative colitis

Feature	Negative	Indefinite	Low-grade dysplasia	High-grade dysplasia	Cancer
Surface maturation	+	Incomplete	–	–	–
Architecture	Intact	Intact or mild alteration[b]	Intact to mild alteration	Intact to marked alteration[c]	Desmoplasia with any architecture
Cytology	Normal or reactive[d] or reparative[e]	Mild or focal marked atypia[f] with inflammation/ulceration; marked atypia limited to crypts but with surface maturation; mild atypia that mostly matures	Mild to moderate atypia (more atypia than indefinite category), ranging from focal to diffuse but with maintained nuclear polarity[g]	Marked atypia (more than low-grade dysplasia), ranging from focal to diffuse; loss of nuclear polarity present in the majority	Mild or marked atypia
Other		Absent surface epithelium preventing assessment of surface maturation	Nuclei may fill the thickness of the epithelium and still be low grade	Nuclei do not have to extend above the halfway point of the epithelial thickness	

a General criteria applying to all grades: (1) biopsies that stand out as different from others, those with mucin loss or hypermucinous change, dystrophic goblet cells or endocrine cell hyperplasia tend to associate with dysplasia but not always; (2) in the case of discrepancy between cytology and architecture, cytology determines the grade; (3) in the presence of severe inflammation, with or without erosion/ulceration, assignment of unequivocal dysplasia is done only in very rare circumstances and the overwhelming majority of such biopsies should be classified as indefinite.
b Mild architectural alteration: glandular crowding, some degree of loss of parallel crypt arrangement.
c Marked architectural alteration: very crowded glands, back-to-back glands, fused or cribriform growth, dilated glands with lumenal necrosis, or zones of coagulative necrosis confined to mucosa.
d Reactive cytological alteration: basal proliferative glands with atypia that matures or slight nuclear atypia extending toward the surface in the presence of active inflammation; chromatin is often normal or open.
e Regenerative cytological alteration: single cell layer at surface repopulating eroded or ulcerated stroma usually devoid of glands or with gland loss; surface cells may have variably bizarre nuclei but they are limited to a single nonstratified pattern with abundant cytoplasm in addition.
f Cytological atypia: nuclear enlargement or hyperchromasia, or stratification, or irregular nuclear contours.
g Maintenance of nuclear polarity: nuclei whose longitudinal axes remain perpendicular to their basement membrane and the nuclei remain orderly and oriented to each other; loss of nuclear polarity refers to nuclei without a perpendicular orientation to the basement membrane and with a disorderly arrangement relative to each other.

Table 137.8 Distinction between polypoid colitis-associated dysplasia and sporadic adenomas arising in inflammatory disease

Feature	Adenoma	Colitis-associated dysplasia
Patient age (years)	Older (> 50)	Younger (< 50)
Disease activity	Active or inactive	Usually active
Disease duration (years)	Shorter (< 10)	Longer (> 10)
Endoscopic appearance	Well marginated	Irregular
Glands	Regular configuration	Irregular configuration
Surface	Homogeneous	Mixture of nonneoplastic and neoplastic glands
Distribution of mucin	Often near lumenal surface	Irregular
Dystrophic (upside-down) goblet cells	Rare	Frequent
Nuclei	Stratified, same level	Stratified, various levels
Stroma	Sparse	Variable
Demarcation from surrounding mucosa	Sharp	Gradual transition
Appearance of surrounding mucosa	Mildly distorted	Prominent architectural distortion
Prominent lamina propria mononuclear cells	No	Yes
Prominent lamina propria neutrophils	+/–	+
Prominent p53 immunohistochemistry	No	Yes (nuclear staining)
β-Catenin	Yes (nuclear staining)	No

"polypoid low-grade (or high-grade) dysplasia versus sporadic adenoma" and note why we favor one or the other (or why we are unable to favor one or the other). As a general rule, lesions arising in the field of inflammatory disease that have an unusual endoscopic appearance are probably best regarded as DALMs. In other words, the gross appearance probably yields more information than the microscopic one. In some cases, we perform p53 and β-catenin immunohistochemistry and find it useful but it does not always prove helpful, in which case we report it as noncontributory.

Adenocarcinomas detected during surveillance have a significantly better prognosis than those found in individuals with clinical symptoms. Total colectomy is then indicated. Resection with subsequent ileoanal anastomosis allows the patient to have relatively normal bowel function by maintaining the anal sphincter following colectomy.

There is an increased risk of adenocarcinoma in patients with Crohn's disease, estimated to be four to 20 times more than those without Crohn's disease. Adenocarcinomas may arise in morphologically normal bowel, in areas of stricture, and within fistula tracts, making diagnosis difficult. Multiple synchronous or metachronous adenocarcinomas may occur and involve the large and small intestines. Well-differentiated tumors can extend from fistula tracts and be misinterpreted as an adenoma. While survival may be good for patients with adenocarcinomas arising in fistula tracts, overall mortality for adenocarcinomas arising in Crohn's disease is high, approximately 80%. This may be due to the similarities between symptoms of Crohn's relapse and symptoms of developing cancer, resulting in delayed diagnosis. Dysplasia does occur in patients with Crohn's disease; however, no standard surveillance strategy has been advocated due to the technical difficulty in surveying the bowel. Unlike ulcerative colitis, resection is not advocated for dysplasia because of the risk of postoperative complications.

Mesenchymal tumors

Benign fibroblastic polyps

These are incidental lesions detected in adult patients undergoing screening colonoscopy. As such the mean age in the reported series was 61.5 years [510]. The present as small polyps at endoscopy (size range 0.2–1.5 cm). The polyps consist of an expansion of the lamina propria by a monomorphic spindle cell population lacking mitotic activity or necrosis (Fig. 137.112). Some are intimately admixed with hyperplastic polyps. Benign fibroblastic polyps are managed by simple polypectomy.

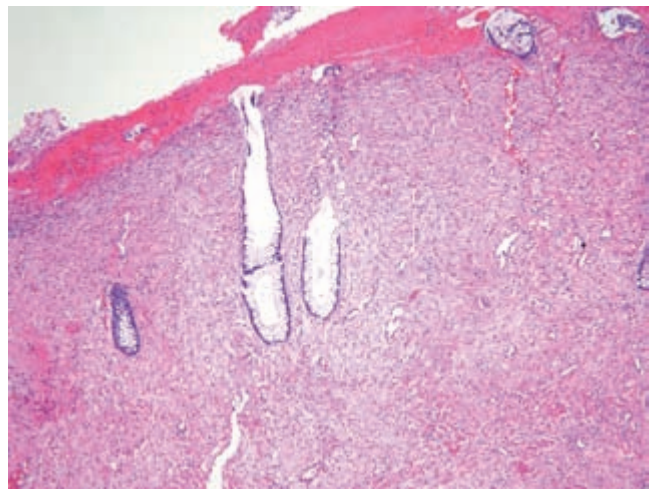

Figure 137.112 Benign fibroblastic polyp of the colon. The lamina propria is expanded by a benign spindle cell proliferation.

Leiomyomas

If a brightly eosinophilic spindle cell lesion with bland cytological features arises in association with the muscularis mucosae, it is in all likelihood an incidental leiomyoma, readily managed by simple polypectomy [511]. Of course most spindle cell tumors of the wall of the GI tract are GISTs (except in the esophagus where leiomyomas prodominate) but muscularis mucosae-associated tumors of the colorectum are usually leiomyomas. Miettinen and colleagues [511] studied 88 such tumors of the muscularis mucosae of the colon and rectum. The lesions, except one, were removed by snare polypectomy as incidental lesions at cancer or polyp surveillance; one small tumor was an incidental finding in the rectal resection specimen. The tumors had a significant male predominance (overall 2.4 : 1) and were found in adults (38–85 years, median 62 years). The lesions were typically small (range 1–22 mm, median 4 mm) and located predominantly in the rectum and sigmoid (72%). All were composed of well-differentiated eosinophilic smooth muscle cells that were seen immediately beneath the mucosa, obliterating the muscularis mucosae layer and merging with it. Two tumors had significant atypia (symplastic leiomyoma); mitotic activity was seen in one of these tumors but not in others. The lesional cells were uniformly positive for SMA and desmin and negative for CD34, CD117, and S100 protein, based on immunohistochemical studies of 20–24 cases with each marker. No GISTs were identified among the tumors of muscularis mucosae, and no CD117-positive cells, except mast cells, were seen in the muscularis mucosae layer. None of the patients had morbidity related to the tumor. Based on the authors' follow-up data on 29 patients, leiomyomas of muscularis mucosae are benign. In daily practice, we do not perform immunohistochemical stains on these lesions, but in any doubtful case a negative CD117 is reassuring.

Gastrointestinal stromal tumors

GISTs are most common in adults in the sixth decade and arise in the ascending and descending colon and usually present with pain or a mass. Except for small subserosal lesions, they are typically transmural tumors with intralumenal and outward-bulging components. Morphologically, they are

heterogeneous with spindle cells in fascicles, palisades or storiform arrangement, and sometimes an organoid patter; a minority has epithelioid cells in varying proportions.

Most (75%) colon GISTs are CD117 positive and 60% have CD34, with c-*kit* mutations in exon 11 in about one-third of cases. Some authors have taken a size of 5 cm and five mitoses per 10 high-power fields as thresholds for malignancy [512,513], but Miettinen and colleagues found lower levels [514]. In their series, tumors smaller than 1 cm did not recur, whereas in larger tumors 20% with minimal mitoses and all with more than five mitoses per 50 high-power fields metastasized or died of disease. Interestingly, the few cases with skeinoid fibers had a better prognosis. These tumors are not commonly encountered on mucosal biopsies as they are typically mural; when they are, performing an immunohistochemical panel is prudent.

Neural tumors

Ganglioneuromas occur in two general settings: as solitary isolated lesions, and syndromically as multiple lesions that either produce multiple exophytic polyps (ganglioneuromatous polyposis) or poorly demarcated transmural proliferations (ganglioneuromatosis) [515]. In solitary examples, there is no gender predominance and lesions have been detected in adults aged 20–90 years, with a peak incidence between the ages of 40 and 60 (mean age 48 years). The majority are found in the colon, usually on the left side. Most patients are asymptomatic and the lesions are detected during routine colonoscopy. Solitary lesions are not associated with genetic syndromes. In contrast, ganglioneuromatous polyposis is associated with FAP and diffuse ganglioneuromatosis with multiple endocrine neoplasia (MEN) type IIB and with NF1. Diffuse ganglioneuromatosis is found in virtually all patients with MEN IIb and often antedates the development of the endocrine neoplasms. Patients with MEN IIb and ganglioneuromatosis present with diverse GI symptoms that may include constipation, diarrhea, difficulty feeding, projectile vomiting, and crampy abdominal pain. Most syndromic GI tract ganglioneuromas are found in the colorectum and in younger patients than sporadic isolated ganglioneuromas (mean ages about 35 years).

Polypoid isolated ganglioneuromas are small sessile or pedunculated polyps that grossly resemble juvenile polyps or adenomas and are 1–2 cm in size. The polyps in ganglioneuromatous polyposis are multiple (20–40) and display greater variability than sporadic ones, ranging in size from 1 mm to over 2 cm. Some are filiform. Diffuse ganglioneuromatosis results in a poorly demarcated whitish thickening that may be transmural.

At low magnification, polypoid sporadic ganglioneuromas often resemble juvenile/inflammatory polyps in that they have disturbed crypt architecture and expanded lamina propria. At higher magnification, the lamina propria is expanded by collections of spindle cells within fibrillary matrix and

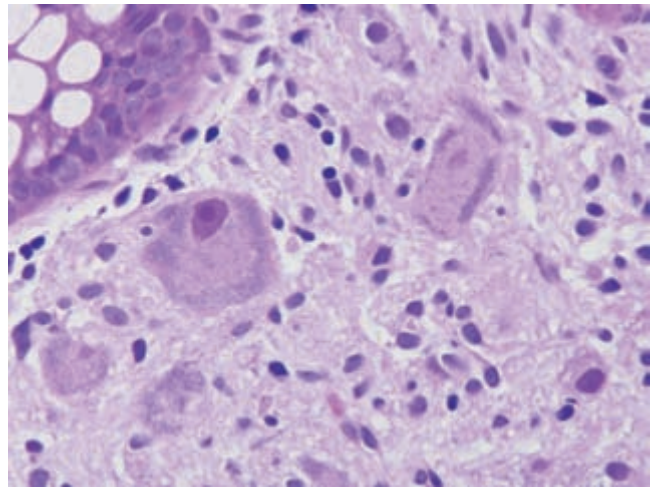

Figure 137.113 Ganglioneuroma. Aberrant ganglion cells and Schwann cells (bland spindled cells) proliferate in the colonic lamina propria. Most examples are isolated sporadic lesions.

irregular nests and groups of ganglion cells (Fig. 137.113). Sporadic examples may also have submucosal extension and a plexiform arrangement involving the submucosal nerve plexus such that they superficially resemble neurofibromas (differing by the presence of many ganglion cells). The ganglioneuromas in ganglioneuromatous polyposis show overlapping features with sporadic ganglioneuromas but tend to be more variable and have more numerous ganglion cells and filiform architecture. In diffuse ganglioneuromatosis, the process is centered around the myenteric plexus and is either diffusely intramural or transmural and consists of fusiform expansions or confluent transmural ganglioneuromatous proliferations.

Sporadic ganglioneuromas are treated by polypectomy and seldom recur. Patients with syndromic ganglioneuromas must be carefully followed based on their specific syndromes. Those with NF1 may develop other neural lesions, including malignant peripheral sheath tumors, and those with MEN IIb may develop endocrine neoplasms. Polypoid ganglioneuromas may herald Cowden disease, tuberous sclerosis, FAP, and juvenile polyposis whereas the diffuse type is most likely to be asociated with NF1 and MEN IIb. This type may cause strictures requiring resections but the ganglioneuromas are all themselves benign.

Schwannomas

Like gastric schwannomas, those of the colon differ from those encountered in the somatic soft tissues by lacking a capsule and having a prominent lymphoid cuff [516,517]. They consistently display strong diffuse S100 protein and lack CD117/c-kit. These tumors differ from conventional schwannomas genetically as well as they have no NF2 alterations whereas conventional schwannomas do [518] but share NF1 alterations with conventional schwannomas. We have

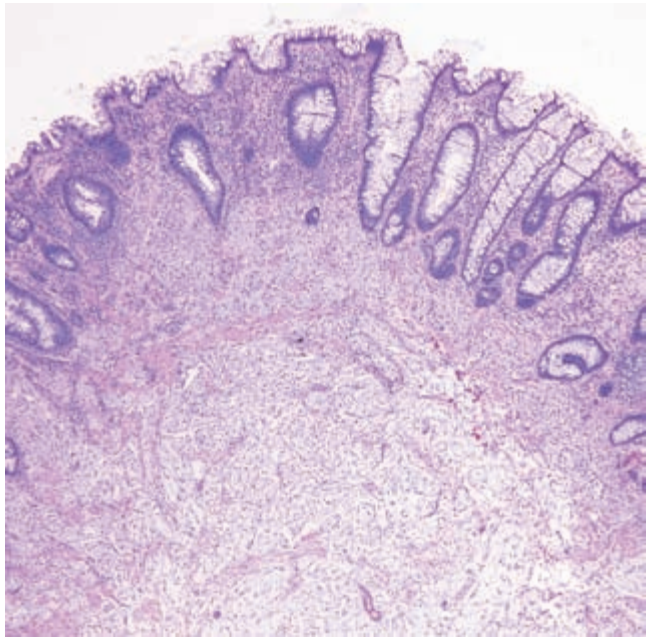

Figure 137.114 Benign epithelioid peripheral nerve sheath tumor. This lesion is centered around the lamina propria and superficial submucosa.

also seen a number of sporadic small epithelioid nerve sheath tumors in our practice that present as small polyps at endoscopy [519] (Fig. 137.114).

Granular cell tumor

Colon granular cell tumors are occasionally encountered in practice and are the second most commonly encountered GI tract granular cell tumor (after esophagus) but are still rare. In our in-house material, we have encountered only two colorectal granular cell tumors in a 20-year period, one of the cecum and the other of the rectum. They display the features of granular cell tumors elsewhere. There are rare reports of multicentric granular cell tumors of the colon [520]. Malignant examples arising in the colorectum were not identified by a PubMed search.

Lipomas

Multiple GI tract and cutaneous lipomas are known in patients with ganglioneuromas of the colon [515] but most lipomas of the colon are sporadic lesions centered around the submucosa. Of course, expanded adipose tissue at the ileocecal junction is sometimes referred to as a lipoma but is probably simply prolapsed submucosal fat. Colorectal lipomas are uncommon and mostly a curiosity (in one study of 5000 sigmoidoscopies, only one lipoma was removed) but those that have been present for some time can result in symptoms based on mechanical problems [521]. Colorectal lipomas are lesions of adulthood and thus are occasionally found at screening and usually recognized by endoscopists by their yellowish color (Fig. 137.115).

Figure 137.115 Colonic lipomas. Surgical specimen.

Carcinoid tumors

In contrast to carcinoid tumors in the stomach, which are often indolent tumors arising in the setting of atrophic gastritis (stimulated by hypergastrinemia), those in the colon arise sporadically. There is a body of literature suggesting a recent explosion in the incidence of such tumors, but one that fails to factor in the advent of colonoscopy and endoscopy during the studied time period [216,217]. Colorectal carcinoids are essentially limited to the rectum and sigmoid. They have a spectrum of appearances just like all carcinoids. A prognostic cutoff size of 2 cm has been suggested in the literature [522].

Other tumors

Prostate and bladder carcinomas can extend directly into the colorectum. Metastatic breast carcinoma can also be encountered, where it mimics primary colonic signet ring cell carcinoma. A host of lymphomas can also be found.

Anus

It is probably most important for pathologists to remember that a biopsy from the anatomical (clinical) anus can display glandular, transitional, or squamous mucosa and a biopsy labeled "anus" showing colonic-type mucosa is not necessarily labeled incorrectly or obtained ineptly. The anal canal, by definition, begins as the rectum enters the puborectalis sling (palpated as the anorectal ring on digital examination) and ends at the squamous mucocutaneous junction with the perianal skin [474]. The anal canal forms during weeks 4–7 of gestation and the upper two-thirds is endodermally derived whereas the lower one-third is ectodermally derived; the dentate or pectinate line is where these zones meet.

The anus can also be divided into the mucosa-lined anal canal and the more distal epidermis-covered anal margin. Where the skin meets the canal, apocrine glands may be prominent. Cancers arising in the anal margin are regarded as skin cancers and are treated with local excision. The anal

canal extends proximally from the anal verge to the rectal mucosa. Most of the anal canal is lined by squamous mucosa, which is present between the anal verge and the dentate (or pectinate) line. The dentate line is a visually identifiable border between the more distal squamous mucosa and a transitional area of squamous and nonsquamous mucosa. The adjacent nonsquamous lining can consist of either transitional (urothelium-like) or rectal glandular mucosa [523,524]. Anal ducts and glands are found at the transition zone and lymphatic spaces are often prominent in these zones.

Biopsies of the anal canal are less frequently performed than those from the remainder of the GI tract. Of course the length of the anus accounts for the minority of the GI tract. For example, in our material, colon biopsy accessions from about 5000 patients each year are sent for pathology and about 5000 from the upper tract, but only about 250 from the anus. We would probably have fewer were it not for a clinic in our hospital directed at detecting HPV-related intraepithelial lesions in high-risk individuals. Anal cancers are uncommon (estimated new cases for 2004 about 4000, in contrast to about 147 000 for colon and rectum [197]) and pilonidal disease, which is common, is seldom biopsied.

Of course the anus can be the site of a host of conditions affecting the skin, a topic beyond the scope of this manual, but, for example, various keratoses can be found in the anal area. When typical seborrheic keratoses present in this area, they raise the possibility of condyloma based on their site despite their lack of viral cytopathic changes. In patients who have been irradiated, the usual radiation-associated changes are seen [525], including hyalinized vessels and collagen.

Nonneoplastic conditions

Hidradenoma papilliferum

This cystic and papillary apocrine neoplasm generally arises in the perianal skin and vulva. The characteristic affected individual is a white woman over the age of 30. The typical sites include labia majora, perineum, and perianal skin [526–528], although extragenital sites appear as case reports. Rare reports of malignant change in these are often contested.

The lesion forms a large epithelial-lined cyst in the middermis that displays elaborate papillary infoldings formed by fibrovascular cores lined by two layers of cuboidal epithelium with foci of bridging and tuft formation (Fig. 137.116). The lesions lack inflammation and the superficial portions of the cyst are often lined by a flattened squamous layer. If the pathologist is concerned that this is a metatastasis, CK5/6 has a relatively limited expression profile, being relatively specific for mesothelium and other pavement-type epithelium such as squamous epithelium, and it stains these tumors [529], a helpful feature in excluding an adenocarcinoma. These lesions also express estrogen receptor [530], a potential problem if the pathologist is concerned that they are metastatic from the breast or female genital tract, although they lack a desmoplastic response and are well marginated.

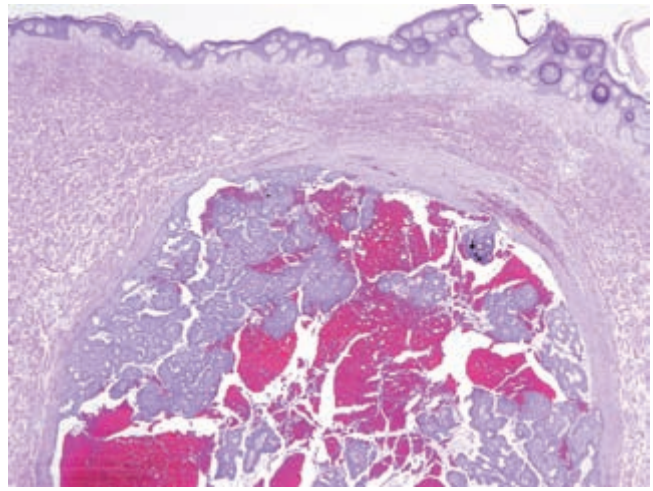

Figure 137.116 Hidradenoma papilliferum. This lesion shows differentiation along sweat gland lineage and is found in the perineum of females.

Hemorrhoids

Hemorrhoids are common and were the subject of both a review and a position statement by the American Gastroenterological Association in 2004 [531,532]. They were traditionally viewed as varicosities of submucosal veins but are now believed to reflect sagging or slippage of normal structures, probably exacerbated by straining at defecation. Hemorrhoids are normal anatomical structures found in the subepithelial space of the anal canal [533]. They consist of connective tissue cushions surrounding the direct arteriovenous communications between the terminal branches of the superior rectal arteries and the superior, inferior, and middle rectal veins. Anal subepithelial smooth muscle arises from the conjoined longitudinal muscle layer, passes through the internal anal sphincter, and inserts into the subepithelial vascular space. There, the smooth muscle suspends and contributes to the bulk of the hemorrhoidal cushions. The hemorrhoidal cushions serve as a "stopper" to ensure complete closure of the anal canal.

Hemorrhoidal vessels are usually located above the dentate line on the left lateral, right anterior, and right posterior aspects of the anal canal. Symptomatic hemorrhoids may be above (internal) or below (external) the dentate line. In some individuals, external hemorrhoids are actually prolapsed internal hemorrhoids.

When hemorrhoids are seen in excised material, they feature large ectatic vessels and the associated mucosa typically displays prolapse changes. Pagetoid dyskeratosis in overlying squamous mucosa, similar to that seen in prolapsed uterine cervices, is also observed [534]. However, we do not suggest reporting this peculiar pattern of dyskeratosis using such terminology (which might prompt excessive concern on the part of the surgeon) but recommend that it is not mistaken for the cytopathic changes of HPV.

Papillary endothelial hyperplasia/vegetant intravascular hemangioendothelioma

Papillary endothelial hyperplasia has been reported in all parts of the body [535–542] but the first report was in hemorrhoidal vessels, imparting a gross appearance that Masson likened to a cherry [543]. This benign reactive process is an unusually exuberant form of organizing vascular thrombus. Papillary endothelial hyperplasia may occur at any age and involve virtually any vessel, including those of neoplastic processes and vascular malformations. However, in its pure form, it typically presents as a solitary, superficial, firm, bluish or erythematous mass, involving the fingers, head and neck, or anorectal region.

Histologically, the lesion shows papillary fronds with a core of fibrin or hyalinized collagen, lined by a single layer of endothelial cells that may be plump or swollen but which lack significant atypia or mitotic activity. Occasionally the fronds appear to be free-floating within the vessel lumen. Extravascular extension may occasionally be seen, although it is usually not extensive.

Herpes simplex virus proctitis

HSV proctitis has the same viral cytopathic features as HSV elsewhere in the body. It is associated with the presence of genital lesions as well as with peno-anal intercourse [544–547]. Biopsies usually show characteristic multinucleated cells with smudged intranuclear inclusions in squamous epithelial cells at the edge of ulcers. Immunohistochemistry can be helpful in confirming an impression of HSV proctitis.

Crohn's disease

In 1978, Hughes proposed an anatomical and pathological classification for perianal Crohn's disease (Cardiff classification) in which each major manifestation of perianal Crohn's disease (ulceration, fistula, stricture) is graded on a scale of 0–2 (0, absent; 2, severe); fistulae are also classified as low (not extending above the dentate line) or high (extending above the dentate line, sometimes to the levator muscles). Other associated anal conditions, the intestinal location of other sites of Crohn's disease, and a global assessment of the activity of the perianal disease are also noted [548]. Despite its descriptive accuracy and comprehensiveness, the Cardiff classification system has never gained widespread acceptance because of the perception by clinicians that it is of limited clinical relevance [549,550].

In ulcerative colitis, involvement of the anal canal is typically nonspecific and unrelated to the inflammatory disease. In contrast, in Crohn's disease the anal canal itself is affected in about 25% of patients with classic small intestinal disease and in up to 80% of those with colonic involvement. Anal disease may be the initial manifestation in about one-third of patients with Crohn's disease. Biopsies may display a patchy active chronic inflammatory process but fissures, fistulae, and striking chronic inflammatory infiltrates are the hallmark. However, unless granulomas are detected, biopsy features are not specific and must be correlated with clinical ones. Anal biopsies are not commonly performed in Crohn's disease simply because they cause a certain amount of morbidity themselves. With anal Crohn's disease, the issue of treatment is far more compelling that than of biopsy interpretation [551].

Polyps

Fibroepithelial polyps

These lesions are also called anal tags and excising them is generally not suggested unless they are uncomfortable or interfere with personal hygiene since removing them may be extremely painful and, in the case of those associated with Crohn's disease, may result in poor healing and additional morbidity. They are essentially projections of anal mucosa and submucosa. When one arises at the leading edge of an anal ulcer or fissure, the term *sentinel tag* is applied. They are often submitted to the pathologist as hemorrhoids but lack vessels, features of hemorrhage, and organized thrombi. They are essentially the same lesion as skin tags (acrochordons).

Fibroepithelial polyps have received little attention in the pathology literature [552,553] but consist of myxoid or collagenous stroma covered by squamous epithelium (Fig. 137.117). Stromal cells with two or more nuclei are found in the majority of examples and large examples often harbor atypical stromal cells. Mast cells are frequent. The stromal cells in these polyps express vimentin, CD34, and sometimes desmin [552,553]. Electron microscopic examination shows fibroblastic and myofibroblastic features. Of interest is that subepithelial connective tissue from the normal anal mucosa shows bizarre multinucleated cells and mast cell infiltration so it has been assumed that fibroepithelial polyps reflect reactive hyperplasia of the subepithelial connective tissue of the anal mucosa.

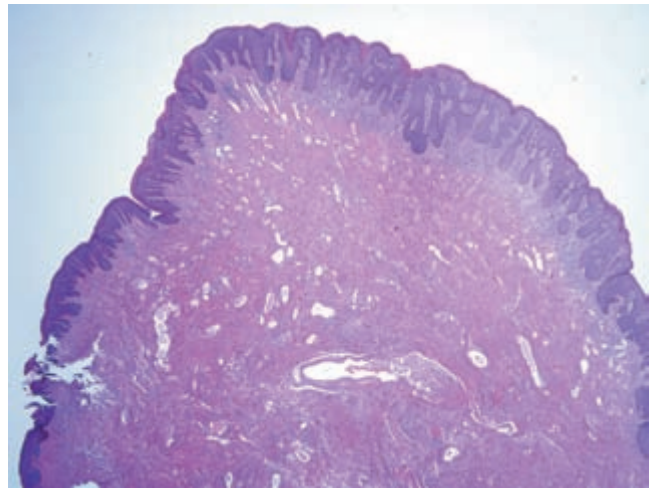

Figure 137.117 Anal fibroepithelial polyp. These appear similar to skin tags elsewhere.

Neoplasia

The bulk of anal neoplastic pathology encountered in daily practice revolves around the diagnosis of squamous carcinoma and its precursors even though such tumors are relatively rare. Resection specimens should be rarer still since most cancers are treated with radiation and chemotherapy. As such, biopsy diagnosis is particularly important. Resected anal specimens are handled differently from colorectal ones [524] and a key feature is that size rather than depth determines T status in anal cancers (T1, ≤ 2 cm; T2, 2–5 cm; T3, > 5 cm; T4, invasion into adjacent organs) [474].

Anal squamous intraepithelial neoplasia

The vast majority of such lesions, like those in the uterine cervix, are related to the various subtypes of sexually transmitted HPV infection, and these are presumed to be the precursors of invasive anal squamous cell carcinoma. The number of individuals in the population exposed to HPV is high; for example, 3% and 12% of US male and female blood donors respectively had positive serology for HPV16 in one study [554]. Since only about 4000 new anal and 10 000 new cervical cancers were reported in 2004 [197], this suggests a very low progression rate. However, it is known that HIV-infected individuals are extremely likely to have anal HPV lesions [555–557] so this is the group in whom screening is presently being evaluated, akin to mass cervical screening, and it is frequently from this population that pathologists receive anal biopsies to evaluate. At least half of the homosexual male HIV population have anal HPV lesions [555–557] and low-grade lesions progressed to high-grade lesions in about 20% of such patients in 2 years in one study [558], although it seems that very few of these in situ lesions progress to invasive carcinoma. However, the reason to treat that has been offered is the aggressive nature of invasive carcinomas in these individuals once invasion ensues. The effect of antiretroviral therapy on these lesions remains unclear but is not necessarily beneficial [559]. Anal intraepithelial lesions are summarized in Table 137.9. As noted above, their common denominator is HPV but the factors that determine whether any given infection manifests as, for example, Bowenoid papulosis versus invasive squamous cell carcinoma remain obscure.

Like the uterine cervix, the anal canal has a transformation zone. This zone cannot be visualized without the use of anoscopy, so a procedure is required even for cytological screening. In biopsies obtained from this area (anoscopically the dentate/pectinate line), it is typical to find fragments of rectal-type mucosa adjoining or separate from the lesions in question. The histology of anal squamous intraepithelial lesions is quite similar to that found in the uterine cervix and associated with the same HPV types as cervical lesions.

Such lesions had been classified as anal intraepithelial neoplasia (AIN) I, AIN II, and AIN III but, as for the cervix, most observers now prefer to separate low- and high-grade lesions, with AIN II subsumed under high grade (Figs 137.118 and 137.119). Variable degree of loss of nuclear stratification and polarity, nuclear pleomorphism and hyperchromatism with or without keratinization or HPV cytopathic changes, with

Table 137.9 Squamous precursor lesions encountered on anal area biopsies

Lesion	Gross appearance	HPV type	Microscopic features	Outcome
Condyloma acuminatum	Protuberant genital wart, cauliflower-like lesion	6, 11	Exophytic lesion with prominent surface viral cytopathic changes	Aggressive transformation, vanishingly rare
Bowenoid papulosis	Multiple brownish to reddish papules	16, 18, 33, 34	Histologically similar to Bowen disease (below, carcinoma in situ) but clinical presentation different (multiple papules vs plaque)	Sometimes resolves spontaneously, but can recur. Essentially never progresses to cancer
Anal intraepithelial neoplasia (AIN)	Inapparent or plaque-like in anal transition zone (area of dentate line). Likely in HIV population	16, 18, others	Variable degree of loss of nuclear stratification and polarity, nuclear pleomorphism and hyperchromatism with or without keratinization or HPV viral cytopathic changes, with increased mitoses high in the epithelium. A two-grade system is used (high and low), but formerly AIN I, II, and III were used. Observer reproducibility can be a problem	Risk of progression to cancer low but unknown. In HIV patients, progression from LSIL to HSIL about 20% in 2 years
Bowen disease (squamous dysplasia at the anal margin; carcinoma in situ)	Reddish-brown plaque of perianal skin, often extending into anal canal	16, 18, others	Full-thickness dysplasia with jumbled nuclei, disorderly maturation, mitoses at all levels and dyskeratosis, sometimes extending into skin appendages (pilosebaceous units)	Progression to cancer reported in 2%–6%

HIV, human immunodeficiency virus; HPV, human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

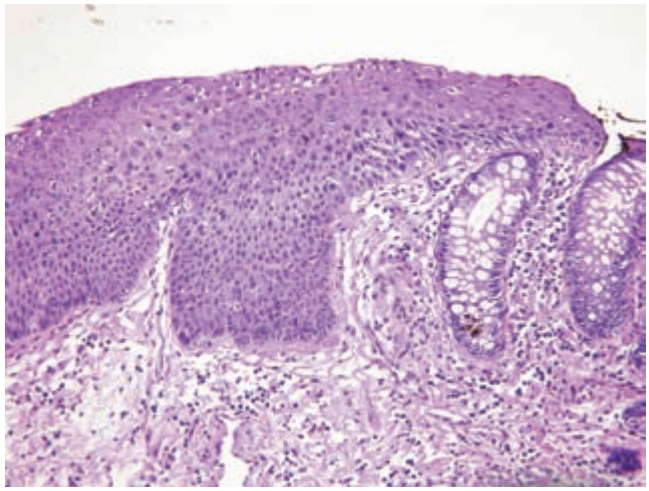

Figure 137.118 Anal intraepithelial neoplasia. This process often occurs at the anorectal transition.

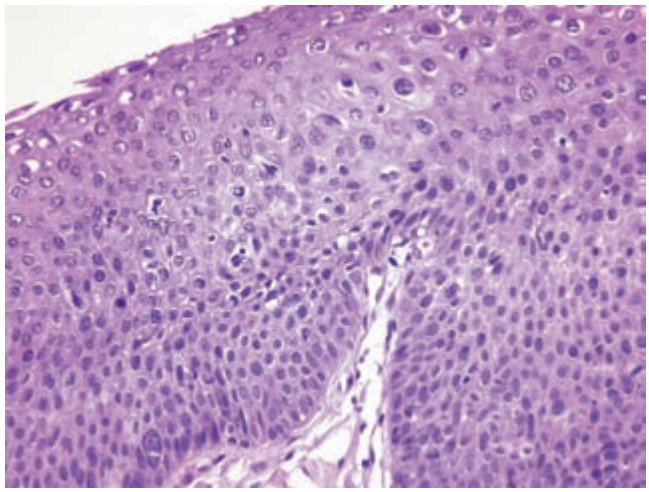

Figure 137.119 Anal intraepithelial neoplasia (AIN). The changes here are those of AIN 2 (moderate dysplasia) and would be subsumed under high-grade in situ lesions.

increased mitoses high in the epithelium are the constellation of findings sought on biopsy. HPV cytopathic changes alone, found in either exophytic or flat lesions, consisting of koilocytes and basal zone proliferation accounting for less than half of the epithelial thickness are classified as low-grade. Lesions with higher proliferation of hyperchromatic cells are regarded as high-grade lesions, as for the uterine cervix. As with the uterine cervix, there can be difficulty in separating AIN from reparative features. In these situations, p16 stains can sometimes be helpful. HPV infection is associated with overexpression of p16, a cyclin-dependent kinase inhibitor. Lu and colleagues studied 29 cases of squamous cell carcinoma of the anorectal region immunohistochemically for the expression of p16 [560]. The tumor cells exhibited a strong and diffuse nuclear stain (with some cytoplasmic positivity) for p16 in all 29 cases. HPV DNA was detected in every case (100%), with 25 cases (86%) harboring type 16. These observations indicate that overexpression of p16 is commonly associated with high-risk HPV infection, which may serve as a useful surrogate biomarker for identifying squamous cell lesions harboring HPV DNA. As such, in intraepithelial lesions, p16 can be a helpful adjunct in separating reactive from AIN lesions. Tandem Ki-67 stain can also be helpful.

Bowen disease is an eponymous term used for squamous carcinoma in situ at the anal margin (the outside part that can be seen by the clinician, in contrast to lesions seen at the transition zone inside the canal at the dentate line) where it presents macroscopically as a brownish plaque. Biopsies of Bowen disease display full-thickness dysplasia with jumbled nuclei, disorderly maturation, mitoses at all levels and dyskeratosis, sometimes extending into skin appendages (pilosebaceous units). Like AIN, it is strongly associated with HPV. It is more likely to progress to invasive cancer than AIN lesions but most cases still do not progress.

Bowenoid papulosis, first described in the penis [561], is a clinicopathological diagnosis. The patient presents with multiple papules, each of which has features of in situ carcinoma, but these essentially never progress to invasive carcinoma and sometimes resolve. These, too, are HPV lesions.

Anal squamous cell carcinoma

Carcinoma of the anal canal accounts for 1.5% of digestive-system cancers in the USA, with an estimated 4000 new cases in 2004 and about 580 disease-associated deaths [197]. The incidence of anal cancer is increasing but it is still uncommon [562]. Thirty years ago, anal cancer was believed to be caused by chronic local inflammation of the perianal area (such as from Crohn's disease). Treatment involved abdominoperineal resection, resulting in a permanent colostomy. However, it is now apparent that development of anal cancer is nearly invariably associated with infection by HPV, and usually sexually transmitted. Moreover, in the majority of patients, concurrent chemotherapy and radiotherapy is curative, without the need for surgery. Data from the Surveillance, Epidemiology, and End Results (SEER) program show similar incidence of anal cancer for men and women between 1994 and 2000 (2.04 per 100 000 and 2.06 per 100 000, respectively), the most recent period for which data are available, whereas men had lower rates than women between 1973 and 1979 (1.06 per 100 000 vs 1.39 per 100 000). In addition, black men had higher incidence rates than did other race-specific and gender-specific groups (2.71 per 100 000). From the earliest period for which data are available to the most recent one, relative 5-year survival improved from 59% to 73% among women, was unchanged among men (~ 60%), and decreased from 45% to 27% among black men; 18% of patients who had distant disease were alive at 5 years compared with 78% of patients who had localized disease [562].

Tumors arising within the anal canal distal to the dentate line are usually keratinizing squamous cell carcinomas, whereas those appearing in the transitional mucosa above the dentate line are frequently nonkeratinizing squamous cell carcinomas. The nonkeratinizing subtypes were once referred to as transitional cell and cloacogenic; however, they are now recognized as variants of squamous cell carcinoma that lack terminal differentiation. One type is composed of large cells and the other is characterized by small cells. The bladder and anus share a common embryological origin, thus giving rise to the similar (i.e., transitional or cloacogenic) morphology. The biology and prognosis of keratinizing and nonkeratinizing tumors of the anal canal are essentially the same, so prior concern about subclassification of anal tumors is probably not warranted (and we do not devote effort to this subclassification) [563,564]. Furthermore, pathologists do not always reproducibly separate the categories, further limiting their utility [565]. Adenocarcinomas of the anal canal are clinicopathologically like rectal cancer and are treated as such.

Lymphatic drainage of anal cancer depends on the location of the tumor. Above the dentate line, drainage flows to perirectal and paravertebral nodes (like rectal adenocarcinoma); below the dentate line, drainage is through inguinal and femoral nodes. Tumor size is the most important prognostic factor for patients with cancer of the anal canal. Mobile lesions less than 2 cm in diameter can be cured in approximately 80% of cases, whereas those 5 cm or more are cured in less than half the time. The probability of nodal involvement is also directly related to the size of the tumor.

Anal squamous carcinoma was classified in 1989 by WHO into the following types [566].

- Squamous cell (cloacogenic): large cell keratinizing; large cell nonkeratinizing (transitional); and basaloid.
- Giant condyloma (verrucous carcinoma).

In this classification, nonkeratinizing carcinomas were said to resemble transitional carcinomas of the urinary bladder (Fig. 137.120) and basaloid carcinoma was composed of solid sheets of small cells, often with peripheral palisading. Striking keratinization is unusual in lesions from the canal but not unknown. Most carcinomas do display the features of squamous carcinoma in other anatomical sites: striking nuclear alterations with nuclear pleomorphism and prominent mitotic activity and scattered squamous intercellular bridges. Anal squamous carcinomas can display the host of patterns found in other sites, including some with clear-cell features.

An exception to the usual striking epithelial changes is the rare verrucous carcinoma, which displays broad-based squamous papillae that are sheet-like in a sclerotic background and often accompanied by many neutrophils and a peculiar pattern of abnormal keratinization at all levels of the markedly thickened epithelial proliferation. Verrucous carcinoma has been termed *giant condyloma of Buschke–Löwenstein* and may involve the external genitalia and perianal skin.

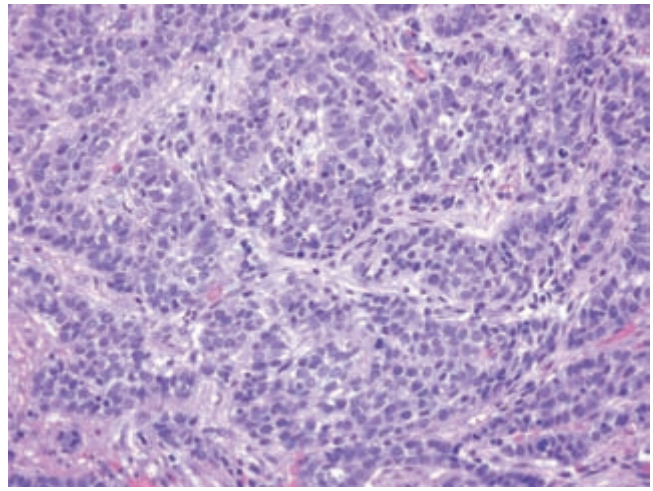

Figure 137.120 Anal squamous cell carcinoma. This example is basaloid (formerly cloacogenic).

It resembles a condyloma but is, if anything, cytologically blander with less striking viral cytopathic changes; koilocytes with nuclear cavities are not seen. Verrucous carcinoma lacks the true papillary configuration with fibrovascular cores seen in large condylomas. Metastases from verrucous carcinomas are vanishingly rare and the primary tumors are treated by local excision (which can be difficult since lesions can be quite large).

Since the reproducibility of diagnosing most of these subtypes is not particularly good [565] and since it bears little impact on outcome (with the exception of attempting to separate verrucous carcinoma), we do not devote much effort to separating anal squamous carcinoma into subtypes. Providing a diagnosis of invasive carcinoma is all that is clinically relevant and awareness of the subtypes is simply a construct to avoid misdiagnosis of squamous carcinoma variants as something else. As such, the 2000 WHO classification simply combined the subsets of squamous carcinoma [567].

Paget disease

Extramammary Paget disease typically affects apocrine gland-rich sites such as the perianal zone. It presents as a slowly growing erythematous eczematoid plaque that may extend internally to the dentate line. On biopsies, part or all of the squamous epithelial thickness is infiltrated by large pale cells, some of which may have signet cell features, and some of which may be large and pink (Figs 137.121 and 137.122). However, if the epithelium is crushed or altered in any way, it can be difficult to separate the Paget cells from atypical keratinocytes or from melanoma. Fortunately, an immunohistochemical panel resolves most doubtful examples. True Paget disease is a lesion with apocrine cell differentation, and the proliferating cells express Cam 5.2, CEA, gross cystic disease fluid protein (GCDFP), and CK7 and have mucin [568, 569]. Of course Merkel cells can also have CK7, a feature

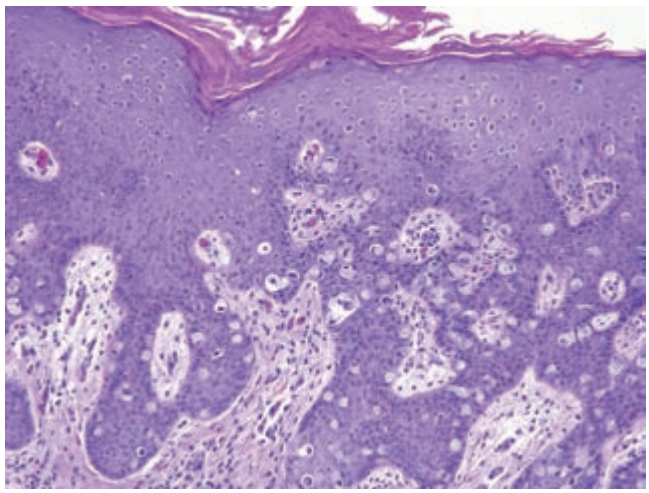

Figure 137.121 Anal Paget disease. Glandular cells proliferate in the squamous mucosa.

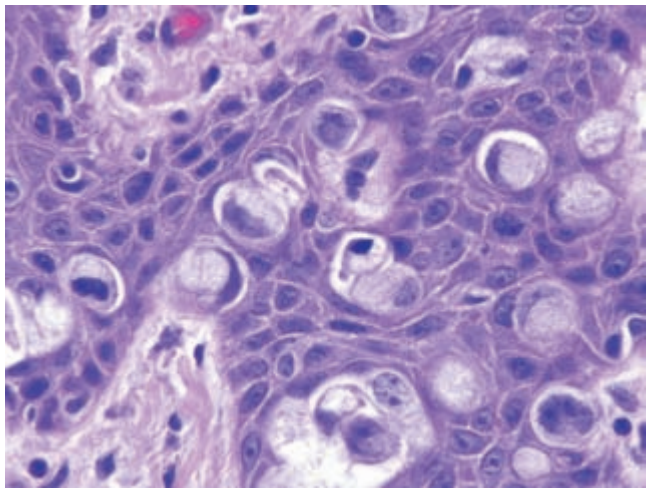

Figure 137.122 Anal Paget disease. At high magnification, intracellular mucin is apparent in these Paget cells.

the pathologist must consider when evaluating these stains. However, about half of cases showing adenocarcinoma cells in squamous mucosa reflect pagetoid extension of an underlying typical colorectal cancer into squamous epithelium and such lesions lack GCDFP and are often $CK20^{+}CK7^{-}$. However, the occasional MSI colorectal carcinoma may lack CK20 [570], although most such tumors are found in the right colon rather than the rectum. However, the best way to determine whether any case of anal Paget disease is associated with an invasive cancer is by a careful clinical examination.

The true Paget disease cases that are epidermotrophic apocrine neoplasms have a high local recurrence rate and can eventually become invasive. Those reflecting epidermotropism of ordinary adenocarcinomas behave and are managed as the associated cancers; thus stage is the primary prognostic marker.

Adenocarcinoma including anal gland adenocarcinomas

Most adenocarcinomas arising in the anus are of the rectal type and arise in the upper zone of the anus (which is normally lined by columnar mucosa). These are identical to true rectal carcinomas and are managed as such. On biopsies they appear identical to rectal adenocarcinomas and their immunohistochemical profile is similarly identical, namely CK20 positive and CK7 (usually) negative. These tumors are characterized by pencillate nuclei, abundant necrosis, and the usual overall pattern of colorectal carcinoma, with frequent association with an adenoma. Like rectal cancers, some are mucinous. These generally pose few diagnostic problems, although the differential diagnosis is with prolapse polyps (inflammatory cloacogenic polyps), which are distinguished by their diamond-shaped glands and blander cytology.

There is a rare subset of anal canal adenocarcinomas believed to arise in association with, or display differentiation toward, anal ducts/anal glands and these are called anal gland (duct) carcinomas. They are sufficiently rare that the AFIP was only able to amass seven convincing cases [571]. These tumors are composed of tubules originating from ducts that open onto the mucosal surface. They are intramural without a lumenal in situ component and they have variable overlying surface ulceration. On immunohistochemical staining they are typically $CK7^{+}$ and $CK20^{-}$ akin to the anal glands and ducts. When these tumors are found, they need to be distinguished from prostate cancer (which is negative for CK7 and CK20). These tumors lack prostate-specific antigen and prostatic acid phosphatase on the small number of tested cases. Many have behaved aggressively.

Gastrointestinal stromal tumors

True GISTs of the anus are rare. An AFIP series of over 100 anorectal GISTS included only three examples [514]. When found, they display the typical staining pattern of GISTs (remember, however, that about 40% of melanomas express CD117/c-kit, so be sure to add S100 protein to such immunohistochemical panels directed at diagnosing GISTs). Far more likely are small leiomyomas that appear similar to those found in the esophagus that tend to occur in women and express hormone receptors. Small leiomyomas may also arise from the muscularis mucosae in the upper part of the anus above the dentate line.

Melanoma

Anal melanoma is rare and usually affects white adults but, like subungual and esophageal lesions, individuals with pigmented skin can also be affected. Patients present with a mass and often with rectal bleeding, which is typically initially attributed to hemorrhoidal bleeding [572]. The lesions

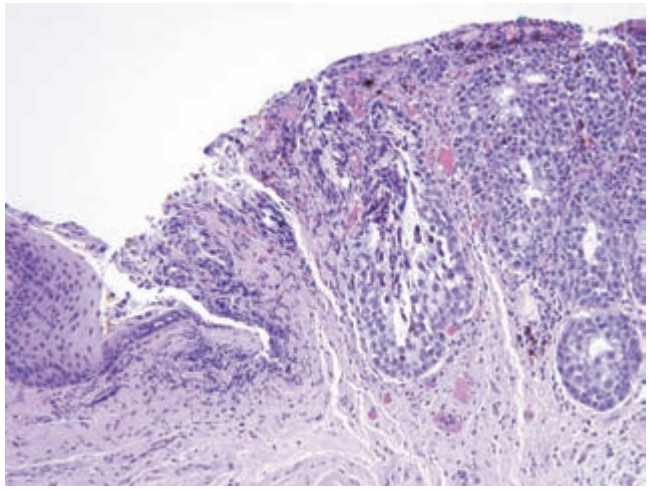

Figure 137.123 Anal melanoma. Note the melanin pigment.

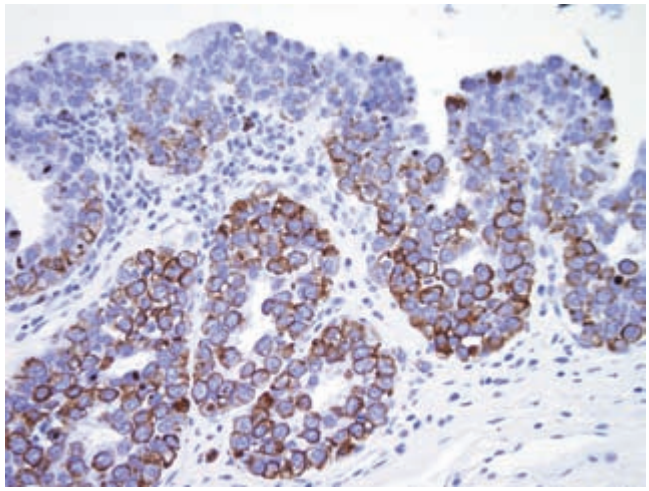

Figure 137.124 Anal melanoma. This immunohistochemical stain (HMB45) is for a relatively melanoma-specific antigen.

may be sessile or polypoid and, unless overt pigment is present (Fig. 137.123), a panel immunohistochemical approach (Fig. 137.124) is usually warranted to exclude poorly differentiated carcinomas and lymphomas. Confirmation that the lesion is primary is best accomplished by identification of an in situ component. The outcome is generally poor, with 5-year survival reported as 10%–45% [573–575]. Surgeons advocate sphincter-sparing surgery with adjuvant therapy rather than operations requiring colostomy [576].

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D GASTROINTESTINAL FUNCTION STUDIES

138 Evaluation of gastrointestinal motility: emerging technologies

John W. Wiley, Chung Owyang

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The methods available to evaluate gastrointestinal (GI) neuromuscular function are advancing rapidly. Conventional technologies often use water-perfused catheters, strain gauge catheters, or balloon devices that measure changes in pressure or volume but provide limited or no direct assessment of muscle tension and elastic properties of the gut wall. Direct assessment of these latter properties can provide more sensitive and specific information relevant to assessment of gut motility under physiological and pathophysiological conditions.

The objective of this chapter is to review selected emerging approaches for monitoring GI motility and visceral sensation. We include assessment of visceral sensation because abnormalities in visceral sensory pathways are thought to be involved in a number of functional GI motility disorders, such as the irritable bowel syndrome and nonulcer dyspepsia. Many of the emerging methods incorporate advances in volumetric measurements, endosonographic imaging technologies, and functional brain imaging to measure lumenal wall muscle structure and function and central nervous system (CNS) response to activation of sensory pathways innervating the viscera. The emerging methodologies to be discussed include:

- impedance planimetry
- intralumenal ultrasound applications to GI motility
- motility measurements of the stomach – scintigraphic emptying, volumetric assessment (e.g., single-photon emission computed tomography [SPECT], three-dimensional (3D) ultrasound, magnetic resonance [MR], and combined emptying volume measurements
- motility measurements of the pelvic floor, especially with MR imaging (MRI); and assessment of visceral sensation using functional brain imaging techniques
- assessment of regional activation in the brain using positron emission tomography (PET) and functional MRI (fMRI) in conjunction with visceral stimulation.

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Background

Relatively few methods are well established for evaluating GI motor function *in vivo*. Manometry is the standard method for clinical motility testing, but in a number of motility disorders manometric results correlate poorly with organ dysfunction and symptoms [1]. Typically, manometry systems use water-perfused hydraulic-capillary systems where pressure at a sensor outside the body can be used accurately to measure pressure at a point along a catheter through a low-flow-rate perfused channel. This approach has been used extensively to measure force by a pressure measurement in lumens and in liquid-filled balloons. However, manometry is suboptimal in compartments where the lumenal diameter does not reach zero. Also, contractions may not be detected if the transducer is facing the lumen instead of the wall and manometric sensors do not directly capture relaxation [2]. Thus, measurement of lumenal pressure is a surrogate measure of changes in force generated by the surrounding tissues [3,4]. Other indirect measures of GI motor function include transit time studies, pH measurements in the esophagus, and radiographic methods using contrast agents.

Impedance planimetry

Since the late 1990s, tools have been developed for studying both the active (phasic and tonic contractions) and passive function using impedance planimetry before and during administration of drugs that effect GI motility [4–6].

Measurements of interest include tension, stress, and strain, because these endpoints give information about the elastic properties of the gut wall and mechanoreceptors respond more directly to these parameters than to pressure and volume [4]. Muscle function can be more accurately evaluated when force and tension are measured rather than the pressure generated by contractions [7,8]. The most commonly employed bag distention technique is the barostat, but techniques such as impedance planimetry and distention combined with imaging techniques give more detailed geometric information that more accurately represents the actual motility pattern. For example, rather than analyzing pressure–volume relationships based on the barostat, impedance planimetry generates data on the cross-sectional area (CSA) of the bag during distention. The CSA provides improved documentation of the dynamic changes in the alimentary lumen diameter during contraction and relaxation compared with volume measurements [6].

The principle of impedance planimetry

Impedance planimetry (IP) allows simultaneous measurement of intralumenal pressure and estimation of the CSA of a bag placed in a tube or hollow organ [9,10]. The CSA is determined from the impedance of fluid inside a bag using electrodes supplied with an alternating current to measure the potential difference between them. The CSA is calculated from the potential difference. The IP data are used to calculate the wall tension, a property that is often altered in a variety of GI motility disorders [6,11]. Specifically, Ohm's law describes the relationship between the potential difference, current, and electrical impedance: $V = IZ$, where V, I, and Z denote the potential difference, the current, and the electrical impedance. The impedance depends on the distance between the electrodes, the conductivity of the liquid in the bag, and the CSA of the bag between the electrodes. The size of the bag should be chosen based on the criteria of obtaining reliable measurements in the physiological range in the region of interest without stretching the bag wall. Before each study, pressures should be calibrated, for example, at 0 and 100 cmH_2O. The calibration curve for impedance planimetry is characteristically nonlinear [6]. Therefore, a multipoint calibration should be performed before each experiment. Also, impedance planimetry measurements are sensitive to temperature changes [7,9]. The fluid temperature should be kept constant at 38°C using a heating device. The coefficient of variation (CV) of the probe in each application should be assessed [12]. To minimize the risk of misattributing in vivo changes caused by variations within the measuring system, the operator should define the threshold for an event, for example, as a CSA deviation of 15% from baseline [12]. The pressure inside the bag can also be measured by a manometry system coupled to an external pressure transducer. Records of CSA and pressure are amplified, analog-to-digital converted, and stored on a computer for later analysis. Therefore, using distention protocols that combine manometry and impedance planimetry during bag distention, it is possible to differentiate phasic and tonic contractions and to obtain data on the force–velocity properties of the intestinal muscle in vivo.

In a typical recording session participants will be fasted for at least 6 h. The impedance planimetry probe is passed into the compartment of interest and the bag positioned using fluoroscopic guidance. The participant is rested for 15 min to adjust to the probe. Depending on whether the study objectives involve recording of fasting or fed motility patterns, a standardized meal may be given to the subjects. The volume-controlled ramp distentions are then initiated. Participants can report the sensation intensity using a validated visual analogue scale (VAS), typically from zero to 10. Prior to starting the study, participants should be trained how to use the VAS, for example, where 0 is no perception, 5 pain threshold, 7 moderate pain, and 10 unbearable pain [9,10,12]. If perception of pain is a primary or secondary endpoint, distentions should not exceed the volume that elicits a VAS value corresponding to moderate pain. The distentions are performed at a standardized infusion rate, for example 25 mL/min, for four times to precondition the tissue and to be used as learning sessions for the sensory ratings [6,10]. Two additional distentions are conducted at the same infusion rate and used for the analysis. Approximately 10 min after completing these distentions to obtain baseline data, they can be repeated during or after administration of a therapeutic intervention, for example, a drug of interest that is hypothesized to increase or inhibit distention-evoked smooth muscle contractions. Recordings should be performed while the effect of the therapeutic intervention is at steady state.

Applications of impedance planimetry to gastrointestinal motility

Impedance planimetry data provide more detailed information than conventional manometry regarding the contractile patterns in the compartment of interest, particular the elasticity of the gut wall. Changes in the elastic properties of the gut wall have been implicated in a variety of GI motility disorders. Some examples of how this technology has been employed to study disorders affecting GI motor function are provided below.

Systemic sclerosis (SS) is a connective tissue disease that affects the gastrointestinal (GI) tract. Previous studies have shown decreased and abnormal intestinal motility, dilation, and a stiffened wall. However, because of the limitations of conventional recording techniques, little is known about the underlying basis for altered muscle mechanics in this population. As discussed previously, impedance planimetry allows the study of several properties of muscle mechanics in vivo, including the wall stretch ratio, tension, shortening velocity, and muscle power, which can be calculated from pressure and CSA data. Patients with SS demonstrated increased wall

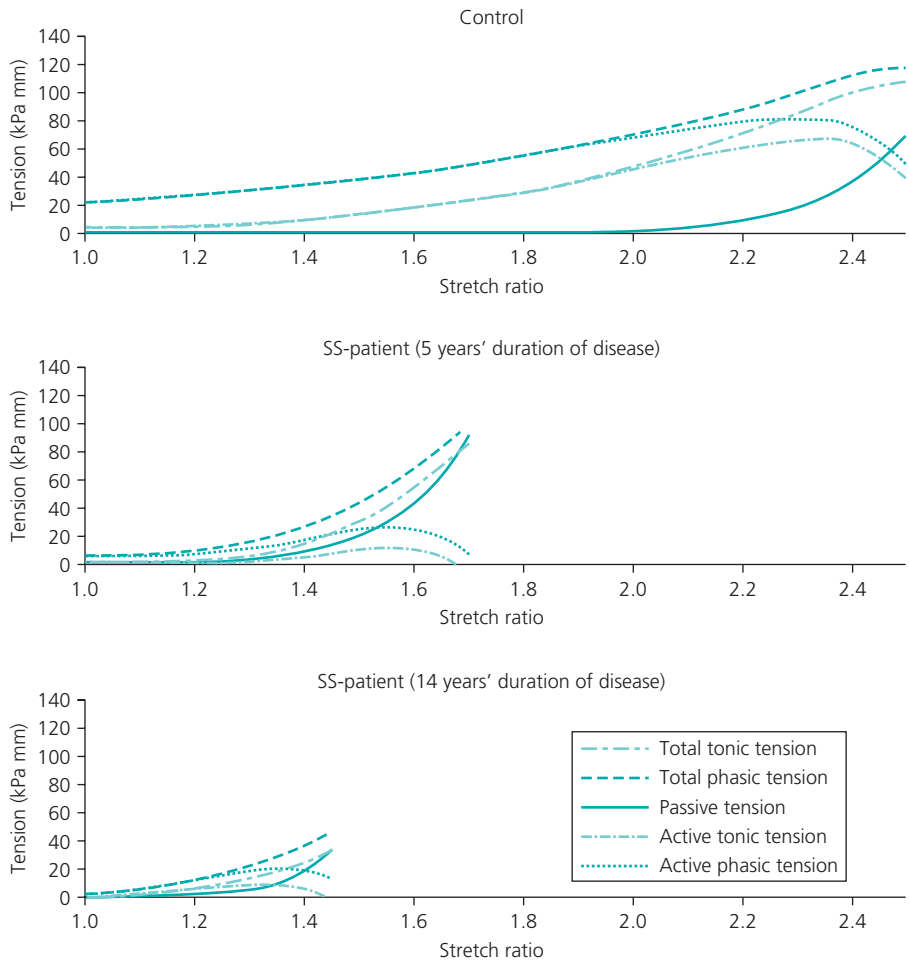


Figure 138.1 Representative length–tension curves, including total phasic tension, total tonic tension, active tonic tension, and passive tension. These data were obtained from a volunteer **(top)**, a patient with a duration of systemic sclerosis for 5 years **(middle)**, and a patient with a duration of systemic sclerosis for 14 years **(bottom)**. The key identifies the meaning of the different line patterns and it applies to all the panels. Reprinted with permission [13].

stiffness and impaired muscle dynamics including reduced power and decreased velocity of the duodenum, which correlated well with clinical symptoms (Fig. 138.1) [13]. The circumferential tension–stretch ratio diagrams showed a similar curve pattern for the controls and the SS patients. Thus, the passive tension curves were exponential and the active tonic and phasic tension curves showed an ascending leg, a local maximum, and the initial part of the descending leg in both the volunteers and patients. However, the tension and stretch ratio clearly decreased in the patients compared to the volunteers. By comparing the tracings in the middle and the bottom curves it appeared that the duration of disease was important. The translation to the left on the stretch ratio axis indicated that the intestine became stiffer in the patients and the low tension indicated that the muscle did not have the ability to contract with the same force as in the volunteers. Both in patients and in the volunteers, the active tonic and phasic tension curves appeared to peak at the same stretch ratio values. Impedance planimetry, however, does not provide information regarding whether the impaired muscle function may be due to reduced contractility of individual muscle cells, a reduced number of muscle cells, or increased stiffness of the matrix surrounding the muscle. In addition, the reduced contractility may also be influenced by lumenal dilation, causing a shift of the tension–stretch curves.

Impedance planimetry has also been used to examine the efficacy of potential therapeutic interventions. For example, theophylline has been proposed as a potentially effective treatment for noncardiac chest pain. The effects of intravenous theophylline or placebo were examined on esophageal sensorimotor function and chest pain, in patients with esophageal hypersensitivity, after intravenous theophylline or placebo [14]. The CSA of the esophagus increased significantly after theophylline compared with placebo. The tension–strain relationship shifted significantly to the right in the group of subjects who received intravenous theophylline but was unchanged in those who received placebo (Fig. 138.2). Based on these data, a second randomized 4-week crossover study was performed. Oral theophylline and placebo were administered to patients with esophageal hypersensitivity. Frequency, intensity, and duration of chest pain episodes were

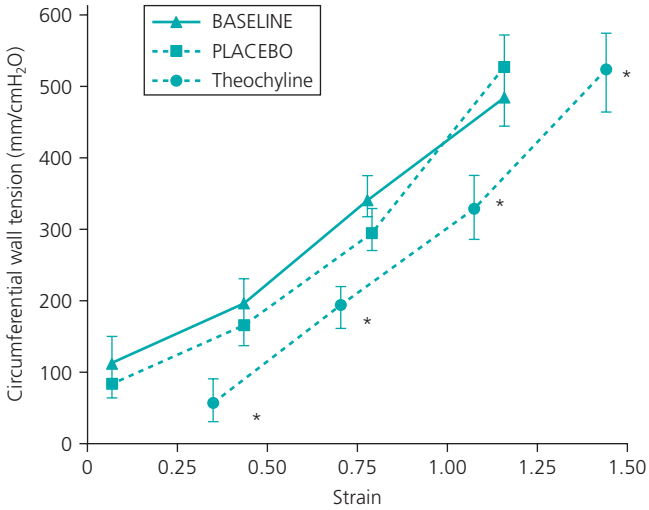


Figure 138.2 Effects of placebo and intravenous theophylline on circumferential wall tension/strain relationship. The curve in subjects that received theophylline (black circle dotted line) is significantly shifted to the right when compared with placebo (black squares, dotted line). These data suggest that the esophageal wall relaxed and became more deformable or more compliant after theophylline infusion (mean ± SEM). Reprinted with permission [14].

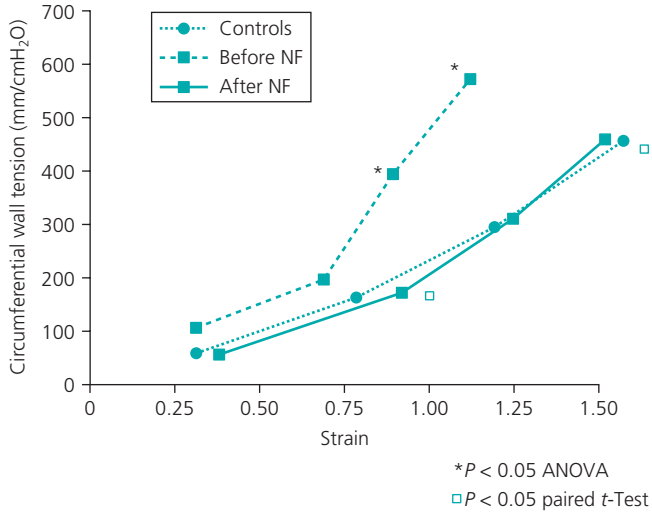


Figure 138.3 Relationship between circumferential wall tension and strain in response to graded balloon distention in control subjects and GERD patients before and after Nissen fundoplication (NF) (mean ± SEM). The curve in GERD patients before surgery significantly shifted to the left compared with control subjects. This suggests that for the same level of tension (force) the degree of strain or the deformability of the esophageal wall was less in GERD patients. However, after NF the curve shifted significantly to the right and was similar to control subjects, suggesting that the deformability of the esophageal wall had improved after NF. Reprinted with permission [15].

evaluated. Theophylline relaxed the esophageal wall, decreased hypersensitivity, and improved chest pain.

In another application, impedance planimetry was used to examine the sensory and biomechanical properties of the esophagus in patients with gastroesophageal reflux disease (GERD) before and after laparoscopic Nissen fundoplication compared with healthy controls [15]. The pathophysiology of persistent GERD symptoms after antireflux surgery is unclear. At baseline, GERD patients had lower thresholds for first perception, discomfort, and pain compared with controls. The esophagus was more reactive and less distensible in patients compared with controls. In GERD patients, before Nissen fundoplication, the curve that expresses the relationship between tension and strain shifted significantly to the left compared with controls (Fig. 138.3). This suggested that the esophageal wall was less distensible, or stiffer, in GERD patients. After Nissen fundoplication, in patients with persistent symptoms, the curve shifted to the right and was similar to controls, suggesting that the distensibility of the esophageal wall had improved. Of interest, some patients demonstrated persistent symptoms after Nissen fundoplication. In this population the sensory thresholds were unchanged but esophageal wall reactivity decreased, and distensibility improved. The authors concluded that laparoscopic Nissen fundoplication improves esophageal biomechanical dysfunction but not the underlying hypersensitivity. These observations highlight the application of this technology to identify and differentiate between abnormalities in the biomechanical properties of gut wall and primary sensory mechanisms.

Summary

Our understanding of the "mechanical" physiology of the GI tract is based on measurement techniques such as manometry. The objective of techniques such as impedance planimetry is to understand better the muscle properties of the gut wall and how they relate to mechanisms involved in symptom generation. The advantage of the impedance planimetric method is improved correlation of the measurements with lumenal wall elasticity based on assessment of changes in balloon CSA compared with changes in balloon volume. As an experimental tool for assessment of distention-induced responses, IP appears to be superior to other available distention techniques because the mechanoreceptors are most likely sensitive to mechanical parameters such as force, tension, and strain rather than to direct variation in pressure or volume. Direct comparisons with traditional barostat recording methods in specific patient populations in the absence and presence of interventions are required to establish the superiority of the impedance planimetric method in clinical research and before general acceptance of this approach as a diagnostic tool in the clinical setting. Similar to the barostat method, this approach is relatively invasive, requiring conscious sedation and intubation for positioning of the recording apparatus.

Endosonography for studying anatomy and motor function in the gastrointestinal tract

Background

Endosonography has emerged as a useful tool for studying the visceral wall and adjacent structures in detail [16]. This section focuses on the utility of endosonography to assess biomechanical and motor function of the gastrointestinal tract.

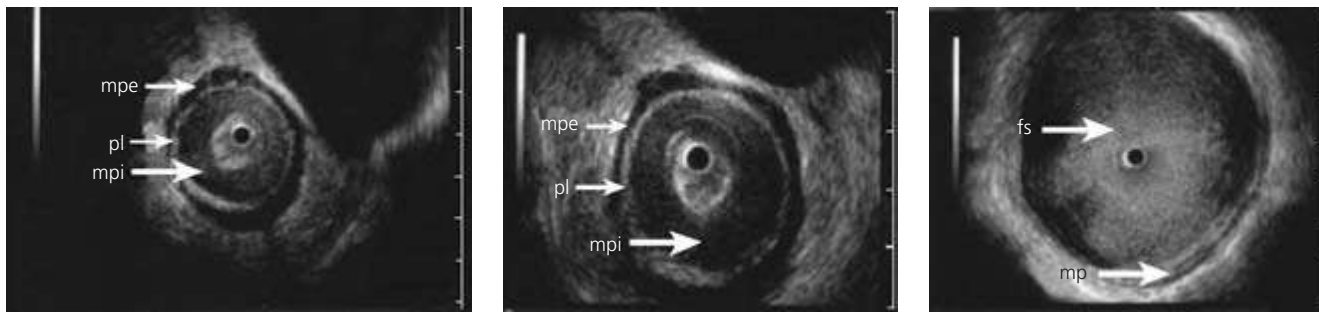
Echoendoscopes combine endoscopy with integrated ultrasound (US) transducers. They may have radial or curvilinear array transducers using frequencies between 7.5 and 30 MHz. Typically, higher frequencies provide more anatomical detail but less penetration. Small transducers or miniprobes have been developed with a diameter that allows the probe to be inserted through the biopsy channel of a conventional endoscope. The small-diameter probes can be passed into stenotic areas or areas with smaller lumenal diameters such as the biliary tree, not traversable with larger echoendoscopes. Ultrasound systems are available that combine linear compound scanning with mechanical sector scanning or M-mode. Doppler sonography can provide important information about vascular flow and separate vessels from other echo-poor structures [17].

Assessment of gastrointestinal motility and biomechanics

Endosonography has been employed in studies of the biomechanical function of the GI tract. Whereas motility considers active properties such as peristaltic contractions, biomechanics typically focuses on the active and passive forces and deformation of the tissue. Biomechanical alterations and abnormal motility in the GI tract are observed in several diseases that may involve the tract itself, the surrounding tissues, or other body systems. Lumenal manometry has so far been the most widely used method to study GI tract physiology. However, despite the development of high-resolution manometry systems problems remain in the investigation of GI motor function. The strength of a muscle contraction may vary considerably, resulting in suboptimal manometric data. Manometric data are also affected by respiration, aortic pulsation, and cardiac contractions.

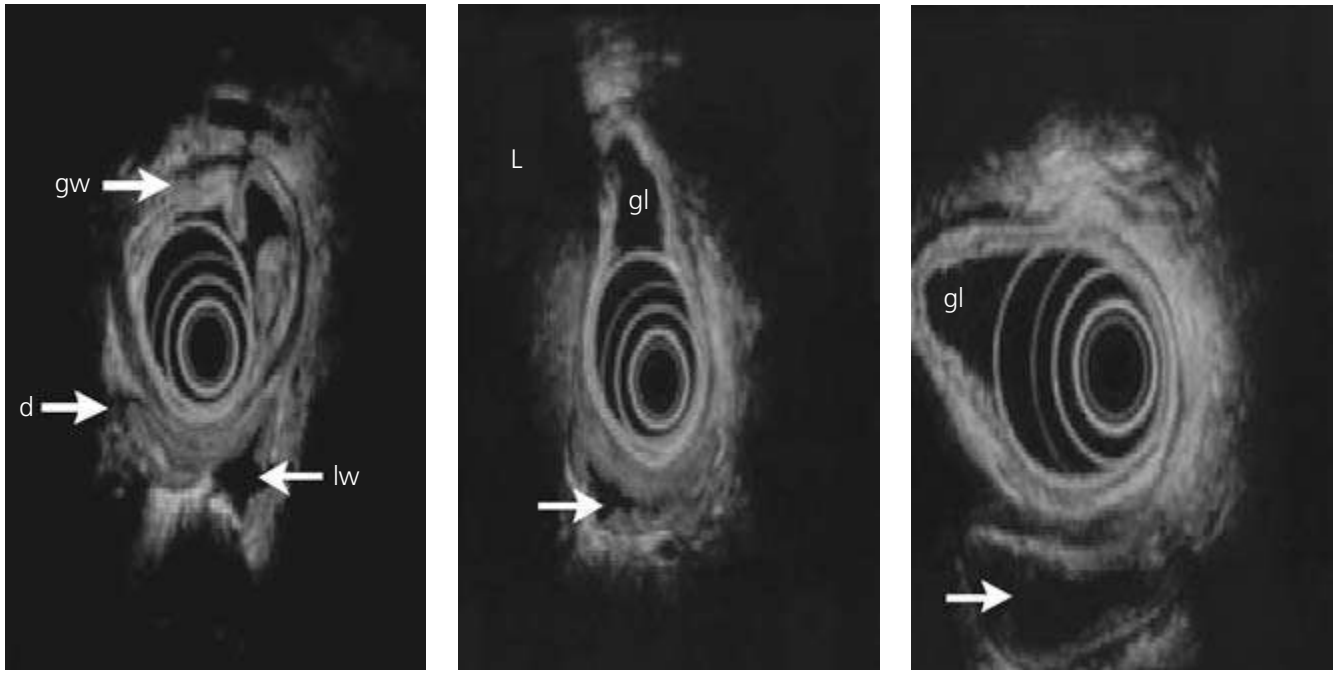
The presence of probes in the GI lumen will influence physiology and biomechanical studies would, ideally, be performed without intralumenal devices. Miniprobes combine the advantages of high ultrasound frequencies with small transducers, allowing detailed images of GI wall layers to be obtained with less influence on the GI lumen than larger echoendoscopes in areas with narrower lumens such as the esophagus (Fig. 138.4). Echoendoscopes can be used to study motility in regions with wider lumens, such as the stomach. Echoendoscopes typically utilize lower ultrasound frequencies and larger ultrasound probes that allow examination of motility in adjacent GI structures (Fig. 138.5).

Correct interpretation of ultrasound images of the GI wall is essential when using this method. Anatomically, the GI wall consists of the mucosa, submucosa, muscularis propria, and adventitia/serosa. The muscularis propria has an inner circular and an outer longitudinal layer that is separated by the myenteric plexus. The normal GI wall is imaged by ultrasonography as a layered structure consisting of three to nine layers depending on the thickness and structure of different layers, ultrasound frequency, and applied pressure on the wall [18,19]. Generally, a five-layer structure of the wall can be demonstrated, as determined by the histological layers and the appearance of interface echoes (Fig. 138.6; see also Fig. 138.4). When the visceral wall is imaged with an endolumenal transducer, the interface between the lumenal contents and the mucosa accounts for the first, echo-rich layer. The rest of mucosa appears as a second, echo-poor layer. The third, echo-rich layer usually includes the interface between the lamina propria and muscularis mucosae, the submucosa, and the interface at the border of the muscularis propria. In a



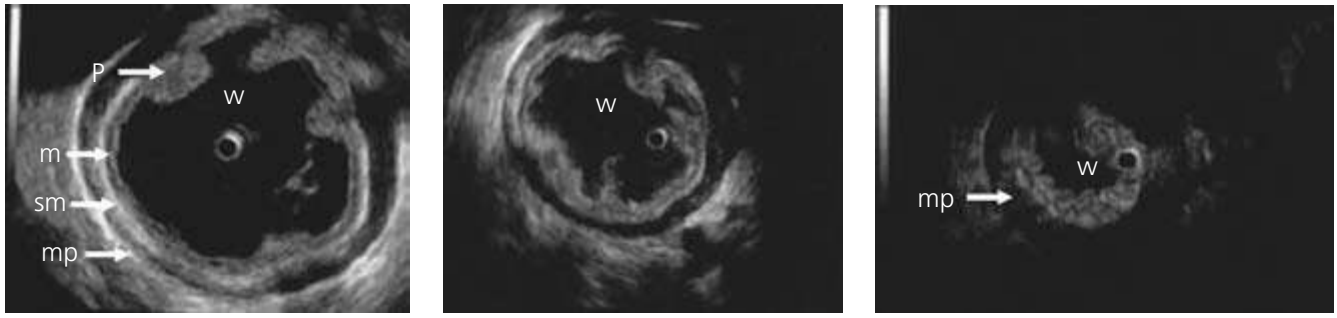
(a) (b) (c)

Figure 138.4 (a,b) Endosonographic images obtained with a 15-MHz miniprobe in a patient with achalasia showing the longitudinal, outer layer of the muscularis propria (mpe) separated from a thickened circular, inner layer (mpi) by an echogenic layer corresponding to the localization of the plexus myentericus (pl). **(c)** A dilated esophageal lumen with food content (fs). A normal aspect of the proper muscle is seen (mp). From Gregersen et al. [11], with permission.



(a) (b) (c)

Figure 138.5 Endosonography with an echoendoscope (12-MHz frequency is used) placed in the gastric lumen (gl) showing the layers of the gastric wall (gw) and gastrointesetinal (GI) loops outside the stomach. The lumen (L) is partly collapsed (d), partly waterfilled (lw) (arrows). Motility and contractions of native GI loops may be studied. High-frequency miniprobes are less suitable for this purpose due to higher frequencies and small transducer diameter. From Gregersen et al. [11], with permission.



(a) (b) (c)

Figure 138.6 (a–c) Contraction sequence demonstrated with a 15-MHz miniprobe in a water-filled (w) stomach. M, mucosa; mp, muscularis propria; p, gastric fold; sm, submucosa. From Gregersen et al. [11], with permission.

five-layer structure, the rest of muscularis propria is imaged as an echo-poor fourth layer. The fifth, echogenic layer represents the outer wall delineation and is an interface echo at the serosa or adventitia level. Sometimes, the use of high-frequency ultrasound produces an interface echo between the different aspects of the muscularis propria, probably reflecting the connective tissue embedding the myenteric plexus. A thick muscularis mucosa may also be identified as an echo-poor layer dividing the third, echogenic layer. Hence, the ultrasonographic wall structure may consist of seven or even nine different layers [18,20].

Applications of endosonography in the clinical setting to study biomechanics and GI motility

Endosonography is regarded as a suitable tool for examination of the esophagus because this portion of the GI tract is a straight tube with a relatively small diameter. Studies of the high-pressure zone between the esophagus and stomach

(lower esophageal sphincter and extrinsic crural diaphragm) in normal subjects have been performed using a dual high-resolution endosonography-manometry catheter [21]. This catheter recorded simultaneously manometric pressures and corresponding ultrasound images while transiting the distal esophagus. Simultaneous high-resolution endolumenal sonography and pressure manometry could separate the two components of the high-pressure zone in the distal esophagus.

Endoscopic ultrasound studies suggest that an association exists between sustained contraction of the longitudinal layer in the esophageal body and chest pain of unknown origin [22]. The combination of manometry with a 20-MHz ultrasound transducer has been used to correlate wall thickness, lumenal diameter, and contractive activity in healthy volunteers [23]. An increase in muscle width detected by ultrasonography correlated with the increase in lumenal pressure by manometry. Hoff et al. [24] have described a multimodal device inserted into the esophagus that combines bag distention, manometry, high-frequency intralumenal ultrasound, laser Doppler flowmetry, and symptom registration. Preliminary results indicate that these modalities can be used in combination without significant technical influence on each other. Some specific applications of endosonography are discussed below.

Achalasia

Achalasia is a disorder in esophageal motility and is defined by its typical manometry pattern. The diagnosis can be supported by findings at barium radiography that may show delayed passage through the lower esophageal sphincter and dilation of the body of the esophagus. At manometry, achalasia is characterized primarily by aperistalsis of the esophageal body and incomplete relaxation of the lower esophageal sphincter. In autopsy studies of patients with achalasia, the thickness of the lower esophageal sphincter is increased. Using high-frequency ultrasound probes, it has been shown that the thickening of muscularis propria appears mainly in the inner circular portion (see Fig. 138.6). The available literature suggests that thickening of the circular layer of the muscularis propria at the gastric cardia may support the diagnosis of evolving achalasia [25–32]. A study of the esophageal wall in patients with newly diagnosed achalasia was performed using a sector scanning echoendoscope with 12-MHz transducer frequency. The endosonographic findings were correlated with manometric features and clinical symptoms, and suggested that in these patients an increase in thickness of the muscularis propria occurs at the level of the lower esophageal sphincter and the proximal 5 cm of the sphincter [25]. Miller et al. [30] used a 20-MHz radial ultrasound transducer to examine the width of the total muscularis propria, and the circular and longitudinal smooth muscles at the lower esophageal sphincter in patients with achalasia, comparing them with 19 normal subjects. They found that the mean width of these muscles was increased in patients with achalasia. However, because of overlap in muscle width thicknesses between these groups it was concluded that a thickened muscularis propria cannot be used to differentiate clinically between patients with achalasia and normal controls. Nevertheless, they found that the mean diameter of both the longitudinal and the circular smooth muscle layers at the lower esophageal sphincter were wider in patients with achalasia than in normal subjects. Trowers et al. [31] used a single crystal transendoscopic probe providing linear images and showed that achalasia patients have a four-fold increase in the thickness of the muscularis propria compared with controls.

Scleroderma

Patients suffering from systemic sclerosis frequently demonstrate clinical gastrointestinal manifestations such as swallowing problems. Endosonography can be useful to define morphological and physiological abnormalities of the esophagus in patients with scleroderma of the esophagus. Liu et al. [33] found that US images in patients with scleroderma correlated with established pathological descriptions like smooth muscle atrophy, variable fibrosis, and collagen deposition. Specifically, increased echogenicity and thinning of the muscularis propria, consistent with fibrosis and atrophy of the smooth muscle were observed. Miller et al. [34] used a 20-MHz ultrasound transducer to evaluate patients with systemic sclerosis and healthy subjects. Postmortem autopsy studies were also performed to compare histopathological abnormalities with sonographic findings in the esophagus. Hyperechoic abnormalities were observed ultrasonographically in the muscularis propria in patients with scleroderma but not in normal controls. These findings corresponded to fibrosis noted in histological examinations. A positive correlation between esophageal hyperechoic and manometric abnormalities was also observed.

Nutcracker esophagus

Patients with nutcracker esophagus (NE) experience dysphagia or chest pain due to strong peristaltic contractions. High-pressure amplitudes are found at manometry. Melzer et al. [35,36] used an echoendoscope to examine the thickness of the muscularis propria at the gastroesophageal junction and in the lower, middle, and upper esophagus in patients with nutcracker esophagus. The muscularis propria was thickened in one-third of the patients. This thickening, however, did not correspond to the location and magnitude of the manometric abnormality and did not correlate with the clinical presentation. They also reported a patient with nutcracker esophagus and esophageal pressures exceeding 800 mmHg in whom endoscopic ultrasonography demonstrated a markedly thickened esophageal muscularis propria. The hypertrophy of the muscularis propria extended from the gastroesophageal junction upward to the mid-esophagus.

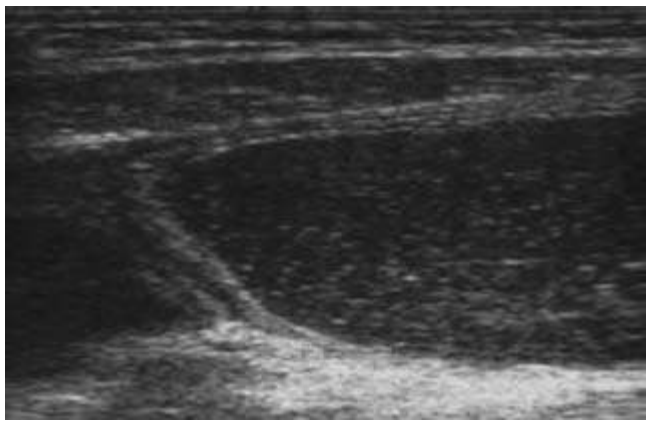


Figure 138.7 Ultrasonogram of the fluid-filled gastric antrum showing the different wall layers and a propagating contraction wave that occludes the lumen. From Gilja et al. [43], with permission.

Gastroduodenal motility

Ultrasonography can provide both qualitative and quantitative information about motility, both in a fasting state and after meal ingestion. Gastric contractions and propagation of waves can be visualized and evaluated by ultrasound [37–43]. Both frequency and amplitude can be monitored; the latter defined as the maximal reduction of antral area induced by a contraction, as a fraction of the relaxed area. High-resolution ultrasound using frequencies in the range 7–15 MHz enables detailed studies of gastric wall layer involvement during peristalsis (Fig. 138.7) [11]. Ultrasound is more sensitive than manometry in detecting antral contractions, particularly nonocclusive contractions [44].

Endosonography can be technically more challenging in wide and curved organs such as the stomach. Transabdominal ultrasound is applied in studying gastroduodenal motility, also in combination with intralumenal pressure devices. Mechanosensory properties in the human gastric antrum were evaluated using transabdominal ultrasound scanning during volume-controlled antral distention [45,46]. Furthermore, a device that combines endosonographic real-time imaging with intralumenal volume-controlled bag distentions has been developed also for use in large lumens. Thus, detailed images of GI wall layers corresponding to the area of bag pressure can be obtained. Arslan et al. [47] investigated the duodenum with high-frequency ultrasound to study wall layers and motility during allergenic provocation. A 20-MHz miniature ultrasound probe was inserted through a nasoduodenal tube and ultrasound examination was performed before and after provocation. Clinical symptoms were recorded and the thickness of mucosa, submucosa, and muscularis propria was measured directly on ultrasonographic images. Endosonographic changes (i.e., detectable wall thickening, contraction, or new echogenic layer) were observed in 75% of the patients. Mucosal thickening, changes in the number of wall layers, and sustained contractions of duodenum were observed in some patients after provocation. The mucosal thickening may represent mucosal edema in response to provocation manifesting in an allergic reaction. The clinical usefulness of endosonography in the investigation of gastroduodenal motility still remains to be determined.

Novel techniques and applications

Three-dimensional (3D) endoscopic ultrasonography (EUS) images are thought to be easier to understand and communicate than the mental reconstruction of 2D images. Three-dimensional techniques are under development for most imaging modalities and 3D-EUS may be applied for improved recognition of the GI anatomy and pathological lesions. Images acquired by echoendoscopes or miniprobes can be used in constructing 3D-EUS images. Typically, a pullback device has been employed to obtain parallel 2D images, which are reconstructed to create a 3D image. Acquired ultrasound images can be stored and digitized using frame grabbing, but direct digitizing of raw ultrasound data is being increasingly used in new scanners. Advanced software programs containing different rendering or other reconstruction algorithms are used for postprocessing and display of 3D ultrasound data, which can be studied using any plane slicing, segmentation, and volume calculation [48,49]. The advanced programs enable the import of image data that are acquired with both mechanical devices and magnetic position sensors as well as endosonographic acquisitions. Manual segmentation or semiautomatic rendering of structures in the 3D data set makes accurate volume estimation and reconstruction of organs or pathological tissue achievable. A representative display from a 3D ultrasound system is shown in Fig. 138.8. Additional information regarding 3D ultrasound systems is available in the literature [50–52].

Strain rate imaging

Strain rate imaging (SRI) is a technique to measure deformation of biological tissue. The technique is based on tissue velocity imaging, which is an ultrasound technique that provides quantitative information on velocities of deformation within a tissue. By color-coded tissue velocity imaging, velocity data from the whole field of view are available simultaneously [53]. This allows extraction of other parameters through spatial and temporal processing of the velocity data. Strain and strain rate are examples of such parameters. The methods have primarily been used in echocardiography, but other uses have also been reported, including measurements of gastric motor function [54]. For example, estimation of relative strain of the muscle layer of the gastric wall by Doppler ultrasonography is feasible (Fig. 138.9) and enables detailed mapping of local strain distribution [55,56]. Strain

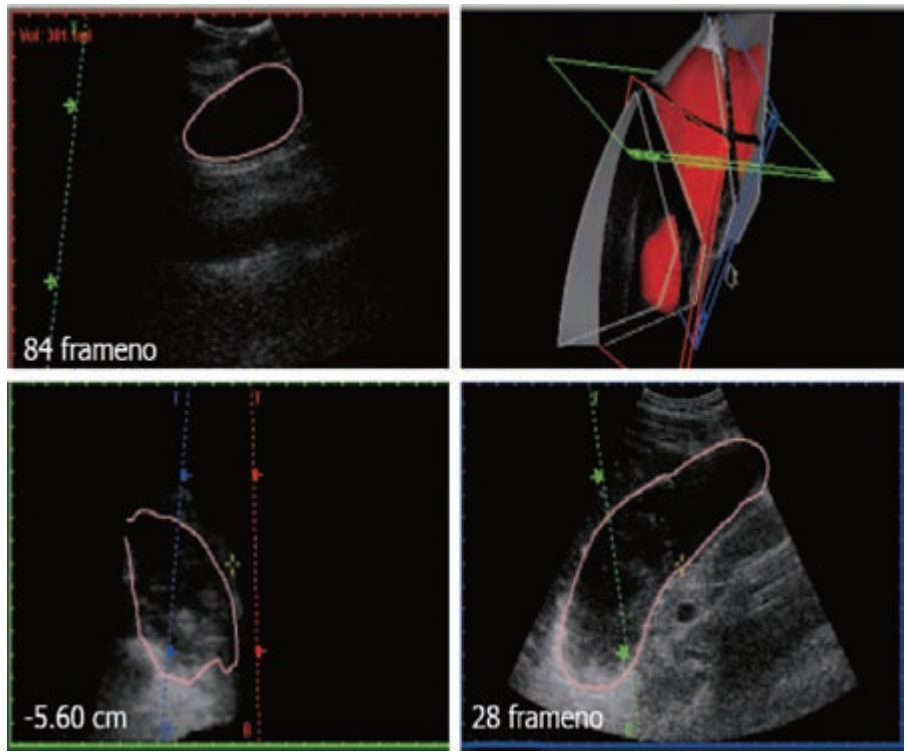


Figure 138.8 A three-dimensional reconstruction of the total stomach volume (red) in the upper left panel. The acquisition is based on magnetic scanhead tracking. Three orthogonal orientation planes are shown in red, green, and blue. The upper left panel is a volume reconstruction window for manual outlining of the structures. From Molin et al. [48], with permission.

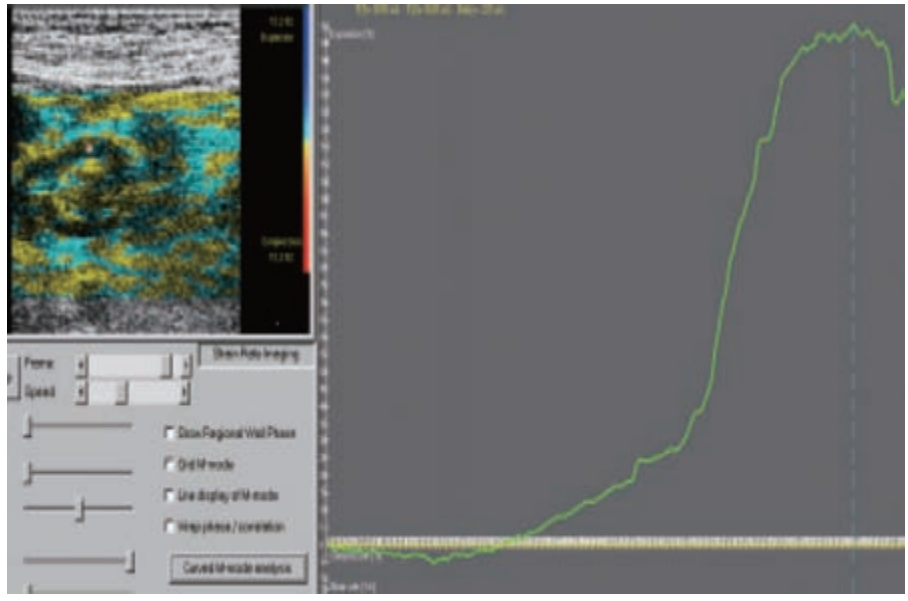


Figure 138.9 Graphics outlining the relative radial strain of the circular muscle layer of the antral wall. The exact sampling point in the gastric wall is denoted with a red marker in the color Doppler ultrasonogram **(left panel)**. **Right panel:** positive strain curve with a maximum of 150% radial elongation of the muscle layer. From Gilja et al. [54], with permission.

rate imaging can distinguish contractile activity of the longitudinal and circular muscle layers, although the two layers cannot be separated visually in the 2D images. It is anticipated that ongoing and future studies will determine whether SRI can be applied to evaluate the relation between symptoms and biomechanical factors in clinical disorders.

Further development of specialized EUS instruments will allow investigators to choose the most appropriate instruments for the specific tasks. Miniature probes, which are less costly and easier to operate, will likely be available in a majority of endoscopy units in the near future. We can anticipate that different modalities will be merged into new

multimodal devices making it possible to obtain multiple motility parameters in one procedure.

Assessment of gastric emptying, accommodation, and volume

Gastric scintigraphy

Assessment of gastric emptying of solids using scintigraphy has evolved over the years. Typically, scans are obtained at hourly intervals for 1–4 h. The longer scanning times potentially provide data on the gastric residuals at 4 h rather than a calculated $t_{1/2}$ (half emptying time for solids) value derived from a computer program-generated estimate of the gastric emptying curve based on 60-min or 90-min data. However, the added cost of extending the scanning time must be considered. Gastric scintigraphy has been standardized for practice and research (including multicenter studies) [57,58]. Interventions can then be evaluated based on documented abnormalities in gastric emptying rather than, for example, response to empirical therapy in patients with subjective symptoms such as postprandial fullness, nausea, bloating, or pain. A well-recognized problem with methods for assessing gastric emptying is that patients' symptoms correlate poorly with abnormal emptying; for example, symptoms are not sufficiently predictive of the nature (slow vs fast vs normal emptying) and the severity of the gastric emptying disorder. It is relevant that the coefficient of variation is lowest for the proportion of ingested food remaining in the stomach at 4 h [59]. Therefore, this time point appears to be a good index for diagnosis and a biomarker in pharmacodynamic studies.

Assessment of gastric accommodation and volume

The gold standard for the measurement of tone in hollow organs remains the barostat [60], which estimates changes in tone by the change of volume of air in a compliant bag maintained at a clamped or constant pressure. A related method is the tensostat [61,62], which corrects in real time for the changes in volume or diameter of the balloon to estimate lumenal wall tension based on the Laplace law. The barostat/tensostat techniques are unequalled for measuring colonic tone, such as in the assessment of megacolon [63] and suspected colonic inertia. It is also possible, for example, to measure tissue mechanical properties or symptoms during tone change, or to assess reflex responses to distention [64,65]. However, a limitation is that the barostat measures a volume within a balloon under constant pressure rather than true tone, volume, or tension. Also, these tests are invasive and unacceptable for some patients who are stressed and uncomfortable during these procedures, which may last over 3 h. These factors may introduce a measure of uncontrolled variation and may lead to erroneous results. Estimates of viscus tension based on the Laplace law are often approximations [66,67]. In addition, intubation and balloon distention under low constant or barostatic pressure may result in reflex relaxation of the stomach; therefore, a true baseline fasting volume cannot be estimated.

Given these concerns in the measurement of gastric accommodation and capacity, volume-based methods have been proposed to measure gastric capacity during fasting and postprandially. A liquid nutrient drink test was proposed as a surrogate for the barostat [68], but the accuracy relative to other volumetric measurements such as SPECT was poor [69]. Imaging-based volume methods include analysis of surface geometry of human stomach using real time 3D ultrasonography or 3D reconstruction of images acquired by ordinary ultrasonography assisted by magnetic scan-head tracking [70]. In an application of ultrasonography [71] an outline of the total stomach volume was visualized after ingestion of a soup meal that serves as a contrast medium.

Single-photon emission computed tomography imaging has been extensively validated in vitro, and for the measurement of gastric volumes during fasting and postprandially in vivo in comparison with the "gold standard," the barostat [72,73]. The effects of liquid and solid equicaloric meals on gastric volumes have been reported, and reproducibility of measurements of gastric volume with the same caloric liquid meal given an average of 9 months apart shows a coefficient of variation of approximately 10% [74]. Single-photon emission computed tomography has been applied to examine the effects of disease on postmeal change in gastric volume, a surrogate of gastric accommodation [75,76], and the effects of medications such as nitrates, erythromycin, glucagon-like peptide 1 (GLP-1) and octreotide [77,78] in health and in diseases such as diabetes [79]. In general, the effects of these interventions were similar to the results observed with the barostat in the published literature.

A novel method has been reported that measures gastric "accommodation" as the percentage change in planar (2D) gastric CSA using a left anterior oblique planar projection and the percentage change in total SPECT gastric voxel counts (by 3D imaging) compared with baseline fasting volume using National Institutes of Health (NIH) image software; see the NIH ImageJ website (http://rsb.info.nih.gov/ij/index.html). The procedure uses anterior and posterior images for estimation of gastric emptying, followed by SPECT imaging with a separate SPECT camera every 20 min [80]. This approach is potentially significant because it allows simultaneous measurements of gastric emptying and volume, thereby providing a means noninvasively to assess the pathophysiology of gastric motility disorders.

Other studies confirm the ability to measure the dynamics of gastric volume and emptying functions in health [81]. One

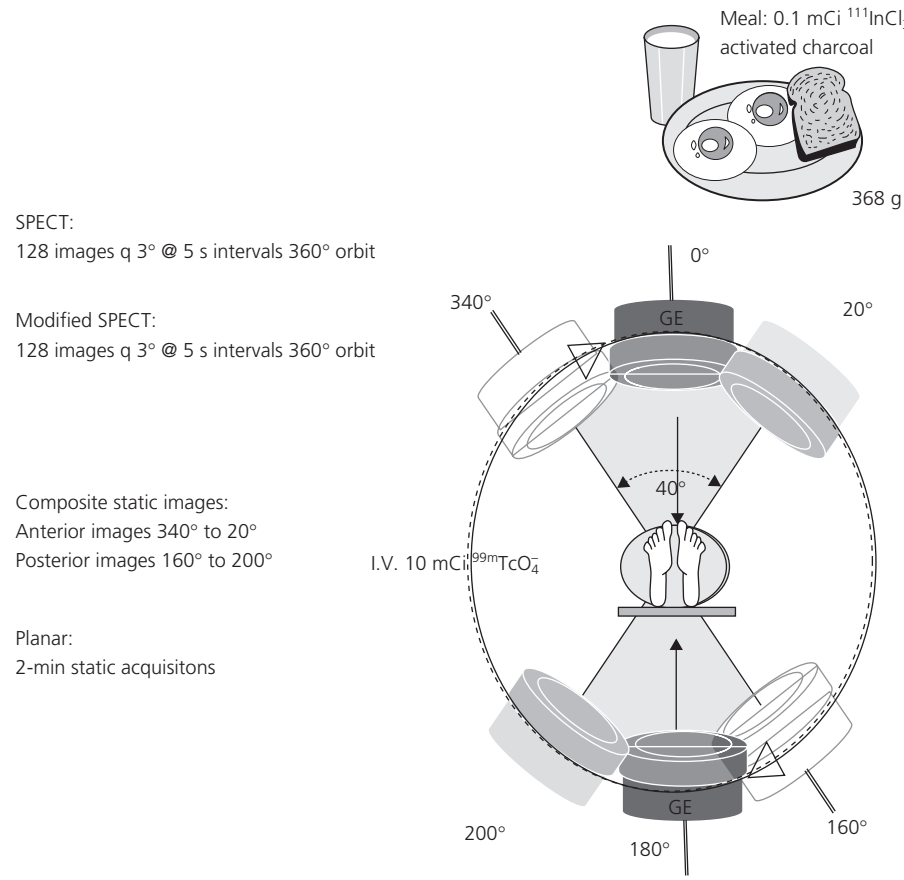


Figure 138.10 Method for combined measurements of gastric volume and emptying. SPECT, single photon emission computed tomography. Camilleri [80], with permission.

approach involves tomographic acquisition over 360° on a dual-head gamma camera SPECT (Fig. 138.10). Specialized software is used to acquire the required planar images from the tomographic data and integrate them into the gastric emptying study and to measure gastric accommodation (Fig. 138.11). The results suggest that the volume measured is not just the volume of the meal in the stomach.

Magnetic resonance imaging (MRI) of the stomach (Fig. 138.12) [82] has the potential of being a safe, noninvasive test that measures emptying, volume change, and wall motion as a surrogate of contractile activity without radiation exposure [83,84]. It is noteworthy that MRI has the ability to assess separately the emptying of fat and water from the stomach [85]. However, MRI of the stomach requires additional validation, such as comparison with barostat, including studies in disease and response to interventions including but not limited to the effects of nutrients and prokinetic drugs. The ability to resolve wall movement and to apply acquisition and projection algorithms developed for dynamic imaging in other indications, such as MR angiography, and the absence of radiation exposure make MRI of gastric function a potentially attractive technology, as long as it is affordable. Magnetic resonance imaging of the small intestine is in the developmental stages [86]. The resolution of abdominal organ imaging is enhanced with techniques such as respiratory gating and multiarray imaging using two surface MRI coils [87].

Motility measurements of the pelvic floor, pelvic organs, and anorectum

Distention-induced deformation of the intestinal wall activates mechanosensitive afferents in the mucosa, muscle layers, and serosa [88]. However, mechanoreceptors are not directly sensitive to volume or pressure [89–91]. Therefore, traditional distention data based on pressure–volume measurements may not be optimal [92–94]. The degree of tissue deformation (strain) and mechanical forces (stress) in the circumferential direction appear to determine activation of mechanoreceptors [95]. Analysis of the contractile state of the smooth muscle may be essential in understanding mechanosensory transduction [96]. As discussed previously, distention technology has been refined by the introduction

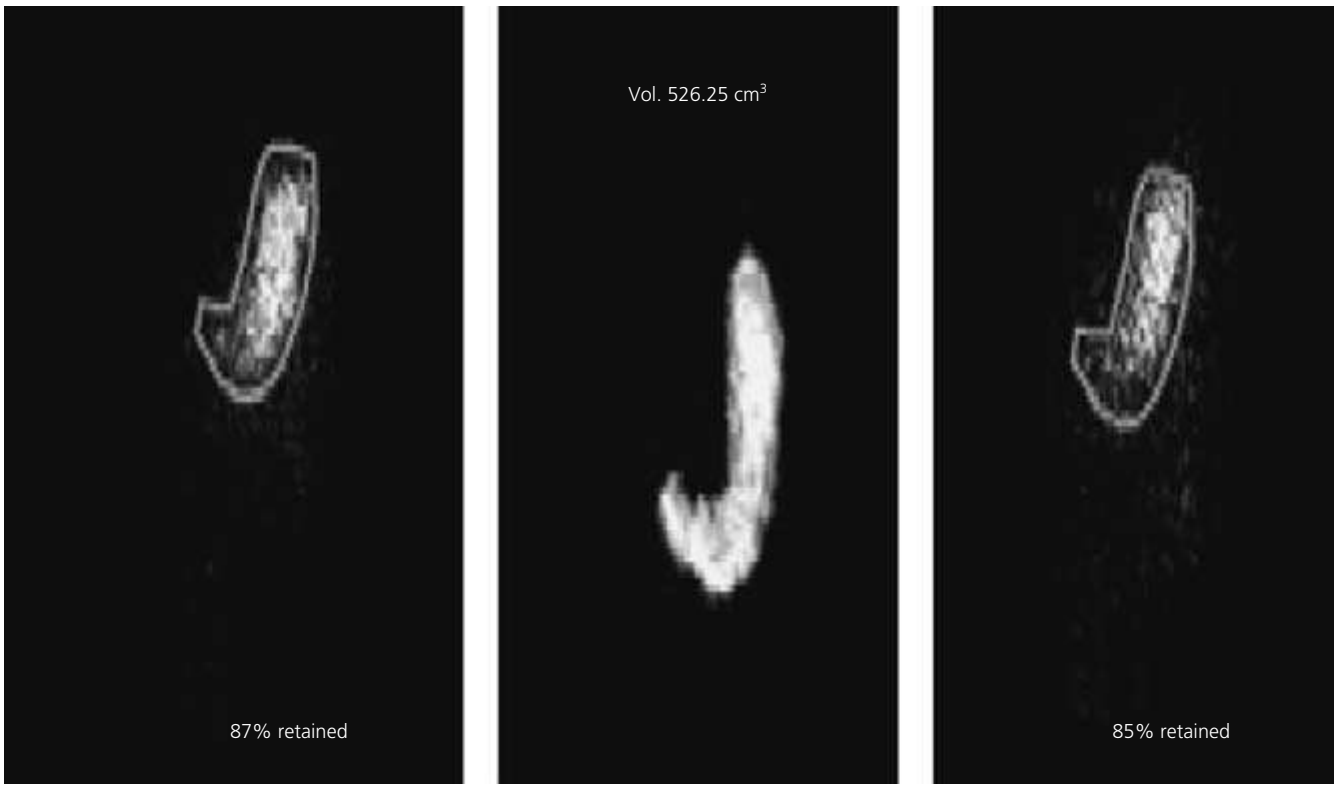


Figure 138.11 Images of the stomach by planar scans, single-photon emission computed tomography (SPECT), and composite SPECT acquisition to construct anterior image using the method described by Burton et al. [81]. Note that the amount of food retained in the stomach is similar when calculated using the traditional two-dimensional or planar anterior and posterior gamma camera images. The central image demonstrates the stomach volume simultaneously estimated using SPECT. From Camilleri [81], with permission.

of impedance planimetric techniques with assessment of circumferential strain, tension, and stiffness [97–104]. However, impedance planimetry using a single CSA measurement has limitations, making it difficult to characterize the biomechanical properties of regions such as the large bowel and rectum because of the complex and dynamic geometry of these segments. Therefore, methods based on cross-sectional imaging have been developed to assess the geometric variables in nontubular segments of the gut, that is, ultrasonography of the stomach and MRI of the gallbladder and rectum [105–108]. Magnetic resonance imaging has an outstanding safety profile and provides excellent soft tissue imaging capabilities. Also, MRI is well suited for studying the gastrointestinal geometry. It is possible to model the 3D geometry and mechanical properties of complex gastrointestinal segments, including multidirectional deformation and the spatial distribution of the 3D principal curvatures, radii of curvature, wall thickness, tension, and stress of the rectum [107]. Cross-sectional imaging and 3D analysis also allow assessment of the contribution and effect of the smooth muscle activity by studying the muscle relaxation during administration of stimulatory or inhibitory drugs.

Therefore, MRI of the pelvic floor has the potential to characterize several components of continence and defecation simultaneously. Dynamic imaging (Fig. 138.13) and the use of magnets that allow for studies in the seated position provide detailed information about structure and integration with function that was not possible using a combination of techniques such as barium defecography and endoanal ultrasound [109,110]. For example, a report demonstrates the occurrence of different phenotypes of obstructed defecation, abnormal structure and function of the pelvic floor and anal sphincters in people with incontinence, and physiological alterations associated with aging [111]. Frokjaer et al. [112] used a flexible PVC catheter equipped with a bag for gut distention and a radiopaque ring allowing proper positioning of the probe to examine sigmoid colon biomechanics in conjunction with MRI (Fig. 138.14). The geometry of the distended sigmoid colon was complex and varied considerably between the subjects, as depicted in Fig. 138.15. The

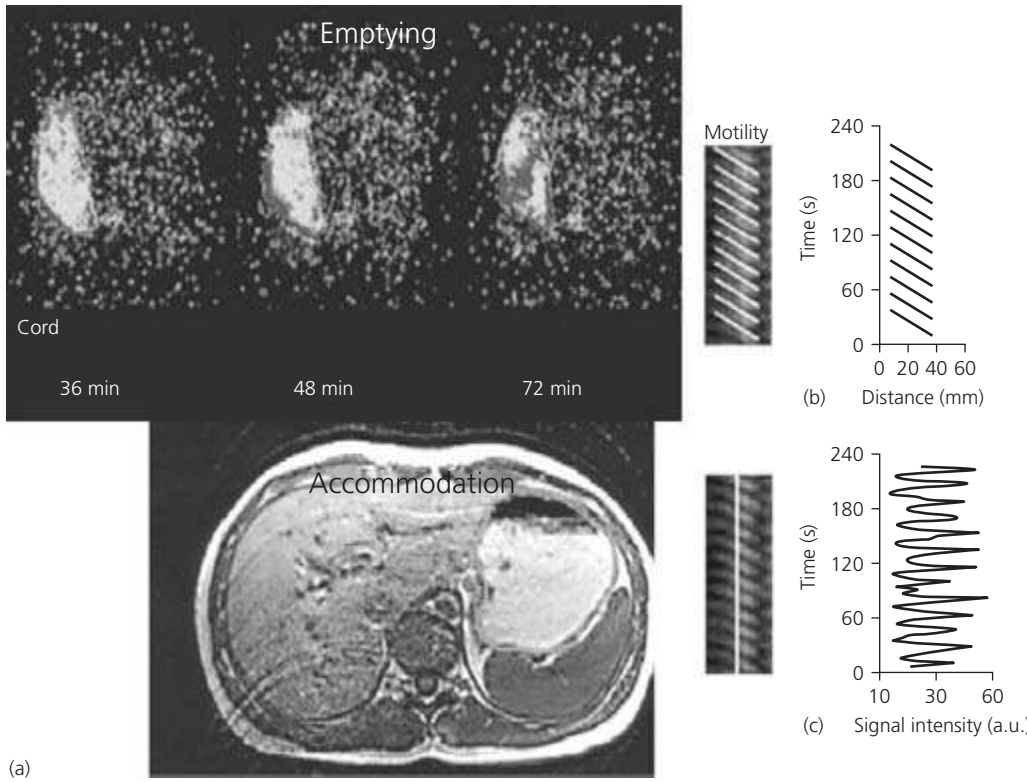


Figure 138.12 Magnetic resonance imaging provides the potential to estimate gastric emptying, accommodation, and wall motion in a noninvasive manner. From Camilleri [81], with permission.

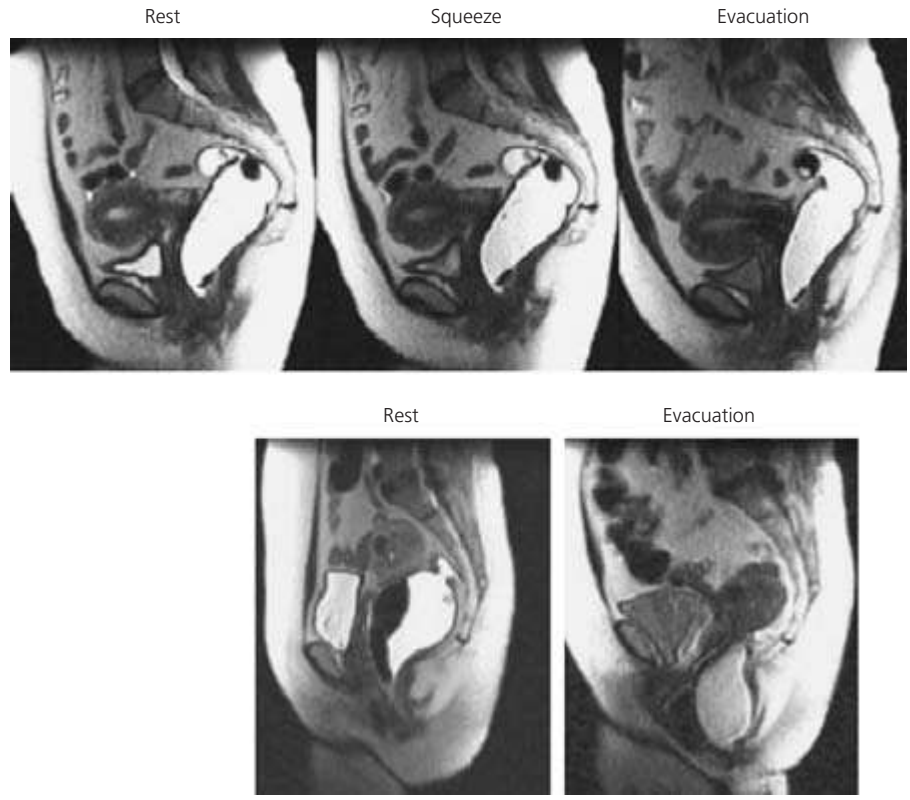


Figure 138.13 Defecation dynamics in patients with evacuation disorders due to immobile perineum **(upper)** or descending perineum syndrome **(lower)** using magnetic resonance imaging. From Bharucha et al. [109], with permission.

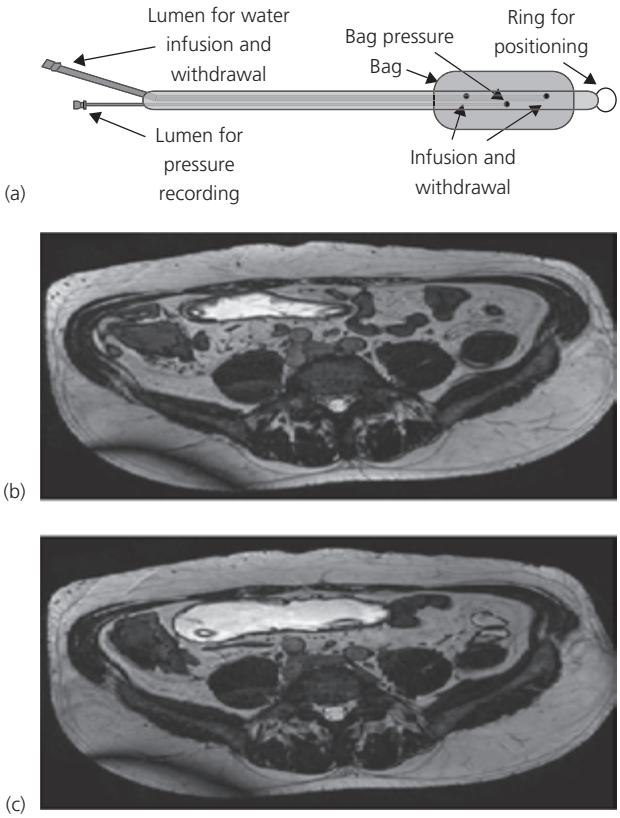


Figure 138.14 **(a)** The probe that allows bag distention and pressure measurements during magnetic resonance imaging (MRI). Transverse MRI shows the distended waterfilled bag (high signal intensity) in the sigmoid colon at 100 mL **(b)** and 300 mL **(c)**. From Frokjaer et al. [110], with permission.

circumferential radii of curvature increased, that is, increased in diameter, during the sigmoid distention but showed a highly inhomogeneous spatial distribution that was observed in all subjects. Histograms illustrating the distribution (frequency diagram) of the circumferential wall stress and wall thickness throughout the sigmoid wall are shown in Fig. 138.16. The histograms reveal "bell-shaped" distributions with an overall increase in wall stress and tendency to a decrease in peak wall thickness during the distention. The sensation induced by distention before and after administration of the muscarinic receptor antagonist butylscopolamine is shown in Fig. 138.17 as a function of bag volume, bag pressure, and the average values of the 3D distribution of the circumferential strain, circumferential wall tension, and stress. Administration of butylscopolamine did not affect the wall thickness, circumferential length, and strain. Bag pressure and wall stress were reduced by smooth muscle relaxation. Therefore, sensory response decreased as a function of circumferential strain, that is, right shift of the stimulus–response curves in the presence of butylscopolamine. The inhomogeneous and complex 3D sigmoid geometry suggest that this type of 3D modeling will likely be useful and provide reliable data for detailed studies of the biomechanical and mechanosensory properties. This technique may be useful in the clinical setting for understanding biomechanical properties and visceral perception in several hollow visceral organs.

Functional brain imaging in assessing visceral sensation in health and disease

We include a section on functional brain imaging because there is general acceptance that selective activation of brain regions occurs in response to regional distention of the gut in health, and emerging data support differential activation of brain regions in patients with functional bowel disorders (FBDs) such as the irritable bowel syndrome (IBS) and nonulcer dyspepsia [113]. For example, several reports of positron emission tomography (PET) or functional MRI (fMRI) suggest that the limbic and emotional motor system in the brain may be differentially activated in patients with functional gastrointestinal diseases [113,114]. However, brain imaging in FBDs requires additional technical and methodological advances to clarify the reported differences in regional brain activation and their relationship to stress, pain, and emotion [115]. The areas of activation often involve neural circuitry of several interacting regions of the brain, which is not addressed in some reports. For example, regions in the cerebellum can be activated during rectal distention without any clear rationale about why this occurs. Other unresolved issues include the imaging differences between PET and fMRI and differences in brain regions activated in males and females in response to painful rectal distention. Methodological concerns include:

- the confounding effects on imaging of the distention itself vs anticipation of the distention
- the potentially significant central effects of placebo controls
- inclusion of clinically heterogeneous patients (e.g., diagnosis and severity of disease)
- existence of comorbidities such as fibromyalgia [116,117].

For example, anterior cingulate cortex activation during somatic pain appears to reflect anticipation [118]. This was confirmed in patients with functional gastrointestinal disorders [116]. However, it has also been reported that actual and anticipated esophageal pain elicits similar cortical responses [119].

Other issues that influence the interpretation of imaging studies include the lack of instrument or protocol standardization, low "signal-to-noise" ratios, and limitations in measuring functionally heterogeneous regions, such as the cingulate and other brain regions. The time course of activa-

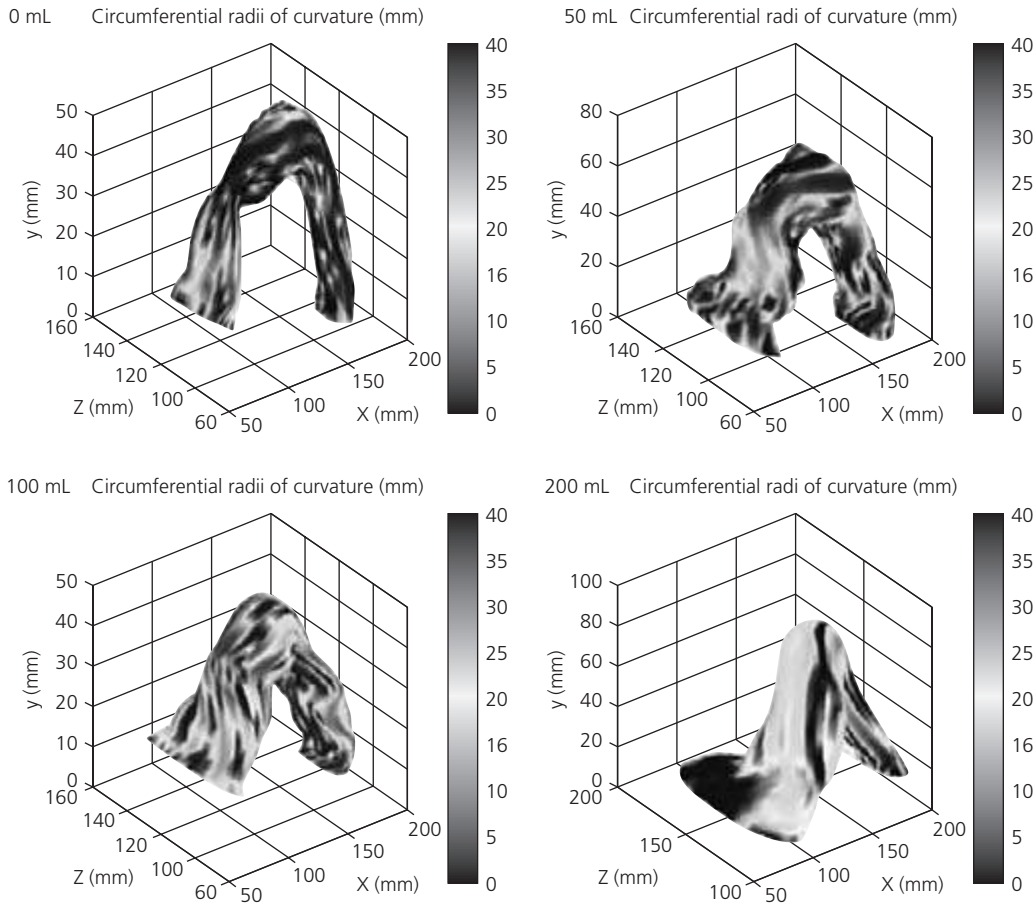


Figure 138.15 The three-dimensional distribution of circumferential principal radii of curvatures in one volunteer before administration of butylscopolamine at infused volumes of 0, 50, 100, and 200 mL. From Frokjaer et al. [110], with permission.

tion of different brain regions in response to stimulation has also resulted in relevant information. For example, cortical neural activity evoked by experimental esophageal pain is processed simultaneously in somatosensory and posterior insula regions. In contrast, activity in the anterior insula and cingulate brain regions that process the affective aspects of esophageal pain occurs significantly later than in the somatosensory regions, and no gender differences were observed with this experimental paradigm [120]. In addition, the timing and intensity of brain activation may be different in health and disease. For example, cortical activity associated with perceived and unperceived esophageal acid exposure in gastroesophageal reflux disease (GERD) patients and healthy controls, respectively, involves multiple brain regions, but it occurs more rapidly and with greater intensity in GERD patients than the activity in response to subliminal acid exposure in healthy controls [121]. Another marker of hypersensitivity in IBS may be detected by an enhanced response to subliminal stimuli [122]. Activity on fMRI was studied in IBS patients and age- and gender-matched healthy controls using a computerized barostat-controlled rectal distention device that generated equal subliminal distention pressures (e.g. 10, 15, and 20 mmHg) in both controls and patients. In all three distention levels, the fMRI activity in IBS patients was significantly greater than in controls, supporting the existence of hypersensitivity in this patient group irrespective of stimulus-related cognitive processes (Fig. 138.18).

In conclusion, the application of functional imaging techniques to neurogastroenterology research has resulted in significant advances in our understanding of the brain–gut axis in health and disease. Some of these methods are associated with significant radiation burden and would benefit from improvements in experimental design and standardization of methods. The experimental designs also suffer from limitations of statistical and experimental approaches to study circuits and networks simultaneously and sequentially

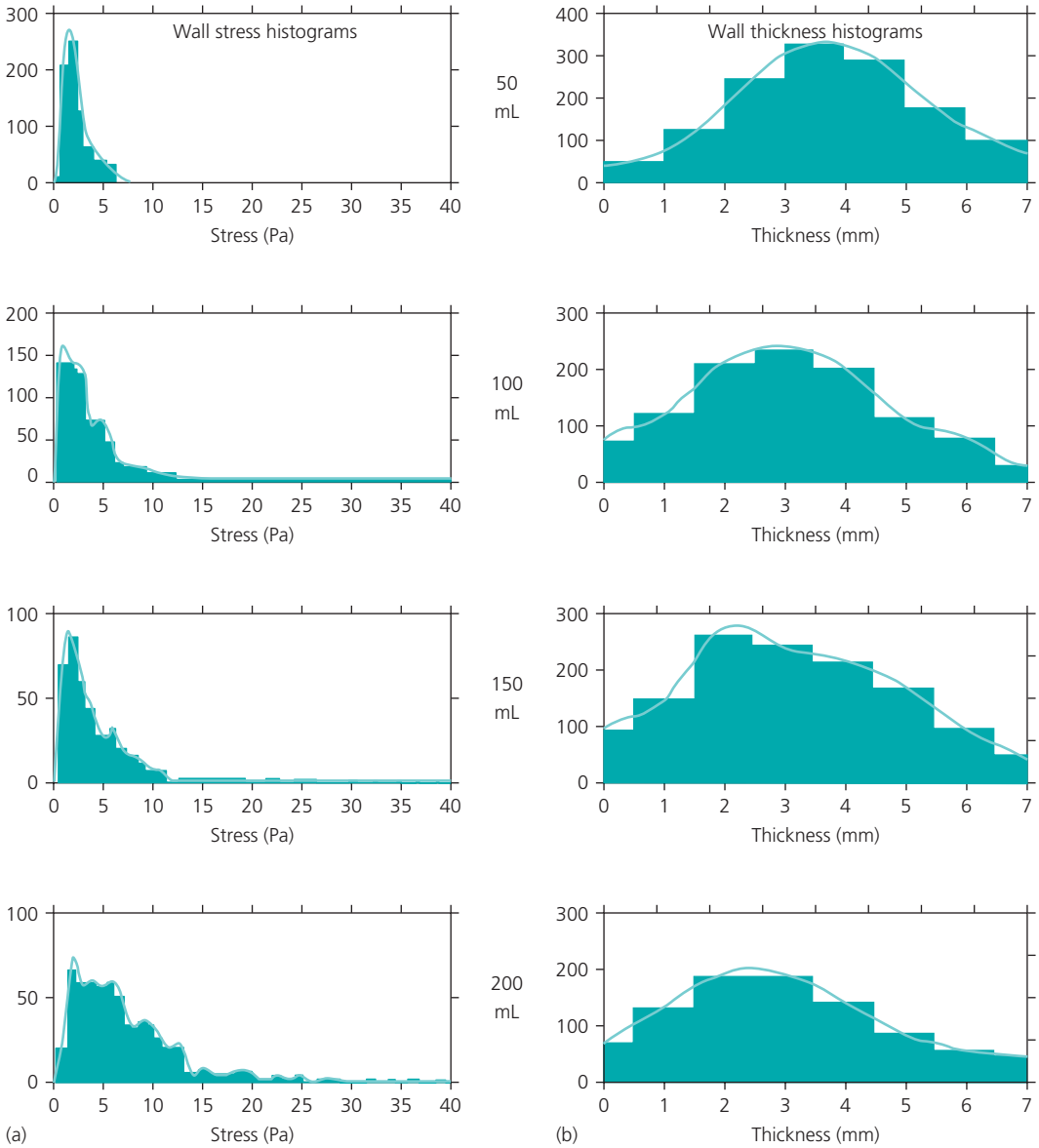


Figure 138.16 Histograms illustrating the distribution of **(a)** wall stress and **(b)** wall thickness before administration of butylscopolamine throughout the three-dimensional sigmoid surface in one subject. The y-axis indicates the number of surface squares. During distention, the distribution of stress clearly shifted to the right, whereas the distribution of wall thickness was not affected to the same degree. The same pattern was seen in all subjects. From Frokjaer et al. [110], with permission.

in "real-time." Advances in technology will likely resolve this problem in the future.

Validation of new technologies

Further validation of the emerging technologies discussed in this chapter is required for research purposes and before their routine application in clinical practice. Validation should include critical appraisal of:

- the internal validity (accurately drawing conclusions about the source population based on information from the study population) and external validity (independent, blind comparison with a reference standard)
- the technology's performance (in particular, reproducibility and coefficient of variation)

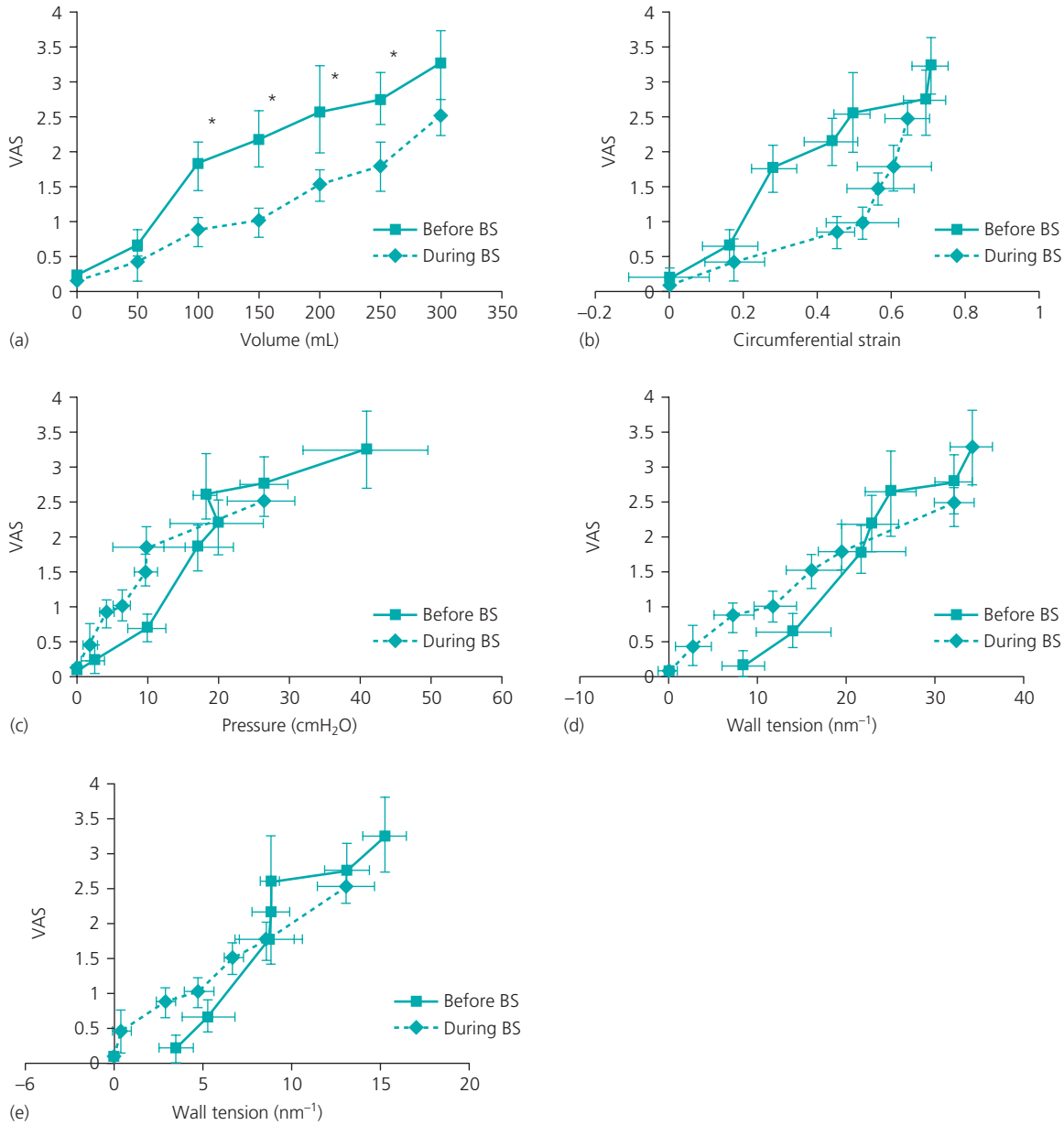


Figure 138.17 The sensory response rated on a visual analog scale (VAS) is illustrated as a function of infused bag volume **(a)**, average three-dimensional circumferential strain **(b)**, bag pressure **(c)**, circumferential tension **(d)**, and stress **(e)** before and after administration of butylscopolamine. Asterisk (*) indicates significant difference for the volume data. BS, butylscopolamine. The error bars indicate standard error of the mean (SEM). From Frokjaer et al. [112], with permission.

- the technology's sensitivity (proportion of true positives of all positives) and specificity (proportion of true negatives of all negatives)
- the likelihood ratios (i.e., the link between the pretest probability of the diagnosis of a disorder and the probability after the test)
- the responsiveness of the diagnostic test to change and the relationship between that change and a change in clinical outcome
- the cost–benefit of the technology either as a diagnostic tool or therapeutic intervention for acceptance in the clinical setting.

These are the standards to which diagnostic tests are held in other areas [123,124].

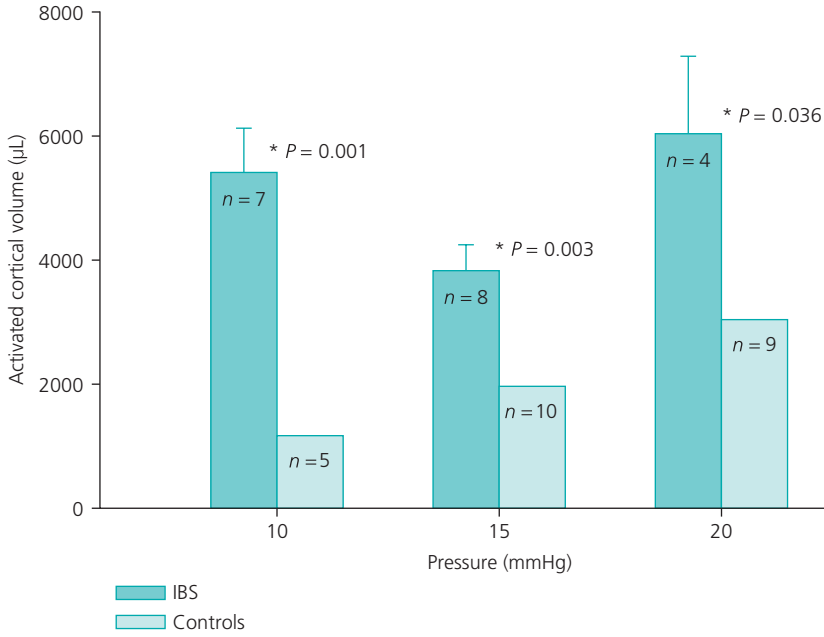


Figure 138.18 Total fMRI cortical activity volume response to three levels of subliminal rectal distention pressures in patients with irritable bowel syndrome and control subjects. In all three subliminal distention pressures the fMRI activity volumes in patients with IBS were significantly larger than those of control subjects. Furthermore, fMRI cortical activity volumes showed a stimulus intensity-dependent relationship in control subjects ($P < 0.001$), but not in patients with IBS for the three analyzed pressure levels. From Lawal et al. [120].

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139 Tests of gastric and exocrine pancreatic function and absorption

William D. Chey, Matthew J. DiMagno, William Y. Chey

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In this chapter, we review the indications and currently available methods of assessing gastric secretory and pancreatic exocrine function. For a detailed review of the physiology of gastric secretory and pancreatic exocrine function, the reader is referred to Chapters 13 and 15.

Tests of gastric secretory function

The clinical applications of gastric acid analysis and prolonged intragastric pH monitoring are limited. However, these tests remain critical for better understanding the effects of various disease states and pharmaceuticals on gastric secretory function. Gastric acid analysis and prolonged intragastric pH monitoring have played a pivotal role in understanding the link between *Helicobacter pylori* infection and the changes in acid secretion documented in patients with peptic ulcer disease decades ago. As the *H. pylori* story has taught us, groundbreaking discoveries of the future will require ongoing reevaluation of our understanding of gastric secretory function. As such, the methodology and strengths and weaknesses of gastric acid analysis and prolonged intragastric pH monitoring continue to be worthy of review.

Gastric acid analysis

Indications

Traditionally, physicians have utilized gastric acid analysis to answer the following questions.

- Does the stomach produce acid?
- If so, what is the basal or unstimulated gastric acid secretion?
- How much acid is secreted in response to physiological or pharmacological stimulation?
- Is the vagal innervation to the stomach intact?

In recent years, the development of less invasive and less cumbersome diagnostic modalities for acid peptic disorders has decreased the relative importance of quantitative gastric acid analysis. In addition, the availability of proton pump inhibitors, which potently and reliably suppress gastric acid secretion, has decreased the number of circumstances in which quantitative gastric acid analysis is necessary. Nonetheless, gastric acid analysis remains the gold standard for evaluating gastric secretory function and is useful for the gastroenterologist or gastrointestinal surgeon in several clinical situations (Table 139.1).

Gastric analysis remains helpful in determining whether an elevated circulating gastrin level is physiological (achlorhydria) or pathological (acid hypersecretory states). Gastric analysis can establish that hypergastrinemia is an appropriate consequence of the inability to produce acid as seen in the setting of atrophy, pernicious anemia, or malignancy involving the body of the stomach [1]. On the other hand, hypergastrinemia can be seen in diseases of excessive acid secretion such as Zollinger–Ellison syndrome [2].

In the past, gastric acid analysis played a crucial role in the diagnosis of Zollinger–Ellison syndrome. However, the development of secretin stimulation testing and accurate imaging modalities such as high-resolution computed tomography (CT), magnetic resonance imaging (MRI), endoscopic ultrasound (EUS), and somatostatin receptor scintigraphy

Table 139.1 Indications for gastric acid analysis

Evaluate an elevated fasting plasma gastrin level
Physiological response (atrophic gastritis)
Pathological response (gastrinoma, ulcerogenic tumor syndrome with nongastrin secretagogue)
Assess the adequacy of proton pump inhibitor therapy in patients with diseases of gastric acid hypersecretion
Assessment of the patient with ulcer recurrence following surgical intervention for peptic ulcer disease
Exclude acid hypersecretion
Assess the completeness of vagotomy (sham feeding)

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have relegated gastric acid analysis to an adjunctive role in the diagnosis of this disease [3,4]. Gastric analysis remains critical to the evaluation of the unusual patient with acid hypersecretion attributable to the ulcerogenic pancreatic tumor syndrome where circulating gastrin levels are typically normal [5,6]. Gastric analysis can be useful in determining the adequacy of acid suppressive therapy after the diagnosis of gastrinoma or the ulcerogenic pancreatic tumor syndrome has been established.

Gastric acid analysis can also be helpful in the evaluation of patients who develop recurrent ulcers following surgery for peptic ulcer disease. In this setting, gastric acid secretion can be quantified and hypersecretion excluded. In addition, the completeness of vagotomy can be determined by sham feeding that evaluates the vagally mediated cephalic phase of acid secretion.

In the age of *H. pylori* and proton pump inhibitors, however, the frequency for which gastric analysis is necessary for this indication is decreasing. Recent epidemiological data suggest that the prevalence of *H. pylori* and peptic ulcer disease in Western countries is declining [7–9]. Operative intervention for peptic ulcer disease has been decreasing in recent years, likely related to improvements in medical and endoscopic management options [10]. Further, most of the classic literature addressing postoperative ulcer recurrence was performed prior to the acceptance of *H. pylori* as one of the major pathogenic factors in the development of peptic ulcer disease [11]. It is likely that *H. pylori* eradication alters postoperative gastric acid secretion or decreases the likelihood of recurrent peptic ulceration following surgery. For these reasons, what was once a common reason to order gastric acid analysis is now an unusual indication for this procedure.

Gastric acid analysis remains an important means of better understanding the effect of various pharmaceuticals and disease states on gastric secretory function. For example, it has long been known that patients with duodenal ulcer have higher levels of basal acid secretion than controls [12,13]. Studies using gastric acid analysis have proven that this abnormality is related to *H. pylori* infection and that eradication of this infection leads to normalization of basal acid output (BAO). On the other hand, pentagastrin-stimulated maximal acid output (MAO) in patients with duodenal ulcer remains elevated even after successful *H. pylori* eradication. This suggests the presence of parietal cell hypersensitivity to gastrin or an increase in parietal cell mass that persists after eradication of *H. pylori* infection [14].

The utility of gastric acid analysis in patients with peptic ulcer disease, but without evidence of *H. pylori* infection or history of nonsteroidal antiinflammatory medication use, has yet to be determined. Other patient populations in whom gastric acid analysis might be useful include those with a nonhealing ulcer despite appropriate acid suppressive therapy and those with ulcer recurrence despite successful *H. pylori* eradication.

Methodology

The most widely practiced means of measuring acid secretion is the aspiration method in which gastric contents are withdrawn through a nasogastric tube at timed intervals under different test conditions. The hydrogen ion concentration in these samples can then be determined provided a quantitative assessment of basal and stimulated acid output and modified sham feeding (MSF) acid secretion.

A point worthy of consideration is the reliability of gastric acid analysis. A great deal of variability in test results has been observed in studies of healthy individuals. Both BAO and MAO tend to be higher in males than females. Patients with functional dyspepsia have similar acid secretion compared with healthy volunteers [12,13]. On the other hand, patients with duodenal ulcer tend to have greater acid secretion than healthy individuals (Fig. 139.1) [12–14]. Unfortunately, because of considerable overlap between populations, gastric analysis does not reliably discriminate between those with peptic ulcer disease and healthy volunteers. Even when Zollinger–Ellison syndrome is suspected, gastric analysis is

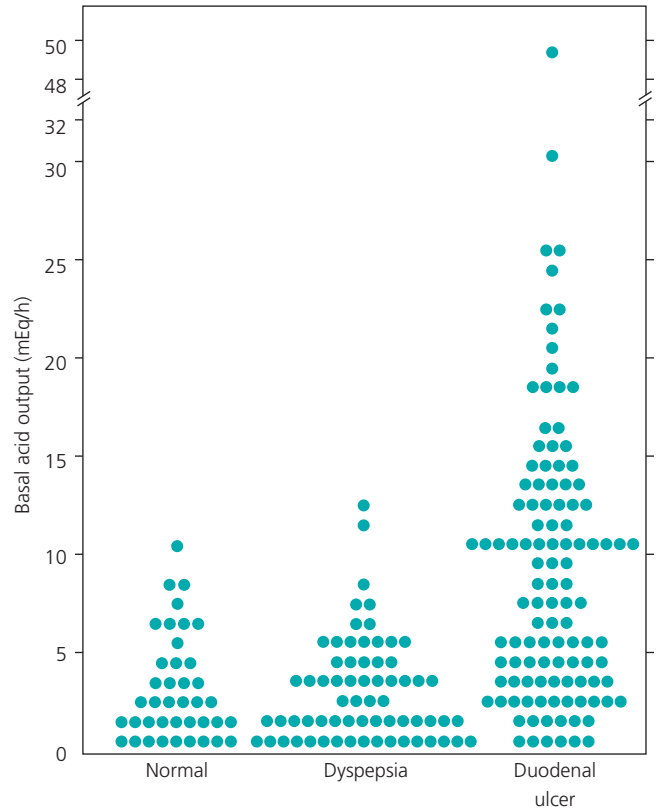

Figure 139.1 Basal acid output (BAO) in 40 healthy controls, 66 patients with nonulcer dyspepsia (NUD), and 114 patients with duodenal ulcer. There was no difference in mean BAO between normals (3.2 ± 2.7 mEq/h) and patients with NUD (2.9 ± 2.7 mEq/h). However, the mean BAO was significantly higher in patients with duodenal ulcer (9.1 ± 7.6 mEq/h) than in either normals or patients with NUD. From Collen and Loebenberg [12], with permission from Springer.

not always a reliable single diagnostic test because of the overlap in results yielded by patients and controls [1].

Aside from the considerable interindividual variability, there is also intraindividual variability when gastric acid analysis is performed on different days [15]. The reproducibility of this test depends on several variables including day-to-day variations in BAO, day-to-day variations in responsiveness of acid output to various secretagogues, and the completeness of gastric contents recovery. One study that evaluated several studies with intraindividual BAO data found the coefficient of variation to be rather poor [15]. This is likely related to the multiple factors (time of day, mental state, duodenal losses of gastric contents, neutralization of acid with swallowed saliva, and neurohormonal effectors of acid secretion) that influence acid secretion and its measurement in the basal state [16]. In contrast to basal acid secretion, acid secretion stimulated by histamine or pentagastrin is much more reproducible from day to day [15].

Recovery of gastric contents can be facilitated by proper tube placement and patient position. The aspiration tube should be of sufficient caliber to allow recovery of the gastric contents but not so large as to be intolerable to the patient. In general, tubes in the range 14–18Fr are best suited to these requirements. Testing should be performed in the seated or left lateral decubitus position. The tip of the tube should be placed into the most dependent portion of the stomach and proper placement is optimally confirmed with fluoroscopy. Even when test conditions are optimized, recovery of the gastric contents is often not complete [17].

The recovery of gastric contents during aspiration can be quantified by the infusion of a nonabsorbable marker such as polyethylene glycol (PEG) or radiolabeled chromic chloride ($^{51}CrCl_3$) [17,18]. Although this technique provides a means of assessing the completeness of gastric aspiration, the infusion and measurement of a nonabsorbable marker, particularly one that is radioactive, makes the procedure much more cumbersome and costly to perform. For these reasons, most centers including ours perform gastric acid analysis without the infusion of a nonabsorbable marker.

Basal acid output

On the evening prior to undergoing gastric acid analysis, patients should take a clear liquid dinner and consume nothing by mouth after midnight. Unless the purpose of the study is to assess the effect of a medication on acid secretion, dosing of agents that affect acid secretion should be modified prior to the test. Histamine (H_2)-receptor antagonists, antihistamines, anticholinergics, and cholinergic agonists should be discontinued at least 24 h before gastric acid analysis. Proton pump inhibitors inactivate the H^+,K^+-ATPase found on the parietal cell [19]. As such, proton pump inhibitors have prolonged effects on acid secretion [20] and should be withheld for at least 7 days before testing. For patients who suffer with recurrent symptoms once their proton pump inhibitor has been discontinued, an H_2-receptor antagonist may be used until 24 h before gastric acid analysis.

On the day of gastric acid analysis, the patient is intubated and tube placement is confirmed as described above. The study starts with a 30-min washout period during which all gastric contents are aspirated and discarded. After this washout period, samples are collected at 15-min intervals. Sample volume is recorded and an aliquot is used to determine the titratable acidity. Titratable acidity (mmol/L) is measured by titrating an aliquot of gastric juice to pH 7 with a base such as NaOH and then correcting this value for the size of the total timed sample. The following equations can be used to determine the titratable acidity:

(mL base to titrate to pH 7) × [concentration of the base] = millimoles H^+ per aliquot

Volume of sample × millimoles H^+ per aliquot

Volume of aliquot = millimoles H^+ per sample

BAO (mmol H^+ per hour) is the sum of the acid output from the four 15-min collections done in the unstimulated state. In healthy unoperated volunteers, BAO should be less than 15 mmol/h. In patients with Zollinger–Ellison (gastrinoma) syndrome or ulcerogenic pancreatic tumor syndrome without gastrinoma, BAO is greater than 15 mmol/h. A BAO of greater than 5 mmol/h is considered abnormal for a patient who has previously undergone partial gastrectomy or vagotomy [8]. In humans, BAO should be approximately 10% of the maximal secretory capacity of the stomach [15].

Alternative endoscopic means of assessing BAO have been described [21,22]. In a study involving 12 patients with gastrinoma, Oh and colleagues found that a 15-min endoscopic gastric acid collection yielded comparable acid concentration and acid output results to standard gastric acid analysis (Fig. 139.2). Volume of gastric contents collected between the two techniques showed poor agreement. For this procedure [22], patients received conscious sedation with meperidine or midazolam. Anticholinergic agents were not allowed prior to this procedure. All gastric contents were aspirated and discarded. This was followed by the collection of gastric contents for a period of 15 min. Hourly BAO was determined by multiplying the acid output of the 15-min sample by 4. Advantages of this technique include improved comfort over traditional gastric aspiration, targeted recovery of gastric contents, and the ability to perform both gastric acid analysis and traditional endoscopy with a single procedure. Further validation of this interesting new technique is eagerly awaited.

Maximal and peak acid output

MAO and peak acid output (PAO) indirectly assess functional parietal cell mass through the measurement of acid secretion following the administration of maximally effective doses of

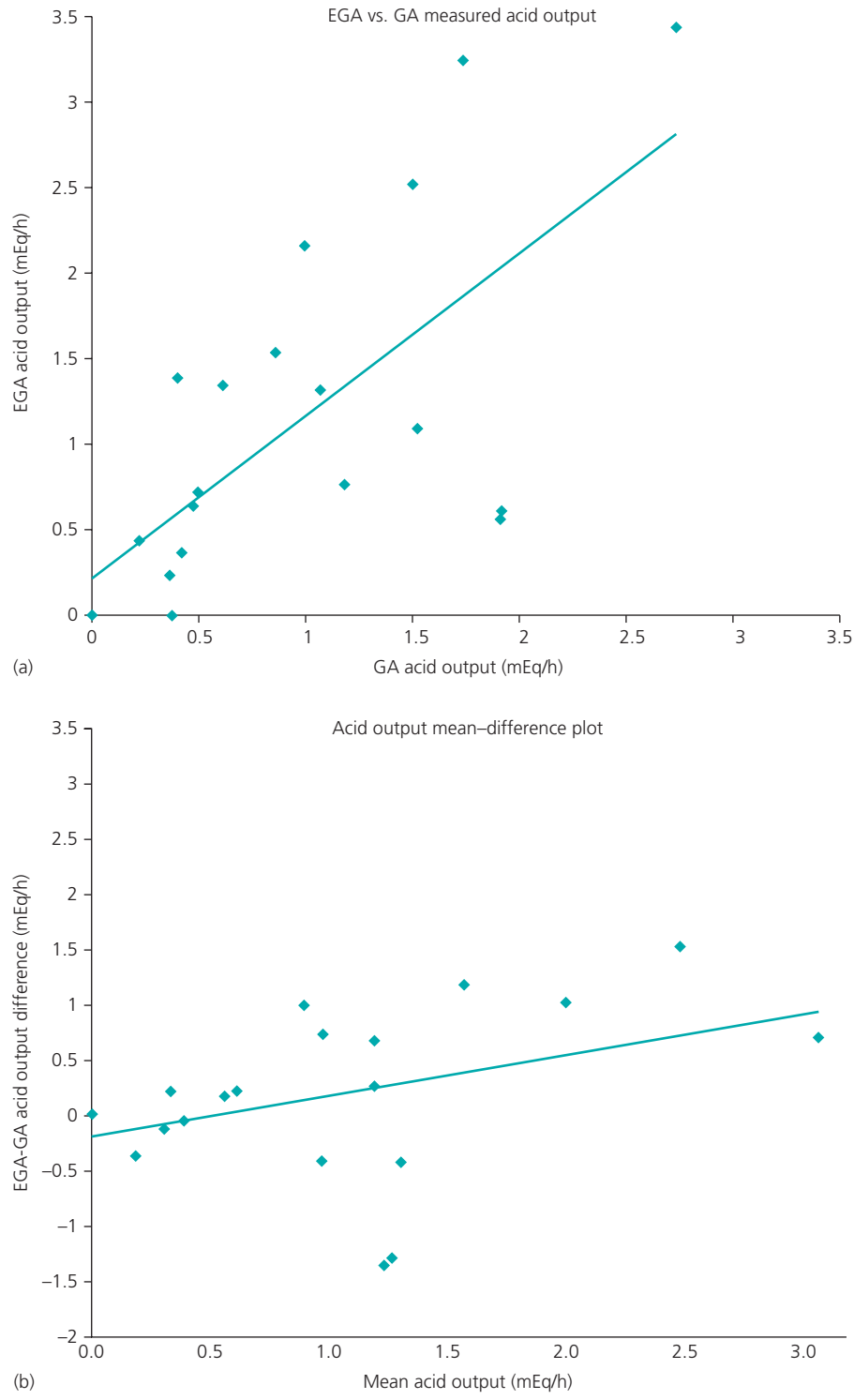

Figure 139.2 **(a)** Plot of endoscopic gastric analysis (EGA)-measured acid output against conventional gastric analysis (GA)-measured acid output with least-squares regression analysis. **(b)** Bland–Altman plot assessing agreement between EGA and GA acid concentration with least-squares regression analysis. The mean difference (horizontal solid line) is plotted. From Oh et al. [22], with permission from Elsevier.

agents that directly stimulate the parietal cell [23,24]. Such stimulants of acid secretion include pentagastrin, histamine, and histamine analogues such as 3-β-aminethylpyrazole. Pentagastrin is the most widely used agonist and is usually administered subcutaneously (6 μg/kg). Pentagastrin can also be given intramuscularly (6 μg/kg) or intravenously (1–6 μg/kg/h). After pentagastrin administration, gastric juice is collected every 15 min for 1–1.5 h. The titratable acidity (mmol H^+ per sample) is then determined for each of the four to six samples obtained after pentagastrin administration. MAO represents the greatest sum of the titratable acid from four consecutive samples (mmol H^+ per hour). PAO is the sum of

the two highest acid outputs multiplied by 2 to yield results in mmol H^+ per hour. MAO and PAO measurements vary widely in healthy subjects, ranging from 0 to 80 mmol/h. MAO is influenced by a number of variables including sex, age, and lean body mass [25]. MAO decreases by approximately 50% after truncal or proximal gastric vagotomy [26,27].

In conjunction with BAO, MAO measurement can suggest the presence of gastrinoma. As the presence of an abnormally elevated gastrin in plasma stimulates parietal cells to produce excessive gastric acid secretion in the basal state, patients with gastrinoma will typically have a BAO/MAO ratio of greater than 0.6 [1].

Another approach to the study of gastric secretory function is to administer gastrin-releasing peptide (GRP) rather than pentagastrin or histamine [14]. GRP leads to the release of gastrin from the antral G-cells and thereby indirectly stimulates gastric acid secretion by the parietal cell [28,29]. In addition, GRP influences the release of counterregulatory hormones such as cholecystokinin (CCK), secretin, gastric inhibitory peptide, vasoactive intestinal peptide, neurotensin, enteroglucagon, and somatostatin [30,31]. Therefore, unlike pentagastrin or histamine, which assesses only the parietal cell response to direct stimulation, GRP theoretically evaluates the sum of both stimulatory and inhibitory pathways responsible for the control of acid secretion. Investigators have used this approach to demonstrate abnormalities in the inhibitory control of acid secretion in patients with *H. pylori* infection and duodenal ulcer [14]. GRP is an interesting clinical investigative tool but is not currently considered part of routine gastric acid analysis.

Testing of vagally stimulated acid secretion

Since the turn of the 20th century, it has been known that the thought, sight, smell, and taste of food stimulate gastric acid secretion. Food-induced stimulation of the senses leads to activation of cerebral cortical sites resulting in increased gastric acid secretion. The so-called cephalic phase of gastric acid secretion is dependent on an intact vagus nerve and is cholinergically mediated [15].

The sham feeding test assesses vagally mediated acid secretion. Most commonly, this test is used to determine the completeness of vagotomy in patients with ulcer recurrence following surgery for peptic ulcer disease [32]. In humans, acid output response to sham feeding is 50%–65% of secretagogue-induced acid output [15]. In addition, some investigators have used this technique to assess the patency of vagal autonomic innervation to the stomach in the patients with diabetes mellitus [33].

During sham feeding, patients are allowed to see, smell, taste, and chew an appetizing meal. Food is then expectorated by the patient, thus avoiding the gastric phase of acid secretion. Gastric juice is collected via a nasogastric tube and the titratable acidity calculated as described above. Feldman and colleagues have shown that a sham acid output (SAO) to PAO ratio of less than 0.1 is abnormal in healthy volunteers. Therefore, an SAO/PAO ratio of more than 0.1 in a patient who has previously undergone peptic ulcer surgery suggests the presence of intact vagal innervation to the stomach [32].

Ambulatory intragastric pH monitoring

Indications

The role of ambulatory intragastric pH monitoring in clinical practice is limited. Like gastric acid analysis, however, the prolonged measurement of intragastric pH is useful in selected clinical and research situations. Prolonged intragastric pH monitoring has gained popularity as an investigative tool because it is better tolerated by patients and less cumbersome to perform than traditional gastric analysis using aspiration techniques. This technique has most often been used to evaluate the effects of duodenogastric reflux [34,35] and drugs that suppress acid secretion (e.g., H_2-receptor antagonists and proton pump inhibitors) on intragastric pH [36–39]. Intragastric pH monitoring can also help to establish a link between pH events and symptoms in patients with gastrointestinal symptoms [37,40,41].

Ambulatory intragastric pH monitoring has been used in a variety of other settings. For example, intragastric pH monitoring has been used to determine the relationship between suppression of gastric acid secretion and peptic ulcer healing [42]. This technique has also been used to define differences in intragastric pH between patients with duodenal ulcer associated with *H. pylori* infection and controls [43,44]. Intragastric pH monitoring has also been used to identify differences in the effectiveness of proton pump inhibitor therapy between those infected and not infected with *H. pylori* [45,46].

It is important to understand the differences in information yielded by gastric acid analysis and intragastric pH monitoring. While gastric acid analysis provides a quantitative measure of gastric secretory function, intragastric pH monitoring yields only qualitative information. As has already been discussed, gastric acid analysis quantitates basal and maximal acid secretion in response to the administration of a specific secretagogue. On the other hand, intragastric pH monitoring allows only the determination of pH, albeit in a more physiological setting than gastric acid analysis. How well results obtained with prolonged intragastric measurement correlate with traditional gastric acid analysis remains controversial. Several studies have reported a good correlation between data obtained from the two techniques [36,47]. However, studies have questioned the validity of drawing conclusions regarding overall intragastric pH solely on the basis of measurements obtained with a single electrode [35,48–50]. Intragastric pH recordings from the body and fundus yield similar results [51] (Fig. 139.3). Simultaneous recordings from the body and antrum have demonstrated significant differences in pH both in the interdigestive and postprandial periods [35,48–50]. In general, H^+ concentration

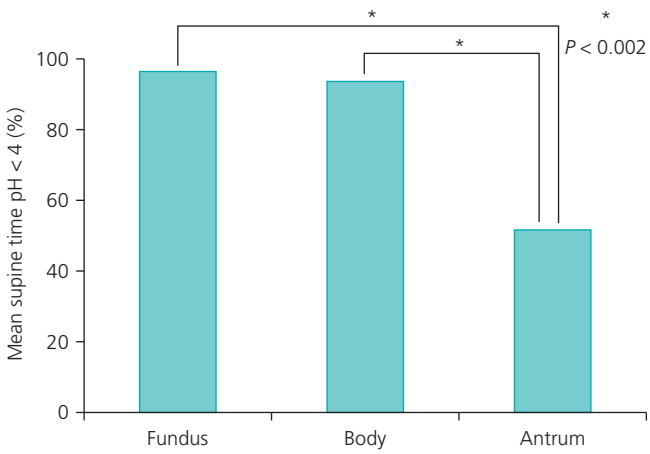

Figure 139.3 Mean percent time pH < 4 during supine periods for five healthy volunteers: comparison of fundus, body, and antrum. From Fackler et al. [51], with permission from Blackwell Publishing.

is higher when measured by intragastric pH monitoring compared with gastric acid analysis [49]. The precise reasons for these differences are not clear, but biophysical phenomena including environmental carbon dioxide concentration, temperature, and technical issues including electrode instability, artifact or interference may be contributory [49,52].

Methodology

There are a variety of ways to measure intragastric pH [51]. The most widely employed method has been to place a catheter containing a single electrode transnasally into the body of the stomach [53,54]. As mentioned above, recent literature has questioned the practice of using a single electrode as a sole means of evaluating intragastric pH. Obtaining pH measurements from more than one part of the stomach appears to yield more complete information. Whether this more complete information has practical relevance to the clinician or investigator remains to be determined. When a single pH electrode is used, recordings from the fundus (10 cm below the lower esophageal sphincter) appear to yield the most consistent and reproducible results [51]. Proper placement should be confirmed with manometry or fluoroscopy. Both antimony and glass pH electrodes have been used and although point-to-point variation in data has been noted, overall these techniques appear to give similar results [55,56]. The catheter is connected to any of a number of commercially available computerized modules that can digitally record changes in intragastric pH. Careful attention should be paid to proper calibration of the pH monitoring equipment prior to each study. The probes used for intragastric pH monitoring reportedly become less accurate with repeated use [52].

A catheterless capsule that can monitor pH events in the esophagus for up to 4 days has also become available [57,58]. Research suggests that this device can successfully record prolonged ambulatory intragastric pH in the cardia [59] and body [60]. Placement of the device in the gastric body can be facilitated by direct visualization via endoscopy [60]. Improved comfort and the ability to engage in daily activities makes this technique more physiological than traditional catheter-based monitoring techniques. Unfortunately, this technique is costly, requires endoscopy for placement, and does not resolve the issue of monitoring from only one location within the stomach. An orally ingested pH and motility capsule has also been evaluated [61]. This device can record intragastric pH until it is emptied from the stomach. An advantage of this technique is that it does not require endoscopy. Unfortunately, because this device is not fixed within the stomach, one cannot be sure from which part of the stomach pH data are being recorded. This device may prove useful as a means of grossly assessing intragastric pH as could be necessary in patients with an elevated fasting gastrin level or those with symptoms despite antisecretory therapy. This capsule has also been used to assess gastric acidification following a meal in patients with diabetic or idiopathic gastroparesis [62].

Data are often expressed as the percentage of time above or below a certain pH. This means of data presentation is simple and easy to understand. Unfortunately, this method of analysis only provides a gross estimation of intragastric acidity. For example, if one were assessing the effectiveness of a new acid-suppressive medication and used an end point of percent time intragastric pH > 4, a patient with a median pH of 4.5 over 6 h would yield identical results to an individual with a mean intragastric pH of 6 over 6 h. It is important to recall that pH is the inverse log of H^+ concentration so this form of analysis can be quite misleading. As such, it is more precise to express data from intragastric pH monitoring as H^+ concentration [49,63] or as integrated gastric acidity [64,65].

Tests of pancreatic exocrine function

Until the 1960s, short of radiological demonstration of pancreatic calcification or exploratory laparotomy, the presence of chronic pancreatic disease was extremely difficult to establish. The use of endoscopic retrograde pancreatography (ERP), CT, MRI, transcutaneous ultrasonography, and EUS has facilitated the diagnosis of chronic pancreatitis. Unfortunately, a definitive diagnosis may not be apparent despite evaluation with structural studies in a subset of patients with chronic pancreatitis. In addition, structural studies by these diagnostic modalities cannot precisely establish the severity of exocrine dysfunction. Although local expertise and availability influence the choice of diagnostic studies, this subset of patients sometimes benefits from functional testing which can uncover previously unidentified abnormalities or complement information yielded by structural studies.

Table 139.2 Pancreatic function tests

Tests requiring gastroduodenal intubation
Direct tests
Secretin test
Secretin/cholecystokinin (CCK-8) test
CCK-8 or cerulein test
Indirect tests
Lundh test
Tubeless pancreatic function tests
Oral tests
Fluorescein dilaurate (pancreolauryl) test
Bentiromide test
Fecal tests
Elastase
Chymotrypsin
Lipase
Fat
Emerging tests of exocrine pancreatic function
Endoscopic pancreatic function tests
Secretin-stimulated magnetic resonance cholangiopancreatography

Tests of pancreatic exocrine function can be divided into several categories:

- tests that quantitate pancreatic exocrine secretion through gastroduodenal intubation
- studies that assess exocrine function indirectly by oral administration of various test substances
- tests that rely on the measurement of fat or pancreatic enzyme activity in feces
- emerging tests that combine more than one modality (Table 139.2).

As we begin this discussion of tests of pancreatic exocrine function, a very important point regarding the interpretation of the available literature deserves mention. Comparisons among tests for chronic pancreatitis are limited by the lack of availability of an accepted gold standard for the diagnosis of this condition. Histological data are rarely available because most patients do not require pancreatic surgery and there may be significant risk in performing core biopsies. Even when available, histological abnormalities are patchy, there is no consensus on grading histological severity, and the importance of fibrotic changes remains unclear in asymptomatic elderly and alcoholic patients without pancreatitis. Many studies comparing the accuracy of diagnostic tests are also limited by ascertainment bias (using the test being evaluated to identify patients for the study) or are confined to only patients with moderate to severe disease (populations in whom the diagnosis is typically not in question).

Tests requiring gastroduodenal intubation

These tests rely on the collection of pancreatic juice from the duodenum before and after intravenous administration of secretagogues including secretin or CCK or its analogues (e.g., CCK-8, cerulein). Compared with histology, tests that measure pancreatic secretion from duodenal contents provide an accurate, sensitive (67%–97%), and specific (78%–100%) means of assessing pancreatic exocrine function [66–75] (Table 139.3). These tests may be more sensitive in detecting pancreatic dysfunction than traditional imaging techniques including ERP but may be less sensitive than EUS. Although these tests are clearly superior to other pancreatic function tests, they are labor intensive and expensive. Moreover, they require specially trained personnel to perform properly. As such, these tests are typically only available at large referral centers and clinical research laboratories.

Secretin and cholecystokinin tests

The secretin test was developed in the early part of the 20th century [76,77] to measure exocrine pancreatic secretion in response to the intravenous injection of purified porcine secretin. The volume of aspirate as well as the concentration of bicarbonate and pancreatic amylase [77,78] are measured before and after the administration of secretin. To minimize contamination of the duodenal aspirate by gastric juice, a double-lumen tube should be used for the secretin test. Using fluoroscopic guidance, gastric aspiration ports are positioned in the antrum and body while duodenal aspiration ports lie in the first, second, and third portion of the duodenum (Fig. 139.4). Both gastric juice and duodenal contents are separately collected by continuous suction. Gastric contents are discarded while duodenal contents are retained for subsequent analysis.

Gastric aspiration is important as contamination of duodenal contents with gastric acid produces falsely low bicarbonate concentration and inaccurate volume measurements. Moreover, acid stimulates the release of secretin from the duodenal mucosa, which can also affect test results. The pH of uncontaminated duodenal fluid should be 7.5 or greater. In clinical conditions associated with gastric acid hypersecretion such as Zollinger–Ellison syndrome, the collection of uncontaminated duodenal fluid is often impossible.

Once the tube is properly placed, duodenal fluid is collected every 15 min for 1 h. Secretin in a dose of one clinical unit (approximately 0.3 μg) per kilogram of weight is then administered as a bolus intravenous injection and duodenal fluid is collected for an additional 1–1.5 h. The duodenal aspirate should lose its bile-stained appearance after secretin injection and become clear and colorless, except in patients with gallbladder dysfunction (e.g., chronic cholecystitis) or in those who have previously undergone cholecystectomy [79].

Normal values for duodenal fluid following secretin injection are provided in Table 139.4. Dreiling favored measuring bicarbonate concentration rather than total output evoked by secretin [80], but others have suggested that total bicarbonate output provides superior discriminant potential for diagnosing chronic pancreatitis compared with several measurements of juice constituents, including bicarbonate

Table 139.3 Subjects with laparotomy and histologically proven clinical pancreatitis

Study	*n*	Etiology	Mild disease (%)	Agonist	Lipase output	HCO_3 output	$[HCO_3]$	PFT sensitivity (%)	PFT specificity (%)	ERCP sensitivity (%)
Dornberger, 1948 [67]	28	Chronic pancreatitis	43	Secretin	Lipase concentration		Yes	82	100	N/A
Dreiling, 1953 [68][a]	71	Chronic pancreatitis	15	Secretin		Yes		97		
Burton, 1960 [69][b]	15	Suspected pancreatic disease		Secretin/CCK	Yes	Yes		84	84	
DiMagno, 1973 [70][c]	32	Chronic pancreatitis, normal controls	18	CCK	Yes			94	94	
Adler, 1976 [71]	52	Suspected pancreatic disease		Secretin			Yes	83		71
DiMagno, 1977 [72]	57	Suspected pancreatic disease		CCK or secretin	Yes	Yes		87	81	N/A
DiMagno, 1977 [73]	70	Suspected pancreatic disease		CCK	Yes			89	78	95
Waye, 1978 [74]	23	Chronic pancreatitis, ≤ 75% alcoholic		Secretin			Yes	83		70
Heij, 1986 [75]	25	Chronic pancreatitis, 76% alcoholic	12	Secretin/CCK		Yes		88		N/A
Hayakawa, 1992 [66]	108	Chronic pancreatitis, 55% alcoholic pancreatic cyst	49	CCK			Yes	67	90	

a Chronic pancreatitis was established by tissue diagnosis (55/71), calcinosis (6/71), tissue diagnosis and calcinosis (5/71), cystic fibrosis (7/11), hemochromatosis (1/71), or by clinical history of repeated attacks of abdominal pain with elevated blood amylase, diarrhea, and diabetes (2/71).

b Chronic pancreatitis was diagnosed by a history of "long-standing attacks, with evidence of permanent glandular damage such as diabetes, steatorrhea, or calcification, and those in whom the surgeon noted a sufficient hardening in the gland to justify without doubt such a diagnosis."

c Chronic pancreatitis was established by tissue diagnosis (10/17) or by calcification on roentgenogram (7/17).

CCK, cholecystokinin; ERCP, endoscopic retrograde cholangiopancreatography; PFT, pancreatic function test.

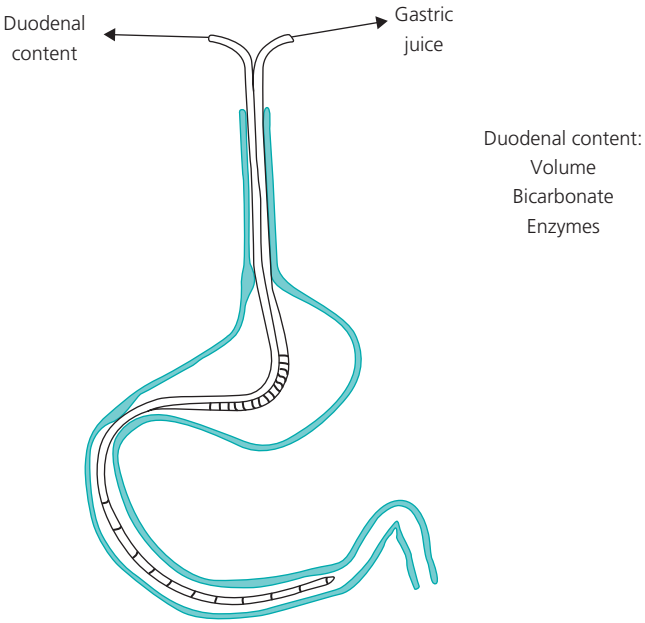

Figure 139.4 Schematic representation of the set-up for pancreatic function tests requiring gastroduodenal intubation. A double-lumen tube is placed into the stomach and duodenum with the assistance of fluoroscopy. Gastric and duodenal contents are then simultaneously aspirated during the basal state and following intravenous administration of pancreatic secretagogues including secretin, cholecystokinin octapeptide, or cerulein alone or in combination. Gastric contents are discarded, while duodenal contents are stored for subsequent analysis as described in the text.

Table 139.4 Normal ranges for results of pancreatic function tests requiring gastroduodenal intubation

Secretin test (mean ± 2SD)[a]
Volume: 107–223 mL/h
Bicarbonate concentration: 92–124 mEq/L
Bicarbonate output: 14–21 mmol/h
Amylase output: 174–1270 U/h
Secretin + CCK (lower limits of normal; mean ± 2SD)[b]
Volume: 151 mL/h
Bicarbonate concentration: 70 mmol/L
Bicarbonate output: 11.3 mmol/h
Amylase output: 131 U during 30 min of CCK
CCK test (range in 16 control subjects)[c]
Trypsin output: 25–54 KU/h
Lipase output: 77–322 KU/h
Lundh test (mean ± 2SD)[d]
Mean tryptic activity: 7–38 IU/L

a Dreiling DA, Hollander F. Studies in pancreatic function. II. A statistical study of pancreatic secretion following secretin in patients without pancreatic disease. Gastroenterology 1950;15:620.
b Howat HT, Braganza JM. Assessment of pancreatic dysfunction in man. In: Howat HT, Sarles H (eds). The Exocrine Pancreas. Philadelphia: WB Saunders, 1979:129.
c From Ref. 70.
d Adapted from Ref. 92.
CCK, cholecystokinin.

concentration [75,81–83]. The sensitivity of bicarbonate concentration/output for diagnosing chronic pancreatitis may be affected by hypersecretion of fluid and bicarbonate, associated with hypertrophy and hyperplasia of the hepatic and pancreatic ductal systems, as seen during the early course of alcoholic chronic pancreatitis and in other conditions including cirrhosis, hemochromatosis, Zollinger–Ellison syndrome and alcoholism [80,83].

It is often difficult to judge the significance of an isolated abnormality in duodenal juice volume as the adequacy of aspiration is often unclear. To avoid this technical error, a nonabsorbable marker can be infused into the proximal duodenum during the course of the study. Quantification of the marker in the samples collected allows one to determine the completeness of duodenal aspiration [84]. When low volume is truly the only abnormality found, the clinician should consider the possibility of cancer in the head of the pancreas. When low values in all parameters including volume, bicarbonate concentration, and amylase output are present, patients virtually always exhibit signs of advanced pancreatic disease such as extensive calcification, steatorrhea, or pancreatic endocrine dysfunction [85]. The secretin test is sensitive enough to detect the presence of mild to moderate chronic pancreatitis. In such patients, low maximum bicarbonate concentration may be the only abnormality.

Using the basic technique described above, testing can be modified by adding or substituting the intravenous injection of CCK [69,79] or its analogue [86]. Whenever CCK is used, measurement of trypsin and lipase output is performed. Normal values for the secretin and CCK tests are given in Table 139.4.

CCK-evoked lipase output is superior to lipase concentration or other enzyme concentrations/outputs (trypsin, amylase) for diagnosing chronic pancreatitis [72,87]. Further, data from two studies that compared lipase and bicarbonate output evoked by CCK or secretin testing indicate that lipase output may be superior [72,87]. Using histology as the gold-standard diagnosis of chronic pancreatitis, CCK-evoked lipase and bicarbonate output had similar accuracy [72] but values for secretin-evoked bicarbonate output overlapped greatly among chronic pancreatitis, controls, pancreatic cancer and other cancer [72]. Burton and colleagues [69] provided early evidence that CCK-evoked enzyme output is the most sensitive indicator of impaired secretory function. Similarly, Bozkurt and colleagues [87] used the secretin/cerulein test and showed that lipase output is the earliest and most sensitive indicator of chronic pancreatitis; patients had dissociated reductions of volume, bicarbonate, amylase and lipase outputs but a reduction in any nonlipase parameter was always coupled to a reduction in lipase output. It is worth noting that administration of CCK by continuous intravenous infusion [72,87] greatly reduces variability of lipase output compared with bicarbonate output following a single injection of CCK [83].

Data from a small number of studies have found that results from these pancreatic function tests correlate well with pancreatic histology [66,75]. An additional study reported general concordance between histology and secretory function but also discordance in a subset of patients [74], perhaps related to nonuniform parenchymal damage. Importantly, some patients with mild histological pancreatitis may have severe pancreatic secretory dysfunction [74].

Lundh test

This test determines the pancreatic enzyme concentration of the duodenal contents in response to a test meal. After an overnight fast, a flexible tube is placed into the duodenum via the oral route with the assistance of fluoroscopy. As described by Lundh [88] the test subject ingests a standardized 300-mL meal composed of dried milk, vegetable oil, and dextrose. Duodenal contents are aspirated at set intervals for measurement of pancreatic enzyme concentration. When chymotrypsin is measured, a concentration below 12.6 IU suggests the presence of pancreatic exocrine insufficiency.

Because this test relies on endogenous secretin and CCK-stimulated pancreatic secretion and these hormones are released mainly from the mucosa of the duodenum and proximal jejunum, results of the Lundh test can be interpreted only in patients with intact gastroduodenal continuity and normal duodenojejunal mucosae [89,90]. Although this test is simpler and less expensive than the secretin test, it allows determination of enzyme concentration only. The sensitivity of this test ranges from 66% to 94% [91,92]. The secretin or CCK test appears to be more sensitive than the Lundh test, particularly in patients without steatorrhea [93,94].

Direct intraductal juice collection

The collection of pure pancreatic juice at the time of ERP is conceptually attractive. Unfortunately, the available data suggest that such collection of pancreatic juice has poor sensitivity and specificity for pancreatic exocrine insufficiency [66]. These findings were confirmed by a study that compared results of traditional secretin-based pancreatic function testing with intraductal secretin testing during ERP. This found that results yielded by intraductal secretin stimulation did not compare favorably with the results from traditional pancreatic function testing [95].

Tests that do not require gastroduodenal intubation

Short of the tests that require duodenal intubation, there are only a limited number of tests available for clinical use. These include the pancreolauryl test, bentiromide test, and tests for fecal pancreatic enzymes and fat.

Oral pancreatic function tests

The pancreolauryl and bentiromide tests are oral indirect tests of pancreatic exocrine function. Because of their limited availability in clinical practice, these tests are of more historical than practical interest. The pancreolauryl and bentiromide tests involve the ingestion of a cleavable test substrate (fluorescein dilaurate or *N*-benzoyl-L-tyrosyl-para-aminobenzoic acid [NBT-PABA] respectively) with or without a standardized meal. For the pancreolauryl test, fluorescein dilaurate is specifically hydrolyzed by pancreatic arylesterase to lauric acid and water-soluble free fluorescein, which is readily absorbed by the small intestine, conjugated in the liver, and excreted in the urine. For the bentiromide test, NBT-PABA, a synthetic peptide, is specifically cleaved by chymotrypsin to yield free PABA, which is absorbed in the small intestine, conjugated by the liver, and excreted in the urine. Fluorescein or PABA can be measured in the serum or urine. In general, serum measurement of both substrates has proven more reliable than urine measurement. These tests reportedly have a sensitivity of 37%–100% and a specificity of 39%–100% [96–104]. Perhaps the most important factor influencing the sensitivity and specificity of this test has been the severity of pancreatic disease in the patient population studied. For example, when data from several studies using the secretin/CCK test as a gold standard were pooled, the sensitivity of the NBT-PABA test with a 6-h urine collection was 71% in patients with severe pancreatic exocrine dysfunction but only 46% in those with mild to moderate exocrine insufficiency [96]. Thus, the utility of these tests appears rather limited in those with mild to moderate exocrine dysfunction. The accuracy of the test can be negatively influenced by previous gastrointestinal surgery, small bowel mucosal diseases (e.g., celiac disease, Crohn's disease), severe liver disease, or bacterial overgrowth.

Tests for fecal pancreatic enzyme output

Fecal pancreatic enzyme determination has been used to identify patients with severe chronic pancreatitis. The enzymes measured for this purpose include elastase, chymotrypsin, and lipase.

Elastase

Human pancreatic elastase 1, a member of the acidic elastase family, was detected by Sziegoleit as a new endoprotease and sterol-binding protein present in both human pancreatic secretion and feces [105]. Unlike chymotrypsin, quantitative studies using immunoelectrophoresis have shown that fecal elastase levels remain unaffected during intestinal transit. Unfortunately, fecal elastase-1 has poor sensitivity and specificity for diagnosing mild to moderate pancreatic insufficiency [106,107]. Hardt and colleagues [108] correlated fecal elastase levels with grades of pancreatitis (Cambridge classification) and showed a sensitivity of 45.3%, 57.4%, and 76.5% for grade 1, 2 and 3, respectively. Similarly, both the monoclonal and the polyclonal ELISA antibody fecal elastase-1 tests have also been shown to have unacceptably low sensitivity for diagnosing exocrine pancreatic insufficiency,

64% for severe disease and 40% for mild to moderate disease [109]. Therefore, if the diagnosis of chronic pancreatitis is in doubt, a direct test of pancreatic function is superior.

Low stool elastase values are not specific for reduced pancreatic exocrine function and occur in 25%–30% of patients with small bowel conditions [110–113] including celiac disease, Crohn's disease, cow milk protein enteropathy, bacterial overgrowth, short bowel syndromes, and enteritis [114]. Indeed, fecal elastase has been promoted as a marker of enteropathy regardless of cause [112]. Another cause of a low stool elastase is diabetes mellitus, a complication of chronic pancreatitis; the prevalence of low stool elastase is 46% in type I disease and 30% in type II disease [115–117]. Because low stool elastase may also be related to dilution of stool, individuals with diarrhea-predominant irritable bowel syndrome who have large-volume stools [118] may have a false-positive test. Thus, because of the poor sensitivity and specificity for exocrine pancreatic insufficiency, fecal elastase is a poor indicator of chronic pancreatitis in the clinic and in epidemiological studies.

Chymotrypsin

Fecal chymotrypsin determination has been used for many years to identify patients with chronic pancreatic disease. The test is typically performed on random stool samples, although prolonged collection may improve accuracy. Fecal chymotrypsin testing has a sensitivity of 45%–100% and specificity of 49%–90% [96,119–123]. A variety of factors including Billroth II gastrojejunostomy, severe hepatic disease, and gastrointestinal diseases that cause diarrhea or malabsorption can reduce the accuracy of this test [96]. Pancreatic enzyme supplementation can cause falsely normal results and should be discontinued 5 days before stool collection. As is the case with other forms of noninvasive pancreatic function testing, fecal chymotrypsin measurement is better able to identify those with advanced disease than those with mild to moderate disease [124]. The marginal performance characteristics of this test limit its usefulness as a screening tool for chronic pancreatitis.

Lipase

Fecal lipase has been measured by a sandwich enzyme immunoassay [125]. Abnormally low values were observed in 46% of patients with chronic pancreatitis, exocrine insufficiency and steatorrhea, and in 13% of patients without insufficiency. The overall sensitivity was a disappointing 34%.

Tests for fecal fat determination

Quantitative stool collection with fecal fat determination is the gold standard for detection of steatorrhea. For this study, patients should be instructed to ingest a diet containing 100 g of fat daily starting at least 3 days before the 48–72 h stool collection. Fecal fat excretion of more than 7 g per 24 h is consistent with steatorrhea [18]. If a patient is ingesting a proper diet, qualitative analysis of random stool samples with Sudan staining also sensitively and specifically detects steatorrhea. Steatocrit, a rapid gravimetric method for measuring stool fat, when performed on a 72-h stool collection is as sensitive and specific as a 72-h quantitative stool fat, and may be as accurate if performed on a 24-h stool collection [126] or random stool samples.

Unfortunately, less than 10% of normal lipase output must be present before fat maldigestion from pancreatic disease can be detected [70]. In addition, many other diseases of the gastrointestinal tract (e.g., Crohn's disease, celiac disease, Whipple disease) can cause steatorrhea. Consequently, the detection of steatorrhea by stool testing is neither sensitive nor specific for pancreatic disease. The specificity of fecal fat testing can be improved by performing a two-stage procedure where a repeat collection is done in conjunction with oral pancreatic enzyme replacement. A significant decrease in fecal fat excretion after enzyme replacement supports the presence of pancreatic exocrine dysfunction.

Comparison between pancreatic exocrine function tests and imaging studies in chronic pancreatic disease

ERP is currently felt to be the most sensitive method for detecting morphological change in the pancreatic duct. The major drawbacks of this procedure are its relative costliness and potential for significant adverse events such as pancreatitis. EUS is another invasive means of imaging the pancreas. High-resolution CT and MRI provide noninvasive, widely accessible means of evaluating the pancreatic parenchyma and duct. Unfortunately, none of the currently available structural or functional tests is always diagnostic in patients with suspected chronic pancreatitis. As such, a combination of structural and functional studies is sometimes required to achieve diagnostic certainty in patients with mild to moderate disease.

There are relatively few studies that have directly compared the ability of structural and functional tests to detect chronic pancreatitis [82,127–140]. The best-designed studies are those that evaluate functional or structural studies against a histological gold standard. For practical reasons, few such studies have been conducted [66,75,131]. When direct comparisons have been made, traditional pancreatic function testing has been found to more closely correlate with histological findings than ERP in patients with chronic pancreatitis [75,131].

A number of published protocols have used one or more structural studies or traditional pancreatic function testing as a gold standard, making meaningful direct comparisons difficult if not impossible. In one study that directly compared invasive pancreatic function testing (secretin/pancreozymin test) with ERP, Lankisch and colleagues found that in the majority of patients with chronic pancreatitis, there was good correlation between the two tests [127]. Of 202 pati-

ents, 129 (64%) had parallel secretin/pancreozymin and ERP test results; 21% had abnormal results on both tests but the degree of abnormalities observed were not consistent; 15% had discordant results with one test abnormal and one test normal. In the group with discordant results, all but one patient had an abnormal ERP but normal secretin/pancreozymin result. Others have also observed discordant ERP and traditional pancreatic function test results in 2%–29% [128–130]. There are several possible explanations for this observation. First, prior episodes of severe acute pancreatitis may have resulted in morphological abnormalities of the pancreatic duct without long-term consequence to pancreatic function [132]. Second, morphological changes of the pancreatic duct without corresponding changes in exocrine function are known to occur with aging [133]. Third, there are likely situations where ERP films are felt to demonstrate changes suggestive of mild chronic pancreatitis when in fact they do not. Finally, it may be that ERP is more sensitive than traditional pancreatic function testing or that traditional pancreatic function testing is falsely normal in some patients with discordant results. This possibility seems less likely based on a study by Lambiase and colleagues [130] which reported in abstract format that 8 of 52 (15%) patients had divergent functional and ERP test results. Six of eight patients had an abnormal traditional pancreatic function test but normal ERP. Of the six patients with an abnormal traditional pancreatic function test but normal ERP, five were subsequently found to have chronic pancreatitis. In contrast, neither of the two patients with an abnormal ERP and normal traditional pancreatic function test was later found to have chronic pancreatitis. This study would suggest that traditional pancreatic function testing may be better able to detect early chronic pancreatitis than ERP.

A number of studies [134–139] have evaluated EUS in the diagnosis of chronic pancreatitis and all but two [135,137] showed excellent accuracy of EUS using ERP or intraductal secretin testing as the gold standard for chronic pancreatitis. EUS and direct pancreatic function testing reach a similar finding in 75% of patients [140,141] and EUS and ERP reach the same diagnosis in 80%, although many patients enrolled in the available studies had advanced structural abnormalities [141]. Unfortunately, criteria for EUS parenchymal and ductal changes [134,136] have not been compared with histology. Most studies use three EUS features as a cutoff for diagnosing chronic pancreatitis [136,138,139]. The problem with these studies is that most patients had moderate to severe chronic pancreatitis and that the intraductal secretin test was used as the gold standard for diagnosis of chronic pancreatitis in three studies [134–136]. Indeed, EUS may be preferable to ERP because it is less expensive, exposes patients to less risk, and potentially allows earlier detection of subtle changes. Unfortunately, the specificity of EUS features for diagnosing early chronic pancreatitis is uncertain [139,142–146]. In one study, the correlation of EUS and ERP was good in advanced disease but poor in mild to moderate chronic pancreatitis [134]. Further, when Sahai and colleagues [138] focused on patients with unexplained abdominal pain without obvious chronic pancreatitis, they had difficulty choosing a cutoff to differentiate pancreatic disease in controls and patients with dyspepsia: EUS abnormalities were commonly seen in "normal" controls and dyspeptics. In addition, it has been reported that abnormal pancreatic EUS features can be identified in a variety of other settings [147], including 58% of asymptomatic alcoholics [144], 89% of alcoholic individuals with pancreatic-type pain [144], acute pancreatitis [134], and as a consequence of aging [146,148]. Further, EUS is operator dependent and suffers from variable degrees of interobserver variation [149]. From this information, it seems fair to conclude that EUS is quite sensitive but may lack specificity for mild chronic pancreatitis.

Emerging methods of assessing pancreatic exocrine function

Two new methods provide a potentially accurate, simple, less cumbersome, less expensive and perhaps better-tolerated method of assessing pancreatic exocrine function than the gold-standard direct pancreatic function test: the endoscopic pancreatic function test and secretin-stimulated magnetic resonance cholangiopancreatography.

Endoscopic pancreatic function test

Recently, a number of studies have assessed simpler, abbreviated, direct exocrine pancreatic function studies performed concurrently with upper endoscopy [150–153]. At the time of endoscopy, either secretin or CCK octapeptide is injected intravenously and pancreatic secretions are collected through the endoscope. In contrast to standard direct tests of pancreatic exocrine function, total pancreatic output of individual juice constituents cannot be quantified because perfusion of a nonabsorbable marker (PEG solution) is not performed.

It is questionable whether shorter collections of juice yield adequate sensitivity and specificity for pancreatic exocrine dysfunction. In a recent study in 412 patients who underwent endoscopic pancreatic function testing [154], collection of pancreatic juice was limited to 10 min after intravenous secretin. Bicarbonate concentrations were deemed unreliable. Although lipase concentrations were significantly lower in patients with chronic pancreatitis and pancreatic cancer compared with those with a normal pancreas, there was too much overlap between groups to be useful in clinical practice (positive predictive value 73%, negative predictive value 85%). These data have been confirmed by several other studies [155–157]. In 633 patients, data from the first 15-min collection of a secretin test had a specificity for chronic pancreatitis of 35%, positive predictive value of 44.0%, and accuracy of 57.3% [155,156]. The authors of another study concluded that endoscopic pancreatic function tests "using brief aspiration periods without marker perfusion or measures

of concentration greatly underestimate pancreatic secretory capacity and misclassify the clinical status of an unacceptably large number of patients [157]."

Whether endoscopic pancreatic function tests have better accuracy using a longer period of pancreatic juice collection remains unclear [153,158]. Preliminary data suggest that an 80-min duodenal collection period following secretin or CCK octapeptide infusion yields high sensitivity for the *peak* juice bicarbonate and lipase concentrations respectively, but is associated with overlap in bicarbonate and lipase values between individuals with and without chronic pancreatitis. Further, the long collection times detract from a major advantage of a short-duration endoscopic pancreatic function test.

Secretin-stimulated magnetic resonance cholangiopancreatography

Secretin-stimulated magnetic resonance cholangiopancreatography (SS-MRCP) is a promising noninvasive test of exocrine pancreatic function. Previously, investigators used semiquantitative measurements to assess pancreatic function, grading the duodenal filling of juice (0–3) based on the furthest downstream location [159–161]. More quantitative measures of pancreatic juice secretion involve translating magnetic resonance signal intensity of fluid in the duodenum and pancreas plotted against time into an actual juice flow rate [162–164]. Unfortunately, the clinical utility of this new procedure remains unclear because only a limited number of patients (nearly all with severe chronic pancreatitis) have been studied [162,164].

The latest modification, diffusion-weighted MRI (DW-MRI), is a calculation of the apparent diffusion coefficient (ADC), which integrates capillary perfusion and molecular diffusion [165]. The hypothesis is that movement of water molecules into the lumen increases during pancreatic secretion and manifests as signal loss on DW-MRI images and hence increased ADC values. Differences in pancreatic ADC values in chronic pancreatitis may be related to diminished blood vessel density [166] and impaired augmentation of pancreatic blood flow in response to secretagogues, described in experimental [167,168] and human [169,170] subjects with chronic pancreatitis. Erturk and colleagues [165] showed that following secretin stimulation, normal pancreatic function is characterized by an early ADC peak, whereas those with substantial alcohol consumption had a markedly delayed peak and those with chronic pancreatitis had no ADC peak. Using a cutoff value of 4 min for determining ADC peaks, the test proved 100% sensitive and 94.7% specific for discriminating the control group from the alcoholics with and without chronic pancreatitis. Although patients with mild to moderate chronic pancreatitis were not included, patients with substantial alcohol consumption and conceivably early chronic pancreatitis had a markedly delayed peak ADC.

Enthusiasm for application of DW-MRCP in clinical practice should be tempered by two major limitations. First, measurement of the ADC peak represents only an indirect assessment of pancreatic function. Secondly, because fewer than 10% of alcoholics develop chronic pancreatitis and reduced pancreatic exocrine function [171], the report that all alcoholics, including those without pancreatitis, had a delayed ADC peak raises questions about whether a low ADC peak truly represents abnormal pancreatic exocrine function. To address these limitations, future investigation of DW-MRCP should include comparative analyses of DW-MRCP with invasive secretagogue-stimulated pancreatic function tests, comparisons between groups of patients with alcoholic and nonalcoholic chronic pancreatitis, and comparisons of test accuracy for patients with mild to moderate and severe chronic pancreatitis.

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D GASTROINTESTINAL FUNCTION STUDIES

140 Natural orifice translumenal endoscopic surgery (NOTES)

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The field of gastrointestinal endoscopy has witnessed major advances since the 1950s. From flexible endoscopy in the 1950s to endoscopic retrograde cholangiopancreatography in the 1970s and endoscopic ultrasound (EUS) in the 1980s, endoscopy has been transformed from a purely diagnostic method to one with significant therapeutic applications. A major advance is the notion of using the flexible endoscope, taking it beyond the beyond the confines of the gastrointestinal tract into the peritoneal and thoracic cavities. Natural orifice translumenal endoscopic surgery (NOTES) offers the exciting potential to be safer, less invasive, and possibly more cost-effective than either traditional open surgery or laparoscopy. The history and principles along with future implications are outlined in this chapter.

Since the first report by Gauderer and colleagues describing a tube-feeding gastrostomy without a laparotomy in 1980 [1], other endoscopic procedures using a transgastric approach have been described [2,3]. However, these procedures have targeted organs that are only in close anatomical relation to the gastric wall. Of the many transformations currently taking place in the field of endoscopy, NOTES stands out and represents a paradigm shift. The traditional view that gastroenterologists should not cause a breach in the integrity of the gastrointestinal wall is challenged by NOTES.

The first report of NOTES was made by Kalloo and colleagues from the Johns Hopkins Hospital in 2000 [4,5]. After standard upper endoscopy, the authors performed gastric wall puncture using a needle knife, extended the incision by balloon dilatation or a pull-type sphincterotome, and advanced a standard gastroscope into the peritoneal cavity in a live porcine model. Examination of the peritoneal cavity along with biopsy of the liver was successful. On completion of the procedure, closure of the gastric wall incision was performed using standard commercially available clips and long-term survival of the animal was successful without significant morbidity or mortality. This was this first study to demonstrate that the peroral transgastric approach to the peritoneal cavity was technically feasible and offered the potential for a wide array of diagnostic and therapeutic interventions.

Cautious but rapid momentum is being gained in the field of NOTES. Since the first publication, several more studies have been performed. Liver biopsy, tubal ligation, lymphadenectomy, gastrojejunostomy; cholecystectomy, and partial hysterectomy have been performed in animal models with successful survival [5–13]. Published nonsurvival studies include reports of splenectomy and abdominal herniorrhaphy [14]. There are also unpublished cases from India of transgastric appendectomy and tubal ligation [15]. Human NOTES procedures have also been performed under institutional review board (IRB)-approved protocols in different parts of the world.

The clinical practicality of NOTES is properly challenged by questions that surround the basic principle of NOTES, namely that access to the peritoneal cavity can be obtained by causing a breach in the gastrointestinal tract and not in the abdominal wall. Some of the critical questions being raised include the following.

- Why perform a perforation of the intact gastrointestinal tract when one can perform laparoscopy or minilaparotomy?
- What is the benefit of this type of surgery compared with currently available minimally invasive techniques?
- Can the procedure be performed without the introduction of gastrointestinal tract flora and hence under aseptic technique?
- Does it have problematic effects on the patient's physiology?
- Can one attain reliable closure of the gastric defect?
- Who will perform NOTES and how will training occur?
- Can it be applied to clinical practice at this stage?

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Potential benefits of NOTES over current surgical techniques

NOTES enables intraperitoneal surgical interventions with the elimination of abdominal wall incisions, resulting in no remnant scars and obviating the possibility of abdominal wall infection and hernia formation. Theoretically, it also allows surgery that results in minimal pain and less potential for adhesion formation compared with currently available surgical approaches.

Several studies that compared laparoscopy to open surgery have demonstrated that laparoscopy results in a reduced neuroendocrine stress response and inflammatory response, as measured by levels of circulating cytokines, and a preserved immune response, as measured by delayed-type hypersensitivity [16–18]. The size of incision, extent of abdominal wall manipulation, and trauma all contribute to the immunosuppression. Laparoscopy also fares better when one evaluates the likelihood that it has an adverse effect on pulmonary function, which is one of the major morbidities that plague open surgery. Several studies have demonstrated that significant deterioration of pulmonary function and atelectasis occurs with open surgery compared with laparoscopy [19]. The conclusion reached from these studies is that smaller incisions and less peritoneal manipulation will result in better postoperative outcomes. Therefore, from a minimally invasive standpoint, there is a conjectural rationale for NOTES, since it would result in no abdominal wall incisions.

Open surgery leads to more damage to the peritoneum and hence more adhesions compared with laparoscopy [20,21]. Subsequent apposition of peritoneal surfaces with inadequate fibrinolytic activity, which is known to exist after surgery, leads to the formation of adhesions [22]. The major consequences of adhesions, such as infertility, chronic abdominal pain, and intestinal obstruction, lead to increased morbidity and also increase the difficulty and risk of repeat surgery. NOTES should result in no or minimal damage to the peritoneum and hence might have a lower likelihood of adhesion formation than open surgery or even, theoretically, laparoscopic surgery. Studies are needed to confirm this supposition.

Laparoscopic techniques were readily accepted by patients because it resulted in minimal scarring, rapid recovery time, and less cost [23,24]. Applying the same inference, the absence of abdominal wall incisions with subsequent reduced pain, less morbidity, faster rehabilitation, shorter hospital stay, and the economic benefit of returning to work earlier may also be more attractive to patients. Currently, advanced endoscopic procedures can be performed using deep sedation, which raises the possibility of NOTES being performed without general anesthesia and endotracheal intubation, unlike laparoscopic and open surgery.

In addition to the above-mentioned factors, the location of many peritoneal organs allows better access via the translumenal route and will thus make NOTES advantageous. Since the first description of transgastric peritoneoscopy, several investigators have confirmed the ease of attaining good intraabdominal visibility and manipulation of organs using currently available endoscopic accessories [8]. NOTES might also be beneficial in environments where performing abdominal wall incisions is less desirable, for example in underdeveloped countries, situations of acute abdominal trauma, and locations remote from health-care facilities. In these situations, NOTES can theoretically provide a safe approach to triage and allow immediate life-saving procedures, providing adequate sterility can be maintained.

Risk of infection in NOTES

The initial animal laboratory experiments resulted in occasional and insignificant intraperitoneal microabscesses [4]. This resulted in the implementation of sterile techniques, including gastric lavage with antiseptic solution, administration of prophylactic antibiotics, and use of sterile instruments. These measures appear to have resulted in better outcomes in subsequent translumenal endoscopic surgical experimental models. The study by Hazey and colleagues evaluated bacterial contamination of the peritoneal cavity after gastrotomy in patients undergoing laparoscopic gastric bypass. Despite the presence of bacteria in the peritoneal cavity after the gastrotomy (from zero colony-forming units [CFU] before gastrotomy to 24 720 CFU post procedure), no clinically evident peritonitis was seen in any of their patients. The authors concluded that the presence of peritoneal contamination after gastrostomy did not result in adverse clinical sequelae [25]. Another study in humans that involved the insertion of an endoscope into the peritoneal cavity along with liver biopsy did not result in any clinical infection in the participants [26]. Ongoing work evaluating the risk of infection and its prevention has demonstrated that the risk of infection depends on the site of access, type of access, and closure methods. When the transgastric access site has been used, the risk of infection has ranged between 0 and 40% [9,10]. These wide rates of infection are a result of a heterogeneous approach to sterilization and use of antibiotics. The transcolonic route has also been plagued with a high rate of infection due to leaks and local peritoneal contamination. In the study by Pai and colleagues, the rate of infection was found to be 20% [27].

The source of infection in NOTES will depend on the route of access and sterility of the accessories used. The transgastric route to the peritoneal cavity has potential for contamination from microorganisms in the buccal cavity, esophagus, gastric lumen, and refluxate from proximal small bowel. The transcolonic and transvaginal routes contain bacterial flora, which if introduced to the sterile peritoneal cavity can result in dire consequences. One study in a porcine model demonstrated that attaining maximal sterile conditions, including steriliza-

tion of equipment and accessories and use of combination systemic and local antibiotics, effectively prevented infection regardless of the closure method used during transgastric surgery [28]. The recommendation based on currently available studies is to attain maximally sterile preoperative conditions regardless of route of peritoneal entry, with secure closure of the access site.

Closure of the gastrointestinal wall after NOTES

In the existing literature, closure of the gastric wall has been performed with clips, endoloops, tags, bioabsorbable plugs, and stapling devices [27,29–32] (Figs 140.1 and 140.2). Kalloo and colleagues did not use any closure device after experiments with transgastric tubal ligation and found no adverse outcome [5]. This is in keeping with what is known about the closure rates of breaches in the gastric wall from various ailments. Studies that have examined closure rates for gastric ulcers randomized to surgery or conservative management in humans have shown that spontaneous closure does indeed take place, with no difference in morbidity or mortality between the two groups [33]. However, other investigators have found that use of closure methods resulted in better outcomes compared with spontaneous closure.

The use of clips as a method of closure of gastric perforations did not start with NOTES (Fig. 140.1). Several studies in humans have shown successful and complete closure of gastric perforations following endoscopic mucosal resection using endoscopic clips [34,35]. Interestingly, an ex-vivo study of leak tests on the different gastric closure methods for NOTES found that the closure afforded by clips was less secure than closure with a prototype full-thickness suturing device or hand-sutured gastric stoma [36].

Figure 140.1 T-bar closure of a gastrotomy.

Figure 140.2 Clip closure of gastrotomy.

Endoloops have also been used successfully as a method of attaining gastric closure (Fig. 140.2). Pai and colleagues first used endoloops as a method of closure following transcolonic peritoneal exploration experiments [27]. Subsequently, a simple variation of gastrotomy site closure using endoloops was reported by Katsarelias and colleagues [29]. These authors used one endoloop on either side of the incision site to create a fold. The two folds were then grasped using a forceps passed through an endoloop. The endoloop was deployed securing the two sides of the incision as one.

Tags and tissue-anchoring devices are gaining popularity for NOTES. This technique uses two sutures individually deployed transmurally and then secured with an anchoring system. This system is being currently refined and can now be deployed through a 2.8-mm accessory channel of an endoscope [30,37]. One downside of this system is that transmural placement of the tag is performed blindly and may lead to adjacent organ damage. The use of EUS for safe deployment of these tags has been described but more research is needed to evaluate the safety margin of this promising technique.

NOTES: current status

Leading figures from the American Society of Gastrointestinal Endoscopy (ASGE) and the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES) convened in New York City in July 2005 to form a working group, the Natural Orifice Surgery Consortium for Assessment and Research (NOSCAR). At this meeting the group discussed issues for the further development of translumenal endoscopic surgery

and listed steps and guidelines needed. A summary of this meeting was jointly published by ASGE and SAGES in February 2006 as a "white paper" [15].

NOSCAR has made recommendations about the direction of future research and steps that need to be taken for the safe introduction of NOTES to clinical practice. Summarized below are significant published studies that address important aspects of NOTES.

Transgastric fallopian tube ligation

Fallopian tube ligation with survival in a porcine model has been performed successfully [6]. In this study of six pigs, tubal ligation was accomplished using an endoscopic detachable loop ligating device (Endoloops, Olympus Optical Ltd, Tokyo, Japan) applied via transgastric endoscopic access to the peritoneal cavity. Endoscopic visualization of pelvic anatomy was excellent. Orientation inside the peritoneal cavity and identification of pelvic structures was technically simple. There were no adverse effects or complications over a 2–3-week survival period. Postprocedure hysterosalpingograms demonstrated complete occlusion of the fallopian tube with histological evidence of obliteration of the lumen with chronic inflammation and no evidence of abscesses. During this experiment, a combination of high-level disinfection, gas sterilization of all equipment, topical and parenteral antibiotics, and a sterile overtube was utilized, resulting in no infectious complications. Furthermore, interventions in the pelvis via a transgastric approach was noted to be technically easier than in other parts of the peritoneal cavity because of the "straight shot" from the gastric wall incision toward the pelvis that eliminates the need for endoscope retroflexion.

Transgastric pelvic organ resection

Wagh and colleagues explored the peritoneal cavity and resected the pelvic organs in nine pigs [10]. They used glutaraldehyde-sterilized single- and double-channel gastroduodenoscopes and accessories and perioperative systemic antibiotics to minimize infection. Similar to our experiments, they applied Endoloops (Olympus Optical Ltd) to the mesentery and then resected the tube along with portions of the uterine horn and ovary. The authors reported no complications after surgery, with all animals tolerating the procedure well. Three animals that underwent survival surgery had no adverse outcome after 24 h.

The same group reported a similar transgastric oophorectomy and tubectomy with an extended survival period of 2 weeks. During this 2-week period there were no adverse clinical events in all animals. Necropsy with histopathological assessment of resected specimens confirmed oophorectomy and tubectomy [9].

Transgastric gastrojejunostomy

Several studies have described endoscopic gastrojejunostomy in a porcine model. The first successful creation of a per-oral endoscopic gastrojejunal anastomosis was published by Kantsevoy and colleagues in 2005 [7]. All procedures were performed with disinfected and gas-sterilized endoscopes and accessories under aseptic conditions. Systemic perioperative antibiotics and gastric lavage with antibiotic solution were used to minimize infection. An endoscopic needle-knife and pull-type sphincterotome were used to create a gastric incision. A small-bowel loop was grasped, pulled into the gastric lumen, and connected to the gastric wall by using a prototype endoscopic suturing device (Eagle Claw, Olympus Optical Ltd). Then, a 15-mm-long incision was made on the small bowel loop by using the needle-knife and the open ends of this incision were connected to the gastric wall with more sutures to complete the gastrojejunostomy. Two animals survived for 2 weeks. Postmortem examination 2 weeks after creation of the gastrojejunostomy revealed the gastrojejunal anastomosis on the border of the anterior abdominal wall and the greater curve of the stomach and normal-appearing small and large bowel and stomach (Fig. 140.3).

A second study by Bergstrom and colleagues published in 2006 confirmed the feasibility of fully endoscopically created transgastric gastrojejunostomy [11]. The authors performed 12 gastrojejunal anastomoses in a porcine model. Access to the peritoneal cavity was achieved in a similar manner to the

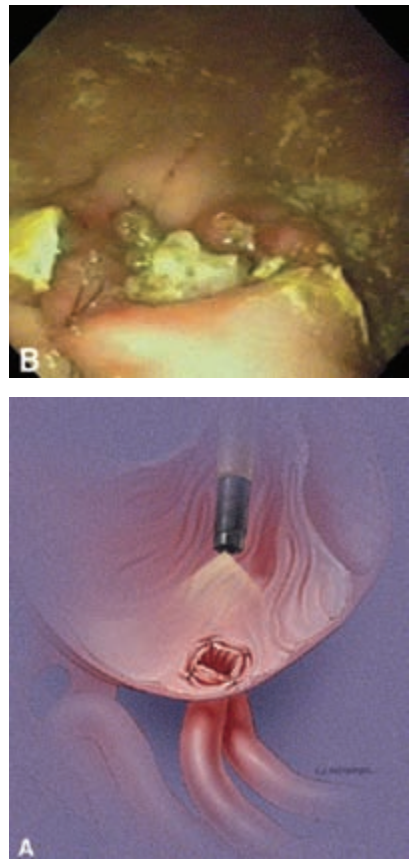

Figure 140.3 Endoscopic gastrojejunostomy.

one described by Kantsevoy and colleagues. The authors then used a snare-over-forceps method to retrieve a loop of small bowel into the gastric lumen for suturing. Incision of the small bowel was made with a needle-knife to open the anastomosis. Suturing was achieved using a new technique in which a metal tag attached to 3.0 polypropylene thread was placed into a 19G Echotip EUS needle, which was then placed into the tissue. The thread and metal tag were released from the needle into the tissue by advancing the stylet. Several (six to eight) placements of pairs of stitches were performed through the small bowel and the gastric wall, after which the pair was tied forming a secure gastrojejunostomy. Interestingly, anastomoses were placed either close to the cardioesophageal junction for bariatric purposes or in the antrum for pancreatic bypass. Survival studies in six pigs demonstrated patent anastomosis at 7–10 days following the procedure.

Biliary surgery

The first transgastric cholecystectomy and biliary anastomosis was published by Park and colleagues in 2005 [8]. They performed acute experiments in a porcine model that demonstrated the feasibility of transgastric cholecystectomy and biliary anastomosis. A single endoscope with percutaneously inserted grasping forceps or two flexible endoscopes were used. Successful cholecystectomies were performed in eight animals within a period of 40–150 min. Cholecystogastric anastomosis was performed successfully in three pigs. The authors accomplished biliary surgery using the transgastric route but emphasized the limitations of flexible endoscopes and instruments, including lack of adequate exposure of the biliary tree in a retroflexed position and reduced triangulation.

Pai and colleagues also reported a survival study that confirmed the feasibility of endoscopic translumenal cholecystectomy [27]. They performed five survival procedures. All pigs were prepared with tapwater enema and colonic antibiotic lavage with a Betadine rinse. The authors used a transcolonic approach to the peritoneal cavity that enabled a "straight shot" toward the gallbladder, making the procedure technically simple. After placement of a double-channel endoscope, an anterior incision was performed in the colon at 15–20 cm from the anus. Successful cholecystectomy was then performed using currently available endoscopic instruments. One animal (20%) developed peritonitis due to incomplete closure of the colonic incision. The authors not only concluded that the transcolonic approach provided a better approach to biliary surgery than the transgastric route but also emphasized the need to attain complete closure of the colonic incision for better outcome.

Transgastric splenectomy and hernia repair

A feasibility study to assess translumenal splenectomy was published by Kantsevoy and colleagues in 2006 [14]. A gastric wall puncture was made using a needle-knife and was extended with a pull-type sphincterotome to create a 1.5-cm incision. After entering the peritoneal cavity, dissection of the spleen from stomach and omentum was performed. Ligation of the splenic artery and vein was done using currently available endoscopic Endoloops and clips. The ligated blood vessels were then cut, freeing the spleen from its attachments. After the spleen was mobilized, the gastric incision was enlarged with the sphincterotome and the spleen was pulled into the stomach. It was found that not only can resection of a large organ be performed using this route but also adequate hemostasis is achievable during translumenal endoscopic procedures.

Using the idea that it may be easier to perform abdominal wall hernia repair using a transgastric approach, Jagannath and colleagues reported, in abstract form, acute experiments in a porcine model that evaluated the feasibility of a transgastric endoscopic approach for repair of abdominal wall hernias [38]. After gaining transgastric access to the peritoneal cavity, an anterior abdominal wall incision was made with a needle-knife to create an animal model of abdominal wall hernia. Using a prototype endoscopic suturing device (Eagle Claw, Olympus Optical Ltd), a 3-cm incision of the abdominal wall (hernia model) was closed without any difficulties. The authors concluded that transgastric endoscopic repair of abdominal wall hernias is feasible and may be technically easier than laparoscopic surgery.

Transesophageal mediastinoscopy

A group from the Mayo Clinic performed mediastinoscopy using a mucosal flap technique. The authors created a submucosal space by injecting high-pressure carbon dioxide that was then accessed using a cap-fitted endoscope. Access to the mediastinum was then gained at a place distant from the site of entry of the endoscope. After the procedure this flap acted as an additional safety barrier and the mucosal entry site was closed using clips [39] (Figs 140.4 and 140.5).

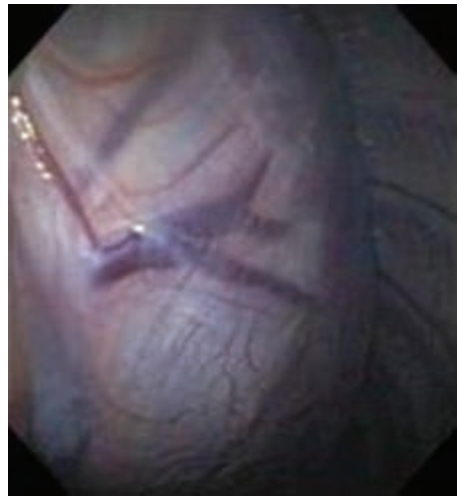

Figure 140.4 Endoscopic view of the heart.

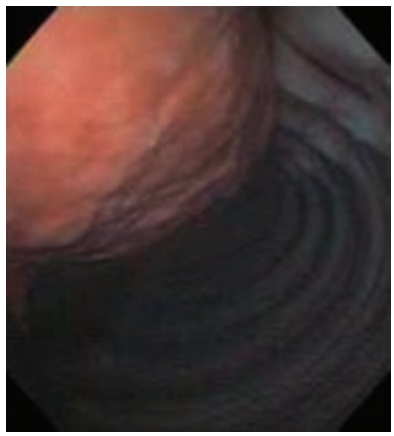

Figure 140.5 Endoscopic view of the hemithorax.

Nongastric translumenal access sites

Because of the limitations of the transgastric route for some procedures, various alternative access sites have been described. Lima and colleagues demonstrated that a different point of access could be used to facilitate entrance to the peritoneal cavity [13]. A ventral cystotomy was made using cystoscopic guidance. Under wire guidance, placement of an overtube followed by a cystoscope into the peritoneal cavity was performed. The authors were able to perform liver biopsy and peritoneoscopy in five survival swine models. Closure of the access site was not performed but the authors left a catheter to assist in decompression of the bladder and hence avoid urinary intraperitoneal leakage. Another study by the same authors demonstrated the feasibility and safety of transvesical thoracoscopy in an animal model. Thoracoscopy with pleural and lung biopsies was performed using a 9.8Fr ureteroscope [40]. The potential advantage of this method is mainly that the bladder is a sterile space and transvesical access may lead to less intraperitoneal infectious complications. Some of the drawbacks of this technique include limited robust accessories that can perform major surgical procedures.

The transcolonic access site is mainly used to obviate the limitation posed by the transgastric route during procedures on organs close to the gastric access site and in the upper abdominal quadrants. Even though results are encouraging, measures to avoid peritoneal contamination and achieve secure closure remain problematic. One study by Wilhelm and colleagues evaluated the use of a rigid sterile overtube to provide a contaminant-free conduit for the endoscope and secure closure [41]. The authors instilled an antibacterial solution into the peritoneal cavity to provide a preliminary hydroperitoneum using a percutaneously placed Veress needle. Postinstallation endoscopic ultrasonography was performed to assist in the identification of a site where there was no adjacent small bowel loops. This site was secured with a pursestring and used to "prolapse" the rectum. Breach of the wall was then achieved under direct visualization to attain a transcolonic route. After peritoneoscopy, closure was achieved using the preplaced pursestring in addition to a surgical stapler. This approach, though promising, needs to be corroborated by further studies.

NOTES: human experience

The first description of a human NOTES procedure, namely transgastric removal of an inflamed appendix, was reported by Rao and Reddy during Digestive Diseases Week in 2004. They performed transgastric peritoneoscopy on a patient using standard endoscope and accessories with no reported complications after the procedure. Following this procedure, NOSCAR has stressed the need for all human studies to be performed with local IRB approval and under a human trial registry. Taking this into consideration several human procedures, some of which are summarized below, are taking place under the auspices of an IRB.

Though not a clinical trial, Marks and colleasgues reported the rescue of a dislodged percutaneous endoscopic gastrostomy (PEG) tube by insertion of an endoscope into the peritoneal cavity and subsequently evacuating intraperitoneal fluid and repositioning the dislodged tube [42]. Subsequently, the first human trial was reported by Hazey and colleagues [43]. They performed peroral transgastric peritoneoscopy in 10 patients who were scheduled to undergo diagnostic laparoscopy. Furthermore, they compared diagnostic findings, operative time, and clinical course between the two modalities. All the patients underwent successful laparoscopy followed by transgastric peritoneoscopy, with a mean duration of 12.3 and 24.8 min, respectively. Closure of the gastric access site was performed by standard surgical and not endoscopic techniques. The authors concluded that human transgastric peritoneoscopy is safe and feasible, with the caveat that more instrumentation and technological innovations are needed before full clinical application.

Transvaginal cholecystectomy has been reported from several independent groups. Bessler and colleagues performed hybrid transvaginal and laparoscopic cholecystectomy on a 66-year-old patient with symptomatic gallstones [44]. Once the endoscope was placed in the peritoneal cavity with the aid of laparoscopy, a shape lock device was used to stabilize and fix the shaft of the endoscope while permitting controlled movement of the tip, allowing stability and precise dissection. In addition, standard laparoscopic clips were deployed through a trocar to ligate the cystic duct.

Marescaux and colleagues from IRCAD-EITS (Institute of Digestive Cancer Research/European Institute of TeleSurgery) performed a hybrid laparoscopic–endoscopic transvaginal cholecystectomy on a 30-year-old patient with symptomatic cholelithiasis [45]. Using a standard double-channel flexible

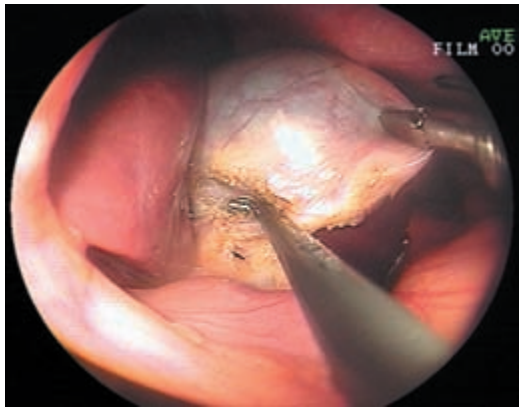

Figure 140.6 "Straight shot" to gallbladder during human transvaginal peritoneoscopy. Courtesy of Professor Ricardo Zorrón.

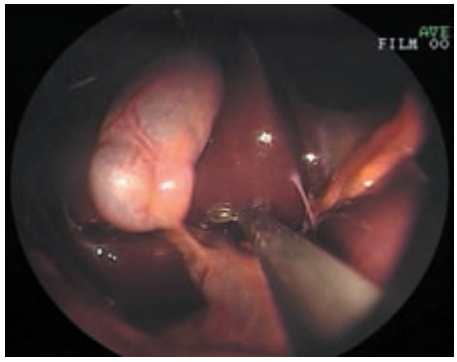

Figure 140.7 Successful human transvaginal cholecystectomy.

endoscope and standard endoscopic instruments along with a 2-mm laparoscopic needle port they performed a successful cholecystectomy using only the endoscope to perform gallbladder dissection. The laparoscopic port was used to give retraction and maintenance and monitoring of pneumoperitoneum. Other investigators have also performed a similar or modified version of transvaginal cholecystectomy with success, again proving the feasibility and safety of a hybrid transvaginal approach for gallbladder surgery [46,47] (Figs 140.6 and 140.7).

Experience with human NOTES has also expanded into other nongastroenterological fields such as urological surgery. A report by Branco and colleagues reported combined transvaginal and laparoscopic nephrectomy of a nonfunctioning kidney in a 23-year-old patient suffering from recurrent urinary tract infection and flank pain. The patient had an uneventful postoperative course and was discharged 12 h after the procedure [48].

All these important steps demonstrate that safe translumenal surgery can take place only if combined with laparoscopic techniques until further research and development provide robust and novel endoscopes and accessories.

Future applications of NOTES

Peritoneal interventions in the intensive care unit

Patients in the intensive-care setting have multiorgan dysfunction that precludes transportation and major surgical intervention. NOTES may avoid transportation to an operating room, allowing for bedside intervention. One example that has been described in the laboratory is diaphragmatic pacing for patients who are difficult to wean from ventilators [49]. NOTES may become a more practical approach for diagnosing ischemic bowel in the intensive-care patient who has contraindications for more definitive imaging such as computed tomography or magnetic resonance imaging [50].

Prehospital management of trauma

Prehospital management of critical patients has resulted in improved outcomes. This is mainly due to the proper resuscitation of patients and appropriate triage [51]. Despite these developments many patients with blunt or other urban-related injuries may not survive because of improper triage and stabilization. NOTES could become an integral and critical step in this setting since all instruments that are required are portable and can fit into current emergency care vehicles. Imagine a scenario where a patient involved in a motor vehicle accident with splenic trauma is given proper triage and hemostasis stabilization using NOTES before reaching the hospital, where traditional and more definitive surgical approaches can be performed. Of course, this could only be attained if there are appropriate tools to enable easy access and closure of the access site with minimal risk of infection and without adding time needed to transport the patient to the hospital.

Acute management of blunt trauma

The use of NOTES as a method for immediate assessment of organ injury and repair is theoretically possible. Its minimally invasive nature, due to the elimination of an abdominal incision and need for general anesthesia, makes NOTES an attractive approach in the prehospital trauma/disaster setting.

In a preliminary study performed at our laboratory we were able to demonstrate the feasibility of NOTES in the assessment of penetrating abdominal injury. We performed a controlled injury to intraabdominal organs (liver, spleen, kidney and small bowel loops) under laparoscopic guidance using laparoscopic shears. Transgastric peritoneal access was then attained using a PEG-like approach by an operator blinded to the sites of organ injury. The findings using NOTES were compared with those using laparoscopy. The peritoneal cavity in all animals was systematically examined, including the anterior abdominal wall, diaphragmatic dome, liver, spleen and both kidneys. The mean duration from time of entry to the peritoneum to identification of lesion was

11 min. Mean duration to identification of site of injury was 12 min for the liver, 15 min for the spleen, 17 min for the small bowel, and 6 min for the kidneys. The time required to identify anterior liver surface injury was significantly shorter than the time required for the identification of small bowel injury. Based on these results, we concluded that NOTES provides rapid and accurate identification of organ injury for penetrating wounds to the abdominal viscera [52].

Our group has also assessed the feasibility of achieving hemostasis after organ injury using nonthermal hemostatic mechanisms. In this study three nonthermal methods of achieving hemostasis were compared. The first group was randomized to QuikClot (QC; Z-Medica, Wallingford, CT), a granular zeolite powder with 1% residual moisture that, when placed on a bleeding wound, adsorbs water in an exothermic reaction, thereby concentrating platelets, erythrocytes, and clotting factors at the site of application. It has been tried on the battlefield with excellent success in achieving hemostasis. Several studies, including a swine liver injury model, have also shown its effectiveness as a hemostatic agent in life-threatening bleeding. The second group was randomized to oxidized regenerated cellulose (ORC), which is also used adjunctively in surgical procedures to assist in the control of capillary, venous, and small arterial hemorrhage when ligation or other conventional methods of control are impractical or ineffective. The third groups of animals was randomized to SHISH, a novel bioabsorbable hemostatic agent (Cook bioengineering prototype material).

The anterior liver surface was then lacerated using a laparoscopic shear to create a grade three (> 3 cm) liver laceration. Modified endoscopic delivery systems were used to deliver the hemostatic agents. The ORC was placed effectively, covering the site of injury in all animals. Delivery of QC granules to the site of bleeding was also straightforward. Accurate placement of the granules to the site of injury was achieved in all animals. In the third group of animals, SHISH was also able to be applied with resulting hemostasis in all animals. There was no statistically significant difference in the time to achievement of hemostasis between the QC and ORC group ($P = 0.33$). The conclusion from these studies is that intraperitoneal hemorrhage can be easily and quickly controlled with these novel endoscopic therapies [53].

NOTES: evolving beyond the traditional operating room

In today's world, where building future operating rooms is a multibillion dollar industry, NOTES maybe a cost-effective solution. Given that the development of accessories and other integral components of NOTES needs to take place, by keeping the focus of sterility to the gastrointestinal tract, instruments and accessories, the need for the sterile operating room environment could be eliminated. The potential cost reduction is staggering, since the cost of using an operating room is obviated as is the use of transportation staff, operating room energy, and operating costs. The fact that NOTES has the potential to be performed under deep sedation rather than general anesthesia will have a great impact in avoiding postoperative ventilator complications including ventilator dependency.

Robots for NOTES

The concept of deploying a mobile robot via NOTES into the peritoneal cavity may appear very futuristic. This limitation may be solved by using robotics and this technique has been shown to be feasible by Rentschler and colleagues [54]. A 12-mm-diameter in vivo robot was advanced into the gastric cavity using a sterile overtube and was able to traverse within the cavity under endoscopic guidance. Using its helical wheel the robot was able to navigate the gastric lumen with no apparent tissue injury. In the peritoneal cavity, the robot successfully navigated and maneuvered several organs, including the liver and small bowel. Development of an in vivo robot with camera and multipurpose arms capable of performing several tasks in addition to endoscopic imaging will enable performance of moderately complex surgery such as cholecystectomy. Furthermore, a robot with visual capabilities will help to overcome the problem of spatial orientation.

Intrauterine fetal interventions

Although transabdominal laparoscopic fetoscopy has proven to be a useful technique in human fetal surgery for such diseases as twin–twin transfusion syndrome and spinal meningomyelocele, it only allows anterior access to the uterine cavity and is limited by the use of rigid instrumentation. In addition, the performance of intraperitoneal procedures by the traditional percutaneous route can be followed by wound dehiscence and induce preterm labor. Theoretically, NOTES can provide unparalleled access to the uterine cavity because of its flexibility and because the transgastric route allows a "straight shot" toward the uterus. Moreover, the absence of abdominal wall incision and general anesthesia also provide an added benefit for NOTES. Based on these assumptions, our group have evaluated the feasibility of NOTES for uterine interventions in pregnant sheep. During this acute nonsurvival experiment NOTES allowed visualization of the anterior, posterior, and lateral walls of the uterus. In addition, all intraperitoneal organs could be identified and were easily accessible. Using EUS, the fetus, various body parts, and the placenta could be easily identified. Amniocentesis and intracardiac fetal interventions were technically feasible with the EUS scope in the peritoneal cavity [55].

Spinal procedures

Surgical spinal procedures most commonly include transthoracic and posterolateral percutaneous approaches. Open surgical techniques require separation of musculoskeletal structures and traction of nerve roots to create an opening large enough to accommodate surgical tools. The morbidities

associated with these surgical approaches include postsurgical neuralgia resulting from traction injuries to nerve roots, lacerations of the dura mater, scars from skin incisions, and muscular atrophy or trauma. Minimally invasive surgical techniques, including thoracoscopy and video-assisted thoracic spine surgery, have reduced the extent of percutaneous incisions and opening of the chest wall. Nevertheless, the consequences of percutaneous access are not totally avoided and complications such as lung atelectasis and retropleural effusions from single lung ventilation are additional morbidities [57].

NOTES provides direct anterior access to the vertebral column by transgastric and transesophageal approaches. In laboratory experiments with NOTES, we found that the proximity of the esophagus to the spine allowed immediate access to the posterior mediastinum and excellent visualization of the entire thoracic vertebrae and intervertebral spaces. This innovative approach to the anterior vertebral column allows the development of novel spinal interventions under direct endoscopic guidance, such as vertebroplasty and kyphoplasty for osteoporotic or pathological vertebral bone fractures, discectomies and interbody fusion for herniated discs, and release of the anterior ligament at different levels of the vertebral column in patients with severe scoliosis. In addition, the lumbosacral spine could also be approached for anterior endoscopic procedures via transgastric access. The advantages of NOTES for spinal interventions are similar to those for anterior laparoscopic spinal surgery but without the limitations of rigid instrumentation. These benefits include maintenance and ease of restoration of intervertebral disc height, avoidance of bone removal from the spine (an integral component of posterior spinal surgery), and preservation of normal spinal anatomy since this approach takes advantage of normal tissue planes with no removal of bone tissue. In addition the complications of posterior spinal surgery, such as injury and damage to nerves from manipulation and retraction of nerves and hematoma around nerves that may cause scarring and chronic pain, may be reduced [58].

NOTES: the road toward clinical applicability

For NOTES to evolve to clinical practice, it will require modification of current endoscopes and development of accessories to overcome the limitations of current endoscopes. The most noticeable limitations include stability of the instrument on retroflexion, small size of the instrument channel, and spatial incongruity [15]. Hybrid techniques combining laparoscopy with NOTES are currently the best solution. The hybrid approach can provide safe intraperitoneal access along with the essential triangulation and retraction. Once experience is gained, coupled with the development of new accessories, pure NOTES can then be tested against currently available techniques. Also important are issues of training, since acquiring advanced endoscopic skills are of paramount importance. Training physicians to perform NOTES will need to incorporate both surgical and advanced endoscopic training. This will result in development of hybrid surgical and endoscopic training programs that will blur the lines of delineation between specialties, challenging traditional training programs. It is equally important that gynecologists and urologists become familiar with NOTES in these early stages of development since successful NOTES may have implications to their routine practice.

In summary, NOTES offers the benefit of incisionless surgery, potentially leading to rapid recovery, less postoperative pain, no abdominal wall scar or hernia formation, and fewer adhesions. It will have definite advantages in situations where the transabdominal route is not optimal, such as in patients with marked obesity where the risk of postoperative wound complications is high and in patients with anterior abdominal wall infection or severe scarring. NOTES provides an exciting opportunity to improve peritoneal intervention using the skills, imagination, and ingenuities of gastrointestinal endoscopists and endoscopic surgeons.

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